Abstract Book

German Biophysical Society Meeting

September 22–25, 2024
Leipzig, Germany

DGfB Deutsche Gesellschaft für Biophysik e.V.

UNIVERSITÄT LEIPZIG

Biennial Meeting of the German Biophysical Society LEIPZIG

Meeting of the German Biophysics Society 2024 Abstract Book

Contents

Program

22 September, 2024

5:00 pm – 7:30 pm

Paulinum

OC | Opening Ceremony

Opening Ceremony, 22 September 2024

05:00 pm Leipzig, the City of Music, Organ (**Stefan Altner**)

- JOHANN SEBASTIAN BACH (*21.03.1685, Eisenach; †28.07.1750, Leipzig)
  - Pièce d'Orgue BWV 572
  - Très vitement – Gravement – Lentement

05:10 pm Welcome from the president of the DGfB (**Klaus Gerwert**)

05:15 pm Welcome from the local organizers (**Daniel Huster**)

05:25 pm Leipzig, the City of the Peaceful Revolution

- **Gisela Kallenbach** | *What happened in the 1980s that triggered the peaceful revolution?*

05:40 pm Leipzig, the City of Music, Organ (**Stefan Altner**)

- MAX REGER (*19.03.1873, Brand/Oberpfalz; †11.05.1916, Leipzig)
  - Benedictus aus op. 59, Nr. 9 *(in the version/edition of Karl Straube)*
  - Adagio – Vivace assai – Adagio

05:45 pm Leipzig, the City of the Peaceful Revolution (**Gisela Kallenbach)**

- **Gisela Kallenbach** | *Leipzig and East Germany after the Reunification*

05:55 pm Leipzig, the City of Music, Organ (**Stefan Altner**)

- JOHANN SEBASTIAN BACH
  - Fantasia BWV 542,1

06:00 pm Leipzig, the City of Science

- **Johannes Krause** | The Journey of Our Genes: a Story About Us and Our Ancestors

06:25 pm Leipzig, the City of Music, Organ (**Stefan Altner**)

- GEORG FRIEDRICH HÄNDEL (*23.02. jul. / 05.03.1685 greg., Halle (Saale); †14.04.1759, London)
  - “Halleluja” – from Oratorio „Messiah“ HWV 56 (1743) – transcription for Organ

06:30 pm Welcome Address from the President of the Indian Biophysical Society, **Sudipta Maiti**

Sudipta Maiti | *Intravesicular Serotonin Facilitates Exocytosis by Promoting Membrane Disorder*

07:00 pm **Anne Imberty |** *Sweet-Talk Between Pathogens and Hosts – from Structural Glycobiology to Antiadhesive Strategies*

07:30 pm Get together, Foyer Paulinum

Intravesicular serotonin facilitates exocytosis by promoting membrane disorder

Debsankar Saha Roy[2], Ankur Gupta[2], Aditi Verma[2], Ankur Chaudhury[2], Parth S. Nayak[2], Sashaina Fanibunda[1], Vidita Vaidya[1], **Sudipta Maiti**[2]

[1] Tata Institute of Fundamental Research, Mumbai, India; [2] Tata Institute of Fundamental Research, Chemical Sciences, Mumbai, India

The research was supported by grant no. RTI4003 from DAE, Govt. of India.

Introduction
Monoamine neurotransmitters, in particular serotonin, can bind to and alter the properties of artificial lipid membranes. Here, we probe whether this also affects the membranes of serotonergic vesicles of live neuronal cells, whose lumens contain a high concentration of serotonin, and whether that can affect the exocytosis probability.
Methods
We built a microscope that could quantify vesicular serotonin concentration using label-free three-photon imaging, and could simultaneously probe vesicular membrane fluidity using confocal spectral imaging of lipophilic dyes.
Results
We find that vesicular membrane fluidity increases with intravesicular serotonin concentration. Upon depolarization, this increased fluidity facilitates the exocytosis of mature vesicles that contain higher serotonin concentrations.
Conclusions
We have discovered a membrane-mediated coupling between intravesicular serotonin and the vesicular exocytosis probability. This provides a mechanism by which neurons can avoid releasing partially filled vesicles and suggests a receptor-independent membrane-mediated pathway of serotonin action.

References

[1] Saha Roy et al., Serotonin Promotes Vesicular Association and Fusion by Modifying Lipid Bilayers, The Journal of Physical Chemistry B, (2024), 128(20):4975-4985

[2] Huster, D., Maiti, S., and Herrmann, A., Phospholipid Membranes as Chemically and Functionally Tunable Materials, Adv. Materials, (2024), https://doi.org/10.1002/adma.202312898

[3] Saha Roy et al., Membrane-Mediated Allosteric Action of Serotonin on a Noncognate G-Protein-Coupled Receptor, J. Phys. Chem. Lett. (2024), 15, 6, 1711−1718

[4] Gupta, A. et al., The unusual robustness of neurotransmitter vesicle membranes against serotonin-induced perturbations, The Journal of Physical Chemistry B 127 (9), 1947-1955 (2023)

[5] Dey, S. et al., Altered Membrane Mechanics Provides a Receptor-Independent Pathway for Serotonin Action, Chem. Eur. J., 2021, 27, 1–10

Sweet-talk between pathogens and hosts – from structural glycobiology to antiadhesive strategies

Anne Imberty

Université Grenoble ALpes, CNRS-CERMAV, Grenoble, France

A large number of pathogenic microorganisms display receptors for specific recognition and adhesion to the glycoconjugates present on human tissues. In addition to membrane-bound adhesins, soluble lectins are involved in lung infections caused by the bacteria *Pseudomonas aeruginosa* and *Burkholderia cepacia* and by the fungus *Aspergillus fumigatus* that are responsible for hospital-acquired diseases. Structural glycobiology on bacterial lectins complexed with human glycans give information on binding site sand multivalency of lectin and neutron crystallographie has been used for fine analysis of hydrogen bond network and role of calcium ions.Accumulated knowledge about the structures of the lectins and the interactions with host glycoconjugates has led to the design of powerful glyco-derived inhibitors that can serve as antimicrobial therapeutic agents, as a complement to or an alternative to antibiotic therapy. Several strategies are developed with development of glycoderivatives and/or multivalent glycostructures.

23 September, 2024

8:30 am – 10:30 am

HS 9

PL-II | Plenary II

PL-II-01

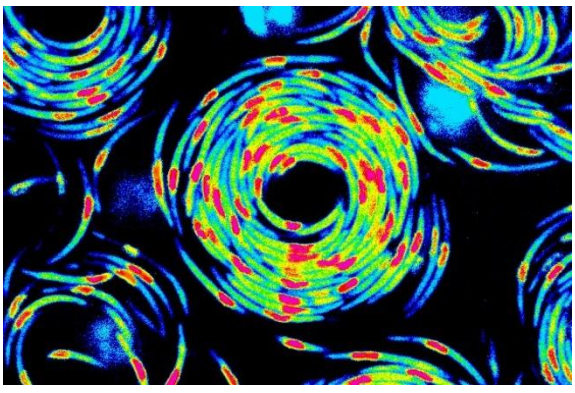
Physics of the malaria parasite

Ulrich S. Schwarz

Heidelberg University, Heidelberg, Germany

The work described in this talk was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) through the Collaborative Research Center 1129 (Integrative analysis of pathogen replication and spread) and the Priority Programme 2332 (Physics of parasitism).

Malaria is possibly the oldest disease of mankind and still causes more than half a million deaths every year. It is caused by the unicellular eukaryotes from the genus Plasmodium, which continuously adapt to the mechanics of the different host environments as they move through their complex lifecyle. Here we discuss two examples for such physical adaptations. Plasmodium sporozoites are the highly motile form of the parasite that is injected by the mosquito into the host skin. We suggest that their crescent shape is an evolutionary adaptation to avoid rotation during gliding motility. Moreover, we show that they can perform collective migration that reveals their mechanical flexibility. For malaria-infected red blood cells, we show how the knob-associated histidine-rich protein (KAHRP) secreted by the parasite leads to remodelling of the cell envelope. The parasite mines actin from the junctional complexes, such that KAHRP can assemble into thousands of knobs. These knobs are adhesion platforms and lead to cytoadhesion, which increases residence time in the vasculature and prevents clearance by the spleen. We also show that the sickle cell mutations of hemoglobin reduce cytoadhesion and therefore increase protection against malaria infections.



Collective migration of malaria parasites
This image shows the rotating vortices that are formed by Plasmodium sporozoites after gentle rupture of the salivary glands of infected mosquitos.

References
[1] Münter, S., Sabass, B., Selhuber-Unkel, C., Kudryashev, M., Hegge, S., Engel, U., Spatz, J.P., Matuschewski, K., Schwarz, U.S., Frischknecht, F. (2009). Plasmodium sporozoite motility is modulated by the turnover of discrete adhesion sites. *Cell Host & Microbe*, *6*(6), 551-562.
[2] Patra, P., Beyer, K., Jaiswal, A., Battista, A., Rohr, K., Frischknecht, F., & Schwarz, U. S. (2022). Collective migration reveals mechanical flexibility of malaria parasites. *Nature Physics*, *18*(5), 586-594.
[3] Lettermann, L., Ziebert, F., Schwarz, U.S. (2024). A geometrical theory of gliding motility based on cell shape and surface flow. *PNAS to appear week of July 15*

[4] Lansche, C., Dasanna, A. K., Quadt, K., Fröhlich, B., Missirlis, D., Tétard, M., Gamain, B., Buchholz, B., Sanchez, C.P., Tanaka, M., Schwarz, U.S., Lanzer, M. (2018). The sickle cell trait affects contact dynamics and endothelial cell activation in Plasmodium falciparum-infected erythrocytes. *Communications Biology* 1, 211.

[5] Jäger, J., Patra, P., Sanchez, C. P., Lanzer, M., & Schwarz, U. S. (2022). A particle-based computational model to analyse remodelling of the red blood cell cytoskeleton during malaria infections. *PLOS Computational Biology*, *18*(4), e1009509.

PL-II-02

Multiwell smFRET measurements reveals small-molecule specific rescue of misfolding of patient mutations in CFTR

Michael Schlierf

TU Dresden, B CUBE, Dresden, Germany

Introduction

Cystic fibrosis (CF) is one of the most common inherited diseases and is caused by mutations in the CFTR protein. Today, over 2,000 mutations have been identified, with more than 300 mutations linked to CF disease. An immense effort in developing small-molecules to counteract misfolding and increase maturation succeeded in the development of a few small molecules for a limited number of CF mutations. Yet, in many cases the state of misfolding and its potential rescue by small molecules remains unclear.

Results

I will introduce our recent development of an open science multiwell, high-content single-molecule FRET platform. Investigations by smFRET often require sampling of a large parameter space, for example, by varying one or more constituent molecular components in ten or more steps to reliably extract distances, kinetic rates, and other quantitative parameters. We show that our multiwell plate assay is on par with conventional single-well smFRET measurements in terms of accuracy and precision, yet enables probing tens to hundreds of conditions in a fully automized manner. We demonstrate the broad applicability of the multiwell plate assay towards nucleic acid dynamics, protein folding, competitive and cooperative protein–DNA interactions, and CF small molecule screening and rescue of misfolding.

PL-II-03

Thermoplasmonic manipulation of liquids and biomolecules

Frank Cichos

Leipzig University, Molecular Nanophotonics Group, Leipzig, Germany

In recent years, new techniques have emerged for manipulating nano-objects and liquid flows in a fluidic environment using remote light control. These methods complement the force generation capabilities of optical tweezers and utilize plasmonic structures [1]. By creating temperature gradients with plasmonic structures at interfaces, localized stresses are generated, allowing for the guidance and manipulation of suspended objects without the need for external forces. This field of thermoplasmonics offers non-invasive, highly precise tools for nanoscale biomanipulation, presenting new opportunities in biophysics, nanomedicine, and molecular biology.
In this contribution, we demonstrate how optically heated plasmonic nanostructures can shape dynamic temperature fields to trap, aggregate, and deform biomolecules and their assemblies. For instance, localized temperature fields can hold amyloid fibrils in liquids [2]. Trapping individual fibrils allows for the observation of their heterogeneous growth at the single fibril level without surface attachment and over extended periods. By introducing additional crowding agents, temperature-induced spatially varying osmotic pressures can interact with biomolecules. These concentration fields of crowders are shown to deform macromolecular conformations, which can be quantified due to precise control over the temperature and concentration fields.This technique also enables the controlled aggregation of fibrillar aggregates into well-ordered structures to explore processes of more complex structure formation.

References
[1] Baffou, G., Cichos, F. & Quidant, R. Applications and challenges of thermoplasmonics. Nat. Mater. 19, 946–958 (2020).
[2] Fränzl, M. et al. Thermophoretic trap for single amyloid fibril and protein aggregation studies. Nat Methods 16, 611–614 (2019).

PL-II-04

Structural odyssey of omyloid-β: oavigating the oonformational oandscape in Alzheimer's oathogenesis

Moritz Schäffler[2,1], Mohammed Khaled[1], Hebah Fatafta[1], **Birgit Strodel**[1,2]

[1] *Forschungszentrum Jülich GmbH, Jülich, Germany;* [2] *Heinrich Heine University Düsseldorf, Jülich, Germany*

Amyloid-β peptide (Aβ) plays a critical role in Alzheimer's disease, particularly in its neurotoxic, smaller oligomeric forms. Experimental challenges complicate the study of these transient structures, which has led us to utilize advanced molecular dynamics (MD) simulations and analysis techniques that include methods to calculate free energies and mean first passage time distributions to identify metastable states and frustrations in the energy landscape. This combination of methods applied to Aβ reveals the structural intricacies of the peptide and reveals its transformative journey from disorder to order through interactions with other biomolecules, including other Aβ peptides. In particular, a stable β-hairpin motif emerges during self-assembly that favors ordered oligomers with a higher propensity to aggregate. Exploring the conformational landscape of Aβ improves our understanding of its dynamics and provides insights that are critical for therapeutic interventions in Alzheimer's disease.

References

[1] M. Khaled, I. Rönnbäck, L.L. Ilag, A. Gräslund, B. Strodel, N. Österlund. A hairpin motif in the Amyloid-β peptide is important for formation of disease-related oligomers. *J. Am. Chem. Soc.* 145:18340-18354 (2023)

[2] M Schäffler, S Samantray, B Strodel. Transition Networks Unveil Disorder-to-Order Transformations in Aβ Caused by Glycosaminoglycans or Lipids. *Int. J. Mol. Sci.* 24:11238 (2023)

[3] B Strodel. Energy landscapes of protein aggregation and conformation switching in intrinsically disordered proteins. *J. Mol. Biol.* 433, 167182 (2021)

[4] H Fatafta, B Kav, BF Bundschuh, J Loschwitz, B Strodel. Disorder-to-order transition of the amyloid-β peptide upon lipid binding. *Biophys. Chem.* 280: 106700 (2022)

[5] H Fatafta, M Khaled, MC Owen, A Sayyed-Ahmad, B Strodel. Amyloid-β peptide dimers undergo a random coil to β-sheet transition in the aqueous phase but not at the neuronal membrane. *Proc. Natl. Acad. Sci. USA* 118, e210621011833 (2021)

11:00 am – 12:05 pm

HS 10

PS-II | Membrane Biophysics I

PS-II-01

Label-free imaging of the polyene macrolide natamycin and its ergosterol-dependent interaction with model and cell membranes

Daniel Wüstner[1], Maria Szomek[1], Vibeke Akkerman[1], Line Lauritsen[1], Jacob M. Egebjerg[1], Katja Thaysen[1], Alice D. Juhl[1], Peter Reinholdt[1], Jacob Kongsted[1], Mohammad Bashawat[2], Peter Müller[2], Holger Scheidt[7], Douglas F. Covey[6], Max Lehmann[3], Pablo Wessig[3], Sebastian Obermayer[5], Alex Foster[5], Bert Poolman[5], Stephan Werner[4], Gerd Schneider[4]

[1] University of Southern Denmark, Odense, Denmark; [2] Humboldt-Universität zu Berlin, Berlin, Germany; [3] University of Potsdam, Postdam, Germany; [4] Helmholtz Zentrum Berlin, Berlin, Germany; [5] University of Groningen, Groningen, Germany; [6] Washington University St. Louis, Washington, Germany; [7] Universität Leipzig, Leipzig, Germany

This research was funded by the Deutsche Forschungsgemeinschaft (DFG), SCHE 1755/4-1 (H.A.S.) MU 1017/14-1 (P.M.), WE1850-12/1 (P.W.) and D.W. as Mercator Fellow, as well as by NWO Graviation program BaSyC to BP. The research of SO and BP was supported by the Marie Curie CoFUND program, oLIFE. The Einstein Stiftung Berlin is acknoweldged for (EJS-2019-565, Einstein Junior Scholar fellowship for M.B.). DFC is thankful for financial support from the NIH, grant NIH 1 P50 MH122379.DW acknowledges funding from the Villum Foundation (grant no. 35865) and from the Danish Research Council (grant ID: 2032-00139B) as well as access to microscope equipment from the Danish Molecular Biomedical Imaging Center (DaMBIC, University of Southern Denmark). Computational resources were provided by the DeIC National HPC Center at the University of Southern Denmark. PR and JK acknowledge the Independent Research Fund Denmark Natural Sciences (DFF-FNU) for financial support (Grant ID no. DFF-7014-00050B).

Introduction

Polyene macrolides like natamycin, are used as food preservative and to treat fungal keratitis, but the molecular mechanism underlying its selectivity for fungal membranes are not understood.

Methods

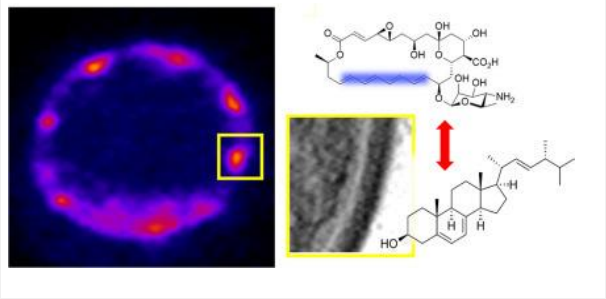
To determine the mode of action of natamycin, we have combined optical spectroscopy with nuclear magnetic resonance (NMR) and electron spin resonance (ESR) spectroscopy as well as molecular dynamics (MD) simulations, label free ultraviolet (UV)-sensitive fluorescence imaging, soft X-ray tomography and computational image analysis.

Results

We found that natamycin forms aggregates in lipid membranes containing ergosterol, causing inhibition of the ergosterol-dependent activity of the amino acid transporter Lyp1 [1]. Natamycin slows the dynamics of ergosterol and cholesterol in lipid membranes to a similar extent but interferes selectively with lipid packing in membranes containing ergosterol [2]. Natamycin binds to the yeast plasma membrane (PM), where it forms aggregates, increases permeability to small solutes and lowers the dipole potential in an ergosterol- and sphingolipid-dependent manner [3]. In contrast to natamycin, intrinsically fluorescent analogs of ergosterol and cholesterol have a homogenous lateral distribution in the yeast PM [3].

Conclusions

Our study shows that ergosterol promotes aggregation of natamycin in the fungal membrane, which is enhanced by inhibitors of sphingolipid synthesis, providing novel drug targets against yeast infections.



Graphical abstract
The image shows label-free imaging of natamycin in the yeast membrane to the left, its structure (right, top) with the polyene part giving rise to its fluorescence in blue. The lower panel in the middle shows an X-ray image of a portion of a natamycin treated cell and the lower right panel shows the structure of ergosterol. From ref. [3].

References

[1] M. Szomek, P. Reinholdt, H.L. Walther, H.A. Scheidt, P. Muller, S. Obermaier, B. Poolman, J. Kongsted, D. Wustner, Natamycin sequesters ergosterol and interferes with substrate transport by the lysine transporter Lyp1 from yeast, Biochim Biophys Acta Biomembr, (2022) 184012.

[2] V. Akkerman, H.A. Scheidt, P. Reinholdt, M. Bashawat, M. Szomek, M. Lehmann, P. Wessig, D.F. Covey, J. Kongsted, P. Müller, D. Wüstner, Natamycin interferes with ergosterol-dependent lipid phases in model membranes., Biochim Biophys Acta Advances, 4 (2023) 100102.

[3] M. Szomek, V. Akkerman, L. Lauritsen, H.L. Walther, A.D. Juhl, K. Thaysen, J.M. Egebjerg, D.F. Covey, M. Lehmann, P. Wessig, A.J. Foster, B. Poolman, S. Werner, G. Schneider, P. Muller, D. Wustner, Ergosterol promotes aggregation of natamycin in the yeast plasma membrane, Biochim Biophys Acta Biomembr, 1866 (2024) 184350.

PS-II-02

The Cluster of Differentiation 95 (CD95) Ligand Configuration and its Induction of CD95 Receptor Oligomerization on the Cell Plasma Membrane Determine Signal Initiation for Cell Apoptosis

Nina Bartels[1], Nicolaas van der Voort[2], Xiaoyue Shang[1], Gajen Thaventhiran[1], Suren Felekyan[2], Oleg Opanasyuk[2], Claus Seidel[2], Cornelia Monzel[1]

[1] Heinrich-Heine University, Physics: Experimental Medical Physics, Düsseldorf, Germany; [2] Heinrich-Heine University, Chemistry: Molecular Physical Chemistry, Düsseldorf, Germany

We are grateful for financial support by German Research Foundation (DFG) – project number 267205415 - CRC 1208, projects A08 and A12; VolkswagenFoundation – Freigeist-Fellowship; and Fonds der Chemischen Industrie.

Introduction

When the membrane receptor Cluster of Differentiation 95 (CD95) couples its trimeric ligand CD95L, a signal primarily leading to apoptosis, but in some cases also leading to proliferation, is initiated. The switch from signaling for death to life was mostly observed in some types of cancer. It was hypothesized to depend on the type and configuration of CD95L as well as on the CD95 activity states [1,2]. For this reason, we investigate the molecular organization of CD95 and CD95L on the cell membrane relative to the initiated signaling.

Methods

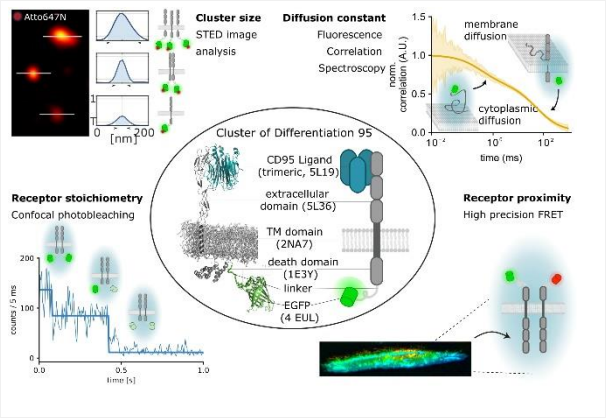
We follow a multiparametric imaging approach to measure the stoichiometry and dynamic changes of CD95 and CD95L on the cell membrane at the single-molecule level.
We use time-resolved FRET live-cell experiments and determine the molecular oligomerization. Super-resolved STED images and confocal Photobleaching-bleaching Step Analysis probe the supramolecular structures and the molecular stoichiometries on the membrane, respectively.

Results

We find that CD95 alone is primarily monomeric (~96%) and dimeric (4%), the addition of CD95L induces oligomerization to dimers/trimers (~15%) leading to cell death. CD95L induces apoptosis most efficiently, when the trimer configuration is stabilized or ligands are crosslinked. We report about the importance of molecular concentrations and on novel experimental/analytical methodologies.[3]

Conclusions

A model how CD95 and CD95L molecular states affect apoptosis signaling is presented.



Determining CD95 Receptor and Ligand States on the Cell Plasma Membrane

Cluster of Differentiation 95 (CD95) and its ligand (CD95L) adapt particular configurations and oligomerization states on the cell plasma membrane to trigger different cell signaling outcomes. We follow a multiparametric imaging approach including time-resolved FRET live-cell experiments, confocal Photobleaching-bleaching Step Analysis, STED microscopy, and FCS to determine these states of CD95 receptor and its ligand.

References

[1] Gülce Gülcüler Balta, et al. 2019, ‘3D cellular architecture modulates tyrosine activity thereby switching CD95 mediated apoptosis to survival’, *Cell Reports*, 29, 2295-2306 https://doi.org/10.1016/j.celrep.2019.10.054

[2] Ricarda M. L. Berger, et al. 2021 'Nanoscale FasL Organization on DNA Origami to Decipher Apoptosis Signal Activation in Cells'. *Small*, 2101678 https://doi.org/10.1002/smll.202101678

[3] Nina Bartels, et al. 2022, 'A Minimal Model of CD95 Signal Initiation Revealed by Advanced Super-resolution and Multiparametric Fluorescence Microscopy' *bioRxiv*, https://doi.org/10.1101/2022.11.29.518370

PS-II-03

Reconciling past and future research: a study on calcium as a protein-free fusogen in negatively-charged cell-sized vesicles

Matilde Accorsi[1], Naresh Yandrapalli[3], Shreya Pramanik[2], Rumiana Dimova[1]

[1] Max Planck Institute of Colloids and Interfaces, Potsdam, Germany; [2] Oregon Health & Science University, Portland, USA; [3] Max Planck Institute of Molecular Cell Biology and Genetics, Dreden, Germany

Introduction

In the late 20th century, calcium took on a new identity as an independent fusogen, when it was found to induce fusion in negatively-charged nanometric vesicles (large unilamellar vesicles, LUVs) even in the absence of proteins [1]. These studies are still a core part of membrane model fusion research and are frequently cited. While cell-sized lipid vesicles (giant unilamellar vesicles, GUVs) gained popularity in the 1990s as membrane model, they have not been the subject of an in-depth study about calcium-induced fusion.

Methods

To investigate this matter, we developed an approach based on confocal microscopy and microfluidics (e.g. Fig. 1) [2][3] and explored how different GUV compositions respond to varying calcium concentrations and environmental conditions.

Results

We find that calcium can easily induce hemifusion with lipid mixing in negatively-charged GUVs, while full fusion with content mixing is rather rare and requires fine membrane composition and environmental control to attempt to curb membrane instability.

Conclusions

Reconciling past research with modern models proves to be difficult in this case, as our results point to calcium as a less ubiquitous and more elusive fusogen than previously thought in pure lipid GUVs. To find a compromise between past and present models, we explore instead calcium as a protein-free fusogen in negatively-charged LUV to GUV fusion, a task that introduces new challenges and questions.

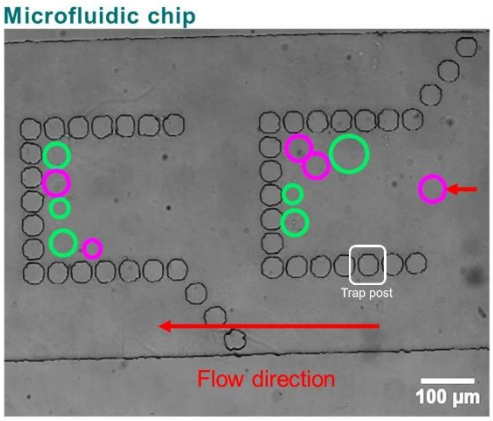


Figure 1: Fusion assay setup
Confocal microscope image portraying two microfluidic traps. The GUVs, represented by circles, are transported by the flow and are confined in a trap made of multiple trap posts. This allows prolonged observations.

References

[1] Wilschut, J, Düzgünes, N, Fraley, R and Papahadjopoulos, D 1980, 'Studies on the mechanism of membrane fusion: kinetics of calcium ion induced fusion of phosphatidylserine vesicles followed by a new assay for mixing of aqueous vesicle contents', *Biochemistry*, 19, 6011-6021, Place of publication: American Chemical Society

[2] Yandrapalli, N, and Robinson, T 2019, 'Ultra-high capacity microfluidic trapping of giant vesicles for high-throughput membrane studies', *Lab on a Chip*, 19, 626-633, Place of publication: Royal Society of Chemistry

[3] Pramanik, S, Steinkuehler, J, Dimova, R, Spatz, J and Lipowsky, R 2022, 'Binding of His-tagged fluorophores to lipid bilayers of giant vesicles', *Soft Matter*, 18, 6372-6383, Place of publication: Royal Society of Chemistry

PS-II-04

Widefield Super-Resolution IR Imaging with Fluorescence Enhanced Photothermal Infrared | Photothermal Spectroscopy Corp

Miriam Unger, Mustafa Kansiz

Santa Barbara, USA; Santa Barbara, USA

Miriam Unger and Mustafa Kansiz
Photothermal Spectroscopy Corp, 325 Chapala St, Santa Barbara, CA, 93101, USA

Optical Photothermal Infrared (O-PTIR) spectroscopy has established itself as a breakthrough vibrational microspectroscopy tool, offering significant advantages over the traditional FTIR/QCL & Raman spectroscopy, providing submicron simultaneous IR+Raman and fluorescence imaging.

However there still exists a demand for rapid and high-resolution widefield IR imaging. To this end, we have developed a novel widefield super-resolution IR imaging approach that utilizes the fluorescent signal directly for IR signal extraction. As the fluorescent signal is captured with a 2D fluorescence camera, this generates, simultaneously, widefield IR as well as widefield fluorescence images.

We have termed this - Fluorescence-Enhanced Photothermal Infrared (FE-PTIR) spectroscopy. The key enabling factor here, is that when the wavelength of the IR pulses is tuned to a molecular vibration of fluorescently labeled molecules, the absorbed heat causes a modulation in the amount of fluorescent light emitted from the fluorophores and it’s surrounds. Coupled with the parallel data acquisition via the 2D (megapixel) visible fluorescence camera, using a standard glass objective of 50x, 0.8NA, single field of view for IR of 70x70um with 200nm pixels are possible. Compatibility with other standard visible glass objectives such like those with higher NA, or even immersion objectives opens up further possibilities for widefield super-resolution IR imaging. FE-PTIR thus allows the IR spectroscopic analysis of specifically labeled regions of biological cells and tissue, for example to study conformational stages of a specifically labeled class of target proteins or protein misfolding associated with neurodegenerative diseases. Various examples from these applications will be provided.

11:00 am – 12:05 pm

HS 11

PS-III | Cellular Biophysics

PS-III-01

Reconstituting DNA immune sensing: a minimal cell-free approach to understand the dynamics of higher-order assemblies and immune receptor activation

Theresia Gutmann, David Kuster, Andrei Pozniakovsky, Anthony Hyman

Max Planck Institute of Molecular Cell Biology and Genetics, 01307, Germany

The detection of DNA in the cytosol induces cellular immune responses. The ubiquitously expressed receptor for cytosolic DNA is the cyclic GMP-AMP synthase (cGAS), which oligomerizes upon DNA binding, condenses into higher-order assemblies, and produces the second messenger cGAMP, leading to proinflammatory signaling. Here, we established a cGAS purification pipeline and explored cGAS assembly and activation by biochemical and biophysical means. With this, we have developed a minimal cell-free reconstitution platform to elucidate cGAS modulation by DNA-protein and protein-protein interactions. This platform enhances our understanding of cGAS-mediated immune responses and could provide a versatile tool to inform drug design targeting aberrant inflammation.

PS-III-02

Growing tumor spheroids from single cells is associated with changes in cell volume and mechanical properties

Vaibhav Mahajan[1], Keshav G. Babu[1], Timon Beck[1,2], Antje Garside[1], Carsten Werner[3], Raimund Schlüssler[1], Anna Taubenberger[1]

[1] TU Dresden, Biotechnology Center, Center for Molecular Bioengineering, Dresden, Germany; [2] Max Planck Institute for the Science of Light, Erlangen, Germany; [3] Max Bergmann Center, Leibniz Institute of Polymer Research Dresden, Dresden, Germany

Tumors are mechanically altered across multiple spatial scales, from the cellular level to complex tissues and these changes are thought to contribute to cancer progression. Effects of mechanically altered microenvironments on tumor cells are well studied in a systematic manner using bioengineered 3D in-vitro models. Previous studies indicate that tumor spheroids adapt their growth and mechanical properties when growing under 3D confinement. Still, the temporal dynamics and molecular basis of this mechanical adaption remain poorly understood. Here we studied single cancer cells forming tumor spheroids in mechanically well-defined 3D hydrogels. Confocal Brillouin microscopy revealed for several cell types consistent increases in the Brillouin frequency shift from single cells, to small clusters and tumor spheroids. These changes coincided with a drastic decrease in the median nuclear volume of up to 60%, together with overall cell volume decreases. The volume changes were not explained by growth-induced compressive stress but rather by both, water efflux from the cells, as well as cell cycle changes evidenced by the FUCCI cell cycle reporter. Specifically, smaller cells that were in the G1 cell cycle phase accumulated in the growing spheroids over time. Taken together, our study provides insights into how tumor cells adapt their cellular/nuclear volumes and mechanical properties when forming multicellular structures in 3D, which is relevant to tumor formation and progression.

PS-III-03

Unprecedented spatial & submolecular spectral chemical imaging of proteins, surfaces of microbes & histological tissue using mid-IR photo-induced force microscopy

Maryam Ali[1,2], **Daniela Täuber**[1,2], Collaboration: COST Action CA21111 OneHealthdrugs

[1] Friedrich Schiller University, Institute of Physical Chemistry, Jena, Germany; [2] Leibniz Institute of Photonic Technology, Microscopy, Jena, Germany

DFG projects no. 439139881 & 542825796
EU COST Action CA21111 OneHealthdrugs

Introduction

Label-free imaging via mid-IR photo-induced force microscopy (PiF-IR) provides submolecular chemical characterization of biopolymers[1] and unprecedented spatial resolution of the chemical contrast in surfaces of microbes[2,3] and histological tissue,[1] a potential to revolutionize chemical nano-imaging in the Life Sciences.

Methods

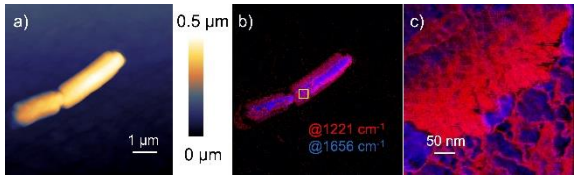
PiF-IR combines a powerful infrared excitation using tunable quantum cascade lasers with mechanical probing of the electromagnetic near-field between a plasmonic tip and the sample. We have applied PiF-IR to materials including biopolymers,[1] nanoparticles,[3] single bacteria cells[3] and human retina.

Results

In PiF-IR hyperspectra of F-Actin we found nanoscale variations in absorption bands related to α-helices and β-sheets.[1] The PiF-IR hyperspectrum of a dipeptide revealed submolecular vibrational components. Nanoscale variations are also found in high-resolution PiF-IR scans of *Bacillus subtilis*[3] and histological liver tissue from a mouse model.[1] We further work on advanced data analysis & theoretical modeling for PiF-IR.[4]

Conclusions

PiF-IR provides access to localized submolecular chemical characterization with high spectral and unprecedented spatial resolution of a few nanometers. This is a potential key to understand chemical processes involved in localized molecular actions on cell surfaces and tissue ex vivo without the need of labeling.



Bacillus subtilis cell visualized using mid-IR Photo-induced Force Microscopy (PiF-IR)
a) Atomic force microscopy topography image of a dividing *Bacillus subtilis* cell and b) cropped images of contrasts in photo-induced force at two excitation frequencies related to proteins (blue) and polysaccharides (red); c) cropped high-resolution scans in the marked area in b).

References

[1] Joseph, J, Spantzel, L, Ali, M, Joseph, DM, Unger, S, Reglinski, K, Krafft, C, Müller, A-D, Eggeling, C, Heintzmann, R, Börsch, M, Press, AT, Täuber, D, 'Nanoscale chemical characterization of secondary protein structure of F-Actin using mid-infrared photoinduced force microscopy (PiF-IR), *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2024, 306, 123612.

[2] Davies-Jones, J, Davies, PR, Graf, A, Hewes, D, Hill, KE, Pascoe, M, 'Photoinduced force microscopy as a novel method for the study of microbial nanostructures', *Nanoscale* 2023, 16, 223.

[3] Krishnakumar, N, Strecker, A, Biehl, P, Schacher, FH, Müller, A-D, Ramoji, A, Neugebauer, U, Täuber, D, 'Nanoscale chemical imaging of cell surface and core-shell nanoparticles using infrared excitation and detection by force microscopy' *70. Mosbacher Kolloquium*, 2019, *Researchgate*: doi: 10.13140/RG.2.2.20000.25601.

[4] Anindo, S, Heintzmann, R, Täuber, D, David, C, 'Photo-thermal expansion of a nanostructured surface in Photo-induced Force Microscopy (PiFM)', *Nano-Scientific Forum Europe NSFE2023*, Barcelona, Spain, September 2023, *Researchgate*: doi:10.13140/RG.2.2.10065.92003

PS-III-04

Tight junctions regulate lumen morphology via hydrostatic pressure and junctional tension

Markus Mukenhirn[1,2], Chen Ho Wang[2], Tristan Guyomar[4,5], Mathew Bovyn[2,3], Michael Staddon[2,3], Rozemarijn E. van der Vee van der Vee[9], Riccardo Maraspini[2], Linjie Lu[4,5], Cecilie Martin-Lemaitre1[1], Masaki Sano[6], Martin Lehmann[9], Tetsuya Hiraiwa[7,8], Daniel Riveline[4,5], Alf Honigmann[1,2]

[1] Technische Universität Dresden, Biotechnologisches Zentrum (BIOTEC) Center for Molecular and Cellular BioengineeringBiotechnologisches Zentrum (BIOTEC) Center for Molecular and Cellular Bioengineering (CMCB) (CMCB), Biophysics, Dreden, Germany; [2] Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany; [3] Max Planck Institute for the Physics of Complex Systems, Dresden, Germany; [4] Université de Strasbourg, IGBMC, Illkirch, France; [5] CNRS, Illkirch, France; [6] Institute of Natural Sciences, School of Physics and Astronomy, Shanghai Jiao Tong University, Shanghai, China; [7] Mechanobiology Institute, National University of Singapore, Singapore, Singapore; [8] Universal Biology Institute, The University of Tokyo, Tokyo, Japan; [9] Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP), Berlin, Germany

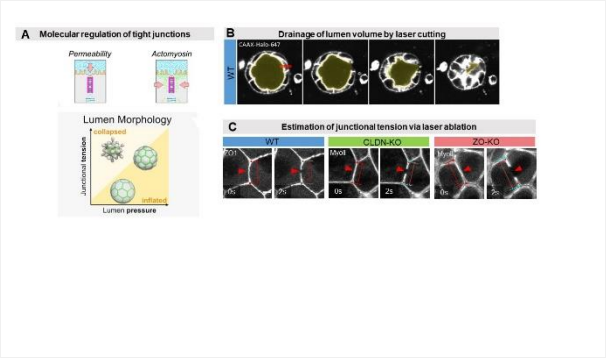
We thank Mikio Furuse, National Institute for Physiological Sciences (NIPS) Japan, for providing the CLDN-5KO MDCKII cell line. We thank Byun Ho Lee for the help with the cyst segmentation. A.H. team thank the Light microscopy facility of the MPI-CBG as well as the Light microscopy facility of the CMCB Dresden for help and support with imaging. D.R. team thanks support from the Imaging Platform of IGBMC. DR team was supported by the Interdisciplinary Thematic Institute IMCBio, as part of the ITI 2021-2028 program of the University of Strasbourg, CNRS and Inserm, was supported by IdEx Unistra (ANR-10-IDEX-0002), and by SFRI-STRAT'US project (ANR 20-SFRI-0012) and EUR IMCBio (ANR-17-EURE-0023) under the framework of the French Investments for the Future Program. AH and M.M. were supported by the Volkswagen Foundation project number A133289. M.J.B and M.F.S were funded by the Max Planck Society, including through an ELBE fellowship. This project was funded by HFSP project number RGP0050/2018.

Introduction The formation of fluid-filled lumens by epithelial tissues is vital for organ development. Essential molecular activities, such as ion pumping[5] and acto-myosin constriction [1], generate the mechanical forces necessary to create luminal cavities between cells. Current models emphasize luminal pressure, considering osmotic, hydraulic, and cortical forces [4]. Lumen inflation is driven by water influx from ion gradients and hydrostatic pressure. However, connecting the mechanical forces that shape lumens to the molecular regulation at cellular and tissue levels remains challenging.

Methods We investigated the role of tight junctions (TJs) in lumen formation using genome-edited MDCKII cells lacking either the cytoplasmic TJ scaffold proteins ZO1 and ZO2 [3]or the five major claudins expressed in MDCKs [2]. Laser dissection measured hydrostatic pressure (B) and apical junctional tension (C) in modified MDCKII cysts, and we modeled the force balance of hydrostatic lumen pressure and junctional tension in TJ mutants (A).

Results Our results show that TJs significantly affect apical-junctional tension and, to a lesser extent, hydrostatic pressure from solute pumping. Additionally, cells control the apical membrane area independently of hydrostatic pressure, junctional tension, and lumen volume.

Conclusions Tight junctions regulate the pressure-tension force balance, crucial for tuning tissue and lumen shapes during morphogenesis.



Tight junctions regulate lumen morphology via hydrostatic pressure and junctional tension
(A) Phase diagram of the inner lumen radius in the apical line tension – hydrostatic pressure parameter space of different tight junction mutants. (B) Lumen volume drainage MDCKII TJ mutant cysts by laser cutting. (C) Laser ablation of cell-cell junctions of MDCK-II mutants.

References
[1] Choi, W. *et al.* Remodeling the zonula adherens in response to tension and the role of afadin in this response. *J. Cell Biol.***213**, 243–260 (2016).
[2] Otani, T. *et al.* Claudins and JAM-A coordinately regulate tight junction formation and epithelial polarity. *J. Cell Biol.***218**, 3372–3396 (2019).
[3] Beutel, O., Maraspini, R., Pombo-García, K., Martin-Lemaitre, C. & Honigmann, A. Phase Separation of Zonula Occludens Proteins Drives Formation of Tight Junctions. *Cell***179**, 923-936.e11 (2019).
[4] Torres-Sánchez, A., Kerr Winter, M. & Salbreux, G. Tissue hydraulics: Physics of lumen formation and interaction. *Cells Dev.***168**, 203724 (2021).
[5] Narayanan, V. *et al.* Osmotic Gradients in Epithelial Acini Increase Mechanical Tension across E-cadherin, Drive Morphogenesis, and Maintain Homeostasis. *Curr. Biol.***30**, 624-633.e4 (2020).

11:00 am – 12:05 pm

HS 8

PS-I | SFB 1423 Session: Structural Dynamics of GPCR Activation and Signaling

PS-I-01

Discovery of Protease-Activated Receptor 4 (PAR4)-Tethered Ligand Antagonists Using Ultralarge Virtual Screening

Jens Meiler

Medizinische Fakultät, Universität Leipzig, Institüt für Wirkstoffentwicklung, Leipzig, Germany

Here, we demonstrate a structure-based small molecule virtual screening and lead optimization pipeline using a homology model of a difficult-to-drug G-protein-coupled receptor (GPCR) target.
Protease-activated receptor 4 (PAR4) is activated by thrombin cleavage, revealing a tethered ligand that activates the receptor, making PAR4 a challenging target. A virtual screen of a make-on-demand chemical library yielded a one-hit compound. From the single-hit compound, we developed a novel series of PAR4 antagonists. Subsequent lead optimization via simultaneous virtual library searches and structure-based rational design efforts led to potent antagonists of thrombin-induced activation. Interestingly, this series of antagonists was active against PAR4 activation by the native protease thrombin cleavage but not the synthetic PAR4 agonist peptide AYPGKF. We will also go into detail on the computational methods employed, including AlphaFold2, Rosetta, and ultra-large library screening

PS-I-02

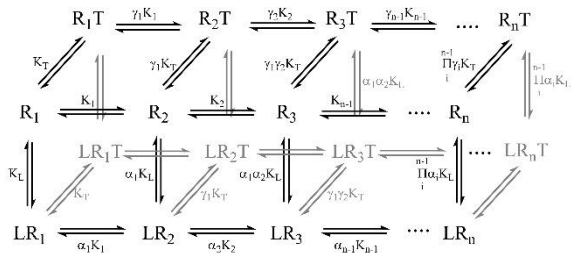
The affinity framework of molecular efficacy

Matthias Elgeti

Leipzig University Medical School, Institute for Drug Discovery, Leipzig, Germany

This work was supported by the German Research foundation, project number 421152132, subproject A03. notionvc: 8cb7ea0d-1774-42c3-8595-8d48d25ad43f

G protein-coupled receptors (GPCRs) are essential signaling proteins and thus prime drug targets. However, a conclusive molecular model of drug action remains elusive. We propose a structural framework for ligand efficacy solely based on ligand affinity: ligands bind to individual receptor conformations with distinct affinity, stabilizing a characteristic equilibrium of conformations each of which exhibits distinct functional properties towards downstream signaling proteins. We integrated an iterative global fitting approach into our analysis of a comprehensive DEER dataset recorded for the type 1 angiotensin II receptor [1] using a generic ternary complex (Ligand-Receptor-Transducer, LRT) framework. Our results show that ligand-induced shifts of conformational equilibria can be solely accounted for by assuming distinct affinities to different receptor conformations. We also applied the LRT framework to include transducer proteins such as G proteins and arrestins in complex with μ-opioid receptors and various ligands [2]. The results demonstrate that biased ligands bind and stabilize the same conformations as the associated transducer protein, but do not reveal heterotropic cooperativity of ligand or transducer binding. This study definitively demonstrates the value of DEER in elucidating the conformational ensemble of GPCRs and paves the way for groundbreaking applications in ligand screening and drug discovery.



The Ligand-Receptor-Transducer framework
The ensemble of receptor conformations makes up the equilibrium R_1, R_2, R_3, ..., R_n with equilibrium constants K_1, K_2, ..., K_{n-1}. A given ligand L exhibits specific affinities to these conformations, leading to an adjusted equilibrium between conformations LRi. Accordingly, transducer T binds to a subset of (active) conformations, further altering the distribution of conformations. DEER provides access to structure and equilibrium populations, and analysis of AT1R and mOR data reveals how ligands bind to specific receptor conformations to introduce functional selectivity (bias).

References
[1] Wingler, LM, Elgeti, M et al. 2019, 'Angiotensin Analogs with Divergent Bias Stabilize Distinct Receptor Conformations.', *Cell*, 176, 468-478
[2] Zhao, J, Elgeti, M et al. 2024, 'Ligand efficacy modulates conformational dynamics of the μ-opioid receptor', *Nature*, 629, 474-48

PS-I-03

Transient ligand contacts of the intrinsically disordered N-terminus of neuropeptide Y_2 receptor regulate arrestin-3 recruitment

Anette Kaiser[1,2], Juan C. Rojas Echeverri[3,4], Claudio Iacobucci[3,4], Maik Pankonin[5], Davide Sala[6], Asat Baischew[3,4], Christian Ihling[3,4], Ronny Müller[2], Annette G. Beck-Sickinger[2], Peter Schmidt[5], Jens Meiler[6], Peter W Hildebrand[5], Andrea Sinz[3,4]

[1] Leipzig University, Medical Faculty, Department of Anesthesiology and Intensive Care, Leipzig, Germany; [2] Leipzig University, Faculty of Life Sciences, Institute of Biochemistry, Leipzig, Germany; [3] Martin Luther University Halle-Wittenberg, Institute of Pharmacy, Department of Pharmaceutical Chemistry and Bioanalytics, Halle-Wittenberg, Germany; [4] Martin Luther University Halle-Wittenberg, Center for Structural Mass Spectrometry, Halle-Wittenberg, Germany; [5] Leipzig University, Medical Faculty, Institute of Medical Physics and Biophysics, Leipzig, Germany; [6] Leipzig University, Medical Faculty, Institute for Drug Discovery, Leipzig, Germany

Previous efforts in delineating molecular mechanisms of G protein-coupled receptor (GPCR) activation have focused on transmembrane regions and ligand-receptor contacts of the extracellular loops. The role of the highly flexible N-termini of rhodopsin-like GPCRs have not been well characterized to date. We hypothesize that transient contacts between the peptide ligand and the intrinsically disordered N-terminus (NT) of the neuropeptide Y (NPY) receptor Y_2 (Y_2R) will affect receptor signaling. We employed cross-linking mass spectrometry to capture ligand-receptor contacts including transient binding modes. A photo-reactive NPY analogue allowed mapping the interaction between NPY and Y_2R NT resulting in a total number of 40 cross-links. The cross-links provide distance constraints for deriving structural models of the interaction. Molecular dynamics simulations highlight the structural flexibility and rapid interconversion of ligand-receptor contacts. Mutagenesis of Y_2R and functional characterization suggest that the cross-linking hotspots in the NT electrostatically control its conformational ensemble. The NT kinetically controls ligand access while transient contacts to the peptide are required for efficient interaction of Y_2R with arrestin-3, but not G_i. We delineate structure-function relationships for the intrinsically disordered Y_2R NT and propose a functional role for transient binding modes involving the NT of a peptide-binding receptor.

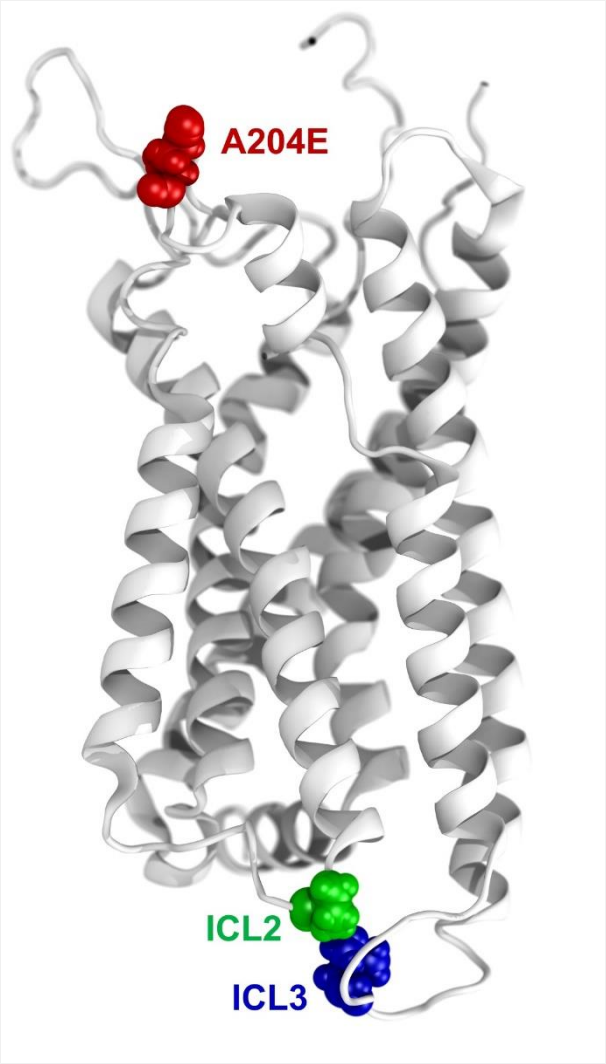
PS-I-04

Influence of the A204E mutation on Ghrelin receptor dynamics investigated by molecular dynamics simulation

Alexander Vogel[1], Albert A. Smith-Penzel[1], Emelyne Pacull[1], Guillermo Pérez Hernández[2], Daniel Huster[1], Peter W. Hildebrand[1]

[1] Leipzig University, Institute for Medical Physics and Biophysics, Leipzig, Germany; [2] Charité – Universitätsmedizin Berlin, Institute of Medical Physics and Biophysics, Berlin, Germany

The Ghrelin receptor (GHSR) is a family A G-protein-coupled receptor that plays a crucial role in food intake, as its ligand Ghrelin is a peptide hormone that is secreted in the stomach when food is required. The GHSR has a high (≈50 %) basal activity, but this basal activity is greatly reduced in the A204E mutant which leads to a short stature phenotype. In this project we conducted extensive all-atom MD simulations of the wild-type and the A204E mutant receptor to elucidate the structural background of basal receptor activity. We simulated the Ghrelin-bound, the antagonist-bound, and two apo states (one starting from the active state and one from the inactive state), summing up to a combined simulation time of approximately one millisecond. Comparison between the wild-type and A204E receptor simulations shows that the short helix in the extracellular loop 2 containing position 204 unfolds in presence of the mutation. Long-range allosteric effects are observed towards the intracellular part of the receptor. In the A204E mutant receptor the basal activity seems to be reduced, measured by a smaller TM6 outward tilt. In addition, intracellular loop 3 tends to fold back and occlude the binding site for G-protein binding. We further rationalize the observed long-range allosteric effects by correlation and network analysis and compare the MD-simulations to NMR-analysis data to corroborate our observations.



Allosteric effects of the A204E mutation on the GHSR

1:00 pm – 2:00 pm

HS 9

PL-III | Plenary III

Petra Schwille

PL-III-01

Resolving heterogeneous biomolecular systems by multi-modal fluorescence spectroscopy and nanoscopy for integrative dynamic structural biology

Claus A.M. Seidel

Heinrich Heine University Düsseldorf, Chemistry: Molecular Physical Chemistry, Düsseldorf, Germany

This study was supported by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) through funding no. SE 1195/16-1 as well as in part by the CRC 1208 (project number 267205415, project A08) and by the European Research Council (ERC) through the Advanced Grant 2014 hybridFRET (Grant No. 671208).

Integrative multimodal fluorescence spectroscopy and microscopy with multiparameter detection [1] in combination with all-atom MD simulations provide rich insights on biomolecular systems with respect to structural and kinetic properties and spatial localization. These methods [2] offer the required nanosecond time resolution to study the motions biomolecules over a large dynamic time range from nano- to milliseconds, to identify the involved states and to decipher the underlying kinetic networks [3]. We assessed the accuracy of this approach in a comparative single-molecule study using two protein systems with distinct conformational changes and dynamics. We obtained an interdye distance precision of ≤2 Å and accuracy of ≤5 Å [4]. Moreover, I will present studies of several conformationally flexible proteins such as a lipase-specific foldase (Lif) [5], GABARAP and the Carbohydrate Binding Module 56 (CBM56), where we characterized the motions and the conformational space that was accessible through flexible protein segments. To enable dissemination of all results according to the FAIR principle, we introduce the flrCIF data representation, which extends established data standards from the Protein Data Bank and allows for archiving fluorescence-aided integrative structures for multiple states together with associated kinetic data on exchange in the PDB-Dev repository.

References

[1] Sisamakis, E. et al.; Methods Enzymol. **475**, 455-514 (2010). doi.org/10.1016/S0076-6879(10)75018-7.
[2] Lerner, E. et al; eLife **10**, e60416 (2021). doi.org/10.7554/eLife.60416
[3] Barth, A. et al.; J. Chem. Phys. **156**, e141501 (2022). doi.org/10.1063/5.0089134 and Opanasyuk, O. et al.; J. Chem. Phys. **156**, e031501 (2022). doi.org/10.1063/5.0095754
[4] Agam, G. et al.; Nat. Methods **20**, 523–535 (2023). doi.org/10.1038/s41592-023-01807-0
[5] Dittrich#, J., Popara#,M. et al. J. Chem. Theory Comput. **19**, 2389-2409 (2023). doi.org/10.1021/acs.jctc.2c01090

PL-III-02

Studying DNA and chromatin replication using integrated force-fluorescence microscopy

Nynke Dekker

TU Delft, Applied Sciences, Delft, Netherlands

Over the past few decades, both single-molecule biophysics, ensemble biochemistry, and structural biology have made substantial contributions to our understanding of molecular machines. This has led to an increased understanding of their mechanochemical cycles and their interplay with accessory proteins that also increases our appreciation of their functioning within the cellular context. An ongoing challenge is to probe the dynamics of complex molecular machines from a biophysical perspective and to do so while maintaining an acceptable degree of experimental yield.

I will describe how we have navigated this challenge in the context of the eukaryotic replisome, the multi-protein protein complex that copies the DNA in all of our cells. While the overall outline of replisome assembly in eukaryotes is understood, much remains to be learned about the dynamics of the individual proteins on the DNA and how these contribute to the formation and activity of proper replisomes. Probing at the single-molecule level can help to investigate such dynamics. Using primarily integrated force-fluorescence microscopy based on optical trapping and confocal microscopy, I will show how we can now dissect how protein binding, diffusion, sequence recognition, and protein-protein interactions play important roles in the assembly of the pre-replication complex and the replicative holo-helicase CMG. Within this context, I will discuss aspects of the workflow that we have optimized that could be useful for monitoring a wide set of DNA-protein interactions. Subsequently, I will discuss our recent data showing how certain of these interactions are altered in the context of chromatin.

2:00 pm – 2:30 pm

HS 9

PS-FL-1 | Posterflashes I

PS-FL-1-01

Investigating the R-loop formation by CRISPR-Cas9 with ultrafast single-molecule twist measurements

Fabian Welzel[1], Julene Madariaga-Marcos[1], Dominik J. Kauert[1], Dina Grohmann[2,3], Ralf Seidel[1]

[1] Universität Leipzig, Peter Debye Institute of Soft Matter Physics / Group Molecular Biophysics, Leipzig, Germany; [2] University of Regensburg, Institute of Microbiology & Archaea Centre, Regensburg, Germany; [3] University of Regensburg, Regensburg Center of Biochemistry (RCB), Regensburg, Germany

Introduction

The CRISPR nuclease Cas9 is a target for genome-editing, since it can be programmed to bind practically any genomic target by its intrinsic RNA component. Cas9 interrogates DNA looking for target sequences that are complementary to the RNA. Upon target recognition, base pairing between RNA and the DNA target strand causes the formation of a so-called R-loop, which has a considerable tolerance for mismatches. To prevent off-targeting, quantitative modelling of the recognition process would be desirable, which requires knowledge about the energy landscapes of the R-loop formation.

Methods

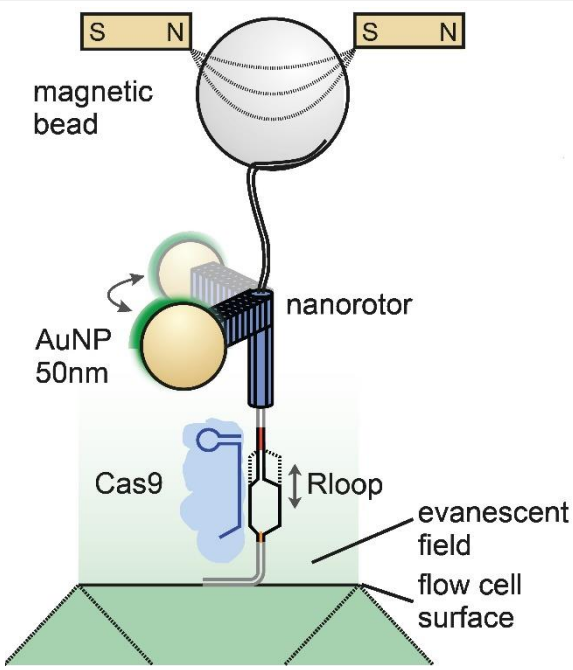
To determine such energy landscapes we measure DNA unwinding during R-loop formation using ultrafast twist measurements. Using DNA origami nanostructures as rotor arms, directly reporting about twist changes on a millisecond time scale, we can resolve real-time R-loop dynamics of CRISPR-Cas effector complexes at the single base-pair level and construct corresponding energy landscapes.

Results

With our approach we achieve unprecendented spatio-temporal resolution which allows to resolve different states during R-loop formation that are in agreement with structural observations. Furthermore, the energy landscapes reveal an uphill bias towards the full R-loop state indicating the protein's intrinsic mechanism of off-target discrimination.

Conclusions

We achieve unprecedented insight in the dynamics of R-loop formation by Cas9 and the impact of mismatches on this process.



Single-molecule nanorotor measurements of R-loop formation by CRISPR-Cas9
Schematic representation of the nanorotor configuration used in a magnetic tweezers setup. The DNA sequence of interest is attached on its bottom end to the surface of the fluidic cell and on its top end to the nanorotor consisting of a DNA origami nanostructure. At the end of the rotor arm a 50 nm AuNP is attached. The top of the nanorotor is connected to a magnetic bead that allows stretching and twisting of the DNA. Imaging the light that is backscattered from the AuNP allows detection of the nanorotor rotations and thus monitoring the DNA untwisting during R-loop formation by CRISPR-Cas9.

PS-FL-1-03

Probing the supramolecular aggregation state of bacterial endotoxin to reveal the basis of biological recognition and endotoxin masking

Andra Schromm[1], Nicolas Gisch[2], Wilmar Correa[3], Frank Steiniger[4], Walter Richter[4], Guillermo Martinez-de-Tejada[5], Klaus Brandenburg[6], Friedrich von Wintzingerode[7]

[1] Research Center Borstel, Leibniz Lung Center, Division of Immunobiophysics, Borstel, Germany; [2] Research Center Borstel, Leibniz Lung Center, Division of Bioanalytical Chemistry, Borstel, Germany; [3] Research Center Borstel, Leibniz Lung Center, Division of Biophysics, Borstel, Germany; [4] University Hospital Jena, Friedrich Schiller University, Electron Microscopy Centre, Jena, Germany; [5] University of Navarra, Department of Microbiology and Parasitology, Pamplona, Spain; [6] Brandenburg Antiinfektiva, Borstel, Germany; [7] Genentech, Inc., San Francisco, USA

Parts of the study funded by ROCHE, Germany (grant to AS, NG, KB). SAXS measurements were performed at the EMBL c/o DESY beamline P12 at PETRA III under a beam time grant to AS

Introduction

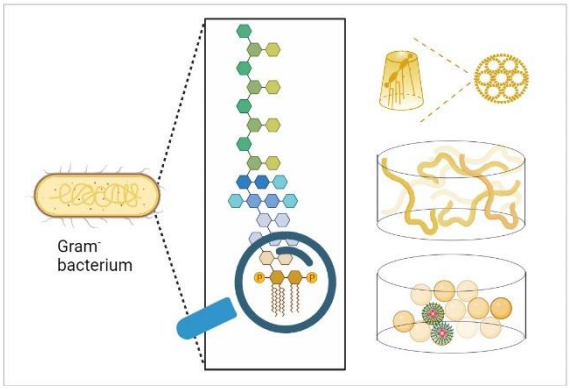
The outer-membrane lipopolysaccharide (LPS, endotoxin) of Gram-negative bacteria is highly immunostimulatory and induces severe pathology in humans. Investigation of a variety of natural and reconstituted membrane systems by small angle X-ray scattering (SAXS) revealed that the supramolecular organization and 3D structure of endotoxin molecules are major determinants for immunological activity. Since endotoxin is ubiquitous, it represents also a harmful contaminant for medical drug products. Masking of endotoxin by surfactant containing drug-formulations poses a severe challenge for drug safety.

Methods & Results:

We have performed a comprehensive study including structural, biophysical, and biological analyses to identify the root cause of endotoxin masking in a drug product. DLS, cryo-TEM, SAXS and ITC analyses revealed a drastic rearrangement of the morphology of LPS aggregates in drug buffer from tube-like structures into a network of interlinked micelles. Our data provide a structure-based explanation for the effect of endotoxin masking. Biochemical detection and responsiveness of different biological detection systems were systematically compared to identify systems suitable for detection of endotoxin in this specific aggregation state.

Conclusions

Our study revealed a new state of bacterial endotoxin aggregation, inaccessible for biochemical detection, but highly accessible for human immune detection.



Aggregation states of bacterial membrane lipopolysaccharide
(Image created by Biorender under licence).

References

[1] **Schromm AB** and Brandenburg K. 2021. TLR4 ligands: Single Molecules and Aggregates. In: The Role of Toll-Like Receptor 4 in Infectious and Non Infectious Inflammation. Rossetti C, Peri F. (Eds.) Springer Nature Switzerland AG.

[2] **Schromm, AB**, Correa W, Gisch, N, Steiniger, F, Richter, W, Martinez-de-Tejada, G, Brandenburg, K, von Wintzingerode, F. 2024. Supramolecular assembly of micellar aggregates is the basis of low endotoxin recovery (LER) in a drug formulation that can be resolved by a whole blood assay. Biomedicine and Pharmacotherapy. 173:116286.

PS-FL-1-05

Raft-partitioning of TM proteins

Nancy Mejía Villagrán[1], Marius F.W. Trollmann[1,2], Cristian R. Popov[1], Rainer A. Böckmann[1,2]

[1] Friedrich-Alexander-Universität Erlangen-Nürnberg, Computational Biology, Department of Biology, Erlangen, Germany; [2] Erlangen National High Performance Computing Center (NHR@FAU), Computational Biology, Department of Biology, Erlangen, Germany

Membrane rafts are small, highly dynamic lipid domains that compartmentalize cellular processes. They are believed to play crucial roles in multiple cellular functions, such as signal transduction. The precise nature, structure, and function of rafts are not well understood, making their characterization challenging and controversial. One major issue is the lack of quantitative methods for characterizing the raft-partitioning of trans-membrane (TM) proteins. Based on fluorescence studies on GPMVs, Lorent *et al.* (2017) developed a model for predicting the raft-partitioning based on protein features [1].

Here, we present a large-scale MD study of nanodomain formation [2] around >70 different TM proteins [1] using different model membranes within the Martini 3 force field. All proteins were modeled employing AlphaFold 3. The simulation results are used to decipher the sequence-specific properties that determine the partitioning to different membrane domains.

References

[1] Lorent, J.H., Diaz-Rohrer, B., Lin, X. et al. *Structural determinants and functional consequences of protein affinity for membrane rafts*. Nat Commun 8, 1219 (2017).

[2] Marius F.W. Trollmann, Rainer A. Böckmann. *Characterization of domain formation in complex membranes*. Methods in Enzymology, Academic Press, (2024).

PS-FL-1-07

Compensatory effects of the disordered region of human harathyroid hormone on amyloid aggregation in crowded conditions

Twinkle Bhatia[1], Shubra Sachan[2], Jochen Balbach[2], Maria Ott[1]

[1] Martin Luther University Halle–Wittenberg, Institute for Biochemistry, Halle, Germany; [2] Martin Luther University Halle–Wittenberg, Institute of Biophysics, Halle, Germany

We acknowledge funding from the German research foundation (DFG, - TRR 102) " Polymers under multiple constraints",TP B12.

Human parathyroid hormone (PTH), comprising 84 amino acids, is secreted by the parathyroid glands and plays a pivotal role in regulating blood serum phosphate and calcium levels. PTH is also characterized as a functional amyloid due to its ability to store itself in the form of amyloid prior to its release.The N-terminal residues (S1-Q29) have a helical propensity, while residues (R25-L37) play a significant role in fibril formation[1]. PTH is largely disordered, as its C-terminus (D30-Q84) constitutes the intrinsically disordered section of PTH.
In this study, we investigated the role of the intrinsically disordered C-terminal region on pre-fibrillar aggregates by comparing sizes and structures of oligomers of the truncated variant and the full length protein using spectroscopic techniques as Fluorescence Correlation Spectroscopy (FCS) and Infrared (IR) spectroscopy
In the conclusion,we found that the absence of the C-terminal region lead to smaller oligomer and nuclei sizes as well as to accelerated fibrillation[2]. Moreover, using macromolecular crowding conditions, we could assign the differences in fibrillation kinetics, sizes, and structural changes to the compensatory effect of the intrinsically disordered region.

References

[1] Lauth, L. M., Voigt, B., Bhatia, T., Machner, L., Balbach, J., & Ott, M. (2022). Heparin promotes rapid fibrillation of the basic parathyroid hormone at physiological pH. FEBS letters, 596(22), 2928–2939. https://doi.org/10.1002/1873-3468.14455

[2] Voigt, B., Bhatia, T., Hesselbarth, J., Baumann, M., Schmidt, C., Ott, M., & Balbach, J. (2023). The Prenucleation Equilibrium of the Parathyroid Hormone Determines the Critical Aggregation Concentration and Amyloid Fibril Nucleation. Chemphyschem : a European journal of chemical physics and physical chemistry, 24(19), e202300439. https://doi.org/10.1002/cphc.202300439

PS-FL-1-09

Carbohydrates with vibrational probes: enabeling IR spectroscopic studies of carbohydrate-protein interactions

Philip Gasse[1], Till Stensitzki[1], Yasemin Mai-Linde[1], Torsten Linker[1], Thomas La Cour Jansen[2], Henrike Müller-Werkmeister[1]

[1] University of Potsdam, Chemistry, Potsdam, Germany; [2] University of Groningen, Faculty of Science and Engineering, Groningen, Netherlands

Protein-carbohydrate interactions play an important role in biochemical processes such as cell signaling, antibody recognition, and glucose metabolism. Although these processes can in principle be studied in molecular detail by IR spectroscopy, they remain largely unexplored by vibrational spectroscopy due to spectral congestion, i.e. strongly overlapping bands. We overcome this obstacle by introducing vibrational reporter groups into carbohydrates, which exhibit peaks in a spectral region free from other protein or carbohydrate signals. Specifically, we have studied mono- and di-saccharides with SCN and azide labels [1], using FTIR, time-resolved IR, and 2D-IR spectroscopy. We found that the position of the label strongly influences the spectra and observed dynamics. Furthermore, we find that the frequency of the reporter group depends significantly on its orientation relative to the ring. With the help of quantum chemical calculations, we attributed this to direct influence of the ring conformation, enabling us to indirectly observe ring-puckering in real-time with 2D-IR spectroscopy. Our results determine the lower speed limit of mono-saccharide ring puckering within ~100 ps.
Using different labelled glucose derivates, we are now targeting the molecular interaction between glucose and the enzyme hexokinase, demonstrating the feasibility of detailed biomolecular studies with these new tools.

References

[1] P. Gasse, T. Stensitzki, Y. Mai-Linde, T. Linker, H. M. Müller-Werkmeister, *2D-IR spectroscopy of carbohydrates: Characterization of thiocyanate-labeled β-glucose in $CHCl_3$ and H_2O*, J. Chem. Phys. 158, 145101 (2023)

PL-FL-1-11

A molecular view into the neuroprotective effects of chlorogenic acids.

Mercedes Alfonso-Prieto[1], Nicolas P. Müller[1,2], Olga A. Sergeeva[3,4], Luciano Navarini[5], Paolo Carloni[1,6]

[1] Forschungszentrum Jülich, INM-9 Computational Biomedicine, Jülich, Germany; [2] Heinrich-Heine University Düsseldorf, Faculty of Mathematics and Natural Sciences, Düsseldorf, Germany; [3] Heinrich Heine University Düsseldorf, Institute of Neural and Sensory Physiology, Düsseldorf, Germany; [4] Heinrich Heine University Düsseldorf, Institute of Clinical Neurosciences and Medical Psychology, Düsseldorf, Germany; [5] illy caffè SpA, Trieste, Italy; [6] RWTH Aachen University, Department of Physics, Aachen, Germany

MAP and PC thank the Ernesto Illy Foundation (Trieste, Italy).

Introduction

Chlorogenic acids (CGAs) are a group of compounds present in many fruits and vegetables, whose consumption has been associated to neuroprotection, among other health benefits [1]. One of the potential targets of CGAs mediating such beneficial effects at the molecular level is the peroxisome proliferator-activated receptor PPARα [2]. This nuclear receptor binds a wide variety of ligands, including fatty acids [3] and their derivatives [4], drugs against diabetes and hyperlipidemia and food molecules such as cinnamic acid [5], the parent compound of CGAs.

Methods

Here, we have combined molecular docking and molecular dynamics simulations to investigate the putative binding modes of cinnamic acid and CGAs in the different pockets of the PPARα binding cavity.

Results

Our computational modeling suggests that CGAs could bind to the receptor similarly to known endogenous agonists and drugs. In particular, cinnamic acid is able to bind to different PPARα pockets simultaneously, in line with the experimentally observed cooperativity effect [5], and exhibits an EC_{50} value comparable to known PPARalpha agonists, as shown by experiments on brain slices.

Conclusions

The molecular information obtained in this work could be used to prioritize CGA compounds for further experimental and computational validation, thus providing a stepping stone for designing future PPARalpha-based neuroprotective therapies.

References

[1] Lu H, Tian Z, Cui Y, Liu Z, Ma X 2020, 'Chlorogenic acid: A comprehensive review of the dietary sources, processing effects, bioavailability, beneficial properties, mechanisms of action, and future directions', *Compr Rev Food Sci Food Saf* 19(6):3130-3158.

[2] Willems S, Zaienne D, Merk D 2021, 'Targeting nuclear receptors in neurodegeneration and neuroinflammation', *J Med Chem* 64(14):9592-9638.

[3] Kamata S, Oyama T, Saito K, Honda A, Yamamoto Y, Suda K, Ishikawa R, Itoh T, Watanabe Y, Shibata T, Uchida K, Suematsu M, Ishii I 2020, 'PPARα ligand-binding domain structures with endogenous fatty acids and fibrates', *iScience* 23(11):101727.

[4] Sergeeva OA, Mazur K, Reiner-Link D, Lutsenko K, Haas HL, Alfonso-Prieto M, Stark H 2022, 'OLHA (Nᵅ-oleoylhistamine) modulates activity of mouse brain histaminergic neurons. *Neuropharmacology*, 215:109167.

[5] Chandra S, Roy A, Jana M, Pahan K 2019, 'Cinnamic acid activates PPARα to stimulate lysosomal biogenesis and lower amyloid plaque pathology in an Alzheimer's disease mouse model', *Neurobiol Dis*, 124:379-395.

PS-FL-1-13

Long-term single-molecule localization microscopy uncovers dynamic co-assembly of Lrp6 and Ror2 into Wnt-signalosomes

Michael Philippi[1], Julia Dohle[1], Isabelle Watrinet[1], Michael Holtmannspötter[1], Oliver Birkholz[1], Yi Miao[2], Ulrich Rothbauer[3], K. Christopher Garcia[2,4], Rainer Kurre[1], Jacob Piehler[1], **Changjiang You**[1]

[1] Osnabrück University, Department of Biology/Chemistry and Center for Cellular Nanoanalytics (CellNanOs), Osnabrück, Germany; [2] Stanford University, School of Medicine, Stanford, USA; [3] Eberhard-Karls-University, Pharmaceutical Biotechnology, Tübingen, Germany; [4] Howard Hughes Medical Institute, Stanford, USA

This project is supported by the DFG (YO 166/1-1, SFB 1557, projects 13 and Z2, and RTG 2900). K.C.G. is supported by the Ludwig Foundation.

Introduction

The conserved Wnt signaling has been classified as two categories of canonical and noncanonical Wnt signaling. With a high promiscuity of Wnt signaling, how receptors from the two distinct pathways re-arrange in multi-protein signalosomes remains elusive. We here developed single-molecule tracking and localization microscopy based on labeling with reversibly binding nanobodies (rbTALM) for imaging receptor dynamics in the plasma membrane for extended time periods. We engineered nanobody-tag pairs with fine-tuned binding stabilities ensuring single-molecule tracking with high fidelity, yet continuous exchange of photobleached labels. Multicolor rbTALM imaging enabled simultaneous tracking and super-resolution imaging of three different Wnt co-receptors in the same cell for more than one hour at video rate. Time-lapse correlation analyses uncovered cooperative association of canonical and noncanonical Wnt co-receptors into a common, hybrid Wnt signalosome, demonstrating the exciting possibilities of rbTALM imaging for exploring nanoscale dynamics across millisecond to hour timescales.

Methods

Single-molecule tracking and localization microscopy

Results

Multicolor, video-rate rbTALM imaging in living cells was developed with uncompromised labeling efficiency and tracking fidelity of transmembrane receptors up to 90 min.

Conclusions

The results uncovered cooperative association of canonical and noncanonical Wnt co-receptors into a common, hybrid Wnt signalosome.

PS-FL-1-15

Molecular dynamics simulations shed light on critical events in the early stages of human IRE1α activation

Elena Spinetti[1,2], Gülsün Elif Karagöz[3,4], Roberto Covino[1,2]

[1] Goethe University, Frankfurt am Main, Germany; [2] Frankfurt Institute for Advanced Studies, Frankfurt am Main, Germany; [3] Max Perutz Laboratories Vienna, Vienna, Austria; [4] Medical University of Vienna, Vienna, Austria

The inositol-requiring enzyme 1 (IRE1) is a highly conserved stress sensor within the endoplasmic reticulum (ER), crucial for mitigating the cytotoxic effects due to the accumulation of unfolded proteins. Dysregulation of the unfolded protein response (UPR), a network of signaling pathways for alleviating ER stress, is implicated in various human pathologies including diabetes, and cancer. IRE1, a transmembrane protein, relies on its core luminal domain (cLD) to sense misfolded protein accumulation and initiate downstream signaling events. While the involvement of IRE1 in recognizing unfolded proteins is well-established, the mechanism underlying this process in human cells remains debated. We conducted extensive molecular dynamics simulations to explore how the dimeric cLD of human IRE1α (hIRE1α) directly interacts with unfolded polypeptides at the atomic level. Our investigations revealed that hIRE1α cLD dimers are stable under non-stress conditions, with peptide binding occurring on the surface of the dimer rather than within its central MHC-like groove, as observed in the yeast homolog. These novel findings support a model where the direct interaction between IRE1 and unfolded proteins represents a crucial early event in the assembly of supramolecular complexes associated with activated IRE1.

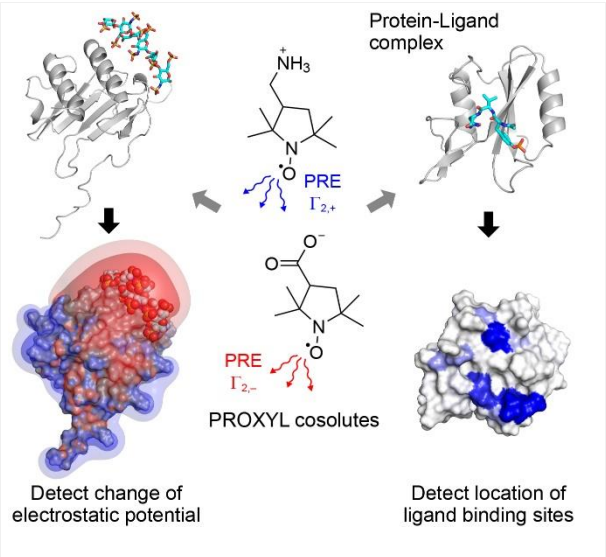
PS-FL-1-17

Detecting protein-ligand interactions with nitroxide based paramagnetic cosolutes

Anja Penk[1], Annemarie Danielsson[2], Margrethe Gaardløs[2], Cindy Montag[1], Andrea Schöler[3], Daniel Huster[1], Sergey Samsonov[2], **Georg Künze**[3]

[1] University of Leipzig, Institute for Medical Physics and Biophysics, Leipzig, Germany; [2] University of Gdańsk, Faculty of Chemistry, Gdańsk, Poland; [3] University of Leipzig, Institute for Drug Discovery, Leipzig, Germany

NMR spectroscopy is a widely used method in drug discovery research. In particular, paramagnetic NMR methods can provide structural restraints for modeling the ligand-target interaction. However, the covalent labeling of proteins with paramagnetic tags or the generation of metal ion-tagged ligands is complicated and bears the risk of perturbing the protein structure or ligand binding mode. Here we tested a tagging-free NMR approach which relies on the measurement of paramagnetic relaxation enhancements (PREs) arising from analogous cationic, anionic or neutral soluble nitroxide molecules, which distribute around the protein-ligand complex depending on near-surface electrostatic potentials. We applied this approach to two protein-ligand systems, interleukin-8 interacting with highly charged glycosaminoglycans and the SH2 domain of Grb2 interacting with less charged phospho-tyrosine tripeptides. The electrostatic potential around interleukin-8 and its changes upon binding of glycosaminoglycans could be derived from the PRE data and confirmed by theoretical predictions from Poisson-Boltzmann calculations. The ligand influence on the PREs and NMR-derived electrostatic potentials of Grb2 SH2 was localized to a narrow protein region which allowed the localization of the peptide binding pocket. Our analysis suggests that experiments with nitroxide cosolutes can be useful for investigating protein-ligand electrostatic interactions and mapping ligand binding sites.



NMR method for detecting protein-ligand interactions with paramagnetic cosolutes
The method relies on the measurement of paramagnetic relaxation enhancements (PREs) arising from analogous cationic, anionic or neutral soluble nitroxide molecules. It allows experimental measurement of the electrostatic potential around proteins and its ligand-induced changes. Ligand binding sites can be localized from the changes of the PRE data.

PS-FL-1-19

oncoGNN: physics guided geometric deep learning in cancer metastasis

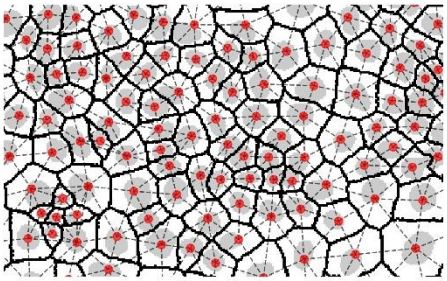
Kolya M. Lettl[1], Pablo Gottheil[1], Eliane Blauth[1], Anne-Sophie Wegscheider[2], Axel Niendorf[2], Josef Käs[1]

[1] Universität Leipzig, Peter Debye Institute for Soft Matter Physics, Leipzig, Germany; [2] MVZ Prof. Dr. med. A. Niendorf Pathologie Hamburg-West GmbH - Institut für Histologie, Zytologie und molekulare Diagnostik, Hamburg, Germany

Geometric deep learning extends machine learning to non-uniformly structured data, most notably graphs, a versatile data structure that can represent various physical systems. We employ this data model to transform data from different experiments into a common format, allowing us to apply insights from one type of data to another using both graph theory and graph neural networks (GNNs).

In this proof-of-concept work, we develop a model to predict cell motility using graphs derived from cell monolayer data (cf. figure “Cell Approximation & Graph Extraction”). Using morphological as well as graph theoretical node features, our GNN model achieves a high correlation between actual and predicted cell motility. Subsequently, we apply the trained model to clinical breast cancer tissue samples to predict the metastatic risk based on cancer cell motility.

This method provides a novel way to transfer knowledge between different types of biological data, offering valuable insights into cell behavior and disease progression. Specifically, it holds potential for improving our understanding of cancer metastasis, ultimately aiding in the development of more effective diagnostic and treatment strategies.



Cell Approximation & Graph Extraction
This figure illustrates the process of approximating the cells and extracting the graph structure. First, the cells (white) are approximated from the segmented nuclei (grey) with a watershed-based algorithm. Next, nodes (red circles) are added to represent each cell. Finally, nodes are connected (dashed lines) if their corresponding cells are neighbors, determined by slightly dilating each cell and detecting overlap with other cells.

3:00 pm – 4:30 pm

Foyer HSG

Poster-1 | Poster Session I

PS-I-01

Viscoelastic Properties of Mono- and Heterotypic Pancreatic Cancer Aggregates

Isis D.V.M. Lima, Mènie Wiemer, Shruti Kulkarni, Manfred Radmacher

Universität Bremen, Institute of Biophysics, Bremen, Germany

This study was financed in part by the Brazilian Research Agency CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), and the experiments were conducted entirely at the Institute of Biophysics within the University of Bremen. Special thanks are extended to the AG Radmacher group, particularly Mènie Wiemer for preparing most of the samples, and to Prof. Dr. Manfred Radmacher for his supervision and guidance.

Introduction

Cells are essentially viscoelastic materials, possessing an internal framework known as cytoskeleton. This structure is involved in many cell processes, such as movement and adhesion. Therefore, assessing viscoelastic properties of living cells and tissues contributes to the advancement of our knowledge related to physiological and pathological processes.

Methods

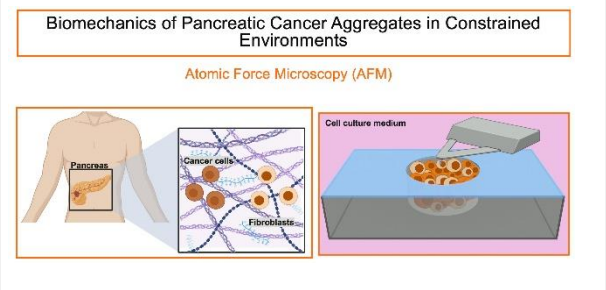
3D cell culture techniques mimic the cellular organization of tissues. Thus we developed a method to prepare multicellular aggregates in confined spaces: we coated Petri dishes with PolyHEMA, which reduces cell adhesion to the coated surfaces. We then drilled holes in the bottom of the Petri dishes and finally seeded cells into these holes to form mono- and heterotypic aggregates. We used PANC-1 or pancreatic cancer cells (originating from a human pancreatic carcinoma) and normal human dermal fibroblasts to investigate the mechanical response of tissues on multiple time scales using an atomic force microscope (AFM).

Results

From this, we developed a protocol to assemble PANC-1 cells to obtain monotypic and mixed aggregates. The latter appeared to be much more stable (potentially due to the extracellular matrix produced by the fibroblasts) and more rigid than pure PANC1 aggregates.

Conclusions

Therefore, AFM can be used to measure the viscoelastic properties of cells and cell aggregates and is able to distinguish between monotypic and mixed aggregates made from cancer cells and fibroblasts.



Investigating Viscoelastic Properties of Multicellular Aggregates in Confined Spaces

References
[1] Lazzari G, Nicolas V, Matsusaki M, Akashi M, Couvreur P, Mura S. Multicellular spheroid based on a triple co-culture: A novel 3D model to mimic pancreatic tumor complexity. Acta Biomater. 2018 Sep 15;78:296-307. doi: 10.1016/j.actbio.2018.08.008. Epub 2018 Aug 10. PMID: 30099198.
[2] Phung YT, Barbone D, Broaddus VC, Ho M. Rapid generation of in vitro multicellular spheroids for the study of monoclonal antibody therapy. J Cancer. 2011;2:507-14. doi: 10.7150/jca.2.507. Epub 2011 Oct 13. PMID: 22043235; PMCID: PMC3204399.
[3] Gnanachandran, K., Kędracka-Krok, S., Pabijan, J., & Lekka, M. (2022). Discriminating bladder cancer cells through rheological mechanomarkers at cell and spheroid levels. Journal of Biomechanics, *144*, 111346.

PS-II-01

Molecular dynamics simulations shed light on critical events in the early stages of human IRE1α activation

Elena Spinetti[1,2], Gülsün Elif Karagöz[3,4], Roberto Covino[1,2]

[1] Goethe University, Frankfurt am Main, Germany; [2] Frankfurt Institute for Advanced Studies, Frankfurt am Main, Germany; [3] Max Perutz Laboratories Vienna, Vienna, Austria; [4] Medical University of Vienna, Vienna, Austria

The inositol-requiring enzyme 1 (IRE1) is a highly conserved stress sensor within the endoplasmic reticulum (ER), crucial for mitigating the cytotoxic effects due to the accumulation of unfolded proteins. Dysregulation of the unfolded protein response (UPR), a network of signaling pathways for alleviating ER stress, is implicated in various human pathologies including diabetes, and cancer. IRE1, a transmembrane protein, relies on its core luminal domain (cLD) to sense misfolded protein accumulation and initiate downstream signaling events. While the involvement of IRE1 in recognizing unfolded proteins is well-established, the mechanism underlying this process in human cells remains debated. We conducted extensive molecular dynamics simulations to explore how the dimeric cLD of human IRE1α (hIRE1α) directly interacts with unfolded polypeptides at the atomic level. Our investigations revealed that hIRE1α cLD dimers are stable under non-stress conditions, with peptide binding occurring on the surface of the dimer rather than within its central MHC-like groove, as observed in the yeast homolog. These novel findings support a model where the direct interaction between IRE1 and unfolded proteins represents a crucial early event in the assembly of supramolecular complexes associated with activated IRE1.

PS-II-03

Free energy landscape of membrane topological transitions during fusion and pore formation

Katharina C. Scherer, Jochen S. Hub

Saarland university, Computational Biophysics Group, Saarbrücken, Germany

Biological membranes undergo significant topological transitions during essential processes like membrane fusion or pore formation. These transitions lead to the reshaping of membranes into non-bilayer conformations. While the general rearrangements of membranes are known, capturing the free energy landscape of these topological transitions remains a substantial challenge. We use MD simulations to calculate the potential of mean force along a reaction coordinate, serving as a measure of connectivity between two compartments. This computationally efficient approach allows us to explore the energetics associated with membrane fusion and pore formation and expansion, not only at coarse-grained level but also with atomistic resolution. [1][2] With this we investigate the impact of lipid composition and membrane-associated proteins, such as transmembrane domains of fusion proteins, on the energetics of membrane reshaping. Further we can apply our techniques to analyze the effect of photoswitchable lipids on pore formation and membrane fusion.

References

[1] Poojari, CS, Scherer, KS, Hub, JS 2021, 'Free Energies of Membrane Stalk Formation from a Lipidomics Perspective', *Nat. Commun.*, 12, 6594

[2] Hub, JS, 2021,'Joint Reaction Coordinate for Computing the Free-Energy Landscape of Pore Nucleation and Pore Expansion in Lipid Membranes', *J. Chem. Theory Comput.* 17, 2, 1229–1239

PS-II-05

Estimating DNA Deformation Free Energy in DNA-Protein Interactions

Christian Sustay Martinez, Martin Zacharias

Technical University of Munich, Center for Functional Protein Assemblies (CPA), Garching, Germany

We would like to thank SFB 1035 for the funding that makes this research possible.

Abstract

During sequence-specific binding of DNA-protein interactions, there are two main mechanisms for the recognition of target DNA, namely base (direct)- and shape (indirect)-readout. The former involves the recognition of the binding site via the formation of hydrogen-bonds and other contacts between the amino acids in the protein and the chemical groups of the DNA. On the other hand, shape-readout makes use of the sequence-dependent flexibility features of DNA where the ensemble of conformations that the oligonucleotide attains provides steric compatibility and promotes protein binding. In the present study we introduce a refactored multi-state Ising model of DNA flexibility that allows for a rapid computation of DNA deformation free energies from a DNA-bound MD trajectory, the decomposition of this energy per base-pair, helical variable and backbone state and extends to high-energy regimes where additional anharmonic terms are required. The model allows us to quickly assess the contribution of direct- and indirect-readout in the complex formation. Additionally, we employ a rigorous scheme for the computation of DNA deformation free energy from advanced sampling simulations. We apply this to the sequence-dependent binding of the FIS-DNA complex, where the sequence mismatches are present in the linker region between the binding sites and, therefore, changes in the binding free energy must originate from variations in DNA elasticity.

PS-II-07

Raft-partitioning of TM proteins

Nancy Mejía Villagrán[1], Marius F.W. Trollmann[1,2], Cristian R. Popov[1], Rainer A. Böckmann[1,2]

[1] Friedrich-Alexander-Universität Erlangen-Nürnberg, Computational Biology, Department of Biology, Erlangen, Germany; [2] Erlangen National High Performance Computing Center (NHR@FAU), Computational Biology, Department of Biology, Erlangen, Germany

Membrane rafts are small, highly dynamic lipid domains that compartmentalize cellular processes. They are believed to play crucial roles in multiple cellular functions, such as signal transduction. The precise nature, structure, and function of rafts are not well understood, making their characterization challenging and controversial. One major issue is the lack of quantitative methods for characterizing the raft-partitioning of trans-membrane (TM) proteins. Based on fluorescence studies on GPMVs, Lorent *et al.* (2017) developed a model for predicting the raft-partitioning based on protein features [1].

Here, we present a large-scale MD study of nanodomain formation [2] around >70 different TM proteins [1] using different model membranes within the Martini 3 force field. All proteins were modeled employing AlphaFold 3. The simulation results are used to decipher the sequence-specific properties that determine the partitioning to different membrane domains.

References

[1] Lorent, J.H., Diaz-Rohrer, B., Lin, X. et al. *Structural determinants and functional consequences of protein affinity for membrane rafts*. Nat Commun 8, 1219 (2017).

[2] Marius F.W. Trollmann, Rainer A. Böckmann. *Characterization of domain formation in complex membranes*. Methods in Enzymology, Academic Press, (2024).

PS-II-09

Crucial role of glycan conformations in the catalytic reaction of CAZymes

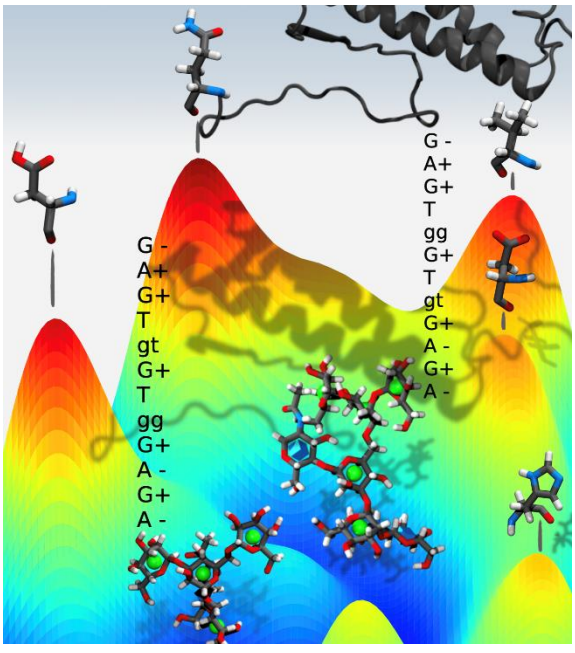
Isabell L. Grothaus[1,2], Lucio Colombi Ciacchi[1]

[1] University of Bremen, Faculty of Production Engineering, Bremen, Germany; [2] Jagiellonian University, Malopolska Centre of Biotechnology, Krakow, Poland

Structural biology has traditionally focused on protein folding, complex formation, and ligand binding. However, the structural elucidation of glycans, which are frequent post-translational modifications on protein surfaces, remains largely unexplored. While still little is known about their function, even less is understood about their structural behavior, especially because state-of-the-art experiments can hardly resolve glycan flexibility. This is to the disadvantage of understanding the synthesis of glycans in the cytoplasm, involving several carbohydrate-active enzymes (CAZymes) whose malfunction is linked to cancer and disease progression [1].

Therefore, we employ molecular dynamics simulation to investigate the correlation between adopted glycan conformers in CAZyme binding sites and the reduction of kinetic barriers for glycan hydrolysis. Our focus is on alpha Golgi-mannosidase 2, a promising but underexplored drug target [2]. Massive enhanced sampling molecular dynamics simulations of the enzyme/glycan complex reveal [3]:

1. a direct correlation between torsion angle settings and monosaccharide ring distortion, often necessary for making hydrolytic reactions energetically feasible.
2. a disruption of this correlation induced by key mutations in the catalytic site, leading to experimentally observed loss of CAZyme function.
3. the ring distortion to be induced by binding of the glycan to the catalytic site of the protein and not adopted prior to substrate binding.



The artistic phase space of an enzymatically bound N-glycan
The high-mannose type N-glycan M5G0 is restricted in its conformational phase space through the surrounding amino acids in the catalytic site. The presence of the protein is shading and altering the free energy landscape. Each conformer is flagged by corresponding conformer labels.

References

[1] Goss, P. E., Reid, C. L., Bailey, D. & Dennis, J. W. Phase IB clinical trial of the oligosaccharide processing inhibitor swainsonine in patients with advanced malignancies. *Clin Cancer Res Official J Am Assoc Cancer Res* **3**, 1077–86 (1997).

[2] Lee, Z. Y., Loo, J. S. E., Wibowo, A., Mohammat, M. F. & Foo, J. B. Targeting Cancer via Golgi α-mannosidase II Inhibition: How Far Have We Come In Developing Effective Inhibitors? *Carbohyd Res* **508**, 108395 (2021).

[3] Grothaus, I. L., Bussi, G. & Ciacchi, L. C. Exploration, Representation, and Rationalization of the Conformational Phase Space of N-Glycans. *J Chem Inf Model* **62**, 4992–5008 (2022).

PS-II-11

Brownian dynamics simulations of the bacterial cytoplasm

Apoorva Mathur, Ariane Nunes-Alves

Technical University Berlin, Institute for Chemistry, Berlin, Germany

Funding from DFG under Germany's Excellence Strategy - EXC 2008/1-390540038 – UniSysCat is gratefully acknowledged. The authors also gratefully acknowledge the computing time made available to them on the high-performance computer LISE at the Nationales Hochleistungsrechnen Center Zuse-Instituts Berlin (ZIB). This center is jointly supported by the Federal Ministry of Education and Research and the state governments participating in the NHR.

Introduction

Cells are crowded with macromolecules like proteins and nucleic acids at very high concentrations, up to 300 g/L. Macromolecular crowding can radically affect the conformation and dynamics of biomolecules inside cells due to excluded volume and weak interactions, which are not present in *in*-vitro assays.

Methods

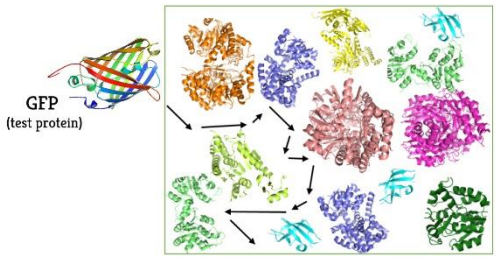
Here, two *in*-silico models of the *E. coli* cytoplasm with a heterogeneous population were built and simulated using Brownian dynamics (BD) simulations to gain mechanistic insights into the dynamics and interactions of proteins in the cellular environment. The first model was composed of the 30 most abundant proteins, and the other with the 46 most abundant proteins and 2 types of tRNA. The number of molecules was determined using proteomics data for *E. coli* cells. Green fluorescent protein (GFP) molecules were added to each system as a tracer protein. The simulations were benchmarked with experimental diffusion rates obtained for GFP inside *E. coli* cells and its curated interactome.

Results

The GFP diffusion rates observed so far in 5 µs of BD simulations, 0.21E-2 $Å^2$/ps and 0.27E-2 $Å^2$/ps, are close to the experimentally determined rates, ranging from 0.06E-2 to 0.14E-2 $Å^2$/ps. Transient interactions of crowder proteins with GFP were also mapped.

Conclusions

These benchmarked models will be used to develop a multi-scale approach to enable the study of dynamics, interactions, and conformational changes for any protein in a crowded cell-like environment.



Diffusion in crowded environments
In crowded environments, excluded volume affects the diffusion of macromolecules, and the interactions with protein crowders can stabilize or destabilize a protein.

PS-II-13

Comparison of different *de novo* modelling approaches for the FRET prediction of structural ensembles of ribonucleic acids

Mirko Weber, Felix Erichson, Richard Börner

University of Applied Sciences Mittweida, Laserinstitut Hochschule Mittweida, Mittweida, Germany

MW, FE and RB received funding from the European Union and the Free State of Saxony [ESF Plus: Young Research Group—LaNa-Sax:100649226]. MW is also funded by a research grant of the University of Applied Sciences Mittweida.

The integrative modelling of biomolecules relies on both a structural ensemble of a biomolecule and reliable experimental data reporting on its binding and folding trajectories. Therein, structural ensembles can be generated by *de novo* modelling tools or by all-atom molecular dynamics (MD) simulations. However, MD simulations are computationally expensive and limited regarding sampling capabilities of the conformational biomolecular ensemble [1]. We therefore use three different tools, RNAcomposer [2], Rosetta's FARFAR2 [3] and Alphafold3 [4], for the *de novo* RNA 3D structure prediction of a ribosomal RNA tertiary contact comprising a kissing loop and a GAAA tetraloop motif, generating a highly diverse structural ensemble. Subsequently we compute the accessible contact volume (ACV) of the FRET pair sCy3 and sCy5 using FRETraj [5] to predict the FRET distribution of each structural ensemble. We use the experimental FRET distribution to weight and analyse the predicted FRET distributions based on the structural ensemble of all tools. Our results indicate that RNAcomposer and Alphafold3 capture only a limited subset of possible structures and cannot replicate the full range of the experimental low FRET unbound state. In contrast, FARFAR2 generates a highly diverse structural ensemble that encompasses a wide range of possible structural conformations and almost perfectly replicates the experimental smFRET distribution.

References

[1] Hospital, A., Goñi, J. R., Orozco, M., & Gelpí, J. L. (2015). Molecular dynamics simulations: advances and applications. *Advances and applications in bioinformatics and chemistry : AABC*, *8*, 37–47. https://doi.org/10.2147/AABC.S70333

[2] Sarzynska, J., Popenda, M., Antczak, M., & Szachniuk, M. (2023). RNA tertiary structure prediction using RNAComposer in CASP15. *Proteins*, *91*(12), 1790–1799. https://doi.org/10.1002/prot.26578

[3] Watkins, A. M., Rangan, R., & Das, R. (2020). FARFAR2: Improved De Novo Rosetta Prediction of Complex Global RNA Folds. *Structure (London, England : 1993)*, *28*(8), 963–976.e6. https://doi.org/10.1016/j.str.2020.05.011

[4] Abramson, J., Adler, J., Dunger, J. *et al.* (2024). Accurate structure prediction of biomolecular interactions with AlphaFold 3. *Nature*. https://doi.org/10.1038/s41586-024-07487-w

[5] Steffen, F. D., Sigel, R. K. O., & Börner, R. (2021). FRETraj: integrating single-molecule spectroscopy with molecular dynamics. In J. Gorodkin (Ed.), Bioinformatics (Vol. 37, Issue 21, pp. 3953–3955). Oxford University Press (OUP). https://doi.org/10.1093/bioinformatics/btab615

PS-II-15

Revisiting experimental active site electric fields in TEM β-lactamases using polarizable AMOEBA MD simulations and computational infrared spectroscopy

Mirjam A. Schulz

Freie Universität Berlin, Experimental Molecular Biophysics, Forschungsbau SupraFAB, Berlin, Germany

We acknowledge the Deutsche Forschungsgemeinschaft for funding this project, and M.A.S. acknowledges helpful discussions with Jacek Kozuch and other members of the Kozuch LAB. Additionally, we thank Prof. Dr. Heberle for hosting this group in the Experimental Physics Department of Freie Universität Berlin.

The rise of resistance to broad-spectrum antibiotics in global healthcare systems is a growing concern as resistance develops faster than new antibiotics. Understanding the molecular mechanisms behind this resistance can help develop new, more effective antibiotics. One area of focus is the enzymes known as TEM β-lactamases, which play a crucial role in conferring resistance to class A β-lactam antibiotics. Recent research using infrared spectroscopy and the Vibrational Stark effect has revealed that these enzymes are highly effective due to electrostatic catalysis, which involves the electrostatic stabilization of the enzymes' active sites. This work aims to explore the experimental findings using molecular dynamics (MD) simulations with fixed-charge AMBER and polarizable AMOEBA force fields. The simulations confirmed the presence of powerful electric fields at the enzymes' active sites of about -170MV/cm, supporting the theory of electrostatic catalysis. Additionally, the results indicated the involvement of non-catalytic binding states at different stages of the enzymes' evolution to broad-spectrum resistance (Figure 1). Computational spectroscopy further helped uncover the origins of previously unexplained spectral features.

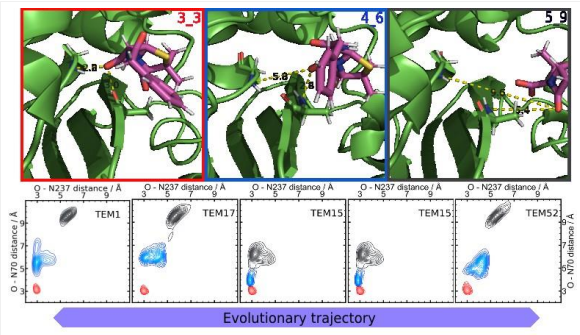


Figure 1. Illustration of different binding modes of PenG observed from AMOEBA MD simulations
a) Different binding modes of Penicillin (PenG) molecule into the oxyanion hole of the wildtype TEM1 β-lactamase. We distinguished three binding modes: 1. the catalytically active binding mode at distances of 3 Å to both active site AS, 2. binding mode at 3/4 Å
and 6 Å to the active site AS donating one hydrogen bond to the oxyanion hole, and 3. the 5+9Å binding mode without hydrogen bonds.
b) 2D correlations of the O-ß-lactam C=O-N between the oxyanion hole composed of the backbone N–H of S70 and A237 and the β-lactam C=O of PenG show the different binding modes found in AMOEBA MD simulations.

References
[1] S.H. Schneider, J. Kozuch, S.G. Boxer, The Interplay of Electrostatics and Chemical Positioning in the Evolution of Antibiotic Resistance in TEM β-Lactamases, ACS Central Science **2021**7 (12), 1996-2008

PS-II-17

Investigation of Gatifloxacin Permeation through Outer Membrane Porins of Gram-Negative Bacteria

Pratik K. Behera, Abhishek Acharya, Ulrich Kleinekathöfer

Constructor University, School of Science, Bremen, Germany

Introduction

Gram-negative bacteria have a distinct outer membrane that creates a formidable barrier to antibiotic permeation. Porins act as diffusion channels, providing a pathway for several antibiotics. Although numerous studies have explored antibiotic permeation through experimental and simulation approaches, a deeper understanding of the underlying mechanisms is still necessary. This investigation examines the permeation of the fluoroquinolone Gatifloxacin through OmpF, a porin found in *Escherichia coli*, and its homolog OmpK35 in *Klebsiella pneumoniae*.

Methods

Temperature Accelerated Sliced Sampling (TASS) is used for the free energy calculations, showing an improved sampling efficiency for the antibiotic permeation and accurate free energy calculations.

Results

These free energy calculations reveal feasible permeation pathways through the two porins and due to structural dissimilarities, expected variations in the free energies are found. These results prompted us to examine the interactions between the antibiotic molecule and the porins along the permeation pathways in detail.

Conclusions

The present study comprehensively analyzes the permeation of antibiotics across porin orthologs, providing insights into their properties and potential modifications to enable permeation through a broader range of diffusion channels.

References

[1] Prajapati, J. D.; Kleinekath öfer, U.; Winterhalter, M. How to Enter a Bacterium: Bacterial Porins and the Permeation of Antibiotics. Chem. Rev. 2021, 121, 5158–5192.

[2] Acharya, A.; Prajapati, J. D.; Kleinekath öfer, U. Improved Sampling and Free Energy Estimates for Antibiotic Permeation through Bacterial Porins. J. Chem. Theory Comput. 2021, 17, 4564–4577.

PS-II-19

Predictive Simulations of DNA Conformations Using NMR Chemical Shifts and Surface Electrostatic Potentials

Julian Holzinger, Petros Mavromatis, Andreas Reichl, Thomas Carell, Anne Schütz, Benjamin Fingerhut

Ludwig-Maximilians-Universität, Faculty of Chemistry, Munich, Germany

Determining structural parameters of the sugar-phosphate backbone of RNA and DNA from NMR chemical shifts remains a challenging task as these parameters do not correlate in a straightforward manner. Therefore, we aim to achieve quantitative accuracy of benchmark simulations and an understanding of the interactions, that govern the chemical shift in aqueous solution. In a first step, we measure NMR chemical shifts in the model system dimethylposphate at different temperatures and ionic conditions to calibrate the measured and simulated NMR parameters. In a second step, we transfer this knowledge to an epigenetically modified DNA model system (5-GCGATXGATCGC-3, where X stands for C in the reference and for 5caC in the modified DNA), on which we determine the NMR chemical shift parameters with and without Mg2+ ions as well as DNA surface electrostatic potentials to improve predictive MD simulations of DNA conformations.

References

[1] Schauss, Jakob, Kundu, Achintya, Fingerhut, Benjamin P., Elsaesser, Thomas 2019, 'Contact Ion Pairs of Phosphate Groups in Water: Two-Dimensional Infrared Spectroscopy of Dimethyl Phosphate and ab Initio Simulations', *J. Phys. Chem. Lett.*, 10, 20, 6281–6286.

[2] Dubini, Romeo C. A., Korytiaková, Eva, Schinkel, Thea, Heinrichs, Pia, Carell, Thomas, Rovó, Petra 2022, '^{1}H NMR Chemical Exchange Techniques Reveal Local and Global Effects of Oxidized Cytosine Derivatives', *ACS Phys. Chem Au*, 2, 3, 237–246.

[3] Yu, Binhan, Wang, Xi, Iwahara, Junji 2022, 'Measuring Local Electrostatic Potentials Around Nucleic Acids by Paramagnetic NMR Spectroscopy', *J. Phys. Chem. Lett.*, 13, 42, 10025–10029.

PS-III-01

Quantum Cascade Laser in IR Difference Spectroscopy: Exploring Photoresponses of Cryptochrome

Manu Diedrich, Jessica L. Klocke, Tilman Kottke

Bielefeld University, Biophysical Chemistry and Diagnostics, Bielefeld, Germany

Introduction

Quantum Cascade Lasers (QCLs) have been established for time-resolved IR spectroscopy as high-power radiation source with a broadly tunable wavelength, complementing existing globar applications. Implemented methods provide an effective tool for the structural and kinetic investigation of protein mechanisms, achieving time resolutions across extensive time scales from nanoseconds up to seconds.

Methods

We introduce an external cavity QCL transmission setup for time-resolved vibrational spectroscopy capable of handling protein samples in a flow cell with perpendicular sample scanning, yielding a very low sample consumption of nanoliters per minute [1]. We recently achieved a coverage in a range of 8 ns to 35 ms at a repetition rate of 2 Hz per acquisition.

Results

We performed characterization experiments on the performance using the irreversible photoreduction of flavin mononucleotide in aqueous solution. Measurements on the blue light receptor protein cryptochrome, featuring the chromophore flavin as a cofactor, are presented. We provide a methodical overview of the setup and the challenges with real-time processing of the acquired data.

Conclusions

Showcasing its application in studying the kinetics of protein photoreactions in aqueous solution, this setup provides a method for unraveling the protonation mechanisms of irreversible systems such as cryptochrome.

References

[1] Klocke, J.L., Kottke T., A quantum cascade laser setup for studying irreversible photoreactions in H2O with nanosecond resolution and microlitre consumption, 2020, 22, *PCCP*, 45, 26459, United Kingdom: Royal Society of Chemistry

PS-III-03

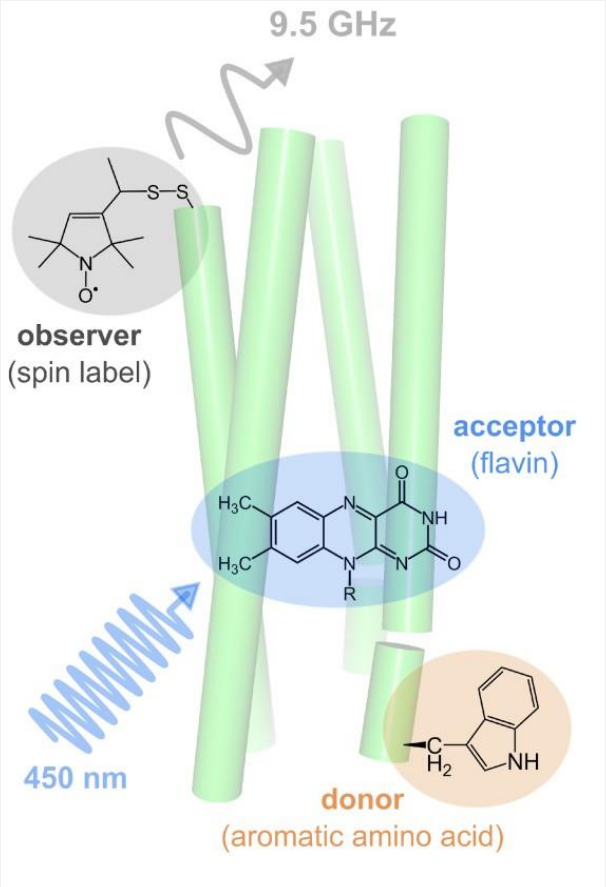
Flavomaquettes with observer spin label for hyperpolarization EPR spectroscopy

Sebastian Krebs[1], Varvara Apet[2], Irene Coin[2], Jens Meiler[1], Matthias Elgeti[1,3]

[1] University of Leipzig, Institute for Drug Discovery, Leipzig, Germany; [2] University of Leipzig, Institute for Biochemistry, Leipzig, Germany; [3] University of Leipzig, Institute for Medical Physics and Biophysics, Leipzig, Germany

This work was funded by the German Research foundation, project number 514664767, subproject B07 to IC, JM and ME.

Electron Paramagnetic Resonance (EPR) and Nuclear Magnetic Resonance (NMR) provide important tools for structural biology with high spatial resolution and picosecond to millisecond timescales. However, sensitivity remains a challenge due to unfavorable Boltzmann ratio and thus low intrinsic polarization. To address this limitation, our research focuses on developing a biological, hypersensitive spin tag for hyperpolarized EPR spectroscopy. We propose the design of a small protein tag, referred to as a “maquette,” which binds a flavin chromophore and carries a stable radical (observer spin). The electron polarization of a spin-correlated radical pair (SCRP), formed upon illumination of the bound flavin molecule and a nearby aromatic amino acid, can be transferred to the observer spin label. Using the stable radical as an "observer" we are able to study the formation and decay of the SCPR. In the initial phase, we focus on the de-novo design of the maquette based on flavoprotein light-sensors (LOV, BLUF, cryptochromes) by using computational tools like Rfdiffusion, MPNN and AlphaFold2. Key milestones are the optimization of the flavin binding pocked, characterization of radical pair formation and enhancing the SCRP yield. The final construct provides a hyperpolarized electron spin system suitable for EPR applications at extremely low concentrations. The novel flavomaquette platform can be used for low signal intensity EPR applications, including *in cell* EPR or EPR imaging.



Flavomaquettes with observer spin label
Example of a maquette protein used for observer spin EPR. Simple, stable protein structure with high affinity flavin binding pocked and covalently attached observer spin label.

PS-IV-01

Hydrophobicity of lipid anchor functionalization dictates the binding efficiency and phase selectivity of DNA origami to lipid membranes

Subhasini Singh, Henri G. Franquelim

Leipzig University, Interfaculty Centre for Bioactive Matter (b-ACTmatter), Leipzig, Germany

DNA origami has been proven to be a remarkable engineering tool when combined with lipid membranes [1]. Most of the described membrane-active DNA nanostructures though rely on the use of commercially available cholesterol modifications to achieve efficient binding to cell and model lipid membranes [2, 3]. However, these highly hydrophobic moieties can induce undesired aggregation hindering the binding efficiency [4]. Thus, we aim to explore new alternative strategies to bind DNA origami to lipid membranes, especially via weakly lipophilic anchors of different hydrophobicity (cLogP) values in order to improve the binding affinity.
Our model DNA origami comprises of linear Atto488-labeled 20-helix bundle functionalized with lipophilic moieties at the bottom facet. The binding affinity of DNA origami exhibiting different numbers and types of strongly to weakly lipophilic anchors were quantitatively studied on various lipid model systems (e.g., GUVs) using primarily confocal laser scanning method. Simultaneously, we were able to distinguish the minimal hydrophobicity required for binding DNA nanostructures to homogeneous membranes depending on the class of lipophilic anchor, along with the influence of combined anchors and their effect on membrane phase partitioning properties.
Overall, our strategy opens new avenues for mapping membrane vesicle properties, demonstrating the unique advantages of DNA nanotechnology for future membrane trafficking and biosensing applications.

References
[1] *Franquelim et al., 2018, Nat Commun, 9(1), 811*
[2] *Czogalla et al., 2016, Biophys J, 110 (8), 1698-1707*
[3] *Khmelinskaia et al., 2023, Methods Mol Biol, 2639, 231-255*
[4] *Ohmann et al., 2019, Nucleic Acids Research, 47(21), 11441-11451*

PS-IV-03

Insertion dynamics of the peptide Gramicidin S into lipid membranes using laser-induced temperature-jump IR spectroscopy

Manuel Oestringer, Karin Hauser

University of Konstanz, Department of Chemistry, Konstanz, Germany

Antibiotic resistance poses a critical threat to global health, necessitating the development of novel antibiotics. Antimicrobial peptides (AMPs) present a promising solution due to their ability to kill bacteria by disrupting cell wall integrity, primarily through membrane insertion. Because they target the cell wall, it is much harder for bacteria to develop resistance. Gramicidin S (GS) is a well-known AMP used for over 80 years with little resistance development. Thus, GS serves as a template for the design of new AMPs that are less harmful to human cells. Despite its well-characterized properties, the mechanism of GS insertion into membranes remains largely unknown. It is known that GS binds to the membrane surface in the gel phase, whereas it inserts into the membrane at temperatures around and slightly above the phase transition temperature [1]. By using a laser-induced temperature-jump we induce a fast phase change of the lipid phase [2,3] and thereby trigger the insertion of GS. This insertion process is monitored by time-resolved quantum cascade laser infrared spectroscopy. The changes in vibrational modes indicate the conformational dynamics of the peptide and the lipid environment.

References

[1] Afonin, S.; Glaser, R. W.; Sachse, C.; Salgado, J.; Wadhwani, P.; Ulrich, A. S. 19F NMR Screening of Unrelated Antimicrobial Peptides Shows That Membrane Interactions Are Largely Governed by Lipids. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **2014**, *1838* (9), 2260–2268

[2] Nagarajan, S.; Schuler, E. E.; Ma, K.; Kindt, J. T.; Dyer, R. B. Dynamics of the Gel to Fluid Phase Transformation in Unilamellar DPPC Vesicles. *J Phys Chem B* **2012**, *116* (46), 13749–13756

[3] Schuler, E. E.; Nagarajan, S.; Dyer, R. B. Submillisecond Dynamics of Mastoparan X Insertion into Lipid Membranes. *J. Phys. Chem. Lett.* **2016**, *7* (17), 3365–3370

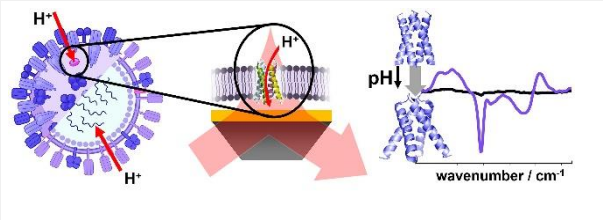
PS-IV-05

Combining vibrational and computational spectroscopy to elucidate the mechanism of the channel opening of Influenza A virus M2

Ronja R. Paschke[1], Swantje Mohr[3], Sascha Lange[3], Adam Lange[3], Jacek Kozuch[1]

[1] Freie Universität Berlin, Fachbereich Physik / Forschungsbau SupraFAB, Berlin, Germany; [2] Freie Universität Berlin, Fachbereich Physik / Forschungsbau SupraFAB, Berlin, Germany; [3] Leibniz-Forschungsinstitut für Molekulare Pharmakologie, Berlin, Germany; [4] Leibniz-Forschungsinstitut für Molekulare Pharmakologie, Berlin, Germany

Small hydrophobic viral proteins with ion channel activity, so-called viroporins, are involved in the virus particle entry into and release from the host cell, and thus present promising targets for antiviral therapy. In fact, it was shown for the M2 proton channel from Influenza A virus (IAV) that its pH-activated proton conductance can be inhibited, which led to its application as an antiviral drug. We employ surface-enhanced infrared absorption (SEIRA) spectroscopy to IAV M2 reconstituted within a solid-supported bilayer lipid membrane, which enables us to track the pH-activated large-scale reorientation of M2's transmembrane α-helices under in situ conditions via changes in its distinctive vibrational fingerprint[1]. By calculating computational SEIRA spectra based on a structural model from nuclear magnetic resonance and simulating a channel opening by tilting the transmembrane helices, we obtained an excellent match with the experimental picture. Combining the computational and spectroscopic results, we were furthermore able to quantify the opening angle of the channel transitioning from closed to the activated state. Intriguingly, M2 inhibitors block this mechanical motion. This combined spectroscopic and computational approach presents a tool to quantify structural changes of viroporins from pathogenic viruses of current relevance, possibly providing information on their function and inhibition.



SEIRA Spectroscopy Reveals Structural Changes During Opening of the Influenza A M2 Proton Channel

References
[1] J. Kozuch, K. Ataka, J. Heberle, *Nat. Rev. Methods Primers* 2023, 3, 70.
[2] R. R. Paschke, S. Mohr, S. Lange, A. Lange, J. Kozuch, *Angew. Chem. Int. Ed.* 2023, e202309069.

PS-IV-07

Stabilizing Black Lipid Membranes Formation on Anodic Aluminum Oxide Substrates Through Surface Optimization

Draia Tambasova[1], Felix Meyer[1], Claudia Steinem[1,2]

[1] Georg-August-Universität Göttingen, Institute of Organic and Biomolecular Chemistry, Göttingen, Germany; [2] Max Planck Institute for Dynamics and Self-Organization, Göttingen, Germany

Introduction

Black lipid membranes (BLMs) have long been a model system for studying the biophysical properties of biological membranes [1]. However, achieving stable and reproducible BLMs on solid substrates like anodic aluminum oxide (AAO) is challenging [2]. Moreover, when using complex lipid compositions to simulate the lipid bilayer of bacterial cells in order to determine the mechanism of action of antimicrobial peptides.

Methods

High-purity aluminum was anodized to create AAO substrates with 60 nm pores. These surfaces were coated with a 20 nm SiO_2 layer and treated with argon or oxygen plasma to enhance the hydrophilicity of surface [3]. E. coli total lipid extract was used to form BLMs on treated surfaces. Impedance spectroscopy monitored membrane formation and stability.

Results

Surface treatments significantly improved AAO substrates the hydrophilicity, aiding stable BLM formation. The SiO_2 coating combined with Ar plasma treatment provided the best results. Impedance spectroscopy confirmed successful membrane stability and reproducibility compared to previous lipid compositions.

Conclusions

Optimizing AAO substrates with SiO_2 coating and plasma treatments improves BLM stability and reproducibility. The use of a lipid composition based on E. coli demonstrates the possibility of using these systems to simulate a bacterial cell. In the future, this system could be used as a model to test the performance of antibacterial peptides such as lugdunin.

References

[1] Römer, W, Steinem, C 2004, 'Impedance analysis and single-channel recordings on nano-black lipid membranes based on porous alumina', *Biophysical Journal*, 86(2), 955-965, USA: Cell Press

[2] Buijnsters, JG, Zhong, R, 2013 'Surface wettability of macroporous anodized aluminum oxide', *ACS Applied Materials & Interfaces*, 5 (8), 3224-3233, USA: American Chemical Society

[3] Gupta, V, Madaan, N 2013, 'Hydrogen Plasma Treatment of Silicon Dioxide for Improved Silane Deposition', *Langmuir*, 29, 3604-3609, USA: American Chemical Society

PS-IV-09

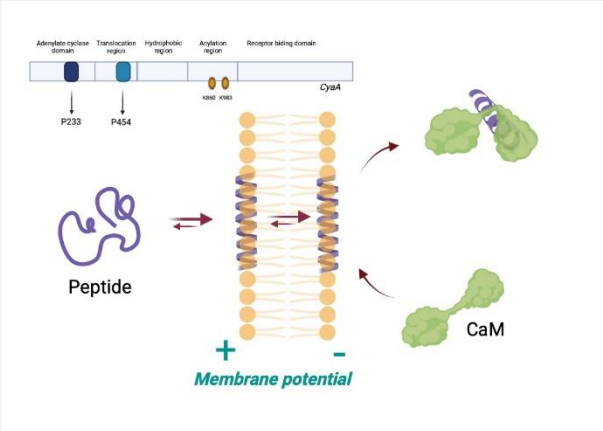
Membrane translocation process of two critical peptides from the CyaA toxin.

Gaia Scilironi[1,2], Nicolas Carvalho[1], Jacinthe Frangieh[1], Dorothée Raoux-Barbot[1], Corentin Léger[1], Sébastien Brulé[3], Iñaki J. Guijarro[4], Patrick England[3], Daniel Ladant[1], Nicolas Rodriguez[2], Sophie Cribier[2], Alexandre Chenal[1]

[1] Institut Pasteur, Biochemistry of Macromolecular Interactions Unit, Paris, France; [2] Sorbonne Université, Laboratoire des Biomolécules LBM, Paris, France; [3] Institut Pasteur, Plateforme de Biophysique Moléculaire, Paris, France; [4] Institut Pasteur, Biological NMR and HDX-MS Technological Platform, Paris, France

We acknowledge SOLEIL synchrotron radiation facilities, and we thank Frank Wien for assistance in using the Disco beamline.

The adenylate cyclase (CyaA) toxin is a major virulence factor produced by *Bordetella pertussis*, the causative agent of whooping cough. CyaA exhibits a unique pathway of intoxication: its catalytic domain is directly translocated across the target cell membrane in a calmodulin-dependent process, producing supraphysiological levels of cAMP, leading to host defence subversion and cell death. Uncovering the CyaA translocation mechanism is critical to understand the fundamental process of protein translocation and to improve biotechnological applications of CyaA-based antigen-delivery vehicles. Here, we investigate the translocation process of two peptides exhibiting membrane-active properties: the P233 peptide from the CyaA catalytic domain, and the P454 peptide from the CyaA translocation region, which is required for the translocation of the catalytic domain into the host cytoplasm. The segments of the catalytic and translocation domains covered by P233 and P454 are both crucial for CyaA translocation and play pivotal roles in the intoxication process. Through a combination of biophysical approaches (fluorescence, NMR, circular dichroism, and droplet interface bilayer model), we characterise the membrane interaction and translocation properties of these two peptides. The integrative analysis of P233 and P454 interactions with membranes provides new insights into the CyaA toxin translocation process.



Visual representation of the P233 and P454 membrane translocation process.

References

[1] Voegele A, Sadi M, O'Brien DP, Gehan P, Raoux-Barbot D, Davi M, Hoos S, Brûlé S, Raynal B, Weber P, Mechaly A, Haouz A, Rodriguez N, Vachette P, Durand D, Brier S, Ladant D, Chenal A. A High-Affinity Calmodulin-Binding Site in the CyaA Toxin Translocation Domain is Essential for Invasion of Eukaryotic Cells. Adv Sci (Weinh). 2021 Mar 8;8(9):2003630. doi: 10.1002/advs.202003630. PMID: 33977052; PMCID: PMC8097335.
[2] O'Brien DP, Durand D, Voegele A, Hourdel V, Davi M, Chamot-Rooke J, Vachette P, Brier S, Ladant D, Chenal A. Calmodulin fishing with a structurally disordered bait triggers CyaA catalysis. PLoS Biol. 2017 Dec 29;15(12):e2004486. doi: 10.1371/journal.pbio.2004486. PMID: 29287065; PMCID: PMC5764468.
[3] Subrini O, Sotomayor-Pérez AC, Hessel A, Spiaczka-Karst J, Selwa E, Sapay N, Veneziano R, Pansieri J, Chopineau J, Ladant D, Chenal A. Characterization of a membrane-active peptide from the Bordetella pertussis CyaA toxin. J Biol Chem. 2013 Nov 8;288(45):32585-32598. doi: 10.1074/jbc.M113.508838. Epub 2013 Sep 24. PMID: 24064217; PMCID: PMC3820891.
[4] Voegele A, Subrini O, Sapay N, Ladant D, Chenal A. Membrane-Active Properties of an Amphitropic Peptide from the CyaA Toxin Translocation Region. Toxins (Basel). 2017 Nov 14;9(11):369. doi: 10.3390/toxins9110369. PMID: 29135925; PMCID: PMC5705984.
[5] Voegele A, O'Brien DP, Subrini O, Sapay N, Cannella SE, Enguéné VYN, Hessel A, Karst J, Hourdel V, Perez ACS, Davi M, Veneziano R, Chopineau J, Vachette P, Durand D, Brier S, Ladant D, Chenal A. Translocation and calmodulin-activation of the adenylate cyclase toxin (CyaA) of Bordetella pertussis. Pathog Dis. 2018 Nov 1;76(8). doi: 10.1093/femspd/fty085. PMID: 30452651.

PS-IV-11

Extracellular calcium modulates metabolic glutamate receptor activation

Sadaf Dadashkhan, Klaus Benndorf, Ralf Schmauder

Friedrich Schiller University, Jena, Germany

Metabotropic Glutamate Receptors (mGluRs), like all Class C G-protein coupled receptors, function as obligatory dimers. Beyond glutamate, calcium ions are suggested as endogenous agonists of mGluR1. This study examines glutamate activation of mGluR1 under different ionic conditions using FRET to monitor receptor dynamics. Constructs with fluorescent proteins in the second intracellular loop of each subunit exhibit an increase in FRET upon ligand binding, thus allow to monitor activation.

Receptor activation is tracked in outside-out cell membrane patches to ensure that only ligand-exposed receptors contribute to the signal while maintaining a native membrane environment and high temporal resolution. A fast 30-channel microfluidic system allows us to map activation and cooperativity under a wide range of conditions.

We demonstrate that Ca^{2+} and glutamate reciprocally increase each other's affinity. However, Ca^{2+} cannot fully activate the receptor at physiological concentrations. We further show that Cl^- facilitates a higher affinity for glutamate while Ca^{2+}modulation is preserved.

This study aims to understand how mGluRs integrate neurotransmitter exposure and ionic conditions as a measure of synaptic activity.

PS-IV-13

Long-term single-molecule localization microscopy uncovers dynamic co-assembly of Lrp6 and Ror2 into Wnt-signalosomes

Michael Philippi[1], Julia Dohle[1], Isabelle Watrinet[1], Michael Holtmannspötter[1], Oliver Birkholz[1], Yi Miao[2], Ulrich Rothbauer[3], K. Christopher Garcia[2,4], Rainer Kurre[1], Jacob Piehler[1], **Changjiang You**[1]

[1] Osnabrück University, Department of Biology/Chemistry and Center for Cellular Nanoanalytics (CellNanOs), Osnabrück, Germany; [2] Stanford University, School of Medicine, Stanford, USA; [3] Eberhard-Karls-University, Pharmaceutical Biotechnology, Tübingen, Germany; [4] Howard Hughes Medical Institute, Stanford, USA

This project is supported by the DFG (YO 166/1-1, SFB 1557, projects 13 and Z2, and RTG 2900). K.C.G. is supported by the Ludwig Foundation.

Introduction

The conserved Wnt signaling has been classified as two categories of canonical and noncanonical Wnt signaling. With a high promiscuity of Wnt signaling, how receptors from the two distinct pathways re-arrange in multi-protein signalosomes remains elusive. We here developed single-molecule tracking and localization microscopy based on labeling with reversibly binding nanobodies (rbTALM) for imaging receptor dynamics in the plasma membrane for extended time periods. We engineered nanobody-tag pairs with fine-tuned binding stabilities ensuring single-molecule tracking with high fidelity, yet continuous exchange of photobleached labels. Multicolor rbTALM imaging enabled simultaneous tracking and super-resolution imaging of three different Wnt co-receptors in the same cell for more than one hour at video rate. Time-lapse correlation analyses uncovered cooperative association of canonical and noncanonical Wnt co-receptors into a common, hybrid Wnt signalosome, demonstrating the exciting possibilities of rbTALM imaging for exploring nanoscale dynamics across millisecond to hour timescales.

Methods

Single-molecule tracking and localization microscopy

Results

Multicolor, video-rate rbTALM imaging in living cells was developed with uncompromised labeling efficiency and tracking fidelity of transmembrane receptors up to 90 min.

Conclusions

The results uncovered cooperative association of canonical and noncanonical Wnt co-receptors into a common, hybrid Wnt signalosome.

PS-IV-15

Lipid Oxidation: where Biophysics and analytical Chemistry meet

Patricia Prabutzki[1], Kathrin M. Engel[1], Marharyta Podliian[2], Eliane Blauth[2], Josef A. Käs[2], Jürgen Schiller[1], **Jenny Leopold**[1,2]

[1] Leipzig University, Medical Faculty, Institute for Medical Physics and Biophysics, Leipzig, Germany; [2] Leipzig University, Soft Matter Physics Division, Peter-Debye-Institute for Soft Matter Physics, Leipzig, Germany

Generated reactive oxygen species (ROS) during inflammatory processes leads to the oxidation of biomolecules. Lipid membranes are known to act as barriers against the diffusion of ROS into the cell and, thus, limit the oxidation of proteins and particularly DNA but become targets for oxidation themselves. Despite many advances, the detection of oxidatively modified lipids (oxLipids) in biological matrices is still a challenging task due to their low abundance, huge chemical diversity, limited stability and pronounced matrix effects. Therefore, sensitive as well as robust analytical techniques like matrix-assisted/laser desorption ionization (MALDI) mass spectrometry (MS) and electrospray ionization (ESI) MS are the methods of choice. In order to identify product patterns of different lipid oxidation model systems, lipid standards, oxidized by either HOCl, $NaMnO_4$ or air, were screened by MALDI and ESI MS. Not only do the different oxidation methods give rise to drastically different oxidation products, some oxLipids were also detected with varying sensitivity between ESI and MALDI MS. Additionally, the impact of the identified oxLipids on membrane properties was investigated. For this purpose, giant unilamellar vesicles (GUV) were prepared via electro-swelling and characterized regarding their size, shape, bending stiffness, and blebbing behavior using different microscopy techniques. The observed differences upon the incorporation of the oxLipids into GUV will be highlighted.

PS-IV-17

A role for lipid bilayer asymmetry in fibroblast growth factor 2 (FGF2) membrane translocation across plasma membranes

Manpreet Kaur, Fabio Lolicato, Walter Nickel

Heidelberg University, Biochemistry Center, Heidelberg, Germany

FGF2 is a peripheral membrane protein secreted via an unconventional secretory pathway. In a cellular context, a single event of FGF2 membrane translocation happens within 200ms, however, translocation *in vitro* occurs at a time scale of minutes. In cells, the first contact of FGF2 with the inner plasma membrane leaflet is the Na,K-ATPase followed by phosphorylation of FGF2 by Tec kinase. Subsequently, $PI(4,5)P_2$-dependent FGF2 oligomerization triggers the formation of a toroidal membrane pore. FGF2 translocation into the extracellular space is completed by GPC1 heparan sulfates chains that capture and disassemble FGF2 oligomers into dimers at the outer leaflet of the plasma membrane. One explanation for the observed difference in the kinetics of FGF2 translocation in cells and *in vitro* could be the asymmetrical distribution of $PI(4,5)P_2$ between the two monolayers in the plasma membrane versus the symmetrical $PI(4,5)P_2$ distribution in liposomes. To mimic the asymmetry of the plasma membrane in reconstitution experiments, we have developed an experimental system to produce liposomes with an asymmetric distribution of $PI(4,5)P_2$ that is based on the enzymatic conversion of PI(4)P to $PI(4,5)P_2$ on liposomal membranesurfaces. This system allows us for studying the rate-limiting steps in FGF2 membrane translocation with the hypothesis of the cellular $PI(4,5)P_2$ asymmetry being a thermodynamic driving force of FGF2 membrane translocation.

PS-IV-19

Lipid phase-dependent membrane and photoreceptor dynamics investigated by QCL-based IR spectroscopy

Paul Stritt, Karin Hauser

University of Konstanz, Department of Chemistry, Konstanz, Germany

Intro

Time-resolved IR spectroscopy reveals the interaction dynamics of membrane proteins and lipid membranes. The protonation and conformational dynamics of the photoreceptor bacteriorhodopsin (BR) were measured with our QCL-based spectrometer (A) [1]. To study the membrane's dynamics, lipids with deuterated alkyl chains were employed, shifting the respective bands into a spectrally silent window within the QCL's coverage (B). By reconstituting BR into DSPC-d_{70} liposomes, the dynamics of the lipid alkyl chains' symmetric and antisymmetric CD_2 modes were resolved, uncovering correlations between lipid transients and protein intermediates [2]. Here, we analyzed the impact of the lipid phase on dynamics.

Methods

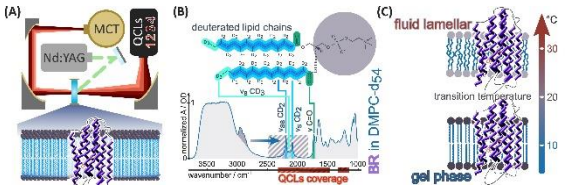
Reconstituting BR into DMPC-d_{54} liposomes – having a suitable phase transition temperature – enabled the analysis of temperature- and lipid phase-dependent dynamics in the gel phase, during the phase transition, and in the fluid lamellar phase (C). The QCL spectrometer provides protein and lipid transients from 100 ns to 20 ms with nanosecond resolution.

Results

The gel and fluid lamellar phases affect the protein's protonation and conformation dynamics differently. Site-specific probes within the membrane show varying correlations to protonation and conformational dynamics in different phases.

Conclusions

Single wavenumber measurements show lipid phase-dependent interplay between protein and membrane dynamics and reveal site-specific dynamics of the lipid membrane.



QCL-IR spectroscopy reveals site-specific membrane dynamics during protein activity
(A) Home-built QCLs-based spectrometer with four different laser heads for single wavenumber measurements with a mercury cadmium telluride (MCT)-detector and a frequency-doubled Nd:YAG laser for photoexcitation. **(B)** FTIR absorption spectrum of BR in DMPC-d_{54} with fully deuterated alkyl chains. Dynamics were probed at different sites of the lipid membrane: lipid ester's C=O (green), alkyl chain's CD_2 (blue) and CD_3 (aquamarine). **(C)** Lipid phase transition from gel phase to fluid lamellar phase of DMPC-d_{54} proteoliposomes with a transition temperature of about 20 °C.

References

[1] Stritt, P., Jawurek, M., Hauser, K. 2020, ’Application of tunable quantum cascade lasers to monitor dynamics of bacteriorhodopsin in the mid-IR spectral range’, *Biomed. Spectrosc. Imaging*, 9, 55-61, DOI: 10.3233/bsi-200195
[2] Stritt, P., Jawurek, M., Hauser, K. 2023, ’Mid-IR quantum cascade laser spectroscopy to resolve lipid dynamics during the photocycle of bacteriorhodopsin’, *J. Chem. Phys.*, 158, 154202, DOI: 10.1063/5.0139808

PS-IV-21

Novel proximity sensitive one-fluorophore biosensing approaches to detect DNA nanostructures binding to lipid membranes

Julian P.M. Kiesel[1,2], Jaqueline T. Mallee[1,2], Henri G. Franquelim[1,2]

[1] Leipzig University, Interfaculty Center for Bioactive Matter, Leipzig, Germany; [2] Leipzig University, Peter Debye Institute for Soft Matter Physics, Leipzig, Germany

J.P.M. Kiesel and J.T. Mallee contributed equally to this work.

Fluorescence microscopy has been a predominant approach for the investigation of biomolecular processes. The plethora of available fluorescent dyes enables the selection of a suitable fluorophore for practically all interests. For instance, when studying reactions such as ligand-receptor binding, researchers commonly opt to label both constituents. Here, we propose a less complex approach where only one of the two components has to be stained by a single proximity sensitive dye. Such fluorophores include for instance those responsive to altering pH-values [1], as well as those classified as photoisomerisation-related fluorescence enhancement (PIFE) active [2]. As a model system we choose to investigate the interaction of functionalised DNA constructs with lipid membranes [3]. We apply the proposed method of a one-fluorophore system to two diametrical cases: At first, staining a lipid membrane and observing the emitted fluorescence when functionalised DNA nanostructures bind to it. Secondly, we place the fluorescent dyes onto the DNA constructs themselves, studying fluorescence parameters when it anchors itself into lipid vesicles. Both systems allow for a microscopic investigation of single-molecule events, as well as ensemble measurements.
Our goal is to provide a less costly and less time-consuming alternative to comparable techniques while maintaining high resolution. Additionally, the systems simplicity promises to be applicable in a wide range of cases.

References
[1] Robison A D, et al. (2013) Fluorescence modulation sensing of positively and negatively charged proteins on lipid bilayers. *Biointerphases;* 8:1
[2] Ploetz E, et al. (2023) A new twist on PIFE: photoisomerisation-related fluorescence enhancement. *Methods Appl Fluoresc;* 12(1): 012001
[3] Khmelinskaia A, et al. (2023) Binding and Characterization of DNA Origami Nanostructures on Lipid Membranes. *Methods Mol Biol;* 2639:231-255

PS-IV-23

Towards probing possible long-range interactions and lipid interactions of the SNARE protein SNAP-25a

Sophia Werner[1,2], Tobias Stief[1,2], Nils-Alexander Lakomek[1,2], Katharina Vormann[1,2], Selcuk Er[1]

[1] Heinrich Heine Universität, Düsseldorf, Germany; [2] Forschungszentrum Jülich, Jülich, Germany

The SNAP-25a (isoform 2) protein is a part of the SNARE complex, a four-helix bundle composed of SNAP-25a and the two other SNARE proteins synaptobrevin-2 and syntaxin-1a. SNAREs are responsible for the transport of vesicles and membrane fusion to exocytosis. SNAP-25a contributes with two α-helices, while synaptobrevin-2 and syntaxin-1a contribute with one helix each to a four-helix bundle. In its monomeric pre-fusion form, SNAP-25a is disordered, except for two α-helices at the N-terminus and displays a high flexibility[1]. SNAP-25a lacks a transmembrane region but has four palmitoylation sites that anchor the protein to the cell membrane.

We aim for deeper insights into the structural dynamics and long-range interaction of the pre-fusion SNAP-25a monomer in the presence and absence of a lipid bilayer. Our current focus is a possible interaction between the N- and C-terminus of SNAP-25a and the palmitoylation site.

NMR spectroscopy is used as the primary technique supported by further biophysical methods. Paramagnetic relaxation enhancement (PRE) data are recorded to obtain long-range distance information relative to a paramagnetic spin-label (MTSL).

High-purity protein samples for a diamagnetic wild-type and a cysteine mutant were obtained, and the paramagnetic spin label was introduced successfully. Both yielded well-resolved NMR spectra. $^{1}HT_2$ relaxation data are currently recorded for the PREs and will be presented at the conference.

References

[1] Stief, T. *et al.* Intrinsic Disorder of the Neuronal SNARE Protein SNAP25a in its Pre-fusion Conformation. *Journal of Molecular Biology* **435**, doi:ARTN 168069 10.1016/j.jmb.2023.168069 (2023).

PS-IV-25

Targeting the endosomal membrane by biomimetic, pH-sensitive polymers

Annika H. Blum[1], Stefanie Schmager[2], Franziska Hausig-Punke[3], Johannes C. Brendel[3,4], Maria Hoernke[1,2]

[1] Martin Luther Universität Halle–Wittenberg, Physical Chemistry, Halle, Germany; [2] Albert-Ludwigs-Univeristät, Chemistry and Pharamcy, Freiburg, Germany; [3] Friedrich Schiller Universität, IOMC, Jena, Germany; [4] Universität Bayreuth, Macromolecular Chemistry, Bayreuth, Germany

Introduction

The interaction of biomimetic polymers with membranes of various lipid composition can cause membrane perturbations. This can be exploited to overcome membranes in the context of drug delivery (endosomal escape). The lowering of the endosomal pH, and the occurrence of the negatively charged lipid BMP can be used to selectively permeabilize endosomal membranes.

Methods

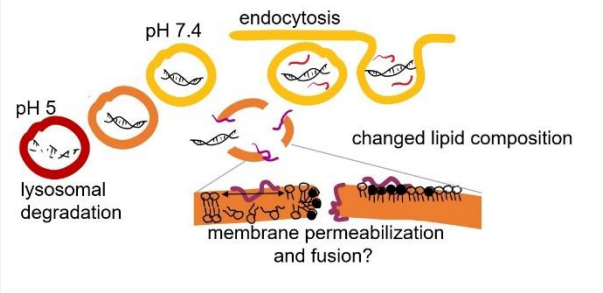
We investigate the principles of pH-sensitive activity and selectivity of membrane-active polycations for membranes of different lipid composition and pH values, representing different cell compartments and stages of endo-lysosomal development. Membrane leakage, electrostatic lipid clustering, and membrane fusion can play important roles for membrane permeabilization. Using fluorescence and filmbalance methods, as well as microcalorimetry, we characterized membrane perturbations caused by the polymers in large/giant unilamellar and multilamellar vesicles and lipid monolayers. The polymer pKa, pH, and lipid composition are varied.

Results

We find that the pH sensitive polymers do not insert into lipid membranes. Our data indicate that the polymers interact electrostatically with negatively charged model membranes and induce local heterogeneities as well as limited permeabilization.

Conclusions

This dominance of polymer and membrane charge underlines the role of the negatively charges endosomal lipid BMP, while the pH change contributes indirectly to endosomal escape by increasing the polymer charge.



Polymer-induced endosomal escape
Principles of endolysosomal development and effect of pH sensitive polymers.

PS-V-01

Regulation of helicase activity in long-range DNA end resection

Martin Mütze[1], Ilaria Ceppi[2], Maria Rosaria Dello Stritto[2], Stefan Braunshier[2], Valentina Mengoli[2], Giordano Reginato[2], Ananya Acharya[2], Megha Roy[2], Aurore Sanchez[2], Swagata Halder[2], Sean Michael Howard[2], Ralf Seidel[1], Petr Cejka[2]

[1] Universität Leipzig, Peter-Debye-Institut für Physik der weichen Materie/ Group Molecular Biophysics, Leipzig, Germany; [2] Università della Svizzera italiana (USI), Faculty of Biomedical Sciences/ Institute for Research in Biomedicine, Bellinzona, Switzerland

Introduction

In the process of repairing DNA double-strand breaks (DSB) through homologous recombination, a crucial step involves DNA end resection, where the 5'-terminated strands at DSB sites undergo controlled degradation. This process is separated in two distinct steps, short-range and long-range DNA end resection. Both involve the interplay of different proteins working together to create the single-stranded DNA overhangs. For our research we focus on the long-range DNA end resection and its regulation through different proteins, especially the tumor suppressor associated with breast cancer BRCA1-BARD1.

Methods

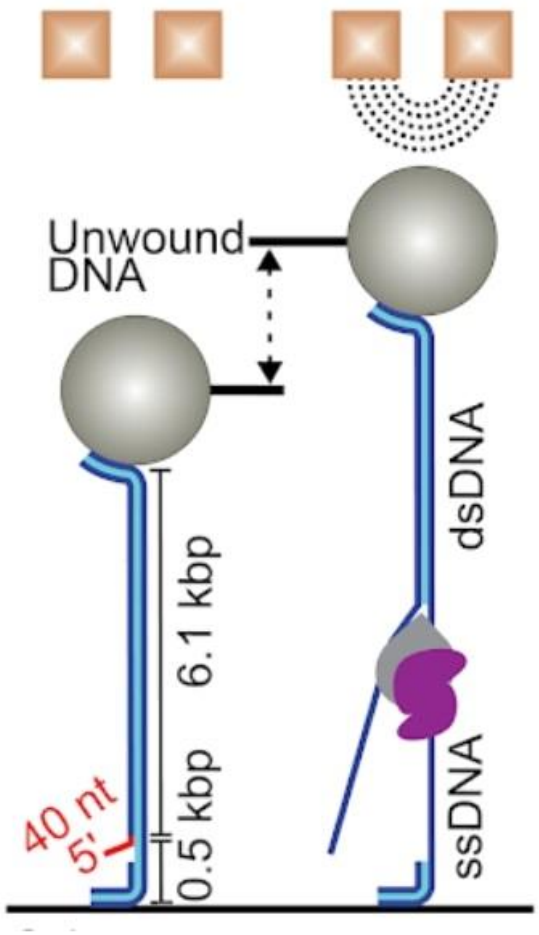
We employed purified recombinant proteins and utilized a combination of bulk-essay data and single molecule measurements conducted with magnetic tweezers.

Results

Our research demonstrates that BRCA1-BARD1 directly facilitates long-range DNA end resection. Specifically, in the DNA2-dependent pathway, BRCA1-BARD1 stimulates DNA unwinding through the WRN/BLM RecQ family helicase. We also found that phosphorylated CtIP drastically stimulates the motor activity of DNA2, and that the MRN-protein complex, normally associated to short-range DNA end resection, also stimulates the motor function of the RecQ helicases.

Conclusions

Our findings show that more proteins are integral to long-range DNA end resection, and it is our hypothesis that the stimulation of proteins through specific interaction partners makes the whole process more robust in vivo.



Investigating Helicase Activity with Magnetic Tweezers

A schematic of the employed single-molecule magnetic tweezers assay setup and the DNA substrate specifically designed to investigate long-range DNA end resection. The helicase complex binds to the 5' overhang and from there starts its motor function, converting double stranded DNA into single stranded DNA. As a result, the length of the DNA substrate increases which can be directly tracked in our setup.

PS-V-03

Multi-color single-molecule localization microscopy to study the SOS response regulation in *E. coli*

Pilar Lörzing[1], Philipp Schake[1,2], Michael Schlierf[1,3]

[1] TU Dresden, B CUBE, Center for Molecular Bioengineering, Dresden, Germany; [2] TU Dresden, Biotechnology Center, Center for Molecular Bioengineering, Dresden, Germany; [3] TU Dresden, Physics of Life, DFG Cluster of Excellence, Dresden, Germany

The SOS regulatory network in *Escherichia coli* orchestrates a multifaceted response to DNA damage, balancing high-fidelity repair mechanisms with error-prone survival pathways. Central to this network is the transcriptional regulator LexA, governing the expression of over 50 genes. Here, we employ 3D super-resolution microscopy to investigate the spatial organisation of LexA. By visualizing LexA-PAmCherry with PALM in conjunction with DNA- and membrane-targeted PAINT labels we observe distinct colocalization patterns providing insights on the LexA distribution within the cellular environment. LexA is mainly associated with the nucleoid, showing a predominant fraction around it. Employing an anisotropic 3D DBSCAN approach, we precisely identify multiple LexA clusters per cell, hinting at their functional significance in SOS response organisation. To further analyse whether these clusters correspond to specific LexA binding sites on the chromosome, we use a *parS*/ParB tagging system to localize specific SOS genetic loci. We provide a more comprehensive understanding of LexA regulation mechanisms within the SOS network and also offer insights into the broader context of bacterial chromosome organization.

PS-V-05

Sequence-Dependent Interaction between the Psoralen Derivative Amotosalen and DNA

Anna T. Ott, Michelle P. Rademacher, Peter Gilch

Heinrich-Heine-Universität, Institut für Physikalische Chemie, Düsseldorf, Germany

This work was founded by the Deutsche Forschungsgemeinschaft, grant number GI 349/6-2. M. P. Rademacher is grateful for a scholarship of the Jürgen Manchot Foundation.

Introduction

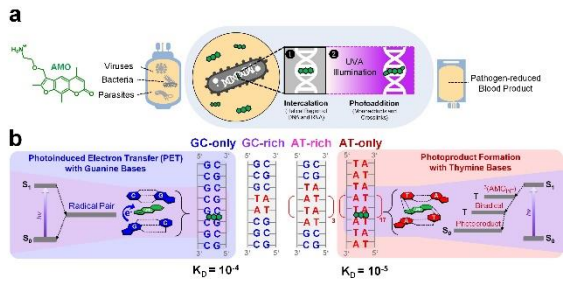
The synthetic psoralen derivative amotosalen (AMO) is a photoactive agent, usedfor pathogen-inactivation of blood products [1]. The mode of action comprises two steps (Fig. 1a): First, AMO reversibly intercalates in between DNA base pairs. Second, it irreversibly bonds with thymine upon UVA-illumination, leading to DNA damage. Earlier work by our group showed that photo-excited psoralens can be quenched by photo-induced electron transfer (PET) in addition to the desired photo-addition, depending on the base pair environment [2]. Unlike other investigated psoralens, AMO intercalates into A,T-only DNA with a higher affinity compared to G,C-only DNA [3], possibly suppressing unwanted photo-induced electron transfer (PET) with guanine (Fig. 1b).

Methods

Here, the interaction of AMO with mixed DNA-sequences, bearing all DNA bases, is studied *via* steady-state and time-resolved spectroscopy on the femto- and nanosecond-time-scale.

Results

Binding assays suggest that a preferential intercalation of AMO into A,T-sites prevails in mixed DNA. Time-resolved measurements show a sequence-dependent superposition of the photoaddition and PET of AMO in “mixed” DNA.



Amotosalen (AMO) and its interaction with DNA. **a)** Pathogen-reduction process for blood products. **b)** Photo-induced electron transfer (PET) from guanine (G) to AMO intercalated into G,C-only DNA (left). Photoaddition of AMO and thymine (T) after irradiation in A,T-only DNA (right). In mixed DNA, either PET or photo-addition is observed separately depending on the intercalation position (middle).

References

[1] Kaiser-Guignard, J, Canellini, G, Lion, N, Abonnenc, M, Osselaer, J C, Tissot, J D 2014, ‘The clinical and biological impact of new pathogen inactivation technologies on platelet concentrates’, *Blood reviews*, *28*.6, 235-241.

[2] Fröbel, S, Reiffers, A, Torres Ziegenbein, C, Gilch, P 2015, ‘DNA intercalated psoralen undergoes efficient photoinduced electron transfer’, *The Journal of Physical Chemistry Letters*, 6.7, 1260-1264.

[3] Rademacher, M P, Rohn, T, Haselbach, W, Ott, A T, Bringmann, P W, Gilch, P 2024, ‘Spectroscopic view on the interaction between the psoralen derivative amotosalen and DNA’, *Photochemical & Photobiological Sciences*, 1-17.

PS-V-07

New insights into the structural dynamics of the intrinsically disordered SNARE protein SNAP25a by high-field NMR spectroscopy

Tobias Stief[1,2], Katharina Vormann[1,2], **Nils-Alexander Lakomek**[2,1]

[1] Forschungszentrum Jülich, IBI-7: Stuctural Biochemistry, Jülich, Germany; [2] Heinrich-Heine-University Düsseldorf, Physical Biology, Düsseldorf, Germany

N.L. thanks the German Science Foundation for funding through the Heisenberg Program (DFG grant number 433700474). We acknowledge generous access to the Jülich-Düsseldorf Biomolecular NMR Center, jointly run by Forschungszentrum Jülich and Heinrich Heine University Düsseldorf (HHU).

Introduction

Intrinsically disordered proteins (IDPs) compose about 30% of the human proteome and are highly dynamic entities well suited for NMR spectroscopy. Recent advances in NMR magnet technology allow for increasingly higher magnetic field strengths, up to 28 Tesla (corresponding to 1.2 GHz Proton Larmor frequency). Those high field strengths offer improved resolution, with particular benefits for studying IDPs. We use the SNARE proteins as a model system. SNARE proteins play a crucial role during neuronal exocytosis by eliciting synaptic vesicle membrane fusion prior to neurotransmitter release.

Methods

We have derived an enhanced set of ^{15}N NMR relaxation experiments with improved water suppression and sensitivity, applicable for high magnetic fields [1]. To assess the structural dynamics of the SNARE protein SNAP25a, field-dependent NMR relaxation experiments at different magnetic field strengths between 600 and 1200 MHz were recorded.

Results

NMR data reveals that the SNARE protein SNAP25a is intrinsically disordered in its monomeric pre-fusion conformation [2]. Interestingly, it exhibits unexpected dynamic behavior, detectable by the field-dependent NMR relaxation measurements (Figure 1).

Conclusions

The examined SNARE protein SNAP25a shows distinct dynamic regimes on the fast ps timescale. Our published [1,2] and unpublished data will be presented at the conference.

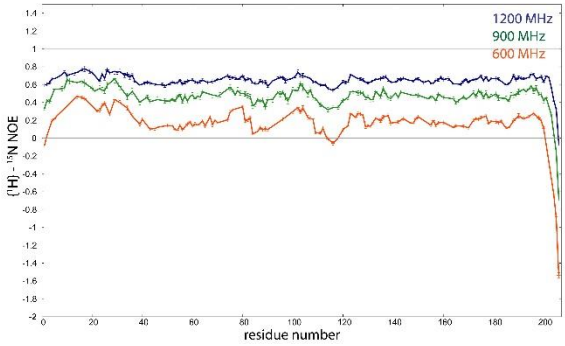


Figure 1: Field-dependent {1H}-15N NOE data of SNAP25a.

References

[1] Stief T, Vormann K, Lakomek NA, 2024, *Methods*, 223, 1-15

[2] Stief T, Vormann K, Lakomek NA, 2023, *J. Mol. Biol.*, *435*, 10

PS-V-09

Ionic shielding in fibrinogen nanofiber formation: A molecular dynamics approach

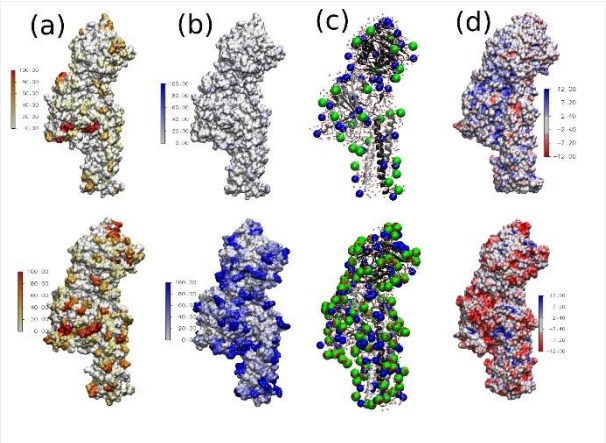
Aparna Sai Malisetty[1], Antoine E. Kwame[2], Dorothea Brüggemann[2], Lucio C. Ciacchi[1], Susan Köppen[1]

[1] University of Bremen, Hybrid Materials Interfaces Group, Faculty of Production Engineering, Bremen, Germany; [2] Hochschule Bremen – City University of Applied Sciences, Biophysics and Applied Biomaterials, Bremen, Germany

We previously reported that fibrinogen (Fg) aggregates into nanofibers when dried in concentrated aqueous solutions of monovalent cations, while divalent cations lead to smooth films [1,2]. Our recent data suggest that both cation and anion type influence Fg aggregation. To rationalize these findings, an atomistic understanding of fiber formation as a function of salt type is essential.
To this end, we studied ion-protein interactions of the Fg-D domain at pH 7 in NaCl, Na_2HPO_4 and $MgCl_2$, at concentrations ranging from 0.1 M to 3 M, with all-atom molecular dynamics simulations.

We find that Na^+ and HPO_4^{2-} have a higher binding specificity for Fg-D than Mg^{2+} and Cl^-, respectively. Upon adsorption, the former free themselves to a significant extent from their hydration shell, while the latter keep their hydration cell mostly or completely intact. Phosphate ions also keep Na^+ ions firmly bound to Fg-D. Analysis of the electrostatic potential revealed that the major ion binding sites on the Fg-D domain are completely occupied by Na^+ and HPO_4^{2-} at higher concentrations, significantly affecting the protein surface charge. In contrast, the indirect interaction with Mg^{2+} ions caused no significant change in surface charge.

This leads to the conclusion that the formation of fibers, especially in the presence of sodium phosphate salts, may be due to ionic shielding effects. These weaken the electrostatic (DLVO) repulsion and promote aggregation via dispersion interactions.



Interactions of Fg-D with Ions and Surface Charge Distribution for NaCl (top) and Na_2HPO_4 (bottom). (a, b) The percentage of time that protein residues interact with ions is shown as a color plot projected onto the Fg-D surface. Cations are represented by a red gradient, and anions by a blue gradient. Higher binding specificity of Na^+ and $HPO4^{2-}$ ((a),(b)-bottom) can be seen. (c) Stern layer composition showing the protein (Newcartoon representation), adsorbed cations (blue), anions (green), and water molecules (licorice representation). (d) APBS electrostatic potential color plot corresponding to the Stern layer in (c).

References
[1] Stapelfeldt, K., Stamboroski, S., Mednikova, P., Brüggemann, D., 2019, 'Fabrication of 3D-nanofibrous fibrinogen scaffolds using salt-induced self assembly', *Biofabrication, 11*(2), 025010.
[2] Stamboroski, S., Boateng, K., Lierath, J., Kowalik, T., Thiel, K., Köppen, S., Noeske, P-L. M., Brüggemann, D., 2021, 'Influence of divalent metal ions on the precipitation of the plasma protein fibrinogen', *Biomacromolecules, 22*(11), 4642–4658.

PS-V-11

AUTOMATED CLUSTERING OF HELICAL FILAMENTS

Janus Lammert[1,2], Gunnar Schröder[1,2]

[1] FZ-Jülich, IBI-7, Jülich, Germany; [2] Heinrich Heine Universität, Düsseldorf, Germany

Recent steps towards automation have improved the quality and efficiency of the entire cryo-electron microscopy workflow, from sample preparation to image processing. Most of the image processing steps are now quite automated, but there are still a few steps which need the specific intervention of researchers. One such step is the identification and separation of helical protein polymorphs at early stages of image processing. Here, we present a new clustering approach based on analyzing partial similarities between 2D class averages. As an automated polymorph separation method, it has the potential to complement automated helical picking, which typically cannot easily distinguish between polymorphs with subtle differences in morphology, and is therefore a useful tool for the image processing and structure determination of helical proteins.

PS-V-13

A thermodynamic characterization of a GAAA tetraloop receptor in the rRNA of bakers yeast

Vanessa Schumann, Richard Börner

University of Applied Sciences Mittweida, Laserinstitut Hochschule Mittweida, Mittweida, Germany

V S and R B received funding from the European Union and the free state of Saxony [ESF Plus: young research group—LaNa-Sax:100649226]

Understanding RNA folding is crucial for a comprehensive understanding of life at the molecular level. Metal ions such as K^+ and Mg^{2+} play an essential role in the folding of RNA and are of special interest in the process of ribosomal maturation[1]. Here, we focus on the *in vitro* characterization of an ribosomal RNA tertiary contact which is built upon binding of a kissing loop motif and a GAAA tetraloop[2]. This tertiary contact has been identified to play a crucial role during ribosomal maturation of bakers yeast especially at lower temperatures, *i. e.* 25°C. We study the folding stability of this rRNA temperature dependent and in the presence of various metal ions such as K^+, Na^+, Ca^{2+} and Mg^{2+} using Förster resonance energy transfer[3] (FRET) and UV thermal melting, both as high-throughput ensemble measurements. To rule out sequence dependencies of the rRNA model construct, we examined the influence of poly adenine (poly(A)) and poly uracil (poly(U)) linkers[4]. Our results indicate that mono- and divalent metal ions progressively stabilize the RNA tertiary contact as expected[1], with K^+ and Mg^{2+} showing the highest increase in stability. Despite an overall similar folding behavior, poly(U) linkers were found to be more sensitive to an increase in temperature compared to poly(A) linkers. With the help of our high-throughput measurements, we have fast and accurate access to the temperature dependent folding and thermodynamic stability of non-coding RNA at various conditions.

References

[1] Börner, R., Kowerko, D., Miserachs, H. G., Schaffer, M. F., & Sigel, R. K. O. (2016). Metal ion induced heterogeneity in RNA folding studied by smFRET. In Coordination Chemistry Reviews (Vols. 327–328, pp. 123–142). Elsevier BV. https://doi.org/10.1016/j.ccr.2016.06.002

[2] Gerhardy, S., Oborská-Oplová, M., Gillet, L., Börner, R., van Nues, R., Leitner, A., Michel, E., Petkowski, J. J., Granneman, S., Sigel, R. K. O., Aebersold, R., & Panse, V. G. (2021). Puf6 primes 60S pre-ribosome nuclear export at low temperature. In Nature Communications (Vol. 12, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1038/s41467-021-24964-2

[3] Förster, Th. (1948). Zwischenmolekulare Energiewanderung und Fluoreszenz. In Annalen der Physik (Vol. 437, Issues 1–2, pp. 55–75). Wiley. https://doi.org/10.1002/andp.19484370105

[4] Downey, C. D., Fiore, J. L., Stoddard, C. D., Hodak, J. H., Nesbitt, D. J., & Pardi, A. (2006). Metal Ion Dependence, Thermodynamics, and Kinetics for Intramolecular Docking of a GAAA Tetraloop and Receptor Connected by a Flexible Linker. In Biochemistry (Vol. 45, Issue 11, pp. 3664–3673). American Chemical Society (ACS). https://doi.org/10.1021/bi0520941

PS-V-15

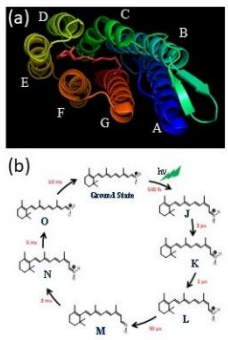
Shedding Light on Photochemistry and Thermal Stability of Acetylated Thermophilic Rhodopsin

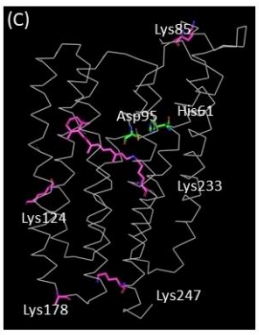
Ramprasad Misra[1], Ishita Das[2], Songhwan Hwang[1], Mordechai Sheves[2], Peter Hegemann[1]

[1] Humboldt Universität, Institute for Biology, Experimental Biophysics, Berlin, Germany; [2] Weizmann Institute of Science, Department of Molecular Chemistry and Materials Science, Rehovot, Israel

Funding from the Kimmelman Center for Biomolecular Structure and Assembly (to MS) and the Cluster of Excellence "UniSysCat" (EXC 2008; Grant No. 390540038; to PH) is greatfully acknowledged.

Microbial rhodopsins consist of seven trans-membrane α-helices (called apoprotein), and an all-*trans* retinal chromophore remains covalently bound to it. Although the role of the bound chromophore, which absorbs in the 440-720 nm region, on the photochemistry and thermal stability of these proteins have been studied expensively, the contribution of the apoprotein is not well-understood due to lack of suitable spectroscopic markers. Here, we studied how acetylation of lysine residues in the protein surface affects the photochemistry and thermal denaturation of thermophilic rhodopsin (TR), the proton pumping rhodopsin of *Thermus thermophilus* bacterium. Although acetylation does not alter the absorption maximum of TR and the change in pKa of the counter-ion is minute, it significantly affects the photocycle of the protein. The difference in the rates of light-catalyzed thermal denaturation and hydroxylamine reaction of TR and AcTR underscores the importance of the interaction among the residues of the apoprotein in these processes. The altered photocycle of TR due to acetylation that leads to faster regenation of the ground state from the O-state correlates well with the difference in rates of their hydroxylamine reaction. Complimentary molecular dynamics simulation revealed the key amino acid residues connecting the retinal binding pocket and the extreacellular region of the protein. Some of the late processes during the thermal unfolding of the protein are also investigated.





Structure and photocycle of thermophilic rhodopsin
(a) Top view of the thermophilic rhodopsin (TR), obtained from it's crystal structure (pdb: 5azd). An all-trans retinal chromophore is attached to the helix-G of the seven trans membrane α-helical protein. (b) A typical photocycle of a microbial rhodopsin. (c) TR consists of five lysine rsidues at 85, 124, 178, 233 and 247 position. Compared to the primary countertion (Asp95) or His61 which forms salt bridge with it, the lysine residues remain quite far from the retinal chromophore.

References
[1] Ernst, O.P., Lodowski, D.T., Elstner, M., Hegemann, P., Brown, L.S., Kandori, H. Microbial and animal rhodopsins: structures, functions, and molecular mechanisms. *Chemical reviews* **2014,** 114 (1), 126-163.

[2] Misra, R., Hirshfeld, A., Sheves, M. Molecular Mechanism for Thermal Denaturation of Thermophilic Rhodopsin. *Chemical Science* **2019**, 10, 7365-7374.
[3] Iyer, E.S.S., Misra, R., Maity, A., Liubashevski, O., Sudo, Y., Sheves, M., Ruhman, S. Temperature Independence of Ultrafast Photo-isomerization in Thermophilic Rhodopsin: Assessment vs Other Microbial Proton Pumps. *J. Am. Chem. Soc.* **2016**, 138, 12401-12407.
[4] Tsukamoto, T., Inoue, K., Kandori, H., Sudo, Y. Thermal and spectroscopic characterization of a proton pumping rhodopsin from an extreme thermophile. *J. Biol. Chem.* **2013**, *288*, 21581– 21592.
[5] Misra, R., Das, I., Hwang, S., Sheves, M., Hegemann, P. Acetylation Sheds Light on Photochemistry and Thermal Stability of Thermophilic Rhodopsin. *(unpublished results)*

PS-V-17

Targeting the extracellular domain of the Ephrin receptor A2 by fragment-based screening

Sattar Khashkhashi Moghaddam, Santosh Gande, Verena Linhard, Kerstin Witt, Sridhar Sreeramulu, Christian Richter, Konstantin Mineev, Harald Schwalbe

Goethe University Frankfurt, department of chemistry,biochemistry and pharamcy, Frankfurt am Main, Germany

The erythropoietin-producing hepatocellular A2 (EphA2) receptor is a member of the Eph tyrosine kinase receptor family and together with the corresponding cell surface-anchored ephrin ligands constitute a cell-cell communication system which is implicated in many physiological and pathological processes such as inflammation, and cancer malignancy. Overexpression of EPHA2 is linked to poor prognosis and the development of metastatic cancers, indicating its potential as a target for cancer treatment. Here, we report the NMR fragment-based screening (FBS) campaign aimed to inhibit the ligand binding domain (LBD) of EPHA2 or ephrin A1 and block the bi-directional Eph signaling. Following the establishment of protocols for the heterologous production of the proteins EphA2 LBD and Ephrin A1, we screened a library of over 600 compounds using NMR. This screening resulted in 104 compounds binding to EphA2-LBD, which were then analyzed with chemoinformatic techniques to prioritize initial hits to optimize titration experiments and extensively sample the chemical space. From these, we prioritized 40 FBS hits and examined them using protein-observed NMR assays to determine their ligand binding modes and affinities. This analysis identified several compounds with moderate affinity that interact either directly with the binding pocket of EphA2 LBD or with adjacent sites. Additionally, the potential of NMR-derived fragments as starting points for developing EphA2 inhibitors is discussed.

PS-V-19

Carbohydrates with vibrational probes: enabeling IR spectroscopic studies of carbohydrate-protein interactions

Philip Gasse[1], Till Stensitzki[1], Yasemin Mai-Linde[1], Torsten Linker[1], Thomas La Cour Jansen[2], Henrike Müller-Werkmeister[1]

[1] University of Potsdam, Chemistry, Potsdam, Germany; [2] University of Groningen, Faculty of Science and Engineering, Groningen, Netherlands

Protein-carbohydrate interactions play an important role in biochemical processes such as cell signaling, antibody recognition, and glucose metabolism. Although these processes can in principle be studied in molecular detail by IR spectroscopy, they remain largely unexplored by vibrational spectroscopy due to spectral congestion, i.e. strongly overlapping bands. We overcome this obstacle by introducing vibrational reporter groups into carbohydrates, which exhibit peaks in a spectral region free from other protein or carbohydrate signals. Specifically, we have studied mono- and di-saccharides with SCN and azide labels [1], using FTIR, time-resolved IR, and 2D-IR spectroscopy. We found that the position of the label strongly influences the spectra and observed dynamics. Furthermore, we find that the frequency of the reporter group depends significantly on its orientation relative to the ring. With the help of quantum chemical calculations, we attributed this to direct influence of the ring conformation, enabling us to indirectly observe ring-puckering in real-time with 2D-IR spectroscopy. Our results determine the lower speed limit of mono-saccharide ring puckering within ~100 ps.
Using different labelled glucose derivates, we are now targeting the molecular interaction between glucose and the enzyme hexokinase, demonstrating the feasibility of detailed biomolecular studies with these new tools.

References

[1] P. Gasse, T. Stensitzki, Y. Mai-Linde, T. Linker, H. M. Müller-Werkmeister, *2D-IR spectroscopy of carbohydrates: Characterization of thiocyanate-labeled β-glucose in $CHCl_3$ and H_2O*, J. Chem. Phys. 158, 145101 (2023)

PS-V-21

Unraveling the role of electrostatics in protein-GAG interactions

Sergey Samsonov[1], **Anja Penk**[2]

[1] University of Gdańsk, Faculty of Chemistry, Gdańsk, Poland; [2] Leipzig University, Institute for Medical Physics and Biophysics, Leipzig, Germany

Glycosaminoglycans (GAGs) represent a class of negatively charged linear polysaccharides binding regulatory proteins (e.g. chemokines/interleukins) and, therefore, actively participate in multiple cell signaling processes. Features like high flexibility, varying sulfation patterns and multivalency make protein-GAG interactions challenging to describe in detail.

To investigate the contributions of electrostatics upon protein-GAG binding, we focused on the homodimeric protein interleukin 8 (IL-8) as an established test system. We used decapeptides with different net charges that potentially mimic the GAG hexamer and applied solution NMR and computational methods as methodological couple. Furthermore, we used heptamers of alginates as ligands. Those oligosaccharides from algae exhibit different conformational space depending on the used blocks - either M-(mannuronate)-blocks (stretched) or G-(guluronate)-blocks (zig-zag structure).

The ^{1}H-^{15}N HSQC (heteronuclear single quantum coherence) NMR titration spectra of IL-8 showed perturbation of the protein backbone signals upon adding the different ligands. Interestingly, the determined binding epitope of IL-8 towards the peptides showed high similarity regarding the GAG binding site, while the affinity for the peptides is orders of magnitude weaker. The same applies for the stretched M-alginate, while for the zig-zag structured G-alginate a completely different binding behavior was observed.

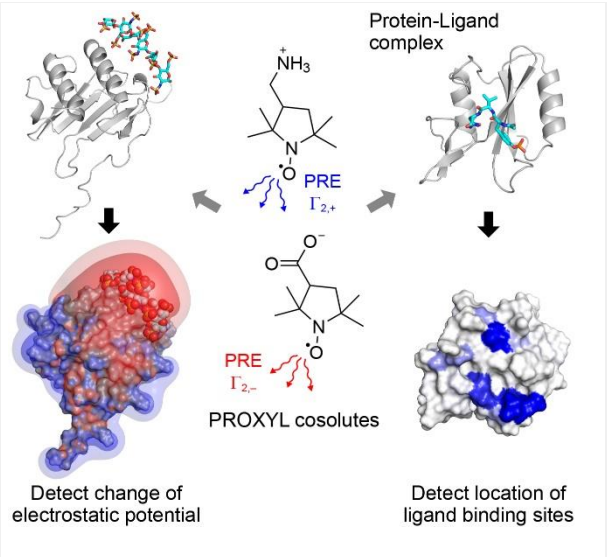
PS-V-23

Detecting protein-ligand interactions with nitroxide based paramagnetic cosolutes

Anja Penk[1], Annemarie Danielsson[2], Margrethe Gaardløs[2], Cindy Montag[1], Andrea Schöler[3], Daniel Huster[1], Sergey Samsonov[2], **Georg Künze**[3]

[1] University of Leipzig, Institute for Medical Physics and Biophysics, Leipzig, Germany; [2] University of Gdańsk, Faculty of Chemistry, Gdańsk, Poland; [3] University of Leipzig, Institute for Drug Discovery, Leipzig, Germany

NMR spectroscopy is a widely used method in drug discovery research. In particular, paramagnetic NMR methods can provide structural restraints for modeling the ligand-target interaction. However, the covalent labeling of proteins with paramagnetic tags or the generation of metal ion-tagged ligands is complicated and bears the risk of perturbing the protein structure or ligand binding mode. Here we tested a tagging-free NMR approach which relies on the measurement of paramagnetic relaxation enhancements (PREs) arising from analogous cationic, anionic or neutral soluble nitroxide molecules, which distribute around the protein-ligand complex depending on near-surface electrostatic potentials. We applied this approach to two protein-ligand systems, interleukin-8 interacting with highly charged glycosaminoglycans and the SH2 domain of Grb2 interacting with less charged phospho-tyrosine tripeptides. The electrostatic potential around interleukin-8 and its changes upon binding of glycosaminoglycans could be derived from the PRE data and confirmed by theoretical predictions from Poisson-Boltzmann calculations. The ligand influence on the PREs and NMR-derived electrostatic potentials of Grb2 SH2 was localized to a narrow protein region which allowed the localization of the peptide binding pocket. Our analysis suggests that experiments with nitroxide cosolutes can be useful for investigating protein-ligand electrostatic interactions and mapping ligand binding sites.



NMR method for detecting protein-ligand interactions with paramagnetic cosolutes
The method relies on the measurement of paramagnetic relaxation enhancements (PREs) arising from analogous cationic, anionic or neutral soluble nitroxide molecules. It allows experimental measurement of the electrostatic potential around proteins and its ligand-induced changes. Ligand binding sites can be localized from the changes of the PRE data.

PS-V-25

Structural stability of the integron synaptic complex

Ekaterina Vorobevskaia[1], Céline Loot[2], Didier Mazel[2], Michael Schlierf[1,3]

[1] TU Dresden, B CUBE Center for Molecular Bioengineering, Dresden, Germany; [2] Institut Pasteur, Université Paris Cité, CNRS UMR3525, Unité Plasticité du Génome Bactérien, Paris, France; [3] TU Dresden, Physics of Life, DFG Cluster of Excellence, Dresden, Germany

We thank all members of the Schlierf lab for lively discussions during the development of this project and in particular César Augusto Quintana-Cantaño for inspiring discussions and Dr. Yujun Zhang for supporting protein mutagenesis and production.

Introduction

The predominant tool for adaptation in Gram-negative bacteria is a genetic system called integron. It rearranges gene cassettes, promoting multiple antibiotic resistances, a recognized major global health threat. It is based on a unique recombination process involving a tyrosine recombinase - integrase IntI - and folded ssDNA hairpins - called *attC* sites. Four recombinases and two *attC* sites form a macromolecular synaptic complex, which is key to the recombination process and the focus of our study. The bottom strand of all *attC* sites shows highest recombination *in vivo*, however, the recombination efficiency still varies five orders of magnitude and the underlying reason remains unknown.

Methods

Here, we established an optical tweezers force-spectroscopy assay that allows to probe the synaptic complex stability. We investigated seven combinations of *attC* sites and two protein variants and determined their *in vivo* recombination efficiencies.

Results

We discovered a strong correlation between recombination efficiency and mechanical stability of the synaptic complex, revealing a new regulatory mechanism of the bacterial integron, mediated through the synaptic complex. Furthermore, we discovered a key structural region of the IntI that greatly destabilizes the formation upon mutation.We designed and probed a competing molecule that could actively destabilize a given synaptic complex, opening a new avenue to reduce the spread of antibiotic resistances among bacteria.

PS-V-27

PI3K SH3 domain - amyloid model protein (un)folding investigated by NMR

Melinda Jaspert[1,2], Luis Gardon[1,2], Leonardo Levorin[1,2], Lothar Gremer[1,2], Nils-Alexander Lakomek[1,2], Henrike Heise[1,2]

[1] Heinrich-Heine-Univeristät, Institute of Physical Biology, Düsseldorf, Germany; [2] Forschungszentrum Jülich, Institute of Biological Information Processing, Jülich, Germany

Introduction

The misfolding and aggregation of proteins is associated with several neurodegenerative diseases. In order to understand the underlying processes, the PI3K SH3 domain is used as a model system. At acidic pH the well folded native structure [1] of the protein unfolds and aggregates into well-folded fibrils. The structure of these PI3K SH3 fibrils was determined recently using cryo-EM [2].

Methods

We use diverse NMR experiments to get insights into the unfolding process of the PI3K SH3 domain itself and its dependencies. Therefor we apply solution-state NMR experiments to obtain information about the secondary structure propensities and the dynamics of the investigated protein to follow the unfolding process with particular regard to its temperature dependency at residue-type resolution.

Results

At acidic pH and lower temperatures an equilibrium between two conformations is present, that upon rising the temperature shifts towards the unfolded conformation. Our developed assignment and the secondary chemical shift analyses of the PI3K SH3 domain at acidic pH and high temperature indicate that the molecule indeed is unfolded under these conditions.

Conclusions

This provides a basis for further examinations of the double conformation state, which will be separated with recently performed experiments, as well as a starting point for ultrafast-MAS-measurements using proton detection, to gain insights into the complexity of the (un)folding process of the PI3K SH3 domain.

References
[1] Booker, G. W. et al. *Cell* **1993**, 73 (4), 813.
[2] Röder, C. et al. *Nature Commun.* **2019**, 10 (1), 3754.

PS-V-29

Coupling Mechanism of the Halogenase PyrH Observed by Light-induced Infrared Difference Spectroscopy

Niklas Diepold[1], Lea Schröder[1], Simon Gäfe[1], Hartmut Niemann[2], Tilman Kottke[1]

[1] Bielefeld University, Biophysical Chemistry and Diagnostics, Bielefeld, Germany; [2] Bielefeld University, Structural Biochemistry, Bielefeld, Germany

Introduction

Halogenases such as PyrH convert tryptophan to 5-Cl-tryptophan only using oxygen and chloride.[1] We aimed to study the structural coupling between the cofactor flavin, the chloride ion and the substrate binding site 10 Å apart.

Method

Enzymatic activity of PyrH could be successfully initiated and driven with blue light by photoreducing the bound flavin.[2] This procedure enables a biophysical investigation of the protein mechanism using light-induced Fourier transform infrared difference spectroscopy. By selective isotopic labeling of the protein moiety, difference signals were assigned to secondary structural changes in the enzyme.

Results

By variation of the presence of different halides we demonstrated a strong structural halide dependency of the flavin binding pocket. Conformational coupling was reduced without chloride. The presence of substrate and product allosterically influenced the structure of flavin binding site. Change of an α-helix upon illumination was identified as marker for the tryptophan binding pocket occupancy. Kinetic studies of the photoreduction in the enzyme have shown a long-lived inactive conformation which could be reversed and assigned to be formed by conformational changes.

Conclusion

We characterized by infrared spectroscopy the allosteric coupling via conformational changes in the enzyme between the cofactor flavin, the chloride and the site of enzymatic conversion active in both directions, despite the large spatial separation.[3]

References

1 Dong, C., Flecks, S., Unversucht, S., Haupt, C., van Pée, K.-H. & Naismith, J. H. Tryptophan 7-halogenase (PrnA) structure suggests a mechanism for regioselective chlorination (2005). Science, 309, 2216–2219.

2 Schroeder L., Frese M., Müller C., Sewald N., Kottke T. Photochemically Driven Biocatalysis of Halogenases for the Green Production of Chlorinated Compounds (2018). ChemCatChem 10, 3336-3341.

3 Schroeder, L., Diepold, N., Gäfe, S., Niemann, H. H., Kottke, T. Coupling and regulation mechanisms of the flavin-dependent halogenase PyrH observed by infrared difference spectroscopy (2024). J. Biol. Chem. 300, 107210.

PS-V-31

Structural investigation of amyloid fibril formation in medical insulin by cryo-EM

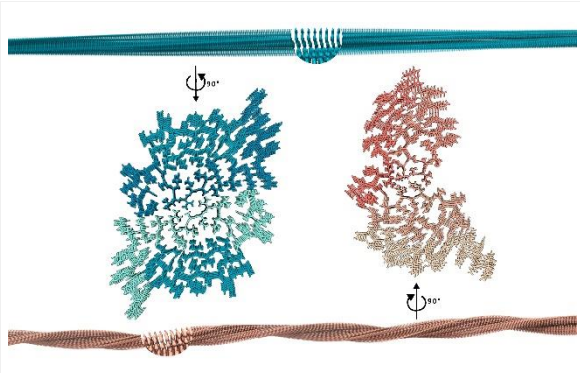
Simon Sommerhage[1], Fernanda S. Peralta Reyes[2], Lothar Gremer[1,2], Gunnar Schröder[1,3]

[1] Forschungszentrum Jülich, Institute of Biological Information Processing, Structural Biochemistry (IBI-7) Jülich Center for Structural Biology, Jülich, Germany; [2] Heinrich-Heine-Universität Düsseldorf, Institut für Physikalische Biologie, Düsseldorf, Germany; [3] Heinrich-Heine-Universität Düsseldorf, Physics Department, Düsseldorf, Germany

Insulin is a peptide hormone involved in the regulation of the glucose level and is the primary drug used to treat diabetes. In recent years, global demand for medical insulin increased in the wake of a worldwide type 2 diabetes pandemic. However, insulin and its different medical analogues have the tendency to aggregate into amyloid fibrils rendering the hormone inactive and pro-inflammatory. This instability is favored by higher temperatures and poses a challenge in both storage and therapeutic application especially in low- and middle-income countries [1].

In this study we used cryo-electron microscopy (cryo-EM) to investigate the molecular structure of amyloid fibrils formed by insulin glargine - a common medical analogue of Insulin - when the vial is agitated at elevated temperatures for several hours. We found five different fibril polymorphs and managed to solve the molecular structure of two, one of them being the most abundant polymorph. Interestingly all fibril types differ significantly from those formed by recombinant human insulin reported earlier [2].

Therefore, the punctual differences in the amino acid sequence of insulin glargine, which were originally introduced to enhance the efficacy of the drug, also seem to affect the energy landscape of amyloid aggregation. The novel structural information can now be leveraged in the development of next-generation insulin analogues, which are less prone to aggregation and will improve global diabetes care.



Schematic depiction of cryo-EM structures of insulin glargine amyloid fibrils
The most abundant fibril polymorph is colored in blue and the second polymorph, that we were able to reconstruct to a high resolution, is colored in shades of red. The reconstructed maps of the coulomb potential are rendered horizontally along the helical axis, while the atomic model is shown as a perpendicular slice. The parallel cross-beta stacking of the individual layers is indicated by the circular zooms into small regions of the cryo-EM maps.

References
[1] Weiss, M. A. Real-world insulin stability and global access. The Lancet Diabetes & Endocrinology (2023)
[2] Wang, L. et al. Structural basis of insulin fibrillation. Science Advances 9, eadi1057 (2023).

PS-V-33

Inherent aggregation mechanisms of Alzheimer peptides - influence of amino acid sequence and confinement

Silvia De Sio, Jana Wägele, **Maria Ott**

Martin-Luther-Universtät Halle-Wittenberg, Biochemistry and Biotechnology, Halle, Germany

We acknowledge funding from the German research foundation (DFG, project ID 189853844 – TRR 102 "polymers under multiple constraints", TP B12, and project ID 391498659 – RTG 2467 "Intrinsically Disordered Proteins – Molecular Principles, Cellular Functions, and Diseases", TP 11).

The disease-related formation of highly ordered amyloid fibrils involves various stages of aggregation and structural conversion. The complexity of the comprised molecular mechanisms, the interplay of different fibrillation pathways and the environmental sensitivity of the processes still require greater understanding of fundamental processes and their mathematical modeling.
We investigated various natural and non-natural variants of the Alzheimer's peptide to reveal the variety of pre-fibrillar oligomeric stages and mechanisms of fibrillar growth. Linear macromolecules such as PEG and Ficoll were used to study the effects of weak non-covalent interactions and confinement effects[1]. Our experimental approach combined ensemble methods such as ThT fluorescence assays with single-molecule fluorescence techniques enabling to relate overall rates of primary and secondary nucleation to pre-fibrillar aggregation stages of the Alzheimer peptides[1,2]. The final fibril morphologies were examined using negative stain transmission electron microscopy and wide-angle X-ray scattering. We further present how the unexpectedly large effects of weak interactions with crowder molecules can be understood by the concept of reactant occlusion, while the entropy gain of the confinement can be quantified by modified crystallization-like models. Finally, we show that the developed analytical tools are of general use and can be successfully applied to other systems[3,4].

References

[1] S. De Sio, J. Waegele, T. Bhatia, B. Voigt, H. Lilie, M. Ott, Inherent Adaptivity of Alzheimer Peptides to Crowded Environments. *Macromol. Biosci.* **2023**, 23, 2200527.

[2] Wägele J, De Sio S, Voigt B, Balbach J, Ott M. How fluorescent tags modify oligomer size distributions of the Alzheimer-peptide. *Biophys. J.* **2019**, 116:227-238

[3] L. M. Lauth, B. Voigt, T. Bhatia, L. Machner, J. Balbach and M. Ott. Heparin promotes rapid fibrillation of the basic parathyroid hormone at physiological. *FEBS Letters***2022**, 596, 2928-2939

[4] Voigt, B., Bhatia, T., Hesselbarth, J., Baumann, M., Schmidt, C., Ott, M., Balbach, J., 2023. The Prenucleation Equilibrium of the Parathyroid Hormone Determines the Critical Aggregation Concentration and Amyloid Fibril Nucleation. *ChemPhysChem* **2023**,24, e2023004

PS-V-35

Exploring heme-enzyme tunability with resonance and surface-enhanced resonance Raman (SERR) spectro-electrochemistry

Patrycja Kielb[1,2], Michelle Mahler[1,2]

[1] University of Bonn, Clausius Institute of Physical and Theoretical Chemistry, Bonn, Germany; [2] University of Bonn, Transdisciplinary Research Area 'Building Blocks of Matter and Fundamental Interactions', Bonn, Germany

The development of artificial metalloproteins designed to catalyze new types of transformations presents a promising avenue in biocatalysis, holding significant potential for technological or medical applications. Enzymes utilizing heme as an active site are particularly attractive due to inherent structural and redox versatility, allowing them for a broad spectrum of small molecule activation. Here, we examine how such structural changes of the heme coordination correlate with the redox properties of the active site and, as such, serve as a parameter governing the biocatalyst catalysis towards specific reactions. Specifically, we explore the redox tuning of Hexameric Tyr-coordinated Heme Protein (HTHP)[1,2] by tailored axial coordination modifications of the heme cofactor. In addition to protein engineering, we employ a combination of electrochemistry with surface-enhanced resonance Raman (SERR) spectroscopy to selectively monitor the vibrational fingerprint of a monolayer of chromophore-containing sample under the control of applied potential.

References

[1] Kielb, P, et al. ‘Switchable redox chemistry of the hexameric tyrosine-coordinated heme protein’, *Journal of Physical Chemistry B*, 121 (16), 3955 - 3964.

[2] Mahler, M and Kielb, P. 'Redox tuning of heme active sites studied by Raman spectro-electrochemistry', *in preparation*

PS-V-39

Retention of the RNA ends provides the molecular memory for maintaining the activation of the Csm complex

Patrick Irmisch[1], Irmantas Mogila[2], Brighton Samatanga[1], Gintautas Tamulaitis[2], Ralf Seidel[1]

[1] University of Leipzig, Peter Debye Institute for Soft Matter Physics, Leipzig, Germany; [2] Vilnius University, Institute of Biotechnology, Vilnius, Lithuania

The type III CRISPR-Cas effector Csm complex functions as a molecular Swiss army knife that provides multilevel defense against foreign nucleic acids. The coordinated action of three catalytic activities of the Csm complex enables simultaneous degradation of the invader's RNA transcripts, destruction of the template DNA and synthesis of signaling molecules (cyclic oligoadenylates cA_n) that activate auxiliary proteins to reinforce CRISPR-Cas defense. While these activities have been thoroughly characterized, their spatiotemporal regulation remained unclear. Here, we employed single-molecule techniques to connect the kinetics of RNA binding, dissociation, and DNA hydrolysis by the Csm complex from *Streptococcus thermophilus*. Although single-stranded RNA is cleaved rapidly (within seconds), our dual-color FCS experiments and single-molecule TIRF microscopy revealed that Csm remains bound to terminal RNA cleavage products with a half-life of over 1 hour while releasing the internal RNA fragments quickly. Using a continuous fluorescent DNA degradation assay, we observed that RNA-regulated single-stranded DNase activity decreases on a similar timescale. These findings suggest that after fast target RNA cleavage the terminal RNA cleavage products stay bound within the Csm complex, keeping the Cas10 subunit activated for DNA destruction. Additionally, we demonstrate that during Cas10 activation, the complex remains capable of RNA turnover, i.e. of ongoing degradation of target RNA.

References

[1] Irmisch, P, Mogila, I, Samatanga, B, Tamulaitis, G, Seidel, G, 2024, 'Retention of the RNA ends provides the molecular memory for maintaining the activation of the Csm complex', *Nucleic Acids Research*, Volume 52, Issue 7, Pages 3896–3910, https://doi.org/10.1093/nar/gkae080

PS-V-01-X

Visualizing Molecular Dynamics with High-Speed Tip-Scanning Atomic Force Microscopy

Thomas Fuhs, Jörg Barner, André Körnig, Thomas Henze

Bruker Nano GmbH, JPK BioAFM, Berlin, Germany

Biological systems exhibit very high structural and functional dynamics on molecular scales. Understanding the principles of the kinetics behind structural changes at that scale is critical when studying samples ranging from single membrane proteins to complex macromolecular systems. We have used high-speed tip-scanning atomic force microscopy (AFM) with a kilohertz linerate to visualize molecular dynamics by enabling temporal resolution on the sub-100-ms scale. The use of a high-spped tip-scanning AFM enables high-resolution correlation experiments with advanced optical techniques. We will give two examples of structural transitions and biomolecular dynamics in samples, triangular DNA origamis and photosensitive surfactants, studied with high-speed AFM.
DNA origami structures serve as a functional template in multiple artificial and native molecular systems. We studied the development of order in 2D DNA triangular Rothemund lattices. Looking at the DNA origami adsorption on mica we studied the temporal dependence between lattice order development and Na+ ion content with a temporal resolution of 1 frame/s.
We monitored the structural photosensitive transition of photosensitive surfactants under external light-induced deformation. By simultaneous high-speed AFM measurements and switching the external wavelength illumination from 365 nm to 546 nm and vice versa, we could monitor and induce a reversible structural transition within the studied sample in real-time.

PS-V-03-Z

Optically driven thermofluidic assembly of bacteria

Desmond J. Quinn[1], Selina Hanisch[2], Rohan Karande[3,2], Frank Cichos[1]

[1] Universität Leipzig, Molecular Nanophotonics Group, Peter Debye Institute for Soft Matter Physics, Leipzig, Germany; [2] Helmholz Centre for Environmental Research (UFZ), Leipzig, Germany; [3] Universität Leipzig, Biophysical Chemistry, Leipzig, Germany

Bacteria in their planktonic state are known to assemble into biofilms in the vicinity of a solid surface. The complex cascade that results in the adhesion of the bacteria to the surface is usually triggered by the diffusion of bacteria to its vicinity. We propose a method that makes use of temperature induced flow fields and depletion interactions for the localised assembly and manipulation of bacteria. We report on the assembly of 3D bacteria clusters assembled at the surface in a time frame of about 15 minutes. We disentangle the role of the contributing thermofluidic processes and investigate the role of activity on the assembly. The highly localised nature of the optically induced heating enables us to control the spatial outcome of the assembly. Additionally, the speed of the heat diffusion, which is orders of magnitude greater than cell diffusion, allows the flow fields to be multiplexed. This enables multiple assemblies to be carried out in parallel. Coupled with feedback-based interactions, the assembly of complex structures involving multiple bacterial species was also demonstrated. Such controlled assembly of bacteria could be useful for technological applications in bioreactors and in understanding the interplay of physics and biology in biofilm formation.

PS-VI-01

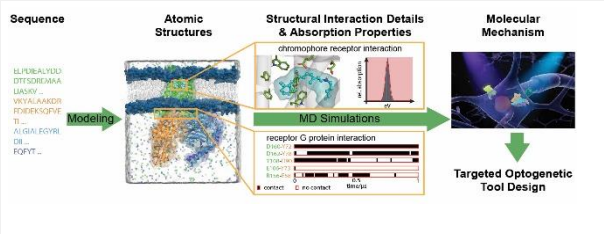
Discover molecular mechanism of GPCRs through computational 4D structural biology

Till Rudack

University of Regensburg, Structural Bioinformatics, Regensburg, Germany

G protein coupled receptors (GPCRs) are photoswitchable proteins that modulate the intracellular signaling of the cell. They are well-suited for optogentic applications that use photoswitchable proteins being genetically targeted to specific cells to control cellular signals by light. Insights into molecular mechanism of GPCRs provide a profound basis for targeted engineering of optogenetic tools. To discover structure function relationships of GPCRs from the electron up to the molecular level, we developed a computational biophysics strategy that combines *ab initio* structure prediction, molecular dynamics simulation, and quantum chemical calculations. The focus lies on discoveries of the structural impact of the retinal binding pocket on retinal absorption and isomerization properties as well as the impact of receptor-effector protein interactions on signaling. Our computational derived structural dynamic models are combined with *in vitro* and *in vivo* experiments of our collaborators to understand how GPCR function is achieved. This understanding advances the design of optimized optonegentic tools through color tuning or changing G protein specificity.

We successfully employed our strategy to investigate molecular mechanism of the GPCRs melanopsin (Tennigkeit et al, ChemBioChem 2019) and parapinopsin (Eickelbeck et al, ChemBioChem, 2019)**.** Our insights on melanopsin function assisted to tailor a variant with improved optogenetic potential (Tennigkeit et al, ChemBioChem 2019).



Protein Engineering Strategy

PS-VI-03

Structural and functional insights into GHS receptor with modified ligands using NMR

Isabel Wehner, Hannes Junker, Emelyne Pacull, Andy A. Smith, Marta Gozzi, Clara Schoeder, Daniel Huster

Leipzig University, Leipzig, Germany

Over the last decades significant progress has been made in structural biology of membrane proteins, especially for G protein-coupled receptors (GPCRs), providing a deeper understanding of their complex structures and activation mechanisms induced by ligand binding. One particularly interesting GPCR is the Growth Hormone Secretagogue Receptor (GHSR), which plays a crucial role in food intake, glucose metabolism, and immune response. As the receptor has a high constitutive activity,1 pharmacological intervention aims at reducing the basal activity of GHSR through the design of an peptide inverse agonist.
In this study we combine NMR, MD simulations and computational protein design to investigate potential modifications of the endogenious peptide ligand ghrelin, a 28 residue octanylated peptide hormone. Initial investigations revolve around a ghrelin analog with mutation Phe4Pro. This mutation has been proposed computationally using a MD simulation of the GHSR-ghrelin complex and the deep learning-based protein design method ProteinMPNN. We hypothesize that this mutation impairs the overall flexibility of the peptide and consequently the activation of GHSR.
In order to substantiate this, GHSR will be expressed cell-free, folded into DMPC bicelles, and analysed using fluorescence-based ligand binding assay and solidstate NMR spectroscopy.
GHSR is expressed with selectively 13C-labeled histidine residues, providing characteristic signals for each site. Ligand binding shifts these resonance providing information on the activation mechanism of GHSR.2 This approach will provide insights into structural and dynamic aspects of GHSR activation, offering new perspectives on its function as phamacological target.

References
[1] [1] GHS-R1a constitutive activity and its physiological relevance; Yves Mear, Alain Enjalbert, Sylvie Thirion
[2] [2] Pacull et al. doi: 10.1002/anie.202302003

PS-VI-05

Using sparse EPR data and Deep Learning to determine all-atom structures of rare protein conformations

Julia Belyaeva, Matthias Elgeti

University of Leipzig, Institute for Drug Discovery, Leipzig, Germany

Abstract

Double electron-electron resonance (DEER) is a powerful experimental technique that provides insights into the structure and population of protein conformations, including those that are sparsely populated. However, DEER can only yield distance information between site-specifically attached spin labels. Thus, molecular modeling techniques are being developed to extrapolate those distances to all-atom structures. Here, we present a computational approach that combines a novel deep learning method [1] with molecular dynamics simulations. Our approach integrates DEER data directly into the process of conformational modeling. For validation, we use published DEER spectroscopy results for the angiotensin II type 1 receptor (AT1R) interacting with a β-arrestin-biased ligand [2], for which no high-resolution structure has been solved yet. Thus the modelled structure of this AT1R conformation should be associated with functional selectivity. Finally, we compare our modeling results with both experimental data and well-established MD simulation methods.

References

[1] Stahl, K., Graziadei, A., Dau, T., Brock, O. and Rappsilber, J. 2023, 'Protein structure prediction with in-cell photo-crosslinking mass spectrometry and deep learning', *Nature Biotechnology*, 41, 1810–1819.

[2] Wingler, L. M., Elgeti, M., Hilger, D., Latorraca, N. R., Lerch, M. T., Staus, D. P., Dror, R. O., Kobilka, B. K., Hubbell, W. L. and Lefkowitz, R. J., 2019, 'Angiotensin analogs with divergent bias stabilize distinct receptor conformations', *Cell*, 176.3, 468-478.

PS-VI-07

Analyzing the Role of Individual Phospholipids in the Activation of the Neuropeptide Y4 Receptor

Rok Ferenc, Marta Gozzi, Oskar Engberg, Albert A. Smith-Penzel, Daniel Huster

University Leipzig, Institute of Medical Physics and Biophysics, Leipzig, Germany

With the cell membrane constituting the majority of the contact surface of G protein-coupled receptors (GPCRs), it is hardly surprising that it influences the signaling of GPCRs, both through variation in bulk properties, as well as specific interaction via individual lipid species. In this regard, the lipids that impact the regulation of human neuropeptide Y4 receptor (Y4R), a member of class A GPCRs, present a potential target for various medical applications. Building on the preliminary results of the ligand binding assays, the solid-state NMR offers a powerful tool to further investigate the role of lipids in the activation of Y4R.

For the solid-state NMR, the nitroxide- and ^{13}C-Trp-labeled Y4R are produced using cell-free expression, and reconstituted in multilamellar vesicles of varied molar ratios of phosphatidylserine (PS). The findings predict that the changes in peak intensities of ^{13}C-Trp residues of Y4R indicate tendencies of the Y4R to inhabit a specific conformational landscape in response to altered PS content. Furthermore, through paramagnetic relaxation enhancement NMR experiments of nitroxide-labeled Y4R, the relaxation rates of individual lipid species can be elucidated for specific regions of the Y4R. The combined results allow to identify preferential interactions of Y4R with PS, as well as its role in the activation of Y4R. Subsequent detector analysis allows characterizing the molecular dynamics of the membrane and receptor-induced changes.

PS-VI-09

The rhodopsin GPCR complex in European Robin: computational construction and validation

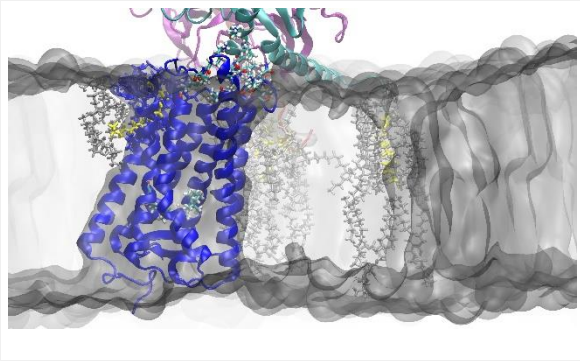
Jonathan Hungerland, Ilia A. Solov'yov

University of Oldenburg, Institute of Physics, Oldenburg, Germany

SFB 1372. NHR project NIP00058.

The biological mechanism of visual perception is a phenomenon that can be realized through the rhodopsin GPCR complex. In some migratory birds, this complex may additionally be involved in magnetoreception. Specifically, the protein cryptochrome 4 located in the eye of the bird European robin may utilize the existing mechanism of rhodopsin to transduce its information on magnetic field orientation into subsequent chemical reactions. An initial step towards this hypothesis is the computational modeling of the rhodopsin GPCR complex of European robin, which represents the first complete avian rhodopsin GPCR structure. Approaches based on single-protein-predicion or direct protein-complex-prediction are compared and their resulting structures are investigated to determine the most promising model for future studies.

Keywords:
GPCR, Alpha-Fold, protein complex, molecular dynamics



Membrane-embedded rhodopsin complex
Highlight on the contact region of the rhodopsin complex between the rhodopsin (blue) and the G-alpha protein (cyan). Important posttranslational modifications (yellow) stabilize the construct, but their positioning is highly sensitive to the modeling procedure.

PS-IV-27

Ionic Liquids vs. Biomembranes: A Romance Gone Toxic

Venus Singh Mithu

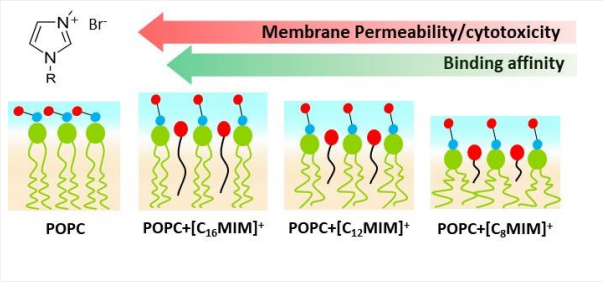
Max Planck Institute for Multidisciplinary Sciences, NMR-based strctural biology, Göttingen, Germany

Introduction:
Ionic liquids (ILs) are "green" solvents with tunable properties like thermal stability and low volatility, but their amphiphilic cations can exhibit significant biological activity. Understanding IL interactions with biomembranes is crucial for creating a structure-property model that links IL design with biological effects, particularly toxicity, for environmental and pharmaceutical applications.

Methods:
We examine the effects of ILs, like1-dodecyl-3-methylimidazolium bromide ($[C_{12}MIM]^+Br^-$), on phospholipid membranes using solid-state NMR spectroscopy, fluorescence dye leakage assays, ζ-potential measurements, and toxicity assessments on cell lines to correlate membrane interactions with cytotoxicity.

Results:
Cholesterol content was found to modulate IL effects, reducing membrane disruption. Membrane composition, IL structure, and concentration were key factors affecting stability and permeability. Dicationic ILs showed lower cytotoxicity than monocationic ILs, with chain length and head-group type influencing membrane interactions. A correlation between membrane disruption and toxicity toward cell lines was established.

Conclusions:
These findings link IL-induced membrane disruption to cytotoxicity, providing a model for designing ILs with targeted properties for biomedical applications. This framework aids in developing safer ILs, optimizing their utility in drug delivery, and therapeutic formulations, and minimizing environmental impact.



Effect of the alkyl chain length of IL cations on the structure and dynamics of POPC bilayer

References
[1] 1. Kumar S, Kaur N, Hitaishi P, Ghosh SK, Mithu VS, Scheidt HA 2024, *J. Phys. Chem. B*, 128, 5407–5418.
2. Kaur N, Fischer M, Hitaishi P, Kumar S, Sharma VK, Ghosh SK, Gahlay GK, Scheidt HS, Mithu VS, 2022, *Langmuir*, 38, 13803–13813.
3. Kaur N, Fischer M, Kumar A, Gahlay GK, Scheidt HS, Mithu VS, 2021, *J. Colloid Interface Sci.*,581, 954–963.
4. Kaur N, Kumar S, Shiksha, Gahlay GK, Mithu VS, 2021, *J. Phys. Chem. B*, 125, 3613–3621.
5. Kumar S, Scheidt HS, Kaur N, Kang TS, Gahlay GK, Huster D, Mithu VS, 2019, Langmuir, 35, 12215–12223.

4:30 pm – 5:35 pm

HS 10

PS-V | Computational Biophysics I

PS-V-01

Cracking the “sulfation code” of glycosaminoglycans using *in silico* tools

Sergey Samsonov

University of Gdańsk, Faculty of Chemistry, Gdańsk, Poland

This research is funded by National Science Centre of Poland, grant number UMO-2023/49/B/ST4/00041.

Glycosaminoglycans (GAGs) represent a class of linear anionic periodic polysaccharides composed of repetitive disaccharide units. Each GAG saccharide building block can be sulfated at different positions contributing to high chemical heterogeneity of these molecules. The amount (net sulfation) and particular positions of the sulfation group (sulfation pattern) constitute “sulfation code”, which is central in the research on GAGs. The “sulfation code” determines the conformational, dynamic, thermodynamic and recognition properties of GAGs, and, consequently, it defines their biological activity which depends on interactions with proteins and other biomolecules in the extracellular matrix of the cell (ECM) that participate in key cellular processes. Thus, deciphering of the “sulfation code” is essential and necessary for understanding GAG function, which is, in turn, of high importance for the development of novel medical approaches based on exploitation of GAG molecular properties [1]. Due to their intrinsic properties, GAGs are very challenging molecules to study experimentally. Therefore, computational approaches are especially promising to investigate GAG containing systems. However, the *in silico* toolset designed specifically for GAGs is limited. In our research, we develop and apply theoretical approaches to understand the physico-chemical nature of GAGs with the ultimate goal to decrypt their “sulfation code” in the context of their biologically relevant funtion.

References

[1] Perez, S, Makshakova, O, Angulo, J, Bedini, E, Bisio, A, de Paz, JL, Fadda, E, Guerrini, M, Hricovini, M, Hricovini, M, Lisacek, F, Nieto, PM, Pagel, K, Paiardi, G., Richter, R, Samsonov, SA, Vives, R, Nikitovic, D, Ricard-Blum, S 2023 'Glycosaminoglycans: What remains to be deciphered?' *JACS Au*, Vol. 323, C1740-C1756.

PS-V-02

Investigating the bottlenecks and pathways for inhibitor dissociation from [NiFe] hydrogenase using simulations and machine learning

Farzin Sohraby, Jing-Yao Guo, **Ariane Nunes-Alves**

Technical University Berlin, Berlin, Germany

Funding from DFG under Germany's Excellence Strategy - EXC 2008/1-390540038 - UniSysCat is gratefully acknowledged. The authors also gratefully acknowledge the Gauss Centre for Supercomputing e.V. (www.gauss-centre.eu) for funding this project by providing computing time through the John von Neumann Institute for Computing (NIC) on the GCS Supercomputer JUWELS at Jülich Supercomputing Centre (JSC).

Introduction

Hydrogenases are important enzymes due to their ability to act as efficient catalysts for hydrogen oxidation and biofuel production. However, some [NiFe] hydrogenases are inhibited by O_2 and CO. One strategy to obtain resistant enzymes is to block the access of inhibitors to the catalytic site by mutation.

Methods

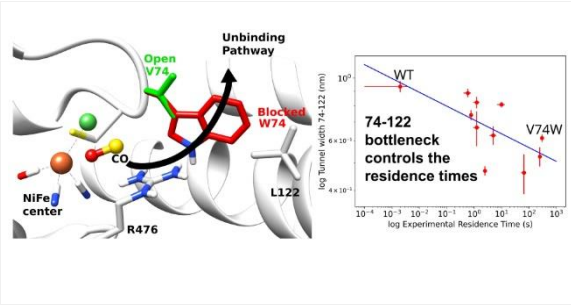
We employed molecular dynamics (MD) simulations, the enhanced sampling method τ-Random Accelerated Molecular Dynamics (τRAMD) and machine learning to study substrate (H_2) and inhibitor (O_2 and CO) unbinding from the wild type and 10 mutants of the *Desulfovibrio fructosovorans* [NiFe] hydrogenase.

Results

The ranking provided by the relative residence times computed with τRAMD was in agreement with experiments [1]. Data analysis revealed that the bottleneck between residues 74 and 122 effectively modulates residence times for CO. We also computed pathway probabilities for the unbinding of different gas molecules and we observed that, while the most probable pathways are the same for different gas molecules, the secondary pathways are different, leading us to propose mutants that increase the access of H_2, but not of the inhibitors, to the catalytic site. Finally, we developed a machine learning model to identify unbinding paths in simulations [2].

Conclusions

The mechanistic insights obtained could be exploited in the engineering of O_2- and CO-tolerant [NiFe] hydrogenases.



Bottleneck for CO diffusion.
The bottleneck between residues 74 and 122 effectively modulates residence times for CO in the *Desulfovibrio fructosovorans* [NiFe] hydrogenase.

References

[1] Sohraby, F, Nunes-Alves, A 2024, Characterization of the Bottlenecks and Pathways for Inhibitor Dissociation from [NiFe] Hydrogenase, *J. Chem. Inf. Model.*, 64, 4193–4203[2] Sohraby, F, Guo, JY, Nunes-Alves, A Manuscript in preparation

PS-V-03

Rotational diffusion of proteins from molecular dynamics simulations

Simon Holtbrügge, Lars Schäfer

Ruhr-Universität Bochum, Center for Theoretical Chemistry, Bochum, Germany

The theoretical description of Brownian rigid-body rotation by Favro (1960) paved the way for extracting the rotational diffusion tensor and the principal axes of rotation from Molecular Dynamics (MD) trajectories. Here, a quaternion-based method that accounts for full anisotropy is used and extended to semi-isotropic and isotropic models, which are statistically more robust. The applicability of the approach is demonstrated with model simulations of an ideal Brownian rotor and also with all-atom MD simulations of Ubiquitin in explicit solvent. The obtained rotational diffusion tensors form the basis for extracting NMR relaxation data from MD simulations.

PS-V-04

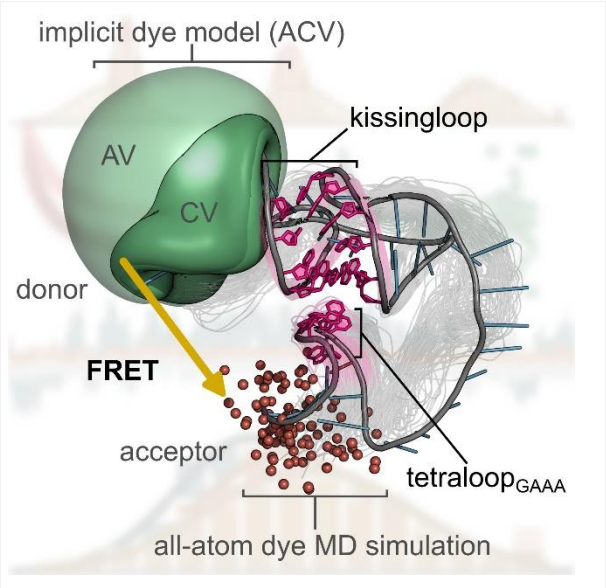
FRET-based integrative modeling for the characterization of an ribosomal RNA tertiary contact

Felix Erichson[1], Fabio D. Steffen[2], Richard Börner[1]

[1] University of Applied Sciences Mittweida, Laserinstitut Hochschule Mittweida, Mittweida, Germany; [2] University of Zurich, Department of Chemistry, Zurich, Switzerland

F E and R B received funding from the European Union and the free state of Saxony [ESF Plus: young research group - LaNa-Sax:100649226]

Tertiary contact interactions between RNA GAAA tetraloops (TL) and their receptors play a crucial role in stabilizing ribosomal RNA folds and facilitating ribosomal maturation [1]. This study uses Förster-Resonance-Energy-Transfer (FRET)-based integrative modeling as an approach to investigate an RNA tertiary contact, comprising a Kissing-loop (KL) which acts as TL receptor [2]. We developed a FRET-assisted modeling pipeline to automate RNA structure prediction. The pipeline includes a knowledge-based modeling approach with PyMOL, MD simulations and the prediction of *in silico* FRET distributions with FRETraj [3] from all-atom dye and a fluorescence anisotropy weighted multiple accessible contact volume (mACV) simulations [4]. The simulations were compared to a cryo-EM structure and experimental *in vitro* single-molecule FRET data [5]. An unconstrained MD simulation showed a slight change in the KL-TL binding pattern compared to the cryo-EM structure, resulting in a mACV-derived FRET distribution that remarkably matches the FRET distribution of an *in vitro* experiment. However, all-atom dye simulations don't represent the dye-RNA interactions leading to deviations in the *in silico* dynamic fluorescence anisotropy and FRET of the Cy3/5 dye pair. Our FRET-guided integrative modeling pipeline has the potential to enhance the understanding of RNA structures and their function, ultimately contributing to the development of more accurate RNA models based on experimental knowledge.



Integrative model of an ribosomal RNA tertiary contact
The knowledge-based modeling results in the bound KL-TL$_{GAAA}$ contact (center) and was characterized by MD and FRET simulations. FRET distributions are derived from all-atom dye (bottom) and accessible contact volume (ACV) simulation (top-left) along the MD trajectory to ensure the comparability to experimental derived FRET data.

References
[1] Nissen, P., et al. 2001, RNA tertiary interactions in the large ribosomal subunit: The A-minor motif, *Proceedings of the National Academy of Sciences,* Vol. 98, Issue 9, pp. 4899–4903
[2] Gerhardy, S., et al. 2021, Puf6 primes 60S pre-ribosome nuclear export at low temperature, *Nature Communications,* Vol. 12, Issue 1, p. 4696
[3] Steffen, F. D., Sigel, R. K. O., & Börner, R. 2021, FRETraj: integrating single-molecule spectroscopy with molecular dynamics, *Bioinformatics,* Vol. 37, Issue 21, pp. 3953–3955
[4] Ploetz, E., et al. 2023, A new twist on PIFE: photoisomerisation-related fluorescence enhancement, *Methods and Applications in Fluorescence,* Vol. 12, Issue 1, p. 012001
[5] Steffen, F. D., et al. 2023, FRET-guided modeling of nucleic acids, *bioRxiv,* 2023.08.07.552238

4:30 pm – 5:35 pm

HS 11

PS-VI | Membrane Biophysics II

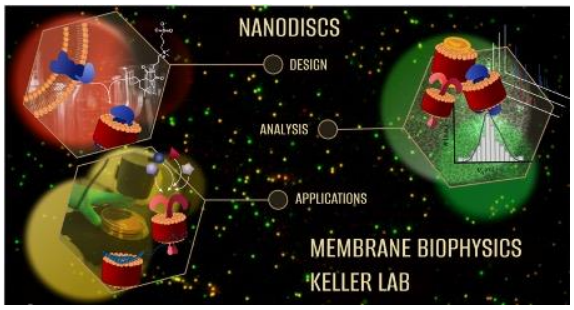
PS-VI-01

New lipid-bilayer nanodiscs for membrane-protein biophysics

Carolyn Vargas[1], Lena Bauernhofer[1], Loretta Eggenreich[1], David Glück[1], Georg Krainer[1], Annette Meister[2], **Sandro Keller**[1]

[1] University of Graz, Graz, Austria; [2] Martin Luther University Halle–Wittenberg, Halle, Germany

Nanodiscs that harbour individual membrane proteins or membrane-protein complexes in a nanoscale lipid-bilayer environment hold great promise for biophysical investigations under well-controlled yet native-like conditions. Our laboratory focusses on new methods for the direct, detergent-free extraction of membrane proteins from cellular membranes into native nanodiscs, which preserve both the overall bilayer architecture and the local lipid composition of the original cellular membrane. Thus, these native nanodiscs render membrane proteins amenable to *in vitro*biophysical investigations without ever removing the proteins from a lipid-bilayer environment. Recently, we have developed and used novel amphiphilic polymers and small-molecule amphiphiles with improved properties for forming native nanodiscs that are compatible with a broad range of ensemble and single-molecule biophysical techniques. In this contribution, we will present selected examples including (i) clinical antibody–receptor interactions (Trastuzumab/HER2) and lipid-specific membrane partitioning of proteins (α-synuclein) studied by microfluidic diffusional sizing (MDS); (ii) native membrane-protein libraries investigated by mass spectrometry (MS); and (iii) cell-free translation of functional membrane proteins (G protein-coupled receptors, GPCRs) with co-translational protein insertion into native nanodiscs.



Nanodiscs

PS-VI-02

Interfacial vs. bulk protons: How proton localization alters ATP synthase activity

Hendrik Flegel, Claudia Steinem

Georg-August-Universität, Institute of Organic and Biomolecular Chemistry, Göttingen, Germany

Many thanks to the IMPRS for Physics of Biological and Complex Systems for the academic and financial support and to the working group of Prof. Nadav Amdursky (Technion, Israel Institute of Technology) for the great collaboration and synthesizing C_{12}-HPTS.

Introduction

The ATP synthase is one of the most crucial proteins responsible for synthesizing the energy currency ATP in prokaryotes as well as in mitochondria and chloroplasts of eukaryotes.[1] It resides in the membrane and uses an electrochemical proton gradient to catalyze ATP synthesis. While the proton pathway within the protein during ATP synthesis is rather understood, the question of how the protons reach the protein entrance is still elusive and highly debated.

Methods

A new experimental approach enables the control of proton release in time and location. Hence, we established a membrane model system containing the F_OF_1 ATP synthase from thermophilic *Bacillus* and use pyranine (8-hydroxy-1,3,6-pyrenetrisulfonate, HPTS) derivatives as photoacids and thus as proton sources.[2] The location of these proton sources either in bulk solution (water-soluble HPTS) or on the membrane surface (amphiphilic C_{12}-HPTS) should allow differentiation between the transfer of bulk or interfacial protons towards the ATP synthase.

Results

Here, we'll show how to reconstitute F_OF_1 ATP synthase into large unilamellar (asymmetric) vesicles containing photoacids at different positions. Furthermore, we'll demonstrate the investigation of the protein activity using luminescence spectroscopy and time-correlated single photon counting.

Conclusions

This strategy is a valuable tool for gaining insights into the question of how proton localization influences the activity of F_OF_1 ATP synthase.

References

[1] Walker, J 2013, The ATP synthase: the understood, the uncertain and the unknown, *Biochem. Soc. Trans.*, 41, 1-16.

[2] Amdursky, N, Lin, Y, Aho, N, Groenhof, G 2019, Exploring fast proton transfer events associated with lateral proton diffusion on the surface of membranes, *PNAS*, 116, 7, 2443-2451.

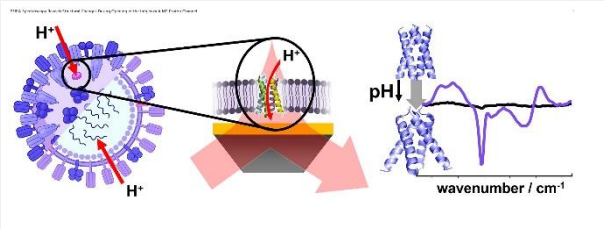
PS-VI-03

Monitoring the opening of the influenza A virus M2 proton channel: combining surface-enhanced infrared absorption and computational spectroscopy

Ronja R. Paschke[1], Swantje Mohr[2], Sascha Lange[2], Adam Lange[2], **Jacek Kozuch**[1]

[1] Freie Universität Berlin, Fachbereich Physik / Forschungsbau SupraFAB, Berlin, Germany; [2] Leibniz-Forschungsinstitut für Molekulare Pharmakologie, Berlin, Germany

Viroporins are small channel-forming proteins in membranes of enveloped viruses that present promising targets for antiviral therapy. The M2 proton channel from Influenza A virus (IAV) has garnered much attention for this family of proteins, when it was shown that its pH-activated proton conductance can be inhibited, thereby, stopping flu infection – an inhibition strategy currently affected by antiviral resistances. Although the chemical steps involved in pH-activation are well understood, the intricate large-scale reorganization of the protein necessary for channel opening remains elusive. To address this gap of knowledge, we employ surface-enhanced infrared absorption (SEIRA) spectroscopy,[1] which enables us to monitor the function of membrane proteins within a single, planar solid-supported bilayer lipid membrane (ssBLM). Upon pH-activation IAV M2, we detected signals characteristic of a reorientation of transmembrane α-helices.[2] Through the integration of experimental and computational spectra, we quantified the mechanical opening of the transmembrane helices, refining the picture of how IAV M2 opens during infection. Interestingly, this very mechanical motion is blocked by M2 inhibitors. In the future, we aim to utilize this approach to enable a combined structural and functional analysis of viroporins of current relevance and contribute to the discovery of new antiviral drugging strategies.



SEIRA Spectroscopy Reveals Structural Changes During Opening of the Influenza A M2 Proton Channel

References
[1] J. Kozuch, K. Ataka, J. Heberle, *Nat. Rev. Methods Primers* 2023, 3, 70.
[2] R. R. Paschke, S. Mohr, S. Lange, A. Lange, J. Kozuch, *Angew. Chem. Int. Ed.* 2023, e202309069.

PS-VI-04

Decoding design principles - membrane remodeling across the tree of life

Felix Frey, Miguel Amaral, Anđela Šarić

Institute of Science and Technology Austria, Klosterneuburg, Austria

Biological cells are defined by lipid membranes, which are constantly remodeled as cells divide or relay materials across them. It is elusive, however, why different membrane designs have evolved across the tree of life. Archaea often live under extreme environmental conditions and have membranes that differ drastically in composition from bacteria and eukaryotes. Typically, archaeal membranes contain mixtures of single-headed bilayer lipids and double-headed bolalipids. However, the physical properties of archaeal membranes and how they react to bending is largely unknown. Here we develop a top-down computational model for archaeal membranes to systematically explore their response to membrane curvature [1]. Similar to living archaea, we can tune the stiffness of bolalipids in our model. We find that both u-shaped (looped) bolalipids and bilayer lipids are enriched in regions of large mean membrane curvature. When individual bolalipids are rigid and therefore preferentially adopt a straight rather than a u-shaped conformation, membrane pores emerge which destabilize the membrane. Our results highlight the similarities and differences between bilayer and bolalipids in their response to membrane curvature, which may shed light on why different lipids and thus membrane designs have evolved.

[1] Felix Frey, Miguel Amaral, and Anđela Šarić, Decoding membrane designs – curvature sorting reveals how membranes remodel, in preparation (2024).

4:30 pm – 5:35 pm

HS 8

PS-IV | Molecular Biophysics I

PS-IV-01

Lipid dependence of the conformational coupling across the membrane bilayer of fulllength epidermal growth factor receptor

Shwetha Srinivasan[1,2], Xingcheng Lin[1], Xuyan Chen[1], Raju Regmi[1], Bin Zhang[1], Gabriela Schlau-Cohen[1]

[1] Massachusetts Institute of Technology, Department of Chemistry, Cambridge, USA; [2] Stanford University, Department of Molecular and Cellular Physiology, Stanford, USA

Membrane proteins regulate several vital cellular processes, establishing them as major drug targets. Epidermal growth factor receptor (EGFR) is one such protein that plays a crucial role in transducing extracellular signal across the membrane bilayer and is implicated in cancer. While the structures of the extracellular and intracellular regions of this protein have been well-elucidated individually, conformational coupling connecting these two regions during signal transduction is challenging to probe due to mixture of hydrophobic and hydrophilic domains. Here, I will discuss the signal transduction mechanism across full length EGFR. We isolate full-length, functional EGFR in a near-physiological environment. Using a multidisciplinary approach involving single-molecule Förster resonance energy transfer, mutagenesis, and molecular dynamics simulations, we observe a compaction in the intracellular domain of EGFR upon extracellular ligand binding in a neutral lipid environment. The ligand-induced extracellular/intracellular conformational coupling is reversed in the presence of anionic lipids and arrested with cholesterol present in the lipid bilayer. Our findings show how the extracellular and intracellular domains are coupled to each other in this critical receptor and the impact of the lipid composition in this conformational coupling. Our results could be universal to other membrane receptors which share the same structural homology and perform other significant functions.

PS-IV-02

Single molecule investigations of the unique DNA lesion search and recognition strategies by the DNA alkyltransferase AGT

Ingrid Tessmer[1], Alan M. Szalai[2], Sarah Kono[1], Lars Richter[2], Giovanni Ferrari[2], Philip Tinnefeld[2]

[1] University of Würzburg, Rudolf Virchow Center for Integrative and Translational Bioimaging, Würzburg, Germany;
[2] Ludwig-Maximilians-Universität, Department of Chemistry and Center for NanoScience, Munich, Germany

This work was made possible through financial support by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation, TE671/7-1 to IT and TI329/14-1 to PT) and the Alexander von Humboldt foundation (Ref 3.2 - ARG - 1220722 - GF-P to AMS).

The O^6-alkylguanine DNA alkyltransferase (AGT) repairs highly mutagenic and cytotoxic O^6-alkylguanine lesions that result from metabolic products but are also deliberately introduced during chemotherapy.
To better understand the working mechanism of this important protein, we used single molecule fluorescence microscopy coupled with a dual-trap optical tweezers system.
We found that AGT preferentially forms and/or stabilises oligomeric complexes or clusters at a target lesion compared to on undamaged DNA. Clusters may thus play a role in lesion processing. Lesion search dynamics on undamaged DNA were comparable for monomers and clusters, in contrast to speculations that clusters may enhance the speed of lesion search by AGT, and showed bi-directional movement on DNA over long distances of thousands of base pairs. This is in contrast to previously reported directionality in lesion repair by AGT on a short DNA substrate, which may be caused by directional cluster growth.

To investigate potential directionality in the short distance regime of lesion search (in the range < 70 base pairs) and the role of AGT monomers and clusters, we recently developed a novel single molecule methodology that is based on single molecule fluorescence quenching by graphene (graphene-induced energy transfer with vertical nucleic acids, GETvNA).

GETvNA has enabled us to directly resolve single base pair steps by AGT monomers and clusters, and to analyse AGT dynamics on different DNA sequences.

PS-IV-03

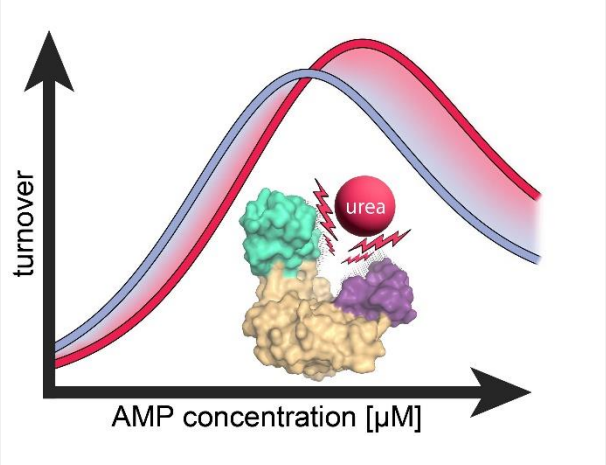
Disclosing a paradox: how urea activates adenylate kinase by modulating conformational dynamics and reducing substrate affinity

David Scheerer, Dorit Levy, Remi Casier, Inbal Riven, Gilad Haran

Weizmann Institute of Science, Chemical and Biological Physics, Rehovot, Israel

Proteins often use large-scale motions of domains and subunits to function effectively. The enzyme adenylate kinase (AK) is a key model for exploring the relationship between conformational dynamics and enzymatic activity. Efficient catalysis is ensured by a large-scale domain motion that encloses the bound substrates. Single-molecule FRET (smFRET) spectroscopy combined with machine learning revealed that domain motion occurs within tens of microseconds, two orders of magnitude faster than the enzyme's turnover [1]. Molecular simulations indicated that the repeated conformational transitions are crucial for the relaxation of incorrectly bound substrates into the catalytically competent conformation [2].

A surprising feature of AK is the activation by urea, a compound commonly acting as a denaturant. We discovered that this activation is linked to substrate inhibition by AMP. Urea alters protein dynamics and AMP binding, reducing AMP inhibition and thus activating the protein. This creates a scenario where a reduction in substrate affinity is beneficial for activity. Furthermore, urea promotes the enzyme's open conformation, what enhances activity by facilitating substrate rearrangement. However, the enzyme is not activated by urea when AMP inhibition is negligible. Our results highlight the crucial role of conformational dynamics in regulating enzymatic activity.



Impact of urea on the enzymatic activity of AK
Urea influences the conformational dynamics and the affinity for AMP. This reduces the activity at low AMP concentrations, but reduces substrate inhbition at high AMP levels, thus activating the enzyme.

References
[1] Scheerer, D. et al., 2023, 'Allosteric communication between ligand binding domains modulates substrate inhibition in adenylate kinase', *PNAS*, 120, e2219855120
[2] Lu, J. *et al.*, 2022, 'Role of repeated conformational transitions in substrate binding of adenylate kinase', *JCP B*, 41, p. 8188

PS-IV-04

A sample efficient qualification method - automated scanning in plate DLS

Arne Meyer, Annette Eckhardt, Karsten Dierks

XtalConcepts GmbH, Hamburg, Germany

In plate dynamic light scattering (in plate DLS) [1, 2] is a powerful tool for protein formulation and stability analysis. The use of standard 96-well plates for DLS provides a high degree of automation, while sample volume/well can be significantly reduced to 80 to 800 nl/well. This method allows rapid analysis of protein buffer response based on aggregate formation. This makes *in plate* scanning DLS the ideal method for formulation approaches. DLS itself is a standard non-invasive biophysical method for determining the dispersity of macromolecular samples. The monodispersity of a sample is a prerequisite for almost all subsequent structure determination methods, e.g. crystallisation, cryo-electron microscopy (cryo-EM), small-angle X-ray scattering (SAXS) and nuclear magnetic resonance (NMR). After purification, biological macromolecules, i.e. proteins, often exhibit a degree of aggregation. Once a formulation has been found, *in plate* DLS can be used to parameterise the formulation in terms of some fundamental criteria such as maximum concentration, storage time, homogeneity, optimum storage temperature, temperature and chemical stability.

[1] J. Birch, D. Axford, J. Foadi, A. Meyer, A. Eckhardt, Y. Thielmann, I. Moraes, The fine art of integral membrane protein crystallization, Methods, Sept. 2018

[2] SpectroLight 600 an in situ DLS system, see www.xtal-concepts.de

24 September, 2024

8:30 am – 10:30 am

HS 9

PL-IV | Plenary IV

PL-IV-01

Allosteric modulation of GPCR signaling

Jean-Louis Baneres

CNRS, Institut des Biomolécules Max Mousseron (IBMM), Montpellier, France

This work was supported by CNRS, Université de Montpellier, Agence Nationale de la Recherche (20-CE92-0028, 21-CE29-0012, 22-CE44-0042) and the Fondation Pour la Recherche Médicale (Equipe FRM EQU202103012736).

Introduction

G protein-coupled receptors (GPCRs) are the largest family of membrane receptors. They elicit diverse signals by coupling to multiple intracellular partners, including different G proteins and β-arrestins. This functional versatility is further allosterically modulated by the cellular environment. However, the molecular mechanisms underlying this allosteric control are unclear. A current hypothesis is that GPCRs exist as a continuum of states, each tuned to different signaling proteins. The equilibria between these states would then be shifted by the environment, resulting in modulation of signaling.

Methods

We have developed a molecular pharmacology strategy based on the use of an isolated GPCR, the ghrelin receptor GHSR, reconstituted *in vitro* in a model membrane-mimicking system, lipid nanodiscs.

Results

Thanks to this nanodisc system and to a combination of computational, biochemical and biophysical approaches, we have been able to illuminate the molecular mechanisms underlying the allosteric control of GPCR efficacy and functional selectivity. In particular, our data indicate that several components of the receptor environment - ions, lipids - modulate the conformational repertoire of GHSR and its ability to selectively interact with its signaling partners.

Conclusions

Taken together, this points to a model in which the receptor environment acts as a selective allosteric modulator of the GHSR, making it an integral part of the GPCR signaling machinery.

PL-IV-02

Dynamic conformational equilibria and biased signaling in GPCRs: insights from β_1-adrenergic receptor NMR studies

Layara A. Abiko, Stephan Grzesiek

University of Basel, Biozentrum, Basel, Switzerland

This work was supported by the Swiss National Science Foundation (CRSK-3_195592 to L.A.A. and 31-201270 and IZLIZ3-200298 to S.G.).

G protein-coupled receptors (GPCRs) are the therapeutically most important receptors in the human genome. GPCR function is regulated by dynamic equilibria between multiple conformatios, influenced by factors such as orthosteric ligands, pressure, and lipid environment.

We showed that pressure shifts the β_1-adrenergic receptor (β_1AR) from a mix of inactive and active states to a fully active state[1]. This implies that the active state is smaller than the inactive state due to collapsed empty cavities. Filling β_1AR voids i.e. with cholesterol, stabilizes the receptor inactive state by blocking the activation of conserved GPCR microswitches[2]. Thus, voids in GPCRs are potential allosteric hot spots.

Orthosteric ligands regulate GPCR signaling to intracellular effectors (G proteins and arrestins). Selective activation of specific signaling pathways (biased signaling) is at the forefront of GPCR-targeted drug development. Our current research focuses on the biased signaling mechanism of carvedilol, a widely used β-blocker. Carvedilol is an arrestin-biased agonist and, simultaneously, a G protein antagonist of βARs[3]. Using NMR, cryo-EM, and biochemical assays, we studied conformational changes in the carvedilol-bound receptor with and without arrestin or G protein-mimicking proteins.

Our findings provide crucial molecular details that explain how GPCRs can selectively activate specific cellular pathways. This may guide the development of selective and safer GPCR-targeted drugs.

References

[1] Abiko, L. A., Grahl, A. & Grzesiek, S. High Pressure Shifts the β_1-Adrenergic Receptor to the Active Conformation in the Absence of G Protein. *J. Am. Chem. Soc.* **141**, 16663–16670 (2019).

[2] Abiko, L. A., Teixeira, R. D., Engilberge, S., Grahl, A., Mühlethaler, T., Sharpe, T., Grzesiek, S. Filling of a water-free void explains the allosteric regulation of the β_1-adrenergic receptor by cholesterol. *Nat. Chem.* 14, 1133–1141 (2022).

[3] Wisler, J. W., DeWire, S. M., Whalen, E. J., Violin, J. D., Drake, M. T., Ahn, S., Shenoy, S. K., Lefkowitz, R. J. A unique mechanism of β-blocker action: Carvedilol stimulates β-arrestin signaling. *Proc. Natl. Acad. Sci.* **104**, 16657–16662 (2007).

PL-IV-03

Unveiling the dynamics of cellular membranes: insights from molecular dynamics simulations

Marius F.W. Trollmann[1,2], Nancy Mejía Villagrán[1], Cristian Popov[1], Paolo Rossetti[1], Matthias Pöhnl[1], **Rainer A. Böckmann**[1,2]

[1] Friedrich-Alexander-Universität Erlangen-Nürnberg, Computational Biology, Erlangen, Germany; [2] Erlangen National High-Performance Computing Center (NHR@FAU), Erlangen, Germany; [3] FAU Profile Center Immunomedicine (FAU I-MED), Erlangen, Germany

The structure and dynamics of cellular membranes are critical for numerous biological functions, with lipids playing a fundamental role in these processes. This talk will delve into the different roles of lipids for membrane structure, dynamics, and function through the lens of scale-bridging molecular dynamics simulations.

We will explore the role of aminolipids for the structure of lipid nanoparticles (LNPs) [1] and discuss how the lipid environment of LNPs modulates the pKa of aminolipids, essential for their function. Regarding membrane dynamics, we reveal a non-universal role of cholesterol for membrane elasticity: we discuss how cooperativity between thermal membrane bending and local cholesterol distribution leads to membrane softening despite cholesterol-induced membrane thickening [2]. Finally, we will investigate how the association of immune receptors drives membrane curving and facilitates antibody recognition by the inhibiting FcgRIIb receptor, crucial for immune responses [3].

These examples underscore the importance of lipids in maintaining membrane structure and function, offering insights into their multiple roles in health and disease, as well as their significance for lipid-based drug delivery systems and therapeutic interventions.

References

[1] Trollmann, MFW, Böckmann, RA 2022. ‘mRNA lipid nanoparticle phase transition’, *Biophys. J.* 121, 3927–3939

[2] Pöhnl, M, Trollmann, MFW, Böckmann, RA 2023, ‘Nonuniversal impact of cholesterol on membranes mobility, curvature sensing and elasticity.’ *Nat. Commun.* 14, 8038

[3] Seeling, M, Pöhnl, M, ..., Böckmann, RA, Nimmerjahn, F 2023, ‘Immunoglobulin G-dependent inhibition of inflammatory bone remodeling requires pattern recognition receptor Dectin-1.’ *Immunity*, 56, 1046-1063

PL-IV-04

A cholesterol switch controls phospholipid scrambling by G protein-coupled receptors

Anant K. Menon

Weill Cornell Medicine, Biochemistry, New York, USA

Introduction

Class A G protein-coupled receptors (GPCRs) moonlight as constitutively active phospholipid scramblases, yet the plasma membrane (PM) of metazoan cells – which is replete with GPCRs -– has a strong trans-bilayer phospholipid asymmetry. To explain this phenomenon, we hypothesized that GPCR-mediated lipid scrambling is regulated by cholesterol, a major constituent of the PM.

Methods and Results

Atomistic molecular dynamics simulations of a GPCR indicate that phospholipid headgroups traverse a dynamically revealed hydrophilic groove between transmembrane helices (TM) 6 and 7 while their tails remain in the bilayer (credit card model). The simulations further indicate that cholesterol inhibits scrambling by populating the TM6/7 interface and stabilizing the closed groove conformation while itself undergoing flip-flop. To test the cholesterol effect experimentally, we supplemented GPCR-containing synthetic vesicles with cholesterol to a level similar to that of the PM. We found that the scramblase activity of two GPCRs, opsin and the b1-adrenergic receptor, is impaired upon cholesterol loading.

Conclusions

Our results indicate that cholesterol acts as a switch, inhibiting scrambling above a receptor-specific threshold concentration to disable GPCR scramblases at the PM.

References

[1] Khelashvili, G., Menon, A.K. (2022) Annu. Rev. Biophys. 51, 39 (PMID: 34932914)

[2] Morra, G. et al. (2022) Structure 30, 1208 (PMID: 35660161)

[3] Menon, I. et al. (2024) J. Biol. Chem. 300, 105649 (PMID: 38237683)

11:00 am – 12:05 pm

HS 10

PS-VIII | Computational and Molecular Biophysics II

PS-VIII-01

Peptide translocation across asymmetric phospholipid membranes

Ladislav Bartoš, **Robert Vácha**

Masaryk University, Central European Institute of Technology (CEITEC) and Faculty of Science, Brno, Czech Republic

Cells are separated from their surroundings by a semi-permeable cytoplasmic membrane. Peptides with specific properties are able to spontaneously cross this barrier and act as drugs or drug carriers. Typically, the permeation of these peptides is studied using symmetric model membranes, even though actual cell membranes are usually asymmetric. It is commonly thought that the permeability of an asymmetric membrane can be approximated from the permeabilities of the corresponding symmetric membranes. Using computer simulations with both coarse-grained and atomistic force fields, we calculated the free energy profiles for the passage of model amphiphilic peptides and a lipid across various symmetric and asymmetric membranes. Our results consistently demonstrate that while the free energy profiles for asymmetric membranes with a small differential stress align with symmetric ones in the region of lipid headgroups. However, the profiles differ around the membrane centre. In this region, the free energy for the asymmetric membrane transitions between the profiles corresponding to two symmetric membranes composed of individual leaflets. We show that peptide permeability through an asymmetric membrane cannot always be predicted from the permeabilities of the symmetric membranes. This suggests that using symmetric membranes does not provide an accurate representation of peptide translocation across asymmetric membranes.

PS-VIII-02

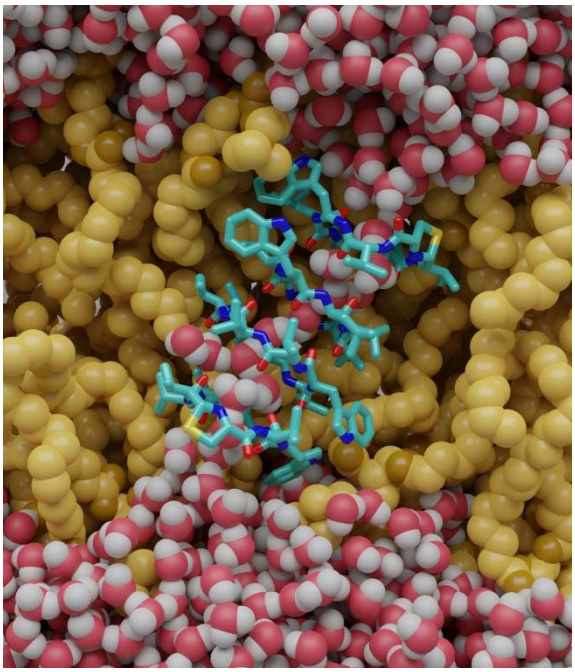
One ring to rule them all: lugdunin's disruptive effects

Marius F.W. Trollmann[1,2], Dominik Ruppelt[3], Claudia Steinem[3,4], Rainer A. Böckmann[1,2]

[1] Friedrich-Alexander-Universität Erlangen-Nürnberg, Computational Biology, Department of Biology, Erlangen, Germany; [2] Erlangen National High Performance Computing Center (NHR@FAU), Erlangen, Germany; [3] Georg-August-Universität Göttingen, Institute of Organic and Biomolecular Chemistry, Göttingen, Germany; [4] Max Planck Institute for Dynamics and Self-Organization, Göttingen, Germany; [5] FAU Profile Center Immunomedicine (FAU I-MED), Erlangen, Germany

We gratefully acknowledge the compute resources and support provided by the Erlangen Regional Computing Center (RRZE) and the Erlangen National High Performance Computing Center (NHR@FAU) under the NHR project b174dc. Special thanks are owed to Alexey Aleksandrov and the colleagues at the Computer Chemistry Center (CCC) at FAU for their invaluable discussions and insights on the parametrization of the thiazolidine moiety.

Antimicrobial resistance is a growing threat to global public health, underscoring the need for novel strategies to counteract the spread of multi-resistant bacterial strains. Antimicrobial peptides have emerged as a promising alternative to common antibiotics by inhibiting bacterial growth inducing hardly any resistance. Recently, the cyclic peptide lugdunin was isolated from nasal *Staphylococcus lugdunensis* and has shown a strong antimicrobial activity against several Gram-positive bacteria. Comprising six D,L-amino acids and a thiazolidine moiety, lugdunin maintains membrane integrity and enables proton translocation across membranes. However, the mechanistic mode of action of lugdunin on membranes is hardly understood. In a recent study [1], we applied atomistic molecular dynamics simulations to investigate lugdunin’s differential interaction with a range of complex model membranes.
Our results suggest that lugdunin easily penetrates the membrane interface region, and is able to advance further into the membrane core. We decipher the driving forces behind lugdunin membrane embedment and in particular the role of the thiazolidine moiety. Microsecond-long simulations indicate the possibility for self-assembly of lugdunin stacks capable of facilitating proton transport across a lipid bilayer. Our simulations provide a basis for the rational design of new macrocyclic thiazolidine peptide antibiotics with enhanced efficacy and safety profiles.



Water-filled lugdunin channel in lipid membrane
Channel composed of four lugdunin peptides embedded in a DOPC membrane. Water molecules diffused spontaneously into the channel to cross the hydrophobic membrane core.

References
[1] Ruppelt, D. et al., 2024, The antimicrobial fibupeptide lugdunin forms water-filled channel structures in lipid membranes, *Nat Commun*, 15, 3521, Nature Portfolio

PS-VIII-03

Ionizable cationic lipids and helper lipids synergistically contribute to RNA packing and protection in lipid-based nanomaterials

David N. Zimmer[1,2], Giovanni Settanni[1,2], Friederike Schmid[2]

[1] Ruhr-Universität Bochum, Institut für Physik und Astronomie, Bochum, Germany; [2] Johannes-Gutenberg-Universität Mainz, Institut für Physik, Mainz, Germany

Lipid-based nanoparticles (LNP) are one of the most effective carriers in mRNA therapeutics. They are made of a mixture of ionizable, helper, and pegylated lipids encapsulating mRNA. While peg tends to remain on the surface of the nanoparticle, the structure of the core has not yet been well characterized. Experimental data point to a relative lack of order. The lipid composition of the formulation plays a key role in determining the effectiveness of the nano carrier. Small changes in the chemical structure of ionizable and helper lipids dramatically affect the efficiency of mRNA transfection. LNPs based on DLinDMA and DLinDAP, two ionizable lipids, showed significantly different transfection efficiencies, notwithstanding the very small difference in structure. Here, using a multiscale modeling approach, the behavior of lipid formulations based on these two lipids, cholesterol and DSPC or DOPE is examined aiming to provide an understanding of the interactions of lipids and mRNA. The simulations show that, despite a more engulfed mRNA in presence of DLinDMA, DLinDAP's binding affinity for mRNA is larger. The hydrophilic linker of DLinDMA leads to more water in-between the bilayer and the mRNA, while the strong binding of mRNA to DLinDAP may hinder the endosomal escape. We show that chemical optimization based only on mRNA-ionizable lipid interactions may not be sufficient for the development of more effective lipid formulations for RNA delivery.

PS-VIII-04

Visualizing and quantifying biomolecular interactions across scales with fluorescence optical tweezers. | LUMICKS

Roman Renger

Amsterdam, Netherlands

Roman Renger
LUMICKS, Paalbergweg 3, 1105 AG, Amsterdam

Biological processes involving proteins interacting with nucleic acids, cell membranes or cytoskeletal filaments are key to cell metabolism and hence to life in general. Detailed insights into these processes provide essential information for understanding the molecular basis of physiology and the pathological conditions that develop when such processes go awry.

The next scientific breakthroughs are relying on direct, real-time observations and measurements of the most fundamental mechanisms and interactions involved in biology. Modern correlative single-molecule technologies offer a powerful opportunity to meet these challenges and to study dynamic protein function and activity in real-time and at unprecedented resolution. Seeing is believing!

Here, we present our efforts to enable discoveries in biology and biophysics using the combination of optical tweezers with correlative fluorescence microscopy and advanced microfluidics capabilities. Our C-Trap allows you to directly visualize and observe biomolecules while simultaneously measuring forces and exposing the biomolecular system to different experimental conditions. We present several examples in which our technology has enhanced the understanding of basic biological phenomena, ranging from DNA repair to structural transition in proteins to intracellular organization and cell mechanics. Furthermore, we demonstrate how advances in hybrid single-molecule methods can be turned into an easy-to-use and stable instrument for laboratories that want to focus on biological and biophysical questions that could not be answered with traditional methods in molecular and structural biology.

11:00 am – 12:05 pm

HS 11

PS-IX | Membrane Biophysics III

PS-IX-01

Membrane permeabilization and other membrane perturbations: the significance of model studies for antimicrobial peptides and polymers

Katharina Beck[1,3], Anja Stulz[1], Shuai Shi[1], **Maria Hoernke**[1,2]

[1] Albert-Ludwigs-Univeristät, Freiburg, Germany; [2] Martin-Luther-Universtät Halle-Wittenberg, Halle, Germany; [3] Universität Augsburg, Augsburg, Germany

Introduction

For several decades, the effects of antimicrobial peptides or their synthetic mimics on model membranes have been investigated, for example with the aim to develop much-needed alternatives to classical antibiotics. Lipid membranes are supposed to be the main target of these natural or designed molecules. Therefore, induced membrane permeabilization or leakage is often taken as an indication for activity. Despite a wealth of natural and designed molecules and high-throughput approaches, a break-through is still missing.

Methods

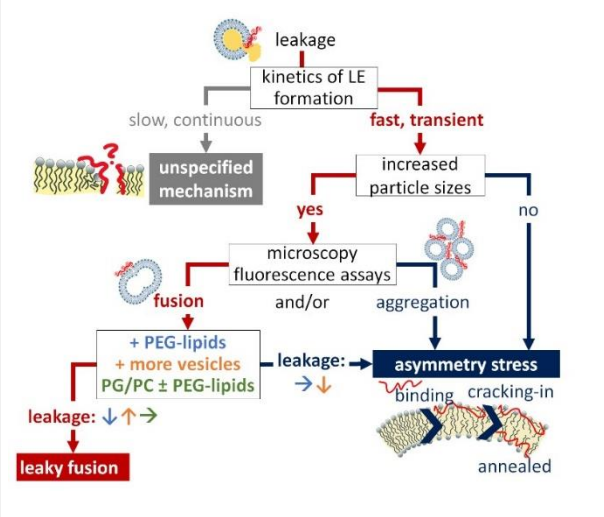
Different mechanisms of membrane permeabilization are distinguished and their relation to other membrane perturbations was examined by a range of biophysical methods. For example, to unambiguously establish a mechanistic link of leakage and fusion, both are studied while aggregation, fusion, and similar effects are inhibited or enhanced.

Results

We found that leakage, fusion and other membrane perturbation often occur at similar conditions, but not be related. A commonly used composition of vesicles (POPG/POPE) is especially prone to leaky fusion, potentially leading to misinterpretation. En route, strategies to recognize, judge, and prevent potential side-effects caused by vesicle aggregation, fusion, or both are compiled.

Conclusions

This detailed mechanistic analysis is expected to improve model studies of membrane-mediated antimicrobial activity and, in turn, future treatments against increasingly resistant microbes.



membrane permeabilization
Different types of membrane permeabilization can be distiguished by experiments on model vesicles. Not all of them are equally relevant for antimicrobial activity.

References

[1] Beck, K.; Nandy, J. & Hoernke, M 2023, Membrane permeabilization can be crucially biased by a fusogenic lipid composition – leaky fusion caused by antimicrobial peptides in model membranes, *Soft Matter*, 19, 2919-2931

[2] Shi, S.; Fan, H. & Hoernke, M. 2022, Leaky membrane fusion: an ambivalent effect *Nanoscale adv.* induced by antimicrobial polycations, *Nanoscale Advances*, *4*, 5109 – 5122

[3] Shi, S.; Markl, A.M.; Lu, Z.; Liu, R. & Hoernke, M. 2022, Interplay of fusion, leakage, and electrostatic lipid clustering: membrane perturbations by a hydrophobic antimicrobial polycation *Langmuir,38*, 2379-2391

[4] Shi, S.; Quarta, N.; Zhang, H.; Lu, Z.; Hof, M.; Šachl, R.; Liu, R. & Hoernke, M. 2021, Hidden complexity in membrane permeabilization behavior of antimicrobial polycations *Phys. Chem. Chem. Phys.,23*, 1475-1488

[5] Stulz, A.; Vogt, A.; Saar, J. S.; Akil, L.; Lienkamp, K.; & Hoernke, M. 2019, Quantified membrane permeabilization indicates the lipid selectivity of membrane-active antimicrobials *Langmuir, 35*, 16366

PS-IX-02

SARS-CoV-2 binding to terminal sialic acid of gangliosides embedded in lipid membranes

Geetanjali Negi

Indian Institute of Technology Kanpur, Chemistry, KANPUR, India

Thanks to my supervisor Dr. Nagma Parveen for her continuous guidance and immense support. Special thanks to our collaborator Dr Krishnan Harshan and IIT Kanpur for the Fellowship (SRF), providing travel grants and family and lab-mates.

Introduction

Multiple reports indicate that the S protein of SARS-CoV-2 interacts explicitly with membrane receptors and attachment factors other than ACE2. They likely have an active role in cellular attachment and virus entry.

Methods

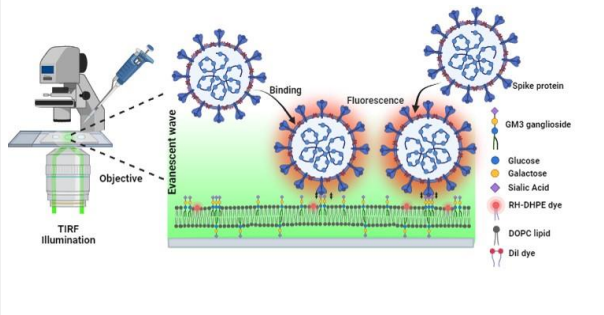
This study used TIRF microscope, Single particle detection, and Surface plasmon resonance.

Results

We examined the binding of SARS-CoV-2 to gangliosides embedded in supported lipid bilayers (SLBs), mimicking the cell membrane-like environment. We show that the virus binds to sialylated (sialic acid (SIA)) gangliosides, i.e., GD1a, GM3, and GM1, as determined from the acquired single-particle fluorescence images using a time-lapse total internal reflection fluorescence (TIRF) microscope. The data of virus binding events, the apparent binding rate constant, and the maximum virus coverage on the ganglioside-rich SLBs show that the virus particles have a higher binding affinity toward the GD1a. Enzymatic hydrolysis of the SIA–Gal bond of the gangliosides confirms that the SIA sugar unit of GD1a and GM3 is essential for virus attachment to the SLBs, and even the cell surface sialic acid is critical for the cellular attachment of the virus. The structural difference between GM3/GD1a and GM1 is the presence of SIA at the main or branched chain.

Conclusions

The number of SIA per ganglioside can weakly influence the initial binding rate of SARS-CoV-2, whereas the terminal/more exposed SIA is critical for the virus binding to the gangliosides in SLBs.



Scheme of basic setup for TIRF imaging of the labeled virus binding on SLB over a cover glass Total internal reflection microscopy was used to study single particle (SARS-CoV-2) binding over the different concentrations of GD1a, GM1 and GM3 ganglioside-rich SLB.

References

[1] Negi, G.; Sharma, A.; Dey, M.; Dhanawat, G.; Parveen, N. Membrane Attachment and Fusion of HIV - 1, Influenza A, and SARS- CoV - 2: Resolving the Mechanisms with Biophysical Methods. Biophys. Rev. 2022, 14, 1109–1140.
[2] Sharma, A.; Negi, G.; Chaudhary, M.; Parveen, N. Kinetics of Ganglioside-Rich Supported Lipid Bilayer Formation with Tracer Vesicle Fluorescence Imaging. *Langmuir***2023**, *39* (33),
[3] Negi, G.; Sharma, A.; Chaudhary, M.; Gupta, D.; Harshan, K. H.; Parveen, N. SARS-CoV-2 Binding to Terminal Sialic Acid of Gangliosides Embedded in Lipid Membranes. *ACS Infect. Dis.***2023**, *9*, 1346–1361
[4] Dey, M.; Sharma, A.; Dhanawat, G.; Gupta, D.; Harshan, K. H.; Parveen, N. Synergistic Binding of SARS-CoV-2 to ACE2 and Gangliosides in Native Lipid Membranes. ACS Infect. Dis. 2024, 10, 907-916.
[5] Petitjean, S. J. L.; Chen, W.; Koehler, M.; Jimmidi, R.; Yang, J.; Mohammed, D.; Juniku, B.; Stanifer, M. L.; Boulant, S.; Vincent, S. P.; Alsteens, D. Multivalent 9-O-Acetylated-Sialic Acid Glycoclusters as Potent Inhibitors for SARS-CoV-2 Infection. *Nat. Commun.***2022**, *13* (2564), 1–12.

PS-IX-03

Do amyloid beta peptides incorporate into lipid membranes during misfolding? Insights by combining SEIRA and computational spectroscopy

Saskia Heermant, Jacek Kozuch

Freie Universität Berlin, Experimental Molecular Biophysics, Forschungsbau SupraFAB, Berlin, Germany

This work was kindly supported by the German Research Foundation.

Introduction

Alzheimer's disease is tightly associated with amyloid beta (Aβ) peptides that misfold at membrane interfaces to form insoluble fibrils. These fibril aggregates lead to physical and electrostatic changes in the membrane and eventually to dysfunction or cell death, which over time causes symptoms such as cognitive decline. While there is strong evidence that negatively charged lipids act as catalyst in the misfolding trajectory, it is not known if the peptide/lipid interaction is superficial or involves the incorporation into the membrane. [1,2]

Methods

We build different tethered bilayer lipid membrane systems and investigate the Aβ (mis)folding process at the interfaces using surface-enhanced infrared absorption (SEIRA) spectroscopy. To quantify the orientation and secondary structure we compare experimental and based on density functional theory (DFT) computed spectra.

Results

Focusing on the amide I and amide II bands in the SEIRA spectra, we monitor the changes in the secondary structures of Aβ during its interaction with the membranes as β-sheet structured intermediates form towards a potentially misfolded state. Changes of the amide I/II ratios indicate that on monolayered membranes, the orientation changes are smaller than on bilayered lipid membranes.

Conclusions

This combined experimental and computational approach shows that peptide/lipid interactions indeed depend on the capability to insert into the lipid membrane.

References

[1] Chen, Gf., Xu, Th., Yan, Y. *et al.* 2017, ‘ Amyloid beta: structure, biology and structure-based therapeutic development ’, *Acta Pharmacologica Sinic* , 38, 1205–1235

[2] Sciacca MFM, Tempra C, Scollo F, Milardi D, La Rosa C. 2018, ‘ Amyloid growth and membrane damage: Current themes and emerging perspectives from theory and experiments on Aβ and hIAPP.’, *Biochim Biophys Acta Biomembr.*, 9, 1625-1638

PS-IX-04

Spatial organization of liquid protein condensates on micro-structured lipid membranes

Chae Yeon Kang, Yoohyun Chang, **Katja Zieske**

Max Planck Institute for the Science of Light, Erlangen, Germany

Introduction

Liquid protein condensates are assembled by proteins or nucleic acids and form through the process of phase separation. They serve as specialized membrane-less compartments, which regulate biochemical reactions in a confined space and recent studies have established associations between lipid membranes and proteins capable of forming liquid condensates. However, little is known about how biophysical parameters of lipid membranes affect liquid condensates.

Methods

Here, we explored how the topography of lipid membranes affects the organization of liquid protein condensates. We implemented an assay to assemble liquid protein condensates on supported lipid membranes with micro-structured topographies.

Results

By employing membrane surfaces designed with various microstructures, we showed that liquid condensates assemble into orderly patterns or defined shapes on topographically structured membranes. We found that capillary forces, mediated by membrane topographies, lead to the directed fusion of liquid condensates.

Conclusions

Our results demonstrate that membrane topography is a potent biophysical regulator for the organization of mesoscale liquid protein condensates.

References

[1] Kang CY, Chang Y, Zieske K 2024, 'Lipid Membrane Topographies Are Regulators for the Spatial Distribution of Liquid Protein Condensates', *Nano Lett.* 24, 15, 4330–4335

11:00 am – 12:05 pm

HS 8

PS-VII | Future Direction in Biophysics I

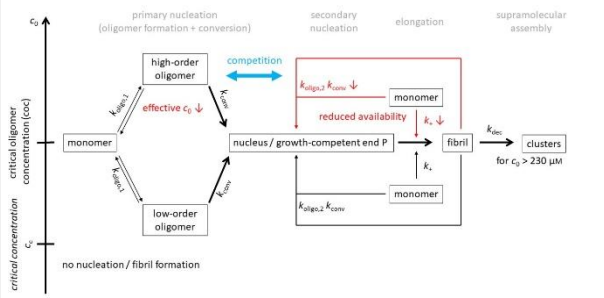
PS-VII-01

A competition of secondary and primary nucleation controls functional amyloid formation of the parathyroid hormone

Jochen Balbach, Bruno Voigt, Shubhra Sachan, Manuel Weber

Martin-Luther-Universtät Halle-Wittenberg, Institut für Physik, Halle, Germany

The parathyroid hormone (PTH) controls the calcium and phosphate homeostasis in human blood. The 84 residue peptide PTH, which is largely intrinsically disordered, gets stored in gland tissue in secretory granules before release. As proposed for many years we could show now by cell biology methods that PTH indeed forms amyloid fibrils in these highly concentrated granules. A premature fibrillation is prevented by a six residue pro-sequence at the N-terminus by coulomb repulsion, which gets cleaved off before granule packing [1]. Combining various biophysical methods including fluorescence and NMR spectroscopy, electron microscopy, X-ray scattering, smFRET and native mass spectrometry, we could elucidate in vitro the fibrillation mechanism of PTH [2-3]. The prenucleation ensemble of states contains PTH trimers and tetramers facilitated by the N-terminal 40 residues at a critical fibrillation concentration of 70 microM as precursors for primary nucleation. The content of states in this highly dynamic ensemble persists also during fibril growth, which is dominated by secondary nucleation. During this phase we find a negative feedback on fibril elongation and secondary nucleation at elevated PTH concentrations. The functional amyloids of PTH are in equilibrium with monomers revealing a thermodynamic stability of – 23.8 kJ/mol Gibbs free energy. Therefore, hormone release into the blood stream as monomers simply occurs by dilution.



Fibrillation mechanism of the parathyroid hormone
Scheme of the concentration dependence of PTH84 amyloid fibril growth. Below the critical concentration cc, PTH84 is monomeric and no fibril formation occurs. Above the cc low-order oligomers or, when exceeding a threshold concentration, coc, high-order oligomers of PTH84 contribute to the formation of primary fibril nuclei. Both oligomer species are on-pathway within the fibrillation network. The equilibrium decreases the concentration of available monomers (apparent c0), negatively affecting elongation and secondary nucleation as the processes requiring monomers (indicated by red arrows).

References
[1] Sachan, S., Moya, C.G., Voigt, B., Köhn, M., Balbach, J. The *pro*-sequence of parathyroid hormone prevents premature amyloid fibril formation. *FEBS Lett.***597**, 995-1006 (2023).
[2] Voigt, B., Bhatia, T., Hesselbarth, J., Baumann, M., Schmidt, C., Ott, M., Balbach, J. The Prenucleation Equilibrium of the Parathyroid Hormone Determines the Critical Aggregation Concentration and Amyloid Fibril Nucleation. *ChemPhysChem***24**, e202300439 (2023).
[3] Voigt, B., Ott, M., Balbach, J. A Competition of Secondary and Primary Nucleation Controls Amyloid Fibril Formation of the Parathyroid Hormone. *Macromol. Biosci.***23**, 2200525 (2023).

PS-VII-02

Assessing the material properties of condensates using pulsed electric fields

Elias Sabri, Agustin Mangiarotti, Rumiana Dimova

Max Planck Institute of Colloids and Interfaces, Sustainable Bio-Materials, Potsdam, Germany

Introduction

Condensates are molecular assemblies formed through liquid-liquid phase separation. In cells, biomolecular condensates are membraneless organelles that participate in many physiochemical processes[1] and exhibit different properties than their surroundings (e.g. viscoelasticity and surface properties) which reflect the specificity of their physiological function[2]. Yet, even in vitro, assessing the material properties of condensates is a challenging task and involves several experimental steps[3] or sophisticated equipment[4].

Methods

The presented approach relies on the tendency of spherical condensates to deform and adopt an ellipsoidal shape when exposed to electric fields. We use digital imaging to monitor the deformation dynamics of various macromolecular, polypeptide and protein-rich condensates under millisecond electric pulses. We estimate their viscosity and interfacial tension by comparing their deformation with the prediction of a well-established theoretical framework[5].

Results

We find agreement between condensate deformation and the prediction of the theoretical model. We further demonstrate with FRAP measurements that the exposure to electric pulses does not alter the mechanical properties of condensates.

Conclusions

We present a novel simple method for measuring condensate viscosity and surface tension simultaneously. Quantitative agreement between our results and reported data for similar systems demonstrate the method reproducibility and reliability.

References

[1] Ye S, Latham AP, Tang Y, Hsiung CH, Chen J, Luo F, Liu Y, and Zhang X 2024 'Micropolarity governs the structural organization of biomolecular condensates.' *Nature chemical biology*, *20*(4), 443-451.

[2] Dai Y, Chamberlayne CF, Messina MS, Chang CJ, Zare RN, You L, and Chilkoti A, 2023, 'Interface of biomolecular condensates modulates redox reactions.' *Chem*, *9*(6), 1594-1609.

[3] Mangiarotti A, Siri M, Tam NW, Zhao Z, Malacrida L, and Dimova R, 2023, 'Biomolecular condensates modulate membrane lipid packing and hydration.' *Nature Communications*, *14*(1), 6081.

[4] Wang H, Kelley FM, Milovanovic D, Schuster BS, and Shi Z, 2021 'Surface tension and viscosity of protein condensates quantified by micropipette aspiration.' *Biophysical Reports*, *1*(1).

[5] Vlahovska PM, 2019 'Electrohydrodynamics of drops and vesicles.' *Annual Review of Fluid Mechanics*, *51*, 305-330.

PS-VII-03

Integrating biophysical dynamics and mechanotransduction in 3D cell culture systems for in-depth understanding of rheumatoid arthritis

Federico Colombo, Mohammadreza Taale, Fereydoon Taheri, Malin Schmidt, Christine Selhuber-Unkel

University of Heidelberg, Institute for Molecular Systems Engineering and Advanced Materials (IMSEAM), Heidelberg, Germany

Introduction

Recent advancements in 3D cell culture systems have revolutionized our understanding of cellular behavior in pathological states such as Rheumatoid Arthritis (RA), a chronic autoimmune disease of the joints. Notably, the extracellular matrix (ECM) and the co-culture between synovial fibroblast and endothelial cells within these models offer critical insights into fundamental biological processes by closely mimicking in vivo conditions, underscoring the influence of the 3D environment and intercellular interactions[1].

Methods

We employed complementary methods to evaluate the stiffness, total mass, and dry mass of synovial fibroblasts within 3D cell cultures, with and without endothelial cells. Immunostainings were used to correlate specific cell markers with biophysical phenotypes. Additionally, we developed a device using Two-Photon Polymerization (2PP) to mechanically stimulate 3D multicellular systems, enabling the study of mechanotransduction with high precision[2].

Results

Our results indicate that fibroblasts in 3D cultures exhibit decreased stiffness and smaller sizes compared to 2D cultures, with a concurrent reduction in mass and an increase in dry mass. The cyclic mechanical stretching of 3D cultures revealed significant alterations in actin morphology and orientation.

Conclusions

Collectively, our findings elucidate the biophysical dynamics of 3D cell cultures and the critical role of mechanical forces and cellular interactions in these environments.

References

[1] K. Wei, I. Korsunsky, J. L. Marshall, A. Gao, G. F. M. Watts, T. Major, A. P. Croft, J. Watts, P. E. Blazar, J. K. Lange, T. S. Thornhill, A. Filer, K. Raza, L. T. Donlin, C. W. Siebel, C. D. Buckley, S. Raychaudhuri, M. B. Brenner, *Nature* **2020**, *582*, 259

[2] F. Colombo, M. Taale, F. Taheri, M. Villiou, T. Debatin, G. Dulatahu, P. Kollenz, M. Schmidt, C. Schlagheck, J. Wittbrodt, C. Selhuber-Unkel, *Adv. Funct. Mater.* **2024**.

PS-VII-04

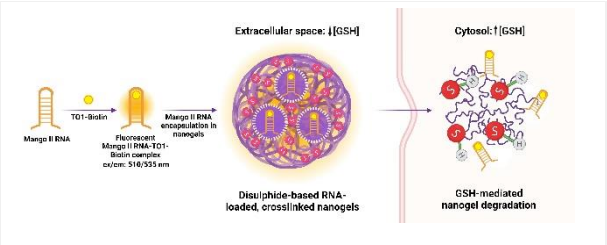
Redox-responsive nanogels for enhanced fluorogenic RNA aptamer delivery

Rupali Dabas[2,4], Haruki Iino[1], Saidjalolov Saidbakhrom[5], Stefan Matile[5], David Carling[2], David Rueda[1,3], Nazila Kamaly[4]

[1] Imperial College London, Single Molecule Imaging Group, MRC Laboratory of Medical Sciences, London, UK; [2] Imperial College London, Cellular Stress Research Group, MRC Laboratory of Medical Sciences, London, UK; [3] Imperial College London, Section of Virology, Department of Infectious Disease, London, UK; [4] Imperial College London, Department of Chemistry, Molecular Sciences Research Hub, London, UK; [5] University of Geneva, Department of Organic Chemistry, Geneva, Switzerland

We thank the MRC-LMS for support. We thank Jun.-Prof. Dr. rer. nat. Jana Eisermann and Dr Naveenan Navaratnam for helpful discussions. We also thank Dilraj Matharu in the Microscopy Facility at the MRC-LMS and Dr Ricardo Aramayo in the electron microscopy (EM) facility at the MRC-LMS. Prof. Stefan Matile thanks the Swiss NSF for financial support (Swiss-ERC Advanced Grant TIMEUP, TMAG-2_209190; Excellence Grant 200020 204175). The Rueda lab is supported by a core grant of the MRC-LMS (UKRIMC-A658-5TY10).

Fluorogenic RNA aptamers, like Mango II RNA, have immense potential for visualising the subcellular localization of diverse RNA types.[1-3] However, their functionality relies on efficient intracellular delivery. Conventional RNA delivery strategies, including lipid nanoparticles, face complex synthesis and insufficient cellular uptake. In contrast, polymeric nanocarriers exhibit considerable chemical versatility, (e.g. conjugating multiple functional groups onto the same polymer backbone), thereby endowing desirable properties. *Nanogels*, a class of polymeric nanoparticles, possess extensive cargo-loading capacities and biocompatibility.[4, 5] We encapsulate *Mango RNA aptamers within disulphide-bearing nanogels*, that can exploit the [glutathione (GSH)] gradient between extracellular (2-20 µM) and intracellular (1-10 mM) environments to enable cytosolic RNA delivery. We show RNA release in response to intracellular (83.3%) compared to extracellular (33.7%) [GSH], thereby emphasising the precise spatiotemporal control over RNA delivery. Moreover, our formulation was ~3.5-fold more effective than commercial reagents in transfection efficiency and maintained RNA integrity for up to 24 h *in vitro*. Furthermore, we evaluate the endolysosomal escape of these nanocarriers, elucidating the cellular delivery mechanism, for the first time. Our redox-responsive nanogels offer a unique strategy for Mango RNA delivery, extending opportunities for intracellular RNA detection and monitoring.



Disulfide-based nanogels mediate intracellular delivery of fluorogenic Mango II RNA aptamers. Figure highlights the encapsulation of fluorogenic Mango II RNA aptamers within redox-responsive polymeric nanogels that degrade at intracellular concentrations of the reducing agent glutathione (GSH), thereby releasing the RNA payload.

References

[1] Autour, A, Jeng S.C.Y., Cawte, A.D., Abdolahzadeh, A., Panchapakesan, S.S.S, Rueda, D., Ryckelynck, M., Unrau, P.J. 2018, 'Fluorogenic RNA Mango aptamers for imaging small non-coding RNAs in mammalian cells', *Nature Communications.*, 9, 656, United Kingdom: Nature
[2] Cawte, A.D., Unrau, P.J., David, R.S. 2020, 'Live cell imaging of single RNA molecules with fluorogenic Mango II arrays', *Nature Communications*, 11, 1283, United Kingdom: Nature
[3] Cawte, A.D., Haruki, I., Unrau, P.J., Rueda, R.S. 2022, 'Single-Molecule RNA Imaging Using Mango II Arrays', *Post-Transcriptional Gene Regulation*, 2404, 267-280, New York: Springer
[4] Kamaly, N., Yameen, B., Wu, J., Farokhzad, O.C. 2016, 'Degradable Controlled-Release Polymers and Polymeric Nanoparticles: Mechanisms of Controlling Drug Release', *Chemical Reviews*, 116, 2602-2663, Boston, Massachusetts: American Chemical Society
[5] Soni, S.K., Desale, S.S., Bronich, T.K., 2016, 'Nanogels: An overview of properties, biomedical applications and obstacles to clinical translation', *Journal of Controlled Release*, 240, 109-126, New England: Elsevier

12:45 pm – 1:15 pm

HS 9

PL-V | Plenary V

Andreas Janshoff

PL-V-01

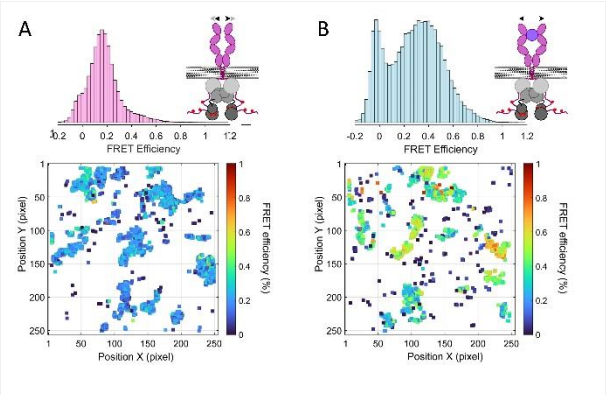
Resolving the dynamics of signaling complexes across spatial and temporal scales

Jacob Piehler

Osnabrück University, Department of Biology/Chemistry and Center for Cellular Nanoanalytics, Osnabrück, Germany

Funding by the DFG (SFB 944, P8 and Z, SFB 1557, P13 and PI 405/15) is gratefully acknowledged.

Signal transduction across the plasma membrane via cytokine receptors requires assembly of signaling complexes from two or more receptor subunits. We have demonstrated the relevance of fine-tuned molecular interactions for signaling specificity and for oncogenic mutations and devised multicolor single-molecule (SM) imaging techniques for probing receptor assembly in the plasma membrane of live cells. Based on engineered nanobodies, efficient cell surface-selective fluorescence labeling of cytokine receptors at physiological expression levels was achieved. We applied SM co-tracking and smFRET to quantify interactions in the plasma membrane of live cells. We identified ligand-induced receptor dimerization as a fundamental principle of class I receptor activation. However, formation of active signaling complexes also involve subtle protein-protein and protein lipid interactions encoded in the transmembrane and cytosolic receptor domains and the associated Janus family kinase (JAK). These interaction sites appear to be evolved for low affinity as we found that oncogenic mutations in these interaction sites enhance ligand-independent receptor dimerization. Time-lapse monitoring of receptor dimers by smFRET enabled quantifying complex lifetimes even for very transiently interacting variants and thus assessment of the dimerization kinetics in the cellular context. We propose a multistep kinetic model explaining activation and dysregulation of class I cytokine receptor signaling.



Conformational dynamics by live-cell smFRET
FRET efficiencies obtained for TpoR dimers formed by the ligand Tpo (A) and by the oncogenic JAK2 mutation V617F (B). Comparision of smFRET efficiency histograms (top) and smFRET efficiency trajectories on the cell surface (bottom).

References
[1] Wilmes S, et al. 2020 Mechanism of homodimeric cytokine receptor activation and dysregulation by oncogenic mutations. *Science* 367(6478):643-652.
[2] Sotolongo Bellon J, et al. 2022. Four-color single-molecule imaging with engineered tags resolves the molecular architecture of signaling complexes in the plasma membrane. *Cell Rep Methods* 2(2):100165.
[3] Tsutsumi N, et al., 2023. Structure of the thrombopoietin-MPL receptor complex is a blueprint for biasing hematopoiesis. *Cell* 186(19):4189-4203 e4122.
[4] Winkelmann H, et al., 2024. Correlative single-molecule and structured illumination microscopy of fast dynamics at the plasma membrane. *Nat Commun* 15(1):5813.

1:45 pm – 2:15 pm

HS 9

PS-Fl-II | Posterflashes II

PS-FI-II-02

Regulation of helicase activity in long-range DNA end resection

Martin Mütze[1], Ilaria Ceppi[2], Maria Rosaria Dello Stritto[2], Stefan Braunshier[2], Valentina Mengoli[2], Giordano Reginato[2], Ananya Acharya[2], Megha Roy[2], Aurore Sanchez[2], Swagata Halder[2], Sean Michael Howard[2], Ralf Seidel[1], Petr Cejka[2]

[1] Universität Leipzig, Peter-Debye-Institut für Physik der weichen Materie/ Group Molecular Biophysics, Leipzig, Germany; [2] Università della Svizzera italiana (USI), Faculty of Biomedical Sciences/ Institute for Research in Biomedicine, Bellinzona, Switzerland

Introduction

In the process of repairing DNA double-strand breaks (DSB) through homologous recombination, a crucial step involves DNA end resection, where the 5'-terminated strands at DSB sites undergo controlled degradation. This process is separated in two distinct steps, short-range and long-range DNA end resection. Both involve the interplay of different proteins working together to create the single-stranded DNA overhangs. For our research we focus on the long-range DNA end resection and its regulation through different proteins, especially the tumor suppressor associated with breast cancer BRCA1-BARD1.

Methods

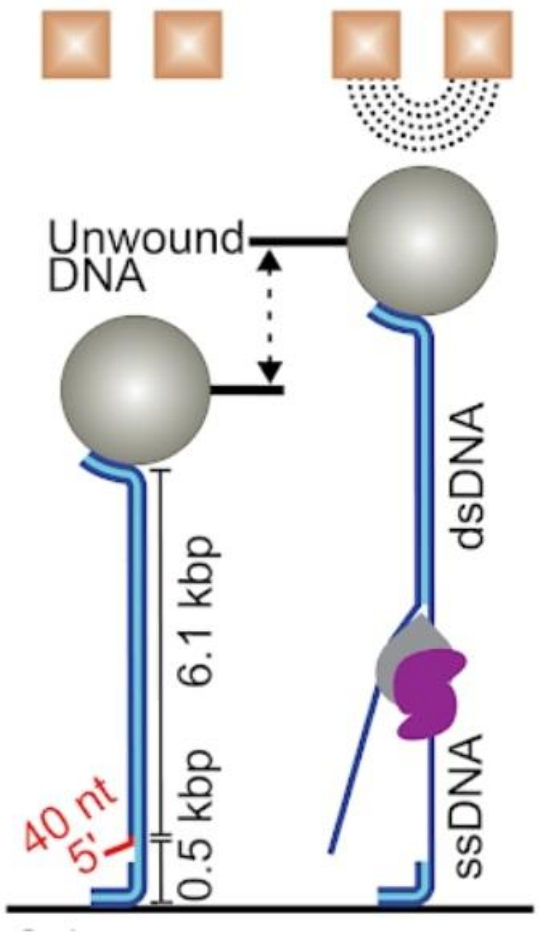
We employed purified recombinant proteins and utilized a combination of bulk-essay data and single molecule measurements conducted with magnetic tweezers.

Results

Our research demonstrates that BRCA1-BARD1 directly facilitates long-range DNA end resection. Specifically, in the DNA2-dependent pathway, BRCA1-BARD1 stimulates DNA unwinding through the WRN/BLM RecQ family helicase. We also found that phosphorylated CtIP drastically stimulates the motor activity of DNA2, and that the MRN-protein complex, normally associated to short-range DNA end resection, also stimulates the motor function of the RecQ helicases.

Conclusions

Our findings show that more proteins are integral to long-range DNA end resection, and it is our hypothesis that the stimulation of proteins through specific interaction partners makes the whole process more robust in vivo.



Investigating Helicase Activity with Magnetic Tweezers

A schematic of the employed single-molecule magnetic tweezers assay setup and the DNA substrate specifically designed to investigate long-range DNA end resection. The helicase complex binds to the 5' overhang and from there starts its motor function, converting double stranded DNA into single stranded DNA. As a result, the length of the DNA substrate increases which can be directly tracked in our setup.

PS-FI-II-04

Intertwined dimer of human PTK6 SH3 domain mediates kinase activity?

Lisa Kupsch, Luisa Buhl, Dilara Oeguetcue, Ines Liebscher, **Sandra Berndt**

University of Leipzig, RSI of Biochemistry, Leipzig, Germany

Introduction

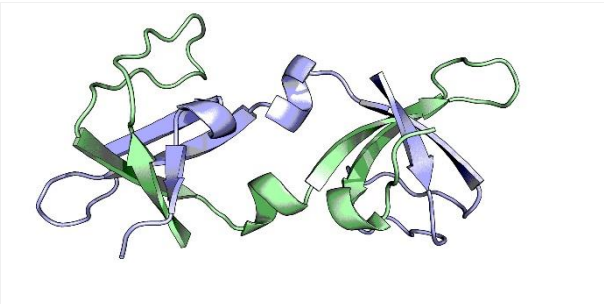
Protein tyrosine kinase 6 (PTK6, or breast tumor kinase, Brk) is abundant in multiple tumor types like breast and prostate cancer [1,2]. PTK6 expression levels correlate with tumor grade and invasiveness. There is only limited structural and functional data of PTK6 available. PTK6 is closely related to Src family kinases, containing functional domains (SH3, SH2, and kinase domain) and mediates regulation of multiple signaling pathways through Src kinase, MAPK p38 and ERK 5 activation [3,4]. However, the activation mechanism is not well understood.

Methods

X-ray crystallography, analytical gel filtration, NanoBiT complementation assay, docking

Results

Here we present a novel intertwined dimer crystal structure of the human PTK6 SH3 domain at 1.75 Å. The dimerization process results in a domain swap architecture, which is a result of a distinctive dimerization mechanism. Comparable dimers were reported for kinases like Src kinase, Eps8, and Nck1. Hence, the functional role of this dimerization process is still to be determined. So far, we found that PTK6 SH3 domain dimerization is inducible *in vitro*. Furthermore, we observed the dimerization *in cells* using multiple cell lines. Applying various PTK6 constructs, we found that the dimerization is SH3 dependent and that multiple amino acids enhance or diminish the dimer formation. A detailed understanding of the dimerization driven PTK6 activation mechanism may allow new a drug targeting approach of this kinase.



Intertwined dimer of human PTK6 SH3 domain
PTK6 SH3 domain crystal structure with an atomic resolution of 1.75Å. The protein was expressed in E. coli and purified using affinity as well as size exclusion chromatography.

The structure model of the dimer was refined to an Rwork of 19.4 (Rfree of 21.9). The overall architecture of the intertwined PTK6 dimer is nearly identical to the monomer, with a RMSD value of 1,007.

References

[1] Ang, HL, Yuan, Y 2021, 'Putting the BRK on breast cancer: From molecular target to therapeutics.', *Theranostics*, 11 (3), 1115-1128.

[2] Alwanian, WM, Tyner, AL 2020, 'Protein tyrosine kinase 6 signaling in prostate cancer', *American journal of clinical and experimental urology*, 8 (1), 1-8.

[3] Locatelli, A, Lofgren, KA, 2012, 'Mechanisms of HGF/Met signaling to Brk and Sam68 in breast cancer progression', *Hormones & cancer*, 3 (1-2), 14-25.

[4] Alwanian, WM, Vlajic, K, 2022, 'Protein tyrosine kinase 6 regulates activation of SRC kinase', *The Journal of biological chemistry*, 298 (11), 102584.

PS-FI-II-06

Standardized evaluation of AMP liposome permeabilization in microfluidic setups

Janina Nandy[1], Marie Wetzel[1], Marcus Fletcher[2], Kareem al Nahas[3], Niels Röckendorf[4], Thomas Gutsmann[1], **Christian Nehls**[1]

[1] Research Center Borstel, Division of Biophysics, Borstel, Germany; [2] Imperial College London, Department of Chemical Engineering, London, UK; [3] Max Planck Institute of Biochemistry, Cellular and Molecular Biophysics, Martinsried, Germany; [4] Research Center Borstel, Division of Mucosal Immunology and Diagnostic, Borstel, Germany

This work was funded by the Phospholipid Research Center, grant number CNE-2022-104/1-1, and financially supported by the Deutsche Forschungsgemeinschaf SPP2225. We are grateful for excellent technical support by Sabrina Groth, Kerstin Stephan and Franziska Kiesow.

Introduction

Antimicrobial resistance is one of the major challenges of the 21st century. There is an urgent need on the one hand for new alternative compounds and on the other hand for platforms for compound identification and analysis.

Methods

In our project, we characterize and compare the membrane permeabilization of a set of antimicrobial peptides (AMPs) that were optimized by a genetic algorithm. As a model system, we use microfluidically generated liposomes, which we trap individually in microfluidic devices [1]. This allows us to monitor the individual permeabilization of hundreds of liposomes simultaneously.

Results

With this approach, we a) evaluate the permeabilizing efficacy of AMPs, b) classify the mode of action of AMPs in combination with other methods, and c) develop a microfluidic platform that allows standardized and routine compound testing. Therefore, we compare our results with microbiologically determined fitness values of AMPs.

Conclusions

We demonstrate the potential of individual liposome trapping for standardized compound testing and add a further piece to the puzzle of developing AMPs as a promising alternative to conventional antibiotics in the fight against AMR.

References

[1] Al Nahas, K, et al. 2022, 'Measuring thousands of single-vesicle leakage events reveals the mode of action of antimicrobial peptides', *Analytical Chemistry*, 94(27), 9530-9539, ACS Publications

PS-FI-II-08

Chondrotin and dermatan sulfates: how small difference lead to huge consequences

Marta Pągielska, Annemarie Danielsson, Sergey Samsonov

University of Gdańsk, Faculty of Chemistry, Gdańsk, Poland

This work was funded by the National Science Centre (grant number UMO-2023/49/B/ST4/00041).

Glycosaminoglycans (GAGs) represent a class of linear anionic periodic polysaccharides which can be sulfated in different positions. The amount (net sulfation) and particular positions of the sulfation group (sulfation pattern) constitute *"sulfation code"*, which determines GAG structure and function. Our study is focused on deciphering the "sulfation code" of chondroitin sulfate (CS) and dermatan sulfate (DS). The only difference between CS and DS is the epimerization of uronic acid unit – GlcA is present in CS while IdoA in DS. Utilizing atomistic molecular dynamics simulations, we analyzed structural, conformational and dynamic properties of these GAGs in several oligomeric forms. We revealed substantial differences in molecular descriptors such as RMSD, molecular volume, glycosidic linkages and hydrogen bonding propensities. These results highlighted the impact of the sulfation position in CS and the type of uronic acid on the properties of these GAGs [1]. We extended our studies to explore the interactions of these GAGs with proteins and determine how the observed structural and dynamic differences in CS and DS affect their binding poses and affinities. Such benchmarking analysis was conducted for the entire family of cysteine cathepsins. These proteins are human proteases which activity is known to be regulated by GAGs. Our work provides valuable insights into the GAGs "sulfation code" which could be exploited in the rational development of GAG-based drugs and therapies.

References

[1] Pągielska, M.; Samsonov, SA. 2023, 'Molecular Dynamics-Based Comparative Analysis of Chondroitin and Dermatan Sulfates'. *Biomolecules*,13, 247.

PS-FI-II-10

PI3K SH3 domain - amyloid model protein (un)folding investigated by NMR

Melinda Jaspert[1,2], Luis Gardon[1,2], Leonardo Levorin[1,2], Lothar Gremer[1,2], Nils-Alexander Lakomek[1,2], Henrike Heise[1,2]

[1] Heinrich-Heine-Univeristät, Institute of Physical Biology, Düsseldorf, Germany; [2] Forschungszentrum Jülich, Institute of Biological Information Processing, Jülich, Germany

Introduction

The misfolding and aggregation of proteins is associated with several neurodegenerative diseases. In order to understand the underlying processes, the PI3K SH3 domain is used as a model system. At acidic pH the well folded native structure [1] of the protein unfolds and aggregates into well-folded fibrils. The structure of these PI3K SH3 fibrils was determined recently using cryo-EM [2].

Methods

We use diverse NMR experiments to get insights into the unfolding process of the PI3K SH3 domain itself and its dependencies. Therefor we apply solution-state NMR experiments to obtain information about the secondary structure propensities and the dynamics of the investigated protein to follow the unfolding process with particular regard to its temperature dependency at residue-type resolution.

Results

At acidic pH and lower temperatures an equilibrium between two conformations is present, that upon rising the temperature shifts towards the unfolded conformation. Our developed assignment and the secondary chemical shift analyses of the PI3K SH3 domain at acidic pH and high temperature indicate that the molecule indeed is unfolded under these conditions.

Conclusions

This provides a basis for further examinations of the double conformation state, which will be separated with recently performed experiments, as well as a starting point for ultrafast-MAS-measurements using proton detection, to gain insights into the complexity of the (un)folding process of the PI3K SH3 domain.

References
[1] Booker, G. W. et al. *Cell* **1993**, 73 (4), 813.
[2] Röder, C. et al. *Nature Commun.* **2019**, 10 (1), 3754.

PS-FI-II-12

Crucial role of glycan conformations in the catalytic reaction of CAZymes

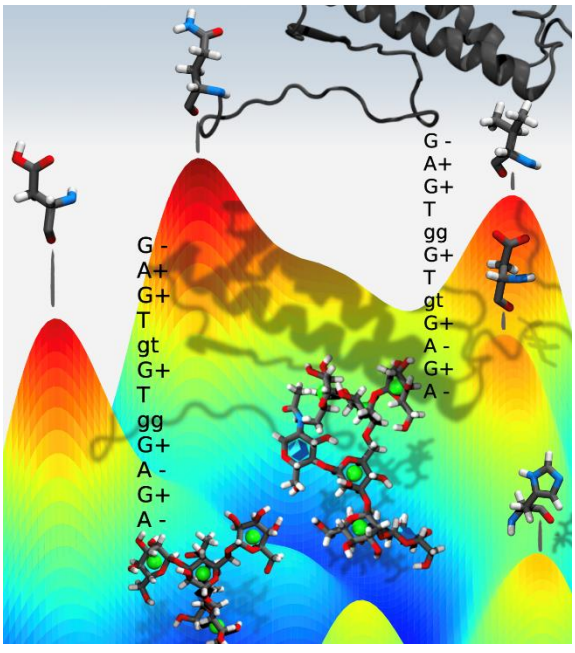
Isabell L. Grothaus[1,2], Lucio Colombi Ciacchi[1]

[1] University of Bremen, Faculty of Production Engineering, Bremen, Germany; [2] Jagiellonian University, Malopolska Centre of Biotechnology, Krakow, Poland

Structural biology has traditionally focused on protein folding, complex formation, and ligand binding. However, the structural elucidation of glycans, which are frequent post-translational modifications on protein surfaces, remains largely unexplored. While still little is known about their function, even less is understood about their structural behavior, especially because state-of-the-art experiments can hardly resolve glycan flexibility. This is to the disadvantage of understanding the synthesis of glycans in the cytoplasm, involving several carbohydrate-active enzymes (CAZymes) whose malfunction is linked to cancer and disease progression [1].

Therefore, we employ molecular dynamics simulation to investigate the correlation between adopted glycan conformers in CAZyme binding sites and the reduction of kinetic barriers for glycan hydrolysis. Our focus is on alpha Golgi-mannosidase 2, a promising but underexplored drug target [2]. Massive enhanced sampling molecular dynamics simulations of the enzyme/glycan complex reveal [3]:

1. a direct correlation between torsion angle settings and monosaccharide ring distortion, often necessary for making hydrolytic reactions energetically feasible.
2. a disruption of this correlation induced by key mutations in the catalytic site, leading to experimentally observed loss of CAZyme function.
3. the ring distortion to be induced by binding of the glycan to the catalytic site of the protein and not adopted prior to substrate binding.



The artistic phase space of an enzymatically bound N-glycan
The high-mannose type N-glycan M5G0 is restricted in its conformational phase space through the surrounding amino acids in the catalytic site. The presence of the protein is shading and altering the free energy landscape. Each conformer is flagged by corresponding conformer labels.

References

[1] Goss, P. E., Reid, C. L., Bailey, D. & Dennis, J. W. Phase IB clinical trial of the oligosaccharide processing inhibitor swainsonine in patients with advanced malignancies. *Clin Cancer Res Official J Am Assoc Cancer Res* **3**, 1077–86 (1997).

[2] Lee, Z. Y., Loo, J. S. E., Wibowo, A., Mohammat, M. F. & Foo, J. B. Targeting Cancer via Golgi α-mannosidase II Inhibition: How Far Have We Come In Developing Effective Inhibitors? *Carbohyd Res* **508**, 108395 (2021).

[3] Grothaus, I. L., Bussi, G. & Ciacchi, L. C. Exploration, Representation, and Rationalization of the Conformational Phase Space of N-Glycans. *J Chem Inf Model* **62**, 4992–5008 (2022).

PS-FI-II-14

Structural basis for the transmembrane signaling of Trk receptors and antidepressant-induced activation of the receptor tyrosine kinase TrkB

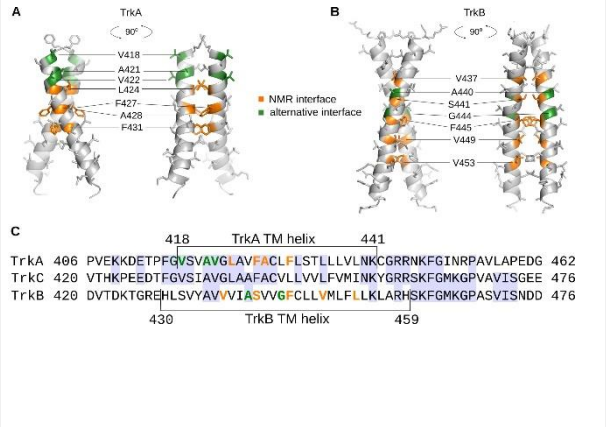
Erik Kot[2], Maria L. Franco[3], Kirill Nadezhdin[5], Daniel McKenzie[4], Kalina Hristova[4], Sergey Goncharuk[2], Marcal Vilar[3], **Konstantin Mineev**[1]

[1] Goethe University Frankfurt, Institute of Organic Chemistry and Chemical Biology, Frankfurt am Main, Germany; [2] Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russia; [3] Instituto de Biomedicina de Valencia-CSIC, Valencia, Spain; [4] Johns Hopkins University, Baltimore, USA; [5] Columbia University, New York, USA

The work of K. Mineev was supported by funding in the group of prof. H. Schwalbe, a project within the Collaborative Research Center #1507.

Neurotrophin receptors of the Trk family play a critical role in regulating brain development and neuroplasticity, making them valuable targets for drugs aimed at treating cancer, stroke, depression, and other conditions. The structures of Trk protein domains in various states upon activation must be elucidated to allow rational drug design. Despite this need, the conformations of the transmembrane and juxtamembrane domains of Trk receptors remained largely unexplored.

In this study, we employed solution NMR spectroscopy to determine the structures of the dimeric transmembrane domains of TrkA and TrkB receptors within a lipid environment. These structures were validated through mutagenesis, functional assays, and fluorescent microscopy, confirming that the identified conformations correspond to the inactive and active states of the receptors, respectively. An alternative active state of TrkA was proposed based on cross-linking experiments. The investigation of TrkA extracellular juxtamembrane regions revealed that they are unstructured and are likely to interact with the neurotrophin in the activated receptor state. Further studies on TrkB interaction with the antidepressant fluoxetine, LSD, and antipsychotic chlorpromazine led to a coherent model describing how fluoxetine activates the receptor by binding to its transmembrane domain.



Comparison of transmembrane domain dimer conformations within the Trk family
A,B - the spatial structures of TrkA (A) and TrkB (B) TM dimers obtained by NMR in DPC micelles and DMPC/DHPC bicelles, respectively. Orange color denotes the residues on the experimentally determined interface. Green color indicates the residues proposed for the alternative dimerization interfaces, assumed from the cross-linking experiments (A) or computer modeling (B) **C** - Sequence alignment of Trk family members based on the Clustal Omega program (http://www.clustal.org/) results for alignment of the transmembrane and intracellular domains.

PS-FI-II-16

cAMP binding to resting pacemaker ion channels is cooperative – a single-molecule study

Stefan Kuschke, Susanne Thon, Christian Sattler, Tina Schwabe, Klaus Benndorf, **Ralf Schmauder**

University Hospital Jena, Friedrich Schiller University, Institute of Physiology II, Jena, Germany

Activation of oligomeric receptors is often fine-tune by cooperativity between subunits. For voltage activated HCN channels, cAMP was shown to regulate channel activation, featuring an intricate cooperativity in binding and activation[1]. These HCN channels generate electric rhythmicity in specialized neurons and cardiomyocytes. Conflicting evidence whether cAMP binding to resting channels is also cooperative was reported previously[2,3].
Here show that cooperativity in ligand binding precedes voltage activation. For this we follow the binding of individual fluorescently labeled cAMP to single, resting, GFP-labeled HCN2 channels in HEK293 derived native membrane sheets using TIRF microscopy. A custom inhibitor protocol was used to block signals from binding to endogenous cAMP-affinities[4].
Kinetic modeling revealed a flip state in both liganded and apo-state. Additionaly, both binding-affinity and flip-state occupation is found to increase with ligand occupancy. The contrast of these finding[4] and previous reports[3] suggest that native membrane environment is required for the observed binding cooperativity. Our data proves that single molecule binding measurements at equilibrium are possible in such membranes.

References

[1] Kusch, J, Thon, S, et al. 2011 “How subunits cooperate in cAMP-induced activation of homotetrameric HCN2 channels.”, *Nature chemical biology* vol. 8,2 162-9. 18 Dec. 2011

[2] Thon, S, Schmauder R, et al. “Conformational Flip of Nonactivated HCN2 Channel Subunits Evoked by Cyclic Nucleotides.” *Biophysical journal* vol. 109,11 (2015): 2268-76.

[3] White, DS, et al. “cAMP binding to closed pacemaker ion channels is non-cooperative.” *Nature* vol. 595,7868 (2021): 606-610.

[4] Kuschke, S, et al. “cAMP binding to closed pacemaker ion channels is cooperative.” *PNAS,* vol. 121,9 (2024): e2315132121.

PS-FI-II-18

Structural stability of the integron synaptic complex

Ekaterina Vorobevskaia[1], Céline Loot[2], Didier Mazel[2], Michael Schlierf[1,3]

[1] TU Dresden, B CUBE Center for Molecular Bioengineering, Dresden, Germany; [2] Institut Pasteur, Université Paris Cité, CNRS UMR3525, Unité Plasticité du Génome Bactérien, Paris, France; [3] TU Dresden, Physics of Life, DFG Cluster of Excellence, Dresden, Germany

We thank all members of the Schlierf lab for lively discussions during the development of this project and in particular César Augusto Quintana-Cantaño for inspiring discussions and Dr. Yujun Zhang for supporting protein mutagenesis and production.

Introduction

The predominant tool for adaptation in Gram-negative bacteria is a genetic system called integron. It rearranges gene cassettes, promoting multiple antibiotic resistances, a recognized major global health threat. It is based on a unique recombination process involving a tyrosine recombinase - integrase IntI - and folded ssDNA hairpins - called *attC* sites. Four recombinases and two *attC* sites form a macromolecular synaptic complex, which is key to the recombination process and the focus of our study. The bottom strand of all *attC* sites shows highest recombination *in vivo*, however, the recombination efficiency still varies five orders of magnitude and the underlying reason remains unknown.

Methods

Here, we established an optical tweezers force-spectroscopy assay that allows to probe the synaptic complex stability. We investigated seven combinations of *attC* sites and two protein variants and determined their *in vivo* recombination efficiencies.

Results

We discovered a strong correlation between recombination efficiency and mechanical stability of the synaptic complex, revealing a new regulatory mechanism of the bacterial integron, mediated through the synaptic complex. Furthermore, we discovered a key structural region of the IntI that greatly destabilizes the formation upon mutation.We designed and probed a competing molecule that could actively destabilize a given synaptic complex, opening a new avenue to reduce the spread of antibiotic resistances among bacteria.

PS-FI-II-20

Exploring heme-enzyme tunability with resonance and surface-enhanced resonance Raman (SERR) spectro-electrochemistry

Patrycja Kielb[1,2], Michelle Mahler[1,2]

[1] University of Bonn, Clausius Institute of Physical and Theoretical Chemistry, Bonn, Germany; [2] University of Bonn, Transdisciplinary Research Area 'Building Blocks of Matter and Fundamental Interactions', Bonn, Germany

The development of artificial metalloproteins designed to catalyze new types of transformations presents a promising avenue in biocatalysis, holding significant potential for technological or medical applications. Enzymes utilizing heme as an active site are particularly attractive due to inherent structural and redox versatility, allowing them for a broad spectrum of small molecule activation. Here, we examine how such structural changes of the heme coordination correlate with the redox properties of the active site and, as such, serve as a parameter governing the biocatalyst catalysis towards specific reactions. Specifically, we explore the redox tuning of Hexameric Tyr-coordinated Heme Protein (HTHP)[1,2] by tailored axial coordination modifications of the heme cofactor. In addition to protein engineering, we employ a combination of electrochemistry with surface-enhanced resonance Raman (SERR) spectroscopy to selectively monitor the vibrational fingerprint of a monolayer of chromophore-containing sample under the control of applied potential.

References

[1] Kielb, P, et al. ‘Switchable redox chemistry of the hexameric tyrosine-coordinated heme protein’, *Journal of Physical Chemistry B*, 121 (16), 3955 - 3964.

[2] Mahler, M and Kielb, P. 'Redox tuning of heme active sites studied by Raman spectro-electrochemistry', *in preparation*

2:45 pm – 3:45 pm

Foyer HSG

Poster-II | Poster Session II

PS-I-02

Sarcomere self-assembly and sarcomere addition

Francine Kolley[1], Clara Sidor[2], Ian D. Estabrook[1,3], Clement Rodier[2], Frank Schnorrer[2], **Benjamin M. Friedrich**[1]

[1] TU Dresden, EXC PoL, Dresden, Germany; [2] IBDM, Marseilles, France; [3] EMBL, Heidelberg, Germany

F.S. and B.M.F. acknowledge financial support from the Human Frontiers Science Program (HFSP, Grant No. RGP0052).

Introduction

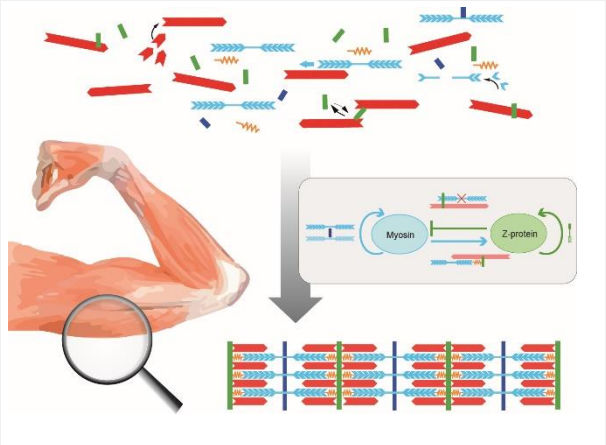
Voluntary motions and heartbeat in animals is driven by contractions of myofibrils, millimeter-long acto-myosin bundles with characteristic periodic patterns of micrometer-sized sarcomere repeat units. The physical mechanisms driving the self-assembly of these “cytoskeletal crystals”, starting from initially unstriated stress-fiber like bundles, are poorly understood.

Methods

In a tight theory-experiment collaboration, we analyze fluorescence microscopy images of the growing insect flight muscle to build data-driven mathematical models.

Results & Conclusions

By analyzing the earliest stages of myofibrillogenesis, we show that myosin and Z-disc proteins establish periodic sarcomeric patterns first, while actin remains disordered and becomes polarity-sorted only hours later [1]. Based on this sequential ordering of sarcomeric components, we postulate a new model of sarcomere self-assembly that relies on mutual feedback between myosin and Z-disc proteins that bind and unbind to a disordered bundle of actin filaments. Agent-based simulations show how interactions between these spatially extended components and tension-dependent catch-bond behavior drive the spontaneous formation of periodic patterns. Second, we report a new, unpublished mechanism of controlled “self-rupture” of sarcomeres in which a mother sarcomere divides into two daughter sarcomeres by splitting its myosin stack, providing an efficient mechanism of sarcomere addition (Rodier et al. unpublished).



Mechanism of sarcomere self-assembly
We propose a new model of sarcomere self-assembly based on data: myosin filaments (blue) and Z-disc proteins (green) attach in an autocatalytic fashion to a disordered bundle of actin filaments (red) that serves as static scaffold. Steric hindrance, interactions via titin, and possibly tension-dependent catch-bonds drive the spontaneous formation of periodic sarcomeric patterns in agent-based simulations [1].

References
[1] Kolley, F, Sidor, C, Dehapiot B, Schnorrer, F, Friedrich, BM, Mechanisms of sarcomere assembly in muscle cells inferred from sequential ordering of myofibril components, *PRX Life*, **2**, 013002, 2024

PS-I-04

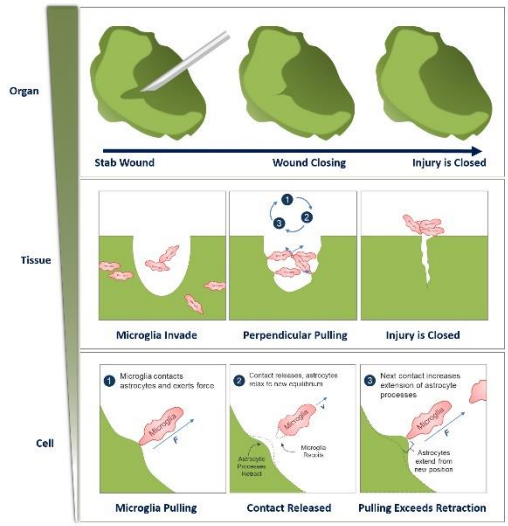
Wound closure after brain injury relies on force generation by microglia in zebrafish.

Francois El-Daher[1,2], Louisa K. Drake[1], Stephen J. Enos[2], Daniel Wehner[3,4], Markus Westphal[2], Nicola J. Porter[1], Catherina G. Becker[2,5], Thomas Becker[1,2]

[1] University of Edinburgh Medical School: Biomedical Sciences, Centre for Discovery Brain Sciences, Edinburgh, UK; [2] Technische Universität Dresden, Center for Regenerative Therapies Dresden, Dresden, Germany; [3] Max Planck Institute for the Science of Light, Erlangen, Germany; [4] Max-Planck-Zentrum für Physik und Medizin, Erlangen, Germany; [5] Technische Universität Dresden, Cluster of Excellence Physics of Life, Dresden, Germany

Funded by the BBSRC and an Alexander-von-Humboldt Professorship (to CGB).

Wound closure after a brain injury is critical for tissue restoration but this process is still not well characterized at the tissue level. We use live observation of wound closure in larval zebrafish after inflicting a stab wound to the brain. We demonstrate that the wound closes in the first 24 hours after injury by global tissue contraction. Microglia accumulation at the point of tissue convergence precedes wound closure and computational modelling of this process indicates that physical traction by microglia could lead to wound closure. Indeed, genetically or pharmacologically depleting microglia leads to defective tissue repair. Live observations indicate centripetal deformation of astrocytic processes contacted by migrating microglia. Severing such contacts leads to retraction of cellular processes, indicating tension. Weakening tension by disrupting the F-actin stabilising gene lcp1 in microglial cells, leads to failure of wound closure. Therefore, we propose a previously unidentified mechanism of brain repair in which microglia has an essential role in contracting spared tissue. Understanding the mechanical role of microglia will support advances in traumatic brain injury therapies.



Wound closure after brain injury relies on force generation by microglia in zebrafish.
We propose a brain repair mechanism in which mechanical forces in the early stages of tissue regeneration are crucial to contracting the tissue and thus facilitate subsequent repair processes. Our findings reveal a new role in the microglial response after an injury. These cells are indispensable for the mechanical action to close the wound.

References

[1] Zulazmi, N. A., Arulsamy, A., Ali, I., Zainal Abidin, S. A., Othman, I., and Shaikh, M. F. "The utilization of small non-mammals in traumatic brain injury research: A systematic review" *CNS Neuroscience & Therapeutics* 27(4), 381–402 (2021).
[2] A, Z. and J., N. "Regeneration of the central nervous system-principles from brain regeneration in adult zebrafish" *World J Stem Cells* 12(1) (2020).
[3] Bollmann, L., Koser, D. E., Shahapure, R., Gautier, H. O., Holzapfel, G. A., Scarcelli, G., Gather, M. C., Ulbricht, E., and Franze, K. "Microglia mechanics: immune activation alters traction forces and durotaxis" *Frontiers in Cellular Neuroscience* 9 (September), 1–16 (2015).
[4] So, W. Y., Johnson, B., Gordon, P. B., Bishop, K. S., Gong, H., Burr, H. A., Staunton, J. R., Handler, C., Sood, R., Scarcelli, G., and Tanner, K. "Macrophage mediated mesoscale brain mechanical homeostasis mechanically imaged via optical tweezers and Brillouin microscopy *in vivo*" *bioRxiv* (2024).
[5] Linehan, J. B., Lucas Zepeda, J., Mitchell, T. A., and LeClair, E. E. "Follow that cell: Leukocyte migration in L-plastin mutant zebrafish" *Cytoskeleton* 79(4-5), 26–37 (2022).

PS-I-06

Influence of transmembrane transport of amines on Insulin Secretory Granule luminal pH

Oleksandra Topcheva[1,2], Martin Neukam[3,2], Andreas Müller[1,2], Klaus Knoch[1,2], Juha Torkko[1,2], Mirko Peitzsch[5], Marcelo L. Zoccoler[4], Anke Sömnez[1,2], Carolin Eckert[1,2], Katharina Ganß[1,2], Carla Münster[1,2], Michele Solimena[1,2]

[1] Technische Universität Dresden, Faculty of Medicine, Dept. Molecular Diabetology, Dresden, Germany; [2] Paul Langerhans Institute Dresden (PLID) of Helmholtz München at the University Hospital and Faculty of Medicine of Technische Universität Dresden, Dresden, Germany; [3] Stanford University School of Medicine, Developmental Biology, Stanford, USA; [4] DFG Cluster of Excellence "Physics of Life", TU Dresden, Dresden, Germany; [5] Institute of Clinical Chemistry and Laboratory Medicine, University Hospital and Medical Faculty Carl Gustav Carus, TU Dresden, Dresden, Germany

Introduction

The lumen of insulin secretory granules (SGs) has a mildly acidic pH maintained by V-ATPase. Previously we revealed that the SG pH dynamically shifts from 5.5 to 6 as SGs age [1]. VMAT1 transports monoamine neurotransmitters such as serotonin into the lumen of SGs. Here, we investigated the potential influence of amine transmembrane transport on the luminal pH of the SGs.

Methods

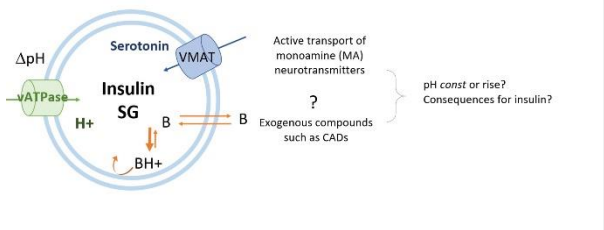
To measure the luminal pH of insulin SGs, we used an insulin SG-specific pH reporter (eCFP fused with RESP18HD) for fluorescence lifetime imaging microscopy (FLIM) in wild-type and *Slc18a1*$^{-/-}$ INS-1 cells. We evaluated the uptake of monoamines into SGs with the fluorescent probe FFN 206. The Serotonin content of INS-1 cells was measured by mass spectrometry.

Results

We found that VMAT1 partially localizes to insulin SGs. The accumulation of serotonin and FFN206 in SGs was abolished by CRISPR/Cas9 deletion of *Slc18a1 (*VMAT1). Moreover, while VMAT1 is not required for the acidification of SGs, we have preliminary evidence indicating that serotonin transport elevates their luminal pH.

Conclusions

In our study, we investigated the potential of insulin SG to accumulate both endogenous and exogenous compounds. We have collected first evidence on how certain pharmacological interventions may induce metabolic adverse effects through alterations in the SG luminal content and deepened our understanding of SG luminal dynamics, highlighting its potential influence on metabolic health.



Transport of organic solutes to insulin SG
Impact of monoamine accumulation on the luminal composition of insulin SG, with an emphasis on their pH.

References

[1] Martin Neukam, Anke Sönmez, Michele Solimena 2017, 'FLIM-based pH measurements reveal incretin-induced rejuvenation of aged insulin secretory granules', bioRxiv , 174391, doi: https://doi.org/10.1101/174391

PS-II-02

A molecular view into the neuroprotective effects of chlorogenic acids.

Mercedes Alfonso-Prieto[1], Nicolas P. Müller[1,2], Olga A. Sergeeva[3,4], Luciano Navarini[5], Paolo Carloni[1,6]

[1] Forschungszentrum Jülich, INM-9 Computational Biomedicine, Jülich, Germany; [2] Heinrich-Heine University Düsseldorf, Faculty of Mathematics and Natural Sciences, Düsseldorf, Germany; [3] Heinrich Heine University Düsseldorf, Institute of Neural and Sensory Physiology, Düsseldorf, Germany; [4] Heinrich Heine University Düsseldorf, Institute of Clinical Neurosciences and Medical Psychology, Düsseldorf, Germany; [5] illy caffè SpA, Trieste, Italy; [6] RWTH Aachen University, Department of Physics, Aachen, Germany

MAP and PC thank the Ernesto Illy Foundation (Trieste, Italy).

Introduction

Chlorogenic acids (CGAs) are a group of compounds present in many fruits and vegetables, whose consumption has been associated to neuroprotection, among other health benefits [1]. One of the potential targets of CGAs mediating such beneficial effects at the molecular level is the peroxisome proliferator-activated receptor PPARα [2]. This nuclear receptor binds a wide variety of ligands, including fatty acids [3] and their derivatives [4], drugs against diabetes and hyperlipidemia and food molecules such as cinnamic acid [5], the parent compound of CGAs.

Methods

Here, we have combined molecular docking and molecular dynamics simulations to investigate the putative binding modes of cinnamic acid and CGAs in the different pockets of the PPARα binding cavity.

Results

Our computational modeling suggests that CGAs could bind to the receptor similarly to known endogenous agonists and drugs. In particular, cinnamic acid is able to bind to different PPARα pockets simultaneously, in line with the experimentally observed cooperativity effect [5], and exhibits an EC_{50} value comparable to known PPARalpha agonists, as shown by experiments on brain slices.

Conclusions

The molecular information obtained in this work could be used to prioritize CGA compounds for further experimental and computational validation, thus providing a stepping stone for designing future PPARalpha-based neuroprotective therapies.

References

[1] Lu H, Tian Z, Cui Y, Liu Z, Ma X 2020, 'Chlorogenic acid: A comprehensive review of the dietary sources, processing effects, bioavailability, beneficial properties, mechanisms of action, and future directions', *Compr Rev Food Sci Food Saf* 19(6):3130-3158.

[2] Willems S, Zaienne D, Merk D 2021, 'Targeting nuclear receptors in neurodegeneration and neuroinflammation', *J Med Chem* 64(14):9592-9638.

[3] Kamata S, Oyama T, Saito K, Honda A, Yamamoto Y, Suda K, Ishikawa R, Itoh T, Watanabe Y, Shibata T, Uchida K, Suematsu M, Ishii I 2020, 'PPARα ligand-binding domain structures with endogenous fatty acids and fibrates', *iScience* 23(11):101727.

[4] Sergeeva OA, Mazur K, Reiner-Link D, Lutsenko K, Haas HL, Alfonso-Prieto M, Stark H 2022, 'OLHA (Nᵅ-oleoylhistamine) modulates activity of mouse brain histaminergic neurons. *Neuropharmacology*, 215:109167.

[5] Chandra S, Roy A, Jana M, Pahan K 2019, 'Cinnamic acid activates PPARα to stimulate lysosomal biogenesis and lower amyloid plaque pathology in an Alzheimer's disease mouse model', *Neurobiol Dis*, 124:379-395.

PS-II-04

Role of amphipathic helices of Ede1 on ENDs formation

Gabriel J. Hella[1,2], Florian Wilfling[3], Roberto Covino[1,2]

[1] Goethe University Frankfurt, Frankfurt am Main, Germany; [2] Frankfurt Institute for Advanced Studies, Frankfurt am Main, Germany; [3] Max Planck Institute of Biophysics, Frankfurt am Main, Germany

Ede1, the yeast homolog of mammalian Eps15, plays a critical role in cellular trafficking and protein homeostasis. Ede1 acts as an early scaffold protein in the clathrin-mediated endocytosis (CME) machinery, forming a liquid-liquid phase-separated (LLPS) environment. In aberrant CME, it clusters to Ede1-dependent endocytic protein deposits (ENDs), recruiting autophagy-related proteins (Atgs)[1] and proteasome regulatory mRNAs[2] .

To gain further insights into the early assembly and membrane binding of ENDs, we aim to identify novel membrane binding sites in the coiled-coil (CC) and low-complexity regions (LCR). The CC-LCR-Fragment is essential for LLPS; resulting punctae show adherence to the plasma membrane as well as ER membranes[1].

First screenings of the CC-LCR-sequence revealed segments with amphipathic properties. Membrane-inserting amphipathic α helices (AH), which can sense membrane charge, curvature, or lipid composition[3], might be pivotal in the CME machinery assembly. To assess the role of the amphipathic segments, we will perform extensive atomistic molecular dynamics (MD) simulations, testing their membrane binding and sensing properties in varying lipid compositions.

In-vivo point mutations in the identified AHs will complement all MD simulations to measure the effect of the membrane binding interfaces on ENDs formation and localization.

We anticipate that our interdisciplinary approach will reveal new mechanistic insight into this critical cellular event.

References

[1] Wilfling, F., Lee, C. W., Erdmann, P. S., Zheng, Y., Sherpa, D., Jentsch, S., Pfander, B., Schulman, B. A., & Baumeister, W. 2020, 'A Selective Autophagy Pathway for Phase-Separated Endocytic Protein Deposits' *Molecular cell*, *80*(5), 764–778.e7

[2] Williams, T. D., Winaya, A., Joshua, I., & Rousseau, A. 2024, 'Proteasome assembly chaperone translation upon stress requires Ede1 phase separation at the plasma membrane', *Iscience*, *27*(1).

[3] Drin, G., Casella, J. F., Gautier, R., Boehmer, T., Schwartz, T. U., & Antonny, B. 2007, 'A general amphipathic α-helical motif for sensing membrane curvature', *Nature structural & molecular biology*, *14*(2), 138-146.

PS-II-06

Membrane-Induced pKa-Shift of Aminolipids

Marius Trollman[1,2], Paolo Rossetti[1], Rainer A. Böckmann[1,3]

[1] Friedrich-Alexander-Universität Erlangen-Nürnberg, Computational Biology, Department of Biology, Erlangen, Germany; [2] Erlangen National High Performance Computing Center (NHR@FAU), Erlangen, Germany; [3] FAU Profile Center Immunomedicine (FAU I-MED), Erlangen, Germany

mRNA-based vaccines have gained significant attention for their role in preventing severe SARS-CoV-2 infections [1]. A critical component of their delivery mechanism are lipid nanoparticles (LNPs), which serve as carriers for bioactive mRNA, shielding it from premature degradation and thereby enhancing protein expression. Additionally, LNPs increase the transfection rate by improving interactions with cell membranes, ensuring efficient delivery and uptake of the mRNA. Experimental and molecular dynamics (MD) [2] studies suggest that pH changes trigger lipid reorganization, leading to LNP destabilization and subsequent release of mRNA cargo from the LNP core.

A major limitation in studying these dynamical processes at the atomistic scale is the lack of dynamic (de-)protonation events in classical MD simulations. To address this issue, we developed parameters for the aminolipid ALC-0315, compatible with the recent constant pH extension of the GROMACS simulation package. Titration curves from these simulations indicate a shift in the apparent pKa of ALC-0315, from approximately 9 in aqueous solution to about 6 in a pharmaceutical lipid mixture. Moreover, simulations with mRNA reveal an increased protonation propensity of the aminolipid around the negatively charged backbone of the polynucleotide.

References
[1] Polack, F. P. et al., *N. Engl. J. Med.* **2020**, *383*(27), 2603-2615.
[2] Trollmann, M. F. W., and Böckmann R. A., *Biophys. J.*, **2022**, *121*(20), 3927-3939

PS-II-08

Permeability calculations of solutes permeation through the bacterial outer membrane

Vasilii Ungurian, Jochen Hub

Universität des Saarlandes, Saarbrücken, Germany

Saarland Graduate Funding Programme (GraduSaar)

Introduction
The development of bacterial resistance to antibiotics, including apearance of superbugs, represents a major health problem, asking for ongoing efforts for finding new antibiotics. Complex organization of the outer membrane of Gram-negative bacteria limits the uptake of many drug candidates. Calculations of permeability can help to describe the interactions of drugs with the membrane at the level of the individual chemical groups and facilitate the drug search.
Methods
We utilize molecular dynamics (MD) simulations in combination with enhanced sampling techniques and Bayesian analysis to derive the free energy and diffusion coefficient profiles. We calculate the permeability following the inhomogeneous solubility diffusion model.
Results
We computed free energy profiles for several small solutes as well as more complex drugl-like compounds for permeation across a model membrane of E. Coli. The obtained free energy profiles reflect the chemical properties of solutes. Furthermore, we currently test various protocols for obtaining the diffusion coefficient.

PS-II-10

Investigation of Mechanism of Calcium Sensitivity Modulation of Cardiac Troponin C by Small Molecules

Steffen Lindert

Ohio State University, Department of Chemistry and Biochemistry, Columbus, USA

Cardiac troponin C (cTnC) binds intracellular calcium and subsequently cardiac troponin I (cTnI), initiating cardiac muscle contraction. Structural studies have shown the binding location of small molecules to be a hydrophobic pocket in the regulatory domain of cTnC (cNTnC) but have not shown the influence of these small molecules on the dynamics of opening this domain. Here we describe an application of an umbrella sampling method used to elucidate the impact these calcium sensitivity modulators have on the free energy of cNTnC hydrophobic patch opening. We found that all molecules lowered the free energy of opening in the absence of the cTnI, with bepridil facilitating the least endergonic transformation. In the presence of cTnI, however, we saw a stabilization of the open configuration due to DPA and dfbp-o binding, and a destabilization of the open configuration imparted by bepridil and W7. Additionally, differences in the free energy of hydrophobic patch opening of hypertrophic (HCM) and dilated cardiomyopathy (DCM) cTnC systems were investigated. Molecular dynamics and umbrella sampling simulations revealed a lower free energy of opening for the HCM mutations. The DCM mutations all exhibited a higher free energy of opening. Our developed simulation protocol presents a novel approach to study calcium sensitivity modulation by small molecules. Additionally, we identified several novel drug candidates for heart failure, using a structure-based drug discovery protocol.

PS-II-12

Protein Environment Hinders Back-Relaxation of Covalently Bound Photoswitchable Kinase Inhibitor

Thilo Duve[1,2], Cristina Gil Herrero[1,2], Sebastian Thallmair[1]

[1] Frankfurt Institute for Advanced Studies, Frankfurt am Main, Germany; [2] Goethe University, Frankfurt am Main, Germany

Photopharmacology is a rapidly evolving field that uses light to control drug activity with high spatial and temporal precision. By designing photoswitchable molecules that change conformation in response to light, drug activity can be modulated, offering innovative therapeutic approaches with reduced side effects. In this study, we investigate a photoswitchable covalent inhibitor for MAP kinase c-Jun N-terminal kinase 3 (JNK3), a target for the treatment of neurodegenerative diseases. The inhibitor can switch reversibly between two (meta-)stable states via photoisomerization of a double bond.

We employ atomistic molecular dynamics simulations to examine how the conformational differences between the two isomers influence their interactions with JNK3 within the ATP-binding pocket. Our results reveal that in the known crystal structure, the inhibitor is in a partially relaxed state. Furthermore, we show that in the stable cis state the distance between the inhibitor and the targeted cystein residue is too great to allow the formation of a covalent bond. Only the metastable trans isomer can form a covalent bond with JNK3, thus permanently inhibiting its function. This bond, in combination with the environment of the protein pocket, hinders the full back-relaxation of the trans isomer. In addition, we employ coarse-grained simulations to estimate the binding affinities of both isomers and the accessibility of the cystein residue.

PS-II-14

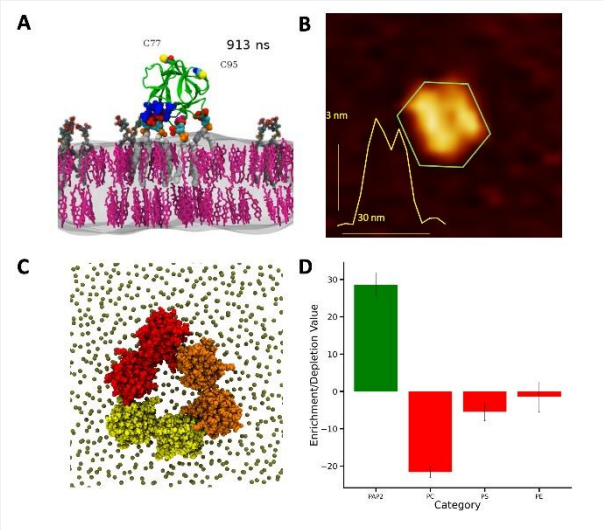
Molecular domino: The interplay between protein oligomerization and lipid sorting in FGF2-dependent membrane pore formation

Alessandra Griffo[1], Daniel Beyer[2], Walter Nickel[2], **Fabio Lolicato**[2,3]

[1] Saarland University, Department of Experimental Physics, Saarbrücken, Germany; [2] Heidelberg University, Biochemistry Center, Heidelberg, Germany; [3] Helsinki University, Department of Physics, Helsinki, Finland

We would like to thank the German Research Foundation DFG (grant LO 2821/1-1, FL). The authors gratefully acknowledge the data storage service SDS@hd supported by the Ministry of Science, Research, and the Arts Baden-Württemberg (MWK), the German Research Foundation (DFG) through grants INST 35/1314-1 FUGG and INST 35/1503-1 FUGG. We acknowledge the computing resources provided by the CSC - IT Center for Science Ltd (Espoo, Finland).

FGF2 is a cell survival factor involved in tumor-associated angiogenesis and is secreted via an unconventional pathway involving direct protein translocation across the plasma membrane. Like other pore-forming proteins, FGF2 binds to the membrane through a specific protein-lipid interaction, with $PI(4,5)P_2$ acting as a trigger for FGF2 disulfide-bridges dimerization and subsequent high oligomer formation[1-3]. Atomic force microscopy data show the formation of ring-like structures as a consequence of this molecular process. Using multiscale molecular dynamics simulations, we examined the surrounding lipids and found that the FGF2 oligomer selectively associates with lipids that have a high negative charge and intrinsic curvature, with over 60% of the surrounding lipids being non-bilayer lipids. This interaction is likely to induce a local lipid phase transition and a charge gradient, resulting in membrane destabilization. This destabilization will lead to the opening of a membrane pore, releasing tension and facilitating protein translocation. Ring-like FGF2 oligomers cause a local accumulation of non-bilayer lipids, which we propose is the key to opening a lipidic membrane pore.



Molecular Domino in FGF2 Pore Formation
FGF2 interacts with the inner leaflet of the Plasma Membrane, accumulating multiple $PI(4,5)P_2$ molecules per monomer (panel A). Subsequently, it oligomerizes into a ring-like structure, as demonstrated by our AFM images (panel B) and self-assembly molecular dynamics simulations (panel C). Our coarse-grained molecular dynamics simulations (panel D) reveal the oligomer's ability to sort non-bilayer and negatively charged $PI(4,5)P_2$ lipid molecules underneath, which we hypothesize is the driving force behind FGF2 membrane pore formation and translocation.

References
[1] Steringer, JP, Lange, S, Čujová, S, Šachl, R, Poojari, C, Lolicato, F, Beutel, O, Müller, H-M, Unger, S, Coskun, Ü, Honigmann, A, Vattulainen, I, Hof, M, Freund, C, Nickel, W 2017, 'Key Steps in Unconventional Secretion of Fibroblast Growth Factor 2 Reconstituted with Purified Components', eLife, 28985
[2] Lolicato, F, Saleppico, R, Griffo, A, Meyer, A, Scollo, F, Pokrandt, B, Müller, HM, Ewers, H, Hähl, H, Fleury, JB, Seemann, R, Hof, M, Brügger, B, Jacobs, K, Vattulainen, I, Nickel, W 2022, 'Cholesterol promotes clustering of PI(4,5)P2 driving unconventional secretion of FGF2', J Cell Biol, 221 (11): e202106123.
[3] Lolicato, F, Steringer, JP, Saleppico, R, Beyer, D, Fernandez-Sobaberas, J, Unger, S, Klein, S, Riegerová, P, Wegehingel, S, Müller, HM, Freund, C, Hof, M, Šachl, R, Chlanda, P, Vattulainen, I, Nickel, W 2023, 'Disulfide bridge-dependent dimerization triggers FGF2 membrane translocation into the extracellular space', eLife 88579.3

PS-II-16

oncoGNN: physics guided geometric deep learning in cancer metastasis

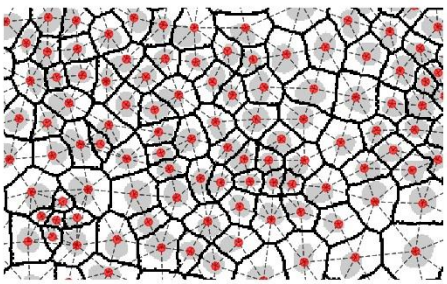
Kolya M. Lettl[1], Pablo Gottheil[1], Eliane Blauth[1], Anne-Sophie Wegscheider[2], Axel Niendorf[2], Josef Käs[1]

[1] Universität Leipzig, Peter Debye Institute for Soft Matter Physics, Leipzig, Germany; [2] MVZ Prof. Dr. med. A. Niendorf Pathologie Hamburg-West GmbH - Institut für Histologie, Zytologie und molekulare Diagnostik, Hamburg, Germany

Geometric deep learning extends machine learning to non-uniformly structured data, most notably graphs, a versatile data structure that can represent various physical systems. We employ this data model to transform data from different experiments into a common format, allowing us to apply insights from one type of data to another using both graph theory and graph neural networks (GNNs).

In this proof-of-concept work, we develop a model to predict cell motility using graphs derived from cell monolayer data (cf. figure “Cell Approximation & Graph Extraction”). Using morphological as well as graph theoretical node features, our GNN model achieves a high correlation between actual and predicted cell motility. Subsequently, we apply the trained model to clinical breast cancer tissue samples to predict the metastatic risk based on cancer cell motility.

This method provides a novel way to transfer knowledge between different types of biological data, offering valuable insights into cell behavior and disease progression. Specifically, it holds potential for improving our understanding of cancer metastasis, ultimately aiding in the development of more effective diagnostic and treatment strategies.



Cell Approximation & Graph Extraction
This figure illustrates the process of approximating the cells and extracting the graph structure. First, the cells (white) are approximated from the segmented nuclei (grey) with a watershed-based algorithm. Next, nodes (red circles) are added to represent each cell. Finally, nodes are connected (dashed lines) if their corresponding cells are neighbors, determined by slightly dilating each cell and detecting overlap with other cells.

PS-II-18

Chondrotin and dermatan sulfates: how small difference lead to huge consequences

Marta Pągielska, Annemarie Danielsson, Sergey Samsonov

University of Gdańsk, Faculty of Chemistry, Gdańsk, Poland

This work was funded by the National Science Centre (grant number UMO-2023/49/B/ST4/00041).

Glycosaminoglycans (GAGs) represent a class of linear anionic periodic polysaccharides which can be sulfated in different positions. The amount (net sulfation) and particular positions of the sulfation group (sulfation pattern) constitute *"sulfation code"*, which determines GAG structure and function. Our study is focused on deciphering the "sulfation code" of chondroitin sulfate (CS) and dermatan sulfate (DS). The only difference between CS and DS is the epimerization of uronic acid unit – GlcA is present in CS while IdoA in DS. Utilizing atomistic molecular dynamics simulations, we analyzed structural, conformational and dynamic properties of these GAGs in several oligomeric forms. We revealed substantial differences in molecular descriptors such as RMSD, molecular volume, glycosidic linkages and hydrogen bonding propensities. These results highlighted the impact of the sulfation position in CS and the type of uronic acid on the properties of these GAGs [1]. We extended our studies to explore the interactions of these GAGs with proteins and determine how the observed structural and dynamic differences in CS and DS affect their binding poses and affinities. Such benchmarking analysis was conducted for the entire family of cysteine cathepsins. These proteins are human proteases which activity is known to be regulated by GAGs. Our work provides valuable insights into the GAGs "sulfation code" which could be exploited in the rational development of GAG-based drugs and therapies.

References

[1] Pągielska, M.; Samsonov, SA. 2023, 'Molecular Dynamics-Based Comparative Analysis of Chondroitin and Dermatan Sulfates'. *Biomolecules*,13, 247.

PS-III-02

Intriguing molecular changes in the brain of leptin-deficient zebrafish detected by state-of-the-art magnetic resonance imaging

Alia Alia[1,2], Rico Singer[1,2], Muhamed N.H. Eeza[1], Narmin Bashirova[1], Alexandra Velthuijzen[2], Herman P. Spaink[3]

[1] Leipzig University, Institute for Medical Physics and Biophysics, Leipzig, Germany; [2] Leiden University, leiden Institute of Chemistry, Leiden, Netherlands; [3] Leiden University, Institute of Biology Leiden, Leiden, Netherlands

Special thanks to Thomas Riemer, Holger Sheidt and Karthick Babu for their help during various NMR/MRI experiments.

Introduction

Leptin is a protein hormone that plays a key role in controlling food intake and energy homeostasis [1-3]. It is currently not clear if and how leptin deficiency can lead to brain-wide microstructural & molecular alterations [4]. In this study, we used state-of-the-art magnetic resonance methods to non-invasively probe the microstructural and molecular changes in the brain of leptin-deficient (lep-/-) zebrafish models.

Methods

Control and Lep-/- mutant zebrafish were used. MRI (T2 weighted, chemical shift selective, diffusion tensor) measurements were performed at 7T & 17.6T magnets. High-resolution magic angle spinning (HR-MAS) NMR was performed at 14.1T magnet.

Results

Chemical shift selective imaging shows high fat accumulation in lep-/- fish brain. An elevated T2 relaxation time and significantly reduced apparent diffusion coefficient (ADC) unveil brain-wide molecular alterations, potentially indicative of inflammation, which was supported by changes in neurometabolites. Multicomponent analysis shows an increase in the slow ADC component indicating restricted diffusion. Diffusion tensor imaging and diffusion kurtosis imaging revealed diminished diffusivity and enhanced kurtosis in various white matter tracts in lep-/- zebrafish.

Conclusions

This study shows that leptin deficiency results in severe microstructural & molecular alterations in several brain regions, signifying inflammation, compromised white matter integrity, and axonal degeneration.

References

[1] Eeza,M.N.H., Singer, R., Ding, Y., He, J., Zuberi, Z., Baelde, H.J., de Groot,H.J.M., Matysik,J., Spaink,H.P., Alia A. PLoS One. 18 (2023):e0284215.

[2] Ding, Y., Haks, M.C., Forn-Cuní, G., He, J., Nowik, N., Harms, A.C., Hankemeier, T., Eeza, M.N.H., Matysik, J., Alia, A., Spaink, H.P. Cell Biosci. 11 (2021) 126.

[3] He, J., Ding, Y., Nowik, N., Jager, C., Hashem Eeza, M.N., Alia, A., Baelde, H.J., Spaink, H.P. J Endocrinol. 249(2021) 125-134.

[4] Morrison, C.D. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease. 1792 (2009) 401-408.

PS-III-04

Screening the swimming motility of commensal bacteria from the human gut

Maria D. Chiotelli, Charlie Pauvert, Thomas Clavel, Marianne Grognot

RWTH Aachen University, Institute of Medical Biology, Aachen, Germany

This work is funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) — Project-ID 403224013 — SFB 1382 ("Gut-Liver Axis")

Introduction

An estimated half of bacterial species can swim, propelled by one or more flagella, displaying a variety of swimming behaviors[1]. It enables them to seek out favorable environments within their ecological niche. Here, we dive into the complex ecosystem of the human gut, which houses trillions of commensal bacteria, and where elevated flagellin (component of the flagella) levels have been linked with intestinal inflammation[2]. However, direct assessment of commensals' motility is lacking beyond the model organism *E. coli*.

Methods & Results

We developed a simple motility screening method compatible with anaerobic cultivation conditions required by many gut bacteria. We applied it to isolates from the Human intestinal Bacterial Collection (HiBC)[3]. We demonstrate a 96% true positive detection rate (relative to predictions based on gene screening) and provide insights bridging motility phenotype and genotype.

We are now characterizing the individual swimming behaviors of selected commensal species and complex communities from fresh faecal samples, using a high-throughput 3D tracking method[4]. Preliminary results suggest elevated motile fraction in a mouse model of chronic intestinal inflammation, and the existence of diverse swimming behaviors other than that of *E. coli*.

Conclusions

Our work paves the way for a quantitative analysis of commensals' motility, to experimentally test the role of bacterial swimming motility in inflammation and subsequent gut diseases.

References

[1] Grognot, M. & Taute, K. M, 2021, 'More than propellers: how flagella shape bacterial motility behaviors', *Current Opinion in Microbiology* 61, 73–81.

[2] Chassaing, B., Ley, R.E., and Gewirtz, A.T., 2014, 'Intestinal Epithelial Cell Toll-like Receptor 5 Regulates the Intestinal Microbiota to Prevent Low-Grade Inflammation and Metabolic Syndrome in Mice', *Gastroenterology* 147, 1363-1377.e17.

[3] Retrieved March 2023, DOI:10.5281/zenodo.7966673

[4] Taute, K. M., Gude, S., Tans, S. J. & Shimizu, T. S, 2015, 'High-throughput 3D tracking of bacteria on a standard phase contrast microscope', *Nature Communications* 6, 8776.

PS-IV-02

Standardized evaluation of AMP liposome permeabilization in microfluidic setups

Janina Nandy[1], Marie Wetzel[1], Marcus Fletcher[2], Kareem al Nahas[3], Niels Röckendorf[4], Thomas Gutsmann[1], **Christian Nehls**[1]

[1] Research Center Borstel, Division of Biophysics, Borstel, Germany; [2] Imperial College London, Department of Chemical Engineering, London, UK; [3] Max Planck Institute of Biochemistry, Cellular and Molecular Biophysics, Martinsried, Germany; [4] Research Center Borstel, Division of Mucosal Immunology and Diagnostic, Borstel, Germany

This work was funded by the Phospholipid Research Center, grant number CNE-2022-104/1-1, and financially supported by the Deutsche Forschungsgemeinschaf SPP2225. We are grateful for excellent technical support by Sabrina Groth, Kerstin Stephan and Franziska Kiesow.

Introduction

Antimicrobial resistance is one of the major challenges of the 21st century. There is an urgent need on the one hand for new alternative compounds and on the other hand for platforms for compound identification and analysis.

Methods

In our project, we characterize and compare the membrane permeabilization of a set of antimicrobial peptides (AMPs) that were optimized by a genetic algorithm. As a model system, we use microfluidically generated liposomes, which we trap individually in microfluidic devices [1]. This allows us to monitor the individual permeabilization of hundreds of liposomes simultaneously.

Results

With this approach, we a) evaluate the permeabilizing efficacy of AMPs, b) classify the mode of action of AMPs in combination with other methods, and c) develop a microfluidic platform that allows standardized and routine compound testing. Therefore, we compare our results with microbiologically determined fitness values of AMPs.

Conclusions

We demonstrate the potential of individual liposome trapping for standardized compound testing and add a further piece to the puzzle of developing AMPs as a promising alternative to conventional antibiotics in the fight against AMR.

References

[1] Al Nahas, K, et al. 2022, 'Measuring thousands of single-vesicle leakage events reveals the mode of action of antimicrobial peptides', *Analytical Chemistry*, 94(27), 9530-9539, ACS Publications

PS-IV-04

Uncovering the distribution of motion in POPC membranes by temperature dependent detector analysis

Kai Zumpfe, Albert A. Smith-Penzel, Oskar Engberg

Leipzig University, Medical Faculty / Institute for Medical Physics and Biophysics, Leipzig, Germany

This project was funded through the Deutsche Forschungsgemeinschaft (DFG) grant 450148812 (A.A.S.), the European Social Funds (ESF) and the Free State of Saxony Junior Research Group UniDyn Project No. SAB 100382164 (A.A.S., K.Z.).

Despite a variety of methods available, the investigation of dynamics in biomolecules remains a challenging task. The complexity of the motions makes it difficult to unify the data into a consistent model, so that a detailed description of the motion and the corresponding timescales remains elusive. The dynamic detector analysis [1] offers a useful tool to investigate motion in timescale-specific windows based on NMR relaxation data with the support of MD simulations. The approach was extended by analysis of temperature dependent relaxation data of POPC bilayers to allow one to observe a shift in the distribution of motion, and draw a sharper picture of the distribution. We could identify three types of motion in the bilayer, which contribute differently to relaxation induced by reorientational dynamics. These include local motions such as libration of H–C bonds, correlated libration and rotation around multiple bonds, and collective motions involving multiple molecules such as tilting and rotation within the lipid bilayer. These motions are represented by distributions of correlation times, where those distributions are fitted globally to relaxation data on the POPC molecule, with amplitudes scaling the influence of each distribution on the behavior of the individual ^{13}C resonances. Our work provides an approach to combine detector analysis with temperature dependent data, and accounts for the impact that distributions of correlation times have on experimental data.

References
[1] K. Zumpfe, A. A. Smith,'Model-Free or NOT?', *Front. Mol. Biosci.***2021**, *8*, 727553.

PS-IV-06

Photoswitchable nanodiscs to study membrane dynamics and peptide insertion by ATR-FTIR spectroscopy

Celine Eckert, Karin Hauser

University of Konstanz, Department of Chemistry, Konstanz, Germany

Membrane proteins play a vital role in cell communication and transport processes. The interactions of these proteins with their surrounding membrane can be examined using a sensitive analytical technique called attenuated total reflection (ATR) Fourier-transform infrared (FTIR) spectroscopy. Nanodiscs provide a suitable and well-established mimetic system for cellular membranes. Nanodiscs are usually composed of a phospholipid bilayer stabilized by scaffold proteins [1]. However, we stabilized the nanodiscs with copolymers for better spectral analysis, as there is no overlap between the IR bands of the scaffold protein and the protein under investigation. We investigated membrane insertion of the model peptide LAH4. The peptide has been shown to insert into the membrane or to bind at the membrane surface in a pH-dependent manner [2]. To actively induce membrane dynamics, about 10% photoswitchable AzoPC lipids were incorporated into POPC nanodiscs. The AzoPC lipids contain azobenzene groups in one of the lipid tails which can be switched between cis- and trans-forms upon light irradiation at different wavelengths [3]. We study how the switchable membrane dynamics influence the pH-controllable insertion of the peptide into the membrane.

References

[1] Malhotra, K, Alder, N N 2014, ’Advances in the use of nanoscale bilayers to study membrane protein structure and function’, *Biotechnology and Genetic Engineering Reviews*, 30, 79-93, DOI:10.1080/02648725.2014.921502

[2] Georgescu, J, Munhoz, V H O, Bechinger, B 2010, ’NMR Structures of the Histidine-Rich Peptide LAH4 in Micellar Environments: Membrane Insertion, pH-Dependent Mode of Antimicrobial Action, and DNA Transfection’, *Biophysical Journal*, 99, 2507-2515, DOI:10.1016/j.bpj.2010.05.038

[3] Crea, F, Vorkas, A, Redlich, A, Cruz, R, Shi, C, Trauner, D, Lange, A, Schlesinger, R, Heberle, J 2022, ’Photoactivation of a Mechanosensitive Channel’, *Front. Mol. Biosci.*, 9, 905306, DOI:10.3389/fmolb.2022.905306

PS-IV-08

In vitro reconstitution of the inhibitory GABAergic post synapse

Lara Dohmen, Leandro Sosnitza, Claudia Steinem

Georg-August-Universität Göttingen, Institute of Organic and Biomolecular Chemistry, Göttingen, Germany

Thanks to the GGNB IMPRS for Physics of Biological and Complex Systems and to the SFB1286 for the academic and financial support. Also many thanks go to the gorup Prof. Dr. Olaf Jahn (Max-Planck-Institute for Multidisciplinary Sciences, Göttingen) for synthesizing the NL2-mimicking peptide.

Introduction

The assembly of the GABAergic post-synapse is a key step in synaptogenesis and crucial for the regulation of neuronal communication. Its formation is dependent on the interplay between a variety of proteins. Owing to its complexity, the regulation of the GABAergic inhibitory post-synapse is extremely prone to disfunctions causing diseases, such as anxiety disorders[1] or epilepsy[2]. Attributed to their interactions, the proteins collybistin 2 (CB2) and neuroligin 2 (NL2) play key roles in GABAergic synapse regulation.

Methods

Here, we present an *in vitro* system allowing the examination of protein-protein interactions located directly at a lipid monolayer. By means of surface plasmon resonance spectroscopy we aim at investigating the interaction between CB2 and phosphoinositides as receptor lipids in solid-supported membranes in dependence of NL2 presence.

Results

In order to establish the model system, a section of the cytosolic loop of NL2 is attached to the solid-supported membrane via an effective Michael-click addition of a cysteine thiol-residue to maleimide-functionalized lipids. The successful fixation of the construct was proven by reflectometric interference and ATR-FTIR spectroscopy.

Conclusions

This approach could be a valuable tool for the investigation of the interactions driving the protein machinery in a highly controled environment, allowing us to gain further insights into this complex process.

References

[1] Crestani, F, Lozer, M, Baer, K *et al.* 1999, 'Decreased GABAA-receptor clustering results in enhanced anxiety and a bias for threat cues', *Nature Neuroscience*, 9, 833-839.

[2] Benarroch, EE 2007, 'GABAA receptor heterogeneity, function, and implications for epilepsy', *Neurology*, 8, 612-614.

PS-IV-10

Functionalized gold nanocavity arrays as support for biomimetic membranes using microcontact printing

Shane Maguire, **Karin Hauser**

University of Konstanz, Department of Chemistry, Konstanz, Germany

Nanocavity supported lipid bilayers are friction-free membranes that span aqueous-filled cavities, preserving the lipid bilayer's high fluidity and preventing contact with the substrate. These platforms combine the fluidity of liposomes and addressability of supported lipid bilayers, making them ideal for studying protein-membrane interactions. Functionalizing gold surfaces is crucial for supporting lipid membranes, particularly since bilayers are usually less stable on gold substrates. In this work, we describe methods of functionalizing gold silicon substrates with hydroxy-terminated self-assembled monolayers (SAMs) to support lipid bilayers. We combine electron beam lithography (EBL) with a soft lithography technique known as microcontact printing (µCP). Microcontact printing is a technique which enables the exterior surface of the nanocavity to be functionalized, without modifying the interior surface of the cavity array, within minutes. Surface functionalization was assessed using cyclic voltammetry (CV) while the formation of the lipid bilayers was confirmed using FTIR spectroscopy. We could demonstrate that the platform was functionalized with alkanethiols to successfully support lipid bilayers. Nanocavity arrays can more closely mimic the natural, fluid environment of cell membranes compared to solid supports, which often limit membrane fluidity and protein mobility.

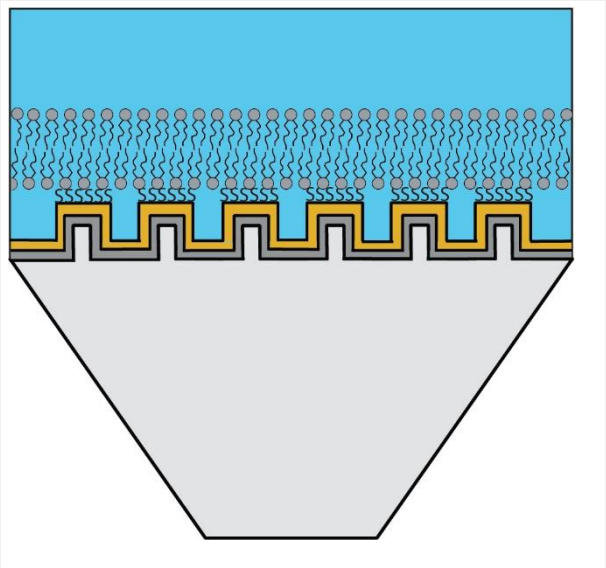


Figure 1:
A tethered lipid membrane on a nanocavity array which enables the membrane to be in a friction-free environment away from the substrate surface.

PS-IV-12

Stay viscoelastic: Studying the impact of actin cortices on membrane tension

Elena L.M. Ackermann, Claudia Steinem, Mara S. Emde

Georg-August-Universität Göttingen, Institute of Organic and Biomolecular Chemistry, Göttingen, Germany

Many thanks to the GAUSS promotional program and DFG for the academic and financial support. Special thanks to Prof. Andreas Janshoff (Institute of Physical Chemistry, Georg-August Universität) for providing the analysis script.

Introduction

Viscoelasticity of membranes is enabled by an actin shell underneath the plasma membrane. During deformation caused by external cues it feedbacks back to maintain the cell shape and leads to mechanic stability and integrity of the cell.[1] The contribution of actin cortices directly attached to the membrane by ERM proteins (ezrin/radixin/moesin) is still elusive. *In vivo* attempts to fathoming the elastic response is difficult and nearly impossible due to the large and complex intertwining of the high number of components.[2,3] Here, we established an *in vitro* bottom-up approach using minimal actin cortices (MACs) formed *via* the innate ezrin-PtdIns[4,5]P_2 (PIP_2) membrane-linkage.

Methods

Porous substrates and ensuing vesicle spreading serve as a tool to constitute pore spanning membranes (PSMs). By specific binding of ezrin to the incorporated anchor lipid PIP_2 in the membrane, ezrin links the fluorescently labeled actin filaments to establish MACs. A combination of atomic force and fluorescence microscopy allows the extraction of the lateral membrane tension of PSMs and MACs attached to PSMs by indentation experiments.

Results

Here, we present a change from superposition to hysteresis between trace and retrace of PSMs compared to membrane-bound actin cortices.

Conclusions

This biomimetic system paves the way to conquer the inscrutable contribution of direct actin linkage on the membrane tension without the highly cellular complexity of cross-linker proteins.

References
[1] Pollard, TD, Cooper, JA 2009, 'Actin, a central player in cell shape and movement', *Science*, 326, 1208-1212.
[2] Lappalainen, P, Kotila, T, Jégou, A, Romet-Lemonne, G 2022, 'Biochemical and mechanical regulation of actin dynamics', *Nature Reviews Molecular Cell Biology*, 23, 836-852.
[3] Waechtler, BE, Jayasankar, R, Morin, EP, Robinson, DN 2024, 'Benefits and challenges of reconstituting the actin cortex', *Cytoskeleton*, 1-21.

PS-IV-14

Probing the supramolecular aggregation state of bacterial endotoxin to reveal the basis of biological recognition and endotoxin masking

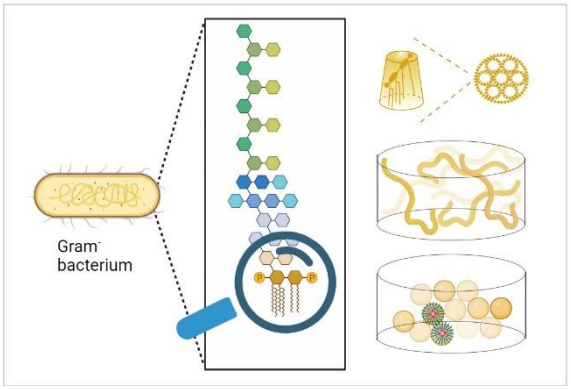
Andra Schromm[1], Nicolas Gisch[2], Wilmar Correa[3], Frank Steiniger[4], Walter Richter[4], Guillermo Martinez-de-Tejada[5], Klaus Brandenburg[6], Friedrich von Wintzingerode[7]

[1] Research Center Borstel, Leibniz Lung Center, Division of Immunobiophysics, Borstel, Germany; [2] Research Center Borstel, Leibniz Lung Center, Division of Bioanalytical Chemistry, Borstel, Germany; [3] Research Center Borstel, Leibniz Lung Center, Division of Biophysics, Borstel, Germany; [4] University Hospital Jena, Friedrich Schiller University, Electron Microscopy Centre, Jena, Germany; [5] University of Navarra, Department of Microbiology and Parasitology, Pamplona, Spain; [6] Brandenburg Antiinfektiva, Borstel, Germany; [7] Genentech, Inc., San Francisco, USA

Parts of the study funded by ROCHE, Germany (grant to AS, NG, KB).
SAXS measurements were performed at the EMBL c/o DESY beamline P12 at PETRA III under a beam time grant to AS

Introduction
The outer-membrane lipopolysaccharide (LPS, endotoxin) of Gram-negative bacteria is highly immunostimulatory and induces severe pathology in humans. Investigation of a variety of natural and reconstituted membrane systems by small angle X-ray scattering (SAXS) revealed that the supramolecular organization and 3D structure of endotoxin molecules are major determinants for immunological activity. Since endotoxin is ubiquitous, it represents also a harmful contaminant for medical drug products. Masking of endotoxin by surfactant containing drug-formulations poses a severe challenge for drug safety.

Methods & Results:
We have performed a comprehensive study including structural, biophysical, and biological analyses to identify the root cause of endotoxin masking in a drug product. DLS, cryo-TEM, SAXS and ITC analyses revealed a drastic rearrangement of the morphology of LPS aggregates in drug buffer from tube-like structures into a network of interlinked micelles. Our data provide a structure-based explanation for the effect of endotoxin masking. Biochemical detection and responsiveness of different biological detection systems were systematically compared to identify systems suitable for detection of endotoxin in this specific aggregation state.
Conclusions
Our study revealed a new state of bacterial endotoxin aggregation, inaccessible for biochemical detection, but highly accessible for human immune detection.



Aggregation states of bacterial membrane lipopolysaccharide
(Image created by Biorender under licence).

References

[1] **Schromm AB** and Brandenburg K. 2021. TLR4 ligands: Single Molecules and Aggregates. In: The Role of Toll-Like Receptor 4 in Infectious and Non Infectious Inflammation. Rossetti C, Peri F. (Eds.) Springer Nature Switzerland AG.

[2] **Schromm, AB**, Correa W, Gisch, N, Steiniger, F, Richter, W, Martinez-de-Tejada, G, Brandenburg, K, von Wintzingerode, F. 2024. Supramolecular assembly of micellar aggregates is the basis of low endotoxin recovery (LER) in a drug formulation that can be resolved by a whole blood assay. Biomedicine and Pharmacotherapy. 173:116286.

PS-IV-16

Membrane remodelling by the cyanobacterial protein IM30

Nadja Hellmann, Dirk Schneider

Johannes-Gutenberg-Universität Mainz, Chemistry/Biochemistry, Mainz, Germany

Introduction

The inner membrane-associated protein of 30 kDa (IM30) is essential for the development and maintenance of the thylakoid membrane (TM) in chloroplasts and cyanobacteria. The structural details of its large (>2 MDa) ring-shaped assemblies have been solved recently (1). However, how it fulfils its task *in vivo* is still an open question.

Methods

Fluorescence spectroscopy, light scattering

Results

In presence of magnesium ions binding of IM30 rings to membranes containing ionic lipids leads to liposome fusion (2). In absence of magnesium ions carpet-like structure are formed which seem to have a stabilizing effect on the membrane with respect to proton permeability (3). A variant which does not form rings anymore (IM30*) is unable to fuse membranes, but the stabilizing effect is even more pronounced. However, dithionite quenching experiments revealed that in absence of magnesium ions NBD-labelled lipids incorporated into liposomes become more accessible in presence of wt IM30 or IM30*. Furthermore, at sufficiently high protein/lipid ratio the scattering intensity of liposomes decreases upon protein addition.

Conclusions

IM30 binding to lipid membranes shows a broad range of effects on the liposomal structure. In absence of magnesium ions both stabilization and destabilization can be observed. The elucidation of the conditions defining these opposite effects are under investigation.

References

[1] Gupta, Tilak Kumar, et al. (2021), "Structural basis for VIPP1 oligomerization and maintenance of thylakoid membrane integrity." *Cell* 184.14 (2021), 3643-3659.

[2] Hennig, Raoul, et al. (2015), "IM30 triggers membrane fusion in cyanobacteria and chloroplasts." *Nature communications* 6.1, 7018.

[3] Junglas, Benedikt, et al. (2020), "IM30 IDPs form a membrane-protective carpet upon super-complex disassembly." *Communications biology* 3.1, 595.

PS-IV-18

Nanodisc reconstitution and NMR analysis of the vesicular SNARE Protein Synaptobrevin-2

Mirko Kraus[1,2], Henrike Heise[1,2], Nils-Alexander Lakomek[1,2]

[1] Heinrich-Heine-Universität, Institute of Physical Biology, Düsseldorf, Germany; [2] Forschungszentrum Jülich, Institute of Biological Information Processing (IBI-7: Structural Biochemistry), Jülich, Germany

M.K. thanks H.H. and the Forschungszentrum Jülich, Institute for Biological Information Processing: Structural Biochemistry (IBI-7) for financial support. N.L. thanks the German Science Foundation for funding through the Heisenberg Program (DFG grant number 433700474). We acknowledge generous access to the Jülich-Düsseldorf Biomolecular NMR Center, jointly run by Forschungszentrum Jülich and Heinrich Heine University Düsseldorf (HHU).

Introduction

Membrane proteins, like the SNARE proteins, play an important role in cellular interactions. The SNARE proteins assemble into a SNARE complex which induces membrane fusion between vesicles and the plasma membrane of the pre-synapses. Synaptobrevin-2 (Syb2) is part of the SNARE complex but is intrinsically disordered in its monomeric pre-fusion form.

Methods

We have optimized the production of isotopically labeled full-length Syb2, which is necessary for structural studies. It is then reconstituted into a lipid nanodisc to mimic a near-physiological lipid environment. Solution and proton-detected solid-state NMR at fast MAS frequencies give insights into the dynamic and interaction of Syb2 with the lipid bilayer. Dynamic nuclear polarization (DNP)-enhanced solid-state NMR measurements provide insights into the characteristics of IDPs.

Results

Measurements indicate that lipid head-group charge influences the structure and lipid-binding site of Syb2. DNP measurements at 100K indicate that the protein is split into two main conformations, α-helical and β-sheet, which average at room temperature and become indistinguishable from random coil.

Conclusions

We aim at structural insights into the SNARE motif and linker domain interacting with the lipid bilayer membrane. We also want to gain insights into the C-terminal transmembrane region of Syb2 in the lipid membrane environment. Next, we want to understand the random coil conformation of an IDP on a structural level.

PS-IV-20

Shifting Perspectives on the Skin Barrier: Unraveling the Complex Dynamic Lipid Landscape in the Stratum Corneum

Oskar Engberg[1], Ferdinand Fandrei[1], Tomáš Havrišák[2], Lukáš Opálka[2], Petra Pullmannová[2], Andrej Kováčik[2], Martin Juhaščik[2], Kateřina Vávrová[2], Daniel Huster[1]

[1] University of Leipzig, Institute of Medical Physics and Biophysics, Leipzig, Germany; [2] Charles University, Skin Barrier Research Group, Faculty of Pharmacy, Hradec Králové, Czech Republic

Introduction

The skin barrier (stratum corneum) is a complex structure of equimolar ratios of the lipids: ceramides (Cer), free fatty acids, and cholesterol. It has been thought to mainly form a rigid crystalline barrier, to protect against pathogens and prevent water dehydration. The study aims to clarify if the skin barrier consists of only a rigid,or fluid-structure, or a combination.

Methods

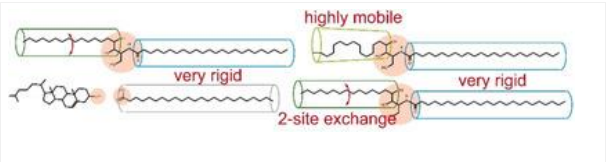
By performing solid-state ^{2}H NMR spectroscopy of skin barrier model membrane systems consisting of differently deuterated lipids, we can acquire anisotropic information about the motions of the individual lipids species or the individual lipid chains. In addition,^{2}H NMR can detect if the lipids or the lipid chains exist in the crystalline phase or a fluid or isotropic phase in a quantitative manner.

Results

We show that specific lipids (sphingosine chain of Cer[NS], cholesterol, and the omega-linked chain of ultralong Cer[EOS]) show a more fluid and isotropic behavior while free fatty acids like Lignoceric acid and the acyl chain of (Cer[NS]) exist mostly in the crystalline phase. We have further explored different ultralong Cer[EOS] with different omega-linked chains (unsaturated lineolate, oleate, saturated stearate, and branched phytanate) to understand how the different lipid structures modify the dynamics and structure of the skin barrier.

Conclusions

We propose improved lipid structural models highlighting that the skin barrier has both fluid and rigid compartments.[1,2].



New model of the stratum corneum

References

[1][2] 1.Engberg, O, Kovacik, A, Pullmannova, P, Juhascik, M, Opalka, L, Huster, D, and Vavrova, K. 2020 "The Sphingosine and Acyl Chains of Ceramide [NS] Show Very Different Structure and Dynamics That Challenge Our Understanding of the Skin Barrier, *Angew Chem Int Ed Engl*. 59, 17383-17387 Wiley-VCH GmbH

2. F. Fandrei, T. Havrisak, L. Opalka, O. Engberg, A.A. Smith, P. Pullmannova, N. Kucerka, V. Ondrejcekova, B. Deme, L. Novakova, M. Steinhart, K. Vavrova, D. Huster, The intriguing molecular dynamics of Cer[EOS] in rigid skin barrier lipid layers requires improvement of the model, *J Lipid Res*, 64 (2023) 100356 Elsevier

PS-IV-22

DNA origami as a dynamic tool for reversible targeting of lipid phases

Rayehe Mamaghaniyeh, Henri G. Franquelim

Leipzig University, Interfaculty Center for Bioactive Matter (b-ACTmatter), Leipzig, Germany

Biological lipid membranes are heterogeneous in composition and can be modeled as liquid-ordered and liquid-disordered phases. These phases exhibit varying levels of rigidity and contribute to cellular functions, such as membrane trafficking. In spite of our increasing knowledge about the topic, challenges about studying lipid-protein functions and their interactions remain. Here, DNA origami is a useful approach to overcome reconstitution challenges in mimicking functional membrane-targeting proteins.
In this study, we developed DNA origami nanostructures functionalized with various lipophilic anchors that are able to recognize different membrane lipid phases and rigidity levels. This technique can be used as a domain selection tool to attach the desired nanostructures to a specific lipid phase. With the aid of fluorescence confocal microscopy as investigation method, many effective factors such as number of moieties on the nanostructure, stiffness of lipid compositions, lipid chain lengths, and DNA origami concentration were studied in order to optimize the membrane targeting conditions. Ultimately, we show that our DNA origami nanostructures can be reversibly transferred between phases, intra- and inter-vesicles, using dynamic strand displacement approaches.
Based on these results, we can design useful toolkits for investigating basic cargo trafficking principles, and physicochemical rules governing lipid phase selectivity.

PS-IV-24

Elucidating conformational dynamics of $LptB_2FGC$ during LPS translocation using solid-state NMR

Aaron Klausnitzer, Jagdeep Kaur, Samuel Seidl, Johanna Becker-Baldus, Clemens Glaubitz

Goethe University Frankfurt, Biophysical Chemistry, Frankfurt am Main, Germany

We thank Melanie McDowell for the excess to their Prometheus Panta to perform the NanoDSF experiments.

Introduction

The lipopolysaccharide (LPS) barrier comprising the outer membrane of gram-negative bacteria is a unique feature, providing impermeability against many toxic compounds such as antibiotics.[1] At the inner membrane $LptB_2FGC$ plays an important role in extruding LPS out of the outer leaflet of the inner membrane handing it onto a periplasmic bridge formed by LptA.[2,3] This bridge transports LPS to the outer membrane, where it is inserted into the outer leaflet.[4] Several structural snapshots of $LptB_2FGC$ reveal a potential mechanism of LPS extrusion, however the role of the LptC transmembrane helix (TMH) and the conformational changes in the ß-jellyroll domains during translocation remain elusive.[2,3,5]

Methods

Here, we co-reconstituted $LptB_2FG$ with LptC for an *in vitro* complex formation. We explore conformational dynamics of ^{13}C, ^{15}N lysine labelled LptC embedded liposomes by recording hNCA spectra under high-field magic angle sample spinning conditions.

Results

We showed activity of $LptB_2FG$ and $LptB_2FGC$ in liposomes by ATPase assays and showed stabilisation upon complex assembly with NanoDSF in DDM micells. To probe the transporter during LPS translocation, we trapped $LptB_2FGC$ in different states of the ATP catalysis in absence and presence of its natural substrate LPS integrated into the liposomes.

Conclusions

Characterising each state, we observed changes in the dynamical behavior of the TMH and the ß-jellyroll domain of LptC within the $LptB_2FGC$ complex.

References

[1] Raetz CR, Whitfield C 2002, 'Lipopolysaccharide endotoxins', *Annu Rev Biochem*, 71, 635-700

[2] Luo Q, Yang X, Yu S *et al. 2017,* 'Structural basis for lipopolysaccharide extraction by ABC transporter LptB2FG', *Nat Struct Mol Biol* , 24, 469–474

[3] Li Y, Orlando BJ, Liao M 2019, 'Structural basis of lipopolysaccharide extraction by the LptB2FGC complex', *Nature,* 567, 486–490

[4] Sherman DJ *et al.* 2018, 'Lipopolysaccharide is transported to the cell surface by a membrane-to-membrane protein bridge', *Science,* 359, 798-801

[5] Tang X, Chang S, Luo Q *et al.* 2019, 'Cryo-EM structures of lipopolysaccharide transporter LptB2FGC in lipopolysaccharide or AMP-PNP-bound states reveal its transport mechanism', *Nat Commun,* 10, 4175

PS-IV-26

Characterizing Immune Receptor Crosstalk: A Molecular Dynamics Simulation Approach

Cristian R. Popov[1], Matthias Pöhnl[1], Marius F. Trollmann[1,2], Falk Nimmerjahn[3], Rainer A. Böckmann[1,2]

[1] Friedrich-Alexander-Universität Erlangen-Nürnberg, Computational Biology, Department of Biology, Erlangen, Germany; [2] Erlangen National High-Performance Computing Center, Erlangen, Germany; [3] Friedrich-Alexander-Universität Erlangen-Nürnberg, Division of Genetics, Department of Biology, Erlangen, Germany

Understanding the intricate interactions between immune receptors, and between receptors and the lipid environment, is crucial for deciphering immune cell activation mechanisms. This study is pivotal in understanding the competitive binding dynamics between the receptors FcγRIII and Mincle, two key receptors in immune response regulation, to the FcεRγ dimer (commonly referred to as the FcRγ-chain).

While FcγRIII receptors are activated by IgG antibody binding, Mincle initiates downstream signaling by directly binding pathogens. However, both receptors cannot signal autonomously and have to associate with the FcεRγ dimer that is equipped with an immunoreceptor tyrosine-based activation motif (ITAM). A thorough understanding of a possible molecular crosstalk of these receptors could inform the development of novel immunotherapies.
Employing coarse-grained and all-atom molecular dynamics simulations, we here explore the spontaneous formation, the configurational flexibility, and the stability of FcγRIII-FcεRγ and Mincle-FcεRγ dimers, and characterize their lipid nanoenvironment. We discuss indications for a molecular crosstalk between the Mincle and activating Fcγ immune receptors, and the role of the lipid environment for receptor dimerization and receptor clustering.

PS-V-02

Kinesin-14 HSET and KlpA are non-processive microtubule motors with load-dependent power strokes

Xinglei Liu[1], Lu Rao[1], Florian Berger[2], Weihong Qiu[3], **Arne Gennerich**[1]

[1] Albert Einstein College of Medicine, Biochemistry, Bronx, USA; [2] Utrecht University, Biology, Utrecht, Netherlands; [3] Oregon State University, Biochemistry, Biophysics and Physics, Corvallis, USA

X. Liu was supported by National Institutes of Health grants R01GM098469 and R01GM127922. L. Rao and A. Gennerich were supported by National Institutes of Health grants R01GM098469 and R01NS114636. W. Qiu was supported by National Institutes of Health grant 1R01GM127922.

Introduction

Accurate chromosome segregation during cell division relies on coordinated actions of microtubule (MT)-based motor proteins in the mitotic spindle. Kinesin-14 motors play vital roles in spindle assembly and maintenance by crosslinking antiparallel MTs at the spindle midzone and anchoring spindle MTs' minus ends at the poles. We investigate the force generation and motility of the Kinesin-14 motors HSET and KlpA, revealing that both motors function as non-processive motors under load, producing single ~25 nm power strokes per MT encounter. Each homodimeric motor generates forces of ~0.5 pN, but when assembled in teams, they cooperate to generate forces of 1 pN or more. Importantly, cooperative activity among multiple motors leads to increased MT-sliding velocities. Our findings quantitatively elucidate the structure-function relationship of Kinesin-14 motors and underscore the significance of cooperative behavior in their cellular functions.

PS-V-04

Spectroscopic Characterization of an Active Site Mutant of the Blue Light Receptor Plant Cryptochrome

Axel Jeibmann[1], Lukas Gött-Zink[1], Amir Taslimi[2], Chandra L. Tucker[2], Tilman Kottke[1]

[1] Bielefeld University, Biophysical Chemistry and Diagnostics, Medical School OWL, Bielefeld, Germany; [2] University of Colorado School of Medicine, Department of Pharmacology, Aurora, CO 80045, USA

Introduction

Plant flowering time is a process, which depends on photoactivation of cryptochrome (CRY) by neutral radical (FADH●) formation from the cofactor flavin adenine dinucleotide (FAD). A new CRY-mutant with D393 exchanged to serine (D393S) showed hyperactivation in yeast-based screens. In wild-type CRY this aspartic acid protonates the FAD anion radical (FAD●–) to FADH● as a key step in formation of the signaling state and subsequent conformational changes [1]. The question arises of how the mutation D393S leads to hyperactivation of CRY. Therefore, this D393S mutation was studied in the photolyase homology region of plant CRY (pCRY) from *Chlamydomonas reinhardtii* to understand the biophysical principles of CRY activation.

Methods

The photoreaction of FAD in pCRY-D393S was investigated via time-resolved flash photolysis. Furthermore, the concentration-dependent clustering behavior and resulting light scattering of this mutant was studied UV-vis spectroscopically, supported by data from size exclusion chromatography.

Results

In contrast to the wild-type, the mutant D393S forms oligomers in the dark supporting hyperactivity. No long-lived signaling state of FAD is observed as the FAD●⁻ decays within milliseconds after illumination without formation of FADH●.

Conclusions

The FAD photoreaction is uncoupled from the protein moiety in hyperactive pCRY-D393S, providing a new insight into the activation mechanism of plant CRY.

References

[1] Thöing, C, Oldemeyer, S, Kottke, T, 'Microsecond Deprotonation of Aspartic Acid and Response of the α/β Subdomain Precede C-Terminal Signaling in the Blue Light Sensor Plant Cryptochrome', *Journal of the American Chemical Society*, 137,5990–5999(2015).

PS-V-06

Dual-comb infrared-spectroscopy as a novel approach in temperature-jump experiments investigating polyQ peptide dynamics

Lorenz Mattes[1], Manuel Oestringer[1], Paul Stritt[1], Raphael Horvath[2], Andreas Hugi[2], Karin Hauser[1]

[1] University of Konstanz, Department of Chemistry, Konstanz, Germany; [2] Sensirion AG, IRsweep, Stäfa, Switzerland

Proteins with extensive polyglutamine (polyQ) repeats tend to aggregate, causing diseases like Huntington's. Our studies use β-hairpin peptides based on Trpzip2, incorporating polyQ chains to investigate their impact on folding [1,2]. Complementary methods of time-resolved infrared spectroscopy are used to investigate the folding dynamics of Trpzip-Q_n β-hairpin peptides. The unfolding is triggered by a laser-induced temperature jump (T-jump). A Ho:YAG laser's nanosecond pulse excites the overtone vibration of the solvent D_2O, inducing a T-jump of about 5 K. Secondary structure changes are monitored in the amide I' region by tunable quantum cascade lasers at single wavenumbers (SW-QCLs) and an MCT-detector with nanosecond time-resolution. Alternatively, the peptide sample can be measured in the same beam path with dual-comb QCLs after a simple mirror flip. Our IRis-F1 dual-comb spectrometer, using heterodyne detection in the MHz region, provides microsecond resolution across 200 comb teeth in the range from 1600 to 1660 cm^{-1} [3]. In T-jump experiments of Trpzip-Q2, both methods point out comparable folding dynamics, enabling complementary use in the future. Dual-comb spectroscopy allows rapid, broad-spectrum data acquisition, while SW-QCL measurements with higher time resolution offer better quantitative analysis. Site-specific isotope labels in the backbone and the side chains will reveal deeper insights into glutamine folding mechanisms.

References

[1] Siu HW, Heck B, Kovermann M, Hauser K. 2021 'Template-assisted design of monomeric polyQ models to unravel the unique role of glutamine side chains in disease-related aggregation', *Chem Sci.*, **12**(1): 412-426, DOI: 10.1039/D0SC05299J

[2] Siu, HW, Hauser, K 2022, 'Observation of oligomeric states indicates a high structural flexibility required for the onset of polyQ fibrillization',.*J Phys Chem Lett.*, **13**(20): 4543-4548, DOI: 10.1021/acs.jpclett.2c00203

[3] Hugi A, Villares G, Blaser S, Liu HC, Faist J 2012, 'Mid-infrared frequency comb based on a quantum cascade laser', *Nature.*, **492**(7428):229-233, DOI: 10.1038/nature11620

PS-V-08

cAMP binding to resting pacemaker ion channels is cooperative – a single-molecule study

Stefan Kuschke, Susanne Thon, Christian Sattler, Tina Schwabe, Klaus Benndorf, **Ralf Schmauder**

University Hospital Jena, Friedrich Schiller University, Institute of Physiology II, Jena, Germany

Activation of oligomeric receptors is often fine-tune by cooperativity between subunits. For voltage activated HCN channels, cAMP was shown to regulate channel activation, featuring an intricate cooperativity in binding and activation[1]. These HCN channels generate electric rhythmicity in specialized neurons and cardiomyocytes. Conflicting evidence whether cAMP binding to resting channels is also cooperative was reported previously[2,3].
Here show that cooperativity in ligand binding precedes voltage activation. For this we follow the binding of individual fluorescently labeled cAMP to single, resting, GFP-labeled HCN2 channels in HEK293 derived native membrane sheets using TIRF microscopy. A custom inhibitor protocol was used to block signals from binding to endogenous cAMP-affinities[4].
Kinetic modeling revealed a flip state in both liganded and apo-state. Additionaly, both binding-affinity and flip-state occupation is found to increase with ligand occupancy. The contrast of these finding[4] and previous reports[3] suggest that native membrane environment is required for the observed binding cooperativity. Our data proves that single molecule binding measurements at equilibrium are possible in such membranes.

References

[1] Kusch, J, Thon, S, et al. 2011 “How subunits cooperate in cAMP-induced activation of homotetrameric HCN2 channels.”, *Nature chemical biology* vol. 8,2 162-9. 18 Dec. 2011

[2] Thon, S, Schmauder R, et al. “Conformational Flip of Nonactivated HCN2 Channel Subunits Evoked by Cyclic Nucleotides.” *Biophysical journal* vol. 109,11 (2015): 2268-76.

[3] White, DS, et al. “cAMP binding to closed pacemaker ion channels is non-cooperative.” *Nature* vol. 595,7868 (2021): 606-610.

[4] Kuschke, S, et al. “cAMP binding to closed pacemaker ion channels is cooperative.” *PNAS,* vol. 121,9 (2024): e2315132121.

PS-V-10

Influence of monovalent salts on fibrinogen self-assembly: Morphological and turbidity analysis

Antoine E. Kwame[1], Aparna S. Malisetty[2], Susan Köppen[2], Lucio C. Ciacchi[2], Dorothea Brüggemann[1]

[1] Hochschule Bremen - City University of Applied Sciences, Biophysics and Applied Biomaterials, Bremen, Germany; [2] University of Bremen, Hybrid Materials Interfaces Group, Faculty of Production Engineering, Bremen, Germany

Our recent reports on salt-induced self-assembly of fibrinogen (Fg) showed that monovalent salts induce the formation of nanofibers while divalent salts yield smooth films. To understand the fundamental principle behind this effect, we present our study on Fg assembly from solutions containing various monovalent salts.

Morphological analysis of dried Fg with scanning electron microscopy revealed that macroporous precipitates were formed in the presence of NaCl and KCl. Highly defined nanofibers were found for $NaPO_4$, and less defined nanofibers for KPO_4, both at low Fg concentrations. With high protein concentrations, spongy porous networks formed for both phosphates. For PBS, which contains all of the above ions, we observed nanofibers only at very low Fg concentrations while high concentrations yielded macroporous networks with few nanofibrous features.

In situ monitoring of the turbidity with UV/Vis-spectroscopy during Fg drying revealed salt-specific profiles. For both phosphates, a 2-3 step profile indicated fiber assembly while 1-2 steps at high Fg concentrations indicated the formation of very few or no nanofibers. For NaCl and KCl, 1-2 step profiles were also associated with no fiber assembly, while 2-3 steps for PBS indicated macroporous Fg precipitates with nanofibrous features.

From this, we conclude that phosphate-based monovalent salts induce Fg fiber formation as a function of the salt: Fg ratio, with turbidity profiles serving as a predictor for fiber assembly.

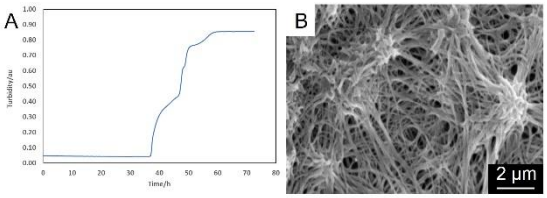


Figure 1: (A) Time-dependent turbidity measurements of fibrinogen drying in the presence of $NaPO_4$ showed a three-step increase, (B) SEM image of fibrinogen nanofibers assembled with $NaPO_4$

Turbidity measurement and SEM image of Fg and NaPO4

PS-V-12

Integrated Structural Characterization of a Novel, Monomeric, Bifurcating Electron Transfer Flavoprotein

Dallas M. Bell[1,4], Hartmut Oschkinat[4], Anne-Frances Miller[3], Berta Martins[2], Maria A. Mroginski[1]

[1] Technische Universität Berlin, Fakultät II – Institut für Chemie, Berlin, Germany; [2] Humboldt-Universität zu Berlin, Department of Biology, Structural Biology, and Biochemistry, Berlin, Germany; [3] University of Kentucky, Department of Chemistry, Lexington, USA; [4] Leibniz-Forschungsinstitut für Molekulare Pharmakologie, Berlin, Germany

It's been established that biology has developed multiple layers of control in redox-active enzymes. Whether it's through large domain-scale movements or seemingly meager interactions as a hydrogen bond, it's critical to understand the synergistic means by which enzymes' structures tailor their functions.

Electron Transfer Flavoproteins (ETFs) are electron carries, found in all kingdoms of life. In certain organisms, there is a subsect called bifurcating ETFs (BfETF), which 'split' a pair of electrons, sending one down an exergonic pathway, paying the thermodynamic price for the other electron to undergo an endergonic reaction. The result is a product with greater reducing power than its reactants, exhibiting a relatively new mechanism of energy conservation. While the reported BfETFs were expressed as heterodimers, we discovered a monomeric BfETF from the thermophilic archeon *Sulfolobus accidocaldarius* (*Sulf*ETF). Initial characterization of *Sulf*ETF shows it to be very stable in a variety of conditions, making it an ideal model to characterize movements, believed to gate electron transfer.

I have employed x-ray crystallography as well as solution and fast MAS solid-state NMR techniques to understand how coenzyme binding and flavin redox state dictate structural changes. Future studies will focus on how the noncovalent interactions in the microenvironment around the two chemically identical flavins tune their reactivity for distinctly different chemistries.

PS-V-14

Thermodynamic characterization of a DNA FRET standard

Josephine Meitzner*, Paul Lehmann*, Vanessa Schumann, Mara Henschel, Richard Börner

University of Applied Sciences Mittweida, Laserinstitut Hochschule Mittweida, Mittweida, Germany

JM, PL, VS and RB received funding from the European Union and the free state of Saxony [ESF Plus: young research group—LaNa-Sax:100649226]. JM and PL would like to thank the "Laserinstitut Mittelsachsen e.V." for the financial support through the "Ausbildungsstipendium". Futhermore JM thanks for the financial support from the "Stipendium zur finanziellen Unterstützung von Masterstudentinnen des Professorinnenprogramms III" of the University of Applied Sciences Mittweida. MH thanks for the financial support within the framework of the ESF Plus doctoral scholarship and the free state of saxony 2023.

*Equally contributing authors

UV-Vis absorption spectroscopy and Förster-Resonance-Energy transfer (FRET) are established methods for the thermodynamic characterization of biomolecules, particularly nucleic acids (NA). Both methods are used to analyse the thermodynamic stability. In addition, UV-Vis spectroscopy is used to study the global folding and FRET reports on specific folding trajectories depending of the chosen labeling position of the FRET pair [1,2].

Here, we study a fluorescently labeled (Cy3/5) DNA duplex closed with a hairpin to observe the temperature-dependent intramolecular NA folding and unfolding. By systematically increasing the concentration of monovalent metal ions (K(I) and Na(I)), the thermodynamic stability, i.e. the melting temperature T_m of the DNA is determined along with the change in Gibbs free energy ΔG and the contribution in enthalpy ΔH, and entropy ΔS [1,3].

Our measurements show the expected two-state folding process, where the global (UV-Vis) and local (FRET) (un-)folding coordinate yield similar T_m, ΔG, ΔH, and ΔS values. Further, we find K(I) and Na(I) to have the same stabilizing effect on the duplex structure as expected. In addition, we characterize the fluorescently labeled DNA via smFRET to determine the labeling efficiency and the two-state folding behavior. This DNA duplex is suitable as a NA-standard to calibrate both temperature dependent UV-Vis absorption and FRET measurements on both, the ensemble and the single molecule level.

References

[1] Mergny, J.-L., Lacroix, L., 2003, 'Analysis of Thermal Melting Curves', *Oligonucleotides*, Vol. 13, Issue 6, pp. 515–537, Mary Ann Liebert Inc

[2] Ha, Taekjip, 2001, 'Single-molecule fluorescence methods for the study of nucleic acids', *Current Opinion in Structural Biology*, Vol. 11, Issue 3, pp. 287–292, Elsevier BV

[3] Böttcher, A., Kowerko, D., Sigel, R. K. O., 2015, 'Explicit analytic equations for multimolecular thermal melting curves', *Biophysical Chemistry*, Vol. 202, pp. 32–39, Elsevier BV

PS-V-16

Compensatory effects of the disordered region of human harathyroid hormone on amyloid aggregation in crowded conditions

Twinkle Bhatia[1], Shubra Sachan[2], Jochen Balbach[2], Maria Ott[1]

[1] Martin Luther University Halle–Wittenberg, Institute for Biochemistry, Halle, Germany; [2] Martin Luther University Halle–Wittenberg, Institute of Biophysics, Halle, Germany

We acknowledge funding from the German research foundation (DFG, - TRR 102) " Polymers under multiple constraints",TP B12.

Human parathyroid hormone (PTH), comprising 84 amino acids, is secreted by the parathyroid glands and plays a pivotal role in regulating blood serum phosphate and calcium levels. PTH is also characterized as a functional amyloid due to its ability to store itself in the form of amyloid prior to its release.The N-terminal residues (S1-Q29) have a helical propensity, while residues (R25-L37) play a significant role in fibril formation[1]. PTH is largely disordered, as its C-terminus (D30-Q84) constitutes the intrinsically disordered section of PTH.
In this study, we investigated the role of the intrinsically disordered C-terminal region on pre-fibrillar aggregates by comparing sizes and structures of oligomers of the truncated variant and the full length protein using spectroscopic techniques as Fluorescence Correlation Spectroscopy (FCS) and Infrared (IR) spectroscopy
In the conclusion,we found that the absence of the C-terminal region lead to smaller oligomer and nuclei sizes as well as to accelerated fibrillation[2]. Moreover, using macromolecular crowding conditions, we could assign the differences in fibrillation kinetics, sizes, and structural changes to the compensatory effect of the intrinsically disordered region.

References

[1] Lauth, L. M., Voigt, B., Bhatia, T., Machner, L., Balbach, J., & Ott, M. (2022). Heparin promotes rapid fibrillation of the basic parathyroid hormone at physiological pH. FEBS letters, 596(22), 2928–2939. https://doi.org/10.1002/1873-3468.14455

[2] Voigt, B., Bhatia, T., Hesselbarth, J., Baumann, M., Schmidt, C., Ott, M., & Balbach, J. (2023). The Prenucleation Equilibrium of the Parathyroid Hormone Determines the Critical Aggregation Concentration and Amyloid Fibril Nucleation. Chemphyschem : a European journal of chemical physics and physical chemistry, 24(19), e202300439. https://doi.org/10.1002/cphc.202300439

PS-V-18

Quantitative hyperpolarized 129Xe NMR on cryptophane-A conjugated SARS-CoV2 receptor binding domain

Thomas Riemer[1,2], Patrick Neckermann[3], Martina Billmeier[3], Wolfgang Kilian[4], Ralf Wagner[3,5], Lorenz Mitschang[1]

[1] Physikalisch-Technische Bundesanstalt / National Metrology Institute, Dep. 8.3, Biomedical Optics, Berlin, Germany; [2] University of Leipzig, Institute for Medical Physics and Biophysics, Leipzig, Germany; [3] University of Regensburg, Institute of Medical Microbiology and Hygiene / Molecular Microbiology (Virology), Regensburg, Germany; [4] Physikalisch-Technische Bundesanstalt / National Metrology Institute, Dep. 8.1, Biomedical Magnetic Resonance, Berlin, Germany; [5] University Hospital Regensburg, Institute of Clinical Microbiology and Hygiene, Regensburg, Germany

This work was supported by the Federal Ministry of Education and Research (No. 03VP08891).

Introduction

Chemical Exchange Saturation Transfer NMR spectroscopy on hyperpolarized 129Xe (hyperCEST) allows nano molar detection of small, xenon-binding host molecules, like cryptophane-A-mono-acid (CrAma), [1]. By experimental variation of radio frequency saturation power and dissolved 129Xe concentration a calibration free quantification of CrAma could be established; only the solubility of Xe in the solvent needs to be known [2]. The so far successfully established model for the quantification of Xe binding to CrAma alone is revised to quantify conjugates of CrAma and the SARS-CoV2 receptor binding domain (RBD-CrA).

Methods

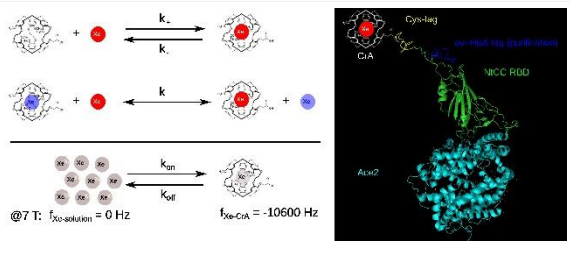
Two RBD-CrA conjugates were synthesized, either with or without an additional GEEK amino acid linker, via a reactive cysteine-containing-tag, using maleimide coupling https://www.sciencedirect.com/science/article/pii/S0939641123002588#s0010 [3]. 129Xe NMR-experiments - standard 1D and hyperCEST - were performed on a 7 T Bruker NMR spectrometer operated inline with a Xe-polarizer featuring the adjustment of the Xe partial pressure [4].

Results

1D 129Xe NMR spectroscopy revealed a successful RBD-CrA synthesis, with 20 to 30 % of the RBD conjugated. 129Xe NMR-lineshape analysis, 1D and hyperCEST, indicated, that the analysis model established in [1] and [2], needs to be completed for T2 relaxation, and distribution of RBD-CrA-Xe -binding sites.

Conclusions

Results towards the absolute quantification of RBD-CrA protein by hyperpolarized 129Xe NMR are presented.



RBD-CrA quantification by 129Xe hyperCEST NMR spectroscopy

The associative and dissociative rate constants k_+ and k_- as well as a degenerate rate constant k are depicted in the upper two reaction equations. The NMR spectroscopically detectable pools for bulk and cage bound Xe are ~ 10600 Hz apart @B_0=7 T and connected by the rate constants k_{on} and k_{off}. At the right the SARS-CoV2 receptor binding domain (RBD) is graphically shown (green) binding to a ACE2 receptor (cyan). RBD is modified by a His-tag for purification (blue) and a N-terminal Cys-tag (yellow) for maleimid conjugation of CrA cage molecule. The latter can reversibly bind a Xe atom.

References

[1]
Sergey E. Korchak, Thomas Riemer, Wolfgang Kilian and Lorenz Mitschang, Quantitative biosensor detection by chemically exchanging hyperpolarized 129Xe, Phys. Chem. Chem. Phys., 2018, 20, 1800–1808.

[2]
Lorenz Mitschang, Sergey Korchak, Wolfgang Kilian, and Thomas Riemer, Comprehensive Quantitative and Calibration-Free Evaluation of Hyperpolarized Xenon–Host Interaction by Multiparametric NMR, Anal. Chem. 2022, 94, 2561–2568.

[3]
Clara Barbey, Jinpeng Su, Martina Billmeier, Nadine Stefan, Romina Bester, George Carnell, Nigel Temperton, Jonathan Heeney, Ulrike Protzer, Miriam Breunig, Ralf Wagner, David Peterhoff, Immunogenicity of a silica nanoparticle-based SARS-CoV-2 vaccine in mice, European Journal of Pharmaceutics and Biopharmaceutics 192 (2023) 41–55.

[4]
Sergey E. Korchak, Wolfgang Kilian, Lorenz Mitschang, Configuration and Performance of a Mobile 129Xe Polarizer, Appl Magn Reson (2013) 44:65–80.

PS-V-20

Intertwined dimer of human PTK6 SH3 domain mediates kinase activity?

Lisa Kupsch, Luisa Buhl, Dilara Oeguetcue, Ines Liebscher, **Sandra Berndt**

University of Leipzig, RSI of Biochemistry, Leipzig, Germany

Introduction

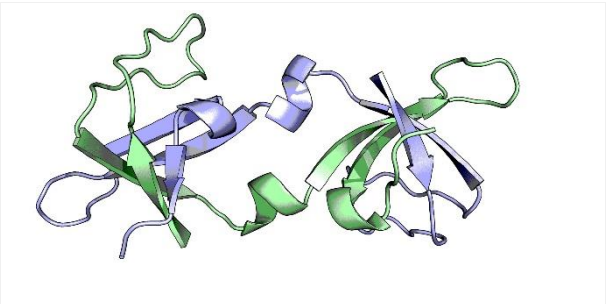
Protein tyrosine kinase 6 (PTK6, or breast tumor kinase, Brk) is abundant in multiple tumor types like breast and prostate cancer [1,2]. PTK6 expression levels correlate with tumor grade and invasiveness. There is only limited structural and functional data of PTK6 available. PTK6 is closely related to Src family kinases, containing functional domains (SH3, SH2, and kinase domain) and mediates regulation of multiple signaling pathways through Src kinase, MAPK p38 and ERK 5 activation [3,4]. However, the activation mechanism is not well understood.

Methods

X-ray crystallography, analytical gel filtration, NanoBiT complementation assay, docking

Results

Here we present a novel intertwined dimer crystal structure of the human PTK6 SH3 domain at 1.75 Å. The dimerization process results in a domain swap architecture, which is a result of a distinctive dimerization mechanism. Comparable dimers were reported for kinases like Src kinase, Eps8, and Nck1. Hence, the functional role of this dimerization process is still to be determined. So far, we found that PTK6 SH3 domain dimerization is inducible *in vitro*. Furthermore, we observed the dimerization *in cells* using multiple cell lines. Applying various PTK6 constructs, we found that the dimerization is SH3 dependent and that multiple amino acids enhance or diminish the dimer formation. A detailed understanding of the dimerization driven PTK6 activation mechanism may allow new a drug targeting approach of this kinase.



Intertwined dimer of human PTK6 SH3 domain
PTK6 SH3 domain crystal structure with an atomic resolution of 1.75Å. The protein was expressed in E. coli and purified using affinity as well as size exclusion chromatography.

The structure model of the dimer was refined to an Rwork of 19.4 (Rfree of 21.9). The overall architecture of the intertwined PTK6 dimer is nearly identical to the monomer, with a RMSD value of 1,007.

References

[1] Ang, HL, Yuan, Y 2021, 'Putting the BRK on breast cancer: From molecular target to therapeutics.', *Theranostics*, 11 (3), 1115-1128.

[2] Alwanian, WM, Tyner, AL 2020, 'Protein tyrosine kinase 6 signaling in prostate cancer', *American journal of clinical and experimental urology*, 8 (1), 1-8.

[3] Locatelli, A, Lofgren, KA, 2012, 'Mechanisms of HGF/Met signaling to Brk and Sam68 in breast cancer progression', *Hormones & cancer*, 3 (1-2), 14-25.

[4] Alwanian, WM, Vlajic, K, 2022, 'Protein tyrosine kinase 6 regulates activation of SRC kinase', *The Journal of biological chemistry*, 298 (11), 102584.

PS-V-22

The coiled-coil of Sla2 acts as a regulatory hub in endocytosis, connecting the plasma membrane to the cytoskeleton

George Draper-Barr, Lucas Defelipe, Maria Garcia Alai

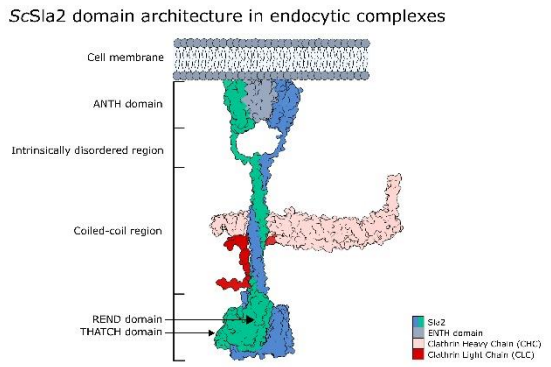
European Molecular Biology Laboratory Hamburg, Hamburg, Germany

Katharina Veith for yeast strain production and validation.

Introduction and current results:
Sla2, an endocytic actin-binding protein homologous to HsHip1R, connects the membrane to the actin cytoskeleton through a network of regulatory protein-protein complexes in the endocytic pit. The regulation of Actin binding by Sla2 is coordinated by Clathrin Light Chain, which in turn modulates the Clathrin coat stiffness. We describe two independent binding sites for CLC within of Sla2 in the micromolar and nanomolar ranges, the nanomolar affinity site is a novel discovery seen in Fungi. This event has an allosteric conformational selection of the Sla2 THATCH domain, providing novel structures of the Apo and Closed states via cryo-EM and completing more to the picture of inhibition of Actin binding by Clathrin Light Chain.

Methods:

- MicroScale Thermophoresis using RED-NHS labelled polypeptide constructs of the coiled-coil of ScSla2 and ScCLC.
- Mass Photometry to captue the stoichiometry of the complex
- QuikChange mutagenesis of Sla2 constructs to remove the binding of CLC to the coiled-coil.
- Cryo-electron microscopy of ScSla2 with and without molar excess of ScCLC to determine the Apo and Closed state of the C-terminal domains of Sla2**Experimental Directions:**
- Fluorescence microscopy of S. cerevisiae to characterise endocytic dynamics. Endogenously knocked-in Ent1-mNeonGreen and ABP1-mTurquoise2 was used alongside transformed plasmids containing the complete ScSla2 5'UTR + cDNA and a C-terminal mScarlet-I tag. (Currently underway)



The complex of Sla2 with the primary interaction partners in the endocytic pit
Sla2 (Hip1R in Metazoa) forms the membrane binding complex with Epsin proteins. The Clathrin heterodimer negatively regulates Actin binding of the THATCH domain in the C-terminus of Sla2. In this study we have probed the properties of this regulation on endocytic dynamics and the interaction mechanisms of the two sites for CLC on the Sla2 coiled-coil region.

References

[1] Garcia-Alai, M. M. et al. Epsin and Sla2 form assemblies through phospholipid interfaces. Nat. Commun. (2018) doi:10.1038/s41467-017-02443-x.
[2] Brett, T. J., Legendre-Guillemin, V., McPherson, P. S. & Fremont, D. H. Structural definition of the F-actin-binding THATCH domain from HIP1R. Nat. Struct. Mol. Biol. 13, 121–130 (2006).
[3] Ren, Y., Yang, J., Fujita, B., Zhang, Y. & Berro, J. A mechanical circuit in End4p coordinates force transmission during clathrin-mediated endocytosis. bioRxiv 2023.10.23.563344 (2023) doi:10.1101/2023.10.23.563344.
[4] Wilbur, J. D. et al. Actin Binding by Hip1 (Huntingtin-interacting Protein 1) and Hip1R (Hip1-related Protein) Is Regulated by Clathrin Light Chain*. J. Biol. Chem. 283, 32870–32879 (2008).
[5] Boettner, D. R., Friesen, H., Andrews, B. & Lemmon, S. K. Clathrin light chain directs endocytosis by influencing the binding of the yeast Hip1R homologue, Sla2, to F-actin. Mol. Biol. Cell 22, 3699–3714 (2011).

PS-V-24

Structural Impact of N-terminal Pyroglutamate in an Amyloid-β(3-42) Fibril Probed by Solid-State NMR Spectroscopy

Luis Gardon[1,2], Nina Becker[1,2], Lothar Gremer[1,2], Henrike Heise[1,2]

[1] Heinrich-Heine University, Institut für Physikalische Biologie, Düsseldorf, Germany; [2] Forschungszentrum Jülich, Strukturbiochemie - IBI-7, Jülich, Germany

The authors gratefully acknowledge experimental assistance by Robin Backer and Celina Schulz, Heinrich-Heine-Universität Düsseldorf (HHU). Access to the Jülich-Düsseldorf Biomolecular NMR Center jointly run by Forschungszentrum Jülich and HHU is acknowledged. HH was supported by the Entrepreneur Foundation at HHU and the DFG (HE 3243/4-1 and INST 208/771-1 FUGG). NMR data are deposited in the Biological Magnetic Resonance Data Bank (BMRB) under accession number 51993. Open Access funding enabled and organized by Projekt DEAL.

Introduction

Extracellular amyloid-β (Aβ) plaques, primarily formed by Aβ(1-40) and Aβ(1-42) fibrils, are a hallmark of Alzheimer's disease. The Aβ peptide undergoes various post-translational modifications including pyroglutamate (pGlu, pE) formation at N-terminal E3 or E11. In contrast to pEAβ(3-42) fibrils, pEAβ(3-40) fibrils and pEAβ(11-40) fibrils have already been well characterized by solid-state NMR [1] [2].

Methods

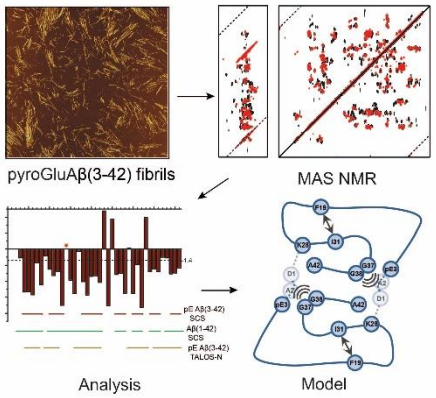
Here we investigated structural similarities and differences between pEAβ(3-42) and LS-shaped Aβ(1-42) [3] fibrils grown under identical conditions (pH 2) using 2D Proton Driven Spin Diffusion (PDSD) ^{13}C-^{13}C, 3D NCACX/NCOCX and water edited T2 filter experiments.

Results

We report a *de novo* resonance assignment for pEAβ(3-42) fibrils at pH 2 except H14. Chemical shift comparison of pEAβ(3-42) and LS-shaped Aβ(1-42) reveal changes at the N- and C-terminus but similarities in the core region. ^{13}C-^{13}C spin diffusion spectra at rotational resonance indicate a close spatial proximity of F19 and I31 sidechains. pEAβ(3-42) fibrils retain their overall structure upon a pH shift from pH 2 to pH ~7.

Conclusions

The central region of pEAβ(3-42) fibrils including the turn region around V24 is almost identical to Aβ(1-42). However, the missing N-terminal residues D1-A2 along with pE3 formation in pEAβ(3-42) preclude a salt bridge between K28-D1' observed in Aβ(1-42) fibrils. The modified N-terminus remains rigid over ~five pH units.



Structural Impact of N-terminal Pyroglutamate in an Amyloid-β(3-42) Fibril Probed by Solid-State NMR

Solid state NMR spectroscopy on fibrils of recombinant, pyroglutamylated [U-^{13}C,^{15}N]- pEAβ(3-42) show highly similar β-strand positions and a conserved turn region around V24 comparable to LS-shaped Aβ(1-42) fibrils. Due to missing D1-A2 and pE3 formation a salt bridge of K28-D1' is absent in pEAβ(3-42) fibrils. G37-G38 act as sensors for the N-terminal modifications in pEAβ(3-42) fibrils.

References

[1] Scheidt H. A. , Adler J., Krueger M. , Huster D., 2016, ‘Fibrils of Truncated Pyroglutamyl-Modified Aβ Peptide Exhibit a Similar Structure as Wildtype Mature Aβ Fibrils’, *Sci. Rep.*, 6, 33531, Leipzig

[2] Scheidt H. A, Adler J, Zeitschel U.,Höfling C.,Korn A.,Krueger M.,Roßner S.,Huster D. 2017, ‘Pyroglutamate-Modified Amyloid β (11- 40) Fibrils Are More Toxic than Wildtype Fibrils but Structurally Very Similar’, *Chem. Eur. J.*, 23, 15834–15838, Leipzig

[3] Gardon L, Becker N, Gremer L, Heise H, 2023, ‘Structural Impact of N-terminal Pyroglutamate in an Amyloid-β(3-42) Fibril Probed by Solid-State NMR Spectroscopy’, Chem. Eur. J., 30, e202303007, Düsseldorf

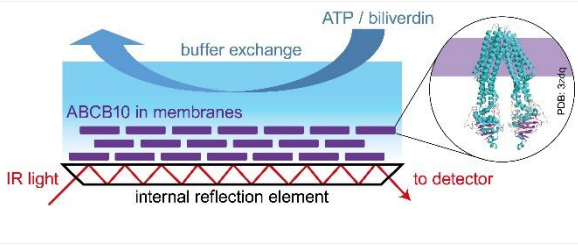
PS-V-26

Perfusion-Induced ATR FTIR Difference Spectroscopy on a Human ABC Transporter

Corinna Strothenke, Lukas Goett-Zink, Tilman Kottke

Bielefeld University, Biophysical Chemistry and Diagnostics, Bielefeld, Germany

ATP binding cassette (ABC) transporters are an important class of membrane proteins using ATP hydrolysis for the active transport of substrates across membranes. Many human ABC transporters are medically relevant, e.g. as key components in multidrug resistance in cancer therapy. Here IR spectroscopy has been applied to investigate the human mitochondrial transporter ABCB10 which protects cells from oxidative stress by exporting biliverdin [1]. Attenuated Total Reflection (ATR) FTIR spectroscopy is a powerful tool for the non-invasive investigation of the mechanism of membrane proteins. The immobilization on the internal reflection element enables to exchange the buffer solution above an adsorbed sample film by perfusion. Detected difference signals can then be assigned to originate from the stimulus by the change of conditions. We developed an attachment for a diamond ATR cell to produce a flow cell with only 500 µL volume and exchanged the buffer using a syringe pump. The activation of ABCB10 by ATP addition was characterized using perfusion-induced ATR FTIR difference spectroscopy. ATP hydrolysis and accompanying changes in α-helical secondary structures were observed and assigned. By measuring under turnover conditions, the response of ABCB10 to biliverdin was analyzed, revealing additional secondary structure changes.
These results by IR spectroscopy show that ABCB10 in membranes undergoes defined structural changes that differ between futile and transport cycles.



Perfusion-Induced ATR FTIR Difference Spectroscopy on ABCB10
ABCB10 in membranes was immobilized on the internal reflection element of an ATR cell. By perfusing ATP and biliverdin, the structural changes of ABCB10 during the transport cycle are observed by IR spectroscopy.

References
[1] Shum, M, Shintre, CA, Althoff, T, Gutierrez, V, Segawa, M, Saxberg, AD, Martinez, M, Adamson, R, Young, MR, Faust, B, Gharakhanian, R, Su, S, Krishnan, KC, Mahdaviani, K, Veliova, M, Wolf, DM, Ngo, J, Nocito, L, Stiles, L, Abramson, J, Lusis, AJ, Hevener, AL, Zoghbi, ME, Carpenter, EP, Liesa, M 2021, 'ABCB10 exports mitochondrial biliverdin, driving metabolic maladaptation in obesity.' *Sci. Transl. Med.*, 13(594).

PS-V-28

Extended or Hairpin? The Conformation of Pyroglutamated Amyloid β (3–40) and (11–40) Monomer in Fibrils

Holger A. Scheidt[1], Alexander Korn[1], Benedikt Schwarze[1], Martin Krueger[2], Daniel Huster[1]

[1] University Leipzig, Institute for Medical Physics and Biophysics, Leipzig, Germany; [2] University Leipzig, Institute of Anatomy, Leipzig, Germany

Introduction

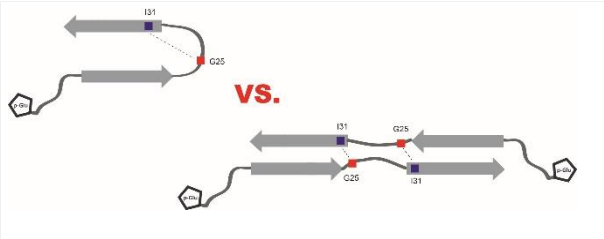
Amyloid β (Aβ) is a hallmark protein of Alzheimer's disease. One physiologically important variant is formed by N-terminal truncation at the glutamic acid in position E_3 or E_{11} and the cyclization into a pyroglutamate. Both peptides are found especially in the core of amyloid plaques. However, the molecular structure of formed fibrils is not clear since a molecular contact between Gly_{25} and Ile_{31}, which disagree with the common hairpin model of WT-$Ab_{1–40}$ fibrils, was reported.

Methods

We investigated the conformation of the monomers of pE_3-Aβ, and pE_{11}-Aβ and WT-Aβ fibrils to determine whether the hairpin or a new extended structure dominates the monomer structure in fibrils. Solid-state NMR spectroscopy was used probing the inter-residual contacts between Phe_{19}/Leu_{34}, Ala_{21}/Leu_{34}, and especially Gly_{25}/Ile_{31}. Additionally, the flexible turn of the $Aβ_{40}$ peptides was replaced by a AMPP-based photoswitch to predefine the peptide conformation to extended (*trans*) or hairpin (*cis*). This allows *in situ* spectroscopic investigation of the conformation of the photoswitch during the fibrillation process in contrast to the structural techniques, which can only be applied to stable conformers.

Results / Conclusions

Both methods confirm an extended structure for the peptide monomers in fibrils of all Aβ variants. Especially the contact Gly_{25}/Ile_{31} is a decisive indicator for the extended structure along with the characteristic absorption spectra of *trans*-AMPP-Aβ.



Extended or Hairpin?

PS-V-30

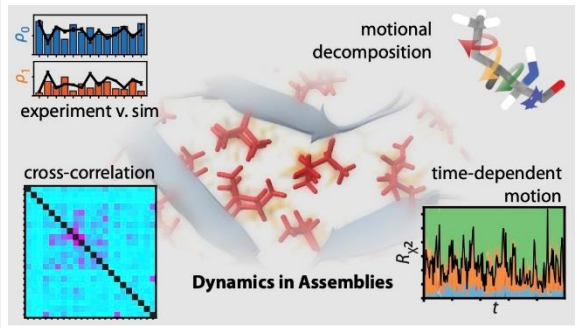
Characterizing Methyl Dynamics in HET-s(218-289) Fibrils with NMR and MD

Kai Zumpfe[1], Mélanie Berbon[2], Birgit Habenstein[2], Antoine Loquet[2], Albert A. Smith-Penzel[1]

[1] Universität Leipzig, Institut für Medizinische Physik und Biophysik, Leipzig, Germany; [2] University of Bordeaux, Bordeaux, France

This project was funded through the Deutsche Forschungsgemeinschaft (DFG) grant 450148812 (A.A.S.), the European Social Funds (ESF) and the Free State of Saxony Junior Research Group UniDyn Project No. SAB 100382164 (A.A.S., K.Z.), and the CNRS.

Side-chain motions are critical to determining protein structure, dynamics, and interactions. However, a lack of analytical tools limits our understanding of such dynamics. We present an approach here combining experiment and simulation (nuclear magnetic resonance relaxation and molecular dynamics simulation) to characterize to a high level of detail the methyl dynamics in HET-s(218-289) fibrils. Experimental data is used to help select optimal simulations, whereas careful separation of motions in simulation is then used to guide our interpretation of experimental results. We are able to characterize methyl dynamics as a function of timescale, further attributing the experimental results to a combination of methyl hopping and libration, and rotameric hops. Based on further MD analysis, we are also able to establish unexpected entanglements between dynamics in different parts of the system, explaining a dependence of backbone dynamics on methyl rotation rates, and also identifying coupling networks within the side-chain rotameric states. The resulting methods offer a useful approach to more complex protein systems where side-chain dynamics play a critical role in signal transduction.



Characterizing methyl dynamics in HET-s fibrils

References

[1] Zumpfe, K. Berbon, M., Habenstein, B., Loquet, A., Smith, A.A. 'Analytical Framework to Understand the Origins of Methyl Side-Chain Dynamics in Protein Assemblies', *J. Am. Chem. Soc.* 146, 8164-8178

PS-V-32

Investigating the R-loop formation by CRISPR-Cas9 with ultrafast single-molecule twist measurements

Fabian Welzel[1], Julene Madariaga-Marcos[1], Dominik J. Kauert[1], Dina Grohmann[2,3], Ralf Seidel[1]

[1] Universität Leipzig, Peter Debye Institute of Soft Matter Physics / Group Molecular Biophysics, Leipzig, Germany; [2] University of Regensburg, Institute of Microbiology & Archaea Centre, Regensburg, Germany; [3] University of Regensburg, Regensburg Center of Biochemistry (RCB), Regensburg, Germany

Introduction

The CRISPR nuclease Cas9 is a target for genome-editing, since it can be programmed to bind practically any genomic target by its intrinsic RNA component. Cas9 interrogates DNA looking for target sequences that are complementary to the RNA. Upon target recognition, base pairing between RNA and the DNA target strand causes the formation of a so-called R-loop, which has a considerable tolerance for mismatches. To prevent off-targeting, quantitative modelling of the recognition process would be desirable, which requires knowledge about the energy landscapes of the R-loop formation.

Methods

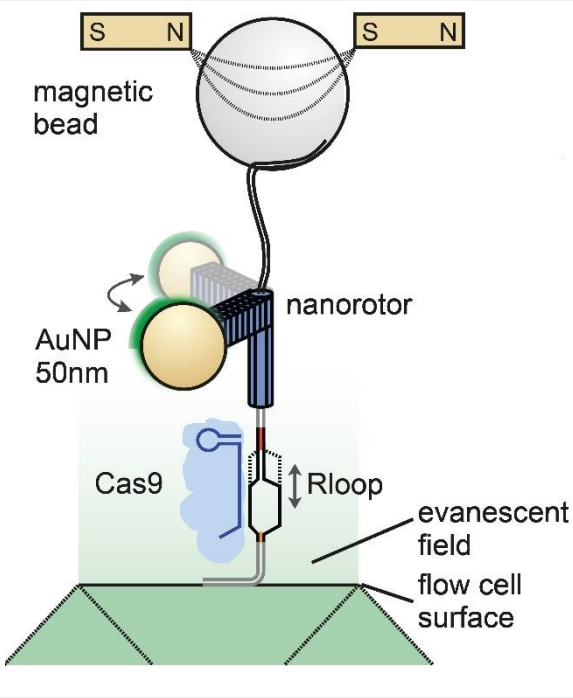
To determine such energy landscapes we measure DNA unwinding during R-loop formation using ultrafast twist measurements. Using DNA origami nanostructures as rotor arms, directly reporting about twist changes on a millisecond time scale, we can resolve real-time R-loop dynamics of CRISPR-Cas effector complexes at the single base-pair level and construct corresponding energy landscapes.

Results

With our approach we achieve unprecendented spatio-temporal resolution which allows to resolve different states during R-loop formation that are in agreement with structural observations. Furthermore, the energy landscapes reveal an uphill bias towards the full R-loop state indicating the protein's intrinsic mechanism of off-target discrimination.

Conclusions

We achieve unprecedented insight in the dynamics of R-loop formation by Cas9 and the impact of mismatches on this process.



Single-molecule nanorotor measurements of R-loop formation by CRISPR-Cas9
Schematic representation of the nanorotor configuration used in a magnetic tweezers setup. The DNA sequence of interest is attached on its bottom end to the surface of the fluidic cell and on its top end to the nanorotor consisting of a DNA origami nanostructure. At the end of the rotor arm a 50 nm AuNP is attached. The top of the nanorotor is connected to a magnetic bead that allows stretching and twisting of the DNA. Imaging the light that is backscattered from the AuNP allows detection of the nanorotor rotations and thus monitoring the DNA untwisting during R-loop formation by CRISPR-Cas9.

PS-V-36

Investigating the structure and dynamics of protein condensate models with infrared spectroscopy

Avinash Chettri[1], Till Stensitzki[1], Anna Zehle[1], Simone Techert[2,3], Henrike Mueller-Werkmeister[1]

[1] University of Potsdam, Institute of Physical Chemistry, Potsdam, Germany; [2] DESY, Photon Science Department, Hamburg, Germany; [3] Georg-August-Universität Göttingen, Institute for X-ray Physics, Göttingen, Germany

Protein condensates are macromolecular biological assemblies formed via liquid-liquid phase separation and play a key role in regulating processes associated with cellular biology. However, the structure and corresponding dynamics of protein condensates at the molecular level are still not clearly understood due to their inherently disordered structure making their study difficult with standard structural biology methods. Reliable information on the structure and dynamics of such condensates can be accessed with infrared spectroscopy, a technique which not only reports on molecular vibrations that are unique to a functional group but also reports on the effect of the local environment on such vibrations, thereby tagging the relevant functional group as a molecular probe. Therefore, in relation to the structure and dynamics associated with protein condensates, this contribution explores the environment-related response of the backbone amide-I stretching vibration in protein condensate models with Fourier-transform and 2D-IR spectroscopy as the key techniques.

PS-V-38

Structural basis for the transmembrane signaling of Trk receptors and antidepressant-induced activation of the receptor tyrosine kinase TrkB

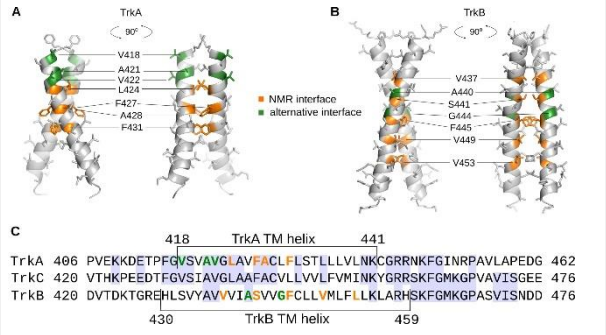
Erik Kot[2], Maria L. Franco[3], Kirill Nadezhdin[5], Daniel McKenzie[4], Kalina Hristova[4], Sergey Goncharuk[2], Marcal Vilar[3], **Konstantin Mineev**[1]

[1] Goethe University Frankfurt, Institute of Organic Chemistry and Chemical Biology, Frankfurt am Main, Germany; [2] Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russia; [3] Instituto de Biomedicina de Valencia-CSIC, Valencia, Spain; [4] Johns Hopkins University, Baltimore, USA; [5] Columbia University, New York, USA

The work of K. Mineev was supported by funding in the group of prof. H. Schwalbe, a project within the Collaborative Research Center #1507.

Neurotrophin receptors of the Trk family play a critical role in regulating brain development and neuroplasticity, making them valuable targets for drugs aimed at treating cancer, stroke, depression, and other conditions. The structures of Trk protein domains in various states upon activation must be elucidated to allow rational drug design. Despite this need, the conformations of the transmembrane and juxtamembrane domains of Trk receptors remained largely unexplored.

In this study, we employed solution NMR spectroscopy to determine the structures of the dimeric transmembrane domains of TrkA and TrkB receptors within a lipid environment. These structures were validated through mutagenesis, functional assays, and fluorescent microscopy, confirming that the identified conformations correspond to the inactive and active states of the receptors, respectively. An alternative active state of TrkA was proposed based on cross-linking experiments. The investigation of TrkA extracellular juxtamembrane regions revealed that they are unstructured and are likely to interact with the neurotrophin in the activated receptor state. Further studies on TrkB interaction with the antidepressant fluoxetine, LSD, and antipsychotic chlorpromazine led to a coherent model describing how fluoxetine activates the receptor by binding to its transmembrane domain.



Comparison of transmembrane domain dimer conformations within the Trk family
A,B - the spatial structures of TrkA (A) and TrkB (B) TM dimers obtained by NMR in DPC micelles and DMPC/DHPC bicelles, respectively. Orange color denotes the residues on the experimentally determined interface. Green color indicates the residues proposed for the alternative dimerization interfaces, assumed from the cross-linking experiments (A) or computer modeling (B) **C** - Sequence alignment of Trk family members based on the Clustal Omega program (http://www.clustal.org/) results for alignment of the transmembrane and intracellular domains.

PS-V-40

The journey to a supertight and stable DNA origami cage for drug delivery

Merle Scherf[1,2], Florian Scheffler[1], Christopher Maffeo[3], Ulrich Kemper[2], Jingjing Ye[2,4], Aleksei Aksimentiev[3,5], Ralf Seidel[2,4], Uta Reibetanz[1]

[1] University of Leipzig, Medical Faculty / Institute for Medical Physics and Biophysics, Leipzig, Germany; [2] University of Leipzig, Peter Debye Institute for Soft Matter Physics/ Faculty of Physics and Earth Science, Leipzig, Germany; [3] University of Illinois at Urbana–Champaign, Department of Physics, Urbana, Germany; [4] University of Dresden, Cluster of Advancing Electronics Devices Dresden, Desden, Germany; [5] University of Illinois at Urbana-Champaign, Department of Physics and Beckman Institute for Advanced Science and Technology, Urbana, Germany

The authors acknowledge funding from the Deutsche Forschungsgemeinschaft (DFG), in particular from grant RE 2681/5-1 to U. Reibetanz as well as grant SE 1646/8-2 and the GRK 2767 (project number 451785257) to R. Seidel.

Introduction

A drug delivery system has to fulfil many criteria. Mainly, it has to avoid opsonization in the blood stream and has to be biocompatible as well as biodegradable in human body. While several different approaches have been investigated over time so far, DNA recently attracted attention, in particular folded as a DNA origami nanostructure.

Results

In our approach, we use a rectangular DNA tube with a cavity that can be filled with a defined number of cargo molecules by binding them to complementary ssDNA strands, which are attached in the inner walls of the tube. Two lids on each side of the tube specifically bind via 2nt overhangs to generate a closed cavity. Moreover, a detachment DNA strand was used to disconnect the cargo molecules, resulting in a freely movable cargo. The stability of the system was enhanced regarding extracellular and intracellular conditions by coating with positively charged polylysine-PEG blockpolymers resulting in electrostatic interactions with the negatively charged DNA. With that, a long time stable carrier system for a freely movable cargo was designed and tested against different media and DNase1 resistance.

Conclusions

Taking advantage of the capability of DNA being easily modified, forming strong hydrogen bonds and allowing electrostatic interaction of the negatively charged backbone, we were able to form an efficient drug delivery system that can trap a high amount of cargo and is resistant against nucleases for a certain time.

References

[1] Scherf, M. et al. 2022, ‘Trapping of protein cargo molecules inside DNA origami nanocages.’, *Nanoscale*, 14, 18041-18050.

PS-V-42

Transmembrane receptors of the apical junctional complex segregate by size

Lennart Kleinschmidt[1], Daxiao Sun[1], Tom Borianne[1], Alf Honigmann[1,2]

[1] Technische Universität Dresden, Biotechnology Center, Center for Molecular Bioengineering, Dresden, Germany;
[2] Technische Universität Dresden, Physics of Life, DFG Cluster of Excellence, Dresden, Germany

In the human body, epithelial tissue serves as the primary physical barrier, protecting it against the external environment and separating functionally distinct compartments within. For epithelial cells, the apical junctional complex (AJC), composed of tight junctions (TJs) and adherens junctions (AJs), plays a crucial role. It regulates their basal-apical polarity, mediates the connection between adjacent cells and seals the emerging tissue by restricting paracellular diffusion. EM imaging and recent studies with super-resolution microscopy have shown the segregation of TJs, positioned further towards the apical site, and AJs, located further to the basal site of Epithelial cells. However, the underlying mechanisms, determining the localization and organization of proteins at the AJC and leading to this segregation have yet to be understood.
Combining bottom-up reconstitution and cell biology approaches, we found that junctional receptors segregate depending on the size of their extracellular domains. Segregated TJs and AJs are essential as they induce condensation of junction-related scaffolds important for the AJCs function. More broadly, our work indicates that physical properties of proteins, like size, sort different membrane-associated complexes in cells and transduce specific downstream signals.

PS-V-02-Y

Super-resolution Infrared Hyperspectral Imaging of Microbial Consortia

Felix H. Patzschke[1], Valentina Schmitz[2], Rohan Karande[2,3], Frank Cichos[1]

[1] Leipzig University, Peter Debye Institute for Soft Matter Physics, Leipzig, Germany; [2] Leipzig University, Institute of Biochemistry, Leipzig, Germany; [3] Helmholz Centre for Environmental Research, Leipzig, Germany

Photothermal Infrared Spectroscopy (PTIR) is a powerful new imaging technique, leveraging infrared absorption spectroscopy and imaging in visible light, enabled through the photothermal effect. Therein, the infrared absorption, which provides chemical resolution but is commonly restricted to a spatial resolution around 10 µm, is enhanced by an optical detection of the IR generated local refractive index changes, achieving a spatial resolution of a few 100 nm. This label-free, high-resolution technique represents a significant advancement in the study of biological systems and their applications in sustainable technologies.
In particular, we investigate mixed-species biofilms, which are a core component in the development of modern bio-reactors aimed at producing cost-effective animal feed whilst combating environmental challenges such as plastic waste and climate change. Through PTIR microscopy we aim to quantify the concentrations of nutrients as well as the chemical signatures of different microbial species to gain precise, quantitative information about the biological composition and growth behaviour of such biofilms. This will facilitate more effective optimization of bioreactor designs than what is feasible with traditional optical methods.

PS-VI-02

Unravelling Structural and Dynamic Changes of the Human Neuropeptide Y Type 4 Receptor (hY_4R) upon Specific Peptide Ligands Activation via Solid-State NMR Spectroscopy and Computational Analysis

Marta Gozzi, Albert A. Smith, Maik Pankonin, Alexander Vogel, Peter W. Hildebrand, Daniel Huster

Leipzig University, Institute for Medical Physics and Biophysics, Leipzig, Germany

We thank the Deutsche Forschungsgemeinschaft (DFG) for financial support (CRC1423, project number 421152132, subprojects A04, C01, Z03).

Introduction

Within the class of rhodopsin-like, peptide-binding G-protein coupled receptors (GPCRs), the human neuropeptide Y receptor (hY_4R) is involved in the regulation of anorexigenic effects and plays a key role in the insurgence of obesity and anorexia. Its complex energy landscape of activation and downstream signalling profiles makes it very difficult to understand the structure–dynamic–function relationships.[1,2]

Methods

We use a combination of solid-state NMR spectroscopy experiments *in vitro*, *INtelligent Fitting Of Spectra* (INFOS) methods[3] and molecular dynamic (MD) simulations to describe the conformational landscape of different Y_4R states (i.e. coupled with different activating peptide ligands), and thus the changes between those states.

Results

We unravelled tremendous changes in the secondary structure of the hY_4R, as well as in the distribution of the equilibrium conformations among the different states, mostly at the intracellular side of the receptor, which are specific to the interaction with a particular peptide ligand.

Conclusions

This approach has the enormous potential to bring us one step closer to understanding the specific receptor–ligand interactions, or, in other words, the structural and dynamic origin of biased signaling of the hY_4R.

References
[1] N. R. Latorraca et al., *Chem. Rev.* **2017**, *117*, 139–155.
[2] X. Deupi, B. K. Kobilka, *Physiology* **2010**, *25*, 293–303.
[3] A. A. Smith, *J. Biomol. NMR* **2017**, *67*, 77–94.

PS-VI-04

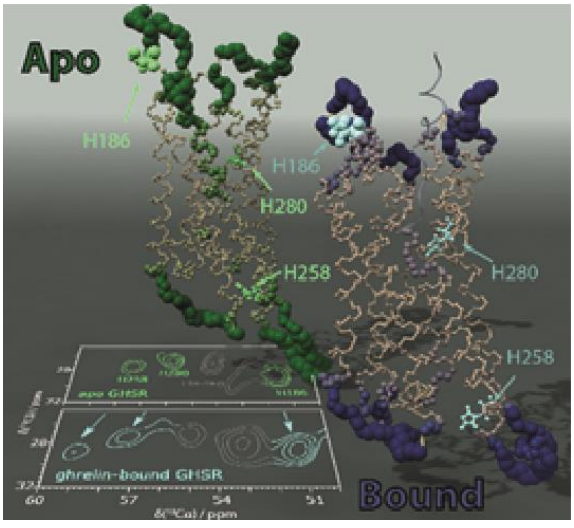
Analysis of the Dynamics of the Human Growth Hormone Secretagogue Receptor Reveals Insights into the Energy Landscape of the Molecule

Albert A. Smith, Emelyne M. Pacull, Sabrina Stecher, Peter W. Hildebrand, Alexander Vogel, Daniel Huster

University of Leipzig, Institute of Medical Physics and Biophysics, Leipzig, Germany

This study was funded by the Deutsche Forschungsgemeinschaft (DFG) through CRC 1423, project number 421152132 and DFG grant 450148812 (AAS).

G protein-coupled receptors initiate signal transduction in response to ligand binding. Growth hormone secretagogue receptor (GHSR), the focus of this study, binds the 28 residue peptide ghrelin. While structures of GHSR in different states of activation are available, dynamics within each state have not been investigated in depth. We analyze long molecular dynamics simulation trajectories using “detec-tors” to compare dynamics of the apo and ghrelin-bound states yield-ing timescale-specific amplitudes of motion. We identify differences in dynamics between apo and ghrelin-bound GHSR in the extracellular loop 2 and transmembrane helices 5-7. NMR of the GHSR histidine residues reveals chemical shift differences in these regions. We eva-luate timescale specific correlation of motions between residues of ghrelin and GHSR, where binding yields a high degree of correlation for the first 8 ghrelin residues, but less correlation for the helical end. Finally, we investigate the traverse of GHSR over a rugged energy landscape via principal component analysis.



Structural Dynamics of GHSR from MD and NMR

References

[1] Albert A. Smith, Emelyne Pacull, Sabrina Stecher, Peter W. Hildebrand, Alexander Vogel, **Daniel Huster** “Analysis of the dynamics of the human growth hormone secretagogue receptor reveals insights into the energy landscape of the molecule” *Angew. Chem. Int. Ed.* **62**(2023) e202302003

PS-VI-06

Dynamic Behavior of the “Toggle Switch” Residue $W^{6.48}$ in Neuropeptide Y1 Receptor upon Ligand Binding

Matthias Voitel[1], Maik Pankonin[1], Karl Leitner[2], Alexander Vogel[1], Anette Kaiser[2], Albert Smith-Penzel[1], Peter Hildebrand[1], Daniel Huster[1]

[1] Leipzig University, Institute of Medical Physics and Biophysics, Leipzig, Germany; [2] Leipzig University, Department of Anesthesiology and Intensive Care, Leipzig, Germany

This work was funded by the German Research Foundation through CRC1423, project number 421152132 (subprojects A04, B03, C01, Z03).

Tryptophan $W^{6.48}$ is a highly conserved residue in G-protein coupled receptors. In the past years, this position was a target of different studies identifying its importance in signal transduction. A common understanding of the role of this residue describes it as part of a “toggle switch”, where it is stabilized in an inactive conformation in the apo state and changes to an active one upon ligand binding. Combining cell culture assays, NMR spectroscopy, molecular dynamics simulations and evaluation of timescale-filtered motions we investigated the dynamic behavior of the “toggle switch” residue $W^{6.48}$ in neuropeptide Y1 receptor. Contrary to the common understanding, we identified three coexisting conformations in the apo form. Neuropeptide Y binding reduces this flexibility to two conformations without creating new ones. Furthermore, we identified correlation of motion between $W^{6.48}$ and other residues of the receptor, which are not necessarily in close proximity, indicating a relationship between this residue and the overall receptor dynamics. These findings help broaden the understanding of receptor activation from a structure-based on/off mechanism to a dynamic interaction.

PS-VI-08

The Role of Conformational Dynamics for Y Receptor Activation

Dilara Ögütcü, Matthias Elgeti

Leipzig University, Institute for Drug Discovery, Leipzig, Germany

GPCR ligands can bind either exclusively to a single receptor subtype or to several subtypes. Additionally, ligands can influence the receptor's conformational balance, potentially directing signaling towards a particular transducer. High-resolution structures of Y2R reveal activating conformational changes concentrated in the second extracellular loop and at the intracellular termini of TM6 and TM7. We are using EPR spectroscopy, and for that reason, the selected positions on functional Y2R will be labeled with site-directed spin labels to perform cw-EPR and DEER measurements. These measurements will help us understand the subtype specificity and functional selectivity of Y2R.

References
[1] Abiko, L.A., Rogowski, M., Gautier, A. et al. Efficient production of a functional G protein-coupled receptor in E. coli for structural studies. J Biomol NMR 75, 25–38 (2021).

[2] Kubatova N, Schmidt T, Schwieters CD, Clore GM. Quantitative analysis of sterol-modulated monomer-dimer equilibrium of the β1-adrenergic receptor by DEER spectroscopy. Proc Natl Acad Sci U S A. 2023;120(7):e2221036120.

PS-IV-28

Structure of Membrane-mediated Amyloid Fibrils of human islet amyloid polypeptide (hIAPP)

Venus Singh Mithu, Karin Giller, Loren Andreas, Stefan Becker, Christian Griesinger

Max Planck Institute of Multidisciplinary Sciences, Department of NMR-based Structural Biology, Göttingen, Germany

Type II diabetes mellitus (T2DM) is characterized by insulin resistance, progressive islet β cell failure, and the presence of extracellular fibrils (islet amyloid) of a 37 amino acid long peptide hormone 'human islet amyloid polypeptide' (hIAPP) in the pancreatic islets of Langerhans. Evidence suggests that the interaction between hIAPP and phospholipid membrane plays a pivotal role in causing β cell failure. A mechanistic understanding of this interaction is thus essential for understanding the therapeutic activity of small molecules. The molecular structure of membrane-mediated hIAPP fibrils, crucial for understanding their role in T2DM, is still unknown. This study aims to determine their structural fold in the presence of model phospholipid membranes.

Small unilamellar vesicles (SUVs) composed of POPC and POPS phospholipids were used as models for β cell membranes in growing hIAPP fibrils. Unlike the evenly dispersed hIAPP fibrils seen without lipids, we observed bundles of fibrils mixed with misshapen lipid vesicles. This complexity challenges structure determination using cryo-EM, thus making solid-state NMR (ssNMR) the preferred method for studying these lipidic hIAPP fibrils. Solution-state NMR and Thioflavin-T (ThT)-based fluorescence assays were used to characterize the aggregation kinetics of hIAPP. The assignment of backbone and side-chain resonances of hIAPP was accomplished through a series of 1H-detected 3D and 13C-detected 2D correlation ssNMR spectra, recorded at 55 kHz magic-angle-spinning (MAS). Chemical shift data was utilized to predict secondary structural and torsional angles using TALOS-N, revealing significant differences from previously reported structures of non-lipidic fibrils.

13C-13C correlation proton driven spin diffusion spectra at slow MAS were used to gather distance constraints. NMR-derived torsional angles and distance constraints, combined with MD simulations, helped construct a model for hIAPP fibrils in membrane environments. These backbone and side-chain assignments will aid in identifying potential binding sites of therapeutic and diagnostic molecules.

Poster-II-108

Event-Graphs to find allosteric pathways in GPCR's from MD simulations

Maximilian Salomon[1,2], Maik Pankonin[2], Franziska Reinhardt[1], Sayan Mukherjee[3], Peter W. Hildebrand[2], Peter F. Stadler[1]

[1] Leipzig University, Bioinformatics Group, Department of Computer Science, and Interdisciplinary Center for Bioinformatics, Leipzig, Germany; [2] Leipzig University, Protein4matics Group, Institute of Medical Physics and Biophysics, Faculty of Medicine, Leipzig, Germany; [3] Max Planck Institute for Mathematics in the Sciences, Learning and Inference Group, Leipzig, Germany

Allostery allows proteins, such as GPCRs, to regulate their activity by binding molecules at distant sites, enabling precise control over essential biological processes. These allosteric signals are transmitted through dynamic changes in the protein structure. Molecular dynamics (MD) simulations provide a way to investigate these dynamic and structural changes in proteins, but isolating the exact signaling pathways from the simulation data remains a challenge. To address this, we construct time-dependent graphs from MD simulation data, where sub-graphs are generated for specific time periods, with nodes representing regions of the protein that move in a coordinated manner (communities). Event edges connect these sub-graphs and nodes to represent transition events between different states. By analyzing this graph system, we aim to trace event chains that map allosteric signal transmission, providing insights into the dynamic processes that drive allosteric regulation in complex molecular systems.

3:45 pm – 4:45 pm

HS 9

PL-VI | Plenary VI

Thomas Gutsmann

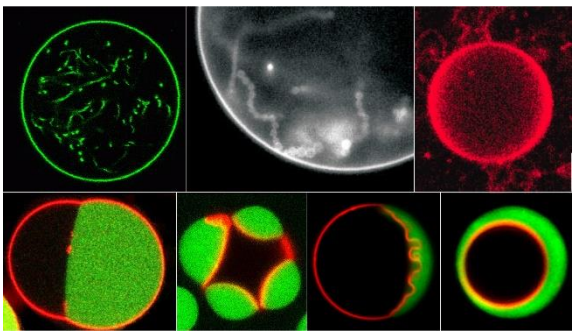
PL-VI-01

Membrane remodeling in artificial cells:when a membrane meets a drop

Rumiana Dimova

Max Planck Institute of Colloids and Interfaces, Sustainable and Bio-inspired Materials, Potsdam, Germany

Cell membranes exhibit a large variation in curvature. While it is commonly perceived that this curvature is primarily driven by specific protein activities, our studies demonstrate that various other asymmetries across the membrane can readily generate curvature. These asymmetries likely play an important role in defining the shapes of membrane organelles. As a workbench for artificial cells, we employ giant unilamellar vesicles (10-100 µm), which serve as a fascinating model system, illustrating the membrane's response at the cell-size scale, see Figure. In this talk, we will first introduce approaches using giant vesicles for the precise quantification of membrane spontaneous curvature. We will provide examples where curvature is induced by the asymmetric distribution of ions across the membrane and by the insertion or adsorption of molecules, as reviewed in [1]. Following this, we will explore the process of membrane wetting by droplets in a molecularly crowded environment [2]. We will show that wetting by biomolecular condensates can dramatically mold the membrane while modulating lipid organization [3], undergo endocytosis [4], and even patch pores in damaged membranes [5]. These examples demonstrate that even without scaffolding proteins or active processes, simple physicochemical factors can readily remodel the membrane.



Membrane morphologies observed on giant vesicles
Giant vesicles exposed to different stimuli can exhibit internal and external tube formation signifying nonzero spontaneous curvature (upper row of images), and their membranes can undergo various morphological transformations when in contact with droplets (lower row of images).

References

[1] Dimova, R 2019, 'Giant vesicles and their use in assays for assessing membrane phase state, curvature, mechanics and electrical properties', *Annu Rev Biophys*, 48, 93.

[2] Mangiarotti, A, Dimova, R 2024, 'Biomolecular condensates in contact with membranes', *Annu Rev Biophys*, 53, 319-341.

[3] Mangiarotti, A, ... Dimova R 2023, 'Wetting and complex remodeling of membranes by biomolecular condensates', *Nature Comm*, 14, 2809;
Mangiarotti, A, ... Dimova R 2023, 'Biomolecular condensates modulate membrane lipid packing and hydration', *Nature Comm*, 14, 6081.

[4] Mangiarotti, A, Aleksanyan, A, Siri, M, Sun, T-W, Lipowsky, R, Dimova, R 2024, 'Photoswitchable Endocytosis of Biomolecular Condensates in Giant Vesicles' *Adv Sci*, 2309864. (in press DOI:10.1002/advs.202309864)

[5] Bussi, C, Mangiarotti, A, Vanhille-Campos, C, Aylan, B, Pellegrino, E, Athanasiadi, N, Fearns, A, Rodgers, A, Franzmann, TM, Šarić, A, Dimova, R, Gutierrez, MG 2023 'Stress granules plug and stabilize damaged endolysosomal membranes' *Nature*, 623, 1062–1069.

PL-VI-02

Life in a crowded environment: regulation of nucleocytoplasmic density

Simone Reber, **Simone Reber**

Max Planck Institute for Infection Biology, Berlin, Germany

The packing and confinement of macromolecules in the cytoplasm and nucleoplasm has profound implications for cellular biochemistry. How intracellular density distributions vary and affect cellular physiology remains largely unknown. We show that the nucleus is less dense than the cytoplasm and that living systems establish and maintain a constant density ratio between these compartments. Using label-free biophotonics and theory, we show that nuclear density is set by a pressure balance across the nuclear envelope in vitro, in vivo and during early development. Nuclear transport establishes a specific nuclear proteome that exerts a colloid osmotic pressure, which, assisted by entropic chromatin pressure, draws water into the nucleus. We propose that the maintenance of a constant N/C density ratio is the biophysical driver of one of the oldest tenets of cell biology: the N/C volume ratio. In summary, we reveal a previously unidentified homeostatic coupling of macromolecular densities that drives cellular organization with implications for pathophysiologies such as senescence and cancer.

25 September, 2024

8:30 am – 10:30 am

HS 9

PL-VII | Plenary VII

PL-VII-01

Time-resolved IR-spectroscopy to unravel the dynamics of disease-related fibril formation

Karin Hauser

University of Konstanz, Department of Chemistry, Konstanz, Germany

Many neurodegenerative diseases are related to protein misfolding and aggregation. While several studies reveal the morphology of fibrils and aggregates, less is known about the structural dynamics and the molecular mechanisms initiating fibril formation. Infrared (IR) spectroscopy is well suited to resolve protein dynamics on the relevant time-scales. In my talk, I will focus on our recent studies on polyglutamine (polyQ) fibril formation. PolyQ diseases, e.g. Huntington's disease, are caused by the aggregation of expanded polyQ tracts in the affected protein. By a combination of laser-excited temperature-jump (T-jump) IR-spectroscopy and appropriately tailored polyQ model peptides, we succeeded in disentangling conformational dynamics in the heterogeneous ensemble of states evolving during aggregation. Individual structural elements could be differentiated by IR-specific signatures, i.e., hairpin monomers, β-structured oligomers, and disordered structure. Submillisecond dynamics were observed for early oligomeric states in contrast to the slow dynamics of the fibril growth. We propose that a high structural flexibility of oligomeric precursor states is required to initiate fibril formation, but not anymore once a fibrillary structure has consolidated and the fibril just grows. Our study reveals that structural dynamics changes at different stages in the aggregation process, from fibril initiation to fibril growth.

PL-VII-02

On concentrations in membrane biophysics research

Olaf S. Andersen, Kelsey Curtin, Radda Rusinova

Weill Cornell Medicine, Physiology and Biophysics, New York, USA

Many drugs are amphiphiles that partition into lipid bilayers to alter lipid bilayer properties such as elasticity, curvature, and thickness. This is important because hydrophobic coupling to the bilayer makes membrane proteins responsive to changes in bilayer properties, which impacts on the bilayer's contribution to the energetics of membrane protein conformational changes. Drug partitioning into bilayers reduces the aqueous concentration to below the nominal concentration (number of molecules/system volume); the difference may be inconsequential for molecules with small partition coefficients or profound for molecules with high partition coefficients, depending on the relative volumes of the aqueous and membrane phases. Therefore, to determine absolute efficacy, it becomes important to know the free drug concentrations in the aqueous phase and, in the case of bilayer-mediated mechanisms, the drug concentrations in the membrane. These concentrations, however, turn out to be somewhat elusive quantities because they depend on both the molecule (its partition coefficient) and the experimental conditions (the volumes of the aqueous and membrane phases plus other factors). This dependence on experimental conditions forms the basis for using isothermal titration calorimetry to measure partition coefficients. A similar strategy, using drug-induced changes in bilayer properties, allows for measuring partition coefficients and bilayer-modifying potency/molecule in the membrane.

References

[1] Rusinova, R., R.E. Koeppe II and O.S. Andersen. A general mechanism for drug promiscuity: studies with amiodarone and other antiarrhythmics. *Journal of General Physiology* **146**:465-475 (2015).

[2] Kapoor, R. T.A. Peyear, R.E. Koeppe II and O.S. Andersen. Antidepressants are modifiers of lipid bilayer properties. *Journal of General Physiology* **151**:342-356 (2019)

[3] Sun, D., T.A. Peyear, W.F.D. Bennett, M. Holcomb, S. He, F. Zhu, F.C. Lightstone, O.S. Andersen and H.I. Ingólfsson. Assessing the perturbing effects of drugs on lipid bilayers using gramicidin channel-based *in silico* and *in vitro* assays. *Journal of Medicinal Chemistry* **63**:11809-11818 (2020)

[4] Rusinova, R., C. He and O.S. Andersen. Mechanisms underlying drug-mediated regulation of membrane protein function. *Proceedings of the National Academy of Sciences of the USA* **118**: e2113229118 (2021).

[5] Ashrafuzzaman, Md., R.E Koeppe II and O.S. Andersen. Intrinsic lipid curvature and bilayer elasticity as regulators of channel function: A comparative single-molecule study *International Journal of Molecular Sciences* **25**:2758 (2024)

PL-VII-03

Lipids, ions and an integral membrane enzyme: an asymmetry story of unexpected allostery

Paulina Piller, Enrico F. Semeraro, Sandro Keller, **Georg Pabst**

University of Graz, Institute of Molecular Biosciences, Graz, Austria

This work was supported by the Austrian Science Funds (FWF) (10.55776/P32514).

Introduction

The asymmetric distribution of lipids across plasma membranes is a distinct feature of all cells. However, the functional implications of this membrane asymmetry, especially regarding membrane protein function, remain poorly understood [1]. We focused on the outer membrane phospholipase A (OmpLA), hypothesizing that differential lateral stress in asymmetric membranes could be exploited by cells to regulate the protein's activity.

Methods

We reconstituted OmpLA in various asymmetric lipid membranes composed of phosphatidylcholine, phosphatidylethanolamine, and phosphatidylglycerol. The kinetics of lipid hydrolysis were monitored using time-resolved high-performance thin layer chromatography and analyzed using rate equations.

Results

Our experiments showed a significant reduction in OmpLA's hydrolytic activity in compositionally asymmetric charge-neutral lipid bilayers compared to symmetric membranes with the same lipids [2]. In charged asymmetric membranes, we observed an overall increase in protein activity, further modulated by Na^{+} ions, which unlike Ca^{2+} ions, do not act as cofactors for the protein.

Conclusions

The observed phenomena can be primarily attributed to an allosteric coupling between protein activity and lateral asymmetry stress, modulated by compositional asymmetry and electrostatic interactions between ions and lipid headgroups. Similar mechanisms may govern the behavior of many other plasma membrane proteins.

References

[1] Pabst, G, Keller, S 2024, 'Exploring membrane asymmetry and its effects on membrane proteins', *TIBS*, 49, 333 - 345.

[2] Piller, P, et al. 2023, ' Allosteric modulation of integral protein activity by differential stress in asymmetric membranes', *PNAS Nexus* 2, 1 – 7.

PL-VII-04

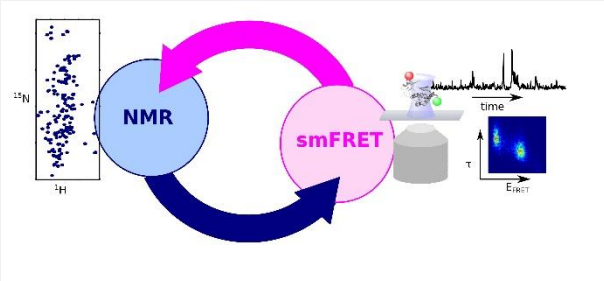
Intrinsically disordered regulators of endocytosis - an integrated NMR/single molecule fluorescence approach

Sigrid Milles

Leibniz-Forschungsinstitut für Molekulare Pharmakologie, Structural Biology, Berlin, Germany

Intrinsically disordered proteins (IDPs) lack clearly defined structure and are therefore highly flexible and easily adaptable to different binding partners. This makes them important players in many biological processes, often with vital regulatory functions. Their dynamic features and broad range of interaction modes, however, render them difficult to study and analyzing their complexes often requires integrated approaches. Integrating complementary parameters from of nuclear magnetic resonance (NMR) and single molecule fluorescence approaches allowed us to describe the conformational landscape of IDPs at molecular resolution and promises to shed new light onto various biological processes.

Among those counts clathrin mediated endocytosis. The early phases of clathrin mediated endocytosis are organized through a highly complex interaction network mediated by clathrin associated sorting proteins (CLASPs) that comprise long intrinsically disordered regions (IDRs). We characterize the IDRs of those CLASPs in their entirety and at molecular resolution, uncovering a plethora of interactions of various strengths and dynamic features with their endocytic interaction partners, proposing a rationale for how first interactions and dynamic rearrangement of partners take place during the uptake of a coated vesicle.



Integration of NMR and smFRET to study intrinsically disordered proteins

References

[1] Naudi-Fabra S, Tengo M, Jensen MR, Blackledge M, Milles S, Quantitative Description of Intrinsically Disordered Proteins Using Single-Molecule FRET, NMR, and SAXS, *JACS*, 2021 Dec 8;143(48):20109-20121

11:00 am – 12:05 pm

HS 10

PS-XII | Membranes and Receptors

PS-XII-01

Remodeling and sensing of lipid membranes using DNA origami

Henri G. Franquelim[1,2]

[1] Leipzig University, Interfaculty Center for Bioactive Matter (b-ACTmatter), Leipzig, Germany; [2] Leipzig University, Peter Debye Institute for Soft Matter Physics, Leipzig, Germany

Biological lipid membranes can have a complex lipid composition, exhibit different curvatures and can be bent and remodeled. Understanding the biophysics of such processes and how to recognize membrane properties is thus crucial, as this knowledge can be used to develop more active molecules that better target lipid bilayers.
Using DNA nanotechnology, my group aims to decipher and artificially recreate minimal features of membrane-active systems in order to develop "à la carte" devices that sense intrinsic membrane properties. To this end, we design customized DNA origami that controllably attach to model lipid membranes [1]. In this context, we have pioneered the development of biomimetic membrane-active and membrane-bending triggerable DNA origami structures [2,3]. To determine membrane properties, we are exploring ways to manipulate the localization of DNA origami, functionalized with lipid anchors, in different membrane environments, and to design DNA origami devices [4] that can recognize different membrane curvatures. In this way, we can gain more insight into lipid phase separation and membrane curvature, with the aim of mimicking features of membrane-active processes (such as those associated with membrane trafficking).
Overall, the biomimetic strategies presented here open new avenues for mapping membrane properties and deciphering common principles underlying membrane remodeling, highlighting the unique advantages of DNA origami in the field of membrane biophysics.

References
[1] Khmelinskaia et al., 2023, Methods Mol Biol, 2639, 231-255
[2] Franquelim et al., 2018, Nat Commun, 9(1), 811
[3] Franquelim et al., 2021, Soft Matter, 17(2), 276-287
[4] Büber et al., 2023, ACS Nano, 17(3), 3088–3097

PS-XII-02

Responses of flavin-based receptors in living human cells Studied by in-cell infrared difference spectroscopy

Lukas Goett-Zink[1], Lennard Karsten[2], Charlotte Mann[2], Kristian Müller[2], Tilman Kottke[1]

[1] Bielefeld University, Biophysical Chemistry and Diagnostics, Medical School OWL, Bielefeld, Germany; [2] Bielefeld University, Cellular and Molecular Biotechnology, Faculty of Technology, Bielefeld, Germany

Introduction

The structural investigation of proteins in cells is important to fully understand their natural function. The intracellular environment affects protein mechanisms via metabolites [1] or macromolecular crowding [2] but this is often neglected by biophysical characterizations because of a limited availability of suitable techniques. We developed the in-cell infrared difference spectroscopy (ICIRD) for human cells to study the receptor domains of light, oxygen or voltage (LOV) proteins and cryptochromes, which are also applied in optogenetic tools [3].

Methods

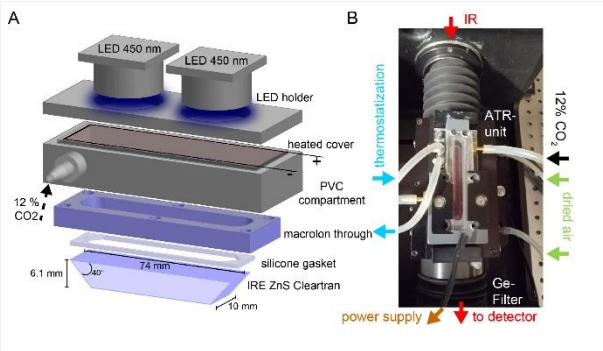
We used a self-built cultivation chamber and the attenuated total reflection approach to cultivate human cells inside an FTIR spectrometer. Growth and viability of the cells is monitored *in situ* by IR spectra. The receptor response to light in living human cells is investigated using the IR difference technique.

Results

With ICIRD we were able to resolve the light-induced responses of plant cryptochrome and LOV in living human cells for the first time. For both receptors, differences of the response between in cells and *in vitro* were observed and assigned to the cofactor flavin. These results demonstrate a clear impact of the cellular environment on flavin-based photoreceptors.

Conclusions

ICIRD expands the range of available in-cell methods by a non-invasive technique. This development encourages further investigations on other receptors in human cells, including those with medical relevance in optogenetics.



Setup for controlled cultivation and investigation of human embryonic kidney cells by ICIRD. (A) The self-built cultivation chamber is composed of a zinc sulphide internal reflection element (IRE). Human cells are seeded on the IRE and incubated over 37 h inside the FTIR spectrometer. Two LEDs are mounted on the top for illumination of the cells for inducing difference spectra. (B) The cultivation chamber inside the FTIR spectrometer is supplied with 12% CO_2 and thermostatized at 37°C.

References

[1] Goett-Zink, L, Toschke, A, Petersen, J, Mittag, M, Kottke, T 2021, 'C-Terminal Extension of a Plant Cryptochrome Dissociates from the β-Sheet of the Flavin-Binding Domain', *J. Phys. Chem. Lett.*, 12, 5558-5563.

[2] Rivas, G, Minton, AP 2018, 'Toward an understanding of biochemical equilibria within living cells', *Biophys. Rev.*, 10, 241-25.

[3] Rost, BR, Scheider-Warme, F, Schmitz, D, Hegemann, P 2017, 'Optogenetic Tools for Subcellular Applications in Neuroscience', *Neuron*, 96, 572-603.

PS-XII-03

Coupling the role of lipids to the conformational dynamics of the ABC transporter P-glycoprotein

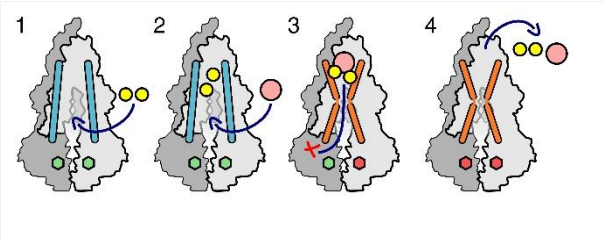
Dario De Vecchis, Lars Schäfer

Ruhr University Bochum, Center for Theoretical Chemistry, Bochum, Germany

This work was supported by the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG) through grant SCHA 1574/6-1.

The ATP-binding cassette (ABC) transporter P-glycoprotein (P-gp) is a multidrug efflux pump overexpressed in cancer cells and associated with drug resistance. P-gp structures solved in detergent and nanodiscs exhibit different transmembrane helix conformations named straight and kinked, respectively, indicating a role of the lipid on the P-gp structural ensemble. We study the conformational dynamics of the straight and kinked P-gp conformers employing all-atom molecular dynamics (MD) simulations in complex bilayers that mimic the hepatocyte membrane where P-gp is expressed.

The two conformers largely differ in the volume and accessibility of the substrate cavity, which is also modulated by cholesterol and ATP. The MD simulations show how different lipid species wedge and enter within the cavity of the straight conformer solved in detergent. However, the access of the kinked conformer solved in nanodiscs is restricted, likely preventing substrate disengagement and transport withdrawal. Therefore, our findings indicate that the straight conformer likely precedes the kinked in the P-gp's working cycle, with the kinked representing a post substrate-bound state. In our unbiased MD simulations, one transmembrane portal helix of the straight conformer underwent a spontaneous conformational transition to a kinked conformation, highlighting the role of the membrane milieu and revealing structural descriptors defining the transition between the two P-gp inward-open conformers [1].



Mechanistic scheme for the straight and kinked inward-open P-gp conformers.
P-gp straight (cyan) and kinked (orange) conformers are represented in cartoon. ATP and ADP are shown as green and red hexagons, respectively. Phospholipids and substrate are depicted as yellow and pink spheres, respectively.

References
[1] De Vecchis, D, Schäfer, LV 2024, Coupling the Role of Lipids to the Conformational Dynamics of the ABC Transporter P-glycoprotein, bioRxiv, doi:/10.1101/2024.04.18.590131

PS-XII-04

Synergistic binding of SARS-CoV-2 to endogenous ACE2 and gangliosides in native lipid membranes

Manorama Dey

Indian Institute of Technology Kanpur, Chemistry, Kanpur, India

Thanks to my supervisor Dr. Nagma Parveen for her continuous guidance and immense support. Special thanks to our collaborator Dr Krishnan Harshan, SERB India, the Fellowship (SRF), IIT Kanpur for providing travel grants, family and lab-mates.

Introduction

Nanometer-sized infectious viruses utilize abundant cell surface glycans and plasma membrane receptors to attain adequate attachment strength for initiating cellular entry. Thus, viruses typically encounter the cell surface glycans before they can engage in short-range and specific interactions with receptors in the plasma membrane. In this article, we focused on how the binding of SARS-CoV-2 to the plasma membrane rich in endogenous ACE2 receptors was affected by the addition of sialylated gangliosides.

Methods

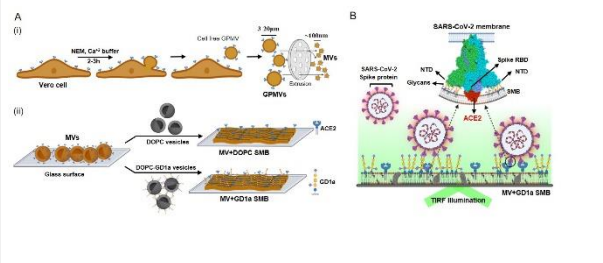
This was explored using supported membrane bilayers (SMBs), which were formed by reconstituting plasma membrane vesicles containing endogenous ACE2 and disialoganglioside-GD1a into artificial lipid vesicles. Total internal reflection fluorescence (TIRF) microscopy technique was used to study the binding kinetics of intact SARS-CoV-2 particles to SMBs.

Results

SMBs have been applied as a membrane-mimicking platform for studying the synergistic binding effect of these gangliosides on the virus binding to the ACE2-rich membrane in a near-native environment. The initial binding rate of SARS-CoV-2 has a high affinity towards the endogenous membrane receptors compared to GD1a.

Conclusions

Using combinations of inhibition assays, we confirmed that adding GD1a in lipid membranes increases the availability of the endogenous ACE2 receptor and results in synergistic binding kinetics of SARS-CoV-2 to the membrane receptors in SMBs.



Schemes of the experimental set up
A) SMBs formation by MVs (extruded Vero GPMVs) and DOPC vesicles with or without GD1a upon their consecutive addition on a planar glass surface. B) These SMBs named as MV+DOPC and MV+GD1a are used to probe the synergistic binding of SARS-CoV-2 (labelled with DiI) to the endogenous ACE2 (from MVs) and GD1 (added vesicles).

References

[1] Negi, G.; Sharma, A.; Dey, M.; Dhanawat, G.; Parveen, N. Membrane Attachment and Fusion of HIV-1, Influenza A, and SARS-CoV-2: Resolving the Mechanisms with Biophysical Methods. *Biophys. Rev.***2022**, *14* (5), 1109-1140

[2] Dey, M.; Dhanawat, G.; Gupta, D.; Goel, A.; Harshan, K. H.; Parveen, N. Giant Plasma Membrane Vesicles as Cellular-Mimics for Probing SARS-CoV-2 Binding at Single Particle Level. *Chem. Sel.***2023**, *8*, e202302608.
[3] Negi, G.; Sharma, A.; Chaudhary, M.; Gupta, D.; Harshan, K. H.; Parveen, N. SARS-CoV-2 Binding to Terminal Sialic Acid of Gangliosides Embedded in Lipid Membranes. *ACS Infect. Dis.***2023**, *9*, 1346–1361.
[4] Dey, M.; Sharma, A.; Dhanawat, G.; Gupta, D.; Harshan, K. H.; Parveen, N. Synergistic Binding of SARS-CoV-2 to ACE2 and Gangliosides in Native Lipid Membranes. *ACS Infect. Dis.***2024**, *10*, 907–916.
[5] Kim, S. H.; Kearns, F. L.; Rosenfeld, M. A.; Votapka, L.; Casalino, L.; Papanikolas, M.; Amaro, R. E.; Freeman, R. SARS-CoV-2 Evolved Variants Optimize Binding to Cellular Glycocalyx. *Cell Reports Phys. Sci.***2023**, *4* (4), 101346.

11:00 am – 12:05 pm

HS 8

PS-X | Membrane Biophysics IV

PS-X-01

Single molecule investigation of liquid-solid-transition of tau protein

Mahaprasad S. Sahu[1], Jiapeng Wei[2], Vinoth Kumar[1], Tuomas Knowles[2], **Kanchan Garai**[1]

[1] Tata Institute of Fundamental Research Hyderabad, Biophysics, Hyderabad, India; [2] University of Cambridge, Yusuf Hamied Department of Chemistry, Cambridge, UK

We acknowledge the Science and Engineering Research Board (SERB) and Tata Institute of Fundamental Research (TIFR) for the funding.

Introduction

While liquid liquid phase separation (LLPS) has emerged as a fundamental principle in biological functions, liquid to solid transition (LST) of the protein rich liquid phase is implicated in the pathology of a spectrum of neurodegenerative disorders. However, molecular mechanisms underlying LST, characterized by conversion of the soluble liquid phase into insoluble amyloid fibrils, is poorly understood.

Methods

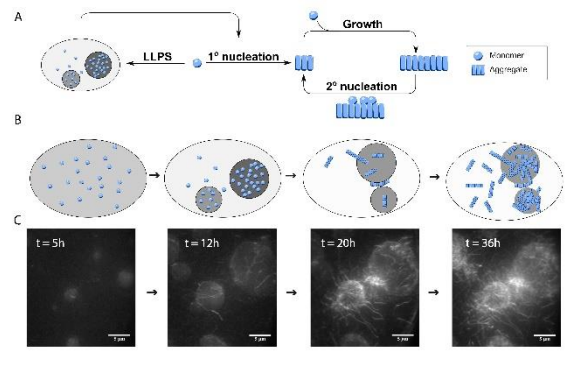
We have used total internal reflection fluorescence microscopy (TIRFM), single molecule tracking, fluorescence recovery after photobleaching, and confocal microscopy to monitor LLPS and the subsequent LST of the intrinsically disordered protein tau in the presence of polyU RNA.

Results

High resolution and high sensitivity of TIRFM enable us to visualize growth of the single fibrils during aging and measure the rates of primary nucleation and elongation of the amyloid fibrils inside the liquid droplets. Time evolution of viscosity and surface tension indicate conversion of the homogeneous liquid droplets into inhomogeneous liquid-solid mixed phases associated with the growth of the fibrils.

Conclusions

Our study accentuates the role of factors such as droplet interface dynamics, protein concentration, and viscosity in dictating the transition from liquid to solid phases, offering valuable insights into these complex biological phenomena.



Aggregation reaction network involving liquid liquid phase separation
A, Tau LLPS and aggregation reaction network, including: primary nucleation, elongation and secondary nucleation. B, Liquid-liquid phase separation and liquid-to solid transition of P301L tau with polyU. Fibrillar aggregates can form inside, outside, and partly inside and partly outside of the condensates. C, TIRF images of Tau and PolyU at t = 5, 12, 20, 36 hours, corresponding to three different stages in B: before LLPS, LLPS, and aggregation following LLPS.

References

[1] Zimmermann MR, Bera SC, Meisl Georg, Dasadhikari S, Ghosh S, Linse S, Garai K*, Knowles TPJ* Mechanism of Secondary Nucleation at the Single Fibril Level from Direct Observations of Aβ42 Aggregation *J. Am. Chem. Soc.* 2021, 143, 40, 16621–16629 https://doi.org/10.1021/jacs.1c07228

PS-X-02

Membrane elastic properties through virtual deformations

Alexey M. Nesterenko[1], Otto Schullian[2], Reinhard Lipowsky[2], Markus S. Miettinen[1,2]

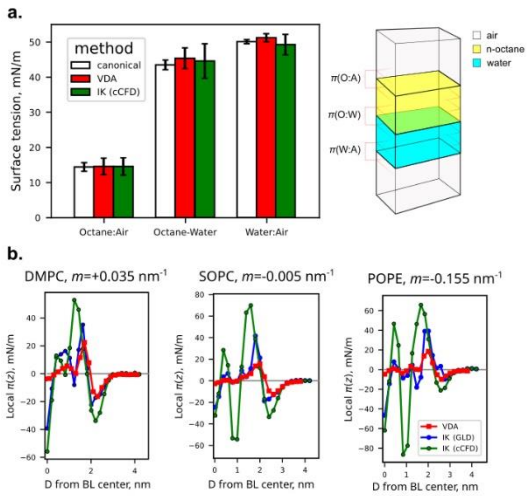
[1] University of Bergen, Computational Biology Unit, Departments of Chemistry and Informatics, Bergen, Norway; [2] Max Planck Institute of Colloids and Interfaces, Department of Theory and Bio-Systems, Potsdam, Germany

The stress computations and simulations were performed on resources provided by Sigma2 — the National Infrastructure for High-Performance Computing and Data Storage in Norway and the LUMI supercomputer, owned by the EuroHPC Joint Undertaking, hosted by CSC (Finland) and the LUMI consortium. We acknowledge UiB (Norway) and Sigma2 for awarding this project access to LUMI resources. M.S.M. was supported by the Trond Mohn Foundation (BFS2017TMT01).

Biological membranes are two-dimensional fluids that can undergo stretching and bending deformations. Stretching (and compression) is governed by the area compressibility modulus, bending by the spontaneous curvature m and the bending rigidity κ. For a planar bilayer comprising a single lipid component, the average shape of individual lipid molecules generates certain intrinsic curvature. In a symmetric bilayer, the two planar leaflets have opposite bending preferences, but cannot follow these preferences – they are “frustrated” by geometrical constraints [1].

The product κm describes the bending preference $-\partial F/\partial m$, with the free energy *F,* of a mono- or bilayer. From molecular dynamics (MD) simulations, this bending preference can be computed as the first moment of the lateral stress profile (LSP) using the Irving–Kirkwood (IK) approach with different decompositions of multi-body forces [2, 3]; these typically lead to differing numerical values of the local stress.

We have developed an alternative thermodynamically-motivated theory, the virtual deformation approach (VDA), based on deformation of a space domain [4]. Applying linear stretching deformations, VDA was shown to reproduce the surface tensions in triphasic systems (Fig. 1a). To find out if the tensionless leaflets of a lipid bilayer have a bending preference, we calculated stress profiles using the IK and VDA methods (Fig. 1b) for lipids with positive, negative, and zero intrinsic curvatures (as reported in Ref. 5).



VDA validation and Lateral Stress Profiles for tensionless leaflets of DMPC, SOPC, and POPE
a. Surface tensions computed for each interface of a triphasic system using VDA and IK approaches compared to surface tensions computed "canonically" (i.e. from the pressure tensors obtained from the total virial of the corresponding biphasic systems). **b.** Lateral Stress Profiles, $\pi(z)$, along the bilayer normal, z, computed from atomistic MD simulations for tensionless leaflets of three lipid bilayers. The $\pi(z)$ from Goetz–Lipowsky [2] (blue) and covariant Central Force [3] (green) Decompositions computed using GROMACS-LS-2016, the VDA profile (red) using our VDA code based on GROMACS-2020.

References
[1] Lipowsky, R, Dimova, R 2021, 'Introduction to remodeling of biomembranes', Soft Matter, 17, 214–221
[2] Goetz, R, Lipowsky, R 1998, 'Computer simulations of bilayer membranes: self-assembly and interfacial tension', J. Chem. Phys., 108, 7397–7409
[3] Vanegas, JM, Torres-Sánchez, A, Arroyo, M 2014, 'Importance of force decomposition for local stress calculations in biomembrane molecular simulations', J. Chem. Theory Comput., 10, 691–702
[4] Schullian, O 2021, 'Consistent Local Stress Theory of Discrete Particle Systems', Dissertation, Mathematisch-Naturwissenschaftliche Fakultät der Humboldt-Universität zu Berlin, Berlin: Humboldt-Universität zu Berlin
[5] Dymond, MK 2021, 'Lipid monolayer spontaneous curvatures: A collection of published values', Chem. Phys. Lip., 239, 105117

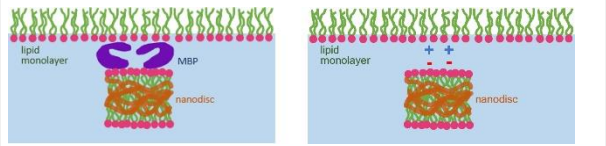
PS-X-03

Adsorption of lipid nanodiscs to monolayers: a new triple layer system for studying membrane proteins

Isabel K. Berg, Jasmin Penker, Vanessa Jerschabeck, Felix Götze, **Christian Schwieger**

Martin Luther University Halle–Wittenberg, Institute for Chemistry, Halle, Germany

Introduction
Lipid monolayers can be used as very simple model system for one membrane leaflet. They have the advantages of being perfectly oriented, completely hydrated, and accessible through the aqueous phase. Thus, they are well suited for studying interactions with peripherally binding proteins or peptides. A variety of methods can be used in these studies, e.g. fluorescence microscopy, infrared reflection-absorption spectroscopy (IRRAS) and X-ray reflectometry. However, no membrane spanning molecules can be studied in this system until now. Therefore, we are exploring methods to assemble lipid bilayers underneath lipid monolayers at the air water interface. These bilayers shall host membrane spanning molecules such as integral membrane proteins. The bilayers will be oriented while still being in a well-hydrated and natural environment..
Methods
Infrared reflection-absorption spectroscopy (IRRAS), epi-fluorescence microscopy, surface potential measurements.
Results
We present first successful attempts in constructing such a model system, i.e. the adsorption of lipid nanodiscs to lipid monolayer at the air water interface. Two different strategies, i.e. protein mediated adsorption and electrostatic adsorption lead to stable and oriented adsorption of polymer stabilized nanodiscs to the monolayer.
Conclusions
We show a new model system, combine the advantages of several well-established membrane models and discuss further perspectives and applications.



Nanodisc adsorption to lipid monolayers
Two strategies for the formation of lipid triple layers at the air-water interface: protein mediated adsorption (left) and electrostatic adsorption (right) of lipid nanodiscs to preassembled lipid monolayers.

PS-X-04

Photoswitchable lipid dynamics in phase-separated membranes

Tsu-Wang Sun[1], Elias Sabri[1], Mina Aleksanyan[1], Joachim Heberle[2], Rumiana Dimova[1]

[1] Max Planck Institute of Colloids and Interfaces, Potsdam, Germany; [2] Freie Universität Berlin, Department of Physics, Berlin, Germany

Introduction

The localization and segregation of specific elements on lipid membranes play a crucial role in their regulation and function. Giant unilamellar vesicles (GUVs) serve as cell-sized model systems. Utilizing azobenzene-phosphatidylcholine (azoPC), which has an azobenzene moiety in one of its lipid tails, in fluidic membranes significantly increases surface area and alters elastic properties[1].

Methods

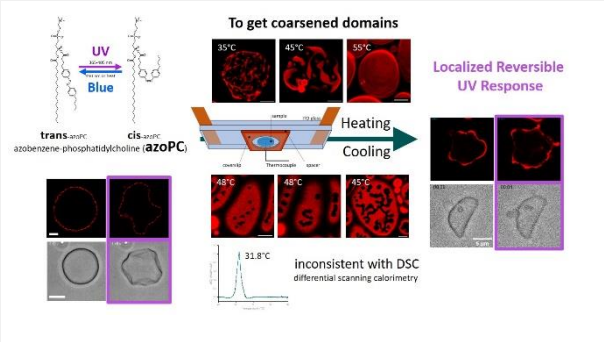
We delve into the phase dynamics of membranes incorporating azoPC and the gel-phase phospholipid 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), which has a melting point of 41°C, exploring the potential for domain-specific photo-response and precise spatiotemporal regulation.

Results

At room temperature, gel domains display irregular patch patterns following electroformation at 60°C and a gradual cooling process (~0.3 K/min). Despite a phase transition at 31.8°C identified by differential scanning calorimetry (DSC) in the equimolar mixture, microscopy observations indicate domain coalescence and homogenization above 45°C. We also examine the light-responsive behavior of GUVs with coarsened azoPC domains. These GUVs show localized membrane protrusions and expansion upon UV irradiation, initially exhibiting twisting or crumpling before coarsening.

Conclusions

This investigation sheds light on the dynamic behaviors of photoswitchable lipids within lipid membranes, enhancing our understanding of cell membrane mechanics and controlled reactions.



From crumpling to budding
Photoswitchable lipid azoPC can soften the membrane under UV light. In phase-separated membranes, after heating and cooling, the behavior transitions from crumpling to budding. Microscopic observation of melting with a specialized heating device reveals a higher temperature than indicated by DSC.

References

[1] Aleksanyan, Mina, Andrea Grafmüller, Fucsia Crea, Vasil N. Georgiev, Naresh Yandrapalli, Stephan Block, Joachim Heberle, and Rumiana Dimova. 'Photomanipulation of Minimal Synthetic Cells: Area Increase, Softening, and Interleaflet Coupling of Membrane Models Doped with Azobenzene-Lipid Photoswitches.' *Advanced Science* 10, no. 31 (2023): 2304336. https://doi.org/10.1002/advs.202304336.

11:00 am – 12:05 pm

HS 9

PS-XI | Computational Biophysics III

PS-XI-01

Machine Learning traits of disordered proteins that undergo liquid-liquid phase separation

Jagannath Mondal, Abdul Wasim

Tata Institute of Fundamental Research Hyderabad, Centre for Interdisciplinary Sciences, Hyderabad, India

The authors acknowledge the funding derived from TIFR Hyderabad for this project.

Introduction

The rules that govern the liquid-liquid phase separation (LLPS) of disordered protein are very complex. An Understanding of the underlying rules would allow potential design of sequences with propensity of LLPS

Methods

In this presentation we will demonstrate a large-language based machine learning(ML) based approach that can scan through the database of the phase separating proteins, understand the context and semnatics and can help generate new sequences that would replicate phase separation behavior.

Results

A sequence analysis of the ML-generated proteins offers a probabilistic insight into the amino acid grammar unique to phase-separating protein. The generated sequences share very low similarity with the existing sequences of phase separating protein. Subsequent validation through multi-chain molecular dynamics simulations further substantiates the phase separatingpotential of the generated proteins. As a promising development, we synthesized a set of ML-generated protein sequences in laboratory, which eventually underwent LLPS

Conclusions

The investigation show-cases the power of generative machine learning models in deriving and interpreting new sequences that can undergo LLPS.

PS-XI-02

Modulating lipid membranes by light: insights from atomistic and coarse-grained molecular dynamics

Cristina Gil Herrero[1,2], Sebastian Thallmair[1]

[1] Frankfurt Institute for Advanced Studies, Frankfurt am Main, Germany; [2] Goethe University, Frankfurt am Main, Germany

Photoswitchable molecules, particularly azobenzene derivatives, have recently found diverse applications in biological systems. Upon light irradiation, these molecules undergo conformational changes, altering their physicochemical properties. A popular application is the integration of azobenzene into lipid tails to modulate membrane properties. Moreover, photoswitchable molecules offer potential in medicinal chemistry, as they can be incorporated into drugs for light-activated control.

While quantum chemical calculations can elucidate their switching behavior, understanding their impact in larger biological ensembles remains challenging. To address this, classical molecular dynamics (MD) simulations at atomistic and coarse-grained (CG) resolution are better suited. In this study, we applied classical atomistic MD simulations to investigate small membrane patches of complex mixtures. In addition, we generated CG models for the trans- and cis-isomer of azobenzene using the Martini force field. We incorporated the models into lipid tails and evaluated and compared membrane properties such as lipid tail order, clustering, and permeability for both states.

Our newly developed CG models for azobenzene-based photolipids effectively capture changes in membrane properties induced by photoswitching. A detailed microscopic description of their mechanism of action will be valuable for future applications of photolipids, such as manipulating cellular membranes for antimicrobial purposes.

PS-XI-03

DNA double helix formation in molecular dynamics simulations

Patrick K. Quoika, Martin Zacharias

Technical University of Munich, Center for Functional Protein Assemblies (CPA), Garching, Germany

Introduction

DNA is possibly the most famous biopolymer. It is commonly known that DNA forms a stable double helix. However, on an atomic scale, the formation process of these double helices is not well understood, yet.

Methods

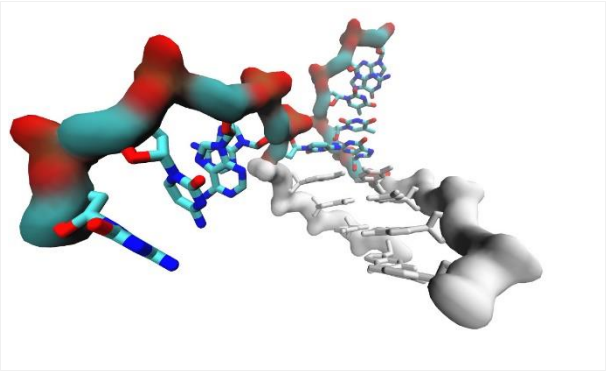
We investigated the formation of the DNA double helix in molecular dynamics simulations. Specifically, we looked into the propagation of the helix formation after the two single strands have formed a few initial base pairs. We were particularly interested in the influence of initial conformation of the single strands at the beginning of the helix formation.

Results

We found that the double helix correctly forms with a very high probability in our simulations, independent of the initial conformation. Furthermore, we investigated the dynamics of the process at multiple temperatures and obtained consistent results. A snapshot of the early onset of the double strand formation is shown in the included figure.

Conclusions

Our preliminary results suggest that the double strand formation happens in a zipper-like manner, i.e., base pairs form consecutively.



DNA folding in molecular dynamics simulations. Here, we show a snapshot from the early onset of the double helix formation. In white, we show the base pairs that model the initial binding of the double helix. The rest of the DNA, which will form the helix, is shown in colors. The side chains are shown atomistically as licorice model, whereas the backbone is shown as less detailed surface model. In the center of the image, the first consecutive base pair is currently forming.

PS-XI-04

Quantitative and mechanistic insights into transmembrane protein dimerization via machine learning-assisted path sampling simulations

Gianmarco Lazzeri[1,2], Emil Jackel[1,2], Peter G. Bolhuis[3], Roberto Covino[1,2]

[1] Goethe University Frankfurt, Frankfurt am Main, Germany; [2] Frankfurt Institute for Advanced Studies, Frankfurt am Main, Germany; [3] University of Amsterdam, Van 't Hoff Institute for Molecular Sciences, Amsterdam, Netherlands

Molecular simulations can provide precise thermodynamic and kinetic information for increasingly complex molecular systems due to the recent advances in modeling and computational power. However, rare events still pose a challenge, as the systems must navigate highly infrequent but critical regions of the conformational landscape to transition between metastable states. Transmembrane protein dimers are peculiar cases due to their extreme asymmetry: while the dimerization process is usually fast, the dissociation is exponentially suppressed by the enthalpic barrier from the dimerized state. To tackle the issue, we build on AI for molecular mechanism discovery - AIMMD. AIMMD leverages machine learning to perform path sampling simulations outside the states while dynamically optimizing a reaction coordinate (RC) model. The model learns from simulated data and controls the upcoming simulations in real time, thus improving the sampling efficiency. The RC and the simulated transitions offer mechanistic insight into the process[1]. Additionally, a reweighting algorithm based on the RC recovers the system's equilibrium thermodynamics and kinetics[2]. Here, we show that AIMMD can accurately measure the free energy profiles and transition rates of epidermal growth factor receptor dimerization/dissociation, outperforming standard molecular dynamics in terms of speed. The results hold promising applications in the study of other rare event-dominated systems.

References

[1] Jung, H, Covino, R, Arjun, A, Leitold, C, Dellago, C, Bolhuis, PG, Hummer, G 2023. 'Machine-guided path sampling to discover mechanisms of molecular self-organization.' *Nature Computational Science*, *3*(4), 334-345.

[2] Lazzeri, G, Jung, H, Bolhuis, PG, Covino, R 2023. 'Molecular free energies, rates, and mechanisms from data-efficient path sampling simulations.' *Journal of Chemical Theory and Computation*, *19*(24), 9060-9076

Index

Author Index

D

E

F

G

H

Author Index

Author Index

Author Index

Author Index

T

U

V

Author Index