12th - 13th June 2025

Niels K. Jerne Auditorium

Panum, UCPH

SYMPOSIUM

CHEMICAL SOLUTIONS TO STRUCTURAL BIOLOGY

SYMPOSIUM DELEGATE BOOKLET

Organising Committee:

Morten Meldal (Chair), Dept. Chemistry, UCPH

Karen Martinez (Vice-Chair), Dept. Chemistry, UCPH

Claus Løland, Dept. Neuroscience, UCPH

Nikos Hatzakis, Dept. Chemistry, UCPH

Hosted by:

The Integrative Structural Biology cluster at the University of Copenhagen

INTEGRATIVE STRUCTURAL BIOLOGY CLUSTER AT THE UNIVERSITY OF COPENHAGEN

Funded by:

Novo Nordisk Foundation, Lundbeck Foundation and Carlsberg Foundation

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PROGRAMME

Day One

11th June
Level 15 Viewing Area, Mærsk Tower

WELCOME NETWORKING SESSION

Sponsored by Novo Nordisk Foundation

16:00 – 18:00	Registration and welcome reception with drinks and snacks
16:30	Welcome speech by Rector David Drejer Lassen

Day Two

12th June
Jerne auditorium, Mærsk Tower

08:30 – 08:45	Registration

SESSION 1: BRIDGING STRUCTURAL BIOLOGY AND DRUG DISCOVERY

Sponsored by Lundbeck Foundation

08:45 – 09:00	Welcome Remarks: Morten Meldal (CSSB Chair)
09:00 – 10:00	Keynote Talk: **Brian Kobilka** (Stanford University) G Protein Coupled Receptors: Challenges and New Approaches to Drug Discovery
10:00 – 10:30	**Coffee Break**
10:30 – 10:55	Invited Talk: **Jörg Rademann** (Freie Universität Berlin) Biomimetics discovery from structure-based design and protein-templated reactions
10:55 – 11:20	Invited Talk: **Annette Beck-Sickinger** (Leipzig University) G Protein-coupled peptides receptors: from structure to innovative ligands and therapeutic concepts
11:20 – 11:45	Invited Talk: **Nieng Yan** (Tsinghua University) From sugar transporters to glycoconjugated ion channels
11:45 – 11:55	Flash Talk: **Sára T. Mellemgaard** (University of Copenhagen) Design and Evolution of Functional l/d-Microproteins Against XIAP to Induce Apoptosis of Cancer Cells
11:55 – 13:15	**Lunch**

SESSION 2: UNRAVELLING DYNAMIC CELL BIOLOGY BY CHEMICAL SYNTHESIS

Sponsored by Novo Nordisk Foundation

13:15 – 14:15	Keynote Talk: **Yamuna Krishnan** (University of Chicago) Intracellular Electrophysiology
14:15 – 14:40	Invited Talk: **Patricia Bassereau** (Institut Curie) Unraveling membrane protein dynamics and interactions at the single-molecule scale
14:40 – 14:50	Flash Talk: **Gerti Beliu** (University of Regensburg) Decoding Hidden Conformational States in Living Cells through Chemical Biology
14:50 – 15:30	**Coffee**

SESSION 3: TAILORING PROTEIN FUNCTION

Sponsored by Lundbeck Foundation

15:30 – 16:30	Keynote Talk: **Don Hilvert** (ETH Zurich) Designing enzymes de novo
16:30 – 16:55	Invited Talk: **Kristian Strømgaard** (University of Copenhagen) Targeting protein-protein interactions of receptor complexes with therapeutic peptides
16:55 – 17:20	Invited Talk: **Knud Jensen** (University of Copenhagen) Selective chemical modification of proteins: His tag acylation
17:20 – 17:30	Flash Talk: **Ciara F. Pugh** (University of Copenhagen) A copolymer for structural and functional characterization of membrane proteins in native lipid nanodiscs
17:30 – 20.00	**Poster session with light bites and drinks**

Day Three

13th June
Jerne auditorium, Mærsk Tower

SESSION 4: SYNTHETIC APPROACHES TO STRUCTURAL BIOLOGY

Sponsored by Carlsberg Foundation

09:00 – 10:00	Keynote Talk: **Ikuhiko Nakase** (Osaka Metropolitan University) Unique intracellular delivery technology based on membrane-penetrating peptides with designed engineering
10:00 – 10:30	**Coffee & Croissants**
10:30 – 10:55	Invited Talk: **Helma Wennemers** (ETH Zurich) Chemistry in the Extracellular Matrix
10:55 – 11:20	Invited Talk: **Thomas Poulsen** (Aarhus University) Precision chemistry – from the protein to the proteome
11:20 – 11:45	Invited Talk: **Christian Adam Olsen** (University of Copenhagen) Targeting epigenetic regulators with peptide macrocycles
11:45 – 11:55	Flash Talk: **Mette Galsgaard Malle** (Aarhus University) Programmable RNA loading of extracellular vesicles
11:55 – 13:00	**Lunch**

SESSION 5: CHEMISTRY FOR FRONTIER IMAGING

Sponsored by Novo Nordisk Foundation

13:00 – 14:00	Keynote Talk: **Markus Sauer** (University of Würzburg) Molecular Resolution Fluorescence Imaging in Cells
14:00 – 14:25	Invited Talk: **Ben Schuler** (University of Zurich) Interaction dynamics of disordered proteins from single-molecule spectroscopy
14:25 – 14:50	Invited Talk: **Nikos Hatzakis** (University of Copenhagen) 4D cellular biology and machine learning analysis of cellular responses in delivery of biologicals
14:50 – 15:20	**Coffee & Cake**
15:20 – 15:45	Invited Talk: **Sonja Schmid** (University of Basel) Bringing structures to life: the broad-range dynamics of biomolecules uncovered by DyeCycling
15:45 – 16:10	Invited Talk: **Jin Zhang** (University California San Diego) Illuminating the Biochemical Activity Architecture of the Cell
16:10 – 16:20	Flash Talk: **Céline Delaitre** (University of Copenhagen) First Selective Fluorescent Ligands recognizing selectively Angiotensin II Receptors subtypes
16:20 – 16:40	**Short Break**

CLOSING SESSION

Sponsored by Novo Nordisk Foundation

16:40 – 17:40	Keynote Talk: **Morten Meldal** (University of Copenhagen) The DS driven intramolecular INAIC and INCIC reactions
17:40 – 17:50	Award Ceremony: For best poster (sponsored by Lundbeck Foundation)
17:50 – 18:00	Closing remarks: Birthe B. Kragelund (ISBUC Chair)
19:00 - onwards	**Gala dinner at UCPH Festsal**

Keynote and Invited Talks

1. **Annette G. Beck-Sickinger:** PEPTIDE RECEPTORS: STRUCTURE, FUNCTION AND INNOVATIVE THERAPEUTIC CONCEPTS
2. **Ben Schuler:** INTERACTION DYNAMICS OF DISORDERED PROTEINS FROM SINGLE-MOLECULE SPECTROSCOPY
3. **Brian Kobilka:** G PROTEIN COUPLED RECEPTORS: CHALLENGES AND NEW APPROACHES TO DRUG DISCOVERY
4. **Christian A. Olsen:** TARGETING EPIGENETIC REGULATORS WITH PEPTIDE MACROCYCLES
5. **Don Hilvert:** DESIGNING ENZYMES DE NOVO
6. **Helma Wennemers:** CHEMISTRY IN THE EXTRACELLULAR MATRIX
7. **Ikuhiko Nakase:** UNIQUE INTRACELLULAR DELIVERY TECHNOLOGY BASED ON MEMBRANE-PENETRATING PEPTIDES WITH DESIGNED ENGINEERING
8. **Jin Zhang:** ILLUMINATING THE BIOCHEMICAL ACTIVITY ARCHITECTURE OF THE CELL
9. **Jörg Rademann:** PHOSPHOTYROSINE BIOMIMETICS DISCOVERY FROM STRUCTURE-BASED DESIGN AND PROTEIN-TEMPLATED REACTIVITY
10. **Knud Jensen:** SELECTIVE CHEMICAL MODIFICATION OF PROTEINS: HIS TAG ACYLATION
11. **Kristian Strømgaard:** TARGETING PROTEIN-PRTOEIN INTERACTIONS IN RECEPTOR COMPLEXES
12. **Markus Sauer:** MOLECULAR RESOLUTION FLUORESCENCE IMAGING IN CELLS
13. **Morten Meldal:** THE DS DRIVEN INTRAMOLECULAR INAIC AND INCIC REACTIONS
14. **Nieng Yan:** FROM SUGAR TRANSPORTERS TP GLYCOCONJUGATED ION CHANNELS
15. **Nikos Hatzakis:** 4D CELLULAR BIOLOGY AND MACHINE LEARNING ANALYSIS OF CELLULAR RESPONSES IN DELIVERY OF BIOLOGICALS
16. **Patricia Bassereau:** UNRAVELING MEMBRANE PROTEIN DYNAMICS AND INTERACTIONS AT THE SINGLE MOLECULE SCALE
17. **Sonja Schmid:** BRINGING STRUCTURES TO LIFE: THE BROAD-RANGE DYNAMICS OF BIOMOLECULES UNCOVERED BY DyeCycling
18. **Thomas B. Poulsen:** PRECISION CHEMISTRY – FROM THE PROTEIN TO THE PROTEOME
19. **Yamuna Krishnan:** INTRACELLULAR ELECTROPHYSIOLOGY

1. Annette G. Beck-Sickinger

PEPTIDE RECEPTORS: STRUCTURE, FUNCTION AND INNOVATIVE THERAPEUTIC CONCEPTS

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Invited talk

Peptides play an important role in the regulation of manifold activities in the body. Many of them transmit their activity through G protein-coupled receptors (GPCR), which are among the most promising drug targets nowadays. However, in addition to their direct activity, indirect mechanisms have been shown to play an important role, e.g. as drug shuttles in tumor targeting. Accordingly, in addition to ligand binding, internalization has to be addressed and to be studied, including arrestin recruitment. Two important pharmacological targets will be discussed.

The neuropeptide Y/pancreatic polypeptide family contains 36 amino acid peptides, which bind to four different so-called Y-receptors in humans. By a combination of structural analysis, molecular modelling and crosslinking combined with mass spectrometry, we have recently identified the distinct binding modes of NPY peptides to their Y-receptors. Neuropeptide Y receptors have been shown to play a relevant role in the regulation of food intake. Furthermore, they participate in adipogenesis and some of them are overexpressed in tumours. By knowing the receptor bound structure, specific ligands as well as peptide-drug conjugates have been designed to selectively address Y receptors in different tissues including allosteric modulators.

In a similar approach, we studied the 135 amino acid protein chemerin, which is recognized by three receptors. Chemerin links adipositas and inflammation but also plays a role in tumour development. By a combination of structural and biochemical methods, the binding and activation mode of the protein, and thereof derived small and stabilized peptides are compared.

2. Ben Schuler

INTERACTION DYNAMICS OF DISORDERED PROTEINS FROM SINGLE-MOLECULE SPECTROSCOPY

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University of Zurich

Invited Talk

The functions of proteins have traditionally been linked to their folded structures, but many proteins perform essential functions without being folded. Quantifying the highly dynamic and conformationally diverse ensembles of these intrinsically disordered proteins (IDPs) and their interaction mechanisms is an important aspect of understanding their functions. A remarkable example are highly charged IDPs, which can form high-affinity interactions but retain their disorder in the complexes. I will illustrate how advanced single-molecule spectroscopy combined with molecular simulations and other biophysical methods can be used to probe the dynamics, interactions, and phase separation of such disordered systems.

References:

Borgia et al. (2018) Extreme disorder in an ultrahigh-affinity protein complex. Nature 555, 61-66

Sottini et al. (2020) Polyelectrolyte interactions enable rapid association and dissociation in high-affinity disordered protein complexes. Nat. Commun. 11, 5736

Heidarsson et al. (2022) Release of linker histone from the nucleosome driven by polyelectrolyte competition with a disordered protein. Nat. Chem. 14, 224-231

Chowdhury et al. (2023) Driving forces of the complex formation between highly charged disordered proteins. Proc. Natl. Acad. Sci. USA 120, e2304036120

Galvanetto, Ivanović et al. (2023) Extreme dynamics in a biomolecular condensate. Nature 619, 876-883

3. Brian Kobilka

G Protein Coupled Receptors: Challenges and New Approaches to Drug Discovery

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Keynote Talk

G protein-coupled receptors (GPCRs) mediate the majority of cellular responses to hormones and neurotransmitters, as well as the senses of sight, smell and taste. They represent the largest class of drug targets for the pharmaceutical industry; however, there are many obstacles to the discovery and development of safe and effective drugs for specific GPCR targets. I will provide an overview of the molecular basis of GPCR signaling, and discuss the challenges in drug discovery for GPCRs using the μ-opioid receptor (μOR) as a model system. The management of acute and chronic pain is one of the greatest challenges in modern medicine. While effective, many of the currently used opioid analgesics are highly addictive and their increased clinical use over the past 20 years is partially responsible for the opioid epidemic. The properties of more recently discovered μOR agonists suggest that it may be possible to separate analgesia from liabilities including addiction, tolerance and respiratory suppression. The μOR can signal through six G protein isoforms (Gi1,2,3, GoA,B, Gz), and through arrestin 2 and 3. We have observed that different μOR agonists differentially activate these signaling pathways. I will discuss what we have learned about the structural basis for G protein isoform and arrestin-biased signaling by the μOR.

4. Christian A. Olsen

TARGETING EPIGENETIC REGULATORS WITH PEPTIDE MACROCYCLES

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Center for Biopharmaceuticals and Department for Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark.

Invited talk

Histone deacetylases (HDACs) are validated targets for treatment of certain cancers and play numerous regulatory roles in biology, ranging from epigenetics to metabolism. Small molecules are highly important as tool compounds to probe these mechanisms as well as for the development of new medicines. Therefore, detailed mechanistic information and precise characterization of the enzyme substrate preference as well as development of chemical probes to investigate the effects of HDAC enzymes are vital.

Through profiling of both sirtuins (SIRTs) and zinc-dependent HDACs, we have developed efficient assay formats for inhibitor characterization and discovered enzymatic activities against novel posttranslational modifications, most recently e-N-lactyllysine. Our insights into the substrate specificity of sirtuins have been exploited for the design of potent inhibitor tools compounds and photo cross-linking probes against SIRT1–3 and 5.

We have also interrogated Nature's arsenal of macrocyclic non-ribosomal peptide HDAC inhibitors by chemical synthesis and evaluation of more than 30 natural products and analogs, enabling the design of highly potent class I and IIb HDAC inhibitors. Our work provides novel inhibitors with varying potencies, selectivity profiles, and mechanisms of inhibition, which we hope will help improve interpretation of the function of HDACs and sirtuins in biology and medicine. In this presentation, I will focus on our most recent advances in the selective targeting of SIRT7 and HDAC11.

5. Don Hilvert

DESIGNING ENZYMES DE NOVO

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Laboratory of Organic Chemistry, ETH Zürich, Zurich, Switzerland.

Keynote talk

Biocatalysis is emerging as a key enabling technology for the development of a greener and more efficient chemical industry. Because natural enzymes are seldom suitable for direct use in chemical processes, however, protein engineering is typically required to optimize their properties for practical applications. For many desirable chemical transformations, natural enzymes may not even exist. Recent advances on the computational design of atomically accurate protein structures, combined with high-throughput evolutionary optimization, are providing a potential roadmap for reliably creating new-to-nature biocatalysts in response to diverse societal demands. In this lecture, the strategies, opportunities, and challenges facing this emerging field will be surveyed.

6. Helma Wennemers

CHEMISTRY IN THE EXTRACELLULAR MATRIX

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Invited talk

Collagen, the most abundant protein in mammals, is a key contributor to the strength and stability of skin, bones, and connective tissue. Collagen formation is thus vital for the integrity of skin, tendons, and the tissue in essentially any organ. Excessive collagen formation is, however, characteristic of fibrotic and malignant diseases, which include major global health issues.

We has used collagen model peptides (CMPs) to understand the stability of collagen at the molecular level and to establish functional synthetic collagen triple helices.1 These include hyperstable triple helices2 and heterotrimeric collagen.3-5 Building on these data, we designed and synthesized chemical probes for the simultaneous monitoring and targeting of lysyl oxidase (LOX)-mediated collagen cross-linking.6 The probes allow for the visualization of collagen formation with spatial resolution in vivo and in tissue sections.6,7

1. a) H. Wennemers, "Peptides – Molecular Allrounders" Chimia 2021, 75, 525.
   b) M. C. Deen, L. B. Boll, H. Wennemers Chimia, 2024, 78, 673.
2. J. Egli, C. Esposito, M. Müri, S. Riniker, H. Wennemers, J. Am. Chem. Soc. 2021, 143, 5937
3. V. Islami, P. Bittner, T. Fiala, N. B. Hentzen, R. Zenobi, H. Wennemers, J. Am. Chem. Soc. 2024, 146, 1789
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5. T. Fiala, P. Bittner, R. Heeb, V. Islami, C. Söll, A. Pruška, R. Zenobi, H. Wennemers, Angew. Chem. Int. Ed. 2025, e202503353
6. M. R. Aronoff, P. Hiebert, N. B. Hentzen, S. Werner, H. Wennemers, Nat. Chem. Biol. 2021, 17, 865
7. P. Hiebert, G. Antoniazzi, M. Aronoff, S. Werner, H. Wennemers, Matrix Biol. 2024, 128, 11

7. Ikuhiko Nakase

UNIQUE INTRACELLULAR DELIVERY TECHNOLOGY BASED ON MEMBRANE-PENETRATING PEPTIDES WITH DESIGNED ENGINEERING

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Keynote talk

Currently, biopharmaceuticals, including antibodies, occupy the top of the market launch order, and there are great expectations for further functionalization of pharmaceuticals and innovation in their diagnostic and therapeutic effects. However, most biopharmaceuticals with relatively large molecular weights target the outside of the cell, and the development of biopharmaceuticals that can target molecules inside the cell, especially those in the cytoplasm after membrane permeation, is an urgent issue. Various cell-permeable peptides (CPPs) [1] have been developed to aid in the cytoplasmic delivery of drugs and are under active clinical investigation. On the other hand, for CPP-based DDS, there is a strong need to establish methodologies to deliver drugs precisely where they should work in the cell and techniques that combine cell targeting and cell membrane permeation. In this talk, we will discuss the technological development of CPP-based drug delivery methods that our research team has been working on, focusing on the development of toxin-based CPPs, pH-sensitive fusion peptides, designed mitochondria-targeted CPPs, photoconcentration techniques using optical tweezers, and delivery of CPPs to target cytosol using inkjet systems delivery to the target cytoplasm from a biophysical perspective [2-4]. The application of CPPs to extracellular vesicles [5-8], including exosomes and microvesicles, such as boron neutron capture therapy (BNCT) will also be presented and future medical applications will be discussed.

References:
[1] Futaki, S., Nakase, I., Acc. Chem. Res., 50, 2449-2456 (2017)
[2] Nakase, I., et al., Chem. Commun., 55, 13955-13958 (2019)
[3] Nakase, I., et al., Nano Lett., 22, 9805-9814 (2022)
[4] Omura, M., et al., ACS Appl. Mater. Interfaces, 15, 47855-47865 (2023)
[5] Morimoto, K., et al., ACS Appl. Mater. Interfaces, 16, 17069-17079 (2024)
[6] Hirase, S., et al., Mol. Pharm., 19, 1135-1145 (2022)
[7] Noguchi, K., et al., Mol. Pharm., 18, 3290-3301 (2021)
[8] Nakase, I., et al., Chem. Commun., 53, 317-320 (2016)
[9] Akishiba. M., et al., Nat. Chem., 9, 751-761 (2017)

8. Jin Zhang

ILLUMINATING THE BIOCHEMICAL ACTIVITY ARCHITECTURE OF THE CELL

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Invited talk

The complexity and specificity of cellular processes require spatial microcompartmentation and dynamic modulation of the underlying biochemical activities, such as dynamic phosphorylation and dephosphorylation catalyzed by specific protein kinases and phosphatases, respectively. We hypothesize that cellular biochemical activities are spatially organized into an “activity architecture” and reorganization and restructuring of this activity architecture lead to disease. In this talk, I will introduce a series of genetically encoded or chemigenetic fluorescent biosensors that we have developed to monitor biochemical events in living cells, and then present a couple of studies where we combine quantitative fluorescence imaging with targeted perturbations as well as biochemical and functional assays to probe the spatiotemporal regulation of cell signaling.

9. Jörg Rademann

PHOSPHOTYROSINE BIOMIMETICS DISCOVERY FROM STRUCTURE-BASED DESIGN AND PROTEIN-Ttemplated REACTIVITY

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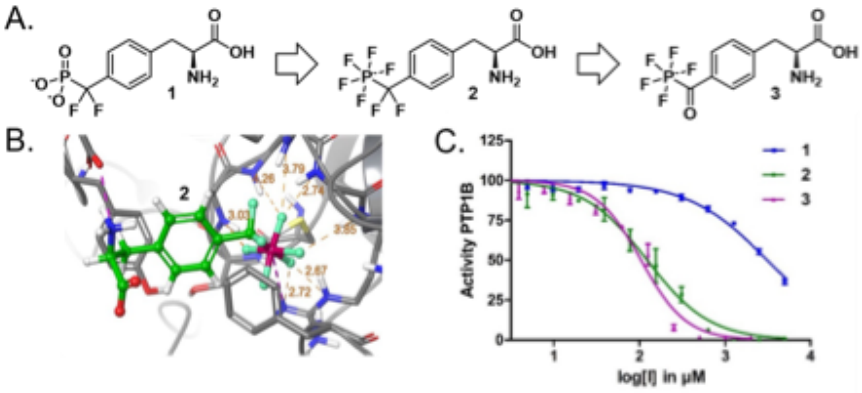
Freie Universität Berlin, Department of Chemistry, Biology, Pharmacy, Institute of Pharmacy.

Invited talk

Phosphate residues in peptides and proteins are essential functional switches in health and in disease. Accordingly, biomimetics of phosphopeptides and -proteins are valuable chemical tools and have been applied successfully in drug development. Here, we report the discovery and investigation of phosphotyrosine biomimetics through fragment ligation screening [1] and structure-based design [2,3]. Pentafluorophosphato-amino acids (PF5-amino acids) were prepared in excellent yields and employed in peptide synthesis and recombinant protein expression. Structures, stability, and fluorine-specific interactions were studied by NMR and vibrational spectroscopy, X-ray diffraction, and in bioactivity assays to assess protein binding. The mono-anionic PF5 motif displays an amphiphilic character binding to hydrophobic surfaces but also showing distinct interactions with water molecules and with phosphotyrosine recognition sites in proteins. Binding of the PF5 motif exploits both charge-charge interactions and fluorine-hydrogen-bonds. The novel biomimetics 2, 3 bind up to 25-fold stronger to the phosphotyrosine binding site of the protein tyrosine phosphatase PTP1B than the structurally analogous, currently used phosphonate biomimetic 1. Our findings were rationalized by computational methods using quantum-mechanical parametrization of a forcefield, docking, and molecular dynamics simulations. Finally, we will discuss applications of fluorine-rich phosphate mimetics in chemical biology and medicinal chemistry.



References

[1] M. Tiemann et al. A Formylglycine-Peptide for the Site-Directed Identification of Phosphotyrosine-Mimetic Fragments. Chem. Eur. J. 2022, 28, e202201282.

[2] M. Accorsi et al. Pentafluorophosphato-Phenylalanines: Amphiphilic Phosphotyrosine Mimetics Displaying Fluorine-Specific Protein Interactions. Angew. Chem. Int. Ed. 2022, 61, e202203579.

[3] A. M. Ambros et al. Genetic Encoding of Pentafluorophosphato-Phenylalanine Provides PF5-Proteins as Phosphoprotein Mimetics, submitted for publication.

10. Knud Jensen

SELECTIVE CHEMICAL MODIFICATION OF PROTEINS: HIS TAG ACYLATION

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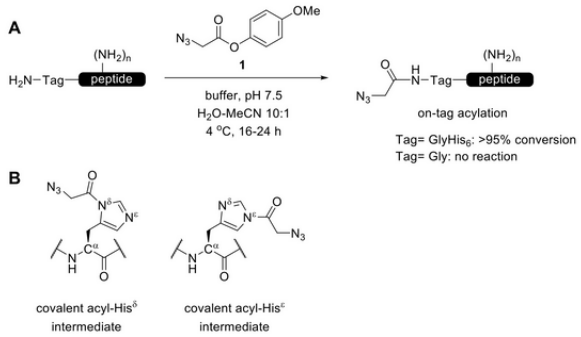
Knud J Jensen, Delphine N. Møller, Tuule Treiberg, Kasper K. Sørensen, Mikkel B. Thygesen, Christian Kofoed
University of Copenhagen, Department of Chemistry

Invited Talk

The chemical modification of peptides and proteins is of great importance in chemical biology, biotechnology, and biopharmaceuticals, as it enables introduction of fluorophores, biotin, half-life extending moieties, and more. We hypothesize that new-to-nature chemical reactivities can be designed in proteins. We have focused on autocatalytic peptide segments that direct their own chemical modification. We developed two methods for using poly-His sequences to direct the highly selective acylation of peptides and proteins, either at the N-terminus or at a specific Lys residue. We developed an N-terminal Gly-His_6 segment (Gly-His tag) that directed acylation of the N-terminal N^a-amine with 4-methoxyphenyl esters resulting in stable conjugates.[1] We have extended His tag acylation by developing an autocatalytic His sequence for highly selective acylation on Lys, which we name a Lys-His tag. We developed the peptide sequences His_n-Lys-His_m (Lys-His tags) that direct the acylation of the designated Lys N^e-amine under mild conditions and with high selectivity over native Lys residues. Both the Gly-His and Lys-His tags maintain the capacity for immobilized metal ion affinity chromatography. We have demonstrated the robustness of these methods by attaching different moieties such as azides, fluorophores, and biotin to different proteins, including antibodies. Here we present Lys-His tag acylation of peptides and proteins, such as antibodies.[2,3] We also present extensive investigations on the mechanism for His tag acylation based on kinetic studies, DFT calculations, and acylation reagents with systematically varied properties. These mechanistic studies have directed the development of new acylation reagents, which will be described.



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2. C. Kofoed et al., Chemistry European Journal, 2022, e202200147.
3. K. J. Jensen et al., ChemBioChem, 2022, cbic 202200359.

11. Kristian Strømgaard

TARGETING PROTEIN-PRTOEIN INTERACTIONS IN RECEPTOR COMPLEXES

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Center for Biopharmaceuticals, Department of Drug Design and Pharmacology, University of Copenhagen

Invited Talk

Protein-protein interactions (PPIs) are essential to vital cellular processes and serve as potential targets for therapeutic intervention. We are particularly interested in the PPIs between integral membrane protein receptors and their intracellular protein partners, so-called ‘receptor complexes’ and examine how modulation of PPIs of such receptor complexes can provide novel biological insight and new therapeutics. We have developed peptide-based inhibitors of the PSD-95/glutamate receptor interaction, as potential treatment for acute conditions such as acute ischemic stroke. Specifically, we have exploited that PSD-95 contains a tandem PDZ1-2 domain and have designed and synthesized dimeric peptides with low nanomolar affinities and have demonstrated that these ligands are potential treatment for ischemic stroke. The lead compound AVLX-144 was recently successfully examined in a Phase I clinical trial. The last part of the talk will cover very recent studies, where we have explored the potential of interrogating both PSD-95 and glutamate receptors as potential means to alleviate obesity.

12. Markus Sauer[1,2]

MOLECULAR RESOLUTION FLUORESCENCE IMAGING IN CELLS

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Keynote talk

Over the past decade, super-resolution fluorescence imaging by single-molecule localization has evolved as a powerful method for subdiffraction-resolution fluorescence
imaging of cells and structural investigations of subcellular structures. However, although refined single-molecule localization microscopy (SMLM) methods can now provide a spatial resolution in the one-digit nanometer range on isolated molecules, that is, well below the diffraction limit of light microscopy, translation of such high spatial resolutions to sub-10 nm imaging in cells or tissues remains challenging. This is mainly caused by the insufficient labeling density and linkage error achieved using standard labeling methods. Furthermore, even if high density labeling can be realized fluorophore communication via different energy pathways can prevent reliable molecular resolution fluorescence imaging in cells. In my contribution I will introduce and discuss different methods to bypass these limitations. One is based on physical expansion of the cellular structure by linking a protein of interest into a dense, cross-linked network of a swellable polyelectrolyte hydrogel. By combining ~8-fold Expansion Microscopy (ExM) with direct stochastic optical reconstruction microscopy (dSTORM) on post-expansion immunolabeled samples we resolve the 8-nm periodicity of a,ß-heterodimers in microtubules and the polyhedral lattice in clathrin-coated pits with nanometer resolution in intact cells. Furthermore, I will demonstrate that 2-color Ex-dSTORM reveals the molecular organization of endogeneous RIM scaffolding proteins and Munc13-1, an essential synaptic vesicle priming protein, in ring-like structures with diameters of 20-30 nm at the presynapse in hippocampal neurons.
Furthermore, I will discuss an alternative approach that uses genetic code expansion (GCE) and click labeling of unnatural amino acids to introduce fluorophores site-specifically into multimeric proteins with minimal linkage error. Using resonance energy transfer between fluorophores separated by less than 10 nm, information about the distance of the fluorophores in cells separated by only a few nanometers can be unraveled using fluorescence photoswitching characteristics. Using time-resolved fluorescence detection in combination with this so-called photoswitching fingerprint analysis interfluorophore distances of only a few nanometers can be reliably resolved, even in living cells. Finally, I will demonstrate that the use of these tools in combination with fixed and live-cell lattice-light-sheet microscopy can be used advantageously to decode the molecular interplay of endogenous CD20 on tumor cells with therapeutic antibodies.

13. Morten Meldal

THE DS DRIVEN INTRAMOLECULAR INAIC AND INCIC REACTIONS

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Keynote talk

The CuAAC click reaction[1] , a Nobel serendipitous discovery will be briefly presented. The intramolecular N-acyl iminium cascade reaction (INAIC) reaction, which is driven by entropy and which in many respects follow click criteria was also a serendipitous discovery. It was discovered by NMR- investigations of peptide aldehydes showing entropy driven quantitative and highly stereo selective reaction with backbone or sidechain amides in peptides. This allowed merger of organic chemistry with peptide diversity. The reaction proceeds through highly reactive N-acyl iminium ions, which in turn react with a second C, O, N or S nucleophile in the peptide to form multiple heterocycles in a stereo and enantioselective manner.[2] The reaction was extended to the intramolecular N-carbamoyl iminium ion cascade reaction which led to a range of interesting environment sensitive fluorophores for investigation of peptide bioactivity. Many examples of these versatile reactions will be presented.

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14. Nieng Yan[1, 2]

FROM SUGAR TRANSPORTERS TP GLYCOCONJUGATED ION CHANNELS

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1. Shenzhen Medical Academy of Research and Translation (SMART)
2. Shenzhen Bay Laboratory

Invited talk

Carbohydrates are the most abundant biomolecules on Earth. Despite their physiological importance, the structural biology of glycans has significantly lagged behind that of proteins and nucleic acids. The crystal structure of the human glucose transporter GLUT3 bound to D-glucose at 1.5 Å resolution clearly demonstrates that the transporter can recognize both α- and β-anomers. This finding underscores the power of high-resolution structures in elucidating the stereochemistry of sugars. While cryo-EM has enabled the structural resolution of glycan chains that modify the extracellular surface of membrane proteins, it has largely been limited to a small number of sugar residues near the modification site and at moderate resolutions. We have been striving to solve high-resolution structures of full glycan chains with little success until recently. By employing a strategy called CryoSeek, we have successfully resolved the high-resolution structures of numerous glycans with higher-order structural assemblies. In this presentation, I will focus on the serendipitous discovery of an 8,000-residue glycoprotein, which we named Mstax. This protein serves as the central shaft for the lateral hairs, known as mastigonemes, that line the cilia of Chlamydomonas. Mstax alone extends over 600 nm from the cilia surface. Notably, it contains a PDK2-like transmembrane domain, which provides an immediate explanation for the previously reported association between mastigonemes and PKD2 subunits. Whether Mstax and PKD2 proteins, along with a third component SIP, form a functional channel remains to be investigated.

15. Nikos Hatzakis

4D CELLULAR BIOLOGY AND MACHINE LEARNING ANALYSIS OF CELLULAR RESPONSES IN DELIVERY OF BIOLOGICALS

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NovoNordisk Center for Optimised Oligo escape University of Copenhagen, Copenhagen, Denmark

Invited talk

Current advances in imaging technologies compounded with, machine learning drivel analysis, have allowed the direct observation of biological phenomena in real time. I will present our single particle assays offering high throughput screening of lipid nanoparticle properties and their interplays with oligonucleotide loading and delivery as well as our 4D readouts for the direct and in real time observation of the escape of genetic material in live cells. I will focus on the advanced the imaging toolboxes offering parallelized tracking of hundreds of individual nanoparticles (viruses Lipid nanoparticles) in live cell as well as on our deep learning approaches that map with up to 95% accuracy -in milliseconds instead of days- the key timepoints of LNPs internalization and genetic material releases. using exclusively diffusional behavior. These advances enable us to directly observe biological processes as they occur, extract quantitative mechanistic information, and leverage this knowledge to control aberrant biological functions.

Relevant publications from my lab

1. Jacob Kaestel- et al. Deep learning assisted single particle tracking for automated correlation between diffusion and function, Nature methods 2025]
2. Pinholt, H. D., et al , N. S. Single-particle diffusional fingerprinting: A machine-learning framework for quantitative analysis of heterogeneous diffusion. PNAS 118, e2104624118 (2021).
3. Stella, S. et al. Conformational Activation Promotes CRISPR-Cas12a Catalysis and Resetting of the Endonuclease Activity. Cell 175, 1856 (2018).
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5. Malle, M. G. et al. Single-particle combinatorial multiplexed liposome fusion mediated by DNA. Nat. Chem. (2022).

16. Patricia Bassereau

UNRAVELING MEMBRANE PROTEIN DYNAMICS AND INTERACTIONS AT THE SINGLE MOLECULE SCALE

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Alicia Damm[1], Kemil Belhadji[1], Raju Regmi[1], Raj Kumar Sadhu[1], Su Jin Paik[1], Jacob Kaestel-Hansen[2], Maxime Dahan[1], Nikos Hatzakis[2], Pierre Sens[1], Daniel Lévy[1], Patricia Bassereau[1]
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[2] Nano-Science Center, University of Copenhagen, Denmark

Invited talk

Cell membranes are highly deformable fluid surfaces that have to be strongly curved to a few tens of nanometers, for instance during intra- or extra-cellular exchanges upon formation of small buds or tubules, or during cell migration with the formation of actin-supported cellular protrusions (filopodia). Membrane-shaping processes always require proteins, in particular proteins with intrinsically-curved shapes or transmembrane proteins with conical shapes. Moreover, since cell membranes are fluid, proteins can diffuse on/in membranes, which allows them to redistribute depending on membrane shape changes. In vitro membrane systems with controlled curvature, combined to theoretical models, and single molecule approaches are instrumental for understanding the rich interplay between membrane shape/tension, protein distribution and lateral diffusion. I will present our recent results obtained with in vitro membrane systems and reconstituted ABC transporters on the effect of membrane curvature on their transport activity and on the effect of membrane tension on proteins diffusion and clustering.

17. Sonja Schmid[1,2]

BRINGING STRUCTURES TO LIFE: THE BROAD-RANGE DYNAMICS OF BIOMOLECULES UNCOVERED BY DYECYCLING

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[2] Swiss Nanoscience Institute, Klingelbergstrasse 82, 4056 Basel, Switzerland

Invited talk

Proteins are the molecular workhorses of life. While static 3D structures of proteins have been extensively characterized – fueling AI-predictions of entire proteomes – the molecular understanding of protein dynamics lags far behind, hampering progress in biomedicine, biotechnology, as well as protein design. Building upon available 3D structures, we therefore focus on capturing biomolecular dynamics with time-resolved single-molecule experiments using fluorescence/FRET as well as nanopore experiments to study cancer-assisting chaperone and kinase proteins, as well as CRISPR-associated proteins.

In this talk, I will primarily focus on our fluorescence-based work, in which we developed the DyeCycling method to overcome the long-standing photobleaching limitation of conventional single-molecule FRET. Previously, the observation of complex dynamics – particularly those involving rare, rate-limiting states – was severely restricted by early photobleaching. DyeCycling addresses this challenge by utilizing reversibly binding fluorescent probes, combined with optimized background suppression strategies, to extend the observation time of single molecules from seconds to over an hour, thereby surpassing conventional single-molecule FRET by two orders of magnitude. This methodological advance enables the long-term monitoring of conformational dynamics, revealing rare and functionally critical events as well as inter-molecular heterogeneities that were previously inaccessible. I will discuss the current capabilities and limitations of DyeCycling, along with potential chemical solutions to further broaden its application range. Overall, our results establish DyeCycling as a transformative approach for elucidating the intricate dynamic behavior of biomolecules, bridging the gap between static 3D structures and the dynamic processes that underlie protein function.

18. Thomas B. Poulsen

PRECISION CHEMISTRY – FROM THE PROTEIN TO THE PROTEOME

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Invited talk

Electrophiles are subject to strong contemporary interest within chemical biology and biomedicine. The discovery and development of novel electrophiles with ability to provide precise functionalization of biomolecules can enable e.g. bioconjugation to native proteins for the preparation of biopharmaceuticals or provide new warhead-classes for targeted covalent inhibitors or covalent fragments for screening. In this talk, I will present examples from our most recent [1,2,3,4,5,6] as well as ongoing projects focused on electrophilic compounds which span from fully synthetic constructs, e.g. based on strained ring systems, to natural products. I will discuss the different synthetic strategies involved but mostly focus on assessment of biological performance in different biochemical and cellular contexts.

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19. Yamuna Krishnan

INTRACELLULAR ELECTROPHYSIOLOGY

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Keynote talk

The chemical milieu within an organelle has been evolutionarily optimized to enable the biochemistry that occurs within. We study how organelle function impacts cell function by mapping ions within the organelle lumens using a chemical imaging technology based on DNA. DNA self-assembles into molecularly precise, synthetic assemblies, commonly referred to as DNA nanodevices. Our DNA nanodevices are ion responsive, fluorescent probes that can be targeted to specific organelles(1). These reporters can then quantitatively image ions in organelles of cells in culture, in live multicellular organisms (2) as well as in cells obtained from blood draws (3) or skin biopsies from human patients (4). I will focus on a recent finding where we solved a thirty-year problem in molecular sensing by mapping lumenal calcium in acidic organelles and in doing so, identified the first example of a human lysosomal Ca^{2+} importer.

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3. Cui, C. et al. A lysosome-targeted DNA nanodevice selectively targets macrophages to attenuate tumours. Nat. Nanotechnol., 2021,
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Flash Talks

1. Céline Delaitre: FIRST SELECTIVE FLUORESCENT LIGANDS RECOGNIZING SELECTIVELY ANGIOTENSIN II RECEPTORS SUBTYPES

2. Ciara Frances Pugh: A COPOLYMER FOR STRUCTURAL AND FUNCTIONAL CHARACTERISATION OF MEMBRANE PROTEINS IN NATIVE LIPID NANODISCS

3. Gerti Beliu: DECODING HIDDEN CONFORMATIONAL STATES IN LIVING CELLS THROUGH CHEMICAL BIOLOGY

4. Mette Galsgaard Malle: PROGRAMMABLE RNA LOADING OF EXTRACELLULAR VESICLES

5. Sára T. Mellemgaard: DESIGN AND EVOLUTION OF FUNCTIONAL L/D-MICROPROTEINS AGAINST XIAP TO INDUCE APOPTOSIS OF CANCER CELLS

1. Céline Delaitre

FIRST SELECTIVE FLUORESCENT LIGANDS RECOGNIZING SELECTIVELY ANGIOTENSIN II RECEPTORS SUBTYPES

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GPCR play fundamental physiological roles in tissues. Once activated by external stimulus, they can trigger various signaling pathways. Developing better drugs requires a better understanding of the different signaling pathways and the development of tools acting selectively on one of the pathways. Here this is the challenge in the case of the ATRs which are GPCRs regulated by AngII: AT1R (angiotensin II type 1 receptor), and AT2R (angiotensin II type 2 receptor). Biased AT1 agonists like TRV027, which selectively activate β-arrestin signaling while blocking Gq pathways, offer promising therapeutic potential (Delaitre et al., 2021). To better explore their effects on cerebral vessels, we designed and synthesized novel fluorescent derivatives of AngII, TRV027, and losartan. Their chemical and pharmacological properties, including receptor selectivity, potency, and pathway-specific activation, were rigorously characterized in both cellular systems and isolated cerebral arteries. Detailed structure–activity relationship studies revealed the first highly AT1-selective fluorescent antagonist based on losartan, and analogues of the biased agonist TRV027 unexpectedly switching selectivity toward AT2 receptors. These chemically engineered ligands now serve as powerful molecular probes to visualize and study AT1 and AT2 receptors in vitro and ex vivo. Quantitative confocal microscopy further showed that losartan binds AT1 with higher affinity at elevated receptor densities (Delaitre et al., 2024). This work illustrates how targeted chemical modifications open new avenues to explore receptor structure, dynamics, and signaling.

Delaitre, C., Boisbrun, M., Lecat, S., & Dupuis, F. (2021). Targeting the Angiotensin II Type 1 Receptor in Cerebrovascular Diseases: Biased Signaling Raises New Hopes. International Journal of Molecular Sciences, 22(13), Article 13. https://doi.org/10.3390/ijms22136738

Delaitre, C., Boisbrun, M., Acherar, S., Dias, A., Kleinclauss, A., Achard, M., Colin, M., Nguyen, T. M., Humbert, N., Chmeis, K., Martinez, K. L., Gilles, N., Robin, P., Lecat, S., & Dupuis, F. (2024). Synthesis and Pharmacological Characterization of Fluorescent Ligands Targeting the Angiotensin II Receptors Derived from Agonists, β-Arrestin-Biased Agonists, and Antagonists. Journal of Medicinal Chemistry, 67(22), 20275–20297. https://doi.org/10.1021/acs.jmedchem.4c01693

2. Ciara Frances Pugh

A COPOLYMER FOR STRUCTURAL AND FUNCTIONAL CHARACTERISATION OF MEMBRANE PROTEINS IN NATIVE LIPID NANODISCS.

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Integral membrane proteins represent an important class of therapeutic targets, necessitating comprehensive understanding of their functional properties and structural attributes to facilitate rational drug development[1]. The development of amphiphilic copolymers capable of extracting membrane proteins directly from lipid bilayers into "native nanodiscs" offers a simplified approach for preparing membrane protein samples for structural and functional studies. Polymer amphiphilicity, length, and composition influence the performance of copolymers, in addition to the protein itself and the purification conditions used[2,3].

In this study, we introduce a copolymer composed of methacrylic acid and styrene, which we term MAASTY4. We leverage the inherent reactivity ratios of the individual monomers to create an anionic copolymer with a statistically determined distribution of monomers. MAASTY copolymers effectively solubilize a broad range of lipid species and a wide array of tested eukaryotic membrane proteins from mammalian cells. We show that MAASTY can be used for high-resolution structural determination of eukaryotic membrane proteins with single particle cryogenic electron microscopy (cryo-EM), preserving endogenous lipids. Additionally, we employ functionalized MAASTY copolymers for immobilization-based binding studies on membrane proteins in lipid nanodiscs. The MAASTY copolymers are promising as effective solubilizers of membrane proteins and offer a new chemical platform for their structural and functional characterization in a detergent-free system.

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3. Gerti Beliu[1,2]

DECODING HIDDEN CONFORMATIONAL STATES IN LIVING CELLS THROUGH CHEMICAL BIOLOGY

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Capturing transient conformational states in their native cellular context remains a key frontier in structural biology. While high-resolution methods provide detailed structural snapshots, they often fail to resolve how functional sites become accessible in dynamic, physiological environments.
We develop minimally invasive chemical strategies to access these hidden states directly in living cells. By integrating genetic code expansion with optimized suppression systems[1] and fluorogenic bioorthogonal labeling[2], we map conformational accessibility at defined sites with high spatiotemporal resolution.
Applying this framework, we revealed that tethered agonists in adhesion GPCRs become exposed through intrinsic GAIN domain flexibility - highlighting a proteolysis-independent gating mechanism for receptor activation[3]. Extending this approach to other protein complexes such as neuronal transmembrane proteins, we uncovered masked epitopes in AMPAR regulatory subunits (TARPs), enabling structural mapping of synaptic receptor assemblies under native conditions[4].
These examples demonstrate how chemical biology can decode dynamic structural states inaccessible by classical methods, offering direct insight into regulatory mechanisms of membrane proteins in situ.

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4. Mette Galsgaard Malle

PROGRAMMABLE RNA LOADING OF EXTRACELLULAR VESICLES

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Currently, synthetic nanoparticles like liposomes and lipid nanoparticles (LNPs) are extensively utilized for drug delivery, yet they suffer from drawbacks such as toxicity and adverse immune reactions. Natural extracellular vesicles (EVs) offer promises as native, safe, and versatile carriers for drugs. However, incorporating or loading of large molecules into EVs remains challenging.

In this study, we introduce a novel method for loading EVs by utilizing DNA-mediated fusion between EVs and liposomes loaded with mRNA. We assess loading and fusion efficiency at the individual particle level using real-time TIRF microscopy.
Liposomes containing mRNA encoding mCherry are DNA-mediated fused with EVs extracted from C2C12 cell culture medium. The EVs are anchored to a passivated surface using a lipidated DNA strand complementary to a biotin-DNA handle on the surface. This enables liposome fusion with surface-tethered EVs, followed by simple removal of non-fused liposomes by thoroughly washing. Fused EVs can then be subsequently released and collected using a DNA sequence for toehold release, providing an inherent fusion and purification strategy.

To evaluate the transfection efficiency of the hybrid EV-liposome particles, we delivered the fused hybrid EV-liposome encapsulated mRNA encoding mCherry to HEK cells. Significant mCherry expression was observed in the hybrid particles, whereas control groups with pure liposomes containing mCherry or traditionally used LNPs showed respectively no expression and significant less expression. We anticipate that this method represents a significant advancement for targeted EV-mediated therapy and natural drug delivery.

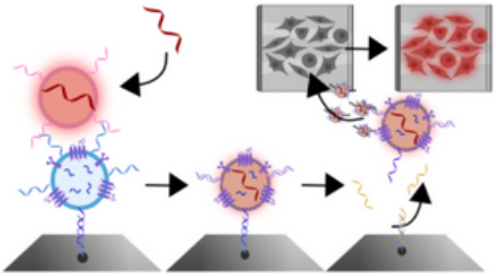

Figure 1: DNA programmable fusion of liposomes and extracellular vesicles for loading of mRNA for an enhanced and non-toxic delivery.

Inglut, C. T. et al. Immunological and toxicological considerations for the design of liposomes. Nanomaterials (Basel) 10, (2020).
Malle, M. G. et al. Single-particle combinatorial multiplexed liposome fusion mediated by DNA. Nat. Chem. 14, 558–565 (2022).

5. Sára T. Mellemgaard

DESIGN AND EVOLUTION OF FUNCTIONAL L/D-MICROPROTEINS AGAINST XIAP TO INDUCE APOPTOSIS OF CANCER CELLS

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Recognition l/d-microproteins, reported in this study are small, compact, atactic, protein-like peptides that function as antibody mimics. These l/d-microproteins are designed to comprise all the key advantages of antibodies, such as high affinity and long serum half-life, while reducing size and immunogenicity. They are designed using mutational molecular dynamics (MMD) and synthesized using automated solid-phase peptide synthesis (SPPS) and by the CuAAC click reaction to enhance structural and metabolic stability. The recognition l/d-microproteins are designed to target the caspase 3/7 binding site (BIR2 domain) and the caspase 9 binding site (BIR3 domain) of the intracellular cancer protein X-linked inhibitor of apoptosis (XIAP). Overexpression of XIAP is observed in tumours leading to increased cell survival and resistance to chemotherapeutics.[1] Inhibition of XIAP's interaction with caspases induces apoptosis, making this approach beneficial for targeting tumour cells. Five XIAP domains have been structurally determined, but the structure of full-sized XIAP is not available. Hence, the spatial organization of these domains is not known. This study reports the expression of the full-size protein XIAP. The XIAP protein was fused with maltose-binding protein (MBP) to stabilise it for expression in E. coli, the full-length XIAP-MBP was successfully isolated. MBP was subsequently cleaved from XIAP using TEV protease.[2] The complete structure of XIAP was further investigated using photo-crosslinking, which may enable more precise designs of inhibitors against XIAP.[3] To assess the designed and synthesised recognition l/d-microproteins, binding and activity assays were performed. Preliminary results show binding to the target protein XIAP, validating our structural model and suggesting the potential for developing more potent inhibitors targeting XIAP and other proteins of interest for the treatment of various diseases.

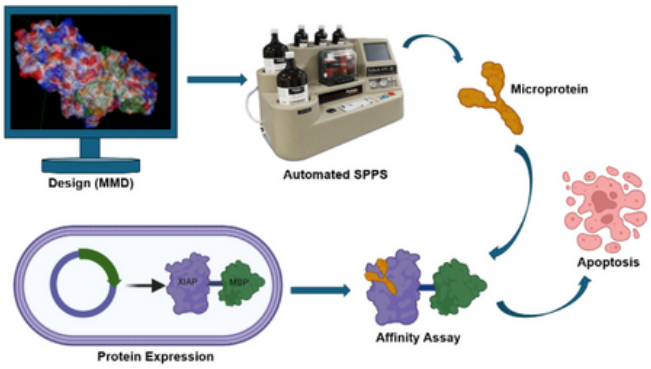

Fig. 1: An overview of the project, highlighting the computational design of microproteins, SPPS, protein expression of XIAP-MBP, and affinity assays, aimed at inducing apoptosis in cancerous tumour cells.

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(3) O'Reilly, F. J.; Rappsilber, J. Nat Struct Mol Biol 2018, 25 (11), 1000-1008.

Posters

1. Adrià Bravo Vidal: MULTI-BODY FLUCTUATION-INDUCED FORCES BETWEEN MEMBRANE PROTEINS: INSIGHTS FROM MESOSCALE SIMULATIONS

2. Alain André: BIOSYNTHESIS WITHIN BIO-INSPIRED ENGINEERED ARTIFICIAL CONDENSATES

3. Andreas Prestel: PROLINE CIS/TRANS CONFORMATIONAL SELECTION CONTROLS 14–3–3 BINDING

4. Anna Ida Trolle: A COARSE-GRAINED MODEL FOR SIMULATIONS OF SALT-DEPENDENT STRUCTURAL TRANSITIONS AND PHASE SEPARATION OF DISORDERED PROTEINS

5. Annamária Tóth: INVESTIGATING THE FUNCTIONAL SELECTIVITY OF THE METALLOREGULATOR AfArsR

6. Artu Breuer: HIGH-THROUGHPUT SINGLE-PARTICLE ANALYSIS OF LIPID NANOPARTICLE BIOPHYSICAL PROPERTIES: A QUANTITATIVE FRAMEWORK FOR OPTIMIZING LNP THERAPEUTICS

7. Beatrice J. Geiger: COMPLEX MECHANICAL RESPONSE OF STOMATOCYTIC MEMBRANES

8. Bo Volf Brøchner: SINGLE-VESICLE TRACKING OF α-SYNUCLEIN OLIGOMERS REVEALS PORE FORMATION BY A THREE-STAGE MODEL MODULATED BY CHARGE, CURVATURE, LIPIDS AND LIGANDS

9. Callum Eke: UNLOCKING STRUCTURAL DYNAMICS OF MEMBRANE TRANSPORTERS WITH SDSL-EPR

10. Carina Beitschek: STRUCTURE AND FUNCTION OF THE LYSOGENY SWITCH IN THE S. AUREUS PHI13 PHAGE

11. Christian Kofoed: PROGRAMMING PROTEIN FUNCTION TO RESPOND TO CELL SURFACE LANDSCAPES

12. Daniel Saar: THE SUBTLE ART OF NUCLEAR RECEPTOR MODULATION CAPTURED BY NMR: MTMR7 AS A NON-CANONICAL PPARΓ MODULATOR WITH POTENTIALLY LOW AFFINITY

13. Daniel Sieme: HIJACKING OF THE GROWTH HORMONE RECEPTOR BY CALMODULIN

14. Daria Gusew: MODELING CHEMICAL EXCHANGE DYNAMICS IN PROTEINS USING MOLECULAR DYNAMICS SIMULATIONS AND NMR RELAXATION

15. Darian Yang: DRIVING MD SAMPLING WITH DYNAMIC EXPERIMENTAL DATA FOR ACTIVE REFINEMENT OF CONFORMATIONAL ENSEMBLES

16. Despoina Kapiki: ELUCIDATING SODIUM SELF-INHIBITION IN EPITHELIAL SODIUM CHANNELS: A MULTIDISCIPLINARY APPROACH TO STRUCTURE AND FUNCTION

17. Dimitrios Kolokouris: DEVELOPMENT OF IBOGAMINALOGS AS THE NEXT-GEN SERT INHIBITORS

18. Elisabeth Asta Sørensen: MINIMAL NUCLEIC ACID-SCAFFOLDED NANOSTRUCTURES FOR ENHANCED MEMBRANE INTERACTIONS

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1. Adrià Bravo Vidal

MULTI-BODY FLUCTUATION-INDUCED FORCES BETWEEN MEMBRANE PROTEINS: INSIGHTS FROM MESOSCALE SIMULATIONS

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The spatial organization of membrane-associated proteins is essential for a wide range of cellular processes, including signal transduction, endocytosis, and cell adhesion. While protein clustering can be driven by direct short-range forces, indirect interactions mediated by the membrane itself, particularly those arising from thermal shape fluctuations, are long-range, generic, and potentially sufficient to drive clustering in the absence of direct binding. In this study, we investigate the capacity of fluctuation-induced interactions to induce clustering using mesoscale simulations based on the dynamically triangulated surface (DTS). We explore the role of protein-induced changes in bending rigidity and Gaussian modulus as well as the effects of membrane tension and protein concentration. Our results systematically explores both low and high bending rigidity regimes and isolate the influence of the Gaussian modulus on many-body interactions. We further extend the model to systems with two types of stiff inclusions and analyze how the introduction of curvature imprinting alters clustering behavior. The nonadditive nature of fluctuation-induced forces poses a challenge to predicting collective behavior, but our simulations provide a comprehensive framework that unifies previous observations. These findings highlight how a few mesoscale physical parameters can control protein self-organization on membranes, offering insights relevant to both cell biology and the design of membrane-based materials.

2. Alain André

BIOSYNTHESIS WITHIN BIO-INSPIRED ENGINEERED ARTIFICIAL CONDENSATES

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Biomolecular condensates have emerged as powerful tools for both biological research and biotechnological applications, due to their inherent ability to sequester molecules. This property makes them ideal candidates for use as reaction crucibles in various enzymatic processes. In this study, we engineered a robust and versatile system using synthetic intrinsically disordered repeat proteins (IDPs). These proteins can be precisely tailored to exhibit specific properties such as stability, responsiveness, and molecular affinity, making them excellent platforms for customized enzymatic environments. We focus on characterizing the material properties of these bio-inspired protein condensates to optimize their functionality and stability for potential use in biotechnological applications. As case study we here study the synthesis of naringin from p-coumarate through a three-enzyme cascade. Our findings aim to advance the development of innovative enzymatic crucibles, enhancing the efficiency and specificity of biochemical reactions.

3. Andreas Prestel

PROLINE CIS/TRANS CONFORMATIONAL SELECTION CONTROLS 14–3–3 BINDING

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Intrinsically disordered protein regions (IDRs) are structurally dynamic yet functional, often interacting with other proteins through short linear motifs (SLiMs). Proline residues in IDRs introduce conformational heterogeneity on a uniquely slow time scale arising from cis/trans isomerization of the Xaa-Pro peptide bond. Here, we explore the role of proline isomerization in the interaction between the prolactin receptor (PRLR) and 14–3–3. Using NMR spectroscopy, thermodynamic profiling, and molecular dynamics (MD) simulations, we uncover a unique proline isomer-dependent binding, with a cis conformation affinity 3 orders of magnitude higher than the trans. MD simulations identify structural constraints in the narrow 14–3–3 binding groove that provide an explanation for the observed isomer selectivity. The cispreference of WT PRLR introduces a slow kinetic component relevant to signal propagation and a steric component that impacts chain direction. Proline isomerization constitutes a previously unrecognized selective component relevant to the ubiquitous 14–3–3 interactome. Given the prevalence of prolines in IDRs and SLiMs, our study highlights the importance of considering the distinct properties of proline isomers in experimental design and data interpretation to fully comprehend IDR functionality.

4. Anna Ida Trolle

A COARSE-GRAINED MODEL FOR SIMULATIONS OF SALT-DEPENDENT STRUCTURAL TRANSITIONS AND PHASE SEPARATION OF DISORDERED PROTEINS

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Intrinsically disordered proteins (IDPs) are highly dynamic molecules and should thus be described by an ensemble of structures rather than an individual conformation. This is often achieved through molecular dynamics (MD) simulations combined with biophysical experiments. The conformational ensembles of IDPs can be highly malleable, so that their conformational ensembles depend on the solution conditions including salt concentrations[1]. In coarse-grained MD simulations, salt effects are usually described solely in electrostatic terms, with charge screening modelled by the Debye-Hückel potential[2]. Oft overlooked are Hofmeister effects: salt-dependent changes in amino acid hydrophobicities. Hofmeister effects are essential when modelling electrolyte effects on protein structure at high salt concentrations: a proper description of salt in molecular dynamics force fields was recently shown to improve predictions of liquid-liquid phase separation of IDRs[1].
Here, we present an optimised CALVADOS model which captures salt-dependent structural transitions and phase separation by refining amino acid hydrophobicities through a new parameter, α, which depends on solution ionic strength. Our iterative, Bayesian optimization framework learns α-values to minimize discrepancies between simulations and experiments. The optimised model provides the foundations for predicting how natural IDPs respond to salt and how we may design new proteins for example to sense salt concentrations.

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5. Annamária Tóth

INVESTIGATING THE FUNCTIONAL SELECTIVITY OF THE METALLOREGULATOR AfArsR

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Metalloid responsive ArsR proteins regulate the As^{III}/Sb^{III} resistance of various bacteria through a derepression mechanism. In this process, the increased concentration of metalloids inside the cells leads to the dissociation of the protein from the DNA, inducing the transcription of genes of the ars operon.[1] The homodimeric protein from A. ferrooxidans (AfArsR) coordinates As^{III}/Sb^{III} at its metalloid binding site (MBS) by three cysteines located at the flexible C-terminal region in each monomer.[2] However, this coordination environment may be preferred by other soft metal ions, too, thus, it is very intriguing how AfArsR selectively recognizes the cognate metalloids. Results of our recent study on an oligopeptide modelling the MBS of AfArsR allowed us to propose the possible origins of metalloid recognition.[3] We then investigated the whole protein and demonstrated the in vitro functional selectivity of AfArsR for As^{III} and Sb^{III}against various non-cognate soft metal ions, i.e. Hg^{II}, Pb^{II}, Cd^{II} and Zn^{II} by electrophoretic mobility shift assays. We monitored the DNA binding of AfArsR by intracellular I-Block assays, too, and the results reflected the selectivity of this protein in the derepression process inside the cells. Furthermore, we attempted to perform crystallographic experiments with various forms of AfArsR.

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6. Artu Breuer

HIGH-THROUGHPUT SINGLE-PARTICLE ANALYSIS OF LIPID NANOPARTICLE BIOPHYSICAL PROPERTIES: A QUANTITATIVE FRAMEWORK FOR OPTIMIZING LNP THERAPEUTICS

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The size and chemical composition of lipid nanoparticles (LNPs) are critical determinants of their therapeutic efficacy. Even subtle variations in lipid ratios or particle size can dramatically affect delivery efficiency, immune activation, and cargo release, underscoring the need for precise, particle-level characterization to inform rational formulation design.

Current analytical methods rely largely on ensemble averages, masking heterogeneity and obscuring how individual LNPs differ in size, cargo content, and lipid distribution—factors that directly influence in vivo performance. To address this gap, we present a high-throughput, fluorescence-based microscopy approach that quantitatively profiles millions of individual LNPs in a single experiment. By leveraging intensity-calibrated total internal reflection fluorescence (TIRF) microscopy, our method enables simultaneous measurement of particle size and encapsulated nucleic acid cargo at single-particle resolution.

Using this platform, we uncover striking heterogeneity within nominally uniform LNP populations, including a bimodal cargo loading distribution that correlates strongly with particle size. Small compositional adjustments—such as changes in cholesterol or ionizable lipid content—significantly shift the relative abundance of these subpopulations, revealing a powerful axis for tuning LNP behavior. Live-cell delivery assays demonstrate that these structural and compositional differences translate into markedly different cellular uptake.

This method provides a scalable and accessible tool for dissecting the structure–function landscape of LNPs. By resolving the heterogeneity that underpins therapeutic performance, our approach supports the development of optimized nanoparticle formulations for RNA therapeutics, vaccines, and precision nanomedicine.

7. Beatrice J. Geiger

COMPLEX MECHANICAL RESPONSE OF STOMATOCYTIC MEMBRANES

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Stomatocyte shapes commonly occur in both organelle and synthetic membranes and are among the fascinating shapes that biomembranes exhibit for example in nuclear membranes and open autophagosomes. They can be visualised as spherical double membranes, with the inner and outer membrane connected by neck-like structures. Stomatocytes characterised by a high topological genus are part of a largely unexplored shape family with a higher degree of polymorphism and new emerging properties that are absent from simple spherical membranes. The nuclear envelope (NE) is a prime example for a high-genus stomatocyte, containing multiple necks. These necks are often occupied by specific biomolecular complexes, like the nuclear pore complex in the NE, which divide the space into three distinct compartments. Understanding how the size of these necks responds to pressure gradients is fundamental to uncover the influence of mechanical stimuli on traffic control through the necks, for example in nuclear mechanosensing. In this work, we use computer simulations and theoretical analysis to investigate how the neck size responds to pressure or tension variations. This reveals a two-phase behavior: surprisingly, necks first constrict as the pressure gradient increases, while above a threshold, they dilate. This response stems from the pure membrane's mechanics and depends on the magnitude of the pressure gradient or tension, the initial neck diameter and the membrane bending rigidity. We also establish a simple equation that links the threshold tension, the neck diameter and the bending rigidity, offering a useful tool to quickly assess different scenarios. Furthermore, when protein complexes occupy a neck they partially counteract both constriction and dilation, stabilising the neck size while preserving the same two-phase response. These findings uncover a promising, previously overlooked membrane property with significant implications for cell organelle function, as well as for biomimetic system design.

8. Bo Volf Brøchner

SINGLE-VESICLE TRACKING OF α-SYNUCLEIN OLIGOMERS REVEALS PORE FORMATION BY A THREE-STAGE MODEL MODULATED BY CHARGE, CURVATURE, LIPIDS AND LIGANDS

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Neurodegenerative disorders like Parkinson's disease (PD) pose significant health challenges, characterized by the progressive accumulation of alpha-synuclein (αSN) protein, forming toxic oligomers and fibrils. Drug development primarily aims to slow disease progression since PD is typically diagnosed after toxic fibrils have formed, but the origin and underlying mechanisms remain unclear. Although αSN functions are not well-defined, its interactions with cellular membranes are crucial in both normal and pathological processes. αSN shows a preference for negatively charged membranes, particularly under fibrillating conditions, forming toxic oligomers (αSO) that disrupt membrane integrity, leading to cellular dysfunction and death. However, the exact mechanisms of αSO and lipid membrane interactions are still not fully understood.

Understanding αSO is challenging due to their heterogeneity. We use a novel single-liposome assay to model αSO and membrane interactions at the single vesicle level. Our findings reveal that αSO membrane association depends on curvature, lipid type, and increases exponentially with negative membrane charge. Real-time imaging shows that αSO incorporates into membranes and acts as pores, confirmed by single-channel recordings on planar lipid bilayers. Small molecules can translocate through αSO pores, while α-synuclein monomers do not disturb the membrane. We propose a three-stage membrane interaction model for αSO: membrane recruitment followed by charge-dependent reorientation and pore formation. Testing nanobodies (NB) and a chemically stabilize αSO, we find that one NB increases translocation, while the ONE modification significantly lowers the translocation. Future perspectives might be to stabilize αSO which seems to prevent the pore formation which could be the driver of toxicity and cellular dysfunction.

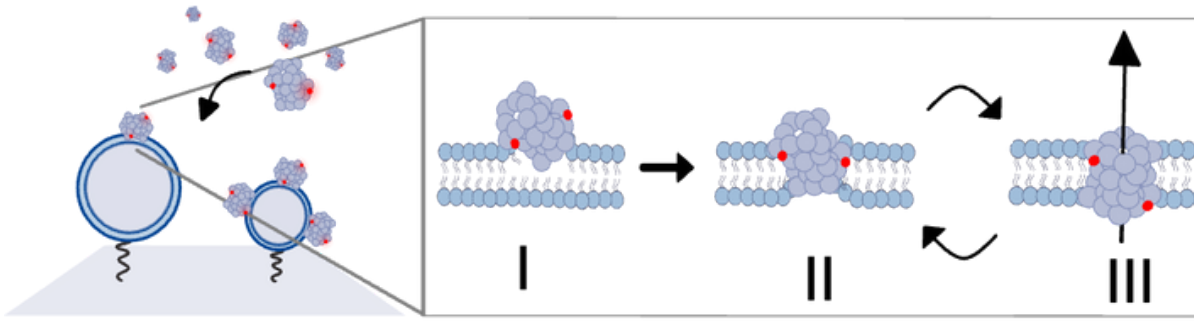

Figure1: Single-vesicle assay enables a comprehensive biophysical understanding of αSO membrane interaction. Results points toward athree-stage model: recruitment, rearrangement and pore formation, which is modulated by charge, curvature, lipids and ligands. Brøchner et al. 2025 (https://doi.org/10.1101/2025.04.10.648112)

9. Callum Eke

UNLOCKING STRUCTURAL DYNAMICS OF MEMBRANE TRANSPORTERS WITH SDSL-EPR

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Electron paramagnetic resonance (EPR) spectroscopy has long been established as the preeminent biophysical method for studying intrinsically paramagnetic systems. The advent of site-directed spin labeling (SDSL) revolutionized its scope, enabling EPR to unveil dynamic and structural insights into a vast array of intrinsically diamagnetic biomacromolecules[1]. Among its most transformative applications is the study of membrane proteins, and membrane transporters in particular, enabling the identification of crucial dynamic information through EPR line shape analysis, topological membrane data via EPR power saturation studies, and coarse-grained structural information in the form of distance restraints from double-electron electron resonance (DEER)[2]. Here, we leverage the EPR-SDSL methodology to investigate the structure and dynamics of the bacterial amino acid transporter LeuT, a well-characterised, prokaryotic homolog of the pharmacologically relevant solute carrier 6 (SLC6) family of Na^+-dependent membrane symporters[3]. This approach probes the dynamics of LeuT in both detergent-solubilised micelles and proteoliposomes, focusing primarily on subtle structural and molecular motions occurring at the extracellular region of transmembrane helix (TM) 10, which are unresolvable using conventional high-resolution crystallographic or cryo-EM techniques. The findings assigned distinct ion/substrate-dependent conformational configurations during substrate transport, quantified the conformational ensembles present in the apo state of the transporter, and highlighted key structural differences based on the lipid environment[4].

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10. Carina Beitschek

STRUCTURE AND FUNCTION OF THE LYSOGENY SWITCH IN THE S. AUREUS PHI13 PHAGE

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Staphylococcus aureus is a major opportunistic pathogen associated with a wide range of infections in humans. The emergence of methicillin-resistant strains (MRSA) has intensified its clinical relevance. In addition to antibiotic resistance, prophages such as phi13 enhance human colonization by encoding virulence factors, including mechanisms of immune evasion, thereby contributing to the pathogen's persistence and adaptability. The temperate bacteriophage phi13 relies on a bi-stable genetic switch to determine whether it enters lysogeny or proceeds with the lytic cycle. This switch is controlled by the interplay between the CI repressor and the modulator of repression (MOR) [1,2].
Both phi13 CI and MOR have been recombinantly expressed in E. coli and purified. CI consists of an N-terminal helix-turn-helix domain involved in DNA binding (NTD), followed by two C-terminal regions: a helical hook (CTD1) and a β-sheet-rich domain with homology to peptidase folds (CTD2). It forms dimers likely via the helical hook, and higher-order oligomers, likely facilitating cooperative DNA binding [2]. Given the structural similarity of CTD2 to the LexA repressor, we are investigating whether CI is a protease undergoing auto-cleavage and/or hetero-cleavage. MOR is a small, helical protein, with the helix-turn-helix structure normally associated with DNA binding, which we are currently verifying. We also used AlphaFold3 to predict interactions within the helical hook region and elsewhere upon dimerization/tetramerization to guide design of a monomer version of CI.
The ongoing investigations will help elucidate the molecular mechanisms at the basis of the phi13 lysogeny switch.

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11. Christian Kofoed

PROGRAMMING PROTEIN FUNCTION TO RESPOND TO CELL SURFACE LANDSCAPES

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Cell surface landscapes vary across cell types and are often reprogrammed in disease[1-3], making them valuable targets for diagnostics, therapeutics, and mechanistic studies. However, current strategies typically focus on single surface markers, which rarely define cell identity with sufficient precision[4,5]. To address this, we present SMART, a programmable molecular system capable of integrating multiple cell-surface cues to trigger user-defined responses[6]. Our platform is based on a proximity-gated protein trans-splicing mechanism that enables the conditional assembly of a functional protein from two inactive polypeptide fragments[7-8]. This "protein actuator" system supports convergent protein synthesis directly on designated cell surfaces, producing active proteins that can either remain tethered to the cell membrane or be released into the extracellular environment. Leveraging its intrinsic modularity, we demonstrate compatibility with diverse inputs, targeting strategies, and functional outputs, enabling localized control over protein activity for selective cell interrogation and manipulation.

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12. Daniel Saar

THE SUBTLE ART OF NUCLEAR RECEPTOR MODULATION CAPTURED BY NMR: MTMR7 AS A NON-CANONICAL PPARΓ MODULATOR WITH POTENTIALLY LOW AFFINITY

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Activation of human transcription factor PPARγ typically involves agonist binding in the ligand binding pocket and co-activator binding via an LXXLL motif after a ligand-induced conformational change [1, 2]. The phosphatase MTMR7 was predicted as a PPARγ co-activator through the action of its coiled-coil domain, impacting metabolic disorders, inflammation, and cancer, but the mechanism was unclear [3]. Our NMR data show that MTMR7 binds PPARγunconventionally, using an alternative surface without involving the co-activator binding site or ligand binding pocket and in the absence of a canonical binding motif. We identify two PPARγ binding sites for MTMR7 on the PPARγ ligand binding domain and show that the coiled-coil domain of MTMR7 stabilizes the ligand binding domain in its active conformation independently of ligand binding, potentially competing with the PPARγ AB domain. Rosiglitazone, a synthetic agonist for PPARγ enhances MTMR7's effect but is not required for MTMR7 binding.

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13. Daniel Sieme

HIJACKING OF THE GROWTH HORMONE RECEPTOR BY CALMODULIN

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The growth hormone receptor (GHR) signals through the JAK/STAT pathway and is mainly known for its role in growth regulation and metabolism. Cellular Ca^{2+} influx resulting from growth hormone (GH) stimulation suggests a link between GHR and cellular calcium metabolism, both playing crucial roles in pathways related to cancer, obesity and cardiovascular diseases. However, mechanistic models of GHR signal transduction and regulation and the direct link to Ca^{2+} metabolism are lacking, hindering causal rather than symptomatic treatments of GHR-related disorders. Using cell biology, isothermal titration calorimetry and NMR spectroscopy, we scrutinize the interaction and reveal multiple interaction sites for CaM on GHR suggesting highly competitive interactions with signaling partners. In addition, we could show a GH-dependent and direct interaction between GHR and CaM in human cells, proving this interaction to be physiologically relevant. This establishes CaM as a novel regulator of GHR signal transduction, linking growth modulations with Ca^{2+}-oscillations.

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14. Daria Gusew

MODELING CHEMICAL EXCHANGE DYNAMICS IN PROTEINS USING MOLECULAR DYNAMICS SIMULATIONS AND NMR RELAXATION

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Understanding the processes that determine conversion between long-lived states is important as they define thedynamics of a protein and protein-ligand interactions. In nuclear magnetic resonance (NMR) spectroscopy, the chemical shifts provide information about the structural details of a pro- tein. Chemical shift fluctuations caused by the exchange between conformational states (chemical exchange) can therefore be used to study the dynamics of proteins. Such information will in turn be essential for benchmarking prediction or generative models for protein dynamics.

Here, we develop a forward model to capture chemical exchange dynamics by mapping the pro- tein dynamics to diffusive motions on a one-dimensional free energy potential [1] extracted from an explicit-water molecular dynamics (MD) simulation [2] of the L99A variant of T4-Lysozyme. This model eliminates assumptions about the number andnature of states, describing the dynamics as oc- curring on the free energy surface by numerical integration of theLangevin equation. These dynamics are subsequently used to evaluate chemical shift autocorrelation functions associated with NMR re- laxation dispersion rates, which are then compared to experimental data.

Applications to T4-Lysozyme demonstrate that we can reproduce the relaxation rates for specific protein residues,which are known to be involved in conformational changes, as determined by NMR relaxation dispersion experiments. In the future, this model could aid in protein design and protein structure prediction methods, potentially leading to better therapeutics in the future by capturing the full range of conformational states, rather than relying on a single static representation.

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15. Darian Yang

DRIVING MD SAMPLING WITH DYNAMIC EXPERIMENTAL DATA FOR ACTIVE REFINEMENT OF CONFORMATIONAL ENSEMBLES

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Objectives: Understanding protein conformational flexibility is essential for structural insights beyond static snapshots. While molecular dynamics (MD) simulations provide dynamic, atomistic insights, they often fail to fully capture experimental observables due to force field inaccuracies and insufficient sampling. Reweighting techniques can improve agreement with experiments but cannot recover completely unsampled states. To address this, we introduce a weighted ensemble (WE) simulation method that adaptively enhances sampling using dynamic experimental observables.

Methods: WE simulations improve sampling efficiency by running multiple unbiased trajectories in parallel, selectively replicating pathways-of-interest while merging redundant ones. Unlike conventional biasing approaches for accelerating MD simulations, our method directly incorporates time-dependent experimental observables, such as NMR relaxation rates, into the resampling step, balancing both trajectory diversity and experimental agreement. This strategy enables guided exploration of dynamically relevant conformations and is broadly applicable to any observable that depends on time correlation functions or multi-frame MD information.

Results: We demonstrate our approach on T4 lysozyme simulations using NMR relaxation data as an example. Our method samples a more diverse set of protein conformations and achieves faster convergence with experimental data compared to standard MD, capturing a more complete and valid conformational ensemble.

Conclusions: Our dynamic observable-guided WE approach uniquely enables the integration of experimental data involving time-dependent molecular motion, overcoming limitations of conventional MD and biasing methods. This framework is broadly applicable to refining protein structural ensembles and improving or validating the accuracy of structure prediction and design models.

16. Despoina Kapiki

ELUCIDATING SODIUM SELF-INHIBITION IN EPITHELIAL SODIUM CHANNELS: A MULTIDISCIPLINARY APPROACH TO STRUCTURE AND FUNCTION

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The epithelial sodium channel (ENaC) is a sodium-selective ion channel that plays a pivotal role in maintaining salt and water homeostasis in tetrapod vertebrates. ENaC regulates sodium absorption in epithelial tissues such as the kidney, colon and lung, thereby influencing electrolyte balance, blood pressure, and fluid clearance, respectively. Disrupted ENaC regulation is associated with a wide range of pathologies, such as hypertension, cystic fibrosis (CF), pulmonary edema, and nephrotic syndrome. The exact molecular mechanisms by which ENaC contributes to such diseases are not yet fully understood, thus constituting a significant challenge for medical research. One of the most intriguing regulatory mechanisms of ENaC is sodium self-inhibition (SSI), a mechanism in which extracellular sodium ions suppress channel activity. The extent of SSI varies in different ENaC subunit compositions (αβγ or δβγ heterotrimer assemblies) and is key to ENaC's ability to modulate excessive sodium uptake in both physiological and pathological microenvironments. In certain vertebrates, the αβγ-ENaC heterotrimer exhibits extensive SSI, whereas δβγ-ENaC is generally characterized by a lower degree of inhibition. Understanding isoform-specific structural adaptations
that govern SSI holds a strong therapeutic potential. ENaC's full structure remains until this date, largely unresolved (PDB:6WTH). Structural insights from its structural homologue, the homotrimeric, proton-gated sodium channel ASIC1 (PDB:4FZ0, 5WKV7CFS) suggest that the β11–β12 linker flexibility might play a crucial role for SSI modulation. By implementing a multidisciplinary strategy that combines site directed mutagenesis, two electrode voltage clamp electrophysiology and molecular dynamics simulations, we have identified that altered isomerization of a residue in the β11–β12 region significantly alters sodium self-inhibition of the channel. By exploring specific protein domains at subunit interfaces, this research aspires to provide further mechanistic insights into the fundamental molecular physiology of ENaC and its structure-function relationships.

17. Dimitrios Kolokouris

DEVELOPMENT OF IBOGAMINALOGS AS THE NEXT-GEN SERT INHIBITORS

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Ibogainalog, a simplified analog of the psychedelic compound ibogaine, was recently identified as a competitive inhibitor of the serotonin transporter (SERT), in contrast to the non-competitive mechanism of ibogaine itself however the mechanism of inhibition remains unknown. We found ibogainalog to induce an occluded state for the transporter with cysteine accessibility assays in an ion-independent mechanism. To further understand this deviation from canonical SERT inhibitor mechanism, we solved a cryo-EM structure of SERT bound to ibogainalog under physiological Na^+ conditions, revealing an outward-facing occluded conformation and providing a structural explanation for conformational selection of the ibogalog class. The structural data was used to build robust relative binding free energy network, recapitulating the observed affinity difference between ibogainalog and ibogaine, further validating the binding model. Structure-guided screening led to the identification of novel ibogalog derivatives, including a new derivative with high in silico affinity and submicromolar experimental Kd values (0.17 μM) with potent dual SERT inhibition and serotonin release activity. Our findings provide a framework for the rational design of SERT-targeting psychedelic derivatives with distinct pharmacological profiles.

18. Elisabeth Asta Sørensen

MINIMAL NUCLEIC ACID-SCAFFOLDED NANOSTRUCTURES FOR ENHANCED MEMBRANE INTERACTIONS

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Limited in their functionality due to the challenging task of penetrating lipid-bilayer membranes, the insertion of DNA-nanopores is still highly inefficient attributed to their highly negatively charged backbone. While a common approach is the attachment of cholesterol and lipids to enhance the hydrophobic interactions of the nucleic acid structures with membranes, cell-penetrating peptides (CPPs) have proved great potential for increasing the membrane insertion efficiency and introduce reversibility of the insertion [1].
Inspired by a previously published minimal nanostructure which self-assembles in a modular fashion [2] we designed an RNA-based nanoscaffold for the peptide-conjugated oligonucleotides which can circularize to increase proximity of the pore-forming.
The aim is to provide insights into the dynamics and mechanics of pore formation of different peptides scaffolded by the minimal nucleic acid scaffold. Using liposomes with differing charges, curvatures, and solvent conditions as model membranes, we will investigate the avidity effects at play in pore-formation by different CPPs and small peptides of interest.

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19. Elisabeth Rexen Ulven

MULTI-COULERED SEQUENTIAL RESONANCE ENERGY TRANSFER FOR SIMULTANEOUS LIGAND BINDING AT GPCRS

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Allosteric modulators have appeared as an important concept in medicinal chemistry, e.g., for amplifying the effect of the endogenous orthosteric agonist. Understanding of ligand binding and interaction between different binding sites on a receptor is therefore important and better methods are needed. Bioluminescence resonance energy transfer (BRET) between a luciferase-tagged receptor and a fluorescent ligand is an effect that can be used as a convenient competition binding assay to probe affinity and kinetics.
The free fatty acid receptor 1 (FFA1) is a G protein-coupled receptor (GPCR) that contains several ligand binding sites that have been confirmed by X-ray and Cryo-EM structures, including one close to the extracellular part and one at the intracellular part (1-2). To study the interplay between simultaneously binding receptor ligands we have developed an approach that relies on sequential resonance energy transfer between two matched fluorescent ligands and a nanoluciferase-tagged receptor and termed it multi-coloured Dual Interaction Sequential Compounds (DISCo)-BRET (3). Using FFA1 as a model system we have shown that this approach can be used to identify novel ligand pharmacology and binding kinetics.

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20. Emil Baltzer Østberg

INTERACTIONS OF THE GLUCURONOYL ESTERASE OTCE15A WITH SACCHARIDE SUBSTRATES

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Glucuronoyl esterases (GEs), classified within the carbohydrate esterase family 15 (CE15) [1], are enzymes capable of aiding in the degradation of recalcitrant plant biomass, making them valuable tools for the sustainable production of renewable energy, materials, and chemicals [2]. GEs catalyze the hydrolysis of the ester bond linking lignin and glucuronic acid (GlcA) moieties on glucuronoxylan, the so-called lignin-carbohydrate complex (LCC). While the catalytic mechanisms of GEs have been studied extensively using monosaccharide GlcA derivatives [3], structural insights into GE interactions with native, polymeric substrates remain limited [4].
The GE from the bacterium Optitutus terrae (OtCE15A) has previously been characterized [3], [4] and shown to crystallize in a reproduceable manner. Leveraging its excellent crystallization and diffraction properties, we determined the structure of OtCE15A in complex with a heptasaccharide, 22-(4-O-methyl-D-glucuronyl)-43-arabinofuranosyl-xylopentaose (XA3XU(4m)2X). This represents the largest ligand observed in complex with a CE15 enzyme to date.
Our structure reveals that the GlcA moiety is tightly bound within the catalytic cleft via a network of hydrogen bonds, underscoring its critical role in substrate recognition. In contrast, the remainder of the oligosaccharide engages primarily through non-hydrogen bond interactions, contributing significantly to the overall binding interface. Furthermore, we demonstrate that OtCE15A tolerates structural variability in glucuronoxylan, allowing arabinose decorations – an advantageous feature for targeting the heterogeneous LCC-rich biomass.

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21. Emil Hundebøll

STRUCTURAL ELUCIDATION OF CARDIOLIPIN SYNTHASE FROM TRYPANOSOMA BRUCEI

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The protozoan parasite Trypanosoma Brucei is the causative agent of the deadly disease African sleeping sickness (Human African trypanosomiasis) and nagana also known as Animal trypanosomiasis. Tb is transmitted by the tsetse fly present in sub-Saharan Africa. African sleeping sickness is on the list of neglected tropical diseases.
Cardiolipin is a lipid with a unique dimeric structure and it is almost exclusively found in the inner mitochondrial membrane where it functions to stabilize protein complex' and in cristae structure. Cardiolipin synthase is the enzyme responsible for formation of this unique lipid and a bacterial and eukaryotic form of this enzyme exists.
Interestingly Trypanosoma has a bacterial-type cardiolipin synthase.
This project therefore has two interests in Tb's cardiolipin synthase; one is from a medical standpoint as it might be a possible target and secondly there is an evolutionary interest as it is a eukaryote with a bacterial-type synthase.
The project wishes to elucidate cardiolipin synthase' structure and perform biophysical analysis. Furthermore, the synthase is found in a large complex which will be attempted to be purified from the Tb's mitochondria.

22. Emma Krindel Beyer

TARGETING A SODIUM LEAK WITH MINI PROTEINS

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Neurons communicate via electrical impulses and, therefore, neuronal signaling is critically dependent on the electrical potential across the cell membrane. The sodium leak channel non-selective (NALCN) contributes to maintaining and regulating this potential. The channel depends on auxiliary subunits UNC79, UNC80, and FAM155A for its function, giving it a distinct molecular architecture compared to other known channels.[1]Mutations in NALCN – or UNC79 and UNC80 – are rare but cause severe neuro-developmental diseases in patients. Most of them result in increased NALCN activity, but so far, no compounds are able to inhibit the channel.
In this study we demonstrate that interactions between NALCN and its auxiliary subunits UNC79 and UNC80 can be outcompeted with mini proteins that fully disrupt wild-type and gain-of-function NALCN activity in electrophysiological recordings. The inhibitory mini proteins are designed based on the UNC79/UNC80 binding sites of NALCN or generated de novo from UNC79/UNC80 target sites. We intend to develop these mini proteins to achieve potent and selective tool compounds for studying NALCN in vitro, and lead candidates for in vivo applications.

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23. Fabian Hink

INHIBITION OF RNA-PROTEIN INTERACTIONS AND PHASE SEPARATION OF HNRNPA1 USING DE NOVO CYCLIC PEPTIDES

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RNA-binding proteins (RBPs) regulate key RNA processes such as splicing, translation, and degradation. Additionally, RBPs like heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1) can form dynamic ribonucleoprotein condensates through phase separation, a process where proteins and RNAs segregate into membraneless organelles essential for cellular functions. Dysregulation of this process, often caused by RBP mutations, underlies neurodegenerative diseases. Despite their critical roles and involvement in diseases, RBPs are challenging drug targets due to their abundance (comprising ~7.5% of the human proteome), structural similarities among RNA-binding domains, and lack of hydrophobic pockets. Macrocyclic peptides could provide a promising strategy to target RBPs, as they have shown exemplary binding towards other challenging protein targets. The RaPID (Random non-standard Peptides Integrated Discovery) system, an mRNA display platform, enables the generation of trillion-membered, macrocyclic peptide libraries and efficient screening for binding hits. Here, we applied RaPID to the RNA recognition motifs (RRM1 and RRM2) of hnRNPA1, a protein whose mutations are linked to amyotrophic lateral sclerosis. While screening with RaPID was unsuccessful to enrich peptide binders for RRM1, it led to the identification of de novo cyclic peptides that bound to RRM2 with nanomolar affinities. The peptide **A1.4** successfully pulled down hnRNPA1 from cell lysates, demonstrating its selectivity. NMR and fluorescence polarization confirmed that **A1.4** bound to RRM2 at its RNA-binding site and was able to displace RNA. Notably, **A1.4** was able to dissolve condensates formed by hnRNPA1 and RNA. This study underscores the potential of macrocyclic peptides to target RBPs and introduces a novel approach to inhibiting RNA-induced phase separation, paving the way for developing therapies for diseases linked to RBPs and aberrant phase separation dynamics.

24. Fabian Schuhmann

EXPERIMENTAL DATA INFORMS COMPUTATIONAL PROTEIN CLUSTER PREDICTION IN MITOCHONDRIA

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Cross-linking mass spectrometry (XL-MS) enables the mapping of protein-protein interactions on the cellular level. When applied to all compartments of mitochondria, the sheer number of cross-links and connections can be overwhelming, rendering simple cluster analyses convoluted and uninformative. To address this limitation, we integrate the XL-MS data, 3D electron microscopy data, and localization annotations with a supra coarse-grained molecular dynamics simulation to sort all data, making clusters more accessible and interpretable. In the context of mitochondria, this method, through a total of 6.9 milliseconds of simulations, successfully identifies known, suggests unknown protein clusters, and reveals the distribution of inner mitochondrial membrane proteins allowing a more precise localization within compartments. Our integrative approach suggests, that two so-far ambigiously placed proteins FAM162A and TMEM126A are localized in the cristae, which is validated through super resolution microscopy. Together, this demonstrates the strong potential of the presented approach.

25. Fan Cao

A COARSE-GRAINED MODEL FOR DISORDERED AND MULTI-DOMAIN PROTEINS

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Multi-domain proteins consist of folded domains connected by flexible linkers. Because of their substantial dynamics, characterising the conformational ensembles of multi-domain proteins by simulations may be difficult. We present a coarse-grained model for multi-domain proteins that provides an accurate description of the global conformational properties, as a starting point for understanding interactions between folded and disordered regions and for possible applications on phase separation.

26. Fernando Salgado Polo

UNCOVERING THE MOLECULAR BASIS FOR THE REGULATION OF CXCR4 ACTIVITY BY CHOLESTEROL

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The chemokine CXCL12 is a 9-kDa signaling protein that controls cell migration in the context of development, immune surveillance, and inflammation through interactions with the chemokine receptors C-X-C chemokine receptor type 4 (CXCR4) and atypical chemokine receptor 3 (ACKR3) at the plasma membrane. CXCR4 is a Gi-coupled receptor and has immunomodulatory effects when binding to CXCL12, which regulates immune cell trafficking, among other processes. ACKR3 does not couple to G proteins, but it regulates CXCR4 by scavenging CXCL12. Importantly, exacerbated CXCL12 signaling has also been linked to several types of cancer, and to neuroinflammation in neurodegenerative diseases (Fig.1). Thus, it is crucial to understand how CXCR4 is regulated.

The cryo-EM structures of ACKR3 and CXCR4 showed their direct interaction with cholesterol molecules. Importantly, it has long been observed that cholesterol can affect CXCR4's signaling activity and function in cells; however, no molecular mechanisms have yet been described.

Here, we show that cholesterol can regulate CXCR4, possibly through direct interactions with the receptor. To study this, we assessed the biological relevance of cholesterol to regulate CXCR4 activity by downstream cell-signaling readouts. We next employed nanodisc models, which provide an adjustable membrane environment that we used in two biophysical techniques: radiometry and fluorescence anisotropy.

27. Florentina Negoita

BDPIC – A NOVEL CHEMICALLY-INDUCIBLE TOOL FOR SITE-SELECTIVE PROTEIN DEPHOSPHORYLATION

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Phosphorylation regulates diverse protein functions, yet the specific contributions of individual phosphorylation sites often remain unclear. Traditional approaches, such as kinase inhibition or site-directed mutagenesis, are limited by off-target effects and potential disruption of native protein conformation. Targeted protein dephosphorylation represents a promising strategy to probe phosphorylation-dependent regulation of proteins with greater specificity.
BDPIC (BromoTAG-dTAG proximity-inducing chimera), a novel heterobifunctional small molecule was developed to enable chemically-induced proximity between target proteins and phosphatases. It bridges the dTAG and the BromoTAG systems.
To evaluate this platform, knock-in cell lines expressing dTAG-Transcription Factor EB (TFEB), a reversible phosphorylation-regulated transcription factor involved in lysosomal biogenesis, and bromoTAG-PPP2CA, the catalytic subunit of protein phosphatase 2 were generated. Following BDPIC treatment, TFEB dephosphorylation was assessed by Western blot and mass spectrometry. Functional consequences were evaluated by microscopy (nuclear translocation) and qPCR (target gene expression). BDPIC induced partial dephosphorylation of TFEB and lead to nuclear accumulation of TFEB and activation of a subset of its transcriptional targets.
BDPIC provides a chemically-inducible and reversible strategy for site-specific dephosphorylation. Future optimization, such as adjusting the linker, may further enhance site specificity, enabling elucidation of site-specific function. When coupled with structural biology techniques, BDPIC can facilitate the generation of proteins with defined phosphorylation states, offering new opportunities to explore the structural and functional consequences of phospho-regulation.

28. Frank H. Schulz

QUANTIFYING ABSOLUTE CYTOPLASMIC DELIVERY OF MRNA USING EGFP EXPRESSION AND MACHINE LEARNING ANALYSIS TO ENHANCE DEVELOPMENT OF OLIGONUCLEOTIDE THERAPEUTICS

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The efficient delivery of mRNA via lipid nanoparticles (LNP) is a critical aspect in the development of mRNA-based therapeutics. Due to low copy numbers, correctly determining mRNA release requires single particle and single cell resolution to access important details hidden by conventional ensemble approaches. This study introduces a robust and easy to use machine learning-based tool for assessing quantised numbers of escaped mRNAs in single cells given a mininmal set of data points describing LNP characteristics and expression of enhanced green fluorescent protein (EGFP) encoded in the mRNA. We train our model using simulated EGFP expression levels in cells under varying conditions across orders of magnitude including LNP loading efficiency, cell entry number, EGFP translation rate, mRNA and EGFP degradation rates, and cell division rates. By applying the tool to data obtained through both spinning disk confocal microscpy and lattice lightsheet microscpy, we prove the robustness of our model and its scalability to be used in various screening setups. This, in turn, provides a framework for evaluating and comparing mRNA-LNP based delivery systems and rationally enhance LNP formulations to enhance therapeutic outcomes in the future.

29. Freja Bohr

CELL MORPHOLOGY FINGERPRINTING: A MACHINE LEARNING FRAMEWORK TO EXTRACT MECHANISMS OF MORPHOLOGICAL CHANGES

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Cellular perturbation and cytotoxic stimuli are known to readily affect the cellular morphology[1-3], yet the mechanisms by which these changes occur remain poorly understood. Traditionally, cytotoxicity is evaluated based on a quantitative endpoint estimate potentially masking important temporal information, cellular variability and fail to capture the exact mechanism of perturbation.
Here, we use state-of-the art fluorescent microscopy to capture the morphological changes of cellular compartments at a single cell level under various stimuli. We introduce a machine-learning methodology designed to segment hundreds of individual cells from a single image, enabling the extraction of over 40 key morphological features linked to observed changes across various conditions. To classify these features, we employ a simple logistic regression classifier combined with permutation importance.
We achieve an overall prediction accuracy of 89.11 ± 2.65% through five-fold cross-validation. This result indicates a clear distinction between the different conditions and highlights the significant key morphological differences that contribute to the separation of classes.
Cell Morphology Fingerprinting is a versatile method that can extract and infer valuable insights across a wide range of different cellular system and under various conditions that might alter the cellular morphology.[4,5]

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30. Haidai Hu

ZORYA ANTI-PHAGE DEFENSE AT THE MEMBRANE BOUNDARY

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Zorya is a recently identified and widely distributed bacterial immune system that protects bacteria from viral (phage) infections. Three Zorya subtypes have been identified, each containing predicted membrane-embedded ZorA–ZorB (ZorAB) complexes paired with soluble subunits that differ among Zorya subtypes, notably ZorC and ZorD in type I Zorya systems1,2. Here we investigate the molecular basis of Zorya defence using cryo-electron microscopy, mutagenesis, fluorescence microscopy, proteomics and functional studies. We present cryo-electron microscopy structures of ZorAB and show that it shares stoichiometry and features of other 5:2 inner membrane ion-driven rotary motors. The $ZorA_5B_2$ complex contains a dimeric ZorB peptidoglycan-binding domain and a pentameric α-helical coiled-coil tail made of ZorA that projects approximately 70 nm into the cytoplasm. We also characterize the structure and function of the soluble Zorya components ZorC and ZorD, finding that they have DNA-binding and nuclease activity, respectively. Comprehensive functional and mutational analyses demonstrate that all Zorya components work in concert to protect bacterial cells against invading phages. We provide evidence that ZorAB operates as a proton-driven motor that becomes activated after sensing of phage invasion. Subsequently, ZorAB transfers the phage invasion signal through the ZorA cytoplasmic tail to recruit and activate the soluble ZorC and ZorD effectors, which facilitate the degradation of the phage DNA. In summary, our study elucidates the foundational mechanisms of Zorya function as an anti-phage defence system.

31. Hendrik Harms

IMPACT OF PHOSPHORYLATION ON FUNCTION AND PHARMACOLOGY OF THE CARDIAC SODIUM CHANNEL REVEALED BY PROTEIN SEMI-SYNTHESIS

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While mutations of the cardiac sodium channel Nav1.5 have long been known to cause severe and potentially life-threatening arrhythmias, it is increasingly recognized that Nav1.5 undergoes dynamic post-translational modification (PTM) in vivo. However, conventional approaches are unable to reliably mimic PTM, such as phosphorylation, hence preventing investigation of their functional implications.
Here, we overcome this limitation by using split intein-based protein semi-synthesis of Nav1.5 in live cells in combination with molecular dynamics simulations. We introduce stable phosphorylation mimics on backgrounds of both WT and patient mutations and decipher functional and pharmacological effects with unique precision.
Our data demonstrate that phosphorylation of Y1495 alters the steady-state inactivation (SSI) of the channel by destabilizing the inactivation particle in its binding site. Surprisingly, we find that the Q1476R patient mutation itself does not alter SSI. Instead, Y1495 phosphorylation of this mutant results in a greatly increased right-shift in SSI compared to the effect on WT channels. Additionally, free energy calculations suggest that unbinding of the inactivation particle is energetically favored when Y1495 is phosphorylated, and that this effect is again exacerbated in the presence of the Q1476R mutation. Lastly, we show that both phosphorylation and patient mutations can impact Nav1.5 sensitivity towards clinically used anti-arrhythmic drugs.
In summary, functional effects of phosphorylation can be exacerbated in the presence of human patient mutations in Nav1.5, highlighting the importance of PTMs for the interpretation of mutational phenotypes.

32. Icaro A. Simon

EXPLORING A DRUGGABLE HYDROPHOBIC TUNNEL IN THE 5-HT_{2A} RECEPTOR WITH POTENT PHENETHYLAMINES

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The serotonin 2A receptor (5-HT_{2A}R) is the most abundant excitatory serotonin receptor in the brain.[1,2] It is also the primary target for serotonergic psychedelics, which have reemerged as potential pharmacotherapies for treating several psychiatric disorders.[3,4] Despite its importance, the receptor's mechanism of action and structure-activity relationships are not fully understood.[5] The experimental 5-HT_{2A}R structures have identified a hydrophobic tunnel lateral to the orthosteric site, situated between the transmembrane helices 4 and 5. Here, we examined this tunnel and its implications for 5-HT_{2A}R agonist pharmacology.[6] The tunnel characteristics and the key roles of residues $Phe234^{5x39}$ and $Gly238^{5x43}$ were delineated by molecular modeling and the synthesis and functional characterization of ten phenethylamine analogs with alkyl/alkenyl/aryl 4-substituents of various sizes and lipophilicities. Molecular dynamics simulations, structure-activity relationship determination, and mutagenesis experiments demonstrate that the agonist potency exhibited by these phenethylamines is rooted in their ability to protrude and interact with this tunnel. Moreover, novel ligand-receptor interaction opportunities were discovered within this tunnel and exploited to further enhance agonist potency. Thus, these findings provide new insights into the molecular basis for phenethylamine-induced 5-HT_{2A}R activation and identify this tunnel, for the first time, as a druggable region to employ in the design of potent 5-HT_{2A}R agonists.

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33. Ikki Yasuda

PARTITIONING MECHANISMS IN BIOMOLECULAR CONDENSATES USING COARSE-GRAINED MOLECULAR DYNAMICS SIMULATION

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Biomolecular condensates function as a compartmentalization mechanism of biomolecules without membranes, playing important roles in the regulation of cellular activities. While various types of biomolecular condensates exist in cells, they can selectively partition specific molecules. This is facilitated by the physicochemical properties of both scaffold and client molecules, but the underlying molecular mechanisms remain not fully understood. In this work, we aim to clarify the molecular partitioning mechanisms of condensates mediated by intrinsically disordered regions. Using coarse-grained molecular dynamics simulations that partially capture chemical differences of amino acids and nucleotides, we study the differential partitioning in two types of condensates, hydrophobic-residue-rich and charged-residue-rich condensates. We elucidate the relationship between partitioning and molecular interaction energies, and subsequently develop a sequence-based model to predict the partitioning.

34. Jakob Madsen

STRUCTURAL AND FUNCTIONAL CHARACTERISATION OF GLP1 VARIANTS: LINKING IN SILICO PREDICTIONS, IN VITRO SIGNALLING, AND IN VIVO BLOOD GLUCOSE CONTROL

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GLP-1 is a key peptide hormone derived from the proglucagon gene (GCG) with a critical role in regulating glucose metabolism and energy homeostasis. While we and others have studied genetic variants in the GLP-1 receptor (GLP1R) and gastric inhibitory polypeptide receptor (GIPR), missense variants in GLP-1 itself remain uncharacterized. We analyze all 22 genetic variants producing alternative GLP-1 peptides from 500,000 UK Biobank individuals. Our analysis spans structural predictions (in silico), cellular signaling (in vitro), mouse studies (in vivo), and population associations (in cohort). We find that gene population constraints show independence from evolutionary effects, suggesting diverse sampling with flexible receptor recognition. Biophysical metrics of peptide-receptor interfaces are predictive of altered signaling profiles in vitro, as demonstrated through fluorescence screening and enhanced random-effects modeling. We verified functional impacts through intraperitoneal glucose tolerance testing (IPGTT) in mice. Variants with impaired in vitrosignaling showed reduced glucose-lowering effects in vivo. Our study provides an in-depth characterization of naturally occurring GLP-1 variants and links their impaired signaling profiles with impaired in vitro glucose tolerance. The framework presented here naturally extends to characterize peptide function and physiological impact more broadly. Our findings may inform future investigations into GLP-1 variants' clinical relevance and therapeutic applications.

35. Jesper Elmsted Dreier

IDENTIFICATION OF A SMALL MOLECULE CAPABLE OF BOTH REVERTING PD-RELATED LIPID CHANGES AND INHIBITING LIPID-INDUCED α-SYNUCLEIN AGGREGATION

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Parkinson's disease (PD) is characterized by a loss of dopaminergic neurons as well as the deposition of protein-lipid inclusions called Lewy bodies (LB) whose main constituent is the pre-synaptic protein α-synuclein (αS) [1]. αS is involved in synaptic plasticity via its binding to vesicles; however, this protein-membrane interaction can also lead to the formation of amyloid fibrils resembling those found in LB. Many findings suggest that anionic lipids not only initiate αS aggregation but also incorporates into the amyloid fibrils[2,3].

Mutations in the gene GBA1, which codes for the enzyme glucocerebrosidase (GCase), are together an important genetic risk factor for PD and is associated with lysosomal disorders, lipid and αS accumulation[4,5]. Consequently, researchers have found small molecules capable of modulating GCase activity and alleviating GBA1 mutation-associated lipid changes. However, these molecules have not been screened for their ability to directly affect αS aggregation.

Using a combination of biophysical methods and kinetic analyses, we identified and characterized a small molecule-GCase modulator that inhibits the lipid-induced aggregation of αS. Moreover, kinetic analyses of Thioflavin-T aggregation curves provided the mechanism of inhibition and showed that the small molecule not only displaced αS from the membrane but slows down the formation of oligomers[6].

These results are highly relevant, not only because they provide a pipeline that could be useful in testing other potential drugs against protein aggregation, but also because they show that a small molecule capable of modulating PD related lipid changes can also directly impact αS by inhibiting its aggregation. This finding is the first evidence for a small molecule to have dual mode of action, acting on both the lipid metabolism and αS aggregation.

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36. Jing Huang

CO-TRANSCRIPTIONAL FOLDING OF CUSTOM RNA ORIGAMI BY SINGLE-MOLECULE FÖRSTER RESONANCE ENERGY TRANSFER

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Understanding how RNA folds is a fundamental question in biology. In this project, we study how RNA molecules fold during synthesis using rationally designed RNA origami nanostructures as a key tool to gain detailed insight into this process. The RNA origami technique allows us to design RNA structures that can be folded from single stranded co-transcriptional RNA with a large sequence freedom. We aim at using Förster resonance energy transfer (FRET) as a powerful approach to investigate co-transcriptional folding dynamics and the transient RNA structures. We present our experimental setup and quantification of RNA synthesis and fluorescent labelling of the designed RNA structure using gel assays.

37. Jing Zhao

CHARACTERIZING THE BINDING OF NOVEL RAPID-DERIVED CYCLIC PEPTIDES USING DEEP MUTATIONAL SCANNING

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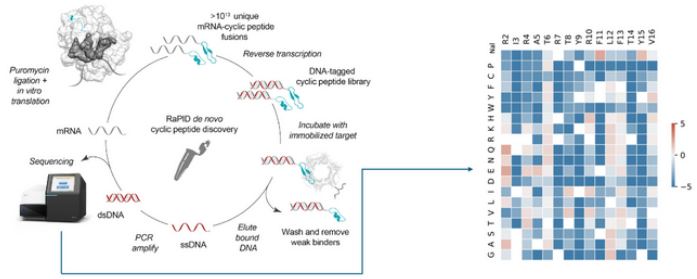
The Random Nonstandard Peptide Integrated Discovery (RaPID) system is a powerful screening approach for discovering cyclic peptides de novo that bind to target proteins. RaPID efficiently screens trillions of DNA-tagged cyclic peptides, rapidly identifying the highest-affinity binders by utilizing randomized sequence mRNA and genetic code reprogramming.
To optimize macrocyclic RaPID hits, a combination of chemical modifications and biophysical measurements is commonly employed. However, this approach relies on extensive chemical synthesis and requires one-at-a-time synthesis and biophysical characterization, making it time-intensive. Computational modeling can aid this process but post-translational modifications (PTMs) may not be well-represented in models. In this project, we developed and implemented RaPID-based deep mutational scanning (DMS), enabling the simultaneous analysis of thousands of peptide variants to identify essential binding residues and guide peptide optimization. By employing saturation mutagenesis on multiple hit cyclic peptides in one-pot experiments, we successfully applied this method across diverse systems, including RNA-binding proteins, and protein-protein interactions. Similar high-throughput approaches could alson be applied to protein design, RaPID-based (DMS) could be employed to systematically refine protein sequences for enhanced activity, specificity, or stability.



38. Julian Storm

STRUCTURAL INSIGHTS INTO MECHANISM OF GLUTAMINE TRANSPORT BY HUMAN SNAT1 AND SNAT3

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Glutamine is the most abundant, conditionally essential amino acid in plasma and cerebrospinal fluid. It is involved in a myriad of biochemical and regulatory processes and is the main precursor for both excitatory and inhibitory neurotransmitters. The sodium-coupled neutral amino acid transporters, SNAT1 and SNAT3, are plasma membrane members of SLC38 family that predominantly translocate glutamine but also other essential amino acids across the cell membrane. In fast dividing cancer cells with a high glutamine demand, SNAT1 and SNAT3 show strong overexpression, and could serve as tumour-cell-selective targets for anticancer drugs with amino acid-based structure. Despite the therapeutical potential, there are no selective inhibitors or research tool compounds targeting SNAT1 or SNAT3. Here we present the first cryo-EM structures of human SNAT1 bound to substrate glutamine, bound to substrate analogue MeAIB and in a substrate free state at 3.0, 2.9 and 3.6 Å resolution, respectively, and of SNAT3 bound to glutamine at 3.0 Å resolution. We show that the substrate and substrate analogue bind to the central pocket of the SNATs, stabilizing the transporter in an inward- open conformational state. We have established the first cell-based transport assays for SNATs revealing the key residues for the substrate recognition, ion-binding and transport activity. Our data form the basis for the development of novel inhibitors and therapeutic strategies for effective targeting of SNATs in a broad range of human diseases including cancer.

39. Junjun Tan

CHEMICAL EVOLUTION IN DYNAMIC PEPTIDE NETWORKS

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The evolutionary process manifests the progressive emergence of higher order from a complex network of molecules under environmental fluctuation and selection pressure to generate intelligent living systems. This process is also accompanied by molecular information selection and transformation. Besides the genetic information flowing from DNA to RNA and then to protein, prion and amyloid define another form of epigenetic heredity. Prion infectious particles are transmitted through the propagation of misfolded cross-β motif. This cross-β folding is also a generic structural form of proteins. We propose to create a diverse and dynamic peptide network to investigate how the selection of both covalent frameworks and conformational information could be achieved and drives chemical evolution.

Several dynamic peptide networks have been constructed using peptide aldehydes and reversible N, O-acetal or N, N-acetal formation reactions. Observation showed that specific sequences can self-select and replicate through progressive nucleation and phase transition processes, similar to prion-like conformational propagation. Additionally, exogenous cross-β template (such as cross-β nanotubes) can guide the formation of the conformations by templating. These discoveries indicated that such processes are widespread and likely a general property of matter, representing a fundamental chemical level of natural selection in chemical evolution.

40. Karen Martinez

NEW INSIGHTS INTO GPCR SIGNALING REVEALED BY A NEW ANALYTICAL TOOL FOR FLUORESCENCE IMAGES

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Growing evidence suggests that the organisation of GPCRs at the plasma membrane in “signaling hot spots”, also called signalosomes, plays a crucial role in GPCR spatial and temporal signaling. Bulk GPCR signaling studies, which provide only average responses across large cell populations, are not well suited to address this question. To overcome this limitation, these studies can be completed with quantitative fluorescence imaging, which offers spatial and temporal resolution at the single-cell level. Unlike bulk measurements, single-cell imaging captures cell-to-cell variability—information critical for understanding the biological noise masked in population-averaged data.

We developed a broadly applicable method that combines automated high-throughput analysis with quantitative measurement of multiple fluorescence signals from confocal images. By acquiring a high density of data points per cell, this approach yields large-scale datasets that enable statistically robust single-cell analyses. These datasets uncover intra-cellular signal heterogeneity and can be leveraged to statistically suppress biological noise at the population level, surpassing the noise inherent to individual cells. As a result, this method enhances the precision of cell signaling studies beyond current state-of-the-art approaches.

Here, we demonstrate how the method can reduce by more than tenfold and hundredfold the biological noise from a monoclonal cell line and a single cell, respectively. We furthermore illustrate how it reveals new insights into several aspects of GPCR signaling, and the influence of protein expression levels on this process.

41. Kristine Salomon

EXPLORING THE MECHANISTIC BINDING DIFFERENCES OF ATYPICAL DOPAMINE TRANSPORTER INHIBITORS AND COCAINE

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Cocaine exerts its addictive effects by binding to the dopamine transporter (DAT) and blocking dopamine reuptake. Atypical DAT inhibitors, such as JHW007, display reduced stimulant properties and lower abuse potential, but the molecular basis of this distinct pharmacology remains unclear[1].
We characterized the binding kinetics and conformational interactions of JHW007 with human DAT (hDAT). The Y156F mutation, which stabilizes the outward-open conformation, reduced JHW007 affinity ~10-fold, while cocaine binding was minimally affected[2]. This suggests JHW007 favors a distinct transporter conformation. Kinetic assays revealed slower binding and a 7.5-fold lower dissociation rate for JHW007 compared to cocaine.
Additionally, incorporation of the environment-sensitive fluorescent unnatural amino acid ANAP allowed detection of ligand- and ion-induced conformational changes[3]. Distinct spectral signatures for JHW007 and cocaine support a model in which JHW007 preferentially stabilizes an inward-facing or intermediate state of hDAT.

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42. Lukas P Feilen

STRUCTURE AND FUNCTION OF THE HUMAN NA^+/H^+ EXCHANGER 6

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Sodium/hydrogen exchangers (NHEs) are ubiquitously expressed transport proteins that reside in cellular membranes and play a pivotal role in maintaining cellular pH and/or cell size and shape. The human Na+/H+ exchanger 6 (HsNHE6) is located in the endosomal trafficking system predominantly in neuronal cells and its disfunction has been linked to several neurological disorders like Christianson syndrome (CS) and Alzheimer's disease (AD).
Here we report the first structure of HsNHE6 at 3.4 Å resolution. Similar to other NHEs, HsNHE6 forms a homodimer in which each protomer is composed of 13 transmembrane (TM) helices. Our structure shows an inward-open conformation with additional densities observed in proximity to side chain residues corresponding to the ion binding site. Additionally, we partially resolve the proximal C-terminal tail of HsNHE6, which forms a short helix lying next to the TM core of HsNHE6. By integrating our cryo-EM structure with NMR and small-angle X-ray scattering (SAXS) data of the distal and flexible part of the C-terminus, we generated a composite model of the (near) full-length HsNHE6.

43. Mads Liep Ramsing

FROM PEPTIDES TO BIOASSAYS: SUSTAINABLE PREPARATION OF PEGA RESINS FOR THE NEXT GENERATION OF CHEMICAL BIOLOGY

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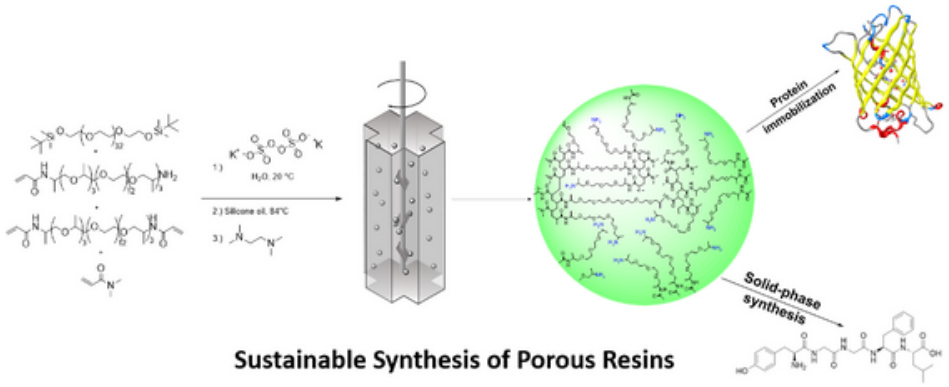
The discontinuation of polar resins such as PEGA has created a bottleneck within peptide chemistry and chemical biology.[1] We present a novel sustainable and scalable approach producing high quality PEGA reins via inverse suspension polymerization in recyclable silicone oil, avoiding highly restricted solvents such as CCl_4, and minimizing waste. Bulk synthesis produced the beaded resin at a size range of 200-400 µm and resulted in over 700 mL of water-swelled resin per batch, suitable for more than 500 medium scale peptide syntheses. These beaded resins are spectroscopically transparent, and act as a stealth polymer that do not interact with proteins, biomolecules or cells. Providing the ideal template for protein immobilization technologies. The bead formation was optimized by obtaining precise temperature control and using PEG_{1500}-$(O\text{-}TBDMS)_2$ as detergent. Flow synthesis were further refined to ensure uniform bead control, yielding the optimal diameters for on-bead bioassays. The swelling capabilities of the resulting PEGA resin was evaluated through a broad range of green solvents, showing excellent solvent compatibility and confirming its suitability for green solid-phase synthesis technologies.

Performance of the resin was validated through successful peptide assembly of Leu-enkephalin utilizing Fmoc-based protocols. Likewise, the performance as a stealth polymer was demonstrated through covalent immobilization of green fluorescent protein confirming uniform biomolecule diffusion.[2]

Incorporation of arginine and ATOTA labeled microparticles in the polymer matrix establishes a foundation for efficient optical encoding, offering the option of efficient synthesis and characterization of substrates in combinatorial libraries.[3]

Our procedures empowers laboratories to maintain an independent and environmentally friendly production of PEGA resin, advancing initiatives in peptide and protein research without compromising on performance.



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44. Malene Hall Jensen

DEVELOPMENT OF FIRST-IN-CLASS INHIBITORS FOR EXTRASYNAPTIC $GABA_A$-RECEPTORS

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Inhibition in the brain is dominated by -Aminobutyric acid (GABA), mainly exerting its effects through pentameric ligand-gated GABA type A receptors ($GABA_A$-Rs). Synaptic and extrasynaptic $GABA_A$-Rs exhibit distinct functional and pharmacological properties, with extrasynaptic -subunit containing $GABA_A$-Rs (-$GABA_A$-Rs) serving as principal mediators of tonic inhibition. Tightly regulated tonic inhibition is critical for both development and functioning of the CNS. Therefore, aberrant tonic inhibition has received great attention as a pathophysiological mechanism underlining various CNS diseases. Despite its significance as a pharmacological target and the therapeutic relevance of -containing $GABA_A$-Rs, no -selective inhibitors have been identified to date. A treatment strategy aiming at inhibition of -containing $GABA_A$-Rs is therefore needed. Utilizing the structural scaffold of a known positive allosteric modulator of -$GABA_A$-Rs, delta-selective compound 2 (DS2), and guided by the discovery of a novel binding pocket within the transmembrane domain of $GABA_A$-Rs, a small compound library was designed in-house. Compounds were screened and characterized for their allosteric modulatory activity using the FLIPR membrane potential assay (FMP) and whole-cell patch clamp electrophysiology. This led to the identification of a new class of selective negative allosteric modulators of d-$GABA_A$-Rs.
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45. Malyasree Giri

THE INTER AND INTRA-MOLECULAR INTERACTIONS DRIVING PHASE SEPERATION OF PROTEIN KINASE A REGULATORY SUBUNIT

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Phase separation of biological macromolecules has emerged as a common mechanism for cellular organization, governing the spatiotemporal regulation of biochemical processes such as signaling pathways. The RIα subunit of cAMP-dependent protein kinase A (PKA) phase separates following cAMP-induced kinase activation, thereby restricting and enhancing PKA activity within a distinct compartment[1,2]. The N-terminal dimerization/docking (D/D) domain and the linker region of RIα have been identified as necessary and sufficient for phase separation, but their interactions have not been studied at atomic resolution[1]. We study the minimal phase separating region of RIα by nuclear magnetic resonance (NMR) spectroscopy and coarse-grained molecular dynamics simulations. The intrinsically disordered regions cause broadening of NMR signals and elevated transversal relaxation rates through-out the protein indicative of dynamics interactions. Mutation of arginine residues in the inhibitory sequence led to small improvement in relaxation properties, but a loss of phase separation. Binding to a helical A-kinase anchoring protein (AKAP) lead to spectral changes indicative of a loss of interactions between the D/D domain and the linker suggesting a competition between intramolecular interactions and partner binding. Simulations suggest pervasive interactions within the intrinsically disordered linker, and between the linker and the D/D domain in agreement with experiment. The simulations further show that the intermolecular interactions in a condensate mirror those found internally in a RIα dimer.

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46. Marco Polimeni

PROBING PROTEIN LIQUID-LIQUID PHASE SEPARATION KINETICS BY MICROFLUIDICS TIME RESOLVED SMALL ANGLE X-RAY SCATTERING AND MOLECULAR SIMULATIONS

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Liquid-liquid phase separation (LLPS) can be a preliminary step on the pathway leading to protein aggregates and amyloid fibrils, the latter being linked to several neurodegenerative pathologies like Parkinson's disease (PD) [1,2]. However, due to a lack of techniques able to look at the early-stage LLPS formation, the molecular interactions, as well as the protein states involved in LLPS formation, are missing. Current strategies only focus on temporal scales where LLPS is already in place (seconds, minutes, hours), preventing access to the early phases (sub-second range) of the condensation. To bridge this gap, we employed time-resolved small-angle x-ray scattering (TR-SAXS) in combination with molecular simulations to monitor the mechanism driving LLPS on protein solutions. TR-SAXS enables real-time investigation of LLPS formation with an unprecedented temporal resolution of up to milliseconds. Simulations complement the experimental outcomes with a molecular view of the phenomena. We validated our approach by investigating LLPS of a bovine serum albumin (BSA) solution induced with polyethylene glycol (PEG). Our TR-SAXS experiments show that a combination of excluded volume effects, salt screening effects, and short-range interactions may be responsible for BSA LLPS. We quantify the importance of such molecular contributions by performing molecular simulations with different levels of coarse-graining. Our protocol is general and applicable to disease-related proteins, potentially providing a powerful tool to investigate key factors determining the onset of the pathologies.

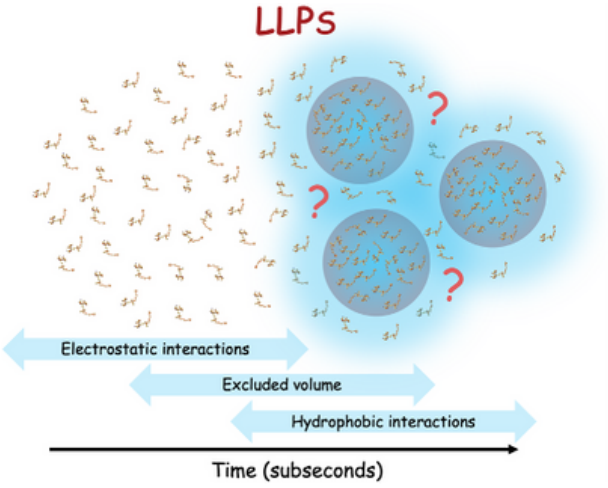

Figure 1. Schematic representation of liquid-liquid phase separation in protein solutions.

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47. Maria F. Vicino

A COPOLYMER-BASED APPROACH FOR STRUCTURAL CHARACTERIZATION OF ACID-SENSING ION CHANNELS

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Human acid-sensing ion channels (hASICs) are membrane embedded proton-gated channels expressed in the central and peripheral nervous systems. Their dysfunction is linked to pain sensing, loss of memory, or neuronal cell death following an ischemic stroke. Therefore, these proteins are particularly attractive therapeutic targets for drug development. Structural insights into hASICs are crucial for understanding their function and for guiding rational drug design.
Our aim is to determine the structures of different hASIC isoforms and to investigate how potential drug candidates bind and modulate their function. To achieve this, it is essential to establish a robust purification protocol. However, membrane proteins present a particular challenge, as traditional purification methods often involve the removal of the native lipid environment, potentially disrupting essential native interaction partners and compromising protein stability or function. One alternative approach that preserves the crucial native lipid environment around the membrane protein is using amphiphilic copolymers.
We use MAASTY copolymers as membrane mimetic systems, which are developed in our group and at DTU. MAASTYs consist of methacrylic acid (MAA) and styrene (STY), which are synthesized by reversible addition-fragmentation chain-transfer (RAFT) polymerization. This allows control over the copolymer length. In these first proof-of-concept experiments we demonstrate that MAASTY copolymers enable successful solubilization and purification of various hASIC homologs. By preserving the native lipid environment, our approach represents a crucial first step towards the structural and functional characterization of hASICs in different conformational and ligand-bound states laying the foundation for future drug discovery efforts.

48. Min Zhang

REAL-TIME DIRECT OBSERVATION OF THE FORMATION OF AMYLOID SPHERULITES BY SUPER-RESOLUTION MICROSCOPY

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The misfolding of native structural proteins into amyloid fibrils is associated with a spectrum ofneurodegenerative diseases such as Alzheimer's and Parkinson's. Recent studies revealed fibrils not to be the only protein aggregate species, but rather the existence of amyloid-like spherical aggregates, named spherulites, ranging from few micrometers to millimeters in diameter. Conventional spectrometric methodsreporting the average growth rates are mostly used to investigate the aggregation mechanism, while microscopy only readouts of final structures, consequently masking the morphological and growth heterogeneity of the aggregates. To solve this, we recently developed a new super resolution method (REPLOM) based on single molecule localization microscopy, and successfully observe directly the formation of insulin spherulites and quantify the existence and abundance of diverse morphologies as well as their heterogeneous growth kinetics of each structure. Our results revealed insulin spherulite growth is not exclusively isotropic, but it may also occur anisotropically. Combined with Machine learning, we associated growth rates to specific morphological transitions and provided energy barriers and the energy landscape for each aggregation morphology. Our method can be extended to more medically- relevant proteins, such as α-synuclein or Aβ peptide. In those cases, our approach may provide unprecedented hitherto information on transient intermediate species, which are nowadays recognized as the cause of the disease progression, both in terms of energetics and morphology.

49. Nadja Joachim

PHOSPHOROUS RECOVERY USING OPTIMISED PHOSPHATE BINDING PROTEINS

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The overall aim of this project is to construct a phosphate-specific chromatographic column that can recover inorganic phosphate (Pi) from wastewater, using the bacterial phosphate binding protein PstS. Phosphorous is essential for plant growth and modern agriculture relies on its use in fertilizers. However, phosphorous reserves are limited, and by the use in farming, it ends up in sewage or washed out of the soil and into our waterways, lakes and oceans mainly as inorganic phosphate, Pi.[1] PstS is a highly selective and high affinity binder of Pi, and could therefore serve as a tool to recover phosphate.[2] By screening a library of 96 diverse PstS sequences, highly stable PstS variants were identified. To engineer a pH sensitive switch to release bound phosphate, we implemented a point mutation in the binding site, which leads to preferred binding of the monobasic compared to dibasic phosphate, and therefore disfavours binding above pH 8.[3] As the binding site is highly conserved, this mutation can also be introduced in the stable homologues identified in the screen, which resulted in a more stable PstS variant with pH sensitive affinity for Pi, demonstrated by ITC. In order to engineer a more sensitive switch and even more stable variants, we study the dynamics of the protein, especially the hinge movement between Pi bound and Pi free state, using NMR methods. We aim to further optimise phosphate release properties as well as protein stability to produce a durable PstS column.

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50. Neda Rahmani

NOVEL DNA-BASED FORCE SENSOR TO MEASURE MEMBRANE-MEDIATED FORCES BETWEEN PROTEINS

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Membrane-mediated interac.ons play a pivotal role in various biological processes, ranging from intercellular trafficking to signaling. These interactions stem from a protein's capacity to disturb the natural state of the membrane. Experimentally tracing these interac.ons is a challenging task.
This project aims to integrate theory and simula.ons to devise a force sensor capable of detec.ng pN forces at the nanometer length scale. We focus on measuring the force between the B-subunit of Shiga toxin on biological membranes.
A DNA-based device has been built to measure the alrac.ve force between two membrane- associated molecules at a distance of a few nanometers.
The geometry of the system has the character of tweezers with two arms and a linker between the arms. Each arm has alached an op.cally ac.ve molecule. We built a supra-coarse-grained model to design and calibrate the force sensor. The molecular dynamics (MD) simula.ons were performed using GROMACS.
Our model predicts that this force sensor can reliably detect membrane-mediated forces in the pN range at nanometer distances. The model's results qualita.vely agree well with the available experimental data. Additionally, our simulations indicate that the soSer the linker, the more sensitive and effective the sensor becomes.

51. Nikolina Sekulic

STRUCTURAL STUDIES ON SPINDLE-ASSOCIATED PROTEIN BUGZ EXPLORING POTENTIAL FOR DRUG TARGETING

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BuGZ, also known as ZNF207 (Zinc Finger Protein 207), is a 478 amino acid long human protein that plays an important role in cell division. BuGZ is a component of the spindle matrix that surrounds the mitotic spindle during open mitosis in eukaryotes. It is an unstructured protein that exhibits concentration-dependent phase separation at physiological temperatures. The N-terminal part of BugZ has two zinc finger domains and is important for the binding of tubulin. Apart from the N-terminal part, the rest of the protein is very unstructured and contains several proline-rich stretches and a GLEBS sequence that interacts with Bub3, an important player in regulating the mitotic spindle assembly checkpoint.
Recently, the Barišić group at the Danish Cancer Institute together with Pittelkow and Meldal, they have used a combination of click chemistry and mass spectrometry to find that the natural compound parthenolide interacts covalently with BuGZ, leading to disruption of KT-MT interactions.
We will describe structural biology approaches and preliminary results on the structure of BuGZ in isolation and in complex with parthenolide and its derivatives. We also speculate on how our results may help in the development of new antimitotic drugs.

52. Phil Rainsford

NUCLEAR MAGNETIC RESONANCE STUDIES OF ACKR3 AND CXCR4

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This project is using nuclear magnetic resonance – NMR – to characterise and explore the relationship between the different functional states of the chemokine receptors ACKR3 and CXCR4, as well as different biologically relevant regulatory mechanisms that can influence their functions, such as the effect of different lipid species. We have expressed both ACKR3 and CXCR4 labelled with ^{13}C-Methionine in Sf9 cells and obtained well-resolved NMR spectra of each receptor in the presence of both an agonist and an antagonist/inverse agonist, identifying distinct conformations for the active and inactive receptor ensembles. To our knowledge, this is the first NMR study of CXCR4.

53. Raminta Venskutonyte

STRUCTURAL STUDIES OF AQUAPORIN INHIBITION

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Aquaporins (AQPs) are membrane proteins, involved in the transport of water, glycerol and other solutes across the cell membrane. They have an important role in metabolism and have been implicated in cancer development. Particularly, aquaglyceroporins AQP7 and AQP3 have been shown to be expressed in breast cancer and are therefore possible drug targets. Several inhibitors binding to AQPs have been reported and shown to block the movement of the solutes through the channel pore. To understand the molecular determinants of the inhibitor-protein interactions in the AQPs we have solved the cryo-EM structures of AQP7 in complex with one inhibitor and two structures of AQP3 with two different promising inhibitor compounds. Moreover, we support the structural data with MD simulations and functional assays. These results provide a good basis for further drug development targeting AQPs.

54. Ranjit Gulvady

A NOVEL DNA-BASED FRET SENSOR TO QUANTIFY CLUSTERING OFNF MEMBRANE PROTEINS

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Membrane proteins play a crucial role in various biological phenomena such as endocytosis and signal transduction [1]. These phenomena are heavily dependent on the clustering of membrane proteins [2]. While the propensity of proteins to cluster on the cell membrane has been well established, the physical mechanisms that govern this behavior are far from clear. Furthermore, the tools to be able to measure the forces responsible for the clustering of membrane proteins have not been developed yet. These measurements are in particular challenging when dealing with weak forces such as membrane-mediated interactions.

To bridge this gap, we have designed a DNA-based FRET sensor. Using the sensor, we are able to, for the first time, directly quantify the clustering of membrane proteins at the single-molecule level. As a proof of concept, the sensor design and measurements have been applied to the B-subunit of the Shiga Toxin (STxB). Pairs of proteins, linked by the sensor, are bound to small liposomes through Gb3 lipids (the STxB receptor). The liposomes, in turn, are tethered to a glass substrate. Our smFRET data provides the first experimental verification testing of a membrane fluctuation-induced force that generates an effective attraction between membrane-bound proteins.

As a proof of concept, the sensor design and measurements have been done applied using to the B-subunit of the Shiga Toxin (STxB). Following measurements on STxB proteins, this novel method shall then be applied to understand the clustering behavior of other membrane proteins.

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55. Ryan Cantwell Chater

STRUCTURAL INSIGHTS INTO ALLOSTERIC MECHANISM OF GLYCINE TRANSPORTER-MEDIATED ANALGESIA

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Chronic neuropathic pain, caused by nerve damage or disease, affects 10% of the population with rising incidence. The burden is exacerbated by ineffective treatments and overreliance on opioids. Neuronal glycine transporter, GlyT2, represents a promising target to restore disrupted inhibitory glycinergic neurotransmission in neuropathic pain. However, most GlyT2 inhibitors have not progressed to clinical use because of significant side effects, partly from irreversible inhibition at analgesic doses. Here, we report cryo- EM structures of human GlyT2 bound to the potent pseudo- irreversible inhibitor ORG25543, a reversible analogue RPI-GLYT2-82, substrate glycine, and in substrate-free state. Both inhibitors bind an extracellular allosteric site, locking the transporter in an outward- open conformation, whereas the glycine-bound and substrate-free GlyT2 adopt an inward-occluded and inward-open conformations, respectively. We demonstrate that RPI- GLYT2- 82 has a faster off-rate, providing analgesia in mouse neuropathic pain models, with no observed mechanism-based side-effects or addiction liability. Our data provide a model for allosteric inhibition of glycine transport, enabling structure-based design of new non-opioid analgesics.

56. Shiv K Sah Teli

REVEALING THE ULTRASTRUCTURE OF CELLULAR GAP JUNCTIONS: A STRUCTURAL BIOLOGY APPROACH

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Gap junctions are specialized cellular structures that enable direct communication between adjacent cells, playing crucial roles in maintaining tissue homeostasis, coordinating cellular functions, and facilitating electrical and metabolic coupling. They are composed of connexins, transmembrane proteins that assemble into hexameric hemichannels which dock with counterparts on neighboring cells to form functional channels. Among the 21 known connexin isoforms in humans, Connexin 43 (Cx43) is the most widely expressed and extensively studied. Although high-resolution structures of a few connexins have been elucidated, the structural details of many isoforms remain unknown. Notably, the C-terminal domain, essential for regulatory interactions and signaling, is structurally uncharacterized across all isoforms. Additionally, the mechanisms governing connexin isoform compatibility for heteromeric assembly and their involvement in specific cellular processes are not fully understood.

Our study aims to uncover the ultrastructure of gap junctions and resolve the architectures of multiple connexin isoforms, including their elusive C-terminal domains. High-resolution structures of different connexin isoforms solubilized or reconstituted in detergents or lipid nanodiscs are investigated using cryo-Electron Microscopy (cryoEM). To capture physiological ultrastructures, connexins are also examined in membrane-mimicking environments, including copolymer-reconstituted systems and membrane vesicles, as well as through in situ cryo-Electron Tomography (cryoET). Furthermore, isoform compatibility for heteromeric assembly and mapping of interaction networks involved in cellular signaling pathways are explored using BioID (proximity-dependent biotin identification) and Affinity Purification Mass Spectrometry (AP-MS). By elucidating the structural and functional roles of connexins in cellular communication, this research advances our understanding of gap junction biology and provides insights into therapeutic strategies for diseases linked to connexin dysfunction, including cardiac arrhythmias, neurodegenerative disorders, and cancer.

57. Sofie Thomsen

ORIENTED FRET WITH FLUORESCENT RNA LIGHT-UP APTAMERS

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Fluorescent light-up aptamers (FLAPs) serve as RNA counterparts to fluorescent proteins[1]. They are short, synthetic RNA sequences that adopt well-defined tertiary structures and selectively bind cognate fluorophores, activating their fluorescence[1,2]. Two FLAPs capable of Förster Resonance Energy Transfer (FRET) can be incorporated in RNA scaffolds to form aptamer-based FRET systems using RNA origami[3]. Aptamer-based FRET systems are interesting tools to probe RNA folding and dynamics, in vitro and in vivo.
Here, we investigate an aptamer-based FRET system. We demonstrate that the aptamer system shows oriented FRET with a minimal RNA construct. Oriented FRET appears as sinusoidal modulations in the FRET efficiency as a function of stem extension, reflecting helical rotation in an A-form RNA helix. We furthermore introduce a FRET prediction tool that takes the orientational FRET factor (κ^2) into account. Experimental FRET aligns well with predicted FRET values, validating our theoretical model. Overall, our findings establish the aptamer-based FRET system as sensitive reporters of RNA structural dynamics.

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58. Stavroula Margaritaki

SHEDDING LIGHT ON MOLECULAR MECHANISMS OF TRANSCYTOSIS WITH 4D IMAGING AND MACHINE LEARNING-BASED ANALYSIS

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This research investigates the molecular mechanisms of transcytosis, with a specific focus on antibody fragments, including Fabs and VHHs, as they navigate cellular barriers such as the blood-brain barrier (BBB). Employing state-of-the-art four-dimensional (4D) imaging techniques and machine learning-driven analytical frameworks, the project aims to understand the interplay between binding kinetics, pH sensitivity, and trafficking efficiency. Endothelial cell models derived from both murine and human sources serve as platforms for these investigations. Advanced imaging methodologies combined with computational analyses, enable a detailed analysis of intracellular pathways. This knowledge is crucial for optimizing therapeutic antibody designs, ultimately enhancing their efficacy in neurodegenerative disorder treatments and refining BBB transcytosis assay methodologies.

59. Tania Ginkel

OPTIMIZED PURIFICATION OF ADENO-ASSOCIATED VIRUS FROM SUSPENSION CELL CULTURE FOR USE IN MAMMALIAN PROTEIN EXPRESSION

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Protein expression and purification are foundational to the investigation of pharmacology and protein structure. A conventional method for the expression of mammalian proteins in suspension cell culture is the BacMam System, which uses baculovirus for transgene delivery.
Here, I explore an alternative gene delivery technique that uses Adeno-Associated Virus (AAV) for transduction to circumvent the elaborate production process, limited storage options, and cell toxicity of baculovirus.
In order to generate the quantities of AAV that are required for this approach, I have optimized a method for AAV expression in suspension cell culture that uses valproic acid and RNA interference to increase AAV yields. Furthermore I have developed a protocol for virus harvest and purification via heparin affinity chromatography.

60. Tereza Kubátová

COMPUTATIONAL PIPELINE FOR POLYPHARMACOLOGICAL PROFILING

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Polypharmacology, where a single compound modulates multiple targets, is a central concept in drug discovery. Modeling such interactions is key to identifying hidden off-targets, optimizing therapeutic profiles, and designing more selective drugs. Traditional approaches include inverse docking, multipharmacophore modeling, and similarity-based profiling; however, quantitative structure–activity relationship (QSAR) modeling remains a highly scalable and interpretable strategy especially when extended with protein and interaction-aware descriptors. We present a modular pipeline that combines ligand-based, protein-based, and cross-term modeling. Ligands are described using physicochemical descriptors, molecular fingerprints, and learned embeddings. Proteins are represented by sequence-based descriptors and, optionally, by binding site–specific features. Feature selection of descriptors integrates both objective and subjective methods, including mutual information, recursive feature elimination, and LASSO. PCA and SHAP-based interpretation are included to enhance model transparency. We applied this pipeline to a curated dataset of 44 psychedelic compounds with experimentally validated G protein-coupled receptor (GPCR) activity, measured by fluorescence-based functional assays. These ligands exhibit diverse and often promiscuous pharmacology, making them ideal for studying multi-target activity and receptor selectivity. This framework supports interpretable, target-aware polypharmacology modeling and enables systematic discovery of multi-target ligands.

61. Thea Schinkel

CRYO-EM AIDED DESIGN OF MULTIVALENT APTAMER NANOSTRUCTURES (CAD-MAN) FOR ENHANCED BIOMOLECULAR TARGETING

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Aptamers targeting therapeutically relevant biomolecules bare great potential in disease treatment and the regulation of cellular processes outperforming widely used antibodies in size, ease of production and immunogenicity. We propose a workflow to design tailored, multivalent RNA-aptamer structures with enhanced targeting and therapeutic potential based on a precise understanding of the aptamers structure and mechanism of action. Here, I focus on aptamers binding to human CD40 ligand (hCD40L), an important checkpoint in immune response, and hemagglutinin, an integral surface protein in influenza virus essential for viral internalization and infection. My goal is to visualize protein-aptamer interactions using cryo-EM structural analysis as a tool to determine important binding interactions and motifs and to evaluate the influence of modified or functionalized nucleobases on structure and binding properties. With the structural information of the protein-aptamer complexes gained by cryo-EM tailored multimeric architectures will be designed using self-assembled RNA nanostructures, which allow precise three-dimensional positioning of monomeric aptamer subunits for enhanced affinity towards the protein targets. Our data shows three aptamers simultaneously binding to trimeric hCD40L, enabling the design of trimeric aptameric RNA nanostructures. Newly designed nanostructures will be structurally validated by cryo-EM and functionally characterized using a variety of cell-based assays and biophysical techniques. If successful, my approach, based on the cryo-EM aided structural design has an enormous innovative potential that will enable the development of high affinity RNA binders for biomedical and sensing applications.

62. Tobias Winckler-Carlsen

AUTOMATED HIGH-THROUGHPUT ANALYSIS OF THE HETEROGENEITY OF AMYLOID AGGREGATION KINETICS

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The stochastic nature of the aggregation processes leads to an intrinsic variability of the measured amyloid kinetics. The standard approach to obtain statistically significant data is based on the detection of Thioflavin T fluorescence of multiple replicates of identical but isolated sample volumes via microplate reader. However, such data contains both information on the intrinsic variability of the process and the experimental well-to-well variability. We have developed a highly automated plater reader method to detect spatially-resolved ThT fluorescence over time within a single microplate well to obtain a quantitative measure of the intrinsic kinetic variability.[1] In addition, we developed a python-based script for the handling and analysis of the generated large data sets, capable of isolating key kinetic parameters of the reactions and generating probability distributions, whose features are linked to the aggregation mechanism. The approach is validated for insulin aggregation and the variability of characteristic time of the reaction can be used as a measure of the competing surface-catalyzed and bulk aggregation. Work is in progress to extend the method to a wider range of proteins undergoing self-assembly and, more in general, to heterogeneous biological reactions that can be monitored by fluorescence.

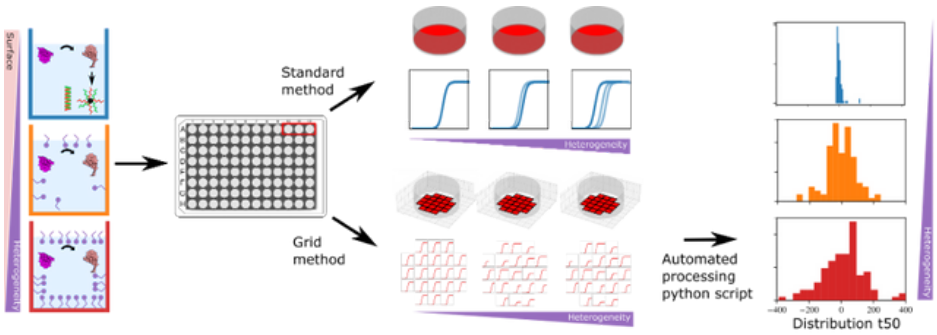

Figure 1. Schematic overview of the grid kinetics method. Collecting spatially resolved ThT fluorescence spectra from multiple points within a single well allows for a quantitative measure of the intrinsic kinetic variability by comparing surface-catalyzed and bulk aggregation processes.

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63. Victor C Yin

DECIPHERING STRUCTURAL AND DYNAMICAL DIFFERENCES IN COMPLEX PROTEIN OLIGOMERIC ASSEMBLIES: A COMBINED HDX-MS AND NATIVE MS APPROACH

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Higher-order protein assemblies play fundamental roles in biological processes. Often, these complexes are made up of repeating copies of only a few subunits. This simple blueprint forms the basis of many viruses, immune complexes, and other molecular machinery. Due to their diverse functionalities, these oligomeric assemblies also form the foundation of several contemporary biotherapeutic formats (e.g. multimeric antibodies, VLPs).
Due to their symmetry, it is often implicitly assumed that subunits within the assembly are structurally and functionally identical. This is reinforced by the practical challenge in detecting the characteristics of individual subunits in these complexes, as most experimental methods (e.g. cryoEM, NMR, bottom-up structural MS) rely on ensemble-averaging. Any individual subunit differences (e.g. from ligand interactions) are therefore "averaged out" and not detectable despite breaking the symmetry of the overall complex. Generally, obtaining detailed higher-order structural properties for such systems is difficult.
In this work, we hope to address this shortcoming using a dual approach by combining native mass spectrometry (native MS) with hydrogen-deuterium exchange mass spectrometry (HDX-MS) to delineate the overall structural and dynamical properties of "symmetric" oligomeric assemblies, using multimeric antibodies such as immunoglobulin M (IgM) as a case study. Using biochemical methods, we will produce a library of truncated IgM variants systematically missing portions of its oligomeric structure (e.g. one or more Fab's), which can be readily detected using native MS. Differences in HDX uptake behaviour between these systematic truncations are expected to provide direct information on the conformational and dynamic differences in this high-symmetry oligomeric system.

64. Yin Kwan Chung

SELF-CLEAVAGE OF THE GAIN DOMAIN OF ADHESION G PROTEIN-COUPLED RECEPTORS REQUIRES MULTIPLE DOMAIN-EXTRINSIC FACTORS

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Adhesion G-protein-coupled receptors (aGPCRs) have emerged as a novel class of biological mechanosensors implicated in numerous force-dependent processes. A significant part of the signalling profile of aGPCRs depends on activation by the encrypted tethered agonist generated by a cleavage event in the extracellular GPCR-autoproteolysis-inducing (GAIN) domain, a highly conserved domain across the aGPCR family. However, investigation of the physiological requirements of GAIN domain cleavage remain challenging due to the fast kinetic of the proteolysis process and the high dynamics of the domain. Here, using ADGRE2/EMR2 as a model aGPCR, we systematically analysed the impact of the seven-transmembrane (7TM) region of aGPCRs on GAIN domain cleavage. By series of truncations and domain replacements, we demonstrate that the 7TM region highly facilitates GAIN domain proteolysis during receptor biogenesis in two means: Firstly, by anchoring the GAIN domain to the lumenal side of the ER membrane; and secondly, by physically stabilising GAIN domain dynamics. The proximity of the GAIN domain to the lumenal side of the ER membrane is important for the proteolysis process of all tested aGPCRs, suggesting its general dependence. Using site-specific photo-crosslinking coupled to mass spectrometry, we discovered that the requirement for membrane proximity is attributed to the vicinity of the GAIN domain with the N-glycosylation machinery. Our findings will be a valuable contribution to understanding GAIN domain self-cleavage across the aGPCR family, and will instruct pharmacological strategies to modulate their activation and signalling.

65. Zhiyu Huang

SERIAL SYNCHROTRON X-RAY CRYSTALLOGRAPHY STUDIES OF LYTIC POLYSACCHARIDE MONOOXYGENASE

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Lytic polysaccharide monooxygenases (LPMOs) are enzymes copper-dependent enzymes that degrade polysaccharides oxidatively and used in second-generation bioethanol production and virulence factors in some pathogens. LPMOs are classified within the Auxiliary Activities (AA) family of CAZymes [1] and histidine brace is highly conserved. The mechanism of LPMOs reaction is highly complex, and it is now widely recognized that hydrogen peroxide serves as a co-substrate in facilitating the catalytic process [2]. However, further in-depth mechanistic investigations are required to elucidate the underlying details. Serial synchrotron X-ray crystallography (SSX) can potentially capture the dynamic action of LPMOs at room temperature in a time-resolved (TR) manner, offering insights into their catalytic mechanism at the molecular level. In this study, LsAA9A, derived from Lentinus similis and classified within the AA9 family, has served as an important model system due to its activity on soluble oligosaccharide substrates. SSX structural analysis of LsAA9A has recently been initiated. Microcrystals of LsAA9A were successfully produced and diffracted. A high-resolution SSX structure of LsAA9A was obtained at 1.55 Å. However, the substrate, Cellotriose, was not observed in the structure, which is inconsistent with the results from single-crystal X-ray cryo-crystallography. Prior to the TR-SSX studies, the reason must be elucidated, and appropriate conditions need to be found. This study provide a solid foundation for future time-resolved serial crystallography experiments on LPMOs.

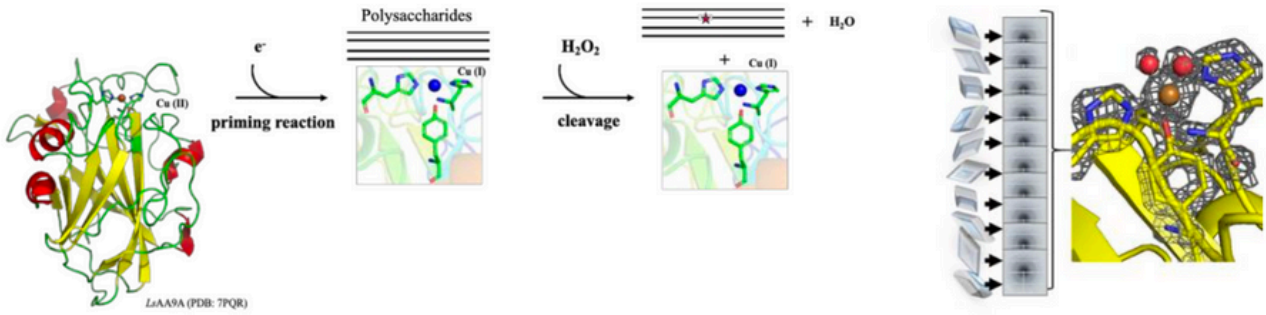

Figure: Left: the “priming reduction” initiates the catalytic cycle of LPMOs reaction. Right: illustration presenting the HALRIC project ‘Towards time-resolved studies of the LPMO reaction’. The structure shown is a preliminary SSX apo structure of LsAA9A from T-REXX data (Banerjee et al, unpublished).

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66. Zimeng Liu

SYSTEMATIC INVESTIGATION OF HIS PROTONATION STATES IN HIGH RESOLUTION X-RAY CRYSTALLOGRAPHY STRUCTURES IN THE PDB

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Histidine residues play a crucial role in biochemical reactions, particularly in proton transfer processes within enzymes. Although high-resolution X-ray crystallography provides detailed structural information, accurately determining histidine protonation states remains challenging due to limitations in electron density maps. To address this, we developed an automated pipeline that combines partially unrestrained refinement[1] and geometric parameter calculations.

High-quality protein structures from the PDB database were selected using strict quality criteria, and histidine residues suitable for analysis were then identified. To validate the predictions, several well-characterized protein groups were analyzed, which highlighted limitations in the linear discriminant analysis (LDA) method[2], prompting the development of an alternative classifier based on the random forest algorithm[3]. The results from both geometry-based methods were then compared with experimentally derived protonation states[4],[5] obtained from neutron crystallography and from estimates based on correlating crystallization pH with residue-specific pKa values determined by NMR spectroscopy. Despite strong overall concordance, certain discrepancies were observed, likely due to inaccuracies in reported crystallization pH. Additional diffraction analyses of lysozyme crystals grown under experimentally controlled pH conditions were conducted to clarify these issues.

Our work establishes a foundation for accurately assessing histidine protonation states, which is important for deepening the understanding of protein function.

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67. Zoë Fisher

DEMAX: THE DEUTERATION AND MACROMOLECULAR CRYSTALLIZATION SUPPORT LAB AT THE EUROPEAN SPALLATION SOURCE

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For small angle neutron scattering (SANS), neutron reflectometry (NR), and neutron protein crystallography (NPX), using deuterium-labeled (partial or fully deuterated) samples has numerous benefits. The molecules that are of most interest include proteins, DNA, lipids, fatty acids, small organic molecules, surfactants, aldehydes, and others. For SANS and NR, selective deuteration is used to enable contrast variation, allowing scientist to “match out” the scattering contribution from certain components of larger complexes. In NPX deuteration is used to boost weak signal-to-noise ratios, reduce the incoherent background due to hydrogen, improve neutron scattering length maps, and enable direct visualization of hydrogen bonds and solvent networks. As such, access to deuterated materials is essential for the neutron scattering scientific community. DEMAX is the ESS Deuteration and Macromolecular Crystallography support platform for users of the European Spallation Source (ESS) and it available to all users of ESS instruments. Support and services provided by DEMAX staff includes chemical deuteration, biological deuteration, and large protein crystal growth. Access to DEMAX support is managed through a peer-reviewed, proposal-based system and is currently free of charge to user upon acceptance. For more details or information, researchers can contact us at demax@ess.eu.

68. Céline Delaitre

FIRST SELECTIVE FLUORESCENT LIGANDS RECOGNIZING SELECTIVELY ANGIOTENSIN II RECEPTORS SUBTYPES

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GPCR play fundamental physiological roles in tissues. Once activated by external stimulus, they can trigger various signaling pathways. Developing better drugs requires a better understanding of the different signaling pathways and the development of tools acting selectively on one of the pathways. Here this is the challenge in the case of the ATRs which are GPCRs regulated by AngII: AT1R (angiotensin II type 1 receptor), and AT2R (angiotensin II type 2 receptor). Biased AT1 agonists like TRV027, which selectively activate β-arrestin signaling while blocking Gq pathways, offer promising therapeutic potential (Delaitre et al., 2021). To better explore their effects on cerebral vessels, we designed and synthesized novel fluorescent derivatives of AngII, TRV027, and losartan. Their chemical and pharmacological properties, including receptor selectivity, potency, and pathway-specific activation, were rigorously characterized in both cellular systems and isolated cerebral arteries. Detailed structure–activity relationship studies revealed the first highly AT1-selective fluorescent antagonist based on losartan, and analogues of the biased agonist TRV027 unexpectedly switching selectivity toward AT2 receptors. These chemically engineered ligands now serve as powerful molecular probes to visualize and study AT1 and AT2 receptors in vitro and ex vivo. Quantitative confocal microscopy further showed that losartan binds AT1 with higher affinity at elevated receptor densities (Delaitre et al., 2024). This work illustrates how targeted chemical modifications open new avenues to explore receptor structure, dynamics, and signaling.

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