NATIONAL HIGH MAGNETIC FIELD LABORATORY

The Symposium on Lipid Nanodiscs: Applications in Structural Biology and Drug Delivery

April 30-May 01, 2026

MagLab (NHMFL), Florida State University,
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Tallahassee, Florida 32310

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OVERVIEW

Symposium on Lipid Nanodiscs: Applications in Structural Biology and Drug Delivery Membrane proteins play central roles in cellular signaling, transport, and metabolism, and represent a major class of therapeutic targets. However, their intrinsic dependence on lipid bilayers has historically posed significant challenges for biochemical/biophysical characterization, high-resolution structural analysis, and translational applications. Lipid nanodiscs have emerged as a powerful and versatile platform to address these challenges, offering a native-like, soluble, and tunable membrane environment for membrane proteins.

This symposium will highlight recent advances in lipid nanodisc technology and explore their expanding applications in structural biology and drug delivery. Nanodiscs are self-assembled nanoscale lipid bilayers stabilized by amphipathic scaffold proteins, peptides, or synthetic polymers. They enable the reconstitution of membrane proteins in well-defined lipid environments. This has facilitated breakthroughs in cryo-electron microscopy, NMR spectroscopy, X-ray scattering, and other biophysical methods, allowing researchers to capture membrane proteins in functionally relevant conformational states.

A key focus of the symposium will be the use of nanodiscs in structural studies of receptors, ion channels, transporters, and multiprotein complexes. Speakers will discuss how lipid composition, bilayer size, and scaffold chemistry influence protein stability, dynamics, and activity, as well as how nanodiscs compare with alternative membrane mimetics such as detergents, liposomes, and bicelles. Emerging hybrid systems and next-generation nanodisc designs will also be presented, with emphasis on their compatibility with high-resolution and time-resolved techniques.

In addition to their impact on fundamental structural biology, lipid nanodiscs are gaining importance as platforms for drug discovery and delivery. Their ability to incorporate hydrophobic drugs, present membrane proteins in native conformations for ligand screening, and interface with biological systems makes them attractive tools for translational research. The symposium will feature discussions on nanodiscs as carriers for small molecules, peptides, and biologics, as well as their potential in targeted delivery.

By bringing together experts in biomedical engineering, biophysics, structural biology, chemistry, and pharmaceutical sciences, this symposium aims to foster interdisciplinary dialogue and collaboration. Participants will gain insight into the current state of the field, technical challenges, and future directions for lipid nanodiscs as enabling tools at the interface of basic research and therapeutic innovation.

ORGANIZING COMMITTEE

Chair

Professor (Rams) Ayyalusamy Ramamoorthy

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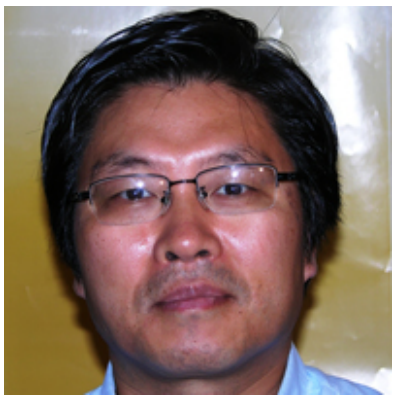
IMB, FSU



Dr. Sungsool Wi

CIMAR, NMR, NHMFL

SPEAKERS

Loren Andreas

Gottingen, Germany

Anirban Banerjee

Huan Bao

Moitrayee Bhattacharyya

Barry Bruce

Tennessee, Knoxville

Wayland Cheng
WashU Medicine

Debasis Das

Science, India

Matthew Eddy
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SPEAKERS

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Institute, San Diego

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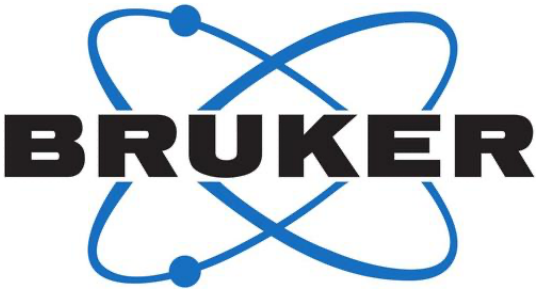
BRUKER

SCHEDULE

Day-1 April 30 (Thursday)
8:15-8:30 AM Meet & Greet
8:30 AM Opening Remarks by **Dr. Stacey Patterson**, FSU Vice President for Research

Session 1 Structural Biology of Membrane Proteins
Chair: (Rams) Ayyalusamy Ramamoorthy
8:35 AM **Franz Hagn**, Technical University of Munich, Germany, "Lipid nanodiscs as a versatile tool for high-resolution NMR and cryo-EM studies of membrane proteins"
9:00 AM **Huan Bao**, University of Virginia, "Designer nanodiscs to probe and reprogram membrane biology"
9:25 AM **Kimmo Rantalainen**, Scripps Research Institute, San Diego, "Virus glycoprotein nanodiscs in vaccine development"
9:50 AM **Wayland Cheng**, Washington University, "Scrutinizing the impact of lipid nanodiscs on the structure and dynamics of a ligand-gated ion channel"

10:15-10:35 AM Coffee Break

Session 2 Biomembranes
Chair: Prof. Elizabeth Stroupe, FSU
10:35 AM **Anirban Banerjee**, NIH, "In vitro Reconstitution of Protein Palmitoyltransferases in Nanodiscs"
11:00 AM **Kallol Gupta**, Yale School of Medicine, "Nanoscale Molecular Cartography of the Cellular Membranes"
11:25 AM **Matthew Eddy**, University of Florida, "How Lipids Modulate GPCR Activation: Insights from MSP Nanodisc Systems"
11:50 AM **John Hackett**, Florida International University, "Applications of small angle scattering to delineate P450-membrane and redox partner interactions"

12:15 AM – 12:45 PM Lunch Break

12:45 – 1:30 PM MagLab Tour by Ms. Malathy Elumalai, MagLab

Session 3 Biophysical Studies
Chair: Dr. Lissa Anderson, MagLab
1:30 PM **Jiansen Jiang**, NIH, "Molecular basis of transport and activity regulation of renal drug transporter OAT1"
1:55 PM **Brandon Ruotolo**, University of Michigan, "Mass Spectrometry Technologies for the Rapid Evaluation of Membrane Protein Structure and Stability"
2:20 PM **Michael Marty**, University of Texas Austin, "Design of Fluorescent Membrane Scaffold Proteins for Nanodiscs"
2:45 PM **Lissa Anderson**, ICR Facilities, NHMFL
2:55 PM **Frederic Mentink-Vigier**, Solid-State NMR Facilities, NHMFL
3:05 PM **Sungsool Wi**, "NMR of Nanodiscs and Bicelles", NHMFL

3:15 – 3:30 PM Coffee Break

Session 4 NMR Structural Studies of Membrane Proteins
Chair: Prof. Yan Yan Hu, FSU
3:30 PM **Clemens Glaubitz**, Goethe University, Frankfurt, Germany, "Studying Integral Membrane Proteins within Lipid Bilayers by Solid-state NMR"
3:55 PM **Loren Andreas**, Max Planck Institute, Gottingen, "A viroporin in Inulin-based nanodiscs"

4:20 PM **Birgit Habenstein**, University of Bordeaux, CNRS, “Assembly mechanisms of plant and bacterial proteins into membrane nanodomains”
4:45 PM **Gary Lorigan**, Miami University, “Comparative Biophysical Analysis of SMA, SMA Derivatives, and VEMA Nanodiscs for Membrane Protein Applications”
5:10 PM **Libin Ye**, University of South Florida, Tampa, “Trade-offs between MNG-3/CHS and Nanodiscs Reconstitutions in GPCR Function and Structure Studies”

5:35 – 7:30 PM **Poster Session, Chair: Dr. Sungsool Wi**

Dinner at the MagLab

Day-2 May 01, 2026 (Friday)

Session 5 Bicelles and Polymer Nanodiscs

Chair: Dr. Loren Andreas, Max Planck Institute, Gottingen

9:00 AM **John Katsaras**, ORNL, “An overview of bilayered micelles as studied by neutron scattering”
9:25 AM **Manuela Zoonens**, Université Paris Cité, CNRS, France, “Amphipols: Versatile tools for the purification and structural analysis of membrane proteins”
9:50 AM **Vladimir Raus**, Czech Academy of Sciences, “PIPOx: A Versatile Synthetic Platform for Designing Copolymers for Membrane Protein Isolation”
10:15 – 10:30 AM Coffee Break

Session 6 Nanodiscs and Lipid-Peptide Interface

Chair: Prof. Thai Thayumanavan, University of Massachusetts Amherst

10:30 AM **Gestél C. Kuyler**, Stellenbosch University, South Africa, “Expanding the Polymer Nanodisc Toolkit: Design and Architectural Expansion of the BzAM Series”
10:55 AM **Debasis Das**, Indian Institute of Science, Bengaluru, India, “Metallo-enzymatic Hydrocarbon Production”
11:20 AM **Yan Yan Hu**, FSU, “Structure and membrane interactions of Two Helices from Mycobacterium tuberculosis FtsL, One Transmembrane and One Amphipathic”
11:45 AM **Dongheon Lee**, FAMU-FSU College of Engineering, "Kinetic Monte Carlo simulation of hetero-multivalent interactions between protein and gangliosides on model membranes"

12:10 noon – 1:00 PM Lunch

Session 7 Drug Delivery and Challenging Membrane Systems

Chair: Dr. Vladimir Raus, Czech Academy of Sciences

1:00 PM **S. Thayumanavan**, University of Massachusetts Amherst, “Designer Materials for Intracellular Protein Delivery”
1 :25 PM **Birgit Strodel**, Heinrich Heine University of Düsseldorf, Germany, “Integrating MD Simulations with Nanodisc Technologies: Atomistic Insights into Lipid Binding and Membrane Protein Dynamics”
1:50 PM **Rituparna Samanta**, University of South Florida, Tampa, “Advancing membrane associated protein complex structure prediction and their design”
2:15 PM **Mandip Sachdeva**, FAMU, Exosomes based Drug Delivery

2:45 PM Poster Prizes & Closing Remarks

A viroporin in Inulin-based nanodiscs

*Abel Varkey, Mookyoung Han, Karin Giller, Stefan Becker, Loren Andreas**

Department of NMR-based Structural Biology, Max Planck Institute for Multidisciplinary Sciences

Abstract

The inulin-based nanodiscs system can solubilize membrane proteins and enables the reconstitution of functional interactions without interfering with electrostatic effects.[1] We explored the use of this nanodisc system to reconstitute a viroporin together with a selected members of a class of host proteins that interact with c-terminal residues of the viroporin. The interaction is screened by the only detergent we found that stabilizes the protein in solution, necessitating an alternative approach. We therefore explored different approaches to reconstitute the interaction, including preparations of liposomes and inulin-based nanodiscs. Initial results suggest that both liposomes and nanodiscs can be used to prepare the complex. Optimization of the reconstitution protocol required careful consideration of the stability of the host factors in preparations containing detergents, as well as accounting for the fate of detergent, which is difficult to remove from the final nanodisc preparation.

References

1. Krishnarjuna, B.; et al. *Anal. Chem.* **2022,** *94* (34), 11908–11915.

In vitro Reconstitution of Protein Palmitoyltransferases in Nanodiscs

Anirban Banerjee

NICHD, National Institutes of Health (NIH)

Abstract

Protein S-acylation, commonly known as protein palmitoylation, is the most common form of protein lipidation with more than 6000 substrates. Protein palmitoylation is catalyzed by the zDHHC family of 23 transmembrane enzymes in humans, that are localized in different organellar membranes as well as the plasma membrane.[1] Protein palmitoylation has been linked to an outstanding number of diseases including neurodegenerative disorders, cardiovascular diseases and various forms of cancer.[2,3] With 23 enzymes and thousands of substrates, the enzyme-substrate network of zDHHC enzymes is complex and very little is understood about the nature of zDHHC-substrate interactions. Even the extent to which zDHHC enzymes are selective for substrates has been questioned. I will describe our work on reconstitution of substrate palmitoylation by zDHHC enzymes in lipid nanodiscs. We have developed different assay modalities. With this and accompanying structural, biochemical and biophysical avenues of investigation, we are beginning to dissect the molecular mechanism of the zDHHC protein palmitoyltransferases.

References

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2. *Signal Transduct. Target Ther.* **2024,** *9* (1), 60.
3. *Nat. Rev. Cancer* **2024,** *24* (4), 240–260.
4. *J. Biol. Chem.* **2026,** *302* (3), 111194.
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Designer nanodiscs to probe and reprogram membrane biology

Huan Bao

Department of Molecular Physiology and Biological Physics, The University of Virginia

Abstract

Membrane scaffold protein-based nanodiscs have enabled unprecedented structural and biophysical analyses of membrane proteins in near-native lipid environments. Our work advances this field through the development of designer nanodiscs that can both probe and reprogram membrane biology. We first established a one-step SpyCatcher-SpyTag strategy for constructing circularized nanodiscs, providing a rapid and modular route to stable membrane mimetics with defined geometries. We next expanded this platform to circularized fluorescent nanodiscs, enabling direct visualization and quantitative analysis of protein-lipid interactions and membrane remodeling. Most recently, we developed DeFrND, a detergent-free method that uses engineered membrane scaffdold peptides to directly reconstitute membrane proteins into native nanodiscs while preserving endogenous lipids. Through systematic engineering and screening, we generated chemically modified apolipoprotein A-I mimetic peptides that enable highly efficient detergent-free nanodisc formation. These engineered nanodiscs support high-resolution single-particle cryo-EM of membrane proteins in native lipid environments and allow direct extraction of diverse membrane signaling proteins with their surrounding membranes for biochemical and biophysical studies. Together, these advances establish an integrated toolkit that bridges synthetic control with native membrane complexity and positions these nanodiscs as a powerful platform for mechanistic membrane biology and next-generation membrane-directed therapeutics.

References

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Nanoscale analysis of membrane proteins in neurodegeneration and pain

Gerard Walker[1], Shailesh Kumar[1], Harry Jung[1], Minh Triet Hong[1], Ariane Huang[1], Moitrayee Bhattacharyya[1]*

[1]Department of Pharmacology, Yale University School of Medicine

Abstract

My lab develops experimental platforms to resolve the molecular and functional organization of membrane proteins in their native environments and applies these tools to study membrane proteins that regulate neuronal signaling and neurodegeneration, chronic pain,[1] and cancer.[2] High-resolution quantitative measurements of the oligomeric organization of membrane proteins in native membranes, and how they change under different conditions, are indispensable for understanding membrane protein biology. However, this remains a challenging problem due to difficulties in preserving the native milieu while achieving nanoscale spatial and precise molecular resolution. This is further complicated by often inadequate sensitivity to analyze proteins at their natural levels of expression. Addressing these challenges, we reported a single-molecule technique, Native-nanoBleach,[3] to determine the oligomeric distribution of membrane proteins from native membranes at ~10 nm spatial resolution and at endogenous levels of expression. We applied Native-nanoBleach to quantify the oligomerization status of structurally and functionally diverse membrane proteins. In unpublished data, we have now extended this general technique to organellar membrane proteins as well as membrane contact sites in the context of neurodegeration and rare developmental diseases. Native-nanoBleach also permits easy adoption with access to commercial TIRF microscopes, without requiring any specialized hardware. This general experimental pipeline will usher in a new era of studying membrane protein organization in their native-membrane environments under various physiological and clinical conditions.

References (#co-first; *corresponding author/s)

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Unlocking Native Membrane Protein Dynamics Through Tunable Copolymer Design

Barry D. Bruce[1], F. Ali[2], G. Ochola[3], N. G. Brady[1], C. E. Workman[3], and B. K. Long[3]*

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Abstract
Membrane protein structure and function are tightly coupled to the surrounding lipid environment, making detergent-free nanodisc platforms increasingly important for capturing native states and dynamics. Styrene-maleic acid copolymers (SMAs) represented a major advance in this area, but their limited universality, variable protein selectivity, and chemical instability have underscored the need for more tunable and robust systems. Here, we describe a stepwise progression in copolymer design that has expanded access to increasingly native and dynamic membrane protein assemblies. Systematic functionalization of SMA with hydrophobic alkoxy ethoxylate sidechains revealed that solubilization efficiency (SE) for trimeric Photosystem I (PSI) from Thermosynechococcus elongatus depends strongly on both sidechain length and degree of substitution. SE increased sharply when more than 50% of maleic acid units were monoesterified, demonstrating that membrane protein extraction can be tuned through controlled modification of polymer hydrophobicity and architecture. These studies also revealed important chemical liabilities, including saponification under basic conditions and sensitivity to temperature, light, and small-molecule impurities, directly linking polymer structure and handling to extraction performance. These insights motivated the development of alpha-olefin-maleic acid copolymers (alphaMAs), which provide improved chemical robustness, tunable hydrophobicity, and enhanced extraction efficiency. These alphaMAs form proteolipid nanodiscs that retain extensive native lipid annuli and preserve near-native PSI activity, enabling access to functional states perturbed or lost during detergent isolation. Small-angle neutron scattering, lipidomics, and ultrafast spectroscopy show that these PSI nanodiscs preserve large native assemblies, retain substantial lipid content, and reveal functional heterogeneity, including previously obscured ultrafast charge separation pathways. Importantly, continued refinement of these copolymers has now enabled isolation of a unique tetrameric form of Photosystem I (Chroococcidiopsis TS-821), permitting cryo-EM analysis of this supercomplex in a highly dynamic, near-native membrane environment previously inaccessible to structural interrogation. Together, these studies establish tunable copolymer design as a powerful strategy for unlocking native membrane protein dynamics.

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Scrutinizing the impact of lipid nanodiscs on the structure and dynamics of a ligand-gated ion channel

*[1]Vikram Dalal, [1]Mark J. Arcario, [1]Wayland W. L. Cheng**

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Abstract

The potential impact of lipid nanodiscs on membrane protein structure and dynamics has not been well-scrutinized. We find that different types and sizes of lipid nanodiscs alter the structure of the pentameric ligand-gated ion channel, ELIC. Notably, copolymeric lipid nanoparticles, SMALPs, limit ligand activation of ELIC, while large, circularized MSP nanodiscs (i.e. 25 nm) significantly deform the structure of the protein.[1] We also find that ELIC preferentially associates with the rim of MSP nanodiscs. MD simulations suggest that contact of ELIC with the nanodisc scaffold significantly perturbs the conformation and dynamics of peripheral transmembrane helices. To further appraise the cryo-EM structures of ELIC, we determined resting, activated and desensitized structures of ELIC in liposomes where the function of the ion channel is confirmed.[2] The structures of ELIC in liposomes are generally less compact than in nanodiscs. Taken together, our results indicate that nanodiscs can significantly impact the structures of membrane proteins, and that copolymeric nanoparticles or large MSP nanodiscs may be particularly problematic for structural studies of pentameric ligand-gated ion channels.

References

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Metallo-enzymatic Hydrocarbon Production

Debasis Das

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Abstract

Biosynthetically produced 1-alkenes hold immense value as sustainable alternatives to fossil fuels and find widespread applications in polymer, lubricant, and detergent industries. UndB is the only known membrane enzyme capable of converting naturally abundant fatty acids to 1-alkenes. However, despite diverse applications, UndB remains poorly understood since its discovery nearly a decade ago. Here, we present insights into the molecular basis of UndB catalysis and the mechanism of UndB reaction at the membrane interface. We unravel UndB as a diiron-enzyme that utilizes a conserved histidine cluster at the active site. We decipher the dependency of UndB activity on molecular oxygen and electrons and identify the most efficient redox partners of UndB. We elucidate the catalytic intricacies of UndB and establish it as the most efficient decarboxylase in producing industrially valuable medium-chain 1-alkenes. Further, we engineered UndB, tremendously improved the enzyme's activity, and developed a novel whole-cell biocatalyst utilizing the engineering UndB for highly efficient conversion (up to 95%) of naturally abundant free fatty acids to 1-alkenes (both aliphatic and aromatic). These findings hold promise for the sustainable high-titre production of 1-alkenes with a multitude of biotechnological applications such as biofuels and commodity chemicals.

References

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How Lipids Modulate GPCR Activation: Insights from MSP Nanodisc Systems

Matthew Eddy

Department of Chemistry, University of Florida, Gainesville

Abstract

Biophysical and biological studies increasingly recognize membrane lipids as endogenous regulators of G protein-coupled receptor (GPCR) signaling, yet the structural mechanisms underlying these observations are incomplete. Lipid nanodiscs assembled using membrane scaffold proteins (MSPs), originally developed by the Sligar lab and subsequently re-engineered by Wagner, Hagn, and additional groups, provide a well-defined and tunable platform for dissecting the contributions of specific phospholipids and cholesterol to GPCR conformational equilibria. We present data from an integrative structural biology approach, centered around NMR spectroscopy, that define how lipids modulate GPCR conformational landscapes and regulate the formation of signaling complexes. We then describe on how insights obtained from this line of research have led us to develop NMR-based methodologies for quantifying drug efficacy that address key limitations of conventional cell-based pharmacological assays. Finally, we present results demonstrating how lipid nanodiscs formed from MSPs enable investigation of the spatial organization of lipids in nanodiscs and how this organization influences structure-activity relationships of GPCRs and likely other membrane proteins.

Studying Integral Membrane Proteins within Lipid Bilayers by Solid-state NMR

Clemens Glaubitz

Centre for Biomolecular Magnetic Resonance and Biophysical Chemistry
Goethe University Frankfurt, Germany

Abstract

The structure and function of integral membrane proteins are intrinsically coupled to their lipid environment. Selecting an appropriate membrane mimic therefore requires a delicate balance between preserving native protein properties and meeting experimental constraints. Here, we discuss the use of liposomes, nanodiscs, and styrene–maleic acid lipid particles (SMALPs) in the context of solid-state NMR spectroscopy, highlighting their suitability for specific applications. For lipid-transporting ABC transporters, such as those involved in intermembrane lipopolysaccharide (LPS) transfer in Gram-negative bacteria, liposomes are advantageous because they provide a sufficiently large reservoir for lipid substrates. LPS precursors are translocated across the inner membrane by the ABC transporter MsbA. Using solid-state NMR and dynamic nuclear polarization (DNP), we investigated the effects of inhibitors and substrates on MsbA, as well as the energy landscape of the in vitro assembled $LptB_2FGC$ complex, which connects the inner and outer membranes.[1,2] A comparative study of nanodiscs and liposomes was conducted on the photoreceptor proteorhodopsin. In this system, lipid order parameters and phase transitions are correlated with the functional photocycle, oligomeric state, and secondary structure of the protein.[3] An example illustrating the use of SMALPs is presented for the neurokinin 2 G protein–coupled receptor (GPCR),[4] demonstrating their applicability for studying membrane proteins in native-like lipid environments. Finally, we demonstrate the application of solid-state NMR to the analysis of lipid nanoparticles and cubic lipid phases, further expanding the range of membrane mimetics accessible to solid-state NMR.

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Nanoscale Molecular Cartography of the Cellular Membranes

Kallol Gupta[1,2]

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Abstract
Cellular membranes present a dynamic molecular landscape where proteins and lipids are organized into spatially restricted assemblies that together govern signaling, trafficking, and membrane remodeling. A central challenge in membrane biology is capturing these macromolecular ultrastructural organizations that are often highly dependent on the native lipid environment. Yet most analytical workflows rely on detergents as membrane mimetics that disrupt this membrane organization, thereby removing the local molecular contexts. Addressing this fundamental limitation, we present an integrated platform that combines membrane-active polymer (MAP)-based native nanodisc extraction, omics-scale profiling, and top-down native mass spectrometry directly from lipid membranes. Leveraging a library of synthetic MAPs and quantitative proteomics, we establish a proteome-scale platform that enables rapid extraction of membrane nanodomains surrounding endogenously expressed target membrane proteins, together with their spatially enriched lipid and protein milieu, directly from defined cellular organellar membranes. We complement this with a mass spectrometry-based omics workflow and native mass spectrometry of targeted systems to render a nanometer-scale spatially resolved, and quantitative molecular view of cellular membranes. Together, the work presents an experimental avenue that can open new opportunities to study how membrane composition, spatial organization, and local interactions collectively regulate membrane protein function in health and disease.

Assembly mechanisms of plant and bacterial proteins into membrane nanodomains

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Abstract

Plant-specific REMORINs (REMs) are crucial proteins involved in plant defense against viral propagation by regulating cell-to-cell connectivity. They are tightly associated with the clustering of nanodomains at the plasma membrane, driven by specific protein-protein and protein-lipid interactions. REMs can be classified into 6 groups, containing a membrane-associating C-terminal anchor (REM-CA), neighboring a coiled-coil domain that is followed by an intrinsically disordered N-terminal region (IDR). We have recently contributed to understanding the precise underlying mechanisms of nanodomain clustering by REMs, involving interactions of REM-CA with specific phosphatidylinositol phosphates (PIPs).[1,2] Moreover, our data have revealed that StREM1.3's nanodomain clustering depends on REM's oligomerization behavior,[3] and on the phosphorylation status in the IDR.[4] We further addressed the role of the structural divergence between the different REM homologous groups and found that REMs rely on diverse sequence motif arrangements and REM-CA sequences. We investigate REM's structural and dynamic organization based on domain-specific analysis and their positioning in the context of the three-domain protein. Bioinformatics analysis suggests implications of motif distribution in the regulation of nanodomain clustering. Based on 3D structure determination of REM-CAs of different REM groups by NMR, we discovered the REM-CA structural diversity, further highlighting the role of sequence adaptation and structure modulation to control membrane association and nanodomain formation.[5] On the other hand, chromosome segregation in Streptococcus pneumoniae requires RocS, a protein that links chromosomal DNA to the membrane. RocS binds DNA via an N-terminal helix-turn-helix motif, oligomerizes through a central coiled-coil domain, and anchors to the membrane via a short C-terminal motif. Unlike the well-characterized partitioning protein ParB, the structural and mechanistic basis of RocS function remains poorly understood. Its membrane association is essential for activity, and the short anchor resembles the conserved MinD membrane-targeting sequence, suggesting a shared strategy for protein localization at the division site. To dissect RocS membrane association, we employed a multiscale approach centered on its membrane-targeting sequence (MTS), with magic-angle spinning (MAS) and wide-line solid-state NMR (ssNMR) as the core techniques.[3] These NMR experiments revealed, at atomic resolution, that the anchor folds into a short kinked helix linked to a flexible segment, locally perturbing the lipid bilayer. Complementary AFM imaging showed that at the mesoscale, the MTS preferentially associates with lipid nanodomains, forming discrete clusters. In vivo, a single glycine mutation demonstrated that the MTS conformation directly controls membrane interaction and proper chromosome segregation. Together, these results highlight the power of NMR to resolve membrane–protein interactions and reveal a conserved mechanism for bacterial membrane targeting, with the kinked helix as a key structural element governing RocS localization and function.

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Applications of small angle scattering to delineate P450-membrane and redox partner interactions

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Abstract

Small-angle X-ray and neutron scattering (SAX/NS) are potent tools for elucidating the structures of biological macromolecules and their complexes. Although resolution is comparatively low compared to methods such as crystallography, electron microscopy, and NMR, SAX/NS offers the advantage of obtaining structural information from readily prepared, small samples without molecular size limitations. When combined with contemporary structure prediction tools and molecular dynamics simulations, SAX/NS becomes an invaluable instrument for investigating conformational ensembles of flexible molecules and the architectures of macromolecular complexes. In this presentation, I will describe the applications of SAX/NS and molecular simulation to pertinent cytochromes P450 and their redox partner interactions. Initially, I will describe how contrast variation SANS was integrated with a molecular dynamics flexible fitting approach to delineate the orientation and interactions of cytochrome P450 reductase with lipoprotein nanodiscs. Subsequently, I will present how SAXS, coupled with size exclusion chromatography, was employed in conjunction with artificial intelligence-based structural predictions and molecular dynamics simulations to derive a novel atomistic model of the interaction between CYP17A1 and cytochrome b5. I anticipate that these case studies will underscore the utility of combining small angle scattering with contemporary computational methods to advance our comprehension of membrane and redox partner interactions within P450 systems.

Lipid nanodiscs as a versatile tool for high-resolution NMR and cryo-EM studies of membrane proteins

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Abstract

This talk will present our recent work on membrane scaffold protein (MSP) lipid nanodiscs and their optimization for high-resolution solution-state NMR spectroscopy, including split-intein- or SortaseA-based segmental isotope labeling approaches. I will outline our strategy of employing size-tunable nanodiscs in the 6-10 nm diameter range that are well suited for both NMR and cryo-EM studies. In addition, I will highlight the advantages of circularized MSP nanodiscs for structural investigations of integral membrane proteins, including neurotensin receptor, a neuropeptide GPCR studied in our laboratory. I will then discuss how this platform has enabled structural studies of the voltage-dependent anion channel (VDAC), the principal gateway for metabolites across the mitochondrial outer membrane. VDAC oligomerization has been implicated in apoptosis triggered by diverse cellular stimuli. Using cryo-EM and NMR, we show that oligomerization of VDAC1 leads to exposure of its N-terminal α-helix (VDAC1-N). Complementary NMR and X-ray crystallography data demonstrate that VDAC1-N forms a complex with the BH3-binding groove of anti-apoptotic Bcl-xL. This mechanism resembles the action of BH3-only sensitizer proteins of the Bcl-2 family, which promote Bax/Bak-mediated mitochondrial outer membrane permeabilization and ultimately apoptosis. Finally, I will present the application of lipid nanodiscs to study tail-anchored inhibitory and pore-forming Bcl-2 family proteins in a native-like lipid environment using NMR, hydrogen-deuterium exchange mass spectrometry (HDX-MS), and other biophysical approaches. Overall, this work highlights the versatility and advantages of MSP-based lipid nanodiscs as a platform for structural and mechanistic studies of both peripheral and integral membrane proteins.

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Structure and membrane interactions of Two Helices from Mycobacterium tuberculosis FtsL, One Transmembrane and One Amphipathic

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Abstract

Many transmembrane (TM) proteins have an amphipathic helix. Amphipathic helices can sense lipid composition, stabilize membrane curvature, and form protein-protein interactions. Here, we combined solid-state NMR spectroscopy, molecular dynamics simulations, and other biophysical techniques to characterize the structure and membrane interaction of a minimal TM and amphipathic construct (residues 101-146) from Mycobacterium tuberculosis (Mtb) FtsL. Aminoacid-specific 15N-labeled oriented-sample NMR spectra in POPC:POPG (at 4:1 molar ratio)membranes uniquely defined the orientation of the TM helix (residues 124-144), including a 16° tilt, and constrained the amphipathic helix (residues 101-114) to a 90° tilt. To determine the helical rotation and membrane burial depth, we calculated the 2-dimensional free-energy surface using umbrella sampling simulations. The free energy surface contained a major minimum, with the sidechains of Leu104, Leu107, and Ile111 projected into the hydrophobic core of the membrane and those of Arg103, Arg107, and Arg114 projected sideways to interact with lipid headgroups. The structure of residues 101-114 was refined by restrained molecular dynamics simulations in a POPC:POPG bilayer. With pure POPC membranes, the free-energy barrier separating the membrane-bound state from the released state was significantly reduced, showing that electrostatic attraction between the Arg sidechains and the acidic POPG headgroup can keep the amphipathic helix membrane-bound. Together, these results demonstrate that the amphipathic helix of FtsL functions as a membrane-interacting element that stabilizes the protein in the membrane environment and facilitates its incorporation into the assembling divisome for Mtb cell division.

Molecular basis of transport and activity regulation of renal drug transporter OAT1

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Abstract

The solute carrier 22 (SLC22) family of transporters plays a vital role in the handling of both endogenous metabolites and therapeutic drugs, governing their absorption, distribution, and excretion. Within this family, organic anion transporter 1 (OAT1) is central to renal function, mediating the basolateral uptake of metabolic waste products and xenobiotics in proximal tubule epithelial cells. OAT1's broad substrate specificity underlies its clinical relevance in transporter-mediated drug interactions. Despite significant progress in OAT1 research, the structural basis of substrate recognition, transport, inhibition, and regulation has remained elusive. Here, we applied cryo-electron microscopy with the composite masking data processing to determine high-resolution structures of OAT1 bound to substrates and inhibitors. These structures capture distinct open conformations of the transporter and reveal that a conserved "π-sandwich" motif within the substrate pocket serves as a common binding site for diverse organic anions. Our analyses further elucidate the molecular mechanism of organic anion exchange and, unexpectedly, identify a chloride-binding site spatially distant from the substrate pocket. Complementary mutagenesis, transport assays, and molecular dynamics simulations support a novel mode of OAT1 regulation by chloride ions. Together, these findings provide critical mechanistic insights into OAT1 function and highlight its importance in drug disposition and transporter-mediated drug interactions.

An overview of bilayered micelles as studied by neutron scattering

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Abstract
Over the past two decades, bilayered micelles, or so-called bicelles composed of long- (e.g., dimyristoyl phosphatidylcholine) and short-chain (e.g., dihexanoyl PC) lipids have emerged as a powerful platform for studying membrane associated macromolecules. Depending on temperature, concentration, and lipid composition, these lipid mixtures can assume a variety of morphologies, some of which when doped with paramagnetic cations, such as thulium, can be aligned in the presence of a magnetic field. Because bilayered micelles are rich in protium and the fact that neutrons scatter very differently from protium (most common stable isotope of hydrogen) versus deuterium (another stable hydrogen isotope), neutrons have been used to elucidate the structure of different composition bicelle mixtures. In today's seminar I will go through a range of bilayered micelle examples where neutrons have been used to great effect.

Expanding the Polymer Nanodisc Toolkit: Design and Architectural Expansion of the BzAM Series

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Abstract

The development of polymer-based nanodiscs remains largely empirical, with limited predictive frameworks linking polymer structure to membrane solubilization and functional performance.[1] Here, we present the poly(styrene-co-maleic acid-co-(N-benzyl)maleimide) (BzAM) series as a modular and tunable platform for the design of polymer-based nanodiscs.[2] Synthesized via RAFT-mediated polymerization, these terpolymers provide precise control over composition, molecular weight, and architecture. Systematic variation within the series reveals that small changes in polymer structural features lead to notable effects on solubilization efficiency and nanodisc properties, establishing BzAM as a platform for interrogating polymer structure–function relationships. We further demonstrate the applicability of selected BzAM polymers for the extraction and functional stabilization of pharmacologically relevant G protein-coupled receptors (GPCRs), with preserved activity confirmed using resonance energy transfer–based assays. To expand the accessible design space, we introduce block copolymer architectures that enhance colloidal stability and enable nanodisc formation under a broader range of experimental conditions and supporting applications in structural biology. Finally, by integrating computational and experimental approaches, we investigate the role of polymer backbone flexibility in membrane solubilization, linking polymer composition to hydrophobicity, chain dynamics, and protein extraction efficiency. Together, this work expands the polymer nanodisc toolkit and demonstrates how controlled polymerization strategies enable systematic exploration of polymer design and architecture for next-generation membrane protein studies.

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Comparative Biophysical Analysis of SMA, SMA Derivatives, and VEMA Nanodiscs for Membrane Protein Applications

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Abstract

The application of styrene maleic acid copolymer (SMA) for the reconstitution of membrane proteins has seen significant growth in recent years. SMA incorporates into the membrane and self-assembles into small discs of bilayer encased by polymer, known as SMA lipid nanoparticles (SMALPs). This approach facilitates the biophysical characterization of lipid bilayers and membrane proteins with greater ease. While SMALPs present several advantages over traditional detergent methods, they also have limitations, particularly their sensitivity to low pH, divalent cations, and the interference of the styrene ring with UV measurements. To tackle these challenges, we have developed SMA derivatives: neutrally charged SMA-Neut, positively charged SMA-Pos, and negatively charged SMA-AE, which are more stable at low pH, and less sensitive to divalent cations. In this study, the charge properties of these SMA derivatives affect model lipid bilayers used for encapsulating membrane proteins, which may also possess charge are explored. To evaluate their impact on bilayers, we employed model membranes consisting of zwitterionic 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), anionic 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG), and a 9:1 molar ratio mixture of POPC and POPG. We utilized techniques such as dynamic light scattering (DLS), continuous wave electron paramagnetic resonance (CW-EPR) spectroscopy, and solid-state nuclear magnetic resonance (ssNMR) spectroscopy to characterize the interactions between the polymers and lipid bilayers. CW-EPR spectroscopy results indicated that the membrane dynamics of the 9:1 POPC-POPG system was better preserved by the SMA derivatives compared to the other lipid systems. Additionally, we developed a novel aliphatic copolymer known as VEMA (Vinyl Ether Maleic Acid) to mitigate the effects of the SMA styrene ring. The biophysical properties of both VEMA and SMA were compared using model membranes composed of POPC and DMPC lipids. Our results show that VEMA successfully solubilizes lipid membranes to form nanodiscs called VEMA lipid particles (VEMALPs).

Design of Fluorescent Membrane Scaffold Proteins for Nanodiscs

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Abstract

Nanodiscs are nanoscale lipid bilayer membrane memetics surrounded by two membrane scaffold proteins (MSP). They are widely used as soluble cassettes for membrane proteins and lipids in diverse applications. The original MSP1 was derived directly from human apolipoprotein A-1, and novel constructs have been adapted from this original design, including nanodiscs with larger sizes and covalent circularization. Here, we developed MSPs with a range of different fluorescent C-terminal protein tags, including a versatile HaloTag fusion. These fluorescent MSP were purified following typical MSP purification procedures with similar yield. Then, we demonstrate that fluorescent MSPs form nanodiscs with similar structure and stoichiometry to conventional MSP nanodiscs. These fluorescent MSP constructs enable a range of different applications of nanodiscs and provide a versatile template for future design of nanodiscs with unique functions.

Virus glycoprotein nanodiscs in vaccine development

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Abstract

Transmembrane glycoproteins of enveloped viruses are key targets of neutralizing antibodies and serve as essential vaccine antigens. However, their biophysical characterization and the in vitro analysis of post-immunization antibody responses typically rely on soluble recombinant proteins. These soluble mimics may not accurately reflect all properties of the immunogens administered—such as those delivered via mRNA–LNP platform encoding transmembrane vaccine designs—and may entirely lack critical epitopes. One such epitope is the membrane-proximal external region (MPER) of HIV Env, which is truncated in soluble HIV Env constructs but is targeted by antibodies exhibiting the broadest known neutralization breadth across HIV strains. The MPER epitope comprises glycan, lipid, and amino acid components and exhibits exceptional complexity and structural flexibility. This complexity, combined with the restricted accessibility of the epitope on the membrane surface, has posed a significant challenge for HIV vaccine development. Here, we present a methodological platform for assembling transmembrane glycoprotein vaccine candidates into lipid nanodiscs with reproducibility and throughput suitable for key vaccine development applications. We demonstrate the utility of this platform in antibody-binding assays using three distinct surface plasmon resonance (SPR) modalities, ex vivo B-cell sorting via fluorescence-activated cell sorting (FACS), and structural determination of a prototypical HIV MPER-targeting immunogen nanodisc in complex with three broadly neutralizing antibodies (bnAbs)—including one targeting the MPER—at 3.5 Å resolution using cryogenic electron microscopy (cryo-EM). Furthermore, we highlight the general applicability of the approach by assembling glycoproteins from COVID-19, Influenza, and Ebola using identical protocols. These approaches provide new templates for structure-based immunogen design and enable characterization of antibody responses to transmembrane glycoproteins in a more native lipid environment. Overall, this platform offers a powerful tool for accelerating the development of next-generation viral vaccines.

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PIPOx: A Versatile Synthetic Platform for Designing Copolymers for Membrane Protein Isolation

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Abstract

Post-polymerization modification of poly(2-isopropenyl-2-oxazoline) (PIPOx) with carboxylic acids, thiols, and amines has recently emerged as a versatile strategy for the precision synthesis of functional polymethacrylamides with broad applicability.[1,2,3] The simplicity, efficiency, and orthogonality of this modification strategy, combined with the easy control of PIPOx molecular weight, makes it an extremely powerful tool for generating libraries of precisely tailored low-molecular weight, amphiphilic copolymers that can potentially facilitate self-assembly of lipid bilayers and enable the reconstitution of membrane proteins in well-defined lipid environments. In this contribution, the superiority of the PIPOx strategy as compared to other synthetic approaches will be highlighted. Further, initial results on designing PIPOx-based copolymers suitable for solubilization of cell membranes and their models (DMPC vesicles) will be presented, including solid-state NMR results showing the nanodisc formation and magnetic alignment. Finally, the huge potential of the PIPOx platform in the polymer nanodisc field will be discussed.

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Mass Spectrometry Technologies for the Rapid Evaluation of Membrane Protein Structure and Stability

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Abstract

Medicines of the future will rely heavily upon our ability to quickly assess the structures and stabilities of complex biomolecules and evaluate the influence of large libraries of conformationally-selective small molecule binders in the context of their native environment. This is especially true for membrane proteins, which act as the gatekeepers of cells and thus account for most drug targets. Current structural biology tools lack the throughput and sensitivity to meet these challenges. In this presentation, I will discuss recent developments surrounding collision induced unfolding (CIU) methods that aim to bridge this technology gap. CIU uses ion mobility-mass spectrometry (IM-MS) to measure the stability and unfolding pathways of gas-phase membrane proteins, without the need for covalent labels or tagging, and consumes 10-100 times less sample than almost any other label-free technology. Recent developments in using an array of membrane mimetics, including lipid nanodiscs and vesicles, will be discussed.

Advancing membrane associated protein complex structure prediction and their design

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Abstract

The oligomerization of protein macromolecules on cell surfaces plays a fundamental role in regulating cellular function, including signal transduction and the immune response. Despite their importance, membrane proteins (MPs) represent only 2% of all protein structures in the protein data bank (PDB), and their complexes are even scarcer. Computational modeling provides a promising alternative to model MP interfaces and predict protein complex structures. Here, we present RosettaMPDock,[1] a flexible transmembrane protein docking protocol that captures binding induced conformational changes. RosettaMPDock is benchmarked on 30 transmembrane-protein complexes of variable flexibility dataset from PDB. RosettaMPDock successfully predicts the correct interface for 67% of moderately flexible targets (unbound-bound backbone motion within 1.5-2.5Å) and 60% of the highly flexible targets (unbound-bound backbone motion greater than 2.5Å), a substantial improvement from the existing membrane protein docking methods, including both physics-based methods as well as deeplearning based structure prediction models. We have also developed a hybrid protocol that refines AlphaFold predicted complex structures with RosettaMPDock and further improves the prediction success rates from 64% to 73%. We have further applied these methods in two case studies: (1) Investigate the interaction between Tim23 (essential component of the mitochondrial inner membrane translocase) and Sfc1 (a succinate-fumarate transporter across the inner membrane) to elucidate a mechanism of metabolic transport regulates protein import across mitochondria inner membrane.[2] (2) Study the role of transmembrane domain in receptor tyrosine kinase (RTK) dimerization across different families.

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Integrating MD Simulations with Nanodisc Technologies: Atomistic Insights into Lipid Binding and Membrane Protein Dynamics

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Abstract

High-resolution structural techniques, such as cryo-EM and NMR spectroscopy, have significantly advanced our understanding of membrane proteins within the near-native environment of nanodiscs. However, capturing the full complexity of the lipid-protein interface remains a challenge, as boundary lipids often exhibit high mobility or transient binding modes that escape traditional structural characterization. In this talk, I will demonstrate how molecular dynamics (MD) simulations, performed in representative lipid bilayer environments, serve as a powerful lens to interpret and augment experimental data from nanodisc studies. By sampling timescales from the nanosecond to the microsecond regime, we can provide an atomistic description of the "solvent" environment provided by the membrane. I will present case studies where MD simulations—ranging from coarse-grained to all-atom resolutions—have been utilized to identify specific lipid-binding sites and characterize the residence times of individual lipid species. These computational insights are directly compared with experimental observables, ultimately showing that the synergy between simulation and nanodisc-based experiments is essential for uncovering the hidden dynamics of the protein-membrane interface.

Designer Materials for Intracellular Protein Delivery

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Abstract

Direct use of proteins, to address a specific deficiency or excess, has the promise to mitigate the off-target effects of typical drug molecules. The show-stopper here is that these therapeutic proteins are not accessible for on-target delivery. Most current approaches are restricted to delivery of extracellular proteins (e.g. insulin). This leaves behind a rather large number of pathologically important intracellular proteins. We report a novel lipids and polymer self-assembly strategy that addresses key shortcomings of prior approaches. Here, the protein itself acts as the template for the polymer to self-assemble around it or the lipids are programmed to dynamically exchange with the cell membrane to be transported across the cell. Complementary approaches taken in this regard will also be discussed.

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Trade-offs between MNG-3/CHS and Nanodiscs Reconstitutions in GPCR Function and Structure Studies

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Abstract

Membrane proteins are targeted by over 40% of FDA-approved medications and remain central to drug development. Detergent MNG-3/CHS and MSP/phospholipid-based nanodisc systems are two main reconstitution systems used to study the structure and function of membrane proteins, including G protein-coupled receptors (GPCRs). However, there is ongoing debate about which system is more effective, with nanodiscs often favored because their names and structures more closely resemble the physiological environment. Using two classic GPCRs, the β2 adrenergic receptor (β_2AR) and the adenosine A2A receptor (A_{2A}R), as examples, and combining 19F-NMR to analyze conformational landscapes and pharmacological assessments, the data showed that both systems have advantages and limitations. We suggest that researchers choose the system based on their specific goals: MNG-3/CHS is better for capturing the receptor's conformational equilibrium with a rational population distribution of substates, which is more aligned with pharmacological output, while nanodiscs are more appropriate for examining fully activated GPCR-G protein complexes and the effects of lipids on receptor function, as they tend to overpopulate the fully activated state in both receptors. Additionally, the scaffold protein might affect NMR chemical shifts in the nanodisc system, whereas MNG-3/CHS does not. This underscores the need to develop a more physiologically relevant nanodisc or other reconstitution system to better understand membrane protein structure and function.

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Amphipols: Versatile tools for the purification and structural analysis of membrane proteins

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Abstract

Over the past three decades, numerous amphipathic scaffolds have been developed to counteract the post-purification destabilization of membrane proteins (MPs) caused by early generation detergents and to provide more membrane-like environments. These scaffolds are now widely used in structural biology, particularly following breakthroughs in cryo-electron microscopy (cryo-EM). By analyzing all MP cryo-EM structures solved at resolutions better than 3 Å, we have recently provided a survey highlighting current trends in scaffold usage.[1] Among these scaffolds, amphipathic polymers have undergone significant expansion. Amphipols (APols) were the first polymers specifically designed to stabilize MPs in aqueous solution,[2] paving the way for the development of subsequent polymers, including the widely used styrene maleic acid (SMA) copolymers. The term "amphipols" derives from the first two syllables of "amphipathic polymers". The prototypical APol, A8-35, is typically used as a post-purification environment, replacing detergents at the transmembrane surfaces once MPs have been purified. We recently showed that its physicochemical properties, distinct from those of the most widely used detergent that is DDM, can be leveraged to optimize cryo-EM grid preparation, particularly with respect to ice thickness and particle orientation.[5] In parallel, polymer development has expanded their use to membrane solubilization prior to MP purification. In this context, we have shown that replacing linear acyl chains by cyclic, hydrophobic groups yields APols–hereafter referred to as CyclAPols–with enhanced membrane-solubilization efficiency while preserving MP integrity.[3,4] We also demonstrated that protein-lipid interactions are maintained during solubilization and purification with CyclAPols, enabling the analysis of the native lipid environment surrounding MPs.

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POSTERS

Quantitative determination of GPCR drug efficacy with ^{19}F-NMR

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Abstract

G protein-coupled receptors (GPCRs) are membrane sensory proteins and targets for more than one-third of all FDA-approved drugs. GPCR drug efficacy is defined as the extent to which a bound ligand activates the GPCR and promotes formation of signaling complexes with intracellular partner proteins. Current methods for measuring ligand efficacy rely primarily on cell-based assays. These approaches typically depend on indirect readouts of receptor activity and can be influenced by variability in transfection efficiency, differences among cell lines, and other factors that complicate accurate and quantitative assessment of drug efficacy. Limitations in cell-based efficacy assays can also observe differences between antagonists, which do not alter receptor basal activity, and inverse agonists, which lower receptor activity from the basal state. In the present work, we show that an improved understanding of GPCR–lipid interactions enabled the development of a method for quantifying drug efficacy using ^{19}F NMR of the A_{2A} adenosine receptor (A_{2A}AR) reconstituted in lipid nanodiscs. Our prior research systematically studying the impact of phospholipids on GPCR activity and conformational equilibria identified experimental conditions and lipid compositions[1] that led to ^{19}F-NMR measurements that quantitatively matched drug efficacies determined from cell-based cAMP accumulation assays.[2] In the present study, we applied these conditions to systematically quantify drug efficacies for a small library of compounds with a wide predicted range of drug efficacies. Ligand selection includes multiple compounds of clinical interest for Parkinson's disease and cancer. We show how ^{19}F-NMR provides a highly sensitive readout that enables us to differentiate between antagonists and inverse agonists among these clinically relevant compounds, providing insights into the molecular structures of molecules that may be therapeutically more beneficial. Our method also enabled differentiation among different degrees of activation, establishing new structure-activity relationships for compounds that activate A_{2A}AR at levels below the maximum signaling output. These data thus provide interesting design criteria for the development of novel compounds with predictable pharmacological properties.

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Ganglioside Lipids Modulate Insulin Amyloid Aggregation Pathways and Reduce Cytotoxicity Through Structural Remodeling

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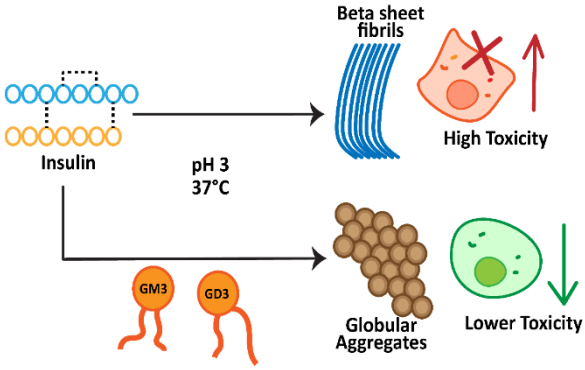
Abstract:

Insulin aggregation is a major pathological and pharmaceutical concern in Type 2 Diabetes (T2D), where amyloid deposition can reduce therapeutic efficacy and trigger β-cell death, leading to local tissue damage. Anionic lipids are known to modulate the amyloidogenic pathway of insulin. Although gangliosides, glycosphingolipids containing sialic acid residues, are also known to modulate amyloid formation in neurodegenerative diseases, their influence on insulin aggregation remains largely unknown. We investigated the effects of gangliosides GM3 and GD3 on insulin aggregation using Thioflavin-T (ThT) fluorescence kinetics, Fourier Transform Infrared (FTIR) spectroscopy, Circular Dichroism (CD), Small-Angle X-ray Scattering (SAXS), Nuclear Magnetic Resonance (NMR) spectroscopy, and Transmission Electron Microscopy (TEM) and cytotoxicity assays. Results show that both GM3 and GD3 accelerated insulin aggregation in a concentration-dependent manner but diverted it from classical fibrillation, yielding short, beaded structures rather than extended fibrils. FTIR and CD analyses revealed distinct non-fibrillar intermediates: β-sheet-rich globular clusters for GD3 and α-helical intermediates for GM3. NMR experiments under low-salt conditions showed rapid insulin precipitation upon exposure to GM3/GD3, accompanied by signal loss in 1H NMR signal intensity, indicating strong electrostatic interactions between positively charged insulin and negatively charged GM3/GD3 micelles. No GM3/GD3 resonances were detected, consistent with the formation of large micelles or co-precipitates. Cytotoxicity assays demonstrated that ganglioside-induced aggregates are markedly less toxic than insulin-only fibrils, yet these species retain seeding capacity. In conclusion, gangliosides modulate insulin amyloid polymorphism through charged, lipid-mediated interactions that favor off-pathway aggregation and reduce toxicity. These findings highlight ganglioside-specific molecular mechanisms and provide new insights into lipid-regulated amyloidogenesis relevant to T2D pathology and therapeutic formulation design.



Characterization of Divisome Membrane Protein FtsB from *Mycobacterium tuberculosis*

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Abstract

Mycobacterium tuberculosis (*Mtb)* is the microorganism responsible for tuberculosis (TB), the leading cause of death from an infectious agent.[1] Due to the existence of multidrug-resistant TB (MDR-TB), there is increasing interest in developing novel therapeutics that target the divisome, the cell division machinery of *Mtb*. FtsB, along with FtsQ and FtsL, forms the QBL complex within the divisome and contributes to the initiation of septation. These three membrane proteins interact via their transmembrane (TM) helices, and their assembly is essential to the cascading recruitment of the other divisome proteins to the bacterium division site. By characterizing the structure and functional interfaces of the QBL proteins, we can identify potential drug targets to disrupt *Mtb* division and slow down the progression of latent TB. Two protein truncations, FtsB(76-106) and FtsB(1-82), were created to characterize the TM and cytoplasmic domains of FtsB, respectively. The tilt and rotation angle of the FtsB TM α-helix has been determined in a native-like membrane environment using oriented-sample solid-state nuclear magnetic resonance (OS ssNMR). FtsB(1-82) is water-soluble and a fully intrinsically disordered protein (IDP) in solution, but an amphipathic helix forms within the IDP when it interacts with acidic lipids that mimic the interior of the *Mtb* cell membrane. Our circular dichroism (CD) data, solution-state NMR data, and biochemical assays reveal that FtsB(1-82) strongly binds to acidic lipids (e.g., POPG) but does not strongly bind to neutral lipids (e.g., POPC). Our results provide insight into the interactions of FtsB with its membrane environment and will guide our future studies on characterizing the interactions between FtsB and other divisome proteins.

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Saponin based Micelles and Nanodiscs Inhibit Amyloid Aggregation and Solubilize Amyloid Fibrils

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Abstract

Amyloidogenic proteins are key targets for study due to their involvement in debilitating diseases such as Alzheimer's and Type-2 Diabetes. One area of importance is their mechanisms of aggregation as oligomers, which are present prior to fibril deposition, are highly toxic but are incredibly difficult to study due to their heterogeneous, transient and highly dynamic nature. Lipid nanodiscs have been shown to stabilize membrane bound amyloid peptide conformations which allows for structural characterization.[1] Saponins are a class of amphipathic glycosides which are commonly found as plant metabolites and have been shown to form nonionic lipid nanodiscs.[2] With their ability to form nanodiscs, their usage in medicine such as adjuvant QS-21, inhibition of amyloid aggregation,[3,4] and reduction of amyloid cytotoxicity,[5,6] we sought to characterize their interaction with the amyloidogenic proteins Amyloid-β (Aβ) and Islet Amyloid Polypeptide (IAPP or Amylin). Through biophysical characterization using ThT, CD, and TEM, we showcase saponins to be effective inhibitors of Aβ and IAPP aggregation while above their critical micelle concentration (CMCs) or when incorporated into DMPC to form nanodiscs. We were also able to demonstrate the loss of ThT Fluorescence with preformed fibrils upon addition of saponin, indicating their ability to solubilize amyloid fibrils and potentially reduce amyloid plaque burden. These findings point toward the potential for saponins as therapeutics leads for reducing plaque burden, potential formulations with existing inhibitors, or as self-assembling nanocarriers for drug delivery.

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Highly Aligned Peptoid Macrodiscs at Various Lipid Compositions

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Abstract

Self-assembled lipid bilayers such as bicelles, peptide belt- and polymer-based lipodiscs can be efficiently used for the structural characterization of membrane proteins in their near-native environments. We have been developing amphipathic, purely synthetic peptoid belts which yield uniform lipid macrodiscs of ca. 30 nm in diameter. Peptoids are essentially polyglycines whose amide hydrogens are replaced by functionalized groups. Here we report on an exceptionally high degree of the magnetic alignment for the macrodiscs formed by peptoids composed of alternating hydrophobic and hydrophilic moieties. Record narrow ^{31}P NMR linewidths of just a few 10's of Hz are observed for mixtures of DMPC with the negatively charged DMPA or DMPG lipids at a 7 T magnetic field. Mixed-composition macrodiscs consisting of DPPC and POPC, as well with cholesterol also exhibit a high degree of magnetic ordering. Moreover, we demonstrate that self-assembling macrodiscs can be formed not only with longer 15-mer peptoids but also with the much shorter 8- and 10-mer constructs, which facilitates their mixing with the lipids often by a simple incubation. Such a control over the disc composition may allow one to study lipid-associated conformational changes of membrane proteins by solid-state NMR and Cryo-EM.

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NMR Spectroscopy of Protein in Emulsions

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Abstract

Protein behavior at fluid interfaces plays an important role in emulsion stability in food and pharmaceutical systems, yet direct measurement of protein diffusion under interfacial confinement remains challenging. Here, we use a broad range of methods including diffusion NMR, circular Dichroism (CD), differential scanning calorimetry (DSC) to assess how protein structure and mobility is affected by the adsorption to oil-water interface. We accomplish this by considering protein behavior in oil-water emulsion. Using lysozyme as a model protein in phosphate buffer, we first established a solution baseline and examined the effect of high-shear homogenization on protein structure and diffusion. Intact and sheared lysozyme exhibited similar diffusion coefficients of 1.0 × 10−10m2/s. Circular dichroism also confirmed retention of α-helical secondary structure at 75-80%, indicating that high-shear processing preserved both protein mobility and native conformation. In contrast, thermally denatured lysozyme diffused about three times faster, with a diffusion coefficient of 3.0 × 10−10m2/s, consistent with substantial structural expansion relative to the native protein. Across the 5 - 0.5 wt% protein concentration range, lysozyme-stabilized high internal phase emulsions (HIPEs) exhibited droplet sizes of about 4 – 7 μm. Diffusion ordered spectroscopy (DOSY) NMR of the HIPEs revealed an apparent diffusion coefficient of 3.5 × 10^{-11} m²/s of the lysozyme protein in HIPEs. This was about three times lower than in bulk solution, consistent with restricted protein mobility under interfacial confinement. Following freeze-thaw cycling, protein recovered from HIPEs exhibited solution-like diffusion coefficients. Circular dichroism again confirmed retention of α-helical secondary structure at 75- 80%. These results suggest that interfacial confinement under these conditions did not cause major structural disruption of the protein. This work establishes high-field DOSY-NMR as a quantitative, noninvasive tool for probing protein mobility in protein-stabilized emulsions, with relevance to interfacial characterization and formulation stability in pharmaceutical and food systems for lysozyme in the HIPEs

Saponin Nanodiscs for NMR studies, membrane protein reconstitution, and future drug delivery applications

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Abstract

Lipid nanodiscs are useful tools for drug delivery and membrane protein research because they are biocompatible, flexible, and closely mimic natural lipid bilayers.[1,2] These small structures, made of lipid bilayers held together by scaffold proteins, peptides, synthetic polymers, or saponins, can carry hydrophobic drugs and membrane proteins.[3,4] They also allow for adjustments in size, surface properties, and lipid exchange. These features make nanodiscs good candidates for targeted drug delivery, controlled release, and use in cancer therapy, antimicrobial treatments, and vaccines.[5] Saponins stand out among stabilizers because they are natural, detergent-free amphiphiles that can form bicelles suitable for NMR studies.[3] In this talk, we examine how crude and purified saponins, when mixed with DMPC, facilitate lipid dissolution, form nanodiscs, and achieve magnetic alignment.[1] Since anionic lipids are common in biological membranes, we also studied how saponin-based nanodiscs with both zwitterionic and anionic lipids form, stay stable, and align magnetically. We tested binary and ternary mixtures of glycyrrhizic acid (GA), hederacoside C (HC), and crude Quillaja saponins (CQS) with DMPC and the anionic lipid DMPG using optical transmittance and temperature-dependent ^{31}P solid-state NMR. We also examined how divalent cations (such as Ca^{2+}, Mg^{2+}) and lanthanide ions (Tm^{3+}) affect bicelle alignment. Our results show that saponin-based nanodiscs can include anionic lipids and still align magnetically under certain conditions. Their distinct responses to biologically important ions indicate that they are tunable and stable. This work demonstrates that natural saponins can substitute for synthetic polymers or MSPs to produce magnetically alignable nanodiscs for NMR, membrane protein studies, and advanced drug delivery.

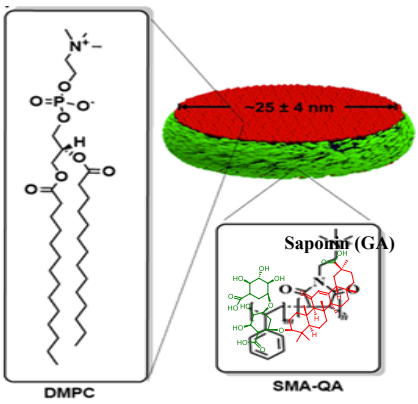

Figure 1. Schematic representation of the formation of Nanodisc from DMPC and Saponins.[4]

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Cholesterol Dependent Optimization of DMPC-Glycyrrhizic Acid (GA) Bicelles Alignment and Structure Revealed by ^{31}P and ^{14}N Solid-State NMR

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Abstract

Bicelles are widely used membrane mimetics for structural studies of membrane-associated systems; however, their formation, alignment, and stability strongly depend on lipid composition and additives such as cholesterol.[1-5] In this study, we systematically investigate the role of cholesterol in regulating the structural organization of DMPC + 0.2 glycyrrhizic acid (GA) bicelles using complementary ^{31}P and ^{14}N solid-state NMR spectroscopy combined with quantitative spectral simulations. The ^{31}P NMR results reveal a temperature-dependent transition from multilamellar vesicles to magnetically aligned bicelles, characterized by the appearance of a narrow resonance near −10 ppm, consistent with established bicelle alignment behavior.[1,2,3] In parallel, ^{14}N NMR provides direct insight into phosphatidylcholine headgroup ordering and enables quantitative determination of aligned bicelles, small bicelles, and isotropic lipid populations.[2] Together, these techniques establish a direct relationship between global membrane alignment and local molecular ordering. Cholesterol is found to exert a non-linear effect on bicelle formation. In the absence of cholesterol, bicelles form but exhibit reduced stability. At higher cholesterol concentrations (≥15–25 mol%), increased membrane rigidity delays alignment and introduces structural heterogeneity.[4,5] In contrast, at 10 mol% cholesterol, bicelles display optimal behavior, including rapid alignment onset, highly aligned bicelle populations (~75–80%), minimal isotropic contributions, and stable performance across a wide temperature range. This optimal composition reflects a balance between bilayer ordering and membrane flexibility. The strong correlation between ^{31}P bicelle alignment and ^{14}N headgroup ordering demonstrates that bicelle formation is governed by coupled structural organization across multiple length scales. These findings provide a quantitative framework for cholesterol-regulated bicelle formation and establish 10 mol% cholesterol as an optimal condition for producing stable, well-aligned bicelles suitable for high-resolution structural studies.

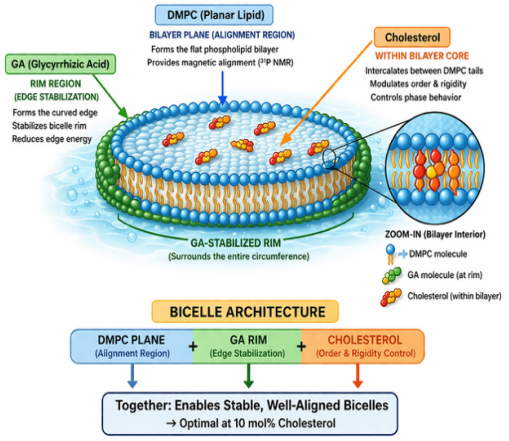

Figure: Cholesterol dependent optimization of DMPC+GA bicelle.

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Structurally distinct gangliosides promote structural heterogeneity in Aβ42 aggregates

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Abstract

The deposition of amyloid beta (Aβ) plaques in the brain is one of the key pathological hallmarks of Alzheimer's Disease (AD). The aggregation of Aβ into oligomers and fibrils is a complex process influenced by a variety of factors, including cell membrane components, metal ions, salts, and interacting proteins. Among these, gangliosides have drawn particular interest due to their reported presence within Aβ plaques, suggesting a direct role in modulating Aβ aggregation. Previous studies have shown that GM1 ganglioside can bind Aβ and promote the formation of both toxic oligomeric intermediates and fibrillar aggregates. However, gangliosides are structurally diverse and vary among different cell types, raising the possibility that this diversity may influence the morphology and toxicity of Aβ aggregates through the formation of structural polymorphs. In this study, we investigated the influence of two structurally distinct gangliosides, GD3 and GM3, on Aβ aggregation. Using solid-state NMR spectroscopy and complementary biophysical techniques, we demonstrate that these gangliosides differentially modulate the aggregation pathway of Aβ, leading to the formation of distinct aggregate species that leads to further seeding and cause cellular toxicity. These findings suggest that the specific structural features of gangliosides can significantly alter Aβ aggregation kinetics and generate polymorphic aggregates. Our study provides new insights into how the cellular lipid environment, particularly the diversity of gangliosides, contributes to the complexity of Aβ aggregation in AD. These findings suggest that the structural features of individual gangliosides can significantly impact the conformational landscape and assembly kinetics of Aβ, potentially contributing to the heterogeneity observed in Aβ aggregates in AD.

Keywords: NMR, Amyloid, Aggregation, Gangliosides, cell toxicity

Translating Injectable Octreotide to Oral Therapy Using Donkey Milk Exosomes: Design, Characterization, and PBPK Modeling

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Abstract

Octreotide (OCT) is currently limited to intramuscular administration due to rapid gastrointestinal degradation and poor absorption. This study aimed to develop the first orally deliverable OCT formulation using donkey milk–derived exosomes, which are naturally enriched with membrane-fusion and transcytosis proteins. The approach integrates pH-gradient loading, in vitro and cellular permeability studies, in silico PBPK modeling, and in vivo pharmacokinetics to establish a translational pipeline for oral peptide delivery. Exosomes were isolated from donkey milk powder via sodium citrate precipitation and ultracentrifugation, followed by characterization using nanoparticle tracking analysis, protein quantification, Western blotting (Alix, CD61, CD83, HSP70), and LC-MS/MS proteomics. OCT was loaded using a pH-gradient method (pH 4.6–7.4). In vitro release was assessed under simulated gastric (pH 1.2) and intestinal (pH 6.8) conditions. Permeability was evaluated using MDCK monolayers (TEER > 500 Ω·cm²). In silico predictions were performed using GastroPlus. Pharmacokinetics and tissue distribution were evaluated in BALB/c mice (2 mg/kg oral dose). Exosomes exhibited a size of 136.2 ± 0.65 nm, zeta potential −33.3 ± 0.23 mV, and protein concentration of 2.5 mg/mL. Proteomics confirmed the presence of uptake-associated proteins (PIGR, MFGE8, ANXA2, CD9, CD63, CD81). OCT loading efficiency was 7.25 ± 0.23%. Controlled release showed reduced burst compared to free OCT. Permeability increased ~37.5-fold (Papp: 17.26 × 10^{-6} vs 0.46 × 10^{-6} cm/s, $p < 0.0001$). Simulations predicted Fa improvement (8.5% → 88.2%), reduced Tmax (5.5 → 2.2 h), and ~4-fold AUC increase. In vivo, OCT-exosomes achieved ~16-fold higher AUC_{0-24} (435,200 vs 27,154 pg·h/mL) with distribution across liver, spleen, kidney, and intestine. Donkey milk exosomes enable effective oral delivery of OCT, significantly enhancing permeability and systemic exposure. This scalable platform offers a promising strategy for oral delivery of peptide therapeutics.

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Probing GCGR Recognition Using Nanodisc-based Peptide Selection

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Abstract

G protein–coupled receptors (GPCRs) adopt multiple conformational states that underlie ligand efficacy and signaling bias. Understanding how ligands selectively recognize these states remains a key challenge in GPCR biology. The glucagon receptor (GCGR), a class B GPCR, plays an important role in metabolic regulation and represents a clinically relevant target. Here, we establish a nanodisc-based platform for selecting peptides that bind GCGR in a native-like membrane environment. Full-length GCGR is expressed in Sf9 insect cells, purified, and reconstituted into lipid nanodiscs. The receptor is fluorescently labeled and used as a target for screening peptide libraries displayed on Pichia pastoris. Negative selection against empty or related nanodiscs is combined with positive selection against GCGR to enrich for specific binders. Fluorescence-activated cell sorting (FACS) is used to isolate peptide populations with increased binding signals across iterative selection rounds. This platform enables the enrichment of peptides that recognize GCGR in a membrane context while reducing non-specific interactions. By integrating nanodisc reconstitution with cell-based display and FACS, this approach provides a general framework for identifying GPCR-binding peptides and may facilitate the development of ligands with improved selectivity.

Lipid organization and GPCR-lipid interactions in lipid nanodiscs: insights from NMR spectroscopy and MD Simulations

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Abstract

Phospholipid nanodiscs are one of the most widely used membrane mimetic systems for in vitro studies of integral membrane proteins as they arguably provide a more native-like environment than detergents. Prior work from the Eddy lab established lipid nanodiscs formed from membrane scaffold proteins (MSPs)[1] as a robust platform for systematic investigations of the roles of phospholipids on the conformational equilibria of G protein-coupled receptors (GPCRs).[2,3] ^{19}F-NMR measurements with the A_{2A} adenosine receptor (A_{2A}AR) in lipid nanodiscs demonstrated that the presence of anionic lipids shifted the A_{2A}AR conformational equilibria toward an active conformation, facilitating the formation of a signaling complex with its partner G protein. We observed that a minimum amount of anionic lipids was required to observe an active conformational ensemble, and this effect was most pronounced with POPS. The current study builds on these data by applying ^{19}F-NMR, solid-state NMR, activity and pharmacological assays, and computational simulations to understand the molecular mechanisms for the lipid-dependence of A_{2A}AR activation. Follow up ^{19}F-NMR experiments with nanodiscs of varying sizes showed that the minimum amount of anionic lipids needed to populate an active A_{2A}AR conformational state increased with overall increase in the size of the lipid nanodisc, with the most pronounced effect observed with the MSP1E3D1 scaffold protein. Pharmacological activity assays with A_{2A}AR showed a qualitatively similar effect, consistent with the ^{19}F-NMR measurements. Based on these data, we hypothesized that non-uniform spatial organization of lipids within nanodiscs could contribute to the observed lipid dependence of activation. We tested this using both solid-state NMR experiments and molecular dynamics (MD) simulations, which revealed that lipid-lipid interactions within nanodiscs influenced lipid-dependent activation of A_{2A}AR. These data suggest that lipid-lipid and lipid-protein interactions within membrane environments can directly influence the conformational equilibria of drug-bound receptor proteins.

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Structure and function of an intermediate GPCR-Gαβγ complex

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Abstract

Unraveling the signaling roles of intermediate complexes is pivotal for G protein-coupled receptor (GPCR) drug development. Despite hundreds of GPCR-Gαβγ structures, these snapshots primarily capture the fully activated complex. Consequently, the functions of intermediate GPCR-G protein complexes remain elusive. Guided by a conformational landscape visualized via ^{19}F quantitative NMR and molecular dynamics (MD) simulations, we determined the structure of an intermediate GPCR-mini-G$\alpha_s\beta\gamma$ complex at 2.6 Å using cryo-EM, by blocking its transition to the fully activated complex. Furthermore, we present direct evidence that the complex at this intermediate state initiates a rate-limited nucleotide exchange before transitioning to the fully activated complex. In this state, BODIPY-GDP/GTP-based nucleotide exchange assays further indicated that the helical domain of the G protein is partially open, allowing it to grasp a nucleotide at a non-canonical binding site, distinct from the canonical nucleotide-binding site. These advances bridge a significant gap in our understanding of GPCR signaling complexity.

Expanding ^{17}O Biomolecular NMR through Cost-Effective ^{17}O Labeling via Bacterial Expression and Novel ^{17}O Relaxation Approach for Probing Water Dynamics

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Abstract

Oxygen plays a central role in biomolecular structure, function, and hydration dynamics, making ^{17}O NMR a uniquely powerful probe of biological systems. However, its widespread application has been limited by the low natural abundance of the NMR-active ^{17}O isotope (0.0373%). Here, we present an integrated approach that expands both the accessibility and analytical capability of ^{17}O NMR for studying proteins and their associated water environments. We introduce a rapid and cost-effective method for amino acid-specific ^{17}O labeling of recombinant proteins, combining standard bacterial expression systems with the fast (30 min) mechanochemical synthesis of ^{17}O-labeled amino acids. Using this strategy, we successfully generated multiple labeled proteins from diverse organisms, including CrgA and FtsQ from *Mycobacterium tuberculosis* and the E protein from SARS-CoV-2 virus, demonstrating the broad applicability. Magic-angle-spinning ^{17}O NMR was used to confirm the efficient incorporation and highlights the sensitivity of ^{17}O chemical shifts to local structural and functional environments. Additionally, we extend the utility of ^{17}O NMR through relaxation measurements that probe water dynamics in complex biological systems. We develop a methodology to disentangle distinct relaxation components, enabling quantitative characterization of heterogeneous water populations. Application of this approach reveals multiple classes of water associated with protein channels, membrane surfaces, and lipid environments, each exhibiting distinct relaxation behaviors. Together, these advances establish ^{17}O NMR as a versatile and accessible tool for simultaneously probing protein structure and water dynamics, providing new insights into oxygen-mediated interactions and the molecular basis of biological function.

CHARACTERIZING THE MEMBRANE-SOLUBILIZING PROPERTIES OF AMPHIPOLS

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Abstract

Cycloalkane-modified polymers (CyclAPols) display membrane-solubilizing properties that differ markedly from those of the reference amphipol A8-35 and of styrene–maleic acid (SMA) copolymers [1, 2]. Yet, the molecular basis of these differences remains poorly understood. Since polymer-mediated membrane solubilization is known to depend on multiple parameters–including lipid and protein composition, membrane fluidity, and protein topology–we conducted a comparative analysis using biological membranes from diverse organisms, together with liposomes of defined lipid composition. Monitoring protein solubilization revealed that CyclAPols are less sensitive than SMA (3:1) to variations in membrane fluidity [1]. Furthermore, we observed that increasing polymer concentrations does not necessarily improve solubilization yields and, for certain types of membranes, both CyclAPols and SMA require an optimal protein-to-polymer mass ratio to achieve efficient solubilization [3]. Investigation of large unilamellar vesicles (LUVs) solubilization revealed striking differences: amphipols with linear octyl chains (A8-35 and A8-50) effectively solubilize DMPC LUVs but fail to disrupt PC/PA/Chol vesicles, likely absorbing onto the vesicle surface without inducing membrane breaking. In contrast, C8C0-50 and SMA (3:1) fully solubilize PC/PA/Chol vesicles, with C8C0-50 promoting vesicle swelling prior to complete solubilization–a phenomenon also observed with SMA (3:1) on DMPC LUVs. While this study aims to identify the key parameters influencing polymer solubilization efficiency, further investigations are needed to elucidate the molecular mechanisms leading to the formation of protein–lipid–polymer particles.

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