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Membrane protein reconstitution : New possibilities for structural biology, biophysical methods, and antibody/drug discovery

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Liguo Wang is the Director of Scientific Operations at the Laboratory for BioMolecular Structure at Brookhaven National Laboratory. With a strong background in cryo-electron microscopy (cryo-EM), structural biology, and computer science, he codeveloped the 'random spherically constrained' cryo-EM method—originally proposed by Dr. Fred Sigworth—to study ion channels reconstituted in lipid vesicles under defined membrane potentials. His lab has resolved important membrane protein structures, including an intermediate state of the BK channel in a native-like membrane environment. Dr. Wang also contributed to artificial intelligence–driven cryo-EM tools and is advancing mega-electron-volt Scanning Transmission Electron Microscope (MeV-STEM) technology for imaging thick biological samples at nanometer resolution.

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Nearly one-third of all proteins in eukaryotes are membrane proteins. Moreover, roughly 60% of Food and Drug Adminstration (FDA)-approved small-molecule drugs act on membrane proteins, which includes G protein—coupled receptors (GPCRs), ion channels, and transporters. The vast majority of these membrane proteins are cell-surface accessible and thus amenable to drug discovery. At the same time, they are considerably more challenging to reconstitute and prepare for structure initiatives, antibody discovery, and drug screening. This series of reviews introduces the reader to current reconstitution systems, biophysical characterization of the membrane proteins and associated lipids, and common applications involving nuclear magnetic resonance (NMR), mass spectrometry (MS), and cryo-electron microscopy (cryo-EM).

Traditionally, membrane protein structures were mainly determined by X-ray crystallography of detergent-solubilized proteins, but with the resolution revolution, cryo-EM has become a commonly used method. Traditionally, detergents such as n-Dodecyl-β-D-maltoside (DDM) and n-Octyl-β-D-glucopyranoside (OG) have been used to extract and stabilize membrane proteins, but these approaches often fail to maintain native conformations and provide only limited stability [1]. To address these limitations, various detergents have been developed [1]: symmetric detergents with two identical tails and head groups were developed, improving protein stabilization, but frequently generating large-micelles [1,2] asymmetric detergents with different tail lengths provided smaller micelles, better solubility, and enhanced stabilization [3], hybrid detergents with different head groups enabled fine-tuning of hydrophilic—lipophilic balance, preserved protein—lipid interactions, and stabilized challenging proteins [1], and [4] asymmetric hybrid detergents with different headgroups and different tail lengths enabled improved performance for extraction, stabilization, and structural studies, particularly in cryo-EM and NMR. Collectively, these innovations enable precise control over micelle size, critical micelle concentration, and hydrophilic—lipophilic balance, thereby expanding the detergent toolbox and offering powerful new approaches for membrane protein structural biology [1].

through a *dynamic ensemble* representation. This has been pursued in the study of a super family of 7-transmembrane receptors called G Protein-Coupled Receptors (GPCRs), and various protein complexes. His lab is also interested in new dynamic and evolvable fragment libraries for membrane protein drug discovery and methods associated with single domain antibody drug discovery. many of the topics covered in this series are crucial to such initiatives - namely, structural biology and biophysical chemistry of membrane proteins and small molecule antibody drug discovery.

Although detergent-based reconstitution methods have revealed many facets of protein structure and function, they cannot fully mimic the native bilayer. In some cases, detergents compromise functional integrity and disrupt native lipid interactions, as shown for the ABC transporter MsbA and pLGIC [2]. Because membrane proteins rely on lipids for stability, dynamics, and function, high-resolution structural studies would ideally be performed in native cellular environments. However, *in situ* single-particle analysis and *in situ* subtomogram averaging are currently limited to naturally abundant molecules. To better preserve protein structure and function, reconstitution of detergent-solubilized membrane proteins has been carried out. Membrane scaffolding protein (MSP)—based nanodiscs provide flat membranes that reveal details not seen in detergent-solubilized proteins and enable control of lipid composition, while proteoliposomes provide curved membranes useful for mechanosensitive channels but are technically challenging for cryo-EM [3]. Detergent-free systems have also been developed, such as cell-derived vesicles and polymer-based nanodiscs. Cell-derived vesicles preserve native-like membranes, but their broad applicability is still under evaluation [3]. Amphiphilic copolymers such as Styrene—Maleic Acid copolymer (SMA) can extract proteins directly with native lipids, but they have limitations including limited extraction efficiency, lipid mixing, distortion, and sensitivity to low pH or divalent cations [3,4]. To overcome these drawbacks, Guo et al. developed the native cell membrane nanoparticle (NCMN) system, which preserves protein—lipid interactions and enables structural and functional studies under broader conditions [4]. NCMNs have been used for high-resolution cryo-EM studies of bacterial and human membrane proteins, enzyme assays, and ligand binding, and protein—protein interaction analyses, while challenges remain in proteoliposome reconstitution and lipid assignment in cryo-EM maps. Finally, the use of a membrane protein scaffold derived originally from saposins can be used to achieve native nanodisc-stabilized receptors and receptor complexes. The Salipro technology and recent advances in this field, particularly for cryo-EM, are reviewed in the context of native nanodisc reconstitution [5].

Despite these developments, MSP-based nanodiscs remain the predominant tool for mechanistic studies as they allow fine control of membrane composition and size, provide sufficient lipid area to avoid constraining proteins, and enable high-resolution cryo-EM. This approach has led to breakthroughs such as the first nanodisc-reconstituted TRPV1 structures, which revealed regulatory lipid interactions [2]. Since then, nanodiscs have become widely adopted, with hundreds of cryo-EM structures showing how they capture conformational dynamics, substrate binding, and lipid modulation. Using NMR, it is sometimes possible to quantify specific conformations within an ensemble that reflect the overall activation propensity of the protein. This is demonstrated in a review by Jain and Eddy who show in studies of a nanodisc-reconstituted human GPCR that distinct negatively charged lipids can integrate with the receptor to regular the stability of specific activation states [6]. To explain how lipids act as triggers, cofactors, or structural supports, the concept of lipidons has emerged [7]. Lipidons are defined sets of lipids enriched in specific membrane zones that function as signals, ligands, or structural scaffolds, guiding proteins into active complexes. Restoring lipidons in nanodiscs or polymer-encapsulated systems is essential for capturing physiologically relevant states. Advances such as cryo-EM, native MS, and

nanodiscs now allow direct visualization of protein–lipid assemblies [7]. Case studies demonstrate lipidons orchestrating membrane organization across plasma membranes, endosomes, mitochondria, and bacterial envelopes, where they regulate trafficking, energy production, fission–fusion dynamics, viral entry, cell death, and ion channel gating.

Many biophysical techniques require precise reconstitution conditions before they can be used in the study of membrane proteins. As micelles and nanodisc systems have been refined, cryo-EM has benefitted both from micelle- and nanodisc-stabilized membrane proteins [2–4]. Similarly, hydrogen–deuterium–exchange mass spectrometry (HDX-MS) has been applied successfully to both micelle and nanodisc systems. HDX-MS can tease out dynamic topologies of membrane proteins or their complexes with other drugs or proteins. This is accomplished by quenching the membrane protein in deuterated buffer and examining residue-specific mass changes after proteolytic digestion, as discussed by Guffick and Politis in their review [8]. Solution NMR has made great strides in obtaining atomic-resolution information on conformational dynamics of membrane proteins in both detergent micelles and nanodiscs, as discussed by Vinogradova [9].

The stable reconstitution of membrane receptors has also helped to accelerate the antibody discovery field since detergent- or nanodisc-stabilized receptors and in particular, GPCRs, can now be used as long-lived antigens in both antibody discovery and antibody panning. Elbaz and Nasr discuss how the stable reconstitution of the gp160 HIV-1 trimer has led to the discovery of more efficacious antibodies and the development of next-generation vaccines [10]. Yao and Thomson also discuss a host of nanodisc formulations that have aided in the stabilization of membrane proteins in functionally relevant states, needed for antibody discovery [11].

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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