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Illuminating Individual Membrane Protein Complexes with Mass Photometry

Michael T. Marty[1,*]

Membrane proteins play critical roles but have been challenging for structural biology. In this issue of *Chem*, Olerinyova et al. apply an emerging technology, mass photometry, to characterize membrane proteins in different solubilization vehicles. Mass photometry streamlines characterization of membrane proteins in different membrane mimetics, enabling rapid analysis of homogeneity.

Every cell is surrounded by a lipid membrane; membrane proteins residing in this lipid bilayer play critical roles in shuttling chemicals and information across this barrier. However, there is a significant gap between the importance of membrane proteins and our ability to study them. For example, membrane proteins make up about 30% of the proteome and around half of all drug targets, but they account for only a tiny fraction of high-resolution structures in the Protein Data Bank.[1] This gap has been driven by several factors. First, there have been limited molecular biology tools for expressing membrane proteins. Second, it has been challenging to preserve the stability and function of membrane proteins during solubilization, purification, and analysis. Finally, the leading structural biology techniques have had significant limitations for membrane proteins, which were often too large for nuclear magnetic resonance spectroscopy (NMR) and did not generally crystallize well for X-ray diffraction.

Recent technological advances have begun to solve each of these limitations. Advances in molecular biology, such as cell-free, yeast, insect, and mammalian expression systems, have enabled more routine expression of functional membrane proteins.[2] A range of relatively new membrane mimetic technologies, including nanodiscs,[3] amphipols,[4] and styrene maleic acid lipid particles (SMALPs),[5] have helped to preserve the structure, function, and interactions of membrane proteins during solubilization and analysis. Finally, advances in cryo-electron microscopy (cryo-EM) have propelled structural characterization of membrane proteins, especially when paired with advanced expression, purification, and solubilization strategies.[6] For example, pioneering cryo-EM on transient receptor potential cation channel subfamily V member 1 (TRPV1) used human embryonic kidney (HEK) cells for expression, and solubilization in nanodiscs provided the best environment for high-resolution EM analysis.[7]

However, significant challenges remain in optimizing the pipeline for membrane protein structural biology. Screening constructs and expression systems can be slow, especially with mammalian expression systems, and yields are often lower than with conventional bacterial expression. Finding the right detergents and membrane mimetics can also be time and material intensive. Because cryo-EM time can be scarce and expensive, we need intermediary techniques to rapidly characterize membrane proteins prior to structural analysis. Conventional methods for characterizing membrane protein complexes embedded in

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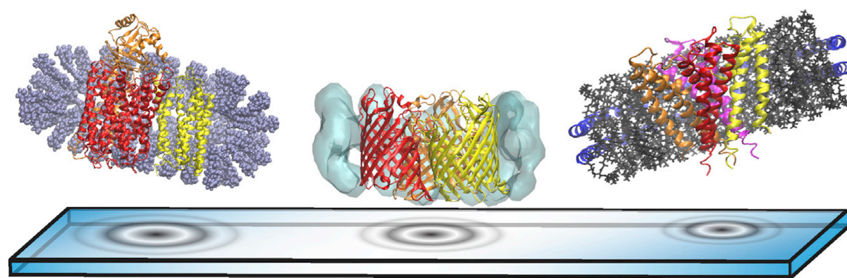

Figure 1. Schematic of Mass Photometry of Membrane Protein Complexes
A schematic (not to scale) shows mass photometry of *bo*$_3$ oxidase in detergent micelles (left), OmpF in amphipols (center), and KcsA in nanodiscs (right). As the complexes approach the glass slide, the contrast of the backscattered light is proportional to the mass.

membrane mimetics, including size exclusion chromatography (SEC), analytical ultracentrifugation, and light scattering methods, generally require high amounts of sample and provide relatively low resolution. A relatively new method, native mass spectrometry (MS), is faster, provides higher resolution, and requires less sample. However, because it generally requires cleaner and more homogeneous samples, native MS might not be ideal for screening highly heterogeneous mixtures.[8]

Another emerging technology for rapid characterization of protein complexes is mass photometry (MP). With MP, a small amount of solution is spotted on a glass slide. As particles in solution approach the back-illuminated glass surface, the contrast of the backscattered light is proportional to the mass of the complex (Figure 1). Thus, interferometric scattering microscopy can be used to monitor individual molecules diffusing to and from the surface to generate a histogram of masses present.[9,10] Importantly, MP uses very small amounts of dilute sample, is tolerant of a wide range of buffer conditions, enables rapid analysis within seconds to minutes, and provides single-particle analysis that reveals the underlying mass distributions for complex ensembles.

In this issue of *Chem*, Struwe, Kukura, and co-workers apply mass photometry to study membrane proteins in a range of different membrane mimetics.[1] The authors first tested detergent micelles, which produced a significant background signal because of the high concentration of empty micelles. Nevertheless, larger membrane protein complexes, such as the *E. coli* respiratory complex I, could be clearly distinguished from the background. Smaller membrane proteins could be analyzed by quick dilution of the detergent immediately prior to analysis to reduce the background. Detergent-free membrane mimetics were much simpler for MP because of their lower background concentrations. MP revealed the ratios of amphipol to protein that best preserved two membrane protein complexes. MP was also used during SMALP purification to find the most homogeneous SEC fraction for negative stain electron microscopy. Finally, the authors used MP to optimize nanodisc assembly for homogeneity prior to NMR studies. Importantly, the higher-resolution MP analysis revealed heterogeneity that was unseen by conventional SEC analysis.

Together, these results have important implications for membrane protein structural biology, especially for cryo-EM analysis. MP allows rapid screening for homogeneity to optimize sample preparation prior to more time-consuming structural analysis, answering key questions like: does the membrane protein retain its correct oligomeric state if we dilute or change the detergent? What is the right amount of amphipol to add? Which SEC fractions should we pool for structural analysis? What are the optimal conditions for nanodisc assembly? Overall, MP provides another powerful technology to the membrane protein structural biology pipeline and will help close the gap in our understanding of membrane protein biochemistry and biophysics.

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