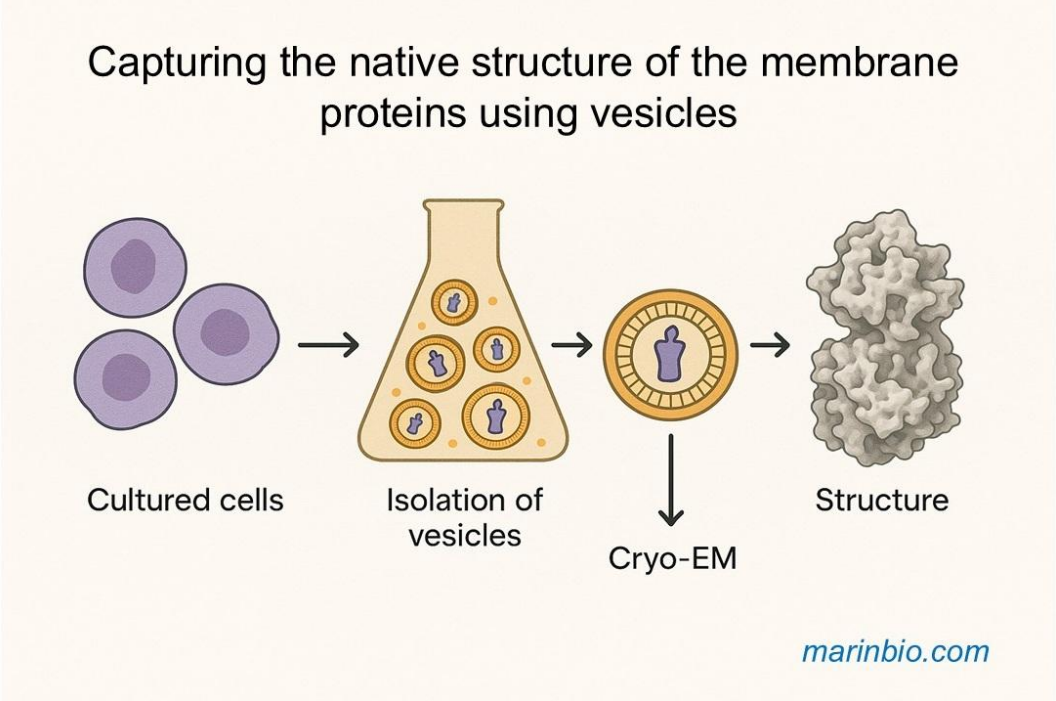
Cutting Edge MarinBio Newsletter

A Vesicle-Based Method for Capturing Native Structure of Membrane Proteins



Membrane proteins form a vital class of macromolecules that govern essential physiological functions, including molecular transport, signal transduction, and cellular recognition. Their biomedical importance is emphasized by the fact that they constitute more than 60% of current drug targets, making them central to modern therapeutic development. Yet, despite their critical roles, these proteins have historically posed major challenges for structural biology, largely because of their amphipathic nature and their strict dependence on the lipid bilayer for stability, folding, and function.

Current Methods for Studying Membrane Proteins

For many years, structural studies have relied on detergent-based solubilization methods. In this approach, membrane proteins are extracted from their native environment with detergents and subsequently purified through affinity chromatography and size exclusion chromatography. While this strategy has enabled the determination of numerous membrane protein structures, it suffers from inherent drawbacks. Identifying appropriate detergents requires labor-intensive screening, as proteins respond unpredictably to different detergents. More significantly,

detergent solubilization strips away the surrounding lipids, preventing investigation into lipid-mediated regulation. Since lipids serve as crucial regulators for most membrane proteins, this absence leaves an incomplete picture of their native function.

To overcome these issues, the last decade has seen the emergence of near-native approaches that aim to preserve the lipid environment. Nanodiscs, stabilized by membrane scaffold proteins or synthetic polymers, encircle lipid bilayers containing target proteins, offering conditions more natural than detergent micelles. Salipro systems and styrene-maleic acid lipid particles (SMALPs) provide detergent-free alternatives by using saposin proteins or copolymers to extract proteins along with their native lipids. Proteoliposome systems reconstitute detergent-purified proteins into artificial lipid vesicles, whereas cell-derived vesicles retain fragments of the original membrane. These innovations have collectively expanded the structural toolkit, allowing researchers to probe membrane proteins in environments that more closely approximate their natural state.

Nevertheless, significant challenges remain. Nanodiscs and proteoliposomes often rely on non-native lipid mixtures, which may distort physiological relevance. Similarly, the extensive purification steps required for many approaches risk the loss of weakly interacting partners, erasing insights into transient interactions that are essential for regulation and function. Thus, while these methods mark substantial progress, they also underscore the continuing need for approaches that capture membrane proteins in their fully native context.

Capturing Membrane Proteins in Their Native Lipid Environments with Vesicle Technology

To overcome the limitations of detergent-based methods, Liu *et al.* (2025) introduced a vesicle-based technology that preserves the native membrane environment. Unlike earlier approaches, this method bypasses detergent extraction and affinity purification, maintaining native lipids and cellular context that are often lost. Table 1 shows a comparison of different methods used for studying membrane proteins.

The workflow begins with vesicle preparation from cells expressing the target protein. In their model system using the E. coli efflux transporter AcrB, harvested cells were treated with lysozyme to remove cell walls and improve imaging contrast. Vesicle formation was then induced with a high-pressure homogenizer, followed by sequential centrifugation steps to remove unbroken cells and soluble proteins. The pellet was resuspended, homogenized, and fractionated by sucrose gradient centrifugation to enrich protein-containing vesicles.

A key advance was optimizing sucrose gradients. Continuous gradients separate vesicles effectively but require overnight runs and dilute samples. The team replaced this with a two-step 20%–40% sucrose gradient, which reduced processing to about one hour and concentrated vesicles at the interface. Combining continuous and noncontinuous gradients allows tailoring for yield or speed, producing vesicles of sufficient quality for cryo-EM.

After isolation, vesicles underwent buffer exchange to reduce sucrose below 1%, minimizing background during imaging. Samples were applied directly to holey carbon grids for cryo-EM. Cryo-EM and cryo-ET characterization revealed vesicles between 20 and 200 nm, with more

Table 1: Comparative methods for studying membrane proteins

Detergent-based (X-ray, NMR, Cryo-EM)	Well-established, high-resolution structures	Removes lipids, destabilizes proteins, loss of partners
Nanodiscs / SMALPs / Proteoliposomes	Mimics lipid environment, stabilizes proteins	Often still needs detergent-purified proteins, non-native lipids
Cryo-ET (in situ)	Visualizes proteins in intact cells	Technical challenges, limited resolution
Vesicle-based (native membranes)	Preserves native lipids and partners, no detergents	Resolution still modest for some proteins, orientation control

than half around 50 nm, ideal for structural studies. Importantly, vesicles were evenly distributed in vitreous ice without air–water interface damage, ensuring preservation of native states for high-resolution analysis.

Validating the Vesicle Method: AcrB as a Model System

To validate their method, the researchers studied AcrB, a trimeric efflux transporter central to multidrug resistance. AcrB expels toxic compounds and serves as a clinically relevant model. Structural determination in vesicles proved difficult because the vesicle membrane introduced strong background signals, limiting resolution to ~7.5 Å with standard cryoSPARC processing.

To overcome this, they devised a micrograph-sorting workflow combined with deep learning particle picking using Topaz. Micrographs were classified into quality tiers, and only those rich in good particles trained the model. Iterative rounds of particle picking, 2D/3D classification, refinement, and CryoSieve filtering ultimately yielded a 3.88 Å cryo-EM structure of AcrB in vesicles.

The structure revealed AcrB as a symmetric trimer with all protomers in the loose (L) state, distinct from the asymmetric LTO state observed with SMA copolymer extraction. This suggests

vesicles better preserve the native protein conformation. Notably, the vesicle-derived map provided superior resolution of transmembrane helices, especially the continuous lα helix spanning TM6–TM7, buried within the bilayer and stabilized by native lipids. Extra densities consistent with lipid molecules were also observed, underscoring the method's ability to capture protein–lipid interactions often lost in detergent-based systems.

Extending Vesicle-Based Structural Analysis to Endogenous Mitochondrial Proteins

To test broader applicability, the researchers generated vesicles from pig heart mitochondria. Compared to bacterial vesicles, these displayed rougher surfaces and size heterogeneity, reflecting higher protein density and membrane curvature, yet remained suitable for cryo-EM.

Random particle picking and iterative classification revealed endogenous proteins, including F-ATPase and respiratory complex III (CIII). Deep learning–based particle picking enriched these classes for further analysis. F-ATPase showed recognizable features such as the stalk, catalytic hexamer, and F0 region, but reconstructions reached only medium resolution, with monomeric forms predominating, but faint dimer-like features were traced back to particle crowding artifacts.

In contrast, CIII was resolved to 3 Å, producing side-chain details and bound ligands. The 45 Å transmembrane span matched native structures, but subunit 9 displayed a continuous density bridging two monomers, heterogeneity resembling detergent-isolated mouse CIII rather than native pig heart complexes. As with AcrB, lipid-like densities surrounded the transmembrane region, though weak signals prevented precise identification. These findings suggest preserved but nonspecific protein–lipid interactions, consistent with reports of lipid substitution in yeast CIII.

Innovative Strategies in Vesicle Preparation and Imaging

The vesicle-based method developed by Liu *et al.* introduces key innovations that overcome persistent challenges in membrane protein structural biology. Their micrograph-sorting strategy markedly improves particle selection for cryo-EM by ranking micrographs by particle quality and training deep learning models on the best datasets. This enhances precision in particle picking, crucial for samples with high membrane background.

Optimization of sucrose gradient centrifugation further strengthens the workflow. A noncontinuous two-step gradient reduces processing time, minimizes dilution, and allows efficient concentration of vesicles. Used alone or in combination with continuous gradients, this approach provides a flexible framework adaptable to diverse protein systems.

Cryo-ET adds another dimension by characterizing vesicle distribution and morphology in vitreous ice. The analysis showed random dispersion without preference for air–water interfaces, indicating minimal preparation damage. Cryo-ET also revealed membrane proteins embedded within vesicles and identified inside-out and inside-in vesicles, offering valuable information to guide downstream data processing.

Advantages and Limitations of the Vesicle-Based Method

The vesicle-based method offers distinct advantages for membrane protein studies. Most importantly, it preserves the native lipid environment, enabling analysis of lipid-mediated

regulation absent in detergent-based systems. By eliminating detergent extraction and multi-step purification, it avoids resource-intensive screening and reduces the loss of weakly interacting partners. The closed vesicle system also allows manipulation of buffer conditions, providing opportunities to probe proteins under stimuli such as pH or ion changes. Compared to proteoliposomes, vesicles more closely mimic natural membranes, stabilizing proteins in physiologically relevant states.

However, the method has limitations. Proteins under 200 kDa remain difficult to resolve, as smaller particles require substantially larger datasets for high-quality reconstructions. Target protein abundance is also lower than in purified samples, making enrichment strategies, such as affinity grids or protein-specific binding partners, valuable for future optimization.

Future Perspectives

This approach enables exploration of proteins under varied conditions, such as pH-regulated channels or mechanosensors influenced by membrane curvature. Vesicle size manipulation could reveal how forces modulate mechanosensitive proteins like PIEZO. Integration with affinity-based enrichment may expand applicability to low-abundance targets.

The vesicle-based method also complements cryo-ET, providing samples ideally sized for single-particle analysis while retaining native membranes. Together, these techniques bridge the gap between biochemical isolation and in situ imaging.

Conclusion

By preserving lipid context and removing detergent dependence, the vesicle-based method advances the structural biology of membrane proteins. Its application to both bacterial and mitochondrial proteins demonstrates versatility. Though challenges remain for small and scarce targets, the method provides a robust foundation for studying proteins in native states, deepening mechanistic insight and supporting future therapeutic discovery.

Reference

Liu H, Tse CM, Dang S. Capturing the native structure of membrane proteins using vesicles. Proc Natl Acad Sci U S A. 2025 Sep 9;122(36):e2423407122. doi: 10.1073/pnas.2423407122.