CellPress

Spotlight

Limitations in membrane protein structure determination by lipid nanodiscs

Chen Zhao [1,*]

Lipid nanodiscs are popular mimetics of biological membranes for determining membrane protein structures. However, a recent study revealed that the choice of nanodisc scaffold directly influenced the structure of an ion channel. This finding prompts us to be cautious and calls for improved membrane mimetics for structure determination.

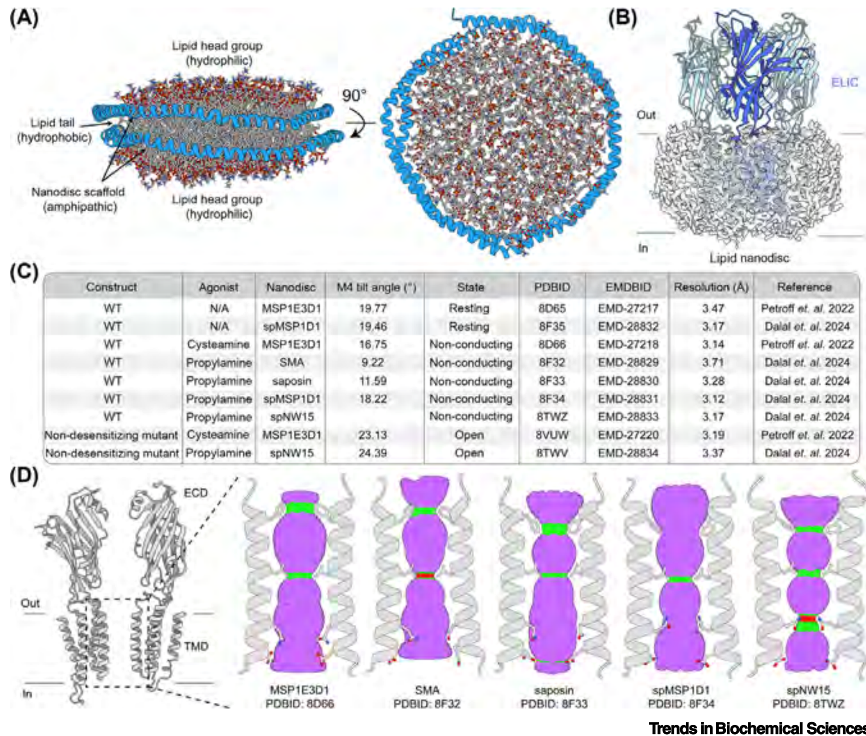
Membrane proteins are naturally embedded in (integral membrane proteins) or anchored to (peripheral membrane proteins) the cell membrane. The physicochemical properties of the cell membrane are generally characterized by its bilayer structure, lipid composition, asymmetry, phase, thickness, fluidity, and local curvature [1]. These properties could influence the distribution, structure, dynamics, and thus the function of the resident membrane proteins [1].

Structural biology of membrane proteins seeks to understand their molecular mechanisms in cells through their atomic structures. Therefore, it is crucial to characterize the structures of the membrane proteins in environments that closely resemble the cell membrane. However, most current high-resolution structural techniques such as X-ray crystallography and single particle cryo-electron microscopy (cryo-EM) require isolating membrane proteins via membrane mimetics for purification and structural determination [2]. Detergents were the dominating membrane mimetics for membrane protein structural biology by X-ray crystallography or cryo-EM for over 30 years until 2016, when 2.9–3.4 Å cryo-EM structures of the ion channel TRPV1 were determined in lipid nanodiscs [3].

Lipid nanodiscs are engineered to comprise a discoidal patch of lipid membrane stabilized by amphipathic scaffolds (‘belts’), with their hydrophobic side shielding the lipid tails and their hydrophilic side keeping them soluble in solution (Figure 1A) [4]. By this design, lipid nanodiscs are considered better membrane mimetics than detergents as they contain a small patch of lipid membrane surrounding the membrane proteins of interest. As a result, substantial efforts have been devoted to creating varieties of lipid nanodiscs that cover a wide range of size distribution by engineering the length of the belt protein Membrane Scaffold Protein (MSP) [4]. At the same time, a new belt protein, saposin, has been introduced to adapt to membrane proteins of any size as multiple copies of saposin can bind simultaneously to accommodate large patches of lipid [5]. In addition, as the protein scaffold-based nanodiscs generally require first extracting membrane proteins by detergent then reconstituting them through detergent removal, varieties of styrene maleic acid (SMA) scaffolds have been developed to directly extract membrane proteins from the cell membrane into the nanodiscs [6]. These SMA-based nanodiscs are viewed as the best cell membrane mimetics for membrane protein structural analyses.

However, the assumption that lipid nanodiscs contain a small patch of lipid bilayer membrane with similar properties to the cell membrane is questionable. Aside from the difference in lipid composition that is relatively easy to address by simply using a defined lipid mixture, lipid nanodiscs are mostly 8–20 nm in size, a diameter not much larger than the membrane proteins enclosed. Additionally, inside the nanodiscs, lipid and protein molecules frequently encounter the belt, which influences their dynamics and structures. Due to the limited size of the lipid patch and the existence of the belt, the physicochemical properties of the lipid membrane, such as lipid packing, have been reported to deviate from what has been measured in lipid vesicles [7], the model system for bilayer lipid membranes. Therefore, it is reasonable to imagine that different lipid nanodiscs could preferentially stabilize distinct conformations of the membrane proteins. In other words, the conformational landscape of a membrane protein in nanodiscs likely deviates from that in the cell membrane or lipid vesicles.

The recent work by Dr Wayland Cheng and his team precisely demonstrated this limitation of lipid nanodiscs. In this work [8] and a related work published previously [9], the authors determined the nanodisc-embedded structures of a ligand-gated pentameric ion channel, *Erwinia* ligand-gated ion channel (ELIC), in agonist free and bound states, stabilized by five different nanodisc scaffolds, including MSP1E3D1, spMSP1D1, spNW15, saposin, and SMA (SMALP 300) (Figure 1B,C) [8]. The authors found that the ELIC channels in the same agonist condition with the same lipid composition showed different degrees of channel activation in different types of nanodiscs (Figure 1B–D) [8]. Specifically, this differential ELIC activation is not only restricted to the transmembrane domain (TMD) characterized by the tilting of the M2 and M4 helices, but the conformational change is also coupled to the extracellular domain that rotates relative to the TMD [8]. The degree of ELIC activation is roughly correlated with the size of the lipid nanodiscs [8]. Through molecular dynamics simulation, the authors found that smaller nanodiscs have reduced membrane thickness and, in smaller nanodiscs, ELIC more frequently interacts with the belt protein MSP [8]. These results provide possible mechanisms underlying the conformation differences of ELIC in nanodiscs with different scaffolds [8]. Based on these results, the authors propose that larger nanodiscs are better membrane mimetics [8].



Construct	Agonist	Nanodisc	M4 tilt angle (°)	State	PDBID	EMDBID	Resolution (Å)	Reference
WT	N/A	MSP1E3D1	19.77	Resting	8D65	EMD-27217	3.47	Petroff et. al. 2022
WT	N/A	spMSP1D1	19.46	Resting	8F35	EMD-28832	3.17	Dalal et. al. 2024
WT	Cysteamine	MSP1E3D1	16.75	Non-conducting	8D66	EMD-27218	3.14	Petroff et. al. 2022
WT	Propylamine	SMA	9.22	Non-conducting	8F32	EMD-28829	3.71	Dalal et. al. 2024
WT	Propylamine	saposin	11.59	Non-conducting	8F33	EMD-28830	3.28	Dalal et. al. 2024
WT	Propylamine	spMSP1D1	18.22	Non-conducting	8F34	EMD-28831	3.12	Dalal et. al. 2024
WT	Propylamine	spNW15		Non-conducting	8TWZ	EMD-28833	3.17	Dalal et. al. 2024
Non-desensitizing mutant	Cysteamine	MSP1E3D1	23.13	Open	8VUW	EMD-27220	3.19	Petroff et. al. 2022
Non-desensitizing mutant	Propylamine	spNW15	24.39	Open	8TWV	EMD-28834	3.37	Dalal et. al. 2024

Figure 1. Lipid nanodisc as a tool for structure determination of membrane proteins. (A) Representative structure of a lipid nanodisc. Membrane Scaffold Protein (MSP)-based nanodisc is shown as an example. (B) Structure of ion channel *Erwinia* ligand-gated ion channel (ELIC) in lipid nanodisc. Cryo-electron microscopy (cryo-EM) density of lipid nanodisc is shown in grey, while ribbon diagram of the ELIC channel is shown in different shades of blue (PDBID: 8D66, EMDID: EMD-27218). (C) A table summarizing the characteristics of all ELIC structures in nanodiscs. (D) Comparison of the ion permeation pathway of wild-type (WT) ELIC with agonist in five different types of lipid nanodiscs. The lipid nanodiscs have the same lipid composition {POPC:POPE:POPG [1-palmitoyl-2-oleoyl-glycero-3-phosphocholine:1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine:1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1′-rac-glycerol)] 2:1:1} but with different scaffolds. The ion channel pores are outlined by solid surfaces using the HOLE program. Subunits in the front and back are hidden for clarity. Abbreviations: ECD, extra-cellular domain; TMD, transmembrane domain. See [8,9].

This work by Dr Wayland Cheng and his team is a timely reminder that we should not automatically accept all structural observations for membrane protein. Quite often, the conformation of our protein of interest is influenced by its environment, most commonly membrane mimetics such as detergents or lipid nanodiscs. When interpreting the structures of membrane proteins, it is crucial to exercise caution and rely on additional evidence from functional analyses.

This work also highlights the importance of studying membrane protein structures in their native lipid membrane environment. Besides utilizing larger nanodiscs as the authors proposed, small lipid vesicles (20–100 nm in diameter) recently emerged as promising tools for membrane protein structure determination. High-resolution membrane protein structures have recently been obtained from both the synthetic lipid vesicles and lipid vesicles directly derived from the cell membrane [10]. While these vesicles cannot completely recapitulate the membrane thickness, lipid phase, asymmetry, fluidity, and local curvature of the cell membrane, I believe they serve as superior membrane mimetics for structural determination. However, it is important to note that structures in lipid vesicles or nanodiscs are not inherently better than structures in detergents: structural biology simply provides a lens through which we examine the atomic and molecular details that govern protein function. As long as the structures provide insights into the molecular mechanism of the membrane protein of interest, all structures are equally valuable for advancing our understanding of biology.

Acknowledgments

The author thanks Drs Maria E. Falzone and Venkata Shiva Mandala for their suggestions on the manuscript. The author regrets the omission of many references due to format limitations.

Declaration of interests

The author declares no competing interests.

[1]Department of Biochemistry and Molecular Biology, University of Florida, College of Medicine, Gainesville, FL 32611, USA

*Correspondence:
chen.zhao@ufl.edu (C. Zhao).

https://doi.org/10.1016/j.tibs.2024.03.010

© 2024 Elsevier Ltd. All rights reserved.

References

1. Levental, I. and Lyman, E. (2023) Regulation of membrane protein structure and function by their lipid nano-environment. *Nat. Rev. Mol. Cell Biol.* 24, 107–122
2. Choy, B.C. *et al.* (2021) A 10-year meta-analysis of membrane protein structural biology: detergents, membrane mimetics, and structure determination techniques. *Biochim. Biophys. Acta Biomembr.* 1863, 183533
3. Gao, Y. *et al.* (2016) TRPV1 structures in nanodiscs reveal mechanisms of ligand and lipid action. *Nature* 534, 347–351
4. Denisov, I.G. and Sligar, S.G. (2016) Nanodiscs for structural and functional studies of membrane proteins. *Nat. Struct. Mol. Biol.* 23, 481–486
5. Frauenfeld, J. *et al.* (2016) A saposin-lipoprotein nanoparticle system for membrane proteins. *Nat. Methods* 13, 345–351
6. Di Mauro, G.M. *et al.* (2021) Benchmarks of SMA-copolymer derivatives and nanodisc integrity. *Langmuir* 37, 3113–3121
7. Real Hernandez, L.M. and Levental, I. (2023) Lipid packing is disrupted in copolymeric nanodiscs compared with intact membranes. *Biophys. J.* 122, 2256–2266
8. Dalal, V. *et al.* (2024) Lipid nanodisc scaffold and size alter the structure of a pentameric ligand-gated ion channel. *Nat. Commun.* 15, 25
9. Petroff 2nd, J.T. *et al.* (2022) Open-channel structure of a pentameric ligand-gated ion channel reveals a mechanism of leaflet-specific phospholipid modulation. *Nat. Commun.* 13, 7017
10. Tao, X. *et al.* (2023) Membrane protein isolation and structure determination in cell-derived membrane vesicles. *Proc. Natl. Acad. Sci. U. S. A.* 120, e2302325120