Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Structural Biology

Nanodisc-reconstitution for single particle cryo-EM structure determination of membrane proteins

Arshay J. Grant[1] and Ingeborg Schmidt-Krey[1,2]

Reconstitution of membrane proteins in nanodiscs has proven to be a highly effective approach to study membrane protein structures in a lipid bilayer, resulting in many recent single particle cryo-EM structures. While most of these studies employed membrane scaffold protein (MSP) nanodiscs, additional types of nanodiscs were developed based on MSPs and provide alternative approaches. Nanodiscs have been particularly effective in solving structures of different protein conformations and of bound lipids, demonstrating key roles of specific lipids in structural integrity and protein function. At the same time, discrepancies of lipid behavior in nanodiscs compared to native membranes and liposomes necessitate careful scrutiny of reconstitution parameters and further evaluation. This brief review covers an overview of types of nanodiscs currently in use for cryo-EM structural studies, their advantages and limitations, as well as examples of the dramatically increased understanding they can reveal.

Addresses
[1] Georgia Institute of Technology, School of Biological Sciences, 310 Ferst Drive, Atlanta, GA 30332, USA
[2] Georgia Institute of Technology, School of Chemistry and Biochemistry, 310 Ferst Drive, Atlanta, GA 30332, USA

Corresponding authors: Schmidt-Krey, Ingeborg (ingeborg.schmidt-krey@biosci.gatech.edu)

Current Opinion in Structural Biology 2025, **93**:103072

This review comes from a themed issue on **Membranes (2025)**

Edited by **Scott Prosser** and **Liguo Wang**

For a complete overview see the Issue and the Editorial

Available online xxx

https://doi.org/10.1016/j.sbi.2025.103072

0959-440X/© 2025 Elsevier Ltd. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

In recent years it has become possible to study membrane protein structure in phospholipid bilayers, which provide key context, especially for specific protein-lipid interactions essential to membrane protein function. Detergents, commonly used to extract membrane proteins during purification and an important approach to study structures of detergent-solubilized membrane proteins, can impact structural and thus functional integrity as well as disrupt important protein-lipid interactions for some membrane proteins [1,2]. Examples include structures of the ABC transporter MsbA that showed lower ATPase activity and pLGIC that may be devoid of several critical lipids [3–5]. The development of nanodiscs and membrane scaffold proteins made it possible to study isolated membrane proteins within a defined membrane bilayer [6–10]. Rat TRPV1 tetramer cryo-EM structures (∼380 kDa) at resolutions of 2.9 Å –3.4 Å were the first structures of a nanodisc-reconstituted membrane protein [11]. The rTRPV1-MSP2N2 structure visualized critical protein-lipid interactions, particularly for regulatory lipids and stabilization of the resting state by phosphatidylinositol. This breakthrough by the Cheng laboratory in 2016 paved the way for membrane protein structural biologists to use nanodiscs [11], including the Moiseenkova-Bell laboratory, where nanodiscs helped to explain channel gating mechanisms of apo-rTRPV2 [12]. Another early example is the 3.5 Å-resolution structure of the multi-subunit yeast vacuolar ATPase V_o proton channel, which provided an isotropic reconstruction where the proton translocation mechanism was further clarified [13].

This concise review provides recent examples of different types of nanodiscs used for single particle cryo-EM integral membrane protein structure determination (Table 1), new applications for peripheral membrane proteins, and an overview of current advantages and limitations. The examples show how nanodiscs can yield structures of several conformations and reveal the high significance of specific lipids.

Nanodisc development

The development of nanodiscs became possible through the extensive characterization of apolipoproteins by Segrest and colleagues [6–8]. Bayburt et al. described membrane protein reconstitution into nanometer-range phospholipid bilayers using human apolipoprotein-A, followed by the development of genetically engineered membrane scaffold protein (MSP) for bilayer formation [9]. MSPs are used to generate nanodiscs, defined membrane bilayers encircled by two MSPs [10]. The MSP type decides the diameter, ranging from ∼8 nm to 17 nm [14–17].

Nanodiscs are now routinely used to study the structure of membrane proteins, particularly by cryo-EM [16–20] (Figure 1) but also with much promise for NMR

Table 1

Examples of recent cryo-EM membrane protein structures solved in different membrane mimetics (2022–2024).

Protein	Membrane mimetic	Lipid composition	Accession ID	Comments
Propofol bound-HCN1 (Human) [48]	MSP1E3	DOPC:POPE:POPS (5:3:2)	EMD-42116	Nanodiscs showed binding sites
TRPV1 (Human) [41]	cNW11	POPC:POPE:POPG (3:1:1) soybean polar extract	EMD-29982 EMD-29981	Higher resolution in mixture, more complete structure in soybean lipids
Channel rhodopsins *Hc*KCR1 & *Hc*CCR (*Hyphochytrium catenoides*) [33]	Peptidisc	Annular lipids	EMD-40062 EMD 40063	First cryo-EM structures in peptidiscs
Pannexin-1 (Mouse) [43]	Salipro DirectMX	Endogenous lipids	PDB 8A3B	First cryo-EM structure using DirectMX[a]
Propylamine bound-ELIC (*Eriwinia chrysanthemi)* [44]	SMA Saposin spMSP1D1 spNW15	POPC: POPE:POPG (2:1:1) used for all systems	EMD-28829 EMD-28830 EMD-28831 EMD-41673	spNW15 shows conformational changes for agonist bound structure
AP2 (Human) [45]	MSP2N2	DOPC:DOPS:PIP_2 (75 %, 15 %, 10 %)	EMD-40035	First cryo-EM[b] structure of peripheral MP[c] with PIP_2 interaction

[a] Propriety technology; currently not generally accessible.

[b] At higher resolution.

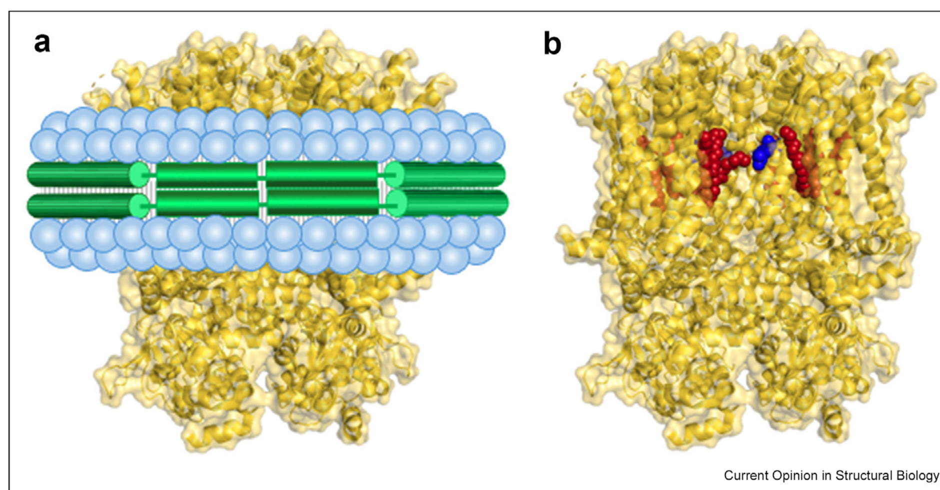
[c] Membrane protein.

spectroscopy [21,22]. While still not trivial to prepare for structure determination, they offer advantages such as the ability to modify lipid composition, choices of diameters, and accessibility of both bilayer sides [14,19,21]. MSP nanodiscs have facilitated cryo-EM of membrane proteins with molecular weights from ∼60 kDa to 2 MDa [23,24].

Preparation of homogeneous nanodiscs requires testing of lipid types, the solubilizing detergent for the membrane protein, and the stoichiometry between protein, lipid, and MSP [25]. Reconstitution efficiency can be controlled by considering lipid chain length and head group diameter [25].

Molecular dynamics simulations are contributing to an emerging description of the impact of lipids on protein structure and their dynamics within nanodiscs [25–27]. Mörs et al. [25] demonstrated increased order of lipids in nanodiscs and thus their bilayer thickness,

Figure 1



An example of a single particle cryo-EM structure of an MSP-nanodisc-reconstituted membrane protein. (a) A schematic of an MSP2N2-nanodisc encircling the HCN1 structure (PDB ID: 8UC7). The schematic MSP α-helices are indicated by green cylinders and the lipid head groups by light blue circles. **(b)** HCN1 without the nanodisc. The presence of a membrane bilayer was essential to obtain the HCN1 structure with propofol (blue) bound [52]. Essential lipids are in red. The figures were prepared with PyMOL [54].

constraining protein dynamics via the lateral pressure of ordered lipids and MSP. Thus, for a given membrane protein, the lateral pressure in the native membranes will ideally need to be considered during nanodisc optimization.

Types of nanodiscs

MSP lipid nanodiscs have laid the foundation to develop additional nanodisc and bilayer mimetic approaches [18]. These include cNW nanodiscs, peptidiscs, and saposins [28–31]. Typically, a particular membrane mimetic is chosen or options are tested to optimally reconstitute a membrane protein.

Circularized nanodiscs (cNWs) are covalently linked variants based on Apolipoprotein-A1 from traditional MSP nanodiscs [28]. This strategy increases nanodisc size homogeneity and offers a choice of diameters between ∼9 nm and 80 nm. SpyCatcherSpyTag technology has enhanced the cNW effectiveness via a 10-fold increase of spNW yield and significantly fewer experimental steps [32]. The homogenous spNWs nanodiscs are up to 100 nm.

Peptidiscs are amphipathic bi-helical peptides (NSP_r) that encircle membrane proteins to replace the bilayer, with a 11.7 ± 1.4 nm peptidisc diameter for MalK2 [30]. This reconstitution strategy does not require additional lipids and size adjustments to fit a membrane protein target and has resulted in recent structures [33,34].

Saposin A is another reconstitution approach based on its distinctive membrane binding properties, yielding square 12 nm × 12 nm nanoparticles [31]. Saposin A reconstitution requires lipids and detergents in the purification buffer. DirectMX further developed this proprietary approach for direct saposin A-lipoprotein nanoparticles (Salipro) protein extraction from crude cell membranes without detergent [35].

Styrene-maleic acid (SMA) copolymers insert into bilayers to extract membrane proteins and native lipids [29]. Kamilar et al. found that SMA lipid particles (SMALPS) do not possess many characteristics of native nanodiscs [36]. Their use results in a mixture of nanodiscs and non-nanodisc lipid-detergent particles, and the differing lipid environments can give rise to various protein states. Thus, careful separation and analysis is recommended [36]. A cryo-EM study revealed that SMA reconstitution can hinder the ability to resolve transmembrane domain regions, possibly due to the heterogeneous nature of SMA polymers [37]. To prevent heterogeneity, RAFT polymerization and SMA modification with benzylamine (SMA-BA) were tested [38]. Dynamic light scattering analysis indicated improved homogeneity. Although SMA-BA appears promising, magnesium sensitivity assays indicate an increase in divalent cation sensitivity.

Ion channel conformational states in MSP-nanodiscs

Crystal structures of the detergent-solubilized ligand-gated ion channel pLGIC (GLIC) provided a first important interpretation but this strategy is not amenable to elucidating conformational changes in the presence of lipids [39]. Cryo-EM structures of MSP nanodisc-reconstituted GLIC at pH 7.5 to 4.0 revealed conformational details [40]. Comparisons between the detergent-solubilized GLIC structure and the GLIC-nanodisc structure showed conformational differences within the extracellular domain (ECD). Most strikingly, at pH 4.0 GLIC demonstrated an intermediate, pre-open, and open state. Lipids were found to be the key driver of these functional states.

TRPV1 in cNW-nanodiscs

The Sobolevsky laboratory solved multiple cryo-EM membrane protein structures with the use of cNWs. To determine human apo and antagonist-bound TRPV1 structures, circularized nanodiscs (cNW11) were used for reconstitution with either a POPC:POPE:POPG mixture or soybean lipids [41]. Interestingly, the later resulted in a lower resolution (2.90 Å) than TRPV1 reconstituted in the synthetic lipid mixture (2.58 Å resolution). Yet while lower in resolution, the structure in soybean lipids allowed for a more complete reconstruction. Thus, structural studies of related or comparable proteins, or proteins with incompletely resolved structures, may benefit similarly in terms of resolution and completeness by studies in different lipid environments. The apo structure included many annular lipids, which impressively allowed for head and tail region models. A phospohatidylinositol in the vanilloid binding site could be distinguished due to the large head group. Interestingly, the agonist binding mechanism was further clarified with a structure of detergent-solubilized protein.

mPANX1 in saposin-nanoparticles

Several structures of membrane proteins such as lipid flippases, mechanosensitive channels and those relevant to SARS-CoV-2 have been characterized using saposin lipid nanoparticles [5,24,42]. Saposins were considered a useful alternative to nanoparticles and other reconstitution methods, especially due to the extensive detergent exposure during purification. Therefore, direct membrane extraction technology (DirectMX) was developed to omit detergent extraction [35]. Membrane proteins are transferred with their bilayer environment directly from crude cell extract into Saposin A nanoparticles, potentially allowing for the selective removal of specific conformations. Successful mPANX1 channel Salipro-reconstitution was demonstrated by cryo-EM and functional assays [43]. Surface

plasmon resonance studies confirmed that Salipro-mPANX1 could be immobilized and was capable of binding to small molecules as opposed to the detergent-reconstituted mPANX1 that displayed decreased stability. A drawback of this promising approach is the proprietary nature of the DirectMX technology, which is currently not generally accessible to the membrane protein structural biology community.

ELIC prefer spNW

Cryo-EM structures combined with molecular dynamics simulations of ELIC (*Erwinia* ligand-gated ion channel) examined the impact of different nanodiscs on protein conformation, resulting in a comparison of the apo vs. the agonist-bound structure and effects of nanodisc type, diameter, thickness, and transmembrane domain interactions with nanodisc scaffolds [44]. The structure of SMA-reconstituted ELIC was compared to structures of ELIC reconstituted in saposin, MSP1E3D1, spMSP1D1, and spNW15 nanodiscs, using POPC:POPE:POPG (2:1:1). The apo structures did not differ, while the agonist-bound structure showed notable deviations. A smaller nanodisc diameter led to structural perturbation attributed to constraints of the limited diameter and/or the direct interactions with the scaffold. The 9 nm-nanodiscs had a thinner bilayer compared to 11 nm-nandiscs and proteoliposomes, which affected the TMD conformation. The authors reasoned that larger nanodiscs allow for more extensive conformational changes in the presence of agonist. Thus, they recommended spNW15 for ELIC, even though ELIC is not necessarily centered and has heterogeneous positions. These findings provide important considerations for studying other membrane proteins in different nanodiscs, where an agonist may or may not have the same impact. Depending on the native environment specific to different proteins, smaller nanodiscs may mimic crowding, or larger nanodiscs may be required for substantial conformational changes. The study also demonstrated the importance of verifying structural data with additional biochemical and biophysical characterization before and after structure determination.

Peripheral membrane protein structures

Nanodiscs also provide an important means to solve peripheral membrane protein structures. The Baker laboratory demonstrated feasibility by solving the structure of a peripheral membrane protein associated with a nanodisc via the 3.3 Å-resolution cryo-EM structure of the AP2 clathrin adaptor complex (AP2) [45]. AP2 bound to ∼9 nm-nanodiscs yielded a low-resolution structure in a nonnative conformation, while larger 17 nm-nanodiscs allowed membrane bound-AP2 and a critical PIP2 head group interaction to be characterized in detail. These findings highlight the importance of testing diverse scaffolds to ensure an appropriate membrane surface area. The availability of various nanodiscs will support the investigation of many more peripheral membrane proteins associated with bilayers.

Conclusions

Hundreds of new cryo-EM structures of nanodisc-reconstituted membrane proteins have been deposited in the EMDB in recent years. Notably, structures of several different conformations often elucidated mechanisms. These structures have expanded our understanding of the frequently high significance of lipids. MSP nanodiscs contributed most of the structures, where the options for diameters could accommodate different membrane protein sizes. At times conformational changes have been shown to require larger nanodiscs than the protein diameter indicates [42,45,46]. cNWs and spNWs offer substantially larger diameters to accommodate integral or peripheral membrane proteins [28–32]. Interestingly, the resolutions of structures from cNWs were higher than 3.0 Å. While this could be linked to cNWs, resolution is bound to increase with optimization of all nanodisc types, optimized lipid contents, and the continuously developing cryoEM strategies. The proprietary DirectMX avoids detergents and retains the native bilayer [35]. Peptidiscs have a similar advantage, without the need to consider size adjustments [30].

The nanodisc application to peripheral membrane proteins in their interactions with membranes will quickly expand [45]. Fusion proteins, Nanobodies, Legobodies, and NabFabs will allow us to understand even smaller membrane proteins in nanodiscs [47–49]. Furthermore, Nanobodies can be used to stabilize proteins or complexes [47,50]. Cell-free expression will undoubtedly assist in studying sensitive membrane proteins.

Nanodiscs can provide key details on annular and integral lipids, lipid–protein interactions, and substrate binding [51,52]. The examples appear to indicate that use may change to larger nanodiscs with space to allow for conformational changes. New membrane protein structures in bilayers and additional research on nanodisc lipid phase behavior, lateral pressure, and the possibly size-dependent curvature of nanodiscs will shed light on the impact of nanodiscs on structure and function and vice versa. Modulation of lipid content and nanodisc sizes was found to be critical. Thus, testing and structure determination with different lipids and nanodisc diameters may become routine to fully understand the possible impact of specific lipids, similarly as done with mutations to understand the detailed role of amino acids in protein structure. Lipids are destined to play an increasingly important role in membrane protein structure investigations as further emphasized by these recent structures, where nanodiscs and other membrane-based approaches will serve as the main tools. Growing focus on nanodisc lipid content and

characteristics will ensure that nanodisc cryo-EM structures are solved under physiologically relevant membrane conditions and within the context of the evolving understanding of membrane zones [53].

Declaration of competing interest

We have nothing to declare.

Data availability

No data was used for the research described in the article.

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
* * of outstanding interest

1. Yang Z, Wang C, Zhou Q, An J, Hildebrandt E, Aleksandrov LA, Kappes JC, DeLucas LJ, Riordan JR, Urbatsch IL, Hunt JF, Brouillette CG: **Membrane protein stability can be compromised by detergent interactions with the extramembranous soluble domains**. *Protein Sci : Publ Protein Soc* 2014, **23**: 769–789, https://doi.org/10.1002/pro.2460.

2. Guo Y: **Be cautious with crystal structures of membrane proteins or complexes prepared in detergents**. *Crystals* 2020, **10**:86, https://doi.org/10.3390/cryst10020086.

3. Hilf RJ, Dutzler R: **X-ray structure of a prokaryotic pentameric ligand-gated ion channel**. *Nature* 2008, **452**:375–379, https://doi.org/10.1038/nature06717.

4. Basak S, Schmandt N, Gicheru Y, Chakrapani S: **Crystal structure and dynamics of a lipid-induced potential desensitized-state of a pentameric ligand-gated channel**. *Elife* 2017, **6**, e23886, https://doi.org/10.7554/eLife.23886.

5. * Kehlenbeck DM, Traore DAK, Josts I, Sander S, Moulin M, Haertlein M, Prevost S, Forsyth VT, Tidow H: **Cryo-EM structure of MsbA in saposin-lipid nanoparticles (Salipro) provides insights into nucleotide coordination**. *FEBS J* 2022, **289**:2959–2970, https://doi.org/10.1111/febs.16327.
An example of saposin-nanoparticle-reconstitution contributing to membrane protein characterization by improving cryo-EM resolution.

6. Segrest JP, Jones MK, Klon AE, Sheldahl CJ, Hellinger M, De Loof H, Harvey SC: **A detailed molecular belt model for apolipoprotein A-I in discoidal high density lipoprotein**. *J Biol Chem* 1999, **274**, https://doi.org/10.1074/jbc.274.45.31755. 31755.-17558.

7. Segrest JP, Li L, Anantharamaiah GM, Harvey SC, Liadaki KN, Zannis V: **Structure and function of apolipoprotein A-I and high-density lipoprotein**. *Curr Opin Lipidol* 2000, **11**:105–115, https://doi.org/10.1097/00041433-200004000-00002.

8. Segrest JP, Jones MK, Mishra VK, Anantharamaiah GM: **Experimental and computational studies of the interactions of amphipathic peptides with lipid surfaces**. *Curr Top Membr* 2002, **52**:397–435.

9. Bayburt TH, Carlson JW, Sligar SG: **Reconstitution and imaging of a membrane protein in a nanometer-size phospholipid bilayer**. *J Struct Biol* 1998, **123**:37–44, https://doi.org/10.1006/jsbi.1998.4007.

10. Bayburt TH, Grinkova YV, Sligar SG: **Self-assembly of discoidal phospholipid bilayer nanoparticles with membrane scaffold proteins**. *Nano Lett* 2002, **2**:853–856, https://doi.org/10.1021/nl025623k.

11. Gao Y, Cao E, Julius D, Cheng Y: **TRPV1 structures in nanodiscs reveal mechanisms of ligand and lipid action**. *Nature* 2016, **534**:347–351, https://doi.org/10.1038/nature17964.

12. Pumroy RA, Samanta A, Liu Y, Hughes TE, Zhao S, Yudin Y, Rohacs T, Han S, Moiseenkova-Bell VY: **Molecular mechanism of TRPV2 channel modulation by cannabidiol**. *Elife* 2019, **8**, e48792, https://doi.org/10.7554/eLife.48792.

13. Roh SH, Stam NJ, Hryc CF, Couoh-Cardel S, Pintilie G, Chiu W, Wilkens S: **The 3.5-å CryoEM structure of nanodisc-reconstituted yeast vacuolar ATPase V_o proton channel**. *Mol Cell* 2018, **69**:993–1004.e3, https://doi.org/10.1016/j.molcel.2018.02.006.

14. Denisov IG, Grinkova YV, Lazarides AA, Sligar SG: **Directed self-assembly of monodisperse phospholipid bilayer Nanodiscs with controlled size**. *J Am Chem Soc* 2004, **126**: 3477–3487, https://doi.org/10.1021/ja0393574.

15. Grinkova YV, Denisov IG, Sligar SG: **Engineering extended membrane scaffold proteins for self-assembly of soluble nanoscale lipid bilayers**. *Protein Eng Des Sel : PEDS* 2010, **23**: 843–848, https://doi.org/10.1093/protein/gzq060.

16. Denisov IG, Sligar SG: **Nanodiscs for structural and functional studies of membrane proteins**. *Nat Struct Mol Biol* 2016, **23**: 481–486, https://doi.org/10.1038/nsmb.3195.

17. Strickland KM, Neselu K, Grant AJ, Espy CL, McCarty NA, Schmidt-Krey I: **Reconstitution of detergent-solubilized membrane proteins into proteoliposomes and nanodiscs for functional and structural studies**. *Methods Mol Biol* 2021, **2302**: 21–35, https://doi.org/10.1007/978-1-0716-1394-8_2.

18. * Young JW: **Recent advances in membrane mimetics for membrane protein research**. *Biochem Soc Trans* 2023, **51**: 1405–1416, https://doi.org/10.1042/BST20230164.
An in-depth review of lipid bilayer mimetics detailing their use for structure–function characterization of membrane proteins.

19. * Pettersen JM, Yang Y, Robinson AS: **Advances in nanodisc platforms for membrane protein purification**. *Trends Biotechnol* 2023, **41**:1041–1054, https://doi.org/10.1016/j.tibtech.2023.02.006.
A detailed review of types of nanodiscs, protocols for nanodisc optimization, and detailed applications.

20. Denisov IG, Sligar SG: **Nanodiscs for the study of membrane proteins**. *Curr Opin Struct Biol* 2024, **87**, 102844, https://doi.org/10.1016/j.sbi.2024.102844.

21. ** Huang SK, Picard LP, Rahmatullah RSM, Pandey A, Van Eps N, Sunahara RK, Ernst OP, Sljoka A, Prosser RS: **Mapping the conformational landscape of the stimulatory heterotrimeric G protein**. *Nat Struct Mol Biol* 2023, **30**:502–511, https://doi.org/10.1038/s41594-023-00957-1.
An exceptional NMR study exploring conformational equilibria of nanodisc-reconstituted GPCR by fluorine nuclear magnetic resonance.

22. Hagn F, Etzkorn M, Raschle T, Wagner G: **Optimized phospholipid bilayer nanodiscs facilitate high-resolution structure determination of membrane proteins**. *J Am Chem Soc* 2013, **135**:1919–1925, https://doi.org/10.1021/ja310901f.

23. Nayak AR, Samsó M: **Ca^{2+} inactivation of the mammalian ryanodine receptor type 1 in a lipidic environment revealed by cryo-EM**. *Elife* 2022, **11**, e75568, https://doi.org/10.7554/eLife.75568.

24. Miller AN, Houlihan PR, Matamala E, Cabezas-Bratesco D, Lee GY, Cristofori-Armstrong B, Dilan TL, Sanchez-Martinez S, Matthies D, Yan R, Yu Z, Ren D, Brauchi SE, Clapham DE: **The SARS-CoV-2 accessory protein Orf3a is not an ion channel, but does interact with trafficking proteins**. *Elife* 2023, **12**, e84477, https://doi.org/10.7554/eLife.84477.

25. Mörs K, Roos C, Scholz F, Wachtveitl J, Dötsch V, Bernhard F, Glaubitz C: **Modified lipid and protein dynamics in nanodiscs**. *Biochim Biophys Acta* 2013, **1828**:1222–1229, https://doi.org/10.1016/j.bbamem.2012.12.011.

26. Plummer-Medeiros AM, Culbertson AT, Morales-Perez CL, Liao M: **Activity and structural dynamics of human ABCA1 in a lipid membrane**. *J Mol Biol* 2023, **435**, 168038.

27. Wang B, Tieleman DP: **The structure, self-assembly and dynamics of lipid nanodiscs revealed by computational approaches**. *Biophys Chem* 2024, **309**, 107231.

28. Nasr ML, Baptista D, Strauss M, Sun ZJ, Grigoriu S, Huser S, Plückthun A, Hagn F, Walz T, Hogle JM, Wagner G: **Covalently circularized nanodiscs for studying membrane proteins and viral entry**. *Nat Methods* 2017, **14**:49–52, https://doi.org/10.1038/nmeth.4079.

29. Knowles TJ, Finka R, Smith C, Lin YP, Dafforn T, Overduin M: **Membrane proteins solubilized intact in lipid containing nanoparticles bounded by styrene maleic acid copolymer**. *J Am Chem Soc* 2009, **131**:7484–7485, https://doi.org/10.1021/ja810046q.

30. Carlson ML, Young JW, Zhao Z, Fabre L, Jun D, Li J, Li J, Dhupar HS, Wason I, Mills AT, Beatty JT, Klassen JS, Rouiller I, Duong F: **The Peptidisc, a simple method for stabilizing membrane proteins in detergent-free solution**. *Elife* 2018, **7**, e34085, https://doi.org/10.7554/eLife.34085.

31. Frauenfeld J, Löving R, Armache JP, Sonnen AF, Guettou F, Moberg P, Zhu L, Jegerschöld C, Flayhan A, Briggs JA, Garoff H, Löw C, Cheng Y, Nordlund P: **A saposin-lipoprotein nanoparticle system for membrane proteins**. *Nat Methods* 2016, **13**: 345–351, https://doi.org/10.1038/nmeth.3801.

32. Zhang S, Ren Q, Novick SJ, Strutzenberg TS, Griffin PR, Bao H: **One-step construction of circularized nanodiscs using SpyCatcher-SpyTag**. *Nat Commun* 2021, **12**:5451, https://doi.org/10.1038/s41467-021-25737-7.

33. Morizumi T, Kim K, Li H, Govorunova EG, Sineshchekov OA, Wang Y, Zheng L, Bertalan É, Bondar AN, Askari A, Brown LS, Spudich JL, Ernst OP: **Structures of channelrhodopsin paralogs in peptidiscs explain their contrasting K^+ and Na^+ selectivities**. *Nat Commun* 2023, **14**:4365, https://doi.org/10.1038/s41467-023-40041-2.

34. Gao Y, Zhang Y, Hakke S, Mohren R, Sijbers LJPM, Peters PJ, Ravelli RBG: **Cryo-EM structure of cytochrome bo_3 quinol oxidase assembled in peptidiscs reveals an "open" conformation for potential ubiquinone-8 release**. *Biochim Biophys Acta Bioenerg* 2024, **1865**, 149045, https://doi.org/10.1016/j.bbabio.2024.149045.

35. * Lloris-Garcerá P, Klinter S, Chen L, Skynner MJ, Löving R, Frauenfeld J: **DirectMX - one-step reconstitution of membrane proteins from crude cell membranes into Salipro nanoparticles**. *Front Bioeng Biotechnol* 2020, **8**:215, https://doi.org/10.3389/fbioe.2020.00215.
DirectMX is a novel, proprietary approach to extract membrane proteins from crude cell extract without the use of detergent while retaining the native lipid environment.

36. Kamilar E, Bariwal J, Zheng W, Ma H, Liang H: **SMALPs are not simply nanodiscs: the polymer-to-lipid ratios of fractionated SMALPs underline their heterogeneous nature**. *Biomacromolecules* 2023, **24**:1819–1838.

37. Catalano C, Ben-Hail D, Qiu W, Blount P, des Georges A, Guo Y: **Cryo-EM structure of mechanosensitive channel Ynal using SMA2000: challenges and opportunities**. *Membranes* 2021, **11**:849, https://doi.org/10.3390/membranes11110849.

38. Akram A, Hadasha W, Kuyler GC, Smith M-P, Bailey-Dallaway S, Preedy A, Browne C, Broadbent L, Hill A, Javaid T, Nazar H, Samra N, Naveed A, Tregunna H, Joshi H, Akhtar N, Javed A, Bowater J, Ravenhill J, … Rothnie AJ: **Solubilisation & purification of membrane proteins using benzylamine-modified SMA polymers**. *Biophys Chem* 2025, **316**, 107343, https://doi.org/10.1016/j.bpc.2024.107343.

39. Dellisanti CD, Ghosh B, Hanson SM, Raspanti JM, Grant VA, Diarra GM, Schuh AM, Satyshur K, Klug CS, Czajkowski C: **Site-directed spin labeling reveals pentameric ligand-gated ion channel gating motions**. *PLoS Biol* 2013, **11**, e1001714, https://doi.org/10.1371/journal.pbio.1001714.

40. Bharambe N, Li Z, Seiferth D, Balakrishna AM, Biggin PC, Basak S: **Cryo-EM structures of prokaryotic ligand-gated ion channel GLIC provide insights into gating in a lipid environment**. *Nat Commun* 2024, **15**:2967, https://doi.org/10.1038/s41467-024-47370-w.

41. Neuberger A, Oda M, Nikolaev YA, Nadezhdin KD, Gracheva EO, Bagriantsev SN, Sobolevsky AI: **Human TRPV1 structure and inhibition by the analgesic SB-366791**. *Nat Commun* 2023, **14**: 2451, https://doi.org/10.1038/s41467-023-38162-9.

42. Zhang J, Maksaev G, Yuan P: **Open structure and gating of the Arabidopsis mechanosensitive ion channel MSL10**. *Nat Commun* 2023, **14**:6284, https://doi.org/10.1038/s41467-023-42117-5.

43. Drulyte I, Gutgsell AR, Lloris-Garcerá P, Liss M, Geschwindner S, Radjainia M, Frauenfeld J, Löving R: **Direct cell extraction of membrane proteins for structure-function analysis**. *Sci Rep* 2023, **13**:1420, https://doi.org/10.1038/s41598-023-28455-w.

44. * Dalal V, Arcario MJ, Petroff JT, Tan BK, Dietzen NM, Rau MJ, Fitzpatrick JAJ, Brannigan G, Cheng WWL: **Lipid nanodisc scaffold and size alter the structure of a pentameric ligand-gated ion channel**. *Nat Commun* 2024, **15**:25, https://doi.org/10.1038/s41467-023-44366-w.
An experimental study comparing nanodisc types, sizes, and compositions and their influence on structures of ELIC.

45. ** Cannon K, Sarsam RD, Tedamrongwanish T, Zhang K, Baker RW: **Lipid nanodiscs as a template for high-resolution cryo-EM structures of peripheral membrane proteins**. *J Struct Biol* 2023, **215**, 107989, https://doi.org/10.1016/j.jsb.2023.107989.
An exceptional study of the first high-resolution structure of a membrane-bound peripheral membrane protein by single particle cryo-EM.

46. Zhao C: **Limitations in membrane protein structure determination by lipid nanodiscs**. *Trends Biochem Sci* 2024, **49**: 475–476, https://doi.org/10.1016/j.tibs.2024.03.010.

47. Wu X, Rapoport TA: **Cryo-EM structure determination of small proteins by nanobody-binding scaffolds (Legobodies)**. *Proc Natl Acad Sci USA* 2021, **118**, e2115001118, https://doi.org/10.1073/pnas.2115001118.

48. Alexander E, Leong KW: **Discovery of nanobodies: a comprehensive review of their applications and potential over the past five years**. *J Nanobiotechnol* 2024, **22**:661, https://doi.org/10.1186/s12951-024-02900-y.

49. Bloch JS, Mukherjee S, Kowal J, Filippova EV, Niederer M, Pardon E, Steyaert J, Kossiakoff AA, Locher KP: **Development of a universal nanobody-binding Fab module for fiducial-assisted cryo-EM studies of membrane proteins**. *Proc Natl Acad Sci U S A* 2021, **118**, e2115435118, https://doi.org/10.1073/pnas.2115435118.

50. Zuo H, Park J, Frangaj A, Ye J, Lu G, Manning JJ, Asher WB, Lu Z, Hu GB, Wang L, Mendez J, Eng E, Zhang Z, Lin X, Grassucci R, Hendrickson WA, Clarke OB, Javitch JA, Conigrave AD, Fan QR: **Promiscuous G-protein activation by the calcium-sensing receptor**. *Nature* 2024, **629**:481–488, https://doi.org/10.1038/s41586-024-07331-1.

51. Feng Z, Alvarenga OE, Accardi A: **Structural basis of closed groove scrambling by a TMEM16 protein**. *Nat Struct Mol Biol* 2024, **31**:1468–1481, https://doi.org/10.1038/s41594-024-01284-9.

52. * Kim ED, Wu X, Lee S, Tibbs GR, Cunningham KP, Di Zanni E, Perez ME, Goldstein PA, Accardi A, Larsson HP, Nimigean CM: **Propofol rescues voltage-dependent gating of HCN1 channel epilepsy mutants**. *Nature* 2024, **632**:451–459, https://doi.org/10.1038/s41586-024-07743-z.
A study showing the importance of using nanodiscs to resolve important drug binding sites.

53. Kervin TA, Overduin M: **Membranes are functionalized by a proteolipid code**. *BMC Biol* 2024, **22**:46, https://doi.org/10.1186/s12915-024-01849-6.

54. The PyMOL molecular graphics system, Version 3.1.3 Schrödinger, LLC.