Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Structural Biology

Advances in native cell membrane nanoparticles system

Weihua Qiu[1,2] and Youzhong Guo[1,2]

The native cell membrane nanoparticles (NCMN) system utilizes membrane-active polymers specifically designed and optimized to extract and stabilize membrane proteins in the form of NCMN particlesfor biochemical and biophysical characterization. The NCMN system is a genuine and advanced detergent-free approach inspired by the membrane activity of the styrene–maleic acid copolymers (SMA), distinguishing it from the nanodisc technology, Salipro technology, and Peptidisc technology. This review introduces the current advancements in the NCMN system, including the development of NCMN polymers, the application of the NCMN system for single-particle cryo-EM analysis, and the functional characterization of membrane proteins.

Addresses
[1] Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond, VA 23298-0540, USA
[2] Center for Drug Discovery, Virginia Commonwealth University, Richmond, VA 23298-0133, USA

Corresponding author: Guo, Youzhong (yguo4@vcu.edu)

Current Opinion in Structural Biology 2025, **94:**103130

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.2025.103130

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

Protein-lipid interactions and the natural structure and function of membrane proteins

Membrane proteins, located in the cell membrane system, play numerous crucial roles in normal physiology and are implicated in various pathological conditions; therefore, they also serve as primary targets for drugs. Both structural and functional information of membrane proteins are essential for understanding the active mechanism or developing better medicines; however, efforts to obtain this information are significantly hindered, essentially because membrane proteins pose notorious challenges to work with *in vitro* due to the lipid environment they rely on for their natural structure and function [1,2]. Traditionally, *in vitro* characterization of membrane proteins depends on small-molecule detergents; however, besides annular lipids, some non-annular lipids are often removed from the transmembrane domain during the extraction and purification process in the presence of detergents [3]. Membrane proteins, particularly those with multiple helices, are noteworthy; the cell membrane environment significantly influences their folding, natural structure, and function. Similarly, membrane proteins from the same protein family in different species may acquire new functions or exhibit distinct protein-lipid interactions for the same function [4]. Several excellent reviews have discussed the importance of protein-lipid interactions. The disruption of these natural protein-lipid interactions can lead to an inaccurate structure and even a misleading interpretation of the active mechanism [3–13]. The most recent and exceptional example demonstrating the essentiality of protein-lipid interactions is the case of the structure-enzyme activity relationships of particular methane monooxygenase (pMMO). Detergent-purified pMMO has no or minimal methane oxidation enzyme activity. In contrast, nanodisc-reconstituted pMMO could only partially but not fully restore the enzyme activity compared with the native cell membrane-associated pMMO [14,15].

Beginning of single-particle cryo-EM analysis of membrane proteins in native cell membrane lipid environments

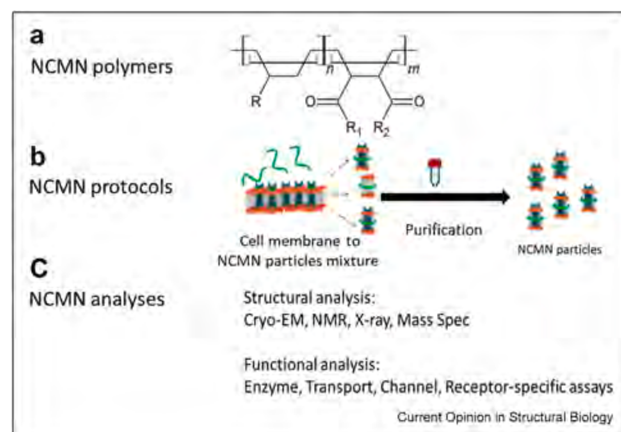
In 2009, styrene–maleic acid copolymer (SMA) was first demonstrated to isolate membrane proteins from the cell membrane as nanoparticles [16]. In 2018, the styrene–maleic acid lipid particle (SMALP) platform was reported for the sub-nm single-particle cryo-EM structure determination of the *E. coli* AcrB [17]; however, the resolution reached only 8.8 Å, and therefore, no structural information on protein-lipid interactions was obtained. Shortly after, the prototype of the native cell membrane nanoparticles system (NCMN system) was used for high-resolution cryo-EM structural analysis of *E. coli* AcrB, which revealed a well-ordered lipid bilayer structure within the transmembrane domain [18]. The asymmetrically native lipid-associated AcrB structures differ remarkably from previously reported symmetric or pseudo-symmetric structures [19–21]. Furthermore, it was shown that detergent application resulted in a loss of the natural lipid patch within the transmembrane

domain, which could lead to significant conformational changes of the transmembrane domain upon a single mutation (D407A)[19]; however, this is not the case in the form of NCMN particles [18]. In the same year, high-resolution structures of Alternative complex III associated with native cell membrane lipids were also determined using the SMA copolymer [22]. The independent structure determination efforts using styrene-maleic acid (SMA) copolymers marked the beginning of the single-particle cryo-EM analysis of membrane proteins in their native lipid environments. The prototypes of membrane-active polymers, such as SMA2000, SMA3000, and (diisobutylene-maleic acid) DIBMA, have significant limitations in their compatibility with low pH conditions and divalent cations [3,23]. Since then, numerous new SMA derivatives and other membrane-active polymers have been developed to overcome these limitations in general membrane protein research [24–38]. However, the developed polymers have rarely been successfully used for high-resolution structure determination, and only a few high-resolution membrane protein structures have been reported, primarily using SMA copolymers [38]. Furthermore, although high-resolution structures were sometimes solved using these polymers, surprisingly, protein-lipid interaction information could not be obtained [39,40]. Considering this situation, a more robust technology development is needed. It was initially known that SMA polymers have limitations in compatibility with low pH conditions and divalent ions; however, another significant limitation was that SMA polymers could also interfere with the transmembrane domain and damage the membrane protein structure [41]. Sometimes, the disruption of the transmembrane domain by SMA or DIMBA copolymers was even more severe than that of small-molecule detergents [3,41,42]. In an earlier review article, Guo suggested that the advanced membrane-active polymers need to meet the following two standards for structural biology [3]: 1) Suitable for maintaining membrane proteins in the same functional states as on the native cell membranes; 2) Suitable for high-resolution structure determination and retaining the structural information of essentially natural protein-lipid interactions.

NCMN system and current advancement

Following the two standards, Guo et al. have focused on developing the native cell membrane nanoparticles system (Figure 1) [3,43–49]. Distinct from SMALP and other detergent-free or detergent-based approaches, the NCMN system emphasizes natural protein-lipid interactions. For example, human membrane proteins should be expressed in human cells but not in insect cells or bacterial cells because the lipid environments do not match those of the membrane proteins. The NCMN system comprises three components: 1) NCMN polymer library. 2) Protocols for making the NCMN particles of

Figure 1



Native cell membrane nanoparticles system (NCMN system). The NCMN system comprises three components: **(a)** NCMN polymers for stabilizing the membrane proteins; **(b)** optimized NCMN protocols for preparation of NCMN particles; and **(c)** customized protocols for various structural and functional characterization of the membrane proteins in the form of NCMN particles.

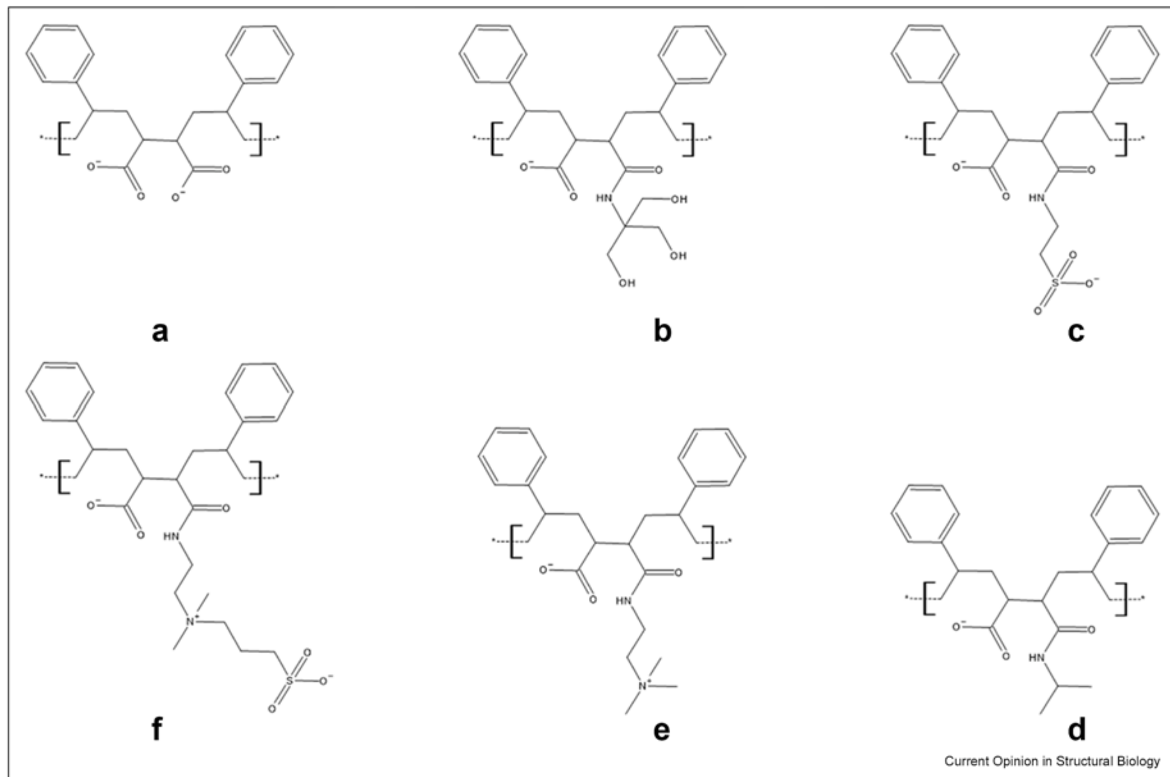
membrane proteins. 3) Analytical approaches for characterization of NCMN particles, including but not limited to cryo-EM analysis, (nuclear magnetic resonance) NMR analysis, X-ray crystallographic analysis, mass spectrometry analysis, and various functional assays for transporters, channels, receptors, and enzymes. In this mini-review, the current technological developments of the NCMN system will be introduced, covering NCMN polymer development, the application of the NCMN system for single-particle cryo-EM analysis of membrane proteins, and biochemical and biophysical characterizations of membrane proteins in the form of NCMN particles. Finally, the perspectives of the NCMN system in membrane protein structure biology and drug discovery will be briefly discussed.

NCMN polymer development

From prototype membrane-active polymers to the NCMNPx-y series library

Prototype membrane-active polymers refer to completely hydrolyzed commercially available styrene–maleic anhydride (SMA) copolymers such as SMA 1000, SMA 2000, SMA 3000, SMA 1440, SMA 2021, and SMA 17352 from Cray Valley, and diisobutylene maleic acids (DIBMA) from BASF (Sokalan® CP 9) and others. Among them, SMA2000 (NCMNP1-1 as indexed in the NCMN polymer library) is the most popular one (Figure 2a); however, the prototype membrane-active polymers including SMA2000 have significant limitations [3,41,50]: 1) They are not compatible with lower pH conditions and usually only work well in solubilized cell membranes at the pH value above 7; 2) They are not

Figure 2



General structures of representative NCMN polymers. (a) NCMNP1-1; **(b)** NCMNP2a-x; **(c)**. NCMNP7-x; **(d)**. NCMNP13-x; **(e)** NCMNP20-x; **(f)** NCMNP21b-x.

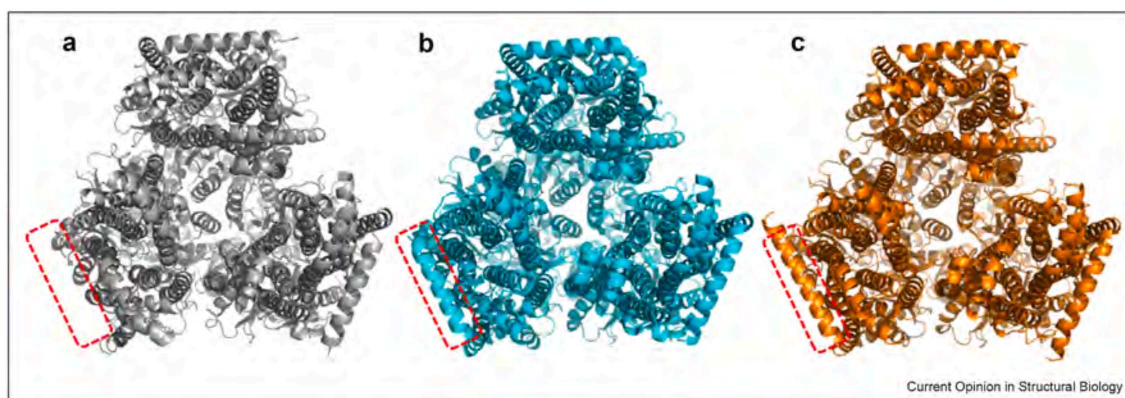
compatible with divalent cations, such as $Mg^{2+}$and Ca^{2+}; 3) They did not work well for membrane proteins with flexible transmembrane domain in terms of high-resolution cryo-EM analysis; 4) They did not work well to maintain the enzyme activities of mammalian membrane protein enzymes.

We developed the first-generation NCMN polymers by fine-tuning the chemical and physical properties of the prototype membrane-active polymers to enhance the compatibility of the NCMN polymer series with divalent ions and low pH conditions. We also ensure their suitability for high-resolution structure determination and functional characterizations of membrane proteins.

NCMNP2a-x series. To overcome the limitations of SMA copolymers and investigate the potential influence of reducing carboxylic acid groups on polymer properties and subsequent membrane protein solubilization, we developed a novel series of NCMNP2a-x copolymers (Figure 2b) [43]. The NCMNP2a-x series polymers exhibit increased compatibility in lower pH conditions: NCMNP2a-5 is compatible with pH 5, NCMNP2a-25 is compatible with pH 4.0, NCMNP2a-50 is compatible with pH 3.0, and NCMNP2a-70 is compatible with pH 2.0. While the compatibility with the pH range has expanded, the NCMNP2a-x polymers do not exhibit significant compatibility with divalent ions, such as calcium. NCMNP2a-5, 25, and 50 are suitable for solubilizing a membrane protein for high-resolution structure determination. The high-resolution cryo-EM structure *of E. coli* AcrB, with associated native cell membrane lipids, was successfully determined. This new structure is superior to the one solved with SMA2000, as previously reported. SMA2000 may have distorted one of the alpha helices (1α helix), which is parallel to the cell membrane. Compared with the SMA 2000, the NCMNP2a-x polymers stabilize the structure of the more flexible region, such as the outer helix (Iα helix), effectively (Figure 3) [43]. Furthermore, the TSPO enzyme activity assay demonstrated that NCMNP2a-50 is suitable for characterizing membrane proteins under lower pH conditions, specifically at pH levels of 3−4 [43]. Membrane-active polymers compatible with low pH conditions are necessary for characterizing membrane proteins in acidic environments, such as those found in locally acidic cellular compartments, including lysosomes and mitochondria.

NCMNP7 series. To overcome the divalent cation compatibility issue and maintain pH compatibility, we

Figure 3



Local structural differences of *E. coli* AcrB resulted from using SMA2000 and NCMNP2a-x. The structural differences in local regions are red-outlined. **(a)** AcrB structure missing the Iα helix resulted from using SMA2000 (PDB: 6BAJ) [18]; **(b)** AcrB structure resulted from using NCMNP2a-50 (PDB: 7RR7) [43]; **(c)** The AcrB structure was obtained using NCMNP2a-5 (PDB: 7RR8) [43].

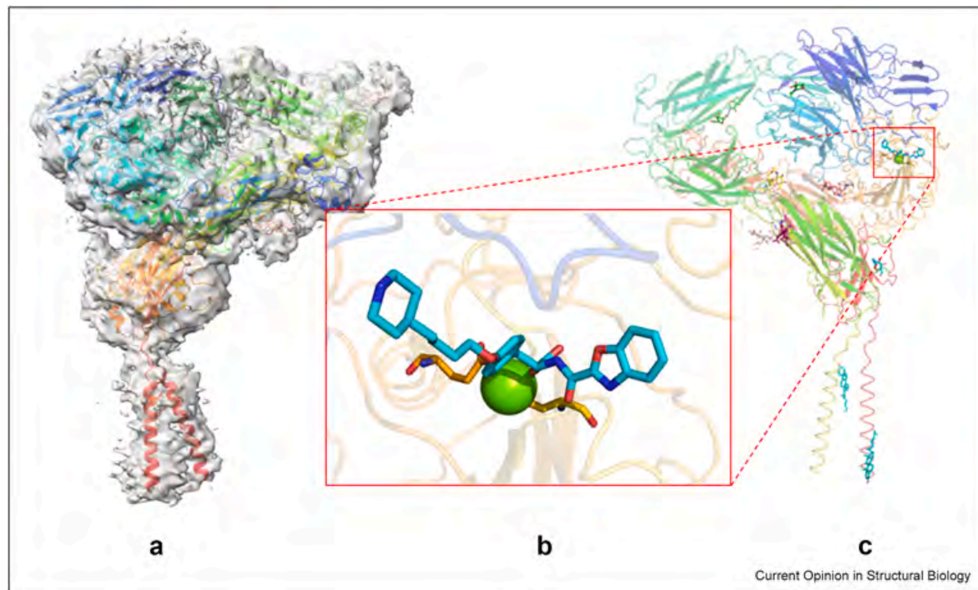
developed the NCMNP7-x series polymer (Figure 2c). The difference between NCMNP7-x and NCMNP2a is the grafted functional group. Instead of using Tris in the case of NCMNP2a-x, we grafted taurine to the carboxyl group in the case of NCMNP7-x (Figure 2b and c). The resulting NCMNP7-x is more compatible with lower pH conditions and divalent ions. It is also suitable for isolating and purifying membrane proteins, as well as for subsequent high-resolution cryo-EM analysis of various membrane proteins [45,47,51,52].

MCMNP13-x, NCMNP21-x, and NCMNP21b-x series and others. To explore the structure—activity relationship, we also designed MCMNP13-x, NCMNP21-x, and NCMNP21b-x series polymers (Figure 2d, e, and f) [44]. The results show that all polymers could solubilize the model membrane proteins. However, the morphologies of the NCMN particles differ significantly. For example, NCMNP13-50 and NCMNP21b-20 produced nice homogeneous single particles; however, NCMNP21-20 produced amorphous precipitates and NCMMP21b-30 produced a mixture of homogeneous single particles and large lipid bilayer patches. The structure—activity relationship analysis suggests that NCMNP21b-x polymers have vastly improved compatibility with divalent ions and a wider pH range, making them suitable for membrane protein structural biology. Furthermore, we have also developed other NCMN polymers, which have been collected into the first generation of the NCMN polymer library, with some having patents pending [49,53,54].

The NCMN system for structural biology and structure-based drug design

Initially, the prototype NCMN system was used to solve a high-resolution single-particle cryo-EM structure of AcrB associated with native cell membrane lipids [18]. The lipid bilayer patch within the transmembrane domain formed a well-organized structure. Since then, multiple other membrane protein structures, including transporters, channels, enzymes, and receptors from bacteria and humans, have also been determined using the NCMN system (to be published separately). Here, the structure determination of human integrin demonstrates the NCMN system's suitability for high-resolution cryo-EM analysis of human membrane proteins and structure-based drug design. Integrins are the primary receptors that animal cells employ to establish a connection with the extracellular matrix. They are heterodimers that are transmembrane linkers between the actin cytoskeleton and the extracellular matrix. Obtaining a high-resolution full-length integrin structure is very challenging. Collaborating with Amin Arnaout et al. , the NCMN system was used to successfully solve the first full-length, high-resolution cryo-EM structure of an integrin family member, specifically the human platelet integrin αIIbβ3 (Figure 4) [51,52]. It is worth noting that the integrin αIIbβ3 was purified from a natural source, the blood platelet membrane, without the use of any affinity tag. This case aligns well with the philosophy behind the NCMN system, namely, creating cell membrane nanoparticles from natural cells. Furthermore, physiologically functional integrin αIIbβ3 complexes need the presence of Mg^{2+} and Ca^{2+}. The cryo-EM structure reveals the binding of Mg^{2+} and Ca^{2+} to the integrin, demonstrating that the NCMN system is superior to the prototype of membrane-active polymers, such as SMA2000, in its higher compatibility with divalent cations (Figure 3b) [52]. Recently, using the NCMN system, we also solved the high-resolution cryo-EM structure of integrins αIIbβ3 with an improved antagonist, m-tirofiban (Figure 4b and c) [52].

Figure 4



Full-length cryo-EM structure of human integrin αIIβ3 in NCMN particles. (a) Full-length apo-state integrin αIIβ3 structure (PDB: 8T2V); **(b)** Enlarged m-tirofiban (cyan color) coordinates with Mg^{2+} (green-colored sphere) binding in the integrin αIIβ3 structure; **(c)** Full-length integrin αIIβ3 complexed with m-tirofiban (PDB: 9DEQ).

This suggests that the NCMN system could potentially be used for structure-based drug discovery.

The NCMN system for other biochemical and biophysical characterizations of membrane proteins

Besides applications for single-particle cryo-EM analysis of a broad range of membrane proteins from bacteria to humans, the NCMN system has also been successfully used for calcium and pH-dependent membrane protein enzyme activity assay [45], proteoliposome reconstitution for functional assay of membrane protein channels [46], membrane protein drug target and ligand binding assay, [47] and membrane protein—protein interaction analysis [48]. Finally, it is worth noting that the NCMN system has also contributed to the discovery of the cholesterol-dependent protoporphyrin-IX oxygenase activity of human mitochondrial TSPO [50].

Perspectives of the NCMN system

The rapid development of cryo-EM technology, mainly single-particle cryo-EM and cryo-EM tomography, has significantly accelerated progress of membrane protein structural biology [55,56]. However, we still have a significant bottleneck: sample preparation. Detergent-based approaches and derivatives, including nanodisc [57], peptidisc [58], and Salipro [59] technologies, will continue to contribute to our understanding of membrane protein structure and function; however, because of the nature of the involvement of the detergents, we have to be cautious with the potential damage to the natural protein-lipid interactions, as recently demonstrated in the case of pMMO [14,15]. Once the natural protein-lipid interactions are damaged, it is challenging to restore the genuine interactions, even with natural cell membrane lipids reconstituted in nanodiscs or Salipro particles [3,14,15]. The NCMN system aims to prepare high-quality membrane protein samples for single-particle cryo-EM analysis and cryo-EM tomographic analysis in native cell membrane lipid environments. At the current stage of technology development of the NCMN system, we have developed an initial NCMN polymer library that is generally compatible with a broad range of pH conditions, divalent cations, and suitable for high-resolution cryo-EM analysis and functional characterization of membrane proteins from both prokaryotic and eukaryotic cells, including some challenging human membrane proteins, such as integrins. However, this is only a promising beginning; there are still many challenges that need to be overcome. For example, 1) Some challenging membrane protein families with dynamic transmembrane domains, such as mechanosensitive channels, remain resistant to forming homogeneous NCMN particles, which hinders high-resolution cryo-EM

structural analysis. 2) The crystallization of the NCMN particles has not been successful yet, which limits current structural analysis of the NCMN particles to single-particle cryo-EM. 3) Proteoliposome reconstitution with NCMN particles remains inefficient because a detergent-free environment is not ideal for the fusion of the membrane protein with preformed liposomes. 4) Accurate lipid assignment to the cryo-EM density remains a challenge; sometimes, due to flexibility, the shape of the density is often inadequate to assign specific lipids. To overcome these challenges, new NCMN polymers need to be developed, and protocols for manufacturing NCMN particles need to be experimentally optimized. The development of membrane-active polymers could be facilitated through rational design, following the accumulation of experimentally validated membrane-active polymers. Furthermore, various creative biochemical and biophysical characterization approaches need to be developed to cater to the detergent-free system.

It is necessary to comment on the current development of the structure-predicting tools. AlphaFold and other evolving structure prediction tools will be beneficial to experimental structural biologists [60]. However, experimental structures will remain essential for accurately interpreting the structure–activity relationship of membrane proteins. Currently, accurate structural information on membrane protein-lipid interactions remains scarce. AlphaFold and other structure prediction tools cannot reliably predict membrane protein structures for basic research or drug discovery practices without an accurate and comprehensive structure database for training. The NCMN system and other genuine detergent-free systems, including SMALP technology [16], cell-derived membrane vesicles [61], and *in situ* cryo-EM tomography [62], may be crucial for advancing the membrane protein structural biology and related drug discovery.

Declaration of competing interest

Y.G. and W.Q. have patents pending for the NCMN system through the Virginia Commonwealth University. Y.G. and W.Q. are founders of NCMNtech..

Acknowledgements

NIH R01-GM132329 to Y.G. supports work on the NCMN system in the Guo laboratory. The VCU School of Pharmacy, Department of Medicinal Chemistry, and the Center for Drug Discovery, formerly known as the Institute for Structural Biology, Drug Discovery, and Development, also support the Guo laboratory through its provision of laboratory space and facilities.

Data availability

Data will be made available on request.

References

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

* of special interest
** of outstanding interest

1. * Li H, Sun X, Cui W, Xu M, Dong J, Ekundayo BE, Ni D, Rao Z, Guo L, Stahlberg H, Yuan S, Vogel H: **Computational drug development for membrane protein targets**. *Nat Biotechnol* 2024, **42**:229–242.
This publication discusses the challenges of membrane protein drug discovery in the era of AI. It highlighted the importance of structural information on protein-lipid interactions and the current limitations of structure prediction for membrane protein drug targets.

2. Carpenter EP, Beis K, Cameron AD, Iwata S: **Overcoming the challenges of membrane protein crystallography**. *Curr Opin Struct Biol* 2008, **18**:581–586.

3. Guo Y: **Detergent-free systems for structural studies of membrane proteins**. *Biochem Soc Trans* 2021, **49**:1361–1374.

4. Cross TA, Murray DT, Watts A: **Helical membrane protein conformations and their environment**. *Eur Biophys J: EBJ (Eur Biophys J)* 2013, **42**:731–755.

5. Lee AG: **How lipids affect the activities of integral membrane proteins**. *Biochim Biophys Acta* 2004, **1666**:62–87.

6. de Mendoza D: **Membranes, where lipids and proteins meet**. *Chem Phys Lipids* 2025, **268**, 105486.

7. Seddon AM, Curnow P, Booth PJ: **Membrane proteins, lipids and detergents: not just a soap opera**. *Biochim Biophys Acta* 2004, **1666**:105–117.

8. * Woubshete M, Cioccolo S, Byrne B: **Advances in membrane mimetic systems for manipulation and analysis of membrane proteins: detergents, polymers, lipids and scaffolds**. *ChemPlusChem* 2024, **89**, e202300678.
This publication reviews the current technologies for stabilizing and analyzing membrane proteins.

9. Young JW: **Recent advances in membrane mimetics for membrane protein research**. *Biochem Soc Trans* 2023, **51**: 1405–1416.

10. Corradi V, Sejdiu BI, Mesa-Galloso H, Abdizadeh H, Noskov SY, Marrink SJ, Tieleman DP: **Emerging diversity in lipid-protein interactions**. *Chem Rev* 2019, **119**:5775–5848.

11. Corradi V, Mendez-Villuendas E, Ingólfsson HI, Gu RX, Siuda I, Melo MN, Moussatova A, DeGagné LJ, Sejdiu BI, Singh G, Wassenaar TA, Delgado Magnero K, Marrink SJ, Tieleman DP: **Lipid-protein interactions are unique fingerprints for membrane proteins**. *ACS Cent Sci* 2018, **4**:709–717.

12. Sych T, Levental KR, Sezgin E: **Lipid-protein interactions in plasma membrane organization and function**. *Annu Rev Biophys* 2022, **51**:135–156.

13. Bolla JR, Agasid MT, Mehmood S, Robinson CV: **Membrane protein-lipid interactions probed using Mass Spectrometry**. *Annu Rev Biochem* 2019, **88**:85–111.

14. Koo CW, Tucci FJ, He Y, Rosenzweig AC: **Recovery of particulate methane monooxygenase structure and activity in a lipid bilayer**. *Science (New York, NY)* 2022, **375**:1287–1291.

15. ** Tucci FJ, Rosenzweig AC: **Structures of methane and ammonia monooxygenases in native membranes**. *Proc Natl Acad Sci USA* 2025, **122**, e2417993121.
This publication discusses the crucial roles that the natural cell membrane lipid environments play in the function of the membrane protein enzyme pMMO. This is an excellent case study highlighting the limitations of nanodisc technology and detergents in membrane protein research, and the authors emphasize the importance of maintaining the native cell membrane lipid environment.

16. 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 co-polymer**. *J Am Chem Soc* 2009, **131**:7484–7485.

17. Parmar M, Rawson S, Scarff CA, Goldman A, Dafforn TR, Muench SP, Postis VLG: **Using a SMALP platform to determine a sub-nm single particle cryo-EM membrane protein structure**. *Biochim Biophys Acta Biomembr* 2018, **1860**: 378–383.

18. Qiu W, Fu Z, Xu GG, Grassucci RA, Zhang Y, Frank J, Hendrickson WA, Guo Y: **Structure and activity of lipid bilayer within a membrane-protein transporter**. *Proc Natl Acad Sci USA* 2018, **115**:12985–12990.

19. Su CC, Li M, Gu R, Takatsuka Y, McDermott G, Nikaido H, Yu EW: **Confirmation of the AcrB multidrug efflux pump in mutants of the putative proton relay pathway**. *J Bacteriol* 2006, **188**:7290–7296.

20. Seeger MA, Schiefner A, Eicher T, Verrey F, Diederichs K, Pos KM: **Structural asymmetry of AcrB trimer suggests a peristaltic pump mechanism**. *Science (New York, NY)* 2006, **313**:1295–1298.

21. Murakami S, Nakashima R, Yamashita E, Yamaguchi A: **Crystal structure of bacterial multidrug efflux transporter AcrB**. *Nature* 2002, **419**:587–593.

22. Sun C, Benlekbir S, Venkatakrishnan P, Wang Y, Hong S, Hosler J, Tajkhorshid E, Rubinstein JL, Gennis RB: **Structure of the alternative complex III in a supercomplex with cytochrome oxidase**. *Nature* 2018, **557**:123–126.

23. Gulamhussein AA, Uddin R, Tighe BJ, Poyner DR, Rothnie AJ: **A comparison of SMA (styrene maleic acid) and DIBMA (diisobutylene maleic acid) for membrane protein purification**. *Biochim Biophys Acta Biomembr* 2020, **1862**, 183281.

24. Esmaili M, Acevedo-Morantes C, Wille H, Overduin M: **The effect of hydrophobic alkyl sidechains on size and solution behaviors of nanodiscs formed by alternating styrene maleamic co-polymer**. *Biochim Biophys Acta Biomembr* 2020, **1862**, 183360.

25. Ravula T, Ramadugu SK, Di Mauro G, Ramamoorthy A: **Bioinspired, size-tunable self-assembly of polymer-lipid bilayer nanodiscs**. *Angew Chem* 2017, **56**:11466–11470.

26. Ravula T, Hardin NZ, Ramadugu SK, Ramamoorthy A: **pH tunable and divalent metal ion tolerant polymer lipid nanodiscs**. *Langmuir : ACS J Surf Colloid* 2017, **33**: 10655–10662.

27. Lindhoud S, Carvalho V, Pronk JW, Aubin-Tam ME: **SMA-SH: modified styrene-maleic acid Co-polymer for functionalization of lipid nanodiscs**. *Biomacromolecules* 2016, **17**: 1516–1522.

28. Esmaili M, Brown CJ, Shaykhutdinov R, Acevedo-Morantes C, Wang YL, Wille H, Gandour RD, Turner SR, Overduin M: **Homogeneous nanodiscs of native membranes formed by stilbene-maleic-acid co-polymers**. *Nanoscale* 2020, **12**: 16705–16709.

29. Hall SCL, Tognoloni C, Charlton J, Bragginton ÉC, Rothnie AJ, Sridhar P, Wheatley M, Knowles TJ, Arnold T, Edler KJ, Dafforn TR: **An acid-compatible co-polymer for the solubilization of membranes and proteins into lipid bilayer-containing nanoparticles**. *Nanoscale* 2018, **10**: 10609–10619.

30. Ravula T, Hardin NZ, Ramadugu SK, Cox SJ, Ramamoorthy A: **Formation of pH-resistant monodispersed polymer-lipid nanodiscs**. *Angew Chem* 2018, **57**:1342–1345.

31. Kopf AH, Lijding O, Elenbaas BOW, Koorengevel MC, Dobruchowska JM, van Walree CA, Killian JA: **Synthesis and evaluation of a library of alternating amphipathic Co-polymers to solubilize and study membrane proteins**. *Biomacromolecules* 2022, **23**:743–759.

32. Smith AA, Autzen HE, Faust B, Mann JL, Muir BW, Howard S, … Appel EA: **Lipid nanodiscs via ordered co-polymers**. *Chem* 2020, **6**:2782–2795.

33. Fiori MC, Jiang Y, Altenberg GA, Liang H: **Polymer-encased nanodiscs with improved buffer compatibility**. *Sci Rep* 2017, **7**:7432.

34. Hardin NZ, Ravula T, Mauro GD, Ramamoorthy A: **Hydrophobic functionalization of polyacrylic acid as a versatile platform for the development of polymer lipid nanodisks**. *Small (Weinheim an der Bergstrasse, Germany)* 2019, **15**, e1804813.

35. Yasuhara K, Arakida J, Ravula T, Ramadugu SK, Sahoo B, Kikuchi JI, Ramamoorthy A: **Spontaneous lipid nanodisc fomation by amphiphilic polymethacrylate Co-polymers**. *J Am Chem Soc* 2017, **139**:18657–18663.

36. Janata M, Gupta S, Čadová E, Angelisová P, Krishnarjuna B, Ramamoorthy A, Hořejší V, Raus V: **Sulfonated polystyrenes: pH and Mg2+-insensitive amphiphilic co-polymers for detergent-free membrane protein isolation**. *Eur Polym J* 2023, **198**, 112412.

37. * Krishnarjuna B, Sharma G, Ravula T, Ramamoorthy A: **Factors influencing the detergent-free membrane protein isolation using synthetic nanodisc-forming polymers**. *Biochim Biophys Acta Biomembr* 2024, **1866**, 184240.

This publication discusses the critical factors that influence the membrane activities of synthetic nanodisc-forming polymers, providing valuable insights into the application and design of new membrane-active polymers.

38. * Krishnarjuna B, Ramamoorthy A: **Detergent-free isolation of membrane proteins and strategies to study them in a near-native membrane environment**. *Biomolecules* 2022, **12**:1076.

This review article comprehensively discusses the current development and application of membrane-active polymers.

39. Tascón I, Sousa JS, Corey RA, Mills DJ, Griwatz D, Aumüller N, Mikusevic V, Stansfeld PJ, Vonck J, Hänelt I: **Structural basis of proton-coupled potassium transport in the KUP family**. *Nat Commun* 2020, **11**:626.

40. Higgins AJ, Flynn AJ, Marconnet A, Musgrove LJ, Postis VLG, Lippiat JD, Chung CW, Ceska T, Zoonens M, Sobott F, Muench SP: **Cycloalkane-modified amphiphilic polymers provide direct extraction of membrane proteins for CryoEM analysis**. *Commun Biol* 2021, **4**:1337.

41. 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.

42. Flegler VJ, Rasmussen A, Rao S, Wu N, Zenobi R, Sansom MSP, Hedrich R, Rasmussen T, Böttcher B: **The MscS-like channel Ynal has a gating mechanism based on flexible pore helices**. *Proc Natl Acad Sci USA* 2020, **117**: 28754–28762.

43. ** Trinh TKH, Cabezas AJ, Joshi S, Catalano C, Siddique AB, Qiu W, Deshmukh S, des Georges A, Guo Y: **pH-tunable membrane-active polymers, NCMNP2a-x, and their potential membrane protein applications**. *Chem Sci* 2023, **14**: 7310–7326.

This publication introduces the methods for NCMN polymer synthesis, optimization, and application, and discusses the mechanism of NCMN particle formation.

44. Hoang Trinh TK, Catalano C, Guo Y: **Fabrication of membrane proteins in the form of native cell membrane nanoparticles using novel membrane-active polymers**. *Nanoscale Adv* 2023, **5**:5932–5940.

45. Dhankhar P, Trinh TKH, Qiu W, Guo Y: **Characterization of Ca^{21} $^{+}$ATPase, LMCA1, with native cell membrane nanoparticles system**. *Biochim Biophys Acta Biomembr* 2023, **1865**, 184143.

46. Yang L, Catalano C, Xu Y, Qiu W, Zhang D, McDermott A, Guo Y, Blount P: **A native cell membrane nanoparticles system allows for high-quality functional proteoliposome reconstitution**. *BBA Adv* 2021, **1**, 100011.

47. Qiu W, Guo Y: **Analysis of the oligomeric state of mycobacterial membrane protein large 3 and its interaction with SQ109 with native cell membrane nanoparticles system**. *Biochim Biophys Acta Biomembr* 2022, **1864**, 183793.

48. Kroeck KG, Qiu W, Catalano C, Trinh TKH, Guo Y: **Native cell membrane nanoparticles system for membrane protein-**

protein interaction analysis. *J Vis Exp* 2020, **161**, https://doi.org/10.3791/61298.

49. Guo, Y. Native cell membrane nanoparticle and methods of use thereof, US 2023/0295349 A1.

50. * Qiu W, Trinh TKH, Catalano C, Mehta A, Desai UR, Kellogg GE, Hendrickson WA, Guo Y: **Cholesterol-dependent enzyme activity of human TSPO1**. *Proc Natl Acad Sci USA* 2025, **122**, e2323045122.

This publication discussed how the NCMN system has been used to reveal the crucial role of cholesterol for the enzyme activity of human mitochondrial TSPO1.

51. ** Adair BD, Xiong JP, Yeager M, Arnaout MA: **Cryo-EM structures of full-length integrin αIIbβ3 in native lipids**. *Nat Commun* 2023, **14**:4168.

This publication demonstrates that the NCMN system plays a crucial role in determining the high-resolution cryo-EM structure of the first full-length human integrin.

52. ** Adair BD, Field CO, Alonso JL, Xiong JP, Deng SX, Ahn HS, Mashin E, Clish CB, van Agthoven J, Yeager M, Guo Y, Tess DA, Landry DW, Poncz M, Arnaout MA: **Platelet integrin αIIbβ3 plays a key role in a venous thrombogenesis mouse model**. *Nat Commun* 2024, **15**:8612.

This publication demonstrates the potential application of the NCMN system for membrane protein structure-based drug design and the compatibility of customized NCMN polymers with divalent cations, such as Mg^{2+} and Ca^{2+}, in the structural analysis of membrane proteins.

53. Guo Y, Trinh TKH, Qiu W: **Native cell membrane nanoparticles**. *Feb* 2024, **20**:758. USSN 18/581.

54. Guo Y, Trinh TKH, Qiu W: **Native cell membrane nanoparticles**. *Feb* 2024, **20**:769. USSN 18/581.

55. Cheng Y: **Membrane protein structural biology in the era of single particle cryo-EM**. *Curr Opin Struct Biol* 2018, **52**:58–63.

56. Sun WW, Michalak DJ, Sochacki KA, Kunamaneni P, Alfonzo-Méndez MA, Arnold AM, Strub MP, Hinshaw JE, Taraska JW: **Cryo-electron tomography pipeline for plasma membranes**. *Nat Commun* 2025, **16**:855.

57. Sligar SG, Denisov IG: **Nanodiscs: a toolkit for membrane protein science**. *Protein Sci : Publ Protein Soc* 2021, **30**:297–315.

58. 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.

59. 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.

60. Malhotra Y, John J, Yadav D, Sharma D, Vanshika, Rawal K, Mishra V, Chaturvedi N: **Advancements in protein structure prediction: a comparative overview of AlphaFold and its derivatives**. *Comput Biol Med* 2025, **188**, 109842.

61a. Li H, Sun X, Cui W, Xu M, Dong J, Ekundayo BE, Ni D, Rao Z, Guo L, Stahlberg H, Yuan S, Vogel H: **Computational drug development for membrane protein targets**. *Nat Biotechnol* 2024, **42**:229–242.

61b. Tao X, Zhao C, MacKinnon R: **Membrane protein isolation and structure determination in cell-derived membrane vesicles**. *Proc Natl Acad Sci USA* 2023, **120**, e2302325120.

62. Ni T, Frosio T, Mendonça L, Sheng Y, Clare D, Himes BA, Zhang P: **High-resolution in situ structure determination by cryo-electron tomography and subtomogram averaging using emClarity**. *Nat Protoc* 2022, **17**:421–444.