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Advances in utilizing reverse micelles to investigate membrane proteins

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Abstract

Reverse micelles (RMs) have emerged as useful tools for the study of membrane associated proteins. With a nanoscale water core surrounded by surfactant and solubilized in a non-polar solvent, RMs stand apart as a unique membrane model. While RMs have been utilized as tools to investigate the physical properties of membranes and their associated water, RMs also effectively house membrane associated proteins for a variety of studies. High-resolution protein NMR revealed a need for development of improved RM formulations, which greatly enhanced the use of RMs for aqueous proteins. Protein-optimized RM formulations enabled encapsulation of challenging membrane associated protein types, including lipidated proteins, transmembrane proteins, and peripheral membrane proteins. Improvements in biological accuracy of RMs using phospholipid-based surfactants has advanced their utility as a membrane mimetic even further, better matching the chemistry of the most common cellular membrane lipids. Natural lipid extracts may also be used to construct RMs and house proteins, resulting in a membrane model that better represents the complexity of biological membranes. Recent applications in high-resolution investigations of protein-membrane interactions and inhibitor design of membrane associated proteins have demonstrated the usefulness of these systems in addressing this difficult category of protein. Further developments of RMs as membrane models will enhance the breadth of investigations facilitated by these systems and will enhance their use in biophysical, structural, and drug discovery pursuits of membrane associated proteins. In this review, we present the development of RMs as membrane models and their application to structural and biophysical study of membrane proteins.

Introduction

Among many functions, membrane proteins act as liaisons and gatekeepers of membranes, the primary barriers of cells [1]. Their central roles make them an important focus for a variety of studies, from basic biology to disease mechanisms and drug discovery. An assortment of membrane protein varieties exists, including transmembrane (TM) proteins that span the bilayer and are primarily of either α-helical bundle or β-barrel types [2],

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monotopic integral membrane proteins that are affixed to a single leaflet [3], proteins that are anchored to membranes through lipidations [4], as well as water-soluble proteins that interact with membranes [5]. *In vitro* investigations of membrane proteins require membrane models that simulate cellular membrane environments while retaining favorable properties for experimentation. Liposomes, nanodiscs, copolymer derived native nanodiscs, bicelles, micelles, amphipols, and other models have emerged as useful tools in the study of membrane proteins [6–8]. Among these, reverse micelles (RMs) stand out as an effective and unique tool in the arsenal of membrane models. RMs consist of nanoscale pools of water, which may contain a protein, solubilized within a hydrophobic solvent, often an alkane. Surfactants form the interface, with their hydrophilic headgroups oriented towards the RM core and the hydrophobic tails interacting with the solvent (Figure 1A)[9]. Due to a resemblance to membrane surfaces, membrane associated proteins may be housed within RMs.

RMs comprise several tunable parameters, which provides adaptability for experimental applications. For example, size can be modified by altering water content or ionic strength of the aqueous core [10–12]. Chemical and physical properties of the inner RM surface may be tuned according to the identity of the surfactant (Figure 1B) [13,14]. Properties of the solvent, such as viscosity, may be adjusted according to the solvent identity [15,16]. While several RM formulations reliably encapsulate most proteins with minimal adjustments, optimization may be necessary [13]. A typical process for constructing RMs is by the addition of a relatively small volume of an aqueous buffer to surfactants that are suspended in an alkane solvent. The water content in RMs, typically called water loading (W_0, molar ratio of water to surfactant), is often between 10 and 30 for protein applications. A cosurfactant, often hexanol, may be necessary to fully form the RMs, depending on the formulation. A typical strategy for encapsulating water-soluble proteins is by including them in aqueous buffer, which is introduced to surfactants in an alkane solvent and results in encapsulation upon RM formation (Figure 1C) [13]. For membrane proteins that are not water soluble, alternate strategies may be necessary such as drying water-solubilized TM proteins housed in micelles, followed by addition of solvent, cosolvent, then finally the aqueous buffer (Figure 1D) [17]. The variety of parameters and delivery methods available for RMs may necessitate an initial investment in optimization for some proteins. On the other hand, these parameters represent a versatility that enables RMs to be used as tunable membrane models in a wide range of studies.

RMs have long been used as models to understand the physical properties of cellular membranes, such as the nature of hydration dynamics at membrane interfaces [18–23], partitioning of small molecules [24–27], and the effect of crowding or confinement near membrane surfaces on molecular conformation [28–30]. In addition, RMs have been used to encapsulate aqueous proteins which permits studies that are otherwise difficult or inaccessible. Low-viscosity alkane solvents may be leveraged to increase rotational diffusion in RMs, essentially breaking the size limit in protein NMR without the need for deuteration or transverse relaxation-optimized spectroscopy (TROSY) techniques [31]. The nanoscale water core provides a means to study the effect of confinement on proteins [32–35] and the impact of solvation on protein motions [36]. RMs have been used as platforms for investigating long-standing open questions in protein biophysics such as cold-denaturation

[37–39] and hydration dynamics [40–42]. While recent development has been driven by a small number of groups, mostly in the protein NMR field, demonstration of RMs as platforms for studying membrane proteins have highlighted their potential. Here, we review the use and development of RMs for studying membrane proteins, including recent advances that promise to improve biological accuracy and facilitate new lines of investigation to this challenging class of protein.

Reverse micelles as platforms to house and study membrane proteins

Foundational research focused on understanding the formation and stability of RMs in various solvent systems [43,44]. RMs are unique membrane models due to the bulk solvent being hydrophobic, often an alkane, which may provide advantages. For example, the use of RMs to promote lipase catalysis takes advantage of this property. [45–49] RMs allowed access to the hydrophobic lipid substrate while stably housing the water-soluble protein within the RM water core, preventing direct exposure to alkanes which may be destabilizing. Solubilizing membrane associated proteins in an *in vitro* membrane model also requires access to hydrophobic and hydrophilic phases through an amphipathic interface. Early studies used RMs to house membrane associated myelin basic protein (MBP) or myelin proteolipid protein (PLP) in a membrane mimetic environment to study the structure of the protein-membrane complex [50–52]. MBP changes to a more ordered α-helical structure within RMs compared to aqueous conditions, reflecting its natural conformation in the membrane [51]. When housed in RMs, specific regions of PLP, such as the ε-amino groups of lysine residues, remained accessible to water, while others, like a key tryptophan residue, became shielded from the solvent, mirroring the expected interactions with biological membranes [50]. These observations highlight that RMs may house membrane proteins and preserve their structural integrity. While many models were initially proposed for how proteins were housed within RMs, the exact mode was not known [53]. It was not known if the hydrophobic regions of membrane proteins are directly solvated by the alkane solvent, if proteins may be adsorbed into the RM surface, or if a protein is solubilized by a single or multiple RMs. Small-angle X-ray scattering (SAXS) measurements helped resolve these questions in regard to transmembrane proteins and revealed the structure of the protein and RM complex [54]. The proteins are encapsulated within two RMs with the hydrophilic portions covered and protected by the aqueous core while the hydrophobic portions interact with either the surfactant tails or the solvent, forming a small bilayer-like environment (Figure 1A) [54,55].

RMs provide means to model interactions between proteins or peptides with membrane surfaces. The chemokine receptor CXCR1 N-terminal domain has a secondary structure similar to its membrane-bound form when encapsulated within bis(2-ethylhexyl) sulfosuccinate (AOT) RMs, as revealed through fluorescence and circular dichroism [56]. Upon increasing the water content, tryptophan residues became more exposed to water, revealing that structure and dynamics of this domain are intertwined with the level of hydration. Monomeric, misfolded “seed” structures of amyloid beta (Aβ) proteins are typically difficult to study due to aggregation effects [57]. However, encapsulation within AOT RMs stabilized these structures. Fourier-transform infrared spectroscopy (FTIR) revealed that extended β-strands formed and may mimic a conformation that is induced

in crowded cellular conditions and the presence of membranes. *In silico* models agree with the structural stability and conformational changes conferred when protein is encapsulated within an RM [58]. Alanine-rich peptide AKA_2 reveals a rigid helical conformation within an RM, unlike the protein in aqueous conditions which forms flexible random coils. Simulations confirmed that RMs are expected to help stabilize membrane associated helices that contain polar and apolar faces. The surface and interfacial properties of RMs well-mimic the amphipathic properties of membranes and aid study of membrane associated proteins and peptides.

High-resolution studies of membrane associated proteins in RMs

While these early studies established RMs as membrane models, detailed structural information regarding encapsulated proteins was still lacking. An early demonstration of RMs as membrane mimics for high-resolution NMR is encapsulation of the neurotransmitter pentapeptide Leu-enkephalin. NMR experiments produced high quality spectra of the peptide encapsulated within AOT RMs where 3D and nuclear Overhauser effect spectroscopy (NOESY) data was collected [59]. The structure of the peptide was similar to that obtained by crystallography. The peptide was adsorbed into the RM surface, mimicking the interaction observed with other membrane models [60]. Another study used NMR to observe gramicidin A (gA), a peptide that forms a dimeric ion-channel in membranes. The peptide was successfully encapsulated within AOT RMs and subsequently NMR diffusion and interresidue NOE experiments showed gA bridging two RMs as expected for the transmembrane dimer [61]. The use of NMR to observe these membrane-associated peptides revealed detail in the structural aspects of membrane interactions and showed promise for utilizing RMs for high-resolution observations of membrane associated proteins.

Using high-resolution NMR with RM encapsulation reveals details about the overall fold and functional competence of a protein. A striking observation from NMR is that the commonly used surfactant AOT unfolds most proteins [13,62]. While it is possible that small membrane-associated peptides may be tolerant to AOT, these observations demonstrated the necessity for alternate RM systems for encapsulating proteins (Figure 1B). Improved surfactant systems were developed and applied to membrane proteins, with careful consideration for retaining fold and function upon encapsulation. The 54kDa transmembrane potassium channel, KcsA, was encapsulated using several different surfactant systems while retaining its native homotetrameric structure [63]. Best spectral conditions were achieved with a mixture of cetyltrimethylammonium bromide (CTAB) and dihexadecyldimethylammonium bromide (DHAB). Retention of function was confirmed by ^{15}N-HSQC, which showed shifting of key residues upon titration with K^+ ions (Figure 2A).

Improved RM formulations have also enabled study of lipidated, membrane-anchored proteins via NMR (Figure 1A). The myristoyl anchor of recoverin embeds into the CTAB/hexanol RM shell upon encapsulation in its Ca^{2+}-saturated, myristoyl-extruded state [64]. Importantly, only the myristoyl sequestered state is water-soluble; myristoyl extrusion typically results in aggregation. Similarly, HIV matrix protein was housed within CTAB/hexanol RMs with the myristoylation embedded in the surfactant shell [64]. Functional competence was demonstrated by binding the activating lipid phosphatidylinositol 4,5-

bisphosphate (Figure 2B). Another example is the pH-dependent myristoyl switch in hisactophilin, which was studied by ^{15}N-HSQC NMR [65]. Encapsulating hisactophilin in a CTAB/hexanol RM revealed that the protein is in the myristoyl-extruded state versus the solution state where the myristol is sequestered. The protective environment of the amphipathic RM shell allowed NMR experimentation for the myristoyl-extruded forms of these proteins, which is not possible in aqueous conditions due to aggregation induced by the exposed lipidations.

Peripheral membrane proteins (PMPs) are a class of membrane associated proteins that are water-soluble, but interact with membranes, often reversibly [5,66]. Due to the relative ease of experiments on water-solubilized proteins versus proteins associated with membranes, structural and *in vitro* experiments performed on PMPs tend to focus on the water-solubilized state. This leaves the functional, membrane bound form of most PMPs less well characterized. RMs have been applied to the study of membrane associated PMPs (Figure 1A). Cytochrome c is a PMP within the mitochondria and movement to the cytosol facilitates apoptosis [67]. Cytochrome c is highly compatible with the decylmonoacyl glycerol and lauryldimethylamino-N-oxide (10MAG/LDAO) surfactant system [68,69]. The NMR structure showed no conformational change upon encapsulation, demonstrating that the protein within RMs is identical to the water-solubilized state [67]. Titration of cardiolipin into the RM induced an interaction between the lipid and cytochrome c, a trigger for apoptosis. The mapped interactions localized to three sites; the previously characterized A- and L-site [70], and a new N-site observed through chemical shift perturbations (CSPs). This study demonstrates the use of RMs to study interactions between PMPs and membranes.

Improving the biological accuracy of RMs as models of cellular membranes.

While RMs were demonstrated to be powerful membrane mimetics for protein studies, often formulations used surfactants that did not fully replicate the chemistry of cellular membranes. To improve their biological accuracy, RMs have been formulated to more closely match lipids found in membranes. By using surfactants such as 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), the high phosphatidylcholine content found in many cellular membranes may be replicated. These formulations have been characterized with molecular dynamics (MD) simulations, SAXS, DLS, and terahertz spectroscopy revealing size, shape, and dynamical properties [71–74]. MD simulations of DOPC RMs were calibrated against experimental data and confirmed a small size that is tunable according to the water content and revealed a roughly spherical shape, qualities that are critical to certain studies (Figure 3)[73]. Size calibration allowed precise exploration of the nature of water dynamics in the aqueous core, which revealed slower hydration dynamics for smaller RMs. This finding that was subsequently reflected by time-domain terahertz spectroscopy combined with MD analysis of water in DOPC RMs that showed the water within hydration shell that contacts the inner RM surface has strong hydrogen bonding with the phosphocholine headgroups[75].

A phosphoethanolamine-headgroup containing surfactant showed promise in encapsulating proteins for NMR spectroscopy, providing a RM surface that more closely resembles

biological membranes [76]. ^{1}H-NOESY NMR experiments concluded that cytochrome c was natively folded after several months in this system, demonstrating robust sample stability. A membrane mimicking RM (mmRM) system was recently developed for use in protein NMR, a mixture of 1,2-dilauroyl-sn-glycero-3-phosphocholine with n-dodecylphosphocholine (DLPC:DPC), allows PMPs to be encapsulated in their membrane embedded state with sample stability spanning months (Figure 4) [77]. A strongly membrane associating PMP, glutathione peroxidase 4 (GPx4), was encapsulated within the NMR optimized 10MAG/LDAO system in its water-solubilized form, with no detectible interaction with the RM shell (Figure 4A–B). The mmRM formulation facilitated embedment of GPx4 onto the interior surface, reflecting the known interaction with membranes (Figure 4C–F), which was also observed with phosphatidylethanolamine binding protein 1 (PEBP1). These mmRMs have been validated by dynamic light scattering (DLS) and SAXS as small and approximately spherical, important characteristics for studies such as protein NMR. Lipid-like surfactants provide an interior RM surface that more closely matches the chemistry of biological membranes, though the use of more diverse lipids could enhance the biological relevance of these homogenous RM systems.

Enhancing the complexity of RMs to better mimic natural membranes.

The use of lecithin from various plant and animal sources achieves a more biologically accurate model by better representing the diversity of membrane lipids. Lecithin is an extract of phospholipids from a natural source, often soybeans, and contains a complex mixture with various phospholipid headgroups and fatty acid tails [78]. RMs may be constructed using lecithin and, despite the complex make-up of the lipid extract, size and shape parameters of lecithin RMs are tunable [79]. For example, in addition to water content driving lecithin RM size [80,81], the addition of oleic acid promotes the water core size to expand [82]. Salts are also known to change the structure of lecithin RMs from spherical to cylindrical and finally to reverse vesicles as shown by the use of DLS, transmission electron microscopy (TEM), UV-Vis spectroscopy, and SAXS [83]. Lecithin RMs support physical measurements of membrane properties, such as probing the nature of water dynamics against membrane surfaces using femtosecond fluorescence and absorption [80]. These experiments revealed that the lecithin RM has three water types: strongly bound, bound, and free, reflecting expected hydration behavior near membranes. Lecithin also shows similar characteristics to DOPC RMs with hydration dynamics being slower compared to AOT RMs [73,80]. This is attributed to the lipids having a stronger interaction with the water molecules than AOT. NMR relaxation measurements of water revealed the presence of a faster and a slower relaxing population, corresponding to water that interacts strongly to the lecithin headgroups and water that is more interior in the RMs [84]. Lecithin RMs have also been used to understand location preferences of encapsulated molecules, modeling their interactions with cellular membranes. Melatonin was encapsulated within lecithin and AOT and in both instances melatonin associated with the surfactant headgroups [85].

Demonstrating a breadth of lipid combinations that may form stable RMs, formulations using native polar lipids extracted from soy lecithin, porcine brain, and bovine heart all form RMs capable of encapsulating proteins for NMR spectroscopy [86,87]. Each formulation contained differing proportions of at least 7 different phospholipid types while

also including fatty acids, cholesterol, and other components, yet native RMs (nRMs) made from each lipid extraction were of similar size and had similar water dependent tunability of size. Protein NMR revealed that interactions of PMPs with the interior surface of the nRMs are similar to the known membrane interactions[87]. Along with phospholipid-based RMs and mmRMs, nRMs are more biologically accurate membrane models which may be used for investigations of protein-membrane interactions.

Use of RMs to screen peripheral membrane proteins for fragment-based drug discovery.

A recent advance in the utility of RMs in the study of membrane associated proteins is applications in early-stage drug discovery. Membrane proteins are a particularly challenging class of protein for target-based drug development. Discovery efforts typically use screening methods that were developed for aqueous protein targets and are therefore limited in application to membrane protein targets. Fragment-based drug discovery (FBDD) is a powerful approach which is based on discovering small, weakly binding molecules (fragments) that may be combined to yield higher affinity inhibitors [88]. Biophysical methods are necessary for fragment screening due to their inherently weak binding, with protein NMR being a particularly useful method due to the structural information it provides [89,90]. The fragment-based approach is attractive for targets such as membrane associated proteins, where new classes of inhibitors may be desirable and the biases common to high-throughput screening libraries may need to be avoided. Existing NMR compatible membrane models such as bicelles and nanodiscs are large and often require extensive deuteration, making them cost prohibitive for screening large numbers of samples. Micelles, while useful, often destabilize proteins and the high concentrations of fragments required for screening can disrupt the micelle structure [91]. Reverse micelles offer a promising solution to these challenges.

Previous studies on encapsulated aqueous proteins allowed for detection of very weak binding fragments and demonstrated stability of RMs in the presence of large concentrations of fragments or other small-molecules [92,93]. A recent development applied mmRMs to successfully screen a PMP for fragment binders [94]. GPx4 is a highly sought after PMP drug target whose inhibition has been implicated in treating a number of cancer types [95]. Current inhibitors are highly reactive, non-specific electrophiles that are not suitable as drug leads; non-covalent inhibition of GPx4 may circumvent this problem. GPx4 is in its active form when embedded in membranes, where it reduces lipid hydroperoxides [96]. Therefore, non-covalent inhibition would therefore target the membrane-engaged form of GPx4 to block processing of the lipid substrate. A fragment screen was performed on GPx4 in mmRMs to understand whether fragment binding could be detected [94]. The screen was successful and yielded a series of hits that bound to GPx4 within the membrane interface (Figure 5). Importantly, the fragments did not bind to water-solubilized GPx4, even at high concentrations, highlighting that embedment onto the mmRM surface was essential for formation of fragment binding sites. A fragment hit with an affinity of ~220 μM was improved by a secondary screen to an affinity of ~15 μM, demonstrating that hits can be developed towards inhibitors and eventually drug leads. The mmRM fragment screening approach serves as an effective entry point for inhibitor and drug development for membrane associated proteins.

Conclusions

These studies collectively highlight advancements in using RMs to house membrane proteins, to investigate protein-membrane interactions, and to initiate drug development efforts. They demonstrate the utility of RMs in providing a biologically relevant environment for detailed structural and biophysical analyses. While RMs have many benefits and unique applications, they are not without their drawbacks. As compared to bicelles or nanodiscs, the degree of curvature in RMs is high, which limits their accuracy as biological membrane models for some applications [97]. Initial optimization of conditions and additional production steps make use of RMs more time-consuming than micelles or bicelles. Finally, from our experience, a small fraction (roughly 10%) of proteins that are otherwise NMR compatible are unsuitable for use with RMs, possibly due to destabilization from exposure to surfactants or alkane. Despite some disadvantages, RMs are a potent addition to the set of tools available for membrane protein studies.

Recently, development of RMs as membrane mimics has been driven by a small number of groups and has mostly been limited to applications in protein NMR. We are optimistic that the latest improvements in biological accuracy of RMs will prove their usefulness for a larger and more diverse community of researchers. Distinctive aqueous protein studies that have been allowed by RMs, such as measurements of protein hydration dynamics and observations of confinement effects, may now be applied to entirely new classes of membrane associated proteins with the recent development of more accurate membrane mimetic RMs. The RM-based fragment screening approach promises to open a range of difficult membrane associated targets to inhibitor development. This body of work underscores the continued evolution and application of RM technology in membrane protein research, offering new insights and methodologies that enhance our understanding of these complex biological systems. Further developments in biological accuracy of RMs as membrane models and applications to new proteins and drug targets promise to advance membrane protein studies.

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Abbreviations

10MAG	decylmonoacyl glycerol
AOT	bis(2-ethylhexyl) sulfosuccinate
CSP	chemical shift perturbation
CTAB	cetyltrimethylammonium bromide

DHAB	dihexadecyldimethylammonium bromide
DLPC	1,2-dilauroyl-sn-glycero-3-phosphocholine
DOPC	1,2-dioleoyl-sn-glycero-3-phosphocholine
DPC	n-dodecylphosphocholine
FBDD	Fragment Based Drug Discovery
gA	gramicidin A
GPx4	glutathione peroxidase 4
LDAO	lauryldimethylamino-N-oxide
MD	molecular dynamics
MBP	myelin basic protein
mmRM	membrane mimicking Reverse Micelle
NOESY	nuclear Overhauser effect spectroscopy
nRM	native Reverse Micelle
PLP	myelin proteolipid protein
PMP	Peripheral Membrane Protein
RM	Reverse Micelle
SAXS	Small-angle X-ray scattering
TEM	transmission electron microscopy
TM	transmembrane
TROSY	transverse relaxation-optimized spectroscopy

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Perspectives

- Study of membrane associated proteins is dependent on the available membrane models, all of which have strengths and weaknesses. Reverse micelles are a promising and unique addition to the toolset for investigating of this challenging class of proteins.
- Reverse micelles provide an adaptable membrane model useful for studying transmembrane, lipid-anchored, and peripheral membrane proteins. Recent work has revealed versatility in the lipids that may be used to construct RMs and has highlighted their use as screening platforms in fragment-based drug discovery for peripheral membrane proteins.
- Promising future directions include leveraging new formulations to further improve biological accuracy of reverse micelles as membrane models and extending use of RMs to new areas in biophysical, structural, and inhibitor development studies for membrane associated proteins.

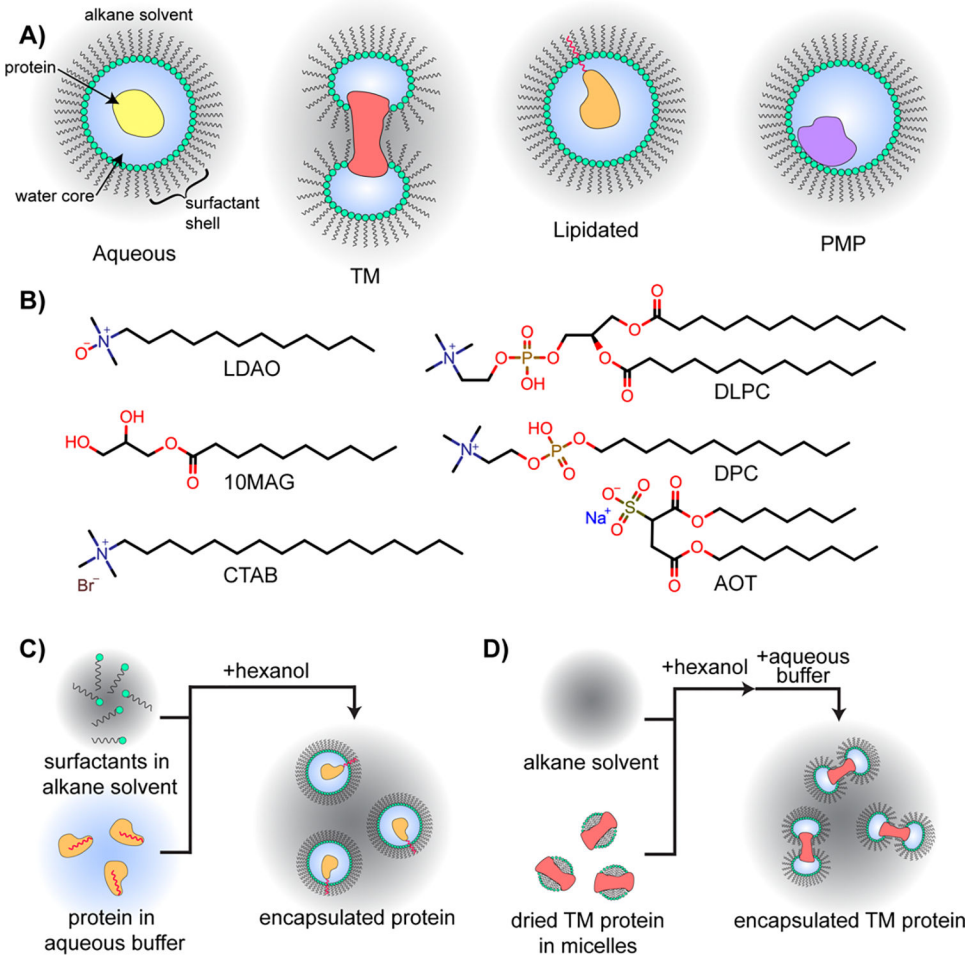

Figure 1.

Depictions of multiple protein types encapsulated within RMs, commonly used RM surfactants, and RM construction workflows. A) RMs may house aqueous proteins within a water core surrounded by a surfactant shell, all solubilized in an alkane solvent. Transmembrane (TM) proteins may be housed within two RMs, each protecting the hydrophilic regions of the protein with the hydrophobic portions interfacing with surfactant tails or alkane solvent. Lipidated proteins may be encapsulated with their membrane anchors embedded into the surfactant shell. Peripheral membrane proteins (PMPs) may reside in the aqueous core of the protein with the membrane interface interacting with the surfactant shell. B) Optimized surfactants that are commonly used for aqueous protein encapsulation include the 10MAG/LDAO binary mixture and CTAB. The DLPC/DPC binary mixture effectively houses PMPs in their membrane embedded state. AOT was historically commonly used, but is now accepted as destabilizing to most proteins. C) General workflow for encapsulation of a water-solubilized proteins, which may be applicable to aqueous proteins, lipid-anchored proteins (depicted here), or PMPs. Small volume of aqueous buffer containing protein is added to the solvent containing lipids and mixed, with hexanol then added if necessary. A detailed protocol may be found in [13]. D) General workflow for TM protein encapsulation.

A TM protein housed in a micelle is lyophilized from aqueous conditions, the alkane solvent is added, followed by hexanol, then the aqueous buffer. Details may be found in [17].

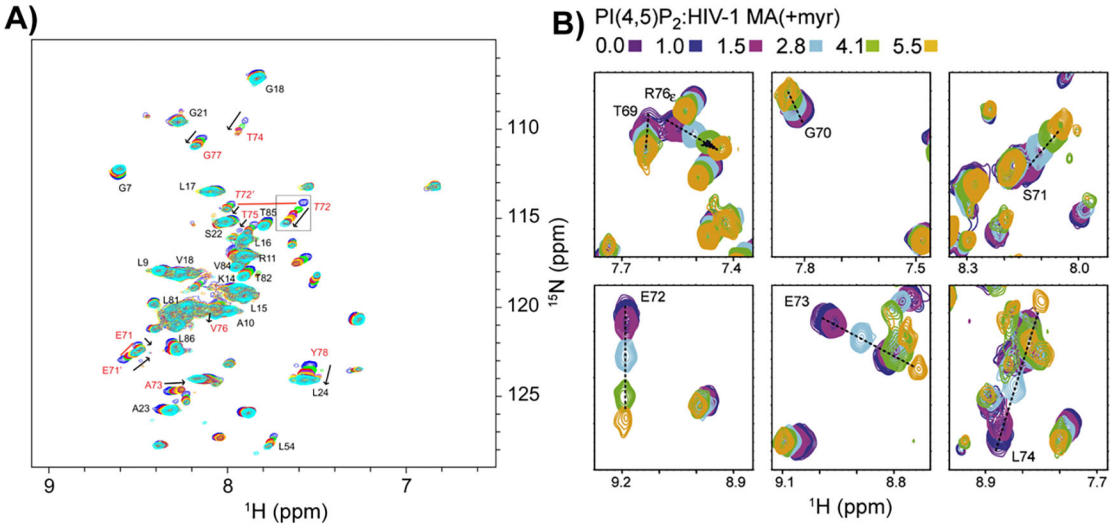

Figure 2.

Reverse micelle encapsulation of functionally competent transmembrane and lipidated proteins. A) Overlay of ^{1}H-^{15}N HSQC spectra of a titration of encapsulated KcsA with potassium, from 0 mM to 28 mM K^{+}. Arrows indicate increasing concentrations. Residues known to be associated with the KcsA selectivity filter are labeled in red, and other assigned residues are labeled in black. Resonances corresponding to the multiple conformers of residues T72 and E71 are connected by red lines. Reprinted from Kielec et al, *Reverse micelles in integral membrane protein structural biology by solution NMR spectroscopy.* Structure. 2009 Mar 11;17(3):345–51, Copyright 2009, with permission from Elsevier. B) Zoomed regions of the overlay of ^{1}H-^{15}N HSQC spectra of a titration of encapsulated myristoylated HIV matrix protein with phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$). Color of spectra corresponding to molar rations of lipid to protein are given. Reprinted from Valentine et al, *Reverse Micelle Encapsulation of Membrane-Anchored Proteins for Solution NMR Studies.* Structure. 2010 Jan 13;18(1):9–16, Copyright 2010, with permission from Elsevier.

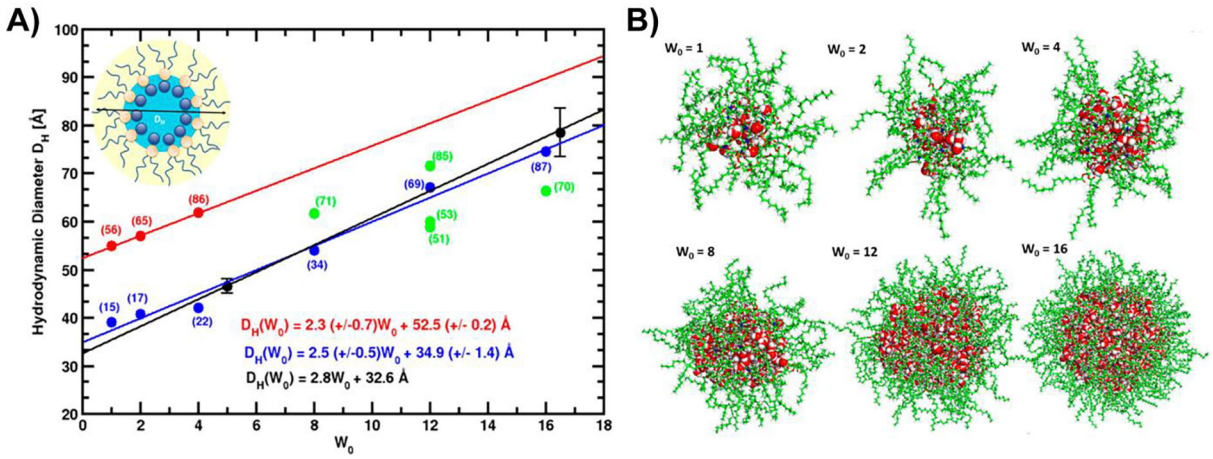

Figure 3.

Experimentally calibrated molecular dynamics simulations of DOPC micelles. A) Hydrodynamic radius as a function of W_0 from DLS measurements (black) [74], from initial MD trials using a fixed DOPC headgroup surface area to determine aggregation number (red), from intermediate trial-and-error attempts to calibrate aggregation number (green), and optimized MD simulations (blue) that reflect experimental measurements. Linear regressions are shown as lines and reported in the figure. Number of DOPC per RM is shown in parentheses. B) Snapshots of typical configurations from optimized MD simulations reflect somewhat ellipsoidal RMs at lower W_0 values and more spherical shapes at larger W_0 values. DOPC is in green, water oxygens are in red, water hydrogens are in white. Adapted with permission from Abel et al, On the structural and dynamical properties of DOPC reverse micelles. Langmuir. 2016 Oct 18;32(41):10610–20. Copyright 2016 American Chemical Society.

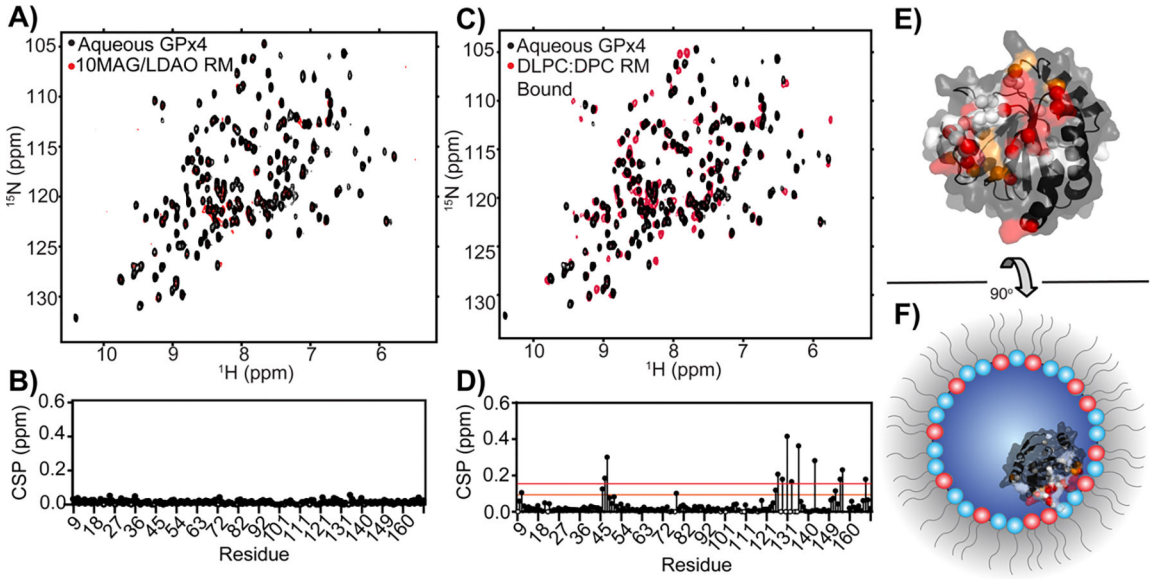

Figure 4.

Demonstration of PMP encapsulation in a RM formulation optimized for aqueous proteins versus a RM formulation optimized to mimic membranes. A) Overlay of ^{1}H-^{15}N HSQCs of aqueous GPx4 (black) and GPx4 encapsulated in the 10MAG/LDAO mixture (red) and B) associated CSPs calculated per residue showing no interaction with the surfactant shell. C) Overlay of ^{1}H-^{15}N HSQCs of aqueous GPx4 (black) and GPx4 encapsulated in the DLPC/DPC mixture (red) and D) associated CSPs calculated for each residue. E) Mapping the residues that have CSPs greater than 1σ (orange) and 2σ (red) above a trimmed mean gives a structural map that may be used to F) estimate the interface of GPx4 with the mmRM which corresponds with the known membrane interface. Unobservable residues are depicted in white and residues with CSPs lower than 1σ are depicted in black. Adapted with permission from Labrecque et al, *Membrane-mimicking reverse micelles for high-resolution interfacial study of proteins and membranes*. Langmuir. 2022 Mar 17;38(12): 3676–86. Copyright 2022 American Chemical Society.

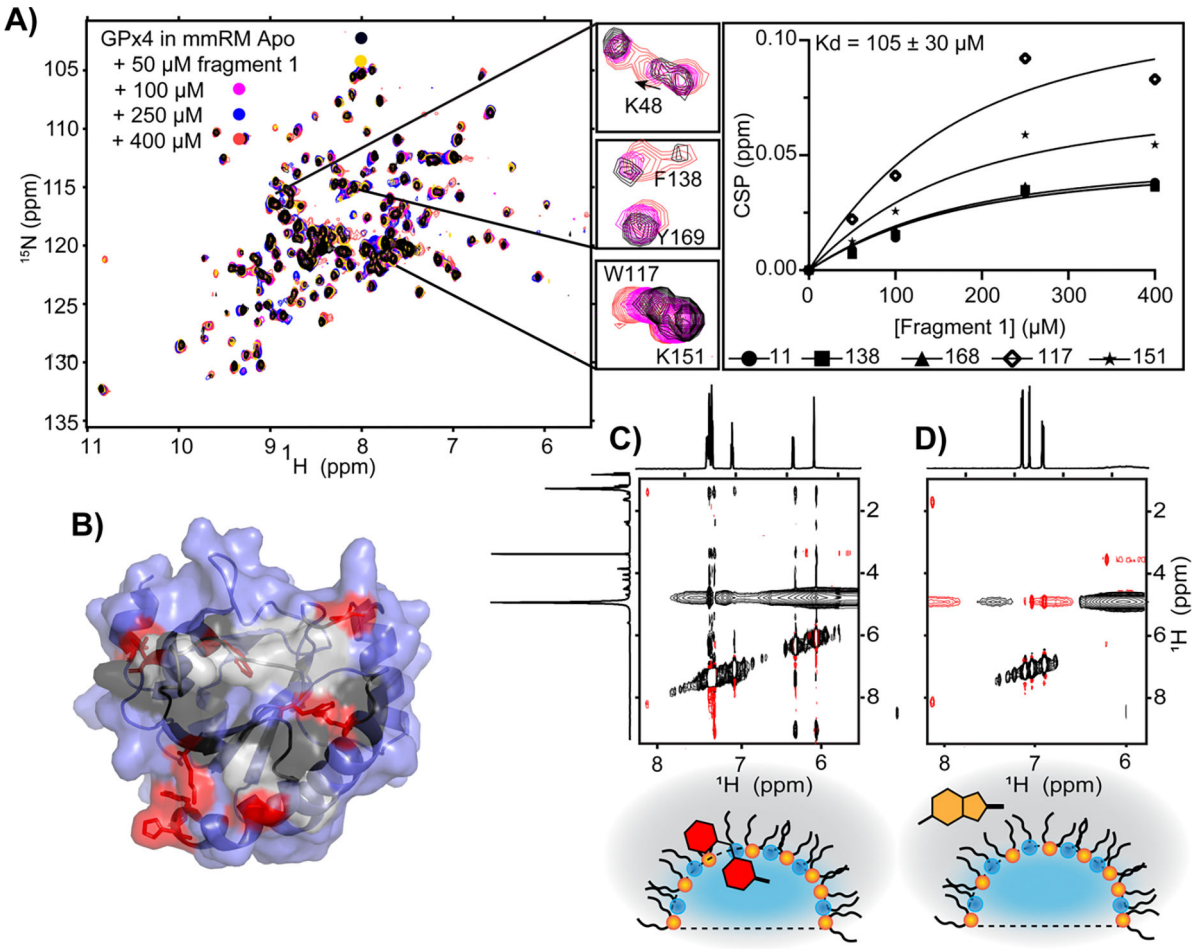

Figure 5.

Use of mmRMs to screen for small-molecule binders for a PMP to initiate inhibitor design. A) A hit, discovered in a screen of a 1911-member library, was titrated against GPx4 encapsulated in mmRMs to characterize affinity and B) reveals binding within the membrane interface. Residues having significant CSPs upon fragment binding are depicted in red, other membrane interface residues in black, unassigned interface residues in gray, and non-interfacial residues are in blue. C) Example of a fragment hit that shows partitioning within the surfactant shell and D) a fragment hit that partitions into the alkane phase in the absence of protein, both demonstrated by ^{1}H-^{1}H NOESY spectra. Both are hits and reveal that multiple modes of partitioning within membranes may acceptable starting points for inhibitor design. All panels are adapted from [94].