French Swimwear for Membrane Proteins

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1. Use of Classical Detergents to Solubilize Membrane Proteins

Integral membrane proteins (IMPs) represent 20–30% of all proteins and well over 50% of the targets for existing drugs.[1, 2] Native IMPs are embedded in the lipid bilayers of biological membranes. The purification of IMPs requires that they first be rendered water soluble. Once solubilized, IMPs may be "reconstituted" back into lipid bilayers or can be directly characterized in soluble form. For example, both 3D crystal growth and solution NMR spectroscopy require the use of solubilized IMPs. Traditionally, membrane proteins are maintained in soluble form by using detergents,[3] which are able to dissolve lipid bilayers to form water-soluble complexes with both lipids and IMPs (Figure 1). Such complexes of detergent with protein and possibly lipid are referred to as "mixed micelles"—one of several different classes of "model membrane" media that can host membrane proteins. The detergent and lipid (if present) components of mixed micelles form a stabilizing annulus around the transmembrane domain of IMPs while allowing normal aqueous solvation of extramembrane domains. Membrane proteins often retain functionality under these conditions.

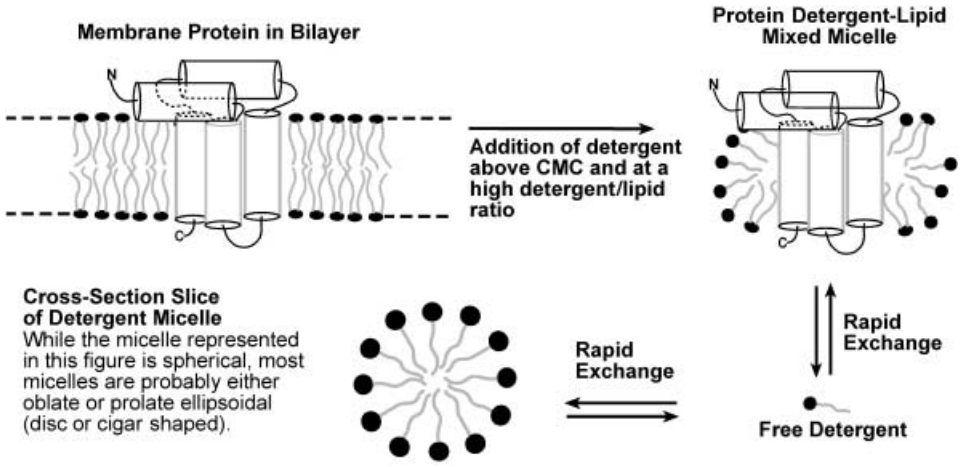

Figure 1. *Bilayers, membrane proteins, detergents, and micelles. For all figures, black indicates molecular hydrophilicity, while gray indicates hydrophobicity.*

Detergents come in a variety of molecular topologies (Figure 2). Moreover, depending upon the detergent used and the detergent/lipid ratio, different mixed micelle shapes and sizes are possible. For simple micelles composed of a single detergent type and no lipid or protein, micelle size is usually described in the form of an "aggregation number", which gives the average number of detergent molecules per micelle.[3] However, aggregation numbers measured for pure detergent cannot always be used to predict the size of the mixed micelles formed by that same detergent with a membrane protein. When the size of an IMP approaches or exceeds the size of the micelle formed by a certain detergent it is the properties of the IMP, especially the transmembrane surface area, that will usually dictate mixed-micelle size.[3, 4]

Detergents are distinguished from lipids because micelles do not have an inner aqueous compartment (unlike liposomes) and because detergent monomers have significant aqueous solubility. Indeed, at total concentrations below the "critical micelle concentration" (CMC) detergents do not form micelles at all. Above the CMC, additional detergent goes into micelles and the free-detergent concentration stays constant at the CMC (Figure 1).[3] Rapid exchange takes place between micellar and free detergent molecules. To sustain membrane-protein solubility, total detergent concentration must be kept above the CMC. Diluting a solution containing IMP/detergent mixed micelles below the detergent's CMC normally results in IMP aggregation and precipitation.

The need to maintain a concentration of detergent well in excess of the amount specifically involved in membrane-protein complexation is not always desirable. Excess detergent can also interfere with protein–protein or protein–ligand interactions that one might be attempting to reconstitute, detect, or

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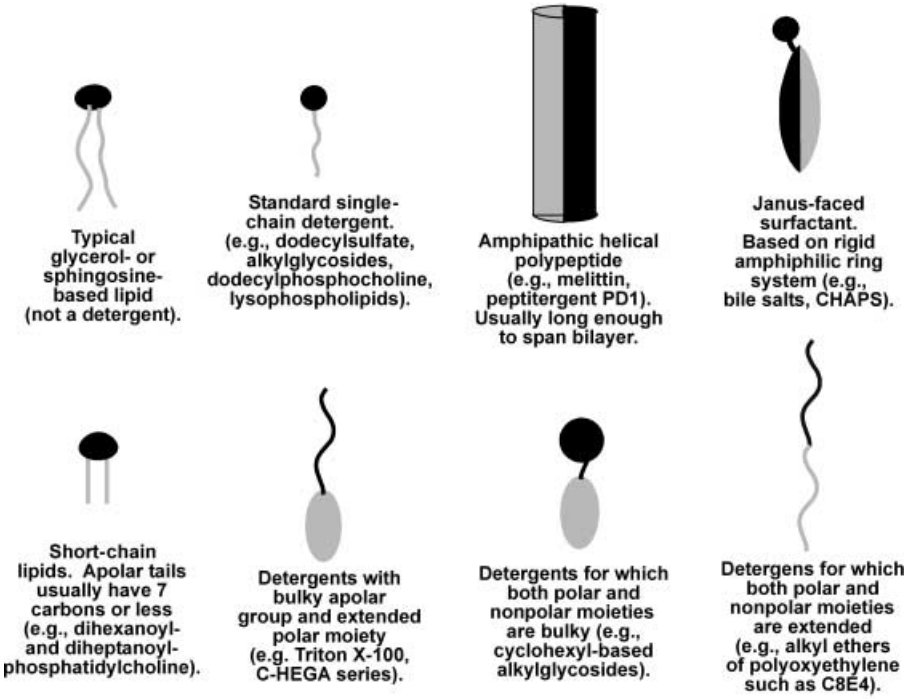

Figure 2. *A nonexhaustive gallery of detergent topologies.*

characterize. There are also advantages to be gained by eliminating the need for high detergent concentrations in studies involving the use of various forms of spectroscopy (e.g., mass, vibrational, circular dichroism, or nuclear magnetic resonance). These considerations underline the need for methods that can solubilize proteins in ways that maintain native-like protein folding and function, but that avoid the use of classical detergents. Here, we review recent progress in the development of model membrane media that offer alternate routes to solubilizing IMPs (see also ref. [5] for another recent perspective).

2. The Latest in Bicelles

Another disadvantage of using detergents to solubilize IMPs is that membrane proteins are often less stable in micelles than in lipid bilayers.[6, 7] This stems, in part, from the fact that the higher molecular order and the lateral surface energy potential of lipid bilayers relative to micelles play a role in stabilizing IMPs. The use of bicelles,[8] also known as nanodiscs,[9] which partially maintain the order of extended bilayers, might provide a more stabilizing environment for soluble IMPs (Figure 3). The most commonly used classes of bicelles involve the use of detergents to stabilize the edges of the discs, and such bicelles would be subject to the usual requirement for relatively high detergent concentrations. In this regard, a welcome recent development is the use of amphipathic protein scaffolds to stabilize the bilayer edges of bicelles without the requirement for excess free material (Figure 3).[9–13] This implies that these "membrane scaffold proteins" might allow studies at assembly concentrations that are much lower than is feasible for detergent-stabilized bicelles. The use of amphipathic proteins in bicelles may also lead to more homogeneous size distribution of the bicelle population. It should be noted that bicelles tend to be more complex than classical mixed micelles in that they may exist over more narrow ranges of temperature and composition. For example, it now appears that, under some conditions, bicelles that use small-molecule detergents for edge stabilization convert into Swiss cheese-like sheets (perforated bilayers) above the gel-to-liquid crystal-phase-transition temperature characteristic of the primary lipid component of the assemblies.[14–17] However, there is emerging data that the new amphipathic protein-stabilized bicelles may retain the bilayered disc morphology even above the phase transition (S. Sligar, personal communication).

Another important development in the use of bicelles has been the recent demonstration that IMPs can be crystallized from bicelles.[18] This development complements innovations both in am-

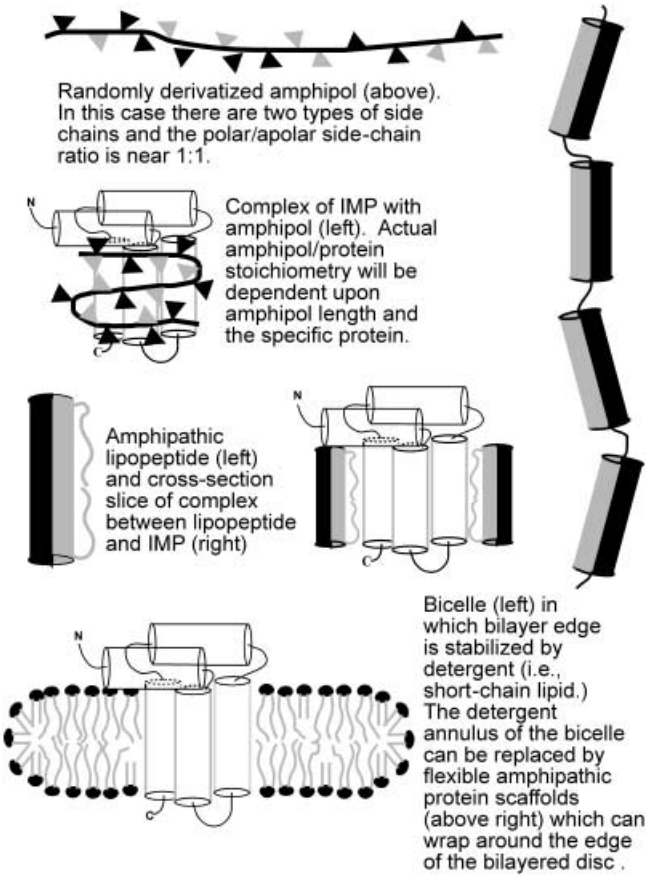

Figure 3. *Amphiphols, amphipathic proteins, lipopeptides, and bicelles.*

phiphile-based approaches to membrane-protein crystallization[19, 20] and in the use of bicelle-related phases by NMR spectroscopists as a medium for aligning both water-soluble and membrane-associated proteins in order to gain access to structurally useful dipolar coupling and chemical-shift-anisotropy data.[21–24] It should be emphasized, however, that under the conditions found to be appropriate for crystallization and for magnetic alignment, the "bicelles" are most likely perforated bilayer sheets rather than bilayered discs.[14–16, 25]

3. Lipopeptides

Lipopeptides have recently been introduced as IMP swimwear by G. Privé and co-workers in Toronto.[26] Extending the previous development of amphipathic helices as detergent surrogates,[27] they have designed molecules in which a helical amphipathic peptide long enough to span a lipid bilayer is adorned on each end by a fatty acid ester (Figure 3). These lipopeptides appear to be able to solubilize IMPs by clustering concentrically around the transmembrane domain of the resident protein. In these complexes, the fatty acyl chains form the interface between the hydrophobic protein surface and the amphipathic peptide that mediates aqueous interactions. The IMP – lipopeptide complexes appear to be favorable in terms of maintaining IMP function and stability. Moreover, unlike classical detergents, the free-lipopeptide concentration in the presence of protein – lipopeptide complexes is very low ($< 10^{-6}$ M). While very early in development, lipopeptides appear to offer distinct advantages for some applications over both classical detergents and amphipathic helical polypeptides.

4. Amphipathic Polymers: "Amphipols"

Several years ago Popot, Audebert, and Tribet in Paris introduced single-chain polymers that were randomly decorated with polar and apolar side chains.[28] These amphipathic polymers are known as "amphipols". Specific amphipols distinguish themselves from each other based on the identity of the parent polymer, the length of the parent polymer, the nature of the polar and apolar side chains, the degree of side-chain derivatization, and the degree of heterogeneity both in side-chain distribution and in main chain length. Amphipols are also in a relatively early stage of development, with progress being summarized in an excellent recent review.[29] Even so, it has been shown that amphipols are able to maintain the solubility of IMPs in the complete absence of excess free amphipol, even when the apolar polymer side chains are relatively short. Such high affinity for membrane proteins by amphipols relative to single detergent molecules can be explained in terms of the smaller entropy loss involved in association of an amphipol with an IMP versus the much larger (unfavorable) entropy loss involved in the association of the many individual detergents molecules required to coat the same amount of exposed hydrophobic surface area.

Amphipols have been shown to confer considerable kinetic stability to at least some membrane proteins.[29] Moreover, their use in sustaining the functionality of IMPs even in the absence of added lipid or detergent has been demonstrated.[30] A prototypical application of amphipols in a biochemical study of an IMP has been published that yielded information on ligand binding that could not readily be obtained in studies in which the same protein was solubilized by using detergents.[31] Although not yet clear from the data, it is very possible that entropy affects might lead to higher thermodynamic stability for amphipol-stabilized IMPs than when classical detergents are employed.

Most amphipols produced to date appear to have only a modest propensity for disrupting lipid bilayers.[29] This suggests that they may be used in cell biological or therapeutic applications as nonperturbative protein-delivery agents. It has been shown, for example, that amphipols can be used to deliver an IMP to preformed lipid bilayers with moderate (ca. 25%) efficiency.[32]

Looking ahead, perhaps the greatest problem for some potential applications of amphipols is the high degree of heterogeneity of amphipols and amphipol – protein complexes. Current synthetic amphipols are based on derivatization of polymers that are not homogeneous in terms of length.[28, 29, 33] Side-chain-modification patterns are also not identical from amphipol to amphipol, but range from completely random to partially ordered sequences. The degree to which molecular heterogeneity will impact the productive use of amphipols depends, of course, on the specific application. An obvious way of avoiding this problem would be to develop structurally homogeneous amphipols. This appears to have already been accomplished in prototype form. The recombinant amphipathic "membrane scaffold proteins" developed by Sligar and coworkers (based on mimicking natural plasma lipoproteins) can be regarded as a class of amphipols. These extended amphipathic proteins have so far been used to stabilize bicelle-like nanodiscs (with or without guest proteins).[9–13] However, it is feasible that proteins of this same basic design might ultimately serve as minimalist swimwear for IMPs even in the absence of additional lipid or detergent.

Beyond the issue of molecular heterogeneity, amphipol – IMP complexes may also be heterogeneous both in terms of stoichiometry and in modes of association within the overall population of complexes.[29] Moreover, interchange between modes is not necessarily rapid. In this regard it should be noted that for classical detergent – IMP mixed micelles, a great deal of instantaneous heterogeneity is likely in terms of mixed micelle sizes and exact protein – detergent interactions. However, because of the small size and high solubility of detergent monomers, interchange between modes is so rapid that, for example, relatively slow spectroscopic techniques, such as NMR, probably see only the average—a great advantage in many cases. The degree to which amphipol – IMP complex heterogeneity is actually a practical problem and whether or not solutions to this problem can be developed remain to be seen.

5. Which Method for Solubilizing IMPs is Best?

Biological membranes represent chemically heterogeneous environments involving hundreds of distinct lipid species. Moreover, the composition of membranes from organism to

organism can vary dramatically, even exotically (c.f. ref. [34]). This is also true from cell to cell within a single organism or even from organelle to organelle within a single cell. The notion that there is a single model membrane medium that is best for all proteins does not conform to biological reality. In general, the choice of model membrane medium is made based on the medium that best lends itself to a particular experimental approach or application. Of course, there will often be a compelling need to provide appropriate control data to demonstrate that the IMP of interest retains native-like structural and functional properties when in a particular model membrane environment. Fortunately, it is already clear that a number of membrane proteins have extremely liberal views regarding what they consider to be appropriate swimwear. For example, *E. coli* diacylglycerol kinase (40 kDa homotrimer with nine transmembrane helices) has been functionally reconstituted in many different types of mixed micelles, bicelles,[35] amphipols,[30] and even (as an insoluble suspension) some organic solvent mixtures.[36]

The recent development of novel classes of model membranes and methods for solubilizing membrane proteins reflect the continuation of decades of innovation in this area. It can be expected that such innovation, if patiently nurtured, will prove critical for future breakthroughs in membrane-protein biology, human therapeutics, and biotechnology.

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