DETERGENTS AND THEIR USES IN MEMBRANE PROTEIN SCIENCE

It's all about purity.

Anatrace

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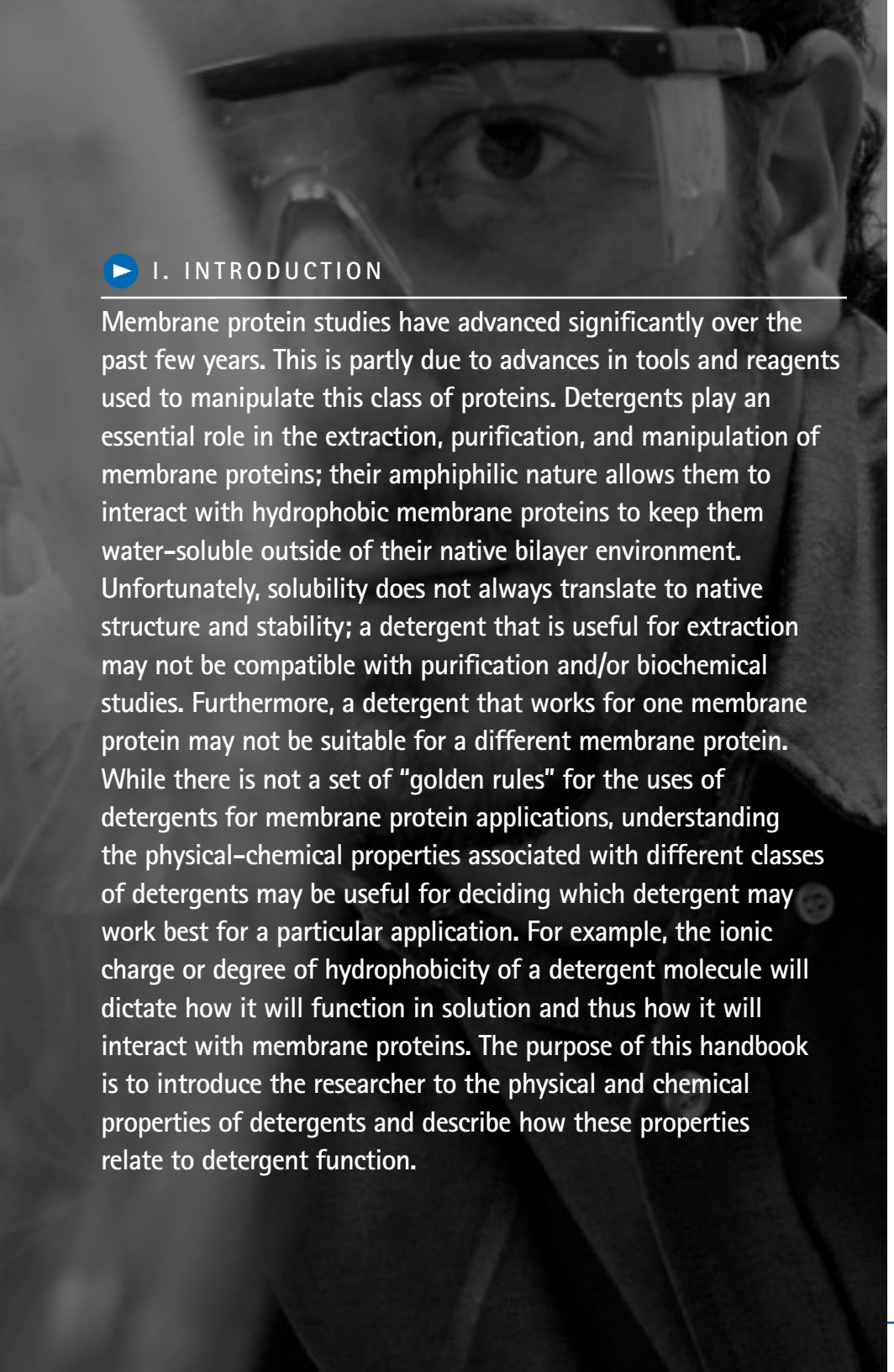


I. INTRODUCTION

Membrane protein studies have advanced significantly over the past few years. This is partly due to advances in tools and reagents used to manipulate this class of proteins. Detergents play an essential role in the extraction, purification, and manipulation of membrane proteins; their amphiphilic nature allows them to interact with hydrophobic membrane proteins to keep them water-soluble outside of their native bilayer environment. Unfortunately, solubility does not always translate to native structure and stability; a detergent that is useful for extraction may not be compatible with purification and/or biochemical studies. Furthermore, a detergent that works for one membrane protein may not be suitable for a different membrane protein. While there is not a set of "golden rules" for the uses of detergents for membrane protein applications, understanding the physical-chemical properties associated with different classes of detergents may be useful for deciding which detergent may work best for a particular application. For example, the ionic charge or degree of hydrophobicity of a detergent molecule will dictate how it will function in solution and thus how it will interact with membrane proteins. The purpose of this handbook is to introduce the researcher to the physical and chemical properties of detergents and describe how these properties relate to detergent function.

II. Structure and Behavior of Detergents

Detergents are amphiphilic compounds with well-segregated polar and apolar domains that have measurable aqueous solubility as both aggregates and as monomers. Detergents belong to a class of compounds called surfactants, which are surface active agents that reduce interfacial surface tension in mixtures (i.e., oil and water) by adsorbing to interfaces [1]. Detergents are useful in a wide variety of applications including: polyacrylamide gel electrophoresis (PAGE), membrane permiabilization, membrane dissolution, inclusion body solubilization, lipid raft preparation, and membrane protein solubilization, biochemistry, crystallization, and manipulation. Detergents are also useful as model membranes for *in vitro* studies and as vehicles for protein/DNA/drug delivery.

The ability of a detergent to participate in a specific biological/biochemical function is related to its structure; the polar hydrophilic portion of the detergent molecule is referred to as the "hydrophilic head group" while the nonpolar hydrophobic, portion is referred to as the "tail" (Figure 1A).

There are, however, a few detergents that have a bean-like molecular shape in the sense that they contain both polar and nonpolar "faces"; these include the bile acid derivatives such as CHAPS and CHAPSO (Figure 1B).

Figure of a detergent monomer

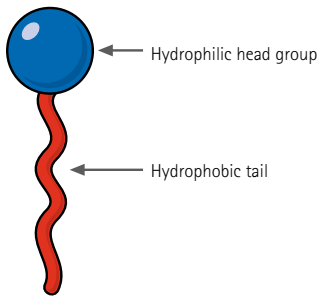


Figure 1A

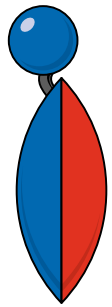


Figure 1B

Traditional detergent monomers are generally cone shaped; the hydrophilic head groups tend to occupy more molecular space than the linear alkyl chains (Figure 2A). Detergents tend to aggregate into spherical or elliptoid micelles that are water soluble (Figure 2B). While lipids also have the same general structure as detergents—a polar hydrophilic head group and a nonpolar hydrophobic tail—lipids differ from detergents in the shape of the monomers, in the type

of aggregates formed in solution, and in the concentration range required for aggregation. Lipids are generally cylindrical; the area occupied by the two alkyl chains is similar to the area occupied by the polar head group (Figure 2C). Lipids have low solubility as monomers and tend to aggregate into planar bilayers that are water insoluble (Figure 2D).

Molecular shapes of detergents and lipids

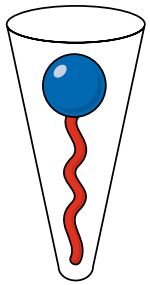


Figure 2A

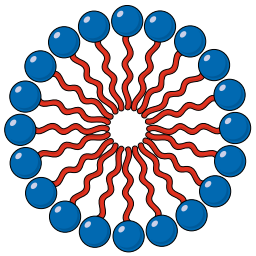


Figure 2B

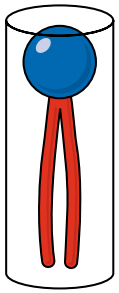


Figure 2C

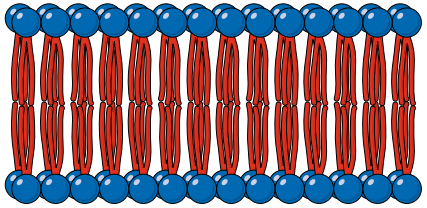


Figure 2D

Effects of the hydrophilic group on detergent function

Water solubility is provided by the hydrophilic portion of a detergent molecule. Hydrophilic groups can be categorized as ionic (cationic or anionic), nonionic, or zwitterionic. Ionic detergents, including sodium dodecyl sulfate (SDS), N-lauryl sarcosine, cetyltrimethylammoniumbromide (CTAB), and sodium cholate are effective at extracting proteins from the membrane. However, these detergents are harsh and tend to be denaturing because they efficiently disrupt both inter- and intra-molecular protein-protein interactions. SDS, for example, is commonly used as a membrane protein denaturant in quantitative protein unfolding/folding studies [2-6]. Bile acid salts (i.e., sodium cholate, deoxycholic acid) are also ionic detergents; however, they tend to be more mild than straight chain ionic detergents [7].

Nonionic detergents, including maltosides, glucosides, and polyoxyethylene glycols are characterized by uncharged hydrophilic head groups. These detergents are mild and nondenaturing because they disrupt protein-lipid and lipid-lipid interactions rather than protein-protein interactions. Short chain (i.e., C_7-C_{10}) nonionic detergents are typically more deactivating than longer chain (i.e., C_{12}-C_{14}) nonionic detergents [7, 8]. A majority of the detergents used in the purification and structural determination of membrane proteins (i.e., lauryl maltoside, octyl glucoside) are nonionic detergents [9-11].

Zwitterionic detergents, including the Zwittergents®, Fos-Cholines®, CHAPS/CHAPSO, and amine oxides contain both a positive and negative charge in their hydrophilic head group. These compounds are electrically neutral like the nonionic detergents, but can often disrupt protein-protein interactions like the ionic detergents; therefore, they tend to be intermediate in their mildness. The zwitterionic detergent lauryldimethyl amine oxide (LDAO) has been used to study the KcsA potassium channel [12] as well as the outer membrane BtuB:TonB complex [13]. Most successful NMR-based structural studies of membrane proteins have been carried out in zwitterionic detergent solutions such as dodecylphosphocholine (i.e., Fos-Choline 12) [14-16].

Effects of the hydrophobic group on detergent function

The hydrophobic portion of a detergent allows the molecule to partition into the apolar lipid bilayer during the solubilization of membrane proteins. It also masks the hydrophobic portions of the membrane proteins once they have been solubilized and thus prevents protein aggregation. The size of the hydrophobic tail is determined by the length of the alkyl chain, the degree of unsaturation within the chain, and whether one or two alkyl chains are present [1]. The physical characteristics of the hydrophobic group (i.e., the length of the alkyl chain, the degree of branching within the chain, the presence of an aromatic nucleus, the number of polyloxyethylene units present, and the presence of fluoroalkyl units) affect the chemical properties of the detergent monomers as well as the aggregates that they form in solution. For example, increasing the hydrophobic chain length decreases the water solubility of the detergent monomer and causes close packing of the monomers within micelles. Branching and unsaturation cause loose packing of detergent monomers in micelles. Polyoxyethylene units tend to decrease the hydrophobicity of the detergent monomer while fluoroalkyl groups increase the hydrophobic character of the detergent monomer [1].

Hydrophilic–Lipophilic Balance (HLB)

Although the hydrophilic head group and hydrophobic tail each affect the properties of the detergent molecule differently, together their total effect is known as the Hydrophilic-Lipophilic Balance (HLB). The HLB is defined by a number that ranges from 0 to 40. In general, an HLB number <10 indicates that a detergent has low solubility in water while an HLB number between 10 and 20 indicates that the detergent is readily soluble in water [17]. Examples of detergents with HLB values between 10 and 40 include: SDS (40), sodium cholate (18), Brij-35 (16.9), Tween 20 (16.7), Tween 80 (15), Triton X-100 (13.5), and Triton X-114 (12.4) [18, 19]. For simple, single-chain detergents, HLB can be determined by the following equation [20, 21]:

$$HLB = \Sigma H - \Sigma L + 7 \qquad \text{(i)}$$

Where H is the contribution from the hydrophilic group and L is the contribution from the lipophilic group

In studies with the human adenosine A_3 receptor, a member of the GPCR superfamily, Berger et al. showed that detergents with an HLB number of 15 correlated with selective extraction of A_3 from the membrane and high activity upon purification [22]. Specifically, A_3 was successfully purified in decyl maltoside (DM), dodecyl maltoside (DDM) and HEGA-10. Detergents with HLB numbers ranging from 12.4 to 13.5 (i.e., Triton X-100) were previously shown to efficiently solubilize and maintain the stability of *B. subtilis* D-alanine carboxypeptidase and *M. luteus* phosphoacetylmuramyl pentapeptide translocase and succinate dehydrogenase [19]. Several other studies have also shown that HLB values may be useful in selecting detergents for membrane protein extraction and purification [23, 24].

The HLB has also been correlated to the detergent packing parameter which can be expressed as:

$$P = v / al \qquad \text{(ii)}$$

Where v is the volume of the detergent chain, l is the length of the chain, and a is the cross-sectional area of the head group.

Packing parameters are assigned to detergent monomers and are useful for predicting the shape of the aggregate (i.e., spherical or lamellar) formed by those monomers. For example, P<1/3 indicates that the detergent will likely form spherical micelles while 1/3<P<1/2 indicates that the detergent will likely form cylindrical micelles [25]. Berger et al. showed that as the HLB value of a detergent decreases, the packing parameter increases [22]. For example, as the hydrophobicity of a detergent increases, there is a tendency for the monomers to assemble into a more lamellar aggregate. These shapes may also influence the effects of a detergent upon a solubilized protein.

III. Micellization

Detergents interact with proteins and membranes as micelles. Micellization occurs when surface active compounds form non-covalent clusters in solution; this process is driven by the hydrophobic effect [1]. When a nonpolar group is introduced into an aqueous solution, the hydrogen bonding network formed by the existing water molecules is disrupted and the water molecules order themselves around the nonpolar entity to satisfy hydrogen bonds (Figure 3A). This results in an unfavorable decrease in entropy in the bulk water phase. As additional nonpolar groups are added to the solution, they self-associate thus reducing the total water-accessible surface of the complex relative to the monodisperse state. (Figure 3B) Now, fewer water molecules are required to re-arrange around the collection of nonpolar groups. Therefore, the entropy associated with the complex is less unfavorable than for the monodisperse

detergents. In short, hydrophobic association and the formation of micelles is driven by the favorable thermodynamic effect on the bulk water phase [26].

Hydrophobic effect and micellization

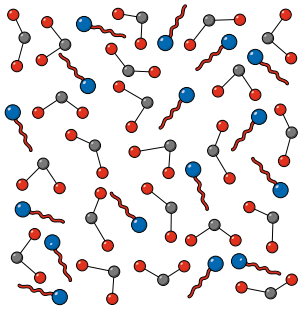


Figure 3A

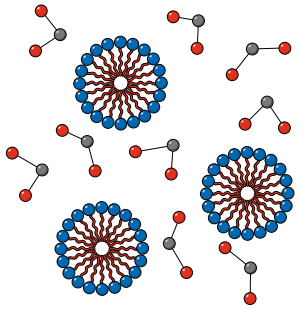


Figure 3B

Solubilization of proteins is dependant upon the formation of micelles in solution. Micelles are colloquially thought to be spherical in shape. However, it is now appreciated that they are asymmetrical and have "rough" surfaces where the alkyl tails are disorganized and transiently poke into the bulk solution (Figure 4A) [27-29].

Micelles are typically a few nanometers in diameter and have a molecular weight of less than 100 kDa. Detergent micelles are dynamic structures; detergent monomers within the micelle are in constant, rapid exchange with free detergent monomers in solution. Although the molecular details of how detergent micelles extract proteins from a membrane are still not completely understood, it is generally accepted that once a protein has been solubilized, the detergent molecules form a torus around the hydrophobic transmembrane domains (Figure 4B) [8].

Micelles and membrane protein extraction

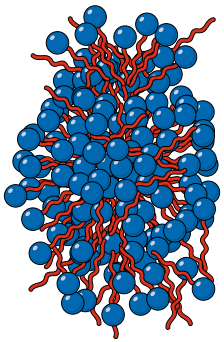


Figure 4A

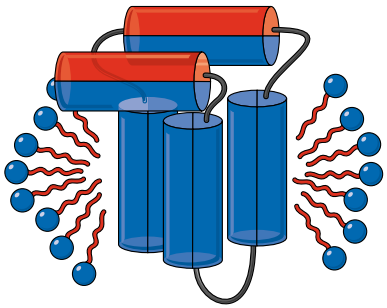


Figure 4B

The critical micelle concentration

Micellization is a critical phenomenon when considering detergent applications. Each detergent can be characterized by its critical micelle concentration (CMC); the concentration of detergent above which monomers self-assemble into non-covalent aggregates (called micelles) [1, 30, 31]. The CMC actually does not occur at a single concentration, but rather, over a narrow concentration range. When the total detergent concentration is below the CMC, detergent monomers are free in bulk solution. However, as more detergent is added above the CMC, all additional detergent monomers will go into micelles. It is important to note that when the total detergent concentration is greater than the CMC, there is a monomeric detergent concentration equal to the CMC and a micellar detergent concentration equal to: [total detergent concentration] – CMC. The CMC can be determined by a variety of methods including surface tension measurements [32] and dye (i.e., anilino-1-naphthalene sulfonic acid [ANS]) binding experiments [33]. When working with membrane proteins, a general rule of thumb is to work at a detergent concentration of *at least* 2X CMC and at a detergent:protein weight-to-weight ratio of at least 4:1. Moreover, when solubilizing proteins from native membranes, it is advisable to work at a detergent concentration well above the CMC as well as at a 10:1 detergent:lipid mol:mol ratio. Therefore, the CMC dictates how much detergent needs to be added to various protein and membrane preparations.

There are several physical-chemical factors that can affect the CMC of a given detergent. Generally, the CMC decreases as the hydrophobicity of the detergent increases. Other properties that directly affect the CMC are the characteristics of the hydrophobic and hydrophilic groups and solution additives such as electrolytes.

Effects of the hydrophilic group on CMC

Variations in the hydrophilic head group affect the detergent CMC. In general, detergents containing ionic head groups have a higher CMC than those containing nonionic head groups [1]. This is due to electronic repulsion between the head groups of neighboring detergent monomers within the micelles. Detergents containing zwitterionic head groups tend to have smaller CMCs than those containing ionic head groups.

Effects of the hydrophobic group on CMC

The physical characteristics of the hydrophobic group can also have varying effects on the CMC of a particular detergent. In general, the CMC decreases as the number of carbon atoms in the alkyl chain increases up to approximately 16 to 18 carbons (for straight chain alkyls) [1]. Above this point, detergents become lipid-like and do not form discrete micelles. As a rule of thumb, for ionic detergents, the addition of a single methylene group to the hydrophobic tail halves the CMC. For nonionic and zwitterionic detergents, the addition of a methylene group reduces the CMC by approximately 80% relative to the parent CMC. In general, carbon atoms on branched hydrophobic chains have about half the effect on the CMC as carbon atoms on straight chains. The addition of a phenyl ring to the hydrocarbon chain is equivalent to approximately 3.5 methylenes. A carbon-carbon double bond increases the CMC compared to the corresponding saturated compound; compounds with *cis* double bonds have a higher CMC than compounds with *trans* double bonds. When an oxygen or hydroxyl group is added to the hydrophobic group, the CMC increases. Methylene groups between these polar groups and the hydrophilic head group have approximately half the effect on the CMC as they would in the absence of the polar group. Fluorocarbons tend to have a lower CMC than hydrogenated carbons [1].

Effects of electrolytes on CMC

Electrolytes tend to reduce the CMC of detergent solutions. For example, the CMC for the anionic detergent SDS is approximately 6 mM; however, in the presence of 150 mM NaCl, the CMC is reduced to 1.4 mM [34]. A further reduction in the CMC to 0.9 mM was found upon the addition of 350 mM NaCl. Similar effects have been shown for other anionic detergents including potassium laurate and sodium decyl sulfate [34]. Reductions in CMC upon salt addition have also been shown for cationic detergents including dodecylammonium chloride, decyltrimethylammoinum bromide, and cetyltrimethylammonium sulfate [34, 35]. The reduction in the CMC in the presence of electrolytes for ionic detergents is likely due to a reduction in the electronic environment surrounding the ionic head groups. Addition of electrolytes decreases the repulsion between similarly charged ionic head groups within a micelle and therefore, the detergent monomers can pack tightly and the CMC is reduced [1].

Addition of salts to solutions containing nonionic detergents also reduces CMC values. For example, the CMC of Triton X-100 in aqueous solution is 0.24 mM. In the presence of 0.5 M or 1.0 M NaCl the CMC is reduced to 0.14 mM and

New thinking for new innovations

0.08 mM respectively [36]. For nonyl glucoside, the CMC is reduced from 6.9 mM in aqueous solution to 2.6 mM in 1.5 M NaCl [37]. The decrease in the CMC for these uncharged detergents is likely due to the effects of electrolytes on the hydrophobic moieties. Electrolytes that are highly hydrated, (i.e., Cl^-) are water structure-makers; they will "salt out" hydrophobic groups and therefore, they tend to decrease the CMC. Electrolytes that have a small charge:radius ratio (i.e., SCN^- and I^-), are water structure breakers; they tend to "salt in" hydrophobic groups. Thus ions may either increase *or* decrease the CMC of a nonionic detergent [1, 36-38].

Cloud point

The cloud point is the temperature above which a nonionic surfactant solution separates into a detergent rich phase and a detergent poor phase [1, 25, 27]. The separation is visualized as turbidity within the solution. An increase in temperature favors micelle formation; the rapid growth of micelles along with intermicellar attraction likely results in the formation of large particles that can precipitate out of solution, thus causing turbidity. This phase separation is reversible upon cooling. Nonpolar additives (i.e., hydrocarbons) tend to increase the cloud point whereas polar compounds (i.e., alcohols) and salts tend to decrease the cloud point [1]. A low cloud point may be useful in membrane protein purification [39-41]. For example, Triton X-114 has a cloud point that is near room temperature. This property makes it possible to carry out two-phase water/detergent extractions to separate water soluble proteins from membrane proteins [39, 42]. However only a very limited number of nonionic detergents have cloud points below 50°C.

Aggregation numbers

Another physical property of the micelle is the aggregation number; the number of detergent monomers present within a micelle [1, 25, 30]. Most detergents used for biochemical applications have aggregation numbers that range from 50 to 100 [8]. Exceptions are some bile acid derivatives like CHAPS, CHAPSO, and Big CHAP which have aggregation numbers of approximately 10. Detergents with smaller aggregation numbers tend to form more spherical micelles while detergents with larger aggregation numbers tend to form elliptoid micelles. In general, aggregation numbers increase as the length of the hydrocarbon chain increases. Aggregation numbers tend to decrease as the size of the hydrophilic group increases and upon the addition of hydrocarbons and polar compounds to the detergent solution [1]. Increasing the temperature of solutions of ionic detergents also causes an increase in the aggregation number. Aggregation numbers can be determined by a variety of methods including light scattering [43], small angle neutron scattering [44], and fluorescent dye binding [45].

With knowledge of the detergent CMC and aggregation number, one can determine several important parameters including the concentration of micelles present in solution and the aggregate molecular weight of the micelle. In ideal, protein-free conditions, the concentration of micelles can be calculated as follows:

$$[\text{micelles}] = [\text{total detergent}] - [\text{CMC}] / \text{AN} \qquad \text{(iii)}$$

where CMC is the critical micelle concentration and
AN is the micelle aggregation number

The aggregate molecular weight (AMW) of a protein-free micelle can be calculated as follows:

$$\text{AMW} = \text{AN} \times \text{monomer molecular weight} \qquad \text{(iv)}$$

where AN is the micelle aggregation number

Typical micelle aggregate molecular weights range from 20 to 100 kDa. It should be noted that determination of the aggregate molecular weight of a protein-detergent complex is more involved and is addressed in Section V.

Detergent removal

The CMC is also important in determining which method should be used to remove excess or unwanted detergent. Detergents may interfere with certain applications and must be removed when reconstituting into liposomes [46, 47]. Detergents with high CMCs are easily removed by dialysis; detergent solutions can be diluted below their CMC so that micelles disintegrate into monomers which can easily pass through dialysis tubing over time [7]. Typically, detergent solutions are dialyzed against a large excess (i.e., 200-fold) of detergent-free buffer for days with several changes of the detergent-free buffer over this time. Detergents with low CMCs are typically removed by adsorption to hydrophobic beads [48]. Detergent bound beads can then be removed by filtration or centrifugation. Detergents can also be removed by various types of column chromatography. Gel filtration can be used to separate detergent micelles from protein-detergent complexes and free protein based on size differences. Detergents can also be removed or exchanged while His-tagged proteins are bound to Nickel resin [7].

IV. Detergents and Biological Membranes

Biological membranes are bilayers of phospholipid molecules; the general architecture of the bilayer is depicted below (Figure 5).

Architecture of the lipid bilayer

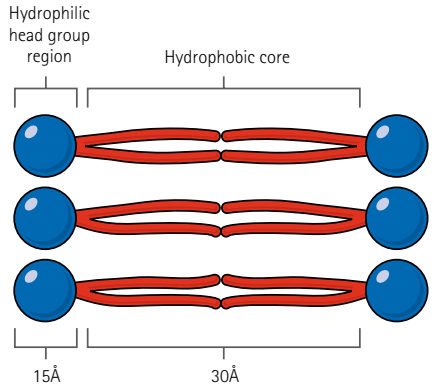


Figure 5

The tails of the lipid acyl chains orient towards each other (creating a non-polar, hydrophobic core) while the polar, phosphoester head groups contact the surrounding bulk water phase. Thus, the bilayer is divided into two distinct regions: the hydrophobic core and the hydrophilic head group region. Each "compartment" has unique properties that differentially affect the proteins that reside within the bilayer. The hydrophobic core of the bilayer, composed of phospholipid acyl chains, is approximately 30 Å thick, and provides the low dielectric environment for the solvation of hydrophobic regions of integral membrane proteins [49, 50]. This region is generally quite fluid at biologically relevant temperatures; bilayer fluidity is often necessary for protein function and lateral diffusion of proteins. The hydrophilic head group region is generally polar and charged. This region interacts with membrane proteins through Columbic forces which stabilize extra-membrane loops and interact with the polar ends of α-helices [49, 50].

Biological membranes are asymmetric with respect to lipids and proteins. For example, the composition of lipids in the different leaflets of red blood cell membranes contributes to the pliability of these cells, permitting their passage through the vasculature (outer leaflet: 76% phosphatidylcholine (PC), 82% sphingomyelin (SP), 20% phosphatidylethanolamine (PE), 0% phosphatidylserine (PS); inner leaflet: 24% PC, 18% SP, 80% PE, 100% PS. Percentages are of total lipid content.) [51]. Additionally, proteins may be preferentially located either on the inner or outer leaflet of the membrane, and in a preferred orientation. This asymmetry can be important when deciding how best to extract a membrane protein and what conditions (i.e., detergents and/or lipids) are best for reconstitution for biochemical studies.

Extracting proteins from the membrane

To study membrane proteins, they must first be extracted from the membrane and maintained in a soluble, native, functional form. During the extraction process, it has been proposed that detergent monomers first partition into the bilayer.

Cooperative detergent-detergent interactions destabilize the bilayer yielding mixed lipid-detergent fragments (Figure 6A). Eventually, further detergent addition leads to bilayer dissolution and protein solubilization (Figure 6B) [8, 52].

Solubilization of membranes

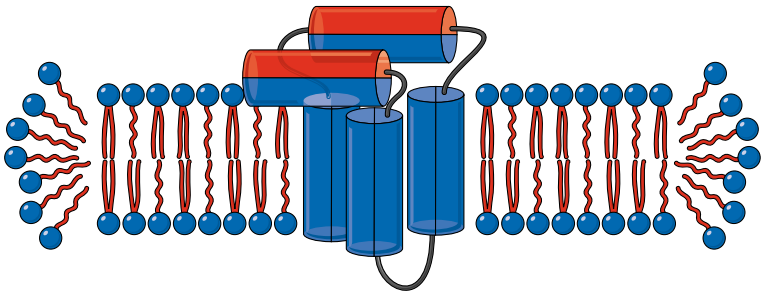


Figure 6A

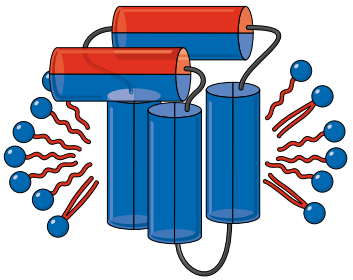
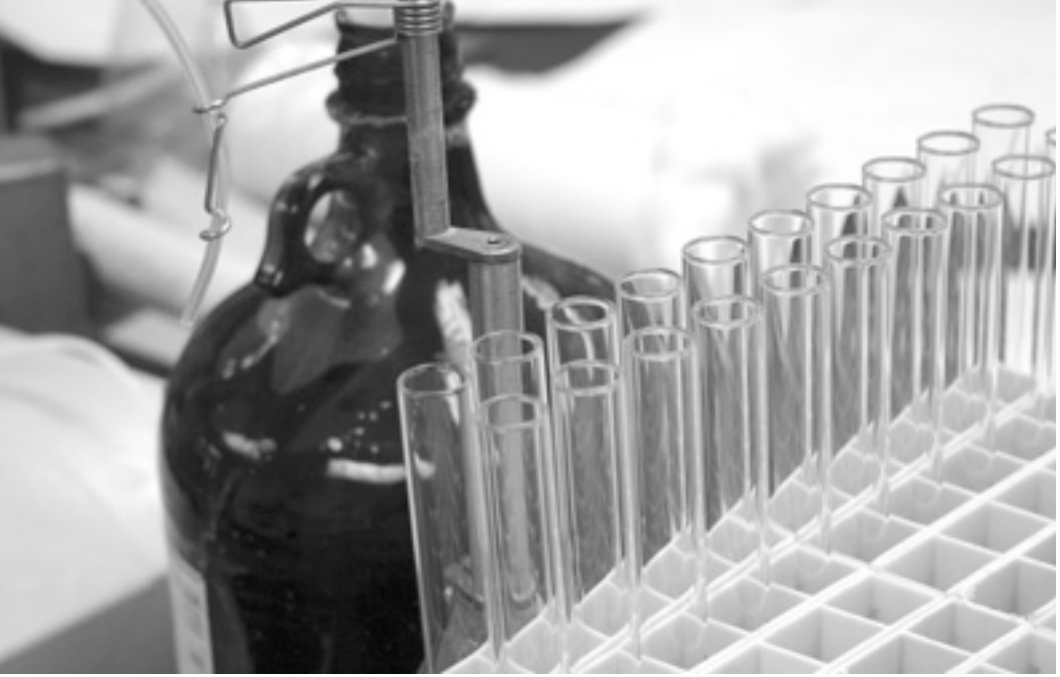


Figure 6B



There are several "degrees" to which a membrane protein can be extracted from the membrane for further study. The protein can be purified in such a way that some native lipids remain bound to the protein. This can be accomplished by using detergents that are not efficient lipid solubilizing agents and by minimizing the duration of detergent exposure during column chromatography. Alternatively, a protein can be completely stripped of native lipids by using stringent detergents. This may be important in applications where homogenous protein preparations are required. Lipids can then be added back to these preparations if necessary for protein activity and/or stability.

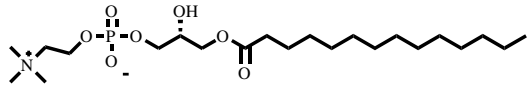
It should be noted that studying the membrane proteins within specialized membrane microdomains, known as lipid rafts, presents a unique problem. Lipid rafts are enriched in sphingolipids, glycerophospholipids, and cholesterol [53-55]. These domains, also called detergent-resistant membranes (DRMs), have been shown to play key roles in cell signaling and protein sorting. Historically, DRMs have been detected by their resistance to solubilization by cold Triton X-100. However, it has been shown that the characteristics of these DRMs are dependant upon the detergents used in their isolation. For example, Schuck et al. showed that the amounts and types of proteins and lipids associated with DRMs varied dramatically when different detergents were used to isolate the membrane domain [53]. Thus, caution should be exercised when choosing an appropriate detergent to isolate proteins from native membranes.

Working with solubilized membrane proteins

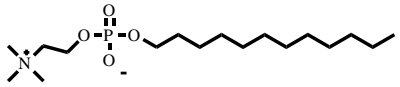
Some of the more common detergents that have been shown to be useful in membrane protein functional and structural studies are the alkyl glycosides [56-58]. For example, short chain alkyl maltosides and glucosides have been successful in the crystallization of membrane proteins [59-63] whereas longer-chain glycosides (i.e., dodecyl maltoside, tetradecyl maltoside, and hexadecyl maltoside) have been shown to stabilize various oligomeric states of the G-protein coupled receptor (GPCR), rhodopsin [64]. Dodecyl maltoside, for example, has been used to crystallize the membrane protein cytochrome *c* oxidase from *Rhodobacter sphaeroides* [65], to study the unfolding of the 4-transmembrane helix protein DsbB from the inner membrane of *E. coli* [66], and to study the light-induced structural changes in mammalian rhodopsin by ^{19}F NMR [67].

Other detergents that are finding an increasing use in membrane protein biochemistry are the lysophospholipids, Fos-Choline® detergents, and short chain phospholipids (Figure 7).

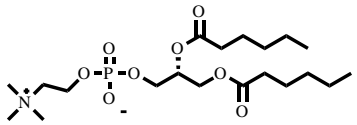
Phospholipid-like detergents



Lysomyristoylphosphatidylcholine



Fos-Choline 12



Dihexanoylphosphatidylcholine

Figure 7

Lysophospholipids are similar to the native phospholipids in which membrane proteins are embedded; they have phospholipid-like head groups however their hydrophobic tails contain only a single acyl chain and they form water-soluble aggregates. Indeed, some GPCRs remain functional after extraction into lysophospholipid micelles [68-70]. Lysophospholipids have also

been used in NMR structural studies of membrane proteins as well as in the purification of the cystic fibrosis transmembrane conductance regulator (CFTR) [71, 72]. As mentioned previously, the Fos-Choline detergents have been successfully used in membrane protein studies by NMR [14-16]. Short chain phospholipids such as dihexanoylphosphatidylcholine (DHPC), have been used to solubilize and reconstitute integral membrane proteins. These compounds form water-soluble micelles in solution and have been shown to maintain native protein structure and function when used in membrane protein purification protocols [73-75]. For example, the NMR structure of the *E. coli* outer membrane protein X (OmpX) was determined in DHPC micelles [76].

Membrane proteins can also be reconstituted into detergent-lipid mixed micelles. This may be the closest representative bilayer-mimetic system. For example, bacteriorhodopsin has been refolded into several different detergent-lipid systems including CHAPS/DMPC and CHAPSO/SDS/DMPC micelles [77, 78].

V. Practical considerations

There are several practical issues to consider when working with detergents and membrane proteins. First, one must determine the degree of detergent purity and homogeneity required for specific applications. For example, when purifying and/or crystallizing proteins, one may choose a detergent that is both pure (i.e., free of contaminating alcohols, amides, or other byproducts of synthesis) and homogeneous (i.e., composed of a single species). Many industrial-grade detergents, including Triton and Tween, may be pure, but are heterogeneous in the composition of their polyoxyethylene chains. These detergents may be less suitable for crystallization screens, but may be sufficient for protein extraction.

Secondly, when determining the molecular weight of a solubilized membrane protein, one must consider the *aggregate molecular weight* of the detergent-protein complex (Figure 8). If it can be assumed that there is one protein molecule per micelle and if the protein is smaller than the micelle, then the aggregate weight of the complex is equal to the protein molecular weight plus the micelle aggregate weight. However, larger membrane proteins will tend to complex with a higher amount of detergent than is present in a free micelle alone. In this case, the detergent concentration must be sufficient to completely coat the exposed regions of the transmembrane domain.

Aggregate molecular weight of protein/detergent complexes

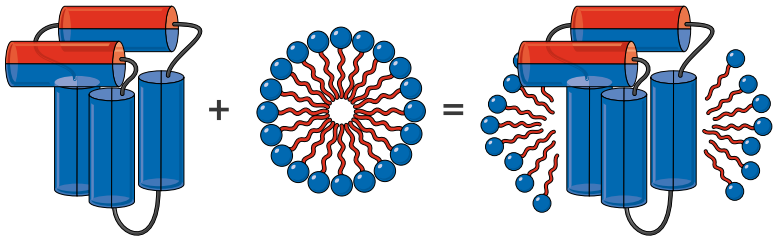


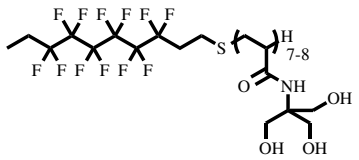
Figure 8

Similarly, it is important to note that when one is concentrating a solution of detergent-solubilized protein, the concentration of empty micelles may also increase as their molecular weight may be greater than the molecular weight cut off of a concentrator membrane. Several methods exist for determining the detergent concentration in solution including colorimetric assays [79], thin layer chromatography [80], refractive index measurements [81], light scattering measurements [81], and analytical ultracentrifugation [82, 83]. Some of these methods are useful for determining the concentration of free detergent in solution [79-81]. Others are useful for determining the amount of protein-bound detergent [79, 80, 83] or the size of a protein-detergent complex [81, 83].

VI. Non-detergent surfactants and other novel detergents

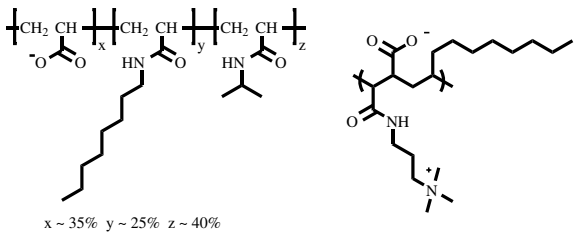
As mentioned previously, membrane proteins can be destabilized or denatured by certain detergents including ionic detergents and short chain nonionic detergents. Hemifluorinated surfactants (Figure 9A) and amphipols (Figure 9B) are two very different non-detergent surfactants that have found use in membrane protein studies.

Non-detergent surfactants



HF-TAC

Figure 9A



A8-35 PMAL-C8

Figure 9B

Hemifluorinated surfactants contain a fluorinated hydrophobic tail and a polar head group [84-86]. Fluorinated chains are unique in that they are not miscible with hydrocarbons (i.e., lipids). Therefore, these compounds cannot be used to solubilize membrane proteins. One compound, HF-TAC, has been shown to maintain the solubility and stability of bacteriorhodopsin and cytochrome b_6f complex [84]. It has been suggested that HF-TAC retains protein-bound lipids better than traditional detergents; this likely contributes to the stability of the cytochrome b_6f complex within these compounds. Other zwitterionic perfluorinated detergents are known to align in a magnetic field and may be useful as tools for NMR studies of membrane proteins [87].

Amphiphols are amphipathic polymers that wrap around membrane proteins to maintain their solubility [88]. Amphiphols are unique in that they bind proteins tightly and protein-amphipol complexes are stable for long periods of time [89]. Due to this tight binding, excess amphipol can often be removed from the bulk solution without affecting protein stability. Several membrane proteins have been studied in complexes with amphipols including the photosynthetic reaction center from *Rhodobacter sphaeroides* [90], the acetylcholine receptor [91], diacylglycerol kinase [92], OmpA, FomA, and bacteriorhodopsin [93].

Several additional novel detergent alternatives have been proposed over the past few years including lipopeptides (Figure 10A) and tripod amphiphiles (Figure 10B).

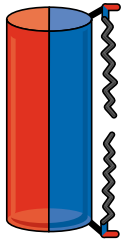


Figure 10A

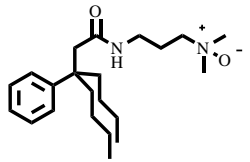


Figure 10B

Lipopeptides contain two hydrophobic alkyl chains separated by a short amphipathic peptide [94]. These compounds self assemble such that the alkyl chains effectively solubilize hydrophobic domains of membrane proteins while the small peptide forms a shell around the complex to render it water soluble. These compounds have been shown to maintain the solubility of bacteriorhodopsin, PagP, and a lac permease-cytochrome b_{562} fusion protein [94].

Tripod amphiphiles are unique amine oxides that have been used in the solubilization and crystallization of bacteriorhodopsin. These compounds contain three rigid chains that have been suggested to promote membrane protein crystallization [95, 96].

VII. Model membrane systems

Several novel model membrane systems incorporating both detergents and lipids have also been used to study integral membrane proteins. Nanodiscs are self-assembling complexes that consist of a phospholipid bilayer core surrounded by an amphipathic membrane scaffold protein (MSP) (Figure 11) [97, 98]. The MSP is a 200-residue protein that is a series of linked amphipathic helices. A target protein can be incorporated into the self-assembly process and theoretically be reconstituted into a native-like environment. A single molecule of bacteriorhodopsin was successfully incorporated into these nanodiscs [97] as was heterologously expressed, functional Arabidopsis cytochrome P450 and P450 reductase [99].

Model membrane systems

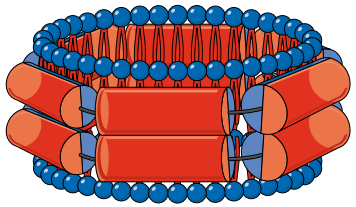


Figure 11

Bicelles are unique model membrane systems composed of both lipids and detergents that have found applications in solution and solid-state NMR [100-103] and more recently in membrane protein crystallography [104, 105]. Bicelles are prepared by mixing lipids such as dimyristoylphosphatidylcholine (DMPC) with edge-stabilizing detergents (i.e., CHAPSO) or short chain lipids such as dihexanoylphosphatidylcholine (DHPC) in 4:1 to 1.5:1 lipid:detergent molar ratios. These edge stabilized planar bilayered assemblies present several advantages over traditional mixed micellar systems; (1) bicelles represent a more native-like environment for structural studies of membrane proteins, (2) the effects of membrane curvature may be less pronounced than seen in pure detergent micelles, (3) for NMR studies, bicelle aggregate sizes are sufficiently small and they can be aligned in a magnetic field, and (4) for crystallization trials, bicelles are easy to manipulate and the crystals produced from them can be easily isolated and mounted for diffraction.

VIII. Conclusions

In conclusion, detergents are indispensable when working with integral membrane proteins. By nature of their amphiphilic character, detergents are able to partition into biological membranes, extract proteins, and maintain protein solubility in solution. Detergents are useful in a wide variety of other applications as well including PAGE, inclusion body solubilization, and lipid raft preparation. Unfortunately, there is not an easy method for choosing which detergent may be best for a particular application. However, several studies have been published comparing the effects of different detergents on membrane protein solubility, activity, and structure [106-113]. These studies can be used as guides for determining which detergents may be most suitable for a particular protein or application. Different detergents display unique physical-chemical properties; the ionic charge, degree of hydrophobicity, and molecular size

each contribute to the function of a detergent in solution. These properties should guide the researcher in choosing an appropriate detergent for their particular application.

IX. Appendix

For additional information on detergent properties including CMC values and Aggregation Numbers see the Technical Data section of our catalogue at: www.anatrace.com/techdata or the individual product pages in our website.

Table 1: Reasons for Detergent Insolubility

Occasionally a detergent solution will precipitate upon cooling or after storage for several days or even weeks. Here are some possible reasons why this may occur:

Problem	Explanation	Solution
Microbial Growth	Sugar derivatives are easily degraded by microorganisms and therefore are an excellent substrate for microbial growth.	Prepare solutions containing sugar-based detergents frequently, store at 4°C, and filter to prevent precipitation. EDTA can also be included at 0.2% as long as the pH is >6.0.
Presence of alcohol	Occasionally a small amount of the alcohol used to prepare alkyl glycosides may be present in the purified detergent. At low temperatures the alcohol may precipitate out of solution. The presence of alcohol may also depress the cloud point of the detergent causing phase separation to occur at a lower temperature than expected.	Check the specifications of your detergent; ANAGRADE® detergents contain <0.005% starting alcohol.
Kinetic Effect	A detergent may "dissolve" as an aggregate at room temperature. Therefore, when it is cooled to 4°C, the aggregate precipitates out of solution; thus, the detergent was never truly dissolved.	Heat the solution to 50°C during solubilization and then cool back to room temperature. This should prevent re-precipitation at 4°C.
Super-saturation	A detergent solution that is supersaturated may appear to be fully solubilized for days. When cooled to 4°C, the detergent may precipitate.	Reduce the detergent concentration to eliminate precipitate or store at room temperature.

Table 2: Factors Affecting CMC and Aggregation Numbers

Factors that Increase CMC	• Carbon-Carbon double bonds • Polar groups within the hydrophobic tail • Ionic head groups
Factors that Decrease CMC	• Increasing number of methylene groups in the alkyl chain • Phenyl rings in the alkyl chain • Fluorocarbons within the hydrophobic tail • Addition of electrolytes to solutions of ionic detergents
Factors that Increase Aggregation Number	• Increasing number of methylene groups in the alkyl chain • Addition of counterions (for ionic detergents)
Factors that Decrease Aggregation Number	• Increasing size of hydrophilic head group • Polar organic additives • Addition of hydrocarbons to solution

Table 3: Useful Equations

Application	Equation
Total detergent concentration	[CMC] + [free micellar] + [protein-associated detergent]
Micelle Concentration	([total detergent] - [CMC]) / AN
Micelle aggregate molecular weight	AN X monomer MW

CMC = Critical Micelle Concentration; AN = Aggregation Number; MW = Molecular Weight.

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