Tutorial on Working with Micelles and Model Membranes

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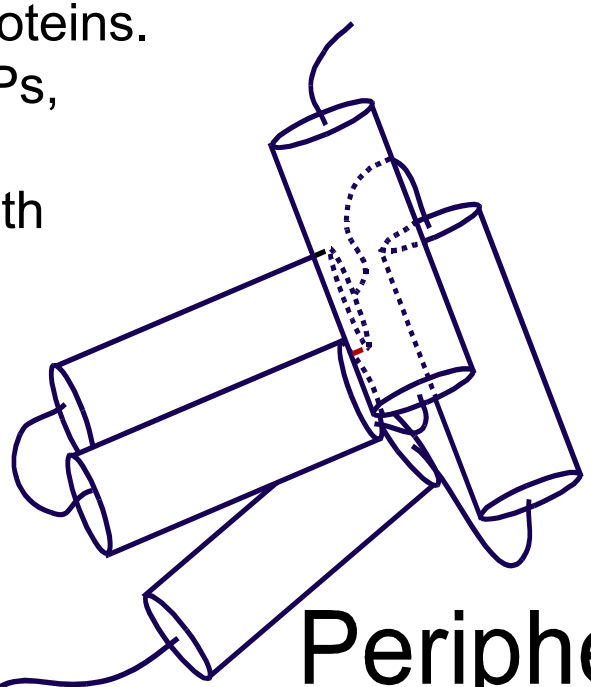
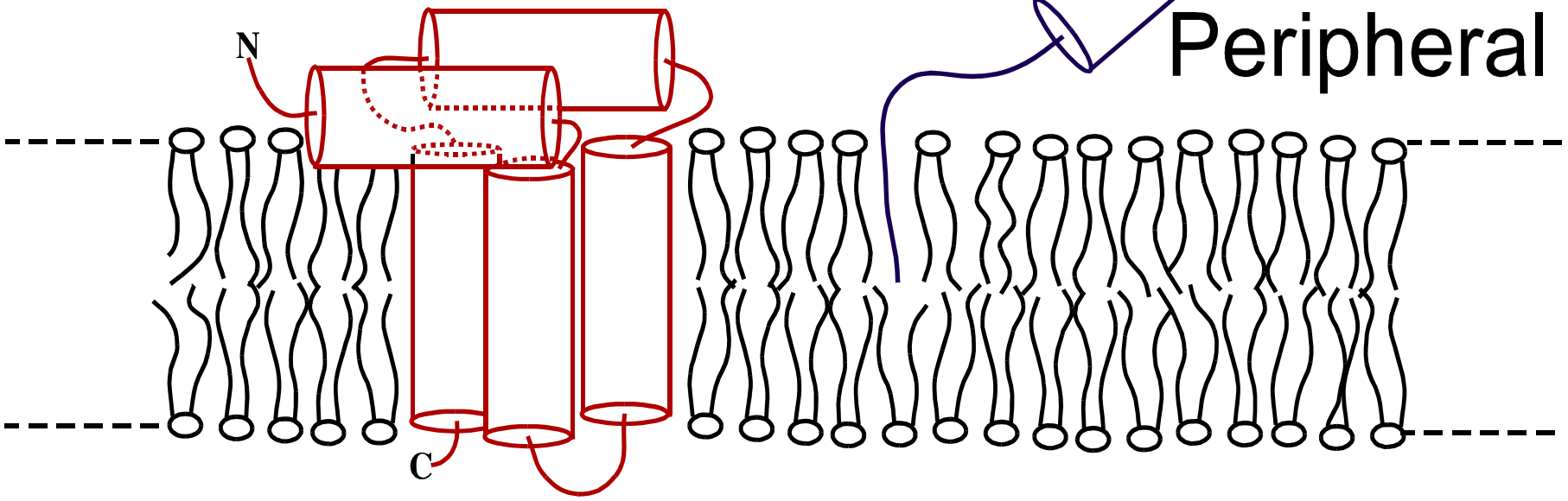
March, 2017

There are two general classes of membrane proteins. This presentation is on working with integral MPs, which traditionally could be removed from the membrane only by dissolving the membrane with detergents or organic solvents.



Peripheral

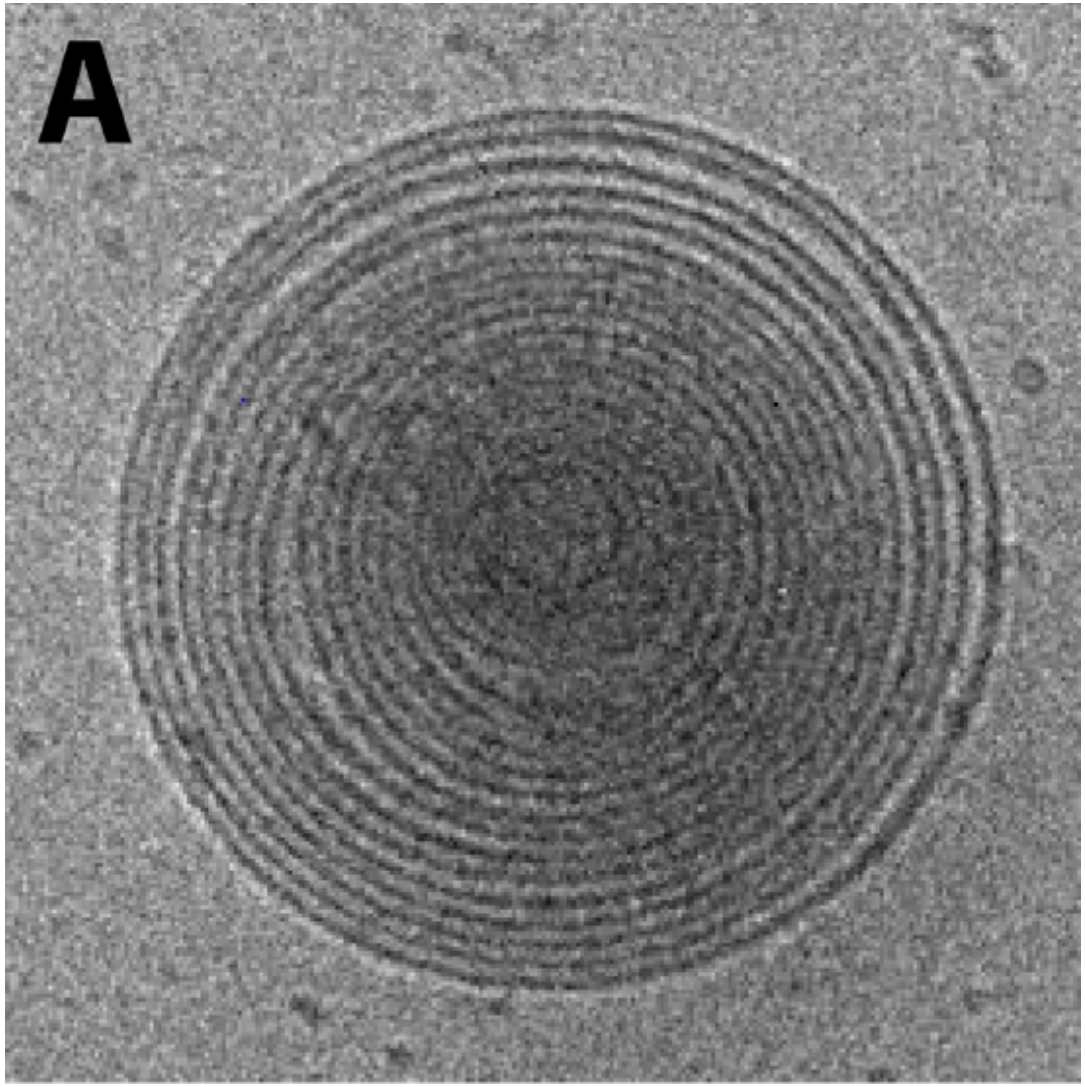
Integral



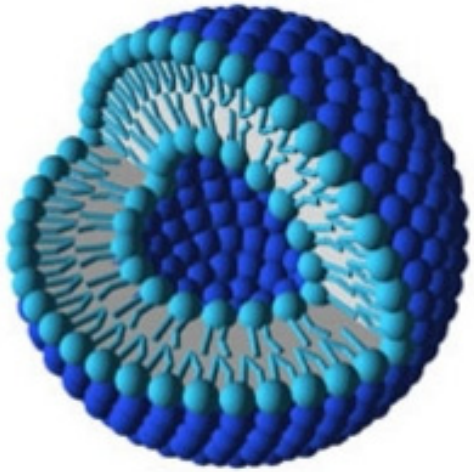
Multilamellar Vesicles: onion-like assemblies.

Each layer is one bilayer. A thin layer of water separates each bilayer.

MLVs are what form when lipid powders are dispersed in water. They form spontaneously.



Cryo-EM Micrograph of a Multilamellar Vesicle
(K. Mittendorf, C. Sanders, and M. Ohi)



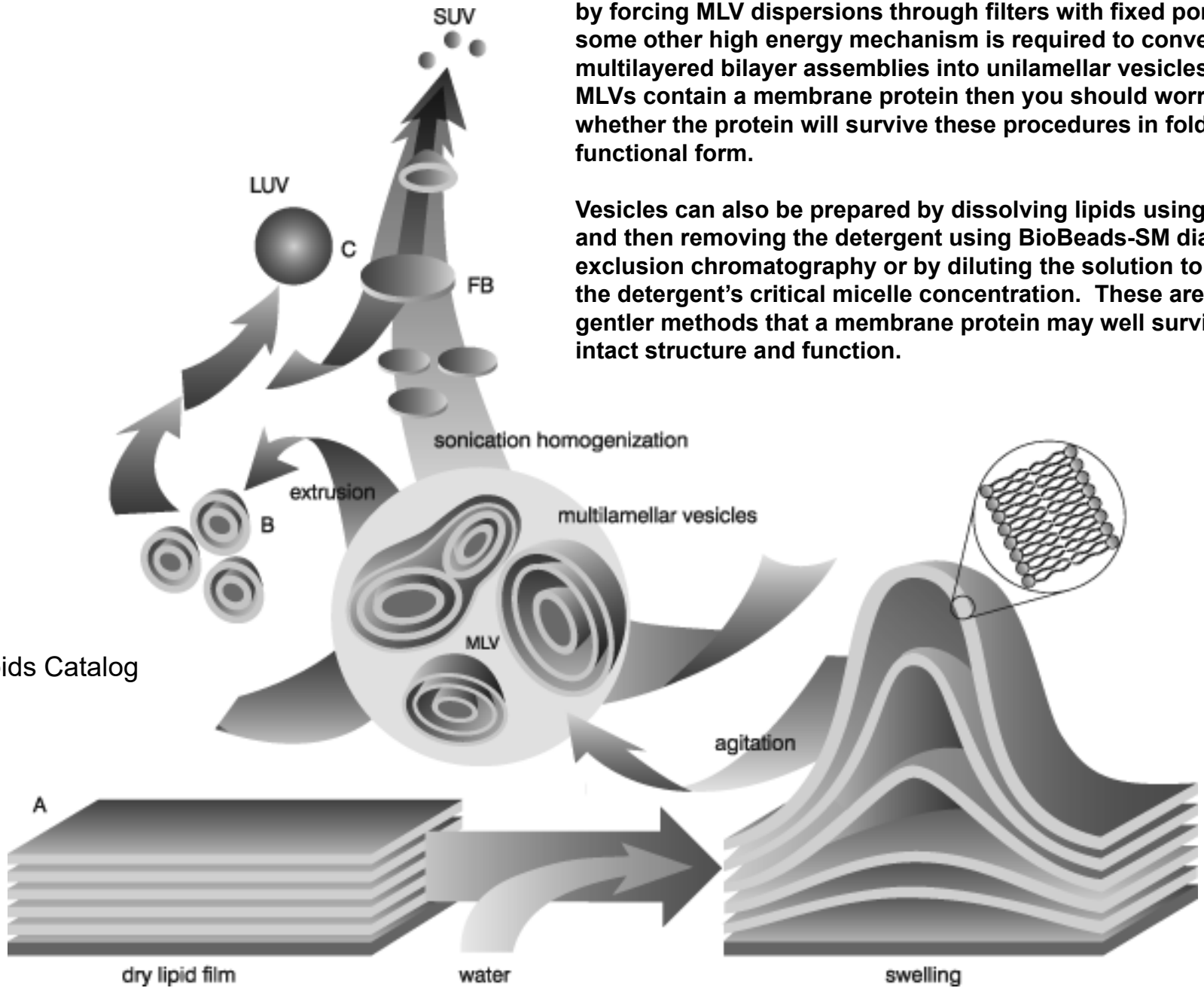
Unilamellar Vesicle

Multilamellar Vesicle

Advances in Anesthesia 32(1):133-147 · 2014

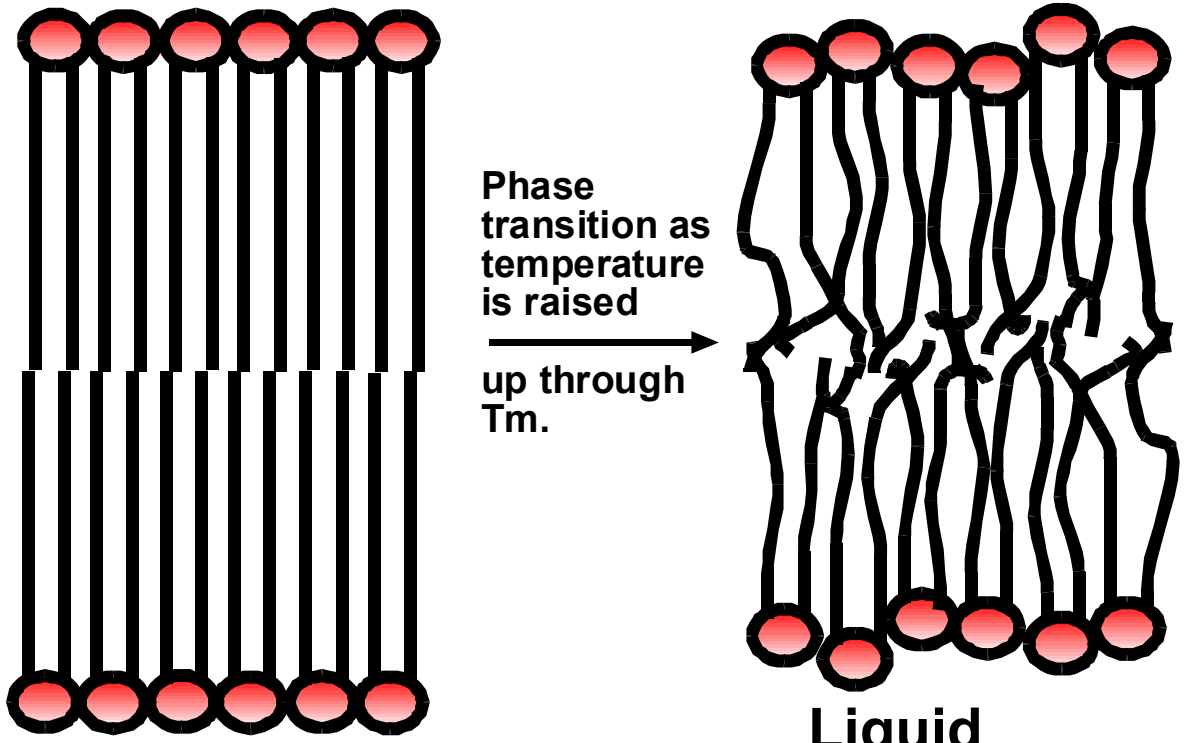
Energy from sonication, physical manipulation (such as extrusion by forcing MLV dispersions through filters with fixed pore sizes), or some other high energy mechanism is required to convert multilayered bilayer assemblies into unilamellar vesicles. If the MLVs contain a membrane protein then you should worry about whether the protein will survive these procedures in folded and functional form.

Vesicles can also be prepared by dissolving lipids using detergents and then removing the detergent using BioBeads-SM dialysis, size exclusion chromatography or by diluting the solution to below the detergent's critical micelle concentration. These are much gentler methods that a membrane protein may well survive with intact structure and function.



From:
Avanti Polar Lipids Catalog

Bilayers can undergo phase transitions at a critical temperature, T_m. Native bilayers are usually in the fluid (liquid crystalline) phase. The T_m for DMPC is 24.5ºC. The T_m for POPC and DOPC are both < 0ºC. The gel phase is so rigid that membrane proteins may not retain native-like structure, dynamics, and function therein. If you are working with lipid vesicles, you will want to make sure you are working above T_m. You can find a good compilation of T_m values in the appendices of the Avanti Polar Lipids Catalog (available on-line)



Gel Phase

Liquid Crystalline Phase ($L\alpha$)

A.K.A.:
"Fluid Phase"
"Disordered Phase"

Bilayer Dimensions: Lewis and Engelman, JMB 1983

DMPC T_m= 24 deg. At 36 deg: Phosphate to Phosphate: 3.4 nm (34 angstroms)
Hydrophobic Thickness: 2.3 nm
Surface area: 66 square angstroms per lipid

DPPC T_m = 41 deg. At 44 deg:
Phosphate to phosphate: 3.7 nm
Hydrophobic Thickness: 2.6 nm
Surface area: 67 square angstroms

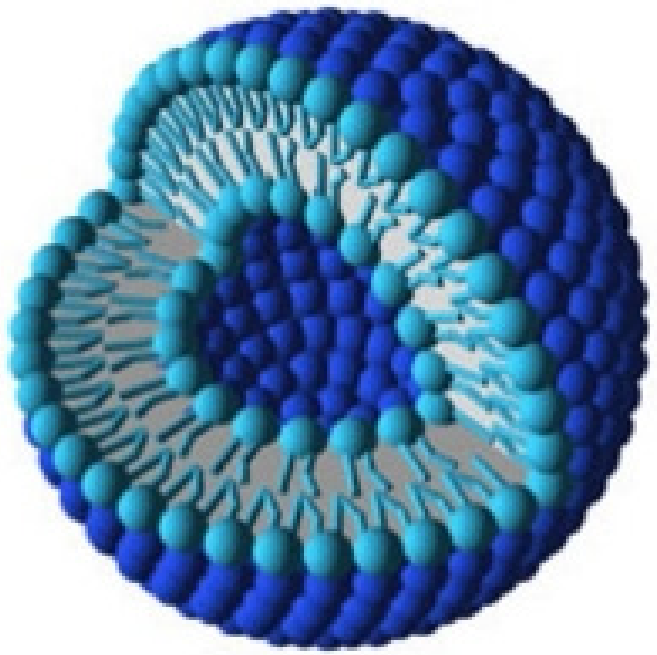
DOPC T_m = -14 deg. At 20 deg.
Phosphate to phosphate: 3.8 nm
Hydrophobic thickness: 2.7 nm
Surface area: 70 square angstroms

EYPC (mostly POPC T_m = -5 deg)
Hydrophobic thickness: 2.8 nm

E. coli lipids
Phosphate to phosphate: ca. 4.2 nm

T_m is the gel to fluid phase transition.
***Fluid phase (above T_m) is the physiologically relevant phase in most cases*.**

The simplest membrane is represented by Unilamellar Lipid Vesicles (ULVs), Also known as "liposomes". When the diameter is < 100 nm, the radius of curvature is relatively high and they are referred to as "small unilamellar vesicles" (SUVs). 100 nm or larger diameter: large unilamellar vesicles (LUVs). But even the smallest SUV have an aggregate molecular weight on the order of 2,000,000 Da.

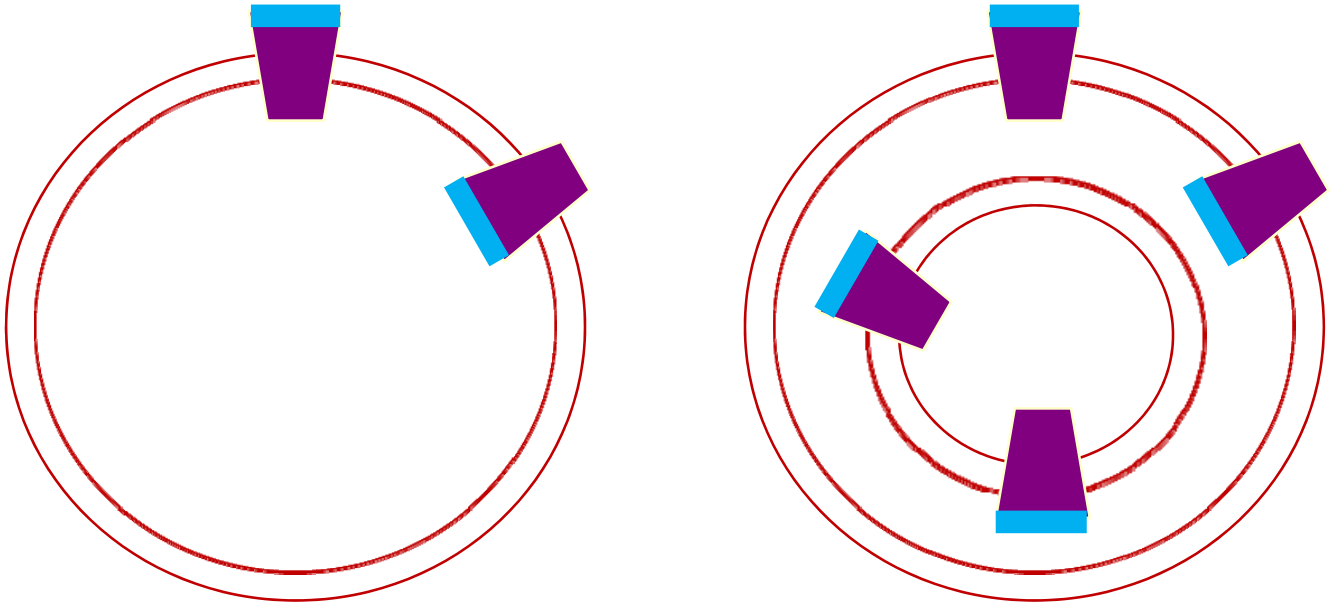


SUVs: can get around problems with light scattering to enable optical spectroscopy (good for CD, UV and fluorescence).

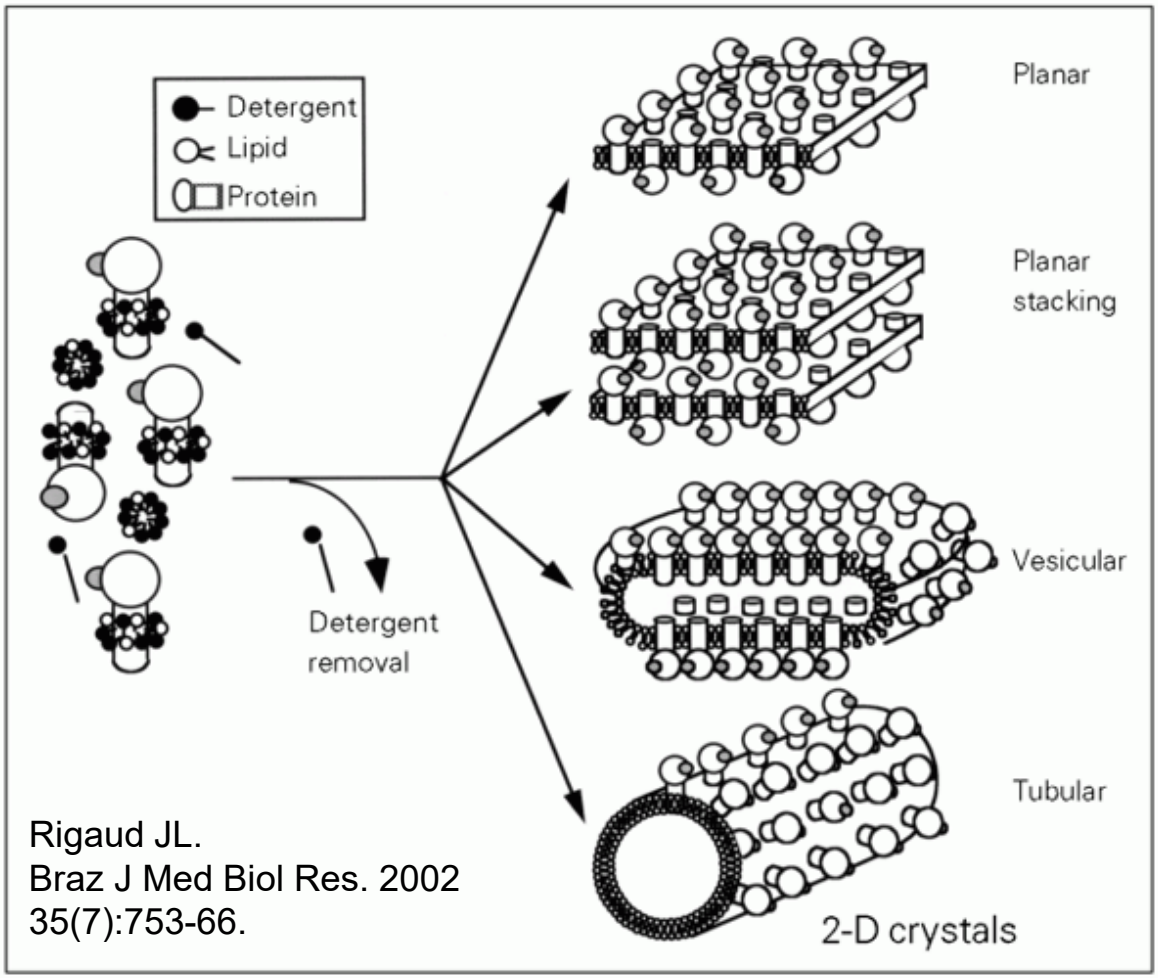
However, high curvature may distort some membrane proteins.

SUV are metastable and tend to spontaneously fuse with time to form larger vesicles.

The process of inserting a purified membrane protein into a lipid vesicle is known as "reconstitution". Vesicles are often used for EPR, solid state NMR , CD, or fluorescence studies of membrane proteins. Vesicles are large enough to be easily visible using EM (although not always also the membrane proteins embedded therein). They also are used for functional/biochemical studies of membrane proteins. However, the sidedness of vesicles can be a problem– often a membrane protein will be oriented with a 50/50 topological distribution with respect to the outside and inside (lumen) of the vesicle, as shown below. Multilayering also complicates some experiments, as some membrane protein will end up in inner bilayers that are inaccessible to water soluble reagents added to the solution.



2-D Crystallization (for EM or AFM)



Rigaud JL.
Braz J Med Biol Res. 2002
35(7):753-66.

Under certain conditions, when membrane proteins are reconstituted in lipid bilayers they will form planar bilayer sheets in which the membrane protein forms a 2-D crystalline array.

2-D crystals of membrane proteins can then subjected to "electron Crystallography" using EM, sometimes leading to a high resolution structure.

In the past, it was crucial to have just a single planar layer (no multilayering of the 2-D crystals). However, methods have recently emerged that allow structures to be determined from EM crystallographic data acquired using multilayered 2D crystals.

Curr Opin Struct Biol. 2007 Aug;17(4):389-95. Epub 2007 Aug 27.

Revival of electron crystallography.

Hite RK[1], Raunser S, Walz T.

Electron cryomicroscopy of **membrane** proteins: specimen preparation for two-dimensional **crystals** and single particles.

Schmidt-Krey I, Rubinstein JL.
Micron. 2011 Feb;42(2):107-16. doi: 10.1016/j.micron.2010.07.004. **Review**.

Example of 2-D Crystals of a Membrane Protein (AFM Images)

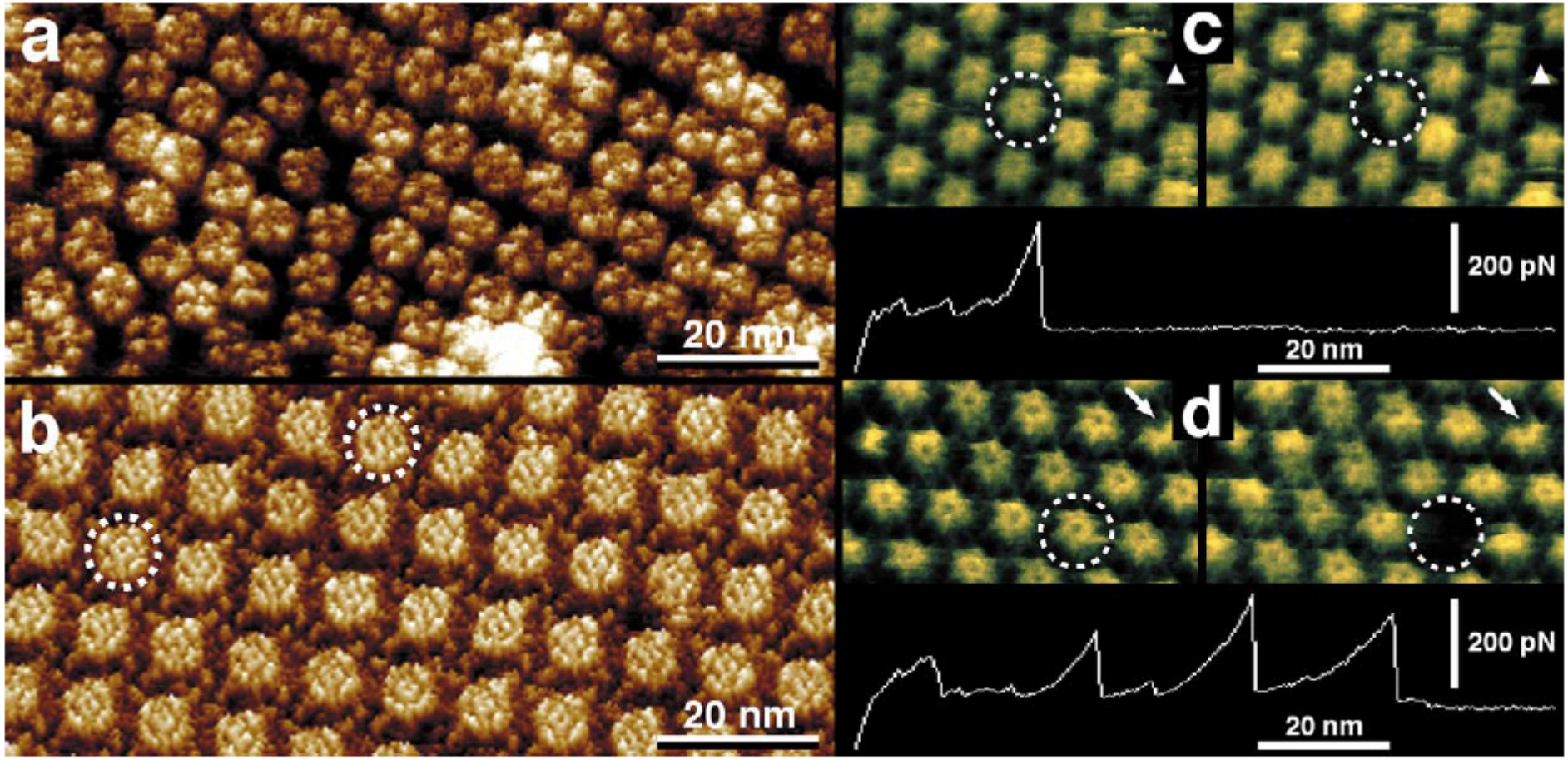
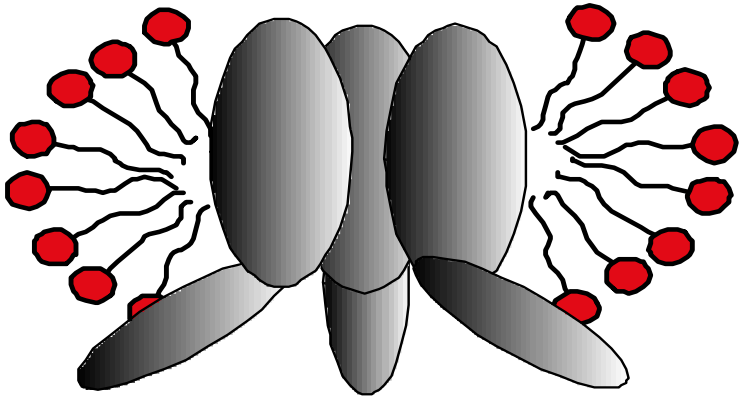


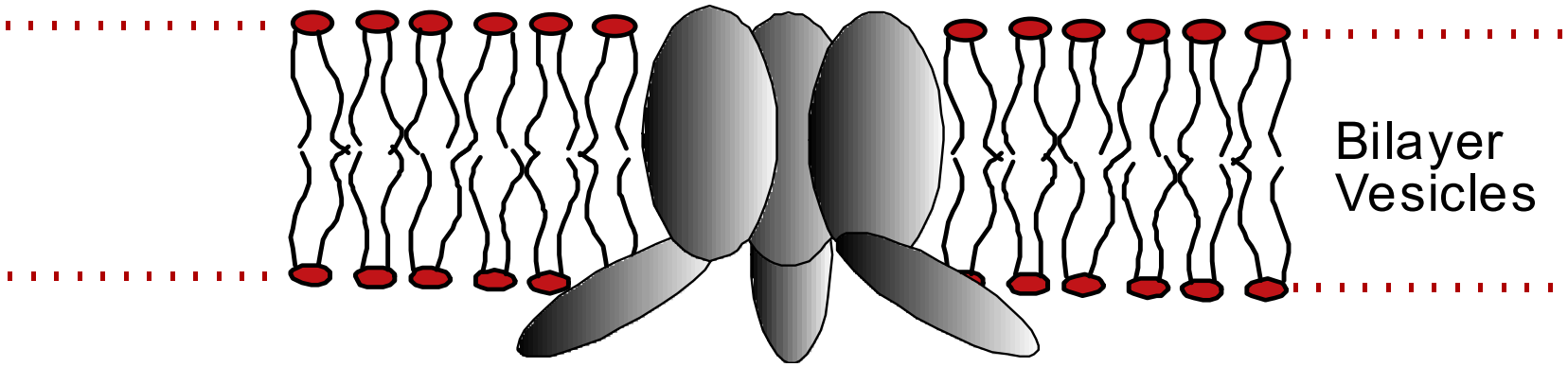
Fig. 3. Atomic force microscopy of biomolecules imaged in aqueous solution. A: Surface topograph of a densely packed vesicle containing tetramers of the major intrinsic protein from lens fiber cells [29]. B: Topograph of a 2D crystal of AqpZ from *Escherichia coli* [13]. Single tetramers exposing the periplasmic side are marked by broken circles. C: Topographs of the surface layer of *Corynebacterium glutamicum* before (left panel) and after (right panel) removal of two protomers [32]. The bottom panel displays the force–distance curve of the induced unfolding giving rise to the surface change (broken circles) observed on the panels above. D: Same as for C, except that here all six protomers were removed. The triangles and the arrows in panels C and D mark defects in the layer that facilitate orientation.

From lab of Andreas Engel

Micelles as Models for Membrane Bilayers

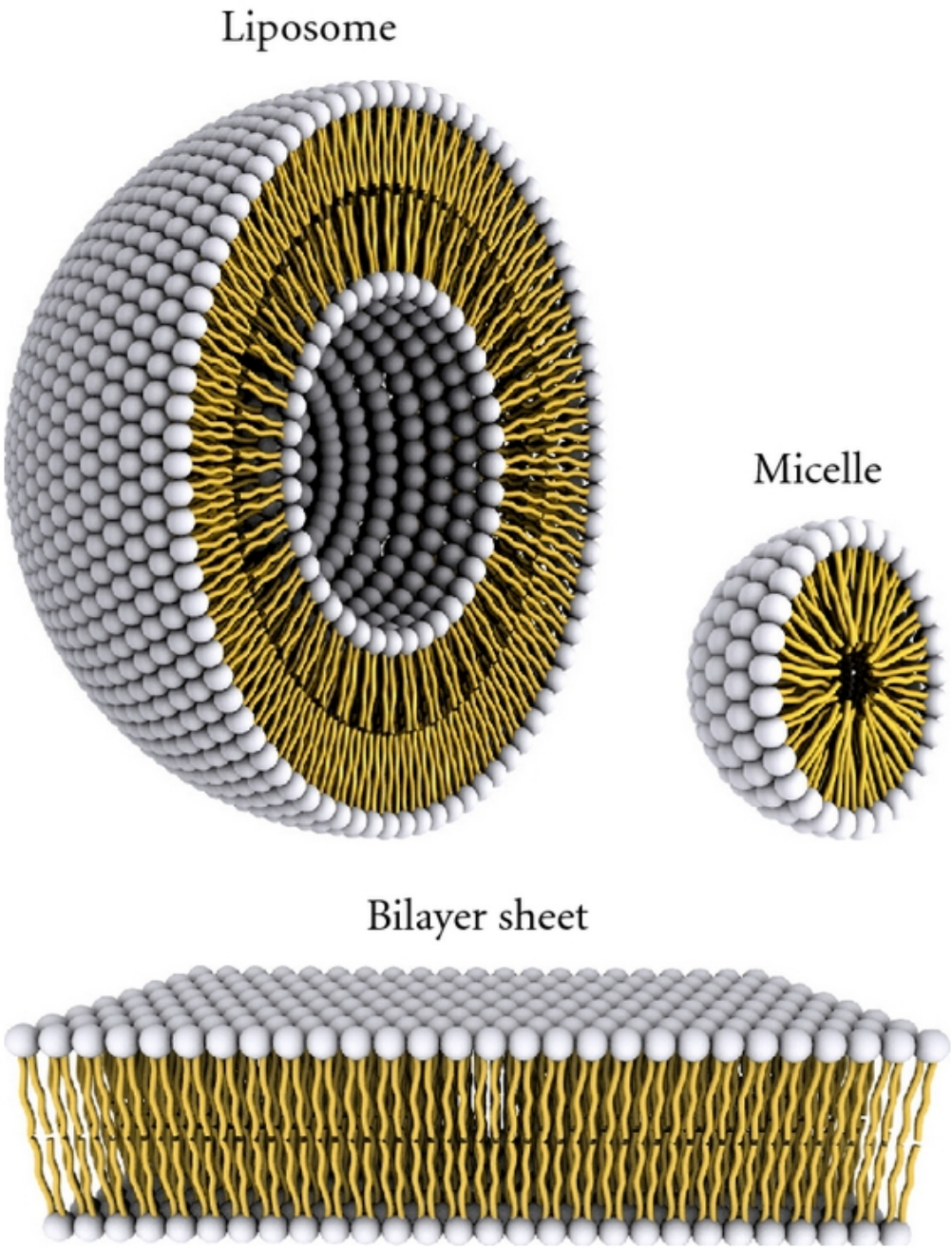


Micelles



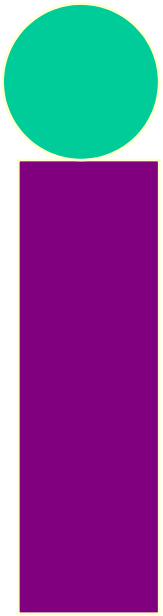
Bilayer
Vesicles

Lipid Bilayers typically span 25-35 angstroms thick (hydrocarbon domain) or 35-45 angstroms (polar headgroup to headgroup). Protein-free micelles will have a similar or slightly smaller diameter.



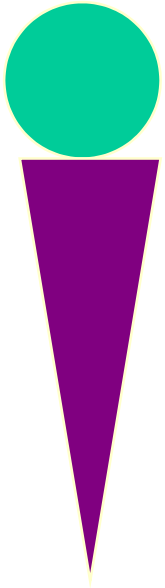
The largest micelles are much smaller than the smallest lipid vesicles. Micelles are water soluble. Lipid vesicles are typically only marginally soluble and can usually be pelleted by ultracentrifugation.

ISRN Pharmaceutics
Volume 2012 (2012), Article ID 738432, 11 pages
http://dx.doi.org/10.5402/2012/738432
Optimizing Druggability through Liposomal Formulations: New Approaches to an Old Concept
Bitounis, Fanciullino, Iliadis, and Ciccolini



Lipid: Cylinder Shape

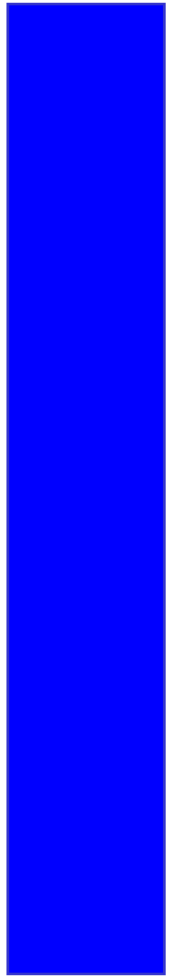
Usually 2 acyl/alkyl Chains, at least 12 carbons each (in humans, usually 16-18 carbon chains)



Detergent: Usually Idealized as Conical in Shape

2 short (6-8 carbons) Unsaturated acyl chains, or 1 alkyl/acyl Chain (8-14 carbons).

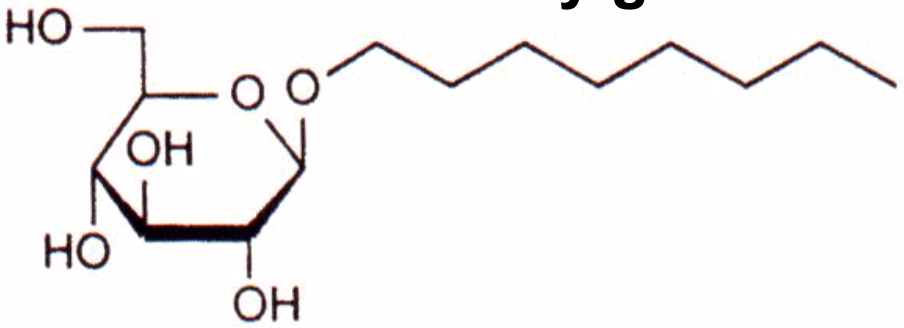
Micro- to millimolar monomer solubility in water.



Transmembrane Helix

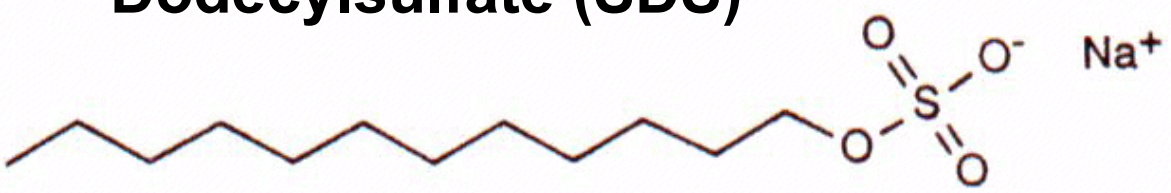
Diameter of cylinder is similar to that of a typical lipid, but twice as long.

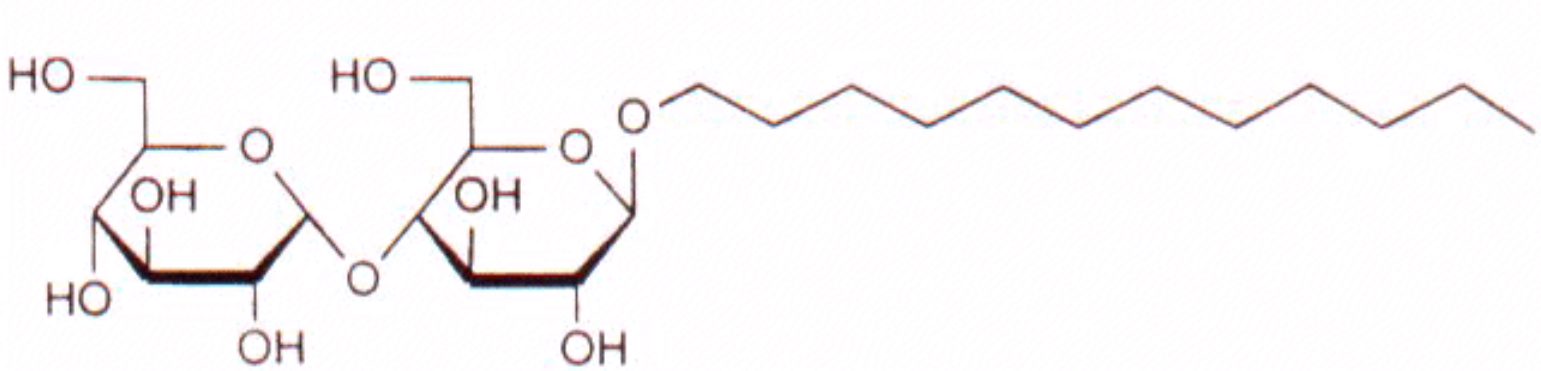
Beta-octylglucoside



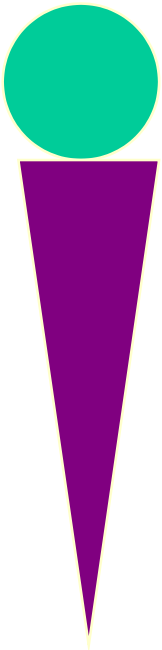
Examples of Classical Detergents

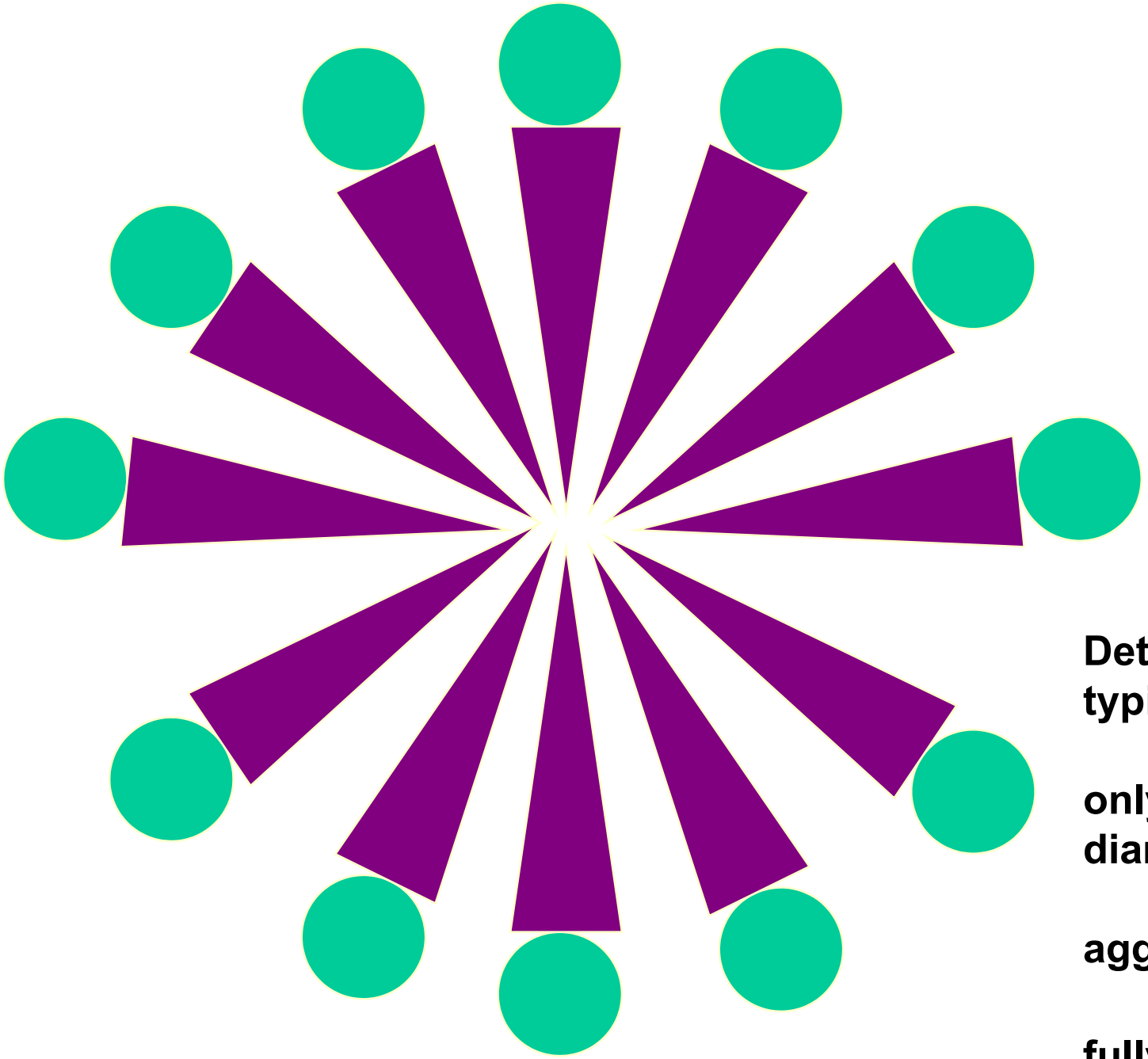
Dodecylsulfate (SDS)





Beta-dodecylmaltoside



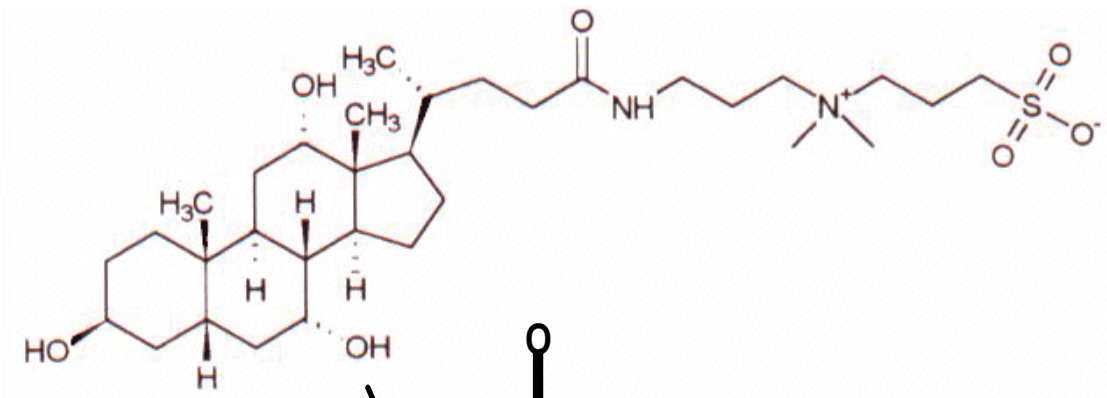


Detergent micelles… typically:

only a few nm in diameter

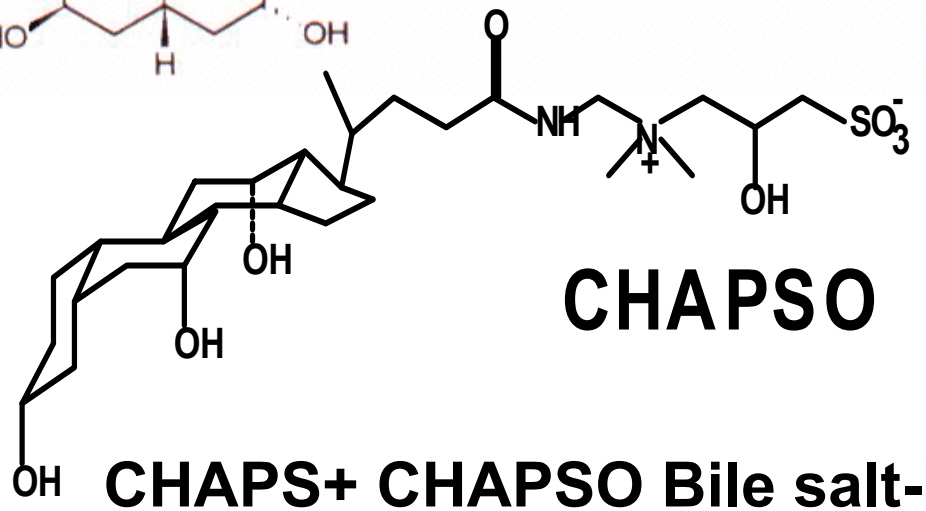
aggregate MW <100 kDa

fully water soluble.

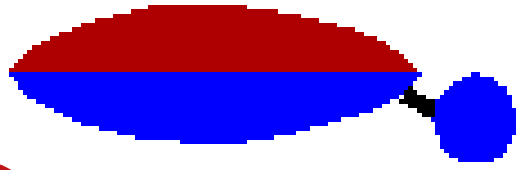


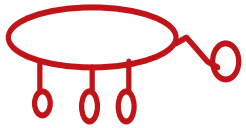
Not all detergents are shaped like ice cream cones.

Also, not all micelles are spherical. Cigar-shaped (prolate ellipsoid) and disoildal shaped (oblate ellipsoid) micelles may be just as common.



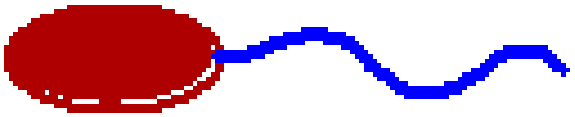
CHAPSO

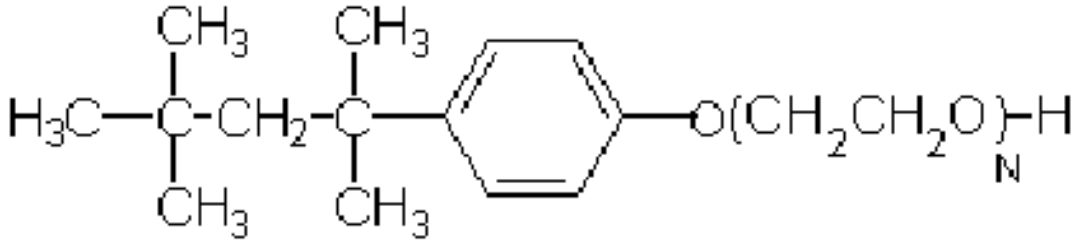




CHAPS+ CHAPSO Bile salt-based detergents (Janus-like)

Triton X-100



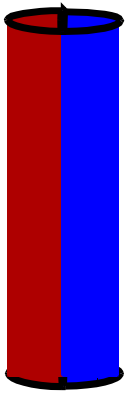


N = approx. 9.5

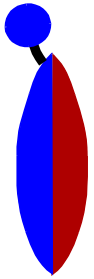
While it is fine to think of detergents As being cone shaped, the reality Is more complicated.

Typical glycerol- or sphingosine-based lipid (not a detergent).

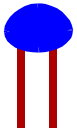
Standard single chain detergent. (e.g., dodecylsulfate, alkylglycosides, dodecylphosphocholine, lysophospholipids).



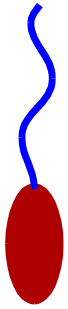
Amphipathic helical polypeptide (e.g., melittin, peptitergent PD1). Usually long enough to span bilayer.



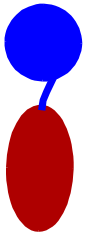
Janus-faced surfactant. Based on rigid amphiphilic ring system (e.g., bile salts, CHAPS).



Short chain lipids. Apolar tails usually have 7 carbons or less (e.g., dihexanoyl- and diheptanoyl-phosphatidylcholine).



Detergents with bulky apolar group and extended polar moiety (e.g. Triton X-100, C-HEGA series).

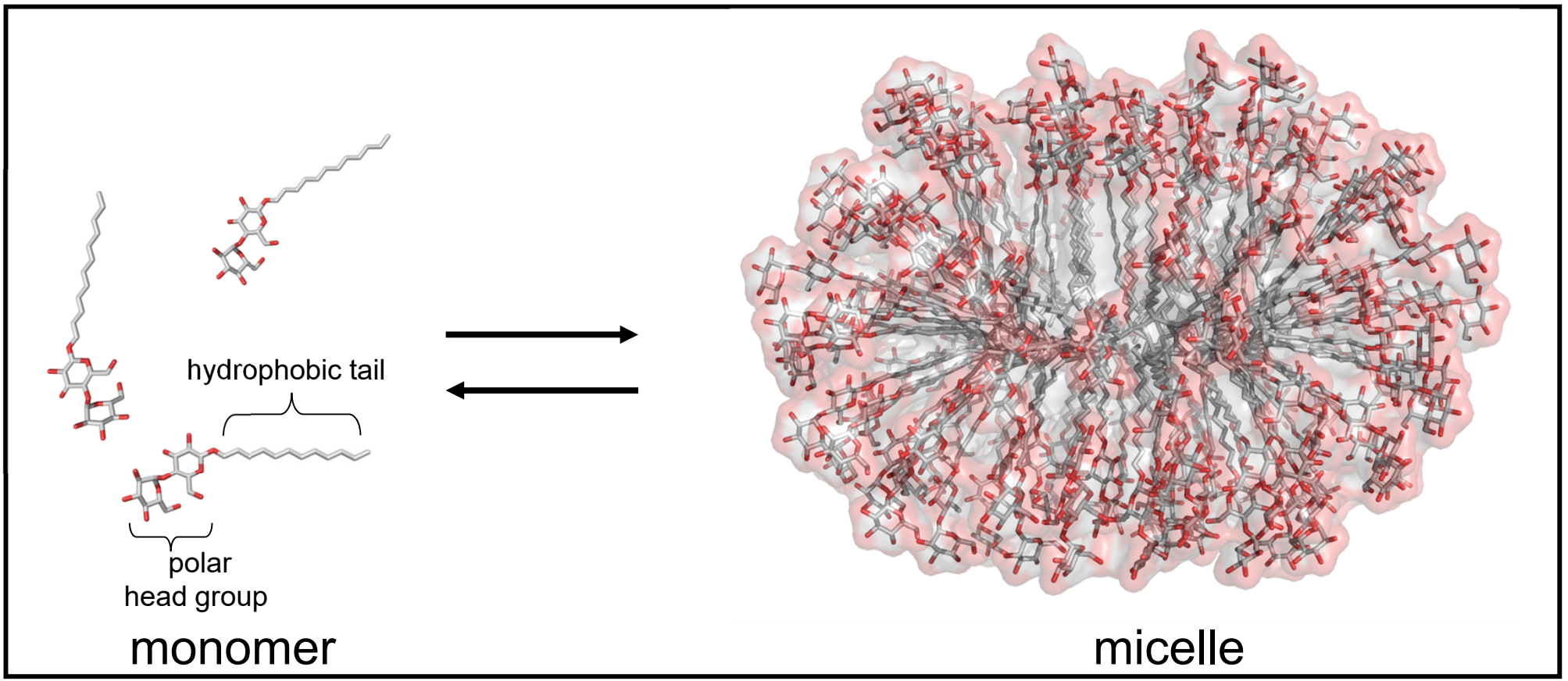


Detergents for which both polar and non-polar moieties are bulky (e.g., cyclohexyl-based alkylglycosides).

Detergens for which both polar and non-polar moieties are extended (e.g., alkyl ethers of polyoxyethylene such as C8E4).

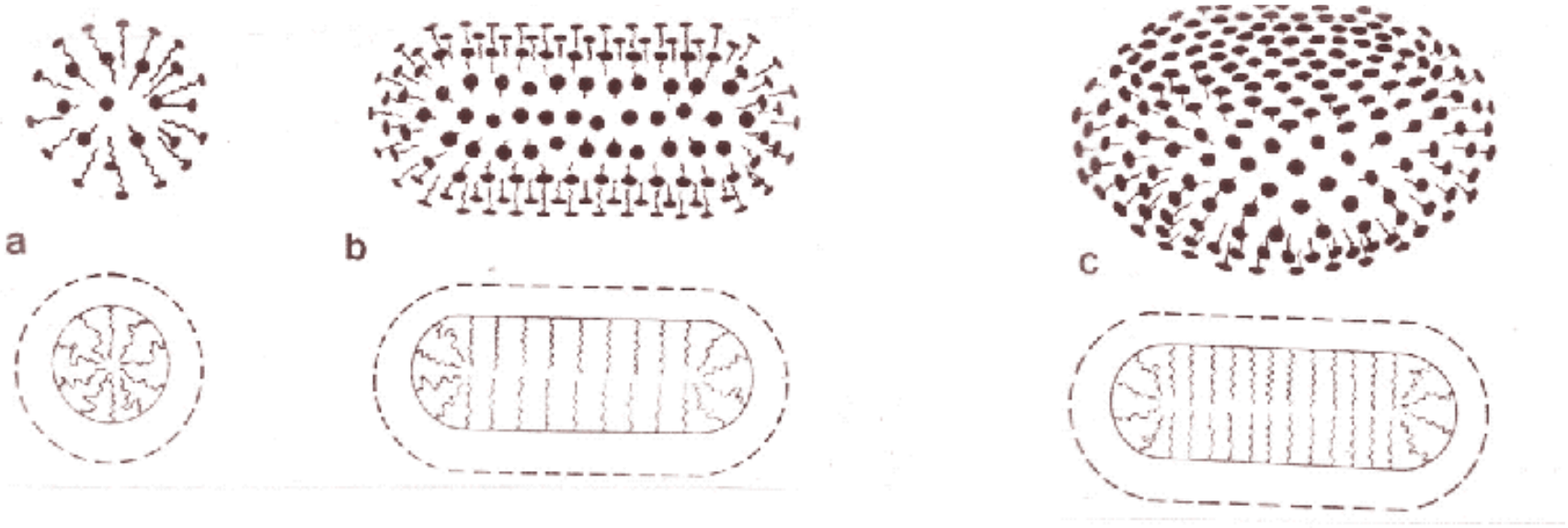
French swimwear for membrane proteins.
Sanders CR, Kuhn Hoffmann A, Gray DN, Keyes MH, Ellis CD.
Chembiochem 2004 5:423-426.

Unlike lipid vesicles, where the lipids are effectively irreversibly assocated with the vesicles (very very low monomer solubility), a hallmark of detergent molecules is that they have significant free monomer solubility in water (in the range 50 uM to 10s of mM). Detergents are in constant and rapid exchange between their free monomer forms and micellar aggregate forms.



Linda Columbus, U of Virginia

Shapes of Micelles: Micelles are often NOT Spherical



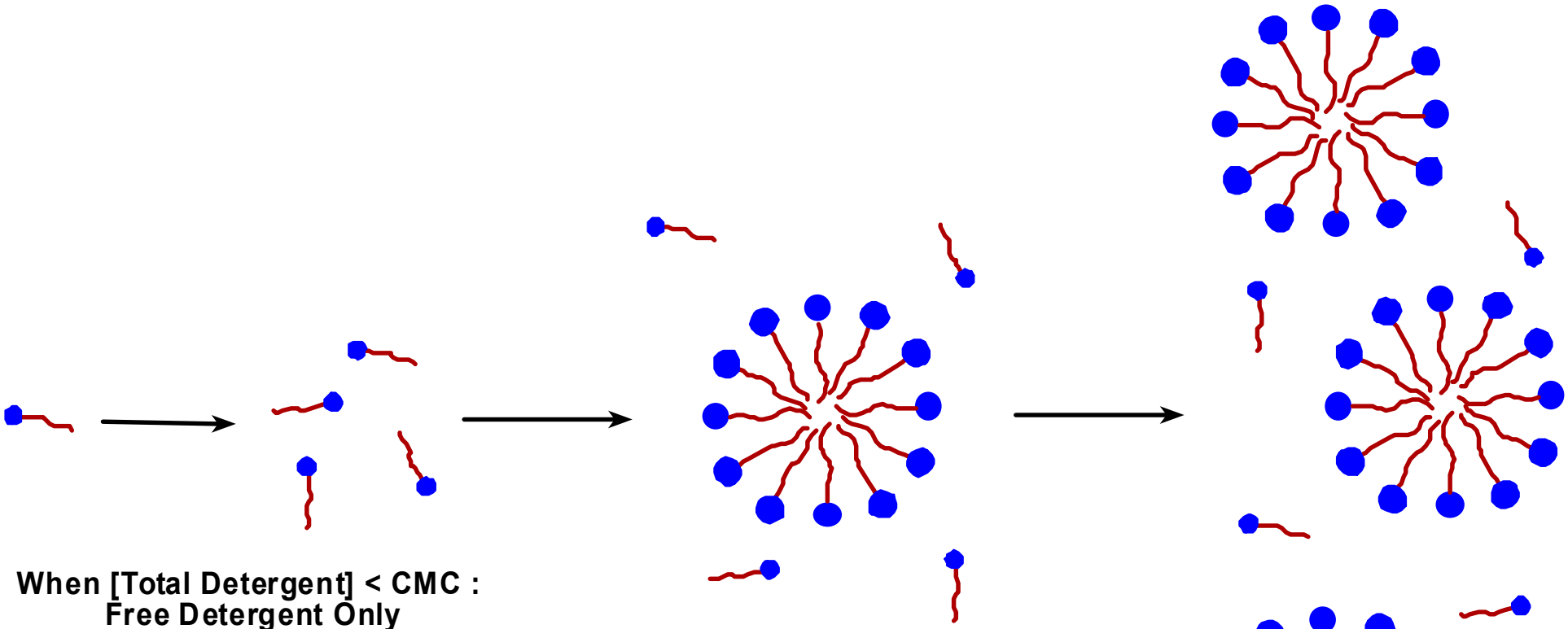
Prolate Ellipsoid

Oblate Ellipsoid

D. Small, *The Physical Chemistry of Lipids*

Each detergent has a “critical micelle” concentration (CMC). When the total detergent conc. is <CMC, it will not form micelles, but will exist only as free monomer. When the total conc. is >CMC, a population of free detergent will be maintained at a CMC concentration and all detergent above that will go into micelles.

Increasing Total Detergent Concentration →



When [Total Detergent] < CMC : Free Detergent Only

When [Total Detergent] > CMC : [Free Detergent] = CMC and all additional detergent goes into micelles. As additional detergent is added, all of it goes into micelles.

French swimwear for membrane proteins.
Sanders CR, Kuhn Hoffmann A, Gray DN, Keyes MH, Ellis CD.
Chembiochem 2004 5:423-426.

Detergents: Vital Information

Detergent Critical Micelle Concentration (CMC):

- **When [total detergent concentration] is below CMC, all detergent molecules are monomeric (free) in solution.**

- **When [total detergent concentration] is greater than CMC there is a monomeric detergent concentration equal to [CMC]**

- **Above CMC there is a micellar detergent concentration equal to: [total detergent concentration – CMC]**

Examples:

β-Octyl glucoside	**25 mM**
Sodium dodecyl sulfate	**7 mM**
Decyl maltoside	**2 mM**
Dodecyl maltoside	**0.2 mM**
Triton X-100	**0.25 mM**
DHPC (D6PC)	**14 mM**
DHePC (D7PC)	**1.5 mM**

The lower the CMC, the harder It is to get rid of the detergent.

If CMC is high, it means you need a LOT of detergent to do anything ($$$).

Detergents: Vital Information

Aggregation Number =

the average number of detergent molecules in a single micelle.

Concentration of micelles = {total detergent conc. – CMC} ÷ aggregation #

***Aggregate Molecular Weight of Micelle* =**

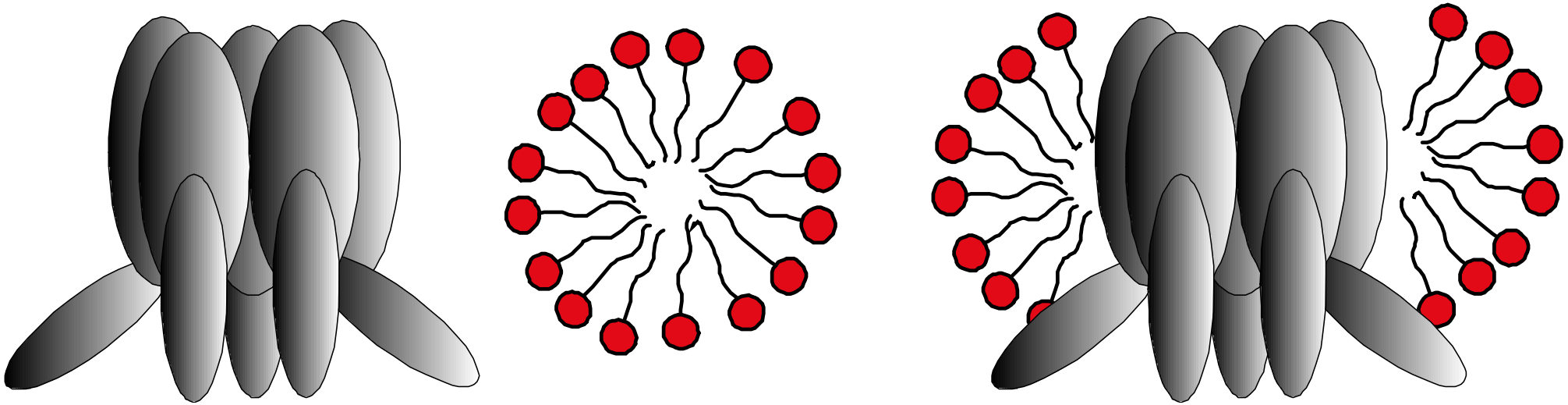
Aggregation number x detergent monomer molecular weight

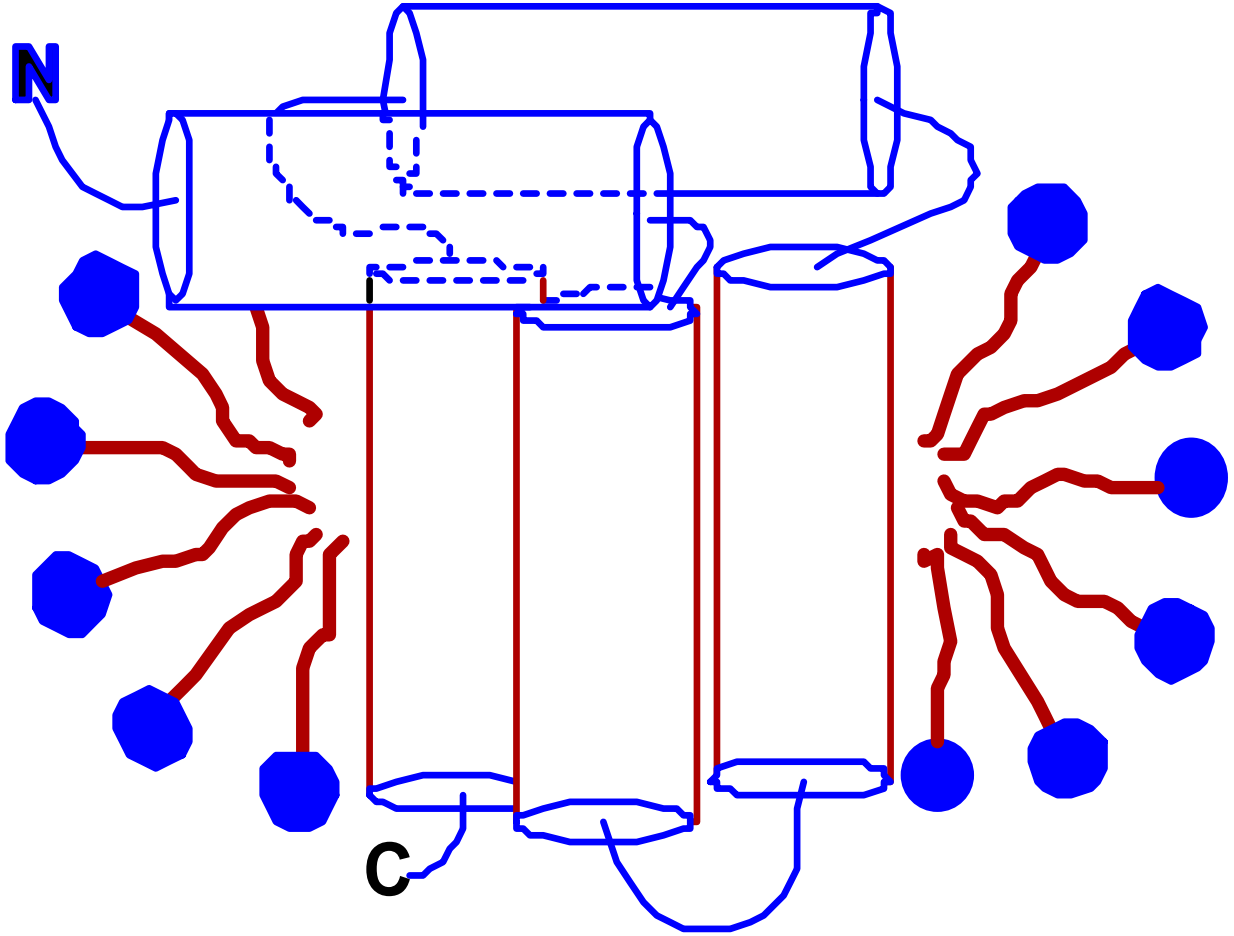
Typical aggregation numbers: 50-200
Typical aggregate MWs: 20-100 kDa

Aggregate MW of Protein/Detergent Complexes

When a protein is smaller than the detergent micelle, the aggregate molecular weight (assuming 1 protein per micelle) can be approximated as the sum of the free micelle aggregate MW and the MW of the protein.

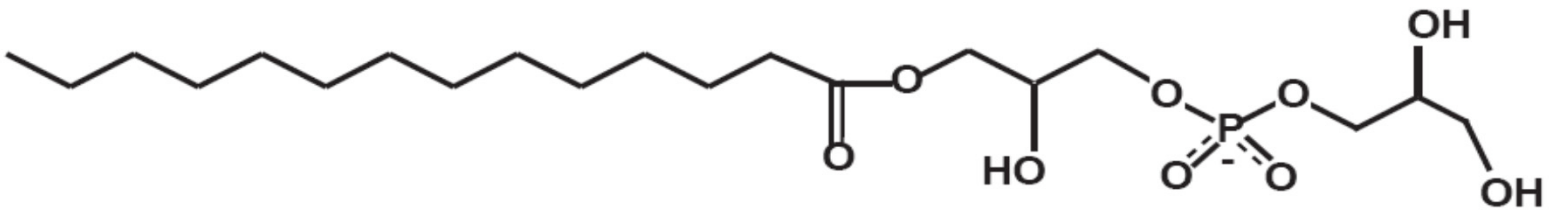
When the transmembrane domain approaches or is larger than the size of the free micelle, the size of the aggregate may be much larger than the sum of the free micelle and protein MWs. Aggregate size will be determined by the hydrophobic surface area of the transmembrane domain which needs to be coated by detergent.





Cross-section of detergent/membrane protein complex. The detergent forms a torus (ring) around the hydrophobic transmembrane domain of the protein, leaving the polar extramembrane domains of the protein (blue) exposed to water.

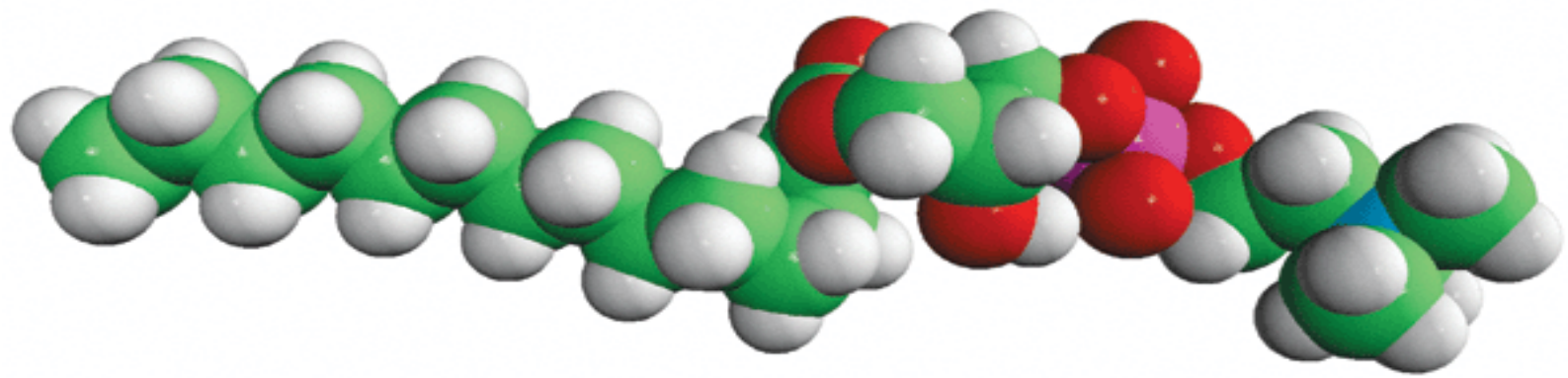
Lyso-Myristoylphosphatidylglycerol (LMPG)



apolar tail

polar, but uncharged spacer

charged head group



The Sanders Lab is a fan of lyso-phospholipids as detergents for use in studies of membrane protein function and for NMR studies. They are phospholipids missing a tail and hence are uniquely close in structure to membrane lipids. Note that with their ester moiety, they are not as chemically stable as most detergents. Never successfully used for crystal growth, however.

Table 1. Properties of selected biological detergents

Detergent	Charge	Molecular Weight	Critical micelle concentration (mM)	Aggregation number	Reference
CHAPS	Zwitterionic	615	6–10	4–14	52,53
CHAPSO	Zwitterionic	631	8	11	53
β-Decylmaltoside	Nonionic	483	2	104	54
β-Dodecylmaltoside	Nonionic	511	0.2	110–140	52
β-Octylglucoside	Nonionic	292	19–25	90	52
Dihexanoylphosphatidyl choline (DHPC)	Zwitterionic	454	15	19–35	55,56
Diheptanoylphosphatidyl choline	Zwitterionic	482	1	42–200	52,57
Decylphosphocholine	Zwitterionic	323	11	ND	58
Dodecylphosphocholine (DPC)	Zwitterionic	352	1.5	50–60	52,59
Tetradecylphosphocholine	Zwitterionic	380	0.12	ND	58
Lyso-lauroylphosphatidyl choline	Zwitterionic	440	0.5–0.7	70[a]	60,61
Lyso-myristoyl phosphatidylcholine (LMPC)	Zwitterionic	468	0.04–09	100[a]	52,60,61
Lyso-palmitoyl phosphatidylcholine (LPPC)	Zwitterionic	496	0.004–0.008	140[a] 186	52,60,61
N-Dodecyl-*N*,*N*-dimethylglycine (empigen BB)	Zwitterionic	272	2	ND	58
Lyso-myristoyl phosphatidylglycerol (LMPG)	Anionic	478	0.2–3 (pH/salt-dependent)	ND	61
Lyso-palmitoyl phosphatidylglycerol (LPPG)	Anionic	506	0.02–0.6 (pH/salt-dependent)	125	56,61
N-Lauroyldimethyl amineoxide (LDAO)	Zwitterionic	229	2	69–73	52
N-Lauroylsarcosine	Anionic	293	*ca* 15	ND	62
Sodium dodecylsulfate (SDS) (precipitates below 15 °C)	Anionic	288	1–7	62–101	52
Lithium dodecylsulfate (soluble below 15 °C)	Anionic	272	Similar to SDS	Similar to SDS	–
Digitonin	Anionic	1229	<0.30	60	–

[a] Estimate, based on ether-linked analogs.[63]

Table 1
Properties[a] of polyoxyethylene glycol detergents (updated from [7])

	Monomer mass (M_r)	CMC (M)	Aggregation number	$\bar{\nu}_D$ (cm^3/g)	Ref.[b]
Homogeneous compounds[c]					
C_8E_4	306	$7–8.5\times10^{-3}$	82	–	[8–10]
C_8E_5	350	$4.3–9.2\times10^{-3}$	–	0.993[e]	[8–11]
C_8E_6	394	1×10^{-2}	32	0.963	[12–14]
$C_{10}E_6$	422	9×10^{-4}	73	–	[12]
$C_{12}E_6$	450	8.2×10^{-5}	105[d]	0.989	[2,12,14]
$C_{12}E_8$	538	9×10^{-5}	90–120	0.973	[2,15–17]
$C_{16}E_6$	506	1.3×10^{-6}	2400	–	[12]
$C_{16}E_9$	638	2.1×10^{-6}	280	–	[12]
$C_{16}E_{12}$	770	2.3×10^{-6}	150	–	[12]
$C_{16}E_{21}$	1166	3.9×10^{-6}	70	–	[12]
p-tert-$C_8\varnothing E_9$	602	3.0×10^{-4}	–	–	[12]
$C_9\varnothing E_{10}$	676	7.5×10^{-5}	–	–	[18]
Heterogeneous compounds[c]					
$C_{12\&14}E_{\langle9.5\rangle}$ (Lubrol PX)	620	1×10^{-4}	100	0.958	[2,12,19,20]
$C_{12}E_{\langle12\rangle}$	710	9×10^{-5}	80	–	[2,12]
$C_{12}E_{\langle23\rangle}$ (Brij 35)	1200	9×10^{-5}	40	–	[12]
$C_{16\&18}E_{\langle17\rangle}$ (Lubrol WX)	1000	4×10^{-6}	90	0.929	[2,15,21]
*p-tert*C8$\varnothing E_{\langle9.5\rangle}$ (Triton X-100)	625	2.5×10^{-4}	75–165	0.908	[16,17,22,23]
p-tert-C8$\varnothing E_{\langle7–8\rangle}$ (Triton X-114)	540	2×10^{-4}	–	0.869	[22,23]
C9$\varnothing E_{\langle10\rangle}$ (Triton N-101)	670	1×10^{-4}	100	0.922	[13,24]
C_{12}sorbitan $E_{\langle20\rangle}$ (Tween-20)	1240	6×10^{-5}	–	0.869	[12,23]
$C_{18:1}$sorbitan $E_{\langle20\rangle}$ (Tween-20)	1320	$0.7–1.2\times10^{-5}$	60	0.896	[12,21,23,25]

[a] Data obtained at 20–25°C by physicochemical methods (surface tension, light scattering, densitometry, analytical ultracentrifugation, fluorescence). Salts should not affect much CMC or aggregation number of non-ionic detergents (however, see [196]).
[b] The references indicated are either for the original data or for data surveys.
[c] Nomenclature: C_xE_y: x refers to the number of C atoms in the alkyl chain and y to the (average) number of polyoxyethylene glycol units; $\varnothing$ denotes a phenyl group. Commonly used trade names are indicated in parentheses.
[d] Measured at 4°C, because of secondary aggregation at 25°C.
[e] Measured for a mixture of C_8E_4 and C_8E_5 [11].

Properties[a] of various types of polar or non-ionic detergents, and of bile salts (updated from [7])

	Monomer mass (M_r)	CMC (M)	Aggregation number	$\bar{\nu}_D$ (cm^3/g)	Ref.[b]
Octyl-β-D-glucoside (OG)	292	$1.9–2.5\times10^{-2}$	≈90	0.859	[8,20,26,27,192]
Decyl-β-D-maltoside	483	2.2×10^{-3}	–	–	[28]
dodecyl-β-D-maltoside (DM)	511	1.8×10^{-4}	110–140	0.81–0.837	[17,20,26,29,192]
Cyclohexyl-hexyl-β-D-maltoside (CYMAL-6)	509	5.6×10^{-4}	63[f]	–	[57]
2-*O*-Lauroylsucrose	524	6.5×10^{-4}	–	–	[30]
Dodecyldimethyl-*N*-amineoxide (DDAO)	229	2.2×10^{-3}	69–73	1.128–1134	[17,31,32,199]
Lauroamido-*N*,*N*-dimethyl-3-*n*-propylamineoxide (LAPAO)	302	3.3×10^{-3}	–	1.067	[33]
Dodecyl-*N*-sulfobetaine (zwittergent 3-12)[c]	336	$1.4–4\times10^{-3}$	55–87	–	[6,20]
Tetradecyl-*N*-sulfobetaine (zwittergent 3-14)	364	$1–60\times10^{-4}$	83–130	–	[6,19]
N-dodecyl-*N*,*N*-(dimethylammmonio) butyrate (DDMAB)	300	4.3×10^{-3}	47	1.07	[34,35]
1-Myristoyl-2-hydroxy-*sn*-glycero-3-phosphocholoine ($C_{14:0}$lysoPC)	468	9×10^{-5}	–	0.97	[6,13]
1-Palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine ($C_{16:0}$lysoPC)	496	1×10^{-5}	–	0.976	[6,13]
N-dodecylphosphocholine (DPC)	352	1.1×10^{-3}	50–60	0.937	[36,215]
1,2 Diheptanoyl-*sn*-glycero-3-phosphocholine (di-$C_{7:0}$PC)	482	$1–1.4\times10^{-3}$	42–200[g]	0.888–0.925	[5,13,58,59]
3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS)[c]	615	$3–10\times10^{-3}$	4–14	0.81	[19,20,37]
Deoxycholic acid[d,e]	393	3×10^{-3}	22	0.778	[3,13]
Cholic acid[d,e]	409	1×10^{-2}	4	0.771	[13,38]
Taurodeoxycholic acid[d]	500	1.3×10^{-3}	20	0.75	[13,38]
Glycocholic acid[d]	466	–	6	0.77	[13,38]
Sodium dodecylsulfate[c]	288	$1.2–7.1\times10^{-3}$	62–101	0.863	[5,19,20,39]
6-*O*-(*N*-heptylcarbamoyl)-methyl-β-D-glucopyranoside (HECAMEG)	335	1.95×10^{-2}	92	–	[40,192]

[a] Data obtained at 20–25°C, pH ≈ 7.

[b] The references indicated are either for the original data or for data surveys.

[c] The lower values of CMC are obtained at 0.1–0.2 M Na^+, the higher values at 0–0.05 M Na^+ (data surveyed by [19,20]). For SDS, the $\bar{\nu}_D$ is lower below the CMC [39].

[d] Data refer to μ=0.15, pH 8.0 (micellar properties are strongly affected, in particular by changes in ionic strength).

[e] Data for CMC refer to the authors' own measurements by the dye uptake method [41].

[f] An Anatrace Inc. measurement.

[g] Not well-defined micelles [58].

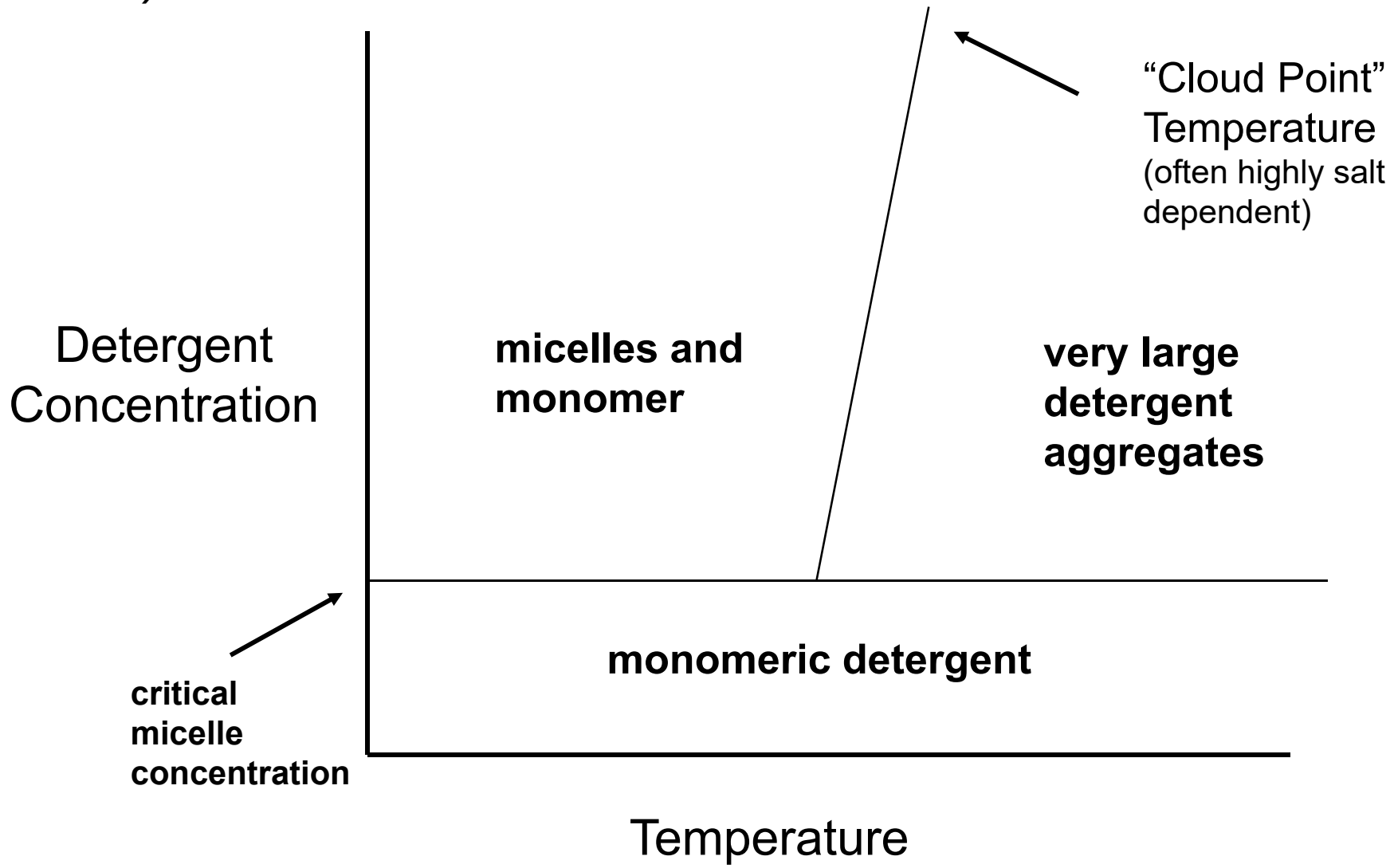
For reference purposes. Do not memorize!

Note on Detergent Homogeneity and Purity

Some detergents are chemically homogeneous– all the detergent molecules are the same molecule. However, others– particularly those that were originally made for industrial or non-scientific applications (e.g., in laundry detergent) are not. For some research applications it will be important to know whether or not your detergent is chemically homogeneous, so you will want to do your homework if you are not sure.

Some detergents that have made their way into biological research are originally prepared for industrial or other large scale applications, where purity is not a big issue. So, the "raw" detergent mixtures (which sometimes are sold without further purification to researchers by vendors) may be contaminated by compounds that are oxidizing agents, have high UV absorbance, or are a nuisance for some other reasons. Often vendors serving the research community will market these detergents both in crude form and in forms where the vendor has take extra step to remove impurities (with varying degrees of vigor). The resulting higher purity forms will, of course, cost extra but can eliminate the potential problems of working with grungy detergents.

A Few (Very Few) Detergents have Cloud Points at Temperatures Near Room Temperature, Leading To Phase-Separated Solutions Above that Temperature
(most notably, Triton X-114– there are some cell biology protocols that exploit this phenomenon)



Note on weighing lipids and detergents:

Note that it can be difficult to weigh mg quantities of powdered lipids and detergents accurately. There are two issues to be aware of.

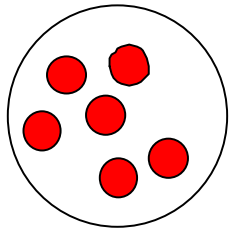
(1) Many lipids (and some detergents) are hygroscopic (they absorb water from air and turn into goo or paste). When you remove a lipid-containing bottle from the freezer, allow it to warm to room temperature before opening and then keep it tightly capped when not actually weighing it out. Seal bottles after use with parafilm before returning to the freezer.

(2) Static electricity can interfere with the weighing process. It is generally best to weigh directly from the commercial bottle to the final bottle/tube/vial your model membrane solution will be formed in (instead of using weighing paper or boats). Static electricity can usually be dissipated by taking damp (but not wet and dripping) paper towel and wiping the bottles/tube/vials, the spatula and the balance pan. Sometime you can even wrap/hold the spatula and source bottle in the damp towel while weighing.

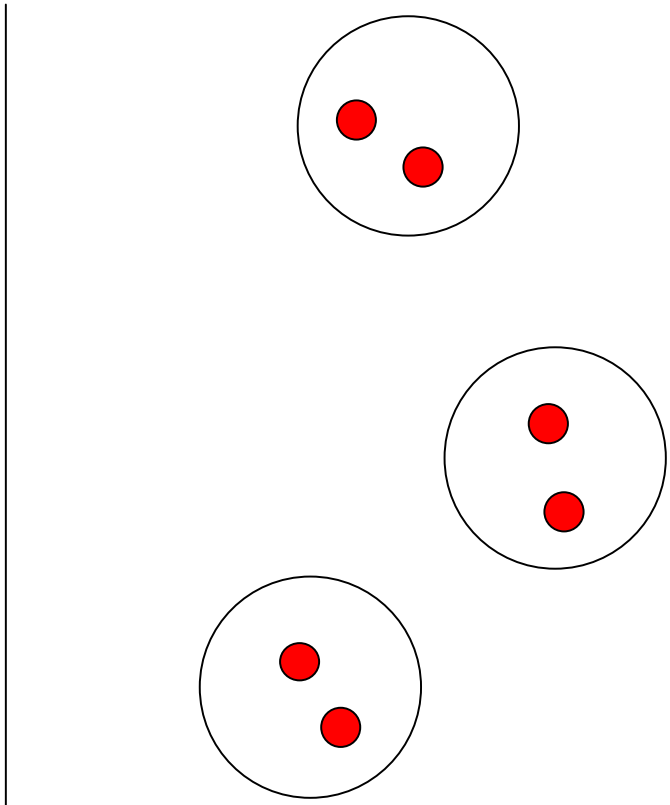
Some detergents are sold as chloroform stock solutions. If using these solutions as used as the source of lipid, it is critical to remove all the chloroform after measuring into a vial/tube/bottle. This may mean more than just blow-drying away the solvent– you may need to place it under high vacuum with an efficient cold trap (not just house vac with no trap) to chase off the residual chloroform.

Surface Dilution

Assume that the total volume of the left compartment is the same as for the right. The red molecule associated with micelles has the same bulk concentration in the left compartment as left as on the right.



However, its concentration in the micelles on the left is 3X as high as on the right because there are 3X as many micelles in the right compartment to distribute the red molecules between.



Surface Concentration:
Expressed in Mol fraction or Mol% Units

Mol fraction for "A" =
{moles of A in the membrane} ÷ {total moles of A + other components of the membrane}

For example: 1 mM C99 in 100 mM LMPG micelles is a 1 mol% C99 solution, whereas 1 mM C99 C99 in 200 mM LMPG micelles is a 0.5% C99 solution.

The Detergent-to-Membrane Protein Ratio as it Relates to the Concentration of Micelles

Consider a detergent, for example, decyl maltoside (DM). DM has a CMC of 2 mM and an aggregation number of 104. If you have a 25 mM DM solution, you will have 2 mM free DM and 23 mM micellar DM. The actual concentration of the micelles will be 23 mM divided by 104 = 0.22 mM.

So, if you are doing an experiment where you do not more than one membrane protein per micelle, the upper limit of your membrane protein concentration would be 0.22 mM. For certain classes of structural biology experiments, such as solution NMR, this is an important consideration.

Harsh vs. Mild Detergents

From a membrane protein's point of view, some detergents tend to be "harsh" in that they partially or fully denature the protein– these are mostly used for membrane protein extraction and the initial stages of purification.

Other detergents are "mild" in that they tend to solubilize membrane proteins in a way which maintains their native function.

In general, non-ionic (uncharged) detergents tend to be the mildest, followed by zwitterionic detergents (charged, but net charge of zero), followed by detergents that have a net positive or negative charge (most harsh). For example, dodecylsulfate is harsh, while dodecylmaltoside is mild.

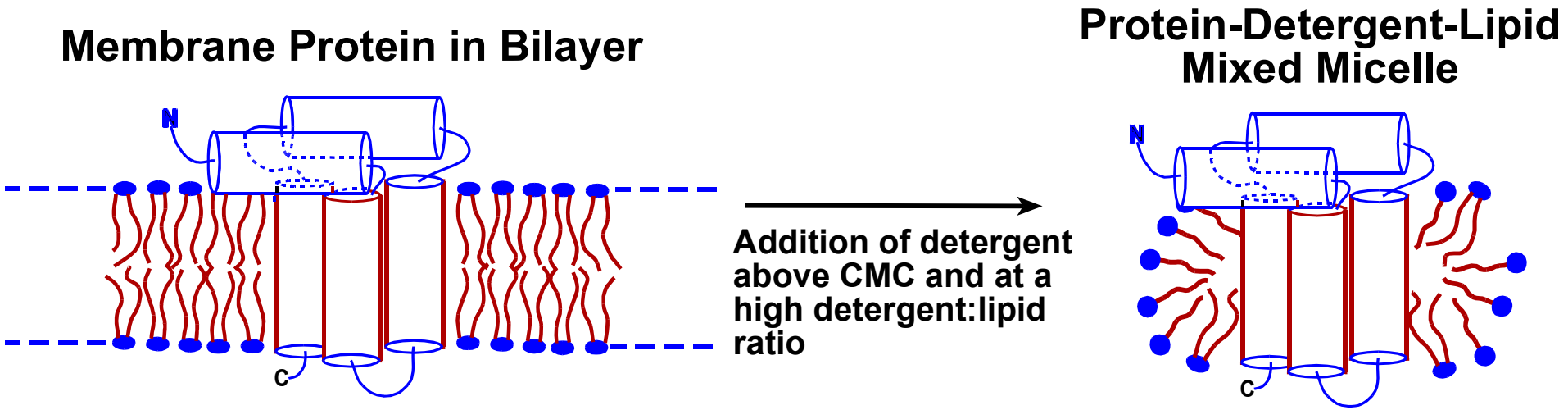
Harsh detergents to use for "universal extraction" (inclusion bodies, etc.):

SDS advantages: will solubilize everything for sure
makes subsequent SDS-PAGE easy, pure/cheap
disadvantages: finicky, may sometimes
not work well with Ni(II)-agarose resin and
His-tagged proteins, anionic

Lauroyl Sarkosine: C_{11}-CO-N(CH_3)-CH_2-COO^-
advantage: not as finicky as SDS– can be used
more easily with Ni(II)-agarose, pure/cheap,
disadvantages: anionic, not as strong a denaturant
as SDS

Empigen: C_{12}-N$(CH_3)_2^+$-CH_2-COO^-
advantages: fully compatible with use of
Ni(II)-agarose, zwitterionic
disadvantages: not as universal a solubilizing
agent as SDS

Extraction of Membrane Proteins from Bilayers



Solubilization of Membranes by Detergents.

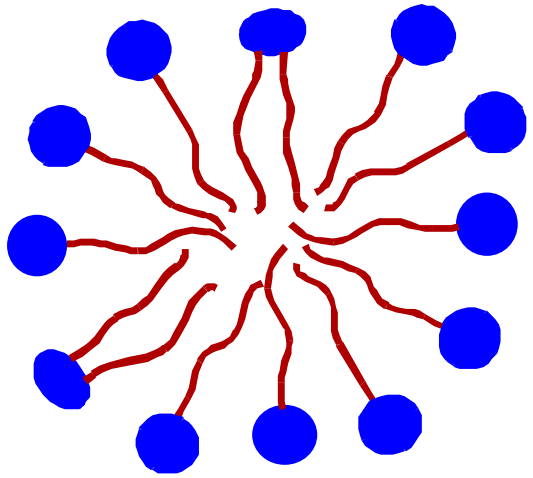
Detergent concentration should be well above CMC

Detergent-to-lipid ratio should be high: typically at least 4:1.

Some detergents are better membrane solubilizing agents than others.

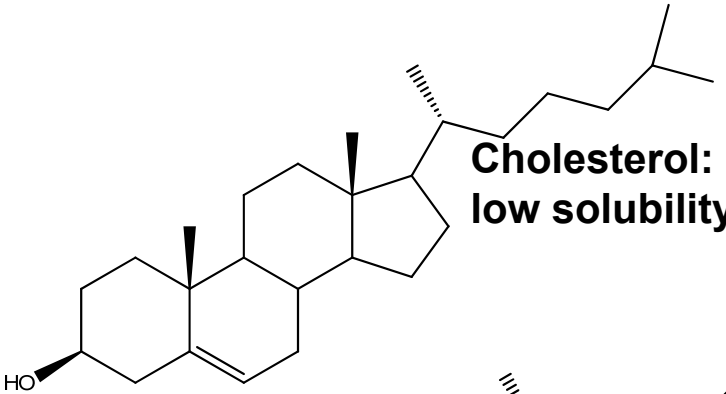
Some lipids are harder to solubilize than others (especially components of lipid rafts.

Freeze thaw cycles alternating liquid nitrogen freezing and a warm water sonicating bath sometimes speed achievement of a homogeneous solution.



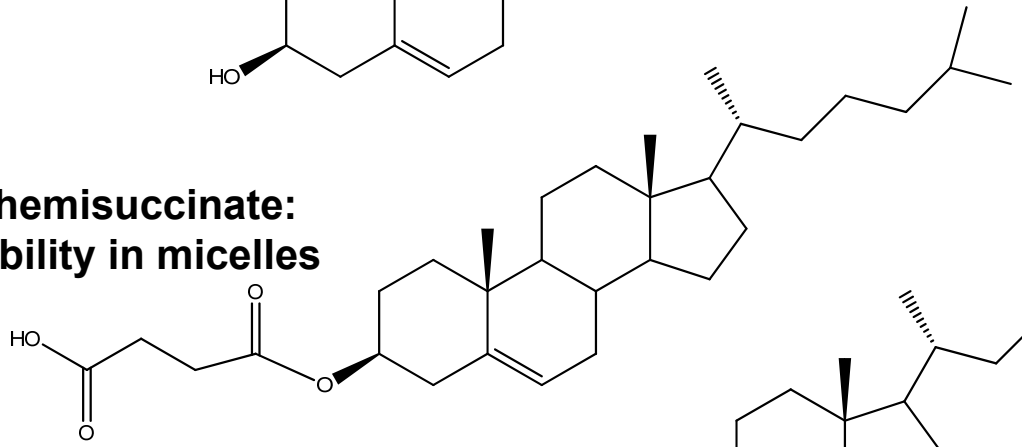
Mixed micelles also contain lipid in addition to detergent. Usually the detergent-to-lipid ratio is in the range of 1:20 to 1:5. Usually, the lipid is a phospholipid (often PC), but sometimes you might want a cholesterol mimic. Cholesterol itself is very hard to solubilize in micelles; therefore, sometimes easier-to-solubilize cholesterol derivatives are used instead of bona fide cholester in mixed micelles.

Detergent-Lipid Mixed Micelles

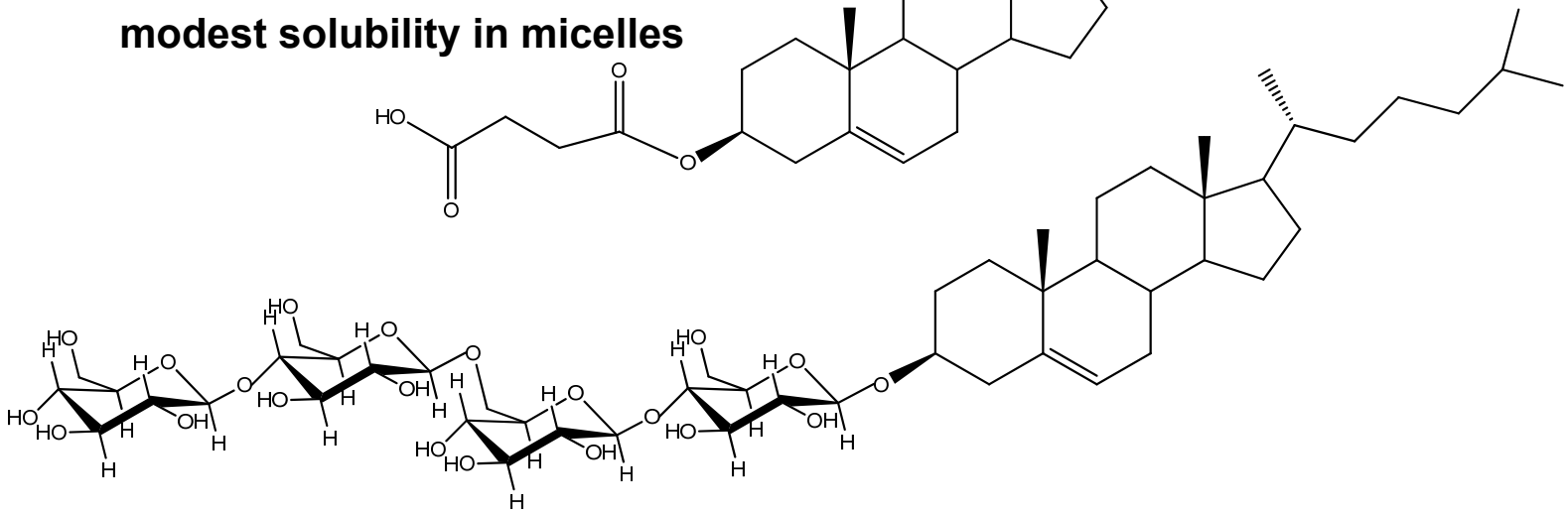


Cholesterol:
low solubility in micelles

Cholesterol hemisuccinate:
modest solubility in micelles



CHOBIMALT:
water soluble



Co-Dissolving Lipids and Detergents or Lipids With Other Lipids

Detergent/lipid mixtures can be difficult to co-dissolve. Cycles of flash-freezing liquid nitrogen followed by warm bath sonication and vortexing and then repeating is a vigorous way to achieve homogeneity of solutions. If this doesn't work then you may need to add additional detergent (at least 4:1 detergent:lipid is by no means an unusual requirement).

Note that if you are going to prepare vesicles with lipid mixtures, it is usually not going to suffice to just mix the lipids and hydrate. The lipids in the resulting vesicles will not be uniformly and ideally mixed from vesicle to vesicle. To achieve uniform mixing, lipid mixtures are typically co-dissolved in an organic solvent, which is then removed, followed by hydration. Chloroform is most often used. This can be removed by air-blowing off excess solvent, followed by chasing the residual solvent away under high vacuum with a good cold trap (house vac with no trap is not sufficient)– the resulting lipid is glassy (or sort of oily). Alternatively, 95:5 benzene:ethanol can be used. In this case you dissolve the lipid mixture, freeze it in liquid nitrogen, and then lyophilize (freeze-dry) off the solvent, which usually results in a nice white powder. Of course, take care not to expose yourself or colleagues to the benzene during or after this procedure (clean the cold trap and freeze-dryer unit afterwards).

Concentrating Detergent and Membrane Protein/Detergent Solutions

- **Blow filtered air over samples to remove water. (Most useful for solutions that have low salt and buffer concentrations since everything in the solution gets concentrated by the same factor.) It helps to have your sample tube sitting in a warm water bath to speed the evaporation process.**

- **Centrifugal ultrafiltration cartridges. Provided the molecular weight cut-off of the membrane is lower than the size of the micelle or of the micelle-protein complex, it is possible to concentrate the micellar assemblies. Note that there can be some loss of monomeric detergent, so if you are using the cartridge for multiple rounds of concentrate-then-dilute (for example, when exchanging from one buffer to another) you may want to include a CMC concentration of detergent in your dilution buffer to make up for detergent lost during the previous concentration step. Whether a true CMC concentration of detergent really passes though any given filter is something that you might want to check experimentally if the exact detergent concentration in your concentrated/exchanged solution is needed. This requires a method for measuring detergent concentration, of course. The Sanders lab, for example, uses NMR to quantitate detergent concentrations.**

Removing Detergent from Solutions

Detergents can be selectively removed from solutions using the following methods:

Dialysis: Monomeric detergent escapes from the sample through the pore of the dialysis tubing into the surrounding bath. The bath needs to be stirred and to be changed periodically (typically 1st thing in the morning and then late in the afternoon). The time it takes to remove all the detergent depends on the MWCO and grade of dialysis tubing used and the CMC of the detergent (the lower the CMC the longer you have to dialyze). Some solutions may require a week of dialysis for removal of all the detergent. You often can tell when all the detergent has dialyzed out when the bath solution no longer foams when poured down the drain after a round of dialysis.

Biobeads-SM: These beads (tiny Styrofoam balls) absorb detergent. You add the beads to your solution and then gently agitate the solution, usually over a period of hours before filtering out beads. Of course if the absorptive capacity of the beads is exceeded by the amount of detergent present you may have to repeat the bead treatment. Note that some (but not all) membrane proteins may be denatured or absorbed by the beads.

Standard "de-salting" methods using size exclusion chromatography resin are usually NOT effective at removing detergents.

SDS can be precipitated from solution by adding K+ salts. Potassium dodecylsulfate is insoluble. See old work by Jean-Luc Popot (JMB, late '80s) for example of its use.

Note that by diluting any detergent-containing solution to the extent that the total detergent concentration reaches <CMC, all micelles will go away. This does not, of course, remove detergent but it does convert it to a fully monomeric form.

Purification of Diacylglycerol Kinase

E. Coli Harboring Overexpressed polyHis-tagged DAGK

Harvest and Lyse

Extract with 3% Empigen

Apply to Ni-Agarose

Wash with 40 mM Imidazole + Empigen

Pure DAGK Bound to Ni-Agarose

Elute with Detergent Solution of Choice and 250 mM imidazole

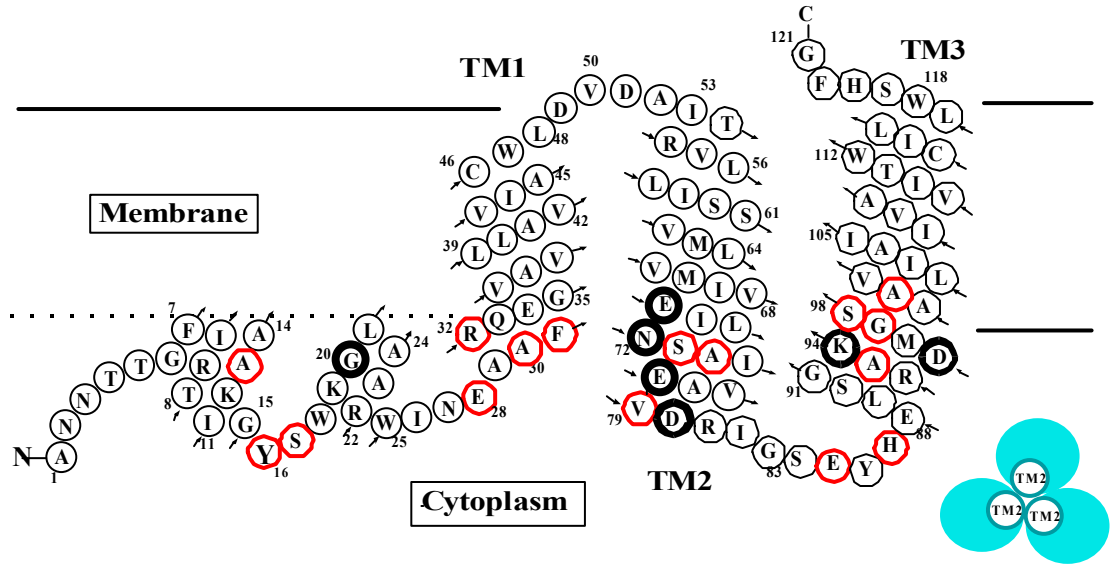
DAGK in Micelles (5-30 mg/ml)

DAGK in Bicelles

DAGK in Mixed Micelles

DAGK in Liposomes

Metal Ion Affinity Chromatography is A Power Tool for Manipulating Membrane Proteins in Terms of Model Membrane Composition



Sanders Lab

Making Detergent-Containing Buffers

- **When possible, make the buffer and adjust the pH before adding detergent, just in case the detergent and the pH electrode don't play well together (as for some ionic detergents).**

- **Addition of a modest amount of EDTA (0.1 mM) to sequester stray metal ions will prevent bacteria from growing in the solutions upon long term storage and will prevent metal ion-catalyzed hydrolysis of lipid.**

- **Thiol reducing agents (CS likes DTT) should be added fresh, as they often have half-lives on the order of a day or even less.**

- **Some detergents (SDS, some lysolipids) precipitate when stored cold.**

- **Guidelines for selecting buffers and chelating agents can be found on pages 36-42 of another Sanders lab tutorial:**

http://structbio.vanderbilt.edu/sanders/Binding_Principles_2010.pdf

Detergent Concentration Following IMAC Purification of a Membrane Protein

Total detergent = [CMC] + [free micellar] + [protein-associated]

If you equilibrate of membrane protein associated with a chromatographic resin with a 0.5% solution of detergent and then elute that protein, the final total detergent concentration will be 0.5% plus the amount of detergent which is associated with the protein.

This needs more study.

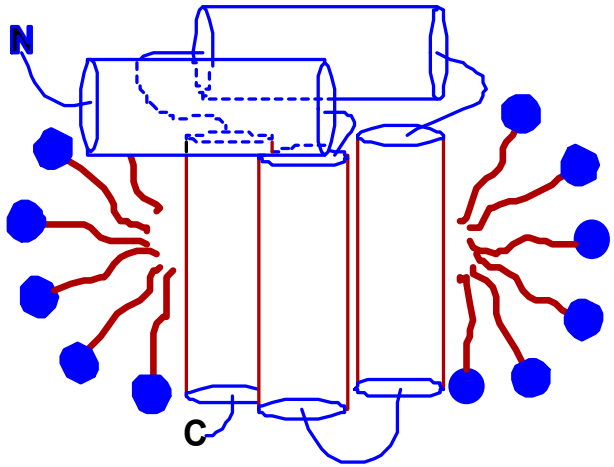
As a very rough guess, you can assume that the membrane-associating domain of a membrane protein binds twice its weight in detergent and/or lipid. (For DAGK, e.g., we know it binds twice its weight in detergent).

So, if you have a 1 mg/ml solution of a MP that has 50% of its sequence involved in membrane interactions, you could guess that the solution would also contain 1 mg/ml of protein-associated detergent.

Micelles and Mixed Micelles as a Medium for Studies of Membrane Proteins

Advantages:

- Low molecular weight/rapid tumbling: often used for solution NMR
- Light scattering not a problem (good for UV, CD, fluorescence)
- Easy to handle and concentrate
- A majority of membrane protein crystal structures have been determined in micelles (most often using dodecylmaltoside)
- Suitable for use in binding studies and biochemical assays (titrations, etc.)



Disadvantages:

- Membrane protein structure can be distorted
- Membrane protein structure can be destabilized (from poking of alky chains into protein fold; there is much more water inside micelles than in bilayers; there is a loss of bilayer lateral pressure and related protein-stabilizing forces.
- Unlike ULVs, not suitable for channel or transport studies (no inside and out)
- Too small for EM unless protein is giant (this could eventually change)
- Dilution below CMC (as in EM sample prep) leads to loss of micelles and protein aggregation

Problems that Can Arise When Working With Micelles or Any Model Membrane

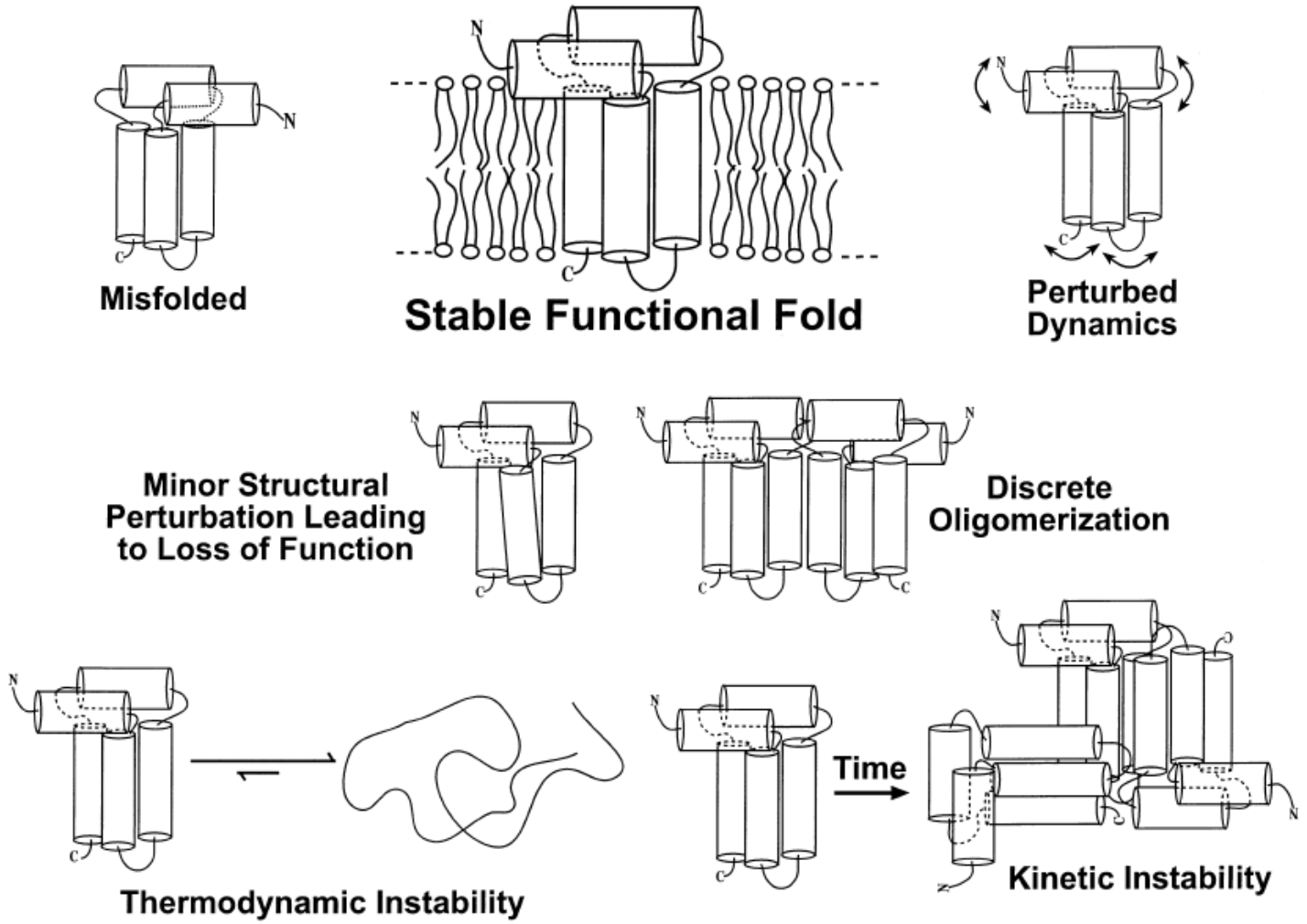


Fig. 3. Undesired fates for a hypothetical membrane protein in model membrane media (membrane-mimetic phases not shown).

Despite concerns about micelle-induced artifacts…

The vast majority of what we know about membrane protein structure derives from studies of membrane proteins in detergent micelles. While micelles are not a perfect model for the incredibly complex milieu represented by a true biological membrane, many membrane proteins retain native-like structure and function in membrane bilayers.

<u>Membrane Reconstitution</u>: Taking purified membrane protein(s) in micelles or mixed micelles and transferring them back into membrane bilayers (lipid vesicles)

Most common methods:

(1) Selectively remove detergent from protein/lipid/detergent mixed micelles using dialysis or BioBeads.

(2) Dilute protein/lipid/detergent mixed micelles to below the detergent's CMC.

<u>Membrane Reconstitution</u>: Taking purified membrane protein(s) in micelles or mixed micelles and transferring them back into membrane bilayers. If successful, protein will function properly in the resulting bilayered lipid vesicles (liposomes).

Most common methods:

(1) Selectively remove detergent from protein/lipid/detergent mixed micelles using dialysis, size exclusion chromatography or some other method. Protein/lipid bilayered vesicles form spontaneously as detergent is removed.

(2) Dilute protein/lipid/detergent mixed micelles to below the detergent's CMC.

(3) Selective binding of detergent to hydrophobic beads leaving protein behind with lipid (although this sometimes results in denaturation of the membrane protein).

Membrane Protein Purification and Reconstitution

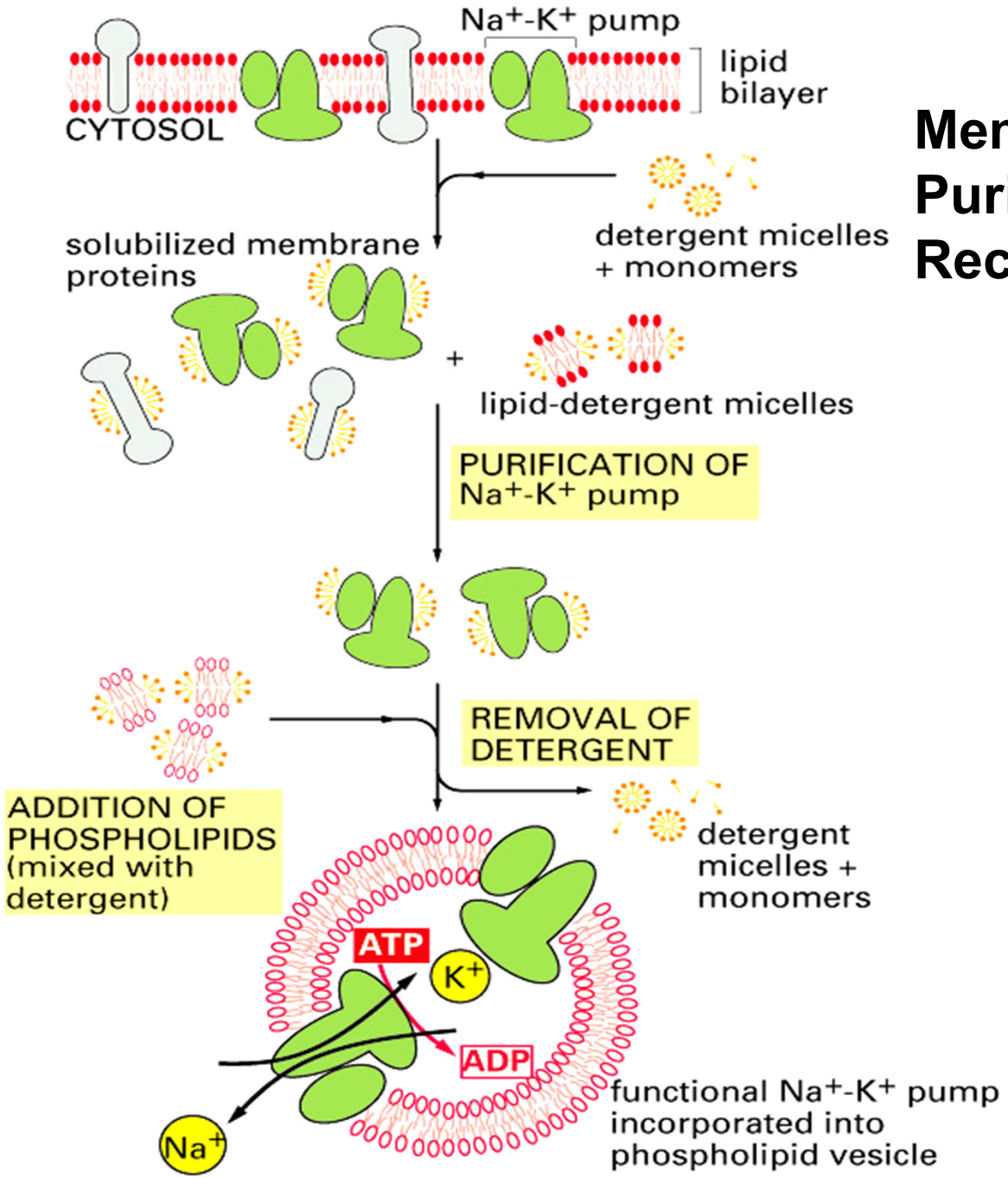
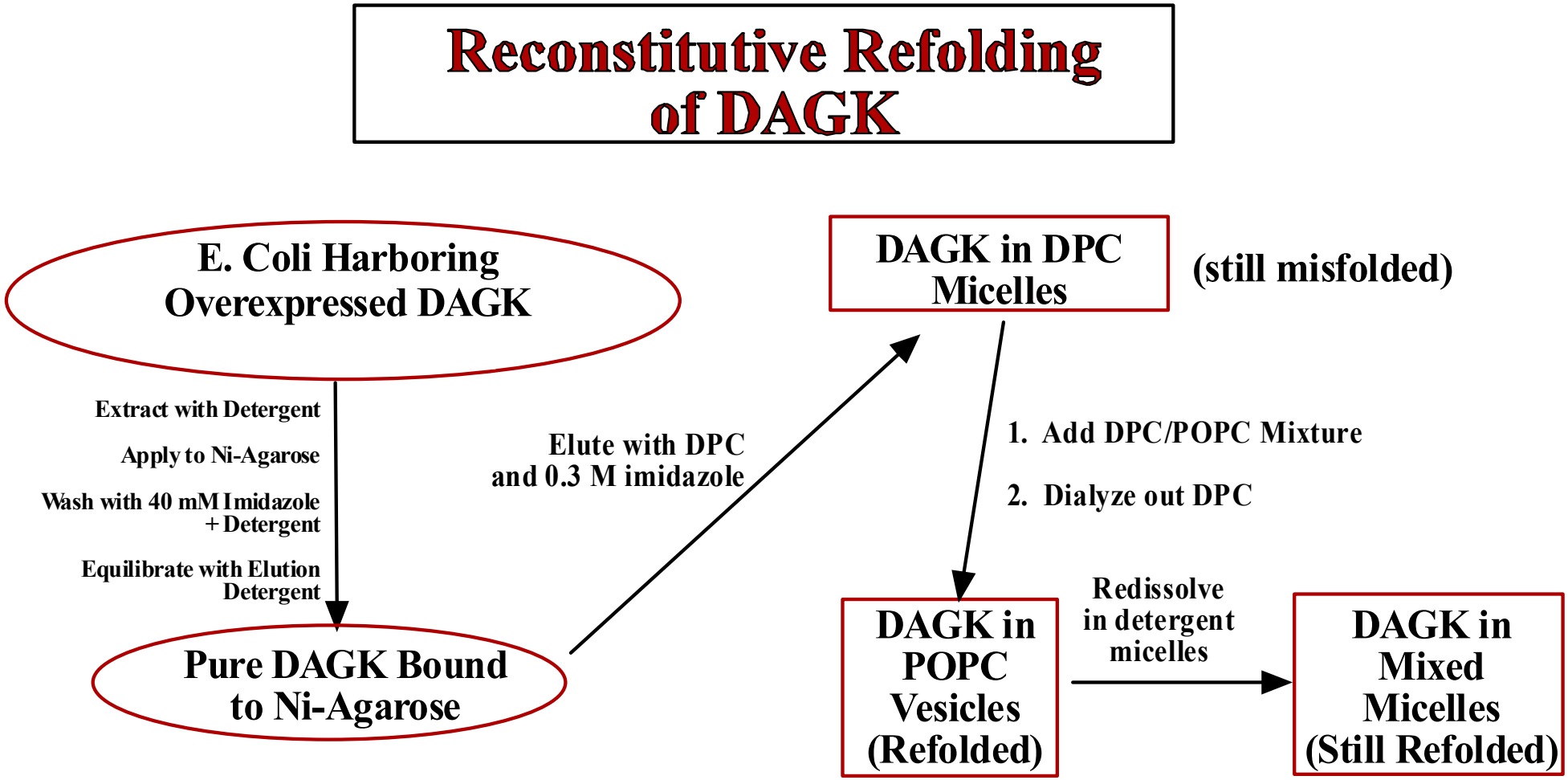


Figure 10–26. Molecular Biology of the Cell, 4th Edition.

Example of a Membrane Reconstitution Protocol



Reconstitutive refolding of diacylglycerol kinase, an integral membrane protein.
Gorzelle BM, Nagy JK, Oxenoid K, Lonzer WL, Cafiso DS, Sanders CR.
Biochemistry. 1999 38(49):16373-82.

Alternative Model Membrane Systems for Use in Studies of Membrane Proteins

Bicelles

Combine some advantages of micelles and bilayers as a medium for membrane proteins

membrane proteins have been crystallized from bicelles

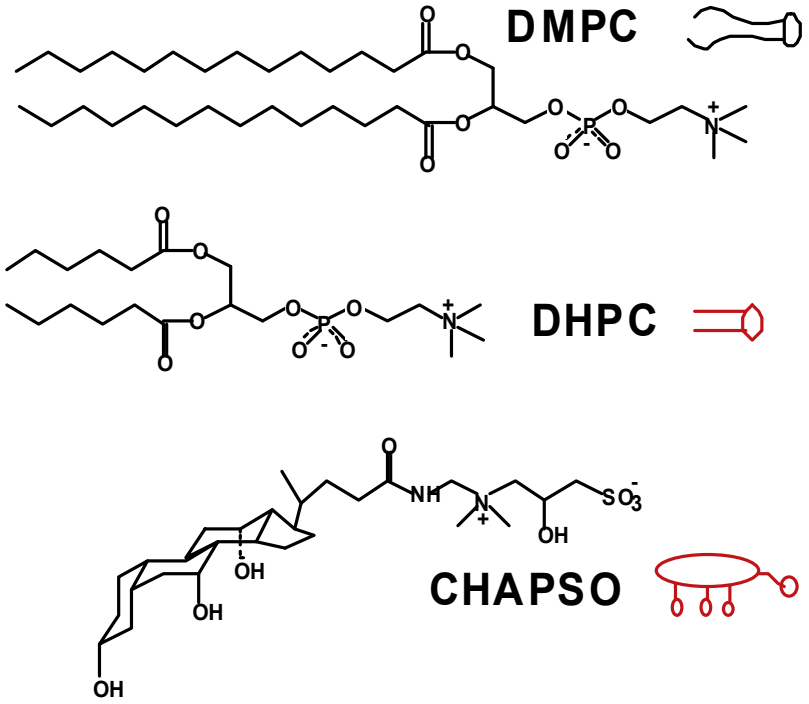
T_m for DMPC is 24.5 deg. C.

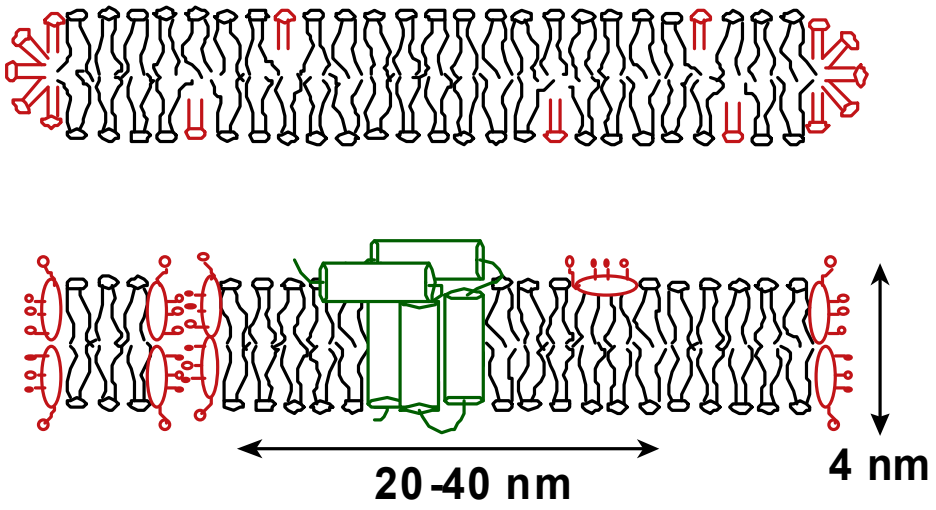
DHPC-DMPC seems to work better for solution NMR.

CHAPSO-DMPC seems to work best for X-ray crystallography (even GPCRs).

Both systems work well for solid state NMR.

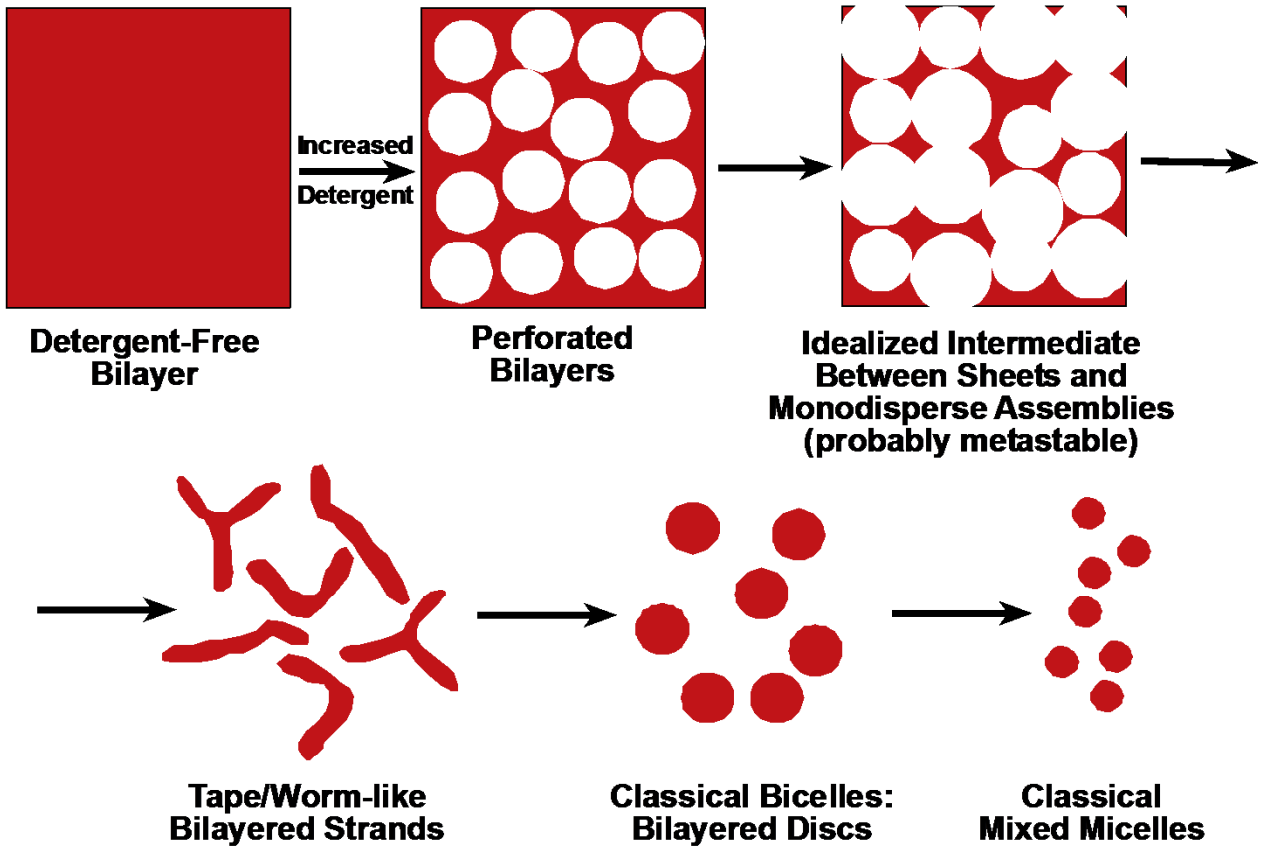
If a negative charge is desired can use DMPG to replace part of the DMPC.





Bicelles: a model membrane system for all seasons?
Sanders CR, Prosser RS. Structure. 1998 6:1227-1234

Intermediate Structures in Membrane Dissolution by Detergents

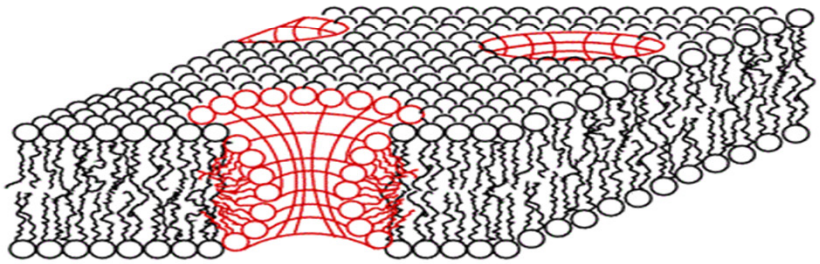


q = moles lipid to moles detergent

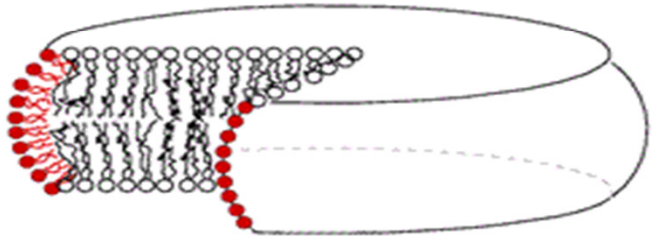
q = 0.5 means 1:2 lipid to detergent = 0.33 mol fraction lipid

The size of bicelles is Determined by the detergent-to-lipid ratio. The higher the detergent the smaller the bilayered discs.

http://structbio.vanderbilt.edu/sanders/bicelles_history_2005_proof.pdf

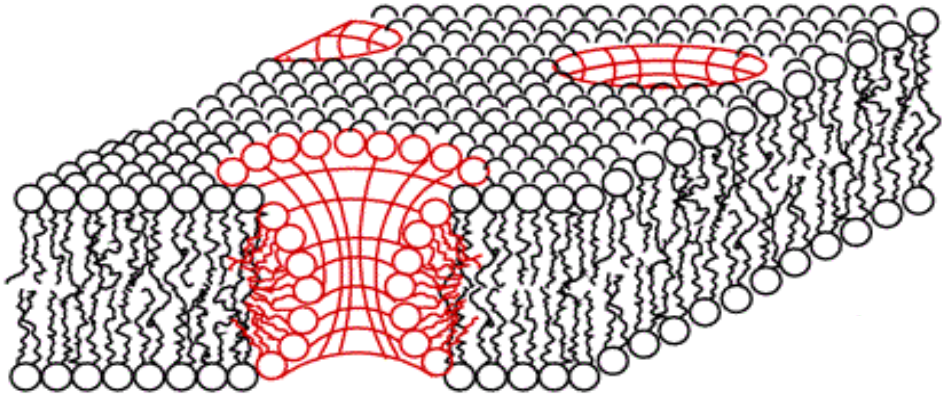


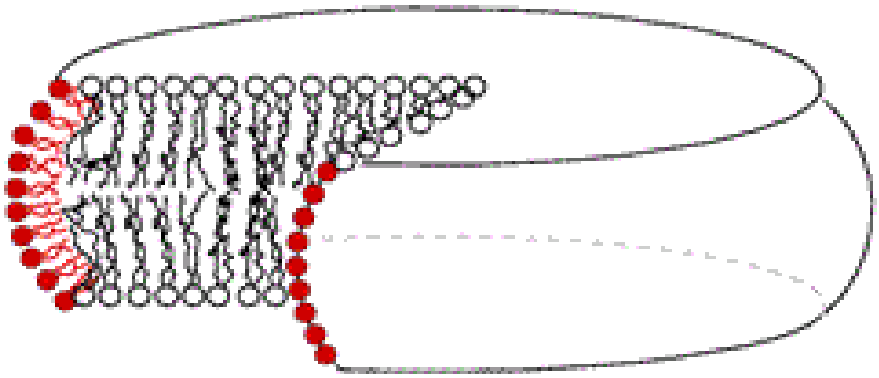
Membrane Permeabilization By Relatively Low Detergent Concentrations.



Membrane Fragmentation to Form Bicelles at Somewhat Higher Detergent Concentrations (typically 4:1 lipid:detergent to 1:1 lipid:detergent)

Intermediate Structures in Membrane Dissolution by Detergents





Above T_m for the lipid
(24.5 deg. C for DMPC)
this phase persists from
q = 2-5 for DMPC/DHPC
or q = 3-8 for DMPC/CHAPSO

Membrane fragmentation to form bicelles at somewhat higher detergent concentrations. Above T_m this phase likely persists q = 0.25 to 1.0 for DMPC/DHPC.

Figure: Biochemistry. 2006 J45:8453-65.
Current applications of bicelles in NMR studies of membrane-associated amphiphiles and proteins.
Prosser RS1, Evanics F, Kitevski JL, Al-Abdul-Wahid MS.

The magic of bicelles lights up membrane protein structure.
Dürr UH, Gildenberg M, Ramamoorthy A.
Chem Rev. 2012 Nov 14;112(11):6054-74.

Naturwissenschaften (2005) 92: 355–366
DOI 10.1007/s00114-005-0641-1

REVIEW

John Katsaras · Thad A. Harroun · Jeremy Pencer · Mu-Ping Nieh

"Bicellar" lipid mixtures as used in biochemical and biophysical studies

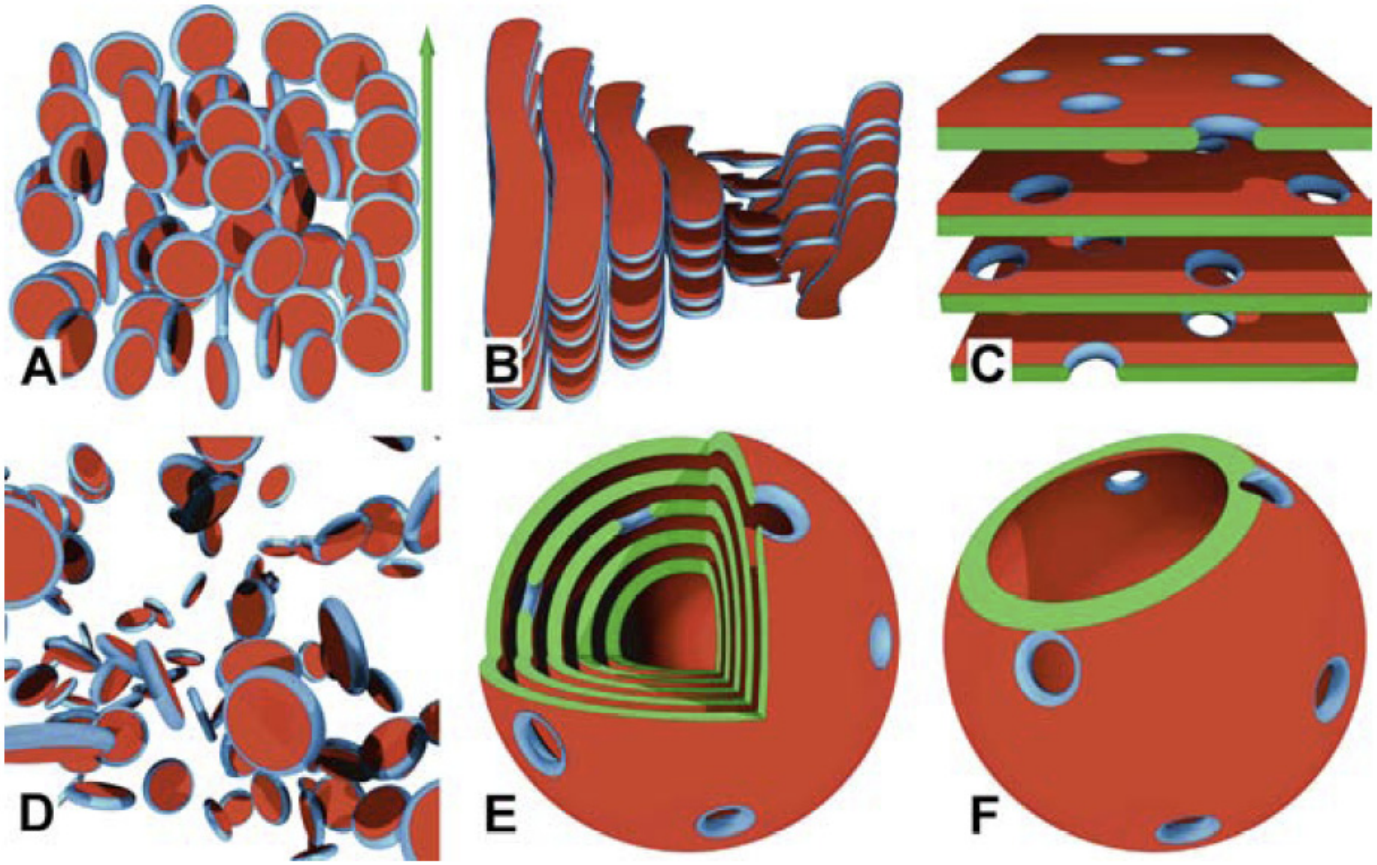
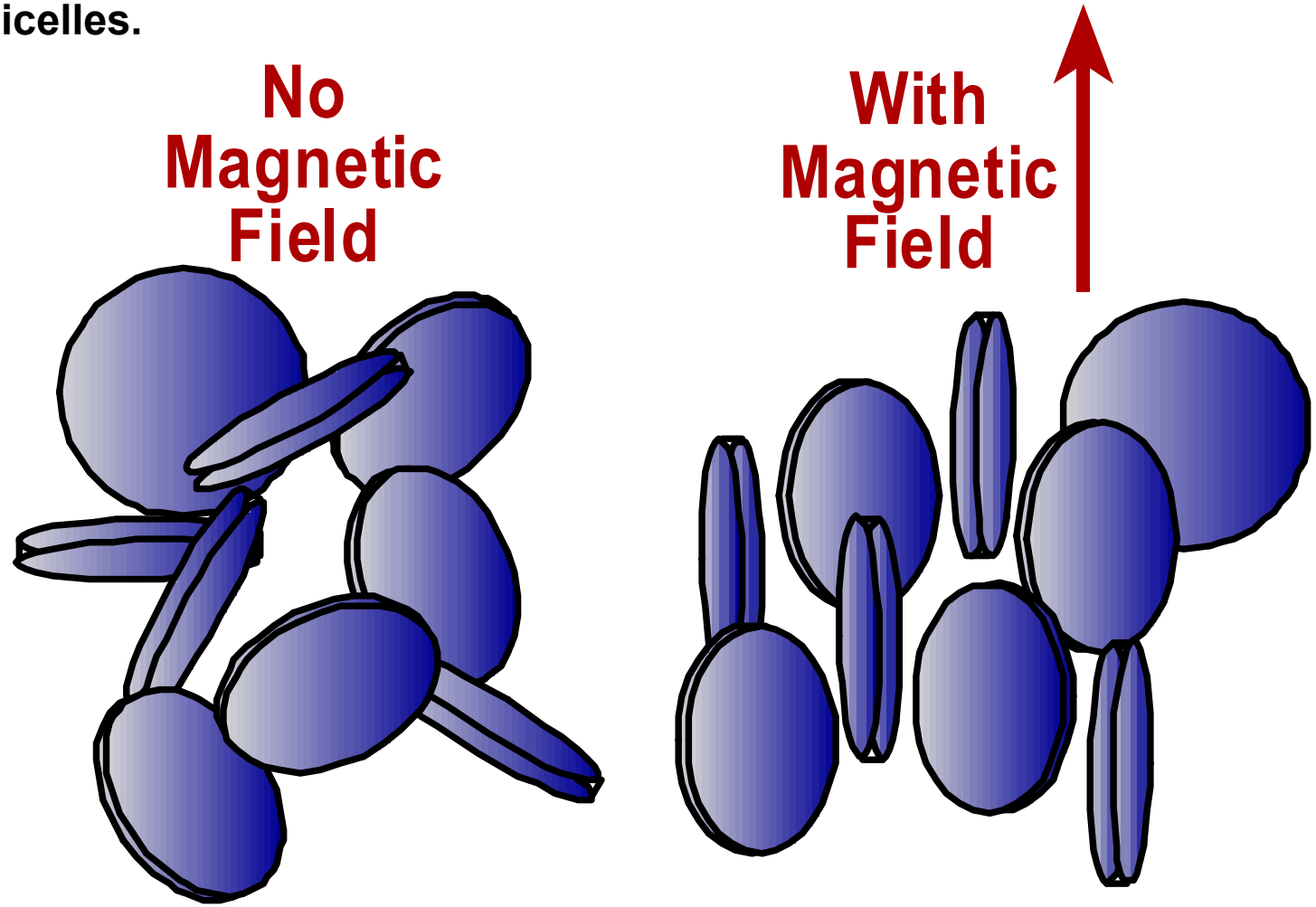


Fig. 1 Morphologies formed by dimyristoyl phosphatidylcholine (DMPC)/dihexanoyl PC (DHPC) lipid mixtures. **A** A nematic phase of bicelles oriented such that, on average the bilayer normals **N** are all pointing at right angles to the externally applied magnetic field (arrow), but the positions of the discs are random. In the literature, this orientation of bicelles was commonly referred to as "negatively aligned". **B** In a cholesteric or chiral nematic phase, the molecular assemblies twist slightly from one layer to the next resulting in a helical formation with a characteristic pitch. On the other hand, the spacings between bilayers lack a well-defined repeat distance, consistent with a nematic phase. The increased viscosity observed in this phase is the result of the entaglement of the elongated bilayered micelles. **C** Extended perforated lamellae, or smectic phase, exhibit both long range positional and orientational order, and can be equated to 1D quasi solids in one direction and 2D liquids (i.e., molecules freely diffusing within the bilayer) in the two orthogonal directions. The appearance of this phase is opaque and fluid. **D** An isotropic dispersion of bicelles. In this phase the bilayered micelles are randomly distributed in the water solvent, exhibiting no long range positional or orientational order. The solution is generally of low viscosity and colorless, and the transition from isotropic to nematic is first order. **E** Multilamellar and **F** unilamellar vesicles (ULV). For all morphologies shown, the colors red, blue and green correspond to the lipids DMPC, DHPC, and the bilayer's interior (hydrocarbon chains), respectively

“Large bicelles” can magnetically aligned. However, it is now realized that it is probably not the ideal bilayered discs that align. Instead it is the “Swiss cheese” bicelles, which form at lipid-rich detergent-to-lipid ratios that align in magnetic fields.

Magnetic alignment of bicelles is useful both for EPR and solid state NMR of membrane proteins in bicelles.



Bicelles: a model membrane system for all seasons?
Sanders CR, Prosser RS. Structure. 1998 6:1227-1234

sumed disklike aggregate. The two domains, a round bilayered center with radius R containing only DMPC and a rim with internal radius r containing only DHPC, are identified in Fig. 1 as I and II, respectively. The surface areas A_I and A_{II} are given by

$$A_I = 2\pi R^2 \quad [1a]$$

$$A_{II} = 2\pi r(\pi R + 2r). \quad [1b]$$

If we now assume that the headgroup areas for the two phosphatidylcholines are identical in the two domains, then the ratio between their surface areas equals the concentration ratio q, i.e.,

$$q = A_I/A_{II} = [\text{DMPC}]/[\text{DHPC}]. \quad [2]$$

An expression for the size of the bicelle, or more precisely, the radius R of the center section, can then be readily obtained from Eqs. [1] and [2]:

$$R = \tfrac{1}{2}rq[\pi + (\pi^2 + 8/q)^{1/2}]. \quad [3]$$

It is easily seen that as $R \to 0$, $A_{II} \to 4\pi r^2$ which is the surface of a sphere with diameter equal to the bilayer thickness. Furthermore, R increases linearly with q once $R > 5r$, or $q > 3$. It is particularly interesting to note that for $R = 2r$, $q = 2/(\pi + 1) \approx 0.5$. In other words, when the bicelles contain twice as much DHPC as DMPC, the diameter of the planar region is roughly 80 Å, or twice the bilayer thickness, which is approximately 40 Å for DMPC. This is well inside the isotropic range of the bicellar solutions and has proven suitable for high-resolution NMR spectroscopy (*11*).

The bicellar radius R is plotted as a function of the phospholipid ratio q in Fig. 2. According to the plot, the commonly used concentration ratio $q = 3$ (*3, 5*) gives rise to bicelles with a planar section with $R = 200$ Å, which may

JOURNAL OF MAGNETIC RESONANCE, Series B **113**, 267–271 (1996)

Magnetically Oriented Phospholipid Bilayered Micelles for Structural Studies of Polypeptides. Does the Ideal Bicelle Exist?

REGITZE R. VOLD* AND R. SCOTT PROSSER

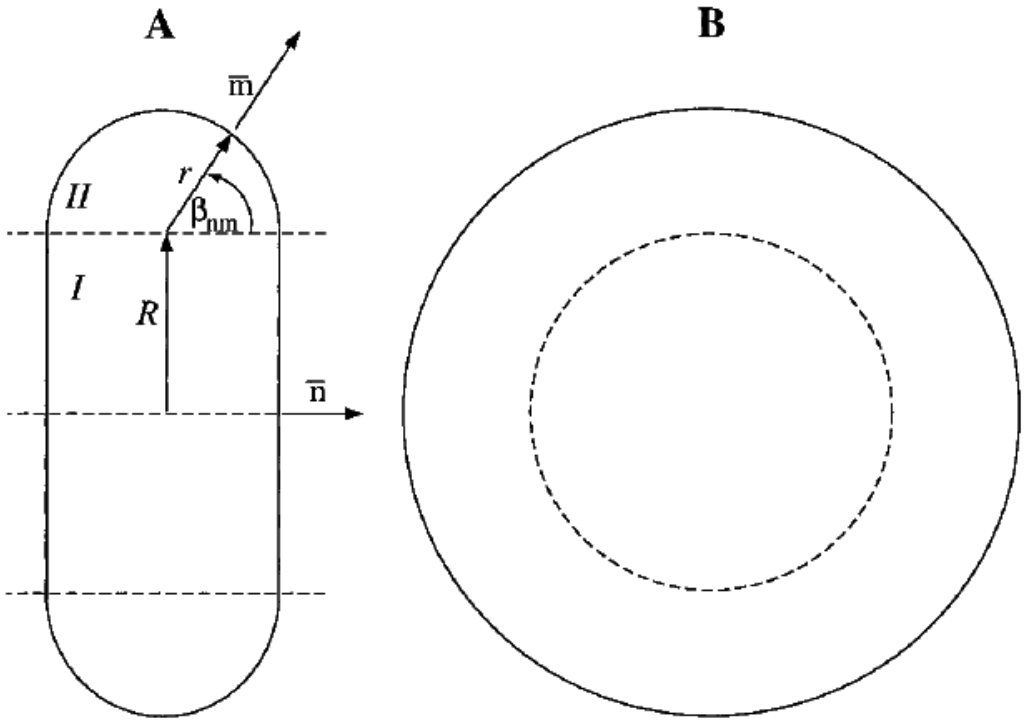


FIG. 1. Simple sketches, (A) cross section and (B) side view, of the ideal mixed phospholipid bicelle. The two domains, plane and rim, are labeled I and II, and the relevant radii, R and r, are indicated along with the molecular axis, **m**, the bicellar normal, **n**, and the angle β_{nm} between them.

Solution NMR studies are usually Carried out using q = 0.3-0.5.

Bilayer in Small Bicelles Revealed by Lipid−Protein Interactions Using NMR Spectroscopy

Donghan Lee,[†] Korvin F. A. Walter,[†] Ann-Kathrin Brückner,[†] Christian Hilty,[‡] Stefan Becker,[†] and Christian Griesinger*,[†]

J. AM. CHEM. SOC. 2008, *130*, 13822–13823

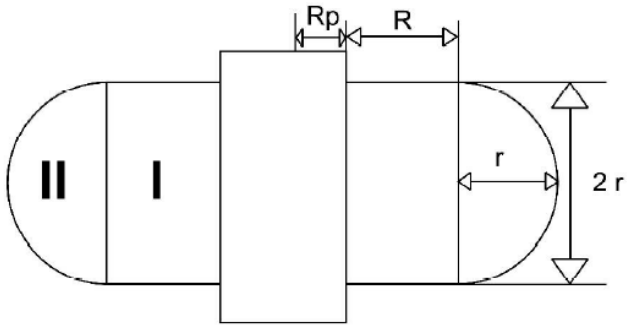


Figure S10. Hemistroidal model of protein-loaded bicelles[7]. The widths of protein, bilayer, and rim are marked as Rp, R, and r, respectively.

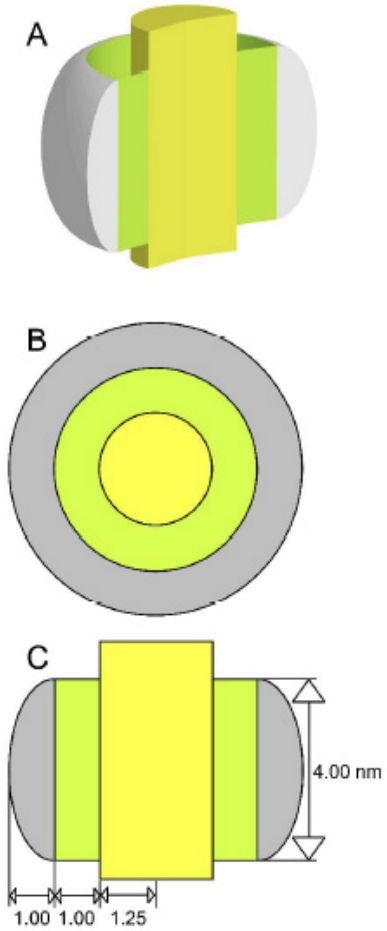


Figure S11. Model of OmpX-loaded bicelles with a molar ratio between DMPC and DHPC of 0.5. (A) 3D and 2D models viewed from (B) top and (C) side are shown. Protein, bilayer, and rim are color-coded in yellow, green, and grey, respectively. The widths of protein, bilayer, and rim are given in nm.

Bicelles in Structural Biology

Much more native membrane-like than micelles. Limited membrane protein-detergent contact.

No compartmentalization, so good for biochemical/titration studies (but not for transport or channel functional studies).

Many membrane proteins (including GPCRs) have been crystallized in relatively large (usually CHAPSO-DMPC) high-q bicelles. (Work pioneered by Faham and Bowie at UCLA)

Small (q = 0.33-0.5) bicelles tumble rapidly and isotropically and have been used for many solution NMR studies of membrane proteins. q is the lipid-to-detergent mole ratio

Larger (q = 2-5) bicelles don't tumble rapidly and can be magnetically aligned for EPR or solid state NMR studies. (These are probably Swiss cheese-like strucutres)

Larger bicelles sometimes scatter light.

Diluting bicelles (lowering the total detergent+lipid concentration) to near or below CMC for the detergent component leads to all the detergent going monomeric and formation of lipid vesicles. So, bicelles are not very useful in EM of membrane proteins.

While DMPC-based bicelles are the best-characterized, it is possible to form bicelles using almost any type of lipids, including bicelles with significant quantities of cholesterol (unlike detergent micelles).

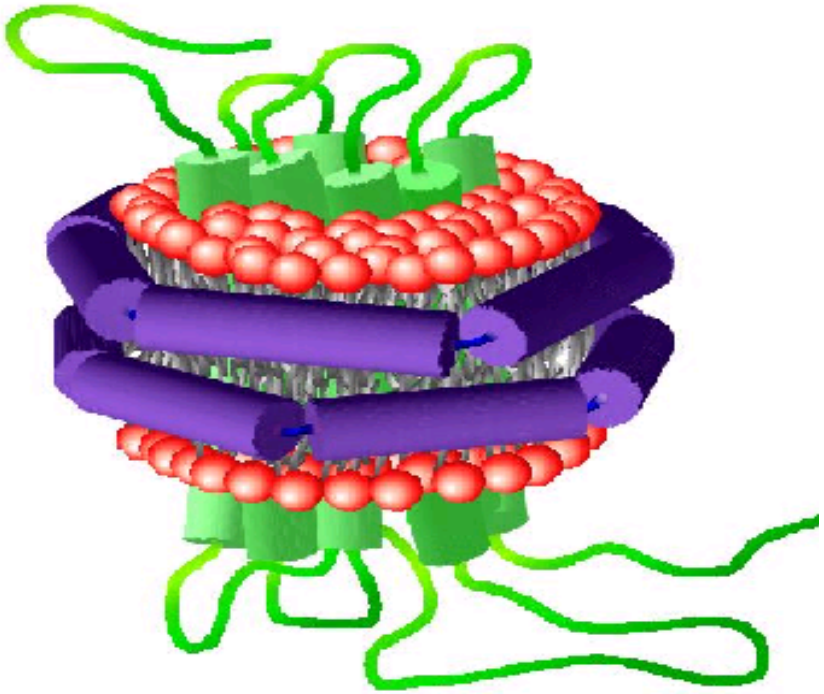
As for lysophospholipids, you have to be concerned about hydrolysis of the ester linkages in bicelle lipids and detergents:

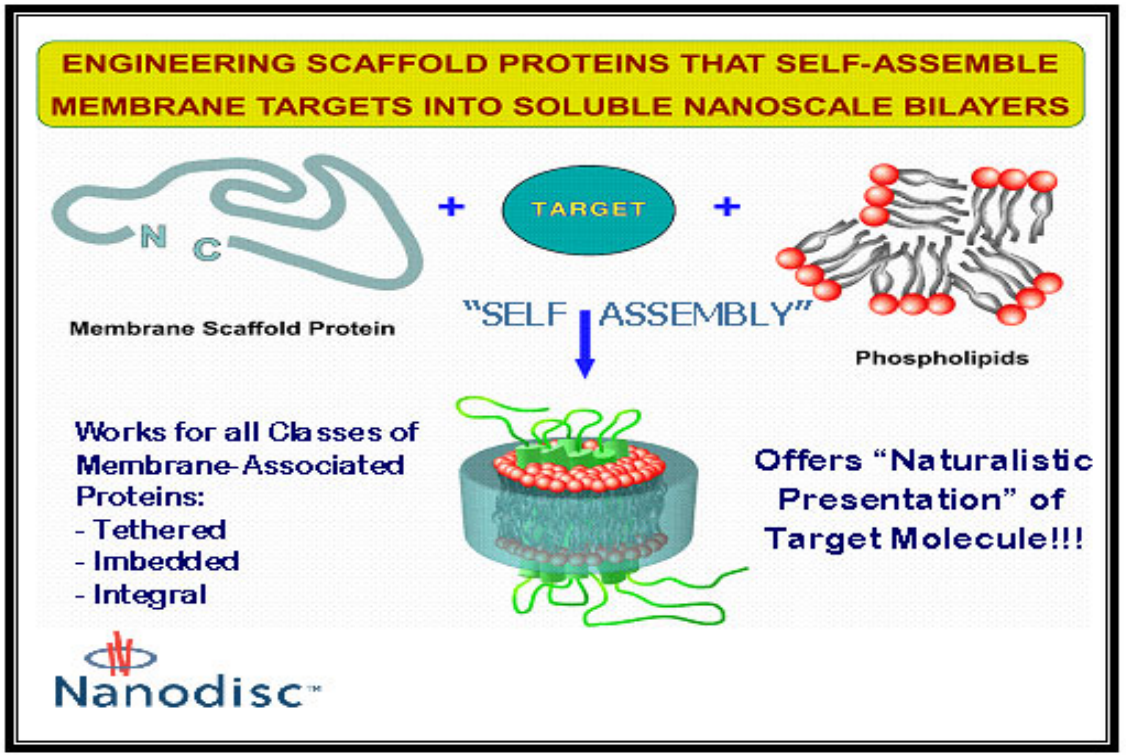
*****Work as close to neutral pH as possible (6.0 – 7.8 should be OK)**

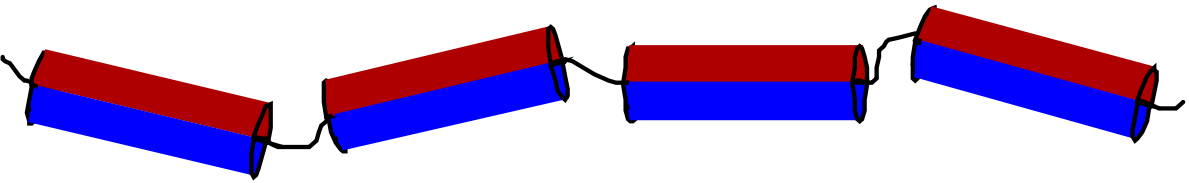
*****Always include a little EDTA (0.5 mM) in bicelle solutions to scavenge any free multivalent metal ions, which can be potent hydrolytic catalysts.**

Nanodiscs

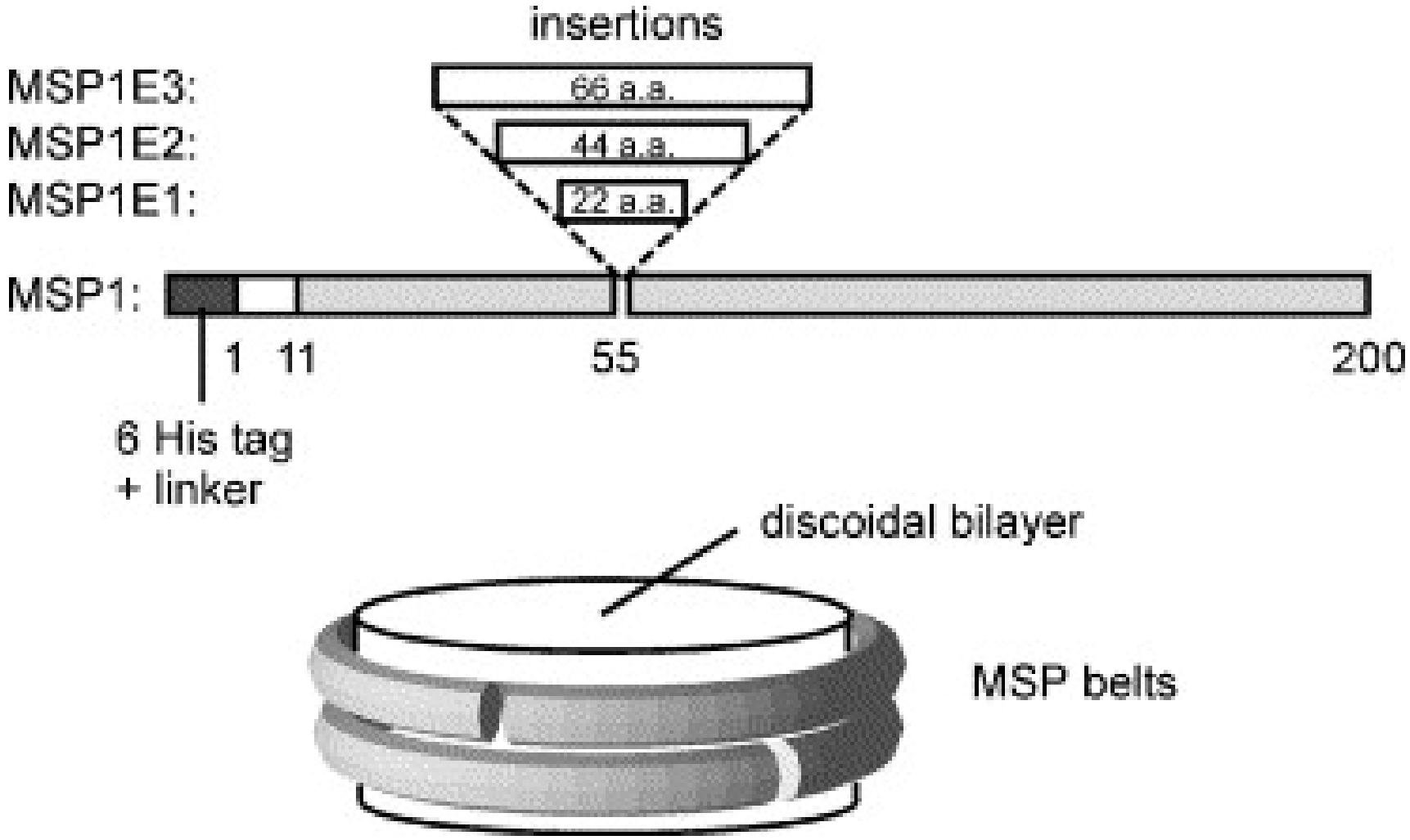
Originally Developed by Steve Sligar, U. of Illinois. There now many variations.







Key ingredient of nanodiscs: Membrane-Scaffolding Protein (MSP): ca. 200 residue apolipoproteins that are comprised of series of linked amphipathic helices. Expressed in *E. coli*. Expression vectors now commercially available.



Unlike bicelles and micelles, nanodiscs persist even at very high dilution. A great property for EM.

Applications of phospholipid bilayer nanodiscs in the study of membranes and membrane proteins.
Nath A, Atkins WM, Sligar SG. Biochemistry. 2007;46:2059-69. (And see other reviews on this topic by Steve Sligar)

Journal of the American Chemical Society

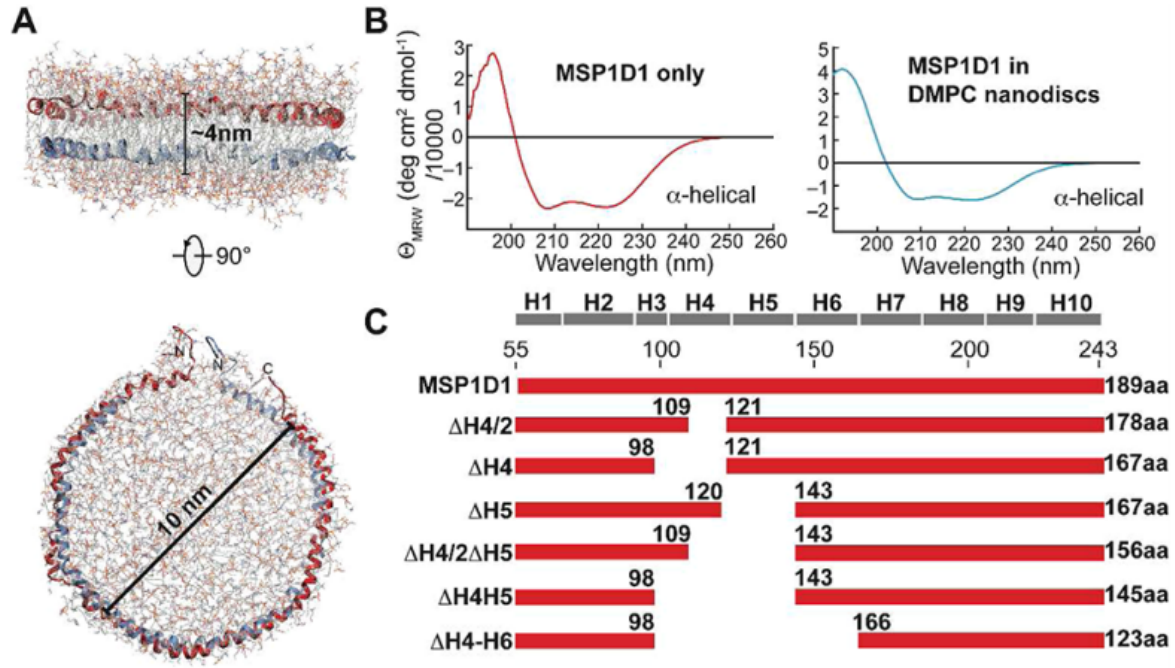


Figure 1. Construction of truncated membrane scaffold protein (MSP) variants. (A) Proposed architecture of a phospholipid nanodisc where two copies of MSPs wrap around a patch of phospholipid bilayer, thereby stabilizing its hydrophobic edge. The most commonly used nanodisc has a diameter of 10 nm. Coordinates of the MSP were taken from ref 4. (B) Far-UV CD spectra of MSP alone (left) or in an assembled nanodisc (right) show that MSP adopts α-helical secondary structure in both cases. (C) Deletion constructs of MSP1D1[5] used in this study. The predicted secondary structure of MSP1D1 is shown on top, and the length of each construct is indicated.

J Am Chem Soc. 2013 Feb 6;135(5):1919-25.
Optimized phospholipid bilayer nanodiscs facilitate high-resolution structure determination of membrane proteins.
Hagn F[1], Etzkorn M, Raschle T, Wagner G.

Smaller bicelles can be formed using shortened membrane scaffolding proteins. Work of lab of Gerhard Wagner.

Advantages and Uses of Nanodiscs in Structural Biology

No detergent at all except for the MSP (an advantage for detergent-sensitive proteins).

Bilayer domain is relatively native membrane-like– they even undergo phase transitions

Well-characterized.

Assemblies remain intact even under conditions of extreme dilution.

Powerful tool for use in single particle cryo-EM of membrane proteins

Useful in biochemical studies, such as those involving ligand titrations

Light scattering not usually a problem (so can use for optical spectroscopy)

Some effort has been made to use them for solution NMR of membrane proteins– recent GPCR data with circularized nanodiscs seems especially promising.

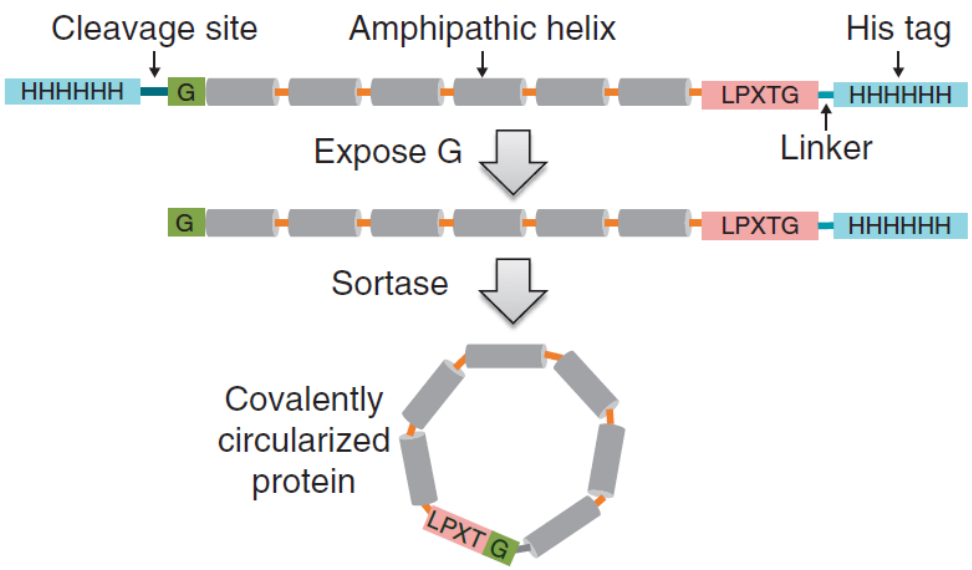
Not used successfully in membrane protein crystallization.

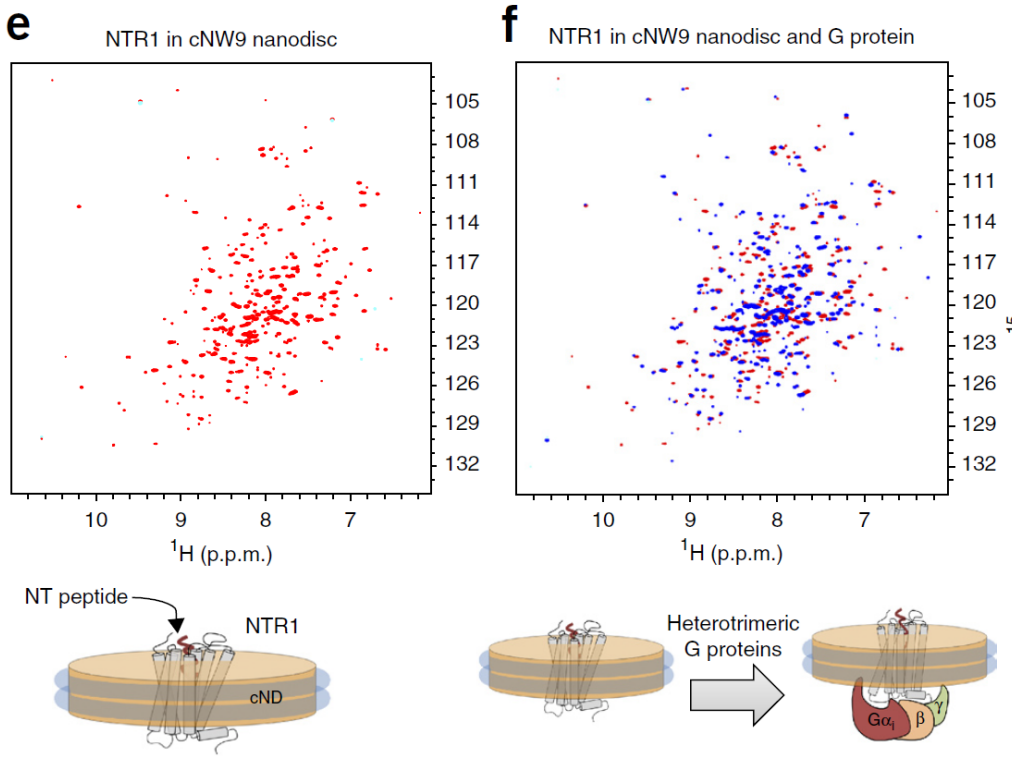
A Major Advance in Nanodiscs: Development of *Circularized* Membrane Scaffolding Proteins

Covalently circularized **nanodiscs** for studying membrane proteins and viral entry.

1. Nasr ML, Baptista D, Strauss M, Sun ZJ, Grigoriu S, Huser S, Plückthun A, Hagn F, Walz T, Hogle JM, **Wagner G**.
Nat Methods. 2017 Jan;14(1):49-52. doi: 10.1038/nmeth.4079.

See also SI section of this paper





Seems to be a huge advance, both for cryo-EM and solution NMR. (Above right: NMR spectra of a GPCR in the new medium, both with and without added G-protein).

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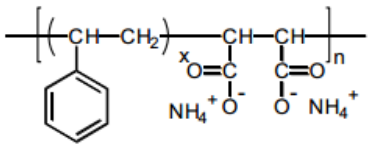
www.CrayValley.com

SMA Polymers and "Lipodisqs"

Technical Data Sheet: SMA® 3000 H Solution

STYRENE MALEIC ANHYDRIDE COPOLYMER SOLUTION



DESCRIPTION
SMA® 3000 H is an aqueous solution of the ammonia salt of SMA 3000.

PRODUCT HIGHLIGHTS
SMA® Resin with the highest styrene content that retains reasonable water solubility

PERFORMANCE PROPERTIES
Water resistance
Viscosity modifier
Adhesion

SUGGESTED APPLICATIONS
Paper sizing
Water-based inks
Polymer modification

SMA® 3000 H Solution
TYPICAL PHYSICAL AND CHEMICAL PROPERTIES

Appearance	Clear liquid
Solids, wt.%	15
Color, Gardner scale	1
Resin Molecular Weight, M_n	3800
Resin Molecular Weight, M_W	9500

Characterizing the structure of **lipodisq** nanoparticles for membrane protein spectroscopic studies.

Zhang R, Sahu ID, Liu L, Osatuke A, Comer RG, Dabney-Smith C, Lorigan GA.
Biochim Biophys Acta. 2015 Jan;1848(1 Pt B):329-33. doi: 10.1016/j.bbamem.2014.05.008.

The styrene-maleic acid copolymer: a versatile tool in membrane research.

Dörr JM, Scheidelaar S, Koorengevel MC, Dominguez JJ, Schäfer M, van Walree CA, Killian JA.
Eur Biophys J. 2016 Jan;45(1):3-21. doi: 10.1007/s00249-015-1093-y. **Review**.

A method for detergent-free isolation of membrane proteins in their local lipid environment. Lee SC, Knowles TJ, Postis VL, Jamshad M, Parslow RA, Lin YP, Goldman A, Sridhar P, Overduin M, Muench SP, Dafforn TR. Nat Protoc. 2016 Jul;11(7):1149-62. doi: 10.1038/nprot.2016.070.

Vol. 30, No. 1, 79–93, http://dx.doi.org/10.1080/02648725.2014.921502

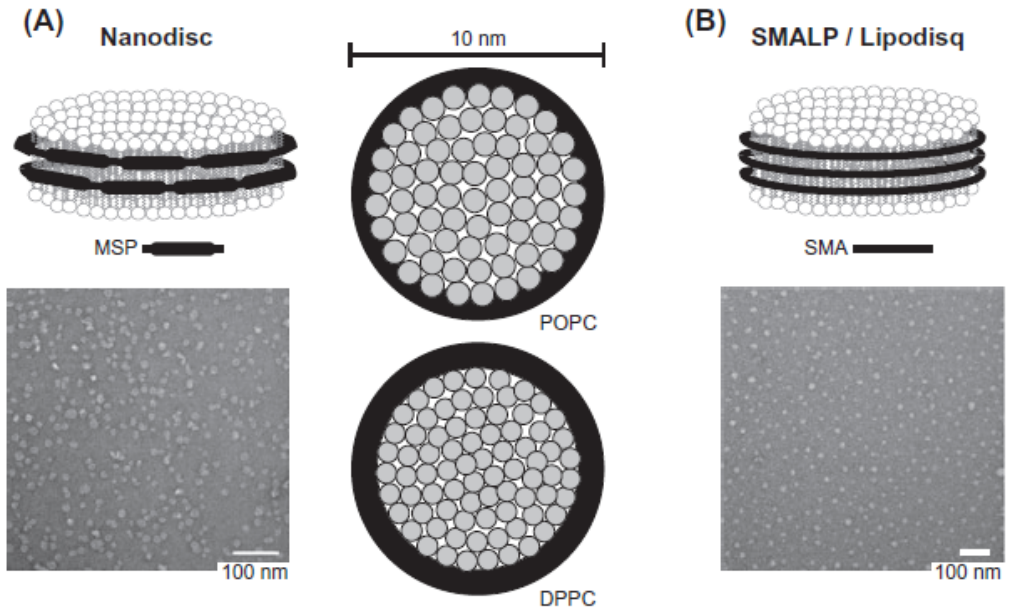


Figure 2. Nanoscale lipid bilayers. (A) Nanodiscs (*left, upper panel*) are lipid discoids bound by two copies of the MSP polypeptide, each one containing amphipathic α-helices (11 or 22 residues in length) that are separated by proline and glycine residues. Commonly used MSP variants include MSP1 (with 10 amphipathic helices, Ø – 9.8 nm) and MSP1E1 (Ø – 10.6 nm), MSP1E2 (Ø – 11.9 nm), and MSP1E3 (Ø – 12.9 nm), containing one, two, and three 22-mer helical inserts in the center of the MSP1 unit, respectively (Denisov et al., 2004; Schuler et al., 2013). *Left, lower panel,* electron micrograph of MSP1E3-bound nanodiscs at a magnification of 180,000 x (Schwall et al., 2012). *Right panels,* top-down schematics showing that the number of lipids per nanodisc depends on the MSP length and the cross-sectional area occupied by each lipid. MSP1 nanodiscs are approximately 10 nm in diameter, producing a lipid bilayer disc with a diameter of 8 nm (bilayer area = 50.2 nm^2). In the liquid crystalline phase, the disaturated lipid 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC, 16:0/16:0 PC) has an area of 0.54 nm^2 and the monounsaturated lipid 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC, 16:0/18:1 PC) has an area of 0.70 nm^2. In agreement with experimental measurements, MSP1 discs contain approximately 78 DMPC lipids and 68 POPC lipids per leaflet. The bilayer thickness of nanodiscs containing DPPC or POPC, measured by SAXS, is ~5.6 nm and ~4.6 nm, respectively, and (B) SMALPs/Lipodisqs® (*upper panel*) are lipid discoids bound by the styrene maleic acid copolymer containing styrene and maleic acid groups in molar ratios of 3:1 or 2:1. *Lower panel*, electron micrograph of 3:1 SMA-bound discs at a magnification of 120,000× (Long et al., 2013).

SMA Polymers and “Lipodisqs”

You can think of these as low budget nanodiscs.

A unique trait is that they seem to be able to dissolve biological membranes and their associated proteins directly into discoidal fragments.

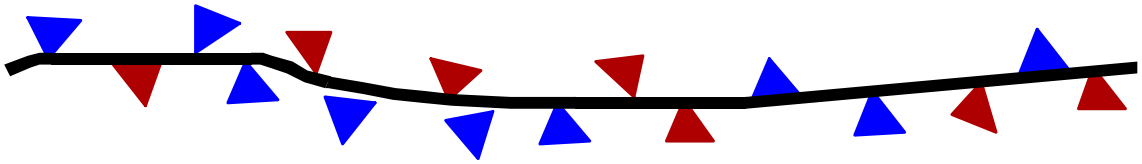
Like nanodiscs they can be diluted to the extreme without changing their morphology.

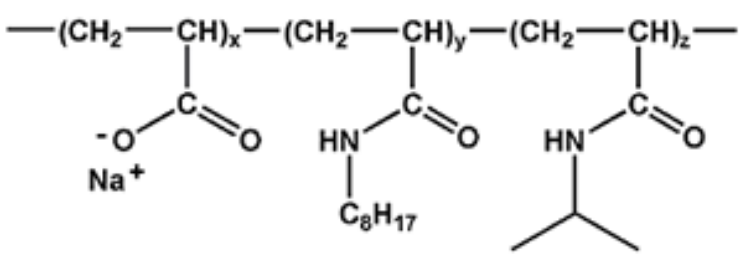
Disadvantages:

- **limited to neutral/basic pH**
- **SMAs have heterogeneous lengths (leading to heterogeneity of lipodisq size**
- **not yet as well characterized as nanodiscs**

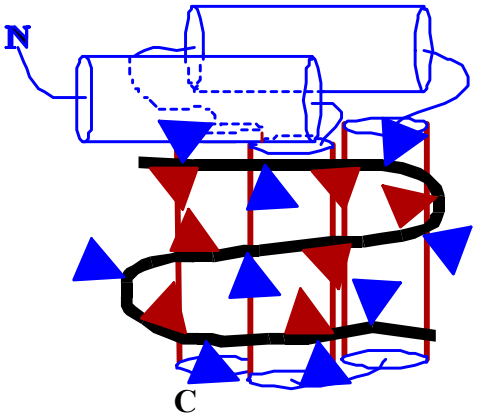
“Amphipols”: Amphipathic Polymers

First developed by Jean-Luc Popot and Co-Workers at CNRS





A8-35



Soluble Membane Protein Complex With Amphipol

Randomly derivatized amphipol (above). In this case there two types of side chains and the polar:apolar side chain ratio is near 1:1.

Popot JL. Annu Rev Biochem. 2010;79:737-75. doi: 10.1146/annurev.biochem.052208.114057.

Amphipols differ from SMA polymers in that they are NOT good at solubilizing lipids or membranes. Unlike micelles, amphipol/MP assemblies are stable even at a very high dilution.

Limited to neutral/basic pH. Heterogeneous in both length and sequence.

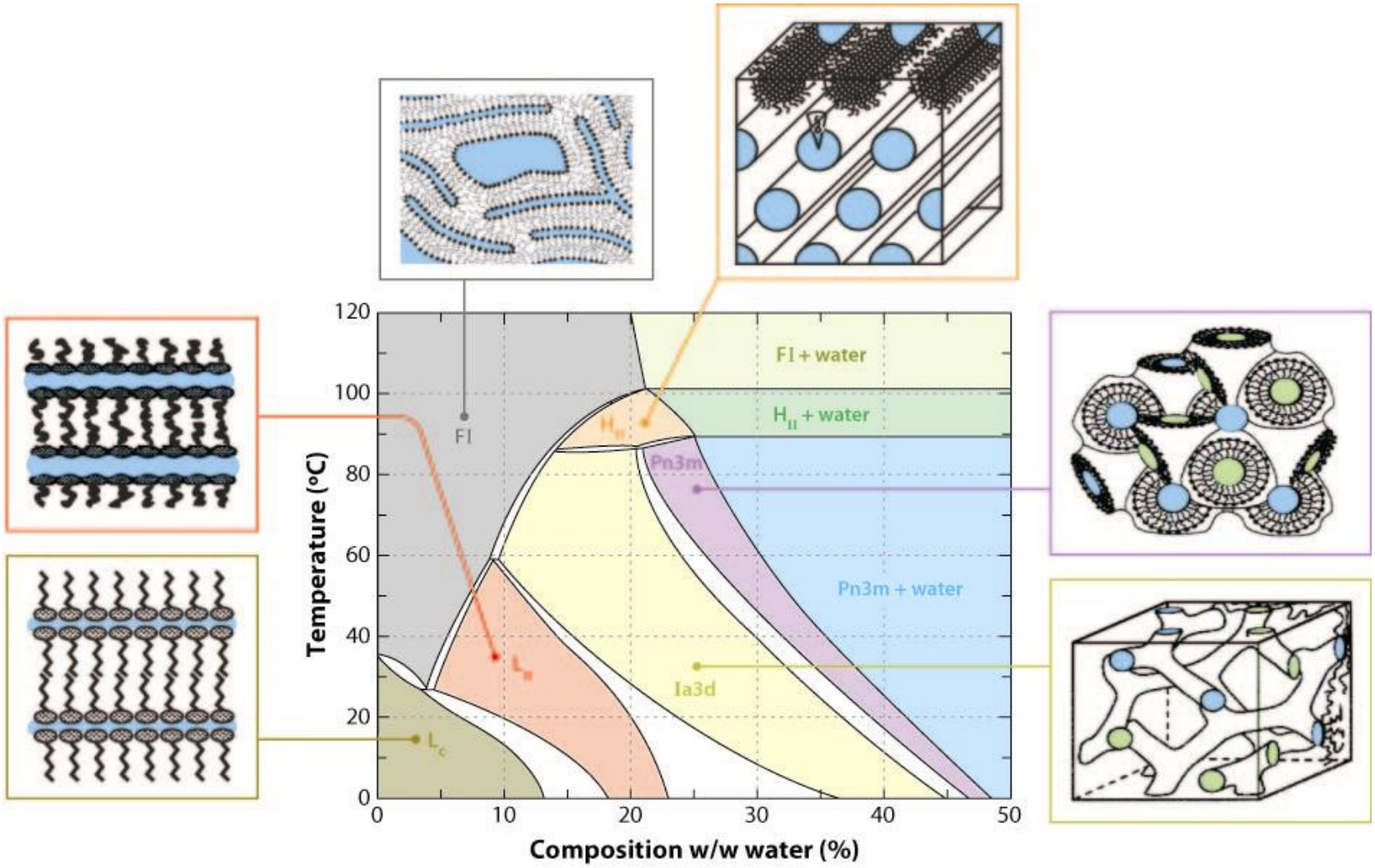
Complexes with membrane proteins can be diluted to the extreme without a problem.

Very well characterized over the years. Used in some recent high resolution cryo-EM studies.

Limited use in NMR. Not used successfully for crystallization.

Zwitterionic amphipols are also commercially available and are soluble even at acidic pH (the PMAL-C8 to -C16 series. But they are not yet as well characterized as the classic Popot A8-38.

Exotic Membrane Phases

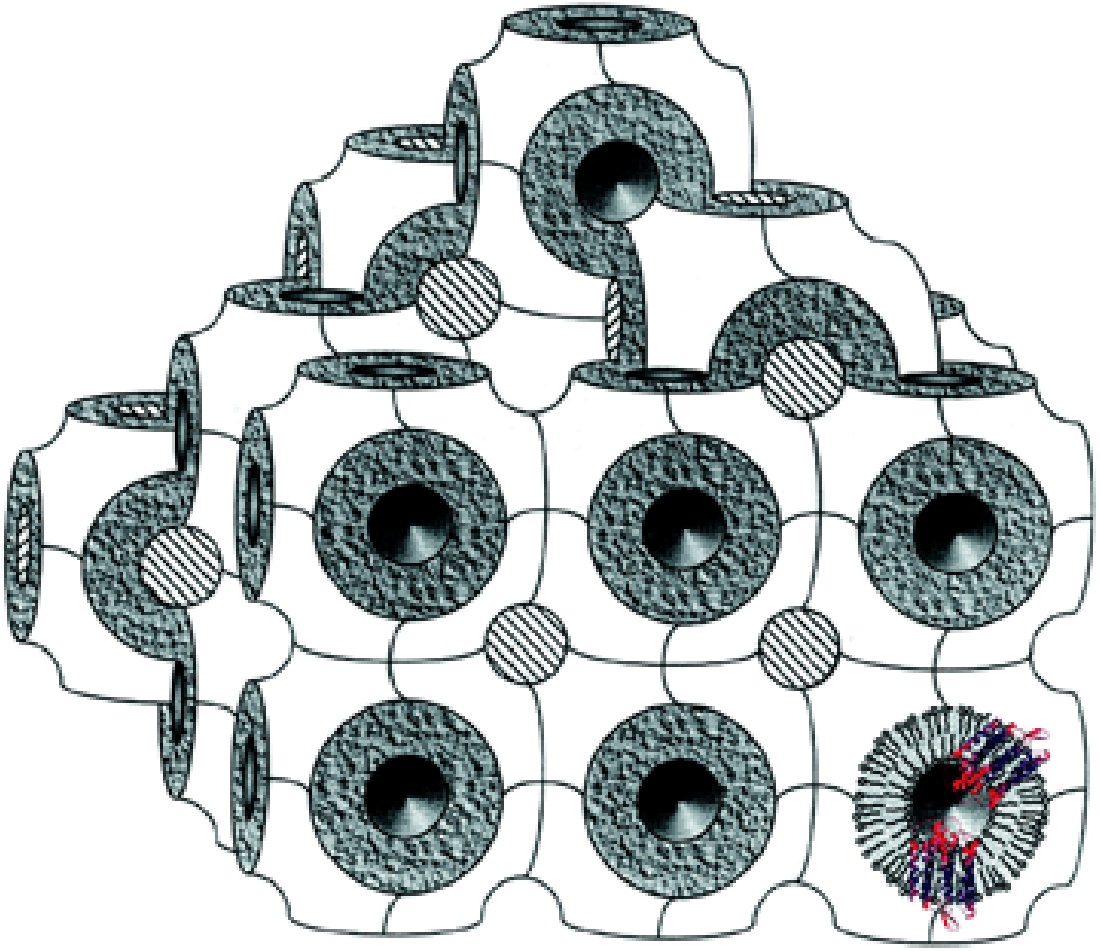


Caffrey M. 2009.
Annu. Rev. Biophys. 38:29–51

Annual Reviews

Lipidic Cubic Phase

(used as a crystallization medium)



Over the past decade the lipidic cubic phase has emerged as a major medium for crystallizing integral membrane proteins, including numerous G protein-coupled receptors and their complexes. This work builds on the pioneering contributions of Ehad Landau, Martin Caffrey, and Vadim Cherezov. The molecules used to make the lipidic cubic phase are typically not native lipids, but are lipid-like. (Although cholesterol is often present!)

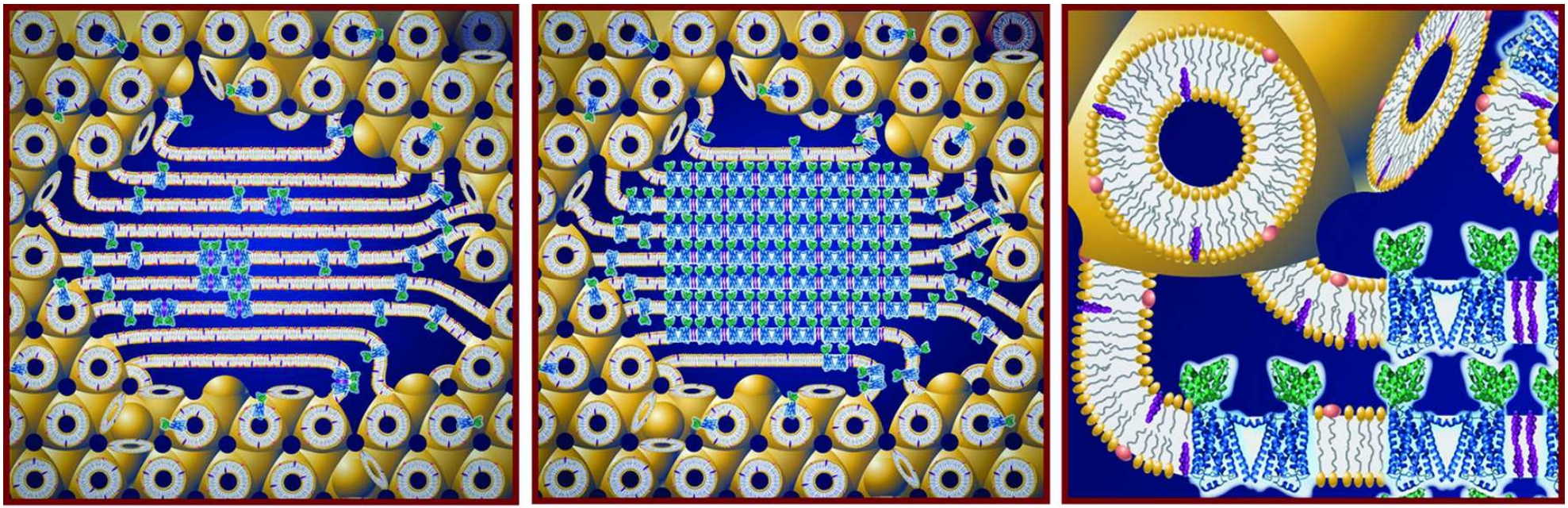
While "exotic", the LCP approach should now be regarded as a standard approach for membrane protein crystallization. (See work of Brian Kobilka and Ray Stevens, for example.)

Nat Rev Mol Cell Biol. 2015 Feb;16(2):69-81. doi: 10.1038/nrm3933. Epub 2015 Jan 15.

Methodological advances: the unsung heroes of the GPCR structural revolution.

Ghosh E[1], Kumari P[1], Jaiman D[1], Shukla AK[1].

Representation of the events proposed to take place during the crystallization of an integral membrane protein from the lipid cubic mesophase.



(*a*) (*b*) (*c*)

Acta Cryst F STRUCTURAL BIOLOGY COMMUNICATIONS

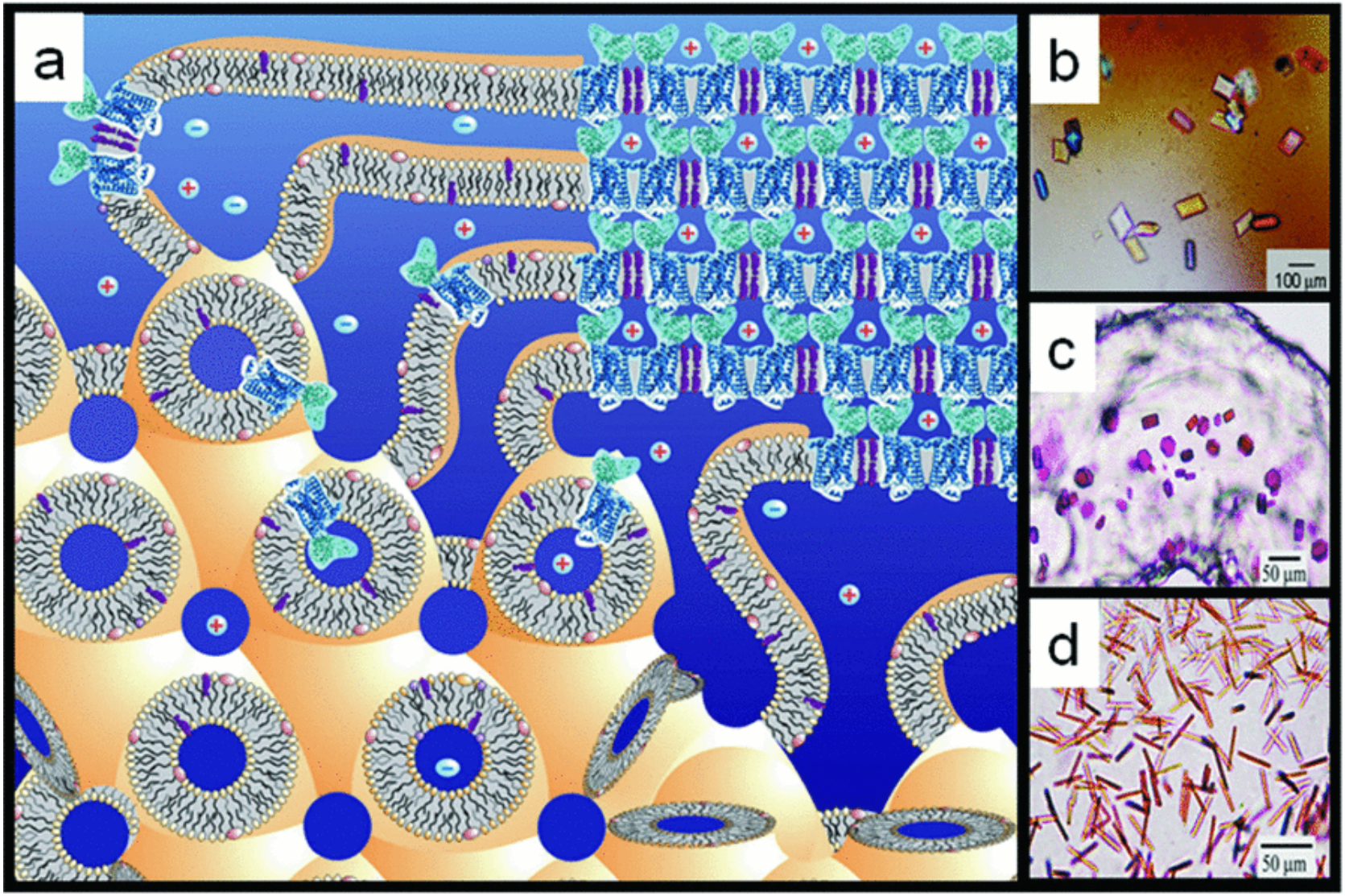
Caffrey

Volume 71 | **Part 1** | January 2015 | Pages 3–18 | 10.1107/S2053230X14026843

Curr Opin Struct Biol. 2011 Aug;21(4):559-66. doi: 10.1016/j.sbi.2011.06.007. Epub 2011 Jul 19.

Lipidic cubic phase technologies for membrane protein structural studies.

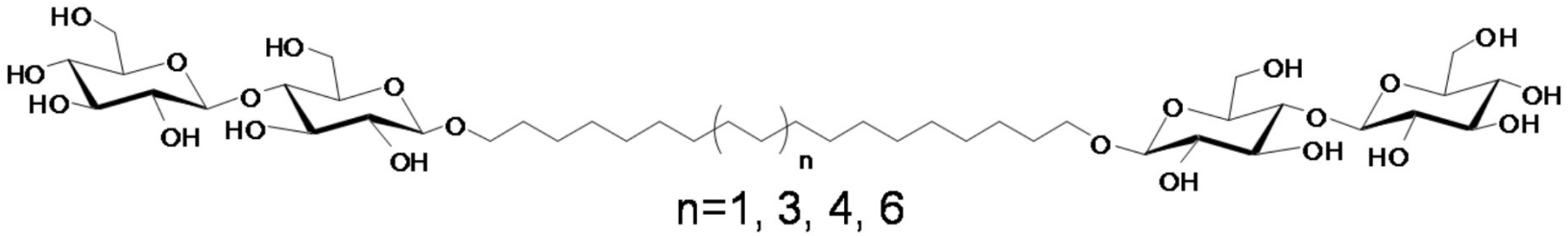
Cherezov V[1].



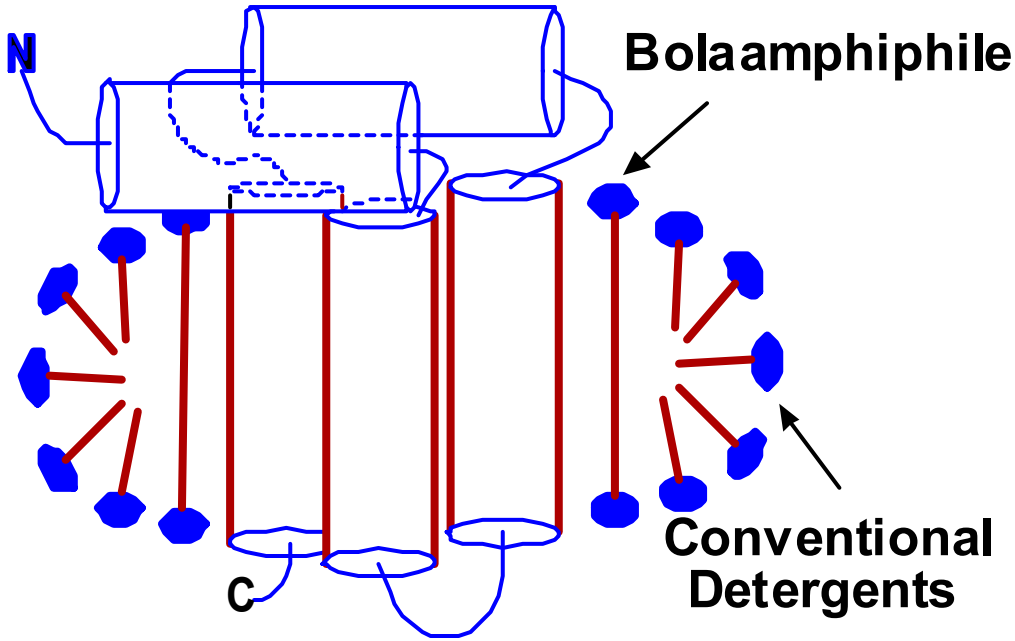
Biochemical Society Transactions (2011) 39, 725-732 - Martin Caffrey

www.biochemsoctrans.org

Bolaamphiphile



n=1, 3, 4, 6

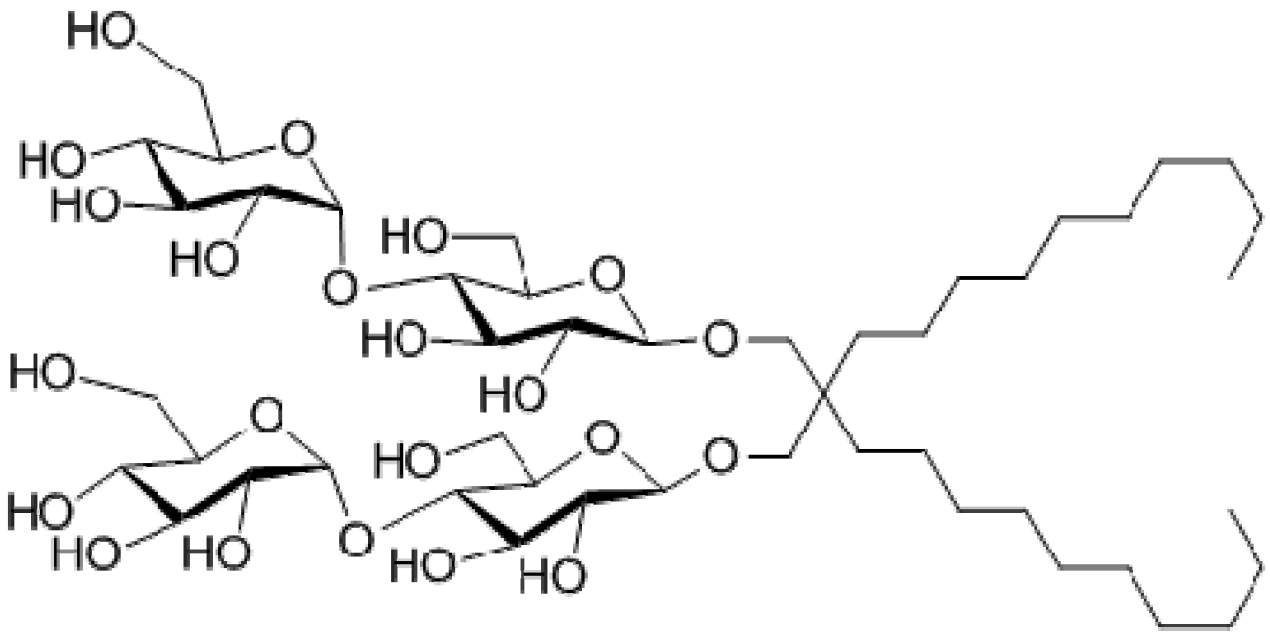


Biochemistry. 2009 Dec 15;48(49):11606-8. doi: 10.1021/bi9018708.

Bolaamphiphile-class surfactants can stabilize and support the function of solubilized integral membrane proteins.

Li Q[1], Mittal R, Huang L, Travis B, Sanders CR.

Laryl maltoside neopentyl glycol



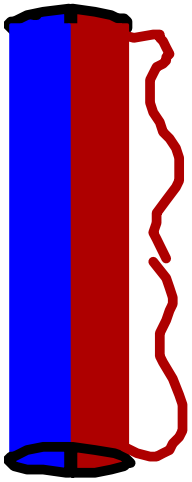
Membrane proteins such as GPCRs seem to be very stable in detergents of this series..

Maltose-**neopentyl** glycol (MNG) amphiphiles for solubilization, stabilization and crystallization of membrane proteins.

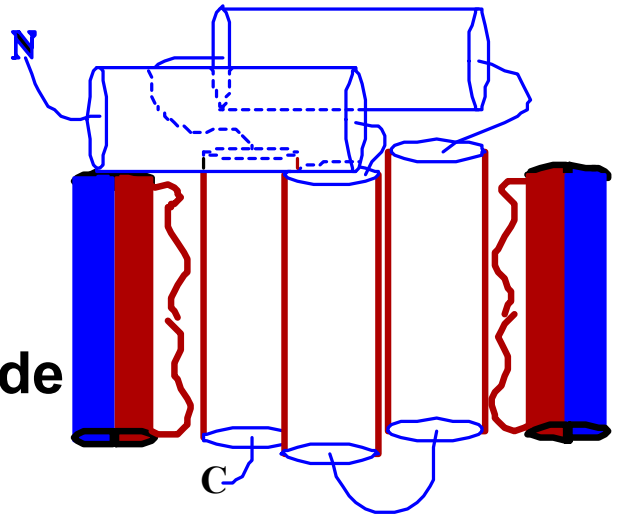
Chae PS, Rasmussen SG, Rana RR, Gotfryd K, Chandra R, Goren MA, Kruse AC, Nurva S, Loland CJ, Pierre Y, Drew D, Popot JL, Picot D, Fox BG, Guan L, Gether U, Byrne B, **Kobilka** B, Gellman SH.
Nat Methods. 2010 Dec;7(12):1003-8. doi: 10.1038/nmeth.1526.

Lipopeptides as Model Membranes

Developed by Gil Prive. U of Toronto



Amphipathic lipopeptide (left) and cross-section slice of complex between lipopeptide and IMP (right)



Curr Opin Struct Biol. 2009 Aug;19(4):379-85. doi: 10.1016/j.sbi.2009.07.008. Epub 2009 Aug 12.

Lipopeptide detergents for membrane protein studies.

Privé GG[1].

Other options: Fully or partially chain-fluorinated detergents and lipids

A fluorinated detergent for membrane-protein applications.
Frotscher E, Danielczak B, Vargas C, Meister A, Durand G, Keller S.
Angew Chem Int Ed Engl. 2015 Apr 20;54(17):5069-73. doi: 10.1002/anie.201412359.

Amphipols and fluorinated surfactants: Two alternatives to detergents for studying membrane proteins in vitro.
Breyton C, Pucci B, Popot JL.
Methods Mol Biol. 2010;601:219-45. doi: 10.1007/978-1-60761-344-2_14.

Fluorocarbons and fluorinated amphiphiles in drug delivery and biomedical research.
Krafft MP.
Adv Drug Deliv Rev. 2001 Apr 25;47(2-3):209-28. Review.

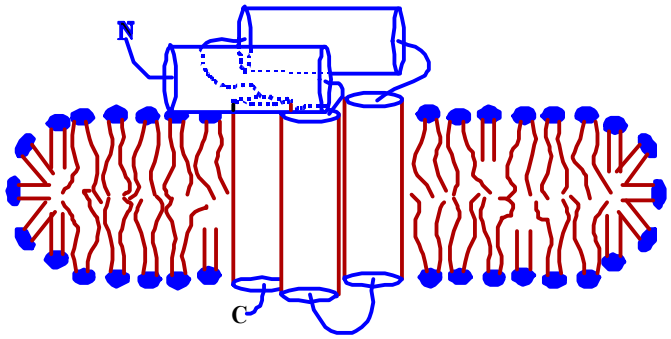
Note: fluorocarbons and water are immiscible, as are fluorocarbons and hydrocarbons. Nevertheless, membrane proteins can sometimes be solubilized in fluorocarbon-based micelles. Note that oxygen is freely soluble to fluorocarbon phases and so potential oxidative damage to proteins is something one may want to worry about.

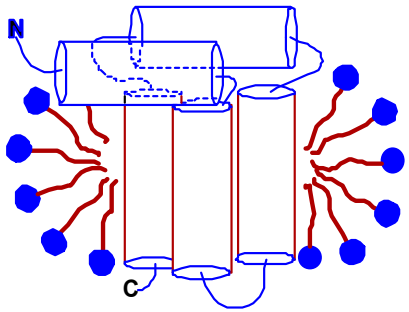
And detergent-like natural products

Biosurfactants and surfactants interacting with membranes and proteins: Same but different?
Otzen DE.
Biochim Biophys Acta. 2017 Apr;1859(4):639-649. doi: 10.1016/j.bbamem.2016.09.024.

And binary mixtures of detergents

Tuning micelle dimensions and properties with binary surfactant mixtures.
Oliver RC, Lipfert J, Fox DA, Lo RH, Kim JJ, Doniach S, Columbus L.
Langmuir. 2014 Nov 11;30(44):13353-61. doi: 10.1021/la503458n.


N
C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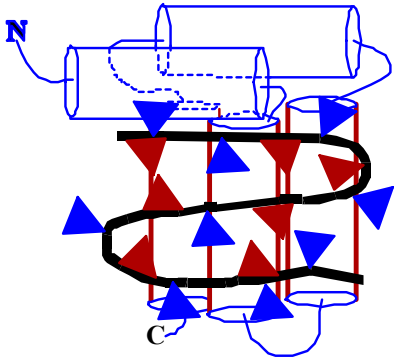
N
C

BIOCHEMISTRY
including biophysical chemistry & molecular biology

Biochemistry(2011) 50:7858-7867.

Current Topic

pubs.acs.org/biochemistry

Tolerance to Changes in Membrane Lipid Composition as a Selected Trait of Membrane Proteins

Charles R. Sanders* and Kathleen F. Mittendorf

Department of Biochemistry and Center for Structural Biology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-8725, United States

ABSTRACT: Membrane lipid composition can vary dramatically across the three domains of life and even within single organisms. Here we review evidence that the lipid-exposed surfaces of membrane proteins have generally evolved to maintain correct structure and function in the face of major changes in lipid composition. Such tolerance has allowed evolution to extensively remodel membrane lipid compositions during the emergence of new species without having to extensively remodel the associated membrane proteins. The tolerance of membrane proteins also permits single-cell organisms to vary their membrane lipid composition in response to their changing environments and allows dynamic and organelle-specific variations in the lipid compositions of eukaryotic cells. Membrane protein structural biology has greatly benefited from this seemingly intrinsic property of membrane proteins: the majority of structures determined to date have been characterized under model membrane conditions that little resemble those of native membranes. Nevertheless, with a few notable exceptions, most experimentally determined membrane protein structures appear, to a good approximation, to faithfully report on native structure.