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Technical Bulletin 100

How to Purify a Membrane Protein

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 how to purify a membrane protein is useful in beginning your research work.

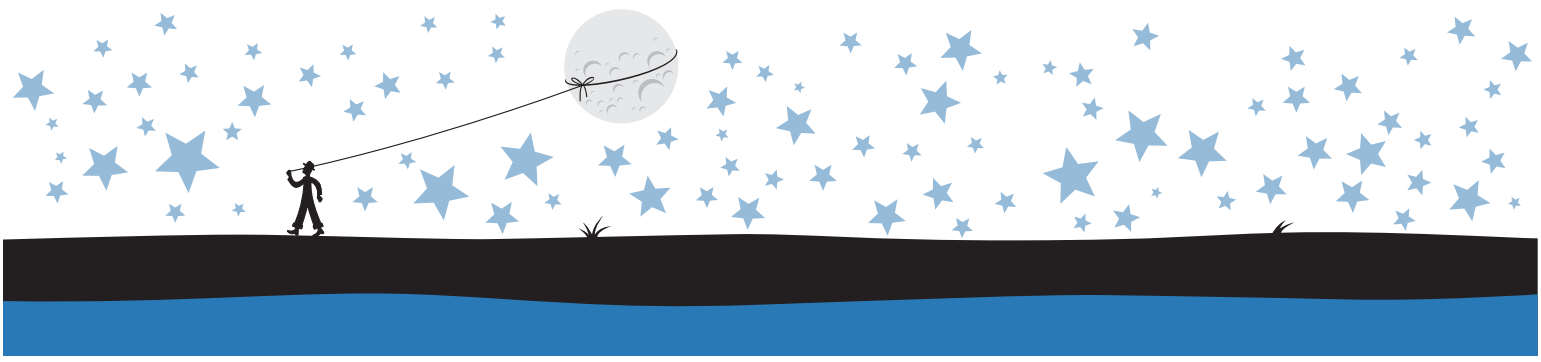
1. Look up other purifications from similar sources and similar membranes. Different tissues, organisms, and membrane types sometimes require different detergents and conditions; e.g., bacterial sources may require distinct solubilization procedures from mammalian sources.

2. Complete dispersion and lipid depletion are critical for successful chromatography. If the protein is not separately solubilized from other proteins, and much of adhering phospholipid removed, the protein will not bind and chromatograph on the basis of its own characteristics. Instead, it will behave heterogeneously on the basis of the other proteins and lipids attached.

3. A detergent that is good for solubilizing intact membranes and removing excess lipid may not be the best for continuing to purify the "naked" protein (may be too harsh). Thus, for the initial steps you may want to choose a relatively cheap, relatively pure detergent that can be readily exchanged for another. For example, Cholate and CHAPS are both strong, charged or zwitterionic, steroid detergents that break up membranes well and help in removal of excess lipid in chromatographic steps such as hydroxyapatite. They also have a high critical micelle which means they can be readily removed by dialysis or exchanged for another on a column.

4. A good dispersing detergent that will also stabilize the protein and is free of contaminants that could inactivate the enzyme, is needed for chromatographic purification. For many, Triton® X-100 has been very suitable, but has the problem of contaminants and 280 nm absorbance. A twelve carbon chain length of hydrocarbon tail is often necessary for good stabilization and dispersion, especially for larger multi-subunit enzymes. Anatrace® Dodecyl-β-D-Maltoside often works equally well or better than Triton, with none of the disadvantages. Shorter chain detergents and some charged detergents tend to dissociate multi-subunit proteins. Trial and error is clearly important at this stage with careful attention to maintaining a high enough detergent-to-protein ratio to make sure the protein is well dispersed (the detergent concentration will be much higher than the CMC).

5. Even if a protein is not active in a particular detergent, if you can show that the inactivity is reversible, by adding back lipid or another detergent, you may still be able to use it.



APX100 **Anapoe®-X-100**

[Triton X-100 / α–[4-(1,1,3,3-Tetramethyl- butyl) phenyl]–ω–Hydroxy-Poly(Oxy-1,2- Ethanediyl)]

Chemical Properties:

FW avg.: 647.0 [9002-93-1]
t-Oct-C_6H_4-$(OCH_2CH_2)_x$OH, x = 9-10
CMC (H_2O): ~ 0.23 mM[1-4] (0.015%) (w/v)
Aggregation number (H_2O): ~ 75-165[5]

Product Specifications:

Low-Oxidant
Purified industrial detergent.
Peroxide: < 20 µM
Supplied in a 10% (w/v) solution under argon gas.

C316 **CHAPS, Anagrade®**

[3-[(3-Cholamidopropyl)-Dimethylammonio]-1-Propane Sulfonate] • N,N-Dimethyl-3-Sulfo-N-[3-[[3α,5β,7α,12α)-3,7,12-Trihydroxy-24-Oxo-cholan-24-yl] Amino]propyl]-1-Propanaminium Hydroxide, Inner Salt]

Chemical Properties:

FW: 614.9 [75621-03-3] $C_{32}H_{58}N_2O_7S$
CMC (H_2O): ~ 8 mM[6] (0.49%)
Aggregation number (H_2O): ~ 10[7]
dn/dc (H_2O): 0.1323 ml/gm[8]

Product Specifications:

Purity: ≥ 99% by HPLC analysis
pH (1% solution in water): 5-8
Solubility in water at 20°C: ≥ 0.5 M
Conductance (0.5 M solution in water): < 50 µS
Percent fluorescence due to a 0.1% solution in water at 345 nm: < 10
Absorbance of a 1% solution in water:
340 nm: < 0.02
280 nm: < 0.04
260 nm: < 0.06

D310 **n-Dodecyl–β–D-Maltopyranoside, Anagrade**

[n-Dodecyl–β–D-Maltoside / Lauryl Maltoside / Dodecyl 4-O–α–D-Glucopyranosyl–β–D-Glucopyranoside / DDM / LM]

Chemical Properties:

FW: 510.6 [69227-93-6] $C_{24}H_{46}O_{11}$
CMC (H_2O): ~ 0.17 mM[10] (0.0087%)
CMC (0.2 M NaCl): ~ 0.12 mM[11]
Aggregation number (H_2O): ~ 78-149[10,11]
dn/dc: 0.1435 ml/gm[8]
Micelle size: 72 kDa[12]

Product Specifications:

Purity (β + α): ≥ 99% by HPLC analysis
For molar volume check reference 13.
Percent anomer: < 2 α (HPLC)
Percent dodecanol: < 0.005 (HPLC)
pH (1% solution in water): 5-8
Solubility in water at 0-5°C: ≥ 20%
Conductance (10% solution in water): < 40 µS
Percent fluorescence due to a 0.1% solution in water at 345 nm: < 10
Absorbance of a 1% solution in water:
340 nm: < 0.02
280 nm: < 0.04
260 nm: < 0.06
225 nm: < 0.1

References:

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