Metadata of the chapter that will be visualized online

Field	Value
Series Title	
Chapter Title	Strategies for the Purification of Membrane Proteins
Chapter SubTitle	
Copyright Year	2011
Copyright Holder	Springer Science + Business Media, LLC

Corresponding Author		
	Family Name	Smith
	Particle	
	Given Name	**Sinead Marian**
	Suffix	
	Division	
	Organization	Institute of Molecular Medicine and Immunology Research Centre, Trinity College Dublin
	Address	Dublin, Ireland
	Email	

Abstract

Although membrane proteins account for 20–30% of the coding regions of all sequenced genomes and play crucial roles in many fundamental cell processes, there are relatively few membranes with known 3D structure. This is likely due to technical challenges associated with membrane protein extraction, solubilisation, and purification. Membrane proteins are classified based on the level of interaction with membrane lipid bilayers, with peripheral membrane proteins associating non-covalently with the membrane, and integral membrane proteins associating more strongly by means of hydrophobic interactions. Generally speaking, peripheral membrane proteins can be purified by milder techniques than integral membrane proteins, whose extraction requires phospholipid bilayer disruption by detergents. Here, important criteria for strategies of membrane protein purification are addressed, with a focus on the initial stages of membrane protein solublilisation, where problems are most frequently encountered. Protocols are outlined for the successful extraction of peripheral membrane proteins, solubilisation of integral membrane proteins, and detergent removal which is important not only for retaining native protein stability and biological functions, but also for the efficiency of later purification techniques.

Key words (separated by '-'): Peripheral membrane protein - Integral membrane protein - Detergent - Protein purification - Protein solubilisation

Author's Proof

Chapter 29 1

Strategies for the Purification of Membrane Proteins 2

Sinead Marian Smith 3

Abstract 4

Although membrane proteins account for 20–30% of the coding regions of all sequenced genomes and 5
play crucial roles in many fundamental cell processes, there are relatively few membranes with known 3D 6
structure. This is likely due to technical challenges associated with membrane protein extraction, solubili- 7
sation, and purification. Membrane proteins are classified based on the level of interaction with membrane 8
lipid bilayers, with peripheral membrane proteins associating non-covalently with the membrane, and 9
integral membrane proteins associating more strongly by means of hydrophobic interactions. Generally 10
speaking, peripheral membrane proteins can be purified by milder techniques than integral membrane 11
proteins, whose extraction requires phospholipid bilayer disruption by detergents. Here, important criteria 12
for strategies of membrane protein purification are addressed, with a focus on the initial stages of membrane 13
protein solublilisation, where problems are most frequently encountered. Protocols are outlined for the 14
successful extraction of peripheral membrane proteins, solubilisation of integral membrane proteins, and 15
detergent removal which is important not only for retaining native protein stability and biological func- 16
tions, but also for the efficiency of later purification techniques. 17

Key words: Peripheral membrane protein, Integral membrane protein, Detergent, Protein purifica- 18
tion, Protein solubilisation 19

Uncorrected Proof

1. Introduction 20

Membrane proteins are associated with the membrane of a cell or 21
particular organelle and are generally more problematic to purify 22
than water-soluble proteins. Membrane proteins represent 23
approximately 20–30% of the open-reading frames of an organ- 24
ism's genome (1) and play crucial roles in basic cell functions 25
including signal transduction, energy production, nutrient uptake, 26
and cell–cell communication. Additionally, many drugs and drug 27
candidates target membrane proteins (2, 3). However, less than 28
2% of the listed 3D structures in the protein data bank (4) are 29

Dermot Walls and Sinéad T. Loughran (eds.), *Protein Chromatography: Methods and Protocols*, Methods in Molecular
Biology, vol. 681, DOI 10.1007/978-1-60761-913-0_29, © Springer Science+Business Media, LLC 2011

Author's Proof

Uncorrected Proof

30 membrane proteins, a fact that is likely due to the technical challenges
31 associated with membrane protein solubilisation and purification
32 in sufficient quantities for crystallisation (5).
33 Membrane proteins are classified into peripheral and integral
34 membrane proteins, which are associated to varying degrees with
35 the phospholipid bilayer (6, 7). Peripheral or extrinsic membrane
36 proteins interact with the membrane surface non-covalently by
37 means of electrostatic and hydrogen bonds. Peripheral membrane
38 proteins can be recruited to the membrane during signalling
39 events or are constitutively localised to the membrane. Integral or
40 intrinsic membrane proteins are more strongly associated with
41 the membrane and interact with hydrophobic moieties of the
42 phospholipid bilayer. They contain one or more characteristic
43 runs of apolar amino acids that span the lipid bilayer (6). Integral
44 membrane proteins are further classified into Type I, which are
45 positioned so that their COOH-terminus is embedded in the
46 cytosol or Type II, which are positioned with the NH_2-terminus
47 in the cytosol. Although there is no single protocol for the purifi-
48 cation of membrane proteins, it is the initial stages of membrane
49 protein solublilisation where problems are most frequently
50 encountered. This chapter addresses important criteria for mem-
51 brane protein extraction and solubilisation. The Subheading 3
52 describes protocols for the successful extraction of peripheral
53 membrane proteins, solubilisation of integral membrane proteins,
54 and detergent removal which is important not only for retaining
55 native protein stability and biological functions, but also for the
56 efficiency of later purification techniques.

57 1.1. Considerations 58 for Membrane Protein 59 Purification

57 The analysis of membrane proteins represents a significant techni-
58 cal challenge in the field of proteomics and there are several rea-
59 sons why the purification of membrane proteins is more difficult
60 than that of water-soluble proteins. Firstly, endogenous expres-
61 sion of membrane proteins is quite low and usually quite large
62 quantities of protein are required for structural investigations.
63 Additionally, integral membrane proteins are extremely hydro-
64 phobic and often require high concentrations of detergents for
65 solubilisation. Membrane proteins have the tendency to form
66 aggregates, even in the presence of detergents, resulting in the
67 reduction of efficiency of subsequent separation techniques (8).
68 The choice of detergent may also affect the efficiency of down-
69 stream protein purification procedures. For example, ion-exchange
70 chromatography (see Chapter 12) should not be carried out in
71 the presence of charged detergents, and hydrophobic interaction
72 chromatography (see Chapter 24) can be problematic in the pres-
73 ence of all detergents (8). In such cases detergents can be removed
74 (see Subheading 1.4). Once solubilised, the membrane proteins
75 are often more susceptible to degradation by proteases. Thus,
76 addition of protease inhibitors such as ethylenediamine tetraacetic

Author's Proof

acid (EDTA), which inactivates metalloproteases, or phenylmethyl sulfonyl fluoride (PMSF), which inhibits serine proteases, needs to be considered.

It is also worthwhile considering the availability of efficient functional assays to detect the protein of interest at different stages during the purification process, for example, measurement of enzymatic activity and immuno- or ligand-binding assays. Given the unique properties of individual proteins, it is usually necessary to determine appropriate assays on a case-by-case basis (8). There is no single protocol for obtaining membrane protein purification; more likely a series of methods are needed, depending on the particular needs of the investigator.

1.2. Peripheral Membrane Protein Extraction

Peripheral membrane proteins can be dissociated using relatively mild techniques that break the electrostatic or hydrogen bonds between the peripheral proteins and the membrane, without total membrane disruption. Common dissociating reagents for the extraction of peripheral membrane proteins are listed in Table 1. Extractions using buffers containing high salts are useful as they decrease electrostatic interactions between proteins and charged lipids (6). Chaotropic ions disrupt hydrophobic bonds present in the membrane surface and promote the transfer of hydrophobic groups from non-polar environment to the aqueous phase (6). Usually, extraction procedures employing high ionic strength NaCl and KCl, alkaline or acidic buffers, and metal chelators result in a relatively distinct separation between solubilised peripheral proteins and membrane-associated integral membrane proteins (7). High pH causes the fractionation of peripheral membrane proteins from integral membrane proteins by disrupting sealed membrane structures without denaturing the lipid

Uncorrected Proof

Table 1
Treatments for the extraction of peripheral membrane proteins

Treatment type	Example
Acidic buffers	pH 3.0–5.0
Alkaline buffers	pH 8.0–12.0 (e.g. 100 mM Na_2CO_3, pH 11.3, see Subheading 3.1)
Chaotropic ions	I^-, ClO_4^-, SCN^-
Denaturing agents	8 M urea or 6 M guanidine hydrochloride
Metal chelators	10 mM EDTA or EGTA
Salt solutions/high ionic strength	1 M NaCl or KCl

Author's Proof

bilayer or extracting integral membrane proteins (9). The high pH method for extraction of peripheral membrane proteins is described in Subheading 3.1 of this chapter. It is worthwhile determining the effect of the buffer on any enzymatic activity the protein of interest may have, and potential interactions the buffer may have with any column matrix that will be used at later stages in the purification process. Additionally, buffer cost may need to be considered if large-scale preparations are to be carried out.

Following extraction (i.e. breaking of electrostatic and H bonds between peripheral protein and the membrane) in the chosen buffer for 10–30 min, the remaining membrane bilayer and its associated integral proteins are separated by centrifugation (30–60 min, 100,000 × *g*) and the released peripheral membrane proteins are recovered in the supernatant (7, 10).

1.3. Integral Membrane Protein Extraction

In order to solubilise integral membrane proteins, it is necessary to disrupt the lipid bilayer, which may be achieved with organic solvents, but is more commonly accomplished using detergents. Extraction using the organic solvent N-butanol (see Subheading 3.2) uses a biphasic system for solubilising proteins from membranes into dilute aqueous buffers. The low solubility of N-butanol in water, combined with its lipophilicity, minimally denatures proteins (9). Detergents are amphipathic molecules that contain both hydrophobic and hydrophilic moieties and form micelles in water. A micelle is a cluster of detergent molecules in which the hydrophilic head moieties face outward. Detergents solubilise proteins by binding to the hydrophobic parts of the protein on one side and interacting with the aqueous parts on the other side (8). The detergent of choice should sufficiently solubilise the membrane protein without irreversibly denaturing it. Detergents can be ionic, non-ionic, or zwitterionic. A list of commonly used detergents for extraction of integral membrane proteins is shown in Table 2. Selection of a particular detergent depends on the properties of the protein of interest and the given aims of subsequent experiments involving the purified protein. If there is little information in the literature on the purification of similar proteins, or if one is purifying a particular protein for the first time, it is often necessary to screen a number of detergents in order to optimise protein solubilisation. Membrane aliquots should be incubated with various concentrations of commonly used detergents and incubation time, buffer concentration, salt solutions, and temperature conditions necessary for optimal solubilisation should be determined.

When screening potential detergents, it is important to be aware of the unique critical micelle concentration (CMC), which is the concentration of free detergent at which the transition from disperse detergent molecules to a micellar structure occurs (10). Since solubilisation corresponds to the removal of the protein

Uncorrected Proof

Author's Proof

Table 2
Detergents used for extracting integral membrane proteins

Detergent type	Name	Alternative chemical name	CMC (mM)
Ionic	CTAB	Cetyltrimethylammonium bromide	1.0
	Sodium cholate		~10
	Sodium deoxycholate		~2
Non-ionic	Big Chap	*N*,*N*-bis(3-D-gluconamidopropyl) cholamide	3.4
	$C_{12}E_8$	Octaethylene glycol monododecyl ether	<0.1
	Triton X-100	Nonaethylene glycol octylphenol ether	0.3
Zwitterionic	CHAPS	3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate	3–10
	CHAPSO	3-[(3-cholamidopropyl) dimethylammonio]-2-hydroxypropane-1-sulfonate	4–8
	LDAO	Dodecyldimethylamine oxide	~1

CMC critical micelle concentration

Uncorrected Proof

from the membrane into the detergent micelle, the CMC is the minimal concentration of detergent necessary to form the required micellar structure for protein extraction (10). CMC values, some of which are listed in Table 2, vary between different detergents, but are usually available from the detergent manufacturer.

Additional considerations when choosing detergents include evaluating the effects of a given detergent on the structural and functional properties of the protein of interest. The effects of detergents on the protein stability may be checked during preliminary screens using different detergents. The compatibility of the chosen detergent with subsequent purification steps should also be considered as certain detergents may affect the efficiency of certain chromatographic techniques. For example, charged detergents may cause problems using assays based on charge difference, such as ion-exchange chromatography (see Chapter 2), and lectin chromatography which may be used to affinity purify subsets of glycoproteins is especially sensitive to high concentrations of a variety of detergents (7, 8). It is often necessary to remove or replace detergents to overcome these problems, thus the ease at which excess detergent can be removed from the solubilised protein fraction should be considered (see Subheading 1.4).

When solubilising integral membrane proteins, buffered stock solutions at a physiological pH environment should be prepared containing the membrane preparation, detergent, and protease inhibitors, such as EDTA, EGTA, and/or PMSF (10). Membrane preparations are used at a final protein concentration of 1–5 mg/mL

Author's Proof

179 and are solubilised by detergent concentrations of 0.1–5% (v/v)
180 (7, 10). The mixture should be stirred gently for 1 h at room tem-
181 perature or 4°C, followed by centrifugation for 1 h at 100,000 × *g* at
182 4°C. Generally speaking, retention of a membrane protein in the
183 supernatant following centrifugation for 60 min at 100,000 × *g* after
184 solubilisation defines this protein as soluble (7). The pellet may sub-
185 sequently be washed to remove residual detergent and finally re-
186 suspended in the appropriate buffer (10). Protein recovery and activity
187 should be investigated in both the pellet and supernatant at this
188 stage. The procedure for solubilising membrane proteins using the
189 non-ionic detergent Triton X-100 is outlined in Subheading 3.3.

190 ***1.4. Removal***
191 ***of Detergents***
192 ***from Membrane***
193 ***Protein Fractions***

The high detergent concentrations that are often required during
the initial extraction of integral membrane proteins could poten-
tially affect the stability and subsequent analysis of the isolated
194 membrane proteins; therefore, excess detergent should be
removed or exchanged for an alternative detergent prior to sub-
195 sequent purification procedures. Examples of methods used to
196 remove or exchange detergents are listed in Table 3. The choice
197 of technique depends on the unique properties of the detergent
198 used and the concentration range of the protein fraction.
199 Successful detergent exchange or removal can be achieved
200 using various chromatographic supports, followed by extensive
201 washing with the desired buffer, containing the new detergent if
202 necessary (6). Alternatively dialysis can be carried out to facilitate
203 detergent exchange or removal. The efficiency of dialysis depends
204 on the CMC and micelle molecular weight, which is determined
205 by the aggregation number of detergent molecules (11).
206 Most detergents with linear alkyl hydrophobic groups (e.g. Triton

Uncorrected Proof

t3.1 **Table 3**
t3.2 **Commonly used techniques for detergent removal/**
t3.3 **exchange**

t3.4	Technique	Reagent
t3.5	Affinity chromatography	Ligand immobilised sepharose
t3.6	Equilibrium dialysis	Appropriate buffer or water
t3.7	Gel permeation chromatography	Sephadex G-25 (GE Healthcare)
t3.8 t3.9	Hydrophobic interaction chromatography	Bio-Beads SM-2 (Bio-Rad)
t3.10	Ion-exchange chromatography	Dowex 1-X2 (Sigma-Aldrich)
t3.11	Precipitation	Acetone
t3.12 t3.13	Ultrafiltration	High molecular weight cut-off membrane

Author's Proof

X-100) have a high micelle molecular weight value and do not pass through dialysis membranes (6). Detergents with a low micelle molecular weight and high CMC (e.g. bile acids and their derivatives) can be removed by dialysis (6). A protocol for dialysis is described in Subheading 3.4 of this chapter. Detergent removal by means of chromatographic supports (see Subheading 3.5) is relatively work-intensive, but is a more rapid procedure than dialysis so can be advantageous in cases where protein stability is an issue.

Following initial extraction of membrane proteins, solubilisation using detergent, and detergent removal or exchange, membrane proteins can then be purified to homogeneity using a variety of protein purification techniques, depending on the particular needs of the investigator and the given properties and abundance of the protein of interest. Because there is no single procedure to characterise membrane proteins, the key importance of membrane purification lies with the initial extraction and solubilisation steps, in order to generate a high yield of pure protein in its native biologically active state. The Subheading 3 of this chapter outlines examples of techniques used during the important initial stages of membrane protein purification.

2. Materials

2.1. Fractionation of Peripheral and Integral Membrane Proteins Using High pH

1. High pH buffer: 100 mM Na_2CO_3, pH 11.3.
2. Dounce homogeniser, e.g. Potter-Elvehjem PTFE pestle and glass tube (Sigma-Aldrich).
3. Ultracentrifuge, e.g. Thermo Scientific Sorvall WX.

2.2. Extraction of Membrane Proteins Using Butanol

1. N-butanol.
2. Cooled bench top centrifuge, e.g. Eppendorf centrifuge 5417R.

2.3. Extraction of Membrane Proteins Using Triton X-100

1. TE buffer: 10 mM Tris–HCl, 2 mM EDTA.
2. 2% Triton X-100 in phosphate buffered saline (PBS) (see Note 1).
3. Ultracentrifuge, e.g. Thermo Scientific Sorvall WX.

2.4. Removal of Non-ionic Detergents by Detergent-Adsorption Chromatography

1. Columns with a bed volume of approx. 5 mL (e.g. Econo-column, Bio-Rad).
2. Commercially available detergent absorption matrix (e.g. Bio-Beads SM-2, Bio-Rad, see Note 2).
3. Blocking buffer: 0.1% (w/v) bovine serum albumin in 50 mM Tris–HCl, pH 7.4, 0.15 M NaCl (see Note 3).
4. Washing buffer: 50 mM Tris–HCl, pH 7.4, 0.15 M NaCl.

Author's Proof

246 247 248 249

2.5. Removal of Detergent with Low Micelle Size and High CMC by Dialysis

1. Dialysis tubing with a molecular weight cut-off of approx. 10,000 Da.
2. Wash buffer: 100 mM $NaHCO_3$, 50 mM EDTA.
3. Dialysis buffer: 20 mM Tris–HCl, pH 7.4, 0.15 M NaCl.

250

3. Methods

251 252 253 254 255 256 257 258 259 260

3.1. Fractionation of Peripheral and Integral Membrane Proteins Using High pH

1. Re-suspend the membrane fraction (see Note 4) at a concentration of <2 mg/mL in high pH buffer (see Notes 5 and 6).
2. Homogenise the suspension in a dounce homogeniser using six to eight strokes.
3. Maintain at 4°C for 30 min. Mix by vortexing three times during this period.
4. Pellet the membrane fraction by centrifugation for 60 min at 100,000 × *g* at 4°C and transfer the supernatant, which contains the peripheral membrane proteins, into a fresh tube and assay for protein (see Note 7).

261 262 263 264 265 266 267 268 269 270 271

3.2. Extraction of Membrane Proteins Using Butanol

1. Add an equal volume of N-butanol to the membrane fraction (see Note 4) and maintain at 4°C.
2. Centrifuge at 500 × *g* at 4°C for 10 min to separate the mixture into an upper phase containing butanol and membrane lipids and a lower aqueous phase containing solubilised proteins. Lipid rich material is localised to the interface.
3. Separate the upper and lower aqueous phases into separate tubes.
4. Dialyze the aqueous phase against a large volume of water or suitable buffer.
5. Assay the dialysed aqueous phase for protein (see Note 8).

272 273 274 275 276 277 278 279 280 281 282 283

3.3. Extraction of Membrane Proteins Using Triton X-100

1. Re-suspend cells in TE buffer at a concentration of 1×10^7 cells/mL.
2. Centrifuge the cells at 40,000 × *g* for 10 min. Remove the supernatant and add fresh TE.
3. Repeat this step and re-suspend the cells in approximately 1 mL of TE.
4. Add cells drop-wise to the 2% Triton X-100 while stirring (see Note 9).
5. Allow to solubilise for 30 min at 4°C.
6. Centrifuge at 100,000 × *g* for 30 min at 4°C.
7. Transfer the supernatant to a fresh tube and assay for protein (see Note 10).

Uncorrected Proof

Author's Proof

3.4. Removal of Non-ionic Detergents by Detergent-Adsorption Chromatography

1. Before starting, ensure that the protein fraction containing the non-ionic detergent (e.g. Triton X-100) has a concentration of >1 mg/mL (see Note 11) and that the molecular weight of the protein to be recovered is large enough to avoid entrapment in the pores of the affinity matrix. 284 285 286 287 288
2. Apply distilled water to the column matrix, followed by blocking buffer. Next, apply washing buffer to the column and repeat wash step. 289 290 291
3. Transfer the protein fraction to the column matrix (see Note 12). 292 293
4. Collect 0.5–1 mL fractions and assay for protein. 294

3.5. Removal of Detergent with Low Micelle Size and High CMC by Dialysis

1. Prepare the dialysis tubing by boiling a section in washing buffer for 10 min (see Note 13). Then boil the dialysis tubing in distilled water for 10 min, followed by washing thoroughly in distilled water. 296 297 298
2. Transfer the solubilised membrane protein fraction into the dialysis tubing (see Note 14) which is securely closed at one end by either tying a double-knot in the tubing or securing it with a plastic clamp (see Note 15). 299 300 301 302
3. Remove air bubbles and seal the dialysis tubing, allowing for a volume increase during dialysis. Check the integrity of the seal to ensure no leakage occurs. 303 304 305
4. Place the tubing in a beaker containing a large external volume (approx. 5 L) of the appropriate buffer. Dialyze with gentle stirring at 4°C. Change the external buffer regularly. 306 307 308
5. When the dialysis is finished, remove the dialysis tubing and wash the outside. Hold the tubing and carefully remove the upper clamp. Using a Pasteur pipette, transfer the protein fraction to a new tube (see Note 16). 309 310 311 312

Uncorrected Proof

4. Notes 313

1. Make a stock solution of 20% Triton X-100 by weighing 2 g Triton X-100 and adding PBS up to 10 mL and stirring gently until fully dissolved. Store the stock solution at 4°C. 314 315 316
2. Bio-Beads are macro-porous polystyrene beads and have a high surface area that adsorbs organics with a molecular weight of <2,000 from aqueous solution. They may be used to remove Triton X-100 from protein fractions. Due to the presence of linear alkyl hydrophobic groups, Triton X-100 has a high micelle molecular weight value and does not pass through dialysis membranes. Detergents with a low micelle molecular weight and high CMC (e.g. bile acids and their derivatives) can be removed by dialysis (see Subheading 3.5). 317 318 319 320 321 322 323 324 325

Author's Proof

3. Bovine serum albumin is used as a bulk carrier protein to saturate non-specific protein binding sites and minimise protein loss during this procedure.
4. The starting material depends on the source from which the membrane proteins are being purified. Membrane proteins can be successfully isolated from plant and animal tissues or cell cultures, bacteria, yeast, and fungi. Animal tissues can be broken easily with a mixer or blender. Due to the presence of robust cell walls, unicellular organisms like yeast or bacteria and plant cells are more difficult to disrupt. Different techniques for breaking down cell walls include glass bead milling, grinding mills, homogenization, ultrasonication, osmotic shock, repeat freeze thawing, and enzymatic lysis (8). If possible, the protein should be prepared from sources where it is in high abundance, as a certain amount of protein may be lost during the purification process. The starting material can be enriched if the target protein is known to be associated with the plasma membrane, mitochondria, or nucleus. During initial steps of membrane protein isolation, cytosolic proteins can be removed to obtain an enriched preparation of membranes containing the protein of interest. Soluble cytoplasmic proteins are extracted by cell disruption in a neutral pH, isotonic, and detergent-free buffer (7), followed by differential centrifugation or purification using sucrose gradient centrifugation.
5. The pH of the working buffer should be tested following addition of any protease inhibitors, as addition of such components may alter the final pH of the buffer.
6. It is worthwhile determining the effect of the high pH buffer on any enzymatic activity the protein of interest may have, and considering potential interactions the buffer may have with any column matrix that will be used at later stages in the purification process.
7. A suitable protein concentration assay should be considered. Options include measuring ultra-violet absorbance at 280 nm, or using one of several commercially available dye-binding assays, such as the Bradford assay, the bicinchonic acid (BCA) assay, or other assays (see Chapter 13).
8. It is worthwhile to keep the butanol phase for protein assays as it may contain extremely hydrophobic proteins that are difficult to solubilise.
9. The effect of the Triton X-100 solubilisation procedure on the structural and functional properties of the protein of interest should be evaluated during preliminary screening experiments. In order to maintain catalytic activity, the membrane protein should be dissolved under optimal conditions for stability at a detergent/protein ratio that is not much above the

Uncorrected Proof

Author's Proof

minimal detergent/protein ratio required for solubilisation (8). Additionally, proteins are more susceptible to protease attack following solubilisation with detergents, so protease inhibitors are necessary to prevent protein degradation. Premixed cocktails of commonly used protease inhibitors are now available commercially from a variety of companies including Roche, Sigma-Aldrich, and Pierce. It is recommended to carry out purification procedures at 4°C in order to minimise proteolysis (see Chapter 4). Additionally, the effects of Triton X-100 on subsequent purification techniques should be evaluated. Replenish protein stabilising additives or protease inhibitors if they are removed or inactivated at any stage in the experiment, for example EDTA is removed by hydroxyapatite chromatography (8). If possible, minimise any purification steps that add new detergents or alter the original detergent/lipid ratio.

10. Due to the presence of aromatic groups, Triton X-100 has substantial UV absorbance at 280 nm, thus an alternative protein concentration assay should be carried out. For the same reason, Triton X-100 is not suitable for subsequent purification steps involving column chromatography with UV monitoring of the fractions. As an alternative, bile salts and their derivatives including CHAPS and CHAPSO can be used for solubilisation.
11. A high concentration is necessary to allow for any loss of protein during the procedure.
12. Use washing buffer to dissolve the protein fraction for optimum detergent binding.
13. As dialysis tubing is susceptible to cellulolytic micro-organisms, gloves should be worn when handling the tubing.
14. A small funnel may be used to aid transfer of the protein fraction into the dialysis tubing.
15. Prior to transferring the protein fraction into the dialysis tubing, the integrity of the membrane and clamp/knot can be tested by applying water or buffer and checking the tubing for leaks.
16. Avoid losing dialyzed samples by carefully opening the tubing over a larger glass beaker to collect any accidental spillage.

References

1. Wallin, E., and von Heijne, G. (1998) Genome-wide analysis of integral membrane proteins from eubacterial, archaean, and eukaryotic organisms. *Protein Sci.* 7, 1029–1038.
2. Mohanty, A. K., and Wiener, M. C. (2004) Membrane protein expression and production: effects of polyhistidine tag length and position. *Protein Expr. Purif.* **33**, 311–325.
3. Gordon, E., Horsefield, R., Swarts, H. G., de Pont, J. J., Neutze, R., and Snijder, A. (2008) Effective high-throughput overproduction of membrane proteins in *Escherichia coli*. *Protein Expr. Purif.* **62**, 1–8.

Uncorrected Proof

Author's Proof

4. Berman, H. M., Battistuz, T., Bhat, T. N., Bluhm, W. F., Bourne, P. E., Burkhardt, K., Feng, Z., Gilliland, G. L., Iype, L., Jain, S., Fagan, P., Marvin, J., Padilla, D., Ravichandran, V., Schneider, B., Thanki, N., Weissig, H., Westbrook, J. D., and Zardecki, C. (2002) The Protein Data Bank. *Acta Crystallogr. D Biol. Crystallogr.* **58**, 899–907.
5. Doerr, A. (2009) Membrane protein structures. *Nat. Methods* **6**, 1.
6. Ahmed, H. (2005) *Principles and Reactions of Protein Extraction, Purification and Characterization*. CRC, Boca Raton, FL.
7. Ohlendieck, K. (1996) Extraction of membrane proteins. In *Protein Purification Protocols* (Doonan, S., Ed.), Humana, Totowa, NJ.
8. Von Jagow, G., Link, T., and Schager, H. (1994) Purification strategies for membrane proteins. In *A Practical Guide to Membrane Protein Purification* (Von Jagow, G., and Schager, H., Eds.), Academic, San Diego, CA.
9. Rosenberg, I. M. (2005) *Protein Analysis and Purification: Benchtop Techniques.* 2nd. ed., Springer, Boston, MA.
10. Schimerlik, M. I. (2001) Overview of membrane protein solubilization. *Curr. Protoc. Neurosci*. Chapter 5, Unit 5.9.
11. Ohlendieck, K. (1996) Removal of detergent from protein fractions. In *Protein Purification Protocols* (Doonan, S., Ed.), Humana, Totowa, NJ.

Uncorrected Proof