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REVIEW ARTICLE

Detergent quantification in membrane protein samples and its application to crystallization experiments

Chelsy C. Prince · Zongchao Jia

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Abstract The structural characterization of membrane proteins remains a challenging field, largely because the use of stabilizing detergents is required. Researchers must first select a suitable detergent for the solubility and stability of their protein during in vitro studies. In addition, an appropriate concentration of detergent in membrane protein samples can be essential for protein solubility, stability, and experimental success. For example, in membrane protein crystallography, detergent concentration in the crystallization drop can be a critical parameter influencing crystal growth. Over the past decade, multiple techniques have been developed for the measurement of detergent concentration using a wide variety of strategies. These methods include colorimetric reactions, which target specific detergent classes, and analytical techniques applicable to a wide variety of detergents. This review will summarize and discuss the available options. It will be a useful resource to those selecting a strategy that best fits their experimental requirements and available instruments.

Keywords Crystallography · Membrane proteins · Detergent · Quantification

Introduction

Membrane proteins represent 20–30 % of all genes, yet structures of these proteins remain severely under represented (Krogh et al. 2001). These proteins are of heightened interest for both structural and functional characterization because they represent over 60 % of current drug targets (Arinaminpathy et al. 2009). Membrane protein purification requires the use of detergents to extract the protein from the phospholipid bilayer and to stabilize the protein in vitro for characterization. The extracted membrane protein is often referred to as a protein-detergent complex (PDC). A wide range of detergents have been applied to membrane research, but a systematic method for choosing which detergent will best stabilize a particular membrane protein does not exist (Prive 2007).

The use of detergents in membrane protein research has been reviewed extensively (Seddon et al. 2004; Prive 2007; Arnold and Linke 2008; Sonoda et al. 2010; Cross et al. 2011), and the intention of this focused review is not to repeat previously published work. This article will focus specifically on the impact of detergents in X-ray crystallography experiments and the importance of quantifying the detergent concentration used for crystallization. Tight control of both protein and detergent concentration may help overcome the "crystallization bottleneck" in structural studies. This review summarizes over ten distinct approaches for detergent quantitation in membrane protein samples, each with their own advantages and disadvantages.

Selection of a detergent for membrane protein research

The detergent in the PDC represents a large portion of the complex surface area, and therefore it is not surprising that the detergent "belt" around proteins can have a strong influence on many properties of the PDC. The particular detergent picked to stabilize a given membrane protein is often chosen empirically after testing a wide range of

C. C. Prince · Z. Jia (✉)
Department of Biomedical and Molecular Sciences, Queen's University, Botterell Hall, 18 Stuart Street, Kingston, ON K7L 3N6, Canada
e-mail: jia@queensu.ca

options. Many efforts have been made to miniaturize the testing of detergents to allow a wide screen to be performed on a minimal amount of sample. Methods include microdialysis (Postis et al. 2008), ultracentrifugation (Gutmann et al. 2007), thermal studies using fluorochromes (Alexandrov et al. 2008; Fan et al. 2011), or size-exclusion chromatography (Hattori et al. 2012). The interaction between detergent and protein has also been more specifically studied. Observation of isolated alpha-helical fragments solubilized in detergent has suggested that it is the interaction of the detergent with the extra-membrane domains that might be the cause of protein denaturation and aggregation in some detergents (Tulumello and Deber 2012).

The classical example of a detergent molecule is amphipathic in nature including a polar head group attached to a single hydrophobic tail. In an attempt to produce better detergents for stabilization and crystallization, researchers have designed detergents with more "lipid-like" hydrophobic tails to better mimic the bilayer environment. Examples of these efforts include the branched-chain maltosides (Hong et al. 2010), the neopentyl glycols (Chae et al. 2010a, 2013a), tripod detergents (McQuade et al. 2000; Chae et al. 2008, 2010b), and the lipopeptides (McGregor et al. 2003; Koutsopoulos et al. 2012). Alternatively, some new detergents have focused on changing the head group chemistry including the trehaloside detergents (Tao et al. 2012), anionic calix[4]arene-based detergents, and carbohydrate based Triton X-100 analogues (Chae et al. 2013b). In addition, alternatives to the solubilization of membrane proteins in detergents continue to be developed (Sanders et al. 2004; Zhang et al. 2011). These include protein-based nanodiscs (Nath et al. 2007), amphipols (Tribet et al. 1996; Gorzelle et al. 2002), and steroid-based facial amphiphiles (Lee et al. 2013). Many of these novel compounds are still expensive, not commercially available, and/or do not have an established track record in crystallization studies. As a result, most laboratories working on membrane protein crystallography still start with traditional detergents, the most common being n-dodecyl-β-D-maltoside (Newstead et al. 2008).

Selection of a detergent for membrane protein crystallization

Even when a detergent is found to stabilize a protein, there is no guarantee that the size of the detergent "belt" around the protein will allow successful crystallization. Productive crystal contacts can only be formed on protein surfaces that are exposed to the solvent (Sonoda et al. 2010). Therefore, the protein to detergent ratio of the final PDC (DaCosta and Baenziger 2002), and the buried surface area of the protein (Gutmann et al. 2007), are important parameters to consider. The amount of detergent bound to a particular membrane protein can be determined using light scattering (Strop and Brunger 2005; Kunji et al. 2008; Slotboom et al. 2008) or analytical ultracentrifugation (Maslennikov et al. 2007; Salvay et al. 2007).

During the development of new detergents for crystallography, a detergent's micelle size is also an important physical parameter which can be measured using dynamic light scattering (Chae et al. 2013a, b), refractive index and light scattering (Strop and Brunger 2005), or neutron scattering (Marone et al. 1999). Practically, smaller detergents are desirable because they reduce the overall PDC size (Marone et al. 1999; Kunji et al. 2008), allow tighter crystal packing (Garavito et al. 1996), and therefore, can produce PDC crystals with above average diffraction (Prive 2007; Sonoda et al. 2010). Although desirable in crystallization, smaller detergents are considered "harsher" and are more likely to denature and destabilize a particular protein or dissociate physiologically relevant oligomers (Prive 2007; Gan et al. 2011). Therefore, the choice of detergent for use in structural studies must take into account the properties of the protein and the requirements of crystallization.

How much detergent?

The minimum free detergent required to keep most membrane proteins stable is the critical micelle concentration (CMC), the lowest detergent concentration that will form micelles. The CMC varies with each specific detergent based on its physical properties, such as the number of methylenes in the aliphatic chain of the detergent (Prive 2007). The composition of the solvent (ionic strength, pH, etc., Ikeda et al. 1978; Kaufmann et al. 2006; Abezgauz et al. 2010) and temperature (Molina-Bolivar et al. 2004) also influences CMC. Various techniques can be used to measure the CMC of detergents including the pyrene 1:3 ratio method (Ruiz and Molina-Bolívar 2011), surface tension measurements (Molina-Bolivar et al. 2004; Kaufmann et al. 2006), isothermal calorimetry (Hildebrand et al. 2004), dynamic light scattering (Pispas 2006), and fluorescence-based methods (Chattopadhyay and London 1984; Jumpertz et al. 2011).

During crystallization, minimizing free micelles in solution could reduce unproductive collisions between molecules, and be beneficial during crystal nucleation and growth (DaCosta and Baenziger 2002; Wiener 2004). In contrast, some researchers have shown a correlation between crystallization success and concentrations of a detergent near its cloud point (i.e., phase boundary where inter-micelle attractive forces drive micelles into a separate

Table 1 Summary of colorimetric techniques for detergent quantitation

Reagents	Detergent class	Sensitivity	Sample volume (μL)	UV–Vis absorption peak (nm)	Interference
Ammonium molybdate Ascorbic acid (1)	Phospholipid derivatives	3.5 μg (FC-12)	10	820	Phosphorylated protein and silicates
Phenol sulfuric acid (2)	Glycosidic detergents	2.5 μg (DDM)	50	490	Glycosylated proteins and glycerol
2,6-Dimethylphenol sulfuric acid (3)	Glycosidic detergents	0.5 μg (DDM)	5	510	Glycosylated proteins and glycerol
Sulfuric acid (4)	Bile salt derivatives	7.65 μg (CHAPS)	50	389	None reported
Methylene blue chloroform (5)	SDS	150 ng	300	651	Many anionic compounds

References: (1) Ames (1966), (2) Dubois et al. (1956); Urbani and Warne (2005), (3) Prince and Jia (2012), (4) Urbani and Warne (2005), (5) Arand et al. (1992)

phase, Hitscherich et al. 2001; Wiener 2004). This has been confirmed by light scattering experiments, which suggest that for both pure detergent micelles and PDCs, the inter-particle forces become significantly attractive well before the actual cloud point is reached (Hitscherich et al. 2000).

During protein extraction and purification, there are numerous stages in which the detergent concentration can be altered. A particularly problematic step is concentration of the protein in centrifugal concentrators, where protein-free detergent micelles are also enriched (Urbani and Warne 2005; Prive 2007; Shi et al. 2008). The detergent concentration factor of centrifugal concentrators has been shown to vary between manufacturers and within units from the same manufacturer (Maslennikov et al. 2007). Therefore, the concentration of detergent is often unknown after protein concentration. In order to improve the screening success and reproducibility of experiments, it is desirable to be able to quickly measure the total detergent present in concentrated samples immediately before crystallization is attempted.

Quantifying detergent in membrane protein samples

Specific colorimetric reactions

Some of the most popular detergents used in membrane protein crystallography can be quantified using chemical reactions that target specific chemical groups in the detergent structure. These techniques are widely accessible because they require no specialized equipment and are relatively quick, although they can only be applied to certain detergent classes (Table 1). They are based on colorimetric methods that measure the UV–Vis absorbance of chemically treated samples. The main disadvantage of these techniques is that many of the reactions involve hazardous chemicals such as sulfuric acid and phenol, so appropriate safety precautions must be observed. These techniques also destroy the original sample, though the high sensitivity of these techniques minimizes the sample loss. The procedure and chemical basis of these reactions are described briefly in the following sections.

Molybdate assay for total phosphate

This procedure was originally described by Bruce Ames in 1966 for the detection of organic phosphates (Ames 1966). It can further be applied to the Fos-choline series of phospholipid-derived detergents. In this procedure, the sample is diluted to <7 mM expected detergent and a 10 μL sample is added to a glass test tube followed by magnesium nitrate in 95 % alcohol. The sample is then ashed over a strong flame and cooled to room temperature. The resulting powder is suspended in hydrochloric acid and heated in a boiling water bath for 15 min. The released phosphate in the sample is quantified by adding a colorimetric mix of ascorbic acid, ammonium molybdate, and sulfuric acid. Incubation at 45 °C for 45 min or 37 °C for 1 h results in a phosphomolybdate complex that is reduced by ascorbic acid to produce the colorimetric product. Caution must be used when applying this technique to phosphorylated proteins as the additional phosphate will be detected. In addition, extremely clean glassware from the same manufacturer must be used because interference has been noted from silicates evolved from the glass during ashing and residue from trisodium phosphate detergents used during cleaning (Ames 1966).

Phenol/sulfuric acid assay for sugar content

A phenol/sulfuric assay originally developed for the measurement of sugars in solution can also be applied to membrane protein samples solubilized in glycosidic detergents, which contain a sugar head group (Dubois et al.

1956; Lau and Bowie 1997; Urbani and Warne 2005). Examples of detergents in this group include *n*-dodecyl-β-D-maltoside, *n*-decyl-β-D-maltoside, and *n*-octyl-β-D-glucoside, the most popular detergents currently used in membrane protein crystallization (Newstead et al. 2008). In addition, some of the newly introduced detergents such as the neopentyl glycols and the trehalose detergents are sugar-based and should be quantifiable by this technique. In this single-step procedure, the sample is mixed with phenol and sulfuric acid in a 1.5 mL tube (designed for thermal applications). A strong exothermic reaction occurs during incubation and results in an acid-catalyzed dehydration of sugar moieties and their condensation with phenol to produce a UV–Vis absorbent species (Mallya and Pattabiraman 1997). This method has been further refined to use 2,6-dimethylphenol as a colorimetric reagent with increased sensitivity (Mallya and Pattabiraman 1997) and applied to the quantification of glycosidic detergents (Prince and Jia 2012).

Assay of bile salt with sulfuric acid

The direct reaction of sulfuric acid with bile acid detergents such as CHAPS and cholate (Urbani and Warne 2005) allows for the quantification of these detergents. In this reaction, a sample of detergent is vortexed with 800 µL of concentrated sulfuric acid. This results in the direct reaction of the sulfuric acid with the hydroxyl group in the cholate ring to produce a colored product with absorption peaks at 389, 450, and 479 nm (Urbani and Warne 2005). The band at 389 nm is used for quantitation as it provides the largest signal.

Methylene blue/chloroform for the detection of SDS

Although SDS is commonly considered a denaturing detergent used in the unfolding of soluble proteins, it is less effective in the case of membrane proteins and has been successfully used for the NMR analysis of some extremely stable targets (Prive 2007). In this assay, methylene blue reagent (methylene blue, sodium sulfate, sulfuric acid) is combined with the sample and a complex is formed between methylene blue and the SDS. Free methylene blue is removed by extraction of the complex into chloroform, after which the absorbance can be measured. Although this assay is extremely sensitive, it suffers from many potentially interfering anionic compounds including sodium chloride, sodium bromide, sodium iodide, and trichloroacetic acid, which also support the transport of methylene blue into the chloroform phase (Arand et al. 1992). Therefore, careful testing of the protein buffer should be conducted before this method is applied to membrane protein samples.

General detergent quantification techniques

While the techniques presented in the above section are based on chemical reactions and absorbance readings that can be done in any laboratory, they are limited in the range of detergents for which they apply. Therefore, more general techniques have been developed that can work on a wider range of detergents. Table 2 provides a summary of detergents for which these general techniques have been explicitly tested, though most should be amenable to an even wider range. Factors to consider when selecting a technique include: its sensitivity, sample volume required, and the processing time. Table 3 provides a direct comparison of the sensitivity of each technique and other parameters that may be of interest.

Radiolabeled detergents

Radiolabelling was one of the first techniques to be used for detergent measurement and has been applied in several studies of protein-detergent complexes (Le Maire et al. 1983; Møller and le Maire 1993; Hannam et al. 1998; Josse et al. 2002). This technique has the advantage of detecting specifically labeled molecules; therefore, the experimenter does not have to worry about the interference from other components of the membrane protein sample. Specific labeling also affords this technique the highest sensitivity. Due to limited availability, high cost, and the ever-increasing safety requirements of radioactive detergents, this technique is often considered impractical for routine measurements despite its accuracy and potential versatility.

FTIR spectroscopy

Fourier transform infrared (FTIR) spectroscopy identifies characteristic absorption bands for proteins, detergents, and lipids in a membrane protein sample (DaCosta and Baenziger 2002). This technique is extremely rapid, with collection times as short as 1 min (DaCosta and Baenziger 2002). Detergent quantitation is achieved by calculating the area under the detergent absorption bands (1,200–900 cm^{-1}) and comparing to a series of standards. A solvent corrected baseline is used, and no common buffer components with problematic absorption bands in this area have been reported. It should be noted that lipids also produce absorption bands in the 1,200–900 cm^{-1} region, but their contribution can be scaled and subtracted when significant (DaCosta and Baenziger 2002). Comparing the area of the unique lipid ester C = 0 band (1,740 cm^{-1}) and the protein amide I band ($\sim$1,650 cm^{-1}) can also detect lipid ratios as low as seven molecules of lipid per molecule of protein (DaCosta and Baenziger 2002). Finally, some protein secondary structure

Table 2 Detergents previously shown to work with the general quantitation techniques

Classification	Detergent	Radiolabeled (1)	FTIR (2)	TLC (3)	HPTLC (4)	Refractive index (5)	Contact angle (6)	NMR (7)	Gas chromatography (8)
Glycosidic	OG		✔	✔	✔		✔	✔	
	OTG				✔		✔		
	NG							✔	
	DM		✔	✔	✔		✔	✔	✔
	DDM	✔		✔	✔	✔	✔	✔	✔
	DDTM				✔				
	FH				✔				
	PCC				✔				
	CYMAL5						✔		
Zwittergents	Zwittergent 3–12		✔						
	Zwittergent 3–14							✔	
Phospholipid derivatives	FC-12			✔			✔	✔	✔
	FC-14					✔		✔	
Glycol ethers	C8E4			✔					
	C8E5			✔					
	C12E8	✔	✔	✔					
	C12E9					✔	✔		
Bile salt derivative	CHAPS	✔					✔		
	Cholate		✔						
	LDAO			✔		✔	✔	✔	✔
	DAO			✔					
	Sarkosyl			✔					
	SDS						✔	✔	
	Triton X-100	✔					✔		

OG *n*-octyl-β-D-glucoside, *OTG* *n*-octyl-β-D-thioglucoside, *NG* *n*-nonyl-β-D-glucoside, *DM* *n*-decyl-β-D-maltoside, *DDM* *n*-dodecyl-β-D-maltoside, *DDTM* *n*-dodecyl-β-D-thiomaltopyranoside, *FH* F4H5-β-maltoside, *PCC* PCC-α-maltoside, *FC-12* Fos-choline 12, *FC-14* Fos-choline 14, *LDAO* *n*-dodecyl-*N*,*N*-dimethylamine-*N*-oxide, *DAO* *n*-decyl-*N*,*N*-dimethylamine-*N*-oxide

References: (1) Le Maire et al. (1983); Hannam et al. (1998); Josse et al. (2002); Kaufmann et al. (2006), (2) DaCosta and Baenziger (2002), (3) Eriks et al. (2003), (4) Barret et al. (2013), (5) Strop and Brunger (2005), (6) Kaufmann et al. (2006), (7) Maslennikov et al. (2007), (8) Shi et al. (2008, 2009)

information can be inferred from the shape of the protein amide absorbances, with alpha-helical folds contributing vibrations at 1,652–1,657 cm^{-1} and beta-sheet folds contributing vibrations at 1,628–1,635 cm^{-1}, and 1,670 cm^{-1} (Jackson and Mantsch 1995). The main disadvantage of this technique is the specialized equipment required, which includes a diamond attenuated total reflectance (ATR) accessory designed for small-volume analysis (DaCosta and Baenziger 2002).

Thin layer chromatography (TLC)

The mobility of different detergents on silica TLC plates in a solvent system of chloroform:methanol:ammonium hydroxide (63:35:5) has been observed (Eriks et al. 2003) and allows many detergents to be distinguished. Detergent is visualized by iodide vapor staining, in a glass desiccator at 60 °C. The degree of staining provides a linear relationship between detergent quantity and optical density for quantitation. Plates can be imaged using laser densitometry (Eriks et al. 2003) or a flatbed scanner (Beebe et al. 2011). During chromatography of membrane protein samples, it has been shown that free detergent migrates while detergent bound to the protein remains at the origin. The intrinsic staining capacity of the protein can be subtracted from the total intensity at the origin, and used to determine the amount of detergent present. Further, total detergent in the sample would be determined by adding the total of the protein bound and free detergent spots (Eriks et al. 2003). The experimenter should also be aware that glycerol does decrease the

Table 3 Comparison of effectiveness of the general quantitation techniques

Technique	Expected sensitivity	Sample volume	Additional benefit	Potential interferences
Radiolabeling (1)	30.63–663 ng/μL (DDM)	n/a	None	None
FTIR (2)	18–585 μg (OG)	10 μL	Secondary structure lipid measurement	High lipid concentration
TLC (3)	13–350 μg (DDM)	5 μL	Identification	Glycerol
HPTLC (4)	0.1–1.6 μg (DDM)	40 μL	Identification	None reported
Refractive index (5)	0.5–10.5 μg (FC-14)	7 μL	Protein oligomeric state	Glycerol, salts
Contact angles (6)	0.2–1.8 μg (DDM)	20 μL	None	Glycerol, lipids, polyethylene glycols
NMR (7)	2 μg (FC-14); 27 μg (DDM)*	520 μL	Identification	Proteins
Gas chromatography-FID (8)	0.05–3 μg (DDM)	1 μL	Identification	None reported
Gas chromatography-MS (9)	0.05–3 μg (DDM)	1 μL	Identification	None reported

References: (1) Kaufmann et al. (2006), (2) DaCosta and Baenziger (2002), (3) Eriks et al. (2003), (4) Barret et al. (2013), (5) Strop and Brunger (2005), (6) Kaufmann et al. (2006), (7) Maslennikov et al. (2007), (8) Shi et al. (2008), (9) Shi et al. (2009)

* Minimum detergent detected

staining of *n*-dodecyl-β-D-maltoside and requires alteration of the standard curve (Eriks et al. 2003).

More recently, an updated high-performance thin layer chromatography (HPTLC) based assay has been described. In this work, an altered solvent system of dichloromethane/methanol/acetic acid (32:7:0.4) is used to improve the separation of similar glycosidic detergents for identification (Barret et al. 2013). In addition, this work explored the use of sulfuric acid staining reagents (Barret et al. 2013) and a modified copper sulfate reagent previously used for lipid detection (Handloser et al. 2008). The modified copper sulfate reagent gives a tenfold increase in the sensitivity of this assay above the previous iodide vapor staining methodology. The limitation of this method thus far is that the new visualization technique has only been optimized for detergents with a sugar moiety (Barret et al. 2013).

Refractive index measurement

Refractive indices can be used to determine the concentration of free detergent in a membrane protein sample after separation of the PDC and micelles by size-exclusion chromatography (Strop and Brunger 2005). This measurement is most commonly done "in-line" with the chromatography step and allows for convenient analysis of any detergent, independent of its chemical formula (Strop and Brunger 2005). Unfortunately, after chromatography some membrane proteins require additional concentration before crystallization, which could further change the ratio of detergent to protein. In addition, refractive index has previously been used to measure both glycerol and salt concentrations (Strop and Brunger 2005), so these buffer components could cause interference in the detergent measurement if appropriate standards are not used. Refractive index, size-exclusion chromatography, and multi-angle laser light scattering (MALLS) can be combined to determine the oligomeric state of the membrane protein and the size of a protein-associated micelle during purification (Strop and Brunger 2005).

Contact angle measurement

Contact angle measurement is a very fast option that relies on the reduced surface tension caused by the presence of detergent. This technique takes three images of a 20 μL sample droplet and uses the change in contact angles between the drop and parafilm to estimate the amount of detergent present (Kaufmann et al. 2006). Standard curves for a range of detergents have been generated and have determined that the difference in surface tension reductions between some detergents is due to differences in the head group chemistry (Kaufmann et al. 2006). Interference in the measurement can be observed when other surface-active reagents like glycerol, polyethylene glycols, or lipids are present in the solution. This technique can only quantitate detergent below its CMC, therefore samples are diluted 50–100 times to determine the concentration of detergent present (Kaufmann et al. 2006). The main drawback of this technique is the requirement for specialized equipment and software to perform both the measurement and calculations (Kaufmann et al. 2006).

Nuclear magnetic resonance

One-dimensional nuclear magnetic resonance (NMR) quantifies detergent by integrating the intensity of signature ^{1}H-signals relative to a 2,2-dimethyl-2-silapentane-5-sulfonic acid (DSS) internal standard (Maslennikov et al.

2007). Data is collected using gradient water suppression, and "background noise" from the protein signal is generally not a problem given that the molar ratio of detergent:protein in a typical PDC sample is 50:1 (Maslennikov et al. 2007). Although many different detergents can be quantified with this technique, the maximum sensitivity varies dramatically depending on the number of protons contributing to the distinctive ^{1}H signal.

Gas chromatography

Gas chromatography can be used to separate many of the detergents frequently applied to membrane protein crystallography, coupled with either flame ionization detection (FID, Shi et al. 2008) or mass spectroscopy (MS, Shi et al. 2009) for quantification. The use of gas chromatography has the advantage of separating out the components of a small-volume sample, minimizing interference caused by other components of the membrane protein buffer, and allowing identification of the detergent present. In contrast, a major disadvantage of this technique is that samples must be precipitated and re-suspended in organic solvent for detergent measurement (Shi et al. 2008). The solubility of the detergent in the organic solvent chosen can also be limited due to the amphipathic nature of detergent molecules (Shi et al. 2008). In the case of FID detection, a quadratic regression curve is used to relate the peak area to different detergent concentrations (Shi et al. 2008). More recently, mass spectrometry has been substituted for FID and provides additional confidence in detergent identification, but does not improve the sensitivity of the quantification measurement (Shi et al. 2009). In this alteration, detergents eluted from gas chromatography are subjected to electron impact ionization, and a specific fragment for each detergent is chosen for quantification after mass spectrometry. All measurements using FID or MS detection require use of an internal standard for consistency (Shi et al. 2008, 2009).

Application of detergent quantitation techniques in crystallography

Currently, almost all protein crystallography projects begin with the expression and purification of the protein of interest. Determining the best conditions for these steps can require a significant time commitment, but is essential for producing monodisperse, soluble, protein samples which are more likely to crystallize (Sonoda et al. 2010). Membrane proteins most likely to crystallize migrate as a single, uniform peak during size-exclusion chromatography. Using this technique in combination with refractive index measurement and light scattering can be very informative for membrane protein stability screening. For example, the oligomeric state of the protein can be assessed and the amount of detergent required for protein stability can be determined. Therefore, this technique is well suited in establishing the best purification conditions for monodisperse protein before crystallization is attempted (Strop and Brunger 2005; Slotboom et al. 2008). During detergent screening experiments, the use of FTIR measurements can also be valuable as a high-throughput technique that determines detergent and lipid ratios simultaneously in addition to providing protein secondary structure information (DaCosta and Baenziger 2002).

Detergents with long aliphatic side chains can be gentler and more appropriate for extracting proteins from the membrane. During purification, some researchers find it valuable to extract membrane proteins using an inexpensive, low CMC, detergent and later change to a potentially more expensive, specialized detergent that is more suitable for crystallization. A recent example of this application is crystallization of membrane-bound pyrophosphatases. In this case, crystals could be obtained from *n*-dodecyl-β-D-maltoside, but crystals with better diffraction were obtained after exchange into alternative detergents, the best being octyl glucose neopentyl glycol (Kellosalo et al. 2013). During these experiments, detergent measurement techniques that both quantitate and identify the detergent are especially valuable because they can be used to continually monitor the exchange for consistency. These techniques include TLC, gas chromatography, and NMR. NMR has been specifically used to monitor the exchange of Foscholine for *n*-dodecyl-β-D-maltoside and *n*-decyl-β-D-maltoside in samples of *E. coli* tyrosine kinase (Maslennikov et al. 2007). In addition, TLC has been applied to monitor the exchange of sarkosyl with *n*-dodecyl-β-D-maltoside in samples of the mitochondrial citrate transport protein (Eriks et al. 2003).

During purification, many membrane protein researchers use a quantity of detergent to extract their membrane protein that is sufficient to completely remove any lipid present. This simplification may not always be prudent, as some membrane proteins absolutely require the presence of lipids for crystallization. The importance of lipid during crystallization has been demonstrated in many cases. Crystals of cytochrome b_6f complex could only be obtained when dioleoylphosphatidylcholine was added to the protein after purification (Zhang et al. 2003). A more recent example is GluCl from *Caenorhabditis elegans*, which has been crystallized with multiple non-native lipids (Hattori et al. 2012). Some membrane proteins also require the presence of native lipids co-purified with the protein to crystallize, examples of which include the glycerol-3-phosphate transporter (Lemieux et al. 2003) and the erythrocyte anion exchanger (Lemieux et al. 2002). The

use of FTIR spectroscopy to monitor the detergent content throughout protein purification may be useful in these cases because of its ability to quantitate lipids simultaneously.

The total detergent content of a membrane protein sample may be just as important as the protein content when attempting to reproduce an initial crystallization lead. Crystallization studies have shown direct correlations between the propensity of a sample to crystallize and its total detergent content. For example, during the crystallization of the membrane protein *E. coli* tyrosine kinase (Etk), crystals were only obtained when a minimum of 6.9 mg/mL protein and a minimum detergent content of 0.8 mg *n*-dodecyl-D-B-maltoside/mg protein was used (Prince and Jia 2012). Ben-Shem et al. (2003) also noticed a similar phenomenon and have reported that the ratio of detergent to chlorophyll during purification of plant photosystem I was essential to obtaining well-diffracting crystals. Based on these observations, it is recommended that the concentration of both protein and detergent be measured for every sample used in crystallization experiments. The phenol/dimethylphenol assays are desirable in this application because they require no pre-treatment, use a small sample volume, and have fast processing times which facilitate their use in routine measurements. The limitation of the phenol assays to sugar-based detergents is mitigated because $\sim$57 % of successful crystallizations have used this class of detergents (Membrane Protein Databank or MPDB—http://www.mpdb.tcd.ie).

Conclusions

Detergent content is a critical component of membrane protein samples that cannot be ignored during in vitro studies. Over the past decade, multiple methodologies for measuring the detergent content of membrane protein samples have been developed, each with their own advantages and disadvantages. The colorimetric methods are quick and simple, but are limited in application to specific classes of detergents. By contrast, more general techniques such as FTIR spectroscopy, TLC, and contact angle measurement are compatible with a wide range of detergents. Some of the methods presented in this review have been cross-validated to confirm their accuracy and precision. TLC provides good accuracy and precision when compared directly with the phenol assay (Eriks et al. 2003). The accuracy of the phenol assay and the contact angles technique have also been confirmed by direct comparison to measurements of radioactive *n*-dodecyl-β-D-maltoside (Urbani and Warne 2005; Kaufmann et al. 2006).

All the techniques presented here have excellent sensitivity and are able to detect the micrograms of detergent present in a small-volume membrane protein sample (Tables 1, 3). In order to use techniques with nanogram sensitivities, samples may need to be diluted before analysis. Unfortunately, the most sensitive techniques, radioactivity and gas chromatography, are often not the most practical for routine measurements. Ultimately, the choice of technique to use will be dependent on the needs of the experiment and many of these techniques could be incorporated into a high-throughput crystallization pipeline. Understandably, controlling detergent content alone is not sufficient to ensure growth of diffraction quality crystals for all proteins, and further adjustments to the PDC such as the addition of lipids may be required (Zhang et al. 2003). However, it should be emphasized that detergent concentration in the final crystallization sample is an important parameter for during membrane protein crystallization and monitoring this variable can improve the reproducibility of crystallization experiments.

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Conflict of interest The authors declare that they have no conflict of interest.

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