Purification of a Membrane Protein with Conjugated Engineered Micelles

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ABSTRACT: A novel method for purifying membrane proteins is presented. The approach makes use of *engineered micelles* composed of a nonionic detergent, *β*-octylglucoside, and a hydrophobic metal chelator, bathophenanthroline. *Via* the chelators, the micelles are specifically conjugated, i.e., tethered, in the presence of Fe^{2+} ions, thereby forming micellar aggregates which provide the environment for separation of lipid-soluble membrane proteins from water-soluble proteins. The micellar aggregates (here imaged by cryo-transmission electron microscopy) successfully purify the light driven proton pump, bacteriorhodopsin (bR), from *E. coli* lysate.

Purification takes place within 15 min and can be performed both at room temperature and at 4 °C. More than 94% of the water-soluble macromolecules in the lysate are excluded, with recovery yields of the membrane protein ranging between 74% and 85%. Since this approach does not require precipitants, high concentrations of detergent to induce micellar aggregates, high temperature, or changes in pH, it is suggested that it may be applied to the purification of a wide variety of membrane proteins.
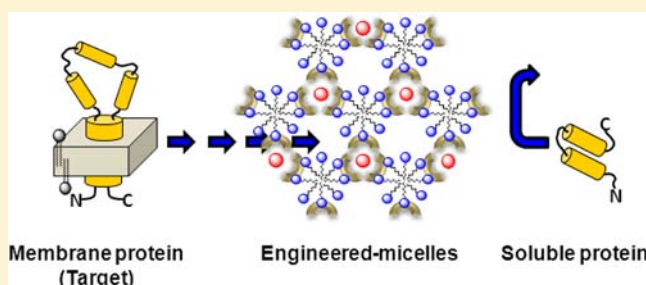
INTRODUCTION

Purification of membrane proteins is essential for their structural determination and commonly requires extraction from biological membranes using detergent molecules.[1,2] The detergents disrupt the membrane and, in parallel, surround and cover the hydrophobic domains of the protein, leading, under favorable conditions, to a water-soluble [detergent-lipid–protein] complex which is then purified by chromatography. However, identification of the particular detergent that will preserve the membrane protein in its functional native state is laborious as dozens of detergents are available and the ideal choice can only be determined experimentally.[3] Once an appropriate detergent is identified, membrane proteins can be purified, similarly to soluble proteins, but in the presence of a detergent. Purification can be accomplished either *via* classical chromatographic methods (e.g., gel filtration, ion exchange chromatography) or by genetically engineered affinity-tags which greatly simplify the isolation process and are commonly placed at the amino- or carboxy-terminal end.[4–7] Affinity tags may include His-tag,[8] Flag-tag,[9] HA-tag,[10] glutathione S-transferase, GST-tag,[11,12] or maltose binding protein-tag[13] and generally lead to a highly purified protein preparation within a single chromatographic step.[3] Among the different affinity-tags, His-tag is probably the most widely used[14] as it possesses sufficient affinity and specificity toward metal chelated resins (e.g., Ni-NTA), as well as requiring only mild

elution conditions.[15] Since affinity-tags may alter the properties of the membrane protein, they are removed at the end of the purification process.[16]

The presence of detergents throughout the purification process deserves special emphasis. Detergents, being amphipathic molecules, are driven by the hydrophobic effect to assemble spontaneously in water into noncovalent assemblies (*micelles*) when the concentration exceeds a broad threshold called the critical micelle concentration (cmc).[2,17–19] Further addition of detergent above the cmc ideally increases only the micelle concentration while keeping the free detergent concentration constant.[20] Micelles may undergo major structural alterations in the presence of an additional detergent, lipid, or protein[21] and will generally not interact with one another in dilute aqueous suspension.[1] However, micelles may cluster once physical or chemical modifications of the medium are induced. Such modifications include: (a) addition of polymeric or inorganic precipitants; (b) increase in ionic strength; or (c) temperature alteration.[1,2,22] Under these conditions, the solution can become turbid and a transient state called the *cloud point*,[2] containing micellar aggregates, is reached. Further micellar aggregation results in phase
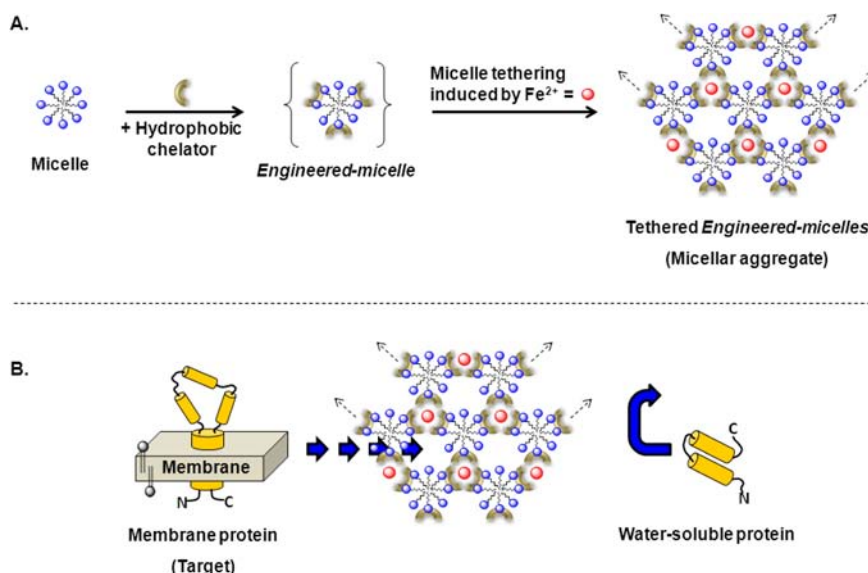
Figure 1. Illustration of tethered *engineered micelles* and their utilization in membrane protein purification. A. Micelles composed of nonionic detergents are transformed into *engineered micelles* upon incubation with a hydrophobic chelator and specifically cluster in the presence of Fe^{2+} ions, thereby forming micellar aggregates interconnected by [metal:chelator] complexes. B. Membrane proteins partition into the micellar aggregate (due to their hydrophobic character) whereas hydrophilic proteins do not.

separation and formation of two distinct phases: a detergent rich phase and a detergent poor phase.[1,2,23] This phenomenon is used for purification of membrane proteins from water-soluble proteins and is called *cloud point extraction*. The approach relies on the preferential partitioning of membrane proteins into the detergent rich phase due to their hydrophobic character.[24] The detergent rich phase is then subjected to an additional chromatographic step to allow purification of the target membrane protein from other membrane proteins that may be present.[24−26]

The key to success, therefore, relies on our ability to induce phase separation under conditions that preserve the functionality of the detergent solubilized membrane proteins. This, however, is a nontrivial task as many detergents used in membrane protein biochemistry phase separate only at (i) very high concentrations or (ii) elevated temperatures, both of which may be deleterious to the protein. However, such barriers may sometimes be successfully suppressed when high concentrations of water-soluble polymers (e.g., dextran, PEG-40000[27−29]) or precipitants (e.g., ammonium sulfate[30,31]) are added to the medium. Under these conditions, the detergent cloud point temperature is lowered and purification can be accomplished while preserving protein functionality.

Obviously, a simplified process would require only the detergent without additional precipitants or polymers. Unfortunately the number of detergents that meet these criteria is limited and those that are commonly used are Triton X-114 or poly(oxyethylene)-4-nonyl phenyl ether[32] where the former is probably the most prominent.[24] Triton X-114 is a nonionic detergent that reaches its cloud point at approximately 20 °C[33,34] and at concentrations compatible with diverse membrane proteins. Therefore, it provided an ideal system for process development by Bordier already in 1981,[35] and since then many membrane proteins have been conveniently purified at room temperate using this detergent.[36] The absence of other nonionic detergents that could be used similarly encouraged us to seek a general solution that would allow

utilization of a broad range of nonionic detergents regardless of their concentration or cloud point temperature.

We recently demonstrated that micelles composed of nonionic detergents and hydrophobic metal chelators, referred to as *engineered micelles,* can be tethered specifically with various divalent metal cations serving as mediators in the aqueous phase, and lead to micellar aggregates[37] (Figure 1A). The fact that such aggregates are generated at room temperature or at 4 °C suggested that they could potentially serve as purification scaffolds for membrane proteins. It was further speculated that gentle agitation of these aggregates with membranes containing the target protein might result in partitioning of the target into the aggregates whereas hydrophilic proteins would be excluded (Figure 1B).

■ EXPERIMENTAL PROCEDURES

Materials. Bathophenanthroline, octyl β-D-glucopyranoside (OG), NaCl, $FeSO_4$, and $ZnCl_2$ were obtained from Sigma-Aldrich (St. Louis, MO).

Preparation of Purple Membranes. *Halobacterium salinarum* was grown from the S9 strain, and purple membranes containing bacteriorhodopsin were isolated as previously described.[38]

Preparation of Conjugated *Engineered Micelles*. Conjugated *engineered micelles* are prepared by adding 10 μL of 20 mM bathophenanthroline in methanol at room temperature to 22 μL of 200 mM OG in water with continuous, vigorous vortexing for 10−15 s. Water (68 μL) is added immediately with continuous vortexing (10 s) and 45 μL aliquots of the resulting mixture are mixed with identical volumes of 1 mM $FeSO_4$ and 400 mM NaCl in water (kept on ice).

Purification of Bacteriorhodopsin (bR) with Conjugated *Engineered Micelles*. Freshly prepared tethered *engineered micelles* (90 μL) were allowed to stand at room temperature for 0−3 min, and only then, were *E. coli* lysate (10−25 μL) and purple membranes containing bR at 6.25 mg/mL (1−2 μL) slowly added without vortexing. The system was

gently agitated with an orbital shaker (300 rounds per minute) for 15 min at 4 °C/room temperature in the dark. Samples were then briefly centrifuged (2 min at 985 × g) and the majority of the supernatant (90 μL) was carefully discarded. The resulting red pellet was briefly washed with cold 200 mM NaCl (90 μL), an additional spin (2 min at 3935 × g) was applied, the supernatant was discarded, and the pellet composition was analyzed by SDS-PAGE.

Light Microscopy. Light microscope images were obtained using an Olympus CX40 microscope.

UV Spectroscopy. Absorption measurements were performed using the HP 8453 UV−Vis spectrophotometer.

Cryotransmission Electron Microscopy (Cryo-TEM). The cryo-TEM images were obtained using an FEI T12 G^2 Cryo-TEM, operating at 120 kV. Images were recorded under low dose conditions as described previously.[39,40]

■ RESULTS

Preparation and Analysis of Red Oily Globules. When the hydrophobic metal chelator, bathophenanthroline (1 mM), was added to an aqueous suspension containing 22 mM OG in 200 mM NaCl, red oily globules appeared a few minutes after addition of Fe^{2+} (0.5 mM) (Figure 2A). The diameter of the globules ranged between 0.1 and 0.01 mm (Figure 2A); the color indicated that they contained the red [(bathophenanthroline)$_3$:Fe^{2+}] complex.
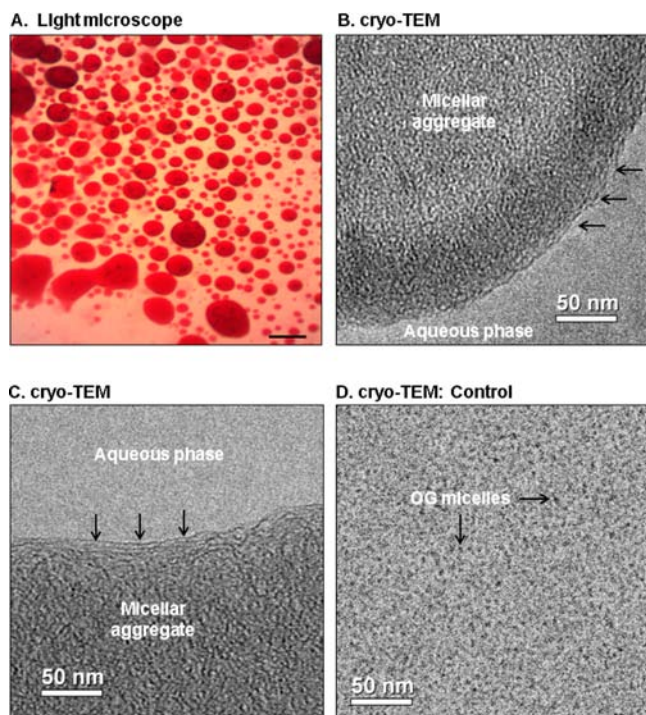

Figure 2. Light microscope and cryo-TEM images of tethered *engineered micelles*. A. Light microscope image of tethered *engineered micelles* consisting of [OG:batho:Fe^{2+}] taken a few minutes after addition of the metal. The scale bar represents 0.1 mm. B−C. Cryo-TEM images of tethered *engineered micelles* described in A. Black arrows point to the micellar aggregate-aqueous phase interface. D. Cryo-TEM image of OG micelles in the absence of the [batho:Fe^{2+}] complex. Numerous, individual OG micelles are observed as dark-shaded dots against a more lightly colored background (arrows point to representative OG micelles).

Analysis of these globules at higher resolution was achieved by cryo-TEM (Figure 2B−D). The results show that conjugation of OG micelles leads to the formation of densely packed, entangled, thread-like micelles with a defined boundary between the micellar aggregate and the aqueous phase (Figure 2B−C). In the absence of the [(bathophenanthroline)$_3$:Fe^{2+}] complex, no red oily globules were observed by light microscopy (data not shown) and cryo-TEM analysis detected the presence of numerous dispersed and independent OG micelles (Figure 2D). Additional control experiments indicated that micellar aggregates are not generated when either the chelator or the metal are absent; only individual, dispersed micelles were obtained (data not shown).

Purification of Bacteriorhodopsin (bR). Purification of the target membrane protein (bR) was performed in two steps: (i) preparation of conjugated *engineered micelles* composed of [OG:bathophenanthroline:Fe^{2+}]; (ii) addition of purple membranes containing bR and *E. coli* lysate (serving as an artificial background) to the preformed micellar aggregate. Efficient partitioning of bR into the detergent aggregate occurred either at room temperature (Figure 3A) or at 4 °C (Figure 3B) and required the presence of both the hydrophobic metal chelator (bathophenanthroline) and the metal ion (Fe^{2+}).

Spectroscopic Characterization of Partially Purified bR. To determine whether bR was affected by the high detergent concentration present within the micellar aggregates, the latter were resuspended at a concentration of 50−100 mM OG and the absorption spectrum of the protein was compared to that of bR present within its native, purple membrane environment. Purification of bR was performed as described above, but, in the presence of Zn^{2+}, so as to avoid the red color of the [(bathophenanthroline)$_3$:Fe^{2+}] complex which was found to overlap the absorption maximum of bR at 568 nm. No significant difference could be found between the absorption spectrum of bR within its native membrane and that of the protein which had partitioned into the micellar aggregate (Figure 3C). Similarly, no major spectroscopic changes were identified when bR was kept within the micellar aggregate overnight at 4 °C (Figure 3C).

■ DISCUSSION

The nonionic detergent β-octylglucoside (OG) was chosen as the test detergent since it is extensively used in the purification, reconstitution, and crystallization of integral membrane proteins. Preparation of the [OG:batho:Fe^{2+}] tethered, engineered micelles, led to the formation of red oily globules, as detected by light microscopy (Figure 2A). Imaging of these red oily globules by cryo-TEM demonstrated that micellar aggregates were formed only in the presence of the [(bath-o)$_3$:Fe^{2+}] complex (Figure 2B−C). The field was covered with micrometer-sized regions of electron-dense material alternating with amorphous ice. The boundaries between these regions and the bulk solvent background were sharp and distinct, showing the presence of thread-like micelles at the edges of the large micellar aggregates (black arrows in Figure 2B−C). In the absence of the [(batho)$_3$:Fe^{2+}] complex, such well-defined electron-dense regions did not form, even when the OG surfactant concentration was raised 5-fold. A control sample contained only small spherical micelles (Figure 2D).

These findings paved the way for purification trials with purple membranes containing the light driven proton pump, bacteriorhodopsin (bR) from *Halobacterium salinarum*.[41,42] bR is a small integral membrane protein (26 kDa) containing a
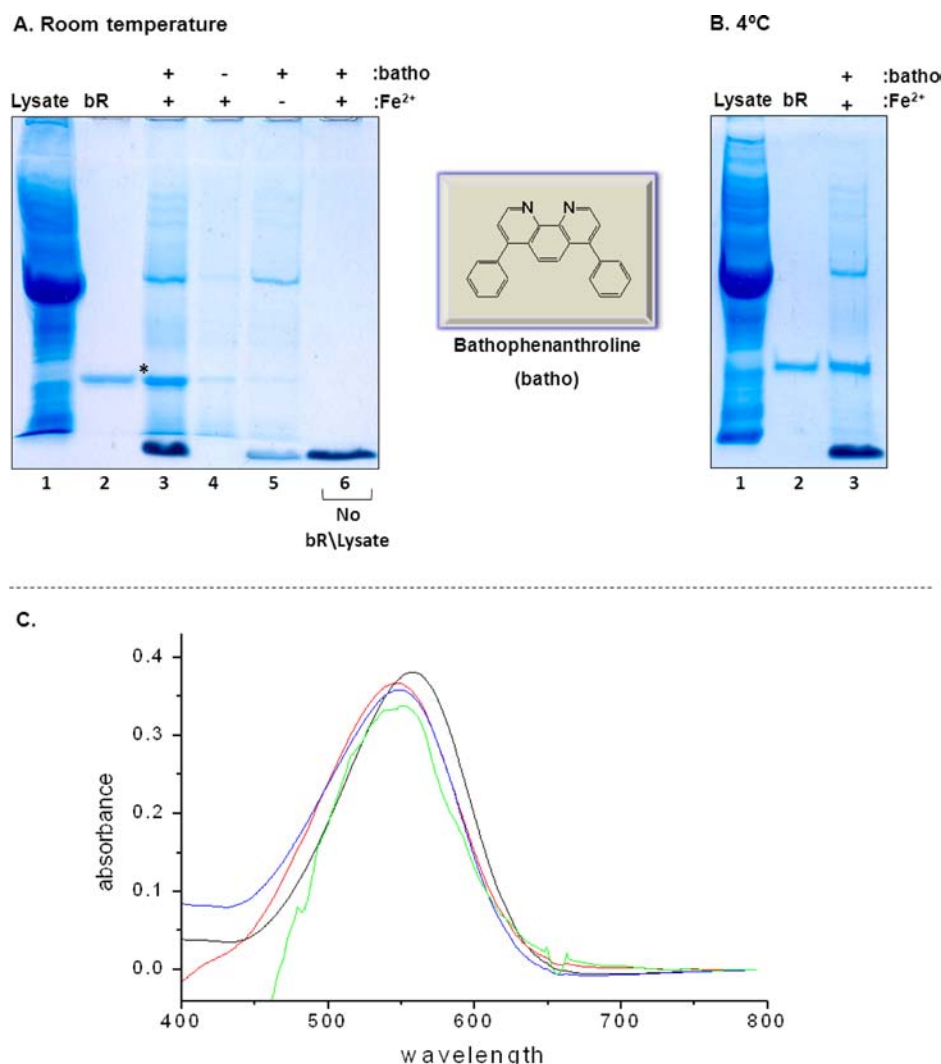
Figure 3. Analysis of the pellet composition by SDS-PAGE (A,B) and the absorption spectrum of purified bR (C). A. Purification of bR at room temperature: lane 1, *E. coli* lysate; lane 2, Purple membranes containing bR; lane 3, Pellet content after purification with the $[(batho)_3:Fe^{2+}]$ complex; lane 4, as in lane 3 but in the absence of batho; lane 5, as in lane 3 but in the absence of Fe^{2+}; lane 6, as in lane 3 but in the absence of added proteins. The asterisk in lane 3 identifies bR as being present in the pellet. B. Purification of bR at 4 °C: lanes 1−3 as in A respectively. C. Absorption spectrum of bR: not subjected to purification in the presence of 100 mM OG (black line); absorption spectra of bR following purification and dissolution in 100 mM OG (blue line), 50 mM OG (green line), or 100 mM OG with incubation overnight at 4 °C (red line). Purification was carried out in the presence of Zn^{2+} rather than Fe^{2+} in order to circumvent the overlapping absorption of the $[(batho)_3:Fe^{2+}]$ complex.

retinal chromophore covalently bound *via* a protonated Schiff-base to Lys216. The protein utilizes light energy for the active transport of protons from the cytosol to the extracellular side of the membrane and uses the generated proton gradient for the synthesis of ATP by ATP synthase. Following micellar aggregate formation, the *E. coli* lysate (serving only as an artificial background and not as an expression host), and purple membranes were added to the aqueous dispersion and were incubated either at room temperature or 4 °C. The presence of the target membrane protein, bR, in the centrifuge pellet was clearly identified by SDS-PAGE (Figure 2A, see asterisk); most water-soluble molecules (>94%, by densitometry) were excluded. These results are consistent with our working hypothesis as they show that a membrane protein does indeed partition into the micellar aggregate, whereas most water-soluble proteins present in the lysate do not. Recovery yields for bR as measured by densitometry ranged between 74% and 85%.

The absolute requirement for the $[(batho)_3:Fe^{2+}]$ complex was demonstrated by repeating the process in the absence of either the chelator (Figure 3A) or the metal ion (Figure 3A). The results clearly indicate that both are critical components for the process. An unexpected band with a very low molecular weight appeared in both lanes 3 and 4, and was shown to be composed of the [detergent:chelator:metal] complex (i.e., $[OG:batho:Fe^{2+}]$ since it could be generated in the absence of bR or the lysate (Figure 3A). The fact that similar results were observed when purification was performed at 4 °C (Figure 3B) suggests that (a) the process may be used with temperature-labile membrane proteins; and (b) purification of membrane proteins via phase separation may now be performed with other detergents of the alkyl-glucoside family in spite of the elevated temperatures (up to 100 °C) generally required to induce their phase separation.[32,43]

The purity of the bR protein is not very high since the method can only separate water-soluble and membrane proteins. Additional bands in the gel pictured in lane 3 of

Figure 3A,B are due to lipophilic macromolecules from the *E. coli* lysate which also partitioned into the micellar aggregates. However, the fact that more than 94% of the background due to the lysate is excluded, and the volume of the sample is reduced ~20-fold should simplify the final polishing step. Pellets containing the partially purified bR could then be dissolved in small volumes (30−75 μL) of 50−100 mM OG in 200 mM NaCl and applied directly to a chromatographic column for the final purification step. However, the fact that bR encountered high detergent concentrations also required demonstration of its integrity. To measure the absorption spectrum of bR, purification was performed in the presence of the colorless $[(batho)_3:Zn^{2+}]$ complex (not shown) instead of the red $[(batho)_3:Fe^{2+}]$ complex as the latter overlaps the absorption maximum of bR at 568 nm. No significant changes in the absorption spectra of purified bR were observed (Figure 3C), indicating that bR is stable following the dissolution process and its integration into the micellar aggregate. Moreover, the exceptional stability of bR[44] *was* apparent when samples containing purified bR were kept overnight in 100 mM OG and 200 mM NaCl at 4 °C (in the dark); again, major changes in the absorption spectrum were not observed (Figure 3C, red line).

These results demonstrate several advantages which seem to be inherent to the approach presented here. They include the following: (a) promoting phase separation under very mild conditions—in this study, OG was induced to undergo phase separation at room temperature or 4 °C and at a concentration (22 mM) close to its critical micelle concentration (18−19 mM);[45] (b) the absence of precipitants (e.g., ammonium sulfate) or water-soluble polymers (e.g., dextran) from the process—no such additives were required to induce phase separation, thus reducing the system complexity; (c) extraction of the target membrane protein by micellar aggregates—bR partitioned into the micellar aggregates without any prior disruption of the membrane with detergents; (d) simple detection of the detergent-rich phase—the red color of the $[(batho)_3:Fe^{2+}]$ complex serves as a convenient indicator for the detergent rich phase containing the target protein. In addition, this feature provides a practical means for evaluating precipitation efficiency and loss of protein during pellet washing.

The method may have three potential limitations. (i) Although micellar conjugation is induced at low detergent concentration, the significantly higher concentration in the conjugated micelles may, in some cases, cause protein denaturation. This difficulty, however, is common to all purification strategies employing detergent phase separation. (ii) Purification of His-tagged proteins in the presence of a hydrophobic metal chelator may require defining conditions for efficient protein binding to the immobilized metals present on chromatographic media. (iii) The hydrophobic chelator should be removed from the protein. This may be accomplished in a similar manner to the way detergents are exchanged for other detergents while the target protein is bound to chromatographic media.

CONCLUSIONS

The utility of tethered *engineered micelles* composed of β-octylglucoside and the chelator:metal complex $[(batho)_3:Fe^{2+}]$ in the purification of bacteriorhodopsin was demonstrated. The fact that other nonionic detergents undergo phase separation under similar mild conditions[37] implies that they may be used

similarly for the successful isolation of diverse membrane proteins. This, however, remains to be shown.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS

batho, bathophenanthroline; bR, bacteriorhodopsin; cmc, critical micelle concentration; cryo-TEM, cryo transmission electron microscopy; OG, β-D-glucoside; PEG, poly ethylene glycol; Ni-NTA, nickel nitrilotriacetic acid

REFERENCES

(1) Loll, P. J., Allaman, M., and Wiencek, J. (2001) Assessing the role of detergent-detergent interactions in membrane protein crystallization. *J. Cryst. Growth* 232, 432−438.

(2) Garavito, R. M., and Ferguson-Miller, S. (2001) Detergents as tools in membrane biochemistry. *J. Biol. Chem.* 276, 32403−32406.

(3) Loll, P. J. (2003) (2003) Membrane protein structural biology: the high throughput challenge (2003). *J. Struct Biol.* 142, 144−153.

(4) Nilsson, J., Stahl, S., Lundeberg, J., Uhlen, M., and Nygren, P. A. (1997) Affinity fusion strategies for detection, purification, and immobilization of recombinant proteins. *Protein Expression Purif.* 11, 1−16.

(5) Sheibani, N. (1999) Prokaryotic gene fusion expression systems and their use in structural and functional studies of proteins. *Prep. Biochem. Biotechnol.* 29, 77−90.

(6) Hearn, M. T., and Acosta, D. (2001) Applications of novel affinity cassette methods: use of peptide fusion handles for the purification of recombinant proteins. *J. Mol. Recognit.* 14, 323−369.

(7) Walian, P., Cross, T. A., and Jap, B. K. (2004) Structural genomics of membrane proteins. *Genome Biol.* 5, 215.

(8) Hochuli, E., Bannwarth, W., Döbeli, H., Gentz, R., and Stüber, D. (1988) Genetic approach to facilitate purification of recombinant proteins with a novel metal chelate adsorbent. *Bio/Technology* 6, 1321−1325.

(9) Brizzard, B. L., Chubet, R. G., and Vizard, D. L. (1994) Immunoaffinity purification of FLAG® epitope-tagged bacterial alkaline phosphatase using a novel monoclonal antibody and peptide elution. *BioTechniques* 16, 730−734.

(10) Chen, Y. T., Holcomb, C., and Moore, H. P. (1993) Expression and localization of two low molecular weight GTP-binding proteins, Rab8 and Rab10, by epitope tag. *Proc. Natl. Acad. Sci. U.S.A.* 90, 6508−6512.

(11) Simons, P. C., and Vander Jagt, D. L. (1981) Purification of glutathione S-transferases by glutathione-affinity chromatography. *Methods Enzymol.* 77, 235−237.

(12) Smith, D. B., and Johnson, K. S. (1988) Single-site purification of polypeptides expressed in *Escherichia coli* as fusions with glutathione S-transferase. *Gene* 67, 31−40.

(13) Kellerman, O. K., and Ferenci, T. (1982) Maltose binding protein from *Escherichia coli*. *Methods Enzymol.* 90, 459−463.

(14) Structural Genomics Consortium (2008) Protein production and purification. *Nat. Methods 5*, 135−146.

(15) Lesley, S. A. (2001) High-throughput proteomics: protein expression and purification in the postgenomic world. *Protein Expression Purif. 22*, 159−164.

(16) Werten, P. J., Rémigy, H. W., de Groot, B. L., Fotiadis, D., Philippsen, A., Stahlberg, H., Grubmüller, H., and Engel, A. (2002) Progress in the analysis of membrane protein structure and function. *FEBS Lett. 529*, 65−72.

(17) Lindman, B., and Wennerström, H. (1980) Micelles. Amphiphile aggregation in aqueous solution. *Top. Curr. Chem. 87*, 1−87.

(18) Tanford, C. (1980) *The Hydrophobic Effect*, Wiley, New York.

(19) Rosen, M. J. (1989) *Surfactants and Interfacial Phenomena*, John Wiley & Sons, Inc., New York.

(20) Gunnarsson, G., Jonsson, B., and Wennerström, H. (1980) Surfactant association into micelles. An electrostatic approach. *J. Phys. Chem. 84*, 3114−3121.

(21) Lambert, O., Levy, D., Ranck, J. L., Leblanc, G., and Rigaud, J. L. (1998) A new "gel-like" phase in dodecyl maltoside-lipid mixtures: implications in solubilization and reconstitution studies. *Biophys. J. 74*, 918−930.

(22) Israelachvili, J. N. (1991) *Intermolecular and Surface Forces*, Academic Press, London.

(23) Neugebauer, J. M. (1990) Detergents: an overview. *Methods Enzymol. 182*, 239−253.

(24) Arnold, T., and Linke, D. (2007) Phase separation in the isolation and purification of membrane proteins. *Biotechniques 43*, 427−440.

(25) Wissing, J., Heim, S., Flohe, L., Bilitewski, U., and Frank, R. (2000) Enrichment of hydrophobic proteins via Triton X-114 phase partitioning and hydroxyapatite column chromatography for mass spectrometry. *Electrophoresis 21*, 2589−2593.

(26) Everberg, H., Leiding, T., Schioth, A., Tjerneld, F., and Gustavsson, N. (2006) Efficient and non-denaturing membrane solubilization combined with enrichment of membrane protein complexes by detergent/polymer aqueous two-phase partitioning for proteome analysis. *J. Chromatogr., A 1122*, 35−46.

(27) Albertsson, P. A. (1973) Application of the phase partition method to a hydrophobic membrane protein, phospholipase A1 from Escherichia coli. *Biochemistry 12*, 2525−2530.

(28) Lukoyanova, M. A., and Petukhova, N. M. (1976) Use of phase separation in polymers for fractionation of hydrophobic membrane proteins. *Biochemistry (Mosc.) 41*, 1468−1475.

(29) Sivars, U., and Tjerneld, F. (2000) Mechanisms of phase behaviour and protein partitioning in detergent/polymer aqueous two-phase systems for purification of integral membrane proteins. *Biochim. Biophys. Acta 1474*, 133−146.

(30) Fricke, B. (1993) Phase separation of nonionic detergents by salt addition and its application to membrane proteins. *Anal. Biochem. 212*, 154−159.

(31) Parish, C. R., Classon, B. J., Tsagaratos, J., Walker, I. D., Kirszbaum, L., and McKenzie, I. F. (1986) Fractionation of detergent lysates of cells by ammonium sulphate-induced phase separation. *Anal. Biochem. 156*, 495−502.

(32) Tani, H., Kamidate, T., and Watanabe, H. (1998) Aqueous micellar two-phase systems for protein separation. *Anal. Sci. 14*, 875−888.

(33) Goldfarb, J., and Sepulveda, L. (1969) Application of a theory of polymer solutions to the cloud points of nonionic detergents. *J. Colloid Interface Sci. 31*, 454−459.

(34) Hinze, W. L., and Pramauro, E. (1993) A critical-review of surfactant-mediated phase separations (cloud-point extractions)-theory and applications. *Crit. Rev. Anal. Chem. 24*, 133−177.

(35) Bordier, C. (1981) Phase separation of integral membrane proteins in Triton X-114 solution. *J. Biol. Chem. 256*, 1604−1607.

(36) Sanchez-Ferrer, A., Bru, R., and Garcia-Carmona, F. (1994) Phase separation of biomolecules in polyoxyethylene glycol nonionic detergents. *Crit. Rev. Biochem. Mol. Biol. 29*, 275−313.

(37) Patchornik, G., Namboothiri, I. I. N., Nair, N. K., Wachtel, E., and Persky, R. (2012) Tethered non-ionic micelles: a matrix for enhanced solubilization of lipophilic Compounds. *Soft Matter 8*, 8456−8463.

(38) Oesterhelt, D., and Stoeckenius, D. (1974) *Methods Enzymol. 31* (Pt A), 667−678.

(39) Danino, D., Bernheim-Groswasser, A., and Talmon, Y. (2001) Digital Cryogenic Transmission Electron Microscopy: an Advanced Tool for Direct Imaging of Complex Fluids. *Colloids Surf., A 183*, 113−122.

(40) Danino, D. (2012) Cryo-TEM of Soft Molecular Assemblies. *Curr. Opin. Colloid Interface Sci. 17*, 316−329.

(41) Subramaniam, S., and Henderson, R. (2002) Molecular mechanism of vectorial proton translocation by bacteriorhodopsin. *Nature 406*, 653−657.

(42) Kühlbrandt, W. (2000) Bacteriorhodopsin-the movie. *Nature 406*, 569−570.

(43) Zulauf, M. (1991) Detergent phenomena in membrane protein crystallization, in *Crystallization of membrane proteins* (Michel, H., Ed.) pp 53−72, CRC Press, Boca Raton.

(44) Essen, L.-O., Siegert, R., Lehmann, W. D., and Oesterhelt, D. (1998) Lipid patches in membrane protein oligomers: Crystal structure of the bacteriorhodopsin-lipid complex. *Proc. Natl. Acad. Sci. U.S.A. 95*, 11673−11678.

(45) Nauli, S., Farr, S., Lee, Y. J., Kim, H. Y., Faham, S., and Bowie, J. U. (2007) Polymer-driven crystallization. *Protein Sci. 16*, 2542−2551.