detergent concentrations can denature protein or limit diffraction (Wiener, 2004). As a general rule, the highest-molecular-weight cutoff membrane that does not permit passage of the target protein should be used, as this will allow the passage of at least some protein-free detergent micelles. Also, any size-exclusion steps should be performed at a relatively high protein concentration (10–20 mg ml^{-1} or higher) to minimize volumes. Purified protein may also be dialysed to exchange non-detergent buffer components, so long as the detergent concentration of the bulk solution is maintained just above the CMC. However, the dialysis of many detergents will proceed slowly (days to weeks). Accordingly, dialysis does not appear to be a widely used method for controlling detergent concentration prior to crystallization (reviewed by Willis & Koth, 2008).

3.2.4. Common purification pitfalls and prioritized alternative strategies

3.2.4.1. Poor solubility of the target protein

If the target protein does not remain soluble during purification, an obvious alternative strategy is to test different detergents or detergent combinations. Given that just a handful of different detergents have been used to solubilize and crystallize most membrane proteins, testing alternatives is usually not a daunting task. The most common method of detergent exchange is SEC (Fig. 3.2.3.1).

The zwitterionic lyso-lipid mimetic detergents such as FOS-CHOLINE 12 (dodecylphosphatidylcholine) or 14 (FC12 and FC14; Anatrace, Maumee, Ohio, USA) will solubilize many membrane proteins (Eshaghi *et al.*, 2005) and there are a few reports of their use in crystallography [MscS mechanosensitive channel (Bass *et al.*, 2002); protein-conducting channel (van den Berg *et al.*, 2004)]. Should solubilization screening with the more commonly used detergents prove ineffective, it may be possible to solubilize first with a 'stronger' more lipid-like detergent, such as FC12, and then exchange to a non-charged detergent, such as DDM, during purification.

3.2.4.2. The isolated target protein does not crystallize

Although an in-depth analysis of membrane-protein crystallization methods is beyond the scope of this chapter (see instead Chapter 4.2), there are many purification parameters that can be varied to improve crystallization outcomes should initial trials fail to yield 'hits' or well diffracting crystals. Perhaps the foremost method is to exchange the protein into an alternative detergent or detergent mixture, typically by SEC, and repeat the crystal trials. Since the vast majority of membrane-protein structures have used one of only a handful of detergents (see above), it is likely that sampling even just a small number of other detergents would dramatically increase the likelihood of obtaining or optimizing crystals. Also, an increase in the alkyl-chain length by one methylene group (*i.e.* nonyl- *versus* octyl-β-D-glucoside) often leads to an increase in protein stability, and this too can improve crystallization outcomes (Wiener, 2004). If significant covalent disulfide aggregation of the protein is observed during purification, alkylation of free cysteines should be considered during the early stages of target-protein isolation. Other parameters that could be varied include the solution pH, salt concentration and temperature, or supplementing with known ligands, to name just a few.

3.2.5. Summary

There are many possible routes for the expression and isolation of membrane proteins for structural studies, and many factors can affect the likelihood of obtaining diffraction-quality crystals. The daunting prospect for almost any new membrane target is that every stage, including expression, solubilization, purification and crystallization, will prove challenging. In this light, it is encouraging that remarkably similar techniques have been employed for many successfully crystallized membrane targets, including, for example, the observation that most structures have been solved using just a handful of different detergents throughout the isolation and crystallization steps. It is equally significant that simple two-step purification strategies predominate. Clearly, new methods for the production of membrane proteins will continue to be developed. Nevertheless, for a field that is quite likely to be entering an acute phase of growth, a solid foundation has been established for reasonable 'first-try' strategies.

References

Bass, R. B., Strop, P., Barclay, M. & Rees, D. C. (2002). *Crystal structure of Escherichia coli MscS, a voltage-modulated and mechanosensitive channel. Science*, **298**, 1582–1587.

Berg, B. van den, Clemons, W. M., Collinson, I., Modis, Y., Hartmann, E., Harrison, S. C. & Rapoport, T. A. (2004). *X-ray structure of a protein-conducting channel. Nature (London)*, **427**, 36–44.

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. & Zardecki, C. (2002). *The Protein Data Bank. Acta Cryst.* D**58**, 899–907.

Carpenter, E. P., Beis, K., Cameron, A. D. & Iwata, S. (2008). *Overcoming the challenges of membrane protein crystallography. Curr. Opin. Struct. Biol.* **18**, 581–586.

Carson, M., Johnson, D. H., McDonald, H., Brouillette, C. & DeLucas, L. J. (2007). *His-tag impact on structure. Acta Cryst.* D**63**, 295–301.

Chang, G., Spencer, R. H., Lee, A. T., Barclay, M. T. & Rees, D. C. (1998). *Structure of the MscL homolog from Mycobacterium tuberculosis: a gated mechanosensitive ion channel. Science*, **282**, 2220–2226.

Chen, Y. J., Pornillos, O., Lieu, S., Ma, C., Chen, A. P. & Chang, G. (2007). *X-ray structure of EmrE supports dual topology model. Proc. Natl Acad. Sci. USA*, **104**, 18999–19004.

Cherezov, V., Rosenbaum, D. M., Hanson, M. A., Rasmussen, S. G., Thian, F. S., Kobilka, T. S., Choi, H. J., Kuhn, P., Weis, W. I., Kobilka, B. K. & Stevens, R. C. (2007). *High-resolution crystal structure of an engineered human beta2-adrenergic G protein-coupled receptor. Science*, **318**, 1258–1265.

Christendat, D., Yee, A., Dharamsi, A., Kluger, Y., Savchenko, A., Cort, J. R., Booth, V., Mackereth, C. D., Saridakis, V., Ekiel, I., Kozlov, G., Maxwell, K. L., Wu, N., McIntosh, L. P., Gehring, K., Kennedy, M. A., Davidson, A. R., Pai, E. F., Gerstein, M., Edwards, A. M. & Arrowsmith, C. H. (2000). *Structural proteomics of an archaeon. Nat. Struct. Biol.* **7**, 903–909.

Cortes, D. M. & Perozo, E. (1997). *Structural dynamics of the Streptomyces lividans K+ channel (SKC1): oligomeric stoichiometry and stability. Biochemistry*, **36**, 10343–10352.

Day, P. W., Rasmussen, S. G., Parnot, C., Fung, J. J., Masood, A., Kobilka, T. S., Yao, X. J., Choi, H. J., Weis, W. I., Rohrer, D. K. & Kobilka, B. K. (2007). *A monoclonal antibody for G protein-coupled receptor crystallography. Nat. Methods*, **4**, 927–929.

Dobrovetsky, E., Lu, M. L., Andorn-Broza, R., Khutoreskaya, G., Bray, J. E., Savchenko, A., Arrowsmith, C. H., Edwards, A. M. & Koth, C. M. (2005). *High-throughput production of prokaryotic membrane proteins. J. Struct. Funct. Genomics*, **6**, 33–50.

Dobrovetsky, E., Menendez, J., Edwards, A. M. & Koth, C. M. (2007). *A robust purification strategy to accelerate membrane proteomics. Methods*, **41**, 381–387.

Doyle, D. A., Morais Cabral, J., Pfuetzner, R. A., Kuo, A., Gulbis, J. M., Cohen, S. L., Chait, B. T. & MacKinnon, R. (1998). *The structure of the*