Unfortunately, general methods for producing substantial amounts of membrane proteins using heterologous expression systems in a native membrane environment do not exist, nor is refolding of membrane proteins from inclusion bodies well established. Once a pure and homogeneous preparation of a membrane protein has been obtained, its stability in various detergents has to be investigated at different pH values. Frequently, a sharp optimum pH for stability is observed with detergents of shorter alkyl-chain length. In crystallization attempts, the usual parameters (nature of the precipitant, buffer, pH, addition of inhibitors and/or substrates, see Chapter 4.1) should be varied. The most important variable, however, is the detergent. Detergents can be exchanged most conveniently by binding the membrane protein to column materials (*e.g.* ion-exchange resins, hydroxyapatite, affinity matrices), washing with a buffer containing the new detergent at concentrations above the CMC under non-eluting conditions, and then eluting the bound membrane protein with a buffer containing the new detergent. The completeness of the detergent exchange should be checked. For this purpose, the use of ^{14}C- or ^{3}H-labelled detergents is recommended. Washing with about 20 column volumes is frequently required for a complete detergent exchange. It is more difficult to exchange a detergent with a low CMC (this means long alkyl chains) against a detergent with a high CMC (shorter alkyl chains). The detergent can often be exchanged during a final step in the purification. Less satisfying methods for detergent exchange are molecular-sieve chromatography, ultracentrifugation or repeated ultrafiltration and dilution. If the amount of membrane protein is limited, it is advisable to restrict the usual parameters to a set of, *e.g.*, 48 standard combinations with respect to precipitating agent, pH, buffer *etc.*, but to try *all* available detergents. If crystals of insufficient quality or size are obtained, trying the antibody Fv fragment approach is highly recommended. Alternatives are to use a different source (species) for the membrane protein under investigation, or to remove flexible parts of the membrane protein by proteolytic digestion. I am convinced that 50% of all membrane proteins will yield well ordered, three-dimensional crystals within five years, once the problem of obtaining a pure, stable and homogeneous preparation has been solved.

References

Allen, J. P., Feher, G., Yeates, T. O., Komiya, H. & Rees, D. C. (1987). *Structure of the reaction center from Rhodobacter sphaeroides R-26: the protein subunits. Proc. Natl Acad. Sci. USA*, **84**, 6162–6166.

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

Buchanan, S. K., Fritzsch, G., Ermler, U. & Michel, H. (1993). *New crystal form of the photosynthetic reaction centre from Rhodobacter sphaeroides of improved diffraction quality. J. Mol. Biol.* **230**, 1311–1314.

Buchanan, S. K., Smith, B. S., Venkatrami, L., Xia, D., Esser, L., Palnitkar, M., Chakraborty, R., van der Helm, D. & Deisenhofer, J. (1999). *Crystal structure of the outer membrane active transporter FepA from Escherichia coli. Nature Struct. Biol.* **6**, 56–63.

Chang, C. H., El-Kabbani, D., Tiede, D., Norris, J. & Schiffer, M. (1991). *Structure of the membrane-bound protein photosynthetic reaction center from Rhodobacter sphaeroides. Biochemistry*, **30**, 5352–5360.

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.

Cowan, S. W., Garavito, R. M., Jansonius, J. N., Jenkins, J. A., Karlsson, R., König, N., Pai, E. F., Pauptit, R. A., Rizkallah, P. J., Rosenbusch, J. P., Rummel, G. & Schirmer, T. (1995). *The structure of OmpF porin in a tetragonal crystal form. Structure*, **3**, 1041–1050.

Cowan, S. W., Schirmer, T., Rummel, G., Steiert, M., Gosh, R., Pauptit, R. A., Jansonius, J. N. & Rosenbusch, J. P. (1992). *Crystal structures explain functional properties of two E. coli porins. Nature (London)*, **358**, 727–733.

Deisenhofer, J., Epp, O., Miki, K., Huber, R. & Michel, H. (1985). *Structure of the protein subunits in the photosynthetic reaction center of Rhodopseudomonas viridis at 3 Å. Nature (London)*, **318**, 618–642.

Deisenhofer, J., Epp, O., Sinning, I. & Michel, H. (1995). *Crystallographic refinement at 2.3 Å resolution and refined model of the photosynthetic reaction centre from Rhodopseudomonas viridis. J. Mol. Biol.* **246**, 429–457.

Doyle, D. A., Cabral, J. M., Pfuetzner, R. A., Kuo, A. L., Gulbis, J. M., Cohen, S. L., Chait, B. T. & MacKinnon, R. (1998). *The structure of the potassium channel: molecular basis of K^+ conduction and selectivity. Science*, **280**, 69–77.

Ermler, U., Fritzsch, G., Buchanan, S. K. & Michel, H. (1994). *Structure of the photosynthetic reaction centre from Rhodobacter sphaeroides at 2.65 Å resolution: cofactors and protein–cofactor interactions. Structure*, **2**, 925–936.

Essen, L. O., Siegert, R., Lehmann, W. D. & Oesterhelt, D. (1998). *Lipid patches in membrane protein oligomers – crystal structure of the bacteriorhodpsin–lipid complex. Proc. Natl Acad. Sci. USA*, **95**, 11673–11678.

Ferguson, A. D., Hofmann, E., Coulton, J. W., Diederichs, K. & Welte, W. (1998). *Siderophore mediated iron transport: crystal structure of FhuA with bound lipopolysaccharide. Science*, **282**, 2215–2220.

Forst, D., Welte, W., Wacker, T. & Diederichs, K. (1998). *Structure of the sucrose-specific porin ScrY from Salmonella typhimurium and its complex with sucrose. Nature Struct. Biol.* **5**, 37–46.

Fromme, P. & Witt, H. T. (1998). *Improved isolation and crystallization of photosystem I for structural analysis. Biochim. Biophys. Acta*, **1365**, 175–184.

Gast, P., Hemelrijk, P. & Hoff, A. J. (1994). *Determination of the number of detergent molecules associated with the reaction center protein isolated from the photosynthetic bacterium Rhodopseudomonas viridis. Effects of the amphiphilic molecule, 1,2,3-heptanetriol. FEBS Lett.* **337**, 39–42.

Gerstein, M. (1998). *Patterns of protein-fold usage in eight microbial genomes: a comprehensive structural census. Proteins*, **33**, 518–534.

Grigorieff, N., Ceska, T. A., Downing, K. H., Baldwin, J. M. & Henderson, R. (1996). *Electron crystallographic refinement of the structure of bacteriorhodopsin. J. Mol. Biol.* **259**, 393–421.

Henderson, R., Baldwin, J. M., Ceska, T. A., Zemlin, F., Beckmann, E. & Downing, K. H. (1990). *Model for the structure of bacteriorhodopsin based on high-resolution electron cryo-microscopy. J. Mol. Biol.* **213**, 899–929.

Henderson, R. & Shotton, D. (1980). *Crystallization of purple membrane in three dimensions. J. Mol. Biol.* **139**, 99–109.

Hirsch, A., Breed, J., Saxena, K., Richter, O. M. H., Ludwig, B., Diederichs, K. & Welte, W. (1997). *The structure of porin from Paracoccus denitrificans at 3.1 Å resolution. FEBS Lett.* **404**, 208–210.

Hjelmeland, L. M. (1990). *Solubilization of native membrane proteins. Methods Enzymol.* **182**, 253–264.

Hunte, C., Lange, C., Koepke, J., Rossmanith, T. & Michel, H. (2000). *Structure at 2.3 Å resolution of the cytochrome bc_1 complex from the yeast Saccharomyces cerevisiae co-crystallized with an antibody Fv fragment. Structure*, **8**, 669–684.

Iwata, S., Lee, J. W., Okada, K., Lee, J. K., Iwata, M., Rasmussen, B., Link, T. A., Ramaswamy, S. & Jap, B. K. (1998). *Complete structure of the 11-subunit bovine mitochondrial cytochrome bc_1 complex. Science*, **281**, 64–71.

Iwata, S., Ostermeier, C., Ludwig, B. & Michel, H. (1995). *Structure at 2.8 Å resolution of cytochrome c oxidase from Paracoccus denitrificans. Nature (London)*, **376**, 660–669.

Kim, H., Xia, D., Yu, C. A., Xia, J. Z., Kachurin, A. M., Li, Z., Yu, L. & Deisenhofer, J. (1998). *Inhibitor binding changes domain mobility in the iron–sulfur protein of the mitochondrial bc_1 complex from bovine heart. Proc. Natl Acad. Sci. USA*, **95**, 8026–8033.

Kimura, Y., Vassylyev, D. G., Miyazawa, A., Kidera, A., Matsushima, M., Mitsuoka, K., Murata, K., Hirai, T. & Fujiyoshi, Y. (1997). *Surface of bacteriorhodopsin revealed by high-resolution electron crystallography. Nature (London)*, **389**, 206–211.

Kleymann, G., Ostermeier, C., Ludwig, B., Skerra, A. & Michel, H. (1995). *Engineered F_v fragments as a tool for the one-step purification*

of integral multisubunit membrane protein complexes. Biotechnology, **13**, 155–160.

Koepke, J., Hu, X., Muenke, C., Schulten, K. & Michel, H. (1996). *The crystal structure of the light-harvesting complex II (B800–850) from Rhodospirilum molischianum. Structure*, **4**, 581–597.

Kreusch, A., Neubüser, A., Schiltz, E., Weckesser, J. & Schulz, G. E. (1994). *Structure of the membrane channel porin from Rhodopseudomonas blastica at 2.0 Å resolution. Protein Sci.* **3**, 58–63.

Kühlbrandt, W., Wang, D. N. & Fujiyoshi, Y. (1994). *Atomic model of plant light-harvesting complex by electron crystallography. Nature (London)*, **367**, 614–621.

Kurumbail, R. G., Stevens, A. M., Gierse, J. K., McDonald, J. J., Stegeman, R. A., Pak, J. Y., Gildehaus, D., Miyashiro, J. M., Penning, T. D., Seibert, K., Isakson, P. C. & Stallings, W. C. (1996). *Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. Nature (London)*, **384**, 644–648.

Lancaster, C. R. D. & Michel, H. (1997). *The coupling of light-induced electron transfer and proton uptake as derived from crystal structures of reaction centres from Rhodopseudomonas viridis modified at the binding site of the secondary quinone, Q_B. Structure*, **5**, 1339–1359.

Lancaster, C. R. D. & Michel, H. (1999). *Refined crystal structures of reaction centres from Rhodopseudomonas viridis in complexes with the herbicide atrazine and two chiral atrazine derivatives also lead to a new model of the bound carotenoid. J. Mol. Biol.* **286**, 883–898.

Landau, E. M. & Rosenbusch, J. P. (1996). *Lipidic cubic phases: a novel concept for the crystallization of membrane proteins. Proc. Natl Acad. Sci. USA*, **93**, 14532–14535.

Lindblom, G. & Rilfors, L. (1989). *Cubic phases and isotropic structures formed by membrane lipids – possible biological relevance. Biochim. Biophys. Acta*, **988**, 221–256.

Locher, K. P., Rees, B., Koebnik, R., Mitschler, A., Moulinier, L., Rosenbusch, J. P. & Moras, D. (1998). *Transmembrane signaling across the ligand-gated FhuA receptor: crystal structures of free and ferrichrome-bound states reveal allosteric changes. Cell*, **98**, 771–778.

Luecke, H., Richter, H. T. & Lamyi, J. K. (1998). *Proton transfer pathways in bacteriorhodopsin at 2.3 Å resolution. Science*, **280**, 1934–1937.

Luong, C., Miller, A., Barnett, J., Chow, J., Ramesha, C. & Browner, M. F. (1996). *Flexibility of the NSAID binding site in the structure of human cyclooxygenase-2. Nature Struct. Biol.* **3**, 927–933.

McDermott, G., Prince, S. M., Freer, A. A., Hawthornthwaite-Lawless, A. M., Papiz, M. Z., Cogdell, R. J. & Isaacs, N. W. (1995). *Crystal-structure of an integral membrane light-harvesting complex from photosynthetic bacteria. Nature (London)*, **374**, 517–521.

Meyer, J. E. W., Hofnung, M. & Schulz, G. E. (1997). *Structure of maltoporin from Salmonella typhimurium ligated with a nitrophenyl-maltotrioside. J. Mol. Biol.* **266**, 761–775.

Michel, H. (1982). *Three-dimensional crystals of a membrane protein complex. The photosynthetic reaction centre from Rhodopseudomonas viridis. J. Mol. Biol.* **158**, 567–572.

Michel, H. (1983). *Crystallization of membrane proteins. Trends Biochem. Sci.* **8**, 56–59.

Michel, H. (1991). Editor. *Crystallization of Membrane Proteins.* Boca Raton, Florida: CRC Press.

Midura, R. J. & Yanagishita, M. (1995). *Chaotropic solvents increase the critical micellar concentrations of detergents. Anal. Biochem.* **228**, 318–322.

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

Ostermeier, C., Harrenga, A., Ermler, U. & Michel, H. (1997). *Structure at 2.7 Å resolution of the Paracoccus denitrificans two-subunit cytochrome c oxidase complexed with an antibody F_v fragment. Proc. Natl Acad. Sci. USA*, **94**, 10547–10553.

Ostermeier, C., Iwata, S., Ludwig, B. & Michel, H. (1995). *F_v fragment-mediated crystallization of the membrane protein bacterial cytochrome c oxidase. Nature Struct. Biol.* **2**, 842–846.

Pautsch, A. & Schulz, G. E. (1998). *Structure of the outer membrane protein: a transmembrane domain. Nature Struct. Biol.* **5**, 1013–1017.

Pebay-Peyroula, E., Rummel, G., Rosenbusch, J. P. & Landau, E. M. (1997). *X-ray structure of bacteriorhodopsin at 2.5 Å from microcrystals grown in lipidic cubic phases. Science*, **277**, 1676–1681.

Picot, D., Loll, P. J. & Garavito, M. (1994). *The X-ray crystal structure of the membrane protein prostaglandin H2 synthase-1. Nature (London)*, **367**, 243–249.

Roth, M., Lewit-Bentley, A., Michel, H., Deisenhofer, J., Huber, R. & Oesterhelt, D. (1989). *Detergent structure in crystals of a bacterial photosynthetic reaction center. Nature (London)*, **340**, 659–662.

Schirmer, T., Keller, T. A., Wang, Y. F. & Rosenbusch, J. P. (1995). *Structural basis for sugar translocation through maltoporin channels at 3.1 Å resolution. Science*, **267**, 512–514.

Song, L., Hobaugh, M. R., Shustak, C., Cheley, S., Bayley, H. & Gouaux, J. E. (1996). *Structure of staphylococcal alpha-hemolysin, a heptameric transmembrane pore. Science*, **274**, 1859–1866.

Stowell, M. H. B., McPhillips, T. M., Rees, D. C., Soltis, S. M., Abresch, E. & Feher, G. (1997). *Light-induced structural changes in photosynthetic reaction center: implications for mechanism of electron–proton transfer. Science*, **276**, 812–816.

Timmins, P. A., Hauk, J., Wacker, T. & Welte, W. (1991). *The influence of heptane-1,2,3-triol on the size and shape of LDAO micelles. Implications for the crystallization of membrane proteins. FEBS Lett*. **280**, 115–120.

Timmins, P. A., Pebay-Peyroula, E. & Welte, W. (1994). *Detergent organisation in solutions and in crystals of membrane proteins. Biophys. Chem.* **53**, 27–36.

Tsukihara, T., Aoyama, H., Yamashita, E., Tomizaki, T., Yamaguchi, H., Shinzawa-Itoh, K., Nakashima, R., Yaono, R. & Yoshikawa, S. (1995). *Structures of metal sites of oxidized bovine heart cytochrome c oxidase at 2.8 Å. Science*, **269**, 1069–1074.

Tsukihara, T., Aoyama, H., Yamashita, E., Tomizaki, T., Yamaguchi, H., Shinzawa-Itoh, K., Nakashima, R., Yaono, R. & Yoshikawa, S. (1996). *The whole structure of the 13-subunit oxidized cytochrome c oxidase at 2.8 Å. Science*, **272**, 1136–1144.

Weiss, M. S., Abele, U., Weckesser, J., Welte, W., Schiltz, E. & Schulz, G. E. (1991). *Molecular architecture and electrostatic properties of a bacterial porin. Science*, **254**, 1627–1630.

Wendt, K. U., Poralla, K. & Schulz, G. E. (1997). *Structure and function of a cyclase. Science*, **277**, 1811–1815.

Xia, D., Yu, C. A., Kim, H., Xia, J. Z., Kachurin, A. M., Zhang, L., Yu, L. & Deisenhofer, J. (1997). *Crystal structure of the cytochrome bc_1 complex from bovine heart mitochondria. Science*, **277**, 60–66.

Yoshikawa, S., Shinzawa-Itoh, K., Nakashima, R., Yaono, R., Yamashita, E., Inoue, N., Yao, M., Fei, M. J., Libeu, C. P., Mizushima, T., Yamaguchi, H., Tomizaki, T. & Tsukihara, T. (1998). *Redox-coupled crystal structural changes in bovine heart cytochrome c oxidase. Science*, **280**, 1723–1729.

Zhang, Z. L., Huang, L. S., Shulmeister, V. M., Chi, Y. I., Kim, K. K., Hung, L. W., Crofts, A. R., Berry, E. A. & Kim, S. H. (1998). *Electron transfer by domain movement in cytochrome bc_1. Nature (London)*, **392**, 677–684.

Zulauf, H. (1991). *Detergent phenomena in membrane protein crystallization.* In *Crystallization of Membrane Proteins*, edited by H. Michel, ch. 2, pp. 53–72. Boca Raton, Florida: CRC Press.