**Table 4.2.1.1**
Compilation of membrane proteins with known structures, including crystallization conditions and key references for the structure determinations

This table is continuously updated and can be inspected at http://www.mpibp-frankfurt.mpg.de/michel/public/memprotstruct.html. The membrane proteins listed are divided into polytopic membrane proteins from inner membranes of bacteria and mitochondria (*a*), membrane proteins from the outer membrane of Gram-negative bacteria (*b*) and monotopic membrane proteins [(*c*); these are proteins that are only inserted into the membrane, but do not span it]. Within parts (*a*), (*b*) and (*c*) the membrane proteins are listed in chronological order of structure determination.

(*a*) Polytopic membrane proteins from inner membranes of bacteria and mitochondria.

| Membrane protein | Crystallization conditions (detergent/additive/precipitating agent) | Key references (and pdb reference code, if available) |
|---|---|---|
| Photosynthetic reaction centre | | |
| from *Rhodopseudomonas viridis* | *N*,*N*-Dimethyldodecylamine-*N*-oxide/heptane-1,2,3-triol/ammonium sulfate | [1], [2] (1PRC), [3], [4] (2PRC, 3PRC, 4PRC, 5PRC, 6PRC, 7PRC) |
| from *Rhodobacter sphaeroides* | *N*,*N*-Dimethyldodecylamine-*N*-oxide/heptane-1,2,3-triol/polyethylene glycol 4000 | [5] (4RCR) |
| | Octyl-$\beta$-D-glucopyranoside/polyethylene glycol 4000 | [6] (2RCR) |
| | *N*,*N*-Dimethyldodecylamine-*N*-oxide/heptane-1,2,3-triol, dioxane/potassium phosphate | [7] (1PCR) |
| | Octyl-$\beta$-D-glucopyranoside/benzamidine, heptane-1,2,3-triol/polyethylene glycol 4000 | [8] (1AIG, 1AIJ) |
| Bacteriorhodopsin | | |
| from *Halobacterium salinarium* | (Electron crystallography using naturally occuring two-dimensional crystals) | [9] (1BRD), [10] (2BRD), [11] (1AT9) |
| | (Type I crystal grown in lipidic cubic phases) | [12] (1AP9), [13] (1BRX) |
| | Octyl-$\beta$-D-glucopyranoside/benzamidine/sodium phosphate (epitaxic growth on benzamidine crystals) | [14] (1BRR) |
| Light-harvesting complex II | | |
| from pea chloroplasts | (Electron crystallography of two-dimensional crystals prepared from Triton X100 solubilized material) | [15] |
| Light-harvesting complex 2 | | |
| from *Rhodopseudomonas acidophila* | Octyl-$\beta$-D-glucopyranoside/benzamidine/phosphate | [16] (1KZU) |
| from *Rhodospirillum molischianum* | *N*,*N*-dimethylundecylamine-*N*-oxide/heptane-1,2,3-triol/ammonium sulfate | [17] (1LGH) |
| Cytochrome *c* oxidase | | |
| from *Paracoccus denitrificans*, four-subunit enzyme complexed with antibody Fv fragment | Dodecyl-$\beta$-D-maltoside/polyethylene glycol monomethylether 2000 | [18] |
| two-subunit enzyme complexed with antibody Fv fragment | Undecyl-$\beta$-D-maltoside/polyethylene glycol monomethylether 2000 | [19] (1AR1) |
| from bovine heart mitochondria | Decyl-$\beta$-D-maltoside with some residual cholate/polyethylene glycol 4000 | [20], [21] (1OCC), [22] (2OCC, 1OCR) |
| Cytochrome $bc_1$ complex | | |
| from bovine heart mitochondria | Decanoyl-*N*-methylglucamide or diheptanoyl phosphatidyl choline/polyethylene glycol 4000 | [23] (1QRC), [24] |
| | Octyl-$\beta$-D-glucopyranoside/polyethylene glycol 4000 | [25] |
| | Pure dodecyl-$\beta$-D-maltoside or mixture with methyl-6-*O*-(*N*-heptylcarbamoyl)-$\alpha$-D-glucopyranoside/polyethylene glycol 4000 | [26] |
| from chicken heart mitochondria | Octyl-$\beta$-D-glucopyranoside/polyethylene glycol 4000 | [25] (1BCC, 3BCC) |
| Potassium channel | | |
| from *Streptomyces lividans* | *N*,*N*-Dimethyldodecylamine/polyethylene glycol 400 | [27] (1BL8) |
| Mechanosensitive ion channel | | |
| from *Mycobacterium tuberculosis* | Dodecyl-$\beta$-D-maltoside/triethylene glycol | [28] |

generally lower at high ionic strength and at high temperatures. The presence of glycerol and similar compounds, as well as that of chaotropic agents (Midura & Yanagishita, 1995), also influences (decreases) the CMC.

### 4.2.3. General properties of detergents relevant to membrane-protein crystallization

The presence of detergents sometimes causes problems. The monomeric detergent itself can crystallize, *e.g.* dodecyl-$\beta$-D-maltoside at 4 °C in the presence of polyethylene glycol. The detergent crystals might be mistaken for protein crystals. Detergent micelles possess attractive interactions (see Zulauf, 1991). Upon addition of salts or polyethylene glycol, or upon temperature changes, a phase separation may be observed: owing to an increase in these attractive interactions, the detergent micelles 'precipitate', forming a viscous detergent-rich phase and a detergent-depleted aqueous phase. The membrane proteins are found exclusively in the viscous phase and crystals – if formed – are difficult to handle. Some detergents, *e.g.* those with polyoxyethylene head groups, undergo a phase separation at higher temperatures. This phenomenon has been used to separate

**Table 4.2.1.1 (continued)**

(*b*) Membrane proteins from the outer membrane of Gram-negative bacteria and related proteins.

| Membrane protein | Crystallization conditions (detergent/additive/ precipitating agent) | Key references (and pdb reference code, if available) |
|---|---|---|
| 16-Stranded porins | | |
| from *Rhodobacter capsulatus* | Octytetraoxyethylene/polyethylene glycol 600 | [29] (2POR) |
| OmpF and PhoE from *Escherichia coli* | Mixture of *n*-octyl-2-hydroxyethylsulfoxide and octylpolyoxyethylene; or *N*,*N*-dimethyl-decylamine-*N*-oxide/polyethylene glycol 2000 | [30] (1OPF, 1PHO), [31] |
| from *Rhodopseudomonas blastica* | Octyltetraoxyethylene/heptane-1,2,3-triol/ polyethylene glycol 600 | [32] (1PRN) |
| from *Paracoccus denitrificans* | Octyl-$\beta$-D-glucoside/polyethylene glycol 600 | [33] |
| 18-Stranded porins | | |
| maltoporin from *Escherichia coli* | Mixture of decyl-$\beta$-D-maltoside and dodecylnonaoxyethylene/polyethylene glycol 2000 | [34] (1MAL) |
| maltoporin from *Salmonella typhimurium* | Mixture of octyltetraoxyethylene and *N*,*N*-dimethylhexylamine-*N*-oxide/polyethylene glycol 1500 | [35] (1MPR, 2MPR) |
| sucrose-specific ScrY porin from *Salmonella typhimurium* | Mixture of octyl-$\beta$-D-glucopyranoside and *N*,*N*-dimethylhexylamine-*N*-oxide/polyethylene glycol 2000 | [36] (1AOS, 1AOT) |
| $\alpha$-Haemolysin | | |
| from *Staphylococcus aureus* | Octyl-$\beta$-D-glucopyranoside/ammonium sulfate, polyethylene glycol monomethylether 5000 | [37] (7AHL) |
| Eight-stranded $\beta$-barrel membrane anchor | | |
| OmpA fragment from *Escherichia coli* | Not yet available | [38] (1BXW) |
| 22-Stranded receptors | | |
| FhuA from *Escherichia coli* | *N*,*N*-Dimethyldecylamine-*N*-oxide/inositol/ polyethylene glycol monomethylether 2000 | [39] (1FCP, 2FCP) |
| | *n*-Octyl-2-hydroxyethylsulfoxide/polyethylene glycol 2000 | [40] (1BY3, 1BY5) |
| ferric enterobacterin receptor (FepA) from *Escherichia coli* | *N*,*N*-dimethyldodecylamine-*N*-oxide/heptane-1,2,3-triol/polyethylene glycol 1000 | [41] (1FEP) |

(*c*) Proteins inserted into, but not crossing the membrane ('monotopic membrane proteins').

| Membrane protein | Crystallization conditions (detergent/additive/ precipitating agent) | Key references (and pdb reference code, if available) |
|---|---|---|
| Prostaglandin $H_2$ synthase 1 | | |
| (cyclooxygenase 1) from sheep | Octyl-$\beta$-D-glucopyranoside/polyethylene glycol 4000 | [42] (1PRH) |
| Cyclooxygenase 2 | | |
| from mouse | Octyl-$\beta$-D-glucopyranoside/polyethylene glycol monomethylether 550 | [43] (1CX2, 3PGH, 4COX, 5COX, 6COX) |
| from man | Octylpentaoxyethylene/polyethylene glycol 4000 | [44] |
| Squalene cyclase | | |
| from *Alicyclobacillus acidocaldarius* | Octyltetraoxyethylene/sodium citrate | [45] (1SQC) |

References: [1] Diesenhofer *et al*. (1985); [2] Diesenhofer *et al*. (1995); [3] Lancaster & Michel (1997); [4] Lancaster & Michel (1999); [5] Allen *et al*. (1987); [6] Chang *et al*. (1991); [7] Ermler *et al*. (1994); [8] Stowell *et al*. (1997); [9] Henderson *et al*. (1990); [10] Grigorieff *et al*. (1996); [11] Kimura *et al*. (1997); [12] Pebay-Peyroula *et al*. (1997); [13] Luecke *et al*. (1998); [14] Essen *et al*. (1998); [15] Kühlbrandt *et al*. (1994); [16] McDermott *et al*. (1995); [17] Koepke *et al*. (1996); [18] Iwata *et al*. (1995); [19] Ostermeier *et al*. (1997); [20] Tsukihara *et al*. (1995); [21] Tsukihara *et al*. (1996); [22] Yoshikawa *et al*. (1998); [23] Xia *et al*. (1997); [24] Kim *et al*. (1998); [25] Zhang *et al*. (1998); [26] Iwata *et al*. (1998); [27] Doyle *et al*. (1998); [28] Chang *et al*. (1998); [29] Weiss *et al*. (1991); [30] Cowan *et al*. (1992); [31] Cowan *et al*. (1995); [32] Kreusch *et al*. (1994); [33] Hirsch *et al*. (1997); [34] Schirmer *et al*. (1995); [35] Meyer *et al*. (1997); [36] Forst *et al*. (1998); [37] Song *et al*. (1996); [38] Pautsch & Schulz (1998); [39] Ferguson *et al*. (1998); [40] Locher *et al*. (1998); [41] Buchanan *et al*. (1999); [42] Picot *et al*. (1994); [43] Kurumbail *et al*. (1996); [44] Luong *et al*. (1996); [45] Wendt *et al*. (1997).

solubilized membrane proteins, which are found in the detergent-rich phase, from the water-soluble proteins. The latter are concentrated in the detergent-depleted phase (Bordier, 1981). Other detergents, *e.g.* octyl-$\beta$-D-glucopyranoside, show this phase separation at lower temperatures. Therefore, if phase separation causes problems, a change of the crystallization temperature may help.

The polar head groups of the detergents influence their usage in many ways. One would like to have a small polar head group, because the head group 'covers' the part of the protein's polar surface that is adjacent to the hydrophobic surface belt. The bigger the head group the more of the polar surface is covered and unavailable for the polar interactions needed to form the crystal lattice. Unfortunately, detergents with small polar head groups are rather denaturing. Detergents with charged head groups cannot be used, but detergents with zwitterionic head groups, *e.g.* sulfobetaines, can be tried with more stable proteins. The head group of a very successful detergent, *N*,*N*-dimethyl-dodecylamine-*N*-oxide, is of zwitterionic nature. I estimate that it can only be used with about 20% of all membrane proteins. Detergents with sugar residues as head groups have been used successfully. Octyl-$\beta$-D-glucopyranoside also tends to be denaturing. The lifetime of many membrane proteins can be prolonged by a factor of three by the use of nonyl-$\beta$-D-glucopyranoside instead of the shorter homologue. Such behaviour is observed within each series of homologous detergents; an increase in the alkyl chain by one methylene group leads to an increase in stability by a factor of three, an increase by two methylene groups leads to an increase in stability by a factor of about ten. Unfortunately, decyl-$\beta$-D-glucopyranoside is too insoluble to be used as detergent. For less stable membrane proteins, alkylmaltoside detergents or alkanoylsucrose deter-

**Table 4.2.2.1**
Potentially useful detergents for membrane-protein crystallizations with molecular weights and CMCs [in water, from Michel (1991) or as provided by the vendor]

The lengths of the alkyl or alkanoyl side chains are given as $C_6$ to $C_{16}$.

| Detergent | Molecular weight | CMC (m*M*) |
|---|---|---|
| Alkyl dihydroxypropyl sulfoxide | | |
| $C_8$ | 238 | 20.6 |
| *N*,*N*-Dimethylalkylamine-*N*-oxides | | |
| $C_8$ | 173 | 162 |
| $C_9$ | 187 | 50 |
| $C_{10}$ | 201 | 20 |
| $C_{12}$ | 229 | 1–2 |
| *n*-Dodecyl-*N*,*N*-dimethylglycine (zwitterionic) | 271 | 1.5 |
| *N*-Alkyl-$\beta$-D-glucopyranosides | | |
| $C_6$ | 264 | 250 |
| $C_7$ | 278 | 79 |
| $C_8$ | 292 | 30 |
| $C_9$ | 306 | 6.5 |
| $C_{10}$ | 320 | 2.6 |
| *n*-Alkanoyl-*N*-hydroxyethylglucamides ('HEGA-*n*') | | |
| $C_8$ | 351 | 109 |
| $C_9$ | 365 | 39 |
| $C_{10}$ | 379 | 7.0 |
| $C_{11}$ | 393 | 1.4 |
| Alkyl hydroxyethyl sulfoxide | | |
| $C_8$ | 222 | 15.8 |
| *n*-Alkyl-$\beta$-D-maltosides | | |
| $C_6$ | 426 | 210 |
| $C_8$ | 454 | 19.5 |
| $C_9$ | 468 | 6 |
| $C_{10}$ | 483 | 1.8 |
| $C_{11}$ | 497 | 0.6 |
| $C_{12}$ | 511 | 0.17 |
| $C_{13}$ | 525 | 0.033 |
| $C_{14}$ | 539 | 0.01 |
| $C_{16}$ | 567 | 0.006 |
| cyclohexyl-$C_1$ | 438 | 340 |
| cyclohexyl-$C_2$ | 452 | 120 |
| cyclohexyl-$C_3$ | 466 | 34.5 |
| cyclohexyl-$C_4$ | 480 | 7.6 |
| cyclohexyl-$C_5$ | 494 | 2.4 |
| cyclohexyl-$C_6$ | 508 | 0.56 |
| cyclohexyl-$C_7$ | 522 | 0.19 |
| *n*-Alkanoyl-*N*-methylglucamides ('MEGA-*n*') | | |
| $C_8$ | 321 | 79 |
| $C_9$ | 335 | 25 |
| $C_{10}$ | 349 | 6 |
| Methyl-6-*O*-(*N*-heptylcarbamoyl)-$\alpha$-D-glucopyranoside ('HECAMEG') | 335 | 19.5 |
| *n*-Alkylphosphocholines (zwitterionic) | | |
| $C_8$ | 295 | 114 |
| $C_9$ | 309 | 39.5 |
| $C_{10}$ | 323 | 11 |
| $C_{12}$ | 315 | 1.5 |
| $C_{14}$ | 379 | 0.12 |
| $C_{16}$ | 407 | 0.013 |
| Polyoxyethylene monoalkylethers ($C_nE_m$) | | |
| $C_8E_4$ | 306 | 7.9 |
| $C_8E_5$ | 350 | 7.1 |
| $C_{10}E_6$ | 422 | 0.9 |
| $C_{12}E_8$ | 538 | 0.071 |
| *n*-Alkanoylsucrose | | |
| $C_{10}$ | 497 | 2.5 |
| $C_{12}$ | 525 | 0.3 |
| *n*-Alkyl-$\beta$-D-thioglucopyranosides | | |
| $C_7$ | 294 | 29 |
| $C_8$ | 308 | 9 |
| $C_9$ | 322 | 2.9 |
| $C_{10}$ | 336 | 0.9 |
| *n*-Alkyl-$\beta$-D-thiomaltopyranosides | | |
| $C_8$ | 471 | 8.5 |
| $C_9$ | 485 | 3.2 |
| $C_{10}$ | 499 | 0.9 |
| $C_{11}$ | 513 | 0.21 |
| $C_{12}$ | 527 | 0.05 |

gents have to be tried. There is one special problem when using alkyl-$\beta$-D-glucopyranosides or alkyl-$\beta$-D-maltosides as detergents: the commercially available detergents are often 'contaminated' with the $\alpha$-anomers in varying, sometimes substantial, concentrations. The $\alpha$-anomers are much less soluble, and appear to prevent crystallization. In the case of photosystem I from a thermophilic cyanobacterium, it has been reported that for a 2% $\alpha$-anomer content in dodecyl-$\beta$-D-maltoside preparations no crystals can be obtained, with a 0.5–2% content the diffraction of the crystals is anisotropic with a reduction in resolution to 5–6 Å, whereas diffraction to better than 3.5 Å resolution in all directions is observed when the content of the $\alpha$-anomer is below 0.1% (Fromme & Witt, 1998). The percentage of $\alpha$-anomer can be determined using NMR spectroscopy or high-performance liquid chromatography. During ageing of detergent solutions, a conversion from the $\beta$-anomer to the $\alpha$-anomer is expected. Therefore, ageing of detergent solutions has to be prevented.

The detergents that have been successfully used to crystallize membrane proteins can also be found in Table 4.2.1.1. The possibilities for developing new detergents for membrane-protein crystallization have not been exhausted. There is a need for new detergents, *e.g.* detergents with head groups with sizes between glucose and maltose are still missing!

It has been observed that crystals of the photosynthetic reaction centre from the purple bacterium *Rhodopseudomonas viridis* could be obtained when *N*,*N*-dimethyldodecylamine-*N*-oxide is used as detergent, but not when *N*,*N*-dimethyldecylamine-*N*-oxide is used. Even crystals formed with the dodecyl homologue lost their order when soaked in a buffer containing the decyl homologue. These observations were found to have an obvious explanation when the location of the detergent molecules bound to the protein was determined using neutron crystallography (Roth *et al.*, 1989); the detergent molecules surrounding neighbouring photosynthetic reaction centres in the crystal lattice are in contact. It is likely that attractive interactions between neighbouring protein-bound detergent micelles contribute to the stability of the crystal lattice. Particularly striking (see Table 4.2.3.1) is the dependence of the crystal quality on the alkyl-chain length in the case of the two-subunit cytochrome *c* oxidase from the soil bacterium *Paracoccus denitrificans*. Well ordered crystals were obtained with undecyl-$\beta$-D-maltoside, but not with the decyl and dodecyl homologues. Table 4.2.3.1 also lists the names of important vendors of detergents.

### 4.2.4. The 'small amphiphile concept'

From the arguments and observations presented above, it is evident that the size and shape of the detergent micelle are very important in membrane-protein crystallization. Detergent micelles can be made smaller (and their curvatures changed) when small amphiphilic molecules like heptane-1,2,3-triol are