

## 4.2. CRYSTALLIZATION OF MEMBRANE PROTEINS

**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 <i>Rhodospseudomonas viridis</i>	<i>N,N</i> -Dimethyldodecylamine- <i>N</i> -oxide/heptane-1,2,3-triol/ammonium sulfate	[1], [2] (1PRC), [3], [4] (2PRC, 3PRC, 4PRC, 5PRC, 6PRC, 7PRC)
from <i>Rhodobacter sphaeroides</i>	<i>N,N</i> -Dimethyldodecylamine- <i>N</i> -oxide/heptane-1,2,3-triol/polyethylene glycol 4000	[5] (4RCR)
	Octyl- $\beta$ -D-glucopyranoside/polyethylene glycol 4000	[6] (2RCR)
	<i>N,N</i> -Dimethyldodecylamine- <i>N</i> -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 <i>Halobacterium salinarum</i>	(Electron crystallography using naturally occurring 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 (epitaxial 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 <i>Rhodospseudomonas acidophila</i>	Octyl- $\beta$ -D-glucopyranoside/benzamidine/phosphate	[16] (1KZU)
from <i>Rhodospirillum molischianum</i>	<i>N,N</i> -dimethylundecylamine- <i>N</i> -oxide/heptane-1,2,3-triol/ammonium sulfate	[17] (1LGH)
Cytochrome <i>c</i> oxidase from <i>Paracoccus denitrificans</i> , 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 <i>bc</i> <sub>1</sub> complex from bovine heart mitochondria	Decanoyl- <i>N</i> -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- <i>O</i> -( <i>N</i> -heptylcarbonyl)- $\alpha$ -D-glucopyranoside/polyethylene glycol 4000	[26]
from chicken heart mitochondria	Octyl- $\beta$ -D-glucopyranoside/polyethylene glycol 4000	[25] (1BCC, 3BCC)
Potassium channel from <i>Streptomyces lividans</i>	<i>N,N</i> -Dimethyldodecylamine/polyethylene glycol 400	[27] (1BL8)
Mechanosensitive ion channel from <i>Mycobacterium tuberculosis</i>	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

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**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 <i>Rhodobacter capsulatus</i> OmpF and PhoE from <i>Escherichia coli</i>	Octyltetraoxyethylene/polyethylene glycol 600 Mixture of <i>n</i> -octyl-2-hydroxyethylsulfoxide and octylpolyoxyethylene; or <i>N,N</i> -dimethyldodecylamine- <i>N</i> -oxide/polyethylene glycol 2000	[29] (2POR) [30] (1OPF, 1PHO), [31]
from <i>Rhodospseudomonas blastica</i>	Octyltetraoxyethylene/heptane-1,2,3-triol/polyethylene glycol 600	[32] (1PRN)
from <i>Paracoccus denitrificans</i>	Octyl- $\beta$ -D-glucoside/polyethylene glycol 600	[33]
18-Stranded porins malto porin from <i>Escherichia coli</i>	Mixture of decyl- $\beta$ -D-maltoside and dodecylnonaoxyethylene/polyethylene glycol 2000	[34] (1MAL)
malto porin from <i>Salmonella typhimurium</i>	Mixture of octyltetraoxyethylene and <i>N,N</i> -dimethylhexylamine- <i>N</i> -oxide/polyethylene glycol 1500	[35] (1MPR, 2MPR)
sucrose-specific ScrY porin from <i>Salmonella typhimurium</i>	Mixture of octyl- $\beta$ -D-glucopyranoside and <i>N,N</i> -dimethylhexylamine- <i>N</i> -oxide/polyethylene glycol 2000	[36] (1AOS, 1AOT)
$\alpha$ -Haemolysin from <i>Staphylococcus aureus</i>	Octyl- $\beta$ -D-glucopyranoside/ammonium sulfate, polyethylene glycol monomethylether 5000	[37] (7AHL)
Eight-stranded $\beta$ -barrel membrane anchor OmpA fragment from <i>Escherichia coli</i>	Not yet available	[38] (1BXW)
22-Stranded receptors FhuA from <i>Escherichia coli</i>	<i>N,N</i> -Dimethyldodecylamine- <i>N</i> -oxide/inositol/polyethylene glycol monomethylether 2000 <i>n</i> -Octyl-2-hydroxyethylsulfoxide/polyethylene glycol 2000	[39] (1FCP, 2FCP) [40] (1BY3, 1BY5)
ferric enterobacterin receptor (FepA) from <i>Escherichia coli</i>	<i>N,N</i> -dimethyldodecylamine- <i>N</i> -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 <sub>2</sub> 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 <i>Alicyclobacillus acidocaldarius</i>	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] Kreuzsch *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*-dimethyldodecylamine-*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-