

Supplemental Online Material Bass *et al.*, published 22 November 2002

Materials and Methods

As in the study of the previously determined MscL structure (S1), multiple homologs of MscS [encoded by the *yggB* gene (S2)] were identified by BLAST searching of the NCBI genome database. Ten homologs from prokaryotes and Archaea were identified and subsequently cloned. The channels were subcloned into expression vectors (pET system, Novagen) and expression screening was carried out. Cells expressing sufficient channels to be identified by Western blotting were subjected to extensive detergent screening utilizing ~50 detergents (Anatrace, Sigma, Aldrich) where both the ability of the channels to be extracted out of the membrane and the ability to remain as a homo-oligomer were determined. Subsequent large-scale expressions, extraction and purification produced sufficient amounts of protein for three channels (*E. coli*, *B. subtilis* and *C. tepidum*) for crystallization trials. Each of these channels was produced recombinantly (vector pet28b, Novagen) in 50-liter fermenter growths in a modified Terrific Broth media containing 1% glucose and 0.4% glycerol. Protein expression was initiated by the addition of 2% lactose and 2 mM IPTG for 2-4 hours, resulting in ~1.5 kg of wet cells. To obtain phase information, selenomethionine-derivatized protein was purified from cells grown in a modified M9 media containing 50 mg/l selenomethionine, and the remaining amino acids at 40 µg/l. Extraction of the *E. coli* MscS was carried out using sonication and solubilization with 1% Foscholine-14. Ni-affinity chromatography, anion exchange, and size exclusion chromatography in the presence of 0.05% Foscholine-14 were used to purify the protein to homogeneity. The apparent molecular mass of the protein, as indicated by size-exclusion chromatography, was in excess of 200 kD, similar to that reported for recombinant MscS by Sukharev (S3). Crystals were obtained with 10-15 mg/ml MscS by hanging drop vapor diffusion with 100 mM pH 7.2 Hepes buffer, 150 mM Na-formate, 8% glycerol, and 16% PEG-3350 as the precipitant. Crystals grew to ~200 µm in each dimension, and were assigned to space group $P4_32_12$ ($a = b = 184.7$ Å, $c = 260.7$ Å) with one MscS oligomer in the asymmetric unit (corresponding to ~71% solvent content). Only residues in the extramembrane (water-soluble) regions of MscS participated in lattice contacts.

References

- S1. G. Chang, R. H. Spencer, A. T. Lee, M. T. Barclay, D. C. Rees, *Science* 282, 2220 (1998).
- S2. N. Levina *et al.*, *EMBO J.* 18, 1730-7 (1999).
- S3. S. Sukharev, *Biophys. J.* 83, 290-8 (2002).

Figure S1

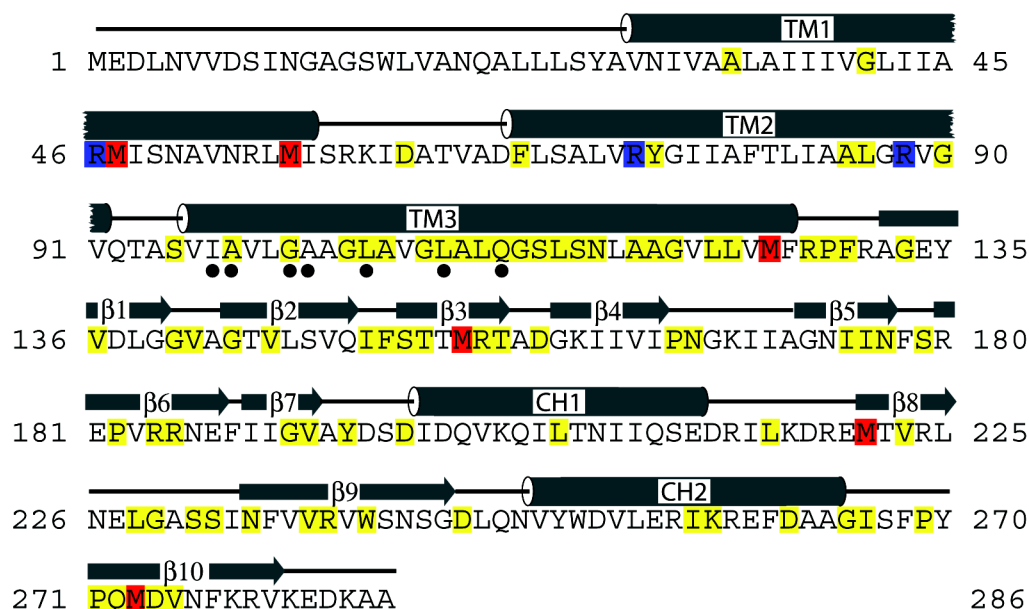


Fig. S1. Amino acid sequence of the *E. coli* MscS (S2). Residues highlighted in yellow are identical in at least 65% of the sequences of 25 yggB homologs in prokaryotes and Archaea (see Fig. S2). The red boxes indicate methionine residues located in the selenomethionine substituted protein used for phasing, while transmembrane arginine residues at positions 46, 74, and 88 are shaded blue. Cylinders above the sequence designate residues in the three transmembrane α -helices, TM1-3, and the two cytoplasmic helices, CH1 and CH2. Black circles below the sequence denote amino acids within residues 96-112 of TM3 that line the permeation pathway. Arrows above the sequence denote residues in β -strands 1-10.

Figure S2 MULTIPLE SEQUENCE ALIGNMENT of yggB
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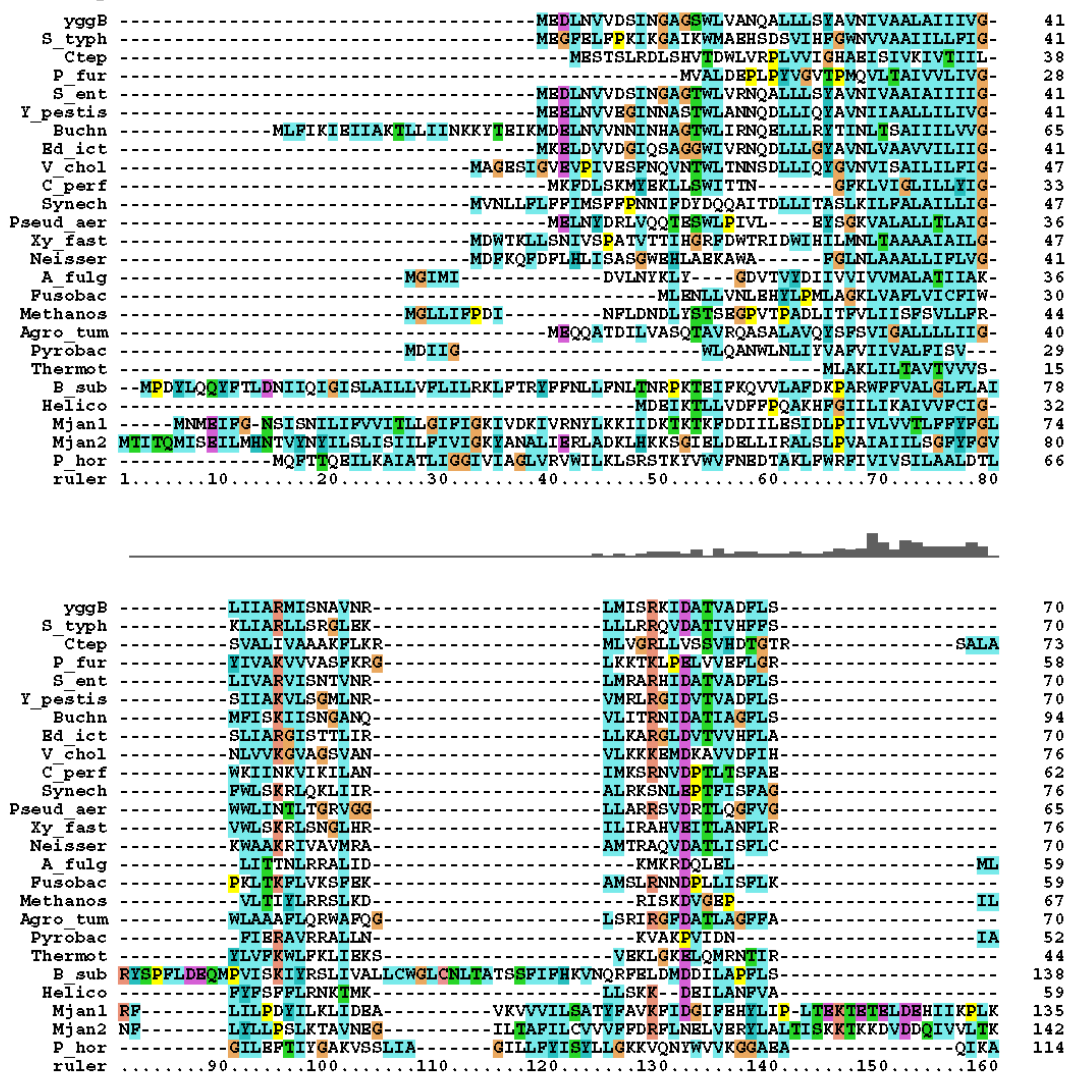


Figure S2 MULTIPLE SEQUENCE ALIGNMENT of yggB

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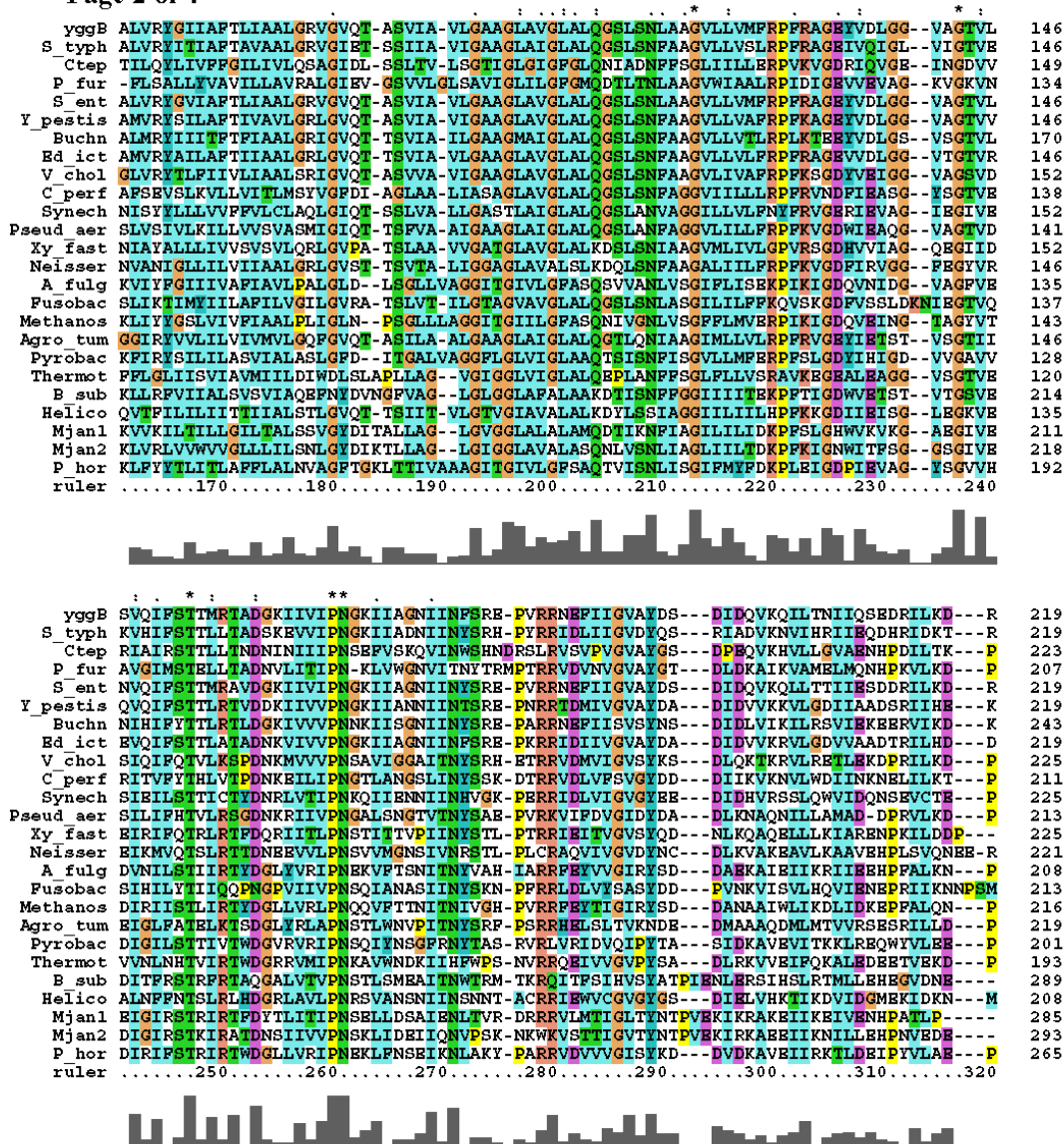


Figure S2 MULTIPLE SEQUENCE ALIGNMENT of yggB

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yggB	EMTVRLNELGASSINFFVVRVWSNS-----GD-LQNVYWDVLERIKREFDAAGISFPYQMDVNFKRVKEDKAA-----	286
S_typh	DITVRLGELAPSSLNFFVVRVWPN-----AQ-YWSYIDLLLENIKKAMDENGINIPYPRMDVVRVENVKSTIP-----	285
Ctep	SPAVLFSDFGNSSLDPELLVWVETR-----IQTPRFLRSELNRYIFDAPRKNIGIPIPPQIDLHIRSSDIPLWEPREKKNP	299
P_fur	APAVVVTTELGDSSINLQLRAWAKT-----EDYWTVKFDLTGKIYBAYRRREGIPIPPQLDVHIKEMPK-----	270
S_ent	EMTVRLNELGASSINFFVVRVWSKS-----SD-LQNVYWDVLERIKREFDAAGISFPYQMDVNFKRVKDNAAE-----	286
Y_pestis	GVTVRLNEMAPSSLNFFVVRVWITN-----GD-AQEVFWDLTENFKRALDAHKIGIPIPPQMDVHLHQVAKABAKDE-----	289
Buchn	DIIVGLSELAPSSLNFFVRCWSKN-----HD-LNTVYWDLMKAFKKELDKNNINIPPPQLDVHVYKKK-----	305
Ed_ict	GVTIIRLNEMAASSLNFFVVRVWQNN-----AD-YWAIYFDLMENFKRALDANNIGIPIPPQMDVHLYQAVKARAB-----	286
V_chol	DMTIGVLTLDASSINFFVVRVWCKT-----SD-YWAVYFDSMQAIKKBALDANGIPIPPQMDVHLNKNIN-----	287
C_perf	EAFVGISQHAASSIDFTVVRVWTKQ-----ED-YWKVHFSLLLEVKLRFDENITIPYQMDVLTIK-----	271
Synech	APTIALGELGDSSVNFVVRVWKS-----ED-YFRLKLQLTBAIKRKLDEENISIPPPQRDVHLIQPETKELDIKAA-----	296
Pseud_aer	APVAVVSNLGSATLTLRLVWVKN-----AD-YWDVMFMFNEKARDALGKEGIGIPIPPQRVVKVQVQAMAD-----	278
Xy_fast	APLVVNNLGSSEVDLLLLAYTQN-----DN-FNPAKSELLEQIHNQLPENGLNIPYPPQDLHLHYHDTNNRKIASLLLP	299
Neisser	QAAAYITLGDNAIEITLWAWANE-----AD-RWTLQCDELNEQVVENLRKVNNINIPPPQDIHIINS-----	282
A_fulg	EPVVVDNLGDSSVNIIVRIWAPS-----TEWYNVKNMELLWKIKTELEKNGIPIPPQRVVWFAN-----ELR-----	271
Fusobac	PTTISLTKQNASLDYMFRAWVRK-----BDYVDIMLDCNIN-VKKPFDKNGIPIPNKLLIMKNNLDIDNKQ-----	281
Methanos	SPSVFVSDLGDSAVKIVRIWAPV-----SEWFGKTRLLWDIKCTLENGIEVPPQRVVLHIKNNSGKKPQEFEGLEKE	290
Agro_tum	APVTVFVSDVTDASATVLRVWVRN-----DN-YFVYTRDVTYKAMRLAFDERKAEVNAQPA-----	273
Pyrobac	EPVVVFAREFADSGIVLEVRWTAG-----VTWFNLYSLATVKKRALDAGIPIPIPPQRVVWFAT-----PLP-----	264
Thermot	APVTVFSAFNSSSIDFIIRFWVNR-----DNPFEGVKRLAFRIKDYLEKEGIYIPIPPQLDVHFDDEEFIRVWKHSGSENK	267
B_sub	IIMVNFDTFADSYINLFFNFYTKT-----VWAENLNIREDNKYKIEILGAEVQFATPGQMVVVKQKHESDQFQVNLNKE	366
Helico	PFIIGITDFGQSSLNFTLRVWAKI-----BDGIFNVRSLETERIKKNALDANRIEIPFNKLDISINKQDSSKZ-----	275
Mjan1	PTRVHFREYGDWSLNLRLVEIFVRN-----MCPDYILNAVDEINLKKEBEFKEGIEMAFPYITVILEKDN-----	350
Mjan2	PTTVYFKBFPGDWSLNQVYVYIKNRYNGYQKYIISINEVNLKIKKEBFDKGIFFAFPTYITLTKRDD-----	361
P_hor	EPTTIVBELGDSSVNLAIRAWAPS-----EKWFDVRIETLKKVKKALDAGIPIPPQHVNVFAB-----ELK-----	328
ruler330.....340.....350.....360.....370.....380.....390.....400	



yggB	-----	286
S_typh	-----	285
Ctep	TEZ-----	302
P_fur	-----	270
S_ent	-----	286
Y_pestis	-----	289
Buchn	-----	305
Ed_ict	-----	286
V_chol	-----	287
C_perf	-----	271
Synech	-----	296
Pseud_aer	-----	278
Xy_fast	DVADES-----	305
Neisser	-----	282
A_fulg	ANVEGKE-----ERRQA-----	283
Fusobac	-----	281
Methanos	GHFEGNEGVLNFEGRGVN-----	308
Agro_tum	ANLKA-----	273
Pyrobac	-----	269
Thermot	S-----	268
B_sub	EKERA-----	371
Helico	-----	275
Mjan1	-----	350
Mjan2	-----	361
P_hor	VKIE-----	332
ruler410.....	

Figure S2 MULTIPLE SEQUENCE ALIGNMENT of yggB
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Abbreviation	Species	Genbank ID
yggB	<i>E. coli</i>	gi 16130825
S_typh	<i>Salmonella typhimurium</i>	gi 16767368
Ctep	<i>Chlorobium tepidum</i>	gi 21648046
P_fur	<i>Pyrococcus furiosus</i>	gi 18977188
M_tub	<i>Mycobacterium tuberculosis</i>	gi 15609571
S_ent	<i>Salmonella enterica</i>	gi 16761848
Y_pestis	<i>Yersinia pestis</i>	gi 16121224
Buchn	<i>Buchnera</i> sp.	gi 15617051
Ed_ict	<i>Edwardsiella ictaluri</i>	gi 2708660
V_chol	<i>Vibrio cholerae</i>	gi 15640507
C_perf	<i>Clostridium perfringens</i>	gi 18309195
Synech	<i>Synechocystis</i> sp.	gi 16331560
Pseud_aer	<i>Pseudomonas aeruginosa</i>	gi 15599590
Xy_fast	<i>Xylella fastidiosa</i>	gi 15837859
Neisser	<i>Neisseria meningitidis</i>	gi 15793305
A_fulg	<i>Archaeoglobus fulgidus</i>	gi 11499141
Fusobac	<i>Fusobacterium nucleatum</i>	gi 19703954
Methanos	<i>Methanosarcina acetivorans</i>	gi 20090570
Agro_tum	<i>Agrobacterium tumefaciens</i>	gi 15888392
Pyrobac	<i>Pyrobaculum aerophilum</i>	gi 18313911
Thermot	<i>Thermotoga maritima</i>	gi 15644311
B_sub	<i>Bacillus subtilis</i>	gi 2633299
Helico	<i>Helicobacter pylori</i>	gi 4155504
Mjan2	<i>Methanococcus jannaschii</i>	gi 1591775
Mjan1	<i>Methanococcus jannaschii</i>	gi 1590923
P_hor	<i>Pyrococcus horikoshii</i>	gi 3256727

Fig. S2. Sequence alignment of MscS homologs. This figure was prepared using ClustalX.