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

Materials and Methods

As in the study of the previously determined MscL structure (S*1*), multiple homologs of MscS [encoded by the *yggb* gene (S*2*)] 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 (S*3*). 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

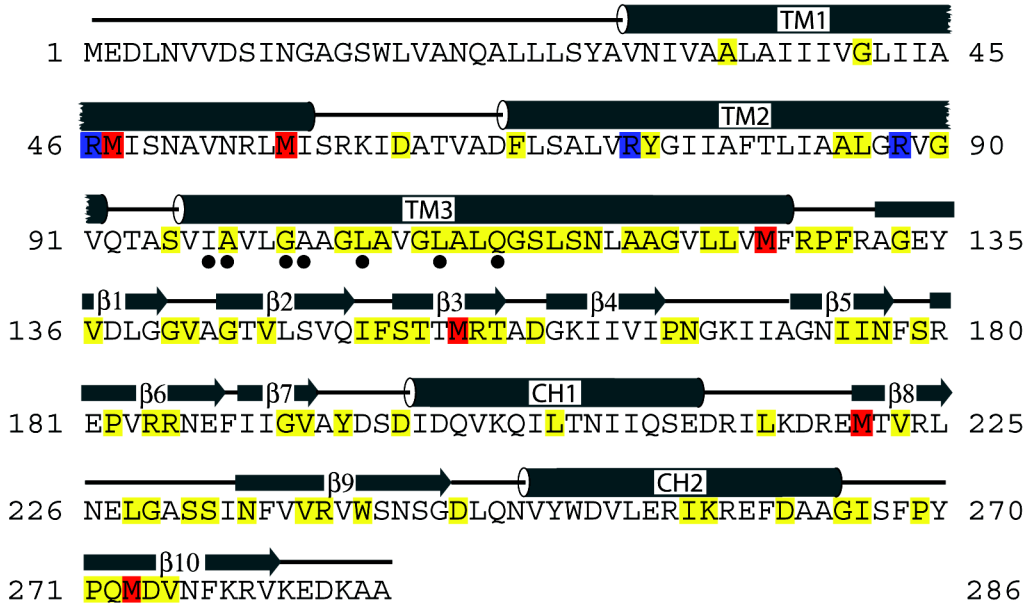

Fig. S1. Amino acid sequence of the *E. coli* MscS (S*2*). Residues highlighted in yellow are identical in at least 65% of the sequences of 25 yggB homologs in prokaryotes and Archaea (see Fig. S2). The red boxes indicate methionine residues located in the selenomethionine substituted protein used for 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
Page 1 of 4

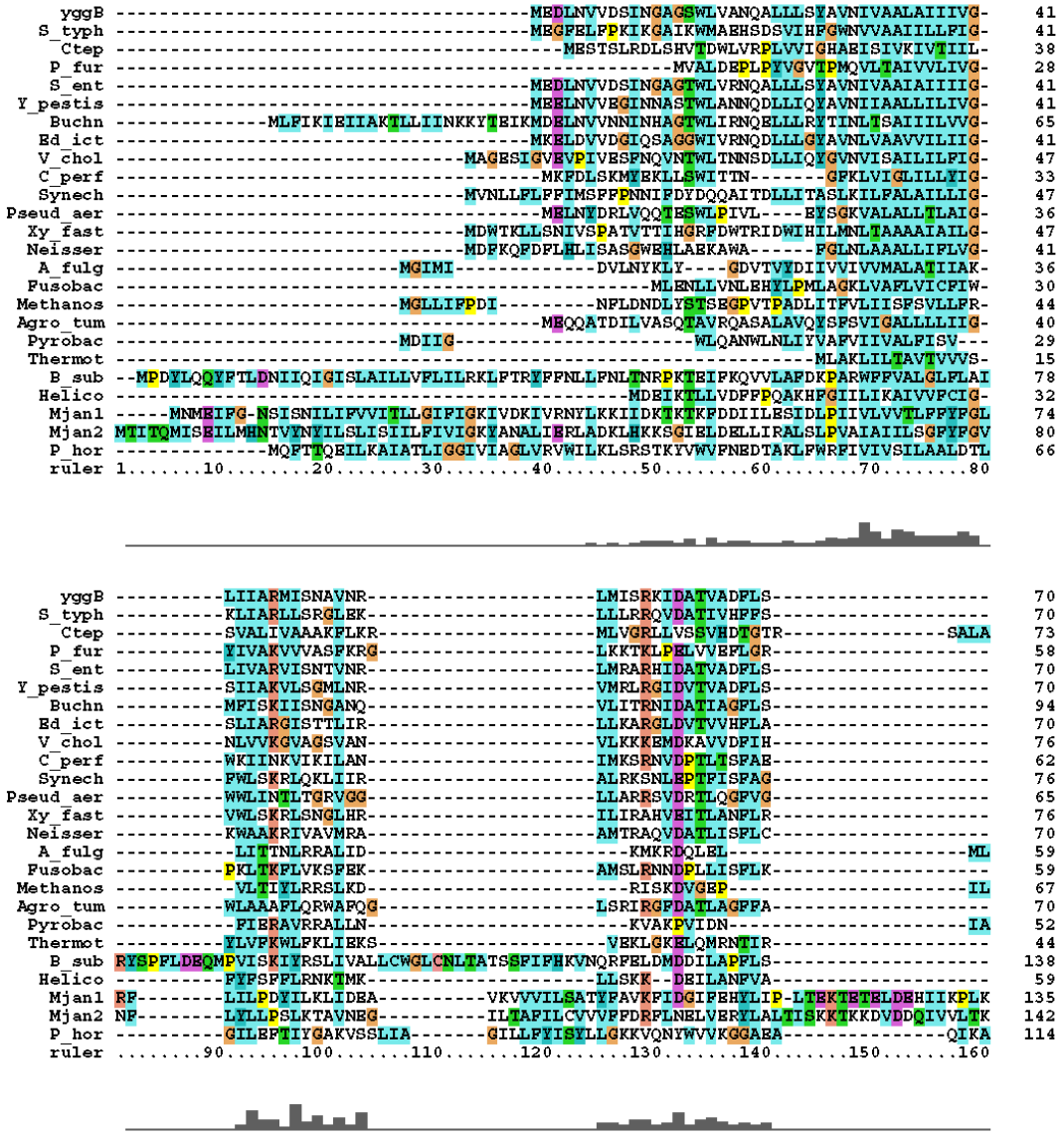

Figure S2 MULTIPLE SEQUENCE ALIGNMENT of yggB
Page 2 of 4

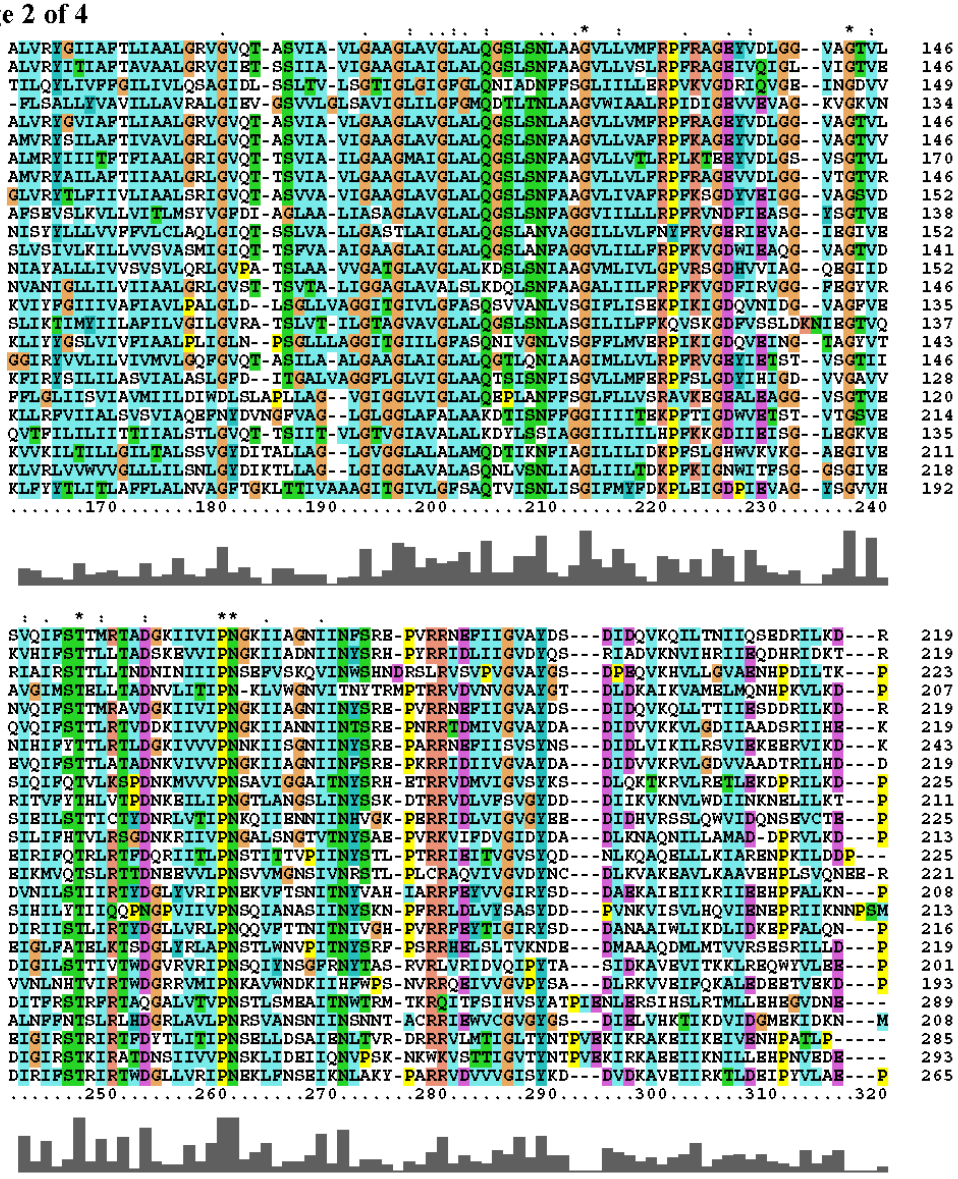

Figure S2 MULTIPLE SEQUENCE ALIGNMENT of yggB
Page 3 of 4

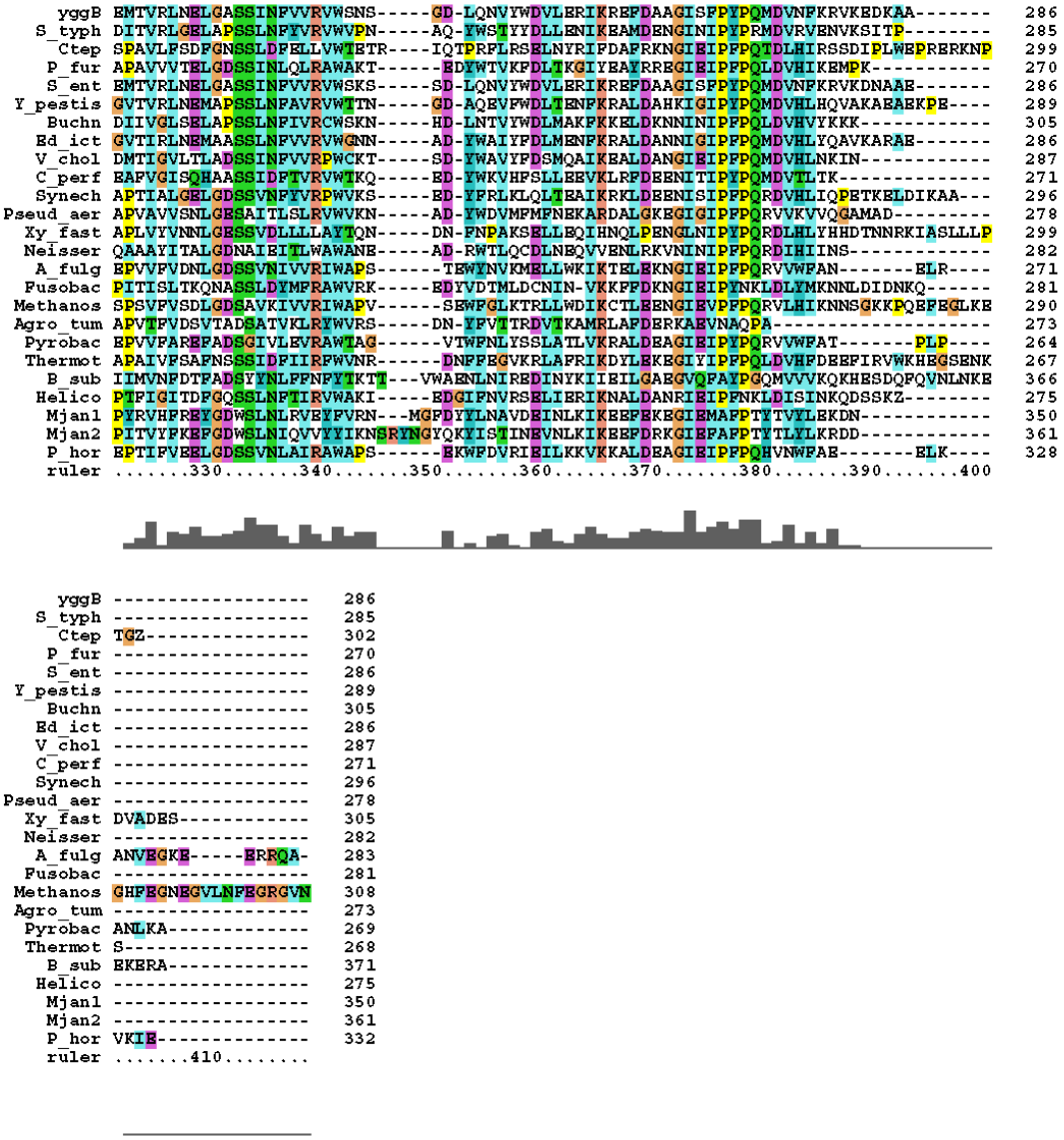

Figure S2 MULTIPLE SEQUENCE ALIGNMENT of yggB
Page 4 of 4

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

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