Supporting Information

Sagermann et al. 10.1073/pnas.0902324106

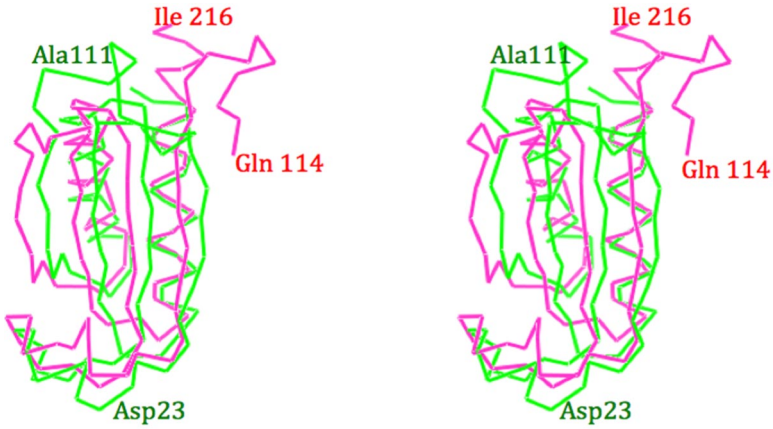

Fig. S1. Stereoview of the backbone (Cα–Cα) structures of the N-terminal (green) and C-terminal domains of EutL superimposed on top of each other. The overall rmsd between the structures is 2.7 Å with the largest differences in the loops.

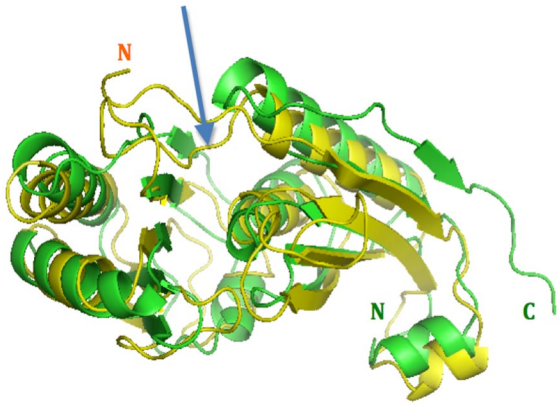

Fig. S2. Superposition of EutL (yellow) with 2 structures of Ccmk1 (green). Both of the Ccmk1 structures are taken directly out of the hexamer configuration and show that the relative positioning of the domains of the EutL structure have remained very similar to the positions of the Ccmk1 proteins despite the low sequence identity. The arrow points to the covalent connection between the 2 domains of EutL.

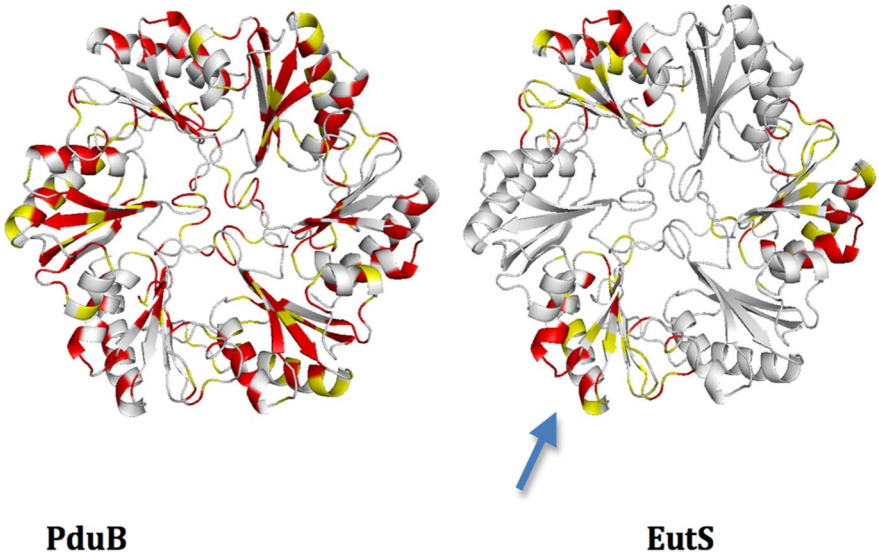

Fig. S3. Mapping of the most conservative residues between PduB and EutL (or EutS and EutL) onto the structure of EutL (ribbon diagram). The modeling is based on the alignment by Kofoid et al. (1). Residues that are strictly conserved are highlighted in red, and similar residues are highlighted in yellow. In both cases, most of the conserved residues are located within the domains of the protein. No reliable alignments can be observed around the channel. Conserved residues that may be involved in lateral contacts between the hexagons are also observed in the EutS model (arrow). Shown here is the modeling of the C-terminal domain of EutL only as it aligned best with the sequence of EutS.

1. Kofoid E, Rappleye C, Stojiljkovic I, Roth J (1999) The 17-gene ethanolamine (eut) operon of *Salmonella typhimurium* encodes five homologues of carboxysome shell proteins. *J Bacteriol* 181:5317–5329.

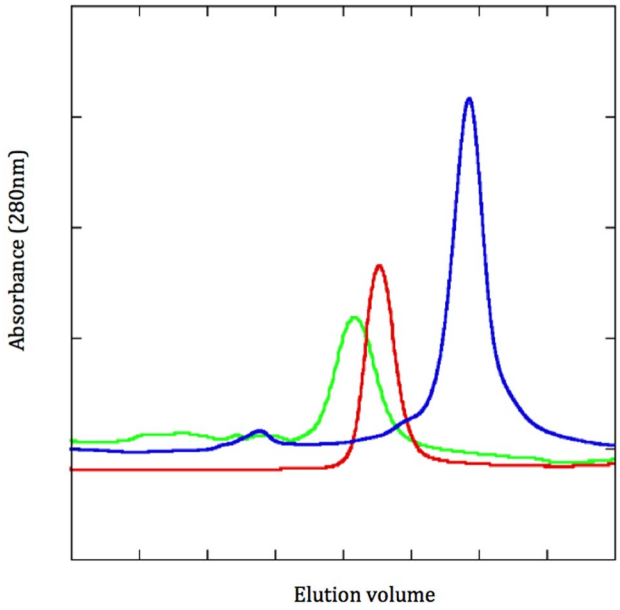

Fig. S4. Sephadex 200 size-exclusion chromatography elution profile of EutL. Shown are the individual elution profiles of the protein at different pH and different time intervals after cell lysis. Blue: pH 8.0 (peak: 67.55 kDa, freshly prepared protein); red: pH 6.2 (231 kDa, freshly prepared protein); green: pH 6.2 (323.7 kDa, 120 h after lysis). The protein preferably crystallized at pH 6.5.

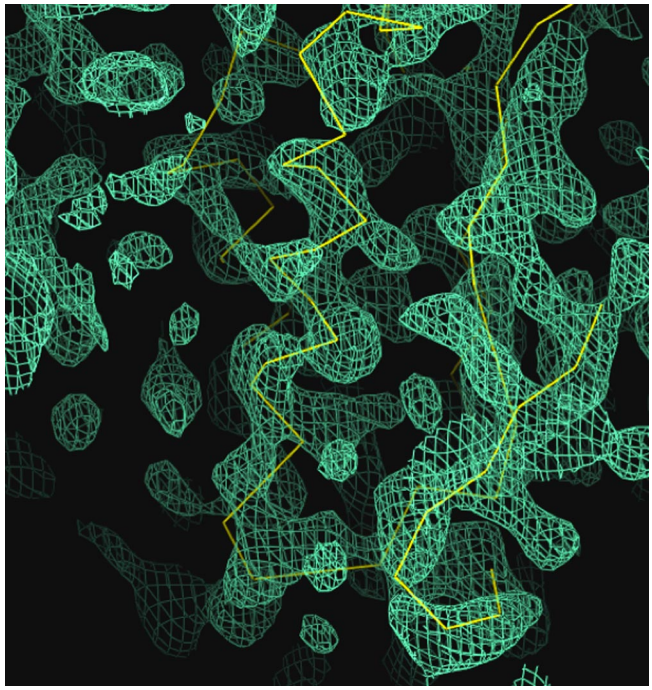

Fig. S5. Initial SIR electron density derived from the mercury chloride derivative. The electron density was calculated to 3.6-Å resolution. The backbone from the refined model of Eut-L is superimposed in yellow.

Table 1. Crystallographic data, phasing, and refinement statistics

Data set	NAT	D11 $HgCl_2$	D4–10 $HgCl_2$	D11–2 $HgCl_2$	SE-1
X-ray source	SSRL 91	SSRL-91	SSRL 11–2	SSRL-91	SSRL-91
Wavelength	0.979462	0.815659	0.97946	0.815659	0.97930
Unit cell					
a, Å	67.57	67.59	67.38	67.59	67.79
b, Å	67.57	67.59	67.38	67.59	67.79
c, Å	80.37	79.85	79.66	79.85	80.74
Space group	P3	P3	P3	P3	P3
Multiplicity	3.4	1.9	3.1	2.9	2.8
Resolution, Å	2.3	3.0	2.2	2.5	3.5
Unique reflections	15,369	7,295	16,696	13,921	12,494
Completeness, %	84.2	89.3	92.7	98.7	86.8
I/Sig(*I*)	9.6	6.4	13.3	3.2	6.5
R_{sym}*	0.129	0.110	0.078	0.092	0.108
No. heavy atoms		3 (Hg)	2 (Hg)	2 (Hg)	3 (Se)
Phasing power† (to 3.5 Å)		1.2	1.1	1.4	
Refinement statistics					
Resolution range, Å	26.0–2.2				
No. reflections (work/test)	34,866/2,944				
R_{cryst}/R_{free}‡	0.223/0.277				
No. atoms	3,489				
rmsd from ideal geometry					
Bond lengths, Å	0.021				
Bond angles, °	2.289				
Ramachandran residue statistics					
	85.1% core				
	13.2% allowed				
	1.6% generously allowed				
	0.0% disallowed				
Average temperature factor, $Å^2$	29.5				
No. water molecules	189				
No. Hg^{2+} ions	2				
PDB ID code	3GFH				

Initial phase calculations were carried out with the datasets NAT, D11, D4–10, and D11–2 and the resulting figure of merit (= 0.58 to 3.5 Å resolution) was calculated with these datasets. Threading of the EutL sequence into the electron density was guided by anomalous dispersion electron density maps calculated with data set SE1. Because of radiation sensitivity, complete multiple wavelength scans of a single crystal failed. The refinement of the model was carried out with dataset D4–10 because of favorable refinement statistics. As this dataset is derived from a mercury-soaked crystal, the final model includes 2 partially-occupied heavy atoms. Additional refinements of the model against native or seleno-metthionine data (Se1) confirmed that no structural changes were induced with the binding of the metal.

*$R_{sym} = \Sigma\, |I - \langle I\rangle| / \Sigma\, \langle I\rangle$, where I is the observed intensity and $\langle I\rangle$ is the statistically weighted average intensity of multiple-symmetry related observation.

†Phasing power was defined in SOLVE: PP = rms(FH)/rms(E) (1, 2).

‡R factors: $R = \Sigma\, ||F_{calc}| - |F_{obs}|| / \Sigma\, |F_{obs}|$,; where F_{calc} and F_{obs} are the calculated and observed structure factors, respectively. R_{free} was calculated by using the same formula with 7% of the observed reflections (3).

1. Collaborative Computational Project 4 (1994) The CCP4 suite: Programs for protein crystallography, *Acta Crystallogr D* 50:760–763.
2. Terwilliger TC, Berendzen J (1999) Automated MAD and MIR structure solution. *Acta Crystallogra*phica *D* 55:849–861.
3. Brunger AT (1992) Free *R* value: A novel statistical quantity for assessing the accuracy of crystal structures. *Nature.* 355:472–475.