Supplementary Information JBC/2009/010058

Table S1: Data collection and refinement statistics

Data set	Carboxin	PCP	Empty Q-site
Beamline	ESRF ID23-1	ESRF ID23-1	ESRF ID23-1
Wavelength (Å)	0.97620	0.97625	0.97620
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Unit cell (Å)	119.4, 178.5, 200.9	120.0, 186.1, 204.0	120.3, 184.8, 204.7
Resolution (Å)	22.96-2.4 (2.53-2.4)	50.06-3.2 (3.37-3.2)	52.85-3.2 (3.37-3.2)
R_{merge} (%)*	9.9 (50.3)	12.5 (54.2)	15.3 (70.3)
Completeness (%)	99.7 (99.9)	99.3 (97.9)	99.8 (100.0)
<<I>/σ<I>>	10.2 (2.6)	9.8 (2.1)	8.7 (2.2)
Observed reflections	597048 (85843)	245632 (33570)	270319 (40189)
Unique reflections	166937 (24260)	76421 (10884)	76081 (11003)
Redundancy	3.6 (3.5)	3.2 (3.1)	3.6 (3.7)
Refinement statistics			
Resolution (Å)	133.63-2.4 (2.462-2.4)	46.52-3.2 (3.283-3.2)	51.85-3.2 (3.283-3.2)
Reflections working set (test set)	158494 (8380)	71558 (3828)	71862 (3844)
R_{cryst} (R_{free}) (%)†	17.1 (20.0)	19.7 (22.8)	20.5 (23.3)
Total number of atoms used	26034	24936	24855
B factors			
From Wilson Plot ($Å^2$)	40.5	51.7¶	55.3¶
Mean atomic B factor ($Å^2$)	47.6	55.0	68.6
Anisotropic B_{11}, B_{22}, B_{33} ($Å^2$)	-3.02, 4.37, -1.34	-3.12, 2.40, 0.72	-4.71, 1.74, 2.97
RMS deviations			
Bond lengths (Å)	0.014	0.009	0.011
Bond angles (°)	1.382	1.066	1.264
Cruickshank's DPI for coordinate error (Å)§	0.190	0.420	0.428
Molprobity scores			
Rotamer outliers (%)	2.03	1.91	2.64

Ramachandran outliers (%)	0.03	0.13	0.13
Ramachandran favored (%)	97.35	96.17	96.20
Clash score (percentile)	9.73 (95)	14.25 (95)	11.45 (95)
Overall quality score (percentile)	1.87 (96)	2.13 (99)	2.15 (99)

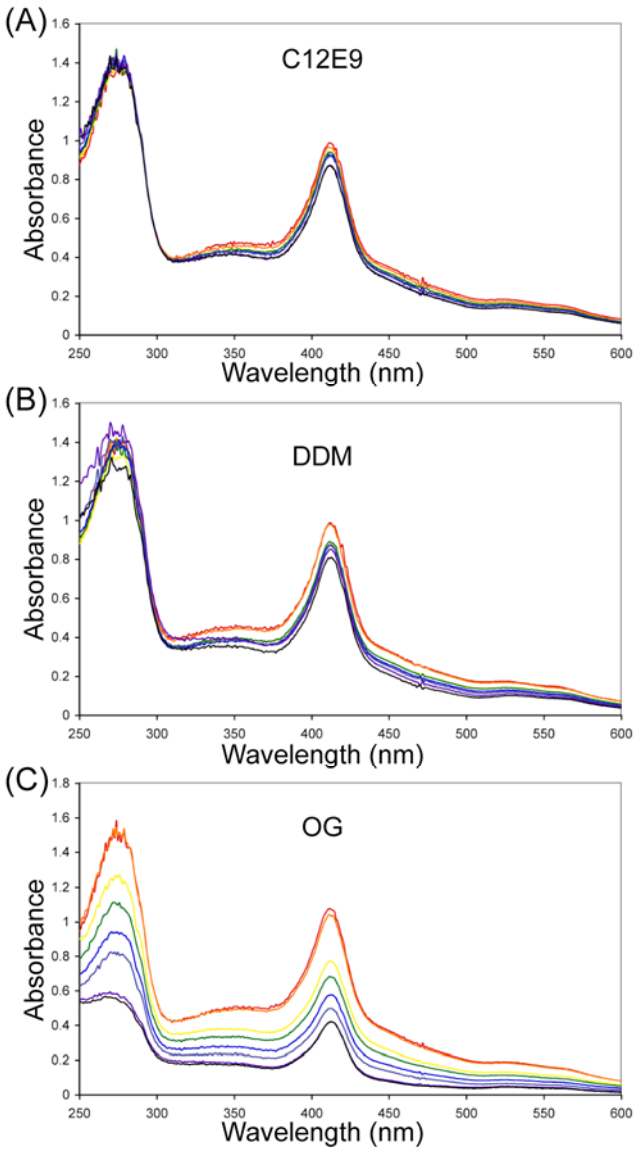
*$R_{merge} = \sum_{\mathbf{h}}\sum_{l}|I_{hl} - \langle I_{\mathbf{h}}\rangle|/\sum_{\mathbf{h}}\sum_{l}\langle I_{\mathbf{h}}\rangle$, where I_{hl} is the lth observation of reflection **h**, and $\langle I_{\mathbf{h}}\rangle$ is the weighted average intensity for all observations l of reflection **h**.

†$R_{cryst} = \sum||F_{obs}| - |F_{calc}||/\sum|F_{obs}|$, where F_{obs} and F_{calc} are the observed and calculated structure factor amplitudes, respectively. R_{free} was calculated as for R_{cryst}, but using the test set of reflections (5% of the diffraction data that were selected randomly and not used during refinement).

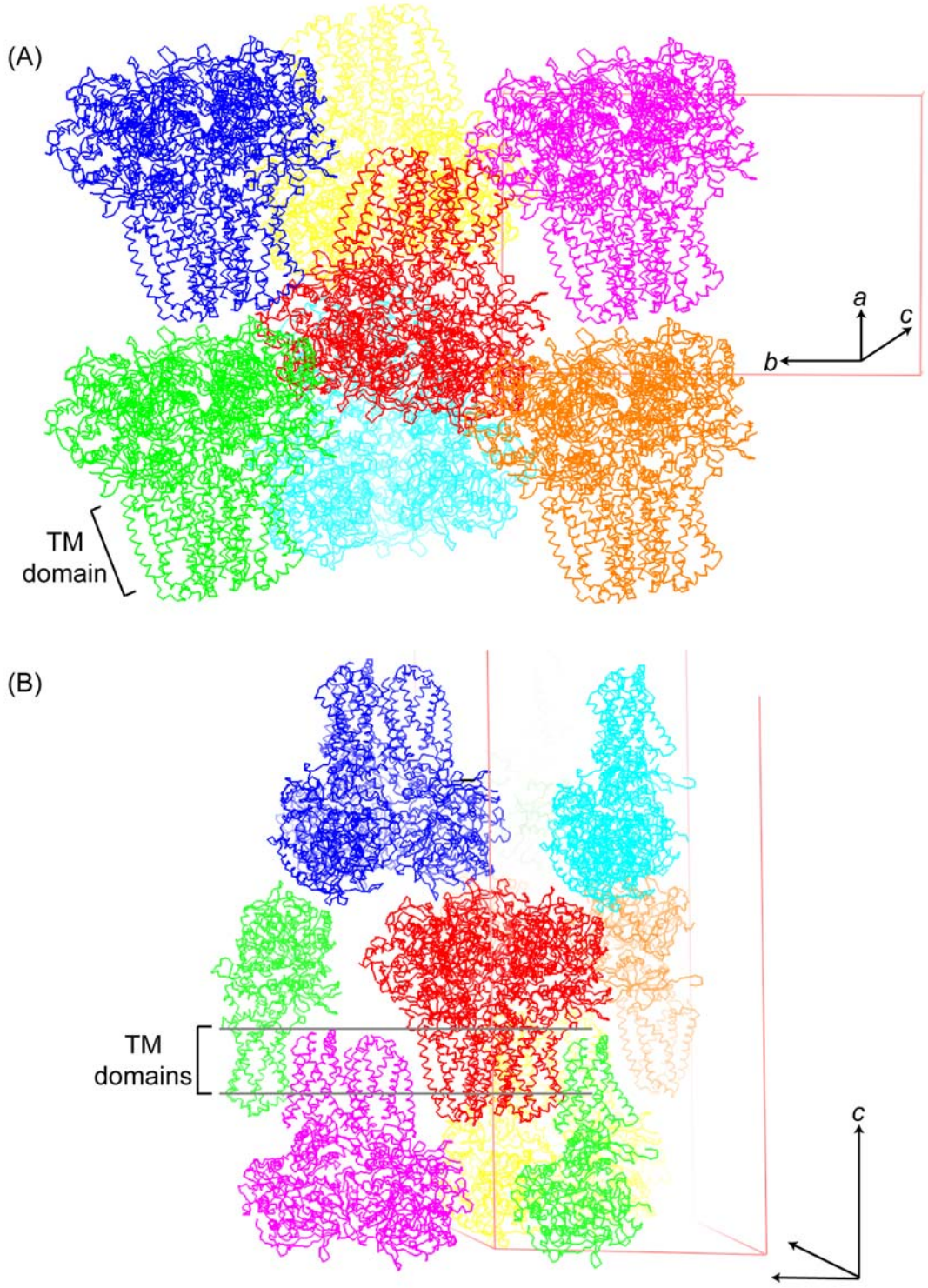
§ $DPI = (N_{atoms}/(N_{obs} - 4N_{atoms}))^{1/2}\, c^{-1/3}\, d_{min}\, R$, where N_{atoms} is the total number of atoms in the unit cell, N_{obs} is the total number of observed reflections, c is the completeness (expressed as a fraction), d_{min} is the minimum d spacing, and R is the conventional R factor. Calculated by the CCP4 program SFCHECK.

¶Calculated by phenix.refine

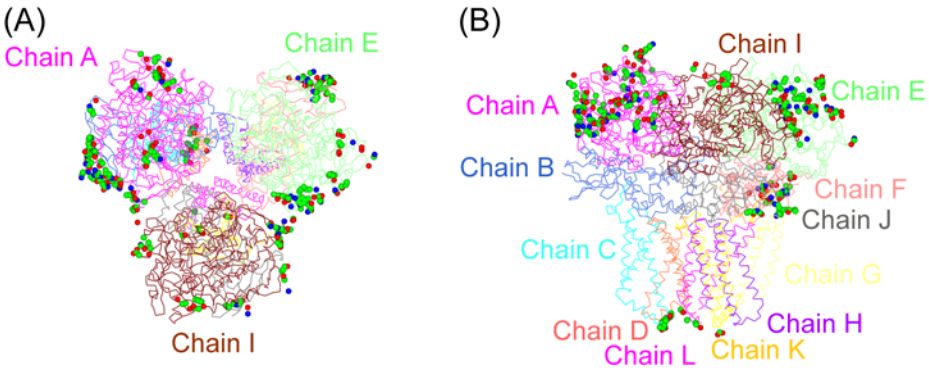
Supplementary Figure S1: Assay for stability of *E. coli* SQR in different detergents. SQR was purified following established protocols (15, 21), and then diluted 50-fold into a range of different buffered detergent solutions. Samples were incubated at 20 °C, and spun at high speed at different time points to pellet aggregated protein. UV/Visible spectrophotometry was used to follow the decrease in SQR concentration in the supernatant due to aggregation. Representative UV/Visible spectra are shown below for the detergents $C_{12}E_9$ (A), which has been used extensively in studies of SQR, DDM (B) and OG (C). Spectra are shown at t=0 (red), t=6 hours (orange), t=23 hours (yellow), t=29 hours (green), t=47 hours (blue), t=53 hours (dark blue), t=70 hours (purple) and t=76 hours (black).



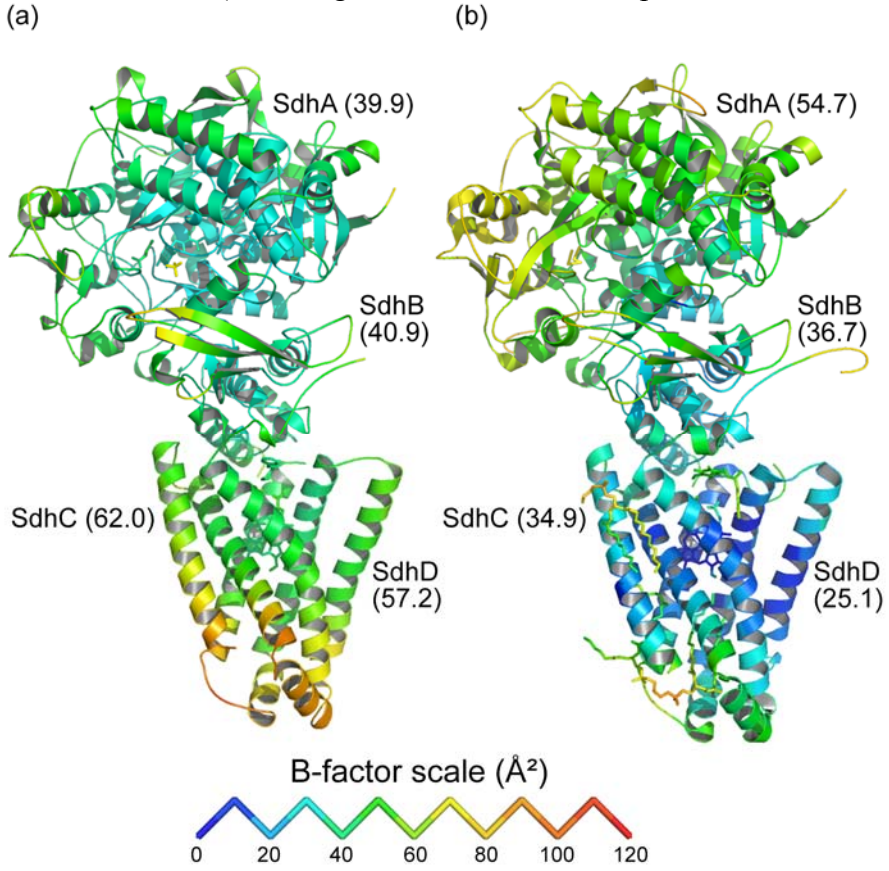
Supplementary Figure S2: Comparison of packing in the orthorhombic $P2_12_12_1$ (A) and trigonal $R32$ (B) crystal forms of *E. coli* SQR (pdb code 1NEK). The Cα atoms of SQR trimers are shown as different colored ribbons.



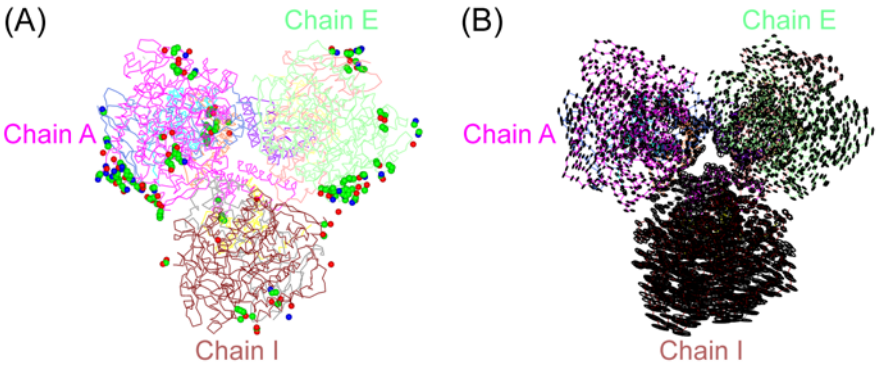
Supplementary Figure S3: Atoms involved in crystal contacts in the orthorhombic crystal form of SQR binding carboxin are shown as coloured spheres. (A) View from the cytoplasm perpendicular to the membrane plane, (B) view parallel to the membrane plane.



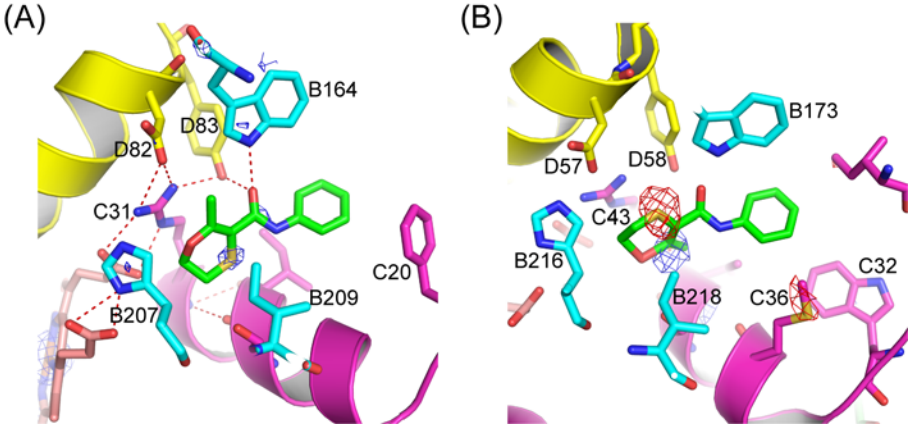
Supplementary Figure S4: Comparison of *B*-factor distributions in (a) the orthorhombic $P2_12_12_1$ and (b) *R*32 (pdb code 1NEK) crystal forms of *E. coli* SQR. The average *B*-factor (whole chain) for each subunit is shown in brackets. For the orthorhombic crystal form, the total *B*-factor is shown (including residual and TLS components after TLS refinement).



Supplementary Figure S5: (A) Crystal contacts in the $P2_12_12_1$ crystal form of SQR with an empty Q-site. Atoms involved in crystal contacts are shown as coloured spheres. (B) TLS-derived anisotropic atomic displacement parameters, shown as black thermal ellipsoids, following TLS refinement of the empty Q-site structure.



Supplementary Figure S6: (A) View of the carboxin-binding site in *E. coli* SQR. Density in blue mesh is a $2mF_o$-DF_c map, contoured at 5σ. (B) View of the carboxin-binding site in avian SQR (pdb code 2FBW). Density is an mF_o-DF_c map, obtained from the coordinates and structure factors deposited at the PDB, contoured at +3σ in blue mesh, and -3σ in red mesh.



Supplementary Figure S7: A view showing the Fe-S clusters and the position of Arg B205 and Lys B230, two residues mutated in order to probe a possible water-mediated proton transfer pathway found in the WT/carboxin structure. The SdhA subunit is colored green, SdhB is cyan, SdhC magenta and SdhD yellow. Carboxin atoms are shown as salmon spheres, heme as red spheres, and the Fe-S clusters as orange and yellow spheres. The side chains of Arg B205 and Lys B230 are shown as spheres, with carbon atoms colored cyan, nitrogen blue and oxygen red.