Supporting Information

Marcia et al. 10.1073/pnas.0904165106

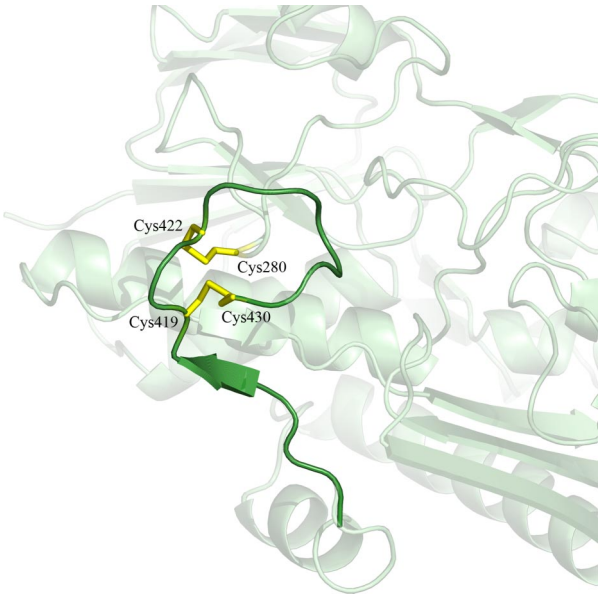

Fig. S1. Disulfide bridges between Cys-419–Cys-430 and Cys-422–Cys-280 stabilizing the C-terminal domain. The protein monomer is represented as a semitransparent cartoon in light green, the last 18-aa main chain of the C-terminal domain is highlighted in dark green, and the side chains of the cystines are represented as yellow sticks.

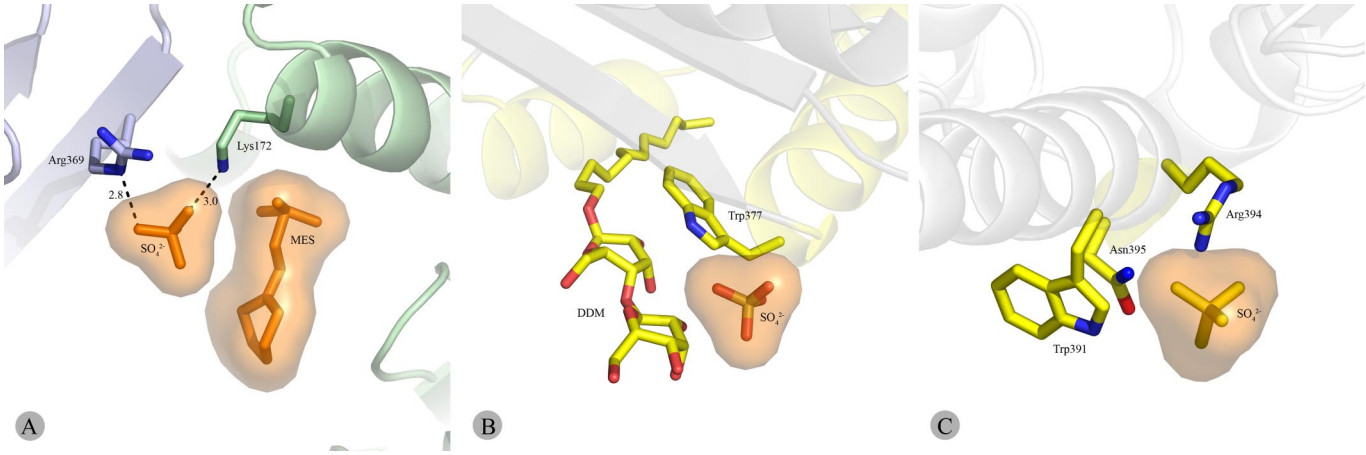

Fig. S2. Putative sulfate ions binding to *A. aeolicus* SQR. The protein is in a cartoon semitransparent representation, relevant amino acids and protein-bound molecules are shown as sticks, and a semitransparent surface of sulfate ions and the Mes molecule is represented in orange. (*A*) One sulfate ion and 1 Mes molecule binding at the monomer–monomer interface in the inner membrane-facing side of the trimer. One sulfate ion is forming ion pairs (distances are in angstrom and are depicted in black dotted lines) between Arg-369 of one monomer and Lys-172 of a neighboring chain. (*B*) The environment of conserved Trp-377, whose side chain interacts with the maltose head group of DDM and with a sulfate ion. (*C*) The environment of Trp-391, whose indole group binds a sulfate ion together with Arg-394 and Asn-395.

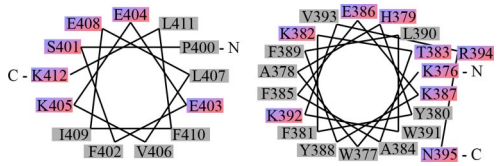

Fig. S3. Helical wheels of the amphipathic helices 376–395 (*Right*) and 400–412 (*Left*). The hydrophobic amino acids are in gray boxes, and the polar residues are in blue-red boxes.

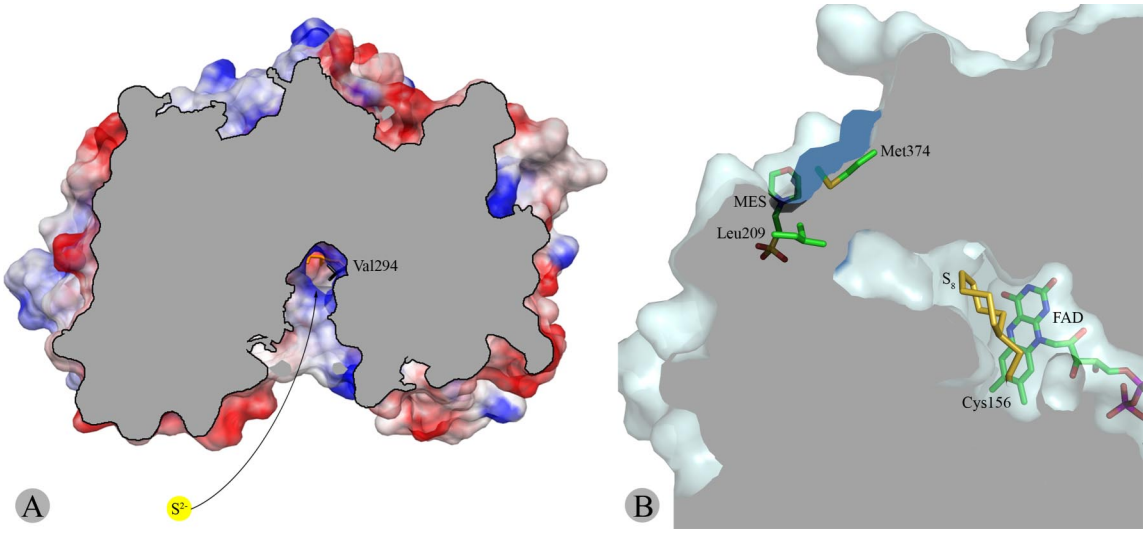

Fig. S4. Sulfide putative access channel and putative cyclooctasulfur release pathways. (*A*) Sulfide (yellow circle) putative access channel (arrow). The surface of the protein is colored according to the electrostatic surface potential calculated with the software GRASP (see Fig. 2). The side chain of Val-294 is represented in black sticks. (*B*) Putative release channel for the product, polysulfur (cyclooctasulfur, S_8). The protein surface is in light cyan. The sulfide oxidation site can be identified by the positions of the FAD molecule, Cys-156, and the polysulfur chain covalently bound to it. The cavity extends toward surface residues Leu-209 and Met-374 (the surface of these residues is shown in blue), and it could potentially open in the inner membrane-facing region of the trimer near the Mes molecule bound to one of the membrane-interacting domains in the crystal structure (see *Results* and Fig. 3). The color code for the residues and molecules shown is described in Fig. 5.

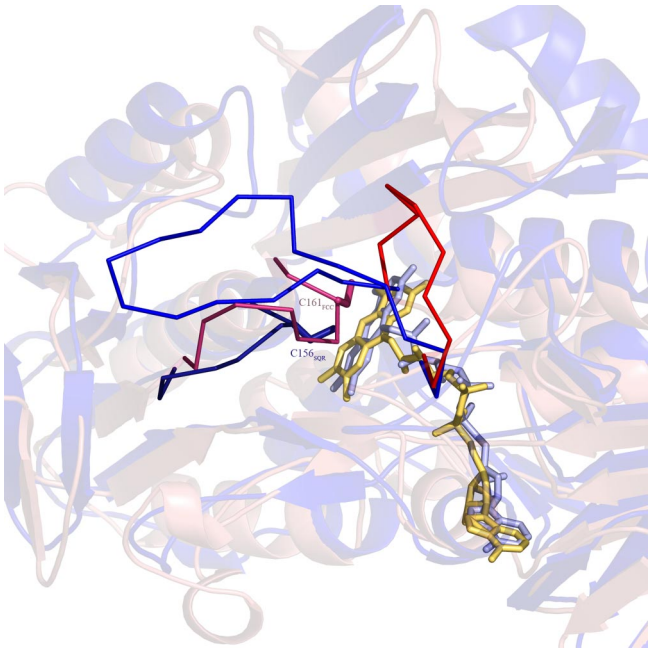

Fig. S5. Superposition of the monomers of the SQR (blue) and of the FCC (red). The FAD molecules are in cyan and yellow for the SQR and FCC, respectively. Two loops around the sulfide oxidation site have a different conformation. Loop 294–312 (SQR) is in bright blue and corresponds to loop 294–303 (FCC) in bright red. Instead, in dark purple (SQR) and dark pink (FCC) are the loops containing Cys-156 (SQR) and Cys-161 (FCC). The side chains of the Cys residues are shown as sticks. A conformational change in the SQR to superimpose the 2 loops to the FCC structure would bring Cys-156 to 4.5 Å away from Cys-347 and might allow the exchange of polysulfur between these 2 residues during polymerization.

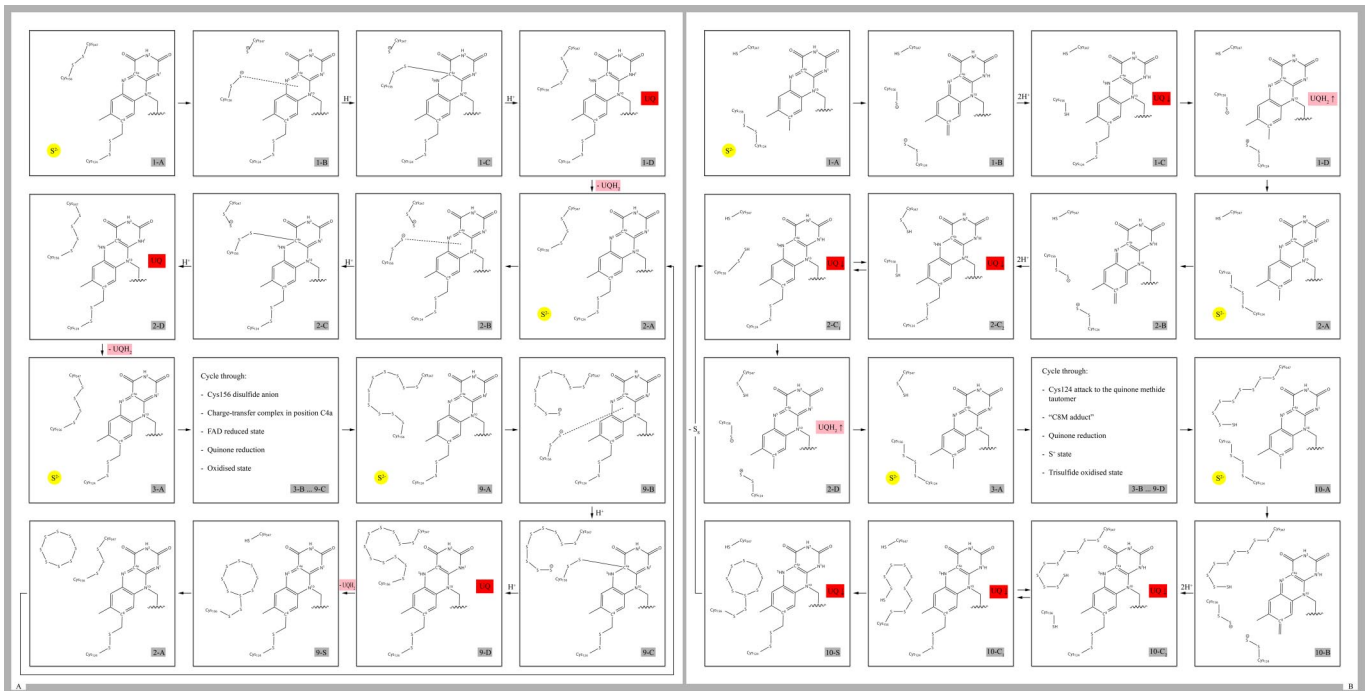

Fig. S6. Detailed steps of the proposed sulfur polymerization reaction following a conventional mechanism of electron transfer with FAD reduction at position C4A (*Left*) or an alternative electron transfer scheme with FAD reduction at position C8M (*Right*). The individual steps are numbered according to the following criteria: the number indicates the number of external S atoms accumulated during polymerization. For example, the steps marked with number 1 relate to the addition of the first sulfide atom, and so on. The letter indicates the oxidoreduction stage of the reaction. Letter A refers to the fully oxidized form, which presents a disulfide bridge in the initial step of catalysis and a polysulfide (*Left*) or a trisulfide (*Right*) bridge state in the following cycles. This state is open for sulfide binding (the sulfide ion is indicated as S^{2-} in a circle with a yellow background). Letter B refers to the state after sulfide binding. (*Left*) Cys-156-S-S$^-$ attacks FAD position C4A, or (*Right*) Cys-124-S-S$^-$ attacks FAD quinone-methide tautomer in position C8M. Letter C refers to the charge-transfer complex (*Left*) or to the C8M adduct described in the text (*Right*). The FAD is in a reduced state, and the protein is open for binding the substrate quinone (indicated as UQ in a box with a red background). In mechanism *Right*, this stage presents an equilibrium, with the growing polysulfur chain exchanged between Cys-156 and Cys-347 after the second cycle. Letter C_1 refers to the conformation in which the chain is attached to Cys-156, and letter C_2 to the one in which the chain binds Cys-347. A significant protein conformational change seems to be required to bring Cys-156 closer to FAD position C4A (*Left*) or favor the S chain exchange between Cys-156 and Cys-347 (*Right*). This possibly involves a rotation of loop 294–312 and of the loop including Cys-156 toward a conformation closer to that of the corresponding loops of FCC (see Fig. S10). Letter D indicates the state in which FAD is reoxidized after electrons are transferred to the quinone (the reduced quinol form is indicated as UQH_2 in a box with pink background). (*Left*) A polysulfide bridge connects Cys-156 and Cys-347, or (*Right*) a transient Cys-124-S$^+$ state is formed. After quinol release, the fully oxidized state (letter A) is formed again. After elongation to a 9-S atom chain, the polysulfur can close on itself to form a cyclooctasulfur still covalently bound to the protein through 1 S atom in a trivalent state, as described in the text and observed in some monomers of the structure. This intermediate is indicated by the letter S. Protonation and deprotonation are mediated by charged residues present in the catalytic pocket of the enzyme but not depicted in this scheme. These might include, for example, Glu-162 on the *re* side [Griesbeck C, et al. (2002) *Biochemistry* 41:11552–11565] and Glu-318 and/or water molecules on the *si* side of FAD.

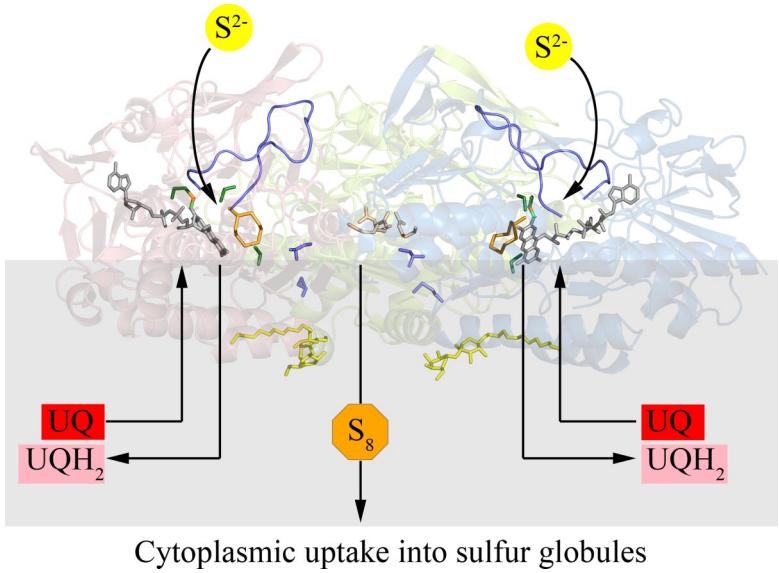

Fig. S7. Overall picture of the proposed SQR reaction coupled to the mode of membrane binding. The protein is shown in a semitransparent cartoon diagram, and details are highlighted only for the 2 monomers in the foreground for higher clarity of representation. Sulfide (S^{2-}) accesses the oxidation site (*re* face of FAD) from a channel controlled by loop 294–312 (blue ribbon). FAD (gray sticks) gets reduced through either a classical charge-transfer complex in position C4A or through an unusual alternative covalent adduct at position C8M. Polymerization occurs over Cys-156 and Cys-347. The mature product (a putative cyclooctasulfur ring; orange sticks) is possibly released below the trimeric central contact (Arg-204; side chains in ochre sticks) directly into the lipidic bilayer through a channel controlled by the side chains of Leu-209 and Met-374 (blue sticks). In *A. aeolicus*, S_8 would then be taken up from the cytoplasmic side to be incorporated into sulfur globules. At every cycle of sulfide oxidation, a quinone molecule (UQ in a red box indicates the oxidized form, UQH_2 in a pink box the reduced form) is reduced in the quinone-binding pocket (*si* face of FAD).

Other Supporting Information Files

Table S1 (PDF)
Table S2 (PDF)