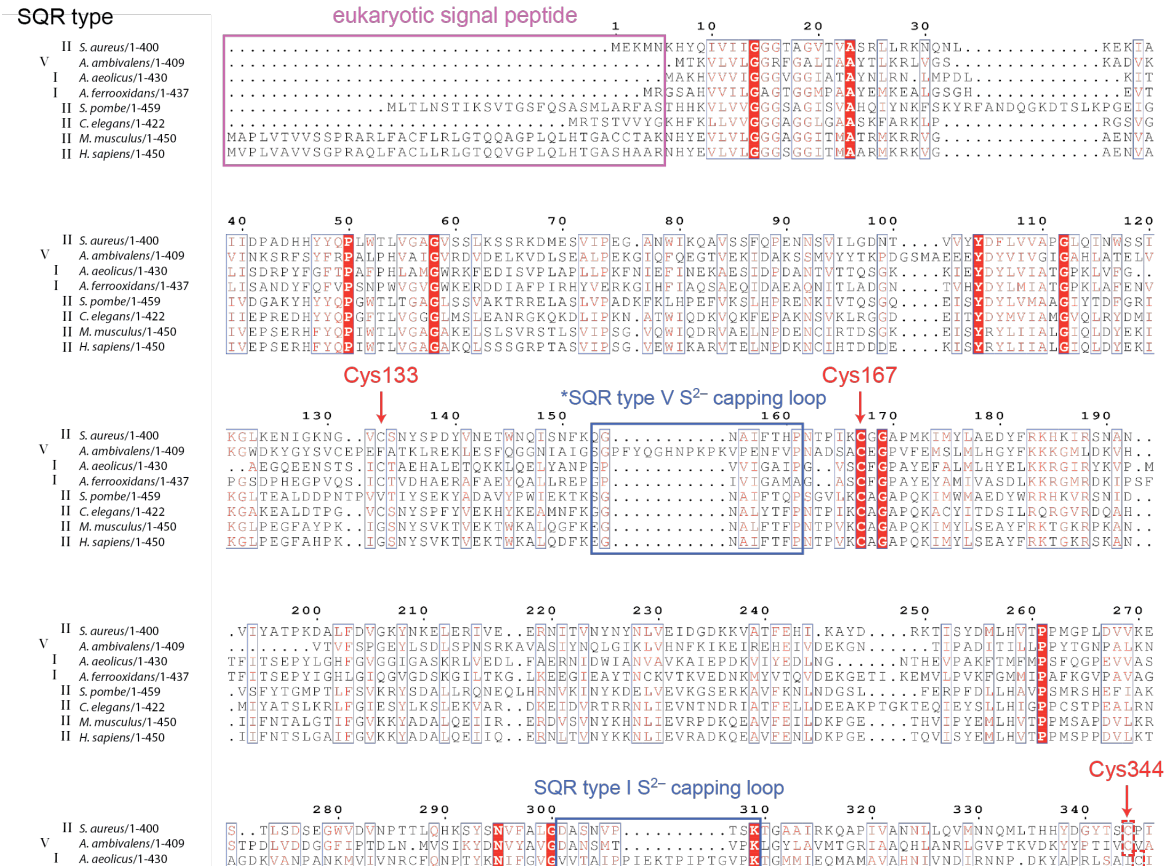
SUPPLEMENTAL MATERIAL

Staphylococcus aureus sqr encodes a type II sulfide:quinone oxidoreductase and impacts reactive sulfur speciation in cells

Shen *et al.*

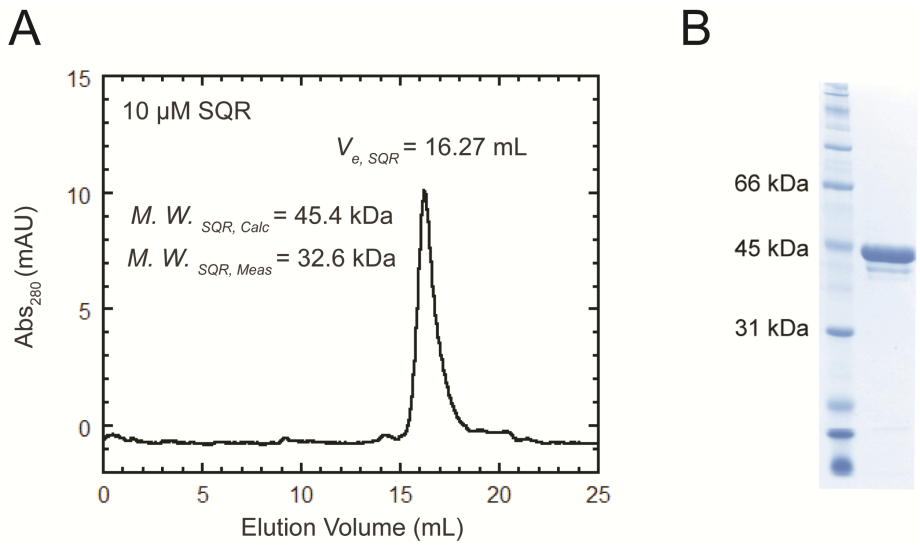
This file contains **Supplementary Figures S1-S7**.



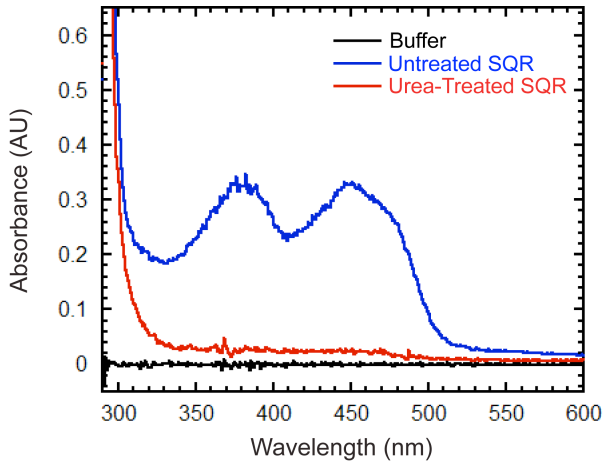
Supplementary Figure S1. Structure-based sequence alignment of *S. aureus* SQR with other biochemically or structurally characterized sulfide:quinone oxidoreductases. The amino acid sequence alignment includes SQR from *S. aureus* strain Newman (NWMN_0029) (*top* entry), the subject of the work presented here, three structurally characterized SQRs from *A. ambivalens* (PDB: 3H8I or 3H8L),[1] *A. aeolicus* (PDB: 3HYV)[2] and *A. ferrooxidans* (PDB: 3T2Z), as well as

predicted or biochemically studied SQRs from eukaryotic organisms (*S. pombe*, *C. elegans*, *M. musculus* and *H. sapiens*). The SQR type (types I-VI) based on a recently reported classification is indicated to the left of each sequence.[3] *Red arrows* mark the positions of the three cysteine residues that correspond to C133, C167 and C344 in *S. aureus* SQR. *Pink box* marks the mitochondrial localization signal. The *blue boxes* mark the two insertion sites which incorporate what have been designated as sulfide (S^{2-}) capping loops in the type V and type I SQRs, respectively, from the N-terminus. As can be seen, *S. aureus* SQR is classified as a type II SQR and lacks both sulfide capping loops, as well as well all previously characterized functionally important features of a type I SQR enzyme.[3] Type II SQRs resemble most closely flavocytochrome c:sulfide dehydrogenases (FCSD), which has been structurally characterized in *Allochromatium vinosum*[4] (see Fig. 1, main text); note that there is no clearly defined functional role established for the cysteine corresponding to C133 in the type II enzymes.[5] The other two Cys, C167 and C344, form the disulfide bond (see text for details). The *light green box* marks the C-terminal amphipathic helical motif which mediates the interaction between SQR protomer in oligomeric enzymes and/or electron acceptor quinone in the membrane. This structure-based alignment was generated in Jalview utilizing Clustal algorithm as default and rendered using ESPript 3.[6]

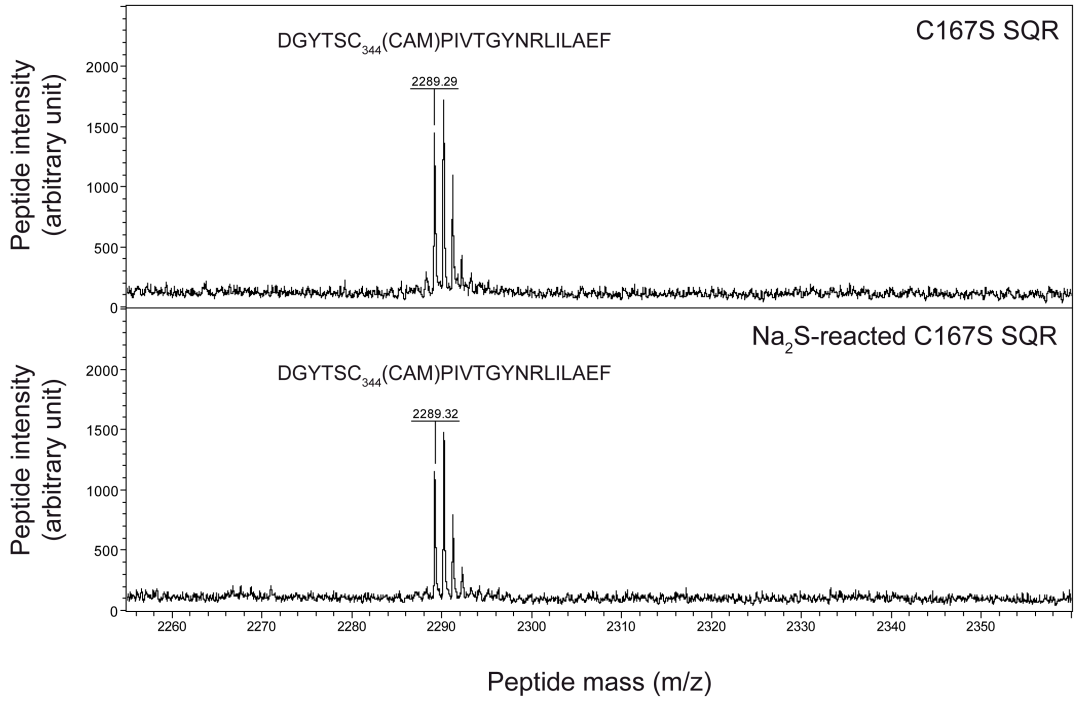
Supplementary Figure S2. Sequence coverage of purified SQR identified by MALDI-MS analysis. Purified SQR protein was proteolytically digested in solution separately by sequencing grade endonucleases (trypsin, Glu-C, Lys-C, Chymotrypsin and Asp-N), with produced peptides enriched by C18 ziptip and analyzed by MALDI-mass spectrometry. Identified peptides are shaded in *grey* and revealing 89.2% (357 of 400 residues over residues 4-400) sequence coverage.



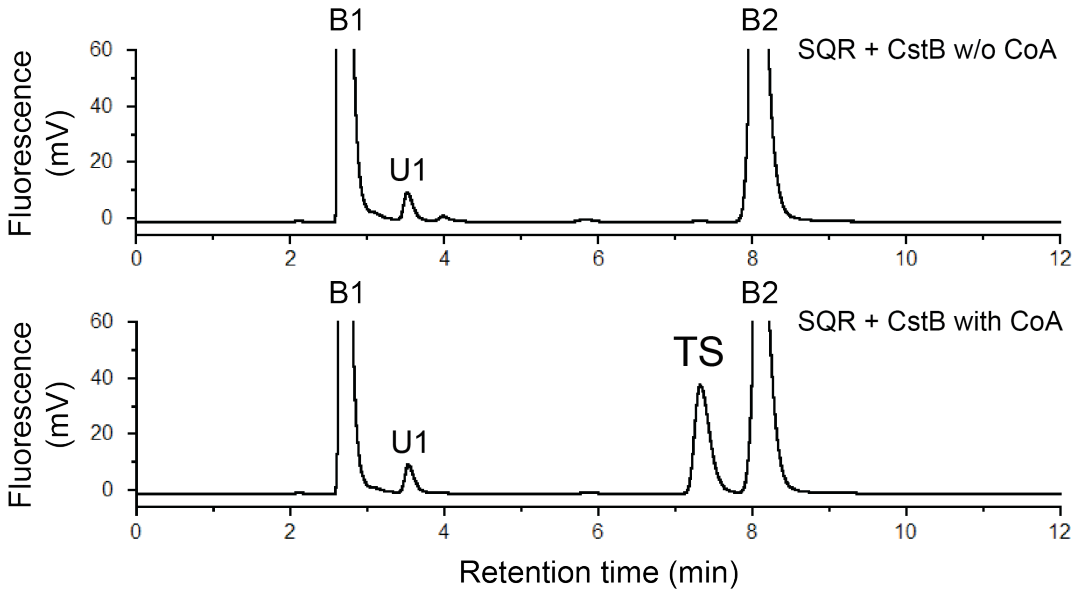
Supplementary Figure S3. Gel filtration profile and SDS-PAGE analysis for wild-type SQR. (A) The gel filtration profile of purified wild-type SQR (V_e = 16.27 mL) was obtained from a Superdex-200 Increase gel filtration column. The standards used for calibration of Superdex-200 Increase gel filtration column were ovalbumin (44 kDa), conalbumin (75 kDa), aldolase (158 kDa), ferritin (440 kDa) and thyroglobulin (669 kDa). Buffer condition: 25 mM Tris-HCl, 500 mM NaCl, 2 mM TCEP, 10% glycerol, pH 8.0. (B) 2 μg of purified wild-type SQR analyzed on a 15% SDS-PAGE on the right of broad range molecular weight standards.



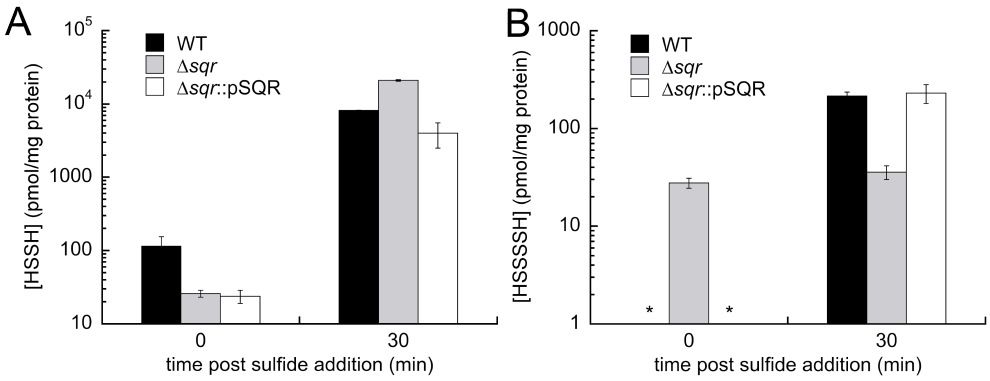
Supplementary Figure S4. Absorption spectra of untreated SQR and urea-treated SQR (50 μM protomer). The *black trace* is for SQR buffer without reducing reagent: 25 mM Tris-HCl, 500 mM NaCl, 10% glycerol, pH 8.0. The *blue trace* is for untreated SQR and the *red trace* is for urea-treated SQR.



Supplementary Figure S5. MALDI mass spectrometry analysis reveals no derivatization of the peak for Cys344-containing peptide from Na_2S-reacted C167S SQR in the absence of electron or S^0 acceptors. The unreacted C167S SQR (*upper panel*) and Na_2S-reacted C167S SQR (*bottom panel*) were alkylated with iodoacetamide, proteolytically digested by Asp-N and analyzed by MALDI mass spectrometry. Cys344-containning peptide displays a mass of 2289.29 Da and 2389.32 Da after reacting with Na_2S, with no detectable derivatized peak found.



Supplementary Figure S6. Representative LC-based assay used to measure the catalytic thiosulfate (TS) production with sulfide as substrate in the absence of CoA (*top trace*) and the presence of 400 μM CoA (*bottom trace*) at *t*=2 min by 2 μM SQR + CstB. Note that B1 and B2 peaks are from the labeling buffer and U1 peak is unidentified species which does not originate with the labeling buffer.[7] A peak (TS) corresponding to thiosulfate was only observed in the presence of CoA.



Supplementary Figure S7. Cellular inorganic polysulfide species profiling of the *S. aureus* strain Newman wild-type (WT), Δ*sqr* and Δ*sqr*::pSQR strains before (*t*=0) and after (*t*=30 min) addition of 0.2 mM Na_2S added to mid-log (OD_{600}≈0.2) cultures. (A) hydrodisulfide (HSSH), also denoted generically as S_2^{2-} (main text); and (B) hydrotetrasulfide (HSSSSH), also denoted as S_4^{2-} (main text) presented in units of pmol per mg total protein. *, n.d., not detected (below the detection limit of 1 pmol per mg protein).

Supplementary References

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(2) Marcia, M., Ermler, U., Peng, G., Michel, H. (2009) The structure of Aquifex aeolicus sulfide:quinone oxidoreductase, a basis to understand sulfide detoxification and respiration. *Proc Natl Acad Sci U S A 106*, 9625-9630.

(3) Marcia, M., Ermler, U., Peng, G., Michel, H. (2010) A new structure-based classification of sulfide:quinone oxidoreductases. *Proteins 78*, 1073-1083.

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(7) Shen, J., Keithly, M. E., Armstrong, R. N., Higgins, K. A., Edmonds, K. A., Giedroc, D. P. (2015) *Staphylococcus aureus* CstB Is a novel multidomain persulfide dioxygenase-sulfurtransferase involved in hydrogen sulfide detoxification. *Biochemistry 54*, 4542-4554.