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Supporting information for article:

Structure of BrxA from *Staphylococcus aureus*, a bacilliredoxin involved in redox homeostasis in Firmicutes

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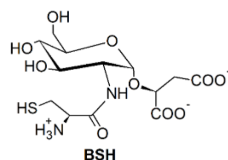

Scheme S1. The structure of bacillithiol

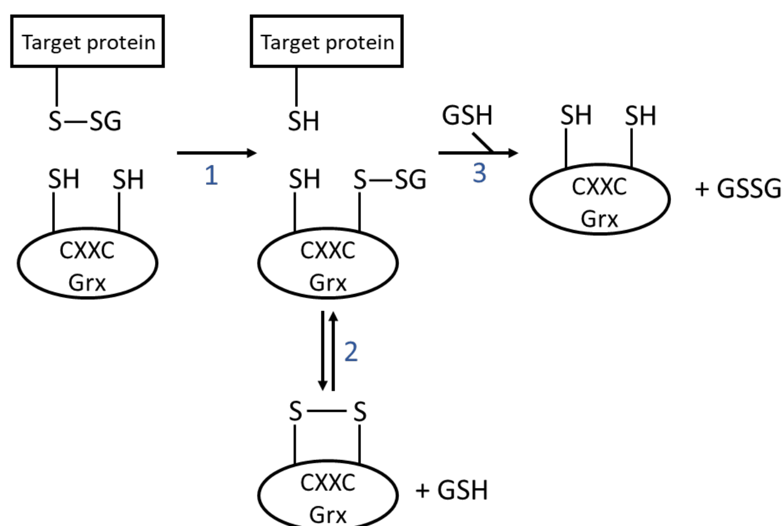

Figure S1. Proposed dithiol reaction for typical CXXC glutaredoxins. **(1)** The N-terminal Cys residue of Grx reacts with the glutathione mixed disulfide of the target protein, transferring the glutathione to Grx and yielding the reduced target protein. **(2)** The C-terminal Cys residue of Grx reacts with the glutathione mixed disulfide, producing a disulfide linkage between the CXXC cysteines and releasing free glutathione (GSH). Although this side reaction may appear to be futile, it may play a role in protecting Grx during oxidative stress or keeping the Grx inactive until it is needed (when GSH levels increase). **(3)** GSH reduces Grx back to its free thiol form, producing glutathione disulfide (GSSG), which can be reduced via an NAD(P)H-dependent reductase. This figure was adapted from Mashamaite, *et al*.[1]

S1. MATERIALS AND METHODS

Gel filtration chromatography. The BrxA enzyme was analyzed via gel filtration chromatography using an Agilent 1200 series HPLC fitted with a Superdex 200 Increase 10/300 gel filtration column (GE). Mobile Phase A consisted of 20 mM HEPES pH 7.0 with 100 mM NaCl and 0.5 mM dithiothreitol (DTT), whereas Mobile Phase B consisted of Mobile Phase A without the addition of DTT. Chromatography was conducted at 21 °C with a flow rate of 0.75 mL/min while monitoring effluent absorbance at 280 nm. The column was calibrated with gel filtration standards (Sigma) with Mobile Phase A. Wild-type and C56S mutant BrxA enzymes were diluted to 0.5-1 mg/mL in Mobile Phase A, after which each enzyme form (50 μL) was analyzed in separate chromatographic runs. Subsequently, wild-type and C56S BrxA (2-4 mg/mL) were incubated in the presence of 5 mM BSSB at room temperature for one hour. The enzymes were diluted to 0.5-1 mg/mL in Mobile Phase B, after which each enzyme form (50 μL) was analyzed in separate chromatographic runs. Finally, wild-type BrxA (2-4 mg/mL) was air-oxidized by incubating it at 4 °C for four days. The preparation was diluted to ~1 mg/mL in Mobile Phase B, after which 50 μL of the sample was analyzed via gel filtration as with the other samples.

SDS-PAGE analysis. Wild-type and C56S BrxA (both at ~0.5 mg/mL) were incubated in the presence of either 1 mM DTT or 5 mM BSSB at room temperature for one hour. Another sample of wild-type BrxA (~0.5 mg/mL) was incubated at 4 °C for four days. Laemmli sample buffer (BioRad) was added without additional reducing agents, and the samples were boiled for 2 minutes. A 12% SDS-PAGE gel (BioRad) was run on all samples and stained with Coomassie.

S2. RESULTS

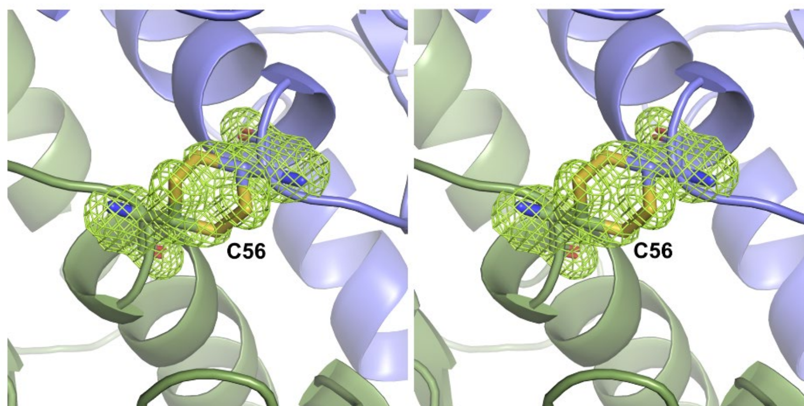

Figure S2. Stereo view of the Cys56 inter-subunit disulfide linkage, colored by subunit. This simulated annealing, $F_o - F_c$ omit map is contoured at 3σ and was generated in Phenix by omitting the coordinates for Cys56.

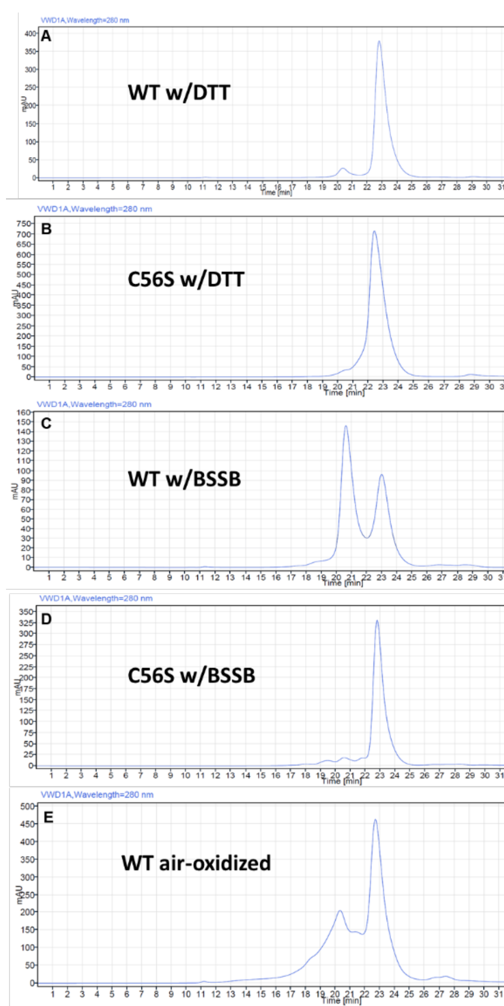

Figure S3. Elution profiles from BrxA gel filtration chromatography. A: wild-type BrxA with DTT added. The major peak occurs at ~22.5 minutes, with a very minor peak at ~20.5 minutes. **B:** C56S mutant BrxA with DTT added. The major peak occurs at ~22.5 minutes. **C:** Wild-type BrxA with bacillithiol disulfide added. The major peak occurs at ~20.5 minutes, with a minor peak at ~22.9 minutes. **D:** C56S mutant BrxA with bacillithiol disulfide added. The major peak occurs ~22.8 minutes. **E:** BrxA WT incubated at 4 °C for four days without added BSSB or DTT (i.e. "air oxidized"). The major peak occurs at ~22.8 minutes, with a minor peak at ~20.4 minutes. The standard curve (not shown) demonstrated that a retention time of ~22.5 minutes is consistent with a BrxA monomer, whereas a retention time of ~20.5 minutes is consistent with a BrxA dimer. Thus, these results suggest that BrxA can form either a monomer or dimer depending on the redox conditions.

Figure S4. Coomassie-stained 12% SDS-PAGE gel of BrxA. Lane 1: Unstained Protein Molecular Weight Marker (Thermo). **Lane 2:** Wild-type BrxA after incubation in 1 mM DTT. **Lane 3:** C56S BrxA after incubation in 1 mM DTT. **Lane 5:** Wild-type BrxA after incubation in 5 mM BSSB. **Lane 6:** C56S BrxA after incubation in 5 mM BSSB. In the presence of DTT, both wild-type and C56S BrxA migrate as their monomeric masses. In the presence of BSSB, wild-type BrxA migrates as mixture of monomer and dimer, whereas C56S BrxA migrates only as a monomer. The faint bands in the BSSB lanes are likely from random disulfide formation after protein denaturation and boiling.

Figure S5. LigPlot+ ligand interaction diagram for BSSP.[2] The BSSP is shown with purple bonds, BrxA amino acid residues are shown with brown bonds, and waters are shown as cyan circles. Potential hydrogen bonds are shown with dashed lines with labeled distances.

REFERENCES

1. Mashamaite LN, Rohwer JM, Pillay CS. 2015. The glutaredoxin mono- and di-thiol mechanisms for deglutathionylation are functionally equivalent: Implications for redox systems biology. *Biosci Rep.* 35(1).

2. Laskowski R A, Swindells M B 2011. LigPlot+: multiple ligand-protein interaction diagrams for drug discovery. *J. Chem. Inf. Model*. 51, 2778-2786.