SUPPLEMENTAL DATA

Crystal structure and biophysical properties of *Bacillus subtilis* BdbD: An oxidizing thiol:disulfide oxidoreductase containing a novel metal site

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SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Reduction potential determinations– The midpoint reduction potential of sBdbD was determined by calculating the fraction of sBdbD reduced, f_r, at each point in the titration and for each selected resonance in the NMR spectrum. f_r was plotted as a function of the system potential, and the data fitted to the Nernst equation (Equ. S1), where E_m is the midpoint potential of sBdbD, n is the number of electrons, F is the Faraday constant, R is the gas constant, T is the temperature and E_h is the potential of the GSSG/GSH couple. A value of -240 mV was used for the standard redox potential of GSSG/GSH at pH 7 and 25 °C (1).

$$f_r = \frac{\exp^{(E_m-E_h)nF/RT}}{1+\exp^{(E_m-E_h)nF/RT}}$$
(S1)

Determination of pH stability range and cysteine thiol pK$_a$ values– Following the reaction of cysteine side chains with alkylating reagents is a well established method for determining the pK_a values of protein cysteine thiol groups, where the observed rate constant is proportional to the extent of thiol deprotonation at a given pH (2-4). sBdbD, pre-reduced by incubation with 1 mM tris(2-carboxyethyl)phosphine (TCEP) for 2 hr, was added (final concentration 0.1 µM) to 3 ml of mixed buffer containing 15 µM 6-bromoacetyl-2-dimethylaminonaphthalene (badan) Emission spectra were recorded every minute for 45 min using an excitation wavelength of 380 nm. Observed rate constants for badan-labeling were obtained by fitting fluorescence changes at 540 nm for each pH value to a single exponential function. pK_a values were determined by fitting the data to a modified form of the Henderson-Hasselbach equation (Equ. S2) (3,5), where k_o is the pseudo-first-rate constant, k_b is the baseline rate and k is the rate constant for cysteine modification reaction.

$$k_o = k_b + \frac{k}{1+10^{pK_{a1}-pH}}$$
(S2)

Protein conformational stability measurements– Fluorescence intensity at 353 nm was converted to fraction unfolded, f_u, where fluorescence intensity in the absence of denaturant and at 6 M denaturant were taken as values for the native and denatured forms of the protein, respectively. f_u was plotted against denaturant concentration and data were fitted to Equ. S3, which describes a two step unfolding process:

$$f_u = \frac{\exp^{(m[D]-\Delta G^o)/RT}}{1+\exp^{(m[D]-\Delta G^o)/RT}}$$
(S3)

where $\Delta G°$ is the Gibbs free energy for unfolding at zero denaturant concentration [D] and m describes the dependence of ΔG on the concentration of denaturant. Note that the Gibbs free energy of unfolding can be converted into that of stabilization by changing the sign. Midpoints of unfolding ($D_{0.5}$) were calculated using Equ. S4.
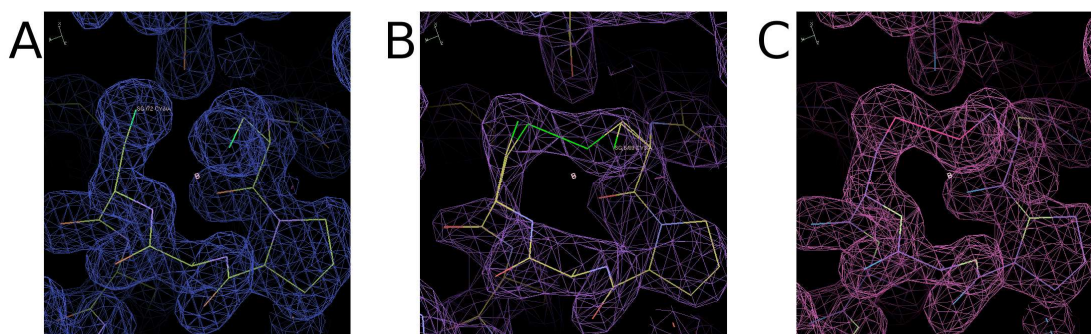
$$D_{0.5} = -\Delta G^o/m$$
(S4)

Fig. S1: 'Unbiased' omit electron density maps confirming the redox status of the active site cyesteine thiols in structures of sBdbD. (A) Reduced BdbD (at 1.5 Å resolution). (B) Oxidized BdbD (at 2.3 Å resolution). (C) Oxidized, EDTA-treated BdbD (at 1.69 Å resolution). In each case, $2|F_o|-|F_c|$ electron density maps were calculated using phases derived from a model in which the active site CPSC residues were removed and a round of restrained refinement applied before map calculation. All maps are contoured at 1.5 σ. The density clearly shows continuous electron density between the sulfur atoms in both 'B' and 'C' indicating the presence of a disulfide bond while the reduced structure, 'A', does not. Careful inspection of the maps during refinement showed that the oxidized structure has a mixture of redox states dominated by the disulfide form, whereas the EDTA-treated oxidized structure represents a fully oxidized population.
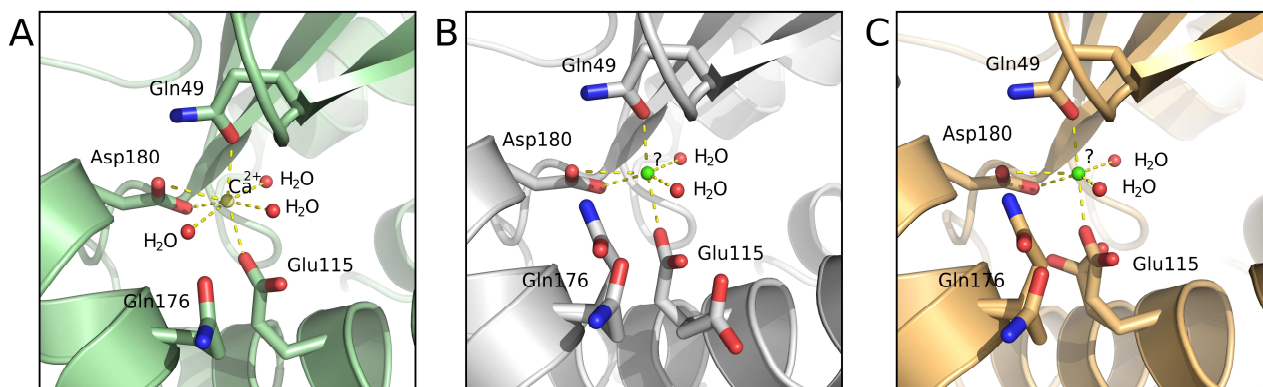
Fig. S2. Comparison of as isolated sBdbD Ca^{2+} site with EDTA-treated sBdbD structures. (A) Reduced BdbD. (B) Reduced EDTA-treated sBdbD. (C) Oxidized EDTA-treated sBdbD. Question marks denote the central ion (known to be Ca^{2+} in reduced BdbD), which may be either a low occupancy Ca^{2+} site, or a high occupancy sodium atom (or both). For reduced BdbD, the Ca^{2+} site is fully occupied and has a 7-coordinate capped-octahedral geometry. In the EDTA-treated structures, one of the waters is lost, and the space is partially occupied by Gln176. In addition, Glu115 adopts multiple side chain conformations, only one of which is close enough to coordinate the central ion.

SUPPLEMENTAL TABLES

Table S1: Data collection and refinement statistics

	Reduced EDTA-treated BdbD	Oxidized EDTA-treated BdbD
Space group	$P2_1$	$P2_1$
Unit cell	a=38.55, b=43.63, c=54.58, β=107.40	a=38.55, b= 43.57, c= 54.56, β=107.40
Unique reflections	33,423	18,095
Resolution (Å)	25.08 -1.40 (1.48-1.40)	36.79 -1.69 (1.78-1.69)
R_{sym}	0.058 (0.351)	0.052 (0.286)
I/σ(I)	9.6 (2.4)	19.9 (3.5)
Completeness (%)	97.8 (99.6)	92.7 (66.2)
Multiplicity	3.4 (3.5)	5.4 (4.0)
R_{work}	0.2088	0.195
R_{free}	0.2544	0.250
rmsAngle (°)	1.207	1.225
rmsBond (Å)	0.008	0.010
Free c.c.	0.918	0.918

Values in parentheses represent the highest resolution shell.
Free c.c. is the correlation coefficient calculated with the free reflection set.

SUPPLEMENTAL REFERENCES

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AUTHOR CONTRIBUTIONS

AC constructed the expression plasmid pACN2, purified proteins, conducted crystallographic work, and measured badan-reactivity profiles, pH stabilities and equilibrium unfolding/refolding of the Ca^{2+}-containing protein. AL measured redox potentials, equilibrium unfolding/refolding of the Ca^{2+}-depleted protein and conducted substrate interaction experiments. OH conducted NMR experiments. MCM purified protein and generated BdbD antiserum. GRM supervised the NMR work. LH and NLB planned and supervised the project. AC, LH and NLB wrote the manuscript.