Methionine sulfoxide reductase B from *Corynebacterium diphtheriae* catalyzes sulfoxide reduction via an intramolecular disulfide cascade

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Running title: *Thiol-disulfide exchange mechanism of Cd-MsrB*

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Supplementary Table 1

Table S1. Structural statistics over the 20 lowest-energy, water-refined NMR structures of the reduced MsrB. [a]Calculated for all residues. [b]Calculated with PROCHECK-NMR.

Distance restraints	
Total	2335
Intra-residue (i - j = 0)	472
Sequential (|i - j| = 1)	618
Medium range (1 < |i - j| < 5)	414
Long range (|i - j| ≥ 5)	825
S-S (Zn cluster)	6
Dihedral angle restraints	
φ and ψ	165
Restraints violations	
NOE, > 0.5 Å	0.0
Dihedral angle, > 5°	1.2
RMSD from average,[a] Å	
Backbone	0.75 ± 0.11
Heavy atoms	1.22 ± 0.14
Ramachandran statistics,[b] %	
Most favored	76.7
Allowed	20.6
Additionally allowed	1.9
Disallowed	0.8

Supplementary Table 2

Table S2: Two disulfide bonds are formed upon MetSO reduction. Free thiol content of Cd-MsrB WT and Cys mutants (C66S, C122S and C127S) in the absence and presence of MetSO was determined using the DTNB assay. * Indicates that the experiment was performed under denaturing conditions.

Cd-MsrB	Without MetSO		With MetSO	
	Expected value	Obtained value	Expected value	Obtained value
WT	3.0	2.5	1.0	0.6
C66S*	6.0	6.1	4.0	4.1
C122S	2.0	1.7	2.0	2.2
C127S	2.0	2.0	0.0	0.0

Supplementary Figure 1

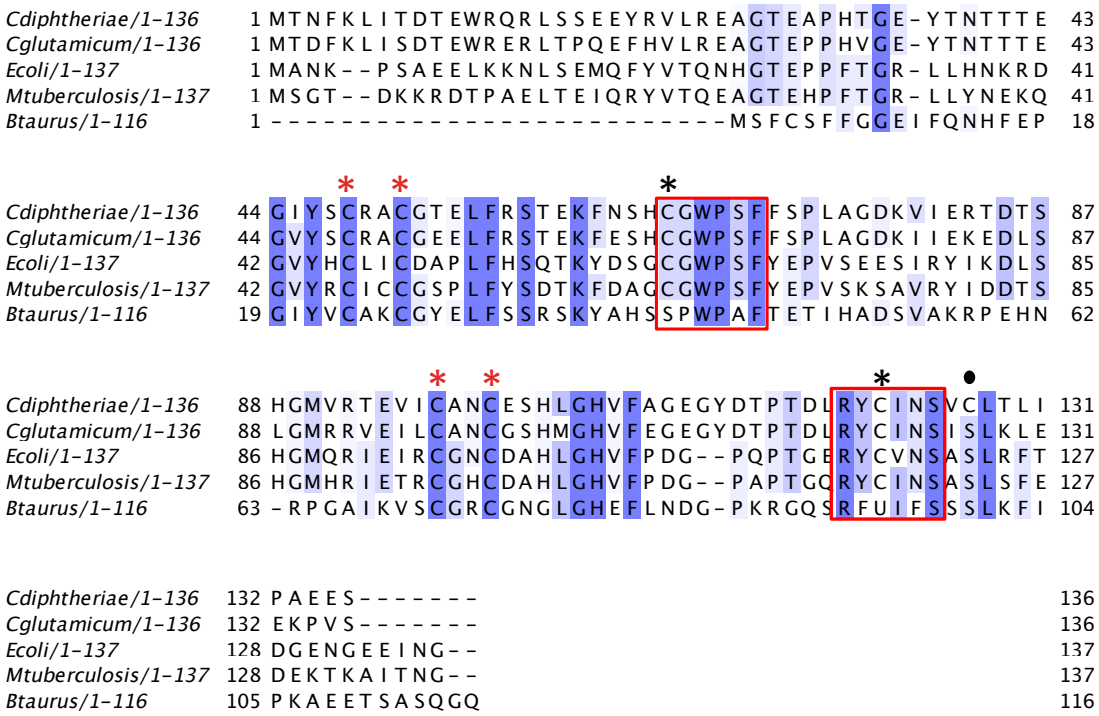

Figure S1. Sequence alignment of MsrB from several organisms. Sequences of MsrB from *B. taurus*, *C. diphtheriae*, *C. glutamicum*, *E. coli* and *M. tuberculosis* are shown (accession numbers: Q3MHL9, ERA55218, SJM49428, YP_006124650 and SGC50423.1). The sequence numbering is according to the *C. diphtheriae* MsrB sequence, and the intensity of the blue color gradient is based on 30% identity. The red asterisks represent the conserved cysteines involved in the coordination of Zn^{2+}; the black asterisks represent the conserved nucleophilic and resolving cysteines, and the black dot represents the non-conserved Cd-MsrB cysteine. The conserved MsrB motifs are boxed.

Supplementary Figure 2

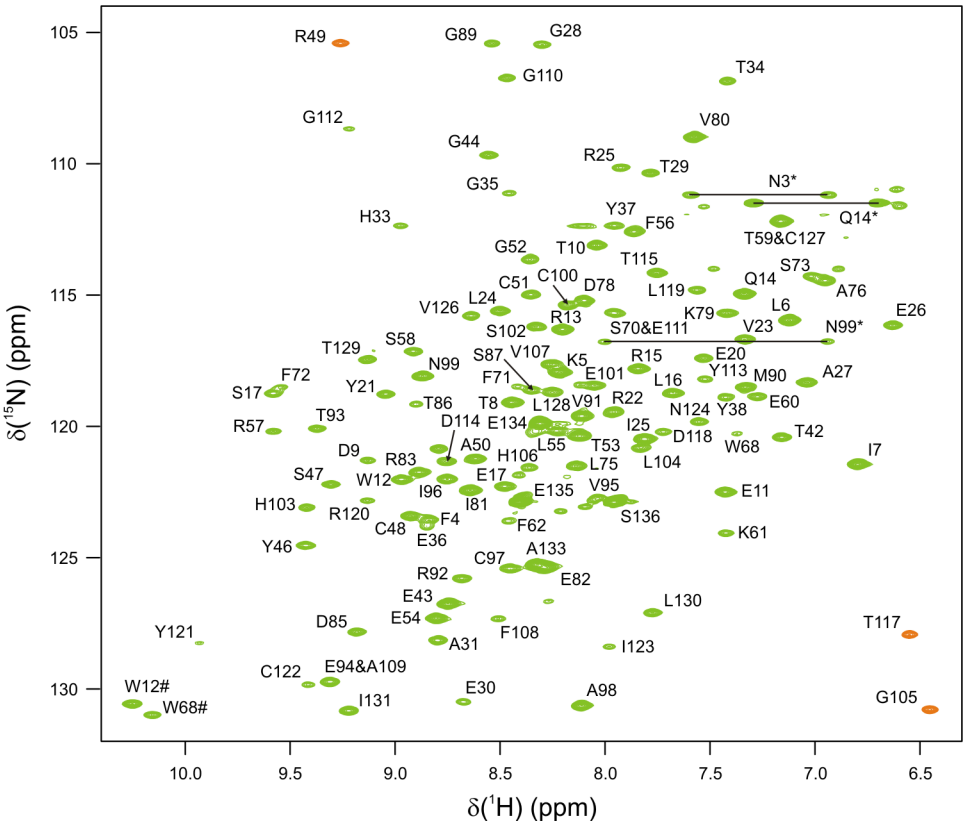

Figure S2. The assigned [^{1}H,^{15}N]-HSQC spectrum of the reduced Cd-MsrB. The labels identify the backbone amides, the side-chain NH_2 groups of Asn and Gln residues (joined by horizontal lines and indicated by asterisks), and the Trp indole amides (labeled by hash symbols). The backbone amide resonances of Arg49, Gly105, and Thr177 (shown in orange) are aliased in the nitrogen dimension, with their real spectral positions being 133.42, 102.73, and 99.87 ppm, respectively.

Supplementary Figure 3

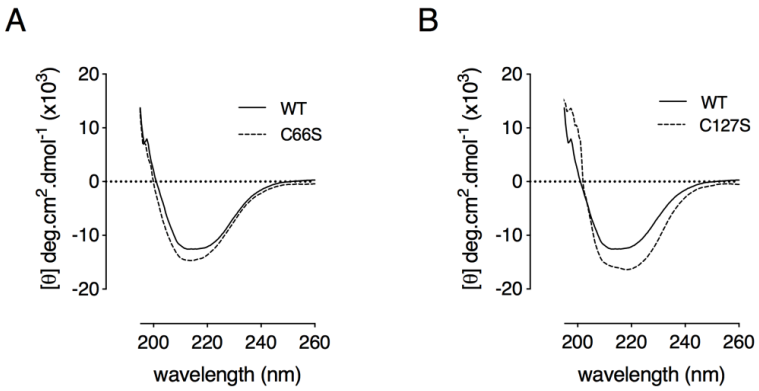

Figure S3. The C66S and C127S mutants have different overall secondary structures compared to WT Cd-MsrB. Circular dichroism far-UV spectra show that both (**A**) the C66S mutant and (**B**) the C127S mutant show changes in the molar ellipticity [θ] at 208 nm and 222 nm, compared to WT. The graphs were generated with Prism8.

Supplementary Figure 4

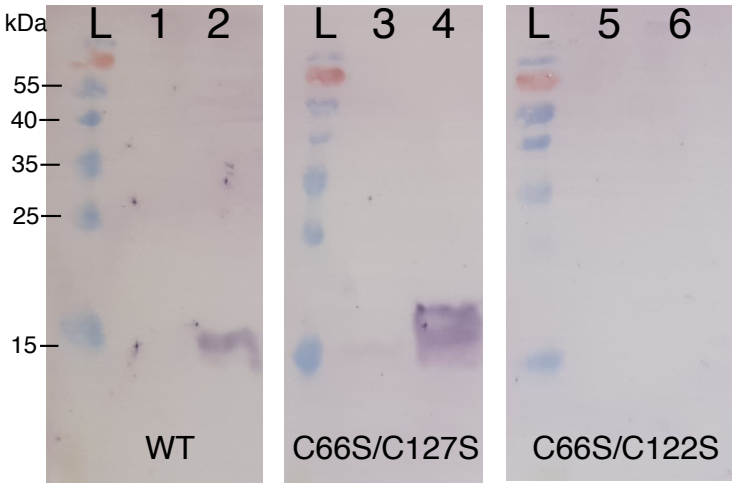

Figure S4: Sulfenic acid is formed on Cys122 after substrate reduction. Cysteine *S*-sulfenylation on an anti-dimedone Western blot is shown. WT Cd-MsrB (lane 2) and the C66S/C127S double mutant (lane 4) show sulfenic acid formation in the presence of substrate, while for the C66S/C122S double mutant no band was observed (lane 6). Lane 1, 3 and 5 are the negative controls in the absence of substrate. L is the molecular weight marker.

Supplementary Figure 5

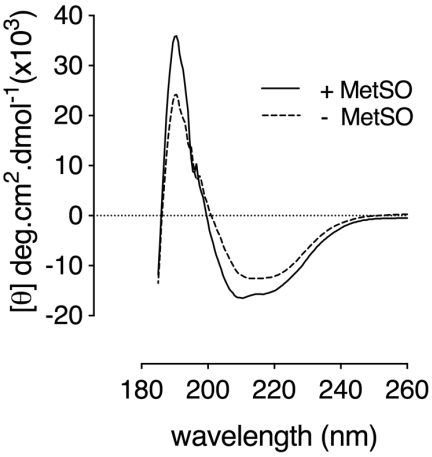

Figure S5. Overall secondary structural changes upon substrate reduction. Circular dichroism far-UV spectra of Cd-MsrB reduced (-MetSO) and MetSO-treated (+MetSO) samples are shown. Overall structural changes are observed at 195, 208 and 222 nm. Graph was generated with Prism8.

Supplementary Figure 6

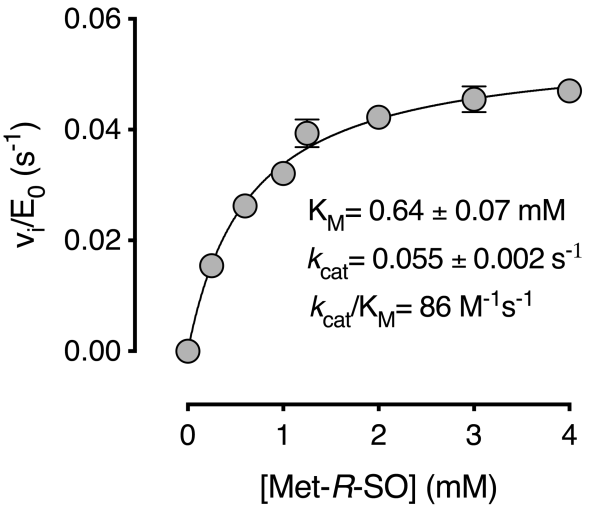

Figure S6: Kinetic parameters of MetSO reduction by WT Cd-MsrB. Michaelis-Menten curve of WT Cd-MsrB is shown. Data are presented as a mean ± SD of two independent technical repeats and the graphs were generated using Prism8.

Supplementary Figure 7

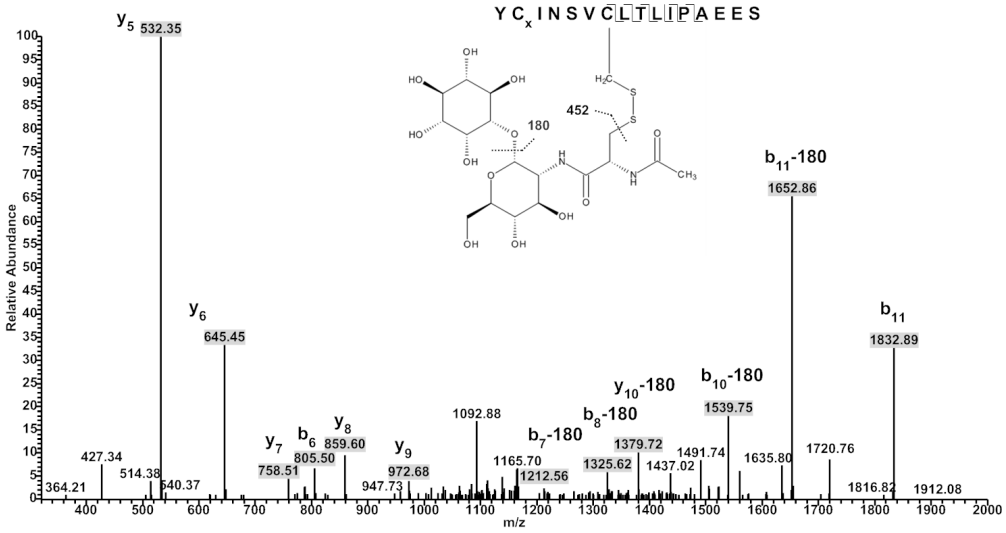

Figure S7. Cd-MsrB is *S*-mycothiolated on Cys127. The multistage activation LC-MS^n spectrum shows MS^2 data obtained from a +2 parent ion with m/z 1182.51 combined with MS3 data obtained from a daughter ion of m/z 1092.48 resulting from a neutral loss of inositol (180 Da). The y- and b- series of ions allows exact localization of the mixed disulfide between mycothiol and Cys127. C_x stands for NEM modified cysteine.

Supplementary Figure 8

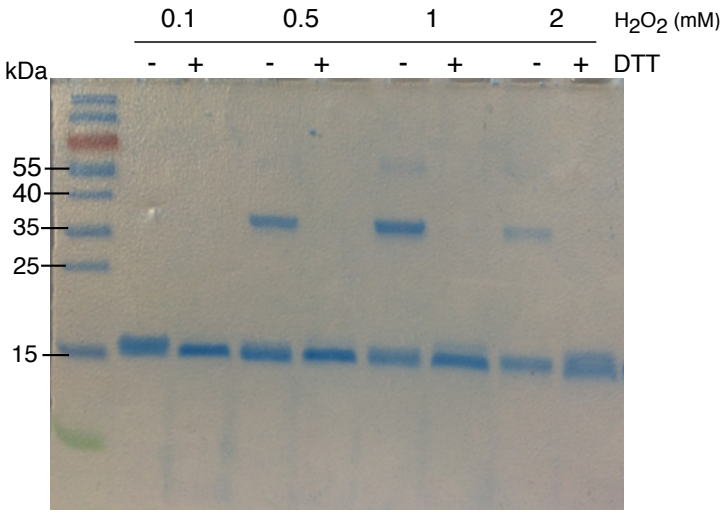

Figure S8. DTT reduces oxidized Cd-MsrB. The SDS-PAGE gel shows Cd-MsrB treatment with increasing H_2O_2 concentrations (0.1 mM, 0.5 mM, 1 mM, and 2 mM). After oxidation, Cd-MsrB migrates as monomeric oxidized (17 kDa), dimeric (34 kDa) and oligomeric (51 kDa) forms. In the presence of DTT, the oxidized bands shift to the reduced monomeric band.