Supporting Information

Mechanistic insight into cadmium-induced inactivation of the Bloom protein

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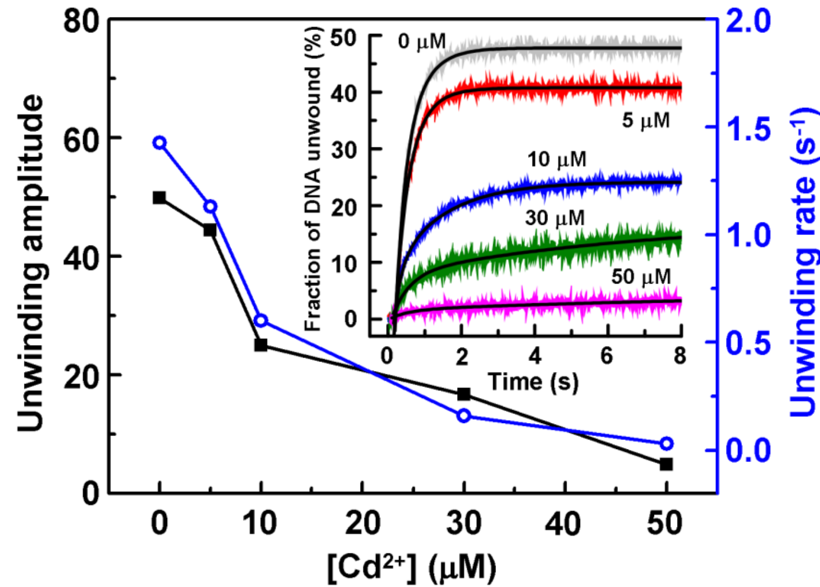


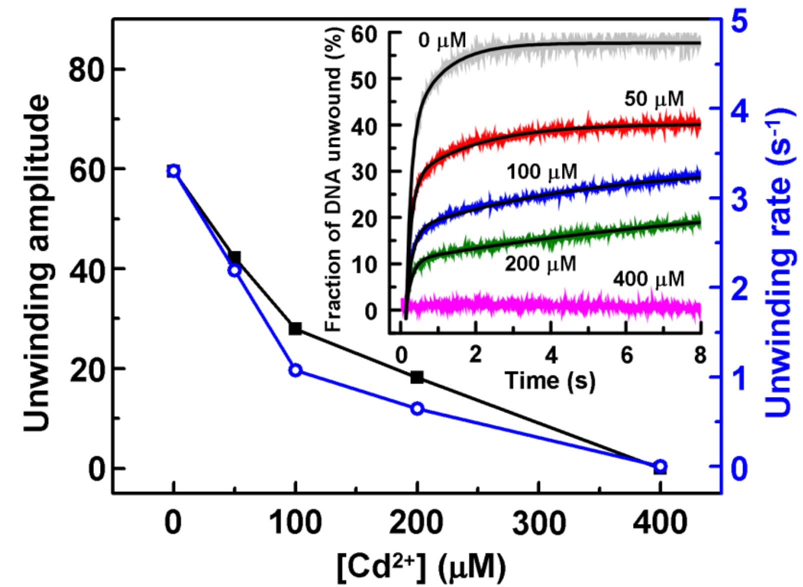
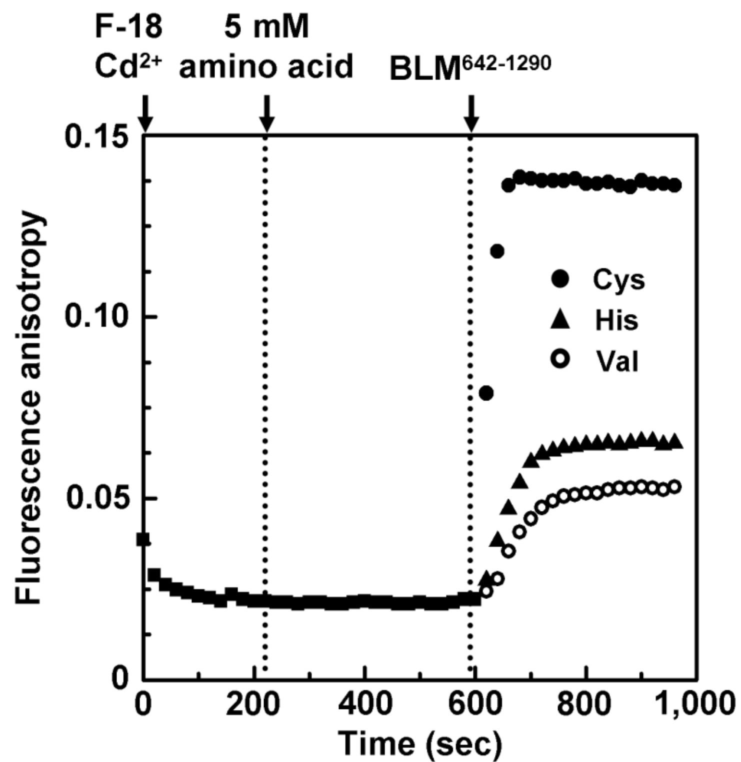


Figure S1: Effect of Cd^{2+} on DNA unwinding activities of $BLM^{full\text{-}length}$ (a) and $RecQ^{E.coli}$ (b) as measured by stopped-flow FRET assay. Each plot shows the dependence of the kinetic rate constant and reaction amplitude as a function of $CdCl_2$ concentration. Proteins (60nM) were first preincubated with varying concentrations of $CdCl_2$ for 5min at 25°C. The DNA substrate (16-bp duplex with a 20-nt 3' tail) was added into the reaction mixture at a final concentration of 4nM, and the reaction was initiated by rapid mixing with 1mM ATP. Insert: typical kinetics for DNA unwinding in the presence of various $CdCl_2$ concentrations. Reactions were performed in Tris-HCl buffer (25mM, pH 7.5) supplemented with 50mM NaCl, 2mM $MgCl_2$ and 1mM DTT at 37°C. The response of $BLM^{642\text{-}1290}$ to Cd^{2+} (explicitly shown in Fig. 6a) was similar to the one observed with $BLM^{full\text{-}length}$.



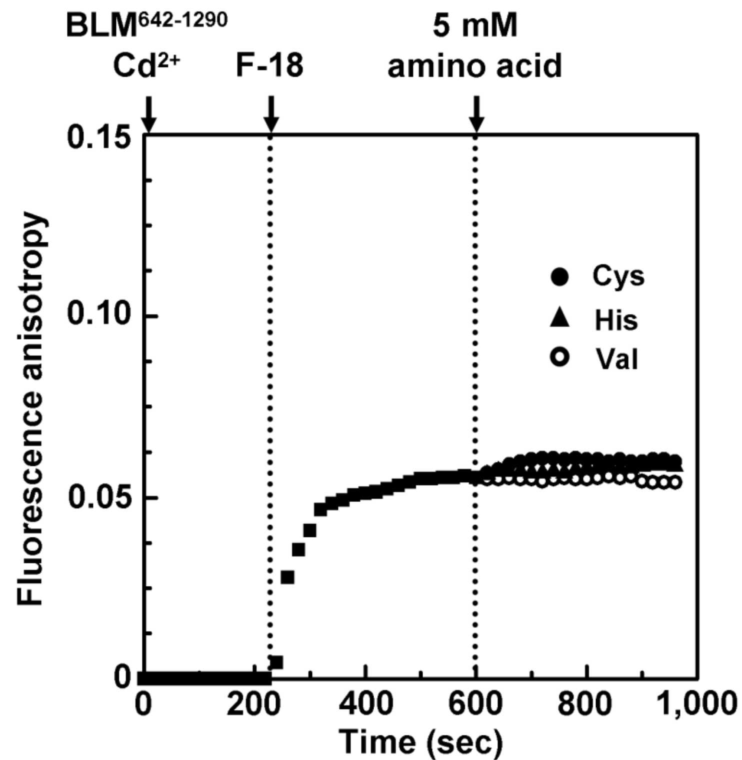


Figure S2: Effect of amino acids on the Cd^{2+}-mediated inhibition of the DNA-binding activity of $BLM^{642-1290}$. $BLM^{642-1290}$ (200nM) was either added after pre-incubation of 10nM 3'-fluorescein-labeled 18-mer ssDNA (F-18), $CdCl_2$ (100μM) and amino acids (5mM) (panel a) or pre-incubated with $CdCl_2$ before addition of F-18 and amino acids (panel b). The steady-state fluorescence anisotropy was then monitored at 25°C as a function of time.

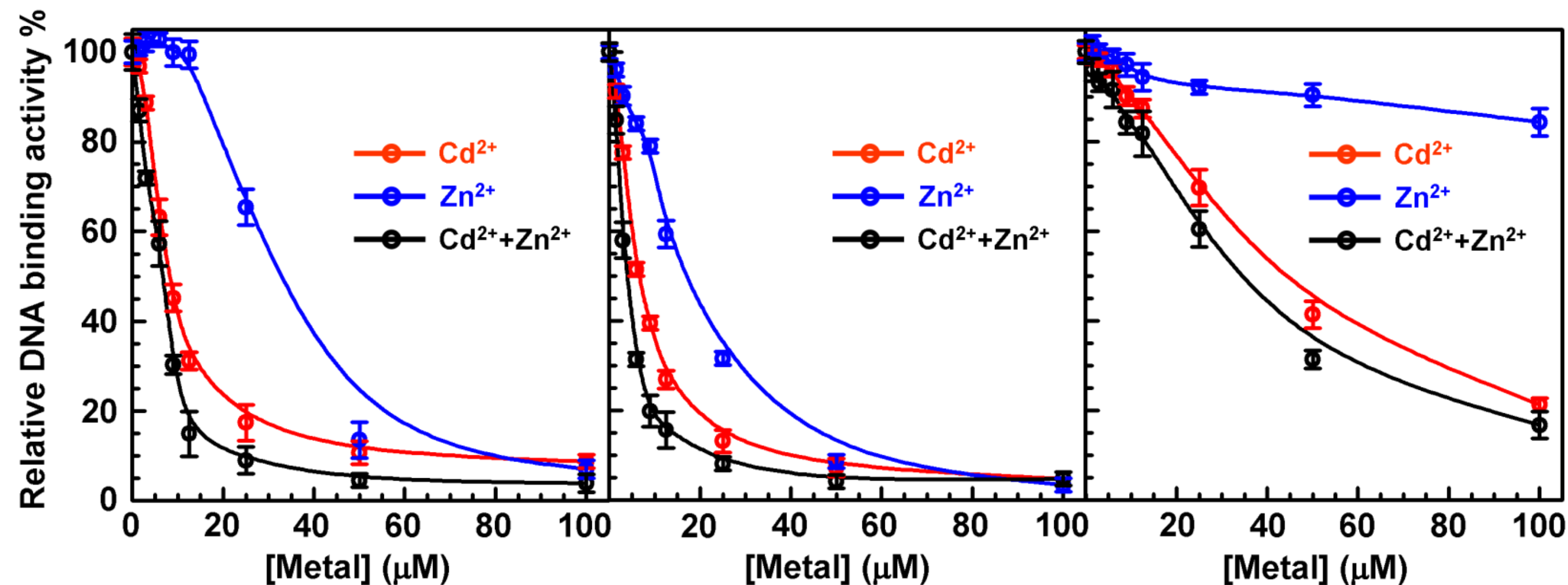


Figure S3: Effect of Cd^{2+}, Zn^{2+} or a combination of both cations on the DNA-binding activities of $BLM^{642-1290}$ (left), $BLM^{full\text{-}length}$ (middle) and $RecQ^{E.coli}$ (right). Increasing concentrations of Cd^{2+}, Zn^{2+} or Cd^{2+}/Zn^{2+} were added on pre-formed helicase/DNA complexes. The concentration relative to Cd^{2+}/Zn^{2+} combination, indicated on the x-axis, corresponds to the total concentration of cations where $[Cd^{2+}] = [Zn^{2+}]$. The fluorescence anisotropy was measured at 25°C using 200nM protein and 5nM F-24 oligonucleotide as described in Methods. The relative DNA-binding activity was calculated according to Eq. 2.

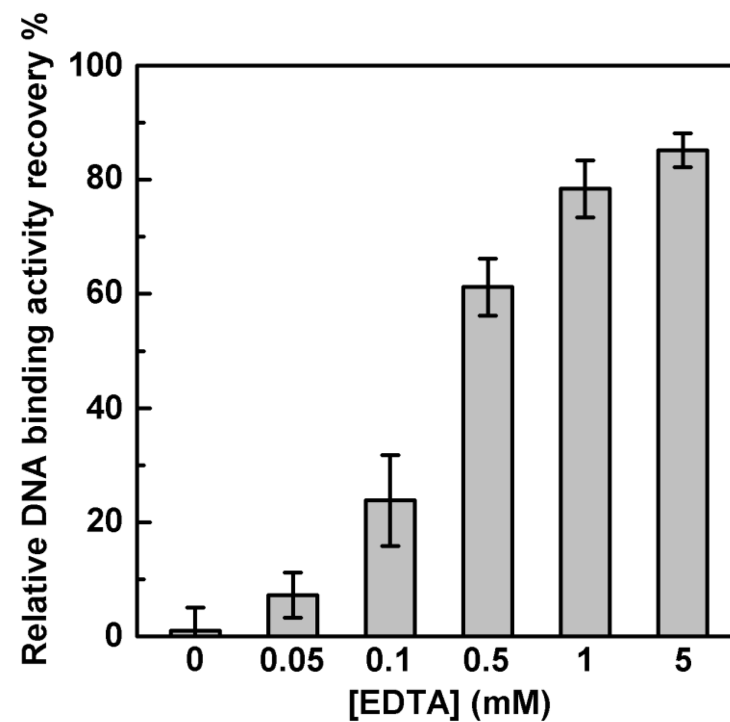


Figure S4: Relative recovery of the DNA-binding activity of $BLM^{642-1290}$ as a function of EDTA concentration after Cd^{2+}-induced dissociation. Concentrations of protein, ssDNA (F-18) and Cd^{2+} were 200nM, 5nM and 50μM, respectively. The relative DNA-binding activity was determined in a Tris-HCl buffer (50mM, pH 8.0, 50mM NaCl, 1mM DTT) according to Eq. 2.

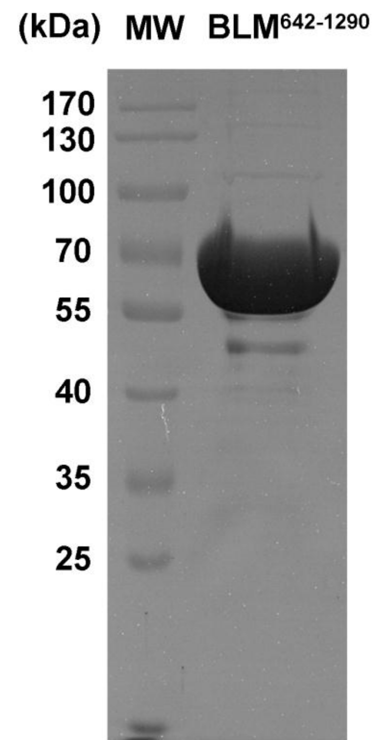


Figure S5: SDS-PAGE analysis of the purified recombinant BLM$^{642\text{-}1290}$ protein. The gel (10% acrylamide) was stained using Coomassie brilliant blue R250.

The amount of BLM$^{642\text{-}1290}$ protein loaded on the gel was 12µg.

Table S1. Structures of the DNA substrates used for the measurement of helicase, ATPase and DNA-binding activities.

Substrates	DNA sequence
Radioactive DNA unwinding assay:	
25-bp duplex with 19-nt 3'-ssDNA tail	5'-GCACTGGCCGTCGTTTTACGGTCGTGACTGGGAAAACCCTGGCG-3'
	3'-AACTTTTTTTTTTCCCCAACCAGCACTGACCCTTTTGGGACCGC-5'
Stopped-flow FRET DNA unwinding assay:	
16-bp duplex with 20-nt 3'-ssDNA tail	5'-CTCTGCTCGACGGATT-F[a]-3'
	5'-HF[b]-AATCCGTCGAGCAGAGtttttttttttttttttttt-3'
ATPase activity assay:	5'-AACCAACAACAACAACAACAACAAC-3'
Fluorescence anisotropy-based DNA binding assay:	
F-18	5'-GCCTCGCTGCCGTCGCCA-F-3'
F-24	5'-GCCCTGCTGCCGACCAACGAAGGT-F-3'
	3'-CGGGACGACGGCTGGTTGCTTCCA-5'
F-40	5'-GCCCTGCTGCCGACCAACGATGGTTACATTCCCGCTGCTG-F-3'

[a]F, fluorescein

[b]HF, hexachlorofluorescein

Table S2. Spatial distribution of cysteine residues in $BLM^{642-1290}$ and $RecQ^{E. coli}$.

$BLM^{642-1290}$		**$RecQ^{E. coli}$**	
Cys position	**Cys distribution**	**Cys position**	**Cys distribution**
685*	Inner	43*	Inner
698*	Inner	56*	Inner
704	Surface	94	Surface
771	Surface	111	Inner
799*	Surface	150*	Surface
878	Inner		
895*	Inner	243*	Inner
901	Inner		
940	Surface		
944	Inner		
989	Inner		
1030	Surface	351	Surface
1036* (ZFD)	Surface	380* (ZFD)	Inner
1055* (ZFD)	Inner	397* (ZFD)	Surface
1063* (ZFD)	Inner	400* (ZFD)	Inner
1066* (ZFD)	Surface	403* (ZFD)	Surface
1067	Surface		
1218	Surface		
1226	Inner		

The spatial conformation distribution of cysteine residues of $BLM^{642-1290}$ and $RecQ^{E. coli}$ were analyzed by PyMol using the X-ray three dimensional structures of $BLM^{642-1290}$ (PDB file, 4CGZ) and $RecQ^{E. coli}$ (PDB file, 1OYW). Cys labeled with stars represent conserved residues at the primary sequence and 3D structural levels.