SUPPLEMENTARY INFORMATION

Methods

Protein refolding

Purified DksA2 and its variants were dialyzed in 8 M urea in 20 mM sodium phosphate (pH 7.5), 50 mM NaCl, 0.1 mM EDTA. The dialyzed protein was separated into two samples, one was serially dialyzed into reducing urea concentrations (8M, 3M, 1.5M, 0.75M, and 0 M) without DTT and the other in the presence of 5 mM DTT. Finally, both samples were dialyzed at the same buffer in the presence of 5 mM DTT. Dialysis was done at room temperature.

NMR analysis

^{1}H-^{15}N HSQC experiments were performed on uniformly ^{15}N-labeled proteins (WT DksA2, DksA2^{C96V}, or WT DksA2 refolded in the presence or absence of 5mM DTT) in 20 mM sodium phosphate (pH 7.5), 50 mM NaCl, 1 mM β-mercaptoethanol, 0.1 mM EDTA and 10% D_2O. The data were acquired at 298K on a Bruker DRX-800 equipped with a 5 mm triple-resonance cryoprobe and z-axis gradient. Data were processed with NMRPipe and analyzed with NMRViewJ. ^{1}H chemical shifts were referenced to the external standard 2,2-dimethyl-2-silapentane-5-sulfonate (DSS).

Crystallization, data processing and determination of crystal structure of PA DksA2.

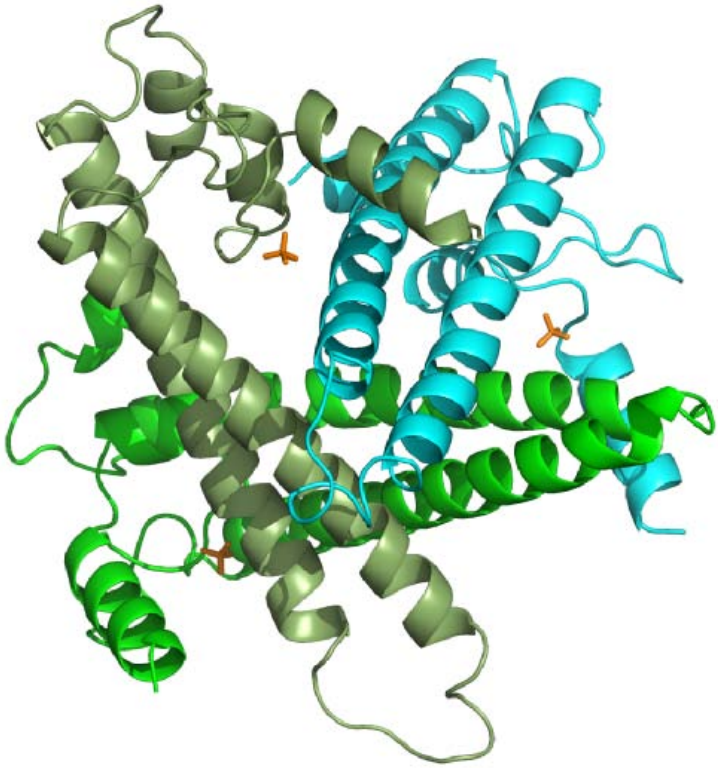
PA DksA2 concentrated to 9.5 mg/ml in 20 mM Tris-HCl pH 6.9, 100 mM NaCl, 2 mM β-mercaptoethanol was used for crystallization by vapor diffusion in sitting drops. Each drop was prepared by mixing 1 μL of concentrated DksA2 with 1 μL of the reservoir solution (100 mM Hepes pH 7.5, 100 mM ammonium sulfate, 16.5% w/v PEG 8,000) and equilibrated against 1 mL of the reservoir solution at 22 °C. The crystals of 0.2 × 0.2 × 0.2 mm in size were formed in 7-14 days. The crystals were gradually transferred into the cryoprotectant solution (100 mM Hepes pH 7.5, 100 mM ammonium sulfate, 16.5% w/v PEG 8,000, 20% glycerol) and rapidly frozen in liquid nitrogen. The X-ray diffraction data were collected at 100 K at beamline 21-ID of the Advanced Photon Source at the Argonne National Laboratories (Argonne, IL). The data were processed with HKL2000 (1)software. The crystal structure of PA DksA2 was determined by molecular replacement by program PHASER (2), where as a search model we used the structure of EC DksA ((3); PDB ID: 1TJL) in which residues 7-13 and 133-151 were deleted. The missing N-terminal and C-terminal regions were built into the Fo-Fc density and the final structure was refined by REFMAC (4) iteratively with manual model corrections in Coot (5). The coordinates and structure factor amplitudes were deposited in the Protein Data Bank under accession number 4IJJ.

Name	Description	Source/reference
TEMPLATES		
pIA536	*rrnB* P1 promoter	(6)
DksA and DksA2 expression vectors		
pIA579	P_{T7} promoter –^{CBD}[HMK]*dksA*	(3)
pVS11	P_{T7} promoter –^{CBD} *dksA*	(3)
pIA923	P_{T7} promoter –$^{His10\text{-}Tev}$*dksA2*	(6)
pRF6	P_{T7} promoter –$^{His10\text{-}}$[HMK]*dksA2*	This work
pIA1153	P_{T7} promoter –$^{His10\text{-}Tev}$*dksA2 [D53N, D56N]*	This work
pIA1154	P_{T7} promoter –$^{His10\text{-}Tev}$*dksA2 [C96V, 117V]*	This work
pIA1185	P_{T7} promoter –$^{His10\text{-}Tev}$*dksA2 [C96V]*	This work
pIA1186	P_{T7} promoter –$^{His10\text{-}Tev}$*dksA2 [C96V, 117A]*	This work
RNAP variants		
pVS10	P_{T7} promoter–*rpoA–rpoB–rpoC*^{His6}*–rpoZ*	(7)
pVS14	P_{T7} promoter–*rpoA–rpoB–rpoC[Δ943-1130]*^{His6}*–rpoZ*	(8)
pVS50	P_{T7} promoter–*rpoA–rpoB–rpoC[V672D, L673D]*^{His6}*–rpoZ*	(8)

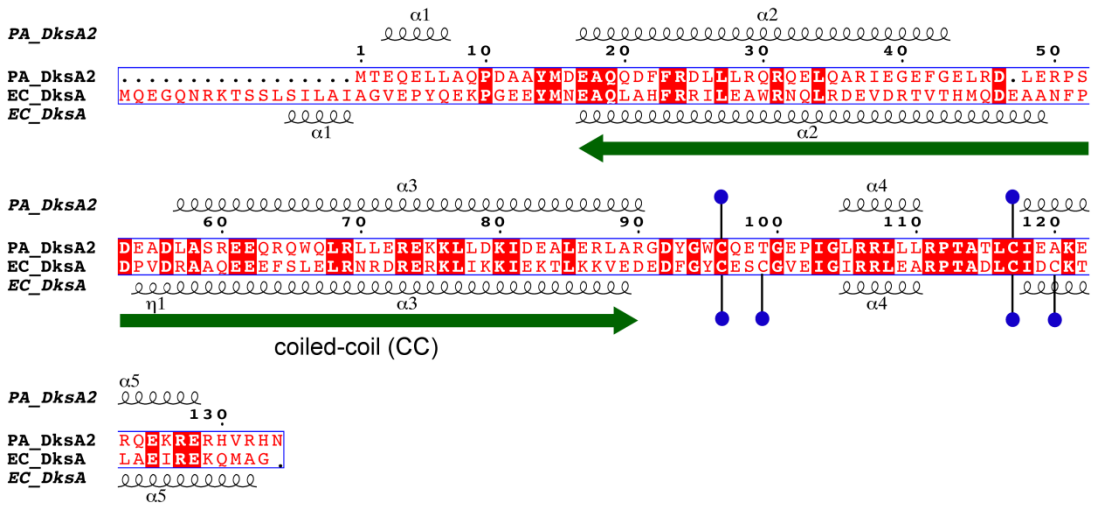
Supplementary Table S1. Plasmids used in this work.

Data Collection	
Space group	P622
Proteins/a.u.	3
Cell dimensions	
a, *b*, *c* (Å)	169.5, 169.5, 92.3
α, β, γ (°)	90, 90, 120
Resolution (Å)	50.0-3.25 (3.29-3.25)[a]
R_{merge} (%)	14.2 (65.8)
$I/\sigma I$	23.3 (2.0)
Completeness	99.4 (93.1)
Redundancy	23.6 (11.9)
Refinement	
Resolution (Å)	40.0-3.25 (3.33-3.25)
No. reflections	12111
R, %	25.7 (33.0)
R_{free}, %	31.0 (40.0)
No. atoms/a.u.	3213
r.m.s. deviations:	
Bond length (Å)	0.007
Bond angle (°)	1.080
Ramachandran plot[b]**:**	
Residues in most favored regions	89.5%
Residues in additionally allowed regions	9.4%
Residues in generously allowed regions	1.1%
Residues in disallowed regions	0

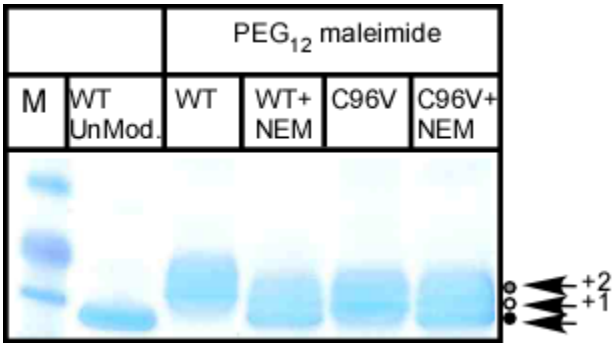
Supplementary Table S2. Data collection and refinement statistics for the structure of PA DksA2. [a]The values given in the parentheses correspond are for the data in the highest-resolution shell. [b]PROCHECK (9) analysis.



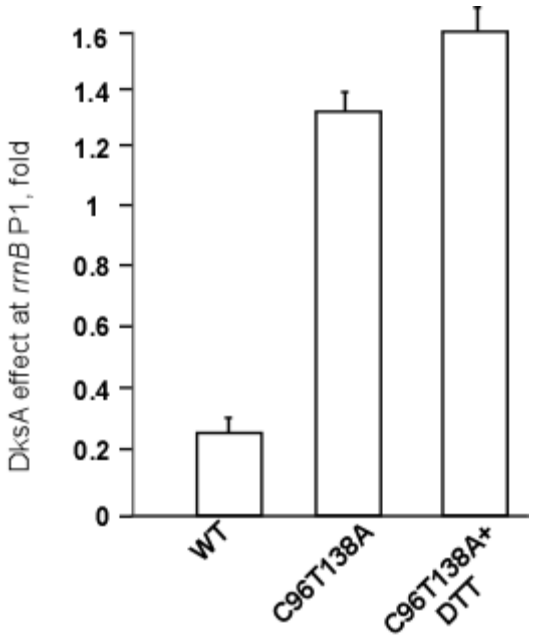
Supplementary Figure S1. Three molecules of DksA2 (contents of an asymmetric unit of the crystal) and three sulfate ions (orange sticks) located at the interfaces between the adjacent protein molecules.



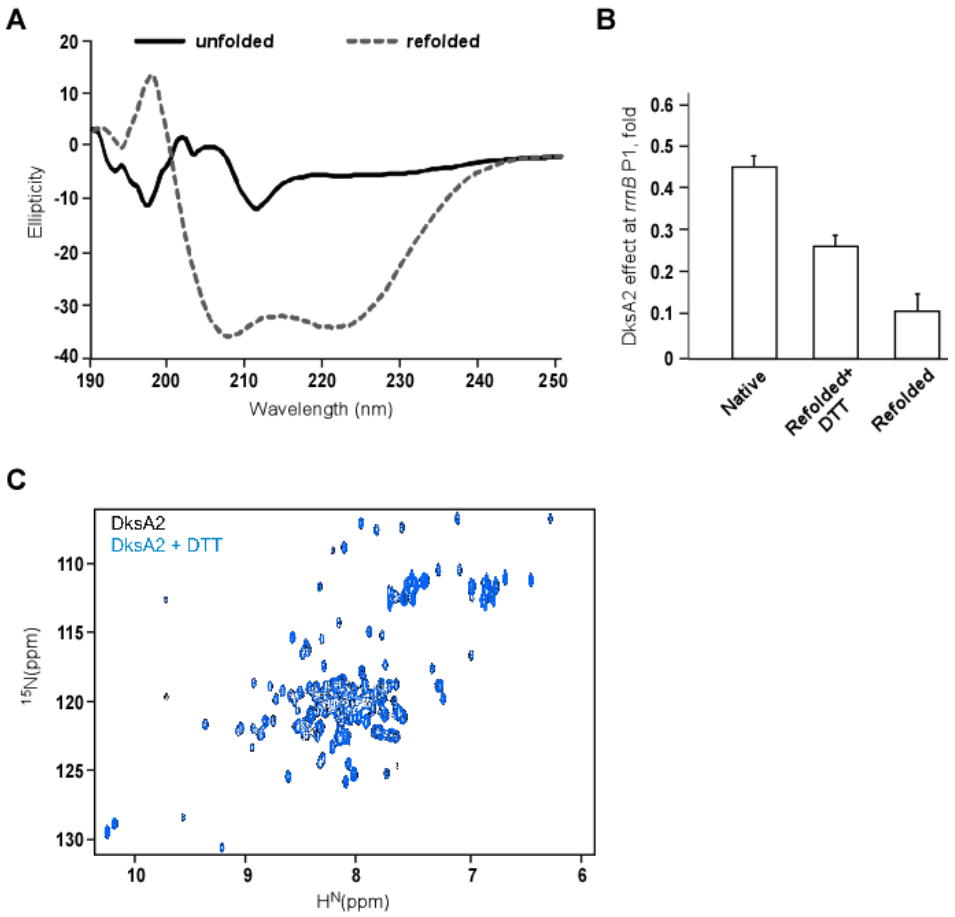
Supplementary Figure S2. Alignment of EC DksA and PA DksA2 with the structural elements indicated. Identical residues are highlighted in red, Cys residues are shown with blue circles.



Supplementary Figure S3. DksA2 cysteine residues can be alkylated in *E. coli cells* suggesting a long-lived disulfide bond is not formed in the cell. *E. coli* cells were treated with N-ethylmaleimide (NEM) as described in Figure 4A. DksA2^{C96V}, which contains a single cysteine and ideally should be modified completely by NEM, was used to roughly estimate the thiol blocking efficiency.



Supplementary Figure S4. Substituting Cys 96 and 138 from EC DksA into the equivalent residues in PA DksA2 (Thr and Ala) abolishes its activity. DksA$^{C96T, 138A}$ was dialyzed O.N in the presence or absence of 2 mM BME. Inhibition of transcription from the *rrnB* P1 was determined in the presence of DksA and DksA$^{C96T, 138A}$. The average was calculated from 3 independent repeats



Supplementary Figure S5. Properties of the refolded DksA2. (A) The CD spectrum of the unfolded using 8 M urea protein indicates that it loses its secondary structure completely. (B) After refolding, DksA2 regains its activity. Transcription inhibition by DksA2 from the *rrnB* P1 promoter was determined as described in Figure 1. The results are an average of 3 independent repeats. (C) The ^{1}H-^{15}N HSQC spectra of DksA2 refolded in the presence or absence of DTT are not significantly different.

References

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