Supporting Information

Supporting Table S1: List of expression constructs examined

Sr. No.	Clone	Vector	MW (kDa)	Protein
1	**σ^K/ $RskA_{cyto}$**	**MCS1-pETDUET1 +RskA**	**21.9 + 8.5**	**Expressed, purified and crystallized in complex with RskA**
2	σ^K (C133S)/ $RskA_{cyto}$	Site directed mutagenesis in pETDUET-1 σ^K/RskA	21.9 + 8.5	Purified as σ^K (C133S) /RskA complex
3	σ^K(C183S)/$RskA_{cyto}$	Site directed mutagenesis in pETDUET-1 σ^K/RskA	21.9 + 8.5	Purified as σ^K (C183S) /RskA complex
4	σ^K(C133S, C183S)/ $RskA_{cyto}$	Site directed mutagenesis in pETDUET-1 σ^K C133S/RskA	21.9 + 8.5	Purified as σ^K (C133S, C183S) /RskA complex
5	RskA (1-232)	pET15b	25.8	Insoluble – purified as a denatured protein.
6	$RskA_{cyto}$ (1-80)	MCS1-pETDUET1	10.3	Purified as a poly-histidine-tagged protein
7.	$\sigma^K{}_4$ (123-187)	pET22b	7.8	Purified by cation exchange chromatography
8	σ^K/RskA (*M. tuberculosis*- 1-232) -- phoA (23-471)	MCS2- pET28a+ σ^K– RskA (*M. tuberculosis* - 1-232) with C-term poly-histidine tag	21.9 + 70.2	Expressed in *E. coli* C43 (DE3)
9	σ^K /RskA (*M. bovis*- 1-232) -- phoA (23-471)	MCS2- pET28a+ σ^K– RskA (*M. bovis* - 1-232) with C-term poly-histidine tag	21.9 + 70.2	Expressed in *E. coli* C43 (DE3)
10	σ^K RskA (*M. tuberculosis*- 1-232) -- phoA (23-471)	MCS2- pET28a+ σ^K– RskA (*M. tuberculosis* - 1-232) without poly-histidine tag	21.9 + 70.2	Expressed in *E. coli* C43 (DE3)
11	σ^K /RskA (*M. bovis*- 1-232) -- phoA (23-471)	MCS2- pET28a+ σ^K– RskA (*M. bovis* - 1-232) without poly-histidine tag	21.9 + 70.2	Expressed in *E. coli* C43 (DE3)

Supporting Table S2: Comparison of the σ^K/RskA complex with other σ/anti- σ structures.

Organism	Domains	Superposed On	PDB ID	RMSD (Å)
Domain 2				
Rhodobacter sphearoides	σ^K_2	σ^E_2	2Z2S	1.06
E. coli	σ^K_2	σ^E_2	1OR7	1.04
Domain 4				
M. tuberculosis	σ^K_4	σ^L_4	1OR7	1.18
E. coli	σ^K_4	σ^E_4	3HUG	0.78
ASD				
Rhodobacter sphearoides	RskA	ChrR	2Z2S	1.87
M. tuberculosis	RskA	RslA	3HUG	1.18

Supporting Table S3: Comparison of the interface in σ/anti-σ complexes

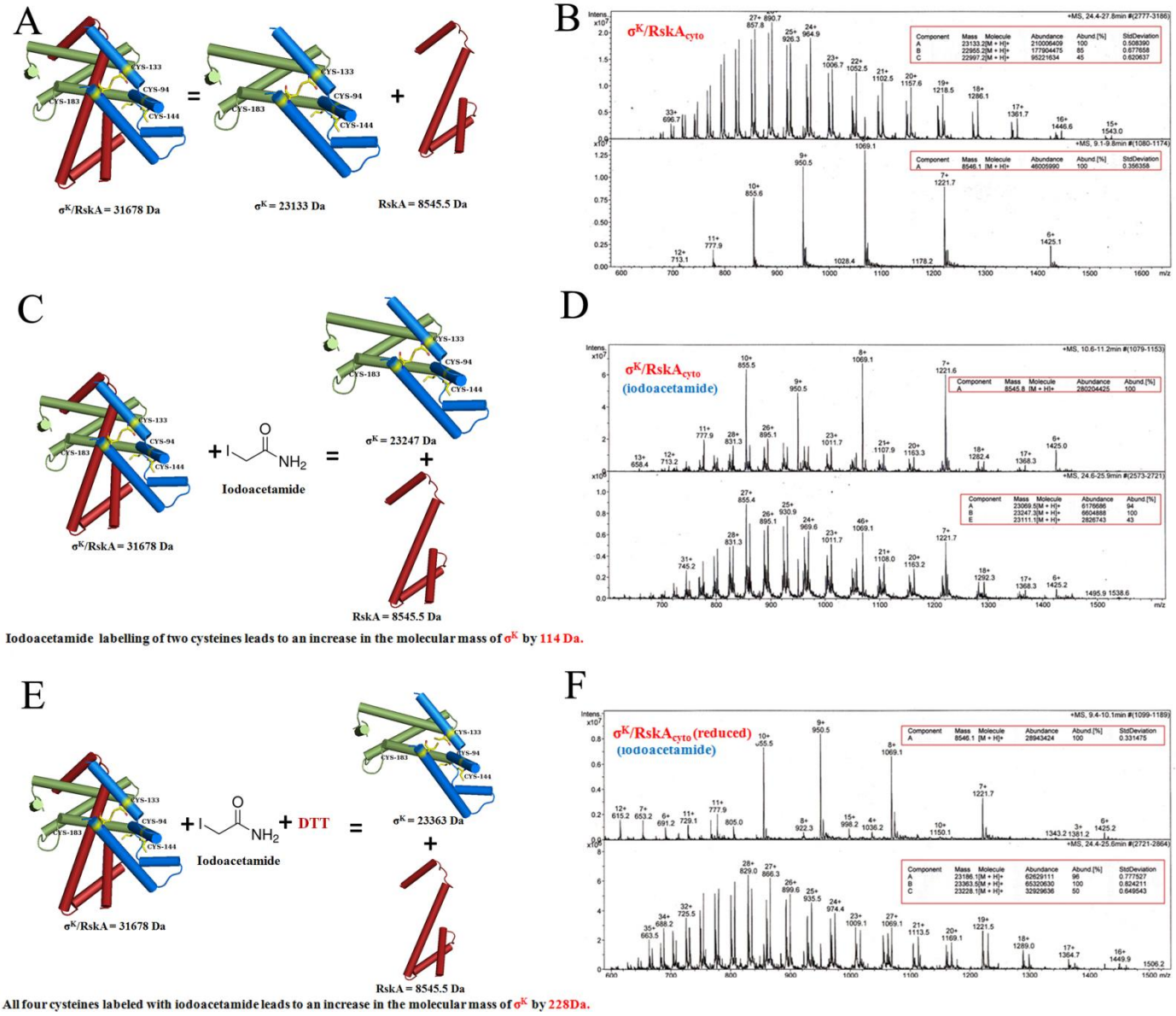
Organism	Name	PDB ID	Interface area (Å^2)	ΔG kCal/mol
M. tuberculosis	**σ^K/RskA$_{cyto}$**	**4NQW**	**2046.9**	**-31.0**
R. sphaeroides	σ^E/ChrR	2Z2S	2638.7	-34.6
E. coli	σ^E/RseA	1OR7	2771.0	-27.0
Aquifex aeolicus	σ^{28}/FlgM	1RP3	1986.8	-30.5
M. tuberculosis	σ^L_4/RslA	3HUG	1339.2	-16.2
B. subtilis	σ^F/SpoIIAB	1L0O	1047.6	-11.9

Footnote: This analysis was performed using the Protein interfaces, surfaces and assemblies (PISA) at the European Bioinformatics Institute (http://www.ebi.ac.uk/pdbe/prot_int/pistart.html)
Reference: E. Krissinel and K. Henrick (2007).*Inference of macromolecular assemblies from crystalline state*. J. Mol. Biol. **372**, 774—797 (18).

Supporting Table S4: Dissociation constants for σ^{K}_{4} interactions with the -35 promoter DNA of *katG* and *mpt70*

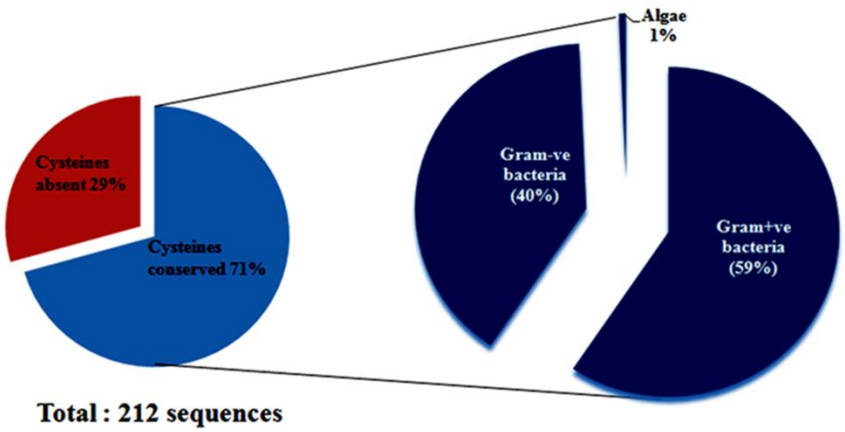
Gene	kD (nM)
katG	**18.0 ± 0.6**
***katG* (1mM TCEP)**	**15.6 ± 0.9**
mpt70	**21.6 ± 0.01**
***mpt70* (1mM TCEP)**	**20.8 ± 0.1**

Supporting Figures

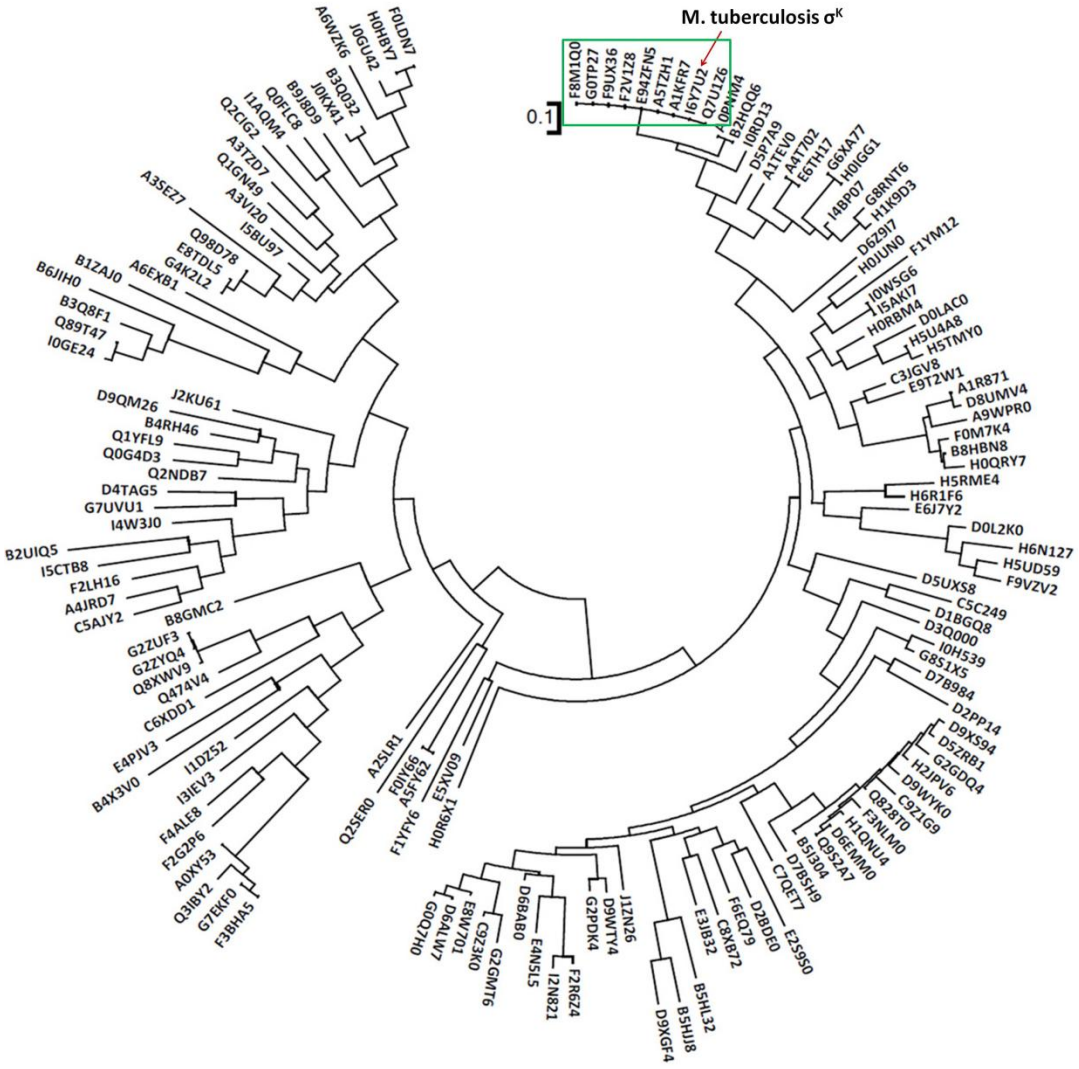


Supporting Figure S1: A and B. Mass spectrometric profile of σ^K/RskA$_{cyto}$ complex. **C and D.** Iodoacetamide labeling followed by mass spectrometry confirmed that Cysteine residues in σ^K_4 are disulfide bonded in the freshly purified σ^K/RskA$_{cyto}$ complex. **E and F.** Iodoacetamide labeling followed by mass spectrometry after reducing the σ^K/RskA$_{cyto}$ protein sample with 1mM TCEP.

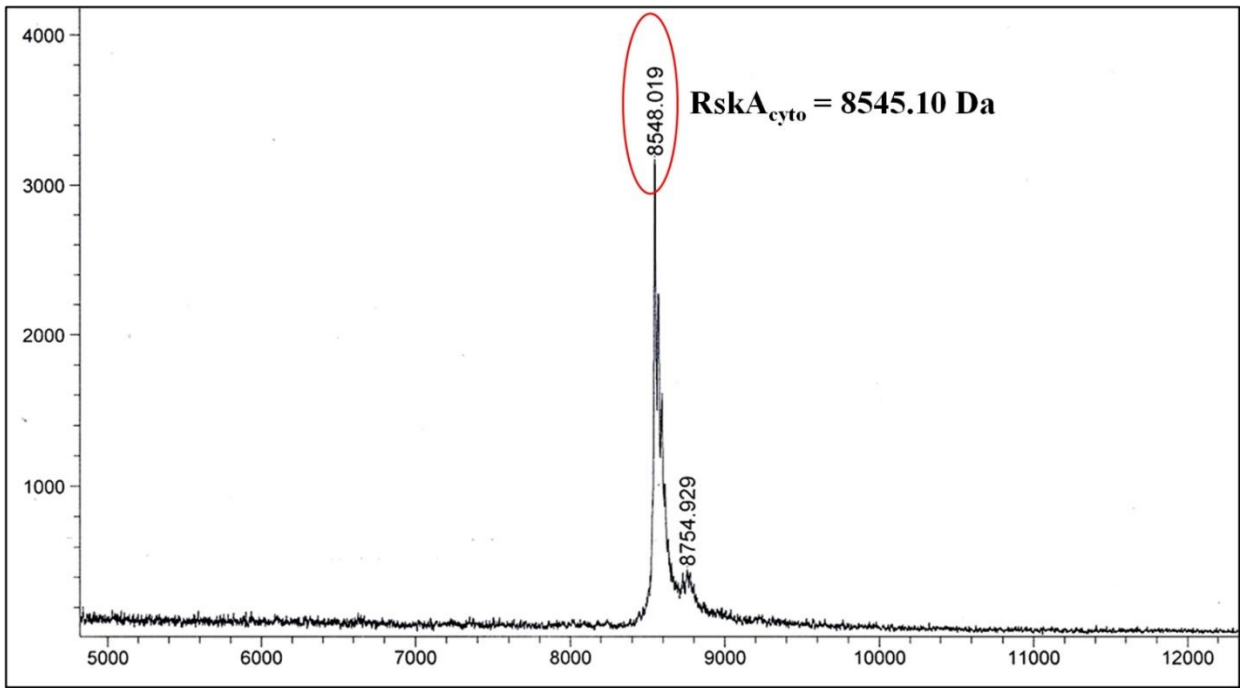
a.


Algae
1%
Gram-ve
bacteria
(40%)
Gram+ve
bacteria
(59%)
Cysteines
absent 29%
Cysteines
conserved 71%
Total : 212 sequences

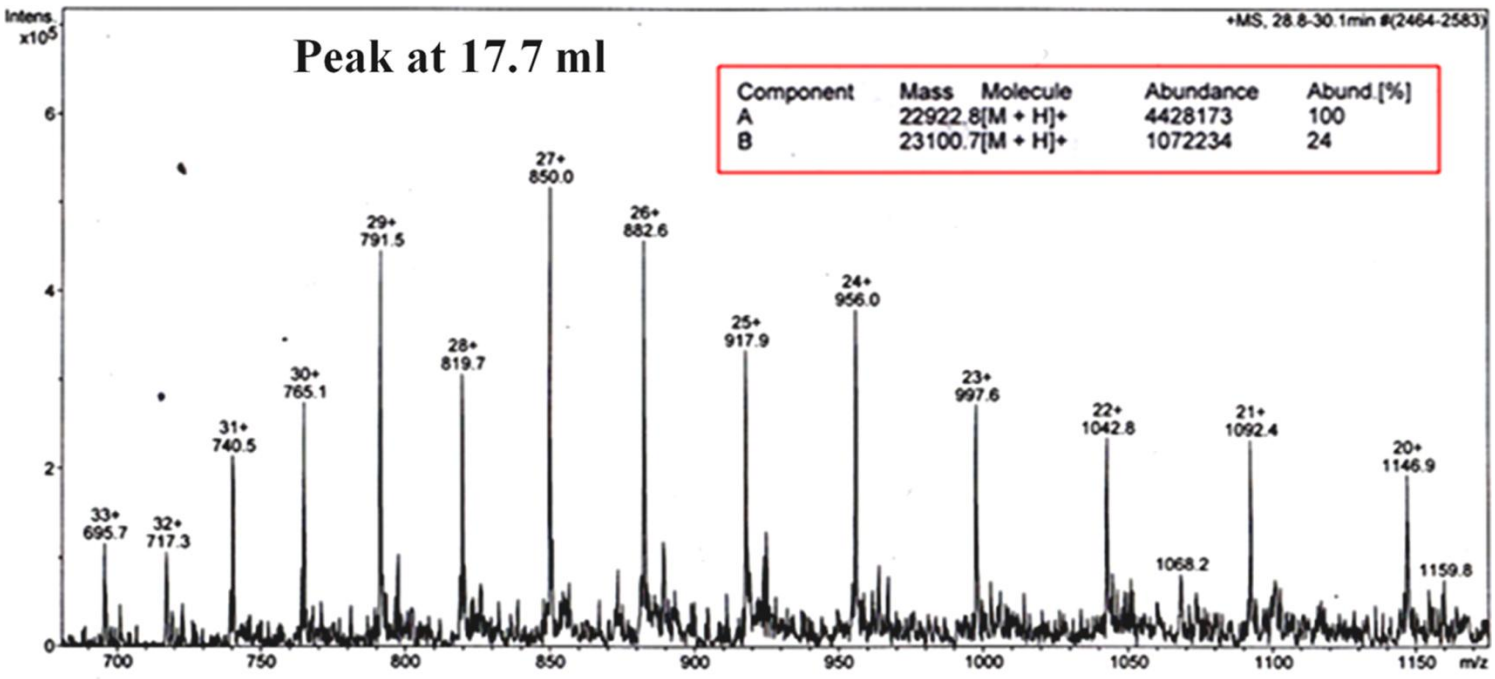
b.


M. tuberculosis σK
0.1

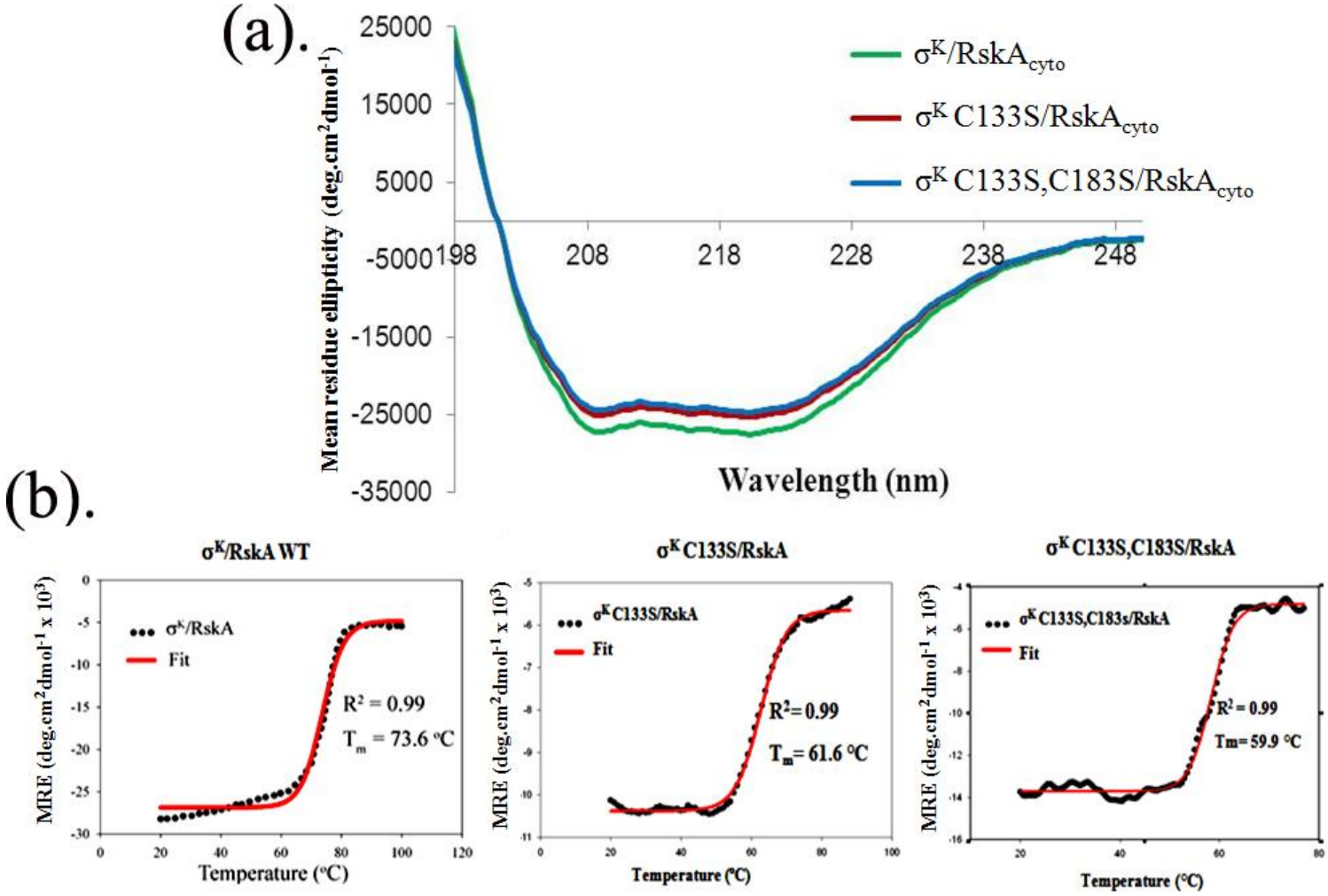
Supporting Figure S2: (a.) The disulfide bonding cysteines (C133 and C183 in σ^{K}_{4}) are conserved across σ factors from diverse organisms. This analysis is based on 212 protein sequences (sequence identity >27%). **(b.)** A cladogram based on neighbor-joining method was constructed for all sequences that had the conserved disulfide forming cysteines (MEGA5 server (Tamura *et al.*, 2011))



Supporting Figure S3: Maldi-TOF mass spectrum of the peak eluting at 20.2 ml (size exclusion chromatography- more details in methods) after treating the σ^{K}/RskA$_{cyto}$ complex with a reducing agent. The mass corresponds to RskA$_{cyto}$. .



Supporting Figure S4: Mass spectrogram for the sample eluting at 17ml. In the case of σ^{K}(C133S,C183S)/RskA$_{cyto}$ complex, this is consistent with the molecular weight of the σ^{K}(C133S,C183S) protein.



Supporting Figure S5. (a). CD spectra of wild type σ^K/RskA$_{cyto}$ and the σ^K(C133S)/RskA$_{cyto}$, σ^K(C183S)/RskA$_{cyto}$ and σ^K(C133S, C183S)/RskA$_{cyto}$ mutants. **(b).** The σ^K/RskA$_{cyto}$ complex is highly stable (T_m *ca* 73.6°C). Thermal denaturation profiles for σ^K(C133S)/RskA$_{cyto}$ (61.6°C) and σ^K(C133S, C183S)/RskA$_{cyto}$ (59.9°C) shows the cysteines are important for the structural stability of the σ^K/RskA$_{cyto}$ complex.

A

B

Mpt70 GGATGAATATCCATCCAAGACCCGGACGGCTCCGAAGAAATCATGTC

KatG ATCTACTGGGGTCTATGTCCTGATTGTTCGATATCCGACACTTCGCG

Supporting Figure S6: (A). The consensus sequence of the -10 and -35 promoter DNA element for genes in the σ^K regulon**. (B).** The promoter DNA sequence for the *mpt70* and *katG* genes used for the fluorescence anisotropy experiments.