

SUPPLEMENTARY MATERIALS

Supplementary Methods

SufS and SufU interaction

6xHis-SufU was incubated for 10 min. with SufS (in stoichiometric amount) and nickel nitrilotriacetic acid (Ni-NTA) resin with and without 5 mM L-cysteine. The mix was then loaded on a column and was left to flow by gravity without any pressure applied. The column was then washed with one column volume of buffer J. Bound proteins containing 6xHis-SufU and the 6xHis-SufU/SufS complex were eluted with buffer J containing 250 mM imidazole. The flow-through and the eluted proteins were analyzed by SDS-PAGE. Based on the SDS-PAGE profile, the concentration of 6xHis-SufU in the flow-through was assumed to be neglected so the protein content in the flow-through was assumed to be the amount of SufS that does not carry a 6xHis-tag and that didn't interact with 6xHis-SufU. The amount of SufS that interacted with SufU was quantified by simple deduction of the amount of SufS quantified in the flow-through from the initial amount of SufS that was used in the experiment.

Supplementary Results

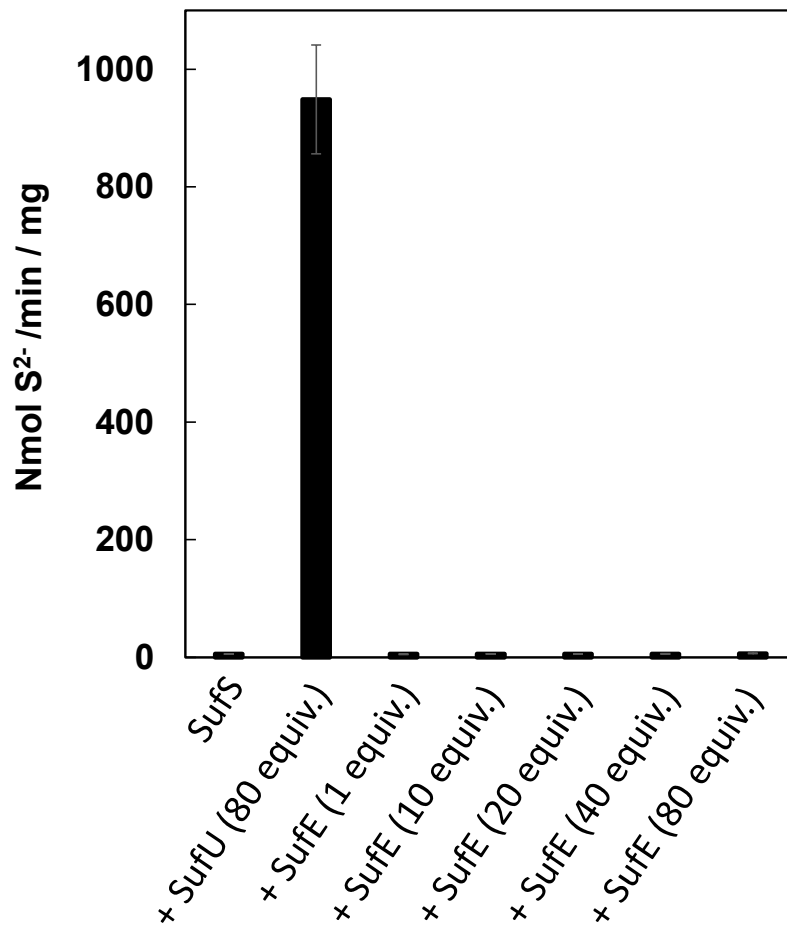


Figure S1. Specific activity of *Mtb* SufS (10 μ M) in the absence and in the presence of different equivalents of *E. coli* SufE (1-80 equivalents) or *Mtb* SufU (80 equivalents). Cysteine at 500 μ M was used in the assay.

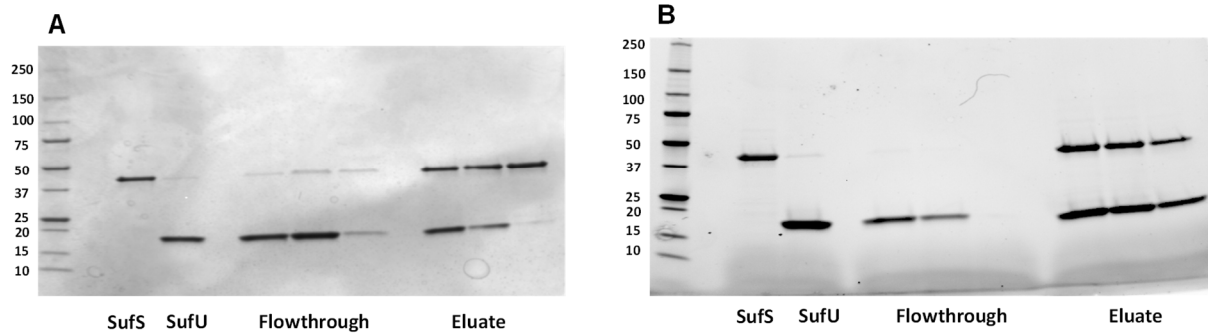


Figure S2. (A) SufS-SufU interaction in solution. (A) SDS-PAGE profile of 6xHis-SufS and SufU interaction in the absence of L-cysteine on affinity column (Ni-NTA). Lane (1) : known size markers (kDa), lane (2) : purified 6xHis-SufS, lane (3): purified SufU, lanes (4-6) flowthrough (FT) samples containing fractions of SufU which doesn't interact with SufS, lanes (7-9): eluate samples containing 6xHis-SufS and SufU. Protein quantification: FT: 63.8% of total SufU; Eluate: 36.2% of SufU. **(B)** SDS-PAGE profile of 6xHis-SufS and SufU interaction in the presence of 5mM L-cysteine on affinity column (Ni-NTA). Lane (1) : known size markers (kDa), lane (2) : purified 6xHis-SufS, lane (3): purified SufU, lanes (4-6) flowthrough (FT) samples containing fractions of SufU which doesn't interact with SufS, lanes (7-9): eluate samples containing 6xHis-SufS and SufU. Protein quantification: FT: 22% of total SufU; Eluate: 78% of SufU. From the quantitation, it seems that the interaction is increased in the presence of L-cysteine.

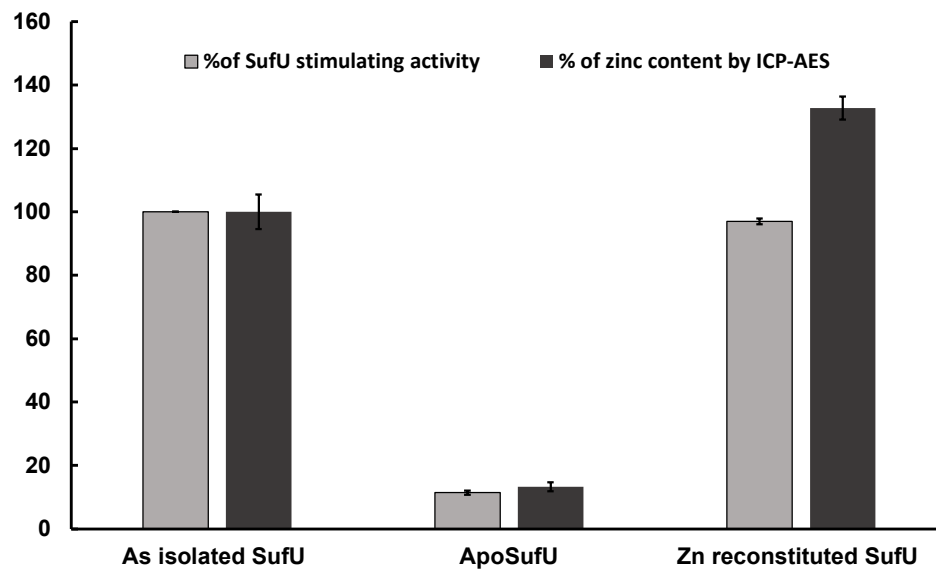


Figure S3. Reconstitution of apo-SufU with zinc. The SufU stimulating activity (%) was determined by measuring the specific activity of SufS (10 μ M) in presence of as-isolated SufU (10 μ M), apo-SufU (10 μ M) and Zn reconstituted SufU (10 μ M). The sulfurtransferase activity with the as-isolated SufU (60 nmoles S^2 /min/mg) was considered as 100%. Apo-SufU displays an activity of 11.4% (6.4 nmoles S^2 /min/mg), while SufU reconstituted with zinc was able to restore 96% (57.7 nmoles S^2 /min/mg) of the as-isolated SufU sulfurtransferase activity. The zinc content was quantified by ICP-AES. The 100% content corresponds to 1.1 Zn/ monomer of the as-isolated SufU. The zinc content in apo-SufU is 13.2% (0.145 Zn/ monomer) and in case of the Zn reconstituted SufU, the zinc content is 132% (1.46 Zn/monomer) reflecting some unspecific zinc.

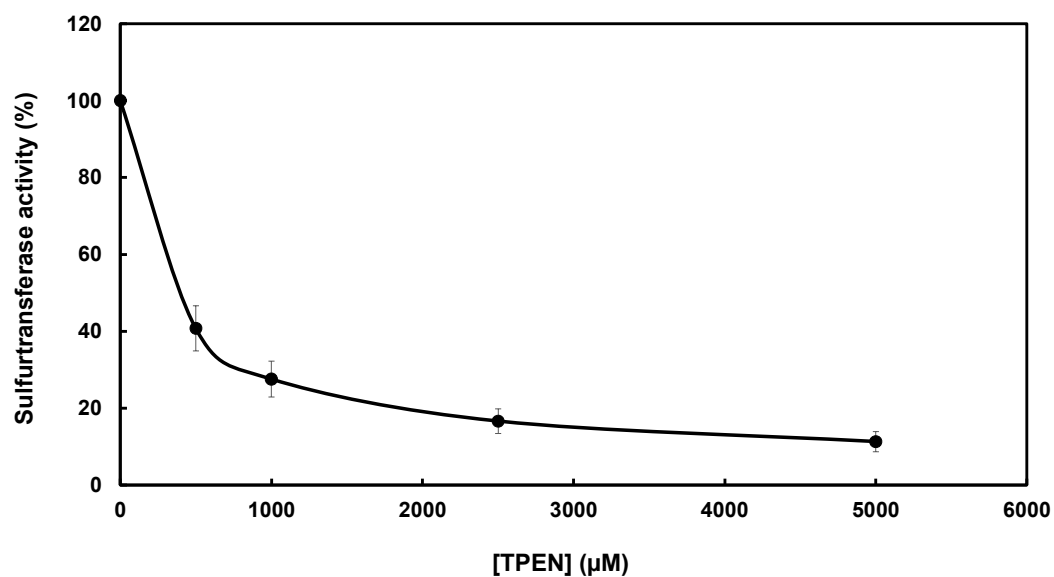


Figure S4. Sulfurtransferase activity of SufU (%). SufU (146 μM) was incubated with increasing concentrations of TPEN (0-5 mM) for 3 h followed by dialysis. The sulfur transfer activity of SufU was then determined by measuring the specific activity of SufS (10 μM) in the presence of each sample (10 μM), L-cysteine (500 μM), DTT (2 mM) for 30 min at 37°C. 100% corresponds to 78 nmoles of S^2 /min/mg of SufS. The curve is superposable to that in Figure 5 showing that the loss of SufU sulfurtransferase activity is due to the gradual decrease in zinc content by increasing TPEN concentration.

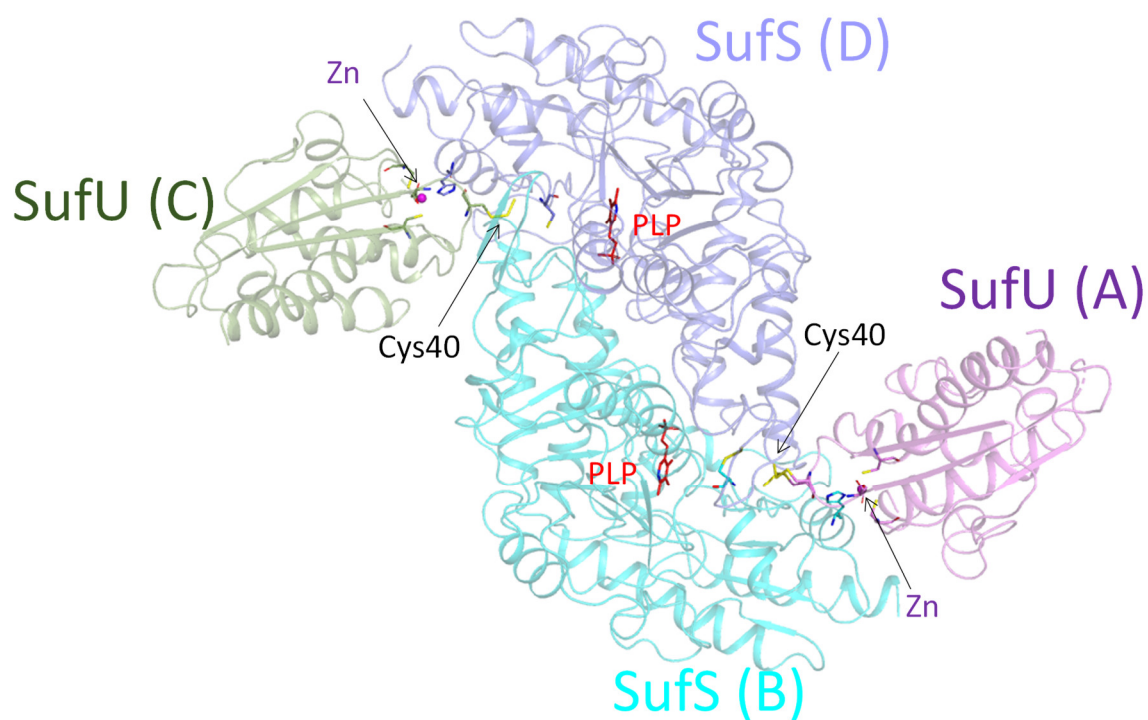


Figure S5. Overall crystal structure of *Mtb* SufS-SufU complex. The complex is represented in cartoon representation. The figure shows the arrangement of the different subunits, which consists as a dimer of complexes SufS-SufU (SufU in green and violet, SufS in cyan and blue). The PLP cofactor is represented in red inside SufS, and the zinc ions in purple at the interface SufS-SufU with their environment drawn in stick representation.

CsdA_Ecoli	-----MNVFNPAQFRAQFPALQD-----AGVYLDASAATALKPEAVVEATQQFYSL--A	47
Rv1464_Mtub	-MTASVNSLDLAAIRADFPILKRI MRGNPLAYLDSGATSQRPLQVLDAREFLTAS--N	57
SufS_Saureus	MNEVAEHSFDVNEVIKDFPILDQKVN-GKRLAYLDSTATSQTPVQVLNVLEDYKKRY--N	57
SufS_Bsubtilis	-----MNITDIREQFPILHQQVN-GHDLVYLDASAATSKPRAVIETLDKYYNQY--N	49
SufS_Efaecalis*	-----MMDAATIRQSFPILFQEVN-DEPLVYLDNAATTQKTAVLDVLRHHYEED--N	50
SufS_Ecoli	-----MIFSVDKVRADFVPLSRREV-NGLPLAYLDSASAQKPSQVIDAEAEFYRHG--Y	51
SufS_MXAN	-----MSGFDVNQVRKDFPILDQEVN-GRPLVYLDASAATQKPQVAIDALVRFYQHD--N	52
	*** *:: * :	
IscS_Ecoli	GNPASRSHRFQWQAEAEVDIARNQIADLVGA-DPREIVFTSGATESDNLAIKGAANF---	89
CsdA_Ecoli	GNVHRSQFAEAQRILTARYEAAREKVAQLLNAPDDKTIVWTRGTESINMVAQCYARP---	104
Rv1464_Mtub	GAVHRGAHQLMEEATDAYEQGRADIALFVGA-DTDELVFTKNATEALNLVSYVLGDSRFE	116
SufS_Saureus	SNVHRGVHTLGLSLATDGYENARETVRRFINAKYFEEIIFTRGTASINLVAHSYGDA---	114
SufS_Bsubtilis	SNVHRGVHTLGLTRATDGYEGAREKVRKF INAKSMAEII FTKGTTSLNMVALSYARA---	106
SufS_Efaecalis*	ANVHRGVHTLAERATKDYEASREKVRQFIHAKETAEVLFTTRGTTSLNWIAKSYGDL---	107
SufS_Ecoli	AAVHRGIHTLSAQATEKMENVRKRASLF INARSAAELVVFVRGTTEGINLVANSWGS---	108
SufS_MXAN	ANVHRGVHVLSEERATEAYEGARETVRRFINARDVKEVVFVRGTTEAINLVAQTYGRK---	109
	. . : *	
IscS_Ecoli	-YQKKGKHIIITSKTEHKAVLDTCRQL-EREGFEVTYLAPQRNGIIDLKELEAAMRDDTIL	147
CsdA_Ecoli	-RLQPGDEIIVSVAEHHANLVPWLMVAQQTGAKVVKLPLNAQRLPDVDDLPELITPRSR	163
Rv1464_Mtub	RAVGPGDVIIVTTELEHHANLIPWQELARRTGATLRWYGVTDGRI DLDLSL--YLDNRVY	174
SufS_Saureus	-NVEEGDEIVVTEMEHHANIVPWQQLAKRKNATLKFIPMTADGELNIEDIKQITINDKTKI	173
SufS_Bsubtilis	-NLKPGDEVVITYMEHHANII PWQQAVKATGATLKYIPLQEDGTISLEDVRETVTNTTKI	165
SufS_Efaecalis*	-AVTAGDEIVISYMEHHSNII PWQQLAQRGTGAILKYIDVTEDGFLDMASARQQITEKTKI	166
SufS_Ecoli	-NVRAGDNIIISQMEHHANIVPWQMLCARVGAELRVIPLPDGTQLQETLPTLFDEKTRL	167
SufS_MXAN	-HIGAGDEVLTQMEHHANIVPWRLMCEQTGAVLKVIPVDDRGLVLDVALLTERTRI	168
	*. : : **:: :	
IscS_Ecoli	VSIMHVNNEIGVVQDIAAIGEMCRARGIIYHVDATQSVGKLPIDLSQLKVDLMSFSGHKI	207
CsdA_Ecoli	LALGQMSNVTGGCPDLARAITFAHSAGMVVMVDGAQAVHFPADVQQLDIDFYAFSGHKL	223
Rv1464_Mtub	VAFTHHSNVTGVLTPVSELVSRAHQSGALTVLDACQSVPHQPVDLHELGVDFAAFSGHKM	234
SufS_Saureus	VAAIAHISNVLGTINDVKTIAEIAHQHGAIISVDGAQAAPHMKLDMQEMNADFYFSFGHKM	233
SufS_Bsubtilis	VAVSHVSNVLGTVPNIKEMAKIAHDNGAVIVVDGAQSTPHMKIDVQDLDCDFALSSHKM	225
SufS_Efaecalis*	VSIAHVSNNVLGVINPIEELTQLAHQNGAVMVVDGAQAVPHMPVDVQADADFYAFSGHKM	226
SufS_Ecoli	LAI THVSNVLGTENPLAEMITLAHQHGAKVLVDGAQAVMHHPVDVQALDCDFYVFSGHKL	227
SufS_MXAN	LAVTHVSNALGTVAPVKELTRRAHAKGIPVLVDGAQAVTHFPVDVQDLGCDFYAFSGHKM	228
	::. : .* * :	
IscS_Ecoli	YGPKGIGALYVRRKPRVRIEAQM HGGGHER-----GMRSGTLPVHQIVGM	252
CsdA_Ecoli	YGPTGIGVLYGKSELLEAMS PWLGGMVHEVSF-DGFTTQSAPWKLEAGTPNVAQVIGL	282
Rv1464_Mtub	LGPNGIGVLYGRRELLAQMP PFLTGGSMIETVTM-EGATYAPAPQREAGTPMTSQVVGL	293
SufS_Saureus	LGPTGIGVLFGRKRELLQKMEPIEFGGDMIDFVSK-YDATWADLPTKFEAGTPLIAQAIGL	292
SufS_Bsubtilis	CGPTGVGVLYGKKALLENMEPAEFGGEMIDFVGL-YESTWKELPWKFEAGTPIIAGAIGL	284
SufS_Efaecalis*	CGPTGIGVLYGKRHLLEQMEPVEFGGEMIDFVHL-QESTWKELPWKFEAGTPNIAGAIAL	285
SufS_Ecoli	YGPTGIGVLYVKEALLQEMPWEFGGSMIATVSLSEGTWTWKAPWFEAGTPNTGGIIGL	287
SufS_MXAN	FGPTGIGVLYGRKERLDAMPYQGGGDMILSVTM-EKVYTNRPYPYRFEAGTPNLEGAVGL	287
	**.*:* : : :	
IscS_Ecoli	GEAYRIAKEEMATEME-RLRGLRNRLWNGIKDIEEVYLNGLD---EHGAPNINLVSNFYV	308
CsdA_Ecoli	SAALEWLADYDINQAESWSRSLATLAEDALAKRPGFRSFRCC---DSSLLAFDFAGVHH	338
Rv1464_Mtub	AAAARYLGAIGMAAVEAHERELVAAAIEGLSGIDGVRILGPTSMRDRGSPVAFVVEGVHA	353
SufS_Saureus	AEAIRYLERIGFDAIHKYEQELTIYAYEQMSAIEGIEIYGP PKD-RRAGVITFNLQDVHP	351
SufS_Bsubtilis	GAAIDFLEEIGLDEISRHEHKLAAVALERFRQLDGVTVYGP---ERAGLVTFNLDDVHP	341
SufS_Efaecalis*	GAAIDYLTIEIGLEAIIHQHEAALVHYVLPKLQAIIEGLTIYGPQNP KDHTGVIAFNIEGLHP	345
SufS_Ecoli	GAALEYVSALGLNNIAEYEQNLMHYALSQLESVPDLTLYGPQ---NRLGVIAFNLGKHHHA	344
SufS_MXAN	AAAI RYLEALGMENVAADHRELLAYATQALESVPGLRMVGTAR---EKSGVLSFMLADIHP	345
	. * :	
IscS_Ecoli	EGESLIMALKDLAVSSGSACTSASLEPSYVLRALGLNDELAHSSIRFSLGRFTTEEEIDY	368
CsdA_Ecoli	SDMVTLLAEY GIALRAGQHCAQPLLA-----ELGV-----TGTLRASFAFYNTKSDVDA	387
Rv1464_Mtub	H DVGQVLD DGGVAVRVGHHCAQLPLHR-----RFLG-----AATARASFAVYNTADEVDR	402
SufS_Saureus	H DVATAVDTEGVAVRAGHHCAQLPMK-----WLVN-----SSTARASFYIYNTKEDVDQ	400
SufS_Bsubtilis	H DVATVLDAEGIAVRAGHHCAQLPMK-----WLDV-----TATARASFYLYNTEEEIDK	390
SufS_Efaecalis*	H DVATALDMEGVAVRAGHHCAQLPLN-----YLSV-----PATARASFYLYNTKEDADR	394
SufS_Ecoli	YDVGSFLDNYGIAVRTGHHCAPLMA-----YYNV-----PAMCRASLAMYNTHEEVDR	393
SufS_MXAN	H DVGTILDREGICIRTGHHCAQPMQ-----HFKV-----PATSRASLALYNTREDVDA	394
	. : :. : * * :	
IscS_Ecoli	TIELVRK SIGRLRDLSPLEWEMYKQGVDLNSIEWAHH 404	
CsdA_Ecoli	LVNAVDRAL ELLVD-----401	
Rv1464_Mtub	LVAGVRRSRHFFGRA-----417	
SufS_Saureus	LINALKQTKEFFSYEF-----416	
SufS_Bsubtilis	LVEALQKTKEYFTNVF-----406	
SufS_Efaecalis*	LVEAIKATKEFFQHGT-----411	
SufS_Ecoli	LVTGLQRIHRLLG-----406	
SufS_MXAN	LVRGLHKVLEVFQ-----407	

Figure S6. Sequence alignment of type I and type II cysteine desulfurases. Type I (*E. coli* IscS) and type II (*E. coli* CsdA, *E. coli* SufS, *S. aureus* SufS, *B. subtilis* SufS, *E. faecalis* SufS, *Myxococcus xanthus* (MXAN) SufS and *Mtb* SufS). Highlight yellow : conserved histidine residue in type II cysteine desulfurases SufS functioning with SufU as sulfurtransferase.

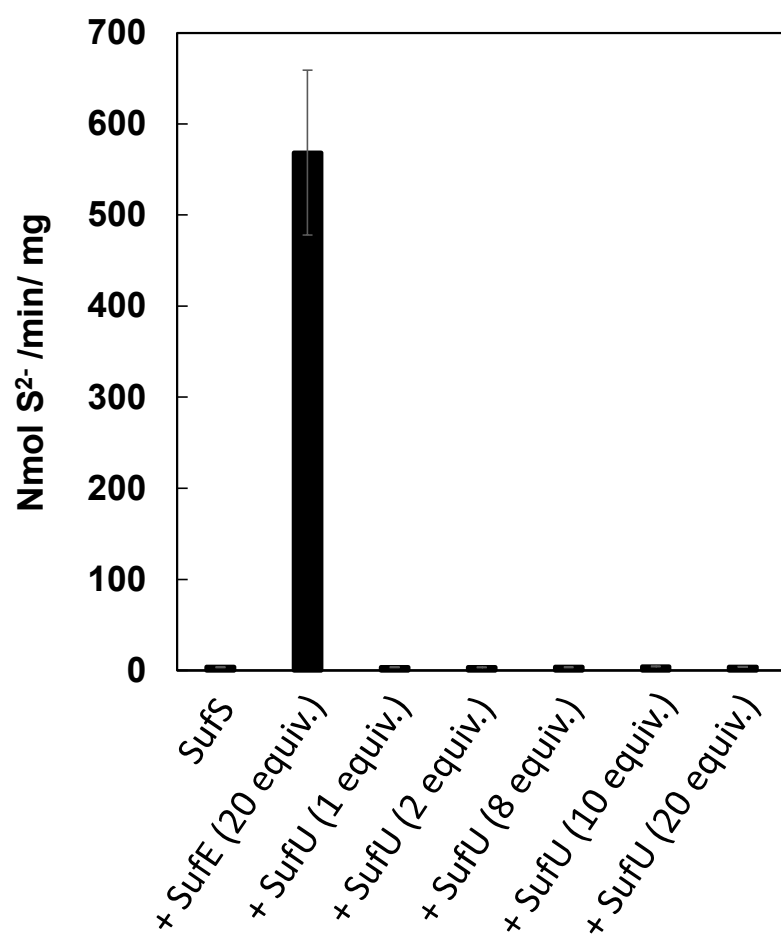


Figure S7. Specific activity of *E. coli* SufS (10 μ M) in the absence and in the presence of different equivalents of *E. coli* SufE (20 equivalents) and *Mtb* SufU (1-20 equivalents) for 30 min. at 37°C in presence of L-cystéine (500 μ M) and DTT (1 mM).

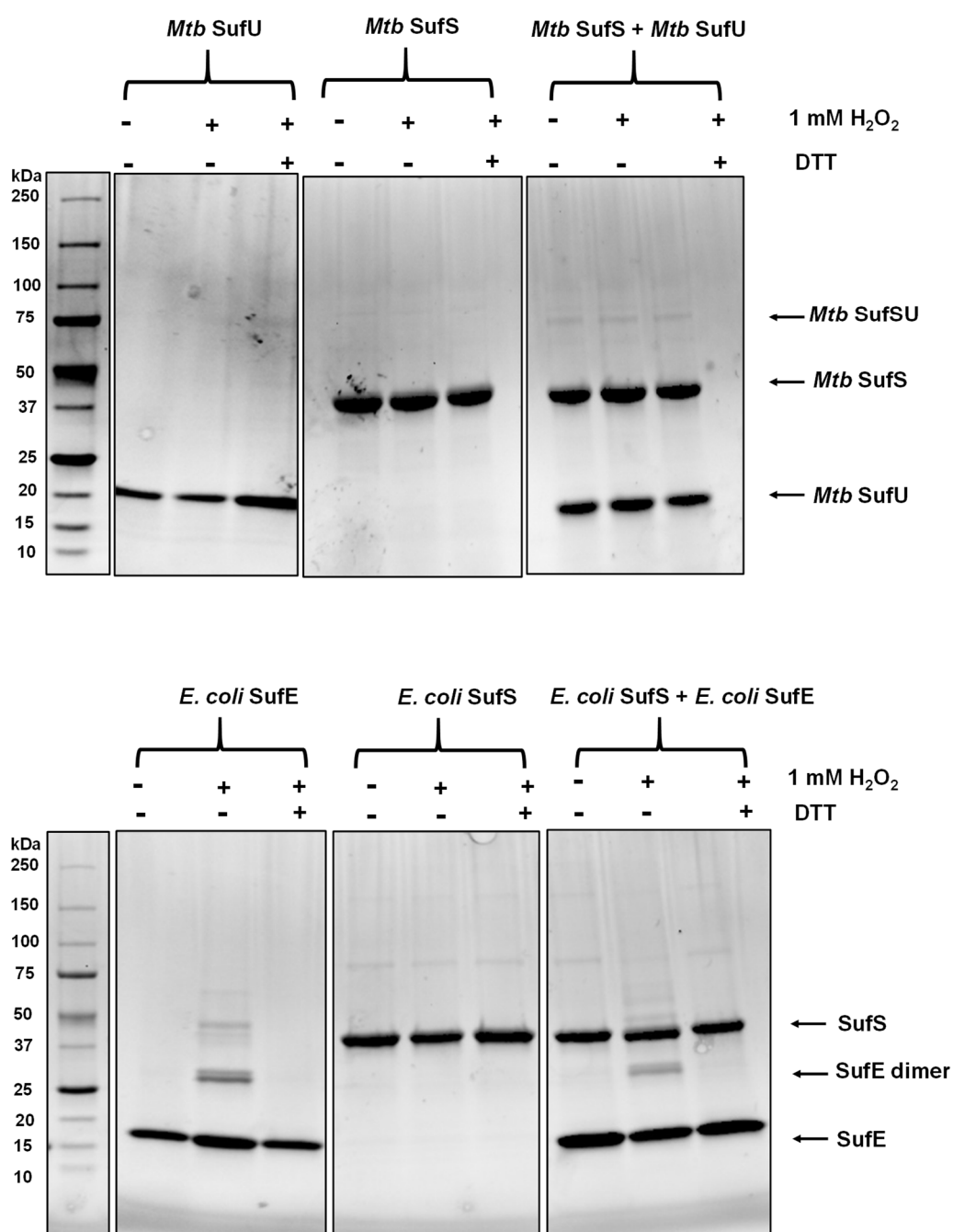


Table S1. X-Ray data and refinement statistics.

Data collection statistics (from the XDS CORRECT file)	
Beamline	ESRF-ID30B
Wavelength (Å)	0.9763
Temperature (K)	100
Space group	<i>P1</i>
<i>a</i> , <i>b</i> , <i>c</i> (Å)	62.3, 75.6, 75.6
α , β , γ (°)	92.4, 109.6, 99.7
Mosaicity (°)	0.166
Resolution range (Å)	50-1.65 (1.75-1.65) [§]
Total No of reflections	422116 (64985) [§]
No of unique reflections	147275 (22934) [§]
Wilson B factor (Å ²)	29.1
Completeness (%)	95.7 (92.4) [§]
Multiplicity	2.9 (2.8) [§]
Mean I/ σ (I)	7.47 (0.96) [§]
<i>R</i> _{meas} (%)	10.4 (117.6)[§]
CC1/2 (%)	99.6 (54.1) [§]
Refinement statistics (from the final refmac log file)	
PDB code of the final refined model	8ODQ
PDB code for initial Molecular	5XT5
No of molecules SufU-SufS in A.U	2
No. of atoms refined	9784
Resolution range (Å)	48.7-1.65Å
No of reflections use for refinement	140036
Refinement Rwork/Rfree (%)	17.9/21.4
Mean B value (Å ²)	27.0
RMSD bond length (Å)	0.01
RMSD bond angle (°)	2.1

§ : in parenthesis, values of high resolution shell

Table S2. Contact interactions (amino-acid involved).

	SufU	SufS	Distance (Å)	
A. Hydrogen bonds	C:SER 68 [OG]	D:ASP 355 [OD1]	2.87	
	C:ARG 128 [NH2]	D:ASP 355 [OD1]	3.12	
	C:TYR 9 [OH]	D:ASP 355 [OD1]	2.59	
	C:SER 68 [N]	D:ASP 355 [OD2]	2.94	
	C:ARG 128 [NH1]	D:GLN 358 [OE1]	2.98	
	C:CYS 40 [S2]	D:VAL 369 [O]	3.29	
	C:CYS 40 [S2]	D:HIS 371 [N]	3.11	
	C:CYS 40 [S2]	D:ARG 368 [NE]	2.84	
	C:CYS 40 [O]	D:HIS 354 [NE2]	2.83	
	C:GLY 41 [O]	D:HIS 352 [NE2]	2.59	
B. Salt Bridges	C:ARG 128 [NH2]	D:ASP 355 [OD1]	3.12	
	C:ARG 128 [NH1]	D:ASP 355 [OD1]	3.92	
	C:GLU 6 [OE2]	D:ARG 409 [NH1]	3.53	
	C:ASP 42 [OD1]	D:HIS 352 [NE2]	3.33	
	C:ASP 42 [OD1]	D:HIS 354 [ND1]	3.33	
	C:ASP 42 [OD2]	D:HIS 354 [ND1]	3.19	
	SufU	SufS		
	Residues	BSA(Å²)	Residues	BSA(Å²)
C. Interfacing residues	C:ARG 4	57.72	D:ARG 25	76.45
	C:LEU 5	59.63	D:GLY 26	17.27
	C:GLU 6	62.27	D:GLY 350	10.91
	C:ILE 8	5.69	D:VAL 351	1.18
	C:LEU 14	35.98	D:VAL 359	12.38
	C:TYR 17	39.12	D:ASP 362	44.81
	C:LYS 18	44.87	D:ARG 368	15.94
	C:ILE 39	28.72	D:GLY 370	15.31
	C:ASP 42	7.20	D:ALA 384	44.38
	C:GLN 65	34.10	D:ALA 385	4.18
	C:GLY 66	9.66	D:ARG 409	37.55
	C:CYS 67	34.94	D:PHE 413	96.72
	C:LYS 124	27.41	D:PHE 414	71.20
	C:TYR 125	52.87	D:GLY 415	1.57
	C:PRO 126	4.36	D:ARG 416	21.40
	C:CYS 131	2.17		

Table S2 : PISA calculation [1] performed on the complex *Mtb* SufS-SufU (subunit C and subunit D, Figure S5). **A.** Hydrogen bonds listed with a cut-off of 3.3 Å. **B.** Salt bridges listed with a cut-off of 4 Å. **C.** Other interfacing residues between SufU and SufS that do not form hydrogen bonds or salt bridges. **BSA** is the residue Buried Surface Area upon SufS-SufU complex formation.

References:

1. Krissinel, E.; Henrick, K. Inference of macromolecular assemblies from crystalline state. *J. Mol. Biol.* **2007**, *372* (3), 774-97.