# SUPPLEMENTARY MATERIALS

## Supplementary Methods

*SufS and SufU interaction*

6xHis-SufU was incubated for 10 min. with SufS (in stochiometric 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-though 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

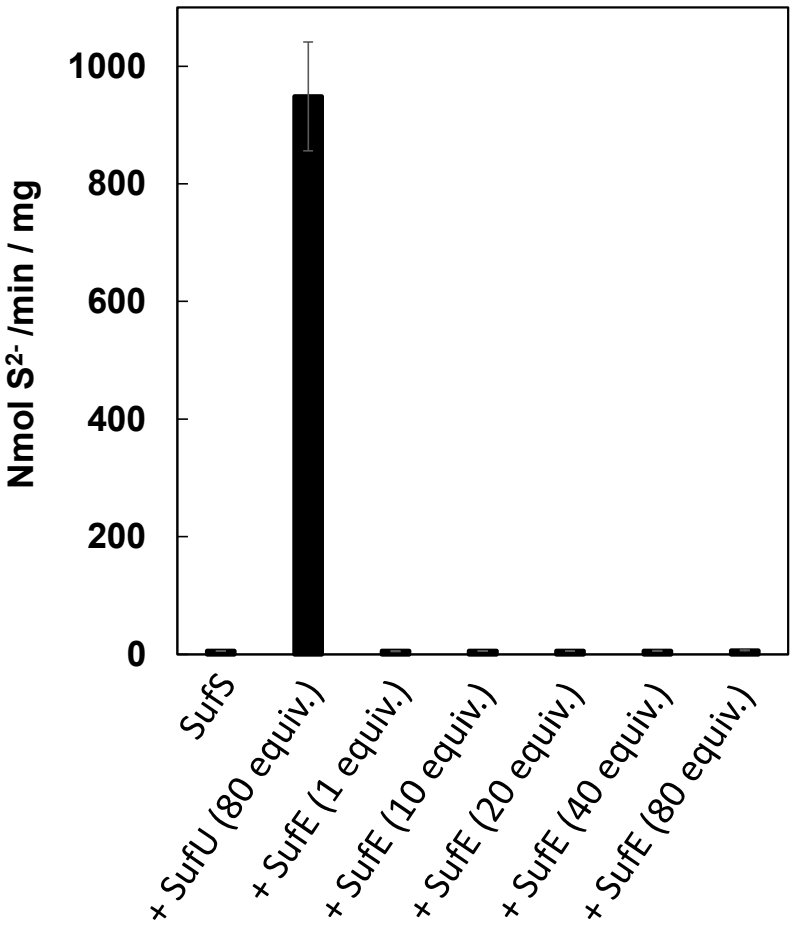


**Figure S1.** Specific activity of *Mtb* SufS (10 μM) in the absence and in the presence of different equivalents of *E. coli* SufE (1-80 equivalents) or *Mtb* SufU (80 equivalents). Cysteine at 500 μM was used in the assay.

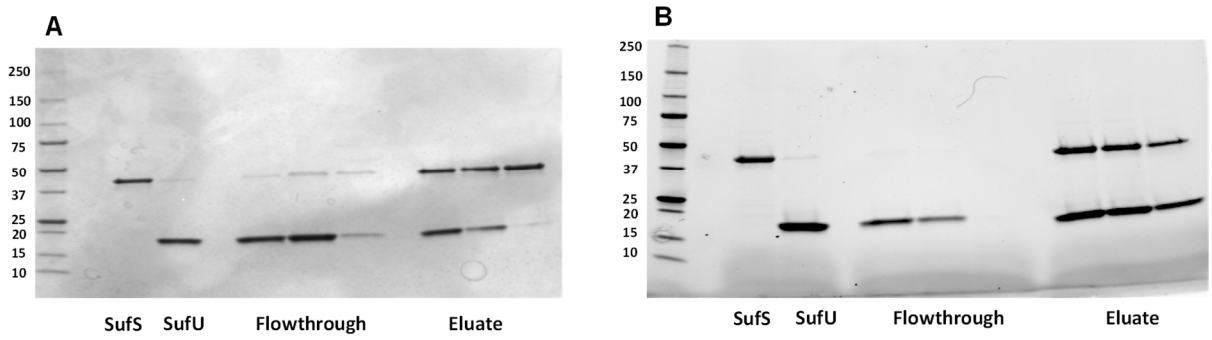


**Figure S2. (A) SufS-SufU interaction in solution. (A)** SDS-PAGE profile of 6xHis-SufS and SufU interaction in the absence of L-cysteine on affinity column (Ni-NTA). Lane (1) : known size markers (kDa), lane (2) : purified 6xHis-SufS, lane (3): purified SufU, lanes (4-6) flowthrough (FT) samples containg fractions of SufU which doesn't interact with SufS, lanes (7-9): eluate samples containg 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 containg fractions of SufU which doesn't interact with SufS, lanes (7-9): eluate samples containg 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.

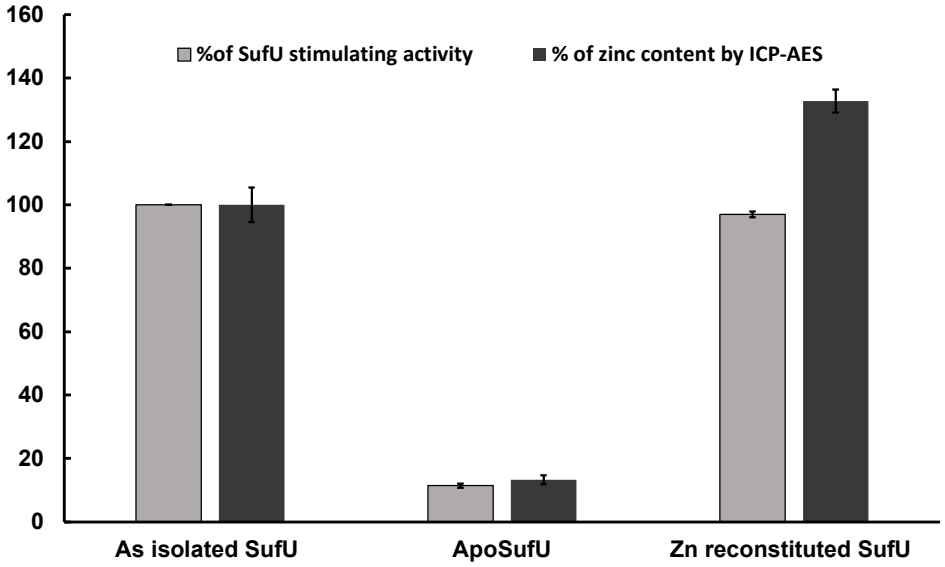


**Figure S3. Reconstitution of apo-SufU with zinc**. The SufU stimulating activity (%) was determined by measuring the specific activity of SufS (10 μM) in presence of as-isolated SufU (10 μM), apo-SufU (10 μM) and Zn reconstituted SufU (10 μM). The sulfurtransferase activity with the as-isolated SufU (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.

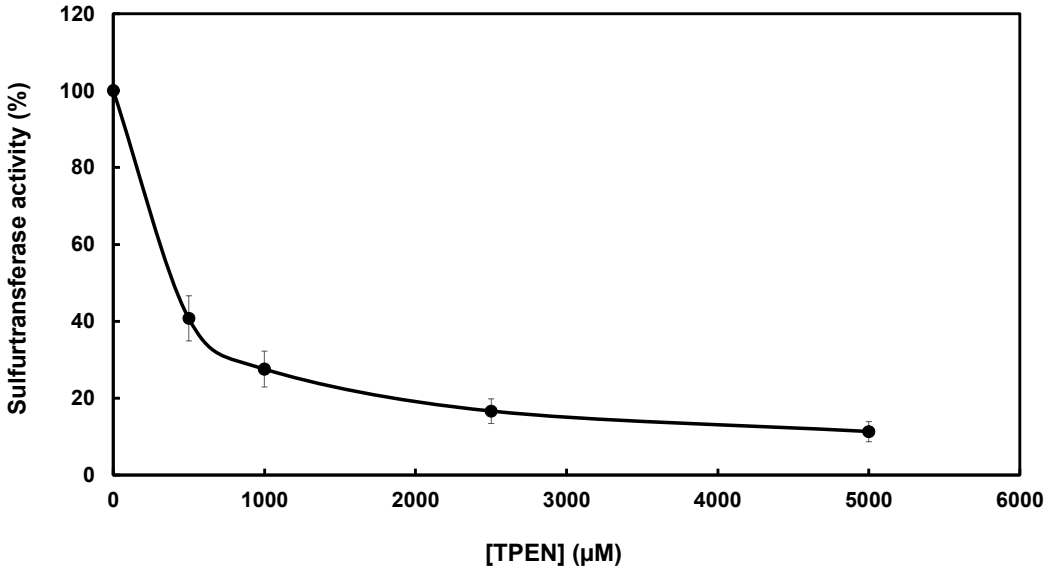


**Figure S4.** Sulfurtransferase activity of SufU (%). SufU (146 μM) was incubated with increasing concentrations of TPEN (0-5 mM) for 3 h followed by dialysis. The sulfur transfer activity of SufU was then determined by measuring the specific activity of SufS (10 μM) in the presence of each sample (10 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.

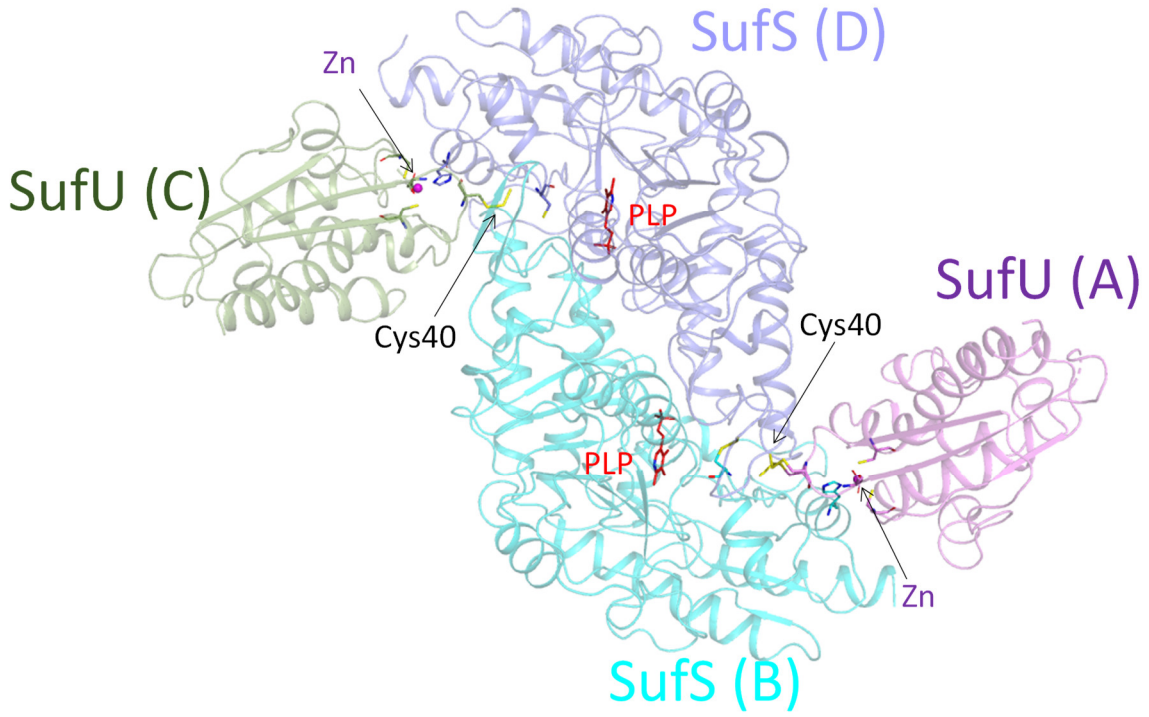


**Figure S5.** Overall crystal structure of *Mtb* SufS-SufU complex. The complex is represented in cartoon representation. The figure shows the arrangement of the different subunits, which consists as a dimer of complexes SufS-SufU (SufU in green and violet, SufS in cyan and blue). The PLP cofactor is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

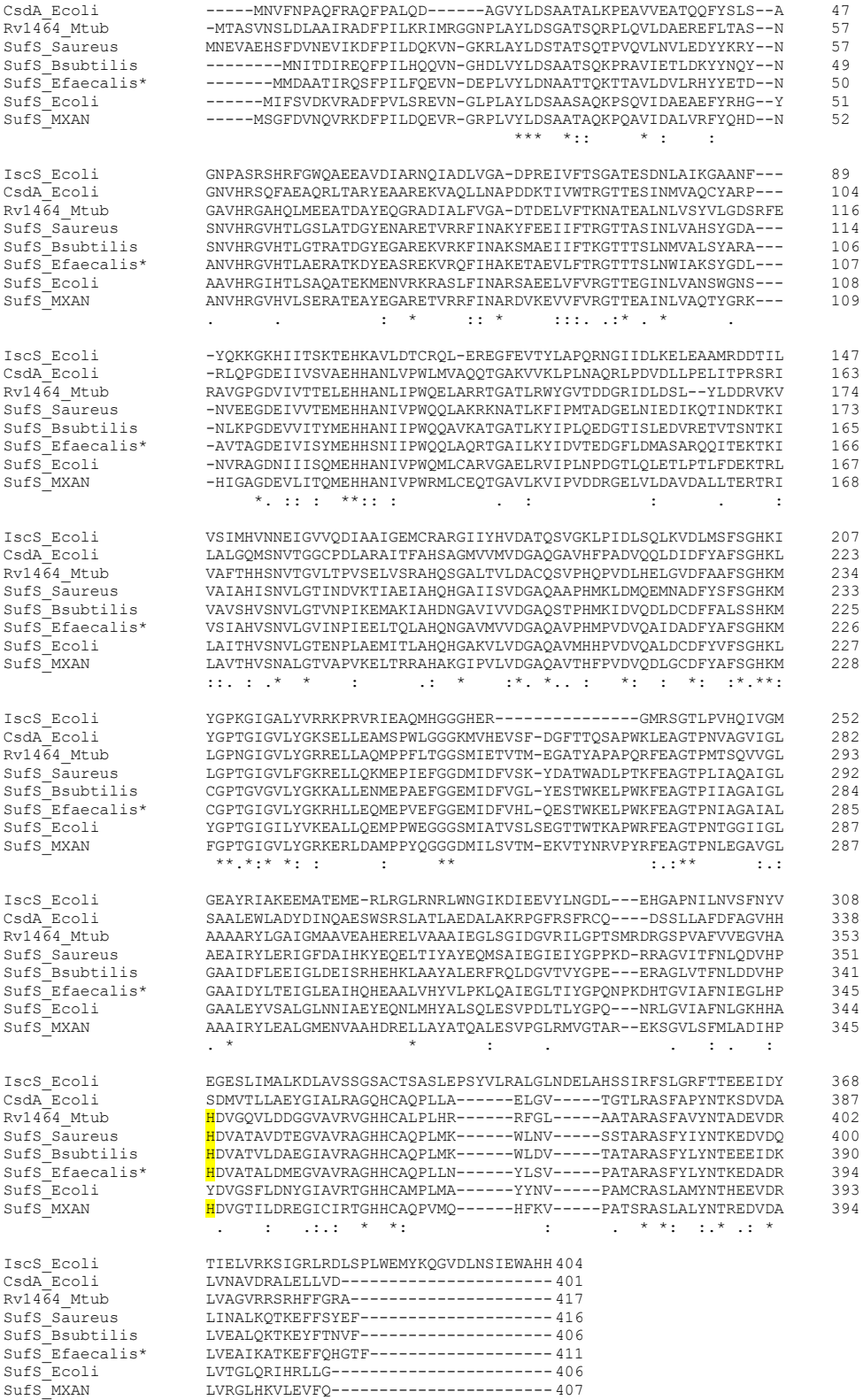

**Figure S6.** Sequence alignment of type I and type II cysteine desulfurases. Type I (*E. coli* IscS) and type II (*E. coli* CsdA, *E. coli* SufS, *S. aureus* SufS, *B. subtilis* SufS, *E. faecalis* SufS, *Myxococcus xanthus* (MXAN) SufS and *Mtb* SufS). Highlight yellow : conserved histidine residue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

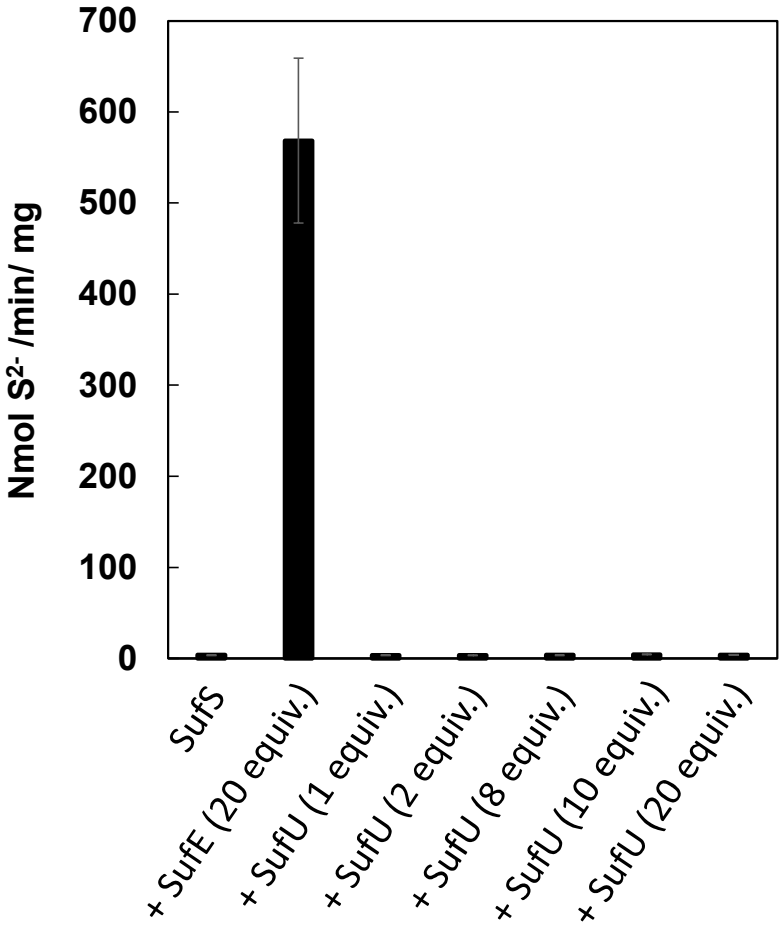


**Figure S7.** Specific activity of *E. coli* SufS (10 μM) in the absence and in the presence of different equivalents of *E. coli* SufE (20 equivalents) and *Mtb* SufU (1-20 equivalents) for 30 min. at 37°C in presence of L-cystéine (500 μM) and DTT (1 mM).

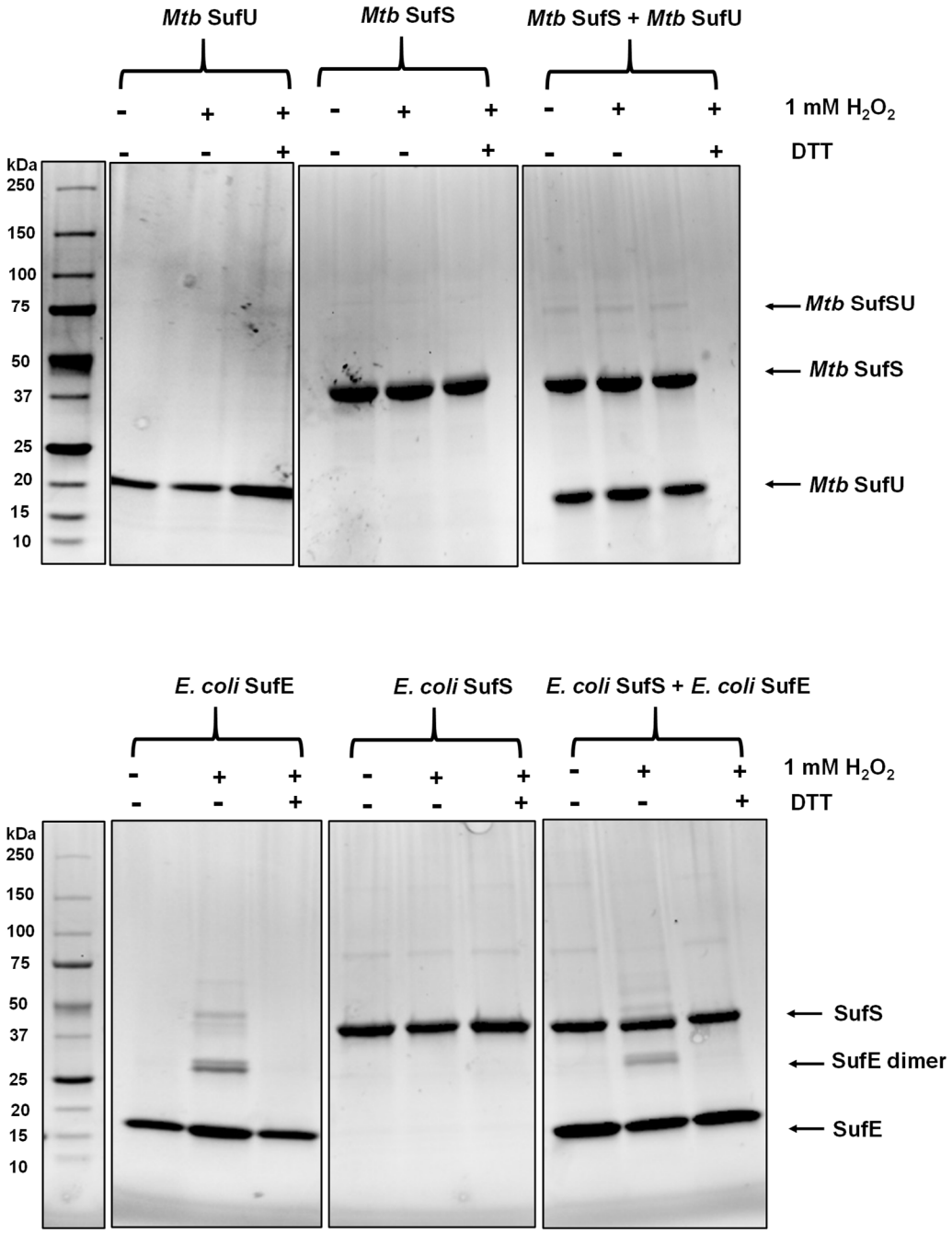


**Figure S8.** SDS-PAGE gel of $H_2O_2$ treated SufS and SufU proteins . SDS-PAGE gels of *Mtb* SufS, SufU, SufS-SufU **(A)** as well as *E. coli* SufS, SufE and SufS-SufE **(B)** in presence of $H_2O_2$ with or without DTT. The gel shows control proteins without any treatment (first lane of each category), proteins treated with 1 mM $H_2O_2$ for 30 mins (second lane of each category), and treated proteins with $H_2O_2$ (30 mins) and 2 mM DTT (third lane of each category). All samples were then dissolved in 1XSDS loading buffer not containing any reducing agents and were heated at 95°C for 15 min. and loaded on the gel. SufE was found to be sensitive to $H_2O_2$ exposure as it tends to form disulfide bridges since dimer (34%) and tetramer (6%) species were detected on gel that disappeared upon treatment with DTT.

**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 | *P1* |
| *a, b, c* (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)[§] |
| $R_{meas}$ (%) | 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 intial 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.