SUPPLEMENTAL INFORMATION

SUPPLEMENTAL FIGURE LEGENDS

FIGURE S1. Cysteine desulfurase activity and zinc occupancy of SufU variants**.** The zinc content was measured through ICP-OES (dark grey) and the specific activity was calculated by the amount of alanine formed in the reaction (light grey). The relative fold was normalized to the zinc content and activity determined for the as-isolated SufU under the same conditions.

FIGURE S2. Far UV CD spectra of SufU and SufU variants. A) Far-UV CD spectra of as-isolated SufU, $SufU^{C41A}$, $SufU^{C66A}$, $SufU^{C128A}$ and $SufU^{D43A}$. B) Far-UV CD spectra of $SufU_{WT}$, $SufU_{apo}$, $SufU_{Zn\text{-}reconstituted}$. Scans were performed with the same concentration (10 μM) in 10 mM Phosphate, pH 7.4 at 25ºC. Table 1 indicated the estimation of secondary structure composition of SufU and SufU variants using SOMCD software.

FIGURE S3. Divalent metal reconstitution of $SufU_{apo}$. $SufU_{apo}$ (0.1 mM) was incubated with 0.5 mM of each respective metal for 2 hrs. The activity of the reconstituted SufU was determined in cysteine desulfurase assays containing 0.5 mM cysteine, 0.28 μM SufS, 2 mM DTT, and 1.39 μM SufU. The percent relative activity was normalized to the rate of sulfide formation when as-isolated SufU was assayed under the same conditions (180 ± 15 nmol sulfide.min^{-1}.mg^{-1}).

FIGURE S4. Fe-S cluster assembly on $SufU^{D43A}$. $SufU^{D43A}$ (70 μM) was incubated with 1μM SufS, 350 μM Fe^{2+}, 400 μM cysteine and 0.5 M DTT in 25 mM Tris pH8 and 10% glycerol. Reaction was incubated for 2 hours in the glove box and purified using Q-sepharose column and elution containing 0.6 M NaCl in the same buffer. The figure shows UV/Vis absorption spectrum of the reconstituted and purified 110 μM $SufU^{43DA}$ containing 1.7 Fe/monomer using a 0.5 mm-pathlength cuvette. The inset shows Vis CD spectrum of the same sample which was calculated from the average of 10 scans.

FIGURE S5. Fe-S cluster assembly on apo-SufU. Apo-SufU (200 μM) was incubated with 5 mM Fe^{2+} and 1 mM DTT for 30 min before the addition of 2 mM Na_2S. The reconstitution reaction was incubated at room temperature for an addition 90 min. The protein was purified using Ni-IMAC column and eluted using 0.5 M Imidazole in 25 mM Tris buffer pH8, 10% glycerol, 0.2 M NaCl. The figure shows UV/Vis absorption spectrum of the reconstituted and purified 97 μM SufU containing 0.3 Fe/monomer using a 1 cm-pathlength cuvette. The inset shows Vis CD spectrum of the same sample using a 0.5 cm-pathlength cuvette which was calculated from the average of 10 scans. Purification of apo-SufU after Fe-S cluster assembly reaction using Q-sepharose or gel filtration yielded similar UV/Vis absorption and CD spectra. Reconstitution reactions using SufS and cysteine as the sulfur source remained clear during the course of the experiment.

FIGURE S6. Isolation of SufU after Fe-S cluster assembly conditions. Vis CD spectrum of SufU after Fe-S cluster assembly described in Figure 6 and purification through Ni-IMAC column as described in materials and methods. Inset shows the relative cysteine desulfurase activity and zinc occupancy before and after cluster assembly and purification. The cysteine desulfurase activity was measured by quantifying the rate of alanine formation and the zinc content was measured by ICP-OES. The relative fold was normalized to the activity and zinc content of the as-isolated protein.

FIGURE S1

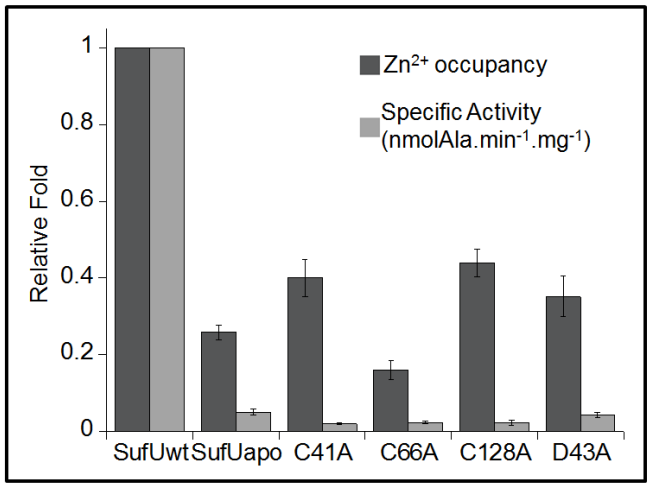

Relative Fold
1
0.8
0.6
0.4
0.2
0
Zn2+ occupancy
Specific Activity
(nmolAla.min-1.mg-1)
SufUwt
SufUapo
C41A
C66A
C128A
D43A

FIGURE S2

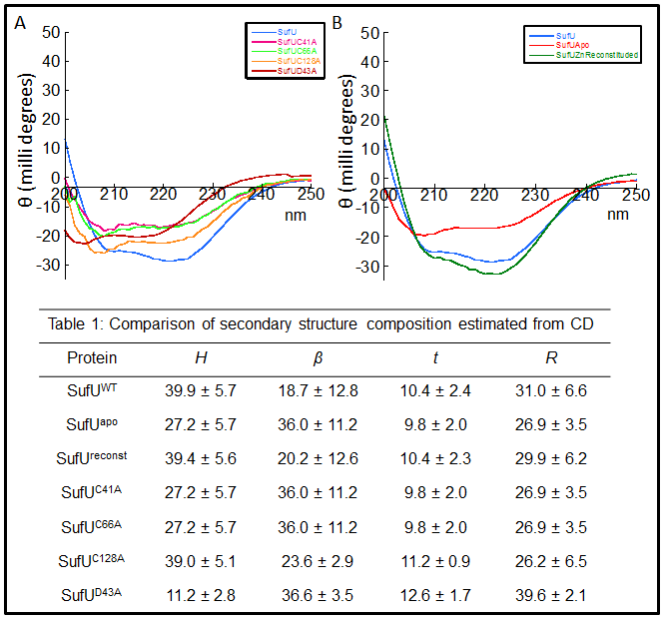


Table 1: Comparison of secondary structure composition estimated from CD

Protein	*H*	*β*	*t*	*R*
$SufU^{WT}$	39.9 ± 5.7	18.7 ± 12.8	10.4 ± 2.4	31.0 ± 6.6
$SufU^{apo}$	27.2 ± 5.7	36.0 ± 11.2	9.8 ± 2.0	26.9 ± 3.5
$SufU^{reconst}$	39.4 ± 5.6	20.2 ± 12.6	10.4 ± 2.3	29.9 ± 6.2
$SufU^{C41A}$	27.2 ± 5.7	36.0 ± 11.2	9.8 ± 2.0	26.9 ± 3.5
$SufU^{C66A}$	27.2 ± 5.7	36.0 ± 11.2	9.8 ± 2.0	26.9 ± 3.5
$SufU^{C128A}$	39.0 ± 5.1	23.6 ± 2.9	11.2 ± 0.9	26.2 ± 6.5
$SufU^{D43A}$	11.2 ± 2.8	36.6 ± 3.5	12.6 ± 1.7	39.6 ± 2.1

FIGURE S3

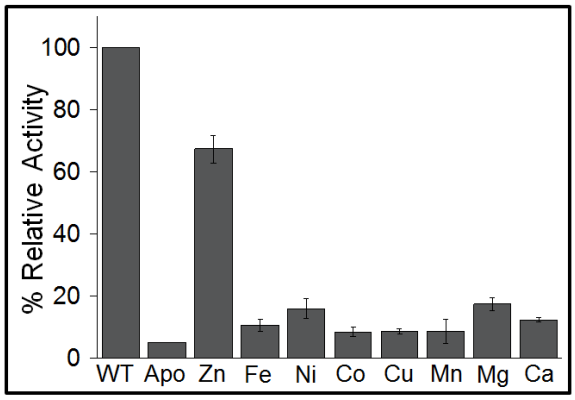


FIGURE S4

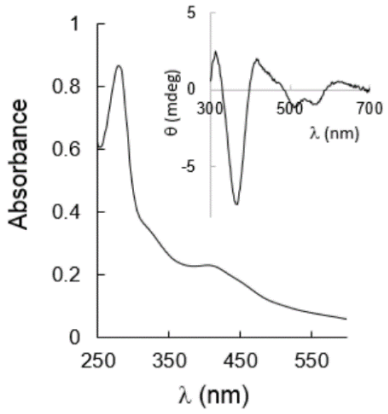

Absorbance
1
0.8
0.6
0.4
0.2
0
250
350
450
550
λ (nm)
θ (mdeg)
5
0
-5
300
500
700
λ (nm)

Figure S5

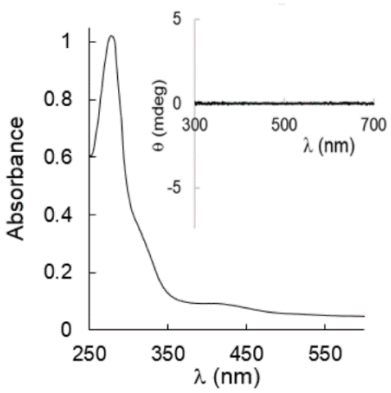
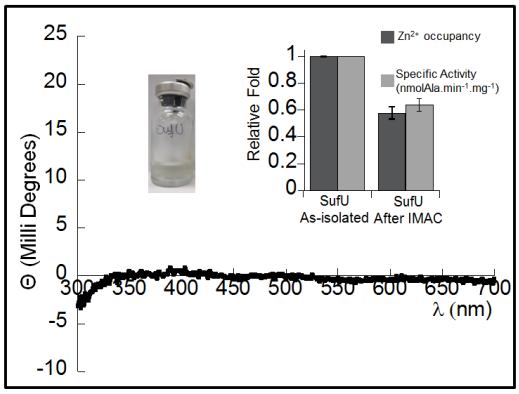

Absorbance
1
0.8
0.6
0.4
0.2
0
250
350
450
550
λ (nm)
θ (mdeg)
5
0
-5
300
500
700
λ (nm)

Figure S6


25
20
15
10
5
0
-5
-10
Θ (Milli Degrees)
300
350
400
450
500
550
600
650
700
λ (nm)
Zn2+ occupancy
Specific Activity (nmolAla.min-1.mg-1)
Relative Fold
1
0.8
0.6
0.4
0.2
0
SufU As-isolated
SufU After IMAC