Supplemental Data

The SufBCD Fe-S Scaffold Complex Interacts with SufA for Fe-S Cluster Transfer

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Figure S1. (A) Increasing amounts of SufA pre-labeled with Mts-Atf-Biotin were incubated for 1 hr with 2 μM of SufC or the SufBCD complex. (B) Label transfer analysis of SufA interaction with the SufBCD complex in the presence of ATP. SufA (4 μM) pre-labeled with Mts-Atf-Biotin was incubated for 1 hr with 2 μM of the SufBCD complex. ATP was present at 0, 1, 10, 50, 100, or 300 μM final concentration during the incubation (increasing concentrations indicated by grey gradient bar above gel). After UV-light induced cross-linking, samples from (A) and (B) were separated by reducing SDS-PAGE and the location of the biotin tag was determined by immunoblot using streptavidin conjugated to horseradish peroxidase.

Figure S2. (A) Surface plasmon resonance analysis of SufE inhibition of SufA-SufS interactions. Increasing amounts of SufE were pre-mixed with 2 μM SufS for 20 min prior to injection over immobilized SufA. The percent binding of SufS to SufA (Y-axis unit) was calculated using the binding of 2 μM SufS to immobilized SufA in the absence of SufE (260 response units) as "100 % binding" and the reduction of binding signal to the baseline value of immobilized SufA alone as "0 % binding". The X-axis is the concentration of SufE divided by the concentration of SufS (molar ratio) for each condition. (B) Comparison of Fe-SufA and ferrous ammonium sulfate as iron sources for SufBCD Fe-S cluster reconstitution. Fe-SufA was prepared as described in Materials and Methods. Fe-SufA or ferrous ammonium sulfate (FAS) were added in excess to provide 5 equivalents Fe per mole SufBCD in a reaction buffer containing SufBCD (300 μM) and SufS-SufE/L-cysteine. The reaction mixture was incubated for 1.5-2 h and then separated by anaerobic gel filtration. SufA and SufBCD were further analyzed for Fe-S cluster content. UV-visible absorption spectra of SufBCD reconstituted with FAS (trace 1, thick black line), SufBCD reconstituted with Fe-SufA (trace 2, thin black line), or SufA after reconstitution (trace 3, grey line). See Results for more details.

Figure S3. (A) Circular dichroism spectra of 280 μM of holoSufBCD (red trace) and holoSufA (black trace) after anaerobic reconstitution as described in Materials and Methods. (B) Stability of the Fe-S clusters in 280 μM holoSufBCD or holoSufA in the presence of increasing EDTA. UV-Visible

absorption spectra of samples were taken at each EDTA concentration. The percent decrease in absorption at 420 nm (SufA) or 408 nm (SufBCD) was calculated using the initial value (- EDTA) as 100 % and is plotted as a function of EDTA concentration.

Figure S1

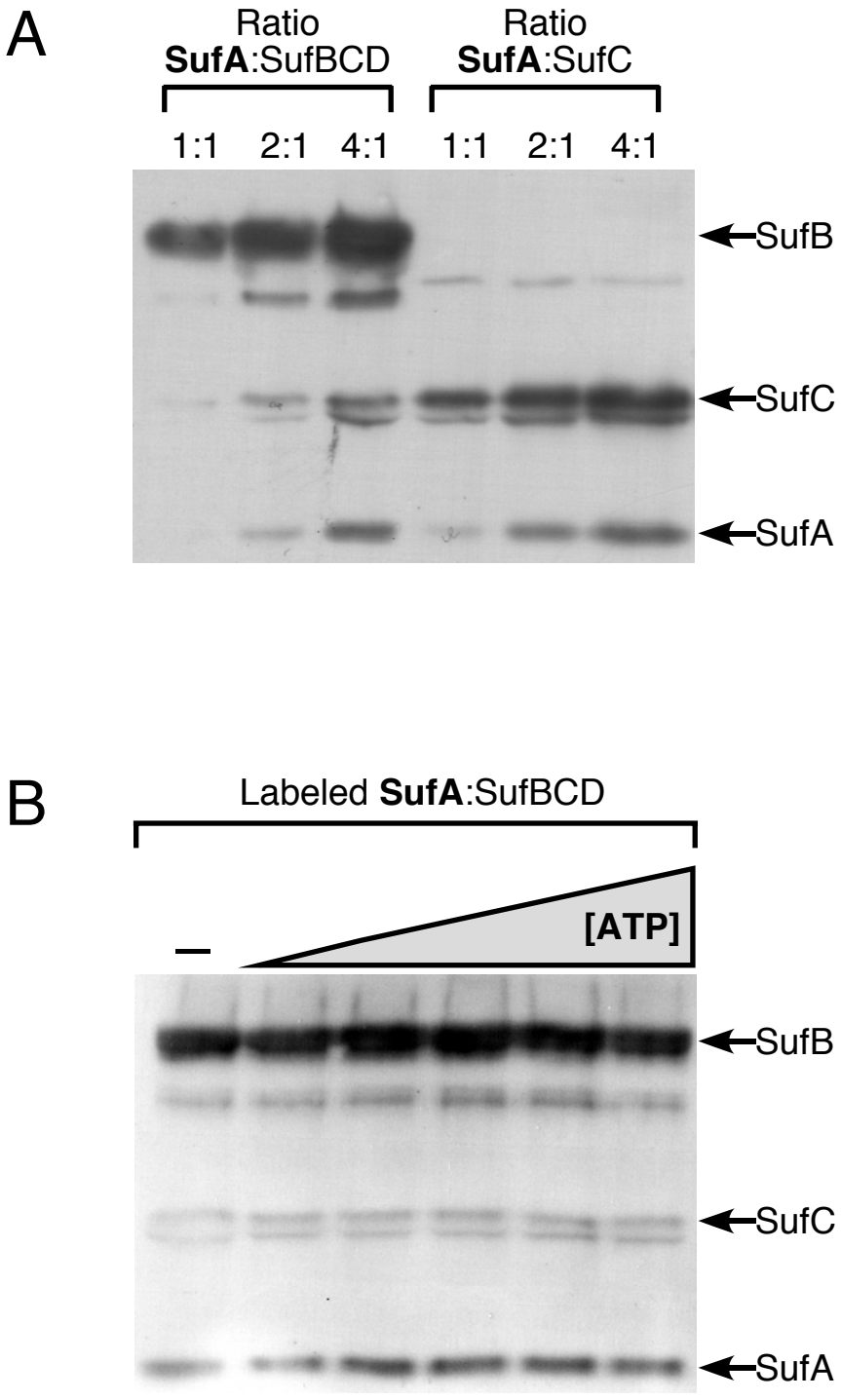
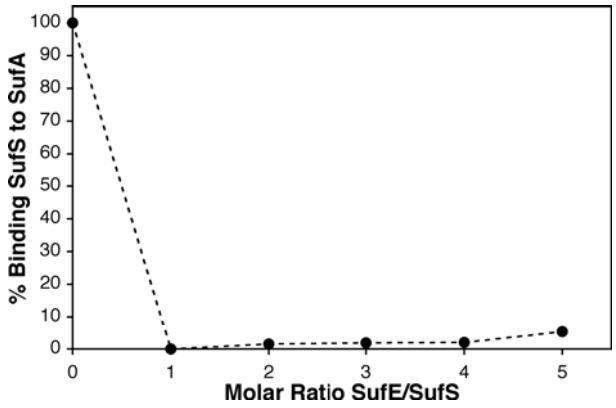

A
Ratio
SufA:SufBCD
Ratio
SufA:SufC
1:1
2:1
4:1
1:1
2:1
4:1
SufB
SufC
SufA
B
Labeled SufA:SufBCD
–
[ATP]
SufB
SufC
SufA

Figure S2

A


% Binding SufS to SufA
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
Molar Ratio SufE/SufS

B

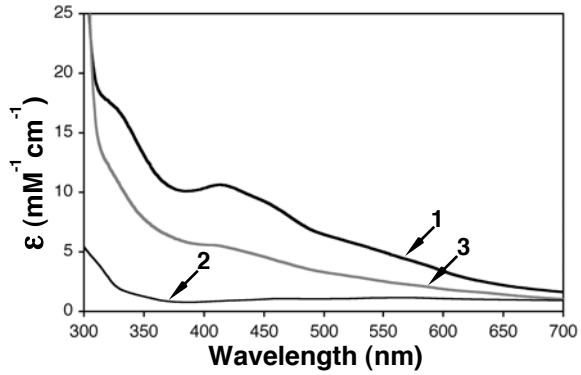
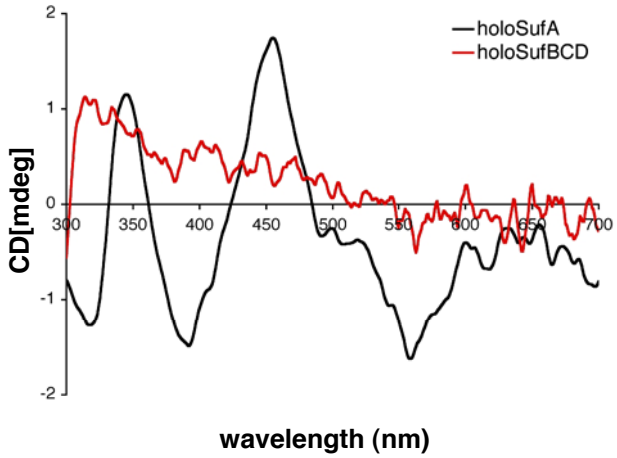
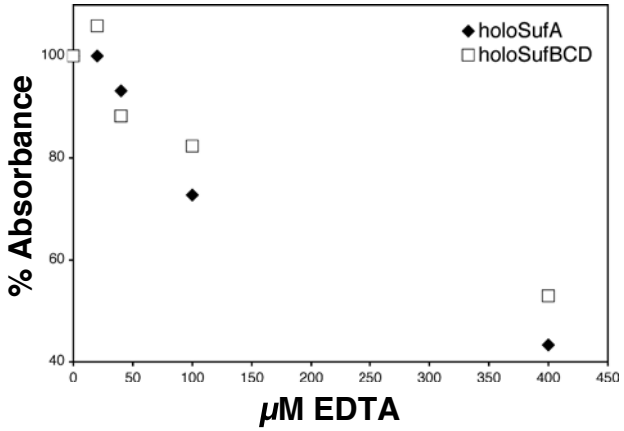

ε (mM-1 cm-1)
25
20
15
10
5
0
300
350
400
450
500
550
600
650
700
Wavelength (nm)
1
2
3

Figure S3

A


holoSufA
holoSufBCD
CD[mdeg]
2
1
0
-1
-2
300
350
400
450
500
550
600
650
700
wavelength (nm)

B


holoSufA
holoSufBCD
% Absorbance
100
80
60
40
0
50
100
150
200
250
300
350
400
450
μM EDTA