Separate Fe-S Scaffold And Carrier Functions For $SufB_2C_2$ And SufA During In Vitro Maturation Of [2Fe-2S] Fdx

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Running title: Fe-S cluster trafficking in the Suf system

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SUPPLEMENTAL INFORMATION

FIGURE LEGENDS

Fig. S1. UV-Visible spectra of holo proteins. *A,* UV-visible spectra of holo-SufB_2C_2 (red trace) or holo-SufBC_2D (black trace). *B,* UV-visible spectrum of holo-SufA. All proteins were reconstituted *in vitro* as described in Experimental Procedures.

Fig. S2. Fdx matured from [4Fe-4S] SufB_2C_2, [4Fe-4S] SufBC_2D, or [2Fe-2S] SufA. *A*, CD spectra of 200 μM of apo-Fdx after 18 h incubation with 300 μM each of holo-SufB_2C_2 (red), holo-SufBC_2D (black), or holo-SufA (blue). Dashed line shows the CD spectrum of 200 μM of holo-Fdx (as-purified from *E. coli*). *B,* CD spectra of transfer at 80 min from holo-SufBC_2D (290 μM) to apo-Fdx (200 μM) in the presence (red) and absence (black) of 60 μM of EDTA (red), reconstitution on apo-Fdx in the presence of FAS (652 μM) and Na2S (687 μM) in the presence of 60 μM of EDTA (blue)

Fig. S3. Separation of proteins following de novo cluster assembly. UV-Visible spectra of (*A)* His_6-SufB_2C_2, (*C)* His_6-SufBC_2D, or (*E*) His_6-SufA (red lines) and Fdx (black line) after co-incubation with iron and sulfide for 20 min and separation via a Ni^{2+}-NTA column. CD spectra of (*B)* His_6-SufB_2C_2, (*D)* His_6-SufBC_2D, or (*F*) His_6-SufA (red lines) and Fdx (black line) after co-incubation with iron and sulfide for 20 min and separation via a Ni^{2+}-NTA column.

Fig. S4. De novo Fe-S cluster assembly on Fdx in the presence of SufB_2C_2, SufBC_2D, or SufA using SufS-SufE-L-cysteine as the sulfur donor. Comparison of cluster formation using FAS (1 mM), SufS-SufE (0.8 μM each), and L-cysteine (1 mM) on Fdx alone (filled triangles) or Fdx in the presence of apo-SufB_2C_2 (squares), apo-SufBC_2D (diamonds), or apo-SufA (circles). Holo-Fdx formation was calculated by comparing the changes in ellipticity at 434 nm with the 434 nm ellipticity of 100% [2Fe-2S] Fdx prepared separately.

Fig. S5. Fdx matured in the presence of catalytic amounts of SufB_2C_2, SufBC_2D, or SufA. *A,* Rate of cluster formation on Fdx in the presence (squares) and absence (triangles) of catalytic amounts of apo-SufB_2C_2. *B*, Rate of cluster formation on Fdx in the presence (diamonds) and absence (triangles) of

catalytic amounts of apo-SufBC_2D. *C*, Rate of cluster formation on Fdx in the presence (circles) and absence (triangles) of catalytic amounts of apo-SufA. Rates of Fdx formation were calculated by monitoring changes in ellipticity at single wavelength 434 nm of a solution containing 10 μM of apo-SufB_2C_2, apo-SufBC_2D, or apo-SufA with 1 mM each FAS and Na_2S and various amounts of apo-Fdx.

Fig. S6. Separation of proteins following Fe-S cluster transfer from SufA to Fdx. *A*, UV-Visible spectra of holo-His_6-SufA (red line) before loading onto the Ni^{2+}-NTA column and spectra of Fdx (black line) and His_6-SufA (dashed line) eluted separately after co-incubation on the Ni^{2+}-NTA column for 20 min. *B*, CD spectra of Fdx (black line) and His_6-SufA (dashed line) isolated after co-incubation on the Ni^{2+}-NTA column for 20 min.

Fig. S1

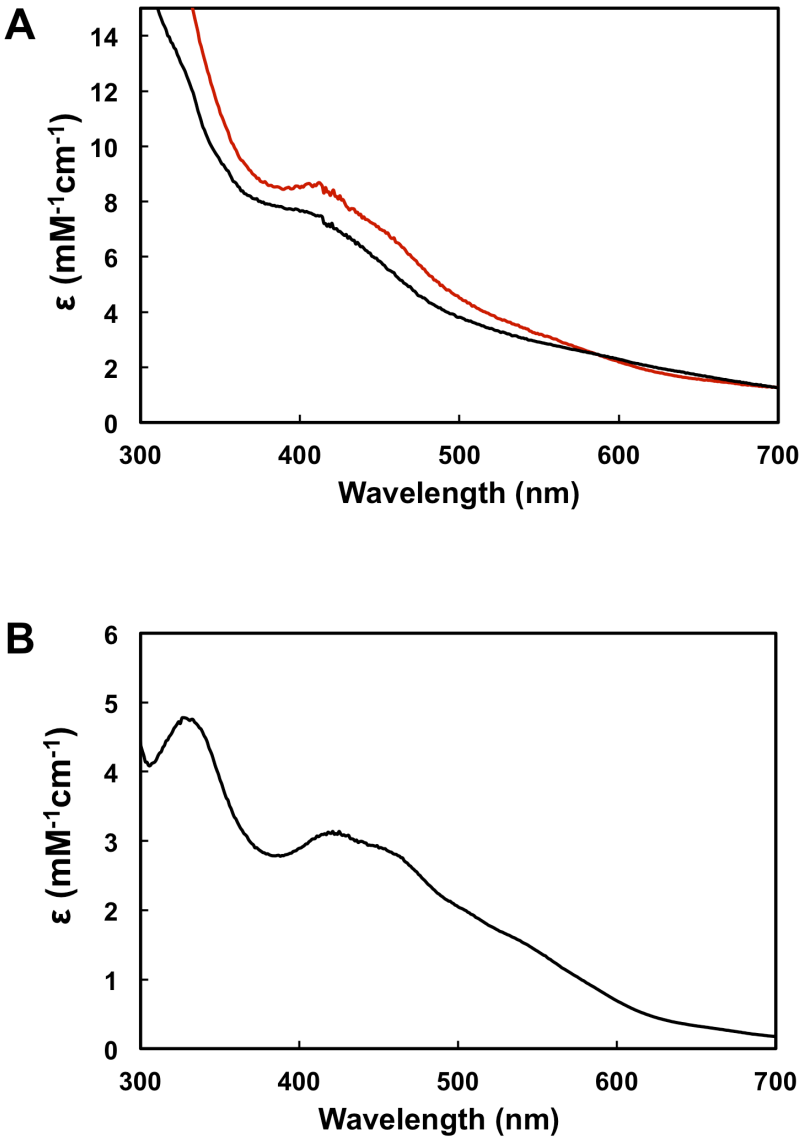
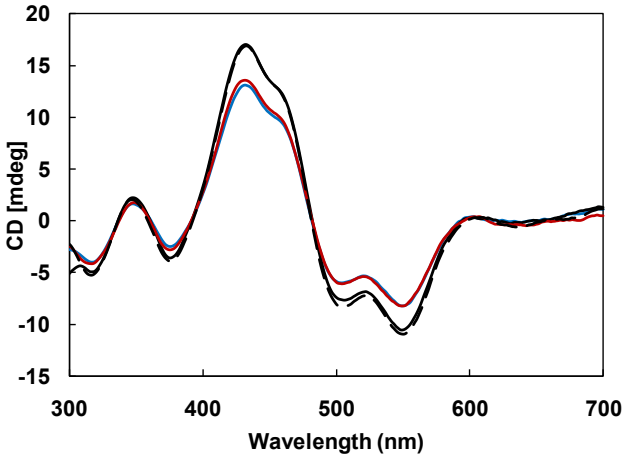

A
ε (mM-1cm-1)
14
12
10
8
6
4
2
0
300
400
500
600
700
Wavelength (nm)
B
ε (mM-1cm-1)
6
5
4
3
2
1
0
300
400
500
600
700
Wavelength (nm)

Fig. S2

A


20
15
10
5
0
-5
-10
-15
CD [mdeg]
300
400
500
600
700
Wavelength (nm)

B

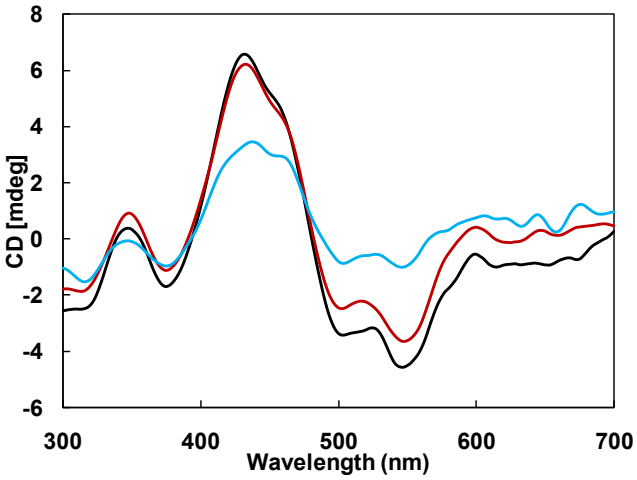

8
6
4
2
0
-2
-4
-6
CD [mdeg]
300
400
500
600
700
Wavelength (nm)

Fig. S3

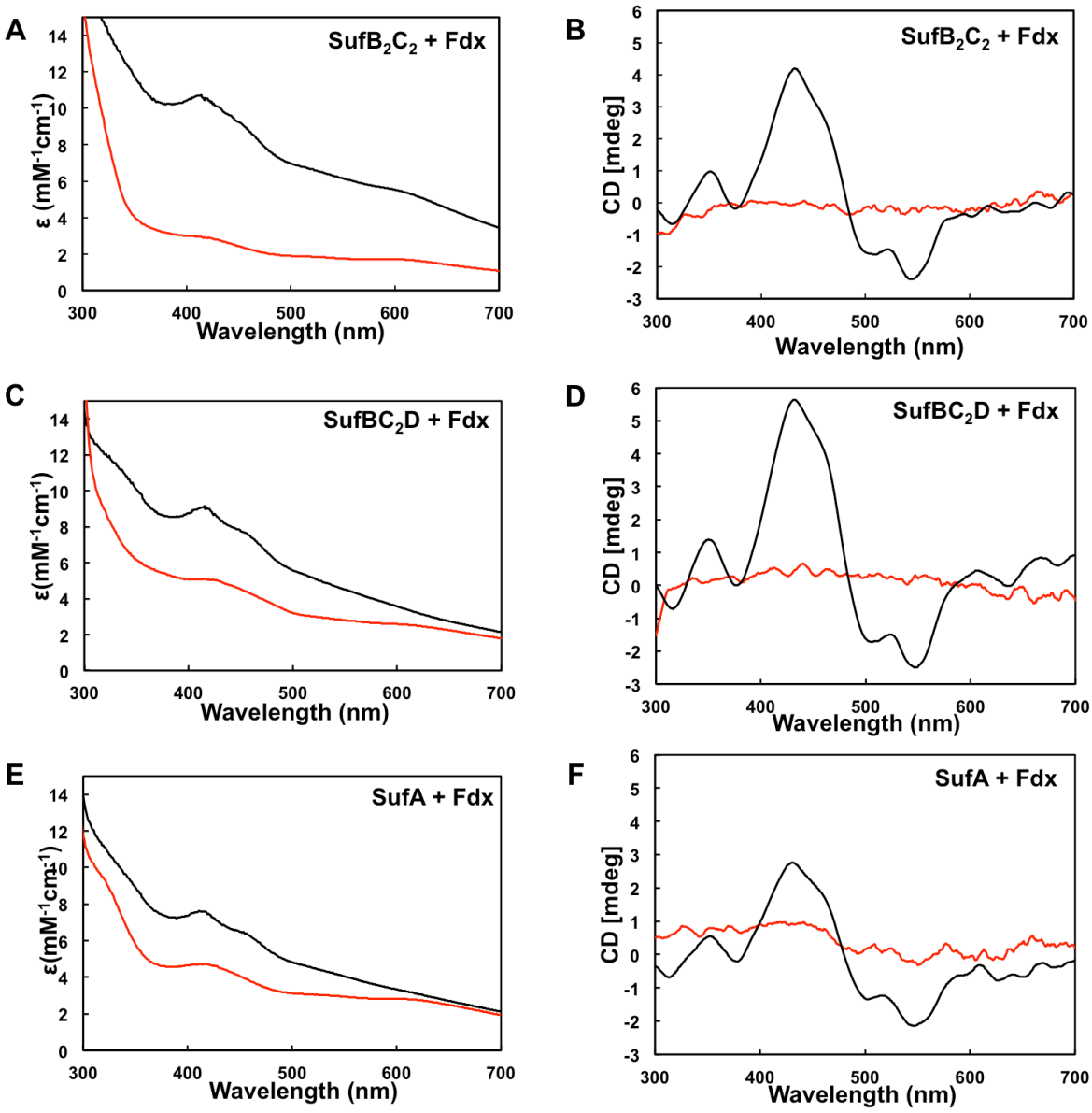

A
SufB2C2 + Fdx
ε (mM-1cm-1)
Wavelength (nm)
B
SufB2C2 + Fdx
CD [mdeg]
Wavelength (nm)
C
SufBC2D + Fdx
ε(mM-1cm-1)
Wavelength (nm)
D
SufBC2D + Fdx
CD [mdeg]
Wavelength (nm)
E
SufA + Fdx
ε(mM-1cm-1)
Wavelength (nm)
F
SufA + Fdx
CD [mdeg]
Wavelength (nm)

Fig. S4

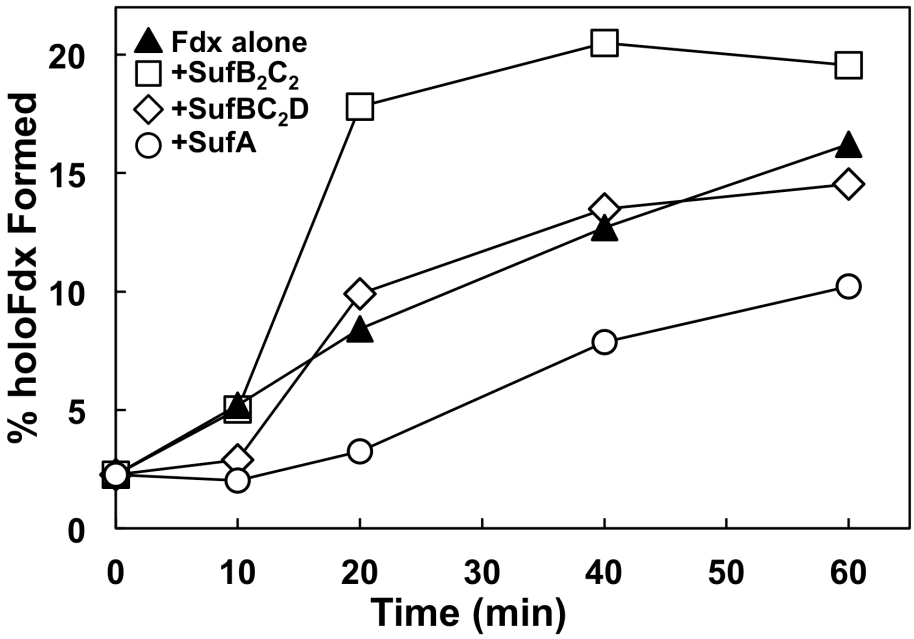
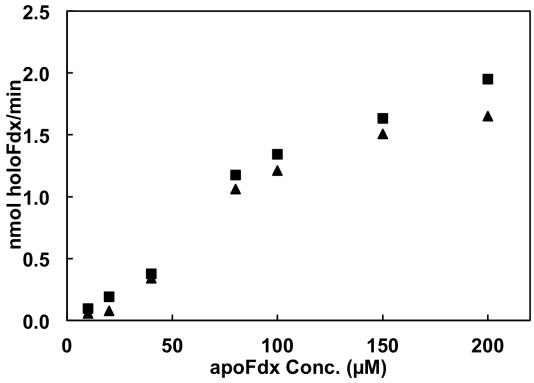
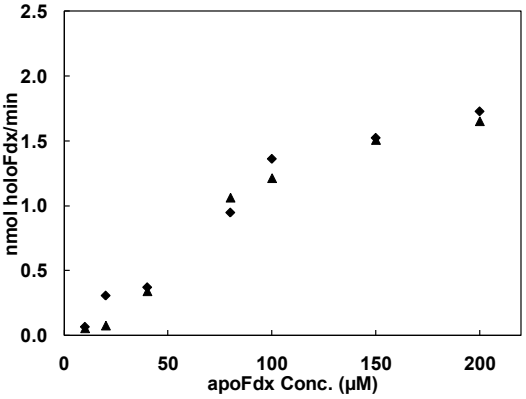

Fdx alone
+SufB2C2
+SufBC2D
+SufA
% holoFdx Formed
Time (min)
0
5
10
15
20
0
10
20
30
40
50
60

Fig. S5

A


nmol holoFdx/min
2.5
2.0
1.5
1.0
0.5
0.0
0
50
100
150
200
apoFdx Conc. (μM)

B


nmol holoFdx/min
2.5
2.0
1.5
1.0
0.5
0.0
0
50
100
150
200
apoFdx Conc. (μM)

C

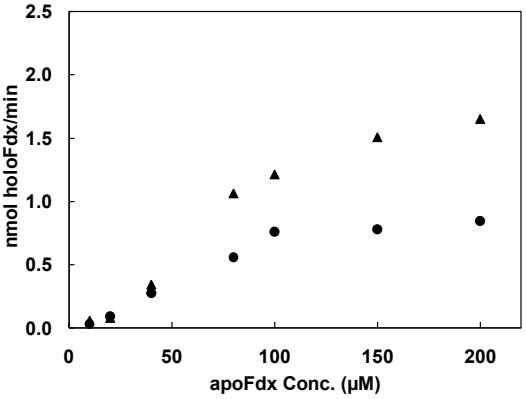
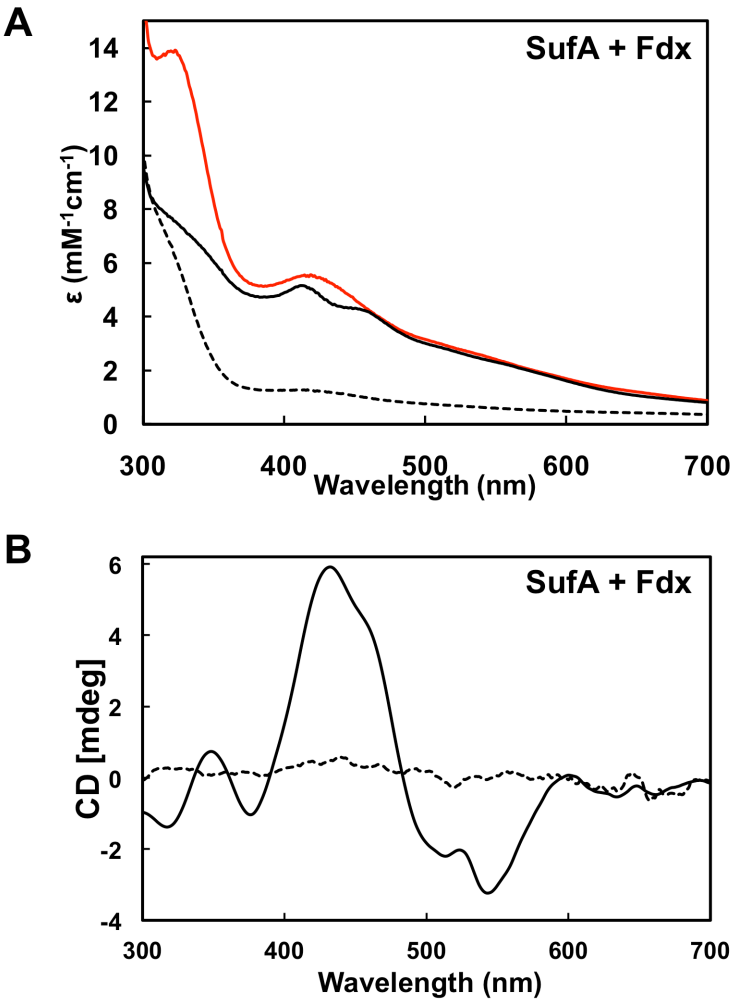

nmol holoFdx/min
2.5
2.0
1.5
1.0
0.5
0.0
0
50
100
150
200
apoFdx Conc. (μM)

Fig. S6


A
SufA + Fdx
ε (mM-1cm-1)
14
12
10
8
6
4
2
0
300
400
500
600
700
Wavelength (nm)
B
SufA + Fdx
CD [mdeg]
6
4
2
0
-2
-4
300
400
500
600
700
Wavelength (nm)