

Supplemental Figures and Tables for

Unique roles of iron and zinc binding to the yeast

Fe-S cluster scaffold assembly protein “Isu1”

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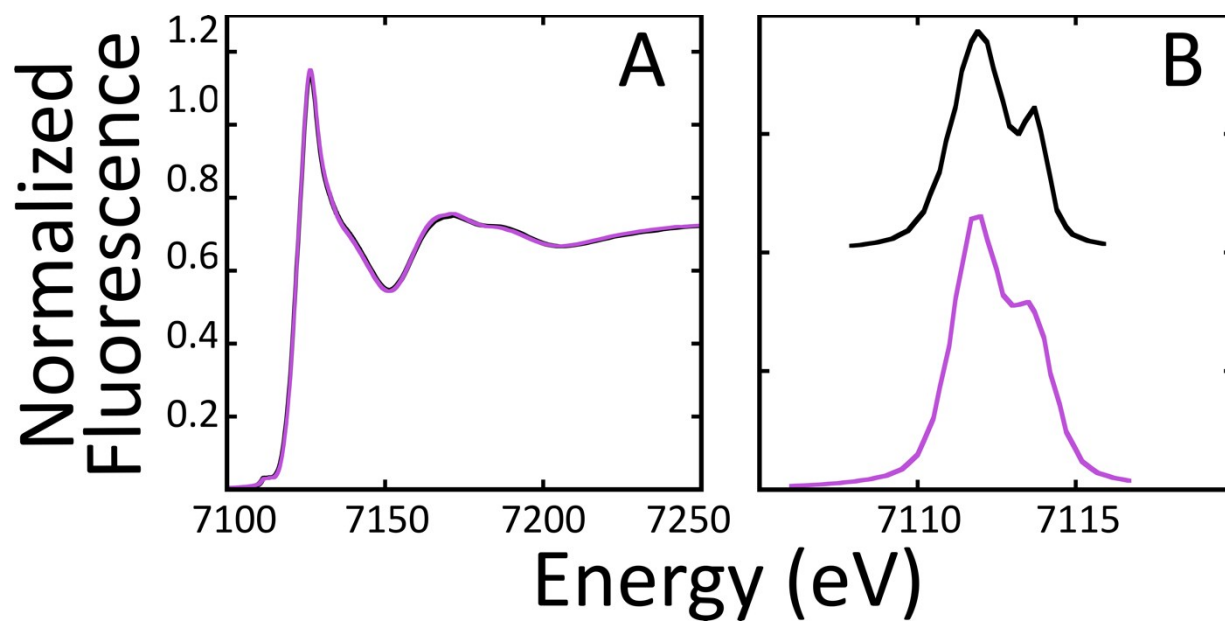
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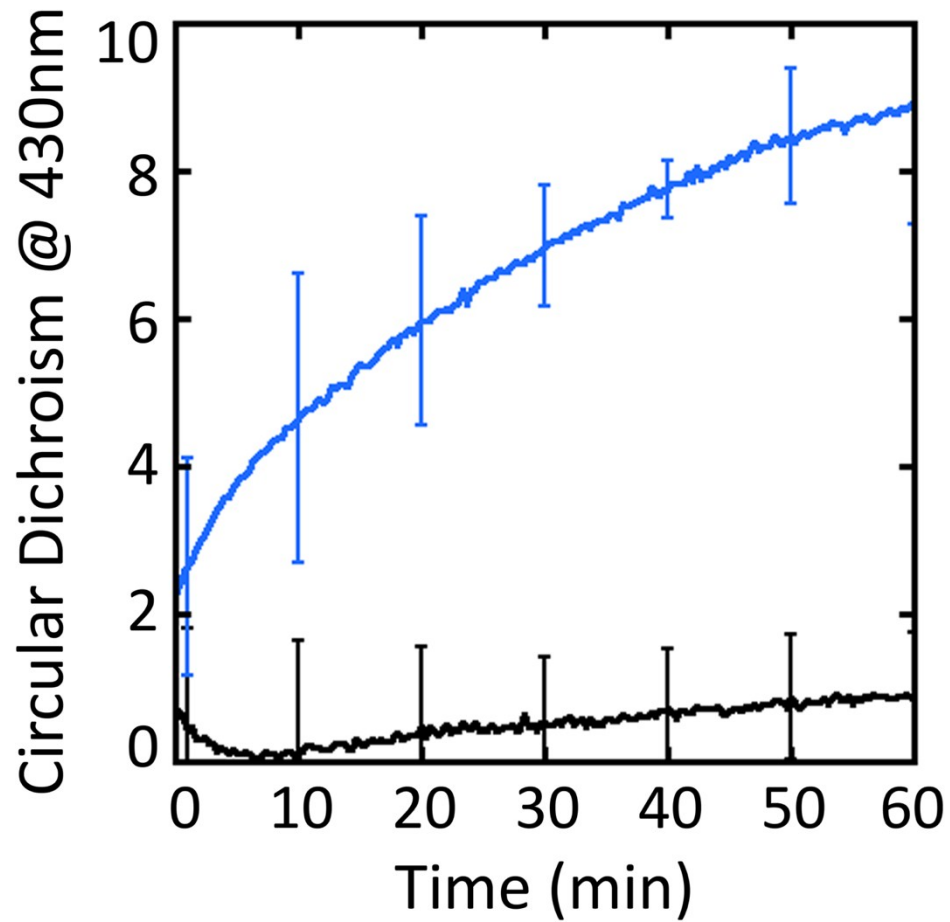
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Supplemental Figure 1. Normalized Fe XANES for Zn/Fe Loaded Isu1 Samples Following a Unique Sequential Loading Order (i.e., Fe then Zn loading, and Zn then Fe loading of Isu1). All samples were eventually loaded with 1 equivalent zinc and 2 equivalents iron, although the loading order was varied. (A) Fe-XANES for iron loaded first shown above in purple while the Zn loaded first sample is shown in black. (B) Respective iron pre-edge features are shown for iron loaded first (purple) and zinc loaded first (black).



Supplemental Figure 2. Fe-S cluster assembly assay. Cluster assembly assay detected by Circular Dichroism Spectroscopy at 430 nm using NIA, Yfh1, Fe(II), *L*-Cys, DTT and either with apo-Isu1 (blue) or Zn-Isu1 (black).

Supplemental Table 1. Summary of ICP-MS analysis of the iron and zinc concentrations found in apo-Isu1, as well as Fe(II), Zn(II) and Fe(II)/Zn(II) loaded XAS samples.

Sample	Isu1 Concentration (μM)	Fe Concentration (μM)	Zn Concentration (μM)
Apo-Isu1 ^a	346.5 \pm 163.5	2.0 \pm 1.1	2.2 \pm 1.2
2Fe-Isu1 ^b	581.5 \pm 56.5	427.1 \pm 38.6	6.0 \pm 3.0
1Zn-Isu1 ^c	546.5 \pm 91.5	11.7 \pm 12.6	582.6 \pm 300.9
1Zn/2Fe-Isu1 ^d	577.0 \pm 122.0	838.7 \pm 354.5	526.7 \pm 328.6

^a Analysis from 4 apo-Isu1 samples isolated from 2 independent reproducible protein purifications.

^b Analysis from 2 independently prepared 2Fe-Isu1 XAS samples.

^c Analysis from 2 independently prepared 1Zn-Isu1 XAS samples.

^d Analysis from 3 independently prepared 1Zn/2Fe-Isu1 XAS samples.

Supplemental Table 2. Fe XANES Pre-edge Area and eV Inflection Analysis from Varying the Fe/Zn Addition Order. Pre-edge areas of the 1s→3d transition signal for Fe(II) within samples measured in dimensionless values.³⁰ Peak inflection energy values were calculated as the edge first derivate of the edge.

	Pre-Edge Area ^a	Peak Inflection (eV)
1Zn-2Fe-Isu1 (Zn loaded first)	4.04 ± 1.34	7122.7 ± 1.3
1Zn-2Fe-Isu1 (Fe loaded first)	3.67	7121.5

^a Dimensionless area values.

