Supplementary Material (ESI) for Chemical Communications


## Electronic Supplementary Information

# Human Frataxin: Iron and Ferrochelatase Binding Structure

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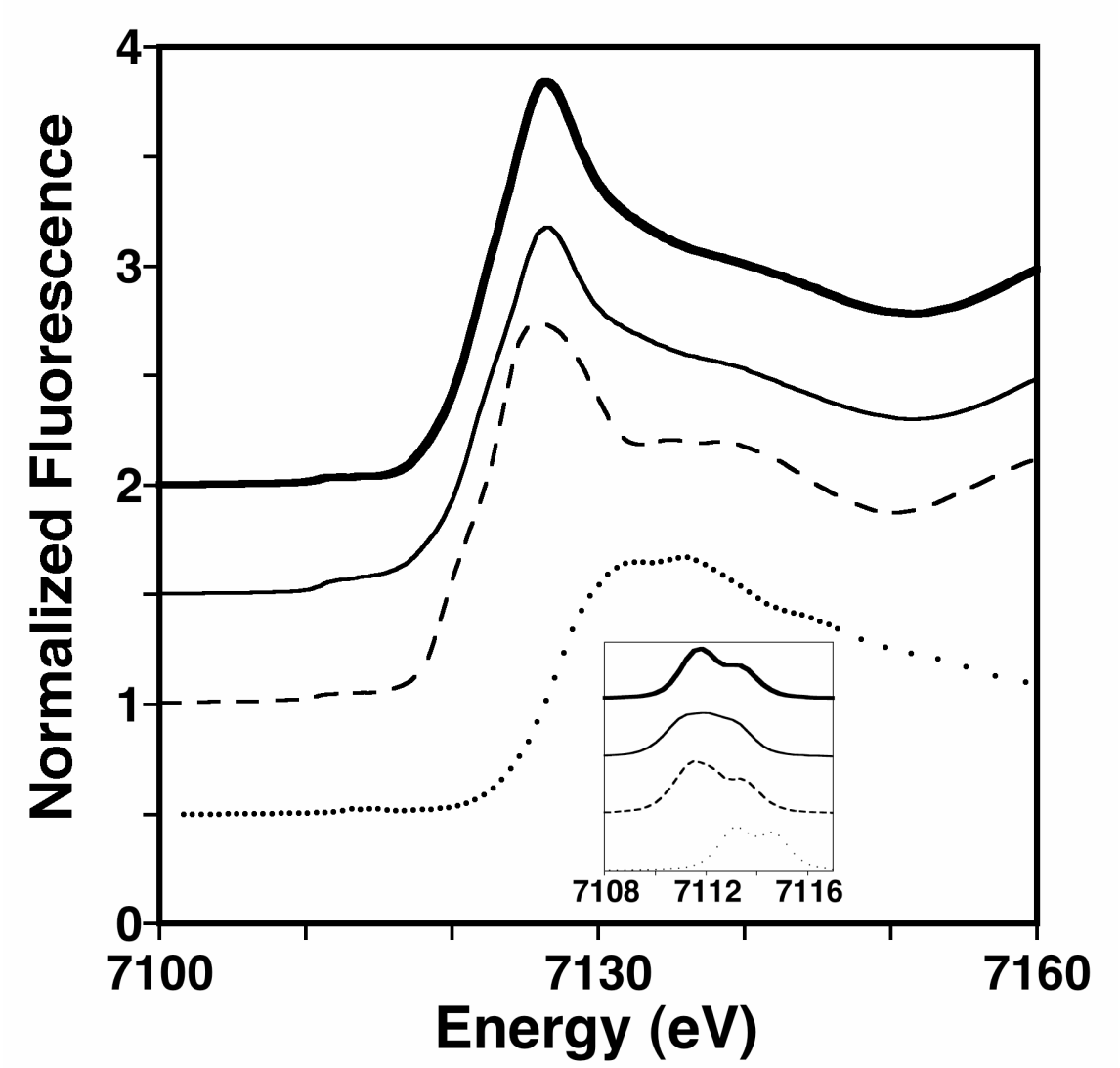
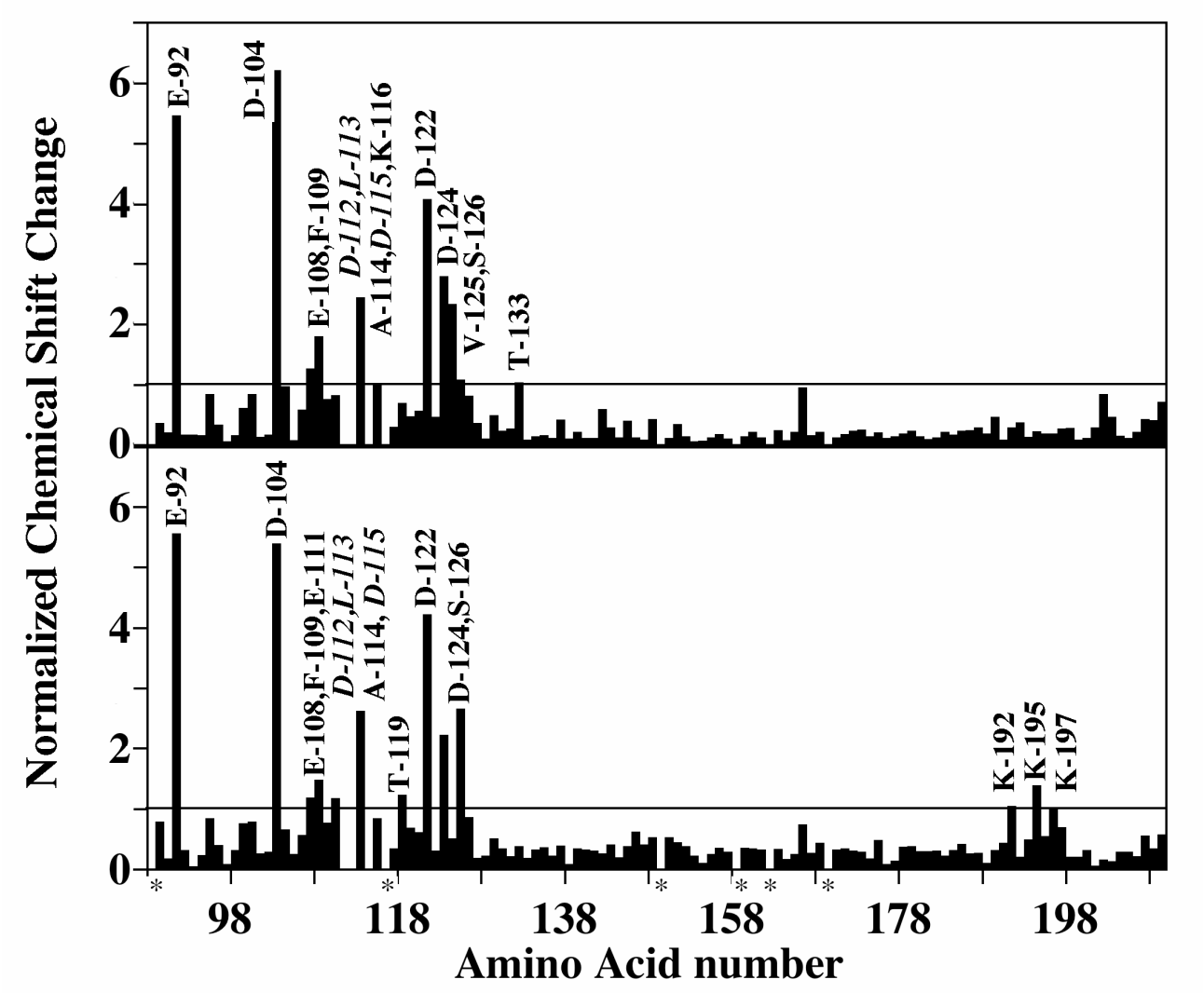
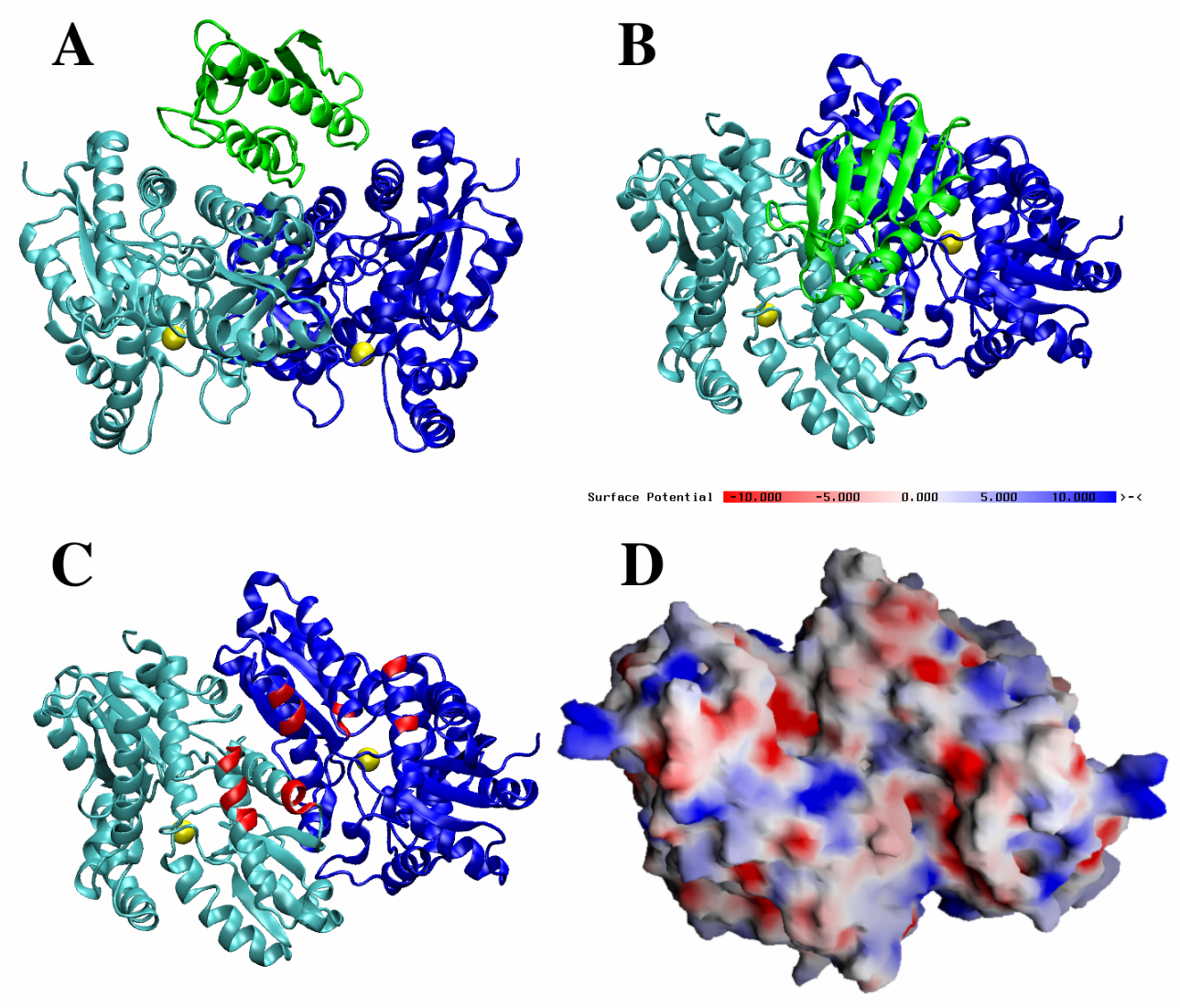



Supporting Figure 1. Normalized XANES spectra of iron loaded monomeric human frataxin with iron models. Solid Line - 2:1 Fe(II) to frataxin ratio; standard line -1:1 Fe(II) to frataxin ratio; dashed line - $Fe(II)(NH_4)_2(SO_4)_2$ model; dotted line - $Fe(III)(NH_4)(SO_4)$ model. Inset: expanded view of background subtracted 1s → 3d transitions for respective samples. Spectra were offset for clarity.




Supporting Figure 2. Perturbations in amide chemical shifts first upon addition of stoichiometric Fe(II) to apo-human frataxin (upper panel) and finally upon further addition of ferrochelatase to the Fe(II) loaded human frataxin sample (lower panel). Stars denote unobservable proline residues and the unassigned G170 resonance. Residues that undergo only chemical shift perturbations are identified in normal text font. Residues identified in italics (D112, L113, D115) disappear upon addition of Fe(II) during the iron titration and hence can not be observed in the ferrochelatase titration. The sequence corresponds to the published solution structure of apo-human frataxin.[1]




Supporting Figure 3. Lowest energy structure of monomeric human frataxin docked to the metal loaded human ferrochelatase. (A) Side view of a single frataxin monomer (green) docked to a ferrochelatase dimer (molecules in dark and light blue). $Co^{2+}$ is bound in the ferrochelatase heme assembly active site close to the four membrane attachment lips at the bottom of this figure. (B) Side view (90° Horizontal rotation of Figure 3A) showing monomeric human frataxin interacts with both units in the ferrochelatase dimer. (C) Ferrochelatase side view with frataxin removed to illustrate the ferrochelatase residues that directly interact with frataxin (shown in red). (D) Electrostatic potential plots of the ferrochelatase side view, calculated using GRASP.[2] The display of molecular structures was performed by VMD[3] while docking was performed with ZDOCK[4] using the frataxin solution structure (PDB ID# 1LY7)[1] and the $Co^{2+}$ loaded human ferrochelatase crystal structure (PDB ID# 1HRK).[5]

Supporting Table 1. Averaged EXAFS fitting analysis for iron loaded monomeric human frataxin with 1 and 2 metals bound, respectively. Data fit over a *k* range of 1.0 to 13.0 $Å^{-1}$ using a scale factor of 0.95 and a $\Delta E_0$ of -10 eV. Text in italics represents the optimal simulation data.

| | | Ligand Environment[a] | | | | Ligand Environment[a] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Sample** | **Fit #** | **Atom[b]** | **R(Å)[c]** | **CN[d]** | **$\sigma^2$ [e]** | **Atom[b]** | **R(Å)[c]** | **CN[d]** | **$\sigma^2$ [e]** | **F' [f]** |
| Frataxin-Fe1 | 1.1[g] | O/N | 2.12 | 5.0 | 4.54 | | | | | 0.81 |
| | *1.2*[g] | *O/N* | *2.12* | *5.0* | *4.59* | *C* | *3.36* | *1.0* | *2.91* | *0.72* |
| Frataxin-Fe2 | 2.1[g] | O/N | 2.12 | 5.5 | 3.97 | | | | | 0.47 |
| | 2.2[g] | O/N | 2.12 | 5.5 | 3.97 | C | 4.06 | 0.5 | 4.09 | 0.45 |
| | *2.3*[g] | *O/N* | *2.12* | *5.5* | *4.00* | *C* | *3.36* | *0.5* | *3.48* | *0.44* |
| | | | | | | *C* | *4.07* | *0.5* | *2.73* | |

[a] Independent metal-ligand scattering environment
[b] Scattering atoms: O (Oxygen), N (Nitrogen)
[c] Metal-ligand bond length
[d] Metal-ligand coordination number
[e] Debye-Waller factor in Å x $10^3$
[f] Number of degrees of freedom weighted mean square deviation between data and fit
[g] Fit using only single scattering Feff 7 theoretical models

## Supporting Material References

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