

## ***Supplementary Information***

### **“Anion clamp” allows flexible protein to impose coordination geometry on metal ions**

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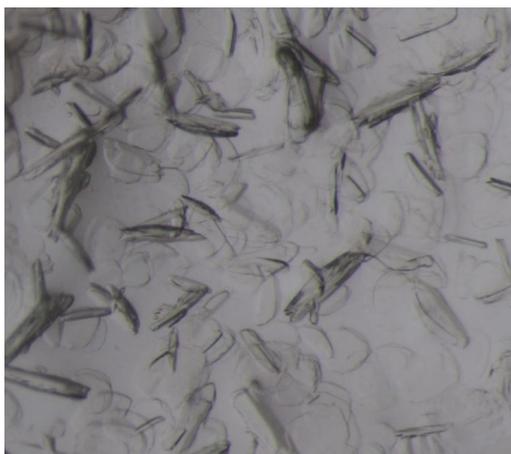
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### **Experimental Details**

Apo-hTF was used as obtained from Sigma-Aldrich. The mono-ytterbium hTF (Yb-hTF) was prepared according to a similar procedure for terbium-bound hTF<sup>1</sup>, buffered in a solution containing 0.01 M HEPES-NaOH pH 7.5 and 0.01 M NaHCO<sub>3</sub>, and concentrated to 1 mM. The sitting-drop method was used for crystallization. The crystallization precipitant medium contained 0.13 M PIPES-NaOH pH 6.0, 8 mM disodium malonate, 17% PEG3350 (w/v) and 18% glycerol (v/v).

The mono-ferric Fe-hTF protein was prepared from apo-hTF and buffered in 0.01 M HEPES-NaOH pH 7.5 and 0.01 M NaHCO<sub>3</sub>, concentrated to 1~1.5 mM. The crystallization condition was the same as for Yb-hTF. Protein crystals appeared after 2-7 days.

Diffraction data were collected at Shanghai Synchrotron Radiation Facility using 0.9793Å radiation. HKL2000 was used for data reduction and scaling. Molecular replacement and model refinement used (the Phenix suite<sup>2</sup>). For the calculation of N1-N2 opening angle (reference model: 1N84), the Superpose tool in the CCP4 suite was used.<sup>3</sup>



(a) Yb<sub>C</sub>-hTF crystals

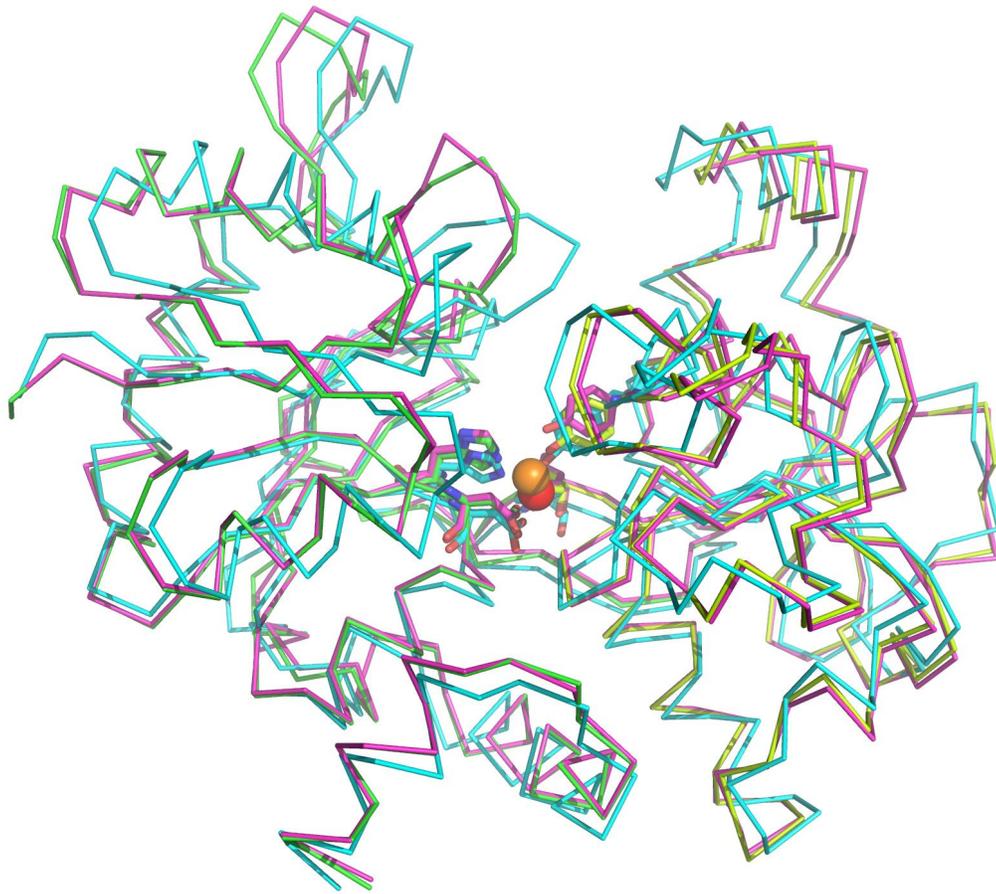


(b) Fe<sub>C</sub>-hTF crystals

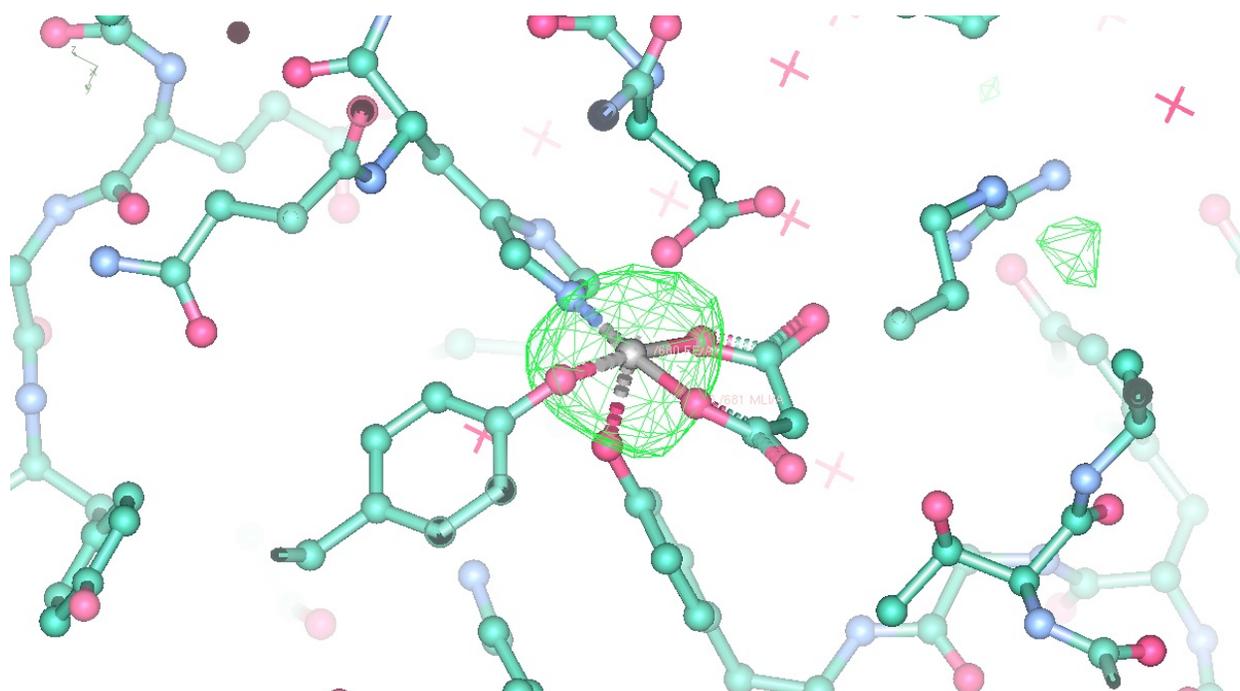
**Figure S1** Photographs of Yb<sub>C</sub>-hTF(a) and Fe<sub>C</sub>-hTF(b) crystals.

**Table S1** Major hydrogen bonds between the N-lobe and C-lobe in Yb-hTF and Fe-hTF.

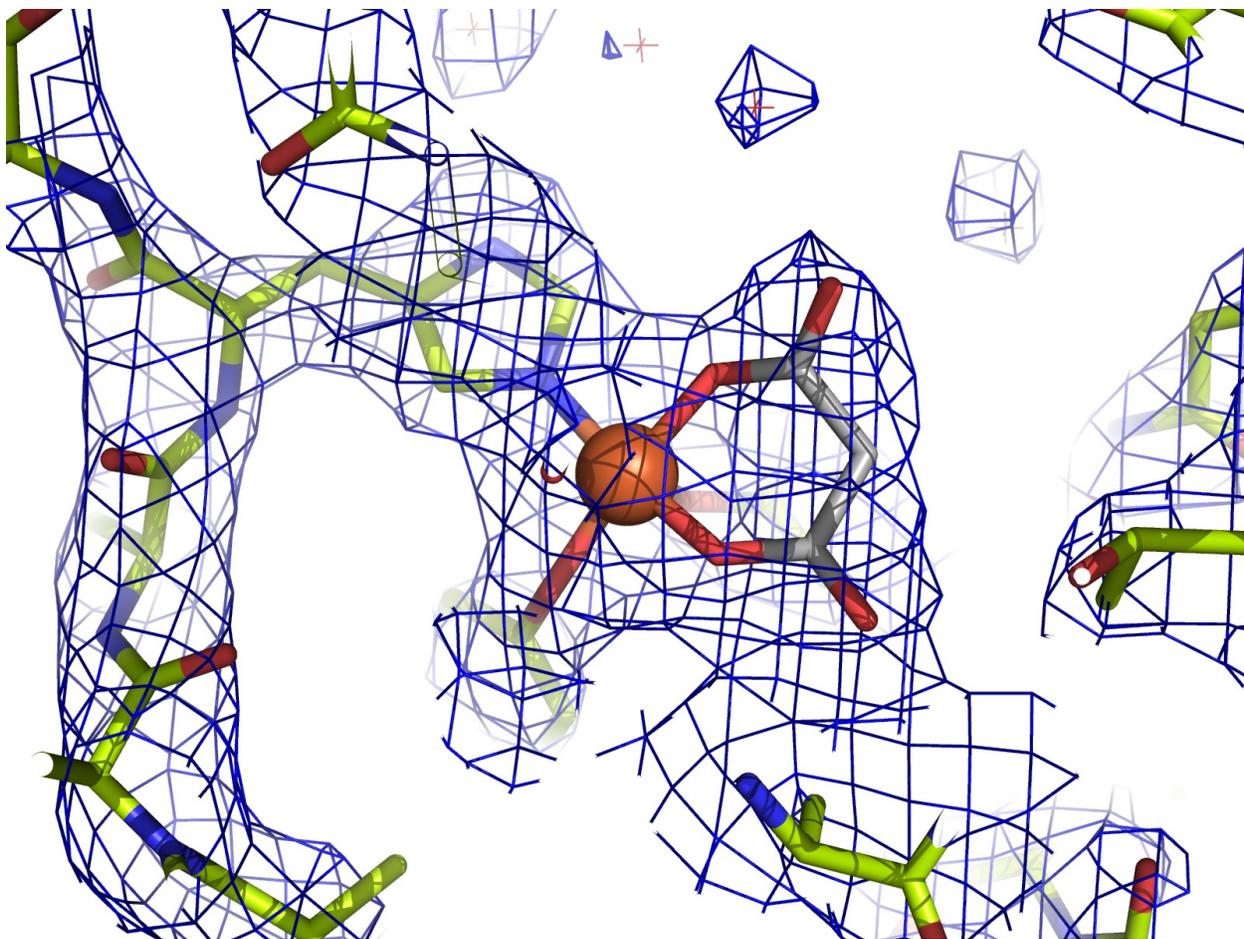
Atom1	Atom2	Å, Fe-hTF	Å, Yb-hTF/A	Å, Yb-hTF/B
Asp240-Oδ1	Arg678-Nη2	3.01	2.46	2.44
Gln245-Nε2	Arg677-O	2.74	2.94	2.95
Arg308-Nη2	Asp376-Oδ1	2.78	3.05	3.07
Lys312-Nζ	Glu385-Oε1	2.78	2.94	2.95
Tyr314-O	Arg677-Nη1	(3.21)	2.86	2.86
Tyr317-Oη	Asp592-Oδ2	2.65	(3.24)	(3.23)



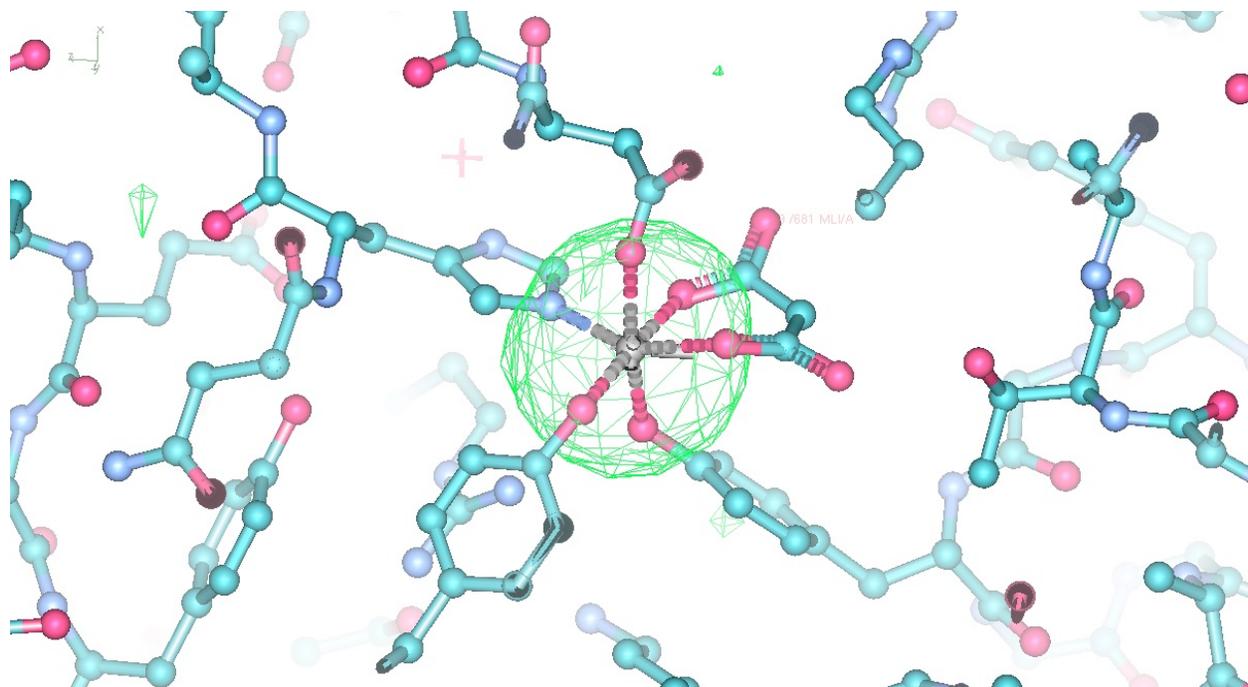
**Figure S2** Superimposition of the fully-closed C-lobes of Bi<sub>N</sub>Fe<sub>C</sub>-hTF (*cyan*, bound to Fe<sup>III</sup> and a bicarbonate in its C-lobe) and Yb<sub>C</sub>-hTF (*pink*) onto Fe<sub>C</sub>-hTF (*green*). (rmsd 1.612 and 0.363 Å, respectively) The structures are shown as ribbons. Introduction of different metal ion (Yb<sup>III</sup>) or anion (malonate) has only a minor effect on the secondary structure of the protein.



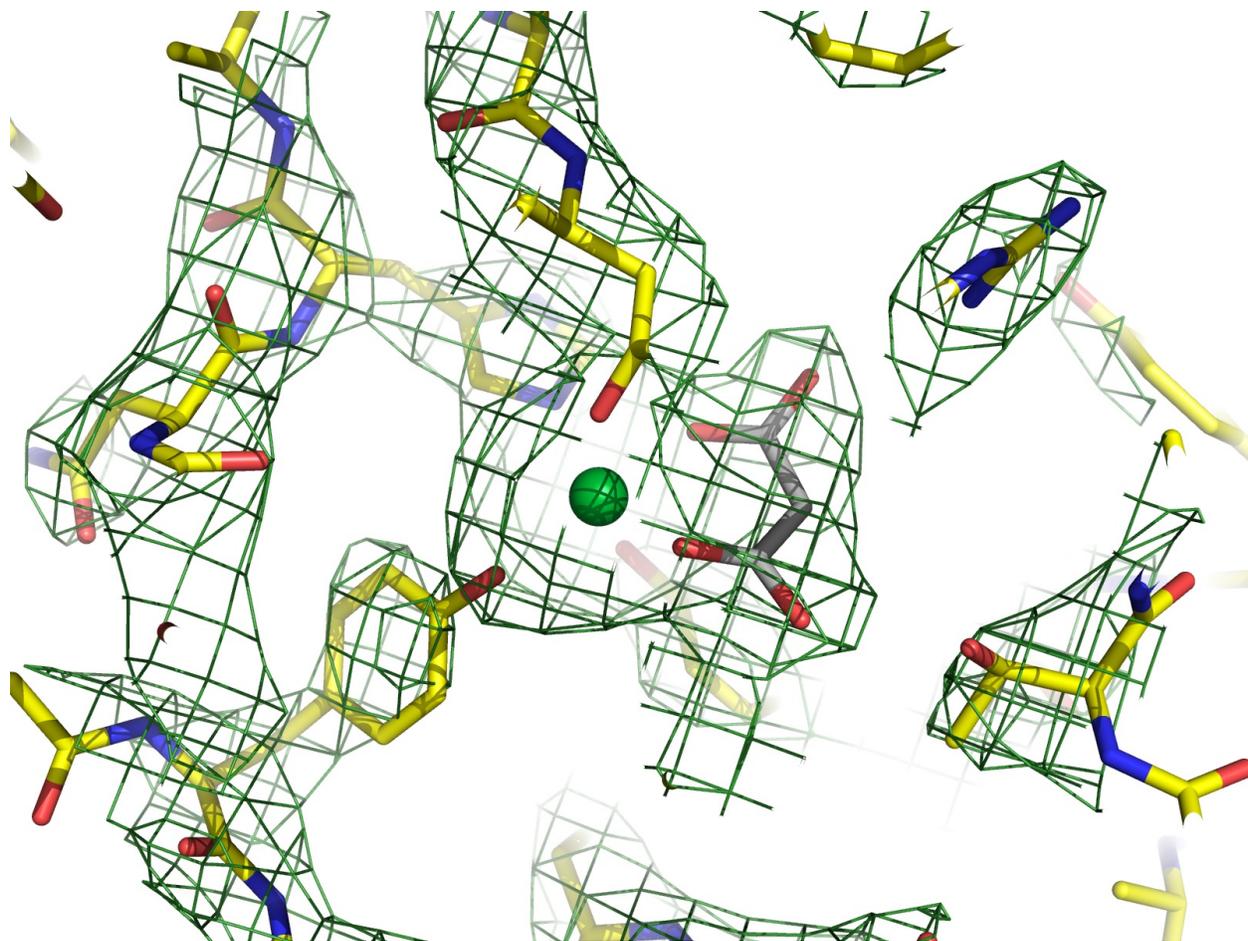
**Figure S3** The anomalous peak (contoured at  $5\sigma$ ) in Fe<sub>C</sub>-hTF before the addition of Fe to the map, shown as mesh. There is a dummy (grey ball) at Fe position.



**Figure S4** The  $2F_o-F_c$  map contoured at  $3.0\sigma$  (shown as mesh) near the metal-binding pocket of Fe<sub>C</sub>-hTF.



**Figure S5** The anomalous peak (contoured at  $5\sigma$ ) in molecule 1 of Yb<sub>C</sub>-hTF before the addition of Yb to the map, shown as mesh. There is a dummy (grey ball) at Yb position.



**Figure S6** The  $2F_o-F_c$  map contoured at  $3.0\sigma$  (shown as mesh) near the metal-binding pocket of Ybc-hTF (molecule 1).

### References

1. L. Yuan, P. Du, K. Wang and X. G. Yang, *J. Biol. Inorg. Chem.*, 2009, **14**, 1243-1251.
2. P. D. Adams, P. V. Afonine, G. Bunkoczi, V. B. Chen, I. W. Davis, N. Echols, J. J. Headd, L. W. Hung, G. J. Kapral, R. W. Grosse-Kunstleve, A. J. McCoy, N. W. Moriarty, R. Oeffner, R. J. Read, D. C. Richardson, J. S. Richardson, T. C. Terwilliger and P. H. Zwart, *Acta Crystallogr D Biol Crystallogr*, 2010, **66**, 213-221.
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