Supporting Information

Formation of [4Fe-4S] clusters in the mitochondrial iron-sulfur cluster assembly machinery

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Table S1. Parameters of the data-driven docking model of dimeric apo human ISCA2.

HADDOCK parameters for Cluster 1 (all)	**Values**
HADDOCK score	-83.1 +/- 1.9
Cluster size	200
RMSD from the overall lowest-energy structure (Å)	0.8 +/- 0.5
Van der Waals energy (kcal mol^{-1})	-44.8 +/- 2.4
Electrostatic energy (kcal mol^{-1})	-206.8 +/- 27.4
Desolvation energy (kcal mol^{-1})	2.8 +/- 5.9
Restraints violation energy (kcal mol^{-1})	3.0 +/- 1.47
Buried Surface Area ($Å^2$)	1240.9 +/- 70.5
Z-Score	0.0

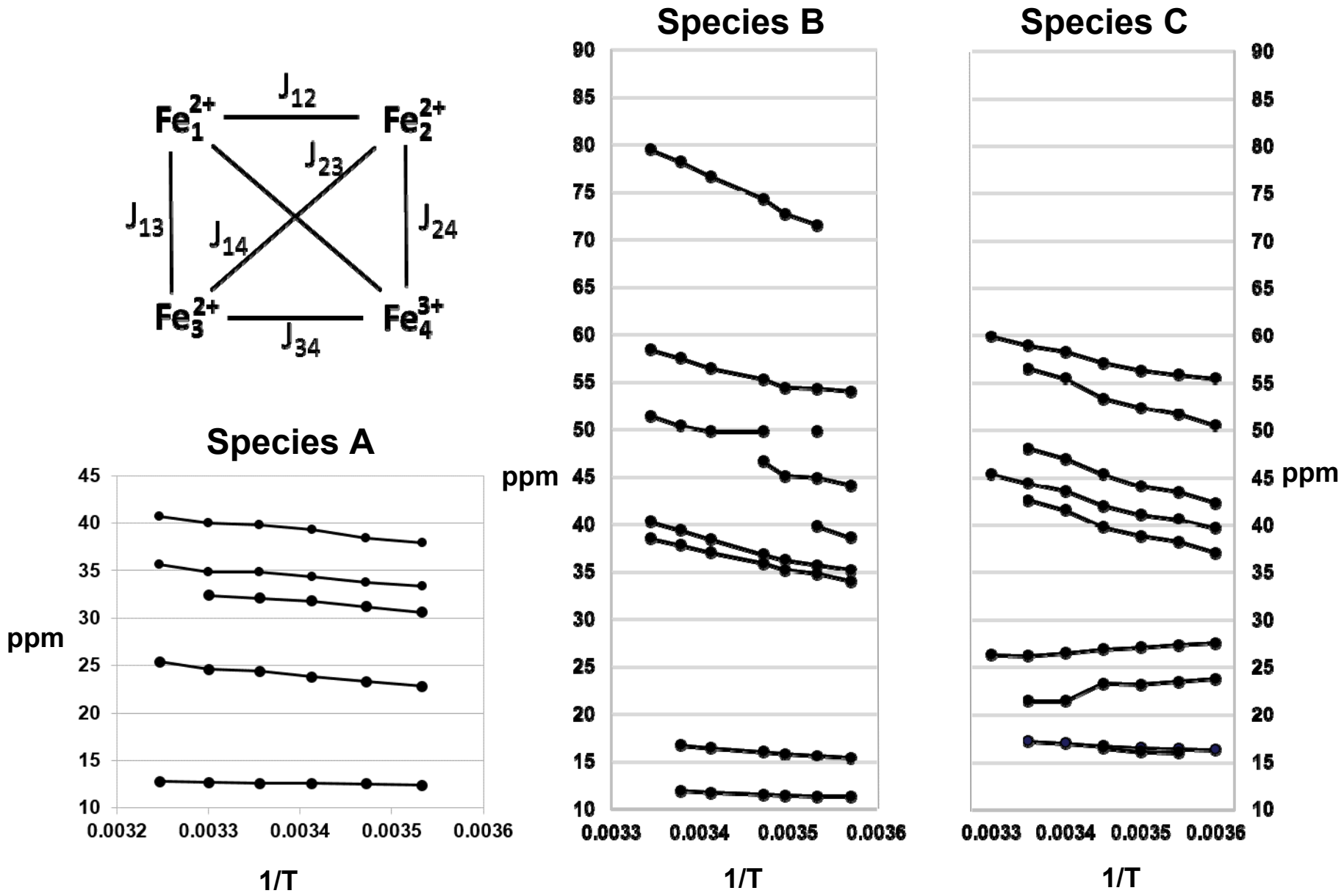

Figure S1. Temperature dependence of the chemical shifts for the hyperfine-shifted signals of the three different species (A,B,C) of chemically reconstituted [4Fe-4S] hISCA2. Experiments were recorded at 600 MHz, pH 7.0 in the temperature range of 280-308 K. A schematic representation of [4Fe-4S] cluster and its coupling scheme in the reduced state $[4Fe\text{-}4S]^{+}$ is also shown (upper left panel).

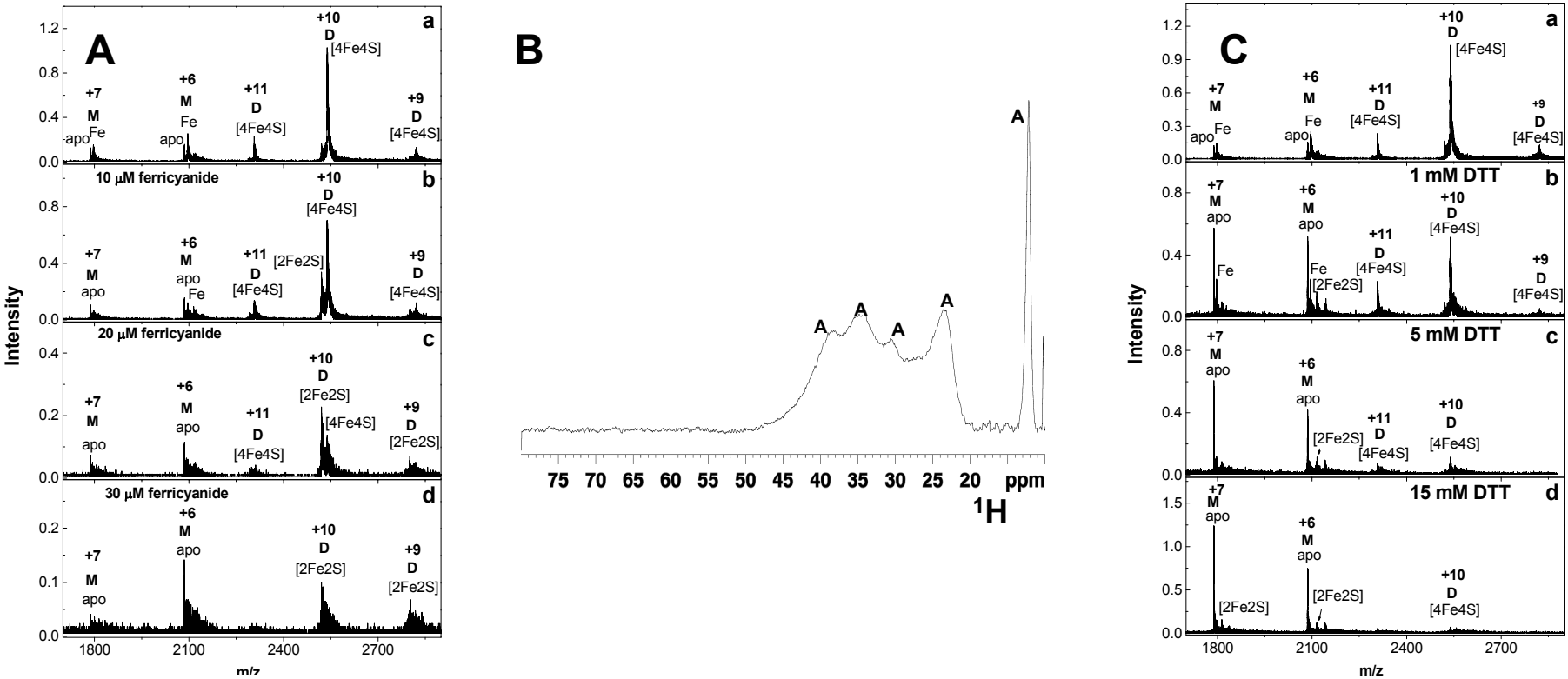

Figure S2. Changes in the redox conditions affect the nature of the cluster bound to hISCA2. (**A**) ESI-MS spectra of [4Fe-4S] hISCA2 in the presence of ferricyanide. Conditions: 20 mM ammonium acetate, pH 7.5, T = 25˚C. Chemically reconstituted 20 μM [4Fe-4S] hISCA2 in the absence (a) and in the presence of 10 μM ferricyanide (b); 20 μM ferricyanide (c) or 30 μM ferricyanide (d). +7 and +6 charge states are presented for apo and Fe hISCA2 monomer (M); +11, +10 and +9 charge states are presented for holo hISCA2 dimer (D). Metal or iron-sulfur cluster contents are indicated on the top of the peaks. (**B**) Paramagnetic 1D 1H NMR spectrum of chemically reconstituted [4Fe-4S] hISCA2 after the addition of 1 equivalent of ferricyanide in 50 mM phosphate buffer pH 7.0 at 600 MHz and 283 K. Signals labeled A arise from Hb and Ha from cysteine ligands coordinated to a $[2Fe\text{-}2S]^{2+}$ cluster. (**C**) ESI-MS spectra of [4Fe-4S] hISCA2 in the presence of DTT. Conditions: 20 mM ammonium acetate, pH 7.5, T = 25˚C. Chemically reconstituted 20 μM [4Fe-4S] hISCA2 in the absence (a) and in the presence of 1 mM DTT (b); 5 mM DTT (c) or 15 mM DTT (d). +7 and +6 charge states are presented for apo and Fe hISCA2 monomer (M); +11, +10 and +9 charge states are presented for holo hISCA2 dimer (D). Metal or iron-sulfur cluster contents are indicated on the top of the peaks.

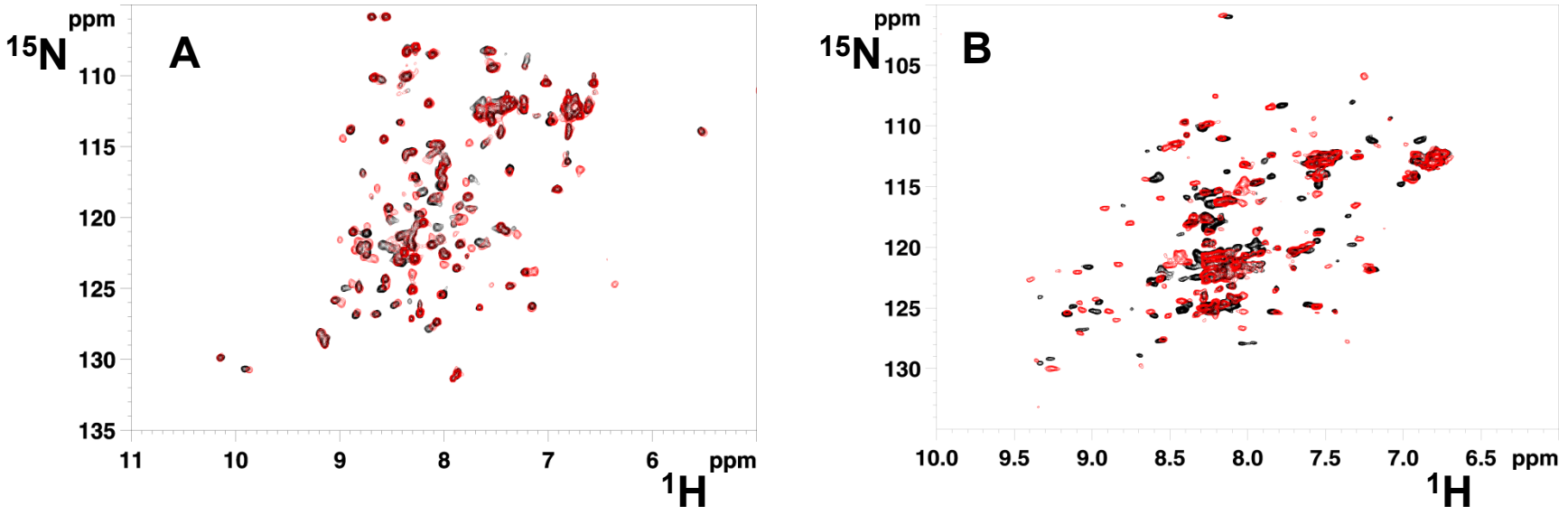

Figure S3. Monitoring the switching from homodimeric hISCA2 to heterodimeric hISCA1/hISCA2 complex by NMR. (**A**) Superimposition of ^{1}H-^{15}N HSQC spectra of ^{15}N-labelled apo hISCA2 free (black) and in a 1:1 complex with unlabelled apo hISCA1 (red). (**B**) Superimposition of ^{1}H-^{15}N HSQC spectra of ^{15}N-labelled apo hISCA1 free (black) and in a 1:1 complex with unlabelled apo hISCA2 (red).