Electronic Supplementary Information:

Monitoring Alkaline Transitions of Natural Isotopic Abundance Yeast Iso-1 Cytochrome c Using Trimethyllysine as Native NMR Probe

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Experimental

Sample preparation

Yeast iso-1 cyt c used in this work was bought from Sigma Aldrich. The protein sample was purified twice with a cation exchange column and a size-exclusion chromatography column orderly. Since wild type ferric yeast iso-1 cyt c was prone to precipitate at alkaline pH, excess sodium ascorbate was added in the buffer solution to maintain the protein in its reduced form during the process of purification. After that, 1.5 mM yeast iso-1 cyt c was exchanged in 10 mM PB buffer of pH 7.0, and concentration of cyt c was then determined at 205 nm using extinction coefficient of 420760 mM^{-1} cm^{-1}. In the pH titration experiments, the pH of the protein solution was adjusted by addition of 0.1 M NaOH solution, and measured after 15 minutes shake to reach a stable condition.

NMR experiments

All NMR experiments were conducted on a Bruker AV600 spectrometer equipped with 5 mm z-gradient BBI probe at 298 K. The pulse sequence used in this work is a standard echo-antiecho mode HSQC. The recycle delay is 1 s for all measurements. In the ^{1}H dimension, 2048 data points were acquired for 6009 Hz and Fourier transformed into 4096 points in the frequency domain with a 90 degree shifted Qsine windows function. In the ^{14}N dimension, 30 (Fig. 2) or 16 (Fig. S1) time increments were recorded for 5202 Hz sweep width and Fourier transformed into 256 points with a 90 degrees shifted Qsine windows function. 512 transients were averaged for each time increment. The J-coupling evolution delay was optimized to 30 ms. The gradient pulse ratio was 80 : 5.78, which equals to the resonance frequency ratio of ^{1}H (600.13 MHz) to ^{14}N (43.35 MHz). No ^{14}N decoupling was applied during data acquisition, since the heteronuclear coupling constant was only 0.6 Hz.

Figures

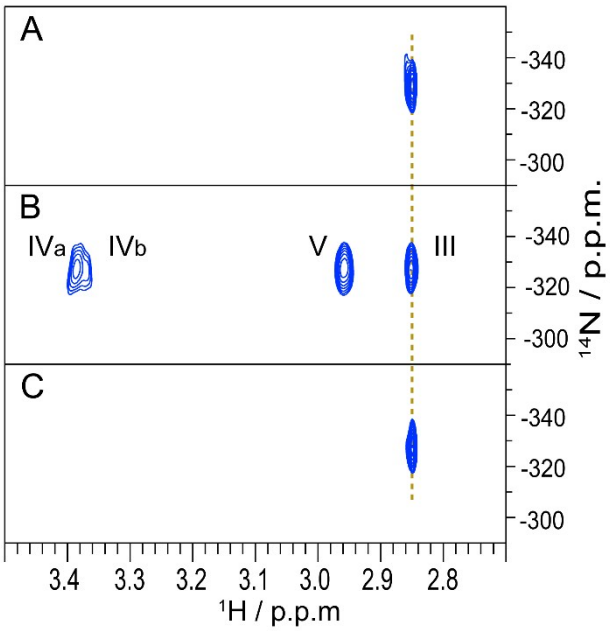

Figure S1. 2D ^{1}H-^{14}N HSQC spectra of yeast iso-1 cytochrome c under different conditions. The experiments were performed at 298 K. A is from yeast iso-1 cytochrome c at pH 10.2 with excess sodium ascorbate, B is from sample A with the addition of 0.7 mM potassium ferricyanide, C is from sample B with the addition of excess sodium ascorbate. Different conformers are denoted (III, V, IV_a and IV_b).

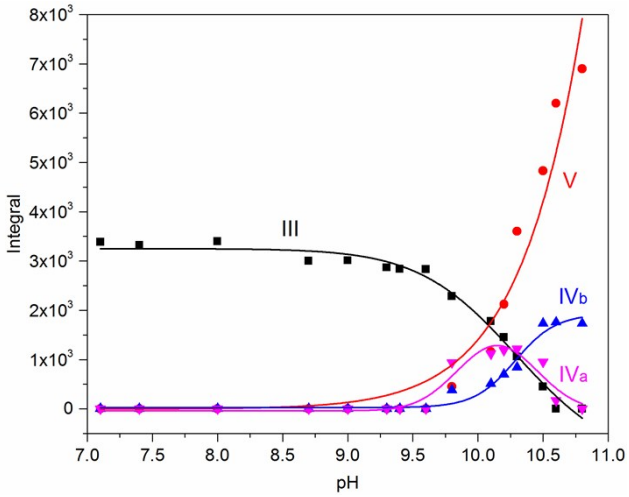

Figure S2. Dependence of the integrals of different resonances in 2D ^{1}H-^{14}N HSQC spectra of yeast iso-1 cytochrome c on pH. The experiments were performed at 298 K. Different conformers are denoted (III, V, IV_a and IV_b).

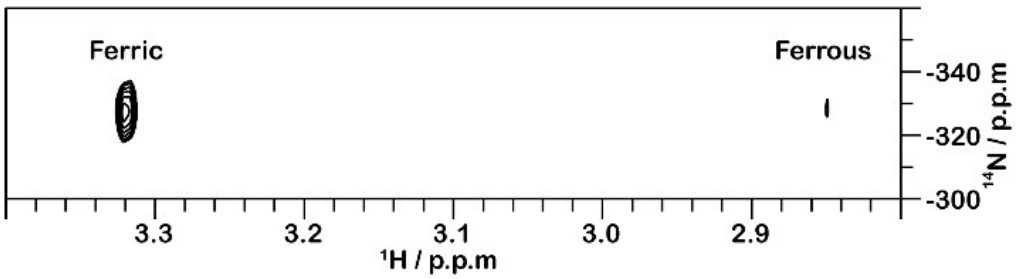

Figure S3. 2D ^{1}H-^{14}N HSQC spectra of ferric yeast iso-1 cytochrome c pH 7.1. The experiment was performed at 298K, The weak signal from ferrous yeast iso-1 cytochrome c might be due to the auto-reduce of cytochrome c.