Using Hyperfine EPR Spectroscopy to define the Proton-Coupled Electron Transfer Reaction at Fe-S Cluster N2 in Respiratory Complex I

Supporting information

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1. Potentiometric titrations of bovine complex I as a function of pH

Supplementary Figure 1. EPR-based potentiometric titrations of cluster N2 in *B. taurus* complex I as function of pH. (A) An example of X-band CW EPR data from a redox titration (pH 6.0). The potentials of the samples are shown vs. the standard hydrogen electrode. Measurements were performed at 25 K, 2 mW microwave power, 100 kHz modulation frequency and 0.7 mT modulation amplitude. Simulations of the experimental spectra are overlaid as red dotted lines. (B) Integral of the simulated N2 EPR signal (i.e. of the red dotted spectra in A) as a function of reduction potential (red: pH 5.0, blue: pH 6.0, green: pH 7.0, magenta: pH 8.0 and cyan: pH 9.0) used to determine the reduction potential of N2 at different pH values. The experimental data points (*) at each pH were fitted to the one-electron Nernst equation (solid lines).

2. Temperature-independent pH buffers (pH 6 – 9)

The pH of many buffer solutions is known to change substantially (more than 1 pH unit) between room and cryogenic temperatures. The effect is thought to arise from the unequal distribution of cations and anions in ice, causing their positive and negative charges to be neutralized by highly mobile OH\(^-\) and H\(^+\) ions during freezing. Such pH changes can lead to erroneous reduction potentials of pH-sensitive redox couples, in our case the Fe-S cluster N2 of complex I (see ref. 4). Our temperature-independent pH buffer system was inspired by the work of Sieracki et al. who formulated a pH 7.0 buffer by exploiting the fact that the pH of HEPES increases as the temperature is lowered while the pH of sodium phosphate decreases.

The compositions of the buffers designed here are summarized in Supplementary Table 1. The buffer composition at pH 7.0 differs slightly from that reported in references 4 and 5 because we detected a small effect from the addition of NaCl. In contrast, and in line with the findings in ref. 2, changing the amount of glycerol in the buffer (30% for titrations, Supplementary Figure 1, or 50% for buffer pH characterization, Supplementary Figure 2) had no significant effect. The addition of 50% (v/v) glycerol enabled the formation of a glass upon freezing and thus colour changes could be observed more accurately during pH-indicator tests (see below).
**Supplementary Table 1. Compositions of buffers at different pH values.** Buffer concentrations were 100 mM (Na$_2$HPO$_4$/HEPES) and 50 mM (pyrophosphate/bicine). In addition, all buffers contained 150 mM NaCl and 30% or 50% (v/v) glycerol for titrations and pH indicator/fluorescence measurements, respectively.

<table>
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<th>7</th>
<th>7.5</th>
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<th>pH</th>
<th>8</th>
<th>8.5</th>
<th>9</th>
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<td>46 %</td>
<td>72 %</td>
</tr>
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<td>Bicine</td>
<td>80 %</td>
<td>54 %</td>
<td>28 %</td>
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</table>

In order to establish the buffer compositions reported in Supplementary Table 1, we used pH indicators$^{1,2,5}$ and fluorescence spectroscopy.$^6$

The pH indicators (bromocresol green, bromophenol blue, bromothymol blue, cresol red, methyl red, thymol blue, xylene blue, all from Sigma-Aldrich) were chosen to cover the range from pH 3.0 to 10.0. Stock solutions of pH indicators at 1% (w/v) were diluted in the buffer under investigation to a final concentration of 0.01% (w/v). The pH of the buffer solution was measured using a micro pH electrode (Orion™ PerpHecT™ ROSS™ Combination) and was unaffected by addition of the pH indicators. The color after flash-freezing in liquid nitrogen was compared by eye to that of reference solutions containing the pH indicator at room temperature. The composition of each pH-independent buffer was deduced from the independent use of at least two different pH indicators.

Comparison of fluorescence measurements at room temperature and 77 K enabled a more quantitative assessment of the suitability of different buffer compositions. The fluorescent dye fluorescein has a pH-dependent excitation spectrum between pH 5 and 9.$^7$ The maximum absorption of the fluorescein anion (protonated form) is around 500 nm while the dianion (deprotonated form) has a weaker absorption spectrum with two maxima around 455 and 478 nm.$^8$ Excitation spectra at room temperature (Supplementary Figure 2A) and 77 K (Supplementary Figure 2B) were recorded at an emission wavelength of 515 nm. The ratios between the intensities at 498 nm (deprotonated form) and 456 nm (protonated form) are plotted in Supplementary Figure 2C and are the same at both temperatures. A linear relationship between the HEPES:phosphate composition and pH became apparent between pH 6 and 8 using both pH indicators and fluorescence measurements (Supplementary Figure 2D).
Supplementary Figure 2. Fluorescence measurements used to verify the temperature independence of buffer pH. (A,B) Excitation spectra of fluorescein at room temperature (A) and at 77 K (B) in buffer mixtures at pH 6.0 (black), pH 7.0 (purple) and pH 8.0 (magenta), normalized to their isosbestic point (478 nm). (C) Ratiometric measurements of fluorescein ($I_{498}/I_{456}$) in different temperature-independent pH buffers at room temperature (black) and two measurements at 77 K (blue and red). (D) Percentage of sodium phosphate as function of the pH obtained using pH indicators (black) and fluorescein (red).

The composition of the second buffer system for pH 8 to 9 (sodium pyrophosphate and bicine) was established using pH indicators only, given their consistency with the fluorescence measurements in Supplementary Figure 2D. The reduction potential of N2 at pH 8.0 was determined using both buffer compositions and no significant difference was observed, showing that the buffer composition itself has no effect on the reduction potential.
3. Echo-detected field-swept spectra of *B. taurus* complex I

**Supplementary Figure 3.** X-band echo-detected field-swept spectra of N2 in *B. taurus* complex I. pH 6.0 (–167 mV), pH 9.0 (–145 mV) and pD 6.0 (–160 mV) at 10 K. Spectra were recorded with the Hahn echo sequence: \( \pi/2 - \tau - \pi - \tau - \text{echo} \) (\( \pi/2 = 16 \text{ ns} \), \( \pi = 32 \text{ ns} \), \( \tau = 200 \text{ ns} \), shot repetition time 1 ms). Arrows indicate the field positions used to record HYSCORE spectra (\( g = 2.051, 1.951, 1.921 \)). The \( g \) values of cluster N2 (\( g_x = 1.921, g_y = 1.927, g_z = 2.054 \)) were found to be unaltered by pH or deuterium substitution, showing that the valence delocalisation is not significantly affected. We note that N2 is the sole paramagnetic Fe-S cluster present at these potentials; the EPR signatures of the Fe-S clusters observed in fully-reduced bovine complex I are well-established\(^9,10\) and readily distinguished from one another.
4. HYSCORE spectra of *B. taurus* complex I

![HYSCORE spectra](image)

**Supplementary Figure 4.** Complete HYSCORE spectra of N2 of *B. taurus* complex I. pH 6.0, –167 mV (A), pH 9.0, –145 mV (B) and pD 6.0, –160 mV (C) at $g_\perp (g = 1.921)$ at 10 K. Spectra were recorded with the 4-pulse sequence: $\pi/2-\tau-\pi/2-\pi/2-\tau$ echo with $\pi/2 = 16$ ns, $\pi = 16$ ns, $\tau = 116$ ns. The intensity of the stimulated echo was measured as function of $t_1$ and $t_2$ with a time increment of 16 ns (A and B) or 24 ns (C). Other experimental conditions: microwave frequency: 9.4224 GHz (A), 9.4549 GHz (B) and 9.4551 GHz (C); shot repetition time 1 ms; acquisition time ca. 14 hrs per spectrum.

The peaks centered along the diagonal in the (+,+)-quadrant at ca. 4 MHz clearly visible in A and B are assigned to protein backbone amide $^{14}\text{N}$ given their characteristic quadrupole parameters ($e^2qQ/h = 3.5$ MHz, $\eta = 0.38$, simulations not shown). The $^{14}\text{N}$ signals in the (+,+)-quadrant in C are masked by the deuterium matrix signal centred at ca. 2.5 MHz that is very large because the blind spot in this experiment was deliberately placed on the $^1\text{H}$ Larmor frequency. The signals observed in the (–,+)-quadrant in A and B must arise from $^{14}\text{N}$ (because $^1\text{H}$ and $^{14}\text{N}$ are the only two magnetic nuclei in detectable quantities in our samples) but cannot currently be assigned. However, we note that these signals were unaffected by pH (frequency coordinates were also found to be independent of measurement conditions and lie neither on the single- or double-quantum $^{14}\text{N}$ lines) and are therefore unlikely to originate from the $^{14}\text{N}$ bearing the exchangeable proton. The maximum intensity peak of the (–,+)-$^{14}\text{N}$ signal at [–0.8, 0.8] MHz was used to obtain difference spectra because this peak was unchanged even in the presence of $^2\text{H}$ cross-suppression effects. We found that choosing this reference signal yielded the best quality difference spectra, but note that other reference points (e.g. the maximum intensity $^{14}\text{N}$ signal in the (+,+)-quadrant) gave rise to similar difference spectra.
Supplementary Figure 5. HYSCORE spectra of N2 of *B. taurus* complex I (proton region), pH 6.0, –167 mV (A), pH 9.0, –145 mV (B) and pD 6.0, –160 mV (C), $^1$H region at $g_{\perp} (g = 1.921)$ at 10 K. Spectra were recorded with the 4-pulse sequence: $\pi/2 - \tau - \pi/2 - t_1 - \pi - t_2 - \pi/2 - \tau$–echo with $\pi/2 = 16$ ns, $\pi = 16$ ns, $\tau = 116$ ns, $t_1 = t_2 = 80$ ns. The intensity of the stimulated echo was measured as function of $t_1$ and $t_2$ with a time increment of 16 ns. Top: contour representation. Bottom: surface representation. Other experimental conditions: microwave frequency: 9.4224 GHz (A), 9.4549 GHz (B) and 9.4551 GHz (C); shot repetition time 1 ms; acquisition time *ca.* 14 hrs per spectrum.

Supplementary Figure 6. HYSCORE [pH 6.0 – pD 6.0] difference spectra of N2 in *B. taurus* complex I at different field positions. See Supplementary Figure 3 for an indication of the field position on the corresponding field-swept spectra. HYSCORE spectra were measured and difference spectra obtained as described in the Experimental Section. Other experimental parameters: $\tau = 116$ ns ($g = 1.921$ and $g = 1.951$), $\tau = 124$ ns ($g = 2.051$).
Supplementary Figure 7. SMART HYSCORE spectrum of N2 in B. taurus complex I at pH 6, 10 K. Experimental conditions: 4 pulse sequence HTA–t1–π–t2–HTA–τ–π–τ–echo with π = 32 ns, HTA = 40 ns, τ = 96 ns, t1 = t2 = 96 ns; microwave frequency 9.4293 GHz; shot repetition time 1 ms; acquisition time ca. 60 hrs. The intensity of the stimulated echo was measured as function of t1 and t2 with a time increment of 8 ns. Because the experiment has no blind spots, the 1H protons at the Larmor frequency (~ 15 MHz) are strong and "starring" is observed.

5. HYSCORE analysis

As a first approximation of the hyperfine coupling of the exchangeable protons revealed by the subtractions (Supplementary Figure 6), a contour line shape analysis of the cross peaks was performed as described previously.15,16 Briefly, the two frequencies from a nuclear spin with I = ½ in the electron spin manifolds can be written for an arbitrary Zeeman frequency v1 using the equation:

\[ v_{\alpha(\beta)} = \left[ \left( \nu_I \pm A \right)^2 + B^2/4 \right]^{1/2}, \tag{1} \]

where A and B are the secular and nonsecular part of the hyperfine coupling, respectively. Equation (1) can be used to recalculate \( v_\alpha \) and \( v_\beta \) corresponding to a different \( \nu_I \) and are derived from Equation (1) as follows:

\[ A = \frac{\nu_\alpha^2 - \nu_\beta^2}{2\nu_I}, \tag{2} \]

\[ B^2 = 2 \left[ \nu_\alpha^2 + \nu_\beta^2 - \nu_I^2 - \frac{\left( \nu_\alpha^2 - \nu_\beta^2 \right)^2}{8\nu_I^2} \right]. \tag{3} \]

For a nuclear spin \( I = \frac{1}{2} \) coupled to an electron spin \( S = \frac{1}{2} \), the contour line shape of an axial hyperfine coupling is transformed into a straight line when plotted as \( v_\alpha^2 \) versus \( v_\beta^2 \), described by17:

\[ v_\alpha^2 = Q_\alpha v_\beta^2 - G_\alpha, \tag{4} \]

where the slope \( Q_\alpha \) is

\[ Q_\alpha = \frac{T + 2A_{iso}}{T + 2A_{iso} + 4\nu_I}, \tag{5} \]

and the intercept \( G_\alpha \) is

\[ G_\alpha = 2\nu_I \left( \frac{4\nu_I^2 - A_{iso}^2 + 2T^2 - A_{iso}T}{T + 2A_{iso} + 4\nu_I} \right). \tag{6} \]

The slope and the intercept can be used to extract two sets of \( A_{iso} \) and \( T \) (isotropic and axial hyperfine coupling respectively) with the same value of \( |2A_{iso} + T| \) and interchanged \( |A_{iso}| = |A_{iso} - T| \) and \( |A_{iso}| = |A_{iso} + 2T| \).

Frequencies \((\nu_\alpha, \nu_\beta)\) were taken along the uppermost contour for each ridge in each HYSCORE difference spectrum in Supplementary Figure 6. To enable plotting all of the data measured at different microwave frequencies on the same graph (Supplementary Figure 8), \((\nu_\alpha, \nu_\beta)\) frequencies were recalculated for a common Zeeman frequency \( \nu_I \) (chosen arbitrarily to be 14.5 MHz) for each field.
position using Equations (1), (2) and (3). Data points were plotted as $v_α^2$ versus $v_β^2$ and the two sets of cross peaks visible in all spectra in Supplementary Figure 6 give rise to two separate straight lines (fitted using linear regression), Supplementary Figure 8. $A_{iso}$ and $T$ were calculated from the slopes and the intercepts using Equations (5) and (6) and are given in Supplementary Table 2.

Supplementary Figure 8. Cross-peak analysis to obtain an estimate of the hyperfine coupling parameters for H1 and H2. Plots of cross peaks 1 (green) and 2 (red) from subtracted HYSCORE (Figure 3 and Supplementary Figure 6) for N2 in B. taurus complex I ([pH 6.0 – pH 9.0] and [pH 6.0 – pD 6.0]) in the $v_α^2$ versus $v_β^2$ coordinates system. Data points were normalised to the same Zeeman frequency (14.5 MHz). The straight lines show the linear fit of plotted data points (parameters shown in Supplementary Table 2).

Supplementary Table 2. Parameters obtained from the contour line-shape analysis of the HYSCORE difference spectra (Figures S6 and S8). $Q_α$ represents the slopes and $G_α$ the intercepts of the lines.

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<td>$Q_α$</td>
<td>$Q_α$</td>
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<td>$-0.946 ± 0.070$</td>
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<td>$G_α$ (MHz$^2$)</td>
<td>$G_α$ (MHz$^2$)</td>
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<td>$421 ± 7$</td>
<td>$414 ± 12$</td>
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<tr>
<td>$A_{iso}$ (MHz)</td>
<td>$A_{iso}$ (MHz)</td>
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<tr>
<td>$-0.6 ± 1.3$</td>
<td>$-0.4 ± 2.5$</td>
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<td>$4.8 ± 0.5$</td>
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<td>$T$ (MHz)</td>
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<td>$-5.4 ± 2.0$</td>
<td>$-2.3 ± 2.0$</td>
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</table>

The best-fit simulations shown in Supplementary Figure 8 and Supplementary Table 3 were obtained as follows. $^1$H HYSCORE simulations were generated with $A_{iso}$, $T$ and the Euler angle $β$ as variables, keeping the values of $A_{iso}$ and $T$ within the boundaries of the error margins reported in Supplementary Table 2 and $β$ was varied between 0° and 90°. Root-mean-square error (RMSE) values were calculated by subtracting the experimental spectra from the simulated spectra for each set of parameters. The lowest resulting total RMSE values were then used to identify the best fit. The parameters thus determined for the two protons (Supplementary Table 3) were then used to fit the deuteron coupling (Supplementary Figure 8D), scaling them by the gyromagnetic ratio and introducing a quadrupole coupling. Because of the time taken to compute the SMART HYSCORE simulations, SMART HYSCORE spectra were not included in the fitting program described above. However, as shown in Supplementary Figure 8E, the parameters determined for H1 and H2 result in a satisfactory fit of the experimental data.
Supplementary Figure 9. Summary of best-fit simulations (red) of all experimental data (black). (A-C) HYSCORE [pH 6.0 – pD 6.0] difference spectra (A: $g = 1.921$, B: $g = 1.951$, C: $g = 2.041$, see Supplementary Figure 6 for further experimental conditions). (D) HYSCORE spectrum of the deuterium region of the pD 6.0 sample measured at $g \perp$. Experimental conditions: 10 K, $\pi/2 = 16$ ns, $\pi = 16$ ns, $\tau = 420$ ns, $t_1 = t_2 = 80$ ns, time increment: 24 ns, shot repetition time: 1 ms. (E) SMART HYSCORE of the pH sample at $g \perp$. Experimental conditions: 10 K, $\pi = 32$, HTA = 40 ns, $\tau = 96$ ns, $t_1 = t_2 = 96$ ns, time increment: 8 ns, shot repetition time: 1 ms. All simulations were performed using Easyspin$^{18,19}$ with the parameters given in Supplementary Table 3. SMART HYSCORE spectra were simulated by defining a custom pulse sequence (HTA–$t_1$–$\pi$–$t_2$–HTA–$\pi$–$\tau$–$\pi$–echo).

Supplementary Table 3. Parameters used for the best-fit simulations shown in Supplementary Figure 9. $A = A_{iso} + [-T, -T, +2T] = A_{iso} + [T_\perp, T_\parallel, T_\perp]$. The quadruple couplings derived for D1 and D2 compare favourably with other hydrogen-bonded deuterons to Fe-S clusters.$^{20}$

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<tr>
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<th>DEUTERIUM 1</th>
<th>DEUTERIUM 2</th>
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<td>n/a</td>
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<td>0.10 ± 0.07</td>
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Supplementary Figure 10. Influence of the β angles of protons H1 and H2 on the SMART HYSCORE simulations. Experimental spectra are in black and simulations are in red. Experimental conditions: 10 K, π = 32, HTA = 40 ns, τ = 96 ns, t1 = t2 = 96 ns, time increment: 8 ns, shot repetition time: 1 ms. Simulations were performed using Easyspin\(^{19}\) with the parameters given in Supplementary Table 3, with the exception of the β angles that are indicated on the spectra.
6. Echo-detected field-swept spectra of *Y. lipolytica* complex I

Supplementary Figure 11. Echo-detected field-swept spectra (EDFS) of NADH-reduced complex I from *Y. lipolytica* wild-type (wt) and H226M mutant at pH 6.5 at 14 K. (Top) EDFS recorded on wt with the Hahn echo sequence $\pi/2-\tau-\pi-\tau$–echo ($\pi/2 = 16$ ns, $\pi = 32$, $\tau = 200$ ns, shot repetition time: 1 ms), showing the presence of both N1b and N2. (Middle) REFINE EDFS recorded on wt with the sequence $\pi-T_\tau-\pi/2-\tau-\pi-\tau$–echo ($\pi/2 = 16$ ns, $\pi = 32$ ns, $T_\tau = 62$ $\mu$s and $\tau = 200$ ns, shot repetition time: 1 ms), showing the presence of only N2. (Bottom) REFINE EDFS recorded the H226M mutant with the sequence $\pi-T_\tau-\pi/2-\tau-\pi-\tau$–echo ($\pi/2 = 16$ ns, $\pi = 32$ ns, $T_\tau = 62$ $\mu$s and $\tau = 200$ ns, shot repetition time: 1 ms), showing the shift in $g_{xy}$ (N2). As noted previously, the $g$ values of cluster N2 ($g_x = 1.921$, $g_y = 1.929$, $g_z = 2.054$ for wt and $g_x = 1.928$, $g_y = 1.936$, $g_z = 2.054$ for the H226M variant, as determined in this study) vary upon substitution of H226, suggesting that the electronic structure and valence delocalization of the N2 cluster are perturbed. Despite these differences, the similarity of the H1 coupling observed in the difference spectra of Yl [wt–H226M], Bt [pH6–pH9] and Bt [pH6–pD6] enabled us to make an unambiguous assignment for H1.

7. Estimation of the hyperfine coupling anisotropy based on structural information

In order to ascertain whether the hydrogen-bonding interaction between H1 and cluster N2 predicted from our spectroscopic experiments is consistent with structural data, we calculated the anisotropic part of the hyperfine coupling using currently available structural models.

$T$ can be estimated using the point-dipole approximation:

$$T = \mu_0e^2\beta N\sum_{ij} k_j \frac{3n_j r_j^{3n_j - 1}}{r_j^3},$$

where $k_j$ is the spin projection factor (SPF) of the nucleus $j$, $n_j$ and $r_j$ are the unit row vector and distance between the proton and the nucleus $j$ and $\dagger$ indicates the matrix transpose.

The reduced N2 cluster $[4Fe-4S]^+$ contains delocalized valence electrons distributed between a Fe$^{2+}$–Fe$^{2+}$ pair and a mixed-valence Fe$^{2.5+}$–Fe$^{2.5+}$ pair. This delocalisation is reflected in the SPFs and we have previously shown that the Fe-S clusters in complex I are well-described by $k(Fe^{2+}) = -0.67$ and $k(Fe^{2.5+}) = +1.17$.10 Dipolar couplings between the Fe atoms in N2 and the histidine H1 proton were
calculated for the six possible arrangements of SPFs using Equation (7). Distances ($r_i$) and unit row vectors ($n_i$) were taken based on atomic coordinates from three different structures: *B. taurus* complex I (PDB ID: 5LDW, 4.27 Å), T. thermophilus complex I (PDB ID: 4HEA, 3.3 Å), and a *B. taurus* complex I model based on the 3.3 Å structure of the hydrophilic domain of *T. thermophilus* complex I. The sets of dipolar couplings obtained are shown in Supplementary Table 4. Possible hydrogen-bonding interactions (including distances) are also included in Supplementary Table 4.

**Supplementary Table 4 – Calculation of dipolar hyperfine coupling (T) based on SPFs and the point dipole model.** The H1 proton of the conserved histidine residue was added using Pymol. Possible H–S hydrogen bonds (Å) are indicated with dashed lines. Donor-acceptor (i.e. N–H…S Cys) distances (not indicated) are 4.0 Å, 2.9 Å and 3.7 Å for *B. taurus* (5LDW), *T. thermophilus* (4HEA) and *B. taurus* (model), respectively.

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<td>-1.1</td>
<td>-2.0</td>
<td>8.7</td>
<td>3.9</td>
<td>5.0</td>
<td>6.2</td>
<td>-3.6</td>
<td>-2.3</td>
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</tr>
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<tr>
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</tr>
</tbody>
</table>

Based on these estimates of $T$, the anisotropy of H1 observed experimentally (Supplementary Table 3) appears plausible. The variation observed between the structures is likely testimony of the medium resolution of currently available density maps. We note that the largest anisotropy (most consistent with the experimental data) is observed with Fe4 bearing a positive SPF, in agreement with our previous assignment of the SPFs in cluster N2. The structure of *Y. lipolytica* complex I was not included in Supplementary Table 4 because the histidine residue was not resolved in the electron density map.
8. *Y. lipolytica* complex I activity measurements

**Supplementary Figure 12. Comparison of the catalytic activity of wild-type and H226M *Y. lipolytica* complex I as a function of pH.** NADH:decyliqueinone oxidoreduction was measured at 32 °C in 100 mM MES, 100 mM MOPS, 100 mM Tris, adjusted to pH with KOH or H2SO4 with 200 μM NADH, 200 μM decyliqueinone and 0.15% asolectin/CHAPS. Values were normalized to the maximum rates observed at pH 7.54 (wild-type 26.4 ± 2.0 μmol min⁻¹ mg⁻¹, H226M 21.0 ± 0.3 μmol min⁻¹ mg⁻¹).

Proteoliposomes containing *Y. lipolytica* complex I and the alternative oxidase from *Trypanosoma brucei* were prepared as described in Jones et al.²⁵ with 0.2 mg *Y. lipolytica* complex I (either wild-type or H226M) and 100 nmol of Q₁₀ to ensure a high and non-rate limiting Q₁₀ concentration. They were assayed in 200 μM NADH at 32 °C in 10 mM Tris-SO₄ (pH 7.5) and 50 mM KCl in the presence of 15 μg/mL alamethicin. The rates observed were 44.3 ± 1.5 μmol min⁻¹ mg⁻¹ (wild-type complex I) and 28.9 ± 2.3 μmol min⁻¹ mg⁻¹ (H226M variant).

9. References


51, L7.


