Supporting Information

Hyperfine and Nuclear Quadrupole Tensors of Nitrogen Donors in the QA Site of Bacterial Reaction Centers: Correlation of the Histidine Nδ Tensors with Hydrogen Bond Strength

Alexander T. Taguchi,† Patrick J. O’Malley,*& Colin A. Wraight,*,†§ and Sergei A. Dikanov*‡

†Center for Biophysics and Computational Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, United States
&School of Chemistry, The University of Manchester, Manchester M13 9PL, U.K.
§Department of Biochemistry, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, United States
‡Department of Veterinary Clinical Medicine, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, United States

* To whom correspondence should be addressed: S.A.D.: email, dikanov@illinois.edu; phone, (217) 300-2209. C.A.W.: email, cwraight@life.illinois.edu; phone, (217) 333-3245. P.J.O.: email, patrick.o’malley@manchester.ac.uk; phone, 00441612004536.
Table S1. Simulated and Experimental Peak Maxima of Q-band $^{15}$N HYSCORE$^a$

|         | $g_x/g_y$ Sim Max (MHz)$^b$ | $g_y$ Exp Max (MHz)$^c$ | $g_z$ Sim Max (MHz)$^d$ | $g_z$ Exp Max (MHz)$^e$ |
|---------|----------------------------|--------------------------|--------------------------|--------------------------|
| N$_8$ His-M219 | (3.58, 6.90)               | (3.59, 6.92)             | (3.58, 6.90)             | (3.59, 6.92)             |
| N$_p$ Ala-M260 | (3.58, 6.90)               | (3.59, 6.92)             | (3.58, 6.90)             | (3.59, 6.92)             |

$^a$His-M219 N$_8$ and Ala-M260 N$_p$ are unresolved in the spectra and therefore have the same peak maxima. No resolvable differences were observed in peak maxima between orientations in-plane with the $g_x$ and $g_y$ axes and along the unique $g_z$ axis;

$^b$Peak maxima from simulations; $^c$Peak maxima from experimental spectra (Figure 5).

Table S2. Simulated and Experimental Peak Maxima of Q-band $^{14}$N HYSCORE

|         | $g_x/g_y$ Sim Max (MHz)$^a$ | $g_y$ Exp Max (MHz)$^b$ | $g_z$ Sim Max (MHz)$^c$ | $g_z$ Exp Max (MHz)$^b$ |
|---------|----------------------------|--------------------------|--------------------------|--------------------------|
| N$_8$ His-M219 | (10.09, 5.08)              | (10.20, 5.01)            | (10.09, 5.08)            | (9.95, 5.25)             |
| N$_p$ Ala-M260 | (10.94, 5.08)              | (10.88, 5.11)            | (10.87, 5.21)            | (10.78, 5.20)            |

$^a$Peak maxima from simulation of 1 and 2; $^b$Peak maxima of 1 and 2 from experimental spectra (Figure 7).

Figure S1. Simulation of the Q-band CW spectrum for estimating the contribution of EPR broadening to the excitation bandwidth. Shown are the first derivative of the field swept 2-pulse echo from Figure 3 (blue), the CW spectrum (red), and the CW simulation (dotted black). The feature at 1237 mT in the blue trace is from the cavity. The field swept 2-pulse echo was not used for simulations, because relaxation rates were found to change with field position. Experimental parameters: 34.631 GHz, 0.2 mT modulation amplitude, 90 K
Figure S2. Q-band $^{15}\text{N}$ Mims ENDOR spectrum of SQ$_A$. Only a single splitting of $\sim$3.2 MHz is observed. Experimental parameters: $\pi/2$-pulse = 32 ns, time between first and second pulses $\tau = 200$ ns, RF $\pi$-pulse length = 70 $\mu$s, microwave frequency 34.222 GHz, temperature 80 K.

Figure S3. Q-band $^{15}\text{N}$ Davies ENDOR simulations of SQ$_A$ with His-M219 N$_8$ assigned the stronger of the two isotropic hyperfine couplings. Simulations failed to reproduce the experimentally observed narrower peak width at $g_X$ than at $g_Z$. Experimental parameters: $\pi/2$-pulse length = 120 ns, time between first and second pulses $\tau = 500$ ns, RF $\pi$-pulse length = 70 $\mu$s, microwave frequency 34.222 GHz, temperature 80 K.
Figure S4. X-band $^{14}$N HYSCORE spectrum of SQ$_A$ in stacked presentation. Intense peaks appear along the diagonal, which are often the result of incomplete inversion of electron spin magnetization by the $\pi$-pulse. Experimental parameters: magnetic field 345.7 mT, time between first and second pulses $\tau = 136$ ns, microwave frequency 9.704 GHz, temperature 80 K.
Figure S5. Nuclear quadrupole tensors for His-M219 N$_\delta$ (purple) and Ala-M260 N$_p$ (green) determined from crystal structure 1DV3 in the same manner as described previously for the Q$_B$ site.\textsuperscript{1} The Euler angles that bring the nuclear quadrupole tensor frame into the g-tensor frame are [-130° 100° -100°] and [170° 80° -110°] for His-M219 N$_\delta$ and Ala-M260 N$_p$, respectively.

Figure S6. Nuclear quadrupole tensors for His-M219 N$_\delta$ (purple) and Ala-M260 N$_p$ (green) as determined from our DFT ORCA calculations. The Euler angles that bring the nuclear quadrupole tensor frame into the g-tensor frame are [-130° 100° -100°] and [140° 80° -110°] for His-M219 N$_\delta$ and Ala-M260 N$_p$, respectively.
Figure S7. Hyperfine tensors for His-M219 N$_\delta$ (purple) and Ala-M260 N$_\rho$ (green) as determined from our DFT ORCA calculations. The Euler angles that bring the hyperfine tensor frame into the g-tensor frame are [0° 120° 0°] and [0° 130° -160°] for His-M219 N$_\delta$ and Ala-M260 N$_\rho$, respectively. For both nitrogens, the calculated tensors were axial so their $A_X$ and $A_Y$ components are not shown.

Figure S8. In-plane ($\theta$) and out-of-plane ($\phi$) angles describing the position of the semiquinone carbonyl oxygen with respect to the histidine N$_\delta$. The figure was reproduced and modified from Fritscher.$^2$
Figure S9. Dependence of $\eta$ on the N· · · O hydrogen bond distance between methylimidazole and benzoquinone from Fritscher.$^2$ The actual data points are shown in yellow. For the three right-most points, the calculated NQI tensor has its maximum component perpendicular to the ring plane. These have been fit with a 2nd order polynomial to extrapolate the N· · · O distance to 2.53 Å, where $\eta = 1$ (red vertical line). Upon further shortening of the H-bond distance, the NQI tensor changes definition, with an exchange of the $Q_{max}$ and $Q_{mid}$ directions. As such, $\eta$ begins to decrease ($\eta = 0.72$ at 2.50 Å). The line drawn from the red vertical line to the point at 2.50 Å has been extended (dashed line) to emphasize the enhanced sensitivity of $\eta$ in this range of H-bond lengths. Regardless of the nqi tensor orientation, $Q_p$ is perpendicular to the imidazole ring plane, $Q_{NH}$ is along the NH bond, and $Q_{pl}$ is orthogonal to both other components (Table 5).
**Figure S10.** Polar plot of calculated nqi tensor for the peptide NH of (a) Thr-M259 and (b) Ala-M260. The nqi tensor is plotted as a surface map showing the sign and symmetry visually, with red representing negative and green representing positive principal values.

**References.**

(1) Taguchi, A. T.; O'Malley, P. J.; Wraight, C. A.; Dikanov, S. A. Nuclear Hyperfine and Quadrupole Tensor Characterization of the Nitrogen Hydrogen Bond Donors to the Semiquinone of the Q₈ Site in Bacterial Reaction Centers: A Combined X- and S-band $^{14,15}$N ESEEM and DFT Study. *J. Phys. Chem. B* **2014**, *118*, 1501-1509.

(2) Fritscher, J. Influence of Hydrogen Bond Geometry on Quadrupole Coupling Parameters: A Theoretical Study of Imidazole–Water and Imidazole–Semiquinone Complexes. *Phys. Chem. Chem. Phys.* **2004**, *6*, 4950-4956.