Supporting Information

Tuning cofactor redox potentials: the 2-methoxy dihedral angle generates a redox potential difference greater than 160 mV between the primary (Q_A) and secondary (Q_B) quinones of the bacterial photosynthetic reaction center.

Alexander T. Taguchi,[†] Aidas J. Mattis,[§] Patrick J. O'Malley,[&] Sergei A. Dikanov,[‡] and Colin A. Wraight*[,†,§]

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

Corresponding author:
Colin A. Wraight - E-mail: cwraight@illinois.edu. Tel: 217 333-3245.

1. Abbreviations

B3LYP, Becke3 Lee−Yang−Parr; SQ, semiquinone; RC, reaction center; EPR, electron paramagnetic resonance; HYSCORE, hyperfine sublevel correlation; Q-10, 2, 3-dimethoxy-5-methyl-6-decaisoprenyl-1,4-benzoquinone; 2-MeO-Q, 2-methoxy-3,5-dimethyl-6-tetraisoprenyl-1,4-benzoquinone; 3-MeO-Q, 3-methoxy-2,5-dimethyl-6-tetraisoprenyl-1,4-benzoquinone.

2. Experimental procedures

Materials, cell growth, and reaction center preparation.

^{13}C-labeled ubiquinone. Headgroup ^{13}C-methyl-labeled ubiquinone was biosynthesized in a strain of *E. coli* as previously described.[1] After growth, ubiquinone was extracted in organic solvents and purified by TLC.[2]

Monomethoxy ubiquinone analogs. The monomethoxy quinones, 2-MeO-Q and 3-MeO-Q, prepared by a modification of an improved method for ubiquinone synthesis,[3] were kindly provided by Professor Bruce Lipshutz (University of California, Santa Barbara).

Cells and RCs. Wild type reaction centers from *Rhodobacter sphaeroides* were engineered with a C-terminal 7xhis-tag on the M subunit[4]. RCs were isolated from wild type cells grown photoheterotrophically on Sistrom's medium with malate as the carbon source, following published procedures.[5] For HYSCORE measurements on the methoxy group orientations, wild type cells were grown in ^{15}N media, to prevent peak overlap and the strong cross-suppression effects of ^{14}N on the ^{13}C modulation.[6] ^{15}N was incorporated by substituting isotopically labeled ammonium sulfate (Cambridge Isotopes) in the medium.

The M265IT mutant RCs with a 6xhis-tag were isolated from mutant cells grown semiaerobically in the dark, also on Sistrom's minimal medium. RC isolation procedures were identical to those for the wild type.

Quinones were extracted from RCs by the method of Okamura et al.[7] as modified by Graige et al.[8], and were replaced with the ^{13}C-methyl labeled ubiquinones for HYSCORE, or the monomethoxy analogs for kinetic measurements.

Sample preparation for HYSCORE measurements. For EPR and related techniques (HYSCORE), the high spin Fe^{2+} coupled to the semiquinones must be replaced with diamagnetic Zn^{2+}. This was achieved according to the procedures outlined by Utschig *et. al*.[5] After metal exchange, RCs were concentrated to ~300-400 μM. Samples for HYSCORE measurements of the semiquinone radicals, SQ_A and SQ_B, were prepared as previously described.[1b, 9]

HYSCORE experiments. Pulsed EPR (HYSCORE) measurements were carried out using an X-band Bruker ELEXSYS E580 spectrometer with an Oxford CF 935 cryostat at 70 K, as previously described.[10] Processed data were imported into Matlab R2010a via the EasySpin package[6] to either be simulated by EasySpin, or be analyzed by a homemade script for fitting data in $(\nu_1)^2$ *vs*. $(\nu_2)^2$ coordinates.[11]

Computational Methods. All density functional calculations were performed using Gaussian 09.[12] All calculations, including geometry optimization, conformational analysis and hyperfine coupling, were performed using the B3LYP functional and the EPR-II basis set. Specific details concerning hyperfine coupling calculations and the SQ_A and SQ_B site models are as previously described.[11, 13] For the present work, one of two new models of ubisemiquinone was used, termed SQ_{M2},[1b] which is hydrogen bonded to a single water molecule (see Fig. S1). Conformational analysis using the SQ_{M2} model was achieved by varying the $C_mO_mC_2C_1$ dihedral angle from 0º to 180º in 20º steps while optimizing all other parameters.

Kinetic measurements. The kinetics of the back reaction (recombination of the light-generated charge separated states, $P^+Q_A^-$ and $P^+Q_B^-$), after a short saturating flash, were monitored as the decay of P^+ at 430 nm in a kinetic spectrophotometer of local design. The decay of the P^+ signal has, in general, two main components - a fast phase reflecting $P^+Q_A^-$ recombination in RCs lacking Q_B activity, and a slow phase due to $P^+Q_A^-$ recombination in RCs with functional Q_B.[14] The two phases can generally be readily deconvoluted to obtain the fraction of slow phase. The initial amplitude, immediately after a flash, and the fraction slow phase (ΔS) corresponding to Q_B, allow for determination of the binding affinity of the quinones in the Q_A and Q_B sites, respectively (see Figures 1 and 2 in the main text).

All kinetic experiments were performed on samples with RC concentrations of approx. 1 μM, in 10 mM Tris buffer, pH 7.8, 0.1% LDAO (lauryl-dimethylamine-N-oxide detergent). Monomethoxy quinones were added from stock solutions in ethanol. The total accumulated ethanol was ≈2% for 2-MeO-Q, and ≈5% for 3-MeO-Q. The latter was high enough to cause some loss of activity towards the end of the titration, accounting for the slight decline in amplitudes seen in Figure 2.

3. Figures

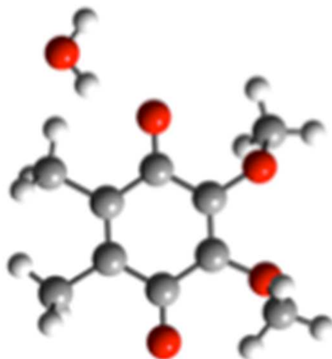

Figure S1. Model SQ_{M2} used for DFT calculations. The quinone is 6-methyl-ubiquinone.

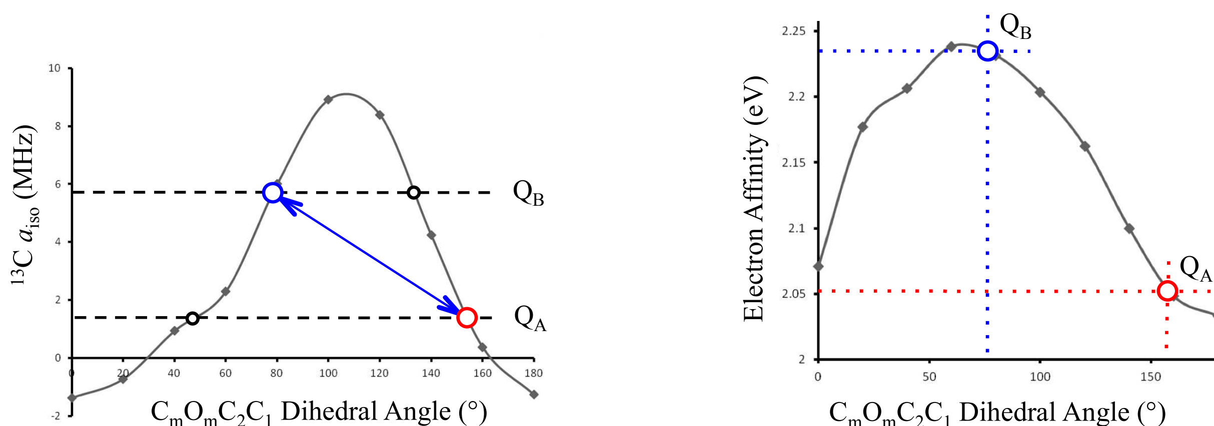

Figure S2. Left: Effect of rotation of the 2-methoxy group on its ^{13}C isotropic hyperfine constant for model SQ_{M2}; the pair of angles supported by the average values in >20 x-ray structures is indicated by the circles and the blue, solid arrow. Right: Effect of rotation of the 2-methoxy group on the electron affinity of the model SQ_{M2}; the values for Q_A and Q_B are indicated by the circles at the intersections of the dotted lines. (Adapted from ref. 1b)

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