Supplementary Material

Theoretical Determination of the Redox Potentials of NRH:quinone Oxidoreductase 2 using Quantum Mechanical/Molecular Mechanical Simulations

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The corrections used in the electron and proton addition reactions are given below.

S1. Electron addition. The thermodynamic cycle for the free energy change for both electron and proton addition processes and their corrections were done following procedure described earlier.[1] Briefly, the uncorrected aqueous standard free energy change ($\Delta G_{uncorr}^{o}(aq)$) for the electron addition (EA) reaction of any reactant X can be obtained directly from the thermodynamic integration method as shown in Scheme S1a, $\Delta G_{uncorr}^{o}(aq) = \Delta G^{TI}(aq)$, but needs to be corrected for the effect of atoms beyond 30 Å (generalized-Born correction), the quantum mechanical effects (electronic and vibrational effect), and the intrinsic (gas-phase) energy correction (difference of energy between SCC-DFTB and a high-level theory).

To account for the effect of atoms beyond 30 Å, we used a general Born correction.[2,3] The charge of the 30 Å spherical zone was kept zero through addition of counter ions. Next, the difference in free energy was computed for the change of charges on the 30 Å sphere as a result of the electron and proton addition on the QM subsystem located at the center of this sphere. According to Born's model, the additional free energy contribution is given by

$$\Delta G_{Born} = -\frac{1}{2}(1 - \frac{1}{\varepsilon})\frac{q^2}{R_0} \tag{S1a}$$

where R_0 is the radius of the spherical region, which in the present study is equal to 30 Å. The overall correction was obtained from the difference between the individual Born corrections of the two states

$$\Delta\Delta G_{Born} = \Delta G_{Born}(Q) - \Delta G_{Born}(P) \tag{S1b}$$

The corrections due to quantum mechanical effects were obtained by combining the vibrational and electronic effects. The vibrational correction was approximated as the difference between the zero-point energies (ZPE) of the two states of the QM-treated atoms calculated previously[1] by gas-phase M06-L[4] calculations:

$$\Delta G_{\text{vib}} = \text{ZPE}(Q) - \text{ZPE}(P) \tag{S2a}$$

The electronic contribution to the correction was computed through consideration of the electronic degeneracies of the reactant and product

$$\Delta G_{\text{elec}} = -RT \ln \frac{d(Q)}{d(P)} \tag{S2b}$$

where d is the electronic degeneracy, P and Q are the two electronically different states, R is the universal gas constant, and T is the temperature. In the present study, ΔG_{elec}, the corrections due to electronic degeneracy difference were within 0.5 kcal/mol and hence were neglected. Therefore, the total quantum mechanical corrections became equal to the correction due to only the zero-point energy differences

$$\Delta G_{\text{quantal}} = \Delta G_{\text{elec}} + \Delta G_{\text{vib}} \cong \Delta G_{\text{vib}} \tag{S2c}$$

The difference between the gas-phase energies of the flavin atoms in the reduction process, computed previously[1] using SCC-DFTB and a high-level DFT calculation with M06-L[4] functional using 6-31+G(d,p) basis set, was taken as high-level corrections

$$\text{HLC} = E_{\text{M06}} - E_{\text{SCC-DFTB}} \tag{S3}$$

where E_{M06} and $E_{\text{SCC-DFTB}}$ stand for the gas-phase energy difference calculated for a specific reaction[1] using M06-L and SCC-DFTB methods. Therefore, the total free energy correction, $\Delta G_{\text{corr}}(\text{EA})$ for the EA process was obtained combining the quantities in eqs S1, S2, and S3

$$\Delta G_{\text{corr}}(\text{EA}) = \Delta\Delta G_{\text{Born}} + \Delta G_{\text{vib}} + \text{HLC} \tag{S4}$$

Specific values of these free energy corrections, $\Delta G_{corr}(EA)$, used for computing the $\Delta G^o(aq)$ for various EA reactions are given in Table S1.

S2. Proton addition. In the case of the proton addition (PA) reaction, the uncorrected standard state free energy change of a proton addition, ΔG^o_{uncorr}, was obtained using four steps of the thermodynamic path shown by the lower half of the thermodynamic cycle of Scheme S1b. During these four steps the anionic part of a molecule, represented by 'A', remained unchanged and completely solvated while only the proton component was changed. The first step (from the left hand side) consisted of changing the solvated proton to the gas-phase. The free energy change for this process is known and is equal to the negative of the solvation free energy of a bare proton at the standard state (1 atm for the gaseous state and 1 mol/L for the solution state), $-\Delta G^o_S(H^+)$, which equals +264.0 kcal/mol.[5-7] In the second step, the gas-phase proton was mutated to a gas-phase 'dummy' atom whose mass and van der Waal's parameters were kept the same as the proton, however, the charge was abolished. The free energy change for such a mutation was taken as zero[1] because despite the proton's charge in the gas–phase, it had no interaction with any atom/molecule, similar to its mutated dummy analog. In the third step, the gas-phase dummy atom was moved back to the solvated phase where it displayed only van der Waals interactions with the anionic part (A⁻) as a result of the uncharged character of the atom. Previous calculations[8] have shown that the free energy change of this process is predominantly due to the build up of bonding interactions between the dummy atom and the solvated anionic A⁻. The free energy contribution $\Delta G_{Bonded}(D)$ was estimated in terms of local properties following the method of Herschbach et al. [9-11]

$$\Delta G_{\text{Bonded}}(\text{D}) = -RT \ln \frac{V_0 \Lambda_\text{D}^{-1}}{(r^2/\sin\Phi)((2\pi k_\text{B} T)/K_\theta)} + \frac{5}{2}RT \qquad (\text{S5})$$

where k_B is Boltzmann's constant, V_0 and Λ_D are the molar volume and thermal wavelength of the dummy atom, respectively; r and Φ represent the distance of the dummy atom to the flavin ring nitrogen atom (N5 or N1) and the angle between the two planes: D–N5(N1)–C7 and D–N5(N1)–C3, respectively; and K_θ is the bending force constants for the two bond angles: D-N5(N1)-C7(C3) and D-N5(N1)-C3.

In the final step, the solvated dummy atom was mutated back to the charged proton, which had quantum mechanical interactions with the anionic part. The free energy change for this step equaled $\Delta G^{\text{TI}}(aq)$, which was computed using thermodynamic integration. Therefore, the uncorrected standard state free energy change was expressed as

$$\Delta G_{\text{uncorr}}^{\text{o}}(aq) = \Delta G^{\text{TI}}(aq) + \Delta G_{\text{Bonded}}(\text{D}) - \Delta G_\text{S}^{\text{o}}(\text{H}^+) \qquad (\text{S6})$$

Various components of the uncorrected standard free energy, $\Delta G_{\text{uncorr}}^{\text{o}}(aq)$, obtained for proton addition reactions are given in Table S2. However, in order to obtain the experimentally comparable $\Delta G^{\text{o}}(aq)$, it is necessary to incorporate all corrections that have been discussed for the electron addition process. Additionally, one more correcting term is necessary to account for the inadequate treatment of proton by SCC-DFTB,[12] which is denoted by $\Delta G_{\text{corr}}(\text{H}^+)$, and is equal to -141.8 kcal/mol. Therefore, the overall correction in the proton-addition (PA) process was expressed as

$$\Delta G_{\text{corr}}(\text{PA}) = \Delta G_{\text{corr}}(\text{H}^+) + \Delta\Delta G_{\text{Born}} + \Delta G_{\text{vib}} + \text{HLC} \qquad (\text{S7})$$

Various components of these free energy corrections, $\Delta G_{corr}(\text{PA})$, used in the calculation of $\Delta G^o(aq)$ for the PA reactions are given in Table S3.

The combined free energy values for the electron and proton transfer reactions are given in table S4.

Table S1. Various correction terms used in eq S4 for calculating the standard free energies of the electron addition reaction (Scheme S1a). The vibrational and high-level-corrections are taken from previous studies[1]

Reaction	Free energy changes	
	ΔG^o	
	(kcal/mol)	
$F + e^- \rightarrow F^{\bullet-}$	$\Delta\Delta G_{Born}$	−5.5
	ΔG_{vib}	−1.9
	HLC	−4.0
$F^{\bullet-} + e^- \rightarrow F^{2-}$	$\Delta\Delta G_{Born}$	−16.5
	ΔG_{vib}	−2.4
	HLC	−1.0
$FH^{\bullet} + e^- \rightarrow FH^-$	$\Delta\Delta G_{Born}$	−5.6
	ΔG_{vib}	−1.3
	HLC	0.9

Table S2. Various components of the uncorrected standard free energy, $\Delta G^{\mathrm{o}}_{\mathrm{uncorr}}(aq)$, used in eq S6 for the proton addition reactions shown in Scheme S1b.

Reaction	Free energy changes ΔG^{o} (kcal/mol)	
$F^{2-} + H^+ \rightarrow FH^-$	$\Delta G^{\mathrm{TI}}(aq)$	−177.4
	$\Delta G_{\mathrm{Bonded}}(D)$	5.7
	$\Delta G^{\mathrm{o}}_{\mathrm{S}}(H^+)$	−264.0
$FH^- + H^+ \rightarrow FH_2$	$\Delta G^{\mathrm{TI}}(aq)$	−142.2
	$\Delta G_{\mathrm{Bonded}}(D)$	5.7
	$\Delta G^{\mathrm{o}}_{\mathrm{S}}(H^+)$	−264.0

Table S3. Various correction terms used in eq S7 for calculating the standard free energies of the proton addition reaction. The vibrational and high-level-corrections are taken from literature.[1]
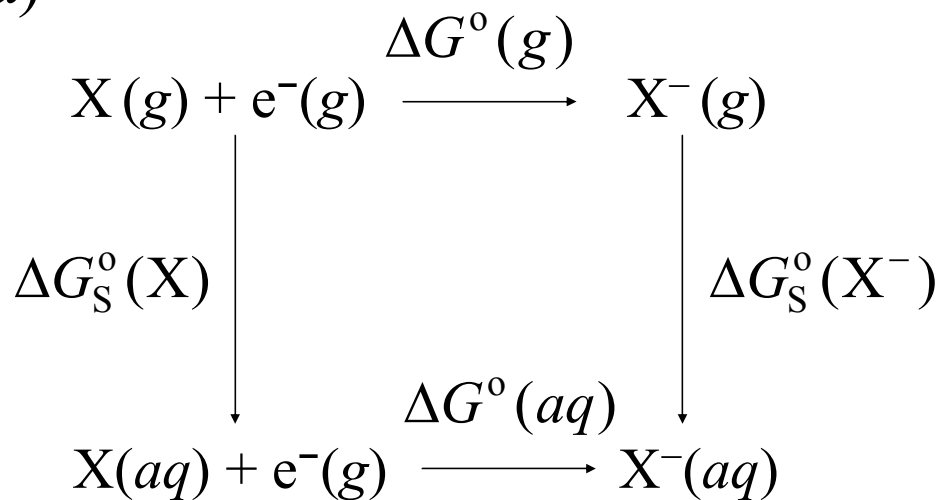
Reaction	Free energy changes ΔG^{o} (kcal/mol)	
$F^{2-} + H^{+} \rightarrow FH^{-}$	$\Delta G_{\mathrm{corr}}(H^{+})$	−141.8
	$\Delta\Delta G_{\mathrm{Born}}$	16.5
	$\Delta\Delta G_{\mathrm{vib}}$	10.2
	$\Delta G_{\mathrm{Bonded}}(D)$	5.7
	HLC	4.0
$FH^{-} + H^{+} \rightarrow FH_{2}$	$\Delta G_{\mathrm{corr}}(H^{+})$	−141.8
	$\Delta\Delta G_{\mathrm{Born}}$	5.5
	$\Delta\Delta G_{\mathrm{vib}}$	8.7
	$\Delta G_{\mathrm{Bonded}}(D)$	5.7
	HLC	1.0

Table S4. Calculated standard free energies $\Delta G^o\,(aq)$ of reaction (kcal/mol) for electron and proton addition reactions for the NQO2-bound FAD at 298 K.
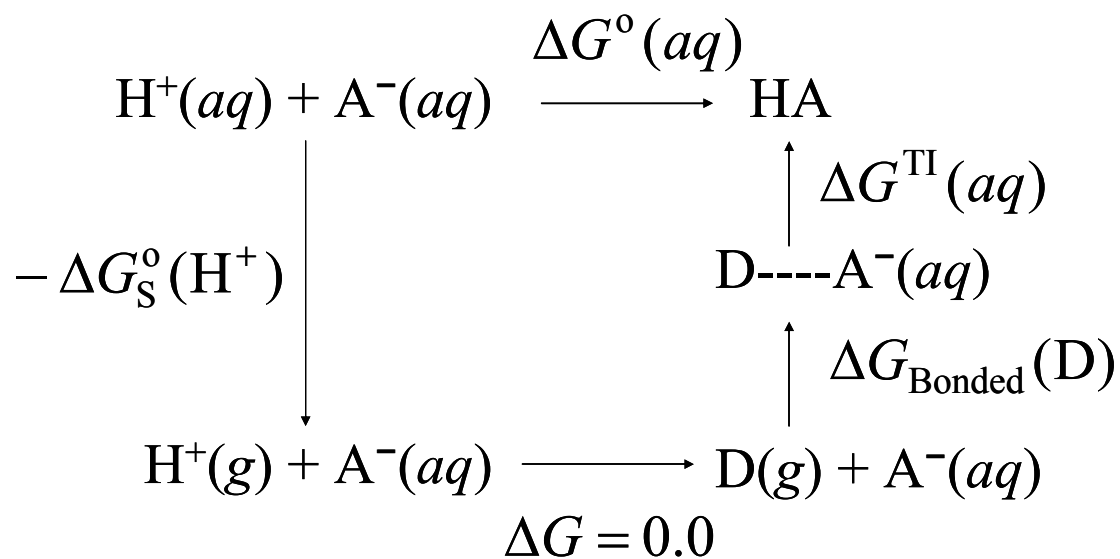
Reaction	Free energy changes		
	$\Delta G^o_{uncorr}\,(aq)$	ΔG_{corr}	$\Delta G^o\,(aq)$
$F + e^- \rightarrow F^{\bullet-}$	−78.3	−11.4	−89.7
$F^{\bullet-} + e^- \rightarrow F^{2-}$	−66.6	−19.9	−86.5
$F^{2-} + H^+ \rightarrow FH^-$	−319.3	+300.4	−18.9
$FH^- + H^+ \rightarrow FH_2$	−284.1	+284.8	+0.7
$FH^{\bullet} + e^- \rightarrow FH^-$	−89.4	−11.8	−101.2
$F^{\bullet-} + H^+ \rightarrow FH^{\bullet}$			−4.2

Scheme S1

a)

$$X(g) + e^-(g) \xrightarrow{\;\Delta G^o(g)\;} X^-(g)$$

$$\Delta G_S^o(X) \qquad\qquad\qquad\qquad \Delta G_S^o(X^-)$$

$$X(aq) + e^-(g) \xrightarrow{\;\Delta G^o(aq)\;} X^-(aq)$$

b)

$$H^+(aq) + A^-(aq) \xrightarrow{\;\Delta G^o(aq)\;} HA$$

$$\Delta G^{TI}(aq)$$

$$-\Delta G_S^o(H^+) \qquad\qquad\qquad D\text{----}A^-(aq)$$

$$\Delta G_{Bonded}(D)$$

$$H^+(g) + A^-(aq) \xrightarrow{} D(g) + A^-(aq)$$

$$\Delta G = 0.0$$

References.

(1)   Bhattacharyya, S.; Stankovich, M. T.; Truhlar, D. G.; Gao, J. *J. Phys. Chem. A* **2007**, *111*, 5729.

(2)   Born, M. *Z. Phys.* **1920**, *1*, 45.

(3)   Cramer, C. J.; Truhlar, D. G. *Chem. Rev.* **1999**, *99*, 2161.

(4)   Zhao, Y.; Truhlar, D. G. *J. Chem. Phys.* **2006**, *125*, 194101.

(5)   Tissandier, M. D.; Cowen, K. A.; Feng, W. Y.; Gundlach, E.; Cohen, M. H.; Earhart, A. D.; Coe, J. V.; Tuttle, T. R. J. *J. Phys. Chem. A* **1998**, *102*, 7787.

(6)   Camaioni, D. M.; Schwerdtfeger, C. A. *J. Phys. Chem. A* **2005**, *109*, 10795.

(7)   Kelly, C. P.; Cramer, C. J.; Truhlar, D. G. *J. Phys. Chem. B* **2006**, *110*, 16066.

(8)   Riccardi, D.; Schaefer, P.; Cui, Q. *J. Phys. Chem. B* **2005**, *109*, 17715.

(9)   Herschbach, D. R.; Johnston, H. S.; Rapp, D. *J. Chem. Phys.* **1959**, *31*, 1652.

(10)   Li, G.; Cui, Q. *J. Phys. Chem. B* **2003**, *107*, 14521.

(11)   Boresche, T. *Mol. Simul.* **2002**, *28*, 13.

(12)   Elstner, M.; Cui, Q.; Munih, P.; Kaxiras, E.; Frauenheim, T.; Karplus, M. *J. Comput. Chem.* **2003**, *24*, 565.