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Improved Density Functional Description of the Electrochemistry and Structure−Property Descriptors of Substituted Flavins

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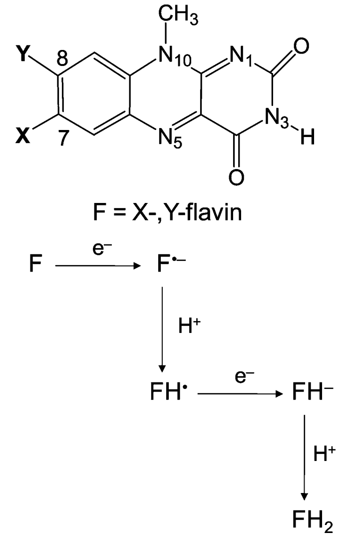
The energetics of electrochemical changes have been investigated for several substituted flavins with the M06-L density functional. The reduction potentials for one- and two-electron reductions of these molecules have been determined and the results are consistent with experimental findings with a mean unsigned error of only 42 mV. It is especially noteworthy that the M06-L density functional makes a significant difference in the computed free energy of the first reduction of lumiflavin, which produces a neutral semiquinone. We also investigate the effects of flavin ring substituents on the geometries, charge distributions, reduction potentials, pK_a's, ionization potentials, electron affinities, hardnesses, softnesses, electrophilic powers, and nucleophilicities.

1. Introduction

Flavins are tricyclic 7,8,10-substituted isoalloxazine ring systems that act as the main electron mediatory cofactor in many enzymes, including most prominently many oxidoreductases.[1−28] As a cofactor, flavins appear predominantly in two forms: (i) flavin adenine dinucleotide (FAD), in which the flavin is conjugated with adenosine diphosphate, and (ii) flavin mononucleotide (FMN), a phosphorylated derivative of riboflavin. Under the influence of certain biological matrices, flavoenzymes behave like light harvesters in which the proton and electron transfer reactions are triggered by light.[29−35] Flavin's photoinduced optical and redox properties are exploited in synthetic molecular assemblies of flavin-wrapped carbon nanotube hybrids (flavin-SWNTs).[36−40] The core chemistry behind the unique physicochemical processes involving flavins stems from a coupled electron−proton transfer involving the isoalloxazine ring.[2,13] The versatility of flavin's chemical behavior is largely due to its unique ability to undergo both sequential (two $1e^-/1H^+$) and simultaneous (one $2e^-/2H^+$) reductions (Scheme 1).[5,41] Enzymes interact with the isoalloxazine ring through a number of noncovalent interactions, and these interactions, especially the electrostatic ones, can be studied by quantum mechanical (QM) electronic structure calculations of the isoalloxazine ring surrounded by a molecular mechanical force field representing the enzyme.[10,11,13−16] Recently, such combined quantum mechanical/molecular mechanical studies of flavin enzymes have been quite successful in unraveling the driving forces,[10,16,24] reorganizations,[42] and kinetics[11,13] of flavin's reduction processes. In these studies, the QM treatment has been either Austin model 1 (AM1)[43] with specific reaction parameters[11,13,44] (SRP) or self-consistent-charge density functional tight-binding[14−16,45,46] (SCC-DFTB). The SRP parametrization did not include terms for van der Waals interactions, although they are included by MM in the MM subsystem and across the QM-MM interface, and the SCC-DFTB method (a tight binding method parametrized against density functional theory [DFT]) is known to perform poorly for van der Waals forces.[45,47−49] In calculations that do not include the enzyme environment, one can more readily use higher level methods such as DFT, and quantum chemical studies have been performed on flavin ring oxidation−reduction[16,51−53] and spectroscopy[52,54−56] using DFT with the Becke 3-paramenter Lee−Yang−Parr[50] (B3LYP) functional; however, B3LYP and many similar functionals are unable to describe general van der Waals interactions.[57−59] Furthermore, it has been observed that B3LYP produces large systematic errors in the heats of formation of many organic molecules.[60,61] Both of these kinds of errors are due to the inaccurate treatment of medium-range correlation (XC) energy.[57,61]

SCHEME 1: Steps in Sequential Electron and Proton Addition Reactions



The new-generation functional, M06-L,[59] employs a new exchange-correlation functional[59] with improved medium-range correlation energy. Recent studies show that M06-L is quite successful for a broad range of systems and properties[16,57,59,62−66] including thermal, electronic, and magnetic behavior in metallic, semiconducting, and organic systems, including systems with

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delocalized π electrons, which are important in flavins. Since M06-L does not have any nonlocal components, calculations on large systems are much faster than for hybrid functionals such as B3LYP[50] or mPW1PW.[67]

In the present work we used the M06-L functional to investigate the energetics and electrochemical behavior of several 7,8-substituted, 10-methyl isoalloxazines (hereafter called 7,8-substituted flavins) in aqueous solution. The reduction potentials in aqueous solutions for both one- and two-electron reductions of these molecules are calculated by implicit solvent methods. The effects of flavin ring substituents on reduction potentials, pK_a's, bend angles, and several structure−property descriptors are investigated.

2. Methods and Computational Details

2.1. Computational Electrochemistry. ***2.1.1. Coupled Electron−Proton Transfer.*** The reduction of a flavin can be described by a coupled electron−proton transfer mechanism in which the electron transfer reactions are rapidly followed by or accompanied by proton transfer reactions.[68,69] The reduction may involve an incomplete $1e^-/1H^+$ process yielding radical semiquinones[70] or a complete $2e^-/2H^+$ process forming stable hydroquinone products.[2,55,71] In the present study, the free energy changes are calculated for sequential electron and proton transfers, as in Scheme 1. Representing the neutral oxidized flavin as F, the first electron transfer results in the formation of an unstable anionic semiquinone, $F^{\bullet-}$:

$$F + e^- \rightarrow F^{\bullet-} \tag{a}$$

$F^{\bullet-}$ can be protonated to site N5 (see numbering in Scheme 1) to form a neutral semiquinone, $FH^{\bullet}$:[55,71]

$$F^{\bullet-} + H^+ \rightarrow FH^{\bullet} \tag{b}$$

The neutral semiquione is not very stable. The second electron transfer results in the formation of anionic hydroquinone, FH^-:

$$FH^{\bullet} + e^- \rightarrow FH^- \tag{c}$$

Finally, depending on the pH of the solution and the pK_a of the neutral hydroquinone, a second proton is transferred to the N1 atom of the anionic hydroquinone to produce neutral hydroquinone, FH_2:

$$FH^- + H^+ \rightarrow FH_2 \tag{d}$$

If $\Delta G^o(X,aq)$ represents the standard-state Gibbs free energy change for a process designated by X, then the net free energy changes for the $1e^-/1H^+$ and $2e^-/2H^+$ processes are

$$\Delta G^o(1e^-/1H^+,aq) = \Delta G^o(a,aq) + \Delta G^o(b,aq) \tag{1}$$

and

$$\Delta G^o(2e^-/2H^+,aq) = \Delta G^o(1e^-/1H^+,aq) + \Delta G(c,aq) + \Delta G(d,aq) \tag{2}$$

The free energy change of the $2e^-/1H^+$ process is also of interest:

$$\Delta G^o(2e^-/H^+,aq) = \Delta G(a,aq) + \Delta G(b,aq) + \Delta G(c,aq) \tag{3}$$

2.1.2. Free Energy Calculations. Gas-phase and implicit-solvation calculations were carried out with the *Gaussian03*,[72] *Gaussian09*,[73] and MN-GSM[74] computer programs. The structures of the 7,8-substituted flavin molecules were optimized in the gas phase by employing B3LYP,[50] mPW1PW,[67] and M06-L[59] density functionals with the 6-31+G(d,p)[75] basis set. In all calculations in this article, we used gas-phase optimized geometries. The gas-phase vibrational−rotational free energies were calculated at 298 K by using the harmonic oscillator-rigid rotor approximation with computed frequencies and moments of inertia. The electronic free energy was approximated as $-RT \ln d$,where R is the gas constant, T is the temperature, and d is the electronic degeneracy of the ground state (1 for singlets, 2 for doublets). The contribution of the electron to the standard-state free energy at 298 K is less than 0.02 kcal/mol[76] (1 meV) and was neglected.

The standard state was defined as an ideal gas at 1 atm for gaseous species and an ideal solution at 1 M for solutes. All solvation calculations were carried out at 298 K with Solvation Model 6 (SM6)[77] implemented in MN-GSM.[74] This implicit solvation model was chosen because it has produced relatively accurate results for solvation calculations involving both neutral and ionic solutes.[77] Furthermore, this model was also found to produce reliable energetics for flavin system.[16] The standard-state free energy changes for electron additions were calculated using Scheme 2a

$$\Delta G^o(aq) = \Delta G^o(g) + \Delta G^o_S(A^-) - \Delta G^o_S(A) \tag{4}$$

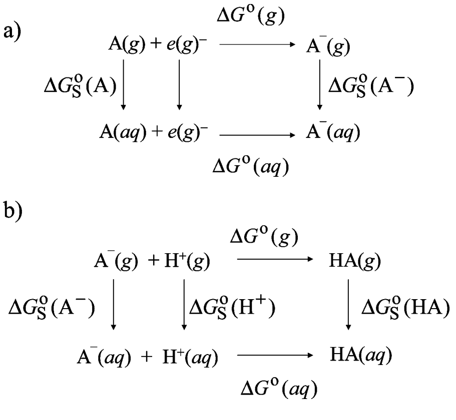
Similarly, the standard-state aqueous free energy change for the proton additions were calculated using Scheme 2b

$$\Delta G^o(aq) = \Delta G^o(g) + \Delta G^o_S(HA) - \Delta G^o_S(A^-) - \Delta G^o_S(H^+) \tag{5}$$

In these schemes,[78,79] ΔG^o represents the standard free energy of reaction either in the gas phase (g) or in the aqueous solution (aq), and ΔG^o_S refers to the standard free energy of solvation.

2.1.3. Standard Reduction Potentials. The experimentally obtained standard reduction potentials are usually expressed relative to the standard reduction potential of the normal hydrogen electrode[80] (NHE), E^o_H, which is taken here as 4.28

SCHEME 2: Thermodynamic Cycles for Electron and Proton Addition Reactions



V.[81] Therefore, the standard reduction potential of any reduction reaction with standard free energy change of $\Delta G^{o}(\text{aq})$ can be expressed as

$$E^{o} = -\frac{\Delta G^{o}(\text{aq})}{nF} - E^{o}_{\text{H}} \tag{6}$$

where n is the number of electrons on the left side of the reaction and F is the Faraday constant, which equals 23.06 kcal mol^{-1} V^{-1}.

The reduction potentials are strongly pH dependent,[68] and if, at a certain pH, the reduction potentials of the oxidized flavin → semiquinone and semiquinone → anionic or neutral hydroquinone steps are denoted by $E_{\text{ox,sq}}$ and $E_{\text{sq,rd}}$, then the midpoint potential, E_{m}, can be defined[55,68] as an average of the two reduction potentials, i.e.

$$E_{\text{m}} = \frac{1}{2}(E_{\text{ox,sq}} + E_{\text{sq,rd}}) \tag{7}$$

If the final protonation step (step d) is favorable, this yields

$$E^{\text{prot}}_{\text{m}} = -\frac{\Delta G^{o}(2\text{e}^{-}/2\text{H}^{+},\text{aq})}{2F} - E^{o}_{\text{H}} \tag{8}$$

The superscript "prot" in eq 8 denotes that the final protonation step is included. To determine if a protonation step is favorable, the $\text{p}K_{\text{a}}$ of a species AH may be calculated from $\Delta G^{o}(\text{H}^{+},\text{aq})$ of the proton addition step using

$$\text{p}K_{\text{a}} = -\frac{\Delta G^{o}(\text{H}^{+},\text{aq})}{2.303RT} \tag{9}$$

If the hydroquinone is deprotonated, the midpoint potential must be computed from the $2\text{e}^{-}/1\text{H}^{+}$ reaction, which yields a midpoint potential for the deprotonated species:

$$E^{\text{deprot}}_{\text{m}} = -\frac{\Delta G^{o}(2\text{e}^{-}/1\text{H}^{+},\text{aq})}{2F} - E^{o}_{\text{H}} \tag{10}$$

2.2. Structure−Property Descriptors. ***2.2.1. Hammett Substituent Effect.*** The Hammett substituent effect[82,83] on the midpoint potential is evaluated for its dependence on the individual σ component of the substitutions at 7- and 8-positions on the flavin ring (Scheme 1) by using

$$E^{\text{prot}}_{\text{m}}(\text{X,Y}) = E^{\text{prot}}_{\text{m}}(\text{H,H}) + (\sigma_{\text{X}} + \sigma_{\text{Y}})\rho \tag{11}$$

where σ_{X} and σ_{Y} are Hammett's σ values corresponding to the effect of substituents X and Y, respectively, of the 7-X,8-Y-flavin and ρ is a proportionality constant.

2.2.2. Electron Affinities and Ionization Potentials. The adiabatic electron affinity (AEA) is calculated from the Born−Oppenheimer energy (E) differences of the optimized neutral isoalloxazine (represented as F) and the optimized negatively charged semiquinone radical ($\text{F}^{\bullet-}$), while the energetic differences between the optimized states of F and F^{+} provide the adiabatic ionization potentials (AIP).

$$\text{AEA} = (E_{\text{neutral}} - E_{\text{anion}}) \tag{12}$$

$$\text{AIP} = (E_{\text{cation}} - E_{\text{neutral}}) \tag{13}$$

In contrast, the vertical electron affinity (VEA) and vertical ionization potential (VIP) denote the difference of the Born−Oppenheimer energies between neutral and the anionic and cationic species, respectively, at the fixed geometry of the neutral. These energies were therefore calculated using

$$\text{VEA} = (E_{\text{neutral}} - E_{\text{anion//neutral}}) \tag{14}$$

$$\text{VIP} = (E_{\text{cation//neutral}} - E_{\text{neutral}}) \tag{15}$$

where $E_{\text{anion//neutral}}$ and $E_{\text{cation//neutral}}$ are the energies of the anionic and cationic species, respectively, calculated using the equilibrium geometry of the neutral species.

2.2.3. Hardness, Chemical Potential, and Electrophilic Power. The hardness (η) is a measure of the tendency of a chemical species to localize charge density.[84,85] It is a companion parameter to electronegativity, and it is calculated by

$$\eta = \frac{1}{2}(\text{AIP} - \text{AEA}) \tag{16}$$

where AIP and AEA are defined in eqs 12 and 13. Species with higher hardness values are less polarizable, and zero hardness corresponds to maximum softness and the most polarizable electron density.[85] The chemical potential (μ) is computed using[84,86,,88]

$$\mu = -\frac{1}{2}(\text{AIP} + \text{AEA}) \tag{17}$$

The electrophilicity index (ϖ) is defined as

$$\varpi = \frac{\mu^{2}}{2\eta} \tag{18}$$

with μ and η defined as above. Electrophilic power is a measure of a molecular system's ability to accept electrons.

2.2.4. Relative Electrophilicity. The Fukui function $f(\mathbf{r})$[84,87] defined in the framework of density functional theory can be represented as[87]

$$f(\mathbf{r}) = \left(\frac{\partial \rho(\mathbf{r})}{\delta N}\right)_{\nu(\mathbf{r})} \tag{19}$$

where $\rho(r)$ is the electron density at point $\mathbf{r}$, N is the number of electrons, and $\nu(\mathbf{r})$ is the external potential in which the N electrons move. The chemically softer region of a molecule consists of atoms with large Fukui function, while the harder regions have a small value of the Fukui function.[89] The Fukui function can be approximated using finite differences to get

$$f^{+}(\mathbf{r}) \approx \rho^{N+1}(\mathbf{r}) - \rho^{N}(\mathbf{r}) \tag{20}$$

TABLE 1: Comparison of the Aqueous Free Energy Changes (kcal/mol) in Electron and Proton Transfer Reactions (Scheme 1) of Lumiflavin Computed Using Various Functionals (All Energies in kcal/mol)

	reaction	B3LYP|SM6	MPW1PW|SM6	M06-L|SM6
	$F + e^- \rightarrow F^{\bullet -}$	−91	−91	−84
	$F^{\bullet -} + H^+ \rightarrow FH^{\bullet}$	−11	−13	−15
subtotal:	$F + e^- + H^+ \rightarrow FH^{\bullet}$	−102	−104	−99
	$FH^{\bullet} + e^- \rightarrow FH^-$	−91	−89	−88
	$FH^- + H^+ \rightarrow FH_2$	−3	−5	−2
total:	$F + 2e^- + 2H^+ \rightarrow FH_2$	−196	−198	−189

TABLE 2: Aqueous Free Energies of Reactions (kcal/mol), Midpoint Potential (mV), and Sum of Hammett Parameters (Unitless) for the Electron and Proton Transfer Reactions of Substituted Flavin As Shown in Scheme 1 and Figure 1[a]

X, Y	$\sigma_X + \sigma_Y$	$\Delta G°$(a,aq)	$\Delta G°$(b,aq)	$\Delta G°$(c,aq)	$\Delta G°$(d,aq)	$\Delta G°$(total,aq)	E_m^{prot}
H, H	0	−85	−16	−84	−7	−192	−117
H, CH_3	−0.17	−85	−15	−85	−9	−194	−95
H, Cl	0.23	−92	−15	−79	−9	−195	−52
H, CN	0.66	−92	−14	−90	−12	−208	230
H, CF_3	0.43	−92	−13	−85	−12	−202	78
H, NH_2	−0.66	−74	−21	−75	−10	−180	−377
H, $N(CH_3)_2$	−0.83	−75	−21	−75	−10	−181	−355
CH_3, H	−0.07	−87	−15	−83	−8	−193	−95
CH_3, CH_3	−0.24	−84	−15	−88	−2	−189	−182
CH_3, NH_2	−0.73	−65	−30	−70	−15	−180	−377
CH_3, Cl	0.16	−89	−14	−85	−8	−196	−30
CH_3, OCH_3	−0.34	−85	−21	−78	−9	−193	−95
Cl, H	0.37	−89	−14	−86	−8	−197	−9
Cl, CH_3	0.20	−88	−13	−85	−9	−195	−52
Cl, $N(CH_3)_2$	−0.46	−82	−15	−83	−10	−190	−160
Cl, Cl	0.60	−90	−13	−88	−9	−200	57
Cl, CN	1.03	−94	−12	−93	−9	−208	230

[a] Values in this table were calculated by M06-L|SM6. $\Delta G°(\mathbf{X},aq)$ represents the free energy of reaction **X** as described in subsection 2.1.1.

$$f^-(\mathbf{r}) \approx \rho^N(\mathbf{r}) - \rho^{N-1}(\mathbf{r}) \tag{21}$$

where $\rho^N(\mathbf{r})$ is the charge density on a molecule with N electrons. ρ^{N+1} is obtained from ρ^N by adding an electron to the lowest unoccupied molecular orbital (LUMO), and ρ^{N-1} is obtained by removing an electron from the HOMO. The densities can be integrated over each atom k, yielding[86]

$$f_k^+ = q_k^{N+1} - q_k^N \tag{22}$$

$$f_k^- = q_k^N - q_k^{N-1} \tag{23}$$

where q_k^N is the partial charge on atom k with N electrons. Frontier molecular orbital theory implies that a high electron density of the highest occupied molecular orbital (HOMO) at a given site in a molecule indicates a high nucleophilicity for that site;[87,90] comparing this statement to eq 23 shows that f_k^- indicates the capacity of atom k to act as an nucleophile or to undergo electrophilic attack. Similarly, f_k^+ indicates the capacity of atom k to act as an electrophile or the tendency to undergo nucleophilic attack. The relative electrophilicity of atom k can be represented as the ratio[86]

$$g_k = f_k^+/f_k^- \tag{24}$$

and a larger value of g_k should indicate a greater tendency to accept a nucleophile such as an electron or a hydride ion.

3. Results and Discussion

3.1. Electrochemistry. ***3.1.1. Free Energy Calculations.*** Table 1 compares solution-phase energies of lumiflavin (7,8,10-trimethylflavin) using the B3LYP, mPW1PW, and M06-L density functionals. Row 3 of the table shows that the B3LYP and mPW1PW density functionals both overestimate the exoergodicity of the first reduction of lumiflavin by 3−5 kcal/mol compared to M06-L. The experimentally determined Gibbs free energy change for the coupled $1e^-/1H^+$ reaction of lumiflavin is −94 kcal/mol[91] and can be used as a test of the accuracy of M06-L. The computed value for M06-L is −99 kcal/mol, as shown in row 3 of Table 1 or as obtained by summing $\Delta G°$(a,aq) and $\Delta G°$(b,aq) in the CH_3,CH_3 row of Table 2, while the computed values with B3LYP and mPW1PW are −102 and −104 kcal/mol, respectively. The improved accuracy with M06-L is encouraging. All further calculated values in the text, tables, and figures will correspond to M06-L.

The aqueous-state free energy changes for reactions a, b, c, and d (Scheme 1) obtained for various substituted flavins are shown in Table 2 and Figure 1. The results reveal that in all cases the free energy changes for the electron additions are substantial and make the dominant contribution to the overall free energy of reduction. The substituent effects on the first and second electron transfers are up to 29 and 23 kcal/mol, respectively; the substituent effects on the proton transfers are smaller but not negligible—up to 18 kcal/mol for the first proton transfer and up to 13 kcal/mol for the second. The table also shows that the energetics of the two sequential electron addition processes are quite similar. The calculations reveal that the free energy change for the second proton addition (process d) is generally less negative than that for the first proton addition

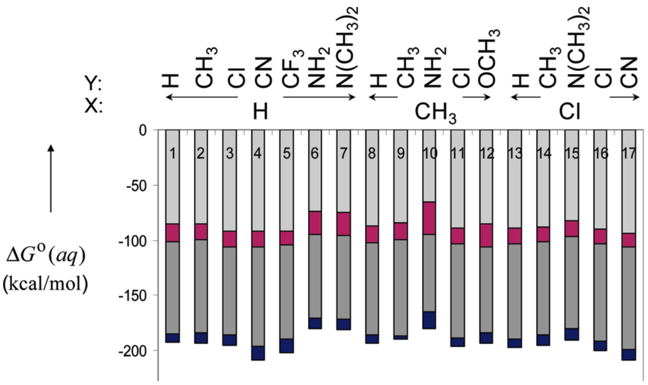


Figure 1. Free energy components of the electron and proton transfer reactions in substituted flavins as calculated by M06-L|SM6. The free energy changes for various reactions shown are color-coded: F + e$^-$ → F$^{\bullet-}$, silver; F$^{\bullet-}$ + H$^+$ → FH$^\bullet$, maroon; FH$^\bullet$ + e$^-$ → FH$^-$, gray; FH$^-$ + H$^+$ → FH$_2$, dark blue.

TABLE 3: Comparison of the Computed (E_m^{deprot}) and Experimental[83] (E_m^{exp}) Reduction Potentials (mV) of Substituted Flavins and Computed pK_a Values of Their Reduced Flavohydroquinones in Aqueous Solution[a]

X, Y	E_m^{prot}	E_m^{deprot}	E_m^{exp}	pK_a of FH$_2$	$\lvert E_m^{deprot} - E_m^{exp}\rvert$
H, H	−117	−266	−210	5.1	56
H, CH$_3$	−95	−304	−242	6.6	62
H, Cl	−52	−260	−188	6.6	72
CH$_3$, H	−95	−273	−222	5.9	51
CH$_3$, Cl	−30	−238	−193	5.9	45
CH$_3$, CH$_3$	−182	−241	−259	1.5	18
Cl, H	−9	−187	−174	5.9	13
Cl, CH$_3$	−52	−260	−205	6.6	55
Cl, Cl	57	−151	−141	6.6	10
mean					42

[a] M06-L|SM6.

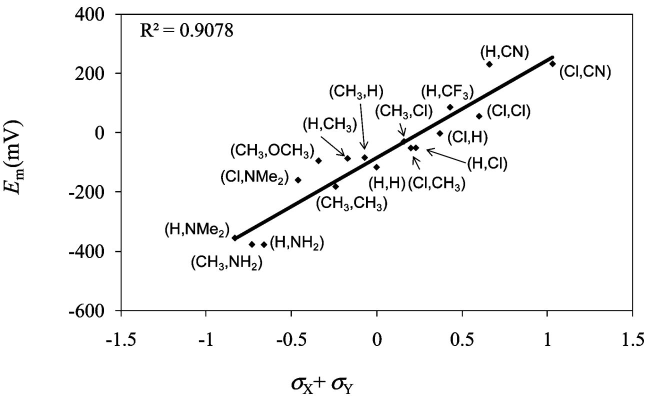


Figure 2. Variation of E_m^{prot} with Hammett constants.

TABLE 4: Gas-Phase Hardness (η), Chemical Potential (μ), Electrophilic Power (ϖ), Ionization Potential, and Electron Affinity of Substituted Lumiflavin Species (All Values in kcal/mol)

X, Y	η	μ	ϖ	AIP	AEA	VIP	VEA
H, H	72.6	−116.9	94.2	189.5	44.4	191.4	39.9
H, CH$_3$	71.6	−114.1	90.9	185.8	42.5	187.8	37.9
H, Cl	71.2	−119.7	100.7	190.9	48.5	193.0	44.2
H, CN	69.4	−128.4	118.8	197.8	59.0	199.7	54.7
H, CF$_3$	70.9	−125.1	110.3	195.9	54.2	198.2	49.0
H, NH$_2$	69.4	−106.5	81.7	176.0	37.1	177.8	30.5
H, N(CH$_3$)$_2$	67.3	−102.7	78.4	170.1	35.4	171.4	30.3
CH$_3$, H	70.5	−113.8	91.7	184.3	43.2	186.5	38.7
CH$_3$, CH$_3$	70.0	−111.3	88.6	181.3	41.4	183.4	37.0
CH$_3$, NH$_2$	70.7	−102.0	73.6	172.6	31.3	175.5	30.7
CH$_3$, Cl	69.3	−116.5	97.9	185.8	47.2	188.5	42.9
CH$_3$, OCH$_3$	64.1	−106.5	88.4	170.6	42.4	184.0	37.7
Cl, H	69.4	−119.1	102.2	188.5	49.7	190.8	45.0
Cl, CH$_3$	69.0	−116.4	98.2	185.4	47.4	187.6	42.9
Cl, N(CH$_3$)$_2$	63.9	−108.0	91.3	171.9	44.1	175.5	38.2
Cl, Cl	68.5	−121.3	107.4	189.7	52.8	191.9	48.3
Cl, CN	90.3	−153.4	130.3	243.8	63.1	198.2	58.7

(process b). This difference is most prominent in the case of 8-NH$_2$ and 8-N(CH$_3$)$_2$ substituents.

3.1.2. *Redox Potentials.* The computed midpoint potentials (eqs 8 and 10) of the substituted flavins are also given in the last column of Table 2 and in Table 3. The pK_a column of Table 3 shows that the computed pK_a's at site N1 of the hydroquinones of substituted flavins are usually close to but less than or equal to 7; this agrees well with the experimental value[83] of 6.5. This suggests that all of them would be substantially deprotonated at pH 7.4, at which the midpoint potentials of Table 3 were experimentally determined. Therefore, the standard reduction potentials of flavins for the 2e$^-$/1H$^+$ reaction producing the anionic hydroquinone are the appropriate ones to compare to experiment. These reduction potentials (eq 10) were computed from the combined free energy change of the 2e$^-$/1H$^+$ reaction. The last column of Table 3 compares theory to experiment. The mean unsigned error is only 42 mV. This corresponds to only 0.96 kcal/mol and constitutes excellent performance for quantum chemical calculations. Li and Fu, using a similar approach but with the B3LYP density functional and a different implicit solvation model, achieved a mean deviation between theory and experiment of less than 60 mV.[53]

3.2. Structure−Property Descriptors. ***3.2.1. Hammett Substituent Descriptors.*** As shown in Scheme 1, the 7- and 8-positions are meta and para with respect to the N5 site, which is the initial hydride receiving site in all biological electron transfer reactions.[4,83] The Hammett substituent effect[82] describes a linear free energy relationship between the various meta and para substitutions, and the effects are additive. Positive σ values are associated with electron withdrawing inductive effects; these stabilize the negative charges developed in steps a and c, and for those steps large negative ΔG^o values are associated with substitutions leading to large positive $\sigma_X + \sigma_Y$, such as substituting an electron withdrawing CN group at the 8-position.[83] Figure 2 shows that, for the 17 compounds studied here, there is a moderate correlation with goodness-of-fit (R^2) equal to 0.91. This is not quite as good as the correlation of E_m^{exp} with σ, which gives $R^2 = 0.97$. Nevertheless, these correlations clearly indicate that electron withdrawing groups such as CN and Cl make the potentials more positive and that the present density functional calculations reproduce the trend by making the flavin ring electron deficient and therefore easier to reduce. Conversely, flavin derivatives in which the 7- and 8-positions are occupied by electron donating substituents (e.g., −NH$_2$ and −N(CH$_3$)$_2$) are comparatively less prone to reduction, and their midpoint reduction potentials are found to be very negative (Figure 2).

3.2.2. Electron Affinities (EAs) and Ionization Potentials (IPs). Previously,[55] ionization potentials of the anionic hydroquinone and the neutral hydroquinone were shown to be well correlated with one- and two-electron reduction potentials. Here we examine ionization potentials and electron affinities of the oxidized flavin. Table 4 shows gas-phase adiabatic and vertical EAs and IPs. The results show expected trends due to substituents. An electron-withdrawing group like CN makes EA and IP larger, while an electron donating group has the opposite effect (Table 4). Low to moderate correlations with the Hammett coefficients were found in all cases, although the EAs were

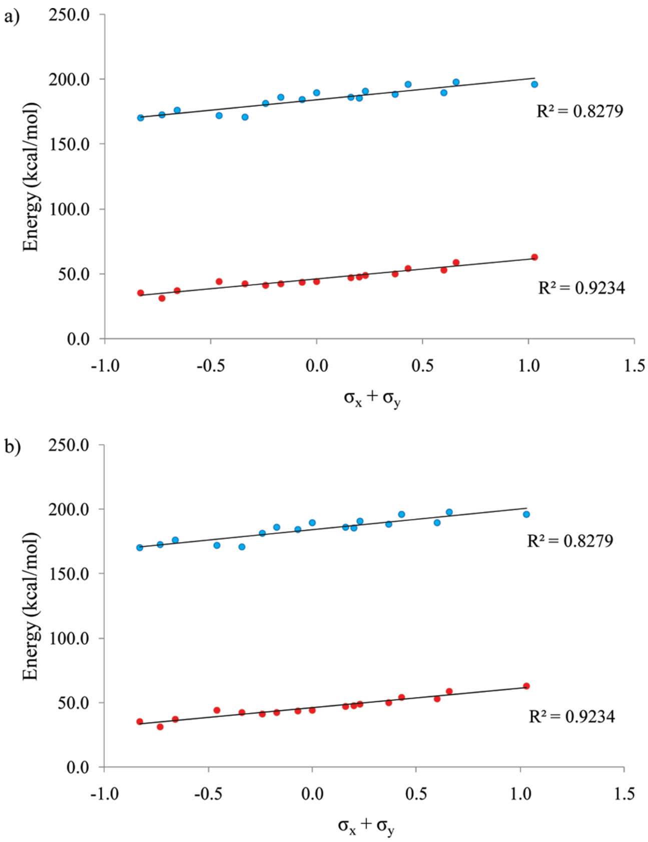


Figure 3. Correlations of electron affinities (red) and ionization potentials (cyan) with Hammett constants: (a) and AIP and (b) VEA and VIP.

found to be more correlated than ionization potentials (Figure 3), with goodness-of-fit ranging from 0.8 to 0.9.

The EAs predicted by M06-L are ∼4 kcal/mol lower than those calculated with B3PW91[86] and 9−11 kcal/mol higher than those calculated with B3LYP.[53] The IPs predicted by M06-L are 25−28 kcal/mol lower than those calculated with B3PW91.[86]

3.2.3. Hardness, Chemical Potentials, and Electrophilicity Indices. The computed hardness, chemical potentials, and electrophilicity indices (eqs 16−18) of the substituted flavins are listed in Table 4. The listed values show that the hardness of these flavins vary 64−90 kcal/mol. The softest molecules are 7-Cl,8-N$(CH_3)_2$− and 7-CH_3,8-OCH_3− flavins with the hardness of ∼64 kcal/mol, while the maximum hardness of 90.5 kcal/mol is found in 7-Cl,8-CN-flavin. The hardness does not appear to be correlated to the Hammett coefficients. In contrast, the chemical potential and the electrophilicity index are well correlated with the Hammett coefficients. The trend of chemical potentials and electrophilicity indices are opposite.

3.2.4. Relative Electrophilicity of Nitrogen and Oxygen. To examine the reactivity indices of the two endocyclic ring nitrogen atoms (N1 and N5) and two exocyclic oxygen atoms (O2 and O4), their relative electrophilicities[86] (eq 24) were calculated. These values are listed in Table 5. The order of the relative electrophilicities (N5 > O4 > O2 > N1) is the same for all 17 substitution patterns. The relative electrophilicity of N1 varies between 0.4 and 0.7 (Table 5), while that for N5 was found to be several times higher, indicating strong electrophilic character for site N5. This is consistent with its role as an acceptor of an electron or hydride ion. The relative electrophilicity is not well correlated with electrophilicity index.

TABLE 5: Gas-Phase Relative Electrophilicities of Endocyclic Nitrogens and Exocyclic Oxygens As Computed Using Eq 24

X, Y	N1	N5	O1	O4
H, H	0.44	1.83	0.83	1.03
H, CH_3	0.44	1.76	0.86	1.06
H, Cl	0.44	1.71	0.85	1.03
H, CN	0.41	1.62	0.77	0.95
H, CF_3	0.41	1.74	0.80	0.84
H, NH_2	0.55	1.60	0.92	1.07
H, $N(CH_3)_2$	0.65	1.68	1.00	1.12
CH_3, H	0.52	2.01	0.92	1.17
CH_3, CH_3	0.53	1.97	0.91	1.18
CH_3, NH_2	0.54	1.64	0.88	1.03
CH_3, Cl	0.50	1.92	0.91	1.15
CH_3, OCH_3	0.60	1.78	0.95	1.31
Cl, H	0.57	1.86	0.93	1.19
Cl, CH_3	0.55	2.05	0.94	1.19
Cl, $N(CH_3)_2$	0.70	1.93	1.04	1.24
Cl, Cl	0.51	1.96	0.93	1.16
Cl, CN	0.49	1.84	0.86	1.06

3.2.5. Butterfly Bending and Distribution of Charges. The butterfly bend angle of the flavin is considered to be a key feature that distinguishes flavin's reduced state from the oxidized one. The difference in geometry (Figure 4) between the two redox states provides a lever arm for the active site to modulate the redox potential.[16] Therefore, in the present study we revisited the bend angle question. The angle of each substituted species was calculated for the reduced state (Table 6) and is compared to key partial atomic charges in Figure 5. The distribution of charges shows that the substitutions have only a minor effect on the partial charges of the oxygen atoms. In contrast, the charges on the nitrogen atoms (N1 and N5) vary significantly (Figure 5) and are correlated with flavin's bending angle. Furthermore, the variation of charges on the nitrogens is opposite to one another, resulting in a charge separation (polarization)

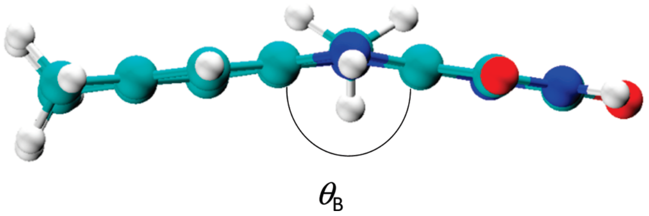


Figure 4. Butterfly bend angles (θ_B) shown for lumiflavin as a representative structure. It is the angle between the planes of pyrimidine and benzene six-membered rings of the flavin.

TABLE 6: Butterfly Angles of Substituted Flavins (Scheme 1) in the Gas Phase

X, Y	θ_B (deg)
H, H	180
H, CH_3	155
H, Cl	180
H, CN	159
H, CF_3	180
H, NH_2	153
H, $N(CH_3)_2$	153
CH_3, H	180
CH_3, CH_3	−154
CH_3, NH_2	−153
CH_3, Cl	180
CH_3, OCH_3	−155
Cl, H	180
Cl, CH_3	180
Cl, $N(CH_3)_2$	154
Cl, Cl	180
Cl, CN	180

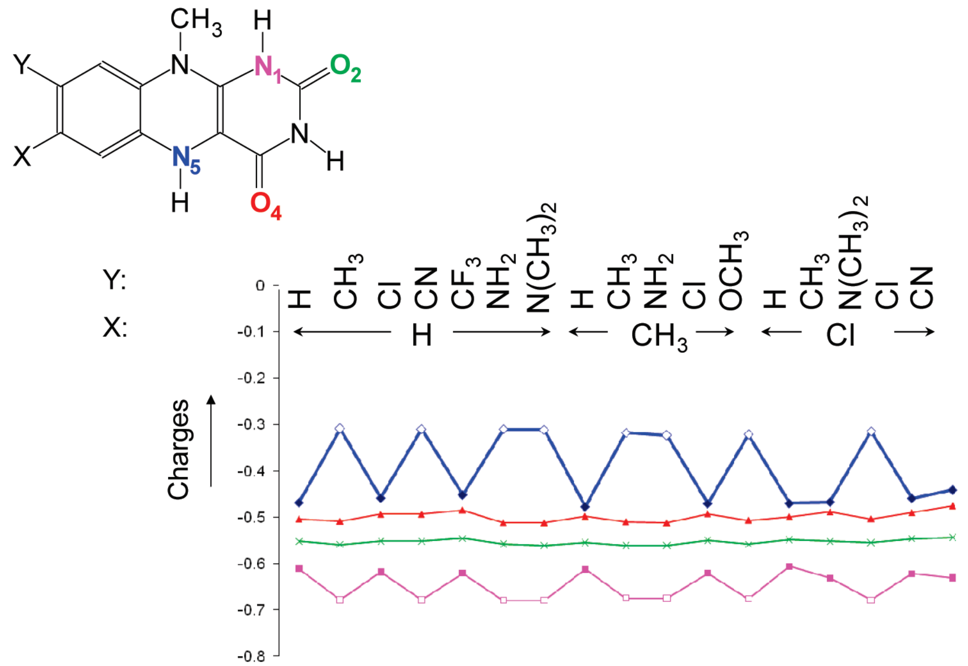


Figure 5. Mulliken atomic charges and their correlation with the bent neutral flavin hydroquinone structures for substituted flavins in the gas phase (as in Table 6). All unfilled points refer to significant changes in the N5 and N1 partial charges as well as substantive bending (~25°) of the flavin ring (Table 5). All calculations in this figure were carried out in the gas phase.

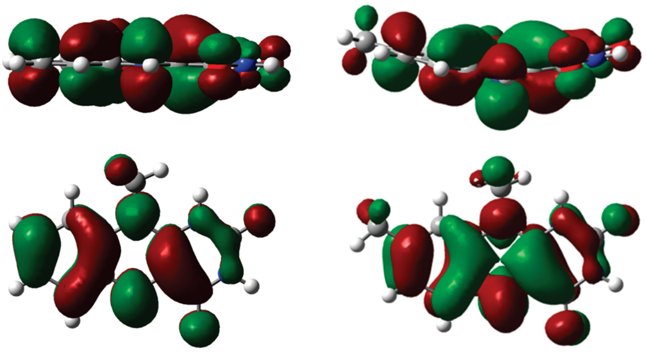

7-H,8-H-flavin 7-H,8-CH_3-flavin

Figure 6. Molecular orbitals of the optimized gas-phase representative structures of a planar (7-H,8-H-flavin) and a bent (7-H,8-CH_3-flavin) neutral flavin hydroquinone. These orbitals are computed for the gas phase.

between N1 and N5. The polarization of the flavin ring was observed to be correlated to the electrophilic power (ϖ) values (Table 4). With the exception of one (7-H-, 8-CN- substitutions), it was found that low electrophilic power of the flavin ring stabilizes a bent hydroquinone structure. The lower electrophilic power in a flavin ring system indicates that it contains larger electron density and, therefore, is relatively less prone to reduction. This happens when the substituents are electron donating, i.e., with negative Hammett constants.

This correlation is also observed for the electron density on the flavin ring for the highest occupied molecular orbitals (HOMO) of planar and bent structures, as shown in Figure 6. In the bent flavin structure (7-H,8-CH_3 flavin), there is a strong delocalized electron density spread out in the central part of the flavin ring as opposed to the planar ring structure in 7-H,8-H flavin. This suggests that the flavin butterfly angle regulates the delocalized electron density in the flavin ring, which can be influenced by 7,8-substitutions.

4. Conclusions

Using M06-L DFT calculations, the energetics of several substituted flavin systems were computed in both the gas and aqueous phase. The computed values are consistent with experimental values with a mean unsigned error of 42 mV, and the first reduction potential of lumiflavin agrees with experiment better than previous calculations. The calculations allowed detailed analysis of the energetics involved in electron and proton transfer to flavins. It was found out that the electron additions are the main contributing factor to the reaction energy for both coupled e^-/H^+ transfer reactions, although the second proton addition is generally much less favorable, than the first one.

The redox potentials, electron affinities, and ionization potentials of these flavin systems were studied and found to be well correlated to the Hammett constants. As a result, the computed chemical potentials and electrophilicities were also found to be related to Hammett constants. This confirms that substitutions at the 7- and 8-positions cause their effects by modulating the electron density on the flavin ring.

A significant effect of these substitutions was observed on the geometry of the reduced flavin, whose planarity was found to be dependent on the flavin ring's electrophilic power, which in turn is controlled by 7,8-substituents. The butterfly bend angle of the reduced flavin and the distribution of charges on the endocyclic ring nitrogen atoms (N1 and N5) and the exocyclic oxygen atoms (O1 and O4) were analyzed. It was found that the separation of charges on these nitrogens correlate well with butterfly bending, being highest in the bent form. This was found to be consistent with the distribution of the HOMO charge density on the reduced flavin rings. The flavin ring with electron donating substituents were bent and showed a more delocalized electron density, while planar structures were found when electron withdrawing functionalities were attached to the flavin ring resulting more localized electron density. As the latter group of flavins does not require a structural change upon reduction, the two-electron reduction process is more favorable in them as supported by their higher Gibbs free energy changes (Table 2).

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