# Modulation of Flavin Recognition and Redox Properties through Donor Atom−$\pi$ Interactions

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**Abstract:** Computational and experimental model studies establish the importance of donor atom−$\pi$ bonding on the recognition and function of flavins. This noncovalent interaction arises from electrostatic attraction between electron-rich (donor) atoms and electron-deficient aromatic systems. The electrostatic origin of this bonding was verified through comparison of binding of model receptors to flavin in both oxidized ($Fl_{ox}$) and radical anion ($Fl_{rad}^-$) forms: Interaction was strongest with the electron-poor $Fl_{ox}$ and was greatly attenuated with the comparatively electron-rich $Fl_{rad}^-$. Other factors determining the magnitudes of these donor−$\pi$ interaction are the size and polarizability of the donor atom.

Hydrogen-bonding,[1] aromatic stacking,[2] and cation−$\pi$[3] interactions are three of the fundamental forces in molecular recognition. Numerous model systems have been devised to examine how these forces contribute to diverse areas such as protein structure, ligand binding, and host−guest chemistry.[4] Although these three specific forces account for a number of known binding and structural motifs, many structural and functional aspects of biomolecular function remain unexplained. Recent literature has shown that consideration of less obvious electrostatic interactions is essential in the determination of biomolecular structure and function.[5]

Redox-active host−guest systems provide a unique milieu for the study of noncovalent interactions. In these systems, recognition is directly linked to redox function. Conversely, since there is often little or no structural change during the redox event, these systems provide an opportunity to isolate and observe the effects of electronic changes on noncovalent interactions.[6] In our research, we have used host−guest chemistry to explore the role of recognition in the redox chemistry of flavoenzymes.[7] Flavoenzymes are redox-active proteins that use a flavin cofactor (generally FAD or FMN) to mediate a wide variety of biological processes over a wide range of redox potentials.[8] An important source of this versatility is the modulation of flavin reactivity through apoenzyme−cofactor interactions. Hydrogen-bonding,[7a] aromatic stacking,[7b] and conformational effects,[9] however, only account for a portion of the modulation of flavin recognition and redox properties seen in the flavoenzymes.

To probe the nature and effects of other flavoprotein−cofactor interactions, we have examined the available crystal structures of these enzymes. A general motif in these structures is an electron-rich (donor) atom positioned adjacent to the electron-deficient flavin nucleus (Figure 1a). These donors take such varied forms as tyrosine hydroxyl oxygens, backbone carbonyl oxygens, and disulfides.[10] Despite their proximity to the flavin nucleus, the role of these donors in flavin processes has not been explored.

To investigate the role of these donor atoms on flavin recognition and function, we first examined the electrostatic surface potential of the flavin nucleus. In recent research, we have used EPR to establish the validity of the B3LYP UKS wave function for the naphthalimide radical anion, a redox-active organic system analogous to flavin.[12] The electrostatic

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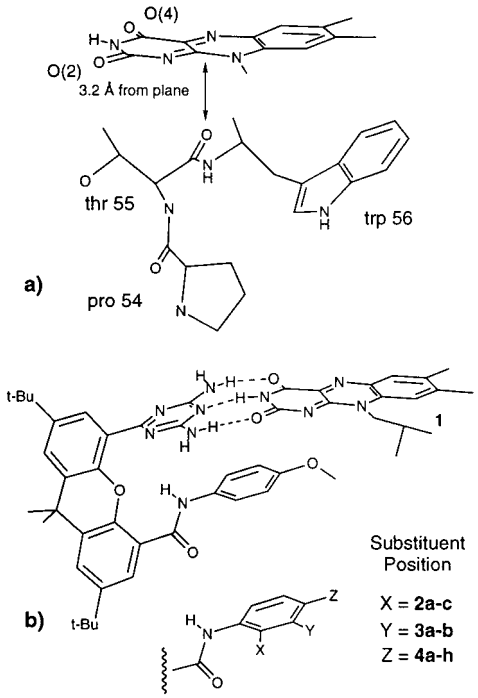


**Figure 1.** (a) Flavin binding site of flavodoxin isolated from red alga *Chondrus crispus*.[10a] (b) Flavin **1**−receptor **4c** complex.[11]

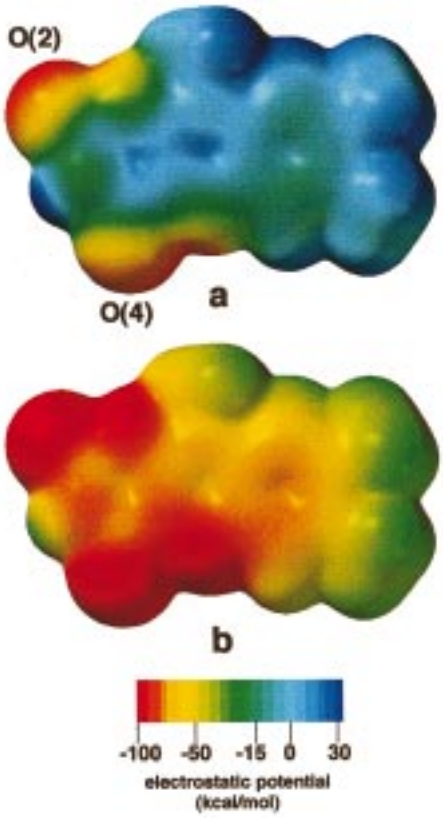


**Figure 2.** Electrostatic potential maps of (a) oxidized flavin ($Fl_{ox}$) and (b) flavin radical anion ($Fl_{rad}^-$).

potential map of fully oxidized flavin ($Fl_{ox}$) (Figure 2a) generated using this methodology shows the highly electron-deficient flavin nucleus with distinct areas of positive potential (blue) adjacent to the C(4a)−C(10a) ring juncture of the flavin.[13,14] These areas would be expected to interact favorably with donor atoms. Reduction of the flavin to the radical anion ($Fl_{rad}^-$) converts this region of the surface to negative potentials

**Table 1.** Binding Constants and Association Energies for Flavin **1**-Receptor Complexes

| receptor | substituent | $K_a$[a] ($M^{-1}$) | $\Delta G_a$ (kcal/mol) | $\Delta G_a Fl_{ox}$(donor−$\pi$) (kcal/mol) |
|---|---|---|---|---|
| none | | | | |
| **2a** | 2-F | 840 | −3.96 | 0.08 |
| **2b** | 2-$OCH_3$ | 1170 | −4.15 | −0.11 |
| **2c** | 2-CN | 780 | −3.92 | 0.12 |
| **3a** | 3-F | 910 | −4.01 | 0.03 |
| **3b** | 3-$OCH_3$ | 1490 | −4.30 | −0.26 |
| **4a** | 4-F | 2910 | −4.69 | −0.65 |
| **4b** | 4-$OCH_3$ | 2210 | −4.53 | −0.49 |
| **4c** | 4-CN | 3940 | −4.87 | −0.83 |
| **4d** | 4-$SCH_3$ | 4830 | −4.99 | −0.95 |
| **4e** | 4-$N(CH_3)_2$ | 3760 | −4.84 | −0.80 |
| **4f** | 4-$OCF_3$ | 1780 | −4.40 | −0.36 |
| **4g** | 4-Br | 3500 | −4.80 | −0.76 |
| **4h** | 4-$CH_3CH_3$ | 960 | −4.04 | 0 |

[a] $CDCl_3/CHCl_3$, 23 °C, all errors ±5%, except **4c** and **4f**, ±20%.

(Figure 2b), diminishing this interaction. This loss of the favorable donor atom-selective binding of $Fl_{ox}$ should make the reduction of the flavin more difficult, resulting in the modulation of the flavin redox potential to more negative values.

To provide experimental evidence for the role of donor atom−flavin interactions in the modulation of flavin redox potentials, we have synthesized receptors **4a**−**g**[15] (Figure 1b). In these hosts, a scaffold[16] positions donor atoms in direct contact with the electron-poor region of the flavin nucleus, analogous to their flavoenzyme prototypes. Because of the modular design of these hosts, the donor atom is readily varied while all other interactions are held constant. Comparison of the recognition properties of receptors **4a**−**g** with ethyl receptor **4h,** or controls **2a**−**c** and **3a,b**, then allows direct measurement of the energetics of specific donor−flavin interactions.

Quantification of donor atom−$\pi$ association with $Fl_{ox}$ was obtained via NMR titration in $CDCl_3$ or fluorescence-quenching titration in $CHCl_3$[17,18] (Table 1) Substantial increases (up to 5-fold) in flavin binding were observed upon the incorporation of a donor atom in receptors **4a**−**g**, relative to the alkyl-substituted receptor **4h**,[19] representing a free energy change ($\Delta G_a Fl_{ox}$(donor−$\pi$)) of −0.95 kcal/mol. This dramatic increase is not observed for the control receptors **2a**−**c** and **3a,b**,[20] where donor atom−flavin interactions are not present.[21] Thiomethyl

(13) Electrostatic potential maps were generated from B3LYP 6-31G* density functional calculations (Gaussian '94, Gaussian, Inc.) using the Spartan V4.1 graphics package (Wavefunction Inc.). We have recently demonstrated the the applicability of the B3LYP hybrid wave function to hydrogen-bonding and redox processes of analogous host−guest systems.

(14) A recent theoretical analysis of crystal structures suggesting donor atom−$\pi$ interactions supports our hypothesis of specific electron-deficient $\pi$ systems complexing with electron-donor atoms. Alkorta, I.; Rozas, I.; Elguero, J. *J. Org. Chem.* **1997**, *62*, 4687−4691.

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(17) Association constants determined for a 1:1 binding process of receptors with flavin using these two methods are equivalent: Tamura, N.; Mitsui, K.; Nabeshima, T.; Yano, Y. *J. Chem. Soc., Perkin Trans. 2* **1994**, 2229−2237.

(18) Nonpolar solvents were chosen to mimic the hydrophobic binding pocket found in flavoenzymes. $K_a$ and $E_{1/2}$ values were determined in similar noncompetitive solvents ($CDCl_3$ and $CH_2Cl_2$) chosen due to their attributes for NMR and electrochemical experiments, respectively. It was found that the presence of carrier electrolyte does not affect the association constants.

(19) Ethyl receptor **4a** was chosen to diminish the possible effect of dispersion interactions on recognition.

(20) Molecular modeling studies[11] indicate the possibility of a donor−$\pi$ interaction for **3b**, providing an explanation for the slightly enhanced recognition of flavin **1** for this receptor.

**Table 2.** Reduction Potential, $\Delta\Delta G$, and $\Delta G_a$ for Flavin **1**−Receptor Complexes

| receptor | substituent | $E_{1/2}{}^{a-c}$ (mV) | $\Delta\Delta G_{red}$ (kcal/mol) | $\Delta\Delta G$(donor−$\pi$)$^d$ (kcal/mol) | $K_a$ $Fl_{rad}{}^-$(donor−$\pi$)$^d$ ($M^{-1}$) |
|---|---|---|---|---|---|
| none | | −1290 | 0 | | |
| **2a** | 2-F | −1268 | −0.51 | −0.02 | 1990 |
| **2b** | 2-$OCH_3$ | −1273 | −0.39 | −0.13 | 2278 |
| **2c** | 2-CN | −1260 | −0.68 | 0.15 | 2529 |
| **3a** | 3-F | −1268 | −0.51 | 0.002 | 2156 |
| **3b** | 3-$OCH_3$ | −1279 | −0.26 | 0.29 | 2293 |
| **4a** | 4-F | −1281 | −0.21 | 0.33 | 4141 |
| **4b** | 4-$OCH_3$ | −1282 | −0.18 | 0.35 | 3024 |
| **4c** | 4-CN | −1295 | 0.12 | 0.65 | 3239 |
| **4d** | 4-$SCH_3$ | −1301 | 0.25 | 0.78 | 3138 |
| **4e** | 4-$N(CH_3)_2$ | −1282 | −0.18 | 0.35 | 5145 |
| **4f** | 4-$OCF_3$ | −1294 | 0.09 | 0.72 | 1522 |
| **4g** | 4-Br | −1293 | 0.07 | 0.67 | 3112 |
| **4h** | 4-$CH_3CH_3$ | −1267 | −0.53 | 0 | 2365 |

[a] Conditions: $CH_2Cl_2$, tetrabutylammonium perchlorate carrier (0.1 M), [**1**] = $1 \times 10^{-3}$ M, 23 °C. [b] [**2**−**4**] = $7 \times 10^{-3}$ M. [c] Referenced to ferrocene as an external standard.[23,24] [d] Relative to **4h**. [e] $-\Delta G_3$, calculated from the relationship, $-\Delta G_3 = \Delta G_1 + \Delta G_2 + \Delta G_4$ (Figure 3).

receptor **4d** showed the largest increase in association, a result that can be attributed to the increased size and polarizability of the sulfur atom, allowing enhanced electrostatic overlap. This hypothesis is further supported by an increase in binding upon changing from a fluorine atom (**4a**) to the larger and more polarizable bromine atom (**4g**).

With the contributions of donor atom to complexation of $Fl_{ox}$ quantified, we next examined the effect of the donor atom on the function of the flavin redox system, specifically the reduction of $Fl_{ox}$ to $Fl_{rad}{}^-$. Cyclic voltammetry (CV) studies [7a,22] were carried out in nonaqueous media to determine the reduction potential of flavin **1** when complexed with receptors **4a**−**h** and control receptors **2a**−**c** and **3a,b** (Table 1). As previously observed, three-point hydrogen bonding by control **4h** stabilizes $Fl_{rad}{}^-$, resulting in a less negative $E_{1/2}$.[7] Receptors **4a**−**g** with available donor atoms all exhibited substantially more negative reduction potentials (up to 34 mV, $\Delta\Delta G_{red}$ 0.78 kcal/mol relative to control **4h**) than the controls **2a**−**c** and **3a,b**. This modulation in reduction potentials is strongly correlated with the recognition of $Fl_{ox}$: The more tightly $Fl_{ox}$ was bound, the more negative the reduction potential. This fully supports the conclusion that reduction to $Fl_{rad}{}^-$ greatly diminishes the favorable donor atom−$\pi$ interactions observed between $Fl_{ox}$ and receptors **4a**−**g** Table 2).[23,24]

The change in free energies of association ($\Delta G_a$) for flavin **1**−receptor complexes upon reduction of $Fl_{ox}$ to $Fl_{rad}{}^-$ can be quantified through use of a thermodynamic cycle (Figure 3).[7] From this cycle, it is apparent that donor atom−$\pi$ interactions with receptors **4a**−**g** are greatly attenuated upon reduction of $Fl_{ox}$ to $Fl_{rad}{}^-$, even becoming unfavorable with the highly negative trifluoromethoxy receptor **4f** (Table 1).[25] This reduction in affinity substantiates the electrostatic origins of the enhanced binding of receptors **4a**−**g** with $Fl_{ox}$.

In conclusion, we have used computational and experimental methods to demonstrate and quantify donor atom−$\pi$ bonding,

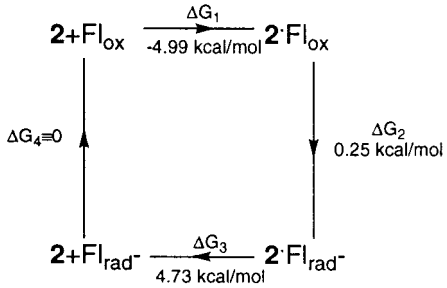


**Figure 3.** Thermodynamic cycle for the receptor−flavin redox system, with representative data for thiomethyl receptor **4d**.

a new noncovalent force. The magnitude of this interaction between flavin and appropriate donors is similar to that of a hydrogen bond, in terms of both recognition and redox modulation. The variety of donor atoms that interact with the electron-poor flavin suggest the wide scope of these interactions in biological systems. Further investigations into the roles of other electrostatic interactions on recognition processes are currently underway, and will be reported in due course.

## Experimental Section

$^1$H NMR titrations were performed using a Bruker 200-MHz NMR spectrometer. Infrared spectra were recorded on a Perkin-Elmer 783 spectrometer. Melting points are uncorrected. Cyclic voltammetry was performed using a Cypress Systems potentiostat.

Electrochemical measurements were obtained in methylene chloride (distilled over calcium hydride), with 0.1 M tetrabutylammonium perchlorate as electrolyte. These studies were performed in an argon-purged temperature controlled cell at 23 °C. The cell consisted of a 1-mm platinum button working electrode, a platinum wire auxiliary electrode, and a silver wire reference electrode isolated by a Vycor frit. Cyclic voltammetry was performed using a 200 mV/s scan rate, with ferrocene (sublimed) as an external standard.

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**Supporting Information Available:** Synthesis and characterization of receptors **2**, **3**, and **4** (8 pages, print/PDF). This material is contained in many libraries on microfiche, im-

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(23) Referenced to ferrocene as an external standard: Gagné, R.; Koval, C. Lisensky, G. C. *Inorg. Chem*, **1980**, *19*, 2854.

(24) All $E_{1/2}$ values are ±3 mV.

(25) Dimethylamino receptor **4e** retains an anomolously large fraction of the interaction energy. We are currently exploring the implications of this favorable amine-$Fl_{rad}$− interaction on the mechanism of the monoamine oxidases.

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