

From Enzyme to Molecular Device. Exploring the Interdependence of Redox and Molecular Recognition

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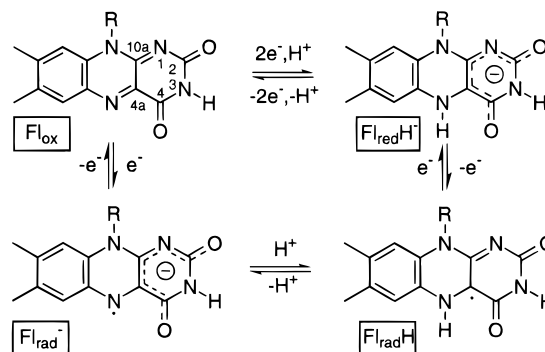
Introduction

The interdependence between redox events and molecular recognition is a prevalent theme in biological systems. Enzymes containing redox-active organic molecules including quinones,¹ flavins,² nicotinamides,³ and pterins⁴ use specific enzyme–cofactor interactions to regulate the reactivity of the cofactor. Through hydrogen bonding, π -stacking, and other electrostatic interactions, these enzymes selectively stabilize specific oxidation and protonation states of the cofactor. Consequently, redox enzymes can be regarded as intricate and efficient molecular devices that utilize molecular recognition to control redox events.

Fundamental principles observed in biological systems are applicable for the design of novel man-made molecular devices.^{5,6} Considerable effort has been devoted to the development of molecular shuttles,⁷ switches,⁸ wires,⁹ and logic gates,¹⁰ which have the potential to replace present day semiconductor technology in the creation of molecular scale computers. Other applications include biosensors^{11,12} and organic ferromagnets.¹³ Various examples have been reported in which a change in oxidation states controls photochemical properties^{8,14} and intermolecular interactions.^{15–17}

Understanding the fundamental principles governing the interplay of redox and molecular recognition is invaluable in the context of both biochemistry and material

Scheme 1. Common Redox and Protonation States of the Flavin Cofactor (lumiflavin, R = CH₃; FMN, R = CH₂((CHOH)₄-phosphate; FAD, R = CH₂((CHOH)₄-pyrophosphate-adenosine; flavin 1, R = CH₂CH(CH₃)₂)



science. In biological systems, the effects of hydrogen bonding, π -stacking, and dipolar interactions are difficult to isolate and quantify individually, and subtle interactions may even go unnoticed. This Account presents studies undertaken in our group using synthetic model systems to parametrically probe the effect of various molecular recognition elements on the redox behavior and physical properties of redox-active organic molecules. The results of these studies are applicable both to explain effects observed in biological systems and in the design of molecular devices.

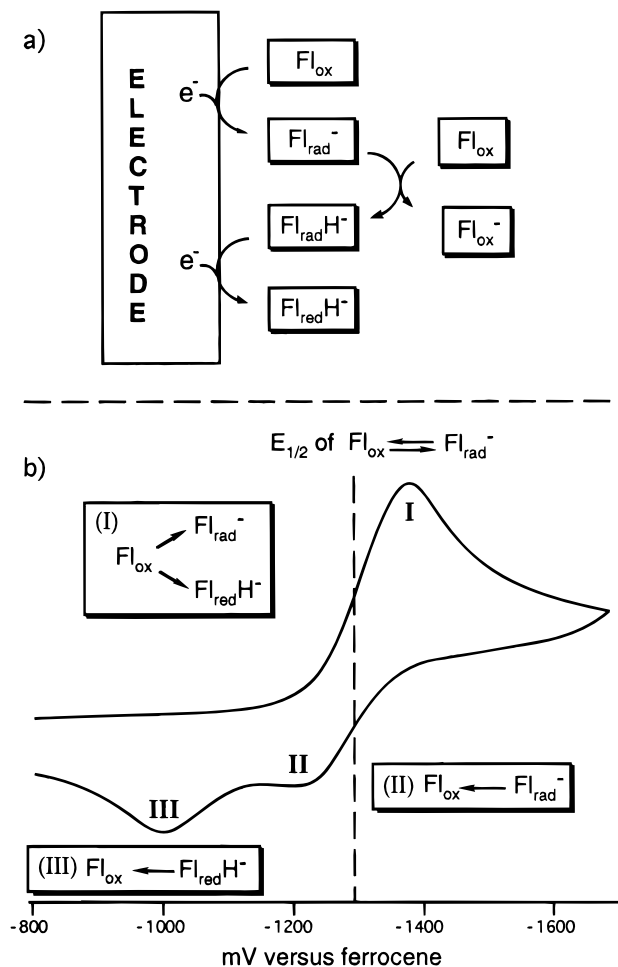
Flavoproteins containing the flavin cofactors FAD (flavin adenine dinucleotide) or FMN (flavin mononucleotide) are ubiquitous redox enzymes involved in metabolic processes, electron transfer, detoxification of xenobiotics, and the regulation of neurotransmitters.^{2,18} These enzymes exhibit considerable variation in flavin redox behavior depending on the active site microenvironment.^{18,19} Analogous to quinones, the oxidized flavin (**Fl_{ox}**) can be reduced in a two-electron process directly to the flavohydroquinone anion (**Fl_{radH}⁻**). Reduction can also occur sequentially via two one-electron processes involving the flavosemiquinone radical, in either the anionic (**Fl_{rad}⁻**) or neutral (**Fl_{radH}**) form (Scheme 1). Flavoenzymes are able to control the redox pathways that the cofactor follows, allowing catalysis of both one- and two-electron redox processes.

Aprotic solvents of low dielectric constant such as CH₂-Cl₂ effectively mimic the hydrophobic protein interior in which the flavin cofactor is buried, providing an appropriate model for flavoenzyme redox behavior. The processes occurring during the reduction of flavins in aprotic organic solvents, however, have been the cause of considerable controversy over the past 30 years.^{20,21} To obtain meaningful insight from model studies, this controversy required resolution. Using a combination of cyclic voltammetry (CV), simultaneous electrochemistry and EPR (SEEP), and UV/vis spectroelectrochemistry, we established that intermolecular proton transfer of the acidic imide proton of oxidized flavin **Fl_{ox}** causes the coupled electrochemical and chemical reactions shown in Scheme 2.²² In this process, **Fl_{ox}** is reduced to the radical anion

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Vincent M. Rotello received his B.S. from the Illinois Institute of Technology in 1985. He obtained a Ph.D. with Harry Wasserman from Yale University in 1990, and then did an NSF Postdoctoral Fellowship with Julius Rebek at MIT. He is currently an Associate Professor in the Department of Chemistry and the Program in Molecular and Cellular Biology at the University of Massachusetts. His principle motivation in research is the fundamental understanding of noncovalent interactions, and the application of this knowledge to biological and materials issues.

Scheme 2. (a) Schematic Representation of the Ece Pathway for Two-Electron Reduction of Flavins in Aprotic Media and (b) Cyclic Voltammogram of Flavin 1 Consisting of the Reduction Wave I Caused by the Ece Process and the Two Oxidation Waves II and III Caused by the Reoxidation of Fl_{rad}^- and $\text{Fl}_{\text{red}}\text{H}^-$, respectively



Fl_{rad}^- at the electrode. A portion of this anionic species is then protonated by the imide of Fl_{ox} in bulk solution, yielding the neutral radical $\text{Fl}_{\text{rad}}\text{H}$. Because $\text{Fl}_{\text{rad}}\text{H}$ has a less negative reduction potential than Fl_{ox} , it is instantly reduced to $\text{Fl}_{\text{red}}\text{H}^-$. This ece (electrochemical–chemical–electrochemical) process gives rise to only one reduction wave in the cyclic voltammogram (Scheme 2b). During the reoxidation process, Fl_{rad}^- is oxidized at more negative potentials than $\text{Fl}_{\text{red}}\text{H}^-$, resulting in the two oxidation waves observed. Because the $\text{Fl}_{\text{ox}} \rightarrow \text{Fl}_{\text{rad}}^-$ couple is essentially reversible, the ΔG for this process can be obtained directly from the half-wave potential $E_{1/2}$ between the reduction and the first oxidation wave via the Nernst equation.

Redox State and Charge Density

In flavoenzymes, the flavin cofactor is normally bound noncovalently to the protein scaffolding.²³ This recognition provides two functions: retention and orientation of the cofactor, and control of cofactor redox behavior through selective stabilization of different oxidation and protonation states. The latter role provides a modulation

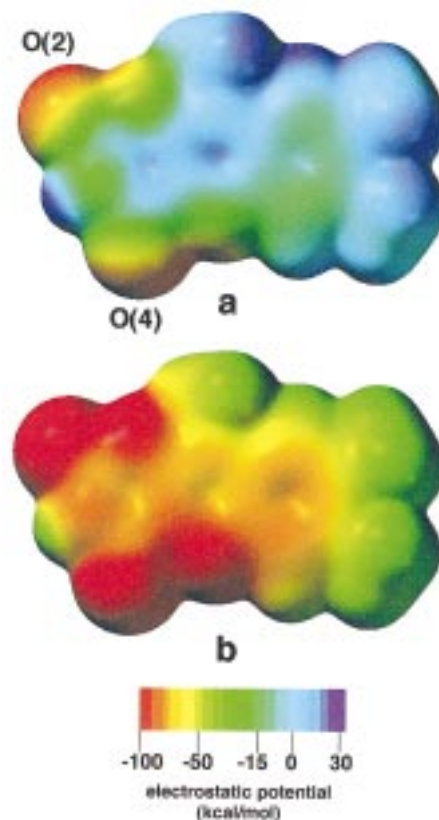


FIGURE 1. Electrostatic potential maps derived from 6-31G* B3LYP-DFT//3-21G UHF calculations for lumiflavin in (a) the oxidized (Fl_{ox}) and (b) the radical anion (Fl_{rad}^-) forms.

of flavin reduction potentials over a 500 mV range (12 kcal/mol),¹⁸ allowing the wide range of processes catalyzed by flavoenzymes. To understand this remarkable diversity, the redox modification of the flavin prosthetic group needs to be related to specific molecular recognition elements present at the active site.

Since most molecular recognition events such as hydrogen bonding, π -stacking, and dipolar interactions are essentially electrostatic in nature, they should be particularly sensitive to changes in charge density distribution within the host–guest complex. Significant changes in charge density distribution of redox-active molecules occur upon change of oxidation state. We have calculated the changes in the electrostatic surface potential between Fl_{ox} and Fl_{rad}^- computationally using B3LYP-DFT calculations on lumiflavin in both the oxidized and radical anion forms (Figure 1). This computational protocol has been shown to accurately reproduce experimental results (vide infra),²⁴ providing access to properties that are not experimentally accessible.

One-electron reduction of flavin increases the negative potential of carbonyl oxygens O(2) and O(4) from -45 kcal/mol (Fl_{ox}) to -116 kcal/mol (Fl_{rad}^-), making them stronger hydrogen bond acceptors. Concurrently, the electron-deficient aromatic framework of Fl_{ox} is converted by reduction into the electron-rich Fl_{rad}^- . The positive electrostatic potential of $+26$ kcal/mol on the central ring allows Fl_{ox} to form favorable π -stacking interactions with

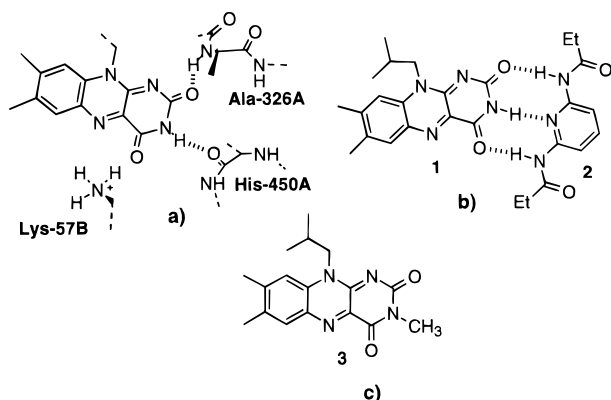


FIGURE 2. (a) Active site of lipoamide dehydrogenase,²⁵ (b) flavin 1–receptor 2 complex, and (c) control *N*(3)-methyl flavin 3.

electron-rich aromatic systems and favorable electrostatic interactions with electron rich functional groups. Fl_{rad}^- , in contrast, possesses a negative electrostatic potential of -77 kcal/mol on the central ring, making interactions with adjacent electron-rich systems highly unfavorable. These gross changes in charge density are accompanied by very few structural differences. The resulting effects are therefore of purely electrostatic nature, greatly simplifying their interpretation.

Redox Modulation through Hydrogen Bonding

Enzyme–cofactor hydrogen bond interactions involving carbonyl oxygens O(2) and O(4), and imide proton N(3)H of flavin are a prevalent motif in flavoproteins,^{25,26} and have been postulated to play an important role in flavin redox modulation.²⁷ Since this hydrogen bonding predominantly involves protein backbone functionality (Figure 2), the effectiveness of mutation studies in probing the role of these interactions on the redox behavior of the cofactor is limited. To address this issue, we have synthesized a series of acylated diaminopyridine receptors including receptor 2 (Figure 2) capable of replicating flavoprotein–cofactor interactions.

Binding to Fl_{ox} was established by plotting the chemical shift of N(3)H of flavin 1 in CDCl_3 versus equivalents of receptor added, with nonlinear least-squares fit of the resulting NMR titration curve to a 1:1 binding isotherm.²⁸ The observation of an intermolecular NOESY cross-peak between flavin 1 N(3)H and the amide protons of receptor 2 further supports the proposed binding geometry.²⁹ Host–flavin recognition was controlled through variation of diaminopyridine acyl substituents, providing association constants from 55 to 540 M^{-1} . This modification of molecular recognition was the result of modulation of both hydrogen bonding (via changes in acidity) and electrostatic through-space field effects arising from the acyl substituents. In later work, we have decoupled these two effects using aryl diaminotriazine-based receptors.³⁰

With host–guest complexation verified, we next studied the effect of hydrogen bonding on the redox behavior of flavin 1.²⁸ Addition of receptor 2 has a 2-fold effect on the CV of flavin 1 in CH_2Cl_2 (Figure 3): the reduction potential of the flavin 1–receptor 2 complex is 155 mV

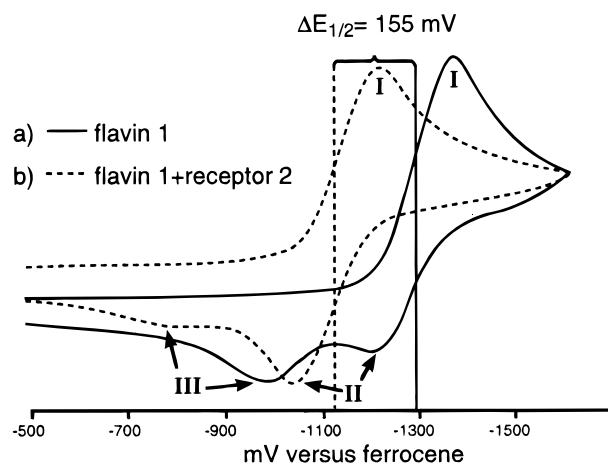
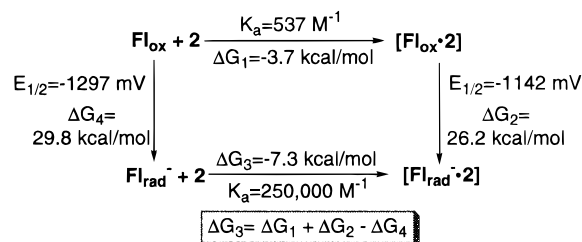


FIGURE 3. Cyclic voltammograms for (a) flavin 1 and (b) the flavin 1–receptor 2 complex showing the single reduction wave (I) and the two oxidation waves corresponding to reoxidation of Fl_{rad}^- (II) and $\text{Fl}_{\text{red}}\text{H}^-$ (III).

Scheme 3. Thermodynamic Cycle Quantifying Radical Anion–Receptor Recognition



less negative than the reduction potential of free flavin 1, and the first oxidative wave corresponding to reoxidation of Fl_{rad}^- is greatly enhanced, indicating that the ece process leading to further reduction of Fl_{rad}^- to $\text{Fl}_{\text{red}}\text{H}^-$ is effectively suppressed in the flavin 1–receptor 2 complex.

The 155 mV shift of the standard reduction potential indicates a substantial stabilization (3.6 kcal/mol) of Fl_{rad}^- , mimicking the shift in flavin cofactor reduction potential observed in flavoproteins that react via the radical anion, such as the electron transferring flavoproteins (ETF). A change in reduction potential was not observed upon addition of receptor 2 to N(3) methyl flavin 3, which cannot form hydrogen-bonded complexes, confirming that *specific* hydrogen bond contacts are required for the stabilization of the flavin 1 radical anion by receptor 2.

During our electrochemical studies, near-maximal shifts occurred at a receptor concentration at which only 22% of the oxidized flavin would be bound, indicating substantially increased binding to receptor 2 upon reduction of Fl_{ox} to Fl_{rad}^- . As predicted computationally, the reduction of Fl_{ox} to Fl_{rad}^- leads to an increase in charge density on the carbonyl oxygens O(2) and O(4) of flavin 1, making them stronger hydrogen bond acceptors. This redox-based enhancement in recognition can be quantified through use of a thermodynamic cycle, where experimentally accessible K_a and $E_{1/2}$ values are used to determine the ΔG and K_a for binding of Fl_{rad}^- to receptor 2 (Scheme 3). Using this cycle, a 500-fold increase in

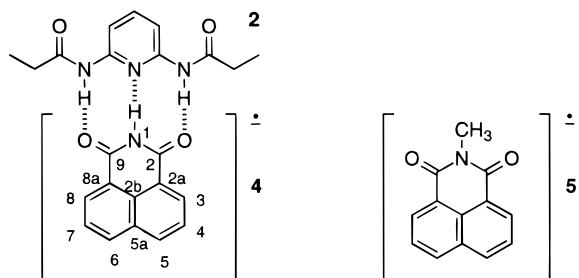


FIGURE 4. Hydrogen-bonded complex between naphthalimide 4 radical anion ($\mathbf{N}_{\text{rad}}^-$) and receptor **2**, negative control *N*(1)-methyl naphthalimide **5**, in which the binding site is blocked via alkylation.

binding constant to receptor **2** was observed upon reduction of \mathbf{Fl}_{ox} to $\mathbf{Fl}_{\text{rad}}^-$.

The relationship demonstrated graphically with the thermodynamic cycle can also be expressed mathematically:

$$K_a(\text{red})/K_a(\text{ox}) = e^{(nF/RT)(E_{1/2}(\text{bound}) - E_{1/2}(\text{unbound}))} \quad (1)$$

where $K_a(\text{ox})$ and $K_a(\text{red})$ are the association constants in the oxidized and reduced form, and $E_{1/2}(\text{unbound})$ and $E_{1/2}(\text{bound})$ ³¹ are the standard reduction potentials in the unbound and receptor-bound form. According to eq 1, enhanced binding ($K_a(\text{red})/K_a(\text{ox}) > 1$) inherently accompanies a positive shift in $E_{1/2}$ ($E_{1/2}(\text{bound}) - E_{1/2}(\text{unbound}) > 1$) and vice versa, an important principle both in the understanding of biological systems and in the design of molecular devices.

Modulation of Spin Density via Hydrogen Bonding

The previous section demonstrates that altered electron density distribution affects hydrogen bonding. We next investigated the inverse effect of hydrogen bonding on the electron density and, specifically, the spin density distribution of radicals, an area of significance in the contexts of biochemistry,^{32,33} chemistry, and material science.¹³ Due to the complexity and lack of symmetry of flavin, EPR is unsuited for the quantification of subtle changes in hyperfine coupling constants (hfc's) of $\mathbf{Fl}_{\text{rad}}^-$.³⁴ The radical anion of naphthalimide **4** ($\mathbf{N}_{\text{rad}}^-$), however, exhibits similar redox-enhanced molecular recognition as $\mathbf{Fl}_{\text{rad}}^-$ (Figure 4)³⁵ yet is simple enough to yield readily interpretable EPR spectra of high resolution.

The spectra of $\mathbf{N}_{\text{rad}}^-$ and the $\mathbf{N}_{\text{rad}}^-$ -receptor **2** complex acquired via simultaneous electrochemistry and EPR (SEEP) are notably different. The spectrum of the hydrogen bound radical exhibits a greater overall signal width and concomitantly less overlap of hyperfine lines (Figure 5).²⁴ Almost complete complexation is achieved at 1 equiv of receptor **2**, providing further evidence of redox-enhanced molecular recognition. Addition of receptor **2** had only negligible effects on the SEEP spectrum of the control, *N*(1)-methyl naphthalimide **5** radical anion, where the binding site is blocked through alkylation, confirming that specific hydrogen bond interactions cause the changes in the spectrum of $\mathbf{N}_{\text{rad}}^-$.

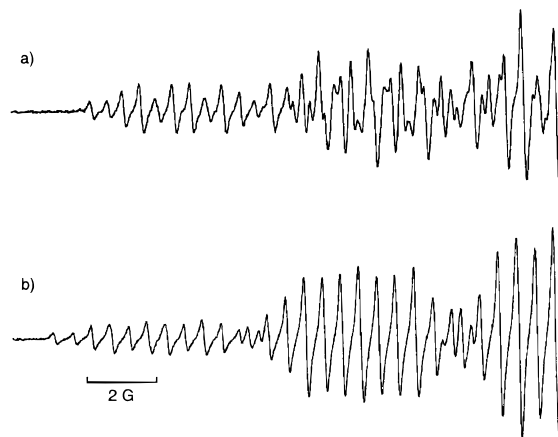


FIGURE 5. Low-field half of the SEEP spectra of (a) $\mathbf{N}_{\text{rad}}^-$ and (b) the $[\mathbf{N}_{\text{rad}}^- \cdot \mathbf{2}]$ complex.

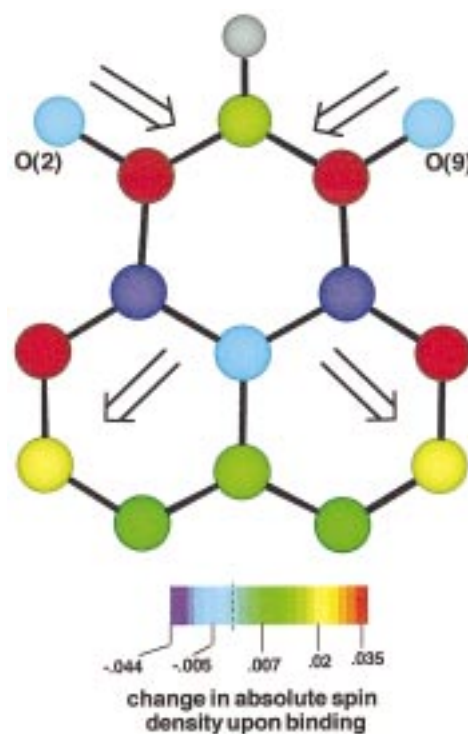


FIGURE 6. Change in the absolute spin density of $\mathbf{N}_{\text{rad}}^-$ upon complexation with **2** as predicted by DFT-B3LYP calculations.

Spectrum simulation and iterative curve-fitting enabled precise determination of hyperfine coupling constants (hfc's) for hydrogen bound and free $\mathbf{N}_{\text{rad}}^-$. Hydrogen bonding to receptor **2** enhances the spin polarization of $\mathbf{N}_{\text{rad}}^-$, which is manifested in the increased spectral width. The experimental hfc's further provide a critical benchmark for the validation of ab initio computational studies of $\mathbf{N}_{\text{rad}}^-$ in the unbound and receptor **2** bound forms. The B3LYP hybrid functional has been shown to accurately model organic π -radicals³⁶ and hydrogen bonding,³⁷ and has been applied to biologically relevant radicals.³⁸ DFT-B3LYP calculations of the $\mathbf{N}_{\text{rad}}^-$ -receptor **2** system were in good overall quantitative agreement with experimental results, and can therefore be used to predict properties not experimentally accessible, such as electrostatic po-

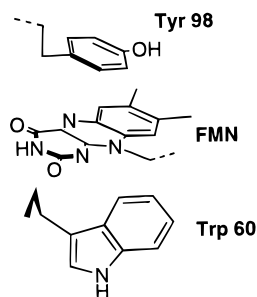


FIGURE 7. Aromatic residues flanking the flavin cofactor in the FMN binding site of *D. vulgaris* flavodoxin.⁴⁰

tential (vide supra), electron density, and spin density distribution.

According to the DFT-B3LYP calculations, complexation by receptor **2** shifts the spin density away from the carbonyl oxygens involved in hydrogen bonding, and toward the carbonyl carbons C(2) and C(9) (Figure 6). The largest increase in spin density, however, is observed in the naphthyl moiety of N_{rad}^- (C(3)–C(8)). Since hydrogen bonding is a two-electron process, it causes a distortion of lower, filled molecular orbitals toward the binding site, leading to an increase in charge density at the carbonyl oxygens. To maintain orthogonality between wave functions, the semioccupied molecular orbital (SOMO) is distorted away from the binding site. A similar effect is expected in the flavin radical anion–receptor **2** complex, causing a movement of spin density away from the hydrogen bonding surface and toward the distal xylene ring. Since flavoprotein electron-transfer events take place through the xylene ring,³⁹ an increase in spin density at the xylene moiety should strongly affect these processes.

Redox Modulation through π -Stacking

Aromatic stacking interactions are important features in a number of flavoproteins such as the flavodoxins,²⁶ in which the two faces of the FMN cofactor are flanked by either a tryptophane/tyrosine or a tryptophane/methionine dyad. These electron-transfer proteins utilize exclusively the $\text{Fl}_{\text{rad}}\text{H}/\text{Fl}_{\text{red}}\text{H}^-$ redox cycle, facilitated by selective stabilization of the neutral radical.⁴⁰ Mutation studies of *Desulfovibrio vulgaris* flavodoxin have shown that Tyr 98 (Figure 7) is involved in both the redox modulation and the stabilization of the neutral radical.⁴¹

To quantify the effect of π -stacking on flavin redox behavior, we have synthesized a series of xanthene-based receptors⁴² that provide a rigid scaffolding for the arrangement of recognition elements in a well-defined geometry (Figure 8). The triazine at the top provides three-point hydrogen bond interactions with flavin, positioning the flavin aromatic face above the bottom aromatic substituent. Effects induced by π -stacking in the complexes formed between flavin **1** and receptors **6b,c** can then be isolated by reference to the receptor **6a**–flavin **1** complex, where only hydrogen bonding is responsible for modulation of flavin properties.

Fluorescence quenching experiments verify the predicted π -stacking interactions in the flavin **1**–receptor **6**

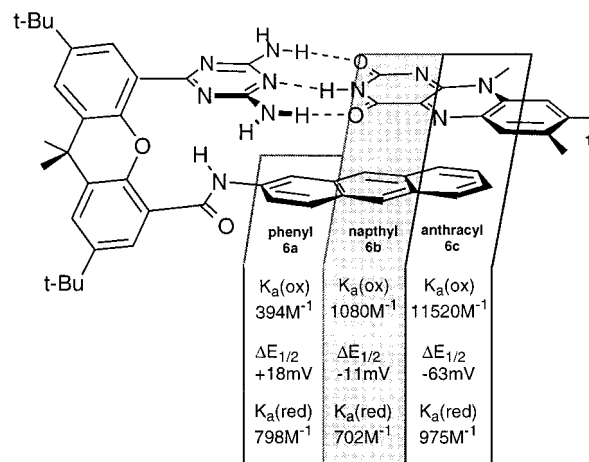


FIGURE 8. Molecular modeling structure (AMBER) of the flavin **1**–receptor **6** complex and thermodynamic constants for host–guest binding.⁴³

complexes.⁴⁴ A progressive decrease in the fluorescence emission of flavin **1** is observed with increasing π -overlap, with almost complete quenching in the presence of the anthracyl receptor **6c**. Addition of receptors **6a–c** causes no quenching in the fluorescence of N(3) methyl flavin **3**, indicating that quenching is the result of close proximity to the π -stacking unit in the preorganized H-bonded complex. NMR titrations of flavin **1** with receptors **6a–c** reveal an up to 44-fold increase in K_a , equivalent to a free energy change of -2.2 kcal/mol, due to favorable π -stacking interactions with Fl_{ox} (Figure 8).

Phenyl receptor **6a**, which cannot form appreciable aromatic–aromatic contacts with bound flavin **1**, causes an 18 mV shift in the $E_{1/2}$ of flavin **1** to less negative values, accompanied by an enhancement of the reversible reoxidation wave. These two effects are indicative of the stabilization of Fl_{rad}^- by hydrogen bonding, as observed in the flavin **1**–receptor **2** complex. The π -stacking receptors **6b,c**, however, shift the flavin **1** $E_{1/2}$ to more negative values, making flavin **1** harder to reduce. Using the flavin **1**–receptor **6a** complex as reference, the unfavorable π -stacking interactions shift the reduction potential by up to -81 mV, causing a change in free energy of up to $+1.5$ kcal/mol. As discussed earlier, one-electron reduction converts the electron-deficient Fl_{ox} into the electron-rich Fl_{rad}^- . While Fl_{ox} undergoes favorable π -stacking interactions with the electron-rich aromatic stacking unit, reduction to Fl_{rad}^- causes two electron-rich systems to be adjacent to each other, resulting in mutual repulsion. Although reduction increases the strength of hydrogen bonding, this increase is more than offset by the unfavorable π -stacking interactions, manifested in a lowering of the association with receptor **6c** from $11\,520\text{M}^{-1}$ for Fl_{ox} to 975M^{-1} for Fl_{rad}^- . In the flavodoxins, analogous unfavorable π -stacking interactions between aromatic amino acid residues and Fl_{rad}^- most likely influence the selective formation of the neutral radical $\text{Fl}_{\text{rad}}\text{H}$, since protonation of Fl_{rad}^- significantly decreases the overall negative charge density of the flavin radical.

To gain insight into the modulation of *submolecular* properties through π -stacking, we explored the effect of

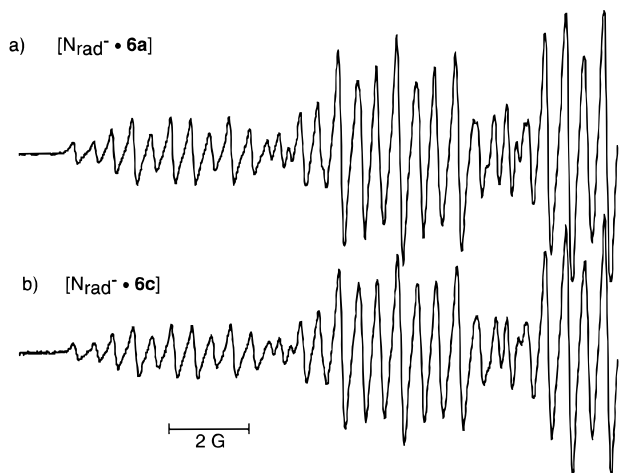
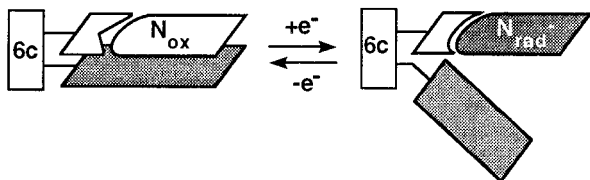


FIGURE 9. Low-field half of the EPR spectra of the (a) $[\text{N}_{\text{rad}}^- \cdot 6\text{a}]$ and (b) $[\text{N}_{\text{rad}}^- \cdot 6\text{c}]$ complex.

Scheme 4. Conformational Switch in the Naphthalimide 4–Receptor 6c Complex upon Reduction of NO_x to N_{rad}^- Due to Repulsion between N_{rad}^- and the π -Stacking Unit (at the same time, H-bonding interactions are increased)



complexation by receptors **6a** and **6c** on the SEPR spectrum of the naphthalimide **4** radical anion (N_{rad}^-). As expected for a predominantly hydrogen bonding receptor, addition of **6a** causes an overall increase in spin polarization of N_{rad}^- and a distortion of spin away from the binding site. Surprisingly, the SEPR spectrum of $[\text{N}_{\text{rad}}^- \cdot 6\text{a}]$ and $[\text{N}_{\text{rad}}^- \cdot 6\text{c}]$ are almost identical (Figure 9). This indicates that there are no interactions between the **6c** anthracyl substituent and bound N_{rad}^- . This supports a structure for the $[\text{N}_{\text{rad}}^- \cdot 6\text{c}]$ complex with divergent aromatic surfaces (Scheme 4), a direct consequence of the repulsion between the two electron-rich systems. Further studies concerning this conformational switch are currently underway.

Redox Modulation through Donor Atom– π Interactions

Another frequently observed molecular recognition motif in flavoproteins is the proximity of electron-rich donor atoms such as oxygen (tyrosyl hydroxyl group,⁴⁰ backbone carbonyl⁴⁵) or sulfur (methionine thioether,²⁶ cysteine thiol, or cystine disulfide²⁵) to the face of the flavin cofactor.⁴⁶ To probe these interactions, we again exploited the preorganized binding geometry provided by the modular xanthene receptors. Using this scaffolding, receptors **7a–e** place functional groups in the required orientation for donor atom– π interactions with the C(4a) and C(10a) positions of bound flavin **1**.⁴⁷ Parametric quantification of the resultant interaction is provided

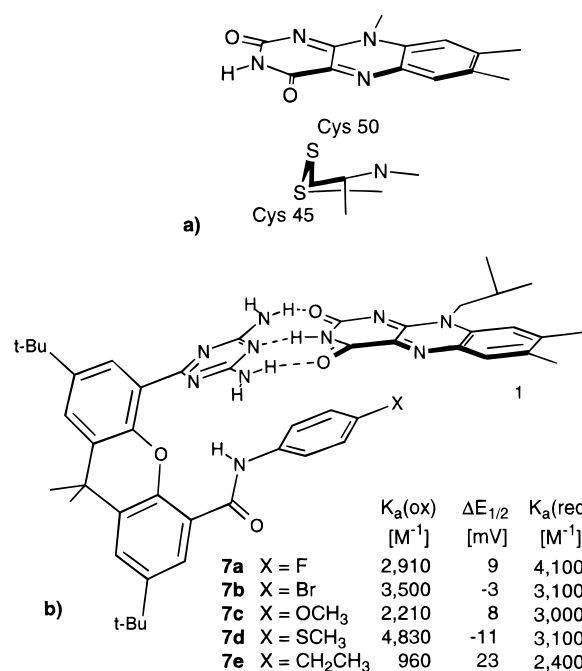


FIGURE 10. (a) Flavin binding site of *Pseudomonas fluorescens* lipoamide dehydrogenase.²⁵ (b) Flavin **1**–receptor **7** complex and thermodynamic constants for host–guest binding.⁴³

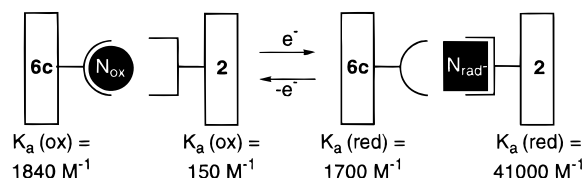
through reference to receptor **7e**, which contains the electrostatically neutral *p*-ethyl group.

Donor atom– π interactions increase the association constant of receptors **7a–d** with Fl_{ox} up to 5-fold, compared to ethyl receptor **7e** (Figure 10), a change in free energy of -0.95 kcal/mol. The efficiency of this interaction is directly correlated with the size and polarizability of the donor atom. This is demonstrated by the enhanced recognition of Fl_{ox} by thiomethoxy receptor **7d** relative to methoxy receptor **7c**, or bromine receptor **7b** relative to fluorine receptor **7a**.

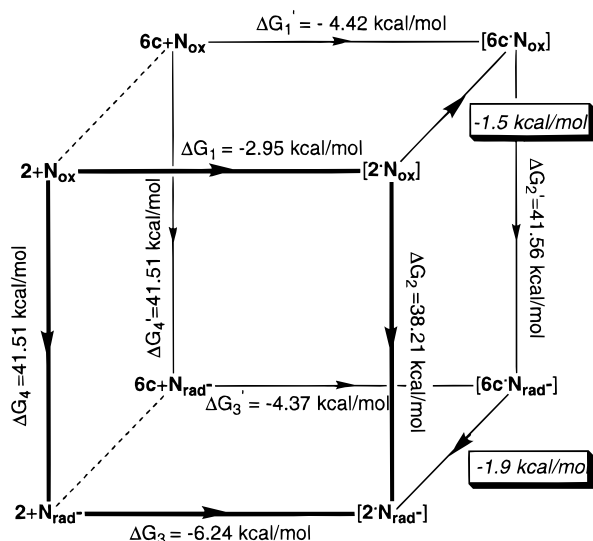
Reduction of Fl_{ox} to the overall electron-rich Fl_{rad}^- should diminish or eliminate donor atom– π interactions. This is evidenced by the shift of the reduction potential of flavin **1** to more negative values upon complexation with receptors **7a–d** relative to control receptor **7e**. This shift corresponds to a maximal change in free energy of reduction due to donor atom– π interactions of 0.78 kcal/mol. For thiomethoxy receptor **7d** this results in a decrease in association constant from 4830 M^{-1} for Fl_{ox} to 3100 M^{-1} for Fl_{rad}^- , more than offsetting the gain in binding strength due to redox-enhanced hydrogen bonding.

Complimentary Redox Modulation Applied in the Design of Molecular Devices

As described in previous sections, we have observed that reduction of Fl_{ox} to Fl_{rad}^- greatly enhances hydrogen bonding. Conversely, π -stacking and donor atom– π interactions become unfavorable. These diametrically opposed effects of redox on molecular recognition can be used to create a molecular switch, where the binding preference to each of the two competing hosts is regulated

Scheme 5. Redox-Mediated Switch in Molecular Recognition⁴⁹

Scheme 6. Redox Cube Quantifying the Binding Preference of Naphthalimide in the Oxidized and Radical Anion Forms



via the redox state of the guest. To explore this possibility, we have investigated the binding of naphthalimide **4** in the oxidized (N_{ox}) and radical anion (N_{rad}^-) forms to the purely hydrogen bonding diaminopyridine receptor **2** and the π -stacking anthracyl receptor **6c**.⁴⁸

Binding to host **2** was enhanced by a factor of more than 250-fold through reduction of N_{ox} to N_{rad}^- (Scheme 5). Host **6c** on the other hand experiences a slight decrease in association constant upon guest reduction, due to the offsetting effects of redox on hydrogen bonding and π -stacking. Combination of the two thermodynamic cycles for the naphthalimide **4**–receptor **2** and the naphthalimide **4**–receptor **6c** systems into a redox cube (Scheme 6) shows that N_{ox} has a 1.5 kcal/mol preference for binding to the π -stacking receptor **6c**, while N_{rad}^- interacts 1.9 kcal/mol more strongly with the purely hydrogen bonding receptor **2**.

Experimental verification of the predicted binding preference in the oxidized state was obtained via NMR. Addition of N_{ox} to a 1:1 mixture of hosts **2** and **6c** caused a large downfield shift of the amine protons of **6c**, yet virtually no shift of the amide protons of **2** was observed. SEEPR provided proof of the opposite binding preference in the radical anion state. Binding to host **2** distorts the spin density distribution of N_{rad}^- to a different degree than binding to host **6c**, resulting in distinct spectra for N_{rad}^- , $[\mathbf{2}\cdot\text{N}_{\text{rad}}^-]$, and $[\mathbf{6c}\cdot\text{N}_{\text{rad}}^-]$. Bulk electrolysis of N_{ox} in the presence of 1 equiv each of **2** and **6c** resulted in a spectrum almost identical to the spectrum of pure $[\mathbf{2}\cdot\text{N}_{\text{rad}}^-]$, and markedly different from the spectrum of $[\mathbf{6c}\cdot\text{N}_{\text{rad}}^-]$ (Figure 11). The ratio of $[\mathbf{2}\cdot\text{N}_{\text{rad}}^-]$ to $[\mathbf{6c}\cdot\text{N}_{\text{rad}}^-]$ in this

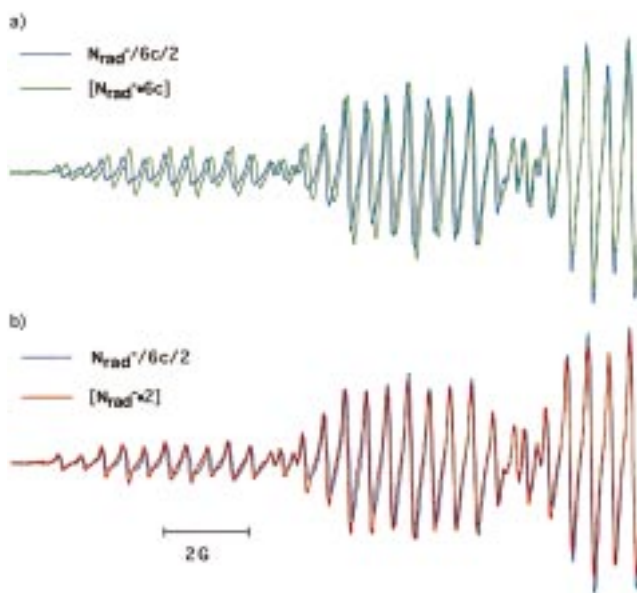


FIGURE 11. SEEPR spectrum of the three-component system overlaid with the spectra of (a) $[\text{N}_{\text{rad}}^- \cdot \mathbf{6c}]$ and (b) $[\text{N}_{\text{rad}}^- \cdot \mathbf{2}]$.

competition experiment was found to be at least 87:13 by spectrum simulation, indicating a substantial preference of N_{rad}^- for binding to **2** even in the presence of **6c**.

The change in binding preference resulting from a change in redox state renders this system a three-component, two-pole molecular switch. We are currently extending our studies to surface-supported analogues, with potential applications in molecular scale logic operations and data storage.

Conclusions and Outlook

Manipulation of redox-active molecules through molecular recognition represents a key process in the regulation of biological redox reactions. To gain insight into how this regulation is achieved, we have conducted a series of parametric studies using synthetic host systems, probing the effect of hydrogen bonding, π -stacking, and donor atom– π interactions on the redox properties of flavin and naphthalimide. The results obtained from our model systems were found to correlate with effects observed in flavoenzymes, demonstrating both the profound and subtle effects brought about via intermolecular interactions.

In a broader sense, the modification of electronic properties through recognition processes is central to the understanding of both redox and nonredox transformations. The ability to uncouple electronic and structural effects using redox-active host–guest systems provides an important and ongoing tool we are using to explore broad issues of enzymatic and nonenzymatic catalysis.

The insight gained through study of the subtle and efficient machinery of biological redox systems is likewise directly applicable to the creation of redox-based molecular devices. The capacity to control redox systems electronically allows direct computer control of these devices. Furthermore, the ability to create electrochemical

sensors and biosensors that need no spectroscopic signal transduction greatly simplifies their design. We are pursuing both of these avenues in our current research.

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