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Model systems for flavoenzyme activity: an investigation of the role functionality attached to the C(7) position of the flavin unit has on redox and molecular recognition properties†‡

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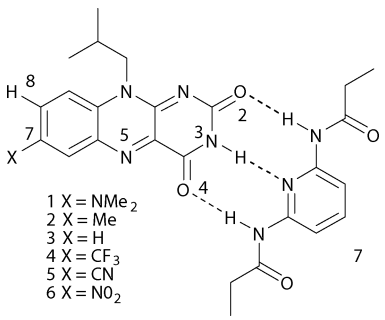
We describe the role functionality attached to the C(7) position of a family of flavin derivatives has in tuning their redox and recognition properties and the subsequent exploitation of two of these derivatives as a three-component electrochemically controllable molecular switch.

Flavoenzymes are a class of proteins that catalyse a range of redox transformations and mediate one- and two-electron transfer processes in biological systems.[1] Supramolecular interactions between the flavin cofactor and the apoenzyme modulate the redox potential of the flavin moiety by over 500 mV, tuning the redox properties of the flavin units to match their biological function.[2] X-Ray crystallography of flavoenzymes has provided important information regarding the nature and relative position of functionality in the flavoenzyme active site; however, little information is provided relating to the role individual interactions play in modulating flavin properties.[3] In other studies, the apoenzymes of flavoenzymes have been reconstituted with artificial flavins to probe the role non-covalent interactions around the flavin binding site play in modulating the reactivity and properties of the cofactor.[4]



Small molecule model system studies of flavoenzymes provide insight into biological processes while simultaneously providing access to new functional molecular systems. Synthetic model systems have been utilised to explore the role of non-covalent interactions in modulating redox properties of flavins.[5] Furthermore, structurally modified flavin units in their C(7) and C(8) positions have been used to quantify the role structural modification of this type has in controlling their physical properties in aqueous and non-aqueous media.[6] However, in these investigations the role functionality attached to the non-biomimetic C(7) position has largely been overlooked. In this communication, we report the role functionality attached to the C(7) position of the flavin unit has in modulating both the redox and recognition properties. We then use this modulation to create a three-component electrochemically controllable molecular switch from flavin derivatives **1** and **6** and complementary diamidopyridine (DAP) derivative **7**.

A family of synthetic flavin derivatives **1–6** have been synthesised with a range of electron donating and withdrawing functionality attached to the C(7) position (see ESI†). Slow evaporation of solvent from a solution of **1** dissolved in toluene resulted in the formation of X-ray quality crystals.§ The X-ray structure of compound **1** is shown in Fig. 1, and unequivocally confirms the presence of a $-NMe_2$ group attached to the C(7) position of the flavin. In the solid state, the flavin units self-assemble *via* two point hydrogen bonding between the imide moieties of adjacent flavin units, resulting in a dimeric structure.

We have investigated the complexation of flavin derivatives **1–6** with complementary derivative **7** using NMR titrations. It has previously been shown that DAP derivative **7** serves as an effective host for flavin derivatives and mimics the three point hydrogen bonding interactions with the complementary residues of the apoenzyme.[7] The titrations resulted in a smooth downfield shift in the hydrogen attached to N(3) of the flavin upon addition of aliquots of **7**. The titration data were successfully fit to a 1 : 1 binding isotherm using non-linear curve fitting methods to afford the association constants (K_a) provided in Table 1. It is clear from these data that the electronic characteristics of the functionality attached to the

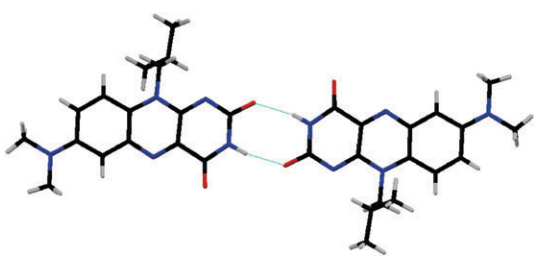


Fig. 1 X-Ray crystal structure of flavin **1**. Hydrogen bonding interactions are highlighted in light blue. The molecular structure was visualised using Mercury 1.4.2.

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‡ Electronic supplementary information (ESI) available: Synthesis of derivatives **1–6**. CCDC 715445. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/b900269n

Table 1 Calculated and LFER predicted binding and electrochemical properties of flavins **1–6**

	K_a/M^{-1}[a]	E, SWV/V	$\Delta G_{binding}$ exp.[b]/ kcal mol^{-1}	$\Delta G_{binding}$ LFER[c]/ kcal mol^{-1}	ΔG_{redox} exp.[d]/ kcal mol^{-1}	$\Delta G_{redox,}$ LFER[c]/ kcal mol^{-1}
1	446 ± 22	−1.40	−3.61	−2.53	32.29	31.78
2	455 ± 23	−1.36	−3.61	−3.15	31.36	31.15
3	460 ± 23	−1.33	−3.63	−3.63	30.67	30.67
4	482 ± 24	−1.21	−3.66	−6.60	27.90	27.70
5	434 ± 22	−1.15	−3.60	−7.50	26.52	26.81
6	436 ± 22	−1.10	−3.60	−9.60	25.37	25.70

[a] Determined in $CDCl_3$ at 298 K. [b] Calculated using: $\Delta G = -RT\ln K_a$. [c] Calculated using: $\Delta G(X,Y) = \rho_m\sigma_m(X) + \rho_p\sigma_p(Y) + \Delta G(H,H)$, where $\rho_m = -6.9$ kcal mol^{-1} σ^{-1} and $\rho_p = -4.6$ kcal mol^{-1} σ^{-1}. [d] Calculated using: $\Delta G = -nFE$.

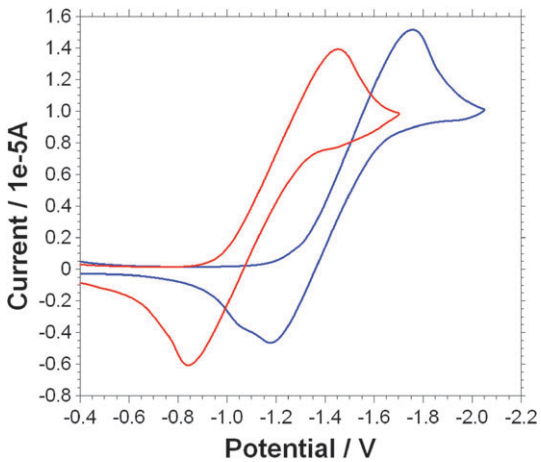

Fig. 2 CVs of compound **1** (blue line) and compound **6** (red line) recorded in CH_2Cl_2 (~1 mmol). Scan rate = 100 mV s^{-1}.

C(7) position plays little role in modulating the electron density of the carbonyl oxygen atoms O(2) and O(4) of the flavin moiety, and thus the binding efficiency with complementary DAP derivative **7**.

We next investigated the electrochemical properties of derivatives **1–6** dissolved in CH_2Cl_2 and upon addition of excess **7** using cyclic (CV) and square wave voltammetries (SWV) (Fig. 2 and Table 1). In contrast to the recognition properties, functionality attached to the C(7) position plays an important role in controlling the redox properties of the flavin unit. Indeed, upon exchanging the electron donating $-NMe_2$ group of compound **1** with an electron withdrawing $-NO_2$ group of compound **6**, over a 300 mV change in the reduction potential to a less negative value was observed. In each case the addition of DAP derivative **7** resulted in a near-identical −100 (±10) mV shift in the reduction of the flavin unit (compared to the free flavin derivative), indicating that significant stabilisation of the flavin radical anion state occurs upon binding to DAP.[7]

Interestingly, for derivative **1**, and to a lesser extent derivative **2**, a single reduction wave and two distinct reoxidation waves were observed for these reductions.[8] The first reduction wave can be attributed to the reversible formation of $\mathbf{1}_{rad}^-$ and $\mathbf{2}_{rad}^-$, whereas the second reoxidation waves are due to an electrochemical–chemical–electrochemical (e–c–e) process where some of the $_{rad}^-$ species formed at the electrode surface rapidly deprotonates the flavin in the bulk solution. The protonated flavin radicals ($\mathbf{1}_{rad}\mathbf{H}$ and $\mathbf{2}_{rad}\mathbf{H}$) formed in this process undergo a further one-electron reduction at the working electrode surface to form the fully reduced flavin anions ($\mathbf{1}_{red}\mathbf{H}^-$ and $\mathbf{2}_{red}\mathbf{H}^-$), which

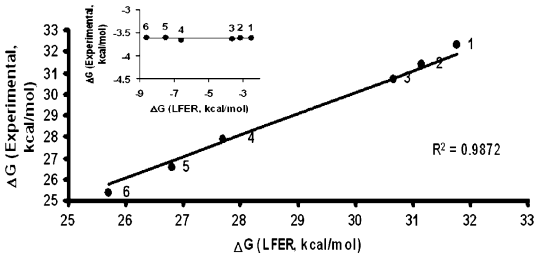

Fig. 3 Graphical representation of the good correlation between experimentally determined and LFER predicted ΔG values for redox properties of **1–6**. Inset shows the near-zero slope of the experimentally determined and LFER predicted ΔG values for binding of **1–6** and **7**.

are subsequently reoxidised at a less negative potential than $\mathbf{1}_{rad}^-$ and $\mathbf{2}_{rad}^-$. However, it must be stressed that the intensity of the second reoxidation wave is suppressed compared to the electrochemical data obtained for flavin derivatives featuring electron donating functionality attached to C(8).[6a] This e–c–e process is largely suppressed with derivatives **5** and **6**, suggesting that the electron withdrawing nature of the $-CN$ and $-NO_2$ groups significantly lowers the pK_a of their radical anions to below that of the imide of the oxidised forms of these derivatives.[6a]

We have used linear free energy (LFER) relationships to further corroborate our experimental work and gain further insight into the role functionality attached to the C(7) position of the heterocycle has upon the redox and recognition properties of the flavin.[6a] The experimental and LFER predicted values for host–guest binding and redox properties were determined using our previously described methodology and are summarised in Table 1 and represented graphically in Fig. 3, also see ESI.‡[6a] Good correlation exists between the experimental and LFER predicted ΔG values for the electrochemical properties of the flavin derivatives, further supporting the importance electronic effects of functionality attached to C(7) have in modulating redox properties. In contrast, a slope of essentially zero was observed for the plot of experimental *versus* LFER predicted ΔG values for host–guest binding, indicating that the electronic characteristics of functionality attached at the C(7) position do not significantly influence recognition of DAP. However, the CV studies suggest that the electronic effects may weakly influence the acidity of the N(5) moiety of the radical anions derived from derivatives **1** and **2**. We attribute the differing role functionality attached to the C(7) position has in modulating recognition and redox properties as follows. Functional groups attached to the C(7) position of flavin are inductively connected to the O(2) and O(4) carbonyls

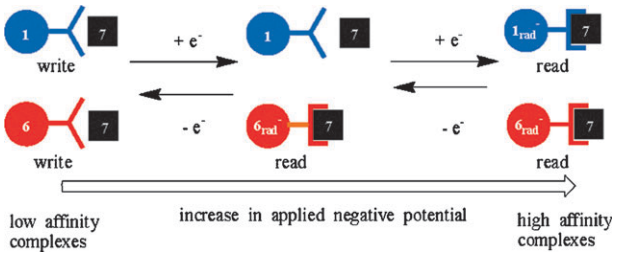

Fig. 4 Schematic representation of the three-component electrochemical switch derived from **1**, **6** and **7**.

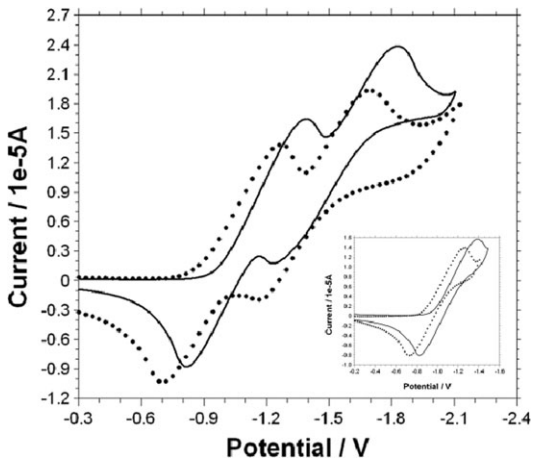

Fig. 5 Cyclic voltammogram of an equimolar mixture of **1** and **6** (~1 mmol) before (—) and after (···) the addition of DAP **7** (~10 mmol). Inset shows CV for cycles between −0.2 and −1.6 V. Scan rate 100 mV s^{-1}.

that dictate binding affinity *via* the "*meta*" position of the phenyl unit. In contrast, the C(7) position is in direct resonance communication with the central redox core of the flavin unit, thereby significantly influencing its redox properties.

In recent years, an enormous amount of effort has been directed towards the development of molecular switches.[9] Flavin–DAP systems are attractive moieties for developing switches as it is well established that the specific DAP–flavin recognition process affords a write state that can be read electrochemically due to the shift in flavin reduction potential that accompanies the hydrogen bonding mediated stabilisation of the flavin $_{rad}^{-}$ state.[7] Systems **1–7** offer the exciting ability to create a novel three-component write–read molecular switch,[10] whereby the binding between **7** and oxidised flavins can be sequentially electrochemically read by adjusting the redox window of the reduction process (Fig. 4). To test this hypothesis we have investigated the formation of a prototype three-component switch from derivatives **1**, **6** and **7**. The CV of an equimolar solution of **1** and **6** is shown in Fig. 5 and clearly shows that the disparate onset of their radical anion states allows the sequential formation of $\mathbf{1}_{rad}^{-}$ and $\mathbf{6}_{rad}^{-}$. Upon the addition of DAP and cycling the applied potential between −0.3 and −2.4 V, both of the redox waves are shifted to a more positive potential (100 mV), which corresponds to a new K_a of ~22 000 M^{-1} for $\mathbf{7.1}_{rad}^{-}$ and $\mathbf{7.6}_{rad}^{-}$.[11] By adjusting the applied voltage to cycle between −0.2 and −1.6 V it is possible to only read the **7.6** binding event in the presence of derivative **1**. Thus, in this system electrochemistry not only provides a direct readout process for the DAP–flavin binding event through the change in voltage of the flavin reduction process but also provides a means of modulating the host–guest binding event.

In conclusion, we have shown that the electronic effects of functionality attached to the C(7) position of flavin derivatives play an important role in modulating the redox properties of the unit. In contrast, they do not significantly modulate the hydrogen bonding affinity for receptor **7**. The similar binding efficiencies of the oxidised flavin derivatives **1** and **6** with compound **7** provide direct access to a prototype three-component redox controlled switch whereby the differing onset of the radical anion states of derivatives **1** and **6** can be sequentially read by the application of increasing negative potential. These results pave the way for the formation of new flavin-based molecular devices addressed by electrochemically controlled host–guest complexation. The results of our investigations in this area will be reported in due course.

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Notes and references

† This article is dedicated to Prof. Seiji Shinkai on the occasion of his 65th birthday.

§ Crystal data for **1**: $4(C_{16}H_{19}N_5O_2)\cdot C_7H_8$: M_r = 1345.58, Z = 1, triclinic, $P\bar{1}$, a = 10.3010(4), b = 11.8400(5), c = 16.1447(7) Å, α = 106.983(2), β = 108.228(2), γ = 106.322(2)°, V = 1631.48(12) Å^3, T = 100(2) K, N_{meas} = 39465, N_{ind} = 5795, R_{int} = 0.041, R_{obs} (all data) = 0.041 (0.068), wR^2_{obs} (all data) = 0.096 (0.104).

1 (*a*) K. Stevenson and V. Massey, in *Flavins and Flavoproteins. 1996*, ed. C. Willams, University of Calgary, Calgary, 1997; (*b*) *Chemistry and Biochemistry of Flavoenzymes*, ed. F. Muller, CRC Press, Boca Raton, 1991, vol. 1–3.

2 For examples see: V. Massey and P. Hemmerich, in *The Enzymes*, ed. P. D. Boyer, Academic Press, New York, 1976, vol. 12, pp. 191–240R. Swenson, G. Krey and M. Eren, in *Flavins and Flavoproteins*, ed. D. Edmondson, D. McKormick de Gruyter, Berlin, 1987, pp. 98–107.

3 S. Ghisla and V. Massey, *Biochem. J.*, 1986, **239**, 1.

4 For examples see: (*a*) M. L. Ludwig, L. M. Schopfer, A. L. Metzger, K. A. Pattridge and V. Massey, *Biochemistry*, 1990, **29**, 10364; (*b*) Y. V. S. N. Murthy, Y. Meah and V. Massey, *J. Am. Chem. Soc.*, 1999, **121**, 5344.

5 For recent reviews see: (*a*) A. Niemz and V. M. Rotello, *Acc. Chem. Res.*, 1999, **32**, 44; (*b*) V. M. Rotello, *Curr. Opin. Chem. Biol.*, 1999, **3**, 747; (*c*) Y. Yano, *Rev. Heteroat. Chem.*, 2000, **22**, 151.

6 For recent examples see: (*a*) Y.-M. Legrand, M. Gray, G. Cooke and V. M. Rotello, *J. Am. Chem. Soc.*, 2003, **125**, 15789; (*b*) J. J. Hasford and C. J. Rizzo, *J. Am. Chem. Soc.*, 1998, **120**, 2251; (*c*) D. E. Edmondson and S. Ghisla, in *Flavins and Flavoproteins*, ed. S. Ghisla, P. Kroneck P. Macheroux and H. Sund, Rudolf Weber, Agency For Scientific Publications, Berlin, 1999, pp. 71–76.

7 E. Breinlinger, A. Niemz and V. M. Rotello, *J. Am. Chem. Soc.*, 1995, **117**, 5379.

8 A. Niemz and V. M. Rotello, *J. Am. Chem. Soc.*, 1997, **119**, 6833.

9 *Molecular Switches*, ed. B. L. Feringa, Wiley-VCH, Weinheim, 2001.

10 For a previous example of a three-component flavin-based molecular switch see: R. Deans, A. Niemz, E. C. Breinlinger and V. M. Rotello, *J. Am. Chem. Soc.*, 1997, **119**, 10863.

11 The redox-based enhancement in recognition can be calculated using a thermodynamic cycle, which can be expressed mathematically using: $K_a(\text{red})/K_a(\text{ox}) = e^{(nF/RT)(E_{1/2}(\text{bound})-E_{1/2}(\text{unbound}))}$. K_a(red) and K_a(ox) are the association constants in the reduced and oxidised forms, and $E_{1/2}$(bound) and $E_{1/2}$(unbound) are the half-wave redox potentials in the receptor bound and unbound states.