ORGANIC
LETTERS

2005
Vol. 7, No. 13
2551−2554

Model Systems for Flavoenzyme Activity: Site-Isolated Redox Behavior in Flavin-Functionalized Random Polystyrene Copolymers

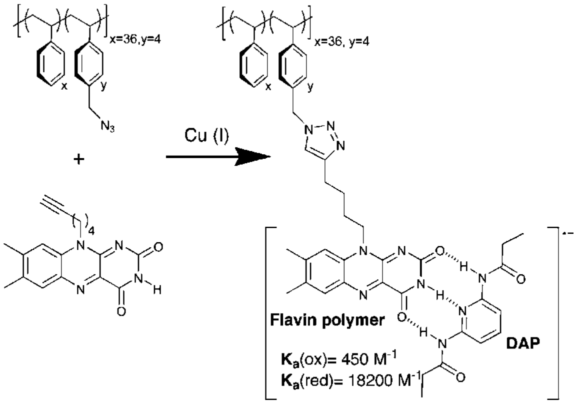
**Joseph B. Carroll,[†] Brian J. Jordan,[†] Hao Xu,[†] Belma Erdogan,[†] Lisa Lee,[†]
Lily Cheng,[†] Christopher Tiernan,[†] Graeme Cooke,[‡] and Vincent M. Rotello*,[†]**

*Department of Chemistry, University of Massachusetts, Amherst, Massachusetts 01003,
and Centre for Biomimetic Design and Synthesis, Chemistry, School of Engineering &
Physical Sciences, Heriot-Watt University, Edinburgh, UK EH14 4AS*

rotello@chem.umass.edu

Received March 11, 2005

ABSTRACT



A model system has been developed to study the redox behaviors of flavin derivatives appended onto random polystyrene copolymers through "click" chemistry strategies. The results demonstrate that flavin units attached onto polymers exhibit site-isolated redox behaviors, yielding new materials with electrochemically tunable associations (K_a(ox) $=$ 450 M^{-1}, K_a(red) $=$ 18 200 M^{-1}) to complementary diamidopyridine (DAP) functionality.

The relationship between complex redox processes and molecular recognition is a central theme in many different biological systems. For example, enzymes composed of redox-active core molecules, including flavins,[1] quinones,[2] nicotinamides,[3] and pterins,[4] use specific enzyme−cofactor interactions to control the activity of a given cofactor. The deployment of different recognition elements, including hydrogen bonding, π-stacking, and electrostatic interactions, in enzymatic processes serves to control the oxidation and protonation states of a cofactor's activity. Accordingly, understanding the cooperative recognition processes that govern complex redox enzyme activity serves as an attractive target in the development of redox-active molecular devices[5] and model systems.[6]

The incorporation of molecular recognition in polymers provides robust materials with applications from sensors to chromatography.[7] The incorporation of flavin functionality

---

[†] University of Massachusetts.

[‡] Heriot-Watt University.

(1) (a) Chaiyen, P.; Sucharitakul, J.; Svasti, J.; Entsch, B.; Massey, V.; Ballou, D. P. *Biochemistry* **2004**, *43*, 3933−3943. (b) Chakraborty, S.; Massey, V. *J. Biol. Chem.* **2002**, *277*, 41507−41516.

(2) (a) Reddy, S. Y.; Bruice, T. C. *Protein Sci.* **2004**, *13*, 1965−1978. (b) Anthony, C. *Biochem. J.* **1996**, *320*, 697−711.

(3) Sundaresan, V.; Chartron, J.; Yamaguchi, M.; Stout, C. D. *J. Mol. Biol.* **2005**, *346*, 617−629.

(4) Pey, A. L.; Thorolfsson, M.; Teigen, K.; Ugarte, M.; Martinez, A. *J. Am. Chem. Soc.* **2004**, *126*, 13670−13678.

10.1021/ol0505407 CCC: $30.25

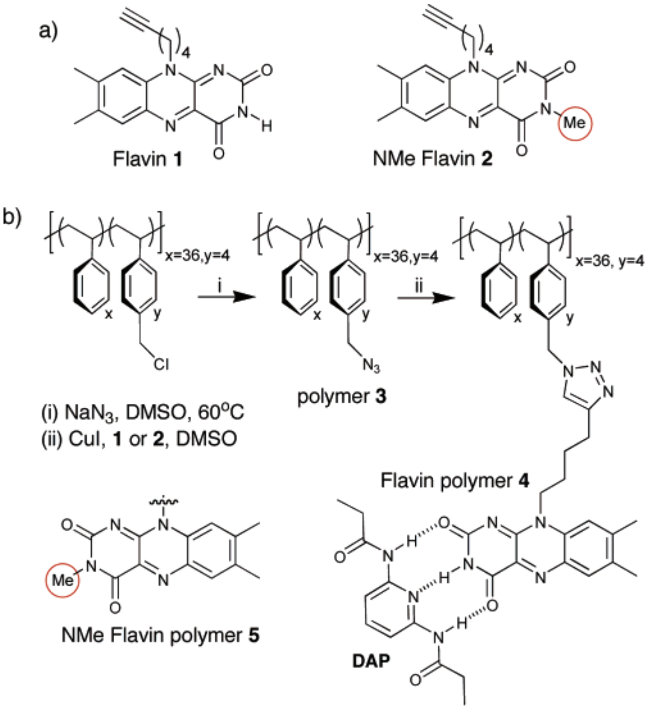
in polymers allows for the study of polymer environmental effects on flavin binding both in neutral and reduced redox states. Here we report the development of a model system seeking to understand flavin-binding interactions within polymeric systems. In this paper, alkyne flavin derivatives were appended to azido-functionalized polystyrene copolymers via Huisgen 1,3-dipolar, "click" cycloadditions.[8] The resultant flavin-functionalized polymers exhibit site-isolated electrochemical behaviors and reversible, redox-modulated recognition with complementary DAP units with a concomitant increase in association constant of ∼40-fold versus neutral binding.

The synthesis of alkyne-functionalized flavin unit **1** (Scheme 1a) began with the substitution of 4,5-dimethyl-1,2-phenylenediamine with 6-chlorohexyne (for synthesis of **1** and **2**, see Supporting Information). The resultant condensation of the monosubstituted alkyne diamine species with alloxan monohydrate yielded alkyne-functionalized flavin **1**. Further reaction of **1** with methyl iodide provides control N-methylated flavin **2**, which cannot participate in three-point hydrogen bonding with DAP.

The synthesis of flavin-functionalized copolymers began with the displacement of chloromethylstyrene copolymer[7e] with sodium azide in DMSO overnight, resulting in polymer **3** (Scheme 1b). To solutions of alkyne-functionalized flavin **1** units and polymer **3** in DMSO was added copper(I) iodide, resulting in flavin-functionalized polymer **4**. Control N-methylated, flavin-functionalized polymer **5** was synthesized in analogous fashion to polymer **4**.

Infrared (IR) spectroscopy of the resultant polymer films was used to monitor transformations before and after "clicking" functionality onto polymer **3**. IR spectra of the azide-functionalized polymer **3** films reveal a pronounced azide stretch at 2100 cm^{-1} (see Supporting Information). Upon triazole formation with **1**, the azide stretch at 2100 cm^{-1} completely disappears, direct evidence of complete functionalization of all of the azide sites of polymer **3**. IR spectra of films of control polymer **5** also display a pronounced carbonyl and aromatic $C{=}N$ stretch in the absence of an N−H stretch at ∼3400 cm^{-1}.

Scheme 1[a]



[a] Key: (a) Alkyne-functionalized flavin **1** and control N-methylated flavin **2**. (b) Synthesis of flavin polymer **4** and control polymer **5** via "click" procedures. Flavin **1** and polymer **4** exhibit specific three-point hydrogen bonding interactions with complementary DAP, where control flavin **2** and polymer **5** demonstrate no hydrogen bonding interactions with DAP.

To probe the association (K_a(ox)) between **1** and flavin-functionalized polymer **4** with complementary DAP, binding interactions were quantified using fluorescence spectroscopy in $CHCl_3$ by monitoring the fluorescence intensity of flavin upon increasing additions of DAP guest (Figure 1). Fluorescence titrations of flavin **1** in the presence of increasing concentrations of DAP show a dramatic quenching of flavin fluorescence corresponding to a $K_a(\mathrm{ox}) \approx 375$ M^{-1}. Titrations with control N-methylated flavin **2**, which cannot participate in hydrogen bonding interactions, show *no* quenching of fluorescence ($K_a(\mathrm{ox}) \approx 0$ M^{-1}) upon addition of an excess of DAP.

Analogous fluorescence titrations of flavin functionalized polymer **4** in the presence of increasing concentrations of DAP also show a dramatic quenching of flavin fluorescence corresponding to a K_a (ox) ≈ 450 M^{-1} (Figure 1). Control titrations with N-methylated flavin polymer **5** and DAP exhibit a slight increase in fluorescence perhaps indicating cooperative flavin interactions; however, no dynamic quenching of polymer fluorescence was observed ($K_a(\mathrm{ox}) \approx 0$ M^{-1}).

In addition to the neutral associations determined via fluorescence spectroscopy, the half-wave reduction potentials of the free flavin **1** and polymer **4** ($E_{1/2}$(u)) and the corresponding potentials for **1** and polymer **4** in the presence of DAP ($E_{1/2}$(b)) were determined in CH_2Cl_2 through cyclic voltammetry (CV). Voltammetric traces of **1**, **2**, polymer **4**,

---

(5) (a) Liu, Y.; Flood, A. H.; Stoddart, J. F. *J. Am. Chem. Soc.* **2004**, *126*, 9150−9151. (b) Lehn, J. M. *Supramolecular Chemistry: Concepts and Perspectives*; VCH: Weinheim, 1995. (c) Kaifer, A. E.; Gomez-Kaifer, M. *Supramolecular Electrochemistry*; Wiley-VCH: Weinheim, 1999.

(6) (a) Hasford, J. J.; Rizzo, C. J. *J. Am. Chem. Soc.* **1998**, *120*, 2251−2255. (b) Kajiki, T.; Moriya, H.; Hoshino, K.; Kuroi, T.; Kondo, S.; Nabeshima, T.; Yano, Y. *J. Org. Chem.* **1999**, *64*, 9679−9689. (c) Deans, R.; Rotello, V. M. *J. Org. Chem.* **1997**, *62*, 4528−4529. (d) Greaves, M. D.; Rotello, V. M. *J. Am. Chem. Soc.* **1997**, *119*, 10569−10572. (e) Legrand, Y. M.; Gray, M.; Cooke, G.; Rotello, V. M. *J. Am. Chem. Soc.* **2003**, *125*, 15789−15795. (f) Gray, M.; Goodman, A. J.; Carroll, J. B.; Bardon, K.; Markey, M.; Cooke, G.; Rotello, V. M. *Org. Lett.* **2004**, *6*, 385−388.

(7) (a) Brunsveld, L.; Folmer, B. J. B.; Meijer, E. W.; Sijbesma, R. P. *Chem. Rev.* **2001**, *101*, 4071−4097. (b) Prins, L. J.; Reinhoudt, D. N.; Timmerman, P. *Angew. Chem., Int. Ed.* **2001**, *40*, 2383−2426. (c) McQuade, D. T.; Pullen, A. E.; Swager, T. M. *Chem. Rev.* **2000**, *100*, 2537−2574. (d) Pollino, J. M.; Stubbs, L. P.; Weck, M. *J. Am. Chem. Soc.* **2004**, *126*, 563−567. (e) Ilhan, F.; Gray, M.; Rotello, V. M. *Macromolecules* **2001**, *34*, 2597−2601.

(8) (a) Huisgen, R. *Pure Appl. Chem.* **1989**, *61*, 613−628. (b) Huisgen, R.; Szeimies, G.; Mobius, L. *Chem. Ber.* **1967**, *100*, 2494−2507. (c) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596−2599. (d) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004.

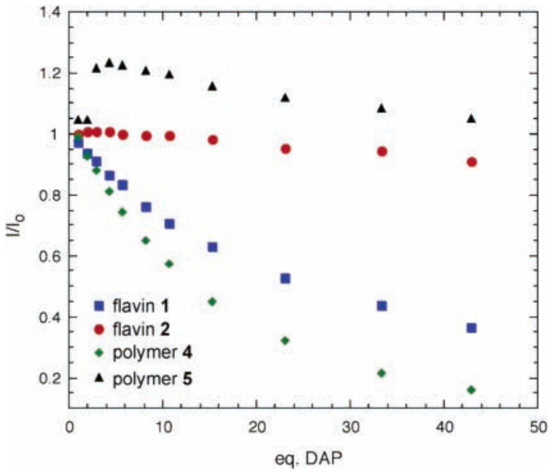


Figure 1. Inverse Stern−Volmer plot showing a dramatic quenching of flavin **1** (■ K_a(ox) = 375 M^{-1}) and flavin-functionalized polymer **4** fluorescence upon addition of DAP (◆) corresponding to a K_a(ox) = 450 M^{-1}, whereas interactions with control N-methylated flavin **2** (●) and control polymer **5** (▲) show no fluorescence quenching (100 μM, λ_{ex} = 445 nm, λ_{em} = 460−650 nm).

and polymer **5** were taken in CH_2Cl_2 with n-$Bu_4N^+ClO_4^-$ (100 μM)[9] as the supporting electrolyte.[10]

Flavin **1** exhibits a single reduction wave followed by two characteristic return oxidation waves (see Supporting Information). In previous studies,[11] this redox behavior was determined to be the result of an electrochemical−chemical−electrochemical (ece) process where the radical anion form of the flavin rapidly deprotonates other oxidized flavins in solution. The newly formed protonated flavin radical then undergoes a further one-electron reduction to form the fully reduced flavin anion. Upon the addition of an excess of DAP, a significant positive shift in the half-wave reduction potential ($E_{1/2}$) of **1** is observed ($\Delta E_{1/2}$ = +100 mV). In addition to the dramatic positive shift, the electrochemical behavior of **1** bound to DAP becomes fully reversible with the disappearance of the second oxidation wave seen with **1** alone.[11] Control CV traces with N-methylated flavin **2** exhibit no appreciable shift upon the addition of an excess of DAP (see Supporting Information).

In contrast to flavin **1**, polymer **4** exhibits very different electrochemical behaviors from its monomeric counterpart. Polymer **4** undergoes a one-electron reduction with only a single return oxidation wave (see Supporting Information). This fully reversible electrochemical behavior demonstrates that the flavin units appended onto the polystyrene polymer are isolated from each other preventing flavin−flavin proton transfer.[12] This observed site isolation is important when engineering electrochemically tunable materials that interact specifically with a given substrate. Upon the addition of an excess of DAP, a significant positive shift in the $E_{1/2}$ of polymer **4** is observed ($\Delta E_{1/2}$ = +95 mV). Control CV traces with N-methylated, flavin-functionalized polymer **5** exhibit no appreciable shift after the addition of an excess of DAP (see Supporting Information).

The large differences in peak shape and relatively small differences in the reduction potential ($E_{1/2}$) observed between flavin **1** ($E_{1/2}$ = −1291 ± 3 mV) and flavin-functionalized polymer **4** ($E_{1/2}$ = −1285 ± 3 mV) prompted further investigation into the thermodynamic behavior of free flavin versus flavins appended onto polymer scaffolds. To probe the thermodynamic contributions to the electrochemical behaviors observed in flavin **1** and polymer **4**, variable-temperature CV (VT-CV) experiments were carried out in dichloroethane (Figure 2). VT-CV traces reveal dramatic differences in the redox behavior of free flavin versus flavins

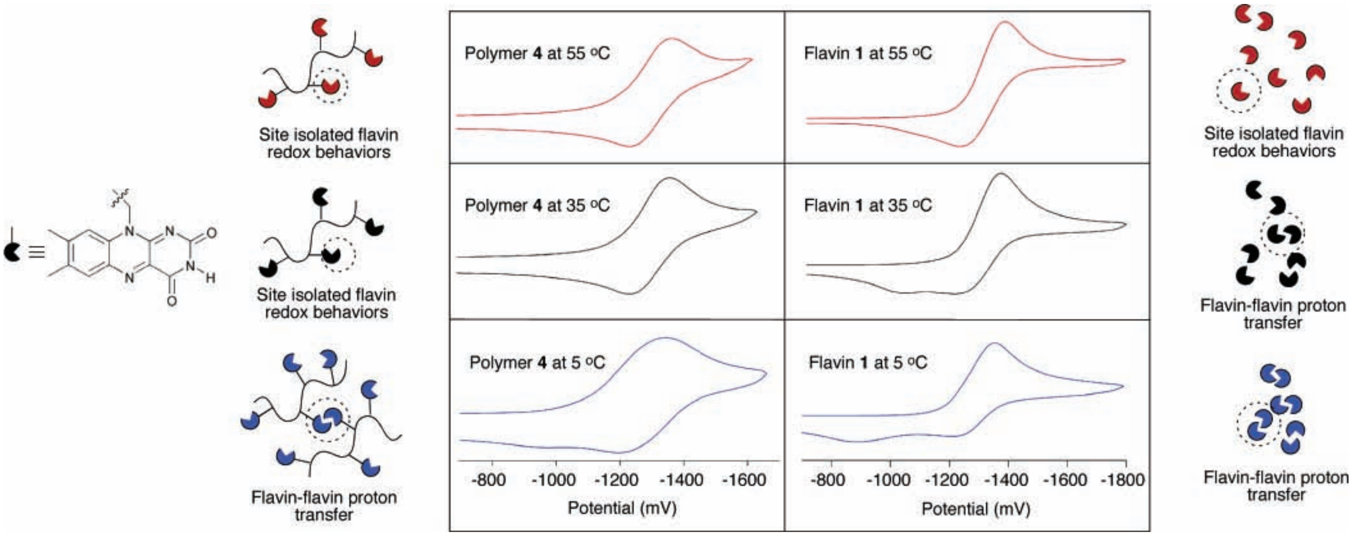


Figure 2. VT-CV traces of polymer **4** and flavin **1** exhibiting different flavin redox behaviors at different temperatures (55, 35, 5 °C). Lowering the temperature (5 °C) induces the presence of a second oxidation in polymer **4**, while elevated temperatures signal the disappearance of the second oxidation in flavin **1** (55 °C).

appended onto polymers. At 35 °C, flavin **1** undergoes a one-electron reduction followed by two characteristic oxidation waves as previously described. Polymer **4** at 35 °C, however, exhibits only one reversible redox couple. At higher temperatures (55 °C), flavin **1** does not exhibit a second oxidation, presumably due to the disruption of flavin−flavin, hydrogen-bonding interactions. Lower temperatures (5 °C) promote flavin−flavin hydrogen bonding interactions as witnessed by the appearance of a second oxidation in polymer **4**. Interestingly enough, these dramatic changes in peak shape with temperature fail to yield large differences in the reduction potential of the flavin systems (see Supporting Information).

In general, at lower temperatures the reduction potential of both flavin **1** and polymer **4** shifts to a more positive potential ($E_{1/2} \approx -1270$ mV at 5 °C), while raising the temperature shifts the reduction potential to a more negative potential ($E_{1/2} \approx -1298$ mV at 55 °C). Using the shifts in the reduction potentials of **1** and polymer **4** ($\Delta E_{1/2}$) obtained from CV (see Table 1) and the association constants (K_a(ox)) as determined using fluorescence spectroscopy, we can determine a binding constant between DAP, flavin **1**, and polymer **4** in the radical anionic form of the flavin (K_a(red)) through the following relationship.

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

Using the above equation, the association constant between DAP and **1** in its reduced state (K_a(red)) was determined to be ~18 500 M^{-1}, resulting in a 50-fold increase in binding and a ~2.3 kcal/mol stabilization of the radical anion of **1**. The K_a(red) between DAP and polymer **4** was determined to be ~18 200 M^{-1}, resulting in a 40-fold increase in binding and a ~2.2 kcal/mol stabilization of the radical anion of polymer **4**.

Table 1. Half-Wave Potential[a] (mV) and Association Constants (M^{-1}) Associated with Flavin Systems

flavin system	K_a(ox)[b]	$E_{1/2}$(u)[c]	$E_{1/2}$(b)[d]	$\Delta E_{1/2}$	K_a(red)
1	375	−1291	−1191	100	18 500
2	0	−1354		0	
polymer **4**	450	−1285	−1190	95	18 200
polymer **5**	0	−1346		0	

[a] Half-wave potentials were taken from square-wave voltammetry measurements ($E_{1/2} \pm 3$ mV vs ferrocene). [b] Neutral association constants (K_a(ox)) determined via fluorescence spectroscopy. [c] Reduction potential of the unbound flavin species. [d] Reduction potential of the bound flavin species with DAP guest.

In summary, we have synthesized flavin-functionalized random polystyrene copolymers through versatile “click” chemistry strategies. Interestingly, flavin units on polymer scaffolds act independently and are site-isolated from each other, yielding unique thermal and electrochemical behaviors not observed with free flavin molecules. The resultant polymer exhibits reversible, redox-modulated hydrogen bonding interactions with complementary DAP units, resulting in a 40-fold increase in binding and a 2.2 kcal/mol stabilization of the radical anion of the polymer. Our further efforts will involve investigating these redox active polymers and their possible applications in electrochemically controlled molecular sensing and surface modification.

Acknowledgment. This research was supported by the NIH (GM 59249) and the EPSRC. J.B.C. would like to thank the Electrochemical Society for the Joseph W. Richards Fellowship.

Supporting Information Available: Synthesis, ^{1}H NMR, and CV of flavins **1** and **2** and polymers **3** and **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0505407

---

(9) Extreme caution was exercised in preparation of electrolyte solutions for CV studies: Sax, N. I.; Lewis, R. J. *Dangerous Properties of Industrial Materials*, 7th ed.; Van Nostrand Reinhold: New York, 1989; Vol. 3.

(10) CV traces were recorded for a CH_2Cl_2 blank, then for a solution of the flavin or flavin polymer (200 μM) and ferrocene (as a reference), and finally for the same samples containing excess DAP. Differences in half-wave potentials were determined using square-wave voltammetry. All scans were recorded at a sweep rate of 100 mV s^{-1}.

(11) Niemz, A.; Imbriglio, J.; Rotello, V. M. *J. Am. Chem. Soc.* **1997**, *119*, 887−892.

(12) (a) Galow, T. H.; Ilhan, F.; Cooke, G.; Rotello, V. M. *J. Am. Chem. Soc.* **2000**, *122*, 3595−3598. (b) Gorman, C. B. *Adv. Mater.* **1998**, *10*, 295−308. (c) Kaifer, A. E. *Acc. Chem. Res.* **1999**, *32*, 62−71. (d) Dandliker, P. J.; Diederich, F.; Gross, M.; Knobler, C. B.; Louati, A.; Sanford, E. M. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1739−1741. (e) Dandliker, P. J.; Diederich, F.; Gisselbrecht, J.-P.; Louati, A.; Gross, M. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2725−2728.