Recognition mediated encapsulation and isolation of flavin–polymer conjugates using dendritic guest moieties†

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Diaminopyridine dendritic scaffolds encapsulate polymeric flavin *via* non-covalent interactions and demonstrate isolation of the redox moiety.

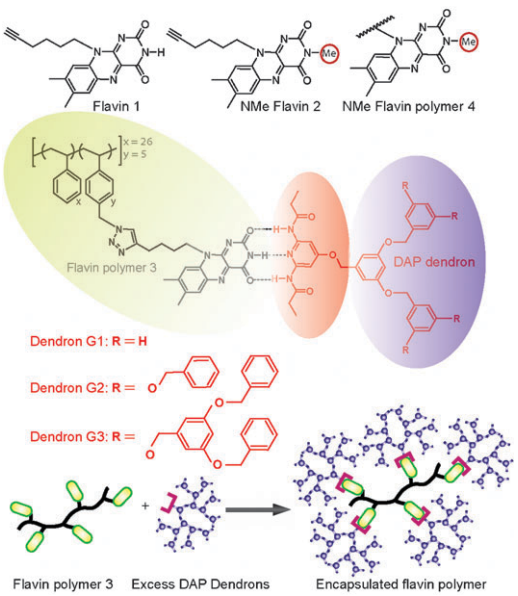
Site isolation and encapsulation of redox-active cofactors control the electron transfer process within many enzymatic systems,[1] protecting the redox center from unwanted interactions with water and other interfering species. Various methods have been used to mimic this effective biological insulation of redox-active core moieties for use in artificial enzymes,[2] catalysts,[3] light harvesting systems,[4] construction of insulated molecular wires,[5] light-emitting diodes[6] and fiber optics.[7] Additional efforts have focused on synthetic systems, such as inclusion complexes and dendrimer cages,[8] for applications as data storage units, sensors, catalysts, and magnets.[9]

Dendritic scaffolds have gained considerable interest due to their unique molecular architecture.[8,9] These dendritic scaffolds have been used for the spatial arrangement of functionalities and tuning the properties of redox-active moieties through the interplay of structural subunits. However, these synthetic systems differ from biological systems because they primarily focus on covalent attachment of dendritic scaffolds to control the encapsulation. Therefore, model systems where modulation of electron transfer properties occurs *via* non-covalent interactions would be more representative of typical enzyme–cofactor systems and would also lead to supramolecular strategies for materials and device applications.

Among various enzyme–cofactor systems, flavoenzymes make attractive model systems because they catalyze a wide variety of biological processes such as redox transformations, signal transduction, and electron transfer. They use cofactors (FMN or FAD) that bind to the active site of the apoenzyme through a series of non-covalent interactions. The interactions are responsible for fine-tuning the $FADH_2$–FAD redox cycle and isolating the cofactor within a hydrophobic pocket. Hence, flavin model systems serve as a useful tool to modulate the effective site isolation and encapsulation of flavin through non-covalent interactions with dendritic scaffolds.

Herein, we report the use of dendritic scaffolds to encapsulate flavin derivatives and tune their electrochemical behavior using molecular recognition. Three generations of dendrons containing diaminopyridine (DAP) unit at their focal point were utilized to probe the supramolecular encapsulation of monomeric and polymeric flavin units. The DAP moiety on the dendrons binds to monomeric and polymeric flavins to produce supramolecular structures. The resultant encapsulated supramolecular assemblies demonstrate distinctly different redox behavior as a function of encapsulation. Monomeric flavins exhibit reversible, redox-modulated behavior consistent with previous flavin model systems.[10] Polymeric flavins, however, demonstrate diffusion-limited redox behavior and site isolation when bound to increasing DAP dendrimer generations. The effective encapsulation of polymeric flavins *via* supramolecular interaction provides a potential tool to modulate redox processes in various systems including artificial enzymes, light harvesting systems, molecular wires, and light-emitting diodes (Scheme 1).

Monomeric and polymeric flavins were synthesized using a previously reported procedure.[11] Briefly, polymeric flavin was



Scheme 1 Alkyne-functionalized flavin **1**, control *N*-methylated flavin **2**, flavin polymer **3** exhibit specific three-point hydrogen bonding interactions with complementary dendrons, control polymer **4**, and a schematic representation of encapsulation of flavin polymer.

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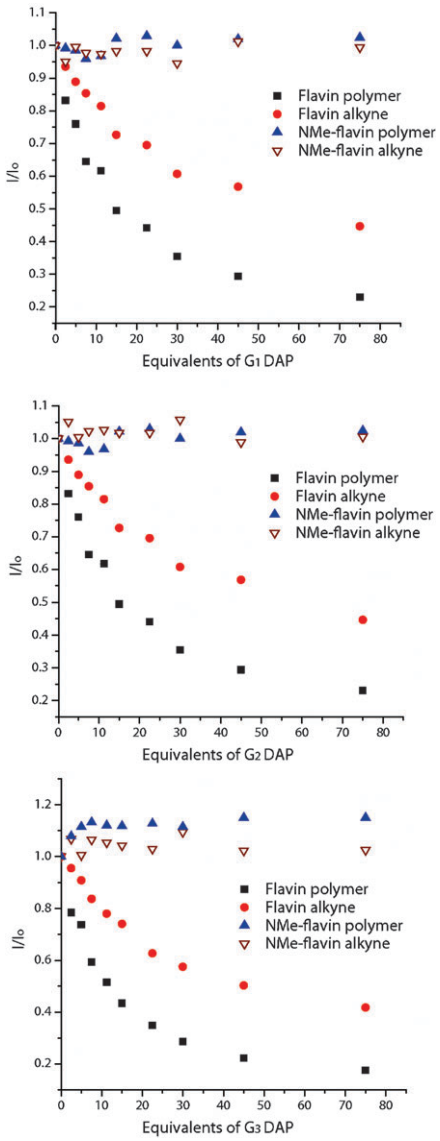

Fig. 1 Inverse Stern–Volmer plot showing a dramatic quenching of flavin **1** and flavin-functionalized polymer **3** fluorescence upon addition of DAP dendrons, whereas interactions with control *N*-methylated flavin **2** and control polymer **4** show no fluorescence quenching (50 μM, λ_{ex} = 445 nm, λ_{em} = 460–650 nm).

synthesized by reacting the monomeric alkyne flavin derivative with azide functionalized polymer *via* Huisgen 1,3-dipolar, "click" cycloadditions.

The association constant (K_a(ox)) of flavin **1** and flavin polymer **3** with complementary DAP dendrons was quantified *via* fluorescence titrations in $CHCl_3$. The fluorescence intensity of flavin was monitored upon addition of guest DAP dendrons (Fig. 1). Fluorescence titrations of flavin **1** in the presence of increasing concentrations of DAP dendrons showed a dramatic quenching of fluorescence, indicative of three point hydrogen bonding between flavin and DAP complexes.[12] Flavin **1** demonstrated relatively similar binding constants (K_a(ox) = 400 M^{-1}) for increasing generations of DAP dendrimer, suggesting that the flavin–DAP complex was independent of dendron size. *N*-Methylated flavin **2**, which cannot participate in hydrogen bonding interactions, was used as a control and showed no quenching of fluorescence upon subsequent additions of excess DAP dendrons.

In contrast to the monomeric flavin, fluorescence titrations of flavin functionalized polymer **3** in the presence of increasing concentrations of DAP dendrons exhibited significant quenching of flavin fluorescence corresponding to K_a(ox) = 600, 700, 550 M^{-1}, respectively, for the G1, G2, and G3 DAP dendrons (Fig. 1). No substantial change in binding affinity was observed for increasing DAP dendrimer generations. However, the enhanced binding affinity recorded for polymer **3** as compared to flavin **1** was attributed to the cooperative non-covalent interactions including hydrogen bonding and π-stacking within the polymeric–dendritic supramolecular complex. As expected, *N*-methylated flavin polymer **4** did not show fluorescence quenching upon additions of DAP dendron.

The redox behavior for the supramolecular complexes was determined in CH_2Cl_2 through cyclic voltammetry (CV). Cyclic voltamograms were obtained for **1**, **2**, polymer **3**, polymer **4** using tetrabutylammonium perchlorate (TBAP) as the supporting electrolyte (ESI†). The half wave reduction potential was calculated for flavin **1** and polymer **3** ($E_{\frac{1}{2}}$ (unbound)) along with the corresponding potentials for **1** and polymer **3** in the presence of DAP ($E_{\frac{1}{2}}$ (bound)).

Flavin **1** showed a single reduction wave followed by two characteristic return oxidation waves (ESI†) typical for an electrochemical–chemical–electrochemical (ece) process. Upon addition of DAP dendrons, flavin **1** became fully reversible with the disappearance of the second oxidation wave (ESI†). A significant positive shift in the half wave reduction potential ($\Delta E_{\frac{1}{2}}$) was observed ($\Delta E_{\frac{1}{2}}$) to be 60 ± 5 mV for the dendrons (Table 1). Hence, the DAP dendrimers modulate the flavin redox behavior which is consistent with previous flavin model systems.[13]

Polymer **3** demonstrated fully reversible electrochemical behavior as well (Fig. 2). The flavin units appended to the polymer produced isolated redox behavior, preventing flavin–flavin proton transfer and the disappearance of a second oxidation wave.[14] However, upon addition of 4 equivalents of DAP dendrons, polymer **3** exhibited distinctly different electrochemical behavior from its monomeric counterpart (Fig. 2). A drastic change in peak shape was observed for polymer **3**. The signal was significantly smaller and broader for each corresponding dendrimer generation, indicating limited electron diffusion as a function of dendrimer size. The peak

Table 1 Half-wave potential[a] (mV) and association constants (M^{-1}) for monomeric and polymeric flavin systems

Flavin	Dendron	K_a(ox)[b]	$E_{\frac{1}{2}}$(u)[c]	$E_{\frac{1}{2}}$(b)[d]	$\Delta E_{\frac{1}{2}}$	K_a(red)
	G1	400	−1280	−1225	55	3400
Flavin **1**	G2	400	−1280	−1215	65	5000
	G3	350	−1280	−1225	55	3000
	G1	650	−1250	−1190	60	6500
Polymer **3**	G2	700	−1250	−1180	70	11 000
	G3	550	−1250	−1190	60	5700

[a] Half-wave potential ($E_{\frac{1}{2}}$) was taken from square-wave voltammetry measurements ($E_{\frac{1}{2}}$ ± 5 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. Association constants have the error range of ≤50.

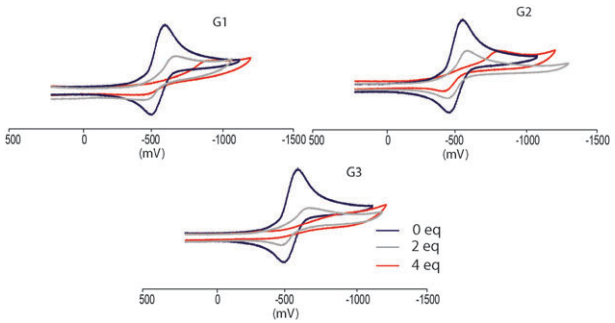

Fig. 2 CV traces of polymeric flavin **3** exhibiting different flavin redox behaviors in the presence of G1, G2 and G3 DAP dendrons.

to peak distances were 200, 225, 440 mV for G1, G2 and G3 respectively. The broadening of the peak to peak distance and the dramatic decrease in the current indicate a slow rate of electron transfer from the flavin to electrode, indicating the effective isolation and encapsulation of appended flavins from outside interfering species (Fig. 2).

Surprisingly, the shift in the polymeric flavin redox potential was not significantly different than the monomeric flavin systems. A positive shift of 60–70 mV was observed for the reduction potential of polymer **3** for all three DAP dendrimer generations. The shifts in reduction potential were only slightly larger than the shifts recorded for the monomeric flavin **1**. Thus, the cooperative non-covalent interactions appeared to modulate the flavin redox potential while the size of the complex determined the encapsulation of the flavin unit.

Association constants in the radical anionic states (K_a(red)) were extrapolated by using the shifts in reduction potentials ($\Delta E_{\frac{1}{2}}$) of **1** and polymer **3** and the association constants (K_a(ox)) through the following relationship.

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

The association constant for the G2 dendron with the polymer bound flavin in reduced state showed a 15.7-fold increase in binding. However, the monomeric flavin showed a 8.5-fold increase in the binding in the reduced state (Table 1). Molecular modeling studies clearly predicted the formation of encapsulated flavin inside the self-assembled structure through hydrogen bonding and aromatic interactions between the flavin polymer **3** and DAP dendron G3 (Fig. 3). Analogous molecular models of flavin polymer **3** with DAP dendrons G1 and G2 have also shown encapsulation of flavin units, while monomeric flavin does not demonstrate any encapsulation of flavin units (ESI†).

Dynamic light scattering experiments corroborated the expected diameter change for the flavin polymer upon addition of DAP dendrons observed in the molecular modeling simulations. The diameter increases (from 4.6 nm to 7.9 nm) as the generation of dendron increased for the flavin polymer **3** whereas the control polymer **4** did not show any appreciable change in diameter (ESI†).

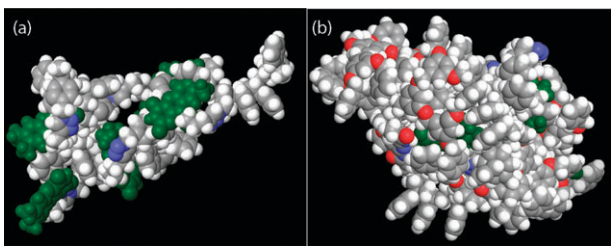

Fig. 3 Molecular dynamics simulation (Amber force field) of (a) polymeric flavin **3** and (b) polymeric flavin **3** with G3 DAP dendrons. (Flavin moieties are highlighted in green.)

In conclusion, we have demonstrated that site isolation of redox active units *via* formation of specific supramolecular complexes can be used as a tool to modulate the redox behavior. In particular, we have shown that DAP containing dendrons showed enhanced binding towards site isolated polymer-bound flavin systems relative to their monomeric counterparts. At higher equivalents of DAP dendrons, a significant insulation of redox activity of polymeric flavin was observed due to the encapsulation of flavin. This supramolecular based modulation of redox behavior of flavin bound polymer motif could be potentially used in the creation of artificial enzymes, light harvesting systems, and molecular wires. Our future work will involve investigating these redox active polymers and their possible applications in redox responsive sensing systems, and photovoltaic devices.

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