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Dendron-based model systems for flavoenzyme activity: towards a new class of synthetic flavoenzyme†

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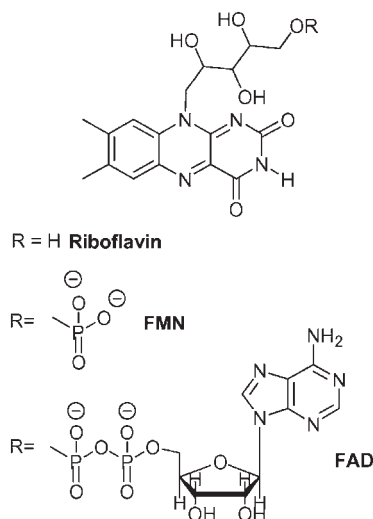
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Three generations of water-soluble flavin dendrons have been synthesized and the role dendrimer generation has on the physical and catalytic properties of these assemblies has been investigated.

Flavoenzymes are an important class of redox enzymes that are involved in a variety of biological processes including electron transfer, redox transformations and signal transduction.¹ The redox active flavin groups (cofactors) (*e.g.* **Riboflavin**, **FMN** or **FAD**) of typical flavoenzymes are usually bound to the active-site of the apoenzyme *via* a range of non-covalent interactions.² The delicate cooperation of these interactions are thought to be responsible for fine-tuning the redox properties of the cofactor, and also for creating a hydrophobic binding pocket to isolate the cofactor from competing interactions with the aqueous environment.



In order for an artificial enzyme to be constructed that adequately simulates the structure and reactivity of the prototype flavoenzyme, a detailed understanding of how

non-covalent interactions tune the properties of the flavin cofactor is desirable. To aid this process, a range of molecular model systems have been synthesized and studied to elucidate the role various supramolecular interactions (*e.g.* hydrogen bonding, π -stacking and donor atom- π interactions) have in modulating the redox properties³ and reactivity⁴ of the flavin moiety. Although these systems have provided some insight into how these interactions modulate the redox potential of the flavin, they have failed to accurately recreate the biomimetic environment that surrounds the flavin cofactor. Polymer-based model systems have also been synthesised by attaching flavin moieties onto biomolecules,⁵ synthetic polymers⁶ and peptides.⁷ However, the lack of active site structural homogeneity and lengthy synthetic protocols have limited their potential applications. Therefore, a highly efficient synthetic flavoenzyme model system still remains a great challenge for the synthetic community.

Recently, we have reported a fully synthetic polymeric flavoenzyme model, where we have incorporated the key attributes of flavoenzymes (cofactor isolation, redox tuning and catalytic activity) by synthesising a water-soluble polymeric system using atom transfer radical polymerisation.⁸ Herein we have turned our attention to take advantage of the microenvironment provided by dendrons in order to generate a new class of synthetic flavoenzyme. Dendron architectures were selected for this study in view of their superior structural characteristics compared to conventional synthetic polymers for creating effective biomimetic environments.⁹ In particular, dendron architectures can be precisely controlled at the molecular level and can confer: (i) a well-defined monodisperse structure; (ii) a large number of terminal functionalities offering effective interactions with their surrounding media, and at the same time, isolating reactive catalytic functionality at their foci.¹⁰

A series of water soluble dendrons featuring triethylene glycol units were designed and synthesised to mimic the flavoenzyme activity (Fig. 1). In the dendritic architecture, the catalytic centre (the flavin residue) was introduced at the focal point of the dendron in order to achieve a substrate accessible biomimetic hydrophobic pocket around the active site. The detailed synthetic protocols for preparing **G1_flavin**, **G2_flavin** and **G3_flavin** are described in the ESI.† Molecular dynamics simulations performed in water for **G1_flavin**, **G2_flavin** and **G3_flavin** indicated that as the dendron generation is increased from G1 to G3, the degree of encapsulation

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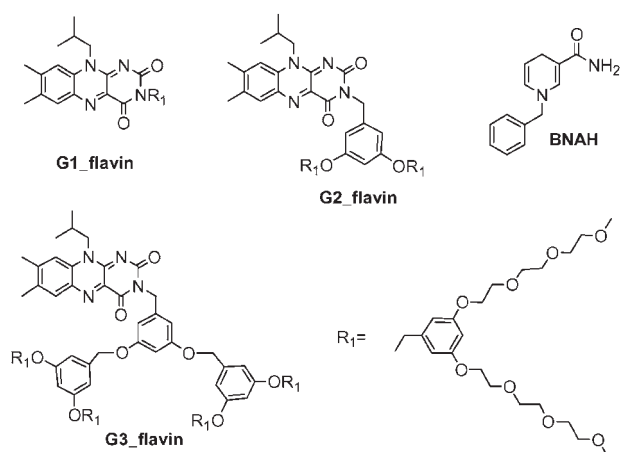


Fig. 1 Structure of the flavin dendrons (**G1_flavin**, **G2_flavin** and **G3_flavin**) and **BNAH**.

increases, with the flavin in **G3_flavin** being partly located in a hydrophobic environment that is near the surface, a feature commonly observed in flavin-based electron-transfer proteins (Fig. 2).

As the flavin unit is a very effective system for probing the polarity of its surrounding microenvironment, we have recorded the UV-vis spectra of **Riboflavin** and the flavin dendrons in pH = 8.0 phosphate buffer–isopropanol (95 : 5).¹¹ It has previously been shown that the S_0 – S_2 transition of the flavin nucleus is solvatochromic and undergoes a bathochromic shift in going from non-polar to polar media, thereby offering a simple method of probing the microenvironment conferred by the dendron units.¹² The overlaid UV-vis spectra of **Riboflavin** and dendrons **G1_flavin**–**G3_flavin** are shown in Fig. 3. The S_0 – S_2 transition for riboflavin and the first and second generation flavin dendrons were very similar and occurred at around 370 nm. However, for the third generation flavin dendron the S_0 – S_2 transition was shifted 20 nm to a shorter wavelength ($\lambda = 370$ nm for **Riboflavin** versus $\lambda = 358$ nm for **G3_flavin**). This blue shift is consistent with the ethylene glycol moieties of the third generation dendron creating a biomimetic hydrophobic pocket for the flavin unit in aqueous media.⁸

Fluorescence spectra of **Riboflavin** and the dendron systems **G1_flavin**–**G3_flavin** were recorded and revealed that the dendron moieties significantly quenched the fluorescence of the flavin moiety (compared to **Riboflavin**), presumably due to

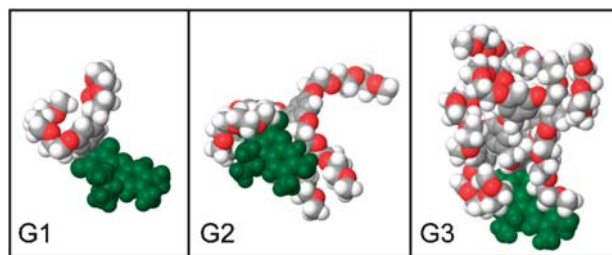


Fig. 2 Molecular dynamic simulations of dendron generations (G1–G3) demonstrating the degree of encapsulation of the flavin unit (green) within the dendron wedges. Water molecules have been omitted for clarity.

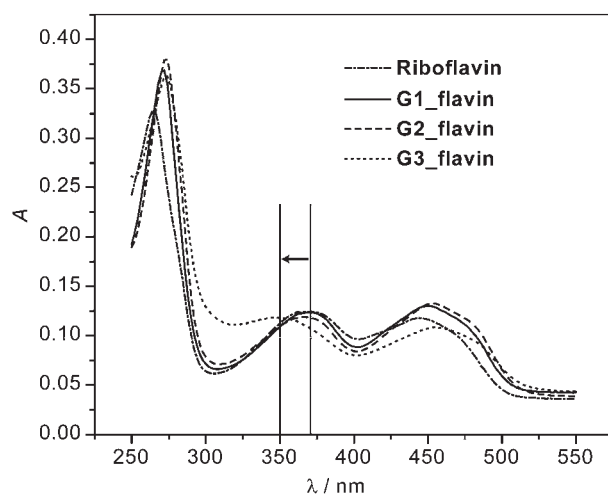


Fig. 3 Overlaid UV-vis spectra of **Riboflavin** and flavin dendrons **G1_flavin**–**G3_flavin** (5 mM solutions in phosphate buffer (pH = 8.0)–isopropanol (95 : 5)). Spectrum of **G3_flavin** shows a hypsochromic shift.

aromatic interactions between the electron deficient flavin and the electron rich aromatic units of the dendron moiety (Fig. 4). We have compared the solution electrochemistry of **Riboflavin** and dendrons **G1_flavin**–**G3_flavin** in pH = 8.0 phosphate buffer–isopropanol (95 : 5) using square wave voltammetry (see ESI†). It is clear from the electrochemical data that the dendron architecture has little influence on the electrochemical properties of the flavin moiety under the conditions examined.

We next investigated the role dendron generation has in the tuning of the catalytic activity of the flavin units of **G1_flavin**–**G3_flavin**. In particular, we have investigated the kinetics of the aerobic oxidation of NADH analogue **BNAH** by **Riboflavin** and the flavin dendrons.¹³ As **BNAH** rapidly oxidised in the presence of flavins, the catalysis was followed by monitoring the decrease in absorbance of **BNAH** at 358 nm. Fig. 5 depicts the plots of initial velocity (v_0) vs. **BNAH** concentration for **Riboflavin** and the flavin dendrons.

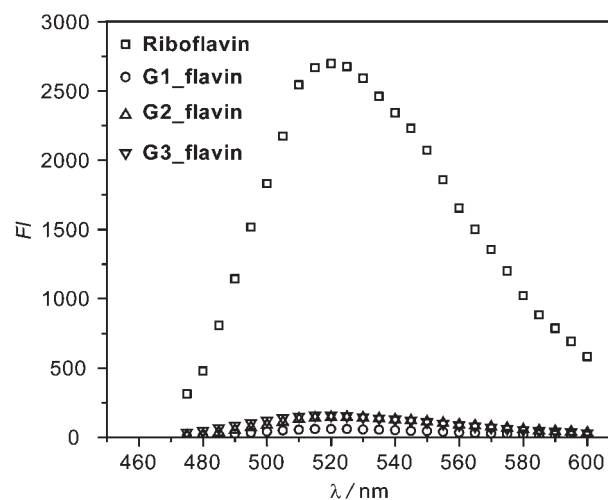


Fig. 4 Fluorescence spectra of the **Riboflavin** and the flavin dendrons in pH = 8.0 buffer–isopropanol (95 : 5) solution at 30 °C ($\lambda_{\text{ex}} = 450$ nm).

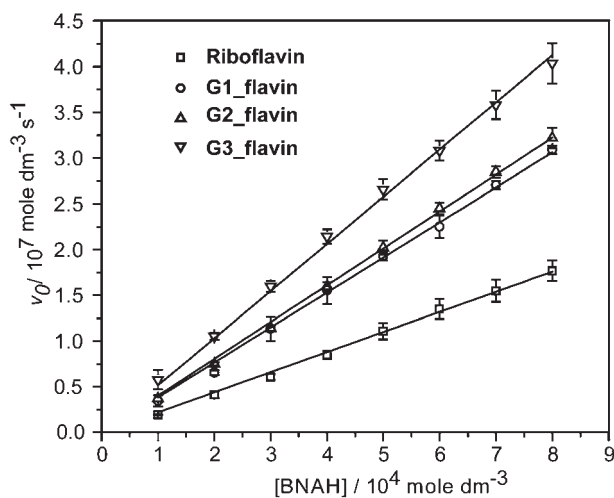


Fig. 5 Plots of initial velocities (v_0) vs. substrate concentration (**BNAH**) for the oxidation by **Riboflavin** and flavin dendrons. The lines represent the best fit of the data using pseudo-first order reaction kinetics.

Table 1 Effect of dendron generation on kinetic parameters for the flavin catalysed oxidation of **BNAH**. The concentration of flavin unit in all the cases maintained at $5 \mu\text{mole dm}^{-3}$

Substrate	Flavin	$k_{\text{pseudo}}/10^{-5} \text{ s}^{-1}$	$k_2/\text{mole dm}^{-3} \text{ s}^{-1}$
BNAH	Riboflavin	22	44
	G1_flavin	38	76
	G2_flavin	40	80
	G3_flavin	52	104

The observation of straight line correlation in the kinetic plots indicates the existence of pseudo-first-order kinetic behaviour for these reactions. Nonlinear least-squares curve fitting analysis was then used to evaluate the pseudo-first order (k_{pseudo}) and second order (k_2) rate constants for the reactions. The rate constants are compiled in Table 1.

The comparison of the rate constants in Table 1 reveals a significant increase of catalytic reaction rate for the flavin dendrons as compared to **Riboflavin**. Relative to **Riboflavin**, an increased second order rate constant of 1.7–2.4 fold was observed in the case of flavin dendrons. Interestingly, within the dendron family, a significant rate acceleration can also be noticed upon increase in dendron generation.^{10c} We believe that the increased reaction rates for the dendron systems compared to **Riboflavin** is likely due to an enhanced association between the substrate (**BNAH**) and the catalyst. We attribute this enhanced association for the dendron systems originates from the π -stacking interactions between the aryl moieties of the dendrons and the reduced form of **BNAH**. As the dendron generation is increased, this feature becomes more prominent with the **G3_flavin** system, presumably aided by the presence of a hydrophobic pocket which helps to localise the hydrophobic **BNAH** near the flavin unit.

In summary, we have prepared a family of water soluble flavin dendrons. We have shown that the dendron moieties provide both a biomimetic binding pocket and a means of tuning the catalytic properties of the flavin unit in aqueous environments. As the dendrons behave similarly to the apoenzyme of natural flavoenzymes, this study paves the way for the synthesis of more elaborate derivatives whereby functionality can be introduced to control the redox properties and reactivity of the flavin moiety. The development of systems displaying electron transfer and improved catalytic properties will be reported in due course.

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