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Electronic Supplementary Information (ESI)

Polymeric model systems for flavoenzyme activity: towards synthetic flavoenzymes

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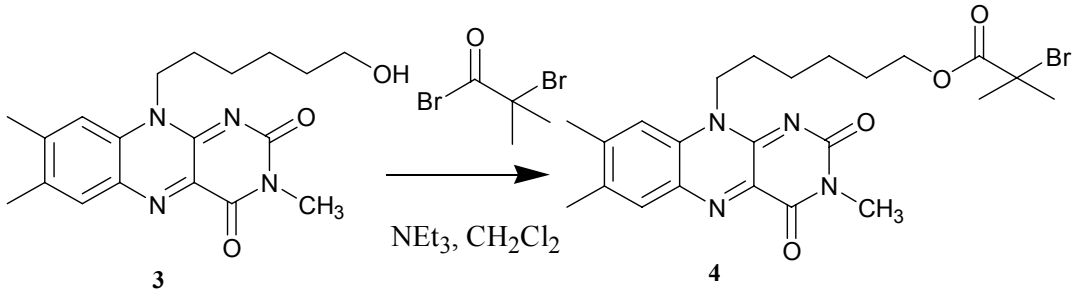
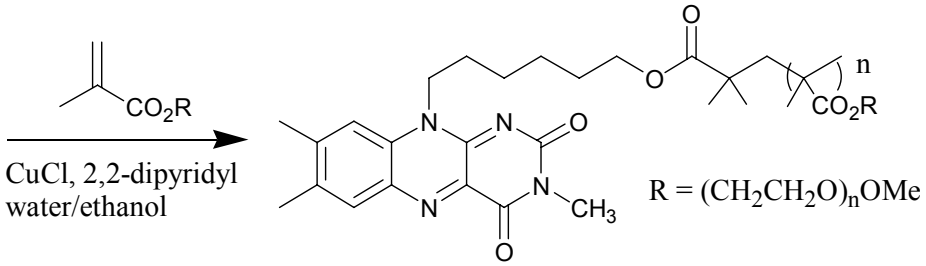
1. Synthesis





Synthesis of compound 4.

To the solution of **3**[1] (0.56 g, 1.57 mmol) in CH_2Cl_2 (10 ml) was added triethylamine (0.48 g, 0.75 ml, 4.8 mmol) and 2-bromoisobutyryl bromide (0.74 g, 0.31 ml, 3.2 mmol). The reaction was stirred at room temperature and in 1.5 h. The solution was washed with water (30 ml), and the CH_2Cl_2 layer was separated and concentrated under reduced pressure. The desired compound was purified by column chromatography on silica gel (eluent: CH_2Cl_2/acetone mixture, 5:1, v/v) to yield an orange solid (0.32 g, 40% yield). Mpt. 147-150 ^{0}C (dec). ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.05 (1H, s), 7.35 (1H, s), 4.67 (2H, t), 4.16 (2H, t), 3.49 (1H, s), 2.52 (3H, s), 2.42 (3H, s), 1.90 (6H, s), 1.89 (2H, m), 1.71 (2H, m), 1.48 (4H, m). C^{13} NMR (400 MHz, $CDCl_3$): δ 171.54; 159.99; 155.98; 148.31; 147.69; 136.57; 135.39;

134.81; 132.61; 130.78; 115.00; 65.57; 60.26; 55.97; 44.48; 28.62; 28.08; 26.96; 26.24; 25.46; 21.54; 19.37; 14.07. IR (KBr pellet, ν, cm^{-1}): 3431, 2936, 2867, 1725, 1705, 1661, 1583, 1543, 1461, 1351, 1273, 1164, 1112, 1040, 970, 806, 763. MS EI (m/z) = 505 Accurate mass: 505.1445 $[M+H]^+$ (505.1445 calc).

Synthesis of polymer 5.

CuCl (4.4 mg, 0.044 mmol), 2,2' bipyridine (18.8 mg, 0.121 mmol) and poly (ethylene glycol) methyl ether methacrylate (Mn = ~ 475 g/mol) (1.65 g, 3.74 mmol) were weighed directly into a 2-necked flask equipped with a magnetic stirrer. Methanol (1 mL) and deionised water (3 mL) were then added to the flask. The flask was then sealed with two glass stop-cock adaptors. One stop-cock was connected to a nitrogen manifold and the second to a vacuum pump. The solution was degassed by freeze-pump-thaw using 3 cycles of vacuum/nitrogen, the vacuum pump was disconnected from the sealed stop-cock and the solution held under nitrogen.

A stock solution of the flavin initiator **4** (0.11 g, 0.218 mmol) in methanol (44 mL) was prepared in a two-necked flask and sealed with two glass stop-cock adaptors. One stop-cock was connected to a nitrogen manifold and the second to a vacuum pump. The initiator solution was degassed by freeze-pump-thaw using 3 vacuum-nitrogen flush cycles. The vacuum pump was disconnected from the stop-cock and the solution held under nitrogen.

The nitrogen pressure was increased to ensure no air entered the reaction vessels during transfer of the initiator solution to the monomer solution. The initiator solution (9 mL, 0.045 mmol) was quickly transferred *via* a nitrogen flushed syringe to the vessel containing the monomer and catalyst in the following manner. The unused stop-cock was opened, the solution removed and the stop-cock closed. The stop-cock

of the monomer solution was then quickly opened, the initiator solution quickly injected to the reaction vessel and the stop-cock closed. The nitrogen pressure reduced to normal and the reaction mixture was stirred at 50°C for 4 hrs.

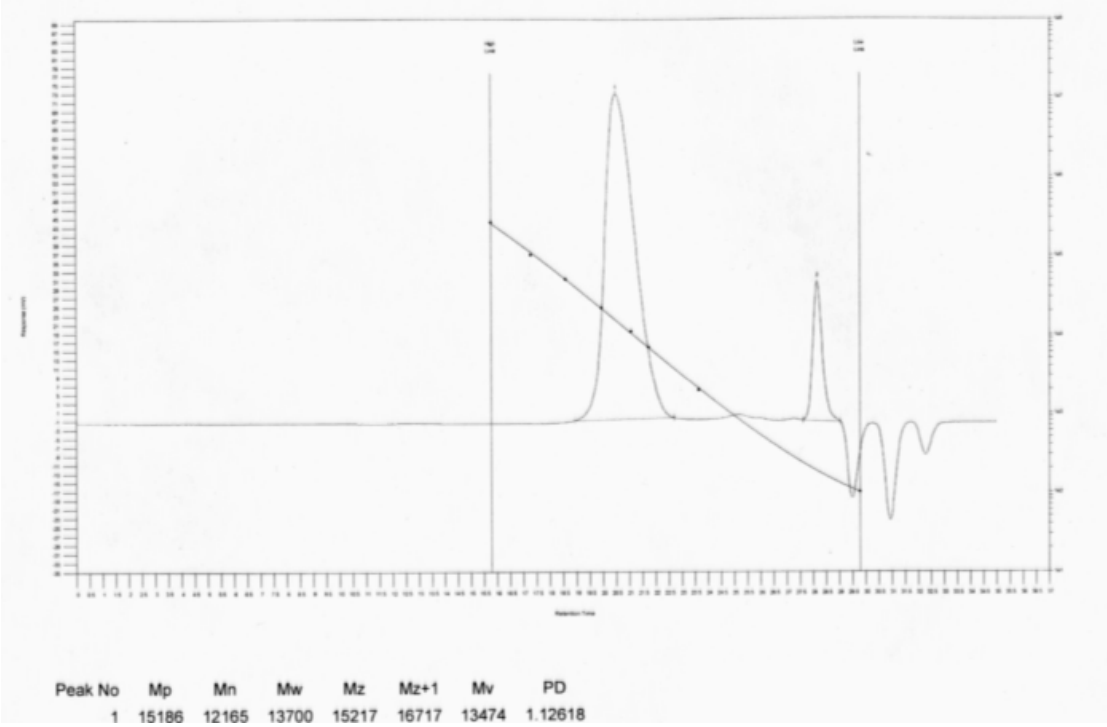
The reaction was allowed to cool, and the solvents were removed under high-vacuum. The crude mixture was dissolved in a minimum amount of CH_2Cl_2, and passed through a short plug of silical gel (methanol as the eluent) and concentrated under high-vacuum. The resulting yellow/green viscous oil was dissolved in a minimum amount of CH_2Cl_2 and precipitated into diethyl ether. This process was repeated until the sample was free of monomer (by ^{1}H NMR). A 30% conversion was achieved.

^{1}H NMR ($CDCl_3$) δ, ppm: 3.92 (broad s, 1.1 H), 3.45 (broad s, 17.5 H), 2.1-2.3 (broad m, 0.4 H), 1.6-1.8 (broad m, 0.5 H), 0.6 - 0.9 (broad m, 1.2 H). The signals corresponding to the flavin initiator were just visible in the spectral baseline.

1.Cooke G, Duclairoir F, John P, Polwart N & Rotello V. *Chem. Commun.* **2003**, 2468.

2. Polymer characterisation

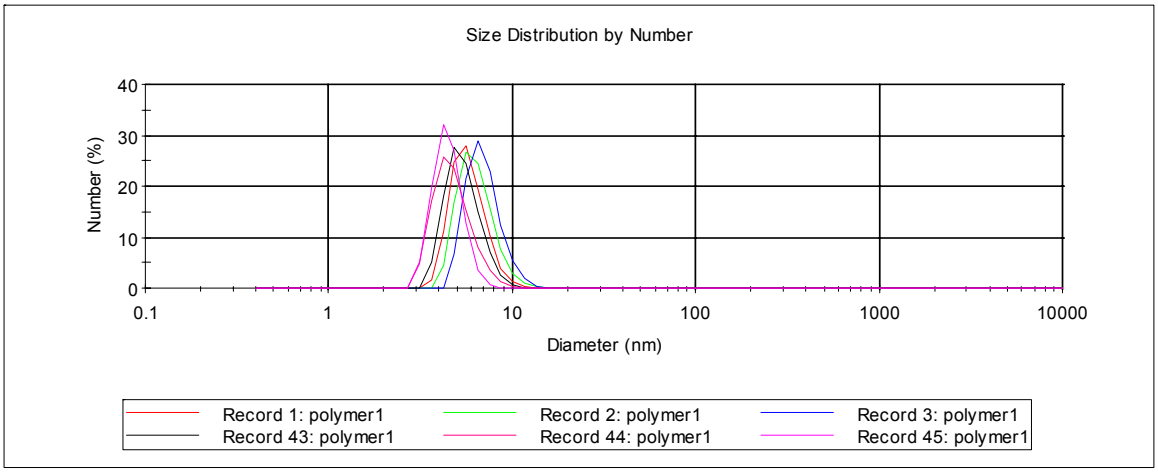
Gel permeation chromatography:



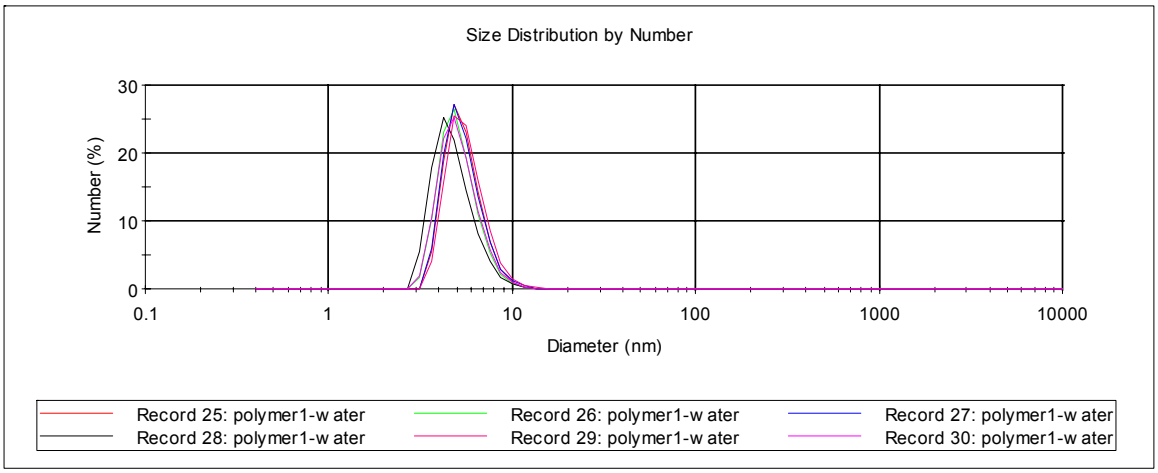
GPC data for derivative **5** performed in THF using PMMA standards.

DLS data for polymer 5:

In chloroform:



In water:



Comparison of DLS data for polymer **5**

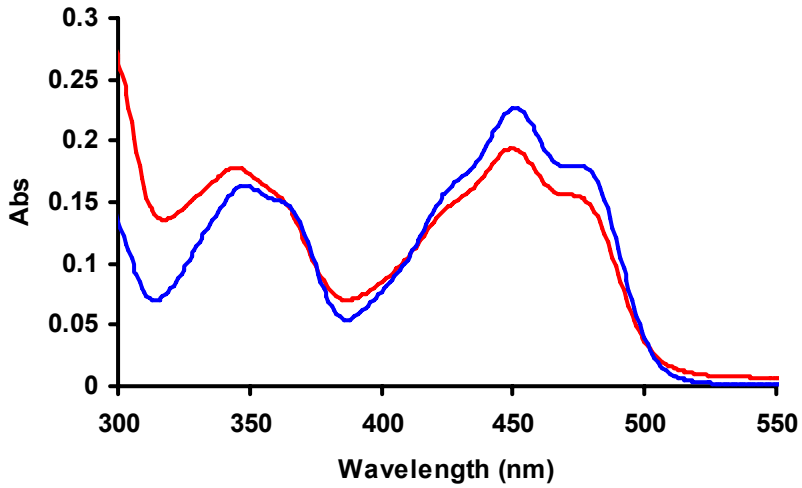
Sample	**$CHCl_3$**	**$CHCl_3$ dev**	**water**	**water dev**
polymer **5**	5.5	0.9	5.0	0.3

3. Molecular modelling

The calculations on polymer **5** were performed using the Amber* force field implemented by the MacroModel programme (subsystem was dynamics). The calculations were performed in a continuum of water as the solvent. Electrostatic treatment was set to the constant dielectric setting. Dielectric constant was set to 1.0. There were no constraints placed on the molecule.

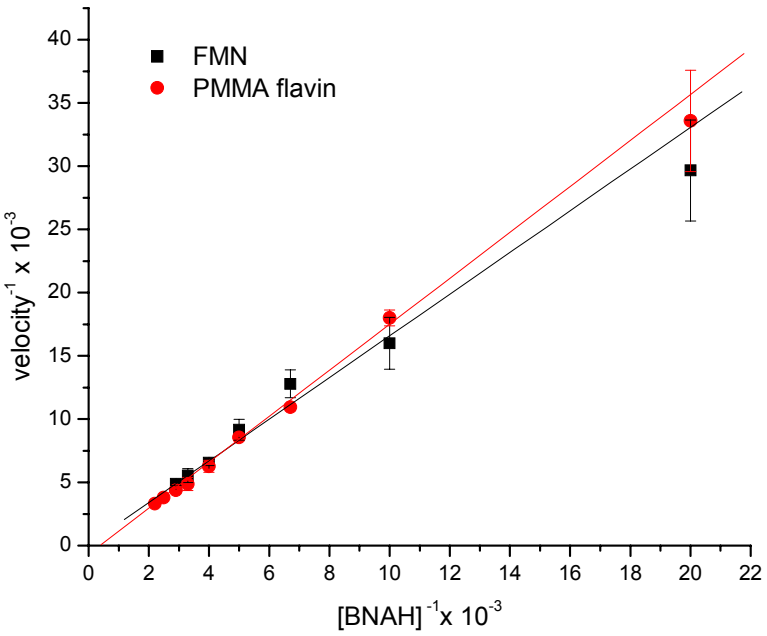
The method used was to TNCG (Truncated Newton linear Conjugate Gradient), with the maximum number of iterations at 500. Convergence was done on a gradient and the threshold was set to 0.0500. Stochastic dynamics were used, and all the bonds were shaken. The simulation temperature was performed over the range of 1000K-100K (decreasing). The time steps (fs) were set to 1.5, the equilibrium time (ps) set to 1.0, and the simulation time (ps) set to 10.0.

4. UV-vis spectrometry studies



UV-vis studies on polymer **5** (—) (1 mg in 3ml) and **6** (—) (0.8 mg in 143 ml) in chloroform.

5. Reactivity of polymer 5 and 1 with 7



Lineweaver-Burk plots of oxidation of **7** by **1** (■) and polymer **5** (●) (5 mM sodium phosphate buffered aqueous solution, pH=8.0). [Flavin] concentrations were kept constant at 3.14 x 10^{-6} while **7** was varied within the range of 5-50 x 10^{-5}.

	5	**1**
k_m	4.48×10^{-3} +/- 0.00136 M	9.83×10^{-4} +/- 0.0005574 M
k_m^{-1}	220 +/- 515	1020 +/- 370
k_{cat}	830 +/- 520 s^{-1}	220 +/- 75 s^{-1}
$[E]_0$ flavin	3.14×10^{-6} M	3.14×10^{-6} M
[**7**]	5 - 50 x 10^{-5} M	5 - 50 x 10^{-5} M

Table showing calculated values (k_{cat} and k_m) for oxidation of **7** by flavins **1** and **5**.

6. Electrochemical Methods

Both cyclic voltammetry and square wave experiments were carried out on a Cypress System Potentiostat. A 1 mm platinum button and a gold-plated electrode were utilized as the working and auxiliary electrodes, respectively. All potentials were referenced via an Ag/AgCl reference electrode using a sweep rate of 100 mV/s. Potassium Chloride (0.1 M) was used as the electrolyte in a 5 mM sodium phosphate buffered solution (pH = 8.0). Solutions of FMN and polymer **5** (1mM) were used to obtain the optimum signal to noise values and the solutions were degassed by bubbling argon through them for 10 minutes. While within the range of redox potentials for most flavin derivatives, the $E_{1/2}$ for FMN differs from our previous recorded value.[2] This difference is attributed to a completely different composition of the buffer system with an extremely low ionic strength (<< 5 mM) that was used in the absence of electrolyte in the previous report.

2. Bayir, A., Jordan, B. J., Verma, A., Pollier, M. A., Cooke, G., Rotello, V. M., *Chem. Commun.*, **2006, 4033**