Supplementary Material (ESI) for Chemical Communications

Supporting Information

Dendrimers as size selective inhibitors to protein-protein binding.

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Experimental: *Chemicals and instrumentation.*

All chemicals and reagents were obtained from either Sigma-Aldrich or Lancaster Co. and used without further purification. Cytochrome *c* from horse heart (MW: 12384) and α-chymotrypsin type-II from bovine pancreas (MW: 25 K) were purchased from Sigma and used without further purification. The fluorescence spectra were recorded on a Hitachi fluorescence spectrophotometer (Model F-4500) and analysed using its attached software.

α-Chymotrypsin inhibition/binding experiment

Unless otherwise stated all solutions were made up using a 0.1M sodium phosphate buffer.

Solution 1. 1.0×10^{-6}M solution of Bovine pancreatic α-chymotrypsin.

Solution 2. 1.0×10^{-6}M solution of all dendrimers.

Solution 3. 4.0×10^{-3}M solution of N-benzoyl-L-tyrosine *p*-nitroanilide (BTNA) in methanol.

The solutions required for the hydrolysis experiments were made up as follows; 10 ml of the α–chymotrypsin solution (solution 1 above) was added to 10 ml of (each) dendrimer solution (solution 2 above). The pH of the resulting solution was then checked to ensure that it remained at 7.4 (which it did in all cases). For the reaction an aliquot (2.00 ml) of this solution was added to a UV cell. 50µl of the BTNA solution were then added (final concentrations were 1.0×10^{-4}M in BTNA, 5×10^{-7}M in dendrimer and 5×10^{-7}M in chymotrypsin). Hydrolysis was followed by monitoring product formation at 410 nm every 20 seconds (for a total of 1200 seconds) using UV/Vis spectrometry. All solutions were kept at 20ºC. Initial velocities (v_o) were calculated from the linear region obtained over the first 150 seconds (Figure 1). Percentage inhibitions were calculated by taking the v_0 ratio of the control to dendrimer reaction and multiplying by 100. The number obtained is the extent of reaction (% of reaction compared to the uninhibited reaction), this was therefore converted to a percentage inhibition by subtracting it from 100. As inhibition is related to binding (i.e. binding blocks the active site entrance), then the relative inhibitions translate to a relative binding efficiency.

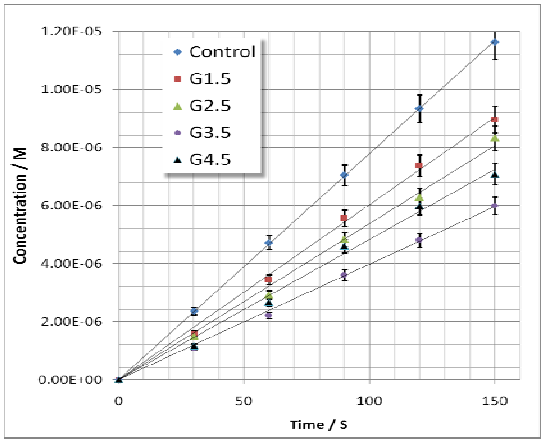

Figure 1: *Initial rate plots for the hydrolysis of BTNA in the presence and absence of dendrimer.*

Dendrimer Gen	None	G1.5	G2.5	G3.5	G4.5
v_0 /x10^{-8}Ms^{-1}	7.78	6.03	5.40	3.15	4.98
Extent of Reaction %	100	78	69	40	65
Rel Binding %	/	22	31	60	35

Table: *Kinetic and inhibition parameters for dendrimer inhibited reactions.*

Cytochrome *c* binding experiment

A tetra(carboxyphenyl)porphyrin (TCPP) stock solution of 5 µM was made up in 0.1M phosphate buffer (pH=7.4). The same TCPP stock solution was used to a make 5 µM cytochrome *c* solution. The PAMAM dendrimer solutions were made up to 0.01M, also using the original TCPP stock solution (i.e. constant porphyrin concentration). For each run 2ml of the TCPP/cytochrome-*c* solution was added to a cuvette and a fluorescence reading taken. The dendrimer solutions (10-50µL) were then titrated in. After each addition a fluorescent emission reading was taken. The fluorimeter was set with a scan speed of 1200 nm/min with emission spectra recorded/measured between 550 and 850 nm (λ_{max} of emision~650nm). The excitation wavelength was set at 420nm. Plots of saturation efficiency (saturation efficiency = (initial emission - final emission)/final emission) *vs* Log dendrimer concentration (molar) were obtained and these were fitted to a 1:1 competitive binding analysis, Figures 2 and 3. The control experiment is shown in Figure 4.

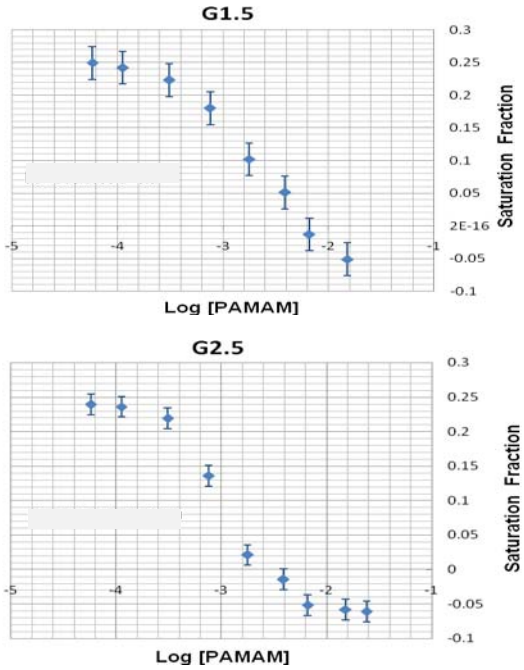

Figure 2: *Competition binding plots for the G1.5 and 2.5 PAMAM dendrimers titrated into a solution of cytochrome-c and the TCPP "hot" ligand. Plots were fitted to a standard 1:1 fitting.*

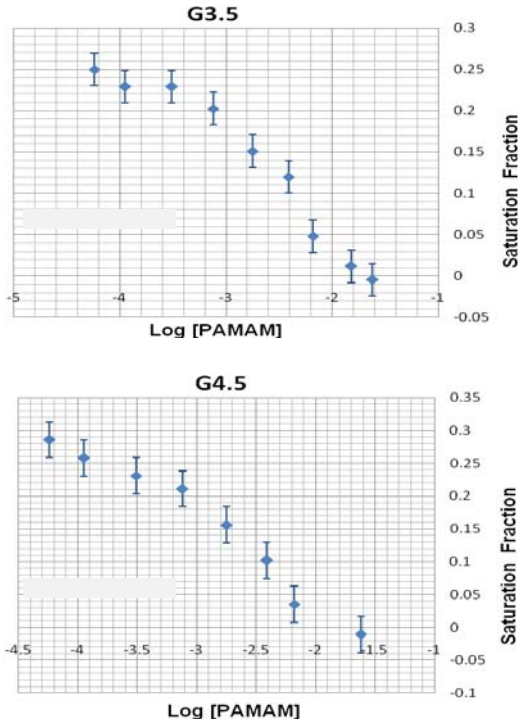

Figure 3: *Competition binding plots for the G3.5 and 4.5 PAMAM dendrimers titrated into a solution of cytochrome-c and the TCPP "hot" ligand. Plots were fitted to a standard 1:1 fitting.*

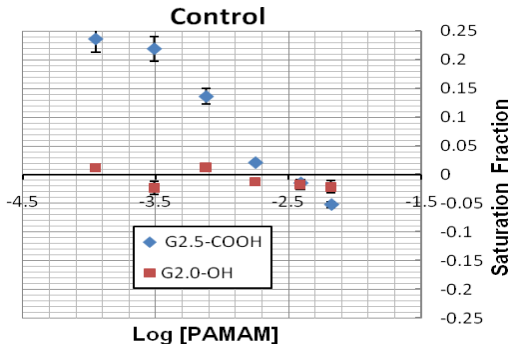

Figure 4: *Control experiment using the neutral G2.0 OH terminated dendrimer titrated into a solution of cytochrome-c and the TCPP "hot" ligand. The fact that TCPP is NOT displaced by the neutral dendrimer (i.e. no change in TCPP's emission) confirms that the increase in porphyrin emission from the original experiments were NOT due to any undesirable porphyrin dendrimer interactions. A plot for the G2.5 dendrimer is shown for comparison.*