Supporting information for: "Using ethidium to probe non-equilibrium states of DNA condensed for gene delivery"

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MATERIALS AND METHODS

Materials. Generation 5 polyamidoamine (PAMAM) dendrimers with and without surface modification through PEGylation were synthesized as previously described (*1-2*) and characterized by ^{1}H and ^{13}C NMR. The 25 % PEG-modified generation 5 PAMAM dendrimers were prepared by connecting PEG550 chains onto the surface of G5 PAMAM dendrimers by the use of a thiourea linker group, and the resulting surface coverage was determined by elemental analysis. All dendrimers were stored as stock solutions at a high concentration (>10 mg/mL) in methanol and were diluted in buffer prior to use. Restriction enzymes for linearization of DNA were obtained from Fermentas (Burlington, Ontario, Canada). All other chemicals and reagents were acquired from Sigma (St Louis, MO, U.S.). The ethidium concentration was determined by absorbance using a Varian Cary 4000 UV-Vis spectrophotometer (Varian Inc) and an extinction coefficient ε_{480} = 5600 M^{-1}cm^{-1} (*3*).

Plasmid DNA preparation. The pEGFP-C1 plasmid was amplified in *E.Coli* strain *XL1 Blue* according to standard procedures and was purified using a QIAfilter Plasmid Giga Kit (Qiagen) according to the manufacturer's instructions. The intactness and identity of the plasmid was confirmed by agarose gel electrophoresis. For linearization, the plasmid was digested with NheI (Fermentas). The efficiency of the reaction was 100% as confirmed by agarose gel electrophoresis. To remove the restriction buffer, the linearized DNA was precipitated in isopropanol, centrifuged, washed once with 70% ethanol, and resuspended in a 10 mM Hepes buffer with 10 mM NaCl (pH 7.4). The plasmid stock solutions were stored at -20°C. For each new experiment, an aliquot of DNA was thawed and diluted in 10 mM Hepes with 10 mM NaCl (pH 7.4). DNA concentrations were determined by absorbance using a Varian Cary 4000 UV-Vis spectrophotometer (Varian Inc) and an extinction coefficient of ε_{260} = 6600 M^{-1}cm^{-1} per base (*4*).

Preparation of complexes. Method 1: Dendriplexes were formed by adding a small volume (2-10 µL) from a dendrimer stock solution into a 30 µM DNA solution, immediately followed by vortexing for 30 seconds. The dendriplexes were incubated for 10 minutes before use. Complexes with N/P ratios of 5 and 10 were investigated. Samples prepared for titration experiments according to method 1 were diluted in buffer to a final DNA concentration of 10 µM prior to the titration with ethidium. For method 2, reversed titration experiments, DNA at a final concentration of 10 µM was first mixed with ethidium at final concentrations ranging from 0.5 µM to 10 µM,

whereafter fluorescence spectra were recorded. A small volume of dendrimers (2-10 µL) yielding a charge ratio of 5 was added and new spectra were recorded. The process was repeated for charge ratio 10. For both methods, UV-Vis spectra were recorded at regular intervals to ensure that no precipitation of the DNA occurred during the course of the experiment.

Ethidium titrations. The binding efficiency of the monovalent cation ethidium to compacted DNA was determined by analysing emission spectra of ethidium upon titration of DNA-dendrimer samples of known concentration with small volumes of EtBr, as previously described ("Method 1") (*2, 5*), or samples of DNA with intercalated ethidium titrated with small volumes of dendrimer ("Method 2"). The typical procedures were as follows: For Method 1, 10 µM of DNA or dendriplexes in 1 mL buffer was titrated with aliquots of 2.5-10 µL 150 µM EtBr. For method 2, DNA at a known concentration was mixed with different concentrations of ethidium in a total volume of 1 mL whereafter 2-10 µL of dendrimer solution was added. Emission spectra were recorded on a Varian Eclipse spectrofluorimeter (Varian Inc.), with excitation and emission bandpass set to 10 nm. Reference spectra were recorded in separate experiments, and that for completely bound ethidium was measured on a sample where DNA at a final concentration of 300 µM was added to a 5 µM EtBr solution. The concentration of bound ethidium in each titration point was determined by least-squares projection of the measured spectra onto reference spectra corresponding to completely bound and completely free ethidium using the pseudoinverse function pinv in the Matlab® software package (see Figure 1 in the original paper). The concentration of free ethidium was determined by subtracting the bound ethidium from the total ethidium concentration The titration data, in the form of Scatchard plots, were fitted to the McGhee and von Hippel conditional probability model of excluded site binding according to Equation 1 (*6*):

$$\frac{\theta_{EtBr}}{c_f} = K_{EtBr}(1 - s\theta_{EtBr})\left[\frac{(1 - s\theta_{EtBr})}{(1 - (s-1)\theta_{EtBr})}\right]^{(s-1)} \quad (1)$$

with K_{EtBr} the apparent binding constant of ethidium to DNA and s the apparent number of binding sites (base pairs) covered by a bound ethidium molecule. The parameter θ_{EtBr}, the ratio of bound ethidium to the number of DNA base pairs, was modified according to Equation 2 by introducing a parameter x representing the apparent

accessible fraction of binding sites on DNA remaining after dendrimer binding:

$$\theta_{EtBr} = \frac{c_b}{x * c_{DNA}} \qquad (2)$$

The number of covered binding sites (s) was calculated to be 2.5 base pairs using titration data for uncondensed, linear DNA (5), which is close to the value 2 of the nearest neighbour exclusion model. It was assumed that s does not change significantly upon dendrimer binding; hence this value was kept constant for the remaining titration data where only values of K_{EtBr} and x were calculated.

References

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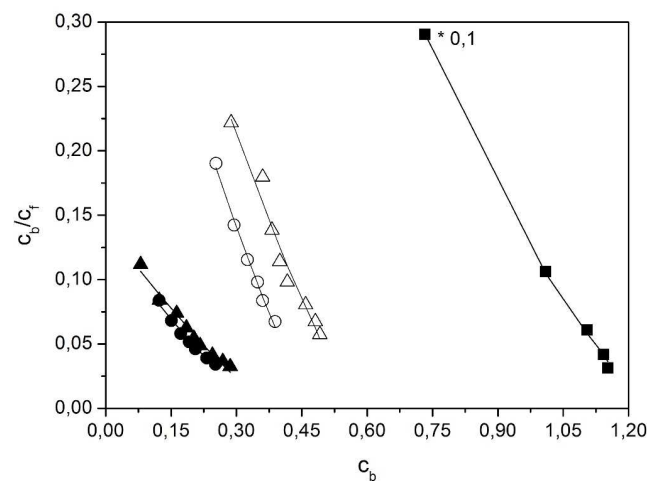6. McGhee, J.; von Hippel, P. *J Mol Biol.* 1974, *86*, 469-489.

Figure S1. Scatchard plots and corresponding simulated data obtained using the least square curve fit of the titration data with the modified McGhee-von Hippel equation (Eq 1). The simulation yields the apparent binding constant K_{EtBr} and the fraction of available binding sites x for dendrimer-condensed DNA. The data represents dendrimer-DNA complexes obtained using PEGylated G5, r = 5 (△) and r = 10 (○), unmodified G5, r = 5 (▲) and r = 10 (●), as well as uncondensed, supercoiled plasmid DNA (■). The resulting parameters K_{EtBr} and x are presented in Figure 4. The data for uncondensed DNA has been multiplied by 0.1 to maintain the clarity of the figure.