

Optically Triggered Release of DNA from Multivalent Dendrons by Degrading and Charge-Switching Multivalency**

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Multivalent binding between nanoscale objects has recently emerged as one of the most powerful methods for the assembly of functional supramolecular materials with applications in nanotechnology.^[1,2] Controlling the self-assembly of nanomaterials by using external stimuli, such as pH, temperature, light, electric potential, or magnetic field, is an important requirement for the preparation of functional and responsive materials for a wide range of potential applications.^[3,4] Stimuli-responsive materials have been extensively pursued, with special focus on medicinal applications; for example, controlled drug and DNA delivery systems,^[5,6] reactivation of caged enzymes,^[7] and switchable membrane proteins.^[8] The use of light as an external stimulus offers a number of advantages, because light is easy to apply, relatively harmless to living organisms, and—importantly—controllable both spatially and temporally.^[5,7,9] DNA-binding compounds that can be manipulated by light are especially interesting in nonviral gene therapy, which relies on synthetic compounds that protect, transport, and release DNA into target cells.^[10] Cationic dendritic systems have been of particular interest in this regard.^[11–13] Pioneering gene-therapy research has been conducted with polyamidoamine (PAMAM)^[14] dendrimers, while dendritic poly(L-lysine)^[15] and poly(propylene imine)^[16] have also been studied. Many of these compounds bind DNA. However, unpacking of the complexes and release of the DNA is difficult to achieve if the binding is very strong, which results in a low transfection efficiency.^[17] DNA release is therefore of direct importance.

There have been some recent studies with a focus on photocleavable dendrimers and dendrons. The studies include

self-immolative dendrimers^[18] and dendrimers based on photocleavable cores^[19,20] or photoactive surfaces.^[21] These systems release covalently bound units from the dendritic structure in a process triggered by UV irradiation. It is worth noting that gold nanoparticles can also be employed in DNA binding and delivery in an analogous manner to dendritic structures.^[9] Despite much progress, systems in which non-covalent multivalent binding could be controlled by external stimuli have not yet been fully developed.

Multivalency is defined as a type of binding in which multiple ligands are attached to a single molecular scaffold and used to interact with another entity that displays multiple binding sites which are complementary to the ligands.^[1,2] In recent studies, we reported a series of Newkome-type^[22] dendritic ligands, with multiple protonated spermine groups on their surfaces, which exhibit multivalent DNA binding.^[17,23,24] Given our interest in multivalent DNA recognition, we decided to explore whether our receptor could be developed in such a way as to achieve photoresponsivity. We therefore modified our previously reported dendrons by attaching the spermine surface groups through an *o*-nitrobenzyl linker (Scheme 1 a). The *o*-nitrobenzyl group undergoes photolytic degradation (Scheme 1 b) when submitted to long-wavelength UV light ($\lambda = 350$ nm), thus allowing a controlled release of the covalently attached spermine surface groups and the noncovalently bound DNA. Once the spermine groups are cleaved from the surface of the dendron, the cationic multivalency effect is destroyed, thereby leaving just individual spermine groups, with only a weak affinity for DNA. In this way, the DNA molecule will be effectively decomplexed upon photolysis. Importantly, as the surface groups are cleaved, they leave behind an anionic carboxylic acid surface that will further repel DNA (Scheme 1 c).

UV-responsive spermine derivatives (Scheme 1 a) were synthesized and characterized by using standard methods (see the Supporting Information). Their photolytic degradation was first studied by means of UV/Vis spectroscopy by irradiating an aqueous solution of **pII-G0**, **pII-G1** (see the Supporting Information), or **pII-G2** (Figure 1) and following the time course of the reaction. Irradiation of these compounds with UV light at 350 nm led to significant changes in the UV/Vis spectra. A decrease of absorbance was observed at 245 nm, along with a clear increase at 268 and 349 nm—changes which typically indicate the photolytic reaction proposed in Scheme 1 b.^[19,25] Figure 1 b shows that the degradation of the dendritic systems reaches a plateau after about 200 s. Longer irradiation times lead to further changes in the absorption spectra, for example, to a decrease of the absorption at 330–400 nm (see the Supporting Information).^[8]

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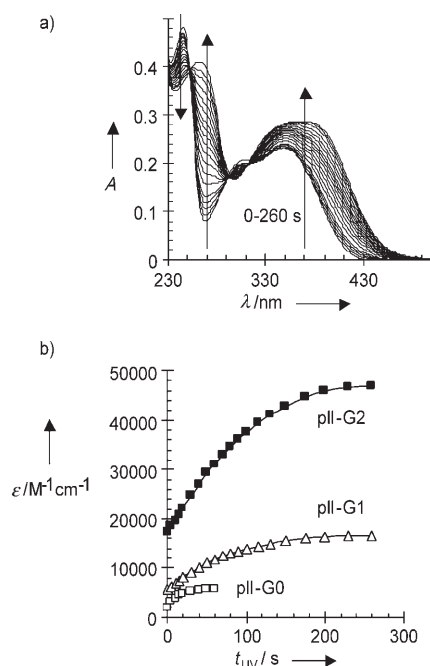


Figure 1. Photolysis of spermine derivatives. a) UV/Vis spectra of **pll-G2** ($40 \mu\text{g mL}^{-1}$) after different UV-irradiation times (0–260 s). b) The molar absorption coefficients of **pll-G0**, **pll-G1**, and **pll-G2** (at 390 nm) plotted against the UV-irradiation time indicate that **pll-G2** releases approximately three times more surface groups than **pll-G1**.

systems. The larger dendron **pll-G2** binds slightly stronger than **pll-G1** ($\text{CE}_{50} = 0.7$ and 1.0 , respectively, Figure 2b and Table 1). These CE_{50} values are in good accordance with—although slightly lower than—our previously reported values for spermine derivatives without the pll-linker.^[23]

Complex relaxation with csB (at a salt concentration of 9.4 mM NaCl) shows that **pll-G0** and spermine pack DNA into weak complexes, which are easily opened by a relatively small amount of csB (Figure 2c). However, **pll-G1** and **pll-G2** form extremely strong complexes with DNA that cannot be opened—not even with very high S/N ratios (Figure 2c). If the salt concentration is increased to 150 mM , the **pll-G1**–DNA complex can be fully relaxed with an approximately 10-fold excess of csB, whereas the **pll-G2** complexes are stronger and can be relaxed with a 50-fold excess (Figure 2d).

The dendron–DNA complex disassembly was then directly monitored as a function of the irradiation time. DNA was first fully complexed with the dendritic polycation ($\text{CE} = 2$), and the resulting complexes were irradiated under UV light. The EthBr fluorescence was then recorded after different time periods (if dendron disassembly occurs, EthBr should be able to compete effectively for DNA binding). At 9.4 mM NaCl , the fluorescence of EthBr increases, which indicates that both pll dendrons release DNA after 90 s (Figure 2e), while at a salt concentration of 150 mM , the dendron **pll-G1** releases DNA after 40 s and **pll-G2** after 55 s

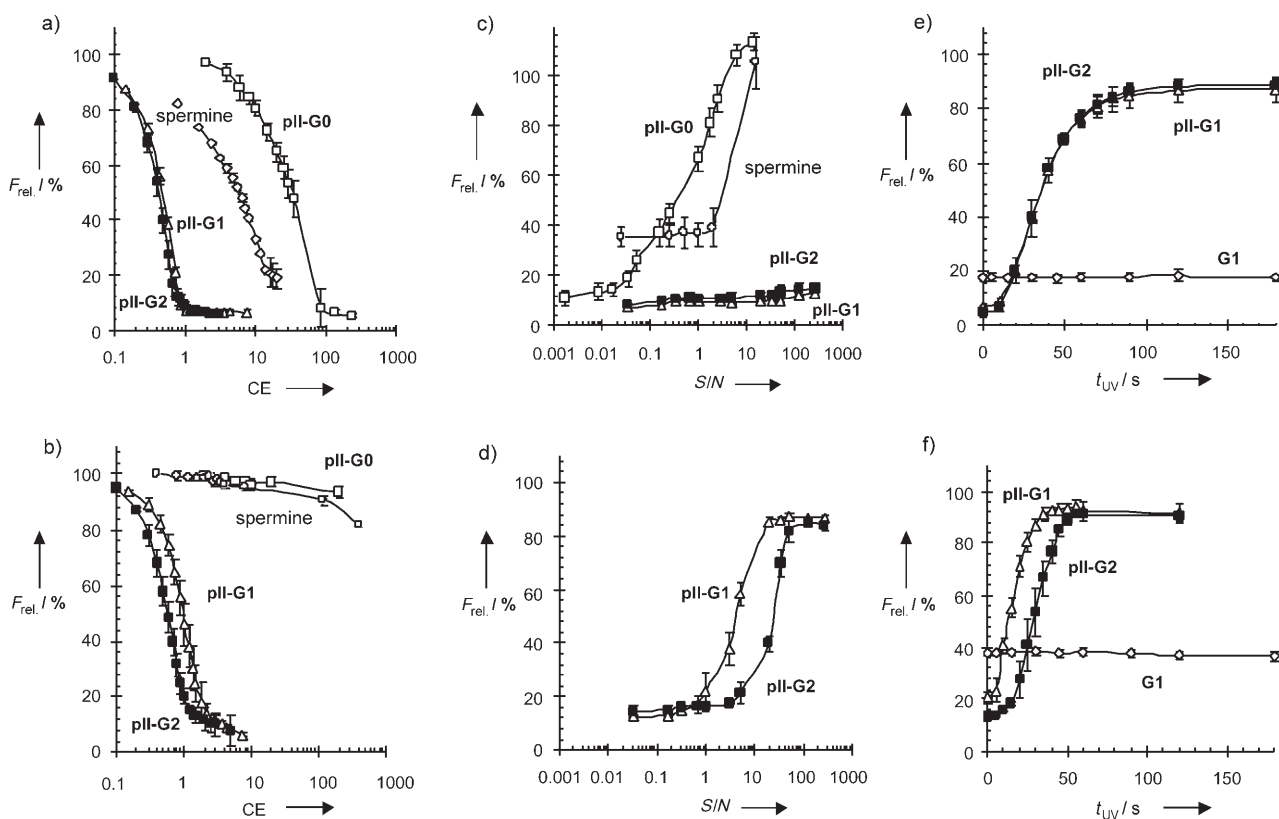


Figure 2. Titration curves for spermine, **pll-G0**, **pll-G1**, or **pll-G2**. EthBr fluorescence quenching in the presence of a) 9.4 mM and b) 150 mM NaCl . DNA–polycation complex relaxation with csB in the presence of c) 9.4 mM and d) 150 mM NaCl . Release of DNA from the complexes by UV irradiation in the presence of e) 9.4 mM and f) 150 mM NaCl . The added polyamine solution did not exceed 5% of the total volume; therefore, no corrections were made for sample dilution. The results are the average of triplicates; error bars: standard deviation.

(Figure 2 f). This faster release might be expected as a result of the slightly weaker complexation between the dendrons and DNA under the high-salt conditions. Dendron G1 (that is, **pII-G1** without the photolabile *o*-nitrobenzyl linker) was used as a reference, and no release of DNA was observed from this complex as a result of UV irradiation. Importantly, this same trend—namely, that the dendrons **pII-G1** and **pII-G2** behave similarly at 9.4 mM NaCl, while the latter compound binds more strongly to DNA than the former one at 150 mM NaCl—was observed with all three fluorescence titration methods.

Light scattering and ζ -potential measurements were used to further investigate the DNA-binding and releasing properties of **pII-G2**. DNA was complexed with **pII-G2** (CE = 2), and the particle-count rate and the ζ -potential were measured before and after one minute of irradiation. Before the UV treatment, the observed count rate was 420.9 kcps (kilo counts per second) and the ζ -potential was (14.7 ± 3.4) mV, which indicates the formation of a large number of positively charged particles. After UV irradiation, however, the particle-count rate dropped to only 8.8 kcps and the ζ -potential to $-(27.8 \pm 7)$ mV. This result confirms the complex breakdown and the formation of anionic species with a high negative charge. The decrease in the count rate is attributed to the change in the refractive index of the dendron–DNA complexes as they undergo a transition from condensed globules to loose coils, which have a lower refractive index.

Finally, DNA binding and release by pII dendrons was confirmed by means of gel electrophoresis. The photolabile dendritic constructs **pII-G1** and **pII-G2** retarded the electrophoretic mobility of pDNA as a consequence of charge neutralization and complex formation, whilst **pII-G0** was ineffective (Figure 3). After UV irradiation, **pII-G1** and **pII-**

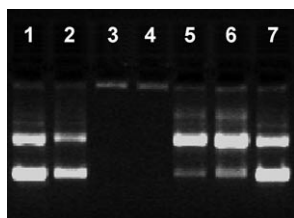


Figure 3. Gel electrophoresis of pDNA (250 ng per lane). Lane 1: pDNA. Lane 2: pDNA + **pII-G0** (CE = 30), no UV irradiation. Lane 3: pDNA + **pII-G1** (CE = 2), no UV irradiation. Lane 4: pDNA + **pII-G2** (CE = 2), no UV irradiation. Lane 5: pDNA + **pII-G1** (CE = 2), 1 min UV irradiation. Lane 6: pDNA + **pII-G2** (CE = 2), 1 min UV irradiation. Lane 7: pDNA, 1 min UV irradiation.

G2 clearly released DNA, thus allowing its free electrophoretic mobility. Interestingly, the conformation of the **pII-G1**- or **pII-G2**-bound pDNA shifted from the supercoiled to the open-circular form (lanes 5 and 6 in Figure 3) after UV irradiation. Importantly, UV-irradiation-induced pDNA fragmentation was not observed.

In conclusion, we have demonstrated a new approach for achieving reversible DNA binding by using photolabile multivalent dendrons, which release DNA by degrading and charge-reversing multivalency. In particular, **pII-G1** and **pII-**

G2 bind efficiently to DNA—through complementary electrostatic interactions—but they can also release their target very rapidly upon long-wavelength UV irradiation ($\lambda = 350$ nm). Effectively, the high-affinity multivalent interactions are “switched off” by UV irradiation. It is therefore possible to control DNA binding and release, which makes these dendrons very promising for applications in nanobiotechnology.

Experimental Section

Full details of the experimental methods can be found in the Supporting Information.

Charge Excess (CE): is defined as the nominal “number of positive charges” of the polyamine divided by the “number of negative charges” present on the DNA. A molecular weight of 330 g mol^{-1} and one negative charge per nucleotide were assumed.

S/N Ratio: is defined as the nominal “number of negative charges” of the csB divided by the “number of positive charges” of the polyamine. A molecular weight of 444 g mol^{-1} and one negative charge per repeat unit were assumed.

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