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Switchable Nanoassemblies from Macroions and Multivalent Dye Counterions

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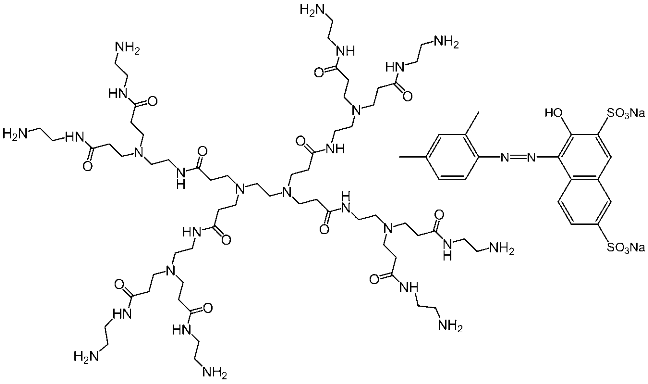
Supramolecular assemblies obtained by self-organization of small building units are of high interest due to the potential for easily designing complex nanostructures with tailored physical, chemical, and biological properties.[1] Inspiration originates from natural supramolecular assemblies with a variety of architectures and functions from cell membranes to DNA–histone complexes. The main advantage of association through noncovalent interactions over a molecular synthetic design is the capability for rearrangements, leading to “self-healing” or “switchable” systems that can respond to external triggers. Possible applications are in medicine, information technology, and optoelectronics. With regard to the growing field of nanotechnology, it is thus highly desirable to develop new concepts for self-assembly and responsive nanostructures.

Classical self-assembly of surfactant molecules into micelles is driven by hydrophobic interactions. Amphiphilic block copolymers widen this concept to a variety of structures and applications from carrier systems to sensors.[1d] Aesthetic supramolecular structures are based on hydrogen bridging, but are restricted to unpolar solvents.[2] New approaches thus focus on specific building blocks for self-assembly in polar solution, for example, bis-zwitterions[3] or biosynthetic copolymers;[4] however, special synthetic design is required. It is therefore of interest to extend the area of self-assembly in aqueous solution to a broader field of structures and functionalities. The challenge in particular is to use a combination of available building units that form supramolecular assemblies. In view of environmental and medical applications the preference is on aqueous solutions. This can be realized with ionic building blocks: Ionic dye molecules can associate with oppositely charged polyelectrolytes in aqueous solution. The binding is cooperative, that is, secondary interaction of the dyes′ π systems causes a preference of adjacent rather than random binding. Spectroscopic studies with monovalent dyes exist since the 1970s, but nanoscale structures of the systems have not been explored.[5] Secondary interaction is also the key to well-defined, solid, nanostructured materials with ionic surfactants, in which hydrophobic interaction is induced upon binding to polyelectrolytes[6] or multivalent organic ions.[7] In contrast, relying solely on π–π interactions for the formation of aggregates yields less structural variety and aggregates often are not stable over time.[8]

Herein we investigate supramolecular structures formed by electrostatic self-assembly of macroions and multivalent dye counterions in aqueous solution. In addition to a certain size and valency the counterions have the possibility to perform mutual π–π interaction and thus allow a “directed” structure formation. Currently we have shown that polyelectrolyte dendrimers and naphthalene dicarboxylic acids in methanol can form cylindrical and spherical aggregates.[9] To develop “switchable structures” and to use aqueous solutions, we have chosen the following model system (Scheme 1): We use fourth-generation (G4) poly(amido-



Scheme 1. Building blocks for electrostatic self-assembly: poly(amidoamine) dendrimer macroion (G1 to visualize the chemical structure, G4 was used in this study) and counterion Acid Red 26.

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amine) (PAMAM) dendrimers as well-characterized molecules with a certain structure yet relatively large flexibility.[10] It should be emphasized that our goal is not to use the dendrimer as a host for guest molecules to be included in their interior as in other studies.[11] In contrast, ionic dyes connect multiple dendrimers to form larger aggregates. The G4 dendrimer is a cationic macroion with 64 charges at pH 6.5 (primary amine groups protonated) and 126 charges at pH 3.5 (also tertiary amine groups protonated).[12] The "linker" is the oppositely charged water-soluble divalent azo dye with two strongly acidic sulfonate groups (Ar26). It has the advantage that the optical spectrum changes upon stacking, but not much when varying other parameters such as pH, solvent polarity, and salt concentration.

Adding increasing amounts of dendrimer to a solution of dye changes the color from red to yellow (Figure 1a). Figure 1b shows the UV/Vis spectra of the pure dye. Upon increasing concentration, band splitting and a blue shift of the absorption maximum is observed. This indicates formation of H-aggregates, that is, stacking of the dye molecules on top of each other.[13] The existence of an isosbestic point shows that only two spectroscopically distinct species are present.[14a,b] This allows us to apply a monomer–dimer model fit (Figure 1b), yielding the dimerization constant of the dye of $K_D = 2400\,\mathrm{L\,mol^{-1}}$ ($\pm 20\,\%$).[14,15] Figure 1c shows spectra for dendrimer–dye solutions obtained at a dye concentration at which the dye does not self-associate on its own. The gradual change in absorption upon dendrimer addition indicates the appearance of "stacked dye", again as H-aggregates, induced by the dendrimer. Upon addition of positively charged dendrimer, the anionic dyes electrostatically associate with the dendrimer, allowing their mutual interaction by providing spatial proximity. An isosbestic point is observed at a "loading ratio" (molar ratio of dye sulfonate groups to dendrimer primary amine groups) of 2. Upon further addition of dendrimer, the absorption increases again, which indicates a change in aggregate type. (A slight increase is observed for loading ratio 1.3:1 in Figure 1c and further increase for even smaller loading ratios.[15])

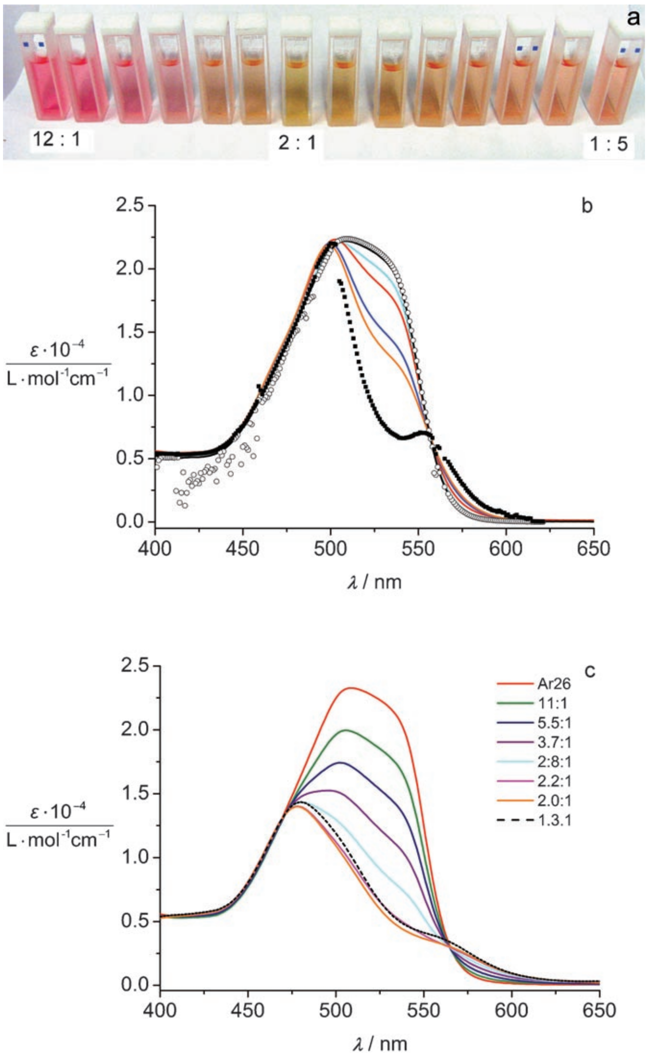

Figure 1. a) Photograph of AR26-G4 samples for different dye:dendrimer ratios. b) UV/Vis spectra of Ar26 at varying concentration of $c = (4\text{–}900) \times 10^{-6}\,\mathrm{mol\,L^{-1}}$ (lines) and calculated spectra of (○) monomer and (■) dimer. c) UV/Vis spectra of dye–dendrimer samples with $c(\mathrm{Ar26}) = 2.2 \times 10^{-6}\,\mathrm{mol\,L^{-1}}$ at varying loading ratios (pH 3.5).

Size and stability of the dendrimer–dye aggregates were analyzed by dynamic light scattering (DLS). Figure 2a shows time-dependent hydrodynamic radii.[15] It is evident that not only the dye attaches to the dendrimer, but thereby larger aggregates of 50–300 nm size are formed that must consist of multiple dendrimer molecules. The behavior strongly depends on dye/dendrimer ratio. In excess of dendrimer, the scattering intensity is low and the predominant species are single dendrimers loaded with dye. For medium loading ratios stable aggregates are formed. The region of stable aggregates depends on pH: It lies around 2:1 for pH 3.5 (between 2:1 and 1.4:1) and around 1:1 (between 1.4:1 and 0.9: 1) for pH 6.5. This is due to the different degree of protonation of the dendrimer that thus can bind twice the amount of dye ions at pH 3.5. Stable aggregates do not show any change in size for at least a month. For excess of dye charges, continuous growth is observed that can lead to precipitation.

The three association regimes are depicted in Scheme 2. At low load, dye ions associate with individual dendrimer molecules in analogy to other systems.[11] Upon increasing load, more dye counterions ionically bind to the dendrimers and thereby connect multiple dendrimers. Usually multivalent ions lead to aggregation of polyelectrolytes, which in our case can take place in a more "defined way" due to building blocks with a certain architecture and interaction capability. Evidently, there is an ideal loading range around charge stoichiometry at which such multidendrimer aggregates are stable in solution. A delicate interplay of electrostatics, π–π interactions, and geometric aspects may be responsible for the size control. Geometric constraints in the binding of dye molecules to dendrimers can cause this aggregation to be incomplete and the aggregate to be charged, as is also the case for the formation of rodlike biomarcomolecule bundles of defined diameter.[16] Thereby these aggre-

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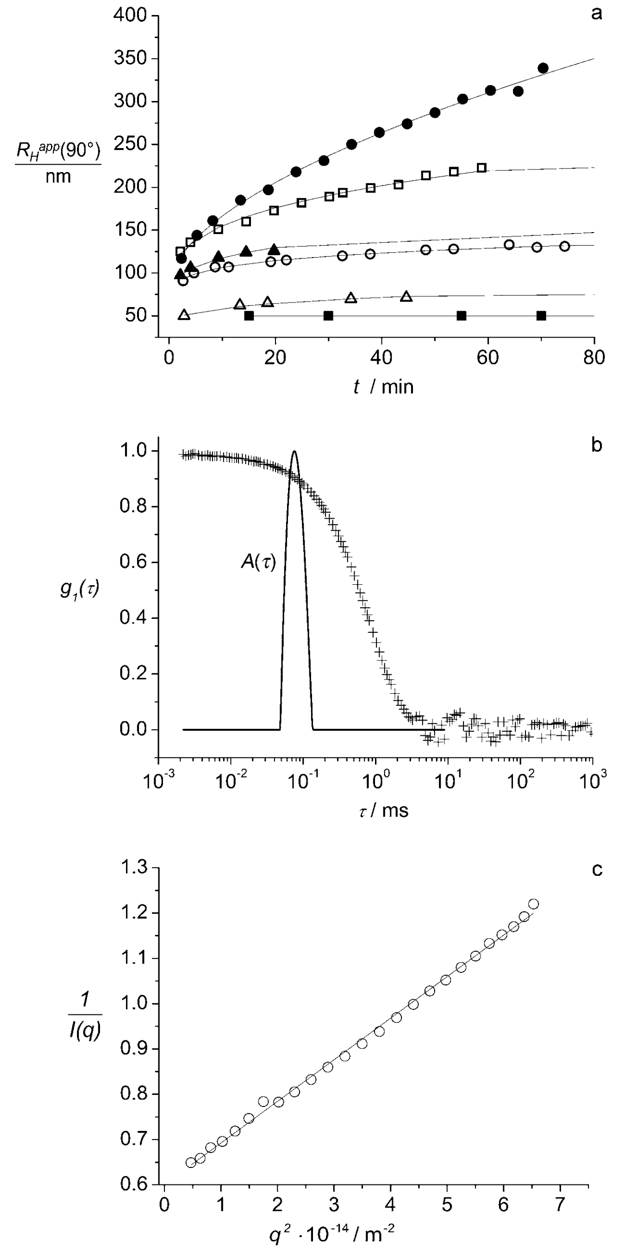
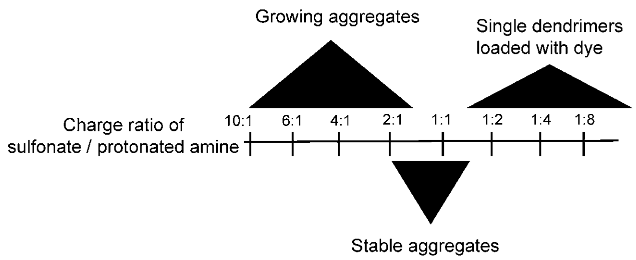


Figure 2. Light-scattering analysis of dendrimer–dye assemblies. a) Time-dependent R_H at pH 3.5 for different loading ratios: (■) 1.8:1; (△) 2.2:1; (●) 2.8:1; (□) 5.2:1; (▲) 6.4:1; (○) 9.3:1. b) Electric-field autocorrelation function $g_1(\tau)$ and distribution of relaxation times $A(\tau)$ (scattering angle $\theta = 90°$) for 1:1 loading at pH 6.5. c) Static light scattering of the same sample ($I(q)$ = scattering intensity in arbitrary units; q = scattering vector).

gates mutually repel each other and are stabilized at a certain size at which attractive and repulsive forces equilibrate.

In the following we will focus on a more detailed characterization of the stable nanoassemblies in solution, with hydrodynamic radii $R_H = 55–85$ nm. Particles show a narrow size-distribution, as is seen from DLS (Figure 2b) and the fact that static light scattering (SLS) yields a linear Zimm plot (Figure 2c). The ratio R_G/R_H (R_G = radius of gyration from SLS) is an indicator for particle shape, here 1.2–1.4,



Scheme 2. Association regimes depending on "charge ratio".

clearly indicates nonspherical aggregates. Further details on particle shape were obtained from small-angle neutron scattering (SANS) measurements (Figure 3). The scattering intensity $I(q)$ (Figure 3a) can be transformed into the pair distance distribution function $P(r)$ (Figure 3b), a function that indicates the particle shape.[17] The $P(r)$ in Figure 3b is typical of cylindrical particles with a length of about 225 nm and a cross-section dimension of about 60 nm. The cross section is not circular, but somewhat flat (30 × 60 nm).[15] Formation of stable anisotropic aggregates with narrow size distribution demonstrates the concept of macroions and counterions forming well-defined supramolecular structures in aqueous

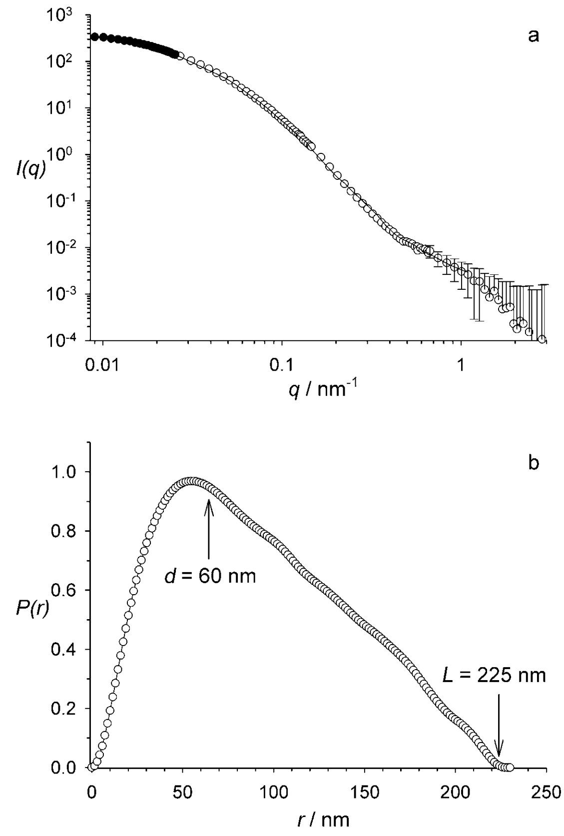


Figure 3. SANS analysis of dendrimer–dye assemblies. a) Scattering curve $I(q)$ combined from (●) SLS and (○) SANS with fit to the data (line) (arbitrary units). b) Corresponding pair distance distribution function $P(r)$ indicating anisotropic particles with a maximum dimension of 225 nm and a cross section in the order of 60 nm.

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solution. It should be noted that both in diameter and length multiple building blocks (4 nm dendrimers and 1 nm dyes) associate. Hence, the structure control is evidently more complex than in surfactant aggregation with a determining "packing parameter". The driving force for the anisotropy in aggregation is the π–π interaction, in analogy to that reported in reference [9], in which it was shown that aggregates have an anisotropic shape only if stacking interaction is involved. In accordance, other divalent ions such as alkane disulfonates did not induce the formation of cylindrical aggregates.[18] It may be concluded that the ionic interaction of dendrimers and dyes induces formation of dye stacks, which again cause the dendrimers to align into cylindrical aggregates. This is consistent with the internal structure that can be deduced from a more detailed $P(r)$ analysis. Dye stacks and aligned dendrimer columns alternate in the inside of the aggregate.[15]

The thermodynamics of the aggregation was investigated by isothermal titration microcalorimetry (ITC). Exothermic peaks on injection were observed up to 61 ± 3 dye molecules per dendrimer, confirming the stoichiometric composition of the assembly derived from UV/Vis spectroscopy and light-scattering measurements. The enthalpy for the binding of one dye molecule to two dendrimer amine groups was $\Delta H = 48\,\mathrm{kJ\,mol^{-1}}$ and the equilibrium binding constant was $K = 5\times10^{7}\,\mathrm{L\,mol^{-1}}$, which is in accordance with the postulate of ionic binding. It is much higher than the dimerization constant of the dye, which means that the tendency to form macroion–dye assemblies is much higher than for the dye alone to form stacks. To further elucidate the nature of the self-assembly, additional dendrimer was added to a stable aggregate to a ratio at which usually no larger aggregates exist: aggregates dissolve, clearly indicating equilibrium structures controlled by the dendrimer/dye ratio.

Well-defined equilibrium supramolecular structures are highly interesting due to the ability to respond to external triggers. In our case this can be realized by uncharging the dendrimer through pH: as the assembly is based on ionic interaction it should dissolve. Figure 4 demonstrates this pH-switching. Upon addition of sodium hydroxide and changing from pH 3 to pH 10, the UV/Vis spectrum shows disappearance of the stacked aggregates and only the monomeric spectrum as without dendrimer (Figure 4, center). Correspondingly, the scattering intensity decreases and almost no autocorrelation is detected (left and right); that is, no larger aggregates are present. Subsequent addition of acid then allows to "switch back" to the aggregated state, with the resulting aggregates having the same aspect ratio as before.[19] UV/Vis measurements show that switching the aggregation "on" and "off" can be repeated at least ten times.

In conclusion, we have successfully demonstrated a novel concept for the formation of responsive supramolecular structures in aqueous solution. Electrostatic self-assembly of macroions and dye counterions was shown to yield stable aggregates with narrow size distribution and anisotropic shape. Aggregation can be repeatedly switched "on" and "off" by pH. This may open a field of versatile functional structures in aqueous solution.

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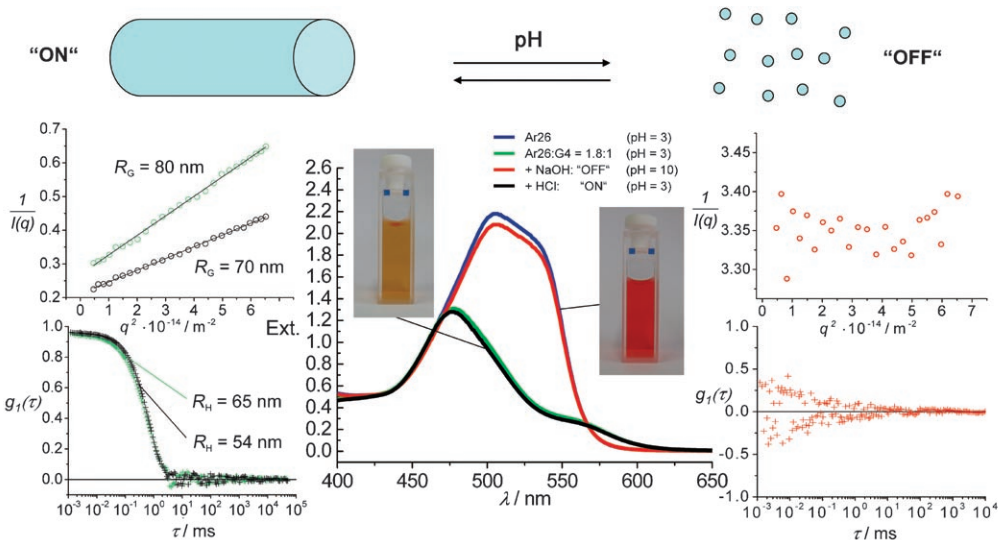

Figure 4. Switching of electrostatically self-assembled structures by pH: "on" and "off" means aggregates and single dendrimers, respectively; center: UV/Vis spectra; left and right, top: static light-scattering, Zimm representation, bottom: dynamic light-scattering, electric field autocorrelation function ($\theta = 90°$).

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[19] The size of the aggregates slightly decreases after switching back on, which may be due to the increased salt content.

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