Switchable hydrogels

DOI: 10.1002/smll.200700366

Controlled Trapping and Release of Quantum Dots in a DNA-Switchable Hydrogel**

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DNA-switchable structures have been under extensive investigation in DNA nanotechnology,[1] where DNA-based supramolecular assemblies are switched between mechanically distinct conformational states through hybridization with DNA "fuel" strands. Reversibility is achieved by displacing previously added fuel strands from the assembly in a branch-migration process.[2] This operation principle has been recently applied to realize a switchable polyacrylamide (PAAm) hydrogel with controllable macroscopic rheological properties.[3] In the present study, we investigate the nanoscopic aspects of this system in detail using fluorescent semiconductor quantum dots (QDs) as probe particles. The diffusion properties of the QDs in the gel are studied using single-molecule fluorescence microscopy and fluorescence correlation spectroscopy. Trapping and DNA-triggered release of the nanoparticles is directly visualized, demonstrating the potential of the DNA-switchable gel as a controlled release system with possible applications in drug delivery.

PAAm gels are usually synthesized by mixing acrylamide monomers, crosslinkers, and water in a certain ratio, followed by initiation of polymerization with a catalyst. The resulting polyacrylamide chains are chemically inert and biocompatible.[4] Numerous crosslinking agents can be used to tune the properties of PAAm gels.[5] Using double-stranded DNA as a reversible crosslinker provides a number of advantages: the resulting gel is biocompatible and sequence programmable; the mechanical properties and the pore size of the crosslinked gel can be adjusted by the length of the DNA crosslinker strands, and the melting temperature of the gel can be controlled by the sequence and length of the DNA crosslinkers.

For the synthesis of the DNA-switchable gel, two non-complementary Acrydite-modified oligonucleotides,[6] A and B, are separately copolymerized with acrylamide (4% w/v) and thereby incorporated into the PAAm chains. Mixing of the two solutions yields a highly viscous fluid, which, in our experiments, is complemented with fluorescent colloidal semiconductor quantum dots as tracer particles. Fluorescent QDs are easy to track with single-molecule fluorescence techniques and they are available with a wide variety of functionalizations for biomedical applications.[7]

Addition of gelation oligonucleotides complementary to the Acrydite strands transforms the fluid PAAm/nanoparticle mixture into a solid gel (Figure 1), trapping the particles. The DNA crosslinker strands are equipped with an additional, unhybridized "toehold" section that acts as a "recognition tag" for DNA release strands.[2] When release strands fully complementary to the crosslinker strands are added to the gel, they attach to the toeholds and remove the crosslinker strands via branch migration. The gel dissolves into a solution, liberating the trapped particles. For a 4% acrylamide "one-dimensional" (1D) gel, that is, for unlinked linear polymer strands, a typical pore size much larger than the particle diameter is expected, which is considerably reduced by the crosslinking process. The crosslinking density used in our experiments corresponds to a value of $\%C = 0.16$ in a conventional PAAm gel crosslinked with *N*,*N*′-methylene bis(acrylamide). Compared to typical polyacrylamide gels used for gel electrophoresis, the DNA–PAAm gel is sparsely crosslinked. For a bis-crosslinked PAAm gel with the same parameters, a pore size of $r_p > 100$ nm would be expected.[8] Nevertheless, we experimentally observe that the DNA-switchable gel is capable of controllably trapping and releasing nanoparticles with a diameter on the order of 10 nm.

To characterize the trapping and release process in detail, the diffusion behavior of our tracer particles was studied in the sol and gel states of the switchable gel. As tracer particles, we used CdSe/ZnS QDs ($\lambda_{em} = 565$ nm) with a "bare" radius of roughly 6 nm. The QDs were made water soluble by a polymer coating[9] that slightly increased their radius. As described previously,[10] the radius of the coated QDs was determined using fluorescence correlation spectroscopy (FCS). FCS measurements on free QDs in water yielded a diffusion constant of $D_{H_2O} = 29 \pm 0.5\ \mu m^2 s^{-1}$ (Figure 2b). Using the Stokes–Einstein (SE) equation this value can be translated into a hydrodynamic radius of the particles of 7.4 ± 0.6 nm. We then determined the diffusion properties of the QDs for the two states of the gel using both single-molecule fluorescence microscopy and FCS.[10,11] In the unlinked hydrogel matrix the diffusion constant dropped to $D_{FCS,unlinked} = 6.6 \pm 0.3\ \mu m^2 s^{-1}$ (Figure 2b). Again using the SE equation, one can estimate the (local[12]) viscosity of the unlinked gel matrix to be $\eta = 4.8 \pm 0.5$ mPa s. Tracking individual QDs in the unlinked gel matrix by fluorescence microscopy (Figure 2c,d) yields a diffusion constant of $D_{Tracking,unlinked} = 6.3 \pm 0.4\ \mu m^2 s^{-1}$, which agrees well with the

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[**] We thank R. Sperling and W. J. Parak for supplying us with quantum dots, T. Schubert for tracking software, and J. O. Rädler, N. A. Langrana, R. A. Neher, and M. Rief for helpful discussions. This work was funded by the IDK-NBT, the DFG (Emmy Noether Grant to FCS and Nanosystems Initiative Munich), and the Bavarian StMWFK (Neue Werkstoffe).

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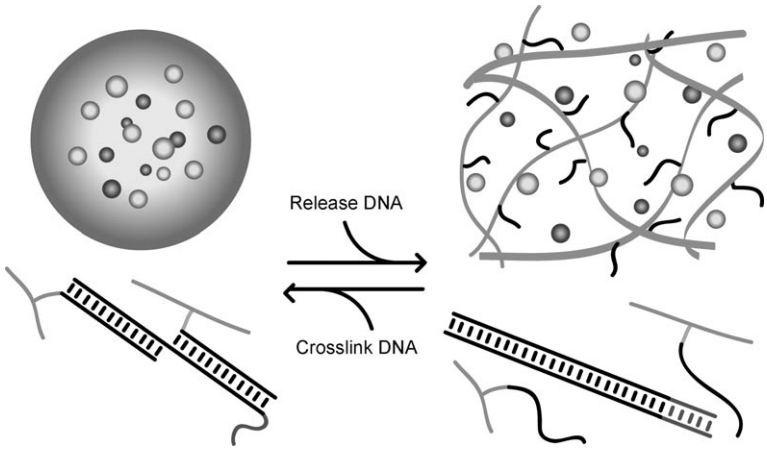

Figure 1. Operation principle of the DNA-switchable gel used for nanoparticle delivery. Water-soluble, polymer-coated nanocrystals trapped reversibly in a DNA-crosslinked polyacrylamide hydrogel (left) can be liberated by unlinking the gel with DNA "release" strands. As a result, the particles can leave the unlinked gel (right), and a DNA waste duplex composed of the crosslinking and the release strand is formed. The unlinked gel can be reorganized by adding the crosslinking strand again.

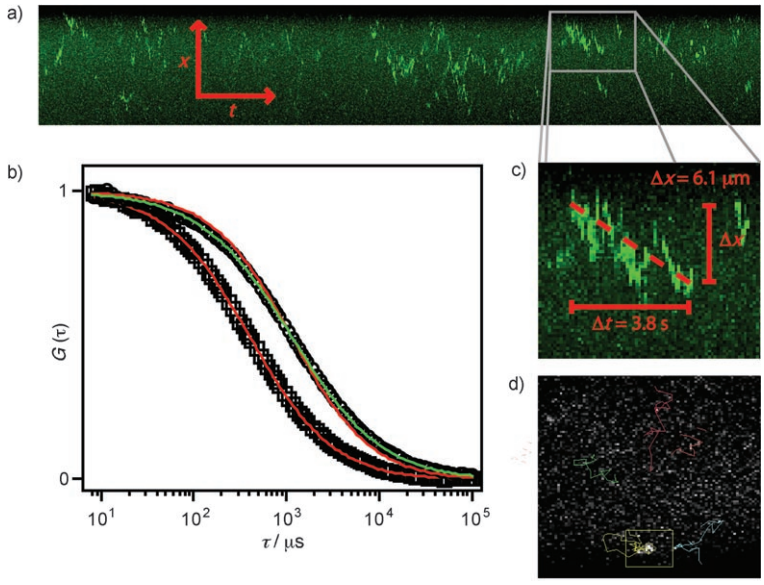

Figure 2. Time trace of QDs in the unlinked gel mixture. The time traces were obtained by stacking 3000 images (128 × 128 pixel^2 = 28 × 28 µm^2, recorded at 35.7 Hz) with a distance of 0.28 pixel and subsequent tilting of the stack by 90° around its *x*- or *y*-axis. The abscissa then corresponds to 100 ms per pixel from left to right, while the ordinate displays the projection of the former *x*- or *y*-axis. b) Autocorrelation functions obtained from FCS measurements of quantum dots in water and in the unlinked gel matrix fitted with Equation (1). The diffusion constant in water is $D_{H_2O} = 29 \pm 0.5$ µm^2 s^{-1} and $D_{FCS,unlinked} = 6.6 \pm 0.3$ µm^2 s^{-1} in the unlinked gel solution. □: water; ○: unlinked solution. Red line: Fit ($\beta = 1$); green line: Fit ($\beta = 0.9$). Already for the unlinked matrix the fit yields a value of $\beta < 1$, an indication of anomalous diffusion (see text). c) The bright traces coincide with 1D diffusion along one axis. By measuring the distances between the points where particles enter and leave the focal plane, one can calculate the diffusion constants with $D = x^2/2t$. By averaging over many particles and traces the obtained value *D* matches $<x^2>/2t$. d) Computer-aided particle tracking yields comparable diffusion constants as obtained by FCS and by the time-trace method described in (c).

value obtained from FCS measurements. The unrestricted mobility of QDs in the unlinked gel is illustrated by the collapsed fluorescence image time traces in Figure 2a.

Addition of the gelation strand L to the PAAm-nanoparticle mixture induces crosslinking of the DNA-modified polyacrylamide strands. The impact of this process on the diffusion of the QDs is observed by fluorescence microscopy. During gelation, an increasing number of nanoparticles ceases to diffuse (Figure 3a); they instead appear as spatially fixed fluorescent spots (Figure 3c) that display the characteristic blinking behavior (Figure 3d) of single quantum dots.[13,14] Complementary FCS measurements reveal decreasing diffusion constants and an increasing contribution of anomalous subdiffusion during gelation (Figure 4a), that is, the mean-square displacement of a particle is no longer simply proportional to time, but scales as $\langle x^2 \rangle \propto t^{\beta}$, with $\beta < 1$. The exponent β was included in the fit function used for the analysis of the FCS data to obtain an estimate of the extent of anomalous behavior, which can be caused by inert obstacles that obstruct particle motion by an excluded volume interaction. Additional binding events to the obstacles may result in a decrease of the apparent diffusion coefficient.[15,16] Depending on the amount of gelation strands added the crosslinking process is completed after 1–2 h. At this point the diffusion constant determined by particle tracking drops to zero. FCS, however, does not allow for an accurate measurement of *D* anymore (Figure 4a and b). All QDs are now tightly trapped in the crosslinked gel. Determination of the position of the center of single fluorescent spots reveals spatial confinement of the particles within 25 nm (Figure 3e), which is on the order of the size of the quantum dots themselves. FCS control experiments with the organic dye

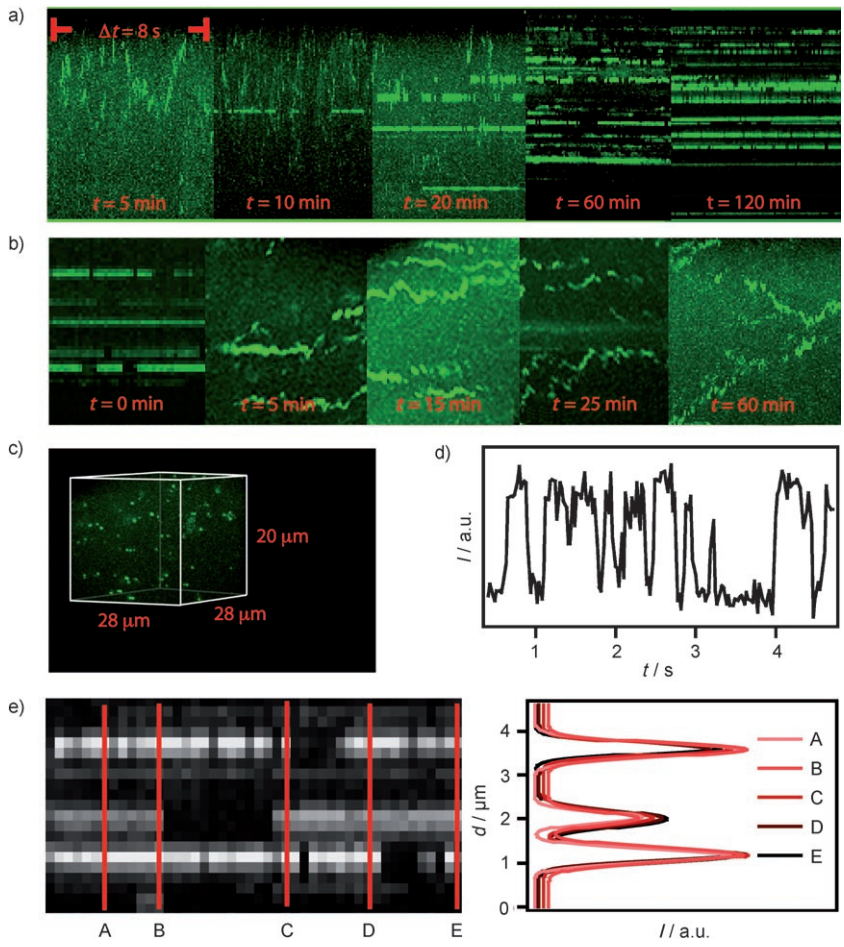

Figure 3. a) Five sections of time traces recorded during the crosslinking process. Each section is approximately 8 s long and was taken 5, 10, 20, 60, and 120 min after the addition of the crosslinking DNA strand. With ongoing crosslinking, more and more particles get trapped. b) Five sections of time traces recorded during the release process. Each section is around 8 s long and was taken 0, 5, 15, 25, and 60 min after the addition of the release DNA strand. With time the particles are uncaged, although the freedom of the unlinked matrix is not completely recovered. c) 1500 images of the crosslinked gel recorded at 35.7 Hz of a *z*-scan at 500 nm s^{-1}were stacked and tilted. Almost all QDs are fixed in the gel. d) On single bright spots, the well-known blinking behavior of QDs can be observed, which indicates the trapping of single nanoparticles. e) Gaussian fitting the fluorescence intensities of the trapped QDs along the *y*-axis allows for the accurate determination of their position with a lateral precision <30 nm. In the crosslinked gel the Gaussian peaks and thus the positions of the nanoparticles are fixed over time at least with this precision. Positions of the peaks $\pm$ maximum error are: 1.18 μm $\pm$ 10 nm, 2.015 μm $\pm$ 15 nm, and 3.585 μm $\pm$ 15 nm.

Alexa 488 with a diameter of only 1 nm revealed no confinement or reduced diffusion constant (data not shown) when the gel was switched between the unlinked and the crosslinked state.

As already mentioned, the confinement of the QDs is stronger than expected from the average pore size of the sparsely crosslinked gel. It is possible that particles are trapped in small pores during the crosslinking process, from which they cannot escape. The distance between two crosslinking points is expected to be of the order of 20 bp, that is, 7.5 nm. It is therefore conceivable that some of the gel pores are much smaller than the average pore size. In addition, particles may be trapped in a gel even when the pore size is larger than the particle diameter, which can be attributed to the high entropic cost of motion in the gel.[17] Finally, we cannot rule out a possible electrostatic interaction between the quantum dots and the charged DNA crosslinkers. However, permanent binding between QDs and DNA-polyacrylamide fibers is not observed.

Addition of the release strand R to the crosslinked gel triggers the liberation of the trapped particles. Strand R displaces the gelation strand L from strands A and B in the PAAm chains by branch migration,[2] resulting in a DNA waste duplex R–L and the gel mixture reverts to the initial fluid state. Due to the slow diffusion of R into the gel the release process takes longer than the gelation process. Released QDs first start to diffuse again within micrometer-sized domains (Figure 3b). A few hours after addition of the release strand, several distinct modes of motion are observed: unhindered, freely diffusing quantum dots, freely diffusing particles confined to volumes of 1 fL–1 μL, and some particles still trapped in undissolved gel clusters.

The release of trapped nanoparticles can also be observed in a bulk experiment (see Figure 4c and videos 1 and 2 of the Supporting Information). We prepared a crosslinked gel loaded with a high concentration of quantum dots and placed it into a buffer solution for several hours. No increase in fluorescence with time could be observed, corroborating the tight trapping of particles in the gel matrix as observed on the single-particle level. Only after addition of the DNA release strand, an increase of fluorescence in the solution surrounding the gel is observed, indicating release of the trapped particles and dissolution of the gel triggered by release DNA.

One of the potential applications of DNA-switchable gels lies in the area of controlled agent release. Hydrogels with variable pore size have been studied extensively as drug-delivery systems.[18] So far, however, gel swelling and component release has been controlled mainly by temperature or pH changes,[19] and only in a few cases by the presence of biologically relevant molecules, such as saccharides

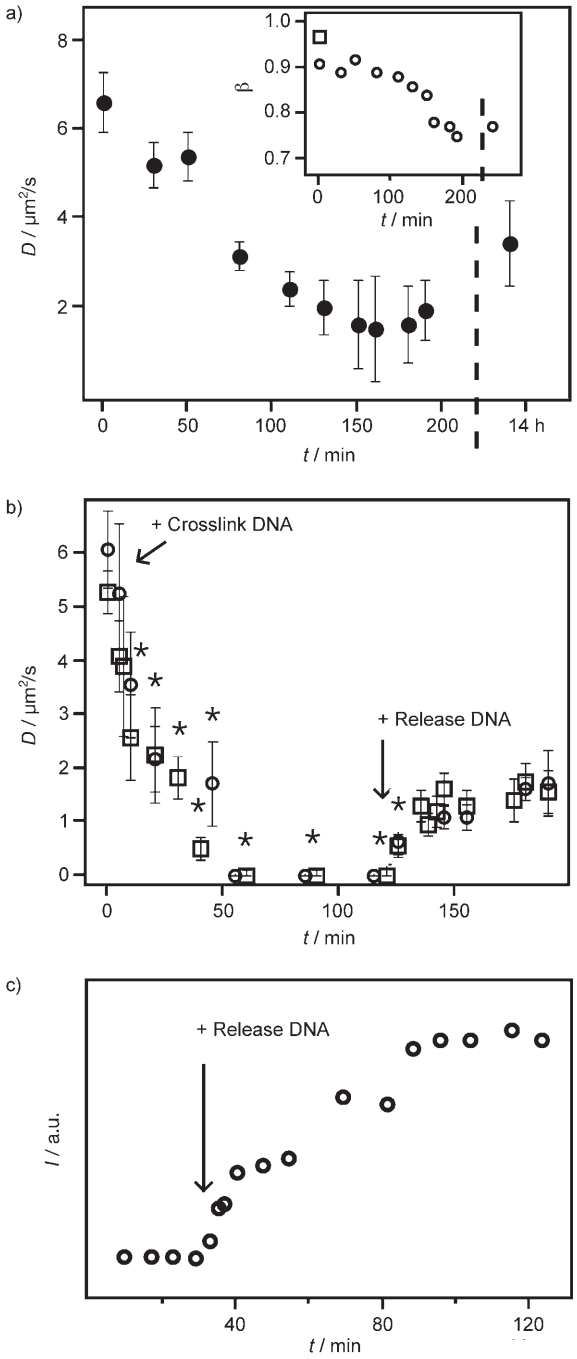

Figure 4. a) Diffusion constants of the QDs during the crosslinking and release process measured by FCS and fitted with Equation (1). Error bars represent the standard deviation obtained from 5–10 single measurements. Inset: β-values obtained from fits with Equation (1), indicating an increasing contribution of anomalous diffusion for increasing crosslinking density. □: water; ○: gel. b) Diffusion constants of the QDs during the crosslinking and release process determined from the time traces recorded by video microscopy (see Figures 2 and 3). □: time trace; ○: tracking; ✱: trapped particles. c) A crosslinked gel with captured fluorescent nanoparticles disintegrates upon addition of the release DNA and dispenses the particles into the buffer solution. The increasing fluorescence was monitored with a fluorescence spectrometer in constant-wavelength mode.

or antigens.[20] Our work demonstrates that a DNA-crosslinked PAAm gel could be used to release nanoparticles as potential drug-delivery vehicles in response to the presence of trigger DNA or RNA molecules. Whether our system—implemented as DNA-crosslinked microgel beads—could be used for in vivo drug delivery depends on a variety of factors. Drug release could be triggered by extracellular DNA or RNA, which is occasionally observed in cancer patients.[21] Response to intracellular RNA signals, however, requires the uptake of the microgel beads by cells. In either case, the concentration of naturally occurring nucleic acids may be too low to trigger drug release efficiently. This could be remedied, for instance, by implementation of a DNA-based signal-amplification scheme.[22] A more realistic scenario involves incorporation of aptamers or allosteric aptazymes.[23] Aptazymes could be used to release DNA or RNA strands in response to extracellular molecular signals. The released nucleic acids would then trigger the release of nanoparticles from the gel. The same strategy could be used to trigger the release of drugs from pure DNA hydrogels[24] with smaller pore sizes.

In conclusion, we have studied the diffusion properties of fluorescent semiconductor nanoparticles in a DNA-switchable hydrogel using single-QD tracking and fluorescence correlation spectroscopy. We have demonstrated that DNA-crosslinked hydrogels are capable of trapping and releasing nanoparticles on demand. Nanoparticles are trapped during the crosslinking process even though their diameter is smaller than the mean pore size. Our system, or a modification thereof, may find application as a switchable material for the controlled release of nanoscale agents.

Experimental Section

Sample preparation: CdSe/ZnS nanocrystals (Quantum Dot Corporation (Invitrogen), Berkeley, CA; emission maximum 565 nm) were capped with a polymer coating as described before.[9] Acrydite-modified DNA (A: 5′-Acrydite-CGG CCT GAA GCC TCC GTG TG-3′, B: 5′-Acrydite-AAG CAC TCT TCT CCT CTC TG 3′) was purchased from IDT (Coralville, IA), linker strand (L: 5′-ATC GCA CGC CCA GAG AGG AGA AGA GTG CTT CAC ACG GAG GCT TCA GGC CG-3′) and release strand (R: 5′-CGG CCT GAA GCC TCC GTG TGA AGC ACT CTT CTC CTC TCT GGG CGT GCG AT-3′) from biomers.net, Ulm, Germany. Stock solutions of strand A and B were prepared separately at 3 mM DNA concentration. The stock solution contained: 10 mM Tris, 1 mm ethylene diamine tetraactetic acid (EDTA; pH 8.0) buffer, 200 mM NaCl, 4% acrylamide, 3 mM DNA strand A or B and deionized water. Directly after mixing, nitrogen was bubbled through this solution for 5 min. Initiator mixture ($(NH_4)_2S_2O_8$ (0.05 g) and N,N,N′,N′-tetramethylethylenediamine (TEMED; 25 μL, Sigma, Germany) in H_2O (0.5 mL)) was added to a final concentration of 1.4% followed by bubbling nitrogen for an additional 5 min. The stock solutions were mixed 1:1 and 20 μL droplets containing 10 nM QDs were placed in coverslip chambers (Nunc, Wiesbaden, Germany) for FCS measurements. For video microscopy, the gel containing 100 pM QDs and was placed into a silicon grease ring of ≈5-mm diameter on a glass coverslip. To crosslink the polyacrylamide solution, linker

strands L were added to a final concentration of 0.5 mM, corresponding to a crosslink density of 33%. Dissolution of the gel was achieved by adding a twofold excess of release strand R.

FCS Measurements: FCS measurements were performed on a commercial FCS setup (microscope Axiovert 200 with 40× C-Apochromat water immersion objective (NA 1.2) and ConfoCor2 module, Carl Zeiss, Jena, Germany). For excitation, the 488-nm line of an argon-ion laser was used. Only 1% of the maximum illumination intensity of 2.91 mW was used to minimize saturation effects.[25] The emitted light was sent through a 50/50 beamsplitter and fed into two identical independent detection channels with 505-nm long-pass filters and pinholes of 70-μm diameter. The signals of the avalanche photodiode detectors were cross correlated to remove artifacts such as detector afterpulsing. A detailed description of FCS theory and techniques has been published before.[26] FCS measurements of fluorescent nanocrystals[10,27] differ from those on organic fluorophores, as QDs exhibit no triplet behavior but show blinking on all timescales from nanoseconds to seconds. The distribution of blinking times follows a power law.[14] As for organic fluorophores, the fluorescence decay time of QDs of a few nanoseconds is too fast to be detected in an FCS measurement. Since, until now, no analytical expression to fit FCS data obtained from fluorophores exhibiting power-law-dependent blinking behavior could be derived, we fitted the curves with a simple FCS model that only takes diffusion into account. For calibration of the sample volume (i.e., to determine the parameters r_0 and z_0, the radius and the longitudinal extension of the focal volume, respectively) the dye Alexa 488 ($D = 316\ \mu m^2 s^{-1}$, Molecular Probes) was used. The triplet behavior of Alexa 488 was taken into account during calibration. For all measurements r_0 was found to be between 180 and 190 nm. The ratio z_0/r_0 was always between 6 and 7. The autocorrelation functions obtained from QD measurements were fitted with an expression for freely diffusing particles without the term describing the triplet behavior:[16,26]

$$G(\tau) = \frac{1}{N} \cdot \frac{1}{1 + \left(\frac{\tau}{\tau_D}\right)^{\beta}} \cdot \frac{1}{\sqrt{1 + \frac{r_0^2}{z_0^2}\left(\frac{\tau}{\tau_D}\right)^{\beta}}} \quad (1)$$

The two free parameters are the mean number of particles N in the focal volume and the diffusion time τ_D. To test the data for signatures of anomalous diffusion, the exponent β was introduced as a third free parameter. Each FCS data point was generated from at least 5 × 30 s measurement times.

Single-molecule fluorescence microscopy: We used a home-built single-molecule, objective-type TIR fluorescence microscope in epifluorescence mode to image single fluorescent quantum dots. Core elements are a sensitive EM-CCD Camera (Andor iXON Du-879) and a high numerical aperture objective (Carl Zeiss, Alpha Plan Fluar, 100× NA 1.45). For excitation we used continuous wave (cw) laser light at 473 nm with a sample plane intensity of approximately 10 $kW cm^{-2}$. Fluorescence emission was collected at 508 ± 10 nm. Kinetic image series were collected with 25 ms exposure time and treated with Image J software in order to calculate collapsed fluorescence image time traces.

Fluorescence spectroscopy: Approximately 5 μL of the crosslinked gel, loaded with 20 nM QDs, were placed on the bottom of a glass cuvette containing 300 μL TE buffer (200 mM NaCl). The cuvette was mounted in a fluorescence spectrometer (Jobin Yvon Fluorolog-322). In order to collect fluorescence only from released particles, the excitation light (480 nm) was precluded from the gel itself. Fluorescence was collected at 525 nm. After 30 min 10 μL of release DNA (3 mM) were added. To overcome slow diffusion of the particles in the comparably large cuvette volume, the cuvette was shaken gently between the measurements. Videos of gel-triggered gel dissolution are available in the Supporting Information.

Keywords:
diffusion · DNA structures · fluorescence · nanoparticles · sol–gel processes

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Received: May 25, 2007
Published online on September 4, 2007

