Nucleic Acid Supercoiling as a Means for Ionic Switching of DNA – Nanoparticle Networks

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Oligomeric nanoparticle networks, generated by the self-assembly of bis-biotinylated double-stranded DNA fragments and streptavidin, have been studied by scanning force microscopy (SFM). SFM imaging revealed the presence within the networks of irregular thick DNA molecules, which were often associated with distinct, Y-shaped structural elements. Closer analysis revealed that the Y structures are formed by condensation (thickening and shortening) of two DNA fragments, most likely through the supercoiling of two DNA molecules bound to adjacent binding sites of the streptavidin particle. The frequency of supercoiling was found to be dependent on the ionic strength applied during the immobilization of the oligomeric networks on mica surfaces. Potential applications of the structural changes as a means for constructing ion-dependent molecular switches in nanomaterials are discussed.

KEYWORDS:

molecular switches · nanostructures · nucleic acids · scanning probe microscopy · supramolecular chemistry

Introduction

The generation of nanoscale structural and functional devices is an important goal of nanotechnology. Amongst the various routes to the fabrication of defined nanostructures, the "bottom-up" approach in which small molecular building blocks self-assemble to form larger entities, is highly attractive.[1] Currently, an increasing number of research reports concern the utilization of biological macromolecules as components for the biomimetic formation of nanostructured elements and materials. For this purpose, DNA is a highly promising construction material.[2–4] Due to its unique recognition capabilities, physicochemical stability, mechanical rigidity, and high-precision processibility, DNA has been extensively used to fabricate nanostructured scaffolds,[3] as well as for the selective positioning of proteins,[5,6] metal or semiconductor nanoclusters,[7] and other molecular devices. Moreover, protein recognition systems, for instance, based on bacterial surface layers[8, 9] and the streptavidin – biotin system,[6, 9] have been used to assemble inorganic nanoparticles into organized arrangements.

Recently, we reported on the self-assembly of oligomeric DNA – protein networks consisting of bis-biotinylated DNA and the biotin-binding protein streptavidin (STV) (Figure 1).[10] Characterization of the oligomers by means of scanning force microscopy (SFM) revealed that the STV functions predominantly as a bivalent or trivalent linker between adjacent double-stranded DNA (dsDNA) molecules, despite its tetravalent binding capacity for biotinylated ligands. As a consequence, the remaining biotin-binding capacity enables the use of the nanostructured DNA – protein networks as powerful reagents in the immuno polymerase chain reaction (immuno-PCR), a method for the highly sensitive trace analysis of proteins and

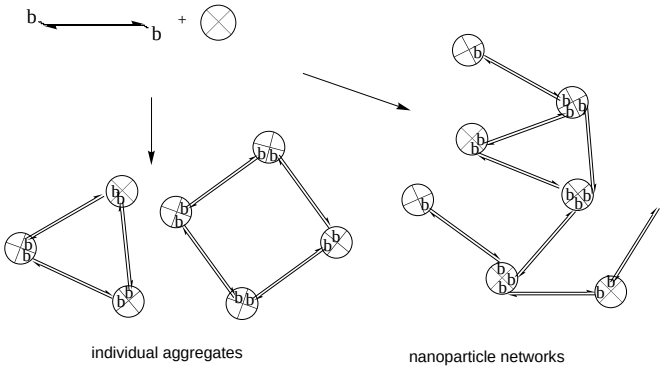

Figure 1. *Schematic representation of the synthesis of nanoparticle networks using double-stranded DNA (dsDNA) as spacer groups. Two binding sites are attached to the two 5′ ends of the dsDNA. The binding sites are biotinyl groups, which allow cross-linking through the biotin-binding protein streptavidin (STV); STV is acting as the model nanoparticle in this report. The assembly of the two components leads to the formation of oligomeric nanoparticle networks, but individual aggregates are also formed which could be isolated by electrophoretic or chromatographic methods. For simplification, complementary DNA strands are drawn as parallel lines with the 3′ ends indicated by arrow heads. b is the binding site and STV is represented by the crossed circles.*

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other antigens. Moreover, the oligomeric networks can be used as a starting material for the synthesis of well-defined nanostructures, for instance, supramolecular DNA circles consisting of a single STV and one dsDNA molecule.[11]

We report here on initial steps towards the development of switchable DNA – nanoparticle networks. We demonstrate that a variation of the ionic strength immediately before immobilization of the oligomeric DNA – nanoparticle aggregates on mica surfaces can be used to induce a tertiary conformational change of the DNA linker fragments attached to individual particles. This condensation (thickening and shortening), in which two DNA backbones are brought into close contact, is probably due to a supercoiling motion of the nucleic acid fragments. The magnesium concentration used for immobilization of the DNA – particle networks on the solid support was systematically altered. These variations led to changes in the relative abundance of characteristic structure elements containing supercoiled DNA fragments. The utilization of these phenomena for the construction of switchable nanoparticle assemblies is discussed.

Results and Discussion

The supramolecular aggregation of nanoparticles by specific nucleic acid hybridization is considered to be a highly promising route to the fabrication of nanostructured materials.[4, 7] For example, Mirkin and co-workers have impressively demonstrated that the specific recognition of complementary DNA fragments can be used to generate oligomeric binary particle networks in which two different kinds of metal clusters are exclusively interconnected in a heterodimeric fashion, thereby forming an oligomeric "$(A-B)_n$" system.[7] Nevertheless, very little is known about the manipulation and tailoring of such particle networks, for instance, about ways to influence the structure and topography of the DNA hybrid materials subsequent to their formation by self-assembly. Within the context of basic studies on DNA-linked nanoparticle networks, the oligomeric aggregates generated from bioorganic STV particles and bis-biotinylated dsDNA are suitable model systems to gain insight into the properties of complex particle networks (Figure 1). The STV functions as a 5 nm model particle which can realize only a limited variation in its connectivity to other particles within the network. Either one, two, three, or four biotinylated DNA fragments can be conjugated with the STV by means of the high-affinity STV – biotin interaction. This simplifies the complexity of the supramolecular particle networks, and allows for the convenient analysis of effects occurring from variations, for example, in the immobilization parameters. In addition, the size of the dsDNA linker fragments, which are typically about 30 – 170 nm in length, allows for convenient direct observation by SFM.[10–12]

Observation and ion-dependency of condensed DNA fragments

During the SFM analyses, irregular thick DNA molecules and the appearance of Y-shaped elements between the STV particles attracted our attention (Figure 2). The section analysis and height measurement revealed that the thick DNA fragments were, on average, 1.97 ± 0.05 times as high as the regular DNA molecules, for instance, 0.9 and 0.45 nm, respectively. Strikingly, the Y-type structure elements were frequently of a highly symmetric structure, comprised of one thick and two regular

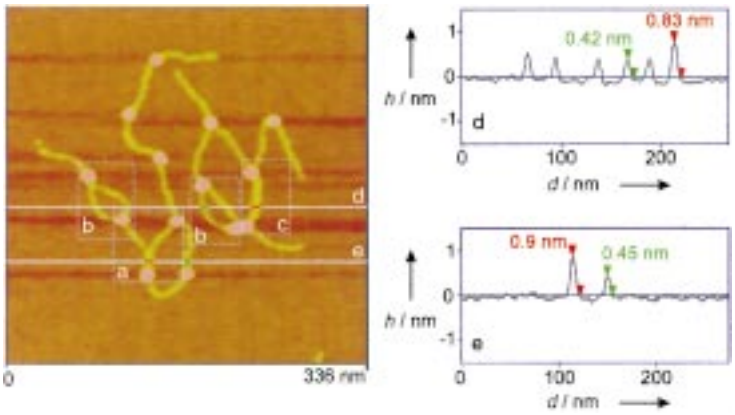

Figure 2. *Scanning force microscopy analysis of a typical oligomeric network obtained from the assembly of STV and bis-biotinylated 169 base-pair dsDNA fragments. Individual structure elements are indicated: a) symmetric Y-type structure; b) binary assembly linked by two regular DNA fragments; c) binary assembly linked by a supercoil of two DNA fragments; d) cross-section through regular and supercoiled DNA; e) cross-section through regular and partially supercoiled DNA. Height measurements* h *along the lines (d) and (e) are shown on the right. Note that the supercoiled DNA has about twice the height of the regular DNA.*

DNA arms. In some cases, the Y structures were comprised of three thick arms. From these observations, we concluded that the thick DNA molecules might contain two double-helical DNA fragments in which the backbones are brought into close contact. It is known that salt will condense double helices and we occasionally observe a shortening and thickening in portions of a DNA fragment where only one strand is present. However, due to the clear appearance of the symmetrical Y-type structures in which two regular strands condense to form a single thick fragment, we assume that this type of condensation is due to a supercoiling of two dsDNA molecules which are twisted around each other, thereby forming a coiled DNA superhelix.

The condensation of double-stranded DNA is a well-known phenomenon occurring in biological systems to compact the genetic material within the cell's nucleus. As a conformational consequence, the contour length of the B-form double-helical DNA is reduced while the thickness of the supertwisted molecule is increased. The system studied here resembles a simplified version of eukaryotic DNA – protein complexes, with a protein bound at regularly spaced intervals of about 55 nm. In this semisynthetic system, ion-dependent condensation was directly observed in the SFM images. As a consequence of the supercoiling, the average contour length of dsDNA of 54.3 ± 4 nm is reduced to about 72%, that is, 39.6 ± 2 nm. Schaper and coworkers had previously applied SFM imaging to compare supercoiled, relaxed, and linearized plasmid DNA.[13] Although their samples were prepared by a different technique (based on the spreading action of benzyldimethylalkylammonium chloride), their height data, obtained by the quantitative analysis of

SFM images, are in good agreement with the values obtained in this study. However, since all of the dsDNA fragments used in this work consist of an identical sequence, it is also be possible that the Y structures observed might contain movable DNA-branched junctions. These might be formed by the non-biotinylated 3′-end nucleotides attacking the neighboring biotinylated 5′-end nucleotides of another dsDNA fragment, bound to an adjacent binding site of the streptavidin.

It is well know that DNA supercoiling is thermodynamically favored at high ionic strength.[14] Thus, we investigated whether the occurrence of supercoiled dsDNA and the abundance of the Y structures might be affected by an external stimulus, such as a variation of the magnesium concentration. For this, samples of the dsDNA – STV oligomers were treated with varying concentrations of Mg^{2+} ions, ranging from 4 to 40 mM $MgCl_2$, immediately prior to the immobilization on mica for the SFM analysis. Statistical evaluation of the SFM images indicates a clear dependency of the supercoiling frequency on the ion strength (Figure 3). Up to 30% of the total DNA molecules incorporated into the nanoparticle networks were found to be engaged in supercoiled structures. Most often, fully condensed cyclic dimers and symmetrical Y-type structures were observed, such as **1d** and **2c** in Figure 4, respectively. This result can be attributed to the compensation of the negative backbone charges by the increased Mg^{2+} ion concentration, which leads to the enhanced formation of condensed double helices. Control experiments with unconjugated dsDNA indicated a similar increase in supercoiled DNA (data not shown).

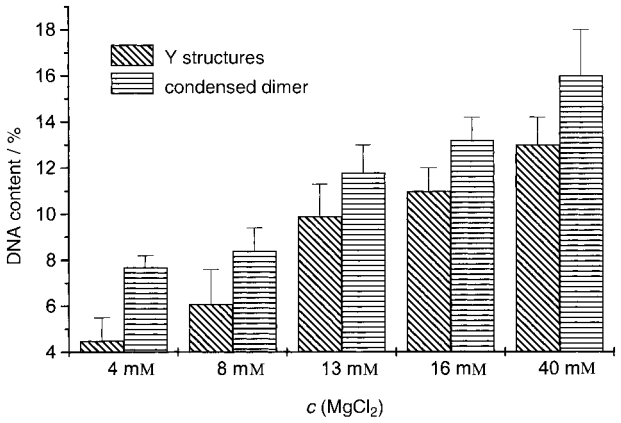

Figure 3. *Influence of the ionic strength on the occurrence of DNA condensation, as determined by statistical analysis of SFM images. The heights of the histograms represent the percentage of Y-type structures (such as **2c** in Figure 4) and condensed DNA fragments (for example, **1d** in Figure 4) within the oligomeric DNA – STV networks according to the concentration of $MgCl_2$ used for immobilization. The total number of dsDNA molecules counted was greater than 2000.*

Towards switchable elements based on Y structures

A favorable property of the DNA double helical molecule is its intrinsic susceptibility to external stimuli. For example, the contour length and the flexibility of a given double helix can be effectively altered by chemical means. As an example, intercalators such as acridinium- or ethidiumbromide derivatives bind in between the stacked nucleobases within the double helix, thereby leading to a significant increase in the DNA contour length.[15] Seeman and co-workers have reported on the change in torque, induced by ethidiumbromide intercalation and applied to a circular DNA molecule containing a partially mobile DNA-branched junction, as a potential supercoiling motion for nanomechanical devices.[16] Moreover, the same group has recently published a powerful approach to fabricate nanomechanical devices from DNA, by using the ion-dependent transition of B-form DNA to the Z conformation to alter the distance between two DNA motifs attached to the switchable double helix. In this device, atomic displacements of about 2 – 6 nm have been attained.[17]

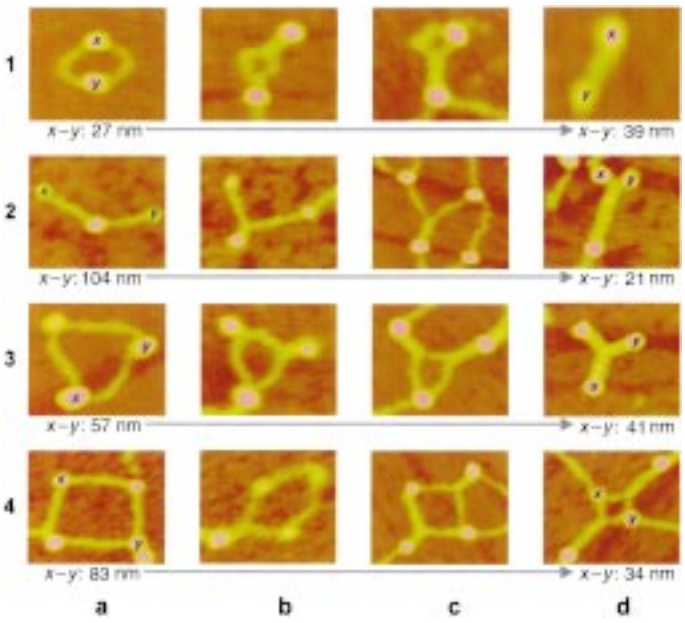

Figure 4. *Ionic switching of nanoparticle networks. The relative orientation of the STV particles is altered by increased condensation of the interconnecting DNA linkers. The SFM images indicate structural changes observed in representative $DNA_2 – STV_2$ (**1**), $DNA_2 – STV_3$ (**2**), $DNA_3 – STV_3$ (**3**), and $DNA_4 – STV_4$ (**4**) elements that occur in the random oligomeric networks. Note that the structures of type **a** represent the extended species, while types **b** and **c** are assigned as intermediates during the formation of type **d**, the species that contains fully condensed DNA fragments.*

We describe here initial approaches for the development of ion-switchable oligomeric nanoparticle networks containing dsDNA linker molecules. The change of the magnesium concentration is a chemical stimulus that leads to an increased occurrence of supercoiled DNA elements within the particle networks. As a consequence, the condensation of two adjacent DNA fragments attached to an individual STV particle alters the relative position of the said particle within its nanoscopic environment (Figure 4). For example, individual cyclic dimer assemblies **1** consisting of two particles interconnected by two dsDNA spacers are frequently observed. In the elliptical aggregates, the particles are located at the termini of the geometric figure, and the interparticle distance observed is

about 30 ± 5 nm. The condensation of the two spacer molecules changes the geometry of the supramolecule, and thereby increases the interparticle distance to about 39 ± 2 nm (conversion of **1a** into **1d** in Figure 3). Another example is illustrated with the cyclic trimer **3**. Without supercoiling (as in **3a**), aggregates containing three particles appear in the SFM as nearly equilateral triangles. Average particle distances are about 54 ± 3 nm. Upon condensation of the interconnecting dsDNA spacers, the particles are drawn towards each other. In the final aggregate containing entirely condensed spacers, the particles are arranged by an even Y-shaped linker, with interparticle distances of about 41 ± 2 nm (conversion of **3a** into **3d** in Figure 4).[18]

Although the increased supercoiling is a statistical phenomenon which cannot yet be controlled on a single-molecule level, statistical analysis of the SFM images clearly revealed the influence of the ionic strength on the relative abundance of the condensed supramolecular species (Figure 5). At low magnesium concentration extended aggregates, such as **1a**, are predominantly present, and at high magnesium concentration the amount of condensed species is significantly increased. Moreover, it is notable that the occurrence of intermediates, such as the structures **1b** and **1c** in Figure 4, is scarce for the cyclic dimer **1**, while in the case of the cyclic trimer **3** and tetramer **4**, the intermediates **3b**, **4b**, and **4c** occur to a significantly greater extent. This suggests that the symmetrical Y-type structure elements present within these species provide enhanced stability.

DNA-linked aggregates consisting of less than five nanoparticles are often observed as individual structures (Figure 4). Such aggregates might be isolated on a preparative scale with, for instance, chromatography or electrophoresis. Subsequently they might be used as well-defined building blocks in the "bottom-up" fabrication of larger nanoelements. Moreover, the binary and ternary particle aggregates described above often occur as distinct structural elements within the oligomeric nanoparticle networks (Figure 2), and thus, they might be used as switchable elements to change the macroscopic properties of nanomaterials by means of ion-induced supercoiling.[18] The DNA – STV aggregates, in particular, are potentially useful as backbone or scaffolding structures which can be further functionalized through coupling of chemical groups with the high-affinity streptavidin – biotin interaction or covalent methods.[5, 11] It has been demonstrated that the alteration of interparticle distances within networks of metal- and semiconductor nanoclusters changes the optical properties of such organized systems.[7] We conclude that further systematic exploitation of the chemically induced manipulation of the DNA conformation and topology might lead to the development of novel controllable molecular nanomaterials.[19] The use of molecular inductors, such as organic and inorganic ions, aromatic intercalators, molecules that bind to the major or minor grooves, or nucleic acid binding proteins, should provide a powerful approach to the further establishment of DNA as a construction material. For instance, the ionic switch described here could be used both to control nanoparticle spacing and to regulate the accessibility of the DNA to enzymes.[18] Potential applications of ion-switchable nanostructures include the manufacturing of addressable supports for bioelectronic devices and sensors, and the use of neural nanoparticle networks as configurable logic gates for novel computing devices,[20] nanoelectromechanical systems, and in other emerging fields of nanotechnology.

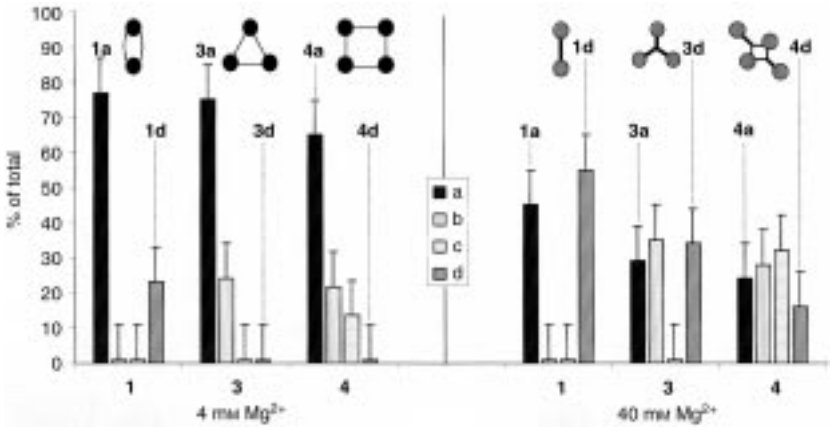

Figure 5. *Statistical evaluation of the ion-dependent relative abundance of the structural intermediates **a** – **d** (Figure 4). The heights of the histograms represent the percentage of the individual species according to the concentration of $MgCl_2$ used for immobilization. The total number of dsDNA molecules counted was greater than 1500. Note the increased abundance of the condensed species at 40 mM $MgCl_2$. The intermediates **b** and **c** are scarce for the dimer **1**, while in the case of the trimer **3** and tetramer **4**, the intermediates **b** and **c**, which contain symmetrical Y-type DNA structures, are present to a greater extent.*

Experimental Section

Bis-biotinylated dsDNA fragments were prepared from M13mp18 (Promega) by preparative PCR, with two biotinylated primers, 5′-biotin-AGC GGA TAA CAA TTT CAC ACA GGA-3′ (bcA) and 5′-biotin-AAG GCG ATT AAG TTG GG-3′ (bG), as previously described.[10] Conjugates of recombinant STV (IBA, Göttingen) and the biotinylated dsDNA were prepared by diluting STV (1 μL; 2 μM in buffer A) with buffer A (13 μL) and subsequently adding the dsDNA (1 μL; 3 μM in buffer A). Buffer A was 10 mM tris(hydroxymethyl)aminomethane (Tris) buffer (pH 7.3) that contained 5 mM ethylenediaminetetraacetate (EDTA). The molar ratio of DNA to STV was about 3:2. The incubation was typically carried out for 48 hours at 4 °C.

Samples for SFM imaging were prepared by placing a drop (6 μL) of solution containing $MgCl_2$ (4 – 40 mM) and DNA – STV conjugates (about 500 nM) onto a parafilm sheet. The drop was adsorbed onto freshly cleaved mica, and left to fix for one minute. The drop was then quickly washed three times with deionized water (50 mL), and immediately blown dry for 5 minutes with nitrogen gas. The SFM inspection was carried out with commercial instruments (Digital Instrument, Dimension 3000, and Multimode III) using Si cantilevers purchased from Nanosensor. The SFM images were taken with instruments operating in high-amplitude dynamic mode with a homemade active feedback circuit,[21] to prevent the onset of intermitted contact (tapping).

With this circuit, SFM can be performed stably within the attractive interaction regime in air, so that the interaction between the scanning tip and the sample is minimized.

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[1] a) G. M. Whitesides, J. P. Mathias, C. T. Seto, *Science* **1991**, *254*, 1312; b) K. E. Drexler, *Nanosystems: Molecular Machinery, Manufacturing, and Computation*, Wiley, New York **1992**; c) J.-M. Lehn, *Supramolecular Chemistry*, VCH, Weinheim, **1995**, p. 193; d) D. Philp, J. F. Stoddart, *Angew. Chem.* **1996**, *108*, 1242; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1154.
[2] N. C. Seeman, *J. Theor. Biol.* **1982**, *99*, 237.
[3] N. C. Seeman, *Trends Biotechnol.* **1999**, *17*, 437.
[4] C. M. Niemeyer, *Curr. Opin. Chem. Biol.* **2000**, *4*, 609.
[5] C. M. Niemeyer, T. Sano, C. L. Smith, C. R. Cantor, *Nucleic Acids Res.* **1994**, *22*, 5530.
[6] C. M. Niemeyer, W. Bürger, J. Peplies, *Angew. Chem. Int. Ed.* **1998**, *110*, 2391; *Angew. Chem.* **1998**, *37*, 2265.
[7] J. J. Storhoff, C. A. Mirkin, *Chem. Rev.* **1999**, *99*, 1849.
[8] U. B. Sleytr, P. Messner, D. Pum, M. Sara, *Angew. Chem.* **1999**, *110*, 1098; *Angew. Chem. Int. Ed.* **1999**, *38*, 1034.
[9] S. Mann, W. Shenton, M. Li, S. Connolly, D. Fitzmaurice, *Adv. Mater.* **2000**, *12*, 186.
[10] C. M. Niemeyer, M. Adler, B. Pignataro, S. Lenhert, S. Gao, L. F. Chi, H. Fuchs, D. Blohm, *Nucleic Acids Res.* **1999**, *27*, 4553.
[11] C. M. Niemeyer, M. Adler, S. Gao, L. F. Chi, *Angew. Chem.* **2000**, *112*, 3183; *Angew. Chem. Int. Ed.* **2000**, *39*, 3055.
[12] The assembly of 5′,5′-bis-thiolated dsDNA fragments and 5-nm gold colloids leads to networks of similar topography, in which the gold nanoparticles are typically connected with two or three DNA molecules.
[13] A. Schaper, L. I. Pietrasanta, T. M. Jovin, *Nucleic Acids Res.* **1993**, *21*, 6004.
[14] For a review, see: A. V. Vologodskii, N. R. Cozzarelli, *Annu. Rev. Biophys. Biomol. Struct.* **1994**, *23*, 609. SFM was applied to visualize cruciforms in negatively supercoiled plasmid DNA (L. S. Shlyakhtenko, V. N. Potaman, R. R. Sinden, Y. L. Lyubchenko, *J. Mol. Biol.* **1998**, *280*, 61). The effects of ionic conditions on the conformations of supercoiled DNA were studied by electrophoresis and theoretical simulations (V. V. Rybenkov, A. V. Vologodskii, N. R. Cozzarelli, *J. Mol. Biol.* **1997**, *267*, 312). It is well known that high concentrations of polycations with charge +3 or greater induce a rapid condensation of DNA fragments to form a tightly-packed, highly ordered toroidal structure. (For a review, see: V. A. Bloomfield, *Biopolymers* **1997**, *44*, 269.)
[15] K. Yoshikawa, Y. Matsuzawa, K. Minagawa, M. Doi, M. Matsumoto, *Biochem. Biophys. Res. Commun.* **1992**, *188*, 1274.
[16] X. Yang, A. V. Vologodskii, B. Liu, B. Kemper, N. C. Seeman, *Biopolymers* **1998**, *45*, 69.
[17] C. Mao, W. Sun, Z. Shen, N. C. Seeman, *Nature* **1999**, *397*, 144.
[18] Since the effects of increasing ionic strength on the average distances between particles are contrary, for example, for the cyclic dimer **1** and the cyclic trimer **3** (Figure 4), the overall effect in a random network is just about a 5% decrease of the average interparticle distance. Thus, future devices will be comprised of well-defined individual elements, such as those shown in Figure 4, or other nanoparticle structures (see refs. [6, 7, 11]). Due to the specificity of nucleic acid hybridization and the high affinity of the streptavidin – biotin interaction, no exchange and rearrangement reactions occur between individual nanostructures (ref. [10]). Thus, such elements might be simultaneously and site-specifically attached to laterally microstructured surfaces, for instance, by DNA-directed immobilization (see ref. [5] and also: C. M. Niemeyer, L. Boldt, B. Ceyhan, D. Blohm, *Anal. Biochem.* **1999**, *268*, 54; T. A. Taton, R. C. Mucic, C. A. Mirkin, R. L. Letsinger, *J. Am. Chem. Soc.* **2000**, *122*, 6305). Otherwise, distinct DNA-based nanostructures might be oligomerized to form larger two-dimensional aggregates (E. Winfree, F. Liu, L. A. Wenzler, N. C. Seeman, *Nature* **1998**, *394*, 539).
[19] DNA supercoiling is a reversible process. The intramolecular condensation of DNA strands induced by polycations occurs in the millisecond time range (D. Porschke, *Biochemistry* **1984**, *23*, 4821), and decondensation has been shown to be complete within a few seconds (J. Widom, R. L. Baldwin, *J. Mol. Biol.* **1980**, *144*, 431). To take advantage of these molecular processes, the fastest switching might be attained with surface-bound nanoparticle materials operated in microfluidic devices. The latter are capable of transporting liquids with a flow speed of several centimeters per second. (For a review, see: S. A. Sundberg, *Curr. Opin. Biotechnol.* **2000**, *11*, 47.) Since complete buffer exchange for a given surface area of about 100 mm^2 is attainable in a few hundred milliseconds, it seems reasonable that nanoparticle switching based on nucleic acid supercoiling might be realized within the second time range.
[20] C. P. Collier, E. W. Wong, M. Belohradsky, F. M. Raymo, J. F. Stoddart, P. J. Kuekes, R. S. Williams, J. R. Heath, *Science* **1999**, *285*, 391.
[21] B. Anczykowski, J. P. Cleveland, D. Krüger, V. Elings, H. Fuchs, *Appl. Phys. A.* **1998**, *66*, 885.

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