Self-assembly of DNA–streptavidin nanostructures and their use as reagents in immuno-PCR

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ABSTRACT

The self-assembly of bis-biotinylated double-stranded DNA and the tetravalent biotin-binding protein streptavidin (STV) have been studied by non-denaturing gel electrophoresis and atomic force microscopy (AFM). The rapid self-assembly reproducibly generated populations of individual oligomeric complexes. Most strikingly, the oligomers predominantly contained bivalent STV molecules bridging two adjacent DNA fragments to form linear nanostructures. Trivalent STV branch points occurred with a lower frequency and the presence of tetravalent STV was scarce. However, valency distribution, size and the exchange dynamics of the supramolecular aggregates were highly sensitive to stoichiometric variations in the relative molar coupling ratio of bis-biotinylated DNA and STV. The largest aggregates were obtained from equimolar amounts while excess STV led to the formation of smaller oligomers appearing as finger-print-like band patterns in electrophoresis. Excess DNA, however, induces a complete breakdown of the oligomers, likely a consequence of the instability of STV conjugates containing more than two biotinylated DNA fragments. It was demonstrated that the oligomers can further be functionalized, for instance by the coupling of biotinylated immunoglobulins. Both pure and also antibody-modified DNA–STV oligomers were used as reagents in immuno-PCR (IPCR), a highly sensitive detection method for proteins and other antigens. Employment of the supramolecular reagents led to an ~100-fold enhanced sensitivity compared to the conventional IPCR procedure.

INTRODUCTION

DNA has a vast amplification potential due to PCR, allowing the detection of nucleic acids at sensitivity levels far below those available for the detection of proteins by conventional immunological methods, such as the antibody-based enzyme-linked immunosorbent assay (ELISA). The detection of a few hundred protein molecules can be attained by a combination of ELISA with the amplification power of PCR. This method, termed immuno-PCR (1) (IPCR) is based on the coupling of specific antibodies with a DNA reporter fragment to be amplified by PCR. The original protocol of IPCR has been adjusted for the extremely sensitive detection of various antigens (2–8), and thus, the method has great potential for innumerable biomedical applications. For example, IPCR has recently been discussed as a high sensitivity diagnostic tool for human prion diseases (9). One critical aspect of IPCR is efficient coupling of the target-specific antibody with the DNA reporter molecule. For instance, this was attained by means of a recombinant protein chimera with specificity for biotinylated DNA and immunoglobulin G (IgG) (1), or else, through direct chemical coupling of the IgG with the DNA reporter (4). However, the establishment of immuno-PCR as a routine method for immunological diagnostics requires convenient and robust experimental protocols, i.e. the avoidance of sophisticated technical equipment and sensitive reagents. Since the former approaches require laborious preparation of the particular reagents, the biotin-binding proteins avidin and streptavidin (STV) have been utilized as a more convenient molecular linker in various IPCR reports published. Typically, assembly of the immuno complex is achieved by successive coupling steps of the several components (3). The major drawbacks of this strategy are the large number of incubation steps required, and more important, the incomplete interphase coupling of the reagents, occurring with an efficiency of only ~10% for each step (8). Also, conjugation of tetravalent STV with biotinylated antibodies and DNA fragments to preform active complexes in solution is not feasible. Mixing of the biotinylated components with STV yields a hardly reproducible, complex mixture of several possible conjugates containing non-productive DNA1–4–STV and IgG1–4–STV complexes and also the free compounds in addition to the mixed conjugates desired. Although this strategy has been described (2,5), it seems unsuitable for a highly standardized routine method since reproducibility in reagent preparation is essential for the high sensitivity of IPCR (10).

On the other hand, synthetic strategies based on self-assembly of molecular building blocks are of growing importance for the fabrication of functional supramolecular composites.
MATERIALS AND METHODS

PCR synthesis

Mono-biotinylated and bis-biotinylated dsDNA fragments of different lengths, 1–4, were prepared from M13mp18 (Promega) and pUC19 (New England Biolabs) DNA templates using PCR. The following oligonucleotide primers (Interactiva) were used for preparation of the DNA oligomers: DNA 1 (M13mp18-based 86 bp fragment), 5'-biotin-AGC GGA TAA CAA TTG CAC ACA GGA-3' (bcA) and 5'-biotin-CAG GTC GAC TCT AGA GGA TCC-3' (bcB); DNA 2 (M13mp18-based 124 bp fragment), 5'-biotin-GAA TTC GAG CTC GGT ACC CGG-3' (bcA) and 5'-biotin-GAG CTC GGT ACC CGG-3' (bcB); DNA 3 (M13mp18-based 169 bp fragment), bcA and bcB; mono-biotinylated 1a, bD and a non-biotinylated analog of bcA; DNA 2 (M13mp18-based 124 bp fragment), 5'-biotin-ACC CTT ACC ACA GGA-3' (bcA) and 5'-biotin-AGC GGA ATT AAG TTG GG-3' (bcB); DNA 3 (M13mp18-based 169 bp fragment), bcA and bcB; mono-biotinylated 3a, bcA and a non-biotinylated analog of bcG; DNA 4 (pUC19-based 256 bp fragment), 5'-biotin-ATT GTT GCC GGG AAG CTA GAG TAA-3' (bBLA1) and 5'-biotin-TAT GCA GTG CTG CCA TAA CCA TGA-3' (bBLA2); mono-biotinylated 4a, bBLA1 and a non-biotinylated analog of bBLA2. PCR was carried out using an initial denaturation step at 95°C (5 min) and subsequently 40 thermal cycles of the following profile: 55°C (1 min), 72°C (2 min), 95°C (1 min). The PCR amplification products were purified on a Superdex 200 gel filtration column (Pharmacia) using buffer A (100 mM phosphate buffer, pH 6.8, containing 150 mM NaCl, 5 mM EDTA) with an FPLC system (Pharmacia), concentrated by ultrafiltration, re-diluted in buffer B (10 mM Tris buffer, pH 7.3, containing 5 mM EDTA) and quantified by photometry.

Oligomeric DNA–protein complexes

Conjugates of recombinant STV (IBA, Göttingen, Germany) and the biotinylated dsDNA fragments were typically prepared by adding 1 µl of STV (2 µM in buffer B) to ~13 µl of buffer B, and subsequently 2 µl of the dsDNA (1–5 µM in buffer B). The final DNA concentration was kept constant at ~0.4 µM, and the molar ratio of DNA to STV was typically in the range of 1:1.
1:10–10:1. The incubation time ranged from 5 min at 37°C up to 4 weeks at 4°C. Conjugates of biotinylated antibody, STV and dsDNA were prepared by addition of 1 molar equivalent of the antibody to an oligomeric conjugate, previously prepared from STV and 1 or 0.5 molar equivalents of dsDNA 3. The mixture was incubated for 1 h at 4°C and the DNA–protein aggregate was purified on an FPLC Superdex 200 gel filtration column (Pharmacia). The aggregate eluting in the void volume of the column was concentrated by ultrafiltration, stabilized with 50% glycerol (Sigma) and stored at –80°C. The samples were analyzed by non-denaturing electrophoresis on 1.5 and 2% agarose gels and subsequent ethidium bromide staining. AFM samples were prepared by placing a 6 μl drop of a solution containing 8 μM MgCl₂ and ~500 nM DNA–STV conjugates on a parafilm sheet. A higher relative STV concentration required a higher conjugate concentration in the drop. The drop was adsorbed onto freshly cleaved mica, and left to fix for 1 min. The drop was then quickly washed three times with 50 ml of deionized water, and immediately blown dry for 5 min with nitrogen gas. At a high relative STV concentration, the samples did not easily spread over the surface. In order to spread these conjugates, the nitrogen was blown at an angle of 45° at a strength that moved the meniscus over the mica at a rate of 30 cm/s (24). The AFM inspection was carried out with commercial instruments (Digital Instruments Dimension 3000 and Multimode III) using Si cantilevers purchased from Nanosensor. The AFM images were taken with instruments operating in high amplitude dynamic mode with a home-made active feedback circuit (25), which prevents the onset of intermittent contact (tapping). AFM can be stably run in the attractive interaction regime in air so that interaction between the scanning tip and the sample is minimized.

Immuno-PCR
Serial dilutions of mouse IgG model antigen (Sigma, St. Louis, MO) were immobilized in wells of TopYield modules (Nunc, Roskilde, Denmark) as previously reported (8). For conventional IPCR employing successive reagent addition, biotinylated goat anti-rabbit antibody (Coulter Immunotech, Hamburg, Germany) was used at 1.5 μg/ml, with 2 μg/ml STV (IBA) and biotinylated dsDNA at 5 pM. Each of the incubation steps was carried out for 1 h at room temperature with 30 μl of reagent dilutions in buffer C (20 mM Tris, 150 mM NaCl, pH 7.3, containing 5 mM EDTA, 0.05% Tween). In some cases (Fig. 5B), mouse IgG antigen was first coupled with an unlabeled primary antibody (rabbit anti-mouse whole sera; Sigma), and subsequently with the biotinylated goat anti-rabbit antibody. After the final wash with buffer C, PCR was carried out using the conditions described above, except that 28 thermal cycles were performed. In addition, a biotinylated primer was used and the PCR reaction mixture (30 μl/well) contained 2 nM 11-digoxigenin-dUTP (Roche) to allow for quantification of the PCR amplification products using the microplate-based fluorescence PCR–ELISA detection assay previously described (8). In order to use the oligomeric DNA–STV complexes as reagents for IPCR, the surface-immobilized antigen was labeled with biotinylated antibody, and subsequently, the oligomeric complex obtained from 3 and 1 molar equivalent of STV, diluted to a final concentration of 5 pM in buffer C, was added to the wells, and the plate was incubated for 30 min. PCR and quantification of the amplification products were carried out as described above. To use the antibody-containing oligomeric reagent in IPCR a solution of the IgG-modified oligomer containing ~20 nM dsDNA 3 was diluted 1:80 in buffer C. Incubation times, PCR and quantification were as described above.

RESULTS
Self-assembly of the DNA–STV complexes
Four bis-biotinylated DNA fragments, 86 (1), 124 (2), 169 (3) and 256 (4) bp dsDNA, were synthesized by preparative PCR using two 5’-biotin-derivatized primer oligonucleotides. Mono-biotinylated dsDNA fragments of 86 (1a), 169 (3a) and 256 (4a) bp were similarly prepared using one 5’-biotin-derivatized and one unlabeled primer. The dsDNA products were purified by gel filtration chromatography, quantified by photometry and characterized by non-denaturing agarose gel electrophoresis in the presence of a DNA molecular weight standard. In a first set of experiments, the DNA products were mixed with various amounts of recombinant STV, ranging from 100:1 down to 0.2:1 molar equivalents of STV and DNA, respectively. A typical gel image obtained from DNA 3 is shown in Figure 2. Samples containing two or more molar equivalents of DNA reveal only a few bands, representing the mono-, bi-, and tris-adducts of the biotinylated DNA and STV, as indicated from the comparison with appropriate controls prepared from mono-biotinylated DNA and STV. Quantitation of the bands obtained from the mono-biotinylated DNA showed that even with a 5-fold molar excess of DNA, the tris- and tetra-adducts are only present in small amounts: ~10 and 5% of the total signal intensity of the STV adducts. This indicates that STV predominantly behaves as a bivalent linker molecule, despite its tetravalent binding capacity. With a typical, sharp change at ~2 molar equivalents of DNA, an increase in the relative amount of STV leads to the formation of a highly characteristic broad, blurry band, appearing in the high molecular weight range. The intensity of this band reaches its maximum at an equimolar coupling stoichiometry. At this point, an immobil high molecular weight band is evident, likely representing highly oligomerized DNA–STV networks. A further increase in the relative amount of STV leads to the formation of a fingerprint-like set of bands, indicating the presence of multiple relatively small DNA–protein complexes comprised of varying amounts of the two constituents. The second change is also quite sharp, and it is reached at ~0.7 molar equivalents of DNA. Thus, the presence of excess STV prevents the formation of highly oligomerized networks, and instead, a variety of individual molecular complexes are generated. The stoichiometry dependence of complex formation is highly reproducible, and the three well-distinguishable states of self-assembly are also evident in similar experiments employing the shorter and longer DNA fragments 1, 2 and 4, respectively, at the same concentrations.

Further electrophoretic experiments were carried out to investigate the kinetics of complex formation and also potential dynamic exchanges between the complexes. No substantial difference in band pattern was observed for mixtures of dsDNA, such as 3, and either 1 or 2 molar equivalents of STV that were allowed to react either for ~2 min at 4°C or ~24 h at room temperature. This indicates that formation of the supramolecular complexes is fast. To further elucidate the
assembly process, experiments were carried out to test whether exchange occurs between pre-assembled DNA–STV complexes. For instance, two different complexes, prepared from either 1 or 3 and 1 or 2 molar equivalents of STV in separate tubes, were mixed and incubated for up to 24 h. Gel electrophoretic analysis shows that only the two fingerprint-like sets of bands of the pre-assembled complexes are present in these mixtures, and no additional bands of mixed species can be detected. It was also observed that a subsequent addition of excess STV to a preformed equimolar complex did not destroy the typical high molecular weight immobile band visible in Figure 2, lane 4. This indicates that exchange of the dsDNA between the complexes is very slow. To investigate the influence of the coupling ratio on such putative exchange processes, similar experiments were carried out with preformed adducts of STV and mono-biotinylated dsDNAs, such as 1a or 3a. In agreement with the results obtained from bis-biotinylated DNA, no exchange was detected when the molar amount of DNA was equal to or greater than that of DNA. In contrast, an excess of DNA leads to the occurrence of novel bands of mixed species containing both DNA and STV already after 5 min. This indicates that free dsDNA induces a fast exchange between the two DNA–STV complexes.

The coupling of further biotinylated compounds with preformed DNA–STV oligomeric complexes was studied by electrophoresis. These experiments are of particular interest with respect to the utilization of the oligomers as reagents in IPCR assays. A low molecular weight compound, i.e. 5 molar equivalents of D-biotin, as well as 2 molar equivalents of a biotinylated antibody (bIgG), were mixed with the oligomeric complexes, previously generated from 3 or 3a and STV (Fig. 3). The complexes obtained from a 1:2 molar ratio of 3 and STV react with bIgG, leading to the formation of an immobile band that indicates the formation of high molecular weight DNA–protein aggregates (Fig. 3, lane 6). The coupling of bIgG with complexes generated from 3 and an equimolar amount of STV, however, leads to partial disruption of the high molecular weight product, and instead, novel bands of lower molecular weight appear (Fig. 3, lane 3). Coupling experiments with monomeric STV adducts containing monobiotinylated DNA 3a reveal that in particular mono-adducts containing one DNA fragment per STV have sufficient residual binding capacity for the additional attachment of a voluminous macromolecule (Fig. 3, lanes 9 and 12). In contrast, the addition of D-biotin neither affects the band patterns obtained from a 1:2 molar ratio of 3 and STV (lanes 4 and 5) nor of 3a and various molar equivalents of STV. However, significant changes can be observed when oligomeric networks pre-assembled from 3 and an equimolar amount of STV are challenged with D-biotin. This leads to almost complete disruption of the immobile band, and instead, complexes of lower molecular weight are visible (Fig. 3, lane 2).

AFM imaging

To study the structures of the oligomeric DNA–protein complexes, samples were imaged using dynamic AFM
working in attractive regime mode. Representative images are shown in Figure 4, and statistical analysis of the complexes observed are given in Tables 1 and 2. As expected, the distance between two adjacent STV molecules varies with the length of the DNA incorporated into the networks. For DNAs 1–4, the distances observed correlate well with the lengths calculated for a DNA double-helix in B-conformation (Table 1). Statistical evaluation indicated that the length of the DNA fragment and the molar ratio DNA:STV influence both the size of the linear oligomers and also the amount and size of supramolecular cycles (Table 1). The largest complexes are observed for DNA 3, containing on average about eight dsDNA fragments per complex. The valency of STV is found to be sensitive only to variations in the coupling stoichiometry (Table 2) and independent of the dsDNA length (Table 1). A decrease in the relative amount of DNA induces a significant decrease in the valency of the STV molecules. This is important, since the monovalent, terminal STV moieties of the supramolecular complexes are the potential binding sites for subsequent coupling with biotinylated antibodies, as determined by gel electrophoresis (Fig. 3). The AFM data provided answers to questions posed by electrophoresis. For instance, immobile structures and fingerprint band patterns found at DNA:STV 1:2, 1:1 and 2:1, respectively (Fig. 2), suggest variations in supramolecular structures. At a 2:1 molar coupling stoichiometry of DNA:STV oligomeric complexes with relatively high amounts of trivalent and tetravalent STV were seen (Table 2). A high valency of STV molecules functioning as branch points within the supramolecular complexes resulted in the largest structures, containing up to 40 dsDNA fragments (Fig. 4A). However, these samples also contain considerable amounts of free DNA. A comparison of the electrophoretic and AFM data indicates the presence of either ~80 or 20% free dsDNA, respectively. With respect to the electrophoretic results of Figure 3, this suggests that the large supramolecular networks are fragile, and thus tend to disrupt during the physical stress of electrophoretic separation. The presence of free DNA, and thus, the appearance of less oligomeric aggregates, also explains the decrease in the intensity of the immobile band, observed in the electrophoresis of oligomers consisting of equimolar amounts of DNA and STV (Fig. 2, lanes 4 and 5). In the AFM images, the formation of supramolecular rings is evident, resulting in geometrically stable arrays (Fig. 4C). The ratio of circular to linear complexes is influenced by variations in the coupling stoichiometry of dsDNA and STV (Table 2) and was found to be at a maximum at around an equimolar concentration. The amount of cyclic structures also increases with decreasing length of the bridging DNA. Furthermore, the number of dsDNA fragments that form such supramolecular rings is higher for short DNA fragments (Table 1).

When the DNA:STV ratio was decreased to 1:2, more linear conjugates were seen (Table 2 and Fig. 4B). As a striking feature, STV dominantly serves as a bivalent linker in these complexes, thus linear aggregates with only a few trivalent STV branch points are present in addition to cyclic species (Fig. 4C). The patterned bands observed in electrophoretic analysis of such samples (Fig. 2) are explainable by a population of rarely branched, linear structures with various numbers of DNA fragments predominantly containing monovalent STV moieties at their termini (Fig. 4D). Antibody-containing complexes have also been imaged by AFM. These aggregates display highly globular structures with significant z-extension. Average particle sizes ranging from 200 to 400 nm in diameter are observed, suggesting that coupling of the polybiotinylated antibody yields an extensive 3-dimensional linkage of several preformed DNA–STV oligomeric complexes, extending as chain-like structures from some of the globular aggregates.
DNA–protein networks as reagents in IPCR

The supramolecular DNA–protein networks were evaluated for their performance as reagents in IPCR (Fig. 5). Initially, the influence of the coupling ratio of dsDNA and STV on performance of the supramolecular complexes as IPCR reagents was tested in a direct IPCR assay. Serial dilutions of rabbit IgG model antigen were immobilized as targets in a microplate. The antigen was coupled with a specific biotinylated antibody, goat anti-rabbit IgG, and subsequently labeled with oligomeric DNA–STV complexes, pre-assembled from DNA 3 and varying relative amounts of STV. The best sensitivity and also the highest signal intensities were obtained using oligomers generated from equimolar coupling mixtures of 3 and STV in which the largest oligomeric networks are present (Fig. 2, lane 4). In a similar set of experiments, the influence of the lengths of the DNA fragments incorporated in the DNA–protein complexes was studied. For this, the IPCR detection of rabbit IgG was achieved with oligomeric aggregates obtained from STV and 1 molar equivalent of the various DNA 1–4. The performance of the supramolecular DNA–protein complexes depended on the length of the DNA incorporated. The use of DNA 3 gave best results, and neither a shorter, e.g. 2, nor a longer dsDNA, e.g. 4, led to increased sensitivity and signal intensities. The control reactions indicated that all DNAs have a similar amplification efficiency during PCR. In agreement with the AFM observation that dsDNA forms the largest oligomers (Table 1), the different IPCR performance thus correlates with structural features of the supramolecular complexes.

Figure 5A demonstrates the power of the supramolecular DNA–STV complexes as reagents in IPCR. Use of the oligomers was compared with a conventional IPCR, in which surface-immobilized antigen was coupled in three steps with a specific biotinylated antibody, STV and DNA 3, or else, with the analogous ELISA, employing alkaline phosphatase as the marker enzyme. Although the sequential IPCR protocol already leads to a significantly enhanced detection sensitivity by about three orders of magnitude, use of the DNA–STV oligomeric complexes still improves the performance of IPCR by enhancing the signal intensities and sensitivity ~10-fold. Following this, pre-assembled DNA–STV complexes containing a biotinylated antibody were tested in highly demanding IPCR applications. In Figure 5B, the detection of mouse IgG model antigen was performed using a specific
unlabeled antibody which is available only as a crude preparation in mouse whole serum. In such cases it is necessary to employ a biotinylated secondary antibody. The sequential two-step coupling of a biotinylated secondary antibody and a supramolecular oligomer of STV and DNA 3 was compared with the use of a preformed, supramolecular complex of the three components. As a control, a regular ELISA was carried out, in which biotinylated secondary antibody, STV and biotinylated alkaline phosphatase were used for antigen detection. The curves shown in Figure 5B clearly indicate a superior performance of the immunoglobulin-containing supramolecular reagent, leading to an ~100-fold enhanced sensitivity. A similar construct, employed as a reagent for the detection of a lectin from mistletoe using a sandwich IPCR assay, revealed similar enhancements in sensitivity and signal intensity.

**DISCUSSION**

**Self-assembly of DNA–STV complexes**

The object of this work was to exploit self-assembled supramolecular complexes, formed from the tetravalent binding protein STV and divalent, bis-biotinylated dsDNA, and to determine whether these complexes can be utilized for applications in molecular biotechnology, such as IPCR. On the assumption that the tetravalent binding capacity of STV combined with its extremely high affinity for biotin would lead to the formation of 3-dimensionally linked networks, our results provide surprising insights into the highly studied (strept)avidin–biotin recognition system. As a prominent conclusion, the tetravalent protein displays a high preference for acting as a bivalent linker molecule, with the formation of tris-adducts being disfavored. Tetra-adducts are rarely detected, neither by electrophoretic analyses nor by AFM imaging. The restricted formation of tetra-adducts, previously reported for the binding of short single-stranded DNA oligomers (26), indicates that electrostatic repulsion of the negatively charged phosphate backbone contributes to destabilization of the STV adducts, in addition to the steric demand of the double helical DNA fragment. Our results also allow conclusions regarding the kinetics of oligomer formation and exchange processes within the DNA–STV networks. Formation of the supramolecular aggregates is rather fast, occurring within minutes, while exchange of dsDNA between preformed complexes is very slow, as long as the amount of bis-biotinylated dsDNA does not exceed 1 molar equivalent of STV. Under these conditions, the system behaves in accord with observations for the binding of biotin by (strept)avidin (27). In contrast, an enhanced exchange dynamic is observed.

**Figure 4.** AFM visualization of the oligomeric DNA–STV complexes investigated in this study. Representative images are shown in this diagram. (A) Overview image of a sample containing STV and dsDNA 3 in a molar ratio of 1:2. (B) Topographical view of a linear DNA–STV oligomer of STV and dsDNA 3, containing a single trivalent STV branch point. (C) Topographical view showing isolated supramolecular cyclic structures consisting of STV and dsDNA 2, indicated by arrows. More than 10% of the total number of complexes reveal such isolated cyclic structures. (D) Topographical view of small linear DNA–STV oligomers obtained from samples of dsDNA 3 and 5 molar equivalents of STV. Note that most of the complexes contain monovalent STV moieties attached to their termini.
when the amount of dsDNA-bound biotin is >2-fold higher than STV. This result suggests that addition of a third dsDNA fragment to a bis-adduct weakens the binding strength of the other fragments, and thus, causes enhanced dissociation. This mechanism is in agreement with both the biphasic dissociation reported earlier for binding of biotin nitroxide derivatives (28) and the affinity decrease for the binding of biotinylated macromolecules (29). The mechanism also explains the lack of high molecular weight complexes in mixtures containing excess bis-biotinylated dsDNA.

**AFM imaging of nanostructures**

Direct analysis of the self-assembled complexes by means of AFM imaging has provided detailed insights regarding the connectivity and composition of the supramolecular DNA–STV networks. This is of great importance for the further development of such nanostructures since it allows conclusions regarding the localization and availability of potential binding sites that can be utilized for the attachment of ligands, for instance, biotinylated antibodies. This work represents the first imaging of a semi-synthetic DNA–protein network. Recently, a few examples of AFM characterization of synthetic 1-dimensional DNA geometrical objects (16) and 2-dimensional periodic DNA arrays (15,17) have been reported. Fortunately, the length of the dsDNA spacers between adjacent proteins can be adjusted in the DNA–STV system to allow for detailed imaging analysis of the nanostructured networks. For instance, the 30 nm spacings obtained with the 86 bp dsDNA 1 has permitted the clear structural discrimination of individual protein molecules, while the use of a 46 bp dsDNA linker was not sufficient. It has been noted that AFM would need far better resolution to obtain detailed information about individual molecules (16), but it can clearly distinguish the components of some biomolecular complexes occurring, for instance, during enzymatic processing of nucleic acids (30,31). This study emphasizes the increasingly important role of AFM for the structural analysis of complex populations of artificial biomolecules formed by self-assembly.

**Applications**

The self-assembled oligomeric DNA–STV networks studied here are suitable reagents for IPCR. A high number of DNA fragments are intrinsically linked with a similar number of target binding sites, the latter allowing for affinity enhancement by increased avidity. The isolated DNA–STV oligomers, however, are not yet optimal for this purpose since binding with bulky biotinylated macromolecules preferentially occurs at the monovalently attached STVs, located at the termini of the supramolecular oligomers. Nevertheless, the coupling of polybiotinylated antibodies produces aggregates that are highly efficient IPCR reagents. The exact composition and structure determination of these complexes, however, is beyond the capabilities of currently available techniques. To generate molecular constructs of even higher versatility, a more distinguishable and possibly more efficient attachment of large numbers of bulky functional moieties might be attained from the incorporation of covalent DNA–STV hybrid molecules (19) as distinct building blocks in self-assembled oligomeric networks. This option supplements the capabilities of the DNA–STV aggregates to serve as a molecular framework for the attachment and selective positioning of biotinylated functional moieties, such as proteins (22), inorganic metal nanoclusters (20) or low molecular weight peptides and fluorophores (26). We anticipate that further exploration of nucleic acid- and STV-based recognition systems will open new routes to the fabrication of man-made biomaterials useful for sensory transduction, preparative biocatalytic transformations, and other applications in supramolecular chemistry and biotechnology.
SUPPLEMENTARY MATERIAL

See Supplementary Material available at NAR Online. Images obtained from: gel electrophoresis of dsDNAs 1–4 with varying amounts of STV; electrophoretic analysis of the exchange processes; AFM imaging of the various dsDNAs; various coupling ratios and preformed, antibody-containing DNA–STV complexes; gel filtration chromatograms; IPCR illustrating the influences of DNA length and coupling stoichiometry; a sandwich IPCR for lectin detection.

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