Nano-biotechnology

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A DNA Nanostructure for the Functional Assembly of Chemical Groups with Tunable Stoichiometry and Defined Nanoscale Geometry**

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DNA is a versatile material in nano-biotechnology[1] and chemical biology. DNA strands can be sequence-specifically assembled into artificial higher-order nanoscale structures[2,3] that are useful in, for example, biotemplating,[4,5] biocomputing,[6] sensing,[7] and biophysical studies.[8] In chemical biology, hybridization of chemically modified strands into DNA duplexes has been utilized to bring chemical groups into defined contact to, for example, enhance their reaction.[9] Herein we describe a new approach which merges DNA-based nanostructures and the chemical modification of DNA. We show that tetrahedron-shaped nanostructures can act as scaffolds to assemble a multitude of different chemical groups at tunable stoichiometry and at geometrically defined sites. The resulting molecular entities exhibit functional properties beneficial in biosensing and diagnostics. Our new strategy for assembling chemical groups at the nanoscale may be expanded to endow other DNA structures with rationally designed functions.

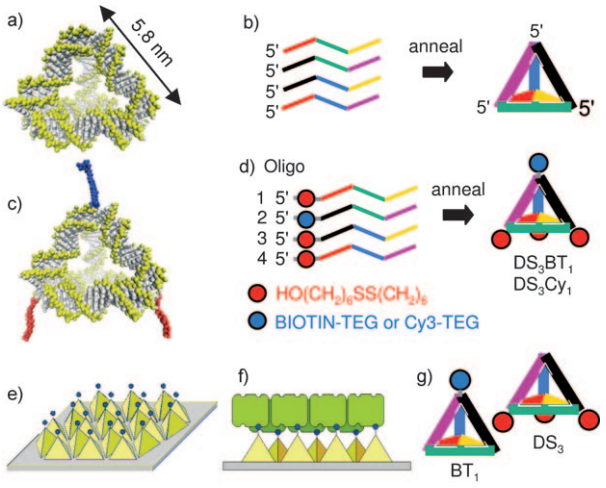

Figure 1. Structure and formation of DNA tetrahedra. a) Molecular model of a DNA tetrahedron formed by b) annealing of four ssDNA each carrying three out of six different, color-coded sequence blocks. The 5′ termini of the ssDNA oligonucleotides are positioned at the four vertices. c) Model of tetrahedron DS_3BT_1 with three DS and one BT tag formed by d) annealing four 5′-modified ssDNA oligonucleotides. Similarly, tetrahedron DS_3Cy_1 carrying the Cy3 fluorophore instead of BT was also generated. The BT and Cy3 tags are attached through a flexible tri(ethylene glycol) (TEG) linker to the 5′ ends and vertices. e) DS_3BT_1 or DS_3Cy_1 bind through their DS legs in an oriented fashion to a gold leaving the BT or Cy3 tag exposed to the ambient. f) A monomolecular film of DS_3BT_1 can capture a layer of streptavidin. g) Tetrahedra BT_1 and DS_3 that carry one BT or three DS groups, respectively.

DNA tetrahedra, as intoduced by Goodman et al, are nanostructures with edges composed of double-stranded DNA (dsDNA; Figure 1a).[10,11] Tetrahedra are obtained by annealing four single-stranded DNA oligonucleotides (ssDNA; Figure 1b); the 55 nucleotide strands used in this study give rise to tetrahedron edges of 5.8 nm in length (Figure 1a).[10] Once assembled, the free 5′ and 3′ termini of the ssDNA are positioned at the vertices of the tetrahedron (Figure 1b). We exploited this structural characteristic and placed a combination of biotin (BT) and disulfide (DS) groups at the four vertices (Figure 1c) by using four ssDNA oligonucleotides that carry the chemical modifications at their 5′ ends (Figure 1d). By placing three DS groups and one BT group at the vertices, the rationally designed structures were expected to exhibit desirable functional properties. In particular, the tetrahedra were anticipated to (1) bind through three

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Experimental procedures and results of the analysis of DNA tetrahedron films by XPS and fluorescence microscopy are provided in the Supporting Information for this article, which is available on the WWW under http://dx.doi.org/10.1002/anie.200804264.

thiol legs with high affinity onto gold substrates and (2) because of their oriented binding, present the BT moiety at a defined surface distance so that the tag is exposed to the ambient (Figure 1e) and is able to capture streptavidin at high density (Figure 1f).

Tetrahedron DS_3BT_1 (with three DS and one BT tags) was generated by annealing four ssDNA oligonucleotides (Figure 1d). The formation of DS_3BT_1 was confirmed by monitoring the position of the DNA bands in polyacrylamide gel electrophoresis. In comparison to the fast-migrating ssDNA bands (Figure 2a, lanes 1–4), hybridization product DS_3BT_1 was at the top of the lane (Figure 2a, lane 5) because the

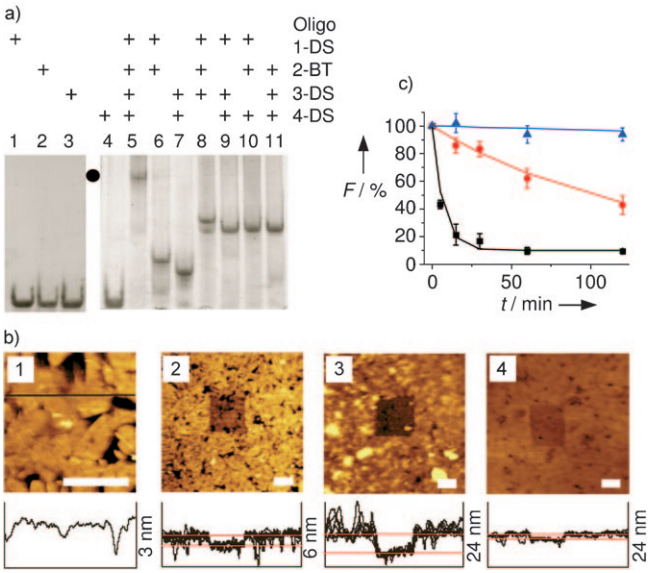

Figure 2. Generation and surface binding of DNA tetrahedra. a) Gel electrophoretic analysis on the formation of DS_3BT_1 (lane 5, dot) from four ssDNA oligonucleotides. Controls comprising monomeric ssDNA, and combinations of two and three ssDNA oligonucleotides are in lanes 1–4, 6–7, and 8–11, respectively. b) AFM topographic images and height profiles of bare TSG gold (panel 1), DS_3BT_1-coated TSG with a hole scratched into the DNA film (panel 2), a DS_3BT_1 film after incubation with streptavidin (panel 3), and a layer of BT-free tetrahedron DS_3 after incubation with streptavidin (panel 4). Scale bars 500 nm. c) Normalized fluorescence counts, *F*, of gold surfaces coated with DS_3Cy_1 (blue triangles), DS_2Cy_1 (red circles), and ssDNA DS–DNA–Cy (black squares) after incubation with DTT (10 mM) for the indicated durations, *t*. These data represent an average of three independent measurements.

increased mass and changed shape of the tetrahedron slows its movement through the gel meshwork.[10] Control experiments with different combinations of two and three ssDNA led to migration heights between ssDNA and the tetrahedron band (Figure 2a, lanes 6–11), which is consistent with the expected size of the hybridization products (see the Supporting Information). Four other DNA tetrahedra were generated: DS_3 and BT_1 (with either three DS or one BT group, respectively; Figure 1g), DS_3Cy_1 (Figure 1d), and DS_2Cy_1. The latter two tetrahedra contain three and two DS groups, respectively, and one Cy3 fluorophore.

The ability of DS_3BT_1 to bind to gold surfaces was assessed by using X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM). In agreement with the formation of a DNA film, XPS analysis showed the attenuation of the Au4f signal as well as the appearance of peaks for C1s, N1s, O1s, and P2p at relative ratios expected for DNA (see the Supporting Information). As would be predicted for the oriented and specific binding, AFM analysis showed that tetrahedron DS_3BT_1 yielded a monomolecular film. Template-stripped gold (TSG) was used for the topographic measurements, and exhibited flat (root mean square (rms) noise, 0.3 nm) facets 100–1000 nm in size, separated by 2–10 nm deep trenches (Figure 2b, panel 1). In comparison, gold surfaces incubated with DS_3BT_1 displayed an increased rms noise of 0.5 nm. In addition, a hole with a depth of 1.5 nm could be scratched into DS_3BT_1 surfaces (Figure 2b, panel 2). This value is lower than the DNA tetrahedron height of 4.5 nm because soft biological material can be compressed in contact mode AFM.[11] AFM analysis showed that, in agreement with the disulfide-specific tethering, gold surfaces incubated with DS-free tetrahedron BT_1 did not form films (data not shown).

AFM was also used to determine whether DS_3BT_1 was able to bind streptavidin while maintaining its adhesion to gold. A DS_3BT_1 film was formed and incubated with streptavidin. Scratching of the surface revealed a profile step of 7 nm (Figure 2b, panel 3), which is consistent with the formation of a monomolecular layer of streptavidin (protein size $4 \times 8 \times 7$ nm) on top of a DNA film. The elevated, bright features around the hole (Figure 2b, panel 3) represent streptavidin and DNA that had been removed from the film. Consistent with a specific biotin–streptavidin interaction, BT-free tetrahedron DS_3 did not capture streptavidin as indicated by the smaller step size (Figure 2b, panel 4).

After confirming the oriented binding of DS_3BT_1, we determined the affinity of the tetrahedra to the gold substrate. Because of the anticipated multivalent enhancement of individual gold–thiol interactions, the DNA structures with three DS legs should bind tighter to the surface than constructs with two legs or a single leg. The differential affinity was experimentally determined by first forming films composed of fluorescence-labeled tetrahedra DS_3Cy_1 or DS_2Cy_1, or single-stranded oligonucleotide DS–DNA–Cy, and then monitoring their time-dependent desorption by using fluorescence microscopy (see the Supporting Information). Desorption was enhanced by incubating the DNA films with dithiothreitol (DTT, 10 mM), which displaces thiolated DNA from the gold surface. The kinetics of the decrease in fluorescence are displayed in Figure 2c. During an observation window of 120 min, very little DS_3Cy_1 was removed (5% removal; Figure 2c, blue triangles) while the coverage of DS_2Cy_1 with two thiol legs fell nearly linearly to below 50% of the starting value (Figure 2c, red circles). DNA that contains a single DS group exhibited the lowest affinity, its signal decayed exponentially to 10% after 30 min (Figure 2c, black squares). The residual constant signal is due to autofluorescence of gold and background fluorescence of desorbed and solvated DNA strands. Comparative analysis of the initial rates of desorption revealed that DS_3Cy_1 (with three legs

bound) has an affinity for gold that is 5000-times higher than monothiolated DNA.

We have presented a new strategy that exploits DNA nanostructures as scaffolds to combine different chemical groups at defined geometrical distances and with tunable stoichiometry. The rationally designed structures exhibit functional properties that may be exploited for the immobilization of DNA or proteins on gold surfaces for biosensing, diagnostics, and cell biological research. Given the great variety of DNA nanostructures,[3] our approach for chemical enhancement has the potential to be extended to other nanostructures and different biochemical tags. For example, it is envisioned that the structures help prepare new templates for chemical reactions, create functional building blocks for defined multimeric enzyme complexes, or build labeling reagents that carry tunable numbers of tags.

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