DNA Arrays

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Addressing Single Molecules on DNA Nanostructures**

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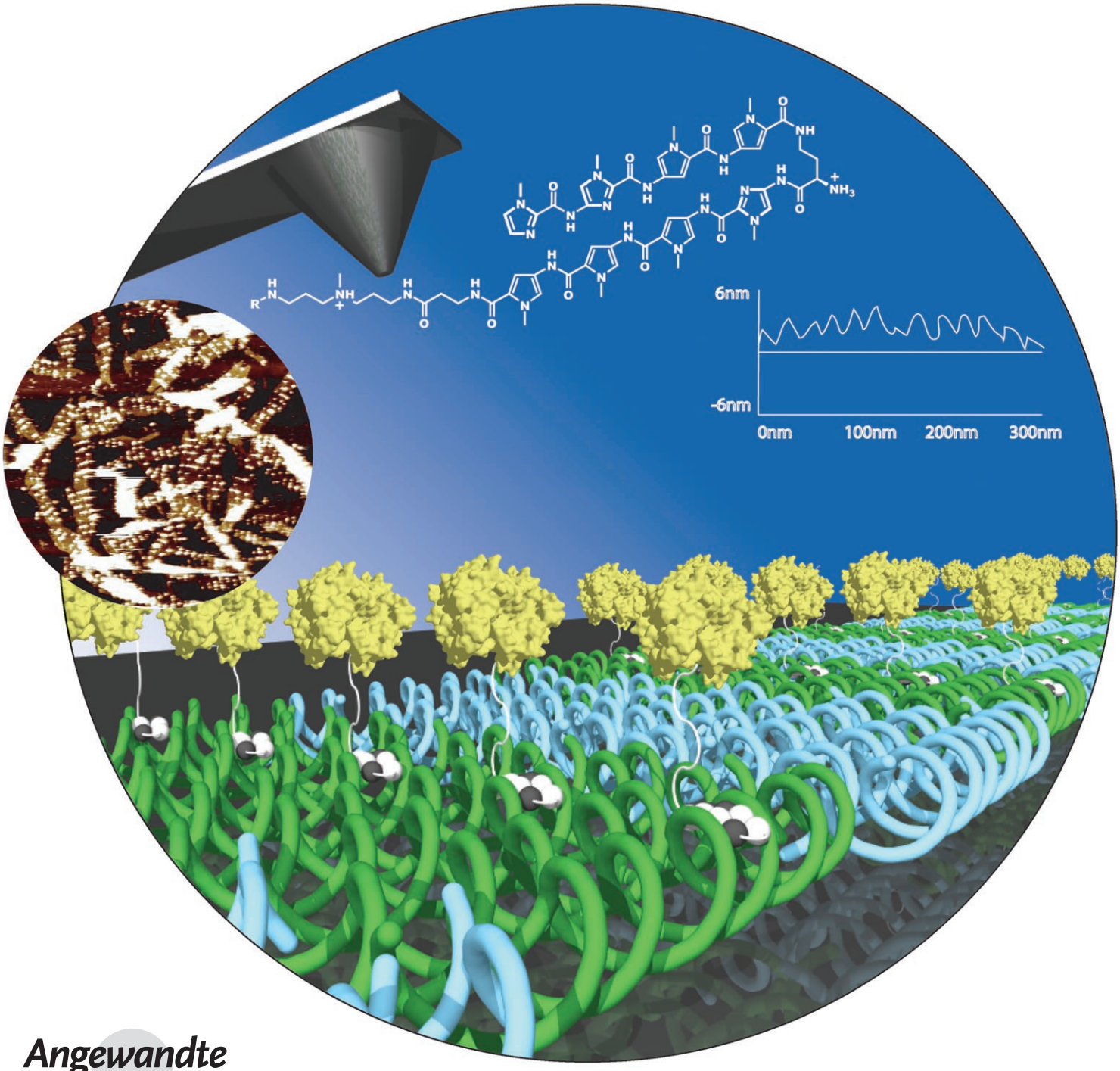

The synthesis of devices and materials from molecular components is a major goal of nanotechnology. Although many such molecular components have been demonstrated previously,[1–3] the ability to combine these components into designed architectures containing significant complexity remains a challenge. By using the hybridization properties of DNA and Watson–Crick base pairing, it has been possible to create well-defined DNA architectures of increasing complexity.[4] The structure of an assembled DNA complex is directly and uniquely determined by the sequence of the DNA bases, which can be designed and manipulated. These methods provide a versatile and programmable way to control the structure and architecture of DNA nanostructures.

A stable and well-behaved DNA structure known as the double-crossover molecule (DX) consists of two helices of DNA connected by two stable crossovers.[4] The termini of each of the four helices contained in this molecule have sticky ends, which give the molecules the ability to self-assemble into a rigid periodic two-dimensional array of DNA.[5] A variety of complex DNA nanostructures have been designed by using or extending the DX motif to create structures such as arrays, cross-tiles, six-helix bundles, and DNA origami.[6–10] Unfortunately, DNA itself lacks the functionality desired for applications such as novel sensors or electronic devices. In order for the DNA nanostructure to be useful as an architecture for molecular assembly, a way to recruit molecular components to the DNA motif is necessary so that the self-assembly of the DNA molecules also serves as a template for the assembly of secondary molecular components.

A variety of methods have been previously used to attach molecules to specific tiles in a DX array. These methods include the incorporation into specific tiles of an additional double-stranded (ds) DNA hairpin which projects perpendicular from the plane of the array,[5,11] the use of biotin-labeled DNA to recruit streptavidin,[5,7,12] the conjugation of DNA to gold nanoparticles,[13,14] the insertion of the thrombin-binding aptamer and subsequent recruitment of thrombin,[15] the insertion of DNA aptamers that can be recognized by single-chain antibodies,[16] and the recognition of a DNA–peptide fusion by an antibody.[17] The modified tiles are easily visualized by the differences in height between the modified and unmodified tiles when viewed with an atomic force microscope. All of these methods, however, rely upon the covalent modification of the DNA modules prior to array assembly to allow visualization.

Pyrrole–imidazole polyamides are a class of oligomers that bind with high specificity to the minor groove of DNA.[18,19] They can be programmed to target a broad repertoire of DNA sequences. By attaching a biotin moiety to the polyamide, the conjugate should be able to recruit streptavidin to individual DX tiles with high specificity and affinity for the labeling of DNA nanostructures (Figure 1).

Polyamide–EDTA–Fe conjugate **1** was programmed to target a single DNA sequence, 5′-WGGWCW-3′,[20] embedded in a previously characterized DX tile[5] (Scheme 1). One DNA strand was radiolabeled and combined with the three unlabeled strands to form tile **A**. The DX tiles formed with over 90% purity, as analyzed by gel electrophoresis, and were found to be stable under the reaction conditions used for affinity cleavage (see Figure S1 in the Supporting Information). Cleavage occurred at the predicted binding site and in the expected orientation (Figure 2). The observed cleavage pattern has a 3′-shift of 2–3 base pairs, which is characteristic of minor-groove-binding polyamides.[21,22] Additional affinity-cleavage experiments were done by using similarly designed DX tiles where the binding site was placed in different locations and orientations along the DX molecule (see Figure S2 in the Supporting Information). In all

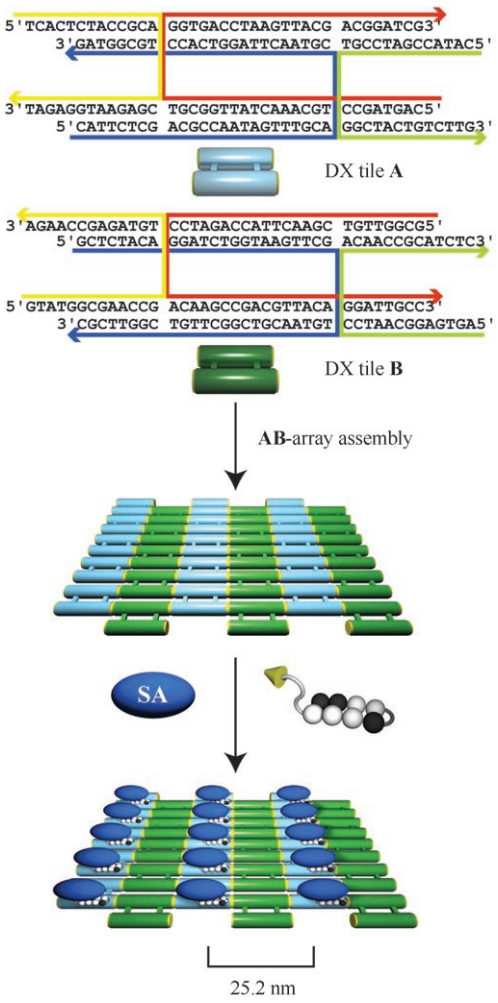

Figure 1. An **AB**-type DX array of DNA modified by a polyamide–biotin conjugate and streptavidin (SA).

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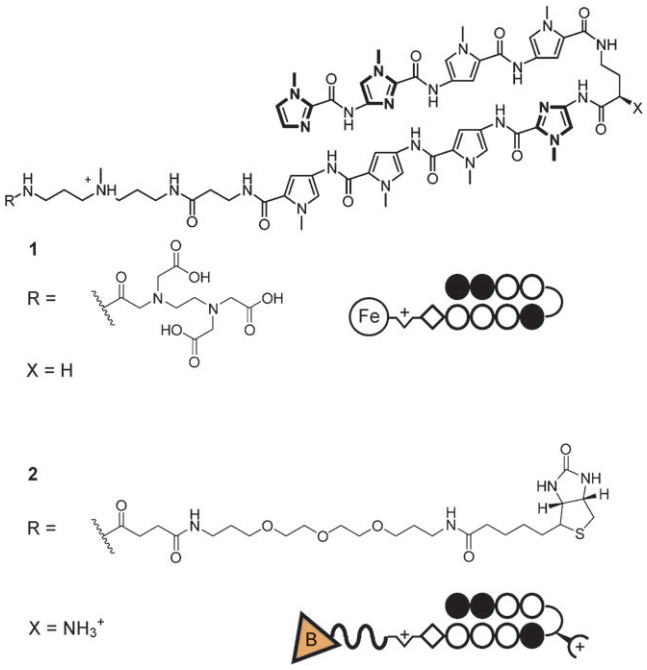

Scheme 1. Structures and schematic models of polyamide conjugates **1** and **2**. Filled circle: *N*-methylimidazole; empty circle: *N*-methylpyrrole; diamond: β-Ala; half-diamond with plus sign: diamino-*N*-methyldipropylamine; half-circle: γ-aminobutyric acid; half-circle with plus sign: NH_3^+; B in triangle: biotin.

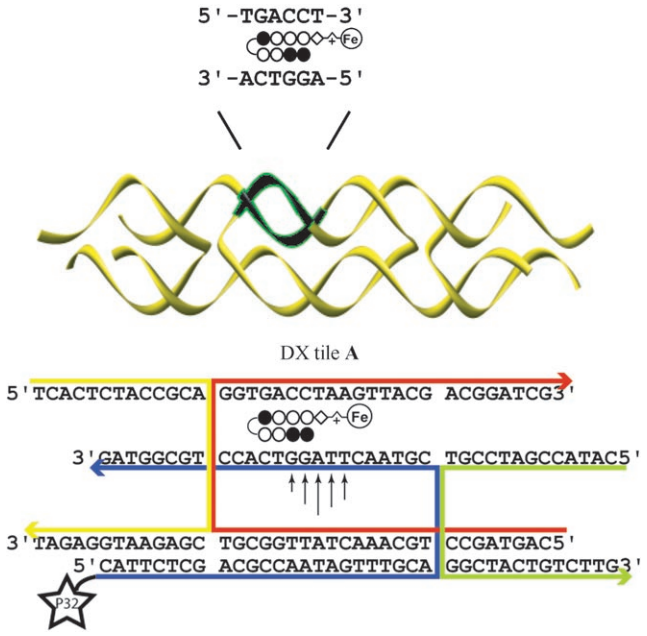

Figure 2. Top: Model of DX tile **A**. The tile consists of four individual DNA strands, shown in yellow with the 6-base-pair polyamide-binding site in black. Bottom: Representation of tile **A** binding with polyamide **1**. The arrows represent the extent of cleavage at the indicated base positions during an affinity-cleavage experiment.

cases, cleavage was observed in the expected location, thereby demonstrating the ability of hairpin polyamides to address a variety of locations along a single DX module (see Figures S3 and S4 in the Supporting Information).

Having shown the ability of polyamides to bind to individual DX tiles, we next addressed the question of whether a polyamide conjugated to biotin would be capable of recruiting streptavidin to DNA and could therefore be used as a labeling agent in AFM studies. DNAse I footprinting was used to ensure that attachment of a biotin molecule did not interfere with the polyamide's ability to bind to DNA (see Figure S5 in the Supporting Information). The ability of the polyamide–biotin conjugate to bind to streptavidin was then tested by using an electrophoretic mobility shift assay (EMSA). A DNA duplex was incubated with polyamide–biotin conjugate **2**, as well as 10 μM streptavidin. The streptavidin concentration was chosen to ensure a tenfold excess of the protein relative to the polyamide–biotin conjugate **2**. As the concentration of **2** is increased to 30 nM, the naked DNA is shifted to a band of lower mobility, which corresponds to the tertiary complex containing polyamide–biotin, streptavidin, and DNA (see Figure S6 in the Supporting Information).

We then examined whether these complexes would be stable and visible by using atomic force microscopy in the context of an **AB**-type DX array. A new DX tile, **B**, which does not contain a match site for the polyamide, was designed to form an **AB**-type array with the previously studied DX tile **A**. In our experiments, we combined tiles **A** and **B** to form an **AB** array in which every other tile has a polyamide-binding site. As shown in Figure 3a, the DX tiles were capable of forming well-defined arrays that could be visualized by AFM.

Addition of polyamide **2** alone or streptavidin alone did not affect the formation or appearance of the DNA arrays (Figure 3a and b). However, addition of polyamide–biotin conjugate **2** and streptavidin led to recruitment of the streptavidin to the **AB** array (Figure 3c and d). Incubation of polyamide **2** with tile **A** prior to array formation, as opposed to after array formation, led to identical results; this indicates that the order of addition is not critical. The streptavidin molecules align with a regular spacing occurring between them, as expected. The distance between the alternating **A** tiles in the **AB** array is expected to be 25.2 nm (Figure 1). A section analysis shows that the average spacing observed between individual streptavidin molecules is (24.1 ± 1.6) nm, in agreement with the expected distance (Figure 3e). For the purposes of this study, it appears that polyamide binding is relatively nondisruptive to array formation and stability.

In our experiments, a polyamide–biotin conjugate was able to recruit streptavidin molecules to one sort of tile (**A**) in an **AB**-type DX array, effectively labeling individual tiles. The ability of polyamides to bind to virtually any sequence of DNA should allow the design of reagents capable of specifically recognizing multiple DNA elements within large arrays. It should be possible to uniquely label different tiles in the same array by changing the polyamide core. Nanoparticles, functional proteins, or other biomolecules could be conjugated to or recruited by polyamides, thereby allowing them to be targeted to specific tiles in an array. The polyamide conjugates would act as a sequence-specific glue or molecular staple that would allow the self-assembly properties of DNA

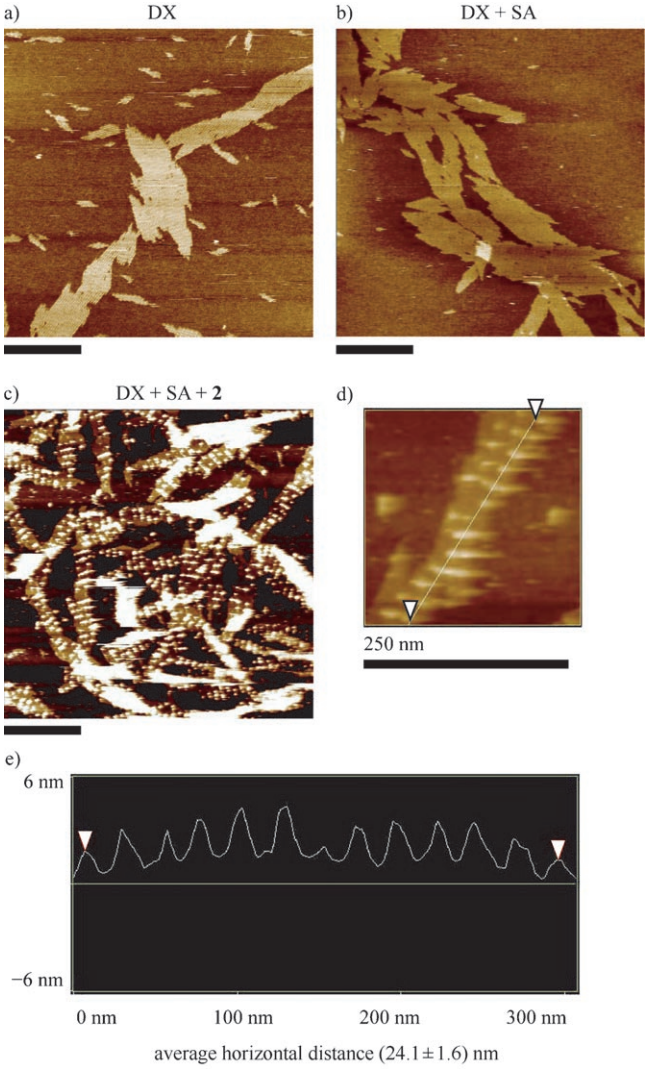

Figure 3. AFM images of combined DX tiles **A** and **B**. The scale bar is equal to 250 nm in length for all images. a) 100 nM DX array. b) 100 nM DX array with 200 nM streptavidin. c) 100 nM DX array with 200 nM streptavidin and 100 nM **2**. d) Close-up image of the DX array with **2**. e) Height profile along the path indicated in (d). The average horizontal distance between the peaks is shown.

to be transferred to complicated functional molecular assemblies.

Experimental Section

Experimental details of the materials, polyamide synthesis, preparation of labeled DX tiles, affinity cleavage on DX complexes, electrophoretic mobility shift assay, and affinity determination by quantitative DNase I footprinting are described in the Supporting Information.

AFM sample preparation: Individual DX tiles **A** and **B** were first annealed by mixing equimolar amounts of each of the 4 required strands in TAEMg buffer (40 mM tris(hydroxymethyl)aminomethane–HCl (Tris-HCl; pH 8.0), 20 mM acetic acid, 1 mM ethylenediaminetetraacetate (EDTA), 12.5 mM magnesium acetate) and heating to 95 °C for 10 min at a concentration of 200 nM. The tiles were then allowed to cool slowly to room temperature over several hours. Equal amounts of DX tiles **A** and **B** were then mixed at a final concentration of 100 nM. Samples were heated to 50 °C and allowed to cool slowly to room temperature over 10–12 h. Polyamide **2** was added to the solution of the DX array and allowed to equilibrate for 1 h at a concentration of 100 nM. This was followed by addition of streptavidin, at a final concentration of 200 nM, which was incubated with the sample for 30 min. In certain cases, the polyamide was incubated with a single tile prior to the 50 °C annealing step, rather than with the already formed array. The sample (5 μL) was spotted on freshly cleaved mica and allowed to absorb for 1 min. Imaging was done with a DI Multimode atomic force microscope.

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