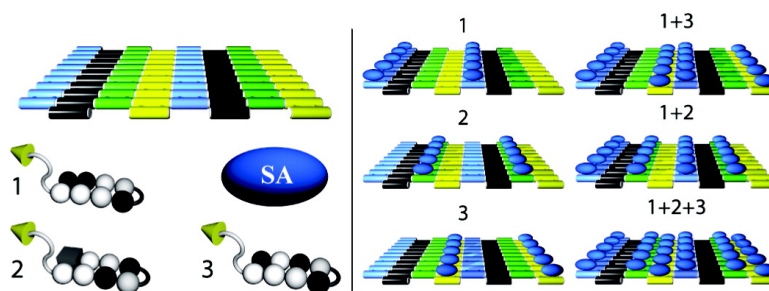Communication

Programming Multiple Protein Patterns on a Single DNA Nanostructure

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Programming Multiple Protein Patterns on a Single DNA Nanostructure

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The ability to create assemblies of proteins with spacing on the nanometer scale has important implications for proteomics, bio-detection, and self-assembly. Structural DNA nanotechnology has led to the creation of a variety of nanostructures which should be capable of serving as an addressable template for the creation of complex molecular assemblies.[1] The goal of such systems is to be able to position proteins or other components in distinct patterns with precise spacing. These systems take advantage of the well-defined structure and spacing of DNA and use these properties to act as a template for secondary components in a bottom-up approach toward self-assembly. Previous work in this area has primarily focused on the use of chemical or structural modifications of the DNA template in order to attach or recruit proteins or nanoparticles.[1,2] We have recently shown that a single polyamide-biotin conjugate is capable of binding to a DX array made from two tiles without any modification of the target DNA.[3]

The highly programmable nature of pyrrole−imidazole poly-amides make them particularly attractive for targeting specific DNA sequences.[4,5] We now demonstrate how the program-mability of polyamide conjugates can be used to target ortho-gonal binding sites on a four tile DX-array. This allows us to use polyamides to arrange proteins into multiple distinct patterns using a common 2-D DNA template.

A periodic array consisting of four DX tiles was designed as shown in Figure 1. Tiles **A**, **C**, and **D** were designed to contain a single polyamide binding site embedded in them while tile **B** does not contain any match sites. Three polyamide-biotin con-jugates **1**−**3** were synthesized with each polyamide core de-signed to address a unique tile in the array. (Figure 2) The binding affinity and specificity of each of these polyamide cores has been previously established. Polyamide **1** is designed to target 5′-WGGWCW-3′,[6] polyamide **2** targets 5′-WTWCGW-3′,[7] and polyamide **3** targets 5′-WGWGCW-3′ where W is an A or T base-pair.[5]

The four-tile DX-**ABCD** self-assembles to give 2-D arrays several μm long. Polyamides **1**, **2**, and **3** were then individually incubated with the **ABCD** DNA array for 1 h prior to AFM imaging. Samples were spotted on mica and allowed to absorb, followed by the addition of streptavidin. As shown in Figure 3, streptavidin recruitment is observed in all three cases. The average spacing for **1**, **2**, and **3** is ∼51, ∼53, and ∼49 nm, respectively, corresponding to a spacing of four tiles as expected.

To demonstrate the ability to target multiple sites on the array simultaneously, we incubated the array with both polyamides **1** and **3**. Since these polyamides target adjacent tiles **A** and **D** on the array, they should give "double-wide" columns of streptavidin. As shown in Figure 4, this is what we observe. The average spacing from the
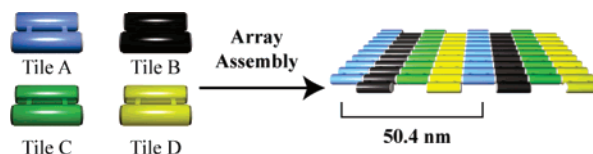

Figure 1. A DX array consisting of four tiles. Tiles **A**, **C**, and **D** contain a binding site for a different polyamide while tile **B** does not contain any binding sites. The predicted spacing between tiles in the array is shown.
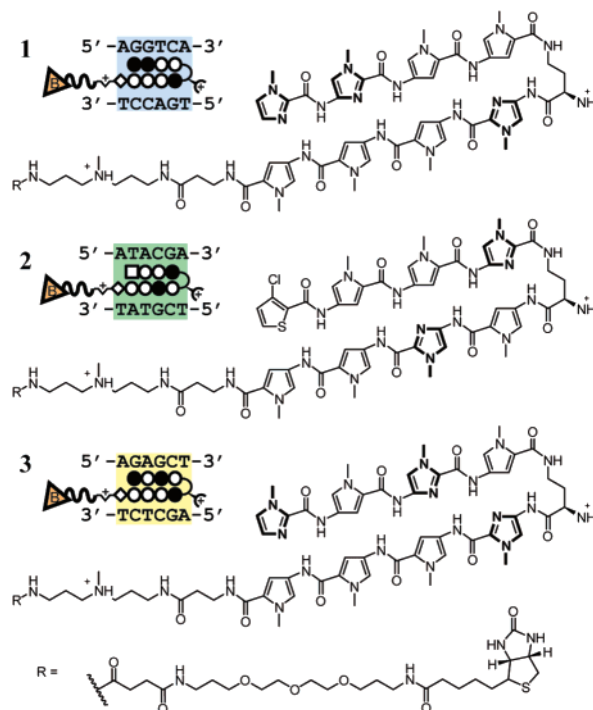

Figure 2. Structure and ball-and-stick models for polyamide−biotin conjugates **1**, **2**, and **3**. The DNA sequence that each is targeted to is shown. The colors correspond to the tile in the array that contains the target sequence as in Figure 1.

center of two adjacent peaks to the next two peaks is ∼53 nm, in agreement with what we predict. We next incubated our array with polyamides **1** and **2** as before. In this case, every other tile in the array is targeted, so we expect to see stripes with a spacing of half that observed for the individual polyamides. We observe a spacing of ∼25 nm. As a final experiment, we incubated our arrays with a combination of polyamides **1**, **2**, and **3**. We observe binding at three sites, with a single unbound site between them as would be predicted. The average spacing between unoccupied sites is ∼47 nm corresponding to a spacing of every four tiles.
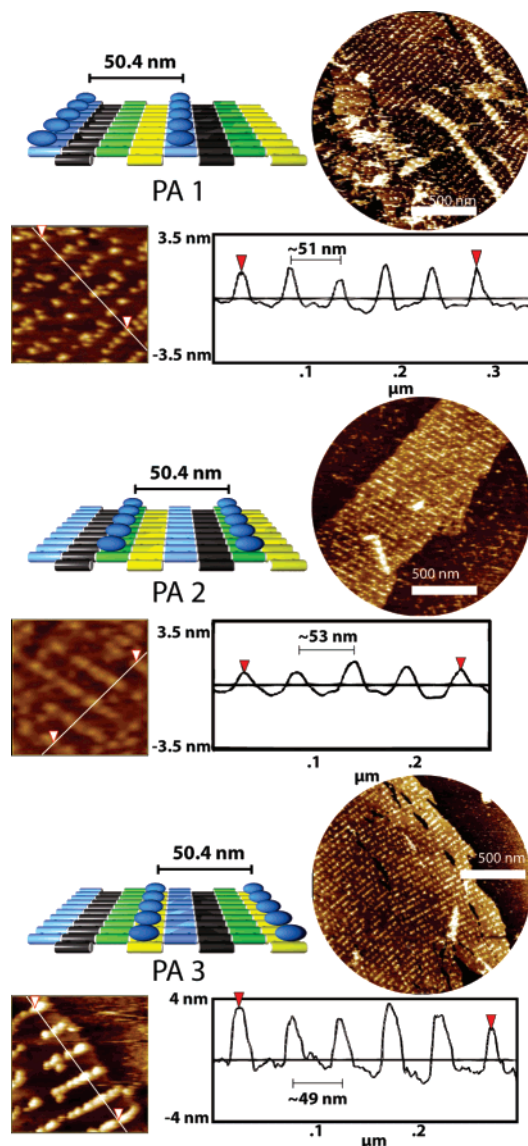
Figure 3. AFM images for individual polyamides incubated with DX-**ABCD** and streptavidin. Section analysis shows the height along the indicated path in the square image.

Figure 4. AFM Images of DX-**ABCD** incubated with combinations of polyamides **1**, **2**, and **3**. Section analysis shows the height along the indicated path in the square image.

In conclusion, we have demonstrated that polyamide−biotin conjugates are capable of addressing specific elements in a multicomponent DX array. We are able to organize streptavidin molecules into distinctly different patterns on the same DNA template, solely by changing the polyamide cores that are used. The synthetic ease in creating polyamides, and the existence of a library of well characterized solutions to target a wide variety of DNA sequences makes these conjugates ideal for arranging proteins at a variety of sites. In addition, the ability to address an array without requiring prior covalent modification increases the flexibility and usefulness of DNA templates, as they could serve to make a variety of increasingly complex assemblies.

Supporting Information Available: Experimental procedures and DNA sequences. This material is available free of charge via the Internet at http://pubs.acs.org.
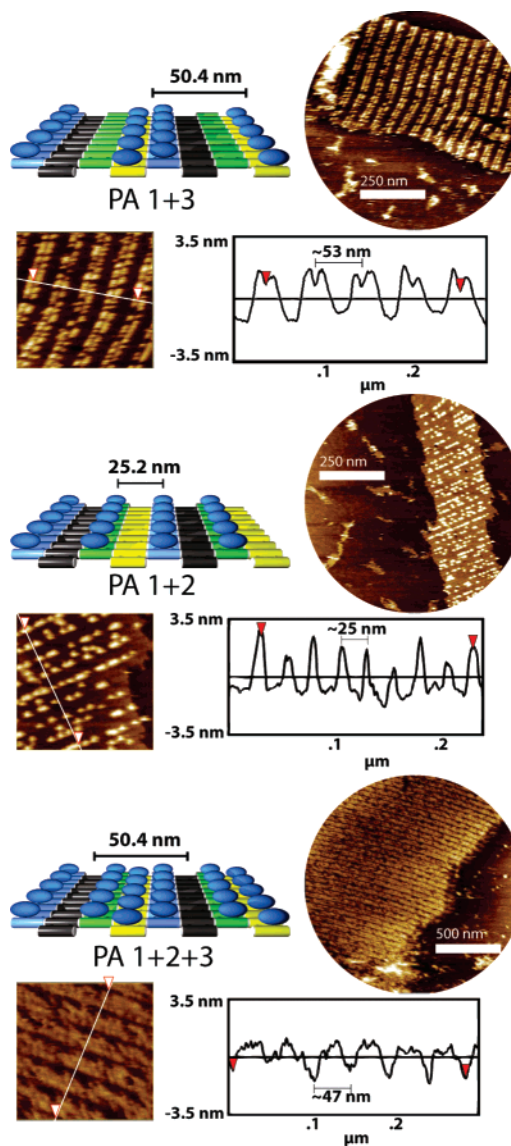
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