Communication

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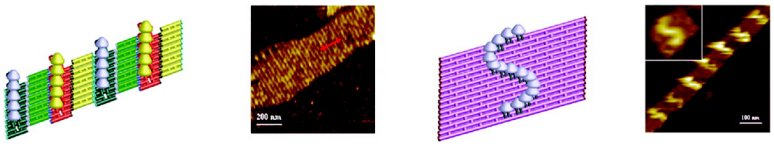

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Spatially Addressable Multiprotein Nanoarrays Templated by Aptamer-Tagged DNA Nanoarchitectures

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Immobilizing proteins with defined nanometer spacing and precision offers great potential in proteomics, tissue engineering, and medical diagnostics.[1] Since all the biomolecular recognition events in nature occur at the nanometer scale regime, arrays of proteins with precisely controlled positions and interprotein spacings can give insight into the fundamental biomolecular interactions. Several techniques have been reported for protein immobilization at the micrometer scale.[2] However, miniaturized protein arrays at nanometer scales with higher protein densities that can be easily modified to bind multiple proteins are very desirable. Herein, we demonstrate the assembly of two-dimensional (2D) nanoarrays of multiple proteins templated by self-assembled DNA scaffolds and deterministic positioning of proteins on fully addressable DNA nanoarchitectures.

DNA directed self-assembly is a biomimetic approach that harnesses the power of DNA to organize molecules in a precisely defined manner. Numerous recent reports demonstrate the successful use of DNA as a structural building material to make a variety of architectures from simple periodic arrays to arrays with complex patterns.[3] These nanoarchitectures have been shown to serve as templates to organize nanoparticles.[4] Some progress has been made in patterning protein arrays using DNA self-assembly. The strategies include streptavidin−biotin interactions,[5a,b] specific antigen−antibody interactions,[5c] Holiday-junction binding proteins,[5d] and covalent coupling of proteins or peptides to DNA by hetero-bifunctional cross-linkers.[5e] In search for a programmable approach, we and others[6] demonstrated that aptamer and aptamer-single chain antibody mediated assembly can be used to organize proteins onto periodic linear DNA nanoarrays, but the use of the aptamer approach to achieve high density and more complex 2D protein nanoarrays is still a challenge. Several important questions remain to be answered: (1) Will the emergence of sequence complexity posed by 2D and fully addressable DNA nanoarrays affect the positioning of preselected aptamer sequences and their individual functions? (2) Will multiple aptamer sequences interfere with each other and function specifically in the same condition of DNA self-assembly? (3) Will the structural rigidity and electrical charge of high-density DNA nanoarray pose any hindrance to the aptamer binding to the protein? By addressing these questions, it could open up exciting possibilities of using DNA nanoscaffolds to investigate distance dependent biomolecular interactions such as the cooperative bindings between multiple aptamers.

As a proof-of-concept, we first used a human α-thrombin binding aptamer[7a] and a platelet derived growth factor (PDGF) binding aptamer[7b] to pattern thrombin and PDGF onto periodic 2D DNA nanoarrays. We used a set of four double crossover (DX) molecules, named the ABCD tile system.[8] Each different DX tile is shown by a different color in Figure 1. The "B" tile is decorated with the thrombin aptamer sequence and the "D" tile with the PDGF aptamer sequence (see Supporting Information for detailed strand structures and DNA sequences). Upon self-assembly, the four-tile system gives 2D arrays displaying parallel and alternate lines of thrombin and

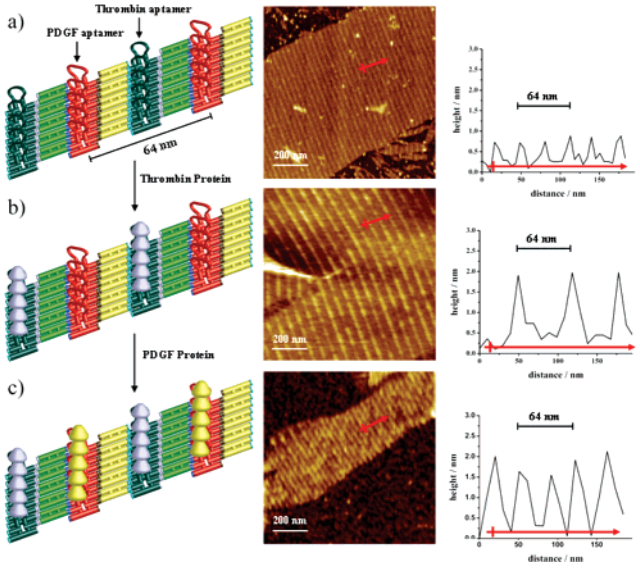

Figure 1. Periodic 2D multiprotein nanoarrays. (left) Schematics showing 2D DNA nanoarrays containing alternate thrombin and PDGF aptamers and binding of their protein targets. The red and green stem-loops represent the thrombin and PDGF binding aptamers, respectively. Grey and yellow balls represent thrombin and PDGF, respectively; (middle) AFM image corresponding to the arrays shown in the left; (right) line cross-section analysis of the AFM images.

PDGF binding aptamers, with a periodic distance between the two different lines of aptamers of ∼32 nm, and a distance between the two adjacent thrombin aptamer arrays of ∼64 nm. After adding only thrombin protein to the DNA array, thrombin molecules specifically bind to the thrombin aptamers. The atomic force microscopy (AFM) image and cross-section profiles clearly demonstrate that thrombin binds specifically to its own aptamer. After binding to one of the proteins, the height corresponding to line-scan across one of the thrombin binding aptamers increases from ∼0.7 to ∼2.0 nm. With a subsequent addition of PDGF protein to the already monofunctionalized nanoarrays, PDGF binds to the PDGF aptamers, and 2D multiprotein nanoarrays are generated displaying both proteins in a periodic pattern.

Native gel analysis confirms the binding of each protein to their respective aptamers attached in the respective DX tiles (see Supporting Information). 2D protein arrays with only PDGF protein binding to their corresponding aptamers were also assembled and characterized by AFM (see Supporting Information). These results unambiguously suggest that in generating the multiprotein arrays, the protein−aptamer pairs are highly specific, and each aptamer array serves as a control for the other aptamer array.

The above experiments demonstrate that multiple aptamer sequences can be successfully adapted into simple periodic 2D DNA nanoarrays. Both aptamer sequences keep their specific binding activities and neither interfere with each other nor with the

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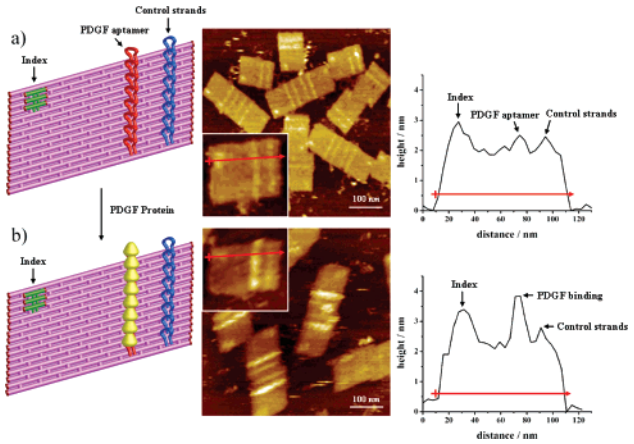

Figure 2. PDGF protein array on DNA origami scaffold. (left) Schematics showing a rectangular shaped DNA origami array with topographic index at the left corner. A PDGF aptamer linear array and an array of strands with random sequences serving as a control were incorporated into the origami array. Yellow balls represent PDGF proteins. (middle) AFM images corresponding to the arrays shown in the left, with zoom-in images (inset); (right) line cross-section analysis of the AFM images show an increase in the height at the sites of protein binding.

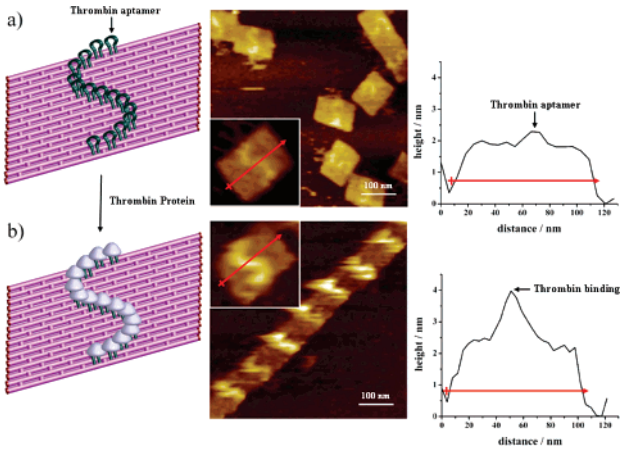

Figure 3. S-shaped thrombin array on DNA origami scaffold. (left) Schematics showing origami array with thrombin binding aptamers arranged in an S-shape. Grey balls represent thrombin proteins. (middle) Images corresponding to the arrays shown in the left, with zoom-in images (inset); (right) line cross-section analysis of the AFM images show an increase in the height at the sites of protein binding. Note that the ribbonlike arrays are caused by stacking of the origami arrays. AFM images showing unstacked arrays can be found in Supporting Information.

sequences already existing in the DNA tile structures. However, it remains to be determined whether or not DNA aptamer sequences can still function when they are incorporated into more complex DNA nanoarchitectures containing larger sequence diversities.

Motivated to answer the above question and to achieve position addressable DNA templated protein nanoarrays, we employed the recently developed DNA origami nanoarrays demonstrated by Rothemund.[9] Rothemund's DNA origami utilizes more than 200 short "helper strands" that fold a long single-stranded viral genome to generate various complex DNA nanostructures. Since each "helper strand" has a unique address on the assembled structure, complete addressability can be achieved.

Here we adapted the DNA origami design and generated rectangular-shaped DNA nanoarrays to contain aptamer sequences at specific positions on the array. In this case, we modified the arrays to show two different patterns, one with PDGF aptamers in a line (Figure 2) and another with thrombin aptamers in an "S"-shape (Figure 3). Corresponding proteins were added to the respective arrays and AFM was used to characterize the binding events. As shown in Figure 2, in the DNA origami array containing the PDGF aptamers, a set of random sequences was also added to the designed features arranged in a line parallel to the PDGF aptamers as a control. To better track the position of modified features on the template, a few dumbbell−loop strands were attached at one corner of the origami array as a topographical index. After the protein addition to the array sample, PDGF binds only to the aptamer sequences and not to the control. Figure 3 shows that the DNA origami with thrombin aptamers can arrange thrombin molecules into the S-shaped pattern. Both experiments indicate that, using aptamers in directed assembly, we can arrange proteins into any geometrical pattern on a DNA array. We found that, in both designs shown in Figure 2 and 3, the aptamer sequences incorporated into the complex nanostructures keep their specific functions.

It is worth noting that in previous reports, we and others[6] have observed that anundesired sandwich of proteins formed between two linear DNA arrays. This phenomenon was proposed to be partly due to the flexibility of the linear nanostructure. In the current study, the use of 2D templates increased the rigidity of the nanotemplates and the DNA density, thus, completely preventing undesired array sandwiches.

In conclusion, we have generated 2D multiprotein nanoarrays using DNA self-assembly encoded with multiple protein-binding aptamers. This study provides clear evidence that the aptamer directed assembly approach can be applied as a programmable strategy to assemble high-density addressable protein nanoarrays with high specificity. Since simplicity and ease of synthesis are important features of aptamer technology,[10] the experiments demonstrated here encourage the use of aptamer-mediated assembly for even a more complex display of protein nanoarrays.

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Supporting Information Available: DNA sequences, experimental methods, additional AFM images, and gel images. This material is available free of charge via the Internet at http://pubs.acs.org.

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