DOI: 10.1002/anie.200603919

Peptide–Antibody Nanoarrays

Self-Assembled Peptide Nanoarrays: An Approach to Studying Protein–Protein Interactions**

Berea A. R. Williams, Kyle Lund, Yan Liu, Hao Yan, and John C. Chaput**

Directed molecular assembly of DNA nanostructures based on Watson–Crick base pairing provides a unique approach to constructing micrometer-scale objects with nanometer-scale features.[1] Early progress in this area showed that DNA could be used to construct a variety of geometrically diverse objects, including cubes,[2] knots,[3] truncated octahedron,[4] and Borromean rings.[5] More recently, DNA has been used as a building-block material to produce large patterned arrays of repeating periodicity. Two-dimensional (2D) arrays composed of thousands to hundreds of thousands of DNA tiles have been made by using positional information encoded in the sticky ends of nonoverlapping DNA to direct the association of one or more individual tiles toward the formation of large lattice structures.[6] Although the design of complex DNA nanostructures is of fundamental importance, the intrinsic value of DNA as a building-block material lies in its ability to organize other molecules with nanometer-scale spacing.[7] This unique property helps distinguish protein nanoarrays from conventional microarrays as proteins deposited onto glass slides are spatially restricted from interacting with one another owing to the micrometer-scale distances between spots. By contrast, protein nanoarrays are assembled in solution on DNA scaffolds that position individual proteins within a few nanometers of each other. The advantages of solution-phase chemistry and site-specific positioning make DNA an attractive candidate for developing high-density protein arrays.

Indeed, many examples now show the general utility of DNA as a molecular scaffold for assembling proteins and metallic particles onto array surfaces.[8] The most common method used to functionalize DNA arrays involves Au–sulfur or biotin–streptavidin attachment chemistry.[7,8] Although these strategies are useful for creating patterned surfaces with one type of molecular component, the design of more-divergent surfaces containing many different types of molecular components, each at well-defined and addressable locations on the same array surface, remains a challenging problem. As a possible solution, we have developed a general method to produce high-density peptide arrays that rely on the addressable information encoded in the nucleic acid portion of a DNA-tagged peptide. This allows positioning of specific amino acid sequences at predetermined locations on the DNA array through hybridization in situ to complementary DNA capture probes. We call this approach "nanodisplay" as the individual peptides are displayed on the surface of a DNA nanostructure. Given the large number of peptide–nucleic acid combinations that can be constructed and the mild conditions under which these arrays form, it should be possible to use this technology as a universal platform for generating program-driven peptide nanoarrays. The peptide nanoarrays produced by nanodisplay could, in theory, be used to study a wide-range of protein–protein and protein–inorganic interactions at the nanometer-scale level. Herein, we demonstrate the feasibility of this approach by capturing the myc-epitope peptide displayed on a 2D DNA array with anti-myc mouse antibody 9E10. By using gel electrophoresis and atomic force microscopy (AFM), we were able to validate each step in the assembly process and demonstrated that the myc-peptide epitope remains functional when displayed on the DNA array.

We began by assembling a synthetic DNA scaffold from a set of 22 synthetic oligonucleotides designed to adopt one of four double-crossover (DX) motifs (Figure 1, tiles A–D).[6a] The array design, called an ABCD tile array, is a common motif in structural DNA nanotechnology. We modified the D tile to contain a DNA capture probe that functions as a tag for positioning DNA–peptide fusions at a specific addressable locations on the DNA array.[8a] The DX tile is an ideal building block material for the construction of DNA nanostructures owing to the rigid nature of the double-crossover motif and the intrinsic nanometer-scale dimensions of the tile: approximately 4×16 nm^2 (width × length) and about 2 nm in thickness. When imaged by AFM, the ABCD tile array is predicted to give a series of regularly spaced topographical features that indicate the location of the probes on the array surface.

The myc-peptide fusion was constructed by using standard peptide coupling chemistry to covalently link the myc-epitope peptide to a 5′-amino-modified DNA strand.[9] This strategy,

[*] K. Lund, Dr. Y. Liu, Prof. Dr. H. Yan
Department of Chemistry and Biochemistry
Center for Single Molecule Biophysics
The Biodesign Institute
Arizona State University
Tempe, AZ 85287-5201 (USA)
Fax: (+1) 480-727-0396
E-mail: hao.yan@asu.edu
B. A. R. Williams, Prof. Dr. J. C. Chaput
Department of Chemistry and Biochemistry
Center for BioOptical Nanotechnology
The Biodesign Institute
Arizona State University
Tempe, AZ 85287-5201 (USA)
Fax: (+1) 480-727-0396
E-mail: john.chaput@asu.edu

[**] This work was supported by grants from the NSF (CCF-0453685, CCF-0453686, and CTS-0545652) to H.Y. and the ONR (N000140710194) to H.Y. and J.C. B.W. is an NSF IGERT Predoctoral Fellow.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

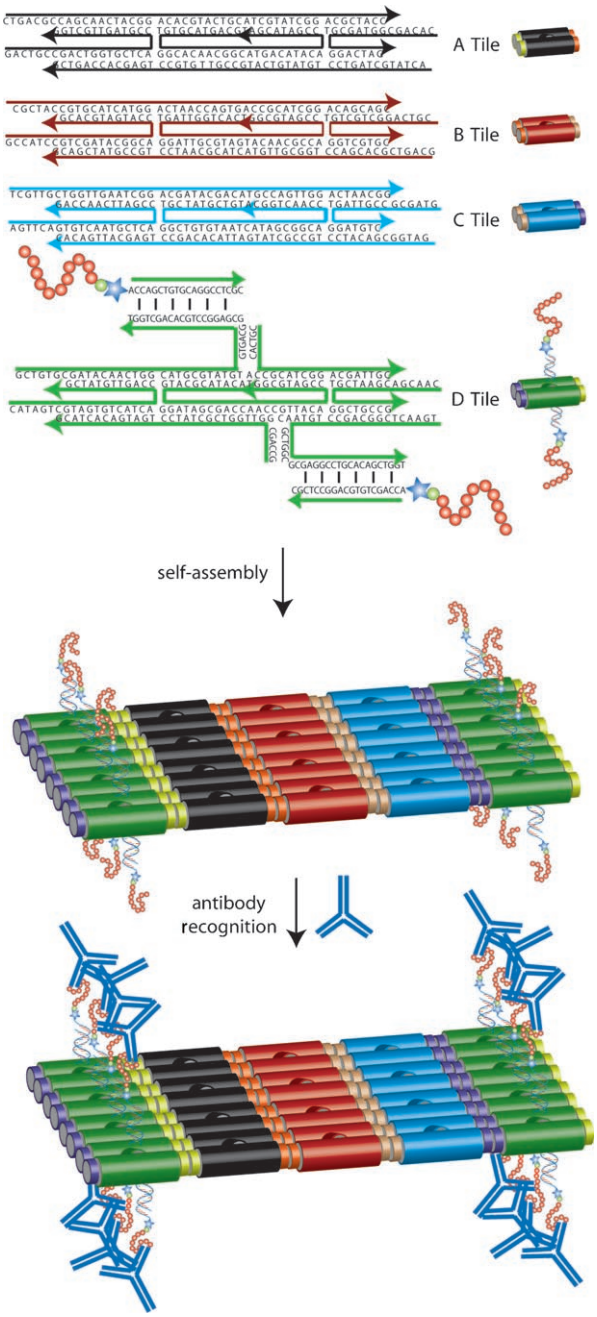

Figure 1. Self-assembled 2D peptide nanostructure. DX tiles A–D are preassembled in solution from a set of 22 DNA strands. The tiles then further assemble into 2D arrays with the D tile displaying a unique capture probe for hybridization to the DNA–peptide conjugate. The peptide conjugated to DNA retains its conformation after hybridization to the DNA nanoarray allowing antibody recognition.

originally designed to improve the binding affinity of antisense oligonucleotides, functions by first attaching a bivalent coupling reagent, 4-(*N*-maleimidomethyl)cyclohexanecarboxylic acid *N*-hydroxysuccinimide ester (SMCC), to the amine-modified oligonucleotide, removing the unreacted SMCC, and coupling the SMCC-modified oligonucleotide to a cysteine residue on the peptide. The DNA portion of the DNA-tagged peptide is complementary in sequence to the capture probe on the D tile.

The myc-peptide array (Figure 1) was assembled from four DX tiles by using sticky-end cohesion. The four tiles were formed separately by heating the strands to 94 °C and slowly cooling to 30 °C. The tiles were then combined with the myc-peptide fusion and further annealed by slowly cooling from 40 °C to 24 °C. During the second annealing step, the four DX tiles assemble into a periodic 2D array and the DNA-tagged peptides hybridize to the DNA capture probes on the D tile. We found that simply heating all of the strands to 94 °C and cooling slowly to 24 °C also led to efficient array formation.

Correct hybridization between the DNA capture probe and the myc-peptide fusion was confirmed by gel-mobility-shift assays performed under native conditions. As illustrated in Figure 2a, the band corresponding to the DNA capture

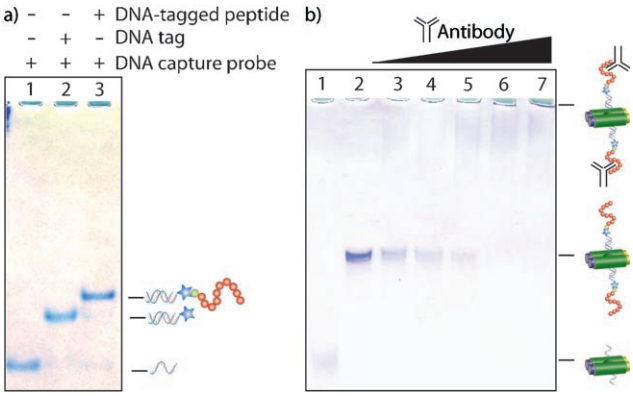

Figure 2. A nondenaturing gel electrophoresis assay showing efficient hybridization of the DNA-tagged peptide fusion to the DNA array. a) A native gel-mobility-shift assay was used to examine the hybridization efficiency of the myc-peptide fusion to the DNA capture probe. Lane 1: single-stranded DNA capture probe; lane 2: SMCC-conjugated DNA tag annealed to the DNA capture probe; and lane 3: DNA-tagged peptide fusion annealed to the DNA capture probe. b) Native gel-mobility-shift assay to demonstrate immunoprecipitation of the myc epitope displayed on the D tile by the anti-myc antibody 9E10. Lane 1: D tile alone; lane 2: myc-peptide fusion annealed to the D tile; and lanes 3–7: binding of the myc-peptide nanoarray (18 pmol) with increasing concentrations (8, 11, 67, 90, and 120 pmol) of anti-myc antibody.

probe shifts to slower mobility when incubated with the complementary DNA tag or the DNA-tagged peptide fusion (lane 1 versus lanes 2 and 3). The change in electrophoretic mobility is consistent with the formation of a double-stranded DNA helix. To demonstrate that the DNA-tagged peptide fusion remains functional when hybridized to the DNA capture probe, we performed a second gel-shift assay (Figure 2b) by using the anti-myc antibody to capture the myc-peptide fusion on the D tile. Myc peptides displayed on D tiles were incubated with increasing concentrations of anti-myc mouse antibody. Complete capture of the myc-peptide fusion by the antibody was visualized by the disappearance of the band corresponding to the D tile displaying the myc-peptide epitope. These two experiments indicate that the myc-peptide fusion anneals to the DNA capture probe and remains accessible to the antibody when localized on the DNA tile.

AFM images were collected on arrays assembled in the absence and presence of the myc-peptide fusion to verify that the DNA array formed properly in the presence of the myc peptide. In each case, a 2-μL aliquot of the final array solution was deposited onto a freshly cleaved mica surface, washed with buffer, and imaged by AFM. Figure 3a–c provides a schematic illustration of the three different states of the DNA surface. The AFM images in Figure 3d–f show complete array formation as indicated by a series of parallel lines separated by approximately 64 nm, which is the approximate distance separating the repeats of the D tile. The lines on the array are due to changes in the surface height when the tip of the AFM probe comes into contact with the DNA capture probe. Comparison of the line height profiles for arrays formed in the absence and presence of the myc-peptide fusion (Figure 3g versus 3h) indicate that arrays formed in the presence of the myc fusion give sharper lines with greater height (≈0.5 nm versus ≈1.5 nm, respectively). We attribute the change in height to the increased rigidity of the DNA probe when annealed to the myc-peptide fusion, and the increased bulk volume owing to the presence of the peptide (Figure 3a, b).

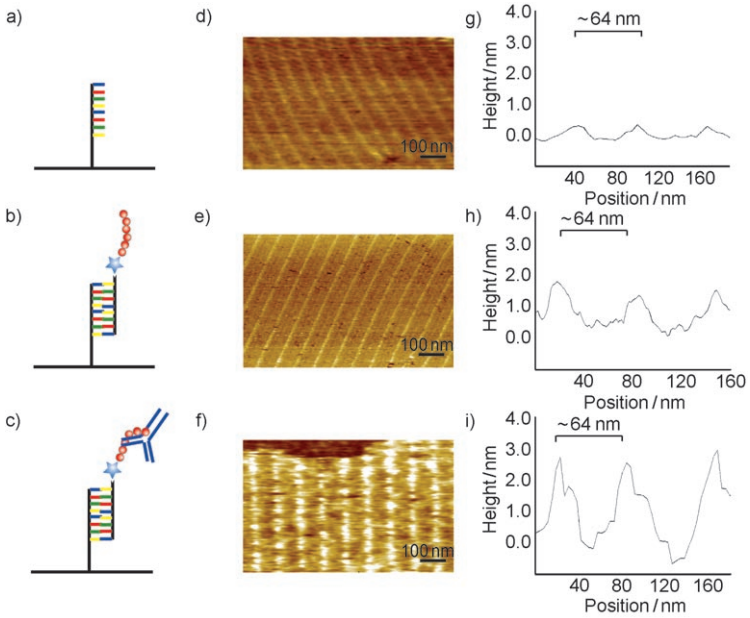

Figure 3. AFM imaging of the peptide nanoarray. a–c) Schematic illustration showing the DNA capture probe on the DNA surface, annealed to the myc-peptide fusion, and immunocaptured by the anti-myc antibody, respectively. d–f) AFM images were collected for the array before hybridization of the myc-peptide fusion, after hybridization of the myc peptide, and following incubation with the anti-myc antibody, respectively. g–i) Height profiles were determined for the array, the array displaying the myc-peptide epitope, and the array with the anti-myc antibody bound to the myc epitope, respectively.

We collected AFM images of the myc-peptide array following a brief incubation with the anti-myc antibody. The resulting array shows a series of parallel lines that signify the location of the antibody bound to the myc-peptide epitope annealed to the ABCD tile array. This is evident by the observation that the lines on the array appear much larger following incubation with the myc antibody than images collected in the absence of the myc antibody (Figure 3e versus 3f). Moreover, almost no antibody binding was observed anywhere else on the array other than where the myc peptide was annealed to the D tile. Comparative height profiles taken before and after the addition of the antibody (Figure 3h and i) show that the line height increases from approximately 1.5 nm to approximately 3.0 nm when the antibody is bound to the peptide array. The increased line height is consistent with the larger size of the anti-myc antibody (≈65 kDa). This experiment confirms that peptides displayed on DNA nanostructures remain accessible to exogenously added proteins. Although we cannot rule out the possibility that some nonspecific interactions between the peptide and the DNA surface may occur at low, perhaps undetectable levels, results from these experiments indicate that such interactions do not impede antibody binding.

In summary, we present a novel approach for constructing tailor-made peptide and protein nanoarrays with addressable surface features. The construction of high-density peptide arrays with nanometer-scale features represents a new strategy for studying protein–protein and protein–inorganic interactions for nanobiotechnology and nanoelectronic applications. A major advantage of nanodisplay over conventional microarray systems is the ability to detect substrate binding in solution at the single-molecule or near single-molecule level. We suggest that this technology in combination with more-diverse DNA surface architectures, such as origami assembly, may lead to self-assembled nanoelectronics and nanobiochips capable of monitoring protein pathways at local concentrations reminiscent of cellular pathways.[10,11]

Received: September 24, 2006
Revised: February 8, 2007
Published online: March 16, 2007

Keywords: DNA · nanostructures · peptide nanoarrays · self-assembly

[1] N. C. Seeman, *J. Theor. Biol.* **1982**, *99*, 237–247.
[2] J. Chen, N. C. Seeman, *Nature* **1991**, *350*, 631–633.
[3] J. E. Mueller, S. M. Du, N. C. Seeman, *J. Am. Chem. Soc.* **1991**, *113*, 6306–6308.
[4] Y. Zhang, N. C. Seeman, *J. Am. Chem. Soc.* **1994**, *116*, 1661–1669.
[5] C. Mao, W. Sun, N. C. Seeman, *Nature* **1997**, *386*, 137–138.
[6] a) E. Winfree, F. Liu, L. A. Wenzler, N. C. Seeman, *Nature* **1998**, *394*, 539–544; b) H. Yan, S. H. Park, G. Finkelstein, J. H. Reif, T. H. LaBean, *Science* **2003**, *301*, 1882–1884.
[7] K. Lund, Y. Liu, S. Lindsay, H. Yan, *J. Am. Chem. Soc.* **2005**, *127*, 17606–17607.
[8] a) J. D. Le, Y. Pinto, N. C. Seeman, K. Musier-Forsyth, T. A. Taton, R. A. Kiehl, *Nano Lett.* **2004**, *4*, 2343–2347; b) H. Li, S. H. Park, J. H. Reif, T. H. LaBean, H. Yan, *J. Am. Chem. Soc.*

2004, *126*, 418 – 419; c) J. Zhang, Y. Liu, Y. Ke, H. Yan, *Nano Lett.* **2006**, *6*, 248 – 251; d) J. Sharma, R. Chhabra, Y. Liu, Y. Ke, H. Yan, *Angew. Chem.* **2006**, *118*, 744 – 749; *Angew. Chem. Int. Ed.* **2006**, *45*, 730 – 735; e) S. Xiao, F. Liu, A. E. Rosen, J. F. Hainfeld, N. C. Seeman, K. Musier-Forsyth, R. A. Kiehl, *J. Nanopart. Res.* **2002**, *4*, 313 – 317; f) Y. Liu, C. Lin, H. Li, H. Yan, *Angew. Chem.* **2005**, *117*, 4407 – 4412; *Angew. Chem. Int. Ed.* **2005**, *44*, 4333 – 4338; g) Y. Pinto, J. D. Le, N. C. Seeman, K. Musier-Forsyth, T. A. Taton, R. A. Kiehl, *Nano Lett.* **2005**, *5*, 2399 – 2400.

[9] J. G. Harrison, S. Balasubramanian, *Nucleic Acids Res.* **1998**, *26*, 3136 – 3145.

[10] H. Yan, T. H. LaBean, L. Feng, J. H. Reif, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 8103 – 8108.

[11] P. W. K. Rothemund, *Nature* **2006**, *440*, 297 – 302.

