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Antibody Nanoarrays with a Pitch of ~20 Nanometers

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This communication reports a DNA-directed antibody self-assembly into two-dimensional (2D) arrays. The resulting antibody arrays present a well-defined tetragonal order with a repeating distance of ~19 nm.

DNA has robust physicochemical properties and predictable Watson−Crick base-pairing ability and provides a versatile molecular system for preparing nanometer-scale structures.[1−3] Recent works have demonstrated that DNA motifs can self-assemble into millimeter-sized, well-ordered 2D arrays with features of nanometer scale, which greatly scales up the DNA-based self-assembly.[2h,i] One potential application of such DNA arrays is to serve as templates to organize nanoparticles[3] and macromolecules. In this direction, streptavidin−biotin interaction is the most studied method,[2l,m] which has been successfully used to construct periodic streptavidin 2D arrays.[2b] Turberfield and co-workers have developed a RuvA array based on that RuvA can bind DNA four-arm junctions.[2f] More recently, Yan and co-workers have introduced a thrombin-binding aptamer into DNA 1D arrays to direct thrombin to form 1D arrays.[2k] However, the exploration of this research field is still quite limited. It would be logical to ask: Are there any other general interactions feasible in these systems? Can we construct highly ordered antibody or enzyme arrays, which might have potential applications in immunodiagnostic or catalysis? This paper takes a step toward addressing these challenges.

Our strategy, as illustrated in Figure 1, relies on a specific antigen−antibody (IgG) interaction. Antigens are conjugated into DNA motifs. Programmed DNA self-assembly will bring antigens into periodic antigen−DNA 2D arrays. Further incubation with antigen-specific antibody will result in DNA−antigen−antibody arrays. Here, a symmetric cross DNA motif was used.[2i] This motif contains nine single strands: one central red strand, four identical blue strands, and four identical black strands (Figure 1a). This motif has been shown to self-assemble into tetragonal 2D arrays with a repeating distance of ~19 nm.[2i] In the current proof-of-concept work, fluorescein was used as the antigen. The fluorescein/anti-fluorescein antibody (IgG) interaction has been extensively studied

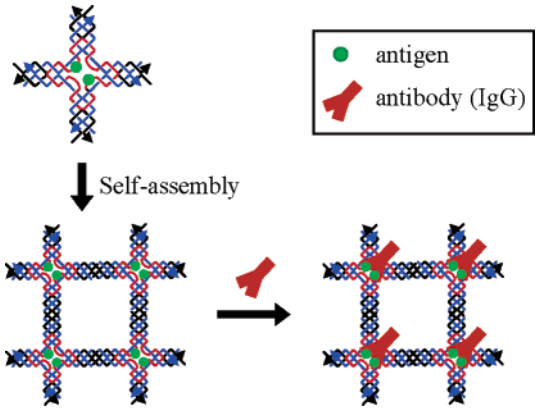

Figure 1. Scheme of the DNA-templated self-assembly of two-dimensional (2D) antibody (IgG) arrays.

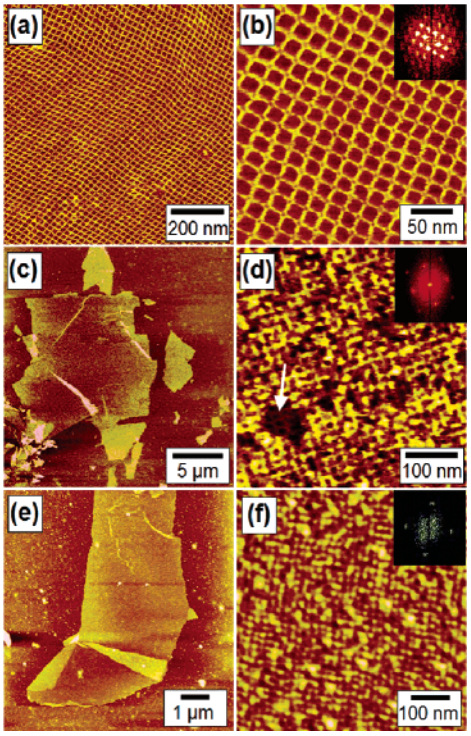

Figure 2. Atomic force microscopy analysis of antigen-modified DNA arrays (a, b) and antibody (IgG) arrays (c−f). Images (a−d) were scanned in solution, and images (e) and (f) were scanned in air. Fourier transform patterns (insets) show the regularities of the arrays. The arrow in (d) indicates a defect where there are no bound antibodies.

as a model system for antigen−antibody interaction.[4] To ensure the binding of antibody to the DNA array, a multivalency strategy has been used. Two fluorescein moieties are covalently conjugated with the central DNA strand at its central loops during solid-phase DNA synthesis. The two fluorescein moieties on the cross motif can simultaneously bind to the two antigen-binding sites of the "Y"-shaped IgG molecule. Such a 2-to-2 binding mode is expected to be strong and hold the DNA cross motif and the IgG protein together tightly.

We characterized the 2D arrays with atomic force microscopy, AFM (Figure 2). The antigen-modified DNA motifs maintained their ability to self-assemble into periodic 2D arrays (Figure 2a,b). The observed repeating distance was ~19 nm, the same as that of DNA 2D arrays without antigens. The domain size of the 2D arrays was up to 60 μm (Figure S1 in Supporting Information).

After self-assembly, the antigen-containing DNA arrays were incubated with anti-fluorescein monoclonal antibody (IgG) in solution. Strong antigen−antibody interaction effectively immobilized IgG proteins onto DNA arrays and resulted in well-defined periodic IgG arrays, as confirmed by AFM imaging (Figure 2c−f, and Figures S2 and S3 in Supporting Information). The IgG molecule is ~9 nm long from the antigen-binding sites to the end

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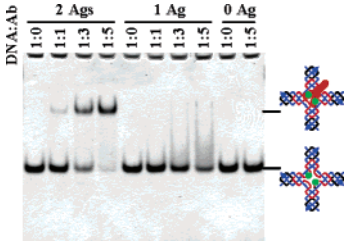

Figure 3. Native polyacrylamide gel electrophoretic (PAGE) analysis of the binding between DNA cross motifs and antibody molecules. Three DNA cross structures are used: with two antigens (2 Ags), one antigen (1 Ag), and no antigen (0 Ag). DNA/Antibody (Ab) ratios are indicated on the top of the gel image.

of constant regions,[5] and the separation between two adjacent IgG proteins in the current arrays is ~19 nm. When imaged in solution (Figure 2c,d), antibodies were well hydrated. They could be easily deformed by AFM probes and swing back and forth, similar to the motion of seaweeds in the ocean. Thus, individual antibody molecules could not be resolved as discrete particles; instead, they appeared as pseudo-continuous bulgy lines. The height of the DNA arrays was 1.7−1.9 nm, while the height of the antibody arrays was 2.7−3.5 nm (Figures S4 and S5 in Supporting Information). The height analysis confirmed the formation of the antibody arrays. Occasionally, we found some spots (indicated by the arrow in Figure 2d) on the arrays where antibodies did not bind to the antigens. The bare DNA array was clearly visible, which was much lower than the surrounding antibody-decorated array. This phenomenon provided a strong evidence for the formation of antibody arrays. When imaged in air (Figure 2e,f), antibodies were dehydrated and fixed on substrate, thus, less deformable. Consequently, individual IgG molecules appeared as discrete particles. The heights of IgG-decorated and bare DNA arrays were 1.6−1.9 and 0.7−0.9 nm, respectively (Figure S6 in Supporting Information). However, it was unclear whether IgGs bound to antigen pairs stoichiometrically in the arrays.

For effectively binding antibodies to the antigen-containing DNA arrays, multivalency played an important role. When only one antigen was conjugated with the cross motif, there was no appreciable antigen−antibody binding under the experimental condition. AFM images showed that IgG molecules randomly distributed relative to the DNA arrays, and the IgG density was quite low (Figure S7 in Supporting Information), indicating no strong, specific antibody binding. We further confirmed this observation by a gel shift experiment with native polyacrylamide gel electrophoresis, PAGE (Figure 3). The DNA motif containing two antigens formed a stable complex with the antibody, and a sharp band appeared. In sharp contrast, the DNA motif containing only one antigen only weakly interacted with the antibody and resulted in smearing tails in the gel.

Antibodies are powerful and flexible tools in proteomics research, drug discovery, and diagnostics because antibodies can bind to antigens with high specificities and high affinities.[6] There is tremendous interest in developing high density, well-orientated antibody arrays.[6] However, most antibody arrays investigated so far are prepared by immobilizing antibodies onto solid substrates. The antibodies are randomly distributed and orientated, and their feature size is generally at the micrometer scale. The antibody arrays reported here have a very high density, and all antibodies have similar orientations. However, the current antibody arrays are unlikely to be useful by their own in the above-mentioned applications because there is no free antigen-binding site available in the antibodies. However, it is possible to modify the current arrays. For example, other active components (such as enzymes) can be conjugated at the constant regions (Fc) of the antibodies. More interestingly, the reported antibody arrays would provide an excellent platform to study multivalency issues involved in antibody−antibody receptor (FcR) interactions.

In summary, we have successfully used antigen-modified DNA arrays as templates to direct antibodies to assemble into very high density nanoarrays. This work introduces a new family of specific interaction into the DNA-directed self-assembly. We would like to point out four features for the current system: (1) Each spot of the nanoarrays has only one singe IgG molecule. It may allow single molecule studies of antigen−antibody interactions. (2) The repeating distance of these 2D arrays is ~19 nm, far beyond the capability of photolithography. (3) All antibody molecules in the arrays have similar orientations, which is important for controlling biochemical activities. (4) The antibody arrays are assembled in solution instead of on solid substrates, which makes it possible to apply these arrays both in vitro and in vivo. We are currently exploring some of these possibilities.

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

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