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Dynamic Patterning Programmed by DNA Tiles Captured on a DNA Origami Substrate

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The aim of nanotechnology is to put specific atomic and molecular species where we want them, when we want them there. Achieving such dynamic and functional could lead to nanoelectronics, nanorobotics, programmable chemical synthesis, and nanoscale systems responsive to their environments. Structural DNA nanotechnology offers a powerful route to this goal by combining stable branched DNA motifs¹ with cohesive ends to produce objects, programmed nanomechanical devices² and fixed³⁻⁵ or modified^{6,7} patterned lattices. Here, we demonstrate a dynamic form of patterning⁸ wherein a pattern component is captured between two independently programmed DNA devices, tailed with cohesive ends that face each other (Figure 1). A simple and robust error-correction protocol has been developed that yields programmed targets in all cases. This capture system can lead to dynamic control either on patterns or on programmed elements; this capability enables computation or a change of structural state as a function of information in the surroundings of the system.

Recently, we reported a DNA cassette that consisted of a sequence-programmable PX-JX₂ device,⁹ combined with a domain for inserting it into a 2D DNA array; the state of the device can be switched when the cassette is inserted into an array.¹⁰ The PX-JX₂ device is a two-state DNA nanomechanical machine; the two states differ from each other by a half-rotation of one end relative to the other. This difference is evident in Figure 1, where the sticky ends are seen to change positions with the different states of the device cassettes. A key element of Figure 1 is that the ‘capture’ molecules are three helical domains thick: The bottom two domains are involved in binding; the third domain both carries the pattern and enforces a top-down direction. If the pattern were attached to the lower domains, the PX-PX arrangement might bind the JX₂-JX₂ target upside down, with the same error possible between binding programmed by the PX-JX₂ and JX₂-PX states. Steric clashes with the third domain prevent upside down binding.

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Supplementary Information Available. Experimental methods, sequences of origami staple strands, molecular features and sequences, non-denaturing gels, additional error correction images, references.

Two cassettes bound in a 2D array and capable of capturing a variety of measurably distinct target species require a lot of surface area. For example, the previous insertion of a single PX-JX₂ cassette with a 5-turn reporter arm required at least six distinct three-helix tiles, and eight tiles were used to allow design flexibility.¹⁰ A convenient alternative that exists today is DNA origami,⁴ which provides approximately three times the addressable surface area as the eight-tile system. As an example, Rinker *et al.* have used origami tiles recently to optimize the spatial features of cooperative binding by aptamers.¹¹ The overall design of the 120 × 50 nm origami tile used here is schematized in Figure 2a(i), and its detailed design is shown in the supplementary information (Fig. S1), along with the sequences of the staple strands. The two key features of the origami tiles are [1] the slots that accommodate the cassettes and [2] the notch on one side that establishes their absolute positions and orientations when viewed by AFM. An AFM image of the tile is shown in Figure 2b(i), demonstrating that the tile forms as designed. Figure 2a(ii) shows the color scheme we use to indicate the state of the cassettes, green for the PX state and purple for the JX₂ state. Figure 2b(ii) shows that it is possible to insert the cassettes into the origami units.

The remaining panels of Figure 2a show schematically the four different capture molecules that the two cassettes are designed to bind in their four different states. The cassettes may be programmed before binding to the origami, or, alternatively, they may be inserted in a default state and then re-programmed after they are bound to the origami; both programming methods have been used here with equal success. The PX-PX arrangement (Figure 2a(iii)) codes to capture a triangle pointing towards the notch, and Figure 2b(iii) contains a captured triangle in that orientation. The PX-JX₂ state (Figure 2a(iv)) is programmed to capture a triangle pointing in the opposite direction, seen by AFM in Figure 2b(iv). Programming for a JX₂-PX pair of cassette states (Figure 2a(v)) leads to the capture of a DNA diamond (Figure 2b(v)), and programming for a JX₂-JX₂ combination (Figure 2a(vi)) leads to the capture of a simple linear three-domain motif that looks like a linear connection between the two cassettes, as seen in Figure 2b(vi). The detailed sequences of the capture molecules are shown in the supplementary information (Figs. S2-S5), and those of the two cassettes are shown in Figure S6. Figure S7 contains non-denaturing gels showing robust formation of the cassettes in both states (S7a), the capture molecules (S7b) and the combination of two cassettes and one of the capture molecules (S7c).

In all cases shown in Figure 2, the capture tiles are added individually with their expected host arrangements. For a meaningful system, it is necessary to deal with competition between capture tiles. This dynamically programmable system confronts the same problem that besets algorithmic assembly,^{5,12} namely that correct capture molecules must compete with half-correct capture molecules. This is in distinct contrast to simple periodic assembly with multiple tiles,³ where correct molecules compete for their positions with completely incorrect molecules. Thus, the fidelity of this system is a central issue. When we load all four capture tiles, we find that the fidelity seems to be a function of the mass of the capture tile: The small line-like capture tile associated with the JX₂-JX₂ state is captured correctly 70-80% of the time, whereas the triangle capture tiles are captured correctly about 60-70% of the time and the diamond capture tile is captured correctly 50-60% of the time. No

completely incorrect binding is observed, but half-correct binding (i.e., one side correct, one side incorrect) occurs frequently.

To deal with this situation, we have developed a simple binding protocol that includes error-correction. We have established that under our conditions half-correct molecules (two sticky-ends attached) are stably bound at a 'permissive' temperature below 35 °C, but they are released at 35-37 °C; by contrast, correct molecules (four sticky-ends attached) are released only at 40 °C. Thus, there is a 'non-permissive' temperature range between 37 °C and 40 °C where correct molecules bind stably, and the binding of half-correct molecules is unstable. The idea behind error correction is simple: After exposure to all four cassettes simultaneously, the system is heated to the non-permissive temperature range where only correct binding is stable, and then cooled to 4 °C over a day. The system is then heated again to the non-permissive temperature range, exposed to one of the possible capture molecules, and put through the cooling protocol. This procedure is repeated until all four species have been added in this fashion. We find that in all cases the correct capture molecule displaces the incorrect capture molecule, but that the incorrect capture molecule cannot displace the correct one. This thermodynamic approach eliminates the kinetic traps of uncorrected assembly, so the order in which the different species are added is unrelated to the success of binding the target molecule.

An example (the worst-case scenario -- the diamond, which is the most massive target molecule) is shown in Figure 3. The other three cases are shown in the supplementary information in Figs S8-S10. Figure 3a shows a sample field following treatment with the mixture. Lines and triangles pointing towards the notch are present, in addition to diamonds. The completely wrong binding (triangles pointing away from the notch) is not visible. Figure 3b follows treatment of the original mixture with the line target; few diamonds are seen. Figure 3c follows treatment of the material in 3b with the triangle pointing towards the notch; again, few if any diamonds are visible. Figure 3d follows treatment of the material in 3c with the diamond; the diamonds have displaced all other targets. This is not changed in Figure 3e, which follows treatment of the material in 3d with the completely wrong target, triangles pointing away from the notch. Panels 3f-3i show the same results, but now the order of single-target treatment has been changed: Panel 3f follows treatment of the initial mixture with the triangle pointing towards the notch; these dominate the image. Panel 3g follows treatment of the material in 3f with the triangle pointing away from the notch; little changes, and the captured molecules are triangles pointing towards the notch. Panel 3h follows treatment of the material in 3g by the line; a large number of lines are present, and virtually no diamonds are seen. Panel 3i follows treatment of the material in 3h by the diamond target; only diamonds are seen. Combined with the data in Figures S8-S10 for the other targets, we find that the completely incorrect target is never bound, i.e., we never see the target with two incorrect binding sides. Likewise, the error correction protocol is able to displace the half-correct target with the completely correct target in every instance. AFM scanning may result in a displaced target molecule, but we find no instances of incorrect tiles following application of the protocol. We noticed that the first step may be unnecessary, so we tested this notion in one case. Figure S11 shows that the idea is correct, and that the four-way competition is not necessary; as soon as the correct molecule is present, the system shows complete fidelity.

We have demonstrated that it is possible to place a specific DNA target species into a selected slot in a dynamically programmed DNA nanotechnological system. Combined with the error-correction system, we are able to achieve this goal in an apparently flawless fashion. The correction of erroneous binding demonstrated here has been applied to a single capture tile at a time. One can envision its application to more tiles along a stepwise-growing front (with selectively deprotected sticky ends) in other types of algorithmic assembly (e.g., ref. 5), so long as distinct permissive and non-permissive temperatures can be identified, as they have been here.

As a prototype, we have used a target consisting exclusively of DNA, as suggested previously.⁸ However, there is no apparent limitation on the ability of the target tile to carry a cargo, such as a nanoelectronic (e.g., ref 13) or biomolecular component (e.g., ref. 14). This ability would allow a given addressable 2D DNA surface to be programmed dynamically for a variety of purposes, ranging from circuit design to multiplexed diagnostic purposes. The key limitation at this time is the small size of the addressable 2D DNA surface. Depending on its design, the area of an M-13 based origami tile is approximately 5000-10000 nm². Multiple origami tiles are not readily combined in large arrays, and they are quite expensive to produce. Progress in the goals enunciated here is likely to be limited by the ability of investigators to increase the size of the specifically addressable 2D surface.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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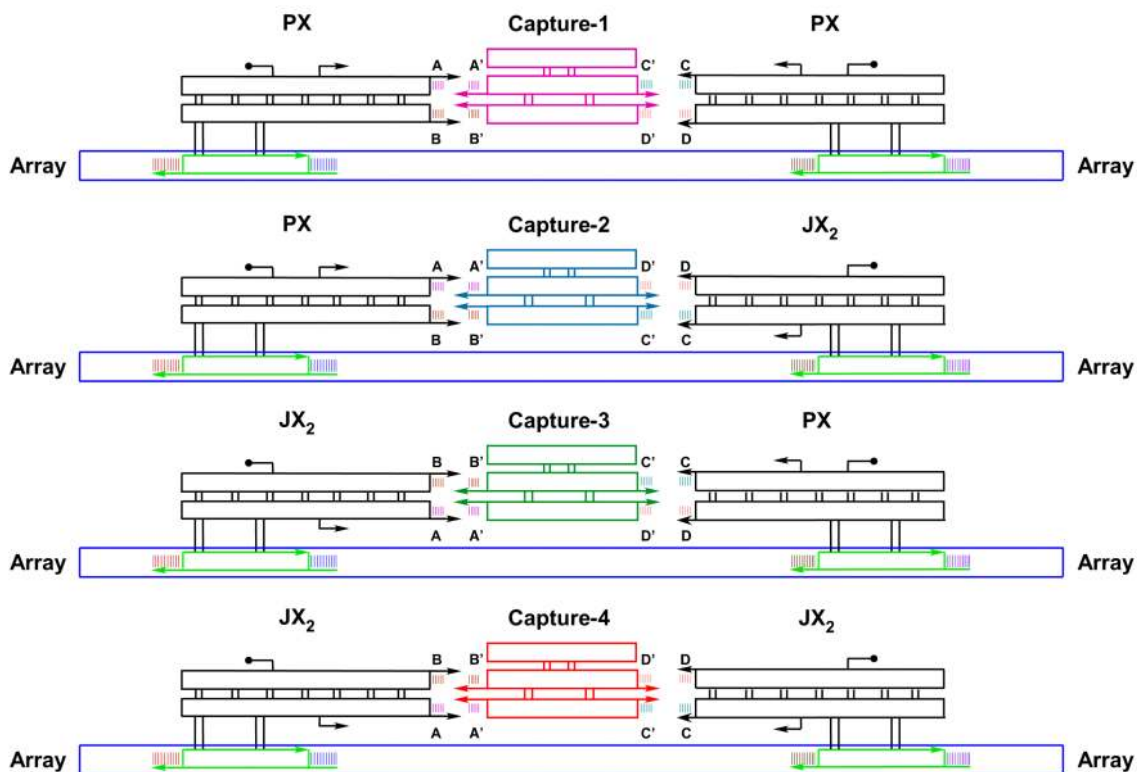


Figure 1.

Schematic Drawings of the Four Different Capture Molecules. In each of the four cases, two PX-JX₂ cassettes that face each other are shown anchored in a blue origami array beneath them by two green domains. The sticky ends are indicated as **A** and **B** (left), or **C** and **D** (right). Their relative positions are established by the state (PX or JX₂) of the cassettes. The four different capture molecules are shown to have sticky ends with primed labels that are complementary to the pairs of sticky ends on the cassettes. The pattern is established by the top domain of the capture molecules. This view, along the direction of origami plane, is perpendicular to the views available in the other figures.

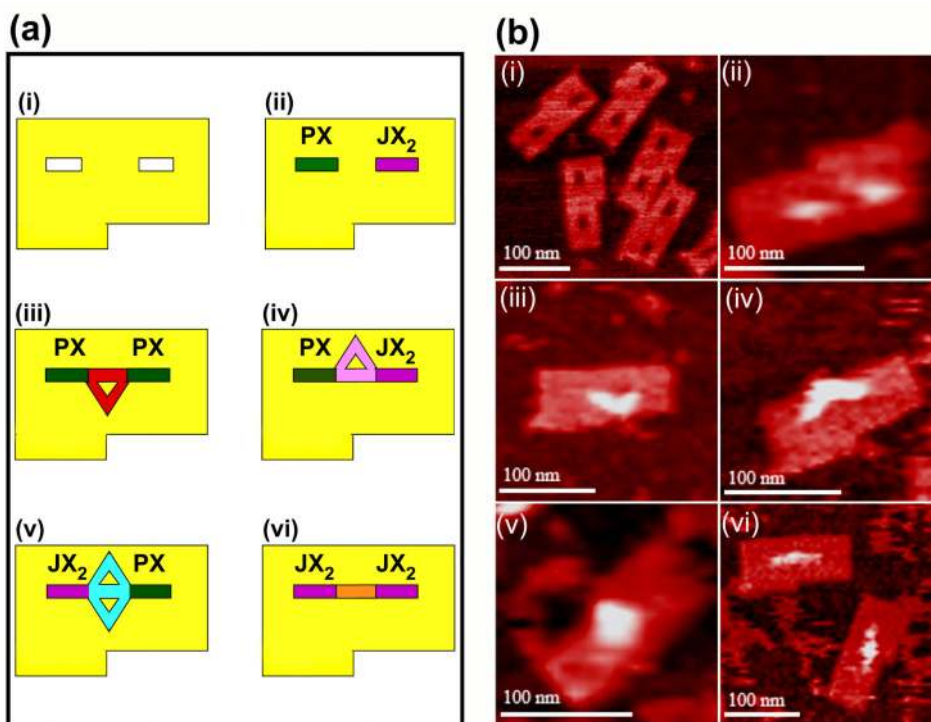


Figure 2. Schematics (a) and Atomic Force Micrographs (b) of the Origami Arrays and Capture Molecules. Panel i of (a) illustrates the origami array containing slots for the cassettes and a notch to enable recognition of orientation; the slots and notches are visible in the AFM in (b). Panels ii show the cassettes in place; the color coding in (a) used throughout the schematics is green for the PX state and violet for the JX₂ state; the presence of the cassettes is evident in the AFM image in (b). Panels iii illustrate the PX-PX state which captures a triangle pointing towards the notch in the schematic (a) and in the AFM image (b). Panels iv illustrate the PX-JX₂ state (a), containing a triangle that points away from the notch, which is evident in the AFM image (b). Panels v illustrate the JX₂-PX state which captures a diamond-shaped molecule (a); its shape is visible in the AFM image (b). Panels vi show the linear molecule captured by the JX₂-JX₂ state, both schematically (a) and in the AFM image (b).

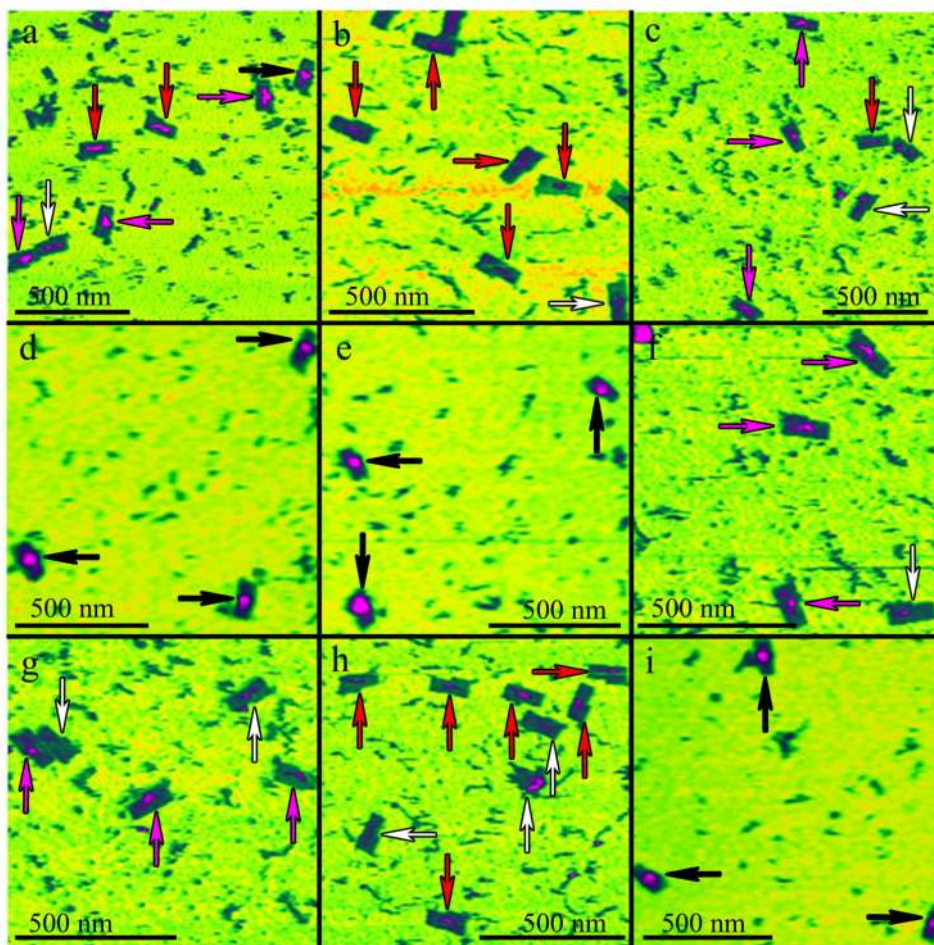


Figure 3. Atomic Force Micrographs of the Correction Procedure for the Diamond-Shaped Capture Molecule. The identity of captured molecules is color-coded by arrows pointing at the origami tiles. The key used here and in the Supporting Online Material is: Diamond -- Black; Line -- Red; Triangle pointing away from the notch -- Blue (none in these images); Triangle pointing towards the notch -- Magenta; Damaged Unit -- White. (a) A mixture of the four capture molecules has been applied to the origami. (b) The linear molecule has been applied, using the binding correction protocol described in the text. (c) The triangle pointing to the notch has been applied to the material in (b) and the correction protocol has been applied. (d) The diamond has been applied to the material in (c) and the correction protocol has been applied. (e) The triangle away from the notch has been applied to the material in (d), and the correction protocol has been applied. Only diamonds are visible in (d) and (e). Panels (f), (g), (h) and (i) show the same procedure, but in a different order: The triangle pointing to the notch, the triangle pointing away from the notch, the linear element and the diamond have been applied, respectively. Again, only diamonds are visible in Panel (i). The other three systems are shown in the Supplementary Data (Figures S8-S10).