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Design and operation of reconfigurable two-dimensional DNA molecular arrays

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Information relay and cascaded transformation are essential in biology and engineering. Imitation of such complex behaviors via synthetic molecular self-assembly at the nanoscale remains challenging. Here we describe the use of structural DNA nanotechnology to realize prescribed, multistep, long-range information relay and cascaded transformation in rationally designed molecular arrays. The engineered arrays provide a controlled platform for studying complex dynamic behaviors of molecular arrays and have a range of potential applications, such as with reconfigurable metamaterials. A reconfigurable array consists of a prescribed number of interconnected dynamic DNA antijunctions. Each antijunction unit consists of four DNA domains of equal length with four dynamic nicking points, which are capable of switching between two stable conformations through an intermediate open conformation. By interconnecting the small DNA antijunctions, one can build custom two-dimensional (2D) molecular 'domino' arrays with arbitrary shapes. More important, the DNA molecular arrays are capable of undergoing programmed, multistep, long-range transformation driven by information relay between neighboring antijunction units. The information relay is initiated by the trigger strands under high temperature or formamide concentration. The array's dynamic behavior can be regulated by external factors such as its shape and size, points of transformation initiation, and/or any engineered information propagation pathways. This protocol provides detailed strategies for designing DNA molecular arrays, as well as procedures for sample production, purification, reconfiguration, and imaging by atomic force microscopy (AFM) and transmission electron microscopy (TEM). The procedure can be completed in 4–7 d.

Introduction

Although the many life forms on Earth differ greatly in size, shape, and lifestyle, they are all made of a few classes of essential biomolecules such as nucleic acids, proteins, and lipids. These basic building blocks form amazingly elegant biological systems, the functions of which are enabled via intimate and detailed interactions among the various biomolecules. Genetic information stored in nucleic acids regulates every aspect of an organism, from self-assembly of a single protein to chemical signal transduction between cells, to the growth and operation of the whole organism. Scientists have long pursued ways to design and control specific molecular interactions as innate biology does. To this end, programmable self-assembly of biomolecules (e.g., nucleic acids, proteins) to imitate complex biological processes has been demonstrated to be one promising route. The rational strategy is to develop biomolecular self-assembly paradigms to generate and regulate complex self-assembled systems.

Owing largely to their unprecedented programmability and self-assembly derived from Watson–Crick base-pairing, nucleic acids have emerged as a versatile material for the construction of static and dynamic 1D[1–9], 2D[4,9–16], and 3D nanostructures[17–28] with precisely controlled physico-chemical properties. Currently, there are two main approaches for the construction of DNA nanostructures: the folding of a long strand ('DNA origami')[29], and the assembly of modular units called DNA tiles[16,25]. Researchers have created diverse synthetic nucleic acid structures such as discrete 2D and 3D objects with defined shapes, extended lattices such as ribbons and tubes, 2D crystals, and macroscopic crystals[30–33]. In addition, dynamic devices have been constructed, such as

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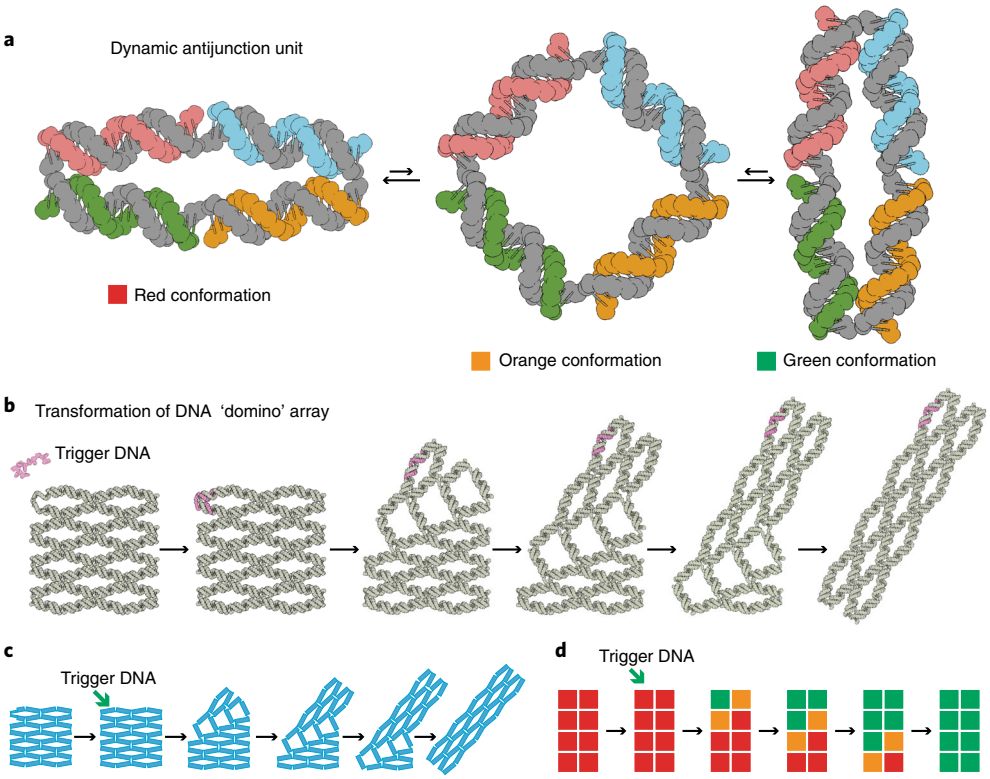

Fig. 1 | Dynamic DNA antijunction and reconfiguration of DNA arrays driven by trigger strands and information relay through antijunction units. a, A dynamic DNA antijunction can switch between two stable conformations (red and green), through an unstable open conformation (orange). **b**, A molecular model showing the transformation of an array consisting of 4 units by 2 units. **c**,**d**, Two simplified diagrams corresponding to the model in **b**, highlighting the molecular structure (**c**) and the switch from red to green conformation (**d**). Adapted from ref. [49].

tweezers[34–37], switches[38,39], walkers[40–46], circuits, and nanomachines[47,48]. However, these dynamic devices have many limitations in scale and complexity. The current dynamic systems often use relatively simple units or contain mainly static segments joined by a few small dynamic units. This lack of sophisticated dynamic behavior limits the advancement of the field. Therefore, we believe that an important challenge for structural DNA technology is to explore new paradigms that will make it possible to study complex dynamic behaviors and transformations.

To address this challenge, we developed a new strategy to build large, scalable DNA structures that can undergo step-by-step, reversible transformations via designated pathways[49]. This new family of dynamic DNA structures is built with switchable DNA antijunction units (Fig. 1)[50]. After trigger-strand-mediated transformation initiation, information transfer propagates through the structure without any need for dehybridization and rehybridization. The transformation is driven solely by the structure's thermodynamic tendency to maximize base-stacking to reduce the overall energy. In principle, DNA structures of arbitrary sizes and shapes can be assembled through the use of interconnected antijunction units to realize structural transformation controlled by information-transfer pathways. To clearly demonstrate the transformation pathway, we describe the principle here with simple rectangular structures. Analogous to a 'domino array', the transformation of interconnected arrays is stimulated by the addition of trigger strands and dictated by a designated trigger position and pathway geometry. Sophisticated on-command on/off pathway control has also been achieved in these molecular DNA domino arrays. A thorough understanding of how design parameters and external conditions affect the structures will support the long-term goals of constructing more complex dynamic DNA structures and explaining other, related chemical reactions and biological systems.

Overview of the protocol

The workflow for the design process and experimental characterization of the DNA domino array is illustrated in Fig. 2. First, the antijunction unit's basic design features, such as domain length and

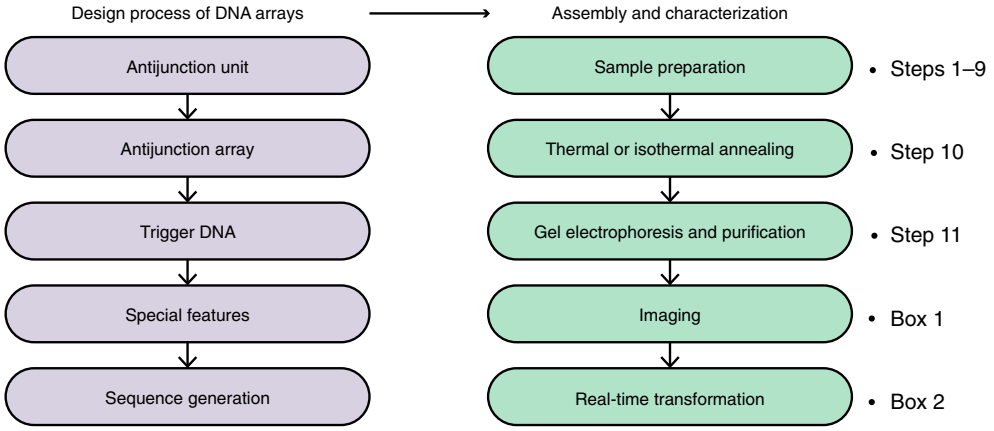

Fig. 2 | The workflow for the DNA relay array design, preparation, and characterization.

nicking-point positions, must be determined (see the Experimental design section for details). The array is then designed to be composed of designated numbers of interconnected antijunction units. The boundaries of the array are left open so that trigger or locking DNA strands can be added. Special design features such as arbitrary shapes and 3D structures may be introduced as well. Once the overall design features have been determined, the sequences of DNA strands can be automatically generated with the caDNAno program[51–53] if a scaffold DNA is involved. For DNA brick designs, a Python script (Supplementary Data 1) run in conjunction with the caDNAno design file will generate the sequences of DNA brick strands. DNA strands of known sequences may be purchased or synthesized in a laboratory. Preparation of the DNA arrays consists of mixing of DNA strands at designated concentrations in aqueous buffer followed by a thermal or isothermal annealing process (Step 10). The assembled DNA arrays are subjected to gel electrophoresis characterization for analysis and purification (Step 11B) and then are directly visualized by AFM (Box 1) or TEM (Box 2). After the addition of triggering strands, real-time transformation can be observed via AFM (Box 3, options C and D). Several regulation strategies are introduced, including blocking or unblocking of information relay by the 'lock' strand or by removal and re-addition of an antijunction unit, reversible conformation transformation by modified trigger strands with toehold extensions and the corresponding releasing strands, and the use of a 3D tube nanostructure.

Comparison with other approaches

There have been a considerable number of studies on dynamic DNA nanostructures over the past two decades. Early studies focused on relatively simple designs composed of a few DNA strands, such as DNA tweezers, or switches, whose dimensions are typically limited to a few nanometers. The development of DNA origami technology allowed hundreds of DNA strands to be assembled into one unit and expanded the dynamic range to ~100 nm. This includes the DNA-origami-based nanorobots[54] and nanomachines[47]. However, these systems either use simple DNA strands moving on the DNA origami static platform or use locally reconfigurable dynamic parts to bridge and regulate large static DNA modules. Recently, Kwon and colleagues demonstrated the reconfigurable DNA accordion rack[55], which changes its structural conformation by making use of external short DNA strands, but this conformation change does not work for cascaded transformation.

Compared with the aforementioned dynamic DNA nanodevices, the DNA molecular array developed here drastically expands the dynamic range of reconfiguration in terms of scale and complexity. It describes a long-range and cascaded information relay in artificial molecular arrays based on a large number of interconnected reconfigurable and modular DNA antijunction units working together according to programmable pathways. The dynamic behavior of a local antijunction unit can change its neighbor's conformation, and this influence will be propagated and eventually lead to global structural change.

Limitations

There are three main limitations to this technique. First, the DNA brick molecular arrays cannot be transformed after folding by either elevated temperature or increased formamide concentration. We tried using both high temperature (>55 °C) and a higher concentration of formamide (>40%)

Box 1 | DNA structure characterization by AFM ● Timing 1–4 h

AFM is a high-resolution imaging modality capable of visualizing molecular structure. Commercial AFM provides resolution down to a few nanometers, which is much greater sensitivity than that offered by diffraction-limited optical tools such as confocal microscopy. AFM measures the force between the cantilever probe and samples to form an image of the 3D shape and thus eliminates the need for external labeling. Therefore, it is widely used in DNA nanotechnology for imaging of DNA origami and DNA bricks. AFM is more useful for 2D DNA nanostructures such as rectangular DNA origami. For 3D DNA nanostructures, TEM is more suitable (see below). AFM has three major capabilities: force measurement, imaging, and manipulation. In this protocol, we focus on the imaging of DNA nanostructures with a Bruker MultiMode VIII microscope. The DNA origami and DNA brick arrays must be adsorbed to a mica surface through electrostatic interactions for AFM imaging to be performed.

Procedure

1 *Sample measurement (Steps 1–15).* Turn on the AFM system, including the computer, MultiMode VIII controller, camera, and Nansoscope software.
2 Fix the mica on the round metal surface with double-sided tape.
3 Cut off the edges of the mica if necessary.
4 Use double-sided tape to remove the top layers of mica until the top surface is satisfactorily smooth and clean.
5 Place 2–3 µl of sample from Step 11 of the main Procedure on the mica. The sample concentration should be ~1–5 nM. Dilute the sample if the concentration is too high.
6 Add 50–70 µl of TE buffer with 12 mM $MgCl_2$.
7 Wash the sample with TE buffer containing 12 mM $MgCl_2$ two to three times by pipetting up and down to remove any unbound sample or impurities.
8 (Optional) Add 2 µl of 100 mM $NiCl_2$ solution to the mica surface to increase the binding of DNA nanostructures to the mica surface.
! CAUTION $NiCl_2$ is toxic and may cause respiratory sensitization. Wear gloves.
9 Assemble a ScanAsyst-Fluid+ AFM tip on the liquid cell.
10 Transfer the sample disk to the AFM scanner and secure the liquid cell on top of the sample disk.
▲ CRITICAL STEP Set the scanner stage at a low position to prevent contact between the AFM tip and the mica surface.
11 Move the AFM tip to the sample surface.
! CAUTION Be careful when moving the tip close to the mica surface, as it is not a visible feature on the mica surface. Find the metal disk first and move the focus up a little, and then move the AFM tip close to the focus.
12 Maximize the SUM signal and adjust the VERT and HORZ values to zero.
13 Open the control software for ScanAsyst in Fluid. Set the initial scan size at 10 nm and the line to 512.
14 Engage the AFM tip.
15 Adjust the parameters to obtain a good image.
? TROUBLESHOOTING
16 *AFM data analysis (Steps 16–20).* Open the NanoScope Analysis software and load the raw data.
17 Execute a flatten function to the image for tilt and bow.
18 One can zoom in to the areas of interest for detailed analysis. Use the Section function to measure parameters such as height and distance.
19 Adjust the color table for optimal contrast.
20 Export the images to JPEG or PNG format for presentation.

Box 2 | DNA structure characterization by TEM ● Timing 30 min–2 h

For 3D nanostructures, TEM is a more suitable imaging approach. We perform TEM imaging with a Hitachi 7700 microscope, but alternatives such as cryo-electron microscopes can also be used.

Procedure

1 Deposit 5 µl of DNA sample solution from Step 11 of the main Procedure onto a carbon-coated copper grid; incubate for 2 min, and then remove the solution from the grid by absorbing it with a piece of filter paper at the edge of the grid.
▲ CRITICAL STEP Do not fully dry the grid; leave a thin layer of solution.
2 Add 6 µl of the staining solution (1% (wt/vol) uranyl formate) to the grid and incubate for 15 s, then remove the solution using a piece of filter paper. Dry the grid for 30 min.
3 Examine the grids immediately or store them in an EM grid case until examination. Image the grids using an electron microscopy operation at 80 kV. Scan at low magnification (10,000–12,000×) to get an idea of the overall sample composition and then examine the finer details of the sample structures at higher magnification (30,000×).
■ PAUSE POINT The EM grids can be stored in an EM grid case at room temperature (15–30 °C) for up to 1 d.

(vol/vol)), but the structural integrity of the DNA bricks was compromised under such conditions. Because DNA bricks do not have a long scaffold strand, they are less stable than DNA origami arrays. This may be one reason that DNA brick arrays cannot transform. We are working to address this

Box 3 | Regulation of DNA molecular array transformation

The regulation of DNA molecular array transformation after folding is performed with the 11 × 4 52-bp DNA origami array because the DNA brick arrays cannot transform after folding and the 32-bp DNA origami array has a higher transformation-energy barrier than the 52-bp design. One should first screen the transformation conditions with different temperatures (option A) and formamide concentrations (option B), and then perform real-time imaging with the optimized conditions (options C and D).

Procedure

(A) **Transformation under high temperature ● Timing 1–24 h**

(i) Add excess trigger strands (~10–20 nM) into the purified DNA samples from Step 11 of the main Procedure (~5 nM).

(ii) Incubate the mixed samples at a constant temperature (from room temperature to 60 °C) for 5 min–12 h.

(iii) Carry out agarose gel electrophoresis (see Step 11B of the main Procedure) or AFM imaging of the samples (Box 1).

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(B) **Transformation in formamide ● Timing 2–4 h**

(i) Add formamide at a concentration of 10–40% (vol/vol) to the purified DNA samples from Step 11 of the main Procedure (~5 nM) together with the trigger DNA (~10–20 nM).

(ii) Incubate the samples at room temperature for 30–60 min.

(iii) Carry out agarose gel electrophoresis (see Step 11B of the main Procedure) or AFM imaging of the samples (Box 1).

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(C) **Real-time imaging of DNA relay array transformation in solution ● Timing 1–4 h**

(i) Mix the purified DNA samples from Step 11 of the main Procedure (~5 nM) with excess trigger strands (generally ~10–20 nM) for 1 min.

(ii) Deposit the samples onto the mica surface.

(iii) Deposit 80 µl of 1× TE 10 mM $MgCl_2$ buffer with the optimized concentration of formamide (from Box 3 option B) on the mica.

(iv) Incubate for 5 min at room temperature.

Measure the sample with AFM (Box 1). Approach the mica surface at a relatively low force. Scan the samples continuously until no further transformation of DNA arrays is observed in the scan area.

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(D) **Real-time imaging using temperature-controlled AFM ● Timing 1–4 h**

▲ **CRITICAL** Measure the sample on the Bruker Icon AFM instrument, which has a temperature controller.

(i) Set the temperature at 60 °C (or the optimized temperature from Box 3 option A) via a resistive heating stage (temperature range, ambient temperature to 250 °C; resolution, 0.1 °C). A cooling water fluid circuit refrigerates the piezo-scanner.

(ii) Scan the DNA array samples until no further transformation of DNA arrays is observed in the scan area.

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limitation. Second, the transformation of relay arrays needs a certain amount of activation energy. In our current study, we used mainly higher temperature or formamide to fulfill this need. However, formamide has been shown to be hemotoxic in animals and is considered hazardous when inhaled or ingested orally. Such reagent and high-temperature conditions limit the use of this approach for many applications, such as in cells. Further studies exploring other reagents or methods for transformation are still worth trying. Third, the 32-bp DNA origami array needs to be incubated at at least 65 °C for noticeable transformations to be achieved, whereas the 52-bp DNA origami array underwent complete transformation at only 55 °C. The different temperatures required for DNA origami arrays with different DNA origami units imply that the 32-bp DNA origami array has a higher transformation energy barrier than the 52-bp DNA origami array.

Future challenges

Using relatively simple antijunction units, we have studied strategies and mechanisms of DNA molecular arrays for (i) controlling the conformational shift of a one-pot assembly, (ii) initiating transformation at designated locations, (iii) controlling information-transfer pathways by using on/off switches, (iv) engineering of information-transfer pathways by means of shape design, and (v) cooperative transformation in tube designs. In addition to the two switch designs (see "Blocking and resuming transformation" in the Experimental design section), it is also feasible to use gold nanoparticles (conjugated with DNA) or proteins (bound directly to DNA or modified DNA) as switches, and to use locking/unlocking strands. Also, the sequence dependence of the conformational shift of a one-pot assembly of DNA bricks implies that it could be possible to lower the energy barrier

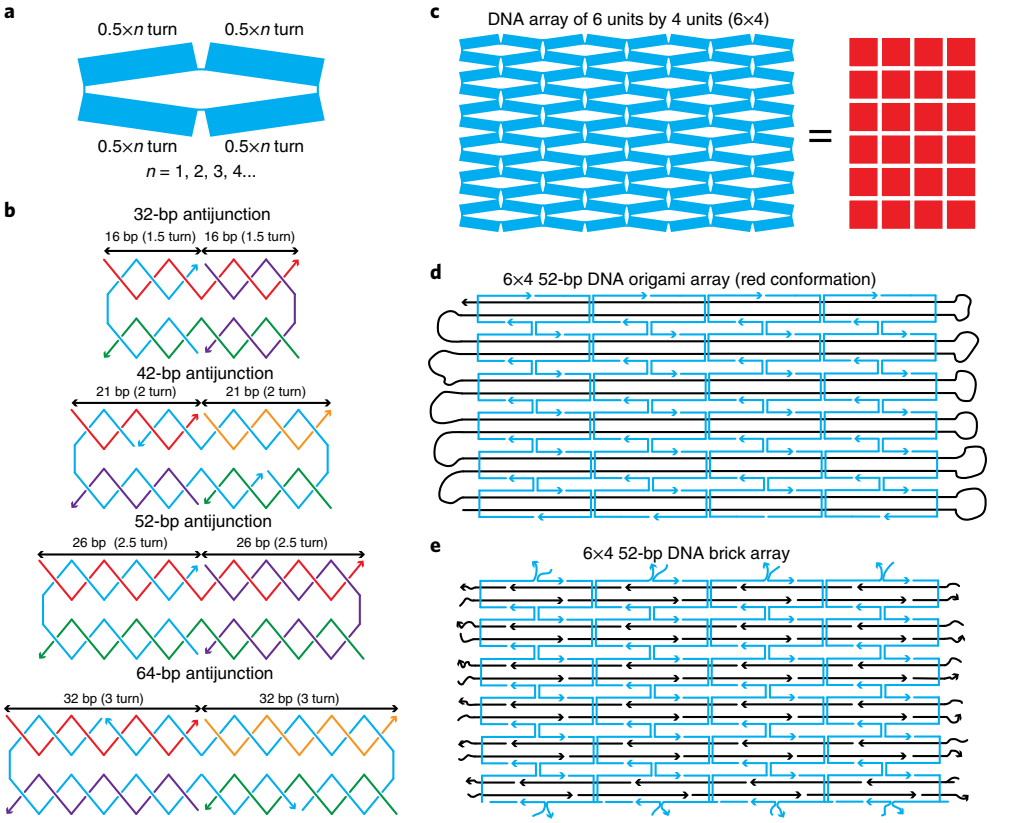

Fig. 3 | Design of DNA antijunctions and DNA arrays. a, A DNA antijunction unit. **b**, Antijunction designs with various arm lengths. **c**, Two diagrams of a 6 × 4 DNA array. **d**, A 6 × 4 52-bp DNA origami array. **e**, A 6 × 4 52-bp DNA brick array.

enough to make conformational transformation of DNA brick arrays possible after assembly. In addition to small 2D domino arrays, the scalability of this new family of dynamic DNA domino arrays is also necessary to test for micrometer-length information transformation. Furthermore, for 3D structures, it should also be possible to construct intricate structures that contain nanoscale features such as cavities and tunnels. Because of the modular nature of DNA origami and DNA bricks, it is fairly straightforward to make a large number of complex 2D and 3D shapes. For instance, in previous work, we successfully produced canonical nonreconfigurable 3D DNA brick structures of up to 12,000 bp[25]. Assuming that reconfigurable structures with similar size could be made, the largest structure could contain close to 142 42-bp antijunction units. Such large, intricate structures would enable the demonstration of complicated transformations. Given that information transfer at the molecular level is fundamental in many chemical reactions and biological process (e.g., transmembrane signaling), we believe that our study on this novel artificial DNA dynamic system will not only have an impact on the field of structural DNA technology and its applications, but also enable a better understanding of information transfer in other chemical and biological systems.

Experimental design

Antijunction unit

A structural unit called an antijunction serves as the basic building block for the construction of large, scalable, and reconfigurable DNA molecular array structures. Named according to the distance (in base pairs) between two opposite dynamic nicking points (e.g., 32-bp antijunction), the antijunction unit consists of four arms of equal-length duplexes and four dynamic nicking points. This antijunction unit is capable of switching between two stable conformations, referred to as 'red' and 'green', through an intermediate 'orange' conformation (Figs. 1a and 3a). The length of each arm is equal to 0.5 × n helical turns, where n is a natural number (Fig. 3b). For DNA origami arrays, the arm of the antijunction unit must be an odd number of turns in length, whereas DNA brick arrays have no such constraint. Using this protocol, we have designed antijunctions of 32, 42, 52, and 64 bp.

DNA molecular array

DNA molecular arrays are formed through the connection of a number of antijunction units into arrays (Fig. 3c). Each DNA array is named according to the number of units ($x \times y$, where x is the number of rows of antijunction units and y is the number of columns of antijunction units) and the antijunction design, for example, a 6×4 52-bp DNA brick array. For the DNA brick array, one of the array conformations is arbitrarily referred to as the red array conformation, and the other is assigned as the green array conformation. For the DNA origami array, the conformation where the scaffold does not cross between DNA helices within the array is assigned as the red array conformation (Fig. 3d), and the other conformation is assigned as the green conformation. Compared with DNA brick arrays, DNA origami arrays are more resilient to denaturing conditions because of the long scaffold strand, but the array size is limited to about ~5 MDa. In most cases, we observed that the red conformation was favored for DNA origami relay arrays in the absence of trigger strands, which suggests that the lack of cross-scaffold routing in the red conformation reduces the energy of the arrays compared with that of the green conformation, which does include cross-scaffold routing.

As illustrated in Fig. 3e, DNA brick arrays do not have scaffold DNA strands. Instead, they consist of interconnected antijunction units that are fully assembled from single-strand tiles[16]. Unlike origami relay arrays, in one-pot isothermal assembly, DNA brick relay arrays typically result in two dominant conformations: red and green array conformations, which correspond to the two lowest-energy states.

Both DNA origami and DNA brick arrays have high tendencies to aggregate, as observed in agarose gel electrophoresis and AFM imaging. The aggregation may be attributed to the nonspecific interactions between exposed single-stranded regions of the DNA nanostructure on the boundary of the desired structures. The addition of poly(T) overhangs to the boundary strands can mitigate this aggregation substantially.

Initiation of transformation

DNA array relay transformation is initiated by the addition of trigger strands under conditions of elevated temperature or increased formamide concentration. The addition of trigger strands at selected locations of the array initiates structural transformation from the selected sites that is propagated through the rest of the array in a stepwise manner. In our present design, the trigger strands are positioned on the edge units of the arrays. Nevertheless, trigger strands may function even if located within the arrays. The principle behind the triggering event is that the trigger strand removes a mobile nicking point from the antijunction unit to initiate conformation transformation. After the antijunction unit transforms, the connecting location between the two units has one stable base-stacking interaction. The neighboring antijunction unit subsequently converts, thus increasing the number of base-stacking interactions to two. The presence of red or green trigger strands will shift the one-pot assembly of reconfigurable structures to the red or green confirmation, respectively. Noticeably, as more trigger strands are added to one-pot assembly, the assembly shifts toward the corresponding array conformation. In comparison, the trigger strands for an 11×4 52-bp DNA origami array (see Supplementary Fig. 1, Supplementary Table 1, and Supplementary Data 1 and 2 for design details) can reversibly transform the array from one conformation to another.

Molecular canvas

A DNA brick array may serve as a molecular canvas to enable the construction of DNA arrays with arbitrary numbers of units. In this protocol, a 20×8 42-bp DNA brick array (see Supplementary Fig. 2, Supplementary Table 2, and Supplementary Data 1 and 2 for design details) with 13,440 bp is used as the molecular canvas. We studied a total of 16 arrays with varied dimensions to investigate how their geometrical features affected the array conformations. In addition, the connectivity between antijunction units can also be controlled by removal of some antijunction units from the 11×4 42-bp DNA brick array. We found that when antijunction units were removed from the array, the structure shifted to the green array conformation.

Arbitrary molecular arrays

In addition to rectangular arrays, arrays of arbitrary shape can also be designed with DNA origami. For example, a rectangular DNA origami array can be converted into tubes or hierarchically assembled into 1D ribbons via placement of linker strands at selected positions. Such linkage would convert the DNA origami to a red-conformation nanotube. The nanotube could then be converted to a green-conformation tube via the addition of green trigger strands at 50 °C. Interestingly, 1D chains

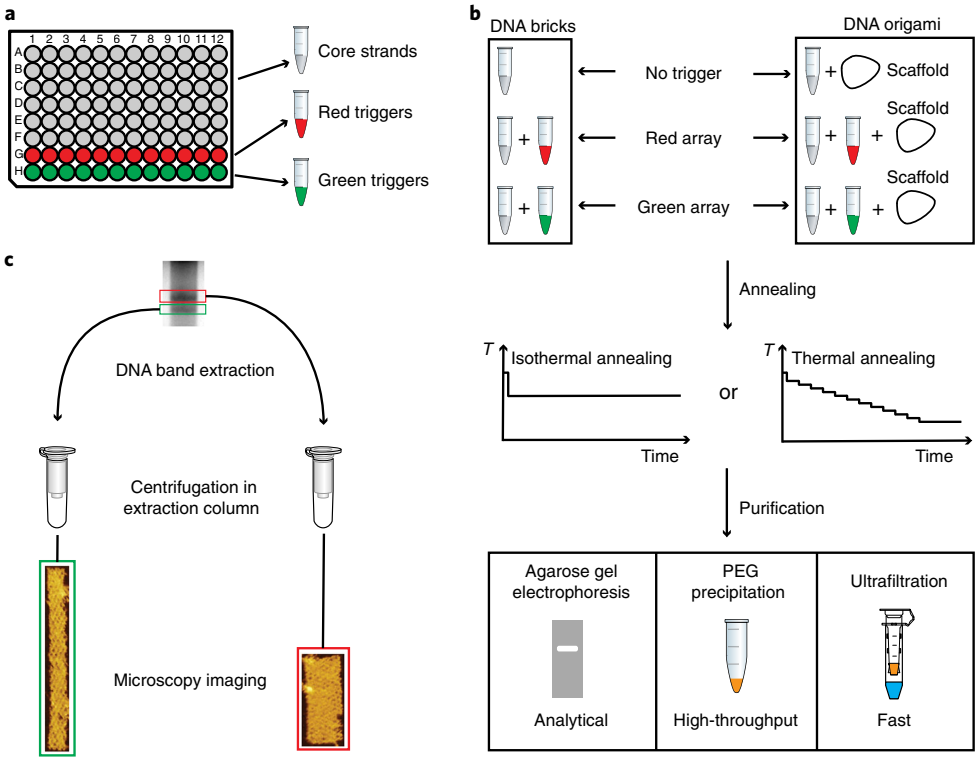

Fig. 4 | Overview of sample preparation for DNA brick or origami molecular arrays. a, 96-well plates were ordered according to the DNA sequences generated by caDNAno or a Python script (Supplementary Data 1 and 2). In the 96-well plates, core staples, green trigger strands, and red trigger strands were pipetted into different tubes to make master mixes. **b**, No-trigger structures and red- and green-conformation structures can be prepared from different master mixes. Both isothermal annealing and thermal annealing can be used to fold the DNA relay arrays. After that, folded structures are separated from free DNA by agarose gel, PEG precipitation, or ultrafiltration purification. **c**, Typically, gel purification is used in this protocol to check the sample quality and separate pure folded structures (green and red array conformations) for AFM imaging. AFM images in **c** adapted from ref. [49].

of arrays can be generated by one-pot assembly of the DNA origami array with the connectors and green triggers.

Blocking and resuming transformation

One can turn the conformation transformation off or on at selected locations by blocking or resuming the information relay. Two approaches may be used for this purpose. In the first approach, one antijunction unit (e.g., strand 11[119] in Supplementary Table 1) is removed from the array during assembly. The information relay will arrest at this location because a local energy minimum will be created. Subsequent addition of the missing unit back into the relay array reinitiates the transformation. In the second approach, a 'lock' strand (6[55]_lock in Supplementary Table 1) is used to turn off the array transformation at certain locations. The lock strand binds to single-stranded DNA extensions from two neighboring units, thereby barring the units from structural transformation.

Assembly of DNA arrays

Isothermal assembly. The folding of DNA origami and DNA bricks is highly cooperative (Fig. 4b). The folding rates of DNA origami and DNA bricks are elevated at constant temperature T_{fold}, whereas deviation from this optimal T_{fold} slows or entirely inhibits structure formation[56]. Therefore, both DNA origami and DNA bricks can be assembled at constant temperature (Step 10A(i)). However, initial denaturation of DNA strands with a heat shock (e.g., 5 min at 90 °C) is still necessary to resolve secondary structures in the template scaffold for DNA origami. Different DNA nanostructures may have different optimal T_{fold}, so it is necessary to determine the optimized isothermal condition for any new DNA nanostructure. For determination of the T_{fold}, DNA origami or DNA bricks can be folded at different constant temperatures (e.g., fold samples at each 1 °C increment from 45 °C to 65 °C with a heat shock at 95 °C for 5 min), and then the samples can be characterized by agarose gel electrophoresis. Successful folding of samples will lead to clear bands. The gel bands can

be further purified and the structure can be characterized by AFM or TEM (Boxes 1 and 2). For a 42-bp DNA brick array, the optimized isothermal condition is 53 °C with 1× TE buffer and 10 mM $MgCl_2$ for 18 h.

Thermal-annealing assembly. For the formation of DNA origami or DNA brick structures with high yield, the DNA strand components are typically annealed in an assembly-favoring buffer by heating of the mixture to a high temperature followed by incremental cooling over the course of several hours to several days (Fig. 4b, Step 10B(i)). For 52-bp and 64-bp DNA brick arrays, an annealing process (95 °C for 5 min, from 85 °C to 24 °C at a rate of 20 min/°C) is used with 50 mM Tris, 1 mM EDTA, and 10 mM $MgCl_2$. For DNA origami relay arrays, a mixture of staple strands (final concentration: 100 nM for each strand) and the scaffold (final concentration: 10 nM) is mixed in 1× TE buffer supplemented with 10 mM $MgCl_2$. The samples are then annealed for 10 h using the following thermal-annealing protocol: 95 °C for 5 min, then 85 °C to 24 °C at a rate of 10 min/°C.

Purification and quality control. After folding of the DNA nanostructures, excess staple DNA should be removed before AFM or TEM imaging. We include three techniques for DNA origami or DNA brick purification and quality control in this protocol (Fig. 4b). PEG precipitation (Step 11A) uses the depleting agent PEG to precipitate the DNA samples and separate the folded objects from staple strands[57,58]. PEG precipitation can concentrate the samples while adjusting the buffer and is suitable for high-throughput sample purification. Agarose gel electrophoresis (Step 11B) is a common method for DNA nanostructure quality control and sample purification. The agarose gel separates DNA samples on the basis of their electrophoretic mobility (Fig. 4c)[22]. The quality of the gel bands provides a first impression of folding quality and shows whether dimerization of DNA nanostructures occurred. The target DNA molecular arrays can be efficiently separated from the free staple DNA by excision of a gel slice containing the species followed by gel-extraction centrifugal filtering. Increasing the agarose gel concentration to ~2.5% (wt/vol) enables the separation of DNA molecular arrays that adopt different conformations. Separation of DNA nanostructures with molecular-weight cutoff (MWCO) membranes (Step 11C) offers residual-free separation but works only for smaller volumes compared with those used for PEG precipitation, because of limits in the size of filtration membranes. DNA molecular arrays are purified by filtering with a 100-kDa MWCO. Generally, ultrafiltration with MWCO filters is an attractive method to separate DNA origami from free DNA strands quickly (≈30 min) and efficiently. Typically, the purification process can be accomplished with three to six centrifugations at 3,000*g* for 10 min. The DNA origami or DNA brick samples are collected after the filter is flipped into a new tube and the sample is subjected to further centrifugation.

Regulation of DNA array transformation. Because the two dominant conformations of DNA relay arrays correspond to minimum-energy states, transformation of the DNA relay array from one conformation to another needs to overcome the energy barrier between the two states. Both elevated temperature and the addition of formamide can assist in this process. We identified different DNA molecular arrays using the two methods. Unfortunately, DNA brick arrays cannot be transformed with either elevated temperature or formamide. However, the 11 × 4 52-bp DNA origami relay array with a p7560 m13 scaffold can be successfully transformed with trigger strands at 55 °C or 10–40% (vol/vol) formamide. The kinetics of the transformation can be accelerated by either increased temperatures or higher concentrations of formamide (Box 3, options A and B), probably because of the reduced energy needed to break the base-stacking interactions under these denaturing conditions. With a temperature-controlled AFM instrument (Bruker), one can observe the transformation of a DNA relay array in real time (Fig. 5a). Alternatively, the denaturing agent formamide can be used to decrease DNA melting temperatures linearly by ~0.6 °C per each 1% formamide added to the buffer[59–61]. The transformation with formamide can be achieved at room temperature, and thus it is more convenient for real-time imaging of DNA array transformation (Fig. 5a). In addition, the transformation can also be accelerated by AFM-tip-enhanced scanning, as the contact force from the AFM tip provides mechanical energy that can aid in transformation.

With real-time AFM (Box 3, options C and D), one can track the information-relay propagation pathway. The trigger strands can be added selectively at the corner or middle of the 11 × 4 52-bp DNA origami relay array, as illustrated in Fig. 5b. Besides the position of the trigger strands, the number of trigger strands can also influence the kinetics of the transformation. The transformation will not start with only three or fewer trigger strands.

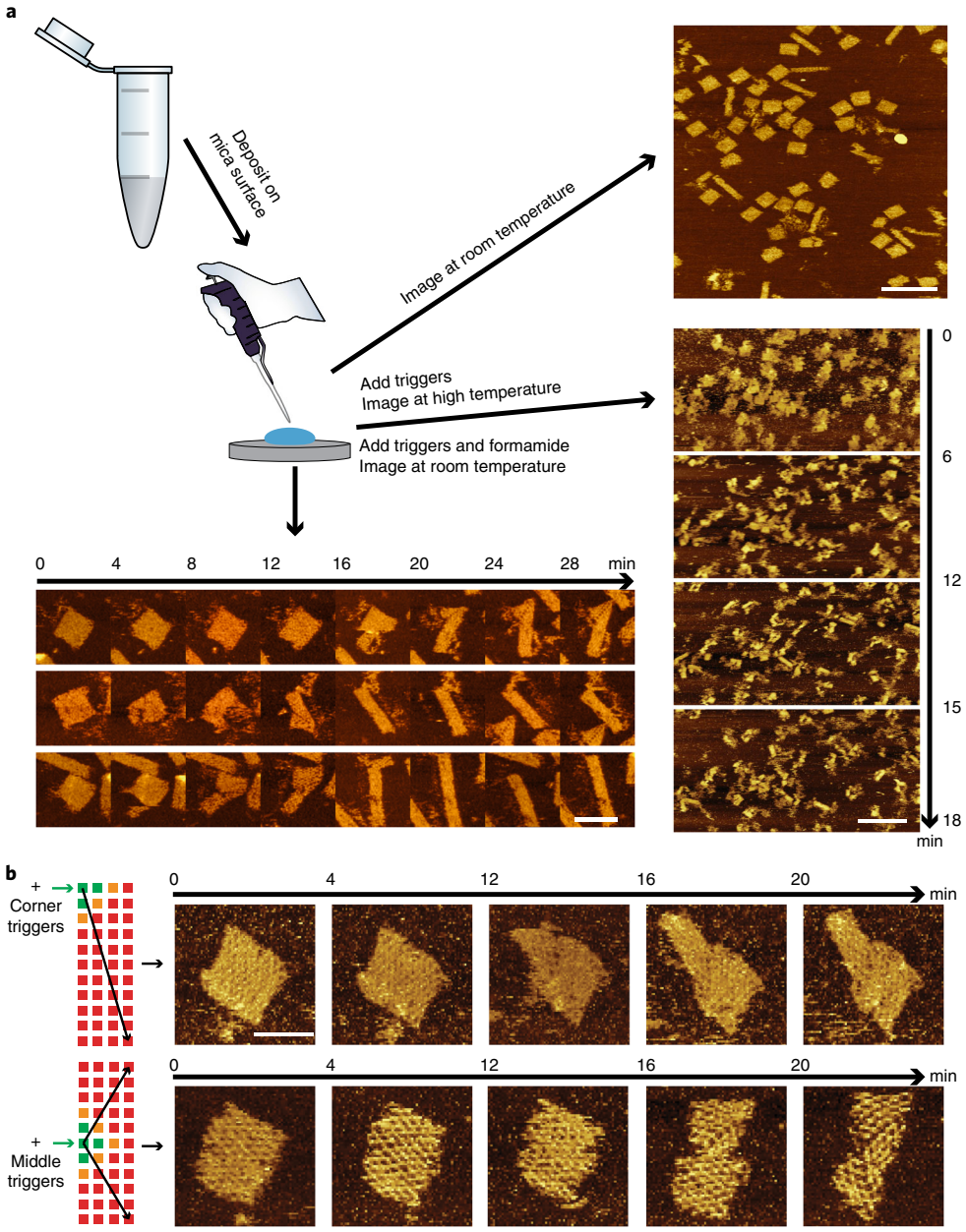

Fig. 5 | AFM imaging process for in situ transformation. a, AFM imaging can be performed in three modes. First, after addition of the sample and buffer to the mica surface, AFM imaging is performed at room temperature for normal imaging (a 15 × 4 42-bp DNA brick array is shown in the upper right; scale bar, 200 nm). Second, the trigger strands are added to the DNA arrays, and sample is then placed on the mica surface for real-time AFM imaging with a temperature-controlled instrument (an 11 × 4 32-bp DNA origami relay array is shown in the lower right; scale bar, 200 nm). Third, green and red triggers are mixed with the DNA array and formamide, after which one can perform real-time AFM imaging at room temperature (an 11 × 4 52-bp DNA origami relay array is shown in the lower left; scale bar, 100 nm). **b**, AFM imaging of the in situ transformation of the 11 × 4 52-bp DNA origami relay array at room temperature with formamide. Green trigger strands were selectively added to the corner (upper row) or middle positions (lower row) of the DNA molecular array. We recorded the transformation by measuring the same local area at different times. Scale bar, 50 nm. AFM data adapted from ref. [49].

The process of transformation is reversible and tunable. Using modified trigger strands with toehold extensions and release strands, one can reversibly switch between the red array conformation and the green array conformation (Fig. 6b). In addition, we used two methods to turn the transformation on or off at desired locations (Fig. 6a). The first method is to remove one unit (e.g., strand 14[119] in Supplementary Table 1) from the relay array to stop the transformation propagation. Interestingly, addition of the missing unit will cause the transformation to resume. In the second method, a lock

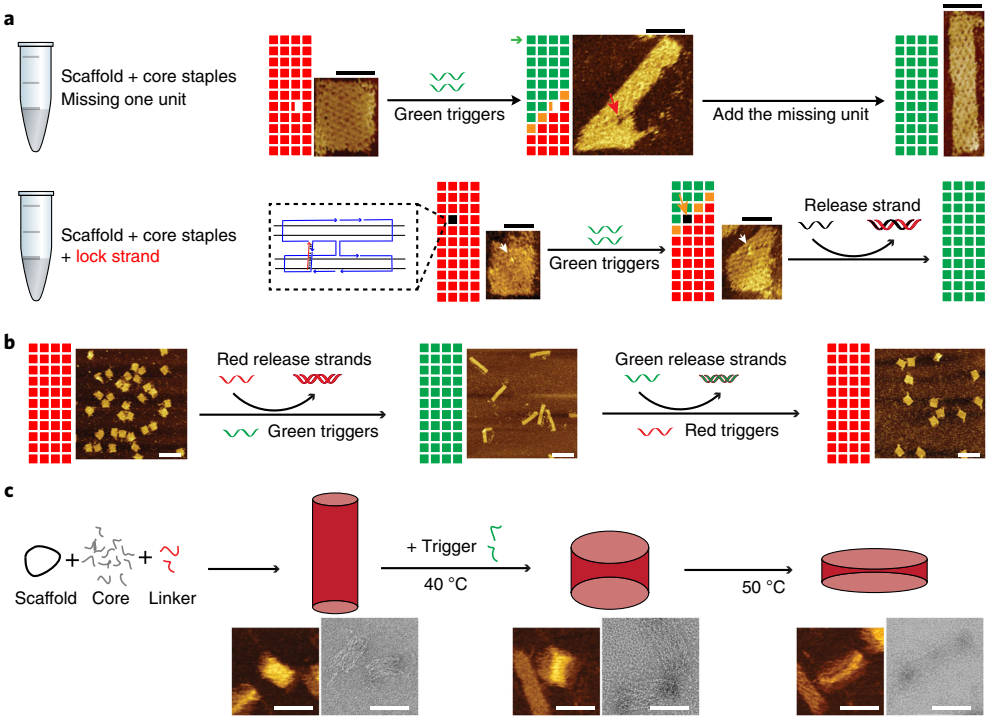

Fig. 6 | Regulation strategies for DNA origami relay arrays. a, The transformation pathways can be blocked or resumed by removal or addition of units (top), respectively, or by lock strands (bottom). Scale bars, 50 nm. **b**, Reversible transformation between the red array conformation and the green array conformation by addition of the corresponding release strands and trigger strands with toeholds. AFM images of the 11 × 4 52-bp DNA origami array transformation are shown. Scale bars, 200 nm. **c**, Transformation of the 11 × 4 52-bp DNA origami array. The 11 × 4 52-bp DNA origami domino array assembled with linker-produced tubes, which convert to a green array conformation tube in a two-step process: the tube first is converted to a stable state in which most units appear partially open at 40 °C, and then fully converts to a green array conformation tube at 50 °C. Scale bars, 50 nm for AFM images or 200 nm for TEM images. AFM data adapted from ref. [49].

strand (e.g., strand 6[55]_lock in Supplementary Table 1) binds to single-stranded DNA extensions from two neighboring units, thus preventing the propagation. The relay array can be induced to assemble in a red-conformation tube by linkage of the top and bottom edges of the DNA origami relay array. The nanotube can be further converted into the green-conformation tube by the addition of green trigger strands (Fig. 6c).

Materials

Reagents

! CAUTION All chemical reagents are potentially harmful. We recommend using protective devices such as lab coats, gloves, and masks during the procedure.

- Trizma base (Sigma-Aldrich, cat. no. T1503)
- Acetic acid (Sigma-Aldrich, cat. no. 695092) **! CAUTION** Wear protective gloves, clothes, and eye/face protection, and keep the material away from heat and open flames. Acetic acid causes severe skin burns and eye damage, and is flammable.
- Boric acid (Sigma-Aldrich, cat. no. B7901)
- Polyethylene glycol 8000 (PEG8000; Sigma-Aldrich, cat. no. P4463-1)
- Magnesium chloride hexahydrate (Sigma-Aldrich, cat. no. M2393) **▲ CRITICAL** We recommend using either magnesium chloride hexahydrate (99.995% (wt/wt)) or magnesium chloride solution of molecular biology grade with a stock concentration of 1.00 ± 0.01 M.
- EDTA (Sigma-Aldrich, cat. no. E9884)
- Sodium chloride (NaCl; Carl Roth, Sigma-Aldrich, cat. no. 3957.1)
- LE agarose/Gold agarose (Biozym, cat. no. 84004/850152)
- GeneRuler, 1 kb plus DNA ladder (Thermo Scientific, cat. no. SM1334)
- Milli-Q ultrapure water (Milli-Q Reference A+, cat. no. FOSA17501E)

- Staple strands (Integrated DNA Technologies, www.idtdna.com). See Supplementary Tables 1 and 2 for the example sequences used in this protocol. Order 2 OD of each staple DNA by PAGE purification.
- Single-stranded M13 bacteriophage-derived scaffold p7560 (Tilibit nanosytems, www.tilibit.com)
- Nickel(II) chloride hexahydrate (Sigma-Aldrich, cat. no. 223387) **! CAUTION** Wear protective gloves. Nickel(II) chloride hexahydrate is irritating upon ingestion, inhalation, and skin contact. Prolonged inhalation is linked to cancer risk to the lungs.
- Formamide (Sigma-Aldrich, cat. no. 11814320001) **! CAUTION** Wear protective gloves. Formamide is moderately irritating to the eyes, skin, and mucous membranes. It has been shown to exhibit hematoxicity in animals and is considered hazardous by prolonged exposure through inhalation or oral intake.
- Gel loading buffer (Sigma-Aldrich, cat. no. G7654)
- Uranyl formate (1 g; Electron Microscopy Sciences, cat. no. 22450) **! CAUTION** Wear protective gloves. Uranyl formate is both radioactive and toxic.
- Ethidium bromide (Sigma-Aldrich, cat. no. E1510) **! CAUTION** Wear protective gloves. Ethidium bromide is toxic as a mutagen. It may potentially cause carcinogenic effects and may cause irritation of mouth, upper respiratory tract, skin, and eyes after exposure.
- 10× loading buffer (Takara, cat. no. AH20611A)

Equipment

- PCR machine (Eppendorf, cat. no. 6331000017)
- Aluminum sealing tape for 96-well plates (Fisher Scientific, cat. no. 11806)
- Centrifuge (Eppendorf, cat. no. 5418R)
- Gel chamber (Bio-Rad, cat. nos. 170 4401-4406 and 170 4481-4486)
- Centrifuge filters (0.5 ml, 100 K; Amicon, cat. no. UFC510096)
- Freeze'n Squeeze spin columns (Bio-Rad, cat. no. 732-6165)
- Parafilm M (Sigma-Aldrich, cat. no. P7793-1EA)
- (Multichannel) pipettes (Eppendorf)
- UV transilluminator (20 × 20 cm, M; VWR GenoView, cat. no. 730-3004)
- Microwave (Severin, cat. no. 7891)
- Erlenmeyer flask (250 ml; Carl Roth, cat. no. NY87.1)
- Razor blade (Carl Roth, cat. no. CK07.1)
- NanoDrop 2000 (Thermo Fisher Scientific, cat. no. S06497)
- Multipette M4 pipette (Eppendorf, cat. no. 4982000314)
- Gel imager (Typhoon FLA 9500; GE, cat. no. 28996943)
- DNA LoBind tube (0.5 ml; Eppendorf, cat. no. 0030 108.035)
- PCR tubes (Trefflab, cat. no. 96.09852.9.01)
- 1.5-ml Eppendorf tubes (Eppendorf, cat. no. 0030 120.086)
- Double-sided adhesive tape (Scotch, cat. no. 665D)
- AFM system (Bruker, Multimode VIII with Nanoscope V controller and Icon)
- Microcantilever SNL-10 (Bruker)
- Mica, 15-mm diameter (Plano)
- Round metal plates, 15-mm diameter (Plano)
- Freezer at −20 °C (Fisher Scientific Isotemp General-Purpose Freezer, cat. no. 13986148)
- Software for DNA nanostructure design and sequence generation (cadnano, http://cadnano.org)

Reagent setup

▲ CRITICAL All buffer solutions should be prepared in deionized water. We suggest that fresh buffers and solutions be prepared regularly.

- *Gel buffer*. The gel buffer is 0.5× TBE containing 45 mM Tris, 45 mM boric acid, 1 mM EDTA, and 12 mM $MgCl_2$. Store the buffer at room temperature for up to 6 months.
- *10× TE-Mg^{2+} buffer*. The 10× TE buffer contains 400 mM Tris, 10 mM EDTA, and 120 mM $MgCl_2$. The buffer can be stored at room temperature for up to 6 months.
- *1× TE-Mg^{2+} buffer*. Dilute 10× TE-Mg^{2+} buffer into a final concentration of 40 mM Tris, 1 mM EDTA, and 12 mM $MgCl_2$. Store the buffer at room temperature for up to 6 months.
- *15% (wt/vol) PEG solution*. The solution contains 15% (wt/vol) PEG8000, 5 mM Tris base, 1 mM EDTA, and 505 mM NaCl. Store the buffer at room temperature for up to 6 months.

• *Staining solution*. The solution contains 1% (wt/vol) uranyl formate. Store the solution at room temperature in the dark for up to 6 months. ▲ **CRITICAL** Centrifuge the solution at 12,000*g* for 5 min at room temperature before use to remove the aggregates and impurities.

Procedure

Folding DNA origami structures ● Timing 6 h–1 d

1 *Staple DNA preparation (Steps 1–4)*. Add dH_2O to each lyophilized oligonucleotide well to make the final concentration ~100 μM in 96-well plates purchased from Integrated DNA Technologies (www.idtdna.com).

2 Seal the plates and vortex at 500*g* for 2 min to dissolve the DNA powder.

3 Spin down the plates at 1,000*g* for 5 min at room temperature.
■ **PAUSE POINT** The dissolved oligonucleotides can be stored at −20 °C for up to 2 years.

4 *Prepare master mix for DNA nanostructures (Steps 4–11)*. Take the 96-well plates containing staple DNA from −20 °C storage and leave at room temperature for 1–2 h to unfreeze the staple DNA.

5 Take a piece of Parafilm. Take 2 μl from each well of the 96-well plates and put the liquid on the Parafilm, using a multichannel pipette. Exclude the wells for trigger strands or modified strands.
▲ **CRITICAL STEP** Each staple DNA should have the same molar concentration.

6 Fuse the droplets on the Parafilm with a 1,000-μl pipette and transfer the solution to a 1.5-ml DNA low-binding tube.

7 Prepare other master mixes of trigger strands or modified strands (Supplementary Tables 1 and 2). The total volume of the master mix should be selected to meet future demands.
■ **PAUSE POINT** Master mixes can be stored at −20 °C for at least 6 months.

8 Seal the plates with aluminum sealing tape for the 96-well plates. Store the plates at −20 °C for up to 5 years.

9 Prepare folding samples in a PCR tube. The sample should have a total final volume of 100 μl and contain the following reagents:

Component	Amount (μl)	Final concentration
Scaffold p7560, 100 nM	10	10 nM
TE buffer, 10×	10	1×
$MgCl_2$, 100 mM	10	10 nM
Master mix, 1 μM	10	100 nM per staple
Red or green trigger DNA, 2 μM each (Supplementary Tables 1 and 2)	5	100 nM
H_2O	55	
Total	100	

▲ **CRITICAL STEP** Magnesium concentration has been observed to have a major effect on the quality of DNA origami and DNA bricks. The optimal $MgCl_2$ concentration may differ depending on the structure of the DNA origami or DNA bricks, and on the specific needs of a given DNA origami structure.
▲ **CRITICAL STEP** Make sure to use the correct concentration of $MgCl_2$ and high-purity magnesium chloride hexahydrate. EDTA is added to 1 mM final concentration in the folding buffer to chelate divalent ion impurities that can compete with magnesium during the folding process.

10 Assemble the DNA origami and DNA brick arrays by either thermal assembly (option A) or isothermal annealing (option B). Thermal annealing can be used for both DNA origami and DNA brick samples. We have tested isothermal annealing only on DNA brick samples, but we anticipate that it could also be used for DNA origami samples.

(A) **Thermal assembly of DNA arrays**

(i) Place the PCR tube into a PCR machine and run the specific temperature program for the DNA nanostructures. Use the following program for 52-bp and 64-bp DNA brick arrays:

Cycle number	Temperature program
1	95 °C, 5 min
2–62	85–25 °C, 20 min per °C
63	4 °C until the next step

Use the following program for DNA origami arrays:

Cycle number	Temperature program
1	95 °C, 5 min
2–62	85–25 °C, 10 min per °C
63	4 °C until the next step

(B) **Isothermal annealing assembly of DNA arrays**

(i) Fold the DNA array samples by incubating them at constant temperatures (e.g., 45–65 °C for every 1 °C with a heat shock at 95 °C for 5 min) to screen the optimal T_{fold}. Correctly folded samples will show clear bands in the agarose gel (Step 11B) and well-defined geometry in the AFM images (Box 1) under the optimal T_{fold}.

▲ **CRITICAL STEP** For 42-bp DNA brick array, the samples can be subjected to a one-step isothermal annealing over 12 h. The optimized isothermal condition is 53 °C with 1× TE buffer and 10 mM $MgCl_2$ for 18 h.

DNA molecular array purification and quality control

11 Purify the assembled DNA structures using agarose gel electrophoresis (option A), PEG precipitation (option B), or ultracentrifugation (option C). See Experimental design for details. Typically, gel electrophoresis is used in this protocol for AFM imaging and quality control. PEG precipitation or ultracentrifugation is also feasible for the purpose of high throughput or fast purification.

(A) **PEG precipitation** ● **Timing 2–3 h**

(i) Adjust the magnesium concentration of the DNA origami or DNA bricks to 20 mM with 1 M $MgCl_2$.

(ii) Add 1× TE and 20 mM $MgCl_2$ buffer to make the total volume 200 µl in a 1.5-ml or 2-ml Eppendorf tube.

(iii) Mix the DNA nanostructure solution with 200 µl of 15% (wt/vol) PEG solution.

(iv) Place the tube in a high-speed centrifuge and centrifuge for 17,000*g* for 30 min at room temperature.

(v) Remove the supernatant with a pipette and add 200 µl of 1× TE and 20 mM $MgCl_2$ to the pellet.

(vi) Insert the tube in a shaker at room temperature for 5 min to dissolve the pellet.

(vii) Repeat Step 11A(ii–v) two to three times.

(viii) Centrifuge at 17,000*g* for 30 min at room temperature and remove the supernatant. Dissolve the pellet with 1× TE and 10 mM $MgCl_2$ to adjust the concentration of DNA nanostructures.

(ix) Measure the concentration of DNA nanostructures with a NanoDrop 2000. The concentration should be ~5–10 nM.

■ **PAUSE POINT** The DNA origami or DNA brick solution can be stored at −20 °C for up to 6 months and at 4 °C for up to 1 d.

(B) **Agarose gel electrophoresis** ● **Timing 3–4 h**

▲ **CRITICAL** The folding quality of DNA origami can be tested by agarose gel electrophoresis. The successful bands can be separated from the short staples for further AFM imaging or TEM imaging.

(i) For a 50-ml gel of 1% (wt/vol) agarose, weigh 0.5 g of agarose and add it to a flask with a total volume of 100–200 ml.

▲ **CRITICAL STEP** To separate the two conformations of DNA arrays, use 2% (wt/vol) or 2.5% (wt/vol) agarose gel.

(ii) Add 50 ml of 0.5× TBE buffer to the flask. Stir the flask to disperse the agarose.

(iii) Put the flask in a microwave at high power for 1–3 min until bubbles appear. Gently swirl the flask to check whether the solution is clear. Repeat this step until the agarose is fully dissolved and the solution is completely clear.

! **CAUTION** Wear heat-insulating gloves to protect your hands from the boiling agarose solution and the hot flask.

(iv) Leave the bottle for 5–10 min to cool the solution before the next few steps.

(v) Add 600 µl of 1 M $MgCl_2$ to a final concentration of 12 mM.
(vi) Add 10 µl of ethidium bromide and swirl it gently until the stain is evenly distributed.
(vii) Pour the gel solution into the casting tray and insert a gel comb.
(viii) Wait for 30–60 min until the gel has been solidified. Remove the gel comb and put the casting tray with gel into the gel box. Fill the gel box with 0.5× TBE containing 12 mM $MgCl_2$. Put the gel box in an ice-water bath to prevent heat damage.
(ix) Add 3 µl of gel loading buffer to 30 µl of samples. Use M13-derived scaffold p7560 or 10k DNA marker as a DNA ladder. Combine 20 µl of sample from Step 10 with 3 µl of loading buffer and load the samples into the gel wells.
(x) Set the voltage at 50–70 V and run the gel for 1.5–2 h. Make sure there are bubbles near the electrode, indicating the flow of current through the gel. Stop running the gel, and use a UV transilluminator to visualize the bands. Adjust the focus and light intensity for good contrast.
? TROUBLESHOOTING
(xi) Cut out the desired bands (~5,000 kDa) with a razor blade, and then cut the gel into pieces and put them into a Freeze'n Squeeze spin column. Freeze the samples for 2–5 min at −20 °C and centrifuge the spin column for 5 min at 1,000*g* at 4 °C. Discard the column with residual agarose and keep the solution obtained in the tube.
(xii) Analyze DNA samples by AFM imaging or TEM imaging (Boxes 1 and 2).
PAUSE POINT The DNA origami solution can be stored at −20 °C for up to 6 months and at 4 °C for up to 1 d.

(C) **Ultrafiltration purification** ● **Timing 30 min–1 h**
(i) Insert a centrifugal filter into an affiliate tube.
(ii) Add 100 µl of the DNA array sample from Step 10 to the filter.
(iii) Add 400 µl of folding buffer to the filter. Seal with the cap.
(iv) Put the centrifugal filter into a centrifuge and spin for 10 min at 3,000*g* at 4 °C.
(v) Empty the tube that contains the folding buffer together with free DNA.
(vi) Repeat Step 11C(iii–v) three to six times.
(vii) Remove the filter set from the tube, flip it, and put it into a new tube.
(viii) Centrifuge the tube for 2 min at 2,000*g* at 4 °C.
(ix) Pipette the 10–30 µl of DNA nanostructure solution into a DNA low-binding tube.
PAUSE POINT The samples can be stored at −20 °C for up to 6 months and at 4 °C for up to 1 d.
? TROUBLESHOOTING

Troubleshooting

Troubleshooting advice can be found in Table 1.

Table 1 | Troubleshooting table

Step	Problem	Possible reason	Solution
11B(xi)	Unable to separate two DNA molecular array conformations	A low gel concentration was used	Use 2–3% (wt/vol) agarose gel. However, do not use too high a concentration of agarose, as it may then take too long to run the gel or the samples might get retarded in the wells
	Gel bands of DNA samples are too weak	Sample volume is too low	Increase the DNA sample concentration and volume
	Sample retarded in the gel wells	The sample aggregated	Add poly(T) overhangs on the boundary strands of DNA molecular arrays
		The agarose gel concentration was too high	Adjust the gel concentration and sample loading volume
11C(ix)	Total volume after filtering is more than 60 µl	Centrifugal speed too low	Increase the speed of centrifugation
		Centrifugal filters too small	Use Amicon 100k centrifugal filters
Box 1, step 15	Cannot find DNA nanostructures	Low DNA origami concentration	Store the DNA samples at −20 °C to minimize DNA adsorption on the tubes. DNA samples can be stored for up to several weeks.

Table continued

Table 1 (continued)

Step	Problem	Possible reason	Solution
	The DNA nanostructure is incomplete	The concentration of magnesium is too low	Increase the concentration of magnesium to at least 10 mM
		Low staple concentration	Adjust the ratio of scaffold to staples to 1:5 or higher
			Keep each staple concentration the same
Box 3, options A–D	Unable to transform conformations of DNA relay arrays	The concentration of formamide is too low	Use 10–40% (vol/vol) formamide. Increase the concentration of formamide if necessary
		The temperature is too low	Increase the temperature to 55 °C or above
		Too few trigger strands are used	Increase trigger strand number or concentration
		DNA brick arrays are used	Because DNA brick arrays cannot transform after folding, a 52-bp DNA origami array may be better for transformation

Timing

Steps 1–10, DNA molecular array folding: ~ 20 h
Step 11A, PEG precipitation of DNA nanostructures: 2–3 h
Step 11B, gel purification of DNA nanostructures: 3–4 h
Step 11C, ultrafiltration purification of DNA nanostructures: 30–60 min
Box 1, AFM imaging of DNA nanostructures: 1–4 h
Box 2, TEM imaging of DNA nanostructures: 30 min–2 h
Box 3, regulation of DNA molecular arrays: 2–24 h

Anticipated results

AFM is used as a main tool in this protocol to characterize DNA molecular arrays. We introduce three modes of AFM imaging (Fig. 5a): normal AFM imaging to check the DNA nanostructure; temperature-controlled AFM imaging for in situ real-time tracking of conformation transformation; and real-time monitoring of conformation transformation at room temperature with added formamide (Box 3). With the use of formamide, the DNA array transformation can be studied after the addition of trigger strands at the corner or middle positions of the 11 × 4 52-bp DNA origami relay array, as illustrated in Fig. 5b. This demonstrates that the conformation transformation can be initiated at the selected positions of the DNA array. It should be noted that the number of trigger strands is a key factor for successful conformation transformation. It is suggested that more than three trigger strands be used for the current 11 × 4 52-bp DNA origami array.

Figure 6 demonstrates the regulation strategies for the DNA array transformation. The transformation can be stopped or resumed at selected positions, as shown in Fig. 6a. Control of the transformation pathway at the selected position is achieved by the use of lock strands or removal of one unit from the DNA relay array. In addition, the transformation of DNA origami arrays can be reversed by the addition or removal of the trigger strands through DNA strand displacement, as illustrated in Fig. 6b. Higher-order DNA origami arrays can be achieved via connection of the edges of the DNA origami array to form tube monomers (red conformation) or 1D tubes (Fig. 6c). Such higher-order DNA origami arrays can be transformed further into the green conformation by the addition of green trigger strands.

Reporting Summary

Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

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Author contributions

J.S. and Y.K. conceived and led the project. J.S., P.W., and Y.K. designed and conducted the experiments. D.W., J.S., P.W., V.P., Y.Z., D.C., and Y.K. contributed to the writing of the manuscript.

Competing interests

A provisional US patent application based on the work described in this paper has been filed.

Additional information

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