We present a simple and effective scheme of a dynamic switch for DNA nanostructures. Under such a framework of toehold-free strand displacement, blocking strands at an excess amount are applied to displace the complementation of specific segments of paired duplexes. The functional mechanism of the scheme is illustrated by modelling the base pairing kinetics of competing strands on a target strand. Simulation reveals the unique properties of toehold-free strand displacement in equilibrium control, which can be leveraged for information processing. Based on the controllable dynamics in the binding of preformed DNA nanostructures, a multi-input-multi-output (MIMO) Boolean function is controlled by the presence of the blockers. In conclusion, we implement two MIMO Boolean functions (one with 4-bit input and 2-bit output, and the other with 16-bit input and 8-bit output) to showcase the controllable dynamics.
In self-assembled DNA nanostructures and especially ones from the scaffolded origami method, designated base pairing can be specified to result in a deterministic structure. However, in most complex DNA nanostructures, a pre-specified segment of base pairing is always static once complemented. On the other hand, toehold mediated strand displacement has enabled many DNA dynamic systems, including switchable devices, walkers, triggered amplification, and circuits. Especially, there are a few examples of dynamic megadalton DNA nanostructures in which the paired segments with toeholds make the systems switchable from one state to another.

Instead of toehold mediated strand displacement, we demonstrate that controlled dynamics can be achieved by the simpler implementation of toehold-free strand displacement. Under such a scheme, when a single-stranded DNA (ssDNA) blocker (e.g., \( n^* \)) fully complementary to a certain segment of paired duplex (e.g., \( NN^* \)) is presented at an excess amount, it can displace the specific segment by pairing competition (e.g., \( nn^* \)) (Fig. 1a). The blocking can be applied to an arbitrary set of segments of paired duplexes (e.g., \( NN^* \), \( N = [n_1, n_2, ..., n_i] \); \( N^* = [n_1^*, n_2^*, ..., n_i^*] \)). When a counterpart set of ssDNA blockers (e.g., \( N^* = [n_1^*, n_2^*, ..., n_i^*] \)) is presented at an excess amount, the original pairing scheme will be outcompeted and displaced (Fig. 1b). In other words, when the original pairing scheme of \( NN^* \) without strand displacement is defined as an ON state, the pairing after strand displacement (\( NN^* \)) can be defined as an OFF state.

In general, our experimental and simulated results exhibit the functional mechanism of toehold-free strand displacement. The controllable dynamics has been applied in a number of DNA nanostructure systems for ON/OFF switch and Boolean functions. We believe the simple and effective implementation could enable advanced computation with DNA nanostructures.

**Results**

**Proof-of-concept system to illustrate the basic dynamics.** To demonstrate the dynamics based on toehold-free strand displacement, we first designed a simple proof-of-concept system. In our polyacrylamide gel electrophoresis (PAGE) assays involving fluorescently labeled strands, we prove the strand displacement indeed happens without toeholds. The toehold-free design results in a reversible strand displacement reaction, which is fundamentally different from the conventional toehold-mediated design.

In this proof-of-concept system, we designed two duplexes (\( D_1 \) and \( D_2 \)) to be connected by a single pair of binding partners (segment \( n \) in \( D_1 \) and segment \( n^* \) in \( D_2 \)). We also introduced a blocker (B, strand \( n^* \)) into this system (Fig. 2a), which can dissociate the two duplexes if toehold-free displacement is kinetically favorable. With fluorophore (e.g., Cy3) modified on the \( 3' \) end of segment \( n \), the different molecule species (e.g., \( D_1, D_1D_2, \) and \( BD_2 \)) can be identified after native PAGE assay. Our results with PAGE purified strands demonstrate that the fraction of \( D_1D_2 \) complex decreases with increasing blocker concentration and that, therefore, the single-pair system indeed functions according to a mechanism involving toehold-free strand displacement. To better understand such mechanism, we developed a probabilistic model based on master equations to simulate the kinetics of the base pairing of \( D_1 \) with \( D_2 \) or a blocker (Supplementary Note 2). This model simulates the kinetic pathways leading to displacement and can help elucidate whether the observed experimental trends can be explained mechanistically from DNA hybridization kinetics at the base-pair level. The model simulations predict that the probability of observing \( D_1 \) in a \( D_1D_2 \) complex reduces with the increasing concentration of blocker according to the equilibrium probability distribution. The predicted time to reach equilibrium from the model simulations of around a hundred seconds matches the experimental fluorescence data at least by order of magnitude (Supplementary Figs. 19 and 20), suggesting that 1 h is sufficient for the toehold-free displacement system to reach equilibrium. Similar experimental results are obtained for blocking in a pre-reaction or post-reaction initial condition (Fig. 2b), which further shows the reversibility of the system. The toehold-free system behaves qualitatively different from a conventional toehold-mediated system. Model simulations predict that the probability of displacement by a blocker essentially vanishes when the length of blocker is minimally reduced, which has been confirmed by an experiment with a blocker that is 1-nt shorter than the binding partner of \( D_2 \) (Supplementary Figs. 18 and 21). Any displacement system with such 1-nt shorter blocker will be less reversible. The probabilistic model based on master equations can broadly explain the observed reversibility of the toehold-free displacement system and its sensitivity with respect to the blocker concentration by simulating the displacement pathways with hybridization kinetics at the base-pair level. However, it cannot describe the equilibrium concentrations of all species in the system. Therefore, we also developed a deterministic reaction pathway model to explain the observed reversibility of the toehold-free displacement system.

**Fig. 1 Schematic diagrams of controllable dynamics of DNA nanostructures based on toehold-free strand displacement.** Tree maps of binding reactions of (a) one species of blocker to displace one species of pre-specified segment (\( nn^* \)) or (b) two species of blockers to displace two species of pre-specified segments (\( nn^*, nn^* \)).
understand the impact of design parameters on the species concentrations at equilibrium. The $\Delta G$ of each pairing or blocking reaction in this model was fitted to experimental data, which reveals that the $\Delta G$ of each reaction is higher compared to the one predicted from the nearest neighbor thermodynamics model (Supplementary Notes 3–6). Such higher $\Delta G$ can potentially be attributed to a lower $\Delta S$ of the pairing reaction of the binding partner of D1 compared to standard duplex formation. Furthermore, the $\Delta G$ for the formation of the D1D2 complex is lower than the $\Delta G$ for the formation of BD1, which suggests that the D1D2 complex is more stable at the tested temperatures. The estimated $\Delta G$ for the formation of BD1 decreases with increasing temperature, which suggests that the OFF state of such system is favored at a higher temperature (Supplementary Fig. 14). This decreasing $\Delta G$ with temperature would suggest that the blocking process is entropy-driven ($\Delta S > 0$), but could also be caused by other temperature-dependent effects that are not captured by the model.

A dual-pair system with two pairs of binding partners was also designed and implemented (Supplementary Fig. 8). The product distribution as function of the blocker concentration is different for the case of such dual-pair system (Supplementary Fig. 22) compared to the single-pair system discussed earlier (Supplementary Fig. 21). A significant dissociation of the D1D2 complex in case of the dual-pair system only occurs when the concentration of the blockers exceeds a certain threshold, whereas the fraction of the D1D2 complex in the product distribution of the single-pair system decreases more gradually with the blocker concentration. The reaction pathway model of the single-pair system can be extended to simulate the dual-pair system after designing and implementing (Supplementary Fig. 8). The product distribution as function of the blocker concentration is different from the single-pair system. One reason for such difference could be an entropy penalty that arises from the internal loop formation of the D1D2 complex with dual binding partners (Supplementary Note 6), which explains why these intermediates could not be separated by PAGE. The low fraction of intermediate structures and the blocker threshold imply cooperativity between the binding partners of multi-pair systems. Finally, the estimated $\Delta G$ suggested that the D1D2 complex is energetically less stable than the blocked duplex, which is different from the single-pair system. One reason for such difference could be an entropy penalty that arises from the internal loop formation of the D1D2 complex with dual binding partners. The cooperativity associated to the dual-pair system implies an improved stability of switches based on multi-pair designs compared to a single-pair design. Therefore, we believe the design of DNA nanostructures with multiple binding partners will benefit the MIMO Boolean functions. Furthermore, the experimental and simulation results show that the higher temperature stabilizes the blocked state better compared to the lower temperature, which allows the switch to be triggered at lower blocker concentrations and is therefore preferred.

The length of binding partner was set as 16-nt for the proof-of-concept systems and also for most systems in the rest of the article. Systems of 8-nt binding partners (e.g., n and n*) were also tested, but the 8 bp binding was too weak and transient to sustain the association of D1 and D2 (Supplementary Figs. 7 and 13).
structures. With different numbers of pairing partners, we successfully demonstrated the controllable ON/OFF switch based on toehold-free strand displacement.

The first example is to control the binding of two preformed 2D origami structures. Each of the two origami rectangles (24 helices × 26 helical turns from close-packed monolayer helices with twist correction34, 35) was designed with sticky ends on one of the long edges for dimerization (Fig. 3a). These two rectangles units (I and II) shared the same core staples but varied by connection staples. A typical connection staple was designed with three segments, a common staple segment complementary to the scaffold, a linker segment (16-nt poly-T), and a sticky end segment (16-nt). Unit I was specified with sticky end set $N (N \subseteq N_{15} = \{n_1, n_2, \ldots, n_9, n_{10}, \ldots, n_{15}\})$ and unit II with sticky end set $N^* (N^* \subseteq N_{15}^* = \{n_1^*, n_2^*, \ldots, n_9^*, \ldots, n_{15}^*\})$. The index of $N_{15}$ represents the total number of sticky ends, $i$ represents the position of a sticky end in $N_{15}$. Such a complementation scheme corresponded to the binding of units I and II as an ON state. A counterpart set of blockers $N' (N' \subseteq N_{15} = \{n_1', n_2', \ldots, n_9', \ldots, n_{15}'\})$, fully complementary to the sticky end set $N$ respectively (NN'), was designed to displace the pairing of $NN^*$ and switch OFF the binding (Fig. 3a).

When the blocker set of only one species ($N_{1}^{*} = \{n_{8}^{*}\}$) was applied at excess amount to the system of preformed units I and II with only one sticky end pair ($N_{i} = \{n_{i}\}$; $N_{1}^{*} = \{n_{8}^{*}\}$), the binding of the two units was significantly reduced when compared to that without blockers as a control. As shown in agarose gel electrophoresis assay, monomer and dimer products ran as separate bands and fluorescent intensities of monomer and dimer bands were used in the analysis of ON/OFF switch efficiency (Fig. 3b, middle row). When product bands were excised out from the gel and eluted samples of purification were subjected to atomic force microscopy (AFM) imaging, purified dimer structures were shown on AFM images (Fig. 3b, top right). Not surprisingly, binding efficiency increased when the number of sticky ends increased from one pair to four pairs ($N_{i} = \{n_{2}, n_{6}, n_{10}, n_{14}\}$; $N_{i}^{*} = \{n_{2}^{*}, n_{6}^{*}, n_{10}^{*}, n_{14}^{*}\}$), seven pairs ($N_{i} = \{n_{2}, n_{4}, n_{6}, n_{8}, n_{10}, n_{12}, n_{14}\}$; $N_{i}^{*} = \{n_{2}^{*}, n_{4}^{*}, n_{6}^{*}, n_{8}^{*}, n_{10}^{*}, n_{12}^{*}, n_{14}^{*}\}$), and all 15 pairs ($N_{15} = \{n_{1}^{*}, n_{15}^{*}\}$) (Fig. 3c-e, top left). An increasingly higher concentration of blockers was necessary to compensate for the kinetic favor of neighboring sticky ends.

To ensure the components react under the scheme of toehold-free strand displacement, a control experiment (Supplementary Fig. 29) with PAGE purified strands serving as sticky ends (seven pairs of sticky end segments locate at 3’ sticky end of the strands respectively) was performed. The results show a similar trend as the counterpart in Fig. 3d, which further confirms that our dynamic systems are toehold-free. Moreover, 8-nt sticky ends were also tested. Dimerization was presented in absence of blockers, but the yield was lower than samples with 16-nt sticky ends. Binding competition from blockers were not available even when blockers were applied in high concentration (Supplementary Figs. 34 and 35), which was presumably due to weak binding of individual 8-nt sticky ends.

### Boolean functions based on dual-unit and quadruple-unit 3D origami systems

Basic dual-unit and more complex quadruple-
unit 3D origami systems then were designed under the scheme of toehold-free strand displacement. With blockers serving as controllers which switch ON/OFF a certain binding by toehold-free strand displacement, a 4-bit input/2-bit output and a 16-bit input/8-bit output Boolean functions are then implemented based on the reliable dynamic switch.

In a dual-unit system, two basic units of $8 \times 8 \times 10T$ cuboids shared the same core staples but varied by connection staples. Twelve connection staples with peripheral sticky ends were arranged along with two of the eight helices for a particular side face of the cuboid (Supplementary Fig. 36). According to a certain complementation scheme, a specific ‘sticky face’ of unit I pairs with its complementary ‘sticky face’ of a matching unit II, and pairing was subjected to displacement at the presence of the corresponding set of blockers (Fig. 4a). When blockers were not available, binding of units I and II was presented upon mixing as an ON state (e.g., sticky face $N$ of unit I pairs with sticky face $N^*$ of unit II; Supplementary Figs. 37 and 38). When the cuboids were mixed with blockers at the excess concentration, on the other hand, the binding was displaced as an OFF state (e.g., sticky face $N$ of unit I pairs with blocker set $N$; Supplementary Figs. 37 and 39). ON/OFF switch (1-bit output) of the system in which the complementation of one face pair (1-bit input) to be controlled by one set of blockers (1-bit input) can be viewed as a Boolean function of 2-bit input and 1-bit output (Supplementary Table 5).

Similarly, when two sets of face pairs in this dual-unit system and the two corresponding sets of blockers are available, a MIMO Boolean function with 4-bit input and 2-bit output can be implemented (Fig. 4b–e and Supplementary Table 6). Notably, it can be viewed as a composite of two Boolean functions of 2-bit input and 1-bit output. Among the 4-bit input in this dual-unit system, the first 2 bits define the system states, representing whether $N$ and $M$ have been blocked by $N'$ and $M'$ before binding reaction, respectively (Fig. 4b–e); and the rest 2 bits serve as the controllers, representing whether blockers $N'$ and $M'$ are presented at excess amount during the binding reaction. The 2-bit output is the new system state after the control operation. In our implementation, 2-bit initial system states were fixed as $N$ not blocked by $N'$ and $M$ not blocked by $M'$ and the 2-bit controllers were variables. This means that among the 4-bit input, the first 2 bits were kept as constant in our experimental setup, and the rest 2 bits were subject to combinatorial specifications. The computation led to 2 $2^2$ types of output: the absence of both $N'$ and $M'$ resulted in the pairing of both face pairs and corresponding formation of zig-zag shaped multimers (Fig. 4b); the presence of either $N'$ or $M'$ resulted in the blocking of either sticky face pair while leaving the remaining pair complemented and the corresponding dimer formation (Fig. 4c and d); the presence of both $N'$ and $M'$ resulted in a total blocking of the two sticky face pairs and the corresponding monomer formation (Fig. 4e). The resulted shapes reflected the new system states and served as reliable output of the Boolean functions. The specific output of computation was characterized in agarose gel electrophoresis assay (Supplementary Fig. 40), in which monomer and dimer showed as discrete bands and multimer showed as ladder-like bands of incremental mobility. Morphologies of the monomer, dimer and zig-zag shaped multimer products under transmission electron microscopy (TEM) further confirmed the results of desired computation (Fig. 4b–e, right panels; Supplementary Figs. 41–44).

With four information-bearing sticky faces for each 3D cuboid origami unit, we then extended the concept in a quadruple-unit system, which involved four basic units of $8 \times 8 \times 10T$ origami cuboids (I, II, III, and IV). Four sticky faces were designed for each unit and sixteen sticky faces in total (eight pairs of sticky faces: $AA^*$, $BB^*$, $CC^*$, ..., and $HH^*$) for the quadruple-unit system. Accordingly, eight sets of blockers ($A'$, $B'$, $C'$, ..., and $F'$) were designed to displace the corresponding sticky face pairs, respectively. A MIMO Boolean function with 16-bit input and 8-bit output was implemented accordingly. Among the 16-bit input, the first 8 bits define the system states, representing whether each of the eight independent sticky face pairs has been blocked before binding reaction, respectively; the rest 8 bits serve as the controllers, representing whether the eight blockers are presented at an excess amount during the binding reaction, respectively. The 8-bit output is the new system state after the control operation. In our implementation, the initial system states before the binding reaction were fixed as none of the eight pairs of sticky face being blocked. Theoretically, there are 256 $2^8$ output combinations in total, and 69 of them correspond to finite shapes (simulated results in Supplementary Fig. 63). Especially, 44 output shapes are tetramers (Supplementary Table 8). Experimentally, the presence of the chosen four or five sets of blockers (as controllers), with the four basic origami units resulted in the formation of one of the seven tetrominoes ($T_-$, $T_-$, $T_-$, $T_-$, $T_-$, $T_-$, $T_-$, and $Z$-tetromino, seven tetramer shapes according to the Tetris game) (Fig. 5). For example, four species of purified cuboid units were mixed chosen sets of blockers at an excess amount (blocker sets $A'$, $E'$, $G'$ and $H'$), which led to...
the blocking of five specific face pairs and the pairing of the remaining three, resulting in the formation of a T shape tetromino (Fig. 5, leftmost column). There are more than one combination sets of blockers to result in a certain tetromino shape, but we only performed experiments of a particular combination for a certain shape. Similarly, six other tetrominoes were generated with the same four basic units but varying sets of blockers, respectively. The successful formations of all seven tetrominoes were verified by gel assay and TEM imaging. Tetramer bands corresponded to the desired tetrominoes in agarose gel electrophoresis results (Supplementary Figs. 45 and 46), and the desired morphologies were observed when the purified samples were subjected to TEM (Fig. 5; Supplementary Figs. 47–53). Moreover, the mirrored shapes (J piece against L piece, and S piece against Z piece) were also specifically identified by adding an extra blocked cuboid to the resulted shapes (Supplementary Fig. 54).

Besides the one-pot reaction pathway, hierarchical reaction pathways can be designed and performed for the same Boolean function. For the J-tetromino as an example (Supplementary Figs. 60–62), alternative pathways of two or three hierarchies of iterations were designed with fewer input and output bits at each round of computation to result in the same outcome of the original Boolean function (Supplementary Note 14), though the overall efficiency might be compromised (Supplementary Fig. 60). Since an output shape can be viewed as a new system state of a certain Boolean function, the sequence information embedded at the shape boundaries from a certain round of Boolean function can be used in successive rounds. In other words, the function implemented on the dynamic system can be extended in an iterative way.

Discussion
The dynamic switch in this study is reversible, which we predicted with reaction model simulations and validated with experiments showing that different initial states led to identical final states. Simulations demonstrate that the reversibility largely vanishes when introducing short toeholds for displacement (Supplementary Figs. 18 and 21). Because of the reversibility, the equilibrium composition can be engineered by simple concentration adjustment of substrates and products to drive reaction directionality. Influx of fuel strands and generation of waste products, which drives the cycling of toehold-mediated strand displacement, are not necessary in our reversible systems. In most of the implementations, ON or OFF states are switched from a pre-reaction (i.e., pre-computation) state in which reactions between binding components are not available. The switch can also be operated from an ON state to an OFF state (Supplementary Note 8) or from OFF to ON (Supplementary Figs. 54–59). Theoretically, the final state is only determined by the ratio between the origami units and the blockers at a certain temperature, but not the initial state. However, an elevated temperature or elongated reaction time may be necessary for a full ON to OFF or OFF to ON switch in a more complex origami system.

Concentration and temperature adjustments are useful kinetic handles to implement the toehold-free strand displacement for reconfiguration of DNA nanostructures. Changes to the solution composition, such as the concentrations of ions, and denaturing and crowding agents, can also be applied to adjust the corresponding energetics and kinetics. Taken together, fine tuning would result in precise control of the desired reaction pathways. Distinguishable shapes readily identifiable can naturally represent specific system states at certain stages of a computation process. Therefore, shape information was adopted as output for most of the Boolean functions demonstrated in this study. Although the multi-round Boolean computation is feasible according to the results J-tetromino from different reaction pathways, implementation of shape information (boundary sequence information) in general Boolean computation is beyond
the scope of this study. At the same time, each origami unit has four programmable sticky faces (four side faces of the origami cuboid) to be arbitrary complementary to designated sticky faces of other partner origami units, and this general architecture is also similar to a typical DNA tile computing system, which fits our systems under the same Wang tile theory paradigm as the other DNA tile based computing systems. The opportunity of using our origami systems in iterated Boolean Circuit for general Boolean computation is rather apparent, though the implementation could be non-trivial. We decide to schematically present two 6-bit input Boolean computation examples using the quadruple-unit system (e.g., the parity and the multiple of 3) without experimental demonstration (Supplementary Figs. 64–66). Since blockers can provide an extra layer of control to the systems, state-of-the-art DNA computation can in theory be pushed one step closer to general computation. For example, a specific Boolean computation could be implemented by a chosen set of blockers at the presence of a shared set of origami units for multiple computation pathways to direct the progression of the selected (Supplementary Fig. 67). The control by blockers could even be applied on the fly for computation process that requires interventions on demand.

Similar dynamic control based on toehold-free strand displacement was also applied to a typical DNA tile system, in which the assembly of more than 300 species of tiles can be controlled with a single set of blockers at the presence of a shared set of origami units for multiple computation pathways to direct the progression of the selected (Supplementary Fig. 67). The control by blockers could even be applied on the fly for computation process that requires interventions on demand.

When DNA dynamics of a higher level of controllability and reliability in conjunction with scalability and hierarchical architecture lead us to the precise execution of advanced computation, such as composition and iteration, one can imagine sophisticated computation tasks to be implemented by DNA nanostuctures.

Methods

Structural and sequence design. Strands used in simple proof-of-concept system to explore the mechanism of toehold-free strand displacement were designed and generated through Uniquimer software (version 1.0)39. In the single-pair system, each unit contains three strands, including a 15-nt strand, a 30-nt strand, and a 15-nt strand with extended segments (16-nt poly-T linker segment and 16-nt sticky end segment). The Cy3 fluorophore was modified at the 3′ end of sticky end segment. The Cy3 fluorophore was modified at the 3′ end of sticky end segment. The Cy5 fluorophore was modified at the 3′ end of blocker. DNA strands were ultra-PAGE synthesized by Sangon Biotech. 2D origami rectangle (24H × 26T) in this study was adapted from an earlier study34. Fifteen connection staples locate along the top boundary helix. For each connection staple, a combined segment of a linker (16-nt poly-T linker segment and 16-nt sticky end fragment) and 30-nt strand with extended segments (16-nt poly-T linker segment and 16-nt sticky end fragment) were sequentially appended to a common staple. 3D origami cuboid (8H × 8H × 10T) was designed by Uniquimer software. Sequences of sticky ends and the corresponding end segment were obtained from earlier reports34, 42 and DNA sequences of the origami cuboid were generated by caDNAno software41. DNA strands were directly used or incubated overnight at 25 °C.

Agarose gel electrophoresis and the related purification. 1% or 1.5% agarose gel was prepared in 0.5 × TBE buffer supplemented with 10 mM MgCl₂ and pre-stained with SYBR Safe (Thermo Scientific). The annealed samples were subjected to native agarose gel electrophoresis at 90 V in an ice-water bath. Then the target gel bands were excised, carefully crushed using the flat end of a plastic pestle in a Freeze‘N Squeeze column (Bio-Rad), and then directly subjected to centrifugation at 106 g for 2 min at 4 °C or room temperature. Samples centrifuged through the column were collected by SynGene GeneTools software (version 4.03.0.05) and used for further analysis by AFM or TEM.

Non-denaturing PAGE analysis. 12% separating gel of non-denaturing PAGE with 4% stacking gel (Shanghai WSHT Inc.) were used to analyze the results of simplified system in 0.5 × TBE buffer. The competitive equilibrium samples were subjected to non-denaturing PAGE at 80 V for 1 h and 120 V for 3 h in an ice-water bath and stained with SYBR Safe. Then the non-denaturing PAGE was observed by amsershiamTM Typhoon™ RGB Biomolecular Imager (GE Healthcare) with different excitation lights and analyzed by Amsershiam Typhoon Control software (version 2.0.0.6).

Polystyrene glycol (PEG) purification. Precipitation buffer PEG 8000 solution was added to the unpurified 3D cube oligo sample solution with an equal volume43. A thorough mix was then subjected to centrifugation at 16,000 g for 25 min at 25 °C. We removed the supernatant and dissolved the obtained pellet in 0.5 × TAE buffer supplemented with 35 mM MgCl₂. Then the purified samples were directly used or incubated overnight at 25 °C.

AFM imaging. AFM images were obtained using a SPM Multimode with Digital Instruments NanoScope V controller (Vecco) and collected by NANOSCOPE ANALYSIS (Bruker Corp.; version 1.50). A 0.5 μL drop of solution for structure assembly and a 5 μL droplet of the sample were applied to a freshly cleaved mica surface and left for ~2 min. The images were captured under liquid tapping mode, with C-type triangular tips (resonant frequency, f₀ = 40–75 kHz; spring constant, k = 0.24 N m⁻¹) from the SNL-10 silicon nitride cantilever chip (Bruker Corporation). For each structure, the AFM image were independently repeated at least three time.

TEM imaging. A 10 μL droplet of the purified sample (2–10 nM) was applied to a plasma-treated, carbon-coated grid (Electron Microscopy Sciences) for 4 min, and then washed and stained for 15 s with 4 μL of stain buffer (2% aqueous uranyl formate with 25 mM NaOH). Then stain buffer was blotted off by filter paper and left on the grid to be air-dried. Sometimes, the grid was coated with 10 μL 10% PAM. For TEM imaging, the grid was first floated on 45 °C water for 1 min, then wicked off and stained for 15 s with 4 μL 100 mM uranyl acetate (pH 4.0), followed by Pequi filter paper and left on the grid to be air-dried. TEM images were collected by TEM Imaging & Analysis software (version 4.6.4). For each structure, the TEM image were independently repeated at least three times.

Yield quantification by gel electrophoresis. Yields were estimated by analyzing specific bands in native agarose gel electrophoresis or PAGE. The ratio between the fluorescent intensity of a target band and that of the sum of the entire lane in were taken as a proportion estimate (version 1.52a)44 and ImageQuant (GE Healthcare, version 8.1) were used to calculate the fluorescent intensity.
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Author contributions
H.K. designed the experimental systems, performed the experiments; T.L. designed and tested the prototype systems; X.X. modeled and simulated the systems. Q.-S.J. and R.L. supervised the project; B.W. conceived and supervised the project. All authors analyzed the data and wrote the paper.

Competing interests
The authors declare no competing interests.

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