Supplementary Materials for

A localized DNA finite-state machine with temporal resolution

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S1 Data and analysis

S1.1 The unintended reactions.

Fig. S1. Possible reactions of the two-input, five-state DNA FSM in a free diffusion system.
Provided no additional constraints, besides the desired reactions (red solid arrows), there would be unintended reactions in the presence of the input (blue dashed arrows) because some processing probes share the same toehold. For example, as P4 has the same active toehold ($x^*$) as P1, the first addition of input A will not only activate P1 which is the desired probe, but also activate P4, the unintended one. Similarly, when input B is first added, there is crosstalk between probes P0 and P3; when the secondary input is introduced, unintended reactions occur between input A-activated P1 and P2, or, between input B-activated P2 and P1.
S1.2 FSM layout on origami.

|   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|
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|   |   |   |   |   |   |   |   |   |

**Fig. S2. Layout for the processing units of FSM on the origami scaffold.** The row and column corresponding to the 3’-end of the modified staple are used to describe the location of the processing probe. The modification site is named as “R(row_value)c(column_value)”: for example, the leftmost modification site, which locates the processing probe P3 is “R11c-6”. The staple strand which has its 3’-end at that site is called “R11c-6” staple.
S1.3 Procedure for processing fluorescence kinetics data.

**Fig. S3. Process workflow to normalize fluorescence data.** All raw data of fluorescence signals are normalized when plotted, which allows us to quantitatively analyze the data despite the difference between batches and instrument performance. Plots on the top panel show the raw data obtained with the spectrophotometer (arbitrary units; a.u., D1 ≈ 10.88 nm, D2 ≈ 22.42 nm). The middle panel is the background corrected data (output = 0 at the start point t = 0) and the bottom panel is the normalized plots (output = 1 for the positive control).
S1.4 Origami purification.

**Fig. S4. Origami purification efficiency verified by gel electrophoresis.** Image of 1% agarose gel in black-on-white format. **Lane L:** 10 kb ladder; **Lane 1:** staple strands (7.5x); **Lane 2:** M13 scaffold (1x); **Lane 3:** Annealed unpurified origami (1x); **Lane 4:** Origami after 8 rounds of purification by Amicon spin filter (0.50x); **Lane 5:** Origami after 1 round of purification by size exclusion chromatograph (SEC) (0.80x); **Lane 6:** Origami after 2 rounds of SEC purification (0.64x). The concentrations of the origami in Lane 4 and Lane 6 are quantified according to their absorption intensities at 260 nm, and the concentration of the origami in Lane 5 is calculated based on the result of Lane 6.
Fig. S5. Origami purification efficiency verified by fluorescence measurements. We use origami with two processing probes placed at a far distance (D2 ≈ 22.42 nm) to test the purification efficiency of different methods to remove excess staples. Input A is added at 300 s.
**S1.5 Higher operating concentration.**

**Fig. S6. The performance when operating at a higher concentration.** We test spatial constraints with 3 nM origami, 300 nM input A. Reactions between probes located within close distances ($D_1 \approx 10.88$ nm, red curve) is observed to exhibit strong signal and process at a rapid speed. The inter-origami interactions (green curve) are minimal, and the intra-origami leaky reactions ($D_2 \approx 22.42$ nm, blue curve) show weak signals. Compared with the results of 1 nM, there is a small increase in the leaky reactions. However, compared with the intensity of the positive sample at the same concentration, these leakages are still neglectable. Together, the result reveals that even working at high operating concentrations, the performance of the spatial constraints on DSD reactions would not be affected.
S1.6 Parallelism.

**Fig. S7. Parallel processing ability.** oriA represents an input A responsive origami, oriB indicates an input B responsive origami. oriAB is responsive to both A and B. ‘oriA+oriB’ represents the mixture of 1 nM oriA and oriB. From the result, we do not observe significant differences in their fluorescence profiles, indicating good parallelism.
S1.7 The curvature of DNA origami.

At first, we use rectangular DNA origami with 10.67 bp per helix turn to build the testing ground. In CanDO simulation (55) result (fig. S8A), it shows a small curvature. When the two units are placed at a short distance ($D_1 \approx 10.88$ nm), addition of input A triggers the DSD reactions at a very fast rate (red curve in fig. S9). When the two processing units are placed at a far distance, the addition of the corresponding input triggers DSD reactions (blue curve in fig. S9). Interactions that occur between two different origamis are minimal (green curve in fig. S9), indicating that the side reactions mainly take place within the same origami. Then we change another origami (10.44 bp/turn) and find that almost no leakage came from intra-origami reactions (Fig. 2C). CanDO simulation provides evidence that origami of 10.44 bp/turn exhibits a flatter surface than the one of 10.67 bp/turn (fig. S8B). We speculate that it is the surface curvature that biases the accuracy and thus recommend 10.44 bp/turn instead of 10.67 bp/turn for better spatial control.

![Fig. S8. CanDo Diagrams showing the flatness of the origami structure. Simulation results of the 10.67 bp/turn (A) and 10.44 bp/turn (B) rectangular origamis.](image-url)
Fig. S9. Reactions on the 10.67 bp/turn origami. Substantial side reactions are observed when the two probes are placed at long distance (D2 ≈ 22.42 nm, blue curve), with minimal inter-origami interactions (green curve), indicating leaky reactions are mainly from intra-origami reactions.
S1.8 AFM Data.

Fig. S10. Wide Field AFM characterization of the FSM for event (no input). A, B. Schematic illustrations of the detailed reaction product on the origami and the anticipated pattern when there is no input. C. Zoom-out AFM image marked with colored rectangles for differentiating the patterns.
Fig. S11. Wide Field AFM characterization of the FSM for event {A}. A, B. Schematic illustrations of the detailed reaction product on the origami and the anticipated pattern when input A is added. C. Zoom-out AFM image marked with colored rectangles for differentiating the patterns.
Fig. S12. Wide Field AFM characterization of the FSM for event \{(A \text{ THEN } B)\}. A, B. Schematic illustrations of the detailed reaction product on the origami and the anticipated pattern when input A is added followed by input B. C. Zoom-out AFM image marked with colored rectangles for differentiating the patterns.
Fig. S13. Wide Field AFM characterization of the FSM for event (B). A, B. Schematic illustrations of the detailed reaction product on the origami and the anticipated pattern when input B is added. C. Zoom-out AFM image marked with colored rectangles for differentiating the patterns.
Fig. S14. Wide Field AFM characterization of the FSM for event \{B THEN A\}. A, B.
Schematic illustrations of the detailed reaction product on the origami and the anticipated pattern when input B is added followed by input A. C. Zoom-out AFM image marked with colored rectangles for differentiating the patterns.
Fig. S15. The statistical analysis of the patterns on the AFM images.
S1.9 Fluorescence Data.

**Fig. S16. The overlayed spectrums of the selected fluorophores.** Four sets of excitation and emission wavelengths are determined to minimize the interference among different fluorophores. Curves are generated by AttBio Spectrum Viewer. Dotted curves show the excitation spectra and solid curves show the emission spectra. To deactivate signals of the previous state, the processing units on the same side of P0 (i.e., P1 and P3, P2 and P4) should be labelled with fluorophores having the same quencher. We thus label P1 with ROX-BHQ2 pair, P3 with Cy5-BHQ2 pair, P2 with FAM-BHQ1 pair, P4 with HEX-BHQ1 pair, respectively.
Fig. S17. Fluorescence emission spectra of the localized two-input, five-state DNA FSM. The fluorescence responses of processing probes P1 (A), P2 (B), P3 (C) and P4 (D) to different temporal events.
Fig. S18. Fluorescence kinetics of the localized two-input, five-state DNA FSM. Kinetics of each processing probe in response to temporal events \{A THEN B\} (A) and \{B THEN A\} (B).
Fig. S19. Side reactions in the localized two-input, five-state DNA FSM. Experiments are conducted to verify that the spurious leaks, including (A) reactions skipping the intermediate processing unit (reactions between P0 and P3, P0 and P4), (B) crosstalk between irrelevant units (reactions between P1 and P2), are inhibited successfully.
S1.10 microRNAs Detection.

**Fig. S20.** Fluorescence responses of the localized DNA FSM to microRNAs. Initiator ssDNAs (bA, bB) are introduced at first, then the microRNAs are added at different orders.
**S2 Supplementary notes**

**Note S2.1**

**Design individual component.**

We utilize two hairpin probes with orthogonal sequences in the loop and toehold regions to encode the identity of inputs (fig. S21A).

The processing units are composed of an initiator probe and a set of transmitter hairpins (fig. S21B). For the initiator probe $P_n$, which has input $I$ as the predefined downstream input, we define it as:

$$P_n[\varnothing, I].$$

It is a single-stranded DNA. Upon the addition of the input $I$, $P_n$ will capture it.

For a transmitter probe, $P_m$, whose predefined upstream input is $I$ and downstream input is $J$, we define it as:

$$P_m[I, J].$$

It is in hairpin conformation. After receiving signal from the captured $I$, it will be activated and ready to capture the next input $J$.

![Figure S21](image)

**Fig. S21. Conformation details for the inputs and processing probes.**
Note S2.2

Spatial constrained DSD reactions.

We define a possible reaction as:

\[ aP_x [r_1, I] \langle I \rangle \langle P_y [I, r_2] \rangle, \quad x \neq y; \]

- \( aP_x \): reactive processing probe, which is activated to capture the next input;
- \( r \): random sequence.

Because both the predefined downstream input of \( P_x \) and the upstream input of \( P_y \) are \( I \), the addition of input \( I \) could probably trigger DSD reactions between them. If the reactions are conducted successfully, we will get a deactivated \( P_x \) and activated \( P_y \) (\( aP_y \)).

In practice, we extend the selected staples of the DNA origami with sequences to recruit corresponding processing probes which have the complementary extensions. In this way, the fluorophore-quencher pair could be labelled at the ends of the probe/staple to indicate the activation/deactivation of the processing unit. Since the initial processing unit is initially activated, we immobilize it by directly extending the corresponding staple (\( P_n \) in fig. S22).
In terms of the fluorescence activation/deactivation, transmitter probe $P_{n+1}$ (fig. S22) is taken as example. Initially, $P_{n+1}$ gives weak signal (fig. S22A). When receiving signal from captured input $I$, the transmitter hairpin is opened, activating the fluorescence signal (fig. S22B). When the next input $J$ is introduced, the activated $P_{n+1}$ captures input $J$. Subsequently, processing probe $P_{n+2}$ reacts with the captured input $J$. The fluorophore on $P_{n+1}$ is quenched by the quencher of $P_{n+2}$ (fig. S22C).

The schematic diagram on fig. S23 shows a base-level description of the reaction. According to the collision theory, only when the base indicated by red arrow falls within the outreaching area of the base indicated by blue arrow (represented by two gray hemispheres), could the two reactive toehold domains collide with each other, resulting in the DSD reaction.
Fig. S23. The base-level description of the spatial constrained DSD reaction.

We denote the length of the reaction product between the two processing units as \( d \); the intermolecular distance between them on the origami is defined as \( D \). Theoretically,

1. for desired reaction between the two processing units,
   \[ D \leq d; \]
2. for undesired reaction,
   \[ D > d. \]

To obtain the distance values, DNA structural parameters and oxDNA simulation are used (fig. S24).

Fig. S24. Distance measurements. The system shown in Fig. 2B is taken as an example here. A. In terms of \( d \) value, we calculate the segments highlighted in yellow using DNA structural parameters, and, simulate the distances between the two circled nucleotides using oxDNA. B. For \( D \) value, we measure the distances between the circled nucleotides of the selected staples on the DNA origami.

For the calculation using DNA structural parameters, their values are defined as below:

\[
d = C_{d\text{DNA}} \times N_{d\text{DNA}} + C_{s\text{DNA}} \times N_{s\text{DNA}}\]
\[ D = [(C_{\text{dsDNA}} \times N_{\text{dsDNA}, H})^2 + (C_{\text{dsDNA}'} \times N_{\text{dsDNA}, V})^2]^{1/2} \]

- \( C_{\text{dsDNA}} \): Constant value, denotes the length of each base pair in a double helix, here 0.34 (nm/bp);
- \( C_{\text{ssDNA}} \): Constant value, denotes the length of each nucleotide in a single strand, here 0.42 (nm/nt);
- \( C_{\text{dsDNA}'} \): Constant value, denotes the sum of the diameter of each base pair (2.0 nm) and the gap between two adjacent helices on the origami plane (0.7 nm), here 2.7 (nm);
- \( N_{\text{dsDNA}} \): Number of base pairs in the generated product's dsDNA part;
- \( N_{\text{ssDNA}} \): Number of nucleotides in the generated product's ssDNA part;
- \( N_{\text{dsDNA}, H} \): Number of base pairs between the two units in the horizontal direction on the origami;
- \( N_{\text{dsDNA}, V} \): Number of helices between the two units on the origami.

To verify the distance control strategy theoretically, we first make analysis on the system shown in Fig. 2B. According to our definitions, the processing probes are

- \( P_0 [\emptyset, A] \),
- \( P_1 [A, r] \).

The potential reaction is

\[ \langle P_0 \rangle (A) \langle P_1 \rangle . \]

Using DNA structural parameters, we obtain the following results:

- \( d \langle P_0 \rangle (A) \langle P_1 \rangle = 14.16 \) nm,
- \( D_{P_0, P_1-D_1} = 10.88 \) nm,
- \( D_{P_0, P_1-D_2} = 22.42 \) nm.

The simulation results by oxDNA (fig. S25) are almost in agreement with the calculation results.

- \( d \langle P_0 \rangle (A) \langle P_1 \rangle = 9.01 \pm 2.62 \) nm,
- \( D_{P_0, P_1-D_1} = 10.78 \pm 0.23 \) nm,
- \( D_{P_0, P_1-D_2} = 22.06 \pm 0.33 \) nm.
Fig. S25. oxDNA simulation of $d$, $D$ value for the verification of spatial control strategy. We record the data from 2000 observations (line graph on the left) and do statistical analysis on it (histogram on the right). Because of the rigidity of the DNA origami plane, the standard deviation values (SD) of $D$ are much smaller than that of $d$, and SD value of $D_2$ is larger than that of $D_1$.

Second, we evaluate the distances. From results of the distance measurements, we conclude that the value of $D_1$ is smaller than $d$ values, while that of $D_2$ is much larger than $d$ (fig. S26).

$$D_{P_0, P_1-D_1} \leq d \ (P_0) \ (A) \ (P_1), \quad (10.88 \text{ nm} \leq 14.16 \text{ nm});$$

$$D_{P_0, P_1-D_2} \geq d \ (P_0) \ (A) \ (P_1), \quad (22.42 \text{ nm} > 14.16 \text{ nm}).$$

Fig. S26. Comparison of the oxDNA simulation results among $d$ and $D$ values. We could observe that the values of $D_1$ fell within the range of $d$ values, while $D_2$ is much larger than $d$.

With these evidences, we speculate that the reaction of $\langle P_0 \rangle \ (A) \ \langle P_1-D_1 \rangle$ would probably occur, while the reaction of $\langle P_0 \rangle \ (A) \ \langle P_1-D_2 \rangle$ is inhibited.

Third, we carry out experiments to test their performances. The final results (Fig. 2C) are in agreement with our predictions.
Note S2.3

Design the localized two-input, five-states DNA FSM.

In the designed FSM:

![Diagram of the FSM](image)

To implement the functionality of the two-input, five-state FSM, we first utilize the following processing probes:

P0 [Ø, A], P1 [A, B], P2 [B, A], P3 [B, r1], P4 [A, r2].

Where, P0 [Ø, A] = aP0 [Ø, A].

Among them, probes with A as their reactive downstream input include:

P_n [r, A] = {P0, aP2}.

Probes with A as the upstream input are:

P_m [A, r] = {P1, P4}.

Possible reactions between these probes in the presence of input A are listed as follows:

〈P0〉 (A) 〈P1〉;
〈P0〉 (A) 〈P4〉;
〈aP2〉 (A) 〈P1〉;
〈aP2〉 (A) 〈P4〉.

Probes with B as their reactive downstream input are:

P_n [r, B] = {P0, aP1}.

Probes with B as the upstream input are:

P_m [P, r] = {P2, P3}.

Possible reactions between these probes in the presence of input B are listed as follows:

〈P0〉 (B) 〈P2〉;
〈P0〉 (B) 〈P3〉;
〈aP1〉 (B) 〈P2〉;
〈aP1〉 (B) 〈P3〉.

To summarize, all possible reactions are:

![Diagram of possible reactions](image)
We next localize these processing probes on the origami (fig. S2) and perform theoretical calculations. For the first operating layer, the potential crosstalks are \(\langle aP2 \rangle \langle P1 \rangle\) and \(\langle aP1 \rangle \langle P2 \rangle\) when the secondary input is added.

Based on the calculation results, we obtain:

\[
D_{P1, P2} > d_{\langle aP1 \rangle \langle P2 \rangle}, \quad (27.00 \text{ nm} > 25.80 \text{ nm});
\]
\[
D_{P2, P1} > d_{\langle aP2 \rangle \langle P1 \rangle}, \quad (27.00 \text{ nm} > 25.80 \text{ nm}).
\]

From the simulation results (fig. S27), we also find that the \(D\) values of these probes are larger than \(d\) values:

\[
D_{P1, P2} > d_{\langle aP1 \rangle \langle P2 \rangle}, \quad ((26.47 \pm 0.31) \text{ nm} > (15.53 \pm 3.84) \text{ nm});
\]
\[
D_{P2, P1} > d_{\langle aP2 \rangle \langle P1 \rangle}, \quad ((26.47 \pm 0.31) \text{ nm} > (15.54 \pm 3.87) \text{ nm}).
\]

**Fig. S27. Simulation results of distances about the crosstalks in the first layer when the secondary input is added.** \(D\) values are larger than \(d\). (For clear clarification, we only show the line graphs.)
**Fig. S28. The fluorescence responses of P1 and P2.** The origami is immobilized with three processing probes: P0, P1 and P2. Among them, P1 is labeled with ROX-BHQ2 pair and P2 is labeled with FAM-BHQ1 pair.

Theoretically, according to the calculation results, the leaky reactions between P1 and P2 should have been inhibited. However, the experimental results (Fig. 3B and fig. S28) are contradictory to the prediction. This might be arising from biased estimates of distances between these probes.

Here, we discuss the main factors which could have consequences on the biases.

(1) DNA breathing.

It is mainly resulted from thermal motion of DNA molecules. Usually, DNA bases will locally melt (breathe) fairly often, especially at the ends of helices because the base stacking forces are relatively weak here. In our cases, the base breathing tends to happen at the end of staples (fig. S29), where only a continuous segment of 7 or 8 nt is paired with the scaffold. It increases the possibility of the immobilized probe to reach the reactant.

**Fig. S29. The zoom-in simulation images of the reaction products on the origami.** The partially dissociation of the staple from the scaffold is indicated with black arrowed lines. The scaffold and the normal staples are colored in white; the selected staples are colored in yellow; the blue strands stand for the extensions of the staples and the red strands represent the modified probes and inputs.

(2) The structural stiffness of DNA origami.

A flatter or stiffer surface would provide more precise spatial constraints. Though compared with the commonly used origami with 10.67 bp/turn, 10.44 bp/turn origami exhibits a stiffer surface (Fig. 2C and fig. S9), it still shows a degree of structural curvature (fig. S8) and could be further stretched or curled under external forces (56, 57). For the leaky DSD reactions between P1 and P2, the difference between $d$ and $D$ values is not very large. Hence, the reaction energy between the two reactants could probably push them closer by bending the origami plane slightly, triggering the reaction and biasing the distance estimates. From the snapshots of the origami simulated with the reaction products on it, we observe that a small curvature occurs on the participated area on the origami plane (fig. S30).
**Fig. S30. Snapshots of the simulation images.** The images on the top are the zoom-out images of the origami (A) and the origami with the reaction products (B). The images on the bottom are the zoom-in images from different perspectives. The green dashed lines indicate the flatness of the plane.

Additionally, we measure $D$ values using origami with or without immobilization of the reaction products and find that the $D$ value becomes slightly smaller when reactions are occurred on the origami compared with that on a pure origami plane (fig. S31), which further supporting our speculation.

\[
D_{P1, P2} = 26.47 \pm 0.31 \text{ nm}, \\
D'_{P1, P2} = 25.10 \pm 0.74 \text{ nm}.
\]

**Fig. S31. Comparison of the $D$ values.** We perform oxDNA simulations for the distance measurements of the two positions ($D_{P1, P2}$) using a pure origami (blue plots) and an origami immobilized with the reaction products (orange plots).

In order to inhibit leaky reactions in the FSM, taking the crosstalk $\langle aP1 \rangle (B) \langle P2 \rangle$ for example, we provide two possible solutions to increase the distances between the P1 and P2.

1. Repeat the intermediate processing step.
A processing probe P1' [A, A] is added between P0 and P1 to propagate input A one more time. Therefore, the distance between P1 and P2 would be increased simultaneously. In this case, there is an additional undesired reaction \( \langle P0 \rangle (A) \langle P1 \rangle \). It could be avoided by carefully positioning these probes. But even this side reaction happens, it would not affect the direction of the overall reaction trajectory.

(2) Split the intermediate processing step.

If solution (1) could not satisfy the demand, we could split P1 into a set of odd-numbered processing probes. The activation of the first probe would trigger the cascade DSD reactions among them automatically. It is worth noting that odd-numbered processing probes had the same directionality (5' to 3') with the original P1, and even-numbered probes have the opposite directionality.

As the crosstalks between P1 and P2 are not severe, we adopt solution (1) to correct the distances by adding two processing probes, P1' [A, A], P2' [B, B].

Then, all the possible reactions in the FSM are:

Among the undesired ones, \( \langle P0 \rangle (B) \langle P2 \rangle \), \( \langle P0 \rangle (A) \langle P1 \rangle \) could be avoided as mentioned before. Besides, since \( D_{P1, P2} > D_{P1, P2'} \), \( D_{P2, P1} > D_{P2, P1'} \), the restrictions of \( D_{P1, P2} > d \langle aP1 \rangle (B) \langle P2 \rangle \) and \( D_{P2, P1} > d \langle aP2 \rangle (A) \langle P1 \rangle \) could be satisfied when \( D_{P1, P2'} > d \langle aP1 \rangle (B) \langle P2' \rangle \) and \( D_{P2, P1'} > d \langle aP2 \rangle (A) \langle P1' \rangle \) are guaranteed. Likewise, inequations
\(D_{P_1, P_4} > d_{(aP_1')} (A) (p_4)\) and \(D_{P_2, P_3} > d_{(aP_2')} (B) (p_3)\) do not need to be taken into account. Thus, reactions to consider are

![Diagram of the FSM]

After arranging these processing probes onto origami surface, we perform theoretical measurements and assessments on the distances (Table S1 and fig. S32).

**Table S1: Theoretical measurements and assessments on the distances of the revised two-input, five-state FSM.**

| #  | Units     | Desired | Distances | Values (nm) | Prediction       |
|----|-----------|---------|-----------|-------------|-----------------|
|    |           |         |           | calculated  | simulated       |                 |
| 1  | P0, P1'   | Desired | \(d_{(P0) (A) (P1')}\) | 14.16       | 9.00 ± 2.55     | \(d \geq D\), allowed; |
|    |           |         |           | D_{P_0, P_1'} | 10.88          | 10.78 ± 0.23    |                 |
| 2  | P0, P2'   | Desired | \(d_{(P0) (B) (P2')}\) | 21.75       | 9.47 ± 4.33     | \(d > D\), allowed; |
|    |           |         |           | D_{P_0, P_2'} | 16.21          | 15.82 ± 0.27    |                 |
| 3  | P1', P1   | Desired | \(d_{(aP_1') (A) (P1)}\) | 25.80       | 15.53 ± 3.84    | \(d \geq D\), allowed; |
|    |           |         |           | D_{P_{1'}, P_1} | 12.15          | 11.90 ± 0.32    |                 |
| 4  | P2', P2   | Desired | \(d_{(aP_2') (B) (P2)}\) | 25.80       | 14.98 ± 2.92    | \(d \geq D\), allowed; |
|    |           |         |           | D_{P_{2'}, P_2} | 19.33          | 18.17 ± 0.66    |                 |
| 5  | P1, P3    | Desired | \(d_{(aP_1) (B) (P3)}\) | 25.80       | 15.48 ± 3.90    | \(d \geq D\), allowed; |
|    |           |         |           | D_{P_1, P_3} | 11.84          | 11.46 ± 0.37    |                 |
| 6  | P2, P4    | Desired | \(d_{(aP_2) (A) (P4)}\) | 25.80       | 15.13 ± 3.47    | \(d \geq D\), allowed; |
|    |           |         |           | D_{P_{2}, P_4} | 14.43          | 14.21 ± 0.72    |                 |
From the theoretical results, the designed localized two-input, five-state DNA FSM should perform the task as anticipated.
Note S2.4

Summary of the design process.

The overall workflow for the localized two-input, five-state DNA FSM is summarized (fig. S33).

Fig. S33. The workflow for the design of the localized two-input, five-state DNA FSM.
**S3 Sequences**

**S3.1 Sequences of the replaced staples.**

The 10.44 bp/turn rectangular origami (twist-corrected) used here is almost the same with the reported work (34), except that we move the large loop of the scaffold from middle to the right edge (fig. S2) in order to create an asymmetry internal label for AFM. The replaced staples are marked with asterisk and listed below.

### Table S2. Annotations and sequences of the replaced staples.

| Staple name | Sequence |
|-------------|----------|
| R22c2 *     | GCCCACTACGTGAACCATCACCC TTAATGC |
| R22c4 *     | TTTTTTGGGTGAGGTGCCGTAA CACAGACA |
| R22c6 *     | ATCGGAACCCTAAAGGGAGCCCC CTTTCTGA |
| R22c8 *     | AGCTTGACGGGGAAAGCCGGCGA AAAGGGA |
| R23c1 *     | AAAGGGCGAAAAACTATCAG GGGCATG |
| R23c3 *     | AAGAGGGAATGGCTATAGTCTTAAATCAAG |
| R23c5 *     | CTGCAACCGTAAGATACGTGA AGCCTAA |
| R23c7 *     | AAATGAAACCAACAGAGATAGAAC CGATTTAG |
| Loop1.0-1 * | ACGTGCGAGAAAGGAAGGAAGGAAGAAAG |
| Loop1.0-2 * | CGAAAGGAGCGGGCGCTAGGGCGCTGG |
| Loop1.0-3 * | CAAGTGTAGCGGTACGCTGCGTGTAAG |
| Loop1.0-4 * | CCACCACACCCCGCGCTTAATGCGC |
| Loop1.0-5 * | CGCTACAGGGCGTGATGATTTGGGC |
| Loop1.0-6 * | TTTGACGAGCAGTATAACGTGCTTT |
| Loop1.0-7 * | CCTCGTTAAGATCAGAGCGGAGCTTA |
| Loop1.0-8 * | AAGGAGGCGGATTAAGGGGATT |
| Loop1.0-9 * | AGACAGGAACGGTACGCGGAGAATCCT |
| Loop1.0-10 *| GAGAAGGTGTTTTTATAATCAGTGAGG |
S3.2 Sequences of the reaction probes.

Table S3. Annotations and sequences of the reaction probes.

| Component | Domains (5′-3′) | Sequence (5′-3′) | Notes |
|-----------|-----------------|-----------------|-------|
| Input:    |                 |                 |       |
| A         | a* s* x s       | TATGTG CTGTGCTATGG CACGAC CCATAGCCACAG |       |
| B         | b* s* y s       | AGTAGG CTGTGCTATGG TGAAAC CCATAGCCACAG |       |
| Biotin-A  | TT a* s* x s    | /5Biotin/ TT TATGTG CTGTGCTATGG CACGAC CCATAGCCACAG | For AFM characterization. |
| Biotin-B  | TT b* s* y s    | /5Biotin/ TT AGTAGG CTGTGCTATGG TGAAAC CCATAGCCACAG | For AFM characterization. |

Initiator ssDNA used for positive control:

| dA | x s |
|----|-----|
|    | CACGAC CCATAGCCACAG |

| dB | y s |
|----|-----|
|    | TGAAAC CCATAGCCACAG |

Selected sites on the origami (AP):

| Biotin-Loop1.0-2 | Loop1.0-2 | /5Biotin/ TT CGAAGGAGCGGGCGCTAGGGCGCGTGG | For AFM characterization. |
| P0 (A)           | s a TTT   | CCATAGCCACAG CACATA TTT TCCCATCAATCTCTGAACAAGAAA GTACGGT | [Ø, A]; A-responsive initiator probe. |
| P0 (B)           | s b TTT   | CCATAGCCACAG CCTACT TTT AAATAAGAAGATAGAAGGCTTA ACCGCACGT | [Ø, B]; B-responsive initiator probe. For parallelism. |
| P0 (A)           | s b s a TTT | CCATAGCCACAG CCTACT CCATAGCCACAG CACATA TTT TCCATCCAGTCTCCTGAACAAGAAA GTACGGT | [Ø, A & B]; A&B-responsive initiator probe. |
| P0 (B)           | s b s a TTT | CCATAGCCACAG CCTACT CCATAGCCACAG CACATA TTT TCCATCCAGTCTCCTGAACAAGAAA GTACGGT | [Ø, A & B]; A&B-responsive initiator probe. |
| AP1’ | R11c-2 TT handle 1* | AATGCTTTCAAAAATCAGGTCTGTA GCTCA TT TGAGAGTTAGGGATG /3BHQ_2/ | To recruit HP1’, HP1-D1, HP1. |
|------|--------------------|-------------------------------------------------|-----------------------------|
| AP1  | R9c-4 TT handle 2* | CAGGACGTCAAACATGCAGATACT ACTGCGG TT CTGTGTGAGGTCAAGG /3BHQ_2/ | To recruit HP1, HP1-D2.    |
| AP2’ | R12c4 TT handle 3* | AATGGTTTTTTAATACTTCCTACCAGC AACATGT TT CTATGTGTGATGATG /3BHQ_1/ | To recruit HP2’, HP2.     |
| AP2  | R6c6 TT handle 4*  | GAATTAACAAACAAAAGTTACCAGAAA CATACAT TT AGTTAGGAGCTGTG /3BHQ_1/ | To recruit HP2, HP(B).  |
| AP3  | R11c-6 TT handle 5* | TGTTCAGAAATAGGAAGGACCCGGG CTCCCTT TT GTAGGAGCTGTGAGG /3BHQ_2/ | To recruit HP3.        |
| AP4  | R11c7 TT handle 6* | AAATAAGAAGATATAAGAAGGCTTA ACCGCACT TT GTCCGTTAGGTGTTTC /3BHQ_1/ | To recruit HP4.    |
| Tu/P0 | s a TTT #80 | CCATAGCCACAG CACATA TTT GAAGCAAAAAAGCGGATTGCATCA GATAAAAA | 10.67 bp/turn rectangular origami. |
| Tu/P1-D1 | #103 TT handle 1* | TTTTACCTGTTCAATAACCTGTATTAT ATCGCGG TT TGAGAGTTAGGGATG /3BHQ_2/ | 10.67 bp/turn rectangular origami; to recruit HP1-D1. |
| Tu/P1-D2 | #128 TT handle 2* | TCTTACCAGCCAGTACAAAATAAA TGAATAA TT CTGTGTGAGGTCAAGG /3BHQ_2/ | 10.67 bp/turn rectangular origami to recruit HP1-D2. |

Immobilized hairpin strands of processing unit probes (HS):

| HP1’ | s a s* x* TT handle 1 | CCATAGCCACAG CACATA CTGTGGCATTGCATCA GATAAAAA | [A, A] |
| Handle     | Sequence                                                                 | Notes                                                                 |
|------------|--------------------------------------------------------------------------|----------------------------------------------------------------------|
| H1-D1; HP1 | \(56-ROXn/ CCATAGCCACAG CCTACTCTGTGGCTATGG GTCGTG TT CATCCCTAACTCTCA\)    | [A, B]; H1-D1: short distance in Fig. 2C; HP1: used in the 1st attempt. |
| H1-D2; HP1 | \(56-ROXn/ CCATAGCCACAG CCTACTCTGTGGCTATGG GTCGTG TT CCTGACCTCACACAG\)    | [A, B]; H1-D2: long distance in Fig. 2C;                               |
| HP2’       | \(CCATAGCCACAG CCTACTCTGTGGCTATGG GTCGTG TT CATCCCTAACTCTCA\)            | [B, B];                                                               |
| HP2; HP(B)| \(56FAM/ CCATAGCCACAG CACATA CTGTGGCTATGG GTTTCA TT ACCATCACACACTAG\)    | [B, A]; Parallelism.                                                 |
| HP3        | \(5Cy5/ CCATAGCCACAG ACCAAA CTGTGGCTATGG GTTTCA TT CACCTACGCTTCAC\)       | [B, r2];                                                             |
| HP4        | \(5HEX/ CCATAGCCACAG CTCATT CTGTGGCTATGG GTTTCA TT GAACCACTCACCGAC\)     | [A, r1];                                                             |

### microRNA detection related probes:

- **miR21**: TAGCTTATCAGACTGATGGTTGA
- **miR122**: TGGAGTGTGACAATGGTGTTTG
- **bA**: CATA TCAACATCAGTCTGATAAGCTA TGA TATGTGCTGTTGCTATGGCAGCAGCACC CATAGCCACAG
- **bB**: TACT CAAAACACCATTGTCACTCTCA TTG AGTAGGCTGTGGCTATGGTGAAACC CATAGCCACAG
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