Electronic Supplementary Information (ESI)
Programmable reaction and diffusion using DNA for pattern formation in hydrogel medium

Keita Abe,[a] Ibuki Kawamata,[a] Shin-ichiro M. Nomura,[a] Satoshi Murata[a*]

a Department of Robotics, Graduate School of Engineering, Tohoku university, 6-6-1 Aobayama, Sendai 980-8579, Japan

Contents

S1. DNA sequences used

The DNA strands (OPC purified) and fluorescence modified DNA strands (HPLC purified) were purchased from Eurofins Genomics. The acrydite modified DNA strands (HPLC purified) were purchased from Integrated DNA Technologies. After delivery, all DNA strands were hydrated with milliQ distilled water.

The small letters under the sequences are names of each domain which is designed as a combination of complementary sequences(like "x" and "$\bar{x}$") with attention to avoid interactions with other domains. The domains function as follows.

Domain "a": Toehold for strand displacement in AND gate reactions

Domain "b": Binding the input A and B

Domain "c": Binding the input A to connector (see Figure S3-2)

Domain "d": Migrated domain for diffusion modulation

Domain "e": Toehold of input A for diffusion modulation

Domain "g": Connecting input B and AND gate

Domain "j": Binding strands and anchor

Domain "k": Toehold for starter (see Figure S3-2)

Domain "l": Toehold of competitor A for diffusion modulation

Domain "h", "i", "m": Domains of diffusion modulation for input B

Other unnamed parts: Spacer to adjust the length of the strand

S2. The AND gate system for pattern formation

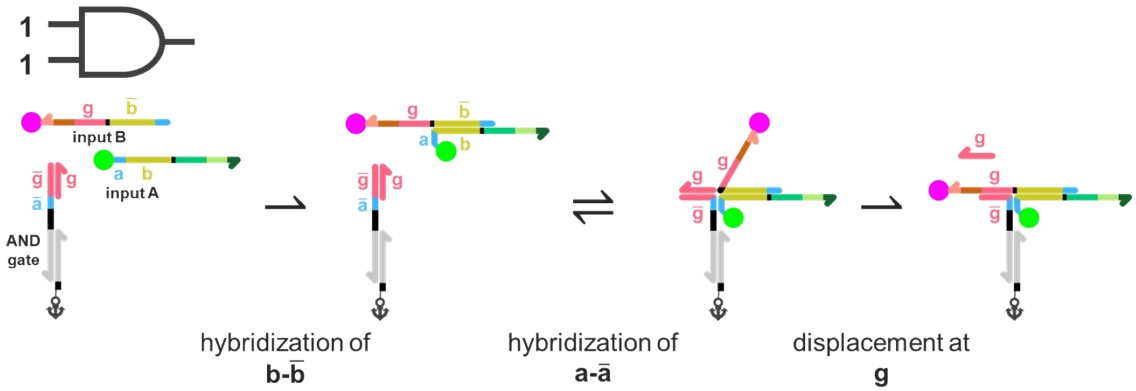
The AND gate consisted of two DNA molecules (Figure S2-1c). The gate is immobilized with acrydite anchor on polyacrylamide. Gate strand 1 has domains for hybridization with input A (light blue) and input B (red). The domain for A is too short (6 nt) to form stable double strand at room temperature. Gate strand 2 hybridizes with Gate strand 1 at a domain for input B to prevent the interaction with the gate and B. Thus, the AND gate will hybridize only with input A-B complex (Figure 1c-d, S2-1a-b).

The AND gate function was confirmed by polyacrylamide gel electrophoresis (100 V, 60 min) (Figure S2-2). The bands were imaged with Chemi Doc MP Imaging system (BIO-RAD) (with Blue Epi illumination and 530/28 filter for green fluorescence; Green Epi illumination and 605/50 filter for red fluorescence; and with UV trans illumination and standard filter for SYBR gold). After imaging the fluorescence modified DNAs, the gel was stained by SYBR gold for 20 minutes.

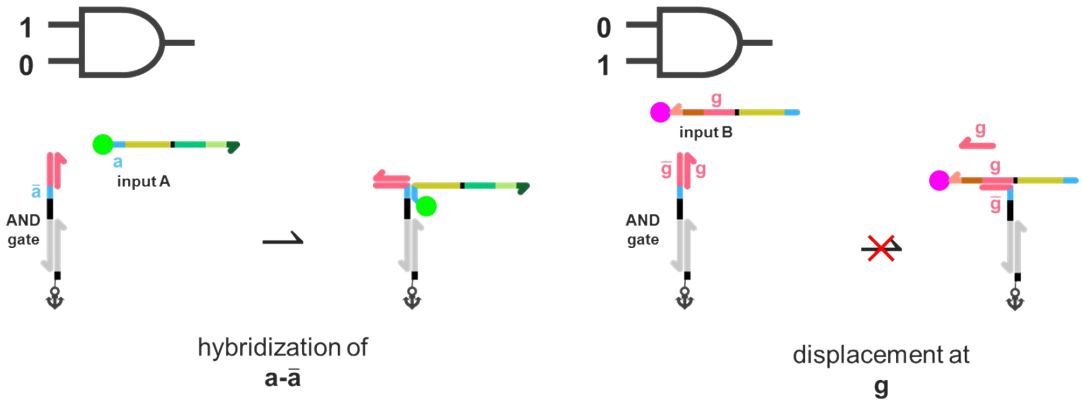
The results showed that the AND gate binds with input A and B if and only if both of them are present. The input A and input B (modified with FAM and TAMRA respectively) hybridize with each other (Lanes 1-3). Gate strand 1 hybridizes with input A-B complex and isolated input B (Lanes 4-7). Especially, the hybridization between the Gate strand 1 and isolated input B (Lane 6) stops the diffusion.

When Gate strand 2 is also present, which does not interact with the input (Lanes 8-11), the AND gate is formed (Lane 12). The AND gate interact with the inputs and the complex of input A, B and Gate strand 1 forms if and only if both of them exist (Lane 12-15). The difference of the red band in Lane 6 and 14 implies the Gate strand 2 prevents the hybridization between the Gate strand 1 and input B. The intensity which corresponds to the Gate strand 1-input B complex in Lane 14 is weaker than that in Lane 6. The difference suggests that AND gate-input B interaction is weaker than the Gate strand 1-input B interaction.

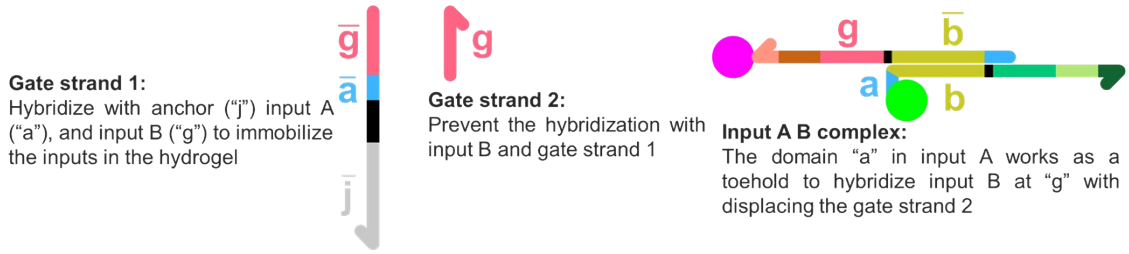
The AND gate also works with the competitor or the trap. In the electrophoresis with adding competitor (16 nt), a band appears at the lowest position close to Gate strand 2 (15 nt). When trap is added, the AND gate also interact with inputs as designed.



a)AND gate with both inputs

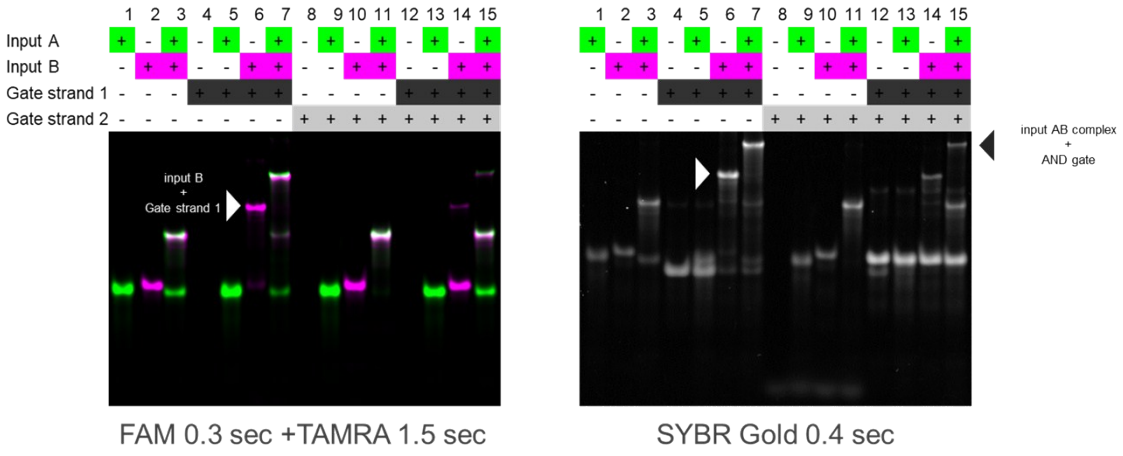


b)AND gate with isolated inputs

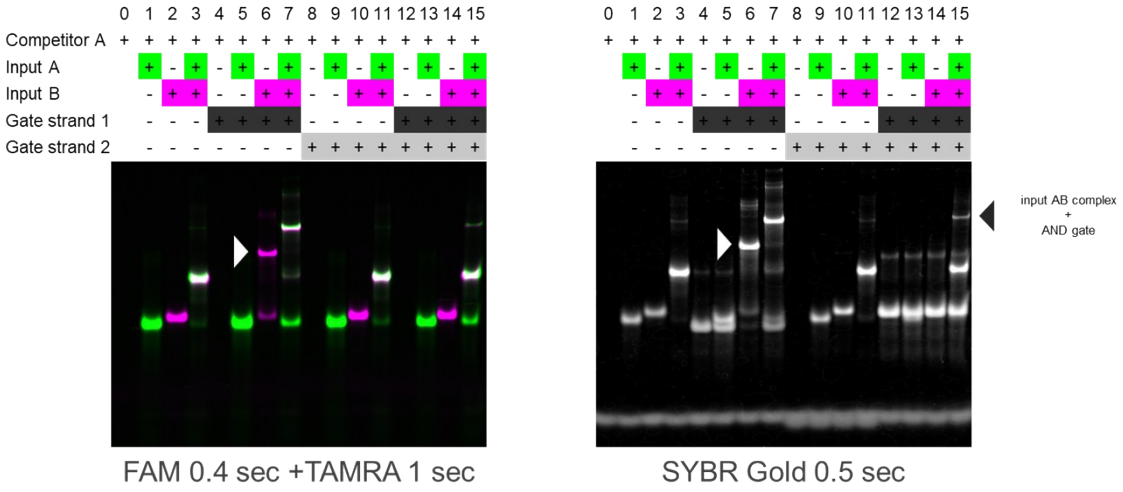


c) Detail of AND gate and input AB complex

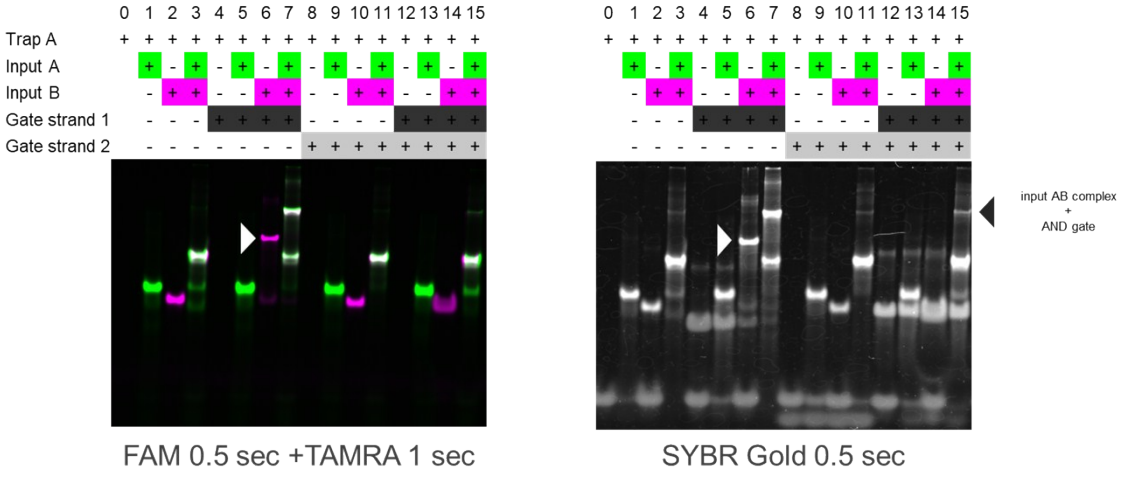
Figure S2-1. Design of AND gate



a) AND gate



b) AND gate with competitor



c) AND gate with trap

Figure S2-2. Performance of the AND gate

S3. Experimental set-up

The pattern formation requires 4 steps (Figre S3-1). In Step 1, the acrydite anchor was synthesized by polymerizing acrydite, acrylamide and bis. In Step 2, the anchor was mixed with DNA solutions. In Step 3, gel beads containing input A or B (their sources) were made from the solutions. In Step 4, the outer of the source beads were filled up by the AND gate solution. By adding starter DNA, the input DNAs is released from the sources and the pattern formation begins (Figure S3-2). The detailed process and materials of each step is as follows.

In Step 1, the acrydite modified DNA (40 μM), 1.5% acrylamide (BIORAD), and 0.5% N,N'-methylenebisacrylamide (BIORAD) were combined together into a Tris-HCl buffer (10 mM) $MgCl_2$(12.5 mM) with 10% TEMED (BIORAD). (Other solutions are also prepared with the same buffer and $MgCl_2$ condition.) After adding 10% APS (Fuji Film Wako Pure Chemical Corporation) to the solution, it was incubated overnight at room temperature.

In Step 2, the acrydite anchor was mixed with solutions including input A, B, or AND gate. To make the source gel beads, the inputs are immobilized with using connector at the initial state. The AND gate, which is a complex of Gate strand 1 and 2, is formed in another solution. The solutions containing inputs or AND gate were mixed with sodium alginate solution (the final concentration is 1.5%, Fuji Film Wako Pure Chemical Corporation) after adding the anchor.

In the Step 3, the solution including inputs bound to anchor (3 μl) is dropped into $CaCl_2$ buffer (0.4 M) to make spherical beads. The solution including sodium alginate gelates rapidly with Ca^{2+} ion. After that, the sources are rinsed two times with buffer without ions.

In the Step 4, the system for observing the pattern formation was made inside a chamber made of silicon rubber sheet on glass. The size of chamber was 7 mm × 6 mm × 1 mm for observation of a pattern formation for a pair of sources, and 5 mm×5 mm×1 mm for four paired sources. After puting the sources, the chamber was filled with the mixture solution of 1.5% sodium alginate, anchored DNA, and the AND gate. The mixture was gelated by adding $CaCl_2$ buffer (0.4 M) and incubated it for 10 minutes. The chamber was set on the microscope (Nikon TE2000-U) and observation started after adding excess amount of starter strands (8 μM×4 μl) to release the inputs from the anchored connector by strand-displacement reaction using the domain k as a toehold (Figure S3-2). The pattern formation was visualized with fluorescent microscopy with Nikon TE2000-U. Green fluorescence and red fluorescence was observed separately and the results are composited after normalizing the brightness to obtain the Figure 2a (Figure S3-3).

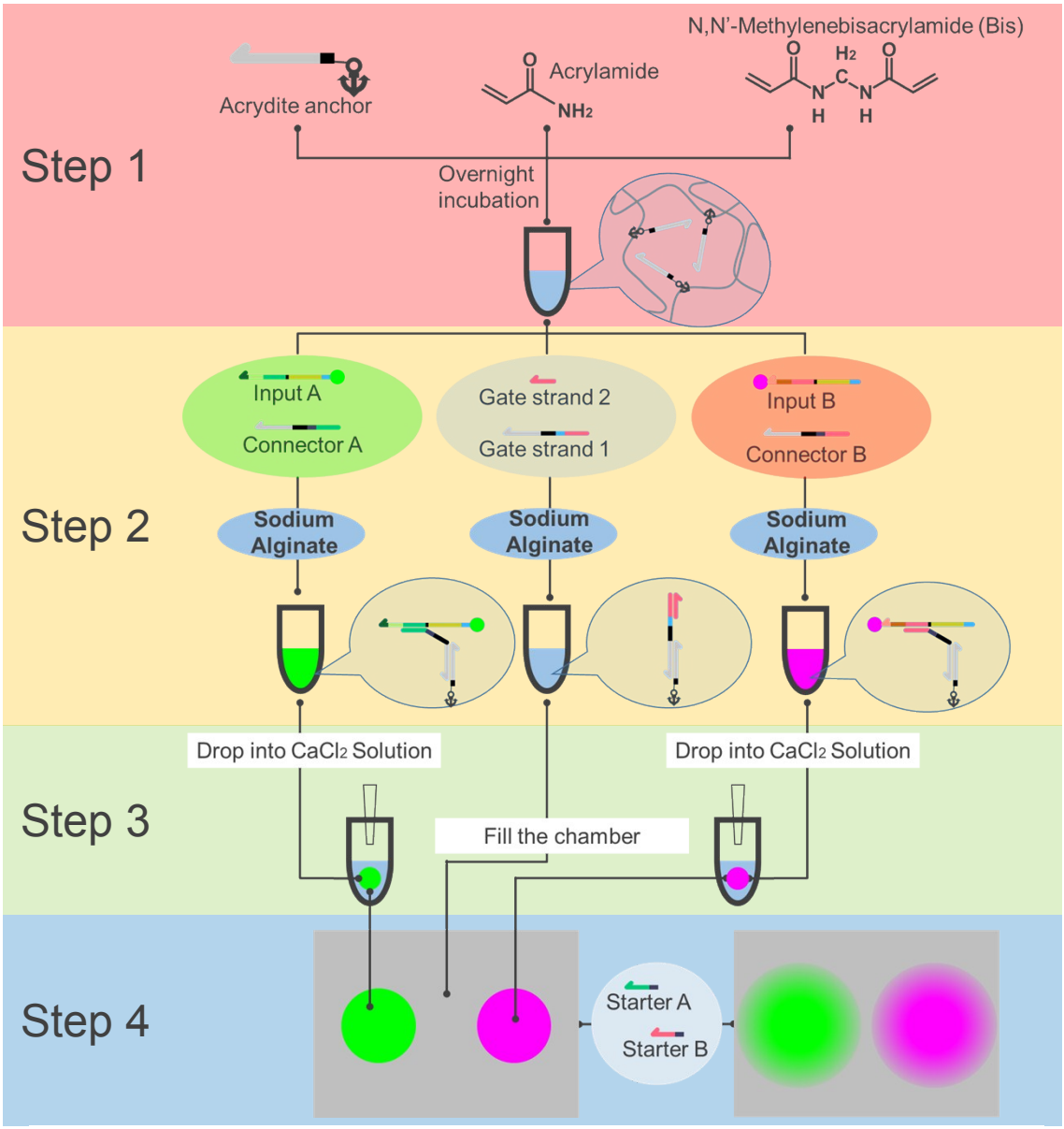


Figure S3-1. Performance of the AND gate

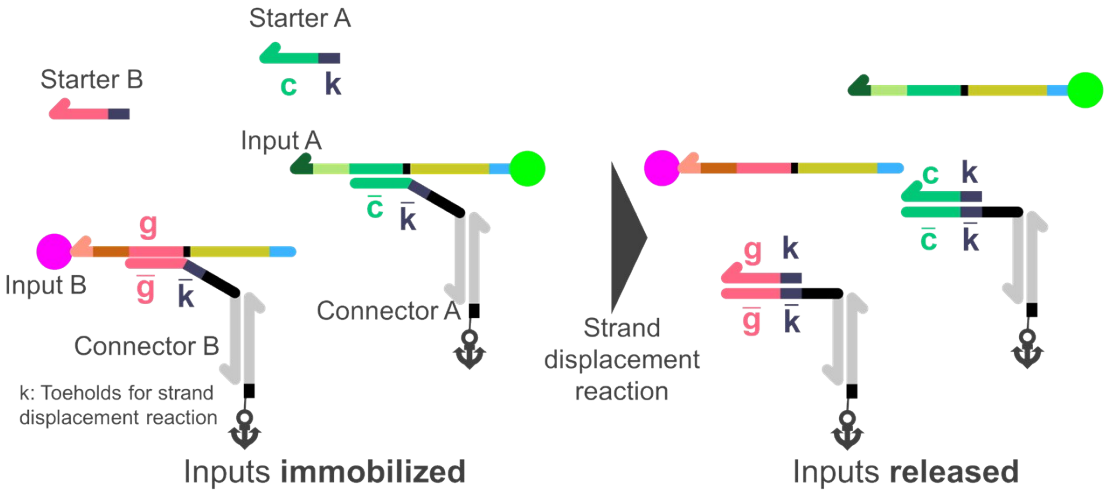


Figure S3-2. Releasing input form immobilization

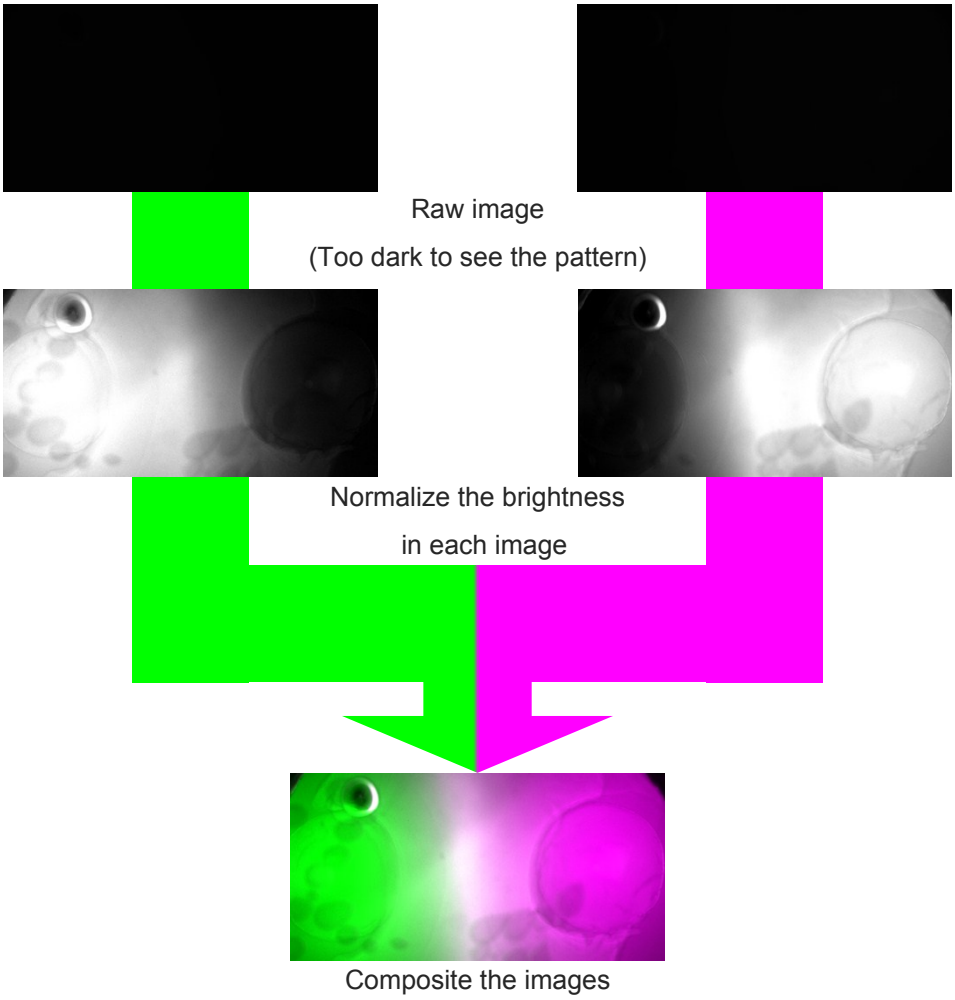


Figure S3-3. Process to obtain Figure 2a

S4. Kymograph

The analysis was performed with using the "Fiji" (image processing software, URL: https://fiji.sc/). First, a line were drawn by hand from the source A to source B and the fluorescent intensities of green and red is measured in each time step. Next, the intensity values were normalized at each time step as follows. The intensity ($F(c, x, t)$) of channel c (green or red) at a position x ($0 \leq x \leq 1$) in t ($0 \leq t \leq 360\ [min]$) is

$$F(c, x, t) = 255 * \frac{F_{raw}(c, x, t)}{\max_x F_{raw}(c, x, t)},$$

where $F_{raw}(c, x, t)$ is the intensity in raw data and $\max_x F_{raw}(c, x, t)$ is the maximum value of the intensity at the time step. Finally the result is plotted as a kymograph with composing red and green channel and resized to 512 pixels ×512 pixels.

From the $F_{raw}(green, x, 360)$ and $F_{raw}(red, x, 360)$, the position of the bisection (x_b) was defined as reached maximum value:

$$x_b = \arg\max_x \sqrt{F_{raw}(green, x, 360) * F_{raw}(red, x, 360)}.$$

where $\arg\max_x (F(x))$ is a value of x where $F(x)$ is $\max_x (F(x))$.

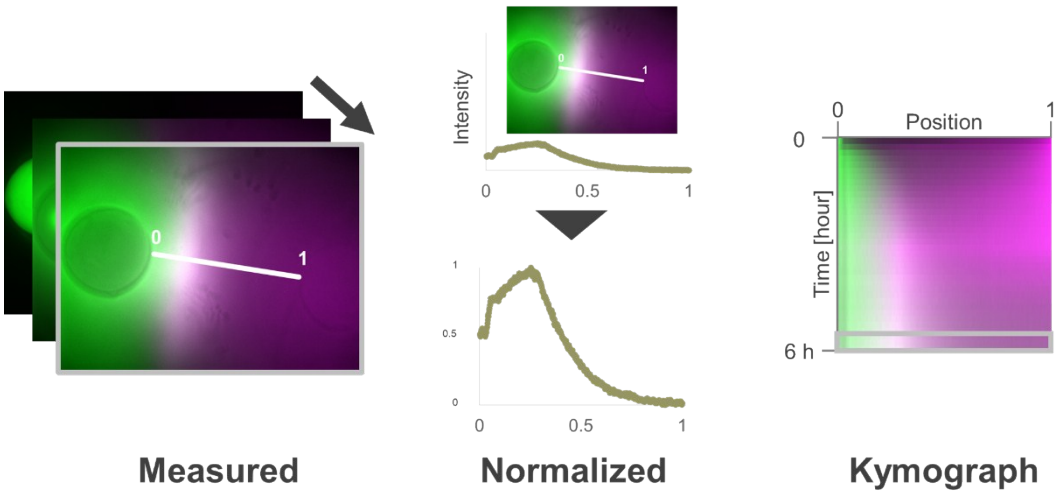


Figure S4-1. Kymograph preparation

S5. Simulation model and parameter

For simulation, the substrates (reactants, products and intermediates) are labeled as follows.

I_A : Input A
I_B : Input B
I_{AB} : Complex of the input A and input B
A_1 : AND gate
$A_{1'}$: Complex of AND gate and input A
A_2 : Complex of AND gate, input A and input B
C : Competitor
T : Trap
T_I : Complex of Trap and input A
T_C : Complex of Trap and competitor

Simulations were performed using the "Ready" (reaction-diffusion simulation software, URL: https://github.com/gollygang/ready). The spatiotemporal development of each substrates was described by partial differential equations as follows:

$$\frac{\partial}{\partial t}[I_A] = D\Delta[I_A] - k_h[I_A][I_B] - k_h[I_A][\mathrm{A_1}] + k_d[\mathrm{A_{1'}}] - k_h[I_A][T] - k_s[I_A][T_C] + k_s[C][T_I],$$

$$\frac{\partial}{\partial t}[I_B] = D\Delta[I_B] - k_h[I_A][I_B] - k_s[I_B][\mathrm{A_{1'}}],$$

$$\frac{\partial}{\partial t}[I_{AB}] = D\Delta[I_{AB}] + k_h[I_A][I_B] + k_s[I_{AB}][\mathrm{A_1}],$$

$$\frac{\partial}{\partial t}[\mathrm{A_1}] = D'\Delta[\mathrm{A_1}] - k_h[I_A][\mathrm{A_1}] - k_s[I_{AB}][\mathrm{A_1}] - k_d[\mathrm{A_1}],$$

$$\frac{\partial}{\partial t}[\mathrm{A_{1'}}] = D'\Delta[\mathrm{A_{1'}}] + k_h[I_A][\mathrm{A_{1'}}] - k_d[\mathrm{A_{1'}}] - k_s[I_B][\mathrm{A_{1'}}],$$

$$\frac{\partial}{\partial t}[\mathrm{A_2}] = D'\Delta[\mathrm{A_2}] + k_s[I_{AB}][\mathrm{A_1}] + k_s[I_B][\mathrm{A_{1'}}],$$

$$\frac{\partial}{\partial t}[C] = D\Delta[C] - k_h[C][T] + k_s[C][T_I],$$

$$\frac{\partial}{\partial t}[T] = D'\Delta[T] - k_h[I_A][T] - k_h[C][T],$$

$$\frac{\partial}{\partial t}[T_I] = D'\Delta[T_I] + k_h[I_A][T] + k_s[I_A][T_C] - k_s[C][T_I],$$

$$\frac{\partial}{\partial t}[T_C] = D'\Delta[T_C] + k_h[C_A][T_A] + k_s[C][T_I] - k_s[I_A][T_C].$$

where $[X]$ is the concentration of species X; k_h, k_d and k_s are reaction rates of hybridization, denaturation and strand displacement reaction respectively. D and D' are diffusion coefficient of diffusible or immobilized DNA.

The diffusion coefficient was computed until the time when the distribution of the input A and B overlapped. At $D = 70\ \mu m^2/sec$, the simulation result was highly consistent with the experiment. The reaction rate appears to be related to the sharpness of the bisector (Figure S5-1). $k_h = 3.3\times10^2\ M^{-1}sec^{-1}$ is the closest condition to the experiment. The anchoring rate has influenced the position of the bisector. The higher the anchoring rate, the nearer the bisector was to the midpoint between the sources (Figure S5-2). Based on the matching between the simulation and experiment, the parameter was set to 80%.

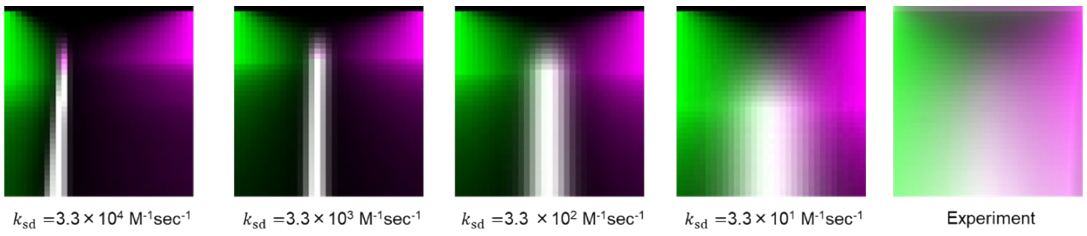


Figure S5-1. Reaction rate effects the sharpness of the bisector

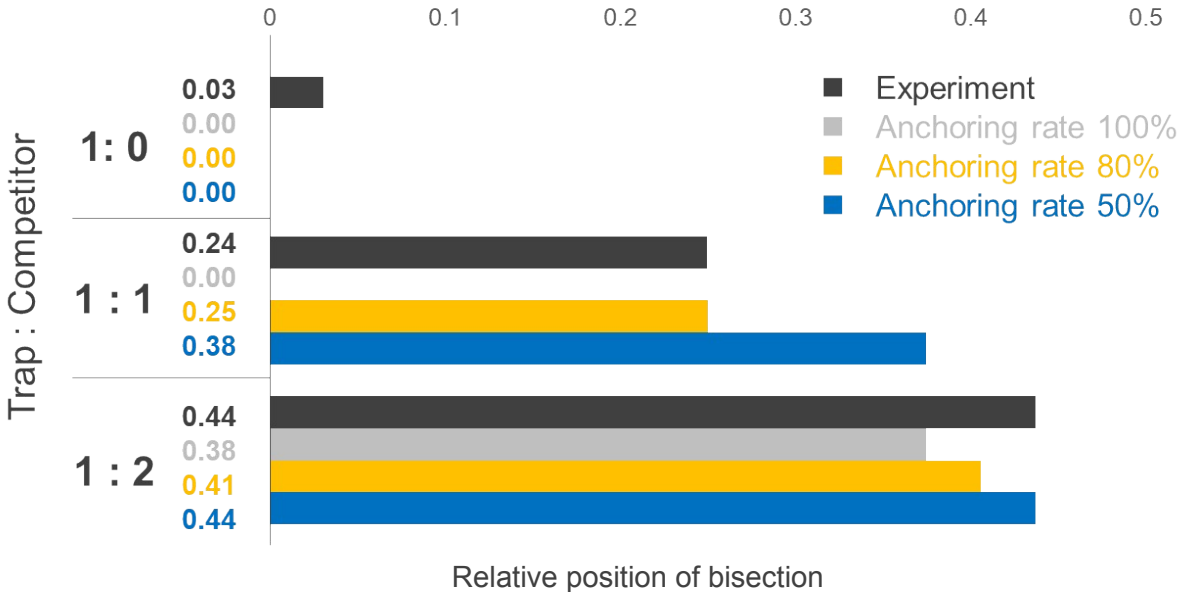


Figure S5-2. Anchoring rate affects the position of the bisection

S6 FRET effects for fluorescent signals.

FAM and TAMRA, which are modified to input A and B respectively, are a well-known pair of fluorophores used for Förster resonance energy transfer (FRET). The effect of FRET to FAM of input A is verified by a fluorescent spectral analysis using a spectrofluorometer (JASCO FP-6200). Figure S6-1 shows a spectrum of fluorescence between the wavelength from 510 nm to 700 nm with 495 nm emission light. The intensity is normalized from 0 to 1 with referring a maximum intensity of excitation light.

Figure S6-1 suggests that FAM excites TAMRA, and the fluorescent intensity of 519 nm decreased by 73.5%. The input A has a spectrum with one peak around 519 nm and the input B did not show a significant spectrum in the case of 495 nm excitation. When input A and B are mixed, two peaks appeared in the spectrum. The left one around the 519 nm which is obtained by a fluorescence of FAM is weaker than that of input A spectrum. The right one around the 580 nm is a fluorescence of TAMRA.

The FRET was not so effective to the position of the bisection (Figure S6-2). The green fluorescent intensity distribution was corrected by considering FRET effect as follows.

$$F_{correct}(green, x, 360) = \begin{cases} \dfrac{F_{raw}(red, x, 360)}{Y} + \left(F_{raw}(green, x, 360) - F_{raw}(red, x, 360)\right) \\ \left(F_{raw}(green, x, 360) \geq F_{raw}(red, x, 360)\right), \\ \dfrac{F_{raw}(green, x, 360)}{Y} \\ \left(F_{raw}(green, x, 360) < F_{raw}(red, x, 360)\right), \end{cases}$$

where $Y =$ 0.265 is the correction constant. There was no significant difference between the position of the corrected and the uncorrected data. The main text employs the uncorrected values.

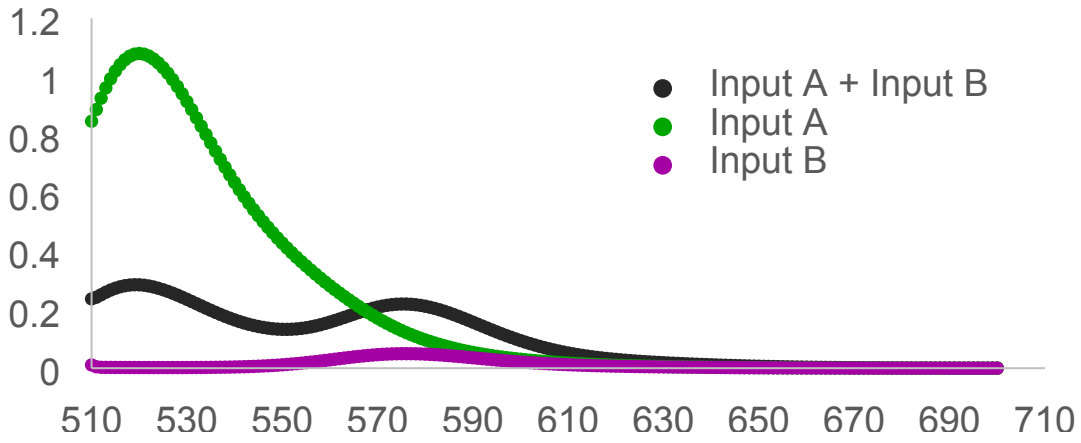


Figure S6-1. Fluorescent spectrum of input A, B and input AB complex.

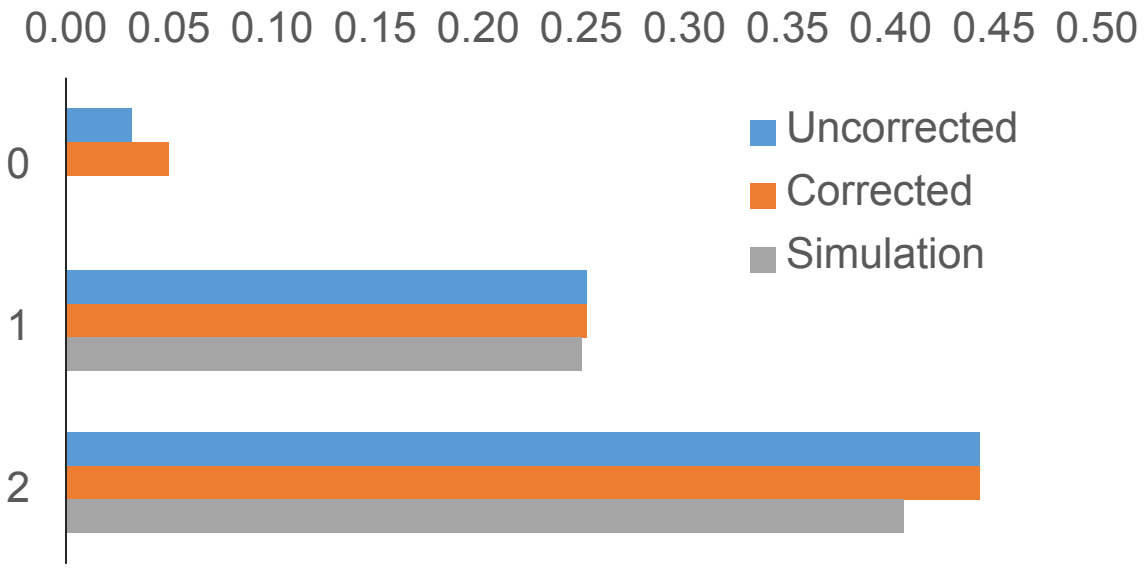


Figure S6-2. Bisection positon of uncorrected and corrected values.