Supporting Information

Cascaded pattern formation in hydrogel medium using the polymerisation approach

S1. DNA sequence

The DNA sequences are presented in Table S1. Six pairs of orthogonal DNA sequences were selected from canonical orthogonal sequences reported in reference [40] in the main text. The connector domain of adjuster DNA is complementary to 15 bases from the 3'-end of L1, whereas the tail domain is filled with poly T. The orthogonality of pairs 1, 2, and 3 was determined using polyacrylamide gel electrophoresis (Figure S1). A 10 % (%C = 5) polyacrylamide gel was used for electrophoresis at 50 V for 120 min. The pair 1 and the pair 2 do not interact each other (Figure S1a). Since L3 behaves as an adjuster of L1, they hybridise each other (Lane 5 in Figure S1b), however, in the presence of R1, the L3 is displaced (Lane 7 in Figure S1b). In the same way, R3 also behaves as an adjuster of R2, which is displaced by L2 (Figure S1c). The results showed that only the correct combination of L1 and R1, L2 and R2, and L3 and R3 were polymerized. We also showed by electrophoresis that the adjuster of L1 binds to L1, changes the size of the molecule, and is released during the polymerization process (Figure S1d).

S2. A hydrogel mould to observe pattern formations

The moulds and combs for the gels were designed using FreeCAD to fit a 5.2-cm diameter Petri dish (Figure S2) and fabricated using a 3D printer (ANYCUBIC Photon, ANYCUBIC, China). The moulds were placed in a Petri dish and 1.6 mL of acrylamide solution (9.5% polyacrylamide, 0.5% bis, 0.01% TEMED, and 0.01% APS) were injected into the moulds while taking care not to introduce bubbles, and then a comb was inserted. Since bubbles may enter when the comb is inserted, 0.4 mL of the acrylamide solution was added from the gap prepared in the mould. To gelate the acrylamide, we kept the solution for 60 min at room temperature(25°C). The combs were then removed, and the petri dish was placed on the microscope. Before the observation, 4 μL of 10 μM DNA solution was injected into the left and right pockets, and 4 mL of liquid paraffin was poured over the gel to prevent evaporation of the gel and DNA solution.

S3. Images in each channel

Although Figure 2 in the main text (superimposed pattern) only shows the fluorescence of FAM and AMCA, other channels were also observed (Figure. S3). In the raw images of superimposed pattern formations, FAM (L1) and Cy5 (R1) were localised at the same location, and the same was true for AMCA (R2) and Cy3 (L2).
Similarly, Figure 3 of the main text (cascaded pattern) only shows the composite values, whereas the raw images are shown in Figure S4.

S4. FRAP experiment

We performed fluorescence recovery after photobleaching (FRAP) experiments to measure the diffusion coefficients of DNA in 10% polyacrylamide gel. To prepare the gel, a solution of 9.5% acrylamide, 0.5% bis, and 5 μM of target DNA with a volume of 10 μL were prepared and kept for 1 h at room temperature. TEMED and APS were then added to a 0.01% solution and reacted for another 1 h at room temperature. The target DNAs differed from the experiments to the experiment, as summarised in Table S2.
The gel prepared in a test tube was placed in a silicon chamber, covered with liquid paraffin to prevent evaporation, and photostimulated using an Olympus FV3000 confocal microscope to measure the recovery of fluorescence. As shown in Figure S5, $I_{bleach}(t)$, $I_{pre}(t)$, and $I_{bg}(t)$ were used to normalise $I_{norm}(t)$ as follows:

$$I_{norm}(t) = \frac{I_{\mathrm{bleach}}(t) - I_{\mathrm{bg}}(t)}{I_{\mathrm{pre}}(t) - I_{\mathrm{bg}}(t)}.$$

No recovery was observed when a pair of DNAs was mixed; therefore, we assumed that the

diffusion coefficient of the polymers was zero. In contrast, fluorescence recovery was observed in single-stranded DNA. Each fluorescence recovery of single-stranded was fitted to the following equation:

$$f(t) = \beta \exp\left(\frac{2\tau_D}{(t-\alpha)}\right)\left(I_0\left(\frac{2\tau_D}{(t-\alpha)}\right) + I_1\left(\frac{2\tau_D}{(t-\alpha)}\right)\right),$$

where I_0 and I1 are modified Bessel functions, α is the time at which the fluorescence intensity is zero, $\beta$is the fluorescent intensity at the plateau level, τ_D is the characteristic time scale for diffusion used to calculate the diffusion coefficient, and t is the time. The first image collected after the bleach in FRAP was set as $t = 0$.

The diffusion coefficient D was calculated using the obtained τ_D and ω, which is the radius of the bleached region.

$$D = \frac{\omega^2}{4\tau_D}$$

The calculated diffusion coefficients are summarized in Table S2.

The differences between the measured values (from 42.3 [μm2/sec] to 84 [μm2/sec]) and the fitted value (20 [μm2/sec]) in simulation may be caused by the effect of our insufficient set up of FRAP experiments. For example, the photobleaching does not reduce the fluorescent intensity to zero and the intensity does not fully recover to one.

S5. Models for reaction-diffusion simulation

Under the assumption that the maximum number of DNA in a polymer is *N*, the partial differential equations of α, β, and γ are as follows:

$$\begin{aligned}
\frac{\partial}{\partial t}[\alpha_{2n-1}] &= D_{2n-1}\Delta[\alpha_{2n-1}] + \sum_{i=1}^{n}\sum_{j=1}^{n-i}(k_h[\alpha_{2i-1}][\alpha_{2j}] + k_h[\alpha_{2i-1}][\beta_{2j}]) \\
&\quad - \sum_{i=1}^{N-n}(k_h[\alpha_{2n-1}][\alpha_{2i}] + k_h[\alpha_{2n-1}][\beta_{2i}] + k_h[\alpha_{2n-1}][\beta_{2i-1}] \\
&\quad + k_h[\beta_{2i-1}][\alpha_{2n-1}]) \\
\frac{\partial}{\partial t}[\beta_{2n-1}] &= D_{2n-1}\Delta[\beta_{2n-1}] + \sum_{i=1}^{n}\sum_{j=1}^{n-i}(k_h[\beta_{2i-1}][\alpha_{2j}] + k_h[\beta_{2i-1}][\beta_{2j}]) \\
&\quad - \sum_{i=1}^{N-n}(k_h[\beta_{2n-1}][\alpha_{2i}] + k_h[\beta_{2n-1}][\beta_{2i}] + k_h[\alpha_{2i-1}][\beta_{2n-1}] \\
&\quad + k_h[\beta_{2n-1}][\alpha_{2i-1}]) \\
\frac{\partial}{\partial t}[\alpha_{2n}] &= D_{2n}\Delta[\alpha_{2n}] + \sum_{i=1}^{n}\sum_{j=1}^{n-i}(k_h[\alpha_{2i}][\alpha_{2j}] + k_h[\alpha_{2j}][\alpha_{2i}] + k_h[\alpha_{2n-1}][\beta_{2i-1}]) \\
&\quad - \sum_{i=1}^{N-n}(k_h[\alpha_{2n}][\alpha_{2i}] + k_h[\alpha_{2i}][\alpha_{2n}] + k_h[\alpha_{2i-1}][\alpha_{2n}] + k_h[\alpha_{2n}][\beta_{2i-1}]) \\
&\quad - k_c[\alpha_{2n}]
\end{aligned}$$

$$\frac{\partial}{\partial t}[\beta_{2n}] = D_{2n}\Delta[\beta_{2n}] + \sum_{i=1}^{n}\sum_{j=1}^{n-i}(k_h[\beta_{2i}][\beta_{2j}] + k_h[\beta_{2j}][\beta_{2i}] + k_h[\alpha_{2n-1}][\beta_{2i-1}])$$
$$- \sum_{i=1}^{N-n}(k_h[\beta_{2n}][\beta_{2i}] + k_h[\beta_{2i}][\beta_{2n}] + k_h[\beta_{2n}][\alpha_{2i-1}] + k_h[\beta_{2n}][\beta_{2i-1}])$$
$$- k_c[\beta_{2n}]$$
$$\frac{\partial}{\partial t}[\gamma_{2n}] = D_{2n}\Delta[\beta_{2n}] + k_c[\alpha_{2n}] + k_c[\beta_{2n}]$$

Here, $[\alpha_n]$, $[\beta_n]$, and $[\gamma_n]$ represent the concentrations of α, β, and γ, which have *n* copies of DNA in each structure. k_h and k_c represent the rate constants of the intermolecular hybridisation and intramolecular hybridisation, respectively. The diffusion coefficients D_n were computed as follows:

$$D_n = \frac{D_1}{n}.$$

To fit the parameters, we computed the error values *E* in each condition as follows:

$$E = \sum \left(I_{\text{experiment}}(t) - I_{\text{simulation}}(t)\right)^2,$$

where $I_{\text{experiment}}(t)$ and $I_{\text{simulation}}(t)$ are the fluorescence intensities of the line in the experiment and simulation, respectively. The values of the parameters and errors are summarised in Table S3. For Figure 4 in the main text, we employed condition 15, where *N*=16, D_1 = 20 [μm^2/sec], k_h = 3.5 × 10^4 [/M/s], and k_c = 3.0 × 10^{-3} [/s] because its error value is minimal in these conditions.

When we use the adjuster, some of the hybridisation is substituted for the strand displacement reaction. The corrected reaction-diffusion coefficients can be described using the rate constant of the strand displacement reaction k_s as follows:

$$\frac{\partial}{\partial t}[\alpha_{2n-1}] = D_{2n}\Delta[\alpha_{2n-1}] + \sum_{i=1}^{n}\sum_{j=1}^{n-i}(k_h[\alpha_{2i-1}][\alpha_{2j}] + k_s[\alpha_{2i-1}][\beta_{2j}])$$
$$- \sum_{i=1}^{N-n}(k_h[\alpha_{2n-1}][\alpha_{2i}] + k_s[\alpha_{2n-1}][\beta_{2i}] + k_s[\alpha_{2n-1}][\beta_{2i-1}]$$
$$+ k_h[\beta_{2i-1}][\alpha_{2n-1}])$$
$$\frac{\partial}{\partial t}[\beta_{2n-1}] = D_{2n}\Delta[\beta_{2n-1}] + \sum_{i=1}^{n}\sum_{j=1}^{n-i}(k_h[\beta_{2i-1}][\alpha_{2j}] + k_s[\beta_{2i-1}][\beta_{2j}])$$
$$- \sum_{i=1}^{N-n}(k_h[\beta_{2n-1}][\alpha_{2i}] + k_h[\beta_{2n-1}][\beta_{2i}] + k_s[\alpha_{2i-1}][\beta_{2n-1}]$$
$$+ k_h[\beta_{2n-1}][\alpha_{2i-1}])$$
$$\frac{\partial}{\partial t}[\alpha_{2n}] = D_{2n}\Delta[\alpha_{2n}] + \sum_{i=1}^{n}\sum_{j=1}^{n-i}(k_h[\alpha_{2i}][\alpha_{2j}] + k_h[\alpha_{2j}][\alpha_{2i}] + k_h[\alpha_{2n-1}][\beta_{2i-1}])$$
$$- \sum_{i=1}^{N-n}(k_h[\alpha_{2n}][\alpha_{2i}] + k_h[\alpha_{2i}][\alpha_{2n}] + k_h[\alpha_{2i-1}][\alpha_{2n}] + k_h[\alpha_{2n}][\beta_{2i-1}])$$
$$- k_c[\alpha_{2n}]$$

$$\frac{\partial}{\partial t}[\beta_{2n}] = D_{2n}\Delta[\beta_{2n}] + \sum_{i=1}^{n}\sum_{j=1}^{n-i}(k_s[\beta_{2i}][\beta_{2j}] + k_s[\beta_{2j}][\beta_{2i}] + k_h[\alpha_{2n-1}][\beta_{2i-1}])$$
$$- \sum_{i=1}^{N-n}(k_s[\beta_{2n}][\beta_{2i}] + k_s[\beta_{2i}][\beta_{2n}] + k_s[\beta_{2n}][\alpha_{2i-1}] + k_s[\beta_{2n}][\beta_{2i-1}])$$
$$- k_c[\beta_{2n}]$$
$$\frac{\partial}{\partial t}[\gamma_{2n}] = D_{2n}\Delta[\beta_{2n}] + k_c[\alpha_{2n}] + k_c[\beta_{2n}]$$

S6. Relationship between the length of DNA strands and pattern formation

The DNA of pair 1 is 46 nt, whereas that of pair 3 is 61 nt. In order to compare the differences, we conducted a non-cascaded pattern formation experiment using pair 3 (Figure S7). Pair 3 was found to form patterns similar to pair 1, but the formation process was delayed compared to pair 1. The intensity of pair 1 reached to 0.05 at 4.3 h, on the other hand, pair 3 took 15 h to reach the same level.

S7. Time development of fluorescent intensity

In order to show that DNA strands move through the hydrogel medium by diffusion, we performed fluorescence observation for 24 hours under the condition that polymerisation does not occur for L1 and R1 (Figure S8a). Based on the results, the distribution of fluorescence intensity at each time point was measured and the position where the fluorescence intensity exceeded the threshold was examined at each time point (Figure S8b). Since the average of the fluorescence intensity of each time point was different, the threshold values of 425, 200 and 20 were set for L1, R1 and simulation, respectively. We plotted the time evolution of the diffusion front and found that the average difference between L1, R1 and simulation was 96 μm and 88 μm, respectively (Figure S8c). Since the resolution of our fluorescence observation is 3.2 μm/pixel (4489 μm/1392 pixels) and the resolution of the simulation is 23 μm/pixel (3000 μm/128 pixels), this error is lower than 5 pixels in the simulation, which suggests that they showed similar dynamics. This simulation reflects only the effect of diffusion, implying that L1 and R1 move through the hydrogel by diffusion.

	Sequence
L1	[FAM]GCATCTACACTCAATACCCAGCCCGTCTATTGCTTGTCACTTCCCC
R1	[Cy5]GGCTGGGTATTGAGTGTAGATGCGGGGAAGTGACAAGCAATAGACG
L2	[Cy3]CGCGACGATTTTAACATTCCTTCAGACACGTTATCAAGCACTTCTC
R2	[AMCA]GAAGGAATGTTAAAATCGTCGCGGAGAAGTGCTTGATAACGTGTCT
L3	[Cy3]GAGTCCGCAAAAATATAGGAGGCTTCGGTTCTCTCCAAAAAAAGCAGGGGAAGTGACAAGC
R3	[Cy5]GCCTCCTATATTTTTGCGGACTCTGCTTTTTTTGGAGAGAACCGAAAGACACGTTATCAAG
A0	GGGGAAGTGACAAGC
A46	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGGGGAAGTGACAAGC
A92	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTTTTTGGGGAAGTGACAAGC

Table S1. DNA sequences

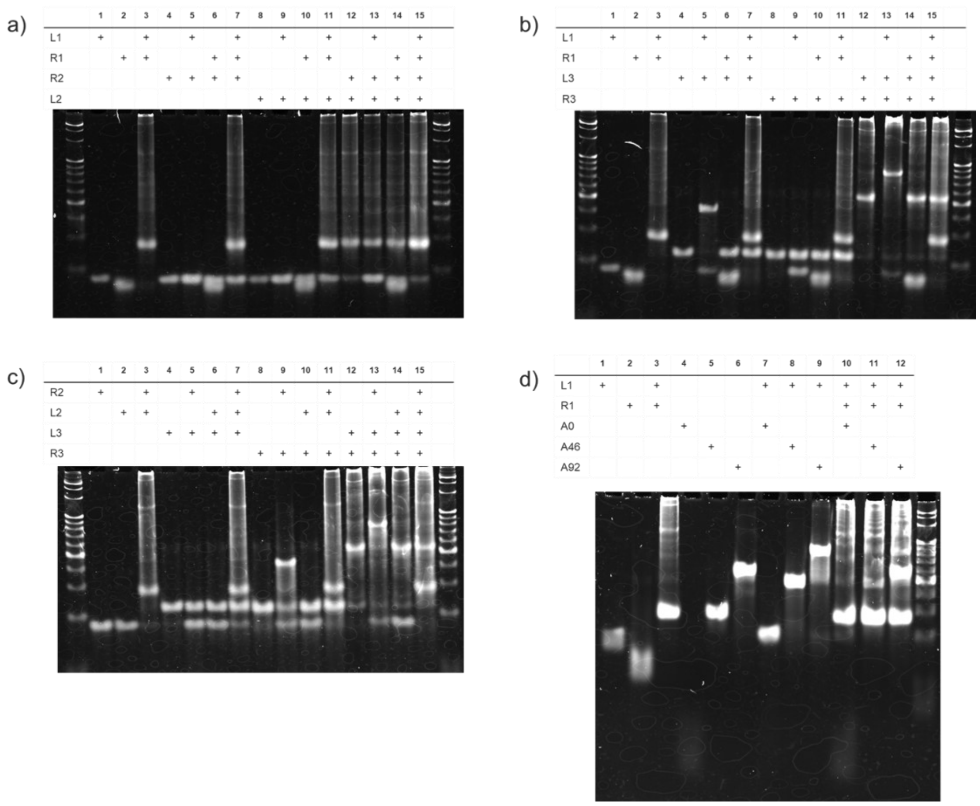


Figure S1. Orthogonality of each pair

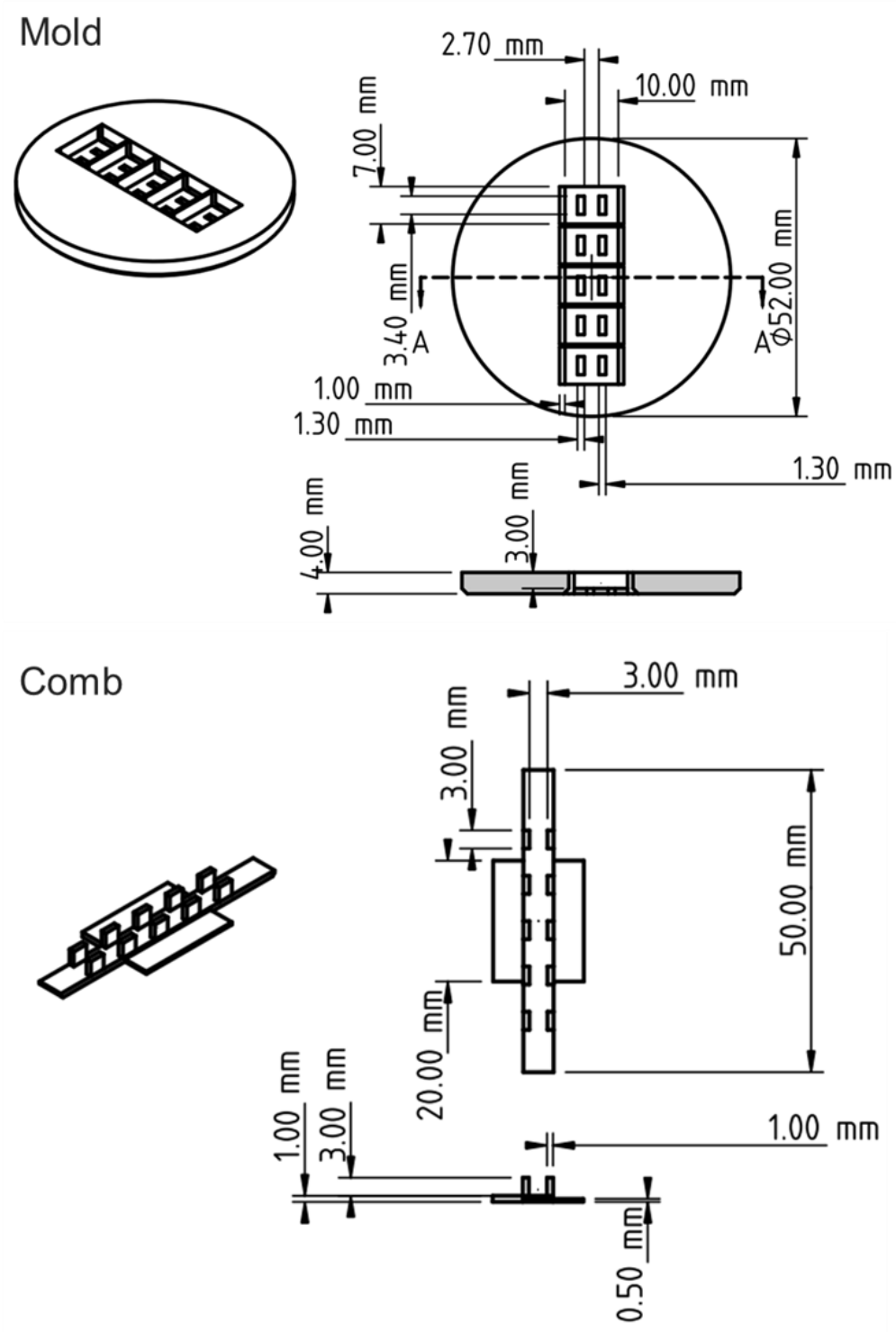


Figure S2. Blueprint of a gel mould for observation

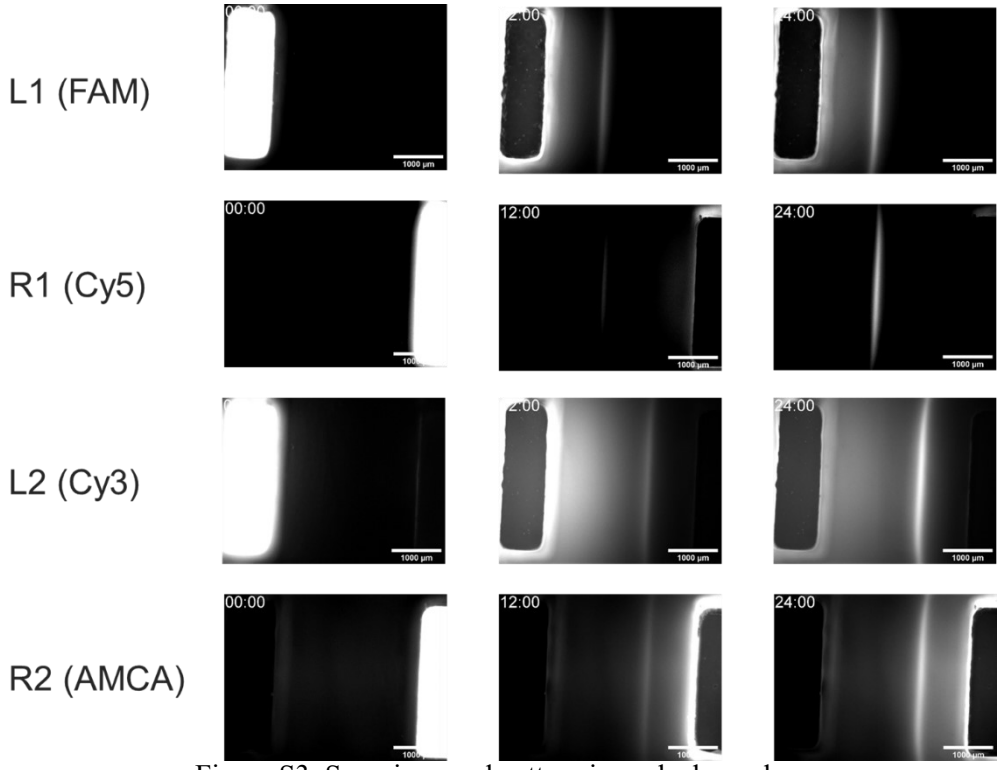


Figure S3. Superimposed pattern in each channel

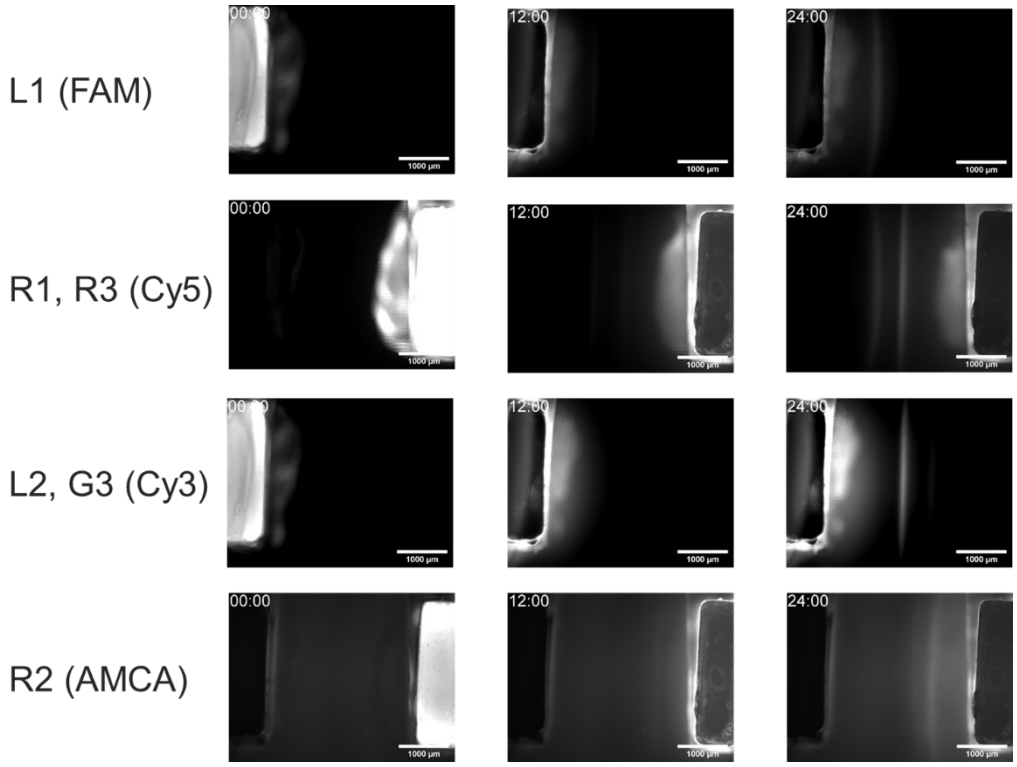


Figure S4. Cascaded pattern in each channel

Condition	Components (Each DNA is 10 μM)	Diffusion coefficient [μm^2/sec]
Experiment L1 a, b, c	L1	42.3
Experiment R1 a, b, c	R1	84.0
Experiment L1 + R1 a, b, c	L1 and R1	No recovery
Experiment L2 a, b, c	L2	46.4
Experiment R2 a, b, c	R2	66.4
Experiment L2 + R2 a, b, c	L2 and R2	No recovery
Experiment L3 a, b, c	L3	65.2
Experiment R3 a, b, c	R3	63.6
Experiment L3 + R3 a, b, c	L3 and R3	No recovery

Table S2. FRAP conditions and diffusion coefficients

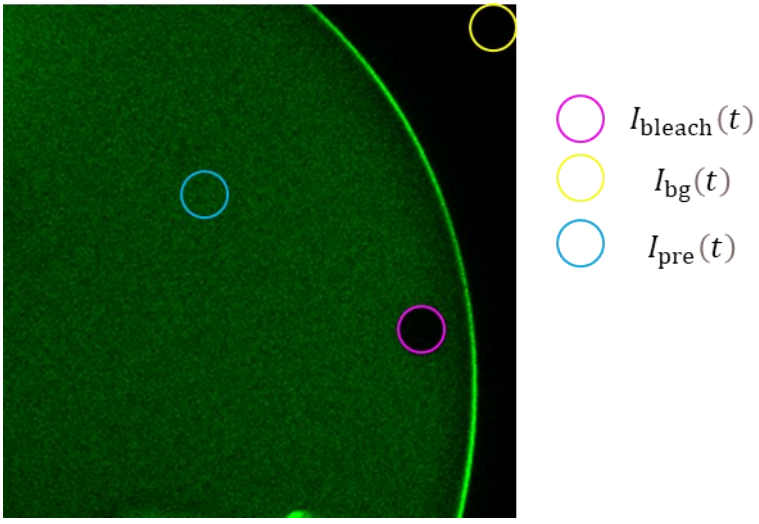
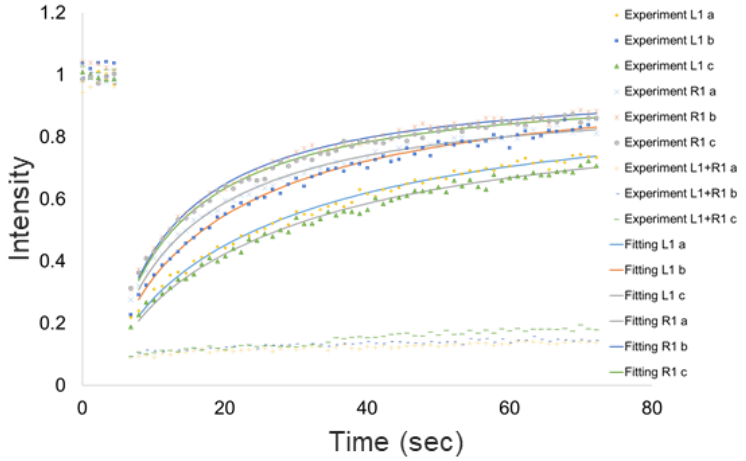
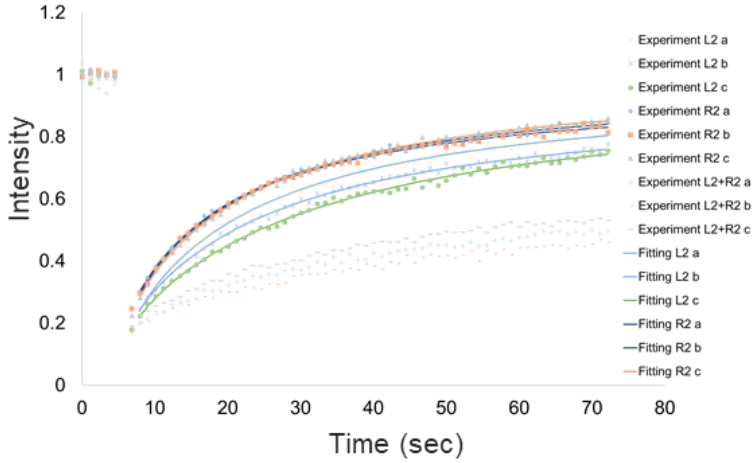


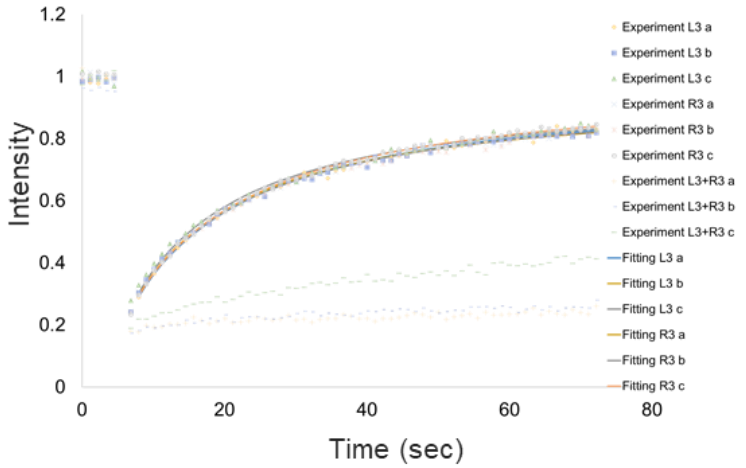
Figure S5. Referring regions for normalization in FRAP



L1 and R1



L2 and R2



L3 and R3

Figure S6. FRAP result

Condition	D_1 [μm²/sec]	k_h [/M/s]	k_c [/s]	Error value
1	10	3.5×10^{3}	1.0×10^{-3}	19.9
2	10	3.5×10^{3}	2.0×10^{-3}	21.5
3	10	3.5×10^{3}	3.0×10^{-3}	22.1
4	10	3.5×10^{4}	1.0×10^{-3}	4.24
5	10	3.5×10^{4}	2.0×10^{-3}	7.62
6	10	3.5×10^{4}	3.0×10^{-3}	11.0
7	10	3.5×10^{5}	1.0×10^{-3}	74.9
8	10	3.5×10^{5}	2.0×10^{-3}	52.1
9	10	3.5×10^{5}	3.0×10^{-3}	36.1
10	20	3.5×10^{3}	1.0×10^{-3}	10.4
11	20	3.5×10^{3}	2.0×10^{-3}	14.5
12	20	3.5×10^{3}	3.0×10^{-3}	16.0
13	20	3.5×10^{4}	1.0×10^{-3}	20.4
14	20	3.5×10^{4}	2.0×10^{-3}	3.69
15	20	3.5×10^{4}	3.0×10^{-3}	0.28
16	20	3.5×10^{5}	1.0×10^{-3}	576
17	20	3.5×10^{5}	2.0×10^{-3}	457
18	20	3.5×10^{5}	3.0×10^{-3}	367
19	40	3.5×10^{3}	1.0×10^{-3}	5.89
20	40	3.5×10^{3}	2.0×10^{-3}	11.5
21	40	3.5×10^{3}	3.0×10^{-3}	13.8
22	40	3.5×10^{4}	1.0×10^{-3}	76.9
23	40	3.5×10^{4}	2.0×10^{-3}	28.0
24	40	3.5×10^{4}	3.0×10^{-3}	9.20
25	40	3.5×10^{5}	1.0×10^{-3}	1302
26	40	3.5×10^{5}	2.0×10^{-3}	1079
27	40	3.5×10^{5}	3.0×10^{-3}	897

Table S7. Parameter fitting in simulation

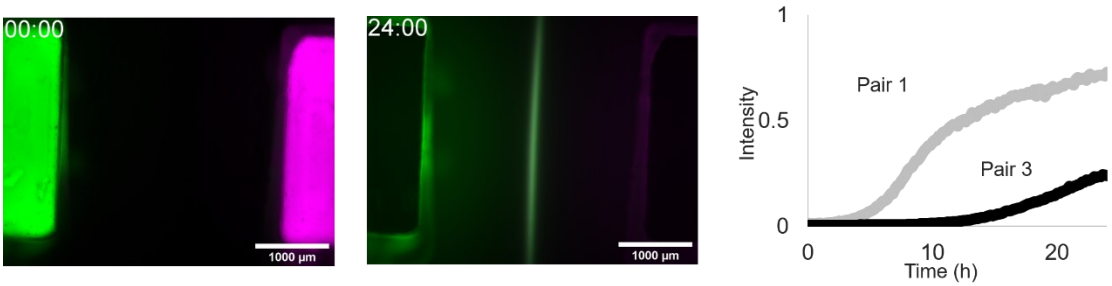


Figure S7. Pattern formation of pair 3

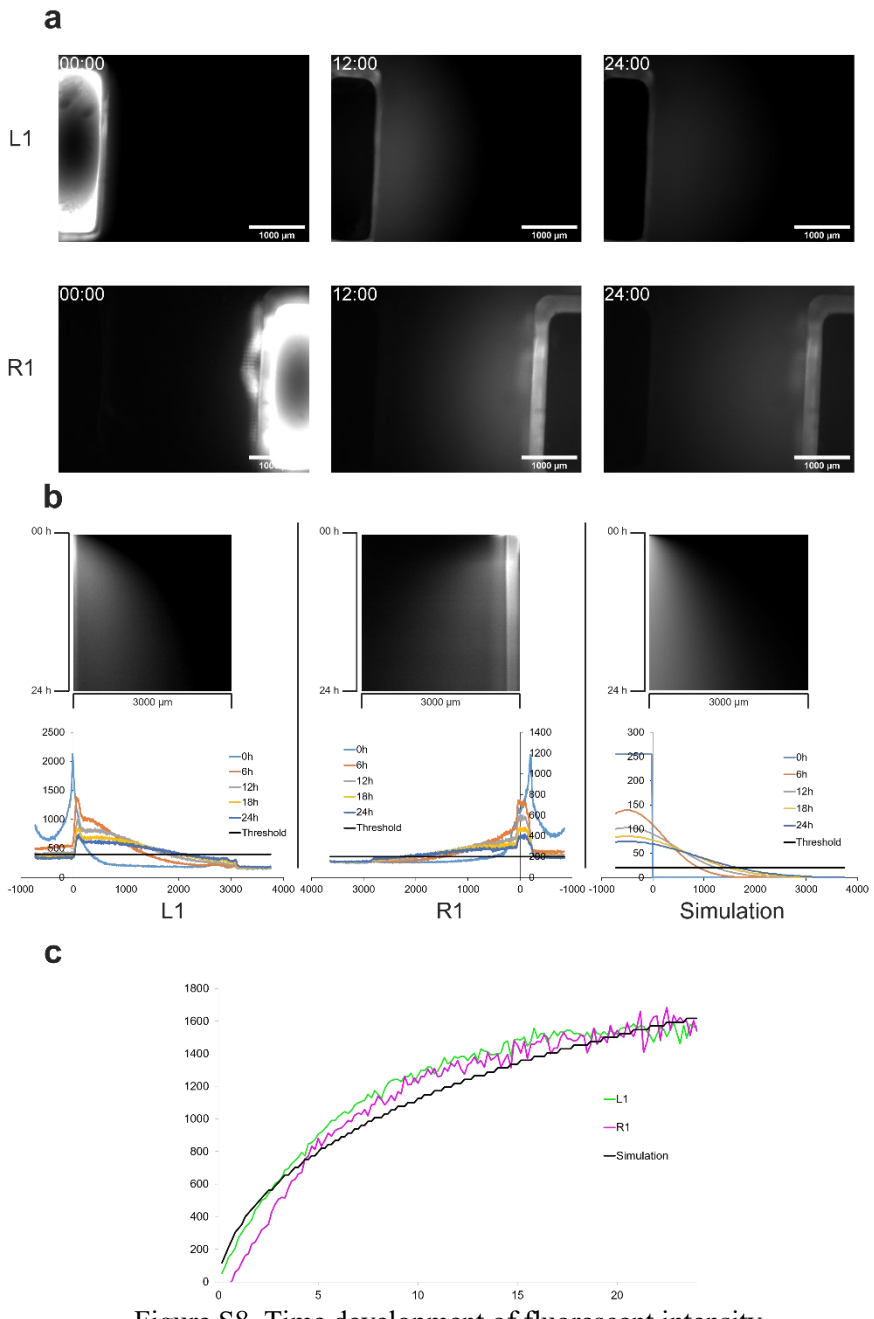


Figure S8. Time development of fluorescent intensity