Supporting Information

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SI Text

This will give complete details about *Materials and Methods* in the main text. In addition, we added supplemental data about probe design and other experimental results.

Chemicals and Reagents. DNA synthesis reagents were purchased from Glen Research. All chemical reagents for buffer preparation, posttreatment for oligo preparation and HPLC purification were from Fisher Scientific. A physiological buffer that resembles physiological conditions contained 25 mM Tris·HCl (pH 7.4), 150 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, and 5% (vol/vol) glycerol for the buffer experiment. Human α-thrombin was obtained from Haematologic Technologies. Fibrinogen was obtained from Sigma–Aldrich. Universal coagulation reference plasma (UCRP) and thromboplastin-DL for human sample testing were purchased from Pacific Hemostasis.

Preparation of Probes. To optimize the number of complementary base pairs and azobenzenes incorporated into the complementary sequences of 15Apt, we designed, prepared, and characterized a series of molecular probes, which are shown in Table S1. The candidates were synthesized by using an ABI 3400 DNA/RNA synthesizer (Applied Biosystems) at 1-μmol scale with the standard synthesis protocol. For complete cleavage and deprotection, overnight incubation with ammonium hydroxide was used. After the deprotection step, ethanol precipitation was performed in the standard procedure. The precipitates were then dissolved in 0.5 mL of 0.1 M triethylammonium acetate (TEAA, pH7.0) for further purification with high-performance liquid chromatography (HPLC). The HPLC was performed on a ProStar HPLC Station (Varian) equipped with a fluorescence detector and a photodiode array detector. A C-18 reverse-phase column (C18, 5 μM, 250 $\times$ 4.6 mm; Alltech) was used.

Real-Time Monitoring of Clotting Reaction. To monitor the clotting time alteration in response to different light treatments, we designed a simple buffer experiment that contained only thrombin, one of the nucleic acid inhibitors, and fibrinogen substrate in physiological buffer. The underlying principle of the experiment is based on the mixture of sample, which becomes nonfluidic and tends to scatter light by fibrin aggregation, the product of thrombin's catalytic reaction. As a result, the clotting process can be measured by the decrease of light penetration through the sample cell. To compare the efficacy of inhibition, each probe was pretreated with UV (365 nm) or Vis (white light). The mixture containing 1 μL of 10 μM thrombin and 1 μL of a 100 μM concentration of each inhibitor in 200 μL of physiological buffer was then incubated for 10 min under either UV (365 nm) or Vis (500 nm) light in the spectrometer. In sequence, 4 μL of 20 mg/ml fibrinogen was quickly added and mixed while the UV spectrometer was monitoring either the UV or the Vis wavelength as a function of time. Reaction mixtures containing only thrombin and fibrinogen with or without 15Apt were always tested together with other samples as internal standards. All clotting times were normalized based on the internal standard and compared with it.

Human Plasma Tests. To evaluate the feasibility of the inhibitor as a potential anticoagulant reagent, we determined PT for each ligand using human plasma samples. Reaction mixtures were prepared in a manner similar to the clotting tests described above, except that the initially scattered light at a 90 degree angle was monitored on a Fluorolog-3 spectrofluorometer (Jobin Yvon) equipped with the circulating temperature controller to maintain a 37 °C reaction temperature. A 100-μL quartz fluorescence cuvette (Starna Cells) was used as a sample container, and the excitation and emission wavelengths were both set at 500 nm for the Vis-treated sample and 365 nm for the UV-treated sample to minimize unwanted isomerization. Procedures applied were those recommended by the manufacturer. For PT determination, 50 μL of UCRP was preincubated at 37 °C with a different amount of each ligand for 2 min; then 50 μL of thromboplastin-L was added to initiate the extrinsic clotting cascade, and the scattering intensity was monitored until the signal reached a plateau. For the calculation of PT, the end time was determined to be the point where scattering signal was half way between the lowest and maximum values. Each sample was repeated twice, and each set of experiments was performed with a single batch of plasma.

Monitoring Site-Specific Activation of Enzymatic Reaction in Microfluidic Channel. To demonstrate site-specific activation of thrombin's activity, we used a microfluidic channel to create a 2-dimensional model of the clotting process using fluorescent fibrinogen to visualize fibrin networks. Fibrinogen was labeled with Alexa Fluor 488. Fibrinogen (10 mg) was dissolved in 1 mL of 0.1 M sodium bicarbonate buffer. Then, 0.5 mg of Alexa Fluor 488 in dimethyl sulfoxide (DMSO) was added to the protein, followed by stirring for 1 h. The conjugated protein was separated from unreacted Alexa Fluor 488 by using a Nap-25 column (GE Healthcare Bio-Sciences). The purified protein had about a single fluorophore on each protein.

Microfluidic channels were prepared by the following procedure. Microscope slides and 18-mm-square no. 1 coverglasses (Fisher) were rinsed with deionized-H_2O and dried under nitrogen. Strips of double-sided tape (3M) were placed 4 mm apart on a microscope slide, and the coverglass was placed on top. Devices were filled by capillary action. Solution exchange was performed by simultaneously pipetting solution at one end and withdrawing fluid from the other end with P8 filter paper (Fisher). An Olympus FV500-IX81 confocal microscope was used to both illuminate and image specific regions of the channel using a 10$\times$ objective and the 494-nm laser line. The length scale of the 10$\times$ field of view was calibrated by using a micrometer, and the image was 1.286 $\times$ 1.286 mm. A human plasma sample (10 μL) was prepared for activated partial thromboplastin time (aPTT) measurement containing 10 μM of *cis*-probes and Alexa-488-labeled fibrinogen, and the mixture was loaded into the channel and incubated at room temperature. The aPTT is a performance indicator for measuring the efficacy of both the "intrinsic" pathway (now referred to as the contact activation pathway) and the common coagulation pathway. The fluorescent time-lapse images were obtained every 15 s until the reaction was completed.

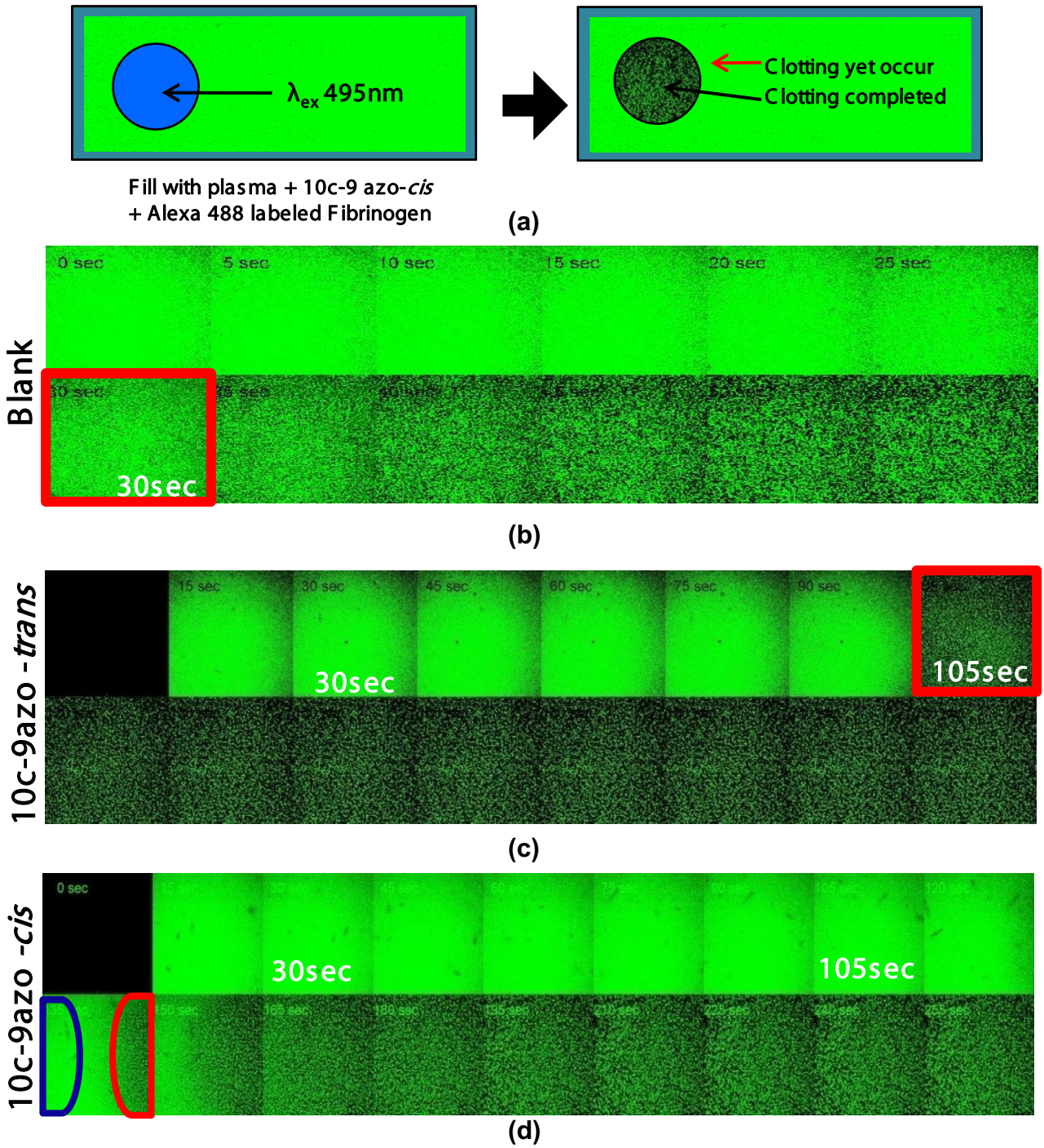

Fig. S1. Imaging clotting reaction in a microfluidic device. The cartoon describes the experiment (*A*), the obtained images (*B–D*) represent no probe (*B*), 10c-9azo-*trans*- (*C*), and 10c-9azo-*cis*-containing (*D*) human plasma samples.

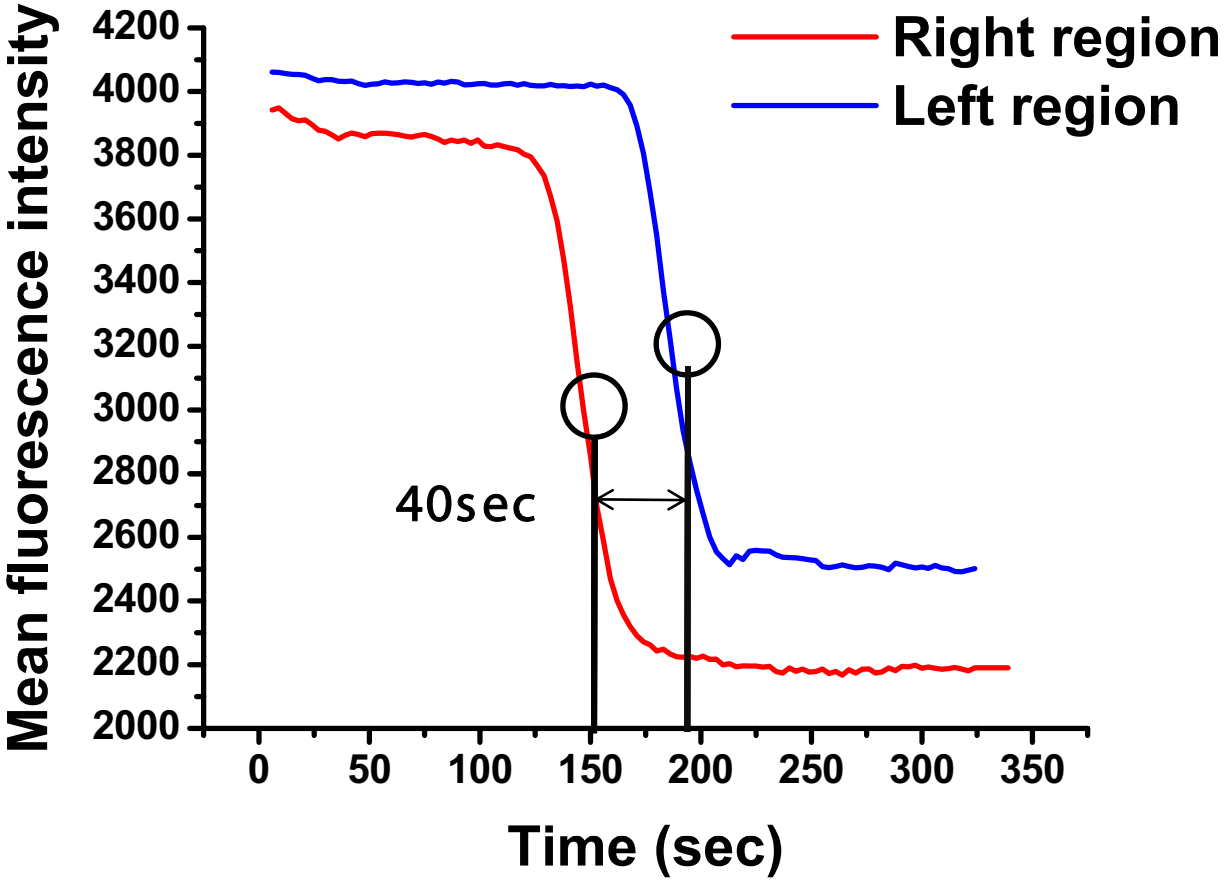

Fig. S2. Fluorescence intensity alteration of different zones in real time.

Table S1. Sequences of probes

Probe	Sequence
16c-15azo	5′-Dabcyl-A**A**C**A**C**A**A**A**A**A**C**A**C**A**A**A**C**A**A**A**C**A**C**A**A**A**A**A**C**A**C-(S)5-GGTTGGTGTGGTTGGT-Fam-3′
16c-14azo	5′-Dabcyl-AC**A**C**A**A**A**A**A**C**A**C**A**A**A**C**A**A**A**C**A**C**A**A**A**A**A**C**A**C-(S)5-GGTTGGTGTGGTTGGT-Fam-3′
14c-13azo	5′-Dabcyl- A**A**C**A**C**A**A**A**A**A**C**A**C**A**A**A**C**A**A**A**C**A**C**A**A**A**A -(S)5-GGTTGGTGTGGTTGGT-Fam-3′
12c-11azo	5′-Dabcyl-A**A**C**A**C**A**A**A**A**A**C**A**C**A**A**A**C**A**A**A**C**A**C-(S)5-GGTTGGTGTGGTTGGT-Fam-3′
10c-9azo	5′-Dabcyl-A**A**C**A**C**A**A**A**A**A**C**A**C**A**A**A**C**A**A-(S)5-GGTTGGTGTGGTTGGT-Fam-3′
10c-4azo	5′-Dabcyl-AC**A**CA**A**AC**A**CA**A**CA-(S)5-GGTTGGTGTGGTTGGT-Fam-3′
9c-8azo	5′-Dabcyl-A**A**C**A**C**A**A**A**A**A**C**A**C**A**A**A**C-(S)5-GGTTGGTGTGGTTGGT-Fam-3′
9c-7azo	5′-Dabcyl-AC**A**C**A**A**A**A**A**C**A**C**A**A**A**C**A**A-(S)5-GGTTGGTGTGGTTGGT-Fam-3′
8c-4azo	5′-Dabcyl-A**A**C**A**CA**A**AC**A**CA-(S)5-GGTTGGTGTGGTTGGT-Fam-3′
7c-6azo	5′-Dabcyl-A**A**C**A**C**A**A**A**A**A**C**A**C-(S)5-GGTTGGTGTGGTTGGT-Fam-3′
7c-4azo	5′-Dabcyl-A**A**C**A**C**A**A**A**A**A**CC-(S)5-GGTTGGTGTGGTTGGT-Fam-3′
7c-3azo	5′-Dabcyl-A**A**CC**A**A**A**A**A**CC-(S)5-GGTTGGTGTGGTTGGT-Fam-3′
6c-5azo	5′-Dabcyl-A**A**C**A**C**A**A**A**A-(S)5-GGTTGGTGTGGTTGGT-Fam-3′
6c-4azo	5′-Dabcyl-A**A**C**A**C**A**A**A**AC-(S)5-GGTTGGTGTGGTTGGT-Fam-3′
6c-3azo	5′-Dabcyl-AC**A**C**A**A**A**AC-(S)5-GGTTGGTGTGGTTGGT-Fam-3′

A in bold type refers to azobenzene.

Table S2. Characterized properties of each probe

Name	Azobenzenes per target, %	T_m average, °C	Response
16c-15azo	48	71 ± 0	O
16c-14azo	47	69 ± 1	O
14c-13azo	48	63 ± 1.5	O
12c-11azo	48	65 ± 0.71	O
10c-9azo	47	62 ± 0.71	O
10c-4azo	29	76 ± 0	X
9c-8azo	47	51 ± 0	O
9c-7azo	44	52 ± 0.71	O
8c-4azo	33	75 ± 0.71	X
7c-6azo	46	48 ± 2.9	O
7c-4azo	36	55 ± 0	X
7c-3azo	30	66 ± 1.2	X
6c-5azo	45	43 ± 2.5	O
6c-4azo	40	51 ± 1.7	Δ
6c-3azo	33	66 ± 1.7	X

Each probe was named Xc-Yazo, where X is the number of complementary sequences, and Y is the number of azobenzene moieties in the regulatory domain. O, Δ, and X represent the difference of relative reaction rate between UV and Vis is greater than 5%, between 5% and 0%, and 0%, respectively.