(Electronic Supplementary Information)

Supramolecular hydrogel containing boronic acid-appended receptor for fluorocolorimetric sensing of polyols with paper platform

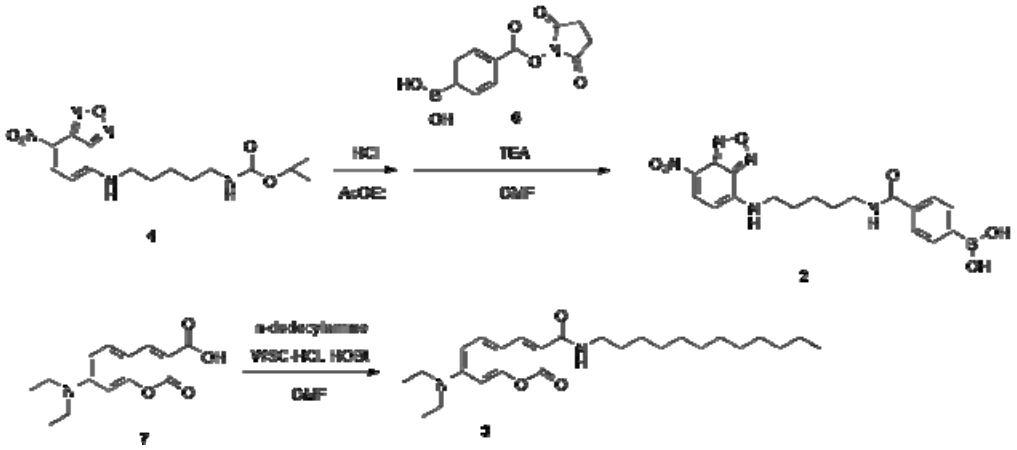
Masato Ikeda, Keisuke Fukuda, Tatsuya Tanida, Tatsuyuki Yoshii, Itaru Hamachi*

[†]Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University, Katsura, Kyoto, 615-8510, Japan. [§]Japan Science and Technology Agency (JST), CREST, 5 Sanbancho, Chiyoda-ku, Tokyo, 102-0075, Japan.

E-mail: ihamachi@sbchem.kyoto-u.ac.jp

Generals. Unless stated otherwise, all commercial reagents were used as received. All water used in the experiments refers to ultra pure water obtained from a Millipore system having a specific resistance of 18 MΩ•cm unless otherwise specified. Thin layer chromatography (TLC) was performed on silica gel $60F_{254}$ (Merck). Column chromatography was performed on silica gel 60N (Kanto, 40–50 μm). ^{1}H NMR spectra were obtained on a Varian Mercury 400 spectrometer with tetramethylsilane (TMS) or residual non-deuterated solvents as the internal references. Multiplicities are abbreviated as follows: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, m = multiplet, dd = double doublet, br = broad. ESI mass spectrometry was performed on a Thermo Scientific Exactive orbitrap mass spectrometer. The absorption and fluorescence spectra were measured using a Shimadzu UV2550 and a Perkin-Elmer LS55 spectrometer, respectively. Fluorescence spectra of hydrogel spots of a filter paper were recorded using an Otsuka Electronics high sensitivity Spectro multichannel photodetector, MCPD-7000.

Synthesis. Compounds **4**[S1], **6**[S2], and **7**[S3] were synthesized according to the method reported for the similar compound.



Scheme S1

Synthesis of NBD-B (2). To a solution of compound **4** (140 mg, 0.33 mmol) in ethyl acetate (AcOEt, 2.0 mL) was added 3 M aqueous HCl (0.4 mL) solution. The mixture was stirred at 60 ºC for 2.5 h. Then the solution was concentrated under reduced pressure and the residue was washed with $CHCl_3$ for several times to give de-protected amine compound (74 mg) as a brown solid. ^{1}H NMR (400 MHz, CD_3OD, room temperature): δ = 1.51–1.58 (m, 2H), 1.73 (quin, J = 7.2 Hz, 2H), 1.84 (quin, J = 7.2 Hz, 2H), 2.95 (t, J = 7.2 Hz, 2H), 3.50–3.64 (m, 2H), 6.36 (d, J = 8.8 Hz, 1H), 8.53 ppm (d, J = 8.8 Hz, 1H). To a solution of the de-protected compound (74 mg, 0.25 mmol) and compound **6** (113 mg, 0.43 mmol) in dry DMF (5 mL) was added TEA (52 μL, 0.38 mmol) and the mixture was stirred at room temperature overnight under an atmosphere of N_2. Then the solution was concentrated under reduced pressure, diluted with AcOEt (50 mL), and washed with water (30 mL × 2) and brine (30 mL). The organic layer was collected and dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated and the residues was purified by column chromatography (SiO_2, $CHCl_3$:MeOH = 15:1) to give compound **2** (10 mg, 7%) as a orange solid. ^{1}H NMR (400 MHz, CD_3OD, room temperature): δ = 1.50–1.58 (m, 2H), 1.71 (quin, J = 7.2 Hz, 2H), 1.84 (quin, J = 7.2 Hz, 2H), 2.95 (t, J = 7.2 Hz, 2H), 3.46–3.64 (m, 2H), 6.32 (d, J = 8.8 Hz, 1H), 7.58–7.84 (m, 4H), 8.47 ppm (d, J = 8.8 Hz, 1H). HR-FTMS (ESI): Calcd. for $[M(C_{18}H_{20}BN_5O_6)+Na]^+$: m/z = 436.1404; Found: 436.1411.

Synthesis of Coum-C_{12} (3). To a solution of compound **8** (66 mg, 0.25 mmol) in dry

DMF (5 mL) was added water soluble carbodiimide hydrochloride (WSC-HCl, 1-ethyl-3-(3-dimethyl aminopropyl)carbodiimde hydrochloride, 69 mg, 0.33 mmol) and 1-hydroxybenzotriazole monohydrate (HOBt•H_2O, 55 mg, 0.33 mmol) and the mixture was stirred at room temperature under an atmosphere of N_2. After 15 minutes, to the solution was added *n*-dodecylamine (66 mg, 0.36 mmol) and *N*,*N*-diisopropylethylamine (DIEA, 131 μL, 0.75 mmol) and the mixture was stirred overnight at room temperature under an atmosphere of N_2. Then the solvent was removed under reduced pressure. The residue was dissolved in $CHCl_3$ (30 mL) and the solution was washed with water (50 mL × 3). The organic layer was collected and dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated to dryness and the residue was purified by column chromatography (SiO_2, $CHCl_3$) to afford compound **3** as a yellow solid (79 mg, 74%). ^{1}H NMR (400 MHz, $CDCl_3$, room temperature): δ = 0.87 (t, J = 7.2 Hz, 3H), 1.21–1.31 (m, 24H), 1.67–1.74 (m, 2H), 3.41–3.64 (m, 6H), 6.57 (s, 1H), 6.63 (d, J = 9.6 Hz, 1H), 7.42 (d, J = 9.6 Hz, 1H), 8.70 (s, 1H), 8.77 ppm (br s, 1H). HR-FTMS (ESI): Calcd. for $[M(C_{26}H_{40}N_2O_3)+Na]^+$: m/z = 451.2937; Found: 451.2944.

Preparation of supramolecular hydrogel containing 2 and 3. A suspension of gelator **1** (2.0 mg) in 50 mM HEPES buffer (pH 7.2, 1.0 mL) was heated to form homogeneous solution and allowed to cool to room temperature. To this solution (976 μL) was added DMSO stock solutions of NBD-B (**2**) (3.5 mM, 13 μL) and coum-C_{12} (**3**) (5.5 mM, 11 μL). The resultant solution (10 μL)) was poured into wells of a 384-well plate (Greiner, Flat Bottom Black) and gelated upon the addition of aqueous $CaCl_2$ solution (32 mM, 1.0 μL) to the each well. The plate was sealed and incubated to complete gelation in a sealed box with high humidity at room temperature for 15 min and each substrate solution (1.0 μL) at designed concentrations was added to the each well. After 15 min, the plate was subjected to fluorescence measurement by using a microplate reader (TECAN, Infinite M200, excitation wavelength = 400 nm).

Preparation of gel-based sensor paper. A similar solution containing **1** NBD-B (**2**) and coum-C_{12} (**3**) prepared as described above was spotted on a filter paper (Whatman, Grade 4, each spot contains 1.0 μL) where aqueous $CaCl_2$ solution (324 mM, 1.0 μL) was spotted beforehand. After drying the paper in the air for several minuets, substrate solution (5.0 μL) at designed concentrations was spotted to a spot. The photographs of

the sensor paper were collected by using a digital camera (Olympus, E-PL2) equipped with a cut-off filter (<440 nm)) in the front of the lens under UV irradiation using a handy lamp (395 nm).

CLSM observation for gel-based sensor paper. A gel-based sensor paper prepared as described above was put into a glass-bottom dish (Matsunami, non-coat, 0.15–0.18 mm-glass bottom). The samples were subjected to observations using an inverted confocal laser scanning microscope (Olympus FV10i).

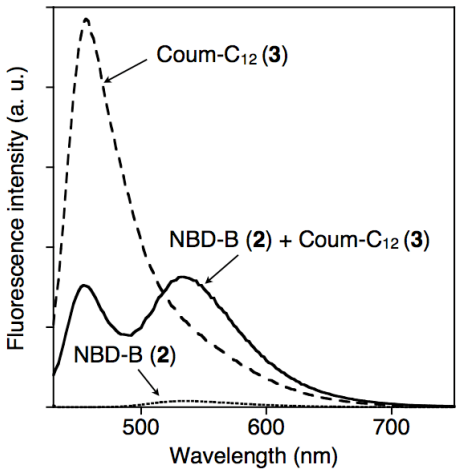


Fig. S1 Fluorescence spectral change (λ_{ex} = 400 nm) of hydrogel **1** containing boronic acid receptor **2** or FRET donor **3** and the both of **2** and **3**. Conditions: [**2**] = 38 μM, [**3**] = 50 μM, [**1**] = 0.2 wt%, [Ca^{2+}/**1**] = 1.0, 50 mM HEPES (pH 7.2) containing 2vol% DMSO, room temperature (RT).

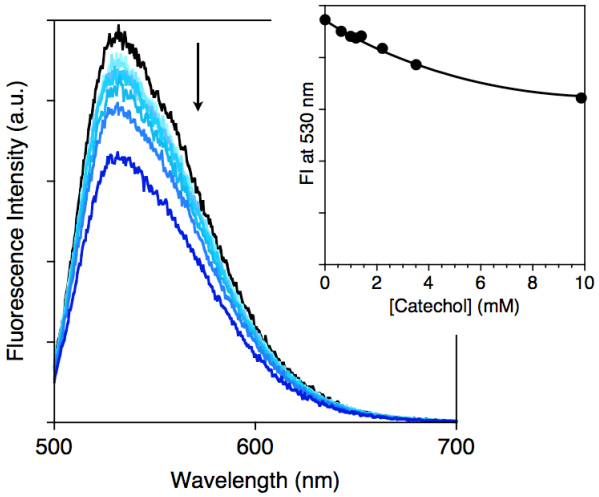


Fig. S2 Fluorescence spectral change (λ_{ex} = 400 nm) of **2** in aqueous buffered solution upon the addition of catechol. Conditions: [**2**] = 38 μM, [catechol] = 0, 0.62, 0.99, 1.2, 1.4, 2.2, 3.5, 9.9 mM, 50 mM HEPES (pH 7.2) containing 1vol% DMSO, RT.

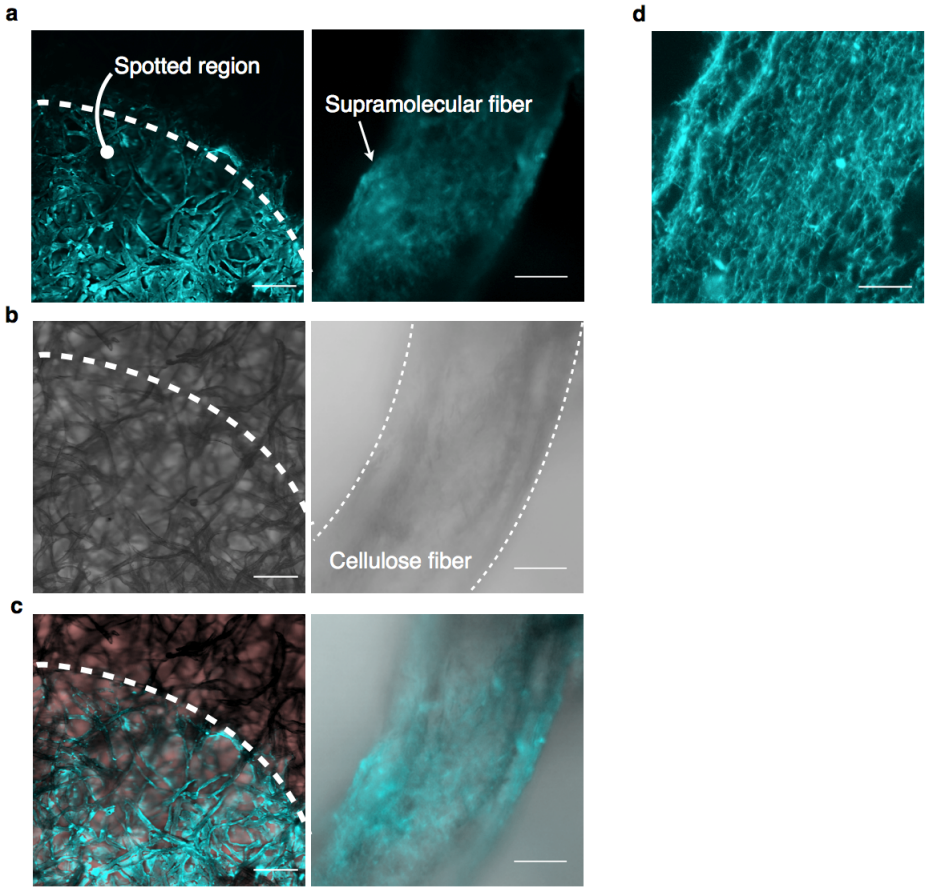


Fig. S3 CLSM images of (a,b,c) a paper containing gel **1**•Ca^{2+} and **3** and (d) gel **1**•Ca^{2+} containing **3** without paper. (a,d: fluorescence image, λ_{ex} = 405 nm, b: phase contrast image, c: merged image). (a,b,c) the scale bars are 200 μm (left) and 10 μm (right). (d) the scale bar is 10 μm. Conditions for (a,b,c): spotted aqueous $CaCl_2$ solution (1.0 μL): [$CaCl_2$] = 324 mM ([Ca^{2+}/**1**] = 100), spotted sol (1.0 μL): [**3**] = 30 μM, [**1**] = 0.2 wt%, 50 mM HEPES (pH 7.2) containing 6vol% DMSO. (d): [**3**] = 50 μM, [**1**] = 0.2 wt%, [Ca^{2+}/**1**] = 1.0, 50 mM HEPES (pH 7.2) containing 6vol% DMSO, RT.

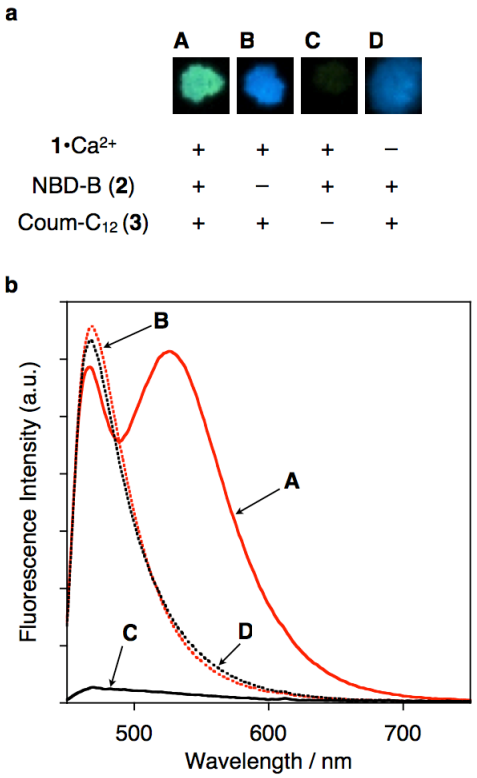


Fig. S4 (a) Photograph (λ_{ex} = 395 nm) of gel-based sensor paper and (b) the corresponding fluorescence spectra (MCPD, λ_{ex} = 395 nm). Conditions: spotted aqueous $CaCl_2$ solution (1.0 μL): [$CaCl_2$] = 324 mM, spotted sol (1.0 μL) for (**A**): [**2**] = 20 μM, [**3**] = 30 μM, [**1**] = 0.2 wt%, 50 mM HEPES (pH 7.2) containing 6vol% DMSO, (**B**): [**2**] = 0 μM, [**3**] = 30 μM, [**1**] = 0.2 wt%, 50 mM HEPES (pH 7.2) containing 1vol% DMSO, (**C**): [**2**] = 20 μM, [**3**] = 0 μM, [**1**] = 0.2 wt%, 50 mM HEPES (pH 7.2) containing 5vol% DMSO, (**D**): [**2**] = 20 μM, [**3**] = 30 μM, 50 mM HEPES (pH 7.2) containing 6vol% DMSO, RT.

References

S1 S. Taliani, F. Simorini, V. Sergianni, C. L. Motta, F. D. Settimo, B. Cosimelli, E. Abignente, G. Greco, E. Novellino, L. Rossi, V. Gremigni, R. Spinetti, B. Chelli and C. Martini, *J. Med. Chem.,* 2007, **50**, 404–407.

S2 L. Hu, K. Maurer and K. D. Moeller, *Org. Lett.,* 2009, **11**, 1273–1276.

S3 A. Song, X. Wang and K. S. Lam, *Tetrahedron Lett.,* 2003, **44**, 1755–1758.