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A supramolecular hydrogel containing boronic acid-appended receptor for fluorocolorimetric sensing of polyols with a paper platform†

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A boronic acid-appended fluorescent receptor was incorporated into self-assembled nanofibers containing a hydrophobic FRET-paired dye to develop a gel-based fluorocolorimetric sensor for polyols. We demonstrated that the gel-based sensor is capable of detecting polyols such as catechol and dopamine not only under semi-wet conditions, but also under dry conditions using a paper platform.

FRET (fluorescence resonance energy transfer)-based fluorescence sensing enables us to monitor a specific analyte accurately by two-wavelength emission ratiometry as well as visually by fluorescence color change.[1] Recently, we have developed a unique approach for constructing a fluorescence sensing system endowed with FRET readout by taking advantage of cooperative action of fluorescent molecular receptors and self-assembled nanofibers in supramolecular hydrogels.[2–4] The sensing principle is simple, that is based on the translocation of the receptors in the gel matrix, when an analyte induces localization change of the receptors between hydrophobic nanofibers and aqueous phase, a ratiometric fluorescence signal change will be observed by the substantial change in the average distance between the fluorescent receptor and a corresponding FRET-paired dye embedded in the nanofibers.

In this study, we introduced a boronic-acid appended fluorescent receptor (**2**) into a supramolecular hydrogel **1**[5] with an aim of sensing polyols such as dopamine and sugars. The boronic acid moiety of **2** can form ester complexes with these polyols in aqueous media concurrently with an anionic charge generation on its boron atom,[6] which makes the receptor more hydrophilic. Therefore, the receptor should migrate from a hydrophobic site of nanofiber **1** to the more hydrophilic sites or aqueous phase upon complexation with polyols, which induces fluorescence color change (Fig. 1c). Here we describe that the gel-based sensing system is capable of fluorocolorimetrically detecting polyols such as catechol derivatives. Moreover, we succeeded in incorporating the gel-based sensing system into a filter paper without notable loss of its sensing ability even after drying, which can be envisioned as a lightweight and portable sensor chip for monitoring the polyols with the naked eye.

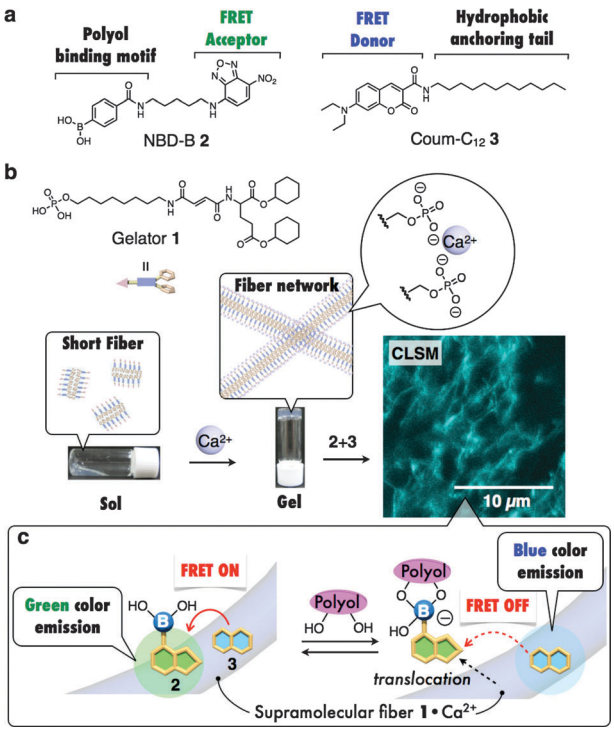

Fig. 1 (a) Chemical structures of boronic acid receptor **2** and FRET donor **3**. (b) Self-assembly of hydrogelator **1** to form supramolecular fibers (supramolecular hydrogel) induced by Ca^{2+} ion complexation. Confocal laser scanning microscopic (CLSM) image of supramolecular nanofiber **1**·Ca^{2+} containing **3** ([**1**] = 0.2 wt%, [Ca^{2+}/**1**] = 1.0, [**3**] = 30 μM, 50 mM HEPES (pH 7.2)). (c) Schematic representation of translocation of receptor **2** upon the binding of a polyol from hydrophobic interior of the nanofiber **1**·Ca^{2+} containing FRET donor **3** to aqueous phase, which leads to the change in FRET efficiency.

As a gel matrix, we employed a phosphoric acid appended supramolecular hydrogelator **1** which forms a stable hydrogel in the presence of a Ca^{2+} ion (Fig. 1b).[5] A boronic acid receptor bearing 7-nitrobenzoxa[1,2,5]diazole (NBD) **2** (NBD-B) for polyol binding and a hydrophobic coumarin dye **3** (Coum-C_{12}) for a suitable FRET donor of NBD were introduced into the

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supramolecular hydrogel **1**·Ca^{2+} (Fig. 1a and c). Fig. 2a shows typical fluorescence spectral change of the supramolecular hydrogel **1**·Ca^{2+} containing NBD-B **2** and Coum-C_{12} **3** upon polyol addition. Without polyols, two emission peaks at 454 nm and 534 nm assignable to coumarin and NBD dye, respectively, were observed with almost the same intensity, typical for the FRET system (F_{454}/F_{534} = 0.95, solid line).[7] With the increase in polyol concentration, the peak at 534 nm gradually decreased and the peak at 454 nm was concurrently intensified, so that the ratio of F_{454}/F_{534} increased to 2.5 for fructose (dotted line) and 2.3 for catechol (dashed line).[8] This seesaw type of spectral change is ascribable to cancellation of FRET upon polyol binding to **2**, suggesting that the average distance between **2** and **3** increased, most probably due to migration of **2** to the more hydrophilic space upon binding of the polyols. In contrast, NBD-B **2** alone in an aqueous solution showed only a slight change in its fluorescence by the addition of catechol (Fig. S2, ESI†). These results indicate that the self-assembled nanofiber **1**·Ca^{2+} is essential as a nano-platform for the present polyol sensing system.

Fig. 2b summarizes titration curves of the gel-based sensing material for various substances, which were obtained using a 384-well plastic micro-plate. The titration curves provided the apparent binding constants for each polyol as follows: 155 ± 28 M^{-1} for catechol, 157 ± 32 M^{-1} for dopamine, 22 ± 2.7 M^{-1} for fructose and 3.3 ± 1.6 M^{-1} for mannitol. The sensing selectivity, in order of catechol, dopamine > fructose, mannitol ≫ glucose, is in good agreement with the selectivity of boronic acid derivatives in aqueous solution reported previously.[6]

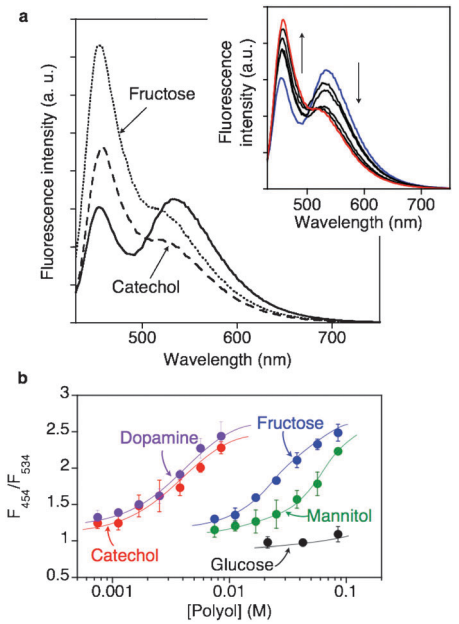

Fig. 2 (a) Fluorescence spectral change (λ_{ex} = 400 nm) of hydrogel **1**·Ca^{2+} containing **2** and **3** upon the addition of polyols ([Fructose] = 85 mM, [Catechol] = 8.5 mM). Inset shows a fluorescence spectral change upon the addition of catechol (0–8.5 mM). (b) Plots of the fluorescence intensity ratio (F_{454}/F_{534}) of hydrogel **1**·Ca^{2+} containing **2** and **3** upon the addition of various substances. *Reaction conditions*: [**2**] = 38 μM, [**3**] = 50 μM, [**1**] = 0.2 wt%, [Ca^{2+}/**1**] = 1.0, 50 mM HEPES (pH 7.2) containing 2 vol% DMSO, room temperature.

Next, we sought to integrate this sensing system into a filter paper to prepare a thin, lightweight, and portable gel-based sensor paper. Integration of a sensing system into a robust solid matrix such as paper and polymer without loss of sensing function is practically important to fabricate sensor devices and is still challenging.[9] Here, the gel-based sensor paper was prepared by simply spotting a gel precursor aqueous solution containing **2** and **3** on a cellulose-based filter paper, on which aqueous $CaCl_2$ solution was spotted beforehand (Fig. 3a).[10] When an aqueous analyte solution (10 mM, 5 μL) was dropped on the gel-based sensor paper, followed by excitation with UV light (a handy LED lamp, λ_{ex} = 395 nm), blue emission at the spots corresponding to catechol, dopamine, and catechin could be distinguished by the naked eye within 15 min, whereas green emission was almost unchanged at spots corresponding to tyrosine, glucose, and fructose (Fig. 3b). This selective color change agrees well with the above-mentioned fluorescence spectral changes, demonstrating that the gel-based sensor paper can sense catechol derivatives visually and rapidly. Even after 1 week, comparable color change was observed, suggesting that the gel-based sensor paper can be stored. In addition, as shown in Fig. 3c, change in the fluorescence colors was clearly distinguished from green to blue in a concentration range of catechol comparable to that of the titration curve in Fig. 2b, suggesting no notable loss of sensitivity under dry conditions. In the absence of the supramolecular nanofiber **1**·Ca^{2+}, the initial color of the spot on the filter paper was not

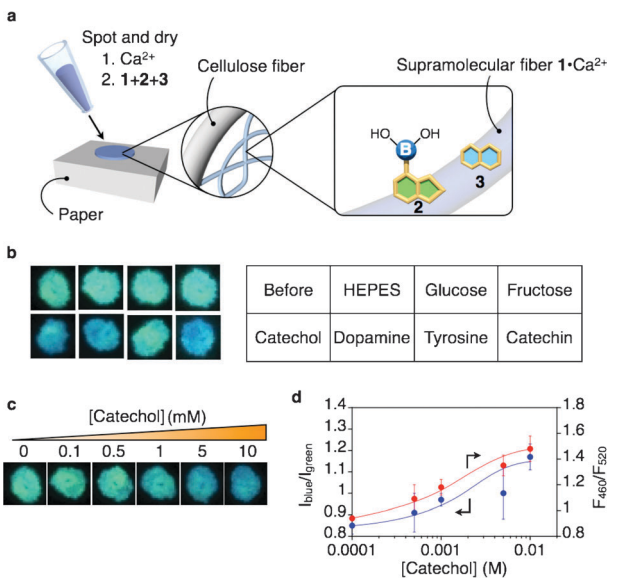

Fig. 3 (a) Schematic illustration showing a fabrication process of a gel-based sensor paper. The sensing spot comprises a porous matrix of hydrophilic cellulose fibers and supramolecular nanofibers **1**·Ca^{2+} containing **2** and **3**. (b) Photograph (λ_{ex} = 395 nm) of a gel-based sensor paper upon the addition of various substances (10 mM, 5.0 μL). The spotted position of substances is shown in the right panel. (c) Photograph of a gel-based sensor paper for monitoring catechol and the corresponding changes in the pixel intensity ratio (I_{blue}/I_{green}) and fluorescence intensity ratio (F_{460}/F_{520}). Error bars represent standard deviation (n = 3). *Reaction conditions*: spotted aqueous $CaCl_2$ solution (1.0 μL): [$CaCl_2$] = 324 mM, spotted sol (1.0 μL): [**2**] = 20 μM, [**3**] = 30 μM, [**1**] = 0.2 wt%, 50 mM HEPES (pH 7.2) containing 6 vol% DMSO.

greenish as that in the presence of nanofiber **1**·Ca^{2+} (Fig. S4, ESI†), supporting that efficient FRET between **2** and **3** didn't occur without the nanofiber **1**·Ca^{2+}. These results demonstrate that the supramolecular nanofiber **1**·Ca^{2+} can function as a robust and versatile nano-platform not only in a semi-wet gel system but also under dry conditions.

We demonstrated that the gel-based sensor is capable of detecting polyols such as catechol, dopamine, and catechin not only under semi-wet conditions, but also under dry conditions using a paper platform. Paper matrices such as paper chromatography, litmus paper, and so on have been used extensively in analytical and clinical chemistry. Very recently, patterned paper devices bearing microfluidic channels inside them have been actively developed to control the flow of solutions.[11] Although the present sensor still needs to be improved further, especially in terms of sensitivity and color discrimination for practical applications,[12] we expect that supramolecular nanofiber platforms enable us to fabricate hydrophobic nano-domains inside paper devices and thus offer a unique opportunity to develop rapid and inexpensive diagnostic tools.

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10 CLSM images of the gel-based sensor paper showed fluorescence of **3** along the surface of cellulose fibers in the filter paper matrix (Fig. S3, ESI†), suggesting that nanofiber **1**·Ca^{2+} is entangled with the cellulose fibers.

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