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Three Distinct Reading-out Modes for Enzyme Activity can be Operated in Semi-wet Supramolecular Hydrogel

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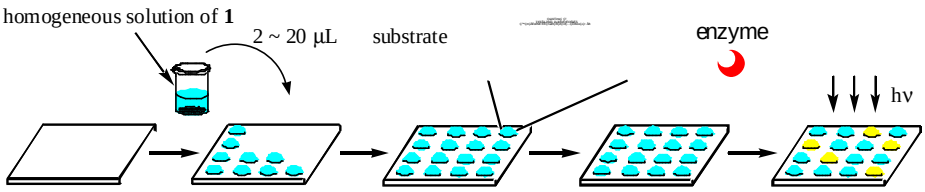
Synthesis of the trypsin inhibitors

4-*n*-Hexylbenzamidine hydrochloride. Following a procedure reported in a literature,[18a] to suspension of NH_4Cl (250 mg, 4.7 mmol) in dry toluene (2 mL) 2 M Me_3Al in toluene (2.15 mL, 4.3 mmol) was dropped slowly via a syringe under a N_2 atmosphere at 0°C, then the mixture was stirred at rt for 1 h to afford a homogeneous solution. After the addition of 4-*n*-hexylbenzonitrile (486 mg, 2.5 mmol) to the solution, the solution was stirred at 80°C for overnight. After cooling to rt, the solution was poured into a suspension of SiO_2 (1.5 g) in $CHCl_3$ (5 mL), and then the mixture was stirred at rt for 5 min. MeOH (5 mL) was added to the mixture, and the mixture was stirred at rt for 1 h. The precipitate was removed by the filtration and was eluated by MeOH. The filtrate and eluate were combined and was evaporated to dryness. After the residue was suspended in ether/hexanes, the insoluble precipitate was collected by the filtration, washed with ether and dissolved in acetone. After the removal of insoluble materials by the filtration, a saturated HCl in MeOH (5 mL) was added to the filtrate and the solution was evaporated to dryness. The oily residue was suspended into acetone to form a precipitate, and the precipitated target compound was collected by filtration, was dried in vacuo to afford a colorless solid (478 mg, 79%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.84 (t, *J* = 6.8 Hz, 3H), 1.23–1.29 (m, 6H), 1.53–1.61 (m, 2H), 2.67 (t, *J* = 8.0 Hz, 2H), 7.43 (d, *J* = 8.4 Hz, 2H), 7.74 (d, *J* = 8.4 Hz, 2H), 9.06 (br s, 2H), 9.28 (br s, 2H).

4-Isopropylbenzamidine hydrochloride. Following a procedure described for 4-*n*-hexylbenzamidine hydrochloride, the reaction of 4-isopropylbenzonitrile with $MeAlNH_2Cl$ in dry toluene followed by the treatment with HCl afforded a colorless solid (315 mg, 63%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.22 (d, *J* = 6.8 Hz, 6H), 2.99 (m, 1H), 7.48 (d, *J* = 8.4 Hz, 2H), 7.77 (d, *J* = 8.4 Hz, 2H), 9.11 (br s, 2H), 9.31 (br s, 2H).

4-*t*-Butylbenzamidine hydrochloride. Following a procedure described for 4-*n*-hexylbenzamidine hydrochloride, the reaction of 4-*t*-butylbenzonitrile with $MeAlNH_2Cl$ in dry toluene followed by the treatment with HCl afforded a colorless solid (433 mg, 81%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.30 (s, 9H), 7.63 (d, *J* = 8.4 Hz, 2H), 7.76 (d, *J* = 8.4 Hz, 2H), 9.04 (br s, 2H), 9.28 (br s, 2H).



Scheme SI-1. Preparation of a semi-wet protein/peptide array using a supramolecular hydrogel (an example for a substrate array). Homogeneous hot solution of **1** is dropped onto a glass plate and incubated to form hydrogel spots. Then, aqueous solution dissolving substrate or enzyme are dropped onto each hydrogel spot and incubated to form substrates or enzymes array. An analyte solution (including an enzyme, in case of the substrate array) is dropped onto the hydrogel spots, and the resulting fluorescence change is monitored.

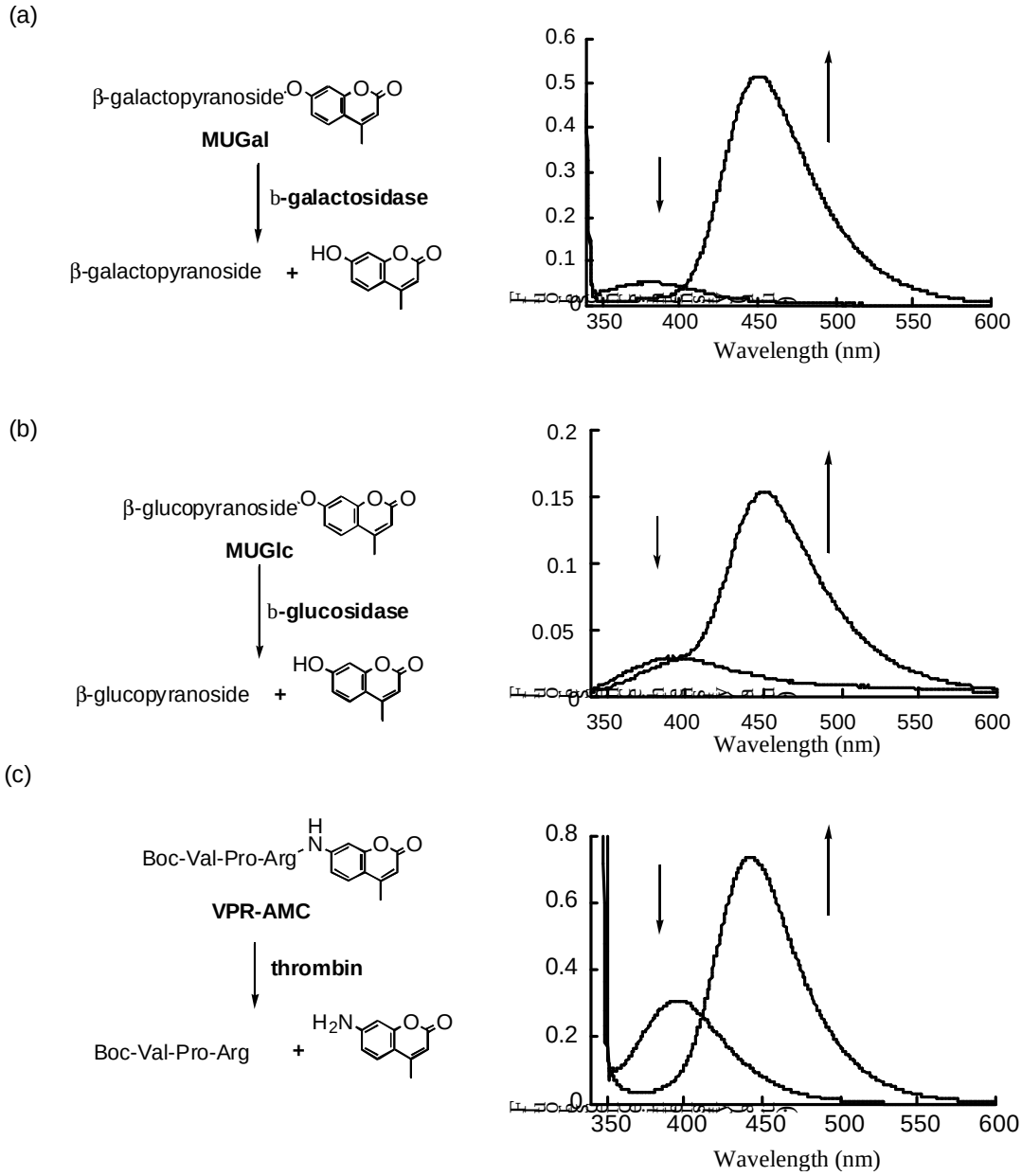
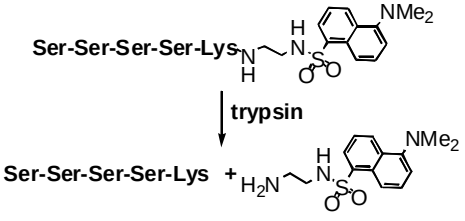
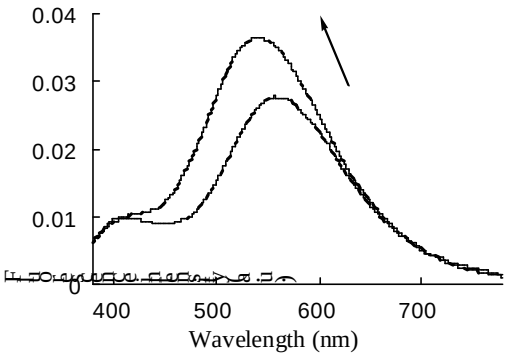


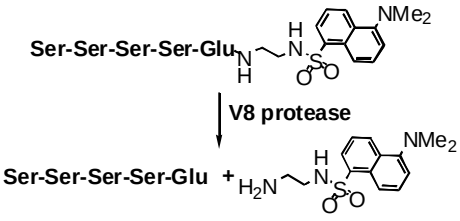
Figure SI-1**.** Fluorescence spectral change of the enzymatic hydrolysis of the fluorogenic substrates in a hydrogel of **1**; [**1**] = 0.25 wt% at rt, (a) [β-galactosidase] = 0.3 μM, [**MUGal**] = 20 μM in 50 mM tris/HCl buffer (pH 8.0), λ_{ex} = 330 nm; (b) [β-glucosidase] = 5 μM, [**MUGlc**] = 20 μM in acetate buffer (pH 5.0), λ_{ex} = 315 nm; (c) [thrombin] = 5 μM, [**VPR-AMC**] = 20 μM in 50 mM tris/HCl buffer (pH 8.0) containing 100 mM NaCl and 10 mM $CaCl_2$, λ_{ex} = 330 nm.

(a)





(b)



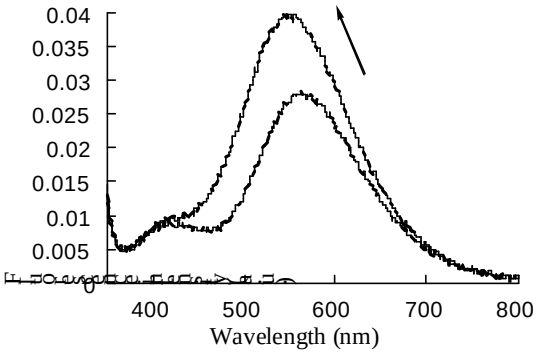


Figure SI-2. Fluorescence spectral change of the enzymatic hydrolysis of the substrates with dansyl group in a hydrogel of **1**; [**1**] = 0.25 wt% at rt, λ_{ex} = 330 nm, (a) [trypsin] = 1 μM, [**SSSSK-DANSen**] = 100 μM in 50 mM tris/HCl buffer (pH 8.0) containing 100 mM $CaCl_2$; (b) [V8 protease] = 25 μM, [**SSSSE-DANSen**] = 100 μM in 50 mM tris/HCl buffer (pH 8.0).

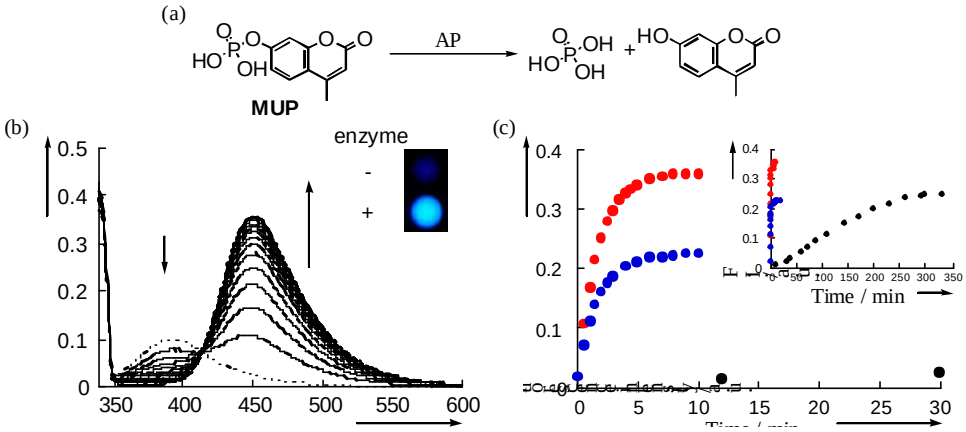


Figure 2. a) Equation of the AP catalyzed hydrolysis of **MUP**. b) Fluorescence spectral change of the AP catalyzed hydrolysis of **MUP** in the hydrogel of **1.** The corresponding emission color change of the hydrogel chip (inset); [**1**] = 0.25 wt%, [AP] = 0.1 nM, [**MUP**] = 20 μM in 50 mM tris/HCl buffer (pH 9) at rt, λ_{ex} = 333 nm. The broken line shows a spectrum for **MUP** in the absence of AP. (c) Time course of the fluorescence intensity change at 450 nm for the AP catalyzed hydrolysis of **MUP**:?20 μL of ηψδρογελ χηιπ of **1** on a glass plate (red), aqueous solution (blue), and 100 μL bulk hydrogel of **1** (black); [**1**] = 0.25 wt%, [AP] = 0.1 nM, [**MUP**] = 20 μM in 50 mM tris/HCl buffer (pH 9) at rt, λ_{ex} = 333 nm.

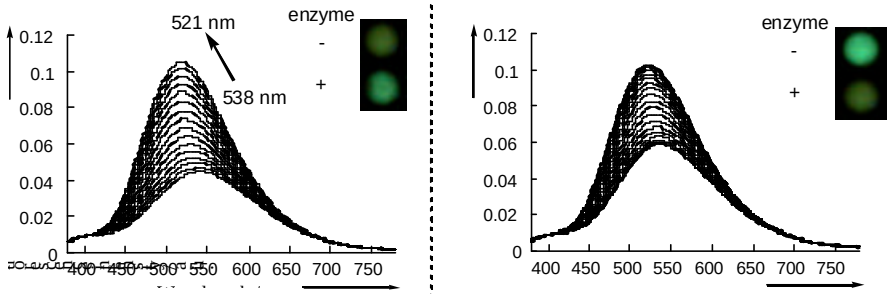


Figure 4. a) Equation of the Chymotrypsin catalyzed hydrolysis of **pep-4**. b) Fluorescence spectral change of the Chymotrypsin catalyzed hydrolysis of **pep-4** in the hydrogel of **1** and the corresponding emission color change of the hydrogel chip (imposed); [**1**] = 0.25 wt%, [Chym] = 1 μM, [**pep-4**] = 100 μM in 50 mM tris/HCl buffer (pH 8) containing 100 mM $CaCl_2$ at rt, λ_{ex} = 330 nm. The spectra were collected every 1 min. c) Equation of the Chymotrypsin catalyzed hydrolysis of **pep-3**. d) Fluorescence spectral change of the Chymotrypsin catalyzed hydrolysis of **pep-3** in the hydrogel of **1** and the corresponding emission color change of the hydrogel chip (imposed); [**1**] = 0.25 wt%, [Chym] = 1 μM, [**pep-3**] = 100 μM in 50 mM tris/HCl buffer (pH 8) containing 100 mM $CaCl_2$ at rt, λ_{ex} = 330 nm. The spectra were collected every 1 min.

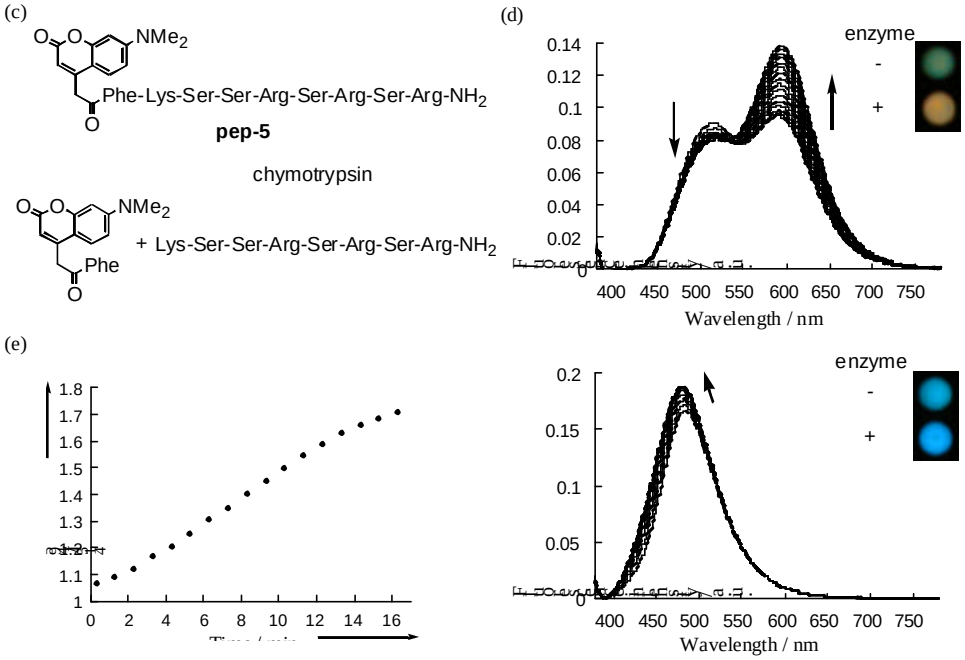


Figure 5. a) Structure of **ASP**. b) Cartoons for the designed FRET process caused by the enzymic reaction in the hydrogel of **1**. The coumarin fragment of substrate is re-distributed into the hydrophobic domain of hydrogel fiber upon the enzymatic hydrolysis and thereby the distance between the coumarin and **ASP** becomes closer to induce the enhanced fluorescence of **ASP** due to FRET. c) Equation of the Chymotrypsin catalyzed hydrolysis of **pep-5**. d) Fluorescence spectral change of the Chymotrypsin catalyzed hydrolysis of **pep-5** in the hydrogel of **1** containing **ASP** and the corresponding emission color change of the hydrogel chip (imposed); [**1**] = 0.25 wt%, [**ASP**] = 200 μM, [Chym] = 1 μM, [**pep-5**] = 100 μM in 50 mM tris/HCl buffer (pH 8) containing 1 vol% of MeOH and 100 mM $CaCl_2$ at rt, λ_{ex} = 351 nm. e) Time course of the fluorescence intensity ratio of 594 nm over 514 nm in Figure 5c. f) Fluorescence spectral change of the Chymotrypsin catalyzed hydrolysis of **pep-5** in the hydrogel of **1** in the absence of **ASP** and the corresponding emission color change of the hydrogel chip (imposed); [**1**] = 0.25 wt%, [Chym] = 1 μM, [**pep-1**] = 100 μM in 50 mM tris/HCl buffer (pH 8) containing 1 vol% of MeOH and 100 mM $CaCl_2$ at rt, λ_{ex}

= 351 nm.