

## Supplementary Information for

### Digital fluorescent pH sensors

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#### Experimental Section

**Materials and Apparatus.** *N*-{2-[(7-*N,N*-Dimethylaminosulfonyl)-2,1,3-benzoxadiazol-4-yl]-(methyl)amino}ethyl-*N*-methylacrylamide (DBD-AA)<sup>S1</sup> and *N*,2-dimethyl-*N*-(2-{methyl[7-(dimethylsulfamoyl)-2,1,3-benzoxadiazol-4-yl]amino}ethyl)propanamide (DBD-IA)<sup>S2</sup> were obtained as previously reported. *N,N*-Diethyl-1,3-propanediamine, *N*-[3-(dimethylamino)propyl]acrylamide (DMAPAM), *N*-isopropylacrylamide (NIPAM) and 3-morpholinopropylamine were obtained from Wako Pure Chemicals. Trisodium 8-hydroxypyrene-1,3,6-trisulfonate (HPTS) was purchased from Invitrogen. Water was purified using a Milli-Q reagent system, Direct-Q 3 UV (Millipore). All other reagents were of guaranteed reagent grade and used without further purification.

Infrared (IR) spectra were measured using a JASCO FT/IR-410 spectrometer. Proton nuclear magnetic resonance spectra (<sup>1</sup>H-NMR, 400 MHz) and carbon nuclear magnetic resonance spectra (<sup>13</sup>C-NMR, 100 MHz) were obtained using a Bruker AVANCE 400 spectrometer. The *J* values are given in hertz. Mass spectra with an electrospray ionization (ESI) system were obtained on a Bruker micrOTOF-05 spectrometer. The gel permeation chromatography apparatus (GPC) consisted of a JUSCO PU-2080 pump, a JASCO RI-2031 refractive index detector, a JASCO FP-2020 fluorescence detector, a JASCO CO-2060 column thermostat and a Shodex GPC KD-806M column. UV-visible absorption spectra were measured using a JASCO V-550 UV/VIS spectrophotometer. Fluorescence spectra were obtained using a JASCO FP-6500 spectrofluorometer with a Hamamatsu R-7029 optional photomultiplier tube (operative range, 200–850 nm) and were corrected using a JASCO ESC-333 substandard light source. The sample temperature was controlled by a JASCO ETC-273T temperature controller.

**Synthesis of *N*-(3-morpholin-4-ylpropyl)acrylamide (MPAM).**<sup>S3</sup> 3-Morpholinopropylamine (500 mg, 3.47 mmol) and triethylamine (485  $\mu$ L, 3.47 mmol) were dissolved in toluene (5 mL). After the addition of acryloyl chloride (281  $\mu$ L, 3.47 mmol) at 0 °C, the mixture was stirred at the same temperature for 1 h. The reaction mixture was then poured into an aqueous NaOH solution (0.5 mol/L, 100 mL) and the product was extracted with dichloromethane (100 mL  $\times$  2). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated to dryness under reduced pressure. Chromatography was performed on the residue on silica gel with dichloromethane-methanol (10 : 1) as an eluent to obtain MPAM (342 mg, 50 %) as a colorless oil:  $\nu_{\max}$ (neat)/cm<sup>-1</sup> 3287, 3076, 2954, 2815, 1660, 1625 and 1556; <sup>1</sup>H-NMR (CDCl<sub>3</sub>,  $\delta$ ) 7.22 (1H, br), 6.26 (1H, d, *J* 17.2), 6.06 (1H, dd, *J* 17.2, 10.4), 5.63 (1H, d, *J* 10.4), 3.72 (4H, t, *J* 4.4), 3.44 (2H, m), 2.48–2.51 (6H, m) and 1.72 (2H, tt, *J* 6.0); <sup>13</sup>C-NMR (CDCl<sub>3</sub>,  $\delta$ ) 165.5, 131.2, 125.9, 67.1, 58.0, 53.7, 39.6 and 24.6; HR-ESI-MS *m/z*: calcd for C<sub>10</sub>H<sub>19</sub>N<sub>2</sub>O<sub>2</sub><sup>+</sup> ([M+H]<sup>+</sup>)

199.1447, Found 199.1447.

**Synthesis of *N*-[3-(diethylamino)propyl]acrylamide (DEAPAM).** *N,N*-Diethyl-1,3-propanediamine (500 mg, 3.84 mmol) and triethylamine (1.61 mL, 11.5 mmol) were dissolved in toluene (5 mL). After the addition of acryloyl chloride (312  $\mu$ L, 3.84 mmol) at 0 °C, the mixture was stirred at the same temperature for 1 h. The reaction mixture was then poured into an aqueous NaOH solution (0.5 mol/L, 100 mL) and the product was extracted with dichloromethane (100 mL  $\times$  2). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated to dryness under reduced pressure. Chromatography was performed on the residue on silica gel with dichloromethane-methanol (100 : 15) as an eluent to obtain DEAPAM (199 mg, 28 %) as a colorless oil:  $\nu_{\text{max}}(\text{neat})/\text{cm}^{-1}$  3279, 3077, 2969, 2803, 1658, 1626 and 1551; <sup>1</sup>H-NMR (CDCl<sub>3</sub>,  $\delta$ ) 7.94 (1H, br), 6.22 (1H, d, *J* 17.2), 6.04 (1H, dd, *J* 17.2, 10.4), 5.59 (1H, d, *J* 10.4), 3.43 (2H, m), 2.49–2.56 (6H, m), 1.68 (2H, tt, *J* 6.0) and 1.04 (6H, t, *J* 7.2); <sup>13</sup>C-NMR (CDCl<sub>3</sub>,  $\delta$ ) 165.4, 131.6, 125.3, 53.2, 46.9, 40.4, 25.0 and 11.9; HR-ESI-MS *m/z*: Calcd for C<sub>10</sub>H<sub>21</sub>N<sub>2</sub>O<sup>+</sup> ([M+H]<sup>+</sup>) 185.1654, Found 185.1650.

**Polymerizations. Copolymers 1 and 3–5.** NIPAM (4.5 mmol), an ionizable monomer (DMAPAM, MPAM, DEAPAM, or AA, 0.5 mmol), DBD-AA (5  $\mu$ mol), and  $\alpha,\alpha'$ -azobisisobutyronitrile (AIBN, 50  $\mu$ mol) were dissolved in 1,4-dioxane (10 mL), and the solution was bubbled with dry nitrogen or argon for 30 min to remove dissolved oxygen. Polymerization was performed at 60 °C for 6 h (for **3** and **4**) or 8 h (for **1** and **5**). The reaction mixture was cooled to room temperature and poured into diethyl ether (200 mL). The obtained copolymer was purified by reprecipitation using 1,4-dioxane (10 mL) and diethyl ether (200 mL). Yields: 83 % (**1**), 82 % (**3**), 71 % (**4**) and 83 % (**5**).

**Copolymer 2.** DMAPAM (5 mmol), DBD-AA (5  $\mu$ mol), and AIBN (25  $\mu$ mol) were dissolved in 1,4-dioxane (5 mL), and the solution was bubbled with dry argon for 30 min to remove dissolved oxygen. Polymerization was performed at 60 °C for 6 h. The reaction mixture was cooled to room temperature and poured into a mixture of *n*-hexane and acetone (9 : 1, v/v, 200 mL). The obtained copolymer was purified by reprecipitation using 1,4-dioxane (3 mL)-diethyl ether (200 mL) and dialysis. The yield was 21 %.

**Characterization of 1-5.** The contents of the NIPAM, DMAPAM, MPAM, DEAPAM, and AA units in the copolymers were determined from <sup>1</sup>H NMR spectra. The proportions of DBD-AA units in the copolymers were determined from the absorbances of the copolymers in methanol, in comparison with DBD-IA ( $\epsilon = 10,800 \text{ M}^{-1}\text{cm}^{-1}$  at 444 nm)<sup>S2</sup> as a model compound. The molecular weights of the copolymers were determined by means of GPC measurements. A calibration curve was obtained using a polystyrene standard, and 1-methyl-2-pyrrolidinone containing LiBr (5 mM) was adopted as the eluent (0.5 mL min<sup>-1</sup>). A small peak at 22.5 min in the chromatograms of **2** and **4** was due to a trace amount of unpolymerized DBD-AA (less than 0.15 % of total area).

**Fluorescence measurements.** The fluorescence spectra of the copolymers (0.01 w/v%) were obtained in a Britton-Robinson buffer with an excitation of 450 nm. After pH adjustments at room temperature, the sample was equilibrated at a specific temperature for at least 500 s. The fluorescence

spectra of HPTS (1  $\mu\text{M}$ ) and DBD-IA (10  $\mu\text{M}$ ) were also measured in a Britton-Robinson buffer with excitations at 454 and 450 nm, respectively. These fluorometric measurements were performed at least in triplicate. The fluorescence quantum yields ( $\Phi_f$ ) were determined by the following equation:

$$\Phi_{f,S} = \Phi_{f,R} F_S A_R n_S^2 / F_R A_S n_R^2 \quad (\text{S1})$$

where  $F$  is the area under the fluorescence spectrum with an excitation of 458 nm,  $A$  is the absorbance at 458 nm,  $n$  is the refractive index of the solvent, and the subscripts R and S represent reference and sample, respectively. 4-Methylamino-7-nitro-2,1,3-benzoxadiazole in acetonitrile ( $\Phi_f = 0.38$  with an excitation at 458 nm<sup>S4</sup>) was used as a reference.

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### References

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