

## Supporting Information

### **A single-step enzyme immunoassay capillary sensor composed of functional multilayer coatings for diagnosis marker proteins**

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#### **1. Synthesis of lipophilic fluorescent substrates**

*Reagents and materials.* 1,3-Dihydroxybenzene, 3-ketoglututaric acid, 4-dimethylaminopyridine (DMAP), dodecylamine, diethyl phosphoryl chloride, trimethylsilyl bromide (TMSBr) were purchased from Tokyo Chemical Industry (Tokyo, Japan). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and triethylamine (TEA) were purchased from Wako Pure Chemical Industries (Osaka, Japan).

*Synthesis of 7-hydroxycoumarin-4-acetic acid (1).* 1,3-Dihydroxybenzene (3.0 g, 27 mmol) was dissolved in 70% sulfuric acid (27 mL) at 0°C, after which 3-ketoglutaric acid (4.0 g, 28 mmol) was added. This mixture was allowed to warm up to room temperature and stirred further for 4 h. The resulting solution was poured onto crushed ice. The white precipitate collected by filtration was washed with water and ethyl acetate, and dried overnight under reduced pressure to yield **1** as a white solid (3.4 g, 56%). <sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.50 (d, *J* = 8.9 Hz, 1H), 6.78 (dd, *J* = 7.6, 2.0 Hz, 1H), 6.68 (d, *J* = 2.1 Hz, 1H), 6.19 (s, 1H), 3.79 (s, 2H)

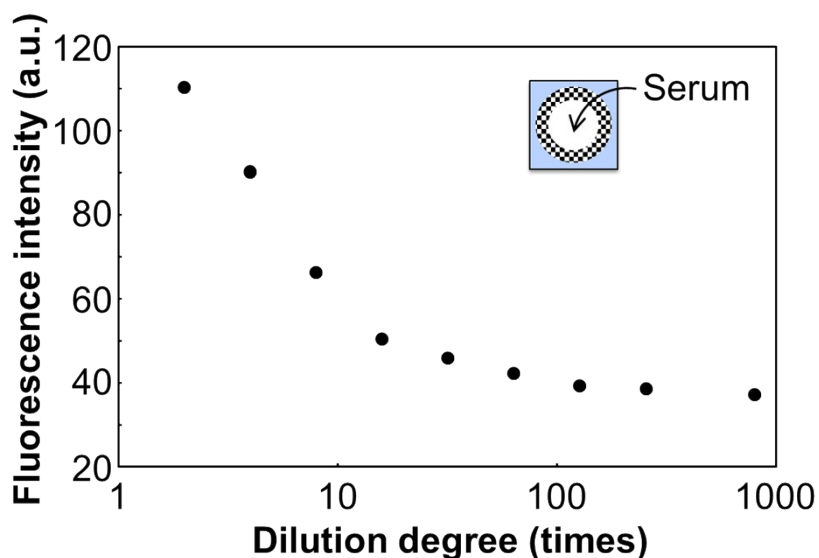
*Synthesis of 4-(N-dodecyl-acetamide) umbelliferone (2).* **1** (2.0 g, 9.11 mmol) was dissolved in *N,N*-dimethylformamide (DMF) (20 mL) at 40 °C, following which DMAP (1.1 g, 9.14 mmol), EDC (1.8 g, 9.55 mmol), and a solution of dodecylamine (2.0 g, 10.8 mmol) in DMF (7 mL) were added, and the resulting mixture was stirred for 12 h. Solvent was removed under reduced pressure, and the residue was dissolved in ethyl acetate and washed with 1 M hydrochloric acid and pure water. The organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure, yielding a yellow oil that was further purified by column chromatography (silica gel, chloroform/methanol, 24:1) to give **2** as a white solid (0.31 g, 8.8 %). <sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.15 (t, *J* = 5.6 Hz, 1H), 7.59 (d, *J* = 8.8 Hz, 1H), 6.77 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.70 (d, *J* = 2.4 Hz, 1H), 6.14 (s, 1H), 3.61 (s, 2H), 3.05 (q, *J* = 6.8 Hz, 2H), 1.40-1.23 (m, 20 H), 0.85 (t, *J* = 6.8 Hz, 3H)

*Synthesis of 4-(N-dodecyl-acetamide) umbelliferyl diethoxy phosphate (3).* **2** (0.19 g, 0.49 mmol) was dispersed in dichloromethane (DCM) (8 mL), and TEA was added until **2** was dissolved at room temperature. Diethyl phosphoryl chloride (0.42 g, 2.44 mmol) was added, and the mixture was stirred for 1 h at 0 °C. Solvent was removed under reduced pressure and the residue was dissolved in ethyl acetate and washed with 1 M hydrochloric acid followed by pure water. The organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure to yield a yellow oil that was further purified by column chromatography (silica gel, chloroform/methanol, 24:1), and size exclusion chromatography (chloroform) to give **3** as a yellow liquid (0.15 g, 61 %). <sup>1</sup>H-NMR(400 MHz, Chloroform-*d*) δ 7.66 (d, *J* = 8.4 Hz, 1H), 7.16-7.20 (m, 2H), 6.33 (s, 1H), 6.10 (t, *J* = 5.6 Hz, 1H), 4.19-4.27 (m, 4H), 3.22 (q, *J* = 6.8 Hz, 2H), 1.48-1.24 (m, 26H), 0.88 (t, *J* = 6.8 Hz, 3H)

*Synthesis of 4-(dodecyl-acetamide) umbelliferyl phosphate (12-PC) (4-b).* TMSBr (0.68 g, 4.41 mmol) was added to a solution of **3** (0.23 g, 0.44 mmol) in DCM (20 mL), and the mixture was stirred at room temperature under argon atmosphere. The resulting solid was collected by filtration, washed with diethyl ether, and dried under reduced pressure to yield a white powder of **4** (0.15 g, yield 73%). <sup>1</sup>H-NMR(400 MHz, DMSO-*d*<sub>6</sub>) δ 8.18 (t, *J* = 5.6 Hz, 1H), 7.76 (d, *J* = 8.8 Hz, 1H), 7.20-7.13 (m, 2H), 6.35 (s, 1H), 3.69 (s, 2H), 3.04 (q, *J* = 6.8 Hz, 2H), 1.44-1.19 (m, 20 H), 0.85 (t, *J* = 6.8 Hz, 3H); MS (FAB): *m/z* 468 [M+1]<sup>+</sup>

## 2. Influence of endogenous human serum ALP

The influence of endogenous human serum ALP was investigated. ALP is an endogenous component of human serum (44 IU/L–147 IU/L normal range)<sup>1</sup> and an increase in background fluorescence due to penetration of the free ALP into hydrogel was a topic of concern. To investigate this, a dilution series of control human serum was introduced into capillaries possessing substrate-immobilized hydrophobic coating and hydrogel film. Results are shown in Figure S1. A 32 fold or more dilution resulted in background levels of response.



**Figure S1** Fluorescence response of a single-step capillary sensor toward serially-diluted control serum samples.

### Reference

1. The U.S. National Library of Medicine and National Institutes of Health, ALP - blood test, <http://www.nlm.nih.gov/medlineplus/ency/article/003470.htm>, (accessed September 2014).