

## Supporting Information

### Latent Fluorophore Based on the Trimethyl Lock

Sunil S. Chandran, Kimberly A. Dickson, and Ronald T. Raines\*

*Departments of Biochemistry and Chemistry, University of Wisconsin, Madison, Wisconsin 53706*

#### General Experimental

Pyridine used was dried by storage for 24 h over activated Linde 4A molecular sieves under Ar(g). Anhydrous DMF was obtained from a CYCLE-TAINER solvent delivery system (J. T. Baker; Phillipsburg, NJ). EDCI was from Novabiochem. Rhodamine 110 was from Aldrich Chemical (Milwaukee, WI). Silica gel 60 (230–400 mesh) for flash chromatography was from Silicycle (Québec City, Québec, Canada).

<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were obtained with a Bruker AC+ 300 spectrometer at the University of Wisconsin–Madison Chemistry Instrument Center. All kinetic evaluations were performed in phosphate-buffered saline (PBS, pH 7.3), which contained (in 1 L) KCl (0.2 g), KH<sub>2</sub>PO<sub>4</sub> (0.2 g), NaCl (8.0 g), and Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O (2.16 g). Pig liver esterase (PLE; MW 163 kDa (ref 1)) was obtained from Sigma Chemical (St. Louis, MO; product number E2884) as a suspension in 3.2 M ammonium sulfate buffer, and was diluted to appropriate concentrations in PBS before use. Stock solutions of pro-fluorophore **3** were prepared in DMSO and added to PBS for the kinetic experiments such that DMSO concentrations never exceeded 1% (v/v). Fluorometric measurements were made with using fluorescence grade quartz or glass cuvettes from Starna Cells (Atascadero, CA) and a QuantaMaster1 photon-counting spectrofluorometer from Photon Technology International (South Brunswick, NJ) equipped with sample stirring.

#### Synthesis of Pro-Fluorophore **3**

Pro-Fluorophore **3** was synthesized by the route shown in Scheme 1. Specifically, acetylated **1** (ref 2; 5 g, 18.8 mmol) was dissolved in a 100 mL of a 1:1 mixture of dry pyridine/DMF at room temperature under argon and treated with EDCI (3.6 g, 18.8 mmol). After stirring the resulting solution at room temperature for 1 h, rhodamine 110 (1.72 g, 4.7 mmol) was added. The reaction mixture was stirred at room temperature for 2 days under Ar(g), during which time the deep red solution slowly turned to a light peach color. The reaction mixture was treated with 100 mL of EtOAc and incubated at –20 °C for 12 h. The clear liquid was removed by decantation and washed with 100 mL of 0.1 M HCl, followed by 100 mL of water. The organic layer was dried over MgSO<sub>4</sub>(s) and concentrated to dryness by rotary evaporation under reduce pressure. The red residue was purified by flash chromatography (*R<sub>f</sub>* 0.44; 4:1 EtOAc/hexanes) to yield pro-fluorophore **3** as a white solid (1.12 g, 29% yield). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.69 (s, 12H), 2.24 (s, 6H), 2.38 (s, 6H), 2.41 (s, 6H), 2.55 (s, 4H), 6.53–6.63 (m, 6H), 6.80 (s, 2H), 7.06 (d, *J* = 6.9 Hz, 1H), 7.34 (s, 2H), 7.42 (s, 2H), 7.59 (m, 2H), 7.97 (d, *J* = 6.9 Hz, 2H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 172.2, 169.8, 153.1, 151.6, 150.1, 140.1, 139.1, 137.3, 134.9, 133.2, 132.8, 129.6, 128.1, 124.9, 123.9, 123.4, 115.1, 113.7, 107.2, 51.1, 40.4, 32.2, 32.1, 25.5, 21.9, 20.2. MS (MALDI) *m/z* 845.3535 (MNa<sup>+</sup> = 845.3414).

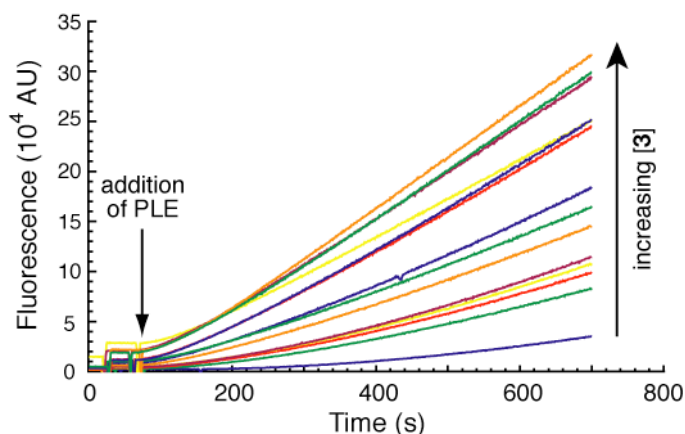
## Supporting Information

### Excitation–Emission Spectra of Pro-Fluorophore **3** in the Absence and Presence of Pig Liver Esterase

A solution of pro-fluorophore **3** ( $5\ \mu\text{M}$ ) in 2 mL of PBS containing PLE (0.25 mg/mL) was incubated at room temperature for 4 h. The fluorescence excitation–emission spectra of the solution were then recorded. The procedure was repeated for a solution of pro-fluorophore **3** treated in an identical manner, except for the presence of PLE.

### Kinetic Parameters for Activation of Pro-Fluorophore **3** by Pig Liver Esterase

All kinetic measurements were performed at room temperature with an excitation wavelength of  $\lambda_{\text{ex}} = 492\ \text{nM}$  and an emission wavelength of  $\lambda_{\text{em}} = 520\ \text{nM}$  in 2.0 mL of PBS containing PLE ( $2.5\ \mu\text{g/mL}$ ). A calibration curve was created by measuring the fluorescence of known concentrations of rhodamine-110 in the aforementioned reaction mixture. The rate of cleavage of pro-fluorophore **3** by PLE was measured adding known concentrations of **3** (50 nM–5 mM) to the reaction mixture and recording the fluorescence as a function of time, as shown in Figure S1. The reaction rate was calculated by using the calibration curve for rhodamine-110. Enzymatic parameters were calculated by fitting the linear portion of the data (which corresponds to the unmasking of the second amino group<sup>3</sup>) to the Michaelis–Menten equation.



**Figure S1.** Raw data showing the change in fluorescence ( $\lambda_{\text{ex}} 492\ \text{nM}$ ,  $\lambda_{\text{em}} 520\ \text{nM}$ ) upon addition of PLE (0.5 mg/mL) to solutions containing various concentrations of pro-fluorophore **3**.

### References

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