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Supporting Information

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Continuous fluorometric assays for acetylcholinesterase activity and inhibition using conjugated polyelectrolytes

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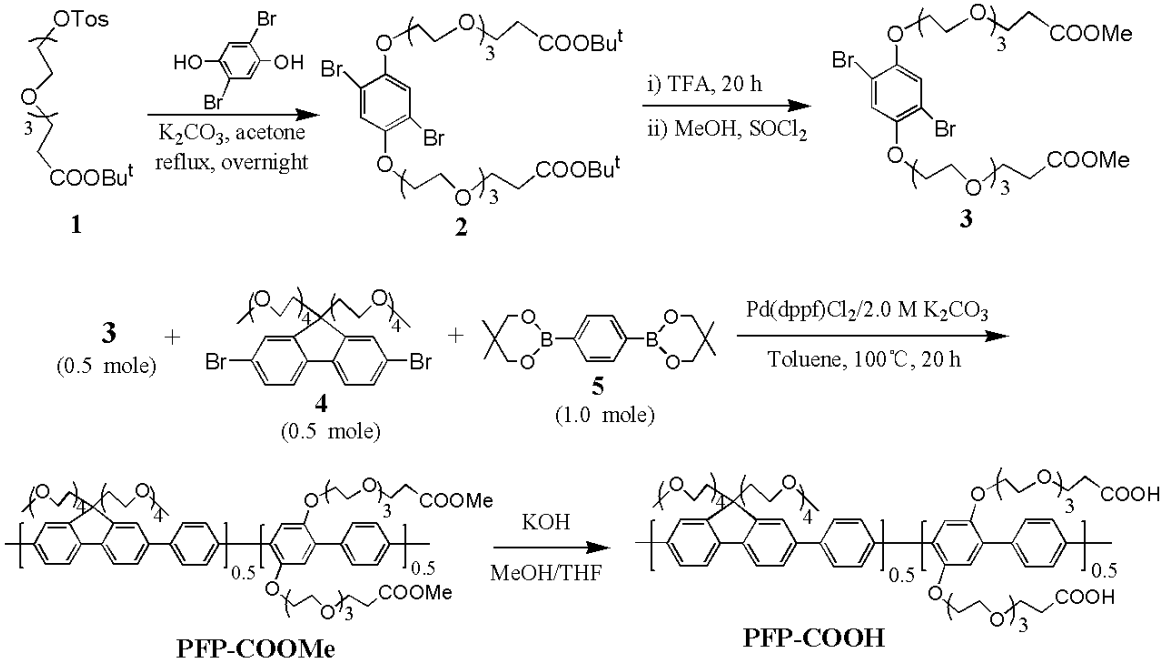

Scheme S1. The synthetic routine of PFP-COOH.

Materials and Instruments.

Starting materials were purchased from Aldrich Chemical Company or Alfa-Aesar and used without further purification. The compounds **1**[1] and **4**[2] were prepared according to the procedures in literatures. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on AV300 or AV400 spectrometer. The gel permeation chromatography (GPC) measurements were performed on Water-410 system against polystyrene standards with THF as eluent.

1,4-Dibromo-2,5-bis(11-(*tert*-butoxycarbonyl)-3,6,9-trioxahendecyloxy)benzene (2)

The mixture of 2,5-dibromohydroquinone (2.0 g, 7.45 mmol), compound **1** (6.9 g, 15.9 mmol), K_2CO_3 (6.7 g, 48.5 mmol) and catalytic quantity of 18-crown-6were in 150 mL of air-free acetone was

heated at reflux for 20 hr under nitrogen. After cooling to room temperature, the reaction mixture was filtered. The filtrate was evaporated to dryness and the residue remaining was taken up in a mixture of CH_2Cl_2 and water. The organic layer was separated, washed with saturated aqueous $NaHCO_3$ and brine, and dried over anhydrous $MgSO_4$. After removal of the solvent, the residue was purified by silica gel chromatography using petroleum ether/EtOAc (2:1) as eluent to afford a clear light yellow oil (3.7 g, 62.6%). ^{1}H NMR ($CDCl_3$, ppm): 1.44 (s, 18H), 2.50 (t, 4H, J = 6.5 Hz). 3.58-3.76 (m, 20H), 3.87 (t, 4H, J = 4.6 Hz), 4.12 (t, 4H, J = 4.6 Hz), 7.15 (s, 2H). ^{13}C NMR ($CDCl_3$, ppm): 28.09, 36.27, 66.89, 69.55, 70.18, 70.39, 70.57, 70.71, 71.09, 80.48, 111.34, 119.10, 150.29, 170.90 ppm. MS (MALDI-TOF) m/z 809 (M^++Na). Anal. Calcd for $C_{32}H_{52}O_{12}Br_2$: C, 48.74; H, 6.65. Found: C, 49.26; H, 6.80 %.

1,4-Dibromo-2,5-bis(11-methoxycarbonyl-3,6,9-trioxahendecyloxy)benzene (3)

The compound **2** (3.2 g, 4.1 mmol) was dissolved in trifloroacetic acid (24 mL) and stirred at room temperature overnight. The reaction mixture was evaporated to dryness, dissolved in water, and re-evaporated under reduced pressure. To the residue were added MeOH (50 mL) and several drops of $SOCl_2$, and the reaction was stirred at room temperature overnight. After removal of the solvent, the residue was purified by silica gel chromatography using petroleum ether/EtOAc (1:3) as eluent to provide a clear light yellow oil (1.4 g, 50.0%). ^{1}H NMR ($CDCl_3$, ppm): 2.60 (t, 4H), 3.64-3.76 (m, 26H), 3.87 (t, 4H), 4.12 (t, 4H), 7.15 (s, 2H). ^{13}C NMR ($CDCl_3$, ppm): 34.87, 51.67, 66.59, 69.57, 70.19, 70.44, 70.55, 70.72, 71.09, 111.34, 119.11, 150.30, 172.03. MS (MALDI-TOF) m/z 725 (M^++Na). Anal. Calcd for $C_{26}H_{40}O_{12}Br_2$: C, 44.33; H, 5.72. Found: C, 44.36; H, 5.74%.

Poly{[2,5-bis(11-(methyloxycarbonyl-3,6,9-trioxahendecyloxy)benzene)]-*co*-alt-[2,7-(9,9-bis(11-methyloxyl-3,6,9-trioxahendecyl))fluorene]} (PFP-COOMe)

The mixture of 5,5,5',5'-tetramethyl-2,2'-*p*-phenylene-bis-[1,3,2]dioxaborinane (214.8 mg, 0.71 mmol), compound **3** (250.0 mg, 0.35 mmol) and 2,7-(9,9-bis(11-methyloxyl-3,6,9-trioxahendecyl))dibromofluorene (250 mg, 0.35 mmol) in 6 mL of

toluene and 4 mL of 2.0 M potassium carbonate aqueous solution was degassed, and then catalytic quantity of $Pd(dppf)Cl_2$ was added. The mixture was vigorously stirred at 100 ℃ under a nitrogen atmosphere for 20 hr. After cooling down to room temperature, it was poured into methanol (200 mL). The precipitate was recovered by filtration, dissolved in THF and reprecipitated in methanol (200 mL). The desired polymer was obtained as nut-brown solid (200.0 mg, 47.2%). ^{1}H NMR (DMSO, ppm): 2.81 (s), 3.18 ~ 3.70 (m), 3.75 (s), 4.21 (br), 7.19 ~ 7.99 (br, m). M_w = 67,000, PDI = 1.2.

Synthesis of PFP-COOH

A solution of KOH (112.0 mg, 1.0 mmol) in methanol (1 ml) was added into the solution of **PFP-1** (150 mg, 0.24 mmol) in THF (50 mL) and stirred overnight. The reaction mixture was acidified with aqueous HCl (7%). The deposit was collected by centrifugation and dried overnight under vacuum at 50 ℃. The desired polymer **PFP-2** was obtained as purple fibroid solid (140.0 mg, 98.0%). ^{1}H NMR ($CDCl_3$, ppm): 2.81 (s), 3.18 ~ 3.75 (m), 4.21 (br), 7.19 ~ 8.01 (br, m), 12.14 (s)

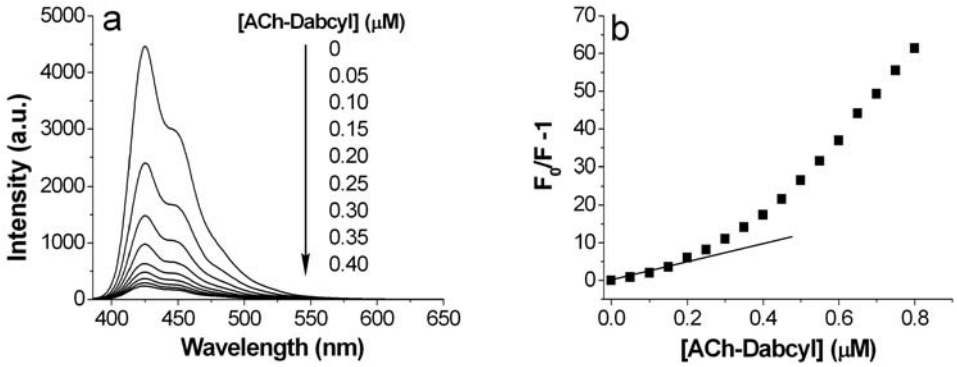

Figure S1. (a) Fluorescence emission spectra of PFP-SO_3^- in buffer solution with successive additions of ACh-Dabcyl. [PFP-SO_3^-] = 2.0 μM in RUs, [ACh-Dabcyl] = 0 to 0.4 μM. (b) K_{sv} plot of PFP-SO_3^- in the presence of ACh-Dabcyl. [PFP-SO_3^-] = 2.0 μM in RUs, [ACh-Dabcyl] = 0 to 0.8 μM. Fluorescence measurements were performed in 25 mM phosphate buffer solutions (pH 8.0) with excitation at 376 nm.

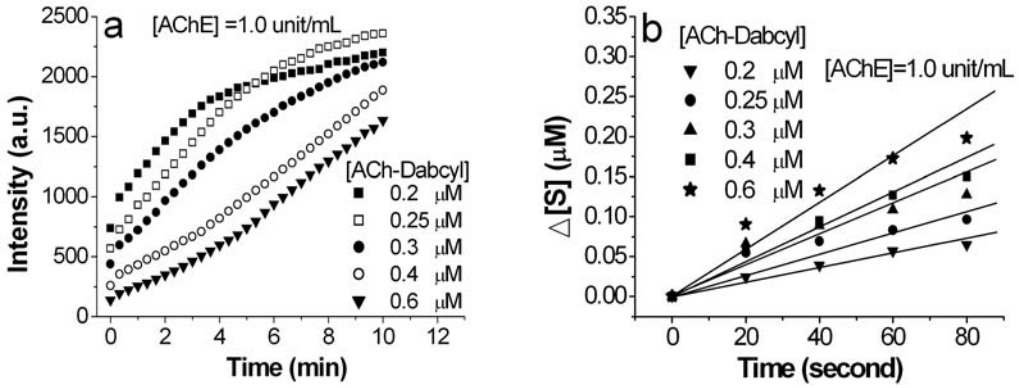

Figure S2. Kinetic data for AChE-catalyzed hydrolysis of ACh-Dabcyl: (a) Emission intensity of PFP-SO_3^- at 424 nm as a function of AChE-catalyzed hydrolysis time with varying ACh-Dabcyl concentrations. (b) Plots of concentration changes of ACh-Dabcyl versus the AChE incubating time. [PFP-SO_3^-] = 2 μM, [AChE] = 1.0 unit/mL, [ACh-Dabcyl] = 0.2, 0.25, 0.3, 0.4, 0.6 μM. The fluorescence measurements were performed in 25 mM phosphate buffer solutions (pH 8.0) with excitation at 376 nm.

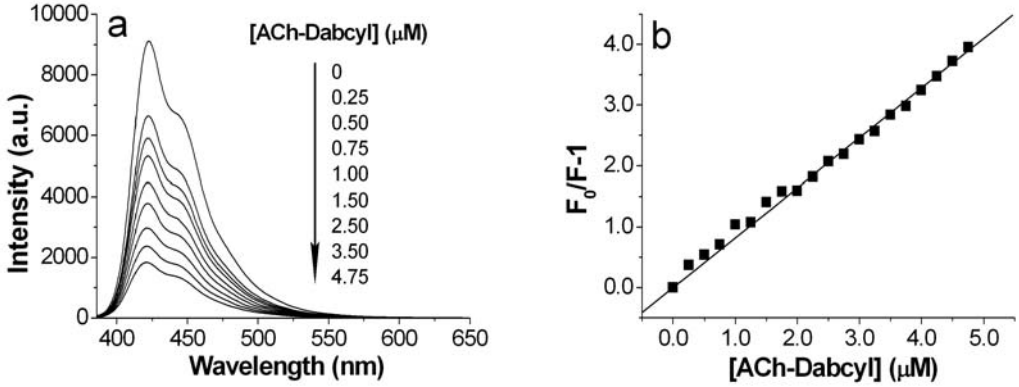

Figure S3. (a) Fluorescence emission spectra of PFP-COOH in buffer solution with successive additions of ACh-Dabcyl. (b) K_{sv} plot of PFP-COOH in the presence of ACh-Dabcyl. [PFP-COOH] = 2.0 μM in RUs, [ACh-Dabcyl] = 0 to 4.75 μM. Fluorescence measurements were performed in 5 mM phosphate buffer solutions (pH 8.0) with excitation at 376 nm.

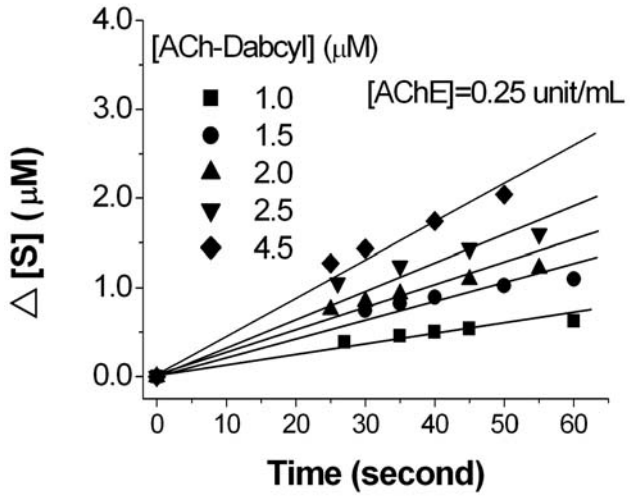

Figure S4. Plots of concentration changes of ACh-Dabcyl versus the AChE incubating time. [PFP-COOH] = 2 μM, [AChE] = 0.25 unit/mL, [ACh-Dabcyl] = 1.0, 1.5, 2.0, 2.5, 4.5 μM. The fluorescence measurements were performed in 5 mM phosphate buffer solutions (pH 8.0) with excitation at 376 nm.

References

[1] O.Seitz, H. Kunz, *J. Org. Chem.* **1997**, *62*, 813.

[2] T. Park, S. A. Haque, R. J. Potter, A. B. Homes, J. R. Durrant, *Chem. Commun*. **2003**, 2878.