## Selective and Sensitive Detection of Melamine by Intra/Inter Liposomal Interaction of Polydiacetylene liposomes

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**Materials and Method.** All solvents were purchased from Sigma-Aldrich Chemicals. 10,12pentacosadiynoic acid (PCDA) was purchased from GFS Chemicals. Dicyclohexylcarbodiimide, 4-dimethylaminopyridine, cyanuric acid, 1,8-diazabicycloundec-7-ene, carbon tetrabromide, triphenylphosphine, 6-bromohexan-1-ol, tri(ethylene glycol) were purchased from Sigma-Aldrich Chemical Co. UV/Vis absorption spectra were taken on a Varian Cary50 UV/Vis spectrophotometer. Fluorescence spectra were obtained using PTI QuantaMasterTM spectrofluorometers equipped with an integrating sphere. Fluorescence images were taken by Olympus BX51 W/DP71 fluorescent microscope.

Amine modified glass slides were prepared according to the reference 15. Briefly, glass slides were cleaned with chloroform, acetone, and 2-propanol for 5 min each. The precleaned glass slides were sonicated in sulfuric acid containing no-chromix. After thorough rinse with deionized water and dry, the glass slides were treated with 2 wt% solution of 3-aminopropyltriethoxysilane in toluene for 1 hour and baked at 130 °C for 30 min. The glass slides were sonicated in toluene, toluene: methanol (1:1) and methanol for 3 min each to remove any unbound silane monomer.

## Synthesis of PCDA Derivatives

The diacetylene monomer PCDA-CA and PCDA-EG-CA investigated in this work were synthesized through the following reaction scheme (Scheme S1).



**Scheme S1:** (A) PCDA-CA synthesis: (a) dicyclohexylcarbodiimide, 4-dimethylaminopyridine, methylene chloride, 0 °C, 2 hours. (b) cyanuric acid, 1,8-diazabicycloundec-7-ene, dimethylformamide, 60 °C, 8 hours. (B) PCDA-EG-CA synthesis: (a) carbon tetrabromide, triphenylphosphine, dichloromethane, 0 °C, 2 hours. (b) 10,12-pentacosadiynoic acid, dicyclohexylcarbodiimide, 4-dimethylaminopyridine, dichloromethane, 0 °C, 2 hours. (c) cyanuric acid, 1,8-diazabicycloundec-7-ene, dimethylformamide, 60 °C, 8 hours.

Synthesis of PCDA-CA



6-Bromohexyl pentacosa-10,12-diynoate 6-Bromohexyl pentacosa-10,12-diynoate To a solution of 10,12-pentacosadiynoic acid (2 g, 5.34 mmol) ) in dichloromethane (30 mL) at 0 °C was added 6-bromohexan-1-ol (1.06 g, 5.88 mmol), dicyclohexylcarbodiimide (1.32 g, 6.41 mmol) and 4-dimethylaminopyridine (0.012 g, 0.10 mmol). After the reaction mixture was vigorously stirred for 2 hours, 20 mL of hexane was poured into the mixture and the white urea solid was filtered off. The filtrate solution was

concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/diethyl ether =10/1) to give 2.24 g (78 %) of desired diacetylene monomer **6-bromohexyl pentacosa-10,12-diynoate** as a colorless oil. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  1.23 (t, J = 6.8 Hz, 3H), 1.26-1.62 (m, 38H), 1.85-1.87 (m, 2H), 2.22-2.32 (m, 6H), 3.41 (t, J = 6.6 Hz, 2H), 4.07 (t, J = 6.6 Hz, 2H). <sup>13</sup> C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  13.98, 19.08, 22.60, 24.88, 25.04, 27.69, 28.24, 28.78, 28.99, 29.52, 31.80, 32.52, 33.52, 34.23, 64.00, 65.21, 173.85, MS (ES): Calcd, 536.3; found (M+Na)<sup>+</sup>, 559.4.



**PCDA-CA** To a solution of **6-bromohexyl pentacosa-10,12-diynoate** (1.7 g, 3.16 mmol) in dimethylformamide (50 mL) was added cyanuric acid (4.08 g, 31.60 mmol) and 1,8-diazabicycloundec-7-ene (0.47 mL, 3.16 mmol). The reaction mixture was heated under 60 °C for 8 hours, poured into the water and extracted with chloroform. The organic layer was washed 3 times with water to eliminate the excess cyanuric acid, dried with MgSO<sub>4</sub> and filtered. Most of

solvent was removed under vacuo. The residue was recrystallized in ethyl acetate to give 1.5 g (81 %) of the desired diacetylene monomer **PCDA-CA** as a white solid. mp 127 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  0.87 (t, *J* = 6.8 Hz, 3H), 1.25-1.64 (m, 40H), 2.21-2.26 (m, 4H), 2.28 (t, *J* = 7.2 Hz, 2H), 3.85 (t, *J* = 6.8 Hz, 2H), 4.05 (t, *J* = 6.6 Hz, 2H), 9.10 (brs, 2H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): 14.07, 19.15, 22.63, 24.88, 25.46, 26.14, 27.58, 28.26, 28.30, 28.42, 28.72, 28.81, 28.86, 29.05, 29.29, 29.42, 29.55, 29.57, 29.59, 31.86, 34.28, 41.85, 64.09, 65.16, 65.24, 77.39, 77.57, 147.81, 148.97, 173.99. MS (ES): Calcd, 585.4; found (M+Na)<sup>+</sup>, 608.4.

## Synthesis of PCDA-EG-CA



## 2-(2-(2-Bromoethoxy)ethoxy)ethanol

**2-(2-(2-Bromoethoxy)ethoxy)ethanol** To a solution of tri(ethylene glycol) (4.08 g, 27.14 mmol) in dichloromethane (50 mL) at 0 °C was added carbon tetrabromide (3.00 g, 9.05 mmol) and triphenylphosphine (2.61 g, 9.95 mmol). The reaction mixture was stirred at room temperature for 2 hours, and the solvent was removed in vacuo. The residue was purified by silica gel column chromatography (hexane/ethyl acetate=1/2) to give 1.61 g (83 %) of desired monomer **2-(2-(2-bromoethoxy)ethoxy)ethanol** as yellowish oil (1.61 g). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  2.18 (brs, 1H), 3.44 (t, *J* = 6.4 Hz, 2H), 3.58 (m, 2H), 3.64 (s, 4H), 3.70 (m, 2H), 3.78 (t, *J* = 6.2 Hz, 2H).



2-(2-(2-Bromoethoxy)ethoxy)ethoy)ethyl pentacosa-10,12-diynoate 2-(2-(2-Bromoethoxy)ethoy)ethyl pentacosa-10,12-diynoate To a solution of 10,12pentacosadiynoic acid (2 g, 5.34 mmol)) in dichloromethane (30 mL) at 0 °C was added 2-(2-(2bromoethoxy)ethoxy)ethanol (1.32 g, 5.34 mmol), dicyclohexylcarbodiimide (1.32 g, 6.41

mmol) and 4-dimethylaminopyridine (0.012g, 0.10 mmol). After the reaction mixture was vigorously stirred for 2 hours, 20 mL of hexane was poured into the mixture and the white urea solid was filtered off. The solvent was removed in vacuo. The residue was purified by silica gel column chromatography (hexane/ethyl acetate =10/1) to give 2.7 g (89 %) of desired diacetylene monomer **2-(2-(2-bromoethoxy)ethoxy)ethyl pentacosa-10,12-diynoate** as a colorless oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  0.84 (t, *J* = 6.8 Hz, 3H), 1.21-1.32 (m, 26H), 1.43-1.59 (m, 6H), 2.20 (t, *J* = 6.8 Hz, 4H), 2.29 (t, *J* = 7.6 Hz, 2H), 3.43 (t, *J* = 6.2 Hz, 2H), 3.62-3.68 (m, 6H), 3.77 (t, *J* = 6.2 Hz, 2H), 4.19 (t, *J* = 4.8 Hz, 2H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  13.99, 19.04, 22.55, 24.72, 28.23, 28.61, 28.77, 28.96, 29.21, 29.34, 29.52, 30.11, 31.78, 34.00, 63.14, 65.17, 69.13, 70.41, 71.12, 77.34, 173.48. MS (ES): Calcd, 568.3; found (M+Na)<sup>+</sup>, 591.3

PCDA-EG-CA To a solution of 2-(2-(2-Bromoethoxy)ethoxy)ethyl pentacosa-10,12-diynoate



(1.9 g, 3.33 mmol) in dimethylformamide (50 mL) was added cyanuric acid (4.30 g, 33.33 mmol) and 1,8-diazabicycloundec-7-ene (0.50 mL, 3.33 mmol). The reaction mixture was heated under 60 °C for 8 hours, poured into the water and extracted with ethyl acetate. The organic layer was washed 3 times with water to eliminate the excess cyanuric acid, dried with MgSO<sub>4</sub> and filtered. Most of solvent was removed under vacuo. The resulting solution was recrystallized from methyl alcohol and ethyl acetate to give 1.56 g (76 %) of desired diacetylene monomer **PCDA-EG-CA** as a white solid. mp 80 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  0.875 (t, *J* = 7.0 Hz, 3H), 1.25-1.47 (m, 26H), 1.49-1.57 (m, 4H), 1.60 (t, *J* = 7.0 Hz, 2H), 2.22-2.25 (m, 4H), 2.32 (t, *J* = 7.6 Hz, 2H), 3.65-3.69 (m, 6H), 3.76 (t, *J* = 5.2 Hz, 2H), 4.07 (t, *J* = 5.2 Hz, 2H), 4.22 (t, *J* = 4.8 Hz, 2H), 9.49 (brs, 2H) . <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  14.07, 19.15, 22.63, 24.78, 28.27,

28.30, 28.73, 28.81, 28.86, 29.05, 29.29, 29.42, 29.59, 31.86, 34.12, 40.46, 63.27, 65.17, 65.24, 67.32, 69.00, 69.80, 70.42, 77.38, 77.55, 148.24, 149.22, 173.97. MS (ES): Calcd, 617.4; found (M+Na)<sup>+</sup>, 640.4.

**Melamine Detection with the PDA Liposome in Solution:** To a solution containing 1 ml of 0.2 mM solution of the PCDA-EG-CA/PCDA (9/1) liposome was added melamine solution to make the each desired concentration from 1 to 40 ppm at room temperature. UV-vis and PL spectra were taken after 5 min incubation at room temperature.

**Microarray of PDA Liposome:** the resulting PDA liposome was spotted onto amine modified glass slide with a manual microarray (V&P Scientitic, VP475A) and stored in a humidity chamber (humidity  $\sim$  70%) for 6 h. Amine modified glass slide was prepared according to the reference.[12] After carefully rinse with 50mM of HEPES buffer pH 7.0, and dried under a stream of nitrogen.

**Fluorescent Microscope Images:** The liposome microarray was incubated with a solution containing various concentrations (0.5, 1, 2.5, 5, 10, 20, 40 ppm) of melamine for 30 min. The fluorescence images were obtained by using a fluorescent microscope (Olympus BX51 W/DP71). The excitation wavelength was 600 nm. The data were obtained with five independent experiments.

**Preparation of the Diacetylene Liposome:** 100  $\mu$ l of PCDA-EG-CA/PCDA (9:1 mole ratio) monomer mixture solution was injected rapidly to 20 ml of 50 mM HEPES buffer pH 7.0. The suspended solution was sonicated for 20 min by probe disembrator and filtered with 0.8  $\mu$ m cellulose acetate syringe filter. The dispersed PDA liposome solution was cooled at 5 °C for 6 hours. The final concentration is 0.2 mM.



**Figure S1.** Colorimetric change of PCDA-CA/PCDA (4/1 mole ratio) in 5 mM (A) and 50 mM HEPES buffer pH 7.0 (B) upon the various concentration of melamine. Buffer concentration study was conducted to optimize the highest polymerization rate of PDA liposomes. 50 mM of HEPES buffer pH 7.0 showed the highest polymerization rate by blue color intensity.



Figure S2. Colour change of 100 % PCDA-EG-CA liposome solution in 50 mM HEPES buffer

pH 7.0 upon the various concentration of melamine.



**Figure S3.** (A) Colorimetric response image of PCDA-EG-CA/PCDA (1/9, 1/6, 1/3, 1/1, 3/1, 6/1, 9/1) liposome upon 10 ppm of melamine. (B) Optical image of aggregated PCDA-EG-CA/PCDA (9/1) liposome upon addition of melamine after 5 min (a) and 1 hr (b).