CHAPTER II

EXPERIMENTAL

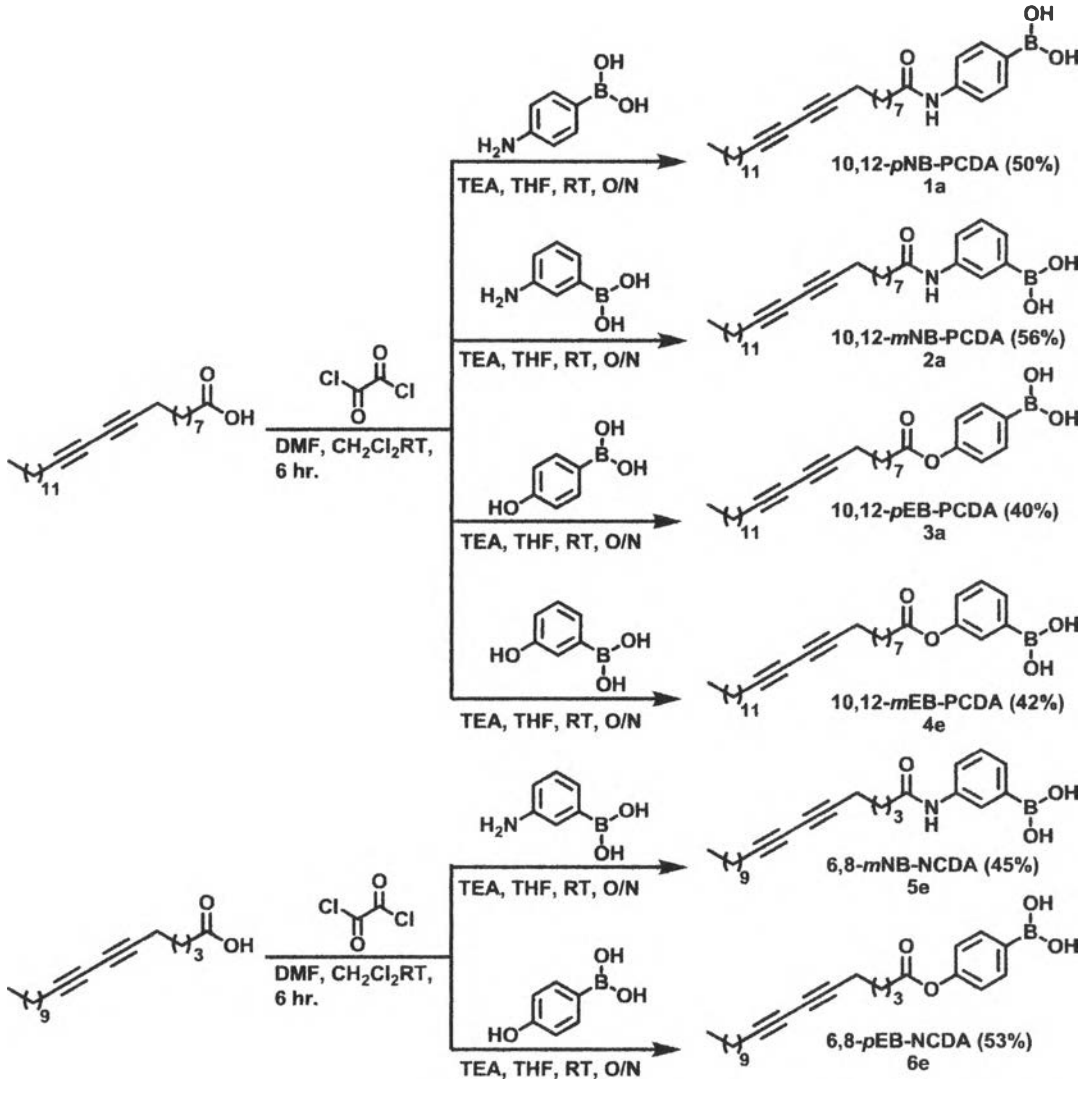
2.1 Materials

10,12-pentacosadiynoic acid (PCDA) and 6,8-nonadecadiynoic acid (NCDA) were purchased from GFS Chemicals (USA). Oxalyl chloride was purchased from Fluka (Switzerland). Triethylamine (AR grade) was purchased from Merck. 3-Amino phenyl boronic acid, 3-Hydroxy phenyl boronic acid, 4-Amino phenyl boronic acid and 4-Hydroxy phenyl boronic acid were purchased from Aldrich (USA). 4-amino salicylic acid and 2,4-dihydroxybenzoic acid were purchased from Aldrich (USA). Triethylene glycol was purchased from Merck. N,N-dicyclohexylcarbodiimide (DCC) was purchased from Aldrich. 4-(Dimethylamino) pyridine (DMAP) was purchased from Aldrich. *p*-Toluenesulfonyl chloride was purchased from Aldrich. Milli-Q water and 10 mM HEPES buffer pH = 7.4 were used in all experiments. Analytical grade solvents such as chloroform and methylene chloride were used without further purification. All organic solvents for monomer synthesis and purification were purchased from RCI Labscan (Thailand). Thin layer chromatography (TLC) was carried out using Merck 60 F254 plates with a thickness of 0.25 mm.

2.2 Analytical instruments

The ^{1}H-NMR spectra were recorded on Varian Mercury 400 MHz NMR spectrometer (Varian, USA) and ^{13}C-NMR spectra were recorded on Bruker Mercury 400 MHz NMR spectrometer (Bruker, German) using the residual solvent proton resonance of $CHCl_3$ and DMSO at 7.26 ppm and 2.50 ppm respectively as the reference. The molecular mass were obtained from a low-resolution quadruple mass analyzer (Quattro Micro API 2000, Micromass) using the electrosprin ionization (ESI) technique. Sonication was carried out in a ultrasonicating bath (Transinic S40H, Elma, Germany). UV-irradiation was conducted using UV light source (TUV 15W/G15 T18 lamp; Philips, Holland). The UV/vis spectra were recorded on a temperature variable UV-Vis spectrophotometer (Varian Carry 100 Bio, USA).

2.3 Boronic acid diacetylene monomers synthesis (1a-6a)



Scheme 2.1 Synthesis of boronic acid diacetylene monomers (**1a-6a**).

General procedure for synthesis of acid chloride polydiacetylene: Oxalyl chloride (158.4 µL, 1.82 mmol) was added dropwise into the solution of 10,12-pentacosadiynoic acid (PCDA) (200 mg, 0.53 mmol) in dry dichloromethane (10 mL). The mixture was stirred for 6 hours at room temperature under N_2 atmosphere. Then, the solvent was removed by evaporator. After that, 4-Amiophenylboronic acid (199.8 mg, 1.46 mmol) was dissolved in dry tetrahydrofuran (THF) (1 mL) and then the boronic acid solution was added dropwise into the solution of acid chloride in dry THF (9 mL) and triethylamine (TEA) (374 µL, 2.19 mmol). The mixture was stirred

for overnight at room temperature under N_2 atmosphere. After that, solvent was removed and purified by recrystallization in methanol to give **10,12-*p*NB-PCDA (1a)** as pale brown solid (148.8 mg, 50 %yield): m.p. = 109-111°C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 0.91 (t, J = 6.8 Hz, 3H; -CH_3), 1.30-1.51 (m, 32H; -CH_2), 2.26-2.34 (m, 6H; -CH_2), 6.72 (d, J = 8.8 Hz, 2H; Ar-H), 7.40 (d, J = 8.9 Hz, 2H; Ar-H), 9.17 (s, 1H), 9.62 (s, 1H)

10,12-*m*NB-PCDA (2a): Synthesized according to above general procedure from oxalyl chloride (395.9 µL, 4.54 mmol), PCDA (500 mg, 1.33 mmol), **3-Amino phenyl boronic acid** (681.4 mg, 3.66 mmol) and TEA (938.8 µL, 5.49 mmol). Purified by recrystallization in methanol to give **10,12-*m*NB-PCDA (2a)** as white solid (489.6 mg, 56 %yield): m.p. = 173-176°C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 0.86 (t, J = 6.8 Hz, 3H; -CH_3), 1.24-1.58 (m, 32H; -CH_2), 2.26-2.30 (m, 6H; -CH_2), 7.24 (t, J = 7.7 Hz, 1H; Ar-H), 7.46 (d, J = 7.3 Hz, 1H; Ar-H), 7.72 (d, J = 7.3 Hz, 1H; Ar-H), 7.82 (s, 1H; Ar-H), 7.97 (s, 2H; -OH), 9.76 (s, 1H; -NH)

6,8-*m*NB-NCDA (3a): Synthesized according to above general procedure from oxalyl chloride (742.9 µL, 5.85 mmol), **6,8-nonadecadiynoic acid (NCDA)** (500 mg, 1.72 mmol), **3-Aminophenylboronic acid** (885.8 mg, 4.76 mmol) and TEA (1220 µL, 7.14 mmol). Purified by recrystallization in methanol to give **6,8-*m*NB-NCDA (3a)** as white solid (365.9 mg, 45 %yield): m.p. = 190-191°C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 0.86 (t, J = 6.8 Hz, 3H; -CH_3), 1.25-1.66 (m, 20H, -CH_2), 2.26-2.35 (m, 6H, -CH_2), 7.25 (t, J = 7.7 Hz, 1H; Ar-H), 7.46 (d, J = 7.3 Hz, 1H; Ar-H), 7.72 (d, J = 8.0 Hz, 1H; Ar-H), 7.82 (s, 1H; Ar-H), 7.99 (s, 2H; Ar-H), 9.81 (s, 1H; -NH)

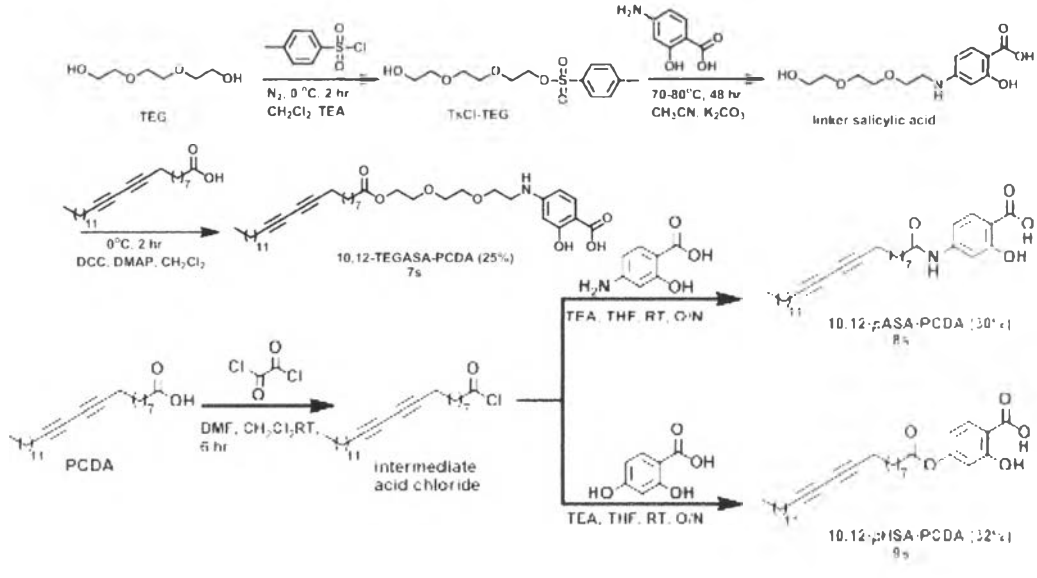
10,12-*p*EB-PCDA (4e): Synthesized according to above general procedure from oxalyl chloride (395.9 µL, 4.54 mmol), PCDA (500 mg, 1.33 mmol), **4-Hydroxyphenyl boronic acid** (262.8 mg, 1.91 mmol) and TEA (325.5 µL, 1.91 mmol). Purified by recrystallization in methanol to give **10,12-*p*EB-PCDA (4e)** as white solid (312.4 mg, 40 %yield): m.p. = 74-75°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.87 (t, J = 5.5 Hz, 3H; -CH_3), 1.25-1.77 (m, 32H; -CH_2), 2.22-2.58 (m, 6H; -CH_2), 7.12 (d, J = 8.3 Hz, 2H; Ar-H), 7.76 (d, J = 8.1 Hz, 2H; Ar-H)

10,12-*m*EB-PCDA (5e): Synthesized according to above general procedure from oxalyl chloride (158.4 µL, 1.82 mmol), PCDA (200 mg, 0.53 mmol), **3-Hydroxyphenyl boronic acid** (266.9 mg, 1.94 mmol) and TEA (490 µL, 2.90 mmol). Purified by recrystallization in methanol to give **10,12-*m*EB-PCDA (5e)** as white solid (160 mg, 42 %yield): m.p. = 50-52°C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 0.85 (t, J = 6.8 Hz, 3H; -

CH_3), 1.34-1.65 (m, 32H; -CH_2), 2.14-2.32 (m, 6H; -CH_2), 7.13 (d, J = 9 Hz, 1H; Ar-H), 7.35 (t, J = 7.8 Hz, 1H; Ar-H), 7.45 (s, 1H; Ar-H), 7.64-7.67 (d, J = 7.5 Hz, 2H; Ar-H)

6,8-*p*EB-NCDA (6e): Synthesized according to above general procedure from oxalyl chloride (742.9 µL, 5.85 mmol), **NCDA** (500 mg, 1.72 mmol), **4-Hydroxyphenyl boronic acid** (722.6 mg, 5.24 mmol) and TEA (1342 µL, 7.86 mmol). Purified by recrystallization in methanol to give **6,8-*p*EB-NCDA (6e)** as white solid (220 mg, 45 %yield): m.p. = 89-90°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.87 (t, J = 6.5 Hz, 3H; -CH_3), 1.26-1.94 (m, 20H, -CH_2), 2.23-2.65 (m, 6H, -CH_2), 7.23 (d, J = 8.2 Hz, 2H; Ar-H), 8.24 (d, J = 8.0 Hz, 2H; Ar-H)

2.4 Salicylic acid diacetylene monomers synthesis (7s-9s)



Scheme 2.2 Synthesis of salicylic acid diacetylene monomers (**7s-9s**).

The synthesis of **10,12-TEGASA-PCDA (7s)** compose of three steps. First, tosylation has used *p*-Toluenesulfonyl chloride as a nucleophile. After that, nucleophilic substitution TsCl-TEG of was reacted 4-aminosalicylic acid. Finally, ester coupling of PCDA was reacted with TEGASA (synthesis route as shown in Scheme 2.2).

Tosylation of TEG: *p*-Toluenesulfonyl chloride (0.3576 g, 18.8 mmol) was added portionwise into the solution of triethylene glycol (11.2250 g, 74.9 mmol) in CH_2Cl_2 (40 mL) and TEA (5 mL, 37.4 mmol). The mixture was stirred for 2 hours at 0°C under N_2 atmosphere. The product was purified by column chromatography with (9:1/$CHCl_3$:MeOH) as an eluent to give the colorless oil of **TsCl-TEG** (4.6996 g,

quantitative yield); ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 2.43 (s, 3H; Ar-CH_3), 3.55-3.70 (m, 10H, -OCH_2), 4.13-4.17 (m, 2H, -OCH_2), 7.33 (d, *J* = 8.1 Hz, 2H; Ar-H), 7.79 (d, *J* = 7.8 Hz, 2H; Ar-H).

Nucleophilic substitution of TsCl-TEG: TsCl-TEG (2.6196 g, 17.8 mmol), 4-aminosalicylic acid (3.2649 g, 21.3 mmol) and K_2CO_3 (2.4559 g, 17.7 mmol) were dissolved in acetonitrile (20 mL). The mixture was reflux for 48 hours under N_2 atmosphere. The solvent was removed by evaporation. The product was purified by column chromatography with (8:2/CH_2Cl_2:MeOH) as an eluent to give the brown viscous oil of **linker salicylic acid**. (2.5011 g, 95 %yield); ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 3.35-3.82 (m, 10H; -OCH_2), 4.43-4.45 (m, 2H; -OCH_2), 6.61 (s, 2H; Ar-H), 7.65 (d, *J* = 9.0 Hz, 1H; Ar-H), 10.82 (s, 1H; -OH).

Ester coupling: *N,N'*-Dicyclohexylcarbodiimide (DCC) (321.9 mg, 1.56 mmol) and 4-Dimethylaminopyridine (DMAP) (2-3 crystals) were portionwise into the solution of 10,12-pentacosadiynoic acid (PCDA) (500 mg, 1.33 mmol) in dry dichloromethane (10 mL). The mixture was stirred for 10 min at 0°C under N_2 atmosphere. The solution of TEGASA (379.4 mg, 1.33 mmol) in dry dichloromethane (5 mL) was added into the mixture. The reaction was stirred for 2 hours. The mixture was filtrated by filtrate paper and then the solvent was removed by evaporation. The crude production was purified by column chromatography with (8:2/CH_2Cl_2:MeOH) as an eluent to give **10,12-TEGASA-PCDA (7s)** as a colorless viscous oil (160.4 mg, 25 %yield): ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.90 (t, *J* = 6.8 Hz, 3H; -CH_3), 1.28-1.55 (m, 32H; -CH_2), 2.24-2.36 (m, 6H; -CH_2), 3.70-3.73 (m, 6H; -OCH_2), 4.24 (t, *J* = Hz, 2H; -OCH_2) 4.47 (t, *J* = Hz, 2H; -OCH_2), 6.26 (d, *J* = 10.2 Hz, 2H; Ar-H), 7.70 (d, *J* = 8.3 Hz, 2H; Ar-H), 10.90 (s, 1H; -OH)

10,12-*p*ASA-PCDA (8s) : Synthesized according to above general procedure from oxalyl chloride (234 µL, 2.72 mmol), PCDA (300 mg, 0.80 mmol), 4-Aminosalicylic acid (308 mg, 2.02 mmol) and TEA (421 µL, 3.02 mmol). Purified by recrystallization in methanol to give **10,12-*p*ASA-PCDA (8s)** as white solid (155 mg, 30 %yield): m.p. = 95-97°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.90 (t, *J* = 6.8 Hz, 3H; -CH_3), 1.28-1.53 (m, 32H; -CH_2), 2.24-2.41 (m, 6H; -CH_2), 6.66 (t, *J* = 7.8 Hz, 1H; Ar-H), 7.18 (t, *J* = 8.3 Hz, 1H; Ar-H), 7.74 (s, 1H; Ar-H)

10,12-*p*HSA-PCDA (9s) : Synthesized according to above general procedure from oxalyl chloride (234 µL, 2.72 mmol), PCDA (300 mg, 0,80 mmol), 2,4-Dihydroxybenzoic acid (344 mg, 2.23 mmol) and TEA (468 µL, 3.35 mmol). Purified by recrystallization in methanol to give **10,12-*p*HSA-PCDA (9s)** as white solid (163.2 mg, %yield): m.p. = 74-76°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.90 (t, *J* = 6.8 Hz, 3H; -CH_3),

1.28-1.55 (m, 32H; -CH_2), 2.24-2.36 (m, 6H; -CH_2), 6.66 (t, *J* = 7.6 Hz, 1H; Ar-H), 7.18 (t, *J* = 8.3 Hz, 1H; Ar-H), 7.74 (s, 1H; Ar-H)

2.5 Preparation of polydiacetylene sols

The diacetylene monomer was dissolved in chloroform (1 mL) in the test tube and then the solvent was evaporated by N_2 gas. The Mill-Q water was added to provide 0.1 mM lipid suspension. The suspension was sonicated at 75-80°C for 2 hours. The suspension was allowed to cool down to room temperature and then filtered through a filter paper. The suspension was kept at 4°C overnight. The vesicle suspension was irradiated by UV irradiation (256 nm, 15 Watt) for 5 minutes at 0°C to generate the PDA blue sols.

2.6 Determination of particle size of polydiacetylene sols

The particle size of polydiacetylene sols were characterized by dynamic light scattering technique. The average size of vesicles and the size distribution were determined by nanosizer (Malvern Instrument). Each sample was repeated measurement for 3 times in order to acquire an average data.

2.7 Thermochromic study of polydiacetylene sols

2.7.1 Study of color transition temperature

The monitoring of PDA sols color transition was taken by using temperature controlled UV-Vis spectrophotometer. The samples were pipetted to immerse in a quartz cell with 1 cm optical path length. The spectrum was collected from 800 to 200 nm with the zero absorbance set at 800 nm. The temperature was heated from 25 to 90°C. The data were collected after the temperature reach to each setting temperature and waited for 5 minutes.

2.7.2 Colorimetric response (%CR)

The quantitative evaluation of colorimetric response (%CR) was determined as percentage of the change of the blue color fraction (FB_0 – FB) against the initial blue color fraction (FB_0) calculated from the following equation

$$\%CR = 100 \times (FB_0 - FB)/FB_0$$

FB is the blue fraction calculated from $A_{blue} / (A_{blue} + A_{red})$ where A_{blue} and A_{red} are the absorbance at the λ_{max} of the blue and the red forms of polydiacetylenes respectively.

2.8 Thermochromic reversibility study of PDA sols

The reversibility of PDA sols was studied by temperature controlled UV-Vis spectrophtotometer. The sample was pipetted 3.0 mL to immerse in a quartz cell with 1 cm optical path length. The temperature was set at 25 and 90°C for ten cycles. The spectrum was collected from 800 to 400 nm. The λ_{max} of the blue and the red phase of each sample were determined at 25 and 90°C of every cycle.

2.8.1 Degree of reversibility (%DR)

To categorize the reversibility of PDA, the blue phase absorption in the cycle experiment were translated into %DR representing percent recovery of the maximum absorption of the blue phase with respect to the first cycle according to the following equation.

$$\%DR = 100 \times \Delta A_{avg}/\Delta A_1$$

ΔA is the difference of absorbance of blue phase between 25°C and 90°C in each cycle. ΔA_{avg} is the ΔA average from the 2nd to 10th cycles and ΔA_1 is ΔA of the first cycle [23].

2.9 Alkalinochromic study of polydiacetylene sols

2.9.1 Acid-base sensing

The stability of PDA sols under various pH were tested. The 2M hydrochloric acid and 2M potassium hydroxide were added into the PDA sols 0.1mM in final concentration for pH adjustment between 2-14. The resulting solutions were kept for 5 minutes before monitoring by digital camera and UV-Vis spectrometry. The spectra were collected from 800 to 200 nm with the zero absorbance set at 800 nm.

2.10 Affinochromic study of polydiacetylene sols

2.10.1 Affinochromism of sugars

The affinochromic properties of all PDAs sols were tested with 8 sugars compose of maltose, lactose, fructose, sucrose, glucose, galactose, mannose and

saccharin at the 1 mM concentration level into PDA sols 0.1 mM in final concentration. The resulting solutions were kept for 5 minutes before monitoring by digital camera.

2.10.2 Affinochromism of surfactans

The affinochromic properties of all PDAs sols were tested with 11 surfactants compose of non-ionic surfactants (Tween20, Brij®58P, TritonX-100), anionic surfactants (SDC, SDS, SDBS) and cationic surfactants (TTAB, DTAB, HTAB, CTAB, HDPB) at the 50 μM concentration level into PDA sols 0.1mM in final concentration. The resulting solutions were kept for 5 minutes before monitoring by digital camera and UV-Vis spectrometry. The spectra were collected from 800 to 200 nm with the zero absorbance set at 800 nm.

2.10.3 Affinochromism of metal ions

The affinochromic properties of all PDAs sols were tested with 16 metal ions compose of Pb^{2+}, Ca^{2+}, Cd^{2+}, Zn^{2+}, Hg^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Na^{+}, Ba^{2+}, Al^{3+}, Li^{+}, Ag^{+}, Mg^{2+} and Sr^{2+} at the 50 μM concentration level into PDA sols 0.1mM in final concentration. The resulting solutions were kept for 5 minutes before monitoring by digital camera.

2.11 Solvatochromic study of PDA coated paper

2.11.1 Preparation of PDA coated paper

Boronic acid diacetylene monomers were fabricated onto filter paper sheets. The fabrication was started by dropping a solution of the DA monomer in THF on filter paper and allowing for air dry in the dark at room temperature. The size of the DA dot can be easily controlled by the solution volume using an auto pipette and the desired numbers of DA dots are created simply by repeating the drop casting process at different locations on the filter paper. The resulting filter paper coated with multiple dots of the DA monomer was irradiated with UV light (254 nm, 500 $\mu W/cm^2$) for 1 min to produce the replicated PDA sensing dots.