Electronic Supplementary Information

Cationic Polythiophenes as Responsive DNA-Binding Polymers

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Experimental section

Materials

Thiophene 3-acetic acid was purchased from Matrix Scientific and was used as received. All solvents used were HPLC grade. Magnesium sulfate and molecular sieves type 3A grade 562 were purchased from Fischer Scientific. Sulfuric acid, ACS grade, was purchased from Acros. Hydrochloric acid, ACS grade, was purchased from Spectrum. Chloroform-d, 99.8%; deuterium oxide, 99.9%; methanol-d_4, 99.8%; and dimethyl sulfoxide-d_6, 99.9% as used for ^{1}H and ^{13}C NMR were purchased from Cambridge Isotope Laboratories, Inc. Anhydrous $FeCl_3$, 98% and 3-(dimethylamino)-1-propylamine, 99% were purchased from Alfa Aesar. Iodomethane, Reagent Plus, 99% stabilized in copper, was purchased from Sigma Aldrich. Agarose optimized grade was purchased from Research Products International, Inc. Perfect DNA^{TM} 50 bp ladder and 6x loading buffer were purchased from Novagen. The plasmid DNA, pUC19, was purchased from New England Biolabs Inc. Whatman filter paper with 110mm pore size was used for filtration. The water used in DNA binding assay was nuclease-free water obtained from QIAGEN Sciences.

Measurements

All ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained from Varian Inova-400 (400 MHz) using $CDCl_3$, DMSO-d_6, methanol-d_4 and D_2O. Fourier transform infrared (FT-IR) spectra were recorded from an Agilent Technologies Cary 630 FTIR with ATR crystal. The molecular weight of polymer **2** was obtained using size exclusion chromatography (SEC) in THF using light scattering and refractive index detectors (Wyatt Technology). All dynamic light scattering (DLS) measurements were run in 1x TBE buffer in triplicate on a Malvern Zetasizer nano series using the cumulants method, with intensity measurements and the standard deviation of the three runs reported. Zeta potential measurements were run on the same instrument under identical conditions and are the results shown are the average and standard deviation of 3-6 measurements. The gas chromatography-mass spectrometry (GC-MS) of the monomer was obtained from Hewlett Packard with HP 6890 series GC system and 5973 mass selective detector. The thermograms of the polymers were measured on TGA Q500 with heating set up to 600 ^oC at a rate of 10 ^oC/min.

Synthesis of methyl 2-(thiophene-3-yl)acetate (1)

3-Thiopheneacetic acid, 3TAA, (2.996 g, 21.08 mmol) was dissolved in dry methanol (17mL, 20 equiv) in a flask charged with magnetic stirring bar. Two drops of concentrated H_2SO_4 was added and the solution was refluxed for 24 h under nitrogen. The reaction was monitored via TLC using 15% ethyl acetate in hexane. After 24 h, the solvent was removed in vacuo and the residue was extracted with diethyl ether (10 mL x 3). The extract was washed with deionized water (20 mL), dried with $MgSO_4$ and

filtered. Compound **1** was purified using silica gel chromatography with gradient elution up to 15% ethyl acetate in hexane. The solvent was removed in vacuo and the product was dried in vacuo overnight. The product was recovered as a pale yellow oil (2.70g, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (1H, dd, J = 2.8, 3.8 Hz), 7.16 – 7.14 (1H, m), 7.04 (1H, dd, J = 1.2, 4.8 Hz), 3.71 (3H, s), 3.67 (2H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.48, 133.53, 128.47, 125.70, 122.83, 77.08, 51.98, 35.61; GC-MS: 9.164 min, m/z 156; TLC 15:85 / Ethyl acetate:hexane, R_f 0.60.

Synthesis of poly(methyl 2-(thiophene-3-yl)acetate) (2)

Anhydrous $FeCl_3$ (8.369 g, 4 equiv) was suspended in dry $CHCl_3$ (30 mL) for 30 min under an N_2 atmosphere at room temperature. A solution of **1** (2.002 g, 12.82 mmol) in dry $CHCl_3$ (30 mL) was added into the $FeCl_3$ suspension dropwise. The reaction mixture was stirred for 24 h under nitrogen. The reaction mixture was concentrated to about 10 mL in vacuo and added dropwise to methanol acidified with 1N HCl (pH = 4, 500 mL) with vigorous stirring to precipitate the polymer. The suspension was allowed to stand, allowing the precipitate to settle, and the solvent was removed by decantation. The precipitate was dried under vacuum at 60 ^{o}C for 24 h. The product was recovered as a brown solid (1.54 g, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23-7.05 (1H, m), 3.91-3.26 (5H, br); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.81, 125-137, 77.02, 52.06, 34.51; FT-IR (neat, cm^{-1}) 2954, 1730, 1435, 1325, 1258 1194, 1155, 1012, 835.

Synthesis of poly(N-(3-dimethylamino)propyl)-2-(thiophene-3-yl)acetamide) (3)

Polymer **2** (105.7 mg, 0.681 mmol based on monomer unit) was added to a round bottom flask together with dry $CHCl_3$ (15 mL) and 3-(dimethylamino)-1-propylamine (0.86 mL, 6.81 mmol, 10 equiv). The reaction mixture was refluxed for 3 days under nitrogen. The reaction mixture was allowed to cool to room temperature then added dropwise to a mixture of $CHCl_3$/diethyl ether (1:1 v/v) (200 mL) with vigorous stirring. The suspension was allowed to stand, allowing the precipitate to settle, and the product was recovered by filtration. The solid was washed with diethyl ether and dried under vacuum at 60 ^{o}C for 48 h. The product was recovered as an orange solid (107 mg, 70%). ^{1}H NMR (400 MHz, MeOD-d_4) δ 7.35-7.20 (1H, m), 3.82-3.51 (2H, m), 3.27-3.06 (2H, m), 2.69-2.53 (2H, br), 2.53-2.27 (6H, br), 1.87-1.67 (2H, br), ^{13}C NMR (100 MHz, MeOD-d_4) δ 172.89, 140-127, 58.28, 54.05, 49.35, 45.12, 39.08, 37.60, 27.80, 25.37, FT-IR (neat, cm^{-1}) 3265, 3057, 2947, 2867, 2820, 2773, 1730, 1644, 1550, 1461, 1338, 1234, 1157, 1036, 842.

Synthesis of poly(N,N,N-trimethyl-3-(2-thiophen-3-yl)acetamido)propan-1-aminium iodide) (4)

Iodomethane (143 μL, 2.30 mmol, 10 equiv) was added dropwise to a mixture of polymer **3** (51.5 mg, 0.230 mmol) in methanol/diethyl ether (2:1 v/v). The reaction mixture was stirred for 24 h under an N_2 atmosphere. Solids were formed during the reaction, and the precipitate was isolated by filtration after adding an excess of diethyl ether to remove unreacted CH_3I. The product was then dried in vacuo. The product was recovered as a dark brown solid (55.0 mg, 100%). ^{1}H NMR (400 MHz, DMSO-d_6/D_2O 1:1 v/v) δ 7.22 (1H, br), 3.68 (1H, br), 3.47 (1H, br), 3.29-3.04 (4H, br), 3.04-2.91 (9H, br), 1.92-1.74 (2H, br); FT-IR (neat, cm^{-1}) 3428, 3260, 3029, 2943, 1731, 1655, 1529, 1474, 1439, 1334, 1228, 1152, 967, 909.

Potentiometric Titration

Polymer **3** was dissolved in 18 MΩ water at a concentration of 1 mg/mL and acidified with 0.1 N HCl. The solution was titrated with 0.1 N NaOH, measuring pH on an Accumet AB15 Plus pH meter (Fig. S11). A pK_a value of 8.3 was calculated using the Henderson-Hasselbalch equation. This was also very close to the value where half of the ammonium groups were ionized.

DNA binding assay

pUC19 plasmid was used in this experiment. Each sample contained a fixed amount of pUC19 (1.0 µg total, made from 25 µL of 0.040 µg/µL), and increasing amounts of polymer **4** (0, 0.37, 0.74, 1.11, 1.48, 3.69, 7.39, 14.78 and 22.17 µL from 1 µg/µL stock solution in nuclease-free water) resulting in increasing N/P ratios (0, 0.50, 1.0, 1.50, 2.0, 5.0, 10, 20, and 30, respectively). 1x TBE buffer was used to adjust the total volume to 50 uL. The equation for N/P calculation is shown below. This charge ratio refers to the ratio of amine nitrogen (N) of the polymer to the phosphate group (P) of the pUC19 DNA. In the equation, x refers to the amount of polymer **4** added in µg, y is the molecular weight of the monomer, Z^+ is the charge of N in each monomer and RU refers to the repeating unit of the plasmid DNA with the average molecular weight of 323.9 µg/µmol. The samples were incubated for 30 min at room temperature. Ten uL of each sample the sample was loaded onto to 2% agarose gel with 0.004% v/v ethidium bromide soaked in 0.5x TBE buffer. The first well in the gel was loaded with ladder DNA (10 µL) and the rest of the samples were added with 6x loading buffer (5µL) prior to loading the samples into the gel. The samples were subjected to an electric field of 120 mAmps for 20mins. The pUC19 and polymer in the agarose gel was visualized under UV light and the photo was taken using digital camera Canon Power Shot A630. Studies using polymer **3** were executed in the same way.

$$\text{Charge ratio} = \frac{\left(\text{x µg}\right)\left(\frac{\text{1 µmol}}{\text{y µg}}\right)\left(\frac{z^+}{\text{1 µmol}}\right)}{\left(\text{1 µg DNA}\right)\left(\frac{\text{1 µmol DNA}_{RU}}{\text{323.9 µg DNA}_{RU}}\right)\left(\frac{1^-}{\text{1 µmol DNA}_{RU}}\right)}$$

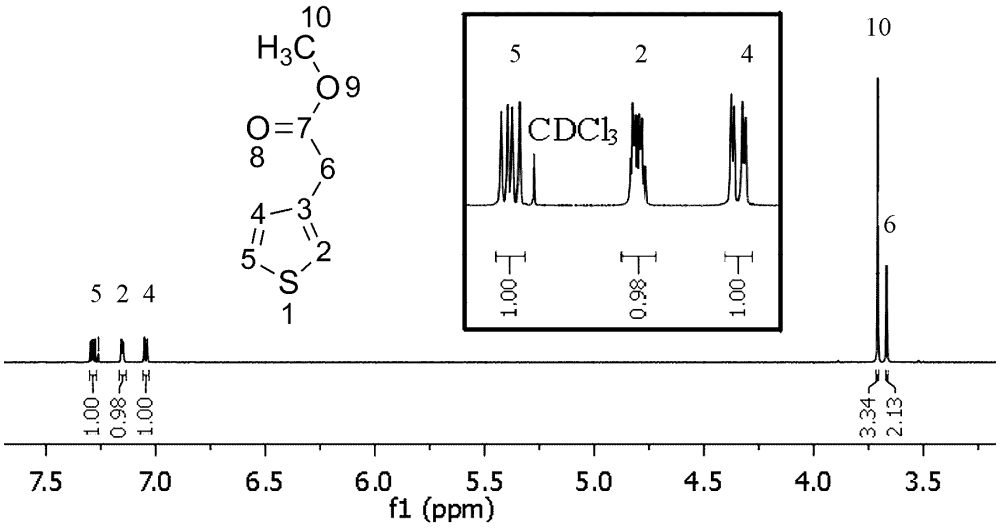

Fig. S1. ^{1}H NMR spectrum of compound **1** in $CDCl_3$.

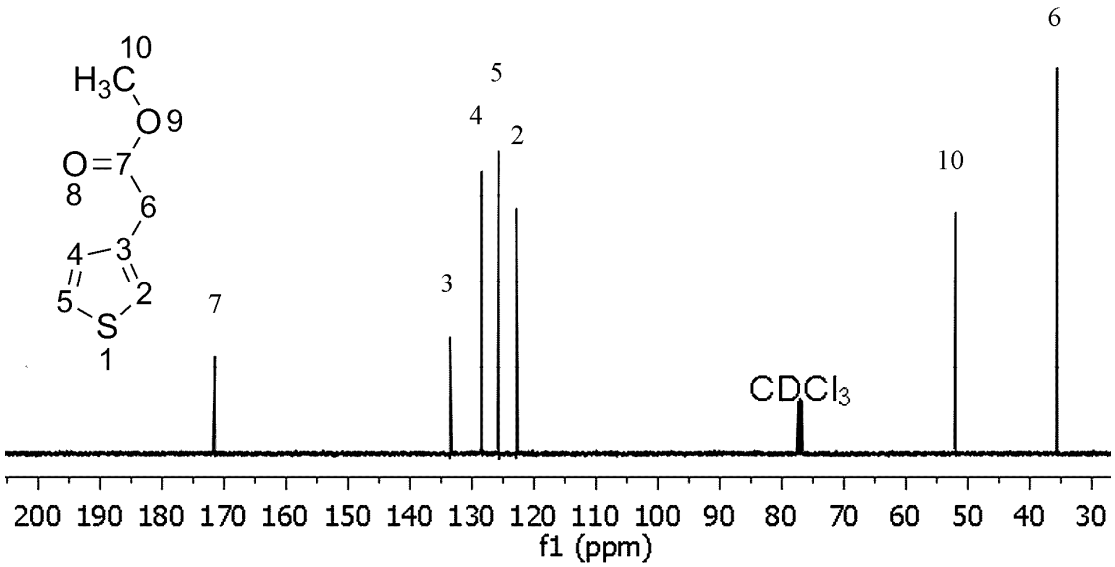

Fig. S2. ^{13}C NMR spectrum of compound **1** in $CDCl_3$.

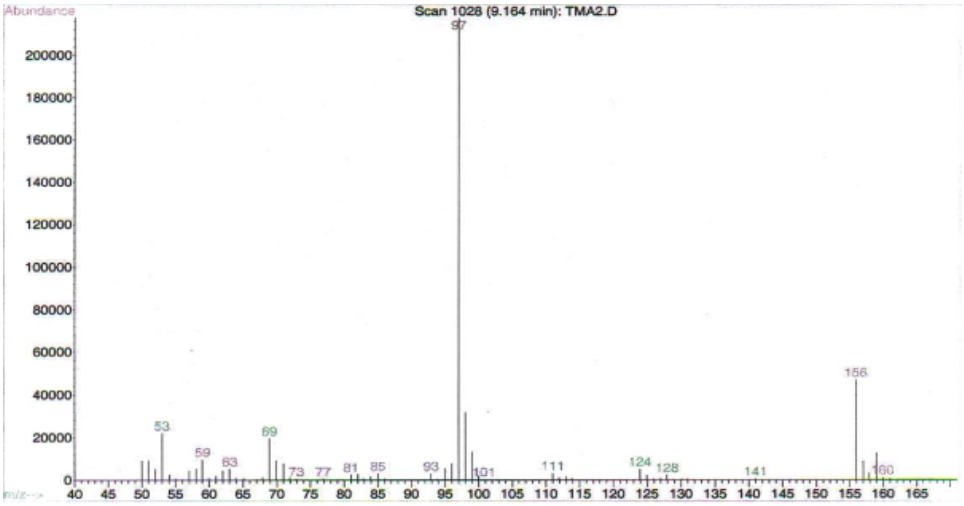

Fig. S3. GC-MS spectrum of compound **1** in $CDCl_3$.

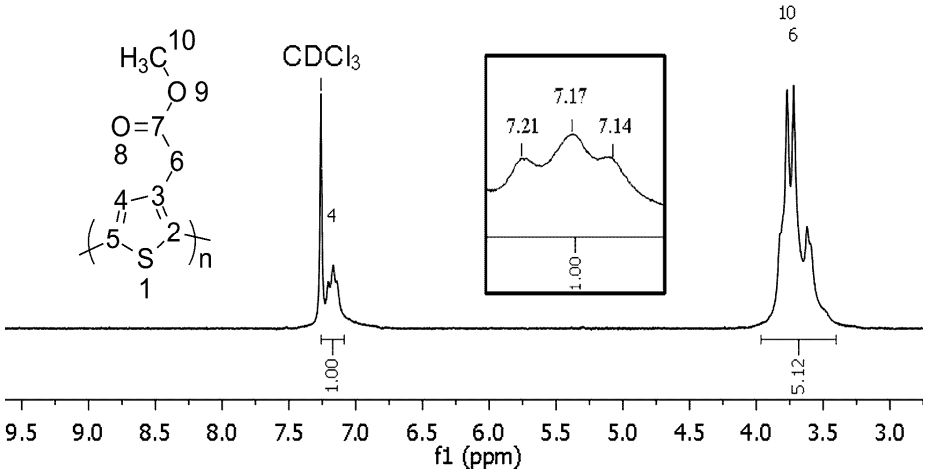

Fig. S4. ^{1}H NMR spectrum of polymer **2** in $CDCl_3$.

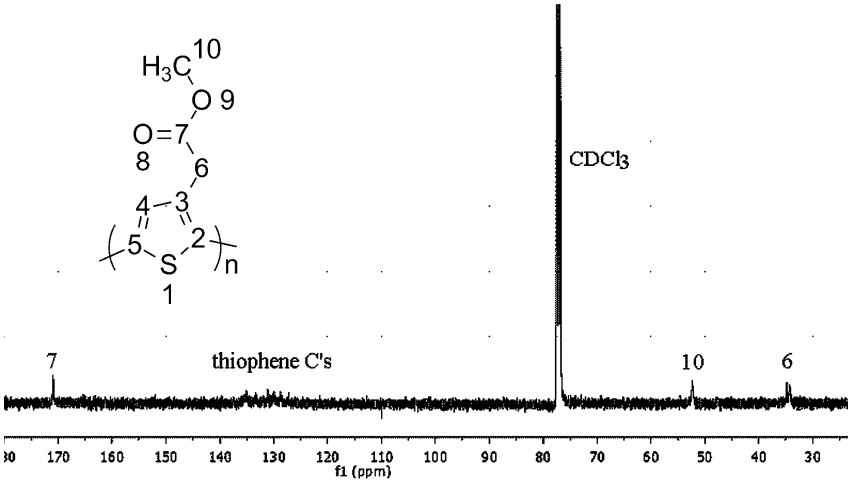

Fig. S5. ^{13}C NMR spectra of polymer **2** in $CDCl_3$.

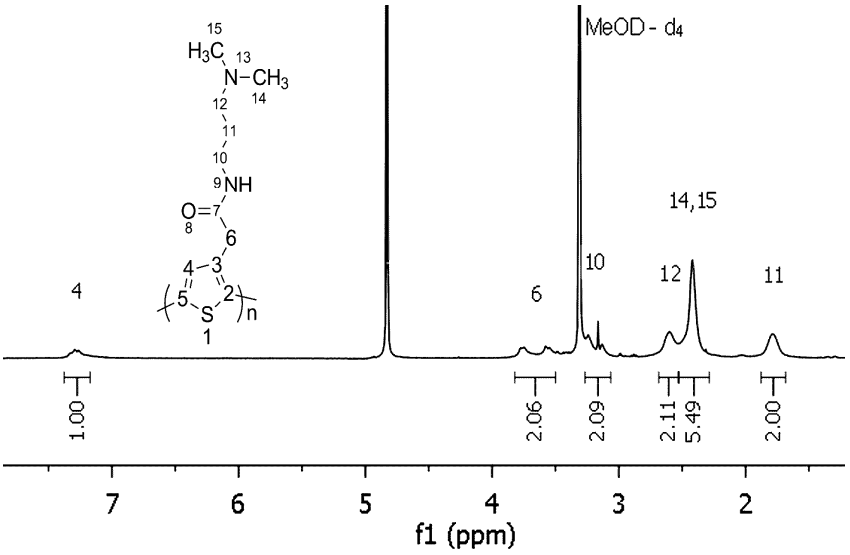

Fig. S6. ^{1}H NMR spectrum of polymer **3** in methanol-d_4.

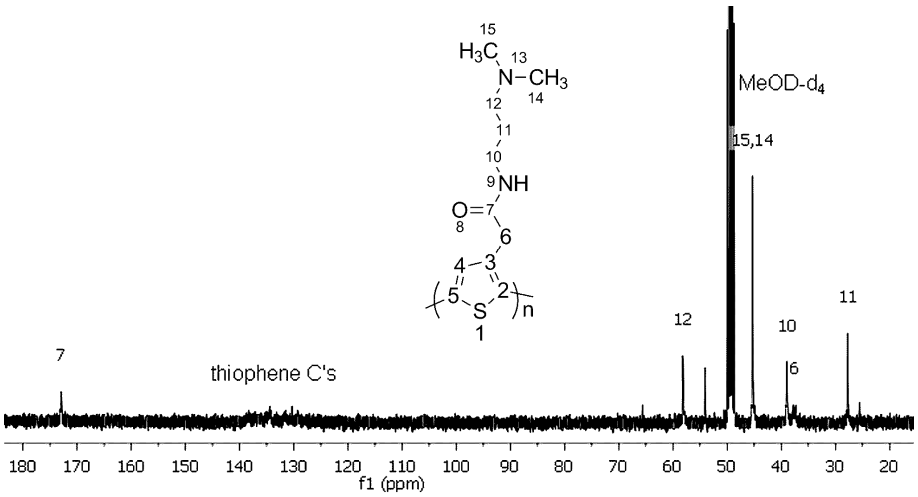

Fig. S7. ^{13}C NMR spectrum of polymer **3** in methanol-d_4.

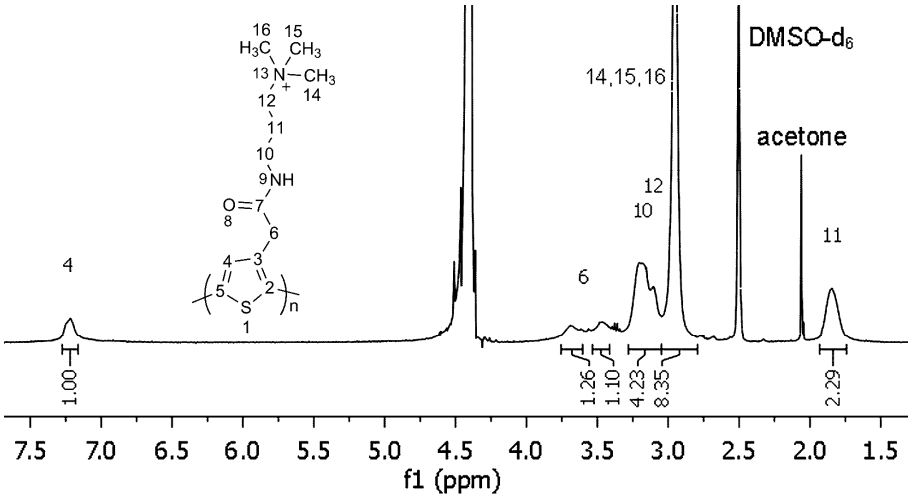

Fig. S8. ^{1}H NMR spectrum of polymer **4** in DMSO-d_6 and D_2O (1:1 v/v).

T**able S1.** GPC data summary of polymer **2** in THF solvent using refractive index detector.

Trial	Mn (g/mol)	Mw (g/mol)	Đ
1	4118	10408	2.53
2	4650	11761	2.53
3	7118	15770	2.22
4	4625	9793	2.12

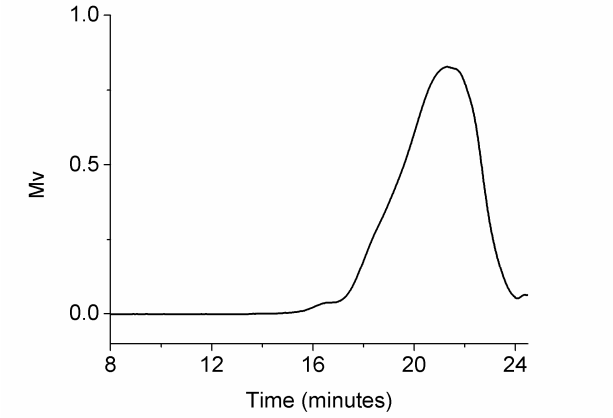

Fig. S9. Representative GPC trace (refractive index detector) of polymer **2** in THF.

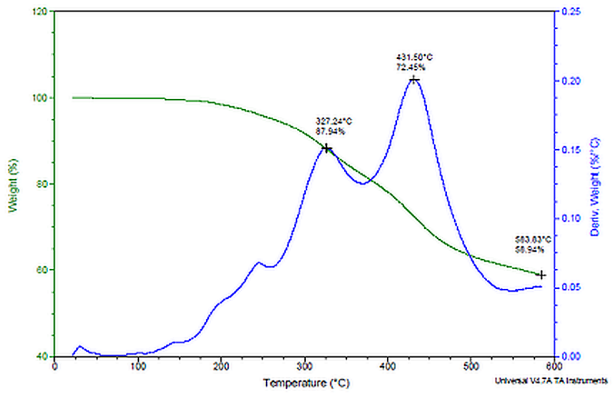

Fig. S10a. TGA thermogram of polymer **2**.

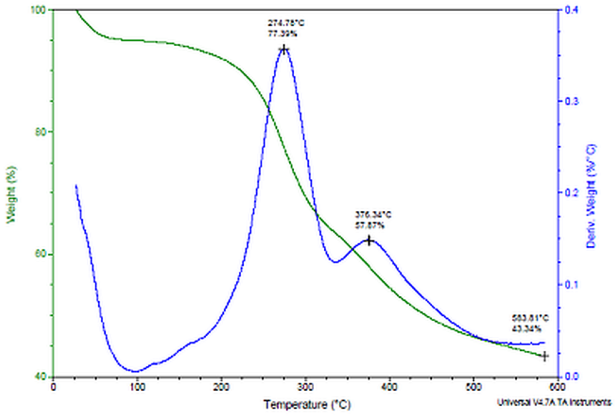

Fig. S10b. TGA thermogram of polymer **3**.

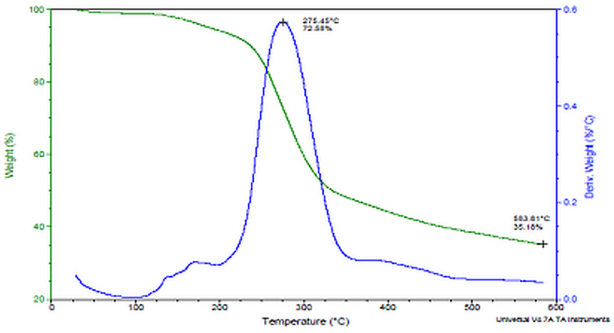

Fig. S10c. TGA thermogram of polymer **4**.

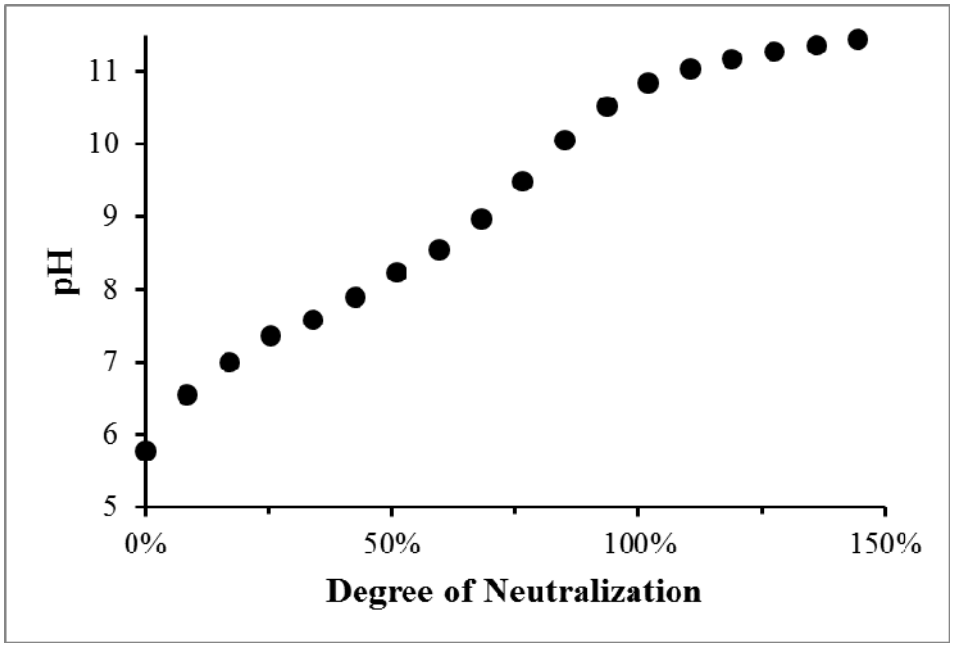

Fig. S11. Titration data of polymer **3**.

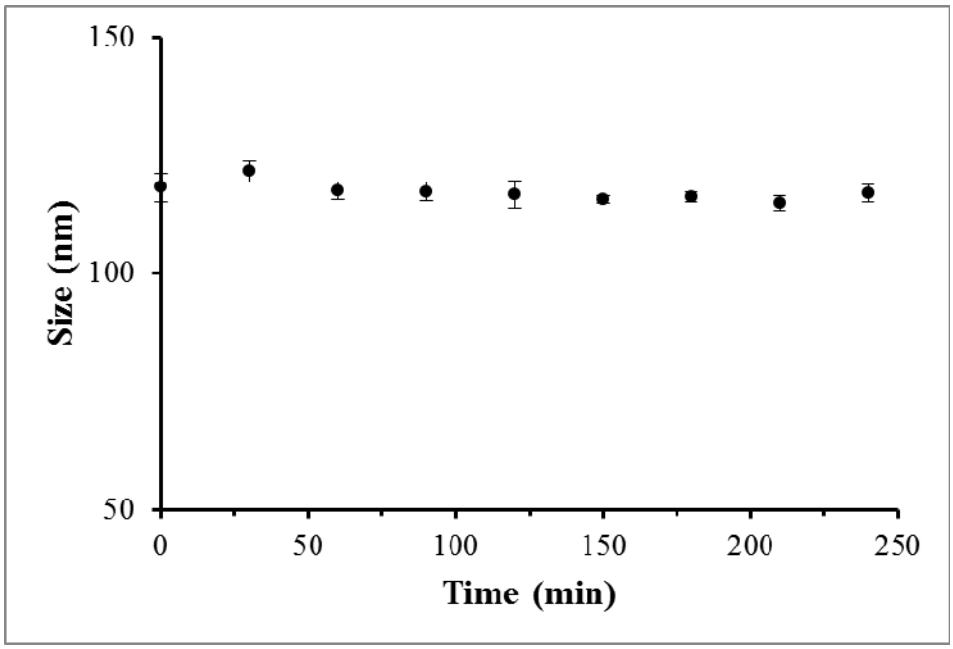

Fig. S12. DLS stability study of polyplexes made from polymer **4** and pUC19 at N/P=30. Note that different batches of polymer **4** and pUC19 DNA were used for this study, resulting in the shift in size compared with the data shown in Figure 3C.