Supporting Information

Influence of Hofmeister I^- on Tuning Optoelectronic Properties of Ampholytic Polythiophene by varying pH and Conjugating with RNA

Radhakanta Ghosh, Dhruba P. Chatterjee[a], Sujoy Das, Titas K. Mukhopadhyay[b], Ayan Datta[b] and Arun K. Nandi*

Polymer Science Unit, Indian Association for the cultivation of Science, Jadavpur, Kolkata-700 032, INDIA

Synthesis of 3[1-Ethyl-2(2-bromoisobutyrate)]thiophene (Thiophene Initiator, TI).

3-thiophene ethanol (2.5 g, 20 mmol) and triethylamine (2.23 g, 22 mmol) were dissolved in anhydrous DCM (30 mL) in a 100 mL round-bottom flask containing a stirrer bar. The mixture was purged with nitrogen and the flask was cooled to 0 °C in an ice bath. Then 2-bromoisobutyryl bromide (BIB, 5.03 g, 22 mmol), diluted with 10 mL of anhydrous DCM, was added drop wise under nitrogen to the stirred solution in the flask over 0.5 h using a pressure-equalizer. After complete addition, the reaction mixture was allowed to warm to room temperature and kept stirring for another 24 h. The triethylamine hydrochloride salt was first filtered and then the filtrate was washed with 1% HCl, saturated $NaHCO_3$, brine solution, and distilled water (ca. 200 mL for each solution). The resulting organic layer was passed through anhydrous Na_2SO_4 to remove any water. The solvent was removed under vacuum. The product was further purified by silica column chromatography in a solvent mixture of hexane:ethyl acetate ((95:5). Finally a brown colour liquid was obtained after the solvent evaporation. (Yield: 3.75 g, 70%). ^{1}H NMR ($CDCl_3$): δ = 1.9 (6H, s), 3.0 (2H, t), 4.3 (2H, t), 6.9−7.2 ppm (aromatic ring protons), (Figure S1a); ^{13}C NMR ($CDCl_3$): δ = 29.4, 30.9, 55.9, 65.9, 121.9, 125.8, 128.4, 137.8, 171.8 ppm (Figure S1b). Molecular weight of TI is obtained using high-resolution mass spectroscopy (HRMS) in DCM which is 277.9 g/mol (Figure S2).

Synthesis of 2,5-Poly(3-[1-ethyl-2(2-bromoisobutyrate)]thiophene) (Polythiophene Macroinitiator, PTI).

Anhydrous $FeCl_3$ (2.5 g, 15 mmol) was dispersed in 30 mL dry chloroform in a 250 mL round-bottom flask containing a stirrer bar under nitrogen atmosphere. Then TI (1.0 g, 3.6 mmol), dissolved in 20 mL of dry $CHCl_3$, was added dropwise into the flask under continuous stirring over 30 min. The solution was stirred for overnight at 25 °C. The mixture

was then added dropwise into 1L of methanol and the resulting mixture was kept for another 2 hour with continuous starring. The solid precipitate was separated with filter paper and was washed with methanol several times. The resulting precipitate polymer was then soxhlet extracted with methanol for a whole day and was dried under vacuum at 40 °C for 3 days. To remove the trace amount of $FeCl_3$, the solid dark brown product was then dissolved in 150 mL of $CHCl_3$ and was refluxed with an additional 100 mL of concentrated ammonia solution. The $CHCl_3$ layer was separated, concentrated by removal of some solvent under rotary evaporation, and precipitated out into an excess amount of methanol. The red precipitate was separated, dried under vacuum at 40 °C for 3 days and was collected as polythiophene macroinitiator, PTI. (Yield: 0.6g, 60%). ^{1}H NMR ($CDCl_3$): δ = 1.9 (6H), 3.2 (2H), 4.4 (2H), 6.9−7.2 ppm (aromatic protons) (Figure S3a); ^{13}C NMR ($CDCl_3$) δ = 29.4, 30.9, 55.9, 65.9, 121.9, 125.8, 128.4, 137.8, 171.8 ppm (Figure S3b). The number-average molecular weight (*M*n) of PTI is about 38 000 g/mol (PDI = 2.7). The number of thiophene units present in the PTI is about 137. The ^{1}H NMR spectrum of PTI (Figure S3) clearly shows that due to head-tail regioirregularity during polymerization, both the peaks assigned as ‘b’ and ‘c’ become broader and splitted than those of TI. The calculated H-T regioregularity from the ratio of signal intensity of these two component signals is ~ 66 %.

Synthesis of 2,5-Poly(3-[1-ethyl-2(2-(poly(*N*,*N*-dimethylaminoethyl methacrylate)-co-poly(tert-butyl methacrylate))]thiophene) (PT-g-pDMAEMA-co-pTBMA).

In a reaction vessel (8 x 2.5 cm), 25 mg of PTI was dissolved in 2 mL of anisole under nitrogen. Then the catalyst CuCl (10 mg), the monomers DMAEMA (0.4 mL) and TBMA (0.6 mL) were injected into the reaction vessel and degassed the reaction vessel by purging with nitrogen. The ligand HMTETA (40 μL) was lastly injected into the reaction mixture and the reaction vessel was placed in a thermo-stated oil bath at 60 °C and was stirred for 6 h.

The polymerization was stopped after 6 h by cooling down the mixture to room temperature and exposing the mixture to air. The solution was poured into 1 L of into the excess petroleum ether to precipitate out the polymer. This process was repeated for 3 times by redissolving the polymer in THF and reprecipitating into excess petroleum ether to remove any trace amount of monomer entrapped within the polymer. Finally the polymer was redissolved in 100 mL THF and was passed through a silica column to remove the catalyst. The polymer was dried by evaporating the THF and was kept it in vacuum oven at 40 °C for overnight. The ^{1}H NMR spectra of PT-g-pDMAEMA-co-pTBMA confirms the structure of the graft copolymers (Figure S4). The signals resonate at 2.27, 2.56 and 4.05 ppm for the corresponding 'j', 'i' and 'h' proton of grafted DMAEMA chains, respectively and the 'e' proton of grafted TBMA chains appear at 1.49 ppm. The molar ratio of grafted TBMA/DMAEMA is 1.48 which is calculated from the ratio of peak area of 'e' proton of grafted TBMA segments and 'h' proton of grafted DMAEMA segments. The molecular weight (*M*n) of the polymer, calculated from ^{1}H NMR spectroscopy is 595520 g/mol. Peaks for 'e', 'h' and 'a' are considered for calculation of PT-g-pDMAEMA-co-pTBMA, namely PT_{137}-g-$pDMAEMA_{1507}$-co-$pTBMA_{2260.}$ Figure S5 shows the GPC traces of PTI and PT-g-pDMAEMA-co-pTBMA. The GPC analysis shows the *M*n of PT-g-pDMAEMA-co-pTBMA are found to be 150,000 g/mol with dispersity 1.7. The molecular weights measured from GPC (here linear polystyrene was used as the calibration standard) for the grafted copolymer may not be accurate due to the non-availability of any grafted/branched copolymer as the GPC standard but the GPC traces show a clean sweep towards lower elution time than that of PTI clearly indicating the formation of graft copolymer possessing larger hydrodynamic volume than that of PTI.

Hydrolysis followed by quaternization of TBMA and DMAEMA residues of PT-g-pDMAEMA-co-pTBMA, respectively, (polythiophene-g-poly[(*N*,*N*,*N*-trimethylamino iodide)ethyl methacrylate-co-methacrylic acid]), (APT).

PT-g-pDMAEMA-co-pTBMA (800 mg) was dissolved in 20 mL of dry DCM in a nitrogen-purged reaction vessel. 1ml of trifluoroacetic acid (TFA) was dropwise injected with continuous stirring at 30 °C. The reaction mixture was kept with continuous stirring for overnight. During the hydrolysis, the resulting hydrolyzed product was precipitated from CH_2Cl_2 gradually. Finally, solvent and excess CF_3COOH were removed by rotating evaporator and the resulting solid precipitate was repeatedly washed with DCM for several times to remove any trace amounts of TFA. Then the product was dried under vacuum for 2 days at 40 °C and a yellow solid PT-g-pDMAEMA-co-pMMA (PTDM) was obtained. The 'e' proton corresponds to the tert-butyl group of grafted TBMA chains at δ 1.49 ppm is totally disappears due to hydrolysis (cf. Figure 1). Then the quaternization of the DMAEMA residues in hydrolyzed PTDM was carried out using methyl iodide (CH_3I). In a nitrogen-purged reaction vessel, 500 mg PTDM was dissolved in 20 ml dry CH_3OH. Then 1 ml of CH_3I was added into the reaction mixture drop wise with continuous stirring. Alkylation step was carried out under reflux for 24 h at 50 °C and during the alkylation the solution became hazy. The resulting product was obtained by removing the solvent and excess of methyl iodide on a rotary evaporator. Furthermore, the polymer was repeatedly washed with CH_3OH to remove any trace amount of CH_3I and the solid product was kept in vacuum at 40 °C for 2 days to get the (polythiophene-g-poly[(*N*,*N*,*N*-trimethylamino iodide)ethyl methacrylate-co-methacrylic acid]), (APT) as the final product.

Characterization

Nuclear magnetic resonance (NMR) spectra

^{1}H and ^{13}C Nuclear magnetic resonance (NMR) spectra of samples were recorded on a 500 MHz Bruker instrument with $CDCl_3$ or D_2O as the solvent. The chemical shifts were expressed in parts per million (ppm) relative to tetramethylsilane.

High-resolution mass spectrum (HRMS)

The high-resolution mass spectrum (HRMS) of TI was recorded by micromass Q-Tof micro Instrument in CH_2Cl_2.

Gel permeation chromatography (GPC)

The molecular weights of the polythiophene macroinitiator (PTI) and the graft co polymer were measured by gel permeation chromatography (GPC) with linear polystyrene calibration kits, used as the calibration standard. The GPC was made employing THF as the eluent, equipped with Waters 1515 pump, Waters 2414 differential refractive index detector, and three μ-Styragel columns. The flow rate for each GPC run was 1 mL/min.

X-ray photoelectron spectrometry (XPS) study

X-ray photoelectron spectrometry (XPS) spectra of APT film was made in an Omicron Nano Technology (model 0571) XPS spectrometer with the help of a focused monochromatized A1 Ka X-ray source (1486.8 eV).

Fourier transform infrared (FTIR) spectra

Fourier transform infrared (FTIR) spectra of the samples in the region of 4000-500 cm^{-1} were recorded at room temperature using KBr pellet in a Shimadzu FTIR instrument (model 8400S).

UV−vis absorption

The UV−vis absorption studies of the samples were taken in aqueous solutions (0.05% w/v) from 190 to 1100 nm using a UV−vis spectrophotometer (Hewlett-Packard, model 8453) at 25 °C using 1.00 cm quartz cuvettes. The spectra were subtracted by the background UV−vis spectra of the same solvent.

Fluorescence Study

Fluorescence spectra of the samples in aqueous solutions (0.05% w/v) were measured using Fluoromax-3 instrument (Horiva Jovin Yvon) in a quartz cell of 1 cm path length at 25 °C. The excitation wavelength is 420 nm and the emission scans were recorded from 440 to 750 nm. Fluorescence lifetimes of the samples at different pH were measured by using a time-correlated single photon counting (TCSPC) fluoremeter (Fluorecule, Horiba Jobin Yvon). The samples (0.05% w/v) were excited with a 440 nm nano LED of Horiba Jobin Yvon having λ_{max} at 532 nm or 547 nm with pulse duration <45 ps. Average fluorescence lifetimes ($<\tau_f>$) were calculated for exponential iterative fitting from the decay times (τ_i) and the relative amplitudes (a_i) using the following relation where a_1, a_2, $a_3$are relative amplitudes and τ_1, τ_2, and τ_3 are the respective lifetimes. $(<\tau_f>) = a_1\tau_1 + a_2\tau_2 + a_3\tau_3$……..... (1).

Zeta (ζ) potential measurement

The zeta (ζ) potential values of the samples in aqueous solution (0.05% w/v) at different pH were measured from a Malvern Zetasizer NANO ZS 90, model No. 3690 at 25 °C.

Circular Dichroism (CD) spectroscopy

Circular Dichroism (CD) spectra of the aqueous solution of each sample (0.05% w/v) at pH 7.4 were recorded in a Jasco J-815 CD spectro polarimeter in a 1 cm quartz cuvette at 25 °C.

Morphology study

The morphology of the samples at various pH were monitored by a high-resolution transmission electron microscope (HRTEM) and a field emission scanning electron microscope (FESEM) instrument. HRTEM study was carried out with a instrument (JEOL, 2010EX) operated at an acceleration voltage of 200 kV and fitted with a charge-coupled device camera. A small drop of aqueous solution of each sample was drop-casted on the carbon-coated copper grid. The samples were dried by slow evaporation of the solvent at room temperature in air, and finally preserved at vacuum before the TEM images were taken. In the case of SEM study, a small amount of the aqueous solution of each sample was drop-casted onto a coverslip and dried by slow evaporation of the solvent at 30 °C, and kept under vacuum at 45 °C for 2 days. Then the dried samples were platinum coated and morphology of each sample was observed by placing them under a Jeol GSM-5800. Optical Microscopy study was made in a Leitz biomed microscope.

Impedance study

Electrical impedance spectroscopy (EIS) of each sample at different pH was carried out using a Solarton SI 1260 impedance analyzer (Solarton, UK) at 25 °C. The spectra were recorded at a frequency range of 32 MHz to 100 mHz with an oscillation voltage of 100 mV. The data were analyzed using the Solarton Z-view software. The equivalent circuit of APT both at pH 2 and at pH 4.5 consists of a resistance (R) and constant phase element (CPE) (Figure 7a) whose values are presented in Table S3. Also, APT at pH 9, the R-C-W circuit (Figure 7b) suggests the presence of resistances (R), Warburg impedance (W) and capacitance (C), where C is parallel to R and W, and the corresponding data are also presented in Table S3. From the CPE values, the capacitance (C) values are calculated using the equation.

$$C = R^{\frac{1-n}{n}} \times Q^{\frac{1}{n}}$$

Where Q is the pre-factor of the CPE, and n is its exponent factor.

Computational Details of Molecular Dynamics Simulations

To explore the characteristics of the APT polymer through molecular dynamics (MD), we have taken a small model consisting of three thiophene rings covalently linked with the side chains containing three ammonium and four carboxylate groups. Two separate cases were considered; in one, carboxylic acids are protonated representing a situation arising at low pH and in the other, carboxylic acids are deprotonated, analogous to the situation at high pH. Each of these polymers was kept at the center of a water box of dimension $4 \times 4 \times 4$ nm^3. In each of the systems three iodide ions were added corresponding to three ammonium groups and the overall charge developed after iodide addition was neutralized with sodium ions. Periodic boundary conditions were applied in all three directions. We performed energy minimization for 5000 steps using conjugate gradient method and equilibration for 5 ns in the canonical (NVT) ensemble at 300 K. After that, a 35 ns production simulation was performed for each system using the isothermal – isobaric (NPT) ensemble at 300 K and 1 atmospheric pressure. Isothermal conditions were maintained through Langevin dynamics with a damping coefficient of 5 ps^{-1}.[1] Langevin piston method was employed to maintain a pressure of 1 atm.[2] To do so, a 100 fs piston period, 50 fs damping time constant and 300 K piston temperature were considered. Particle mesh Ewald (PME) method with 1 Å grid was used to calculate the periodic electrostatic interactions.[3] A 2 fs time step was used to integrate classical equations of motions using the Velocity Verlet algorithm. The SHAKE algorithm was engaged to hold rigid covalent bonds with hydrogen atoms.[4] Non-bonded interactions were calculated having a cut off distance of 12 Å. Packmol utility was used for the preparation of all initial configurations, NAMD 2.10 for all classical molecular dynamics simulations, VMD for

visualization and our in house Tcl scripts for the analysis of data.[5-7] We have used TIP3P model for water and CHARMM General Force Field (CGenFF) parameters (version 3.0.1) for the APT polymer.[8-9]

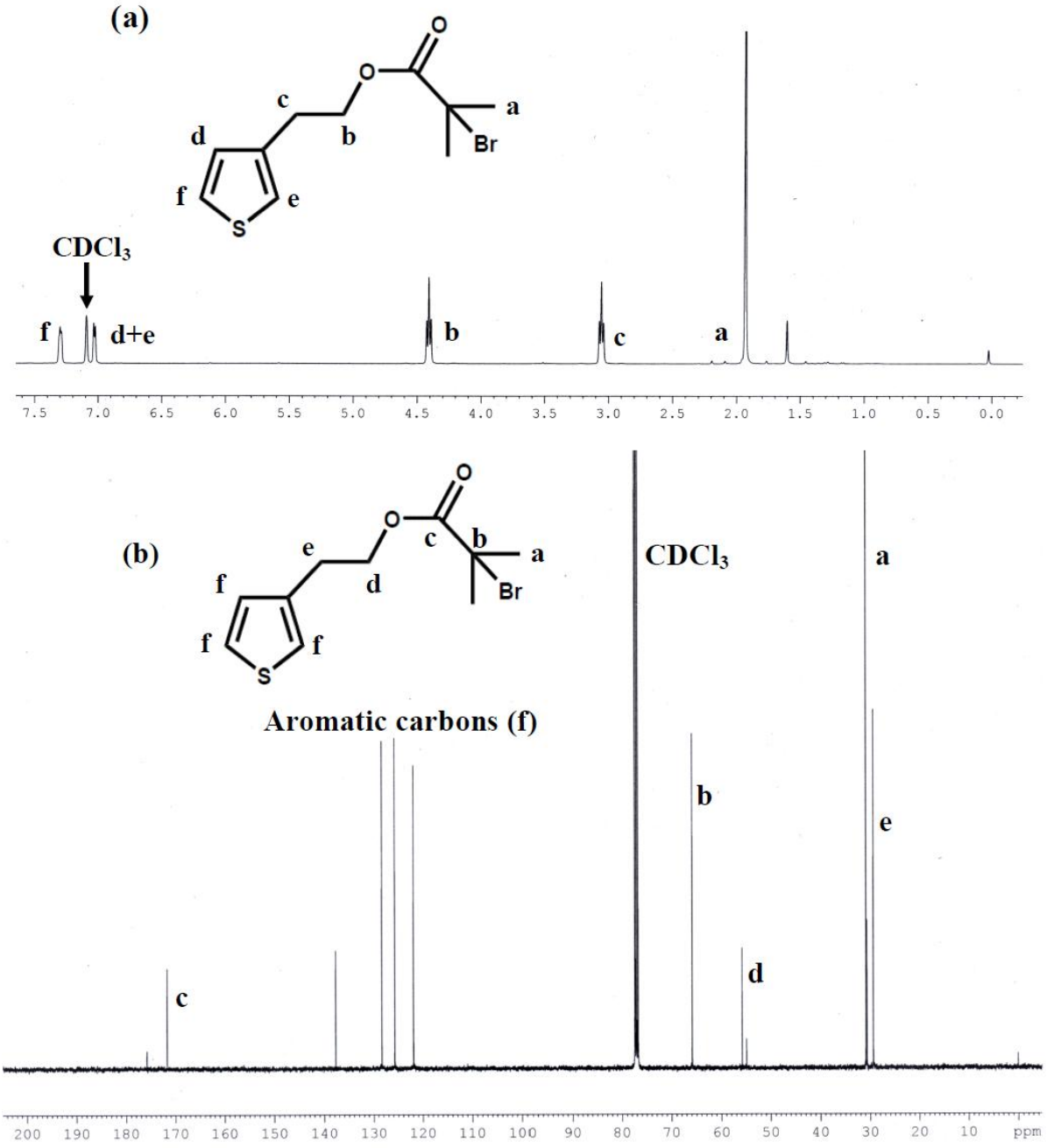


Figure S1. (a) ^{1}H NMR and (b) ^{13}C NMR spectrum of TI along with their peak assignments in $CDCl_3$.

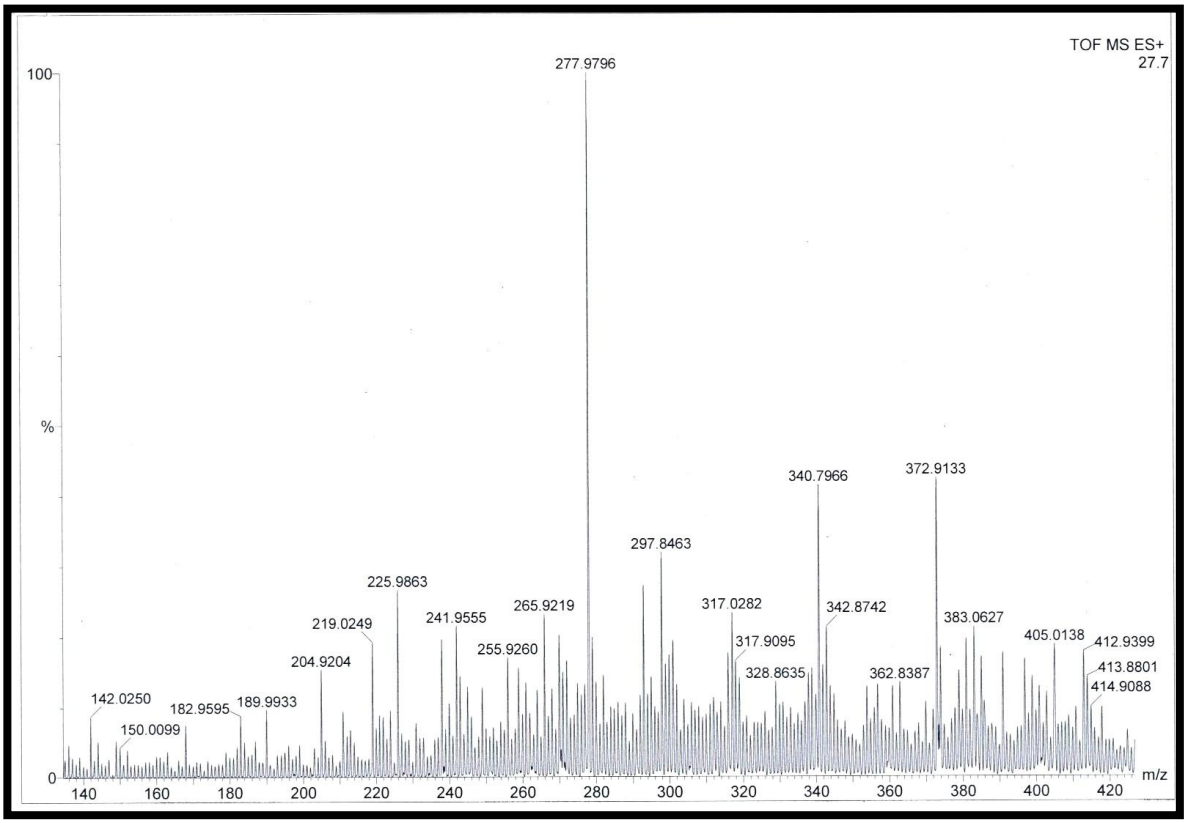


Figure S2. HRMS spectra of TI in CH_2Cl_2.

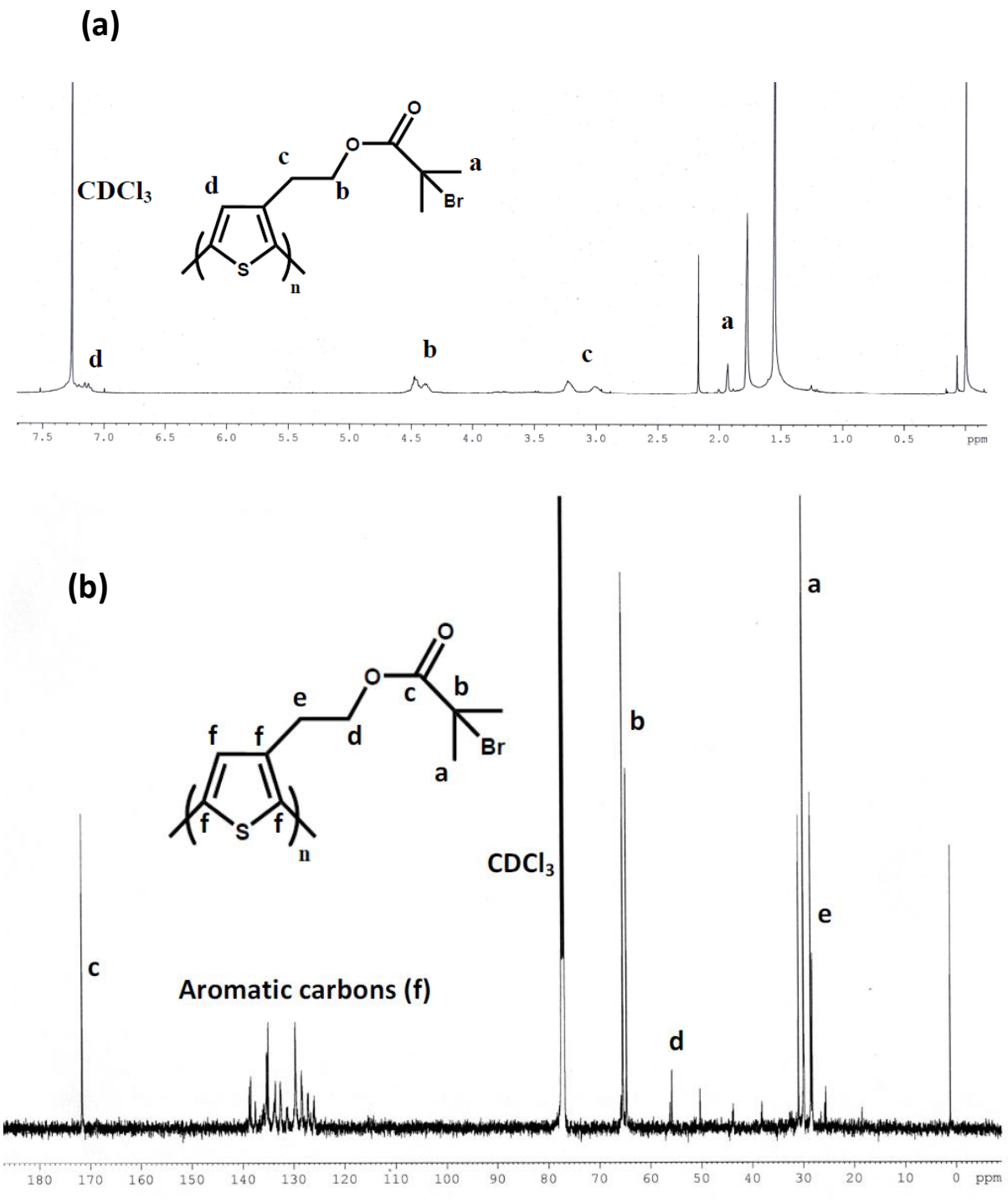


Figure S3. (a) ^{1}H NMR and (b) ^{13}C NMR spectrum of PTI in $CDCl_3$ along with their peak assignments.

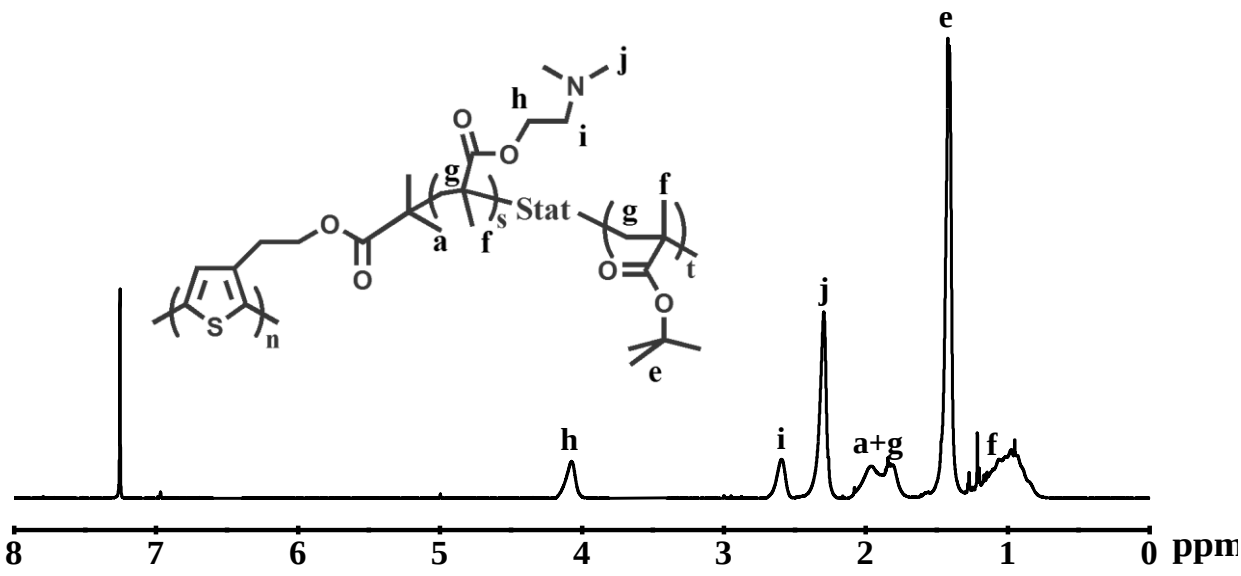


Figure S4. ^{1}H NMR of PT-g-pDMAEMA-co-pTBMA in $CDCl_3$ along with their peak assignments.

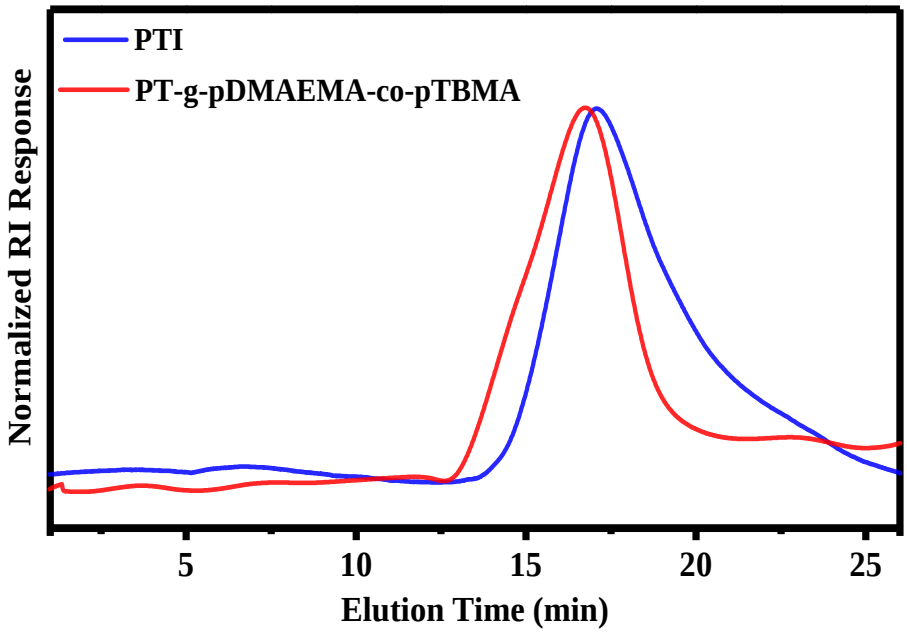


Figure S5. GPC traces of PTI and PT-g-pDMAEMA-co-pTBMA. Eluent: THF, Flow rate: 1ml/min

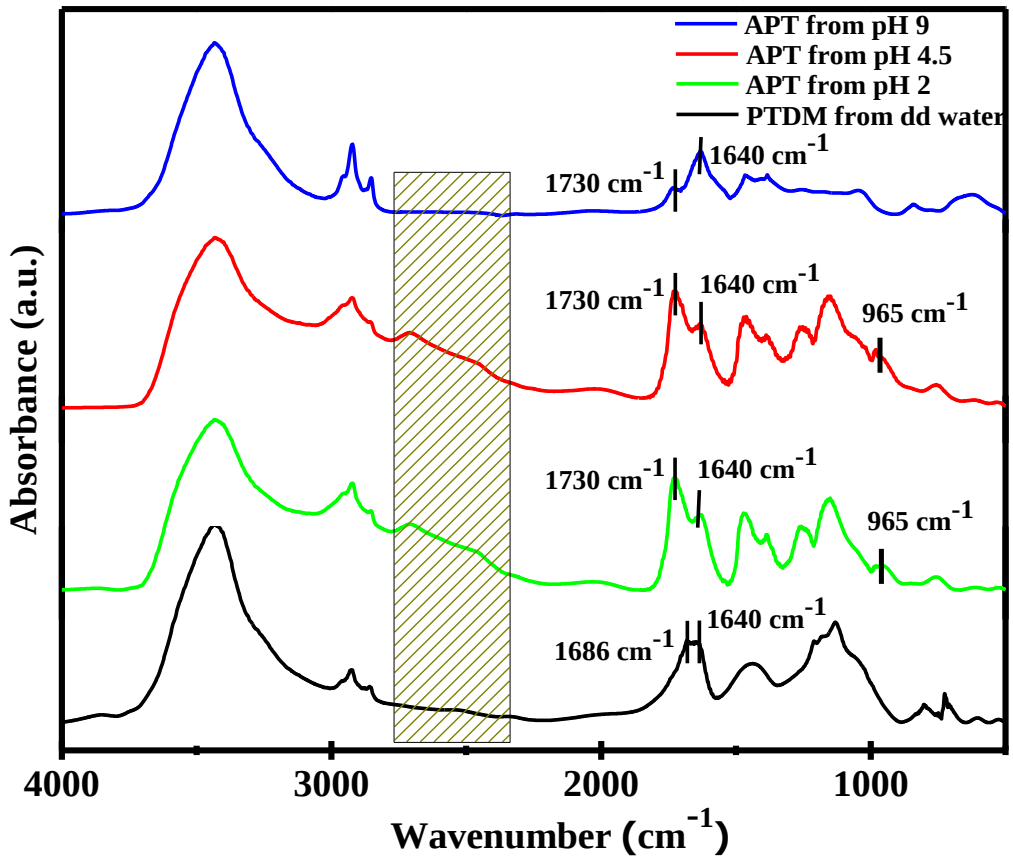


Figure S6. FTIR spectra of freeze dried samples of PTDM from dd water solution and APT from pH 2 solution, from pH 4.5 solution and from pH 9 solution (as indicated).

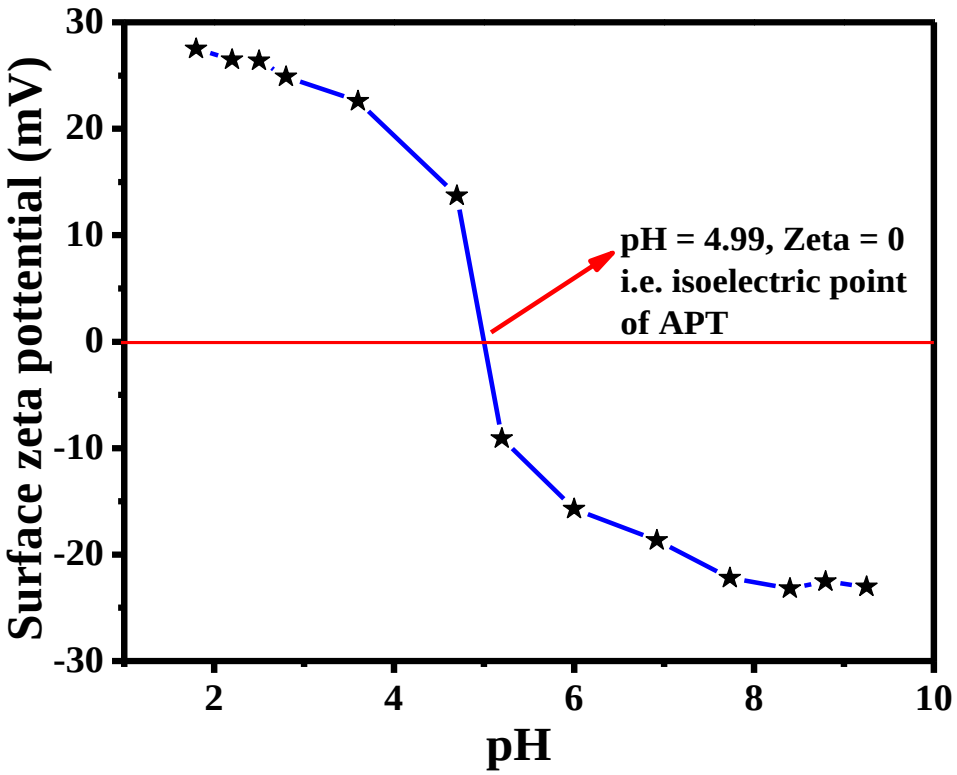


Figure S7. Variation of zeta potential with pH of APT showing isoelectric point determination of APT.

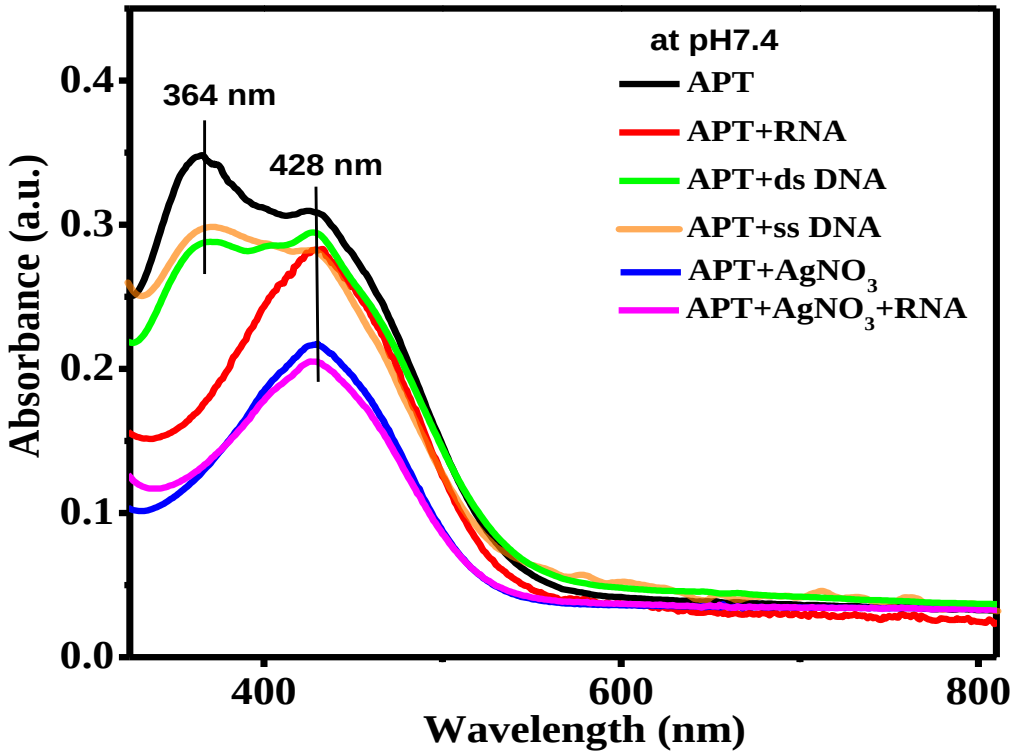


Figure S8. UV-vis absorption spectra of different APT system (as indicated) at pH 7.4 (APT: 0.05% w/v, RNA: 0.083% (w/v), DNA: 0.00044% (w/v) in 2 ml solution).

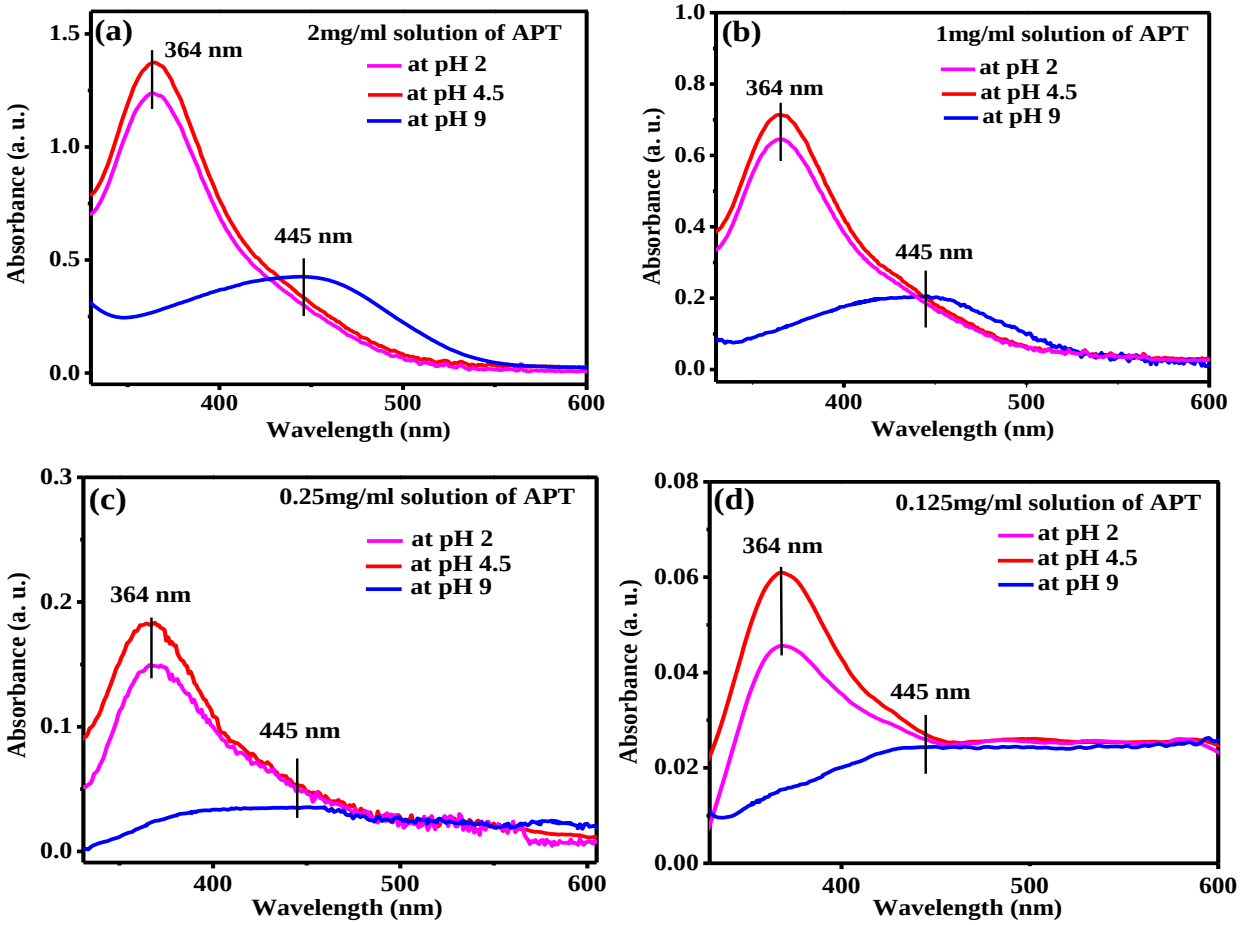


Figure S9. UV-vis absorption spectra of APT solutions of different concentrations at different pH (as indicated).

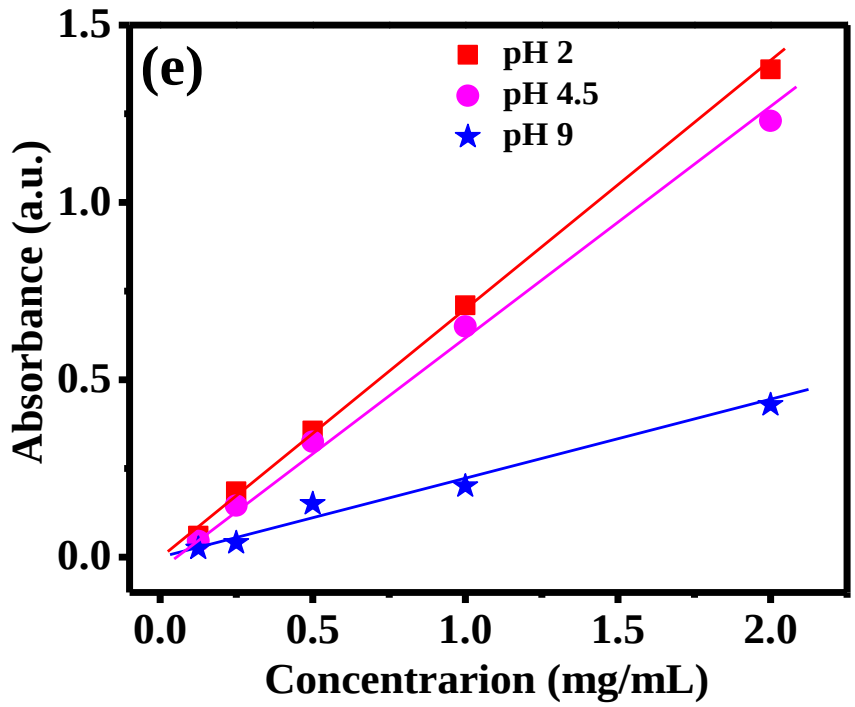


Figure S10. Lambert-Beer plot of UV-vis absorbance at 364 nm for pH2 & pH 4.5 and at 445 nm for pH 9 with concentration of APT at different pH (as indicated).

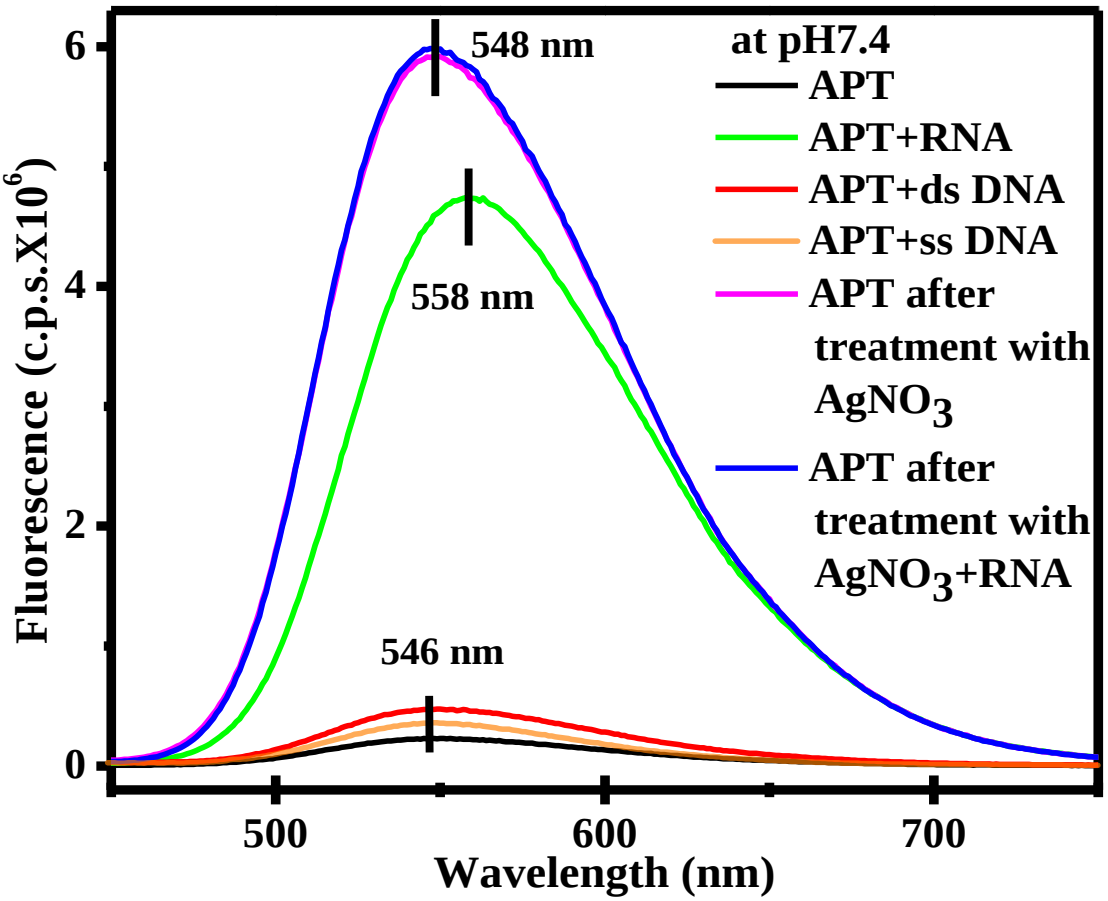


Figure S11. Fluorescence spectra of different APT system (as indicated) at pH 7.4 (ATP: 0.05% w/v, RNA: 0.083% (w/v), DNA: 0.00044% (w/v) in 2ml solution).

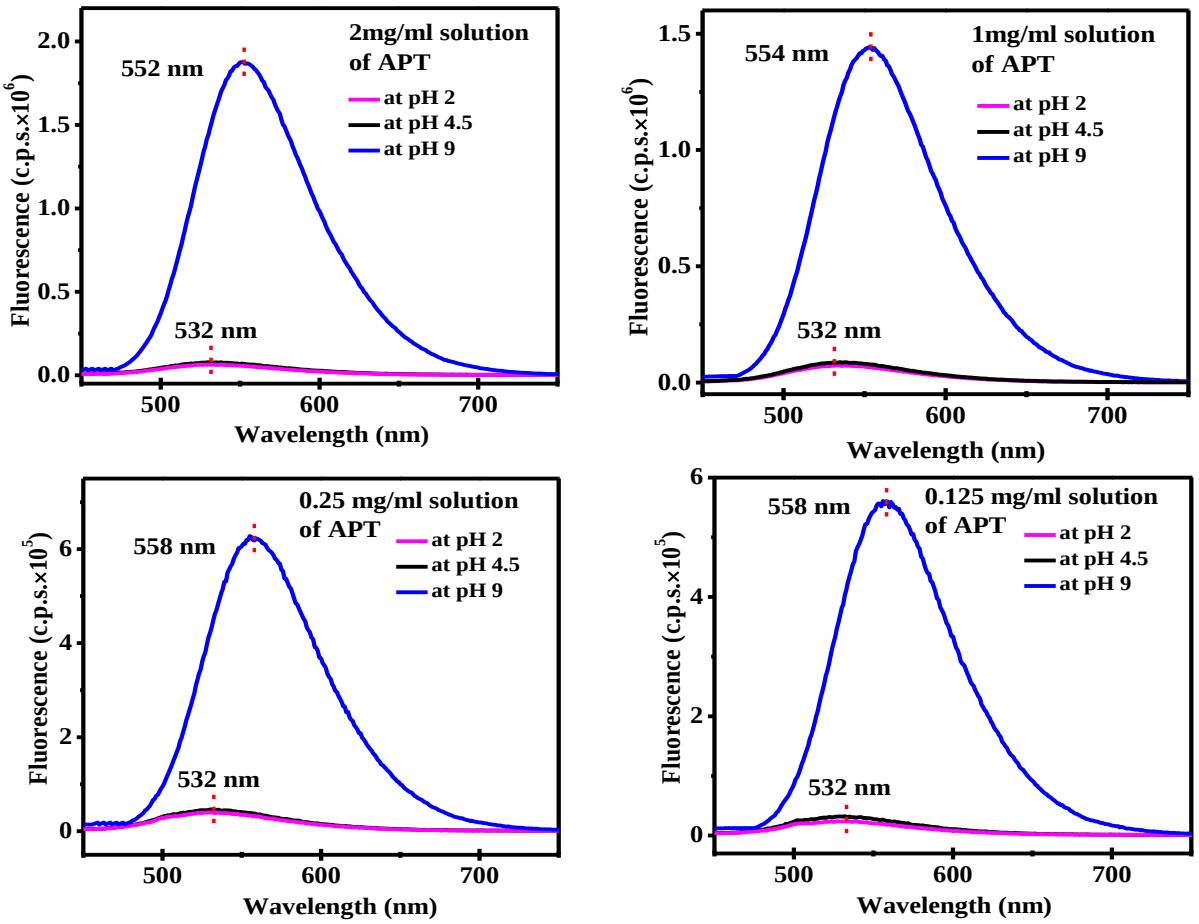


Figure S12. PL spectra of APT solutions with various concentrations at different pH (as indicated) for excitation at 420 nm.

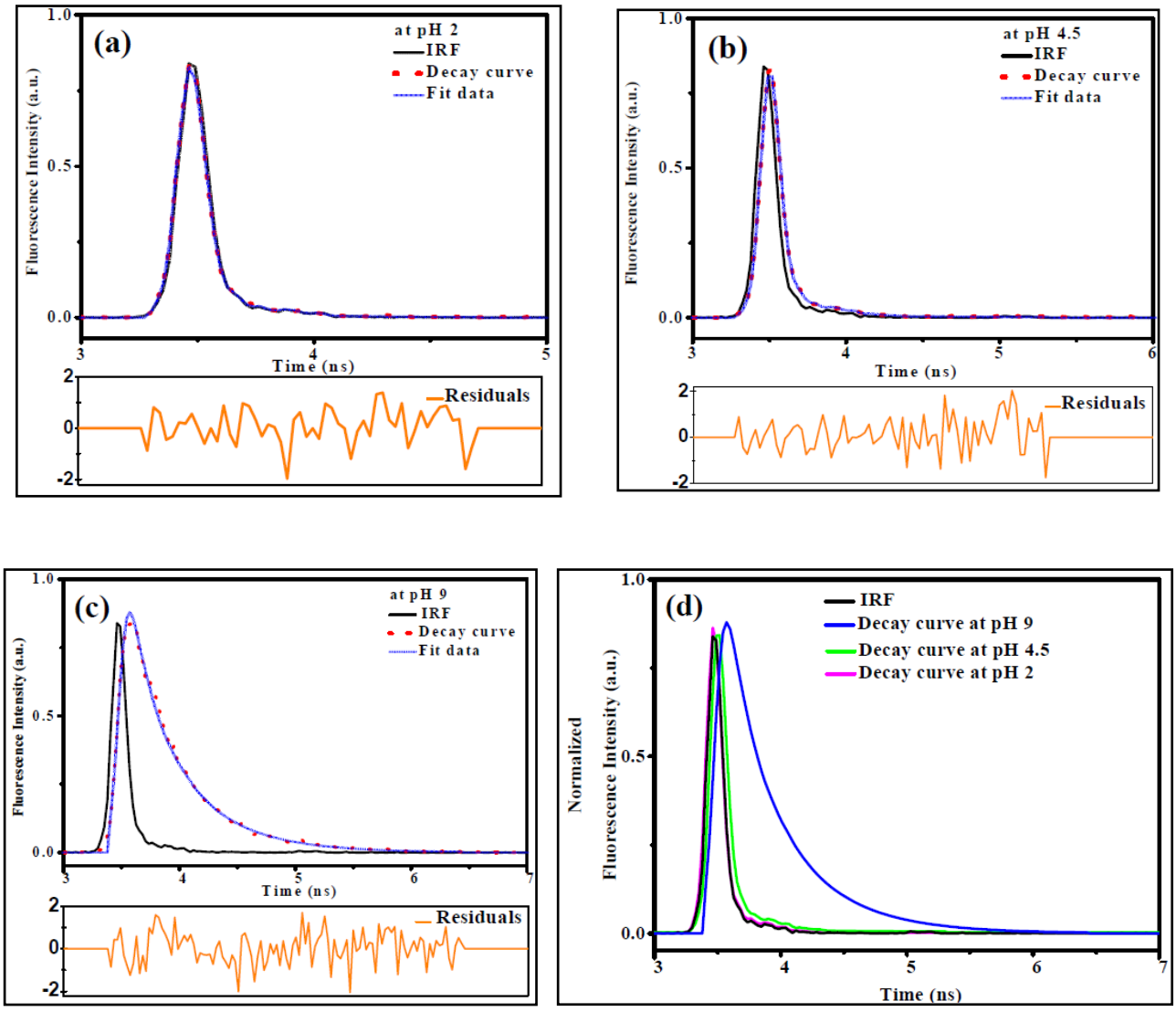
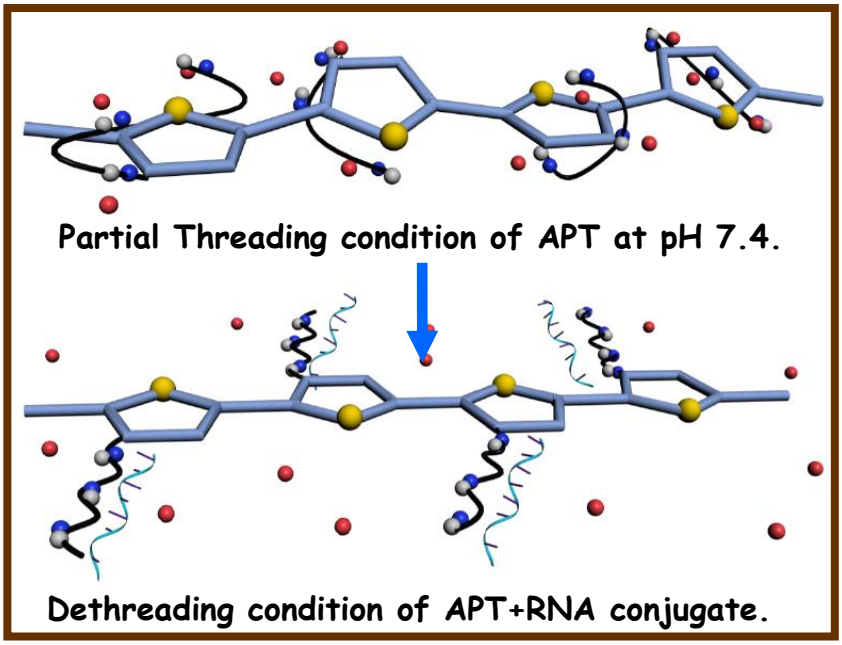


Figure S13. Fluorescence decay spectra of aqueous solution of APT (0.05% w/v) at (a) pH 2, (b) pH 4.5, (c) pH 9 and (d) comparisons of all these three pH. For simplicity of comparing the decays, all the data have been normalized at the maximum intensities.

Table S1. Summary of the Lifetimes (τ_1,τ_2) and Relative Amplitudes (a_1,a_2) extracted for the decay measured of APT at pH9 through reconvolution data fitting ($\chi 2$ is the reduced chi-square).

Solution pH	Lifetime [τ_1 (ps)]	Relative Amplitude [a_1 (%)]	Lifetime τ_2(ps)	Relative Amplitude a_2 (%)	Reduced chi-square ($\chi 2$)	Average fluorescence lifetime ($<\tau_f>$) (ps)
pH 9	165	29	500	71	1.01	402.8



Scheme S1. Schematic representation of partial threading-complete dethreading processes of grafted chains of APT with RNA at pH 7.4.

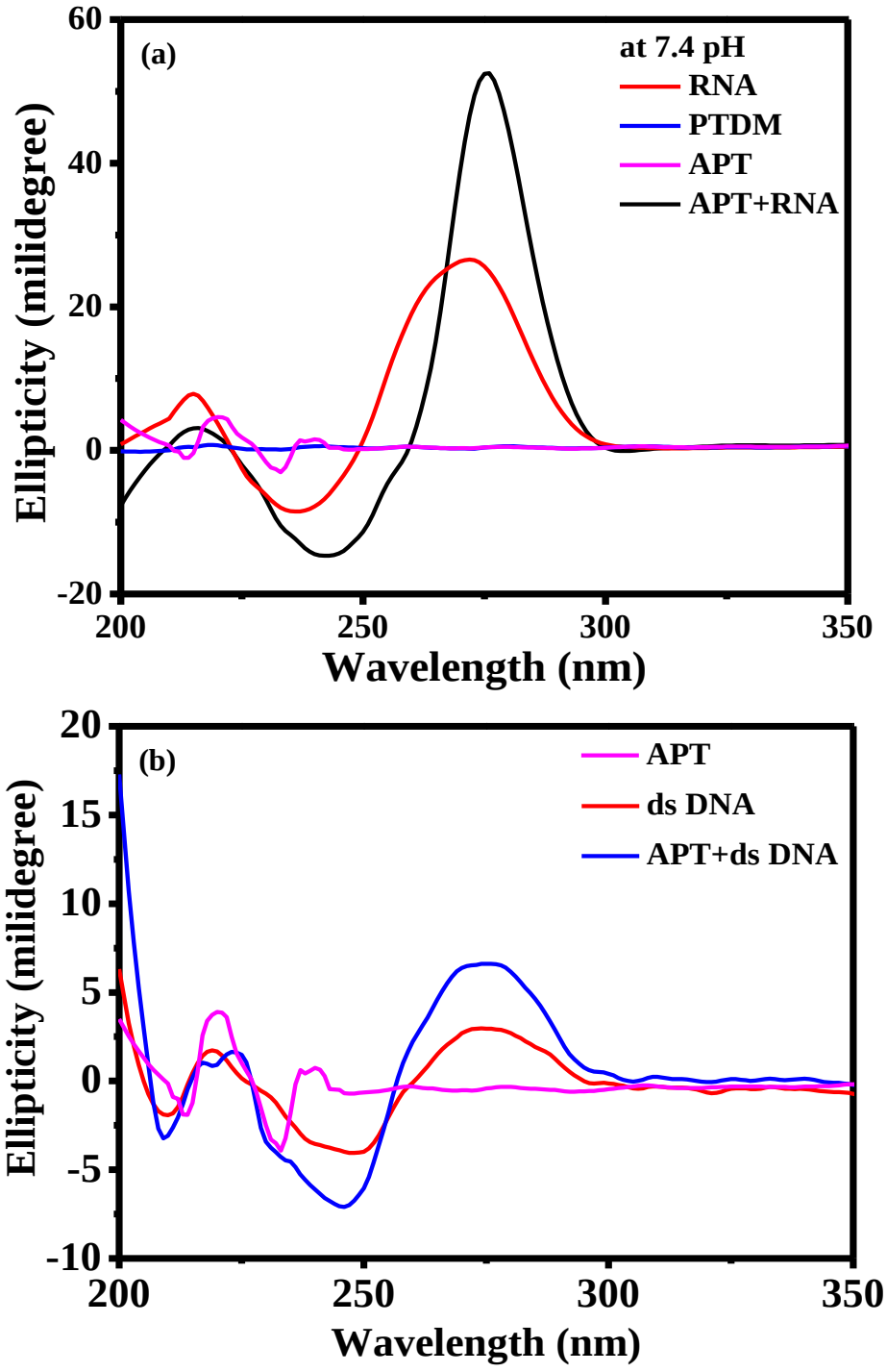


Figure S14. (a) Circular dichroism (CD) spectra of aqueous solution of PTDM, APT, pure RNA, APT+RNA system at pH 7.4. (ATP: 0.05% w/v, RNA: 0.083% (w/v) in 2ml solution). **(b)** Circular dichroism (CD) spectra of aqueous solution of APT, pure dsDNA, APT+ds DNA system at pH 7.4. (ATP: 0.05% w/v, DNA: 0.00044% (w/v), 2ml solution).

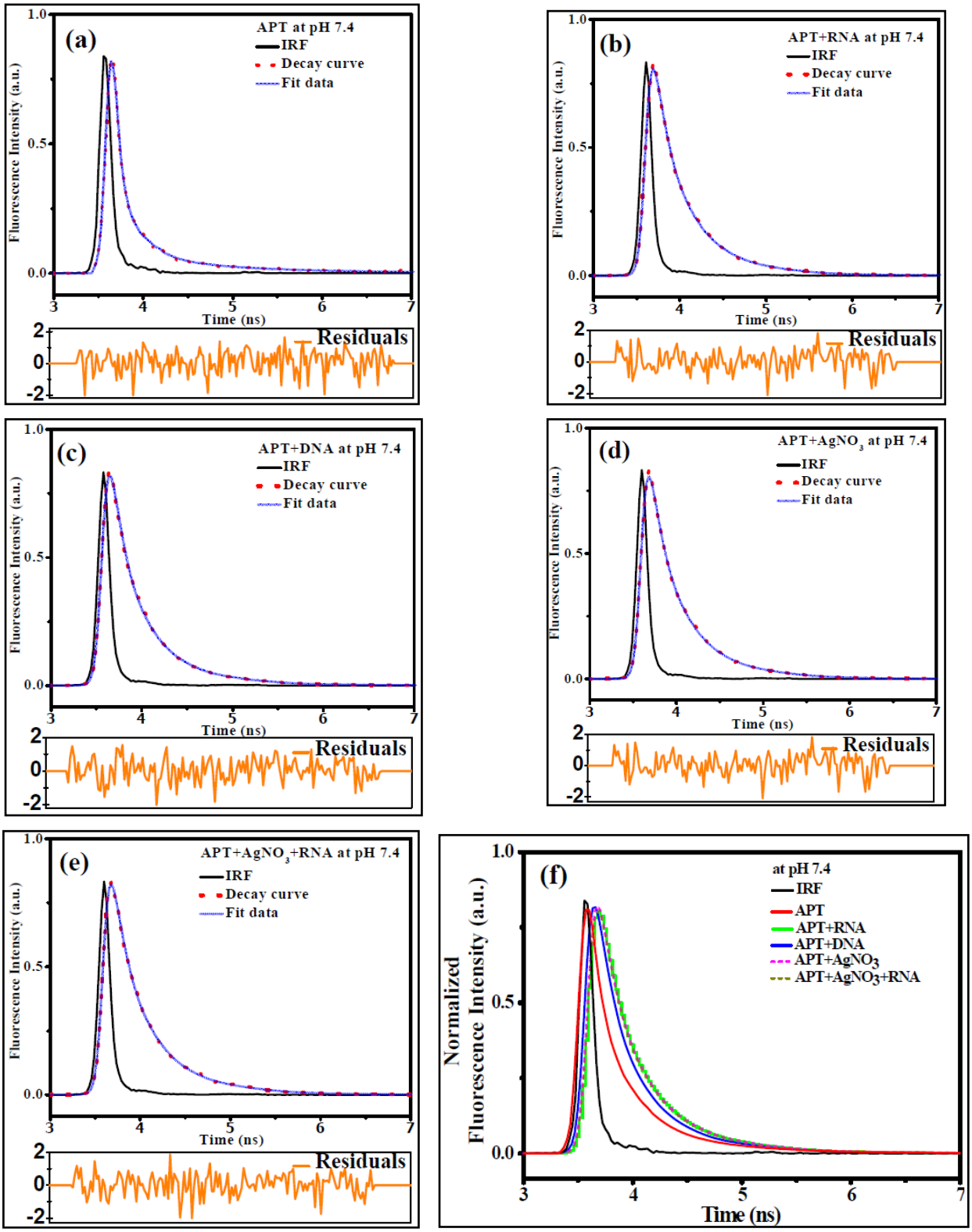


Figure S15. Fluorescence decays measured at pH 7.4 for (a) APT, (b) APT+RNA, (c) APT+DNA, (d) APT+$AgNO_3$, (e) APT+$AgNO_3$+RNA, and (f) comparison of different APT systems (as indicated). For simplicity of comparing the decays, all the data have been normalized at the maximum intensities.

Table S2. Summary of the Lifetimes (τ_1-τ_3) and Relative amplitudes (a_1-a_3) extracted for the decays measured for different APT system (as indicated) through reconvolution data fitting ($\chi 2$ is the reduced chi-square).

Samples (at pH 7.4)	τ_1 (ps)	a_1 (%)	τ_2(ps)	a_2 (%)	τ_3 (ps)	a_3 (%)	($\chi 2$)	Average fluorescence lifetimes ($<\tau_f>$) (ps)
APT	49	25	213	43	665	32	1.14	317
APT+RNA	160	36	538	64	-	-	1.11	402
APT+DNA	159	41	488	59	-	-	1.01	353
APT+$AgNO_3$	165	36	500	64	-	-	1.09	380
APT+$AgNO_3$+RNA	168	36	498	64	-	-	1.08	379

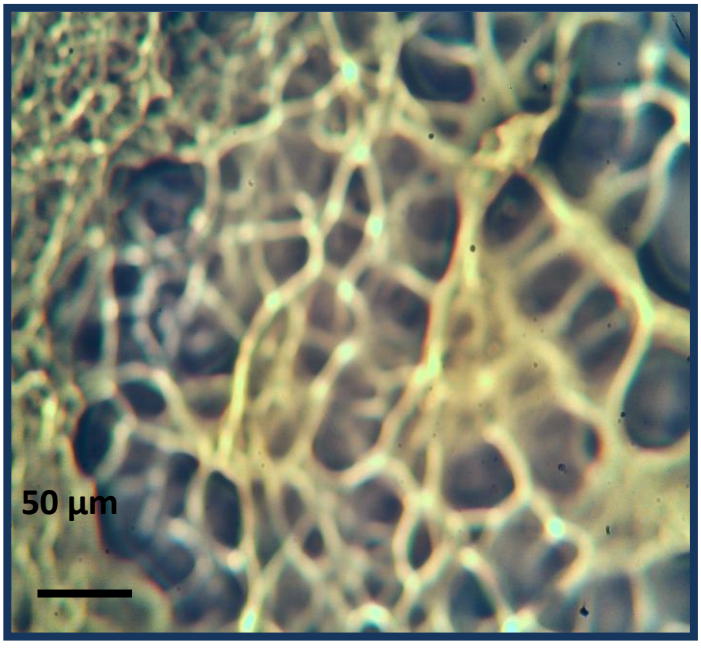


Figure S16. Optical microscopic image of APT+dsDNA system at pH 7.4.

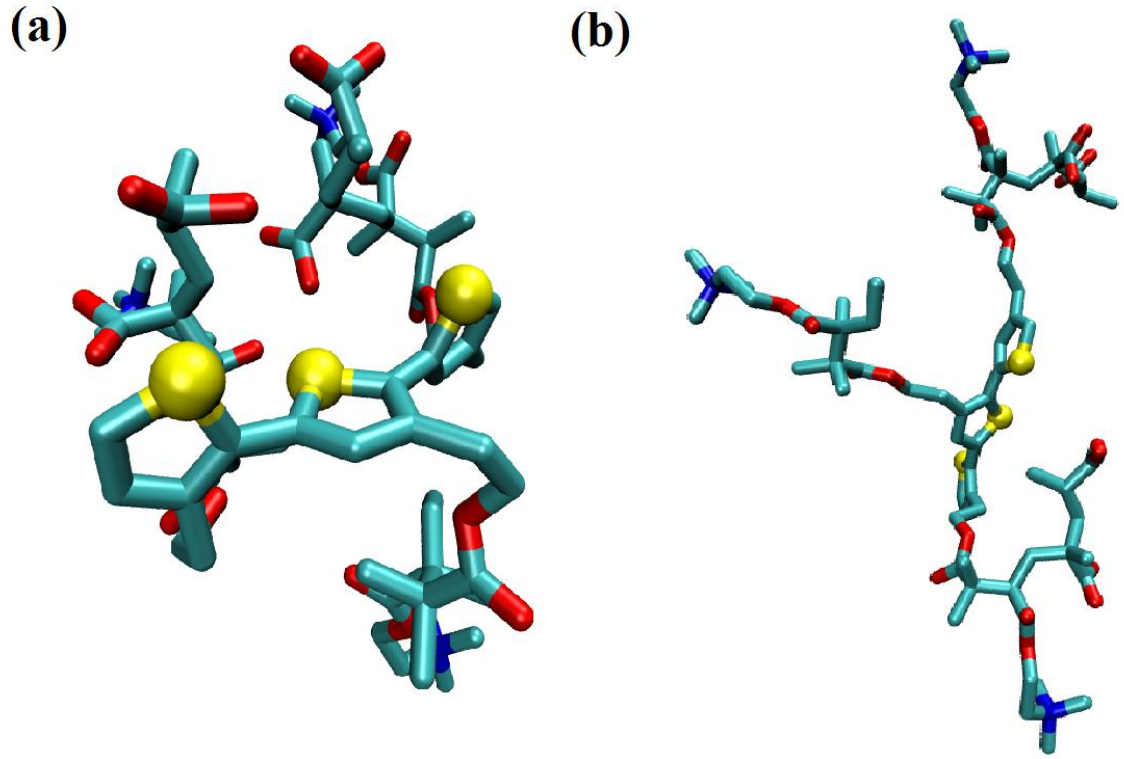


Figure S17 (a) Energy minimized structure of the protonated form (threading) and (b) Energy minimized structure of the deprotonated form (dethreading) of the model polymer of APT.

Table S3. EIS parameters of APT at different pH. (R_1= contact resistance, R_2 = electronic resistance, CPE= constant phase element, W= Warburg impedance, n = exponent factor).

APT from	**R_1 (Ω)**	**R_2 (Ω)**	**CPE**	**W(Ω)**	**Capacitance (F)**	**n**
pH 2	35.23	5.14×10^{7}	3.199×10^{-9}	-	1.94×10^{-9}	0.783
pH 4.5	44.69	2.8×10^{7}	20.62×10^{-12}	-	7.95×10^{-12}	0.887
pH 9	35.89	3739	0.61×10^{-9}	109062	2.42×10^{-10}	0.934

References

(1) Martyna, G. J.; Tobias, D. J.; Klein, M. L. Constant-Pressure Molecular-Dynamics Algorithms. *J. Chem. Phys.* **1994**, *101*, 4177−4189.

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