Fluorescent Micellar Nanoparticles by Self-Assembling of Amphiphilic, Nonionic and Water Self-Dispersible Polythiophenes with "Hairy Rod" Architecture

Supplementary Information

Luminita Cianga[‡a], Anca-Dana Bendrea[‡a], Nicusor Fifere[a], Loredana Elena Nita[a], Florica Doroftei[a], Didem Ag[b], Muharrem Seleci[b], Suna Timur[*b] and Ioan Cianga[*a]

[a] "Petru Poni" Institute of Macromolecular Chemistry, 41A Grigore-Ghica Voda Alley, Iasi, 700487, Romania; E-mail: ioanc@icmpp.ro; Fax: +40-232-211299; Tel: +40-232- 217454

[b] Department of Biochemistry, Faculty of Science, Ege University, Izmir 35100, Turkey; E-mail: suna.timur@ege.edu.tr ; Fax: +90-232-3438624; Tel: +90-232-3115425

‡- Both authors contributed equally as first authors to this work.

Supporting Experimental Details

Synthesis of intermediates and macromonomers

Synthesis of 2,5-dibromothiophene-3-carboxylic acid (1)

The reaction was performed using a method adapted from literature.[1]

8.15g (0.0637 mol) of 3-thiophene carboxylic acid were placed in a 500 ml round-bottom flask equipped with a magnetic stirrer, condenser, dropping funnel and N_2 inlet-outlet and 159.8 ml of glacial acetic acid were added. Then, 17.6 ml Br_2 were added from the dropping funnel in about 30 min. The reaction was heated at 60°C and maintained at this temperature overnight. The reaction mixture was placed in an ice-bath and about 300 ml of deionized cold water and some Na_2SO_3 was added to decolorize. The mixture was filtered and the white solid was recrystallized twice from a mixture of water-ethanol 2/2 (v/v).

^{1}H-NMR signals, acetone d_6, (δ, ppm):10.86 ppm (COO*H*), 7.4 ppm (Ti*H*).

Synthesis of 2, 5-dibromothiophene-3-acetic acid (2)

2,5-Dibromothiophene-3-acetic acid was prepared in a similar manner with **1** using a method adapted from literature.[2,3] 5g (0.0351 mol) 3-thiophene acetic acid and 60 ml mixture of solvents (50:50 v/v dry $CHCl_3$/ acetic acid glacial) were introduced, under nitrogen, in a 100 ml three –necked round bottom flask, equipped with magnetic stirrer. 14.35g (0.080 mol) NBS were slowly added to the solution. The mixture was stirred overnight at 70°C. After slowly cooling under nitrogen, the solution was diluted with $CHCl_3$ and washed with water several times. The organic layer was dried over anhydrous $MgSO_4$ and the solvent was removed. The crude product was dissolved in THF and passed through a silicagel column. Final product was obtained after recrystallyzation from hexane (yield 78%).

^{1}H-NMR signals, acetone d_6, (δ, ppm): 6.94 (1H, Ti*H*), 3.63 (2H, Ti-C*H*$_2$).

Synthesis PEG macromonomers functionalized with 2, 5 dibromothiopene moieties (M1, M2)

8g (0.004 mol) of **PEG$_{2000}$** and 1.716g (0.006 mol) of 2, 5-dibromothiophene-3-carboxylic acid (for **M1**) or of 2, 5-dibromothiophene-3-acetic acid (for **M2**) were placed into a three-neck round-bottom flask equipped with a dropping funnel, under N_2. 70ml of CH_2Cl_2 and 0.0726g of DMAP 0.00006 mol) were added to the flask. 1.2312g (0.006mol) of DCCI in 6 ml CH_2Cl_2 were placed in the dropping funnel and added in about 15 min. The mixture was stirred at room temperature for three days. The

resulting solution was filtered and precipitated in cold diethyl ether to remove the catalyst and the unreacted reagents. After filtration and drying a white solid was obtained.

^{1}H-NMR signals, acetone d_6 (δ, ppm): **M1**: 7.40 ppm (Ti*H*), 4.45 ppm; (CO-OC*H*$_2$), 3.82-3.67 ppm (C*H*$_2$O from PEG, 3.40 (OC*H*$_3$); M$_{n\,(GPC)}$=2400, PDI=1.03

M2: 7.15ppm Ti*H*, 4.24ppm (CO-OC*H*$_2$), 3.75-3.4ppm (C*H*$_2$O from PEG and Ti-C*H*$_2$), 3.291ppm (OC*H*$_3$); M$_{n\,(GPC)}$=2400, PDI=1.03.

Synthesis of PEG substituted quinquethiophene (5T)

A 100 mL three neck round bottom flask equipped with a condenser, a rubber septum, nitrogen inlet-outlet and magnetic stirrer was charged with 10 mL 1M NaHCO$_3$ aqueous solution and 15 mL THF. The solvents were previously deaerated by bubbling nitrogen over a period of 30 minutes and the mixture was refluxed under nitrogen for 3 hours.

A 20 mL three neck round bottom flask equipped in the same way as the previous one was charged under inert atmosphere with 0.957g (0.416 mmol) dibrominated macromonomer **M1,** 0.4 g (1.368 mmol) 2,2'-bithiophene-5-boronic acid pinacol ester and 0.0192 g (0.0166 mmol) Pd(PPh$_3$)$_4$. The mixture of solvents was introduced with a syringe through the septum. The reaction was maintained under vigorous stirring and with the exclusion of oxygen and light. The mixture was refluxed under nitrogen for 3 days. After that period the reaction was stopped and the mixture was extracted several times with dichloromethane. The organic layer was dried over MgSO$_4$, concentrated at rotaevaporatory and precipitated in cold diethyl ether. After filtration and drying under vacuum, **5T** was obtained as an orange powder. The product was passed through a silicagel filled column and reprecipitated in cold diethyl ether using CH$_2$Cl$_2$ as solvent.

^{1}H-NMR signals, acetone d_6 (δ, ppm): 7.8-7.05 ppm (aromatic protons of quinquethiophene), 4.48-4.36 ppm (CO-OC*H*$_2$); 3.9-3.44 ppm (C*H*$_2$O from PEG); 3.42 ppm (OC*H*$_3$). Mn, $_{(GPC)}$= 2120, PDI=1.03.

Supporting Discussion Concerning to the IR Characterization of the Reported Compounds

IR spectra of macromonomers **M1, M2** and **5T** and those of the corresponding polymers **P1**, **P2** and **P5T** (Fig. S4) are dominated, as expected, by the strong absorptions originating from PEG at 2884 cm^{-1} (ν_{as}CH$_2$), 2738 cm^{-1}(ν_sCH$_2$), 1464 cm^{-1}(δCH$_2$), 1361 cm^{-1}, 1342 cm^{-1}(CH$_2$ wagging), 1277 cm^{-1}(CH$_2$ twisting), 1241 cm^{-1}(CH$_2$ twisting and C-O-C stretching), 1150 cm^{-1}(ν CO), 1114 cm^{-1}(C-O-C

stretching), 1061 cm^{-1}(CH$_2$ rocking and C-O-C stretching), 964 cm^{-1}(CH$_2$ rocking), 841 cm^{-1}(r (CH$_2$)+ ν (C-O-C) and 528 cm^{-1}.[4] The peaks characteristic to the thienyl rings in the synthesized compounds (marked with asterisks in Fig. S4) are also present but with a lower intensity, as the thiophene ratio in their structure is lower than that of PEG. The peaks of νC=O in the ester linkage between the thiophene rings and PEG side chains are present in all the synthesized compounds, their positions ranging from 1740 cm^{-1} to 1708 cm^{-1}. For the 2,5-dibromo-thiophene moiety the absorbance due to the aromatic β(C–H) stretching vibration appeared at 3093 cm^{-1} in macromonomer **M1** and at 3097 cm^{-1} in **M2**. The peaks at 1526 cm^{-1} and 1425 cm^{-1} in **M1** and at 1544 cm^{-1} and 1326 cm^{-1} in **M2** are related to antisymmetric and symmetric stretching vibrations of the thienyl rings.[5,6] In the IR spectrum of **M1** the absorbance at 1006 cm^{-1} should be attributed to ν (C-Br),[7] that at 769 cm^{-1} to the out-of-plane δ (C-H) vibration of 2,5-disubstituted thiophene ring,[7,8] while C-S bending and C-S-C ring deformation vibrations appeared at 673 cm^{-1} and 479 cm^{-1}, respectively.[9] In the spectrum of **M2** a shallow peak appeared at 1010 cm^{-1} for ν (C-Br) and only C-S-C ring deformation vibration at 473 cm^{-1}was discernible. The effect of polymerization can be recognized as a general broadening of the absorption bands. So, in the spectra of **P1** and **P2** the broad peaks in the range 3138 cm^{-1} – 3072 cm^{-1} and 3145 cm^{-1} – 3073 cm^{-1} respectively could be attributed to both aromatic β(C–H) stretching vibration and α(C–H) stretching vibration, the last one corresponding to the unbrominated thienyl rings of the chains ends, for **P1** in concordance with ^{1}H-NMR spectrum. For antisymmetric and symmetric stretching vibrations of the thienyl rings peaks at 1620 cm^{-1} and 1405 cm^{-1} appeared in spectrum of **P1**, while for **P2** these peaks were centered at 1579 cm^{-1} and 1400 cm^{-1}, respectively. In both **P1** and **P2** spectra no peaks for ν (C-Br) must be noticed, proving that the polymerization reactions took place. While in the spectrum of **P1**, in the low weavenumbers region, only peaks at 693 cm^{-1} and 673 cm^{-1} characteristic to C-S bending are discernible, for **P2** a peak at 744 cm^{-1} characteristic to out-of-plane δ (C-H) vibrations of 2,5-disubstituted thienylenes and another one at 473 cm^{-1} related to C-S-C ring deformation vibration are present.

By comparing with the spectrum of the starting material (**M1**), the IR spectrum of **5T** shows several new absorption peaks, due to the presence of oligothiophene sequence. Thus, the signal from 3106 cm^{-1} and that from 3073 cm^{-1} belong to the aromatic α(C–H) stretching vibration and to β(C–H) stretching vibration, respectively. The peaks at 1650 cm^{-1} and at 1616 cm^{-1} could be attributed to the conjugation between the thiophene five rings. For antisymmetric and symmetric stretching vibrations of the thienyl rings, peaks at 1524 cm^{-1} and 1434 cm^{-1} could be attributed. The absorbance at 810 cm^{-1} is due to the ring β(C–H) out of plane bending vibration, and that at 745 cm^{-1} belong to α(C–H) out of

plane deformation. At 796 cm^{-1} the out-of-plane δ (C-H) vibrations of 2,5-disubstituted thiophene ring appeared, whereas C-S bending and C-S-C ring deformation vibrations appeared at 694 cm^{-1}, 671 cm^{-1} and 477 cm^{-1}. For **P5T** the broadest IR spectrum was registered. It should be noted that the absorbances at 3106 cm^{-1} and 745 cm^{-1} in the spectrum of **5T** attributed to aromatic α(C–H) stretching and to out-of-plane α(C–H) ring deformation vibration disappeared in the spectrum of **P5T**, fact that proves that the polymerization took place successfully through the α-positions of the thiophene rings.

Supporting Data and Disscusion for GPC Measurements and HLB Calculation

Table S1. The GPC experimental values of the compounds and the calculated theoretical values for M_n of the macromonomers and polymers HLB

Code	$M_{n, theoretical}$ (g/mol)	$M_{n, GPC}$ (g/mol)	M_w / M_n [a]	HLB[b]
M1	2268	2400	1.03	
M2	2282	2400	1.03	
5T	2430	2120	1.03	0.82
P1	-	10700	4.18	0.96
P2	-	5620	1.13	0.95
P5T	-	3060	1.09	0.82

a- determined by GPC measurements; b- calculated for the repeating units (RU), based on molar mass of the hydrophilic and hydrophobic blocks respectively, using a formula reported in reference [10].

GPC traces of the synthesized thiophene macromonomers and derived polymers using THF as eluent are presented in Figure S3. As can be seen, for all the investigated compounds excepting polymer **P1**, these traces are unimodal and narrow for all macromonomers and unimodal and symmetrical in shape for **P2** and **P5T**. The obtained values for the polymers are higher than those of the staring macromonomers and despite the uncontrolled nature of the polymerisation methods used for their synthesis they show narrow polydispersities. The GPC trace of **P1** is bimodal and a higher polydispersity index was determined (Table S1).

However the apparent molecular weights values found from GPC cannot be very reliable. The GPC measurements of PEG-containing compounds using columns calibrated with polystyrene standards can furnish overestimated values due to the difference in polarity of the two polymers. On the other hand linear polystyrene has a different hydrodynamic volume by comparison with the investigated " hairy rods" which have compact shape making the measured values to be lower than the real absolute molecular weight. Similar observations were noticed also in the case of other PEG-containing molecular brushes.[11]

The values reported in Table S1 for the HLB were calculated taking in account the molar mass of the hydrophilic and hydrophobic blocks in each compound repeating unit (RU) respectively using a reported formula.[10]

Table S2. Photophysical properties of polythiophenes substituted at the 3-th position with PEGs of different lengths, reported in the literature

PEG side chains	Connected to thiophene ring through:					
No. of R.U.	$-(CH_2)_a-$			-O-		
	λ^a_{abs} (nm)	λ^a_{em}(nm)	Ref.	λ_{abs}(nm)	λ_{em}(nm)	Ref.
2	439^1	570^1	12	602	-	14
	418^2	-	13	420^b	-	15
3	429^1	568^1	16	578	657	17
4	409^2	562^2	18	465	-	19
				432^b	-	20
7	359^1	-	21	426^b (THF)	-	22
				426^b; 550^b (MeOH)	542^b (ex. 434)	22
				510^b; 540^b; 595^b(water)	-	23
4*				508; 550; 602 (water)	612; 662 (water)	24

a- number of CH_2 moieties between thiophene ring and PEG side chain; b- CH_3 substitute is present in 4-th position of thiophene ring; * The PEG side chains substitutes both 3-rd and 4-th position of thiophene ring.
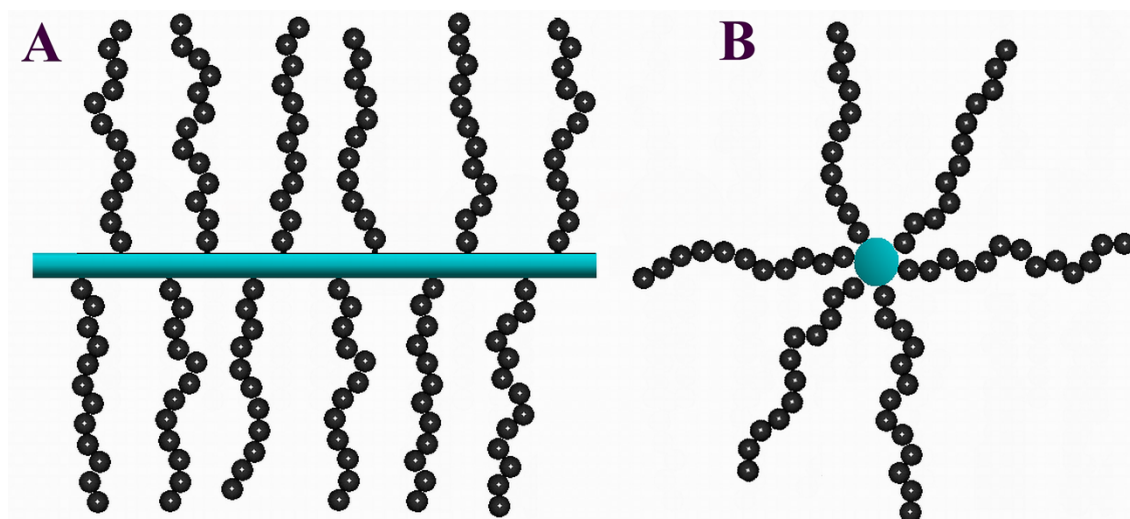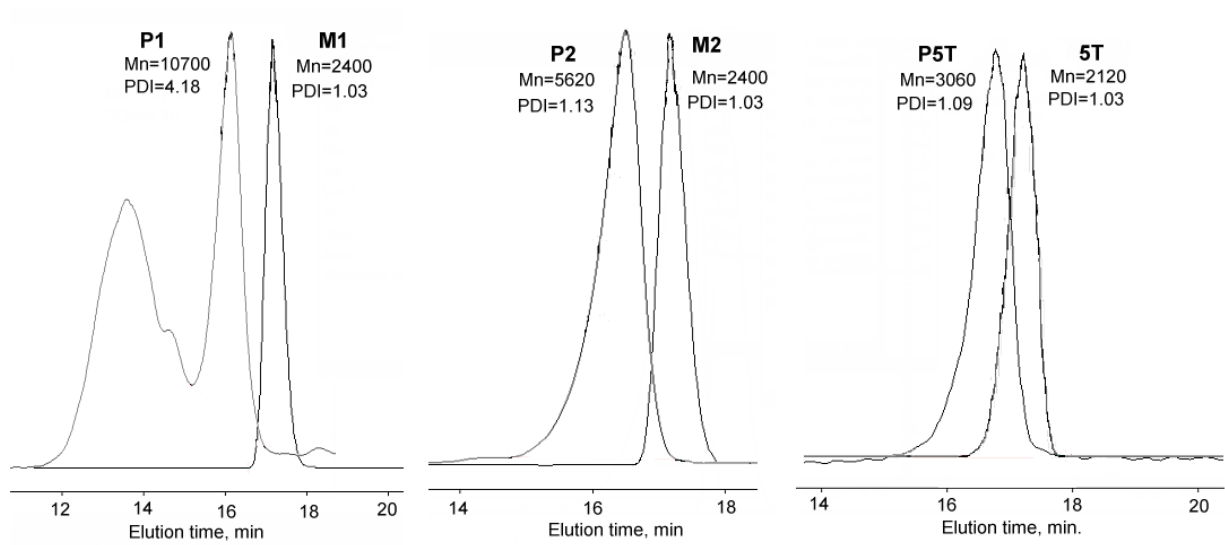
Figure S1. Pictorial drawing of "Hairy-Rods"- (A)-Side view; (B)-Front view
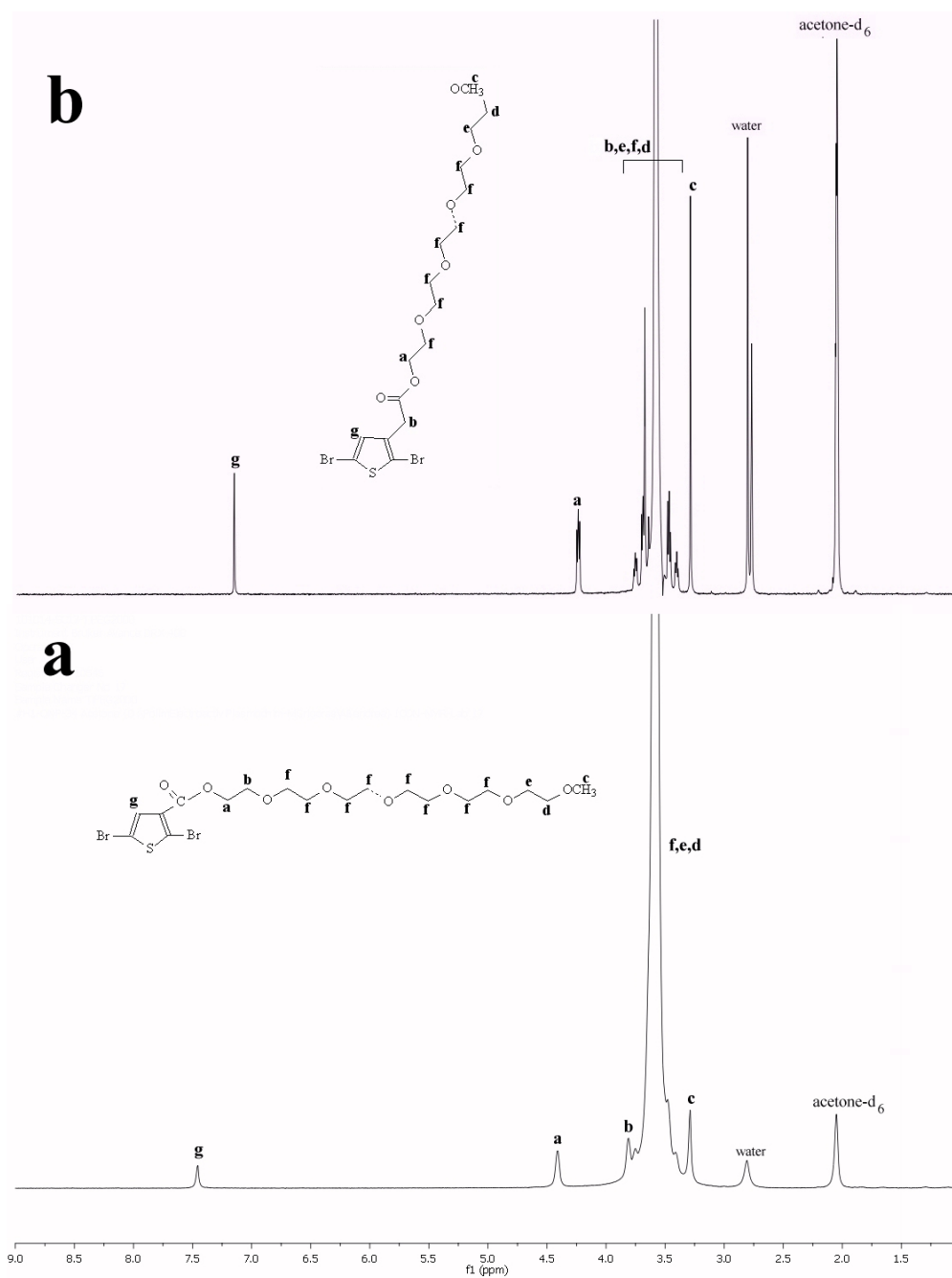
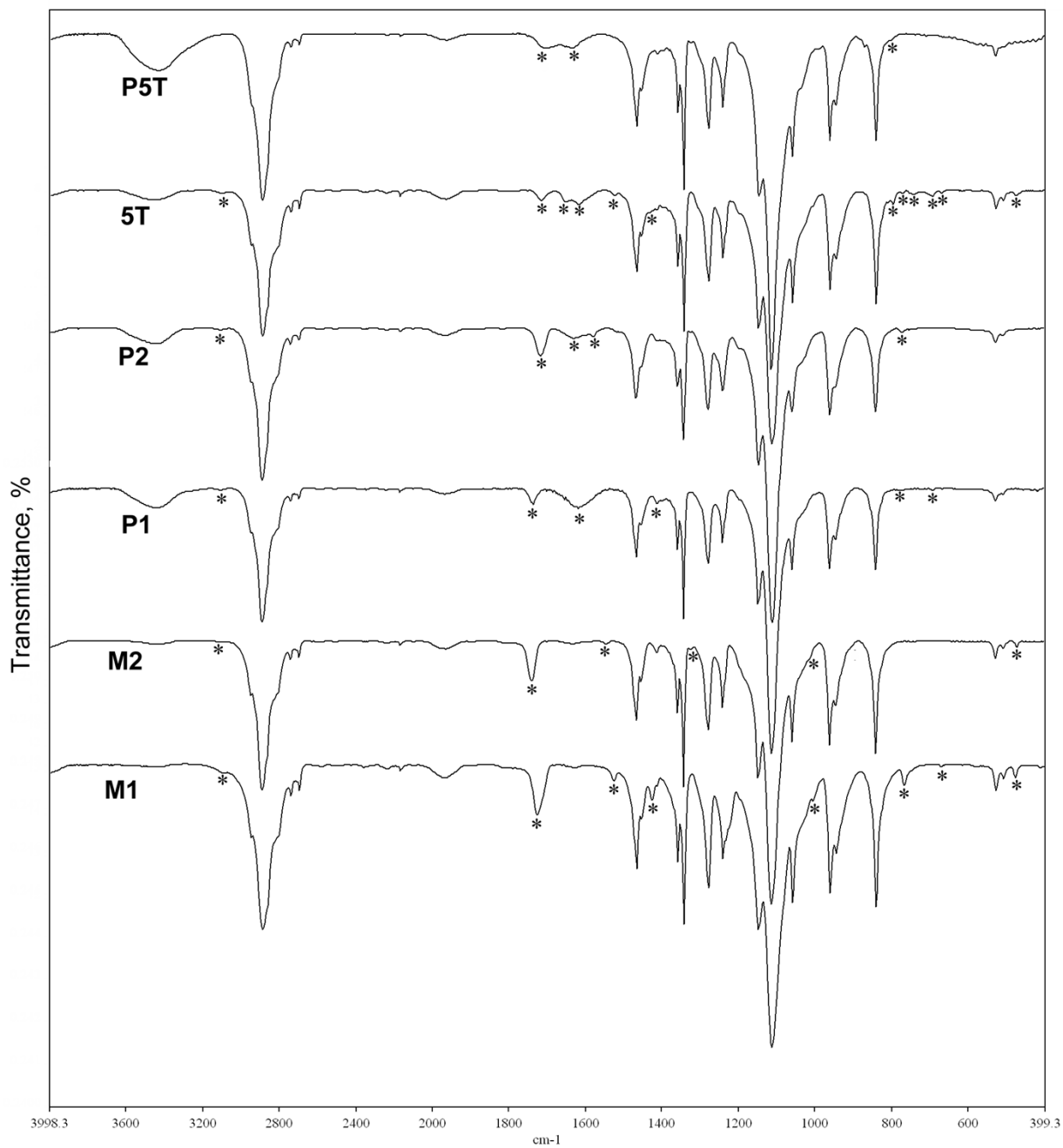**Figure S2**. (a)- ^{1}H-NMR spectrum of **M1** in *acetone-d$_6$*; (b)- ^{1}H-NMR spectrum of **M2** in *acetone-d$_6$*
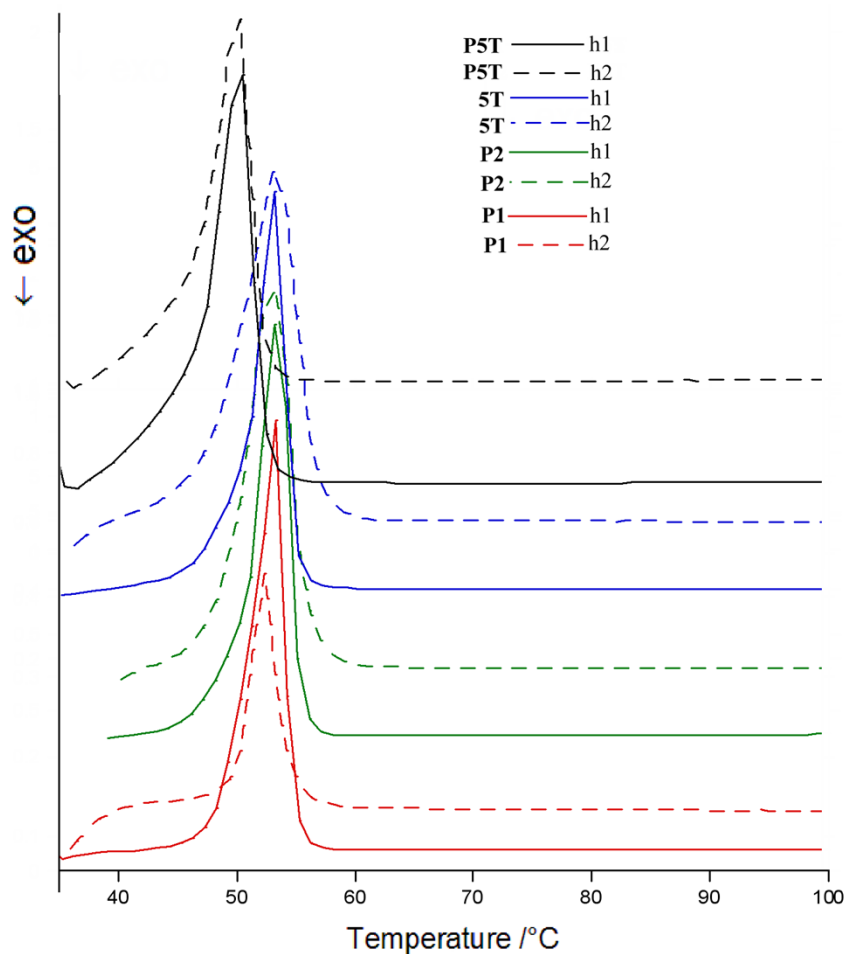
**Figure S3**. GPC traces of the synthesized thiophene macromonomers and derived polymers

Figure S4. IR spectra of the synthesized thiophene macromonomers and derived polymers

Figure S5. DSC traces of the investigated polymers: h_1 represent the first heating at 5 °C/ min; h_2 represent the second heating performed at 10 °C/ min after a cooling cycle performed at 5 °C/ min. For simplicity of the figure the thermograms are presented in the range 35 °C -100 °C, even if the experiment was performed between 35 °C and 250 °C.
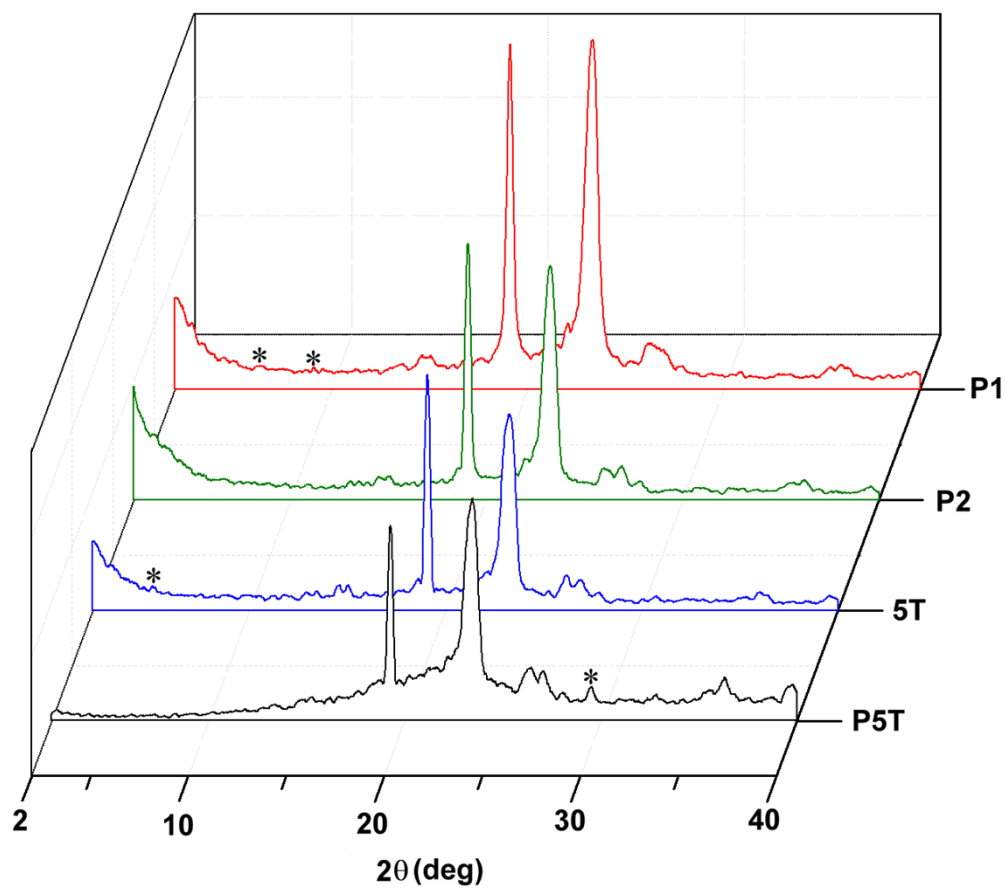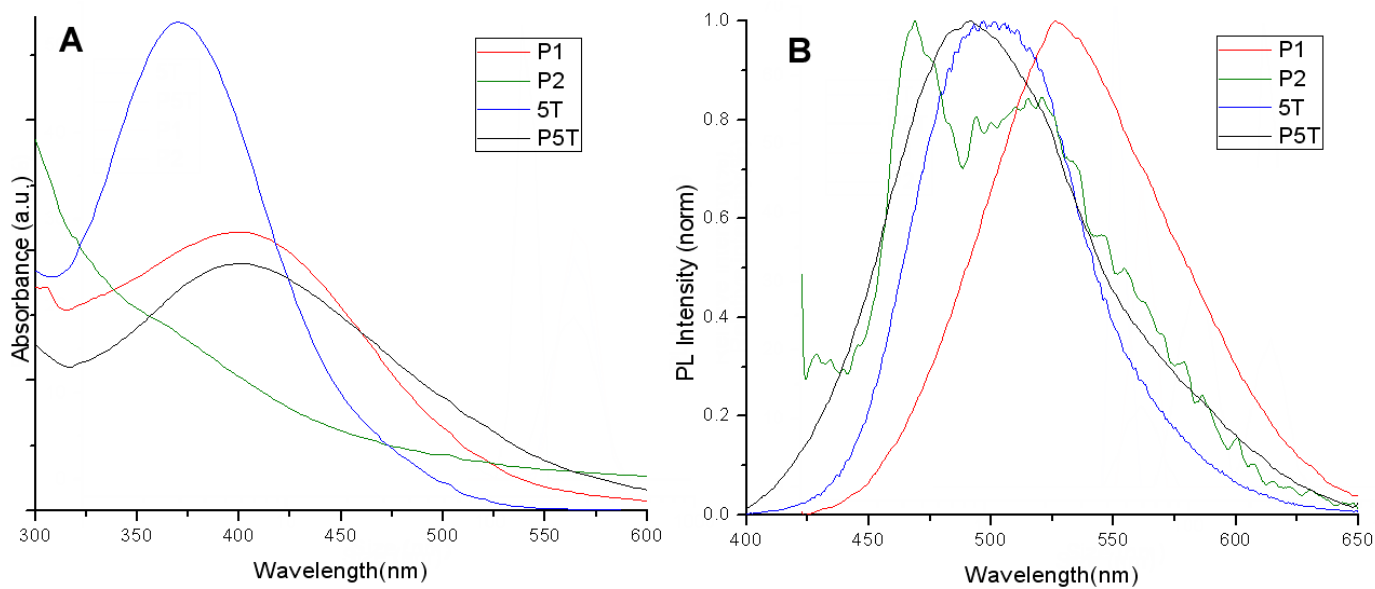
**Figure S6**. X-ray diffractograms of the investigated polymers in powders
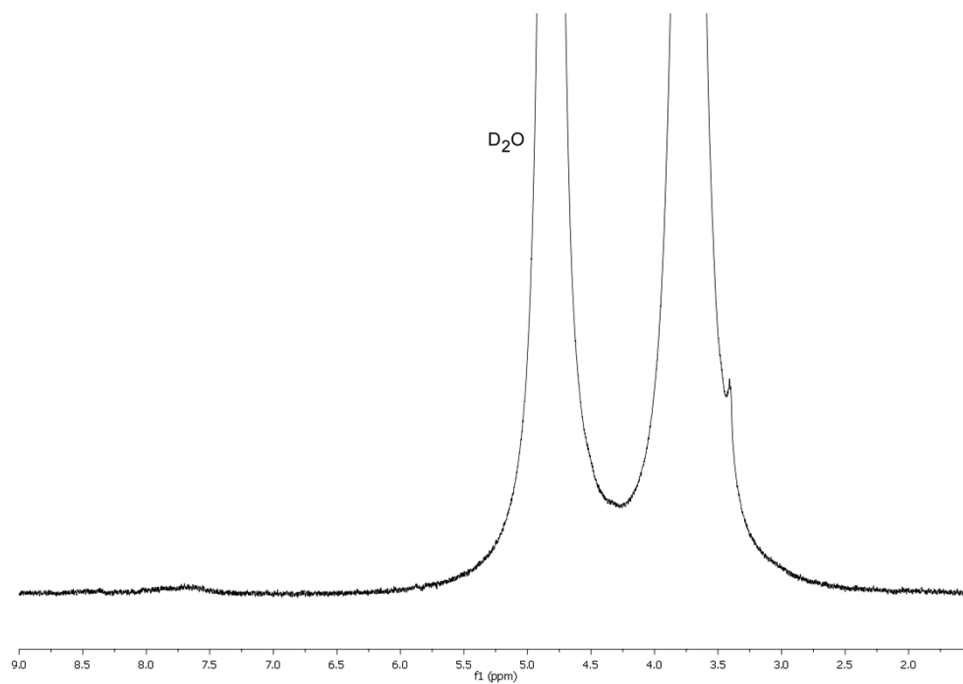
Figure S7. The ^{1}H-NMR spectrum of polymer **P1** in D$_2$O
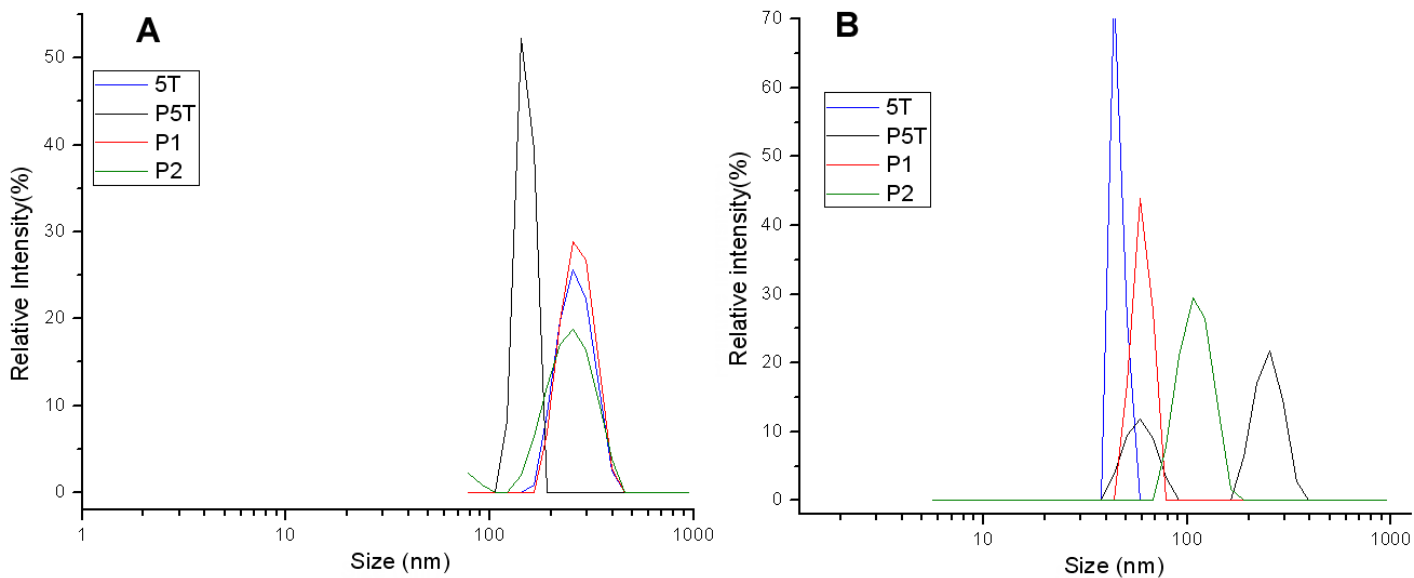
Figure S8. DLS traces of polymers: (A) in water and (B) in PBS at concentration $5 \times 10^{-4} \mu M$ based on repeating unit (RU)

Figure S9. (A)- UV-Vis traces of polymers in water; (B)- Normalized fluorescence curves of polymers in water; Solution concentration $5 \times 10^{-4} \mu M$ based on repeating unit (RU)
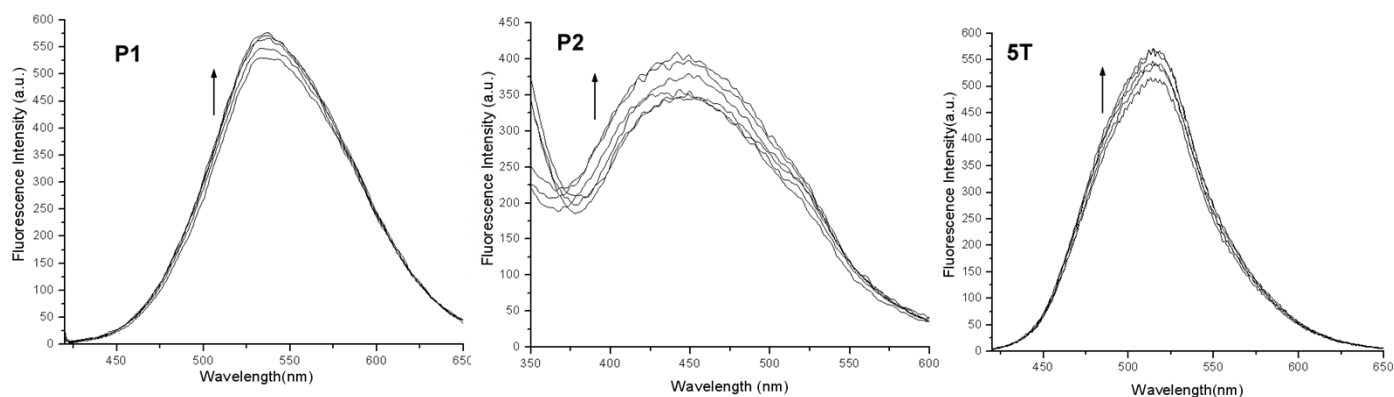
Figure S10. The fluorescence spectra of **P1**, **P2** and **5T** in the presence of BSA solutions in 25mM PBS; The BSA concentrations varied from 0 to 0.25μM in steps of 0.05 μM and the arrows indicate the increasing of BSA concentration. The polymers concentration in solutions was 5×10^{-4}μM based on repeating unit (RU)

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