Formation of Polyelectrolyte Complexes from Cationic Polyfluorenes and ssDNA

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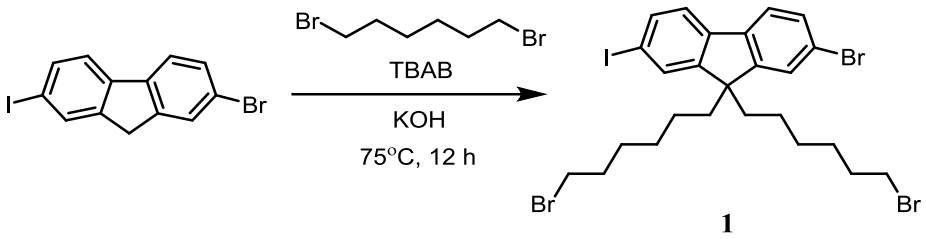
Materials

2-Iodo-7-bromofluorene (purity: 98+%), tetrabutylammonium bromide (TBAB, 99+%), and bis(diphenylphosphino)propane (dppp, 98+%) were purchased from Tokyo Chemical Industries Ltd. (Japan), and used as received. Tetrahydrofuran (THF, 99.5+%), dimethyl sulfoxide (DMSO, 99.5+%), *N*,*N*-dimethylformamide (DMF, 99.5+%), and potassium hydroxide (85+%) were purchased from Wako Pure Chemical Industries Ltd. (Japan), and used as received. *iso*-Propylmagnesium chloride·lithium chloride complex solution (^{i}PrMgCl·LiCl, 1.3 M in THF), nickel acetylacetonate ($Ni(acac)_2$, 95+%), and trimethylphosphine ($P(Me)_3$, 1.0 M in THF) were purchased from Sigma-Aldrich (Japan), and were used as received. Chloroform and dichloromethane (Wako Pure Chemical Industries Ltd.) were purified using conventional methods. 1,6-Dibromohexane (Tokyo Chemical Industries Ltd.) was purified by reduced-pressure distillation. 1,4-Bishexyloxybenzene (DHB) was synthesized according to ref. 1.

The bio-sensing properties of the prepared polymers (see below) were evaluated using low molecular weight deoxyribonucleic acid (DNA) from salmon sperm, ribonucleic acid (RNA) from torula yeast, which were purchased from Sigma-Aldrich, which were used as received. Oligomers based on thymine, adenine, guanine, and cytosine were purchased from Gene Design Inc., Japan.

Synthesis of monomer 1 (Scheme S1)



Scheme S1. Synthesis of monomer **1**.

2-Iodo-7-bromo-9,9-bis-(6-bromohexyl)-fluorene (1). A solution of KOH (55 g) in H_2O (100 mL) was heated to 75°C, then 2-iodo-7-bromofluorene (1.620 g, 5.0 mmol), 1,6-dibromohexane (12.20 g, 50 mmol), and tetrabutylammonium bromide (0.161 g, 0.5 mmol) were added and the resulting mixture stirred vigorously for 12 h at 75°C. After this time, the reaction mixture was extracted with dichloromethane (150 mL), and the organic layer was washed with HCl aq. (100 mL), brine (100 mL), and H_2O (100 mL) prior to drying over anhydrous $MgSO_4$, and removing the solvent and excess 1,6-dibromohexane under reduced pressure at 100°C. The resulting yellow oil was passed through a column of silica gel (Wakogel C-300) using chloroform/hexane (1:9, *v/v*) as the eluent, and evaporation of the solvent provided the desired product as a white crystalline solid (2.93 g, 90%, melting point: 275°C). ^{1}H NMR (300 MHz, $CDCl_3$) 0.578 (4H, m, CH_2), 1.08 (4H, m, CH_2), 1.20 (4H, m, CH_2), 1.64 (4H, quin, CH_2), 1.92 (4H, m, CH_2), 3.29 (4H, t, CH_2), 7.43 (2H, d, J_{HH}=1.8 Hz, CH), 7.46 (2H, dd, J_{HH}=8.0 Hz, J_{HH}=1.8 Hz, CH), 7.53 (2H, d, J_{HH}=8 Hz, CH). ^{13}C NMR (125 MHz, $CDCl_3$) 23.67, 27.52, 27.99, 29.18, 32.72, 32.82, 33.92, 40.27, 55.75, 121.47, 121.80, 126.31, 130.56, 139.27, 152.39. Found: C, 62.95; H, 4.25; Br, 34.18. Calc. for $C_{25}H_{32}Br_3I$: C, 43.07; H, 4.34; Br, 34.39.

General procedure for preparation of the cationic PTMPHF homopolymer by catalyst-transfer polycondensation

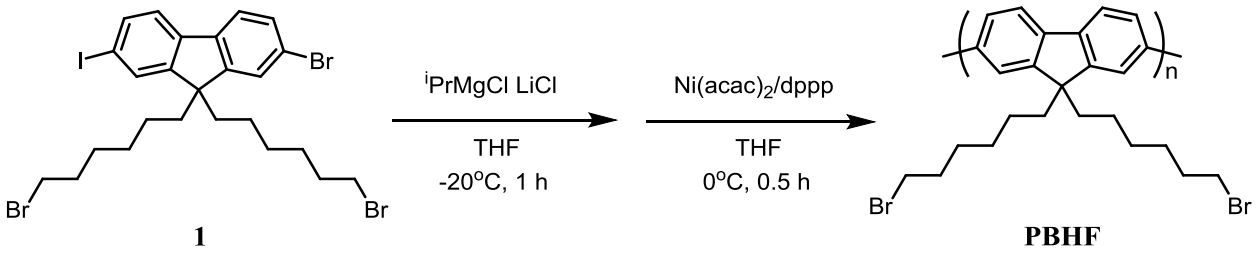
Preparation of the $Ni(acac)_2$/dppp solutions

In a typical experiment, THF was added to a mixture of $Ni(acac)_2$ and dppp ($Ni(acac)_2$/dppp=1/1.02, mol/mol) in an argon-filled glove box (see Table S1), and the resulting solution was stirred at 25°C for 20 min prior to direct use in the polymerization reaction.

Table S1: Conditions for the preparation of $Ni(acac)_2$/dppp solution

Entry	$Ni(acac)_2$ / g (mmol)	Dppp / g (mmol)	THF / mL
1	0.102 (0.20)	0.168 (0.204)	20

Synthesis of PBHF



Scheme S2. Synthesis of **PBHF**.

iso-Propylmagnesium chloride lithium chloride complex (^{i}PrMgCl·LiCl, 1.3 M in THF) was added via a syringe to a solution of the monomer **1** in a glove box under argon. After removal from the glove box, the mixture was stirred at −20°C for 1 h, and the above-prepared $Ni(acac)_2$/dppp THF solution was added via a syringe (see Table S2). After stirring at 0°C for 30 min, the reaction was quenched using a 5 M HCl aqueous solution (5 mL), and the insoluble material was washed with methanol and collected by suction filtration. The polymer was further purified by precipitation in hexane.

Table S2. Conditions for the synthesis of PBHF(*n*)						
Sample	Monomer **1** / mg (mmol)	^{i}PrMgCl·LiCl / mL (mmol)	Ni(acac)$_2$/dppp / mL (mmol)	THF / mL	[Monomer]/ [Ni(acac)$_2$/dppp]	Yield / g (%)
PBHF(12)	670 (1.03)	0.79 (1.03)	1.5 (0.030)	20	32	0.55 (79)
PBHF(20)	670 (1.03)	0.79 (1.03)	2.0 (0.040)	20	24	0.50 (71)
PBHF(23)	699 (1.00)	0.77 (1.00)	2.5 (0.050)	20	20	0.58 (81)
PBHF(26)	697 (1.00)	0.77 (1.00)	3.0 (0.060)	20	16	0.52 (74)

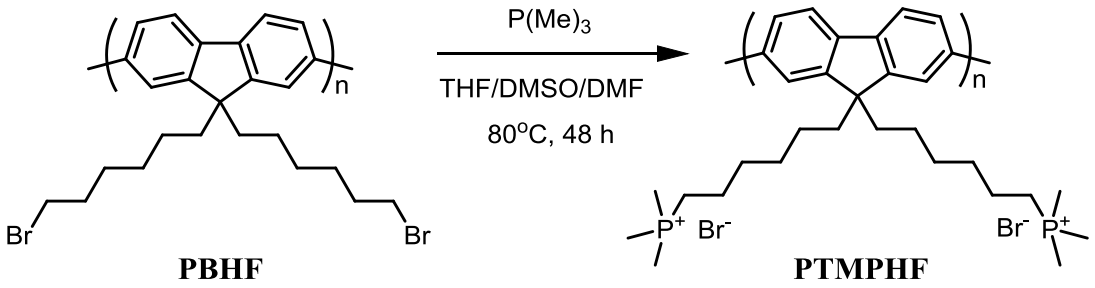
PBHF(12): IR ν_{max}/cm^{-1} 669 (C-Br stretching), 950 (Aromatic C=C bending), 1425 (C-H bending), 1525 (Aromatic C=C stretching), 2930 (C-H stretching); ^{1}H NMR (300 MHz; $CDCl_3$) 7.84 (2H, dd, J_{HH}=8.0 Hz, J_{HH}=1.8 Hz, CH), 7.72 (2H, d, J_{HH}=8.0 Hz, CH), 3.30 (4H, t, CH_2), 2.60 (4H, m, CH_2), 1.69 (4H, quin, CH_2), 1.32 (4H, m, CH_2), 0.90 (4H, m, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) 23.70, 27.77, 29.07, 32.62, 34.02, 40.28, 55.33, 120.19, 121.31, 126.37, 140.13, 140.50, 150.89; Found: C, 60.78; H, 6.74; Br, 33.42. Calc. for $C_{300}H_{385}Br_{25}$: C, 60.89; H, 6.61; Br, 32.50.

PBHF(20): IR ν_{max}/cm^{-1} 670 (C-Br stretching), 936 (Aromatic C=C bending), 1425 (C-H bending), 1520 (Aromatic C=C stretching), 2941 (C-H stretching); ^{1}H NMR (300 MHz, $CDCl_3$) 7.82 (2H, dd, J_{HH}=8.0 Hz, J_{HH}=1.8 Hz, CH), 7.73 (2H, d, J_{HH}=8.0 Hz, CH), 3.32 (4H, t, CH_2), 2.61 (4H, m, CH_2), 1.67 (4H, quin, CH_2), 1.31 (4H, m, CH_2), 0.88 (4H, m, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) 23.71, 27.77, 29.07, 32.62, 34.02, 40.28, 55.33, 120.19, 121.33, 126.38, 140.13, 140.51, 150.89; Found: C, 60.80; H, 6.80; Br, 32.39. Calc. for $C_{500}H_{641}Br_{41}$: C, 60.84; H, 6.72; Br, 32.44.

PBHF(23): IR ν_{max}/cm^{-1} 672 (C-Br stretching), 940 (Aromatic C=C bending), 1428 (C-H bending), 1517 (Aromatic C=C stretching), 2938 (C-H stretching); ^{1}H NMR (300 MHz, $CDCl_3$) 7.81 (2H, dd, J_{HH}=8.0 Hz, J_{HH}=1.8 Hz, CH), 7.70 (2H, d, J_{HH}=8 Hz, CH), 3.30 (4H, t, CH_2), 2.58 (4H, m, CH_2), 1.65 (4H, quin, CH_2), 1.33 (4H, m, CH_2), 0.91 (4H, m, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) 23.72, 27.77, 29.07, 32.62, 34.02, 40.27, 55.33, 120.19, 121.32, 126.37, 140.11, 140.51, 150.90; Found: C, 60.97; H, 6.42; Br, 32.50. Calc. for $C_{575}H_{737}Br_{47}$: C, 61.02; H, 6.33; Br, 32.65.

PBHF(26): IR ν_{max}/cm^{-1} 675 (C-Br stretching), 934 (Aromatic C=C bending), 1425 (C-H bending), 1522 (Aromatic C=C stretching), 2940 (C-H stretching); ^{1}H NMR (300 MHz, $CDCl_3$) 7.83 (2H, dd, J_{HH}=8.0 Hz, J_{HH}=1.8 Hz, CH), 7.73 (2H, d, J_{HH}=8 Hz, CH), 3.30 (4H, t, CH_2), 2.61 (4H, m, CH_2), 1.66 (4H, quin, CH_2), 1.34 (4H, m, CH_2), 0.89 (4H, m, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) 23.70, 26.49, 27.77, 29.07, 32.62, 34.02, 40.30, 55.33, 120.18, 121.33, 126.38, 140.12, 140.52, 150.88; Found: C, 61.01; H, 6.34; Br, 32.58. Calc. for $C_{650}H_{833}Br_{53}$: C, 61.04; H, 6.30; Br, 32.66.

Synthesis of PTMPHF(Schme S3)



Scheme S3. Synthesis of **PTMPHF**.

Trimethylphosphine ($P(Me)_3$, 1.0 M in THF) was added via a syringe to a solution of **PBHF** in a mixture of THF, DMF, and DMSO (1:1:1, *v/v/v*) (see Table S3 for volumes) and the resulting solution was stirred at 80ºC for 48 h. After cooling to 25ºC, the yellow/green reaction solvent was removed under reduced pressure at 80ºC. After cooling to 25ºC, the resulting residue was then diluted with methanol (15 mL) and the obtained solution was poured into diethyl ether (300 mL). The insoluble material was collected by suction filtration to give

PTMPHF(*n*) as a yellow powder. Detailed synthetic conditions are outlined in Table **S3**.

Table S3. Conditions for the synthesis of PTMPHF(*n*)

Sample name	**PBHF**/g(mmol [a])	$P(Me)_3$ [b]/mL (mmol)	THF/mL	DMF/mL	DMSO/mL	Yield/g(%)
PTMPHF(12)	0.30 (0.047)	1.45 (0.047)	10	10	10	0.26 (86)
PTMPHF(20)	0.10 (0.011)	0.82 (0.011)				0.80 (80)
PTMPHF(23)	0.30 (0.026)	1.5 (0.026)				0.24 (80)
PTMPHF(26)	0.30 (0.023)	1.0 (0.023)				0.25 (83)

[a] Calculated from the molecular weight (M_n=6,400, 9,300, 11,500, 12,900).

[b] A 1.0 M solution in THF.

PTMPHF(12): IR ν_{max}/cm^{-1} 825 (C-P stretching), 1253 (Aromatic C=C bending), 1458 (C-H bending), 1560 (Aromatic C=C stretching), 2460 (P-H stretching), 2934 (C-H stretching); ^{1}H NMR (300 MHz, DMSO-d_6) 7.96 (2H, dd, J_{HH}=8.0 Hz, J_{HH}=1.8 Hz, CH), 7.82 (2H, d, J_{HH}=8 Hz, CH), 2.45 (18H, t, CH_3), 1.69 (8H, m, CH_2), 1.41 (4H, m, CH_2), 1.31 (4H, m, CH_2), 0.90 (4H, m, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) 12.18, 12.72, 25.78, 27.01, 27.53, 28.74, 34.98, 120.19, 121.33, 126.38, 140.13, 140.52, 151.50; Found: C, 57.40; H, 8.20; Br, 24.67. Calc. for $C_{372}H_{601}P_{24}Br_{25}$: C, 57.55; H, 7.90; Br, 24.88.

PTMPHF(20): IR ν_{max}/cm^{-1} 1030 (C-P stretching), 1250 (Aromatic C=C bending), 1498 (C-H bending), 1602 (Aromatic C=C stretching), 2430 (P-H stretching), 2948 (C-H stretching); ^{1}H NMR(300 MHz, DMSO-d_6) 7.94 (2H, dd, J_{HH}=8.0 Hz, J_{HH}=1.8 Hz, CH), 7.86 (2H, d, J_{HH}=8 Hz, CH), 2.42 (18H, t, CH_3), 1.68 (8H, m, CH_2), 1.40 (4H, m, CH_2), 1.32 (4H, m, CH_2), 0.88 (4H, m, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) 12.18, 12.71, 25.75, 27.01, 27.53, 34.94, 120.19, 121.33, 126.38, 140.13, 140.52, 151.50; Found: C, 57.22; H, 8.16; Br, 24.81. Calc. for $C_{620}H_{1001}P_{40}Br_{41}$: C, 57.42; H, 7.93; Br, 29.42.

PTMPHF(23): IR ν_{max}/cm^{-1} 1025 (C-P stretching), 1244 (Aromatic C=C bending), 1503 (C-H bending), 1583

(Aromatic C=C stretching), 2435 (P-H stretching), 2942 (C-H stretching); ^{1}H NMR (300 MHz, DMSO-d_6) 7.94 (2H, dd, J_{HH}=8.0 Hz, J_{HH}=1.8 Hz, CH), 7.84 (2H, d, J_{HH}=8.0 Hz, CH), 2.61 (18H, t, CH_3), 1.70 (8H, m, CH_2), 1.43 (4H, m, CH_2), 1.33, (4H, m, CH_2) 0.92 (4H, m, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) 12.18, 12.71, 25.76, 27.01, 27.54, 33.90, 34.95, 120.19, 121.33, 126.38, 140.13, 140.52, 151.50; Found: C, 57.88; H, 7.62; Br, 24.50. Calc. for $C_{713}H_{1151}P_{46}Br_{47}$: C, 58.01; H, 7.54; Br, 24.70.

PTMPHF(26): IR ν_{max}/cm^{-1} 1032 (C-P stretching), 1253 (Aromatic C=C bending), 1497 (C-H bending), 1551 (Aromatic C=C stretching), 2427 (P-H stretching), 2950 (C-H stretching); ^{1}H NMR (300 MHz, DMSO-d_6) 7.94 (2H, dd, J_{HH}=8.0 Hz, J_{HH}=1.8 Hz, CH), 7.85 (2H, d, J_{HH}=8.0 Hz, CH), 2.44 (18H, t, CH_3), 1.69 (8H, m, CH_2), 1.42 (4H, m, CH_2), 1.29 (4H, m, CH_2), 0.87 (4H, m, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) 12.18, 12.72, 25.75, 27.02, 27.53, 33.89, 34.96, 120.19, 121.33, 126.38, 140.13, 140.52, 151.50; Found: C, 58.22; H, 8.04; Br, 24.25. Calc. for $C_{806}H_{1301}P_{52}Br_{53}$: C, 58.40; H, 7.96; Br, 24.33.

Characterization of the bio-sensing properties

Biomolecules (DNA from salmon sperm, RNA from torula yeast, ATP, ADP, and AMP) were added separately to **PTMPHF** in Tris-HCl buffer solutions containing 1 vol% DMSO. After sonication for 5 min, the sensing properties of **PTMPHF** were examined by monitoring changes in the absorption and fluorescence of the polymer in the presence and absence of the biomolecules.

Characterization of the polymers

All ^{1}H NMR and ^{13}C NMR spectra of the monomers and polymers were acquired using Lambda 500 and 300 (JEOL) instruments, respectively. Elemental analyses were conducted using a PE2400-II elemental analyser (PerkinElmer Inc.) at 975°C. The molecular weights of the **PBHF** samples were determined by gel permeation chromatography (Tosoh HLC-8320GPC) equipped with two Shodex LF-804 columns. Measurements were carried out at 40°C using THF as the eluent at a flow rate of 1.0 mL min^{-1}. All molecular weights were estimated from a calibration curve constructed using polystyrene standards.

References

1. Plater MJ, Sinclair JP, Aiken S, Gelbrich T (2004) Hursthouse. Tetrahedron 60: 6385.

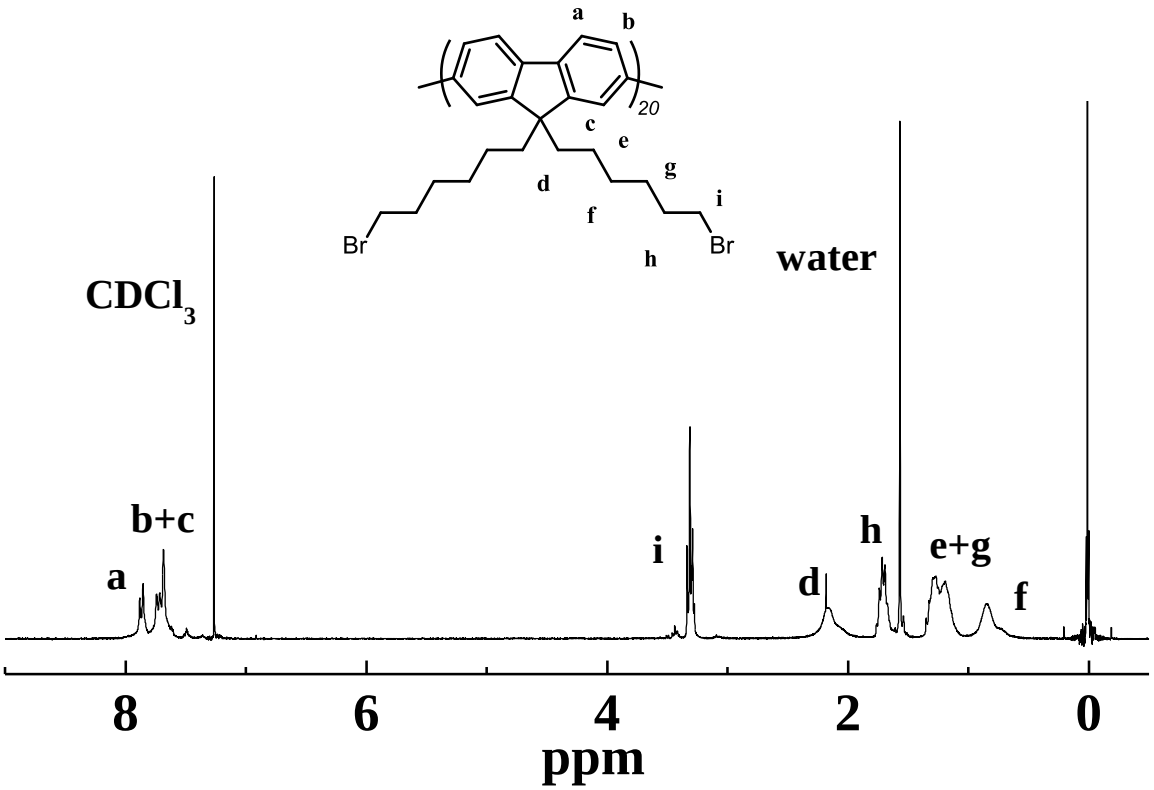


Figure S1. ^{1}H NMR spectrum of **PBHF(20)** in $CDCl_{3.}$

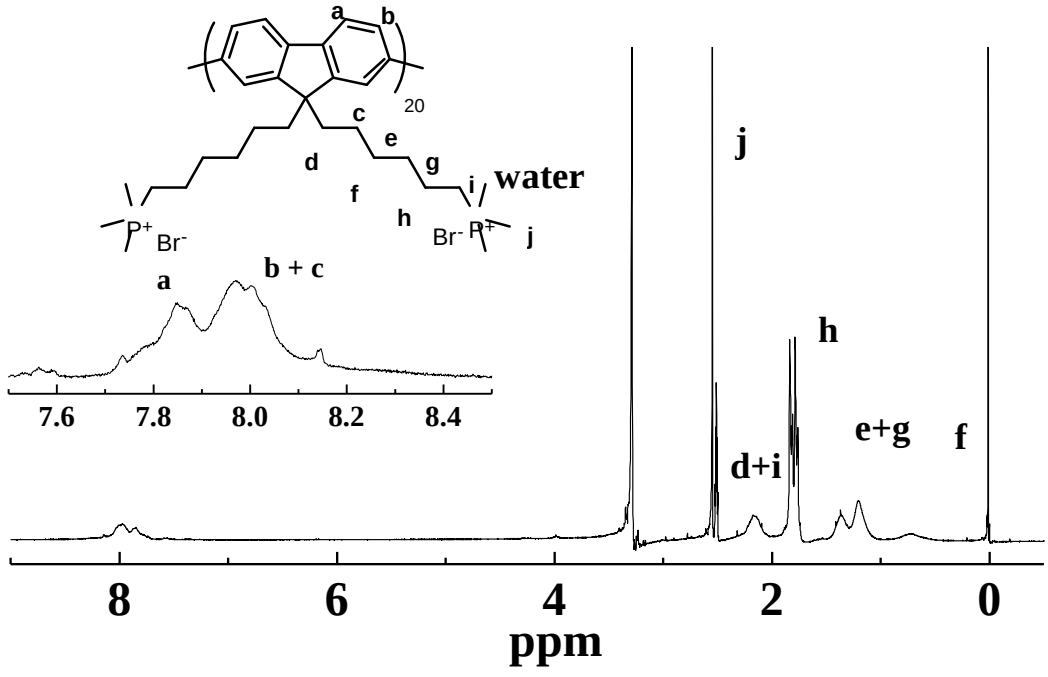


Figure S2. ^{1}H NMR spectrum of **PTMPHF(20)** in DMSO-d_6.

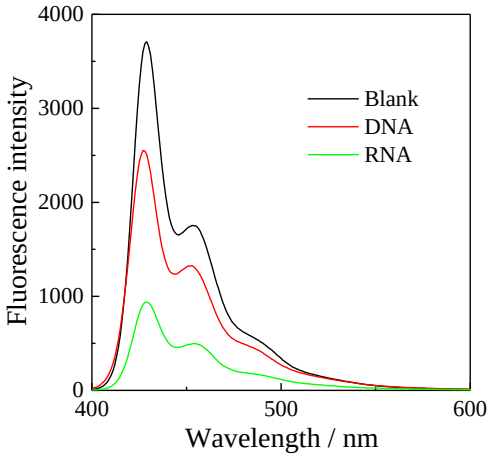


Figure S3. Fluorescence (λ_{ex}=395 nm) spectra of **PTMPHF** (*n*=26, 5.0×10^{-3} g L^{-1}) in a Tris-buffer solution containing 1 vol% DMSO at pH 8.0 in the presence of ds-DNA and RNA (5.0×10^{-2} g L^{-1}).