Fluorescence turn-on detection of DNA based on the aggregation-induced emission of conjugated poly(pyridinium salt)s

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Synthesis and characterization data of compounds 1-5.

Synthesis of 3,3'-(1,4-Phenylene)bis(1,5-diphenyl-1,5-pentadione) (1)

A mixture of terephthalaldehyde (10.0 g, 0.075 mol) and acetophenone (54.3 g, 0.45 mol) was stirred in 250 mL of 95% ethanol and heated to 65°C. After the starting compounds dissolved in aqueous ethanol, a solution of KOH (10.5 g, 0.19 mol) in 10 mL of water was added dropwise over 30 min with vigorous stirring. A yellow precipitate formed immediately. The heterogeneous reaction mixture was then heated at reflux until it turned pink that occurred over a period of 4-5 h. The reaction mixture was filtered hot, and the tan solid was collected by filtration to afford 41.0 g of the crude product. It was recrystallized from toluene to afford 35.0 g (yield 81%) of off-white crystals. m.p. 205-206°C. ^{1}H NMR (400 MHz, d_6-DMSO, ppm): 7.96 (d, J = 7.6 Hz, 8H, ortho Ar-C=O), 7.38-7.56 (m, 12H, meta and para Ar-C=O), 7.19 (s, 2H, center Ar-H), 4.02 (t, 2H, Ar-CH), 3.23-3.51 (m, 8H, CH_2-C=O).

Synthesis of 4,4'-(1,4-phenylene)bis(2,6-diphen-yl-pyrylium) ditosylate (2)

The triphenylmethanol (7.8 g, 0.030 mol) and p-toluenesulfonic acid monohydrate (5.8 g, 0.030 mol) were added to 100 mL of $(CH_3CO)_2O$, followed with stirring at room temperature for 3 h. Then the solid tetraketone **1** (7.2 g, 0.012 mol) was added to the reaction mixture, and the mixture was heated to 100°C for 1 h. The heterogeneous reaction mixture became clear. Upon cooling, yellow crystals appeared and were collected by filtration, washed carefully with $(CH_3CO)_2O$ and ethanol, respectively, and dried in air to afford the crude **2**. It was then recrystallized from acetic acid and dried in vacuum to afford 7.9 g of orange colored **2** (*yield*: 75%). ^{1}H NMR (400 MHz, d_6-DMSO, ppm): 9.35 (s, 4H, aromatic meta to O^+), 9.21 (s, 4H, 1,4-phenylene), 7.58-8.93 (m, 20H, phenyl), 7.46-7.47(d, J = 6.7 Hz, 4H, tosylate), 7.09-7.10 (d, J = 7.7 Hz, 4H, tosylate), 2.27 (s, 6H, CH_3). Anal. Calcd for $C_{54}H_{42}O_8S_2$: C, 73.45; H, 4.79. Found: C, 73.25; H, 4.71.

Synthesis of 9H-N-butylcarbazole (3)

Carbazole (8.36 g, 0.05 mol), KOH (4.2 g, 0.075 mol) and 18-Crown-6 (0.1 g) as phase transfer catalysis were added to 90 mL of toluene and refluxed for 4 h. The solution of 1-bromobutane (0.075 mol. 8.1 mL) in 30 mL toluene were slowly added to the reaction mixture. The mixture was stirred at 110°C for 18 h and then cooled to the room temperature. Then, the reaction mixture poured into the 200 mL of ethanol and stripped off by a rotary evaporator. The product was recrystallized from methanol and dried in vacuum desiccators. *Yield*: 89.7%. ^{1}H NMR (400 MHz, $CHCl_3$-*d*, ppm): 8.10 (d, 2H, Ar-H); 7.62 (d, 2H, Ar-H); 7.44 (t, 2H, Ar-H); 7.22 (m, 2H); 4.30 (t, 2H, –N–CH_2–); 1.86 (m, 2H); 1.40 (m, 2H), 0.94 (t, 3H, R-CH_3). ^{13}C NMR ($CHCl_3$-*d*, ppm): 141; 108; 126; 117; 119; 123; 44; 31; 14.

Synthesis of 3, 6-dinitro-9H-N-butylcarbazole (4)

$Cu(NO_3)_2 \cdot 3H_2O$ (7.73 g, 0.032 mol) was added into a mixture of acetic acid (45 mL) and acetic anhydride (90 mL) at room temperature. The mixture was stirred for 30 min, and then N-butylcarbazole **3** (6.69g, 0.03 mol) was slowly added to this solution. After 15 min, 30 mL of acetic acid was added to the reaction mixture. The mixture was further stirred at this temperature for 2 h and then poured into distilled water (500 mL). The yellow precipitate was collected by filtration, washed with water (300 mL × 3) and dried at 60°C under vacuum. *Yield*: 93.7%. ^{1}H NMR (400 MHz, $CHCl_3$-*d*, ppm): 9.09 (s, 2H, Ar–H); 8.49 (d, 2H, Ar–H); 7.55 (d, 2H, Ar–H); 4.43 (t, 2H, –N–CH_2–); 1.94 (m, 2H), 1.43 (m, 2H), 0.99 (t, 3H, R-CH_3). ^{13}C NMR ($CHCl_3$-*d*, ppm): 147; 109; 127; 144; 116; 124; 43; 31; 14.

Synthesis of 3,6-diamino-9H-N-butylcarbazole (5)

Pd/C (10%, w/w) (0.1 g) was added to the solution of 3-6-dinitro-9-N-butylcarbazole **4** (6.26g, 0.02 mol) in 200 mL of ethanol at room temperature. Then the mixture was heated to reflux temperature for 1.5 h. 15 mL of hydraziniumhydroxide in 30 mL ethanol was then added to the solution as dropwise for 45 min. The mixture was stirred at 40°C for 24 h and then the Pd/C was filtered off. Ethanol was striped by rotary evaporator and remaining solid was crystallized on toluene two times. The solid was dried at 50°C under vacuum. *Yield*: 63%. ^{1}H NMR (400 MHz, $CHCl_3$-*d*, ppm): 7.35 (s, 2H, Ar–H); 7.17 (d, 2H,Ar–H); 6.90 (d, 2H,Ar–H); 4.20 (t, 2H, –N–CH_2–); 3.71 (broad, 2H, –NH_2); 1.81 (m, 2H); 1.38 (m, 2H); 0.95 (t, 3H, R-CH_3). ^{13}C NMR($CHCl_3$-*d*, ppm): 147; 109; 127; 144; 116; 124; 43; 34–21; 14. Anal. Calcd for $C_{16}H_{19}N_3$: C, 75.85; H, 7.56; N, 16.59. Found: C, 75.72; H, 7.61; N, 16.55.

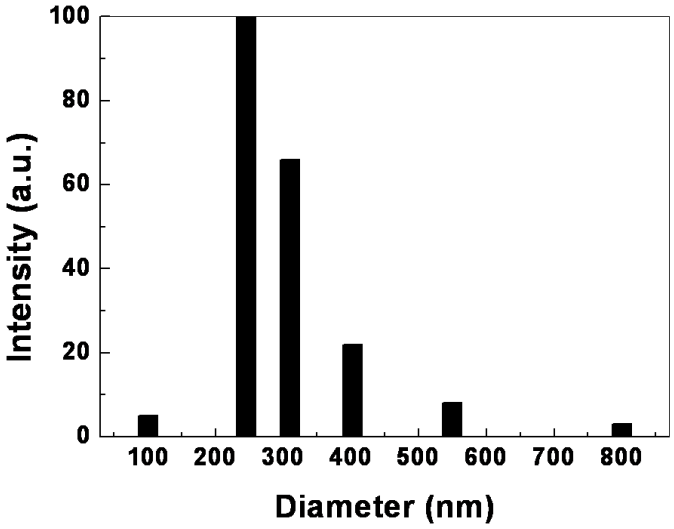

Fig. S1 Size distribution of the aggregates of **Poly1** with the concentration of 50 μM in aqueous solution.

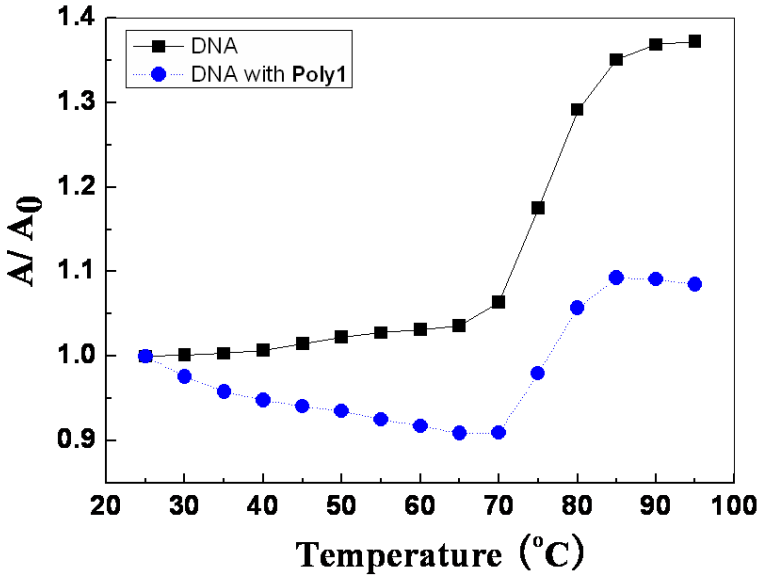

Fig. S2 Calf thymus DNA (30 μM) melting curves at 260 nm in the absence (squares) and presence of **Poly1** (10 μM) (circles) in PBS solution (2mM, pH 7.4). A_0 and A denote the initial absorbance and the recorded absorbance at different temperatures, respectively.

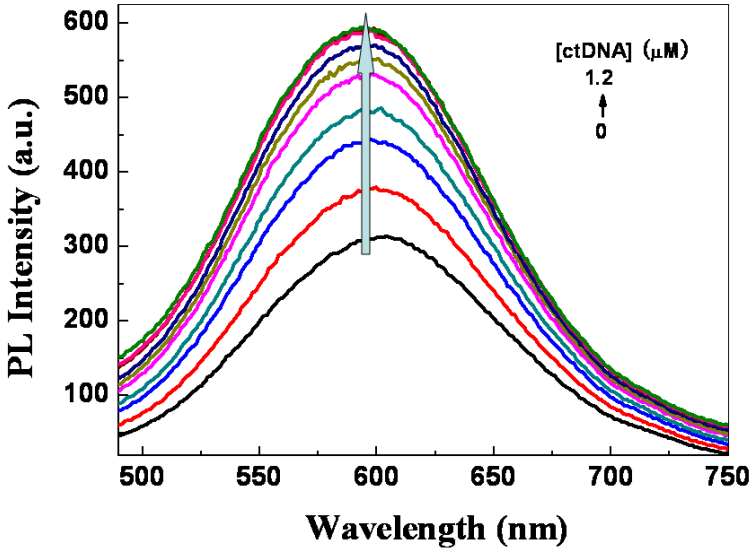

Fig. S3 PL spectra of **Poly1** at [**Poly1**] = 50 μM in PBS (2 mM, pH 7.4) solution in the presence of different concentrations of calf thymus DNA (excitation at 400 nm), [ctDNA] = 0.1, 0.2, 0.3, 0.5, 0.6, 0.7, 0.9, 1.0, 1.2 μM.

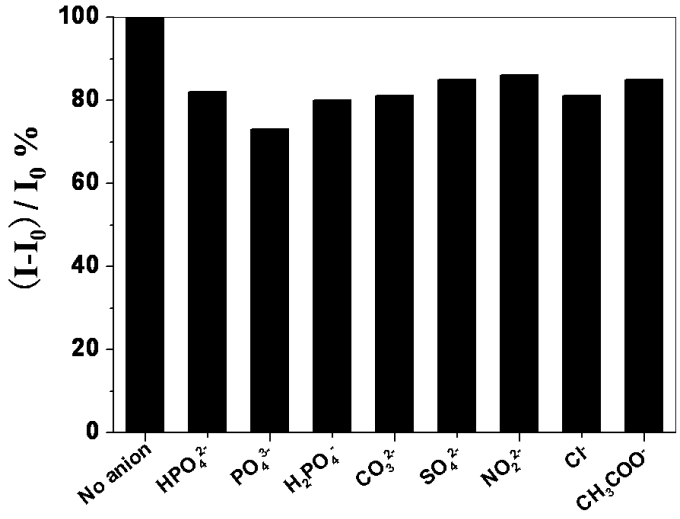

Fig. S4 $(I-I_0)/I_0$ ratio of fluorescence intensity of **Poly1** (50 μM) upon the addition of 2 equivalents ctDNA in the presence of 10 equivalents various background anions in aqueous ethanol solution (ethanol-water, 1:1, v/v)