Supporting Information

On the Binding of Cationic, Water-Soluble Conjugated Polymer to DNA: Electrostatic and Hydrophobic Interactions

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1. The thermo-stability of the molecular beacons

We have tested thermo-stability of the molecular beacons via thermal denaturation from 20 °C to 80 °C (Figure S1). We find that the most stable molecular beacon, No. 3, is only partly opened at 80 °C, and the increase in fluorescence intensity we observe for the thermally-unfolded state is similar to that observed when the beacon is opened by PFP-Br. This later observation indicates that PFP-Br can open even the most stable of our molecular beacons.

Figure S1. The thermo-stability of the molecular beacons we have employed. The cyan, blue, orange and red curve represent the fluorescence of molecular beacons at 20 °C, 40 °C, 60 °C and 80 °C, respectively.

2. The interactions between PFP-Br and molecular beacons are not competed by double-stranded DNA

We also found that the interaction between PFP-Br and molecular beacons is not inhibited by adding a 10-fold excess of double-stranded DNA (Figure S2).

Figure S2. The interactions between PFP-Br and the most stable molecular beacon (No. 3) are not inhibited by adding a 10 fold excess of double-stranded DNA.

3. Photographs taken under UV light

Figure S3. (Top) FRET from PFP-Br to single-stranded DNA is more efficient than that with double-stranded DNA. Photograph of single-stranded DNA with PFP-Br (Left) and double-stranded DNA with PFP-Br (right) taken under UV light ($\lambda = 365$ nm). (Bottom) FRET from PFP-Br to the split cocaine aptamer is higher in the absence of cocaine, conditions under which the split aptamer dissociates into two largely-single stranded fragments. Photograph of cocaine aptamer without cocaine with PFP-Br (Left) and cocaine aptamer with cocaine and PFP-Br (right) taken under UV light ($\lambda = 365$ nm).

4. The reduction of the hydrophobic effect prevents PFP-Br from opening molecular beacons

Figure S4. In the presence of the organic solvent NMP, which reduces the hydrophobic interactions between PFP-Br and molecular beacons, the PFP-Br can no longer “open” molecular beacons. (A), (B)

and (C) show fluorescence spectra (excited at 490 nm) of 96 nM molecular beacons without (blue lines) and with (red lines) PFP-Br (154 nM) in 10 % NMP solution.

5. Chemical structure of Cy3

6. FRET from PFB-Br to single stranded DNA (tagged with Cy3) at different concentration of NMP

Figure S5. Emission spectra from solutions containing PFB-Br and single stranded DNA-Cy3. The spectra are normalized with respect to the emission of PFB-Br.

6. Experimental Section

Materials: Cocaine was used as received. PDDA and PAH were obtained from Sigma-Aldrich. The sequences of the involved oligonucleotides, which are all purchased from Biosearch Technologies, Inc. (Novato, CA, USA), purified by HPLC, and confirmed by mass spectrometry, are listed following. The Fluorophore/quencher labeled stem-loop DNA was synthesized by Biosearch Technologies, Inc. (Novato, CA, USA), purified by HPLC. The three sequences have been shown in Figure 1. The water-soluble conjugated polymer, poly[(9,9-bis(6'-N,N,N-trimethylammonium)hexyl)fluorene-alt-1,4-phenylene] bromide (PFP-Br) was prepared according to the published references.^{13, 14}

The Sequences of the DNA probes and targets used in this work.

34-mer-Linear-Probe:

5'-(Cy3)-TGGATCGGCGTTTTATTCTTGTTTCAGATATTCAA-3'

34-mer-Linear-Target:

5'-TTGAATATCTGAACAAGAATAAAACGCCGATCCA-3'

5' fragment for anti-cocaine aptamer:

5'-(Cy3)-TCCTTCAATGAAGTGGGTCG-3'

3' fragment for anti-cocaine aptamer:

5'-AGACAAGGAAAA-3'

Assay protocol: The DNA (molecular beacons, single-stranded DNA and double-stranded DNA) were dissolved in a salt solution (112.5 mM NaCl) to form 1.25 μ M DNA solution at room temperature. Such solution (5 μ L) was added to DI water (50 μ L), and the resulting solution was incubated for 2 min at room temperature. PFP-Br (10 μ L, 1 μ M) was then added to the above solution, followed by fluorescence characterization. Finally, the concentrations of DNA and PFP-Br in the solution were 96 nM and 154 nM, respectively. In the cocaine assays, the 3' fragment and 5' fragment were dissolved in a salt solution (112.5 mM NaCl) to form a 0.625 μ M 3' fragment and 0.625 μ M 5' fragment probe solution at room temperature. Cocaine was then added to the above solution. This solution (5 μ L) was then added to DI water (50 μ L) and the obtained solution was incubated for 2 min at room temperature. PFP-Br (10 μ L, 1 μ M) was then added, followed by fluorescence characterization.

Fluorescence measurements: Fluorescence was measured with a Peltier block, using quartz fluorescence cuvettes, and with the following settings: 5 nm slit, PMT detector voltage = 650 V.