Design of a novel G-quenched molecular beacon: A simple and efficient strategy for DNA sequence analysis

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**Figure S1:** Structures of the MB2-5 and MB7

**General experimental:**

**Oligonucleotide synthesis and characterisation:** All reagents for DNA synthesis were purchased from Glen Research. MB1 was synthesized by automated DNA synthesizer. The fluorophores were post-synthetically incorporated into MB1 to get desired molecular beacons, MB2, 3, and 4. As a target loop strands, we synthesised unmodified ODN 11, a fully matched sequence, and ODN 8-10, one-base-mismatched sequences at the central position of the loop sequence. ODNs were purified by reverse phase HPLC on a CHEMCO-BOND 5-ODS-H column (10 x 150 mm, elution with 50 mM ammonium formate buffer (AF), pH 7.0, linear gradient over 50 min from 3% to 40% acetonitrile at a flow rate 2.0 ml/min). ODNs containing modified nucleotides were fully digested with calf intestine alkaline phosphatase (50 U/mL), snake venom phosphodiesterase (0.15 U/mL), and P1 nuclease (50 U/mL) at 37 °C for 3 h. Digested solutions were analysed by HPLC on a CHEMCO-BOND 5-ODS-H column (4.6 x 150 mm), elution with a solvent mixture of 50 mM ammonium formate buffer (AF), pH 7.0, linear gradient over 60 min from 3% to 50% acetonitrile at a flow rate 1.0
mL/min). The concentration of each ODNs was determined by comparing peak areas with standard solution containing dA, dC, dG, and dT at a concentration of 0.1 mM. Mass spectra of ODNs purified by HPLC were determined with a MALDI-TOF mass spectroscopy, Shimadzu, AXIMA-LNR.

Table S1: MALDI-TOF Mass Spectral data for the ODNs

<table>
<thead>
<tr>
<th>ODNs</th>
<th>MALDI-TOF Mass cald. for</th>
<th>MALDI-TOF Mass found</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 MB1</td>
<td>7870.25</td>
<td>7870.35</td>
</tr>
<tr>
<td>2 MB2</td>
<td>8101.52</td>
<td>8101.04</td>
</tr>
<tr>
<td>3 MB3</td>
<td>8073.42</td>
<td>8073.87</td>
</tr>
<tr>
<td>4 MB4</td>
<td>8090.47</td>
<td>8090.94</td>
</tr>
<tr>
<td>5 MB5</td>
<td>8230.57</td>
<td>8230.74</td>
</tr>
<tr>
<td>5 MB7</td>
<td>9323.34</td>
<td>9323.74</td>
</tr>
</tbody>
</table>

Synthesis of modified G-quenched molecular beacons (MBs): The fluorophores were post-synthetically incorporated into MB1 to get desired molecular beacons, MB2, 3, and 4. Thus, active esters (1.0 mg) were dissolved in a small amount of dry DMF (20 μL) and added to the 5'-amino modified MBs (20 μL) in a total volume of 150 μL of 1.0M sodium NaHCO3 and incubated for 8 hours at 37 °C. Purification and the characterization of the products were performed according to the standard process as described above.

Melting temperature (Tm) measurements: All Tms of the ODNs (2.5 μM, final duplex concentration) were taken in 50 mM sodium phosphate buffers (pH 7.0) containing 100 mM sodium chloride. Absorbance vs temperature profiles were measured at 260 nm using a Shimadzu UV-2550 spectrophotometer equipped with a Peltier temperature controller using 1 cm path length cell. The absorbance of the samples was monitored at 260 nm from 4 °C to 90 °C with a heating rate of 1 °C/min. From these profiles, first derivatives were calculated to determine Tm values.

UV absorption measurements: ODN solutions were prepared as described in Tm measurement experiment. Absorption spectra were obtained using a Shimadzu UV-2550 spectrophotometer at room temperature using 1 cm path length cell.

Fluorescence measurements: ODN solutions were prepared as described in Tm measurement experiment. Fluorescence spectra were obtained using a Shimadzu RF-5300PC spectrophotometer at 25°C using 1cm path length cell. The excitation and the emission bandwidth was 1.5 nm.
**MB1** with or without target ODNs  

**Figure S2:** UV-visible spectra and thermal melting curve of the probe **MB1** alone (MB) and with their targets (ODN 8-11) [2.5 μM, 50mM sodium phosphate, 0.1M sodium chloride, pH 7.0, RT]

**MB2** with or without target ODNs  

**MB4** with or without target ODNs  

**Figure S3:** UV-visible spectra of probes **MB2** and **MB4** alone (MBs) and with their target (ODN 8-11) [2.5 μM, 50mM sodium phosphate, 0.1M sodium chloride, pH 7.0, RT]
**Figure S4**: Thermal denaturation curves of probes MB2 and MB4 alone (MBs) and with their target (ODN 8-11) [2.5 μM, 50mM sodium phosphate, 0.1M sodium chloride, pH 7.0, RT]

**Figure S5**: Fluorescence excitation spectra of probes MB2 and MB4 alone (MBs) and with their target (ODN 8-11) [2.5 μM, 50mM sodium phosphate, 0.1M sodium chloride, pH 7.0, RT]
**Figure S6:** UV-visible spectra of probes MB3 and MB5 alone (MBs) and with their target (ODN 8-11) [2.5 μM, 50mM sodium phosphate, 0.1M sodium chloride, pH 7.0, RT]

**Figure S7:** Thermal denaturation curves of probes MB3 and MB5 alone (MBs) and with their target (ODN 8-11) [2.5 μM, 50mM sodium phosphate, 0.1M sodium chloride, pH 7.0, RT]
Figure S8: Fluorescence excitation spectra of probes MB3 and MB5 alone (MBs) and with their target (ODN 8-11) [2.5 μM, 50mM sodium phosphate, 0.1M sodium chloride, pH 7.0, RT]
Figure S9: UV, thermal denaturation curves and fluorescence excitation spectra of probe MB7 alone (MBs) and with its target (ODN 8-11) [2.5 μM, 50 mM sodium phosphate, 0.1 M sodium chloride, pH 7.0, RT].

Figure S10: Structures of the MB8 and MB9

Figure S11: Fluorescence excitation spectra of hairpin MB8 (a) and MB9 (b) [(2.5 μM) and the different duplexes formed by hybridization with ODN 8-11 (2.5 μM, 50 mM sodium phosphate, 0.1M sodium chloride, pH 7.0, RT)]. Excitation wavelength was 403 nm. "MB" denotes the hairpin states.
Figure S12: Fluorescence spectra of hairpin MB8 (a) and MB9 (b) [(2.5 μM) and the different duplexes formed by hybridization with ODN 8-11 (2.5 μM, 50 mM sodium phosphate, 0.1M sodium chloride, pH 7.0, RT)]. Excitation wavelength was 403 nm. "MB" denotes the hairpin states.