Supplementary Material (ESI) for Chemical Communications

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

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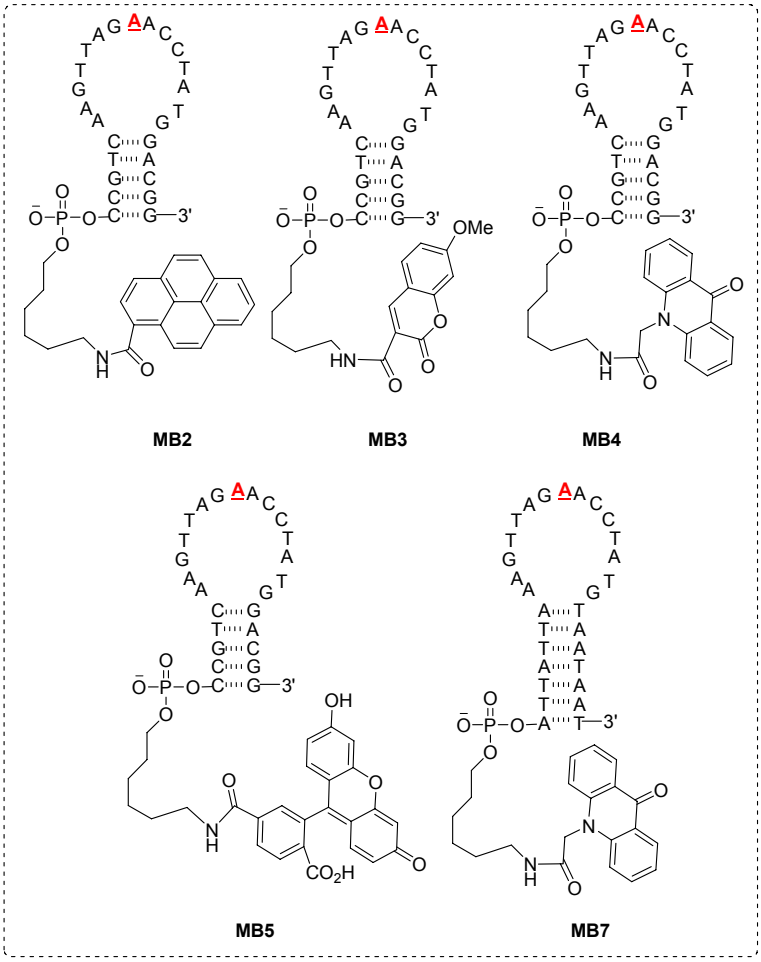


Figure S1: Structures of the **MB2-5** and **MB7**

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 ^{o}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

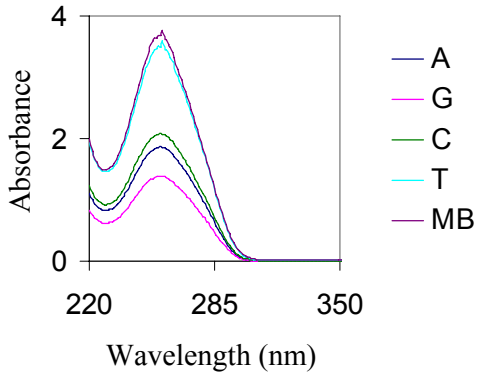
ODNs		MALDI-TOF Mass cald. for	MALDI-TOF Mass found
1	**MB1**	7870.25	7870.35
2	**MB2**	8101.52	8101.04
3	**MB3**	8073.42	8073.87
4	**MB4**	8090.47	8090.94
5	**MB5**	8230.57	8230.74
5	**MB7**	9323.34	9323.74

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 $NaHCO_3$ and incubated for 8 hours at 37 ^oC. Purification and the characterization of the products were performed according to the standard process as described above.

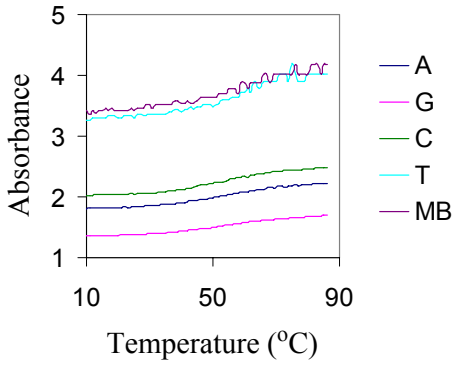
Melting temperature (T_m) measurements: All T_ms of the ODNs (2.5 μM, final duplex concentration) were taken in 50 mM sodium phosphate buffers (pH 7.0) containing 100 mM sodium chloride. Absorbance *vs* temperature profiles were measured at 260 nm using a Shimadzu UV-2550 spectrophotometer equipped with a Peltier temperature controller using 1 cm path length cell. The absorbance of the samples was monitored at 260 nm from 4 ^oC to 90 ^oC with a heating rate of 1 ^oC/min. From these profiles, first derivatives were calculated to determine T_m 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 T_m measurement experiment. Fluorescence spectra were obtained using a Shimadzu RF-5300PC spectrophotometer at 25^oC using 1cm path length cell. The excitation and the emission bandwidth was 1.5 nm.

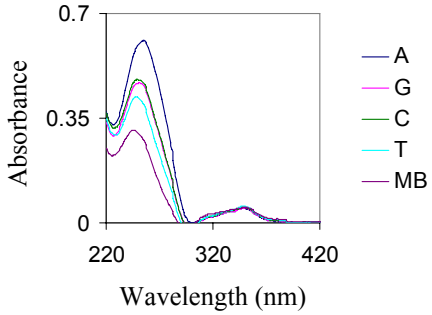


MB1 with or without target ODNs

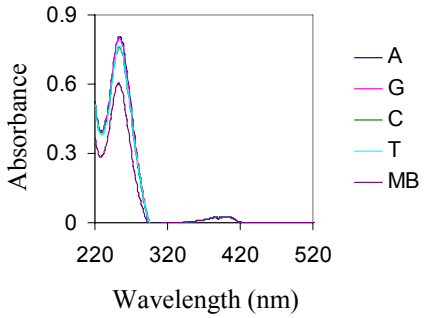


MB1 with or without target ODNs

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]

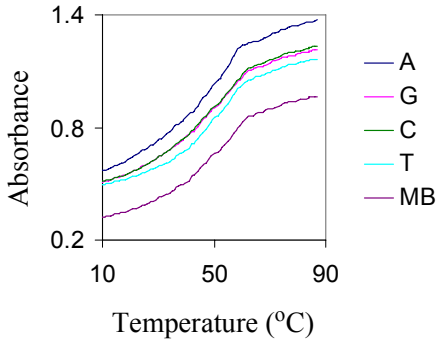


MB2 with or without target ODNs

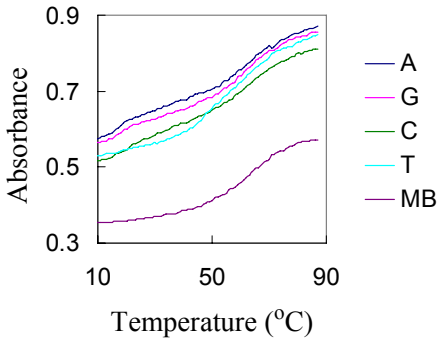


MB4 with or without target ODNs

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]

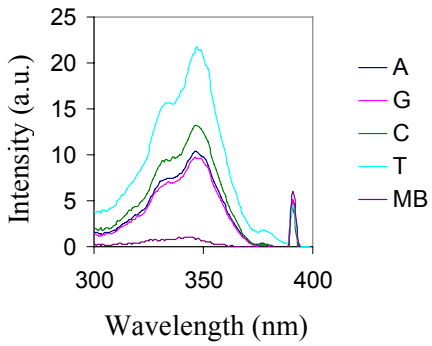


MB2 with or without target ODNs

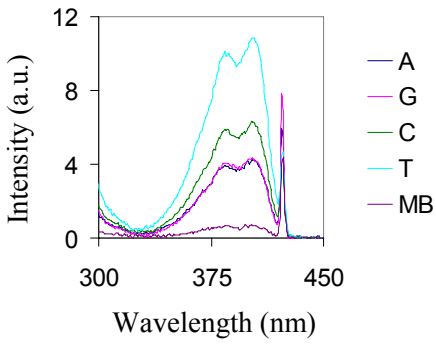


MB4 with or without target ODNs

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]

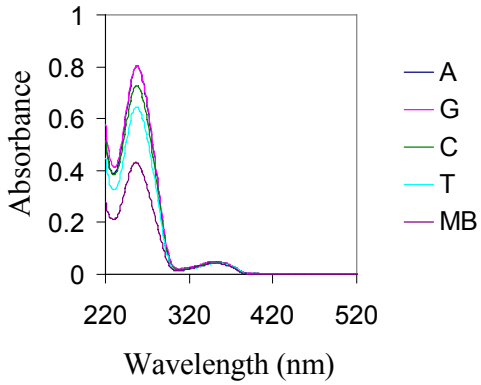


MB2 with or without target ODNs

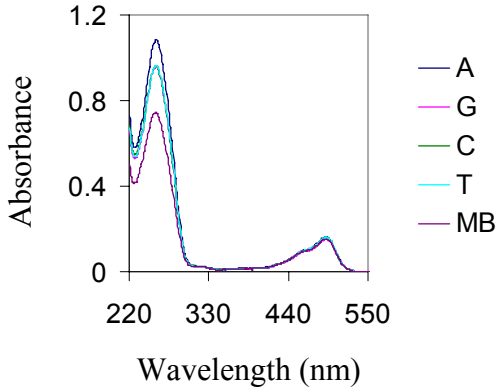


MB4 with or without target ODNs

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]

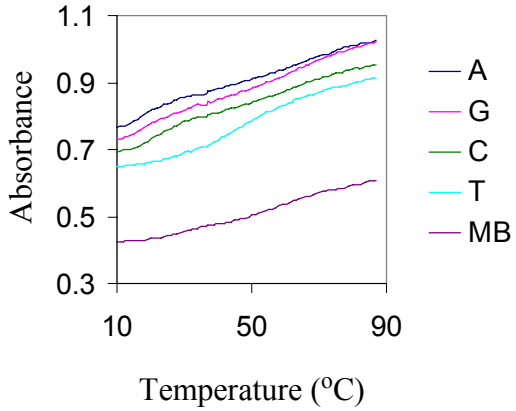


MB3 with or without target ODNs

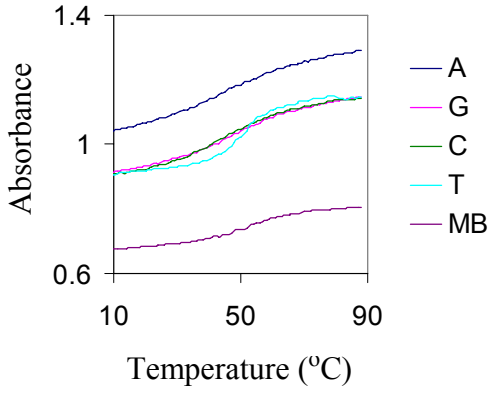


MB5 with or without target ODNs

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]

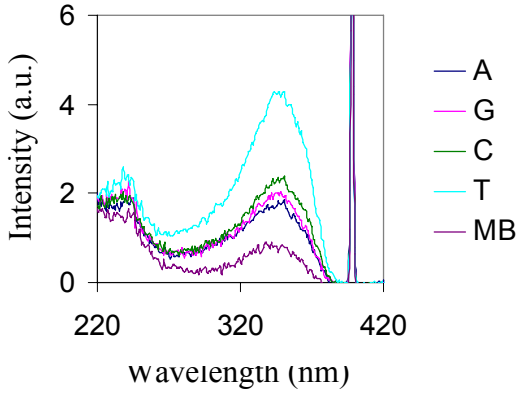


MB3 with or without target ODNs

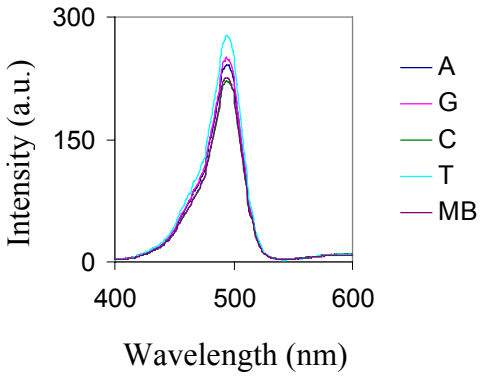


MB5 with or without target ODNs

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]

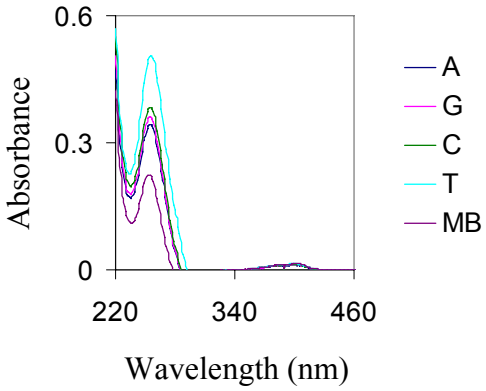


MB3 with or without target ODNs

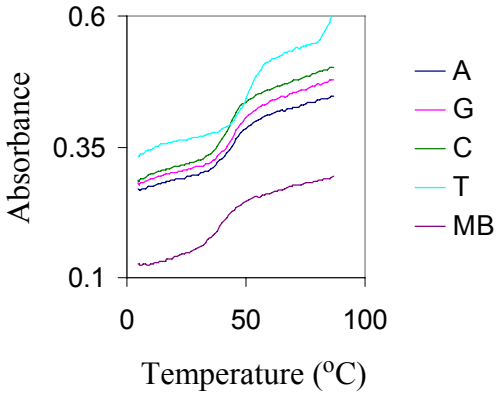


MB5 with or without target ODNs

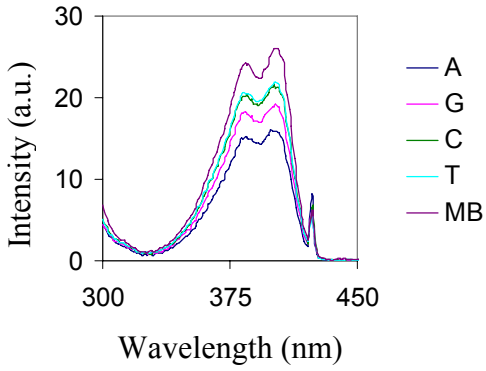
Figure S8: Fluorescence excitation spectra of probes **MB3** and **MB5** alone (MBs) and with their target (ODN **8-11**) [2.5 μM, 50mM sodium phosphate, 0.1M sodium chloride, pH 7.0, RT]



MB7 with or without target ODNs



MB7 with or without target ODNs



MB7 with or without target ODNs

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].

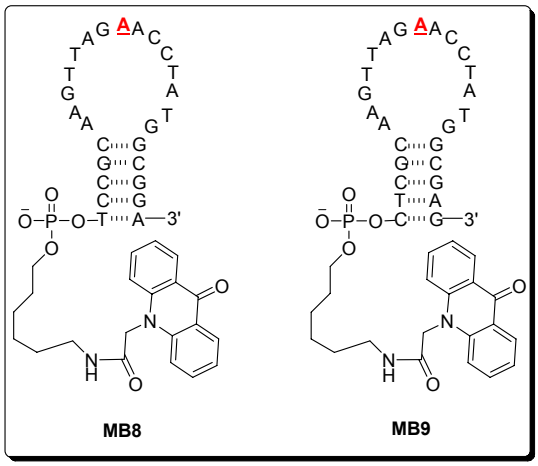


Figure S10: Structures of the **MB8** and **MB9**

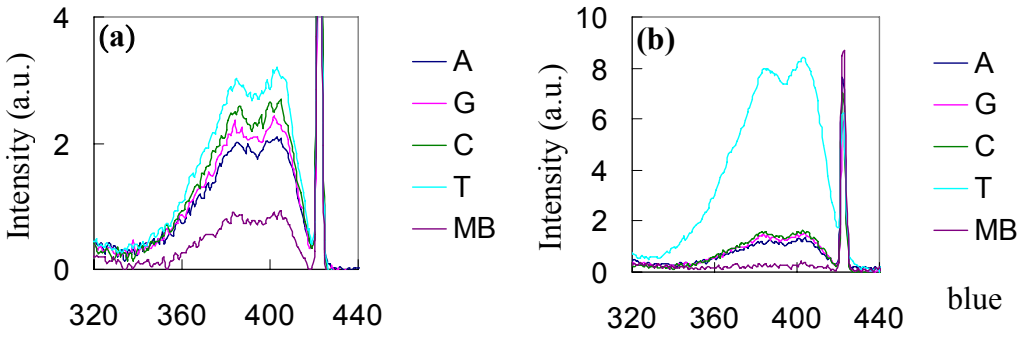


Figure S11: Fluorescene excitation spectra of hairpin **MB8** (a) and **MB9** (b) [(2.5 μM) and the different duplexes formed by hybridization with ODN 8-11 (2.5 μM, 50 mM sodium phosphate, 0.1M sodium chloride, pH 7.0, RT)]. Excitation wavelength was 403 nm. "MB"denotes the hairpin states.

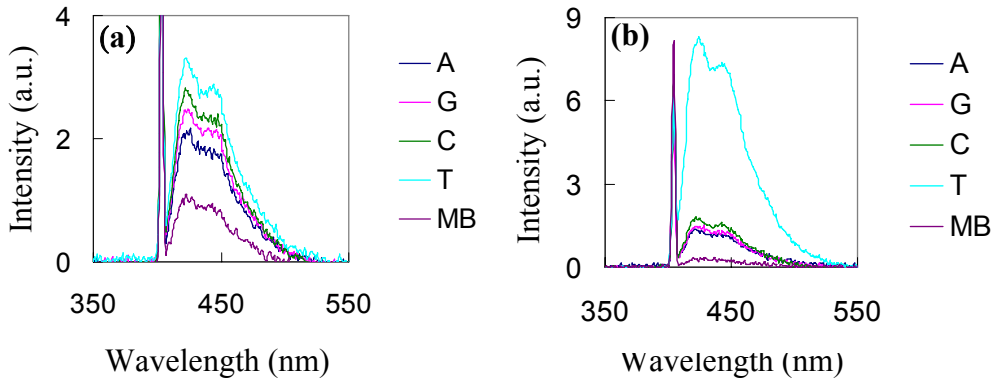


Figure S12: Fluorescence spectra of hairpin **MB8** (a) and **MB9** (b) [(2.5 μM) and the different duplexes formed by hybridization with ODN 8-11 (2.5 μM, 50 mM sodium phosphate, 0.1M sodium chloride, pH 7.0, RT)]. Excitation wavelength was 403 nm . "MB"denotes the hairpin states.