

Supporting Information

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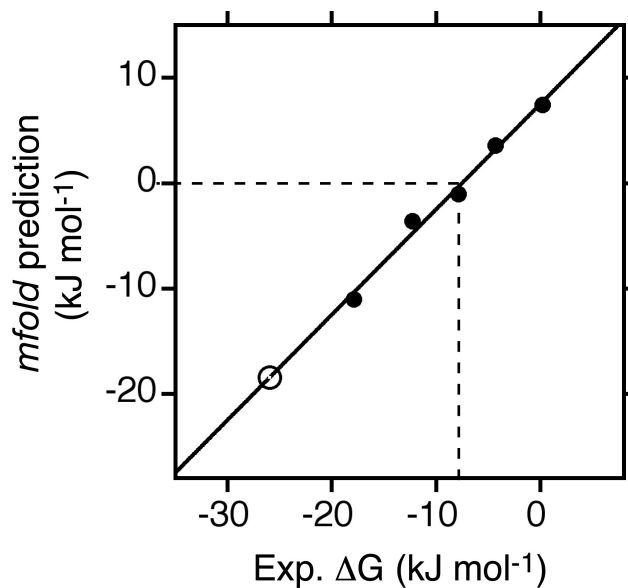


Fig. S1. Molecular beacon 5GC is very stable as such, as 8 M urea does not fully unfold it (Fig. 2), rendering it difficult to measure its switching thermodynamics experimentally. Instead we estimated its switching thermodynamics (open circle) using the strong relationship observed between the *mfold* predicted and experimentally observed free energies of the 5 less stable molecular beacons (slope: 1.00 ± 0.06 , $R^2 = 0.98$). Of note, the stability of all 5 of the less stable molecular beacons were offset by 7.6 ± 0.6 kJ mol⁻¹ from their predicted *mfold* values (dashed lines), presumably due to a strong, favorable interaction between the attached fluorophore (FAM) quencher (BHQ-1) pair (51).