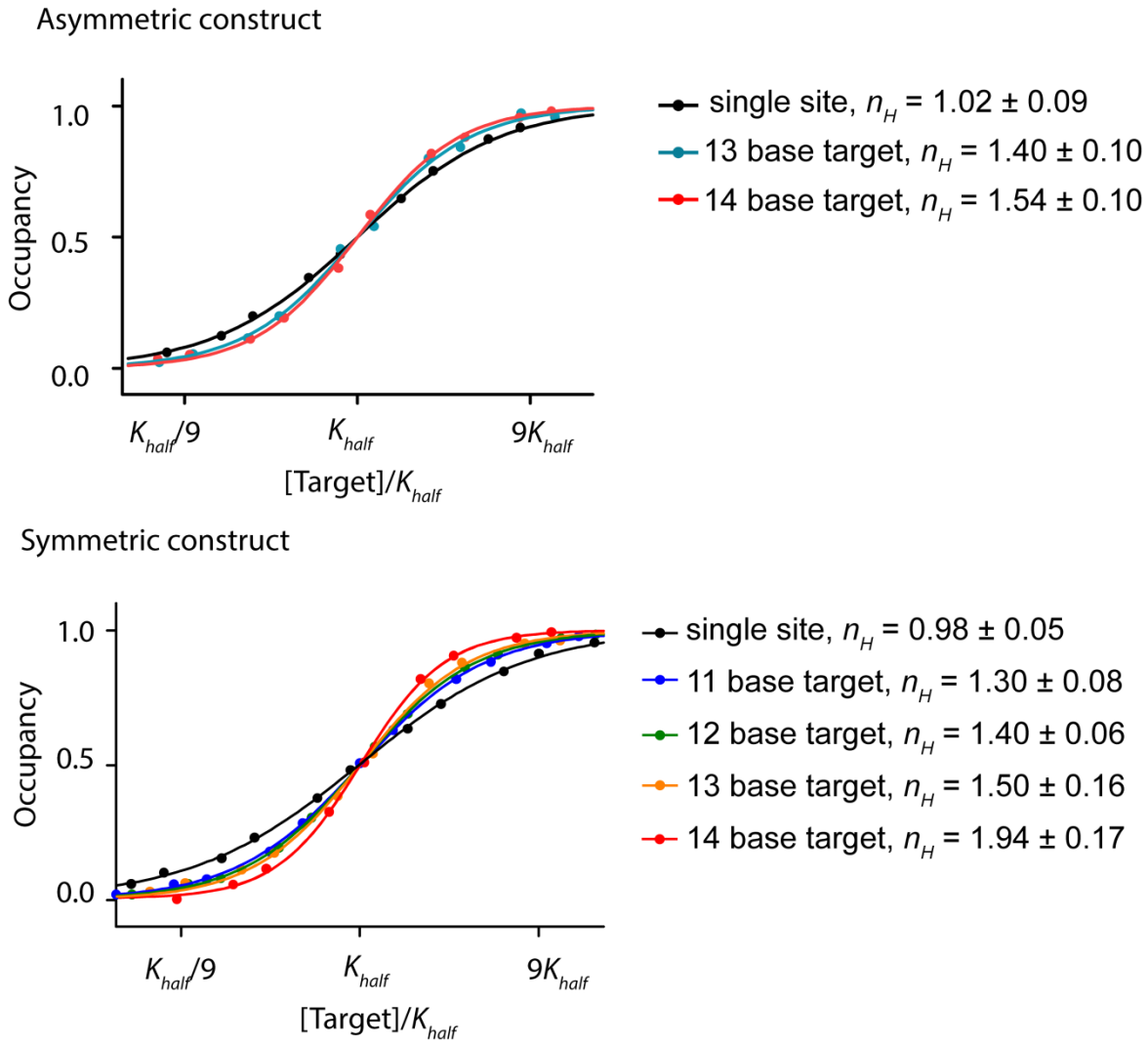

Figure SI1: The cooperativity of both our asymmetric (i.e., tailed) and symmetric molecular beacons increase monotonically as the length of the target oligonucleotide increases, as this increases the size of the energy gap between the first and second binding events.

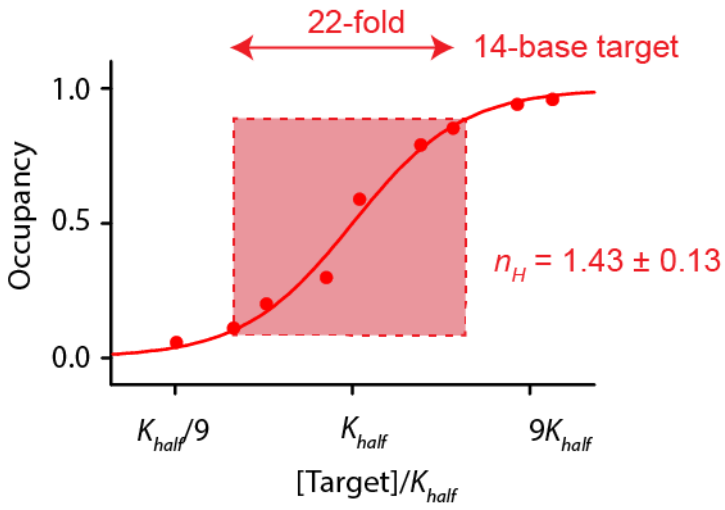

Figure SI2: A symmetric, cooperative molecular beacon employing a shorter, weaker stem (five base-pair, four GC) exhibits a Hill coefficient of 1.43 ± 0.13. A construct employing a longer, more stable stem (six base-pair, five GC), in contrast, achieves a Hill coefficient of 1.94 ± 0.17 (Fig. 3).

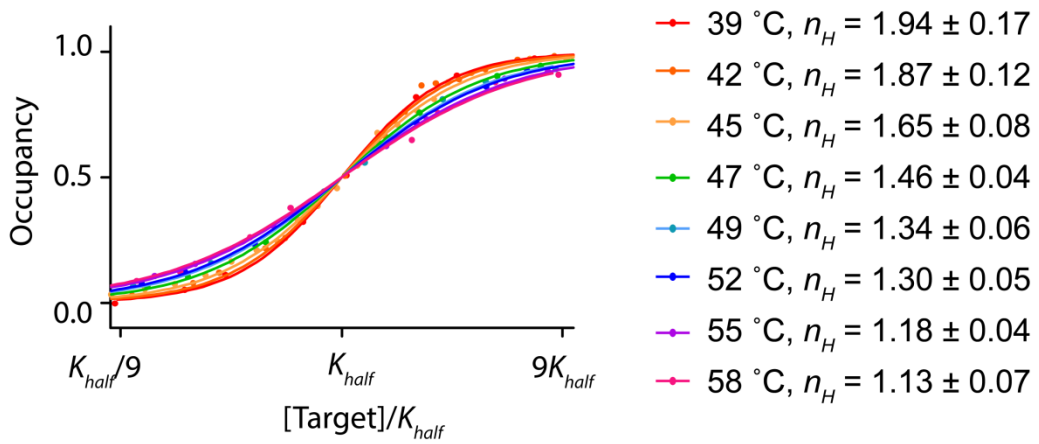

Figure SI 3: As the temperature falls the stability of the stem in our symmetric molecular beacon increases, thus increasing the energy gap between the first and second binding events and, in turn, increasing its cooperativity.

Materials and Methods

All reagent-grade chemicals, including cocaine hydrochloride, mercury chloride, sodium phosphate monobasic and, sodium phosphate dibasic (all from Sigma-Aldrich, St. Louis, Missouri) were used as received. DNA probes modified with a carboxyfluorescein (FAM) and a black-hole-quencher-1 (BHQ) and unmodified DNA target sequences were used as purchased (BioSearch Tech, Novato, CA). The sequences are as follows, with (BHQ) and (FAM) representing the black hole quencher and the carboxyfluoroscein fluorophore conjugated directly to the 3' hydroxyl, or via 6-carbon linkers attached to either the 5' hydroxyl or on the 5-position of an internal thymine, respectively, as indicated.

Single-site molecular beacon:
5' (FAM)-C6-TTGTGGATCGGCCGTTTTACACAA-(BHQ)

Asymmetric cooperative molecular beacon:
5'CGTAATTTGTCTCTTCCTCT(FAM)TGGCAGTCTCTTTCCTCTTGCCAT(BHQ)GAGTC TTCTAGTACATTCGA

Asymmetric cooperative molecular beacon targets:
14-base target: 5' AAGAGGAAAGAGACT
13-base target: 5‘ AGAGGAAAGAGACT

5 GC Symmetric cooperative molecular beacon:
5'(FAM)-C6-GGGTGGGTTCACTGTCTTAGTTCACTGTCTTACCACCC-(BHQ)

4 GC Symmetric cooperative molecular beacon:
5' (FAM)-C6-AGGTGGGTTCACTGTCTTAGTTCACTGTCTTACCACCG-(BHQ)

Symmetric cooperative molecular beacon targets:
14-base target: 5’ GTAAGACAGTGAACC
13-base target: 5’ TAAGACAGTGAAC
12-base target: 5’AAGACAGTGAAC
11-base target: 5’AGACAGTGAAC

Control (non-cooperative) two-site molecular beacon:
5’ (FAM) GGTGGGCTTGAGTCTCTTAGTTCACTGTCTTACCCACC

Control target sequence:
5’ TAAGAGACTCAAG

We collected all fluorescence measurements using a Cary Eclipse Fluorimeter (Varian) with excitation at 485 (± 5) nm and acquisition at 515 (± 5) nm. We performed all measurements in 150 mM sodium chloride, 50 mM sodium phosphate buffer pH 7. All measurements were conducted at 39˚C unless otherwise noted. We incubated all samples for at least 15 minutes with each target concentration at the appropriate temperature prior to taking measurements; kinetic traces collected at all target concentrations indicate that this is more than sufficient to achieve equilibration. To determine the Hill coefficient, we measured fluorescence at five replicate points per titration. We used GraphPad Prism plotting software to fit the traces to the Hill equation. Error bars represent 95% confidence intervals based on standard errors derived from the fits.