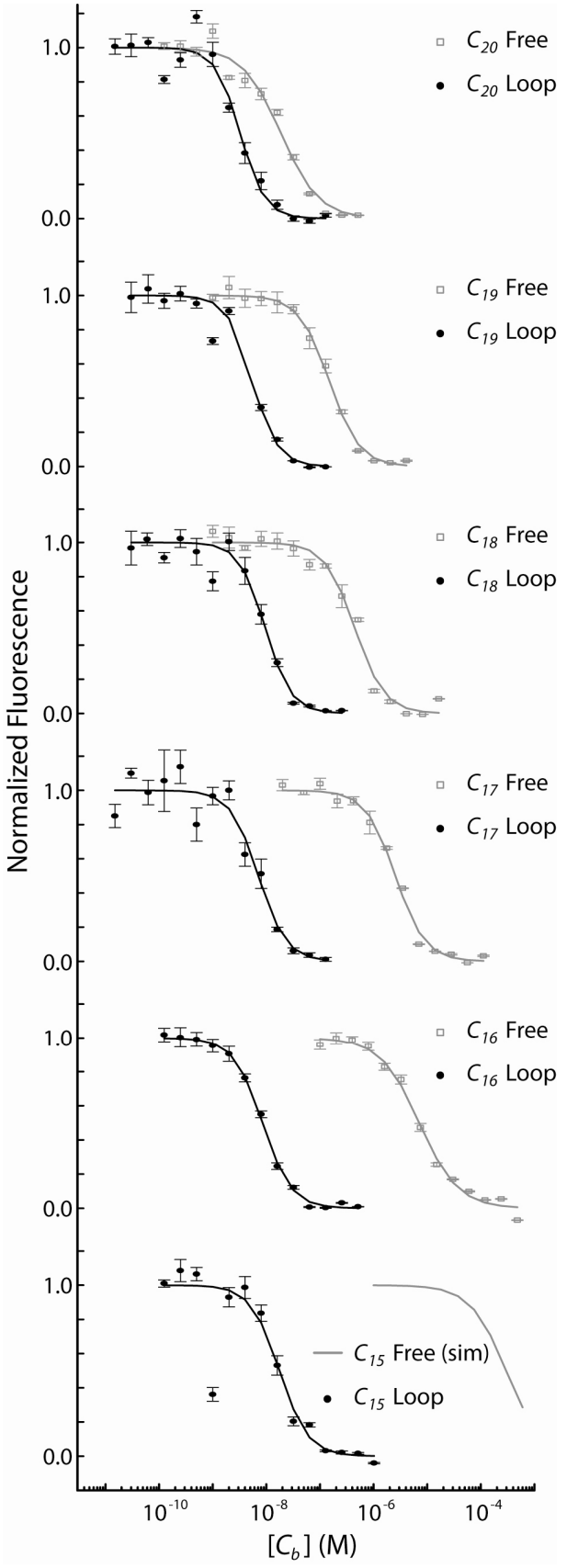
SUPPORTING INFORMATION FOR:

Asymmetric connector hybridization improves sensitivity and dynamic range in proximity ligation assays

Joonyul Kim[1], Jiaming Hu[1], Rebecca S. Sollie, and Christopher J. Easley[1,*]
[1] Auburn University, Department of Chemistry and Biochemistry, 179 Chemistry Building, Auburn, AL 36849, USA

CONTENTS

Figure S-1: Complete set of results from experimental model of proximity hybridization. Fluorescence response curves for the signal (*Loop*, black circles) and background (*Free*, gray open squares) complexes when titrated with C_{15} through C_{20}. NLLS fits to the Hill equation (Eq. 2) are shown as solid curves. It is clear from this depiction that as the connector affinity decreases, the background stability is drastically decreased, while the signal stability is only moderately decreased.



Synthesis of *Loop* strands. *Loop* strands (**Table 1**) were synthesized by ligation of $Free_A$ and $Free_B$ using T4 DNA ligase. The Linker strand was used to hybridize with and adjacently position the strands for ligation. Ligation was carried out by T4 DNA ligase (New England Biolabs) in a total volume of 100 µL for 3 h at 16°C with a Linker:$Free_{A,B}$ molar ratio of 20:1. Ligated products were further concentrated to 20 µL total volume and mixed with 100µl of 1.2x DNA loading dye (0.5mg/ml bromophenol blue, 8M urea, 1% (v/v) NP-40, 1mM Tris-HCl pH 8; Nucleic Acids Res. 37, e112). Then, samples were denatured at 90 °C for 5 min, and quickly loaded onto 1 M urea containing TAE 5% agarose gel. Gel electrophoresis was run on a VWR Mini Gel apparatus at 70 volts for 5 h with a 100 nt ssDNA as well as the 50 nt $Free_A$ and $Free_B$ strands for markers (**Figure S-2**). Under UV light, the ligated product bands were excised and eluted using Wizard® SV Gel and PCR Clean-Up Systems (Promega) under the manufacturer's protocol. Ligated products were quantified using both UV absorbance at 260 nm and FAM absorbance at 490 nm with a Nanodrop 1000 small-volume spectrophotometer (Thermo Scientific).

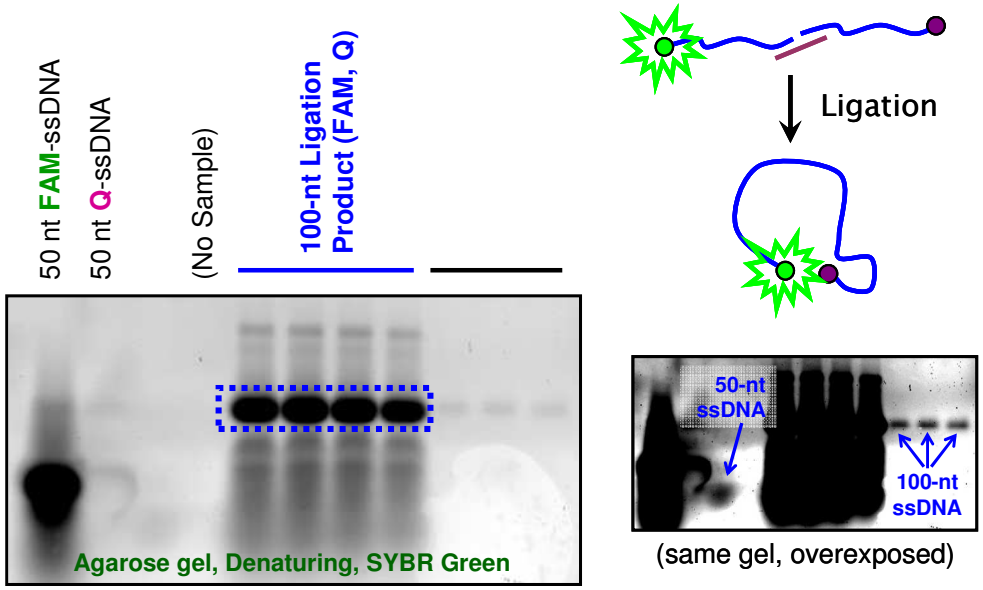


Figure S-2: Schematic of *Loop* strand synthesis by ligation. ssDNA separation by agarose gel electrophoresis was used to purify ligation products.

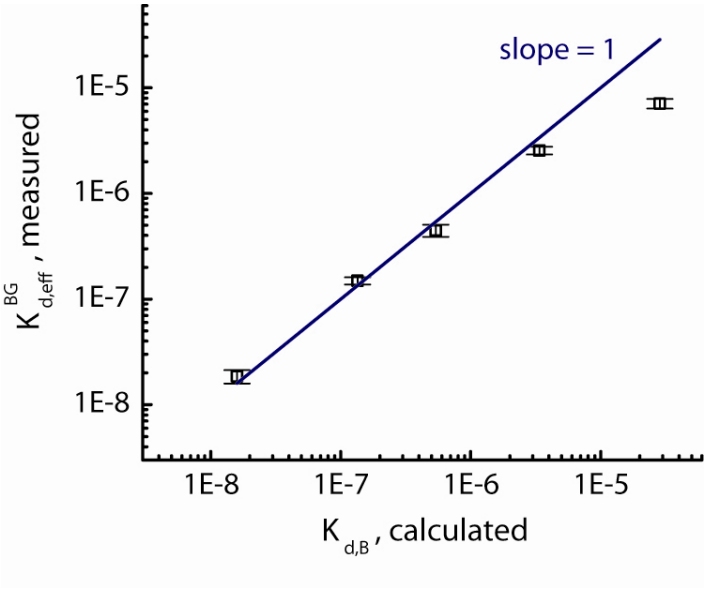


Figure S-3: Data from **Table 2** is shown in graphical form to highlight the experimental confirmation of SantaLucia's[12] nearest-neighbor calculations of $K_{d,B}$. Ideally, $K^{BG}_{d,eff}$ should equal $K_{d,B}$. Since the majority of the points follow a slope of unity (blue line), the measured and calculated values are shown to be well correlated.

DNA melting curve measurement. The melting temperatures (T_m) of DNA oligomers C_{16} through C_{20} (sequences in **Table 1**) were determined using a Bio-Rad CFX96 qPCR instrument. Working solutions consisted of varying concentrations of oligomers and 200 nM of the DNA intercalating dye PicoGreen (em ~520 nm; Invitrogen) in 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, and 1 mM $MgCl_2$. Oligomer concentrations were intentionally adjusted to approximately $3 \times K_{d,B}$ from **Table 2** (calculated values). In this way, a constant T_m for C_{16} through C_{20} inferred accuracy of SantaLucia's[12] thermodynamic nearest-neighbor modeling approach for estimating $K_{d,B}$ values. Fluorescence was measured as a function of temperature, and the derivative of the fluorescence was fit to Gaussian functions using nonlinear least squares fitting to determine T_m values (peak center).

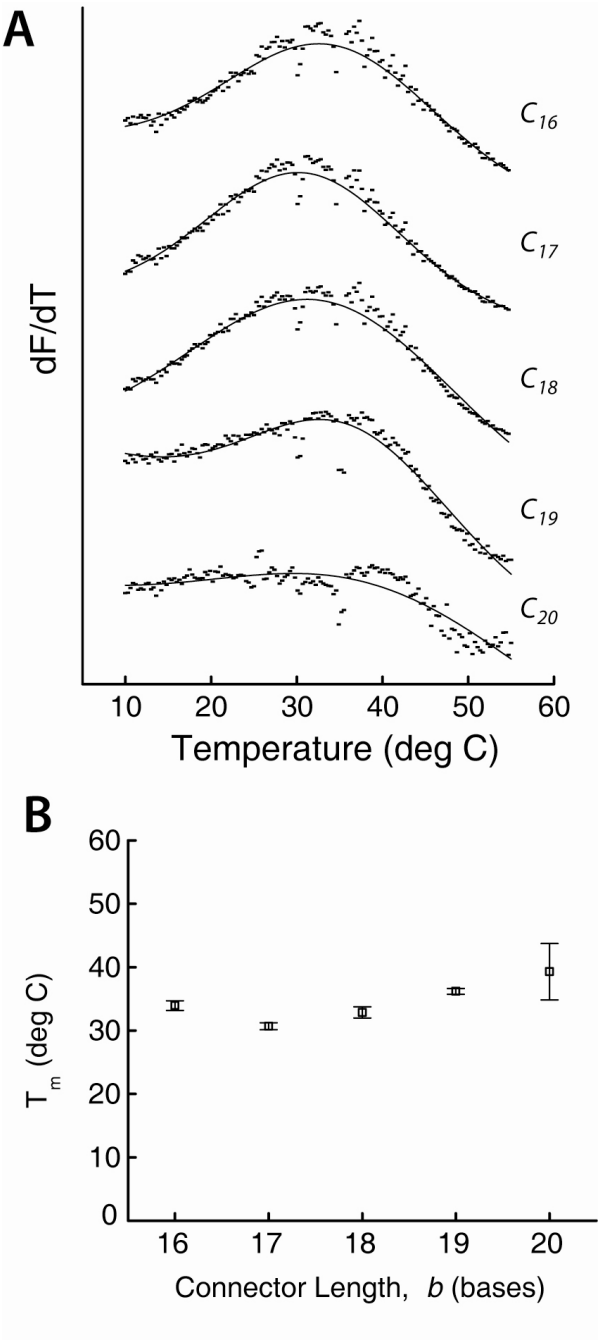


Figure S-4: (**A**) Fluorescence melting curves of DNA oligomers from **Table 1**. (**B**) Melting temperatures (T_m) were kept essentially constant using variable oligomer concentrations of $3 \times K_{d,B}$.