Supporting Information for Gartner and Liu Manuscript

DNA synthesis. DNA oligonucleotides were synthesized on a PerSeptive Biosystems Expedite 8909 DNA synthesizer using standard protocols and purified by reverse phase HPLC. Oligonucleotides were quantitated spectrophotometrically and by denaturing polyacrylamide gel electrophoresis (PAGE) followed by staining with ethidium bromide or SYBR Green (Molecular Probes) and quantitation using a Stratagene Eagle Eye II densitometer. Phosphoramidites enabling the synthesis of 5'-NH_2-dT, 5' tetrachlorofluorescein, abasic backbone spacer, C3 backbone spacer, 9-bond polyethylene glycol spacer, 12-bond saturated hydrocarbon spacer, and 5' biotin groups were purchased from Glen Research. Thiol-linked oligonucleotide reagents were synthesized on C3 disulfide controlled pore glass (Glen Research).

Template functionalization. Templates bearing 5'-NH_2-dT groups were transformed into a variety of electrophilic functional groups by reaction with the appropriate electrophile-NHS ester (Pierce). Reactions were performed in 200 mM sodium phosphate pH 7.2 with 2 mg/mL electrophile-NHS ester, 10% DMSO, and up to 100 μg of 5'-amino template at 25 °C for 1 h. Desired products were purified by reverse-phase HPLC and characterized by gel electrophoresis and MALDI mass spectrometry.

DNA-templated synthesis reactions. Reactions were initiated by mixing equimolar quantities of reagent and template in buffer containing 50 mM MOPS pH 7.5 and 250 mM NaCl at the desired temperature (25 °C unless stated otherwise). Concentrations of reagents and templates were 60 nM unless otherwise indicated. At various time points, aliquots were removed, quenched with excess β-mercaptoethanol, and analyzed by denaturing PAGE. Reaction products were quantitated by densitometry using their intrinsic fluorescence or by staining followed by densitometry. Representative products were also verified by MALDI mass spectrometry.

***In vitro* selection for avidin binding**. Products of the library translation reaction were isolated by ethanol precipitation and dissolved in binding buffer (10 mM Tris pH 8, 1 M NaCl, 10 mM EDTA). Products were incubated with 30 μg of streptavidin-linked magnetic beads (Roche Biosciences) for 10 min at room temperature in 100 uL total volume. Beads were washed 16 times with binding buffer and eluted by treatment with 1 μmol free biotin in 100 uL binding buffer at 70 °C for 10 minutes. Eluted molecules were isolated by ethanol precipitation and amplified by standard PCR protocols (2 mM $MgCl_2$, 55 °C annealing, 20 cycles) using the primers 5'-TGGTGCGGAGCCGCCG and 5'-CCACTGTCCGTGGCGCGACCCCGGCTCCTCGGCTCGG. Automated DNA sequencing used the primer 5'-CCACTGTCCGTGGCGCGACCC.

DNA sequences. Sequences not provided in the figures are as follows: matched reagent in Fig. 1b SIAB and SBAP reactions: 5'-CCCGAGTCGAAGTCGTACC-SH; mismatched reagent in Fig. 1b SIAB and SBAP reactions: 5'-GGGCTCAGCTTCCCCATAA-SH; mismatched reagents for other reactions in Figs. 1b, 1c, 1d, and 3a: 5'-FAAATCTTCCC-SH (F = tetrachlorofluorescein); reagents in Figs. 1c and 1d containing one mismatch: 5'-FAATTCTTACC-SH; E template in Figs. 1a, 1b SMCC, GMBS, BMPS, and SVSB reactions, and 3a: 5'-(NH_2dT)-GGTACGAATTCGACTCGGGAATACCACCTTCGACTCGAGG; H template in Fig. 1a: 5'-(NH_2dT)-CGCGAGCGTACGCTCGCGATGGTACGAATTCGACTCGGGAATACCACCTTCGACTCGAGG; H template in Fig. 1b SIAB, SBAP, and SIA reactions: 5'-(NH_2dT)-CGCGAGCGTACGCTCGCGATGGTACGAATTC; clamp oligonucleotide in Fig. 3b: 5'-ATTCGTACCA