Supporting Information

Aptamer Recognition of Multiplexed Small-Molecule-Functionalized Substrates

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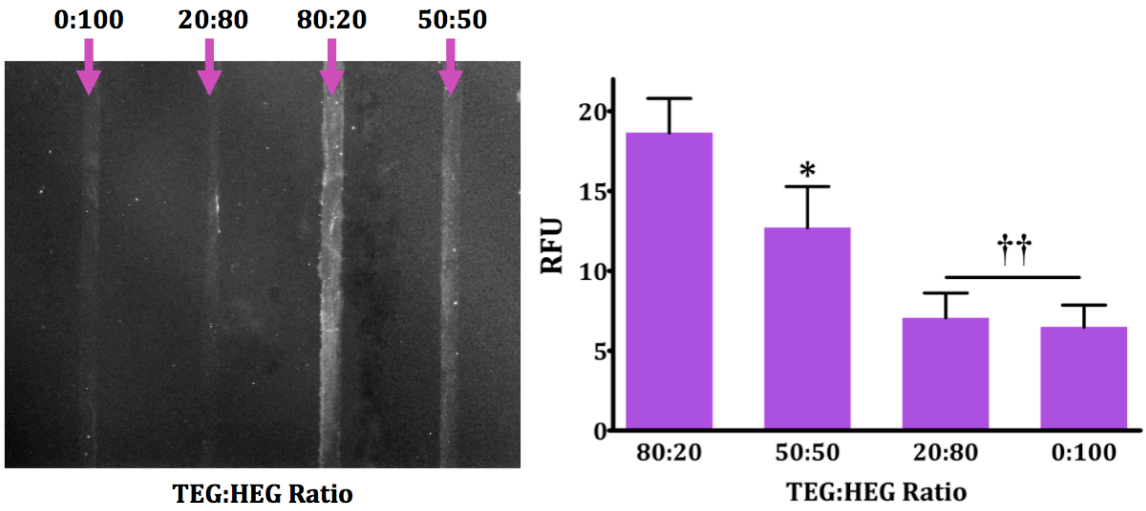

Figure S1. Optimizing surface-tethered small molecule densities. **(Left)** Different ratios of hydroxyl-terminated alkanethiols (background molecules; hydroxyl triethylene glycol (undecanethiol) (TEG)) *vs.* carboxyl-terminated alkanethiols (small-molecule tethers; carboxyl hexa(ethylene glycol) undecanethiol (HEG)) were incubated in each channel. Representative fluorescence image for dopamine aptamer recognition of dopamine functionalized channels. **(Right)** Maximal relative fluorescence intensities were observed for 80% TEG:20% HEG. This ratio was used for all subsequent experiments. Error bars are standard errors of the means for N=3 substrates. Group means were significantly different *P<0.05 and ††P<0.01 *vs.* the 80:20 TEG:HEG ratio.

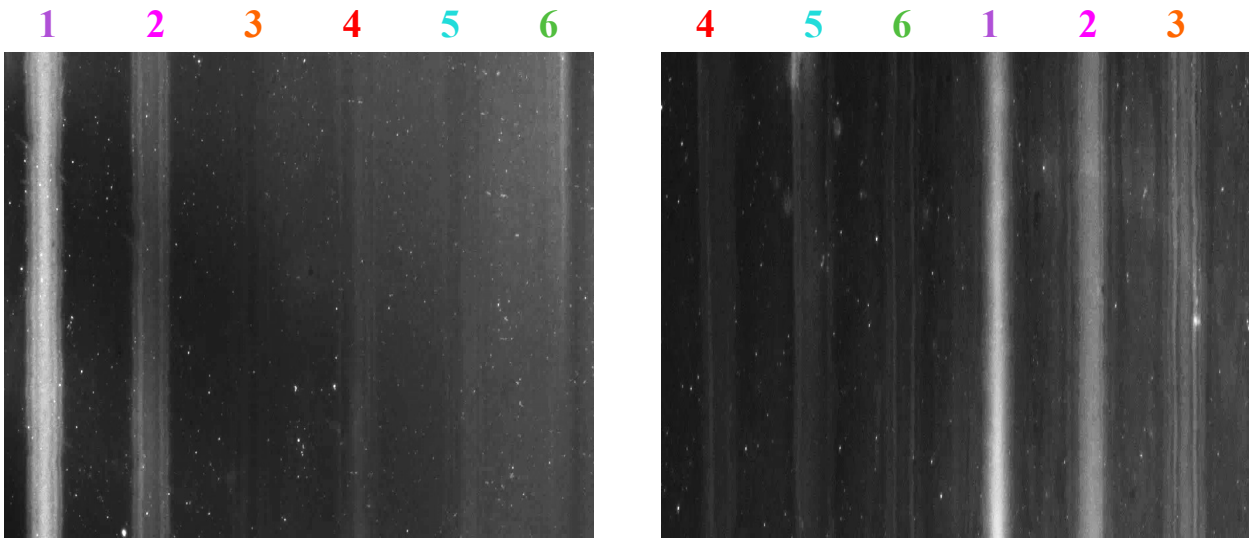

Figure S2. Representative fluorescence images for the competitive displacement experiment to investigate reversible binding of the dopamine (DA) aptamer to surface-tethered dopamine. A different concentration of free dopamine (0-50 µM) was added to each channel in different orders on different substrates. Binding was quantified for *N*=4 substrates in Figure 3A.

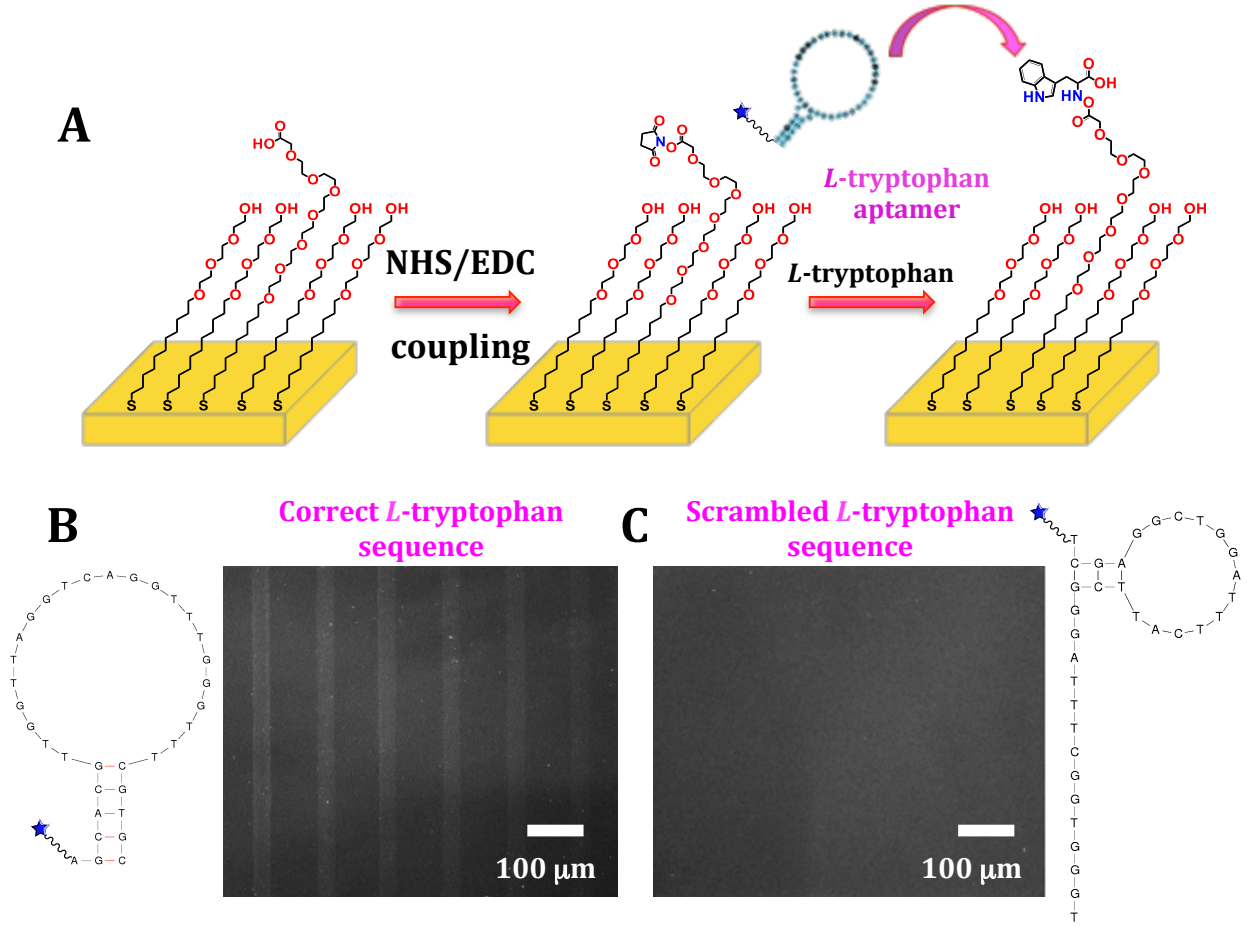

Figure S3. *L*-Tryptophan aptamer capture on patterned substrates. **(A)** Schematic (not to scale) of patterning and functionalization of *L*-tryptophan. **(B)** Substrates were incubated with the 34-base *L*-tryptophan-specific aptamer sequence or **(C)** a scrambled sequence with the same numbers of each nucleotide as the correct sequence but randomized to generate a different secondary structure. The secondary structure of the correct and scrambled sequences were generated using *Mfold.* Substrates were imaged at an emission wavelength of 525 nm for AlexaFluor® 488 (excitation at 490 nm).

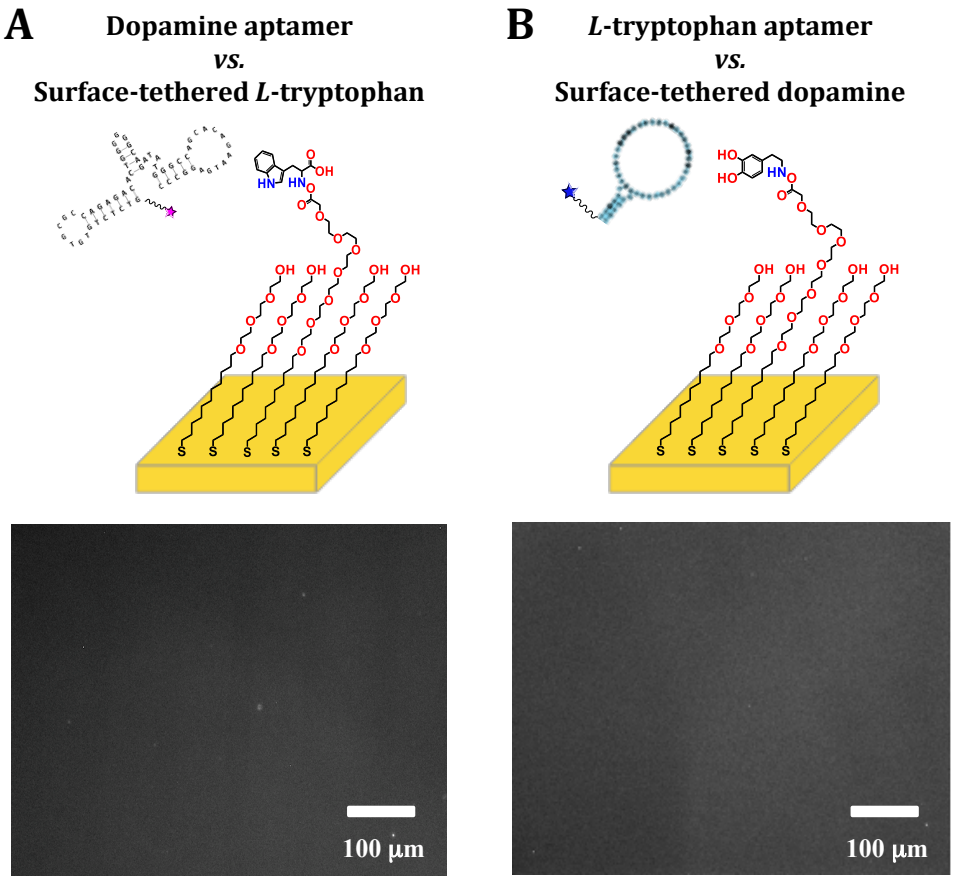

Figure S4. Selectivity of dopamine and *L*-tryptophan aptamers. **(A)** Patterned *L*-tryptophan-functionalized substrates imaged at an emission wavelength of 605 nm for AlexaFluor® 546 (excitation at 556 nm) to visualize bound dopamine aptamers. **(B)** Patterned dopamine-functionalized substrates imaged at an emission wavelength of 525 nm for AlexaFluor® 488 (excitation at 490 nm) to visualize bound *L*-tryptophan aptamers. In both cases, no observable patterns were detected indicating minimal cross-reactivity for the incorrect targets.