Supplemental Information

Characterizing the Incorporation of DNA into Single NIPAm Hydrogel Nanoparticles with Surface Plasmon Resonance Imaging Measurements

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I. Fluorescence Loss Measurements

Fluorescence spectra were obtained using a JASCO FP-6300 Spectrofluorometer (JASCO Analytical Instruments, Easton, MD). For SYBR Green staining experiments, parallel solutions of a 1 to 10 dilution of D1-HNPs were mixed with either 10 nM D1c or D1nc, and permitted to sit for 30 minutes. Following, SYBR Green was added to a final concentration of 0.003x, and allowed to sit for an additional 30 minutes. To remove excess unbound ssDNA or SYBR Green, Amicon Ultra – 0.5 mL Centrifugal Filter Devices 50k NMWL (MilliporeSigma, Burlington, MA) were used. Filters were pre-rinsed with buffer and centrifuged at 14,000 × g for 15 minutes. 200 µL of D1-HNP mixtures were then added to the filters, and centrifuged three times at 14,000 × g for 15 minutes. Each wash cycle was resuspended to 200 µL total volume, and D1-HNPs were recovered from the filters by spinning at 1,000 × g for 2 minutes after the final wash. The collected mixture was brought to a total volume of 200 µL, and fluorescence measured. For fluorescence loss measurements, a standard curve was made using D1c fluorescently labeled DNA (D1c*) from 0 to 10 nM. Then, three 10 nM D1c*, 1 to 10 dilution of D1-HNPs mixtures were prepared and brought to a final volume of 1 mL. After half an hour, the mixtures were spun at 14,000 rpm for 15 minutes to create a HNP pellet at the bottom of the vial. The supernatant was removed, and measured.

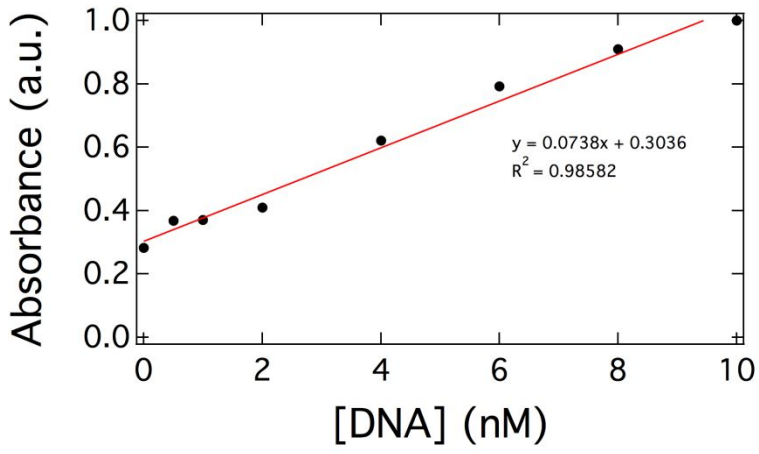

Figure S1. A fluorescence curve was plotted to calculate the fluorescence of the supernatant. Approximately 35% of fluorescently labeled DNA was hybridized into D1-HNP, equal to 3.47 nM of accessible ssDNA within the HNP.

II. Statistical Analysis of SPRI Microscopy of Complementary DNA Hybridization

Table S1. SPRI Microscopy for D1c Binging to D1-HNPs

D1c Concentration (nM)	$<\Delta\%R_{NP}>$	**Standard Deviation (s)**	**95% CI**	**# of NPs**
0.1	5.60	1.84	0.22	276
1	5.94	2.20	0.26	282
3	6.75	2.60	0.30	414
5	7.02	3.11	0.35	304
10	6.98	2.40	0.27	307
100	7.11	2.20	0.25	314