Supporting Information

Measuring Protein Binding to Individual Hydrogel Nanoparticles with Single-Nanoparticle Surface Plasmon Resonance Imaging Microscopy

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Table of Contents

I. Digital Adsorption Curves for mHNP SPRI Adsorption Experiments

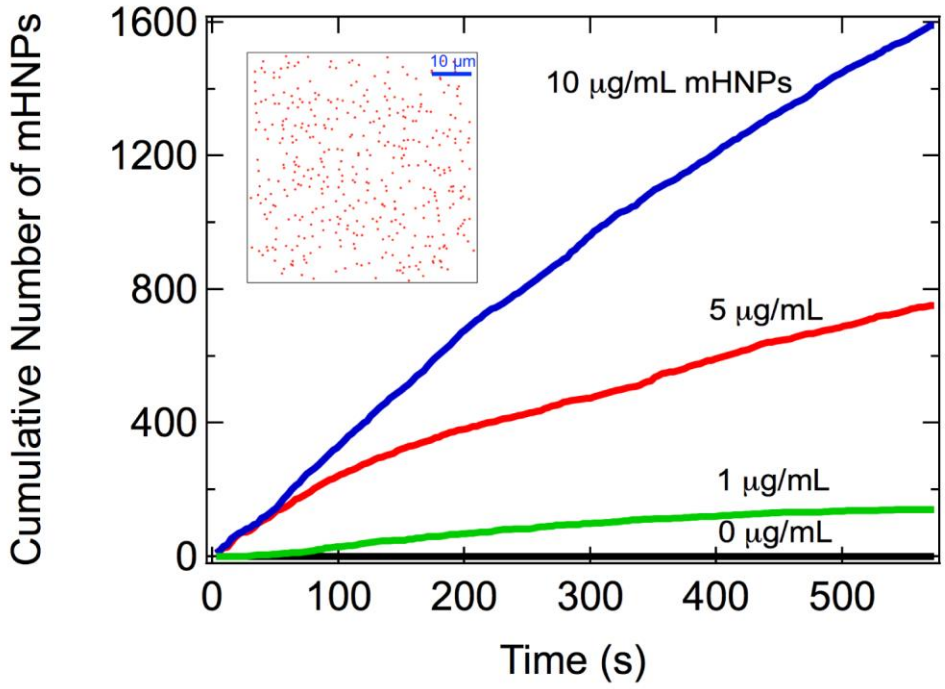

Figure S1. Real-time digital adsorption curves depicting the cumulative number of mHNPs irreversibly adsorbing to the hydrophobic C11 surface. Concentrations of 10 μg/mL, 5 μg/mL, and 1 μg/mL were measured in 10-minute experiments. Additionally, a control with no mHNPs (0 μg/mL) was measured. The inset shows the digital adsorption map for mHNPs at a concentration of 5 μg/mL. Each red dot represents a single mHNP adsorption event.

II. Statistical Analysis of SPRI Microscopy and DLS Experiments

Table S-1. SPRI Microscopy and DLS data for Con A binding to mHNPs.

Con A Concentration (nM)	$<\Delta\%R_{NP}>$	Standard deviation ($\Delta\%R_{NP}$)	95% Confidence interval ($\Delta\%R_{NP}$)	N	d_{DLS} (nm)	Standard deviation (nm)
0	0.51	0.18	0.02	395	178.8	7.0
5	0.50	0.22	0.02	340	180.0	4.2
50	0.64	0.23	0.02	506	185.2	2.5
100	0.94	0.49	0.06	258	212.3	2.8
200	1.29	0.77	0.09	321	274.1	6.8
300	1.3	0.85	0.1	249	334.1	5.1
500	1.31	0.80	0.08	407	345.7	5.6
700	1.4	1.00	0.1	384	328.0	6.4
1000	1.4	0.81	0.1	266	323.4	7.1

III. Con A-Mannose Control Experiments

HNPs without the mannose monomer were synthesized in order to confirm the specificity of the mannose- Con A interaction and thus ensure that Con A does not interact with the HNPs if no mannose is present in the nanoparticle. HNPs were synthesized following the same procedure as the mHNP synthesis with the following monomer ratio: NIPAm (65 mol %), TBAm (20 mol %), AAc (5 mol %), and BIS (10 mol %). As expected, we saw no change in the SPRI microscopy signal when 1 μM Con A was mixed with HNPs, compared to the SPRI microscopy signal of HNPs without Con A (Figure S2).

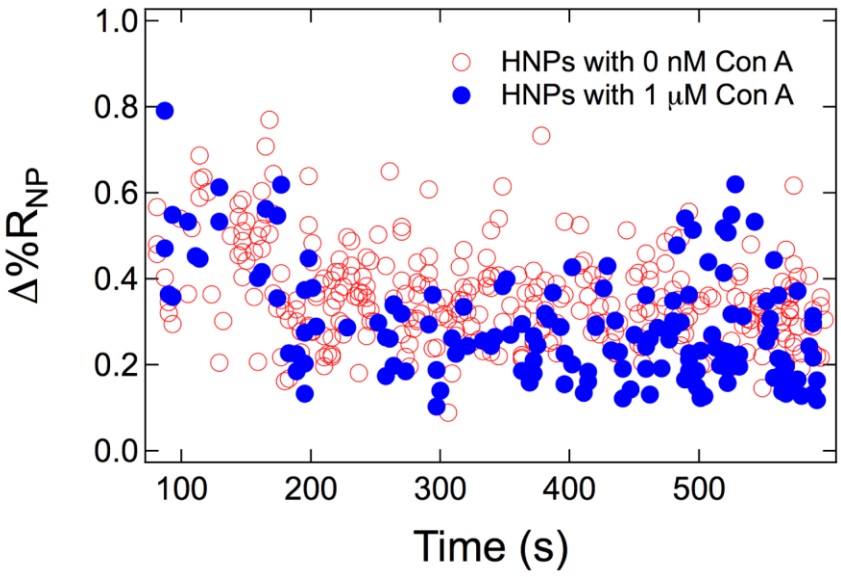

Figure S2. HNPs were synthesized without the mannose monomer in order to confirm Con A does not interact with HNPs without mannose incorporated into the polymer. SPRI microscopy measurements were performed and plotted here are the time-dependent distribution of $\Delta\%R_{NP}$ values for HNPs without Con A (0 nM, open red circles) and in the presence of 1 μM Con A (solid blue circles). For both experiments, all $\Delta\%R_{NP}$ values fall below 1%.