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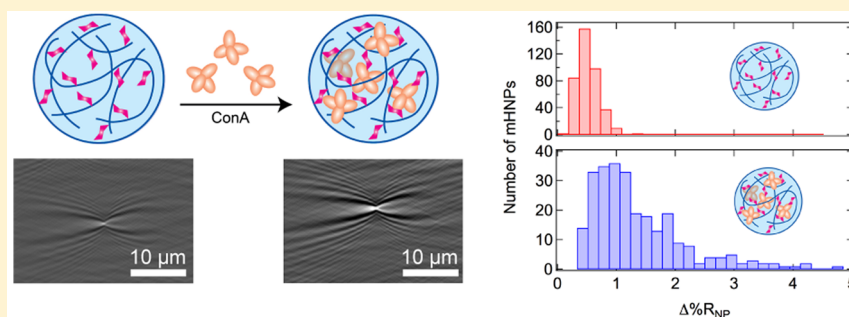
Measuring Protein Binding to Individual Hydrogel Nanoparticles with Single-Nanoparticle Surface Plasmon Resonance Imaging Microscopy

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S Supporting Information



ABSTRACT: The specific binding and uptake of protein molecules to individual hydrogel nanoparticles is measured with real-time single-nanoparticle surface plasmon resonance imaging (SPRI) microscopy. Nanoparticles that adsorb onto chemically modified gold thin films interact with traveling surface plasmon polaritons and create individual point diffraction patterns in the SPRI microscopy differential reflectivity images. The intensity of each point diffraction pattern depends on the integrated refractive index of the nanoparticle; an increase in this single nanoparticle point diffraction intensity ($\Delta\%R_{\text{NP}}$) is observed for nanoparticles that bind proteins. SPRI adsorption measurements can be used to measure an average increase in $\Delta\%R_{\text{NP}}$ that can be correlated with bulk dynamic light scattering measurements. Moreover, the distribution of $\Delta\%R_{\text{NP}}$ values observed for individual nanoparticles can be used to learn more about the nature of the protein–nanoparticle interaction. As a first example, the binding of the lectin Concanavalin A to 180 nm *N*-isopropylacrylamide hydrogel nanoparticles that incorporate a small percentage of mannose sugar monomer units is characterized.

INTRODUCTION

Hydrogel nanoparticles (HNPs) are unique synthetic nanomaterials that can incorporate various chemical functionalities specifically designed to capture and release proteins, peptides, or other small molecules. These capabilities have led to a significant interest in the potential use of HNPs in biomedical applications such as targeted drug delivery, medical diagnostics, and biosensing.^{1–6} For example, NIPAm-based (*N*-isopropylacrylamide) HNPs have been utilized for detection of various biomolecules, such as DNA,^{7,8} proteins,^{9–11} and other biologically relevant small molecules.^{12,13} Additionally, the specific uptake of proteins into HNPs can also be used as a model system for studying various biological phenomena such as multivalent lectin–carbohydrate interactions.^{14–20} For all of these applications, it is essential that the uptake of proteins into individual nanoparticles be quantitated and analyzed. For the case of fluorescently labeled proteins, single nanoparticle fluorescence imaging can be used to monitor affinity uptake into single HNPs.^{21–23} For nonfluorescent proteins, the

average particle size and molecular weight of HNPs can be obtained by a combination of dynamic light scattering (DLS)^{24,25} and multiangle light scattering (MALS),²⁶ but measurements of single HNPs are more difficult, typically requiring methods such as cryo-TEM or atomic force microscopy.^{27–29}

Recently, we have demonstrated that real-time single-nanoparticle surface plasmon resonance imaging (SPRI) microscopy can be used to detect single HNPs *in situ* and quantitatively monitor the specific uptake of nonfluorescent biomolecules into the individual nanoparticles.³⁰ SPRI microscopy has been used previously to study single metallic nanoparticles, membrane proteins, cells, and viruses;^{31–42} an example of the SPRI microscopy experimental setup is shown in Figure 1a. When a nanoparticle adsorbs onto a chemically

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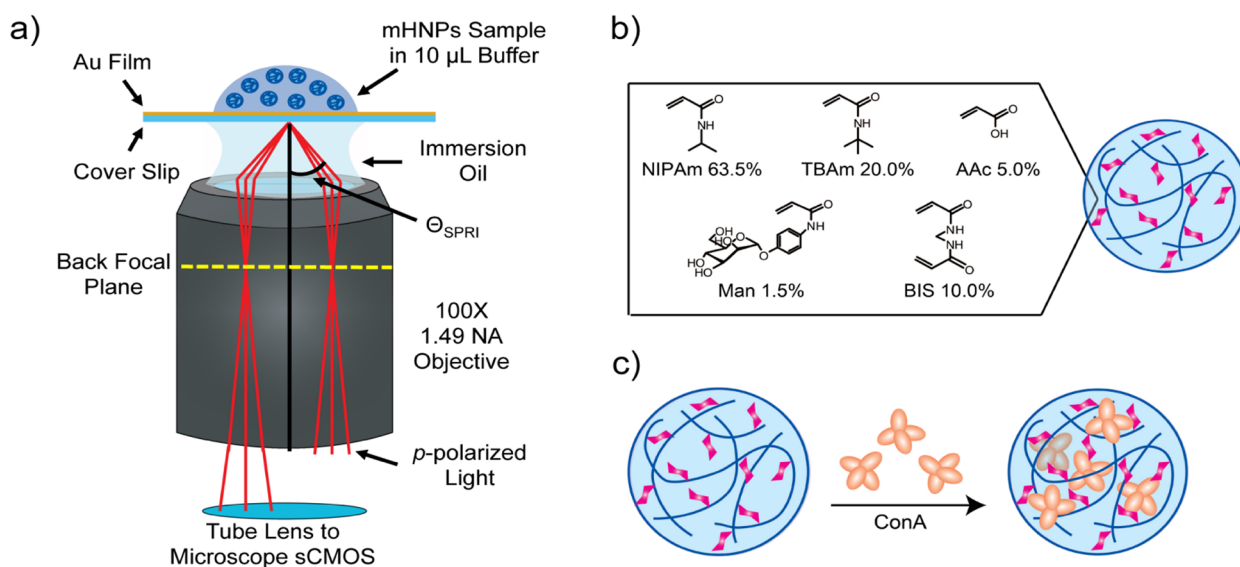


Figure 1. (a) Schematic diagram of the SPRI microscope. A knife-edge mirror was used to send collimated p -polarized light through the objective at the SPRI angle of 30% reflectivity. The reflected images were collected by the sCMOS camera. (b) Mannose-incorporated hydrogel nanoparticles (mHNPs) were synthesized from N -isopropylacrylamide (NIPAm, 63.5 mol %), N -*tert*-butylacrylamide (TBAm, 20 mol %), acrylic acid (AAc, 5 mol %), N,N' -methylenebis(acrylamide) (BIS, 10 mol %), and p -acrylamidophenyl- α -D-mannopyranoside (Man, 1.5 mol %). (c) The uptake of Concanavalin A (Con A) into mHNPs was monitored by SPRI microscopy. Con A specifically binds to mannose sugar units (pink) in the mHNPs.

54 modified gold thin film from solution, a large point diffraction
 55 pattern in the SPRI microscopy image is created from the
 56 interaction between the nanoparticle and the traveling surface
 57 plasmon polaritons. This single-nanoparticle point diffraction
 58 intensity can be quantitated and is expressed as a change in
 59 percent reflectivity ($\Delta\%R_{NP}$).^{30,41} The value of $\Delta\%R_{NP}$ for a
 60 single nanoparticle depends on its integrated refractive index
 61 and thus, in the case of HNPs, on the amount of protein
 62 adsorbed and incorporated to the nanoparticle. In our recent
 63 paper, we synthesized NIPAm-based HNPs with specific
 64 affinity for the peptide melittin.³⁰ SPRI microscopy was then
 65 used to quantify the average uptake of melittin into these HNPs
 66 by calculating average $\Delta\%R_{NP}$ values from individual HNPs.
 67 We showed that although the average HNP size (as measured
 68 by DLS) did not change with melittin concentration the
 69 average $\Delta\%R_{NP}$ varied linearly due to melittin uptake into the
 70 HNPs.

71 In this paper, we extend our use of single-nanoparticle SPRI
 72 microscopy to monitor the specific adsorption and uptake of
 73 proteins to individual HNPs. We have synthesized NIPAm-
 74 based HNPs that incorporate a small percentage of monomers
 75 modified with mannose sugar units into the hydrogel polymer
 76 as shown in Figure 1b. We then used SPRI microscopy to
 77 monitor the interactions of the lectin Concanavalin A (Con A)
 78 to these mannose-incorporated HNPs (mHNPs), shown in
 79 Figure 1c. Both the average and distribution of $\Delta\%R_{NP}$ values
 80 for single mHNPs in the presence of Con A were quantitated;
 81 an increase in the average $\Delta\%R_{NP}$ due to the combination of
 82 Con A binding to the mHNPs and Con A-induced specific
 83 aggregation of mHNPs was observed for solutions up to 200
 84 nM. We also found that the interaction of Con A with the
 85 mHNPs led to a significant increase in the distribution of $\Delta\%$
 86 R_{NP} values that we attribute to variations of mannose sugar unit
 87 availability for Con A binding in individual mHNPs. At Con A
 88 concentrations above 200 nM, a saturation of binding and
 89 mHNP aggregation led to an observed leveling off of the $\Delta\%$
 90 R_{NP} values for the single mHNPs.

EXPERIMENTAL METHODS

91

Hydrogel Nanoparticle Materials. NIPAm, acrylic acid 92
 (AAc), sodium dodecyl sulfate (SDS), and V-501 were 93
 obtained from Sigma-Aldrich, Inc. (St. Louis, MO). N,N' - 94
 Methylenebis(acrylamide) (BIS) was obtained from Fluka (St. 95
 Louis, MO). N -*tert*-Butylacrylamide (TBAm) was obtained 96
 from Acros Organics (Geel, Belgium). NIPAm was recrystal- 97
 lized from hexane before use. All other chemicals were used as 98
 received. 99

Hydrogel Nanoparticle Synthesis. The sugar unit p - 100
 acrylamidophenyl- α -D-mannopyranoside (Man) was synthe- 101
 sized using methods reported previously.^{24,43} mHNP synthesis 102
 was adapted from previous HNP synthesis methods.^{44,45} The 103
 monomers NIPAm (63.5 mol %), TBAm (20 mol %), AAc (5 104
 mol %), BIS (10 mol %), and Man (1.5 mol %) and 2.5 mg (8.7 105
 mmol) of SDS were dissolved in 50 mL of nanopure water for a 106
 total monomer concentration of 65 mM. TBAm was dissolved 107
 in 1 mL of ethanol before addition to nanopure water. Nitrogen 108
 gas was bubbled through the mixture for 30 min. Following the 109
 addition of V-501 (131.3 μ mol/0.5 mL of DMSO), the 110
 polymerization was carried out in an oil bath at 70 $^{\circ}$ C for 3 h 111
 under a nitrogen atmosphere. The resulting solution was 112
 purified by dialysis using a 12–14 kDa molecular weight cut off 113
 dialysis membrane against an excess amount of nanopure water 114
 (changed more than 3 times a day) for 4 days. The yield and 115
 concentration of HNPs was obtained by gravimetric analysis of 116
 lyophilized polymers. The hydrodynamic diameter of mHNPs 117
 was determined in 1X PBS at 25 $^{\circ}$ C using DLS equipped with 118
 Zetasizer Software (Zetasizer Nano ZS, Malvern Instruments 119
 Ltd., Worcestershire, U.K.). 120

Substrate Preparation. The Au substrates were coated by 121
 thermal vapor deposition of a 1 nm Cr adhesion layer and 45 122
 nm Au onto Borosilicate No. 1.5 coverslips (Fisherbrand, 123
 Pittsburgh, PA). The Au surface was immobilized with 1- 124
 undecanethiol (C11) by immersing the Au substrate into a 1 125
 mM C11/EtOH solution. The Au surface was partitioned using 126

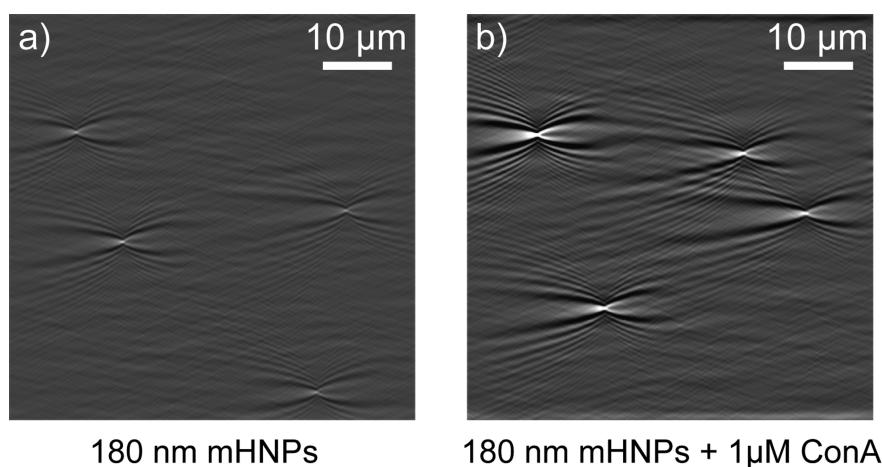


Figure 2. SPRI microscopy differential reflectivity images of (a) 180 nm mHNPs without Con A and (b) 180 nm mHNPs in the presence of 1 μM Con A. Each image is $58.5 \mu\text{m} \times 58.5 \mu\text{m}$.

127 adhesive silicone isolation wells (Electron Microscopy Sciences,
128 Hafield, PA).

129 **SPRI Microscopy Measurements.** The SPRI microscope
130 setup was described in a recent publication.⁴¹ Briefly, the
131 microscope was built into the frame of an IX51 inverted
132 microscope (Olympus, Tokyo, Japan). A 1 mW near-infrared
133 (814 nm) diode laser (Melles Griot, Carlsbad, CA) was
134 expanded and collimated using a spatial filter (Newport Corp.,
135 Newport Beach, CA). The beam was polarized and then
136 focused with a lens ($f = 200 \text{ mm}$). The beam was directed onto
137 the back focal plane of a $100\times$ 1.49 high numerical aperture
138 objective (Olympus) with a gold-coated knife-edge mirror
139 (Thorlabs, Newton, NJ). The reflected image was passed to an
140 Andor Neo sCMOS camera (South Windsor, CT). Each three-
141 second reflectivity image was acquired by accumulating 30 11-
142 bit, 0.1 s exposures.

143 Solutions of mHNPs were diluted in 1X PBS (11.9 mM
144 phosphates, 13 mM sodium chloride, 2.7 mM potassium
145 chloride, pH 7.4, Fisher) to concentrations specified in the
146 experiments. Solutions of Concanavalin A (Sigma-Aldrich)
147 were prepared in 1X PBS before mixing with mHNPs. SPRI
148 microscopy experiments were performed after mixing mHNPs
149 and Con A typically for 12 h at room temperature, though
150 incubation times as short as 1 h worked equally well. For each
151 experiment, 10 μL of mHNP solution was pipetted into the
152 isolation well immediately preceding the image acquisition
153 process.

154 ■ RESULTS AND DISCUSSION

155 To quantitate the binding of the lectin Con A to hydrophobic
156 mHNPs, real-time SPRI microscopy was used to characterize
157 the irreversible adsorption of single mHNPs, in both the
158 presence and absence of Con A, onto gold films functionalized
159 with hydrophobic undecanethiol (C11) monolayers. In each
160 SPRI adsorption measurement, SPRI microscopy reflectivity
161 images were recorded every three seconds for a total time of 10
162 min, and then the images were sequentially subtracted from
163 one another in order to obtain a series of differential reflectivity
164 images. Figure 2 shows two typical SPRI microscopy differential
165 reflectivity images that were obtained during the adsorption of
166 mHNPs from different solutions: first, a solution of mHNPs (5
167 $\mu\text{g}/\text{mL}$) in the absence of Con A (Figure 2a), and second, a
168 solution of mHNPs (5 $\mu\text{g}/\text{mL}$) in the presence of 1 μM Con A

(Figure 2b). As demonstrated previously, the adsorption of a
single mHNP appears in the SPRI microscopy differential
reflectivity image as a point diffraction pattern. These patterns
are due to the interaction of the mHNPs with the traveling
surface plasmon polariton waves. Each image in Figure 2 shows
four distinct point diffraction patterns (each spanning an area of
at least $30 \mu\text{m} \times 10 \mu\text{m}$) that indicate the irreversible
adsorption of four individual mHNPs onto the surface during
these particular three-second time periods. As seen in Figure
2b, when Con A is present with the mHNPs, more intense
point diffraction patterns are observed. We attribute this change
in intensity to an increase in the refractive index of the mHNPs
due to the various interactions (adsorption, uptake, and
induced aggregation) of Con A with the mHNPs.

In order to quantify the observed increase in the individual
mHNP point diffraction intensities in the presence of Con A,
hundreds of point diffraction patterns from every SPRI
adsorption measurement were analyzed. For every point
diffraction pattern, the percent change in reflectivity, $\Delta\%R_{\text{NP}}$,
was calculated from the region of maximum diffraction intensity
in the image. We have used the same analysis method for
calculating the $\Delta\%R_{\text{NP}}$ values as described in our previous
publication.³⁰ Approximately 400 $\Delta\%R_{\text{NP}}$ values were obtained
from each SPRI adsorption experiment (the cumulative number
of adsorbed nanoparticles is plotted as a function of time for
SPRI adsorption measurements at several mHNP concen-
trations in the Supporting Information). Figure 3 plots all of
the individual $\Delta\%R_{\text{NP}}$ values measured during two different
SPRI adsorption experiments: one experiment of mHNP
without Con A (0 nM, open red circles) and the other
experiment of mHNPs with 1 μM Con A (solid blue circles). It
is readily apparent from the data in Figure 3 that the binding of
Con A to mHNPs greatly increased the range of the individual
 $\Delta\%R_{\text{NP}}$ values; some point diffraction patterns had $\Delta\%R_{\text{NP}}$
values as large as 5%. In the absence of Con A, almost all of the
 $\Delta\%R_{\text{NP}}$ values were less than or equal to 1%. This increase in
the range of $\Delta\%R_{\text{NP}}$ values can also be seen in Figure 4, which
plots histograms representing the distribution of $\Delta\%R_{\text{NP}}$ values
obtained in the presence of 0 nM, 100 nM, and 1 μM Con A.
Additionally, the average $\Delta\%R_{\text{NP}}$ value for each experiment is
plotted as a dotted black line in each histogram. This average
 $\Delta\%R_{\text{NP}}$, denoted as $\langle\Delta\%R_{\text{NP}}\rangle$, increased from a value of $0.51 \pm$
 0.02% for mHNPs without Con A present to a value of $1.4 \pm$

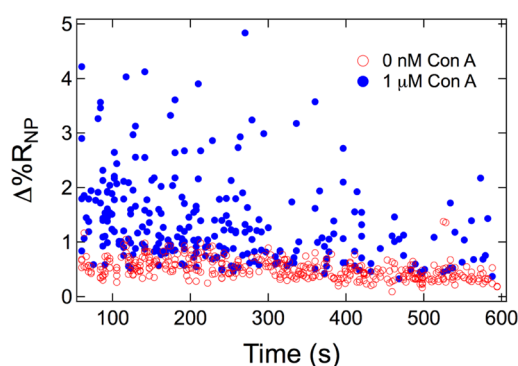


Figure 3. Time-dependent distribution of $\Delta\%R_{\text{NP}}$ values for mHNPs without Con A (0 nM, open red circles) and in the presence of 1 μM Con A (solid blue circles). Each circle represents the $\Delta\%R_{\text{NP}}$ for a single mHNP irreversibly adsorbing to the C11-functionalized surface.

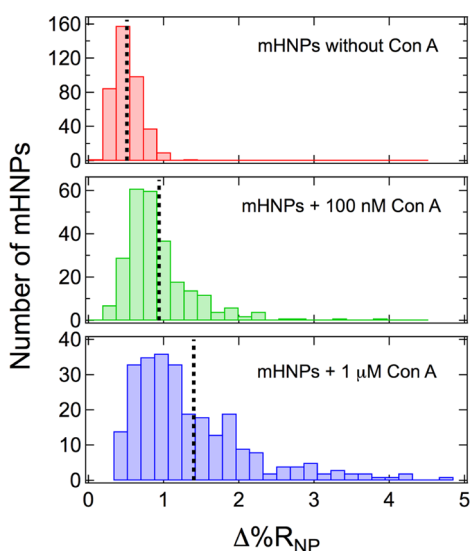


Figure 4. Distributions of $\Delta\%R_{\text{NP}}$ values for mHNPs mixed with no Con A (top), 100 nM Con A (middle), and 1 μM Con A (bottom), all plotted as histograms. The average $\Delta\%R_{\text{NP}}$ value for each experiment is plotted as a black dotted line in each histogram. For mHNPs mixed with no Con A, 100 nM Con A, and 1 μM Con A, average $\Delta\%R_{\text{NP}}$ values are $0.51 \pm 0.02\%$, $0.94 \pm 0.06\%$, and $1.4 \pm 0.1\%$, respectively.

212 0.1% for mHNPs in the presence of 1 μM Con A. The error
 213 bars stated in this paper are the 95% confidence intervals, $\pm 2\sigma/
 214 (N)^{1/2}$, where σ is the standard deviation and N is the number
 215 of mHNPs measured in the SPRI microscopy experiment. A
 216 complete table of statistical data for these experiments is
 217 available in the [Supporting Information](#). In order to confirm the
 218 specificity of the Con A binding to mHNPs, HNP with no
 219 incorporated mannose units were mixed with Con A. No
 220 change in $\langle\Delta\%R_{\text{NP}}\rangle$ or the distribution of $\Delta\%R_{\text{NP}}$ values was
 221 found compared to HNP in the absence of Con A (see
 222 [Supporting Information](#)).

223 The observed increase in $\langle\Delta\%R_{\text{NP}}\rangle$ in the presence of Con A
 224 is further examined in [Figure 5](#), which plots the $\langle\Delta\%R_{\text{NP}}\rangle$
 225 values as a function of Con A concentration. The $\langle\Delta\%R_{\text{NP}}\rangle$
 226 values increase linearly from 0.51% to 1.4% at low Con A
 227 concentrations but then level off and do not change at
 228 concentrations above 200 nM. The highest concentration of
 229 Con A used in these experiments was 1 μM because Con A
 230 precipitated out of solution at concentrations above 1 μM .⁴⁶
 231 We attribute this increase in $\langle\Delta\%R_{\text{NP}}\rangle$ to two effects: first, an

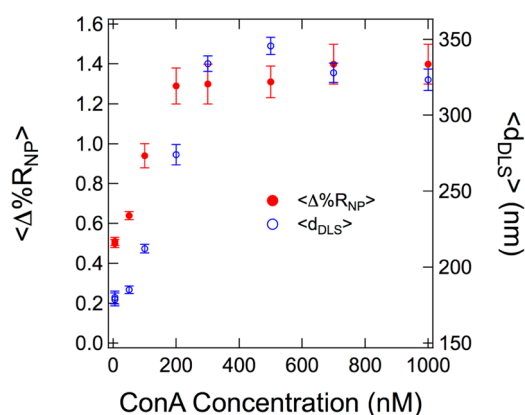


Figure 5. Average $\Delta\%R_{\text{NP}}$ values (solid red circles) from SPRI adsorption measurements and average hydrodynamic diameters (open blue circles) from DLS measurements for mHNPs mixed with varying concentrations of Con A, both plotted as a function of Con A concentration. Error bars are the 95% confidence intervals for the average $\Delta\%R_{\text{NP}}$ values and are the standard deviations for the average hydrodynamic diameters.

increase in the refractive index of the mHNPs due to the 232
 binding of Con A to the mannose in the mHNPs, and second, 233
 the specific aggregation of mHNPs induced by the presence of 234
 Con A. The lectin Con A contains four mannose binding sites, 235
 and thus mHNP aggregation can occur when Con A molecules 236
 that are attached to the outer portions of the mHNP cross-link 237
 by binding to more than one mHNP. The specific aggregation 238
 of sugar-modified nanoparticles due to Con A cross-linking has 239
 been reported previously.^{47–49} In the presence of Con A, a 240
 Poisson distribution of the $\Delta\%R_{\text{NP}}$ values for individual 241
 mHNPs was not observed, further confirming that the changes 242
 in $\Delta\%R_{\text{NP}}$ values are due to a combination of both Con A 243
 binding to mHNPs and the specific Con A-induced 244
 aggregation. Above Con A concentrations of 200 nM, the 245
 observed leveling off of $\langle\Delta\%R_{\text{NP}}\rangle$ values is attributed to a 246
 saturation in both Con A binding and Con A-induced mHNP 247
 aggregation. 248

The measured increase of $\langle\Delta\%R_{\text{NP}}\rangle$ with Con A concen- 249
 tration can be compared with bulk DLS measurements in order 250
 to further characterize the Con A uptake process. The average 251
 hydrodynamic diameter of the nanoparticles, d_{DLS} , is also 252
 plotted as a function of Con A concentration in [Figure 5](#) and is 253
 found to increase in a similar manner as $\langle\Delta\%R_{\text{NP}}\rangle$. This 254
 observation is different from the results reported previously for 255
 the study of melittin uptake into HNP.³⁰ For those 256
 measurements, a linear increase in $\langle\Delta\%R_{\text{NP}}\rangle$ was observed for 257
 SPRI adsorption measurements in solutions with melittin 258
 concentrations between 0 μM and 2.5 μM , but no changes were 259
 observed in the d_{DLS} . In order to explain the data in [Figure 5](#) for 260
 Con A binding to mHNPs, we conclude that the 104 kDa 261
 protein Con A, unlike the smaller peptide melittin, is unable to 262
 access the interior mannose groups of the hydrogel polymer 263
 and thus binds primarily to the outer regions of the mHNP. In 264
 addition, Con A can induce cross-linked aggregation by binding 265
 to mannose groups on more than one mHNP. Both the binding 266
 of Con A to the outer regions of the mHNPs and the specific 267
 aggregation of mHNPs induced by Con A cross-linking will 268
 lead to an increase in both $\langle\Delta\%R_{\text{NP}}\rangle$ and d_{DLS} . 269

Finally, in addition to changes in $\langle\Delta\%R_{\text{NP}}\rangle$ in the presence of 270
 Con A, the changes in the distribution of $\Delta\%R_{\text{NP}}$ values in the 271
 presence of Con A can be used to learn more about the lectin– 272

nanoparticle interactions. As seen in Figures 3 and 4, for mHNPs without Con A, the $\Delta\%R_{NP}$ values are tightly distributed in a range between 0.1 and 1.0%. However, this range of $\Delta\%R_{NP}$ values greatly expands in the presence of Con A, with $\Delta\%R_{NP}$ values as large as 5% at high Con A concentrations. In order to quantitate this expansion, we have arbitrarily divided the histogram distributions into the three subsets (labeled A, B, and C) as shown as an example in Figure 6a. These subsets include mHNPs with $\Delta\%R_{NP}$ values in the

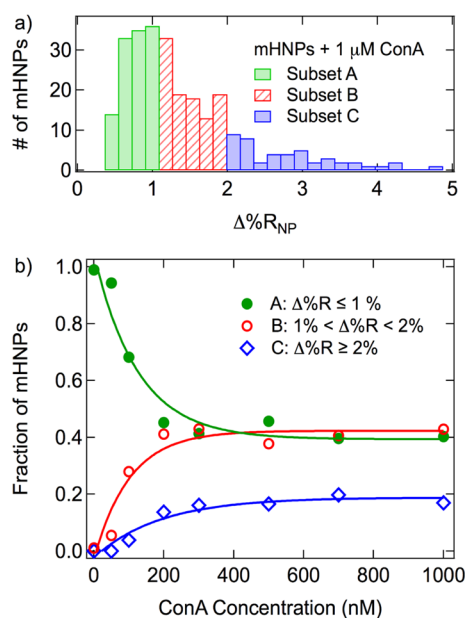


Figure 6. MHNPs were divided into three subsets based on their $\Delta\%R_{NP}$ values. (a) As an example, the histogram for mHNPs mixed with $1\ \mu\text{M}$ Con A is shown again with the mHNPs divided into the three subsets. Subset A (green) contains all mHNPs with $\Delta\%R_{NP} \leq 1\%$; subset B (red) contains all mHNPs with $\Delta\%R_{NP}$ between 1 and 2%; and subset C (blue) contains all mHNPs with $\Delta\%R_{NP} \geq 2\%$. (b) The fraction of mHNPs in each subset is plotted as a function of Con A concentration. The saturation point for each curve is 200 nM.

ranges of 0 to 1% (subset A), 1 to 2% (subset B), and above 2% (subset C). The fraction of mHNPs in each subset is plotted as a function of Con A concentration in Figure 6b. In the absence of Con A, nearly 100% of the mHNPs are in subset A ($\Delta\%R_{NP} \leq 1\%$). As Con A concentration increases, the number of mHNPs in subset A decreases, while the number of mHNPs in subsets B and C increases. The interaction of Con A with the mHNPs is strong: at a 200 nM Con A concentration, 60% of the mHNPs have a $\Delta\%R_{NP}$ value that is higher than the range of the values observed when no Con A was present (40% in subset B and 20% in subset C). Both the binding of multiple Con A molecules to mHNPs and the resulting specific aggregation of mHNPs substantially change the refractive index of individual mHNPs and thus the SPRI microscopy response. For example, the mHNPs in subset C have point diffraction intensities 6 to 10 times larger compared to the $\langle\Delta\%R_{NP}\rangle$ for mHNPs in the absence of Con A. These larger point diffraction intensities are most likely due to the adsorption of multiple aggregated mHNPs. Additionally, no changes in subset populations are seen at concentrations above 200 nM, suggesting that both the binding of Con A and the Con A-induced aggregation of mHNPs saturated at this point.

CONCLUSIONS AND FUTURE DIRECTIONS

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In this paper we have demonstrated how real-time single-nanoparticle SPRI microscopy can be used to characterize the binding of the lectin Con A to mannose-incorporated HNPs. By measuring both the average magnitude and distribution of the single-point diffraction pattern intensities for mHNPs as a function of Con A concentration, we observed a significant binding of Con A to mHNPs that varied substantially from particle to particle. The SPRI microscopy data were used in conjunction with the observation of a concomitant increase in d_{DLS} as a function of Con A concentration. This allowed us to develop a model in which Con A protein primarily bound to the mannose units on the outer portions of the mHNPs and also induced aggregation of mHNPs by cross-linking with mannose units on multiple mHNPs. This Con A binding and mHNP aggregation process saturated at a Con A concentration of 200 nM. A large increase in the distribution of individual $\Delta\%R_{NP}$ values is observed and is attributed to a combination of mannose availability in mHNPs and Con A-induced aggregation of the mHNPs. Because the mHNPs both adsorb Con A and aggregate in its presence, the binding affinity between individual Con A molecules and mannose groups incorporated into the mHNPs cannot be determined from the SPRI data alone. In the future, we will apply our single-nanoparticle SPRI adsorption measurements to NIPAm-based HNPs that incorporate multiple types of sugar monomers in order to characterize the specificity, binding strength, and multivalency of other lectin-carbohydrate interactions.

ASSOCIATED CONTENT

332

Supporting Information

333

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpcc.6b05700.

Cumulative adsorption curves for SPRI adsorption measurements, statistical data for SPRI adsorption and DLS measurements, and mHNP control experiments (PDF)

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Author Contributions

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[§]These authors contributed equally.

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Notes

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The authors declare no competing financial interest.

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348

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REFERENCES

353

- (1) Hamidi, M.; Azadi, A.; Rafiei, P. Hydrogel Nanoparticles in Drug Delivery. *Adv. Drug Delivery Rev.* **2008**, *60*, 1638–1649.
- (2) Peppas, N. A.; Hilt, J. Z.; Khademhosseini, A.; Langer, R. Hydrogels in Biology and Medicine: From Molecular Principles to Bionanotechnology. *Adv. Mater.* **2006**, *18*, 1345–1360.
- (3) Nayak, S.; Lyon, L. A. Soft Nanotechnology with Soft Nanoparticles. *Angew. Chem., Int. Ed.* **2005**, *44*, 7686–7708.

360

- (4) Seliktar, D. Designing Cell-Compatible Hydrogels for Biomedical Applications. *Science* **2012**, *336*, 1124–1128.
- (5) Vermonden, T.; Censi, R.; Hennink, W. E. Hydrogels for Protein Delivery. *Chem. Rev.* **2012**, *112*, 2853–2888.
- (6) Karimi, M.; Ghasemi, A.; Sahandi Zangabad, P.; Rahighi, R.; Moosavi Basri, S. M.; Mirshekari, H.; Amiri, M.; Shafaei Pishabad, Z.; Aslani, A.; Bozorgomid, M.; et al. Smart Micro/Nanoparticles in Stimulus-Responsive Drug/Gene Delivery Systems. *Chem. Soc. Rev.* **2016**, *45*, 1457–1501.
- (7) Baeissa, A.; Dave, N.; Smith, B. D.; Liu, J. DNA-Functionalized Monolithic Hydrogels and Gold Nanoparticles for Colorimetric DNA Detection. *ACS Appl. Mater. Interfaces* **2010**, *2*, 3594–3600.
- (8) Li, J.; Mo, L.; Lu, C. H.; Fu, T.; Yang, H. H.; Tan, W. Functional Nucleic Acid-Based Hydrogels for Bioanalytical and Biomedical Applications. *Chem. Soc. Rev.* **2016**, *45*, 1410–1431.
- (9) Yonamine, Y.; Hoshino, Y.; Shea, K. J. ELISA-Mimic Screen for Synthetic Polymer Nanoparticles with High Affinity to Target Proteins. *Biomacromolecules* **2012**, *13*, 2952–2957.
- (10) Lifson, M. A.; Carter, J. A.; Miller, B. L. Functionalized Polymer Microgel Particles Enable Customizable Production of Label-Free Sensor Arrays. *Anal. Chem.* **2015**, *87*, 7887–7893.
- (11) Wang, Y. Z.; Li, D. Y.; He, X. W.; Li, W. Y.; Zhang, Y. K. Epitope Imprinted Polymer Nanoparticles Containing Fluorescent Quantum Dots for Specific Recognition of Human Serum Albumin. *Microchim. Acta* **2015**, *182*, 1465–1472.
- (12) Yan, L.; Zhu, Z.; Zou, Y.; Huang, Y.; Liu, D.; Jia, S.; Xu, D.; Wu, M.; Zhou, Y.; Zhou, S.; et al. Target-Responsive “Sweet” Hydrogel with Glucometer Readout for Portable and Quantitative Detection of Non-Glucose Targets. *J. Am. Chem. Soc.* **2013**, *135*, 3748–3751.
- (13) Zhao, H.; Jiang, G.; Weng, J.; Ma, Q.; Zhang, H.; Ito, Y.; Liu, M. A Signal-Accumulating DNzyme-Crosslinked Hydrogel for Colorimetric Sensing of Hydrogen Peroxide. *J. Mater. Chem. B* **2016**, *4*, 393–4648–4651.
- (14) Miura, Y. Synthesis and Biological Application of Glycopolymers. *J. Polym. Sci., Part A: Polym. Chem.* **2007**, *45*, 5031–5036.
- (15) Bernard, J.; Hao, X.; Davis, T. P.; Barner-Kowollik, C.; Stenzel, M. H. Synthesis of Various Glycopolymers Architectures via RAFT Polymerization: From Block Copolymers to Stars. *Biomacromolecules* **2006**, *7*, 232–238.
- (16) Ghadban, A.; Albertin, L. Synthesis of Glycopolymers Architectures by Reversible-Deactivation Radical Polymerization. *Polymers* **2013**, *5*, 431–526.
- (17) Chen, Y.; Espeel, P.; Reinicke, S.; Du Prez, F. E.; Stenzel, M. H. Control of Glycopolymers Nanoparticle Morphology by a One-Pot, Double Modification Procedure Using Thiolactones. *Macromol. Rapid Commun.* **2014**, *35*, 1128–1134.
- (18) Zhang, Q.; Collins, J.; Anastasaki, A.; Wallis, R.; Mitchell, D. A.; Becer, C. R.; Haddleton, D. M. Sequence-Controlled Multi-Block Glycopolymers to Inhibit DC-SIGN-gp120 Binding. *Angew. Chem., Int. Ed.* **2013**, *52*, 4435–4439.
- (19) Gou, Y.; Geng, J.; Richards, S. J.; Burns, J.; Remzi Becer, C.; Haddleton, D. M. A Detailed Study on Understanding Glycopolymers Library and Con A Interactions. *J. Polym. Sci., Part A: Polym. Chem.* **2013**, *51*, 2588–2597.
- (20) Yilmaz, G.; Becer, C. R. Precision Glycopolymers and Their Interactions with Lectins. *Eur. Polym. J.* **2013**, *49*, 3046–3051.
- (21) Su, S.; Wang, H.; Liu, X.; Wu, Y.; Nie, G. iRGD-Coupled Responsive Fluorescent Nanogel for Targeted Drug Delivery. *Biomaterials* **2013**, *34*, 3523–3533.
- (22) Wolfbeis, O. S. An Overview of Nanoparticles Commonly Used in Fluorescent Bioimaging. *Chem. Soc. Rev.* **2015**, *44*, 4743–4768.
- (23) De Koker, S.; Cui, J.; Vanparijs, N.; Albertazzi, L.; Grooten, J.; Caruso, F.; De Geest, B. G. Engineering Polymer Hydrogel Nanoparticles for Lymph Node-Targeted Delivery. *Angew. Chem., Int. Ed.* **2016**, *55*, 1334–1339.
- (24) Hoshino, Y.; Nakamoto, M.; Miura, Y. Control of Protein-Binding Kinetics on Synthetic Polymer Nanoparticles by Tuning Flexibility and Inducing Conformation Changes of Polymer Chains. *J. Am. Chem. Soc.* **2012**, *134*, 15209–15212.
- (25) Cho, E. J.; Holback, H.; Liu, K. C.; Abouelmagd, S. A.; Park, J.; Yeo, Y. Nanoparticle Characterization: State of the Art, Challenges, and Emerging Technologies. *Mol. Pharmaceutics* **2013**, *10*, 2093–2110.
- (26) Smith, M. H.; Lyon, L. A. Tunable Encapsulation of Proteins within Charged Microgels. *Macromolecules* **2011**, *44*, 8154–8160.
- (27) Ballauff, M.; Lu, Y. Smart Nanoparticles: Preparation, Characterization and Applications. *Polymer* **2007**, *48*, 1815–1823.
- (28) Karg, M.; Hellweg, T. New “Smart” poly(NIPAM) Microgels and Nanoparticle Microgel Hybrids: Properties and Advances in Characterisation. *Curr. Opin. Colloid Interface Sci.* **2009**, *14*, 438–450.
- (29) Yagmur, A.; Helvig, S.; Azmi, I.; Moghimi, S. Recent Advances in Cryo-TEM Imaging of Soft Lipid Nanoparticles. *AIMS Biophysics* **2015**, *2*, 116–130.
- (30) Cho, K.; Fasoli, J. B.; Yoshimatsu, K.; Shea, K. J.; Corn, R. M. Measuring Melittin Uptake into Hydrogel Nanoparticles with Near-Infrared Single Nanoparticle Surface Plasmon Resonance Microscopy. *Anal. Chem.* **2015**, *87*, 4973–4979.
- (31) Rothenhäusler, B.; Knoll, W. Surface-Plasmon Microscopy. *Nature* **1988**, *332*, 615–617.
- (32) Zybin, A.; Kuritsyn, Y. A.; Gurevich, E. L.; Temchura, V. V.; Überla, K.; Niemax, K. Real-time Detection of Single Immobilized Nanoparticles by Surface Plasmon Resonance Imaging. *Plasmonics* **2010**, *5*, 31–35.
- (33) Weichert, F.; Gaspar, M.; Timm, C.; Zybin, A.; Gurevich, E. L.; Engel, M.; Müller, H.; Marwedel, P. Signal Analysis and Classification for Surface Plasmon Assisted Microscopy of Nanoobjects. *Sens. Actuators, B* **2010**, *151*, 281–290.
- (34) Giebel, K. F.; Bechinger, C.; Herminghaus, S.; Riedel, M.; Leiderer, P.; Weiland, U.; Bastmeyer, M. Imaging of Cell/Substrate Contacts of Living Cells with Surface Plasmon Resonance Microscopy. *Biophys. J.* **1999**, *76*, 509–516.
- (35) Jamil, M. M.; Denyer, M. C.; Youseffi, M.; Britland, S. T.; Liu, S.; See, C. W.; Somekh, M. G.; Zhang, J. Imaging of the Cell Surface Interface Using Objective Coupled Widefield Surface Plasmon Microscopy. *J. Struct. Biol.* **2008**, *164*, 75–80.
- (36) Wang, S.; Shan, X.; Patel, U.; Huang, X.; Lu, J.; Li, J.; Tao, N. Label-Free Imaging, Detection, and Mass Measurement of Single Viruses by Surface Plasmon Resonance. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 16028–16032.
- (37) Gurevich, E. L.; Temchura, V. V.; Überla, K.; Zybin, A. Analytical Features of Particle Counting Sensor Based on Plasmon Assisted Microscopy of Nano Objects. *Sens. Actuators, B* **2011**, *160*, 1210–1215.
- (38) Wang, W.; Foley, K.; Shan, X.; Wang, S.; Eaton, S.; Nagaraj, V. J.; Wiktor, P.; Patel, U.; Tao, N. Single Cells and Intracellular Processes Studied by a Plasmonic-Based Electrochemical Impedance Microscopy. *Nat. Chem.* **2011**, *3*, 251–257.
- (39) Wang, W.; Wang, S.; Liu, Q.; Wu, J.; Tao, N. Mapping Single-Cell-Substrate Interactions by Surface Plasmon Resonance Microscopy. *Langmuir* **2012**, *28*, 13373–13379.
- (40) Wang, W.; Tao, N. Detection, Counting, and Imaging of Single Nanoparticles. *Anal. Chem.* **2014**, *86*, 2–14.
- (41) Halpern, A. R.; Wood, J. B.; Wang, Y.; Corn, R. M. Single-Nanoparticle Near-Infrared Surface Plasmon Resonance Microscopy for Real-Time Measurements of DNA Hybridization Adsorption. *ACS Nano* **2014**, *8*, 1022–1030.
- (42) Yang, Y.; Yu, H.; Shan, X.; Wang, W.; Liu, X.; Wang, S.; Tao, N. Label-Free Tracking of Single Organelle Transportation in Cells with Nanometer Precision Using a Plasmonic Imaging Technique. *Small* **2015**, *11*, 2878–2884.
- (43) Toyoshima, M.; Miura, Y. Preparation of Glycopolymers-Substituted Gold Nanoparticles and Their Molecular Recognition. *J. Polym. Sci., Part A: Polym. Chem.* **2009**, *47*, 1412–1421.
- (44) Terada, Y.; Hashimoto, W.; Endo, T.; Seto, H.; Murakami, T.; Hisamoto, H.; Hoshino, Y.; Miura, Y. Signal Amplified Two-Dimensional Photonic Crystal Biosensor Immobilized with Glyco-Nanoparticles. *J. Mater. Chem. B* **2014**, *2*, 3324–3332.

- 497 (45) Debord, J. D.; Lyon, L. A. Synthesis and Characterization of pH-
498 Responsive Copolymer Microgels with Tunable Volume Phase
499 Transition Temperatures. *Langmuir* **2003**, *19*, 7662–7664.
- 500 (46) Ding, L.; Qian, R.; Xue, Y.; Cheng, W.; Ju, H. In Situ
501 Scanometric Assay of Cell Surface Carbohydrate by Glyconanopar-
502 ticle-Aggregation-Regulated Silver Enhancement. *Anal. Chem.* **2010**,
503 *82*, 5804–5809.
- 504 (47) Hone, D. C.; Haines, A. H.; Russell, D. A. Rapid, Quantitative
505 Colorimetric Detection of a Lectin Using Mannose-Stabilized Gold
506 Nanoparticles. *Langmuir* **2003**, *19*, 7141–7144.
- 507 (48) Schofield, C. L.; Haines, A. H.; Field, R. A.; Russell, D. A. Silver
508 and Gold Glyconanoparticles for Colorimetric Bioassays. *Langmuir*
509 **2006**, *22*, 6707–6711.
- 510 (49) Kober, M.; Moros, M.; Franco Fraguas, L.; Grazu, V.; de la
511 Fuente, J. M.; Luna, M.; Briones, F. Nanoparticle-Mediated
512 Monitoring of Carbohydrate-Lectin Interactions Using Transient
513 Magnetic Birefringence. *Anal. Chem.* **2014**, *86*, 12159–12165.