Adsorption and Unfolding of a Single Protein Triggers Nanoparticle Aggregation

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1. Supplementary Figures and Tables

**Table S1.** Hydrodynamic radius ($R_h$) of MUTAB-AuNRs before and after addition of BSA at different concentrations.

| Sample                        | $R_h,\text{ trans (nm)}$ | $R_h,\text{ rot (nm)}$ | $R_h,\text{ theor (nm)}$ |
|-------------------------------|---------------------------|-------------------------|---------------------------|
| MUTAB-AuNRs alone             | 24 ± 2                    | 25 ± 2                  | 24                        |
| MUTAB-AuNRs + 2 nM BSA        | N/A**                     | N/A**                   | -                         |
| MUTAB-AuNRs + 10 µM BSA       | 32 ± 3                    | 33 ± 3                  | -                         |
| MUTAB-AuNRs + 20 µM BSA       | 31 ± 3                    | 33 ± 3                  | -                         |
| MUTAB-AuNRs + 100 µM BSA      | 30 ± 3                    | 33 ± 3                  | -                         |
| MUTAB-AuNRs + 500 µM BSA      | 31 ± 3                    | 34 ± 3                  | -                         |

*Average values ± standard deviations from three independent measurements.*

**Poor fitting, as shown in Fig. 1**

**Figure S1. Electric field enhancement for a 20 nm x 58 nm AuNR.**

The electric field enhancement is calculated at the excitation wavelength of 638 nm in an aqueous environment using a commercially available finite-difference time domain (FDTD) package. $E$ is the radiated electric field and $E_0$ is the incident electric field. The frequency-dependent dielectric function of gold was adopted from the tabulated values for bulk gold. Minimum grid size of 1 nm was used and default convergence criteria were adopted. The calculations were carried out using the Shared Tightly-Integrated Cluster (STIC) provided by Rice University.
Figure S2. Enhanced BSA fluorophore emission intensity near AuNRs.

To measure the plasmonic fluorescence enhancement of the Alexa647-labeled BSA when adsorbed to MUTAB-AuNRs, the intensity of single Alexa647-labeled BSA molecules alone on the substrate were compared to single Alexa647-labeled BSA molecules adsorbed to MUTAB-AuNRs. The intensity contribution of MUTAB-AuNRs alone in a single frame was comparable to the background intensity and therefore considered negligible for this calculation. Samples of Alexa647-BSA on the substrate alone were prepared by incorporating 750 pM of Alexa647-BSA into the 500 µM unlabeled-BSA solution that was deposited on the glass coverslips for passivation against unspecific protein adsorption, as described in the preparation of the substrate samples (Figure S5). No MUTAB-AuNRs were added to the slides, the samples were sealed with the custom flow chamber, and buffer was injected. Data was collected under identical imaging conditions to the Alexa647-labeled BSA molecules adsorbed to MUTAB-AuNRs. Figure S2 reports the cumulative distributions \( P(I' > I) \) of Alexa647-labeled BSA in the absence and presence of MUTAB-AuNRs obtained from the intensity \( I \) of molecules identified in the first frame for three different trials, showing a 2.5 fold fluorescence enhancement. Enhanced fluorophore emission is consistent with the electric field enhancement at the surface of an AuNR (Figure S1) and the spectral overlap between dye fluorescence (\( \lambda_{\text{max}} = 668 \text{ nm} \)) and surface plasmon resonance (\( \lambda_{\text{max}} = 664 \text{ nm} \)).

Figure S3. Co-localization of single MUTAB-AuNRs with fluorescently labeled BSA in an alternative area of the sample.

Fluorescence images are 10 frames binned with the same intensity; scale bar 500 nm.
Figure S4. Representative SEM images of the sample area analyzed in Figure 3. A high number of monomer MUTAB-AuNRs (>99%) are observed, confirming that the data was collected from single MUTAB-AuNRs. The scale bar is 1 μm and applies to all images.
Figure S5. **Passivation of substrates for luminescence correlation spectroscopy (left) and single molecule imaging experiments (right).**

The passivation of the cleaned coverslips depended on the type of experiment and the analyte of interest. For correlation spectroscopy (left), the success of the experiment required minimal interaction of the positively charged MUTAB-AuNRs with the substrate. The coverslips were immersed in a 2% solution of Vectabond reagent (Vector Labs) for 5 minutes followed by rinsing with DI water. Vectabond ensured minimal interaction with the MUTAB-AuNRs in solution. Negligible adsorption of MUTAB-AuNRs was observed by fluorescence imaging on these Vectabond passivated coverslips (left). Single protein binding experiments (right) on the other hand, required free diffusion of Alexa647-labeled BSA molecules and negligible non-specific adsorption to the coverslip surface without affecting their binding interaction with immobilized MUTAB-AuNRs. Using a custom silicon isolator with a small elliptical opening (43018C, Grace Biolabs), the cleaned glass coverslips were passivated with 30 μl of 25 mg/ml unlabeled BSA in buffer and dried at room temperature for three hours. Excess BSA was washed with 15 soft rinses of 1 ml Molecular Biology H2O. This BSA layer acted as an excellent passivating agent against Alexa647-labeled BSA as negligible non-specific binding of Alexa647-labeled BSA was observed by fluorescence imaging (right). Both of these fluorescence images demonstrate the effectiveness of these passivation protocols and were collected under the same conditions of the single-protein binding experiments (incident excitation intensity of 5 mW/cm², an integration time of 100 ms, frame rate of 7.5 Hz, and electron multiplying gain of 300).
Figure S6. Evidence for the duration of the BSA dissociation time being limited by photobleaching.

Single molecule fluorescence images were collected by varying the collection frame rate where unnecessary excitation/emission cycles of the fluorophores -when data is not being collected-was prevented using an acousto-optic modulator and frequency generators within the total internal reflection fluorescence microscope setup. Using a 30 ms integration time, the frame collection rate was varied between 0.25-16 Hz. The BSA dissociation times (on-times) on single MUTAB-AuNRs were extracted by first identifying BSA adsorption events and then measuring the time until fluorescence was no longer observed in consecutive frames from the same adsorption site. These on-times of all identified events are plotted as cumulative distributions $P$ as a function of frame rate. It is expected that when decreasing frequencies produce the same distribution of dissociation times, the data collection frequency is optimized to observe desorption and not photobleaching of adsorbed molecules. However, the dissociation time distributions for BSA on MUTAB-AuNRs did not converge (i.e. 0.25 Hz shows events much longer than any greater frequency), indicating that photobleaching and not BSA desorption dominates the measured residence times. Beyond 0.25 Hz stage stability and focal drift did not allow for accurate quantification of the dissociation times. Therefore, photophysics of the fluorophore labels on the protein are observed, and based on these experiments we can only estimate that the dissociation time is on the order of 10’s of seconds or greater.
Figure S7. Adsorption of BSA onto 50 nm MUTAB-AuNPs also leads to unfolding of BSA. Far UV and visible CD spectra of BSA (black, 0.1 mg/ml), MUTAB-AuNPs (blue, ~0.75 nM), MUTAB-AuNPs mixed with an equal volume of BSA (red, “BSA+MUTAB-AuNPs”) keeping the final concentrations of BSA and AuNPs identical to the control experiments, and the same BSA+MUTAB-AuNPs but after the liquid supernatant was separated and the centrifuged pellet was re-suspended in the same volume of the original solution (green, “Centrif. BSA+MUTAB-AuNPs”, 7500 rpm, 10 min). All solutions were suspended in the same phosphate buffer used to record CD of MUTAB-AuNRs. The decrease of the UV CD signal (black vs red lines) indicates BSA unfolding and not a dilution effect, as both spectra were recorded for the same BSA concentration. The absence of surface plasmon coupled CD signal despite AuNP aggregation (Figure S8) is currently not fully understood, but could be due to the shape of the nanostructures and the resulting aggregate geometries as well as electromagnetic hot spots.
Figure S8. Unfolding of BSA onto MUTAB-AuNPs also leads to nanoparticle aggregation.
UV/vis extinction spectra of BSA (black, “BSA”), MUTAB-AuNPs (blue, “MUTAB-AuNPs”), MUTAB-AuNPs mixed with an equal volume of BSA (red, “BSA+MUTAB-AuNPs”), and the same BSA+MUTAB-AuNPs but after the liquid supernatant was separated and the centrifuged pellet was re-suspended in the same volume as the original solution (green, “Centrif. BSA+MUTAB-AuNPs”). These extinction spectra correspond to the CD spectra shown in Figure S7 (measured simultaneously). The redshift, decrease in maximum intensity, and appearance of a longer wavelength shoulder for the surface plasmon resonance of the MUTAB-AuNPs with BSA added (red) compared to only MUTAB-AuNPs are clear signatures of nanoparticle aggregation.

While the lack of surface plasmon coupled CD signal does not allow us to correct for the amount of BSA adsorbed to the MUTAB-AuNPs and lost after the supernatant was removed (see below the corresponding discussion of the CD measurements for AuNRs), we can instead use the decrease in extinction.

This simple calculation yields a loss of $\alpha$-helical structure of BSA adsorbed onto MUTAB-AuNPs of 70%, similar to what was found for BSA adsorbed onto MUTAB-AuNRs.

$$1 - \frac{CD_{209, \text{BSA}}}{\text{Ext}_{450, \text{BSA}}} \times \frac{\text{Ext}_{450, \text{BSA+MUTAB-AuNPs}}}{\text{Ext}_{450, \text{Centrif. BSA+MUTAB-AuNPs}}} = 1 - \frac{1.08}{0.21} = 0.70$$

where:

$CD_{209, \text{BSA}}$: CD signal in mdeg of BSA in the buffer conditions of the experiment at 209 nm (a typical wavelength used to measure $\alpha$-helical content of proteins).\(^{12}\)
$CD_{209,\text{centrif.BSA+MUTAB-AuNPs}}$: CD signal in mdeg of a mixture of BSA and MUTAB-AuNPs after the liquid supernatant was removed and the pellet was re-suspended in the same buffer conditions of the experiment at 209 nm.

$\text{Ext}_{450,\text{BSA+MUTAB-AuNPs}}$: Extinction of the mixture of BSA and MUTAB-AuNPs at 450 nm (a wavelength where the extinction intensity is attributed to the amount of gold in solution only).

$\text{Ext}_{450,\text{centrif.BSA+MUTAB-AuNPs}}$: Extinction at 450 nm for the mixture of BSA and MUTAB-AuNPs after the liquid supernatant was removed and the pellet was re-suspended in the same buffer conditions of the experiment.
Figure S9. Lack of aggregation of anionic citrate-AuNPs (d = 48 nm) in the presence of BSA at low and high protein-to-nanoparticle ratios. The slight redshift and increase in extinction intensity of the plasmon resonance peak is indicative of BSA binding.
Figure S10. Additional extinction and circular dichroism (CD) spectra of controls and samples presented in Figure 5a.

a, Control far UV CD spectra of BSA (0.1 mg/ml, \( \approx 1.5 \) µM) in the presence of free MUTAB ligand in solution (0.1 mg/ml) and BSA (same concentration) with spherical citrate-capped Au nanoparticles (citrate-AuNPs, concentration = 75 pM, diameter \( d = 48 \pm 4 \) nm). No change in the CD spectrum of BSA is observed under these conditions when compared to BSA in buffer at the same concentration. Note that BSA binds to citrate-AuNPs and starts to form a monolayer at micromolar concentrations.
b, Far UV CD spectra of MUTAB-AuNRs (75 pM) mixed with an equal volume of BSA (0.1 mg/ml, \( \approx 1.5 \) µM, “BSA + MUTAB-AuNRs”) and of the liquid supernatant that was removed from the MUTAB-AuNRs by centrifugation (7,500 rpm, 10 min, “Supernatant”). The overlaying spectral features suggest that the majority of the CD signal corresponding to the secondary structure characteristics of the BSA + MUTAB-AuNR sample arises from the free, native BSA in solution.
c, UV/vis extinction spectra of the same samples, for which the CD spectra are shown in Figure 5 (measured simultaneously). Details regarding sample preparation and concentrations can be found in the methods section.

“BSA”: BSA in the buffer conditions of the experiment.
“BSA+MUTAB-AuNRs”: Mixture of equal volume of BSA and MUTAB-AuNRs.
“Centrifuged BSA+MUTAB-AuNRs”: Same BSA+MUTAB-AuNRs after the liquid supernatant was removed by centrifugation (7,500 rpm, 10 min), and the pellet was re-suspended in the same buffer used to dilute the BSA for CD experiments.
“MUTAB-AuNRs” : Nanorods mixed with an equal volume of the same buffer.
Note that approximately 50% of the MUTAB-AuNRs were lost during the centrifugation step (red vs green lines) because MUTAB-AuNRs easily stick to the walls of the centrifuge tube even at low centrifugation speeds. This decrease in extinction is also observed in Figure 5 where the corresponding CD signal decreases by ≈50% after this centrifugation step. In contrast to Figure 1d where nM concentrations of BSA caused a blueshift in the extinction spectra of MUTAB-AuNRs, the higher BSA concentration required by the spectropolarimeter (0.1 mg/ml, ≈1.5 µM) causes a slight redshift of the extinction spectra of the MUTAB-AuNRs (0.75 nM, blue and red curves), probably indicating the formation of larger end-to-end MUTAB-AuNR aggregates according to plasmon hybridization theory. Given that aggregation is a strongly non-equilibrium process, it is not surprising that for distinct experiments different aggregate geometries can be encountered.

Based on this data, the loss of α-helical structure of BSA is estimated to be as high as 69%:

\[
1 - \frac{CD_{209,\text{BSA}}}{CD_{209,\text{BSA}+\text{MUTAB-AuNRs}}} \times \frac{CD_{645,\text{BSA+MUTAB-AuNRs}}}{CD_{645,\text{BSA+MUTAB-AuNRs},\text{centrif}}} = 1 - \frac{1.66}{13.57} \times \frac{34.78}{14.05} = 0.69
\]

where:

\(CD_{209,\text{BSA}}\): CD signal in mdeg of BSA in the buffer conditions of the experiment at 209 nm (a typical wavelength used to measure α-helical content of proteins).

\(CD_{209,\text{BSA}+\text{MUTAB-AuNRs}}\): CD signal in mdeg of a mixture of BSA and MUTAB-AuNRs after the liquid supernatant was removed and the pellet was re-suspended in the same buffer conditions of the experiment at 209 nm.

\(CD_{645,\text{BSA+MUTAB-AuNRs}}\): CD signal in mdeg of the mixture of BSA and MUTAB-AuNRs at 645 nm (a wavelength where the surface plasmon coupled CD signal peaks in Figure 5b).

\(CD_{645,\text{BSA+MUTAB-AuNRs},\text{centrif}}\): CD signal in mdeg of a mixture of BSA and MUTAB-AuNRs after the liquid supernatant was removed and the pellet was re-suspended in the same buffer conditions of the experiment at 645 nm.

In an ideal case without gold losses in the centrifugation step, this empirical calculation should have only considered the ratio of the CD signals in the UV (at 209 nm) before (\(CD_{209,\text{BSA}}\)) and after mixing BSA with MUTAB-AuNRs and removing the liquid supernatant (\(CD_{209,\text{BSA}+\text{MUTAB-AuNRs}}\)). However because some AuNRs with BSA adsorbed onto their surface was lost during the centrifugation step, such a calculation would be an overestimation of the loss of α-helical structure. To differentiate between BSA that truly lost secondary structure due to adsorption onto the MUTAB-AuNRs, and BSA bound to MUTAB-AuNRs lost during the centrifugation step, we take into account the ratio of CD signals in the visible region at the peak of the surface plasmon coupled CD spectrum (Figure 5) at 675 nm

\[
\frac{CD_{645,\text{BSA}+\text{MUTAB-AuNRs}}}{CD_{645,\text{BSA+MUTAB-AuNRs},\text{centrif}}} = 0.74
\]

Note that this calculation predicts the loss of α-helical content of BSA due to adsorption to be as high as 69%, which is approximately 2.5 times higher than the 20% loss predicted by the DichroWeb K2d neural network secondary structure algorithm if only BSA and BSA+MUTAB-AuNR samples were considered, ignoring the presence of excess folded-BSA in solution. If instead of the changes in the visible CD spectra the decrease in extinction is considered (Figure S10) a similar value of ~74% is obtained. At the moment we cannot rule out that some of the visible CD signal could also arise from chiral AuNR assemblies in addition to surface plasmon coupled CD. While this interpretation requires
further experiments, also in light of the AuNP data, the calculation for the loss of BSA α-helical structure is not affected by it.

| Sample                          | $R_h$ (nm) |
|---------------------------------|------------|
| BSA in buffer                   | 4 ± 1      |
| BSA in 0.05 mg/ml MUTAB         | 4 ± 1      |
| BSA – supernatant               | 4 ± 2      |

**Figure S11. Free BSA in solution does not increase its hydrodynamic dimensions in the presence of free MUTAB.**

These control experiments were performed to further verify that BSA unfolding is not induced by free MUTAB in solution. Normalized autocorrelation curves are shown for 7.5 nM Alexa-647 BSA in buffer (black triangles), in 0.05 mg/ml MUTAB solution (red squares), and after mixing with MUTAB-AuNRs and separating the supernatant via centrifugation (green circles, corresponding to conditions used in Figure S10). The MUTAB concentration tested in this control experiment (0.05 mg/ml) is at least five times higher than the concentration of free MUTAB present in the MUTAB-AuNR solutions used for luminescence correlation spectroscopy experiments. The overlap of the autocorrelation curves indicates equal hydrodynamic dimensions of BSA in these three environments, demonstrating that BSA does not unfold under these conditions. Hydrodynamic values extracted from the autocorrelation fits are shown in the table (right panel).
Figure S12. Magnified view of UV/CD spectra shown in Figure 5a.
Figure S13. Separation distances between nearest-neighboring BSA proteins using a single particle identification algorithm. Histogram of 27,586 point spread functions observed on seven Au nanowires, identified using a particle identification algorithm, as described in Figure S14.

Figure S14. Super-resolution analysis of BSA separation by localization and cross-correlation analysis in Figure 5c and demonstration of single BSA proteins in grouped pixels by cross-correlation analysis. First, for the results shown in Figure S14, we applied a particle identification algorithm to localize every point spread function (PSF) in each frame. Each PSF corresponded to an adsorbed BSA. We searched for the nearest neighboring localized centroid of each BSA and calculated the BSA-BSA distance. The distribution of all the BSA-BSA distances is illustrated in Figure S14. We considered the peak distance (~ 384 nm) as the most likely smallest separation between multiple adsorbed BSA molecules on the Au nanowire. The broad distribution was caused by fitting error limited by the diffraction limit and noise (width of distribution < 384 nm), and low photon counts due to photobleaching and photoblinking of fluorescent molecules on BSA (width > 384 nm).
Because of these limitations and the density of molecules on the surface of the nanowire, we applied a cross-correlation algorithm to further improve our spatial resolution. Cross-correlation analysis was performed as followed. The intensity-time trace at each pixel was correlated with its eight neighboring pixels. The averaged correlation coefficient at each pixel was used to indicate the possibility of the pixel being a center position of an adsorbed BSA (Figure S14a). Starting from each correlation peak, we calculated the cross-correlation between this peak and its nearby pixels, and grouped the pixels together based on the correlation value (Figure S14b; adjacent groups are labeled in different colors). To prove each group corresponded to a single BSA, we analyzed the number of photobleaching steps in the intensity-time trace of the center pixel of each group using a change-point algorithm (Figure S14c). Figure S14c shows the fluorescence intensity-time traces (blue lines) for the four sets of grouped pixels selected (labeled as 1-4) in the top of Figure S14b with the corresponding intensity states and photobleaching steps identified by our change-point algorithm (red lines), showing an average of ~3 steps per molecule. The smallest separation we identified using cross-correlation analysis is about 250 nm, below the diffraction limit of ~320 nm. Therefore, both protein localization and cross-correlation analyses prove the smallest BSA-BSA separation is larger than the MUTAB-AuNR size of 20 x 58 nm. Given that BSA is composed of 585 amino acids, and each one has on average a length of ~0.8 nm (as estimated from about five bonds per amino acid with an average bond length of ~160 pm), it is physically possible for the protein to spread such a large distance across the nanowire.
Figure S15. Control nanoparticle aggregation measurement of 2 nM BSA/MUTAB-AuNRs flowed over a blank coverslip.

(Left) Cartoon for comparison to cartoons in Figure 6. (Right) Representative SEM image, scale bar 1 µm. A small amount of aggregates that fell out of solution in the control measurement is seen, but most of the substrate did not have any aggregates. Further quantification is reported in Table S2.

**Table S2. Particle and aggregate sizes analyzed in Figure 4**

|                  | (i)         | (ii)        | (iii)        |
|------------------|-------------|-------------|--------------|
| Number particles | 152         | 333         | 20           |
| Area particles (nm^2) |           |             |              |
| mean ± std      | 1300 ± 300  | 20000 ± 50000 | 30000 ± 60000 |
| max             | 2884        | 593041      | 204248       |
| min             | 961         | 961         | 1682         |
| area particles/total area (%) |           |             |              |
| mean ± std      | 0.084 ± 0.007 | 2 ± 1     | 0.3 ±0.3     |
| max             | 0.096       | 4.03        | 0.57         |
| min             | 0.79        | 1.12        | 0.007        |

**Table S2. Quantitative analysis of aggregate size from Figures 6, S15.**

The “area particles” is the analysis described in the main text and data shown in Figure 6. The “area particles/total area” represents the percent coverage of the substrate imaged (total area) with aggregates (area particles). Overall, the data shows that for case (i), isolated single MUTAB-AuNRs are observed. For case (ii), large aggregates are seen covering ~2% of the substrate. Finally, for case (iii) a very small number of aggregates from solution are observed, but most of the substrate is bare, with coverage as low as 0.007%. 
Figure S16. Unlike BSA, fibrinogen and gamma-globulin induce nanoparticle aggregation at low and high protein-to-nanoparticle ratios. Globulin and fibrinogen are the second and third most abundant proteins in serum after albumin. UV/Vis extinction spectra of MUTAB-AuNRs in the presence of 2 nM and 10 μM of a, fibrinogen and b, gamma-globulin. The red-shift and broadening of the extinction spectra of MUTAB-AuNRs at both low and high protein concentrations is indicative of aggregation. Strong, irreversible adsorption of fibrinogen to MUTAB-AuNRs is confirmed by single molecule imaging. c, Photoluminescence image of single immobilized MUTAB-AuNRs. d, 2 nM Alexa 647-labeled fibrinogen adsorbed onto single immobilized MUTAB-AuNRs is observed as an increase in fluorescence intensity at particle locations. e, Fibrinogen remained bound onto MUTAB-AuNRs even after rinsing with buffer for 15 minutes.
Figure S17. Extinction spectra of CTAB and MUTAB-AuNRs.
Extinction spectroscopy measurements in solution were carried out using an Ocean Optics SD1024DW spectrometer, and a 10 mm pathlength glass cuvette (Starna Cells, California, USA). Replacement of CTAB with MUTAB caused a small blueshift of the extinction peak (<5 nm) and a small loss of AuNRs as seen from the decrease in the extinction.

Figure S18. Resistance against aggregation in buffer for MUTAB-AuNRs.
Almost identical spectra of MUTAB-AuNRs in water and in buffer show that no colloidal aggregation occurred when changing to the buffer conditions of the experiments (20 mM HEPES, 20 mM NaCl in Molecular Biology Grade H₂O, Thermo Scientific).
Figure S19. Distribution of sizes of AuNRs and representative transmission electron microscopy (TEM) image.
Distributions of length (top right), width (bottom left), and aspect ratio (bottom right) of the AuNRs used for this study were obtained from analyzing more than 600 nanoparticles in TEM images such as the one shown in the top left. TEM micrographs were obtained with a JEOL 2010.
Figure S20. Negligible auto-fluorescence background of unlabeled BSA. Luminescence intensity trajectory of ~10 nM BSA in buffer at the same excitation power used for the correlation spectroscopy experiments shown in Figure 1c.

Figure S21. Calibration of the focal volume for luminescence correlation spectroscopy experiments using 36 nm fluorescent beads. Each fluorescence correlation spectroscopy calibration measurement was analyzed with the known diffusion time of the 36 nm fluorescent beads to yield the radial dimension of the focal volume. Figure S21 shows a histogram of all values obtained and a corresponding Gaussian fit of the distribution.
Figure S22. Number of cells/mL under the two different incubation conditions used for the nanoparticle uptake experiments.

BSA-FBS: 1% BSA followed by 10% FBS; FBS: 10% FBS. Cells were also counted without MUTAB-AuNRs as a control.

BSA-FBS: 1% BSA followed by 10% FBS; FBS: 10% FBS. Cells were also counted without MUTAB-AuNRs as a control.
Figure S23. Normalized LDH release under the two different incubation conditions used for the nanoparticle uptake experiments.
BSA-FBS: 1% BSA followed by 10% FBS; FBS: 10% FBS only. LDH were also monitored without MUTAB-AuNRs as a control. These results demonstrate that the MUTAB-AuNRs under the two protein incubation conditions tested are not significantly cytotoxic to MCF-7 cells.

Figure S24. Concentration of Au (ppb) in MCF-7 cells under the two different incubation conditions used for the nanoparticle uptake experiments determined via ICP-MS.
BSA-FBS: 1% BSA followed by 10% FBS; FBS: 10% FBS. MCF-7 cells alone were also measured without MUTAB-AuNRs as a control.
Figure S25. Super-localization of BSA on MUTAB-AuNRs supports single protein adsorption.

(insets) Spatially mapped 2D histograms of super-localized single BSA locations on single MUTAB-AuNRs are quantified by taking the standard deviation of centroids grouped within 200 x 200 nm² of one another. The spread of super-localized points for 337 single-BSA/MUTAB-AuNR systems is shown in the histogram, with an average σ = 22 ± 2 nm. Rather than mapping the AuNR dimensions, a spread of ~22 nm expresses the measurement resolution. One interpretation is that only a single BSA protein binds to a single AuNR. These results are also consistent with emission from many dye-labeled BSA proteins though, if the emission is coupled into the far-field through the plasmon resonance. Based on the photobleaching analysis of time transients (Figure 3), we confirm the small, resolution-limited spread is due to single BSA proteins. Scale bar, 50 nm.

2. Descriptions of Supplementary Videos.

Video S1. Wide field fluorescence imaging of adsorption of single BSA molecules to single MUTAB-AuNRs.

At the start of the video, MUTAB-AuNR photoluminescence is observed only. At ~ 4.5 s, fluorescently labeled BSA is introduced to the sample and high affinity adsorption is observed. Image measures 12.8 x 12.8 μm² in size. The frame rate is 7.5 Hz. Screenshot provided above; video provided as additional supplementary material available for download.
Video S2. Unlabeled BSA from the substrate does not interact with the MUTAB-AuNRs. Side-by-side comparison of Alexa647-BSA interaction with MUTAB-AuNRs either (left) previously exposed to unlabeled BSA or (right) not exposed. No Alexa647-BSA is present at the start of the video, and is then introduced to both samples after 20 frames. Images measure 10 x 10 µm in size. To confirm that unlabeled BSA from the substrate does not interact with the MUTAB-AuNRs, a control experiment was performed by first flowing unlabeled BSA over the MUTAB-AuNRs and then introducing labeled BSA. A side-by-side video comparison of the MUTAB AuNRs with and without being exposed to unlabeled BSA is shown in Movie S2, demonstrating that the labeled BSA does not interact with the AuNRs once unlabeled BSA is adsorbed. The figure below the screenshot of Movie S2 illustrates our observation. This data further supports our observations of irreversible adsorption of only a single BSA molecule. In addition, interference from the BSA used to passivate the coverslip surface can be ruled out as well as fluorescence quenching of an initial layer of dye-labeled BSA followed by further BSA adsorption.
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