

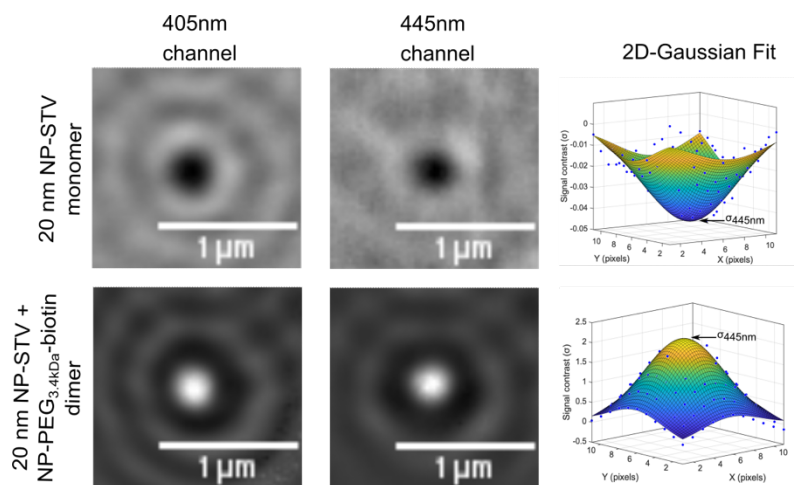
# Two-color Interferometric Scattering (iSCAT) Microscopy Reveals Structural Dynamics in Discrete Plasmonic Molecules

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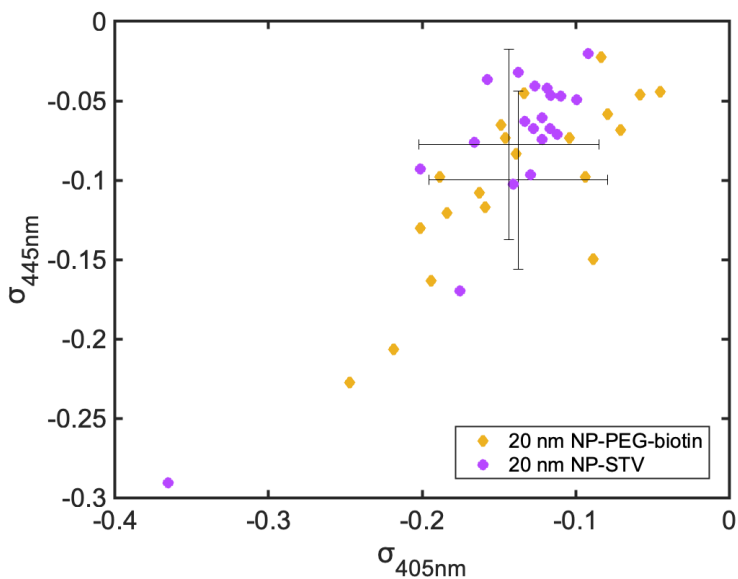
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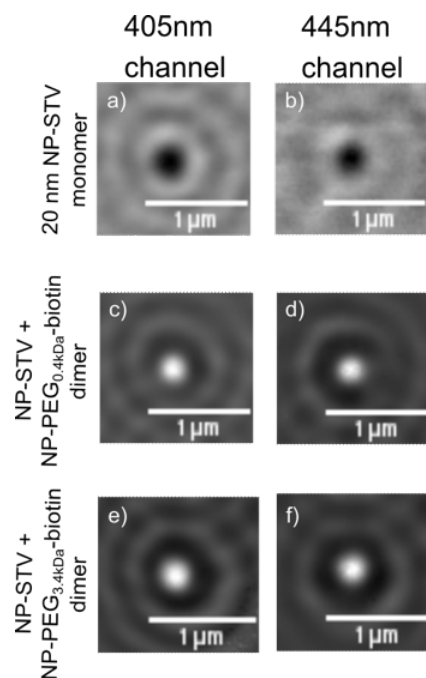
**Electronic Supplementary Information**



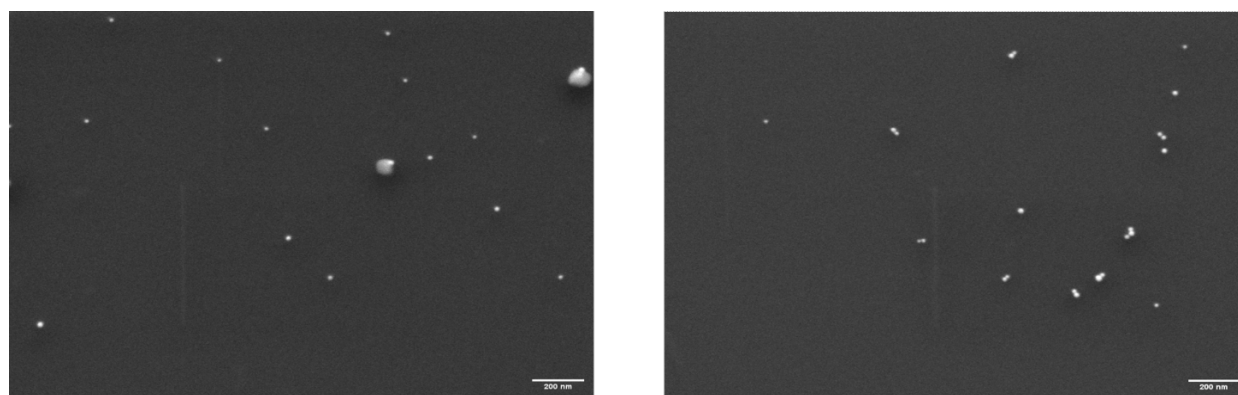
**Figure S1:** Overview of two-color iSCAT signal of 20 nm NP-STV monomer (top row) and 20 nm NP-STV + 20 nm NP-PEG<sub>3.4kDa</sub>-biotin dimer (bottom row). The last column shows two-dimensional Gaussian fits for signal on the 445 nm channel for monomer (top) and dimer (bottom) respectively.



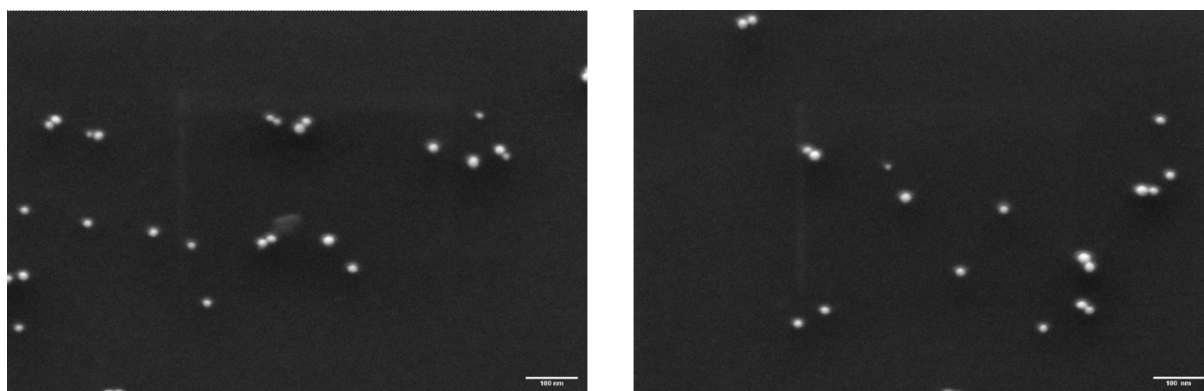
**Figure S2:**  $\sigma_{405}$ ,  $\sigma_{445}$  plots for 20 nm NP-STV and 20 nm NP-PEG<sub>3.4kDa</sub>-biotin. The averages  $\pm$  standard deviation (STD) are included. NP-STV particles were immobilized in a BSA-Biotin treated flow chamber. NP-PEG<sub>3.4kDa</sub>-biotin particles were immobilized in a BSA-Biotin and streptavidin treated flow chamber as described in methods.



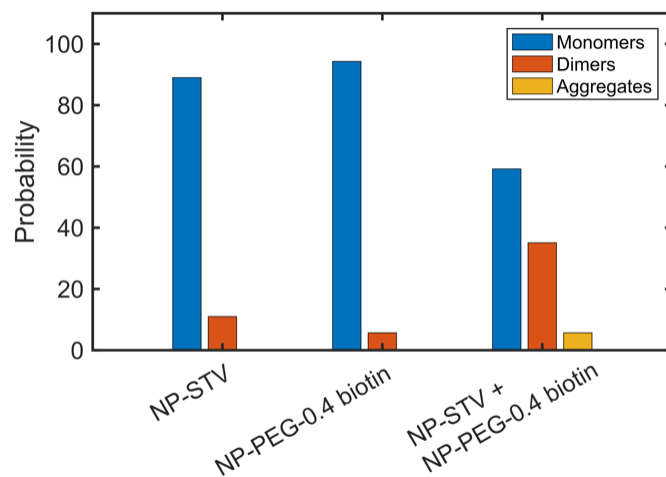
**Figure S3:** iSCAT signal of 20 nm NP-STV monomer (top row) and 20 nm NP-STV + 20 nm NP-PEG<sub>0.4kDa</sub>-biotin dimer (middle row), and 20 nm NP-STV + 20 nm NP-PEG<sub>3.4kDa</sub>-biotin dimer (bottom row).



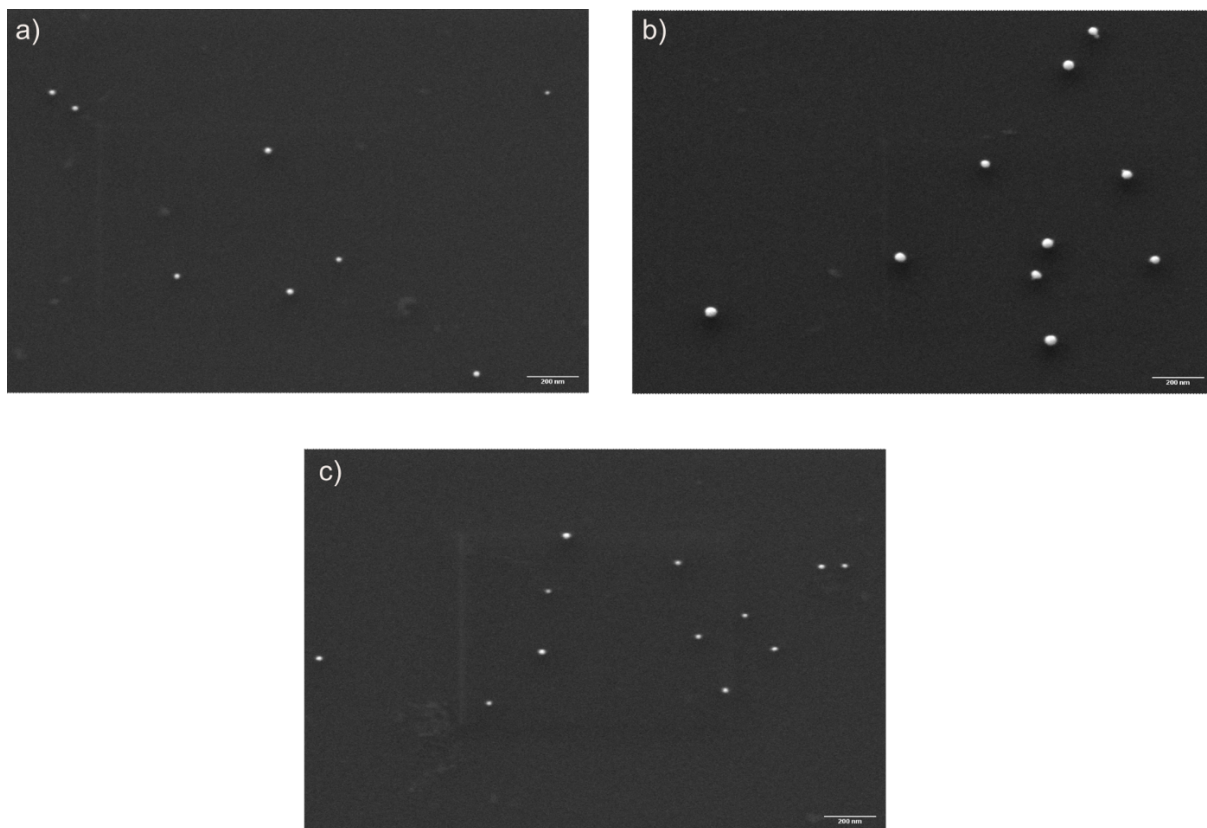
**Figure S4:** Representative SEM images at 50,000x magnification taken of 20 nm NP-STV (monomer) on left and 20 nm NP-STV + 20 nm NP-PEG<sub>3.4kDa</sub>-biotin (dimers) on right with stage tilted by 30 degrees.



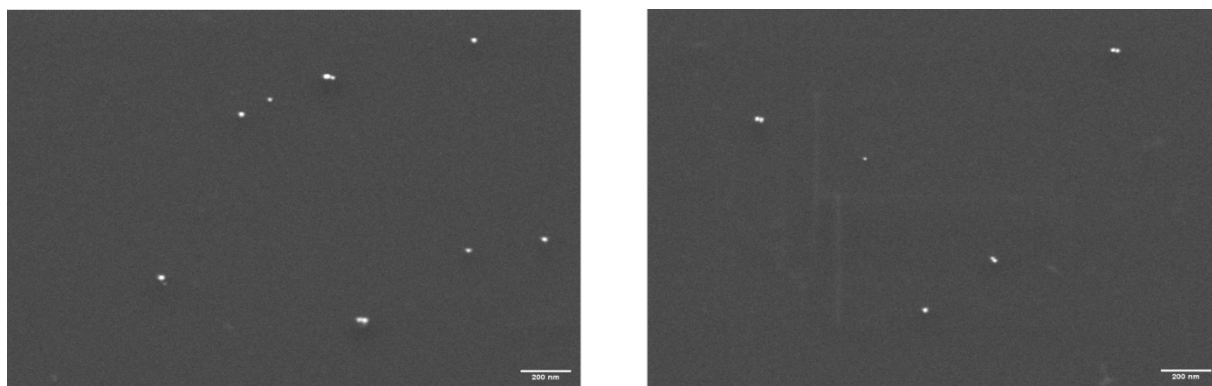
**Figure S5:** Representative SEM images at 100,000x magnification taken of 20 nm NP-STV + 20 nm NP-PEG<sub>0.4kDa</sub>-biotin (dimers) with stage tilted by 30 degrees.



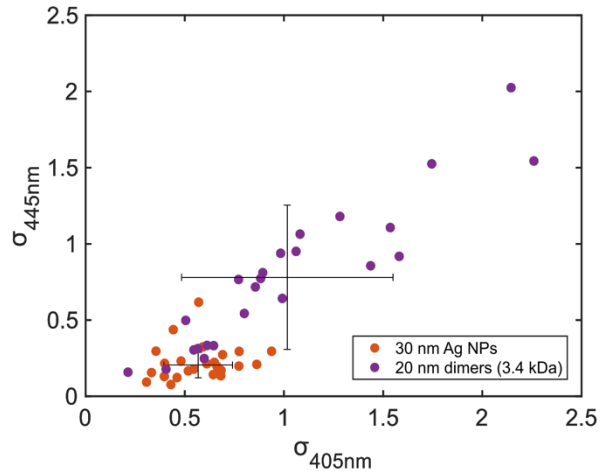
**Figure S6:** Histogram of 20 nm Ag NP aggregation state (monomers, dimers, aggregates) as determined by SEM for NP-STV, NP-PEG<sub>0.4kDa</sub>-biotin, and NP-STV + NP-PEG<sub>0.4kDa</sub>-biotin.



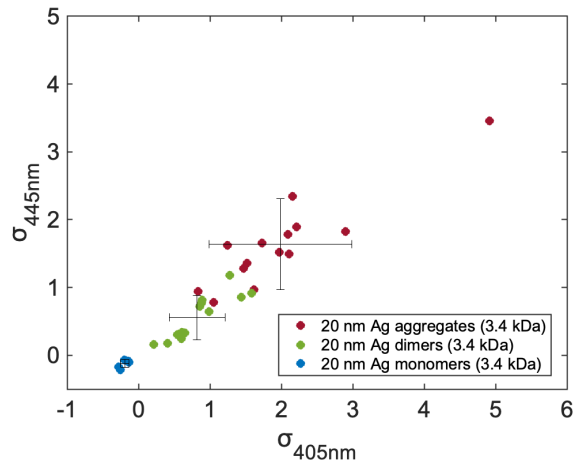
**Figure S7:** SEM images of control NP-PEG-biotin particles at 50,000x magnification. a) 20 nm NP-PEG<sub>3.4kDa</sub>-biotin monomers b) 40 nm NP-PEG<sub>3.4kDa</sub>-biotin monomers c) 20 nm NP-PEG<sub>0.4kDa</sub>-biotin monomers with stage tilted at 30 degrees.



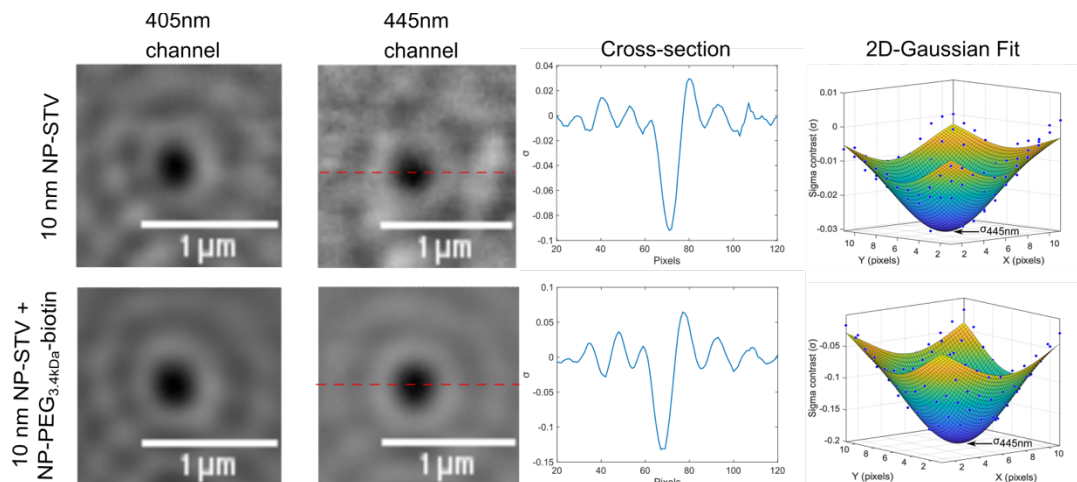
**Figure S8:** Representative SEM images at 50,000x magnification taken of 20 nm NP-STV + 40 nm NP-PEG<sub>3.4kDa</sub>-biotin heterodimers with stage titled at 30 degrees.



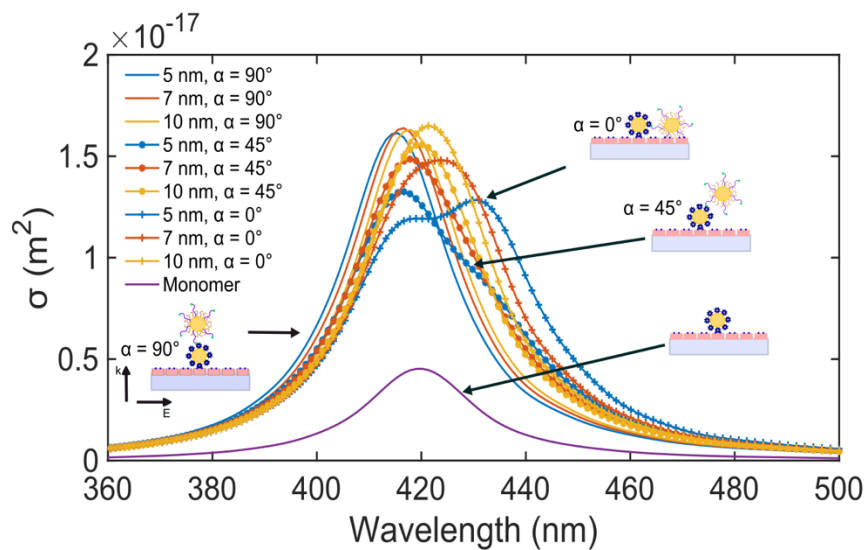
**Figure S9:**  $\sigma_{405}, \sigma_{445}$  plot for scatterers imaged after incubating 20 nm NP-STV with 20 nm NP-PEG<sub>3.4kDa</sub>-biotin (dimers) and immobilized 30 nm Ag NP monomers. The averages  $\pm$  standard deviation (STD) are included.



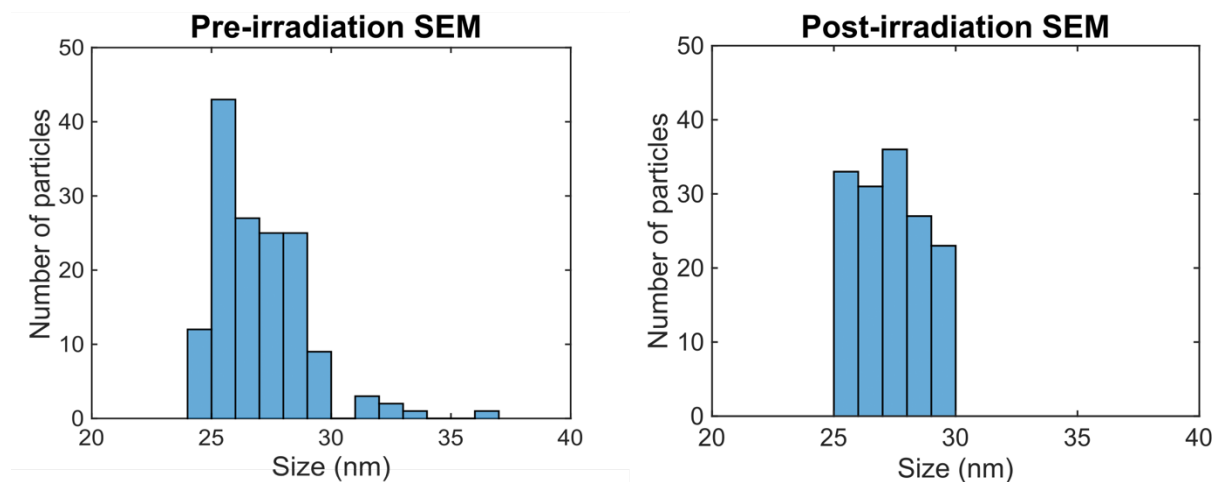
**Figure S10:**  $\sigma_{405}, \sigma_{445}$  plot for scatterers imaged after incubating 20 nm NP-STV with low (dimers) and high (aggregates) concentration of 20 nm NP-PEG<sub>3.4kDa</sub>-biotin. Low concentration of tether particles were  $10^9$  NPs/mL and high concentration  $10^{10}$  NPs/mL. The averages  $\pm$  standard deviation (STD) are included.



**Figure S11:** iSCAT signal of 10 nm NP-STV (top row) and 10 nm NP-STV + 10 nm NP-PEG<sub>3.4kDa</sub>-biotin (bottom row). Cross section of the signal along the red dashed line from the 445 nm channel is plotted in the third column. The last column shows two-dimensional Gaussian fits for signal on 445 nm channel for monomer (top) and dimer (bottom) respectively.



**Figure S12:** Simulated scattering spectra of 10 nm Ag NP monomer and dimers observed with circular polarized light in  $n = 1.5$  refractive index medium. For dimers three different elevations  $\alpha = 0^\circ, 45^\circ, 90^\circ$  and interparticle separations of 5 nm, 7 nm, and 10 nm were evaluated.



**Figure S13:** Size histograms (n=150) taken of Ag NP-PEG<sub>3.4kDa</sub>-biotin before and after illumination for 10s under identical conditions than in the iSCAT experiments. The average size of a sample of 150 particles before illumination was  $27.02 \pm 1.919$  nm. The average size of a sample of 150 particles after illumination was  $27.35 \pm 1.307$  nm.