Insights from a Nanoparticle Minuet: Two-Dimensional Membrane Profiling through Silver Plasmon Ruler Tracking

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Supporting Information

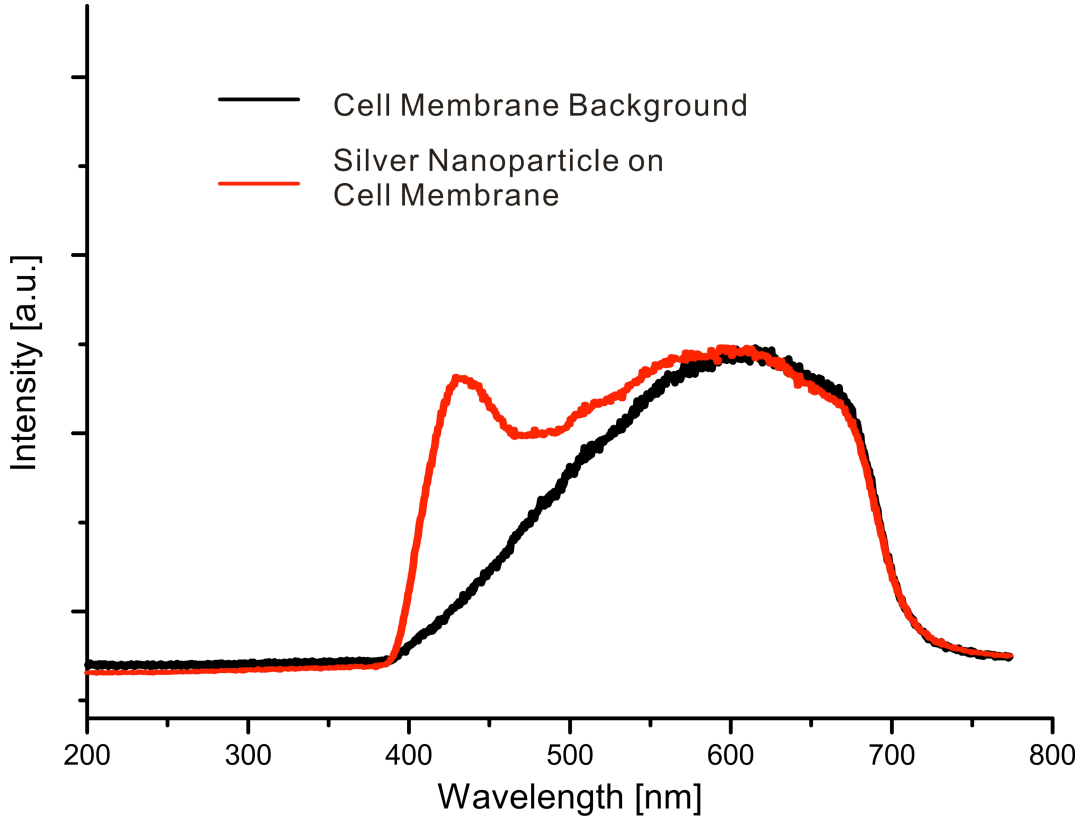


Figure S1: Unprocessed scattering spectra of an individual 30 nm diamter silver nanoparticle on a lysed HeLa cell (red) and lysed cell without silver nanoparticle (black).

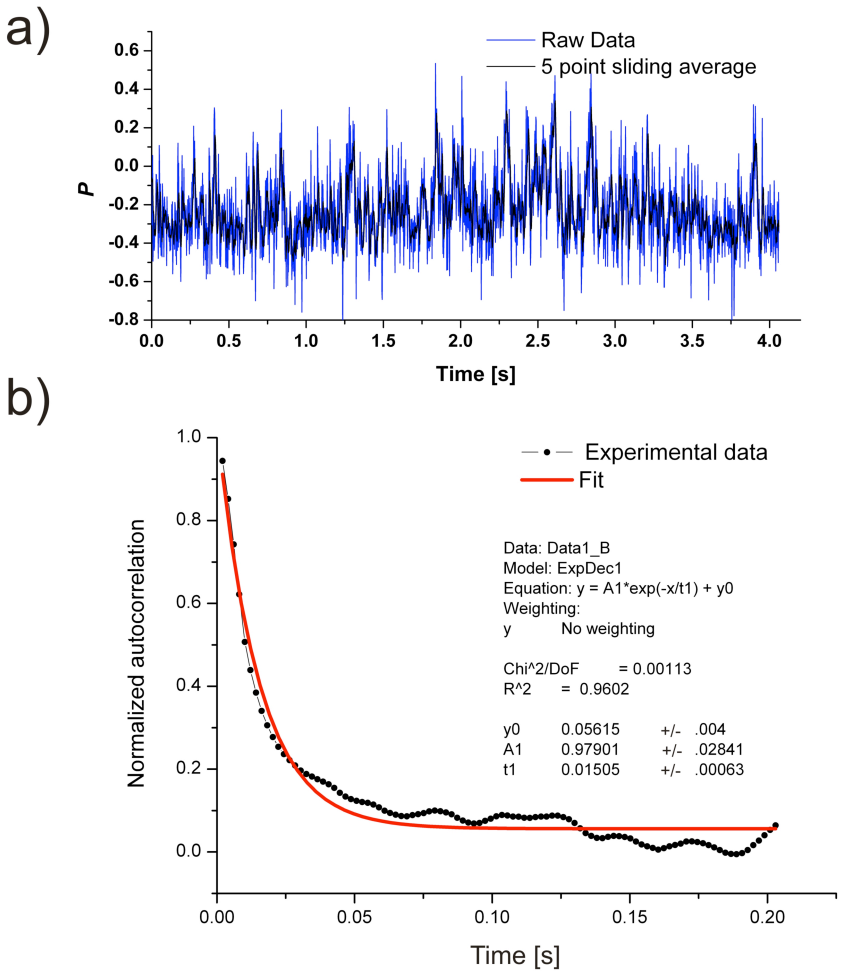
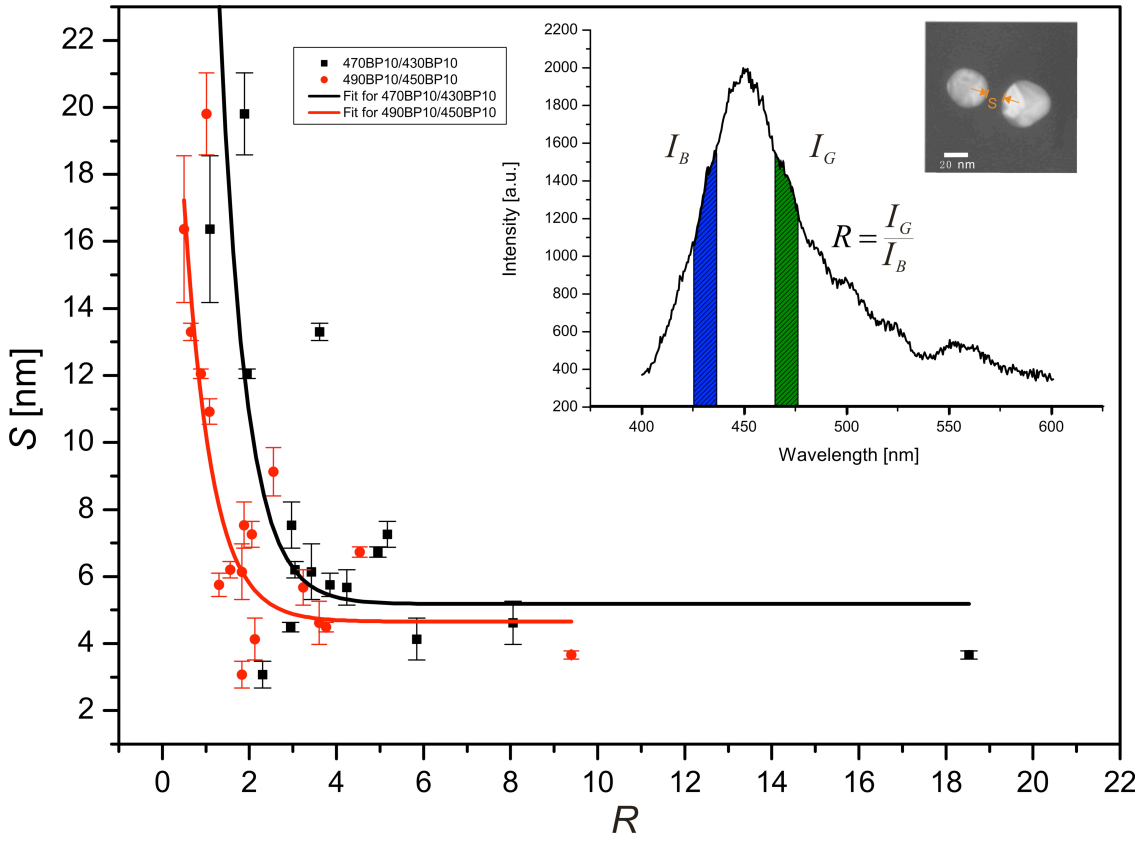


Figure S2: The scattering intensities of individual plasmon rulers are collected on two orthogonal polarization channels which allows the calculation of a reduced linear dichroism *P*. In a) the *P* values of a single plasmon ruler tracked with a frame rate of 500 Hz is shown. In b) we plot the normalized autocorrelation of the *P* trajectory shown in a) together with an exponential fit that describes the decay of the autocorrelation as function of time. The rotational correlation time τ is obtained as characteristic decay constant from the fit (and labeled here as t1).



$$470\text{BP10/430BP10}: S(nm) = 156.73559 \times e^{\frac{-R}{0.60118}} + 5.18306(nm)$$

$$490\text{BP10/450BP10}: S(nm) = 27.69357 \times e^{\frac{-R}{0.62575}} + 4.6561(nm)$$

Figure S3: Calibration of the *S*(*R*) relationship for two different filter combinations: 430BP10/470BP10 and 450BP10/490BP10. The *R* values were obtained from the scattering spectra of dimers with defined interparticle separation *S*. This scheme is illustrated for one representative dimer in the inset. The scattering intensities obtained through integration across the spectral width of the filters' passbands were corrected with the transmission characteristics of the filters as provided by the manufacturer. The resulting corrected intensities (I_B,I_G) were then used to calculate $R = I_G/I_B$.

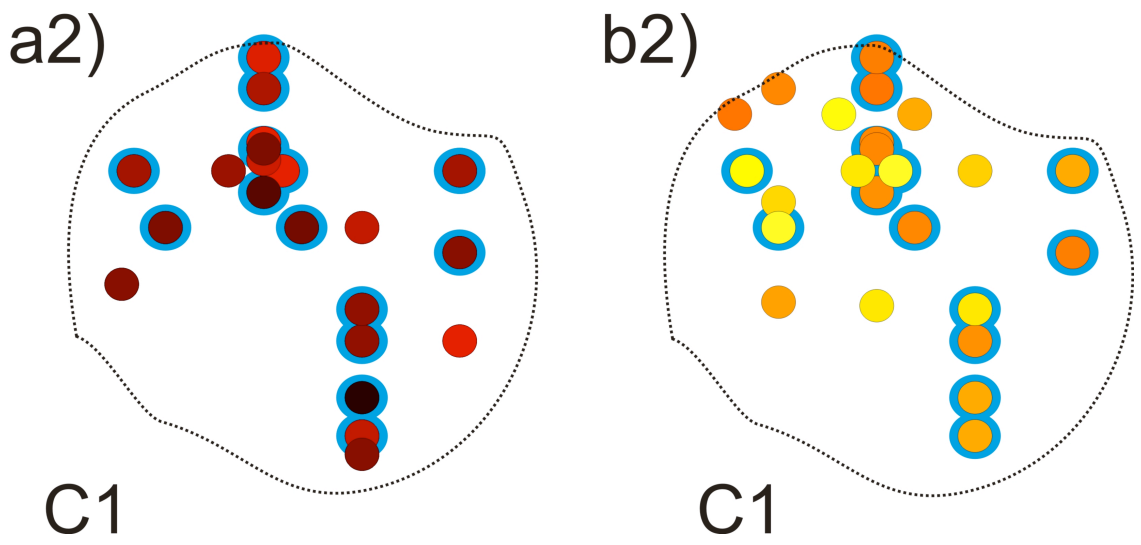


Figure S4: Magnification of Sub-Area C1 from Figures 6a2 and b2. *S* and *P* data points that superimpose are marked with a blue halo.

Methods

DNA Programmed Assembly of the Probes: Silver Nanoparticle Dimers. In a first step of our dimer assembly approach two batches of commercial citrate stabilized silver nanoparticles with average diameters of *d* = 30 ± 4 nm were reacted with partly complementary 5' thiolated DNA handles: Seq1: HS-AAA AAA AAA ATA GTT CGA TAT CGG ATG TGG TGT CAG TCG TAG CGT GAG; Seq2: HS-AAA AAA AAA ACT CAC GCT ACG ACT GAC ACC. After incubation of the particles with DNAs in a ratio 1:20 in 40 mM phosphate buffer, pH8 overnight, both batches were passivated by adding the PEGs (alkyl-[polyethylene glycol(PEG)]-acetate (HS-$(CH_2)_5$-$(OCH_2CH_2)_6$-OCH_2-COOH)) in excess. After another night of incubation, the PEG stabilized silver nanoparticle-oligonucleotides conjugates were cleaned by repeated centrifugation and finally resuspended in 80 mM phosphate buffer. In the last reaction step the two batches were combined to assemble into dimers. Formed dimers were then purified by gel-electrophoresis in a 1% agarose gel using 0.5x tris borate EDTA (TBE) as running buffer and isolated from the gel by electroelution. The isolated plasmon rulers were stored in running buffer at 4℃. For the imaging experiments the plasmon rulers were then diluted in Hanks' balanced salt solution.

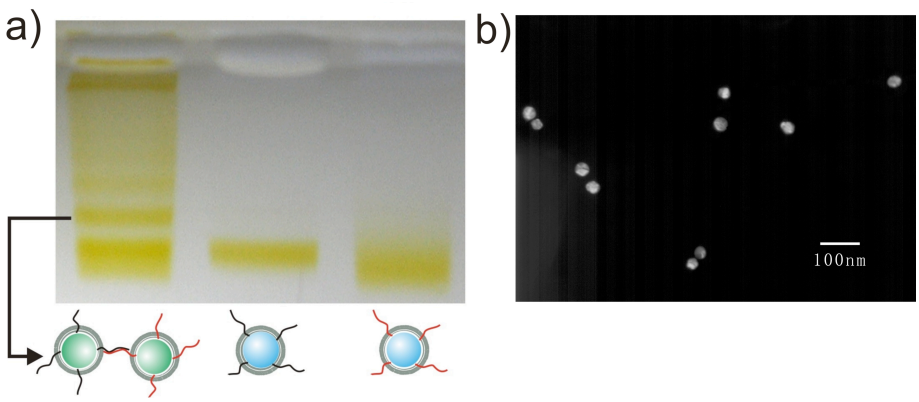


Figure S5: Purification of silver plasmon rulers. a) The band containing the hybridization mixture (left lane) contains a ladder structure indicative of successful hybridization of the silver nanoparticles functionalized with complementary DNA handles (middle and right lanes). b) TEM micrograph of isolated plasmon rulers. The resulting plasmon rulers contained typically ~75% dimers.

End-to-End Distance of the DNA Tether. The DNA tether contains both single and double-stranded regions:

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HS-AAA AA AAA ATA GTT CGA TAT CGG ATG TGG TGT CAG TCG TAG CGT GAG
                                                  || || | | | || | || | | | | | | |
                                                  CC ACA GTC AGC ATC GCA  CTC AAA AAA AAA A-SH
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This single-stranded and double-stranded segments that have different flexiblities. We modeled the complete DNA tether as a hybrid comprising one 20 base pair long double-stranded and one 38 nucleotide long single stranded DNA segment using the methodoly derived by Rivetti et al. [1] for polymers comprising segments of different flexibility. All imaging experiments were performed in Hanks' balanced salt solution (~138 mM NaCl). At this salt concentration the persistence length (L_P) of single stranded DNA is $L_P \approx 2.5$ nm[2] and its contour length is L_c = 0.64 nm / nucleotide. For the double stranded segment we used L_P = 53 nm and L_c = 0.34 nm / basepair.[3] With these parameters we obtain an approximate end-to-end distance of the DNA tether of S_0 = 13.7 nm.

1. Rivetti, C., C. Walker, and C. Bustamante, *Polymer chain statistics and conformational analysis of DNA molecules with bends or sections of different flexibility.* Journal of Molecular Biology, 1998, **280**, 41.
2. Murphy, J.C., I. Rasnik, W. Cheng, T.M. Lohman, and T. Ha, *Probing Single-Stranded DNA Conformational Flexibility Using Fluorescence Spectroscopy.* Biophysical Journal, 2004, **86**, 2530.
3. Smith, S.B., Y.J. Cui, and C. Bustamante, *Overstretching B-DNA: The elastic response of individual double-stranded and single-stranded DNA molecules.* Science, 1996, **271**, 795.