SUPPORTING INFORMATION

Probing DNA Stiffness through Optical Fluctuation Analysis of Plasmon Rulers

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Materials

The following materials were used as obtained from the vendors: trichloro(octadecyl)silane (Sigma-Aldrich); 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) (Avanti Polar Lipids Inc.); 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(cap biotinyl) sodium salt (biotin-PE) (Avanti Polar Lipids Inc.); thiol-alkyl-PEG,HS-$C_{11}H_{22}$-EG_6-COOH, EG=OCH_2CH_2 (NANOCS Inc.); PAMAM dendrimer generation 4 (Sigma-Aldrich). All DNA strands are purchased from Integrated DNA technologies.

Assembly of DNA tethered Gold NP Dimers (Plasmon Rulers)

Citrate stabilized gold NP monomers (56±2.4nm diameter) were synthesized by a Turkevich synthesis and then functionalized through a sequential ligand exchange procedure.[1-3] The gold colloid with a concentration of approximately 9×10^{10} particles/mL was first stabilized by addition of bis(*p*-sulfonatophenyl)phenylphosphine (BSPP) dipotassium salt at a concentration of 1mg/mL. The mixture was incubated in a water bath at 45 °C water overnight. The particles were then washed once (4,000 rpm, 10 min) by centrifugation and resuspension in T20 (20 mM NaCl/10 mM Tris, pH8.0). The final concentration of the particles was 3×10^{12} particles /mL.

The particles were split into two batches to allow the functionalization with different thiolated single-stranded oligonucleotides (HS-ssDNA) that either hybridize with each other or to a third ssDNA linker strand to form DNA tethered dimers. The DNA sequences are shown in Table S1.

Table S1. Detailed Structure and Sequence of DNA use. Note: DNA sequences are printed with the same color code used for the corresponding in Figure 1a of the manuscript.

	Sequence (5' →3')
PR1 Handle 1	HS - AG GCT GGA GGT TGG TTC ACT TCA TAC ATC AAC CAC AAG TCT CCT ACA CCT GC
PR1 Handle 2	HS - GC AGG TGT AGG AGA CTT GTG GTT GAT GTA TGA AGT GAA CCA ACC TCC AGC CT
PR2 Handle 1	HS - TC AGT CGT AGC GTG AGG CTA GAT GAT CGT A
PR2 Handle 2	HS - TA CGA TCA TCT AGC CTC ACG CTA CGA CTG A
PR1/2 Matrix DNA	SH - A AAA AAA AAA CTC ACG CTA CGA CTG ACA CC
PR3 Handle 1	SH –AAA AAA AAA AAG GCT GGA GGT TGG TTC ACT
PR3 Handle 2	GTG ACT ATG TAA CTG GCT GAA AAA AAA AAA - SH
PR3 Linker	TCA GCC AGT TAC ATA GTC ACG AAT GGA CGC AGG TGT AGG AGA CTT GTG GTT GAT GTA TGA AGT GAA CCA ACC TCC AGC CT
PR1/2/3 HS-DNA-Biotin	SH- AAA AAA AAA AGA CCT ACT AAG ACT ACT ACA CAA CCA GAG A-biotin

For PR1 and PR2, three different ssDNAs were used. One NP building block for the PRs was functionalized with Handle 1 (Table S1), HS-ssDNA-biotin, and a third 30 nucleotide "matrix" DNA to assemble a DNA brush and passivate all available surface sites. The molar ratios of the DNAs was 50:30:20. A total amount of 1 µL of 100 µM ssDNA mixture was added into each centrifuge tube containing 30µL NP solution and incubated overnight at room temperature. The second NP was incubated with Handle 2, HS-ssDNA-biotin and matrix DNA (DNA ratio 1:30:69). In order to obtain NP dimers with reasonable yield and, at the same time, reduce the probability of multiple tether formation in the PRs, NPs were functionalized with a high surface concentration of DNA Handle 1, but the surface concentration for Handle 2 was lower. In all cases 1 µL of 10 mM thiol-alkyl-PEG-COOH was added into each tube after the DNA functionalization and incubated overnight to further stabilize the gold NPs. The functionalized NP monomers were washed three times by centrifugation (4,000 rpm, 10 min) and resuspension in DDI water followed by one wash in T90 (90 mM NaCl/10 mM Tris, pH8.0). The two flavors of monomers with complementary strands were then combined to anneal overnight at room temperature.

For PR3, a third ssDNA linker was used to form tethers, and the procedure was slightly altered. After passivation with BSPP and redispersion in T20, both handles were mixed with biotinylated HS-ssDNA (70:30 molar ratio). After functionalization with DNAs and PEGs, the particles were washed three times with water and one time with T90. 1 µL of 1 µM linker ssDNA solution was added to each tube of NPs with handle 1 and annealed overnight. The particles were washed four times with T90 before they were combined with Handle 2 functionalized NPs.

The gold NP dimers were separated from monomers and larger clusters by electrophoresis in a 1% agarose gel in 0.5x TBE Buffer under 120V voltage for 40 minutes (**Figure S1a**).[1] The dimer band was cut out of the gel and submerged in a small volume of T50 (50 mM NaCl/10 mM Tris, pH8.0) buffer overnight to let the dimers diffuse out of the gel. The dimer purity was characterized by SEM (one representative micrograph of surface immobilized dimers is shown in **Figure S1c**) and summarized in **Figure S1b**. The obtained samples were mainly composed of

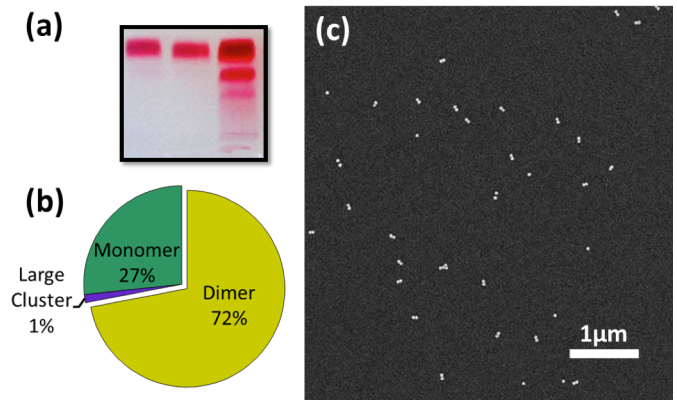

Figure S1. (a) 1% Agarose gel containing (from left to right): NP monomers functionalized with PR1 Handle 1, NP monomers with PR2 handle 2, and their mixture. (b) Contributions from NP monomer, dimers and larger clusters to the final sample as determined by SEM. (c) SEM picture of final sample.

NP dimers with a yield of over 70%. The remaining 30% were made up of monomers and larger agglomerates.

Lipid Membrane Assembly

1x1 cm^2 quartz chips were cleaned by piranha solution before silanization. Chips were submerged in 2mM trichloro(octadecyl)silane solution in hexane and carbon tetrachloride (80/20 v/v) for one hour at room temperature. After silanization, the chips were rinsed with an excess of acetone and baked at 80°C for one hour. Subsequently, the chips were stored in a desiccator until further use.

The lipid chloroform solution containing 99.5% POPC and 0.5% (w%) biotin-PE was rotary evaporated and dissolved in decane to obtain a final concentration of 2mg/mL. A silanized substrate chip was first mounted on a 3x1 $inch^2$ plastic slide and submerged in T50 (50 mM NaCl/10 mM Tris, pH8.0). 2 µL lipid decane solution was then applied directly onto the hydrophobic quartz substrate surface. An air bubble was introduced and scanned across the whole substrate surface for three times to form a uniform layer of decane covering the whole substrate. A lipid monolayer was formed through lipid self-assembly. A home-made chamber top was then assembled onto the 3x1 inch plastic slide to form a flow chamber adaptable to operation in a microscope.

Prior to plasmon ruler sample loading, 0.5 mL of 0.2 mg/mL NeutrAvidin in T50 was flushed into the flow chamber and incubated for 10 min to allow PR binding to biotin-PE contained in the lipid monolayer. Unbound protein was then flushed out with excess T50 buffer. A dispersion of plasmon rulers (approximately 10 pM) in T50 was flushed into the chamber and incubated for 10 min before it was flushed out with T50. In some experiments 6 pM G4 PAMAM dendrimer in

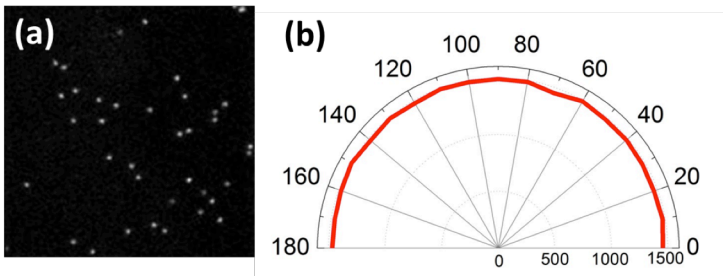

Figure S2. (a) Darkfield microscope image of 200 nm PS particles. (b) Polar plot of intensity at different linear analyzer angles recorded from individual particles.

T50 was flushed into the flow chamber to compact the plasmon rulers diffusing on the lipid membrane.

Optical Characterization

Darkfield experiments were performed with an Olympus IX71 inverted microscope. A 100 W tungsten burner was used as illumination source. The samples were illuminated through an oil darkfield condenser (NA 1.2-1.4). Scattering signals from plasmon rulers in the sample plane were collected with a 60× oil objective (Olympus RMS60X-PFOD) with NA 0.65. The signal was further magnified by a 1.6× lens and then split into two beam paths (530 nm channel and 585 nm channel) using a dichroic mirror and two bandpass filters. The signal of each channel was collected on a separate electron-multiplying charge coupled devices (EMCCDs). We used Andor IxonEM$^+$ detectors with a maximum detection area of 40 x 40 μm^2 and a resolution of 128×128 pixels. The two cameras were triggered to start simultaneously with an external pulse generator.

The polarization of the incident light was analyzed with spherical polystyrene particles. A linear analyzer was inserted in front of the EMCCD camera. **Figure S2b** shows an analyzer angle independent intensity distribution, which confirms that the excitation light in the sample plane is unpolarized.

Data Processing and Controls

Darkfield movies were recorded from 5-20 different locations within one chamber. The movies were 20s in length and typically include tens of plasmon rulers in each movie. The raw intensity data of each particle in each frame of each channel was fitted with a three dimensional Gaussian peak whose volume was used as the particle intensity by a home written Matlab code.[4]

We used 80nm gold NPs as control and measured the dependence of $\sigma^2(R)$ to the signal intensity by systematically varying the incident light intensity. The 80nm NPs were functionalized using matrix DNA and HS-ssDNA-biotin (70:30 molar ratio) and measured on a self-assembled membrane using identical experimental procedures as for the PRs. A total of 23,000 trajectories of various signal intensities were collected. The $\sigma^2(R)$ values for NPs recorded at different intensities were histogrammed (total intensity bin size 3000 a.u.) and fitted to a normal distribution within each bin. We only included PR data in our analysis whose variance was at

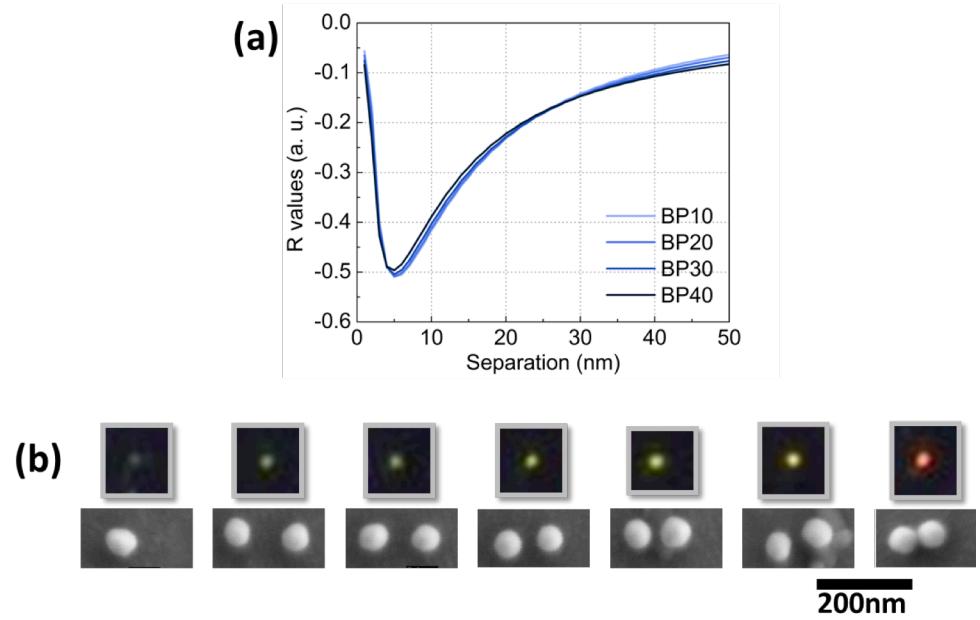

Figure S3. (a) Simulated *R*(*S*) functions; lighter to darker blue represents increasing widths of the bandpass filters used in each channel. (b) Color recorded by digital camera (top row) and corresponding SEM (bottom row) pictures of dimers of various interparticle separations.

least three standard deviations (>99.7%) higher than the NP control in the respective intensity bin.

FDTD simulations

The numerical simulations of gold dimers were performed by finite-difference time domain (FDTD) calculations using the commercial software Lumerical. All models were analyzed in a homogeneous medium with refractive index n=1.33 using literature reported dielectric function of gold.[5] The longitudinal and transverse resonance modes for dimers with different interparticle separations were simulated with incident light polarizations parallel or perpendicular to the long dimer axis. The scattering cross section was calculated as the average of these two modes and multiplied with the spectrum of the light source to simulate experimental scattering spectra under our experimental condition. The *R* values of individual Plasmon Rulers were calculated by integrating their simulated scattering spectrum in each color channel. The simulations show that the *R*(*S*) dependence is relatively insensitive to the width of the bandpass filters used for ratiometric imaging (**Figure S3a**). We, therefore, choose a wide bandpass filter width of approximately 40 nm to optimize signal-to-noise ratios.

Experimental Calibration

Calibration dimers were assembled on an ITO coated glass support using a template guided self-assembly approach.[4] The samples were immersed in water and the *R* values of the dimers were subsequently measured using the ratiometric imaging set-up described above. After that, the interparticle separations of the imaged surface-bound dimers were obtained from their inspection

in the SEM (**Figure S3b**).

Calculation of PR Potentials

Each individual trajectory was converted into a PR potential through the following steps. Each S trajectory was first converted into an extension (x) trajectory by subtracting the equilibrium separation $\overline{S_0}$. The probability distribution P(x) was then obtained by histogramming the x values of individual trajectories and dividing by the number of frames in the trajectory. The potentials shown in Fig. 5a was constructed using Eq. 4 $\varphi(x)/kT = -\ln(P(x))+C$. Since our primary goal is a comparative analysis of the PR tether stiffness, which depends on the derivative of the potential, we C was adjusted to yield $\varphi(x=0) =0$.Since the potentials were well described by parabola fits, especially at small displacements from the equilibrium point x=0, we treat the plasmon ruler potentials as Hookean spring and fit the lower part of each potential with the equation $\varphi(x) = \frac{1}{2} k_{eff} x^2$, generating a k_{eff} for each PR.

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