Published in final edited form as:

J Phys Chem C Nanomater Interfaces. 2010 March 1; 114(11): 4901–4908. doi:10.1021/jp911858v.

Calibration of Silver Plasmon Rulers in the 1-25 nm Separation Range: Experimental Indications of Distinct Plasmon Coupling Regimes

Linglu Yang, Hongyun Wang, Bo Yan, and Björn M. Reinhard

Department of Chemistry and The Photonics Center, Boston University, Boston, Massachusetts 02215

Abstract

In this manuscript pairs of flexibly linked silver nanoparticles, so called silver plasmon rulers, are synthesized using a rational DNA programmed self-assembly procedure. The plasmon resonance energy (E_{res}) *versus* distance relationship is calibrated for dimers comprising sphere-like silver nanoparticles with diameters of 41.0 ± 4.6 nm and surface-to-surface separations between 1-25 nm. Single dimer Rayleigh scattering spectra are correlated with structural information of the same dimers obtained through transmission electron microscopy with 1 nm spatial resolution. The calibration reveals different plasmon coupling regimes. For larger separations the plasmon resonance energy red-shifts continuously with decreasing center-to-center distance (L) until the L to diameter (D) ratio reaches a value of $L/D \approx 1.05$. For shorter interparticle separations E_{res} does not further red-shift; instead the slope of $E_{\text{res}}(L/D)$ levels off, and the measured resonance energies become broadly distributed. Overall, the spectral response of nearly touching dimers indicates that the plasmon coupling does not continue to intensify with decreasing interparticle separation at very short separations. The performed characterization of the distance dependent plasmon coupling forms the quantitative foundation for applications of plasmon rulers in plasmon coupling microscopy and nanoplasmonic devices with defined near- and far-field properties.

Keywords

Noble metal nanoparticle; Plasmon coupling; Plasmon rulers; Active nanostructures; Nanoplasmonics

1. Introduction

Gold and silver nanoparticles are versatile materials that exhibit strong optical responses at the respective plasmon resonance frequencies at which the incident light excites collective oscillations of the conduction band electrons.[1,2] The resonance frequency depends on the particle size and shape as well as on the refractive index of the surrounding medium, which makes noble metal nanostructures multifunctional materials for biological and chemical sensing.[1,3] Due to resonant interactions of the incident light with the conduction band electrons, noble metal nanoparticles act as nanosized optical antennas that enhance the E-field in their immediate vicinity. If two nanoparticles are brought into close proximity these fields

bmr@bu.edu .

Supporting Information Available. Figures S1. This material is available free of charge via the Internet at <http://pubs.acs.org>.

overlap and the individual particle plasmons couple in a distance dependent fashion resulting in a field confinement and enhancement in the interstitial space.[4-10]

The classical electromagnetic interaction regime of plasmon coupling has been investigated for gold nanoparticles of different sizes and shapes, nanofabricated particle arrays as well as in pairs of DNA linked spherical gold nanoparticles, so called gold plasmon rulers, [7,8, 11-16] and general scaling laws have been derived.[17] With decreasing interparticle separation the coupling efficiency increases monotonically, and the predominantly dipolar longitudinal plasmon mode red-shifts, while the E-field confinement and enhancement increases. In the case of silver nanoparticles, Gunnarsson *et al.* investigated the interactions between nanofabricated silver nanodiscs and observed shifts in the resonance wavelength (λ_{res}) that were much larger than observed before in similar gold structures.[18] This finding raised our interest in silver nanoparticles as building blocks for dynamic molecular rulers that can report changes in the interparticle separation and as probes in plasmon coupling microscopy.[16,19-26] A larger dynamic range in λ_{res} combined with the sharper resonances of silver nanoparticles when compared with gold would improve the sensitivity and resolution of plasmonic molecular rulers. Gunnarsson *et al.* correlated the optical spectra of immobilized, nanofabricated silver discs with their interparticle separation as obtained by scanning electron microscopy (SEM).[18] Nanodiscs have the advantage that they can be conveniently generated with controlled distances by using electron beam lithography. However, for dynamic distance measurements in biological systems, anisotropic particles are less useful since the resonance wavelength of the coupled particles depends not only on the interparticle separation but also on the orientation of the probes which often cannot be controlled. This has been illustrated, for instance, in coupled nanorods whose optical response depends strongly on the orientation of the rods in the dimers.[27] In addition, due to limitations in the experimental spatial resolution, interparticle separations below 10 nm as well as structural details of the dimer junction could not be resolved in the work of Gunnarsson *et al.*. For many biological sensing applications, [3] nanoantenna design, as well as from a fundamental point of view,[4,28-32] the regime of strong coupling with surface-to-surface separations < 10 nm is, however, of high relevance.

Whereas for interparticle separations on the length scale of the particle diameter, plasmon coupling is dominated through dipole-dipole coupling, at short separations additional interparticle interaction mechanisms become relevant.[4,27-34] Higher order multipole modes contribute to the coupled dimer plasmon and finite size effects become important.[4,18,29, 30,31,32,35] When two nanoparticles touch, intraparticle charge neutrality is no longer required and new modes that are non-physical in separated particles become available.[29] The optical responses of dimers change abruptly upon contact formation and touching nanoparticles with so called “hourglass” structures show unique features which have been discussed, for instance, by Atay *et al.*[31] and Romero *et al.*[29]. Kim *et al.* have performed apertureless near-field scanning optical microscopy (ANSOM) of pairs of nearly touching gold nanocubes and found that E-field and phase distributions indicated a capacitive coupling between the cubes.[36] The spatial resolution of the ANSOM in this study was limited to ~10 nm and consequently changes in the coupling mechanism as function of surface-to-surface separation were not accessible.

In general the regime of nearly touching nanoparticles is very difficult to access experimentally and much of our knowledge about the change in the physical nature of plasmon coupling stems from theoretical studies. Zuluoga *et al.* have recently performed time dependent density functional theory studies of plasmon coupling in nearly touching noble metal nanoparticle dimers and found indications for a direct charge transfer between the particles. [28] This charge transfer results in a weakening of the plasmon coupling and thus a relative spectral blue shift. The work performed by Zuluoga *et al.* was limited to very small nanoparticles with diameters below 1.3 nm, but de Abajo observed a similar relative blue shift in the spectral response of

dimers of 20 nm diameter gold particles at short interparticle separations using a non-local dielectric function in a specular reflection model (SRM).[30,35,37] Overall these theoretical studies imply a strong change in the nature of the plasmon coupling mechanism in nearly touching noble metal nanoparticles. The predicted change in the distance dependence of the optical response has also been associated with reduced scattering cross-sections and quenched near-fields.[30] The latter would also have important implications for the design of nanoplasmonic devices with defined optical responses.

One important aim of this study is to experimentally verify the predicted change in the plasmon coupling mechanisms in nearly touching dimers and to quantify at what interparticle separation it occurs. In order to be able to access interparticle separations of a few nanometers, which is an absolute necessity for this endeavor, we assembled dimers of silver nanoparticles using a DNA programmed self-assembly approach. The spectral response of individual dimers was then correlated with their structure through combination of single dimer Rayleigh scattering spectroscopy and transmission electron microscopy (TEM).[34] We routinely achieve a spatial resolution of 1 nm for the determination of the interparticle separation in correlated spectroscopic/TEM measurements. The second aim of this paper is to provide a calibration curve for the distance dependent optical response of flexible linked silver nanoparticles in the classical electromagnetic interaction range. This relationship is the quantitative foundation for silver plasmon ruler applications.

2. Experimental Section

DNA Programmed Assembly of the Probes: Silver Nanoparticle Dimers

Two batches of commercial citrate stabilized silver nanoparticles with an average diameter of $d = 41.0 \pm 4.6$ nm, as measured by TEM, were reacted with partly complementary 5' thiolated DNA handles (Integrated DNA Technologies, IDT). We used the following sequences; 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 oligonucleotides in a ratio of 1:1 in 40 mM phosphate buffer, pH 8 overnight both batches were passivated by adding mixture of alkyl-PEG acetates ($\text{HSC}_{11}\text{H}_{22}(\text{OC}_2\text{H}_4)_6\text{OCH}_2\text{COOH}$) and biotinylated PEGs ($\text{HSC}_2\text{H}_4\text{CONHC}_2\text{H}_4\text{O}(\text{C}_2\text{H}_4\text{O})_8\text{C}_2\text{H}_4\text{NHCOC}_4\text{H}_8\text{-Biotin}$) (molar ratio: 24:1) in excess. After another night of incubation, the PEG stabilized silver nanoparticle-oligonucleotides conjugates were cleaned by repeated centrifugation and re-suspension (3x) and finally dispersed in 80 mM phosphate buffer, pH 8. In the last reaction step, the two batches were combined for ~1 h 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 at 170 Volts for 15 minutes. Finally the dimer band was isolated from the gel. Dimers were recovered by electroelution and stored in TBE buffer at 4°C.

TEM Sample Preparation

5 μL of a 1 mg/mL BSA-Biotin solution were incubated on a formvar coated TEM finder grid (100 mesh Gilder binary finder grid, SPI Inc.) for 5 min in an atmosphere saturated with water. The remaining solution was removed with filter paper and the grid was rinsed with T50 (50 mM NaCl, 10 mM Tris-HCl, pH 7). Then the grid was incubated with 1 mg/mL Neutravidin in T50 for 5 min. After that the grid was washed with T50 and subsequently 5 μL silver plasmon ruler solution, as obtained through electroelution, were incubated on the grid for 5 min. Finally the grid was washed with T50 and then with DI water. After washing, the TEM grids were dried under ambient atmosphere.

Experimental Set-up and Spectrum Processing

All optical measurements were performed in an Olympus BXWI microscope under darkfield illumination. The samples were illuminated with unpolarized whitelight from a 100 W Tungsten halogen lamp through an oil darkfield condenser (numerical aperture, NA = 1.2 - 1.4). The scattered light of individual dimers was collected with a 60x oil immersion objective (NA = 0.65) and recorded with a back-illuminated CCD camera (Andor, DV437-BV); the distribution of the dimers in this image served as map that guided the identification of individual dimers in TEM micrographs at lower magnification. After recording an image of all the scattering objectives in the field of view, individual dimers were then analyzed spectroscopically. Using a switchable mirror the scattered light of individual dimers was injected into a 303 mm focal length imaging spectrometer (Andor, Shamrock) with a 150 lines/mm grating with blaze wavelength at 500 nm and back-illuminated CCD detector (Andor, DU401-BR-DD). A variable slit in front of the detector was used to selectively record the scattering spectra of individual scattering sources. For polarization resolved measurements an analyzer was inserted into the beampath and stepwise rotated in 15 degree steps.

The recorded dimer spectra were background corrected through subtraction of the spectrum of an adjacent area of the CCD chip that did not contain a scatterer and then corrected for the excitation profile of the whitelight by dividing through the spectrum of an ideal whitelight scatterer. In case of the polarization resolved measurements, separate excitation profile corrections were performed for each analyzer orientation.

FDTD Simulations

FDTD simulations have been shown to reliably predict optical properties of coupled nanoparticles.[38] In this manuscript all FDTD simulations of silver dimers were performed with Lumerical FDTD solutions (version 6.5.4). Two 40 nm silver nanoparticles were created with edge-to-edge separations from 3.2 nm to 27.2 nm. The dielectric function of silver was generated through a four coefficient fit to the CRC database in the software, the refractive index of the surrounding environment was set to $n_r = 1.474$. The FDTD area was defined as a cube in a 3-D space with 400 nm length in each side. Within this area a mesh override region of 160 nm side length with 3 nm mesh size was defined to contain the dimers in its center. A total field scattered field (TFSF) source was injected from 140 nm below the dimer plane. The simulated light source had a wavelength range between 300 nm to 600 nm, with the polarization parallel to the long dimer axis. The power of the scattered light was detected by a group of six 2-D power detectors which corresponded to the 6 sides of the mesh override region. The total power was normalized to the source power and plotted against the wavelength. In each spectrum the peak position of the plasmon resonance was obtained by fitting the global scattering maximum.

3. Results and Discussion

Our preparative route to silver plasmon rulers is outlined in Figure 1. Oligonucleotides and silver nanoparticles (average diameter: $d = 41.0 \pm 4.6$ nm) were mixed in a ratio of 1:1 and then passivated and stabilized by assembling a monolayer of short polyethylene glycol (PEG) molecules on the particle surface (see Figure 1a). The PEGs were mixed with a small percentage of biotinylated PEGs to enable their efficient immobilization on Neutravidin functionalized surfaces. Assembled dimers were purified from the hybridization mix through gel electrophoresis and isolated from the gel through gel-electroelution (see Figures 1b&c). The recovered dimers were then immobilized on Neutravidin functionalized TEM grids. After that, the samples were immersed in index matching glycerine ($n_r \approx 1.474$), sandwiched between two glass coverslips and transferred to the optical microscope to record the scattering spectra of individual dimers.[16] After spectroscopic characterization, the glycerine was removed from

the TEM grids through a quick rinse with methanol and the grids were inspected under the TEM. The sequence of the experiments, first optical then structural characterization, was deliberately chosen to avoid any changes in the optical properties of the nanoparticles due to electron beam exposure. [34] In test experiments with gold plasmon rulers we had confirmed that the sample handling procedure does not change the interparticle separation of surface immobilized plasmon rulers. We demonstrated this by measuring the interparticle separations before and after immersion in glycerin and subsequent washing with methanol (Figure S1). For detailed information about the dimer assembly, the TEM sample preparation, the optical set-up, and the processing of the recorded spectra, please refer to the Experimental Section.

The correlation of the spectra of an individual dimer with its structure as obtained by TEM was achieved by using the spatial distribution of the particles on the TEM grid as internal marker to correlate the electron micrograph with the image of the optical microscope. Patterns of individual scatters observed in the optical microscope (Figure 2a) were found again in the TEM (Figure 2b) at low magnification (8000x). Structural details of the assigned dimers such as interparticle separation and the particle diameters were then determined at high magnification (Figure 2c). In a typical experiment the dimers' structures were characterized at a magnification of 100 000x at which we achieved a spatial resolution of ~1 nm.

Colloidal nanoparticles always contain a natural distribution in sizes and shapes and the commercial silver particles used in our studies were clearly not ideal spheres. TEM images of the particles typically show spheroids, polyhedra and distorted polyhedra at higher magnification. Especially in the case of some asymmetric dimers containing strongly non-spherical particles the spectra (see, for instance, Figures 2d1&d2) can contain multiple features or shoulders indicating the presence of several modes. Since the focus of this study is the distance dependence of plasmon coupling, we only included dimers comprising sphere-like particles in our analysis to minimize the influence of the particle shape. We define sphere-like particles in this work as spheroids and polyhedra with aspect ratios < 1.2. Asymmetric dimers for which the diameters of the two particles deviated by more than 20 % were also not considered. We emphasize that only dimer c4 in Figure 2 fulfills our selection criteria and was included in our analysis. Dimers with irregular or strongly non-spherical particles (for instance, c1 and c2 in Figure 2) were excluded. The spectra of these more complex dimers show interesting features and our correlated spectroscopic/structural experimental approach is potentially useful to elucidate the underlying structure-spectrum relationships. In the current work we focus, however, entirely on the distance dependent plasmon coupling of sphere-like particles. Unless otherwise noted, the resonance energies (E_{res}) were obtained by fitting the global peak intensities of the scattering spectra obtained with unpolarized white light.

At high magnification the TEM images show that the silver particles contain areas with different contrast indicative of polycrystallinity. This polycrystallinity could cause a reduction of the plasmon lifetime in individual particles but based on previous studies we anticipate that the effect on the distance dependent plasmon coupling is small. [18]

3.1. Distance Dependence of the Plasmon Resonance in DNA Tethered Silver Nanoparticle Dimers

In the classical electromagnetic coupling regime a plot of the ratio of resonance wavelength shift ($\Delta\lambda$) to monomer resonance wavelength (λ_0) *versus* the ratio of interparticle separation to particle diameter is particularly useful to illustrate the “universal” scaling of the plasmon coupling since this relationship is particle size invariant over a wide range.[7,17,18] In this study we only investigate silver particles with an average diameter of 41nm, and it is therefore more instructive to plot the resonance energy (E_{res}) against the ratio of center-to-center separation (L) to effective diameter (D). D is obtained as average of the individual particle diameters d_1 and d_2 in each dimer which are themselves obtained by taking the mean of two

perpendicular particle axes. The L values are then computed from the particle diameters and the surface-to-surface separation (S) measured at the closest contact between two particles:

$$L = S + \frac{1}{2}(d_1 + d_2) \quad (1)$$

For calculation of the L/D ratio we divided the center-to-center separation (L) through the effective diameter (D):

$$L/D = \frac{L}{\frac{1}{2}(d_1 + d_2)} \quad (2)$$

In solution the plasmon ruler interparticle separation is variable and the plasmon ruler has an average interparticle separation that is determined by the length of the DNA tether. Upon binding of the biotinylated plasmon ruler on the Neutravidin functionalized TEM grid, the plasmon ruler is “captured” in one specific configuration. The immobilization of many DNA tethered silver nanoparticles on a TEM grid leads to a broad distribution of interparticle separations and enables the investigation of the distance dependent optical properties of plasmon rulers one dimer at a time. In Figure 3a we show the fitted peak resonance energies (E_{res}) as function of L/D for all measured dimers; the included errors represent our peak fitting precision as ΔE_{res} and the difference in the particle size in individual dimers as $\Delta(L/D)$. The plot also contains the asymptotic limit of individual silver nanoparticles ($E_0 = 2.77$ eV) as horizontal dashed line. In previous studies of plasmon coupling, the distance dependence of the resonance energy was well described by a single exponential fit in the classical electromagnetic interaction regime.[7, 16-18] For the silver dimers investigated here, we observe that a single exponential fit describes the $E_{\text{res}}(L/D)$ dependency insufficiently across the whole investigated L/D range. The goodness of fit is significantly improved when dimers with nearly touching nanoparticles are excluded. This is demonstrated in Figure 3a by including two different exponential fits to the experimental data. While the green dashed line is a fit to all measured E_{res} data points (coefficient of determination, $R^2 = 0.50$), the red dashed line is an exponential fit ($R^2 = 0.78$) only to the data in the range $L/D > 1.05$. The red fit describes the experimental data for $L/D > 1.05$ clearly much better. It is significantly steeper than the green fit and consequently all resonance energies with $L/D < 1.05$ lie above the resonance energy predicted by the red fit at $L/R = 1.05$.

E_{res} does not further red-shift at very short interparticle separations. The slope in the measured $E_{\text{res}}(L/D)$ relationship levels off at very short interparticle separations and the distribution of the measured E_{res} values broadens. The measured resonance energies for $L/D < 1.05$ vary between $E_{\text{res}} = 2.35$ eV and $E_{\text{res}} = 2.63$ eV. The observed change in slope and the broad spread of the measured resonance energies indicate the occurrence of additional interaction mechanisms between the particles at short separations. Before we analyze those in more detail, we first want to review the behavior of the dimers with $L/D > 1.05$, since this is the separation range relevant for most applications of the DNA tethered silver noble metal nanoparticles, for instance, as plasmon rulers.

For $L/D > 1.05$ the resonance energies continuously red-shift with decreasing L/D and this behavior is best described by a single exponential (red dashed line in Figure 3a) with the formula:

$$E_{\text{res}}^{\text{fit}} = \left(2.77 - 457.36 e^{-\frac{(L/D)}{k}} \right) \text{ eV} \quad (3)$$

with $k = 0.16 \pm 0.03$. The observed spread of the individual data points around this global trend arises from variations in size and shape of the nanoparticles. Although we excluded particles with strongly non-spherical shapes in our analysis, this selection cannot completely compensate discrepancies in the resonance energies of the individual particles due to more subtle differences in particle size and shape. Variations in the resonance energies of the individual particles within one dimer influence the coupling efficiency [39] and increase the spread of the measured E_{res} values for a defined L/D value.

To account for the error in the experimental fit we included an error range ΔE in the $E_{\text{res}}^{\text{fit}}$ plot in Figure 3 obtained from fits with k values in the range of 0.13-0.19 as yellow shaded area. We emphasize that the observed continuous red-shift of E_{res} with decreasing L/D for $L/D > 1.05$ does not depend on the data point at $L/D = 1.07$. This data point is strongly red-shifted and consistent with a short interparticle separation but could also be an outlier. If this data point is omitted from the fit, k increases to $k = 0.19$ which is still within the plotted error range. Despite the spread in the experimental E_{res} values, equation (3) confirms a systematic distance dependence of the resonance energy for $L/D > 1.05$ and calibrates the silver plasmon ruler. This relationship can be utilized for optical distance measurements between individual silver nanoparticles and Rong *et al.* used this approach to map the lateral heterogeneity of mammalian cell surfaces [25].

In Figure 3b we compare this calibration relationship for DNA linked silver nanospheres with the calibration curve obtained for nanofabricated silver nanodiscs by Gunnarsson *et al.* [18], finite-difference time-domain (FDTD) simulations for 40 nm silver spheres, and the universal scaling law in gold nanoparticles derived by Jain and El-Sayed. [17] The comparison of the silver dimer curve (red dashed line) with the gold universal scaling curve (black line) shows that the fractional shift ($\Delta E/E_0$) is larger for silver than for gold dimers, making silver dimers more sensitive molecular rulers for most practical applications. Jain and El-Sayed and Gunnarsson *et al.* determined decay constants of the single exponential fit of gold and silver nanoparticle dimers of 0.18 and 0.22 respectively [17, 18]. The k value observed for silver plasmon rulers in the classical electromagnetic interaction regime in this work $k = 0.16 \pm 0.03$ is in good agreement with these previous studies. Our studies thus confirm a universal scaling of the plasmon coupling distance decay between gold and silver dimers. The $\Delta E/E_0$ curve for silver nanodiscs reported by Gunnarsson *et al.* (black dashed line) is shifted to lower $\Delta E/E_0$ values, corresponding to a larger magnitude of the fractional wavelength shift, but overall has a similar slope as the curve we obtained for silver spheres. The finite-difference time-domain (FDTD) simulations for dimers of 40 nm silver spheres performed in a homogenous medium of $n_r = 1.474$, which are included as black dotted line in Figure 3b, lie between the experimental curves for silver spheres and discs. The simulations are in reasonable agreement with the experimental observations but do not provide additional insight into the differences between the sphere and the nanodisc curves.

Multiple factors such as the shape of the particles and the different environments can cause differences in the spectral response of silver nanospheres and nanodiscs. The silver spheres investigated in this study were immobilized through proteins on formvar and immersed in glycerine, whereas the nanodiscs in the work of Gunnarsson *et al.* were measured on a glass substrate at the glass-air interface. Furthermore, the polarizability of nanoparticles is shape dependent which can influence the distance dependent plasmon coupling. In addition, the silver plasmon rulers are assembled from commercial colloids which will show some degree of oxidation. It is possible that the solution based self-assembly process of the DNA linked silver nanoparticles is accompanied by an additional partial oxidation of the silver nanoparticles. The resulting “poisoned” silver dielectric function could contribute to a weaker coupling between the spheres and needs to be considered when comparing silver plasmon ruler data with calibration relationships obtained from pure silver particles. Differences in the particle shape,

non-consideration of the substrate and inaccuracies in the dielectric function can also account for the observed differences between the FDTD simulations and the experimental data.

3.2. Optical Response of “Nearly Touching” Dimers

Our studies of the distance dependence of the plasmon coupling between DNA tethered silver nanoparticles revealed that the resonance energy red-shifts continuously with decreasing interparticle separation until a threshold of $L/D \approx 1.05$ is reached where the slope in the measured $E_{\text{res}}(L/D)$ relationship levels off and the distribution of the measured resonance energies broadens. For some of the dimers with $L/D < 1.05$ in Figure 3a we cannot exclude that particles are directly touching, since under our experimental conditions the TEM could only resolve interparticle gaps with surface-to-surface separations (S) > 1 nm. For most investigated dimers we can, however, unambiguously identify a gap between the particles and thus exclude that the particles are directly touching. This is illustrated in Figure 4, in which we compare the scattering spectra of representative nearly touching dimers (Figures 4a&b) with those of two dimers that are still coupled but have larger interparticle separations (Figures 4c&d).

The scattering spectra of all dimers in Figure 4 were recorded in a polarization sensitive fashion. These measurements were performed by rotating an analyzer in front of the detector and measuring the scattering spectra as function of the analyzer angle. We recorded one spectrum every 15° between 0° and 180° . All dimers exhibit at least two distinct plasmon modes, a low and a high energy mode, whose resonances are indicated as dotted vertical lines in the spectra (middle column in Figure 4). As shown in the full spectrum sweep in Figure 4a, the relative intensities of the two modes changes in an anti-correlated fashion and the maxima of the modes are phase-shifted between them by 90° . Due to their relative orientations with regard to the long dimer axis, the modes are tentatively assigned to the longitudinal mode, which dominates the scattering spectrum in case of a non-polarization sensitive detection, and the vertical plasmon mode, respectively. The vertical mode is often convoluted with the longitudinal mode because the polarization axis is not perfectly aligned with the short dimer axis in our scans. However, in most cases a deconvolution into separate modes is possible (figure 4c & d) and confirms the presence of two modes. We determined the energetic difference ΔE_{LV} between high and low energy modes as function of L/D for 9 randomly chosen dimers which we recorded polarization resolved scattering spectra. The plot in Figure 5 reveals that ΔE_{LV} increases continuously with decreasing L/D . In the classical dipolar interaction regime the vertical mode is expected to blue-shift (relative to the asymptotic value of an individual spherical particle of ~ 2.77 eV) with decreasing interparticle separation. [12,29] We observed resonance energies of the vertical plasmon mode as high as ~ 3.0 eV in some of the nearly touching dimers.

At very short interparticle separations the increase in ΔE_{LV} is mainly due to a blue-shift of the high energy (vertical) plasmon mode. Although the low energy (longitudinal) mode of the silver dimers red-shifts initially with decreasing interparticle separation, there is no further red-shift after the interparticle separation has decreased to $L/D \approx 1.05$. The observation that the slope in the experimental $E_{\text{res}}(L/D)$ relationship levels off at $L/D \approx 1.05$, which corresponds to a surface-to-surface separation of ~ 2 nm in the investigated dimers, is experimental evidence for a change in the nature of the plasmon coupling mechanism for the longitudinal mode in pairs of nearly touching silver nanoparticles. In periodic arrays of gold disc (150 nm diameter, 17 nm height) dimers at the glass/air interface a dramatic splitting of the plasmon resonance was observed if the conducting junction between two touching nanoparticles was removed through a 100 fs laser pulse. [31] One of the resulting resonance peaks in these nanogap separated dimers was blue-shifted and the other one red-shifted with regard to the resonance in dimers with large interparticle separations. The shifted resonances observed in the nearly touching discs could be assigned to a high-frequency mode of higher multipolar character and

a low-frequency dipolar mode. [29] For the DNA linked spherical silver nanoparticles with a diameter of ~40 nm investigated in this study, we do not observe a plasmon resonance splitting of comparable magnitude as observed in the larger gold nanodiscs. Instead, we observe a gradually increasing energy difference between the high and low energy modes of the dimers with decreasing interparticle separation (see Figure 5).

We cannot exclude that the strongly red-shifted data point at $L/D = 1.07$ indicates the onset of a new interaction regime of strongly coupled particles in which the dipolar mode is – in analogy to the findings in ref [31] – strongly red-shifted. In this model, the blue-shifted resonance energies in the range $L/D < 1.05$ would then belong to quadrupolar modes while the dipolar modes would have shifted out of our detector range. This interpretation requires that between $L/D = 1.07$ and $L/D = 1.05$ the longitudinal dipolar mode jumps from ~2.2eV to below the detection threshold of our detector (1.57eV). While this is a possibility, the large discontinuous jump of the dipolar mode is unexpected. Simulations that could explain the observations in ref [31] have predicted that the dipolar mode continuously red-shifts with decreasing interparticle separation until the particles touch.[29] We also want to mention that the investigated plasmon rulers were clearly visible in the microscopy “by eye” and showed a vivid blue-greenish color. Consequently, we prefer an alternative interpretation of our experimental observations for the longitudinal mode based on the theoretical work by Abajo and Zuluoga *et al.* Both of these groups have recently investigated the distance dependent spectral response of strongly coupled noble metal nanoparticles theoretically and predicted a reduced plasmon coupling between particles in very close proximity. [28,30]

In nearly touching metal spheres, the longitudinal mode can induce a large charge pile-up around the point of closest separation. Due to the high charge density in the particle junction the non-local character of the metal dielectric function needs to be considered to obtain a correct description of the particles’ optical properties.[30,35] The work of de Abajo has shown that a non-local treatment of nearly touching nanoparticles leads to a reduced magnitude of the effective dielectric function of induced charges, which is equivalent to a strong screening and effective increase of the interparticle separation.[30] The non-local model predicts that the short-distance interactions between induced charges is weaker than that in locally described pairs of nanoparticles. In his paper de Abajo concluded that a non-local treatment results “in a softening of characteristic effects attributed to narrow gaps between metal nanoparticles”. [30] In addition, the work of Zuluoga *et al.* implies that direct charge transfer between the particles becomes possible at very short interparticle separations, which can further weaken the interparticle plasmon coupling.

Both theories predict a decreased slope in $E_{\text{res}}(L/D)$ at very short interparticle separations and are thus in qualitative agreement with our experimental observations. de Abajo predicted that for 20 nm diameter nanoparticles non-local effects should become significant at separations of about 2 nm, which is in good agreement with our observations. For two nanoparticles in vacuum, Zuluoga *et al.* predicted the charge transfer to set-in at separations of about 1 nm. Our experimental system comprises PEG coated particles that are connected by DNA and immersed in glycerin. In molecular systems effective charge transfer is possible over longer distances than in vacuum due to efficient charge-transfer mechanisms, such as superexchange or charge hopping. [40-42] We can therefore not exclude that both predicted effects, an increased screening and direct charge transfer, contribute to the experimentally observed stagnation of $E_{\text{res}}^{\text{fit}}$ for $L/D < 1.05$.

L/D is an important parameter that influences the spectral response in strongly coupled silver nanoparticles but it is not the only one. Other factors such as the size and shape influence the resonance energies of individual particles as well, and these additional dependencies explain the spread of the experimental $E_{\text{res}}(L/D)$ data. The sensitivity of the spectral response on

structural details in nearly touching nanoparticles does, however, not interfere with the overall change in the distance dependence of the plasmon coupling at close contact. For $L/D < 1.05$ the data points are asymmetrically spread; all data points lie energetically above the predictions from the $E_{\text{res}}^{\text{fit}}$ fit (equation 3) derived in the classical electromagnetic interactions regime ($L/D > 1.05$). Our experimental observations are therefore consistent with previous theoretical studies by de Abajo and Zuluoga *et al.* that predicted a change in the plasmon coupling mechanism at very short interparticle separations and confirm the existence of at least two distinct plasmon coupling regimes.

4. Conclusions

Using a DNA programmed self-assembly process and gel purification protocols we generated dimers of DNA tethered silver nanoparticles in solution. We calibrated the distance dependent plasmon coupling in these assemblies by correlating the plasmon resonance energy (E_{res}) obtained by single dimer scattering spectroscopy with the ratio of center-to-center particle separation (L) to diameter (D) as obtained by transmission electron microscopy of individual dimers. For $1.05 < L/D < 1.6$ we observe a continuous red-shift in E_{res} with decreasing L/D . The distance dependence of the resonance energy for dimers of DNA tethered silver nanoparticles in this classical electromagnetic interaction regime is best described by a single exponential function:

$$E_{\text{res}}^{\text{fit}} = \left(2.77 - 457.36 e^{-\frac{(L/D)}{k}} \right) \text{eV}$$

with $k = 0.16 \pm 0.03$. Stabilized silver nanoparticles have tremendous potential for applications in biosensing and imaging due to their advantageous photophysical properties [26]. Silver particles have even larger optical scattering cross-sections than gold nanoparticles with similar size. The performed $E_{\text{res}}(L/D)$ calibration for distances $L/D > 1.05$ provides now the quantitative foundation for applications of flexibly linked silver nanoparticles as dynamic molecular rulers, so called plasmon rulers.

Our analyses also reveal that the dynamic range of $E_{\text{res}}(L/D)$ is higher for silver than for gold dimers, [16] and that silver plasmon rulers achieve a higher sensitivity for detecting small distance changes at constant refractive index. In dimers with very short interparticle separations, $L/D < 1.05$, the slope in the measured $E_{\text{res}}(L/D)$ relationship levels off and E_{res} does not further red-shift with decreasing interparticle separation. This observation is consistent with the interpretation that the plasmon coupling does not further increase in nearly touching nanoparticles due to more effective screening and direct charge transfer.[28,30] The existence of two distinct plasmon coupling regimes has important implications for the design of nanostructures with maximized field enhancement, for instance, for applications in surface enhanced Raman spectroscopy (SERS).

Supplementary Material

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Acknowledgments

This work was financially supported by the National Institutes of Health through grants 5 R21 EB008822-02 and 1 R01 CA138509-01 and the National Science Foundation through grant CBET-0853798.

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Figure 1.

Assembly and characterization of silver plasmon rulers. a) Silver nanoparticles are functionalized with complementary single-stranded DNA (ssDNA) and pegylated with a mix of short thiolated alkyl-PEG-acetates and biotinylated PEGs. Finally, the particles are combined to hybridize. b) 1% Agarose gel of hybridization mix (left lane) and silver monomers with complementary DNA (two right lanes). The hybrid mix contains new bands indicative of successful hybridization. The new band (arrow) running closest to the monomers is cut out of the gel, and the particles are recovered through gel electroelution. c) A TEM micrograph of the recovered particles from this band confirms that the band is enriched in dimers.

Figure 2.

Correlation of scattering spectroscopy and transmission electron microscopy (TEM) through pattern matching. a) The distribution of individual scattering sources are captured in an optical scattering image and define a characteristic pattern. b) This pattern can be found again under the TEM at low magnification; the background on the TEM originates from proteins applied to the grid to immobilize the silver nanoparticles. TEM images obtained at high magnifications (c1-c4) then reveal structural details of the individual plasmon rulers. d) Rayleigh scattering spectra of the four identified scattering objects.

Figure 3.

Distance dependence of the plasmon resonance energy in dimers of ~41 nm silver nanoparticles. a) Fitted resonance energies E_{res} as function of the ratio L/D of center-to-center separation (L) to particle diameter (D). Two exponential fits for the experimental data are included; one for all data points (green dashed line) and the other for the data in the range $L/D > 1.05$ (red dashed line). In b) the shift in resonance energy (ΔE) normalized by the isolated monomer energy (E_0) for dimers of silver nanoparticles is compared with gold dimers from the work of Jain and El-Sayed (black continuous line), silver nanodiscs from the work of Gunnarsson *et al.* (black dashed line), as well as with FDTD simulations for silver spheres (black dotted line).

Figure 4.

Correlation of TEM images with polarization resolved optical spectra. The spectra were recorded with an analyzer in the beam path in front of the detector allowing a polarization sensitive detection. Left column: TEM images of individual silver nanoparticle dimers. Middle column: Corresponding scattering spectra. For dimer a) we included a full sweep of scattering spectra for all analyzer orientations (bottom to top: 0° - 180° in 15° steps). For dimers b) - d) we included only the spectra recorded for analyzer angles closest to the parallel or perpendicular orientation with respect to the interparticle axis. All dimer spectra contain indications for at least one high and one low energy mode whose resonance energies are marked by the dashed vertical lines in the spectra. In c) and d) the modes are partially overlapping, but can be

separated through deconvolution. Right column: Normalized intensity distribution as function of analyzer angle for the low (black) and high energy mode (red, a) only) as polar plot.

Figure 5. Energy difference ΔE_{LV} between longitudinal and vertical plasmon modes as function of L/D .