Light Extinction by Agglomerates of Gold Nanoparticles: A Plasmon Ruler for Sub-10 nm Interparticle Distances

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ABSTRACT: Plasmon rulers relate the shift of resonance wavelength, $\lambda_0$, of gold agglomerates to the average distance, $s$, between their constituent nanoparticles. These rulers are essential for monitoring the dynamics of biomolecules (e.g., proteins and DNA) by determining their small (<10 nm) coating thickness. However, existing rulers for dimers and chains estimate coating thicknesses smaller than 10 nm with rather large errors (more than 200%). Here, the light extinction of dimers, 7- and 15-mers of gold nanoparticles with diameter $d_p = 20–80$ nm and $s = 1–50$ nm is simulated. Such agglomerates shift $\lambda_i$ up to 680 nm due to plasmonic coupling, in excellent agreement with experimental data by microscopy, dynamic light scattering, analytical centrifugation, and UV–visible spectroscopy. Subsequently, a new plasmon ruler is derived for gold nanoagglomerates that enables the accurate determination of sub-10 nm coating thicknesses, in excellent agreement also with tedious microscopy measurements.

INTRODUCTION

Clusters (agglomerates) of Au nanoparticles (NPs) attract scientific attention for their unique optical properties that have a strong wavelength dependence due to oscillating surface electrons (i.e., plasmons). Such clusters exhibit a longitudinal surface plasmon resonance at a wavelength $\lambda_l$ due to plasmonic coupling in addition to their transversal light extinction maximum, $\lambda_p$, at 530 nm. The Au $\lambda_l$ shifts to longer wavelengths as the interparticle distance, $s$, between two Au NPs (dimers) is reduced down to 1 nm. This shift is achieved by coating the NPs with nanothin organic or inorganic films. Reducing $s$ below 1 nm does not affect $\lambda_l$ due to quantum tunneling and a quenched near-field intensity.

Plasmon rulers describe the dependence of Au $\lambda_l$ on $s$. They are used to select the film thickness onto these particles that are extracted using tedious microscopy or unstable Förster resonance energy transfer measurements. Such rulers enable monitoring of the conformational dynamics of bovine serum albumin, fibrinogen, $\gamma$-globulin, histone, insulin, mouse matrix metalloproteinase, the heat shock protein 90, and single and double stranded DNA molecules. A plasmon ruler has been used also to detect DNA molecules using surface-enhanced Raman spectroscopy. Furthermore, plasmon rulers are essential for the optimization of the Au agglomerate efficiency in the photothermal treatment of cancers.

Empirical plasmon rulers have been derived based on UV–visible spectroscopy of single or dimer Au NPs deposited on flat surfaces. Such relations facilitate the detection of organic coatings or films as thin as 0.4 nm but have been used only for in vitro biomedical applications. This can be partly attributed to the wet chemistry methods used for the Au dimer synthesis that have not been scaled up yet and limit their translation to clinical applications. To this end, a plasmon ruler was obtained by discrete dipole approximation (DDA) simulations of Au dimers with $s > 10$ nm. This ruler was used to accurately estimate thicknesses of DNA coatings larger than 10 nm. However, at small $s$ (<10 nm), the Au $\lambda_l$ redshift ($\Delta \lambda = \lambda_l - \lambda_0$) of 90 nm is up to 40% smaller than that measured from Au dimers. In addition, the use of this ruler for in vivo detection of organics is not trivial, as dimers further coagulate into larger agglomerates during their administration into the blood stream and their internalization by cells.

The morphology of such agglomerates is typically quantified by the fractal dimension, $D_f$, that rapidly attains its asymptotic value within 100 ms for the typical Au suspension concentrations employed in drug delivery. During in vivo administration of gold NPs, hydrodynamic (e.g., shear), van der Waals, or electric forces could break and reassemble these agglomerates, increasing their $D_f$ up to about 2 (Figure 8 in ref 24). The extinction spectra of such...
agglomerates show a characteristic broad peak\textsuperscript{26} with a $\lambda_1$ of 650–700 nm. Khlebtsov et al.\textsuperscript{27} simulated the light absorption of gold NP chains ($D_e = 1$) having 4–36 NPs at $s = 1, 2, 5, 10,$ and 20 nm. In addition, the light absorption by realistic agglomerates with $D_e = 2$ having 2–200 NPs was investigated for $s = 0.05$ and 1 nm. These coarse-grid DDA\textsuperscript{28} and T-matrix\textsuperscript{27} simulations underestimate the measured\textsuperscript{29} $\lambda_1$ redshift by up to 55%. This is due to the low dipole resolution used in DDA that limits the optimization of the $\lambda_1$ of agglomerates based on the s of their constituent gold NPs.

Here, a new plasmon ruler for gold agglomerates having $s < 10$ nm is derived, for the first time to the best of our knowledge, by determining the light extinction of agglomerates in the presence of organic\textsuperscript{29} and inorganic\textsuperscript{5} coatings that are used between 1 and 50 nm by adjusting their center of mass to account for organic\textsuperscript{29} and inorganic\textsuperscript{5} coatings may enhance experimental to optimize the optical properties of gold agglomerates. Quantum tunnelling effects are negligible for the present s range (1–50 nm).\textsuperscript{42} The composition of such coatings may enhance $\lambda_1$ by 5–9% depending on their refractive index.\textsuperscript{1} The $n_p, d_h,$ and $d_d$ of these DEM-derived agglomerates are determined\textsuperscript{23,35} accounting for their detailed morphology. In particular, the $d_h$ of NP agglomerates having $n_p < 100$ is\textsuperscript{43}

$$d_h = d_p n_p^{0.46}$$

while their $d_d$ is\textsuperscript{44}

$$d_d^2 = 4 \sum x_i^2 m_i \sum m_i$$

where $m_i$ is the mass, and $x_i$ is the distance of primary particle i to the agglomerate center of mass. The agglomerate $d_h$ is related to $d_p$ and $n_p$ by a power law\textsuperscript{45}

$$n_p = k_d \left( \frac{d_h}{d_p} \right)^{D_f}$$

where $D_f$ and $k_d$ are the fractal dimension and prefactor, respectively. The morphology of DEM-derived agglomerates has been validated already with helium ion microscopy,\textsuperscript{33} light scattering,\textsuperscript{36} and mass-mobility measurements of nascent\textsuperscript{35} and mature\textsuperscript{36} soot, zirconia,\textsuperscript{37} silica,\textsuperscript{38} and gold\textsuperscript{39} NPs.

2. METHODS

2.1. DEM of Gold NP Agglomerates. Brownian coagulation or agglomeration of gold NPs in the absence of sintering or coalescence is described by DEM neglecting van der Waals, electric, and hydrodynamic forces.\textsuperscript{23} Such DEM-derived coagulation dynamics have been validated in detail for organic (e.g., nascent\textsuperscript{35} and mature soot\textsuperscript{46}) and inorganic (e.g., zirconia,\textsuperscript{37} silica,\textsuperscript{38} and gold\textsuperscript{39}) agglomerates dispersed in air and water\textsuperscript{40} at a wide range of particle concentrations.\textsuperscript{41} Here, 1000 monodisperse gold primary particles with a diameter $d_p = 20–80$ nm (that cover the entire range of gold NPs used in the UV–vis spectroscopy data\textsuperscript{4,5,29,33,34} presented in Figures 2, 3, and 5) are randomly distributed in a cubic cell containing water at 1 atm and 300 K, applying periodic boundary conditions.\textsuperscript{35} These conditions are commonly used for the wet-phase synthesis of gold NP agglomerates.\textsuperscript{29} Therefore, agglomerates consisting of 2–15 monodisperse NPs are formed. The distance s between constituent gold NPs is varied between 1 and 50 nm by adjusting their center of mass to account for organic\textsuperscript{29} and inorganic\textsuperscript{5} coatings that are used

![Figure 1](https://doi.org/10.1021/acs.analchem.1c05145)

**Figure 1.** (a) Evolution of $D_e$ during agglomeration of gold NPs as a function of their $d_p$ (bottom abscissa) and $n_p$ (top abscissa) derived here by DEM (line and inset agglomerate images) along with microscopy data\textsuperscript{31} (symbols). (b) Evolution of $d_h$ (solid line, symbols) and $d_d$ (broken line) of gold NP agglomerates as a function of their $n_p$ derived by DEM (lines and inset images) and measured using dynamic light scattering\textsuperscript{32} (DLS; symbols).
extinction coefficient, $Q_{\text{ext}}$, that accounts for both light absorption and scattering of gold NPs. Owing to the high light extinction of gold NPs in the NIR, $x$ is varied for different $\lambda$ and RI to have a constant ratio of $2\pi n R / \lambda = 0.0192$. This precision criterion is up to 16 times smaller than those used for other strongly absorbing materials (e.g., soot) and results in 100,000 dipoles per gold primary particle.

3. RESULTS AND DISCUSSION

3.1. Validation of Au Agglomerate Morphology and Optical Properties. Agglomerates of gold NPs of diameter $d_p = 30$ nm with well-defined morphology characteristics were created by DEM. Figure 1a shows the evolution of their $D_f$ (line and insets) during growth by coagulation as a function of their average diameter of gyration, $d_g$ (bottom abscissa), or the number of constituent NPs per agglomerate, $n_p$ (top abscissa). The shaded area indicates the statistical variation of DEM. These $D_f$ dynamics are compared to microscopy data of gold NP agglomerates with similar $d_p$ (symbols) from microscopy images based on the measured bounding length. The DEM-derived $D_f$ evolution shown in Figure 1a was obtained using $d_p = 30$ nm to be consistent with the $d_p$ range that is relevant for UV–vis spectroscopy measurements. The $D_f$ was measured by Grogan et al. for NPs with $d_p = 5$ nm. Decreasing $d_p$ from 30 to 5 nm increases $D_f$ by 3–9% (as shown in Figure 5a of ref 23). This is on par with the statistical variation of the DEM simulations (shaded area) and experiments shown in Figure 1a.

As single spherical Au NPs coagulate and form agglomerates, their $D_f$ decreases from 3 to about 1.6–1.7 for $d_p > 40$ nm or $n_p > 3$. The $D_f$ is slightly smaller than the classic limit of $D_f = 1.78 \pm 0.05$ obtained by diffusion-limited cluster agglomeration as their $n_p$ is rather small ($n_p < 20$) for this asymptotic $D_f$. Nevertheless, the evolution of $D_f$ (Figure 1a, line) is in excellent agreement with microscopy data of gold agglomerates of similarly small $n_p$ (Figure 1a, symbols) pointing out the validity of DEM.

Figure 2. Normalized $Q_{\text{ext}}$ as a function of $\lambda$ of gold (a) spheres (dotted line, circles), dimers (dot-broken line, squares, inverse triangles), and 15-mers (solid and double dot-broken lines, triangles) with $D_f = 1.67$ (dot-broken, broken, and solid lines) or 1.9 (double dot-broken lines) estimated here by DDA (lines) and compared to those measured by Zook et al. (circles, diamonds, and triangles) and Eshashita et al. (squares and inverse triangles). The DDA-derived $Q_{\text{ext}}$ of gold NPs increases as single spheres (dotted line) form dimers (dot-broken line), 7- (broken line), and 15-mers (solid line) by agglomeration shifting $\lambda$ from 530 to 680 nm, in excellent agreement with data (symbols).

Figure 1b shows the hydrodynamic diameter, $d_h$ (solid line, symbols), and $d_g$ (broken line) of Au agglomerates as a function of $n_p$ derived by DEM (lines) and measured using dynamic light scattering (DLS; symbols). The $d_h$ and $d_g$ are the diameters of drag- and inertia-equivalent spheres, respectively. For example, single spheres of $d_h = 30$ nm have $d_g = d_h / 1.29$. During coagulation, $d_h$ and $d_g$ increase with increasing $n_p$ based on eqs 1 and 3, respectively (see Methods). This results in 7- and 15-mers having $d_h = d_g$ and $d_h = 0.9 d_g$, respectively. The latter is consistent with Stokesian dynamics of agglomerates having similar $n_p$. The $d_h$ of all agglomerates is smaller than 120 nm, the desirable $d_h$ range for drug delivery. The good agreement of DEM-derived $D_f$ and $d_h$ with microscopy (Figure 1a) and light scattering measurements (Figure 1b), respectively, further validates the present simulations.

Next, these realistic agglomerates were used for determining their light extinction by DDA. Figure 2 shows the normalized extinction spectra of gold (a) spheres (dotted line, circles) and dimers (dot-broken line, squares, inverse triangles), as well as of (b) the above 7- (broken line, diamonds) and 15-mers (solid & double dot-broken lines, triangles) with $D_f = 1.67$ (dot-broken, broken and solid lines) or 1.9 (double dot-broken lines). The $D_f$ is selected as an average between the $D_f = 1.78$ and 2 of agglomerates obtained by coagulation and break up in the presence of van der Waals, hydrodynamic (e.g., shear) or electric forces, respectively, at long residence times during drug delivery. Lines correspond to present simulations and symbols to data by Zook et al. (circles, diamonds, and triangles) and Eshashita et al. (squares and inverse triangles). All extinction spectra were obtained from Au agglomerates with the NP $d_h = 20–30$ nm and $s = 1.4$ nm. The shaded areas indicate the DDA variation between $d_h = 20$ and 30 nm for four dimers (Figure 2a) and 7-mers (Figure 2b). The measured and simulated light extinction spectra are normalized with the maximum extinction efficiency, $Q_{\text{ext}}$. The DDA-derived $Q_{\text{ext}}$ of single gold NPs with $d_p = 30$ nm (Figure 2a) attains its maximum at a transverse $\lambda$ of about 530 nm (dotted line), consistent with measurements (circles)
The light extinction spectrum obtained by DDA for dimers of such gold NPs also exhibits a peak at $\lambda_1 = 600$ nm due to plasmonic coupling. This peak becomes more significant with 7- and 15-mers (Figure 2b, broken and solid lines), shifting $\lambda_1$ up to 680 nm. The $\lambda_1$ increases only by 10% as 7-mers grow into 15-mers, in good agreement with the data. The small sensitivity of $\lambda_1$ on the gold agglomerate $n_p$ suggests that the light extinction derived here by DEM-DDA for $n_p = 7$ and 15 is also valid for larger agglomerates consistent with Khlebtsov et al. and may not depend strongly on individual agglomerate $n_p$ and $D_1$. However, previous simulations using a single dipole per NP showed that the $\lambda_1$ of gold agglomerates increases only up to 600 nm. Here, the maximum $\lambda_1 = 680$ nm obtained from 15-mers using 100’000 dipoles per NP (lines) is in excellent agreement with the measured $\lambda_1$ range of 650–700 nm (Figure 2b, triangles). Increasing $D_1$ from 1.67 to 1.9 broadens the light extinction spectra of 15-mers (again in agreement with data) but hardly affects their $\lambda_1$ (double dot-broken line). Therefore, the plasmon ruler derived here based on $\lambda_1$ is not affected by van der Waals, hydrodynamic, and electric forces.

The electric field enhancement by plasmonic coupling depends on $d_p$ and $s$, affecting the light extinction of Au agglomerates. Figure 3a shows the normalized extinction spectra of gold dimers with $d_p = 30$ (dotted line, diamonds) and 50 nm (broken line, triangles) at $s = 2$ nm derived here by DDA (lines) and compared to UV–visible spectroscopy and microscopy measurements (symbols). Increasing $d_p$ from 30 to 50 nm enhances the plasmonic coupling between gold NPs and increases their $\lambda_1$ from 580 to 620 nm, in good agreement with the data (symbols).

Similarly, Figure 3b shows the normalized extinction spectra of gold dimers ($d_p = 50$ nm) at $s = 50$ (solid line, circles), 2 (broken line, triangles), and 1 nm (dot-broken line, squares) estimated here by DDA (lines) along with UV–visible spectroscopy and microscopy measurements (symbols). The $Q_{ext}$ of dimers of spheres with $d_p = 50$ nm each at $s = 50$ nm that practically corresponds to single ones is in excellent agreement with that measured by UV–visible spectroscopy from single spheres for all $\lambda$. As the $s$ of these dimers decreases to 2 and 1 nm, the $Q_{ext}$ exhibits a second maximum at the longitudinal $\lambda_1$ from the electric field enhancement induced by plasmonic coupling. The $\lambda_1$ redshifts with decreasing $s$, consistent with previous DDA simulations and is in excellent agreement with data (symbols). This redshift of $\lambda_1$ increases the light extinction of gold dimers in the NIR, consistent with the light absorption measurements of Au NPs separated by silica coatings of various thicknesses, further validating the present DEM-DDA methodology.

### 3.2. Plasmon Ruler for Au Nanoagglomerates

Figure 4 shows the $\lambda_1$ as a function of $s/d_p$ by DEM-DDA for dimers of monodisperse (squares and inverse triangles) and bidisperse (circles) NPs, and DEM-derived 7- (diamonds) and 15-mers (triangles) of monodisperse NPs with $d_p = 20–50$ nm (squares, circles, diamonds, triangles) or 75 and 80 nm (inverse triangles) and $s = 1–50$ nm. Dimers with $d_p = 75–80$.

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**Figure 3.** Normalized $Q_{ext}$ as a function of $\lambda$ of gold dimers with $d_p = 30$ (a: dotted line) or 50 nm (b) at $s = 50$ (solid line), 2 (broken & dotted lines, triangles, and diamonds), and 1 nm (dot-broken line, squares) estimated here by DDA (lines) and measured using UV–visible spectroscopy and microscopy (symbols).

**Figure 4.** Au agglomerate $\lambda_1$ as a function of normalized interparticle separation, $s/d_p$, estimated by DDA using dimers of monodisperse (squares and inverse triangles) and bidisperse (circles) NPs, and DEM-derived 7- (diamonds) and 15-mers (triangles) of monodisperse NPs with $d_p = 20–50$ (squares, circles, diamonds, and triangles) or 75 and 80 nm (inverse triangles) and $s = 1–50$ nm. A new plasmon ruler (eq 4, solid line and shaded area) is derived by regressing the DDA-derived $\lambda_1$ evolution.
nm have 7% larger $\lambda_l$ than those with $d_{p} = 20−50$ nm at $s/d_{p} = 0.025$. Similarly, increasing the NP polydispersity decreases the $\lambda_l$ of dimers by about 8% at $s/d_{p} = 0.02−0.04$. The above small reductions are within the statistical variability of the DEM-DDA simulations (shaded area). By regressing $s/d_{p}$ to the ratio of $\Delta \lambda = \lambda_s − \lambda_i$, to $\lambda_s = 530$ nm corresponding to a Au sphere suspended in water,3 a new plasmon ruler is created (Figure 4, solid line):

$$\Delta \lambda/\lambda_s = 0.004 \left( \frac{s}{d_{p}} \right)^{-1.1}$$

Equation 4 has been derived for gold NPs with $d_{p} < 100$ nm and is not affected by dynamic depolarization and structural retardation.66 The light extinction of such NPs is given only by their dipole mode resulting in $\lambda_s \sim 530$ nm.27 Therefore, the critical diameter for this ruler is 100 nm, as the light extinction of gold NPs with $d_{p} > 100$ nm is determined by the sum of both the dipole and quadrupole modes and increases their $\lambda_s > 530$ nm.27 Furthermore, eq 4 is valid for agglomerates with $s \geq 1$ nm that are not affected by quantum tunneling6 and the quenched near-field intensity.

Next, eq 4 is compared to the experimental data and previously reported plasmon rulers. Figure 5 shows the estimated $s$ (lines) as a function of the normalized $\lambda_i$ shift, $\Delta \lambda/\lambda_s$ using plasmon rulers for chains27 (dotted line), dimers3 (broken line), and 7- and 15-mers (eq 4, solid line) of gold NPs with $d_{p} = 34$ (a), 50 (b), 60 (c), and 80 nm (d) compared to microscopy and UV–vis measurements of dimers (triangles,34 and squares4) and agglomerates (circles5). Jain et al.3 derived and validated their plasmon ruler for dimers at $s > 10$ nm. Below 10 nm, this ruler (broken line) results in an error of 220% on average. The plasmon ruler of Khlebtsov et al.27 was derived for chains with $D_f = 1$ at $s = 1−20$ nm (dotted line) and is in better agreement with data. Nevertheless, it still overestimates the measured $s$ by 70% (on average) due to the neglect of the realistic structure of agglomerates ($D_f > 1$) that are commonly present in measurements. In contrast, the plasmon ruler derived here with $D_f = 1.67−1.9$ (solid line and within its shade) is in better agreement with the data for all $d_{p}$ studied here.

Therefore, eq 4 can be used to measure and accurately select sub-10 nm organic and inorganic coatings of gold nano-agglomerates and to monitor the dynamics of biomolecules. For example, during protein adsorption on the NP surface, a corona monolayer is formed.57 The monolayer thickness may vary from 3.358 to 16 nm59 depending on the adsorbed protein57 and its concentration,60 as well as the NP size59 and surface charge.63 The protein corona monolayer is formed within 10−50 min and covers up to 80% of the particle
At such long residence times, gold NPs coagulate into agglomerates with various \( s \).

In specific, Figure 6 shows the \( s \) of gold agglomerates with \( d_p = 30 \) nm coated by fibrinogen (red), histone (green), albumin (orange), and \( \gamma \)-globulin (blue) estimated using UV–vis spectroscopy data\(^{11} \) with plasmon rulers for dimers\(^3 \) (open bars), chains\(^{27} \) (lines bars) and agglomerates (eq 4, filled bars).

The evolution of gold agglomerate morphology and optical properties during agglomeration is investigated here by coupling DEM with DDA. The morphology and hydrodynamic diameter of DEM-derived gold NP agglomerates are validated with microscopy\(^{31} \) and light scattering\(^{12} \) measurements, respectively. The evolution of gold light extinction during agglomeration reveals that the longitudinal surface plasmon resonance wavelength, \( \lambda_p \), increases up to 680 nm as single gold NPs coagulate to 15-mers, in excellent agreement with data from UV–visible spectroscopy.\(^4,29\)

The \( \lambda_p \) shift of gold dimers, 7-, and 15-mers increases with decreasing \( s \) and can be described by a universal power law resulting in a new plasmon ruler (eq 4) that enables the estimation of Au nanoagglomerate coating thickness, \( s \), in excellent agreement with the microscopy data.\(^4,3,44\)

In contrast, existing, widely used plasmon rulers for dimers\(^3 \) and chains\(^{27} \) estimate coating thicknesses smaller than 10 nm with an average error of 220 and 70%, respectively. Therefore, the new plasmon ruler obtained here for agglomerates of Au NPs can be used instead of tedious microscopy measurements to determine the thickness of sub-10 nm organic and inorganic coatings. This can facilitate monitoring of the dynamics of biomolecules, such as proteins\(^{10} \) and DNA,\(^{12} \) and the optimization of gold agglomerate coating thickness for photothermal therapy of cancer.\(^7\)

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