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Zooming in on the Physical and Optical Properties of Gold Nanostars: An Experimental and Computational Study

T. V. Tsoulos^a, L. Han^b, J. Weir^a, H. L. Xin^b and L. Fabris^a

A combined experimental and computational study was carried out to design a semi-empirical method to determine volume, surface area, and extinction coefficients of gold nanostars. The values obtained were confirmed by reconstructing the nanostar 3D topography through high-tilt TEM tomography and introducing the finite elements in Comsol Multiphysics, through which we have also calculated the morphology-dependent extinction coefficient. Doing so we have, for the first time, also modeled the heat losses of a real, experimentally synthesized nanostar, and found the plasmon resonances to be in excellent agreement with those obtained experimentally. We believe that our approach could substantially improve the applicability of this remarkable nanomaterial.

Introduction

Gold nanostars (NS) have recently emerged as powerful nanostructures for surface enhanced Raman scattering (SERS)^[1] and MRI imaging,^[2] and promise to lead to exciting new devices driven by plasmonic hot electrons.^[3] In SERS-based experiments, primarily by virtue of the lightning rod effect,^[4] Au NSs have been shown to provide enhancement factors (EF) close to 10^{10} ,^[5] which, based on recent EF recalculations carried out by Etchegoin *et al.*, should closely approximate the maximum EF values experimentally achievable.^[6] These EFs can be further increased when the nanostars are coupled to other nanoparticles in regiospecific core-satellite assemblies,^[7, 8] in which junction-driven hot spots can be employed, for instance, to observe the formation of individual amide bonds.^[7] Furthermore, numerical simulations and photocatalytic experiments have shown that, in TiO₂-capped nanostars, the location of the hot spots at the tip of the spike enables the localization of hot electrons at the metal-semiconductor interface, facilitating hot electron injection into the latter, as compared to what possible with spherical and rod-shaped particles.^[3] However, looking closely, one realizes that the fundamental work necessary to truly enable the application of nanostars as imaging tags, sensors, or photocatalysts, is still scarce. Most importantly, the fundamental physical and optical parameters describing Au NSs, such as volume, surface area, and extinction coefficients, have not yet been evaluated for all types of NSs. Recently, Hamad-Schifferli and coworkers have estimated, for the first time, the extinction coefficient of NSs synthesized via HEPES-based seedless protocols;^[9] this value is extremely important to estimate the concentration of NSs in suspension and enable their quantitative

application. However, surfactant-free NSs and HEPES-capped NSs drastically differ in morphology, thus granting a closer look at NSs synthesized with seed-mediated approaches.

Recently, we have shown how Au NSs can be grown via seed-mediated bottom up protocols to possess extremely sharp spikes if ascorbic acid (AA) is employed both as the reducing and the capping agent.^[5] The morphology of AA-capped nanostars can be tuned primarily by varying the concentration of gold seeds and AgNO₃, leading to multiply-spiked NSs, in which the spikes can further branch out in a characteristic snowflake-like geometry, if aging is conducted in particular experimental conditions or the order of addition of the reagents is modified. The AA ligands can be easily replaced by silanols to grow uniform silica shells that can be then etched with NaBH₄ to expose only the tips and lead to NSs with further increased EFs.^[10] However a better understanding of NS morphology is still needed and would dramatically improve the applicability of these nanoparticles in catalysis, sensing, and plasmonics. For example, the ability to programmatically correlate the synthetic parameters to the number and length of the tips produced, the overall surface area, and the nanoparticle volume, would give us the opportunity to quantitatively calculate SERS EFs, which have until now only been estimated,^[5] often in extremely conservative terms.

In this work, we have initiated a two-pronged study in which simulations and experiments have been employed jointly to predict and confirm the fundamental physical and optical properties of gold nanostars synthesized following seed-mediated bottom up protocols. Namely, we have determined the volume, surface area, and extinction coefficient by first developing a semi-empirical model in which their values were estimated. We have then carried out high-resolution (HR) TEM experiments, inclusive of STEM tomography, to confirm them and determine whether our proposed simplified approach could be employed to rapidly estimate these parameters and generalized to calculate them for nanostars of varying dimensions. Finally, we have exported the 3D reconstructed NS topography into the software Comsol Multiphysics, to reconstruct the octahedral elements needed

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to build the NS finite element geometry, calculate intensity and distribution of local scattered electric fields, and determine the particle's morphology-dependent extinction coefficient. Our results provide, for the first time, a computational model describing scattered electric fields in real Au NSs. Importantly, our work provides a practical method that can be used to easily estimate the fundamental physical and optical parameters of nanostars of any given diameter, spike length and density, and material composition. Although herein we do not discuss explicitly mode assignments on the optical parameters, these considerations are closely taken into account in the fundamental physics built within the 3-dimensional approach we use to solve Laplace's homogeneous time-averaged wave equation in the non-symmetric nanostar geometry. With this work, our overarching goal was to provide a simplified yet accurate and reliable method that could be used by any scientist needing to confidently employ these particles in sensing, imaging, or catalysis.

Experimental Section

Synthesis of Au NSs

All chemicals were ACS reagent grade and were purchased from Acros Organics, unless otherwise noted. Au NSs were synthesized using a surfactant-free, seed-mediated growth method adapted from Yuan *et al.* [11] Briefly, a 25 mM gold salt solution was prepared by dissolving 10 mg hydrogen tetrachloroaurate (III) trihydrate (HAuCl₄·3H₂O) per mL of MilliQ water. To prepare a seed solution of citrate capped gold nanospheres approximately 6 nm in radius, 4 mL of 25 mM HAuCl₄ solution were added to 250 mL of MilliQ water and brought to a boil; 1% trisodium citrate solution was then prepared by dissolving 500 mg of citric acid trisodium salt dehydrate 99% (pure grade) in 50 mL of MilliQ water, and 15 mL of the citrate solution were added to the boiling HAuCl₄ solution.

After 35 minutes of vigorous boiling, the solution was cooled to room temperature, centrifuged for 45 minutes at 10,000 g, and resuspended in 20 mL of MilliQ water. 142 µL of the seed solution (A₅₁₉: 1.052) were added to a large centrifuge tube containing 10 mL MilliQ water, 400 µL of 25 mM HAuCl₄ solution, and 40 µL of 1 N HCl (diluted from 37 wt% certified ACS Plus grade hydrochloric acid purchased from Fisher Scientific). A 100 mM ascorbic acid (AA) solution was prepared by dissolving 35.2 mg of AA in 2 mL of MilliQ water and sonicating, while a 3 mM AgNO₃ solution was prepared by dissolving 12.7 mg of silver nitrate ≥ 99% (Sigma-Aldrich) in 25 mL of MilliQ water. 200 µL of the AA solution and 400 µL of the AgNO₃ solution were simultaneously added, and the solution was gently agitated for 2 minutes before centrifuging for 15 minutes at 3,000 g and resuspending in 2 mL of MilliQ water.

Characterization

UV-Vis-NIR spectra of Au NS suspensions were obtained using a Thermo Scientific Evolution 300 Spectrometer. The NSs were imaged with high-resolution transmission electron microscopy (HR-TEM) at the Center for Functional Nanomaterials, at Brookhaven National Laboratories, employing a FEI Titan 80-300 kV. The highest spatial resolution achieved was 0.14 nm. NS dimensions were measured using ImageJ software developed by the National Institutes of Health. To calculate the volumes of Au NSs, the cores were modeled as spheres and their spikes as eccentric cones with hemispherical tips; the spherical cap where each spike intersected with the core was subtracted. The volume of an individual Au NS modeled with this shape is given by **Equation 1**.

$$(1) \quad V = \frac{4}{3}\pi a^3 + \sum_{i=1}^n \left\{ \frac{h_i}{3}\pi(R_i^2 + R_i r_i + r_i^2) + \frac{2}{3}\pi r_i^3 - \frac{\pi}{6} \left(a - \sqrt{a^2 - R_i^2} \right) [3R_i^2 + (a - \sqrt{a^2 - R_i^2})^2] \right\}$$

Where a is the radius of the spherical core, R_i is the base radius of a spike, r_i is the radius of the hemispherical tip of a spike, and h_i is the distance between R and r . The number of atoms in a nanostar was determined from its volume and gold density.

Similarly, the surface area of a gold NS is calculated according to **Equation 2**, where, once again, a is the radius of the spherical core, R is the base radius of a spike, r is the radius of the hemispherical tip of a spike, and h is the distance between R and r .

$$(2) \quad A = 4\pi a^2 - \sum_{i=1}^n \left\{ 2\pi a \left(a - \sqrt{a^2 - R_i^2} \right) \right\} + \sum_{i=1}^n \left\{ \pi (r_i + R_i) \sqrt{(R_i - r_i)^2 + h_i^2} + 2\pi r_i^2 \right\}$$

The molar concentration of NSs in solution was calculated as the ratio between the total atomic gold concentration and the previously determined number of atoms per NS as determined via inductively coupled plasma mass spectrometry (ICP-MS). The extinction coefficient was determined from Lambert Beer's Law (**Equation 3**):

$$(3) \quad \epsilon = \frac{Abs}{bC}$$

Where ϵ is the NS extinction coefficient, Abs is the absorbance of the plasmon peak, C is the molar concentration of NSs, and b is the cell path length of the spectrophotometer (0.1 cm). The concentration of gold in solution was determined via ICP-MS, using a ThermoScientific Neptune Plus instrument.

Annular dark-field STEM tomography and 3D Reconstruction

A project image series of the NS was acquired from -70 degrees to +70 degrees with two-degree intervals in the high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) imaging mode. The use of HAADF-STEM signals reduces diffraction contrast and renders image intensities approximating those of the projected atomic mass of the NS. The 3D tomograms of the NS were subsequently reconstructed using a custom-written Matlab code with the multiplicative simultaneous iterative reconstruction technique. The isosurface rendition of the 3D reconstructed NS was performed in Avizo 6.0.

Modeling

The 3D electromagnetic response of NSs under the electric field of a linearly polarized (LP) incident light (**Equation 4**) was simulated using a model developed in the RF Module of the software COMSOL Multiphysics 5.0. This model was based on our newly developed approach, in which a 22,000-points geometrical topography was reconstructed from the high tilt STEM tomography data collected as described above. The size of the modeled nanostar was fit to match the real size of the NS studied, as seen in **Figure S1**. The simulation was based on solving **Equation 5** in three concentric geometry domains: 1) An outermost fully absorbing spherical layer, 2) a spherical water layer which is assigned the dielectric properties of water and 3) a core nanostar domain, characterized by the dielectric properties of bulk gold from the Lorentz-Drude model by Johnson and Christy. [12]

$$(4) \quad \mathbf{E} = e^{-jk_0 z} \hat{x}$$

$$(5) \quad \nabla \times \frac{1}{\mu_r} (\nabla \times \mathbf{E}) - k_0^2 \left(\epsilon_r - \frac{j\sigma}{\omega\epsilon_0} \right) \mathbf{E} = \mathbf{0}$$

This layered geometry was meshed employing randomly distributed tetrahedra, whose size was kept between 0.1 and 2 nm within the NS domain and at less than 5 nm in the surrounding medium domain. The solution of Equation 5 was reached through an iterative method in which the difference between individual solutions in the constituent tetrahedral elements is minimized within a tolerance of 10⁻⁶. The relative permeability μ_r was taken to be 1 in all geometry

domains without loss of generality. The real and imaginary parts of the dielectric constant ϵ_r and conductivity σ were obtained from Johnson and Christy.^[12] The angular frequency ω was calculated from the input wavelength values.

$$(6) \quad I = I_0 e^{-2\alpha z}$$

$$(7) \quad \alpha = \omega \sqrt{\frac{\mu\epsilon}{2} \left[\sqrt{1 + \left(\frac{\sigma}{\omega\epsilon}\right)^2} - 1 \right]}$$

The absorption of the incident LP electric field is described by **Equation 6** and **7**, where α is the absorption coefficient. In this work we calculate the heat losses from the obtained solution of Equation 5 by integrating over the nanostar volume according to **Equation 8**.

$$(8) \quad \text{Heat Losses} = \iiint_{xyz} \left[\frac{1}{2} \text{Re}[\mathbf{J} \cdot \mathbf{E}^*] \right] dx dy dz \quad (\text{W})$$

We correlate this physical quantity to the experimentally measured absorbance as defined by Beer-Lambert's law (**Equation 9**). Both heat losses and absorbance depend on the conductivity of the studied system but heat losses spectra also depend on $|\mathbf{E}|^2$ as seen in **Equation 8**, by knowing that $\mathbf{J} = \sigma \mathbf{E}$.

$$(9) \quad \text{Abs} = \log_{10} \frac{I_0}{I}$$

We provide plots of the normalized scattered electric field $(1 + E_s/E_0)$, in order to focus exclusively on the plasmon enhancement properties of the NSs, along with the heat losses spectra, that are a physical quantification of the thermal effects that arise from light absorption by the illuminated NS. This approach provides a descriptive platform for the spatial localization of the electric field, its correlation to the morphological characteristics of the nanoparticle and its optical properties.

Additionally, as a holistic approach to the optical properties, we calculated the morphology-dependent extinction coefficient of the 3D reconstructed nanostar directly from the solution of **Equation 5** for the scattered electric field. The scattering and absorption cross sections are defined by the energy rates scattered and absorbed by the nanostar volume and divided by the incident irradiance, as defined in **Equation 10**:

$$(10) \quad P_{inc} = \frac{1}{2Z_0} |E_0|^2$$

Where Z_0 is the impedance of free space and E_0 is the amplitude of the incident electric field, imposed to be one in **Equation 4**. Scattering and absorption cross sections are given in **Equation 11**:

$$(11) \quad \sigma_{abs} = \frac{W_{abs}}{P_{inc}} \quad \sigma_{sca} = \frac{W_{sca}}{P_{inc}}$$

Where W_{abs} and W_{sca} are the energy rates at which the absorptive and scattering losses are occurring within the nanostar volume, as defined in **Equations 12** and **13** respectively:

$$(12) \quad W_{abs} = \frac{1}{2} \iiint_{xyz} [\text{Re}[(\sigma \mathbf{E} + j\omega \mathbf{D}) \cdot \mathbf{E}^*] + j\omega \mathbf{B} \cdot \mathbf{H}^*] dx dy dz \quad (\text{W})$$

$$(13) \quad W_{sca} = \iint [\mathbf{E} \times \mathbf{H}^*] \cdot \mathbf{n} dS \quad (\text{W})$$

The addition of scattering and absorption cross sections gives the extinction cross section as seen in **Equation 14**:

$$(14) \quad \sigma_{ext} = \sigma_{abs} + \sigma_{sca}$$

Given the extinction cross section, we can then calculate the shape- and concentration-dependent extinction coefficient by multiplying the

Figure 1. HRTEM micrographs of a gold nanostar seed with 12 nm diameter (left) and of the tip of a nanostar spike (right). Both scale bars are 2 nm. Atomic arrangement in agreement with a FCC crystallographic structure is highlighted on the nanostar spike.

extinction cross section with the number density of nanostars as seen in **Equation 15**:

$$(15) \quad \epsilon_{dep} = \sigma_{ext} n$$

Dark Field Scattering Spectroscopy

Dark field scattering spectra were obtained using a Nikon Optiphot 66 optical microscope and an Andor Shamrock SR 303i imaging spectrometer equipped with a 250 μm slit. A CCD camera attached to the microscope was employed to collect $110 \times 83 \mu\text{m}^2$ images of the samples.

Results and Discussion

We have synthesized AA-capped AuNSs *via* a bottom up seed-mediated approach in which spherical nanoparticles are employed to seed the growth of variably sharp spikes. The HR-TEM micrograph in **Figure 1** (left panel) shows the morphology of a typical 12 nm citrate-capped seed. A face centered cubic crystal structure (FCC), commonly observed in spherical gold nanoparticles within this size regime, characterizes these seeds. A typical FCC atomic packing can be also observed for the AA-capped NS spike (right panel), thus we can confidently assume a density of 59 atoms/ nm^3 for the overall NS.^[13] We observe the crystal planes in both structures and we recognize five – fold twinning in both the seed and the nanostar spike. Five – fold twinning is quite common in gold nanoparticle synthesis in general as reported by Grzelczak *et al.*^[14] The growth of NS spikes is related to and affected by twinning.^[15] In the inset of Figure 1 we present a further magnified HR-TEM micrograph where we can recognize the FCC crystal structure as captured on a {100} plane. As {111} planes are known to be the crystal facets of lowest energy in FCC gold nanoparticles,^[13] we can expect that these {100} planes are not the result of a thermodynamic-driven reaction, but may be due to kinetic traps occurring during the NS growth. Both {111} and {100} planes are dominating in the spherical Au nanoparticle size regime around 12 nm, while other crystallographic facets appear for larger spherical particles or when the symmetry is broken, as demonstrated experimentally^[16] and calculated via Wulff construction.^[17, 18]

Dimensional analysis of the NSs was performed using the ImageJ software, including the spherical core size, the tip base *radius*, the tip's hemispherical cap *radius*, and the distance between the two *radii* (i.e. base and cap), for all spikes that appeared normal to the beam. Tips that were observed but not measureable were accounted for, and their number assumed to be equal by symmetry on the side of the NSs opposite to our viewpoint. Based on the calculated averages and standard deviations of spike volumes and the volumes of spherical

Figure 2. Tilted STEM micrographs of a gold nanostar. Note the crystallographic details that appear at each orientation.

caps formed by the intersection of the spikes with the spherical core, the volumes for the unmeasured spikes were simulated assuming a normal distribution of dimensions. After statistical analysis (see ESI), we modeled our nanostars as possessing 18 spikes with normally distributed lengths, even though we realize that this does indeed represent only a simplified model.

The average AA-capped NS volume was calculated according to **Equation 1** (Experimental Section) to be $4.141 \times 10^4 \text{ nm}^3$, leading to approximately 2.443×10^6 atoms per NS on average. The exact NS concentration was determined taking into account ICP-MS experimental results, in which the concentration of gold in the NS suspension was calculated to be 23.54 mg/mL, leading to a NS yield of 74% for this synthetic protocol, in line with values reported by Hamad-Schifferli and Murphy.^[9, 19] Based on these values, the molar concentration of Au NSs was estimated to be $[(5.355 \times 10^{-3} \text{ M} / 2.443 \times 10^6 \text{ NS}^{-1}) \times 0.74] = 1.622 \text{ nM}$. The average nanostar surface area was calculated as according to **Equation 2** to be $4.772 \times 10^3 \text{ nm}^2$.

Starting from the calculated concentration value and the absorbance of the plasmon peak using Beer-Lambert's Law from **Equation 3**, the extinction coefficient was estimated to be $3.14 \times 10^9 \text{ M}^{-1} \text{ cm}^{-1}$, in agreement with the values reported in the literature.^[9] A per-atom estimation of the NS extinction coefficient results in a value of $9.77 \times 10^9 \text{ M}^{-1} \text{ cm}^{-1}$, where the extinction coefficient of a single gold atom is equal to $4000 \text{ M}^{-1} \text{ cm}^{-1}$, as shown by Link *et al.*^[20] This differs from the value determined using our semiempirical method and the Beer Lambert's law by a factor of 3.11, which could be due to an overestimation of the number of spikes or to our assumption of a normal spike-length distribution. Nonetheless, the close proximity of the two values leads us to consider the semi-empirical model we developed a valid approach to calculate extinction coefficient, volume, and surface area of gold nanostars synthesized from seed mediated bottom up protocols starting from the basic NS dimensions determined from TEM analyses.

To complement and further validate these experimental results, we have employed the software Comsol Multiphysics to calculate the physical and optical properties of our NSs. Whereas we usually employ finite element simulations to exclusively study the optical properties of the nanomaterials we synthesize,^[7, 10] in this work we exploited them from an additional standpoint, i.e. to also calculate the total volume and surface area of the NSs and corroborate our proposed simplified approach. By collecting electron micrographs at high tilt ($+70^\circ/-70^\circ$) (**Figure 2**) and reconstructing the 3D topography of the NS (**Figure 3**), we have introduced, after a size adjustment to exactly match the dimensions of the real and simulated NS (**Figure S1**), the 22,000 topographical tetrahedra contained in the tomography into Comsol's RF module, employing Rhinoceros (Rhino3d) software to convert the Virtual Reality Modeling (VRML) topography to a Stereolithography (STL) topography. Through this approach, we have estimated the volume of the 3D reconstructed NS to be 5.551×10^4

Figure 3. 3D tomogram (left), nanostar topography reconstructed employing 22,000 tetrahedral elements (center), and scattered electric field distribution (right) calculated using Comsol Multiphysics starting from the finite elements extracted from the tomogram.

nm^3 , with a surface area of $1.345 \times 10^4 \text{ nm}^2$. The volume calculated is impressively similar to that estimated according to Equation 1 ($4.141 \times 10^4 \text{ nm}^3$), while the surface area is roughly 2.5 times larger than the $5.055 \times 10^3 \text{ nm}^2$ calculated with Equation 2, consistent with the fact that the semi-empirical model does not take into account the local roughness at the surface of the NSs, which can instead be picked up by the tomographic analysis. Based on these numbers, a per-atom calculation of the extinction coefficient led to a value similar to that reported above ($1.31 \times 10^{10} \text{ M}^{-1} \text{ cm}^{-1}$), slightly higher than what calculated by Hamad-Schifferli and coworkers.^[9] These results are consistent with the fact that our NSs are, on average, larger than those analyzed by Hamad-Schifferli and her group (71 nm vs. 22-55 nm spike length), and are thus characterized by higher per-particle extinction coefficients. Compared to values that have been reported in the literature for gold nanospheres of similar volume (e.g. $2.93 \times 10^9 \text{ M}^{-1} \text{ cm}^{-1}$ for nanospheres with volume of $70,000 \text{ nm}^3$), the values we calculated for nanostars employing the 3D reconstructed model are ca. 4.5 times higher than those of nanospheres with 1.3 times larger volumes.^[21] Thus, when normalizing for nanoparticle volumes, we obtain a value for the extinction coefficient of nanostars that is 5.9 times larger than that of nanospheres, thus confirming the superior performance of nanostars in their interaction with light.

We also determined the morphology-dependent extinction coefficient of the 3D reconstructed nanostar according to **Equation 15**. We first calculated the extinction cross section for 8 different orientations at 720 nm and then determined its weighted average according to **Figure 4**, leading to a value of $\sigma_{\text{ext}} = 5.268 \times 10^{-15} \text{ m}^2$. Assuming again 59 atoms/ nm^3 with a $5.551 \times 10^4 \text{ nm}^3$ volume per nanostar we obtained 3.275×10^6 atoms per nanostar. Having estimated (via ICP-MS) the total concentration of gold to be $3.9627 \times 10^{-3} \text{ M}$, and having determined the NS volume via tomographic reconstruction, we could determine the final concentration of NSs to be 1.210 nM. The number density of nanostars was found as $C \times N_A = 1.210 \text{ nM} \times 6.02214 \times 10^{23} = 7.287 \times 10^{14} \text{ NS} / \text{L}$ or $7.287 \times 10^{17} \text{ NS} / \text{m}^3$. According to **Equation 15** the concentration dependent extinction coefficient was found to be $\epsilon_{\text{dep}} = (5.268 \times 10^{-15} \text{ m}^2) \times (7.287 \times 10^{17} \text{ m}^{-3}) = 3.839 \times 10^3 \text{ m}^{-1}$. Finally, the concentration independent extinction coefficient was calculated as $\epsilon = \epsilon_{\text{dep}} / C = (3.839 \times 10^3 \text{ m}^{-1}) / (1.210 \text{ nM}) = 3.173 \times 10^{12} \text{ M}^{-1} \text{ m}^{-1}$ or $\epsilon = 3.173 \times 10^{10} \text{ M}^{-1} \text{ cm}^{-1}$. This morphology-dependent extinction coefficient value, significantly larger than those determined with the other methods, stresses the importance of taking into account the role of morphology in particles such as nanostars.

To correlate not only the intensity but also the spectral features of the calculated and experimental data, we have then calculated the scattered E-field maps and the associated heat losses and, following the approach we have previously reported,^[10] compared the heat losses spectra to the experimental UV-Vis-NIR absorption spectra. We have calculated the heat losses spectra of the 3D reconstructed

Figure 4. Electric field plasmonic enhancement maps of the 3D reconstructed nanostar under 8 different orientations along with their respective heat losses spectra.

nanostar under eight different orientations, when keeping the x-axis as the polarization axis of the incident electric field. By assuming every orientation as equally probable, we double the probability of the 0° orientation, as the 180° orientation gives the exact same spectrum. This case is not applied for the 45° and 135° cases, as seen in **Figure 4**, as the NS does not possess a C_4 axis of symmetry. Each individual orientation appears to excite different plasmonic modes on the spikes, as observed in Figure 4. Interestingly, only select spikes localize a *hot spot* at each orientation, with some spikes localizing the highest enhanced scattered fields at slightly offset positions with respect to the tip of the spikes, as we observed before.^[10] Symmetric nanostructures have been reported to exhibit multiple resonant modes of different order, studied as extension to the dipolar image of plasmon resonances.^[22] In the case of this highly asymmetric nanostars, it is noteworthy how plasmonic modes of different order, contributing to the resonances seen in the spectra, can still be observed. Several dominant resonances can be identified in the simulated spectra, at 675 nm, 720 nm, and 800 nm, which can be assigned to dipolar (720 nm and 800 nm) and multipolar (675 nm) plasmon modes of the spikes. The broad resonance around 550 nm is attributed to the spherical “bumps” on the nanostar body (most likely non-fully grown or reconstructed spikes). As opposed to other computational studies carried out by us and others, in this simulation it is difficult to assign a mode to the spherical core, as it is not possible to determine whether or not it has remained intact during the growth of the spikes, even though it most likely does contribute in part to this resonant band.

We have then considered a weighted average of all the heat losses spectra in an attempt to reproduce the peak broadening observed in UV-Vis-NIR ensemble-averaged measurements, as presented in **Figure 5**. In addition to the broadening due to tumbling of the nanoparticles in solution, we need to take into account the morphological differences intrinsic to the sample, as AA-capped NSs are known to be very inhomogeneous, as observed in **Figure S2**. Small differences in the shape, length, and orientation of the spikes result in large differences in the absorption profiles, as seen in **Figure 4**. Thus, even in the case of a very uniform batch of NSs with multiple spikes, small differences result in large peak broadenings. Nonetheless, despite the fact that we are herein comparing an individual nanostar modeled under eight different orientations to

thousands of nanostars of significantly different shape under many possible orientations, the comparison provides an excellent insight into the nature of the plasmonic resonances of nanostars. By carrying out additional TEM tomography experiments over a larger number of nanostars per sample and reconstructing the heat losses spectra at different orientation with respect to the incoming light, as demonstrated herein, one could take further into account the shape effects on the peak broadening and build an even more detailed model. Our purpose here was, however, to demonstrate the applicability and reliability of the method and identify ways in which it could be used for nanostars of different overall diameter, spike size and density, and nature of the metal.

The UV-Vis-NIR spectrum of the nanostar suspension (**Figure 5 and S4**) exhibited a plasmon resonance peak centered at 778 nm at

Figure 5. Computational simulation and experimental characterization of the optical properties of the nanostars studied. Weighted average of the heat losses spectra of the 3D reconstructed nanostar (a), UV-Vis-NIR spectrum of aqueous nanostar suspensions when TEM tomograms were collected (b, red line), and when dark field spectra were recorded (b, green line). Dark field scattered light spectrum (black line scattered light intensity signal and red line mean average) (c), UV-Vis-NIR spectrum of nanostars deposited on a microscope slide (black line signal and red line mean average) (d).

the time of the synthesis. At the time of the microscopy studies we repeated the measurement noticing a blue-shift of the plasmon resonance peak to 672 nm, due to a dynamic shrinkage of the NS spikes, as reviewed by Jiang and coworkers.^[23] Based on an additional UV-Vis-NIR spectrum taken 18 months after the synthesis, which exhibits a maximum at 676 nm, we can confidently state that the nanostars studied possess the final, kinetically-trapped morphology and that their properties can be analyzed side-by-side.

In **Figure 5** (a) we compare the weighted average of the heat losses spectra of the 3D reconstructed nanostar presented in **Figure 4**, along with the UV-Vis-NIR spectra of aqueous nanostar suspensions (b), a dark field scattered light spectrum (c), and the UV-Vis-NIR spectrum of nanostars deposited on a microscope slide (d). In general we see good agreement among the experimental spectra collected for dried nanostars ((c) and (d)), in which the plasmon resonance appears redshifted compared to what observed in suspension. This fact is consistent with the higher dielectric function for glass, onto which the nanostars are deposited, compared to water and air. By comparing the experimental data with the heat losses spectra of nanostars in water we can observe how the latter display a much narrower plasmon band which is centered at 715 nm. While we can confidently justify the broadening as due to the heterogeneous nanostar morphology and their brownian motions in suspension, the discrepancy concerning the peak position deserves more insight. Overall, we believe this to be due to the fact that our 3D reconstruction has only focused on one nanostar, which is non representative of the entire sample; for instance, by examining a larger sample composed of nanostars with more numerous, shorter spikes, we would have observed a substantial blue shift of the plasmon resonance peak (data not shown).

Nonetheless, despite the mild mismatch between our modeled and experimental data, we can observe an overall agreement in the observed trends, which can justify the use of our proposed, simplified model for the study of morphology and optical properties of gold nanostars synthesized from seed-mediated protocols. In prospective, one can imagine to be able to use the semi-empirical or tomography-based tools we described herein to study not only nanostars of different morphology and constituting metal, but also, more broadly, nanoparticles of any arbitrary shape and composition, thus further strengthening our understanding of the structure-property relationships of nanoparticles and link them to the various protocols used to synthesize them.

Conclusion

In conclusion, in this study we developed a semi-empirical, simplified model to calculate the fundamental physical and optical parameters (i.e. volume, surface area, and extinction coefficient) for nanostars grown in solution using seed-mediated protocols. Starting from nanostar dimensions and dominant morphologies determined from TEM and STEM analyses, we calculated quite accurate extinction coefficient values, that we confirmed by comparison with the literature^[9] and carrying out combined UV-Vis-NIR and ICP-MS experiments. Based on the close agreement among the various values, we are confident that our approach could be generalized for nanostars of various dimensions synthesized in solution using seed-mediated protocols. In addition, we have further validated our calculations by introducing into Comsol Multiphysics the 22,000 tetrahedra constituting the nanostar's 3D topography reconstructed via STEM tomography, and calculated volume and surface area values, and morphology-dependent extinction coefficient. The remarkable agreement between these and the values determined semi-empirically once more confirms the validity of our simplified model and highlights how NS morphology plays a non-negligible role in enhancing the value of the extinction coefficient, as clearly evidenced

Table 1. Fundamental physical and optical properties characterizing gold nanostars as estimated in this work and reported by others^[9]

Approach	Volume [nm ³]	Surface Area [nm ²]	ϵ [M ⁻¹ cm ⁻¹]	ϵ^b [M ⁻¹ cm ⁻¹]
Semi-empirical Method	4.141×10 ⁴	5.055×10 ³	3.14×10 ^{9a)}	9.77×10 ⁹
STEM Tomography	5.551×10 ⁴	1.345×10 ⁴	3.17×10 ¹⁰	1.31×10 ¹⁰
Literature	1.0×10 ³ 3.2×10 ^{3 c)}	3.0×10 ³ 1.2×10 ⁴	- -	5.7×10 ⁸ 2.7×10 ^{9 d)} 7.8×10 ⁸ 3.5×10 ^{9 e)}

^{a)}Beer-Lambert law; ^{b)}Per-atom; ^{c)}AutoCAD reconstruction; ^{d)}Per-atom; ^{e)}Discrete Dipole Approximation.

by a comparative analysis of the values reported in **Table 1**. Overall, we believe that our approach could drastically improve the application of gold nanostars by providing the fundamental parameters necessary to calculate concentration, ligand density, or SERS enhancement factors and thus finally enabling the quantitative application of this remarkable nanomaterial.

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