Manipulation of the Geometry and Modulation of the Optical Response of Surfactant-Free Gold Nanostars: A Systematic Bottom-Up Synthesis

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Supporting Information

ABSTRACT: Among plasmonic nanoparticles, surfactant-free branched gold nanoparticles have exhibited exceptional properties as a nanoplatform for a wide variety of applications ranging from surface-enhanced Raman scattering sensing and imaging applications to photothermal treatment and photothermal therapy. The effectiveness and reliability of branched gold nanoparticles in biomedical applications strongly rely on the consistency and reproducibility of physical, chemical, optical, and therapeutic properties of nanoparticles, which are mainly governed by their morphological features. Herein, we present an optimized bottom-up synthesis that improves the reproducibility and homogeneity of the gold-branched nanoparticles with desired morphological features and optical properties. We identified that the order of reagent addition is crucial for improved homogeneity of the branched nature of nanoparticles that enable a high batch-to-batch reproducibility and reliability. In addition, a different combination of the synthesis parameters, in particular, additive halides and concentration ratios of reactive Au to Ag and Au to Au seeds, which yield branched nanoparticles of similar localized surface plasmon resonances but with distinguishable changes in the dimensions of the branches, was realized. Overall, our study introduces the design parameters for the purpose-tailored manufacturing of surfactant-free gold nanostars in a reliable manner.

INTRODUCTION

Anisotropic nanoparticles (NPs) have been investigated over many years due to their unique morphology-dependent physical, chemical, and optical properties.1–8 In particular, synthetic routes for the rational design and optimization of bottom-up syntheses have received great interest as translational applications in biology, medicine, and energy industry are expanding exponentially.9–14 Gold nanoparticles (Au NPs) that exhibit optical absorption in the near-infrared (NIR) have gained notable attention for diagnostic and therapeutic applications as biological tissues are optically transparent in the NIR region, particularly, those that exhibit higher electromagnetic field enhancement for enhanced optical imaging and those that exhibit enhanced photon-to-heat conversion for effective photothermal therapies.15–19 Highly branched Au NPs are of particular interest among other anisotropic nanoparticles, as they offer a wide range of optical tunability by subtle changes in their geometry and a higher optical signal enhancement due to the highly intense nanoantenna effect from the sharp features of the branches.20–26

As it is common among anisotropic nanoparticle syntheses, a variety of synthetic approaches have been developed for branched nanoparticles via surfactant-mediated or capping agent-guided methods,9,27,28 whereas very few surfactant-free routes have recently been developed.14,23,29,30 Our group first introduced a surfactant-free route that does not require toxic cetyltrimethylammonium bromide for producing biocompatible nanoparticles with multiple sharp branches protruding from a spherical core, which are referred to as “gold nanostars” (GNS).23 The biocompatible surface chemistry that requires no ligand exchange or extensive purification protocols, ease of direct surface functionalization, and higher surface area available for molecular recognition or transporting therapeutic/functional entities are major advantages offered by GNS that have led to the versatility of their broad-ranging biological and sensing applications.17,23,24 For instance, in combination with the excellent plasmonic properties, intense surface-enhanced Raman scattering and two-photon luminescence, GNS have provided an exceptional platform for in vitro and in vivo diagnostics,31,32 stem cell tracking,33 bioimaging,34,35 photodynamic and photothermal tumor treatments,36,37 and immunotherapy.38

As surfactant- and capping-agent-free synthesis of GNS offers many advantages, a more mechanistic understanding of the modulation of the morphological features of GNS is yet to be
explored. This is of utmost importance, as the effectiveness and reliability of GNS in biomedical applications rely strongly on the consistency and reproducibility of physical, chemical, optical, and therapeutic properties of GNS, which are mainly governed by the morphological features of branches and the homogeneity of the nanoparticles.

The original protocol of seed-mediated, surfactant- and capping-agent-free GNS synthesis (first generation GNS) introduced by Vo-Dinh group yields individual nanoparticles with significant morphological inhomogeneity and broad absorbance spectra. The original protocol involves simultaneous addition of AgNO₃ and ascorbic acid (AA) to an acidic mixture of polycrystalline spherical Au NPs (d = 12 nm gold seeds) and gold chloride. The reaction kinetic for the formation of branched nanoparticles by this method is fast, completing in less than 5 s, and results in a 100% yield of GNS with a highly three-dimensional (3-D) nature in which multiple branches protrude from a spherical core in multiple planes. Although this synthetic method results in no to negligible byproducts, a careful analysis of the transmission electron microscopy (TEM) micrographs reveal a significantly high variation in the morphological features of the branches that results in the overall size inhomogeneity, which, in turn, leads to broad absorbance spectra. This ultimately affects the reproducibility, effectiveness, and reliability of the biomedical applications of GNS. For instance, a significant variation in the average surface areas of the nanoparticles affects the loading of therapeutic molecules or bioactive molecules for therapy applications.

In this study, we conducted a systematic investigation to identify the critical synthesis parameters that determine the homogeneity of the geometric features of branched nanoparticles and modulate the morphology, particularly the branch density and dimensions to obtain the desired optical properties. The investigation presents the design rules to optimize the bottom-up synthesis, and hence, improve the reproducibility and homogeneity of the surfactant-free GNS.

## RESULTS AND DISCUSSION

Addition of Shape-Directing Agent, Ag⁺, before Reducing Agent, Ascorbic Acid, Improves the Homogeneity of Branched Gold Nanoparticles. The underpotential deposition of Ag atoms on the defect sites of polycrystalline spherical Au NPs has been suggested as the underlying mechanism for the overgrowth of protrusion from spherical Au seeds leading to branched structures. Based on that growth mechanism, we identified that simultaneous addition of AgNO₃ and AA should not be necessary, but doing so might instead generate different subpopulations of nucleation sites in the same growth solution leading to a higher degree of variation in the overall morphology.

Therefore, the original synthesis protocol was revised such that the AgNO₃ is added to the growth solution (a mixture of Au NP seeds and an acidic gold chloride solution), let the solution mix for ~5 s to create a homogeneous reaction mixture and then introduce AA. Although this did not produce any significant changes in the reaction rates or color in the colloidal solution, a significant improvement in the quality of absorption spectra and morphology of GNS was observed (Figure 1a–d). The TEM image analysis of GNS showed a narrow size distribution, i.e., a significantly improved homogeneity in the size distribution for the spherical core diameter as well as the average tip-to-tip distance (Figures 1b and S1). This could also explain the improved features observed in the absorbance spectra of GNS. In general, for the new (second) generation of GNS, (i) the localized surface plasmon resonance (LSPR) peaks are noticeably more symmetric and (ii) the full width at half-maxima (fwhm) of the plasmon peaks occur in the NIR region are significantly narrower at any given LSPR maxima in comparison to that of the GNS synthesized from the original
These properties are important indications of significant improvements in the quality of nanoparticles, i.e., better homogeneity in size/morphology distribution. GNS now display improved morphological features as evident in their well-defined branches with narrower tip curvatures (Figure 1).

The batch-to-batch reproducibility and repeatability of the optical response of GNS are also improved with the modified protocol (Figures 1 and S2). Figure 1c shows very high reproducibility and repeatability of the LSPR peak position (649 ± 4 nm) and fwhm of the absorption spectra of GNS synthesized by different laboratory members on different days.

Less prominent spherical core and the more branched nature of the second generation GNS contribute to the negligible short band/shoulder ~520 nm in comparison to the first generation GNS from the original protocol. The sequential addition of AgNO3 and AA also improved the monodispersity of the GNS colloidal suspension, which is reflected by the narrower fwhm (on average ~136 nm) of the main LSPR peak (~650 nm) in the NIR region, in comparison to the fwhm ~200 nm in a comparative first generation GNS. The high-resolution TEM images and the EDX maps show that the presence of Ag (7 wt %) and Au (93 wt %) in single surfactant-free GNS (Figures 1e and S3) and the growth of branches along the {111} lattice planes (Figure 1f).

In addition to the main LSPR mode in NIR (mode 1, \(\lambda_1\)), second generation GNS show a higher-order plasmon mode (mode 2, \(\lambda_2\)) at higher wavelengths (Figure 2). These higher-order plasmon modes were not observable in the absorbance spectra of first generation GNS, which could be due to the high polydispersity of GNS colloidal suspensions. GNS with multiple plasmon modes in the NIR region has also been observed in branched NPs synthesized by other synthetic approaches. These higher-order LSPR modes (\(\lambda_2\)) become more prominent and also red-shift as the GNS becomes larger in size with long branches and higher branch densities (Figures 3, S4 and S5). However, it should be noted that only the first LSPR mode (mode 1, \(\lambda_1\)) was considered for the study reported in this article. We expect that an in-depth understanding of the higher-order plasmon modes could provide valuable information on the plasmonic properties of GNS, however, that is beyond the scope of this study.

The currently accepted mechanism for the seed-mediated formation of branched nanoparticles proposes that the underpotential deposition of Ag atoms on the defect sites of polycrystalline spherical Au NPs catalyzes and promotes the growth of protrusions, whereas reduced Au atoms contribute to the extension of protrusion and the growth of the Au core. This mechanism suggests two important factors that could determine the branch density of nanoparticles, (1) the number of surface exposed defect sites on Au seeds and (2) the amount of Ag+ available in the growth solution. To deconvolute the individual contributions of the defect sites and the Ag+ content, two sets of experiments were carried out, where (1) the size of the polycrystalline Au NPs seeds and (2) the amount...
The optical absorption or the LSPR maxima tunability can be achieved in the visible to NIR region regardless of the size of the Au seeds, both core and tip-to-tip distance, are analyzed. Table 1 provides the approximated branch dimensions by analyzing the TEM micrographs of GNS. It should be noted that the provided values are considered underestimations, as it is challenging to obtain more accurate estimations from these nanoparticles of highly 3-D nature from the 2-D projections from the TEM images (Figure S1).

Based on the TEM analysis, two significant differences between GNS synthesized using 5 and 12 nm Au seeds were observed. (i) The size of particles, both core and tip-to-tip diameter, is smaller for GNS synthesized from 5 nm Au seeds at any given LSPR maximum considered in this study (Table 1). (ii) The branches are relatively long for GNS synthesized using 5 nm Au seeds. The latter fact could contribute to more morphological heterogeneity in individual GNS, which collectively could be more pronounced.

The optical absorption or the LSPR maxima tunability can be achieved in the visible to NIR region regardless of the size of the Au seeds (Figure 2c). In general, branched nanoparticles of similar LSPR maxima can be obtained from the Au seeds of varying diameters, which was evident by GNS synthesized using \( d = 5 \) and 12 nm Au seeds in this study. Regardless of the size of the Au seeds, three general trends in the morphology of GNS were observed as the number of Au seeds to moles of \( \text{Au}^{3+} \) available in the growth solution increases: (i) the branch length and the branch aspect ratio, i.e., the branch length to width, decreases, (ii) the size of the Au core decreases, which collectively leads to a decrease in the overall size of the branched nanoparticles (Table 1, Figures S5 and S6), and (iii) an overall decrease in the branch density of GNS (as visible in the two-dimensional (2-D) plane from the TEM micrographs) is observed with increasing Au seeds-to-Au\(^{3+}\) concentration ratio. These observations could be attributed to the limited \( \text{Au}^{3+} \) available in the growth solution for the growth of core and protrusions as the amount of seeds increases. This also explains the presence of nanoparticles with incomplete growth of protrusions or GNS bearing much shorter and wider protrusions (less prominent branched nature) at elevated seed concentrations that exhibit optical absorbance <650 nm. It should also be noted that a general trend of peak broadening was observed with the red-shift in the LSPR peak position. This could be attributed to the morphological heterogeneity that GNS acquire, as it gets larger in size with multiple branches. In addition to morphological changes, three prominent trends in the optical absorption of GNS (originate from both \( d = 5 \) and 12 nm Au seeds) were observed when the number of Au seeds to amount (moles) of \( \text{Au}^{3+} \) available in the growth solution decreases: (i) the main LSPR mode in the NIR red-shifts, (ii) the peak symmetry gradually decreases, and (iii) fwhm of the main LSPR mode increases. This could be attributed to the morphological anisotropy that originates from the increase in branch densities and the branch lengths in GNS that possess LSPR modes at wavelengths approximately above 750 nm in comparison to GNS with the main LSPR modes at wavelengths below 750 nm.

For further examination, GNS obtained from \( d = 12 \) and 5 nm Au seeds that exhibit similar LSPR peak position were compared for any relationship between the branch characteristics and the size of the Au seeds. Analysis of the TEM micrographs of four pairs of nanoparticles from 12 to 5 nm Au seeds that exhibit LSPR maximum \( \sim 650, 700, 750, \) and 800 nm was carried out (Figure 3). Table 1 provides the approximated branch dimensions by analyzing the TEM micrographs of GNS. It should be noted that the provided values are considered underestimations, as it is challenging to obtain more accurate estimations from these nanoparticles of highly 3-D nature from the 2-D projections from the TEM images (Figure S1).

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Surprisingly, no significant difference in the branch density was observed among the set of nanoparticles and LSPR peak regions considered. Therefore, it is concluded that at least under the physical parameters we studied, the size of the Au seeds do not make a noticeable contribution to the tuning of the branched nature of particles.

### Table 1. Dimensions of the Features of GNS and Their LSPR Peak Positions As Determined by the TEM Analysis

| AuCl\(_3\)/mol | Ag\(^+/\)/mol | amount of seeds/particles | LSPR\(_{\text{max}}\) (nm) | core diameter (nm) | tip–tip distance (nm) | branch density |
|----------------|---------------|---------------------------|---------------------------|-------------------|----------------------|---------------|
| \(2.5 \times 10^{-6}\) | \(1.02 \times 10^{-7}\) | \(2.5 \times 10^{10}\) | 800 | 93 ± 11 | 145 ± 30 | \(\sim 7\)–8 |
| \(2.5 \times 10^{-6}\) | \(1.02 \times 10^{-7}\) | \(5.0 \times 10^{10}\) | 738 | 70 ± 9 | 105 ± 28 | \(\sim 8\)–10 |
| \(2.5 \times 10^{-6}\) | \(1.02 \times 10^{-7}\) | \(1.0 \times 10^{10}\) | 690 | 57 ± 8 | 80 ± 16 | \(\sim 5\)–6 |
| \(2.5 \times 10^{-6}\) | \(1.02 \times 10^{-7}\) | \(2.0 \times 10^{10}\) | 658 | 46 ± 7 | 67 ± 15 | \(\sim 5\)–6 |
| \(2.5 \times 10^{-6}\) | \(1.02 \times 10^{-7}\) | \(3.0 \times 10^{10}\) | 638 | 39 ± 6 | 59 ± 14 | \(\sim 4\)–5 |

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### Amount of Reactive Au\(^+/\)/Ag\(^+/\) Determines the Branch Density and Dimensions of Nanoparticles

The contribution of Ag on the anisotropic growth of nanoparticles has been reported in literature.\cite{40,41,44} Figure 4 shows that as the Au "to-
Ag⁺ molar ratio is changed, a significant change in the branched morphological features is observed. The branch density is increased on average from 3 to 10 as the available Ag⁺ content is increased by 8 times. In addition, the average branch length is increased, leading to an increased overall size of the particle, as defined by the average tip-to-tip distance. Based on the catalytic Ag theory, we hypothesized that more defect sites on the polycrystalline seeds are activated and catalyzed by the Ag deposition with increasing Ag⁺ content, and having more activated surface sites on Au seeds leads to the growth of more protrusions during the growth process, hence a higher branch density. The distribution of Au₅⁺ among an increasing number of branches leads to relatively shorter branches in comparison to longer branches as observed in nanoparticles with lower branch density. Table 1 summarizes the dimensions for various morphological features of GNS synthesized under varying Au³⁺-to-Ag⁺ molar ratios.

**pH and Additive Halides Play an Important Role in the Formation of Branched Nanoparticles.** Halides have been demonstrated to affect the anisotropic growth of surfactant-mediated Au and Ag NPs.⁴⁻⁵ There are few fundamental studies that have demonstrated the Au–halide chemical affinities and halide-concentration-dependent morphological and spectral tuning of Au NPs in different contexts.⁴⁷ Our method for the surfactant-free, seed-mediated growth of GNS uses HCl to adjust the pH of the growth solution, which introduces 2 mM final concentration of chloride (Cl⁻) into the growth solution. To the best of our knowledge, no studies have been reported to understand the influence of halides on the surfactant-free/capping agent-free synthesis of anisotropic nanoparticles. Therefore, we conducted a systematic investigation of the effect of Cl⁻, bromide (Br⁻), and iodide (I⁻) on the growth mechanism of the surfactant-free GNS, in terms of the GNS overall size, branch characteristics, homogeneity of particles, and optical properties.

Neither pH nor Cl⁻ alone is critical for the growth of surfactant-free GNS, but together they affect the subtle modulations in the shape and optical properties of GNS. We conducted the syntheses in which (a) pH was adjusted to 3 using a non-halide-containing inorganic acid, nitric acid (absence of Cl⁻), and (b) no pH adjustment was made, but the same amount of Cl⁻ was introduced to the growth solution as NaCl (pH of the growth medium is 6). The resultant GNS were compared to that from the original growth conditions, where the pH is 3 and Cl⁻ is present. Both these conditions resulted in the GNS whose morphology and optical response were similar to those from the original protocol (pH 3, 2 mM Cl⁻), indicating that the presence of Cl⁻ is not necessary or critical for the shape evolution of GNS (Figures 5 and S3).

Changing the pH of the growth solution from 3 to 6, however, resulted in significant differences in the optical response and also a few noticeable changes in the morphology. In particular, growth media at less acidic pH (pH 6) resulted in GNS that reflected less defined, relatively shorter branches (Figure 5a,c). Growth media at basic pHs have also reported to result in GNS that are more spherical in shape with less defined branches.⁴⁹ The pH of the growth solution is important for the equilibrium concentration of AuCl₄⁻, and the acidic pHs employed in this study is considered adequate to drive the equilibrium reaction to yield all of the reactive Au³⁺. However, the LSPR maxima and the overall size of the GNS were smaller for the syntheses carried out at pH 6 compared to that of the GNS for the syntheses at pH 3 (Figure 5a,c). This can be attributed to the reduction of Au³⁺ to Au(0) to a lesser extent at pH 6 compared to that at pH 3, ultimately affecting the growth of GNS in size (both core and branch extension in size) and hence the blue-shifted LSPR maxima. To estimate the reduction yield of Au³⁺ at each pH, we compared the absorbance ~295 nm, which is the characteristic absorbance band for AuCl₄⁻ for the as-synthesized GNS.⁴⁸ Based on the absorbance values at 295 nm for a representative GNS obtained at pH 3 (A = 0.584) and pH 6 (A = 0.624), we estimated that the reduction of AuCl₄⁻...
probably takes place to a lesser extent at pH 6 than at pH 3 (Figure S7). This could be attributed mainly due to the reducing power of ascorbic acid (higher reducing power at pHs lower than the $pK_a$ of ascorbic acid and vice versa) being affected by the pH of the growth solution. It could affect the complete reduction of Au$^{3+}$ but not the Ag$^+$ ions present in the growth solution. As a result, the complete nucleation of Ag$^+$ and incomplete reduction of Au$^{3+}$ could dominate at pH 6, which, in turn, affect the growth of GNS, resulting particles that are relatively smaller in size compared to those from the GNS obtained at pH 3. In addition, it is also reported that as the pH increases, the equilibrium concentration of AuCl$_4^{-}$ decreases due to the formation of gold hydroxyl complexes.

This could also reduce the available amount of Au$^{3+}$ for the GNS growth at pH 6. Therefore, the collective influence of the above-discussed pH-dependent changes in the growth solution could yield GNS that are relatively smaller in size at pH 6. Therefore, based on our results, an acidic growth solution is more favorable for the complete growth of surfactant-free GNS. The fact that the morphology evolution of GNS is not significantly impacted by pH is advantageous for an effective subsequent pH-sensitive postsynthesis modifications such as functionalization with oligonucleotides or silica/silver shell formation.

However, subtle modulation of the optical absorbance of GNS in a narrow wavelength range was observed as the final concentration of Cl$^-$ in the growth solution changes. As shown in the TEM micrographs, an increase in the branch density is noticeable as the [Cl$^-$] in the growth solution decreases, and this effect is more prominent in the growth solutions at pH 3 (Figure 5c). Though Cl$^-$ is not a critical experimental factor that contributes to the origin of branches, we have demonstrated that Cl$^-$ could be used to fine-tune the morphological features, and thereby modulate the LSPR peak maxima of the surfactant-free GNS in a narrow range of wavelengths. It is noteworthy that the morphological and optical tuning of GNS mediated by Cl$^-$ is not significant and effective as that by Au seeds and AgNO$_3$. But the presence of Cl$^-$ in the growth solution should be taken into consideration as an important experimental factor to ensure reproducible optical and morphological properties of GNS.

Addition of Br$^-$ significantly affects the shape evolution of branched nanoparticles, and this effect is both pH- and concentration-dependent. In the presence of Br$^-$, at the same concentration and pH as in the original protocol (pH 3, 2 mM), the resultant GNS exhibit truncated branches with more prominent spherical core, whereas GNS synthesized at pH 6, 2 mM Br$^-$ exhibit only a very few, broad protrusions (Figure 5d). However, longer and narrower branches with higher density, even comparable to the original GNS, can be observed from the growth solutions containing nanomolar concentrations of Br$^-$ at pH 3 and to a lesser degree at pH 6. In addition, it should be noted that in the presence of Br$^-$ all of the concentrations and pHs investigated in this study, the batch-to-batch reproducibility of LSPR peak position is not as consistent as it is in the presence of Cl$^-$ (Figures 5b and S7). The shape evolution and morphology tuning trends in the presence of Br$^-$ could be explained by chemisorption of Br$^-$ on the crystallographic facets of the Au seeds with higher Au–Br$^-$ affinity than Au–Cl$^-$, which elevates the reduction potential of Au and Ag for the emergence of protrusions and their growth.

The presence of $\Gamma^-$, even in nanomolar concentration, causes drastic morphological and optical changes in the surfactant-free GNS synthesis. At the various $\Gamma^-$ concentrations (10 nM to 100 $\mu$M) investigated in this study, the growth of branches is completely diminished, resulting spherical nanoparticles that exhibit optical absorption in the visible region with a broader fwhm (Figure S8). This could be attributed to the chemisorption of $\Gamma^-$ with high affinity to Au (the highest among halides) that increases the reduction potential of Au and passivates the crystallographic facets of polycrystalline Au seeds, which, in turn, suppress the emergence of protrusions from the Au seeds.$^{27,45,46}$

**CONCLUSIONS**

In conclusion, we have identified several experimental parameters that determine the geometrical features of branches, which, in turn, affect the overall morphology, optical response, and, most importantly, the batch-to-batch reproducibility. This latter feature is essential for the reliable performances of surfactant-free GNS-based applications. We have demonstrated that the addition of shape-directing AgNO$_3$ followed by ascorbic acid addition ensures improved homogeneity and high reproducibility of surfactant-free GNS synthesis. The LSPR peak position tuning was demonstrated to be achievable by changing the relative concentration of Au seeds, reactive Ag$^+$, and also by tuning the chemical affinities of Au–halide. Branch characteristics of GNS were found to depend strongly on the reactive Ag content, whereas the shape evolution of branched nanoparticles was sensitive to the presence of halides. These synthetic design rules provide multiple routes for manufacturing GNS that exhibit a specifically desired optical response and have varying morphological characteristics, using a simple surfactant-free approach. Finally, the findings of this study lay the foundation for predetermining and synthesizing the best-performing GNS for an application of interest.

**MATERIALS AND METHODS**

**Chemicals and Materials.** Gold chloride solution, silver nitrate (AgNO$_3$), l-ascorbic acid (AA), sodium bromide (NaBr), sodium chloride (NaCl), and sodium iodide (NaI) were purchased from Sigma-Aldrich. Hydrochloric acid (HCl, 1 M) and 1 M nitric acid (HNO$_3$) were purchased from VWR. Citrated capped gold colloid solution (5 nm diameter, concentration = $5 \times 10^{13}$ particles/mL) was purchased from BBI Solutions (Figure S9) and thiol-poly(ethylene glycol) (PEG)-COOH MW 5000 from Nanocs Inc. All of the syntheses were performed in 20 mL polyethylene scintillation vials (VWR) using Ultrapure Millipore Milli-Q water. Magnetic stir bars and all of the the glassware used in Au NP syntheses were cleaned by aqua regia before use. Citrate capped 12 nm diameter Au NPs seeds (concentration = $1.5 \times 10^{11}$ particles/mL) for GNS synthesis were synthesized according to a modified version of the Turkveth method (Figure S9).$^{53}$

**Surfactant- and Capping-Agent-Free GNS Synthesis.** The GNS synthesis was carried out using a modified version of surfactant-free GNS synthesis described by Vo-Dinh et al.$^{23}$ 492 $\mu$L of gold chloride solution (5.08 mM) was added to 10 mL of Milli-Q water and mixed under magnetic stirring for 1 min. 20 $\mu$L of 1 M HCl was then added and 5 $\mu$L of 12 nm diameter Au seeds (concentration = $1.5 \times 10^{11}$ particles/mL) 30 s later. After 5 s, 34 $\mu$L of 3 mM AgNO$_3$ followed by 100 $\mu$L 100 mM AA, was added with a 5 s delay between them, and the reaction was allowed to proceed for 30 s under moderate stirring conditions. Formation of GNS begins immediately after the addition of AgNO$_3$, which is observable by the color change
from colorless to bluish-green to purple (depending on the AgNO₃ and seed amounts). The resulted GNS were immediately purified by centrifugation at 3000g for 10 min, and the resulted pellet was resuspended in about 1 mL of Milli-Q water. The purified GNS were either functionalized with ligands of interested or stabilized with thiol-PEG-COOH and stored at 4 °C until further use. For stabilization of as-purified GNS by PEGylation, 10 μL of 1 mg/mL aqueous solution of thiol-PEG-COOH was added to 1 mL of purified GNS and incubated at room temperature for 1 h. Then, PEGylated-GNS was purified by centrifugation at 3000g for 10 min and the resulted pellet was resuspended in about 1 mL of Milli-Q water and stored at 4 °C covered in aluminum foil. These functionalized GNS were used for further experimentation within 2 weeks, as GNS exhibited better optical and morphological stability within that time (Figure S10). The same protocol was used to synthesize GNS using 5 nm diameter Au seeds (concentration = 5 × 10¹⁵ particles/mL).

Changing Halide Concentration and pH. GNS were synthesized by varying halide concentration in the growth solution between 10 nM and 1 mM by adding 10 μL of the relevant stock solutions (10 μM to 1 M) of NaCl, NaBr, and NaI. The pH of the growth solution was adjusted to pH 3 by adding 20 μL of 1 M HNO₃ whereas pH 6 was obtained by not adding acids to the growth solution.

Characterization. The absorbance spectra of the purified GNS (both PEGylated and bare) suspended in Milli-Q water were recorded using a multimode microplate reader, FLUOstar Omega (BMG Labtech), and a UV-3600 (Shimadzu) spectrophotometer. The morphology and the particle size distribution of Au NPs were evaluated by using FEI Tecnai G² Twin transmission electron microscope, and HAADF STEM images and EDX maps were acquired using FEI Titan 80-300. Hydrodynamic particle size distribution and nanoparticle concentration were measured by nanoparticle-tracking analysis using a NanoSight NS500 instrument (Malvern, Worcestershire, U.K.).

Determination of the Dimensions of GNS. Dimensions of GNS were determined using a marker-controlled watershed segmentation algorithm on MATLAB (Supporting Information). This process involves segmenting the TEM images into foreground objects (nanoparticles) and background. Segmentation is carried out in a two-step process by first performing a distance transform on the image and subsequently applying a watershed transform on the resulting image. Briefly, these two functions separate the foreground objects by quantifying the pixel intensity differences between different particles and the overall background. This watershed transform is function that turns an image into a surface where low-intensity pixels can be thought of as troughs or catchment basins and high-intensity pixels are the ridges that separate these basins. The process effectively separates objects from each other and forms the background. Afterward, another function is defined to quantify the pixel lengths of each detected particle’s estimated perimeter. The pixel values are subsequently converted to real lengths by using a calibration from a scale bar in the same image. The core diameter was estimated as the equivalent diameter of a circle with the same area as the particle. The tip-to-tip length was estimated using an algorithm that finds the maximum distance of any two points on the particle’s perimeter.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.7b01700.

Characterization of surfactant-free GNS synthesized under varying experimental condition, description of Matlab code (PDF)

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Author Contributions
The manuscript was written through contributions of all of the authors. All of the authors have given approval to the final version of the manuscript.

Notes
The authors declare no competing financial interest.

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