Nanoscale Surface Curvature Effects on Ligand—Nanoparticle Interactions: A Plasmon-Enhanced Spectroscopic Study of Thiolated Ligand Adsorption, Desorption, and Exchange on Gold Nanoparticles

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ABSTRACT: The interfacial adsorption, desorption, and exchange behaviors of thiolated ligands on nanotextured Au nanoparticle surfaces exhibit phenomenal site-to-site variations essentially dictated by the local surface curvatures, resulting in heterogeneous thermodynamic and kinetic profiles remarkably more sophisticated than those associated with the self-assembly of organothiol ligand monolayers on atomically flat Au surfaces. Here we use plasmon-enhanced Raman scattering as a spectroscopic tool combining time-resolving and molecular fingerprinting capabilities to quantitatively correlate the ligand dynamics with detailed molecular structures in real time under diverse set of ligand adsorption, desorption, and exchange conditions at both equilibrium and nonequilibrium states, which enables us to delineate the effects of nanoscale surface curvature on the binding affinity, cooperativity, structural ordering, and the adsorption/desorption/exchange kinetics of organothiol ligands on colloidal Au nanoparticles. This work provides mechanistic insights on the key thermodynamic, kinetic, and geometric factors underpinning the surface curvature-dependent interfacial ligand behaviors, which serve as a central knowledge framework guiding the site-selective incorporation of desired surface functionalities into individual metallic nanoparticles for specific applications.

KEYWORDS: Surface capping ligands, metallic nanoparticles, nanoscale surface curvature, plasmon-enhanced spectroscopy, ligand dynamics, ligand exchange

Almost all chemically synthesized colloidal inorganic nanoparticles, regardless of their sizes, shapes, and compositions, are coated with organic surface capping ligands, which play pivotal roles in guiding the nanocrystal growth during bottom-up colloidal syntheses. The functionalization of nanoparticle surfaces with deliberately designed ligands, either through ligand-guided nanocrystal growth or postsynthetic ligand exchange, may introduce interesting modifications to the optical, electronic, and surface properties of the nanoparticles, remarkably influencing a series of important interfacial processes, such as surface charge transfers, catalytic molecular transformations, targeted delivery in biological systems, and programmed assembly of nanocrystals into suprastructures.

Rational optimization of nanoparticle surfaces for specific applications essentially relies on detailed mechanistic understanding of the dynamic interactions between the inorganic nanocrystal cores and the organic ligand shells.

It has long been known that a plethora of organothiol molecules spontaneously chemisorb onto macroscopic Au substrates exhibiting atomically flat surfaces through strong Au–S interactions to form structurally ordered and thermodynamically stable self-assembled monolayers (SAMs). The energetics and dynamics of the ligand-surface interactions, however, are drastically modified when nanoscale curvatures are introduced to the planar Au surfaces. The effects of such nanoscale surface curvature become remarkably more pronounced when the thiolated ligands interact with ultrasmall, sub-5 nm Au nanoparticles whose surfaces are highly curved and dominated by undercoordinated surface atoms, giving rise to a unique set of intriguing phenomena that are otherwise not observable on flat Au surfaces, such as phase separation of subnanometer ligand domains, altered pKa of surface ligands, enhanced macro-molecular crowding effect, chemical patchiness-guided self-assembly, and kinetic modulation of regioselective photoreactions. To better understand the surface curvature effects, a series of in situ spectroscopic techniques, such as electron paramagnetic resonance, nuclear magnetic resonance, second harmonic generation, X-ray absorption spectroscopy, and surface plasmon resonance spectroscopy, have been used in combination with calorimetry to resolve the complex kinetic and thermodynamic profiles of the ligand-
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We selectively synthesized subwavelength Au quasi-spherical nanoparticles (QSNPs) and surfaceroughened nanoparticles (SRNPs) with tunable overall particle sizes by kinetically maneuvering the seed-mediated nanocrystal growth following a protocol we previously developed. Slow nanocrystal growth resulted in the formation of thermodynamically stable, multifaceted Au QSNPs (Figure 1A), whereas Au SRNPs enclosed by highly curved surfaces (Figure 1B) represented the kinetically favored products under fast nanocrystal growth conditions. Here we tuned the sizes of QSNPs and SRNPs to be both around 130 nm (Figure 1C) to ensure that the strikingly distinct interfacial ligand behaviors were intrinsically tied to the difference in surface curvatures rather than overall particle sizes.

As revealed by detailed electron microscopy characterizations, the surfaces of SRNPs were enclosed by a mixture of undercoordinated and close-packed surface atoms at the highly curved and locally flat surface regions, respectively, whereas QSNPs were primarily enclosed by {100} and {111} facets covered with close-packed surface atoms with a small fraction of undercoordinated surface atoms located at the particle edges, corners, and surface defects. A strong correlation existed between the characteristic surface oxidation potentials and the surface atomic coordinations, allowing us to use cyclic voltammetry (CV) to characterize the atomic-level surface structures of QSNPs and SRNPs (Figure 1D).

The as-synthesized QSNPs and SRNPs were both capped with positively charged CTAC ligand bilayers, which could be completely displaced by organothiol ligands, such as 4-thiolated aromatic ligands on surface-textured Au nanoparticles using surface-enhanced Raman scattering (SERS) as an ultrasensitive plasmon-enhanced spectroscopic tool. SERS has unique time-resolving and molecular fingerprinting capabilities, enabling detailed correlation of ligand dynamics with interfacial molecular structures in real time both at equilibrium and nonequilibrium conditions without separating the colloidal nanoparticles from their native, ligand-present environments. Previous spectroscopic studies on the interactions of organothiols with Au surfaces, however, have been focusing on either macroscopic planar Au substrates or sub-5 nm Au nanoparticles, neither of which possess desired plasmonic properties exploitable for SERS, whereas the ligand dynamics on Au nanoparticles in the size range from 5 to 100 nm remains much less explored.

Here, we are particularly interested in subwavelength Au colloids in a mesoscopic size regime (50–200 nm) with well-preserved colloidal stability under a wide range of ligand adsorption, desorption, and exchange conditions. These subwavelength Au nanoparticles exhibit tunable plasmon resonances with intense field enhancements optimizable for SERS-based quantification and characterization of surface ligands on individual colloidal nanoparticles, circumventing the irreproducibility issues caused by the interstitial “hot spots” in nanoparticle aggregates. As shown in this work, atomically flat facets and nanoscale textures can be selectively created on the nanoparticle surfaces while still keeping the overall particle sizes in the plasmonically active, subwavelength regime.
aminothiophenol (4-ATP). Upon complete surface ligand exchange of CTAC with 4-ATP, the $\xi$-potentials of QSNPs and SRNPs both decreased by $\sim$60% while the hydrodynamic sizes remained essentially unchanged (Figure S1A in Supporting Information). The introduction of nanoscale surface roughness to subwavelength Au nanoparticles caused significant spectral red-shift of the plasmon resonance and further intensified local-field enhancements. After ligand exchange, the plasmon resonances of both the QSNPs and SRNPs red-shifted by $\sim$5 nm while the plasmon bandwidths and spectral lineshapes remained essentially unchanged (Figure S1D in Supporting Information), verifying that both Au QSNPs and SRNPs well-maintained their colloidal stability, free of aggregation. The temporal evolution of the $\xi$-potentials of colloidal nanoparticles dispersed in pure water (Figure S1C in Supporting Information) indicated that the dissociation of 4-ATP from the particle surfaces was far slower than that of CTAC, exhibiting no detectable decrease in $\xi$-potentials over 30 days because the covalent Au–Si interactions were remarkably stronger than the Au–CTAC interactions.

We used SERS to quantify the surface coverages and characterize the structures of chemisorbed 4-ATP ligands. We focused a near-infrared laser (785 nm, excitation volume of $\sim$100 fL) on the colloidal samples using a confocal Raman microscope such that each freely diffusing nanoparticle was exposed to the excitation laser for a short time period limited by diffusion, effectively eliminating photothermal sample damage and plasmon-driven photoreactions (e.g., photocatalytic coupling of 4-ATP). As shown in Figure S2 in Supporting Information, a broad SERS band corresponding to the C–H stretching mode of CTAC was observed around 2800 cm$^{-1}$ on the CTAC-coated QSNPs, which completely disappeared after the CTAC ligands were fully displaced with 4-ATP. When the surfaces of QSNPs were saturated with 4-ATP, the SERS signatures of 4-ATP were clearly resolved (see detailed peak assignments in Table S1 in Supporting Information), whereas no Raman signals of 4-ATP were detectable on 100 $\mu$M 4-ATP solution without Au nanoparticles. The absence of the characteristic peak of S–H bond stretching at 2530 cm$^{-1}$ strongly indicated that the SERS signals were from the 4-ATP ligands chemisorbed on nanoparticle surfaces with negligible interference from the unbound 4-ATP ligands remaining in the bulk solution. The Raman band corresponding to the O–H stretching mode of H$_2$O (3230 cm$^{-1}$) exhibited essentially fixed intensity and peak position for all the samples we measured, thereby serving as an internal reference for quantifying the relative SERS intensities of various Raman modes. At 785 nm excitation, 4-ATP-coated SRNPs exhibited SERS signals about 60 times stronger than those on the 4-ATP-coated QSNPs (Figure S3 in Supporting Information). The empirical average Raman enhancement factor was estimated to be on the order of 10$^6$ on SRNPs, about 1 order of magnitude higher than that on QSNPs, which was in excellent agreement with previously reported finite-difference time domain calculations and the SERS data collected on surface-immobilized single nanoparticles. Details of the enhancement factor estimation were presented in Supporting Information.

We titrated the Au QSNPs and SRNPs with 4-ATP to displace the surface-bound CTAC (Figure 2A) and used SERS...
to quantify the apparent surface coverages of 4-ATP at equilibria, $\theta_{eq}$. We incubated CTAC-coated QSNPs and SRNPs with various concentrations of 4-ATP for a sufficiently long time, that is, 24 h, to ensure that the ligand adsorption/desorption equilibrium was fully established at each titration point prior to the SERS measurements. As the 4-ATP concentration, $C_{4\text{-ATP}}$, progressively increased, the Raman peaks signifying the chemisorbed 4-ATP became increasingly more intense until reaching a plateau upon the saturation of the nanoparticle surfaces with 4-ATP (Figure 2B,C). The $\theta_{eq}$ at various $C_{4\text{-ATP}}$ were calculated by normalizing the intensities of the two major Raman peaks, the C=S stretching mode at 1078 cm$^{-1}$ and the benzene ring stretching mode at 1588 cm$^{-1}$, against those corresponding to saturated 4-ATP surface coverages (obtained after incubation of QSNPs or SRNPs with 100 $\mu$M 4-ATP). The $\theta_{eq}$ at various $C_{4\text{-ATP}}$ were calculated by normalizing the intensities of the two major Raman peaks, the C=S stretching mode at 1078 cm$^{-1}$ and the benzene ring stretching mode at 1588 cm$^{-1}$, against those corresponding to saturated 4-ATP surface coverages (obtained after incubation of QSNPs or SRNPs with 100 $\mu$M 4-ATP for 24 h). The apparent ligand adsorption isotherms (plots of $\theta_{eq}$ versus $C_{4\text{-ATP}}$) obtained from the 1078 and 1588 cm$^{-1}$ modes were shown in Figure 2D,E, respectively. Assuming the surface area of each SRNP to be 4.5 times of that of a nanosphere with a diameter of 130 nm and the ligand binding affinity to be infinitely high, we estimated that the surfaces of SRNPs could be fully saturated with a close-packed ligand monolayer at $C_{4\text{-ATP}}$ of $\sim$1 $\mu$M. These estimations also involved the assumption that the binding footprint size of each 4-ATP molecule on the SRNPs was the same as that for 4-ATP SAMs on a planar Au surface, which was previously measured to be 0.39 nm$^2$ per molecule. Considering the finite ligand binding affinity, the orientational flexibility of ligands at unsaturated ligand coverages, and considerably lower molecular packing density on highly curved surfaces than that on planar surfaces, the fraction of ligands bound to nanoparticle surfaces should be much smaller than that of the unbound ligands in solution in the ligand concentration range we explored. Therefore, it was reasonable to assume the free ligand concentration, $[4\text{-ATP}]$, to be roughly equal to the total ligand concentration, $C_{4\text{-ATP}}$.

We fitted the 4-ATP adsorption isotherms on QSNPs with the Hill equation shown below

$$\theta_{eq} = \frac{K_1C^\eta}{1 + K_1C^\eta}$$

where $K_1$ was the equilibrium constant, $\eta$ was the Hill coefficient, and $C$ was the ligand concentration. The fitting results showed that 4-ATP strongly adsorbed to the faceted surfaces of QSNPs with micromolar-scale affinities in a noncooperative manner with Hill coefficients very close to 1, obeying the classical Langmuir adsorption model which involved the ligand binding to independent surface sites with a uniform binding affinity.

The 4-ATP binding isotherms on Au SRNPs, however, exhibited much more complicated profiles that cannot be interpreted by a simple Langmuir adsorption model. Because each SRNP was enclosed by both locally flat and curved surfaces, the contributions of two types of binding sites with remarkably different affinities and cooperativities must be considered using a two-component Hill equation.
\[ \theta_{eq} = a \times \frac{K_C C^{eq}}{1 + K_C C^{eq}} + (1 - a) \times \frac{K_C C^{eq}}{1 + K_C C^{eq}} \]  

where \( K_1 \) and \( n_1 \) are the equilibrium constant and Hill coefficient, respectively, for the ligand binding to the locally flat regions on SRNP surfaces. \( K_2 \) and \( n_2 \) are the equilibrium constant and Hill coefficient describing the binding of 4-ATP to the high-curvature sites on SRNP surfaces. The fractional coefficient, \( a \), refers to the fraction of ligands bound to flat surface regions. We fitted the binding isotherms of 4-ATP on SRNPs with eq 2 to get \( K_1, K_2 \) and \( n_1 \), while adopting the same \( K_1 \) and \( n_1 \) values for QSNPs obtained from curve fitting using eq 1. The binding affinity of 4-ATP to the high-curvature sites was approximately 1 order of magnitude lower than that to the locally flat Au surfaces (Figure 2D,E). The low-affinity ligand binding to high-curvature sites provided the major contribution (\( \sim 77\% \)) to the overall binding isotherm of 4-ATP on SRNPs, whereas the low-affinity component was not observed on QSNPs because the locally curved edges, corners, and defects only accounted for a negligibly small fraction of the surface areas of QSNPs. The relative areas of locally flat regions versus the highly curved regions, however, should not be simply estimated based on the relative fractions of the low-affinity and high-affinity binding components because the local field enhancements are also strongly dependent upon the local surface curvatures. Distinct from the noncooperative adsorption on flat Au surfaces, the adsorption of 4-ATP onto nanoscale curved surfaces was highly cooperative with Hill coefficients around 2 (Figure 2D,E, and Figure S4 in Supporting Information). Such surface curvature-dependent ligand adsorption behaviors were also observed on 4-mercaptobenzoic nitrile (4-MBN), another representative thiolated aromatic ligand (see adsorption isotherms in Figure S5 and SERS peak assignments in Table S2 in Supporting Information).

The SERS-based ligand titration assay allowed us to further correlate the structural ordering with the \( \theta_{eq} \) of chemisorbed ligands. We found that the SERS peak intensity at 1480 cm\(^{-1}\), which was assigned to a coupled \( \nu_{C-N} + \nu_{C-H} + \nu_{C=O} \) mode, \(^5\) was sensitively dependent on the degree of structural ordering of chemisorbed 4-ATP. On QSNPs, the intensity ratios of the 1480 to 1588 cm\(^{-1}\) peaks, \( I_{1480\text{cm}^{-1}}/I_{1588\text{cm}^{-1}} \) progressively increased with \( C_{4\text{ATP}} \), reflecting the transition from randomly oriented molecules at low \( \theta_{eq} \) to more ordered monolayer structures at higher \( \theta_{eq} \) (Figure 2F). At low \( \theta_{eq} \), the randomly oriented 4-ATP ligands might also interact with Au surfaces through Au–amine interactions, which were typically considered to be much weaker than the Au–thiol interactions. The \( I_{1480\text{cm}^{-1}}/I_{1588\text{cm}^{-1}} \) on SRNPs also increased with \( C_{4\text{ATP}} \) reaching a plateau upon saturation of the flat regions with 4-ATP at \( C_{4\text{ATP}} \) around 2.5 \( \mu \)M. However, \( I_{1480\text{cm}^{-1}}/I_{1588\text{cm}^{-1}} \) did not further increase at higher \( C_{4\text{ATP}} \), strongly suggesting that 4-ATP on the nanoscale curved surfaces were significantly less ordered than those on the flat surfaces.

We further used SERS as an in situ spectroscopic tool to monitor the kinetics of thiolated ligand adsorption (Figure 3A–D). Widely divergent kinetic results exist in the literature, as the time it takes to form densely packed, pseudocrystalline organothiol SAMs on Au surfaces has been reported to vary over a diverse range of time scales from seconds \(^5\) to minutes \(^5\) to hours \(^5\), and even up to several days. \(^5\) The lack of consensus on the ligand adsorption kinetics is believed to stem from the structural diversity of the ligand molecules being studied and the heterogeneous distributions of ligand binding sites on the substrates with varying local surface curvatures. In the context of Langmuir adsorption kinetics, both first- and second-order rate laws have been commonly used to describe ligand adsorption kinetics, and the claims of which one is better than the other is quite often made based on a marginal difference in the correlation coefficients of fitting. \(^60\) A more general form of the Langmuir kinetics can be expressed as a varying-order rate equation using the following polynomial expression \(^60\)

\[ \frac{d\theta}{dt} = k_1(\theta_{eq} - \theta) + k_2(\theta_{eq} - \theta)^2 \]  

where \( \theta_{eq} \) is the ligand coverage at equilibrium, \( \theta \) is ligand coverage at a reaction time, \( t \), and \( k_1 \) and \( k_2 \) are the first- and second-order rate constants, respectively. The relative magnitude of \( k_1 \) over \( k_2 \times (\theta_{eq} - \theta) \), which is dependent on the initial concentration of ligands, governs the simplification of the overall kinetics to either first- or second-order rate laws. Here we studied the ligand adsorption kinetics at a sufficiently high \( C_{4\text{ATP}} \), of 50 \( \mu \)M, far beyond that required for monolayer saturation of the particle surfaces. As shown by the theoretical derivations in Supporting Information, the ligand adsorption in this high ligand concentration regime can be simplified to a first-order kinetic process well-described by a two-component first-order rate equation shown as follows

\[ \xi_{ad} = \frac{I_i}{I_{4\text{at}}} = f_{\text{slow}}(1 - e^{-\lambda_{\text{slow}}t}) + (1 - f_{\text{slow}})(1 - e^{-\lambda_{\text{fast}}t}) \]  

where \( \xi_{ad} \) is the apparent progress of the ligand adsorption, which was equal to the SERS intensities, \( I_i \), normalized against the equilibrium values (24 h incubation), \( I_{4\text{at}} \), of the 1078 cm\(^{-1}\) mode. \( k_{\text{fast}} \) and \( k_{\text{slow}} \) are the first-order rate constants of the fast and slow kinetic components, respectively. \( f_{\text{slow}} \) is the fractional coefficient for the slow component. Significantly less satisfactory results (Figures S6 in Supporting Information) were obtained when fitting the kinetic data with a two-component second-order rate equation shown below

\[ \xi_{ad} = \frac{I_i}{I_{4\text{at}}} = f_{\text{slow}} \frac{k_{\text{slow}}^t}{1 + k_{\text{slow}}^t} + (1 - f_{\text{slow}}) \frac{k_{\text{fast}}^t}{1 + k_{\text{fast}}^t} \]  

The kinetics of 4-ATP adsorption on QSNPs was found to be described by the slow component with \( f_{\text{slow}} \) of \( \sim 0.8 \), whereas the fast component provided more than 70% contribution to the overall kinetics of 4-ATP adsorption on SRNPs, strongly indicating that the surface adsorption of 4-ATP occurred more rapidly on high-curvature sites than on the atomically flat facets. Such surface curvature-dependent adsorption kinetic profiles were further confirmed when switching the organothiol ligands from 4-ATP to 4-MBN (Figures S7 and S8 in Supporting Information).

We also kinetically monitored the structural ordering of the chemisorbed 4-ATP based on the temporal evolution of \( I_{1480\text{cm}^{-1}}/I_{1588\text{cm}^{-1}} \) (Figure 3E and Figure S9 in Supporting Information). Upon exposure of QSNPs to 50 \( \mu \)M 4-ATP, \( I_{1480\text{cm}^{-1}}/I_{1588\text{cm}^{-1}} \) remained around 0.08 until \( \theta_{eq} \) reached \( \sim 85\% \) after 400 min, indicating that the chemisorbed 4-ATP remained partially ordered in this subsaturation coverage regime. A sharp increase of \( I_{1480\text{cm}^{-1}}/I_{1588\text{cm}^{-1}} \) was observed when \( \theta_{eq} \) exceeded 98% until reaching another plateau at \( I_{1480\text{cm}^{-1}}/I_{1588\text{cm}^{-1}} \) of \( \sim 0.24 \), which signified the formation of the ordered SAMs at saturated \( \theta_{eq} \). In contrast, the 4-ATP chemisorbed on SRNPs,
irrespective of $\theta_{\text{th}}$ exhibited $I_{1480 \text{cm}^{-1}}/I_{1588 \text{cm}^{-1}}$ values around an intermediate value of $\approx 0.1$ throughout the entire ligand adsorption process, suggesting that the chemisorbed 4-ATP on the highly curved surfaces of SRNPs remained much less ordered than those on the flat facets of QSNPs. We further studied the ligand adsorption kinetics on SRNPs at significantly lower $C_{\text{4-ATP}}$ where only partial surface coverages were achieved at equilibrium. As shown in Figure 3F, the SERS intensity of the 1078 cm$^{-1}$ mode rapidly increased to a maximum value within 3 min after exposure of the SRNPs to 2.0 $\mu$M 4-ATP, followed by a gradual decrease over a time scale of minutes before reaching the equilibrium. The rapidly increasing SERS signals at the initial stage came from the 4-ATP molecules chemisorbed on the highly curved surface regions of SRNPs, which then migrated to the flatter surface regions of SRNPs to form thermodynamically more stable ligand domains. Because the local plasmonic field enhancements at the highly curved surface regions are remarkably stronger than those on the flatter surface regions, the SERS intensity gradually decreased as the 4-ATP ligands underwent the surface migration process. The mechanistic complexity of the NaBH$_4$-induced desorption of 4-ATP ligands was manifested by several noteworthy observations. (1) An induction time was observed before the thiolated ligands started to desorb from the SRNP surfaces most likely due to the presence of dissolved oxygen in water, which rapidly oxidized the surface hydride species derived from NaBH$_4$ and thereby suppressed the hydride-driven ligand desorption. A similar induction time was previously observed during Au nanoparticle-catalyzed hydrogenation of aromatic nitro compounds by borohydride, which was also driven by the active surface hydride species. The origin of this induction time had long been under intense debate until recently Neretina and co-workers uncovered the underlying correlation between the dissolved oxygen and the induction time for the catalytic hydrogenation reactions. For NaBH$_4$-induced desorption of organothiol ligands, only when the dissolved oxygen was consumed to a critical depletion level can the hydride species reach a surface abundance sufficient for driving the ligand desorption. By increasing the NaBH$_4$ concentration, $C_{\text{NaBH}_4}$, the consumption of the dissolved oxygen became faster, resulting in shortening of the induction time (Figure 4D). (2) During the ligand desorption, the SERS peak
Intensities progressively decreased while all the characteristic Raman modes of 4-ATP were well preserved, indicating that NaBH₄ induced the ligand desorption rather than reacting with the chemisorbed ligands to form other molecules. Interestingly, both the 1078 cm⁻¹ mode and 1588 cm⁻¹ mode upshifted by 2−3 cm⁻¹ (Figure 4C) when surface hydride and 4-ATP were coadsorbed on the SRNPs. The formation of surface hydride species involved interfacial transfer of electrons mediated by the Au nanoparticles, resulting in increased electron density at the nanoparticle surfaces.⁶⁴ The electron enrichment at the nanoparticle surfaces led to an increase of the electron densities in the chemisorbed 4-ATP, causing the spectral upshift of the SERS peaks and in some cases even the emergence of transient, anomalous Raman features for some specific organothiol ligands.⁶¹ (3) When NaBH₄ was in excess with respect to the chemisorbed 4-ATP, the desorption kinetics followed a pseudo-first-order rate law described by the following equation

\[ \xi_{\text{des}} = \frac{I}{I_{t=0}} = e^{-k_{\text{des}}(t-t_{\text{ind}})} \]  

where \( \xi_{\text{des}} \) is the apparent progress of ligand desorption, which was quantified by normalizing the SERS intensities of the 1078 cm⁻¹ mode, \( I_p \) against the initial values, \( I_{t=0} \), \( k_{\text{des}} \) is the pseudo-first-order rate constant and \( t_{\text{ind}} \) is the induction time. The curve fitting results in Figure 4E,F clearly showed that \( k_{\text{des}} \) increased while \( t_{\text{ind}} \) decreased as \( C_{\text{NaBH₄}} \) increased. While two kinetic components were clearly resolved for the ligand adsorption on SRNPs, the kinetics of NaBH₄-induced ligand desorption from SRNPs could be fitted adequately well with a single exponential component, strongly suggesting that the ligand desorption from the locally flat and curved surface regions of SRNPs was cooperative and synergistic in nature and thus became kinetically irresolvable under the current experimental conditions.

In striking contrast to the case of SRNPs, no apparent ligand desorption was observed upon exposure of 4-ATP-coated QSNPs to 5 mM NaBH₄ even over several hours (Figure 4D). Further increasing \( C_{\text{NaBH₄}} \) from 5 to 15 mM caused aggregation of the QSNPs. The SERS intensities tremendously increased immediately after mixing the 4-ATP-coated QSNPs with 15 mM NaBH₄ due to the formation of interstitial SERS hotspots in the interparticle junctions of aggregated nanoparticle clusters (Figure S11A in Supporting Information). As the aggregation further proceeded, larger scale aggregates started to precipitate out of the colloidal suspensions, resulting in decrease of the SERS intensities when the excitation laser was still focused in the solution. However, strong SERS signals of chemisorbed 4-ATP were detected when focusing the excitation laser on the precipitated nanoparticle aggregates (Figure S11B in Supporting Information), indicating that the 4-ATP ligands were still present on the particle surfaces. It remained challenging, however, to quantitatively study the kinetics of ligand desorption from QSNPs at this high \( C_{\text{NaBH₄}} \) due to the complication caused by nanoparticle aggregation.

Figure 5. Thiolated ligand exchange on highly curved nanoparticle surfaces. (A) Schematic illustration of ligand exchange of 4-ATP with 4-MBN on Au nanoparticle surfaces. (B) Time-resolved SERS spectra of 4-ATP-coated SRNPs (obtained through incubation of CTAC-coated SRNPs with 100 μM 4-ATP for 24 h) after exposure to 250 μM 4-MBN at room temperature. (C) Representative snapshot SERS spectra collected at 1, 10, 30, and 50 min. The SERS spectra of SRNPs fully covered with 4-MBN (obtained after incubating the CTAC-coated SRNPs with 100 μM 4-MBN for 24 h) was also shown for comparison. The Raman signals were normalized using the O–H stretching mode of H₂O as the internal reference. The spectra were offset for clarity. (D) Temporal evolution of SERS intensities of the 2226 cm⁻¹ mode upon exposure of 4-ATP-coated Au SRNPs to various concentrations of 4-MBN. The peak intensities were normalized against the intensity of the 2226 cm⁻¹ peak of the SRNPs with saturated 4-MBN coverages. The error bars represent the standard deviations obtained from five independent experimental runs. The solid black curves show the least-squares curve fitting results. (E) \( k_{\text{ex}} \), (F) \( f_{\text{ex}} \) and (G) \( t_{\text{0}} \) at various \( C_{\text{4-MBN}} \).
The drastically different desorption behaviors of 4-ATP ligands observed on the QSNPs and SRNPs were rooted in the nanoscale surface curvature effects. On one hand, the 4-ATP ligands bound to the atomic flat surfaces are thermodynamically more stable and thereby more resistive against desorption than those on the highly curved surfaces. On the other hand, borohydride exhibited significantly stronger binding affinities to the undercoordinated surface atoms than to their close-packed counterparts exposed on the \{111\}/\{100\} facets,\textsuperscript{65} thereby propelling the formation of surface hydride species and thus the ligand desorption from the highly curved surfaces.

The surface curvature-dependent ligand adsorption and desorption behaviors further translated into the kinetic complexity of the exchange between thiolated ligands on Au nanoparticle surfaces. The exchange of thiolated ligands on Au surfaces is mechanistically complex and exhibits sophisticated overall kinetic profiles with widely divergent and even contradictory results reported in the literature.\textsuperscript{66} On planar Au surfaces, the exchange of thiolated ligands has been observed to occur rapidly at some minority surface sites, hypothetically at the surface defects, terrace edges, and grain boundaries, even though the complete ligand exchange is accomplished over much longer time scales up to a few days.\textsuperscript{28} Fast ligand exchange on the time scale of minutes has also been observed on sub-5 nm Au nanoparticles, presumably at the particle corners and edges.\textsuperscript{36} Such complex kinetic profiles implicate the coexistence of multiple pathways involved in the ligand exchange that are intimately tied to the local surface curvatures of the ligand-binding sites. Previous kinetic studies have shown that the thiolated ligands may undergo either associative or dissociative exchange processes, or a combination of the two,\textsuperscript{28,35,66,67} depending on the molecular structures of the ligands, the surface structures of the Au nanoparticles, the solvents, the temperature, and the molar ratios between the incoming and outgoing ligands. Here we found that the ligand exchange of 4-ATP with 4-MBN on the Au nanoparticle surfaces (Figure 5A) was essentially a multistep process exhibiting multiple kinetic components far more complicated than a simple associative or dissociative exchange process. We first achieved saturated surface coverage of 4-ATP on the SRNPs by incubating the CTAC-coated SRNPs with 100 μM 4-ATP for 24 h, then separated the 4-ATP-coated SRNPs from the unbound 4-ATP ligands through centrifugation, and finally exposed the 4-ATP-coated SRNPs to 4-MBN to initiate the ligand exchange. Because the energy barrier for the 4-ATP desorption from the nanoparticle surfaces was relatively high and both 4-MBN and 4-ATP exhibited comparable molecular sizes and adsorption affinities to Au surfaces, 4-MBN must be kept in great excess with respect to the 4-ATP chemisorbed on SRNP surfaces to kinetically boost the ligand exchange and thermodynamically shift the equilibrium toward 4-MBN chemisorption.

We monitored the ligand exchange in real time using time-resolved SERS (Figure 5B,C). As the ligand exchange proceeded, the intensity of the characteristic SERS feature of the C≡N bond of 4-MBN at 2226 cm$^{-1}$ progressively increased, accompanied by weakening of the SERS peak at 1015 cm$^{-1}$, which was assigned to a coupled ring deformation mode of 4-ATP. The C≡C ring stretching mode at 1588 cm$^{-1}$ was down-shifted by ∼5 cm$^{-1}$ when 4-ATP was displaced with 4-MBN, and such spectral down-shifts could be interpreted as a consequence of decreased bond energies due to the substitution of an electron donating amino group with an electron withdrawing cyano group on the para-position of the thiolphenol derivatives. We monitored the progress of the ligand exchange based on the temporal evolution of the SERS peak intensity at 2226 cm$^{-1}$ (Figure 5D).

Several intriguing phenomena were observed by time-resolved SERS during the ligand exchange. (1) We observed a pre-exchange time during which the SERS intensity of the 2226 cm$^{-1}$ mode increased slowly following a zero-order kinetics until reaching a threshold value above which much faster ligand exchange occurred. As shown in Figure S12 in Supporting Information, during this pre-exchange time, the intensity of the 1015 cm$^{-1}$ mode for 4-ATP remained almost unchanged, indicating the formation of an associative pre-exchange complex comprising both 4-ATP and 4-MBN bound or partially bound to the same binding sites or in close proximity to the same binding sites. Only when the coverage of the associative pre-exchange complex was built up to a certain threshold value could the rapid ligand exchange start to occur. Such pre-exchange time was not observed during desorption of 4-MBN onto the surfaces of SRNPs and QSNPs with unsaturated 4-ATP coverages (Figure S13 in Supporting Information). (2) Upon initiation of the ligand exchange, the peak intensity of the 2226 cm$^{-1}$ mode increased much more rapidly than during the pre-exchange time until asymptotically attaining a nonunity value corresponding to the fraction of ligand exchange at equilibrium, $f_{ex}$. The ligand exchange obeyed pseudo-first-order kinetics described by the following rate law

$$
\frac{\dot{e}}{e_{ex}} = \frac{I}{I_{sat}} = f_{ex} \left(1 - e^{-k_{ex}(t-t_0)}\right)
$$

(7)

where $\dot{e}_{ex}$ referred to the apparent progress rate of ligand exchange, which was equal to the SERS intensity of the 2226 cm$^{-1}$ mode, $I_0$ normalized against that of the SRNPs with saturated 4-MBN coverages, $I_{sat}$, $t_0$ is the duration of pre-exchange time and $k_{ex}$ is the pseudo-first-order rate constant. The $k_{ex}$ and $t_0$ values at various 4-MBN concentrations, $C_{4-MBN}$ obtained from curve fitting were shown in Figure 5E−G, respectively. (3) Regardless of $C_{4-MBN}$ the ligand exchange was always initiated when the apparent coverage of the associative pre-exchange complex exceeded a threshold values around 16% of $f_{ex}$. This was analogous to the NaBH$_4$-induced 4-ATP desorption, which required the accumulation of hydride to a certain threshold surface coverage prior to the occurrence of 4-ATP desorption. A linear relationship existed between $t_0$ and $f_{ex}$ as shown in Figure S14 in Supporting Information, further verifying that the formation of the associative pre-exchange complex was a surface controlled process obeying zero-order kinetics. (4) The ligand exchange never reached 100% $f_{ex}$ even at $C_{4-MBN}$ as high as 250 μM ($f_{ex}$ of ∼72%). We hypothesized that only the 4-ATP chemisorbed on the highly curved surface regions of SRNPs could be displaced with 4-MBN while the ligand exchange on the locally flat regions was kinetically so slow that it became not observable on the experimentally probed timescales. Our SERS results further implied that phase segregation of the ligands occurred on the nanoparticle surfaces when the ligand exchange reached equilibrium with 4-ATP and 4-MBN bound to the locally flat and highly curved surface regions, respectively. An $f_{ex}$ around only 20% was achieved when exposing 4-ATP-coated QSNPs to 250 μM 4-MBN (Figure S15 in Supporting Information), strongly indicating that only the 4-ATP bound to the corners, edges, and surface defects of
QSNPs was readily exchangeable with 4-MBN under the current conditions.

In summary, the quantitative insights gained from this work shed light on the complicated thermodynamic and kinetic landscapes underlying the surface curvature-dependent adsorption, desorption, and exchange behaviors of thiolated ligands on Au nanoparticle surfaces, providing an important knowledge framework that guides us to fine-tailor the surface chemistry on nanoparticles for specific applications. The surface curvature-dependent interfacial ligand behaviors form the keystone for the site-selective functionalization of nanoparticle surfaces, allowing markedly different chemical reactions and intermolecular interactions to occur at distinct surface sites on the same nanoparticles. The incorporation of multiple surface functionalities, sometimes even dynamic molecular moieties, into individual multifaceted metallic nanoparticles, especially anisotropic nanostructures with highly curved surface features, such as nanorods,6,68 nanoprisms69 and multibranched nanostars,70 represents a key step toward the construction of versatile nanocarriers for targeted drug delivery and multimodality bioimaging as well as the assembly of dynamically reconfigurable, active plasmonic metamaterials for sensing and photonic applications. While our SERS results allow us to resolve the markedly distinct interfacial ligand dynamics on highly curved surfaces versus atomically flat facets of Au nanoparticles, several fundamentally intriguing but intrinsically complicated issues still remain unresolved and open to further scrutiny. For example, the highly curved nanoparticle surfaces are enclosed by a variety of local high-index facets that correlate to the degree of local curvatures. Each type of high-index facets has its characteristic surface atomic coordinations and spatial distribution of surface atomic steps.71 How such atomic-level surface curvature on the subnanometer length-scale affects the interfacial ligand behaviors still remains elusive. Local regions with positive, negative, and neutral curvatures are all present on the nanotextured surfaces of SRNPs. The negative surface curvature may not only exhibit a nanocrevise effect analogues to the plasmonic nanogap effect on SERS72,73 but also play crucial roles distinct from those of the positive surface curvature in determining the interfacial ligand dynamics. We believe this work provides important implications stimulating future work that will push the understanding of nanoparticle surface chemistry toward a higher level of comprehensiveness and depth.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.nanolett.7b01593.

Experimental details, additional discussions, tables listing Raman peak assignments, and additional figures as noted in the text, including hydrodynamic sizes, ξ-potentials, TEM images, optical extinction spectra, SERS spectra, ligand adsorption isotherms, kinetic results, and correlation between \(t_0\) and \(f_{ex}\) (PDF)

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Notes

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Nanoscale Surface Curvature Effects on Ligand-Nanoparticle Interactions: A Plasmon-Enhanced Spectroscopic Study of Thiolated Ligand Adsorption, Desorption, and Exchange on Gold Nanoparticles

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S1. Experimental Details

S1.1. Chemicals and Materials:

Gold(III) chloride trihydrate (HAuCl$_4$·3H$_2$O, ACS grade) was purchased from J.T. Baker. Sodium borohydride (NaBH$_4$, 99%), hydrochloric acid (HCl, 37%), and L-ascorbic acid (AA, 99.5%) were obtained from Sigma-Aldrich. Cetyltrimethylammonium chloride (CTAC, 96%), 4-aminothiophenol (C$_6$H$_7$NS, 4-ATP, 97%), and 4-mercaptobenzonitrile (C$_7$H$_5$NS, 4-MBN, 99%) were obtained from Alfa Aesar. All reagents were used as received without further purification. Ultrapure water (18.2 MΩ resistivity, Barnstead EasyPure II 7138) was used for all experiments.

S1.2. Nanoparticle Synthesis:

Au surface roughened nanoparticles (SRNPs) and quasi-spherical nanoparticles (QSNPs) were synthesized using a kinetically controlled, seed-mediated nanocrystal growth method previously developed by our group.$^{1,2}$ Colloidal Au seeds (~3 nm in diameter) were first prepared by reducing HAuCl$_4$ with NaBH$_4$ in the presence of CTAC. Briefly, 0.25 mL of HAuCl$_4$ (10 mM) was introduced into an aqueous solution of CTAC (10 mL, 100 mM) under magnetic stir. Then 0.30 mL of ice cold, freshly prepared NaBH$_4$ (10 mM) was quickly added to the solution containing both CTAC and HAuCl$_4$. The mixture solution was stirred for 1 min, then left undisturbed for 2 h, and finally diluted 1000-fold with 100 mM CTAC. The diluted seed colloids were used for the subsequent seed-mediated growth of Au SRNPs and QSNPs.

The growth solution for SRNPs was prepared by sequentially adding 0.50 mL of HAuCl$_4$ (10 mM) and 0.10 mL of AA (100 mM) into 10.0 mL of CTAC (100 mM) solution. To prepare Au SRNPs with average diameter of ~ 128 nm, 40 μL of the diluted Au seed solution was added into the growth solution. The reactants were gently mixed for 30 s and then left undisturbed at room temperature for 4 h. The as-obtained Au SRNPs were washed with water 3 times through centrifugation/redispersion cycles, and finally redispersed in water. The Au QSNPs were fabricated following a similar protocol for the Au SRNPs except for the addition of HCl. The growth solution for QSNPs was prepared by sequentially adding 0.50 mL of HAuCl$_4$ (10 mM), 0.20 mL of HCl (1.0 M), and 0.10 mL of AA (100 mM) into 10.00 mL of CTAC (100 mM) solution. After gently mixing the reactants for 30 s, the growth of QSNPs was initiated by adding 35 μL of the diluted Au seed solution, and then left undisturbed at room temperature for 4 h. The obtained Au QSNPs were washed with water 3 times and finally redispersed in water.

S1.3. Structural Characterizations of Nanoparticles:

The structures of the nanoparticles were characterized by scanning electron microscopy (SEM) using a Zeiss Ultraplus thermal field emission scanning electron microscope. The samples for SEM measurements were dispersed in water and drop-dried on silicon wafers. The optical extinction spectra of the NPs were measured on aqueous colloidal samples at room temperature using a Beckman Coulter Du 640 spectrophotometer. The hydrodynamic sizes and ζ-potentials of the colloidal NPs were measured at room temperature using ZETASIZER nanoseries (Nano-ZS, Malvern). The samples for ζ-potential measurements were all freshly prepared, centrifuged, and dispersed in water. The concentration of the colloids for ζ-potential measurements was kept at $2 \times 10^9$ particles mL$^{-1}$ for all the samples and pHs of the colloidal suspensions were measured to be around 7.4. In our ζ-potential measurements, we used the zeta potentiometer to measure the free mobility and effective hydrodynamic sizes of NPs, which were then converted into ζ-potentials using simple theoretical formulas approximating the NP as a hard sphere homogenously coated with a charged thin layer.$^3$
The electrochemical surface oxide stripping measurements were performed using a CHI 660E workstation (CH Instruments, Austin, Texas) at room temperature with a three-electrode system composed of a Pt wire as the auxiliary, a saturated calomel electrode (SCE) as the reference, and a glassy carbon electrode (GCE, 3 mm diameter) as the working electrode. The GCE was first polished with 0.3 mm alumina slurry, followed by washing with water and ethanol before use. 20 μL of colloidal suspensions of Au QSNPs or Au SRNPs with the same particle concentration of 1.0 x 10^{10} particles mL^{-1} were dropped and air-dried on the pretreated GCE at room temperature. 2 μL of Nafion solution (0.2 wt%) was then drop-dried to hold the NPs on the electrode surfaces. Therefore, there were nominally ~ 2.0 x 10^{8} particles on each GCE. Cyclic voltammetry (CV) measurements for oxide stripping were conducted in N2-saturated 0.5 M H_{2}SO_{4} solution at a potential sweep rate of 5 mV s^{-1}. The total charges associated with the Au oxide stripping were calculated based on the integrated areas of the stripping peaks at ~ 0.94 V (vs. SCE). Assuming the specific charge associated with Au oxide stripping to be 450 μC cm^{-2},^4 the electrochemically active surface area (ECSA) of Au QSNPs was estimated to be 2.2 x 10^{4} nm^{2} per particle, which was approximately ~43 % of the physical surface area of an ideal nanosphere with a diameter of 132 nm (5.5 x 10^{4} nm^{2}). The average ECSA of Au SRNPs was estimated to be 1.0 x 10^{5} nm^{2} per particle, about 4.5 times of the ECSA of the Au QSNPs.

S1.4. Surface-Enhanced Raman Scattering (SERS) Measurements:
The SERS measurements were performed using a Bayspec Nomadic confocal Raman microscope built on an Olympus BX51 reflected optical system with a 785 nm continuous wave excitation laser. Excitation laser was focused on colloidal samples with a 10x objective [Numerical Aperture (NA) = 0.30, working distance (WD) = 11.0 mm, Olympus MPLFLN, excitation volume of ~100 fL].^5 The laser power for Raman excitation was 10.0 mW.

S1.5. Thiol Ligand Adsorption Isotherms:
The equilibrium surface coverages of thiol ligands on Au SRNPs and QSNPs were characterized as a function of thiol ligand concentrations. Colloidal Au SRNPs or QSNPs suspended in water were incubated with various concentrations of 4-ATP or 4-MBN at room temperature for 24 h to reach the equilibria. The total volume of each incubated sample was 200 μL, and particle concentrations of the SRNPs and QSNPs were kept at 1.0 x 10^{9} particles mL^{-1} and 4.5 x 10^{9} particles mL^{-1}, respectively, such that the total apparent surface areas of SRNPs and QSNPs were nominally the same. The adsorption of 4-ATP or 4-MBN on the Au NPs was quantified by SERS. The integration time for each spectrum was 25 s and 100 s for SRNPs and QSNPs, respectively. The apparent surface coverages of 4-ATP on the Au SRNPs and QSNPs at various 4-ATP concentrations were quantified based on the SERS intensities of the 1078 cm^{-1} (C-S stretching mode) and 1588 cm^{-1} (aromatic C-C stretching mode) peaks normalized against those of the nanoparticles with saturated 4-ATP monolayer coverages (nanoparticles incubated with 100 μM 4-ATP for 24 h). The apparent surface coverages of 4-MBN at various 4-MBN concentrations were quantified based on the normalized peak intensities at 1078 cm^{-1} and 2226 cm^{-1} (C≡N stretching mode).

S1.6. Thiol Ligand Adsorption Kinetics:
We used SERS to monitor the thiol ligand adsorption kinetics on the surfaces of Au SRNPs and QSNPs in real time at room temperature. The thiol ligand adsorption was initiated by introducing 4 μL of ethanolic solutions of 4-ATP or 4-MBN at certain concentrations into 200 μL of colloidal Au SRNPs
(1.0 x 10^9 particles mL⁻¹) or Au QSNPs (4.5 x 10^9 particles mL⁻¹). Immediately after the thiol ligands and the nanoparticles were mixed, SERS spectra were collected in real time with various time intervals and spectral integration times of 5 s for SRNPs and 50 s for QSNPs, respectively, until reaching the maximum SERS intensities after sufficiently long incubation times. The apparent surface coverages of 4-ATP and 4-MBN were quantified as a function of incubation time based on the temporal evolution of SERS intensities of the 1078 cm⁻¹ mode for 4-ATP and the 2226 cm⁻¹ mode for 4-MBN, respectively, normalized against those of the nanoparticles with saturated equilibrium monolayer ligand coverages (nanoparticles incubated with 100 µM of 4-ATP or 4-MBN for 24 h).

**S1.7. NaBH₄-Induced 4-ATP Desorption:**

Au SRNPs and QSNPs with saturated surface coverages of 4-ATP (nanoparticles incubated with 50 µM of 4-ATP for 24 h) were centrifuged and redispersed in water. 200 µL of 4-ATP-coated Au SRNPs (1.0 x 10^9 particles mL⁻¹) or Au QSNPs (4.5 x 10^9 particles mL⁻¹) were then mixed with 10 µL of NaBH₄ at various concentrations to initiate the 4-ATP desorption. The spectral integration times were 5 s for SRNPs and 50 s for QSNPs, respectively. The peak intensities at 1078 cm⁻¹ were normalized against the initial peak intensities.

**S1.8. Ligand Exchange of 4-ATP with 4-MBN:**

Au SRNPs and QSNPs with saturated surface coverages of 4-ATP (nanoparticles incubated with 100 µM of 4-ATP for 24 h) were centrifuged and redispersed in water. 200 µL of 4-ATP-coated Au SRNPs (1.0 x 10^9 particles mL⁻¹) or Au QSNPs (4.5 x 10^9 particles mL⁻¹) were then mixed with various concentrations of 4-MBN to initiate the thiol ligand exchange. The total volume of each sample was kept at 210 µL. SERS spectra were collected with an excitation power of 10.0 mW. The temporal evolution of the peak intensities at 2226 cm⁻¹ was monitored to track the ligand exchange kinetics. The spectral integration times were 5 s for SRNPs and 50 s for QSNPs, respectively.

The ligand exchange measurements were also performed on Au SRNPs and QSNPs with unsaturated surface coverages of 4-ATP under the same experimental conditions. Au SRNPs with unsaturated 4-ATP coverage were fabricated by incubating the CTAC-coated Au SRNPs with 10.0 µM 4-ATP for 24 h. Au QSNPs with unsaturated 4-ATP coverage were fabricated by incubating the CTAC-coated Au QSNPs with 2.0 µM 4-ATP for 24 h.
S2. Additional Discussions

S2.1. Estimation of Enhancement Factors (EFs) of Raman Signals:

We estimated the enhancement factors (EFs) of Raman signals using the following equation:

\[ EF = \frac{I_{\text{SERS}} \times N_{\text{normal}}}{I_{\text{normal}} \times N_{\text{SERS}}} \quad (S1), \]

where \( I_{\text{SERS}} \) is the intensity of a specific band in the SERS spectra of 4-ATP; \( I_{\text{normal}} \) is the intensity of the same band in the normal Raman spectra of 4-ATP under the same condition; \( N_{\text{normal}} \) is the number of probe molecules in the excitation volume for the normal Raman measurements; \( N_{\text{SERS}} \) is the number of adsorbed molecules on the nanoparticles whose Raman signals are being enhanced.

We chose the Raman modes of 4-ATP at 1078 cm\(^{-1}\) for the EF calculations. \( N_{\text{normal}} \) was calculated by using the following expression:

\[ N_{\text{normal}} = \frac{V \times D}{M} \times N_A = 1.80 \times 10^{11} \text{ molecules} \quad (S2), \]

where \( V \) is the excitation volume (100 fL), \( D \) is the density of 4-ATP (1.17 g mL\(^{-1}\)), \( M \) is the molar mass of 4-ATP (125 g mol\(^{-1}\)) and \( N_A = 6.02 \times 10^{23} \text{ mol}^{-1} \) is the Avogadro constant.

To determine \( N_{\text{SERS}} \), a monolayer of 4-ATP molecules with molecular footprint size of 0.39 nm\(^2\) was assumed to be closely packed on the surface of each Au particle. The surface area of a QSNP was estimated as the surface area of a sphere that is 130 nm in diameter. The surface area of a SRNP was estimated as 4.5 times of that of a QSNP. We kept the concentrations of 4-ATP-coated QSNPs and SRNPs both at 1.0 \( \times 10^9 \) particles mL\(^{-1}\) such that there were on average 0.1 particles in the laser focal volume.

The ensemble averaged EFs on the Au SRNPs and QSNPs were estimated to be \( 3.0 \times 10^6 \) and \( 2.4 \times 10^5 \), respectively, which were in very good agreement with our previous results obtained on surface-immobilized single nanoparticles.\(^1\)

In these calculations, we assumed that the binding footprint size of each 4-ATP on the nanoparticles was the same as that on a planar surface, which was previously measured to be 0.39 nm\(^2\) per molecule.\(^6\)

Considering the fact that the molecular packing density on highly curved surfaces should be lower than that on planar surfaces, the average enhancement factors on SRNPs might be underestimated.

S2.2. Simplification of the Langmuir Kinetics of Ligand Adsorption to First-Order Rate Laws:

The polynomial expression of the varying-order rate equations for the ligand adsorption were derived by Liu and coworkers.\(^7\) The ligand adsorption/desorption on nanoparticle surfaces can be considered as a reversible process described below:

\[ L + S \xrightarrow{k_a} S-L \quad (S3), \]

in which \( L \) is the free ligand in the bulk solution, \( S \) refers to the ligand binding sites on the nanoparticle surfaces, and \( S-L \) is the ligand bound to the nanoparticle surfaces. \( k_a \) and \( k_d \) represent the adsorption and desorption rate constants, respectively. The surface coverage of the ligands at any time, \( \theta_t \), is defined as

\[ \theta_t = \frac{q_t}{q_{\text{max}}} = \frac{C_0 - C_t}{q_{\text{max}} \times x} \quad (S4), \]

in which \( q_t \) and \( q_{\text{max}} \) are the adsorption capacities of nanoparticles at any time and its maximum value, respectively. \( C_0 \) and \( C_t \) are the respective concentrations of ligands in solution at time zero and time \( t \), while \( x \) represents the dosage of adsorbent. The overall rate equation for ligand adsorption can be further expressed as follows:
\[ \frac{d\theta_t}{dt} = k_a C_i (1-\theta_t) - k_d \theta_t \]  
\hspace{2cm} (S5).

Equation S5 has been often referred to as the rate law for Langmuir adsorption kinetics, which has been widely applied to describe adsorption of ligands at solid surfaces. Inserting Equation S4 into Equation S5 yields
\[ \frac{d\theta_t}{dt} = k_a q_{\text{max}} x \theta_t^2 - (k_a C_0 + k_a q_{\text{max}} x + k_d) \theta_t - k_a C_0 \]  
\hspace{2cm} (S6).

Equation S6 shows that the adsorption rate is a quadratic function of \( \theta_t \) at given \( C_0 \) and \( x \). When adsorption reaches equilibrium, \( d\theta_t/dt \) becomes zero, and \( \theta_t \) reaches its value at equilibrium, \( \theta_{eq} \). Solving Equation S6 under the equilibrium conditions gives
\[ \theta_{eq} = \frac{K_{eq} (q_{\text{max}} x + C_0) + 1 - \sqrt{\Delta}}{2K_{eq} q_{\text{max}} x} \]  
\hspace{2cm} (S7),

where \( \Delta = k_a^2 (C_0 - q_{\text{max}} x)^2 + 2K_{eq} (C_0 + q_{\text{max}} x) + 1 \), and \( K_{eq} = \frac{k_a}{k_d} \).

If we define that \( k_1 \equiv \sqrt{\Delta} \times k_d \) (S8), and \( k_2 \equiv k_{eq} q_{\text{max}} x k_d \) (S9), then
\[ \frac{d\theta_t}{dt} = k_1 (\theta_{eq} - \theta_t) + k_2 (\theta_{eq} - \theta_t)^2 \]  
\hspace{2cm} (S10).

Equation S10 shows that the overall adsorption rate, \( d\theta_t/dt \), is the combination of the first-order term \( k_1(\theta_{eq} - \theta_t) \) and the second-order term \( k_2(\theta_{eq} - \theta_t)^2 \). The relative magnitude of \( k_1 \) over \( k_2 \times (\theta_{eq} - \theta_t) \), which is dependent upon \( C_0 \), governs the simplification of the overall kinetics to either the first- or the second-order rate laws.

By combining Equations S8, S9, and S10, we can obtain the follow equation:
\[ \theta_{eq} = \frac{k_1}{k_2} \left( \frac{K_{eq} (q_{\text{max}} x + C_0) + 1 - 3\sqrt{\Delta}}{2K_{eq} q_{\text{max}} x} \right) \]  
\hspace{2cm} (S11).

When the ligand concentrations are far higher than that required for monolayer saturation of the particle surfaces, i.e. \( C_0 \gg q_{\text{max}} x \), \( \Delta \approx (K_{eq} C_0 + 1)^2 \). Therefore,
\[ \theta_{eq} = \frac{k_1}{k_2} \approx \frac{K_{eq} C_0 + 1 - 3K_{eq} C_0 - 3}{2K_{eq} q_{\text{max}} x} = -\frac{K_{eq} C_0 + 1}{K_{eq} q_{\text{max}} x} \]  
\hspace{2cm} (S12).

If \( C_0 \) is sufficiently high, then \( \theta_{eq} \approx \frac{k_1}{k_2} \ll \theta_t \), based on which we can further get
\[ k_1 \gg k_2 (\theta_{eq} - \theta_t) \]  
\hspace{2cm} (S13).

Therefore, at sufficiently high ligand concentrations, the Langmuir kinetics of ligand adsorption can be simplified to a first-order rate law shown as follows:
\[ \frac{d\theta_t}{dt} \approx k_1 (\theta_{eq} - \theta_t) \]  
\hspace{2cm} (S14).
S3. Additional Figures

Figure S1. Hydrodynamic diameters of (A) Au SRNPs and (B) Au QSNPs before 4-ATP adsorption and after incubation with 50 µM of 4-ATP for 24 h. (C) ζ-potentials of freshly prepared CTAC-coated and 4-ATP-coated NPs and the NPs after being store in water for 30 days at room temperature. (D) Extinction spectra of colloidal CTAC-coated and 4-ATP-coated Au SRNPs and Au QSNPs. The vertical dash line shows the wavelength (785 nm) of the excitation laser for SERS measurements.

Figure S2. Raman spectra of pure water, 100 µM of 4-ATP in water, and SERS spectra of freshly prepared CTAC-coated Au QSNPs and Au QSNPs incubated with 50 µM 4-ATP after 24 h. The concentrations of the colloidal QSNPs were kept at 4.5 x 10⁹ particles mL⁻¹. The spectra were obtained at 785 nm excitation with a laser power of 10 mW and an integration time of 100 s. The SERS peaks at 1078 cm⁻¹ and 1588 cm⁻¹ were assigned to the C-S stretching mode and benzene ring stretching mode of surface-adsorbed 4-ATP, respectively. The two broad Raman band centered around 2870 cm⁻¹ and 3230 cm⁻¹ were assigned to the C-H stretching mode of surface-adsorbed CTAC and the O-H stretching mode of water, respectively. The spectra were off-set for clarity.
Figure S3. SERS spectra of 4-ATP-coated Au SRNPs (top panel) and Au QSNPs (middle panel) and normal Raman spectrum of neat 4-ATP solid film (bottom panel). The spectra were obtained at 785 nm excitation with a laser power of 10 mW and an integration time of 100 s. The concentrations of SRNPs and QSNPs were both kept at $1.0 \times 10^9$ particles mL$^{-1}$.

Figure S4. 4-ATP adsorption isotherms obtained on the basis of SERS intensities of (A) 1078 cm$^{-1}$ and (B) 1588 cm$^{-1}$ modes. Least squares curve fitting were conducted on the experimental results (i). Curves ii show the curve fitting results using Equation (2). The fitting results were further decomposed into a high binding affinity component (curves iii) and a low binding affinity component (curves iv), respectively. Curves v show the least squares fitting results assuming non-cooperative adsorption at the low affinity sites (assuming $n_2$ of 1). The $R^2$ values for curves v were 0.927 and 0.929 in panels A and B, respectively.
**Figure S5.** SERS spectra collected on colloidal (A) Au QSNPs and (B) Au SRNPs after the nanoparticles were incubated with various concentrations of 4-MBN for 24 h. The peak intensity at 3230 cm⁻¹ (normal Raman peak corresponding to the O-H stretching mode of H₂O) was used as the internal reference for signal normalization. 4-MBN adsorption isotherms (plots of apparent surface-coverage of 4-MBN, \( \theta_{4\text{-MBN}} \), vs. concentration of 4-MBN, \( C_{4\text{-MBN}} \)) on the surfaces of Au QSNPs and SRNPs obtained from the (C) 2226 cm⁻¹ mode and (D) 1588 cm⁻¹ mode in the SERS spectra. The error bars represent the standard deviations obtained from 5 replicate samples prepared under identical conditions. The solid black curves show the least squares curve fitting results. The \( R^2 \) values were all greater than 0.98.

**Figure S6.** Least squares curve fitting results using the second order Langmuir rate law (Equation (5)) for 4-ATP adsorption on (A) Au QSNPs and (B) Au SRNPs at 4-ATP concentration of 50 µM.
Figure S7. Kinetics of 4-MBN adsorption on the surfaces of Au SRNPs. (A) Color-coded plots showing the time-resolved SERS spectra obtained on colloidal Au SRNPs incubated with 50 µM 4-MBN at room temperature. (B) Representative SERS spectra at incubation times of 0 min, 5 min, 30 min, and 70 min. The SERS intensities were normalized using the O-H stretching mode of water as an internal reference. (C) SERS intensities of the 2226 cm\(^{-1}\) mode (\(v_{C≡N}\)) plotted as a function of incubation time after exposure of Au SRNPs to 50 µM 4-MBN. The SERS intensities of the 2226 cm\(^{-1}\) mode were normalized against the values obtained after the adsorption-desorption equilibrium was reached (24 h incubation). The error bars represent the standard deviations obtained from 5 independent experimental runs. The solid black curves show the least squares curve fitting results using Equation (4). The curve fitting results and \(R^2\) values were labeled in the figure.

Figure S8. Kinetics of 4-MBN adsorption on the surfaces of Au QSNPs. (A) Color-coded plots showing the time-resolved SERS spectra obtained on colloidal Au QSNPs incubated with 50 µM 4-MBN at room temperature. (B) SERS intensities of the 2226 cm\(^{-1}\) mode (\(v_{C≡N}\)) plotted as a function of incubation time after exposure of Au QSNPs to 50 µM 4-MBN. The SERS intensities of the 2226 cm\(^{-1}\) mode were normalized against the values obtained after the adsorption-desorption equilibrium was reached (24 h incubation). The error bars represent the standard deviations obtained from 5 independent experimental runs. The solid black curves show the least squares curve fitting results using Equation (4). The curve fitting results and \(R^2\) values were labeled in the figure.
**Figure S9.** Temporal evolution of the SERS spectra showing the intensity ratio of the 1480 cm$^{-1}$ mode to the 1588 cm$^{-1}$ mode upon exposure of (A) Au QSNPs and (B) Au SRNPs to 50 µM 4-ATP.

**Figure S10.** (A) SERS spectra of freshly prepared 4-ATP-coated Au QSNPs (CTAC-coated Au QSNPs incubated with 100 µM 4-ATP for 24 h) and 4-ATP-coated Au QSNPs redispersed and stored in water at room temperature for 30 days. (B) SERS spectra of freshly prepared 4-ATP-coated Au SRNPs (CTAC-coated Au SRNPs incubated with 100 µM 4-ATP for 24 h) and 4-ATP-coated Au SRNPs redispersed and stored in water at room temperature for 30 days. The Raman intensities were normalized using the O-H stretching mode of water as an internal reference. The spectra were off-set for clarity.
Figure S11. (A) Temporal evolution of the intensity of the 1078 cm\(^{-1}\) mode upon exposure of 4-ATP-coated Au QSNPs to 15 mM NaBH\(_4\) at room temperature. The SERS intensities of the 1078 cm\(^{-1}\) mode were normalized against the initial value before mixing the ATP-coated Au QSNPs with NaBH\(_4\). The error bars represent the standard deviations obtained from 5 independent experimental runs. (B) A representative SERS spectrum collected on aggregated Au QSNPs settled down at the bottom of the reactant container after exposing the ATP-coated Au QSNPs to 15 mM NaBH\(_4\) for 1 h.

Figure S12. Temporal evolution of SERS intensities of the 2226 cm\(^{-1}\) mode of 4-MBN and the 1015 cm\(^{-1}\) mode of 4-ATP upon exposure of 4-ATP-coated Au SRNPs to 250 µM 4-MBN. The intensities of the 2226 cm\(^{-1}\) peak were normalized against the intensity of the SRNPs with saturated 4-MBN coverages (obtained after incubating the CTAC-coated SRNPs with 100 µM 4-MBN for 24 h). The intensities of the 1015 cm\(^{-1}\) peak were normalized against the intensity of the SRNPs with saturated 4-ATP coverages (obtained after incubating the CTAC-coated SRNPs with 100 µM 4-ATP for 24 h). The error bars represent the standard deviations obtained from 5 independent experimental runs.
**Figure S13.** SERS intensities of the 2226 cm$^{-1}$ mode ($v_{C≡N}$) plotted as a function of incubation time after exposure of (A) Au SRNPs or (B) Au QSNPs with unsaturated 4-ATP coverage to 250 µM 4-MBN. The SERS intensities of the 2226 cm$^{-1}$ mode were normalized against the values obtained of nanoparticles with saturated 4-MBN coverages (incubation of CTAC-coated NPs with 100 µM 4-MBN for 24 h). The error bars represent the standard deviations of 3 experimental runs. Au QSNPs and Au SRNPs with unsaturated 4-ATP coverages were obtained through incubations of the QSNPs with 2 µM 4-ATP and the SRNPs with 10 µM 4-ATP, respectively, for 24 h. The experimental results were fitted with the first-order rate law and the fitting results were shown as solid black curves.

**Figure S14.** Plots of pre-exchange time ($t_0$) vs. fraction of ligand exchange ($f_{ex}$) for the ligand exchange of 4-ATP with 4-MBN on Au SRNPs.
Figure S15. Temporal evolution of the SERS intensities of the 2226 cm⁻¹ mode (νC≡N) upon exposure of Au QSNPs with saturated 4-ATP coverage to 250 µM 4-MBN. The SERS intensities of the 2226 cm⁻¹ mode were normalized against the values of the QSNPs fully covered with monolayer 4-MBN (obtained after incubation of CTAC-coated Au QSNPs with 100 µM 4-MBN for 24 h). The error bars represent the standard deviations of 3 experimental runs. The experimental results were fitted with Equation (7) and the fitting results were shown as solid black curve.
**S4. Tables Listing the Raman Peak Assignments**

*Table S1.* Assignments of the Major SERS peaks of 4-ATP

<table>
<thead>
<tr>
<th>Raman Shift / cm(^{-1})</th>
<th>Peak Assignments*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1015</td>
<td>(\alpha_{\text{CCC}} + \alpha_{\text{CC}}^{8-12})</td>
</tr>
<tr>
<td>1078</td>
<td>(\nu_{\text{C-S}} + \nu_{\text{CC}}^{8-12})</td>
</tr>
<tr>
<td>1173</td>
<td>(\beta_{\text{C-H}} + \nu_{\text{CC}}^{11})</td>
</tr>
<tr>
<td>1480</td>
<td>(\nu_{\text{C-N}} + \beta_{\text{C-H}} + \nu_{\text{CC}}^{8-10})</td>
</tr>
<tr>
<td>1588</td>
<td>(\nu_{\text{CC}}^{8-12})</td>
</tr>
</tbody>
</table>

* \(\nu\): stretching; \(\beta\): bending; \(\alpha\): ring deformation.

*Table S2.* Assignments of the Major SERS peaks of 4-MBN

<table>
<thead>
<tr>
<th>Raman Shift / cm(^{-1})</th>
<th>Peak Assignments*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1074</td>
<td>(\nu_{\text{C-S}} + \nu_{\text{CC}}^{8-12})</td>
</tr>
<tr>
<td>1173</td>
<td>(\beta_{\text{C-H}} + \nu_{\text{CC}}^{11})</td>
</tr>
<tr>
<td>1203</td>
<td>(\beta_{\text{C-H}} + \nu_{\text{C\equiv N}}^{13, 15-17})</td>
</tr>
<tr>
<td>1582</td>
<td>(\nu_{\text{CC}}^{8-12})</td>
</tr>
<tr>
<td>2226</td>
<td>(\nu_{\text{C\equiv N}}^{14-17})</td>
</tr>
</tbody>
</table>

* \(\nu\): stretching; \(\beta\): bending; \(\alpha\): ring deformation.
S5. References for Supporting Information