Distinct Adsorbed States of DNA and RNA Bases Investigated by Flocculation-Assisted Surface Enhanced Raman Scattering*

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We have succeeded in forming flocculates of gold nanoparticles (AuNPs) using DNA and RNA bases to obtain their surface enhanced Raman spectra. Adenine, guanine and cytosine molecules which possess a primary amino group (–NH₂) formed flocculates at much lower concentration than uracil and thymine without an –NH₂ group, suggesting a crucial role of an –NH₂ group for adsorption of these base molecules on neighboring AuNPs. Detailed adsorption structure of base molecules was investigated using their SERS spectra at various pH in solutions and those for deuterated bases, comparing with those for bulk state and also by DFT calculations.

Keywords: Flocculation; Localized surface plasmon; Gold nanoparticles; Surface enhanced Raman scattering; A gap mode

1. INTRODUCTION

Attainment of enormous enhancement with high reproducibility is critical for the utilization of SERS in quantitative analysis. Single molecule detection by Raman scattering has been investigated using a coupled localized surface plasmon (LSP) of adjacent metal nanostructures, by controlling the nanogap size and the adsorption of target molecules [1, 2]. For this purpose, we have studied flocculates of silver and gold nanoparticles (AgNPs, and AuNPs), which are a few closely adjacent nanoparticles, providing various molecules with enormous SERS intensity in solutions [3–7]. Theoretical calculations suggest that one of AgNPs or AuNPs in flocculates can be replaced with metal substrates [8], such as Pd, Fe, Ni, Zn, Al and Pt [9–12] having large damping factors of surface plasmon, while providing enormous enhancement factors in SERS equivalent to those obtained in flocculates of AgNPs and AuNPs. Furthermore, MNPs can be attached to the tip of cantilevers or sharpened optical fiber probes for near-field Raman spectroscopy. Such fabrication is expected to provide us with Raman images in single molecule sensitivity and a spatial resolution of a few nanometers [13, 14].

However, we have experimental limitations in the utilization of the gap mode of metal nanostructures. For instance, addition of target molecules or salts to suspension of MNPs for fabrication of flocculates often causes coagulation or precipitation of MNPs. Coagulation of MNPs may give rather large SERS enhancement but fails in getting good reproducibility. In general, MNPs are isolated by electrostatic repulsion between counter ions in an electrical double layer on MNPs, as rationalized by the DLVO (Derjaguin-Landau-Verway-Överbeek) theory [15]. Indeed, the surface of AgNPs or AuNPs prepared by chemical reduction using trisodium citrate is covered with residual citrate anions through sodium cations in close proximity to the MNP surfaces. Substitution of such surface residuals with suitable chemicals is necessary for us to suppress steric hindrance by citrate anions for efficient adsorption of cationic xanthene and triphenyl methane dyes [3, 4, 6, 8]. A larger amount of surface residuals such as a layer of citrates and oxides on AgNPs does not allow the chemisorption of neutral molecules. Indeed only cationic xanthene and triphenylmethane dyes are adsorbed on AgNPs to form flocculates [3, 4]. Formation of self-assembled monolayers (SAM) of thiol molecules with functional groups such as carboxylic and amino groups is a promising way to form flocculates of AgNPs and to attain enormous SERS enhancement by trapping various molecules between neighboring AgNPs coated with SAM-film [5, 16].

In contrast to AgNPs, neutralized R123 molecules adsorb on AuNPs via a coordination bond of Au–N in a tilted orientation in sufficiently low coverage of citrates, whereas cationic rhodamine 123 (R123) molecules adsorb on AuNPs through electrostatic interaction in parallel to the interparticle axis [6, 7]. Similarly, AuNPs are incubated into a living cell to monitor pH distribution [1], and used to detect hybridization of DNA [17]. Although single molecule detection is required in DNA sequencing [18], Raman spectroscopy has not been used in such challenging purpose. Indeed, DNA bases or other biomolecules are not directly characterized in such applications, but monitored by the faint differences in conductivity at a nanogap for individual DNA bases [18]. This is partly due to intrinsic difficulty in detecting and distinguishing Raman spectra for individual DNA bases passing through a nanogap. In principle, the same base molecules in different sequences can provide distinct Raman spectra in a flocculation of AuNPs, due to different enhancement for different distance from Au surfaces, and also due to distinct interaction with neighboring molecules, albeit the differences are inherently small [19]. For precise sequencing in DNA molecules by Raman spectroscopy, one should start with characterization of individual DNA and RNA bases, adenine (A), guanine (G), cytosine (C), thymine (T) and uracil (U) molecules, adsorbed on AuNP surfaces, which has not been sufficiently elucidated as seen in recent reports [20-22]. In the next step, nucleotide and DNA molecules will be studied to detect inherently faint differences in Raman spectra of DNA bases under quite similar circumstances. Here we focused ourselves on distinct adsorption structure of various DNA and RNA bases on Au

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II. EXPERIMENTAL DETAILS

Gold nanoparticles were prepared by a citrate reduction method [6, 7]. Shortly, AuNPs were prepared using the citrate reduction method reported by Frens [23]. Briefly, a trisodium citrate solution (20 mg in 10 mL solution, i.e. 1% aq. solution) was added to a HAuCl₄ - 4H₂O (20 mg in 200 mL) aqueous solution under reflux, and then the reacting solution was kept at 100°C for 5 min. The AuNPs thus prepared (called ‘as-prepared AuNPs’) were mostly spherical with a diameter of ca. 20 nm according to SEM measurements (data not shown), although AuNPs larger than 100 nm were occasionally observed owing to the limited incubation time. As-prepared AuNP suspension showed pH ~4.3, which is mostly governed by initial concentration of strong acid HAuCl₄ (~0.3 mM). Extinction spectra were measured for the AuNP dispersed solutions after cooling to room temperature (20-23°C).

Surface of AuNPs are covered by citrate anions, which stabilize AuNPs via electrostatic repulsion between cationic layers, consisting of hydrated counter ions, surroundings of each AuNP. As explained by DLVO theory, the stability of MNPs is governed by the concentration of counter ions in solution for AuNPs with definite amount of citrates anions on their surfaces. Namely, at the higher concentration of cations in solutions, the thinner layer of the counter ions surroundings of each AuNP. Accordingly, van der Waals attractive force becomes crucial to form flocculates. Consequently, one should adjust the concentration of salt in solutions to detect extremely low concentration of target molecules. Also, note that flocculation is a quite slow kinetic process, yielding gradual growth of flocculates with time. Although surface residuals with negative charges like citrate anions stabilize AuNPs suspensions by the electrostatic repulsion between cationic ion layer surroundings of AuNPs, too much amount of citrates on AuNPs result in deficient adsorption sites for neutral molecules. Surface coverage of citrates on AuNPs is determined by the initial amount which would be used for reducing HAuCl₄, and also by reaction conditions such as temperature and reaction time. One must compromise or find appropriate surface coverage of citrate between stability of AuNPs suspension and adsorbed quantity of target molecules.

In this study, we used as-prepared AuNP suspensions, with pH ~4.3, mixed with different DNA and RNA base solutions at 2×10⁻³ M, 1×10⁻⁷ M, and 5×10⁻⁶ M, respectively. As mentioned in experimental section, ccf values are inherently affected by the amount of negative charge on each AuNP and concentration of AuNPs, we used the same batch of AuNPs suspension for these experiments. Hence, distinct ccf values observed for different base molecules are intrinsic, which result from different molecular structures and chemical properties. Thus, bases of A, G, and C with a primary amino group (~–NH₂) showed much lower critical concentrations than those of T and U without an –NH₂ group. Higher ccf values for A, G, and C suggest much stronger interaction of these bases with Au surfaces compared with T and U, which is consistent with former reports on thermal stability of DNA bases adsorbed on gold [27]. Preferential adsorption of A, G and C is presumably due to coordination of a lone pair electrons located in purine and pyrimidine rings. Also it is possibly due to distinct electronic state of these bases.


FIG. 1. Molecular structure of DNA and RNA bases: (a) adenine A, (b) guanine G, (c) cytosine C, (d) thymine T, and (e) uracil U. At pH < pK\textsubscript{A1} (3.3-4.6), imino groups at N\textsubscript{1} (A), N\textsubscript{7} (G), and N\textsubscript{3} (C) are protonated. Similarly, imino groups at N\textsubscript{9} (A), N\textsubscript{1} (G), N\textsubscript{1} (C), N\textsubscript{3} (T, U) are deprotonated at pH > pK\textsubscript{A2} (ca. 9-10).

FIG. 2. Extinction spectra of AuNP (as prepared, pH=4.3) suspensions mixed with solutions containing DNA bases: (a) A, (b) G, (c) C, (d) T, and (e) U.

of which adsorption on AuNPs is stabilized by an \textit{–NH\textsubscript{2}} group. However, the situation for distinct flocculation of AuNPs by different base molecules is much more complicated than anticipated.

To clarify the origin of distinct ccf values, we investigated the effect of protonation on ccf values of DNA bases. Occasionally, pH of our sample solutions containing AuNPs and DNA bases is around 4.3 determined by initial concentration of HAuCl\textsubscript{4} (~0.3 mM), which is close to pK\textsubscript{a} of the imino groups (>N) A (4.2), G (3.3), and C (4.6) [29]. Protonation at an imino group (>N site), next to the carbon combined with an \textit{–NH\textsubscript{2}} group in A, G and C molecules occurs at such low pH probably due to electron donating effect of an \textit{–NH\textsubscript{2}} group. Indeed, T and U molecules without an \textit{–NH\textsubscript{2}} group have no such pK\textsubscript{a}, while they have pK\textsubscript{a} of 9.4-9.5 for an imino group (>N: at N\textsubscript{3} site). Accordingly, A, G and C molecules with an \textit{–NH\textsubscript{2}} group are mostly protonated to be cationic species (>N–H\textsuperscript{+}) in AuNP suspensions of pH~4.3. Here, A, G, and C molecules adsorb on AuNPs at pH < pK\textsubscript{A} with electrostatic interaction between positive charge in DNA bases and negatively charged AuNPs (see also Experimental Details), in addition to chemisorption \textit{via} a lone pair electrons at nitrogen atoms in base molecules. Electrostatic interaction between cationic molecules and anionic charges on AuNPs are much stronger (~1000 kJmol\textsuperscript{-1}) than thermal energy (2.5 kJmol\textsuperscript{-1} at 300 K), and comparable with those of coordination bond \textit{via} lone pair electrons [15]. To derive the contribution of chemisorption \textit{via} lone pair electrons to flocculation of AuNP, pH in solutions containing AuNPs and these base molecules was tuned between 1.8-12.2. For instance, the critical concentration for flocculation of AuNPs (Fig. 3a) was quite similar at pH=6.8 (2\times10\textsuperscript{-6} M) to those at pH=1.8 (3\times10\textsuperscript{-7} M) and 4.3 (3\times10\textsuperscript{-7} M) for adenine, indicating the possibility that chemisorption \textit{via} lone pair electrons at amino groups in base molecules plays a crucial role in flocculation of AuNPs irrespective of their protonated or deprotonated state, rather than electrostatic interaction between protonated adenine and negatively charged AuNPs. This possibility was supported by the observation of a Raman band at 223-238 cm\textsuperscript{-1} for adenine on AuNPs at acidic and neutral pH conditions, which is assigned to a \textit{\nu_{Au-N}} stretching mode as described in the next section. In contrast to the observations in acidic and neutral pH solutions, much higher concentration was necessary for starting flocculation at pH=12.2 (9\times10\textsuperscript{-4} M, Fig. 3b). This result is clearly due to electrostatic repulsion between deprotonated adenine, of which pK\textsubscript{A2} is 9.8, and negatively charged AuNPs. Essentially the same results were observed for guanine and cytosine as those for adenine that ccf values for these bases are similar at acidic and neutral pH, such as 5\times10\textsuperscript{-6} M (pH=4.3), and 5\times10\textsuperscript{-6} M (pH=6-7) for cytosine (Figs. 2c, 3c).

In contrast, T and U molecules showed significantly higher ccf values in acidic pH conditions compared to neutral pH, such as 5\times10\textsuperscript{-5} M (pH=6.8) and
5×10−4 M (pH=4.3) for T, and 3×10−5 M (pH=6.8) and 1.5×10−3 M (pH=4.3) for U molecules. In the DLVO theory, stability of colloidal particles in solution is determined by a competition of electrostatic repulsion between counter ions of surface charges, and van der Waals attractive interaction between nanoparticles [15]. According to this theory, ccf values should be diminished by increasing ionic strength, which decreases the thickness of double layer formed by counter ions. Nevertheless, addition of sulfuric acid to mixed solution of AuNPs and T (or U) molecules up to ∼10 mM increased the ccf of AuNPs. Recalling that T and U are neutral molecules at pH regions between 2.0 and 7.0, the observed pH dependence of ccf values for U and T result from their adsorbed structures. For instance, if T and U adsorb on Au surfaces via an >N–Au coordination bond, deprotonation of an N–H bond is prerequisite (Fig. 1), which is likely inhibited in acidic solutions. This assumption was supported by experimental Raman band for a νAu−N mode, such as at ∼220 cm−1 for U and T, and DFT calculations for these bases adsorbed on AuNPs (Section 3.3, see also [20]). Much lower ccf values observed for T and U bases at neutral pH occasionally reduces differences in ccf values against A, G and C molecules. Nevertheless, absolute ccf values for A, G and C bases increased only by a factor of 6 at neutral pH (vide infra). This observation is again caused by the molecular structure of A, G, and C having >N and >N–H bonds, since deprotonation is unnecessary for A, G and C to adsorb on AuNPs with a coordination bond >N–Au.

Consequently, flocculation of AuNPs observed for different DNA and RNA bases are primarily caused by the formation of an Au–N coordination bond. Adsorption of these base molecules on Au surfaces are facilitated by an –NH2 group, while those for base molecules without an –NH2 group shows pH dependent ccf values. In the next section, we describe details on adsorbed structures of distinct bases A and U on the basis of their Raman spectra observed in bulk powder, in flocculates of AuNPs, and DFT calculations.

B. Adsorbed state of adenine molecules on AuNPs

First, we investigated adsorbed state of adenine, representing base molecules with an –NH2 group. Adenine on AuNPs showed quite similar Raman spectra at acidic (pH=4.3, and 1.8) and neutral pH (6.8) in solutions, which are almost identical to those of bulk powder (Figs. 4a-4c, Table I). In solutions, adenine is protonated (>N3H+, >N10H+) at pH lower than pKa1 (=4.2), neutral (>N1, >N10H) at pH between 4.3 and 9.3, whereas deprotonated ( (>N1, >N5) at pH higher than pKa2 (=9.3). As described above, adenine anions formed flocculates of AuNPs at quite high concentrations. Protonated adenine showed Raman bands at 1648, 1567, 1514, 1460, 1399, 1342, 1312, 1230, 1108, 1013, 958, 730, 616, 540, 313 and 238 cm−1, while neutral adenine showed quite similar Raman bands at 1643, 1543, 1510, 1455, 1394, 1372, 1335, 1317, 1268, 1233, 1023, 960, 730, 621, 554, 323, and 223 cm−1. Only faint changes in peak positions and intensity were observed for SERS bands at 1372, 1268 and 1023 cm−1 in neutral pH, compared to those in acidic condition. We observed a νAu−N mode at 238 cm−1 (223 cm−1), which proves formation of a N–Au coordination bond in acidic and neutral pH irrespective of protonated or neutral state of adenine. Red-shift of Au–N stretching mode from 238 (pH=4.3) to 223 cm−1 (6.8), which was detected only for adsorbed state, suggests slightly weaker Au–N bond for neutral state compared with protonated state. Quite similar Raman spectra observed in acidic and neutral pH solutions indicate that adsorbed state of adenine molecules on AuNPs are inherently identical for cationic and neutral state which is governed by coordination of lone pair electrons at an imino group, as supported by their similar critical concentration for flocculation.

We performed DFT calculations for a free-standing ade-
| TABLE I. Experimental and calculated Raman peak wavenumber for free-standing and adsorbed adenine and uracil molecules (cm\(^{-1}\)). |
|---------------------------------------------------------------|
| Bulk SERS at SERS for Free-neutral Adsorbed | Assignment\( ^{*} \) |
| powder pH=4.2 pH=6.8 deuterated >N-D (obsd.) state state (calcd.) (calcd.) |
|---------------------------------------------------------------|
| Adenine           | 1648 | 1642 | 1639 | 1643, 1641 | \( \delta_{\text{N=C}}(-\text{NH}_2) , \nu(\text{N}_{10}-\text{C}_6) \) |
| 1609, 1594        | 1543 | 1537, 1509 | 1622, 1605 | 1614 | \( \nu(\text{N}_3-\text{C}_4), \nu(\text{N}_1-\text{C}_6) \) |
| 1514              | 1510 | 1510 | 1510 | 1527 | \( \nu(\text{N}_2-\text{C}_6), \delta(\text{C}_8-\text{H}) \) |
| 1479, 1457        | 1440 | 1440 | 1492 | 1486, 1473 | \( \delta(\text{C}_6-\text{H}), \nu(\text{N}_1-\text{C}_6) \) |
| 1414              | 1373 | 1423 | 1457 | \( \nu(\text{C}_4-\text{N}_6), \delta(\text{N}_9-\text{H}) \) |
| 1366              | 1372 | 1367 | 1403 | \( \delta(\text{C}_2-\text{H}), \nu(\text{C}_8-\text{N}_9) \) |
| 1326, 1308        | 1299 | 1328 | 1317 | \( \nu(\text{N}_3-\text{C}_2), \nu(\text{C}_2-\text{N}_1) \) |
| 1208              | 1262 | 1267 | 1270 | \( \nu(\text{N}_2-\text{C}_8), \delta(\text{C}_8-\text{H}) \) |
| 1230              | 1233 | 1238 | 1230 | \( \nu(\text{C}_5-\text{N}_7), \delta(\text{NH}_2) \) |
| 1244              | 1186 | 1143 | 1168 | \( \nu(\text{N}-\text{C}(\text{R}6)), \delta(\text{CNC}) \) |
| 1120              | 1099 | 1097 | 1118 | \( \nu(\text{C}_8-\text{N}_9), \delta(\text{N}_9-\text{H}) \) |
| 1017              | 1023 | 1007 | 1006 | \( \delta_{\text{rock}}(\text{NH}_2), \nu(\text{N}_{11}-\text{C}_6) \) |
| 938               | 960  | 946  | 949, 947 | \( \delta(\text{R}5) \) |
| 895               | 851  | 901  | \( \delta(\text{R}6) \) |
| 720               | 725  | 724  | 746  | \( \delta(\text{R}5), \delta(\text{R}6) \) |
| 620               | 611  | 618  | 636, 619 | \( \delta(\text{R}5), \delta(\text{R}6) \) |
| 554, 531          | 541  | 525  | 560, 555, 546 | \( \omega_{\text{wag}}(-\text{NH}_2) \) |
| 323               | 319  | 279  | 303  | \( \delta(\text{R}5), \delta(\text{R}6) \) |
| 238               | 223  | \( \nu(\text{N}-\text{Au}) \) |
| Uracil            | 1642 | 1749, 1698 | 1675 | \( \nu(\text{C}_2=\text{O}), \nu(\text{C}_4=\text{O}) \) |
| 1643              | 1619, 1576 | 1667 | 1603 | \( \nu(\text{C}_5-\text{C}_6), \delta(\text{C}_8-\text{H}) \) |
| 1502              | 1520 | 1508 | 1574 | \( \delta(\text{N}_1-\text{H}), \nu(\text{N}_1-\text{C}_6) \) |
| 1454, 1415        | 1434 | 1426, 1416 | 1472 | \( \delta(\text{C}_6-\text{H}), \delta(\text{N}_3-\text{H}) \) |
| 1390              | 1367 | 1390 | 1392 | \( \delta(\text{N}_3-\text{H}), \delta(\text{C}_5-\text{H}) \) |
| 1366              | 1302 | 1360 | \( \nu(\text{N}_1\text{C}_2-\text{C}_2\text{N}_3), \delta(\text{ring}) \) |
| 1230              | 1230 | 1237 | 1292 | \( \delta(\text{C}_5-\text{H}), \nu(\text{N}_1\text{C}_2-\text{C}_2\text{N}_3) \) |
| 1209              | 1136 | 1211 | 1181 | \( \delta(\text{C}_6-\text{H}), \nu(\text{C}_2\text{N}_3-\text{N}_3\text{C}_4) \) |
| 1093              | 1032 | 1099 | 1126 | \( \nu(\text{C}_6-\text{N}_1), \delta(\text{C}_5-\text{H}) \) |
| 983               | 1036 | 985  | 1025, 1009 | \( \nu(\text{N}_1-\text{C}_2), \nu(\text{N}_3-\text{C}_4) \) |
| 825               | 808  | 957  | \( \omega_{\text{wag}}(\text{C}_6-\text{H}, \text{C}_5-\text{H}) \) |
| 786               | 794  | 780  | 792, 716 | Ring breathing |
| 573               | 604, 573 | 563 | 594 | \( \delta(\text{R}) \) |
| 551               | 554  | 547  | 564 | \( \delta(\text{R}) \) |
| 530               | 526  | \( \delta(\text{R}) \) |
| 423               | 433  | 405, 385 | 427 | \( \delta(\text{R}) \) |
| 221               | 221  | 211  | \( \nu(\text{N}-\text{Au}) \) |

\( ^{*} \) Calculated for adsorbed structure of \( \text{N}_3\text{Au}_1\text{N}_{10-}\text{Au}_2 \) for adenine, and \( \text{N}_3\text{Au}_1\text{N}_1\text{C}_2=\text{O-}\text{Au}_2 \) for uracil using a DFT method (see the main text in detail). Each Raman band was primarily assigned on the basis of free-standing molecules with the software VEDA, and former report [20–22].
remarkably strong out-of-plane bending mode, an Au atom (Fig. 1a). Among these structures, adenine adsorbed on 7

746, 689, 635, 619, 555, 463, 303 and 200 cm\(^{-1}\) by DFT calculations (Fig. 5a, Table I) for a free-molecule with slight modifications in peak positions and intensity, indicating no drastic changes occurred in molecular structure upon adsorption on AuNPs. In contrast to a free-standing state of adenine, DFT calculation for adenine in flocculates of AuNPs is not so straightforward. This is because we have various molecular structures for DNA bases such as proton tautomers shown by keto-enol isomers, and also protonated and deprotonated state at primary amino (–NH\(_2\)) and imino (>N–H) groups. DNA bases with such different structures likely chemisorb on AuNPs via amino or imino groups in A, C, G, T and U, and carbonyl groups in C, T and U, while base molecules also physisorb on AuNPs. DFT calculations for DNA bases in flocculates of AuNPs should cover a large number of configurations for all these structures, in which bases adsorb on AuNPs via at least two sites. Furthermore, if we take into account hydration using explicit molecular structures of water [24], instead of using a continuous media model, or metal clusters like Ag\(_{3}\) [25] or Au\(_{4}\) [26] to get insight into electronic state of metals and adsorbates, vast amount of computing resources are prerequisite. These considerations are beyond our present scope that deduces plausible adsorbed structures of DNA and RNA bases on AuNPs.

For the first adsorption site in adenine, we adopted three distinct structures coordinating on an Au atom via lone pair electrons at N\(_1\), N\(_3\) and N\(_7\) amino groups (Fig. 1a). Among these structures, adenine adsorbed on an Au atom via N\(_3\) provided Raman spectra most similar to those observed (Data not shown). Other sites yielded significantly different spectra, such as adsorption at an N\(_1\) site gave larger intensity for Raman bands at higher wavenumber region, and that at N\(_7\) site yielded remarkably strong out-of-plane bending mode, \(\gamma_{C–H}\), at \(\sim 800 \text{ cm}^{-1}\) (Data not shown). To get insight into the second adsorption site, isotope shift of SERS spectra was examined for adenine on AuNPs. We experimentally observed that deuteration of amino groups in adenine gave pronounced shift in scissoring mode of –NH\(_2\) at 1600 cm\(^{-1}\) to 1186 and 1099 cm\(^{-1}\) and a rocking mode of –NH\(_2\) from 1233 to 851 cm\(^{-1}\), as well as that in bending mode for N\(_3\)–H from 1394 to 1262 cm\(^{-1}\) [20]. These spectral changes by deuteration indicate that an N–H bond in adenine remains even after adsorption on AuNPs. By taking this observation into account, N\(_{10}\) (in –NH\(_2\) at C\(_6\)) atom which weakly interacts with Au surfaces, abbreviated as N\(_3\)Au\(_{1}\)N\(_{10}..Au_{2}\) structure, is examined for the second adsorption site. Thus, DFT calculations were performed for adenine adsorbed on AuNPs via an N\(_3–\)Au coordination bond (first site) and via –N\(_{10}H_2\) (second site), abbreviated as N\(_3\)Au\(_{1}\)N\(_{10}..Au_{2}\) structure (Fig. 5), which provided SERS spectra at 1641, 1614, 1486, 1473, 1403, 1386, 1317, 1270, 1230, 1168, 1118, 1006, 947,
adsorbed structure of U on Au surfaces. SERS spectra of U in floculates of AuNPs observed in neutral pH (7.3) at 1642, 1625, 1500, 1383, 1366, 1250, 1209, 1095, 1036, 796, 613, 585, 554, 438, and 221 cm$^{-1}$ are corresponded well with those for bulk powder sample at 1712, 1643, 1502, 1454, 1415, 1390, 1248, 1230, 1093, 983, 825, 786, 573, 551, 530, 423 cm$^{-1}$ (Figs. 6a and 6b, Table 1). Interestingly, SERS spectra of U on AuNPs showed a $\delta_{\text{Au}_1\text{N}}$ band at 221 cm$^{-1}$, a definite evidence for deprotonation of an $>\text{N-H}$ group followed by the formation of Au-N. To get further insight into adsorbed state of U on Au, we used deuteration of amino groups in U molecules as well as for adenine. Interestingly, significant SERS spectral changes in 1600–1100 cm$^{-1}$ region were observed for the deuteration of imino groups ($>\text{N-H}$) in U molecule adsorbed on AuNPs. For instance, Raman bands at 1642, 1625, 1383, 1366, 1250, 1209, 1095, and 1036 cm$^{-1}$ for U with $\text{-NH}$ groups were modified to those at 1619, 1576, 1520, 1434, 1367, 1302, 1230, 1136, and 1032 cm$^{-1}$ for U with $\text{-ND}$ groups (Figs. 6a and 6c). Such spectral changes definitely identify that at least one of $>\text{N-H}$ bonds is retained after deuteration of U molecules adsorbed on AuNPs. Indeed, a $\delta_{\text{N-H}}$ bending mode blue-shifted from 1210 cm$^{-1}$ ($>\text{N-H}$) to 1230 cm$^{-1}$ ($>\text{N-D}$). Similar peak shift was observed for bulk powder sample of U molecules from 1230 cm$^{-1}$ ($>\text{N-H}$) to 1260 cm$^{-1}$ ($>\text{N-D}$) (Figs. 6b and 6d). These experimental observations suggest plausible adsorbed structures of U molecules on AuNPs as presented in the following section.

In DFT calculations, keto forms that are thermally more stable than enol forms gave Raman spectra for free-standing U molecules, which are consistent with those experimentally observed for bulk powder samples (Figs. 6 and 7a). Keto structures have various adsorption sites in a pyrimidine ring such as two amino groups ($>\text{N-H}$) at N$_1$ and N$_3$, and two carbonyl groups ($>\text{C=O}$) at C$_2$ and C$_4$. One of N-H bonds in U should be deprotonated to form coordination bonds with Au surfaces. We adopted a plausible candidate for adsorption structure of U, in which a lone pair electrons at N$_1$ coordinate to an Au atom, while N$_3$ and C$_4=\text{O}$ bond weakly interact with another Au atom, abbreviated as $\text{N}_1\text{Au}_1\text{N}_3\text{C}_4=\text{O}..\text{Au}_2$ (Fig. 7), on the basis of experimental observations. This adsorption structure provided theoretical SERS spectra at 1675, 1603, 1574, 1472, 1360, 1181, 1126, 1025, 1009, 792, 716, 624, 594, 564, 427 and 214 cm$^{-1}$ corresponded well with those observed (Figs. 6a and 7a). DFT calculation for the adsorbed structure $\text{N}_1\text{Au}_1\text{N}_3\text{C}_4=\text{O}..\text{Au}_2$ of U on AuNPs gave spectral changes by deuteration of $>\text{NH}$ groups, such as blue shift of $\delta_{\text{N-H}}$ mode from 1181 cm$^{-1}$ to 1227 cm$^{-1}$, corresponding to the observed peak at 1209 and 1230 cm$^{-1}$ (Figs. 7a and 7b). Another adsorbed structures such as those with two coordination bonds ($>\text{N-Au}$) by deprotonation of both $>\text{N-H}$ groups, which are obviously inconsistent with experimental observation in SERS for deuterated U molecules (vide supra), did not have stable structures.

IV. CONCLUSION

We have succeeded in forming floculates of gold nanoparticles (AuNPs) using DNA and RNA bases to obtain their surface enhanced Raman spectra. Adenine, guanine and cytosine molecules which possess a primary amino group ($-\text{NH}_2$) formed floculates at much lower concentration than uracil and thymine without an $-\text{NH}_2$ group, which is inherently related to their adsorbed structures. We found that similar adsorbed structure for these distinct base molecules such as A and U bases, which is described by one coordination bond through a lone pair electrons at an $>\text{N}$-$\text{H}$ group, and the other weak adsorption nearby another $>\text{N}$-$\text{C=O}$ and $-\text{NH}_2$ groups. Such adsorption is facilitated by the existence of an $-\text{NH}_2$ group in A, G and C by stronger van der Waals interaction, whereas inhibited in acidic conditions due to prerequisite deprotonation for T and U molecules.

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[1] K. Kneipp, M. Moskovic, and H. Kneipp, Eds., Surface Enhanced Raman Scattering (Springer, Berlin, 2006).
[2] E. C. Le Ru and P. G. Etchegoin, Principles of Surface-Enhanced Raman Spectroscopy (Elsevier, 2009).

http://www.sssj.org/ejssnt (J-Stage: http://www.jstage.jst.go.jp/browse/ejssnt/)
[3] M. Futamata, T. Yanatori, T. Kokubun, and Y. Yu, J. Phys. Chem. C **114**, 7502 (2010).
[4] M. Futamata, Y. Yu, and T. Yajima, J. Phys. Chem. C **115**, 5271 (2011).
[5] Y. Yu, S. Handa, T. Yajima, and M. Futamata, Chem. Phys. Lett. **560**, 49 (2013).
[6] T. Yajima, Y. Yu, and M. Futamata, Phys. Chem. Chem. Phys. **13**, 12454 (2011).
[7] T. Yajima, Y. Yu, and M. Futamata, J. Raman Spectrosc. **44**, 406 (2013).
[8] P. K. Aravind and H. Metiu, Surf. Sci. **124**, 506 (1984).
[9] K. Ikeda, K. Takahashi, T. Masuda, H. Kobori, M. Kanehara, T. Teranishi, and K. Uosaki, J. Phys. Chem. C **116**, 20806 (2012).
[10] K. Kim, H. B. Lee, J-Y. Choi, and K. S. Shin, J. Phys. Chem. C **115**, 21047 (2012).
[11] H. Suzuki, H. Chiba, and M. Futamata, Vibrational Spectrosc. **72**, 105 (2014).
[12] M. Futamata, M. Ishikura, S. Handa, and C. Iida, Faraday Discussions **178**, in press (DOI: 10.1039/c4fd00188e).
[13] T. Yano, T. Ichimura, S. Kuwahara, F. H’Dhili, K. Uetsuki, Y. Okuno, P. Verna, and S. Kawata, Nat. Commun. **4**, 2592 (2013).
[14] Z. Liu, S-Y. Ding, Z-B. Chen, X. Wang, J-H. Tian, J. R. Anema, X-S. Zhou, D-Y. Wu, B-W Mao, X. Xu, Bin Ren, and Z-Q. Tian, Nat. Commun. **2**, 305 (2011).
[15] J. N. Israelachvili, *Intermolecular and Surface Forces* (Academic Press, London, 1991).
[16] S. Handa, Y. Yu, and M. Futamata, Vibrational Spectrosc. **72**, 128 (2014).
[17] For example, N. E. Marotta, K. R. Beavers, and L. A. Bottomley, Anal. Chem. **85**, 1440 (2013), and A. Barhoumi, and N. J. Halas, J. Am. Chem. Soc. **132**, 17292 (2010).
[18] L. D. Menard, C. E. Mair, M. E. Woodson, J. P. Alarie, and J. Michaely, ACS Nano **6**, 9087 (2012).
[19] S. Bell, Proc. ICORS 2014, WeP-O-007.
[20] B. Giese and D. McNaughton, J. Phys. Chem. B **106**, 1461 (2002), and *ibid.* **106**, 101 (2002).
[21] R. Aroca and R. Bujalski, Vibrational Spectrosc. **19**, 11 (1999).
[22] E. Papadopoulou and S. E. Bell, J. Phys. Chem. C **114**, 22644 (2010).
[23] G. Frens, Nature Phys. Sci. **241**, 20 (1973).
[24] R. Huang, L-B. Zhao, D-Y. Wu, and Z-Q. Tian, J. Phys. Chem. C **115**, 13739 (2011).
[25] R. Huang, H-T. Yang, L. Cui, D-Y. Wu, B. Ren, and Z-Q. Tian, J. Phys. Chem. C **117**, 23730 (2013).
[26] E. S. Kryachiko and R. Remacle, Nano Lett. **5**, 735 (2005).
[27] M. Ostblom, B. Liedberg, L. M. Demers, and C. A. Mirkin, J. Phys. Chem. B **109**, 15150 (2009).
[28] M. H. Jamroz, Spectrochimica Acta A **114**, 220 (2013).
[29] V. Verderolina, R. Cammi, B. H. Munk, and H. B. Schlegel, J. Phys. Chem. B **112**, 16860 (2008) and references therein.