Scott R. Smith, Ryan Seenath, Monika R. Kulak, Jacek Lipkowski “Characterization of a Self-Assembled Monolayer of 1-Thio- β -D-Glucose with Electrochemical Surface Enhanced Raman Spectroscopy using a Nanoparticle Modified Gold Electrode” *Langmuir*, **2015**,*31*,10076-10086

Characterization of a Self-Assembled Monolayer of 1-Thio- β -D-Glucose with Electrochemical Surface Enhanced Raman Spectroscopy using a Nanoparticle Modified Gold Electrode

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KEYWORDS: electrochemical surface-enhanced Raman spectroscopy, 1-thio- β -D-glucose, gold nanoparticles, biomimetic membrane, generalized 2D correlation spectroscopy

ABSTRACT

Preparation of a nanoparticle modified gold substrate designed for characterization of hydrophilic self-assembled monolayers of 1-thio- β -D-glucose (TG) with electrochemical

surface-enhanced Raman spectroscopy (EC-SERS) is presented. Citrate stabilized gold nanoparticles were deposited on a polycrystalline gold electrode and subjected to an electrochemical desorption procedure to completely remove all traces of adsorbed citrate. Complete desorption of citrate was confirmed by recording cyclic voltammetry curves and SERS spectra. The citrate-free nanoparticle modified gold electrode was then incubated in a 1 mg mL⁻¹ aqueous solution of TG for 16 hours prior to being characterized by EC-SERS. The SERS spectra confirmed that at potentials more negative than -0.10 V vs. SCE thioglucose forms a monolayer in which majority of the molecules preserve their lactol ring structure and only a small fraction of molecules appear to be oxidized. At potentials more positive than -0.10 V the oxidation of TG molecules becomes prominent and at potentials more positive than 0.20 V vs. SCE the monolayer of TG consists chiefly of oxidized product. The SERS spectra collected in the double layer regions suggests the SAM of TG is well hydrated and hence can be used for hydrophilic modifications of a gold surface.

INTRODUCTION

Phospholipid bilayers that mimic biological membranes are commonly deposited on metal surfaces to study changes in the membrane-protein interactions upon exposure to an applied electrical field.^{1,2} Due to protein denaturation when in direct contact with metal surfaces, the substrate surface must first be modified prior to the bilayer deposition. Specifically, development of floating lipid membranes on gold electrodes for biomimetic investigations requires the hydrophilic modification of the gold surface to retain fluidity/integrity of the model membrane and to prevent protein denaturation. For that purpose, self-assembled monolayers (SAMs) of 1-thio- β -D-glucose (TG) have been successfully employed as a simple and effective method for

modifying gold substrates as a precursor to lipid membrane deposition and to ensure a uniform water layer remains between the solid support and the biomimetic lipid membrane.¹⁻⁵ Modification of the gold electrode with TG is also preferred over many other adsorbates, i.e. citrate, as this monolayer is stable over a larger potential window.

The formation of SAMs of TG from aqueous solutions at gold surfaces is a result of the spontaneous formation of a covalent Au-S bond through the thiolate of the TG molecules:⁶

Kycia et al. have previously investigated the formation of SAMs of TG from aqueous solutions on a Au(111) electrode surface using electrochemistry and electrochemical scanning tunneling microscopy (EC-STM).⁴ The results of their experiments are summarized in Figure 1. They observed that an ordered monolayer is formed within the potential region between -0.20 to -0.60 V, as suggested by the stable charge density curve in this potential region. However, the monolayer was observed to undergo a phase transformation to a transition state at $E > -0.20$ V vs. SCE, followed by further transformation to a complete disordered state at higher potentials. They suggested that oxidation of the adsorbed TG molecules took place at these positive potentials. Reductive desorption of the TG SAM was also observed at $E < -0.60$ V vs. SCE. However, no spectroscopic studies of TG adsorbed at the gold surface were performed to characterize the stability of the TG SAM as a function of the applied potential. Therefore, the objective of the present work is to apply surface enhanced Raman spectroscopy (SERS) to provide molecular level information about the nature of the TG molecules adsorbed at a gold electrode surface.

Vezvaie et al. have successfully employed SERS to determine the stability of the SAM of TG formed at a nanocavities patterned silver electrode.⁷ The adsorption of TG molecules to the silver surface occurs through a Ag-S bond formation, similar to Equation 1. The SERS studies demonstrated that the TG monolayer is partially oxidized and that a fraction of the β -TG undergoes anomerization at the metal surface to its α -anomer. Most importantly for the biomimetic studies, the SERS investigations demonstrated a large retention of water in close proximity of the silver surface suggesting that a successful hydrophilic modification was accomplished. However, silver is well known to give larger surface enhancement in SERS than gold and the nanocavities patterned gold substrates failed to provide spectra with a sufficient S/N ratio. The difficulty with detection of TG adsorption to electrode surfaces is related to a combination of the small normal Raman scattering cross-section, which has been reported for the 1126 cm^{-1} of glucose to be approximately $5.6 \times 10^{-30}\text{ cm}^2\text{ molecules}^{-1}\text{ sr}^{-1}$,⁸⁻¹⁰ and the low surface concentration expected to be on the order of $10^{-10}\text{ mol cm}^{-2}$.

Achieving surface enhancement through the use of gold nanoparticles (AuNPs) has become a simple and reliable method for characterizing metal adsorbates with Raman spectroscopy. However, their implementation for achieving enhancement of Raman scattering from adsorbates is complicated by place exchange reactions with the capping ligands used during nanoparticle synthesis. Typically, gold nanoparticles are synthesized following the well-established citrate reduction method where HAuCl_4 is reduced to Au^0 by decarboxylation of citrate to acetoacetate.^{11,12} The adsorbed citrate acts as a capping ligand preventing further growth of the nanoparticles and increases nanoparticle stability by preventing agglomeration through a high negative surface charge which results in electrostatic repulsion of the AuNPs with each other. Unfortunately, upon attempting to passively displace adsorbed citrate with

alkylthiols, Park and Shumaker-Parry observed coadsorption leading to a mixed monolayer of the alkylthiol and citrate on the AuNPs.¹¹ Therefore, complete displacement of citrate must be performed prior to the self-assembly of a monolayer of alkylthiols.

In this work we have successfully employed SERS to identify characteristic bands of TG adsorbed on AuNPs and to determine the extent of oxidation of the TG monolayer as a function of the applied electrode potential. To acquire spectra of TG on the SERS active electrode we have developed a procedure to completely remove citrate from the AuNPs. We describe electrochemical and spectroscopic investigations after deposition of the citrate stabilized AuNPs on a gold electrode, followed by complete electrochemical desorption of the adsorbed citrate, and further modification of the SERS active electrode with TG. This constitutes an important element of novelty in the studies of SAMs at AuNPs. Furthermore, the description of the adsorption behavior of TG on gold surfaces is significant because the SAM of TG provides a stable and reproducible hydrophilic surface modification, important for further biomimetic investigations using gold as a solid support. This monolayer may also find application in other areas of research that requires a hydrophilic electrode surface.

MATERIALS AND METHODS

Substrate Preparation. Citrate stabilized gold nanoparticles (AuNPs) were prepared following the procedure previously described by Li et al.^{13–15} The AuNPs were grown in one step process, 40-50 nm dia., following the typical sodium citrate reduction method at ~90.0°C using hydrogen tetrachloroaurate (III) trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$, Alfa Aesar, 99.99%). Centrifugation of the AuNPs suspension at 1500 RPM for 15 mins was performed and deposition of approximately 20 μL of the concentrated citrate-AuNPs solution was placed in the form of a droplet onto the

desired substrate and allowed to dry under vacuum. The SERS spectra were recorded by depositing the citrate-AuNPs on either a clean silicon wafer or a polycrystalline gold rotating disk electrode (RDE, PINE Instruments, 99.99%). A polycrystalline gold RDE was selected as the electrode surface for EC-SERS studies due to; (i) the ease of polishing the RDE surface to remove the AuNPs and regeneration of a clean surface for repetitive investigations, (ii) ease of electrochemical desorption of citrate from the AuNPs modified surface upon applied potential and rotation (if needed), and (iii) convenient configuration with the custom built Teflon spectroelectrochemical Raman cell. The gold RDE was polished with successive grades of diamond suspension (6, 3, 0.25 μm , LECO), with excessive rinsing with Milli-Q water (18.2 $\text{M}\Omega\text{ cm}$) between each grade.

Prior to, and following deposition of citrate-AuNPs, the modified RDE (Au/AuNPs/Citrate) was gently cleaned of all adsorbed citrate and other surface contaminants in a three-electrode glass cell with an argon purged 0.1 M sodium fluoride (NaF, Sigma Aldrich, >99%) electrolyte solution ($\text{pH} \sim 8.4$), by repetitive cycling between -0.90 and +1.25 V vs. SCE, at a scan rate of 20 mV s^{-1} and electrode rotation of 500 RPM, until a cyclic voltammetry curve (CV) and a SERS spectrum indicative of a stable and clean surface was obtained. After a sufficient number of cycles were completed, the removal of all adsorbed citrate was confirmed by recording a CV at 20 mV s^{-1} between -0.90 and +0.40 V vs. SCE, without rotation of the electrode. A coiled gold wire (Alfa Aesar, >99.9%) and a saturated calomel electrode separated from the electrolyte solution through a salt bridge were employed as the counter and reference electrode respectively. Electrochemical investigations were conducted using a HEKA PG 590 potentiostat coupled with a dual lock-in amplifier and all electrochemical information was recorded with a National Instruments data acquisition card and in-house Labview written

software provided by Prof. Ian Burgess. All glassware was acid-washed (3:1 v/v mixture of concentrated sulfuric and nitric acid) and rinsed with a copious amount of Milli-Q water.

Modification of the gold electrode surface for SERS investigations was performed by immersing the electrochemically cleaned Au/AuNPs electrode in a 1 mg mL⁻¹ 1-thio- β -D-glucose sodium salt (TG, C₆H₁₁O₅SNa•2H₂O, Sigma Aldrich) dissolved in Milli-Q water for 16 hours, followed by repetitive rinsing in Milli-Q water to remove excess adsorbates.

Instrumental Setup. UV-vis absorption spectra of freshly prepared citrate-AuNPs in an aqueous suspension and in air (after vacuum deposition on a clean glass slide) were recorded in the 400-800 nm spectral range using a Cary 300 UV-vis spectrophotometer. Collection of all scanning electron microscopy (SEM) images was achieved with a FEI Inspect S50 equipped with a tungsten filament and a Everhart-Thornley secondary electron detector with a working distance of approximately 9.6 cm, a spot size of 3.5, and set to a 20 kV electron beam energy.

A Renishaw model 2000 imaging microscope furnished with a near IR laser (785 nm wavelength, 300 mW power output) and a CCD array detector was employed for collection of all Raman spectra. A spectral resolution of 1 cm⁻¹ was achieved employing a holographic grating, 1200 grooves mm⁻¹, and a slit width of 50 μ m. Calibration of the spectrometer to the vibrational Raman band of silicon ca. 521 cm⁻¹ was performed prior to every investigation. All EC-SERS investigations were performed with a custom built Teflon spectroelectrochemical cell designed to fit a PINE RDE in an inverted, stationary configuration. A gold wire ring that encircled the working electrode, a SCE separated from the cell through a salt bridge, were used as the counter and reference electrodes, respectively, and a PVC film (DuraSeal, VWR) was used as the transparent window for focusing of the microscope objective. SERS spectra were recorded with a

LEICA immersion lens (63x), separated from the electrolyte solution with a polyethylene-based film, by focusing the laser spot size onto the substrate surface. Normal Raman spectra were collected in a single acquisition between 3200 to 100 cm^{-1} , using 100% laser power, a 100 second exposure, and 10 accumulations per cycle. SERS spectra were recorded at 25% laser power ($\sim 2 \times 10^7 \text{ mW cm}^{-2}$), 10 s exposure, and 3 accumulations to decrease the collection time of each spectra and reduce the possibility of laser induced changes in the sample substrate. All investigations were performed in triplicate to ensure reproducibility of spectra obtained. Generalized two-dimensional correlation spectroscopy (2DCOS) spectra^{16–19} were generated from a collection of sequentially recorded EC-SERS spectra in which the spectral band intensities changed relative to the time-average spectrum as a function of the applied potential.

Molecular Simulations. Following a procedure outlined by Vezvaie et al.,⁷ the structure and assignment of the molecular vibrations observed as a result of TG adsorption on the SERS active gold electrode were determined through ab initio density functional theory (DFT) calculations performed using the Gaussian 03W program suite. In brief, optimization of geometry of TG bonded to a three gold atom cluster were obtained using the DFT-B3LYP/LanL2DZ basis set. The calculated vibrational wavenumbers for the optimized structure were corrected according to Yoshida et al.²⁰ who introduced the wavenumber-linear scaling method: $\nu_{\text{obs}}/\nu_{\text{calc}} = 1.0 - \kappa\nu_{\text{calc}}$, where ν_{obs} and ν_{calc} , are the wavenumbers (in cm^{-1}) of the observed and calculated normal mode vibrations, respectively, and κ is the appropriate scaling factor.²⁰ It was found that the same scaling factor, i.e. $\kappa = 0.00001880$, that was reported by Vezvaie et al.⁷ gave excellent agreement between the calculated and experimentally obtained spectra reported here.

RESULTS AND DISCUSSION

Characterization of Citrate Stabilized Gold Nanoparticles. Figure 2A displays the UV-Vis absorbance spectrum of citrate-AuNPs suspended in Milli-Q water. The spectrum matches well with the spectrum reported by Mabuchi et al. in the literature.²¹ Figure 2A displays a single band positioned ca. 535 nm, indicative of a narrow size distribution of AuNPs consistent between 40 and 50 nm in diameter.²¹ Upon deposition onto a glass substrate, the position of the absorbance band in Figure 2A shifts to 552 nm with the emergence of a shoulder at 591 nm suggesting the AuNPs have agglomerated into larger clusters on the glass surface. Figure 2B shows a SEM image of the 3D agglomeration of the AuNPs, upon drying of the aqueous suspension of citrate-AuNPs on a clean gold RDE surface. The average diameter of the AuNPs on the RDE surface in Figure 2B was measured to be 48.6 ± 9.3 nm consistent with the UV-vis band position observed in Figure 2A of citrate-AuNPs suspended in solution. It is expected that the size and distribution of the AuNPs on the gold surface will provide sufficient surface enhancement for detection and characterization of the adsorbed molecules in EC-SERS investigations.

Spectrum 1 in Figure 3 displays the baseline corrected SERS spectrum of freshly prepared citrate-AuNPs after vacuum deposition onto a clean silicon wafer, washing with Milli-Q H₂O, and re-immersion into Milli-Q water (Si/AuNPs/Citrate/H₂O). The SERS spectra of the citrate-AuNPs surface reveal many distinct bands indicative of adsorbed citrate, which assignment is given in Table 1. To assist band assignment, spectrum 2 displays the expanded 2000 - 600 cm⁻¹ region. Previously, several spectroscopic reports have suggested citrate adsorbs to gold with deprotonated carboxylic groups preferentially oriented towards the gold surface.^{22,23} Whereas, some have suggested that adsorbed citrate exhibits partially protonated carboxylic groups that are either hydrogen bonded with a next-neighbor or are oriented outwards toward the bulk solution.¹¹ Figure 3 exhibits several bands characteristic of citrate adsorbed to the surface of

the AuNPs. For example, a large broad band is observed ca. 253 cm^{-1} , likely associated with gold-citrate interactions through the deprotonated carboxylic groups.²⁴ Nichols et al.²³ and Floate et al.²⁵ tentatively assigned the bands at ca. 1686 and 1580 cm^{-1} as the $\nu_{\text{as}}(\text{COOH})_{\text{ads}}$ and $\nu_{\text{as}}(\text{COO}^-)_{\text{ads}}$ of citrate bound to the Au surface. The 1382 cm^{-1} band originates from carboxylic groups coordinated to the AuNPs surface as an η -2 adsorbate.^{23,25-27} Other bands indicative of citrate adsorbed to the AuNPs are observed ca. 883, 997, 1440, and 2988 - 2782 cm^{-1} attributed to $\nu(\text{CO}, \text{CC})$, $\nu(\text{CC})$, $\delta(\text{CH}_2)/\omega(\text{CH}_2)$, and $\nu(\text{CH}, \text{CH}_2)$ respectively.^{12,21,23,25,27-30} Many of bands of adsorbed citrate correlate well, albeit slightly shifted, with the Raman band positions observed from citrate molecules in an aqueous solution (spectra not shown) as summarized in Table 1. Furthermore, the band positioned at 1640 cm^{-1} likely stems from a combination of $\delta(\text{H}_2\text{O})_{\text{bulk}}$ and $\nu_{\text{as}}(\text{COO}^-)_{\text{ads}}$, making it difficult to determine the extent of hydration or the quantity of water that is in close proximity with the Au/AuNPs surface. It is important to note that the intense band ca. 521 cm^{-1} originates from the underlying Si substrate.

After deposition of the citrate-AuNPs onto the gold electrode, the adsorbed citrate molecules were successfully desorbed by applying repetitive electrochemical desorption cycles between the potential limits of -0.90 and +1.25 V vs. SCE, using a 20 mV s^{-1} sweep rate and a 500 RPM electrode rotation in a 0.1 M NaF electrolyte until a CV curve characteristic of a clean gold electrode was obtained. The continuous red line in Figure 4 plots the CV curve collected with the clean Au/AuNPs stationary electrode free from adsorbed citrates. For comparison, the dotted black curve plots the CV curve recorded at the AuNPs free, freshly-polished polycrystalline gold surface that was not exposed to citrate ions. The two curves have similar shape, however, much larger currents are recorded at the AuNPs modified surface illustrating an increase in the surface area due to the presence of AuNPs. The inset in Figure 4 displays the

CVs recorded in the double layer region, i.e. between -0.90 V and +0.40 V vs. SCE. The two curves have similar shape indicating that the electrochemical cleaning procedure was successful in removing citrates from AuNPs.

Behavior of Adsorbed 1-Thio- β -D-Glucose (TG) in the Ordered Region. The Au/AuNPs electrode that was citrate free was rinsed with a copious quantity of Milli-Q water. Removal of all traces of citrate and other contaminants was then confirmed by recording a SERS spectrum (shown as spectrum 1 in Figure 5) of the clean Au/AuNPs electrode surface in an argon purged 0.1 M NaF electrolyte at the open circuit potential (E_{OCP}), ~ 0.15 V vs. SCE. The clean Au/AuNPs electrode was then removed from the cell and incubated in a 1 mg mL⁻¹ TG solution for 16 hours, rinsed with Milli-Q water to remove physisorbed TG molecules, and then reinserted into the Raman cell with a freshly prepared argon purged 0.1 M NaF electrolyte. SERS spectra of the TG modified Au/AuNPs surface were then collected as a function of applied potential starting at E_{OCP} , followed by -0.10 V vs. SCE and then stepping every 0.10 V negatively for every sequentially acquired spectrum. For potentials between E_{OCP} (~ -0.08 V vs. SCE) and -0.60 V vs. SCE corresponding to the ordered state of the monolayer (see Figure 1), the shape of the spectra was invariant with applied potential. For this region, seven spectra were averaged to improve S/N and plotted as spectrum 2 in Figure 5. This spectrum is dominated by a very strong $\nu(\text{Au-S})$ band at ~ 265 cm⁻¹ coupled with a strong shoulder at 295 cm⁻¹. Therefore, spectrum 3 in Figure 5 plots bands in the 2000 - 400 cm⁻¹ region with the spectral intensity enhanced 5 times. All band positions in the SERS spectrum of the SAM of TG on gold and their respective assignments are summarized in Table 2. The assignment was assisted by computation of the Raman spectra of TG coordinated to a cluster of three Au atoms. The calculated vibrational frequencies were visualized through animation with Gaussview 3.09. Several examples of such

animations are included in the Supporting Information. The assignment is in good agreement with the literature concerning the Raman spectra of reducing sugars.^{31–33} The spectral intensity in the spectra collected negative of $E = -0.60$ V vs. SCE was observed to uniformly decrease until all spectral features were lost at $E = -0.80$ V vs. SCE, consistent with reductive desorption of TG molecules reported by Kycia et al. from Au (111) electrodes.⁴

To facilitate discussion, Figure 6A plots the SERS spectra recorded of TG adsorbed on the silver nanocavity substrate (Ag/TG/0.1 M phosphate buffer), previously published by Vezvaie et al.,⁷ and their assignments of observed bands is given in Table 2. Figure 6B plots the averaged spectrum of TG adsorbed on the Au/AuNPs electrode surface presented earlier in Figure 5. In addition, Figure 6C also plots the subtractively normalized (SN) Raman spectrum collected of TG dissolved in Milli-Q H₂O for reference. The normalized Raman spectrum of TG in solution, $I_{TG}^N(\nu)$, was calculated by determining the spectral intensity difference between the raw Raman spectrum of TG solution, $I_{TG/H_2O}(\nu)$ and the reference spectrum of Milli-Q water, $I_{H_2O}(\nu)$, spectra not shown, according to Equation 2:

$$I_{TG}^N(\nu) = I_{TG/H_2O}(\nu) - I_{H_2O}(\nu) \left[\frac{I_{TG/H_2O}(1640 \text{ cm}^{-1})}{I_{H_2O}(1640 \text{ cm}^{-1})} \right] \quad (\text{EQ 2})$$

Disappearance of the water bending vibration, ca. 1640 cm^{-1} , in Figure 6C indicates complete removal of the convoluting water bands and Raman background. The observed spectral band positions observed in Figure 6C and corresponding assignments are also summarized in Table 2.

The spectra recorded at the silver electrode (Figures 6A) and the AuNPs (Figure 6B) show a broad band centered at $\sim 1600 \text{ cm}^{-1}$ that is absent in the SN spectrum of TG in the aqueous solution (Figure 6C). This band is characteristic for $\nu_{as}(\text{COO}^-)$ and its presence indicates

a partial oxidation of the TG monolayer. The band at 1725 cm^{-1} in the spectrum Figure 6A is characteristic for the carbonyl group vibration. This band is absent in the spectrum Figure 6B recorded at AuNPs suggesting that in this case the oxidation of TG molecules is somewhat less pronounced. The band at 1650 cm^{-1} at the Ag electrode and a shoulder at $\sim 1640\text{ cm}^{-1}$ at the AuNPs are characteristic for the bending band of water, $\delta(\text{H}_2\text{O})_{\text{bulk}}$. Their presence indicates that monolayers of TG adsorbed on electrode surfaces retain water molecules very near the electrode surface. This is important information in view of the application of TG SAMs for hydrophilic modifications of gold or silver surfaces. Overall, the spectra of TG at AuNPs and at the Ag nanocavities electrodes display many similarities. However, in contrast there are a few apparent differences between the two SERS spectra and the Raman spectrum of TG in solution. Vezvaie et al.⁷ attributed the differences between the SERS spectra and Raman spectrum of TG in solution to the interaction of the TG molecule with the metal and to surface induced anomerization. In the absence of water, thioglucose exists in the lactol ring form. In an aqueous solution, the lactol ring form and the aldehydo acyclic form are in equilibrium. On relactolization, the sugar undergoes anomerization involving disposition of the OH group attached to the anomeric C-1 carbon.³² The SERS spectra recorded at silver nanocavities electrode by Vezvaie et al. resembled a spectrum of a 38:62 mixture of α - and β -TG anomers.⁷

To assess the effect of anomerization of the SERS spectra for the SAM of thioglucose at AuNPs, calculations were performed for β -TG and α -TG coordinated to three gold atoms (β -TG-3Au and α -TG-3Au). To calculate the spectra, the ab initio density functional theory (DFT) and Hartree-Fock (HF) methods were used and implemented in the Gaussian 03W program suite, using the procedure described in details in ref ⁷. The calculations show that the coordination of TG to Au has a very significant impact on the spectrum. Figure 7 plots the simulated Raman

spectrum of (A) α -TG and (B) β -TG coordinated to a cluster of three Au atoms, which matches quite well with the simulated spectrum of aurothioglucose reported by Karabulut and Leszczynski.³⁴ The averaged SERS spectrum recorded with the Au/AuNPs electrode surface for the potentials corresponding to the ordered state of the monolayer is also plotted in Figure 7 for direct comparison. The bands observed in Figure 7C positioned ca. 890 and 800 cm^{-1} , which relate to $\nu_s(\text{COC})_{\text{ring}}/\rho(\text{CH}_2)/\nu(\text{CC})$ and $\nu(\text{CS})$ coupled with ring vibrations (see animations in the Supporting Information), correspond very well with β -TG spectrum, Figure 7B, suggesting that the SAM of TG on the Au/AuNPs electrode surface is predominantly comprised of β -TG.⁷ Overall, the SERS spectrum of TG at AuNPs is in a good agreement with the calculated spectrum for 3Au/ β -TG suggesting that the anomerization was negligible in this case. The small differences can be explained by the fact that the SERS spectrum corresponds to a hydrated sample while the calculated spectrum was computed for an isolated molecule. For example a broad band at $\sim 700 \text{ cm}^{-1}$ is present in the SERS spectrum and is absent in the computed spectrum. However, Raman spectrum in Figure 6C also shows a broad band at these frequencies. In addition, due to surface selection rules relative intensities in the SERS spectrum may be different than in the calculated spectrum or in the spectrum of TG in solution.

The vibrations in hydrocarbons are usually coupled, making it difficult to assign individual bands in the Raman spectra to a specific group vibration. Therefore, the vibrational spectra of sugars are analyzed by dividing the spectrum into regions.^{31,33} The 1500-1200 cm^{-1} region contains bands corresponding to coupled vibrations of methylene and hydroxyl groups.³³ For example strong bands at ~ 1437 and 1370 cm^{-1} correspond to combination of $\delta(\text{CH}_2, \text{COH}, \text{CH})$ and $\omega(\text{CH}_2)$. These bands are present both in the SERS spectra and in the Raman spectrum of TG in solution. The bands in the 1200-950 cm^{-1} region are due predominantly to C-O and C-

C stretching with stronger contributions from C-O stretches and a small part due to bending of the OH functional groups. The 950-700 cm^{-1} region is frequently referred to as the anomeric region because it contains vibrations due to the anomeric carbon C(1). Finally the region below 700-300 cm^{-1} contains bands that may be described roughly as out-of-plane ring deformations. This is nicely illustrated by animations of vibrations corresponding to these regions and presented in the Supporting Information. The presence of strong bands at frequencies below 1200 cm^{-1} such as (1080, 1050, 1002, 890, 800, 609, 538, 414 cm^{-1}), that are characteristic of the lactol ring vibrations, is a strong indication that in the ordered state of the monolayer the lactol ring is preserved in the majority of the TG molecules. Therefore, it seems reasonable to conclude that in the ordered region of the SAM; (i) TG molecules are adsorbing to the SERS active gold electrode, (ii) a partial quantity of molecules in the SAM undergo oxidation, (iii) the SAM appears to retain some quantity of water in close proximity of the gold-electrolyte interface, and (iv) a significant fraction of the adsorbed and not oxidized TG molecules retains their closed lactol ring.

Behaviour of Adsorbed TG in the disordered region. Successful description of TG adsorption at the Au/AuNPs in the ordered region allows for further SERS characterization of the stability and response of the SAM to an applied electric field across the disordered region defined in Figure 1. Figure 8 compares the averaged SERS spectra for the ordered region to the spectra recorded upon polarization of the SAM covered electrode surface to potentials more positive than 0.20 V vs. SCE. It is important to note that all of the spectra were collected successively one after another in the same location on the electrode surface. In this way the spectral changes induced as a function of potential are independent of morphological changes of the substrate surface, i.e. same number and arrangement of AuNPs sampled. Increasing the applied potential up to +0.50 V

vs. SCE results in the appearance of several bands indicative of oxidized TG at 1581, 1210 and 998 cm^{-1} . These bands become very pronounced and increase in intensity with increasing applied potential. There are several strong bands in the 1200-1100 cm^{-1} region characteristic of $\nu(\text{CC})/\nu(\text{COC})/\delta(\text{OH})$ vibrations. However, their positions are shifted relative to the bands observed for the TG in the ordered state of the monolayer and coupled with the disappearance of the bands between 1100-1000 cm^{-1} that are typical of the lactol ring of glucose. This behavior suggests that the lactol ring was opened at these higher potential as they likely correspond to the $\nu(\text{CC})/\nu(\text{COC})/\delta(\text{OH})$ vibrations in the opened ring configuration. It is important to note that the intensity of bands at $\sim 290 \text{ cm}^{-1}$ does not change at the positive potentials. These bands correspond to $\nu(\text{Au-S})$ and their presence indicates that the gold-thiol bond remained intact at the positive potentials.

Investigations of glucose oxidation at positive potentials applied to gold electrode surfaces have been performed in the past. The oxidation of TG molecules observed on gold at E_{OCP} may be speculated as the result of oxidation of the glucose moiety to any one of a number of different products. For example, previous literature on the electrocatalytic oxidation of glucose on metal electrode surfaces, including AuNPs, have been proposed gluconolactone, gluconic acid, gluconate, and/or formic acid as oxidation products.^{24,35-45} The disappearance of the 1100-1000 cm^{-1} bands in Figure 8A, typical of the lactol ring of glucose moiety, may suggest that the TG molecules undergone a ring-opening oxidation reaction likely producing a thiolated form of gluconate still adsorbed to the surface. This is consistent with Largeaud et al.⁴⁶ and others⁴⁷⁻⁴⁹ that also observed a band at low potentials in IR and Raman spectra positioned between 1587-1595 cm^{-1} and assigned it as the asymmetric O-C-O vibration of gluconate.

To better understand the complex behaviour observed upon anodic polarization of the Au/AuNPs electrode, generalized 2DCOS spectra were calculated from the sequentially recorded spectra collected as a function of applied potential, found in Figure 8A. The 2DCOS spectra illustrates the synchronous, Figure 8B, and asynchronous, Figure 8C, changes that occur with respect to the potential-average spectrum, included for reference in Figure 8B and 8C along the x - and y -axis. It is important to note that only the order of observed changes in the spectral intensities recorded in the SERS spectra can be determined and not the distribution of related species. For example, synchronous 2DCOS spectra can determine which spectral features change coincidentally. Auto-correlation peaks are found as positive bands on the diagonal position, i.e. $\nu_1 = \nu_2$, which result from changes in the spectral intensities of two bands with the applied perturbation (applied potential in the present case). Cross-correlation peaks can be observed in the off-diagonal positions when either $\Phi(\nu_1, \nu_2) > 0$, the spectral intensity of the observed SERS bands positioned at ν_1 and ν_2 simultaneously change in the same direction, or $\Phi(\nu_1, \nu_2) < 0$ indicating that the spectral changes are in opposite direction. Asynchronous 2DCOS spectra, $\Psi(\nu_1, \nu_2)$, represent the sequential changes in spectral intensities of two observed features relative to one another. No auto-correlation bands are observed in asynchronous 2DCOS spectra. However, the sign of the variation in spectral intensity of the cross-correlation peaks in asynchronous spectra can be either positive or negative as in synchronous spectra. If the sign of the asynchronous and synchronous cross-correlation peaks match then the change in the spectral intensity at ν_1 occurs first and is followed by a change in spectral intensity at ν_2 . If the signs of the synchronous and asynchronous cross-correlation peaks are opposite, then the change in the band intensity at ν_2 takes place before the change at ν_1 .¹⁹

Auto-correlation bands are observed along the diagonal positions in the synchronous 2DCOS plot seen in Figure 8B, i.e. $\nu_1 = \nu_2$, at 1581, 1210, and 998. Strong positive cross correlation bands in Figure 8B are observed in the off-diagonal positions, ca. (1581, 998), (1581, 1210), (1210, 998), (1210, 1581), (998, 1581), and (998, 1210), illustrating the changes in spectral intensity that occur simultaneously with respect to a change in the applied potential. Strong positive cross correlation bands in the asynchronous spectra of Figure 8C reveal bands positioned ca. $(\nu_1, 1581)$, $(\nu_1, 1210-150)$, $(\nu_1, 998)$ where ν_1 correspond to bands in the frequency range 1220-1500 cm^{-1} . Since positive cross correlations are observed in the synchronous spectrum they indicate that the bands at in the 1220-1500 cm^{-1} change before changes of the bands at 1581, 1210, and 998 take place.

In the asynchronous 2DCOS spectrum the bands at (1210,1002), (1581,1002), (1210,1028), (1581,1028), (1210,1050) and (1581,1050) are negative. Since the corresponding cross correlation bands in the synchronous spectrum are positive this result indicates that changes at 1002, 1028 and 1050 cm^{-1} that correspond to the lactol ring vibrations (see animation in the Supporting Information) take place before changes at 1210 and 1581 cm^{-1} bands that corresponds to the symmetric and asymmetric $\nu(\text{COO}^-)$ vibrations. Therefore, from the SERS and 2DCOS spectra it may be reasonably concluded that anodic polarization of the electrode surface induces oxidation of the TG SAM on the Au surface involving disappearance of the lactol ring vibrations observed at 800, 890, 1002, 1028 and 1050 cm^{-1} indicating ring opening first, followed by an oxidation of the TG molecules evidenced by the appearance of the $\nu(\text{COO}^-)$ bands at 1581 and 1210 cm^{-1} .

The formation of an oxidized TG to possible products, like thiogluconate or similar species, at potentials between 0.40 to 0.6 V vs. SCE, see Figure 1, indicates that its cathodic desorption by stepping the potential to -0.90 V vs. SCE leads to the formation of a dianion (with one negative charge at the sulfur atom and the second at the carboxylic group). This leads to the apparent increase in the charge density curve at positive potentials as evidenced in Figure 1. In contrast, the cathodic desorption of TG at potentials between -0.10 and -0.50 V vs. SCE, within the region of the ordered film results in the formation of a thiolate with a single negative charge, hence lower values are observed in Figure 1 in this region. Thus the Raman studies reported in aid in the understanding of why the charge corresponding to the formation of the monolayer shown in Figure 1 is doubled by moving from the ordered to the disordered film. By moving the potential in the positive direction, the surface concentration of TG molecules is not changed. However, its oxidation state is changed.

CONCLUSION

A simple SERS active AuNPs modified gold electrode, free of adsorbed contaminants, was successfully employed to identify and characterize the electrochemical behaviour of a SAM of TG in an aqueous electrolyte. Citrate used in the synthesis of the gold nanoparticles was successfully removed from the gold electrode using the electrochemical desorption procedure. The AuNPs modified substrates exhibited good mechanical stability for EC-SERS experiments, however to avoid deterioration, the AuNPs modified substrates were prepared fresh prior to each investigation. The AuNPs modified gold electrode was observed to offer increased sensitivity through; (i) tuning of the AuNPs size to achieve optimal localized surface plasmon resonance, and (ii) spatial distribution leading to a larger quantity of hotspots in a confined space relative to

traditional substrates, for example electrochemically roughened electrodes. The SERS spectra demonstrated that at potentials more negative than -0.10 V vs. SCE thioglucose forms a monolayer in which majority of the molecules preserves its lactol ring structure and only a small fraction is oxidized. At potentials more positive than -0.10 V the oxidation of TG molecules becomes prominent and at potentials more positive than 0.20 V vs. SCE the monolayer of TG may chiefly consist of thiogluconate. The present study shows that a monolayer of thioglucose can be used in biomimetic research as a hydrophilic film that promotes the interaction with the hydrophilic components of the membrane and prevents direct interaction of proteins with the metal surface. However, this film may contain oxidized groups particularly at positive electrode potentials.

ASSOCIATED CONTENT

Supporting Information. Animations of the molecular vibrations at calculated positions ca. 1216, 1176, 1139, 1070, 1048, 1022, 1000, 894, and 788 cm^{-1} of TG coordinated to three gold atoms is included in the supporting information. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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ACKNOWLEDGEMENTS

This work was supported by the Discovery Grant from Natural Science and Engineering Research Council of Canada.

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Figure 1. Charge density curves recorded with a Au(111) electrode in a 0.1 M NaF electrolyte before (black squares) and after (red circles) external β -TG SAM formation. Also included are EC-STM images (31 x 31 nm, tunneling conditions; $I_t = 0.39$ nA, $V_{\text{bias}} = +200$ mV) collected in a 0.05 M phosphate buffer + 2 mM β -TG electrolyte at potentials identified by dashed grey lines. STM images reproduced from ref⁴.

Figure 2. (A) UV-vis absorbance spectra of freshly prepared citrate-AuNPs (black line) suspended in Milli-Q H_2O , and (red line, absorbance multiplied 5x) vacuum deposited on a clean glass surface. (B) SEM image (15 000x mag) illustrating the surface morphology of the SERS active gold RDE after the deposition of citrate-AuNPs (ca. 48.6 nm dia.).

Figure 3. Baseline corrected SERS spectrum of freshly prepared citrate stabilized AuNPs vacuum deposited on a clean Si wafer, washed, and re-immersed in Milli-Q water (Si/AuNPs/Citrate/H₂O), 25% laser power, 10 s exposure, 3 accumulations. Inset: wavenumber axis enlarged for the 2000 to 600 cm⁻¹ region with marked band positions.

Table 1. Observed Raman band positions of a 0.1 M sodium citrate solution and citrate adsorbed to AuNPs with corresponding band assignments.

Vibrational Mode Assignment	Observed Band Positions (cm ⁻¹)		References
	0.1 M Citrate/H ₂ O	Si/AuNPs/Citrate/H ₂ O	
$\nu(\text{OH})$	> 3001	-	28
$\nu(\text{CH}, \text{CH}_2)$	2982-2894	2885	30
$\nu(\text{C}\equiv\text{O})_{\text{ads}}$	-	2118	21
$\nu_{\text{as}}(\text{COOH})_{\text{bulk}}$	1710	-	23,25,26
$\nu_{\text{as}}(\text{COOH})_{\text{ads}}$	-	1686	
$\delta(\text{HOH}) / \nu_{\text{as}}(\text{COO}^-)_{\text{ads}}$	1640	1640	23,25,28
$\nu_{\text{as}}(\text{COO}^-)_{\text{ads}}$	-	1580, 1532	23,25,26
$\delta(\text{CH}_2) / \omega(\text{CH}_2)$	1434, 1410	1440	21,26,27
$\nu_{\text{s}}(\text{COO}^-)_{\text{ads}}$	-	1382	23,25-27
$\nu_{\text{s}}(\text{COO}^-)$		1351	27
$\nu(\text{CO}) / \delta(\text{OH})$	-	1290, 1255	26,27
$\nu(\text{CO}, \text{CC})$	1050	1140, 1116, 1046	26,27
$\nu(\text{CC})$	945	997	21,27
$\nu(\text{CO}, \text{CC})$	838	883	21,27
$\nu_{\text{s}}(\text{CO}) / \rho(\text{CH}_2)$	794	800	27
$\nu(\text{Si-Si})$	-	521	-
$\nu(\text{Au-COO}^-) / \delta(\text{CCC})$	-	294, 253	-
$\delta(\text{CCC}) / \nu(\text{Au-C}\equiv\text{O})$	124	140	21

ν_{s} - symmetric stretching

ν_{as} - asymmetric stretching

δ - bending

ω - wagging

Figure 4. CV curves (5 mV s^{-1} , 0 RPM) of a polycrystalline gold RDE in an argon purged 0.1 M NaF electrolyte before (black straight line) and after (dashed red line) modification with AuNPs.

Figure 5. Spectrum 1: Baseline corrected SERS spectra recorded of the electrochemically cleaned Au/AuNPs substrate in a 0.1 M NaF electrolyte. Spectrum 2: average spectrum in the ordered region of TG SAM formed by incubation in a 1 mg mL⁻¹ solution of TG for 16 hours (25% laser power, 10s exposure, 3 accumulations). Spectrum 3: the 2000-400 cm⁻¹ region of the SERS of TG SAM plotted with intensities increased five times.

Figure 6. SERS spectra in the 2000 - 400 cm⁻¹ wavenumber region of the (A) Ag/TG/0.1M phosphate buffer and (B) the Au/AuNPs/TG electrode recorded at E_{OCP} . (C) Subtractively normalized and baseline corrected Raman spectra of TG dissolved in Milli-Q H₂O, calculated by determining the difference of the normal Raman spectra recorded of TG dissolved in Milli-Q H₂O and the background spectrum of Milli-Q H₂O.

Figure 7. Simulated Raman spectra of (A) 1-thio- α -D-glucose (α -TG), and (B) 1-thio- β -D-glucose (β -TG) coordinated to three gold atoms and plotted in comparison with the experimental average SERS spectrum of the TG modified Au/AuNPs electrode immersed in a 0.1 M NaF (argon purged) electrolyte between E_{OCP} and -0.60 V vs. SCE.

Table 2. Observed solution and adsorbed Raman bands of a 1-thio- β -D-glucose (TG) and corresponding band assignments.

Vibrational Mode Assignment	Observed Raman Bands (cm ⁻¹)		
	TG Solution	Average Spectrum of Au/AuNPs/TG/0.1M NaF	Ag/TG/0.05M Phosphate Buffer ⁷
$\nu(\text{CH}, \text{CH}_2)$	2940 - 2800	2876 (broad)	3000 - 2830 (broad)
$\nu(\text{C}=\text{O})$	-	not seen	1725
$\delta(\text{H}_2\text{O})_{\text{bulk}}$	1640 (broad)	1643 (broad)	1650 (broad)
$\delta(\text{H}_2\text{O})_{\text{interface}} / \nu_{\text{as}}(\text{COO}^-)$	not seen	1595 (broad)	1600 (broad)
$\omega(\text{CH}_2) / \delta(\text{CH}_2, \text{COH}, \text{CH})$	1457	1437	1440
$\delta(\text{COH}, \text{CH})$	1381, 1363	1370	1366
$\nu_{\text{s}}(\text{COO}^-)$	not seen	not seen	1295
* $\nu_{\text{as}}(\text{COC})_{\text{ring}} / \nu(\text{CO}) / \delta(\text{COH}, \text{CH})$	1238	1218	1212
	not seen	1183	1178
	1116	1139	1127
* $\nu(\text{CC})_{\text{ring}} / \nu(\text{COC})_{\text{ring}} / \delta(\text{COH})$	1071	1080	1080
	1051	1050	1065
	1019	1028	1035
	1000 (broad)	1002	1001
* $\nu(\text{CC}) / \nu_{\text{s}}(\text{COC})_{\text{ring}} / \rho(\text{CH}_2) /$	914, 879	917, 890	928, 890
* $\nu(\text{CS})_{\text{ring}}$	826	800	798
$\delta(\text{OH}) / \tau(\text{CCC})_{\text{ring}}$	687 (broad)	696 (broad)	700
	not seen	609	not seen
	578	574	583
	-	538	540
	503	505	510
$\nu(\text{Au-S}, \text{Ag-S}) / \delta(\text{CCC})_{\text{ring}}$	427	437	433
	412	414	411
	358	not seen	362
	-	295	-
	-	265	-
	-	-	247
$\delta(\text{CCC})_{\text{ring}}$	173	not seen	not seen

* Simulated molecular vibrations can be found in supplemental information

ν_{s} -symmetric stretching, ν_{as} -asymmetric stretching, δ -bending, ω -wagging, τ -twisting, ρ -rocking

Figure 8. (A) Baseline corrected SERS spectra recorded of the TG modified Au/AuNPs electrode in a 0.1 M NaF electrolyte as a function of increasing applied anodic potential (25% laser power, 10s exposure, 3 accumulations). (B) Synchronous and (C) asynchronous 2DCOS spectra generated as a function of immersion time; red – positive bands, blue – negative bands. Time-averaged spectra are included for reference. The 2DCOS analysis was performed on spectra recorded in the 0.00 to 0.50 V vs. SCE range.