

# Characterization of COOH-Molecules Self-Assembled on Gold Nanoparticles

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## Abstract

11-MUA solution of the concentration of 10mM was deposited on a gold surface through the self-assembled molecules. The gold surface was characterized by AFM-topography and XRD-spectrum. Both results show that the gold surface was composed of gold nanoparticles (AuNPs) piling up on each other and the most significant peak, centered at  $2\theta = 38.2^\circ$ , is originated from the face centered cubic gold, respectively. COOH-functional molecules were self-assembled onto AuNPs surface through the reaction between thiol molecules and AuNPs to form the Au-S binding. The IR spectrum of a COOH-terminated AuNP sample shows the existence of carbonyl (C=O) and hydroxyl (OH) molecules on AuNP surface. Furthermore, COHN-FITC@SiO<sub>2</sub> bonding to AuNPs, observed on a confocal microscope, also revealed the COOH-molecules self-assembled to AuNP-sample.

## 1. Introduction

Biosensors have become important and practical tools in the field of healthcare, chemical and biological analysis, environmental monitoring, food safety control, and homeland security. The performance of biosensors depends on their components, among which the matrix material, i.e., the layer between a recognition layer of biomolecule and a transducer, plays a crucial role in defining the stability, sensitivity and shelf life of a biosensor. However, most of research work has focused on the recognition layer that directly determines a target like antigens. The recognition layer consists of an antibody bound to a functional molecule that is attached to electronic circuits made by gold metals.

Up to now, there have been much research working on how to deposit functional molecules to gold surface through self-assembled molecules. Bifunctional ligands of the type R-(CH<sub>2</sub>)<sub>n</sub>-SH (R = COOH, OH, NH<sub>2</sub>) so that the surface is terminated with these functionalities. The ligands bind to the gold surface through their thiol (-SH) molecules [1-4].

In the present work, we present methods for preparing prototypical gold nanoparticle systems surface coated with bifunctional ligands of the type COOH-(CH<sub>2</sub>)<sub>n</sub>-SH. The gold surface is known to preferentially bind to the -SH group and hence the coated gold particles are expected to have COOH-termination [3-4]. The COOH functional molecules deposited on the gold-surface were confirmed by using an atomic force microscope (AFM) and FITC-dots covered SiO<sub>2</sub>-nanoparticles. Characterization results revealed the existence of COOH-terminated gold surface.

## 2. Experimental procedures

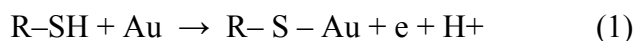
Gold films were sputtered on a silicon wafer using a hand-made sputtering machine equipped in a laboratory. The film deposition was described in papers somewhere. The deposited gold-film of less

than 18nm ~ 20 nm in thickness on a silicon substrate was determined by an optical profilometer (Wyko NT9100, Veeco Co.).

The interaction of sulfur and gold atom is strong enough to immobilize thiol-group containing molecules to the gold nanoparticles (AuNPs) to produce highly stable AuNPs published [ ]. The sputtered thin film was annealed in an oven at temperature of 500<sup>0</sup>C in air, for 30min, and then was gradually cooled in air to room temperature

Topography of gold-deposited film on a silicon-wafer sample was measured on a 5500 scanning probe microscope (SPM), Agilent Technologies Co. Imaging 3 x 3 μm and interaction data were an AuNP-deposited silicon substrate well was cleaned by its immersion in piraha solution for 30min at 80<sup>0</sup> C, subsequently, sonicated in acetone solution for 10 min and dried at 60<sup>0</sup>C. The topography and structure of deposited AuNPs was revealed using an atomic force microscope (AFM) operating in tapping mode (Acoustic AC mode) suitable for both gold-deposited surface and SAM-terminated AuNPs and a Bruker D8 Advance X-ray diffractometer (XRD).

All gold-deposited samples, ultrasonically cleaned in acetone solvent for 10 min, were dried in a vacuum oven at 60<sup>0</sup>C for 15min prior to their immersion in the 11-mercaptoundecanoic acid (11-MUA) solution of 10mM. This 10mM-solution was prepared by diluting the 11-MUA powder in absolute ethanol (EtOH) solvent. During the stay in the solution, thiol (–SH) molecules of the 11-MUA solution were self-assembled on AuNP-surface through the interaction of thiol (–SH) and Au, resulting in the formation of COOH-terminated AuNP-layer with an Au-S bond. Details of the chemical reaction during the time of self-assembled alkanethiols had been clearly described in the previous paper [3-4]. The following formation of the sulphur-gold (S-Au) bond:

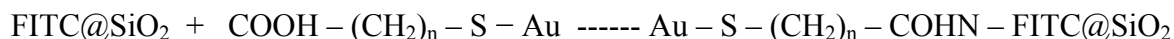
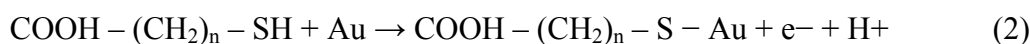


After that, the COOH-terminated sample (SAM sample) was thoroughly washed with ethanol to remove the residuals of 11-MUA solution on the sample's surface. And then, the sample was dried naturally, kept at room temperature for the characterization of COOH-terminated molecules deposited on the AuNPs.

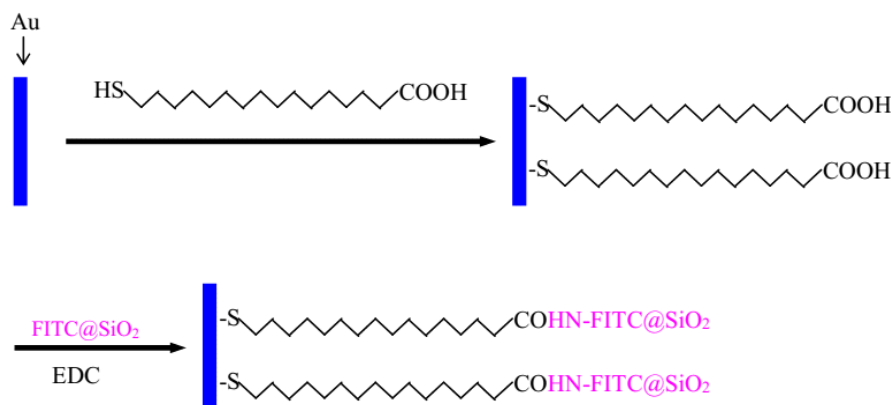
To prove the interaction of COOH-terminated alkanethiol molecules with AuNP surface, we employed a Fourier transform infrared (FTIR) spectrophotometer (GX-Perkin Elmer, USA) using the reflection mode at a resolution of 4 cm<sup>-1</sup> over the 4000–500 cm<sup>-1</sup> spectral region to reveal the Au–S bond and functional groups such as carbonyl (C=O) and hydroxyl (OH) groups belonging to COOH-terminated alkanethiol SAMs. The presence and disappearance of the thiol (S–H) bond, before and after dipping AuNP surface samples in 11-MUA 10mM solution, were characterized to confirm the formation of Au–S bond. Moreover, a COOH-terminated SAM on Au surface was revealed by a labeling technique. In this technique silica-nanoparticles composed of FITC dot covered by a silica shell. Silica shell's surface was functionalized and has a functional primary amine (NH<sub>2</sub>) at an end.

COOH-derived Au films and Au-films on silicon wafer were both immersed in solution of 1-etyl-3-carbodiimide hydrochloride (EDC) for 15 min to stimulate COOH functional group to be active. And then, these samples were kept for 24 hours in a stirred solution containing FITC-dot encapsulated silica-nanoparticles that have 60-80 nm in size and their concentration of 6.10<sup>12</sup> FITC-

dots per ml. Moreover, each. After that, these samples were taken out of the solution, and then thoroughly rinsed and dried in a vacuum oven for 15 min. at 60°C.

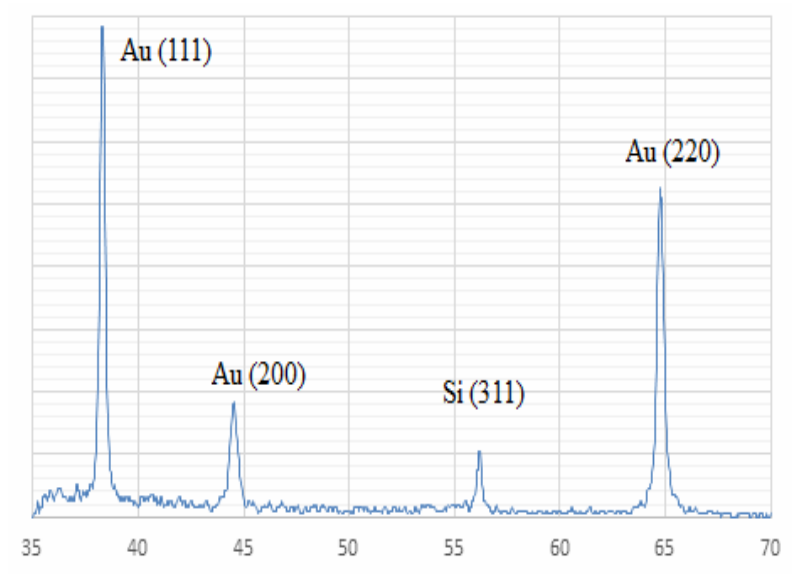


Finally, the gold surface was observed using a laser confocal scanning microscope (LCSM) at magnification scale of 10 with excited wavelength of 480 nm. Observing luminescence emission of FITC-dots emitted at a wavelength of 530 nm, we could determine whether NH<sub>2</sub>-terminated silica/FITC dots are bonded to Au films. Observation results on the confocal microscope revealed whether the functional COOH groups were bonded to gold/silicon samples.



*Chemical reaction of COOH functional group of 11-MUA with NH<sub>2</sub> of FITC@SiO<sub>2</sub>*

### 3. Results and Discussion



*Fig. 1. X-ray diffraction pattern of AuNPs deposited on a silicon substrate*

The crystalline structure of AuNPs deposited was determined by a X-ray diffraction (XRD) spectrum, measured at diffractometry angle ( $2\theta$ ), ranging from 35° to 70°, on a D8 advance x-ray diffractometer (Bruker, Germany). As we can see, the XRD pattern of AuNPs, shown in Fig.1, exhibits three distinct peaks, located at  $2\theta = 38.2^\circ$ ,  $44.6^\circ$  and  $64.8^\circ$  which could be assigned to standard Bragg reflections (111), (200) and (220) of face center cubic (fcc) lattice, respectively.

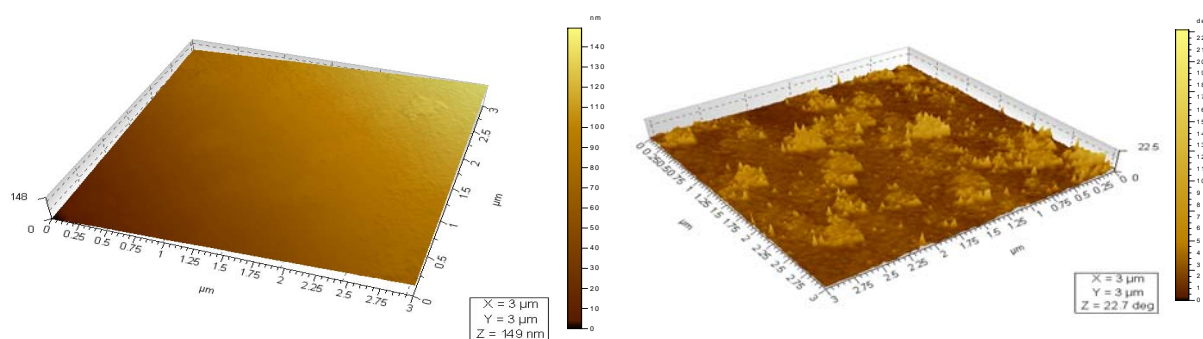
Moreover, the XRD pattern showing the most significant peak, centered at  $2\theta = 38.2^\circ$ , is originated from the face centered cubic gold. This XRD pattern is typical of pure Au nanocrystals. Besides these peaks mentioned-above, there is a the lowest-intensity peak, located at  $2\theta = 56.2^\circ$ , that is ascribed to Si (311). This reveals AuNPs were deposited on a silicon substrate.

Calculating the size of AuNPs, we used the Debye-Scherrer's equation expressed as follows:

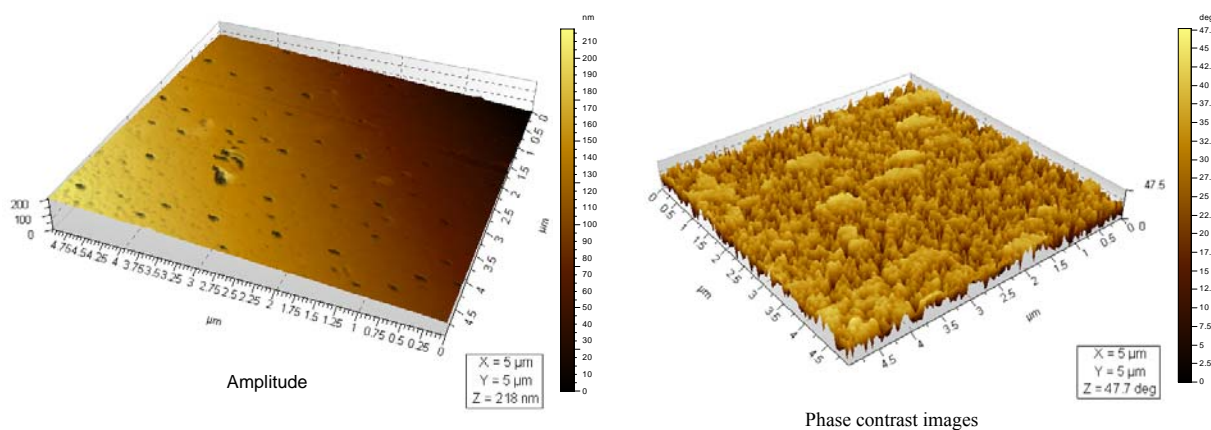
$$d = 0.9 \lambda / \beta \cdot \cos\theta_\beta \quad (3)$$

Where:  $d$  is particle size,  $\beta$  is denoted as full-width at half-maximum (FWHM) of the XRD peak,  $\lambda$  of  $1.54056\text{\AA}$  for Cu-K is denoted as the wave length of the X-ray source. The FWHM value of the peak oriented to (111) was estimated to be 0.0017 rad, and  $\theta$  is the Bragg angle. Therefore, the average size of AuNPs was approximately 86 nm.

Deposited surface of AuNPs was measured on an atomic force microscope (AFM) named Agilen (USA), model 5500 prior to



**Fig. 2. The AFM images of AuNP-layer deposited on a silicon substrate**

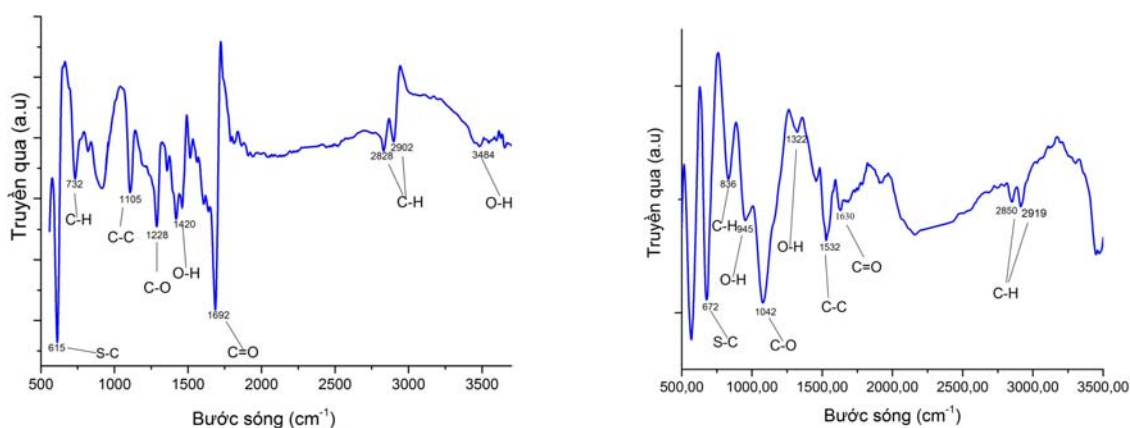


**Fig. 3. The AFM imaged of COOH-terminated AuNPs on a silicon substrate**

Images of the AuNP-surfaces before and after being coated with COOH-terminated SAM recorded either in amplitude or phase mode exhibited contrast variations (Fig. 2 and 3) that were highly effective for estimating structural inhomogeneity of materials. The interaction between the AFM tip and the different structures on the monolayer varies between attractive and repulsive depending on measurement parameters. The results of an AFM surface roughness analysis over two-scanning  $3\mu\text{m} \times 3\mu\text{m}$ -areas for Au thin films, and two-scanning  $5\mu\text{m} \times 5\mu\text{m}$  areas for the COOH-terminated AuNP-layer, respectively are shown in Fig.2 and 3, respectively. It can be seen that the surface topography of both samples are quite different. The latter looks more rough surface due to the

attachment of SAM-molecules to AuNPs surface. Also the maximum roughness of 218 nm for SAM-attached AuNP-surface is higher than that of 149 nm for the AuNP-layer. This can confirm that COOH-terminated molecules were binding AuNP-surface through bonding Au-S interaction [1-2]

### Determination of COOH-molecules in infrared spectra



**Fig. 4. Infrared spectra of 10 mM solution of 11-MUA (left) and COOH-terminated AuNPs**

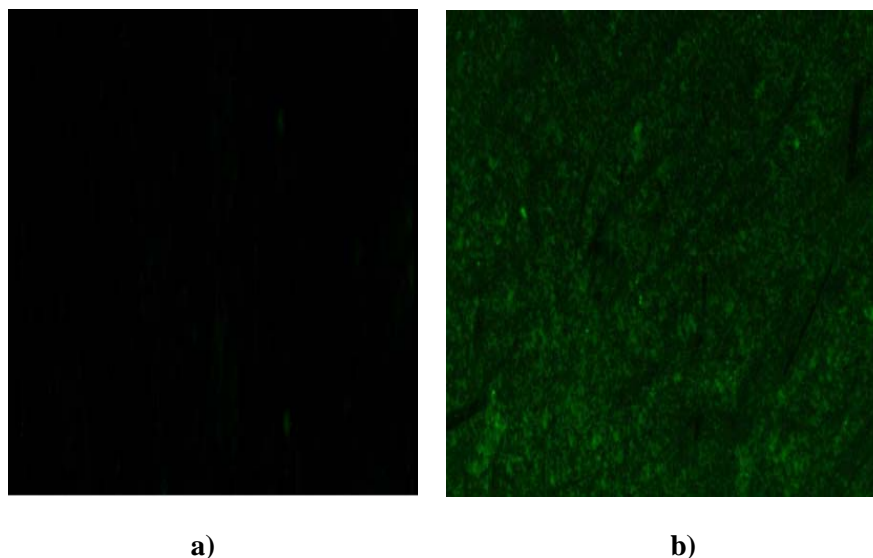
The IR-spectrum of COOH-terminated AuNPs-layer compared to that of 11-MUA solution at the 10mM-concentration shows that both carbonyl (C=O) and hydroxyl (OH) groups belonging to 11-MUA solution also appeared on the COO-terminated AuNPs sample. However, the shift of IR-peaks and the broaden of peak's FWHM reveals the interaction between AuNPs and the thiol (SH) terminated 11-MUA molecule, resulting in the formation of Au-S bonding in the range of  $2530\text{ cm}^{-1}$  -  $2600\text{ cm}^{-1}$  and the disappearance of SH stretching peak at  $2590\text{ cm}^{-1}$ . Further, the S-H bond is cleaved upon S-Au chemisorption, in agreement with the study done by Y. Xue *et. al.* [1]. This suggests that in all cases the bonding of the bifunctional ligands to the Au surface takes place through the S-H end that confirmed the COOH-terminated molecules already deposited on the AuNP-layer.

### Labeling COOH-terminated SAM on AuNP-surface by fluorescent molecules

Fluorescent images of the AuNP-deposited sample (a) and the COOH-terminated AuNP-sample (b) were observed and taken on a Confocon microscopy, Biophotonics Lab, IOP-VAS. We can see that the left image was black (non-fluorescent emission) while the right is a fluorescent image. This phenomenon can be explained by the chemical reaction of  $-\text{NH}_2$  in the  $\text{SiO}_2@\text{NH}_2\text{-FITC}$  solution with COOH-terminated AuNP-sample, resulting in the formation of the bonding COHN that kept a  $\text{SiO}_2@\text{FITC}$ -segment on the COOH-terminated sample. Though later, this sample was washed thoroughly in distilled water, the  $\text{SiO}_2@\text{FITC}$ -segment was still linked to AuNP-surface through the COHN segment. When we observed the  $\text{FITC}@\text{SiO}_2$ -terminated sample on the LCSM with the 480nm-excited laser beam, we can see the sample's emission of 530nm-fluorescence (Fig. 5(b)).

Quite the contrary, the leftside image shows the black color on its surface (Fig. 5a). This means that there is not any  $\text{SiO}_2@\text{NH}_2\text{-FITC}$  molecule attached to AuNPs due to a non-bonding

between the silica-FITC molecule and the AuNP-surface because, resulting in this solution washed out of AuNP-surface by EtOH solvent.



*Fig. 5. Fluorescent image of (a) the AuNP-surface and (b) COOH-terminated AuNPs*

#### **4. Conclusions.**

Carboxylic-acid molecules bound to AuNPs deposited on a silicon substrate were characterized by AFM images, XRD spectrum and SiO<sub>2</sub>@NH<sub>2</sub>-FITC labeling. The results obtained confirmed the existence of COOH-molecules on the AuNP-surface through stable chemical bonding Au-S. These COOH molecules would be used for binding biological molecules like antibodies, protein, cells to form probes of biosensors.

#### **References**

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