

*Dedicated to Professor Ionel Haiduc,  
President of The Romanian Academy at his 70<sup>th</sup> anniversary*

## LYSINE MEDIATED ASSEMBLY OF GOLD NANOPARTICLES

OSSI HOROVITZ<sup>1</sup>, AURORA MOCANU<sup>1</sup>, GHEORGHE TOMOAIA<sup>2</sup>, LIVIU BOBOS<sup>1</sup>, DIANA DUBERT<sup>1</sup>, IULIA DAIAN<sup>1</sup>, TRAIANOS YUSANIS<sup>3</sup> AND MARIA TOMOAIA-COTISEL<sup>1</sup>

**ABSTRACT.** This paper reports the data of an investigation of the gold nanoparticles assembly mediated by organic molecules, such as lysine. Gold nanoparticles were synthesized in citrate aqueous solution and characterized by UV-Vis spectroscopy and a strong absorption band at 528 nm is found for surface plasmon resonance. Transmission electron microscopy (TEM) images indicate a mean gold nanoparticle size of about 14 nm. The aqueous colloidal gold solution is stable in time, indicating a good electrostatic stabilization of gold nanoparticles through the adsorbed monolayer of citrate anions on nanoparticle surface. The interaction between the citrate capped gold nanoparticles in aqueous solution and lysine was investigated monitoring their UV-Vis spectra. At a certain lysine/gold ratio a new, large absorption band appears between 600 and 700 nm, which increases in time, while the color of the colloidal gold solution changes from red to blue. The 528 nm band decreases, as a consequence of the assembly formation of gold nanoparticles mediated by lysine. The nanostructure of the lysine mediated assembly of gold particles is analyzed by TEM and atomic force microscopy (AFM). The TEM and AFM images evidence the assembly of gold nanoparticles, where particles are not in direct physical contact. This effect could be explained primarily through the zwitterion-type electrostatic interactions between the charged amine and acid groups of the lysine molecules adsorbed and bound to different gold nanoparticles. These findings provide new insights into the precise control of interfacial interactions between organic molecules anchored on gold nanoparticles.

**Keywords:** *gold nanoparticles, lysine, self-assembly, UV-Vis spectra, TEM, AFM.*

### Introduction

One of the problems which has attracted the attention of both science and industry is the integration of nanoparticles and organic molecules, in order to develop new materials for electronics and optics and new applications in biomedical and bioanalytical areas, such as controlled drug delivery, medical diagnosis devices and biosensors [1-6].

Amino acids are a promising class of organic compounds to be used in biofunctionalization of gold nanoparticles, as protective layers and for their

<sup>1</sup> Babes-Bolyai University of Cluj-Napoca, Faculty for Chemistry and Chemical Engineering, 400028 Cluj-Napoca, Romania,

<sup>2</sup> Iuliu Hatieganu University of Medicine and Pharmacy, Department of Orthopedic Surgery, 400015 Cluj-Napoca, Romania,

<sup>3</sup> Aristotle University of Thessaloniki, 54006 Thessaloniki, Greece

assembly. Functional groups such as -SH and -NH<sub>2</sub> present a high affinity for gold, and since amino acids contain some of these groups, they are expected to stabilise gold nanoparticles. Their capacity of generating structural diversity was recognised [7], and gold surfaces capped with amino acids are considered to represent the simplest mimics for protein surfaces [8]. However, there are relatively few reports in the literature on surface modifications of gold nanoparticles with amino acid molecules [9–14], but no systematic study of amino acid interactions with gold nanoparticles is available.

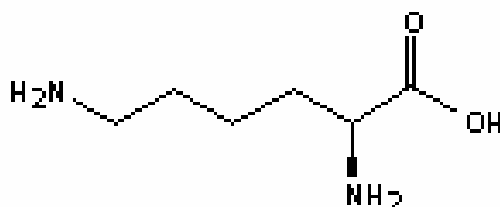
Amino acids can be used in the assembly formation of inorganic nanoparticles. If such molecules are adsorbed and bonded to the nanoparticle surface, amino acids belonging to two different nanoparticles can be connected through a condensation reaction with peptide bonding formation, thus leading to a peptide-linked assembly of nanoparticles. The properties of such assemblies could be designed rationally by choosing the initial amino acid.

Alternatively, amino acids can be adsorbed on the metallic particle surface already during the formation of nanoparticles, using the amino acid itself as reduction agent [14–18], or latter, by ligand exchange reactions or binding on the former adsorbed stabilising molecules. In this way protecting homogeneous or heterogeneous, mixed monolayers of metallic nanoparticles can be obtained.

In our previous work, we synthesized gold nanoparticles in aqueous solutions of citrate and used them to be functionalized with biomolecules, such as protein extracted from aleurone cells of barley [19]. How citrate anions, capping agents of gold nanoparticles, are involved in the interaction between gold nanoparticles and amino acids and how colloidal solution pH influences the nanoparticle assembly are questions to be answered.

The aim of this investigation is to gain insights into the assembly formation of gold nanoparticles and interparticle interactions in the presence of lysine, which could have potential application for the analytical detection of amino acids found in various media. The investigation of the effect of lysine concentrations on the surface plasmon resonance (SPR) band evolution of gold nanoparticles is examined to describe the lysine effect on electrostatic interactions and on interparticle binding properties. The results provide important information on the affinity of gold nanoparticles to lysine molecules and its optical applications.

Lysine (three-letter code: Lys or one-letter code: K) is an amino acid with a  $\epsilon$ -amine group on the side chain (Fig.1). It is a basic, positively charged, polar amino acid, with hydrophilic properties [20]. Its pK<sub>a</sub> values are: 2.20 (for the carboxylic group), 9.20 (for the  $\alpha$ -amine group) 10.1 (for the  $\epsilon$ -amine group); and the isoelectric pH is about 9.65. Therefore, lysine is a monocationic molecule in a large interval of pH from about 4 to almost 8.



**Fig.1.** Lysine formula

The understanding of the gold interparticle binding properties, in the presence of lysine, where both hydrogen bonding and electrostatic interactions could be operative in the assembly process of gold nanoparticles, is an important focus in this investigation.

### Experimental part

An aqueous colloidal gold solution was prepared by  $\text{HAuCl}_4$  reduction with sodium citrate, in variants of the Turkevich method, as adapted from [19, 21]. Deionized water with resistivity of  $18 \text{ M}\Omega\cdot\text{cm}$  was used in all experiments and it was obtained from an Elgastat water purification system. A 200 mL 0.005% (w/w)  $\text{HAuCl}_4\cdot 3\text{H}_2\text{O}$  solution stirred vigorously was refluxed. To the boiling solution 15.3 mg trisodium citrate ( $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7\cdot 2\text{H}_2\text{O}$ ), solved in a minimum amount of water, was added. After color change, the heat was turned off and the solution was allowed to cool overnight to room temperature. The gold content in the final colloidal solution ( $c_{\text{Au}}$ ) is 25 mg/L. The resulting solution of colloidal gold nanoparticles was stored in a brown bottle and kept at  $4^\circ\text{C}$ .

Solid L-lysine was purchased from Sigma and used without further purification. Lysine was dissolved in deionized water.

The UV/Vis absorption spectrum of all solutions was studied using a Jasco UV/Vis V-530 spectrophotometer with 10 mm path length quartz cuvettes in the 190 – 900 nm wavelengths range.

The molar concentrations ( $c_K$ ) of lysine solutions used in the experiments were the following: 0.01, 0.10 and 0.54.

The investigated mixtures were obtained from the gold colloidal solution ( $c_{\text{Au}}$ ) and the lysine solutions (concentration  $c_K$ ), by successive removal of small amounts of the previous mixture and the adding of equal amounts of amino acid solution. The gold and lysine contents in the resulting mixtures and their ratios, used in most of the determinations, are given in Table 1.

The gold nanoparticles suspension in the absence and in the presence of lysine was air-dried on the specimen grid and observed with a transmission electron microscope (TEM: JEOL – JEM 1010). TEM

specimens consist of carbon or collodion coated copper grids. TEM images have been recorded with a JEOL standard software.

**Table 1.**  
Gold / lysine ratios in the investigated mixtures. The concentration of the colloidal gold solution is  $c_{Au}$ ; the concentration of the lysine solution is  $c_K$ .

Gold content reported to $c_{Au}$	Lysine content reported to $c_K$	Content Au/K ratio reported to $(c_{Au}/c_K)$
0.833	0.167	5 / 1
0.695	0.306	2.2 / 1
0.579	0.421	1.4 / 1
0.483	0.518	1 / 1.1
0.402	0.598	1 / 1.5
0.335	0.665	1 / 2
0.322	0.679	1 / 2.1
0.214	0.786	1 / 3.7

Atomic force microscopy (AFM) investigations were executed on the gold nanostructured films without and with lysine using a commercial AFM JEOL 4210 equipment with a 10 x 10 (x-y)  $\mu\text{m}$  scanner operating in tapping (noted ac) mode on thin ordered gold adsorbed films on glass plates, that are optically polished and silanized with 3-aminopropyl-trietoxysilane or are prior treated to be positively charged. Standard cantilevers, non-contact conical shaped of silicon nitride coated with aluminum, were used. The tip was on a cantilever with a resonant frequency in the range of 200 - 300 kHz and with a spring constant of 17.5 N/m. AFM observations were repeated on different areas from 30 x 30  $\mu\text{m}^2$  to 250 x 250  $\text{nm}^2$  of the same gold film. The images were obtained from at least ten macroscopically separated areas on each sample.

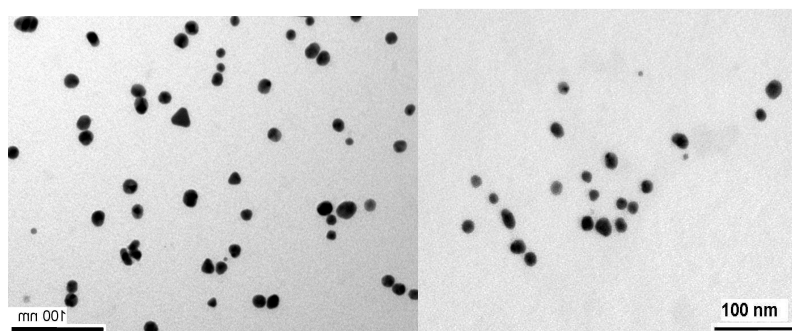
All images were processed using the standard procedures for AFM. The sizes of nanoparticles were measured directly from AFM 2D-topographic images and their 3D-views. The thickness (vertical distance) variations were estimated from vertical linear cross sections and height distributions on AFM images [22-26]. AFM images consist of multiple scans displaced laterally from each other in y direction with 512 x 512 pixels. Low pass filtering was performed to remove the statistical noise without to loose the features of the sample. All AFM experiments were carried out under ambient laboratory conditions (about 20 °C) as previously reported [23, 25].

## Results and discussion

### *Characterization of the colloidal gold solution.*

The size of the colloid gold particles has been measured by TEM imaging. Two representative TEM images of these gold particles are shown in Fig.2. The particles show mostly spherical or elliptical (ovaliform) shape,

just a few triangles, pentagons or hexagons are observed. From the sizes of a great number of particles, measured on the TEM images, an average size (diameter) of about 14.2 nm with a standard deviation of 2.6 nm were calculated as well as the extreme values of the sizes, from 8.5 to 24 nm.



**Fig.2.** TEM images of gold nanoparticles

From these, the approximate average mass of a nanoparticle (considered spherical) is estimated as  $2.9 \cdot 10^{-17}$  g, the number of gold atoms in a particle  $8.8 \cdot 10^4$  and the number of particles per  $\text{cm}^3$  of solution:  $8.6 \cdot 10^{11}$ .

The visible absorption spectra of the aqueous colloidal gold solution presents a well-defined absorption maxima at the wavelength  $\lambda_{\text{max}} = 528 - 529$  nm (Fig. 3). This value is characteristic for plasmon absorbance for nanometric gold particles. The wavelength was not significantly modified during a year after preparation.

Thus, the colloidal gold solution proved to be very stable in time, without observable modifications in the UV-Vis spectrum for a year after preparation. This indicates electrostatic stabilization via citrate anions adsorbed on the surface of gold nanoparticles and the gold nanoparticles are negatively charged.

#### *Interactions of the colloidal gold with lysine.*

The UV-Vis spectrum of lysine solutions presents no absorption bands in the range of wavelengths investigated here.

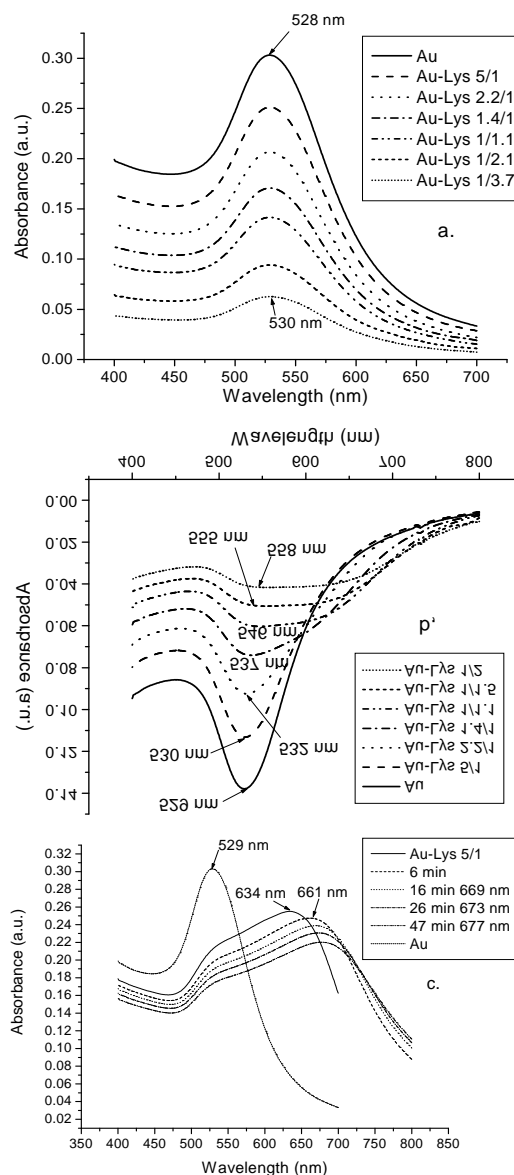
The adding of a diluted 0.01 M lysine solution (Fig. 3a) produces no or little modification in the UV-Vis spectrum of the colloidal gold solution. Besides the lowering of the absorbance due to the dilution of the gold solution, the absorption maxima are lightly shifted towards longer wavelength. This shift is due to the changes in the dielectric constant in the adsorption layer anchored on the gold nanoparticles surface. This effect, probably, is due to an increase

of the average refractive index of the environment surrounding the gold nanoparticles and to the size increase of particles by the adsorbed layer.

For a more concentrated lysine 0.1 M solution (Fig. 3b) the shift towards higher wavelengths is much more important. For higher quantities of lysine added to the colloidal gold solution the SPR band becomes substantially larger, suggesting an increasing assembly formation of gold nanoparticles.

Thus, these data (Fig. 3b) show the appearance of the broad peak at longer wavelengths in the UV-Vis spectrum. This fact results from the coupling of surface plasmon resonance of two adjacent nanoparticles. This finding is an indication of the anisotropic optical properties of the gold nanoparticles self-assembled or aggregated in agreement with other published data on related systems [27].

With a very concentrated (0.54 M) lysine solution, the band for the assembly formation of gold nanoparticles mediated by lysine appears at once (maximum at about 634 nm, Fig. 3c), while the 529 nm band for individual gold particles decreases and appears only as a shoulder in the spectrum. The maximum of the broad absorption band continues its shift towards higher wavelengths in time (Fig. 3c).

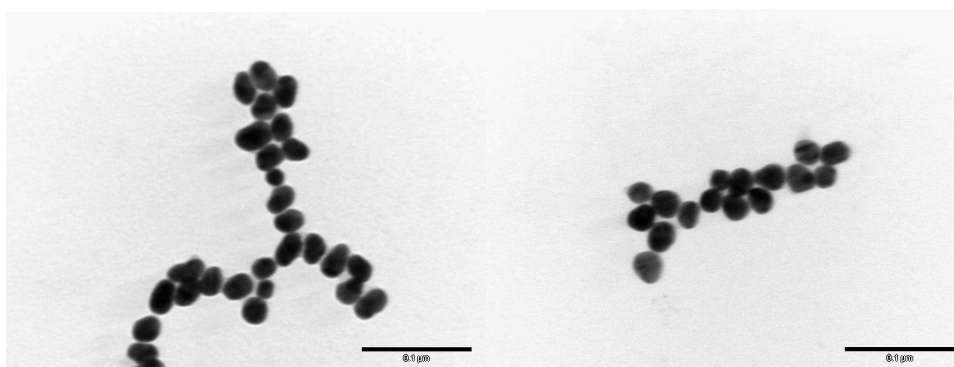


**Fig. 3.** Optical spectrum of colloidal gold solution with added 0.01 M (a) and 0.1 M (b) lysine solution at different ratios, and with 0.54 M lysine solution in time (c).

The color of the solution is changed from reddish to blue. This kind of color change is considered as an effect of the self-assembly or aggregation phenomenon [28, 29]. When the interparticle distance in the assemblies or aggregates decreases to less than about the average nanoparticle diameter, the electric dipole–dipole interaction and the coupling between the plasmons of neighboring particles result in the bathochromic shift of the absorption band.

Lysine adsorption on gold nanoparticles has been investigated [30], using 13 nm diameter gold particles and a  $7 \cdot 10^{-4}$  M L-lysine solution at pH about 10.7. The UV-Vis spectrum showed a SPR peak at 520 nm, similar to that found for citrate coated nanoparticles. This result is similar to our results, using the most diluted lysine solution. By adding a condensing agent, the authors [30] observed that gold particles were aggregated by the formation of peptide bonds between two lysine molecules adsorbed on different gold nanoparticles. In the UV-Vis spectrum the SPR band of gold nanoparticles was shifted to 527 nm and a new broad peak appeared at 633 nm. For this situation, the spectrum is similar to that obtained by us at high concentrations of the lysine solution, pleading for assembly formation of gold nanoparticles mediated by lysine.

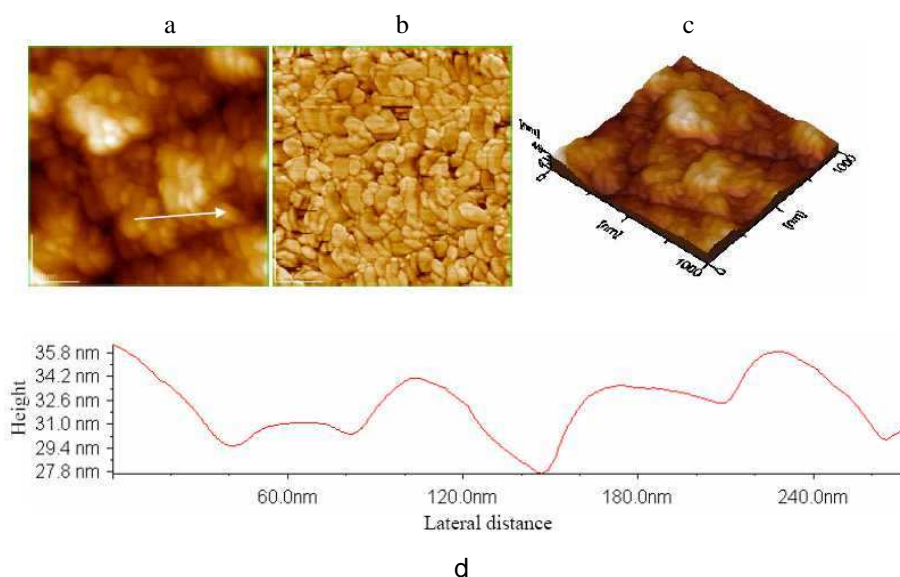
TEM images for gold nanoparticles with a 0.1 M lysine solution (Fig. 4) show both linearly ordered nanoparticles and more complex arrangements. Finally, these arrangements generate an almost ordered assembly of gold nanoparticles, mediated by lysine molecules, as also evidenced by AFM observations.



**Fig.4.** TEM images of gold nanoparticles capped with lysine (0.1 M), for the volume ratio 1:1 of aqueous solutions, at 30 sec after the mixing process.

The atomic force microscope allows simultaneous acquisition of multiple images, such as topography and phase images, under tapping mode operation. The assembly of gold nanoparticles, mediated by lysine,

was deposited on planar hydrophobic glass or on positively charged glass surfaces. Then, the assembly was observed by AFM under tapping mode and the structural features are visualized in Fig. 5. The two-dimensional topographic image (Fig. 5a) shows the morphology of an almost ordered



**Fig. 5.** 2D-topographic (a) and phase (b) AFM images of the assembly of gold nanoparticles, mediated by lysine, deposited on glass after one hour deposition time; scanned area 1000 x 1000 nm<sup>2</sup>; 3D-view (c) of the topography (a); (d) cross section profile along the arrow in panel a; RMS of image panel (a) is 2.6 nm.

structure within domains, with small defects at domain boundaries. For this assembly of gold nanoparticles mediated by lysine, immobilized on planar glass surface, individual gold nanoparticles are visible from 2D-topography (Fig. 5a) and 3D-view (Fig. 5c). The image phase (Fig. 5b) show also that the assembly of gold nanoparticles mediated by lysine is almost compact.

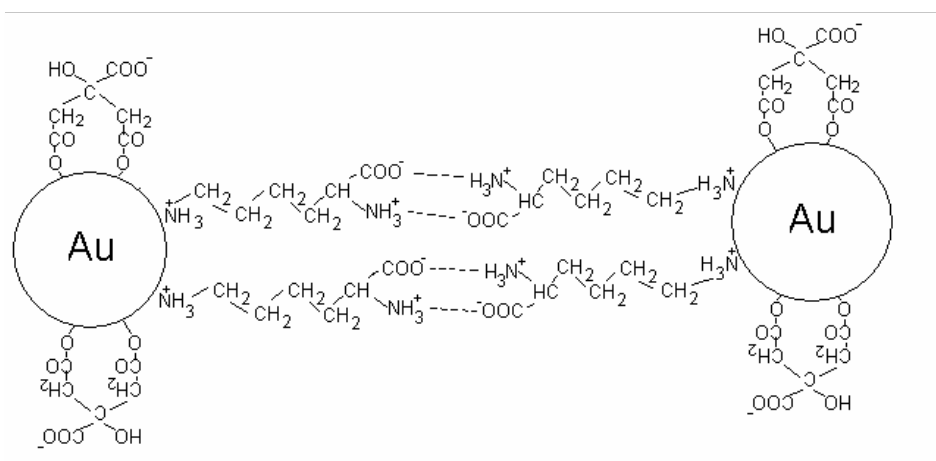
The AFM images were acquired with high resolution as in the case of imaging assemblies of lipid molecules [22-26], assemblies of protein molecules [31, 32] or assemblies of gold nanoparticles mediated by different organic molecules [19], despite the structural complexity of molecules or of gold nanoparticles arrangements.

The AFM images evidence both the selectivity of lysine adsorption and its orientation on gold surface and the self-assembly process of gold nanoparticles within a compact network, as observed by UV-Vis spectroscopy (Fig. 3). Also, the gold nanoparticles appear almost ordered both in AFM images and in cross section profile (Fig. 5d) in substantial agreement with TEM images.



For lysine the binding with the gold nanoparticles surface may be achieved through the  $\epsilon$ -amine group. The binding could occur through the electrostatic interaction of the lysine protonated  $\epsilon$ -amine group ( $-\text{NH}_3^+$ ) with the negatively charged citrate anions adsorbed on gold surface. On the other hand, a direct bonding of the amine group to the gold surface can not be excluded (Fig. 6).

All the charged amino acids present such strong interactions with gold surface, so are the amino acids that are usually *positively charged* (i.e. protonated), such as lysine (K), arginine (R) and histidine (H). A possible amino acid binding to gold nanoparticles and the formation of particle aggregates are schematized for lysine in Fig.6.



**Fig. 6.** Schema of lysine binding to citrate capped gold nanoparticles and bonds formation between gold nanoparticles.

### Conclusions

Our investigations indicate that the assembly of gold nanoparticles can be induced by lysine, an amino acid possessing an additional functional group, i.e.  $\epsilon$ -amine, besides the  $\alpha$ -amine group. The correlation between physical and chemical properties of amino acids, such as hydrophobicity and acidity or basicity in various protolytic equilibria, and their molecular structure is in substantial agreement with their assembly effect on the gold nanoparticles. This effect could be explained primarily through the zwitterion-type electrostatic interactions between the charged amine and acid groups of lysine adsorbed and bound to different gold nanoparticles.

The affinity of gold nanoparticles towards lysine can lead to the development of new detection methods for analytical purposes, medical diagnostics and biosensors and to potential controlled drug delivery applications. On the other hand, the use of amino acids both in the

functionalization of gold nanoparticles and in the cross-linking of amino acid capped gold nanoparticles leading to stable self-assemblies are promising ways to the synthesis of nanostructured biomaterials. The stabilization of gold nanoparticles through amino acids is also important for the understanding of complex phenomena involved in the formation of new biomaterials by binding of proteins with gold nanoparticles with important implications in nanoscience and nanotechnology.

In future studies, the nanoengineering approach will be extended to explore the possible improvement in nanofabrication methods to reach amino acids and protein immobilization at molecular precision to control both the lateral and perpendicular orientations of functionalized gold nanoparticles. Definitely, the high-resolution engineering and imaging studies reveal the nanoscale and molecular level details for supramolecular chemistry and self-assembly processes, e.g., the influence of size, orientation and the local orders, with applications in bioscience [32]. Thus, the assemblies can be designed to contain the desired charged or chemical functionalities that will affect the intermolecular interactions such as van der Waals forces, hydrogen bonding, polar attractions and hydrophobic interactions.

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