

Gold nanoparticles plasmonic enhancement for decoding of molecule-surface interactions

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Amanece y la luz se expande...

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Abbreviations

AgNP:	Silver nanoparticles (spheres)
AOT:	Dioctyl sulfosuccinate sodium salt
APTES:	3-aminopropyltriethoxysilane
AuNP:	Gold nanoparticles (spheres)
AuNR:	Gold nanorods (rods)
AuNS:	Gold nanostructures (general, involving any shape)
AFM:	Atomic Force Microscope
CB:	Cucurbit[7]uril
CHCl ₃ :	Chloroform
CTAB:	Cetyl-Trimethyl-Ammonium Bromide
CuNP:	Copper nanoparticles
DDAB:	Didodecyldimethylammonium bromide
DLS:	Dynamic Light Scattering
EDS:	Energy-Dispersive X-rays Spectroscopy
EtOH:	Ethanol
FLIM:	Fluorescence Lifetime Imaging Microscopy
G:	Enhancement Factor; SERS enhancement
I2959:	Irgacure® 2959
IR:	Infrared
LOD:	Limit of Detection
LOI:	Limit of Identification

LSPR:	Localized Surface Plasmon Resonance
MB:	Methylene Blue
MeOH:	Methanol
MPA:	3-Mercaptopropionic acid
MPS:	Sodium 3-mercapto-1-propanesulfonate
NP:	Nanoparticles (general, involving any shape)
PEF:	Plasmon-Enhanced Fluorescence
RM:	Reverse Micelles
SDS:	Sodium dodecyl sulfate
SEM:	Scanning Electron Microscopy
SERS:	Surface Enhanced Raman Scattering/Spectroscopy
S-naph:	2-Napthalene-thiol
S-ph:	Thiophenol
TEM:	Transmission Electron Microscopy
TERS:	Tip-Enhanced Raman Scattering/Spectroscopy
THF:	Tetrahydrofuran
UV-Vis:	Ultraviolet-Visible Spectroscopy

Abstract

In this research, the use of gold nanostructures (AuNS) was explored to evaluate the interaction between molecules and the nanoparticle (NP) surface. In that way, three different projects were developed; one project using fluorescence and two projects using Raman spectroscopy as measuring technique.

The fluorescence spectroscopy project used the fluorescence lifetime imaging microscope (FLIM) to evaluate the relative position of the molecules methylene blue (MB) and cucurbit[7]uril (CB) on the gold nanoparticle (AuNP) surface. Although the inclusion complex is favored in solution, it was found that MB forms an exclusion complex with CB, when CB is attached to the AuNP surface.

The first project utilizing Raman spectroscopy, specifically surface enhanced Raman scattering (SERS), took advantage of a confined system (a reverse micelle) to evaluate the Raman signal of water molecules in close proximity to the AuNP surface. It was observed that the SERS water signal had a big shift to higher energies compared with the Raman signal of the bulk water; indicating the water molecules in the system are subjected to different bond-stretching energies.

The second Raman project studied the modification of two different AuNS (specifically AuNP and gold nanorod -AuNR) with thiols. Different thiols were used to evaluate the kinetics of the modification of the AuNS surface, also the different AuNS presented different ligands on their surface. In general, and considering the difference in the bonding strength of the ligands present on the AuNS surface (by synthesis) and the size of the thiol, at least 2 h are required to modify the complete AuNS surface.

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1. Introduction to plasmonics. Plasmon-enhanced fluorescence and Raman scattering enhancement

1.1. Nanoparticles and localized surface plasmon band

Nanoparticles (NP) are defined as 3D structures with two or three dimensions greater than 1 nm and smaller than 100 nm.¹ Typically, the most studied NP are spherical but almost any shape can be synthesized, including cubes,² rods,³ bipyramids⁴ and flowers;⁵ only changes in the synthesis conditions are required to obtain different shapes. Many efforts have been focused on how to get the ideal conditions in order to acquire the desired shape and size.⁶⁻⁸ It is important to notice that NP can be made of many materials; however, this work focuses on the use of gold nanoparticles (AuNP) in order to take advantage of its plasmonic properties.

In the group of plasmonic materials the metallic nanoparticles of the three first elements in the 11 group, specifically Cu, Ag and Au are normally explored.⁹ The plasmonic material group also includes other metallic NP like Al and Pt¹⁰ or semiconductors.^{11,12} From these materials, Ag was observed to have the best plasmonic properties^{10,13} since its properties in the visible range provide the best performance,¹⁰ just like a silver mirror is better than a gold or a copper mirror.

The use of copper in plasmonics is limited due to the low stability of the copper nanoparticles (CuNP); while Au has been used the most since its high stability and that at longer wavelength ($\lambda > 600$ nm) its enhancement capabilities are comparable with Ag.^{10,13} These are the reasons of focusing on using gold in this research.

The plasmonic properties of noble metals (Ag and Au) have been known since antiquity, when colloids of these metals were used to color glasses.¹⁴ The color presented by the colloidal metal nanoparticles is due to the oscillation of delocalized electrons on the surface of the NP, induced by an external electromagnetic field (light). The oscillation of the electrons produces the

polarization of the NP surface (Figure 1.1); the nuclei then attract the electron cloud restoring the original state. This collective oscillation of electrons is known as localized surface plasmon resonance (LSPR) and it is responsible for the optical properties of the nanoparticles. The location of the LSPR depends on the metal (Au, Ag, Cu), the dielectric constant of the medium, shape and size of the NP.^{14,15}

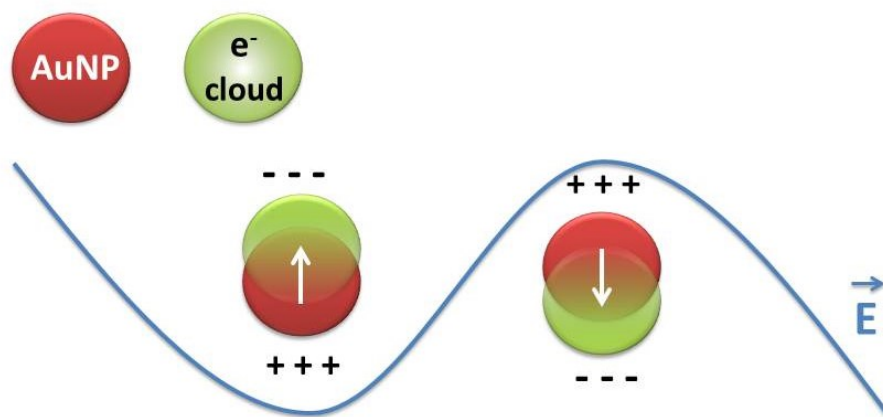


Figure 1.1. Localized surface plasmon resonance (LSPR) theory used to explain the optical properties in nanoparticles.

One of the main consequences of the presence of the LSPR is the generation of an induced electromagnetic field (IEF) on the NP surface. Thus molecules in close proximity to the NP surface can interact with this IEF and the effects of such interactions are discussed in the following sections.

1.2. Raman spectroscopy and surface enhanced Raman scattering (SERS)

The discovery of Raman scattering is well documented and was observed by Krishna and Raman in 1928 in India, by looking into the sun light with a simple telescope.¹⁶ The Raman spectrum normally has three parts that are represented in the Jablonski diagram in Figure 1.2. The

Rayleigh line located at zero Raman shift (0 cm^{-1}) is at the same wavelength as the excitation source and corresponds to the elastic scattering of light. Raman is defined as inelastic scattering, where the photon gives part of its energy to the molecule (Stokes) or the molecule to the photon (Anti-Stokes). In this way, the Stokes lines have a positive Raman shift, while the Anti-Stokes lines have a negative Raman shift (Figure 1.2). It is important to note that in general, when someone talks about Raman they usually present the Stokes part of the Raman spectrum, which is more probable to occur than the Anti-Stokes and, in that way, it is more intense. In this work any Raman spectrum refers to the Stokes lines.

Physically, for the molecule to undergo Raman scattering it needs to absorb light from the electronic ground state into a virtual state, and then scatter (inelastic) into a higher vibrational state in the electronic ground state (Stokes lines, Figure 1.2). The Raman shift is an indication of energy in the vibrational state, and has molecular information similar to infrared spectroscopy (IR). This explains why Raman spectroscopy is classified as a vibrational spectroscopy and it is complementary to the information that can be obtained by IR.

The probability of observing Stokes lines versus Anti-Stokes lines was discussed although the probability of the Raman phenomenon itself also needs to be explored. When compared with UV-Vis absorption, a typical sample for an absorption experiment (1 mM , $\epsilon = 1000\text{ M}^{-1}\text{cm}^{-1}$ and 1 cm optical path) absorbs 90% of the incident photons, whereas only 1 in 10^{10} photons will undergo Raman scattering. Raman scattering is about 10^{-10} less intense than the corresponding IR signals. Additionally, fluorescence is competitive with Raman phenomena (especially important when using excitation $\lambda < 650\text{ nm}$) since it is more probable and can easily overlap any Raman signal.¹⁶

Lasers are the preferred light source in Raman spectroscopy, since similar to fluorescence, and contrary to IR, increasing the intensity of the excitation light increases the intensity of the Raman signal because the signal intensity is proportional to the source intensity. Thus, the introduction

of lasers as excitation source in the Raman instrument was a key step in the development process.^{9,16}

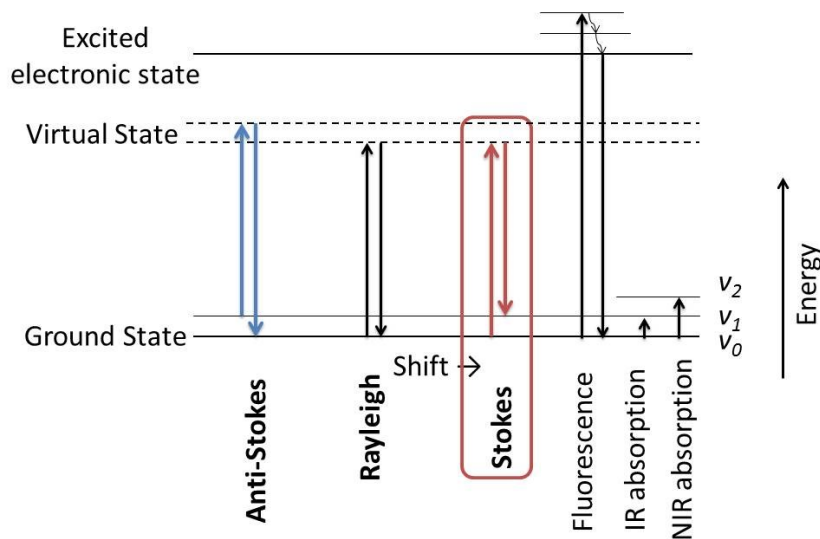


Figure 1.2. A simplified Jablonski diagram showing that molecules can have different transitions in response to incident light. Raman scattering is referred to as the Anti-Stokes and Stokes lines, while Rayleigh is a natural process observed during Raman scattering. Mid infrared (IR) and near infrared (NIR) absorption are other vibrational spectroscopies while fluorescence is a competitive phenomenon.

The limit of detection (LOD) and limit of identification (LOI) of an analyte in Raman are very important factors that must be determined, and they depend on the analyte itself, the laser power, other instruments settings and the background signal.¹⁷ Knowing that a very intense source can damage the sample, or facilitate more fluorescence, typical LOD and LOI in Raman are very poor, and require several micrometers or high concentration of material to achieve good Raman signals.¹⁸ To increase the Raman sensitivity, surface enhanced Raman scattering (SERS) is frequently employed. This is a sample preparation technique that involves the use of the optical properties of a plasmonic material, frequently a NP (LSPR and IEF) to enhance the Raman signal of molecules in the NP surface, and normally allows enhancement factors (G) below 10^{10} with the right combination of NP size,¹⁹ source²⁰ and analyte.²¹ The appropriate calculations of G will be developed in section 1.4.

Surface enhanced Raman scattering (SERS) depends on the interaction of the molecule with the LSPR of the NP, so distance is a key factor.^{9,10,22,23} In order to achieve a better enhancement, the molecule must be located as a monolayer at the NP surface, which has the advantage of reducing competitive fluorescence by surface quenching by the NP.^{10,13} The creation of a monolayer of the analyte on the NP surface is also important because it has been reported that in some cases the formation of multilayers decrease the SERS intensity.^{18,24} This is the reason why the majority of researches focus on monolayers and it is an important factor in tip-enhanced Raman spectroscopy (TERS).

Once the molecule is on the NP surface, it can be physically or chemically adsorbed. These two possibilities have different results, since factors higher than 10^{11} are reported for chemical enhancements.²⁵ Additionally, chemical absorption has been associated with more significant differences between the Raman signals of the molecule in the bulk and those found when the molecule is chemisorbed onto the NP surface. Therefore, Raman peaks shift when the molecule is on the NP surface, and the bigger shifts correspond to tighter interaction between the NP surface and the molecule.²⁶

Higher enhancements can be achieved with the creation of IEF hot-spots: thus, intense IEF can be found in between two nanoparticles and therefore molecules located there will be subjected to higher enhancement –this has been reported as single molecules analysis using SERS.^{27,28} The creation of the hot-spots can be achieved by different synthetic methods²⁹ or using molecules as linkers between two NP.³⁰ The enhancement factor can also be improved by the use of asymmetric NP (like flowers or triangles) that provide sharp edges with more intense IEF^{5,9,10} which produce higher enhancements.

Another factor that it is important to consider is the NP size and the location of the LSPR band, since the location of the maximum relative to the laser wavelength has been reported as relevant in SERS.²⁰ It has been reported that different NP sizes have different G values,¹⁹ having optimal diameter sizes around 50 nm. In the case of the laser wavelength location, it should be in the tail

(front or back) of the NP absorption band and never at the maximum; since at the maximum the absorption of light by the NP diminishes the molecule Raman scattering.²⁰

Finally, it is also important to note the orientation of the molecule on the NP surface.³¹ Consider that a molecule with an average length of 1 nm is much smaller than the NP, and the NP surface can be approximated as a flat surface. As such, the metallic surface is like a mirror in which the molecule and its dipole can be reflected. As can be observed in Figure 1.3, when the molecule is parallel to the surface, its dipole and the reflection gets cancelled; while when the molecule is perpendicular to the surface, the mirror is additive and a signal can be obtained.⁹

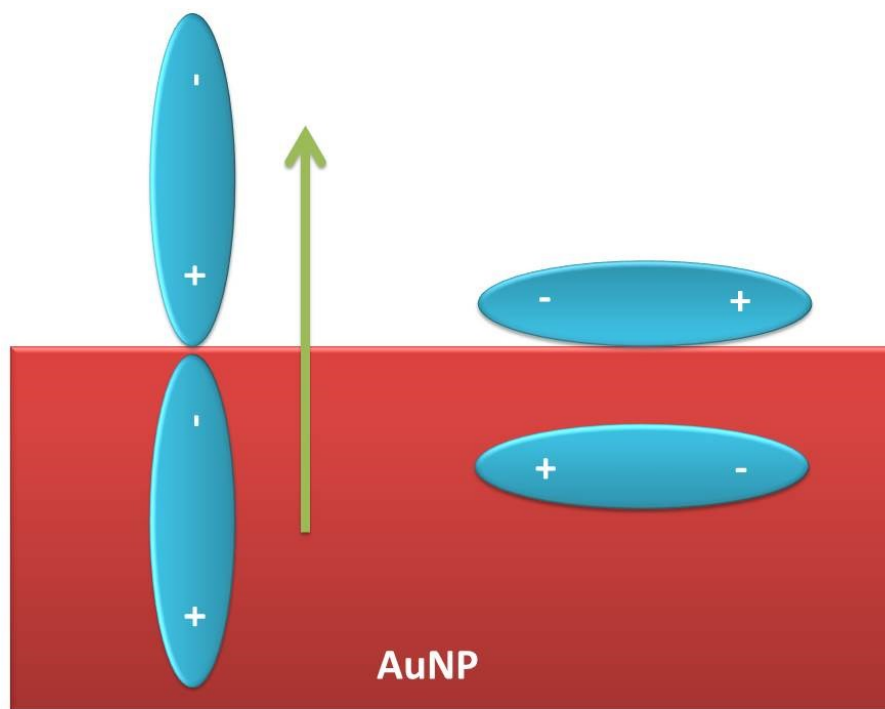


Figure 1.3. Picture of dipoles and their reflection on a metallic nanoparticle surface. The dipoles parallel to the surface cancel by its reflecting image, while the perpendicular position and its mirror are additives and a signal can be achieved. Adapted from reference [9].

This anisotropy effect has been used to determine the orientation of the molecules on the NP surface and it is important to consider for any spectroscopy technique using metallic structures; thus it will be discussed again in section 3.2.4.

1.3. Fluorescence quenching and enhancement

As previously mentioned, fluorescence and Raman are competitive phenomena. In the case of fluorescence, the molecule absorbs energy reaching an excited electronic state (Figure 1.2). From there, it relaxes by non-radiative paths to the ground vibrational state of the first excited electronic state (Kasha's rule³²) and finally, the molecule can release the energy through non-radiative (internal conversion) or radiative processes (fluorescence) into the ground electronic state. This process has limited vibrational information and, thus, fluorescence is not classified as a vibrational spectroscopy. Very broad bands are frequently observed in fluorescence spectroscopy, typical of its nature as an electronic spectroscopy and with typical lifetime of about 10 ns³²—that vary from molecule to molecule.

All the projects presented in this work involve the interaction between the molecule and the NP surface. In fluorescence, just like in Raman, the distance between NP and the analyte is very important; since it is observed that when varying the molecule-NP distance there is a transition between fluorescence quenching to enhancement.^{33–35} Therefore, having the molecule in direct contact with the NP surface increases the Raman signals while quenching the fluorescence, but increasing the distance molecule-NP fluorescence enhancement is observed (Figure 1.4).

The process of fluorescence quenching is well documented.³² Generally, it requires an interaction between the fluorophore and the quencher, so there are two types of quenching process: dynamic and static. In the case of fluorophore-NP (or molecule-NP) there are two theories that can be applied; the molecule associate with the NP surface creating a “new molecule” that does not fluoresce (just like a static quenching process)³² or that the molecule transfers the energy to the NP with a “quenching” net effect.³⁵ When the molecule is not in direct contact with the NP surface, then the energy transfer is not facilitated and the quenching effect diminishes.

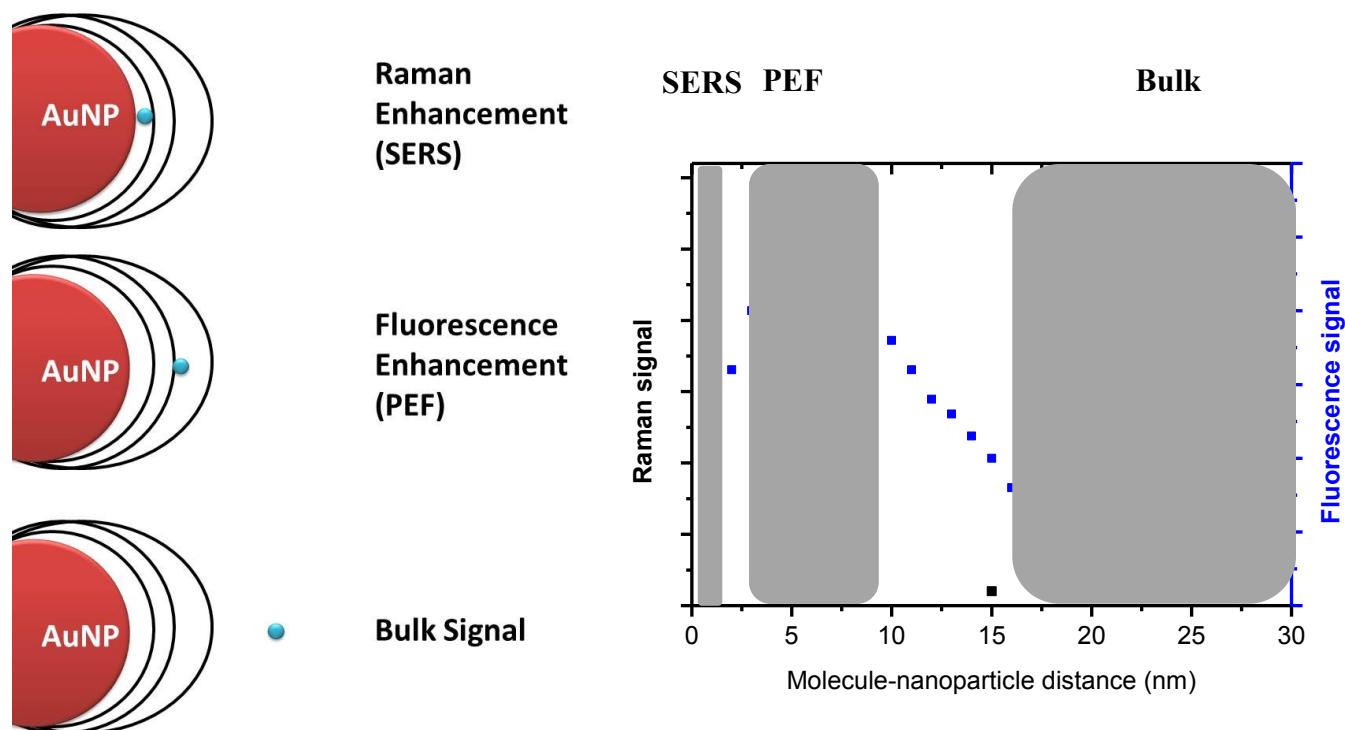


Figure 1.4. Representation of the spectroscopy signal variation with the molecule-NP distance. When the molecules are at the NP surface they undergo surface enhanced Raman scattering (SERS); at distance of about 5 nm they undergo plasmon-enhanced fluorescence (PEF); while at longer distances, the spectroscopy signal of the bulk material is observed. Plot adapted from references [23 and 33].

Increasing the molecule-NP distance so that fluoresce enhancement can be observed, reaches a maximum after which there is not more enhancement (Figure 1.4). Anger *et al*³³ completed a very extensive research for this process. They found that for a AuNP of 80 nm in diameter, the maximum enhancement is observed at 5 nm distance between molecule-NP. The enhancement also decays very quickly after reaching the maximum point and cannot be observed past 20 nm. This last observation is in agreement with the decrease of the LSPR of the NP indicating that the molecule needs to have an interaction with the LSPR in order to produce plasmon-enhanced fluorescence (PEF). The creation of hot-spots also improves PEF facilitating G in the order of 200, while typical values are lower than 100.^{34,35}

1.4. Enhancement factor (G)

It is important to know the enhancement factor (G) of a system. In this case, the G values for the SERS enhancement will be calculated and not for PEF, since the fluorescence measurements were completed with a fluorescence lifetime imaging microscope (FLIM) that does not have concentration information.⁹

The calculation of G was completed by adapting the equation from Lui *et al.*³⁶ As can be observed in Equation 1, the G corresponds to the ratio of the instrumental signal (in this case the Area) of the analyte on the NP surface versus the analyte signal in the bulk material, multiplied by the ratio of both concentrations.

Equation 1. Calculation of the enhancement factor (G) for Raman or fluorescence spectroscopy

$$G = \frac{Area_{AuNP}[Analyte]_{Bulk}}{Area_{Bulk}[Analyte]_{AuNP}}$$

It is necessary to measure the signal (area or intensity) of two different solutions of the analyte, one using the NP and the other corresponding to the bulk material –normally in higher concentration. The final values for the G factor are presented in the different projects (section 4.2.5 and 5.2.7), while Table 1.1 present the typical values observed for G.^{9,10}

Table 1.1. Typical values for the enhancement factor (G) in surface enhanced Raman scattering (SERS) and plasmon-enhanced fluorescence (PEF).

Typical G values	
SERS	$10^7 - 10^{10}$
PEF	< 100

2. Materials and Methods

2.1. Materials

All chemicals were purchased and used as received. Unless otherwise specified, the chemicals were purchased from Sigma-Aldrich. Hexadecyltrimethylammonium bromide (CTAB) 99%; gold as $\text{HAuCl}_4 \times 3\text{H}_2\text{O}$ 99%; sodium citrate tribasic 99%; sodium borohydride (NaBH_4) 99%; l-ascorbic acid 99+%; didodecyldimethylammonium bromide (DDAB) 98%; methylene blue (MB) hydrate 97%, cucurbit[7]uril (CB) hydrate 99%; thiophenol (S-ph) 99%; sodium dodecyl sulfate (SDS) 99% was purchased from Fluka; silver nitrate (AgNO_3) 99.9% was purchased from Alfa Aesar. Irgacure® (I2959) 98% was a gift from BASF.

Hydrogen peroxide (H_2O_2) at 50% and HPLC grade solvents like: CHCl_3 , MeOH, EtOH, THF, acetone, toluene, hexane, heptane, iso-octane (2,2,4-trimethylpentane), dodecane, were purchased from Fisher Scientific. Ultrapure water from a MQ system (18 M Ω).

2.2. Concentration calculation and synthesis of gold nanostructures (AuNS)

2.2.1. Calculation of the AuNS concentration

The concentration of all AuNS presented in this work was calculated by estimating that the volume of one AuNS is completed by a defined number of gold atoms. To complete calculations, the estimations include the approximation that all atoms of Au^{3+} are reduced into Au^0 , and that the particles are monodisperse and perfect spheres (or rods, section 2.2.5). With equations adapted from R. Johnston;³⁷ the detail calculations are as follows:

The volume of a NP is expressed as the number of atoms that it contains times the volume taken by each atom (0.024 nm^3 for metallic Ag or Au), thus the number of atoms per NP is a ratio of volumes (Equation 2), where the volume of a NP is calculated from its radius according to the shape (e.g. sphere).

Equation 2. Calculation of the number of atoms presented in a NP, using the atom volume (0.024 nm^3 for metallic Ag or Au) and the NP volume (V_{NP}) calculated from its diameter (in nm).

$$n_{atoms \text{ NP}^{-1}} = \frac{V_{NP}}{v_{Au}} = \frac{V_{NP}}{0.024 \text{ nm}^3} \quad \text{with } V_{NP} \text{ in } \text{nm}^3$$

According to the original concentration of metal used during the NP preparation (Equation 3) the total number of atoms in solution is the multiplication of the concentration by Avogadro's number (AN). With this calculation, the resulting value of atoms is per liter.

Equation 3. Calculation of total quantity of atoms per liter present in the solution as initially prepared.

$$atoms_{sol \text{ L}^{-1}} = \frac{mol_{atoms}}{V_{sol}} AN_{atoms \text{ mol}^{-1}} \quad AN: \text{Avogadro's number}$$

Therefore, the total number of NP (N_{NP}) present in the solution is the ratio of the atoms of the metal in solution ($atoms_{sol}$) divided by the number of atoms per NP (Equation 4). Since the calculating from Equation 3 is the number of atoms per liter, the value of number of NP is per liter too.

Equation 4. Calculation of number of NP (N_{NP}) per liter formed during the reduction process, estimating that all atoms were reduced into metallic form.

$$N_{NP \text{ sol L}^{-1}} = \frac{atoms_{sol \text{ L}^{-1}}}{n_{atoms \text{ NP}^{-1}}}$$

Therefore the molar concentration of NP in solution is the number of NP in solution ($N_{NP\ sol}$) per liter divided by Avogadro's number (AN) (Equation 5).

Equation 5. Concentration of NP in solution, as mol of NP in solution.

$$C_{NP}(M) = \frac{N_{NP\ sol\ L^{-1}}}{AN_{NP\ mol^{-1}}}$$

Finally, all calculations can be simplified into one equation (Equation 6).

Equation 6. Final equation for the calculation of NP concentration in solution as mol of NP per liter.

$$C_{NP}(M) = \frac{mol_{atoms}}{V_{sol}} \times \frac{0.024\ nm^3}{V_{NP}} \quad \text{with } V_{NP} \text{ in } nm^3$$

All NP concentrations presented in this work were calculated with the method shown, just considering the different NP volume presented by the shape (rods vs. spheres). As it can be observed in Equation 6, the concentration of NP in the final solution is proportional to the original concentration of metal used for the preparation and inverse to the NP volume.

2.2.2. *Unprotected AuNP (I2959 method)*

Unprotected gold nanoparticles (AuNP) were prepared by a photoreduction method, using Irgacure® 2959 (I2959) from BASF.³⁸ This method provides very uniform AuNP with an approximated diameter of 15 nm and calculated concentration of 2.5 nM.³⁹ The detailed preparation is as follows:

Mix 10 mL of Au^{3+} (0.33 mM) solution with 10 mL of I2959 (1.0 mM) in a glass flask. Place the mixture into the photoreactor without disturbing for 15 min with black lamps (UVA). After the reaction is completed the solution should be an intense pink/red solution, indication of AuNP presence.

It is important to note that during this work, these AuNP were prepared only on a glass cover slip surface (section 3.2.1), which is why only the AFM was obtained (Figure 3.7) and no UV-Vis spectra or TEM images.

2.2.3. Naked AuNP (H_2O_2 method)

The preparation of naked AuNP is a challenge since, normally, there are residues of the reductive agent or the medium used to grow the nanomaterial on the NP surface. In order to obtain naked AuNP the photoreductive method developed at the Scaiano group⁴⁰ was followed. For that, hydrogen peroxide (H_2O_2) was used as a reductive agent. The detailed procedure is as follows:

Preparation of AuNP:

1.98 mL of a 10 mM HAuCl_4 solution is mixed with 52 mL of water followed by the addition of 6 mL of 0.1 mM H_2O_2 . The final solution has a yellow color and 2.5 mL of the solution is transfer into each cell of a 24 well plate and placed into a photoreactor with UVA lamps for 10 min. The final solution has a violet/pink color with maximum absorbance at 549 nm (Figure 2.1).

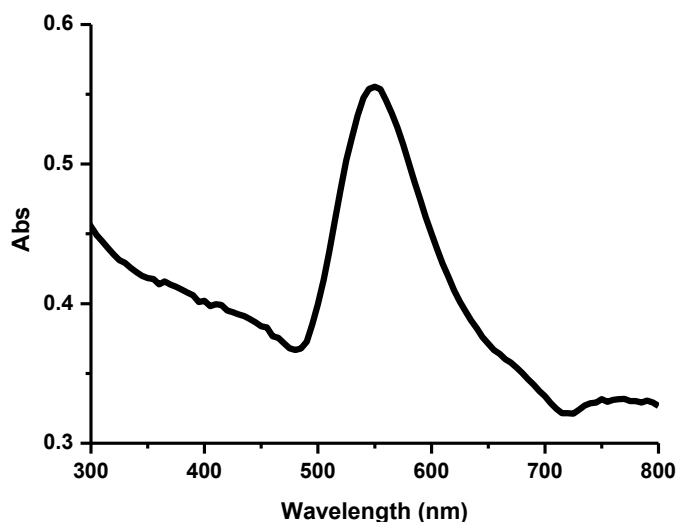


Figure 2.1. UV-Vis spectra of naked AuNP prepared by the H_2O_2 method before laser ablation.

The resulting solution of naked AuNP contains very polydisperse AuNP when using this method. To obtain a monodisperse suspension of AuNP, the solution was transferred into a laser ablation system (laser drop) developed by the Scaiano group,⁴¹ as follows:

Laser ablation:

The solution of AuNP is placed into a plastic syringe where drops are generated to ablate the AuNP using a 532 nm Nd:YAG laser, at 45 mJ and 1 Hz. After a determined number of shots per drop, the drop of ablated AuNP falls into a glass vial with a receptor solution (e.g. surfactant, section 4.1). The final concentration of AuNP in solution must incorporate this dilution factor. This method provides different sized AuNP according with the number of shots per drop (Table 2.1); the different sizes also produce different calculated concentration of AuNP before the dilution.

Table 2.1. Size of AuNP by laser drop ablation as function of number of shots per drop and calculated final concentration in water without dilution factor.

Shots per drop	Diameter (nm)*	Concentration (M)**
7	5.1 ± 1.0	6.3×10^{-8}
14	4.1 ± 0.8	1.23×10^{-8}
28	3.5 ± 0.8	5.0×10^{-7}

* Based of TEM images of AuNP@TiO₂. Adapted from reference [41]

** Calculation before dilution

This procedure was used in two of the projects evaluated in this work. One of them with 7 shots per drop (section 3.1), while the other with 28 shots per drop (section 4.1).

2.2.4. AuNP@citrate (citrate reduction method)

A polymorphic solution of AuNP with median size of 65 nm (Figure 2.2 and Figure 2.3) and an estimated concentration of 31 pM (assuming that all AuNP are perfect spheres and $d=65$ nm) was prepared following the procedure from Vanegas *et al.*⁴² details as follows:

In an oil bath, reflux for 10 min 40 mg of gold ($\text{HAuCl}_4 \times 3\text{H}_2\text{O}$) dissolved into 300 mL of MQ water in a 500 mL round bottom flask. Then, slowly add 20 mg of tri-sodium citrate (previously dissolved in 20 mL of water) and allow it to cool down slowly, which takes approximately 60 min after the color change. The resulting suspension is stable for about 4 months and contains a polymorphic mixture of AuNP covered with citrate (AuNP@citrate) with maximum absorption at 553 nm (Figure 2.4)

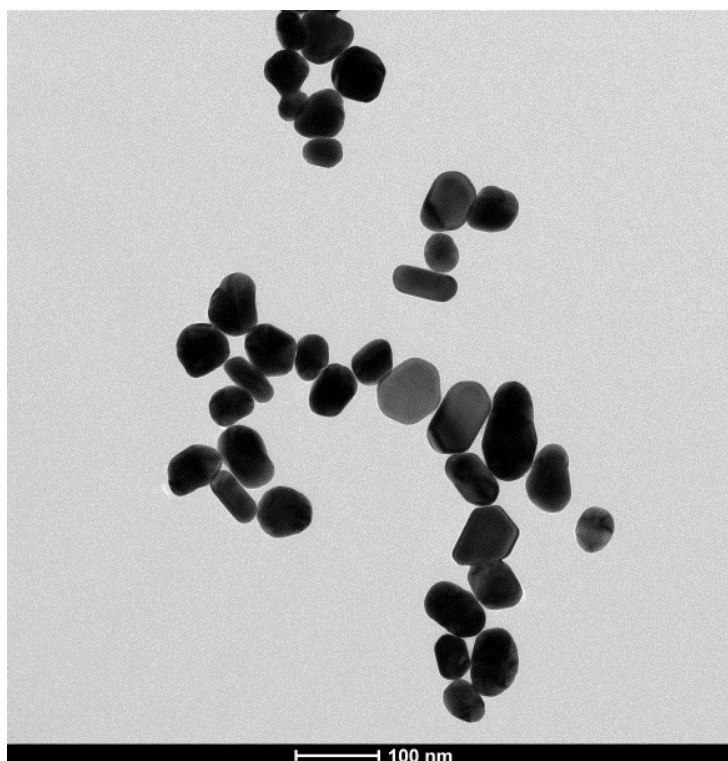


Figure 2.2. TEM image of AuNP@citrate. Scale bar, 100 nm.

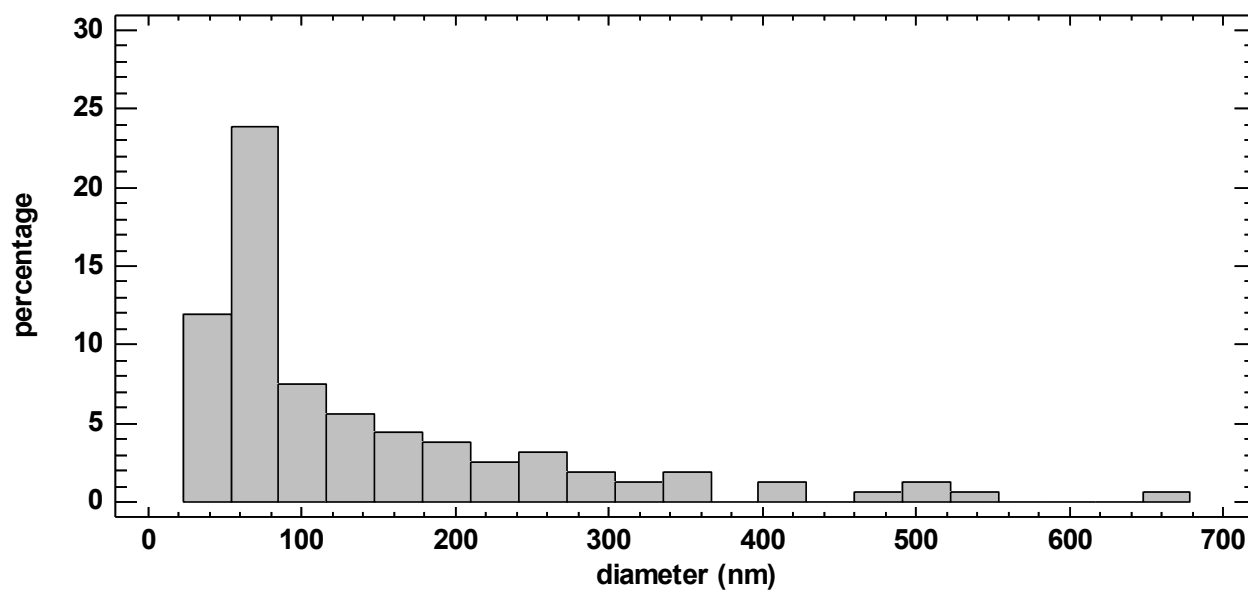


Figure 2.3. Percentage histogram of more than 100 AuNP@citrate particles. Maximum size at diameter of 65 nm, assuming that all AuNP are perfect spheres.

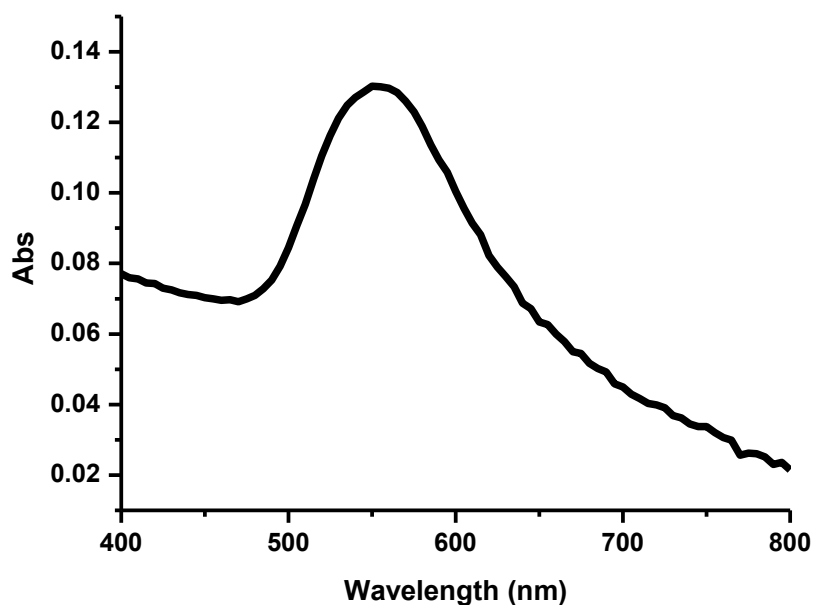


Figure 2.4. UV-Vis spectrum of AuNP@citrate in 1:10 diluted solution (calculated concentration of 3.1 pM in water).

2.2.5. AuNR@CTAB (seed growth method)

Gold nanorods (AuNR) were prepared by the seed growing method in cetyl-trimethyl-ammonium bromide (CTAB), following the procedure by Marquez *et al.*⁴³ The details are as follows:

Seed Solutions:

Start by dissolving 364.5 mg of CTAB in 10 mL of water. After completely dissolved, add 25 μ L of 0.1 M HAuCl₄ and then add 0.6 mL of ice-cooled 0.01 M NaBH₄. Stir for 2 min and then age for 30-45 min at 27 °C. The final suspension should have a dark brown color.

Growth Solution:

Dissolve 3.6 g of CTAB in 100 mL of water with the help of magnetic stirring and warm to 55 °C. Once the temperature is stable, add 0.44 g of 5-bromosalicylic acid and wait until completely dissolved. Then, add 1.92 mL of AgNO₃ 0.01 M and keep undisturbed for 15 min; slowly cooling down the temperature until 27 °C.

After the 15 min, add 100 mL of HAuCl₄ 1 mM while stirring slowly, after which, the solution should change into an orange color. At this point, add 0.512 mL of 0.1 M ascorbic acid solution and stir at maximum speed for 30 s. The solution should turn colorless because of the reduction of Au³⁺ to Au¹⁺. Stop the stirring and add 0.32 mL of seeds follow by another stirring of 30 s at maximum speed. Finally, keep undisturbed for 12 h at 27 °C. The rods solution should change color from brown to purple and finally dark red-purple color. The AuNR@CTAB are stable for about one month when stored at 27 °C.

AuNR Purification:

The excess of CTAB molecules remaining in solution after synthesis can be removed without aggregation of the AuNR. For that, 10 mL of AuNR solution is centrifuged in a plastic centrifuge tube twice, the first time at 8000 rpm for 20 min at 20 °C, and the second time at 8000 rpm for 30 min at 13 °C.

The resulting AuNR are covered with CTAB (AuNR@CTAB) and have two maxima in the UV-Vis spectrum (Figure 2.5) corresponding to the longitudinal (755 nm) and the transversal plasmon (513 nm) bands. They are also monodisperse with an approximated aspect ratio of 1:3 (Figure 2.6), 2861 nm² of surface and a calculated concentration of 1.5 nM.

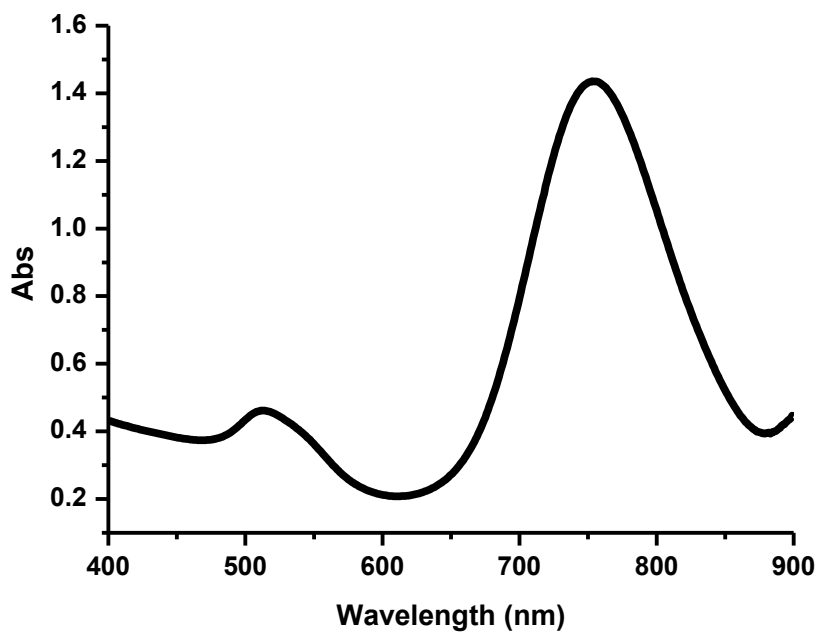


Figure 2.5. UV-Vis of AuNR@CTAB prepared by seed growth method. Calculated AuNR concentration of 1.5 nM in water.

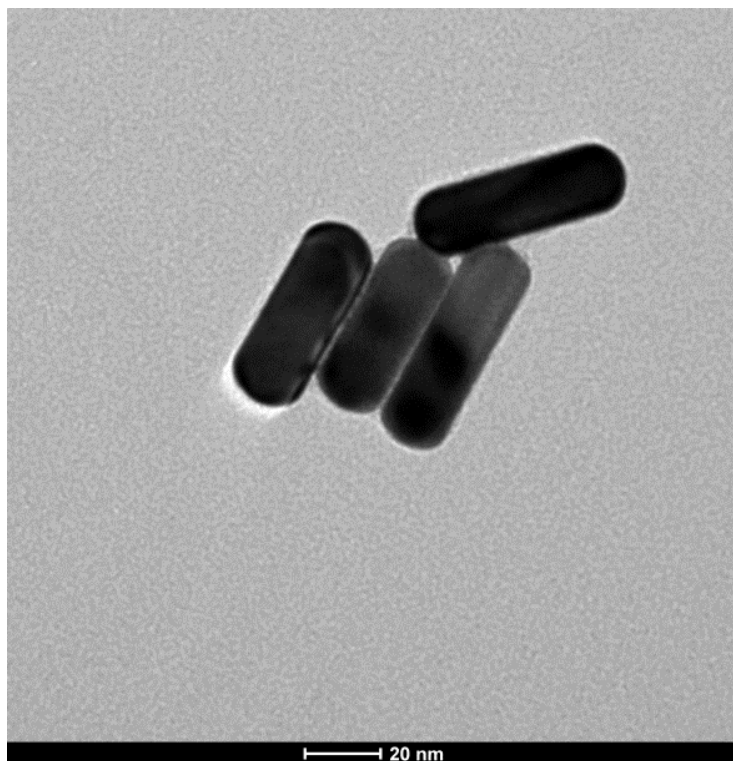


Figure 2.6. TEM image of AuNR@CTAB prepared by seed growth method with 1:3 aspect ratio and average long axis of 50 nm. Scale bar, 20 nm.

2.3. Instrumentation

2.3.1. *UV-Vis measurements*

In this work, for UV-Vis measurements, it was used a Cary 50Bio, a Cary 60 and a Cary 7000; all instruments from Agilent. The Cary 7000 has the advantage of measuring in the infrared region, as well as an accessory for solid analysis (integrating sphere). All measurements were completed in absorption mode (Abs) with the appropriate cell (quartz, glass or plastic), doing one scan per sample or scan kinetics experiments (section 5.2.2) as required.

2.3.2. *Raman measurements*

The Raman instrument that is available at the Catalysis Centre at the University of Ottawa is an Xplora microscope from HORIBA with two lasers (532 and 785 nm), two objectives (10x and 100x), four possible grating, and different aperture of the microscope slit and hole. Table 2.2 shows the different possibilities and the ones that were selected during this work. More detailed information will be presented case by case, including the number of accumulations and integration time –two critical acquisition parameters.

The software (LabSpec 6) allows for mapping and kinetic experiments. The Raman kinetics experiments are the base for the project presented in section 5. The Xplora microscope is also connected with an atomic force microscope (AFM) allowing tip-enhanced Raman scattering (TERS) measurements. The Xplora instrument is verified daily using the natural Raman band of silicon located with at 520 cm^{-1} .

Table 2.2. Possible configuration of the Raman Xplora microscope and selected ones.

	Possibilities	Selected
Laser (nm)	532 and 785	532 and 785
Laser power (%)	0.1, 1, 10, 25, 50, 100	25 and 10
Objective (x)	10 and 100	10
Grating (gr/mm)	600, 1200, 1800, 2400	600
Slit (μm)	50, 100, 200	200
Hole (μm)	100, 300, 500	500

The final item to explore is the power of the laser used in the Raman instrument. Table 2.3 include the measured power of the laser with and without objective when the power is set at 100%.

Table 2.3. Measured power of both lasers (532 and 785 nm) in the Raman Xplora microscope with and without objectives, using 100% of power.

	Power (μW)	
	532 nm	785 nm
No objective	26	60
10x	24	50
100x	18	27

2.3.3. *FLIM measurements*

The Scaiano group fluorescence lifetime imaging microscope (FLIM, MicroTime 200 from PicoQuant) is equipped with four picosecond pulse diode lasers including the 635 nm laser (635 nm, 100 ps, 40 MHz, LDH-D-C-485, PicoQuant) that was used in this work. The laser beam was collimated and focused through a fibre-coupling unit. A beam splitter Z638rdc (Chroma) was used to reflect the excitation light into the oil immersion total internal reflection (TIR) objective (100x, NA1.45, Olympus, PLAPO). The excitation dose (average power) is about 0.5 μ W for all samples. A band pass filter (690/70) was used to eliminate the excitation from the final FLIM images.

The images obtained in the FLIM are a 2D representation of the fluorescence intensity (and lifetime) of each sample point. This facilitates the location of fluorophores on the sample surface. Then, points of the sample can be selected and the fluorescence spectrum can be collected by the spectrograph (SR-163 from Andor). All fluorescence spectra were acquired with 100 accumulations at 1s and electron multiplier (EM) of 100. The spectrograph was calibrated with 488 nm and 637 nm lasers. The use of the instrument is the base of the methylene blue project discussed in section 3.

2.3.4. *AFM measurements*

As previously mentioned, the Catalysis Centre at the University of Ottawa has an atomic force microscope (AFM, SmartSPM 100 from AIST-NT) that can be coupled to the Raman Xplora microscope. The instrument is a stand-alone instrument that can work on the following modes: contact, semi-contact and non-contact topography, phase, lateral force, force modulation, Kelvin (surface potentials) and TERS (Raman).

In this work, all measurements were completed with a regular silicon tip (NanoAndMore, with resonance frequency of 65 KHz and force constant of 0.5 N/m) on non-contact mode.

2.3.5. *Size analysis*

Two different techniques were implemented in order to analyze the NP size:

2.3.5.1. Dynamic light scattering (DLS)

Dynamic light scattering (DLS) analyses were carried on a Zetasizer Nano from Malvern, by triplicate (three different solutions, measured three times for a total of nine values per sample) and monitoring the number of particles per size. Proper adjustments were introduced into the software, such as the cuvette (quartz and rectangular) and the solvent refractive index. This analysis was of major importance on the reverse micelles project discussed in section 4.

The result of the DLS measurement is the hydrodynamic size (diameter) of the particles that are present in solution. The instrument does not need to be calibrated and use the Mie equation to calculate the NP size, estimating that all NP are perfect spheres. In this way, DLS analysis is a very useful for spherical NP and not for others shapes.

2.3.5.2. Transmission electron microscope (TEM)

As mentioned before, DLS results are the hydrodynamic diameter of the NP which is normally a little higher than the actual core NP size. Thus, for actual NP size determination a transmission electron microscope (TEM) was used.

The Catalysis Centre at the University of Ottawa has a G² Spirit TWIN TEM from Tecnai, which allows a magnification from 18 to 650,000 with working acceleration voltages from 20 to 120 kV. The instrument also has energy-dispersive X-ray spectroscopy (EDS) capability and was used at room temperature in all the cases presented in this work.

Finally, all samples preparation will be detailed in the experimental parts of each one of the projects.

3. Mapping the position of methylene blue on nanoparticles surface by plasmon-enhanced fluorescence (PEF)

One of the basic questions presented in this research is the interaction of molecules with the NP surface. What happens to the molecules, how strong the interaction is and how the molecules are oriented; all of these are examples of typical questions that need to be answered. In this chapter, the use of methylene blue (MB) and cucurbit[7]uril (CB) is presented for the exploration of one of those questions: How the molecules are oriented on the AuNP surface.

Methylene blue (MB, Figure 3.1 left) is a very common and widely studied dye that absorbs in the visible region of the spectrum (Figure 3.2). At high concentration, MB has the tendency to form dimers and trimers in solution which have an effect on its absorption properties;^{44–46} this can be avoided by using very low concentrations or solvents other than water.⁴⁷

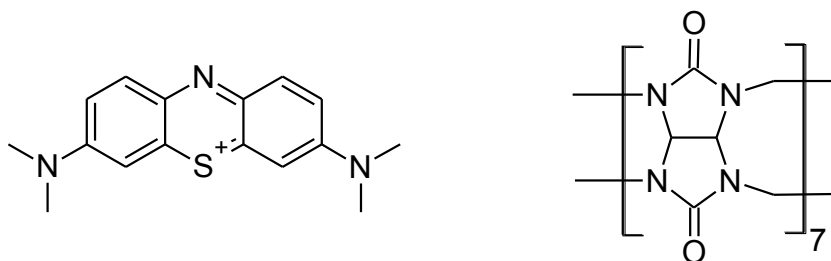


Figure 3.1. Structure of methylene blue (MB, left) and cucurbit[7]uril (CB, right).

Methylene blue (MB) molecules arrange in a 2D well-ordered system on Au(111),⁴⁸ with distances of 1.4 and 2.5 nm between molecules, depending on their direction. Similar to other molecules that lie flat on the gold surface, no enhancement properties should be expected⁴⁹ for MB on AuNP unless that the molecule is forced to be stand vertical on the surface and, in that way, to have a better interaction with the AuNP plasmon,^{9,31,34,35} just like it was discussed before (Figure 1.3).

In the case of CB ($C_{42}H_{42}N_{28}O_{14}$; Figure 3.1 right), the CB is part of the family of cucurbiturils that are macrocyclic molecules made of glycoluril ($C_4H_4N_4O_2$) monomers linked by methylene groups. As can be expected, the molecular size depends on the number of glycoluril monomers present, and in that way, cucurbiturils with 5 to 14 monomers has been synthesized.^{50,51} The cucurbit[7]uril (with 7 monomers) has an approximated high of 0.9 nm and an internal diameter of 0.7 nm with perfect size to host one (and only one) MB molecule, which make it very attractive for MB@CB complex studies.

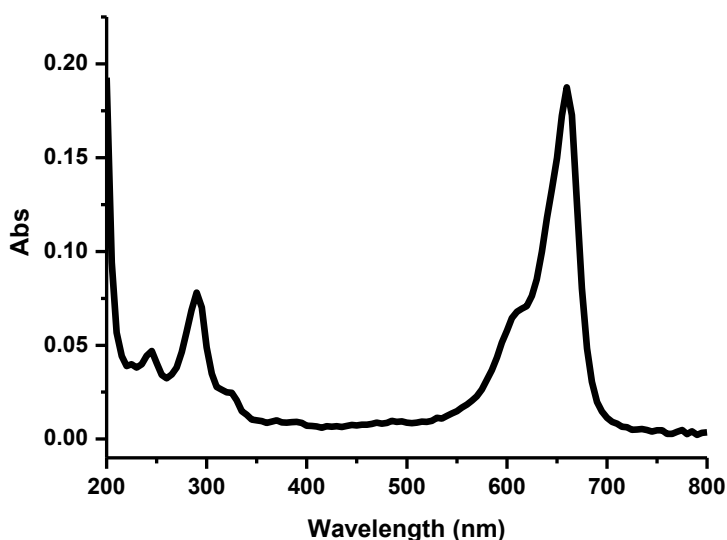


Figure 3.2. UV-Vis absorption spectrum of methylene blue (MB) 0.4 μ M in water.

Cucurbit[7]uril (CB) can form a complex with many different molecules^{50,51} by dipole or hydrophobic interactions. As can be seen in the structure (Figure 3.1 right and Figure 3.3), the CB rim is formed by carbonyl groups while the interior of the cavity does not have any charge or electron pair pointing in that direction and becomes hydrophobic. As a result, MB and CB can form an inclusion or an exclusion complex (Figure 3.3).

The inclusion (or host-guest) complexes of CB are known for changing the properties of the guest molecules, because of the change of the surrounding environment. In this way, NMR, absorption and fluorescence spectra of the guest molecules are normally shifted; such is the case of MB@CB complex where the fluorescence spectrum has a blue shift of approximately 20 nm (Figure 3.4). Under the experimental conditions described below and using a 635 nm laser as excitation source, the emission of MB in solution has a maximum at 687 nm and the band is blue shifted to 670 nm when the complex is formed. In theory, this simple shift should be big enough to recognize whether the inclusion or the exclusion complex forms, and is the main objective for the project presented in this chapter.

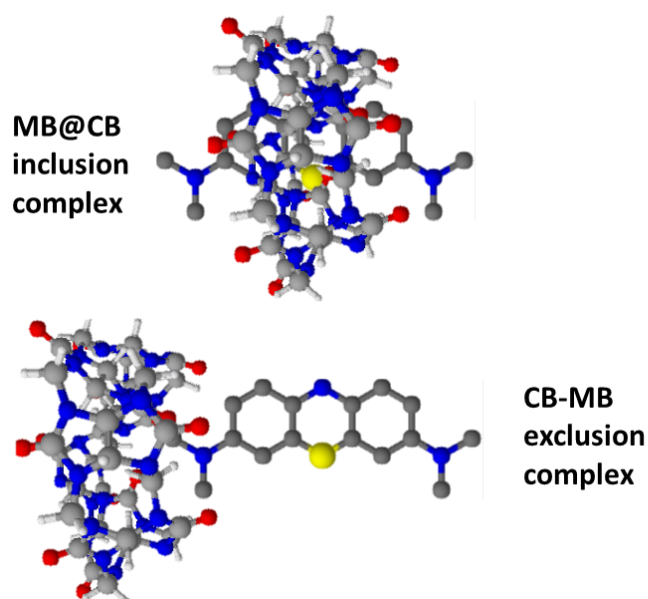


Figure 3.3. Possible complexes formed by methylene blue and cucurbit[7]uril. Inclusion or host-guest complex (1:1) and exclusion complex (1:1). Draw on ChemSketch with 3D optimization.

It has been observed that CB attaches to the AuNP by the carbonyl groups, and when concentrations allows it, it can form a repetitive assembly of AuNP separated by the CB distance (0.9 nm) with the CB as link of two AuNP³⁰ (Figure 3.5). At very low AuNP concentration and high concentration of CB, the probability is that many CB will be present at the AuNP surface and interacting with only one AuNP. Additionally, the use of AuNP fixed to a surface will avoid

any change in the AuNP distribution, which will lead to changes in the hot-spots and affecting the enhancement factor (G).³⁵

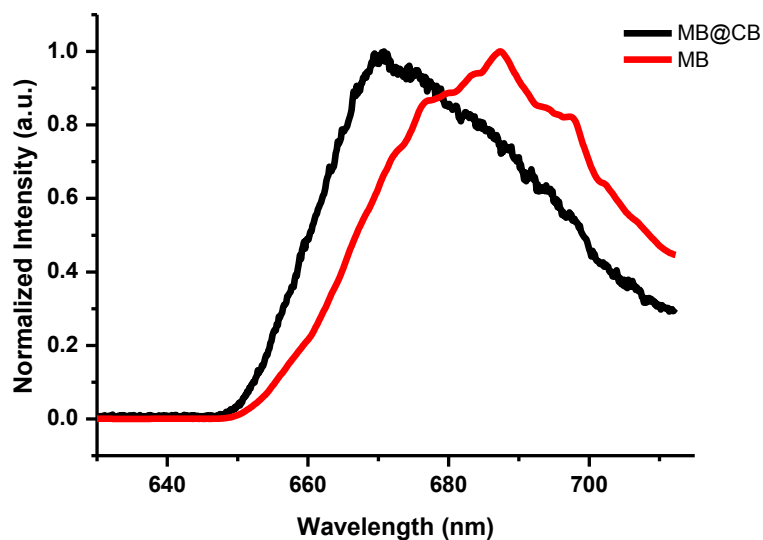


Figure 3.4. Normalized fluorescence spectra of MB and MB@CB in solution over glass coverslips. The emission maximum of MB in solution is located at 687 nm, while after forming the inclusion complex (host-guest) the emission is blue shifted to 670 nm. Spectra measured with 635 nm laser as excitation source and a band pass filter (690/70).

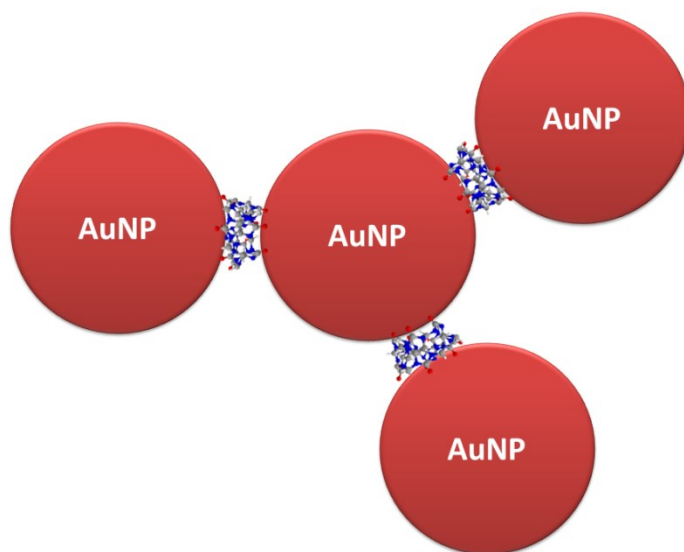


Figure 3.5. Schematic interaction between gold nanoparticles (AuNP) and cucurbit[7]uril (CB) forming hot-spots with 0.9 nm distance between particles.

In theory, the CB attached to AuNP prevents the formation of a host-guest complex of MB@CB, and instead will create an exclusion complex by the interaction of the CB carbonyl groups with the MB amines.^{51–53} This leads to a complex where MB could interact with one or two CB molecules at the same time, to form a 1:1 or 2:1 exclusion complex; since both exclusion complex explain the results observed in this chapter, for simplicity, the 1:1 exclusion complex is presented (Figure 3.3).

The formation of an exclusion complex will also minimize of the fluorescence quenching created by the AuNP surface (section 1.3, Figure 1.4), while avoiding changes in the spectrum itself. This is in addition to the fact that fluorescence of MB is enhanced after forming MB@CB host-guest complex,⁵⁴ and will provide a very good opportunity to perform the study at very low concentrations.

Finally, working with very low concentrations, the conditions are present to study the molecules position on AuNP surface by plasmon-enhanced fluorescence (PEF) using fluorescence lifetime imaging microscopy (FLIM) on a system where the AuNP are supported on a glass coverslip.

3.1. Experimental

In this work, naked AuNP were prepared as indicated in section 2.2 and ablated for a final average diameter of 5 nm (7 laser shots per drop). The particles solution was diluted for a final concentration of 3.6 nM and used to modify glass coverslips. The glass coverslips surface was previously functionalized with APTES (3-aminopropyltriethoxysilane) to increase the AuNP retention. The detail procedure for functionalization is as follows:

Glass coverslips functionalization with APTES:

The surface of the glass coverslips is activated by contact with piranha solution ($\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2$ (50%) 3:1) for 30 min, followed by rinsing with abundant MQ water, drying with Argon and bake at 120 °C for 5 min. After cooling down, the glass

coverslips are submerged into a 4% APTES solution in toluene and stirred for 90 min in an orbital shaker. Finally, the excess of APTES is removed by successively washing with toluene, acetone, ethanol and finally MQ water. The glass coverslips were stored in MQ water.

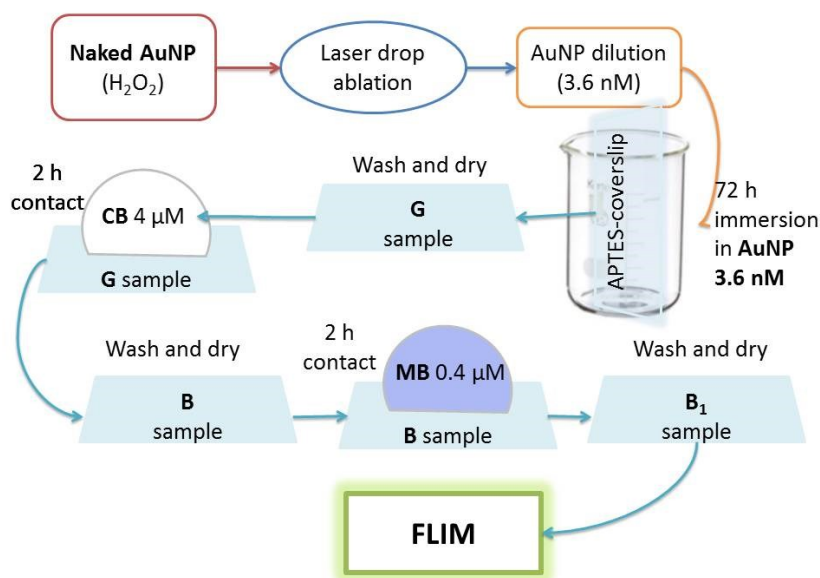


Figure 3.6. Experimental design for sample preparation of MB@CB on AuNP supported on a glass coverslip. Sample name as presented in Table 3.1.

The final samples taken to the FLIM (section 2.3.3) were prepared by immersing the APTES functionalized coverslip into AuNP 3.6 nM in water for 72 h; followed by placing the MB, CB and MB@CB solutions (0.4; 4 μM and 1:10, respective) on top of the coverslips, and allowing contact for 2 h, washing with MQ water and drying with air between solutions as indicated in Figure 3.6. The sample name and preparation order is indicated in the Table 3.1.

After finding the laser focus on the coverslip surface in the FLIM system and using two detectors with a 50/50 polarization cube, different images of different sizes and locations were recorded within the same sample, followed by selecting a few points in the image to measure the emission spectra through the spectrograph. All spectra were smoothed using a Savitzky-Golay algorithm with window size of 20 points.

Table 3.1. Sample name and preparation order for measurement of MB@CB on AuNP by FLIM.

Sample		Contact of the glass coverslip			
		1 st	2 nd	3 rd	4 th
Au-MB	A	AuNP	MB		
Au-MB-CB	A₁	AuNP	MB	CB	
Au-CB	B	AuNP	CB		
Au-CB-MB	B₁	AuNP	CB	MB	
Au-CB-MB-CB	B₁₁	AuNP	CB	MB	CB
Au-CB-MB@CB	B₂	AuNP	CB	MB@CB	
Au-MB@CB	C	AuNP	MB@CB		
Au	G	AuNP			
MB	H	MB			
MB@CB	I	MB@CB			

All solutions are in water, AuNP 3.6 nM, MB 0.4 μ M, CB 4 μ M, MB@CB 0.4:4 μ M; washing with MQ water and drying with air between solutions.

3.2. Results and Discussion

3.2.1. Synthesis of AuNP on glass coverslip (*in situ*)

An ideal condition is to functionalize the coverslip with a monolayer of AuNP, in an arrangement that could be observed in the FLIM system. For such a goal, AuNP were synthesized directly on a glass coverslip by modification of two photochemical methods, using I2959 and H₂O₂ (described in section 2.2.2 and 2.2.3 respectively).

In both cases, 200 μL of the final solution containing Au^{3+} and the corresponding reducing agent (I2959 or H_2O_2) was spin coated on the glass coverslip; the coverslips were then introduced into the photoreactor for 15 min with a quartz $5 \times 5 \mu\text{m}$ square mask to create a pattern that could be observed in the FLIM.

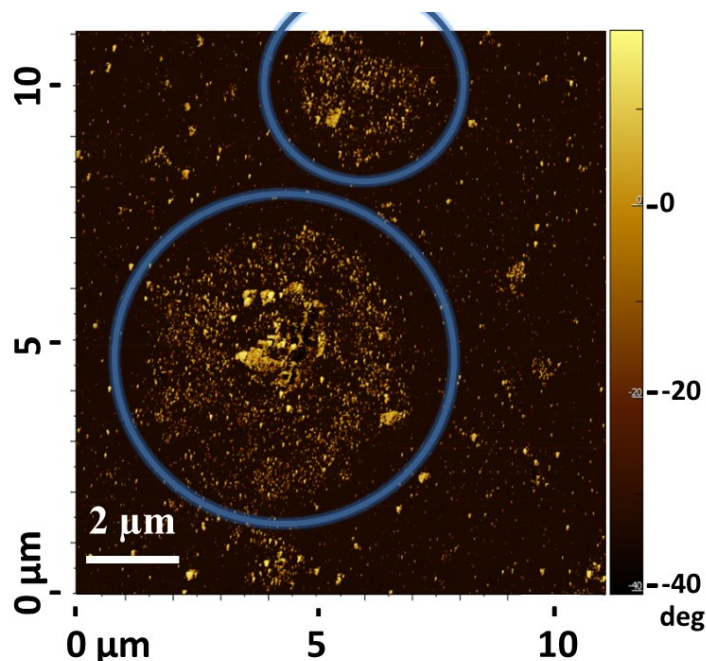


Figure 3.7. AFM phase image of AuNP on glass coverslip synthesized *in situ* by I2959 method using a $5 \times 5 \mu\text{m}$ square quartz mask. Scale bar: 2 μm . The circles highly islands of AuNP formed during the synthesis.

During the evaluation of the topography by atomic force microscope (AFM) it was observed islands of AuNP about the size of the mask (highlighted in circles in Figure 3.7) when the AuNP were synthesized by the I2959 method, while in the case of the H_2O_2 synthesis method there was a very good dispersion of the AuNP on the surface (Figure 3.8) and no clear pattern or islands. Following these excellent results, the topography was analyzed once again after the glass coverslips were washed with MQ water and dried with air. Unfortunately, the majority of AuNP were lost in the washing process leaving glass coverslips with very small population of AuNP, indicating that this synthesis method cannot be used.

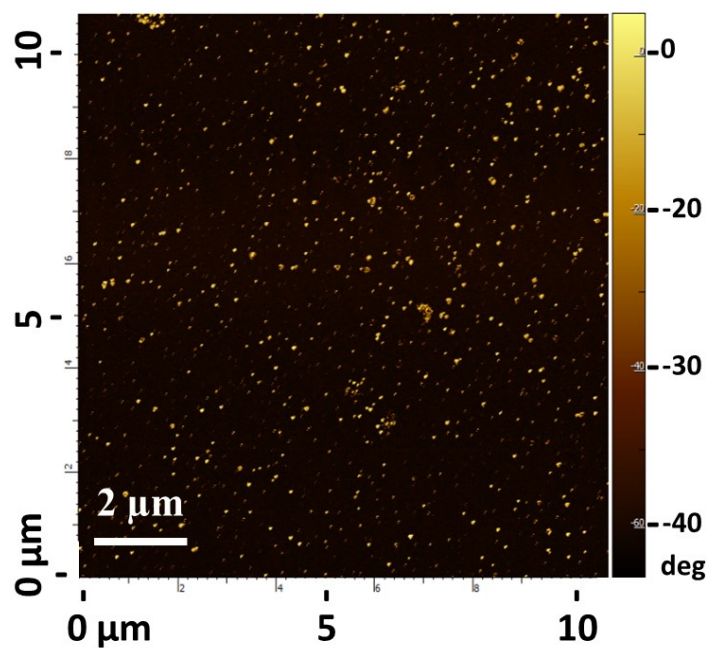


Figure 3.8. AFM phase image of AuNP on glass coverslip synthesized *in situ* by H_2O_2 method using a $5 \times 5 \mu\text{m}$ square quartz mask. Scale bar: $2 \mu\text{m}$. Very dispersed distribution of AuNP is observed on the surface.

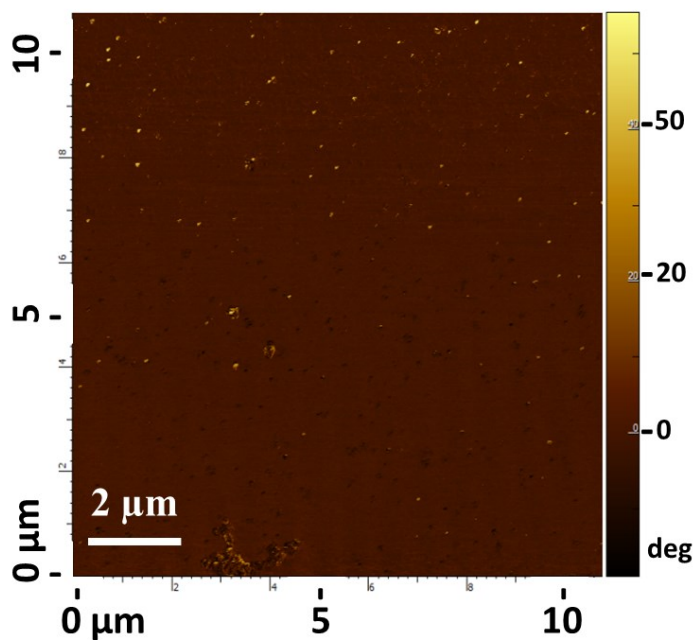


Figure 3.9. AFM phase image of AuNP on APTES functionalized coverslip synthesized *in situ* by I2959 method using a $5 \times 5 \mu\text{m}$ square quartz mask. Scale bar: $2 \mu\text{m}$. Very small quantity of AuNP is observed on the surface.

In order to retain the AuNP, APTES functionalized coverslips were used in the synthesis process. Unfortunately, a very small population of AuNP was observed on these coverslips (Figure 3.9) and the AuNP synthesis *in situ* was discarded. The final decision was to continue with synthesized AuNP (by H₂O₂ method, section 2.2.3), ablated and fixed on the APTES functionalized coverslips as previously described (section 3.1).

3.2.2. Selection of conditions

In order to create a very homogeneous monolayer of AuNP, CB, MB and MB@CB on the APTES functionalized coverslip surface different conditions were evaluated.

Four different samples were prepared for the modification of glass coverslips with AuNP, as follows: three samples with different contact time between the 3.6 nM AuNP solution and the coverslip, specifically: 2 h, 12 h and 72 h of contact; and one sample where the AuNP solution was left to dry on the coverslip (dry). From these samples, only the samples corresponding to 72 h contact presented the ideal proportion of size and quantity of AuNP on the coverslip. While the 2 h and 12 h contact samples presented a very small population of AuNP and the dry sample presented big agglomerations of AuNP with heights higher than 100 nm (Figure 3.10 and Figure 3.11). The observed agglomeration could occur in solution, during the drying process or a mixture of both, and only the deposition of particle over particle can explain the observed heights.

As mentioned before, the optimal balance of AuNP quantity, distribution and size was obtained by the immersion of the APTES functionalized coverslips into the AuNP solution (3.6 nM) for 72 h (Figure 3.12). The height profile for this image is presented in Figure 3.13, and average height under 6 nm can be observed which agrees with the expected heights for the synthesized AuNP.

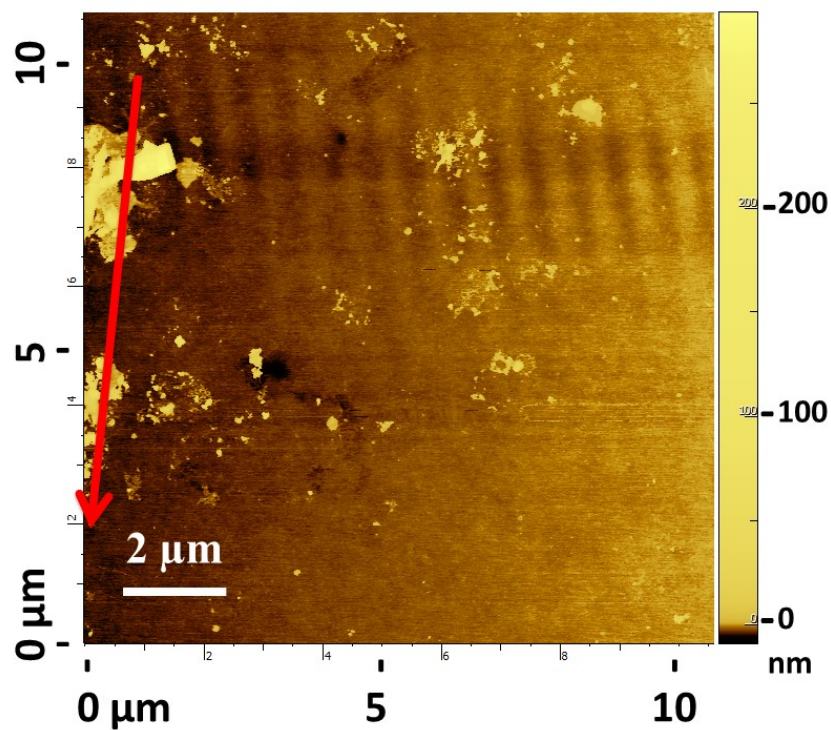


Figure 3.10. AFM non-contact topography image of AuNP on glass coverslip after drying a solution of AuNP 3.6 nM. Scale bar: 2 μm.

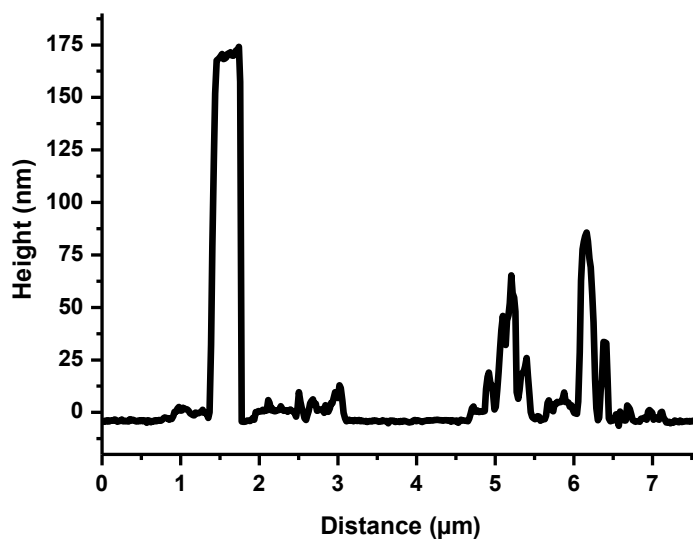


Figure 3.11. AFM height profile of the orange arrow in Figure 3.10, for the AuNP on coverslips functionalized with APTES.

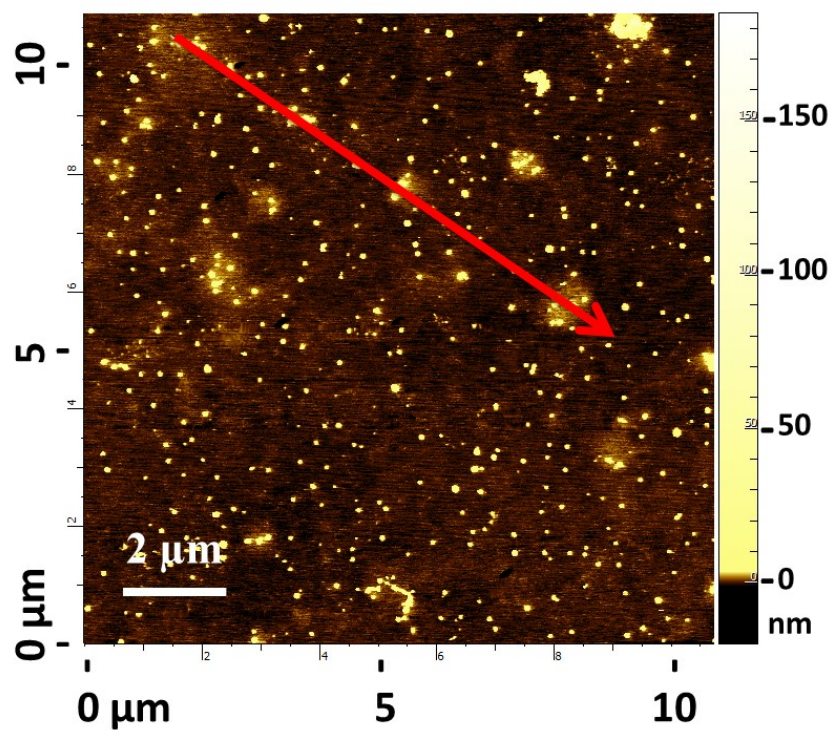


Figure 3.12. AFM non-contact topography image of AuNP on glass coverslip after 72 h contact with a 3.6 nM AuNP solution. Scale bar: 2 μm .

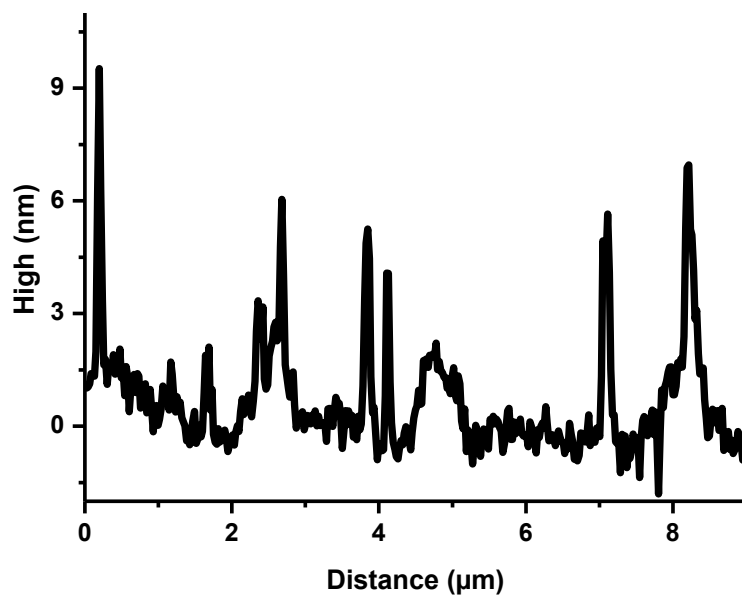


Figure 3.13. AFM height profile of the orange arrow in Figure 3.12, for the AuNP on coverslips functionalized with APTES.

In the case of CB, MB and MB@CB solutions two different conditions were evaluated: 30 min and 2 h contact of each solution with the AuNP modified coverslip. The 30 min contact shows FLIM images with low intensity signals, and although almost no spectra was observed this indicated that a very low quantity of material had deposited on the surface. The sample prepared by 2 h contact of the solutions with the AuNP modified coverslips (sample G) was sufficient to obtain FLIM images and fluorescence spectra of the different samples.

With the final conditions fixed, all the different samples (Table 3.1) were evaluated by AFM in order to measure the differences in height. Samples Au-MB to Au-MB@CB (A to C) did not present any difference from the original AuNP on the coverslip (sample G), indicating that the modification of the surface (after adding the AuNP) could not be followed by AFM, probably because the molecules distribute equally on the surface and the differences from peak to valley are indistinguishable by AFM.

3.2.3. *Fluorescence lifetime image microscope (FLIM) analysis*

Different areas of the same sample (Table 3.1) were analyzed by FLIM; samples Au-MB, Au-CB, Au, MB and MB@CB (samples A, B, G-I) presented FLIM images with very low fluorescence intensity (counts). The study of the fluorescence spectra in different point of each image did not differ from the background spectrum. As an example, Figure 3.14 presents one FLIM image of sample Au-MB (sample A) while in Figure 3.15 shows the spectra obtained for each location examined in the FLIM image.

Since the MB directly located on the AuNP surface suffers a strong quenching, the absence of a emission spectrum in sample A (Au-MB) is expected. The absence of fluorescence spectrum in samples B (Au-CB) and G (Au) is expected because there is an absence of any fluorescent

species. In the case of samples H (MB) and I (MB@CB), the concentration of MB and MB@CB present on the coverslip is very low and possibly lower than the instrument detection limit.

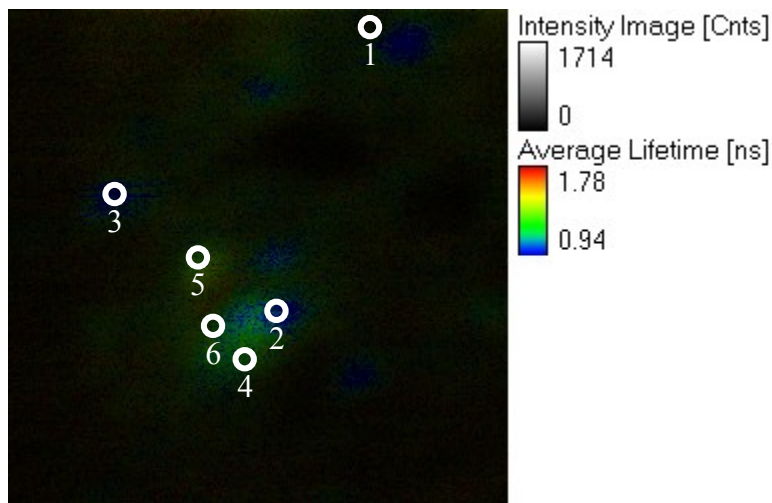


Figure 3.14. FLIM image of sample A (Au-MB) on coverslip. Size 9.5 x 9.5 μm ; lifetime max. 0.74 ns. Notice each circle represent the locations where the spectra in Figure 3.15 were recorded.

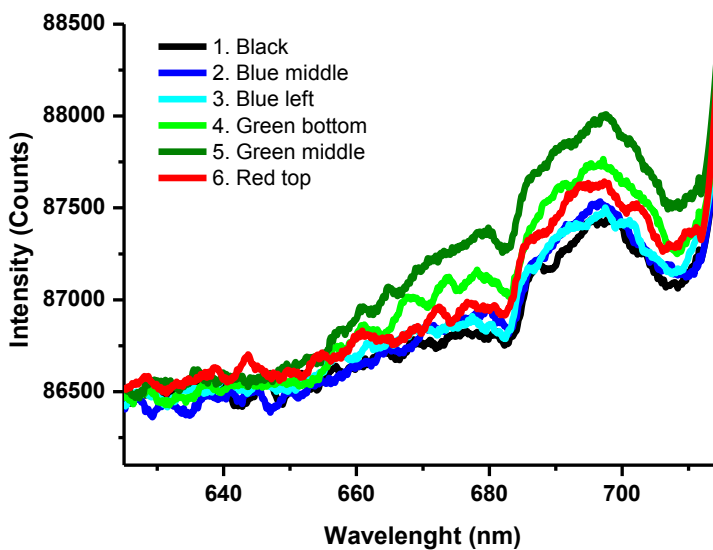


Figure 3.15. Fluorescence spectra recorded at different locations in Figure 3.14 (sample A, Au-MB). All spectra present similar features to the background. Spectra measured with 635 nm laser as excitation source and a band pass filter (690/70).

In the case of sample B₁ (Au-CB-MB Figure 3.16), the different locations present a spectrum similar to a mixture of the MB and MB@CB spectrum (Figure 3.17A), indicating that both complexes (inclusion and exclusion) of MB with CB can be formed when CB is already on the AuNP surface. Notice that the intensity is slightly higher on the MB alone area (Figure 3.17B), suggesting that are more molecules in the exclusion complex configuration, as it was theorized.

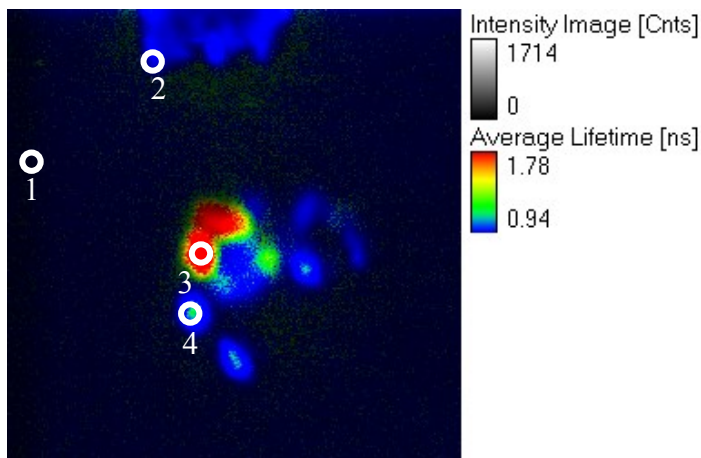


Figure 3.16. FLIM image of sample B₁ (Au-CB-MB) on coverslip. Size 5.2 x 5.2 μm; lifetime max. 1.31 ns. Notice each circle represent the locations where the spectra in Figure 3.17 were recorded.

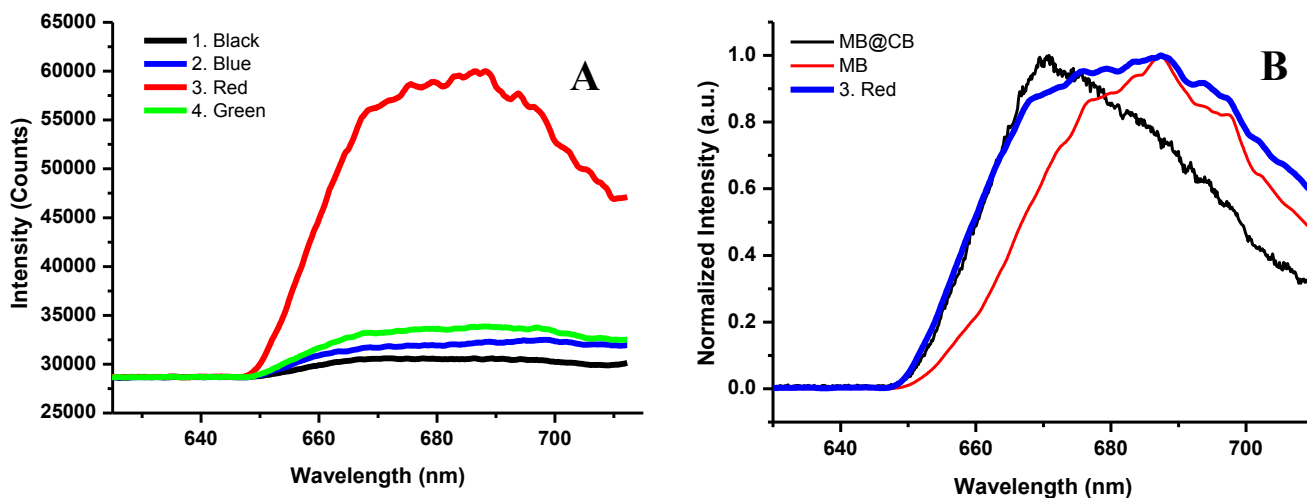


Figure 3.17. A: Fluorescence spectra recorded at different locations in Figure 3.16 (sample B₁, Au-CB-MB). The spectrum presents similar features to the mixture of MB and MB@CB, indicating the formation of both complexes when CB is already present in the AuNP surface, whit slightly more presence of the exclusion complex (MB alone). B: Normalized fluorescence spectra of inclusion complex (MB@CB, black), exclusion complex (MB, red) and location 3 in Figure 3.16 (blue); it can be observed a slightly higher intensity on the MB fluorescence for location 3. Spectra measured with 635 nm laser as excitation source and a band pass filter (690/70).

Similar observations are made with the sample B₁₁ (Au-CB-MB-CB) presented in Figure 3.18, where the spectrum present features similar to the mixture of both complexes (Figure 3.19Figure 3.19).

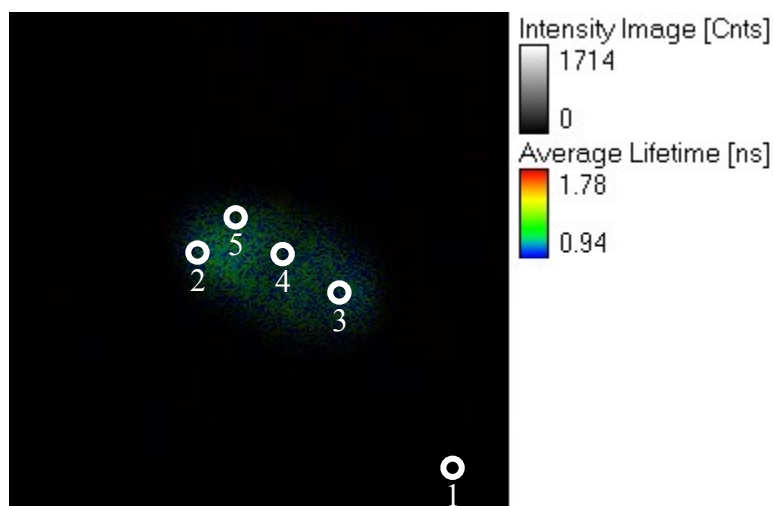


Figure 3.18. FLIM image of sample B₁₁ (Au-CB-MB-CB) on coverslip. Size 5.9 x 5.9 μm ; lifetime max. 1.26 ns. Notice each circle represent the locations where the spectra in Figure 3.19 were recorded.

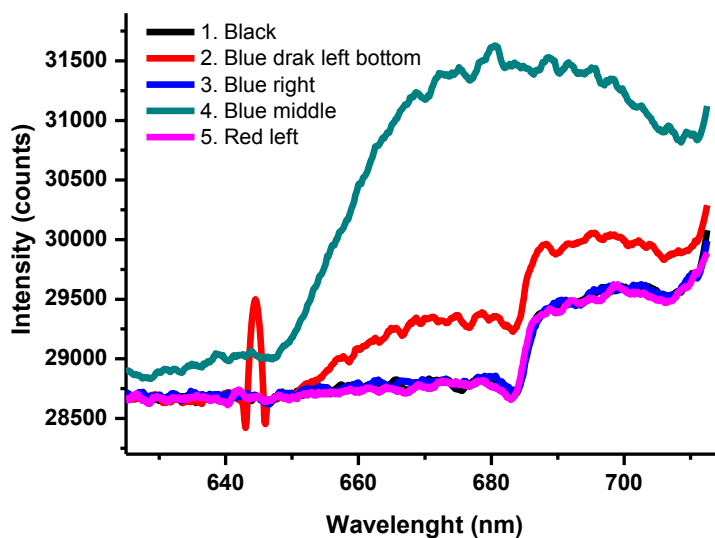


Figure 3.19. Fluorescence spectra recorded at different locations in Figure 3.18 (sample B₁₁, Au-CB-MB-CB). The spectrum presents similar feature to the MB alone, indicating the preference for forming the exclusion complex. Spectra measured with 635 nm laser as excitation source and a band pass filter (690/70).

In the case of samples B₂ (Au-CB-MB@CB Figure 3.20) and C (Au-MB@CB Figure 3.22), the spectra of the different locations (Figure 3.21 and Figure 3.23, respectively) show features that are more similar to the MB alone, indicating the preferred formation of the exclusion complex.

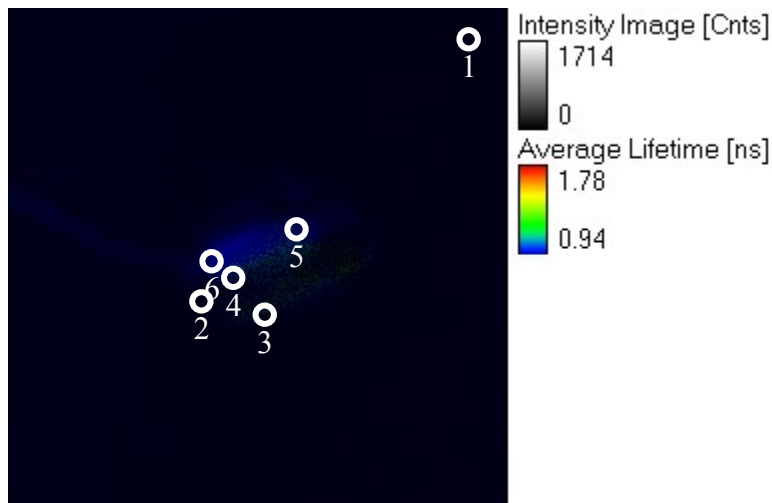


Figure 3.20. FLIM image of sample B₂ (Au-CB-MB@CB) on coverslip. Size 9.8 x 9.8 μm ; lifetime max. Notice each circle represent the locations where the spectra in Figure 3.21 were recorded.

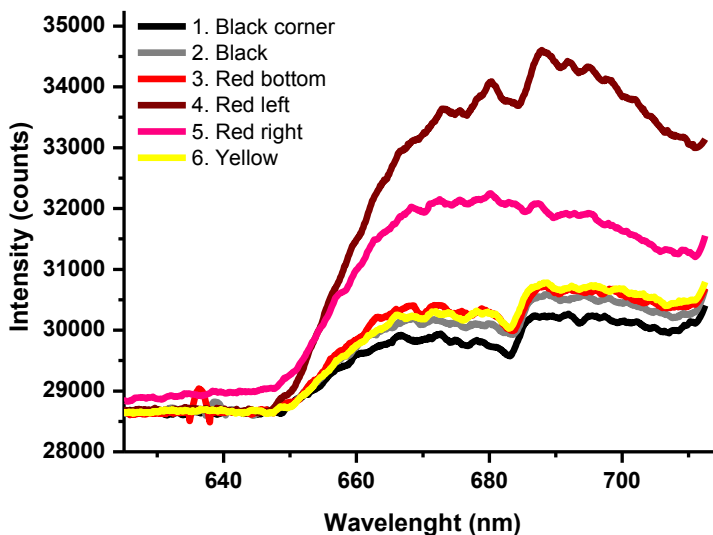


Figure 3.21. Fluorescence spectra recorded at different locations in Figure 3.20 (sample B₂, Au-CB-MB-CB). The spectrum in location 4 presents similar feature to the MB alone, indicating the preference for forming the exclusion complex. While the spectrum in location 3 presents similar features to the MB@CB host-guest complex, revealing the need of analyzing at the microscope level. Spectra measured with 635 nm laser as excitation source and a band pass filter (690/70).

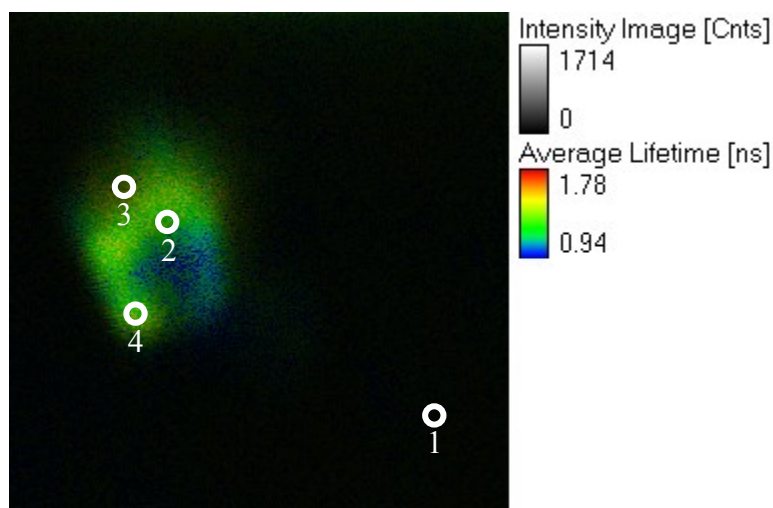


Figure 3.22. FLIM image of sample C (Au-MB@CB) on coverslip. Size 6.4 x 6.4 μm ; lifetime max. 1.26 ns. Notice each circle represent the locations where the spectra in Figure 3.23 were recorded.

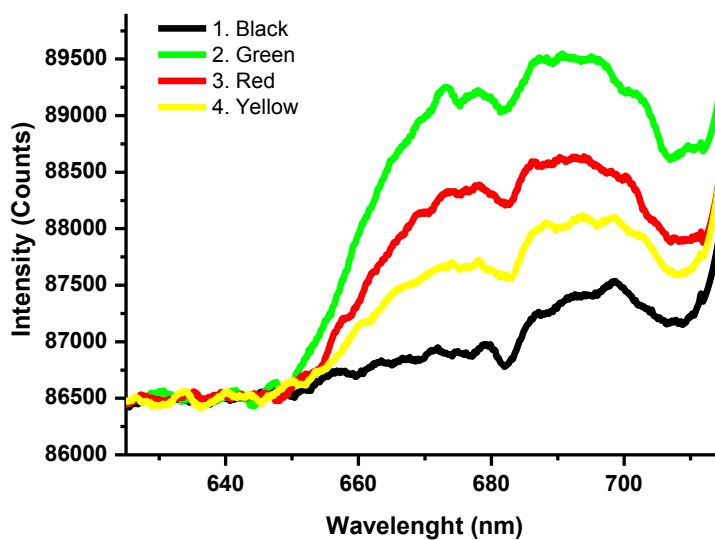


Figure 3.23. Fluorescence spectra recorded at different locations in Figure 3.22 (sample C, Au-MB@CB). The spectrum presents similar feature to the MB alone, indicating the preference for forming the exclusion complex. Spectra measured with 635 nm laser as excitation source and a band pass filter (690/70).

It is important to highlight that one of the locations examined in sample B₂ has a spectrum that differ from the group and it is more similar to the inclusion complex –MB@CB (location 5, Figure 3.21) with a maximum at shorter wavelength. This kind of result was observed in many of the areas; Figure 3.24A is a good example where location 3 differs from the group (Figure 3.25A). This observation reveals the need of measuring at the microscopic level.

Experiments to see the mobility of the molecules on the AuNP surface were completed by placing a drop of water on top of the sample. Since no FLIM image or spectra was observed in these cases, the following experiment was placing a drop of MB@CB solution (1:10) on the coverslip. As can be observed in Figure 3.24, the FLIM image remained similar (A: dry, B: with MB@CB solution). A series of spectra were recorded at the same locations for both images (Figure 3.25A and B respectively) and while in Figure 3.25A the different spectra present features of the complex and a mixture of MB and MB@CB, all the spectra that were obtained after adding the drop of MB@CB (Figure 3.25B) present features of a mixture of both: MB and MB@CB.

It could be expected that after adding the MB@CB solution to the glass slide, all spectra will have the appearance of the complex. In fact that was the case in about 50% of the cases, while the other 50% presented a mixture of both or very flat spectra as indicated in Table 3.2. This results corroborate the preference of the CB of being located at the AuNP surface, while MB has the preference to form the exclusion complex even when the inclusion complex is placed in solution during the FLIM measurements.

It is expeted that the interaction of the MB and CB with the AuNP surface is similar, and that after adding any of the solutions the molecules will remain in the same position. To test this, a final sample A₁ (Au-MB-CB) was prepared to see how the spectrum was influenced by the preparation method.

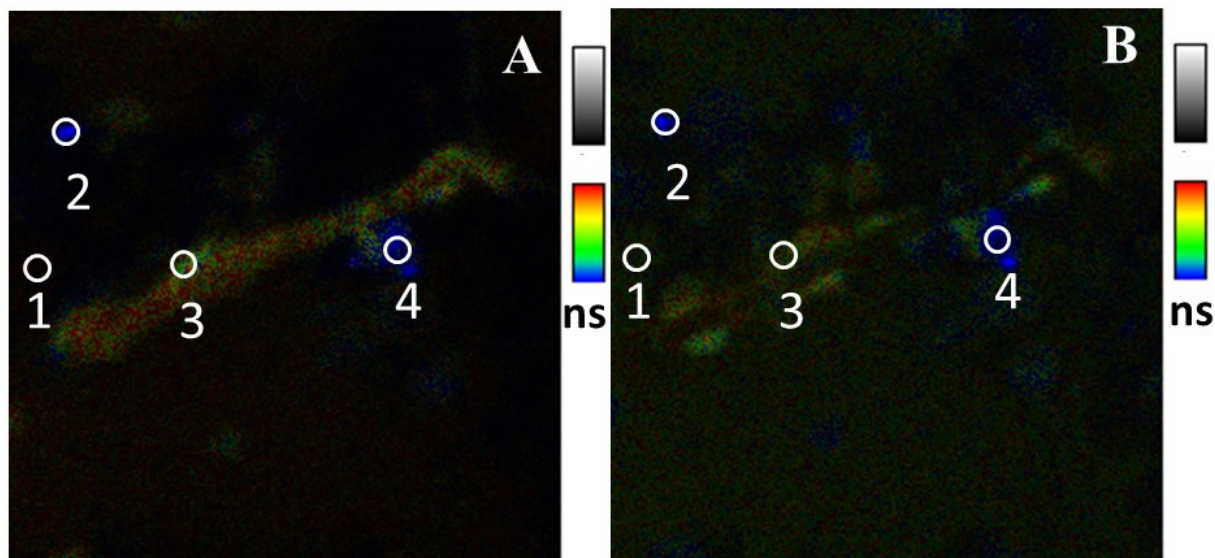


Figure 3.24. FLIM image of sample B₁₁ (Au-CB-MB-CB) on coverslip. Size 16.10 x 16.10 μm ; **A:** dry, lifetime max. 1.26 ns. **B:** after adding a drop of MB@CB on top of the coverslip, lifetime max. 1.15 ns. Notice each circle represent the locations where the spectra in Figure 3.25 were recorded.

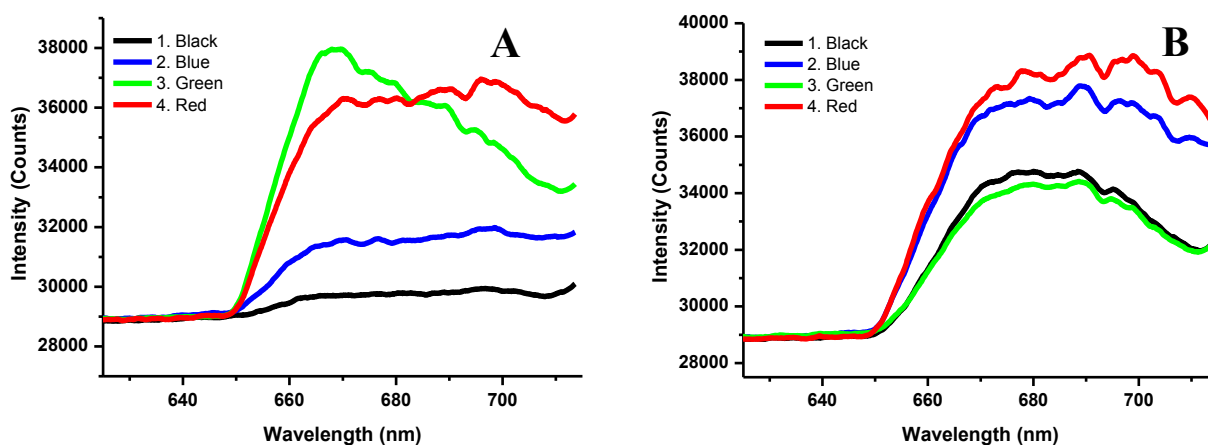


Figure 3.25. Fluorescence spectra recorded at different locations in sample B₁₁ (Au-CB-MB-CB). **A:** from Figure 3.24A, **B:** from Figure 3.24B. In Figure A: locations 2 and 4 presents spectra with features similar to a mixture of the inclusion complex (MB@CB) and MB alone, while location 3 shows features similar to inclusion complex (MB@CB). In Figure B: all spectra present features similar to a mixture of MB and MB@CB. Spectra measured with 635 nm laser as excitation source and a band pass filter (690/70).

Figure 3.26 presents a FLIM image of sample A₁ (Au-MB-CB) and indicates the location of the different spectra. As can be observed in Figure 3.27, all the spectra present the clear features of the inclusion complex (MB@CB) in contrast with sample B₁ (Au-CB-MB, spectra in Figure 3.17) that shows features similar to the MB alone. Indicating that when MB is already on the AuNP surface the formation of the host-guest complex is favored, more likely because the MB is already in a good position to form the complex.

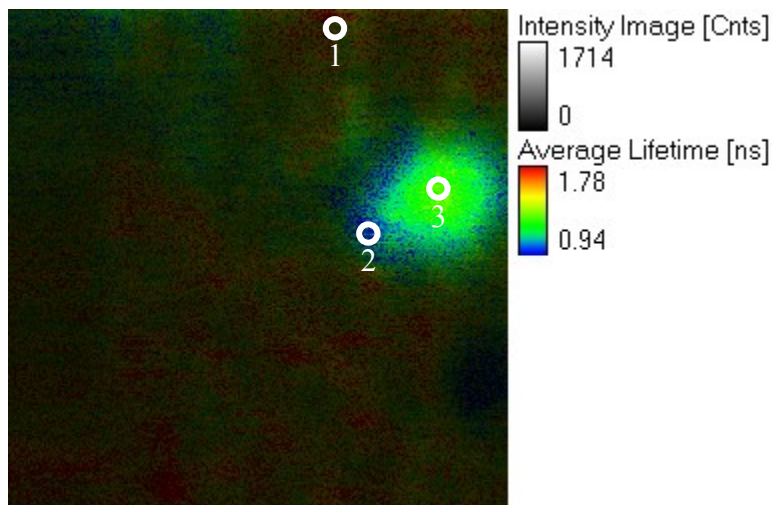


Figure 3.26. FLIM image of sample A₁ (Au-MB-CB) on coverslip. Size 5.1 x 5.1 μm ; lifetime max. 1.20 ns. Notice each circle represent the locations where the spectra in Figure 3.27 were recorded.

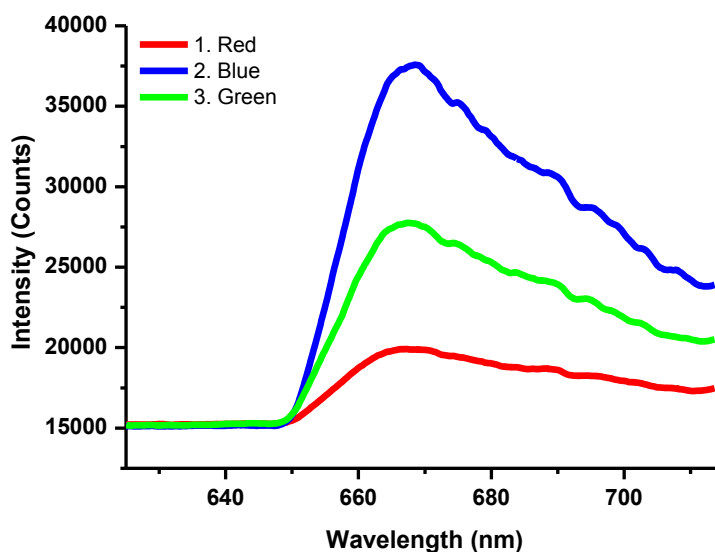


Figure 3.27. Fluorescence spectra recorded at different locations in Figure 3.26 (sample A₁, Au-MB-CB). All spectra present similar features to the inclusion complex (MB@CB). Spectra measured with 635 nm laser as excitation source and a band pass filter (690/70).

As previously mentioned, it has been reported that dry MB on Au(111) remains flat on the surface,⁴⁸ while the results of this project indicate that after putting the system in contact with a CB solution, the MB moves to a vertical position where it can form the host-guest complex. This position also favors the interaction with the AuNP plasmon band, providing better enhancement (Figure 1.3).

Table 3.2. Summary of spectra observations on the different samples measured by FLIM.

Sample		Observation	
		Dry	+ MB@CB _(sol)
Au-MB-CB	A ₁	MB@CB	MB@CB
Au-CB-MB	B ₁	MB and mixture	MB@CB and mixture
Au-CB-MB-CB	B ₁₁	MB@CB and mixture	Mixture
Au-CB-MB@CB	B ₂	MB and mixture	MB@CB
Au-MB@CB	C	MB	Mixture

Table 3.2 presents a summary of the spectra observed in all samples, and considering all different areas measured. As can be observed in many cases a mixture of both (MB and MB@CB) features was observed as a very flat spectrum. Also, in the majority of the cases, the spectrum in some locations differs from the rest of the group (as sample B₁₁), while only in one case (sample A₁, Au-MB-CB) the spectrum of the inclusion complex was observed in all the occasions. The preference of the CB of interacting directly with the AuNP surface becomes very significant when the inclusion complex is added to the AuNP surface and only the spectrum corresponding to the exclusion complex was observed (sample C, Au-MB@CB); this preference is also observed after the addition of MB@CB solution to the coverslip, since this did not shift the spectrum to the inclusion complex and rather a mixture was observed in many cases; corroborating that MB prefers to interact with CB by the exclusion complex.

3.2.4. Anisotropy studies

As mentioned before, the use of a 50/50 polarization cube in the FLIM allows the measurement of anisotropy of the molecules on the surface. The molecules in a parallel or perpendicular position have preference to get excited and emit with the same component (parallel or perpendicular) of the electromagnetic spectrum. The instrument measures the intensity of light that arrives into each of the detectors (parallel and perpendicular) and compares it into a plot where positive numbers indicate events in the parallel plane, while negative numbers indicate perpendicular events. It is important to notice that in solids the molecules are fixed and rotate very slowly, while in liquids the molecules rotate constantly; therefore, anisotropy can be observed only if the molecules rotation is slower than the fluorescence lifetime and having fixed molecules favor the observation of anisotropy.

Table 3.3. Anisotropy events for different sample measured by FLIM.

Sample		Max. events	
		Dry	+ MB@CB _(sol)
Au-MB-CB	A ₁	-0.30	-0.20
Au-CB-MB	B ₁	-0.20	-0.20
Au-CB-MB-CB	B ₁₁	-0.25	-0.20
Au-CB-MB@CB	B ₂	-0.30	-0.20
Au-MB@CB	C	-0.25	-0.20

As can be seen in Table 3.3, all the events maximize with negative numbers, indicating preference for events perpendicular to the polarization of the excitation light. In fact, very little

positive (parallel) events were observed in all samples and the majority presented very narrow (and Gaussian) distributions.

As can be observed in Figure 3.28, there are more possibilities for molecules to be located in the perpendicular plane to the excitation light than in the parallel one; just as the results indicate that all the observed molecules are located in the perpendicular plane (negative values). Also in the perpendicular plane to the light, there is the possibility of creating hot-spots by interaction between AuNP. It has been observed that the creation of hot-spots increase the enhancement factor (G) in fluorescence.³⁵ This subject could be investigated by carefully controlling the particle inter distance.

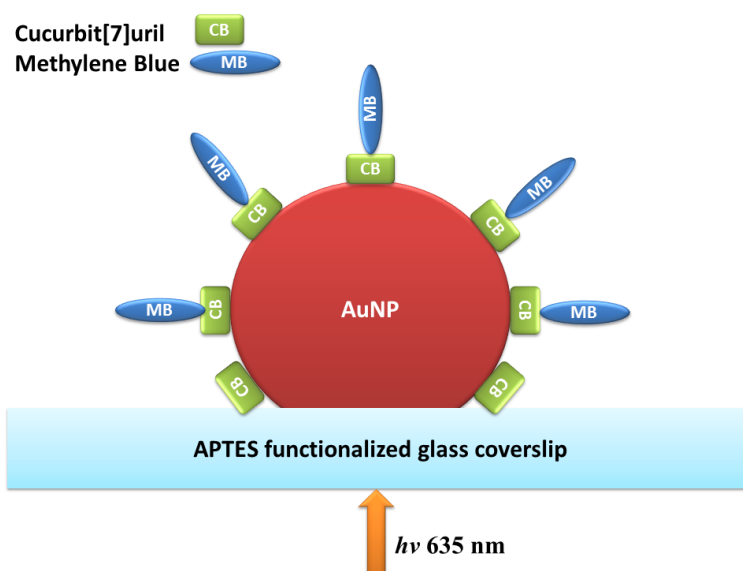


Figure 3.28. Schematic arrangement of cucurbit[7]uril (CB) and methylene blue (MB) exclusion complex on AuNP surface.

Table 3.3 shows that after putting the complex (MB@CB) solution in contact with the system, all the maxima changes to the same location, indicating that the rotational time of the molecules in liquid is larger than the fluorescence lifetime. Since the value is negative, it also indicates that

the molecules prefer the perpendicular position just like in the dry case. Unfortunately, no correlation between the observed spectrum and the anisotropy was found, and therefore the anisotropy study did not provide further information.

3.3. Conclusions

In conclusion, the proposed method was very useful to investigate the position of molecules on AuNP surface. Using a simple shift in the fluorescence spectra of MB, it was possible to observe that in the presence of AuNP, MB has a preference for interacting with CB as an exclusion complex, while the CB interacts with the AuNP surface, at the point that when the host-guest complex is already formed, the MB could exit the CB to form the exclusion complex (1:1 or 2:1) with one or two CB.

While the comparison between A_1 (Au-MB-CB) and B_1 (Au-CB-MB) shows that a simple change in the sample preparation procedure leads to a change in the resultant spectra, providing a simple way to tune the fluorescence spectrum.

Finally, the use of FLIM proved to be a simple way to study the orientations of molecules on a surface, increasing the use of the valuable instrument in chemistry; while the anisotropy study did not provided further information.

4. Water-Nanoparticle interaction measured inside reverse micelles

While studying nanoparticles (NP) on surfaces the Scaiano group observed emission-like signals that were tentatively attributed to water on the nanoparticles (NP) surface. Since water is a very attractive option as a probe for nanostructures analysis, the idea of measuring the Raman signal of water on the NP surface was born.

Looking for a system that would allow selectively measuring only the water molecules on the NP surface and differentiating them from bulk water, this project explores the use of reverse micelles (RM). A RM is a constrained system, that forms water droplets surrounded by the hydrophilic head of the surfactant, while the hydrophobic tail remains in the non-polar solvent.^{55,56}

A key factor in the RM system is the cavity radius, which is proportional to the ratio of water molecules to the surfactant,^{55,56} also known as W_0 ($=[\text{water}]/[\text{surfactant}]$). In this way, a higher W_0 produces bigger RM with more water molecules per droplet (or pool).

Several experiments were performed in order to fabricate AgNP and AuNP inside RM,⁵⁷⁻⁵⁹ principally using dioctyl sulfosuccinate sodium salt (AOT) in hydrocarbons, which is an attractive system given the importance of water in the petroleum industry. These methods produce very monodisperse NP, where the NP surface can have a weak interaction with the internal RM surface.⁵⁵ It is important to notice that NP can also be fabricated independently and added in a second step into the surfactant – organic solvent solution to form the RM. In both cases, the W_0 must and can be controlled since the NP size will depend on the selected W_0 .

In a system where NP are inside a RM (NP@RM), the NP will have the preference of being inside the RM if its size allows it and, with a carefully controlled relation of NP size and W_0 , a layer of water molecules should be present between the NP and the surfactant.

As it was mentioned before (section 1.2); any Raman signal obtained from water molecules in the NP@RM system will have an intensity that depends on the enhancement factor (G). Once again, very large SERS enhancements (G) can be achieved with the appropriate combination of NP size,¹⁹ excitation source²⁰ and probe molecule;²¹ typically lower than 10^{11} , higher enhancements are associated with other processes like the creation of hot spots²⁷ or chemical enhancement.²⁵ Even while water does not have an intense Raman signal –to the point that it is considered an ideal transparent solvent in Raman, which means that low G values can be expected.

Finally, this work proposes a system to evaluate the interaction between water and metals NP surface using surface enhanced Raman spectroscopy (SERS), bringing more information into the nanotechnology world.

4.1. Experimental

In this work, naked AuNP prepared as described in section 2.2.3 were ablated with a 532 nm laser at 45 mJ and 28 shots per drop, for a final particle size of approximately 3.5 nm. The water droplets (containing AuNP) were collected on a 90 mM dioctyl sulfosuccinate sodium salt (AOT) solution in heptane under vigorous stirring to create the RM (Figure 4.1). The resultant solution was analyzed by DLS followed by Raman spectroscopy on the same day. TEM samples were prepared by placing 20 μ L of the solution on the carbon side of the TEM grid.

The Raman spectrum was acquired by placing 4 mL of the sample on a glass vial and measured from the open top under the following conditions: 532 nm laser, 10% power, 10x objective, 1 s acquisition time, 5 accumulations, 600 gr/mm grating and complete open pin holes (200 and 500 μ m –slit and hole) of the Xplora microscope.

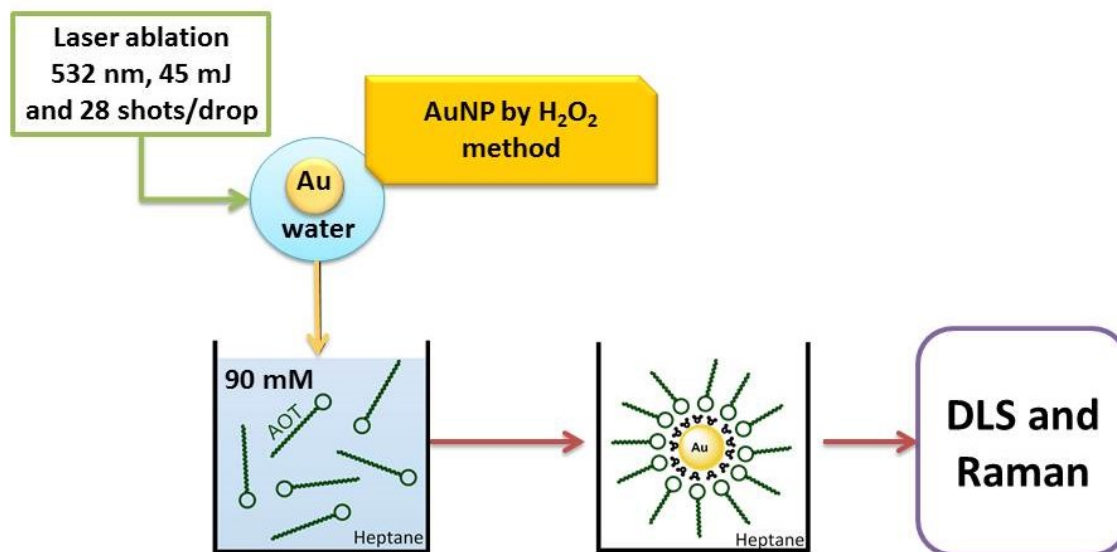


Figure 4.1. Experimental design for sample preparation of AuNP@RM. Using ablated naked AuNP in water, droplets were collected into AOT-Heptane solution (under vigorous stirring) to form the RM, followed by DLS and Raman spectroscopy analysis.

4.2. Results and Discussion

4.2.1. Drop size

The quantity of water introduced into the RM is dependent on the size of the drop. In this regard, different quantities of drops were collected and weight by triplicate. The result (Figure 4.2) is a linear plot with a slope of (15.24 ± 0.05) mg per drop and $r = 0.9999$.

The weight of the drop can be used to determine the drop volume, and considering a water density of $0.998 \text{ mg}/\mu\text{L}$ (22.1°C) the calculated volume is $\sim 15.27 \mu\text{L}$ per drop; in agreement with values previously reported for this system.⁴¹

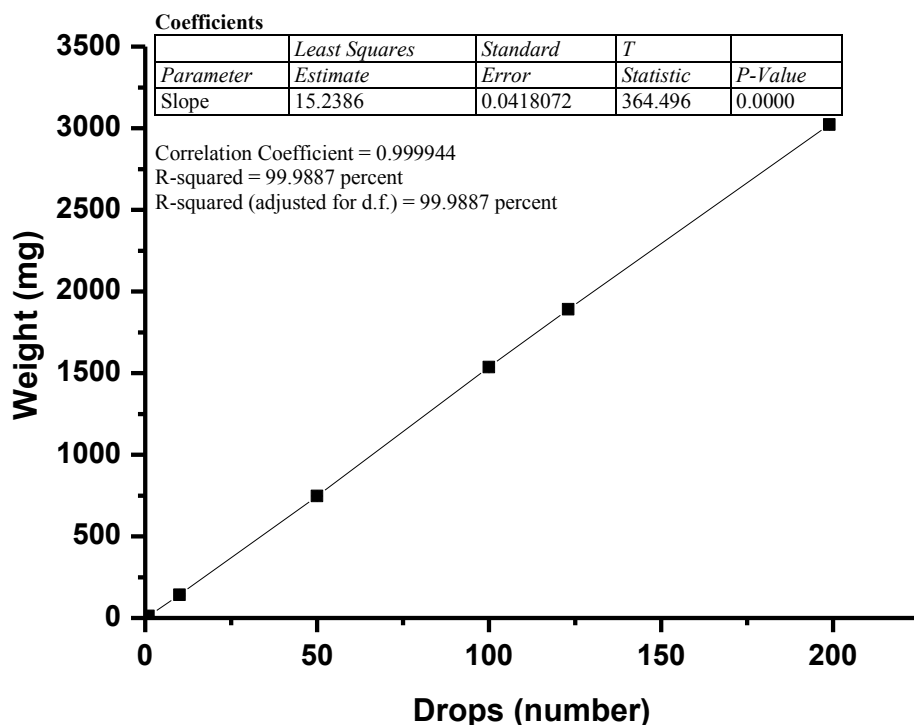


Figure 4.2. Determination of drop weight (mg/drop) in the laser ablation system.

4.2.2. *Selecting the NP, surfactant and organic solvent*

Starting with AgNP, different NP were evaluated; as it is presented in Table 4.1 where the big majority are AuNP synthesized by different methods, inside or outside of the RM.

Table 4.1 also lists a variety of organic solvents and surfactants that were evaluated. The majority of the experiments were completed using AOT – heptane; chloroform was also evaluated in order to increase the RM size, while other surfactants were evaluated in the original search.

The only combination of NP – surfactant – organic solvent that produced positive results was the AuNP prepared by the H_2O_2 method (naked, 2.2.3) with 90 mM AOT in heptane and following the procedure described in section 4.1. The DLS and Raman results from those dissolutions are presented below.

Table 4.1. Combinations of different NP synthesis method, surfactants and organic solvents used in this work. The only combination that produced positive results (water Raman signal) was AuNP (by H_2O_2) ablated and dropped on 90 mM AOT-heptane.

NP method	Surfactant	Organic solvent
AgNP by NaBH_4 inside and outside	Brij30	Heptane
AgNP by NaBH_4 inside	SDS	Iso-octane
AgNP by NaBH_4 inside and outside	AOT	Heptane
AgNP by N_2H_4 inside and outside	AOT	Dodecane
AuNP by NaBH_4 inside and outside	DDAB	Toluene
AuNP by NaBH_4 inside and outside	Brij30	Heptane
AuNP by NaBH_4 inside	SDS	Iso-octane
AuNP by NaBH_4 inside and outside	AOT	Hexane
AuNP by NaBH_4 inside and outside	AOT	CHCl_3
AuNP by NaBH_4 inside and outside	AOT	Heptane
Naked AuNP by H_2O_2 outside, before and after ablation	AOT	Dodecane
Naked AuNP by H_2O_2 outside, after ablation	AOT	Octane
Naked AuNP by H_2O_2 outside, after ablation	AOT	Heptane

4.2.3. Size analysis by DLS

All solutions were measured by DLS as indicated in section 2.3.5.1, in order to observe the approximate RM hydrodynamic size. Figure 4.3 present the variation of size vs W_0 , where it is observed that W_0 smaller than 10 presented almost the same RM size, a small increase between $W_0 = 10$ -15 and bigger increase after that.

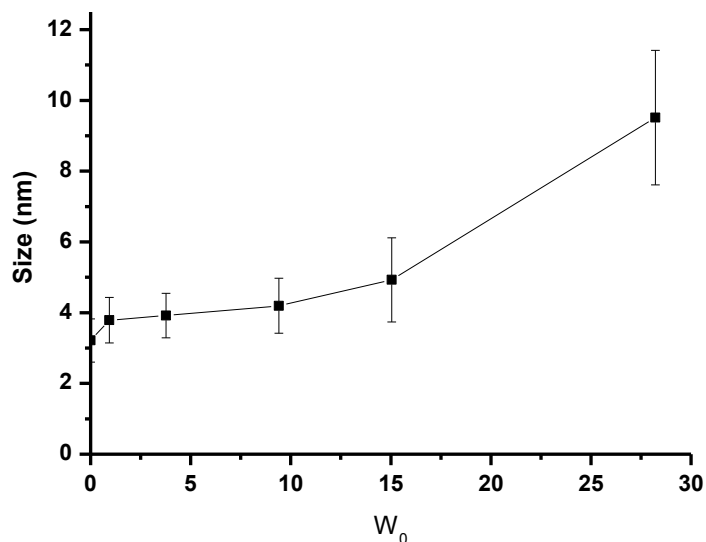


Figure 4.3. Size dependence of AuNP@RM in AOT (90 mM) – heptane RM system with W_0 . The hydrodynamic size (determined by DLS) of the RM remains the same for W_0 smaller than 10, with a small increase between 10 and 15, and bigger increase when W_0 is higher than 15.

4.2.4. TEM analysis

The DLS analysis provide very quick and reproducible information about the hydrodynamic size of the RM it is useful information resulting from a theory model that consider the NP and the layers that surrounds it. In order to evaluate the core AuNP size, TEM analyses were completed.

Due to the low concentration of AuNP in the RM and huge amount of organic material in the system; Figure 4.4 shows one of the few images that included at least one AuNP where AuNP presented sizes under 3 nm. This is consistent with having one AuNP inside the RM.

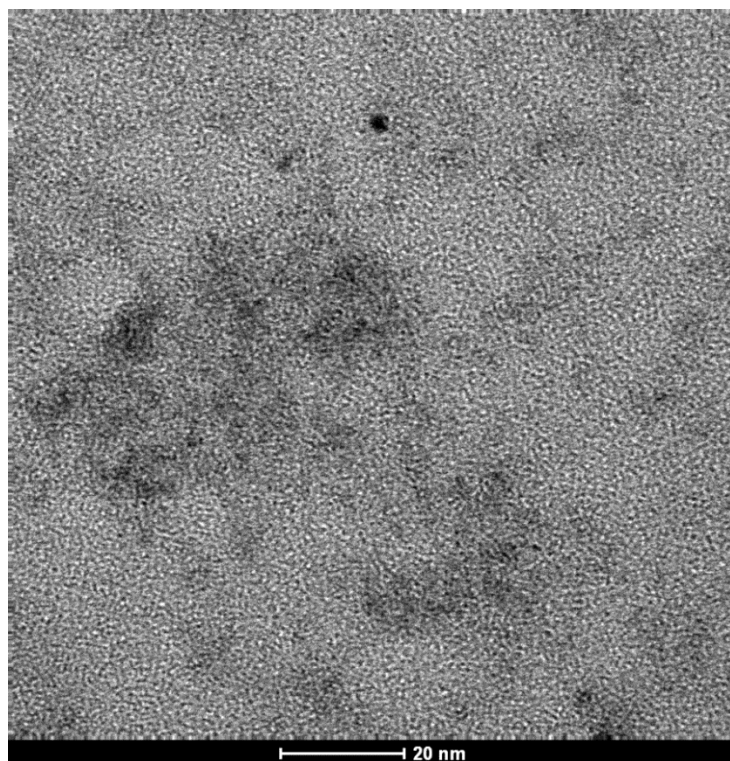


Figure 4.4. TEM image of AuNP@RM (AOT 90 mM – heptane). Scale bar: 20 nm.

4.2.5. Raman Analysis

The vast majority of the samples have a Raman spectrum that does not differ from the RM without AuNP (blank), and the Raman signal of water was observed only in the case of AOT-heptane with RM of similar size as the AuNP. Figure 4.5 presents the Raman spectrum obtained of the blank RM, AuNP@RM (H₂O 85 mM) and bulk water (55 M).

The band presented in both, the RM and AuNP@RM, located around 2900 cm^{-1} was observed in AOT and heptane, and was used as a reference with an integration area between 2788 and 3085 cm^{-1} . In the case of the H₂O band, it is observed a significant blue shift from the bulk water (O-H stretch) and in AuNP@RM was integrated between 3085 and 4035 cm^{-1} as indicated.

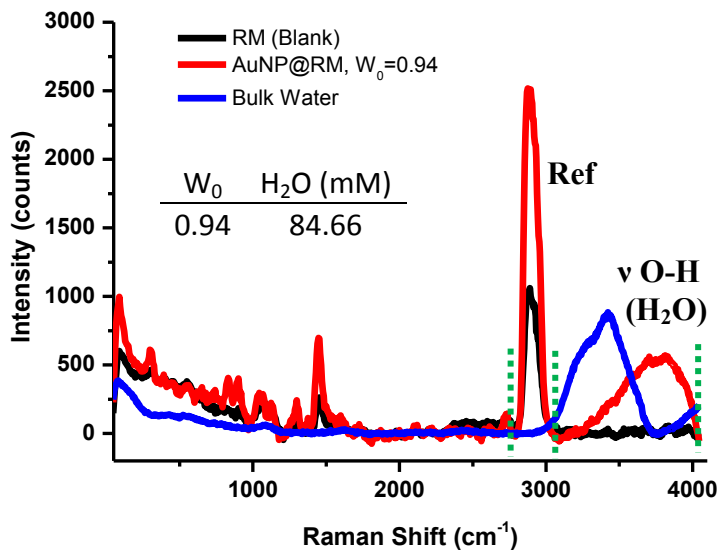


Figure 4.5. Raman spectra of a RM solution without AuNP (black), AuNP@RM with $W_0 = 0.94$ (red) and bulk water (blue).

This significant blue shift has the advantage of providing a way to easily identify and differentiate water molecules that are part of the bulk from those that are part of this system, without the need of recurring to calculation of concentration and G —even that could be completed.

It is very well known that the presence of metal NP produce changes in the Infrared (IR) and Raman peaks of a molecule.⁹ The reason why it was observed such a huge shift in the water peak could be due to the presence of the AuNP inside the RM, because the constrains that water molecules suffer inside the RM system itself, or both.

Contrary to these work observations, others studies have reported a red shift (lower energy) in the O-H band of water on a metallic surface⁶⁰ associated with the formation of an O-H $\cdots$ M hydrogen bond in a salty system (0.1 M NaClO₄). The same study calculated a very small blue shift of the O-H band when water is absorbed on Ag. While Li *et al*⁶¹ did observe a blue shift in the water peak when analysing the water inside an AOT-heptane RM by infrared (IR). They also found that the IR signal of water inside the RM is similar to the bulk only when the W_0 is higher than 16, and in fact, it was been reported that water molecules are essentially frozen⁶² when the

W_0 is close to 1. The study presented in this work involves W_0 smaller than 6, and gets maximum G at W_0 close to 1 (Figure 4.6). Since the shift observed during the realization of this project is higher than the one observed by Li *et al* in an AOT-heptane RM without AuNP, it can be deduced that the observed shift is due to the combination of the constrained system and the presence of AuNP.

As mentioned before, the evaluation of the SERS signal was carried with different W_0 ; Figure 4.6 presents the dependence of the water signal with W_0 , observing that the signal decrease at increasing W_0 . Since the AOT concentration was fixed during all the experiments, increasing W_0 reflects an increase in the concentration of water. This result can be explained since the dependence of the SERS signal with the molecule-NP distance (section 1.2 and Figure 1.4). The maximum enhancement is found when a monolayer of molecules is present on the NP surface and it is lost after adding several layers of the same molecule. In this way, increasing the W_0 could produce several layers of water on the AuNP surface and might facilitate a direct interaction of the AuNP with the internal surface of the RM (AOT heads).

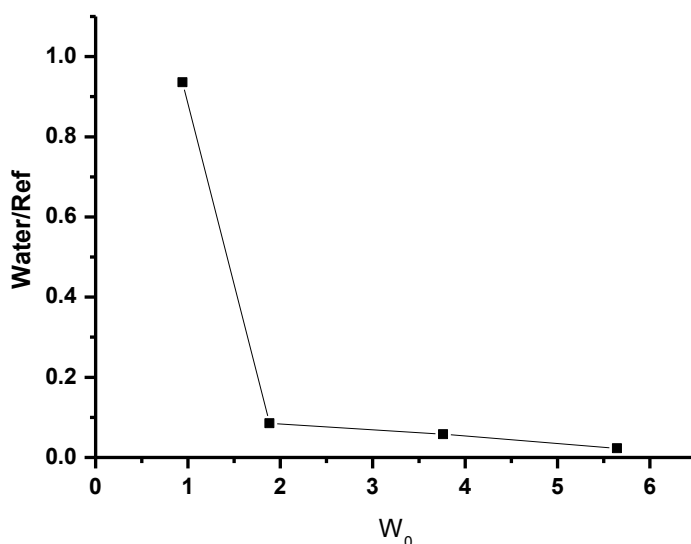


Figure 4.6. Dependence of the area of water (ν O-H) on AuNP@RM with W_0 (AOT 90 mM – heptane).

These results indicate that the interaction of water with the AuNP surface is very weak, and having any other compound leads into a more stable surface-molecule interaction. In that way, the perfect balance between AuNP size and concentration, RM size and W_0 must be achieved in order to obtain SERS. The consequence of this was lack of reproducibility. Other questions that need to be answered for this project are: quantity of RM and AuNP; and ratio of AuNP/RM

Finally, sugars and rhodamines were evaluated as probes inside the RM, without any success. The use of different Clays modified with AuNP for SERS of water and pyridine were also evaluated as part of this project. No Raman signal was observed in any of those cases. The list of Clays tested is: Kaolin (KGa-1b), Hectorite (SHCa-1), Attapulgate (PFI-1), Barasym SSM-100 (SYn-1), Na-Montmorillonite (SWy-2), Montmorillonite KSF, Montmorillonite K10 and one MCM-22 zeolite.

Since the concentration of AuNP in the RM system is an important factor, a calculation of the estimated AuNP concentrations was performed in the working system as approximately 1 pM (for $W_0 = 0.94$), low enough to explain the absence of any characteristic signal (Figure 4.7) in the UV-Vis spectrum. Thus, it was difficult to monitor the AuNP concentration.

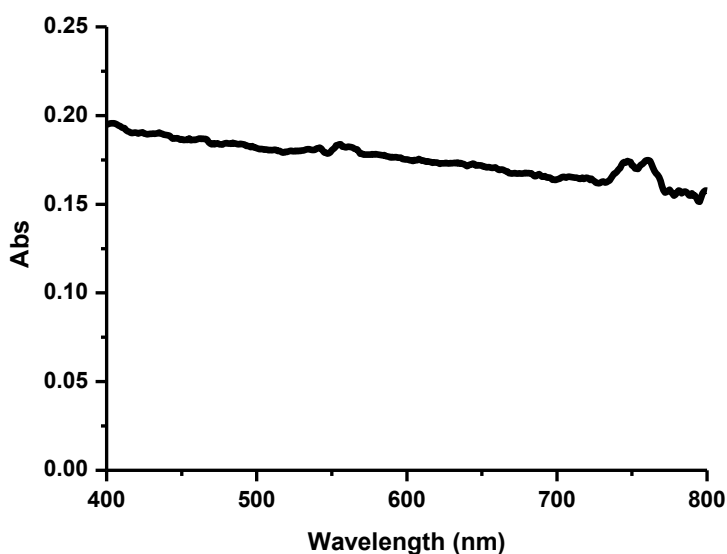


Figure 4.7. UV-Vis spectrum of AuNP@RM in a 20 cm optical path liquid cell.

Finally, the enhancement factor (G) was calculated by following the procedure mentioned in section 1.4. The integration of the O-H of bulk water (55 M) was carried between 2962 – 3751 cm^{-1} and comparing with the area of the shifted band on the AuNP@RM for the $W_0 = 0.94$ ($[\text{H}_2\text{O}] = 85 \text{ mM}$). In this way, the enhancement factor for water on AuNP@RM is 4.8×10^2 . Not surprisingly, a relatively low enhancement factor was obtained. The enhancement factor also depends on the molecule nature and water, *per se*, has low Raman absorption.

4.3. Conclusions

In a system with many variables that are difficult to monitor makes it challenging to find the optimal conditions. Some results of SERS from water molecules were obtained: The Raman water signal (ν O-H) on AuNP@RM is shifted into higher energies compared to the bulk water, indicating a higher strength on the bond that can be attributed to the combination of a highly constrained system and the presence of the AuNP inside the RM. The shift is significant enough that could easily be used for differentiation water molecules in a bulk or a RM system.

The enhancement factor has a maximum at lower W_0 and small RM size, probably when a monolayer of water molecules was located on the AuNP surface, and the AuNP is completely surrounded by water molecules limiting the interaction between AuNP-AOT.

5. Thiol modifications of gold nanostructures followed by surface enhanced Raman spectroscopy (SERS)

A typical strategy to modify the surface of gold nanoparticles (AuNP) to use thiols, since it is known that they have a strong interaction with the Au surface.⁷ While many studies involve the synthesis of molecules with a thiol ending (e.g. DNA⁶³), little is known about how quickly a ligand on the AuNP surface is modified or displaced by thiols. It is very important to evaluate and understand the kinetics of the process in order to determine when the nanoparticles (NP) are ready to be used, given the different applications of the thiol-modified gold nanostructures (AuNS). To follow this process by SERS there are at least two conditions that should be met: first, the analyte's Raman signal should be enhanced enough to measure concentrations lower than the LOD in the absence of NP; and second, the NP should not aggregate during the modification process.

Some studies have been completed in an organic solvent (such as DMF⁶⁴) to avoid aggregation. This is a simple strategy that cannot be used in many cases since changing the NP solvent could lead to its instability. Further, water is a much more desirable solvent for Raman and biological analysis. Also, it has been observed that the exchange kinetics differs in different solvents,⁶⁵ indicating that an experimental study should be completed in the desired solvent, such as water.

Gold nanorods (AuNR) are known for their instability in different media as soon as their capping agent (e.g. CTAB) is removed or exchanged. A strategy to avoid possible aggregation of AuNR is having them supported in another substrate, which would provide the possibility of working with different thiols, a larger range of concentrations and different solvents if required.

The best way to monitor if there is any aggregation present during the surface modification in solution is to record the UV-Vis spectrum of the system, since aggregation of AuNR or AuNP is denoted by a red shift of the plasmon band.^{66,67}

As previously mentioned, AuNR are covered by CTAB from the synthesis process.⁴³ It is known that CTAB binds better along the length of the rod due to better interaction with the Au(100), in contrast with the tips where the gold facet is predominately Au(111).^{26,65,68} This is one of the reasons why AuNR are usually more reactive at the tips. In contrast, spherical AuNP do not present defined facets,⁶⁵ and thiols do not have a preference for binding on a particular gold facet, just like the citrate present on the AuNP surface after synthesis⁴² that do not have a facet preference either.^{26,65} Therefore, the modification of rods is expected to occur first on the tips, and then along the rod length; while in the case of spherical AuNP there is no preference and the modification should occur simultaneously around the surface.

Some studies have been completed in the modification of AuNS with thiols. It has been shown that the kinetics and activation energy is affected by the solution pH,^{69,70} not a surprising result since the actual thiol structure (S-H bond) is pH dependent.

In this study, there are two types of molecules tested; small molecules that can fit between the CTAB molecules on the surface of AuNR and exchange it, and aromatic molecules that have a big Raman enhancement especially for the peaks related with the ring vibration.¹³ The molecules used in this project are presented in Figure 5.1.

It has been suggested that thiols form a covalent-like bond with Au;⁷¹ respecting this, the bibliography is contradictory since the peak that has been reported as Au-S ($\sim 254\text{ cm}^{-1}$) bond in some studies^{70,72} is reported as C-S (combination band) in others;^{65,73} and some studies even report a Ag-S bond.⁷² In this study, Raman peaks are observed in the pure compound at the same locations that the ones described as Au-S and C-S (combination band), therefore the latter description will be used.

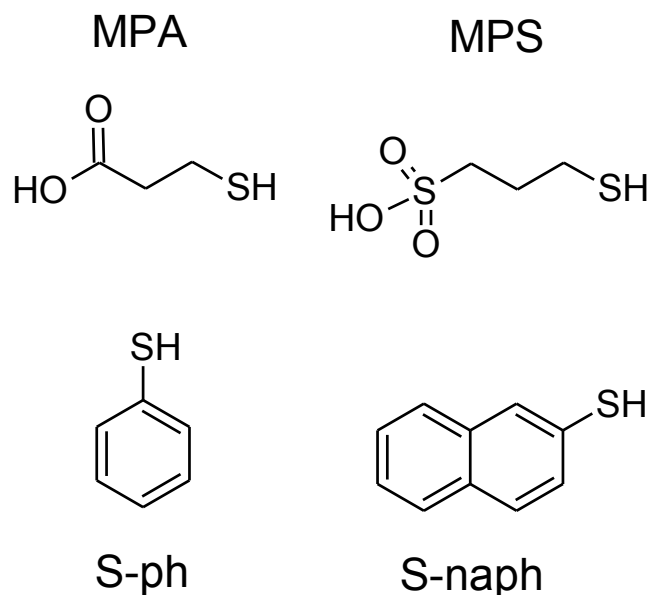


Figure 5.1. Structure of: 3-mercaptopropionic acid (MPA), 3-mercaptopropylsulfonic acid (MPS), thiophenol (S-ph) and 2-naphthalene-thiol (S-naph).

A similar discrepancy can also occur with a peak present in AuNR solutions, which is associated with Au-Br interactions by some authors^{26,65} while others reported an interaction between the CTAB positive head (ammonium group) and the negatively charged Au surface.⁶⁸ In the work presented in this chapter, the previous Au-Br interaction will be used to explain the observed Raman peak.

It is important to highlight that the interaction of the thiol with Au surface is described as a strong interaction that has preference over other Au-ligand interactions and therefore the thiol should displace any ligand present at the Au surface, and thus naked AuNP are not required for this study. Since the thiol-Au interaction is very strong, different alternatives to remove thiols from gold surface had been developed including a recent proposal by the Scaiano group to remove thiols from AuNP⁴² that involves the use of lanthanide salts.

Finally, this project proposes the study of the modification of AuNS surface followed by Raman Scattering in three different systems: 1) AuNP in water solution; 2) AuNR in water solution and 3) AuNR supported in APTES functionalized glass coverslips. Additionally, the elimination of thiols from AuNP surface by the addition of a lanthanide (Yb^{3+}) was also evaluated.

5.1. Experimental

In this work AuNP and AuNR synthesized according to the method presented in sections 2.2.4 and 2.2.5, respectively, were used to monitor the modification of the gold surface with a thiol. The AuNP are covered with citrate (from synthesis) and have a mean size of 65 nm and calculated concentration of 31 pM, while the AuNR are covered with CTAB (from synthesis) and have an aspect ratio of 1:3 and calculated concentration of 1.5 nM.

Different thiols were tested, including small thiols like: 3-mercaptopropionic acid (MPA), sodium 3-mercapto-1-propanesulfate (MPS) and aromatics like: thiophenol (S-ph) and 2-naphthalene-thiol (S-naph). Stock solutions of MPA, MPS and S-ph were prepared and diluted in MQ water daily; while the stock solution of S-naph was prepared in EtOH and kept in the fridge for a maximum of one week, followed by an appropriate daily dilution in a 1:1 EtOH/Water mixture.

The test was completed in two different media, in 1:10 dilution of the AuNP or AuNR solution and in AuNR supported in an APTES functionalized glass coverslip, just like the ones used in the MB project (section 3.1). In this case, the APTES modified glass coverslips were placed in contact with the AuNR concentrated solution for 2 h (washed with MQ water and dried with air), after that the coverslips presented a blue color and good UV-Vis spectra (Figure 5.2 and Figure 5.3, respectively) indication of the presence of AuNR in high concentration.

The thiol-modification was monitored by two methods: SERS and UV-Vis spectroscopy. The SERS spectra were recorded with the instrument described in section 2.3.2 and the following acquisition conditions: 785 nm laser (25% power), 5 s integration time, 10 accumulations per spectrum and 60 s measurement interval time. The areas of different peaks were calculated by the software LabSpec (HORIBA) between two valleys where all the spectra converge as indicated below. In the case of the kinetics followed by UV-Vis kinetics, the spectra were recorded every 60 seconds and following the maximum absorbance at 554 nm for AuNP and 755 nm for AuNR.

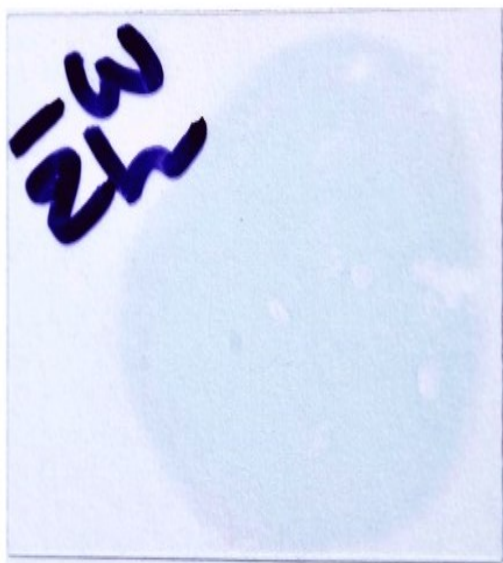


Figure 5.2. Picture of an APTES functionalized glass coverslips modified with AuNR. The coverslips takes a blue color (in dry conditions) because of the modification with AuNR. Top-left marking is a sample identifier.

For the displacement of thiols, solutions of Yb^{3+} were prepared daily in MQ water from YbCl_3 , and added to the AuNP previous modified with 100 nM of S-ph. All kinetics experiments were fitted using the algorithm BoxLucas1 in the software OriginPro 8, corresponding to the first-order Langmuir adsorption model, as follows:

Equation 7. First order Langmuir absorption model.

$$\text{Area} = A(1 - e^{-k't}) \quad \text{where } k' \text{ correspond to the observed rate constant.}$$

5.2. Results and Discussion

5.2.1. UV-Vis spectra of AuNS and Raman spectra of pure compounds

All materials were characterized before the kinetics was examined. The UV-Vis spectra of AuNP and AuNR are presented in Figure 2.4 and Figure 2.5, respectively. In the case of supported AuNR (Figure 5.3) they present two maxima located at 512 nm and 678 nm corresponding to the transversal and longitudinal band, in contrast with the AuNR in solution (Figure 2.5) where the bands are located at 513 and 755 nm, respectively. The change in the position of the longitudinal band corresponds to the change in the diffraction index of the medium and has been previously studied.^{15,74}

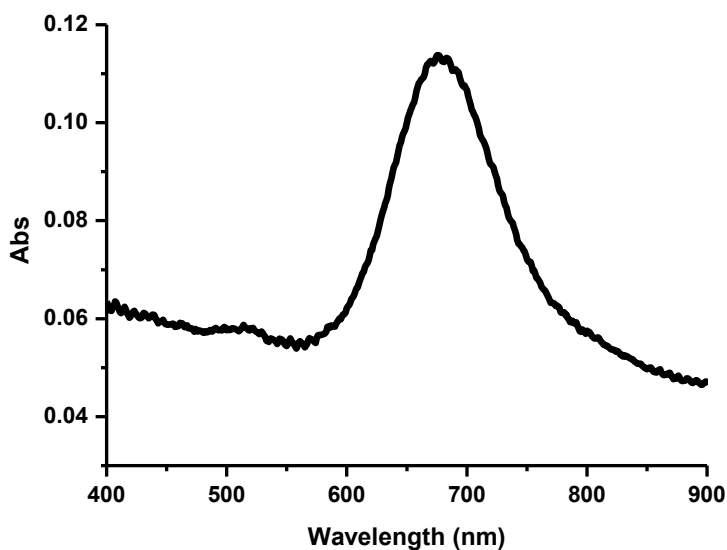


Figure 5.3. UV-Vis spectrum of AuNR supported on APTES functionalized glass coverslip (dry). The location of the plasmon band is slightly blue shift with respect to the same AuNR in water solution, effect produced by the change in the refraction index of the media.^{15,74}

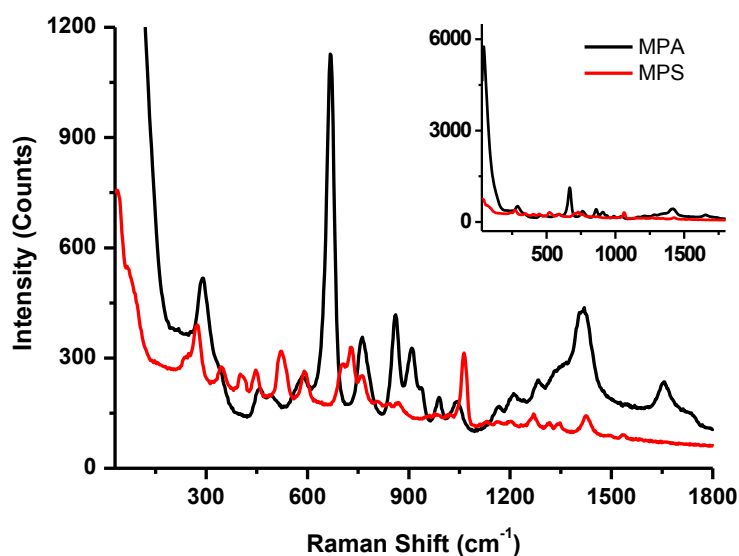


Figure 5.4. Raman spectra of pure compounds: 3-mercaptopropionic acid (MPA) and sodium 3-mercapto-1-propanesulfonate (MPS). Insert: complete intensity range of the spectra, where it can be observed the high intensity peaks located under 100 cm⁻¹.

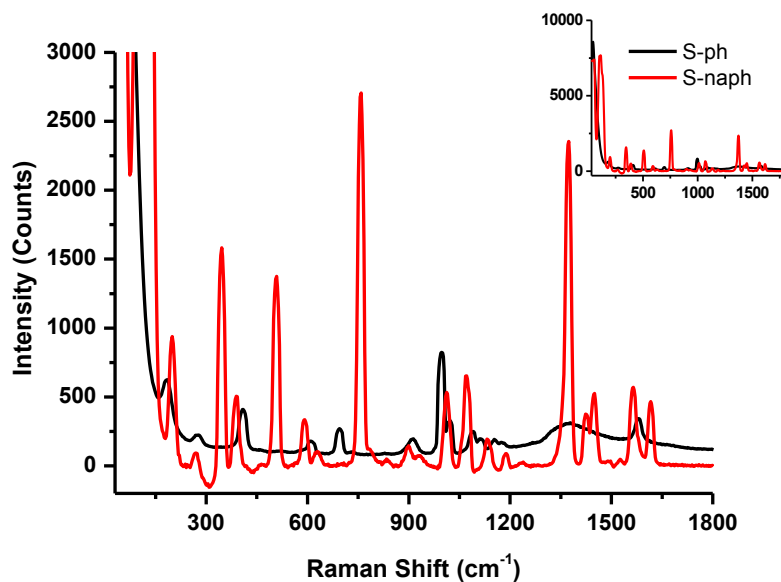


Figure 5.5. Raman spectra of pure compound: thiophenol (S-ph) and 2-naphthalene-thiol (S-naph). Insert: complete intensity range of the spectra, where it can be observed the high intensity peaks located under 150 cm⁻¹.

The selected thiol-based molecules, specifically, two linear molecules (MPA and MPS) and two aromatic (S-ph and S-naph) were characterized by Raman spectroscopy (Figure 5.4 and Figure 5.5). It can be noticed that MPA and S-ph have a very intense peak located below 100 cm^{-1} , while S-naph has two peaks below 150 cm^{-1} . However, as shown later in Figure 5.19 and Figure 5.22, the AuNR (in solution and supported) also presents a peak in that region, and therefore these signals were not useful for kinetics analysis.

The assignment^{65,73} of the different peaks observed in the Raman spectra was completed and is presented in Table 5.1; where the values presented between parenthesis are the location of the peak in the pure compound. Notice that the peak corresponding to the strength of the C-S was not observed in S-ph on AuNS, while the bending of the C-S in MPA was nor observed in the pure compound.

Table 5.1. Raman assignment of major peaks in different compounds on AuNS surface and (pure).

Assignment	Peak Location (cm^{-1}) on AuNS and (Pure)		
	Compound		
	MPA	S-ph	S-naph
$\delta_{\text{C-S}}$ (combination)	258 (288)	258 (271)	284 (264)
$\delta_{\text{C-S}}$ (and γ_{ring})	404 (--)	408 (413)	357 (386)
$\nu_{\text{C-S}}$	683 (664)	-- (993)	754 (757)
$\delta_{\text{C-H}}$	891 (865)	1067 (1071)	1054 (1070)
$\nu_{\text{C=C}}$ (ring)	-- (--)	1565 (1584)	1370 (1374)

5.2.2. Thiol-modification followed by UV-Vis (AuNR and AuNP in solution)

As previously mentioned, the challenge of working with gold nanostructures (AuNS) in solution is that they must remain in the solution without any aggregation in order to ensure that any SERS enhancement is produced by the modification of the surface with the thiol and not particle aggregation. For this goal, 1:10 diluted solution of AuNR and AuNP (section 2.2.5 and 2.2.4, respectively) was found as the optimal dilution to monitor the modification after adding different concentrations of thiol.

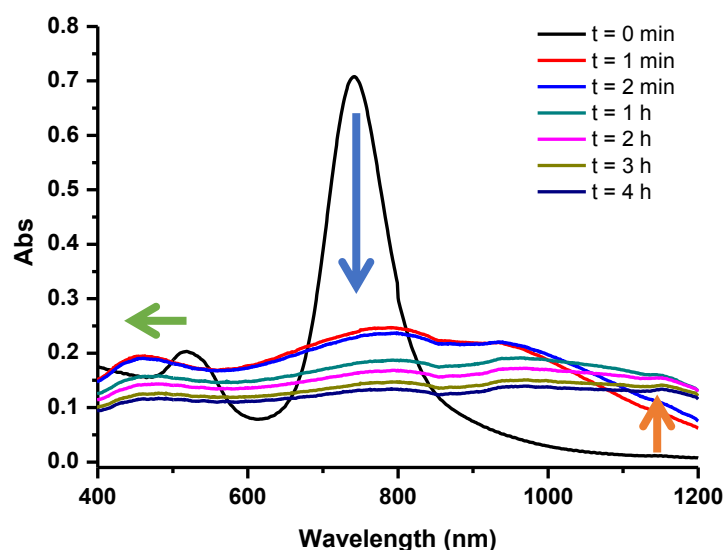


Figure 5.6. Selected spectra from UV-Vis kinetics experiment of AuNR in solution with 1 mM MPA, monitoring for 4 h. The spectrum change dramatically within the first minute, decreasing the intensity of the longitudinal plasmon band, while there is a shift in the transversal band and the increase in absorbance at 1150 nm.

Figure 5.6 presents selected spectra corresponding to 4 h kinetics followed by UV-Vis (each spectrum was taken, 1 min apart) for the modification of AuNR with 1 mM MPA. As can be observed, the spectrum changed dramatically within the first minute, indicating a very rapid modification of the AuNR tips, since the longitudinal band decreases in intensity and shifts to longer wavelengths (from 755 to 790 nm), at the same time that the AuNR assembly⁷⁴ (increase in absorbance at 933 and 1150 nm) using the MPA as a link between two AuNR -similar to what it was presented for the CB case (Figure 3.5). The transversal band is also affected, presenting a shift to shorter wavelengths (from 513 to 457 nm).

The use of S-ph with AuNR was more promising, as can be observed in Figure 5.7 where the UV-Vis spectra remains basically the same after 4.5 h of kinetics; indicating the that the colloidal system remains in solution during the modification process and therefore suitable for monitoring by SERS.

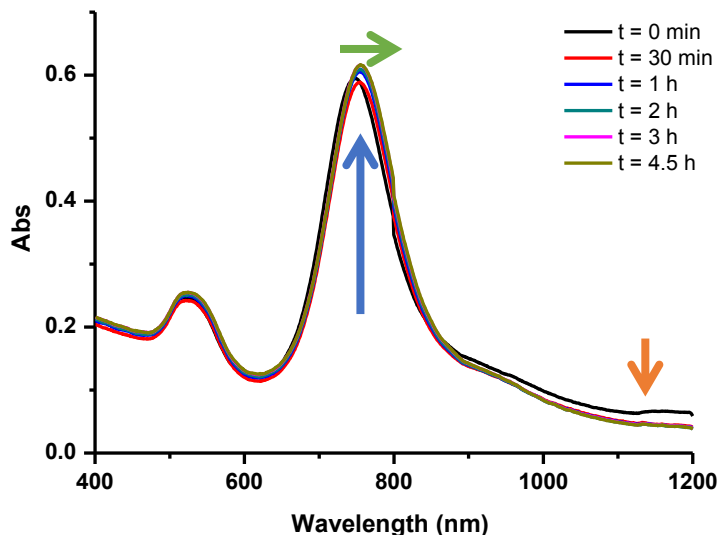


Figure 5.7. Selected spectra from the UV-Vis kinetics experiment of AuNR in solution with 1 mM S-ph, monitoring for 4.5 h. The spectrum remains basically the same during all the time, just slightly decrease of absorbance at 1180 nm while there is a small red shift in the longitudinal band, this small change could be produced only by the surface modification and does not indicate aggregation.

The second nanostructure evaluated was AuNP. In this case, thiophenol (S-ph) was evaluated in a lower concentration (100 nM). As can be seen in Figure 5.8, very little change in the UV-Vis spectra was found in 4 h kinetics. The last thiol test with AuNP was S-naph. As can be seen in Figure 5.9, the maximum located at 551 nm decreases in intensity while a little of aggregation is found (increase of absorbance at 700 nm) within the 3 h of kinetics monitoring. This result, although not optimum, it is still good to evaluate the system by SERS.

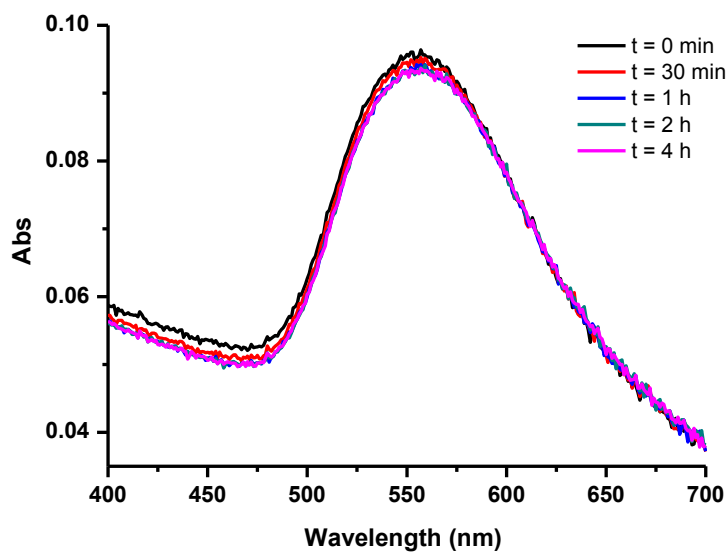


Figure 5.8. Selected spectra from the UV-Vis kinetics experiment of AuNP in solution with 100 nM S-ph, monitoring for 4 h. The spectrum remains the same during all the experiment.

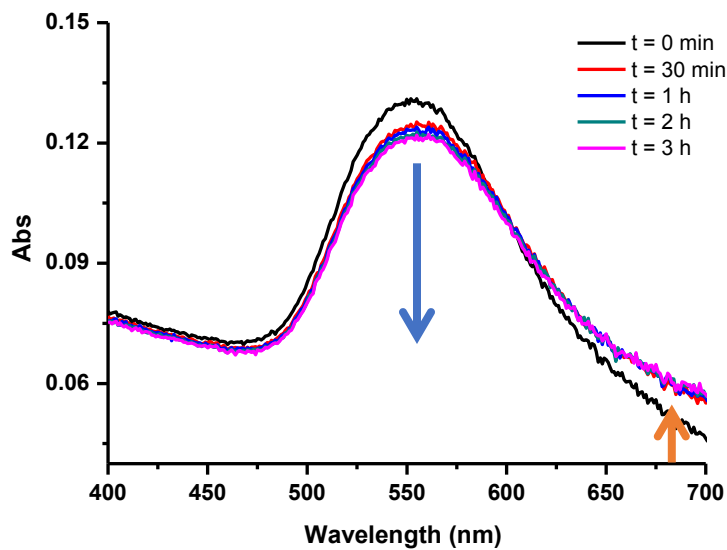


Figure 5.9. Selected spectra from the UV-Vis kinetics experiment of AuNP in solution with 100 nM S-naph, monitoring for 3 h. There is a small early decrease in the plasmon band intensity while increasing the absorbance at 700 nm.

5.2.3. AuNP in solution, thiol-modification followed by SERS

Two different thiols were evaluated with AuNP: the thiophenol (S-ph), used also with AuNR, and 2-thiol-naphthalene (S-naph). In the case of S-ph on AuNP, the S-ph signals increased with the thiol concentration (Figure 5.10) until about 220 nM, after which the signal started decreasing and concentrations higher than 300 nM produced very poor signals, similar to what was obtained with a 40 nM concentration.

When the AuNP surface is completely covered with the analyte a linear increase of the Raman signal is anticipated before arriving to a plateau. In all the Raman experiments presented in this thesis it can be observed that after a point the Raman signal decreases, which could be associated one or more theories: 1) the formation of a multilayer on the surface observing a net effect of bulk signal instead of the local signal -theorized and discussed in section 1.2, 2) precipitation of the AuNS –even that it is not observed in the UV-Vis spectrum, 3) increase of molecule-molecule interactions and successive decrease of molecule-NP interaction.

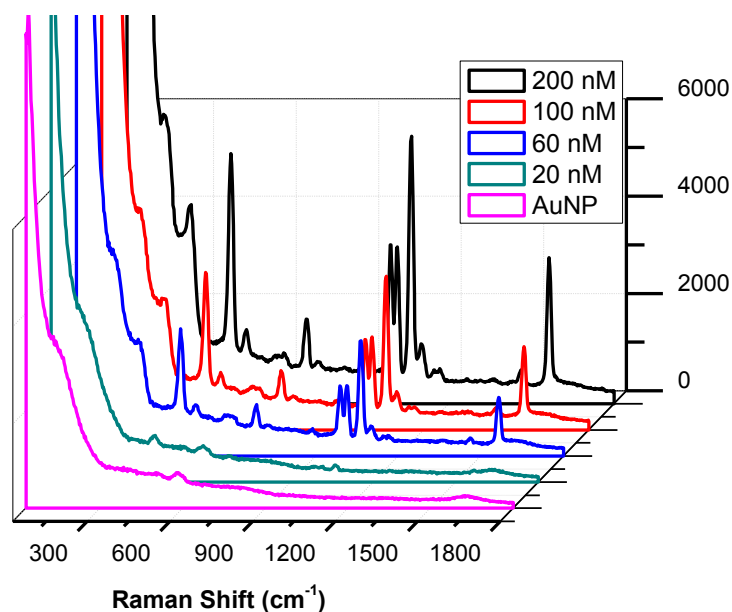


Figure 5.10. Raman spectra of AuNP after 30 min of modification with different concentration of S-ph. The intensity of the peaks increase proportional with the analyte concentration.

The effects of the different S-ph concentration were more noticeable with the Raman kinetics experiments (Figure 5.11). After about 30 min of monitoring, the signal arrived at a maximum and the behaviour was the same in all the different peaks evaluated. In agreement with the previous observation (Figure 5.10), the signal of 220 nM started decreasing after 4000 s and did not arrive to the maximum observed for 200 and 220 nM when the S-ph concentration was higher than 250 nM.

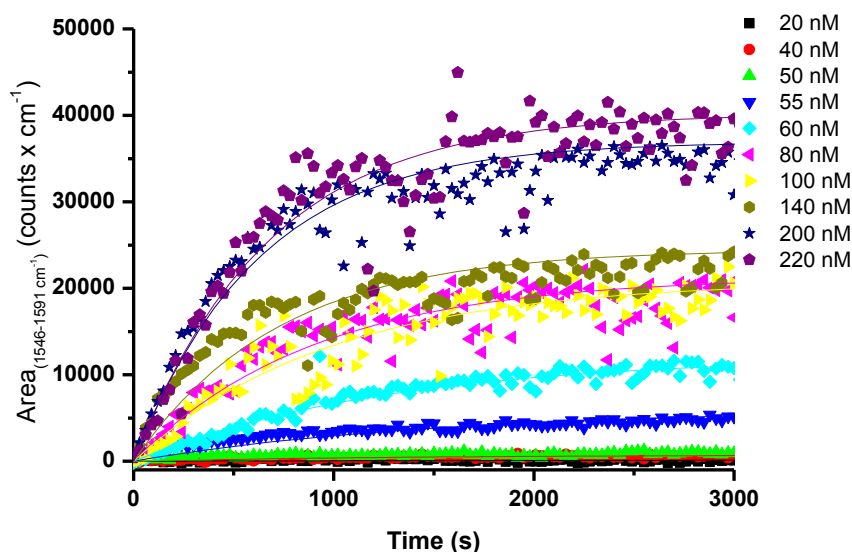


Figure 5.11. Kinetics experiment for the AuNP surface modification with different concentrations of S-ph in water, monitoring the area of the C=C peak at 1565 cm^{-1} .

After doing the fit for the first order Langmuir in the evaluated peaks, it can be observed that the constant increased with the concentration (Figure 5.12) until about 200 nM when it reach the saturation point. A linear fit of the plot is found in Table 5.2 (ignoring the 220 nM point), where it is noted the similar constants obtained between the different peaks with an average value of $1.44 \times 10^{-5} \text{ s}^{-1} \text{ nM}^{-1}$.

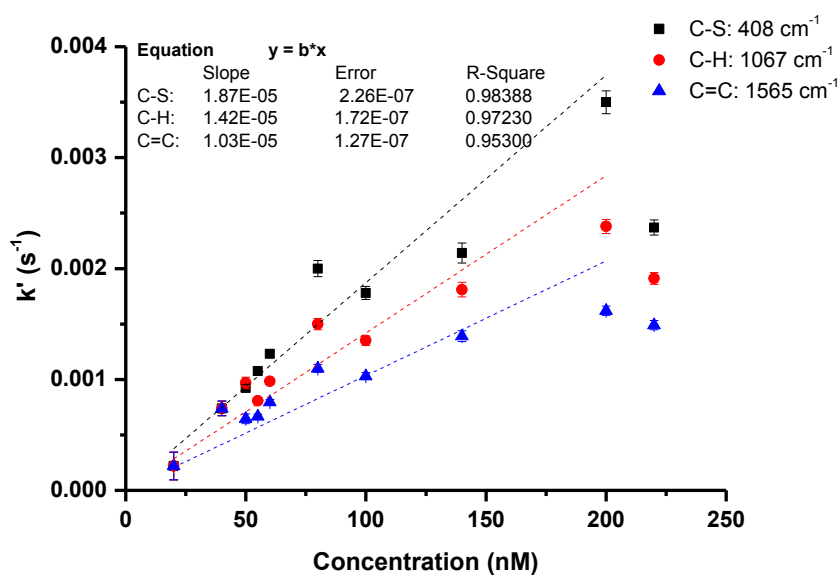


Figure 5.12. Variation of the observed kinetics constant for the modification of AuNP surface by different concentration of S-ph, monitoring the area of three different peaks. Fit from 20 to 200 nM of S-ph, excluding the last point (220 nM).

Table 5.2. Dependence of the constant rate (k) with the thiol peak, after linear fit of observed rate (k') vs S-ph concentration. Fit from 20 to 200 nM of S-ph.

Peak	k (s ⁻¹ nM ⁻¹)	Error	Adj. R-Square
C-S: 408 cm ⁻¹	1.87×10^{-5}	2.26×10^{-7}	0.98388
C-H: 1067 cm ⁻¹	1.42×10^{-5}	1.72×10^{-7}	0.97230
C=C: 1565 cm ⁻¹	1.03×10^{-5}	1.27×10^{-7}	0.95300

In the case of S-naph, the decrease in the signal was observed after just 200 nM, as can be appreciated in Figure 5.13 where some of the peaks of 200 nM already have a lower intensity than the peaks at 100 nM. This result is not surprising since the S-naph is a bigger molecule and the AuNP surface must be completely covered with a lower concentration of S-naph than S-ph.

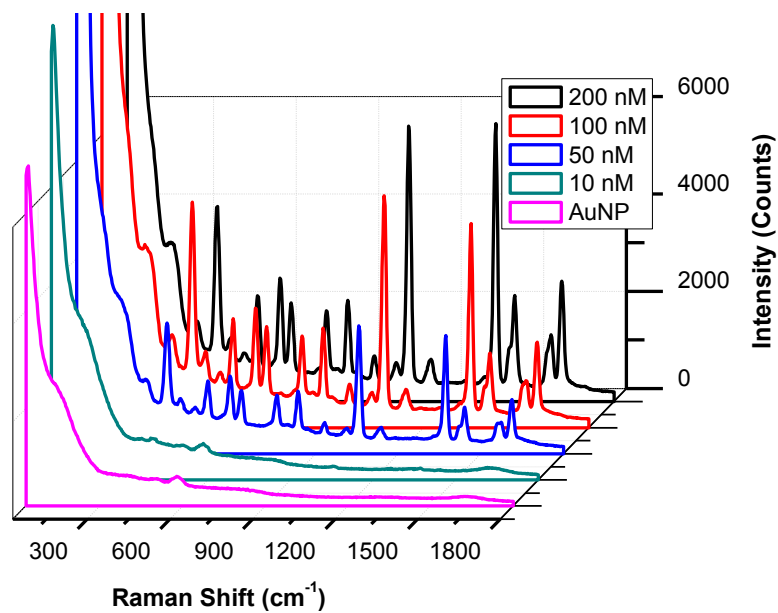


Figure 5.13. Raman spectra of AuNP after 90 min of modification with different concentration of S-naph. The intensity of the analyte signal increases with concentration reaching a maximum after which the signal decreases as can be observed for the C-S peak (357 cm^{-1}) of 200 nM that is less intense than the same peak with 100 nM,

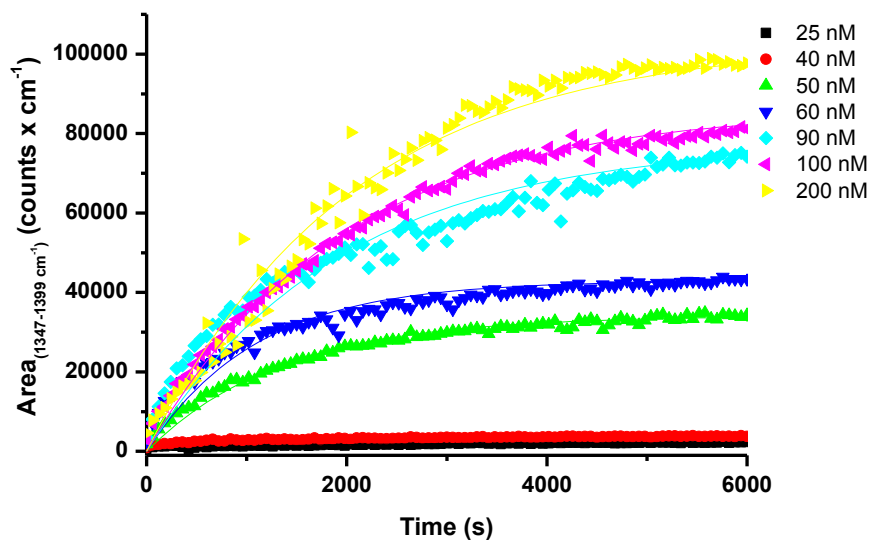


Figure 5.14. Kinetics experiment for the AuNP surface modification with different concentrations of S-naph in water, monitoring the area of the C=C peak at 1370 cm^{-1} .

The SERS kinetics of S-naph also shows a good exponential profile (Figure 5.14), needing at least 90 min to reach the maximum, which again indicates the difference of using a bulkier analyte.

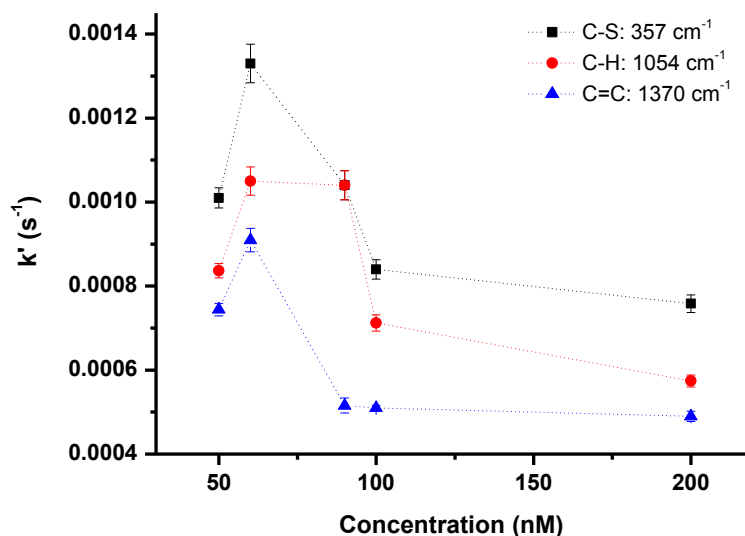


Figure 5.15. Variation of the observed kinetics constant for the modification of AuNP surface by different concentration of S-naph, monitoring the area of three different peaks.

The fit of the kinetics is presented in Figure 5.15. At difference from S-ph, the observed rate for S-naph does not increase or decrease with the concentration of analyte. The behavior is basically random indicating that the rate is controlled by the molecules diffusion, in agreement with previous studies.^{69,72}

5.2.4. Thiol displacement by Lanthanides

As previously mentioned, Lanthanides have been used for removing the thiols from a gold surface. In that way, the Raman spectrum of a solution of AuNP that was previously modified with 100 nM of S-ph was evaluated with 100 μ M of YbCl₃. The result presented in Figure 5.16

is very promising since the loss of thiols should lead to a decrease in the Raman intensity, as observed.

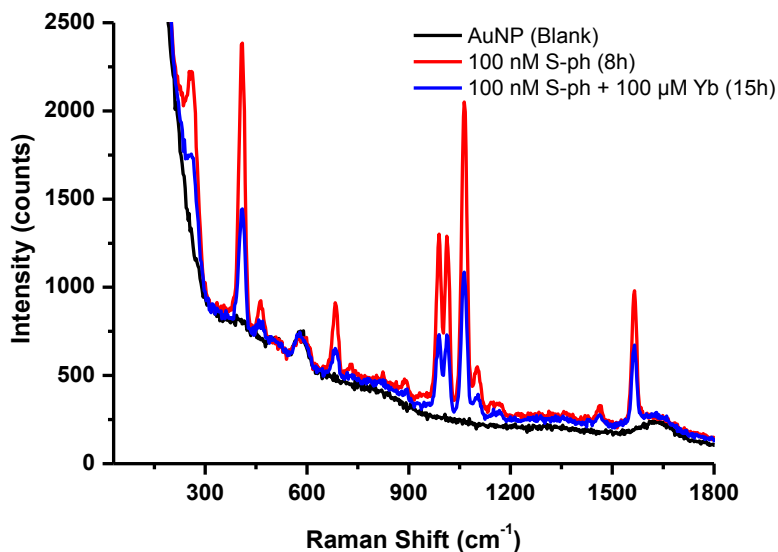


Figure 5.16. Raman spectra of AuNP after modification of surface with 100 nM of S-ph and after displacement of the thiol with 100 μM of YbCl_3 .

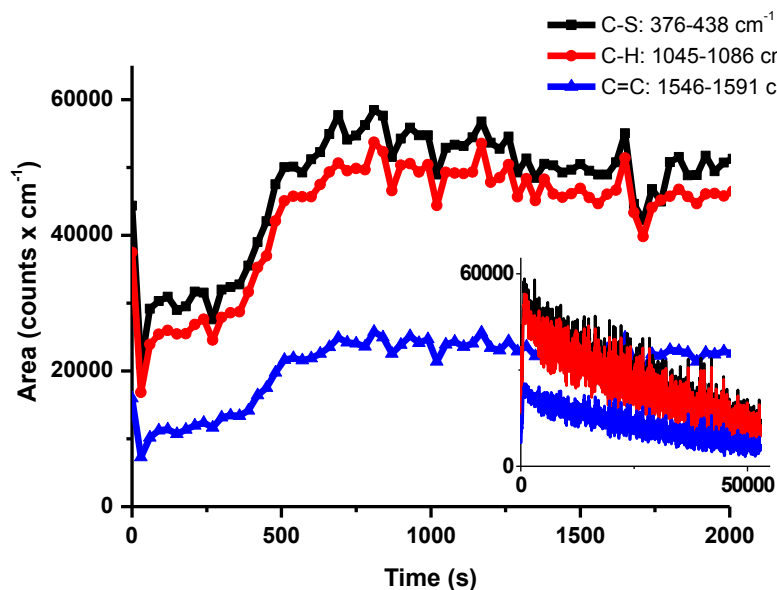


Figure 5.17. Raman kinetics of the displacement of S-ph from AuNP surface with 100 μM of YbCl_3 . Insert: complete time dependence of the signal, it can be observed that after reaching a maximum, the Raman signal decrease with the precipitation of AuNP.

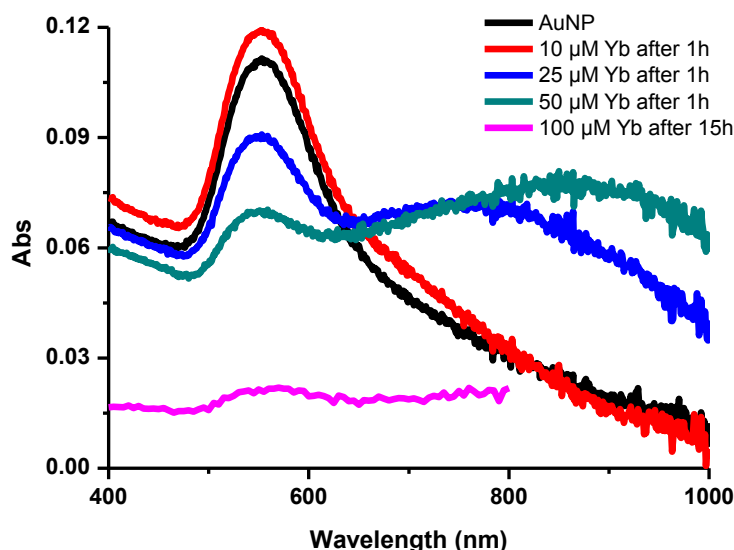


Figure 5.18. UV-Vis spectra of AuNP after displacement of S-ph from the surface with different concentrations of YbCl_3 .

The kinetics of the thiols displacement was followed by Raman Scattering (Figure 5.17) and it was observed that the signal increased within the first few minutes, followed by a slow decrease in the signal while the AuNP precipitated.

The issue is that the agglomeration of the AuNP produces an enhancement in the signal therefore the agglomeration should be avoided and different concentrations of Yb^{3+} were evaluated. As can be observed in Figure 5.18, with only 10 μM YbCl_3 solution there was little change in the UV-Vis spectra, indicating that little to no precipitation of AuNP is present in the system and therefore the possibility to follow the kinetics by SERS.

Unfortunately, 10 μM of Yb^{3+} did not produce any change in the Raman signal, while all others concentration evaluated produced results similar to the ones presented in Figure 5.16, where there was an increase in the signal followed by a slow decrease.

5.2.5. AuNR in solution, kinetics followed by Raman scattering

The experience with the UV-Vis kinetics of MPA (Figure 5.6) indicates that the tips of the rods modify very quickly; also, it was noted indications of undesired aggregation. Although Raman kinetics was evaluated with different concentrations of MPA; generally, very little enhancement was observed in the different concentrations. As can be observed in Figure 5.19, just a few peaks are developed during the kinetics and the more intense ones are located at 404 and 891 cm^{-1} and previously assigned (Table 5.1).

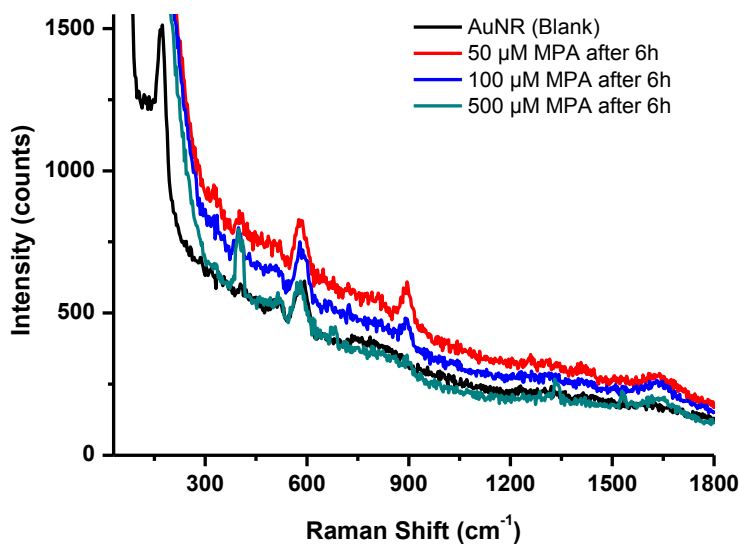


Figure 5.19. Raman spectra of AuNR before (Blank) and after modification with different concentrations of MPA.

In the case of the S-ph, more quantity and intense peaks are developed during the kinetics, as observed in Figure 5.20 that includes some Raman spectra before and after 4 h of kinetic, where the three more intense peaks were monitored during the modification (408, 1067 and 1565 cm^{-1} ; Table 5.1).

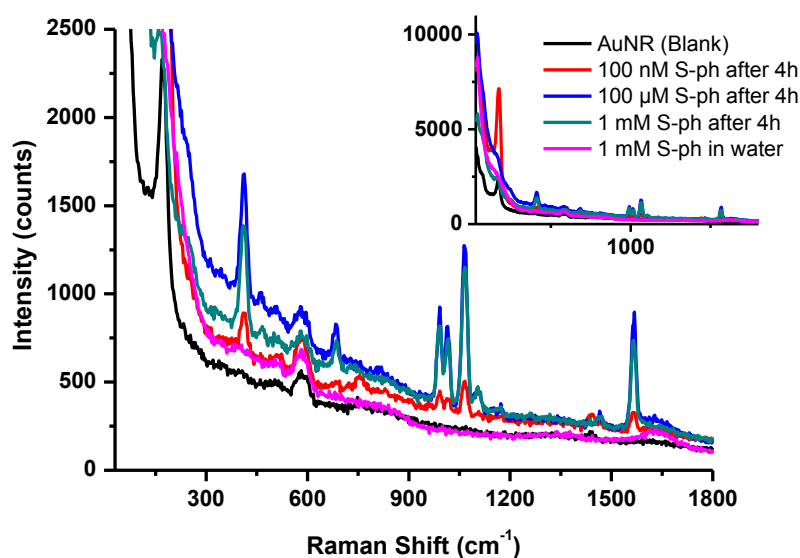


Figure 5.20. Raman spectra of AuNR before (Blank) and after modification with different concentrations of S-ph and Raman spectrum of 1 mM S-ph in water for reference. Insert: complete intensity range where it can be observed the high intensity of the peak located below 300 cm^{-1} , including the Au-CTAB peak.

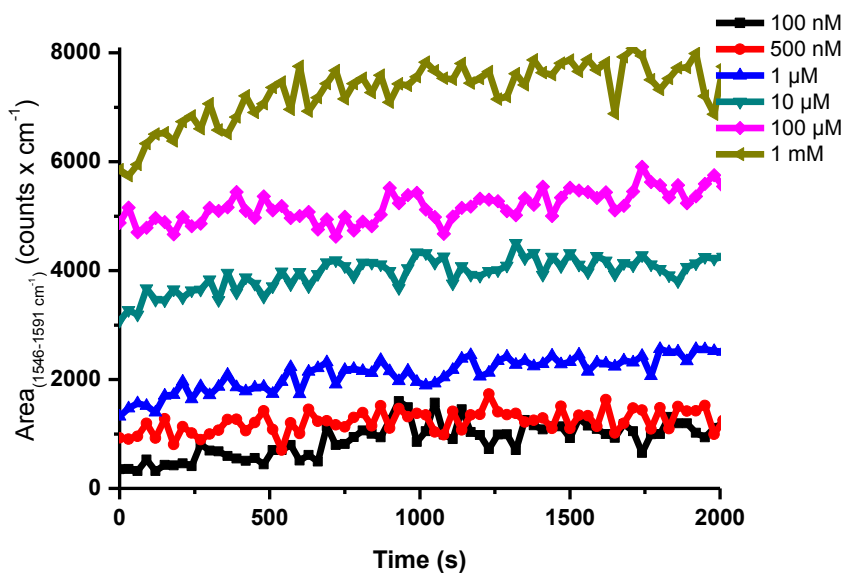


Figure 5.21. Time dependence of Raman signal for the AuNR surface modification by different concentrations of S-ph in water, monitoring the area of the C=C peak at 1565 cm^{-1} . The modification occurs very quickly in the AuNR tips and only very high concentrations of S-ph (1 mM) show kinetics behavior.

It can also be observed that a solution of 1 mM S-ph in water (Figure 5.20) does not have a Raman signal different from water itself, indicating that any signal observed during the modification is due to SERS.

On the modification with S-ph, the peak with more differences between concentrations was the corresponding to the vibration of the C=C (ring), which kinetics is presented in Figure 5.21. As can be observed, the area increased with the concentration while the modification is very fast, finishing within the first few minutes just like it was observed on the UV-Vis kinetics of MPA (Figure 5.6).

The rate of the modification of AuNR surface makes it very difficult to monitor and it probably happen in the tips as preference. The facet of AuNR on the long edge is Au(100) with strong binding to the CTAB molecules^{26,65} and becomes difficult to exchange by the thiol. Additionally, the location of the laser wavelength compared with the plasmon band indicates that changes in the tips will be observed with preference over the change in the rod body.

5.2.6. AuNR supported on APTES functionalized glass coverslip

Since the experiment with AuNR in solution and MPA demonstrated a quick agglomeration of the AuNR; supported AuNP on glass coverslips were evaluated as a medium for the AuNR surface modification. In this way, different solutions of MPA and MPS in water were placed in contact with the coverslip for 4 h, washed with MQ water and dried. As can be observed in Figure 5.22, the AuNR have a peak located at 175 cm^{-1} corresponding to the interaction between bromide (Br^- from the CTAB) and the AuNR surface.⁶⁵ This peak is a useful resource in order to find a good location to monitor the kinetics modification of the AuNR on the coverslip with the Xplora microscope. Also the disappearance of this peak is an indication of ligand exchange.

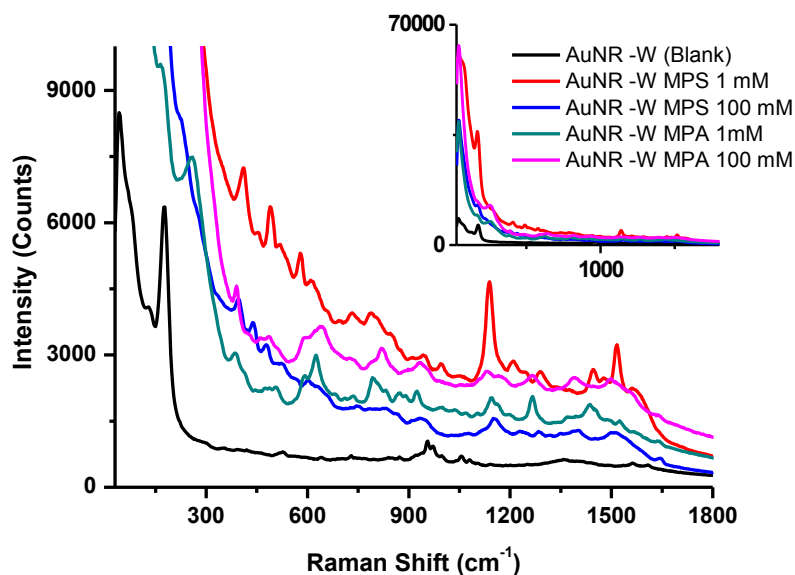


Figure 5.22. Raman spectra of MPA and MPS on AuNR supported on glass coverslip, after 4 h contact of solution with the coverslip. Insert: complete intensity range where it can be observed the high intensity of the peak located below 300 cm^{-1} , including the Au-CTAB peak.

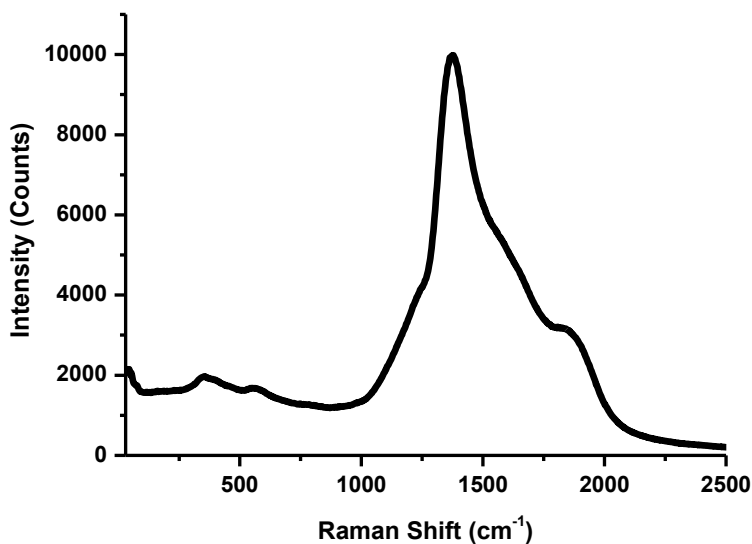


Figure 5.23. Raman spectrum of glass measured with 785 nm laser. Broad peak with maximum at 1373 cm^{-1} . The intense signal overlaps any Raman signal of molecules on glass.

In the case of MPS, with a 1 mM solution the peak of Au-CTAB is very clear, and it is also found after contact with a 100 mM solution indicating a poor exchange of the ligand in the surface. In contrast, with MPA the Au-CTAB peak disappears and peaks corresponding to the MPA are found including one located at 258 cm^{-1} in the AuNR (290 cm^{-1} in pure compound) assigned in Table 5.1. Thus, MPA was selected to monitor the modification in the following test.

Unfortunately, glass has a very high Raman signal when using 785 nm laser (Figure 5.23), that cannot be avoided, and although in the previous experiments it was not significant, after adding the drop of solution, the water worked as a lens and helped to increase the light that arrives into the sample, increasing the background signal. In cases like this, when there is a very high background signal, the modification cannot be followed using this system.

5.2.7. SERS enhancement factor

Table 5.3. Average enhancement factor (G) for different molecules in AuNR and AuNP and their relative standard deviation (%RSD, $n = 3$). Signal of the C=C peak in 5 M solution of S-ph and S-naph in THF and C-S peak in 1 M solution of MPA in water used for calculations.

Compound	Enhancement Factor (G)	
	AuNR	AuNP
S-ph	$3.70 \times 10^{11} (\pm 2.5\%)$	$2.19 \times 10^{12} (\pm 2\%)$
S-naph	--	$2.65 \times 10^{13} (\pm 2\%)$
MPA	$6.31 \times 10^4 (\pm 5\%)$	--

The enhancement factor (G) was calculated for the evaluated molecules (Table 5.3) in both AuNS following the procedure described in section 1.4 and comparing with the signal of 5 M solutions of S-ph and S-naph in THF and a 1 M solution of MPA in water. The enhancement factor for all aromatics are similar and in agreement with values reported for chemical

enhancement,²⁵ and as expected, MPA has lower enhancement and falls into the typical G values for purely electromagnetic enhancement.¹⁰

5.3. Conclusions

In conclusion, the modification of the AuNR happened very quickly in the tips where MPA and similar molecules also bind to create an arrangement that changes the hot-spots and the enhancement factor. To avoid the agglomeration observed with AuNR, the use of the supported AuNR on APTES functionalized glass cover-slips was evaluated. Unfortunately the big Raman background of glass with a 785 nm laser overlapped any signal that could be developed during the surface modification. MPA and MPS were not found as good analytes to monitor the AuNS surface modification; instead S-ph and S-naph provided excellent results with better enhancement.

In the case of AuNR, the location of the Raman laser wavelength (exciting the longitudinal plasmon band of the AuNR) is perfect to monitor the modification in the tips, but does not help monitoring the exchange in the rod body.

Also, as it was found in previous studies,^{69,72} the dynamics of the modification of AuNP with two different aromatic compounds (S-ph and S-naph) in water is controlled by the diffusion of the molecules.

Finally, the displacement of the thiols from the AuNP surface with lanthanides was evaluated. The results indicate that the initial agglomeration of the AuNP encumbered the thiols loss, by creating new hot-spots with larger enhancement of the thiols signal. In this way, it is not viable to follow the displacement of thiols with lanthanides by SERS.

6. General conclusions and future work

This thesis focuses on three different projects aimed to study interactions between molecules and the AuNP surface. The systems considered were: i) emission properties of dyes close to the Au surface, ii) Raman properties of water molecules in close proximity to Au surface, and iii) thiol modification of Au surface for different NP geometries.

The first system, composed by a dye and a macrocycle close to the Au surface was studied by fluorescence lifetime imaging microscopy (FLIM). It is known that dyes such as methylene blue (MB) form complexes with host molecules like cucurbit[7]uril (CB), which can be classified as inclusion or exclusion complexes (MB@CB). The blue shift of the emission band showed by the MB@CB inclusion complex was used as a tool to determine the position of the dye related to the NP surface. The modification of the AuNP surface with CB moieties, prior to the addition of dye, served as a spacer between the particle surface and the MB molecules. Based on the emission spectra obtained under different conditions, it was possible to determine the formation of an exclusion-type complex between MB and CB whenever the particle is present. Even if the inclusion complex is being performed, the interaction with the Au favours the exclusion complex formation on the surface.

The second system studied includes the interaction of water molecules with the Au surface, which was studied by surface enhanced Raman spectroscopy (SERS) utilizing 532 nm laser as excitation source. The test system was a reverse micelle (RM) made of dioctyl sulfosuccinate sodium salt (AOT) in heptane, where the water molecules are trapped inside the RM. The small amount of water molecules in this system (~ 90 mM) is negligible for Raman spectroscopy. However, when AuNP are introduced into the RM, SERS signals of water become significant shifted from what is expected for bulk water signal; providing a simple way to differentiate the water molecules that are close to Au surface from those found in bulk water samples. The

enhancement factor found for these water molecules on AuNP inside the RM system was around 4.8×10^2 .

Finally, the third project focused on the study of the thiol-Au surface interactions was carried out with different gold nanostructures (AuNS); specifically, gold nanoparticles (AuNP) and gold nanorods (AuNR). Different thiol molecules in solution were mixed together with the AuNS and their SERS signals followed versus time of modification using 785 nm laser as excitation source. The increase on the SERS signals of C=C, C-S, and C-H bonds helped to follow the kinetics of the surface reaction. It was determined that the surface process depends upon several conditions including ligand-exchange interactions – which depends on the passive agent previously loaded on the AuNS surface, geometry of the thiol molecules, and shape of the AuNS. In general, at least 2 h are required for complete surface modification of AuNS with a thiol. The enhancement factors found for aromatic thiols were in the order of 10^{12} , allowing analysis of small concentrations of analyte. Additionally, aromatic molecules act as better stabilizing agents, producing more stable AuNS – especially important for AuNR that are normally unstable.

In the Scaiano group, gold nanoparticles (AuNP), silver nanoparticles (AgNP) and copper nanoparticles (CuNP) are frequently used in catalytic reactions. This provides a scenario where the advances in this thesis can inspire future work. As it was mentioned before, all of them have plasmonic properties and AgNP have similar SERS enhancement than AuNP at wavelength longer than 600 nm. AgNP and CuNP can easily oxidize under air and therefore their low stability limits the expansion of their use. However, knowing that silver has a similar behaviour than gold in terms of SERS, different molecules can be evaluated to improve the stability of AgNP, such as thiophenol and 1,2-benzenedithiol⁷⁵ – molecules that have proven useful to stabilize several NP. This more stable AgNP could be tested as catalyst for reactions like the Sonogashira C–C coupling.⁷⁶ Later, the stability of CuNP could also be improved by the use of thiolate molecules, using aromatic thiol molecules as capping agent.⁷⁷ catalytic activity of this CuNP could be evaluated with a click reaction.⁷⁸ The kinetics of AgNP surface modification should be similar to AuNP, while more variations could be expected for CuNP and its kinetics can be followed by SERS.

Since during this thesis it was shown that FLIM allowed to determine the position of the molecules on the Au surface, a catalytic reaction could be completed on the surface of self-assembled AuNP with defined hot-spots. A possibility for this kind of arrangements are the gold nanoarrows (AuNArS) developed by Wang *et al*⁷⁹ from the growth of AuNR. This self-assembly nanomaterial is highly anisotropic, presenting different arrangements that could direct the catalytic reaction in one specific way. With this kind of material, it could also be interesting to measure using tip-enhanced Raman spectroscopy (TERS) – available at the Catalysis Centre – Since the Au in the AuNArS present different facets, the use of TERS could allow a direct measurement of the preference of molecules over the Au facets within the same material and evaluate if the ligands are completely exchange in the surface; additionally, it may be possible to differentiate the catalytic activity of the Au facets (Figure 6.1), for this, it could be useful to have a product molecule that after completion of the reaction would attach to the Au surface (e.g. formation of a thiol) allowing to measure the product after the reaction is complete.

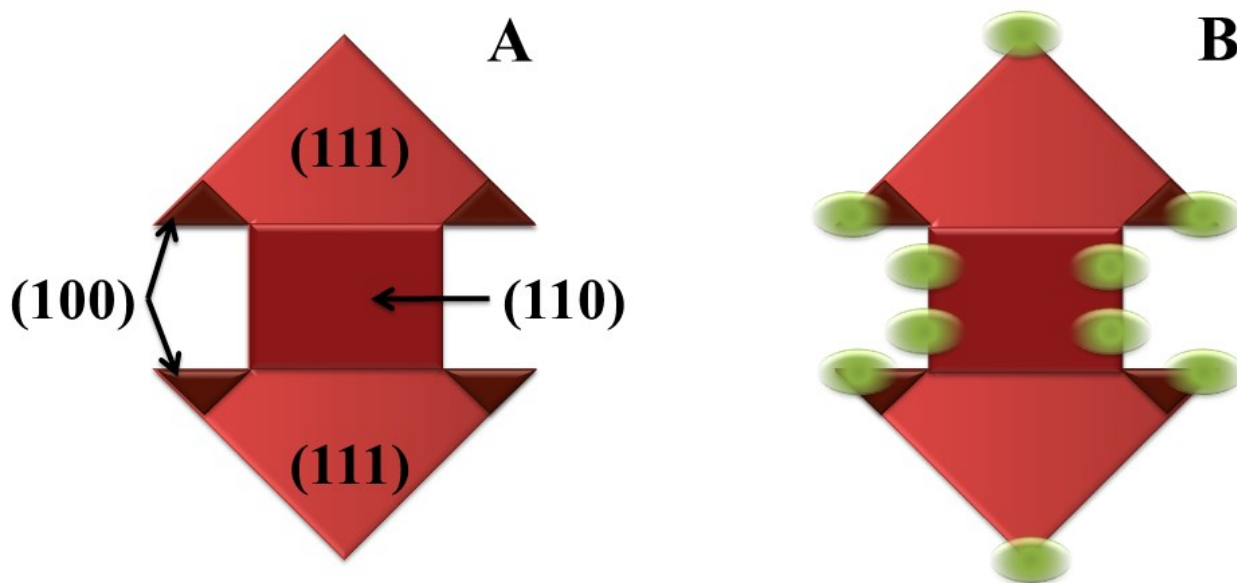


Figure 6.1. Schematic of the gold nanoarrows (AuNArS). **A:** Location of the different Au facets, on the arrows the predominant facet is Au(111), while the central body is Au(110) and the internal tips of the arrows are Au(100). Adapted from reference [79]. **B:** Predominant location of the molecules after catalytic reaction on the AuNArS surface, the molecules could be located on the tips and the central body of the AuNArS.

Nanoparticles are known for their tendency to aggregate and grow, this is especially important for small and naked NP. A way to improve the stability of the nanoparticles (NP) -specially in small AuNP- is the use of metal organic frameworks (MOF) as supporting material⁸⁰ where the AuNP would be located inside or outside the MOF pores –similar to the RM project and it could allow an ideal material for heterogeneous catalysis. The use of AuNP@MOF could allow naked (or unprotected) very small stable NP that can be used for a catalytic reactions or other purposes; in any case, it is important to know the mobility of the molecules in these new porous materials and in that way, SERS could be used to evaluate the kinetics of the system.

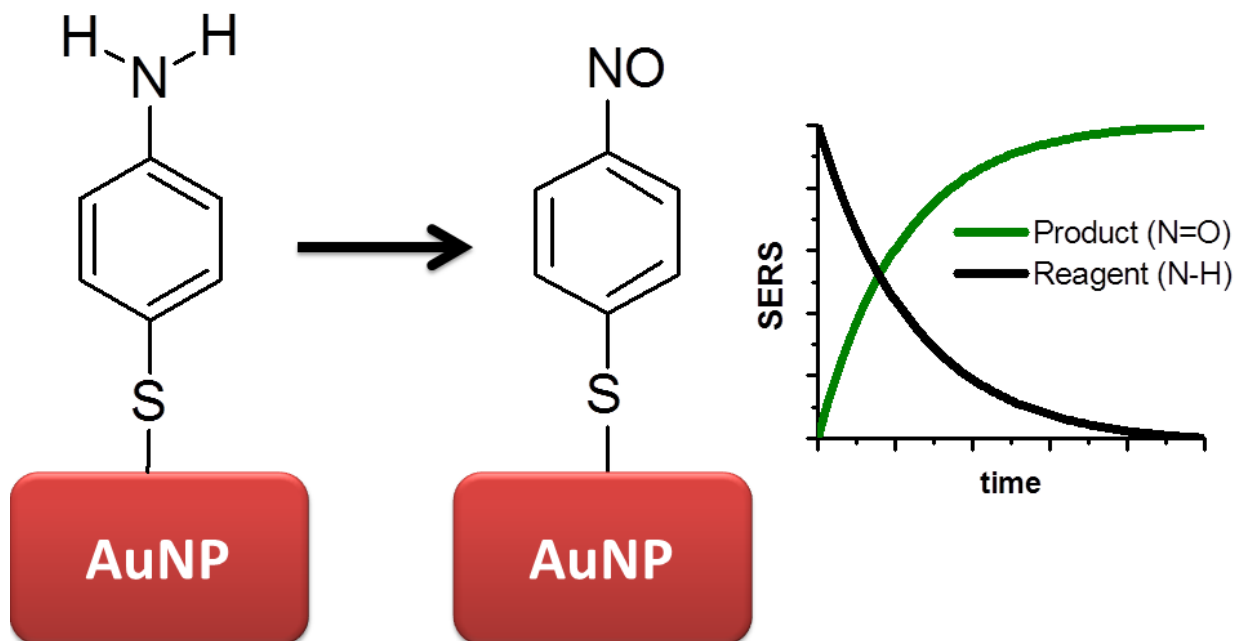


Figure 6.2. Schematic of reaction completed on gold nanoparticles supported on metal organic framework (AuNP@MOF) for the oxidation of aniline followed by surface enhanced Raman spectroscopy (SERS).

The ideal case is having the AuNP inside the MOF to form AuNP@MOF, their preparation can be completed similar to the RM project, where the AuNP can be prepared inside or outside the pores. To prepare the AuNP outside the MOF could be completed by laser ablation (section 2.2.3) and an estimated final NP size smaller than 3 nm so the AuNP could travel inside the pores after its formation; to prepare the AuNP inside the porous a method has been proposed,

similar to the preparation the AuNP inside the RM and known as a “two solvent” method,⁸¹ where the MOF is suspended in an hydrophobic solvent (e.g. heptane) and the gold and reduction agent are in an hydrophilic solvent (e.g. water) in a quantity equal or less than the internal pore volume of the MOF. In this way the procedure assures that all the AuNP are formed inside the hydrophilic internal pores of the MOF. Once the AuNP are formed inside the MOF, the material can be used to evaluate: i) the mobility of aromatic thiol molecules inside the MOF that have high SERS enhancement allowing to be followed by SERS in low concentrations, ii) experimental evaluation of the enhancement factor of this material with different molecules, after this it could be measured iii) real time catalytic activity of the AuNP@MOF monitored by time dependence SERS in solution where it could be observed the change on the peaks corresponding to the reagent to product formation (Figure 6.2). A simple reaction that could be used to test this hypothesis is the oxidation of aniline, an aromatic compound that should have very good SERS enhancement and that also should present a few Raman characteristic peaks different between reagent and product.

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