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National University of Ireland



Highly sensitive Surface Enhanced Raman Scattering (SERS) detection platforms formed by large area selfassembled Au nanorod arrays.

A Thesis Presented to the National University of Ireland for the degree of Doctor of Philosophy

by

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Supervised by Dr. Daniela Iacopino



Tyndall National Institute

University College Cork

October 2014

Declaration

The work presented in this thesis is Alfonso Martin's own work except for the dark field spectra of *Figure 88* that have been plot by Carola Schopf and the electric field enhancement simulations of *Figure 92* that have been performed by Andrea Pescaglini. The work presented in this thesis has not been submitted for another degree, either at University College Cork or elsewhere.

The present thesis is submitted for the degree of Doctor in Philosophy at University College Cork.

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Abstract

This thesis explores a new method to fabricate SERS detection platforms formed by large area self-assembled Au nanorod arrays. For the fabrication of these new SERS platforms a new droplet deposition method for the self-assembly of Au nanorods was developed. The method, based in the controlled evaporation of organic suspensions of Au nanorods, was used for the fabrication of horizontal and vertical arrays of Au nanorods over large areas (100µm²). The fabricated nanorods arrays showed a high degree of order measured by SEM and optical microscopy over mm² areas, but unfortunately they detached from the support when immersed in any analyte solutions. In order to improve adhesion of arrays to the support and clean off residual organic matter, we introduced an additional stamping process. The stamping process allows the immobilization of the arrays on different flexible and rigid substrates, whose feasibility as SERS platforms were tested satisfactory with the model molecule 4ABT. Following the feasibility study, the substrates were used for the detection of the food contaminant Crystal Violet and the drug analogue Benzocaine as examples of recognition of health menaces in real field applications.

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As an end, I want just to remark that these four years have been a wonderful journey.

Alfonso Martín Ruano

This dissertation is dedicated to my late grandfather Crescencio, who would have been so proud and happy of seeing his grandson working as a scientific.

Self-Assembly and Optical Applications of Au Nanostructures

In this initial chapter a brief introduction to the optoelectronic properties of Au nanostructures is given with special attention to nanorod shaped structures. Different methods for the synthesis of nanostructures and their assembly in ordered arrays are also discussed. Finally, current applications of Au nanorods ordered arrays are presented with special emphasis on sensing based on Surface Enhanced Raman Scattering.

1.1 Opto-electronic properties of nanostructures

The intriguing properties of metal nanostructures, reflected by their intense colour, are attributed to their unique interaction with incident light. Noble metal nanostructures are able to selectively absorb frequencies (in the visible and near IR region) of incident electromagnetic radiation and convert the energy into a coherent oscillation of their conduction electrons, which is called Localised Surface Plasmon Resonance (LSPR). As shown in **Figure 1a**, this oscillation induces a charge separation between the free electrons and the ionic metal core, which in turn exert a restoring Coulomb force to make the electrons oscillate back and forth on the particle surface, resulting in a dipole oscillation. The LSPR oscillation induces a strong absorption of light, as seen in the UV-vis spectrum of **Figure 1b**, which is the origin for the observed colour of noble metal colloidal suspensions.



Figure 1 a) Schematic of the plasmonic electron oscillations and b) UV-vis absorbance spectra of gold nanoparticles.

In the case of Au nanorods electron oscillations can occur in two directions, depending on the polarisation of the incident light: the short and long axes of the rod. **Figure 2** shows that the excitation of the surface plasmon oscillation along the short axis induces an absorption band in the visible region at wavelengths close to 521 nm, referred to as transverse band. The excitation of the surface plasmon oscillation along the long axis induces a stronger absorption band in the near – infrared (NIR) region of the spectrum, referred to as the longitudinal band. While the transverse band is mainly insensitive to the size of the nanorods, the longitudinal band is red-shifted largely from the visible to the NIR with increasing aspect ratios of the Au nanorods. The position of this band is also extremely sensitive to changes in shape, size, composition, refractive index and local dielectric environment of the medium^[1, 2].



Figure 2 UV-vis absorbance spectrum of Au nanorod aqueous suspensions and schematic of the plasmonic electron oscillations.

1.1.1 Electromagnetic origin of the plasmons

The interaction of light with a nanoparticle and its response can be calculated by solving Maxwell's equations. In 1908 Gustav Mie found an analytical solution for spherical particles of arbitrary size^[3, 4] When enounced, the theory was meant to explain the different colours present in the Au colloidal suspensions. The results obtained by Mie were compiled into a theory explaining how visible light frequencies were selectively absorbed by colloidal particles as a function of the particle size, material and surrounding environment.

Assuming particles much smaller than the wavelength of light allows the application of the dipole approximation that excludes higher polar plasmon modes that appear in larger particles. This approximation leads to simplified expressions for the extinction and scattering cross section (eq 1-3)

$$\boldsymbol{\sigma}_{ext} = \frac{18\pi\varepsilon_m^{3/2}V}{\lambda} \frac{\varepsilon_2(\lambda)}{[\varepsilon_1(\lambda) + 2\varepsilon_m]^2 + \varepsilon_2(\lambda)^2}$$
(eq1)

$$\boldsymbol{\sigma}_{sca} = \frac{24\pi^3 \varepsilon_m^2 V^2}{\lambda^4} \frac{(\varepsilon_1(\lambda) - \varepsilon_m)^2 + \varepsilon_2(\lambda)^2}{[\varepsilon_1(\lambda) + 2\varepsilon_m]^2 + \varepsilon_2(\lambda)^2}$$
(eq 2)

$$\sigma_{abs} = \sigma_{ext} - \sigma_{sca} \qquad (eq 3)$$

where λ is the incident wavelength, $\varepsilon_{\rm m}$ is the dielectric constant of the medium where particles are embedded, $\varepsilon_{\rm I}$ and $\varepsilon_{\rm 2}$ are the dielectric functions of the metal and V is the volume of the spherical particles. It has to be noted that σ_{sca} is proportional to V^2 , while σ_{ext} is proportional to V. As a result small particles will mainly absorb light, while larger particles will mainly scatter light.

The dielectric functions of a metal ε_1 and ε_2 are related to the refractive index **n** and to the absorption coefficient **k** of the metal by the following equations (eq 4-6).

$$\varepsilon(\lambda) = \varepsilon_1(\lambda) + i\varepsilon_2(\lambda) \qquad (eq \ 4)$$

$$\mathbf{\varepsilon}_1 = \mathbf{n}^2 - \mathbf{\kappa}^2 \tag{eq 5}$$

$$\varepsilon_2 = 2\mathbf{n}\kappa$$
 (eq 6)

The values of ε_1 and ε_2 determine respectively the resonance wavelength of the nanostructure and the damping coefficient that determines the width of the plasmon band. For metallic materials working under large frequency ranges (wavelengths up to near IR) the imaginary term ε_2 of the dielectric constant is negligible compared to its real counterpart ε_1 . Simplification that applied to *eq3* and *eq4*, the value of σ when

$$\varepsilon_1(\omega) = -2\varepsilon_m \qquad (eq 7)$$

The expression of eq 7 allows the calculus of the resonance frequency ω that maximize the cross section of a small noble metal sphere, and therefore match the maxima of its plasmon band. Expressions of eq 5 and eq 6 implies that changes in the refractive index of a metal will changes its dielectric constant and therefore the resonant frequency obtained in eq 7. This dependence is used to detect changes in the refractive index of a noble metal nanoparticle through the position of its plasmon band.

Following the Mie's theory, in 1912 $\text{Gans}^{[5]}$ predicted that the LSP of small ellipsoid particles (where the dipole approximation still holds) would split in two distinct modes. A transversal mode with a small scattering cross section associated to the dipole oscillations of the conduction electron following the transversal axe of the ellipsoid, and a longitudinal mode with a very high scattering cross section associated to the dipole oscillations following the longitudinal axis of the ellipsoid. Applying again the dipole approximation for particles much smaller than the wavelength of light the equivalent equations to *eq 1* and to *eq 2* for ellipsoids are:

$$\sigma_{ext} = \frac{2\pi\varepsilon_m^{3/2}V}{3\lambda} \sum_{j=A}^{C} \frac{\left(\frac{1}{P_j^2}\right)\varepsilon_2(\lambda)}{\left[\varepsilon_1(\lambda) + \left(\frac{1}{P_j} - 1\right)\varepsilon_m\right]^2 + \varepsilon_2(\lambda)^2}$$
 (eq 8)

$$\sigma_{sca} = \frac{8\pi^3 \varepsilon_m^2 V^2}{9\lambda^4} \sum_{j=A}^C \frac{\left(\frac{1}{P_j^2}\right) (\varepsilon_1(\lambda) - \varepsilon_m)^2 + \varepsilon_2(\lambda)^2}{\left[\varepsilon_1(\lambda) + \left(\frac{1}{P_j} - 1\right)\varepsilon_m\right]^2 + \varepsilon_2(\lambda)^2} \tag{eq 9}$$

The depolarisation factors P_j along the main axes of the ellipsoid are defined by:

$$P_A = \frac{1 - e^2}{e^2} \left[\frac{1}{2e} \ln\left(\frac{1 + e}{1 - e}\right) - 1 \right]$$
 (eq 10)

$$P_B = P_C = \frac{1 - P_A}{2} \qquad (eq \ 11)$$

with the eccentricity calculated from the length and diameter of the ellipsoid $e = \left(\frac{L^2 - d^2}{L^2}\right)^{\frac{1}{2}}$. In case of a spherical particle the eccentricity is zero and the polarisation factors for all axes become 1/3, which leads back from eq 8 and eq 9 to eq 1 and eq 2.



Figure 3 a) Comparison of the experimental and theoretical position of the longitudinal band of Au nanorods of different aspect ratio dispersed in water. b) Absorbance spectra of Au nanorods dispersed in solvents of different refractive index. Adapted from Mulvaney and co-workers^[6]

Gans theory predicted also a quantified response that would allow the tunability of the absorption spectrum through changes in the aspect ratio of the ellipsoids. According to the theory, experimental data shows that the LSP absorption maximum (λ_{max}) for Au nanorods is linearly proportional to their aspect ratio (*Figure 3a*). According to the theory, the LSP band is also sensitive to the dielectric constant of the surrounding medium. This sensitivity, defined as the shift in LSP wavelength relative to the refractive index change of the surrounding medium, increases also linearly with the increasing aspect ratio. *Figure 3b* reports an experimental example of red shift occurring in Au nanorods (A.R = 3) when dispersed in increasing refractive index media.

1.2. Synthesis and post-synthesis modifications of Au nanorods

The three main methods used to synthesize Au nanorods are template methods, electrochemical methods and seed-mediated methods, and they will be discussed in details below.

The template method is a dry chemistry route, where neither solvents nor surfactants are used for the synthesis and therefore Au nanorods surfaces present no other molecules, but gold. The method was developed by Martin and co-workers^[7], who used nanoporus polycarbonate and alumina membranes as templates for the electrochemical growth of Au rods (**Figure 4a**). The method requires that a small amount of Ag or Cu sputtered at the back on the porous template to provide a conductive film (**Figure 4b**). This is then used as a foundation onto which the Au nanorods can be grown by electro-deposition (**Figure 4c**). The third stage is the chemical removal of the alumina (**Figure 4d**) and dispersion of the Au nanorods in aqueous or organic solvents (**Figure 4e**). The diameter of the Au rods corresponds to the diameter of the used porous template and the length is controlled by the electro-deposition process time.



Figure 4 Schematic of the synthesis of Au nanorods by template methods^[7].

Unlike the dry route used by the template method, the electrochemical and seed mediated methods use an aqueous chemistry route, to produce rods formed by Au^0 metal core and an ionic double layer shell formed by cationic surfactants and its counter anions. The methods synthesize Au nanorods by reduction of Au(III) halogeno-aurates (AuBr₄⁻ and AuCl₄⁻) compounds complexed with cationic surfactant.

The synthesis of Au nanorods by electrochemical methods is conducted within a simple two electrode type cell with a sacrificial Au metal plate as anode and a platinum plate as cathode (*Figure 5*), both immersed in an electrolytic solution.



Figure 5 Schematic of the synthesis of Au nanorods by electrochemical methods. Adapted from ref $^{[6]}$.

The method was firstly demonstrated by Wang and co-workers^[8], who used a mix of organic surfactant (hexadecyltrymethyl-ammonium bromide CTAB and tetradodecyl-ammonium bromide, TOAB) as both supporting electrolyte and stabiliser for the nanoparticles). The synthesis proceeds as follows. Firstly, the Au anode is dissolved in an aqueous solution containing the cation surfactants CTAB and TOAB. Oxidation of the Au anode in this solution leads to the formation of the complex species AuBr₄⁻ - CTA⁺ and AuBr₄⁻ - TOA⁺ inside micelles formed due to the CTAB and TOAB long organic chains. Previous to electrolysis these micelles are loosed by the addition of acetone and cyclohexane to enhance the rod formation. Once the reduction current is passed through the electrodes, the AuBr₄⁻ species are reduced into Au⁰ rods being not clear if nucleation of the Au⁰ clusters occurs on the cathode surface or within the micelles. Afterwards, sonication is used to break the rods off the cathode surface, as well as to shear the nanorods that formed away from the surface. The method provides a synthetic route for preparing high yields of Au nanorods, but has poor control of shape and size of obtained structures.

1.2.1. Seed mediated synthesis Au nanorods

Among the three methods, the seed mediated synthesis of Au nanorods is the most used due to the high yield, low cost and flexibility to tune the dimensions of the rods through changes of the concentration of chemicals used in the synthesis. The seed mediated synthesis of Au nanorods is based on a two stages process (**Figure 6**). In the first stage small Au colloids (seeds) are synthesized through a fast redox process, in the second stage the Au seeds are used as nucleation points to growth the Au nanorods through a slow redox process.



Figure 6 Redox mechansim of the silver free seed mediated synthesis of Au nanorods. First step, reduction of Au^{III} to Au^{I} . Second step, reduction and desproportion of Au^{I} to Au metal initiated by injection of Au seeds. Adapted from Perez-Juste and co-workers^[9]

The first seed mediated synthesis of Au nanorods was reported in 2001 by Jan and coworkers^[10]. They used citrated capped Au particles as seeds and successive growth steps to fabricate different aspect ratio nanorod. Good yield was obtained but several nanoparticle and other nanostructures were also obtained as by-products.

In 2003 El-Sayed and co-workers^[11] developed a variation of the method where formation of by-products was avoided by the use of CTAB capped seeds and AgNO₃ as additive in the growth solution. The method produced very uniform-sized nanorods with high yield (99%) and very small percentage of by-products. Although some improvements have been reported in recent years^[12], the method developed by El-Sayed is to date the most widely used for the synthesis of Au nanorods. The method is schematically depicted in **Figure 7a**.

Chapter 1



Figure 7 a) Schematic of the two steps seed mediated synthesis of Au nanorods. b) TEM images of Au nanorods. c) UV-vis spectra of Au nanorods synthetized with different Ag^+ concentrations (1 lowest to 5 highest) inside the growth solutions. d) Position of the longitudinal plasmon band of Au nanorods synthetized with different Ag^+ and $HAuCl_4$ concentrations. c) and d) Adapted from El-Sayed and co-workers^[11].

In the first stage, Au seeds (< 4 nm) are generated by a quick reduction of HAuCl₄ by NaBH₄. In the second stage, seeds are used as nucleation points to grow the Au nanorods by slow reduction of HAuCl₄ with Ascorbic Acid in the presence of Ag^I ions. The method typically produces nanorods of ca. 11x40 nm dimension, as shown in the TEM of **Figure 7b**. The synthesis also allows for a fine tuning of the aspect ratio (1.5 to 5) of the nanorods through control of the concentration of HAuCl₄ and AgNO₃ in the growth solution and through the volume of seeds injected (**Figure 7c,d**).

The mechanism of formation of rod-shaped nanoparticles is still unclear. Based on the idea that CTAB forms a bilayer on the nanorod surface, Perez-Juste and co-workers^[9] proposed that the silver free growth of Au nanorod is governed by preferential adsorption of

CTAB to different crystal faces during the growth. Therefore they proposed a "zipping" mechanism taking into account the van der Waals interactions between surfactant tails within the surfactant bilayer might promote the formation of longer nanorods from more stable bilayers.

The mechanism by which the addition of Ag^{I} allows control of the aspect ratio of formed nanorods is neither totally understood. There are two theories proposed^[13]. The first theory hypothesizes that the AgBr (formed in solution immediately after AgNO₃ addition) is adsorbed over the CTAB shell of the nanorods reducing even more the growth of the facets where CTAB is strongly complexed. The second and most accepted theory^[14] suggests that Ag^{I} ions undergo to an under-potential deposition on the {110} crystallographic facets of the seeds leading to an anisotropic growth of the {111} facets (see **Figure 8**).



Figure 8 Hypotetic underpotential deposition of the Ag^{I} cations in the[110] facets of the seeds leading to the synthesis of Au nanoords by anisotropic growing.

Regarding the influence of other parameters, if an excess of ascorbic acid is added to the growth solution a decrease in the length and the yield of the rods synthesized is obtained. Finally the role of the stabilising surfactant has been further explored by Murphy and co-workers^[15] who found that the length of the surfactant tail is critical for controlling the length of the rods and its yield, and that the bromide ion works much better than other halides as rod inducing agent in the presence of C_nTA^+ .

1.2.2. Post-Synthesis modifications of seed mediated nanorods

We have described in details the origin and characteristics of Au nanorod longitudinal surface plasmon, which is highly sensitive to structural and medium/surrounding changes. The tunability of the longitudinal surface plasmon wavelength is a very useful property for biochemical sensing, biological imaging and medical diagnostic applications^[6]. Moreover, synthetic control on the nanorod resonance wavelength is also highly desirable for engineering of nanorod and nanorod ensemble properties for sensing and photonic applications.

Once synthesised by standard seed mediated methods, the optical characteristics of Au nanorod solutions can be tailored by shortening their size (anisotropic oxidation) or by increasing the Au nanorods dimensions by overgrowth.

Anisotropic oxidation was pioneered by Stucky and co-workers^[16] and consisted into mixing Au nanorods with aliquots of 1 M HCl at 70-90 °C under O₂. The oxidation rate was controlled by the acid concentration and the reaction temperature. Wang and co-workers^[17] developed another anisotropic oxidation method using a mix of H_2O_2 and HCl as oxidizing agent, whose results are showed in **Figure 9**.



Figure 9 a)UV-vis spectra obtained at varying stages of the oxidation process. b-d) SEM images obtained at different stages of the oxidation process. Adapted from Wang and co-workers^[17]

Figure 9a shows extinction spectra acquired at varying stages of Au nanorod oxidation. As synthesised Au nanorods exhibited two extinction peaks, but as oxidation proceeded, the longitudinal band shifted to the blue while the transversal band decreased in intensity and eventually disappeared, suggesting the conversion of nanorods into nanoparticles. SEM images of **Figure 9b-d** show the progressive size decrease of nanorods.

It is also possible to increase the size of synthesised Au nanorods by different methods. Wang and co-workers^[18] induced a transversal overgrowth of Au nanorods by the selective binding of glutathione and cysteine thiol molecules onto the Au nanorods ends (**Figure 10**).



Figure 10 Schematic of the mechanism of the overgrowth of Au nanorods by cysteine-glutathione binding onto the ends.

On the other hand Liz –Marzan and co-workers^[19] were able to overgrow nanorods by stepped additions of reducing agent ascorbic acid to a solution of nanorods. The excess ascorbic acid reduced the Au (I) ions left unreacted in the nanorod solution, leading to an amplification of the nanorods dimensions from 10x45 nm to 25x75 nm (**Figure 11**).



Figure 11a) *SEM image of Au nanorods after overgrowth process. b) UV-vis spectrum of Au nanorods dispersed in water after overgrowth process*^[19]

1.2.3. Surface modification and phase transfer of Au nanorods

Once synthesized, it is possible to tune the stability of the nanorods in different media^[20] by encapsulation of the nanorods, chemical modifications of the shell and or exchanges of the stabilizing surfactants.



Figure 12 Wrapping of anionic polyelectrolyte over Au nanorods and immobilization on negative charge surfaces. Adapted from Murphy and co-workers^[21]

Several authors have reported methods to improve the biocompatibility of the Au nanorods and its stability in aqueous phases. For example, Murphy and co-workers^[21] reversed the ζ -potential of the Au nanorods by deposition of an anionic polyelectrolyte over the positively charged CTAB molecules (**Figure 12**). Using similar layer by layer approaches Niidome and co-workers^[22] prepared non cytotoxic nanorods by encapsulation and substitution of the CTAB molecules for polyethylene glycol thiol terminated (PEG-SH) and Wang and co-workers^[23] deposited an inorganic coating based on silica compounds.

Au nanorods have also been modified to enable their dispersion into organic media. Mitamura and co-workers^[24] have used a three step method: binding of mercaptopropylsilane (MPS) to the rods, followed by hydrolysis of the bound MPS and a silane-polymerization to develop hydrophobic Au nanorods stable in polar organic solvents as chloroform (**Figure 13**).



Figure 13 Phase transfer of Au nanorods from water to chloroform by binding of organosilane and posterior polymerization process. Adapted from Mitamura and co-workers^[24]

Using a different approach Yang and co-workers^[25] developed a method to transfer the Au nanorods to a wide variety of organic solvents by exchanging the CTAB aqueous soluble surfactant by the TOAB surfactant in presence of mercaptosuccinic acid (MSA).



Figure 14 Schematic of the link of TOA⁺ to the Au core. Adapted from Wang^[26].

The use of TOAB as a replacement surfactant to transfer Au nanostructures into organic phases was furthered investigated by Wang and co-workers^[26]. The authors found that negative stabilized nanoparticles form spontaneous ion pairs with the TOAB molecules inducing the transfer of nanoparticles to the organic solvent (**Figure 14**). They also found that the TOAB induced phase transfer process is a size dependent process, where the small particles are favoured over the large ones, thanks to the higher surface/volume ratio.

1.3. Fabrication of ordered assemblies

The main approaches for the fabrication of ordered nanostructure arrays can be broadly classified into top-down, ie. lithographic fabrication, and bottom-up, ie. selfassembly processes. In this chapter we will focus our attention to self-assembly process, as such approach was used for the fabrication of ordered structures presented in this thesis.

The anisotropic shape confers interesting assembly characteristics to Au nanorods leading to a number of possible ordered superstructure configurations such as horizontal side by side, horizontal end by end or vertical side by side. Crystallographic differences in the facets of Au nanorods induce a denser packing of the CTAB molecules at the sides than at the ends of the nanorods, but theoretical calculations of the potential curves^[27] have shown that the side by side alignment configuration is lower in energy. According to this, the spontaneous assembly of Au nanorods has usually resulted in horizontal side by side configurations, while end by end configurations have being forced through specific linkers^[28], external forces^[29] or template assisted substrates^[30].

Methods explored so far for the assembly (either side by side or end by end) of nanorods into ordered arrays can be classified into assembly on substrates, at interfaces and in solutions and are schematically described in **Figure 15**.



Figure 15 Schematic of the diverse techniques used to Self-Assembly Au nanorods. Adapted from review article^[31].

Self-assembly on substrates can be spontaneous, being the process driven by simple droplet evaporation (discussed in detail in section 1.3.2) or directed, being the process driven by external forces^[29] or template assisted methods^[32-34]. Directed evaporation techniques are generally used for the formation of horizontal end by end alignments. For example, using electrophoresis techniques in combination with micro-electrodes Pesacaglini and co-workers^[29] were able to self-assemble chains of Au nanorods in end to end configuration within sub-micrometer gaps (**Figure 16**). Similar geometries were obtained also by Rusell and co-workers^[30] using substrates with channels formed by block co-polymers.



Figure 16 a) schematic of the experimental set-up. b) micro-photograph of the electrodes used to align the Au nanorods. c) Scanning microscope image of the Au nanorods aligned in the gap between electrodes. Adapted from Pescaglini and co-workers^[29]

Nanorod assemblies have also been obtained at phase interfaces, either liquid-liquid or air-liquid by Langmuir-Blodgett or other techniques (*Figure 17*). The assembly of nanorods on the liquid-liquid interface between two immiscible fluids is driven by the decrease in the interfacial energy between the two phases when nanostructures are placed between them. The stability of the nanostructures on the interface depends on their sizes^[31], while other factors as shapes of the particles, or hydrophobicity/hidrophilicity of the particle shell plays also an important role in the stability of the nanostructure at the interface.



Figure 17 Schematic of the self-assembly of rod shape nanostructure though a Langmuir-Blodgett technique. Adapted from Zhang and co-workers^[31]

The assembly of nanostructures at air-liquid interfaces can be driven by the decrease in the interfacial energy between the liquid and the gas, by the insolubility of the nanostructures in the solvent or by a contribution of both factors.

The self-assembly on interfaces either liquid-liquid or air-liquid has been proven as a method able to produce compact monolayers over large macroscopic areas with exceptional long-range order. The method has been widely used for the self-assembly of semiconductor nanostructures dispersed in organic solvents^[35], but only one papers have reported its use with noble metal nanostructures^[36].

Finally, Au nanorods have been assembled in solution by chemical binding and linker assisted methods. The chemical assisted methods are usually based on the substitution of the surfactant molecules of the shell of the nanostructures by bi- functionalized species as diamines or di-thiols, which promote the self-assembly in solution by linking one nanostructure to each other. Using this technique *Thomas* and co-workers^[28] fabricated in solution chains of AuNRs assembled end by end through chemical substitution of the CTAB α - ω dithiol compounds (**Figure 18**). Following the same line Pradeep and co-workers^[37] used dimercaptosuccinic acid to fabricate one, two and three dimensional superstructures of Au nanorods depending of the concentration of the acid.



Figure 18 a) Transmission electron micrographs of nano-chains. b) Mechanism of nanochains formation through dithiol binding.

1.3.1. Evaporation mediated techniques: droplet deposition

Recently, droplet deposition has emerged as a promising technique to organise metal nanostructures into 2D and 3D superstructures. This simple process consists of depositing a droplet of a suspension on a substrate (usually silicon) and letting the solvent evaporate under controlled conditions (**Figure 19**).



Figure 19 Schematic of self-assembly of Au nanorods by droplet evaporation. a) Au NRs dispersed in suspension. b) Self-Assembly of Au nanorods inside the droplet. c) Deposition of the Au nanorods on the substrate after full evaporation of the solvent.

The droplet deposition process involves inter-particle and external forces. The interparticle forces are the Van der Waals attractions between the particles metal cores and the repulsive forces between the surfactant molecules on the shell of the nanostructure. The external forces are the depletion and capillary forces^[27, 38-40] induced by the evaporation of the solvent.

The Van der Waals forces are electrostatic interactions between temporal multi-poles generated by the random movements of charges inside the solids. They are weak attractive forces usually depreciable between solids at the macro scale, but very relevant for nanoparticles at close range) due to their high surface in volume ratio. Without any counterpart van der Waals forces would make colloidal suspensions instable, and on absence of surfactants to provide any repulsion they produce along the time the irreversible clustering and coalescence of the particles dispersed in a suspension.

The repulsion forces need to stabilize particles in colloid suspensions are provided by the surfactant molecules of the shell of the nanostructures (**Figure 20**). The nature of these repulsion forces depend of the type of surfactants. Ionic surfactants generate long range electrostatic forces, while polymer-type surfactants generate close range steric forces. Some of the surfactants as the CTAB or TOAB combine polar heads together with long alkane chains to provide both electrostatic and steric repulsions of variable strength depending of the distance.



Figure 20 a) Schematic of Au nanoparticle stabilised with negative (blue) and positive (red) surfactants. b) Electrostatic interaction potentials between the Au nanoparticles of a).

The stability and phase state of a colloid solution depends on the balance between the van der Waals and the repulsion forces. This balance is a very complex equilibrium depending of the volume fraction of the suspension, the intensity and range of the forces, and the internal energy of the system (mainly temperature)^[41, 42]

One of the first works reporting the formation of Au nanorods arrays by droplet deposition was done by Murphy and co-workers^[43] in 2005 (**Figure 21**). By controlling the CTAB concentration they were able to produce different self-assembly patterns of Au nanorods and other Au nanostructures coated also by CTAB.



Figure 21 Au nanorods assembly patterns obtained by droplet evaporation of different Au nanorods suspensions. Adapted from Murphy and co-workers^[43].

Wang and co-workers^[39] reported also the self-assembly of several Au nanostructures through control of the temperature evaporation range plus control over the particle and surfactant concentration. Evaporation of aqueous suspensions with Au nanorod concentration 20mM and CTAB concentration over 1mM results in the formation of nematic and smectic superstructures of ordered Au nanorods (**Figure 22**) within 50µm of the periphery of the droplet. They found also that shorter Au nanorods often align vertical and that two kinds of nanorods evaporated together formed separated domains one from each other.



Figure 22 Smectic assemblies formed by droplet evaporation of aqueous suspension with different type of Au nanorods dispersed. Adapted from Wang and co-workers^[39].

But both works present arrays of very limited size localized only at the periphery of the droplets, a problem that is common for several works reporting the self-assembly of Au nanorods by droplet deposition.

Droplet deposition and coffee ring effect

The self-assembly of Au nanorods by droplet deposition is a flexible and low cost method, but the ordered assemblies are usually localized exclusively at the periphery of the droplets and limited in size to only a few micrometers, being this the main issue for the fabrication of highly ordered assemblies over large areas by droplet deposition



Figure 23 a) multiple exposures photography illustrating the motion of colloidal particles in a water droplet. b) Schematic illustrating the different evaporation rates per unit area (J) along a droplet profile and the outward flow (v) that causes the coffee ring effect. Adapted from Degaan^[44].

This is a well known problem of the colloidal suspensions, whose causes where discovered in 1997 by *Degaan* and co-workers^[44]. When a droplet of a colloidal dispersion dries, the colloidal particles, initially dispersed over the entire droplet, accumulate in multilayer deposits along the perimeter leaving a ring-like deposit (**Figure 23a**). This pattern obtained from slow droplet evaporation is known as "coffee ring" and it is due to a capillary flow in which no volatile solutes (like nanostructures) are carried from the inside to the edge of a droplet by the outward flowing of the solvent^[44-46].

The causes that trigger this flow are explained through the evaporation mechanisms of colloidal suspensions as follows. The periphery of the droplets is the first area to be fully evaporated due to the thin layer of solvent present there. In standard solutions, this effect leads to a gradual shrinking of the droplet along the evaporation process, but in colloidal suspensions it causes the settling down of the fraction of colloidal particles dispersed at the periphery of the droplet within the first instants of the evaporation process. Once deposited on the substrate, the particles pin the contact line of the droplet avoiding, therefore, its usual shrinking. The pinning of the contact line of the droplet triggers an outward flowing of the solvent in order to replenish the liquid that is being evaporated at the pinned edge (**Figure 23b**), and as an indirect effect carry the dispersed material from the inside to the periphery of the droplet.

Assembly of nanorods by modified droplet evaporation methods

Since the assembly of nanorod structures over large areas is crucial for numerous photonic and sensing applications, many efforts have been recently devoted to overcome the formation of coffee ring during droplet evaporation and to produce different assemblies geometries, overall standing nanorods^[27, 38, 39, 46,47].



Figure 24 Schematic of the assembly process and SEM images of the formed vertical assemblies a) using gemmini surfactant (adapted from Liz-Marzan and co-workers^[48]) and b) using OH-terminated alkanethiol (adapted from Xie and co-workers^[27]).

One of the most effective methods to produce large area vertical arrays consists into increasing inter-nanorod attraction forces by decreasing the repulsion exerted by CTAB molecules. For example, *Liz-Marzan* and co-workers^[48] (Figure 24a) were able to fabricate

standing Au nanorods superlattices using gemini surfactants instead of CTAB. Similar standings arrays were also fabricated by Xie and co-workers^[27] (**Figure 24b**). They fabricated three dimensional structures scattered over the droplet surface, with vertical arrays of Au nanorods stacking one over the other by partial exchange of the CTAB molecules with an OH-terminated alkanethiol to reduce the repulsion forces between nanorods.

The group of Xie reported also a second work^[38] (**Figure 25**) where they fabricated more homogenous vertical arrays through a two staged droplet evaporation method, where the droplet was first pinned to the substrate by a fast evaporation stage followed by a slow evaporation step (lasting around 8h) where the nanorods self-assembled in vertical arrays. However, in spite of the high degree of ordered obtained the ordered area was still limited to the external rim of the droplet



Figure 25 (*a*) Formation of pinned edge in the first stage and (*b*) formation of the near-equilibrium environment in the second stage. *c*) SEM image at low magnification shows the self-assembly area restricted to the droplet periphery. Inset of high magnification SEM image shows the vertical arrays. Adapted from Xie and co-workers^[38].

1.4. Au nanorod ordered arrays and SERS detection

As a consequence of the Localised Surface Plasmons, surface of noble metal nanostructures presents strong electric fields^[49]. The intensity of this electric fields greatly depends of the curvature radius of the nanostructure, having enormous values in singular points as the peaks of the nano-pyramids, corners of the nano-cubes, or ends of the nanorods, whose simulated electric field is shown in **Figure 26a**.



Figure 26 Simulation of the electric field enhancement obtained for a) single Au nanorod, b)two Au nanorods close a few nanometers one to each other when enlighted by a 650 nm and 620 nm light source respectively.

When two or more nanorods are brought into close proximity or assembled into ordered arrays with controlled inter-nanorod spacing of less than 10 nm the plasmons of neighbouring particles couple. This coupling localizes and reinforces the LSP in the gaps between nanorods leading to the formation of so-called "Hot Spots" (**Figure 26b**)^[50]. The characteristics and enhancement ratio of the electric fields within the hot spots depend crucially on the geometry and inter-nanorod spacing of the nanostructure arrays, and therefore the key to unlocking the potential of plasmonic structures is the control of these features at the nanometer and sub-nanometer scale^[51].

The high density of "hot spots" generated in ordered Au nanorod arrays make such superstructures particularly suitable for SERS applications. For instance, Finite-Difference Time Domains (FDTD) calculations have shown^[47] that hexagonally packed nanorods possess strong and uniform electromagnetic fields highly enhanced along the length of adjacent nanorods (**Figure 27**), strongly decreasing with the increase of the inter-nanorod gap.



Figure 27 Simulations of electric fields enhancements produced at the gaps of vertical nanorods. Adapted from Peng at al^[47].

The formation of high density hotspot has been vastly exploited for sensing applications based on Surface Enhanced Raman Spectroscopy (SERS). SERS is the amplification of Raman signals experienced by molecular species adsorbed on a rough metal surface. Although SERS has been reported for single metal nanostructures, the highest enhancement have been observed for ordered assembled metal nanostructures (Ag, Au, Cu) that, depending on the particle size and gap between particles, lead to signal enhancements so high that even single molecules maybe detected^[52]

Substrates with reproducibly and coherent high SERS enhancement factors (EF) thus appear to be highly desirable and are a prerequisite for a wider application of SERS beyond fundamental studies, but before going into details of molecular SERS detection on ordered Au nanostructures we will briefly introduce the basics of Raman and SERS spectroscopy in the next paragraph.

1.4.1. Surface Enhancement Raman Spectroscopy

Raman is a spectroscopy technique based on inelastic scattering of monochromatic light, usually from a laser source. Photons of the laser light are absorbed by the sample and then reemitted. Frequency of the reemitted photons is shifted up or down in comparison with the original frequency, which is called the Raman effect. The resulting Raman spectrum consist of bands corresponding to vibrational and rotational transitions specific to the molecular structure, and therefore provides chemical fingerprints to identify the analyte.



Figure 28 Energy diagram of the a) Stokes, b) Raylegth and c) anti-Stokes scattering.

Specifically, Raman scattering^[53, 54] is an inelastic process where an incoming photon from a laser excites a vibration mode of a molecule while emitting a scattered photon. The process can be read as the promotion of an electron from a vibrational base state to a higher "virtual energy state" absorbing the photon emitted by the laser on the process, followed by a demotion of the electron from the virtual state to a vibrational state that can be the same of the original (Rayleight scattering) or a different one at a higher energy level (Stokes scattering) or at a lower level (anti-Stokes scattering) (**Figure 28**).

Unfortunately, about 99.999% of all incident photons undergo elastic Rayleigh scattering, which is useless for practical purposes of molecular characterization. For this reason up to the 1960s Raman measurements has been limited to the analysis of solvents and some bulk materials.

This changed in the 1970s when Jean Marie and Van Duyne^[55] reported that molecular absorption into or near a roughened noble metal surface led to drastically increased Raman signal intensity due to electromagnetic enhancement mechanisms. A series of subsequent experiments confirmed that noble metal films with roughened surfaces or nanoscale features can dramatically increase Raman scattering signals of analytes and produce enhancement factors of 10^4 – 10^8 over normal Raman scattering^[56]. This phenomenon was associated with the plasmonic field enhancement generated by rough metal surfaces and nanostructures and it was called Surface-Enhanced Raman Scattering (SERS).
1.4.2. Enhancement mechanisms of SERS

The enhancement factors of SERS as compared to normal Raman scattering are attributed to two mechanisms: an electromagnetic mechanism and a chemical mechanism^[49, 57-59].

The electromagnetic mechanism (EM) is a wavelength-dependent effect arising from the excitation of the localised surface plasmon resonance. When a molecule is adsorbed onto a noble metal nanostructure the intensities of the Raman incident photon and of the scattered photons are susceptible to be enhanced if their wavelengths are in resonance with the plasmon mode of the nanostructure^[49]. Ignoring any further interaction between the incoming and scattered field, the total electromagnetic enhancement produced for the nanostructure can be calculated as the product of the enhancement of both photons. Taking in account that the local field intensity at a specific point is proportional to the square of the electric field amplitude at that point, the Enhancement Factor expression results

$$\mathbf{EFtotal} = [\mathbf{Einc}(\mathbf{r}) / \mathbf{Eo}(\mathbf{r})]^2 * [\mathbf{Esct}(\mathbf{r}) / \mathbf{Eo}(\mathbf{r})]^2 \qquad (eq \ 12)$$

Where [Einc(r) / Eo(r)] is the enhancement of the amplitude of the incoming field and [Esct(r) / Eo(r)] is the enhancement of the amplitude of the scattered field. Differences between the excitation and emission fields can be usually ignored, so *eq 13* can be further simplified into

$$\mathbf{EFtotal} = [\mathbf{E}(\mathbf{r}) / \mathbf{Eo}(\mathbf{r})]^4 \qquad (eq \ 13)$$

Expression that justify the common assumption that enhancement of the Raman signals in the SERS technique depends to the fourth power of the amplitude of the electric field used to irradiate the sample. Furthermore, since the radiation amplitude scales down with the third power of the distance $[E(r) \sim r^{-3}]$, the SERS enhancement will scale down with the twelve power of the distance $[EF(r) \sim r^{-12}]$, making of SERS a truly surface dependent process (**Figure 29a**).



Figure 29 a) Schematic illustrating the sensitive of the enhancement factors to the size of the gap between nanostructures. b) Schematic of the charge transfer mechanism between the HOMO and LUMO orbitals of the molecule and the electrons at the Fermi level of the metal surface.

The chemical mechanism (CM)^[59-64] contributes to the enhancement through the chemisorption of the molecule to the noble metal surface, allowing the molecular orbitals of the molecule to interact with the conduction electron band of the metal. These interactions becomes of special importance in systems where the Fermi level of the metal is localised between the HOMO and LUMO orbitals of the molecule (**Figure 29b**). In these systems, the vibrational Raman transitions of the molecule are enhanced by energy transferred from the electronic transitions of the molecule using the Fermi level of the metal as an intermediary.

Furthermore, in some molecules (as the 4-ABT) some vibrational modes forbidden as Raman transitions because of its symmetry (as the b2 modes) become active thanks to the energy transfer process and as a result, new peaks (associated with the vibration mode of the forbidden transitions) appear in the SERS spectra of those molecules

It is important to remark that while the electromagnetic enhancement is independent of the molecule and has a range of action of around 10nm (some weak effects can extend up to 100 nm), the chemical enhancement depends of the match between the molecular orbitals of the molecule and the Fermi band of the metal and is relevant only when the molecule is physisorbed or chemisorbed to the metal.

1.4.3. Use of arrays as SERS platforms

Compared to Au nanoparticles, Au nanorods arrays exhibit a stronger plasmon band, an a reduced plasmon damping, which produces a stronger enhancement of the electromagnetic fields, overall at their tips^[57, 65, 66]. These characteristics make arrays of closely packed nanorods one of the best systems for SERS platforms designing. Confirming its potential, several authors have reported its use for SERS detection of several analytes as biological species^[67] and hazardous chemicals^[6, 68, 69] to mention these ones only.

Biological Sensing

The coupling of plasmonic nanostructures with biomolecules has recently attracted increasing attention. One of the most active areas is the development of new immune-sensing assays using Au nanostructures conjugated with biomolecules as DNA-plasmids^[70] or prions^[19]. For example Liz-Marzan and co-workers^[19] used vertical arrays of Au nanorods to detect structural changes in mutated prions In particular, protein structural changes from α -helix to β -sheet were detected in the SERS spectra as transition from a triplet signal to a singlet signal (**Figure 30**).



Figure 30 Schematic showing the *a*) biological active (α -helix protein) and the *b*) mutated (β -sheet) prion. *c*) SERS spectra of active protein (blue curve) and scrambled prion (yellow curve). Adapted from Liz-Marzan and co-workers^[19].

While most of the studies have been done in-vitro, the combination of high sensitive and no interference from biological fluids have allowed also the use of in-vivo SERS techniques. As a milestone, in 2006 *Van Duyne* and co-workers^[71] demonstrated the first in vivo application of the SERS technique by monitoring the glucose levels in rats with a subcutaneous nanostructured membrane.

On field detection of hazardous chemical traces

Detection of hazardous substances is in high demanded due to the current harmful environments and terrorist attacks. The detection of these substances in the field requires a robust method able to operate in a wide variety of surfaces and chemical environments as the plasmonic Au nanostructures, which have already been satisfactory used for the fast detection of several health menaces such as explosives^[72, 73], pesticides^[74], drugs^[75], or food contaminants^[47].

The detection of explosives has been overall focus in the TNT detection thanks to the formation of complex species between either cysteine or 4ABT functionalized Au nanostructures and the TNT molecule. For example, Chandra Ray and co-workers^[73] reported the selective detection of TNT at pM level with a portable Raman system using cysteine-Au nanoparticle.

The detection of individual pesticides from complex samples is favoured by the ability of the SERS technique for the multi-analyte detection. For example Saute and co-workers^[74] used Au nanorods treated with HCl to detect nM concentrations of Ferbam, Ziram, and Thiram mixed together in aqueous solution. Despite the similarity of the SERS spectra of the three species, the authors were able to distinguish individual concentration of every pesticide in the mixed mixtures by two dimensional Principle Component Analysis (PCA), also known as "barcode" analysis (**Figure 31**).



Figure 31 SERS spectra of a) Ferbam and b) Ziram at different concentrations. c) two dimensional PCA to discriminate Ferbam, Thiram and Ziram from multicomponent mixtures.

Au nanostructures were also used to detect traces of drugs by filtering, swabbing and other techniques suitable for real life applications. For example White and co-workers^[68] shown the detection of Cocaine by filtration of solution through Ag nanoparticles previously adhered to filter papers.

In the food contaminant field Peng and co-workers^[47] reported femtomlar detection of melamine and plasticides in orange juices using vertical arrays of Au nanorods cleaned by UV Ozone (**Figure 32**). Furthermore, the ozone cleaning treatment proved also the reusability of their substrates.



Figure 32 SERS spectra of BBP on vertical arrays of nanorods at different concentrations and after the UV Ozone cleaning (red curve). b) SERS spectra of several contaminants detected on vertical arrays of nanorods at fM concentration.

Monitoring nanoscale chemical and biochemical Phenomena

The high sensitivity of SERS technique, together with its ability to provide structural information of the analytes bound to the plasmonic nanostructures has been used to follow different chemical process as the formation of self-assembled monolayers (SAM) of alkanethiols on Au and Ag substrates^[76]. Its high spatial resolution plus the possibility to work in aqueous fluids without interferences from the water molecules have also allowed the monitoring of important biochemical process. For example, *Meule* and co-workers^[77] monitored the release of a neurotransmitter at the single cell level, thanks to the ability of SERS to provide specific structural information of compounds released in specific sites.

Use of low-cost flexible SERS for in-situ sample analysis

On the last years low cost flexible SERS platform for on field practical detection has become an important alternative^[78] to the usual substrates. Flexible plasmonic SERS substrates have been fabricated using plastic^[79-81], paper^[82-84], polymer materials^{8,9[85, 86]}, and TLCs^[87] as supported substrates, in combination with metal nanostructures constituting SERS active layers. These hybrid systems have the potential to combine low cost and flexibility while still maintaining sensitivity and robustness of rigid substrates, as well as cost and ease of fabrication.



Figure 33 Detection of pollutants in water by dropping of the samples on TLC plates, followed by chromatographic separation of the analytes and posterior in situ SERS analysis with a portable Raman system. Adapted from Long and co-worker^[87]

Due to flexibility and permeability, these substrates allow sample collection by swabbing of surfaces or filtering of solutions, maintaining the ability to detect analytes mixed in complex matrix. Both characteristics make them particularly suited for real world sample analysis. Results have been further improved by physical separation of the diverse analytes through chromatography and similar separation techniques performed straight on the plasmonic substrates (**Figure 33**). For example White and co-workers^[88] have detected individual dyes from a complex dyes mixture using a two steps chromatographic separation process on silver nanoparticles printed on standard chromatographic paper (**Figure 34**). The same group reported^[68] also the detection of malathion and cocaine by swabbing traces from surfaces with SERS dipsticks and posterior pre-concentration of the analyte by capillarity techniques.



Figure 34 Comparison of the SERS spectrum of each separated dye to the respective reference spectrum after chromatographic separation. Adapted from White and co-workers^[88].

1.5. Objectives of this thesis

For future integration in photonic and sensing devices, ordered arrays of Au nanorods will have to show a good homogeneity over mm² areas, together a good chemical and physical stability in a wide range of substrates against harsh environments. The goal of this thesis was to develop a low-cost process to fabricate ordered Au nanorods arrays over large areas for sensing applications. The milestones of the project carried out in this thesis are shown below:

- 1) Fabrication of Horizontal Au Nanorods Arrays.
- 2) Fabrication of Vertical Au Nanorods Arrays.
- Immobilization of Horizontal and Vertical Au Nanorods Arrays on rigid substrates and testing of their feasibility as SERS platforms.
- 4) Immobilization of Au Nanorods Arrays on flexible substrates and use as SERS platforms in on field applications.

Droplet evaporation was chosen as the self-assembly method to fabricate the ordered gold nanorods arrays due to its inherent simplicity, versatility and low cost. In order to overcome coffee ring limitations (see Section 1.3.1) and obtain a homogeneous distribution of the nanorods we developed a new method, based on controlled evaporation of Au nanorods organic suspensions, for the self-assembly of Au nanorods at the air-liquid interface of the droplets.

Different orientational order was achieved by using nanorods obtained from different synthetic routes, in particular, controlled evaporation of seed mediated Au nanorods suspensions led to the development of horizontal arrays, while evaporation of overgrowth Au nanorods suspensions led to the development of vertical arrays. The conditions and key parameters necessary for the fabrication of these horizontal and vertical arrays are presented in the Chapter 3 and Chapter 4.

Upon solvent evaporation, Au nanorods arrays with a high degree of order were fabricated, but it was impossible to use them as SERS platforms due to their poor adhesion to the support. To overcome this, a stamp method was developed. Once immobilized on cover slips, feasibility of horizontal and vertical arrays as SERS platforms was assessed using 4-aminobenzenethiol (4ABT) as test molecule. The description of the stamping process and the

study of the SERS performance of the horizontal and vertical arrays are presented in the Chapter 5.

Taking further advantages of the stamping method, in the Chapter 6 we used flexible sheets made of different materials as receiving substrates of the Au nanorods arrays to fabricate flexible plasmonic SERS substrates. The SERS performance of these flexible plasmonic substrates was assessed again with model molecule 4-ABT. Finally, detection of food contaminant Crystal Violet in solution and traces of the drug marker benzocaine on cover slips were achieved with pPET active substrates as a proof of field based applications.

1.6. References

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CHAPTER 2

Synthetic methods and post-synthesis modifications of Au nanorods

This chapter presents the synthetic methods used in this thesis to synthesize three different types of Au nanorods: the seed mediated Au nanorods, the overgrowth Au nanorods, and the Au nanorods synthesized using seed mediated Au nanorods as seeds. Each type of nanorod was characterized by a high magnification SEM, a UV-vis spectrum obtained from an aqueous suspension, and histograms of their geometrical dimensions. In this chapter is also presented the methods used in this thesis to oxidize anisotropically the Au nanorods and to transfer them from aqueous suspensions to organic solvents.

2.1. Seed mediated synthesis of Au nanorods

Au nanorods used in this thesis have been synthesized by the El-Sayed seed mediated method^[1] (*Figure 35*). The process comprise two steps, in a first step Au seeds (< 4 nm) were generated by a fast reduction of HAuCl₄ by NaBH₄ (*Figure 35* top row). In the second step, seeds were used as nucleation points to grow the Au nanorods by slow reduction with ascorbic acid (*Figure 35* bottom row). The synthesis allows for a fine tuning of the aspect ratio of the nanorods through control of the following parameters: concentration of HAuCl₄ and AgNO₃ in the growth solution and through variations of the seeds added.



Figure 35 Schematic of the two steps seed mediated synthesis of Au nanorods discovered by El-Sayed and co-workers^[1]

Increments of the AgNO₃ concentration inside the growth solution produced Au nanorods of slightly bigger aspect ratio. Specifically, additions of 1 ml, 0.5 ml and 0.3 ml of AgNO₃ 4 mM to 25ml of growth solution produced Au nanorods of aspect ratio 4.1, 3.7 and 3.4, respectively. Changes in the seeds injected to the growth solution produced a greater effect over the size and aspect ratio of the nanorods synthesized. Reduction of the NaBH₄ concentration used to generate the seeds produced thicker Au nanorods of smaller aspect ratio. Specifically, the addition of 300 μ l of NaBH₄ 6.6 mM into 5 ml of seed solution generates seeds, which once injected into the growth solution produced Au nanorods of size 23 x 55 nm, aspect ratio 2.1. In comparison, the seeds generated by addition of 300 μ l of NaBH₄ 10 mM into 5 ml of seed solution produced Au nanorods of size 11 x 41 nm, aspect ratio 3.7. **Figure 36b** shows a table illustrating the concentrations of Au(III), AgNO₃ and NaBH₄ used to synthesize four different kinds of nanorods, and **Figure 36a** shows the UV-vis spectra of the four kinds of Au nanorods dispersed in water.



Figure 36 Table with the volumes (Y) of AgNO₃ added to the growth solutions and the concentration (X) of NaBH₄ added to the seed solutions for the fabrication of the Au nanorods A1, A2, A3, and B1. a)schematic of the synthesis of seed mediated Au nanorods. b) UV-vis spectra of the Au nanorods A1(green line), A2(magenta line). A3(red line), and B1(blue line) dispersed in water.

2.1.1. Materials employed

The synthesis and phase transfer of Au nanorods used Hexadecyltrimethyl amonium bromide (CTAB) BioXtra with a purity equal or greater than 99.9 %, Tetraoctylammonium bromide (TOAB) with a purity equal or greater than 99.9%, tetracloro Auric acid tri-hydrate (HAuCl₄ 3H₂O), Silver Nitrate (AgNO₃) trace metal basis with a purity equal or greater than 99.9999%, Mercaptosuccinic acid (C₄H₆O₄S) with a purity bigger or greater to 99% HPLC grade, Ascorbic acid (C₆H₈O₆) 20-200 mesh and Sodium borohidride (NaBH₄) trace metal basis with a purity equal or greater than 99.99%. All of them commercial product bought from Sigma-Aldrich.

2.1.2. Synthesis of the seed solution and growth solution

For the synthesis of Au nanorods two seed solution were used. Both solutions shared the same recipe, except for the concentration of the NaBH₄ employed to reduce the Au(III) precursor. Specifically, a CTAB solution (3.75 mL, 0.15 M) was mixed with 1.25 mL of 1 mM HAuCl₄ at 30°. To the stirred solution **0.3 mL of ice-cold NaBH₄** of concentration **X**

was added under vigorous stirring (able to produces a vortex), which resulted in the formation of a pale brown solution. Stirring of the seed solution was continued vigorous for 1 min and gentle (approximately, one revolution of the stirring bar per second) after further use.

The synthesis of the different **Au nanorods** shared the same recipe, except for changes in the quantities of AgNO₃ added, and the type of seeds used as nucleation points. Specifically 0.91 g of CTAB were dissolved in 12.5 ml of deionized water at 30 °C. Once all the CTAB just solved **Y mL of AgNO₃ (4 mM)** and **12.5 mL of HAuCl₄ (1mM)** dissolved in deionized water were added. After gentle mixing of the solution **0.175 mL of ascorbic acid** (**0.0788 M**) were injected in once. Upon addition of ascorbic acid the solution color changed from intense orange to colorless as a result of the reduction of the Au^{III} ions (yellow-orange color) to Au^I ions (colorless). Finally, **35 µL of seed solution** generated by addition of **0.3 mL of ice-cold NaBH**₄ of concentration **X** were injected also in once at 30 °C. After the seed injection color of the solution gradually changed from colorless to intense brown-red (10-20 min) as a result of the reduction of the Au^I ions (colorless). The solution was kept under mild stirring at 30 °C until growth process was complete (90-120 min), which was indicated by further absence of spectral changes in the solution UV-Vis spectrum.

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Figure 37 a,b) Histograms of the Au nanorods a) diameter, b)length obtained by statistical measures over 100 rods. c) High magnification SEM image of the Au nanorods deposited onto a SiO₂ substrate. d) UV-vis spectrum of the Au nanorods dispersed in water.



Figure 38 a,b) *Histograms of the Au nanorods a) diameter, b) length obtained by statistical measures over 100 rods. c) High magnification SEM image of the Au nanorods deposited onto a SiO₂ substrate. d) UV-vis spectrum of the Au nanorods dispersed in water.*





Figure 39 a,b) *Histograms of the Au nanorods a) diameter, b) length obtained by statistical measures over 100 rods. c) High magnification SEM image of the Au nanorods deposited onto a SiO*₂ *substrate. d) UV-vis spectrum of the Au nanorods dispersed in water.*



Figure 40 a,b) *Histograms of the Au nanorods a) diameter, b) length obtained by statistical measures over 100 rods. c) High magnification SEM image of the Au nanorods deposited onto a SiO₂ substrate. d) UV-vis spectrum of the Au nanorods dispersed in water.*

2.2. Overgrowth synthesis of Au nanorods

Au nanorods were overgrown by reduction of the Au (I) remaining in solution after a typical seed mediated synthesis, according to a method described by Marzán and co-workers ^[2]. The process consisted in stepped additions of reducing agent ascorbic acid to a solution of Au nanorods obtained by seed mediated method. The excess ascorbic acid reduced the Au (I) ions left unreacted in the nanorod solution leading to a growth of the nanorod. The overgrowing process was monitored by the gradual increase in intensity of the longitudinal plasmon band in combination with a slight blue shift (*Figure 41a*). The suspension was stirred until no unreacted Au (I) ions were left in the solution, as estimated by the absence of further changes in the UV-vis spectrum upon addition of ascorbic acid.



Figure 41 Table with the volumes of $AgNO_3$ added to the growth solutions and the concentration of $NaBH_4$ added to the seed solutions for the fabrication of the Au nanorods A4, A5, and B2. a) Evolution of the UV VIS absorbance spectrum of the gold nanorods water suspensions along the overgrowth process. b) Schematic of overgrowth process of Au nanorods.

In order to assure enough Au^{I} ions were left in solution for the posterior overgrowth step, growth solution used to synthesize the Au nanorods 2.2A 3.2A and 4.2A (*Figure 41b*) contained double quantity of Au^{III} salt and $AgNO_{3}$ than the growth solutions used to synthesized the Au nanorods 1.1, 2.1 3.1 and 4.1 (*Figure 36*). Consequently were also reduced with double quantity of ascorbic acid. Seed solutions and the volume of seeds injected into the growth solution were left unmodified.

The synthesis of the different overgrowth **Au nanorods** shared the same recipe, except for changes in the quantities of AgNO₃ added, and the type of seeds used as nucleation points. Specifically 0.91 g of CTAB were dissolved in 12.5 ml of deionized water at 30 °C. Once all the CTAB just solved **Y mL of AgNO₃ (4 mM)** and **25 mL of HAuCl₄ (1mM)** dissolved in deionized water were added. After gentle mixing of the solution **0.350 mL of ascorbic acid (0.0788 M)** were injected in once. Upon addition of ascorbic acid the solution color changed from intense orange to colorless as a result of the reduction of the Au^{III} ions (yellow-orange color) to Au^I ions (colorless). Finally, **35 µL of seed solution** generated by addition of **0.3 mL of ice-cold NaBH**₄ of concentration **X** were injected also in once at 30 °C. After the seed injection color of the solution gradually changed from colorless to intense brown-red (10-20 min) as a result of the reduction of the Au^{II} ions (colorless) into Au⁰ nanorods (color depending of the aspect ratio). The solution was kept under mild stirring at 30 °C until growth process was complete (90-120 min), which was indicated by further absence of spectral changes in the solution UV-Vis spectrum.

Once normal growing of Au nanorods was completed, **ascorbic acid solution (0.0788 M**) was added at a rate of 5 μ l every 5 minutes until no further changes in the UV-vis spectrum (*Figure 41b*) were detected upon addition of ascorbic acid.





Figure 42 a,b) Histograms of the Au nanorods a) diameter, b) length obtained by statistical measures of 100 rods. c) High magnification SEM image of the Au nanorods deposited onto a SiO_2 substrate. d) UV-vis spectrum of the Au nanorods dispersed in water.



Figure 43 a,b) Histograms of the Au nanorods a) diameter, b) length obtained by statistical measures over 100 rods. c) High magnification SEM image of the Au nanorods deposited onto a SiO_2 substrate. d) UV-vis spectrum of the Au nanorods dispersed in water.





Figure 44 a,b) *Histograms of the Au nanorods a) diameter, b) length obtained by statistical measures over 100 rods. c) High magnification SEM image of the Au nanorods deposited onto a SiO₂ substrate. d) UV-vis spectrum of the Au nanorods dispersed in water.*

2.3. Synthesis using Au nanorods as seeds

Large Au nanorods were also synthesized by injection of Au nanorods previously synthesized into a new growth solution as seeds (*Figure 45*). The use of Au nanorods as seeds has already been reported by Murphy and co-workers^[3] to fabricate high aspect ratio rods (some of them of aspect ratio 10 or bigger). However, on our case the process led to the formation of bigger rods with the same rounded ends but of smaller aspect ratio than the ones used to seed the second growth process.

Droplet evaporation of aqueous suspensions of Au nanorods C1 and C2 results in the aggregation of the rods at the periphery of the droplet of Unfortunately, it was no possible to study their self-assembly behaviour when dispersed in Chlorobenzene. Due to their size, it was not possible to transfer the Au nanorods C1 and C2 from the aqueous media to Chlorobenzene following the method used with all the other nanorods (see section 1.5).



Figure 45 Schematic of the fabrication of the Au nanorods C1 and C2 using Au nanorods A2 and B1 as seeds, respectively.

The synthesis of the different overgrowth **Au nanorods** shared the same recipe, except for changes in the quantities of AgNO₃ added, and the type of seeds used as nucleation points. Specifically 0.91 g of CTAB were dissolved in 12.5 ml of deionized water at 30 °C. Once all the CTAB just solved **1 mL of AgNO₃ (4 mM)** and **12.5 mL of HAuCl₄ (1mM)** dissolved in deionized water were added. After gentle mixing of the solution **0.175 mL of ascorbic acid (0.0788 M)** were injected in once. Upon addition of ascorbic acid the solution color changed from intense orange to colorless as a result of the reduction of the Au^{III} ions (yellow-orange color) to Au^I ions (colorless).

Finally, **2 mL** of previously synthesized **Au nanorods A2** or **B1** were injected also in once at 30 °C. After the seed injection color of the solution gradually changed from colorless to intense brown-red (10-20 min) as a result of the reduction of the Au^I ions (colorless) into Au⁰ nanorods (color depending of the aspect ratio). The solution was kept under mild stirring at 30 °C until growth process was complete (90-120 min), which was indicated by further absence of spectral changes in the solution UV-Vis spectrum.

After the nanorod injection color of the solution gradually changed from colorless to pale reddish during the first 20-30 min. After the first 30 minutes, color intensity increased together with the turbidity. The solution was kept under mild stirring at 30 °C until growth process was complete (90-120 min), which was indicated by further absence of spectral changes in the solution UV-Vis spectrum



2.3.1. Characterization of Au nanorods C1

Figure 46 Histograms of the Au nanorods a) diameter, b) length obtained by statistical measures over 100 rods. c) High magnification SEM image of the Au nanorods deposited onto a SiO_2 substrate. d) UV-vis spectrum of the Au nanorods dispersed in water.



2.3.1. Characterization of Au nanorods C2

Figure 47 Histograms of the Au nanorods a) diameter, b) length obtained by statistical measures over 100 rods. c) High magnification SEM image of the Au nanorods deposited onto a SiO_2 substrate. d) UV-vis spectrum of the Au nanorods dispersed in water.

2.4. Anisotropic oxidation of Au nanorods

Au nanorods were anisotropically oxidized by addition of external redox agents^[4]. Specifically, a mixture compose of 200 μ l of HCl 1M and 50 μ l of H₂O₂ 30% were added to 10 ml of Au nanorod solution and left under mild stirring during several hours. The mix shortened the nanorods (**Figure 48a**) by a selective oxidizing of the tips. **Figure 48b** shows a gradual decrease in the intensity of the longitudinal band of the nanorods in combination with a slight blue shift. These changes allowed the monitoring of the oxidizing process which could be stopped at any time by centrifugation of the solution and re-dispersion in fresh deionized water. After the oxidizing, Au nanorods were reduced to dimensions 11(1) nm x 25(2) nm.



Figure 48 a) Schematic of the anisotropic oxidizing process. b) Evolution of the UV VIS absorbance spectrum of the gold nanorods water suspensions along the oxidizing process. c) SEM image of Au nanorods deposited onto SiO₂ substrate after being oxidizing during 2 hours.

2.5. Phase transfer of Au nanorods.

Once synthesized, nanorods were transferred from its original water phase where synthesis was developed to a variety of organic solvents, following a method reported by *Chen* and co-workers^[5]. The method transfer the nanorods form water to organic solvents by exchanging the organo-phobic CTAB molecules of the shell by the organo-philic TOAB molecules.

The reaction mechanism of this specific process has not been reported yet. However, Wang and co-workers^[6] studying similar transfer processes hypothesized two different mechanisms depending if the TOAB molecule bind a positive or a negative stabilized Au particle. Their results showed that Au particles stabilized by negative anions (ie. Citrate) were able to bind straightforward the TOAB molecule through ion pairs, while Au particles stabilized by positive cations (ie.CTA⁺) need a second specie (usually a thiol-carboxylate) to act as a bridge between the TOAB molecules and the Au particle. Based in this study we hypothesize the possible mechanism of the method used along this thesis to transfer Au nanorods from water to Chlorobenzene (**Figure 49**).



Figure 49 Possible mechanism of the phase transfer of the Au nanorods. a) Binding of the Succinic acid to the nanorods surface in the water phase. a) Ionic interaction between the acid groups of the

succinic molecule and the polar nitrogen head of the TOAB molecules transport the nanorods to the organic phase.

First, Au nanorods in water solution were centrifuged and re-dispersed in water so that the final CTAB concentration was adjusted between 0.1 mM and 0.35 mM. When CTAB concentrations were smaller than 0.1 mM Au nanorods aggregated during phase transfer and when CTAB concentrations were larger than 0.35-0.40 mM Au nanorods did not transfer into the organic phase. After the CTAB concentration was adjusted into the correct range, 3 ml of the Au nanorods aqueous suspension were added under vigorous stirring to an emulsion formed by Mercaptosuccinic acid (3 mL, 10 mM, pH 9) in aqueous solution and TOAB (1.5 mL, 50 mM) in chlorobenzene solution. The resulting mixture was left under vigorous stirring for an average time of 90 min until the water phase discolored and the organic phase became intense red. Au nanorods in chlorobenzene were separated from the water phase only immediately before use to prevent nanorod aggregation induced by chlorobenzene evaporation.



Figure 50 a) Photo of solutions containing Au nanorods dispersed in different solvents after phase transfer from water. b) UV-vis spectra of Au nanorods dispersed in water (black curve), chloroform (green curve), toluene (cyan curve), and chlorobenzene (red curve). c) Plot comparing the refractive index of the solvent and the position of the longitudinal plasmon band of the Au nanorods.

The position of the plasmon band of the Au nanorods was shifted in function of the the refractive index of the solvent were they were dispersed. *Figure 50*b,c show the red shift of the longitudinal plasmon band of Au nanorods from 789 nm when dispersed in water (black curve) to 815 nm when dispersed in chloroform (green curve), 839 nm when dispersed in toluene (cyan curve) and 838 nm when dispersed in chlorobenzene.

According with the results reported by Wang^[6], the transferring of Au nanorods from aqueous to organic media was strongly dependent of the nanorod size.

The small Au nanorods types A1, A2 and A3 were transferred with no problems for a wide range of nanorods and surfactant concentration. Colour transfer from water to organic phase occurs within minutes of the solvent mixing

The transfer of the larger Au nanorods types B1, A4 and A5 was a less straight forward process. In this case, the colour of the water-organic emulsion disappeared few minutes after the mixture was prepared, pointing to a possible Au nanorods aggregation. The emulsion stays totally white-uncoloured during 15-30 minutes until the characteristic redbrown colour begins to appear in the organic phase again. Despite the vanishing of the colour, once the stirring was stopped 90 minutes later, water and organic phase separated neatly, being the water phase transparent and the organic phase strongly red-brown coloured.

Finally the transfer of the Au nanorods types B2, C1 and C2 was not possible. In this case the colour of the water-organic emulsion also disappeared but did not return at any time. Once the stirring was stopped (after several hours) the organic and water phases were transparent and Au nanorods were found aggregated as black particles at the bottom of the recipients.

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2.6. Calculation of CTAB concentration

CTAB concentration in nanorods water dispersions was calculated through the UV VIS absorbance spectrum. CTAB molecule presents a characteristic UV VIS absorbance peak with a maximum at 195 nm, whose height and shape are affected by the CTAB concentration. The highest is the CTAB concentration the longest is the tail of the peak, and the further to the blue is shifted its inflexion point. This inflexion point is hard to appreciate in the absorbance spectrum (**Figure 51a**), but is clearly represented as a minimum in the first derivative of the curve (**Figure 51b**), whose position and value is used to estimate the CTAB concentration in the gold nanorods water dispersions.



Figure 51 a) *UV-vis spectrum of a Au nanorod water solution showing the region where is localised the peak associated to the CTAB. b) Calibration plot with the derivatives curves obtained from UV-vis spectra of different solutions with known CTAB concentrations.*

In order to estimate the CTAB concentration of our solutions we prepared a calibration plot with the derivatives curves obtained from the UV-VIS spectra of different dispersions with known CTAB concentrations (Figure 51b). Red and green curves of Figure 51b are the derivatives curves obtained from dispersions with CTAB concentrations 0.1mM and 0.4mM, respectively. Only between these two values was possible to transfer the nanorods from water to the different organic solvents. AuNRs dispersed in water with CTAB concentrations lower than 0.1mM collapsed before being transferred to chlorobenzene, and GNRs dispersions with CTAB concentration higher than 0.4mM did not transfer to the chlorobenzene phase.

2.7. References

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CHAPTER 3

Self-Assembly of Au Nanorods into Horizontal Superstructures

A new droplet evaporation method is presented for the fabrication of Au nanorods superstructures. Specifically, large domains of nanorods arranged horizontal to the substrate in a close-packed side-to-side fashion were obtained by controlled evaporation of Au nanorod chlorobenzene dispersions. Experimental parameters controlling the self-assembly process were carefully investigated and will be discussed in detail. Optical microscopy analysis during the evaporating process revealed that nanorods assembled at the interface between solvent and air forming domains that grew in size with the evaporation of the droplet. The high degree of internal order generated within individual domains was visualized by electron and optical microscopy and resulted in markedly anisotropic *characteristics* highlighted polarized optical microscopy imaging. by

^{(1) &}lt;sup>1</sup> * This work has been partially published as: "Au Nanorod Plasmonic Superstructures Obtained by a Combined Droplet Evaporation and Stamping Method", C.Schopf, A.Martín, M.Burke, A.J.Quinn, D.Iacopino, J. Mater. Chem. C 2014, 2, 3536-3541.

⁽²⁾ This work has been partialy published as: "Synthesis, optical properties and selfassembly of gold nanorods", A. Martin, C. Schopf, A. Pescaglini, A. O'Riordan, D. Iacopino. Journal of Experimental Nanoscience, 2012, 7, 688-702.

⁽³⁾ Au nanorods horizontal arrays have been used for photoconductivity measures in the article: "Polarization dependent, surface plasmon induced photoconductance in gold nanorod arrays", S. Diefenbach, N. Erhard, J. Schopka, A. Martin, C. Karnetzky, D. Iacopino, A. W. Holleitner Physica Status Solidi (RRL)), 2014, 8, Issue 3, pp 264-268.

3.1. Introduction

Au nanorods possess unusual optical properties desirable for a number of applications ranging from photonic to sensing^[1, 2]. The dimensional anisotropy of Au nanorods results in the strong polarization of its surface-plasmon optical properties. Therefore, Au nanorod assemblies constitute ideal candidates for the preparation of optically anisotropic superstructures for the manipulation of light on the nanoscale^[3]. Moreover, the enhanced optical phenomena arising from inter-nanorod coupling in close ordered arrays have potential applications in fields such as sensing, negative refractive index meta-materials or information technologies^[4, 5].

In order to exploit this potential, great efforts have been devoted to the development of suitable techniques to organize Au nanorods into designed assemblies^[6], such as template methods^[7, 8], drying at an interface^[9], association in solution^[10], capillary assembly^[11], chemical modification of nanorod surfaces^[12, 13],electrophoresis^[14] and droplet evaporation^[15-18].

Droplet evaporation has been widely recognised as a suitable route to self-assembly different nanostructures. The technique consists simply of depositing a droplet of a nanorod suspension (usually water) on a substrate (usually silicon) and let the solvent evaporate under controlled conditions. The assembly of nanostructures is driven by a balance of attractive forces (van de Waals, dipole-dipole interactions), repulsive electrostatic forces and capillary forces that become more apparent as the volume of the droplet decreases^[6, 19]. However, assembly area is limited to a thin ring at the periphery of the droplets (the coffee ring effect)^[20]. This ring form when non-volatile solutes are carried to the pinned droplet edge by outward flowing of solvents from the interior to the edge.

In recent years several authors ^[8, 16, 23, 24] have reported fabrication of ordered nanorod arrays by variations of the droplet deposition technique. Pioneering work was performed by Murphy and El-Sayed who investigated the formation of self-assembly patterns resulting from evaporation of aqueous droplets onto TEM grids^[15, 25]. More focused work toward higher degrees of organization was performed by Wang and co-workers^[16], who were able to induce organization of Au nanorods into nematic and smectic multilayer superstructures by evaporation of aqueous droplets of precisely controlled nanorod and CTAB concentrations. Chemical modification of nanorod surfaces was also used to promote selective one and two-

dimensional assembly^[17]. Also interface-induced assembly and template assisted assembly were used in combination with controlled evaporation for the assembly of Au nanorods into surfaces^[23, 26]. However, most of the above methods only provided ordered structures with limited area and inhomogeneous thicknesses. Fabrication of large size assemblies, with homogeneous thickness and highly controlled geometrical order still remains a key challenge in this field.

In contrast to the vast literature dedicated to theoretical and experimental investigation of the mechanisms regulating the droplet evaporation-induced self-assembly of Au nanorods from aqueous suspensions, limited attention has been dedicated to self-assembly processes occurring in Au nanorod organic suspensions. Mitamura and co-workers^[27] have developed protocols for phase transfer of Au nanorods into organic phase and have investigated their self-assembly by droplet evaporation onto TEM grids. Nanorods assembled on horizontal or vertical structures with average distance 3-4 nm, depending on the concentration. However, further investigation of the self-assembly process would be highly desirable as the ability to produce large area ordered superstructures from organic suspensions would extend applications of Au nanorods, potentially leading to development of hybrid devices with enhanced optoelectronic properties^[28].

This chapter describes the formation of Au nanorod parallel superstructures by droplet evaporation of Au nanorod chlorobenzene solutions. Under controlled evaporation such suspensions led to formation of domains where nanorods assembled horizontal to the substrate in a close-packed side-by-side fashion. Domains measured over 100 μ m² and extended as a monolayer over the entire droplet surface. Optical polarized imaging of individual domains revealed markedly anisotropic characteristics, confirming the high degree of internal order generated by the aligned nanorods.

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3.2. Experimental Section

3.2.1. Synthesis and phase transfer of Au nanorods

In this chapter nanorods type A1, A3 and B1 of dimensions 13x44 nm, 10x41nm and 23x49 nm respectively, were used. All of them were synthesized by seed mediated methods^[29] described in the second chapter of this thesis, section 2.1.3, 2.1.5 and 2.1.6.

After synthesis, Au nanoords were transferred from the original aqueous media to chlorobenzene following a phase trasnfer method^[30] described in the second chapter of this thesis, section 2.5.

3.2.2. Droplet evaporation of Au nanorod solutions

A small aliquot (10 μ L) of Au nanorod cholobenzene solution ([Au] = 1 to 12 nM) was deposited on SiO₂ substrate, covered with a petri dish and then left to evaporate at room temperature over a time of 3 h. Controlled solvent evaporation resulted in formation of horizontal assemblies.

3.2.3. Characterization of horizontal superstructures

Scanning electron microscopy (SEM) images of nanorod 2D lattices were acquired using a field emission SEM (JSM-6700F, JEOL UK Ltd.) operating at beam voltages of 5 kV. Polarized optical images were acquired with an optical microscope (Zeiss axioscop II) equipped with a CCD camera (Optronics DEI-750) using a 100 W halogen lamp as illumination source.

3.3. Results and Discussion

3.3.1. Synthesis of Au nanorods

Au nanorods were synthesized by a standard seed-mediated method described by El Sayed and co-workers^[23]. The as-prepared Au nanorod suspension (1 mL) was centrifuged twice to remove excess CTAB and re-dispersed in 1 mL of Millipore water (18M Ω). The final CTAB concentration was kept between 0.1 and 0.35 mM. *Figure 52a* shows UV-vis spectra of synthesized nanorods dispersed in water (black curve) and chlorobenzene (red curve). The spectra were characterised by a transversal plasmon mode at 521 nm and an intense longitudinal plasmon mode localised at 737 nm when nanorods were dispersed in water and at 785 nm when nanorods were dispersed in chlorobenzene. *Figure 52b* shows a SEM image of nanorods deposited on a SiO₂ substrate. The nanorods mean diameter and length were 13 ± 2 nm and 44 ± 3 nm respectively, aspect ratio (AR) = 3.4



Figure 52 a) UV-vis spectrum of Au nanorod aqueous suspension; b) SEM image of Au nanorods deposited onto a SiO₂ substrate.

Prior to deposition on solid substrates, nanorods were transferred into chlorobenzene following the method reported by Chen and co-workers^[24] and described in the chapter two of this thesis, section 2.5.

3.3.2. Formation of horizontal superstructures by droplet evaporation of Au nanorods dispersed in chlorobenzene suspensions

Assembly of nanorods into horizontal superstructures was achieved by droplet deposition of chlorobenzene suspensions into SiO_2 substrates, which were covered by a petri dish to assure a slow evaporation process (3h) (process illustrated in the schematic of *Figure* 53a).



Figure 53 a) Schematic of the self-assembly of Au nanorods into horizontal superstructures. b-e) SEM images of horizontal superstructures formed by evaporation of Au nanorod chlorobenzene suspensions. b) Low magnification image of the assembly area; c) Domain formation in dried droplet; d) High magnification image of an individual domain. Insets: top) Fast Fourier transform; bottom) detail of formed superstructure; e) 45° tilted image of a nanorod domains.

Figure 53b shows a low magnification SEM image of the dried droplet obtained by deposition of (10 μ l, 6 nM) Au nanorods chlorobenzene suspensions onto a SiO₂ substrate showing deposition of compact and homogeneous material over the whole explored surface.

Closer observation (*Figure 53c*) showed that Au nanorods assembled into domains c.a. 100 μ m², which covered the entire area of a 20 mm² droplet. Domains were packed close to each other as a result of strong capillary forces becoming dominant in the last phases of droplet evaporation. SEM images of an individual domain (**Figure 53d**) revealed the formation of a solid Au nanorod monolayer phase, ordered both orientationally and positionally. Fast Fourier transform analysis, shown in the inset of **Figure 53d**, displays diffuse streaks corresponding to side-by-side alignment of nanorods. The regular spacing in vertical and horizontal directions between the streaks of the FFT confirmed the disposition of the Au nanorods under smetcic order inside the horizontal arrays^[16]. Evidence of monolayer formation is shown in **Figure 53d** depicting a 45° tilted SEM image of the superstructure

In order to clarify the role played by the organic solvent during the assembly mechanism, we prepared analogous Au nanorods aqueous suspensions and performed droplet evaporation maintaining the same experimental conditions (10µl, slow evaporation). The evaporation process followed a coffee stain mechanism in the explored nanorod concentration range (1.5 nM to 12 nM)^[20] with nanorods mainly depositing in the external ring of the droplet. *Figure 54* shows low and high magnification SEM images of structures formed by evaporation of aqueous Au nanorod suspensions (10 µL, 6 nM). Nanorods accumulated at the periphery of the droplet in a ring between 10 to 100 µm width, depending on the concentration of deposited solution (*Figure 54*a). Within the ring degree of order was low with mixed parallel and perpendicular multilayer structures formed, as highlighted in *Figure 54*b. Very low nanorod density was found in the center of the drop, with formation of small and sparse nanorod domains (see *Figure 54*c).



Figure 54 a) Low magnification SEM image of Au nanorod superstructures formed by aqueous droplet deposition on SiO_2 substrates. The arrow indicates the width of the area where nanorods are assembled; b) High magnification SEM image of assembled nanorods close to the edge; c) SEM image of small size assemblies formed in the center of the droplet.

These results suggest that the use of chlorobenzene as solvent was essential for the generation of long-range ordering observed in our superstructures. The compactness and high degree of order obtained were attributed to the physico-chemical differences between chlorobenzene and water^[19, 31]. The lower viscosity and surface tension of the chlorobenzene compared to water (0.75 *vs* 0.9 mPa×s and 33 *vs* 72 dyn/cm, respectively) promoted a higher degree of ordering by offering less resistance to domain movement. The lower dielectric constant of chlorobenzene compared to water (5.7 vs 88 ε_0) promoted the long range order on the assembly by extending the range of action of the electrostatic repulsion forces between nanorod and nanorod^[32].

3.3.3. Effect of experimental parameters

In order to gain a deeper understanding of the mechanism regulating the formation of Au nanorod superstructures, investigation of the effects played by the following parameters was undertaken: nanorod aspect ratio and substrate, nanorod concentration, surfactant concentration, and evaporation rate. Electron microscopy and polarized optical microscopy were used to investigate the influence of these parameters on the assembly process and to gain insights on the degree of assembly-induced internal order.

Influence of the substrate and nanorod size on the assembly process

Horizontal Au nanorods superstructures of comparable high order were obtained by droplet evaporation on SiO₂, glass and glass/ITO substrates (contact angles 52° , 40° and 72° respectively).

Droplet evaporation of Au nanorods dispersed in chlorobenzene generated also ordered assemblies with nanorods of different sizes. Large areas of ordered monolayer superstructures with nanorods assembled side-by-side to form 2D monolayers were successfully obtained using Au nanorods of aspect ratios 2.1, 3.4 and 4.1.

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Figure 55 a-c) statistical analysis of diameter and length of Au nanorods. : a) mean diameter 23 ± 2 nm, mean length 49 ± 3 nm, AR = 2.1, SPR = 656 nm ,b) mean diameter 13 ± 2 nm, mean length 44 ± 2 nm, AR = 3.4, SPR = 737 nm; c) mean diameter 10 ± 2 nm, mean length 41 ± 2 nm, AR = 4.1, SPR = 781 nm. d) UV-vis spectra. e-g) SEM images of vertical arrays formed under controlled evaporation of chlorobenzene droplets (10μ l, 6nM) of nanorods with aspect ratio e) 2.1; f) 3.4, g) 4.1.

*Figure 55*a-c shows statistical analysis of the nanorod used, which had the following characteristics: mean diameter 23 ± 2 nm, mean length 49 ± 3 nm, AR = 2.1; mean diameter 13 ± 2 nm, mean length 44 ± 2 nm, AR = 3.4; mean diameter 10 ± 2 nm, mean length 41 ± 2 nm, AR = 4.1. UV-vis spectra of nanorods dispersed in aqueous solutions are shown in *Figure 55*d. The position of the longitudinal peak was 656, 737 and 781 nm for aspect ratios of 2.1, 3.4 and 4.1, respectively. SEM images of horizontal arrays obtained by controlled evaporation of droplets (10 µl, 6 nM) of the three different Au nanorods chlorobenzene suspensions (*Figure 55e-g*) show that the domain size, compactness and order of the nanorods inside the domains were comparable.

Influence of nanorod concentration on the assembly process

Ordered assemblies formed across a relatively large range of Au nanorod concentrations, between 1.5 nM and 12 nM. Nanorod concentration affected the size of the deposited dried droplets. Photographs of *Figure 56* show an increase from 2 mm diameter to 5 mm diameter for droplets obtained by deposition of 10 μ L solution of concentration 1.5 nM – 12 nM at room temperature.



Figure 56 Photographs of dried droplets obtained from Au nanorods chlorobenzene solutions of increasing concentration (1.5 nM to 12 nM).

Initial concentration of Au nanorods also affected the size of the domains formed. SEM images of horizontal arrays formed by slow evaporation (3h) of nanorod chlorobenzene suspensions showed gradual increase of domain size as Au nanorod concentration increased from 1.5 nM to 12 nM *Figure 57a-d*). In particular, at concentrations 1.5 nM isolated domains were obtained with sizes ranging from 0.2 to 1 μ m² (*Figure 57a*), at concentrations 3 nM domain size ranged from 2 to 10 μ m² (*Figure 57b*), and finally at concentrations 6 nM and higher, domain size reached up to 100 μ m² and space between domains decreased until horizontal arrays covered the entire surface of the droplet (20 mm²) as a quasi-continuous monolayer (*Figure 57 c,d*). In spite of the different size of domains formed at different nanorod concentrations, the extent of organization within each domain was similar with nanorods found closely arranged into side-by-side rows and rows aligned parallel to each other in the whole range of explored concentrations (*Figure 57* bottom row).



Figure 57 Low and high magnification SEM images of horizontal superstructures obtained from Au nanorod suspensions of concentrations a) 1.5 nM; b) 3 nM; c) 6nM; d) 12nM.

In order to analyse the coverage of the droplet surface by horizontal arrays and the grade of order of these arrays over larger areas we used optical microscopy. Due to the high degree of internal order, domains appeared strongly green and red coloured under polarised light illumination, which allowed easy individuation of domain boundaries and dimension measurements.

Figure 58a-c shows polarised microphotographs of three samples prepared by controlled evaporation of Au nanorods chlorobenzene suspensions of concentrations 3 nM, 6 nM and 12 nM. Nanorod domains appeared red and green, depending on the relative alignment of domain main axes with the excitation polarisation direction. Empty areas appeared bright yellow. Black areas mainly formed at high concentrations and were due to deposition of organic residues. The initial concentration of Au nanorods affected the surface coverage, which increased with the increase of nanorod concentration. Surface coverage was defined as the ratio between the area occupied by horizontal arrays and the total area of the

droplet. Statistical analysis were performed by image J program on optical images equivalent to **Figure 58a-c** (area of 5000 μ m²) and gave a surface coverage of 55%, 95% and 95% for Au nanorod concentration of 3 nM, 6 nM and 12 nM, respectively.



Figure 58 Polarised microscopy images of domains obtained by deposition of 10 μ l droplets of Au nanorod chlorobenzene suspensions of concentrations a) 3 nM; b) 6 nM; c) 12 nM.

Close observation of data collected from electron and optical microscopy led to the conclusion that 6 nM was the optimum concentration to produce highly ordered assemblies. In fact, in samples prepared with lower concentrations, horizontal arrays did not cover the whole droplet surface and samples prepared with higher concentrations presented a greater number of defects. In particular, at the periphery of the droplet samples prepared with Au nanorod concentration 12nM presented a ring of c.a. $20 - 100 \mu m$ (**Figure 59**) where almost all the nanorods assembled vertical to the substrate.



Figure 59 SEM images of the Au nanorod superstructures on SiO₂ substrate formed at the periphery of a dried droplet (10µl, 12nM) a) Low magnification; b) High magnification.

In agreement with reports from other authors using water suspensions^[32], we ascribe this behaviour to a singular and quick increase of the density of Au nanorods in the peripherical areas of the droplet caused by the small volume of solvent in these areas (due to

the much lower height of the droplet at the periphery) and by a faster evaporation rate compared with the center of the droplet. These two effect should occur for every droplet, but apparently only when the Au nanorods concentration was 12nM or bigger the density of the nanorods reach the critical value that triggered its assembly in vertical arrays. Again, it should be remarked that all the samples prepared by evaporation of suspensions of concentration 6nM or smaller did not present any area where the Au nanorods aligned vertical to the substrate.

Influence of surfactant concentration on the assembly process

All experiments discussed in the previous section were carried out with a concentration of CTAB equivalent to 0.1 mM. In order to assess the influence of the surfactants droplet evaporation experiments were repeated using suspensions of higher CTAB concentration (0.35 mM). The CTAB concentration was adjusted and calculated using the optical method described in the second chapter of this thesis, section 2.6. Coverage and domain size remained unchanged, but the order inside the domains resulted slightly worse (*Figure 60*). In particular, nanorods still aligned side by side but they appeared loosely packed with a number of cracks forming within the domains. This is consistent with a higher surfactant concentration which likely prevented nanorods from coming in close contact due to enhanced steric and repulsive forces.



Figure 60 SEM images of horizontal arrays on SiO2 substrate formed by controlled evaporation under a petri dish (3h) of chlorobenzene droplets (10µl, 6 nM) with CTAB concentration 0.35mM.

A wider range of CTAB concentration could not be explored. At CTAB concentrations smaller than 0.1 mM Au nanorods aggregated during phase transfer and at CTAB concentrations larger than 0.35-0.40 mM Au nanorods did not transfer into organic phase. Variation of tetraoctylammonium bromide (TOAB) concentration from the 50 mM

value used for phase transfer did not affect the final degree of order obtained. However, the concentration of TOAB affected the stability of nanorod chlorobenzene suspension, which was stable for over two weeks with [TOAB] = 50 mM and for less than a week at lower TOAB concentrations.

Influence of evaporation rate on the assembly process

In order to assess the influence of the evaporation rate chlorobenzene suspensions of various concentrations were left to evaporate at room temperature under free air without petri dish, which decreased the evaporation time from 3 h to ca. 20 minutes.



Figure 61 Low magnification SEM images of horizontal arrays on SiO₂ substrate formed by a) fast evaporation (20 minutes) and b) controlled evaporation (3h) of 10 μ l droplets with concentrations a1,b1) 3 nM; a2,b2) 6 nM; a3,b3) 12 nM.

Low magnification SEM images of *Figure 61* show that domains formed by fast evaporation were smaller than domains formed by slow evaporation for the whole explored concentrations range. Specifically, in samples prepared by fast evaporation of 6 nM and 12 nM Au nanorod suspensions produced domains of c.a. 1-5 μ m² (*Figure 61a2,a3*) whereas domains larger than 100 μ m² (*Figure 61b2,b3*) were obtained by slow evaporation of the same suspensions.





Figure 62 High magnification SEM images of horizontal arrays on SiO₂ substrate formed by a) fast evaporation and b) slow evaporation (3h) of 10 μ l droplets with concentrations a1,b1) 3 nM ; a2,b2) 6 nM ; a3,b3) 12 nM

The degree of internal order was also significantly lower for the samples prepared by fast evaporation. Disorder increased with the increase of Au nanorod concentration. High magnification SEM images of *Figure 62a2,a3* show assemblies obtained by fast evaporation of 6 nM and 12 nM suspensions where several areas present gaps, misalignments and multi-layered structures. The presence of all these defects is minimized in the horizontal arrays obtained at 3 nM at fast evaporation. Comparison with equivalent superstructures formed under slow evaporation for the same range of concentrations is shown in *Figure 62*, row b

Polarised optical microscopy was used again to compare the size of the domains obtained by slow and fast evaporation over large areas. **Figure 63** shows that the size of domains (red and green areas) obtained by fast evaporation was smaller than the size obtained by slow evaporation for the whole range of explored concentrations. As shown in SEM images the size difference became significant when high Au nanorods concentrations were used.

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Figure 63 Polarised microscope images of horizontal arrays on a glass cover slip formed by a) fast evaporation and b) controlled evaporation under a petri dish (3h) of 10 μ l droplets of Au nanorod chlorobenzene suspensions of concentrations a1,b1) 3 nM ; a2,b2) 6 nM ; a3,b3) 12 nM.

Domain size comparison for fast and slow evaporation is summarized in the plot of **Figure 64**. At Au nanorod concentration 3 nM domain size measured less than 10 μ m² and c.a. 30μ m² when droplets were dried by fast and slow evaporation respectively. At Au nanorod concentration 6 nM and 12 nM domain size measured c.a. 15 μ m² and 5 μ m², respectively when droplets were dried by fast evaporation (blue curve). In contrast, at Au nanorods concentration 6 nM and 12 nM the suspensions produced coloured areas that were measured more than 100 μ m² when droplets were dried by slow evaporation (red curve)



Figure 64 Size comparison of the Red and Green areas generated by slow (red curve) and fast (blue curve) evaporation of a 10 μ l droplet of Au nanorod chlorobenzene suspensions of concentrations 3, 6 and 12 nM.

Finally, samples prepared by fast evaporation were also less uniform than samples prepared under controlled evaporation. Occasional formation of small assemblies of 5-10 nanorods with no order between them were founded (*Figure 65a*) together with areas where Au nanorods assembled perpendicular to the substrate (*Figure 65b*). Both kinds of defects appeared overall in samples prepared from suspensions with AuNRs concentration 6nM and higher. This general loss of order and reduction of domain size at high Au nanorod concentration and shorter evaporation times was ascribed to the reduction of the time available for the nanorods to order themselves in conditions far to the equilibrium.



Figure 65 SEM images of superstructures obtained by fast evaporation of $10\mu l$ droplets of 12 nM Au nanorod concentration.

In conclusion, fast evaporation of chlorobenzene suspensions led to formation of smaller size domains which were also characterised by lower degree of order, especially evident for nanorod concentrations higher than 6 nM.

3.3.4. Mechanism of Au nanorod self-assembly of into horizontal superstructures

In order to clarify the mechanism of superstructure formation, the evaporation process was recorded on video films and photographs were obtained by optical microscopy.



Figure 66 Snapshots from the video recorded along the evaporation process.

Snapshots from the evaporation process video (*Figure 66*) showed progressive formation of a gold ring at the periphery of the droplet. The ring increased in thickness as the solvent evaporated, eventually covering the entire area of the droplet. Towards the end of the process shrinkage of the droplet from its original 7 mm to ca. 5 mm at the end was observed, with the assembled material covering the full surface of the droplet.



Figure 67 Images of the different stages during the evaporation of a droplet deposited on glass cover slip. The edge of the drop is indicated by the white arrow in a,b). Sample was illuminated with cross-polarised light from above.

The process was also followed by optical microscopy. In order to reproduce a slow evaporation rate, the 10 μ L nanorod droplet was enclosed into an evaporation chamber and the microscope illumination source was directed away from the sample when measurements

were not taken. Figure 67a shows that a highly birefringent deposit started forming as the solvent evaporated ca. 30 min after droplet deposition. Width of this deposit increased inwards as a function of time (see Figure 67a-c and snapshots of Figure 66a-c). Deposit was formed by domains that appear to form and accumulate at the edge of the droplet. Changes in the birefringence of the deposit indicate the formation of a liquid crystal phase driven by phase separation induced by drying and densification processes. According to the coffee stain effect^[20] the bright birefringent ring width should increase with time until complete evaporation of the droplet. However, from $t_0 + 80$ min a suppression of the coffee stain mechanism resulting in movement of domain material towards the center of the drop was observed (Figure 67e). We speculate that this was due to the adhesion of formed domains to the air-solvent interface before they reached the contact line. As domains grew in size, they produced a surface viscosity much larger than the bulk viscosity, facilitating nanorod resistance to radial outward flow. As a result, the drop edge depinned and started moving inward. At the end of the process, when the droplet completed dried, a thin ring of aligned nanorods was found at the edge of the drop whereas the majority of nanorod domains settled in the core of the droplet (*Figure 67f*).



Figure 68 Schematic of the self-assembly process of Au nanorods into horizontal arrays. a) Nanorods initially dispersed inside the droplet. b) Au nanorods trasnported by solvent streams to the periphery of the droplet. c)Au nanorods assembled into horizontal arrays on the air-solvent interface at the periphery of the droplet. d) Shrinking of the droplet and compacting of the domains

The droplet evaporation mechanism can be thought as comprising of two main stages (**Figure 68**).

During the first stage (**Figure 68a,b**) a pseudo coffee ring effect occurred. Nanorods, initially uniformly dispersed in the chlorobenzene suspension (**Figure 68a**), were transported towards the periphery (**Figure 68b**) by solvent streams produced inside the droplet to balance out the faster evaporation rate of the periphery. Due to this faster evaporation rate, plus the decreasing height of the meniscus toward the edge nanorods reach at some point the air-solvent interface during its movement towards the periphery. The consequent increase of local nanorod concentration triggered its assembly into horizontal arrays in the periphery area of the droplet. This early formed arrays can be seen as the birefringent ring in the optical images of *Figure 67* **a,b** and are also illustrated in the schematic of **Figure 68b**. The localization of the nanorods on the 2D air-solvent interface when the assembly is triggered promoted its order as smetic monolayers inside the horizontal arrays. Once formed on the interface, horizontal arrays tended to be stabilized by micelle effects induced by the CTAB/TOAB surfactants at the nanorod surfaces.

During the second stage (**Figure 68c,d**) shrinkage of the droplet (*Figure 67* **e,f**) brought the formed domains close to each other (**Figure 68c**) resulting in formation of compact assemblies upon droplet evaporation (**Figure 68d**).

3.3.5. Optical properties of horizontal Au nanorods arrays

In order to explore optical properties, superstructures deposited on glass coverslips were examined by optical transmission imaging and spectroscopy. Optical images of nanorods aligned within domains of an average size of 100 μ m² were acquired under polarized excitation (*Figure 69*). Domains appeared green and red depending on the orientation of the domain main axis respect to the light polarization direction. In particular green/red domains were formed by nanorods with long axes oriented parallel/perpendicular to the polarization excitation. The clear colour transition from one domain to the other was associated to the high degree of internal order within each domain, as previously shown in high magnification SEM images. The origin of this colour shift can be explained by the selective excitation of the longitudinal and transversal surface plasmon resonance (SPR) modes of the nanorods within the domain, respectively. Individual domains changed colour from green to red as the polarization direction was rotated from parallel to perpendicular to the nanorod long axes, respectively.



Figure 69 Polarized transmission images of Au nanorod domains showing red to dark green colour transition associated to the relative orientation of polarized light with the long axes of nanorods that constitute the domains.

3.4. Summary and Conclusions

In conclusion, horizontal superstructures were obtained by droplet evaporation of Au nanorod dispersed in organic solvents. Controlled evaporation led to formation of large area ordered arrays where nanorods assembled side-to-side into monolayer domains with an average size of 100 μ m². Experimental parameters regulating the formation of ordered assemblies were carefully investigated. The process resulted mainly regulated by the concentration of deposited nanorods and the evaporation time. Specifically, slow evaporation, low CTAB (0.1 mM) and 6 nM nanorods concentration emerged as optimum conditions to form large and highly ordered horizontal arrays. Following of the droplet evaporation by polarized microscopy revealed a two stage mechanism. Horizontal arrays firstly formed at the periphery of the droplet and were transported back to the center of the droplet at the last stages of the evaporation process. Optical characterization of formed superstructures revealed marked optical anisotropic behaviour, confirming that nanorods were aligned within individual domains with a high degree of internal order.

3.5. References

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CHAPTER 4

Self-Assembly of Au Nanorods into Vertical Superstructures

A droplet evaporation method will be discussed for the fabrication of vertical arrays by fast evaporation of Au nanorods dispersed in chlorobenzene suspensions. Droplet evaporation is known to produce ordered nanorod arrays of desired geometry only under narrow experimental conditions and controlled evaporation. The assembly of nanorods into ordered arrays usually requires slow evaporation times, in order to allow nanorod organization. The proposed evaporation method is based on evaporation of organic suspensions with minimum control on evaporation conditions. Droplet evaporation occurs in 20 min and produces ordered vertical arrays over large areas (mm²). The mechanism leading to formation of vertical arrays will be discussed in details, together with the role played by key experimental parameters.²

² This work have been published as : "*Facile Formation of Ordered Vertical Arrays by Droplet Evaporation of Au Nanorod Organic Solutions*", A. Martín, C. Schopf, A. Pescaglini, J. J. Wang, and D. Iacopino, *Langmuir*, **2014**, *30* (34), pp 10206–10212

4.1. Introduction

The elongated shape of Au nanorods and their consequent intriguing optical properties are highly attractive for catalytic, plasmonic and biomedical applications^[1-3]. The controllable assembly of Au nanorods into large scale ordered superstructures is also attractive, as it can generate intriguing properties arising from the collective inter-particle coupling of the assembled components^[4]. Among possible assembly geometries, the 2-dimensional (2D) hexagonal sheet (honeycomb) structure that is formed from vertical superstructures is regarded as the ideal assembly configuration for many technological devices, such as solar cells and magnetic memory devices^[5]. Potential applications such as Surface-Enhanced Raman Scattering (SERS), cavity resonators^{2[4]}, nanoscale light polarizers^[6] and ultrafast non-linear optics^[7] have been envisaged for vertically aligned nanorod superstructures.

Common approaches used for fabrication of Au nanorod vertical arrays on substrates include template mediated methodologies, oblique angle deposition^[8] and self-assembly on patterned substrates^[9, 10]. Recently, droplet evaporation has been proposed as a simple and relative fast method for formation of large area nanorod superstructures^[11]. The process has been discussed in detail in chapter 1 of this thesis. Briefly, it consists in depositing a droplet of nanorod suspension (usually water) onto a substrate (usually silicon) and let the solvent evaporate under controlled conditions. The assembly is driven by relatively weak attractive forces (van de Waals, dipole-dipole interactions), becoming relevant as the solvent evaporates. Also electrostatic repulsive forces, hydrophobic interactions, capillary forces and entropic depletion interactions play a role that mediate nanostructure's self-assembly.

The evaporation mediated process is affected by several parameters (such as nanorod concentration, surfactant, evaporation rate, nanorod aspect ratio and substrate, among others) which have to be controlled in order to obtain the desired assembly geometry. For example, nanorod surfactant exchange has been used by *Marzán* and co-workers^[12] who used cationic Gemini surfactants instead of CTAB for the growth of Au nanorods. Droplet evaporation of such suspensions produced self-assembly of nanorods into highly ordered, multilayer vertical arrays on substrates. In a similar manner, *Liu* and co-workers^[13] produced nanorod vertical assembly by substituting CTAB with a less electrostatically repulsive glycol/thiol. Using a two steps controlled evaporation rate, Xie and co-workers^[14] achieved reproducible vertical

nanorod arrays over large areas. Finally, Xiong and co-workers^[15] fabricated closely spaced vertical nanorod arrays (ca. 7 nm) by altering the ionic strength of the deposited nanorod solution. Thus, the simplicity and flexibility of the method makes droplet evaporation processes highly suitable for fabrication of nanorod ordered arrays. However, the ability to induce high degree of order in the self-assembly process is still limited by the necessary use of appropriate, often long, drying conditions and warrants careful control of several parameters. Moreover, the use of droplet evaporation techniques for fabrication of structures with a high degree of order that extends over large areas has, to date, proven challenging.

In this chapter, we present a facile and fast approach for fabrication of large area vertical nanorod superstructures. We used droplet evaporation of overgrown nanorod chlorobenzene suspensions as method for the assembly process. The simple evaporation of a droplet under uncontrolled conditions (free air, 20 min) led to the formation of vertical nanorod monolayer superstructures covering the entire area of the deposited droplet (20 mm²). The assembly process was found to be prevalently dependent on nanorod concentration, whereas the influence of other parameters was found negligible. At low concentrations nanorods assembled vertically into domains, with the area between domains occupied by nanorods deposited horizontally to the substrate. At high concentrations density and size of domains increased until the entire area of the droplet was covered by vertical superstructures. Observation of droplet evaporation process under microscope light revealed that nanorods assembled at the interface between solvent and air, forming domains that grew in size and density as the solvent evaporated.

4.2. Experimental Section

4.2.1. Synthesis and phase transfer of Au nanorods

In this chapter Au nanorods type A4.2 and A5.3 of respectively dimensions 20x60 nm 23x55 nm were used. All of them were generated by a post-synthesis modification of nanorods obtained from seed mediated reported by Liz-Marzan and co-workers^[16] and described in the second chapter of this thesis, section 2.2. For comparison reason Au nanoords type A4.1, synthesized by the usual seed mediated method were also used.

After synthesis, Au nanoords were transferred from the original aqueous media to chlorobenzene following a phase trasnfer method^[17] described in the second chapter of this thesis, section 2.5

4.2.2. Droplet deposition of Au nanorods solution

A small aliquot (10 μ L) of Au nanorod chlorobenzene solution ([Au] = 1-5 nM) was deposited onto SiO₂ substrate, and left to evaporate in free air over 20 min. Solvent evaporation resulted in formation of vertical assemblies

4.2.3. Characterization of vertical array

Scanning electron microscopy (SEM) images of nanorods vertical arrays were acquired using a field emission SEM (JSM-6700F, JEOL UK Ltd.) operating at beam voltages of 5 kV. Optical image were acquired with an optical microscope (Zeiss axioscop II) equipped with a CCD camera (Optronics DEI-750) using a 100 W halogen lamp as illumination source.

4.3. Results and Discussion

4.3.1. Synthesis of overgrown nanorods

Au overgrown nanorods (ONRs) were synthesized by a combined seedmediated/overgrowth method described by Marzán and co-workers^[16]. The process consisted in step additions of reducing agent ascorbic acid to a solution of nanorods obtained by seed mediated method. The excess ascorbic acid reduced the Au (I) ions left unreacted in the nanorod solution, leading to isotropic growth of the nanorods.



Figure 70 a) Evolution of the UV VIS absorbance spectrum of the gold nanorods water suspensions along the overgrowth process. b, SEM image of Au nanorods after overgrowth process, c,d) histograms of the Au nanorods c) diameter anddc) length before (red bars) and after overgrowth (blue bars).

Figure 70a shows UV-vis spectra of nanorods solutions after subsequent additions of ascorbic acid. A gradual increase in the intensity of the longitudinal band was observed in combination with a slight blue shift from 720 nm (black curve) to 690 nm (pink curve). These changes allowed the monitoring of the growth process, which was considered complete when no further changes in the UV-vis spectrum were detected upon addition of ascorbic acid.

Figure 70 b,c show statistical analysis of nanorod dimensions obtained from SEM images of suspensions of Au nanorods before (red bars) and after (blue bars) overgrowth process. Nanorods grew from average size $15 \pm 2 \text{ nm} \times 40 \pm 3 \text{ nm}$ (AR) = 2.7 to a final mean size of $23 \pm 3 \text{ nm} \times 55 \pm 3 \text{ nm}$, aspect ratio (AR) = 2.3. Together with the changes in

dimension, original Au nanorods rounded ends were transformed into flat ends along the process, as can be appreciated in the SEM images of Figure 70d,e.

Prior to deposition on solid substrates, nanorods were transferred into chlorobenzene following the method reported by Chen and co-workers^[24] and described in the chapter two of this thesis, section 2.5.

4.3.2. Formation of vertical superstructures

Ordered arrays were obtained by deposition of overgrown nanorods chlorobenzene solutions onto an arbitrary substrate (usually SiO_2), followed by evaporation of the droplet under free air over 20 min (process illustrated in the schematic of *Figure 71a*).

Figure 71b shows a low magnification SEM image of the dried droplet obtained by deposition of (10µl, 14 nM) Au nanorod chlorobenzene solutions onto a SiO₂ substrate showing uniform distribution of material within the droplet. A closer observation of formed structures (Figure 71c) revealed that nanorods assembled into domains of average size 20 μ m², uniformly covering ca. 95% of the droplet surface. High SEM magnification images of an individual domain (Figure 71d) showed formation of a 2D solid and highly ordered phase where nanorods aligned with their long axes perpendicular to the substrate. The nanorods were organized into closely packed hexagonal monolayer arrays, as revealed by the fast Fourier transform analysis showed as inset in Figure 71d. Evidence of monolayer formation is shown in Figure 71e, depicting the edge of an isolated nanorod domain.





Figure 71 a) Schematic of the self-assembly of Au nanorods into vertical arrays. b-e) SEM images of vertical arrays formed on SiO₂ substrate by evaporation of Au nanorod chlorobenzene suspensions; b) Low magnification image of the dried droplet; c) Domain formation within the dried droplet; d) High magnification image of an individual domain showing ordered hexagonal assemblies. Inset: Fast Fourier transform of the image. e) Image of the border of a domain showing evidence of monolayer formation.

In order to clarify the role played by the organic solvent in the assembly mechanism we performed droplet evaporation of aqueous suspensions (10 μ l, 14 nM) under the same experimental conditions (ie. free air). Droplet evaporation of aqueous suspensions resulted in formation of disordered multilayer vertical and horizontal aggregates which accumulated at the periphery of the droplet, in agreement with coffee-stain mechanisms^[18] (see **Figure 72**), and low density mixed parallel and vertical assemblies formed in the central area of the droplet (not shown).



Figure 72 SEM images of nanorods deposited on a SiO₂ substrate after evaporation of a (10 μ l, 14nM) aqueous suspension. a) Low magnification. b) High magnification.

The compactness and high degree of order obtained using chlorobenzene were attributed to their physico – chemical differences with the water^[19, 20]. We attributed the results obtained with chlorobenzene to the lower surface tension, viscosity and dielectric constant of this solvent. These factors promoted a higher degree of ordering by offering less resistance to the domain movement thus allowing more nanorods to be at an equilibrium position and assemble together in the solution before droplet evaporation.

4.3.3. Effect of experimental parameters

With the aim to understand the mechanism of vertical superstructure formation, investigation of the effects played by the following parameters was undertaken: nanorod aspect ratio and substrate, nanorod concentration, surfactant concentration, and evaporation rate.

Influence of the substrate and nanorod size on the assembly process

Regarding the effect of substrates, vertical superstructures of comparable high order were obtained from droplet evaporation on SiO₂, glass and glass/ITO substrates with calculated contact angles 52° , 40° and 72° respectively (data no shown).

Chapter 4



Figure 73 a,b) statistical analysis of the diameter and length of Au nanorods *a*) mean diameter 23 ± 2 nm, mean length 53 ± 3 nm, AR = 2.3, SPR = 670 nm; *b*) mean diameter 19 ± 2 nm, mean length 59 ± 2 nm, AR = 3, SPR = 711 nm; *c,d*) SEM images of vertical arrays formed in free air evaporation of chlorobenzene droplets (10µl, 14nM) of nanorods with aspect ratio *c*) 2.3; *d*) 3.

The droplet evaporation of Au nanorods dispersed in chlorobenzene generated also ordered assemblies from nanorods of different sizes. Vertical arrays were successfully obtained using Au nanorods of aspect ratios 2.3 and 3. Nanorods were synthesized by small variation of the basic seed-mediated syntheses and were subsequently overgrown by addition of ascorbic acid. **Figure 73a,b** shows statistical analysis of nanorod dimensions obtained from SEM images of diluted suspensions. Nanorods displayed the following sizes: 23 ± 2 nm $\times 53 \pm 3$ nm, AR = 2.3, 19 ± 2 nm, $\times 60 \pm 2$ nm, AR = 3.. The position of the maximum of the longitudinal mode was 670 and 711 for aspect ratios of 2.3 and 3, respectively. SEM images of vertical arrays obtained from the three nanorods are shown in *Figure 73c-d*. Overall the nanorod dimension and aspect ratio did not affect the assembly process. Equally ordered vertical arrays were produced in all cases proving the robustness of the assembly method in the range of size and aspect ratio experimented.

Influence of nanorod concentration on the assembly process

Ordered assemblies formed across a relatively large range of Au nanorod concentrations, between 1.4 nM and 14 nM. The increase in concentration affected the size of the deposited dried droplet, which varied from 1.5 mm in diameter to 5 mm in diameter, respectively (see photographs of the droplet in *Figure 74*).
-			ALL A
$ \rightarrow $	\leftrightarrow	-	0
5 mm	4mm	3mm	1.5mm
14 nM	6 nM	3 nM	1.4 nM

Figure 74 Photographs of dried droplets obtained from Au nanorods chlorobenzene solutions of decreasing concentrations (14 nM to 1.5 nM)

Domain size, density and compactness were also found to be dependent on the initial nanorod solution concentration. *Figure 75* reports increasing magnification (top to bottom) SEM images of droplets obtained from Au nanorod solution concentrations 1.5 nM, 3 nM, 6 nM and 14 nM (left to right). At low concentrations, nanorods arranged vertically into domains of 2 μ m² average size (*Figure 75a*). The density of formed domain was low and high density of empty areas was found upon droplet evaporation. At concentrations 3 nM and 6 nM domains grew to an average size of 20 μ m². Also domain concentration grew and was accompanied by a reduction of empty areas between domains (*Figure 75b*,c). Finally, for concentrations of 14 nM individual domains merged uniformly covering the entire area of the droplet (*Figure 75d*). Overall, the increase in nanorod concentration caused the formation of a higher number of domains with larger size.

In contrast with the changes occurring in domain size and compactness, the ordering of nanorods inside the domains was not affected by changes in nanorod concentration, resulting in formation of highly ordered vertical arrays in the whole range of explored concentrations (1.5 nM - 14 nM) (**Figure 75** bottom row). This suggests that the assembly of nanorods into vertical configurations was highly favourable and occured on a fast time scale even at low nanorod concentrations. Therefore high nanorod concentration is no necessary to trigger the assembly but to promote the formation of more compact and larger domains. Our findings are in contrast with what is reported in literature where application of long and controlled evaporation times are reported to be essential to the formation of ordered arrays^[14]



Figure 75 Low and high magnification SEM images of vertical arrays on a SiO₂ substrate formed by evaporation in free air (20 min) of 10 μ l droplets of Au nanorod chlorobenzene suspensions of concentrations a) 1.5 nM ; b) 3 nM ; c)6nM; d) 14nM.

In order to calculate the increase in surface coverage correlated to the progressive increase of Au nanorod solutions, reflection optical microscopy images of dried droplets were acquired. **Figure 76** shows optical images of dried droplets obtained from Au nanorod solutions between 1.5 nM and 14 nM. Three areas were identified with different color intensities. Bright gold areas corresponded to areas where the nanorods aligned vertical to the substrate. Dark gold areas corresponded to nanorods lying flat on the substrate. Black areas where empty spaces, corresponding to background reflection. Surface coverage was defined as the ratio between the area occupied by the vertical arrays (bright gold areas) and the total area of the image and was calculated from the optical images by Image J program. Domain surface coverages of 34 %, 65%, 70% and 94% were calculated for Au nanorod concentrations of 1.5 nM, 3 nM, 6 nM and 14 nM, respectively.



Figure 76 Optical microscope images of vertical arrays on a glass cover slips formed by evaporation in free air (20 min) of 10 μ l droplets of Au nanorod chlorobenzene suspensions of concentrations a) 1.5 nM; b) 3 nM; c)6nM; d) 14nM

Influence of surfactant concentration

The concentration of CTAB is known to play an important role on the self-assembly process of Au nanorods with reports in literature that higher CTAB concentrations are favorable to formation of better assemblies^[14]. In contrast, our data showed that the best quality of assemblies (in terms of surface coverage and ratio between vertical nanorod domains and deposited horizontal nanorods) formed when the CTAB concentration was kept closer to the low value of 0.1 mM. *Figure 77b-e* show optical reflection images of dried droplets obtained from Au nanorod solutions between 1.5 nM and 14 nM. The images were used to estimate the surface coverage occupied by formed domains for nanorod solutions of increasing concentrations at CTAB concentration 0.35 mM. Domain surface coverage of 32%, 51%, 57% and 66% were calculated for Au nanorod concentrations of 1.5 nM, 3 nM, 6 nM and 14 nM, respectively. Surface coverage obtained for nanorod of increasing concentrations at CTAB on 0.35 mM is summarized in plot of *Figure 78*.



Figure 77 Optical microscope images of vertical arrays on a glass cover slips formed by evaporation in free air (20 min) of 10 μ l droplets with CTAB concentration 0.35mM and Au nanorod concentrations a) 1.5 nM; b) 3 nM; c) 6nM; d) 14nM. Insets: High magnification SEM images of the vertical arrays.

The plot of the figure reports that at 0.1 mM CTAB concentration the surface covered by vertical arrays increased from 34% to 94% as nanorod concentration increased from 1.4 nM to 14 nM, when CTAB concentration was increased at 0.35 mM surface coverage values increased only from 34 to 66% as nanorod concentration increased from 1.4 nM to 14 nM. The fact that nanorods could not be successfully transferred into organic phase at CTAB concentrations lower than 0.1 nM or higher than 0.35 nM, prevented the exploration of larger CTAB concentration ranges.



Figure 78 Surface Coverage of vertical arrays obtained by free air evaporation of 10 μ l droplet with variable Au nanorods concentration and CTAB concentrations 0.1mM (blue curve) and 0.35 mM (red curve).

Variation of TOAB concentration from the 50 mM value used for phase transfer did not affect the final degree of order obtained. However, the concentration of TOAB affected the stability of nanorod chlorobenzene suspension, which was stable for over two weeks with [TOAB] = 50 mM and for less than a week at lower TOAB concentrations

Influence of evaporation rate

In order to investigate the effect of the evaporation rate, deposited droplets were covered with a petri dish in order to slow down the evaporation to ca. 3 h. Under these conditions nanorods self-assembled into compact and well ordered vertical arrays only on the periphery of the droplet, in an area corresponding to ca. 25% of the entire droplet area.



Figure 79 a) SEM image of assembly obtained by slow evaporation of nanorod chlorobenzene solutions ([Au nanorods] = 6 nM). b) SEM images of assemblies obtained at the periphery (top right). c) SEM image of assemblies obtained at the central area.

Figure 79 displays a low magnification SEM image of a dried droplet obtained by slow evaporation of a 6 nM Au nanorods chlorobenzene suspension. As can be seen, nanorods assembled vertically at the periphery of the ring, within the marked area (see *Figure 79b*). Outside the marked area, nanorods mainly aligned side-to-side horizontal to the substrate were found, as shown in *Figure 79c*.

Nanorod ordered vertical arrays formed at the periphery of the droplet in the whole range of concentrations (1.5 nM - 14 nM). Domain size and compactness were affected by the initial concentration of nanorods as already reported for the fast evaporation process.



Figure 80 Low and high magnification SEM images of vertical arrays on a SiO₂ substrate formed by controlled evaporation under a petri dish (3h) of 10 μ l droplets of Au nanorod chlorobenzene suspensions of concentrations a) 1.5 nM; b) 3 nM; c)6nM; d) 14nM.

SEM images obtained from the periphery of slow evaporated dried droplets (*Figure* 80) show that at concentration 1.5 nM nanorods arranged vertically into domains of 2 μ m² average size with gaps between domains of ca. 200 nm (*Figure 80*a). At concentrations 3 nM domains grew to an average size of 10 μ m² and gaps were reduced to a size of ca. 10 nm (*Figure 80*b). Finally, for concentration 6 nM and higher domains merged covering the periphery of the droplet with almost no gaps (*Figure 80*c,d). As occurred with the fast evaporation process, nanorods inside the domains were highly ordered in the whole range of concentrations explored (*Figure 80*, bottom row).

Analogously to statistical analysis performed on fast evaporation droplets, optical miscroscopy in combination with image J program was used to calculate the surface coverage obtained from the slow evaporation process at different nanorod concentrations. Calculations were performed with optical images taken at the periphery and center of the droplet, respectively. Optical images used for statistical analysis are reported in *Figure 81*a (periphery) and *Figure 81*b (center). At the periphery of the droplet coverage increased slightly from 78% to 83% when nanorod concentration increased from 1.4 nM to 14 nM, at the center of the droplet the area occupied by vertical arrays grew from 16% to 81% in the same range of concentrations.



Figure 81 optical microscope images of a) Periphery; b) central area of dried droplets obtained by slow evaporation of Au nanorod chlorobenzene suspensions of concentrations a1,b1) 1.5 nM; a2,b2) 3 nM; a3,b3) 6nM; a4,b4) 14nM.

Surface coverage values achieved with slow evaporation method for different nanorods concentration ([CTAB] = 0.1 mM) are summarized in **Figure 82** (black curves). From these data it was evident that at high nanorod concentrations the surface coverage of vertical arrays in the center of the droplet (black dashed curve) was equivalent to the coverage obtained at the periphery (continuous black curve), resulting in coverage of entire droplet by vertical arrays. For comparison the coverage calculated for free air assembly is also reported in **Figure 82** as a green line. Again at high nanorod concentration the two evaporation methods produced equivalent surface area coverage.



Figure 82 Percentage of the droplet covered by vertical arrays obtained by slow evaporation, at the periphery (black continuous curve) and the center (black dashed curve) of the droplet. Green curve shows the coverage obtained by fast (free air) evaporation.

4.3.4. Mechanism of Au nanorod self-assembly

Our experiments showed than vertical superstructures with a high degree of order can be obtained from chlorobenzene suspensions of nanorods. Observation of the evaporation process revealed that nanorods organized into vertically aligned domains at the interface between solvent and air.



Figure 83 a) Snapshots of drying stages of a ONRs droplet $(10\mu l, 14nM)$ deposited on glass coverslip; b optical reflection images of drying droplet showing progressive formation and growth of nanorod domains.

Snapshots of evaporation stages for a droplet (10 μ l, 14nM) deposited on a glass surface (**Figure 83a**) displayed a colour transition from red to gold associated to the progressive organization of nanorods into vertical arrays. A modest shrinking of the droplet diameter from 7 mm to ca. 5 mm was also observed upon solvent evaporation. *Figure 83b* shows optical reflection images of an evaporating droplet, highlighting the formation and growth of nanorod domains.

The assembly of the nanorods side to side into vertical superstructures was ascribe to a balance between repulsive electrostatic and steric forces and van der Waals attractive hydrophobic interactions between the CTAB/TOAB alkyl chains deposited on the surface of Au nanorods. Supporting our observations *Xie* and co-workers^[14] and *Liu* and co-workers^[13] have reported studies where the nanorods side to side assembly configuration resulted energetically favourable over the end to end configuration

From images of **Figure 83** and all information gathered from optical and electron microscopy analysis of dried droplets we hypothesise the following mechanism of nanorod assembly and domain growth.



Figure 84 Schematic of the self-assembly process of Au nanorods into vertical arrays. a) Nanorods initially dispersed inside the droplet. b) Formation of the first vertical arrays and phase segregation at the interface from the bulk solvent. c) Growth of former domains and addition of new ones to the interface. d) Shrinking of the droplet and compacting of the domains.

Initially nanorods were uniformly dispersed in the chlorobenzene suspension (*Figure 84a*). Due to fast evaporation rate, the meniscus of the droplet decreased quickly and produced a local increase of nanorod concentration at the liquid-air interface. This local increase in concentration triggered the assembly of nanorods. As result within 5-10 minutes of droplet deposition a phase segregation process^[21] occurred where vertical arrays can be seen at the air-solvent interface as continuous gold layer (**Figure 83 b1,b2**) and is illustrated in *Figure 84b*. This same kind of self-assembly on interfaces by phase segregation have already been reported using other nanostructures by other authors^[20, 21]

Once on the interface, vertical arrays of nanorods are kept perpendicular thank to the torque force of surfactant molecules occupying the external surface of nanorods (**Figure 84b**). Finally, as the solvent evaporation continuous, formed assemblies grew in size and density and new domains reached the air-solvent interface forming a continuous gold layer as can be seen in **Figure 83b3** and illustrated in **Figure 84c**. The high electrostatic repulsion between the end of the nanorods is thought as the most probable cause of

monolayer formation^[14]. Towards the end, shrinkage of the droplet brought formed domains close to each other, resulting in formation of compact assemblies upon droplet evaporation (**Figure 84d**).

4.4. Summary and Conclusions

In conclusion, we have used droplet evaporation methods for the facile and fast fabrication of vertical superstructures. Droplet evaporation of nanorods dispersed into chlorobenzene solutions led to formation of large area ordered arrays where nanorods assembled vertical into monolayer domains. The assembly process proceeded on a fast time scale (20 min) and did not necessitate strict control of experimental parameters. Extensive investigation of experimental parameters was carried out showing that vertical arrays formed over wide nanorod concentration ranges and with nanorods of slightly different aspect ratios. Fast evaporation, low CTAB and high nanorods concentration emerged as the optimum condition to form the vertical arrays. The mechanism of droplet evaporation was followed by optical microscopy and was explained on the basis of fast organization of nanorods into domains at the interface between solvent and air.

4.5. References

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Chapter 5

SERS performances of Au nanorod superstructures immobilized on rigid substrates

A stamping process has been developed to immobilize horizontal and vertical Au nanorod arrays fabricated on arbitrary rigid substrates. The method, based on a simple stamping process, was developed to improve the otherwise poor adhesion of nanorod arrays on rigid supports. The potential of Au nanorod immobilized substrates as SERS probes was investigated using model molecule 4-aminobenzenethiol (4-ABT), for which enhanced signals where obtained compared to the Raman signals of the bulk molecule. Enhancement factors of the order of 10^4 and 10^5 were calculated for horizontal and vertical arrays, respectively. Quantitative Raman detection of 4-ABT was obtained with detection limits in the nM concentration range. Fabricated arrays displayed good stability and uniformity, showing their potential as sensing platforms for plasmon-induced optical molecular detection ³.

³ This work has been published as: "Surface-Enhanced Raman Scattering of 4-Aminobenzenethiol on Au Nanorod Ordered Arrays", Martín.A, Pescaglini.A, Schopf.C, Scardaci.V, Coull.R, Byrne.L, Iacopino.D, J. Phys. Chem. C, 2014, 118(24), pp 13260-13267.

5.1. Introduction

Surface-Enhanced Raman Scattering (SERS)^[1-3] of analytes adsorbed on metal nanostructures is a powerful tool for collecting molecular chemical information. It is widely accepted that SERS enhancement comes from two contributions: chemical effects (CM) and electromagnetic effects (EM)^[4-11]. CM effects result from charge transfer mechanisms^[4, 5, 8, 12] between analyte molecules chemisorbed at the metal substrate. EM enhancements are due to the collective excitation of the surface plasmons of the metal nano-structures by incident light^[13-15]. Although it has been shown that SERS can be observed for molecules adsorbed on isolated metal nanostructures, the largest enhancement factors are obtained in the gaps of closely spaced nanostructures (named "hot spots"), due to the coupling of localized surface plasmons^[16]. Of all possible nanostructure shapes, Au nanorods are especially suitable for SERS applications as they offer strong plasmonic fields while exhibiting excellent optical tunability and biocompatibility^[17, 18]. Theoretical work by Schatz and co-workers^[19] has calculated the greatest E-field enhancement at the end of isolated nanorods compared to other nanoparticle shapes. Moreover, its dimensional anisotropy gives nanorods the ability to assemble into different alignment configurations. Thus, horizontal or vertical arrays with endto-end or side-to-side assembly, where large E-fields between neighboring nanorods can be coupled, constitute attractive geometries for SERS.

One of the key challenges for the use of Au nanorods as SERS platforms is the limited availability of methodologies for the rational organization of nanorods into well-defined arrays, which would allow fabrication of SERS substrates with the high level of uniformity and robustness required for analytical detection. To date, a number of self-assembly techniques have been used for the fabrication of ordered Au nanorod arrays such as capillary and convective assembly^[20], electrostatic interactions^[21], template methods^[22], covalent bonding^[23] and oblique angle deposition^[24, 25]. Recently droplet deposition has emerged as a promising technique for fabrication of SERS substrates^[26-29]. For example, detection of cocaine derivatives and food contaminants has been achieved using vertical arrays formed by slow evaporation of highly concentrated Au nanorod aqueous solutions^[27, 29]. However, further progress on the order, assembly area and reproducibility are necessary to enable metal nanorod-based SERS substrates to provide low limits of detection, chemical stability and reproducibility of results required for analytical detection.

This chapter describes a stamping process to immobilize and clean horizontal arrays (HA) and vertical arrays (VA) of Au nanoords and overgrown Au nanorods prepared by droplet deposition of chlorobenzene suspensions (see Chapter 3 and 4). The potential of such arrays as SERS substrates was investigated using model molecule 4-aminobenzenethiol (4-ABT). Both substrates gave enhanced signals compared to the signals of the bulk molecule. Finite-difference time-domain (FDTD) methods were used to simulate the electromagnetic fields in horizontal and vertical array and enhancement factors (EF) were obtained. Finally Quantitative detection of 4-ABT was performed on vertical arrays which showed intensity/concentration logarithmic dependence between 0.1 mM and 100 pM. Arrays also displayed good uniformity and good temporal stability under continuous laser irradiation.

5.2. Experimental section

Two kinds of nanorods were used along this chapter, **nanorods A3** of dimensions 13x44 nm (A.R: 3.1) synthesized by seed mediated methods (detailed synthesis in section 2.1.5), and **nanorods A5.2** of dimensions 23x55 nm (A.R:2.4) generated by overgrowth of previously seed mediated synthesized Au nanorods of dimensions 15x40 nm (see Chapter 4).

5.2.1. Stamping Process

Horizontal gold nanorods arrays were fabricated by controlled evaporation (see Chapter 3) of 13x44nm AuNRs chlorobenzene dispersions (10 μ l, 6nM). Fabricated arrays were transferred intact onto transparent supports by placing a glass cover slip onto the original cover slip support and pressing the two surfaces together during c.a. 5 s. Excess of organic matter was removed by immersing glass-nanorod arrays in isopropanol for 2 h, followed by multiple rinses with fresh isopropanol.

Vertical Au nanorods arrays were fabricated by droplet deposition (see Chapter 4) of 23x55 nm Overgrown AuNRs chlorobenzene dispersions (10µl, 14nM) nanorods. Fabricated arrays were also transferred onto cover slips by stamping method and cleaned following the same procedure used with the horizontal arrays.

5.2.2. Characterization of arrays

Electron Microscopy

Scanning electron microscopy (SEM) images of vertical and horizontal nanorods arrays immobilized on cover slips were acquired using a field emission SEM (JSM-6700F, JEOL UK Ltd.) operating at beam voltages of 5 kV.

Optical characterization

Transmission spectra of gold nanorod arrays were acquired with an inverted IX-71 Olympus microscope with a 100X objective. The sample was illuminated with a 100 W halogen lamp. The light collected by the objective was directed into the entrance of slit of a monochromator (SP-300i, Acton Research) equipped with a thermoelectrically cooled, back illuminated CCD (Spec10:100B, Princeton Instruments) for spectra acquisition. The sample polarization rate was determined by a polarizer placed between the lamp and the sample. Spectra were typically recorded using an integration time of 1-10 s. The extinction was

calculated according to Lambert-Beer's law: $A = -\log 10(I/I_0)$ with I being the sample spectrum and I_0 being the blank spectrum taken from a nearby clean area on the substrate.

5.2.3. SERS acquisition

Raman spectra were obtained from a Renishaw Raman system with excitation wavelength at 514 nm and from a Horiba Jobin-Yvon system with excitation wavelength at 633 nm. Laser power values were 1.5 mW and 15 mW, respectively. In both systems the laser beam was focused onto a spot of 5 μ m in diameter with an objective microscope at a magnification 50X. Acquisition time was usually 30 s. To obtain SERS spectra, Au nanorod arrays deposited on glass cover slips were immersed in 4-ABT solutions (MeOH, 0.1 mM – 100 pM, 20 h). Substrates were rinsed with MeOH in order to remove unbound 4-ABT and gently dried under N₂ stream.

5.3. Results and Discussion

5.3.1. Stamping of gold nanorods arrays on glass substrates

Horizontal and vertical Au nanorods arrays (HA) and (VA) were fabricated following a method illustrated in **Figure 85a,b**. For simplicity only the formation of VA is schematically represented. The formation of HA followed an analogous mechanism. The fabrication process comprised two steps: a) formation of the nanorods arrays by controlled evaporation of a droplet (10 μ l) of Au nanorods chlorobenzene suspension (**Figure 85a**), and b) stamping of the resulting nanorod array on a receiving support (**Figure 85b**).



Figure 85 Formation of vertical arrays by combined droplet evaporation/stamping technique; a1-3) droplet evaporation process; b1-3) stamping and cleaning of Au nanorod arrays on a receiving substrate. c) Images of the VA on a cover slip c1) before stamping, c2) after stamping.

Nanorod arrays were obtained by droplet evaporation of Au nanorod chlorobenzene solutions, as described in details in chapters 3 and 4 (**Figure 85a**). Solvent evaporation of the chlorobenzene solutions resulted in formation of highly ordered superstructures. However, during solvent evaporation a phase separation process occurred resulting in deposition of excess organic surfactant and organic residues between the nanorod array and the support (**Figure 85a3**). This residual deposit made fabricated arrays labile, easy to damage and easy to detach from the support. In order to improve adhesion of arrays to the support and clean off residual organic matter an additional stamping process was introduced, as shown in **Figure 85b**. Specifically, a receiving support (glass coverslip) was pressed on the original cover slip containing the arrays for 5 s (**Figure 85b1**). Nanorod arrays were transferred intact onto the glass support along with part of the residual organic matter (**Figure 85b.2**), which

was eliminated by immersion in isopropanol, followed by multiple rinses with clean isopropanol (Figure 85b.3).



Figure 86 a,b) SEM images of arrays obtained by droplet evaporation of Au nanorods chlorobenzene suspensions and stamping of the dried droplet on glass coverslip support; a) horizontal arrays formed by Au nanorods (24× 44 nm); b) vertical arrays formed by overgrown nanords (23× 55 nm). c) SEM images of nanorods arrays before stamping. c1) Horizontal, c2) Vertical

Figure 86b1 shows a low magnification image of vertical arrays stamped on a glass coverslip showing how the entire area of the deposited droplet was transferred onto the host support. A closer inspection (Figure 86b2) confirmed that nanorod arrays were successfully transferred on the host support with minimum alteration of their geometrical order. In comparison with droplet evaporated superstructures (Figure 86c2) only occasional irregularities and formation of crack patterns occurred on the lattice as result of the stamping process. Figure 86a shows equivalent SEM images for horizontal arrays, whereas Figure 86c1 shows SEM image of droplet deposited horizontal arrays. In general, both vertical and horizontal stamped nanorod arrays were chemically stable, did not suffer from handling damages and showed resistant to harsh environments and chemical conditions (ie. contact with concentrated acid and strong oxidizing solutions)

5.3.2. SERS of 4-ABT deposited on horizontal and vertical arrays

Potential use of horizontal and vertical arrays as SERS platforms was investigated using 4-Aminobenzenethiol (4ABT) as test molecule. 4-ABT is a molecule frequently used to investigate the potential use of noble metal nanostructures as SERS platforms ^[9, 11, 30, 31] because its Raman spectrum is qualitatively different than its SERS spectrum. When linked to appropriate nanostructures the 4-ABT SERS spectrum presents two kinds of peaks. The peaks associated to vibrational modes of symmetry a1 (Raman active) and some peaks associated to vibrational modes of symmetry b2 (theoretically not Raman active).

These newly appeared b2 peaks are present in the SERS spectra thanks to charge transfer phenomenon between the 4ABT molecule and the noble metal nanostructure, which is also the mechanism behind the chemical enhancement (CM) of the SERS technique^[4, 8, 10]. Almost all the analytes are sensitive to the CM effect, but the rising of new peaks in SERS spectra is a quiet rare property that makes 4-ABT an ideal molecule to study the EM and CM produced by noble metal nanostructures.

a) 20 [°] 0115 tul uame tul tul tul tul tul tul tul tul	HORIZON POWDI	TAL TAL ER 1400 1500 1600 er (cm ⁻¹)	b) SH
Peak Position	Peak Position	Peak Position	Assignments
4-ABT powder	4-ABT/Au perpendicular	4-ABT/Au parallel	
1591	1578	1576	νCC, 8a (a ₁)
1569			νCC, 8b (b ₂)
1495	1478	1475	ν CC + dCH, 19a (a ₁)
	🗙 1435	1434	ν CC+ δ CH, 19b (b ₂)
	🛨 1388	1387	δ CH+vCC, 3(b ₂)
	1304	1297	ν CC+ δ CH, 14b (b ₂)
1178	1178	1180	δ CH, 9a (a ₁)
	★ 1139	1139	δ CH, 9b (b ₂)
1086	1074	1073	νCS, 7a (a ₁)
1007	1004	1005	γCC+γCCC, 18a, (a ₁)

Figure 87 a) SERS spectra obtained from parallel and vertical arrays immersed in 4ABT methanol solution (0.1mM) for 20h. b) 4-ABT molecule. c) Table with Raman band frequencies of bulk 4-ATP and SERS band frequencies of 4-ABT adsorbed on parallel and perpendicular substrates; [*] v =

stretching, δ , γ = bending, π = wagging. Letters in parenthesis indicate the vibrational symmetry. b2 modes are label with a green star.

*Figure 87***a** (black spectrum) shows the Raman spectrum of solid 4-ABT deposited on glass coverslips obtained using 633 nm radiation excitation source. The spectrum was dominated by a prominent peak at 1086 cm⁻¹ assigned to the C-S stretching mode. A medium peak at 1591 cm⁻¹ assigned to C-C stretching modes was also observed. The spectrum also shown three very weak peaks at 1007, 1178 and 1495 cm⁻¹ assigned to C-C and C-C-C bending, pure C-H bending and a combination of C-C stretching and C-H bending, respectively. All detected peaks were associated with the characteristic a₁ vibrational modes in plane, in phase modes of the 4-ATP molecule, as summarized in the first column of the table of **Figure 87c**.

In order to obtain SERS spectra, fabricated nanorod arrays were immersed in 4-ABT solutions (MeOH, 0.1 mM) for 20 h. SERS spectra obtained from horizontal and vertical arrays using a 633 nm laser as excitation source are shown in **Figure 87a** (blue and red spectra). SERS spectra obtained from horizontal and vertical arrays showed markedly different characteristics compared to the Raman spectrum of the bulk material. The two a1 modes appearing at 1086 cm⁻¹ and 1591 cm⁻¹ in the powder spectrum were also found in the SERS spectra but shifted at 1074 cm⁻¹ and 1578 cm⁻¹ in both arrays, respectively. The weak mode originally observed at 1178 cm⁻¹ was found at 1180 cm⁻¹ in the powder spectrum was not seen in the SERS spectra, indicating that this mode might have been buried under the background originated from the Au substrates.

In addition, three new peaks were found in the SERS spectra at 1139, 1388 and 1435 cm⁻¹. These peaks were assigned to b_2 in plane, out of phase vibrational modes and were attributed to metal-molecule charge transfer related to a chemical enhancement process.^[4, 7-11]. A closer examination of the SERS spectra obtained from vertical and from horizontal arrays revealed that the relative intensities of various Raman bands changed between the spectra. We hypothesized that the observed changes in relative intensities were due to CM mechanisms (explained in more detail in section 4.3.4 of this thesis). The enhancement of b_2 modes is also indicative of an orientation of 4-ABT molecules perpendicular to the Au arrays^[11]

5.3.3. Extinction spectra of Au nanorods Arrays

The examination of SERS spectra reported in **Figure 87** showed a higher absolute intensity of the SERS spectra obtained with vertical arrays compared to the SERS spectra obtained with horizontal arrays. This difference could be ascribed to the different position and intensity of the plasmon resonance peak of the two arrays. In order to prove this point, we measured extinction spectra of horizontal and vertical arrays (**Figure 88**).

The extinction spectrum of horizontal arrays (**Figure 88a**, black curve) measured with un-polarized light was characterized by a broad peak with a maximum centered at 580 nm. Spectra recorded with light polarized perpendicular and parallel to the direction of the nanorod longitudinal axes were characterized by narrower peaks centered at 580 nm (**Figure 88a**, green curve) and 750 nm (**Figure 88a**, blue curve) respectively. The extinction spectrum of vertical arrays (**Figure 88b**) was characterized by a broad SPR peak centered at 640 nm, which due to the vertical orientation stayed unmodified when recorded under polarized light.



Figure 88 a) Extinction spectra of nanorods arranged in horizontal arrays measured under no polarized excitation conditions (black curve), polarized excitation perpendicular to the nanorod long axes (green curve) and polarized excitation parallel to the nanorod long axes (blue curve). b) Extinction spectrum of nanorods arranged in vertical arrays measured under un-polarized excitation conditions. Red lines indicate the wavelength of the excitation light fixed at 633 nm. Insets: corresponding SEM micrographs of horizontal and vertical arrays.

The higher extinction value (0.75 versus 0.3) and closer position of the SPR peak to the laser excitation wavelength (red lines in **Figure 88a,b**) explain the higher absolute intensity of SERS signals obtained with the vertical arrays.

5.3.4. Improvement of SERS enhancement by chemical cleaning of stamped arrays

In an attempt to further improve the SERS responses, fabricated arrays were subjected to a chemical cleaning in order to remove CTAB and TOAB surfactants stabilizing nanorod surfaces. Surfactants, present as a double layer on the shell of the nanorods, generate electrostatic and steric repulsion forces than difficult the binding of target molecules to the nanorods. Their removal resulted in generation of increased SERS signals due to i) generation of higher electromagnetic field between nanorods of decreased inter-nanorod distance and ii) better infiltration of analyte due to stronger capillary action^[29, 31].



Figure 89 SEM images of vertical and horizontal Au nanorods arrays obtained after acid treatment with HCl concentrated for 2 minutes. a1-3) Vertical arrays; b1-3) Horizontal arrays

Figure 89a depicts different magnification SEM images of vertical arrays after cleaning with concentrated HCl for 2 min, showing how the overall orientation of the nanorods was not significantly affected by the acid treatment (**Figure 89a3**). Acid treatment was also used on horizontal arrays where loss of order caused by partial detachment of the AuNRs from the substrate occurred (**Figure 89b**). As result the corresponding SERS spectra did not show a significant improvement compared to the signals obtained with un-treated arrays.

Chapter 5



Figure 90 SERS spectra obtained from 4-ABT solutions absorbed on a) IPA (1h) cleaned vertical arrays b) IPA (1h) and HClconc (2min) cleaned vertical arrays.

Figure 90b shows SERS spectra of acid cleaned overgrown AuNRs vertical arrays immersed for 20 h into 4-ABT solutions of decreasing concentrations. SERS signals were detected from solution concentrations as low as 100 pM, two orders of magnitude lower than the lowest concentration that could be detected with untreated vertical arrays (**Figure 90a**).

A comparison between the 4-ABT SERS spectra obtained with the three substrates: horizontal, vertical and acid clean vertical showed than the relative intensities of various Raman bands changed between the spectra (**Figure 91**). We hypothesized that the observed changes in relative intensities were due to the chemical mechanism.

60 -	a)	b)	Horizontal	Vertical	Vertical
t / 10 ³					Acid-treat
man Ir	Mannh	 1139 1074	0.7	1.1	1.5
²⁰ 10		1388/ 1074	0.7	0.6	0.9
0 - 10	00 1100 1200 1300 1400 1500 1600 Wavenumber (cm ⁻¹)	1435 / 1074	0.6	0.8	1.2

Figure 91 a) SERS spectra of different arrays immersed in 4ATP MeOH solutions (0.1mM, 20hours). Acid cleaned vertical arrays (red); IPA cleaned vertical arrays (green); IPA cleaned horizontal arrays (blue); Klarite commercial substrate (magenta); and 4ATP powder on a cover slip (black) as reference. b) Table with the intensity of the b2 peaks modes normalised against the intensity of the v_{7a} (1074 cm⁻¹) peak.

In order to quantify the degree of CM we divided the intensities of the b₂ bands by the intensity of the C-S stretching band, v_{7a} (1074 cm⁻¹). Since v_{7a} is enhanced mainly by electromagnetic effects^[7, 9, 10] this operation should normalized the spectra and factor out any EM effects to quantitatively evaluate the extent of chemical contribution. Table 2 of *Figure 91* shows the intensities of the b₂ modes normalised to the v_{7a} band for horizontal, vertical, and acid clean vertical arrays. These values (0.7, 1.1, 1.5) represent a comparative measure of the strength of the CM versus the EM for the different substrates. A comparison of the value obtained with vertical arrays (1.1) and with acid cleaned vertical arrays (1.5) shows that after the acid cleaning, relative intensity of the b2 peaks against the a1 peak at 1074cm⁻¹ was increased by a 30% (table of *Figure 91*).

We argue that this difference in the relative intensity between b2 and a1 peaks is related to the relative intensity between the chemical and the electromagnetic enhancement, respectively. To fully understand this comparison, it should be remarked that the EM affect the intensity of every peak and the CM affect mainly the intensity of the b2 peaks.

Experiments reported by *Kawata* and co-workers^[7] have reported than the intensity of the b2 peaks in the 4ABT, is very sensitive to the chemical environment of the areas where the 4-ABT molecule bind to the nanostructure. In a similar fashion, we argue than the acid clean treatment of our substrates reinforced the chemisorption of the 4ABT molecules to the nanorods surfaces, increasing the efficacy of the charge transfer mechanism and therefore the CM effect, which increased overall the signals of the b2 peaks.

On the other side, the removal of CTAB molecules also generated a greater number of active sites available to bound 4ABT molecules, increasing therefore the average number of molecules that contributed to the signal of every peak.

5.3.5. Enhancement of the electromagnetic field on the Au nanorods arrays

As complementary data, distribution of the electromagnetic field around the nanorods in the arrays was estimated from FDTD simulations. *Figure 92* displays the local electromagnetic field enhancement surface plots for parallel and perpendicular arrays with inter-nanorod distance fixed at 2 nm calculated with an incident plane wave along the z direction (perpendicular to the substrate). The local electric field enhancement factor is defined as $|E/E_0|^2$, where |E| and $|E_0|$ are magnitudes of the local electric field and the incident field, respectively.

For horizontal arrays (*Figure 92a*,b) cross sectional areas of a horizontal plane (x-y) were considered with an incident wave plane polarized along the x and y direction, respectively. According to the plots, the local electromagnetic field was localized in the area between adjacent nanorods, with the highest enhancement calculated between nanorod tips and a more moderated enhancement between the nanorods sides (*Figure 92b*).



Figure 92 Electromagnetic field enhancement surface plots of nanorod arrays for wave plane incident along the *z* direction. *a*,*b*) parallel arrays on *x*-*y* plane with incident wave plane polarized along the *x* and *y* direction; perpendicular arrays on *c*) *x*-*z* plane and *d*) *x*-*y* plane with incident wave plane polarized along the *x* direction.

For vertical arrays (*Figure 92c,d*) the cross sectional areas of vertical (x-z plane) and horizontal plane (x-y) were considered, with a plane wave polarized along the x direction. These plots showed that in VA the local fields were highly enhanced between the tips of adjacent nanorods.

5.3.6. Calculation of the enhancement factor of vertical and horizontal arrays

A further evaluation of the SERS performances of our fabricated arrays was achieved by calculating the Enhancement Factor (EF), defined as the ratios of the intensities of the scattered radiation for SERS and normal Raman scattering per molecule

$EF = (I_{SERS}/N_{SERS})/(I_{Raman}/N_{Raman})$

Where I_{SERS} and I_{NR} are the integrated intensities of the SERS and normal Raman scattering spectra for 4-ABT, respectively; N_{SERS} and N_{NR} are the number of molecules found in the laser excitation area adsorbed on nanorod arrays and in bulk powder form, respectively.

It should be stressed that a precise evaluation of the EF is extremely difficult for large area substrates of slightly irregular morphology. A precise evaluation should involve a reliable determination of the number of molecules adsorbed on the surface within the area probed by the exciting radiation. In our calculations we estimated that only molecules adsorbed in the areas of highly enhanced field (hot spots) contributed to the EF. FDTD simulations of the electric field around the nanorods (*Figure 92*) were used to estimate the size of the hot spots area drawn in **Figure 93**



Figure 93 Schematic of the hot spot areas in nanorods arranged in parallel and perpendicular arrays.

For horizontal arrays four rectangle and four semi-spheres shared by every two nanorods were considered, so the hot spot area is equal to two rectangles and two semispheres for each nanorod:

$$A_{horizontal} = 380 \text{ nm}^2$$

For vertical arrays six rectangles along the nanorod shared by six nanorods were considered, so the hot spot area is equal to three rectangles for each nanorod:

$$A_{vertical} = 225 \text{ nm}^2$$

The sampling volume used to obtain the Raman spectrum of neat 4-ABT powder was calculated assuming that the laser spot formed a cylinder of 10 μ m in diameter and 40 μ m in length (penetration depth). Therefore the illuminated volume was:

Volume illuminated = 3.14×10^{-9} cm³.

Since the density of 4-ABT is 1.18 g/cm^3 and its molecular weight (MW) is 125.19 g/mol, the number of 4-ABT molecules illuminated by the laser light is calculated to be:

$$N_{Raman} = 1.78 \times 10^{13}$$
.

Assuming that the surface illuminated by the laser is $\pi r^2 = 3.14 \times (5)^2 = 78.5 \ \mu m^2$. The calculated number of nanorods/ μm^2 is 1500 and 2200 for horizontal and vertical arrays, respectively. So the number of nanorods in the illuminated area is :

NRs horizontal arrays illuminated = 1.1×10^5

NRs vertical arrays illuminated = 1.7×10^5

The calculated surface area of each nanorod hot spots was 380 nm² and 225 nm² for horizontal and vertical arrays, respectively. So the total nanorod hot spot surface area under the laser spot is:

Area hot spot horizontal = 4.1×10^7 nm²

Area hot spot vertical $= 3.9 \times 10^7 \text{ nm}^2$

Considering that each 4-ABT molecule occupies an area of ca. 0.2 nm² the effective number of molecules illuminated by the laser light is $(4.1 \times 10^7/0.2)$:

 $N_{SERS hor} = 2.1 \times 10^8$

N_{SERS ver =} 2.0×10⁸

The intensity ratio of the 7a bands at 1080 cm⁻¹ in **Figure 87** was measured to be:

I_{SERS} / I_{Raman} horizontal	= 1.09		
I_{SERS} / I_{Raman} vertical	= 1.5		
I_{SERS} / I_{Raman} acid clean vertical = 5			

Therefore the enhancement factor (EF) should be:

$(I_{SERS} / I_{Raman})x(N_{Raman} / I_{SERS})$ horizontal	$= 6.9 \times 10^4$
$(I_{SERS} / I_{Raman})x(N_{Raman} / I_{SERS})$ vertical	$= 8.9 \times 10^4$

 $(I_{SERS} / I_{Raman})x(N_{Raman} / I_{SERS})$ acid clean vertical = 4.45×10^5

Values that are in agreement with the enhancement factors reported in literature for aggregates metal nanoparticle and nanorods systems^[32].

5.3.7. Quantitative SERS detection of 4ABT

SERS detection achieved with horizontal and acid-treated vertical arrays was also used to obtain quantitative information regarding the concentration of 4-ABT in solution. Before each spectrum acquisition, instrumentation was calibrated using an internal Si reference at 521 cm⁻¹ to ensure measurement comparability.

Plots of **Figure 94** show the intensity of the 1074 cm⁻¹ band against 4-ABT concentration. Signal became saturated at higher concentrations, which arose from the saturation of the active sites of the nanorod arrays. **Figure 94b**, reports log plots of I_{1074} against a series of 4-ABT concentrations. Good logaritmic relationship was found with $R^2 = 0.99$ in the concentration range $10^{-4} - 10^{-8}$ M.

Chapter 5



Figure 94 Variation of the relative intensity of C-S stretching band, v7a, with 4-ABT concentration measured for a,b) horizontal arrays, b,c) vertical arrays. a,c) Plots with red line as a log fit to the data; b,d) log plots with red line as a linear fit to the data.

Uniformity of the fabricated arrays necessary to provide reproducible results was evaluated by measuring 4-ABT SERS spectra in 12 random points over an area of 5 mm diameter of vertical (acid-treated) array. The obtained spectra is shown in **Figure 95c** and **Figure 95a**, respectively.



Figure 95 a) 4-ABT SERS spectra measured in 12 random points across a 5 mm diameter of vertical array (SD = 5.9%.) b) Evolution of the 4ABT SERS spectra along 2.2 min laser continuous illumination in vertical arrays (SD = 6.1%).

The intensity change of the Raman band at 1074 cm⁻¹ was used to calculate the relative standard deviation of the intensity from 12 points. A value of 5.9% was obtained, which showed the remarkable good uniformity of the array. This values was lower than reproducibility values reported in literature for nanoparticle aggregates and printed

nanoparticle arrays and comparable to values reported for ordered nanowire arrays obtained by nanosphere lithography^[30, 32, 33].

To investigate temporal stability of fabricated substrates SERS spectra of 4-ABT absorbed on vertical acid treated arrays were recorded under continuous laser irradiation for 2.2 min, laser power of 3 mW. Spectra were recorded every 12 s and the integration time was 10 s. **Figure 95b** shows the intensity change of SERS spectra over 12 measurements. A relative standard deviation (SD) of 6.1% was calculated by considering the variation in intensity of the Raman band at 1074 cm⁻¹. This result indicated that the array had good temporal stability under standard illumination conditions, which could meet requirements of routine SERS measurements.

5.4. Summary and Conclusions

In conclusion, a combined droplet evaporation/stamping method was used for the fabrication of SERS substrates based on Au nanorods horizontal and vertical arrays. SERS response of both arrays was investigated using model molecule 4-ABT. Improvement of SERS response was obtained by cleaning the vertical arrays with HCl. EFs between 6.9×10^4 and 2.2×10^5 were obtained for all fabricated arrays. Quantitative Raman detection was obtained for vertical arrays with detection limits in the nM concentration range. Finally fabricated arrays displayed good stability and uniformity, showing their potential to be widely used as sensing platforms for plasmon-induced optical molecular detection.

5.5. References

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Feasibility of Au Nanorods plasmonic flexible substrates as SERS platforms

SERS active flexible substrates were fabricated by droplet evaporation of Au nanorod solutions on rigid surfaces and subsequent stamping of assembled Au nanorods arrays on photographic paper (pPET) and different plastic surfaces, The SERS feasibility of flexible active substrates was investigated using model molecule 4-aminobenzenethiol (4-ABT), for which detection in the μ M concentration range and Enhancement Factors (EF) of 10.⁵ As real-world applications detection of food contaminant Cristal Violet (CV) to sub-nM detection limits and trace detection of benzocaine powder by swabbing were achieved on pPET active substrates⁴.

⁴ This work has been published as: *"Flexible SERS active substrates from ordered vertical Au nanorod arrays"*, A. Martín, J.J. Wang, D. Iacopino, RSC Advances, **2014**, 4, 20038-20043.

6.1. Introduction

Large efforts have been aimed at translating the capabilities of SERS to a practical detection system that could be utilised for routine analysis in the field. Generally two main approaches are used to produce SERS active substrates: top-down approaches such as lithography and bottom-up approaches such as self-assembly. In both cases, despite the tremendous signal enhancements achieved, the high cost fabrication procedures or limited reproducibility and shell life have limited the use of these SERS active substrates in common analysis.

Flexible substrates can become an alternative ^[1] as they have the potential to combine low cost and flexibility while still maintaining sensitivity and robustness of rigid substrates. Flexible plasmonic SERS substrates have been fabricated using plastic,^[2-4] paper^[5-7] and polymer materials^[8, 9] as surfaces in combination with metal nanostructures constituting SERS active layer. Numerous methods of deposition of nanostructures have been tried, such as physical deposition^[10] and laser induced- photothermal methods^[6]. Although highly sensitive SERS substrates are fabricated with these techniques, the requirement for high laser power or expensive equipment has limited their production in large-scale. In contrast, solution fabrication processes such as in situ synthesis on porous paper^[10], printing^[11] and dip-coating^[5] are preferred, since plasmonic SERS substrates are obtained with small amount of materials and inexpensive deposition techniques.

As well as cost and ease of fabrication, flexible substrates have been shown efficiency of sample collection particularly suited for real world sample analysis. For example, White and co-workers have achieved detection of analytes from complex samples using chromatographic separation on SERS inkjet-printed cellulose paper^[12], and SERS active filter paper substrates have been used for trace detection of explosives^[13]. Swabbing the surface under investigation with a flexible substrate has also been proposed as a highly practical and efficient method to maximise sample collection. Using this technique SERS dipsticks and swabs fabricated by SERS inkjet-printed paper have been proposed by White and co-workers for efficient detection of trace chemical detection of malathion and cocaine^[14].

Taking advantage of the stamping method described in the previous chapter, we used flexible surfaces as receiving substrates during droplet deposition/stamping method to fabricate flexible plasmonic SERS substrates. Large arrays of vertically aligned Au nanorods were formed by droplet evaporation on glass and were subsequently stamped on photographic paper (paper covered with a layer of polyethylene terephthalate (pPET) and polyvinyl chloride (PVC) surfaces. The SERS activity of the fabricated plasmonic substrates was investigated with model molecule 4-ABT. PPET and PVC active substrates were robust and could be immersed in analyte solutions for up to 20 h without degradation. Enhancement factors on the order of 10⁵ were achieved for these active substrates. Finally, to demonstrate field-based applications, sub-nM detection of food contaminant crystal violet and traces detection of drug-marker benzocaine traces by swabbing technique was achieved.

6.2. Experimental section

Nanorods 3.2B of dimensions 22x55 nm (A.R:2.4) were utilised along this chapter (detailed synthesis in section 2.2.2 of this thesis)

6.2.1. Stamping process

Vertical gold nanorods arrays were fabricated by droplet deposition (see Chapter 4) of 22x55 nm overgrowth nanorods in chlorobenzene dispersions (10 μ l, 14nM). Fabricated arrays were transferred intact onto flexible supports by placing a plastic sheet (PET, PVC or PDMS) on the original coverslip support and pressing the two surfaces together for c.a. 10 s. Excess organic matter was removed by immersing plastic-nanorod arrays in isopropanol for 2 h, followed by multiple rinses with fresh isopropanol.

6.2.2. Plasmonic substrates with Thin Layer Chromatography (TLC) plates

Another type of plasmonic substrates was developed by simple deposition of a droplet $(5\mu l, 14nM)$ of overgrown Au nanorods in aqueous suspensions into Thin Layer Chromatography (TLC) plates. After a few seconds the droplet was dried leaving the Au nanorods dispersed in the silica matrix of the TLC plate

6.2.3. Characterization of arrays

Electron Microscopy

Scanning electron microscopy (SEM) images of vertical and horizontal nanorods arrays immobilized on cover slips were acquired using a field emission SEM (JSM-6700F, JEOL UK Ltd.) operating at beam voltages of 5 kV. To avoid charge phenomenon from the substrate, a tiny quantity of gold was sputtered by an Edwards sputter coater over some of the sample before SEM analysis.

6.2.4. SERS experiments

SERS spectra were obtained from a Renishaw in Via Raman system. A 632.8nm helium-neon laser was employed as an excitation source. The laser beam was focused onto the sample through a Mitutoyo M Plan Apo 100X objective with 0.7 N.A. Measured power at the sampling level was controlled at about 5mW. Acquisition time was usually 30 s

6.3. Results and Discussion

6.3.1. Immobilization and cleaning of gold nanorods arrays on flexible substrates

Taking advantage of the stamping method described in the previous chapter, we used flexible surfaces as receiving substrates during droplet deposition/stamping method to fabricate flexible plasmonic SERS substrates. Vertical Au nanorod arrays were fabricated following a method illustrated in **Figure 96**. The fabrication process comprised two steps: a) deposition of a nanorod droplet solution on a rigid surface followed by evaporation under controlled conditions (**Figure 96a**) and b) stamping of the obtained nanorod array on a receiving flexible surface **Figure 96b**.



Figure 96 Formation of vertical arrays by combined droplet evaporation/stamping technique; a1-3) droplet evaporation process; b1-3) stamping and cleaning of Au nanorod arrays on a receiving flexible substrate.

Specifically, a droplet of Au nanorod chlorobenzene solution was deposited on a glass coverslip and left evaporate under free air conditions (T = 20 °C, Humidity = 70%, evaporation time = 20 minutes). As solvent evaporated, nanorods assembled forming a gold film at the interphase between solvent and air. Following solvent evaporation, assembled nanorods deposited on the glass surface covering the entire area of droplet (process detailed in chapter 4 of this thesis). Formed nanorod arrays were transferred onto flexible surfaces by stamping, following the method described in **Figure 96b**. Specifically, a flexible surface was pressed on the original glass surface containing nanorod arrays for 5 s (**Figure 96b.1**). Nanorod arrays transferred intact on the flexible surface together with some residual organic matter, which deposited on

top of the arrays (Figure 96b2). Nanorod arrays were cleaned by immersion in isopropanol (2 hours), followed by multiple rinses with clean isopropanol (Figure 96b3). It should be noticed that formation of vertical arrays by direct droplet deposition on flexible supports was achieved but the concomitant deposition of excess organic surfactants arising from synthesis and phase transfer processes prevented further practical use of such substrates. In fact, the arrays lifted from the support when immersed in analyte solutions, making impossible their use as SERS active substrates. In contrast, after stamping nanorods arrays strongly attached to flexible surfaces, thus allowing removal of excess organic matter

Figure 97 shows SEM images of nanorods vertical arrays stamped on different plastic substrates. Stamped arrays kept the same degree of pattern regularity than their no stamped counterparts, although occasional irregularities in the lattice and formation of crack patterns occurred as result of the stamping process.



Figure 97 SEM images of arrays obtained by droplet evaporation of ONRs chlorobenzene suspensions and stamping of the dried droplet on flexible substrates. a) on pPET; b) on PVC; c) on PDMS and photography of the substrates as insets.

6.3.2. Feasibility of plastic-nanorods-arrays as SERS substrates

Performance of the plastic-NRs-arrays as SERS platforms was investigated using 4-aminobenzenethiol (4ABT) as test molecule. Fabricated nanorod arrays were immersed in 4-ABT solutions (MeOH, 0.1 mM) for 20 h. and SERS spectra were obtained with a 633 nm laser as excitation source.

Figure 98 shows a comparison between 4-ABT spectra measured on flexible substrates (green and blue curve) with spectra measured on stamped glass substrates (red curve) and commercially available Klarite substrates (black curve).



Figure 98 Raman spectrum (black curve) of powder 4-ABT and SERS spectra obtained from vertical arrays on different substrates immersed in 4ABT methanol solution (0.1mM) for 20h

Absolute scattering intensity of glass substrates was higher than flexible substrates by a factor of ca. 3 in agreement with lower detection limits demonstrated with such substrates (see Chapter 5). However, SERS spectra recorded with flexible substrates showed an increase of the absolute scattering intensity of the a_1 1074 cm⁻¹ peaks of a factor of ca. 2 compared to klarite, confirming that the electromagnetic field generated by rough Au surfaces is lower in intensity than the field generated by strongly coupled Au nanorods. An even higher enhancement factor was observed for the 1139 cm⁻¹ b₂ peak (factor 8 and 16 for PVC and pPET respectively). This enhancement is likely associated with the strong chemical bound of the 4-ABT molecules linked to high surface area Au vertical arrays compared to rough Au surfaces (see Chapter 5).

A further evaluation of SERS performances of plasmonic flexible substrates was achieved by calculating the Enhancement Factor (EF), defined as the ratios of the intensities of the scattered radiation for SERS and normal Raman scattering per molecule

$EF = (I_{SERS}/N_{SERS})/(I_{Raman}/N_{Raman})$

Where I_{SERS} and I_{Raman} are the integrated intensities of the SERS and normal Raman scattering spectra for 4-ABT, respectively; N_{SERS} and N_{Raman} are the number of molecules found in the laser excitation area adsorbed on nanorod arrays and in bulk powder form, respectively. For calculations the intensity of the band at 1074 cm⁻¹ from spectra of *Figure 98* was used. PPET and PVC active substrates gave EF values of 1.4×10^5 and 9.2×10^4 , respectively. Values that are in agreement with enhanced factors reported in literature for SERS active flexible substrates^[15, 16]. For example, *Bruno* and co-workers obtained Enhancement Factors of 4×10^4 by direct sputtering of Au nanoparticles on PET.

6.3.3. Use of highly ordered flexible plasmonic SERS substrates in field applications

To demonstrate the use of these active substrates for field applications, we performed detection of crystal violet (CV), a toxic cationic dye largely used as food colouring agent and food additive, and benzocaine, a readily available marker for drug with physico-chemical similarities to cocaine.

Detection of Crystal Violet at nM range

CV has been classified as recalcitrant molecule since it is poorly metabolized by microbes, is not bio-degradable and can persist in a number of environments^[16]. For these reasons the minimum required performance limit (MPRL) was set for 2 mg L⁻¹ (ca. 4.9 nM) in European Commission and US^[17].

Detection of CV is shown in **Figure 99** for concentrations between 0.1 μ M and 1 nM. SERS spectra were recorded by immersing pPET active substrates in CV solutions (water) for 4 h. As comparison the Raman spectrum of bulk CV in powder form is also shown in **Figure 99**. All spectra were recorded with a 633 nm excitation source.

crystal violet Raman spectrum (*Figure 99*c, black curve) is dominated by three peaks in the region from 1000 cm⁻¹ to 1750, a narrow and strong peak at 1617 at cm⁻¹ assigned to the benzene rings C-C stretching and two medium and broader peaks, one at 1387 cm⁻¹ assigned to the C-N stretching and another at 1178 cm⁻¹ assigned to the C-H in plane bending



Figure 99 a) schematic of CV molecule. b) photo of a 10⁻⁶M CV water solution containing a pPETplasmonic active substrate stamped with nanorods vertical arrays. c) SERS spectra of CV adsorbed on pPET active substrate. CV concentration was 100 nM (red curve), 10 nM (green curve), 1 nM (blue curve). Black curve is the bulk Raman spectrum of powder CV. Inset: sinusoidal change in intensity of the 1171 cm-1 peak with CV concentration.

SERS spectra obtained from CV deposited on pPET plasmonic substrates shown clearly those three peaks in the range of concentration from 10^{-5} M to 10^{-9} M. The detection limit of CV with pPET was lower than 1nM, because even the spectrum of the 1 nM sample had a good signal-to-noise ratio and one of the peaks at 1617 cm⁻¹ was still clearly observable. A sigmoidal relationship between the SERS intensity and concentration of CV was observed (inset **Figure 99**) showing concentration detection from 10 μ M to 1 nM. For the calculations the change in intensity of the 1171 cm⁻¹ peak with CV concentration was used. The SERS intensities were mostly linear at low CV concentration. At higher concentration(0.1 μ M) a non linear response emerged, and at concentrations higher than 0.1mM saturation of the SERS signal occurred indicating that the adsorption of CV onto the arrays become saturated beyond this level^[5]. The demonstrated ability to detect CV below the currently accepted levels would make possible such field-based application of our plasmonic flexible substrates.

Detection of traces of Benzocaine by surface swabbing

We also performed detection of benzocaine by swabbing a surface containing traces of the analyte. Specifically, small amounts of benzocaine were deposited and crushed on a glass slide. A nitrogen gun was used to remove as much benzocaine as possible, leaving only traces amounts. A drop of MeOH was added to a pPET substrate which was swabbed against the glass slide containing benzocaine traces. The pPET was subsequently dried with N_2 gun, rinsed again with MeOH and dried



Figure 100 a)Photo illustrating the swabbing of a glass slide with benzocaine traces using a pPETplasmonic active substrate b) SERS spectrum of benzocaine swabbed on a pPET active substrate (red curve); Raman spectrum of benzocaine powder (blue curve); Raman spectrum of pPET active substrate (black curve).

Figure 100 (red curve) shows how small amounts of benzocaine swabbed from the glass slide could be detected with pPET subtrates. For comparison the Raman spectrum of bulk powder benzocaine (blue curve) is shown together with the blank featureless spectrum of pPET active substrate recorded before swabbing.

Benzocaine Raman spectrum (**Figure 100**, blue curve) presented three main peaks in the 800 cm⁻¹ to 1400 cm⁻¹ region. Two of the peaks, one at 861 cm⁻¹ and one at 1171 cm⁻¹ were assigned respectively to a stretching mode and a bending in plane mode of the C-H of the benzene ring. The third peak, at 1277 cm⁻¹, was assigned to the C-N stretching mode.

The three peaks were present (slightly shifted to shorter Wavenumbers) in the benzocaine SERS spectrum obtained by swabbing the surface with our pPET plasmonic substrates. The peak at 861 cm⁻¹ became very broad and was barely visible from the noisy baseline. The peak at 1171 cm⁻¹, although quiet broad, was perfectly visible over the

background. Finally the peak at 1277 cm⁻¹ was greatly enhanced and is dominant over the full spectrum. The great enhancement in the intensity of the peak at 1277 cm⁻¹ assigned to the C-N bound suggest than the Benzocaine molecule bound our plasmonic-pPET substrate by the amine group as it was expected.

6.3.4. Plasmonic-TLC substrates for fast SERS measures.

A new kind of plasmonic flexible substrate was developed by simple deposition of a droplet (5 μ l, 14nM) of overgrown Au nanorods in aqueous suspensions into Thin Layer Chromatography (TLC) plates. Thanks to the porous texture of the silica of the TLC, droplets were dried within a few seconds and nanorods were uniform dispersed over a 5mm area. The method produced dense clusters of nanorods, instead of ordered arrays, on the TLC surface (*Figure 101b*).



Figure 101 a) photo of the portable Raman system used. b) SEM images of overgrown nanorods aggregates on the TLC substrate. c) SERS spectra of overgrown nanorods (10 μ l, 14 nM) deposited on top of c1) 4-ABT 10³⁴ M on TLC; c2) CV 10⁻⁵M on TLC.

To prepare the samples, fresh TLC plates were firstly stung with a capillary tube to deposit the analytes on the TLC surfaces. Quantities deposited were ca. $1\mu l \ 10^{-3}$ M of 4-ABT aqueous solution for one of the samples and $1\mu l \ 10^{-5}$ M of CV aqueous solution for the other. Once dried, a droplet (5µl, 14 nM) of overgrown Au nanorods aqueous solution was deposited over each spot of the TLCs previously stung. Once dried, samples were analysed with a portable Raman system (*Figure 101a*) without further treatment.

Due to the lack of order in the disposition of the nanorods on the TLCs (*Figure 101b*) neither reproducible measures nor greatly enhanced spectra were intended or expected. Nevertheless, SERS spectra of 4-ABT and CV (*Figure 101*c1,c2) obtained with this protocol shown perfectly clear the characteristic Raman peaks of the molecules, allowing therefore the identification of both analytes. This ability to detect molecules on TLCs plates by simple dropping of Au nanorods suspensions is an application that could streamline the workflow in synthetic laboratories and industries.

6.4. Summary and Conclusions

Plasmonic SERS substrates were fabricated by stamping on flexible surfaces vertical nanorod arrays obtained by droplet evaporation of Au nanorod solutions on glass surfaces. The SERS performances of fabricated substrates were assessed with model molecule 4-ABT. Detection down to 1 μ M and EF of the order of 10⁵ were obtained for pPET and PVC active substrates. In order to show real field-based applications, detection of food contaminant CV was achieved below the recommended limit of 5 nM on pPET active substrates. Also detection of benzocaine traces was achieved by swabbing contaminated glass slides with pPET active substrates. These examples show that our fabricated substrates can be suitable for real-world applications, where flexibility on sample collection, fast responses and low limits of detection are highly desired.

6.5. References

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Conclusions and Future Work

7.1 Conclusions

The scope of this thesis was to develop novel methodologies for the fabrication of large area Au nanorods arrays to test their feasibility for sensing applications.

Chapter 1 outlined the main areas of research involved in this thesis: the optical properties of the noble metal nanostructures, the assembly of nanostructures by droplet evaporation and the application of these assemblies to enhance coupled lights phenomena like the Surface Enhancement Raman Spectroscopy.

Droplet evaporation is a well known technique to fabricate ordered nanostructure. It is simple and can be applied to a wide variety of substrates, but it usually produces small area assemblies with poor reproducibility due to the coffee ring effect. In order to overcome these limitations we developed a new self-assembly process using chlorobenzene as a solvent instead of water. Controlled droplet evaporation of chlorobenzene dispersions led to controlled formation of horizontal and vertical arrays.

Chapter 2 of this thesis describes the different methods used to synthesize different types of Au nanrods used in this thesis and the method used to transfer the Au nanorods from water to organic phases.

Chapter 3 of this thesis addressed a detailed study of the key parameters for the fabrication of Au nanorods horizontal arrays. Nanorods assemblies were obtained from evaporation of chlorobenzene suspensions of nanorods seed mediated under slow evaporation conditions. The assembly process was clearly favoured by slow evaporations (3h) and average Au nanoro concentration (6nM).

Chapter 4 of this thesis addressed the study of the key parameters for the fabrication of Au nanorods vertical arrays. In this case, formation of vertical arrays occurred selectively with overgrown nanorods. The assembly process was found to be favoured by a fast evaporation and occurs over a large range of nanorods concentration.

Nanorods arrays fabricated with this new droplet deposition method showed a high degree of order over mm² areas, but unfortunately they detached from the cover slip support when immersed in any analyte solutions. In order to improve adhesion of arrays to the support and clean off residual organic matter, we introduced an additional stamping process.

This stamping process allowed the immobilization of nanorods on rigid substrates as glass, ITO or SiO_2 and on flexible substrates as pPET, PVC and PDMS. Once stamped, arrays were attached strong enough to be cleaned by usual solvents as MeOH, Acetone or ITO. On glass substrates, vertical arrays kept ordered even after acid cleaning with HCl. Feasibility of the stamped arrays to work as SERS platforms was checked in Chapter 5 and Chapter 6 of this thesis.

Chapter 5 of this thesis showed a comparative study of the SERS response of horizontal, vertical and acid clean vertical arrays using 4ABT as model molecule. Enhancements factors of 6.9×10^4 (horizontal arrays), 7.8×10^4 (vertical arrays) and 2.2×10^5 (vertical acid-treated arrays) were obtained. Relative intensities between the b2 and a1 peaks of the 4ABT spectra obtained for horizontal (0.7), vertical (1.1) and vertical acid cleaned (1.5) showed evidences than the vertical and overall acid cleaned vertical substrates produced a stronger chemical enhancement of the SERS modes of the 4-ABT . Finally, quantitative detection of the 4ABT was achieved with the vertical arrays, with detection limits within the nM range and a SD value of 6%.

Chapter 6 of this thesis showed real field applications of the vertical arrays immobilized on flexible substrates. Detection of food contaminant Cristal Violet (CV) was achieved up to sub-nM detection limits by immersion of substrate in solution. Flexible pPET plasmonic active substrates habilitate the detection of benzocaine by direct swabbing of traces left on surfaces.

7.2 Future work and potential applications

The main novelty of the assembly method developed in this thesis is the possibility to fabricate ordered Au nanorods arrays over mm² areas and to immobilize them on arbitrary substrates to resist harsh environments. Future work should be directed to the exploitation of these two properties: the possibility to support extensive plasmonic arrays on a wide variety of substrates and the possibility to subject these arrays to a wide variety of chemical environments without a loss of order in the assemblies.

Plasmonic nano-structures similar to the ones developed in this thesis have been proof useful mainly in three different fields: nano-catalysis^{[1],} Surface Enhancement Raman Scattering (SERS) and Surface Enhancement Fluorescence (SEF) ^[2]. SERS capacity has already been investigated in this thesis (see Chapter 5 and 6), meanwhile the other two application field are still unexplored.

In the SERS field, we have showed in this thesis the use of flexible active substrates to detect chemicals without pre-treatment by simple sample swabbing, this capacity open a very promising field for in situ detection of a variety of drugs, pollutants and other health menaces. Furthermore, the strength of the developed substrates against harsh chemical environments enable the use of pre and post chemical treatments to improve molecule binding of the analytes to the nanostructures, thus widening the possible target analytes.

SEF is produced by the strong electromagnetic fields generated at the surface of the nanostructures, but in contrast with the SERS technique, if chromophores are within 5nm to the nanostructure fluorescence becomes strongly quenched. Being the optimum distance 7-10 nm, devices designed for SEF have to present very homogeneous assembly geometry to assure than chromophores stay at the correct range. This requirement, make Au nanorods arrays a very promising candidate for SEF. By spin coating, an ad-hoc layer of transparent polymer could be applied over our AuNRs arrays to provide the required 7-10 nm spacing. The tuning of the polymer thickness combined with the possibility to select the nanorods orientation would make of these devices ideal candidates for testing this new technique.

Several authors have also reported the use of Au nanostructures as efficient catalyst for diverse reactions, as the oxidation of CO into $CO_2^{[1]}$. Crystal facets, shapes and sizes of the nanostructures have been reported as key parameters for an efficient reaction yield. Therefore, the mutually exclusive vertical or horizontal nanorods assembly make our

substrates very suitable for studying the effect of different crystallographic facets on the result of the reactions. Another important parameter to the yield of the catalysis is the surfactant molecules on the shell of the nanostructures, which have been reported^[3] as detrimental agents for a correct catalyst performance. Thus, the ability of our substrates to be cleaned from the excess CTAB and TOAB could be of great relevance on the nano-catalysis field.

7.3 References

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Appendix

Appendix A.

Glossary

4-ABT	4-Aminobenzenethiol
СМ	Chemical mechanism
СТАВ	Hexadecyltrimethyl ammonium bromide
EM	Electromagnetic Mechanism
FDTD	Finite Difference Time Domain
НОМО	Highest Occupied Molecular Orbital
ITO	Indium Tin Oxide
LUMO	Lowest Unoccupied Molecular orbital
MPS	Mercaptopropyl Silane
MSA	Mercaptosuccinic Acid
PCA	Principle Component Analysis
PEG	Polyethylene Glycol
SEM	Scanning Electron Microscopy
SERS	Surface Enhancement Raman Spectroscopy
TEM	Transmission Electron Microscopy
TNT	trinitrotoluene
ТОАВ	Tetraoctylammonium bromide
TLC	Thin Layer Chromatography

Appendix B.

Peer reviewed publications

- "*Synthesis, optical properties and self-assembly of gold nanorods*", A. Martín, C. Schopf, A. Pescaglini, A.O'Riordan, D. Iacopino. Journal of experimental nanoscience, 2012, 7, 688-702.
- "Facile Formation of Ordered Vertical Arrays by Droplet Evaporation of Au Nanorod Organic Solutions", A. Martín, C. Schopf, A. Pescaglini, J.J. Wang, and D. Iacopino, Langmuir, 2014, 30 (34), pp 10206–10212.
- *"Surface-Enhanced Raman Scattering of 4-Aminobenzenethiol on Au Nanorod Ordered Arrays",* A. Martín, A. Pescaglini, C. Schopf, V. Scardaci, R. Coull, L. Byrne, D. Iacopino, J. Phys. Chem. C, 2014, 118(24), pp 13260-13267.
- *"Flexible SERS active substrates from ordered vertical Au nanorod arrays"*, A. Martín, J.J. Wang, D. Iacopino, RSC Advances, 2014, 4, 20038-20043.
- "*Polarization dependent, surface plasmon induced photoconductance in gold nanorod arrays*", S. Diefenbach, N. Erhard, J. Schopka, A. Martín, C. Karnetzky, D. Iacopino, A. W. Holleitner, Physica Status Solidi (RRL), 2014, Vol 8, Issue 3, pp 264-268.
- "Au Nanorod Plasmonic Superstructures Obtained by a Combined Droplet Evaporation and Stamping Method", C. Schopf, A. Martín, M. Burke, A. Quinn, D. Jones. A. Pescaglini, D. Iacopino, J. Mater. Chem. C 2014, 2, 3536-3541.
- "Hot-Electron Injection in Au Nanorod-ZnO Nanowire Hybrid Device for Near-Infrared Photodetection", A. Pescaglini, A. Martín, D. Cammi, C.Ronning, A.Cantarero, N. Garro, D. Iacopino, Nano Lett., 2014, 14 (11), pp 6202–6209.

Conferences

- Discovery Exhibition, Cork, November 2011
- BT Young Scientist and technology Exhibition, Dublin, 2012
- BCFN nanomaterials workshop, University of Bristol, March 2013
- 11th International Conference on Materials Chemistry (MC11) University of Warwick, July 2013

Posters

- "Synthesis, Chemical modification and Self-assembly of Gold Nanords", Alfonso Martin, Daniela Iacopino, Tyndall postgraduate poster competition, Cork, July 2011.
- "Tuneable Device-Scale Assemblies of Gold Nanorods in Organic Solvent", Alfonso Martin, Carola Schopf, Daniela Iacopino, Tyndall postgraduate poster competition, Cork, July 2012.
- "Highly ordered Au nanorods arrays: optical properties and applicattions", Carola Schopf, Alfonso Martin, Daniela Iacopino, Tyndall postgraduate poster competition, Cork, July 2013.

Talks

- " Self-assembly of Au nanorods by controlled droplet evaporation of organic suspensions", BCFN nanomaterials workshop, University of Bristol, March 2013.
- "Gold Nanorod Superstructures for Sensing Applications" MC11, University of Warwick, July 2013
- "*Gold Nanorod Superstructures for Sensing Applications*", Tyndall postgraduate talks, May 2013.
- "Self Assembly of Au Nanorod Superstructures for Sensing Applications", University College Cork, chemistry day, August 2014.