Short Visit Grant 3822
Scientific Report: Gold nanotriangles for LSPR detection of antibody/antigen binding.

Leonor Soares
FCUP, Faculdade de Ciências da Universidade do Porto
FCT/UNL, Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa, Portugal

Purpose of the Visit:

Environmental biosensors are gaining importance because of ever-growing environmental concerns and their ability to provide rapid information on the identification and/or quantification of contaminants or toxic compounds in samples. For this purpose, metallic nanoparticles, such as gold and silver have been widely used as an efficient probe, due to their strongly enhanced surface plasmon resonance at optical frequencies, that makes them excellent scatterers and absorbers of visible light. The LSPR dependence of these nanoparticles on their dielectric environment has been studied over the past few years, being successfully used as a mechanism in the detection of biological molecules. LSPR biosensors are based on sensing changes in the surface refractive index and transducing these changes into wavelength shifts of the LSPR extinction maximum as light is shined onto the patterned sensing platforms. The electric field component of the incident light interacts with the electrons of each metallic particle, causing the collective electron density to oscillate at a frequency that is resonant with the incoming light. This collective oscillation is what we call localized surface plasmon resonance. The versatile nature of LSPRs can be attributed to their strong dependence on a nanoparticle's composition, shape, size, interparticle spacing, as well as its dielectric properties and those of the local environment.[1]

Therefore, the main objective of this visit was to combine the preparation of bionanoconjugates (BNCs) with LSPR measurements, to confirm the variations on the nanoparticle surface, at a single-particle level. Molecules adsorbed to the particle surface lead to a spectral shift of the LSP resonances. The magnitude of the shift is related to the local optical field strength, with strong fields causing strong shifts and thus high sensitivity in sensor applications.

The BNCs used are made of gold nanotriangles, previously functionalized with small peptides in solution, and they are able to immobilize proteins, in this case, the mushroom tyrosinase (TYR), a copper enzyme that is responsible for the catalysis of oxidation reactions with vast utilization in phenol detection and detoxification.[2] The binding of the corresponding tyrosinase antibody was also studied using this usefull technique, at the host institute. The gold nanotriangles were synthesized by a photocatalytical method, developded by the nanochemistry group in FCUP. The immobilization of the protein and the enzymatic activity of the BNCs was studied in

FCT/UNL group, as well as other characterization techniques to ascertain the formation of BNCs, such as UV-Vis spectrophotometry and zeta potencial measurements.

It was already reported [3] that this approach is advantageous in terms of the performance of the enzyme when it is attached to the nanoparticle, instead of free, making these BNCs promising in biosensing and bioremediation.

Work carried out and main results

Gold nanotriangles were previously synthesized in FCUP using a photocatalytical method.[4] The method used allows obtaining nanotriangles with controlled size over a large range of sizes. The most interesting aspect in this method is that uses a photocatalyst (a tin (IV) porphyrin) that is responsible for the size control, together with the capping agent, CTAB. The nanotriangles have lenghts between 100 and 150 nm, as evaluated by TEM. In order to modify the nanotriangles surface to improve the binding of proteins, two small peptides were used (cationic, CALKK and anionic, CALNN). 11-Mercaptoundecanoic acid (MUA) was also used as a control for electrostatic interactions. Then in Lisbon, activity assays with the tyrosinase either alone, or immobilized in the nanotriangles were perfomed, using L-tyrosine as substrate. The present enzymatic reaction can be followed by UV-Vis spectrophotometry, due to the formation of a coloured product (dopachrome) that absorbs at 475 nm.[5] The preliminary results obtained show that in the BNCs formed with CALKK-AuNTs the tyrosinase activity increases in comparison with the enzyme alone.

At the host institution, the nanoparticles were optically characterized by acquiring single particle scattering spectra. A ZEISS Axio Imager.Z1m microscope in combination with a fibre-optically connected monochromator of type SpectraPro 2300i of Princeton Instruments was used. In this setup, a glass substrate with deposited nanostructures is illuminated from below using a dark field immersion condenser (NA = 1:2 - 1:4), such that only light scattered from the particles on the top side is collected by a microscope objective (100x NA = 0:9). For spectral analysis, the structure plane is imaged on a fibre coupled to the spectrometer. The detection spot-size is approximately 2 μm in diameter, allowing real single-particle measurements. Some previous procedures were carried out before the immobilization and spectral analysis of the nanoparticles, such as the cleansing treatment of the chips, their surface activation (plasma etching) and silanization to ensure a higher adsorption of the particles to the glass surface of the chip.

In order to investigate the variation of the LSPRs due to changes in the refractive index in the immediate surrounding medium, a procedure with three steps was devised. The first step was carried out in solution; briefly, the selected peptide (CALKK, CALNN) or MUA was added to gold nanotriangles (after removal of excess CTAB) and this solution was used to dip the glass chip. In a second step, the chips were dipped in solutions containing different concentrations of protein (TYR) in order to bind the protein to the deposited gold nanotriangles it was immobilized. The same procedure was used in the third step for its corresponding antibody. After every step, scattering spectra of the same particles were acquired.

According to the characteristics of the synthesis, it is quite likely to have nanospheres in addition to the nanotriangles, as well as some truncated triangles, so it

is necessary to know exactly what is the morphology of the nanoparticle that is measured, which was done by AFM imaging (figure 1). Figure 2 shows the scattering spectra of two nanotriangles chosen to have similar side lengths (~150 nm each, morphology confirmed by AFM measurements, see figure 1).

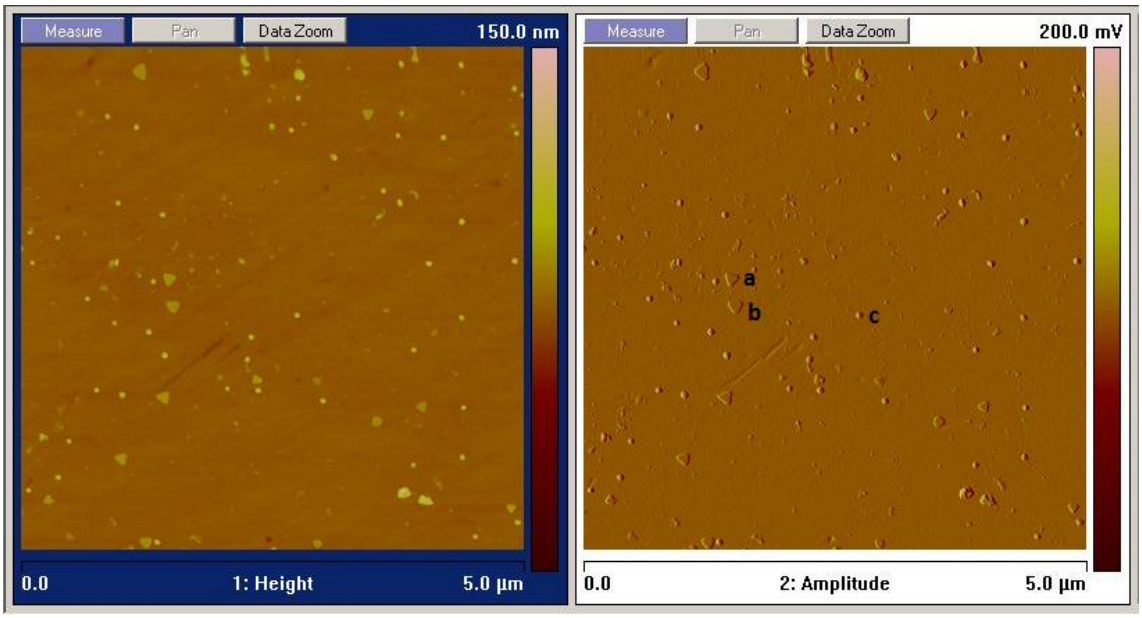

Figure 1: AFM image A) Topography; B) Amplitude of a given area of the chip with different nanostructures: a) Regular gold nanotriangle, b) Truncated gold nanotriangle, c) Gold nanosphere. (AuNTs-CALKK sample)

The resonance peaks of the nanotriangles with those dimensions and characteristics are typically centered at around 750 nm and 580-600 nm.[6] The intensity of this second peak is usually assigned to the truncated profile of some triangles, the more it resembles to a sphere, the more intense is the peak.

In both analyzed gold nanotriangles, the scattering spectra obtained after each step seems to indicate some variation of the dielectric constant of the close environment. A red-shift of the more intense peak (between 700-800 nm) is observed when the protein is immobilized on the surface of the CALKK capped AuNT and also by binding of the correspondent antibody.

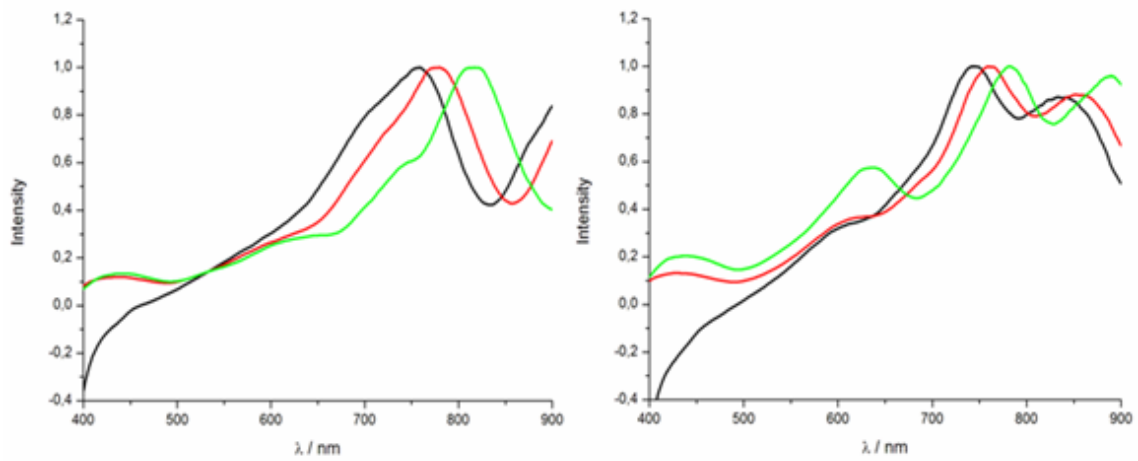

Figure 2: Single nanoparticle scattering spectra of the two selected nanotriangles (figure 1a and 1b, respectively) on the differents steps of the BNCs formation and antibody binding: CALKK capped AuNT (—); TYR immobilized on CALKK-AuNT (—) and Antibody-TYR binding to the BNC (—). [TYR] = 240 U/mL and [Ab-TYR] = 0,04 mg/mL.

The same procedure was carried out with the other capping agents (CALNN and MUA, both negatively charged). The average relative shifts of the LSP resonances for the three different capping agents are graphically depicted in figure 3. The average shifts were calculated with the results obtained for xx, yy, zz particles for CALKK, CALNN and MUA, respectively. CALNN-AuNTs show a similar behaviour to CALKK-AuNTs, but with lower shifts. This result is probably due to a lower adsorption of the protein to the negative peptide, as was also suggested by the results obtained by zeta-potential measurements. MUA capped nanoparticles show a completely different behaviour, with a significant blue shift upon protein immobilization. More experiments are necessary to elucidate this different behavior.

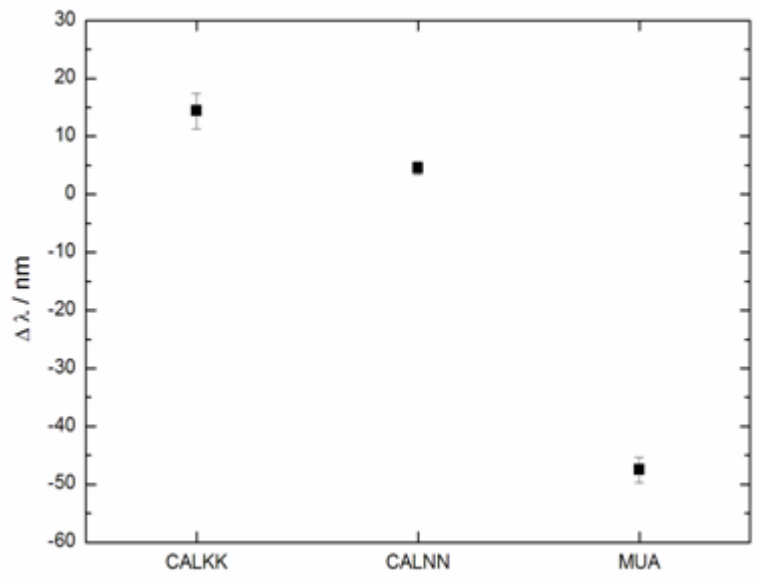

Figure 3: Average relative shifts of LSP resonances of nanotriangles with the studied molecules (CALKK, CALNN and MUA) upon protein adsorption (about 12 nanotriangles were analyzed). The bars in each point represent standard errors.

Future collaboration and projected publications

Further studies to understand the results obtained are necessary, namely a more detailed study of the adsorption of protein on the nanoparticles, and the influence of the capping agent on the properties of the protein adsorbed. Future collaboration with the hosting institution is planned to further investigate the immobilization behaviour of other biological molecules, such as oligonucleotides and nucleic acids, with the gold nanotriangles. It will be interesting also to continue a parallel study of the formation of BNCs using the same peptides and enzyme with silver nanotriangles, synthesised in the host laboratory, that was initiated in the last few days there. A publication in a field related journal is projected.

References

[1] E. Ringe, J. M. McMahon, K. Sohn, C. Cobley, Y. Xia, J. Huang, G. C. Schatz, L. D. Marks and R. P. Van Duyne; *Unraveling the Effects of Size, Composition, and Substrate on the Localized Surface Plasmon Resonance Frequencies of Gold and Silver Nanocubes: A Systematic Single-Particle Approach*, J. Phys. Chem. C, 2010, **114** (29), pp 12511–12516.

[2] G. Kim, J. Shim, M. Kang, S. Moon; *Optimized coverage of gold nanoparticles at tyrosinase electrode for measurement of a pesticide in various water samples*. J Hazard Mater, 2008, **156**, 141–147.

[3] J. Cortez, E. Vorobieva, D. Gralheira, I. Osório, L. Soares, N. Vale, E. Pereira, P. Gomes and R. Franco; *Bionanoconjugates of tyrosinase and peptide-derivatised gold nanoparticles for biosensing of phenolic compounds.* J. Nanopart. Res., DOI 10.1007/s11051-010-0099-8.

[4] A. Miranda, E. Malheiro, E. Skiba, P. Quaresma, P. Carvalho, P. Eaton, B. Castro, J. A. Shelnutt and E. Pereira; *One-pot synthesis of triangular gold nanoplates allowing broad and fine tuning of edge length*. Nanoscale, 2010, **2**, pp 2209–2216.

[5] I. Kubo, K. Nihei and K. Shimizu; *Oxidation products of quercetin catalyzed by mushroom tyrosinase*, Bioorg. Med. Chem., 2004, **12**, pp 5343–5347.

[6] N. Félidj, J. Grand, G. Laurent,J. Aubard, G. Lévi, A. Hohenau, N. Galler, F. R. Aussenegg and J. R. Krenn; *Multipolar surface plasmon peaks on gold nanotriangles*, J. Chem. Phys., 2008, **128**, pp .