SCIENTIFIC REPORT OF A SHORT VISIT TO DR. DAWSON'S AND DR. LYNCH'S LABORATORY @ UCD, Dublin, Ireland.

Program: "Mapping the detailed composition (epitope exposure) of surface-adsorbed protein layers on biomaterials and nanoparticles - an alternative approach to biocompatibility and nanotoxicity (EpitopeMap)"
Dates: June 15-18, 2010
Visitor: Ricardo Franco, Assistant Professor, REQUIMTE, FCT/UNL, Caparica, Portugal

Purpose of the visit: To determine size distributions of conjugates of gold nanoparticles with an antibody/antigen system for malaria detection.

Description of the work carried out during the visit:
In the beginning of my visit, I gave a seminar entitled "Interactions of biomolecules with gold nano-surfaces for biosensor development" presenting the main research lines in which collaborations could be established.

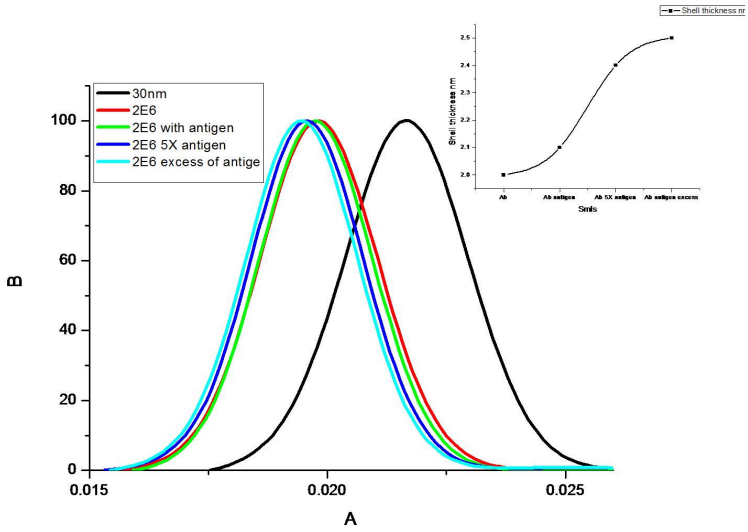
As for laboratory experiments I worked in collaboration with Dr. Marco Monopoly. As planned, techniques such as DLS and a CPS Disc Centrifuge were utilized to determine size distribution and protein corona properties of nano-conjugates. Nano-conjugates were formed by gold nanoparticles (AuNPs) with 15 nm (citrate-synthesized; functionalized with a negatively-charged thiolated ligand (MercaptoUndecanoic Acid) and 30 nm (citrate-capped; NIST standard); and a monoclonal antibody against *Plasmodium falciparum* Heat Shock Protein 70 (*Pf*Hsp70). Coupling of the antibody to the 15 nm AuNPs was performed either by simple incubation of both components, or by cross-linking the antibody to MUA-functionalized AuNPs via NHS/EDC chemistry. Antibody-30 nm AuNP conjugates were prepared by simple incubation of both components. The intended antigen (Hsp70) was then added to the AuNP-antibody nanoconjugates.

Description of the main results obtained
DLS results of 15 nm AuNP-antibody conjugates indicated the formation of AuNP-antibody conjugates, as judged by an increase of hydrodynamic diameter comparatively with AuNPs alone, but some larger aggregates (average diameters of 100 nm) could also be detected. These were probably originating from non-conjugated antibody that aggregated under the

low-ionic strength solution conditions. Further experiments will have to be performed in order to eliminate these large aggregates that seriously interfere with measurements of the smaller particles .

CPS Disc Centrifuge results corroborated DLS results for the 15 nm AuNP-antibody conjugates. CPS experiments also corroborated the formation of aggregates for antibody alone under the same concentration and solution conditions, as observed by DLS.

Nevertheless, CPS results for the 30 nm AuNP-antibody conjugates were more clarifying as a much lower antibody/AuNP concentrations ratio was used to prepare the respective conjugates. From the latter experiments, the AuNP-antibody conjugates proved to be less dense than the 30 nm AuNPs alone, and density diminished further upon increase of the amount of conjugated antibody. Small but significant variations of density were further observed upon incubation with increasing amounts of the respective antigen. CPS data is presented in the following Figure, in which the inset represents a calculation of the protein shell thickness around the AuNP (protein corona) for increasing amounts of the antigen.



Future collaboration with host institution

Informal meetings were held with Professor Kenneth Dawson and with Dr. Iseult Lynch, defining the continuation of the presently initiated collaboration work. It will be based on a larger Project of great impact related to the interaction of antibodies with nanoparticles and its influence in epitope recognition. Immediate applications are envisaged in immunoassays while a wider scientific perspective in protein-nanoparticle interactions is sought.

An exchange visit of a student from Portugal was planned for a three month period, possibility starting October 2010.

I took the opportunity of being in Dublin to accept the invitation to visit the Focas Research Institute, at the Dublin Institute of Technology, made by Focas' Head, Prof. Hugh J. Byrne. The visit went very well and a few lines of possible collaboration were defined, based on Focas as a facility with state-of-the-art biophotonic instrumentation. A possible joint Project would be based on the utilization of functionalized anisotropic nanoparticles for Surface-Enhanced Raman Spectroscopy (SERS) studies of adsorbed proteins and the nanoparticles surfaces, with great potential for protein structural elucidation.