Project: 'Development of nanogels for drug delivery and vaccination: characterization of the protein corona' within the framework of the European Science Foundation (ESF) activity entitled 'Mapping the detailed composition of Surface-Absorbed Protein Layers on Biomaterials and Nanoparticles (EpitopeMap)'

Dates: 1st September – 20th December, 2010

Visitor: Sílvia Ferreira, PhD Student, in IBB – Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, Minho University, 4710-057 Braga, Portugal, silviarmferreira@deb.uminho.pt, within thesis project *"Development of multifunctional Nanoparticles"* supervised by Professor Dr. Francisco Miguel Portela da Gama, Minho University, and co-supervised by Professor Dr. Manuel João Rua Vilanova, Porto University, funded by International Iberian Nanotechnology Laboratory (INL) and FCT, trough POCTI

Host: Dr. Tommy Cedervall, Department of Biophysical Chemistry, Lund University, Box 124, 221 00 Lund, Sweden, tcedervall@yahoo.com; and Professor Dr. Sara Linse, Department of Biochemistry, Lund University, Box 124, 22100 Lund, Sweden, sara.linse@gmail.com

Purpose of the visit

The project was developed in Lund University, Lund, Sweden in collaboration with Minho University, Braga, Portugal and ICBAS, Porto University, Portugal. The immediate goals for the visit were to produce new data, transfer knowledge between the institutes, and provide an opportunity for the student to work in a different environment using a different set of experimental techniques. A long-term goal was to develop methodology that connects and integrates the concept of a biomolecular corona with studies aimed for medical applications.

Description of the work carried out during the visit

Materials. Self-assembled nanoparticles made of mannan were synthesized in University of Minho (unpublished data, ongoing PhD project); animal and human plasma proteins were purchased from commercial suppliers or purified from plasma in Lund. Human plasma was obtained from blood donors.

Protein Corona in Plasma. After incubating self-assembled nanoparticles made of mannan with plasma the complex of nanoparticles and bound plasma proteins were separated from unbound proteins both by centrifugation or gel filtration. Bound proteins were desorbed from the nanoparticles with SDS and separated by SDS-PAGE. Only a few proteins bind the nanoparticles with high affinity. These proteins will be identified with mass spectrometry.

Size and Surface charge. Dynamic light scattering (DLS) techniques were used to measure hydrodynamic diameter, polydispersity and zeta potential of the nanoparticles alone or protein corona.

Affinities, kinetics and structural changes. Structural changes of human serum albumin (HSA), lysozyme and apolipoprotein A-I were observed after binding to the nanoparticles by circular dichroism spectrometry (CD) and intrinsic tryptophan fluorescence. Furthermore the affinities were determined by isothermal titration calorimetry (ITC) and intrinsic tryptophan fluorescence after titration of particles into the protein solutions.

Protein Fibrillation. Thioflavin T fluorescence assay was performed using both human protein β2-microglobulin and Alzheimer's disease-associated amyloid *β* peptide to examine the role of nanoparticles in protein fibrillation.

Description of the main results obtained

Protein corona. Only a few proteins were found in nanoparticle–protein complex, which shows that the surfaces of nanoparticles made of mannan bind very selectively to plasma proteins upon incubation of nanoparticles into plasma. Same protein patterns are seen whether bound proteins were separated from unbound proteins by gel filtration or by centrifugation. Changes in protein corona were observed over time up to 24h of incubation. Longer time of incubation, higher nanoparticles concentrations, or bigger amounts of plasma produced larger pellets during centrifugation experiments. In contrast, plasma or nanoparticles alone did not pellet at same centrifugation force. The identity of the proteins will be determined by mass spectrometry, but it is likely that two of the protein bands are albumin and apolipoprotein A-I. Therefore we used these proteins in the structural studies.

CD measurements show that incubation of apolipoprotein A-I with the nanoparticles induced an increased CD signal, which is indicative of increased helical structure in the protein. When the apolipoprotein A-I was incubated in increasing concentrations of nanoparticles the wavelength of maximum intrinsic tryptophan fluorescence was blue shifted. This shift can be explained either by that tryptophans are buried when protein interacts with the nanoparticle or by structural changes in the protein changing the hydrophobic interactions in the protein after binding to nanoparticles. The changes in the intrinsic tryptophan fluorescence are not the same after 1h and 24h of incubation. Experiments in which nanoparticles were titrated into apolipoprotein A-I suggest that the protein binds with higher affinity after 24h.

No significant changes were observed in mean hydrodynamic diameter of the nanoparticles made of mannan, measured by DLS, after 1h or 24h of incubation of nanoparticles with or without HSA in phosphate buffer saline (PBS) or just phosphate buffer pH 7.5. CD measurements of HSA together with nanoparticles made of mannan revealed small structural changes with bigger changes after 24h of incubation than after 1h. The intrinsic tryptophan fluorescence of the HSA together with increasing concentrations of nanoparticles undergo a blue shift after 1h and 24h of incubation, both in PBS or phosphate buffer pH 7.5. Titration of particles into HSA solutions in PBS indicated a low affinity between HSA and the nanoparticles and no differences were seen after 1h or 24h of incubation. The affinity was also measured by

ITC. Only a small signal was obtained, which means that HSA binds the nanoparticles with low affinity and/or that there is low change in ΔH after binding.

Lysozyme is in opposite to apolipoprotein A-I and HSA a positively charged protein. Therefore it is interesting to compare the binding of lysozyme to the nanoparticles made of mannan with the other proteins. CD data from lysozyme in PBS showed that the CD signal increased with increasing nanoparticle concentrations. This was not seen in phosphate buffer without salt. Also in the intrinsic tryptophan fluorescence there was a difference between buffers with or without salts. In phosphate buffer pH 7.5 there was a blue shift in increasing concentrations of nanoparticles. In contrast, no changes were observed after incubation in PBS.

Protein Fibrillation. The Thioflavin T fluorescence assay performed using both human protein β2-microglobulin and Alzheimer's disease-associated amyloid *β* peptide revealed that nanoparticles delayed protein fibrillation. This was more pronounce when the nanoparticle concentrations were above critical micellar concentration.

Future collaboration with host institution

The collaboration has been very fruitful and the data obtained in the project during the grant period are very interesting and suggest many future projects. New projects that include collaborations between the three participating laboratories are already planned.

Projected publications/articles resulting or to result from the grant

The project has experimentally been very successful. The results obtained within the time period of this grant will be the substantial data in a manuscript that is to be submitted during 2011.