Scientific report of a short visit on June 1-10, 2010 entitled

„The biocompatibility of magnetic fluids and the toxicity of magnetite nanoparticles"

at the UCD Centre for BioNano Interactions, School of Chemistry and Chemical Biology
within the framework of **ESF network program Epitopmap**

- Purpose of the visit

The aim of the visit was to initiate collaboration and discussion between the Centre for BioNano Interactions in Dublin and Aqueous Colloids Group in Szeged. During the short visit areas of common interest were identified and ideas for collaborative work between our two groups were discussed and developed within the framework of EpitopeMap and beyond. Additionally, the visit was used to review the progress and further refine the details of the proposed research project of Ms. Angéla Hajdú, which is also being undertaken within the EpitopeMap framework.

- Description of the work carried out during the visit

Dr. Francesca Baldelli organized **a seminar** on 3rd of June for the researchers at the Centre for BioNanoInteractions (CBNI), where I presented our work on magnetic nanoparticles and their colloidal stability in composite aqueous media in general; the title of my lecture was "Colloidal stability of magnetic nanoparticles in biorelevant media". The stability of aqueous systems, especially their electrolyte tolerance are well-studied, however, the lack of experience with biological systems and methods is our weakness although the particle interactions with proteins, cell membranes relevant to the living systems are of crucial importance. This is a critical area where the exchange and sharing of knowledge developed in the Centre for BioNano Interactions will benefit my Aqueous Colloids Group.

I attended the regular seminar of **the CBNI group**. I was acquainted with some methods in protein and cell laboratories, as well as some facilities (confocal microscopy, PCR amplifying, HPLC-MS, etc.), which Dr. Anna Salvati showed me around and explained the various approaches to me.

I met with Dr. Iseult Lynch, and besides having an effective talk on the organization and ongoing research in the group; she explained briefly some formal rules of **ESF networking programmes**.

During the short visit several fruitful scientific **discussions** including a great one with Prof. Kenneth Dawson, took place, focussing on my main interest here, the biocompatibility of magnetic fluids and the toxicity of magnetite nanoparticles in general within the framework of the ESF Research Networking Programme EpitopMap. We discussed also the results of Ms. Angéla Hajdú on **human serum protein adsorption on carboxylated MNPs**. She made a great progress during her 3-month-exchange visit.

- Description of the main results obtained

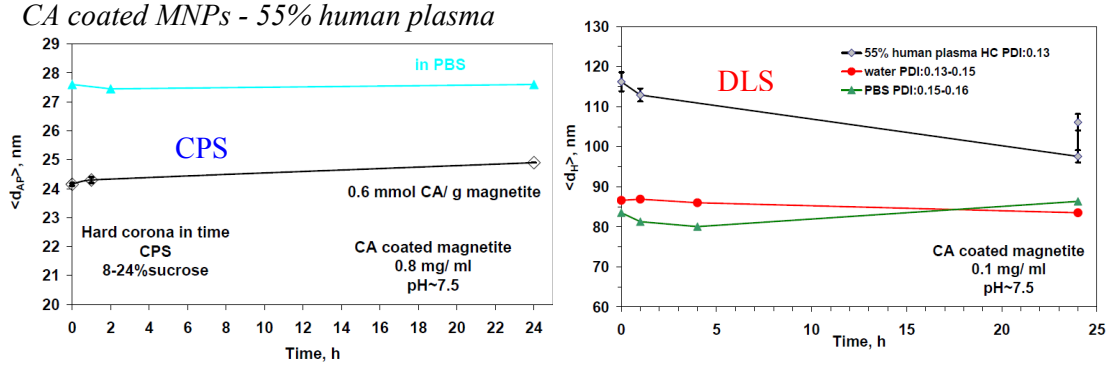
The **interaction of magnetic nanoparticles** with numerous components in blood, and specifically with plasma proteins and the membrane of healthy and diseased cells, has not been well-studied as yet.

Briefly, the magnetite nanoparticles (MNPs) synthesized in Szeged were stabilized with different carboxylated compounds, each of which can bind chemically to ≡Fe-OH sites of the MNPs, but which have systematic differences such as citric acid (CA) is a small molecular complexant, while poly(acrylic acid) (PAA) is a macromolecular compound, and sodium oleate (NaOA) is a surfactant with hydrophobic alkyl chain. The chemical interactions between the ≡Fe-OH sites and the adsorbed carboxylic groups are similar, but

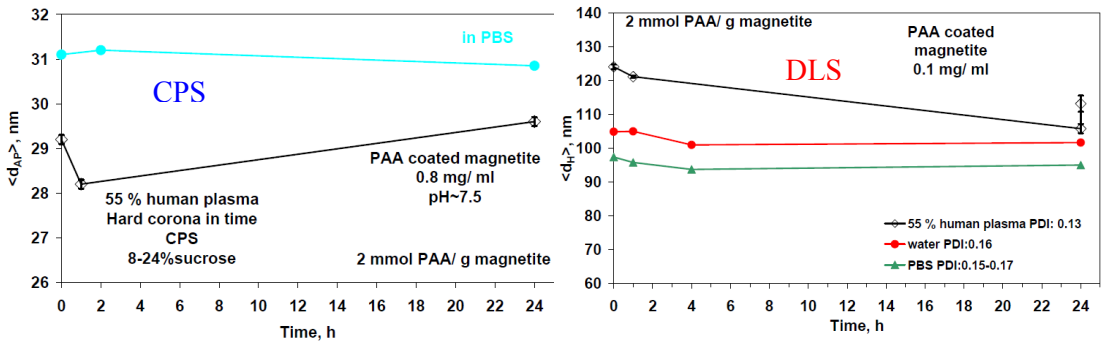
their bond strengths differ significantly from each other, and their stabilizing efficiency changes definitely due to the different structure and thickness of the coating layer on the MNPs, i.e., the composition of the aqueous interface at the particle surface (adsorption has been measured). Although each carboxylated MNP holds excess negative charges around pH~7 (pH-dependent electrophoretic mobility has been measured), the CA coating is thin and its density is low (less than 1 CA per nm^2), the PAA is adsorbed in a thicker layer (the density of monomer units is above 2 AA per nm^2), while oleate forms a thick double layer with quite high packing density (~2.5 OA per nm^2 in 1st and ~5 OA per nm^2 in the 2nd layer) on MNPs. Well-stabilized magnetic fluids (MFs) were prepared by using these compounds. The pH-dependent colloidal stability of MFs has been tested and their electrolyte (NaCl) tolerance has been characterised by coagulation kinetic measurements. The PAA and NaOA coated MNPs fulfilled the stability criteria of physiological condition regarding the pH and salt concentration, however, the CA coating does not provide suitable resistance against salt.

Angela Hajdú studied here in Dublin the interaction of these carboxylated MFs with human plasma in two different dilutions to identify the quality and thickness of the **protein corona** formed **on the CA, PAA and NaOA coated MNPs** using sedimentation in a density gradient (CPS) and dynamic light scattering (DLS) methods in parallel. She learned the methods and data evaluation procedures rapidly and well. Each sample was acceptably stable under the experimental conditions (different media such as mainly PBS, complete medium with 10% foetal calf serum (cMEM) were used) and easy to re-disperse during washing with buffer. Significant differences in the protein coronas were revealed. The trends of results obtained from CPS and DLS methods for the CA, PAA and NaOA coated MNPs were more or less the same. I show some examples for hard corona only, since the details will be in her report.

CA coated MNPs - 55% human plasma



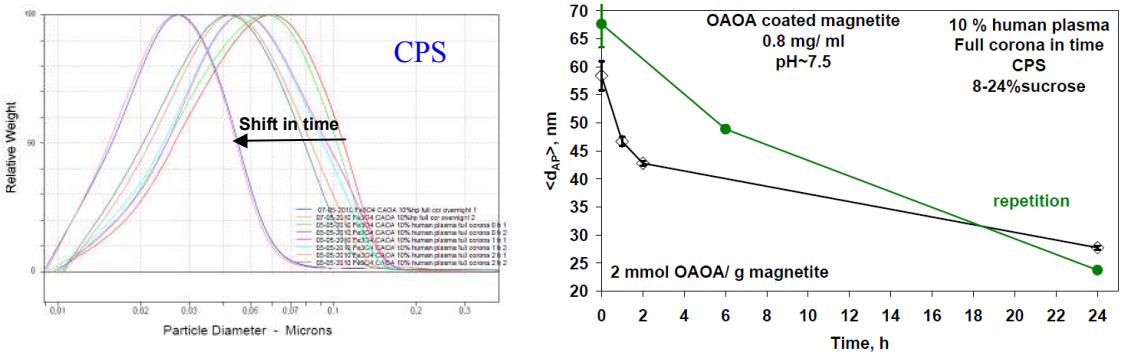
PAA coated MNPs - 55% human plasma



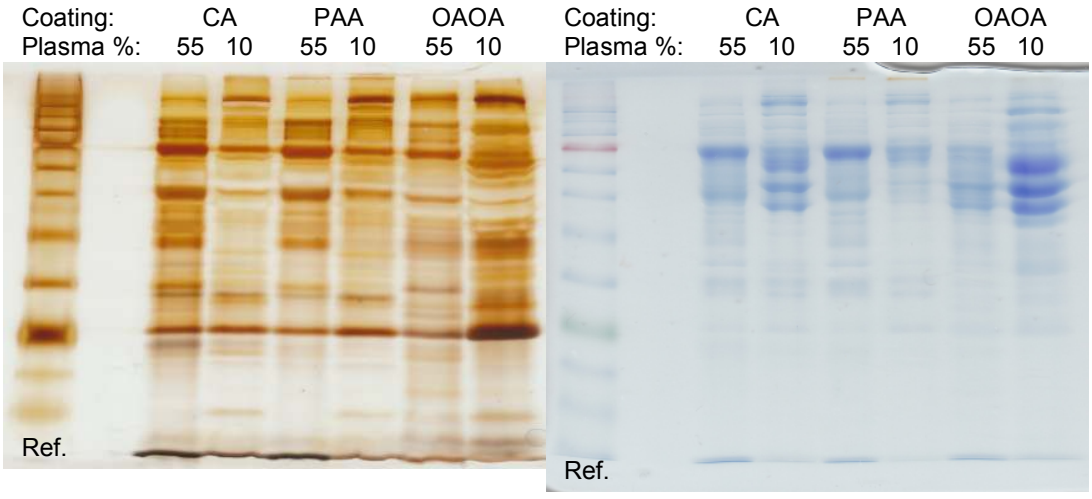
The **differences in size values** of the given MNPs with or without corona from the CPS and DLS measurements, however, were beyond the experimental errors of these methods. We should analyse these sizing methods in the future to answer why the DLS sizes (80-160 nm) are significantly different from CPS (25-30 nm). We should consider the facts that both methods have different "contrasts" (difference in refractive index and density for DLS and CPS, respectively), which are not well defined for the core-shell type particles in question; and their weakness (DLS – polydispersity and aggregation in part, validity of Γ vs. q^2 dependence for translation diffusion; CPS – density correction, the effect of shear force). Although the reproducibility of both sizing measurements was good indeed, we cannot preclude the possibility of any systematic mistake. Additional size information will be obtained by Transmission Electron Microscopy, although this also has limitations, including the need for drying the particles, which can itself lead to aggregation.

Interesting findings are that **oleate ions release** from the OAOA double layer in phosphate buffer presumably phosphate ions displace oleate ones due to stronger complexation on ≡Fe-OH surface sites; and that during contact with human plasma the OAOA coated MNPs showed a **systematic shift in time** (see below some CPS results as an example).

OAOA coated MNPs - 10% human plasma



Investigation of the **proteins bound in corona: gel electrophoresis** was used and several bands appeared over the broad range of molecular weights related to the reference proteins (Ref.) ranging from 10 to 230 kD.



The patterns (see above) showed very different features in general. This finding itself is interesting for MNPs coated by carboxylic groups and uniformly negatively charged. Only glancing at the gels, it is obvious that the type and quantity of the adsorbed proteins depends on the human plasma concentration present in the tests, and that OAOA coated MNPs can retain the greatest number of proteins probably due to the hydrophobic inner part of surfactant double layer. It can be supposed that proteins displace oleate ions bound in the second layer by hydrophobic interaction, and further work, perhaps using a fluorescent marker, will be used to follow this exchange. Apart from the layer thickness, considerable similarity between the CA and PAA coating has been achieved, hence we supposed very similar behaviour in relation to their interaction with proteins. The experiments did not support this assumption at all; definite differences can be identified in the patterns as shown in the gel above.

- Future collaboration with host institution (if applicable)

We would like to continue the collaboration with host institute the Centre for BioNano Interactions, where the excellence and experience are related mainly to the interaction of nanoparticles with proteins and cells.

We have great experience in the preparation and stabilization of water based MFs. All of our products are pre-tested in respect of the pH and salt tolerance. We will go further to test the effect of Ca^{2+} and phosphate as specific ions in biological media. Besides improving MNPs stability, we will prepare MNPs with different sizes and less polydispersity.

On our part we would like to strengthen this collaboration in order to have access to the advanced methods to test interactions in biological media such as testing protein adsorption on MNPs and MNPs' uptake in cells.

- Projected publications/articles resulting or to result from your grant

As great preliminary results suggest, it will be worth publishing in a good journal, although additional work is needed. Angela Hajdú has now received an Eotvos fellowship for 6 months from the Hungarian ministry for Science, which she will also undertake at the Centre for BioNano Interactions in UCD, in order to further progress our joint-work.

- Other comments (if any)

We also agreed to see a mechanism for joint funding for Angela Hajdú to perform postdoctoral research across and between our two groups, thereby further strengthening the linkages facilitated by the EpitopeMap grant, and building on the research progress.