Thursday Afternoon, October 31, 2013

Nanoparticle-Liquid Interfaces Focus Topic
Room: 201 B - Session NL+AS+BI-ThA

Nanoparticles with Proteins and Cells: Modelling and Measurement

Moderator: D.G. Castner, University of Washington

2:00pm **NL+AS+BI-ThA1 Nanoscale Interface Between Engineered Matter and Living Organisms: Understanding the Biological Identity of Nanosized Materials**, ***K. Dawson***, University College, Dublin **INVITED**

Nanoscale materials can interact with living organisms in a qualitatively different manner than small molecules. Crucially, biological phenomena such as immune clearance, cellular uptake and biological barrier crossing are all determined by processes on the nanometer scale. Harnessing these endogeneous biological processes (for example in creation of new nanomedicines or nanodiagnostics) will therefore require us to work on the nanoscale. This ensures that nanoscience, biology and medicine will be intimately connected for generations to come, and may well provide the best hope of tacking currently intractable diseases. These same scientific observations lead to widespread concern about the potential safety of nanomaterials in general. Early unfocussed concerns have diminished, leaving a more disciplined and balanced scientific dialogue. In particular a growing interest in understanding the fundamental principles of bionanointeractions may offer insight into potential hazard, as well as the basis for therapeutic use. Whilst nanoparticle size is important, the detailed nature of the nanoparticle interface is key to understanding interactions with living organisms. This interface may be quite complex, involving also adsorbed proteins from the biological fluid (blood, or other), leading to a 'protein corona' on the nanoparticle surface that determines its "biological identity."We discuss how this corona is formed, how it is a determining feature in biological interactions, and indeed how in many cases can undermine efforts at targeting nanoparticles using simple grafting strategies. Thus, nanoparticle interactions with living organisms cannot be fully understood without explicitly accounting for the interactions with its surroundings, i.e. the nature of the corona.

·Monopoli,M. P.; Aberg,C.;Salvati, A.;Dawson,K. A. Biomolecular Coronas Provide the Biological Identity of Nanosized Materials. *Nature Nanotechnology* **2012**,*7*,779-786.

Kim, J. A.;Aberg,C.; Salvati,A.;Dawson,K.A. Role of Cell Cycle on the Cellular Uptake and Dilution of Nanoparticles in a Cell Population. *Nature Nanotechnology* **2012**,*7*,62-68.

·Monopoli, M. P.; Walczyk, D.;Campbell,A.; Elia,G.; Lynch, I.;Baldelli Bombelli, F.;Dawson,K. A. Physical-Chemical Aspects of Protein Corona: Relevance to in Vitro and in Vivo Biological Impacts of Nanoparticles. *Journal of the American Chemical Society* **2011**,*133*,2525-2534.

·Cedervall, T.; Lynch, I.;Lindman,S.; Berggard,T.; Thulin, E.;Nilsson,H.;Dawson,K. A.;Linse,S. Understanding the Nanoparticle-Protein Corona Using Methods to Quantify Exchange Rates and Affinities of Proteins for Nanoparticles. *Proceedings of the National Academy of Sciences* **2007**,*104*,2050-2055.

2:40pm **NL+AS+BI-ThA3 *In Silico* Modelling and Prediction of the Biological Effects of Nanoparticles**, ***D.A. Winkler***, *V.C. Epa, F.R. Burden,* CSIRO Materials Science & Engineering, Australia, *C. Tassa, R. Weissleder,* Harvard Medical Center, *S. Shaw*, Massachusetts General Hospital and Harvard Medical School **INVITED**

Products are increasingly incorporating nanomaterials because of their superior properties. It is estimated that 50,000 products will contain nanomaterials by 2015. However, we have a poor understanding of their potential adverse effects on workers, the public, and the environment. To assess risk, regulatory authorities need more experimental testing of nanoparticles. Computational models play a complementary role to experiments in allowing rapid prediction of potential toxicities of new and modified nanomaterials. We have generated quantitative, predictive models of cellular uptake and apoptosis induced by surface modified metal iron oxide nanoparticles for several cell types using sparse feature selection and optimal machine learning methods. We illustrate the potential of computational methods to make a contribution to nanosafety.

3:40pm **NL+AS+BI-ThA6 Quantitative Characterization of Bacterial Cell Loading with Nanoparticles**, *C. Sousa, D. Sequeira, P.M. Martins,* University of Minho, Portugal, *Y.V. Kolen'ko,* International Iberian Nanotechnology Laboratory, Portugal, *S. Lanceros-Méndez,* University of Minho, Portugal, ***D.Y. Petrovykh***, International Iberian Nanotechnology Laboratory, Portugal

The primary analytical challenge in characterizing bacterial cells loaded with nanoparticles (NPs) is that the various methods that are traditionally used to measure cells or NPs separately are not readily applied to the mixed samples. These complex samples may contain, for example, a mixture of free NPs, NP-loaded cells, and cells without NPs, while the relative concentrations of NPs and cells or the average number of NPs loaded in one cell is not always known or readily established. Accordingly, methods for separating the different sample components have to be developed and validated before the component of interest (NP-loaded cells in most cases) can be characterized. The final challenge is determining the localization of NPs in and around the cells, as for some applications in sensing and nanomedicine NPs bound to cells externally can be the goal, whereas for exploiting physical properties of NPs, e.g., to induce hyperthermia, maximizing the internalization of NPs by cells can be advantageous.

Our approach to investigating these complex analytical challenges is based on using model systems that are amendable to quantitative characterization by complementary methods, both separately and when mixed as indicated above. Specifically, we are using *Staphyloccus aureus* as model bacterial cells, in part because the typical 500 nm diameter of *S. aureus* cells is within the size range of NP aggregates or large NPs, therefore, the same microscopy and spectroscopy methods can be applied to both components of mixed cell-NP samples. We use gold NPs as the primary model NPs because the strong plasmon peak enables their characterization in solution, while the high density and atomic number of gold can be helpful during separation and for characterization by electron microscopy and spectroscopy. Superparamagnetic iron-oxide NPs with different organic shells are used as a second type of model NPs. We will describe the use of multiple complementary microscopy and spectroscopy techniques for developing, validating, and quantifying protocols for cell-NP separation and for characterization of cell loading by NPs.

4:00pm **NL+AS+BI-ThA7 Protein-Corona: A New Gateway to Disease Therapeutics**, ***K. Giri***, *P. Mukherjee, M. Zimmermann, S. Khader, B. Madden, D. McCormick*, Mayo Clinic

Nanomedicine is a burgeoning field with immense potential in disease therapeutics, diagnosis and imaging. However, an inevitable phenomenon regarding the use of nanoparticles (NPs) *in vivo* is the adsorption of proteins to its surface to form a layer called the "protein corona". The concept of synthetic vs. biological identity of the NPs has emerged. Studies have reported that the acquired biological identity of NPs due to its protein corona influences not just the interaction of the NPs with its targets but also its fate. Among all the NPs that are currently being investigated in nanomedicine, gold nanoparticles (GNPs) are unique in that they posses strong affinity to bind to SH and NH2 containing molecules. Therefore, proteins by virtue of having cysteine and lysine residues function as unique substrates to bind to GNPs. We hypothesize that the proteome and secretome of cancer cells may include low abundance proteins that escape detection by conventional methods. Enrichment and identification of these proteins may play a critical role in understanding the pathophysiology of disease development and open new avenues for treatment. Our aim was to study the formation of protein corona on GNP surface as a unique way to enrich and identify low abundance proteins that can serve as new therapeutic targets for ovarian cancer. Understanding the interaction of proteins on GNP surface is important as it will guide modulation of protein corona formation for protein enrichment based on physicochemical properties and structure. Here, we present a systematic study of protein corona using 20 nm GNPs. We studied the binding of proteins from lysates derived from two ovarian cell lines, namely OSE (non-cancerous) and A2780 (cancerous). We followed the evolution of the corona for 24 hrs to account for the dynamic and competitive binding of proteins on the NP surface. We characterized the corona at 5 mins, 15 mins, 1hr, 6hrs and 24 hrs using UV-vis spectroscopy, dynamic light scattering, electron microscopy and ζ-potential measurements and identified corona constituents by mass spectroscopy. We focused on understanding what drives protein adsorption to the NP surface. Lastly, we identified low abundance proteins from the A2780 cell line that were enriched on GNP surface as a proof of concept study to demonstrate that protein corona can be effectively utilized for study of disease and its therapeutics.

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