Biomaterial Interfaces
Room: 201 B - Session BI+AS+BA+NL-TuM

Biointerface, Energy and Environmental Applications of QCM
Moderator: L. Hanley, University of Illinois at Chicago

8:00am **BI+AS+BA+NL-TuM1 QCM-D for Energy and Environmental Applications**, ***B.H. Kasemo***, Chalmers University of Technology, Sweden **INVITED**

QCM-D has over the past ca. 15 years matured to a measurement technique with a manifold of applications for liquid or gas phase applications. "D" stands for *dissipation* or damping of the sensor oscillation. It yields new information about sample visco-elastic properties, in addition to the mass changes at the ng/cm^2 level obtained from the QCM frequency shift. New information is obtained when the overlayer or film that is studied, causes significant energy dissipation. This is e.g. the case with viscous or visco-elastic films and molecular adlayers. In such cases the two independent quantities, the frequency shift Δf and the dissipation change ΔD , via modeling, allow unique new information to be extracted from the measurements, compared to conventional QCM. In addition, the magnitude of ΔD provides an immediate hint if the Sauerbrey relation, converting Δf to a proportional change in mass, is applicable or not. Major application areas of QCM-D in the past and currently are biomolecule adsorption on surfaces, e.g. on medical implant materials, supported lipid bilayers mimicking cell membranes, polyelectrolytes e.g. layer-by-layer growth, polymer coatings and their curing and phase changes, and more recently cell and bacterial studies. Well over 1200 QCM-D publications have been produced in these areas, cited over 15 000 times. More recently studies related to applications in the energy and environmental areas have rapidly increased. Energy technology examples include solar cells (dye impregnation of DSSC), fuel cell electrode corrosion, studies related to fossil fuel properties and processes, hydrogen storage and CO2 capture/sorption. In the environmental area many applications relate to nanoparticle safety and toxicity, e.g. measuring (surface) affinities between NPs and other materials or agglomeration between NPs. Yet another growing area is to use supported lipid membranes as up-stream model and screening systems, mimicking cell membranes, for testing of NP affinity to such membranes. The method is also used for other aspects of waste water cleaning, such as measuring affinities to filtering materials and membranes of heavy metal ions and other impurities.

9:00am **BI+AS+BA+NL-TuM4 Accounting for Unintended Binding Events in the Analysis of Quartz Crystal Microbalance Kinetic Data**, ***G. Heller***, *T. Zwang, M. Sazinsky, A. Radunskaya, M.S. Johal*, Pomona College

Previous methods for analyzing protein-ligand binding events using the Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D) fail to account for unintended binding that inevitably occurs during surface measurements and obscure kinetic information. In this talk, I present a system of differential equations that accounts for both reversible and irreversible unintended interactions. This model is tested on three well-characterized protein-ligand systems, each of which has different features, to establish the feasibility of using the QCM-D for protein binding analysis. The first system presented is the binding of hemin to human serum albumin. The second is the binding of Fe (III) 2,5-dihydroxybenzoic acid complex to neutrophil gelatinase-associated lipocalin tagged with glutathione S-transferase. The third system presented is the interaction of caffeine and bovine serum albumin. Characteristics of the QCM-D binding data for these three systems that are inconsistent with previous QCM-D kinetic models are 1) a non-constant deposition rate in the association phase, 2) a non-zero mass near the steady state of the rinse phase, 3) a non-linear dependence on ligand concentration, and 4) a non-constant ligand concentration for runs lasting short periods of time. Our model accounts for these factors and demonstrates the feasibility of using QCM-D to extract kinetic information and accurately determine affinity constants (K_d) for protein-ligand complexes.

9:20am **BI+AS+BA+NL-TuM5 Silica Nanoparticle – Lipid Membrane Interaction Studies Towards Nano(Q)SAR?**, *L. De Battice, R. Frost,* Chalmers University of Technology, Sweden, *A. Sundblom, M. Persson,* AkzoNobel PPC, Sweden, *M. Wallin, J. Sturve,* University of Gothenburg, Sweden, ***S. Svedhem***, Chalmers University of Technology, Sweden

To improve on the performance of silica-based nanomaterials, and to reduce environmental and health risks related to this development, it is important to learn about how engineered nanomaterials interact with e.g. biomolecules and biological barriers. We are also interested in the development of a generic screening methodology for nanoparticles, and to identify nanoparticle features which are likely to lead to effects in cells. The present results have been obtained with a set of five silica nanoparticles, four of which were spherical (about 20 nm in diameter) and one of which had an elongated shape (roughly 4 x 20 nm). Size and zeta potential measurements were performed, and the adsorption profiles for the nanoparticles when interacting with each of four model lipid membranes of different composition and net charge were monitored in real time using the quartz crystal microbalance with dissipation monitoring (QCM-D). We found clear differences in adsorption profiles on the model membranes with respect to surface coating, and particle shape. These results were compared to the results obtained when exposing frog cells to the same particles, using a conventional assay detecting cellular damage and cytotoxicity (through cell lactate dehydrogenase (LDH) release) and as well in experiments where the function of frog cells cultured on QCM-D sensors was studied by QCM-D (the method is published in Frost et al., Analytical Biochemistry, in press). In general, there were small effects on the cells.

The results will be discussed in the perspective of establishing (Q)SAR for nanoparticles.

9:40am **BI+AS+BA+NL-TuM6 Using Real-Time Acoustical Sensing by QCM-D to follow Dynamic Processes in Live Cell Morphology and Cell-Surface Interactions**, ***E. Nilebäck***, Biolin Scientific, Sweden, *N. Tymchenko, A. Kunze,* Chalmers University of Technology, Sweden, *L. Enochson,* University of Gothenburg, Sweden, *P. Wallin, J. Gold, S. Svedhem,* Chalmers University of Technology, Sweden, *A. Lindahl*, University of Gothenburg, Sweden

The mechanical properties and morphology of living cells are dynamic and regulated by cell signaling pathways that can be triggered by both external and internal stimuli. The dynamic nature of these cellular shape changes leaves a great potential for real-time techniques to reveal new time-resolved information in addition to microscopy methods based on fluorescence that are typically end-point measurements. By using quartz crystal microbalance with dissipation monitoring (QCM-D), the nano-mechanical properties at the cell-surface interface can be studied. How the cells interact with the surface greatly influences the QCM-D response, particularly at cell adhesion and when the cells undergo morphological changes due to internal or external stimuli.

To explore the potential of acoustically sensing the cell-surface interface in real-time, we have used QCM-D as the main technique in several cell studies:

i) Changes in cell morphology were studied simultaneously by QCM-D and light microscopy as 3t3 and human derived fibroblasts were subjected to the actin disrupting agent cytochalasin D that depolymerizes actin in the cytoskeleton. This resulted in a dramatic change in cell morphology that was reversible upon rinsing and could repeatedly be detected as significant changes in the energy dissipation. [1]

ii) Cell adhesion and cell-surface interactions were studied for human derived chondrocytes as they were subjected to well-defined layers of the glycosaminoglycan (GAG) hyaluronan (HA). HA is present in e.g. extra cellular matrix of cartilage and the chondrocytes could be seen in the QCM-D signal to degrade the GAG layer in 2 hours.

iii) Cell adhesion and fixation studies of 3t3 fibroblasts were performed on silicon dioxide coated surfaces with and without a coating of serum proteins. This revealed that the protein layer greatly affected the QCM-D response from the cells. The later fixation by formaldehyde was performed *in situ* and from the QCM-D data it was shown that the viscoelastic behavior of the cells was to a large extent retained after fixation.

1. Tymchenko, N., Nilebäck, E. et al., *Reversible Changes in Cell Morphology due to Cytoskeletal Rearrangements Measured in Real-time by QCM-D,* Biointerphases, 2012. (1): p. 1-9.

11:00am **BI+AS+BA+NL-TuM10 QCM-D as a Novel Technique to Investigate Nuclear Pore Transport**, ***M. Sorci***, Rensselaer Polytechnic Institute, *R. Hayama, B.T. Chait, M.P. Rout,* Rockefeller University, *G. Belfort*, Rensselaer Polytechnic Institute

A quartz crystal microbalance (QCM-D) is a simple and highly sensitive mass and dissipation sensor which has been used to study interfacial adsorption reactions and conformational changes on a variety of supports in real time. In this paper we aim to apply this technique to gain a better understanding of nuclear transport. In particular, we are investigating the transport of proteins through the Nuclear Pore Complex (NPC), which is the sole mediator of exchange between the nucleus and the cytoplasm in all

eukaryotic cells[1]. Recent publications have further improved our understanding of the architecture and evolutionary origins of this macromolecular gate,[2,3] yet the molecular transport mechanism remains unclear. Transport across the NPC is fast, energy-dependent (to give directionality) and often receptor-mediated. While small molecules pass through the NPCs unchallenged, large macromolecules (>40 kDa) are excluded unless chaperoned across by transport factors collectively termed Karyopherins (Kaps). The translocation of the complexes of Kaps and their cargo proteins/RNAs occurs through the specific affinity and binding between Kaps and particular nuclear pore complex proteins (nucleoporins) called FG-Nups, which share a degenerate multiple-repeated "Phe-Gly" motif. In an attempt to better understand the transport and the selective process under crowding conditions, we immobilized Nsp1 and truncated variations of it onto QCM-D sensors. The binding and unbinding of Kap95, other binding proteins, as well as control proteins (e.g. BSA), was studied in order to investigate specificity, kinetics rate constants, effect of competitive binding. Ultimately we aim to gain sufficient understanding of the molecular scale engineering principles behind nuclear transport to allow us to design the next generation of synthetic selective nanosorters capable of purifying any protein that we desire.

1. Grünwald, Singer and Rout, Nature 2011, 475, 333

2. Alber et al., Nature 2007, 450, 683

3. Alber et al., Nature 2007, 450, 695

11:20am **BI+AS+BA+NL-TuM11 Using QCM-D and Ellipsometry to Determine the Orientation and State of Hydration of Antibodies Adsorbed on a Hydrophobic Surface**, ***C.W. Frank***, Stanford University, *M.E. Wiseman*, DSM Research **INVITED**

Adsorbed antibodies can take several orientations: end-on/fab-up, end-on/fab-down, side-on, and flat-on. Since the accessibility of antigens will depend on the antibody orientation, we have used QCM-D to monitor transient adsorption and have determined the orientation as a function of coverage. In addition, we have used simultaneous QCM-D and ellipsometry to distinguish between the "wet" mass consisting of protein plus coupled water and the "dry" mass consisting only of the protein. Finally, we have applied an alternative protocol for determining the state of hydration using only QCM-D. This involves a D_2O exchange that allows determination of the dry mass. We conclude that the QCM-D signal of proteins in liquids contains a major component from coupled water.

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