AuPS/ASB Meeting - Adelaide 2010

Free communications: Imaging and structural analysis of lipids and proteins

Monday 29th November 2010 - Broughton Room - 11:00

Chair: Joe Shapter

Small molecule diffusion in inverse cubic phase lipid systems

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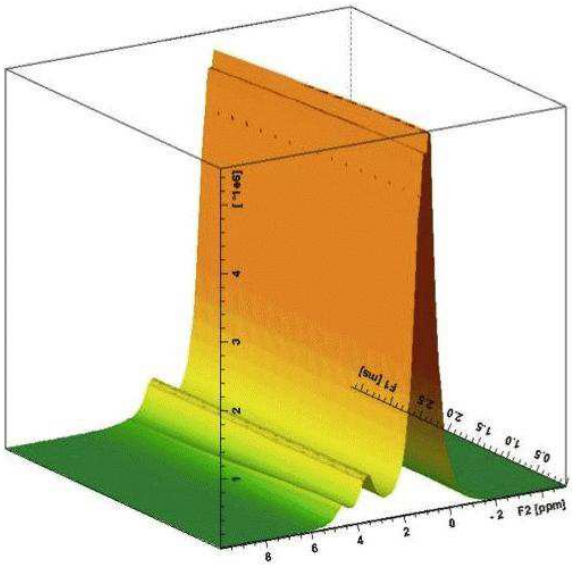
The cubic phases formed by amphiphiles are an important class of nanostructured self-assembled material. Colloidally stable, particulate dispersions of the inverse cubic phase, termed cubosomes, are of particular interest due to their thermal stability, large surface-area to volume ratio (up to $400m^2.g^{-1}$ of cubic gel), their ability to incorporate functional molecules, and a viscosity approximately equal to that of water. In such structurally complex, multi-component systems, diffusion NMR is a powerful technique for measuring small molecule diffusion within cubosomes and, importantly, allows simultaneous acquisition of diffusion coefficients for free molecules in solution or bound to a receptor embedded in the lipid matrix. The model system used here is the biotin-Neutravidin system (K_D = ~10^{-15} M), with the biotin chemically attached to a lipid incorporated into the cubosomes, whilst the Neutravidin is added in solution. The results reported here indicate a multi-component diffusion of water and proteins through the pores of cubosomes. NMR diffusion measurements show signal attenuation in the water and protein resonances whilst the lipid components of the system remain unaffected (Figure). The decrease in diffusion constants for water and protein in the cubosome system in comparison to free solution indicates that diffusion is dependent on the porous nature of the cubosome and protein binding.



DOSY proton NMR spectra of the phytantriol inverse cubic phase at 25°C.

Detection of proteins in the pathological deposits in Pseudoexfoliation syndrome using Atomic Force Microscopy

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Protein aggregation is of significant interest to various disciplines; it can be the cause of debilitating diseases, or the foundation of advanced nanomaterials. One ocular disease hallmarked by protein aggregation is known as Pseudoexfoliation Syndrome (PEX). This condition is caused by the formation of insoluble aggregates, and is characterised by deposition of fibrillar proteinaceous material on the anterior lens capsule. PEX deposits in the eye block the aqueous outflow mechanisms, which can lead to an elevation in intraocular pressure and subsequent glaucoma. Glaucoma is the second leading cause of irreversible blindness worldwide, and PEX is the most common known risk factor for glaucoma.

Proteomic analyses have revealed an association of various genetic markers and protein expression with PEX; however a complete explanation for disease susceptibility is not yet available. As the aggregates are a complex arrangement of proteins, the ultrastructure is poorly characterised and many protein constituents of the aggregates remain unknown. This study addresses the critical issue of determining the molecular nature of PEX on lens capsules in their native state by atomic force microscopy (AFM) based antibody recognition imaging. The particular focus of this study is on a type of AFM methodology referred to as topography and recognition imaging (TREC). Proteins identified by proteomic data as being implicated in the PEX pathophysiology, such as clusterin and LOXL1, are detected by an AFM probe modified with the appropriate antibody. Topographical AFM images and antibody recognition images are obtained simultaneously to determine the specific location of proteins in and around PEX aggregates on the lens capsule anterior surface. These data, combined with data from alternative antibody-recognition imaging techniques, proteomic and genetic analyses, are leading to an improved understanding of the pathophysiological basis of PEX. A more complete understanding of the pathophysiological basis for the disease will lead to the development of earlier detection methods and treatments that target the disease instead of the subsequent glaucoma.

Visualisation of bacterial hydrodynamics

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Bacteria and other microorganisms live in an aqueous, low-Reynolds number environment where viscous forces dominate. The means by which many microorganisms propel themselves through such an environment are not well understood. Additionally, understanding the way in which fluid couples physical interactions between micro-scale objects and surfaces has received increasing attention in a number of biological and technological fields over recent years. The traditional approaches to investigating these problems are mathematical simulations and particle image velocimetry (PIV) experiments. While modeling can be done in three dimensions, the vast majority of experimental investigations have been restricted to only two. This is primarily due to the reduced depth of field available at the high magnifications required to see microorganisms.

I have used some novel approaches to investigating hydrodynamic interactions on the micro-scale, specifically in the context of the bacterial flagellar motor. I have adapted in-line holographic PIV techniques (Cheong *et al.*, 2009) for use in a biological environment under high magnification. Assays of motile *Escherichia coli* are seeded with microsphere tracer particles and illuminated with a collimated laser beam. The coherent light scattering off a microsphere interferes with non-scattered light and results in a characteristic interference pattern. From this pattern, microsphere positions in three dimensions can be determined. This technique allows for greater depth of field than other techniques, and, when combined with high-speed video microscopy, can generate a full three-dimensional map of a flow field in a dynamic micro-system.

Independently, microspheres can also be used as passive detectors of dynamic behaviour. In a separate experiment, a microsphere is held in an optical trap in close proximity to a second microsphere attached to the motor of an immobilised bacterium. The resulting hydrodynamic interactions are recorded through video microscopy. The ultimate aim of this approach is to develop a non-contact method of detecting the motion of dynamic systems which are invisible under normal microscopy.

These methods hold promise for exploring and visualising not only bacterial systems, but any dynamic, aqueous micro-scale environment.

Cheong, FC, Sun, B, Dreyfus, R, Amato-Grill, J, Xiao, K, Dixon, L & Grier, DG (2009). Flow visualization and flow cytometry with holographic video microscopy. *Optics Express*, **17**(15), 13071–13079.

Shedding light on liposomes: Using lipid-mimetic metal complexes for fluorescent labeling

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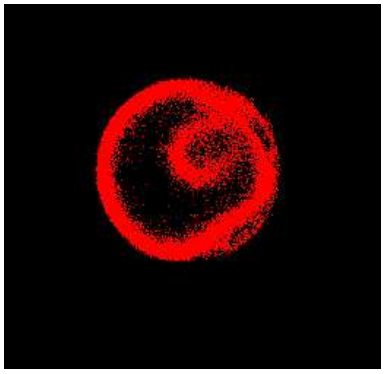
For the study of biophysical systems involving phospholipid membranes, such as membrane lysis by antimicrobial peptides, supported membrane deposition, or liposome size and shape studies, it is often desired to apply a fluorescent label to the lipid phase. However, lipid membranes are dynamic systems that are highly dependent on the strength of headgroup interactions, internal pressure and lipid mobility. If either of these factors is changed, the properties of the membrane (surface tension, liposome diameter, ability to attach to surfaces) might change as well, compromising the measurements. Unfortunately, fluorescent labeled lipids and membrane-specific dyes typically do change the physicochemical properties of the membrane. Our aim was to develop a fluorescent dye that is similar to the lipid molecules in shape and size, and to use it for liposome imaging.

Two metal complexes, $(Ru(2,2\text{-bipyridine})_2(4,4\text{-dinonyl-}2,2\text{-dipyridyl})(PF6)_2)$ and $(Ir(2\text{-phenylpyridine})_2(4,4\text{-dinonyl-}2,2\text{-dipyridyl})(PF6))$ have been synthesized, both with the same molecular structure: a "head group" made of the metal coordinated by bpy and ppy, respectively, and a "tail group" with two alkane chains. The lipid-mimetic complexes have been successfully reconstituted into DMPC liposomes. Importantly, when making the liposomes, extrusion was omitted; only a gentle vortexing and 30 s weak sonication was employed and the liposome size distribution was left to evolve. As a result, liposomes of a relatively broad size distribution were created, tending towards a bimodal distribution containing small unilamellar liposomes (SUV; ~100nm of diameter) and large (up to micron size) liposomes, as revealed by DLS measurements. Importantly, the size distribution of the liposomes labeled with the metal complexes is very similar to DMPC liposomes without the fluorescent label, thus the presence of the complex does not alter significantly the physicochemical properties of the membrane. It is regularly assumed that if larger liposomes form they must be multilamellar "onion" structures with an SUV core. However, confocal microscopic imaging of the metal complex labeled liposomes shows hollow structures with occasional encapsulated smaller liposomes. The figure shows an example of a confocal microscopic cross-section picture of a large liposome enclosing a smaller one. Remarkably, the metal complexes suffer minimal, practically negligible photobleaching, making longer time lapse studies feasible. The two metal complexes fluoresce at different wavelengths opening the door for dichroic measurements with this new labeling method.



A liposome- in- liposome system, labeled with $Ru(bpy)_2$dinone. The diameter of the outer liposome is ~3.5μm.

Shedding light on neurodegeneration: small angle X-ray scattering and misfolded proteins

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The misfolding pathways that lead to cytotoxic species vary between diseases such as Alzheimer's (AD), Huntington's and Parkinson's but there is a common link in that they all involve some form of oligomeric species, in contrast to the extra- or intra-cellular fibrillar deposits of aggregated protein that are regarded as end products of the pathological process (Villemagne *et al.*, 2010). To elucidate possible folding pathways for the amyloid β peptide of AD (Aβ) we made time-resolved, stopped-flow SAXS measurements at the Australian Synchrotron on Aβ 1-40 and Aβ 1-42 peptides in dilute NaOH (13mM) that were rapidly mixed with pH 6.9 phosphate buffered saline containing Cu^{2+} ions. These showed that protofibril formation occurred in less than one second in either control or Cu^{2+}–containing buffer and that evolution of the fibrils in subsequent seconds followed a non-linear pattern. Static measurements on the peptides that had been reacted with sub-micellar concentrations of the lipid mimetic, N-lauroylaminopropyl-N′,N′- dimethylamine oxide (LDAO) and a dipeptide formed of tyrosine 10 cross-linked Aβ 1-40 (Kok *et al.*, 2009), however, gave a stable well-defined "Y" shaped structure for both the di-tyrosine linked peptide and LDAO- associated Aβ 1-42. The "Y" shape is reminiscent of the Fc antibody fragment. Since the di-tyrosine linked peptide is neurotoxic, as in the case of cytotoxic antibodies, its two arms may carry ligands able to cross-link cell membrane receptors to initiate a cytotoxic cascade.

Villemagne VL, Perez KA, Pike KE, Kok WM, Rowe CC, White AR, Bourgeat P, Salvado O, Bedo J, Hutton CA, Faux NG, Masters CL, Barnham KJ (2010) *Journal of Neuroscience* **30**: 6315-22.

Kok WM, Scanlon DB, Karas JA, Miles LA, Tew DJ, Parker MW, Barnham KJ,Hutton CA (2009) *Chemical Communications (Cambridge, England)* **7**: 6228-30.