

## FARFIELD PUBLICATIONS

2011

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Lane, T.J., Fletcher, W.R., Gormally, M.V. and Johal, M.S., **Dual-Beam Polarization Interferometry Resolves Mechanistic Aspects of Polyelectrolyte Adsorption. *Langmuir*, (2008), 008 ASAP Article, Web Release Date: September 10, 2008.**

**Abstract:** The electrostatically driven binding dynamics of a polyelectrolyte multilayer (PEMU) film was investigated in real-time using dual-beam polarization interferometry (DPI) and independently supported by quartz crystal microbalance with dissipation monitoring (QCM-D) studies. Multilayer assemblies of the polyanions poly[1-[4[(3-carboxy-4-hydroxyphenylazo)benzenesulfonamido]-1,2-ethanediy] sodium salt] (PAZO) and poly(styrene sulfonate) (PSS) were respectively constructed with the polycation poly(ethylenimine) (PEI) on anionic functionalized substrates using the layer-by-layer electrostatic self-assembly method. DPI measurements indicate that polyelectrolyte adsorption occurs in three distinct stages. In the first stage, for ~5 s, coil-like segments of polyanion partially tether to the surface of the oppositely charged PEI. In the second stage, these coils unfurl over a period of ~10 s to cover the surface resulting in an increase in average density of the film. During the final adsorption step, the surface-bound polyelectrolyte diffuses into the multilayer assembly, exposing the surface to further deposition. This last step occurs over a much longer time period and results in a highly interpenetrated film containing a charge-overcompensated region at the film surface.

Edmondson S, Vo C.D., Armes S.P., Unali G.F., Weir M.P., **Layer-by-Layer Deposition of Polyelectrolyte Macroinitiators for Enhanced Initiator Density in Surface-Initiated ATRP, *Langmuir*, (2008), 15; 24(14), 7208-15.**

**Abstract:** The layer-by-layer (L-b-L) deposition of oppositely charged polyelectrolytic macroinitiators has been demonstrated on planar silica substrates. The build-up of the macroinitiator multilayers was monitored by ellipsometry (up to 21 layers) and dual polarization interferometry (up to 17 layers) and good agreement was found between these techniques. The increase in L-b-L thickness was approximately linear, with an average thickness of 2.3 Å per layer of deposited macroinitiator. Surface-initiated ATRP of a model nonionic methacrylic monomer, 2-hydroxyethyl methacrylate (HEMA) in a 1:1 methanol/water mixture was conducted at ambient temperature. Increasing the number of macroinitiator layers led to a significant increase in PHEMA brush thickness up to 110 nm, which is attributed to the greater surface grafting density. PHEMA brush thicknesses obtained after 22 h showed a linear dependence on the number of layers of deposited macro-initiator, with all layers exhibiting near-identical growth kinetics. X-ray photoelectron spectroscopy was used to monitor L-b-L assembly and also to confirm PHEMA growth. This technique indicated the loss of small counterions from the multilayers during L-b-L deposition and confirmed an increase in the surface density of bromoester initiator groups as the number of deposited macroinitiator layers was increased. For 17 macroinitiator layers, the bromoester initiator density is estimated to be approximately 4.9 +/- 0.2 nm<sup>-2</sup> from the DPI data. This is comparable to that calculated for ATRP initiator monolayers obtained by either thiol or silane chemistry. Ellipsometry suggested that the macroinitiator multilayers were weakly hydrated prior to the in situ HEMA polymerization. AFM studies indicated that the PHEMA brushes had appreciable surface roughness, but this roughness became negligible compared to the brush thickness with increasing macroinitiator layers.

Morley, S., Cecchini, M., Zhang, W., Virgulti, A., Noy, N., Atkinson, J., Manor, D., **Mechanisms of ligand transfer by the hepatic Tocopherol Transfer Protein, *J. Biol. Chem.*, (2008), 283(26), 17797-804.**

**Abstract:** Alpha-tocopherol is a member of the vitamin E family that functions as the principal fat-soluble antioxidant in vertebrates. Body-wide distribution of tocopherol is regulated by the hepatic alpha-tocopherol transfer protein (aTTP), which stimulates secretion of the vitamin from hepatocytes to circulating lipoproteins. This biological activity of aTTP is thought to stem from its ability to facilitate the transfer of vitamin E between membranes, but the mechanism by which the protein exerts this activity remains poorly understood. Using a fluorescence energy transfer methodology, we found that the rate of tocopherol transfer from lipid vesicles to aTTP increases with increasing aTTP concentration. This concentration dependence indicates that ligand transfer by aTTP involves direct protein-membrane interaction. In support of this notion, equilibrium analyses employing filtration, dual polarization interferometry, and tryptophan fluorescence demonstrated the presence of a stable aTTP•bilayer complex. The physical association of aTTP with membranes is markedly sensitive to the presence of vitamin E in the bilayer. Some naturally-occurring mutations in aTTP that cause the hereditary disorder Ataxia with vitamin E deficiency (AVED) diminish the effect of tocopherol on the protein-membrane association, suggesting a possible mechanism for the accompanying pathology.

Khan, T.R., Grandin, M., Morozov, A., Mashaghi, A., Textor, M., Reimhult, E., Reviakine, I., **Lipid redistribution in phosphatidylserine-containing vesicles adsorbing on Titania. *Biointerfaces*, (2008), 3(2), FA90 - FA95.**

Mashaghi, A., Swann, M., Popplewell, J., Textor M., and Reimhult, E., **Optical anisotropy of supported lipid structures probed by waveguide spectroscopy and its application to study of supported lipid bilayer formation kinetics**, *Anal. Chem.*, (2008), 80 (10), 3666–3676.

Aulin C, Varga I, Claesson, P.M, Wågberg, L, Lindström, T., **Buildup of polyelectrolyte multilayers of polyethyleneimine and microfibrillated cellulose studied by in situ dual-polarization interferometry and quartz crystal microbalance with dissipation**, *Langmuir*, (2008), 18; 24(6), 2509-18.

Abstract: Polyethyleneimine (PEI) and Microfibrillated cellulose (MFC) have been used to buildup polyelectrolyte multilayers (PEM) on silicon oxide and silicon oxynitride surfaces at different pH values and with different electrolyte and polyelectrolyte/colloid concentrations of the components. Consecutive adsorption on these surfaces was studied by in situ dual-polarization interferometry (DPI) and quartz crystal microbalance measurements. The adsorption data obtained from both the techniques showed a steady buildup of multilayers. High pH and electrolyte concentration of the PEI solution was found to be beneficial for achieving a high adsorbed amount of PEI, and hence of MFC, during the buildup of the multilayer. On the other hand, an increase in the electrolyte concentration of the MFC dispersion was found to inhibit the adsorption of MFC onto PEI. The adsorbed amount of MFC was independent of the bulk MFC concentration in the investigated concentration range (15-250 mg/L). Atomic force microscopy measurements were used to image a MFC-treated silicon oxynitride chip from DPI measurements. The surface was found to be almost fully covered by randomly oriented microfibrils after the adsorption of only one bilayer of PEI/MFC. The surface roughness expressed as the rms-roughness over  $1 \mu\text{m}^2$  was calculated to be 4.6 nm (1 bilayer). The adsorbed amount of PEI and MFC and the amount of water entrapped by the individual layers in the multilayer structures were estimated by combining results from the two analytical techniques using the de Feijter formula. These results indicate a total water content of ca. 41% in the PEM.

Azemi, E., Stauffer, W.R., Gostock, M.S., Lagenaur, C.F., Cui, X.T., **Surface immobilization of neural adhesion molecule L1 for improving the biocompatibility of chronic neural probes: In vitro characterization**, *Acta Biomater.*, (2008), 1208-17.

Abstract: Silicon-based implantable neural electrode arrays are known to experience failure during long-term recording, partially due to host tissue responses. Surface modification and immobilization of biomolecules may provide a means to improve their biocompatibility and integration within the host brain tissue. Previously, the laminin biomolecule or laminin fragments have been used to modify the neural probe's silicon surface to promote neuronal attachment and growth. Here we report the successful immobilization of the L1 biomolecule on a silicon surface. L1 is a neuronal adhesion molecule that can specifically promote neurite outgrowth and neuronal survival. Silane chemistry and the heterobifunctional coupling agent 4-maleimidobutyric acid N-hydroxysuccinimide ester (GMBS) were used to covalently bind these two biomolecules onto the surface of silicon dioxide wafers, which mimic the surface of silicon-based implantable neural probes. After covalent binding of the biomolecules, polyethylene glycol (PEG)-NH<sub>2</sub> was used to cap the unreacted GMBS groups. Surface immobilization was verified by goniometry, dual polarization interferometry, and immunostaining techniques. Primary murine neurons or astrocytes were used to evaluate the modified silicon surfaces. Both L1- and laminin-modified surfaces promoted neuronal attachment, while the L1-modified surface demonstrated significantly enhanced levels of neurite outgrowth ( $p < 0.05$ ). In addition, the laminin-modified surface promoted astrocyte attachment, while the L1-modified surface showed significantly reduced levels of astrocyte attachment relative to the laminin-modified surface and other controls ( $p < 0.05$ ). These results demonstrate the ability of the L1-immobilized surface to specifically promote neuronal growth and neurite extension, while inhibiting the attachment of astrocytes, one of the main cellular components of the glial sheath. Such unique properties present vast potentials to improve the biocompatibility and chronic recording performance of neural probes.

Boudjemline, A., Clarke, D.T., Freeman, N.J., Nicholson J.M., Jones, G.R., **Early stages of protein crystallization as revealed by emerging optical waveguide technology**, *J. Appl. Cryst.*, (2008), 41, 523–530.

Keywords: protein crystallization; dual polarization interferometry; protein aggregation; protein crystallization detection; nucleation; protein adlayers; crystal growth.

Abstract: A highly sensitive method for studying the onset of protein crystallization in real time using an optical-waveguide-based technique is reported. Dual polarization interferometry uses light from sensing and reference waveguides to produce an interference pattern, which when the sensing waveguide is immersed in a protein solution supplies information on the thickness and density of any protein adlayer on the sensing waveguide's surface. This technique provides evidence that crystallization proceeds via large protein aggregates but, more strikingly, shows dramatic light loss from the sensing waveguide at a very early stage during crystallization. The technique proves relatively insensitive to the crystallization of small molecules or poorly formed protein crystals and affords a method of distinguishing crystal formation from the formation of other protein aggregates or salt crystals. Our experimental setup currently necessitates crystallization using the batch method, and precipitant mixing at high supersaturation is known to introduce a greater variability compared with methods such as vapour diffusion or

dialysis, but first results promise to bridge the paucity of real-time methods available to distinguish the onset of protein crystallization from other forms of aggregation.

Sonesson, A. W., Callisen, T. H., Brismar H., and Elofsson, U. M., **Adsorption and activity of Thermomyces lanuginosus lipase on hydrophobic and hydrophilic surfaces measured with dual polarization interferometry (DPI) and confocal microscopy** *Colloids and Surfaces B, Biointerfaces*, (2008), 15:61(2)208-15.

**Abstract:** The adsorption and activity of Thermomyces lanuginosus lipase (TLL) was measured with dual polarization interferometry (DPI) and confocal microscopy at a hydrophilic and hydrophobic surface. In the adsorption isotherms, it was evident that TLL both had higher affinity for the hydrophobic surface and adsorbed to a higher adsorbed amount (1.90mg/m<sup>2</sup>) compared to the hydrophilic surface (1.40-1.50mg/m<sup>2</sup>). The thickness of the adsorbed layer was constant (approximately 3.5nm) on both surfaces at an adsorbed amount >1.0mg/m<sup>2</sup>, but decreased on the hydrophilic surface at lower surface coverage, which might be explained by partially unfolding of the TLL structure. However, a linear dependence of the refractive index of the adsorbed layer on adsorbed amount of TLL on C18 surfaces indicated that the structure of TLL was similar at low and high surface coverage. The activity of adsorbed TLL was measured towards carboxyfluorescein diacetate (CFDA) in solution, which upon lipase activity formed a fluorescent product. The surface fluorescence intensity increase was measured in a confocal microscope as a function of time after lipase adsorption. It was evident that TLL was more active on the hydrophilic surface, which suggested that a larger fraction of adsorbed TLL molecules were oriented with the active site facing the solution compared to the hydrophobic surface. Moreover, most of the activity remained when the TLL surface coverage decreased. Earlier reports on TLL surface mobility on the same surfaces have found that the lateral diffusion was highest on hydrophilic surfaces and at low surface coverage of TLL. Hence, a high lateral mobility might lead to a longer exposure time of the active site towards solution, thereby increasing the activity against a water-soluble substrate.

Johnson, S., Evans, S., Laurenson, S., Paul, D., Davies, A.J., Ferrigno, P.K., Walti, P., **Surface-Immobilized Peptide Aptamers as Probe Molecules for Protein Detection**, *Analytical Chemistry*, (2008), Vol. 80, No. 4.

**Abstract:** We demonstrate the use of surface-immobilized oriented peptide aptamers for the detection of specific target proteins from complex biological solutions. These peptide aptamers are target-specific peptides expressed within a protein scaffold engineered from the human protease inhibitor stefin A. The scaffold provides stability to the inserted peptides and increases their binding affinity owing to the resulting three-dimensional constraints. A unique cysteine residue was introduced into the protein scaffold to allow orientation-specific surface immobilization of the peptide aptamer and to ensure exposure of the binding site to the target solution. Using dual-polarization interferometry, we demonstrate a strong relationship between binding affinity and aptamer orientation and determine the affinity constant  $K_D$  for the interaction between an oriented peptide aptamer STMpep9 cys+ and the target protein CDK2. Further, we demonstrate the high selectivity of the peptide aptamer STMpep9 by exposing surface-immobilized STMpep9 cys+ to a complex biological solution containing small concentrations of the target protein CDK2.

**2007**

Gengler, S., Gault, V.A., Harriott, P., and Hölscher, C., **Impairments of hippocampal synaptic plasticity induced by aggregated beta-amyloid (25-35) are dependent on stimulation-protocol and genetic background**. *Experimental Brain Research*, (2007), Volume 179, pp. 621-630 (10).

**Keywords:** Learning Memory Neurodegeneration LTP Rat

**Abstract:** The aggregation of beta-amyloid to plaques in the brain is one of the hallmarks of Alzheimer disease (AD). Numerous studies have tried to elucidate to what degree amyloid peptides play a role in the neurodegenerative developments seen in AD. While most studies report an effect of amyloid on neural activity and cognitive abilities of rodents, there have been many inconsistencies in the results. This study investigated to what degree the different genetic backgrounds affect the outcome of beta-amyloid fragment (25–35) on synaptic plasticity in vivo in the rat hippocampus. Two strains, Wistar and Lister hooded rats, were tested. In addition, the effects of a strong (600 stimuli) and a weak stimulation protocol (100 stimuli) on impairments of LTP were analysed. Furthermore, since the state of amyloid aggregation appears to play a role in the induction of toxic processes, it was tested by dual polarisation interferometry to what degree and at what speed beta-amyloid (25–35) can aggregate in vitro. It was found that 100 nmol beta-amyloid (25–35) injected icv did impair LTP in Wistar rats when using the weak but not the strong stimulation protocol ( $P < 0.001$ ). One-hundred nano mole of the reverse sequence amyloid (35–25) had no effect. LTP in Lister Hooded rats was not impaired by amyloid at any stimulation protocol. The aggregation studies showed that amyloid (25–35) aggregated within hours, while amyloid (35–25) did not. These results show that the genetic background and the stimulation protocol are important variables that greatly influence the experimental outcome. The fact that amyloid (25–35) aggregated quickly and showed neurophysiological effects, while amyloid (35–25) did not aggregate and did not show any effects indicates that the state of aggregation plays an important role in the physiological effects.

Aulin, C., **Preparation, characterisation and wetting of fluorinated cellulose surfaces**, PhD Thesis, Royal Institute of Technology, 2007.

Rekas A, Jankova L, Thorn DC, Cappai R, Carver, A., **Monitoring the prevention of amyloid fibril formation by alpha-crystallin Temperature dependence and the nature of the aggregating species**, *FEBS J.*, (2007), 274(24), 6290-304.

**Abstract:** The molecular chaperone, alpha-crystallin, has the ability to prevent the fibrillar aggregation of proteins implicated in human diseases, for example, amyloid beta peptide and alpha-synuclein. In this study, we examine, in detail, two aspects of alpha-crystallin's fibril-suppressing ability: (a) its temperature dependence, and (b) the nature of the aggregating species with which it interacts. First, the efficiency of alpha-crystallin to suppress fibril formation in kappa-casein and alpha-synuclein increases with temperature, despite their rate of fibrillation also increasing in the absence of alpha-crystallin. This is consistent with an increased chaperone ability of alpha-crystallin at higher temperatures to protect target proteins from amorphous aggregation [GB Reddy, KP Das, JM Petrash & WK Surewicz (2000) *J Biol Chem* 275, 4565-4570]. Second, dual polarization interferometry was used to monitor real-time alpha-synuclein aggregation in the presence and absence of alphaB-crystallin. In contrast to more common methods for monitoring the time-dependent formation of amyloid fibrils (e.g. the binding of dyes like thioflavin T), dual polarization interferometry data did not reveal any initial lag phase, generally attributed to the formation of prefibrillar aggregates. It was shown that alphaB-crystallin interrupted alpha-synuclein aggregation at its earliest stages, most likely by binding to partially folded monomers and thereby preventing their aggregation into fibrillar structures.

Hassler, K., Rigler P., Blom, H., Rigler, R., Widengren, J. and Lasser, T., **Dynamic Disorder in Horseradish Peroxidase Observed with Total Internal reflection Correlation Spectroscopy**, *Optics Express*, (2007), 15(9), 5366-5375.

**Abstract:** This paper discusses the application of objective-type total internal reflection fluorescence correlation spectroscopy (TIR-FCS) to the study of the kinetics of immobilized horseradish peroxidase on a single molecule level. Objective-type TIR-FCS combines the advantages of FCS with TIRF microscopy in a way that allows for simultaneous ultra-sensitive spectroscopic measurements using a single-point detector and convenient localization of single molecules on a surface by means of parallel imaging.

Swann, M.J., Freeman N.J., Cross G. H., **Dual Polarization Interferometry: A Real-Time Optical Technique for Measuring (Bio)Molecular Orientation, Structure and Function at the Solid/Liquid Interface**, *Handbook of Biosensors and Biochips*, 2 Volume Set, (2007). Eds: R. S. Marks, C. R. Lowe, D. C. Cullen, H. H. Weetall, I. Karube, Hardcover, 1500 pages.

Fresquet, M., Jowitt, T., Ylöstalo, J., Coffey, P., Meadows, R., Ala-Kokko, L., Thornton D. J., Briggs M.D., **Structural and Functional Characterisation of Recombinant Matrilin-3 A-Domain and Implications for Human Genetic Bone Diseases**, *Journal of Biological Chemistry*, (2007).

**Keywords:** Multiple epiphyseal dysplasia, matrilin-3, A-domain, conformational change, type IX collagen, cartilage oligomeric matrix protein

Lee, T.H., Hall, K., Mechler, Martin, L., Popplewell, J., Ronan, G., Aguilar, M., **Molecular Imaging and Orientational Changes of Antimicrobial Peptides in Membranes**, *American Peptide Society*, (2007).

Edmondson, S., Vo, C-D., Armes S.P., Unali, G-F., **Surface Polymerization from Planar Surfaces by Atom Transfer Radical Polymerization Using Polyelectrolytic Macroinitiators**, *Macromolecules*, (2007), 40(15), 5271-5278.

**Abstract:** The one-pot synthesis of a new anionic polyelectrolytic macroinitiator based on esterification of a poly(glycerol monomethacrylate) precursor is described. Electrostatic adsorption of this macroinitiator onto an aminated (cationic) planar substrate is monitored by dual polarization interferometry. Controlled surface-initiated polymerization of five hydrophilic methacrylic monomers from this macroinitiator adsorbed onto aminated silicon wafer surfaces is achieved by atom transfer radical polymerization (ATRP) in protic media. The thickness, uniformity, and hydrophilicity of the resulting polymer brushes are characterized by ellipsometry, atomic force microscopy and contact angle studies, and the hydrophilic surface polymerization kinetics is modeled. Microcontact printing is used to produce patterned surfaces with micrometer-sized features. In summary, polyelectrolytic macroinitiators allow the facile synthesis of well-defined polymer brushes on commercially relevant metal oxide surfaces.

Thompsett, A.R., Brown D.R., **Dual polarisation interferometry analysis of copper binding to the prion protein: Evidence for two folding states**, *Biochimica et Biophysica Acta (BBA) - Proteins & Proteomics*, (2007), 1774(7), 920-927.

Keywords: Copper; Prion; Metal; Scrapie; Dual polarisation interferometry

Abstract: The prion protein is a copper binding glycoprotein expressed in neurones and other cells. Conversion of this protein to an abnormal isoform is central to the cause of prion diseases or transmissible spongiform encephalopathies. Detecting slight structural differences between different forms of the prion protein could be essential to understanding the role of the protein in health and disease. Dual polarisation interferometry (DPI) is a new method that allows detection of small structural differences. We used this technique to evaluate the effectiveness of DPI in the analysis of metal binding to recombinant mouse prion protein. DPI was able to measure mass change in the prion protein following addition of copper and could identify reproducible differences in the structure of prion protein dependent on how metal was added to the protein. These slight structural differences were confirmed by the use of circular dichroism spectroscopy and Fourier-transformed infra-red spectroscopy. These results suggest that DPI can provide important information on both transitory and stable structural difference that are induced in the prion protein. This technique could be important not only for the study of metal-protein interactions but also small structural differences that could define prion strains.

Sonesson A., **Dynamics of Enzymes at Interfaces: Lipase adsorption and mobility on solid surfaces**, PhD Thesis, Royal Institute of Technology, 2007.

Keywords: Biophysics, surface chemistry, diffusion, enzymes, lipases, adsorption, mobility

Abstract: This thesis aimed to give more insight in the dynamics of enzymes at interfaces. The adsorption and mobility of adsorbed proteins can e.g. give a better understanding of structure-function properties of interfacially active enzymes. Studied enzyme was the lipase from *Thermomyces lanuginosus* (TLL).

Adsorption of TLL to surfaces of different hydrophobicity was studied by Dual Polarization Interferometry (DPI), Surface Plasmon Resonance (SPR) and ellipsometry. It was found that TLL had highest affinity and adsorbed to largest adsorbed amount on a hydrophobic, C18 terminated surface. Moreover, activity studies of adsorbed TLL suggested that a larger fraction of the lipases were orientated with the active site facing the surface on hydrophobic surfaces.

Mobility of adsorbed enzymes was studied by means of Fluorescence Recovery After Photobleaching (FRAP) with Confocal Laser Scanning Microscopy (CLSM). CLSM was also used as a tool to image the role of TLL in the detergency of lipids from single cotton fibers. The TLL surface mobility was measured on model surfaces of different hydrophobicity. The rate of TLL surface diffusion was strongly dependent on the surface density of lipase, which was explained by sterical hindrance and intermolecular repulsion. The diffusion was both lowest and decreased as a function of time after adsorption on the most hydrophobic surface. This was thought to be due to a larger fraction of adsorbed TLL oriented with the active site towards the hydrophobic surface and that this fraction increased as a function of time.

The presence of surfactants affected the TLL mobility on hydrophobic surfaces. The diffusion increased more than tenfold when TLL was coadsorbed with C12E6/LAS above the critical micellar concentration (cmc) of the surfactant. This was thought to be due to a surfactant induced desorption-rebinding mechanism of TLL. Total Internal Reflection Fluorescence Correlation Spectroscopy (TIR-FCS) supported this theory and was implemented as a technique to quantify kinetic processes of protein-surfactant interactions at surfaces. The surface mobility of TLL was higher on a trimyristin substrate surface compared to the model hydrophobic surface. Single particle tracing of lipases could be performed by conjugation of TLL to Quantum Dots (QDs). The microscopic behavior of QD-lipases on trimyristin suggested that the enzyme operated in two different modes on the surface, which gave the trajectories of single lipase molecules a "bead on a string" appearance.

Horgan, C. P., Oleksy, A., Zhdanov, A., V., Lall, P. Y., White, I. J., Khan, A.R., Futter, C. E., McCaffrey, J.G., McCaffrey M.W., **Rab11-FIP3 is critical for the structural integrity of the endosomal-recycling compartment**, *Traffic*, (2007), 8, 414-430.

Keywords: Rab11-FIP3/Rab11/Endosomal-recycling compartment/Nuf/Coiled-coil

Abstract: Rab11-FIP3 is an endosomal-recycling compartment (ERC) protein that is implicated in the process of membrane delivery from the ERC to sites of membrane insertion during cell division. Here we report that Rab11-FIP3 is critical for the structural integrity of the ERC during interphase. We demonstrate that knockdown of Rab11-FIP3 and expression of a mutant of Rab11-FIP3 that is Rab11-binding deficient causes loss of all ERC-marker protein staining from the pericentrosomal region of A431 cells. Furthermore, we find that fluorophore-labelled

transferrin cannot access the pericentrosomal region of cells in which Rab11-FIP3 function has been perturbed. We find that this Rab11-FIP3 function appears to be specific, since expression of the equivalent Rab11-binding deficient mutant of RCP does not perturb ERC morphology. In addition, we find that other organelles such as sorting and late endosomes are unaffected by loss of Rab11-FIP3 function. Finally, we demonstrate the presence of an extensive coiled-coil region between residues 463 and 692 of Rab11-FIP3, which exists as a dimer in solution and is critical to support its function on the ERC. Together, these data indicate that Rab11-FIP3 is necessary for the structural integrity of the pericentrosomal ERC.

Karim, K., Taylor, J.D., Cullen, D.C., Swann M.J., Freeman N.J., **Measurement of Conformational Changes in the Structure of Transglutaminase on Binding Calcium Ions using Optical Evanescent Dual Polarisation Interferometry**, *Anal. Chem.*, (2007).

Claesson, P., Dedinaite, A., Mszros, R., and Varga I., **Interfacial Adsorption: Dual Polarisation Interferometry. In: Colloid Stability and Application in Pharmacy**, *Colloids and Interface Science*, Ed. Tharwat F Tadros, (2007), Vol. 3 pp 379-381.

Sonesson, A. W., Callisen, T.H., Brismar H., Elofsson, U.M, **U A comparison between Dual Polarisation Interferometry (DPI) and Surface Plasmon Resonance (SPR) for protein adsorption studies Colloids and Surfaces B**, *Biointerfaces*, (2007), 54(2) 236-240.

Keywords: Dual polarization interferometry; Surface plasmon resonance; Protein adsorption; Lipase; C18-surface

Abstract: This work was performed with the aim of comparing protein adsorption results obtained from the recently developed dual polarization interferometry (DPI) with the well-established surface plasmon resonance (SPR) technique. Both techniques use an evanescent field as the sensing element but completely different methods to calculate the adsorbed mass. As a test system we used adsorption of the lipase from *Thermomyces lanuginosus* (TLL) on C18 surfaces. The adsorbed amount calculated with both techniques is in good agreement, with both adsorption isotherms saturating at 1.30–1.35 mg/m<sup>2</sup> at TLL concentrations of 1000nM and above. Therefore, this supports the use of both SPR and DPI as tools for studying protein adsorption, which is very important when comparing adsorption data obtained from the use different techniques. Due to the spot sensing in SPR, this technique is recommended for initial kinetic studies, whereas DPI is more accurate when the refractive index and thickness of the adsorbed layer is of more interest.

Popplewell, J. Swann, M., Freeman, N., McDonnell C., Ford R., **Quantifying of the Effects of Melittin on Liposomes**, *Biochimica et Biophysica Acta*, (2007), 1768 13-20.

Keywords: Liposome; Phospholipid; Bilayer; Melittin; Lysis

Abstract: Melittin, the soluble peptide of bee venom, has been demonstrated to induce lysis of phospholipid liposomes. We have investigated the dependence of the lytic activity of melittin on lipid composition. The lysis of liposomes, measured by following their mass and dimensions when immobilised on a solid substrate, was close to zero when the negatively charged lipids phosphatidyl glycerol or phosphatidyl serine were used as the phospholipid component of the liposome. Whilst there was significant binding of melittin to the liposomes, there was little net change in their diameter with melittin binding reversed upon salt injection. For the zwitterionic phosphatidyl choline the lytic ability of melittin is dependent on the degree of acyl chain unsaturation, with melittin able to induce lysis of liposomes in the liquid crystalline state, whilst those in the gel state showed strong resistance to lysis. By directly measuring the dimensions and mass changes of liposomes on exposure to melittin using Dual Polarisation Interferometry, rather than following the fluorescence of entrapped dyes we attained further information about the initial stages of melittin binding to liposomes.

## 2006

Furutania, M., Tsujitaa, K., Itoha, T., Ijuina T., Takenawa, T., **Application of phosphoinositide-binding domains for the detection and quantification of specific phosphoinositides**, *Analytical Biochemistry*, (2006), 355, 1, 8-18.

Abstract: In mammals, seven phosphoinositides are known to play crucial roles as signaling molecules in a variety of cellular processes. Their synthesis and degradation are thought to be strictly controlled by metabolic enzymes such as phosphoinositide kinases and phosphatases, and their aberrant activities cause diseases. Thus, there is great interest in convenient and high-throughput measurement of such activities for the screening of drugs that enhance or block them. To date, radioactive labeling and colorimetric detection of released inorganic phosphates are mainly used to measure phosphoinositide kinase and phosphatase activities, respectively. Here, we describe a novel method for detecting and quantifying individual phosphoinositides via phosphoinositide-binding domains that exhibit high specificity and affinity toward this lipid. Enzyme-linked immunosorbent assay wells are modified with alkyl chains (C16), which enables more uniform and quantitative immobilization of phosphoinositide-containing

liposomes onto the well surfaces. Phosphoinositides, as the substrate or the product, are detected by pleckstrin homology domains that specifically bind to each phosphoinositide. By this method, phosphoinositide contents are measured with higher sensitivities than those by conventional methods. More importantly, both phosphoinositide kinase and phosphatase activities can be measured for purified enzymes and crude cellular lysates. This assay is easy, sensitive, and quantitative and thus may have a variety of applications in the development of diagnostic tests or the screening of therapeutic treatments for diseases such as cancer and diabetes which may be caused by abnormal phosphoinositide metabolism.

Lord M. S., Stenzel, M.H., Simmons, A., Milthorpe, B. K. **Lysozyme interaction with poly(HEMA)-based hydrogel.** *Biomaterials*, (2006), 27(8), 1341-1345.

Keywords: Hydrogels; Protein adsorption; Lysozyme; Contact lens

Abstract: Lysozyme interaction with an acrylic-based hydrogel, poly(2-hydroxyethyl methacrylate) co-methacrylic acid (P(HEMA-MAA)), was investigated using a combination of quartz crystal microbalance with dissipation (QCM-D), surface plasmon resonance (SPR) and dual polarisation interferometry (DPI). This combination of techniques demonstrated that lysozyme initially absorbed into the hydrogel matrix and displaced water from the hydrogel while subsequent lysozyme additions were adsorbed onto the surface of the hydrogel material. QCM-D, being sensitive to bound water, showed an overall decrease in mass and stiffening of the layer after lysozyme addition. SPR, a water insensitive technique, showed a net mass increase after addition of lysozyme and buffer rinses. DPI showed that the first exposure of lysozyme to P(HEMA-MAA) was consistent with lysozyme absorption while subsequent lysozyme exposures were consistent with lysozyme adsorption.

Yates, E., Terry, C., Rees, C., Rudd, T., Duchesne, T., Skidmore, M., Levy, R., Thanh, N., Nichols, R., Clarke, D., Fernig, D., **Protein-GAG Interactions: New Surface-based Techniques, Spectroscopies and Nanotechnology Probes**, *Biochem. Soc. Trans.*, (2006), 34 427-430.

Keywords: dual polarization interferometry, fibroblast growth factor, nanoparticle, protein-glycosaminoglycan interaction, quartz crystal microbalance-dissipation, synchrotron radiation CD spectroscopy.

Abstract: New approaches, rooted in the physical sciences, have been developed to gain a more fundamental understanding of protein-GAG (glycosaminoglycan) interactions. DPI (dual polarization interferometry) is an optical technique, which measures real-time changes in the mass of molecules bound at a surface and the geometry of the bound molecules. QCM-D (quartz crystal microbalance-dissipation), an acoustic technique, measures the mass and the viscoelastic properties of adsorbates. The FTIR (Fourier-transform IR) amide bands I, II and III, resulting from the peptide bond, provide insight into protein secondary structure. Synchrotron radiation CD goes to much shorter wavelengths than laboratory CD, allowing access to chromophores that provide insights into the conformation of the GAG chain and of  $\beta$ -strand structures of proteins. To tackle the diversity of GAG structure, we are developing noble metal nanoparticle probes, which can be detected at the level of single particles and so enable single molecule biochemistry and analytical chemistry. These new approaches are enabling new insights into structure-function relationships in GAGs and together they will resolve many of the outstanding problems in this field.

Ricard-Blum, S., Peel, L., Ruggerio, F., Freeman, N., **Dual Polarization Interferometry Characterization of Carbohydrate-Protein Interactions**, *Anal. Biochem.*, (2006), 352 252-259.

Keywords: Collagen V; Carbohydrate-protein interaction; Heparin binding; Protein-carbohydrate interaction; Dual polarization interferometry, Quantitative analysis

Abstract: Dual polarization interferometry (DPI) is an analytical technique that allows the simultaneous determination of thickness, density, and mass of a biological layer on a sensing waveguide surface in real time. The technique was applied to the analysis of carbohydrate-protein interactions. The selected system involved a 12-kDa recombinant fragment of collagen V (HepV) and heparin, a complex polysaccharide. Here we report on the analysis of thickness, density, and mass of surface structures obtained during the binding of HepV to heparin, which is a useful model compound for the sulfated, protein-binding regions of heparan sulfate. This system, which was initially studied for its biological relevance, displayed anomalous behavior in kinetic studies using surface plasmon resonance (SPR) assays that has been attributed to putative conformational changes. It was this putative conformational change that prompted us to investigate the binding using an alternative analytical approach. While using DPI to monitor binding events, a streptavidin layer (surface coverage  $2.105 \text{ ng mm}^{-2}$ ) was bound to the sensor surface (92% coverage), which captured  $0.105 \text{ ng mm}^{-2}$  of biotinylated heparin (a stoichiometric ratio of 1:6 heparin-streptavidin). The heparin inserted into the streptavidin layer but was still found to be capable of binding  $0.154 \text{ ng mm}^{-2}$  of HepV, which was also observed to insert into the streptavidin layer. This allowed the reliable calculation of the stoichiometric ratio for the HepV-heparin complex ( $\sim 1.7:1.0$ ), which has proved to be difficult to evaluate by SPR assays. Furthermore, real-time analysis of the heparin-HepV interaction by DPI suggested that there was some surface loss (probably of streptavidin) while the binding was occurring rather than the putative

conformational change that has been suggested on the basis of kinetic data alone. This gives further insight into the binding mechanism of HepV to heparin.

Lillis, B., Manning, M., Berney, H., Hurley, E., Mathewson, A., Sheehan, M., **Dual Polarisation Interferometry Characterisation of DNA Immobilisation and Hybridisation on a Silanised Support**, *Biosensors & Bioelectronics*, (2006), 21 1459-1467.

Keywords: Dual polarisation; Immobilisation; Hybridisation

Abstract: Dual polarisation interferometry is an analytical technique that allows the simultaneous determination of thickness, density and mass of a biological layer on a sensing waveguide surface in real time. We evaluated, for the first time, the ability of this technique to characterize the covalent immobilisation of single stranded probe DNA and the selective detection of target DNA hybridisation on a silanised support. Two immobilisation strategies have been evaluated: direct attachment of the probe molecule and a more complex chemistry employing a 1,2 homobifunctional crosslinker molecule. With this technique we demonstrate it was possible to determine probe orientation and measure probe coverage at different stages of the immobilisation process in real time and in a single experiment. In addition, by measuring simultaneously changes in thickness and density of the probe layer upon hybridisation of target DNA, it was possible to directly elucidate the impact that probe mobility had on hybridisation efficiency. Direct covalent attachment of an amine modified 19mer resulted in a thickness change of 0.68 nm that was consistent with multipoint attachment of the probe molecule to the surface. Blocking with BSA formed a dense layer of protein molecules that absorbed between the probe molecules on the surface. The observed hybridisation efficiency to target DNA was ~35%. No further significant reorientation of the probe molecule occurred upon hybridisation. The initial thickness of the probe layer upon attachment to the crosslinker molecule was 0.5 nm. Significant reorientation of the probe molecule surface normal occurred upon hybridisation to target DNA. This indicated that the probe molecule had greater mobility to hybridise to target DNA. The observed hybridisation efficiency for target DNA was ~85%. The results show that a probe molecule attached to the surface via a crosslinker group is better able to hybridise to target DNA due to its greater mobility.

Lin, S., Lee, C-K., Lin, Y-N., Lee, S-Y., Sheu, B-C., Tsai J-C., and Hsu, S-M., **Homopolyvalent Antibody-Antigen Interaction Kinetic Studies with the Use of a Dual Polarisation Interferometric Biosensor**, *Biosensors & Bioelectronics*, (2006), 22(5) 715-72.

Keywords: Dual-polarization interferometry; C-reactive protein; Binding constant; Affinity; Stoichiometry; Homopolyvalence

Abstract: We used dual-polarization interferometry (DPI) to study the interaction kinetics between a 'homopolyvalent' antigen (Ag) and a monoclonal antibody (Ab). A model system, which uses a monoclonal Ab against a homopentameric Ag, C-reactive protein (CRP), is presented with principle and experiments for the study of the interactions between an Ab and an Ag that has multiple identical epitopes. This allows evaluation of the dissociation constant (KD) and of the binding stoichiometry by DPI based on measurements of phase changes of Ab-Ag complexes in the transverse magnetic (TM) and transverse electric (TE) polarization modes. The average experimental value of KD found by the DPI technique for anti-CRP Ab was shown to be in close agreement with the value obtained by an indirect competition-enzyme-linked immunosorbent assay (ELISA). Moreover, the total number of Ab combining sites on the DPI sensor chip was calculated, and the binding stoichiometry of the surface Ag-Ab complex was obtained. This study illustrates the advantages of the DPI method in biosensing in its capacity for simultaneous evaluation of the thickness and refractive index (density, mass) of adsorbed layers. This allowed a comprehensive analysis of affinity reactions between an Ab having two binding sites and a multi-sited Ag.

Lin, S., Lee, C-K., Wang, Y-M., Huang, L-S., Lin, Y-H., Lee S-Y., Sheu, B-C., Hsu, S., **Measurement of dimensions of pentagonal doughnut-shaped C-reactive protein using an atomic force microscope and a dual polarisation interferometric biosensor**, *Biosensors & Bioelectronics*, (2006), 22(2) 323-7.

Keywords: Dual polarization interferometry; Atomic force microscopy; C-reactive protein

Abstract: In order to develop the C-reactive protein (CRP) sensor chips for clinical detection of atherosclerosis and coronary heart disease, we used an atomic force microscope (AFM) and a dual polarization interferometric (DPI) biosensor to probe the surface ultrastructure and to measure the dimensions of CRP. A single pentagonal structure was directly visualized by AFM, and quantitative measurements of the dimensions of the protein were provided. The average height calculated for each pentagonal CRP particle was approximately  $3.03 \pm 0.37$  nm, which basically corresponds to that ( $36^\circ$  A in protomer diameter) previously obtained from the structure of CRP determined by X-ray crystallography. Moreover, an experiment using dual polarization interferometric (DPI) as a biosensor was then performed, and the average monolayer thickness value ( $3.18 \pm 0.43$  nm) that was calculated basically corresponds to that obtained from the experimental value ( $3.03 \pm 0.37$  nm) of the height measured by an AFM method for CRP. Further investigations will be performed to study the surface ultrastructure of a single pentagonal CRP molecule, and for this purpose a CRP sample (at low concentration) was scanned in vacuum by AFM. The higher-resolution images clearly revealed the presence of doughnut-shaped CRP molecules. In addition, phase images of CRP

molecules were captured simultaneously with their height images, and the lateral dimensions of the doughnut-shaped CRP molecules were then measured. It was found that the average values calculated for the outer diameter ( $11.13 \pm 1.47$  nm) and pore diameter ( $3.52 \pm 0.42$  nm) are respectively close to those ( $102$  Å in outer diameter and  $30$  Å in pore diameter) previously obtained from the structure of CRP determined by X-ray crystallography. This study represents the first direct characterization of the surface ultrastructure and dimensional measurement of the CRP molecule on the sensor chip.

Terry, C. J., Popplewell, J. F., Swann, M. J., Freeman, N. J., Fernig, D.G., **Characterisation of Membrane mimetics on a dual polarisation interferometer**, *Biosensors & Bioelectronics*, (2006), 22(5) 627-32.

Keywords: Dual polarisation interferometry; Hybrid bilayer membranes

Abstract: Dual polarisation interferometry (DPI) has been used to characterise the formation of hybrid bilayer membranes (HBM) on a silicon-oxynitride surface. This technique allows the simultaneous determination of multiple physical properties of an HBM, as the HBM is being formed in a single experiment: mass, thickness in the z-direction (normal to the surface), tilt angle of the first layer and refractive index. Decanoic acid was covalently attached to an amine modified silicon-oxynitride sensor chip surface via 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride condensation reaction. The decanoic acid layer was  $0.92 \pm 0.12$  nm thick, indicating a tilt angle of  $57^\circ$  from surface normal, and possessed a mass of  $1.05 \pm 0.10$  ngmm<sup>-2</sup> and a refractive index (RI) of  $1.450 \pm 0.020$ . Phospholipid vesicles made from 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) or 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) were injected over the fatty acid surface to form an HBM. The DPPC HBM was  $4.32 \pm 0.68$  nm thick, with a total mass of  $3.18 \pm 0.60$  ngmm<sup>-2</sup> and a RI of  $1.404 \pm 0.007$ . The DMPC HBM was  $2.12 \pm 0.34$  nm thick, with a total mass of  $2.25 \pm 0.51$  ngmm<sup>-2</sup>, and a RI of  $1.435 \pm 0.007$ . DPI thus provides an insight into HBM formation and differences between the structural organisation of HBMs of different composition.

Poptoshev, E., and Claessen, P., **Adsorption of dimethyldodecylamine-N-oxide at the mica-solution interface studied by ellipsometry** *Colloids and Surfaces A., Physicochem Eng. Aspects*, (2006), 291(1-3) 45-50.

Keywords: Dimethyldodecylamine-N-oxide; Ellipsometry; Mica; Adsorption; Surface excess; Dual polarization interferometry

Abstract: The surface excess of dimethyldodecylamine-N-oxide (DDAO) surfactant at the mica-water interface has been determined using phase modulated ellipsometry. Surface excess versus bulk surfactant concentration isotherms were constructed at different pH values. The plateau adsorbed amount reaches a maximum in the pH range 6–7.5, which includes the native pH of DDAO solutions at the cmc (pH 7.5). At low pH values, where the surfactant is essentially cationic, the surface excess is reduced due to decrease of the mica surface charge, and due to that electrostatic repulsion between charged head groups prevents formation of tightly packed surfactant bilayers. Adsorption from solutions at pH 9 also renders a lower plateau surface excess than that obtained at neutral pH. In this case the surfactant-substrate affinity is greatly reduced due to complete deprotonation of the head group of the surfactant in bulk solution. The results suggest that the degree of protonation of DDAO in the adsorbed layer differs from its bulk value. Close to the negatively charged mica surface the protonation of the head group is increased in order to adjust its charge to that of the surface, whereas the uncharged form is expected to be enriched in the outer part of the bilayer.

Lord, M.S., **Biomolecular and Cellular Interactions with Surfaces**, PhD Thesis, University of New South Wales, 2006.

Thibault, G., Yudin, J., Wong, P., Tsitrin, V., Sprangers, R., Zhao R., and Houry, W. A., **Specificity in substrate and cofactor recognition by the N-terminal domain of the chaperone ClpX**, *Proc Natl Acad Sci U S A.*, (2006), 103(47) 17724-9.

Keywords: NMR \_ SspB \_ zinc-binding domain

Abstract: Clp ATPases are a unique group of ATP-dependent chaperones supporting targeted protein unfolding and degradation in concert with their respective proteases. ClpX is a representative member of these ATPases; it consists of two domains, a zinc-binding domain (ZBD) that forms dimers and a AAA+ ATP-binding domain that arranges into a hexamer. Analysis of the binding preferences of these two domains in ClpX revealed that both domains preferentially bind to hydrophobic residues but have different sequence preferences, with the AAA+ domain preferentially recognizing a wider range of specific sequences than ZBD. As part of this analysis, the binding site of the ClpX dimeric cofactor, SspB2, on ZBD in ClpX was determined by NMR and mutational analysis. The SspB C terminus was found to interact with a hydrophobic patch on the surface of ZBD. The affinity of SspB2 toward ZBD2 and the geometry of the SspB2-ZBD2 complex were investigated by using the newly developed quantitative optical biosensor method of dual polarization interferometry. The data suggest a model for the interaction between SspB2 and the ClpX hexamer.

Harish, A., **Nanomechanical cantilever systems for biological sensing**, PhD Thesis, University of Newcastle upon Tyne, 2006.

Suetsugu, S., Kurisu, S., Oikawa, T., Yamazaki, D., Oda A., and Takenawa, T., **Optimization of WAVE2 complex-induced actin polymerization by membrane-bound IRSp53, PIP<sub>3</sub>, and Rac**, *Journal of Cell Biology*, (2006), 173(4), 571-585.

Abstract: WAVE2 activates the actin-related protein (Arp) 2/3 complex for Rac-induced actin polymerization during lamellipodium formation and exists as a large WAVE2 protein complex with Sra1/PIR121, Nap1, Abi1, and HSPC300. IRSp53 binds to both Rac and Cdc42 and is proposed to link Rac to WAVE2. We found that the knockdown of IRSp53 by RNA interference decreased lamellipodium formation without a decrease in the amount of WAVE2 complex. Localization of WAVE2 at the cell periphery was retained in IRSp53 knockdown cells. Moreover, activated Cdc42 but not Rac weakened the association between WAVE2 and IRSp53. When we measured Arp2/3 activation in vitro, the WAVE2 complex isolated from the membrane fraction of cells was fully active in an IRSp53-dependent manner but WAVE2 isolated from the cytosol was not. Purified WAVE2 and purified WAVE2 complex were activated by IRSp53 in a Rac-dependent manner with PIP<sub>3</sub>-containing liposomes. Therefore, IRSp53 optimizes the activity of the WAVE2 complex in the presence of activated Rac and PIP<sub>3</sub>.

Tsujita, K., Suetsugu, S., Sasaki, N., Furutani, M., Oikawa, T., Takenawa, T., **Coordination between the actin cytoskeleton and membrane deformation by a novel membrane tubulation domain of PCH proteins is involved in endocytosis**, *The Journal of Cell Biology*, (2006), 172(2), 269-279.

Abstract: The conserved FER-CIP4 homology (FCH) domain is found in the pombe Cdc15 homology (PCH) protein family members, including formin-binding protein 17 (FBP17). However, the amino acid sequence homology extends beyond the FCH domain. We have termed this region the extended FC (EFC) domain. We found that FBP17 coordinated membrane deformation with actin cytoskeleton reorganization during endocytosis. The EFC domains of FBP17, CIP4, and other PCH protein family members show weak homology to the Bin-amphiphysin-Rvs (BAR) domain. The EFC domains bound strongly to phosphatidylserine and phosphatidylinositol 4,5-bisphosphate and deformed the plasma membrane and liposomes into narrow tubules.

Most PCH proteins possess an SH3 domain that is known to bind to dynamin and that recruited and activated neural Wiskott-Aldrich syndrome protein (N-WASP) at the plasma membrane. FBP17 and/or CIP4 contributed to the formation of the protein complex, including N-WASP and dynamin-2, in the early stage of endocytosis. Furthermore, knockdown of endogenous FBP17 and CIP4 impaired endocytosis. Our data indicate that PCH protein family members couple membrane deformation to actin cytoskeleton reorganization in various cellular processes.

Halthur, T., Claessen, P., Elofsson, U., **Immobilization of Enamel Matrix Derivate Protein onto Polypeptide Multilayers. Comparative in Situ Measurements Using Ellipsometry, Quartz Crystal Microbalance with Dissipation, and Dual-Polarization Interferometry**, *Langmuir*, (2006), 22 (26) 11065-71.

Abstract: The buildup of biodegradable poly(L-glutamic acid) (PGA) and poly(L-lysine) (PLL) multilayers on silica and titanium surfaces and the immobilization of enamel matrix derivate (EMD) protein was followed by utilizing in situ ellipsometry, quartz crystal microbalance with dissipation, and dual-polarization interferometry (DPI). The use of the relatively new DPI technique validated earlier published ellipsometry measurements of the PLL-PGA polypeptide films. The hydrophobic aggregating EMD protein was successfully immobilized both on top of and within the multilayer structures at pH 5.0. DPI measurements further indicated that the immobilization of EMD is influenced by the flow pattern during adsorption. The formed polypeptide-EMD multilayer films are of interest since it is known that EMD is able to trigger cell response and induce biomineralization. The multilayer films thus have potential to be useful as bioactive and biodegradable coatings for future dental implants.

Freeman, N., **Dual Polarisation Interferometry: An Optical Technique to Measure the Orientation and Structure of Proteins at the Solid-Liquid Interface in Real Time**, *Proteins at Solid-Liquid Interfaces*. Ed. Philippe Déjardin, (2006).

Chen, X., Pelton, R. **Pre-Adsorption of Amphiphilic Polymers on Synthetic Surfaces for Biofouling Retardation**, *Adv. Materials Research*, (2006), 11(12) 363-366.

Keywords: Surface treatment, Amphiphilic polymer, Protein adsorption

Abstract. Polystyrene (PS), polyethylene (PE), polypropylene (PP), glass and stainless steel were exposed to aqueous solutions of a series amphiphilic polymers at room temperature, including N-isopropylacrylamide (NIPAM)-based polymers, polyvinylpyrrolidone (PVP), polypropylene oxide (PPO)-polyethylene oxide (PEO) block copolymers and PEO. Dynamic contact angle measurements of the material surfaces before and after the treatment indicate that only NIPAM-based polymers can adsorb on both hydrophobic and hydrophilic surfaces. The

surface morphologies of the materials before and after polymer adsorption were investigated by profilometry. Protein adsorption on the surfaces pre-adsorbed NIPAM-based polymers was investigated by dual polarisation interferometry (DPI) and profilometry using lysozyme as the model protein. The results obtained indicate that NIPAM-based polymers can significantly improve the biofouling resistance of synthetic surfaces.

Suetsugu, S., Murayama, K., Sakamoto, A., Hanawa-Suetsugu, K., Seto, A., Oikawa, T., Mishima, C., Shirouzu, Tadaomi, M., Yokoyama, S., **The RAC Binding Domain/IRSp53-MIM Homology Domain of IRSp53 Induces RAC-dependent Membrane Deformation**, *J. Biol. Chem.*, (2006), 281(46), 35347-35358.

Abstract: The concave surface of the crescent-shaped Bin-amphiphysin- Rvs (BAR) domain is postulated to bind to the cell membrane to induce membrane deformation of a specific curvature. The Rac binding (RCB) domain/IRSp53-MIM homology domain (IMD) has a dimeric structure that is similar to the structure of the BAR domain; however, the RCB domain/IMD has a "zeppelin-shaped" dimer. Interestingly, the RCB domain/IMD of IRSp53 possesses Rac binding, membrane binding, and actin filament binding abilities. Here we report that the RCB domain/IMD of IRSp53 induces membrane deformation independent of the actin filaments in a Rac-dependent manner. In contrast to the BAR domain, the RCB domain/IMD did not cause long tubulation of the artificial liposomes; however, the Rac binding domain caused the formation of small buds on the liposomal surface. When expressed in cells, the Rac binding domain induced outward protrusion of the plasma membrane in a direction opposite to that induced by the BAR domain. Mapping of the amino acids responsible for membrane deformation suggests that the convex surface of the Rac binding domain binds to the membrane in a Rac-dependent manner, which may explain the mechanism of the membrane deformation induced by the RCB domain/IMD.

Yuji, O. K., Yoji, S., Tomoharu, M., Kota, K., Kazuo, K., **Metal Ion binding to Prion Protein**, *Biophysics*, (2006), 46(2), S439.

Abstract: Prion diseases are fatal neurodegenerative diseases caused by conformational transition of the cellular isoform of prion protein (PrP<sup>C</sup>) to the pathogenic scrapie isoform PrP<sup>Sc</sup>). Function of the PrP<sup>C</sup> is still not clear. But, it is known that the prion protein is a copper binding protein and it is suggested that PrP<sup>C</sup> is involved in the copper metabolism. We have applied Dual Polarisation Interferometry (DPI) to study the interaction between the recombinant mouse prion PrP<sup>C</sup> and nine different divalent metal ions. Mass, thickness and refractive index changes induced by the metal ion binding were observed. We have estimated the binding constants, association and dissociation rate constants, and molecular ratios of protein to metal ions. We found that copper has the highest affinity of PrP<sup>C</sup>, but some other metal ions also have a certain degree of affinity to this protein. This result suggests that PrP<sup>C</sup> may participate in the metabolism of not only copper but also some other divalent metal ions.

Yoji, Y., Toshifusa, T., **Studies of metal ion interactions with calmodulin using Dual Polarization Interferometry (DPI)**, *Biophysics*, (2006), 46(2), S271.

Abstract: A number of optical biosensors have been developed in rapid succession for the past decade. They have been adopted as a tool for drug discovery and interaction analysis in a wide area of biological researches. In the present study, calmodulin (CaM) interactions with metal ions were investigated using the dual polarisation interferometry (DPI), a novel technology of optical biosensor to measure thickness, refractive index and mass on a solid-liquid interface. CaM, a key protein in intracellular calcium signalling, was immobilised on the sensor surface in a ca. 1.4 nm layer. Subsequently, the CaM layer was subjected to interaction by exposing to micro-flow of metal ions at several concentrations. The metal ions used in this study were Na<sup>2+</sup>, Mg<sup>2+</sup>, Al<sup>3+</sup>, Ca<sup>2+</sup>, Mn<sup>2+</sup>, Fe<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>, Cd<sup>2+</sup>, Sn<sup>2+</sup> and Hg<sup>2+</sup>. In addition to Ca<sup>2+</sup>, as a result, Co<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup>, Cu<sup>2+</sup> and Cd<sup>2+</sup> showed strong interactions with CaM. When CaM interacted with Co<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup>, Cu<sup>2+</sup> and Cd<sup>2+</sup>, the thickness of the CaM layer was decreased and the refractive indices were increased, whereas Ca<sup>2+</sup> interaction showed increase in thickness and decrease in refractive index. This suggests that the conformational changes to CaM by Ca<sup>2+</sup> are different from those by other ions. The dissociation constant, association rate constant and dissociation rate constant of CaM with Ca<sup>2+</sup> were estimated to be 26 μM, 3.5 × 10<sup>3</sup> M<sup>-1</sup>s<sup>-1</sup> and 0.091 s<sup>-1</sup>, respectively. The present study proved the DPI technique to provide significant information about protein structures on metal ion interaction.

Lee, T. H., Aguilar, M.I., **Trends in the development and application of functional biomembrane surfaces**, *Biotechnology Annual Review*, (2006), Volume 12, pp 85-136.

Keywords: bilayer lipid membranes; supported lipid monolayer; tethered lipid bilayers; immobilised phospholipid monolayer; gel-entrapped liposomes; membrane microarray; nanoarray.

**2005**

Rudd, T., Skidmore, M.A., Yates, E. A., **Surface Based Studies of Heparin/Heparan Sulfate-Protein Interactions: Considerations for Surface Immobilisation of HS/Heparin Saccharides and Monitoring their**

**Interactions with Binding Proteins**, *Chemistry and Biology of Heparin and Heparan Sulfate*, (2005), eds., H.G. Garg, R.J Linhardt and C.A. Hales pp.345-366.

Lin, Y-H., Chen, J\_H., Wang, Y-M., Wang, A-B., Huang, L-S., Lee, C-K., Lin, S., **Dual Polarisation Interferometry for Measurement of Biomolecule at Nanometer Scale**, *Instruments Today*, (2005), 26(4), 55–62.

Abstract: From decade the application of surface plasmon resonance technique was broadly on molecular dynamics, however, Fairfield Sensor invented the new method, dual polarisation interferometry, which is used on analysis of changes of diameter, thickness, density, surface concentration, and dynamics on solid-liquid interface at real time. The application of dual polarisation interferometer includes single molecule structure and conformation change, interactions between molecules, nanomedicine diagnosis, and nano-technology etc. The invention of dual polarisation interferometer proves the study on molecular structure conformation change.

Daud, S, **Feasibility study of a Porin Based Logic Device**, PhD Thesis, University of Newcastle upon Tyne, 2005.

Halthur, T., **Multilayer Structures for Biomaterial Applications**, Doctoral Thesis in Chemistry, Royal Institute of Technology, Stockholm, Sweden (2005).

Popplewell, J., Freeman, N., Carrington, S., Ronan, G., McDonnell, C., Ford R. C., **Quantification of the effects of melittin on liposome structure**, *Biochem. Soc. Trans.*, (2005), 3 931–933.

Keywords: dual-polarization interferometry, lipid–peptide interaction, liposome structure, melittin, membrane mimic.

Abstract: An optical technique, dual-polarization interferometry, has been used to examine lipid structures at the solid/liquid interface. Changes in the lipid structures, in real time, were examined as a consequence of challenging them with a peptide (melittin) that is known to induce liposome rupture. This work suggests that it should be possible to obtain a better understanding of the detail of the melittin rupture process.

Toda, T., Nakamura, T., Yoshimi, Y., Yoshitani, N., Swann M., **Small Molecule – Protein Interactions Using the New Technique DPI**, *Practical Guide for Protein Structure and Function Analysis*, (2005), Chapter 2, pp.118-125.

West, D., Rees, C., Duchesne, L., Patey, S., Terry, C., Turnbull, J., Delehedde, M., Heegaard, C., Allain, F., Vanpouille, C., Ron, D., Fernig D, **Interactions of Multiple Heparin Binding Growth Factors with Neuropilin-1 and Potentiation of the Activity of Fibroblast Growth Factor-2**, *J. Biol. Chem.*, (2005), 280 13457-13464.

Abstract: The hypothesis that neuropilin-1 (Npn-1) may interact with heparin-binding proteins other than vascular endothelial growth factor has been tested using an optical biosensor-based binding assay. The results show that fibroblast growth factor (FGF) 1, 2, 4, and 7, FGF receptor 1, hepatocyte growth factor/scatter factor (HGF/SF), FGF binding protein, normal protease sensitive form of prion protein, antithrombin III, and Npn-1 itself are all able to interact with Npn-1 immobilized on the sensor surface. FGF-2, FGF-4, and HGF/SF are also shown to interact with Npn-1 in a solution assay. Moreover, these protein-protein interactions are dependent on the ionic strength of the medium and are inhibited by heparin, and the kinetics of binding of FGF-2, FGF-4 and HGF/SF to Npn-1 are characterized by fast association rate constants ( $270,000\text{--}1,600,000\text{ M}^{-1}\text{s}^{-1}$ ). These results suggest that Npn-1 possesses a “heparin” mimetic site that is able to interact at least in part through ionic bonding with the heparin binding site on many of the proteins studied. Npn-1 was also found to potentiate the growth stimulatory activity of FGF-2 on human umbilical vein endothelial cells, indicating that Npn-1 may not just bind but also regulate the activity of heparin-binding proteins.

Johnson, D., **Molecular Level Investigations of Coiled-Coil Proteins PhD Thesis**, University of Nottingham, 2005.

Berney, H., Oliver, K., **Dual Polarization Interferometry size and density characterisation of DNA Immobilisation and hybridization**, *Biosensor & Bioelectronics*, (2005), 21(4) 618-26.

Keywords: DNA immobilisation; Hybridisation efficiency; Persistence length; Direct real-time measurement; Dual polarization interferometry

Abstract: Investigation of nucleic acid interactions was performed using dual polarization interferometry, a novel approach to elucidating molecular interactions. This paper presents a preliminary study of adsorption of single stranded DNA onto functionalised silicon oxynitride, compared with covalent linkage, and avidin–biotin immobilisation. The effect of probe concentration on hybridisation efficiency was also examined. We found that increasing the electrolyte concentration resulted in a decrease of adsorbed DNA and that capture of a biotinylated duplex DNA on an adsorbed avidin layer resulted in four times fewer molecules per cm<sup>2</sup> than for duplex DNA covalently bound via an amine end terminal. The rate of thickness increase of a biotin probe layer on an adsorbed

avidin capture layer increased 10-fold when the probe concentration was increased from 0.1  $\mu$ M to 1  $\mu$ M. The close grafting density of the higher concentration probe meant that the immobilised probes were unavailable for hybridisation.

2004

J., Shukla, R., Popplewell, J.F., Swann, M.J., Freeman, N.J., Clark, J.F., Biehle, S. J., Carrozzella, **Apolipoprotein E Isoprotein Specific Interactions with Tissue Plasminogen Activator**, *Biochimica et Biophysica Acta – Molecular Basis of Disease*, (2004), 1689 244-251.

Keywords: Apolipoprotein E; Tissue plasminogen activator; Thrombolysis; Protein–protein interaction; Mechanism; Dual polarization interferometry

Abstract: Apolipoprotein E (Apo E) is an important genetic risk factor for multiple neurological, vascular and cardiovascular diseases. Previously, we reported Apo E isoprotein-specific modulation of tissue plasminogen activator (tPA) using an in vitro blood-clotting assay. Here, we studied the conformational changes of Apo E2, E3 and E4 in the presence of tPA and vice versa using circular dichroism (CD) and dual polarization interferometry (DPI). We report isoprotein and state-specific intermolecular interactions between the Apo E isoforms and tPA. Apo E2 interaction with immobilized tPA leads to significant conformational changes which are not observed with Apo E3 or E4. Additionally, tPA induces changes in helicity of lipidated Apo E2 whereas no detectable changes were observed in Apo E3 or E4. The Tukey's test for interaction indicated a significant ( $P < 0.001$ ) interaction between tPA and Apo E2 in the lipidated environment. These results may be important regarding the mechanism by which Apo E has isoprotein-specific effects on many biological processes and diseases involving blood clotting, proteolysis and perfusion.

Freeman, N. J., Peel, Swann, M.J., Cross, G.H., Reeves, A., Brand S., Lu, J.R. **Real Time, High Resolution Studies of Protein Adsorption and Structure at the Solid-Liquid Interface Using Dual Polarisation Interferometry**. *J. Phys.: Condens. Matter*, (2004), 16 S2493-S2496.

Abstract: A novel method for the analysis of thin biological films, called dual polarization interferometry (DPI), is described. This high resolution ( $<1 \text{ \AA}$ ), laboratory based technique allows the thickness and refractive index (density) of biological molecules adsorbing or reacting at the solid–liquid interface to be measured in real time (up to 10 measurements per second). Results from the adsorption of bovine serum albumin (BSA) on to a silicon oxynitride chip surface are presented to demonstrate how time dependent molecular behaviour can be examined using DPI. Mechanistic and structural information relating to the adsorption process is obtained as a function of the solution pH.

Armstrong, J., Salacinski, H.J., Mu, Q., Seifalian, A. M., Peel, L., Freeman, N., Holt, C.M, Lu J.R. **Interfacial Adsorption of Fibrinogen and its Inhibition by RGD Peptide: A Combined Physical Study**, *J. Phys.: Condens. Matter*, (2004), 16 S2483-S2491.

Abstract: The Arg–Gly–Asp (RGD) peptide sequence is known as a cell recognition site for numerous adhesive proteins present in the extracellular matrix (ECM) and in blood. Whilst surface immobilized RGD groups enhance cell attachment, RGD components present in solution can effectively inhibit cell attachment by competing with endogenous ligands for the same recognition site. In contrast to the widely reported binding to cell integrin, this study demonstrates a new RGD feature: its inhibitive effect on fibrinogen adsorption. Through a combined analysis of spectroscopic ellipsometry, neutron reflection and dual polarization interferometry, we show that the kinetic process of fibrinogen adsorption as a model pro-coagulant at the silica/solution interface and in the absence of any cells can be substantially reduced by the addition of RGD in solution and that the extent of the reduction is dependent on the relative concentration of RGD.

Lu, J.R., Swann, M. J., Peel, L.L., Freeman, N., J. **Lysozyme Adsorption Studies at the Silica-Water Interface using Dual Polarisation Interferometry**, *Langmuir*, (2004), 20 1827-1832.

Abstract: Lysozyme adsorption at the silica/water interface has been studied using a new analytical technique called dual polarization interferometry. This laboratory-based technique allows the build up or removal of molecular layers adsorbing or reacting on a lightly doped silicon dioxide (silica) surface to be measured in terms of thickness and refractive index changes with time. Lysozyme adsorption was studied at a range of concentrations from 0.03 to 4.0  $\text{g dm}^{-3}$  and at both pH 4 and pH 7. Adsorbed layers ranging from 14 to  $43 \pm 1 \text{ \AA}$  in thickness and 0.21 to 2.36 ( $0.05 \text{ mg m}^{-2}$ ) in mass coverage were observed at pH 4 with increasing lysozyme concentration, indicating a strong deformation of the monolayer over the low concentration range and the formation of an almost complete sideways-

on bilayer toward the high concentration of 4 g dm<sup>-3</sup>. At pH 7, the thickness of adsorbed layers varied from 16 to 54 ±1 Å with significantly higher surface coverage (0.74 to 3.29±0.05 mgm<sup>-2</sup>), again indicating structural deformation during the initial monolayer formation, followed by a gradual transition to bilayer adsorption over the high concentration end. The pH recycling performed at a fixed lysozyme concentration of 1.0 g dm<sup>-3</sup> indicated a broadly reversible adsorption regardless of whether the pH was cycled from pH 7 to pH 4 and back again or vice versa. These observations are in good agreement with earlier studies undertaken using neutron reflection although the fine details of molecular orientations in the layers differ subtly.

Swann, M. J., Peel, L.L., Carrington, S., Freeman N. J., **Dual Polarisation Interferometry: An Analytical Technique to Measure Changes in Protein Structure in Real Time, to Determine the Stoichiometry of Binding Events and to Differentiate Between Specific and Non-Specific Interaction**, *Analytical Biochemistry*, (2004), 329 190-198.

Abstract: The study of solution-phase interactions between small molecules and immobilized proteins is of intense interest, especially to the pharmaceutical industry. An optical sensing technique, dual polarization interferometry, has been employed for the detailed study of a model protein system, namely, D-biotin interactions with streptavidin immobilized on a solid surface. Changes in thickness and density of an immobilized streptavidin layer as a result of the binding of D-biotin have been directly measured in solution and in real time. The results obtained from this approach are in excellent agreement with X-ray crystallographic data for the structural changes expected in the streptavidin-D-biotin system. The mass changes measured on binding D-biotin also agree closely with anticipated binding capacity values. Determination of the density changes occurring in the protein adlayer provides a means for differentiation between specific and nonspecific interactions.

Oikawa, T., Yamaguchi, H., Itoh, T., Kato, M., Ijuin, T., Yamazaki, D., Suetsugu, S., Takenawa T., **PtdIns(3,4,5) P<sub>3</sub> Binding is Necessary for WAVE2-induced Formation of Lamellipodia**, *Nature Cell Biology*, (2004), 6 420-426.

Abstract: Polarized cell movement is triggered by the development of a PtdIns(3,4,5)P<sub>3</sub> gradient at the membrane, which is followed by rearrangement of the actin cytoskeleton. The WASP family verprolin homologous protein (WAVE) is essential for lamellipodium formation at the leading edge by activating the Arp2/3 complex downstream of Rac GTPase. Here, we report that WAVE2 binds to PtdIns(3,4,5)P<sub>3</sub> through its basic domain. The amino-terminal portion of WAVE2, which includes the PtdIns(3,4,5)P<sub>3</sub>-binding sequence, was localized at the leading edge of lamellipodia induced by an active form of Rac (RacDA) or by treatment with platelet derived growth factor (PDGF). Production of PtdIns(3,4,5)P<sub>3</sub> at the cell membrane by myristoylated phosphatidylinositol- 3-OH kinase (PI(3)K) is sufficient to recruit WAVE2 in the presence of dominant-negative Rac and latrunculin, demonstrating that PtdIns(3,4,5)P<sub>3</sub> alone is able to recruit WAVE2. Expression of a full-length mutant of WAVE2 that lacks the lipid-binding activity inhibited proper formation of lamellipodia induced by RacDA. These results suggest that one of the products of PI(3)K, PtdIns(3,4,5)P<sub>3</sub>, recruits WAVE2 to the polarized membrane and that this recruitment is essential for lamellipodium formation at the leading edge.

Cross, G. H., Reeves, A., Brand, S., Swann, M. J., Peel, L., Freeman, N.J., Lu, J.R., **The Metrics of Surface Adsorbed Small Molecules on the Young's Fringe Dual-Slab Waveguide Interferometer**, *J. Phys. D: Appl. Phys.*, (2004), 37 74-80.

Abstract: A method for analysing thin films using a dual-waveguide interferometric technique is described. Alternate dual polarization addressing of the interferometer sensor using a ferroelectric liquid crystal polarization switch allowed the opto-geometrical properties (density and thickness) of adsorbed layers at a solid-liquid interface to be determined. Differences in the waveguide mode dispersion between the transverse electric and transverse magnetic modes allowed unique combinations of layer thickness and refractive index to be determined at all stages of the layer formation process. The technique has been verified by comparing the analysis of the surface adsorption of surfactants with data obtained using neutron scattering techniques, observing their behaviour on trimethylsilane coated silicon oxynitride surfaces. The data obtained were found to be in excellent agreement with analogous neutron scattering experiments and the precision of the measurements taken to be of the order of 40 pm with respect to adsorbed layer thicknesses. The study was extended to a series of surfactants whose layer morphology could be correlated with their hydrophilicity/lipophilicity balance. Those in the series with longer alkyl chains were observed to form thinner, denser layers at the hydrophobic solid/aqueous liquid interface and the degree of order attained at sub-critical micelle concentrations to be correlated with molecular fluidity. The technique is expected to find utility with those interested in thin film analysis. An important and growing area of application is within the life sciences, especially in the field of protein structure and function.

Brennan, D., O'Brien, P., O'Brien, J., Freeman, N., Swann, M., **Development and test of an integrated Microsystem for HPLC separation and detection using Refractive index measurements**, *Sensors & Actuators*, (2004), 103 184-189.

Keywords: Micro-fluidics; Micro-HPLC; Packaging; Refractive index

Abstract: In recent years optical waveguide sensors have been used for applications as biosensors and chemical sensors. Often the fluidic element of the sensor has a large working volume (L) and full device integration is not undertaken. We look at a number of fabrication routes available to integrate microfluidic and optical detection components to achieve a fully integrated microsystem and highlight some of the technical issues arising in different integration strategies. Wafer and chip integration strategies using anodic, thin film and polymer bonding for the fluidic module and the optical sensor were investigated. The integrated microsystem was evaluated for fluidic and optical performance, integration showed no significant impact on performance of either component. The fluidic system presents a nanolitre detection volume offering reduced sample volumes in HPLC applications. Initial optical performance of the integrated structure was carried out using a refractive index measurement system, the Farfield Analight@Bio250.

Reeves, A., **Theoretical Studies of One-dimensional and Two-dimensional Photonic Structures**, PhD Thesis, University of Durham, (2004).

Freeman, N., **From microstructures to measurements in the pharmaceutical industry**, *The IEE Seminar on MNT in Medicine*, (2004), 2004/10743, 79-92.

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Toshifusa, T., Rosser D, Nakamura, M., Swann, M.J. **Real-time detection of conformational changes in calmodulin on oxidation and calcium binding by dual polarization interferometry**, *Nippon Bunshi Seibutsu Gakkai Nenkai Puroguramu*, (2003), Koen Yoshishu, 26 447.

Swann, M.J., Freeman, N.J., Carrington, S., Ronan, G., Barrett, P. **Quantifying Structural Changes and Stoichiometry of Protein Interactions Using Size and Density Profiling**, *Letters in Peptide Science*, (2003), 10 487-494.

Alternative reference

Swann, M., Freeman, N., Carrington, S., Ronan, G., Barrett, P., **Quantifying structural changes and stoichiometry of protein interactions using size and density profiling**, *International Journal of Peptide Research and Therapeutics*, (2003), 10 (5-6) 487-494.

Koopmann, J. O., Blackburn, J., **High Affinity Capture Surface for Matrix-Assisted Laser Desorption/Ionisation Compatible Protein Microarrays**, *Rapid Commun. Mass Spectrom.*, (2003), 17 455-462.

Abstract: A surface for the capture of biotin-tagged proteins on matrix-assisted laser desorption/ionization (MALDI) targets has been investigated. Binding of a poly-L-lysine poly(ethylene glycol)-biotin polymer to glass and gold surfaces has been demonstrated using dual wavelength interferometry. Biotinylated proteins were captured onto this surface using tetrameric neutravidin as a multivalent bridging molecule. Biotin tagging of proteins was achieved by chemical biotinylation or by expressing a protein with a biotinylation consensus sequence in *E. coli*. The specificity of the surface for biotin-tagged proteins allowed the purification of biotin-tagged glutathione-S-transferase from a bacterial lysate directly onto a MALDI target. Subsequently, the protein was digested on the MALDI target and a protein fingerprint analysis confirmed its presence directly, but no *E. coli* proteins were detected. Therefore, we conclude that this surface is highly specific for the capture of biotin-labelled proteins and has low non-specific binding properties for non-biotinylated proteins. Furthermore, protein-protein interactions using biotinylated lectins were investigated, and the selective capture of the glycoprotein fetuin with wheat germ agglutinin was demonstrated. Also, immobilised *Arachis hypogea* agglutinin recognised a minor asialo component of this glycoprotein on the array. The high affinity immobilisation of proteins onto this surface allowed effective desalting procedures to be used which improved the desorption of high molecular weight proteins. Another aspect of this surface is that a highly ordered coupling of the analyte can be achieved which eliminates the search for the sweet spot and allows the creation of densely packed protein microarrays for use in mass spectrometry.

Cross, G.H., Brand, A. S., Popplewell, J. F., Peel, L. L., Swann, M.J., Freeman N. J., **A New Quantitative Optical Biosensor for Protein Characterisation**, *Biosensors and Bioelectronics*, (2003), 19 383-390.

Keywords: Optical biosensor; Protein characterisation; X-ray crystallography

Abstract: A new optical biosensor is described based on a dual waveguide interferometric technique. By addressing the waveguide structure with alternate polarisations the optogeometrical properties (density and thickness) of adsorbed protein layers at the sensor (solid)-liquid interface have been determined. Differences in the waveguide mode dispersion between the transverse electric (TE) and transverse magnetic (TM) modes allow unique solutions for adlayer thickness and refractive index to be determined at all stages during the formation process. The technique has been verified using standard protein systems and by comparing the data with published work using X-ray crystallography and neutron reflection techniques. The data obtained was found to be

in excellent agreement with previously reported X-ray experiments given that typical film thicknesses for streptavidin layers were in the range 5.5\_ 6.5 nm compared with the short axis crystal structure of between 4.8 and 5.6 nm. The precision of the measurements taken was of the order of 40 pm with respect to adsorbed adlayer thicknesses. This biosensor approach provides measurements of both thickness and density of adlayers to a high precision, simultaneously and in real time enabling detail of the structure and function of proteins to be elucidated. From such data it is possible to obtain information on the orientation, distortion and efficiency of immobilisation procedures as well as the interaction event of interest. The technique is expected to find utility with those interested in protein structure and function. This is an area of growing importance within the life sciences as the demand for quantitative analytical techniques increases with the growth in 'proteomics'.

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Cross, G.H., Ren Y., Swann, M.J., **Refractometric Discrimination of Void-Space Filling and Swelling During Vapour Sorption in Polymer Films**, *The Analyst*, (2000), 125 2173-2175.

Abstract: Thin polymeric films have been deposited as upper cladding layers on a new integrated optical interferometer fabricated from layers of silicon oxynitride on a silicon wafer. The evanescent field of the probing waveguide mode transduces refractive index changes in the polymer layer into the measured phase changes in the device. Real-time measurement of index change and its sign is obtained. Upon exposure to humid air, we record water sorption by films of poly(vinyl pyrrolidone) by a rapid positive index change for void-space filling followed by a slow negative index change for swelling. Sorption of water vapor into a thin film of the viscous liquid polymer polyethylenimine shows only swelling mode behaviour and a simple constitutive model can be applied to give the fractional water occupied volume.

Cross, G.H., Ren, Y., Freeman, N.J., **Young's Fringes from Vertically Integrated Slab Waveguides: Applications to Humidity Sensing**, *Applied Physics*, (1999), 86 6483-6499.

Abstract: Using a multiple layer optical waveguide system consisting of two vertically slab waveguides, classical Young's fringes may be obtained in the far-field diffraction plane. In agreement with the simple theory of diffraction interference the spacing of the far-field fringes is easily observed on mm to cm dimensions without further transformation of the output light. The simple methods of fabrication and means of optical coupling should provide a readily adaptable method for examining the principles of interferometry in an integrated optical format. The structure acts to transform polarized incident plane wave input light into separate slab modes of the device which emerge as two closely spaced and coherent sources at the output. The elements required for a classical Young's fringe demonstration are therefore all embodied in this approach. The basic concept can be applied to an optical method for sensing. In one example of this we demonstrate measurement of the phase difference induced between the upper and lower propagating modes in structures due to water vapor diffusion into the layers which are formed from hydrophilic polymers. The Young's fringe patterns exhibit a spatial intensity distribution which is sensitive to water vapor introduced over the surface of the structure. Differences in the effective index between the modes of the two waveguides during the diffusion of the vapor causes phase shifts which result in redistribution in the fringe pattern. The anticipated limit of detection of these devices is lower than 1 ppm for water vapor.