



**ANALYTICAL
CHEMISTRY**
TRUST FUND

**RSC / EPSRC Analytical Science Studentships
Summary Reports 2010**

These annual summary reports are published by permission of the supervisors of the projects listed below. Many other supervisors have withheld permission to publish.

PROJECT: The Development of Nanoporous Metallised Membranes for Analytical Separations

SUPERVISOR: Professor Colin Boxall, Lancaster University

STUDENT: Mr Michael Bromley

Fast, controllable and selective separation of metal ions from complex solutions plays a key role in a wide range of activities including analytical separation in the nuclear industry. Novel, surface-metallised, ion-selective membranes are being developed for use in the separation and preparation of pure isolates of a range of metal ions prior to quantitative analysis.

Specific metal ions can be separated using commercially available PVDF ion-selective membranes with a supported ligand. The ligand is chosen to have an affinity for the target ion and hence facilitates transport across the separation membrane.

This project aims to develop enhanced ion-selective membranes via surface metallisation i.e. the fabrication of in-situ electrodes on the membrane surfaces. Metallisation of ion-selective membranes will allow electrochemical control of the oxidation state of target ions in complex

solutions and a ligand is chosen with an affinity for this oxidation state. The combination of this increased ligand specificity and an electrochemically tunable system offers increased separation rates and selectivity over conventional supported ligand membranes with time and cost benefits. The focus of the project has been to develop the required production techniques for making nanoporous metallised membranes. These techniques include a novel method of depositing metal onto an insulating surface and a process of inducing ordered porosity into the deposited metal. These techniques have been first developed on glass substrates before being transferred to PVDF membrane surfaces.

PROJECT: Quantitative Nanosensing of Multiple Protein Interactions in Real Time

SUPERVISOR: Professor Duncan Graham, University of Strathclyde

STUDENT: Mr Derek Craig

The study of the human genome has yielded few results which allow a further understanding of how biomolecules interact with biological systems in various disease states. Proteomics is the study of every protein within an organism and how these proteins interact and change dependent on the biological system in which they are found. Information gained from the genome and proteome, which highlight specific protein interactions with certain disease states, can be used to define new drug targets.

In recent times a great interest has grown into how the interactions of biomolecules such as proteins and DNA can be detected. This has led to the revolution of detection science, resulting in a growing number of techniques becoming available, which can follow these interactions.

SER(R)S is one of the leading techniques in this field due to the ease of which biomolecules can be conjugated to SER(R)S active analytes. These biomolecules become conjugated to SER(R)S active surfaces via linker groups which are bound to the surface of the enhancing material.

SER(R)S has been used for detection in a wide range of Immunoassays allowing real time detection of the interaction between proteins such as antibodies and antigens.

This project is focused on the synthesis of novel linker group and nanoparticle conjugates to be used in a detection assay for three cardiac biomarkers.

PROJECT: Nanosensing of Multiple Protein Interactions in Real Time.

SUPERVISOR: Professor Duncan Graham. University of Strathclyde

STUDENT: Ms Jane Gallagher

In recent years much effort has focused on the understanding of biological systems in order to gain insight of certain disease states. This has been achieved through research of the genome. Although the field of genomics had been fundamental in advancing this area it is now becoming apparent that further information is required.

Proteomics is advancing as a complementary field to genomics. As proteins are the main components of metabolic pathways in cells, interest within this area is vast. Currently there are many challenges in this area, in terms of identity of the protein interacting, distances at which specific proteins interact and the location of the proteins within a single cell. It is the aim of this project to address these issues by investigating a new approach for the analysis of protein interactions. It is hoped this can be achieved by labelling of proteins via nanoparticles and through the use of SERRS.

In order to identify multiple protein interactions coded nanoparticles have to be used. This involves the synthesis of various dyes which can be used for multiplexing. Several dyes have been investigated and proven to have great multiplexing abilities. Work has focussed upon synthesising a dye capable of conjugation to nanoparticles. This has been achieved on a few occasions, however, further reactions have been unsuccessful and conjugation to nanoparticles has yet to be achieved. One of the main issues in the synthesis of these dyes is the low yields in the final steps. Therefore, various methods have been investigated and employed. One of these methods has been solid phase synthesis which has so far provided a higher rate of success. However, conjugation to nanoparticles is ongoing. Also, we have started looking at the detection of fluorescent proteins using SERS in an unlabelled situation. As these fluorescent proteins are important biomarkers within cellular biology, the ability to detect them using SERS could introduce this technique into the cellular biology field.

PROJECT: Investigation of Protein Functionalized Nanoparticles as a New Approach to Proteomics

SUPERVISOR: Professor Duncan Graham, University of Strathclyde

STUDENT: Leanne Gibson

The detection of proteins and their interactions with other species including proteins are of much interest to scientists in order to gain further insight into disease state, drug design and biological pathways.

The interaction of protein A and protein G with an IgG antibody is well established and is commonly studied but immunoassays. However, this work shows how these proteins have been interacted with each other by conjugation via a polyethylene glycol (PEG) linker to metallic nanoparticles. Protein A binds to the IgG antibody via interaction with its Fc region. Protein G has also been used as it can interact with the Fc and Fab regions of an IgG antibody.

The use of functionalised nanoparticles is becoming a popular route for labeling of biomolecules, disease detection and diagnostics. When nanoparticles aggregate, through interaction of biomolecules, a colour change is observed and this can also be detected by optical techniques such as UV – Visible Spectroscopy and Surface Enhanced Resonance Raman Spectroscopy, SERRS.

In UV – Visible spectroscopy, aggregation of these nanoparticles causes a bathochromic shift accompanied by a colour change from red to blue when using gold nanoparticles. In SERRS, aggregation of the nanoparticles through a protein interaction causes an increase in signal intensity allowing a lower concentration of analytes to be detected. SERRS can, therefore, offer better sensitivity as an analytical technique.

So far, work has concentrated primarily on the interaction of these two proteins. However, future work will include applying this concept to other protein systems such as streptavidin-biotin interactions.

PROJECT: Production of Nano-Arrays of Proteins to Study Cellular Interactions using Dip-pen Nanolithography and SERRS

SUPERVISOR: Professor Duncan Graham, University of Strathclyde

STUDENT: Ms Stacey Laing

Since proteins are the main functional output of the cell, and because of their dynamic nature, proteomic-based analysis can give a vast insight into biological processes. Through the study of protein expression, protein interactions and the detection and quantification of proteins in biological fluids, we can develop biomarkers for early disease diagnosis, gain an understanding of disease processes, and improve drug development. However, in order to achieve this, we require the development of sensitive and high-throughput techniques.

Resonance Raman spectroscopy (RRS) is a sensitive technique which provides unique vibrational fingerprints in the form of sharp, well-resolved bands. It is capable of selectively detecting analytes from complex mixtures such as biological samples and offers the possibility of multiplexed analysis. Dip-pen nanolithography (DPN) is a highly scalable method of producing high density nano- and microarrays. This project combines the two techniques to allow sensitive and high-throughput analysis of proteins.

PROJECT: SERRS active nanoparticles as versatile reagents for quantitative bioanalysis

SUPERVISOR: Professor Duncan Graham, University of Strathclyde

STUDENT: Ms Louise Rocks

In recent years there has been an increase in demand for real-time detection of specific biological species and interactions within a meaningful environment. Fluorescence has been the leader in tagging technology for many years. Quantum dots (QD) have been pivotal in advancing the sensitivity and photostability of biological detection systems. Metallic nanoparticles provide an alternative spectroscopic substrate for the *in vivo* detection and imaging of biological targets. Utilising Surface Enhanced Raman Scattering (SERS) from metallic nanoparticles provides narrower bands of information rich spectral output. Sensitivity of this detection system can be further increased by combination with resonance Raman scattering resulting in Surface Enhanced Resonance Raman Scattering (SERRS). The use of both QD and metallic nanoparticles for *in vivo detection* is hindered by both non specific adsorption and uncontrolled aggregation. Silica encapsulation of nanoparticles should afford a biocompatible, robust substrate with consistent surface chemistry.

PROJECT: Investigation of Immune-genomic Sensing using Functionalised

Nanoparticles

SUPERVISOR: Professor Duncan Graham, University of Strathclyde

STUDENT: Ms Sarah Rooney

A study carried out by the Global Burden of Disease Study (GBD) in 1996 ranked chronic obstructive pulmonary disease (COPD) as the 12th leading cause of chronic morbidity and mortality worldwide, and projected it to be ranked as high as 5th by the year 2020. Environmental factors are the most common causes of development of COPD and emphysema, however, a study carried out in 1963 looking at genetic factors distinguished alpha-1-antitrypsin (A1AT) deficiency as a hereditary condition which can lead to these types of lung diseases.

If an individual is suspected of being deficient in A1AT, both quantitative and qualitative tests can be carried out to confirm that this is the case. Qualitative tests include alpha-1-antitrypsin phenotype test which separates out the different types of A1AT proteins produced and then compares them to known patterns, and alpha-1-antitrypsin DNA testing which is a genetic test that is done to identify which protease inhibitor gene mutations on the gene alleles that are present. This type of test is carried out to help evaluate people with the disease and their family members. Quantitative tests can also be carried out such as rocket immunoelectrophoresis, radial immunodiffusion and nephelometry can be used to screen plasma levels of A1AT.

Lateral flow platforms were developed by BBInternational in order to detect the A1AT antigen. A secondary A1AT antibody is conjugated to a nanoparticle via a dye linker that is SERRS active. Once a positive result has been obtained (in the form of a colour change on the test line) the lateral flow platform can be further analysed using SERRS. With the use of SERRS detection of the antigen should be able to be obtained as low as nanomolar concentrations.

PROJECT: UltraDots : Stimuli-Responsive Analytical Sensor Capsules

SUPERVISOR: Professor Lisa Hall, University of Cambridge

STUDENT: Mr Jamie Walters

Ultradots are delivery vehicles that carry analytical response capability and can be stimulated to deliver a payload. These vehicles are fabricated through a sacrificial template self-assembly mechanism, and comprise of a hollow core with a mesoporous organosilica shell. These vehicles, UltraDot microcapsules, are sensitive to ultrasound (US). Upon insonation at low acoustic pressures, the capsules resonate non-linearly. This results in a large backscatter

echo, which allows for their visualisation using conventional US imaging equipment. As the pressure increases to a critical acoustic pressure, the amplitude of rarefaction is large and the capsule ruptures.

In addition to this, a method of preferentially assembling analyte responsive agents within the capsule shell has been developed. The capsules can be interrogated optically. As a consequence of the analyte responsive agent's localisation within a thin capsule shell, the analyte diffusion length is significantly smaller comparative with similar sized particle sensors. The response time is thus two orders of magnitude faster than any published sensor particle of similar diameter. UltraDots provide a unique system capable of both localised detection of selected analytes and ultrasound controlled delivery of functional materials such as drugs and antibiotics. This research holds promise in a range of applications from drug delivery and microreactors to sensing technologies.

PROJECT: Novel microanalytical tools for malaria research

SUPERVISOR: Dr Clemens Kaminski, University of Cambridge

STUDENT: Mr Jakob Mauritz

Noel microscopy tools were developed to study malaria infected red blood cells. Using a battery of state of the art analytical imaging tools we could track morphology, elasticity and protein species content of infected red blood cells during the intraerythrocytic cycle of malaria falciparum infected red blood cells. These novel measurement techniques allowed for the first time the experimental validation of a key hypothesis in malaria research, the colloidosmotic hypothesis providing a mechanistic model for the unexpected stability of red blood cell throughout the time course of the intraerythrocytic cycle. The results led to publication of 6 articles in high impact journals, including Biophysics Journal, PLoS Computational Biology, PLoS One, and Journal of Biomedical Optics and 2 more articles are being prepared for submission.

PROJECT: Imaging Mass Spectrometry in the Life Sciences

SUPERVISOR: Professor Cameron McLeod, University of Sheffield

STUDENT: Mr John Pugh

John Pugh has made very significant contributions to the emerging field of imaging (elemental) mass spectrometry. Main activities concerned methodological studies on laser ablation of biological tissue together with imaging of MRI contrast agents (Gd, Mn) and metallotherapeutics (Pt, Gd) in microtomed sections. The latter work involved close collaboration with MRI imaging groups and medical scientists (IC/Royal Hammersmith Hospital, UCL and Bristol University) and the complementary value of elemental imaging in pre-clinical MRI research was clearly demonstrated eg improved sensitivity/resolution, signal quantitation and ability to establish ultimate fate/spatial location of administered agents. It is projected that these groundbreaking studies will stimulate new interest in the field.

PROJECT: Chemometric Analysis of Metabolic Spectral Profiles

SUPERVISOR: Professor Elaine Holmes, Imperial College

STUDENT: Ms Judith Fonville

The interrogation of metabolic information is crucial to understanding the functioning of a biological system. Analytical chemistry methods can be used to acquire spectroscopic profiles of biofluids containing latent information about the metabolic state. Nuclear magnetic resonance spectroscopy and mass spectrometry techniques generate vast amounts of highly complex data, and ways to analyse and interpret these data successfully are investigated. The evaluation of *J*-resolved spectroscopy in magnetic resonance profiling and the statistical techniques required to extract maximum information from the projections of these spectra are discussed. In particular, correlation and regression methods are investigated with respect to their ability to enhance model interpretation, and the effects of several pre-processing techniques are evaluated. It is shown that non-linearities of metabolic spectra and the response being studied can be effectively modelled with kernel orthogonal partial least squares, for which an automated optimisation was implemented. Finally, the enormous amount of data generated with mass spectrometry imaging has been investigated in terms of processing, and the advantages of applying multivariate chemometric techniques to these data have been illustrated, especially in terms of interpretation and visualisation.

This body of work therefore demonstrates new means of increasing the amount of obtainable information from metabonomic techniques. Approaches to extract a maximum amount of useful data from spectral profiling of a given set of samples in biological applications are detailed, by

investigating various analytical and statistical methods in a wide variety of metabolic spectroscopic profiling studies.

PROJECT: MALDI-Mass Spectrometry Imaging of Tissue Sections for Spatial Metabolic Profiling

SUPERVISOR: Professor Elaine Holmes, Imperial College

STUDENT: Mr Panagiotis Vorkas

Mass Spectrometry Imaging (MSI) has gradually been introduced as a technique for profiling of a wide molecular weight range of compounds, but by providing, in addition to molecular information, the spatial location of each spectrum or distribution of metabolites. One of the techniques used to a large extent is MALDI-MSI. MALDI-MSI offers the advantages of reduced fragmentation, wide m/z range of application, and the ease of use of the MALDI-TOF instrumentation. Consequently, applications of MALDI-MSI have recently been employed for metabolic profiling studies. In the present study the capabilities of MALDI-MSI for metabolic profiling studies is assessed.

Additionally, several data analysis methods have been applied to MSI data and adjusted in order to cover the additional requirements of metabolic profiling in a spatial mode. Therefore, the capabilities of two data analysis methods, namely Principal Component Analysis (PCA), and Hierarchical Clustering Analysis (HCA) have also been assessed.

For the development of methodology for MALDI-MSI analysis a section obtained from a calcified human aortic valve was used. Sectioning and mounting the tissue on a microscopy glass slide, was followed by application of α -cyano-4-hydroxy-cinnapinic acid as a matrix. Spectra were acquired using an API, Q-Star, Pulsar-*i* (Applied Biosystems). The tissue section was then subjected to Haematoxylin and Eosin staining.

Results from the MSI experiment were subjected to PCA and HCA. For this, new algorithms and codes were developed, using Matlab programming language, in order to represent data analysis results in a spatial mode. Additionally, a code to exclude matrix spectra from data analysis was also developed.

In conclusion, MALDI-MSI analysis provided additional information to the standard applied metabolic profiling techniques. Moreover, the results emphasized the importance of data analysis tools, and the value of applying such tools in order to assist in obtaining comprehensive results.

DAF 08.11.10