



## MOLECULAR MEDICINE IRELAND COURSES & WORKSHOPS

### TECHNIQUES & STRATEGIES IN MOLECULAR MEDICINE

11-12 & 15-16 Dec 2008 (0930-1300 each day)

Venue: Joly Lecture Theatre, Hamilton Building, TCD, Main Campus

This course, running over four mornings, is designed to give bioscientists and clinicians a broad overview of research techniques and their application. Basic molecular biology laboratory experience is assumed, but you should not need prior knowledge of the techniques covered in the course.

#### Day 1 (Thurs 11 Dec; 0930-1300)

*Session Chair: Dr Ross McManus (TCD)*

- 0930      **RNA Detection and quantitation**  
            Dr Shane Duggan, TCD
- 1015      **Differential gene expression: overview of relevant methods**  
            Prof William Gallagher, UCD
- 1100      Coffee/Tea
- 1130      **Polymorphism association with disease**  
            Prof. Denis Shields, UCD
- 1215      **DNA cloning strategies**  
            Dr Ross McManus, TCD

#### Day 2 (Fri 12 Dec; 0930-1300)

*Session Chair: Dr Niamh Moran (RCSI)*

- 0930      **Model organisms**  
            Dr Breandán Kennedy, UCD
- 1015      **RNA interference**  
            Dr Jane Farrar, TCD
- 1100      Coffee/Tea
- 1130      **Protein expression and purification**  
            Dr Henry Windle, TCD
- 1215      **Determining protein: protein interactions in biology**  
            Dr Niamh Moran, RCSI



**Day 3 (Mon 15 Dec; 0930-1315)**

*Session Chair: Dr Leonie Young (RCSI)*

- 0930 **Cell imaging and sorting – flow cytometry**  
Prof. William Watson, UCD
- 1015 **Introduction to Mass Spectrometry**  
Dr Giuliano Elia, UCD
- 1045 Coffee/Tea
- 1100 **Expression proteomics: biomedical applications**  
Prof. Steve Pennington, UCD
- 1145 **Immunodetection methods on cell and tissue extracts**  
Dr Leonie Young, RCSI
- 1230 **Transgenics and knockouts**  
Dr Rosemary Kane, UCD

**Day 4 (Tues 16 Dec; 0930-1300)**

*Session Chair: TBC*

- 0930 **Laser Capture Microdissection and in situ hybridisation**  
Dr Orla Sheils, TCD
- 1015 **High Content Analysis of nanoparticle/cell interactions**  
Dr Yuri Volkov, TCD
- 1100 Coffee/Tea
- 1130 **Stem Cells - Biology and Applications**  
Dr Linda Howard, NUI Galway
- 1215 **Medical Systems Biology - The case of the cancer cell not able to die**  
Prof. Jochen Prehn, RCSI

## Abstracts

### Day 1

#### **RNA Detection and quantitation**

Dr Shane Duggan (Institute of Molecular Medicine, TCD)

The protein components of the cell are derived by numerous processes indirectly interpreted from a genetic element known as the “gene” which is coded in the cellular DNA. This element is interpreted by the cell in a process called “transcription” where the genetic code for a particular gene is converted into a molecular code known as messenger RNA (mRNA). This mRNA molecule can now be utilised in the creation of a new protein via the translation process. In this lecture the nature and analysis of Ribonucleic acid (RNA) in biological systems will be explored. The understanding of this has allowed the laboratory scientist to interrogate and explore gene expression as it may relate to diseases or cell signalling. Extraction and quantitation of good quality RNA will be discussed as they are the first step in any investigation of gene expression. Standard techniques in common use such as Northern blotting and cycle limited RT-PCR shall also be described as well as more modern techniques such as real time RT-PCR analysis. This lecture will allow the interpretation of published literature utilising these techniques and introduce the steps involved in performing RNA related techniques in your laboratory.

#### **Differential gene expression: overview of relevant methods**

Prof William Gallagher (UCD Conway Institute of Biomolecular & Biomedical Research)

This lecture will summarise the main approaches used to determine alterations in gene expression at the RNA level. Emphasis will be placed in this context on global approaches that attempt to map differences in the transcriptome, i.e. entire complement of transcripts in a cell. Methodologies that will be addressed include differential display, subtractive hybridization, high-throughput sequencing (ESTs and SAGE), and DNA microarray technologies. Key examples from the literature will be utilised to illustrate examples of investigators applying these technologies to understand biological phenomena, with a focus on disease-related processes. An indication of the relevant infrastructure and expertise to carry out this work within the DMMC will be presented.

#### **Review articles**

Lennon, G. G. (2000).

#### **High-throughput gene expression analysis for drug discovery.**

Drug Discovery Today, 5, 59-66.

Schulze, A. and Downward, J. (2001).

#### **Navigating gene expression using microarrays – a technology review.**

Nature Cell Biology, 3, E190-E195.

#### **Polymorphism association with disease**

Prof Denis Shields (UCD Conway Institute of Biomolecular & Biomedical Research)

Different strategies are required to identify rare and common genetic variants underlying both rare and common diseases. For common genetic variants, there is now a very rich dataset of identified common single nucleotide polymorphisms (SNPs). These can be investigated in disease groups (compared to controls) in candidate genes, or by whole genome association analysis. Analysis of these genes requires careful attention to the patterns of association of SNPs that are chromosomally adjacent (in linkage disequilibrium). Linkage analysis (tracking in families the disease co-inheritance with widely spaced gene markers) is the traditional approach of choice for rare mutations that have strong phenotypic effects. High throughput sequencing of candidate regions (and in future whole genomes) are accelerating the rate of data accumulation.

#### **Introduction to cloning**

Dr Ross McManus (Institute of Molecular Medicine, TCD)

Even in the post genome era, DNA cloning is essential to the manipulation and stable propagation of genetic material. This talk will cover the basic aspects of DNA cloning, ranging from the anatomy of cloning vectors to the choice of vectors based on the cloning strategy employed. The strategy employed will depend on the overall objectives of the project and the nature of the starting information or material available. Thus different choices and approaches would be employed for a sequencing project compared with a genome mapping project or production of RNA or protein. I will discuss a number of basic and specialised cloning strategies to illustrate some of the options and possibilities available.



## Day 2

### **Model organisms**

Dr Breandán Kennedy (UCD Conway Institute of Biomolecular & Biomedical Research)

The goal of this lecture is to discuss animal models that are routinely applied to biomedical research. The advantages of using *Drosophila* (fly), *Xenopus* (frog), *Danio* (zebrafish), *Gallus* (chicken) and *Mus Musculus* (mouse) as model organisms will be described. The life-cycle, generation time, embryo development and amenability of these organisms to genetic manipulation will be discussed. An emphasis will be placed on describing mutagenesis screens. This technique, in which the genes in the genome are randomly inactivated, has been extensively applied to the fly/fish models and has accelerated our understanding of gene function (functional genomics).

### **Protein expression and purification**

Dr Henry Windle (Institute of Molecular Medicine, TCD)

This lecture will cover the basics of protein expression and purification. Emphasis will be placed on alternative strategies and issues that should be considered prior to selection of specific expression systems and purification strategies. As protein purification methodologies are generally well described and accessible, only a brief overview of these will be given but with emphasis on common problems that can arise, particularly for those about to attempt purification for the first time. The following books from The Practical Approach series by IRL Press are an invaluable aid with detailed and reliable protocols: *Protein Purification Applications*; *Protein Purification Methods* (2001, Editor Simon Roe). *Gallus* (chicken) and *Mus Musculus* (mouse) as model organisms will be described. The life-cycle, generation time, embryo development and amenability of these organisms to genetic manipulation will be discussed. An emphasis will be placed on describing mutagenesis screens. This technique, in which the genes in the genome are randomly inactivated, has been extensively applied to the fly/fish models and has accelerated our understanding of gene function (functional genomics).

### **Determining protein:protein interactions in biology**

Dr Niamh Moran (RCSI Research Institute)

Whereas the Human genome Project has successfully identified all human genes and their corresponding proteins, the next challenge in biology is to understand how these proteins interact with each other in a dynamic cell system. My lab is interested in the molecular mechanisms of integrin activation in the human platelet in order to better understand thrombotic disease. Using the platelet as a model system therefore, we explored various mechanisms of elucidating relevant protein interactions with integrin cytoplasmic tails. Current conventional methods are discussed in this presentation including co-immunoprecipitation, FRET, yeast-two hybrid system, TAP-Tags and protein chip technology. In addition, the emerging protein interaction websites provide a useful tool in this process. Verification of newly identified interactions is the next bottleneck in the process and needs to be tailor made for each specific protein. This involves a combination of literature-searching, bio-informatics and basic cell-biology techniques. The conclusion of this presentation will highlight the importance of choosing an appropriate system for exploring protein:protein interactions; identifying relevant association and eliminating non-specific interactions, and finally emphasising the importance of relevant verification in this process. We have termed this process Protein ENONOMICS from the Greek term for interaction and claim that it is the next emerging 'omic' after proteomics!

## Day 3

### **Cell imaging and sorting – flow cytometry**

Professor William Watson (UCD Conway Institute of Biomolecular & Biomedical Research)

Flow cytometry is a method for quantitating components or structural features of cells, primarily by optical means. Although it makes measurements on one cell at a time, it can process thousands of cells in a few seconds. Since cell types can be distinguished by quantitating structural features, flow cytometry can be used to count prokaryotic or eukaryotic cells of different types in complex mixtures.

### **Transgenics and knockouts**

Dr Rosemary Kane (UCD)

This lecture will provide a broad overview of the strategies used to generate both transgenic and knockout mice, starting from the generation of the DNA constructs using cDNAs or genomic DNA, and proceeding through embryonic stem cell biology, to aggregation and chimeric mouse generation. Details on genotyping of transgenic animals, as well as phenotype characterization will be discussed. Specific examples will be cited.

### **Introduction to Mass Spectrometry**

Dr Giuliano Elia (UCD Conway Institute of Biomolecular & Biomedical Research)

Mass spectrometry is a powerful technique for the precise and accurate determination of the molecular mass of a vast range of chemical compounds. The introduction, about two decades ago, of soft ionization techniques like MALDI (Matrix-Assisted Laser Desorption-Ionization) and ESI (ElectroSpray Ionization) allowed the widespread application of mass spectrometry to biological macromolecules, including proteins and peptides. Mass spectrometry is truly at the origin of the enormous success of proteomic science.

This lecture will present basic concepts in mass spectrometry, introduce the most common types of mass spectrometers used in biological research and discuss some simple mass spectrometry-based proteomic approaches.

### **Expression proteomics: biomedical applications**

Prof Stephen Pennington (UCD Conway Institute of Biomolecular & Biomedical Research)

Following the sequencing of the whole genomes of a large number of organisms including the human genome it has become increasingly apparent that the study of the encoded proteins on a genome-wide scale is required – this is the field of proteomics. When, in 1994, Marc Wilkins coined the term 'proteome', defined as the protein complement of a genome, the methods for investigating protein expression on such a scale were in their infancy and even today (nearly a decade and a half later) they are still in evolution. The technique of two-dimensional gel electrophoresis (2-DE) remains a core technology of choice for many applied proteomic projects due to its ability to separate simultaneously thousands of proteins and to indicate post-translational modifications that result in alterations in protein pI and/or Mr. Moreover, recent developments for 2-DE including the use of fluorescent dyes that facilitate the multiplex analysis of samples make it possible to achieve greater proteomic coverage combined with more accurate differential expression analysis.

Liquid chromatography (LC) based methods have emerged as powerful approaches for protein expression analysis. Multi-dimensional LC potentially has the advantage of higher coverage and sensitivity but as yet is not routinely applied to the separation and quantification of very complex mixtures of proteins. In this presentation several biomedical applications will be presented to demonstrate the utility of gel and LC based proteomics approaches including the use of isotope coded affinity tags based LC approaches. These applications include analysis of drug-induced toxicity, identification of biomarkers of pancreatic cancer, prostate cancer and inflammatory arthritis. Together, these should illustrate the diversity of approaches available for the measurement of protein expression.

### **Review article**

Cravatt BF, Simon GM, Yates JR 3rd. The biological impact of mass-spectrometry-based proteomics. *Nature*. 2007 Dec 13;450(7172):991-1000. Review.

PMID: 18075578 [PubMed - indexed for MEDLINE]

### **Expression proteomics: biomedical applications**

Prof Stephen Pennington (UCD Conway Institute of Biomolecular & Biomedical Research)

Following the sequencing of the whole genomes of a large number of organisms including the human genome it has become increasingly apparent that the study of the encoded proteins on a genome-wide scale is required – this is the field of proteomics. When, in 1994, Marc Wilkins coined the term 'proteome', defined as the protein complement of a genome, the methods for investigating protein expression on such a scale were in their infancy and even today (nearly a decade and a half later) they are still in evolution.

The technique of two-dimensional gel electrophoresis (2-DE) remains a core technology of choice for many applied proteomic projects due to its ability to separate simultaneously thousands of proteins and to indicate post-translational modifications that result in alterations in protein pI and/or Mr. Moreover, recent developments for 2-DE including the use of fluorescent dyes that facilitate the multiplex analysis of samples make it possible to achieve greater proteomic coverage combined with more accurate differential expression analysis.

Liquid chromatography (LC) based methods have emerged as powerful approaches for protein expression analysis. Multi-dimensional LC potentially has the advantage of higher coverage and sensitivity but as yet is not routinely applied to the separation and quantification of very complex mixtures of proteins. In this presentation several biomedical applications will be presented to demonstrate the utility of gel and LC based proteomics approaches including the use of isotope coded affinity tags based LC approaches. These applications include analysis of drug-induced toxicity, identification of biomarkers of pancreatic cancer, prostate cancer and inflammatory arthritis. Together, these should illustrate the diversity of approaches available for the measurement of protein expression.

### **Review article**

Cravatt BF, Simon GM, Yates JR 3rd. The biological impact of mass-spectrometry-based proteomics. *Nature*. 2007 Dec 13;450(7172):991-1000. Review.

PMID: 18075578 [PubMed - indexed for MEDLINE]

### **Immunodetection methods on cell and tissue extracts**

Dr Leonie Young (UCD Conway Institute of Biomolecular & Biomedical Research)

The use of antibodies to detect and characterise proteins has been well established. With the development of high through-put techniques such as tissue microarrays (TMA), a real challenge now exists to determine the cellular location, level of expression and the function of these identified proteins. In this lecture, principles fundamental to immunodetection will be outlined. Common pitfalls and measures to avoid these will be discussed. Applications of immunodetection in a modern molecular context will be illustrated, including: western blotting, ELISA, immunohistochemistry/ immunofluorescence, tissue microarrays, co-immunoprecipitation, Electromobility shift assays, chromatin immunoprecipitation (ChIP) and antibody arrays.

### **Cell imaging and sorting – flow cytometry**

Professor William Watson (UCD Conway Institute of Biomolecular & Biomedical Research)

Flow cytometry is a method for quantitating components or structural features of cells, primarily by optical means. Although it makes measurements on one cell at a time, it can process thousands of cells in a few seconds. Since cell types can be distinguished by quantitating structural features, flow cytometry can be used to count prokaryotic or eukaryotic cells of different types in complex mixtures.

### **Transgenics and knockouts**

Dr Rosemary Kane (UCD)

This lecture will provide a broad overview of the strategies used to generate both transgenic and knockout mice, starting from the generation of the DNA constructs using cDNAs or genomic DNA, and proceeding through embryonic stem cell biology, to aggregation and chimeric mouse generation. Details on genotyping of transgenic animals, as well as phenotype characterization will be discussed. Specific examples will be cited.



## Day 4

### **Laser Capture Microdissection and in situ hybridisation**

Dr Orla Sheils (Institute of Molecular Medicine, TCD)

Laser Capture Microdissection is a method for procuring pure cells from specific microscopic regions of tissue sections. Under the microscope, tissues are heterogeneous complicated structures with hundreds of different cell types locked in morphologic units. In disease pathologies, the diseased cells of interest are surrounded by these heterogeneous tissue elements. Laser Capture Microdissection constitutes an essential upstream technology to molecular analysis methods studying evolving disease lesions in actual tissue.

In Situ Hybridization techniques allow the demonstration of specific nucleic acid sequences within their cellular environment. A logical extension of early in situ hybridization (ISH) techniques, which exploited the ability to label DNA with high-energy fluorophores, is FISH. This technique is now applied in an increasing number of molecular diagnostic areas, including karyotype analysis, gene mapping, disease diagnosis, and therapeutic targeting.

### **High Content Analysis of nanoparticle/cell interactions**

Dr Yuri Volkov (Institute of Molecular Medicine, TCD)

Fluorescent organic tags have represented one of the major tools in the arsenal of researchers working in the biomedical sciences for more than two decades. The progress in development of new fast and efficient research and diagnostic methods is largely dependent on the availability of fluorescent probes with desired cell receptor- and organelle specificity and optimised experimental protocols for their utilization.

A unique opportunity to generate a wide spectrum of such probes suitable for applications in living cells is offered by semiconductor quantum dots (QDs). As fluorescent probes QDs have several advantages over organic dyes, including wide absorption profiles, tunable emission spectra, and superior photostability. QDs have been shown to readily distribute across animal cells, tissues and organs. Today, QDs with different physico-chemical properties and functionalities are readily available worldwide. However, further exploitation of QDs in biomedical studies has been hindered by the absence of adequate technological platforms capable of performing multi-parametric quantitative analysis of individual responses in specific cell types.

Recent years have witnessed a rapid progress in the development of novel methods permitting high-resolution visualisation of cell receptor dynamics and intracellular biochemical processes utilizing fluorescent probes. Among these, high content screening (HCS) technology allows to perform analysis of molecular interactions in individual cells and their populations at the sub-cellular level under physiological conditions. This technology not only facilitates development of a better understanding of the true functionality of target molecules in the living cells, but it can also promote designing of highly informative screens for novel therapeutic drugs, including inhibitors at small molecule and gene therapy level applicable in inflammation and cancer.

Here we will discuss the data demonstrating the influence of such factors as QDs size, charge and selective functionalisation on their membrane and subcellular localisation specificity and present an overview of advantages and hurdles on the way of merging nanotechnology and high content cell analysis.

### **Stem Cells - Biology and Applications**

Dr Linda Howard (NUI Galway)

Stem cells are relatively unspecialised cells which lack tissue-specific characteristics. Under the right conditions they can give rise to one or multiple specialised cell types in a process called 'differentiation'. Stem cells show enormous therapeutic potential based on their ability to generate cells for repair or regeneration of damaged tissues and organs.

This lecture will describe the stem cells sources and the characteristics and properties of stem cells from different sources. Finally it will give details of current and future clinical trials designed to evaluate the safety and efficacy of stem cell therapies in the clinic.

### **Medical Systems Biology - The case of the cancer cell not able to die**

Prof Jochen Prehn (RCSI)

Apoptosis is a key biological process important for the removal of unwanted or damaged cells. The ability of cells to evade apoptosis is a key process underlying radio- and chemotherapy resistance. In this presentation, we will discuss approaches to develop computational models of biological process that help explain how key biological processes such as apoptosis are switched 'on' or 'off', by looking at several rather than individual genes or proteins. We will also discuss the increasing importance of systems biology approaches as a predictive tool for the development of prognostic and therapeutic approaches.