DCU'S SCHOOL OF BIOTECHNOLOGY

9TH RESEARCH DAY 2017

Programme of Abstracts

JANUARY 27TH

LONSDALE BUILDING

BRS BIOLOGICAL RESEARCH SOCIETY

DCU

8:30 - 9:00	**Registration**
9:00 - 9:10	**Opening Remarks** *Mary Rose Sweeney, Associate Dean for Research in the Faculty of Science and Health.* *Jonathan Loftus, Chairperson of the Biological Research Society.*
9:15 - 10:20	**Session 1**
Daniel McPartlin	We need to talk about our toxic waters
Brian Freeland	Nanoparticle fabrication via pulsed laser ablation in liquids (PLAL)
Arun Decano	Population Phylogenomic Analysis of the Origin and Spread of the Pandemic E. coli Sequence Type 131 (ST131)
Catherine Allen	Health Impacts of Phthalates and the use of Sewage as a Biomarker
Charles O'Doherty	Cell culture models for micronutrient interactions and tolerance
Flávio Ferreira	The use of recombinant lectins for the bioanalysis of Cell surface Glycosylation
Paloma Ozores	Oestrogenic leachable in water solar disinfection: Are they a problem?
Sarah Lynch	Selenium source impacts protection of porcine jejunal epithelial cells from cadmium-induced DNA damage
	Anne Parle-McDermott, DCU Ambassador for Biochemical Society.
10:20 - 11:00	**Coffee Break and Poster Session**
11:00 - 11:10	**Company Speaker: Dr. John Synnott, Sales & Marketing Executive, Bio-Sciences Limited.**
11:10 - 11:35	**Session 2**
Donal Monaghan	Glyco-profiling of Colorectal Carcinoma cell lines using commercial and recombinant lectins
Thayse Marques Passos	Antimicrobial photodynamic therapy: a non-antibiotic multi-target approach to disinfect water
Ali Coyle	The role of selenium in cancer
Burcu Güneş	Enhancement of Anaerobic Digestion of Whiskey Distillery Co-Products
11:35 - 12:30	**Session 3**
Kara Moran	Mycotoxins - Problems and Detection

Alan Costello	Control of miRNA in Chinese Hamster Ovary Cells
Orla Coleman	Identification, validation and characterisation of specific tumour markers for pancreatic adenocarcinoma Session 1
12:30 - 13:10	**Guest Speaker: Dr. Jonathan Bones, National Institute for Bioprocessing Research and Training (NIBRT), UCD.**
13:10 - 14:00	**Lunch Break and Poster Session**
14:00 - 15:50	**Session 4**
Gemma Moore	Investigating the role of the tumour microenvironment in the behaviour of pancreatic cancer cells using indirect co-culture
Nicola Gaynor	The immune response to trastuzumab in breast cancer
Keith Rochfort	Perfusion of the lung ex vivo – the shear stress of it all!
Kevin Kellner	Molecular Impact of the miRNA 23 cluster on bioprocess relevant attributes of Chinese Hamster Ovary cells
Neil Conlon	Novel mechanisms of resistance to HER2-targeted therapies in breast cancer
Ricardo Valdés-Bango Curell	Evaluation of miR-toehold switches as transgene expression modulators in CHO cells
Ray Moran	Major genetic innovations in animal evolution: gene remodeling in the Metazoa
Caroline Murphy	Strategy for enhancing immunosensor performance by optimally engineering antibody binding and format
Prashant Kaushik	Proteomics and posttranslational regulatory networks in Chinese Hamster ovary cells.
Antonio Alarcon Miguez	Effects of heteroplasmy inside mitochondria in productive CHO-Cell lines
15:50 - 16:30	**Coffee Break and Poster Session**
16:30 - 17:10	**Guest Speaker: Dr. Tobias Knoch, Head of Biophysical Genomics, Erasmus MC, Rotterdam, The Netherlands.**
17:10 - 17:20	**Conference End and Awards Ceremony**
18:00	**Reception in The Ivy House, Drumcondra.**

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Flash 5 Oral Presentations

We need to talk about our toxic waters

Daniel McPartlin, Dr. Caroline Murphy and Prof. Richard O'Kennedy
School of Biotechnology, Dublin City University, Glasnevin, Dublin 9.

Ocean temperatures changes and increasing atmospheric CO_2 concentrations have led to increasing occurrences of harmful algal blooms. In particular, rising temperatures in the North Atlantic has seen the increase in populations of Azadinium spinosum, the primary producer of the shellfish-poisoning toxins, Azaspiracids (AZAs). Filter-feeding shellfish accumulate AZAs and the consumption of such contaminated shellfish leads to severe gastrointestinal disorders. This has major economic implications for global fisheries.

The primary detection method for AZAs is reliant on expensive and laborious analytical methods. An alternative to such methods is using point-of-need (PON) devices. These cheap, sensitive and rapid detection systems often use antibodies as a biological recognition element. This project focuses on developing recombinant antibodies against AZA1 and A. spinosum and incorporating them into a PON device. This device will be deployed on a marine-monitoring buoy in Galway Bay, Ireland, thereby allowing for autonomous, in-situ monitoring, and early-warning of AZA1 and A. spinosum.

Nanoparticle fabrication via pulsed laser ablation in liquids (PLAL).

B.Freeland, G.Foley, D.Brabazon
Advanced Processing Technology Research Centre

The field of nanotechnology has expanded rapidly over the past ten years. The use of nanoparticles has seen applications in fields diverse as printing technology, semiconductor sensors, surface coatings, surface functionalization, water treatment, chemical separations and bio-diagnostics. However considerable work needs to be performed to offer a ligand free “green synthesis” approach to the manufacture of these nanoparticles. This work suggests such a green and ligand free manufacturing technique using pulsed laser ablation in liquids (PLAL) to fabricate the particles. This technique offers narrower size distributions and “clean” ligand free particles compared with chemical synthesis techniques, opening the way for single step functionalization.

Health Impacts of Phthalates and the use of Sewage as a Biomarker

Allen, Catherine[a,d], Jones, Lisa[a,d], Staines, Anthony[b,d], Regan, Fiona[c,d] and Lawler, Jenny[a,d]
[a]School of Biotechnology
[b]School of Nursing and Human Sciences
[c]School of Chemistry
[d]DCU Water Institute
Dublin City University

Phthalate esters are synthetic organic chemicals commonly used as plasticizers in PVC materials. Phthalates are ubiquitous within the environment, and are often detrimental to human health. Research into the human health effects of phthalates is far from complete. While some phthalates have been banned or limited in manufacturing, new research indicates that substitute plasticizers have similar adverse health effects. This research is timely as the extent of phthalate contamination within Ireland, and their impacts on human health, are unknown.

This project will pioneer the use of sewage as a biomarker to determine phthalate exposure, assessing the feasibility of this method for future compliance monitoring. Metabolites from 11 priority phthalates are proposed for investigation. This information, in conjunction with meta-analysis and modelling will form an Irish risk assessment, informing environmental policy. This study will contribute to a large scale project assessing potential sources and environmental fates of priority phthalates in Ireland.

Cell culture models for micronutrient interactions and tolerance

Charles O'Doherty, Joanne Keenan, Martin Clynes, Finbarr O'Sullivan
NICB, Alltech

Many unknowns remain regarding how the small intestine processes essential mineral micronutrients, such as iron, copper and zinc. The aim of the project is to generate small intestine-like models that will mimic the responses of in vivo tissues when exposed to a range of micronutrient compounds to better understand requirement, utilisation and toxicity.

Alongside traditional immortalised cell line models, induced pluripotent stem cells (iPSCs) are being differentiated in vitro towards small intestine-like tissues to determine how cells react under different combinations and concentrations of micronutrient exposure.

A variety of techniques are used to measure changes in the models. Micronutrient compounds are applied in the form of inorganic and organic sources and acquisition, storage and transport of the minerals are examined in the systems. The effect on gene and protein expression is also examined by RT-PCR and Western blotting.

The use of recombinant lectins for the bioanalysis of Cell surface Glycosylation

Flávio Ferreira, Brendan O'Connor, Dermot Walls
School of Biotechnology, DCU

Cell surface glycosylation is an important indicator of cell health. These specific glycans or sugars are attached to surface proteins, called glycoproteins. Our group has developed a number of specific recombinant lectin probes that have the ability to interrogate the state of glycosylation on the cell surface by selective binding to glycoproteins. As cells become stressed or diseased the earliest changes that occur are in cell surface glycosylation. CHO cells are the host cell of choice of the rapidly emerging biopharmaceutical industry for the production of Glycoprotein therapeutics. This study intends to investigate the interaction between recombinant lectin probes with the membrane glycoproteins of normal CHO cells and compare this to CHO cells that have been subjected to several stressing conditions. Our aim is to develop a rapid and accurate bioanalytical method to monitor the cell health during a bioprocess using Flow Cytometry, Fluorescence microscopy and Western blotting.

Oestrogenic leachable in water solar disinfection: Are they a problem?

Paloma Ozores
WATERSPOUTT project & EU horizon 2020

Solar disinfection (SODIS) of water is a low-cost, simple, effective and environmentally friendly technique that has gained popularity in developing countries. This technique involves filling transparent containers with contaminated water which are then exposed to sunlight for 6h to be disinfected through the synergistic effects of heat and UV radiation. The most commonly used containers are 2L plastic polyethylene-terephthalate bottles. A range of large volume (≥20L) SODIS technologies are being developed to produce a minimum of 20L of treated water per day. Manufacture of these novel technologies will involve the use of plastics. However, research has shown that most currently available plastic products release oestrogenic chemicals that are implicated in toxic endocrine disruptive events affecting human development. In order to mitigate toxicity concerns, further research is required to test new solar prototype reactors for efficacy in both disinfection and reduction of xenoestrogens in water. The current state of knowledge for detecting and assessing xenoestrogens will be reviewed.

Selenium source impacts protection of porcine jejunal epithelial cells from cadmium-induced DNA damage

Sarah Lynch[1], Karina Horgan[2], Blanaid White[3] and Dermot Walls[1]
1 School of Biotechnology, 2 Alltech Ltd, 3 School of Chemical Sciences

Selenium (Se) is found in inorganic and organic forms, both of which are commonly used in animal feed supplements. The aim of this study was to determine the impact of the chemical form of Se on its associated ameliorative effects on cadmium (Cd) induced DNA damage in a porcine gut model. The results showed that Se antioxidant effects were both composition- and dose-dependent as evident from cell viability and DNA damage assays. Organic Se sources had protective effects against Cd-induced DNA damage whereas inorganic Se compounds had no protective effects and in fact these enhanced the negative effects of Cd-induced DNA damage. Additional experimentation revealed differences in the ameliorative capacity of different organo-selenium sources and indicated a potential role for the modulation of cellular DNA repair mechanisms. We conclude that nutritional supplementation with organoselenium may protect porcine gut integrity from damage induced by Cd.

Glyco-profiling of Colorectal Carcinoma cell lines using commercial and recombinant lectins

Donal Monaghan
School of Biotechnology, NCSR, ISSC

Glycosylation is one of the most important post translational modifications. In recent years the study of cell surface glycosylation has become increasingly important in respect to cells in a diseased state. Many diseased cells, such as various types of cancer, exhibit different levels of glycosylation on the cell surface. Due to this fact, analysis of the specific changes on cell surface glycans can be compared between diseased and healthy cells to try and identify novel biomarkers.

In this project we can use commercial and recombinant lectins to probe cells at different stages of cancer to try and identify significant differences to the cell surface glycosylation. SW480 and SW620 colorectal carcinoma cell lines were used in this project as they are stage 2 and stage 3 carcinoma cell lines, respectively. Through the use of fluorescent microscopy and flow cytometry the aim of the project was to qualitatively and quantitatively compare the cell surface glycans between these two cell lines.

Antimicrobial photodynamic therapy: a non-antibiotic multi-target approach to disinfect water

T. Passos[1], M. Pryce[2] and B. Quilty[1]
[1]School of Biotechnology, Dublin City University (DCU), Dublin 9, Ireland.
[2]School of Chemical Sciences, Dublin City University (DCU), Dublin 9, Ireland.

The interest in finding alternative processes to eradicate waterborne pathogens is growing in recent times. Antimicrobial photodynamic therapy is a promising option for water disinfection. It uses visible light as an energy source combined with a photosensitiser (PS) to transfer energy/electrons to a substrate or molecular oxygen, generating reactive oxygen species, which causes cidal effects towards cells. Some of the advantages of applying PDI in water treatment are the fact that this technique does not induce bacterial resistance and the low cost, since sunlight can be the energy source. The aim of this study was to investigate the efficacy of different concentrations of 5,10,15,20-tetrakis(N-methyl-4-pyridyl)-21H, 23H-porphine (TMPyP) against 12 bacterial strains and its mutagenicity, using the Ames Test. TMPyP showed good inactivation of the bacterial strains, with the responses varying among the strains and the concentrations used. The higher concentration of TMPyP used in the experiments, 10μM, was shown to be mutagenic when tested against Salmonella in the Ames test.

The role of selenium in cancer

Ali Coyle, Joanne Keenan, Finbarr O' Sullivan, Karina Horgan, Martin Clynes.
NICB, Alltech

Selenium, a trace element consumed in our diet, plays multiple key roles in normal health, including antioxidant protection, normal thyroid and immune function. Both clinical, animal and in vitro cancer studies have suggested anti-carcinogenic benefits to increasing selenium in the diet. My project investigates the molecular mechanisms behind this anti-cancer protection associated with increased selenium intake.

Enhancement of Anaerobic Digestion of Whiskey Distillery Co-Products

Burcu Gunes[a], Khaled Benyounis[b], Paul Davis[c], Joseph Stokes[b], Cathal Connolly[d], Jenny Lawler[a]
a School of Biotechnology DCU
b School of Mechanical & Manufacturing Engineering DCU
c DCU Business School
d Alltech European Bioscience Centre, Dunboyne, Co. Meath

The focus of this research is on energy recovery from distillery co-products such as pot ale, spent barley and spent yeast. Due to the characteristics of these waste streams, the alcoholic beverage industry is a highly polluting industry, with approximately 3.4 million tonnes of solid wastes and over 40 billion litres/year of liquid effluent produced per year[1,2] . Distillery waste streams are an ideal feedstock for the process of anaerobic digestion (AD) - biological breakdown of organic material by microorganisms, including methanogenic bacteria, resulting in the production of methane rich biogas. The application of AD could render most distilleries self-sufficient in terms of energy and low-grade heat requirements.
However, due to the lignocellulosic nature of distillery co-products, pretreatment is needed in order to maximise bio-methane yield. This work details the application of two pre-treatments (mechanical and chemical) prior to AD, to optimise CH_4 production using distillery co-products as a substrate.

[1] Mohana, S., Acharya, B. K., & Madamwar, D. (2009). Distillery spent wash: Treatment technologies and potential applications. Journal of Hazardous Materials, 163(1), 12-25
[2] Aliyu, S., & Bala, M. (2011). Brewer's spent grain: A review of its potentials and applications. African Journal of Biotechnology, 10(3), 324-331

Oral Presentations

Mycotoxins - Problems and Detection

Kara Moran, Richard O' Kennedy
School of Biotechnology, BDI

Mycotoxins are naturally occurring food adulterants produced by *Aspergillus* and *Fusarium* species. These toxins have huge implications for food safety, readily contaminating at least 25% of globally consumed basic food commodities such as rice, maize and wheat (Boutrif and Canet, 1998). When ingested, mycotoxins may cause mycotoxicosis which can provoke acute or chronic disease. Chronic exposure has a greater impact on health stimulating carcinogenic, mutagenic, teratogenic, estrogenic, hemorrhagic, immunotoxic, nephrotoxic, hepatotoxic, dermotoxic and neurotoxic effects in humans and animals.

This study describes the bacterial expression, purification and use of an anti-AFB1 recombinant antibody Fab fragment and anti-T2 polyclonal antibodies in an optical-planar waveguide biosensor device for sensitive 'point-of-site' mycotoxin detection to EU maximal limits in food. Therefore, this system will take sensitive mycotoxin analysis out of the traditional laboratory-based setting and directly into the field permitting monitoring and identification of unsafe food for human and animal consumption.

References:

[1] BOUTRIF, E. and CANET, C., 1998. Mycotoxin prevention and control: FAO programmes. *Revue de Médecine Vétérinaire*, 149(6), pp. 681-694.

Control of miRNA in Chinese Hamster Ovary Cells

Alan Costello, Nga Lao, Clair Gallagher, Colin Clarke, Niall Barron, Martin Clynes
NICB, DCU

miRNA play a pivotal role in gene and pathway regulation through their intervention during RNA processing. Utilizing targeted genetic engineering approaches, it is feasible to increase or deplete the free abundance of these molecules in the cell. Numerous miRNA demonstrated ability to improve bio-process relevant phenotypes; growth, increased product titre, delay of apoptosis, through the stable manipulation of these molecules in Chinese Hamster Ovary (CHO) cells. To take this a step further, the aim of this research is to add control to stable miRNA manipulation. A TET-On inducible expression system is used to timely trigger the over-expression of a specific miRNA or knockdown via a miRNA sponge decoy. Bioprocess control through process analytical technologies (PAT) is a cornerstone of the bio-pharma sector. The process development undertaken by this research investigates the potential for Bio-process control on a cellular level.

Identification, validation and characterisation of specific tumour markers for pancreatic adenocarcinoma

Orla Coleman[1*], Michael Henry[1], Fiona O'Neill[1], Sandra Roche[1], Niall Swan[2], Martin Clynes[1] and Paula Meleady[1]
[1]Dublin City University
[2] St. Vincent's University Hospital, Dublin

Pancreatic cancer is the 4th leading cause of cancer related deaths worldwide and develops in a relatively symptom-free manner leading to rapid disease progression and usually a dismal prognosis at the time of diagnosis. Targeted therapeutics has dawned a new era of cancer treatments such as the introduction of antibody-drug conjugates (ADCs) which take advantage of the specificity of antibodies to deliver cytotoxic agents to cancer cells expressing a tumour specific protein marker. This project is focused on the identification of membrane proteins associated with pancreatic cancer for their use as novel targets for ADC therapy. Label-free LC-MS/MS proteomic analysis of 10 patient tumours, 10 normal-adjacent tissues and 22 patient-derived xenograft identified over 300 differentially expressed proteins with ≥2-fold change in abundance compared to the normal-adjacent samples. A number of strong target candidates were taken forward for validation of expression by both immunoblotting and immunohistochemistry. Immunohistochemistry validation is necessary to confirm cell surface expression of a potential target for ADC specificity and efficiency. Proteins identified in this study may also be useful as diagnostic, prognostic or predictive targets for pancreatic cancer. This study provides relevant and necessary potential targets for pancreatic cancer therapy which have been identified from patient material thus underlying their value.

Investigating the role of the tumour microenvironment in the behaviour of pancreatic cancer cells using indirect co-culture

Gemma Moore, Fiona O'Neill, Gerard McVey, Michael Moriarty, Laura Breen and Martin Clynes
National Institute for Cellular Biotechnology, Dublin City University
St. Luke's Radiation Oncology Network, St. Luke's Hospital, Rathgar, Dublin 6.

Pancreatic cancer is one of the most lethal cancers worldwide. The 5-year survival rate is approximately 6%, due to late diagnosis by which time usually metastasis has occurred. Despite an influx of research on the disease in recent years, very little progress has been made and the development of new, more effective therapies is needed. The role of the tumour microenvironment in the progression of pancreatic cancer is becoming the focus of more and more pancreatic cancer research. This in part is due to the realization that the stromal cells surrounding tumours play an important role in the progression and metastasis of this cancer, and the fact that pancreatic cancer is one of the most stroma-rich cancers. This project investigates the interaction between the tumour microenvironment and pancreatic tumour cells. The aim is to further understand the mechanism by which the fibroblasts and stellate cells in the tumour influence the behaviour of the cancer cells.

Molecular Impact of the miRNA 23 cluster on bioprocess relevant attributes of Chinese Hamster Ovary cells

Kevin Kellner, Dr. Nga Lao
NICB

Chinese Hamster Ovary (CHO) cells are the prominent cell line used in biopharmaceutical production. To improve yields and find beneficial bioprocess phenotypes genetic engineering plays an essential role in recent research. Previous work showed that stable depletion using sponge decoy technology of the miRNA-23 cluster or single miRNA members can result in beneficial bioprocess phenotypes. In this work we aimed to disrupt miRNA function using miRNA sponges and to implement the recently developed CRISPR/Cas9 system for the same purpose.

The depletion of single miRNA members or the whole cluster showed certain beneficial bioprocess attributes including elevated product titers and growth in two different CHO cell lines. Furthermore, this work highlights that miRNAs can successfully be targeted using CRISPR/Cas9 in CHO cells to improve the bioprocess phenotype, whilst obtaining the characteristics achieved by using sponge decoy technology.

Major genetic innovations in animal evolution: gene remodeling in the *Metazoa*

[1]Ray J. Moran, [2]Jananan Pathmanathan, [3]Christopher J. Creevey, [3,4]Karen Siu-Ting, [2]Eric Bapteste, [5]James O. McInerney, [1,4]Mary J. O'Connell[*]
1. Bioinformatics & Molecular Evolution Group, School of Biotechnology, Faculty of Science and Health, Dublin City University;
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3. Institute of Biological, Environmental and Rural Sciences (IBERS), Aberystwyth University, Aberystwyth, Ceredigion SY23 3FG, UK;
4. 250 Great Minds University Academic Fellow, School of Biology, Faculty of Biological Sciences, University of Leeds, UK;
5. Department of Biology, National University Ireland, Maynooth, Ireland, Faculty of Life Sciences, The University of Manchester, Manchester, United Kingdom;

Animals range from single tissue organisms like sponges to complex systems of tissues and organs like humans. A major question in biology has been what genomic changes have occurred to facilitate the emergence of novel phenotypes or major transitions such as the transition from non-symmetrical to bilaterally symmetrical body plan? We use a novel approach that combines 63 *Metazoan* genomes, phylogenomics and sequence similarity networks to understand the contribution of 2 interrelated processes in the evolution of animal life. Firstly, we use a novel phylogenetic based approach to determine the role for gene duplication, loss and point mutation in the evolution of gene families in the *Metazoa*. Secondly, we use network based approaches to study the non-phylogenetic processes of novel gene family evolution by gene fusion and fission. We show that gene remodeling is present right across the metazoan tree, and is a major contributor to the emergence of novel gene families. However, there is a striking difference in the processes involved in the evolution of protein-coding gene families depending on the animal lineage, for example *Deuterostomes* evolve composites primarily by using existing material and *Protostomes* by using 'new' material. We show that in extant *Deuterostomes* like human remodeled genes tend to reuse and recycle already existing remodeled material. On a broader scale, this work provides a first glimpse into how the protein coding elements of animals have evolved through time and how this correlates with major evolutionary transitions in phenotypes from sponges to humans.

Effects of heteroplasmy inside mitochondria in productive CHO-Cell lines

Antonio Alarcón Miguez
NICB, DCU

Mitochondrial metabolism studies has been rising on interest during the last years , mainly due to the role of such metabolism in several human hereditary diseases, but also due to its implications on pharma-producing cell lines, such as Chinese Hamster Ovary (CHO) cells. The phenomena known as Heteroplasmy involves the existence of different mitochondrial-DNA copies inside the same mitochondria, although is a not well-known nowadays, recently several studies have highlighted its potential relationship with mitochondrial oxygen metabolism, raising the necessity of studying the heteroplasmy phenomena.

In this study, several producing CHO-cell lines showing different degrees of heteroplasmy are being assessed for mitochondria and metabolic parameters. The goal is to establish a relationship, if any, between the behaviour, growth and productivity of the cells and their degree of heteroplasmy observed in genes concerning the respiratory chain, such as CYTB or COX2.

Video Presentations

The immune response to trastuzumab in breast cancer

Gaynor N [1], O'Donovan N [1], Crown J [2], Collins DM [1].
1. National Institute for Cellular Biotechnology, Dublin City University, Glasnevin, Dublin 9. 2. St. Vincent's University Hospital, Elm Park, Dublin 4.

Therapeutic monoclonal antibodies (mAb) can recognize and bind to specific tumour antigens. Trastuzumab, brand name Herceptin ®, is a mAb which targets the tumor antigen HER2. Patients with HER2-positive tumours receive chemotherapy and trastuzumab. Some immune cells such as NK cells and macrophages have receptors that recognize the Fc portion of mAbs like trastuzumab. This interaction starts a process that releases cytotoxic granules from the immune cells which induce tumor cell death. This mechanism is known as antibody dependent cell-mediated cytotoxicity (ADCC).

A strong ADCC response may contribute to the effectiveness of trastuzumab but there are multiple factors which may affect the ADCC capacity of this mAb in patients. My work is examining the effects of the chemotherapy given with trastuzumab and the immune modulatory elements present in the tumour microenvironment on trastuzumab-mediated ADCC using patient white blood cells and cancer cells grown in the lab.

Perfusion of the lung ex vivo – the shear stress of it all!

Keith Rochfort[1], Simon Coyle Rowan[2], Lucie Poiuceau[2,] Phil Cummins[1], Paul McLoughlin[2]
1. School of Biotechnology/NICB, DCU 2. School of Medicine, Conway Institute, UCD

Isolated perfused lung (IPL) preparations have considerably advanced our knowledge and understanding of physiological lung function. Recently, ex vivo lung perfusion (EVLP), a technique similar to IPL, has facilitated the reconditioning of 'marginal' donor lungs enabling successful transplantation. Consequently, there is significant interest in optimising perfusate solutions used in both techniques to preserve physiological lung function ex vivo.

The pulmonary capillaries are the primary site of oedema formation. By mimicking the viscosity and flow rate of the perfusate to that of blood, the hemodynamic environment observed in the capillary bed was recapitulated in in vivo and in vitro models with data obtained indicating solution viscosity has a direct effect on endothelial barrier function, and as such, pulmonary health.
Our data demonstrates perfusion of an isolated lung ex vivo with a physiological viscosity solution can attenuate oedema development, thus prolonging the duration that physiological lung function could be preserved ex vivo.

Novel mechanisms of resistance to HER2-targeted therapies in breast cancer

[1]Neil Conlon, [1]Martina McDermott [1,2]John Crown, [1]Norma O'Donovan
[1]National Institute for Cellular Biotechnology, Dublin City University, Glasnevin, Dublin,
[2]Department of Oncology, St. Vincent's University Hospital, Elm Road, Dublin 4

HER2-positive breast cancer accounts for approximately 20% of all breast cancers and is characterised by over-expression of HER2. Despite the success of HER2-targeted therapies, such as trastuzumab and lapatinib, innate and acquired resistance remains a clinically relevant problem. The aim of this study was to examine the potential therapeutic benefit of PP2A inhibition in HER2-positive breast cancer.

Previous work in our lab showed that PP2A plays an important role in acquired lapatinib resistance. Two lapatinib resistant cell lines were generated by continuous exposure to lapatinib *in vitro*. Both of these resistant cell lines showed increased sensitivity to PP2A inhibition, with LB-100, compared to their parental cell lines. LB-100 was also synergistic in combination with lapatinib in the resistant cell lines. This combination prevented or delayed the development of resistance to lapatinib in long term culture.

Evaluation of miR-toehold switches as transgene expression modulators in CHO cells

Ricardo Valdés-Bango Curell[1], Nga Lao[1], Nicole Borth[2], Niall Barron[1]
1) National Institute for Cellular Biotechnology, Dublin City University, Ireland
2) Department of Biotechnology, BOKU University of Natural Resources and Life Sciences Vienna, Austria.

Current methods for controlled gene expression CHO cells include a few inducible promoters/transcription factors, most of which require the addition of chemicals to the culture medium to switch ON/OFF gene expression (e.g. TET-Promoter-responsive elements). MicroRNAs (miRNAs) play an important role in the regulation of gene expression at a post-transcriptional level. Their main mechanism of action implies binding to the UTRs of their target mRNA molecules and down-regulate gene expression by inhibiting translation. The ability of these molecules to regulate complex gene networks has made them very interesting targets for cell engineering and can potentially be used as an endogenous trigger for controlled gene expression. In this work, different types of synthetic genetic constructs containing miRNA-responsive elements were designed and characterized in CHO cells, in order to assess their ability to drive miRNA modulated gene expression.

Strategy for enhancing immunosensor performance by optimally engineering antibody binding and format

Caroline Murphy, Edwina Stack, Svetlana Krivelo, Mark Breheny, and Richard O'Kennedy.
School of Biotechnology and National Centre for Sensor Research

Immuno-sensory devices utilise antibody-based sensors for analyte detection. In the case of environmental-toxin monitoring applications, recombinant antibodies have great utility as their affinity or production can be refined and optimized. Herein, two aspects of the binding of harmful algal-toxin microcystin-leucine-arginine (MC-LR) to a single chain fragment variable (scFv) recombinant antibody were investigated. Firstly, the antibody-antigen-binding interactions were analyzed *in-silico* by examining the detailed sub-structure of the scFv, and secondly the effect of the introduction of an auxiliary antibody domain to ascertain any change in binding was demonstrated.

Taken together, these measures provided a deeper knowledge of the scFv sub-structure and identified key amino acids and regions essential to the toxin-antibody binding interaction. The results obtained demonstrated the enhancement of a biorecognition element due to single amino acid changes, as well as the improvement of immunoassay range by changing the scFv to a single chain Antibody (scAb).

Proteomics and posttranslational regulatory networks in Chinese Hamster ovary cells.

Prashant Kaushik, Michael Henry, Paula Meleady.
National Institute for Cellular Biotechnology, Dublin City University.

Although more the 70% of biotherapeutics are currently being produced in Chinese hamster ovary (CHO) cells, cell signalling networks in this cell factory are poorly understood. Identifying phosphoproteins is key to understanding such pathways as phosphorylation is one of the major mechanisms of cell signaling. However, low abundant phosphoproteins are often masked by high abundant proteins or total unphosphorylated protein present in the cell. Hence, it is necessary to enrich proteome fractions for phosphoproteins/phosphopeptides prior to LC-MS/MS analysis. In this study we present an optimized method for phosphopeptide enrichment using Metal oxide affinity (TiO_2) and Immobilized metal affinity (Fe-NTA) chromatography, which provides phosphopeptide coverage by up to 75% allowing us to study low stoichiometric phosphorylation events. Using this method, an initial temperature shift study on CHO SEAP cells revealed more than 400 uniquely differentially expressed phosphopeptides.

Poster Presentations

Control of miRNA in Chinese Hamster Ovary Cells

Alan Costello, Niall Barron, Martin Clynes
NICB, DCU

Chinese hamster ovary (CHO) cells are the workhorse of the biopharmaceutical industry. Manipulation of miRNA to enhance CHO cell productivity has successfully been implemented (Kelly et al, 2015, Fischer et al, 2014, Sanchez et al, 2013). The use of miRNA-sponges (Ebert et al, 2007; 2010) is one such engineering strategy. Recent findings highlighting the abundance of endogenous circular RNA (circRNA) in human and mouse (Jeck et al, 2013) and functional validation of numerous circRNA species as miRNA-sponges (Hansen et al, 2013, Memzcak et al, 2013) point towards new genetic engineering targets. Naturally occurring circRNA sponges demonstrate greater stability over their linear counterparts, and artificial circularization of exonic sequence has been achieved (Hansen et al, 2013, Li et al, 2015). Coupling this with the advantage of reduced translation burden exhibited by currently used exogenous miRNA-sponge decoys, endogenous and artificial circRNA sponges have potential to join the CHO cell engineering toolbox.

Biological Evaluation of Lipoxin A_4 Analogues

[1]Alisha McLoughlin, [1]Catherine Maingot, [2]Monica deGaetano, [3]Catherine Tigue, [3]Denise Moran, [2]Catherine Godson, [3]Patrick Guiry, [1]Christine Loscher
[1]Immunomodulation Research Group, School of Biotechnology, Dublin City University, Glasnevin, Dublin 9, Ireland.
[2]School of Medicine and Medical Sciences, Diabetes Complication Research Centre, UCD Conway Institute, University College Dublin, Belfield, Dublin 4, Ireland.
[3]Centre for Synthesis and Chemical Biology, School of Chemistry, UCD Conway Institute, University College Dublin, Belfield, Dublin 4, Ireland.

Objective: To monitor the anti-inflammatory capabilities of chemically synthesised imidazole- and isoquinoline-containing lipoxin A_4 analogues to the native-derived eicosanoid.

Methods: Synthetic analogues of lipoxin A_4 were derived by replacing certain functionalities with imidazole and isoquinoline stabile motifs. Murine T-cell subsets were treated for 24 hrs with 0.1 and 1000 nM concentrations of the generated lipoxins, to which cell viability was tested using MTS assays and cytokine secretion measured using immunoassays.

Results: Both imidazole- and isoquinoline-containing lipoxin A_4 analogues proved non-toxic to naïve T-cells following 24 hrs treatment. The immunoassays confirmed that the imidazole- analogues were effective in damping the Th1 immune response, as confirmed using naïve, Th1 and Th17 subsets, whilst the isoquinoline analogues recorded a significant reduction of the Th2 stimulating cytokines using the upper dose of 1000 nM.

Conclusion: The synthesis of imidazole- and isoquinoline lipoxin A_4 analogues show promising indications of dampening both the Th1 and Th2 immune response in murine T-cells. It is imperative that overall immune balance is achieved in order to model an effective anti-inflammatory treatment strategy.

Generating an anti-AGR2 x CD3e bispecific T-cell engager (BiTE) for the improved targeted treatment of pancreatic cancer

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Pancreatic cancer (Pa) is a lethal malignancy with a 5-year survival rate of ~5%. Only ~15% of patients present with resectable cancer. The majority suffer a recurrence, accounting for the near 100% mortality rate. Pa has the capability to evade the immune system and is resistant to traditional chemotherapy regimes. Alternative treatment options must be explored. A bispecific T-cell engager redirects the immune system towards the tumour cells by linking them with a T-cell. This activates the T-cell and produces a cytolytic synapse between the cells resulting tumor cell death. Anterior gradient 2 (AGR2) is a protein that is absent from normal tissue and overexpressed in Pa cancerous tissue rendering it an ideal marker for targeted therapy of Pa. This project aims to generate a BiTE that binds AGR2 on a tumour cell and CD3e on a T-cell for the improved targeted treatment of Pa.

Sensing Crop Pathogens: A novel surveillance system for crop diseases of economic importance

Arabelle Cassedy, Richard O'Kennedy
Teagasc, Tyndall Institute UCC

The potato and barley industries have suffered substantial losses due to reduced yields as a result of viral and fungal infections. Large amounts of chemicals are often used as the main method of preventing such crop diseases. Unfortunately, these chemical methods have a severe impact on the environment and, therefore, the use of other methods to prevent crop diseases is strongly encouraged by bodies such as the EPA and the EU. My project aims to generate an antibody-based biosensor which could easily be used as a point-of-use diagnostic method, so that diseased crops would be identified immediately in the field and could be removed from the crop population before spreading. The research carried out in this project could directly benefit potato and barley production, reduce pesticide use and enhance food quality, in Ireland and globally.

Development of a serum-free media for CHO cell lines

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NICB

Single-cell cloning is an essential step used by the biopharmaceutical industry in the upstream development of transformed cell lines for therapeutic protein production, enabling the selection of the most productive clones from mixed populations. Low cell densities observed at this early stage, resulting from lowered cloning efficiencies or even lack of cell growth, cost the industry time and money. To address this problem, we seek to develop a highly-efficient serum-free media suitable for single cell cloning of several CHO cell lines. Combining a standard basal media with selected concentrations of supplements, we have developed a media which displays improved cell density performance when compared to commercial media. Further analysis of metabolism and productivity will be done to optimise the product, which will be then tested for single-cell cloning assays.

DCU Policy on the Use of Animals for Scientific or Educational Purposes

Damian O'Donohue, MVB, MVM, MRCVS (Animal Care & Welfare Officer)
WATERSPOUTT project & EU horizon 2020

Dublin City University (DCU) places a high value on maintaining the highest standards of animal welfare, while still recognising the benefit, to man, animals and the environment, that can be derived from the use of animals for scientific or educational purposes. In September 2016 the University formally adopted a Policy that provides guidance on how best to balance these potentially conflicting ideals. The poster will be used to present that policy, to elaborate on the "3Rs" on which the policy (and European and Irish legislation) is based, and to outline the system that is used to implement the policy at a practical level in the University.

The protective effect of TRAIL on RANKL-induced vascular calcification in an *in vitro* co-culture model

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Vascular calcification (VC), often referred to as “hardening of the arteries”, is prevalent in type-2 diabetic patients, causing elevated risk of cardiovascular events such as heart attack or stroke. Three key circulatory glycoproteins, namely osteoprotegerin (OPG), receptor-activator of NF-kB ligand (RANKL) and tumour necrosis factor-related apoptosis-inducing ligand (TRAIL), are believed to co-interact to regulate VC. Specifically, RANKL promotes VC, whilst OPG ameliorates it; however, although TRAIL is believed to be anti-calcific, its vascular function is less defined. We therefore hypothesized that TRAIL exerts a “vasoprotective” influence on the vasculature by attenuating RANKL-induced calcification. Using a physiologically relevant *in vitro* co-culture model of human aortic endothelial and smooth muscle cells, **we demonstrate that TRAIL has the potential ability to block several of the pro-calcific effects of RANKL on the blood vessel wall (e.g. RANKL-induced BMP-2, NF- B/p52, alkaline phosphatase, and Sox9),** yielding valuable information on VC pathogenesis and on the potential therapeutic value of TRAIL in this context.

ClickGene: Click Chemistry for Future Gene Therapies to Benefit Citizens, Researchers and Industry

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Gene therapy is a growing field that aims to discover new molecules and therapeutics for personalised use within molecular medicine. In this context, the Marie Curie Innovative Training Network, ClickGene, is focused on the development of the next-generation gene silencing therapeutics and epigenetic DNAprobes. This project focuses on artificial metallonuclease (AMN) development, representing an intriguing choice of gene silencing agent given their ability to both target and oxidatively damage DNA. A new class of compounds has been designed to offer a unique mechanism for DNA cleavage compared with the current state-of-art gene editing nucleases. Furthermore, these newly developed AMNs will be tethered by “click” chemistry to sequence-selective nucleic acid targeting molecules such as zinc finger proteins and triplex forming oligonucleotides which can discriminate epigenetic bases. This project aims also to create customised liposomal nanoparticle and nanocontainer delivery vehicles for highly specific delivery of these AMN bioconjugates to the nucleus of selected human cells.

Potential Sources and Environmental Fates of Certain Phthalates

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Phthalate esters are a group of synthetic organic chemicals that are most commonly used as plasticizers in polyvinyl chloride (PVC) materials. Common end uses for PVC, and therefore phthalates, include tubing, blood bags, medical devices, clothing, flooring, packaging, toys, automobile parts, flooring, and roofing. As phthalates are so commonly used, their impact on the environment and human health has been extensively studied. Phthalates have been found to be recalcitrant, ubiquitous within the environment, and in many cases, detrimental to human and animal health. This project represents an important collaboration between three research centres (DCU, ASU, & NIVA) with support from Irish Water, Panda and Fingal Co. Co., to assess the potential sources and environmental fates of priority phthalates in Ireland. This project is supported by several by studies carried out in Ireland already (DCU) and a vast array of literature in the area of priority pollutant monitoring. The impact of such study would be the analysis of these eleven phthalates from source to fate, in order to inform environmental policy on the risks posed by phthalate usage.

The phthalates proposed for investigation are Benzylbutylphthalate (BBP), Dibutylphthalate (DBP), Dipentylphthalate (DPP), Diisopentylphthalate (DIPP), Diethylhexylphthalate (DEHP), Dihexylphthalate (DHP), Diisobutylphthalate (DIBP), Di-n-octylphthalate (DNOP), Diisononylphthalate (DINP), Diisodecylphthalate (DIDP) and Dimethylphthalate (DMP). Research into the human health effects of phthalates is far from complete, and while phthalates including DBP, BBP, and DEHP have been banned or limited in manufacturing (in particular for items such as children's toys), new research is emerging which indicates that substitute plasticizers have similar deleterious health effects. This research is timely as the extent of phthalate contamination within Ireland, and the impacts on human health, are unknown.

RNAi screening with self-delivering, synthetic siRNAs for identification of genes that regulate primary human T cell migration

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Screening of siRNA libraries in primary T cells is labour-intensive and technically challenging because these cells are hard-to-transfect. Chemically-modified, self-delivering siRNAs offer a solution to this problem, as they enter into hard-to-transfect cell types without the need of a delivery reagent and are available in library format for siRNA screening. We have screened a library of chemically-modified, self-delivering siRNAs targeting the expression of 72 distinct genes in conjunction with an image-based HCA platform as a proof-of-principle strategy to identify genes involved in LFA-1-mediated migration in primary human T cells. Our library screening strategy identified the small GTPase RhoA as being crucial for T cell polarisation and migration in response to LFA-1 stimulation and other migratory ligands. This study therefore demonstrates the ease and benefits of conducting siRNA library screens in primary human T cells using self-delivering siRNAs and their utility for elucidating signalling pathways that regulate T cell function.

Hypoallergenic and Immunomodulatory Hydrolysates for Infants Diagnosed with Cow's Milk Protein Allergy

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Cow's milk protein allergy (CMPA) is the most common food allergy associated with infants and can affect 2-3% of children worldwide. Persisting CMPA can lead to asthma, allergic rhinitis and anaphylaxis in later life. Strict cow's milk (CM) protein avoidance has been the standard therapy of CMPA, but oral immunotherapy (OIT) seems to provide an alternative treatment. OIT results in avoidance of clinical symptoms by providing the infant with hydrolysed proteins that lack the IgE binding epitopes. OIT can result in tolerance which enables the mucosal immune system to remain in a non-activated state when it encounters harmless proteins, such as CM, while retaining its ability to mount an immune response to a potential pathogen.
The aim of this study is to identify novel bovine milk-derived protein hydrolysates that can modulate the immune response away from an allergic type 2 helper T-cell (Th2) response and towards a regulatory T-cell (Treg cell) phenotype in vitro. Treg cells are the key cells involved in the induction of tolerance. The candidate hydrolysates will then be brought forward for in vivo testing in a murine model of allergy in order to determine their effectiveness to induce oral tolerance to food allergy.

Influenza and Secondary Bacterial Infections: How to Tackle Antimicrobial Resistance?

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Predictions for causes of death imply that mortality due to antimicrobial resistance will surpass cancer worldwide by 2050. It is well accepted that influenza A virus predisposes individuals to more severe superinfections with bacteria such as *Streptococcus pneumonia (Sp)*. However, the mechanisms are not clearly understood. We aim to increase our understanding of how influenza may alter immune responses to *Sp*.

We found that primary human immune cells infected with influenza significantly showed impaired defence against Sp. Cells that were not infected with influenza mounted robust immune responses to *Sp*.

These results suggest that immunological suppression by influenza prevents appropriate immune priming to *Sp*. They also infer a mechanism whereby the immune deficiency may be tackled by reinstating immunological arms of defence rather than using antibiotics. This may be used in patients with compromised immunity and in cases of infection with multidrug resistant strains of bacterium.

eCHO Systems – Enhancing the CHO Cell Factory through Systems Biology

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The eCHO Systems Innovative Training Network (ITN) is a collaborative effort of four academic and 15 industrial partners, jointly training 15 PhDs, seeking to significantly advance the CHO cell platform for production of biopharmaceuticals. Chinese hamster (CHO) ovary cells are the production host for a +50 billion €/yr biopharmaceuticals market. Our primary goal is the development and application of synthetic and systems biology tools to develop the next generation of these cell factories.