



conway

focus

Enzyme chemistry research wins 2014 UCD Conway Festival medal

PhD student, Jennifer Cleary has been awarded the 2014 UCD Conway Festival medal for research using a technique called nuclear magnetic resonance (NMR) to figure out how chymotrypsin works and how to inhibit its action.

Chymotrypsin is a digestive enzyme that breaks down proteins in the gut and falls into the category of serine proteases. Jennifer is using chymotrypsin as a model of serine proteases.

Frequently, research involves doing extensive ground work using a molecule or organism that is similar to what you actually want to investigate in humans. Scientists can then apply learnings from working with a model to human cells.

Jennifer Cleary is interested in how cancer spreads in the body. In particular, she wants to find out how the serine protease, urokinase or urokinase-type plasminogen activator (uPA) is involved in the process of tissue breakdown that can allow cancer cells to invade and spread around the body.

"The research findings that I presented at the 2014 UCD Conway Festival are a small

part of my wider doctoral research project focusing in on the active site or boiler room of the chymotrypsin molecule".

There are three key amino acids or residues located in this active site that make chymotrypsin function; His-57, Ser-195, Asp-102. The NMR technique allows Jennifer to actually see the connections between residues. She identified hydrogen atoms involved in hydrogen bonds between His-57 and Ser-195 as well as between His-57 and Asp-102.

"I also used proton NMR to see what happens at the active site to these residues when I added an aldehyde inhibitor to chymotrypsin to try to stop it working". In order to make the serine residue reactive, the histidine residue needs to pull away a proton from the serine hydroxyl group. Histidine needs to increase its affinity for the serine proton to pull it away. This measure of affinity for the proton is the pKa. Jennifer's research has shown that the level of affinity or pKa does not have to be as high as previously thought to increase the reactivity of the catalytic serine by ~6000.

For the first time, using a particular

software programme with the NMR technique, Jennifer was able to determine the pKa of free chymotrypsin at 25^o C. This is a more biologically relevant temperature than the previous studies done at 3-4^o C.

Jennifer Cleary works in the research group of Conway Fellow, Professor J. Paul G. Malthouse of UCD School of Biomolecular & Biomedical Science and is funded through the Irish Research Council.



Professor Andrew Deeks, UCD President & Jennifer Cleary, 2014 UCD Conway Festival medal winner

Director's Message

Welcome!

The 2014 UCD Conway Festival of Research & Innovation took place on September 10th & 11th in the UCD Charles and UCD Conway Institutes. This year, the Festival was expanded to give particular focus to the excellent work being done by our early career researchers. The new poster review session gave our graduate and postdoctoral researchers a platform to discuss their research with teams of

senior scientists and was an overwhelming success. Professor Andrew Deeks, UCD President presented Jennifer Cleary, PhD student from the Malthouse group with the 2014 UCD Conway Festival gold medal sponsored by Cruinn Diagnostics.

I would like to welcome Dr Eoin O'Cearbhaill, UCD School of Mechanical & Materials Engineering as a new Conway Fellow. Eoin works in the area of medical devices with a particular focus on targeted,

minimally invasive delivery of next-generation therapeutics. He has a number of existing collaborations with Conway Fellows and I have no doubt that he will be strengthening and extending those through his Institute fellowship.

Professor Walter Kolch
Director

Highly organised protein structure implicated in schizophrenia

Schizophrenia is associated with enormous personal, familial and societal cost. Exactly how genetic and environmental risk factors act together to lead to the development of schizophrenia is as yet unknown. As a result, current treatments target symptoms rather than underlying causes and do not achieve complete remission.

Researchers in UCD, the Royal College of Surgeons in Ireland and Beaumont Hospital have published findings in *Molecular Psychiatry* that implicate proteins and genes associated with the postsynaptic density (PSD) in schizophrenia. This improved characterisation may suggest novel treatments to target this structure in the future.

The PSD is a complex network of proteins with roles in cell scaffolding and signalling that are critical to the normal transmission of neural messages and to adaptive behaviours like learning and memory.

There is strong evidence to support the view that the synaptic neurotransmitter, N-methyl-D-aspartate (NMDA) receptor hypofunction contributes to the pathophysiology of schizophrenia. It has been proposed that the PSD may contribute to this through dysregulation of NMDA receptor recycling.

The team firstly enriched for the PSD in the anterior cingulate cortex in schizophrenia and control tissue obtained from the Stanley Medical Research Institute before using liquid chromatography-mass spectrometry to quantify differential protein expression.

"We combined proteomic and genomic methods to characterise postsynaptic density-associated protein expression in schizophrenia. We found more than 700 protein identifications and 143 differentially expressed proteins in the PSD. Our pathway analysis of these differentially expressed proteins implicated the cellular processes of endocytosis, long-term potentiation and calcium signalling", said Conway Fellow, Dr Gerard Cagney.

The resulting data provides robust evidence implicating PSD-associated proteins and genes in schizophrenia and suggest that NMDA interacting and endocytosis related proteins within this multiprotein complex contribute to disease pathophysiology.

"Our study may provide the first evidence pointing towards pharmacological manipulation of (Clathrin-mediated) endocytosis as a novel treatment for schizophrenia", outlined Dr Melanie Föcking, first author and postdoctoral researcher with Prof David Cotter in the Department of Psychiatry, Royal College of Surgeons in Ireland.

Reference
Föcking M, Lopez LM, English JA, Dicker P, Wolff A, Brindley E, Wynne K, Cagney G, Cotter DR. Proteomic and genomic evidence implicates the postsynaptic density in schizophrenia. *Mol Psychiatry*. 2014 Jul 22. doi: 10.1038/mp.2014.63.

Starting investigator research grant awards

Research Fellow, Dr Alex von Kriegsheim in Systems Biology Ireland (SBI) has recently been awarded two substantial grant awards from Science Foundation Ireland and the UK charity, Breast Cancer Campaign that will see him become a fully independent researcher.

Dr Alex von Kriegsheim joined SBI as a postdoctoral researcher in 2009 from the Cancer Research UK Beatson Laboratories in Glasgow, where he worked under current SBI and UCD Conway Institute Director, Prof Walter Kolch. The molecular mechanisms driving cancer initiation and progression are at the core of his research interests.

Under a Science Foundation Ireland Starting Investigator Research Grant (SIRG) award, Alex aims to develop new treatments to help prevent bowel cancer in patients with colitis and Crohn's disease. Both conditions lead to chronic inflammation of the gut that can in turn increase the risk of bowel cancer.

Alex hopes that his research can identify the ways in which this inflammation causes the growth of cancer cells and how

the process can be halted through the release of enzymes known as hydroxylases that are blocked in chronically inflamed tissues. He will investigate if a novel strategy of treatment using modulators of hydroxylase activities is effective in preventing the progression from inflammation to cancer.

Breast Cancer Campaign awarded Alex von Kriegsheim £200,000 to fund research on the role played by a small protein in breast cancer metastasis. Cancer mortality is closely linked to the ability of tumour cells to spread throughout the body and form secondary tumours. During this process, individual cells detach from the primary tumour mass, invade the surrounding tissue and spread throughout the body by infiltrating the lymph and blood system.

The three year study, in collaboration with SBI and UCD Conway Institute colleagues Dr Lan Nguyen, Dr Alfonso Bolado and Prof Liam Gallagher, will combine mathematical modelling with biomolecular and cell biology techniques to understand how over-expression of a small protein, ISG15, affects breast tumour and metastasis-promoting

signalling pathways. It is hoped that a better understanding of how ISG15 works will potentially pave the way for drugs that inhibit its activity and that of the proteins it works with.



Dr Alex von Kriegsheim, Systems Biology Ireland

Major new resource for fungal research

Researchers led by Conway Fellow, Prof Geraldine Butler have constructed a set of tools that will be of huge benefit to the scientific community as they try to understand how the fungal pathogen, *Candida parapsilosis* causes infection.

Candida species are among the most common causes of fungal infection globally. *C. parapsilosis* causes infection outbreaks in neonatal wards and is one of the few *Candida* species that is transferred from the hands of healthcare workers.

C. parapsilosis grows as biofilms or living mats of cells on the surface of indwelling medical devices such as feeding tubes. Until now, very little was known of the virulence properties of the pathogen and current treatment regimens involve removing the infected indwelling device.

"Our aim in this study was to characterise

the genes needed for biofilm development using a genetic approach. In order to find out how a gene works, a geneticist will often remove it or knock it out to see what happens as a result. We created a collection of 200 *C. parapsilosis* strains carrying knockouts in regulatory genes, and we identified those required for biofilm development", explained Prof Geraldine Butler.

The research team then looked at the impact on growth under various conditions such as temperature and the presence of anti-fungal drugs when particular regulatory genes are missing.

"We found similarities between *C. parapsilosis* and the most commonly isolated species, *C. albicans* but some regulators have major roles only in *C. parapsilosis*. Two particular transcription factors, Cph2 and Bcr1 are major biofilm

regulators in *C. parapsilosis* only. Perhaps more importantly, we created a major research resource that will be accessible to the fungal research community globally", said Prof Butler.

Reference

Holland, LM et al. Comparative Phenotypic Analysis of the Major Fungal Pathogens *Candida parapsilosis* and *Candida albicans*. *PLoS Pathogens* September 18, 2014 doi: 10.1371/journal.ppat.1004365.



Candida parapsilosis strains growing in the presence of antifungal drugs

A novel therapy for common and aggressive brain tumour

New research findings from an international collaboration involving Conway Fellow, Dr Margaret Mc Gee and researchers at Vall d'Hebron Institute of Oncology (VHIO), Spain may ultimately provide glioma patients with new therapeutic avenues. The work also reveals the potential of exploiting our innate oncosuppressive mitotic catastrophe programme in cancer therapy.

Glioma is a highly aggressive tumour of the adult central nervous system that outsmarts current anti-cancer therapies. Typically, these tumours have elevated levels of Myc protein.

The Myc protein plays an important role in regulating gene transcription, controlling the expression of up to 15% of human genes. It is also implicated in vital cellular processes such as proliferation, differentiation and

apoptosis. However, Myc is deregulated in the majority of cancers where it triggers uncontrolled cell proliferation. This study provides the most conclusive preclinical results to-date validating Myc inhibition as a therapeutic strategy in glioma.

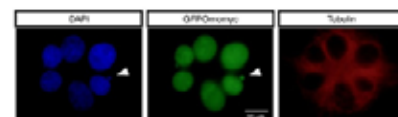
The researchers applied the Myc inhibitor, Omomyc to human glioblastoma cell lines and mice with patient-derived tumour xenografts. With a similar structure to Myc, Omomyc can block the transcription of genes controlled by this protein. Thus, loss of Myc function blocks the ability of tumour cells to divide efficiently.

"In my laboratory, we focus on understanding the molecular mechanisms involved in mitotic catastrophe, an innate mechanism to remove genomically unstable cells, with a view to exploiting the process in new anti-cancer therapies. In this study, the team showed that Myc

inhibition leads to aberrant mitosis and the formation of multinucleated cells that eventually died through the process of mitotic catastrophe, clearly highlighting the therapeutic potential in this very aggressive cancer", explained Dr McGee.

Reference

Annibali D et al. Myc inhibition is effective against glioma and reveals a role for Myc in proficient mitosis. *Nat Commun.* 2014 Aug 18;5:4632. doi: 10.1038/ncomms5632. PMID: 25130259



A representative image of a multinucleated Omomyc-expressing cell: GFP (green) immunofluorescence and DAPI nuclear counterstain (blue) show GFP-Omomyc expression, α -tubulin staining (in red) shows that all nuclei belong to the same cell. The arrowhead indicates a typical micronucleus.

Targeting survival protein in immature leukaemia

New research has shown that a high risk form of leukaemia is dependent on a survival protein BCL-2 and is very sensitive to treatment with drug specific to this protein target.

Research Fellow, Dr Triona Ní Chonghaile used a new technology, BH3 profiling to measure how sensitive mitochondria are to cell death. Given that mitochondria are the power house within cells, their destruction leads to cell death.

Leukaemia arising in an immature cell (early T-cell progenitor acute lymphoblastic leukaemia or ETP-ALL) is a treatment resistant subtype of this

disease of blood cells. There is a pressing need for more effective treatment options for patients to improve response along with minimising the adverse side effects of the cytotoxic agents.

"Using BH3 profiling, we discovered that ETP-ALL is dependent on a survival protein BCL-2 and is very sensitive to treatment with a BCL-2 specific inhibitor named ABT-199, a drug well tolerated in clinical trials. This is the first time that the maturation stage of a cancer has been shown to determine dependence on a survival anti-apoptotic protein", said Dr Ní Chonghaile.

"We used patient derived samples and leukaemia cell lines in this study, the next step is to assess the clinical benefit of ABT-199 in patients with this high-risk disease".

Dr Triona Ní Chonghaile joined the research team of Conway Fellow, Professor Liam Gallagher earlier this year from the Dana-Farber Cancer Institute and Harvard Medical School.

Reference

Ní Chonghaile et al. *Cancer Discovery* September 2014 4:1074-1087

Targeting angiogenesis in eye disease

It is common in eye diseases such as proliferative diabetic retinopathy or wet age-related macular degeneration to develop abnormal or leaky blood vessels. Current treatments to treat this problem are costly and require injections into the eye area with increased risk of infection as a result.

The process of developing of new blood vessels from pre-existing vessels (angiogenesis) is a tightly controlled cascade of molecular events. One particular signalling pathway, the phosphatidylinositol (PI3K)/Akt/mammalian target of rapamycin (mTOR), is a candidate therapeutic target for angiogenesis.

Some PI3K/Akt/mTOR pathway drugs have successfully progressed into clinical use in human cancer. However, there are issues in relation to toxicity that arise after systemic delivery. These drugs may be more useful in treating eye disease given their potential for local delivery as eye drops.

Conway Fellow, Dr Breandan Kennedy and his team have recently published findings in PLoS One of research evaluating the safety and efficacy of various combinations of these pathway drugs targeting angiogenesis in a disease model.

"We proposed that we could enhance

the effectiveness of drugs targeting angiogenesis within the eye by simultaneously hitting multiple nodes or points in the signalling pathway that controls the process. We ranked NVP-BEZ235 + PI-103 as the most effective and safest combination. Now, we need to quantify these effects on human retinal endothelial cells in vitro", said Dr Kennedy.

Reference

Sasore T, Kennedy B (2014). Deciphering combinations of PI3K/AKT/mTOR pathway drugs augmenting anti-angiogenic efficacy in vivo. PLoS ONE 9(8):e105280. doi:10.1371/journal.pone.0105280

UCD Images of Research 2014

Emer Conroy, Research Engineer in the Cancer Biology & Therapeutics laboratory, co-led by Conway Fellow, Prof William Gallagher and Dr David Orr, UCD Bat Research Centre led by Conway Fellow, Prof Emma Teeling were one of twelve shortlisted entries in the 2014 UCD Images of Research competition.

Their image is of a lesser horseshoe bat viewed from two angles and taken using

a 3D x-ray technique called computerised tomography, which makes the bone structure and soft tissue information visible. In certain species of bats, the

pre-maxilla (bone at the tip of the upper jaw) is free floating and potentially movable, whereas in most other bats and in all other mammals, the pre-maxilla is fused. This means that bats are potentially a great model to explore the development of cleft palate.



Na, na, na, na, na, na..... Bat-scan!

Success for obesity research in Thesis in 3

Aoife Murphy, PhD student with Conway Fellow, Prof Helen Roche won 2nd place at the 2014 Thesis in 3 UCD regional competition heat with her talk; 'Obesity: It's InFATuating'. Aoife will now represent UCD at the national final on October 20th in the Sugar Club, Leeson St, Dublin.

Aoife describes obesity as being similar to having a small tummy bug that never goes away. Certain fatty foods cause stress in our body the same way that a nasty bug does. The immune system tries to defend itself by inflaming our body. In

obesity, this ongoing inflammation leads to problems such as type 2 diabetes.

Thesis in 3 competition, led by Systems Biology Ireland and Insight Centre, challenges postgraduate students from research institutions across Ireland to present their research in a series of concise, rapidly paced talks consisting of 3 slides, in 3 minutes.

Free tickets on <http://www.thesisin3.com>



(L-R) William Fitzmaurice, SBI; Philip Smyth, UCD Access Centre; Suzanne Hayden, SMMS; Dr Barbara Dooley, Dean of Graduate Studies & Deputy Registrar; Aoife Murphy (2nd); Darragh Whelan (1st), Humberto Corona (3rd); Barry Brophy, SMME; Dr Aoibheann Bird, Insight Centre

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