A proteomic future

Associate Professor Sze Siu Kwan Newman discusses his exciting multidisciplinary work at the Nanyang Technological University, which primarily focuses on quantitative proteomics research

As an introduction, could you describe what inspired your passion for proteomics?

My curiosity about the beauty and complexity of protein structures, properties and functions led me to this field. While the protein amylase was discovered and studied as early as 1833, protein in general remains a class of complex molecules whose function and interconnections are not fully understood. For example, protein function is critically regulated by post-translational modifications (PTMs). This cannot be explained at the genomics level at this time and must be studied at the protein level. It may take another 100 years to fully understand the connections behind protein PTMs and their roles in human diseases.

Mass spectrometry-based proteomics is currently the most powerful technology to decode protein structure, regulation, PTM and functions. Despite the strengths of this technique and the great progress made over the past decade, research in proteomics remains challenging, requiring diverse skills with instrumentation, protein chemistry, bioinformatics, etc. I have benefited from my multidisciplinary research background in chemistry, chemical physics, protein chemistry and mass spectrometry.

What is the focus of your current research project?

Our goal is to develop new proteomic methods to uncover answers pertaining to biological and biomedical questions that are difficult or impossible to study with traditional biochemistry methods. We conduct multidisciplinary research which applies proteomics to: deciphering human disease, including cardiovascular diseases, stroke and cancer; elucidating epigenetic regulatory mechanisms and chromatin structure by quantitative profiling of the chromatin proteome; and characterising microbial biocatalysts for viable biorefinery processes, as well as producing renewable bioenergy and valuable fine chemicals.

What influenced your decision to study the three major lethal diseases – heart disease, cancer and stroke?

Over 60 per cent of annual deaths in Singapore and other developed countries can be attributed to these diseases. It seemed reasonable to study them, as the ultimate aim of our research is to apply proteomic technology to solve biomedical and biological problems which impact on human health and benefit the widest population.

Can you outline some of the novel proteomic methods you have developed?

We aim to develop proteomic methods to address the most challenging and important biological questions. We focus on methods for investigating membrane protein conformational changes, during ligand-receptor interaction in relation to its biological functions, and studying protein PTMs such as deamidation, glycosylation and phosphorylation in normal physiology, disease pathology and ageing.

To this end, we have introduced laser photolytic hydroxyl radical footprinting for elucidating conformational changes of membrane proteins directly on the plasma membrane of living cells. In addition, electrostatic repulsion-hydrophilic interaction chromatography (ERLIC) – a mixed-mode method originally developed by Dr Andrew Alpert – has been further developed in my laboratory for comprehensive profiling of proteomes and various PTMs.

With your findings recognised by researchers and prominent institutions, what is your proudest achievement to date?

I am proud of many of the research achievements of our group in a number of areas. When I joined Nanyang Technological University (NTU) in 2006, I was lucky to work with a great team of PhD and FYP students, who built the foundation and infrastructure for our subsequent studies on hypoxia and reperfusion of various disease models.

We are also the first laboratory to quantitatively profile the secretomes and cellular proteomes of cancer cells at various instances of hypoxia and reoxygenation stresses. The quantitative proteomic results revealed that the tumour cells not only secreted proteins involved in angiogenesis, focal adhesion, extracellular matrix-receptor interaction and immune modulation, but

Detecting fatal disease

Researchers from the Nanyang Technological University, Singapore are developing proteomic methods that could help to diagnose and prevent a wide range of diseases, and even contribute to the development of better biofuel production processes in the future

also secrete exosomes with the potential to modulate the tumour microenvironment and facilitate angiogenesis and metastasis.

Since the establishment of your laboratory in the School of Biological Sciences, how has your research progressed? How do you hope your research will develop in the next few years?

I am fortunate to work with an excellent team of students, staff and collaborators, and to have had continuous funding support from various grant agencies. These favourable factors have given me opportunities to develop, as well as to initiate new directions in our research projects. Our research in the School of Biological Sciences is multi-dimensional, but is focused within the framework of proteomics research. In these areas, our research has been successful and highly productive so far. Our quantitative proteomic studies of ischaemia/reperfusion of heart disease, cancer and stroke in the past six years have revealed many hypoxia-perturbed pathways and have generated testable hypotheses.

For our research effort in the coming years, I hope to validate our findings, prove our hypotheses and translate the results to clinical applications.

PROTEINS ARE INCREDIBLY important in the body, acting as oxygen transporters, messengers, cell reproduction regulators and chemical catalysers, to name but a few. The study of all of the different proteins produced by a given organism is called proteomics, and is a relatively new field of study which, in the case of humans, encompasses over 2 million protein isoforms each performing a different role. There is currently a great deal of excitement in this field as the potential for proteomic technologies is vast: by analysing the human proteome for biosignatures in the body, indicating specific cell presence of certain proteins, scientists believe that they could one day link the underlying causes of various diseases.

Research headed by Associate Professor Sze Siu Kwan Newman at the School of Biological Sciences, Nanyang Technological University (NTU) is leading the way in creating an innovative path to help proteomic technology reach its full potential. Newman joined the School of Biological Sciences in 2006, after spending time working on emerging proteomic technology at the Genome Institute of Singapore. He is a keen innovator, and spent his formative years of research working with Professor Fred McLafferty at Cornell Univeristy, who pioneered numerous mass spectrometric and proteomic researches, and with Professor John Polanyi at the University of Toronto, who won the 1986 Nobel Prize in Chemistry for his work with chemical kinetics.

DEVELOPING TOOLS

Proteomic technology is still in its infancy as a disciplinary tool, and so establishing interdepartmental partnerships and adaptable, high-specification technological platforms is key to pushing the research to its full potential in diagnosing and treating patients. Newman's team works closely with clinicians and clinical scientists from the National University Hospital, the National Cancer Centre and have collaborated with a number of overseas researchers, including Professor Dominique de Kleijn from the Interuniversity Cardiology Institute of The Netherlands (ICIN). This pairing helps the researchers to translate their experimental output quickly into clinical applications, making their work more efficient and producing fast, high-impact results in real-world applications.

Another key development Newman and his colleagues are aiming to establish is a 'biobank' of clinical samples to aid the detection and cataloguing of biomarkers which signal the presence of disease. This biobank will contain patients' medical records and clinical data, allowing in-depth analysis of all of the biomarkers present and the ability to identify the presence of specific biomarkers, which can then be added to a proteomic library which will be widely available, allowing cross-referencing and international collaboration to become commonplace and vastly used. This, in turn, could lead to the identification of new biomarkers, and improve existing proteomic technologies and analysis.

DISEASE BIOMARKERS

Beyond the clinical biobank, Newman's group is in the process of detecting and analysing biosignatures related to three high-profile diseases – stroke, cancer and cardiovascular disease (CVD). This investigation has been extremely challenging, using a combination of cell lines and animal models alongside clinical data through their collaborations with clinical researchers. A key example of this is the analysis of hypoxic tumour cell excretions, which modulate their microenvironment to facilitate tumour angiogenesis and metastasis. Alongside the standard hypoxic conditions required to simulate this environment, the cells must also be cultured in an environment completely clear of foetal calf serum, a difficult task successfully completed by the team.

The argument for analysing biomarkers for CVD is particularly compelling. CVD is commonly caused by the development of atherosclerosis, where plaques made up of

VALIDATING CANDIDATE BIOMARKERS IN PLASMA MICROVESICLES FOR THE DIAGNOSIS AND PROGNOSIS OF LACUNAR INFARCTION

OBJECTIVES

To develop new methods in proteomic technology capable of uncovering answers pertaining to biological and biomedical questions that are difficult or impossible to study using traditional biochemical and molecular biology methods. These methods are being employed to:

- Decipher human diseases, including cardiovascular diseases, stroke and cancer
- Elucidate epigenetic regulatory mechanisms and chromatin structure by quantitative profiling of the chromatin proteome
- Characterise microbial biocatalysts for a viable biorefinery and for producing renewable bioenergy and valuable fine chemicals

PARTNERS

Chuen Neng Lee; **Vitaly Sorokin**; **Christopher Chen**; **Mitchell Lai**, National University Hospital, Singapore • **Dominique de Kleijn**, Interuniversity Cardiology Institute, The Netherlands • **Sai Kiang Lim**, Institute of Medical Biology, Singapore • **Kon Oi Lian**, National Cancer Center, Singapore • **Lim Swee Han**, Singapore General Hospital, Singapore • **Andrew Alpert**, PolyLC Inc, USA

FUNDING

National Medical Research Council • Biomedical Research Council • National Research Foundation of Singapore • Ministry of Education of Singapore • Johnson and Johnson

CONTACT

Associate Professor Sze Siu Kwan Newman
Director of Proteomics Facility

School of Biological Sciences
Nanyang Technological University
60 Nanyang Drive
Singapore 637551

T +65 6514 1006
E skszе@ntu.edu.sg

ASSOCIATE PROFESSOR SZE SIU KWAN NEWMAN received his PhD from the University of Hong Kong in 1995. Postdoctoral positions at the University of Toronto and the University of Waterloo in Canada, and Cornell University in the US followed before moving to Singapore in 2002 as a Group Leader at the Genome Institute of Singapore. In 2006, Newman joined Nanyang Technological University, Singapore. He is currently Associate Professor in the School of Biological Sciences.

NANYANG TECHNOLOGICAL UNIVERSITY

Proteomic technology is still in its infancy as a disciplinary tool, and so establishing interdepartmental partnerships and adaptable, high-specification technological platforms is key to pushing the research to its full potential in diagnosing and treating patients

fatty materials such as cholesterol builds up on the inner walls of arteries. Some patients will then develop unstable plaques which rupture, blocking the blood supply to downstream tissues and causing stroke, organ damage or acute myocardial infarction. This is known as thrombosis, and can initially appear similar to a heart attack during diagnosis. The ability to distinguish between the two during early stages of chest pain or to identify the likelihood of an attack will visibly improve survival rates of cardiovascular patients whilst simultaneously easing the pressure of diagnosis and treatment for emergency services: "Together with de Kleijn of ICIN, we have discovered a biosignature of protein biomarkers from atherosclerotic plaque and plasma microvesicles that contains information predictive of secondary cardiovascular events on top of traditional risk factors," enthuses Newman.

HYPOXIC TUMOUR ANALYSIS

This is not the only work that the group is doing which could have huge implications on the healthcare of critically ill patients. They have also been using biomarkers to examine the treatment of tumours using radiotherapy. Hypoxic solid tumours are known to be resistant to both chemotherapy and radiotherapy, which can lead to poor outcomes and difficulties during treatment. The underlying reasons for this resistance are not yet fully understood and so any progress in this field is extremely important.

It has been suggested that cells with low oxygen have increased changes in the incidence of DNA strand breaks, cross-linking between DNA strands and base damage, but until now this had never been proven. By using quantitative proteomic studies, the group has managed to finally reveal the mechanisms underlying these phenomena. They have demonstrated that hypoxia upregulates the nonhomologous end-joining (NHEJ) pathway, which plays a central role in repairing the DNA damage of irradiated cells.

BIOFUELS

In parallel with their health and medical investigations, Newman and his team have also been using their proteomics expertise to tackle wider problems. Examining the problems associated with the production of biofuel, they have focused specifically on the speed and efficiency of converting biomass to useable fuel sources.

Biofuels have been cited by many as a way to alleviate the pressures of the looming energy crisis, substituting dwindling fossil fuels for more abundant sources of biomass. Newman identified that one of the biggest challenges in this process is the bottleneck caused by the conversion of lignocelluslosic biomass to monomeric sugars and co-utilisation of lignin. By using proteomics to study microbial biocatalysts, the Singapore researchers hope to ease this pressure by helping to develop quicker and more efficient methods of conversion. They have particularly focused on biomass-degrading microbes and have identified some highly active examples from the rainforest and mangrove areas in Singapore. This discovery could help biofuels attain a desirable efficiency of production, and live up to the production targets needed to make a true impact on the current energy shortage.

PROTEIN POST-TRANSLATIONAL MODIFICATION

Future studies by Newman's group will focus on protein post-translational modification (PTM), including its implications on the ageing of the human body. They have already developed a reverse-phase coupling to electrostatic repulsion-hydrophilic interaction chromatography mass spectrometry (RP-ERLIC-MS/MS) strategy for the study of protein deamidation, which is strongly believed to be involved in ageing and degenerative diseases. However, protein PTM is drastically understudied due to its complexity, despite the significant benefits promised by a greater understanding of its occurrence and implications.

Protein PTMs are strongly related to some diseases' initiation and progression and many researchers have speculated that they are a molecular-level source of disease dysfunctions. A particular area of focus is the non-enzymatic deamidation of asparagine and glutamine. This process has been linked to the onset of many human degenerative diseases, including those affecting the liver and kidneys. The use of the team's extensive biobank of data means they are able to analyse clinical samples from patients with these diseases and fully characterise the deamidation proteome responsible. "This could have a meaningful impact on the prevention and treatment of degenerative diseases, leading to better healthcare and wellbeing in an ageing society," comments Newman.