Annual Scientific Meeting
7th – 8th of November, 2019
South Australian Health and Medical Research Institute (SAHMRI), Adelaide

Dear colleagues,

On behalf of the 2019 committee, and the Australian Society for Molecular Imaging, I would like to warmly welcome you to Adelaide for the Annual Scientific meeting of the Australian Society for Molecular Imaging.

South Australia has invested heavily in the past decade in biomedical research, resulting in different hubs in what is now called Adelaide BioMed City (adelaidebiomedcity.com), a hub for health and life sciences. It co-locates institutions from research, education and clinical care in a precinct in Adelaide: the University of South Australia, University of Adelaide, Flinders University, the South Australian Health and Medical Research Institute (SAHMRI) and the Royal Adelaide Hospital.

SAHMRI is our proud sponsor. In 5 years SAHMRI has grown into a thriving community of more than 450 staff and 300 partners. In the context of molecular imaging, there is a small animal imaging facility in North Terrace in addition to the Large Animal Imaging and Research Facility at PIRL (the South Australian Node of the National Imaging Facility) with the opportunity for pre-clinical molecular imaging. The TGA licensed cyclotron facility in the basement (MITRU) provides capability for labeling different molecules and a variety of PET agents for delivery throughout Australia. Adjacent to SAHMRI there is a clinical molecular imaging facility featuring the latest scanners with dedicated research time (CRIC.org.au). Feel free to ask for a tour on Friday afternoon!

In the century of advances in imaging, genomics, transcriptomics, proteomics, and metabolomics we are geared towards a healthcare system that will be adopting personalised medicine. Imaging, and in particular molecular imaging, will play an important role in this process, both in preclinical and clinical setting. It is exciting to have an active community in this lovely part of the world and have a meeting where we can share science and experience.

This year we have invited high-class speakers with very diverse backgrounds that will highlight different aspects of the multidisciplinary field of Molecular Imaging. We are very much looking forward to all of their contributions. We are happy to say that we have had a great response in terms of abstracts.

A meeting like this doesn't just 'happen' and I must sincerely thank the members of ASMI 2019 committee and ASMI board who have all contributed towards making this a successful meeting. Our committee has worked hard to make this a pleasurable and memorable conference.

Importantly, we also would like to express our gratitude to our sponsors. Without their support this small conference would not have been possible and while maintaining a friendly registration fee. We also want to thank the South Australian Health and Medical Research Institute for hosting and facilitating us at their amazing venue.

With warmest regards,

Dr. Johan Verjans, on behalf of the co-chairs in the committee,

A/Prof. Sally Plush, Dr. Patrick Hughes, Dr. Marianne Keller, Dr. Ryan O'Hare Doig and Ms. Nicole Dmochowska

Thank you to the sponsors of ASMI 2019

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Program

DAY ONE: Thursday 7th November Auditorium, South Australian Health and Medical Research Institute (SAHMRI)			
Time	**Speaker**	**Theme**	**Session Chair**
9:00 -9:45	Registration		
9:45 – 10:00	*Welcome and opening from Co-Chairs and CEO SAHMRI,* ***Steve Wesselingh***		
10:00 – 11:00	**Mark Hutchinson** University of Adelaide *How quantifying how you know you are sick can change how we understand pain.*	Plenary Speaker	Johan Verjans & Jiarun Li
11:00 – 11:30	**Karine Mardon** University of Queensland *Molecular Imaging Markers of Neuroinflammation*	Invited	
11:30 -12:00	**Willy Gsell** KU Leuven *Preclinical simultaneous PET-MRI*	Sponsor	Dylan Bartholomeusz & Ta Hang
12:00 – 12:15	**Jiawen Li** University of Adelaide *Multimodal optical coherence tomography + fluorescence catheter for intravascular imaging*	Oral	
12:15 – 12:30	**Jath Palasubramaniam** Baker Institute *Targeting activated platelets: Molecular imaging and theranostic approaches in cancer and cardiovascular disease*	Oral	
12:30 – 13:30	Lunch		
13:30 - 14:00	**Steven Meikle** University of Sydney *Total Body PET: Current Status and Future Directions*	Invited	Christina Bursil & Nicole Dmochowska
14:00 - 14:15	**Patrick Hughes** University of Adelaide *Pro-MMP9 immunoPET detects inflammation induced fibrosis of the intestine and kidney*	Oral	
14:15 -14:30	**Mary-Anne Migotto** University of Queensland *Imaging and Biodistribution of a Zirconium-89 Labelled Anti-CD20 Antibody Following Subcutaneous and Intravenous Administration in Experimental Autoimmune Encephalomyelitis (EAE) Mice*	Oral	
14:30 - 14:45	**Mitch Klenner** ANSTO *Rhenium Complexation-Dissociation Strategy for The Radiosynthesis of Fluorine-18 Labelled PET Molecular Probes*	Oral	
14:45 - 15:00	**Courtney Hollis** SAHRMI *Rapid and automated production of [68Ga]GaCl3 and [68Ga]Ga-DOTA-TATE on a medical cyclotron*	Oral	

15:00 – 15:30	Afternoon tea		
15:30 – 16:00	**Mitra Safavi-Naeini** ANSTO *Neutron Capture Enhanced Particle Therapy (NCEPT): A new frontier in hadron therapy*	Invited	Marianne Keller & David Hartnell
16:00 – 16:30	**Max Massi** Curtin University *Imaging with Luminescent Metal Complexes: from Cells to Bacteria*	Invited	Marianne Keller & David Hartnell
16:30 - 16:45	**Jiarun Lin** University of Sydney *Towards the multimodal analysis of lysosomal redox states*	Oral	
16:50 – 17:10	Poster slam		
17:10 – 18:00	Poster presentations & Tours		
18:30 – late	Cocktail Reception West Oak Hotel, Hindley St		

DAY TWO: Friday 8th November SAHMRI			
Time	Speaker	Theme	Session Chair
9:00 – 10:00	**Connie Wong** Monash University *Intravital microscopy of leukocytes.*	Plenary Speaker	Andrew Abell & Mitch Klenner
10:00 – 10:30	**Marten Snel** SAHRMI *Ion Mobility Separation – Added Specificity in Mass Spectrometric Imaging*	Invited	
10:30 – 11:00	Morning Tea		
11:00 - 11:30	**Andrew Abel** University of Adelaide *New on/off probes for in vivo sensing and drug delivery*	Invited	Connie Wong & Ryan Doig
11:30 – 11:45	**Ta Hang** University of Queensland *Advancing the Diagnosis and Treatment of Cardiovascular Diseases with Nanotechnolgy*	Oral	
11:45 - 12:00	**Shadrack Mutuku** University of Adelaide *Mass Spectrometry Imaging: An Emerging Spatial Tool for Discovery Lipidomics of Prostate Cancer*	Oral	
12:00 – 12:15	**Ashleigh Hull** University of South Australia *The current status of radioimmunotherapy in pancreatic cancer*	Oral (ECR)	
12:15 – 12:30	**David Hartnell** Curtin University *Spectroscopic Alterations in Lipofuscin during Aging*	Oral	
12:30 – 12:45	**Krzysztof Mrozik** University of Adelaide *The novel vascular disruption agent LCRF-0006 synergistically enhances response to bortezomib to inhibit multiple myeloma progression*	Oral	
12:45 - 13:00	**Mufarreh Alazmi** University of Queensland *Simultaneous measurement of the blood-brain barrier (BBB) permeability and cerebral 2-deoxy-2[18F]-Fluoro-D-glucose (FDG) uptake in mice using PET-MRI with single echo DCE-MRI technique*	Oral	
13:00 – 13:45	Lunch		
13:45 – 14:45	**Eva Bezak** University of South Australia *Radiation biology and the quest for personalized cancer therapies: in silico approaches.*	Plenary	Sally Plush & Mufarreh Alazmi
14:45 – 15:00	Closing and Prizes		
16:30 - 16:45	ASMI Annual General Meeting		

Prof. Mark Hutchinson

Plenary speaker – 7th November, 10am

Professor Hutchinson is the Director of the ARC Centre of Excellence for Nanoscale BioPhotonics (CNBP) and a Professor within the Adelaide Medical School at the University of Adelaide.

Professor Hutchinson's research explores the "other brain" or the other 90% of cells in the brain and spinal cord. These immune-like cells are termed glia. Mark's research has implicated the brain immune- like cells in the action of drugs of dependence and the negative side effects of pain treatments. He has pioneered research which has led to the discovery of novel drug activity at innate immune receptors. His work has enabled the translation of compounds at the lab bench to clinical agents used at the bedside.

He has now added Director of the CNBP to his roles. The CNBP is an ARC Centre of Excellence with $50M of funding committed for 7 years, headquartered at The University of Adelaide, with nodes at Macquarie University, Sydney and the RMIT, Melbourne. We are partnered with universities and companies in Europe, the US and China, as well as other Australian institutions. Prof Hutchinson's work with the CNBP is to "Discover new approaches to measure nano-scale dynamic phenomena in living systems" and allow the first minimally invasive realtime visualisations of the "other brain".

Title: **How quantifying how you know you are sick can change how we understand pain.**

Abstract: The aetiology of persistent pain in humans is comprised of a complex, twisted and multi factorial journey that culminates in a "cancer of the soul". Recent advances in the basic science underpinning our mechanistic understanding of persistent pain have embraced "the other brain" as an integrator of multiple life stimuli. This complex integration of life experiences, which are translated into neurokine signals cause the neuroimmune cells of the central nervous system to adapt and change the environment in which the neuronal system operates. If these adaptations present in the somatosensory neuroanatomical locations then this can present as hypernociception and eventual persistent pain. Our appreciation for this neuroimmune signalling and its contributions to the health and disease of the brain has its origins in the study of the illness response. It is now apparent that these specialised brain-immune processes are engaged in a range of other disparate responses, including the rewarding properties of drugs of abuse. However, no one has yet visualised the working neuroimmune synapse in a behaving clinical or preclinical model. This also means that the molecular origins of pain have yet to be quantified. This presentation will summarise recent studies conducted within the Australian Research Council Centre of Excellence for Nanoscale BioPhotonics in this field and equip the attendees with further insights of the complexity and power that visualising and sensing the "other brain" with next generation light science and related technologies can brings to understanding persistent pain and drug responses.

Dr. Connie Wong

Plenary speaker – 8th November, 9am

The focus of Dr. Connie Wong's research is investigating the pathophysiology of stroke and the subsequent host inflammatory response. After completing her PhD at Monash University in 2008, Connie was trained in the Snyder Institute for Chronic Diseases at the University of Calgary in Canada (2008- 2012) and returned to Monash University in 2013, before heading her own lab in 2015. Connie has published >45 journal articles, including first/senior author in Science, Nature Immunology and Nature Medicine.

Connie was awarded "The Centenary Institute Lawrence Creative Prize" in 2013 and Victorian Tall Poppy award in 2017. Her research is funded by NHMRC and National Heart Foundation. She is a current recipient of the CSL Centenary Fellowship.

Title: **Intravital microscopy of leukocytes.**

Abstract: The capacity of leukocytes to move between blood and tissues or organs, interact with diverse types of other cells within the body, and adjust behaviour and morphology upon environmental changes, are all crucial features for securing the survival of an organism as a whole. As such, an ability to image motile leukocytes in 3 dimensions in situ in tissues and organs over time (the 4th dimension) in a living organism presents a unique and powerful tool that allows for real-time investigation of their function and behaviour. This approach of “seeing” cells in a live animal is called intravital imaging or in vivo microcopy. Although intravital imaging is considered a new tool in biomedical research, in reality, its roots originated in the 19th century. By the end of the 20th century, in an era of fluorescent and confocal microscopy, the technique was substantially enhanced embracing not only new microscopic technologies but also the development of new surgical approaches which expanded the repertoire of tissues and organs that can be imaged. In this talk, I will present the latest advances in research on leukocytes that were made possible with the application of intravital microscopy.

Prof. Eva Bezak

Plenary speaker – 8th November, 1.45pm

Eva Bezak is a Professor in Medical Radiations and Centre Director for Translational Cancer Research at the University of South Australia. Previously she was Chief Physicist at the Department of Medical Physics, Royal Adelaide Hospital, providing services to radiation oncology in South Australia. She has authored and co-authored over 140 papers, 250 conference presentations and co-authored books on medical physics and supervised over 30 HDR students.

Bezak and her group are national leaders in radiation biology modelling using Monte Carlo algorithms. These were either developed in-house or using the existing Monte Carlo packages: SRIM, EGSnrc, GEANT4, MCNP. Other research interests include targeted alpha therapy, microdosimetry and artificial intelligence in health care. At UniSA, Prof Bezak and her group have established themselves as national and international leaders in radiation biology modelling using Monte Carlo computational algorithms. Some of the models developed are world class, first of its kind, attracting praise from international referees, e.g. their work was considered to be "light years ahead of everyone else in the field".

They have developed the most advanced and sophisticated 4D (temporal and spatial) in-silico tumour model presently available in the world that has true biological and radiobiological properties of specific cancers (e.g. a head and neck cancer), including tumour growth, cellular hierarchy (including cancer stem cells and differentiated cells), spatial distribution and chaotic tumour vasculature (and therefore can predict oxygenation of cells as a function of distance from blood vessels). Following irradiation, DNA cluster damage is calculated and cell death predicted based on radiation damage to DNA. Tumour growth restarts post “virtual irradiation”. Treatment regimens can be thus be simulated and treatment outcomes predicted.

Title: Radiation biology and the quest for personalized cancer therapies: in silico approaches.

Abstract: At present, quite correctly, radiation therapy for cancer is delivered based on departmental protocols derived from published clinical trials. However, reported data show that radiotherapy response varies from patient-to-patient, despite using uniform treatment protocols. This is due to a number of patient specific factors, like comorbidities and lifestyle as well as due to other factors (interaction of radiation, uncertainties in organ motion, etc.). But perhaps the most dominant factor (up to 80%) in radiation response of individual patients, is their own genetic predisposition, dictating how radiosensitive an individual is. This in turn means that using uniform solutions/protocols does not benefit all patients.As such, more personalized radiation oncology approaches are needed based on understanding and utilization of broad data currently available. How can we resolve all these challenges?

We can have a) more clinical trials (but these are time consuming and costly, and sometimes not beneficial to patients), b) additional R&D (in vitro, animal models) or c) computational (or in silico) modelling. Computational modelling of various treatment regimens and their input parameters can offer a comprehensive understanding of the radiobiological interactions and also the treatment outcome, without the involvement of lengthy trials.

Computational models allow us to explain observations, for example compare clinical trial results; they can predict clinical outcomes under conditions not previously measured (e.g. alternative schedules); allow us to optimise radiotherapy and other treatments (e.g. chemotherapy) and identify and evaluate risks; e.g. radiation/chemo side effects. As such those treatment protocols that can be identified as not beneficial to patients can be eliminated immediately and do not have to progress to clinical trials. Only those treatment regimens that show promise and benefit can be then trialled in a clinical setting.

Dr. Karine Mardon

Invited speaker – 8th November, 9am

Dr. Mardon is the Facility Fellow for the preclinical PET/CT for the Queensland Node of the National Imaging Facility (NIF), based at the Centre for Advanced Imaging (CAI), University of Queensland. Dr Karine Mardon obtained her PhD in radiopharmacology from University Paris XII in 1994. In 1995, she pursued postdoctoral studies at ANSTO (Sydney) in the Radiopharmaceutical Division where she gained experience in the development and characterization of radiopharmaceuticals for SPECT and PET. She has extensive experience in in vitro and in vivo preclinical research particularly in the evaluation of drugs developed for the study of movement disorders as well as in the evaluation of radiolabelled ligands targeting receptors expressed in activated microglia during neuroinflammation. Her other interest, more recently, is in the field of oncology with the development of multimodal imaging probes for diagnostic and theranostic applications. She moved to Brisbane in 2000 and joined The University of Queensland where she gained valuable experience working in the field of drug metabolism and preclinical drug development in the ADME division of TetraQ. She has published 30 research articles and over 45 conference papers in the field of molecular imaging and nuclear medicine. She has collaborated with many scientists leading the field in nuclear medicine and molecular imaging in Australia and internationally. She joined CAI in October 2010 as NIF facility Fellow in Molecular Imaging. Since 2014, she has also taken the role of Course Coordinator for the Master of Molecular Imaging and Technology (MMIT) offered at The University of Queensland.

Title: **Molecular Imaging Markers of Neuroinflammation**

Abstract: Inflammation is part of the immune system's response to damage or infection. In the central nervous system (CNS), microglia, astrocytes and macrophages play an important surveying role by using pattern recognition receptors (PRRs) that sense pathogen specific proteins (PAMPs) and damage associated proteins (DAMPs) from pathogenic agents. An important subgroups of cytosolic PRRs are called inflammasomes and are primarily expressed by microglia, astrocytes and macrophages. After activation by various stimuli (tissue damage, pathogen, protein aggregates), chronically activated microglia will produce inflammatory cytokines and reactive oxygen/nitrogen species which can lead to neuronal death. Various cell surface, cytosolic and mitochondrial receptors expressed in microglia cells are involved in the regulation and function of microglia, and some of these receptors have been targeted for the development of ligands for imaging. In vivo imaging technologies (PET, CT and MRI) have progressed enough to be able to study the molecular mechanisms involved in neuroinflammation at various disease stages. The long-term goal being the development of imaging markers for neuroinflammation which will help to identify new targets for therapeutic intervention to alter the neurotoxic inflammatory pathway. The presentation will focus on molecular imaging markers targeting the translocator protein (TSPO), the cannabinoid CB2 receptors, the purinergic P2X7 receptors and PRRs receptors and their involvement in the activation of microglia during neuroinflammation in various animal models.

Prof. Steven Meikle

Invited speaker – 7th November, 1.30pm

Steven Meikle is Professor of Medical Imaging Physics at the University of Sydney and Head of the Imaging Physics Laboratory at the Brain and Mind Centre (BMC). He received his Ph.D. from the University of New South Wales in 1995. He was a medical physicist at Royal Prince Alfred Hospital in Sydney from 1987-2004, a visiting research associate at the Division of Nuclear Medicine and Biophysics, UCLA School of Medicine from 1991-2 and a post-doctoral research scientist at the MRC Cyclotron Unit in London from 1995-6, before joining the University of Sydney in 2004. He is best known for his contributions to the development of quantitative emission computed tomography and small animal molecular imaging. He has published more than 200 original research articles which have attracted over 5,700 citations (h- index: 42). He has received $14 million in competitive grant funding over the last 10 years and a further $10.3 mill for the USyd/ANSTO node out of $70 mill awarded to the National Imaging Facility for research infrastructure over the next 5 years. He has supervised 20 postgraduate research students to successful completion who have secured postdoctoral fellowships at UCLA, Yale and MIT/Harvard among others, and 8 postdoctoral scientists who have established successful careers in academia and industry. Steven is a Senior Member of the IEEE, a Fellow of the Australian Institute of Physics, Vice President and President-elect of the IEEE Nuclear and Plasma Sciences Society and an Editorial Board member of the journal Physics in Medicine and Biology.

Title: **Total Body PET: Current Status and Future Directions**

Abstract: Current generation clinical positron emission tomography (PET) scanners have an axial field of view (FoV) of up to 26 cm. They are able to achieve relatively high spatial resolution (~3 mm FWHM), excellent coincidence timing resolution (~220 ps) and adequate sensitivity for single organ imaging. However, to image the distribution of a radiopharmaceutical throughout the entire body, the subject must be imaged in segments using sequential overlapping scans while the couch is moved through the PET gantry. This results in less than 1% of the available signal being captured which is a very inefficient use of the radiation dose administered to the subject. Recently, the first total body PET scanners were developed which have an axial FoV sufficiently long (>1 metre) to image all organs of the body simultaneously, resulting in an order of magnitude increase in sensitivity and dose efficiency, as well as the ability to capture dynamic, physiological information from every tissue in the body simultaneously. This is expected to lead to an expanded range of applications for PET in chronic systemic diseases, such as diabetes and infectious disease, paediatrics and foetal medicine (taking advantage of the low dose capability) and drug discovery to name a few. This presentation will describe the technical advances and challenges of total body PET, discuss the first human studies and explore potential applications and future developments of the technology.

Dr. Mitra Savafi-Naeni

Invited speaker – 7th November, 3.30pm

Title: Neutron Capture Enhanced Particle Therapy (NCEPT): A new frontier in hadron therapy

Abstract: Neutron Capture Enhanced Particle Therapy (NCEPT) is a radical new paradigm in radiotherapy being developed by an international team led by ANSTO. NCEPT combines the precision of particle therapy with the cancer-specific targeting capability of neutron capture therapy (NCT). NCEPT magnifies the impact of particle therapy by capturing neutrons - produced internally at the target as a by-product of treatment - inside cancer cells, where they deliver extra dose to the tumour (Fig. 1). NCEPT uses low-toxicity agents containing boron-10 and gadolinium-157 which concentrate in cancer cells, already approved or under development for other medical applications.

There is increasing evidence that in many tumour types, tumour stem cells are residing in niches outside the gross target volume (GTV) and are resistant to low LET radiation currently used with x-ray and proton therapy. These cells could be a source of local- regional and distal recurrence. Through the use of tumour selective boron or gadolinium agents which accumulate in tumour cells both inside and outside the GTV, NCEPT makes use of the thermal neutron component prevalent inside and outside of each proton beam and targets these cells specifically. If successfully translated, NCEPT would be a paradigm shift in particle therapy addressing an important clinical problem in cancer therapy.

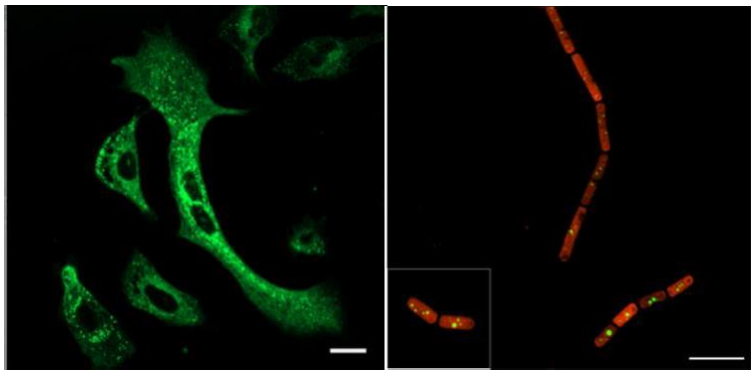
Simulations and experiments on cancer cells have yielded extremely compelling results, indicating of the radiation dose compared to particle therapy alone. NCEPT has generated considerable excitement within the radiation oncology communities in Australia, USA, and in particular in Japan, where it has been dubbed “the future of ion-beam radiotherapy”. Initial discussions regarding the first clinical trials in Japan are currently in progress.

A/Prof. Max Massi

Invited speaker – 7th November, 4pm

Title: Imaging with Luminescent Metal Complexes: from Cells to Bacteria

Abstract: The advantages offered by photoactive metal complexes in life science have been well demonstrated. Luminescent metal complexes can be exploited as cellular probes with complementary properties to those offered by more traditional organic fluorophores.[1] On the other hand, photochemically active metal complexes can be designed for therapeutic purpose.[2] For the past decade, our group has been interested in designing luminescent complexes based on transition metals, especially rhenium and iridium, in order to gain insights into their structure-activity relationship and elucidate their diagnostic potential as cellular and tissue markers.[3] More recently, we have started to investigate whether metal complexes could be suitable to be exploited as diagnostic agents in bacterial imaging, an area that is exclusively dominated by organic fluorophores. This presentation will highlight our recent discoveries in bacterial imaging using metal complexes.



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Dr. Marten Snel

Invited speaker – 8th November, 10am

Dr. Marten Snel heads SAHMRI's Proteomics, Metabolomics and MS-Imaging Core Facility. He has been active in the field of mass spectrometry for over 20 years. He completed his PhD focused on the MS analysis of synthetic polymers at Edinburgh University in 1999.On completion of his studies he worked for Waters Corporation a manufacturer of analytical instrumentation for nine years in both a commercial and scientific capacity, including spearheading the development of MALDI imaging combined with ion mobility separation. His current research interests are in comprehensive high throughput characterisation of patient derived samples as well as in situ analysis of lipids and other small molecules in tissue using laser- based mass spectrometry.

Title: Ion Mobility Separation – Added Specificity in Mass Spectrometric Imaging

Prof. Andrew Abell

Invited speaker – 8th November, 9am

Andrew is Professor of Chemistry and Adelaide node director of the ARC Centre of Excellence for Nanoscale Biophotonics (CNBP) at the University of Adelaide. He is also currently an Australian Fulbright Ambassador and Chief Scientific Advisor and co- founder of Calpain Therapeutics. He has strong connections with the pharmaceutical industry, having been a visiting scientist, consultant and senior Fulbright scholar with Smithkline Beecham (now GSK) in Philadelphia, USA. An author of some 300 publications, a past Head of School of Chemistry and Physics at Adelaide and recipient of Royal Australian Chemical Institute Adrien Albert Prize and the Alexander R. Matzuk Prize and Lecture in Drug Discovery (Baylor College of Medicine, Houston).

Title: New on/off probes for in vivo sensing and drug delivery

Abstract: The problem with many biological probes and sensors is that they lack an ability to be switched on and off and to present dual functionality, e.g. delivery of a therapeutic response following sensing or provide extended imaging capacity. Organic fluorescent probes also generally lack the photostability required for extended intracellular imaging.

A hybrid nanomaterial (PNS) is reported with an organic fluorescent probe bound to a nanodiamond for concurrent and extended cell-based imaging and ratiometric detection of H2O2. Far-red fluorescence of the nanodiamond offers continuous imaging without photobleaching, while green fluorescence of the attached probe detects H2O2 on demand. The sensor detects basal production of H2O2 within macrophages and does not affect growth during prolonged co- incubation. This nanosensor can be used for extended bio-imaging not possible with a standalone organic fluorescent probe.A sensor can also be incorporated into a lipid extract-based liposome to convert a cell permeable probe into one capable of selective detection of extracellular Ca2+, GSH or Zn2+. This allows a range of intracellular fluorescent sensors to be repurposed to specifically detect extracellular analytes and to improve biocompatibility in a wide range of biomedical applications.

Finally, drug delivery capability can be added to these platforms using a new spiropyran-based nanoparticle that responds to Zn2+ to release an encapsulated payload to target diseased cells. The component spiroparticles self-assemble into a micelle-like structure on nanoprecipitation to trap a payload within its core. A linear relationship is apparent between added Zn2+ and fluorescence of the payload, while imaging with confocal microscopy shows that released payload and spiroparticles are co-located in the same cellular region. This allows inhibition of caspase activity on Zn^{2+} promoted release of encapsulated azure B (AzB) in dying cells to be imaged using time- lapsed microscopy. These new spiroparticles represent a unique Zn^{2+} responsive delivery tool, with an ability to provide real time sensing of the spiroparticle release event.

Multimodal optical coherence tomography + fluorescence catheter for intravascular imaging

Jiawen Li[1,2*], Bryden C. Quirk[1,2], Rodney W. Kirk[1,2], Loretta Scolaro[1,2], Christina Bursill[1,3], Johan W. Verjans[1,3,4], Robert A. McLaughlin[1,2]

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Background

Cardiovascular disease (CVD) is the leading cause of death worldwide. Although significant progress has been made in our understanding of CVD, accurate diagnosis of high-risk atherosclerotic plaques before they become life-threatening remains a significant challenge. This is mainly due to the lack of tools to measure plaques at the molecular and microscopic level over time. To address this challenge, we are developing a new, multimodal optical coherence tomography (OCT) and fluorescence imaging catheter that can directly study plaques in living organisms, in real-time, and so provide molecular and microstructural insights into the development of plaques.

Methods and Results

We have developed a multimodal imaging catheter that consists of a double clad fibre (DCF) terminated in a side-viewing microlens fabricated by splicing exact lengths of no-core fibres and gradient-index fibre to focus and redirect the light beam. The single mode core of the DCF carries the OCT signal to and from the sample. The same core also carries the fluorescence excitation light. Emitted fluorescence is then collected within the inner cladding of the DCF and passed to a double-clad-fiber coupler where it is separated from the OCT signal for detection. The fibre assembly was fixed inside a thin-wall torque coil. The torque coil allows rotational and linear motion to be precisely transferred from the proximal end to the distal end of the imaging probe, thus achieving 3D scanning. The fibre probe with torque coil rotates freely inside a catheter sheath (outer diameter 0.457 mm), which remains stationary and protects the biological tissue during 3D scanning.

A portable multimodal system was developed by integrating a 1310nm-OCT sub-system and a 785nm-fluorescence sub-system, optimised to detect indocyanine green (ICG) uptake. ICG is an FDA approved contrast agent that has been shown to detect high-risk features in plaques. In a proof of principle animal study, we imaged an atherosclerotic mouse thoracic aorta *in situ,* preserving anatomical configuration to emulate *in vivo* conditions. The vessel wall structure, along with three fluorescent hotspots, which were created by locally injecting a low concentration of ICG, were clearly imaged by our multimodal imaging catheter.

Conclusion and Outlook

To the best of our knowledge, we have developed the thinnest intravascular OCT + fluorescence catheter that is capable of simultaneous molecular and microstructural imaging. This technology can be further used for imaging other delicate narrow luminal organs (e.g., small airways).

Targeting activated platelets: Molecular imaging and theranostic approaches in cancer and cardiovascular disease

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[2] Baker Heart and Diabetes Institute, Australia

Background – Platelets play a major role in both cardiovascular disease and cancer. Glycoprotein (GP) IIb/IIIa is the most abundant platelet surface receptor, responsible for adhesion and aggregation. We have developed conformation-specific single-chain antibodies (scFv) binding specifically to activated GPIIb/IIIa, which retain the binding specificity of a full antibody whilst being more readily functionalized for molecular imaging. We hypothesized that targeting activated platelets, we can offer diagnostic and theranostic agents for a range of imaging technologies including ultrasound, MRI, PET/CT and optical imaging.

Methods and Results – Single-chain antibodies specific for activated GPIIb/IIIa were conjugated with appropriate contrast agents; 1) microbubbles (Targ-MB) for ultrasound, 2) near-infrared dyes (Targ-Cy7) for fluorescence imaging, 3) iron oxides (Targ-MPIOs) for MRI and 4) radiotracers (Targ-Cu^{64}) for PET/CT. In a murine carotid thrombosis model, we successfully imaged thrombi using both Targ-MB and Targ-Cy7. After thrombolysis with fibrinolytic drugs, both agents revealed a reduction in thrombus size ($p<0.001$). In a theranostic approach, administration of urokinase coated Targ-MBs resulted in a similar reduction in thrombus size ($62.91\pm5.6\%$ vs $2.84\pm4.3\%$, $p<0.001$). In a murine myocardial infarction model, we demonstrated that Targ-MPIOs identify the area of infarction. Targeted cancer imaging was assessed in four human tumour xenograft models. We showed specific targeting of activated platelets within the tumour microenvironment using Targ-Cy7 fluorescence (2.89 ± 0.32 vs 1.26 ± 0.12; $p<0.001$), Targ-Cu^{64} PET/CT (5.94 ± 1.19 vs 2.33 ± 0.33;
$p<0.05$) and Targ-MB ultrasound (55.16 ± 11.58 vs 5.67 ± 0.14; $p<0.001$).

Conclusions – Activated platelets are ideal targets for molecular imaging of atherothrombosis and cancer. Our scFv binds specifically to activated platelets and allows molecular imaging of these diseases across a range of imaging modalities. Our thrombosis studies demonstrate that we can directly diagnose acute arterial thrombosis as well as monitor treatment *in vivo*. Our theranostic construct allows a single agent theranostic approach. Further, MRI can be used to identify myocardial infarction using our Targ-MPIOs. Finally, our cancer study provides proof of concept for localization of tumours by molecular targeting of activated platelets.

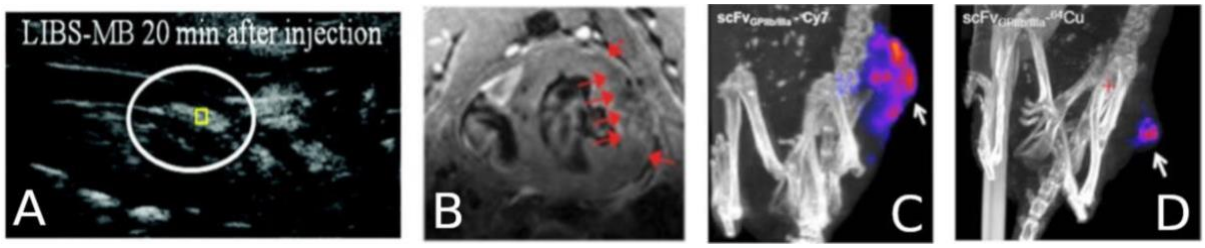

Figure 1. A) Targ-MBs identify thrombus on ultrasound; **B)** Targ-MPIOs highlight the area of infarction in an MI model; **C)** Targ-Cy7 localization to tumour xenograft; **D)** Targ-Cu^{64} localization to tumour xenograft.

pro-MMP9 immunoPET detects inflammation induced fibrosis of the intestine and kidney

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Introduction: Intestinal fibrosis is a common complication of inflammatory bowel disease (IBD) but remains difficult to diagnose and treat. Matrix metalloproteases (MMPs) and tissue inhibitors of matrix metalloproteases (TIMPs) have key roles in fibrosis and are therefore potential targets for detection of fibrosis by immunoPET.

Methods: Mice were administered 2% DSS treated water for 5 days+normal water for 3 days (inflamed), 3 cycles of DSS+8 days normal water (fibrotic), or untreated (control). Colonic and kidney collagen content, innate cytokine, MMP and TIMP-1 and faecal MPO concentrations were analysed by multiplex / ELSIA. α-pro-MMP-9 F(ab')2 fragments were engineered, conjugated to ^{89}Zr and administered i.v. o/n before PET imaging. Bio-distribution was determined ex-vivo by Cherenkov imaging (IVIS spectrum) and gamma-counts.

Results: Colonic collagen concentrations were increased in fibrotic mice ($P<0.01$, $N=6$). Colonic IL-1α, IL-1β, IL-6 and TNF-α concentrations were increased in inflamed mice ($P<0.05$, $N=6$) but did not differ between fibrotic and control mice. MMP-2, -3, -8, pro-MMP- 9 and TIMP-1 were increased in inflamed relative to control mice ($P<0.001$, $N=6$). Only pro- MMP-9 remained increased in fibrotic relative to inflamed mice. ^{89}Zr-pro-MMP-9 F(ab')2 uptake was increased in the intestine ($P<0.01$, $N=5$) but also in the kidney ($P<0.001$) of fibrotic mice. Collagen and pro-MMP-9 concentrations were increased in the kidney in fibrotic mice ($P<0.01$, $N=6$).

Conclusion: ^{89}Zr-pro-MMP-9 F(ab')2 detects colitis induced intestinal fibrosis and associated kidney fibrosis. This is the first immunoPET study of fibrosis in any tissue, and highlights the utility of coupling antibody specificity to PET sensitivity for detecting disease targets in tissues that are distant to disease origin.

Imaging and Biodistribution of a Zirconium-89 Labelled Anti-CD20 Antibody Following Subcutaneous and Intravenous Administration in Experimental Autoimmune Encephalomyelitis (EAE) Mice.

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B-cell directed immunotherapies using monoclonal antibodies are emerging targets in the treatment of multiple sclerosis and recombinant human myelin oligodendrocyte glycoprotein (rhMOG)-induced EAE mouse model involves B-cell-mediated inflammation and demyelination. The lymphatic system likely plays a crucial role linking the peripheral immune system and the central nervous system (CNS) and lymphatics could possibly provide a route for B-cell trafficking that bypass the peripheral circulation.

C57BL/6J mice (12-15 weeks old; n=39) were EAE-induced using rhMOG. At peak of disease on Day 14 post EAE induction, ^{89}Zr-labelled-anti-CD20 monoclonal antibody (mAb) was injected in control and EAE mice subcutaneous (s.c.) in the right lower flank or intravenous (i.v.) via tail vein. Positron emission tomography/computed tomography (PET/CT) imaging and gamma counting (ex vivo) were performed on Days 1, 3 and 7 to quantify tracer accumulation in the major organs, lymphatics, and CNS.

Results demonstrated that ^{89}Zr-labelled anti-CD20 mAb was effectively absorbed from both s.c. and i.v. injection sites and distributed to all major organs in the EAE and control mice. Correlation between in vivo PET-CT data and ex vivo biodistribution studies confirmed sensitivity and specificity for in vivo CD20 targeting.

Gamma counting biodistribution studies showed initial tracer uptake within the lymphatic system was found to be higher in the draining lymph nodes (subiliac and sciatic) following s.c. versus i.v. administration. Across all disease states in EAE mice, a significant correlation was observed for ^{89}Zr-labelled-anti-CD20-Ab uptake within the spinal cord and central nervous system (CNS) following s.c. and i.v. administration.

This study highlights that mAb based therapies administered through the s.c. route which target B-cell rich LN's can be a potential therapeutic option to treat autoimmune diseases such as MS.

Rhenium Complexation-Dissociation Strategy for The Radiosynthesis of Fluorine-18 Labelled PET Molecular Probes

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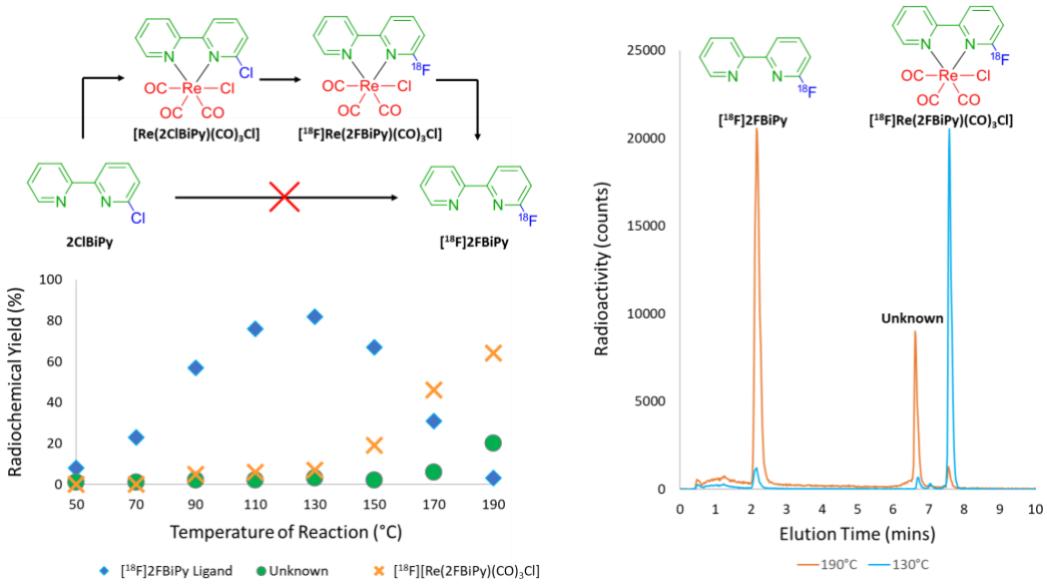
Fluorine-18 radiotracers are commonly employed in positron emission tomography (PET) imaging to investigate biochemical systems and to diagnose disease pathologies. The discovery of new fluorine-18 radiolabelling methods is thus critical to the improved synthesis of new tracers which can image different biochemical interactions or diagnose untreated disease states. Herein we report on such a radiolabelling method which relies on the complexation of a ligand to a rhenium center, followed by thermal decomplexation, to radiosynthesise novel fluorine-18 labelled molecular probes of interest. An example of this complexation-dissociation strategy is shown in the figure below, whereupon a [18F]2,2'- bipyridine ligand was produced in greater than 60% radiochemical yield (RCY). This ligand, among other examples, was unable to be synthesised without this rhenium mediated approach. Not least the fluorine-18 labelled rhenium complexes also formed in greater than 80% RCY in most cases and may have far reaching applications as PET-optical multimodal probes.



Towards elucidating the reaction mechanism, density functional theory (DFT) calculations have suggested that the surprising efficiency of this rhenium complexation-dissociation strategy may be attributed to the formation of an acyl fluoride intermediate species which anchors the fluorine-18 radioisotope in proximity of the site for substitution. This enhanced effect is likely also complimented by electron withdrawal of the rhenium center, as evidenced by our nuclear magnetic resonance (NMR) data, which may improve nucleophilic substitution for [18F]fluoride. This approach has been applied to the radiosynthesis of the [18F]CABS13 Alzheimer's disease PET imaging agent and ongoing work is currently being undertaken to expand the scope of potential radiotracers and alternative decomplexation routes.

Rapid and automated production of [^{68}Ga]GaCl$_3$ and [^{68}Ga]Ga- DOTA-TATE on a medical cyclotron

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The demand for ^{68}Ga for labelling PET radiopharmaceuticals has recently increased. ^{68}Ga is obtained from the decayed parent radionuclide ^{68}Ge using commercial ^{68}Ge/^{68}Ga generators. The principal limitation of commercial generators is that only a limited and finite quantity of ^{68}Ga (<1.85 GBq at the start of synthesis) may be accessed

The focus of this study was to investigate the use of a low energy medical cyclotron to produce greater quantities of ^{68}Ga than are currently available via the use of a ^{68}Ge/^{68}Ga generator and to develop an automated and rapid procedure for processing the product.

Isotopically enriched ^{68}Zn was electroplated onto the target shuttle using the Comecer electrochemical deposition system. The ^{68}Zn target was irradiated on a GE PETtrace 880 cyclotron at 14.5 or 12.0 MeV for 8.5 min. Purification was performed on the Comecer Taddeo with single-use cassettes using an octanol resin to give [^{68}Ga]GaCl$_3$. Radiolabelling and purification of [^{68}Ga]Ga-DOTA-TATE was subsequently performed on the same cassette.

^{68}Ga was obtained in 6.30 ± 0.42 GBq after an 8.5 min bombardment and with low radionuclidic impurities (66Ga (<0.005%) and ^{67}Ga (<0.09%)). Purification on a single octanol resin gave 82% recovery with the resulting [^{68}Ga]GaCl$_3$ obtained in 3.5 mL of 0.2 M HCl. To highlight the utility of the automated procedure, [^{68}Ga]Ga-DOTA-TATE labelling was incorporated to give 1.56 GBq of the labelled peptide with radiochemical yield of >70%.

The production time from ^{68}Zn irradiation to [^{68}Ga]Ga-DOTA-TATE was 1 hour (Figure 1). Although the reported process times cannot match those obtained from a ^{68}Ge/^{68}Ga generator they are reasonable and do allow for routine use of cyclotron ^{68}Ga. Furthermore, the 6–7 GBq obtained from a short bombardment is significantly greater than the quantities accessible with a generator.

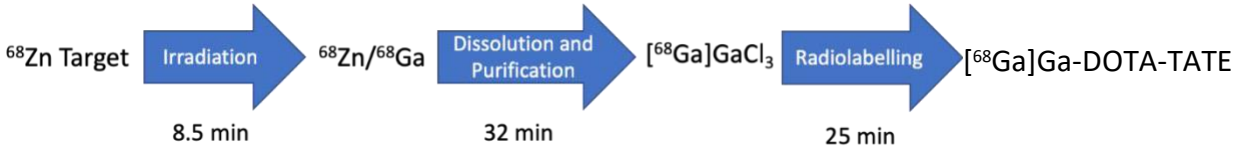

Figure 1. Overview of [^{68}Ga]GaCl$_3$ and [^{68}Ga]Ga-DOTA-TATE automated process.

Towards the multimodal analysis of lysosomal redox states

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Understanding biochemical changes in specific organelles is a pivotal part of understanding disease processes. It is thus necessary to develop tools to both identify and monitor the chemistry of organelles *in vivo*. Fluorescent probes have become important biosensing and imaging tools, as they can be targeted to specific organelles and detect changes in their chemical environment. However, the sensing capacity of fluorescent probes is highly specific and often limited to a single analyte of interest. A novel approach to imaging organelles is to combine fluorescent sensors with 3D vibrational spectroscopic imaging techniques; the latter provides a comprehensive map of the relative biochemical distributions throughout the cell, gaining a more complete picture of the biochemistry of organelles.

The lysosome is ideally suited for such multimodal investigation – whilst known as the organelle responsible for cellular digestion, we have yet to gain a complete understanding of the chemical environment of the lysosome. Lysosomal dysfunction is often linked to oxidative stress, and both have links to pathological conditions, including neurodegenerative and metabolic diseases.

Several lysosomal targeted fluorescent probes were developed. The first examples of lysosomally-targeted fluorescent reversible redox sensors were synthesized based on the NpFR scaffold [1], capable of reversibly measuring redox changes in the lysosomal environment (NpFR3.1 and NpFR3.2). Two non-sensing control probes using a naphthalimide scaffold were also developed. Confocal microscopy experiments showed that the novel probes exhibited good lysosomal localisation. The probes were also successfully used in flow cytometry studies to differentiate between populations of cells with different lysosomal numbers and redox states. These probes were then used to investigate the lysosome in a novel multimodal approach involving Raman spectroscopy, the first study of any kind involving sensor fluorescent probes and vibrational spectroscopy.

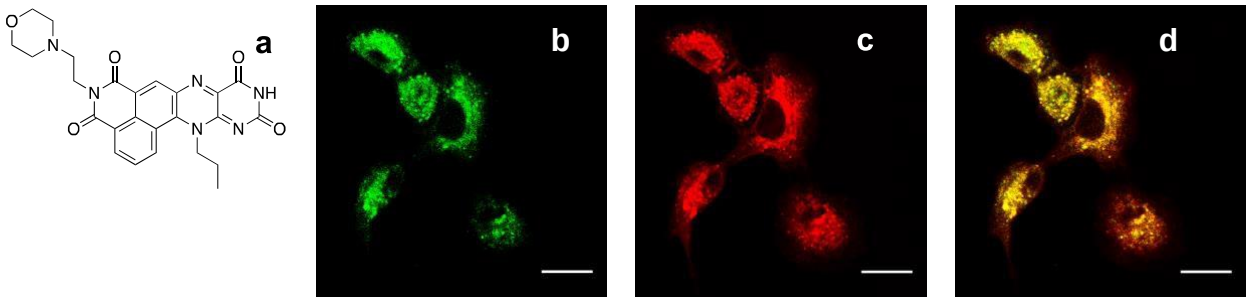

Figure 1: Chemical structure of NpFR3.1 (a) and confocal microscopy images of A549 cells treated with NpFR3.1 (40 μM, 1 h) and LysoTracker Deep Red (100 nm, 5 min). Live cells were imaged with an Olympus FV1000 microscope with (b) λ_{ex} = 488 nm, λ_{em} = 500-600 nm (c) λ_{ex} = 633 nm, λ_{em} = 650-750 nm and (d) merged images of a and b. Scale bar = 20 μm.

[1] Yeow J. Kaur, A. Anscomb M D. New E J. "A novel flavin derivative reveals the impact of glucose on oxidative stress in adipocytes", *Chemical Communications*, Vol. 50, (2014), pp 8181-8184.

Advancing the Diagnosis and Treatment of Cardiovascular Diseases with Nanotechnolgy

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Background:
The most common form of cardiovascular disease (CVD) and also the leading cause of sudden death is atherosclerosis, a chronic progressive inflammatory disease of the arterial vessels. Unstable, vulnerable atherosclerotic plaques can rupture and cause thrombosis, resulting in myocardial infarction (MI) and stroke. Magnetic resonance imaging (MRI) has been used as a powerful and indispensable tool in medical research and clinical diagnosis due to its high spatial resolution and non-limited penetration depth.

Aims:
We aim to develop innovative nanotechnology approaches for advanced and reliable diagnosis and treatment of CVD such as thrombosis and atherosclerosis. The central aim of the project is to develop novel targeted contrast agents with improved functionality and efficacy based on well-known non- or low toxic materials and materials approved by FDA, which enables easy translation to clinical use.

Methods:
We have designed and synthesized different targeted nanoparticles based on metal complex and metal oxide such as iron oxide (well-known low toxic) and Gadolinium complex (Gd-DOTA, FDA approved).

Results
We have developed both targeted negative contrast agents and targeted dual positive/negative contrast agents for molecular imaging of atherothrombosis. The simultaneous use of positive and negative MRI imaging that employs the same contrast agents will significantly improve the detection accuracy. Using these dual contrast agent, both T1- and T2-weighted MRI of thrombosis can be recorded simultaneously which enables self-confirmation of images and leads to a greater diagnostic accuracy. We have also designed and developed smart MRI nano- sensors that can not only detect, but also sense and report the stage or progression of CVD such as thrombosis. The early detection and accurate characterization of life-threatening diseases such as CVD and cancer are critical to the design of treatment. Knowing whether a thrombus in a blood vessel is new/fresh or old/constituted is very important for physicians to decide a treatment protocol. Theranostic nanoparticles based on iron oxide and cerium oxide have also been developed in our group as potential materials for diagnosis and treatment of reactive oxygen species related inflammatory diseases such as CVD.

Conclusions:
Our data have shown the potential use of metal and metal oxide based nanomaterials for diagnosis and treatment of cardiovascular diseases.

Mass Spectrometry Imaging: An Emerging Spatial Tool for Discovery Lipidomics of Prostate Cancer

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Metabolomics has been extensively exploited to uncover biomarkers of prostate cancer (PCa). However, the highly heterogenous nature of PCa, makes the spatial information of altered lipids indiscernible with this conventional technique. Moreover, current screening of PCa using the prostate specific antigen test suffers from sub-optimal sensitivity and has led to unnecessary treatment. Mass spectrometry imaging (MSI) offers a highly targeted approach to visually characterise the lipidomic composition of solid tumours. Our project utilises matrix assisted laser/desorption ionisation (MALDI) MSI where molecules are detected by their mass/charge (m/z) ratio at discrete loci that can be coordinated into a spatial intensity ion map.

In a cohort of 20 patients, frozen prostate tissue sections were thaw-mounted on glass slides for haematoxylin and eosin (H&E) staining and lipid imaging. Tissue sections for imaging were either coated with α-CHCA matrix using a sublimation device or SunCollect sprayer. MSI data was acquired on a Waters MALDI SYNAPT Mass Spectrometer in positive ion mode, m/z 400-990, with a step size of 60 µm. Data was processed into 0.03 Da bins using HDImaging v1.4. Spatial analysis was achieved by R package *Cardinal* and SCiLS Lab MVS v2019c Pro. Statistical analysis was performed using R *MetaboAnalyst*.

Pathological assessment of H&E scans delineated key histological features such as normal, inflammation and tumours of varying Gleason grades across the patient set which corresponded closely with adjacent MSI data. The predominant m/z in the respective segments (clusters) ranked by t-statistics were subjected to a data base search on Lipid Maps with a mass tolerance of 0.01 Da and validated by MS/MS imaging. Segmentation analysis showed that PC(36:4)/PC(34:1), m/z 782.56, was associated with epithelial regions whilst SM(d34:1), m/z
725.53 abundant in stroma. Region of interests (ROI) were then equivalently selected to compare lipid profiles between annotated pathological features. PCA/PLS-DA analysis plots showed aggregation of mass spectra of similar cell types and disease state with tumours separating from normal tissue. ANOVA heatmaps also revealed certain lysophospholipids to be preferentially localised in normal/benign epithelium compared to cancerous regions.

MALDI MSI is an emerging *in situ* imaging approach that can reveal lipids that correlate with PCa aggressiveness and has translational potential to compliment current diagnostic tools.

Spectroscopic Alterations in Lipofuscin during Aging

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Lipofuscin deposits are bundles of oxidised, cross-linked and aggregated nucleic acid, lipid and protein residues formed in lysosomes. Found commonly in brain cells, and considered a waste product, their interaction with the cellular environment, and correlation with aging and neurodegenerative disease has fueled further studies into their characterisation over the course of aging. Lipofuscin is auto-fluorescent and easily observed inside cells using fluorescence microscopy, however, characterising the chemical composition of lipofuscin and identifying changes in lipofuscin chemical composition during ageing is more difficult. Application of direct spectroscopic methods in combination with fluorescence microscopy may enable the chemical composition of lipofuscin to be studied in more detail during the ageing process, which could provide important insight into altered intracellular chemical environments that occur during ageing.

HYPOTHESIS: Lipofuscin deposits interfere with autophagic processes, causing increased accumulation of damaged cellular components, and increased cellular vulnerability to oxidative stress.

AIM: Characterise the biochemical profile of lipofuscin *in situ*, to determine if markers of increased oxidative stress occur during the ageing process.

METHODS: Tissue was generated from the senescence accelerated mouse prone strain 8 (SAMP8) from mice aged 4, 20 and 40 weeks. Raman spectroscopy was used to image individual brain neurons *in situ* within tissue sections, at sub-micron spatial resolution to reveal tissue-autofluorescence and biochemical composition.

RESULTS: Raman spectroscopic analysis revealed the size, abundancy and chemical composition of lipofuscin deposits change during ageing. Specifically, the biochemical profile of lipofuscin appears to be enriched in nucleic acid material in young SAMP8 mice, with data indicating a transition towards protein accumulation in older SAMP8 mice.

CONCLUSIONS: Our data shows unique, direct biochemical insight into the biochemical composition of lipofuscin during ageing. The increased accumulation of protein aggregates in aged SAMP8 mice is consistent with elevated protein oxidation and decreased function of the proteasome.

The current status of radioimmunotherapy in pancreatic cancer

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Background: Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive malignancy with few curative treatment options. Several anatomical and physiological factors, including the cellular heterogeneity and hypoxic nature of PDAC cells, attribute to the lack of curative treatments. Radioimmunotherapy (RIT), using either alpha- (α) or beta- (β) emitting radioisotopes, is a rapidly developing field with potential to improve the prognosis of low survival cancers such as PDAC. However, the value of α- or β-RIT is yet to be fully recognised in PDAC. The aim of this study was to evaluate the current preclinical and clinical trial evidence of α- and β-RIT for PDAC.

Methods: A search of MEDLINE® and Scopus was performed in August 2019 to identify relevant articles on RIT in PDAC. A total of 33 RIT studies (n=28 preclinical studies and n = 5 clinical trials) published across six countries were evaluated in this review.

Results: At a preclinical level, both α- and β-RIT demonstrated improvements in tumour control compared to untreated controls. Typically, tumour control was enhanced when RIT was used in combination with other treatments (e.g. chemotherapy). For both RIT types, the primary side-effect observed at the in vivo level was transient weight loss. Despite promising preclinical results for both α- and β-RIT, only β-RIT has progressed to clinical trial in PDAC. Phase I and II clinical trials observed disease control rates of 11.2 – 57.9%, whilst an unpublished phase III trial demonstrated β-RIT did not significantly improve patient survival.

Conclusions: Preclinical evidence suggests RIT may be an effective treatment for PDAC, particularly when used in a combination therapy. However, advancing these findings in clinical trials has been challenging. Careful development and optimisation is needed to improve the clinical translation of α- and β-RIT in PDAC.

The novel vascular disruption agent LCRF-0006 synergistically enhances response to bortezomib to inhibit multiple myeloma progression

[1,2]Mrozik, K.M., [1,2]Cheong, C.M., [1,2]Hewett, D.R., [1,2]Noll, J.E., [1,2]Opperman, K.S, [3]Blaschuk, O.W., [1,2]Vandyke, K. & [1,2,4]Zannettino A.C.W.

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Introduction: N-cadherin is a cell adhesion molecule that plays an important role in endothelial barrier integrity and controlling vascular permeability by maintaining adhesive junctions between endothelial cells. Previous studies have demonstrated that the N-cadherin antagonist peptide ADH-1 increases vascular permeability and chemotherapeutic drug delivery to the tumour microenvironment.

Aims: To (1) evaluate the effects of the small molecule ADH-1 peptidomimetic, LCRF-0006, on blood vessel integrity and permeability and (2) assess the efficacy of LCRF-0006 as a monotherapy and in combination with the standard-of-care multiple myeloma (MM) agent bortezomib in a mouse model of MM.

Methods: *In vitro* vascular disruption and permeability assays were performed using the human bone marrow endothelial cell line TrHBMEC. Drug-induced cell apoptosis was assessed by flow cytometry using Annexin V and 7-AAD. FITC-dextran extravasation studies were performed *in vivo* using whole-mount retinal angiography. The therapeutic utility of LCRF-0006 (100mg/kg) alone, and in combination with a sub-therapeutic dose of bortezomib (0.5mg/kg), was evaluated in the syngeneic C57Bl/KaLwRij/5TGM1 mouse model of systemic MM disease using bioluminescence imaging.

Results: LCRF-0006 rapidly disrupted cell-cell junctions in confluent endothelial monolayers in a transient and reversible manner and increased monolayer permeability to FITC-dextran *in vitro*. In addition, LCRF-0006 disrupted pre-formed immature (5 hr old) and established (24 hr old) endothelial tubes *in vitro* and increased the extravasation FITC-dextran *in vivo*, suggesting enhanced vascular permeability following LCRF-0006 treatment. While LCRF- 0006 potently induced 5TGM1 cell apoptosis *in vitro*, LCRF-0006 monotherapy did not inhibit tumour progression in an established MM disease setting *in vivo*. However, LCRF- 0006 synergised with bortezomib, significantly inhibiting MM tumour progression and leading to regression of disease in 5 of 5 mice ($P < 0.001$; co-efficient of drug interaction < 0.7).

Conclusion: These findings highlight the potential utility of LCRF-0006 to increase the effectiveness of bortezomib to treat MM tumours in patients. Future studies will assess the utility of LCRF-0006 to reduce the incidence and severity of bortezomib-induced side effects (i.e. peripheral neuropathy) and to increase the effectiveness of other commonly used MM drugs using mouse models.

Simultaneous measurement of the blood-brain barrier (BBB) permeability and cerebral 2-deoxy-2[18F]-Fluoro-D-glucose (FDG) uptake in mice using PET-MRI with single echo DCE-MRI technique

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Brain diseases and normal ageing can change the structure and physiology of brain tissues. Combined MR-PET multimodal imaging, for the first time, allows simultaneous dynamic measurement of the cerebral glucose uptake, via FDG-PET and blood brain barrier (BBB) permeability, via Gadolinium (Gd) enhanced MRI in old mice.

Initial experiments investigated the optimum amount of Gadovist for injection. Three volumes, 10, 20 and 50µl of Gadovist mixed with saline to give a total volume of 200µl were investigated. The 50µl dose showed the stronger effect on the signal time course during dynamic scanning. To investigate potential changes in brain with aging, 50 µl Gadovist, approximately 10 MBq of FDG, and saline was mixed in a total volume of 200 µl. Following
~2 min of dynamic MRI and PET baseline scanning, the mixture was injected into a tail vein cannula placed in the mouse before positioning in the MR-PET system for scanning.

Using standard uptake value ratio analysis of the FDG PET scans showed no significant difference between two age groups. No evidence for altered brain perfusion was evident from the Gd enhanced MRI scanning. This work illustrates the potential of simultaneous dynamic MR-PET for investigating normal and pathological neurological conditions.

Poster #1

Novel mesoporous silica nanoparticles for the targeted delivery of anticancer agents to breast cancer

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Breast cancer is the second cancer to cause death in women. Conventional chemo-agents work in a non-specific manner, attacking both healthy and cancerous cells, causing at least 36 toxic effects. Therefore, any technology proven to reduce the toxicity caused by the lack of selectivity of current anticancer drugs will greatly enhance the treatment efficacy.

To overcome the selectively issues of chemotherapy, mesoporous silica nanoparticles (MSNs) were employed as drug carries, to deliver Doxorubicin (DOX) to solid breast tumours, whilst protecting healthy cells from toxic effects. MSNs were decorated with a targeting ligand that can bind specifically to breast tumours. A pH-sensitive component was grafted onto the surface of MSNs, ensuring that drug release will strategically occur at tumour pH.

HR-TEM, XRD, FT-IR and BET techniques were employed to confirm the successful fabrication of native MSNs, with high surface areas (>1000 m_2/g) and uniform morphology (Fig. 1). Resulting MSNs have a mean particle size of 180 nm, indicating their ability to penetrate into the premature blood vessels surrounding breast tumours (EPR effect). Successful surface functionalization was supported by FT-IR, XPS and TGA techniques, with a grafting ratio of 29%.

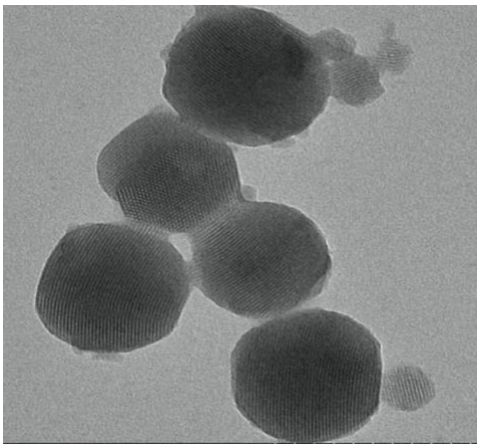

Figure 1: Uniform MSNs with clear porous channels.

Final nano-system achieved an impressive drug encapsulation efficiency of 97.5%, and 32.8% loading capacity. The resulting formulation demonstrated a pH-dependent *in-vitro* drug release pattern, with noticeably higher amount of DOX released at tumour pH. Overall, this intelligent nano-system is a promising solution to the selectivity issues of conventional chemotherapy, offering a novel treatment for breast cancer with high efficacy and low toxicity.

Poster #2

Halogenated BODIPY Probe Intermediates Highlight Lipophilic Regions in Rodent Brain Tissue

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Fluorescent probes are synthetically designed to exhibit native affinity to target biomolecules of interest. Due to this design, detection of target biomolecules can be markedly more time efficient than other methods (e.g, antibody stains). Common synthetic strategies include the attachment of a targeting moiety to a fluorophore. Highly conjugated fluorophores are easily halogenated allowing for subsequent attachment of the targeting moiety via palladium cross coupling reactions. This rapidly leads to probes suitable for biological application. However, these halogenated intermediate species are rarely explored for potential biological applications. In our research, we have synthesised a variety of halogenated probes based on the 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY) fluorophore. In order to assess potential for biological applications, the synthesised compounds were applied to brain tissue and imaged using fluorescence microscopy.

AIM: To investigate biological affinity and evaluate potential applications of halogenated intermediate BODIPY based probes. This will be achieved by photophysical analysis and fluorescence microscopy to elucidate probe-biomolecule bonding in tissue samples.

METHODS: BODIPY probes were synthesised as per literature procedures. Brain tissue sections (10 μm thick) were generated from healthy rat cerebellum. Tissue was fixed in formalin and stained in probe solution. Following staining, slides were imaged on an upright fluorescence microscope using a wide blue excitation and a wide green emission filter. Photophysical analysis includes measurement of absorption, emission, excitation, quantum yield and lifetime of the probes in the solid state and 10^{-5} M phosphate buffered saline solutions. The *n*-octanol/water partition coefficient of the compounds were determined using the shake-flask method.

RESULTS: Application of halogenated intermediate BODIPY probes to brain tissue resulted in brightly fluorescent images displaying strong lipid affinity. Specifically, these probes appear to localise in the lipid-rich regions of brain white-matter. Consisting of myelinated axons, visualisation of this region of tissue may be useful for pathological studies of brain disease and injury such as multiple sclerosis and traumatic brain injuries. Further tissue analysis displayed a possible clinical link with the BODIPY probes, revealing pathological changes in the rodent corpus callosum following diffuse traumatic brain injury.

CONCLUSIONS: Our results display the capacity for halogenated BODIPY synthetic intermediates to be effective biological probes, potentially as a novel myelin stain. This highlights the importance of thorough investigation of intermediate species for potential biological applications, which may enhance discovery of useful fluorescent probes for biological studies and clinical application, as shown by our results.

Poster #3
A reaction based Fluorescent Iron(II) Probe

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Sensing intracellular Fe(II) is crucial to understand cellular physiology and pathophysiology including ferroptosis, an iron-dependent cell death. Here we present a rhodamine-based probe selective for Fe(II). We postulate that the mechanism of the probe in sensing iron works via Fenton-induced hydrolysis. Our pilot studies had demonstrated that the probe detects labile Fe(II) in *cellulo* across multiple cell lines. Further work will include modifying the probe to sense labile Fe(II) in respective organelles. This will provide insights, and hence, a holistic view of iron trafficking and ferroptosis.

Poster #4
Fluorescent probes for copper

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Copper is an essential trace metal required for a variety of enzymatic and cellular processes in human body. However, excessive copper causes neurodegenerative diseases, like Alzheimer's disease. In order to better understand the physiological functions and pathological effects of copper, fluorescent probes for copper are needed. We have developed two types of fluorescent probes for copper (II):one is turn-on probe based on rhodamine;and anotheris a ratiometric probe based on coumarin. Our probes provide effectivetools to visualize copper in living cells and tissues.

Poster #5

INSANE IN THE MEMBRANE: AMPHIPHILIC COUMARIN METAL CHELATORS AS MEMBRANE-ACTIVE ANTIBACTERIAL AGENTS

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Antibacterial resistance continues to remain an imminent threat and by 2050 is predicted to be the direct cause of 10 million deaths annually.[1] In order to combat antibacterial resistance, constant discovery and development of new antibiotics is required. Current areas of interest include membrane-active antibacterial agents which permeabilise or destabilise bacterial membranes. Traditionally, this is achieved by generating amphiphiles with cationic heads to facilitate phospholipid association and hydrophobic, lipid-like tails to facilitate membrane perturbation.

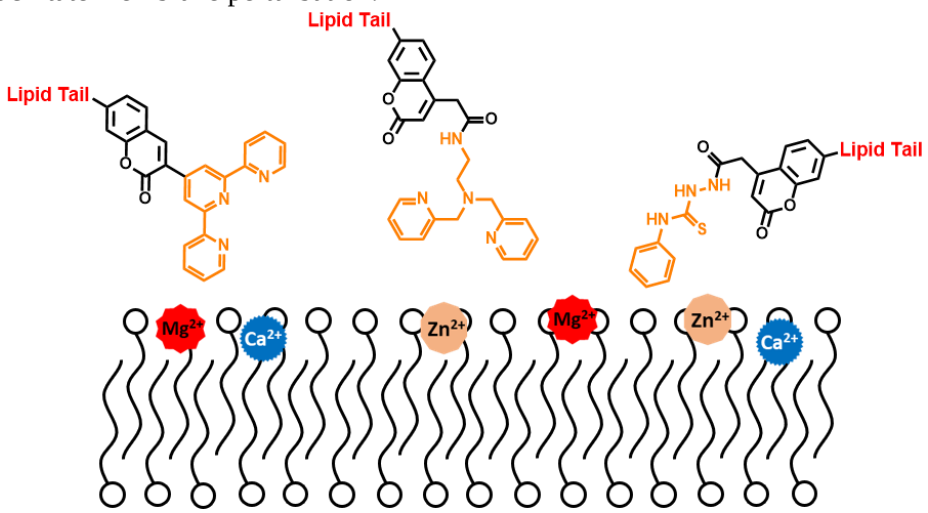

Figure 2: Amphiphilic coumarin metal chelators binding metals in outer membrane diagram.

Divalent cationic metal ions are important co-factors in stabilising proteins and performing functional roles within biological membranes. Competitive displacement of lipopolysaccharide-associated (LPS-associated) Mg^{2+} and Ca^{2+} in Gram-negative bacteria has been proposed as a model for the initial interaction of membrane-active antibacterial peptides with bacterial membranes.[2] We propose to achieve metal-centred targeting by generating small fluorescent molecular amphiphiles to selectively bind metals within bacterial membranes generating novel antibacterial agents (**Figure 2**). Coumarin represents the perfect scaffold due to its vast use in medicinal chemistry and as a fluorescent chemosensor for metal ions.[3]

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Poster#6
Shining a light on effective cancer therapy

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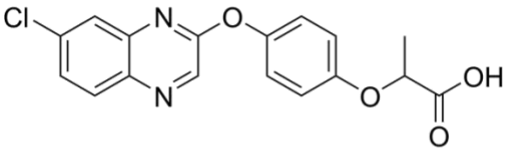
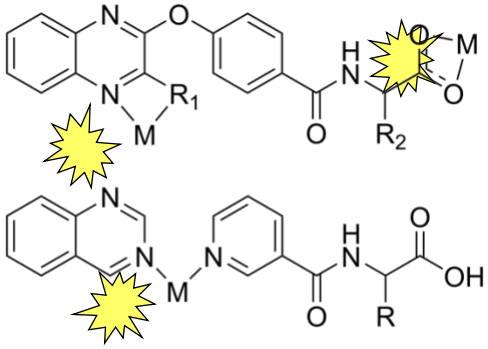
Background: The incorporation of metal-ion complexes in existing drug structures provides new opportunities for therapeutic optimisation and expanded diagnostic capabilities. [1-3] Through selection of the complexed metal ion and coordinated ligands, drug properties including stability and target specificity can be modified while the drug is further enhanced with a phosphorescent capability to allow the determination of cellular drug uptake and intracellular localisation. [4] This strategy therefore has the potential to produce viable analogues of drugs that initially showed potential in testing but were subsequently rejected due to undesirable side effects. As an example, the drug candidate XK469 showed promising antitumour effectiveness in preclinical testing but was withdrawn from further testing due to causing myelosuppression in phase I trials. Using XK469 as a lead compound, we envisage that the incorporation of a metal ion complex will allow the modification of derived drugs specificity and efficacy while adding theranostic capabilities. **Objectives:** To explore how altering the structure of XK469 derivatives with metal ion complexes effects therapeutic activity and interaction with the activity site.



A. XK469 lead compound.



B. Options for metal complexation of derived compounds.

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Poster #7

Sensing macrophage derived Nitric oxide using a Ruthenium-based fluorescent sensor

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Macrophage-derived nitric oxide (NO) plays a critical role in the onset and progression of atherosclerosis. Detecting NO within atherosclerotic plaque macrophages is immensely desirable for understanding the role of NO in plaque biology.
To assess the uptake and to detect a Ruthenium-based NO fluorescent sensor in macrophages using *in vitro* and *in vivo* atherosclerosis models. *In vitro*, human THP1 monocytes and differentiated macrophages were incubated with 50μM sensor.
Flow-cytometry, confocal microscopy and mass-spectrometry (CyTOF) were used to detect sensor internalisation of endogenous NO-dependent changes in fluorescence. *In vivo*, the sensor was injected intravenously into C57BL6/J mice at low (0.6μg/kg) and high (2.4μg/kg) concentrations for toxicity testing. Sensor biodistribution was also investigated in time course studies (5 min, 2, 4 and 6 hrs post-injection) using flow cytometry on single-cell suspensions of macrophage-rich tissues. Macrophage uptake of the sensor was further assessed in a thioglycollate-induced peritonitis model and in atherosclerosis-prone apoE$^{-/-}$ mice fed a high-fat diet for 12 weeks.
The Ruthenium-based NO sensor is internalised by macrophages and can be detected *in vitro* and *in vivo*. The fluorescent signal of the NO sensor has future implications for tracking plaque progression.