Medical Imaging
EPSRC Centre for Doctoral Training

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Theme 2

- 102 Noninvasive Molecular Biopsy – Focused Ultrasound Delivery of Targeted Contrast Agents and Detection by Magnetic Resonance Imaging

Aim of the project

This project has elements of both stream 1 and stream 2 topics so could suit students with strengths in either physics/bioengineering or chemistry. We seek the best candidate who wants to do some synthetic chemistry, alongside the bioengineering and image analysis.

The overarching aim of the project is to develop a noninvasive biopsy system that can image the molecular signatures of a disease in its in vivo native state, in 3D, and throughout the progression of the disease. A focused ultrasound-based drug delivery system will be used to noninvasively and locally deliver normally impermeable targeted MRI contrast agents across the capillaries of a target disease (such as breast cancer). MRI pulse sequences will then be optimised to detect the bound targeted MRI contrast agents in vivo from the surrounding tissue and unbound contrast agents. The sub-aims of this project are (1) to design ultrasound parameters for delivering targeted MRI contrast agents, (2) to identify and/or design targeted MRI contrast agents that could provide sufficient signal contrast when delivered by ultrasound, and (3) to optimise MRI pulse sequences to identify bound targeted contrast agents.

Project description/background

1st supervisor: James Choi
2nd supervisor: Tobias Schaeffter
3rd supervisor: Nick Long

In stratified medicine, diseases are diagnosed and treated according to biological markers in a patient, allowing for targeted treatments and preventing unnecessary treatments. The current diagnostic gold standard is the needle biopsy, whereby a thick needle penetrates through healthy tissue to cut a tissue volume for histological examination. This procedure has its limitations: potential side effects (eg damaging of healthy tissue, pain, bleeding, infection) and can lead to incorrect diagnoses due to poor spatial sampling of the tissue volume. A needle biopsy is also not feasible for certain organs (eg brain), and therefore cannot be used to identify certain diseases. Molecular Imaging by PET, MRI, CT, or ultrasound aims to provide an alternative – if not advantageous – method for diagnosing diseases. “Tagged” targeting contrast agents (CAs) are systemically administered and circulate throughout the entire body. If the agent sufficiently accumulates in the diseased region, it has the potential to provide a unique molecular signature detectable by an imaging modality, such as MRI. However, many targeted CAs fail to sufficiently accumulate at the target and those that do, require high administration doses that elicit side effects too extreme for clinical use. Thus diagnosis of deadly diseases, such as cancer, remains noninvasively unidentifiable making treatment selection inaccurate and its associated side effects unnecessary.
In recent years, ultrasound technologies have been developed to open capillaries and thereby deliver normally impermeable molecules and nanoparticles into a tissue volume. Ultrasound is a mechanical wave, which can propagate through soft tissue and bone. When emitted from a focussed transducer, ultrasound converges to a small volume on the order of cubic millimetres while leaving the surroundings intact. Systemically administered clinically-approved microbubbles are driven by ultrasound to expand and contract against the capillary walls and create a transient permeability increase. Specific parameters have been shown to homogeneously deliver a high dose of molecules to the target volume without cellular damage [1, 2] and the Choi Group has further optimised these sequences [3].

Fig 1. Safe in vivo capillary opening. The (A, C) left hippocampus was sonicated using short US pulses during systemic microbubble circulation while the (B, D) right region was not sonicated. (A, B) Fluorescently-labeled 3-kDa dextran was delivered to the left region (C, D) without detectable damage as assessed using H&E staining. The white bar in (A) depicts 1 mm.

Highly paramagnetic metals, such as gadolinium, held within a stable chelating ligand, have been used successfully as CAs in MRI, enhancing signal intensity to aid in the diagnosis of various disease states, such as inflammation and cancer. The design of targeted Gd-based CAs, usually based around the classical macrocyclic motif DOTA [4], and targeted nanoparticle (NP)-based CAs, are expanding research areas eg for imaging proteins involved in disease. The Long group has developed Gd-D03A compounds peripherally functionalised with targeting peptides for formyl peptide receptors [4] and Fe3O4 SPIOs functionalised for cancer targets, with NP-surface ligand receptors such as a cyclopentapeptide for CXCR4 and the RGD peptide for tumours over-expressing the αvβ3 integrin [5]. In the proposed research, these targeted agents or similar bioconjugation strategies of CAs will be utilised, particularly for cancerous tumour targets.

In this project, the state-of-the-art techniques in ultrasound-mediated capillary opening, targeted MRI-CAs, and MRI pulse sequences will be combined to create a noninvasive molecular biopsy system that can be assessed by MRI.

Fig 2. Targeted contrast agent. A new Gd(III) DOTA conjugate of cFLFLFK has been synthesised which targets and visualises FPR1 in the inflammatory responses using magnetic resonance imaging (MRI).

References:
[3] Pouliopoulos A, Bonaccorsi S, Choi JJ. Exploiting flow to control the in vitro spatiotemporal distribution of...

- 204 Developing new targeted molecular contrast agents for imaging inflammation of vulnerable plaques

**Aim of the project**

This PhD project is uniquely situated between chemistry (ICL-Chem), bioengineering (ICL-Bioeng) and imaging (KCL-Imag), and aims are divided according to departments. Aim 1: Development of a bespoke nanotechnology platform for optimal imaging of vulnerable plaques (ICL-Chem). Aim 2: Identification of new biological targets for vulnerable plaques detection on the basis of recently performed genome-wide screening of a unique animal model (ICL-Bioeng). Aim 3: Testing of the new targets in murine and pig tissue with MRI/PET/CT. We will test (i) the passive uptake, (ii) distribution and (iii) active and specific uptake of the molecular contrast agents in vulnerable plaques (KCL-Imag).

**Project description/background**

1st supervisor: Rob Krams
2nd supervisor: Rene Botnar
3rd supervisor: Nick Long

This project is multi-faceted so it would suit students with strengths in either physics/bioengineering or chemistry. We seek the best candidate who wants to carry out some synthetic chemistry, alongside the bioengineering and image analysis.

Coronary heart disease (CHD) is the global leading cause of death. In the UK, acute coronary syndromes (ACS) cause ~60% of CHD deaths and lead to ~240,000 hospitalisations each year, incurring direct healthcare costs of ~£1.7 billion annually. The majority of the mortality of CHD is related to the rupture of a thin cap fibro-atheroma (TCFA). The characteristics of a rupture-prone plaque are that of a large and soft lipid-rich necrotic core covered by a thin and inflamed fibrous cap. Associated features include large plaque burden, expansive remodelling preventing luminal obstruction (mild stenosis by angiography), neo-vascularization, plaque hemorrhage, adventitial inflammation, and a "spotty" pattern of calcifications. Despite considerable world-wide efforts, specific diagnostic tools for detection of vulnerable plaques are currently lacking.

Molecular imaging enables detection beyond (vascular) anatomy and is of special interest for detection of plaque vulnerability, as structural changes are considered relatively stable (weeks to months), as compared to the kinetics of macrophage uptake (hours to days). The dynamic accumulation of activated macrophages, associates with plaque vulnerability through release of activated MMPs. Site-specific accumulation and concentration of macrophages occur through surface receptors expressed on the endothelium. This process may lead to high local activation of MMPs, local weak spots in the extra-cellular matrix and when these spots coincide with high peaks in mechanical stress, rupture may occur.

Several contrast agents have been proposed to target proteins and cell surface receptors of interest, including targeted micelles, liposomes, superparamagnetic iron oxide particles (SPIOs) and gold/silica particles. Nanotechnology e.g. the design of nanoparticles (1-100 nm), is emerging as a new area within the field of molecular imaging, partially through superior properties over other contrast material, including high payloads, high contrast, high retention and short washout kinetics from plaques, efficient coupling to proteins and antibodies and custom-made composition. This latter property enables design of probes for MRI, CT and OCT...
(optical coherence tomography), the main imaging modalities for cardiovascular diagnosis. In this project, the new nanoparticle materials will be synthesised (ICL-Chem) and imaged with microscopic systems available in Imperial College (ICL-BioEng) and subsequently, with MRI/PET/CT in murine models and a novel atherosclerotic pig model (KCL-Imag).

The Long group have expertise in the design and synthesis of a range of nanoparticulate contrast materials – ranging from functionalised iron and manganese oxide nanoparticles (Fig. 1), to gold- and silica-containing materials (refs: 1, 2). The Krums group has long-lasting experience in quantitative imaging of vulnerable plaques in animal models, in the isolation and imaging of isolated macrophages, and in the generation of novel antibodies (refs: 3, 4). The Botnar group has experience with the development of cardiovascular MRI sequences and validation of novel contrast agents for preclinical and clinical molecular imaging of atherosclerosis (Fig. 2) (refs: 5, 6).

The collaboration of these groups enables evaluation of the hypothesis that “molecular imaging offers a new and unique method to identify progress and rupture-risk of vulnerable plaques”. It is expected that by adding extra functionality to standard imaging, molecular imaging will improve precision/stratification of diagnosis and aid therapy.

References:
205 Novel theranostic targeted anti-cancer probe for multi-modal imaging on multiple scales

Aim of the project
In this project we will develop cancer cell-targeted theranostic compounds for visualizing tumours at a whole-body (using SPECT/PET) and microscopic (using optical imaging) level; at the same time the compound stabilizes guanine-rich DNA/RNA structures (G-quadruplexes) that are inhibitory to cancer cell proliferation and oncogene expression. The probes will be built by coupling a known platinum-based cytotoxic agent (which also acts as an optical probe) and the radioisotope chelator DOTA to a peptide or antibody that selectively targets cancer cells. We will also aim to modify the platinum-based cytotoxic agent such that it is only activated in the reducing environment found within targeted cells thereby reducing potential off-target activity risks. In this way, our theranostic agent will be highly selective against cancer cells and allow tracking the tumour uptake of the cytotoxic probe on a whole-body level as well as visualize targeted cells on a microscopic level.

Project description/background
1st supervisor: Gilbert Fruhwirth
2nd supervisor: Ramon Vilar

G-quadruplexes (G Qs) are non-canonical secondary structures that can be adopted by guanine-rich DNA and RNA sequences. Such DNA sequences are frequently found in telomeres and regulatory regions of oncogenes (e.g. MYC), thus playing important roles in cancer biology. Furthermore, G Qs of messenger RNA were very recently identified to be responsible for translational control and immune evasion of viruses (e.g. Epstein-Barr virus). Various small molecules were designed to interact with a variety of G Qs most notably some with promising anti-cancer activity in tumour xenograft models, which lately entered phase II clinical trials on cancer patients. However, as with many small molecule drugs systemic administration has significant undesired side-effects. Targeting the drug to tumour cells can resolve this issue with a particular promising concept represented by so-called Antibody-Drug-Conjugates (ADC); they consist of an antibody for tumour targeting, a highly cytotoxic agent, and a linker that must not interfere with the functions of either of the other components; the first ADCs were recently licensed and have entered the market.

The goal of this PhD project is to build on the ADC concept and develop a theranostic probe that will allow tumour imaging at a macroscopic (whole-body) and microscopic level and at the same time deliver selectively a highly cytotoxic agent into tumours. The student will combine a G-quadruplex binder previously developed in the Vilar group, which acts both as an optical probe and cytotoxic agent, with a tumour-targeting moiety and a probe that allows whole-body radionuclide imaging (PET/SPECT). The product will be the first tumour-targeted G Q that can be tracked by in vivo imaging. In addition, we plan to further refine the approach converting the cytotoxic drug into a pro-drug to further reduce off-target cytotoxicity.

This ESPRC-funded CDT PhD studentship covers basic research spanning several disciplines with the candidate benefitting largely from being embedded within an active and truly multidisciplinary research environment at Imperial College London (Department of Chemistry and Institute of Chemical Biology) and King’s College London (Division of Imaging Sciences & Biomedical Engineering embedded into the Comprehensive Cancer Imaging Centre, the Medical Engineering Centre and the Biomedical Research Centre). In particular, the student will obtain experience in targeted drug design and chemical synthesis, cell biological drug evaluation, advanced fluorescence microscopy, preclinical multi-modal whole-body imaging, as well as animal models for oncology.

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**Development of New [11C]CO2 Incorporation Methodologies and application to the development of imaging probes for the gammahydroxybutyric acid receptor**

**Aim of the project**
The aims of this PhD project are to: synthesise organozinc reagents and apply them to the synthesis of 11C-carboxylic acid-containing molecules; perform model reactions for synthesising 11C-carboxylic acids from cyclotron-produced 11C-carbon dioxide; optimise synthesis of model reactions; synthesise substrates for GHB ligands; use the above methods/substrates in the targeted synthesis of PET radiotracers for imaging putative GHB receptors in the brain; characterise and evaluate labelled GHB radioligands in cell based assays and by autoradiography and to use radiolabelled GHB radioligands to investigate the expression and function of the putative GHB receptor in vivo (microPET in rodents).

**Project description/background**
1st supervisor: Tony Gee  
2nd supervisor: David Nutt

Positron emission tomography (PET) imaging is a powerful tool for disease diagnosis, understanding in vivo biomechanisms, and in drug discovery and development. PET imaging probes are labelled with cyclotron-produced short-lived positron-emitting radionuclides (e.g., carbon-11 and fluorine-18, radioactive half-lives 20 min and 110 min, respectively) using rapid labelling techniques. A major confounding factor in developing new radiolabelled PET probes is the limited number of labelled starting materials available from the cyclotron.

Radiolabelling of carboxyl functionalities, a key component of many biologically active molecules, is currently limited to using organolithium and Grignard reagents. However, these organometallic reagents show low compatibility with many functional groups, precluding the synthesis of functionalised molecules. Recent studies have shown that the carboxylation of organozinc reagents with carbon dioxide allows the synthesis of functionalised carboxylic acids. These compounds are highly compatible with many functional groups and react with carbon dioxide in polar aprotic solvents (e.g., dimethylformamide, dimethyl sulfoxide and acetonitrile) in the presence of lithium chloride or strong Lewis acids.

We propose to investigate the use of organozinc reagents in the synthesis of carboxylic acid-containing carbon-11 ligands for the putative GHB receptor, implicated in drug abuse, narcolepsy, schizophrenia and depression:  
**Target molecules: GHB analogues – labelling in the carboxylic acid moiety**

Gamma-Hydroxybutyric acid (GHB) is an endogenous compound in the mammalian brain with both low- and high-affinity receptor targets. GHB is used clinically in the treatment of symptoms of narcolepsy and alcoholism, but also illicitly abused as the recreational drug Fantasy. Major pharmacological effects of exogenous GHB are mediated by GABA subtype B (GABAB) receptors that bind GHB with low affinity. The existence of GHB high-affinity binding sites has been known for more than three decades, but the uncovering of their molecular identity has only recently begun. The development of PET probes for this target may elucidate the in vivo pharmacology of this class of compounds and shed light on the physiological role of the putative GHB receptor.

The project will be run jointly with Prof David Nutt, whose laboratory has a long track record in assessing the effects of examining the role of GABA-related receptor function in brain. He has developed many CNS radiotracers over the last 2 decades and the laboratory will play a key role in the in vitro and in vivo evaluation of ligand candidates.

The training on this project will provide the student with unique skills in radiochemistry, radiochemistry instrumentation, radioanalytical techniques, purification science, synthetic medicinal chemistry, in vitro and in vivo radiobiology (e.g., autoradiography, microPET scanning) and pharmacology in addition to unique national and international networking opportunities.
207 Imaging glycogenesis as a novel biomarker of drug-induced quiescence and senescence

Aim of the project
Having recently discovered a new probe to trace glycogenesis, the aim of this PhD project is to establish the biological rationale and specificity of imaging cancer-specific quiescence/senescence through measurement of enhanced glycogenesis by positron emission tomography.

Project description/background
1st supervisor: Eric Aboagye
2nd supervisor: Ralph Sinkus

Quiescence and by extension senescence are important cancer phenotypes that have not to date been described by molecular imaging. Of the targets known to be regulated by this phenotype, glycogen synthesis is perhaps the most optimal for non-invasive ‘tracing’. Glycogen is a multi-branched glucose polysaccharide that in humans is synthesised (glycogenesis) and stored primarily by liver and muscle cells, and physiologically functions as a secondary energy source. While the high rate of glucose uptake to fuel the bioenergetic and anabolic demands of proliferating cancer cells is well recognized, and exploited with 18F-2-fluoro-2-deoxyglucose positron emission tomography (18F-FDG-PET) to image tumours clinically, enhanced glycogenesis in cancer is less well understood. Distinct from our perception of the cancer cell as a cell deficient in energy stores requiring glucose uptake and glycolysis to meet energy demands, emerging data suggest that the quiescent state (high proportions of cancer cells normally exist in this state) can induce glycogen storage in cancer cells and buffer bio-energetic stress. To provide biologic insights into physiologic regulation of glycogen metabolism, we recently developed a non-invasive method to image glycogen storage via 18F-N-(methyl-(2-fluoroethyl)-1H-[1,2,3]triazole-4-yl)glucosamine positron emission tomography (18F-NFTG-PET) (Witney et al Cancer Res 2014).

Specificity of glycogen labelling was demonstrated by isolating 18F-NFTG-associated glycogen and with stable knockdown of glycogen synthase 1 (GYS1), which inhibited 18F-NFTG uptake, while oncogene (Rab25) activation-associated glycogen synthesis led to increased uptake. We have presented preliminary data to show that the rate of glycogenesis is cell cycle-regulated, enhanced during the non-proliferative state of a cancer cell line. In this cell line, 18F-NFTG but not 18F-FDG uptake increased proportionally with cell density and G1/G0 arrest.

208 Structural imaging of tumour viscosity/Novel probes to correlate microscopic and macroscopic tumour structures

Aim of the project
Tumours are complex structures, which are defined by diverse parameters such as cellular organisation, rigidity, intracellular and extracellular viscosity, diffusion, or interstitial pressure. Several of those impact on tumour cell proliferation/migration, on drug delivery, and consequently on treatment options. Currently, little is known about intracellular and extracellular microviscosity/rigidity and how the latter affect macroscopic structures and diffusion accessible by MRI. Here, we will develop and characterize new targeted microviscosity/rigidity sensors. We will build on our existing fluorescent microviscosity sensors to develop new probes targeted to cancer cells and the tumour matrix. We will characterize and validate them by photophysical and biochemical methods in vitro and in vivo including the use of a subcutaneous tumour model. Importantly, we will use MRI for assessing macroscopic tumour structure and diffusion properties and correlate the latter with microviscosity/rigidity quantified by the newly designed probes. This work will improve our understanding of tumour micro- and macrostructures and how they impact on drug delivery and ultimately on therapy.

Project description/background
1st supervisor: Marina Kuimova
2nd supervisor: Gilbert Fruhwirth

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Structural parameters of tumours include cellular organisation, rigidity, intracellular and extracellular viscosity, diffusion, or interstitial pressure with several of those impacting on tumour cell proliferation/migration, drug delivery and treatment options. Currently, little is known about how microstructures such as (sub-)cellular organisation as well as extracellular matrix components (ECM) affect overall tumour structure and growth as well as treatment options. The physical properties of the ECM refer to its rigidity, porosity, insolubility, spatial arrangement and topography, and other physical features that together determine its role in scaffolding to support tissue architecture and integrity. Some of those parameters are accessible via various microscopic or macroscopic imaging techniques; however, no systematic correlation of micro- with macrostructure has been reported, largely due to a lack of suitable sensors.

In this project we will develop and characterize new probes to visualize the tumour microstructure, namely microviscosity/rigidity. We will build on existing so-called fluorescent molecular rotor technology. Molecular rotors are small synthetic fluorophores that allow quantitative microviscosity measurements of individual organelles of live cells [1-3]. Recently we were successful in synthesize a plasma membrane-specific molecular rotor, Figure 1 [3], which is a water soluble molecule and shows good retention properties to tumour tissue in vivo.

The goal of this PhD project is to develop new tumour targeting probes, for quantifying intracellular and extracellular matrix microviscosity. We will exploit synthetic modification of molecular rotors to achieve conjugates with targeting groups for cancer cells and the ECM. We will then characterize and validate them in vitro in cells and spheroid cultures as a simple tumour model using FLI and RFI; validation will include experiments affecting matrix rigidity, porosity, and viscosity as well as disturbances thereof in live cultures (by using validated small molecule inhibitors or biologicals). The student will use an animal tumour model for in vivo validation and to measure drug treatment impact on tumour micro- and macrostructure (viscosity, rigidity, diffusion).

This studentship covers basic research spanning several disciplines with the candidate benefitting largely from being embedded within an active and truly multidisciplinary research environment at Imperial College London (Faculty of Natural Sciences) and King’s College London (Division of Imaging Sciences & Biomedical Engineering embedded into the Comprehensive Cancer Imaging Centre, the Medical Engineering Centre and the Biomedical Research Centre).

In particular, the student will obtain hands-on experience in probe design and basic chemical synthesis, biochemistry, advanced fluorescence imaging and microscopy, preclinical MR imaging, and cancer cell and spheroid culture as well as animal models for oncology (including training in accordance with UK legislation).

References:
209 Targeting the Progesterone Receptor using [C-11]Tanaproget

Aim of the project

This project will assess the non-steroidal progesterone receptor (PR) antagonist C-11 Tanaproget as PET tracer for imaging breast cancer. To date, several F-18 derivatives of Tanaproget have been reported but suffer from complex and poor yielding radiochemistries, and/or undesirable metabolic profiles. Using a novel C-11 radiolabelling technique, C-11 derivatives of the Tanaproget molecule will be labelled which should lead to a more robust tracers with improved metabolic profiles. It is anticipated that this will result in a PET tracer that will provide more accurate estimates of the levels PR in breast tumours and thus enable better therapeutic guidance.

Project description/background

1st supervisor: Phil Miller
2nd supervisor: Eric Aboagye

Positron Emission Tomography (PET) is an important imaging modality for the clinical diagnosis and staging of a wide range of conditions such as cancer, neurodegenerative illnesses and cardiovascular diseases. The targeting of biological receptors using positron emitting radiotracers has enabled the extraction of important quantitative information relating to metabolism, biodistribution, physiology and pathology in vivo. The steroidal hormone receptors are an important and well-studied class of receptor that regulate numerous cellular functions, including gene expression. It is now well established that the level and activities of steroid receptors are inextricably linked to the growth of hormone responsive cancers such as breast cancer and prostate cancer. The implicated hormone receptors, estrogen receptor (ER) and progesterone receptor (PR) in breast cancer, and androgen receptor (AR) in prostate cancer have therefore become targets for both diagnosis and therapeutic intervention. Although the clinical PET imaging of ER in breast cancer and AR in prostate cancer is now well established, limited clinical progress has been made in imaging PR. Knowledge of PR levels in breast tumours can be used to predict patient response to ER-targeted therapies, hence a non-invasive PET tracer that can provide quantitative information on PR levels could prove important for therapeutic guidance.

To date, most PR PET radioligands are based on steroidal compounds that have been labelled with either F-18, I-123 or Br-76. Several of these promising steroidal radioligands have unfortunately failed when translated to human. The purported reasons for the failures of these ligands include cross reactivity with other steroid receptors, inactivating metabolism and increased non-specific binding due to the high lipophilicity of steroids. Recently several non-steroidal progestins with high binding affinities and selectivities have been reported, of these Tanaproget (figure below) has been singled out because it was discovered to be exceptionally potent, with very high binding affinities (KD in sub nM range). Tanaproget is a relatively simple organic molecule that has a dihydrobenzoxazinethione core, in addition to a pyrrole ring and nitrile functional group. Recent strategies to develop an F-18 derivative have focused on tagging an F-18 prosthetic group to the pyrrole ring and replacing the methyl groups on the dihydrobenzoxazinethione core with F-18 fluorooethyl or a fluoropropyl groups. The caveats of these methods are that the first route gives a tracer that has unfavourable metabolism and the second is synthetically very challenging, requiring at least three transformations which results in low (<5%) radiochemical yields in a reaction time >2 hrs (Bioconjugate Chem., 2010, 21, 1096). It is our aim to develop a rapid one step protocol to generate C-11 Tanaproget. We will attempt 2 routes: 1. firstly labelling within dihydrobenzoxazinethione core using novel 11CS2 radiochemistry in order to generate an ‘exact’ C-11 Tanaproget derivative; and secondly 2. Using a completely new C-11 reagent, C-11 ammonium thiocyanate (NH4S11CN), we will radiolabel on the periphery of the molecule in one step to generate a labelled thiocyanate C-11 Tanaproget derivative (see figure inset). Based on previously published structure-activity-relationships, modification of the Tanaproget molecule at ring methyl positions does not significantly inhibit its binding. Although it may possible to label on the pyrrole ring using standard C-11 methylation chemistry all indications suggest that this is a metabolically susceptible position, hence the reasoning for labelling within the core of the molecule.
210 Development of Novel 18F-PET Tracers for Direct Detection of Myocardial Oxidative Stress

Aim of the project
Reactive oxygen species (ROS) induced oxidative stress closely associates with many cardiovascular pathological conditions such as the evolution of atherosclerotic plaques, the progression of cardiac hypertrophy to heart failure, and the cardiotoxicity as the major side effect of doxorubicin cancer chemotherapy. As undisputed mediators of cardiac pathologies, it would be highly desirable to have a non-invasive means of visualising and quantifying the pathologically elevated ROS in patients for both diagnostic and prognostic purposes. In this research program, we will develop 18F-labelled molecular probes for direct measurement of the overproduced ROS in vivo. Such novel imaging agents would have broad clinical applications to detect the advanced atherosclerotic plaques, personalise dose titration of chemotherapeutic drugs, and evaluate the response to anti-ROS therapies using positron emission tomography (PET).

Project description/background
1st supervisor: Ran Yan
2nd supervisor: Rick Southworth

Oxidative stress induced by the elevated reactive oxygen species (ROS) such as superoxide (•O2−), hydroxyl radical (•OH), hydrogen peroxide (H2O2), hypochlorous acid (HOCl), nitric oxide (•NO) and peroxynitrite (ONOO−) closely associates with many cardiovascular pathologies including ischemia/reperfusion injury, atherosclerotic plaque evolution, and cardiotoxicity in cancer chemotherapy. Current imaging methods such as Electron Paramagnetic Resonance spectroscopy and near-infrared fluorescence technique are limited by both tissue penetration depth and lack of whole-body human scanners. In contrast, Positron emission tomography (PET) is a non-invasive nuclear imaging technique that allows quantitative measurement of whole-body distribution and metabolism of radiolabeled tracers in vivo without the limitation of detection depth. By employing sub-pharmacological doses of radiotracers, it enables the study of biological processes without perturbing them, and is largely unaffected by toxicity issues. As a dynamic imaging technique, it also allows the serial investigation of biological processes over time in the same subject such as the evolution of pathologies and response to treatment. Moreover, clinical PET scanners are widely distributed and routinely employed for diagnosis of various diseases.

The PhD students will carry out investigation and receive training as following:
Stage 1: Tracer library preparation and chemical specificity testing (12 months)
For systematic screening, a library of 18/19F-labelled tracer candidates will be prepared. To investigate their chemical specificity, all 18F-labelled compounds will be reacted with a range of bio-relevant ROS. The degree of tracer oxidation and the identity of the oxidised products will be analysed by HPLC and LC-MS. We will identify candidates that can rapidly and selectively react with one or a type of ROS. Their logD will also be measured.
Training on: a variety of organic synthesis methods, safely handling radioactive material and 18F-labelling techniques; analytical methods such as MRI, HPLC, LC-MS etc.

Stage 2: Tracer evaluation in an isolated heart gamma-detector system (8 months)
We will employ a well-established protocol that reproducibly induces a burst of ROS in isolated perfused rat hearts to evaluate the tracer candidates. The radioactive species trapped within the myocardium will be extracted for HPLC analysis. Successful candidates that can detect the elevated ROS will progress forward.
Training on: handling live animals to obtain personal animal licences; isolated heart gamma-detector system; pharmacokinetics analysis
Stage 3: Evaluation of tracer biodistribution & metabolism in healthy rats (4 months)
The tracer candidate biodistribution at different time points will be carried out to determine their distribution pattern and metabolic pathways in healthy rats. Candidates with low lung, heart, and bone uptake, rapid blood clearance and sufficient blood stability will progress forward.
Training on: radiotracer biodistribution and metabolite analysis; animal dissection techniques; gamma-counter

Stage 4: PET imaging of elevated ROS in doxorubicin-induced cardiotoxicity (12 months)
We established doxorubicin induced ROS-dependent cardiotoxicity in rats. We will evaluate tracer candidate’s capacity to detect ROS with PET scans after doxorubicin administration. Promising candidates should have low cardiac uptake in the pre-doxorubicin baseline scan and a greater than three-fold increased cardiac uptake after doxorubicin treatment. We will further evaluate the promising candidates in other ROS-dependent cardiovascular disease models including atherosclerotic, diabetic, and cardiac hypertrophy mice. If successful, we will approach the cardiologists and oncologists at Guy’s and St Thomas’ Hospital for their translational research.
Training on: inducing ROS-dependent cardiotoxicity in rats with doxorubicin; small animal PET imaging and data analysis.

References:

211 New routes to carbon-11 based molecular imaging agents for in vivo PET imaging

Aim of the project
The aims of this project are to optimise the conversion of cyclotron-produced 11C-carbon monoxide to 11C-carbon dioxide using chlorosilanes as trapping and reducing agents; to use the labelled silacarboxyllic acids as alternative reagents for 11C-CO mediated carbonylation reactions; to use micro/milli-fluidic systems developed by King’s and PMB to implement the efficient synthesis of compounds for in vivo PET imaging application using the developed chemistry; to use the above methods in the targeted synthesis of PET radiotracers of interest for imaging brain receptors and enzymes; to develop methods for synthesising libraries of carbon-11 compounds in order to optimise in vivo stability and binding properties.

Project description/background
1st supervisor: Tony Gee
2nd supervisor: Ran Yan
Industry partner: PMB

Positron emission tomography (PET) imaging is a powerful tool for disease diagnosis, understanding in-vivo biomechanisms, and in drug discovery and development. PET Imaging probes are labelled with cyclotron-produced short-lived positron-emitting radionuclides (eg. carbon-11 and fluorine-18, radioactive half-lives 20 min and 110 min, respectively) using rapid labelling techniques. A major confounding factor in developing new radiolabelled PET probes is the limited number of labelled starting materials available from the cyclotron. Recently the use of labelled carbon monoxide has generated much interest in the synthesis of PET imaging probes because the versatility of the metal-mediated carbonylation chemistry allows access to a plethora of labelled carbonyl compounds of biological interest. However, the availability of labelled carbon monoxide as a synthetic starting material at PET sites internationally is limited to a few research sites because of the need for specialist infrastructure for its conversion from cyclotron-produced carbon monoxide, precluding the wider utilisation of this attractive labelling approach.
This proposal aims to overcome this obstacle by harnessing the properties of Chlorosilanes (Friis et al. J. Am. Chem. Soc. 2011, 133, 18114–18117) as carbon dioxide trapping and reducing agents, obviating the need for specialist infrastructure to perform carbon monoxide radiolabelling procedures (a small scale pilot study in our lab indicate that this may be a viable approach and warrents further investigation). The project will aim to synthesis a library of chorosilanes which will be examined for their propensity to trap and reduce $^{11}$C-carbon monoxide and release $^{11}$C-CO in a rapid and convenient manner. The technique will then be tested in the radiosynthesis of carbonyl-containing compounds of biological interest and will involve a medicinal chemistry component to the project. The imaging properties of the radiolabelled candidates will be initially evaluated in vitro. Promising $^{11}$C-labelled compounds may be evaluated in vivo during the course of the project.

The project will be run in close collaboration with PMB, a subsidiary of the group ALCEN engaged in various sectors, such as the healthcare, the aerospace, the defence the electronics, the nuclear power and the scientific research. PMB is specialized in the design and manufacturing of particles accelerators for medical applications, with a specific focus on the cyclotron and associated technologies used for the Positron Emission Tomography. PMB is engaged in the development of an innovative PET Imaging Biomarkers production solution, based on a new fully micro & milli-fluidic synthesize system, to be proposed to researchers and clinicians.

Based on King’s expertise in radiochemistry and its ability of developing tracers for PET, PMB & King’s have decided to join efforts to co-develop the innovative solution, on the technology, on the radiochemistry and on the integration of new tracers into microfluidic systems. PMB & KCL believe that the field of PET needs a new generation of scientists and clinicians with a deeper and wider understanding of the real value of PET.

This PhD program will provide the student the expertise and skills sought after by this scientific community. The training will provide the student with unique skills in radiochemistry, microfluidics, radiochemistry instrumentation, radioanalytical techniques, purification science, synthetic medicinal chemistry, in vitro and in vivo radiobiology (eg autoradiograophy, microPET scanning) and pharmacology in addition to unique industrial and international networking opportunities.

- 214 Cell and liposome tracking by PET with zirconium-89

**Aim of the project**

Standard radiolabelling methodology for cell tracking by scintigraphy has exploited non-specific assimilation of lipophilic, metastable complexes of indium-111 (eg. with oxine). New developments in cellular medicine are creating new applications for cell tracking in humans, including some with small lesions/cell numbers below the sensitivity of SPECT with 111In (eg coronary artery disease, diabetes, neurovascular inflammation and thrombus), creating a need for positron-emitting analogues to bring the benefits of PET to cell tracking. The first feasible solution to this, using Zr-89, has been developed by the Blower group and has shown great promise in early preclinical in vivo evaluation. In addition, Torres has shown that these complexes are able to radiolabel liposome drug carriers by similar mechanisms. This project will optimise the design and use of the labelling compound and evaluate the effects on radiobiology and survival of the labelled cells and their in vivo behaviour, as a preparation for clinical application in imaging inflammation in diseases such as atherosclerosis. We will also use the optimised tracers for labelling of drug carrying liposomes to track their fate and drug delivery in vivo. 89Zr is a long half-life
positron emitter that could meet this need.

**Project description/background**

1st supervisor: **Rafa Torres**
2nd supervisor: **Nick Long**
3rd supervisor: **Phil Blower**

This project is ideally suited to a student who wants to carry out synthetic chemistry, alongside radiochemistry and biological cell assays and analysis.

The favoured oxidation state of zirconium is 4+ (compared to 3+ for indium), but the parallels between the two metals in reactivity and preferred ligand types suggest that the mechanism exploited to label cells with 111In (i.e. lipophilic metastable chelates entering cells and subsequently dissociating) might be exploited in the case of 89Zr. Tetravalent zirconium forms ZrL4 complexes with monobasic bidentate ligands such as oxinate, tropolonate and hydroxamates, analogous to InL3. On this basis we developed the first synthesis of [89Zr]-Zr(oxinate)4, and compared it with [111In]-In(oxinate)3 for labelling several cell lines and human donor leukocyte, and tracking GFP-5T33 myeloma cells in mice. The resulting efficiency of labelling with Zr-89 was similar to that of In-111, but the Zr-89 showed reduced loss of radiolabel from cells over several days post labelling, both in vitro and in vivo, and initial comparison of toxic effects showed no notable differences (Figure 1). In addition, preliminary work by the Torres group (unpublished) has shown that the oxine complex can label liposomes with 89Zr, with 45% efficiency with stability for at least 48h in human serum.

![Figure 1. Distribution of Zr-89-labelled myeloma cells 2 days and 7 days after injection into mice. Uptake in the liver, spleen and bone marrow is seen as expected.](image)

We therefore propose to convert this preliminary methodology into a clinical translatable PET cell-tracking tool. This requires a survey of the most effective chelating agents to improve on the current use of oxine, both to improve the synthesis of the initial complex and to improve the efficiency of radiolabelling cells (this part of the project has the potential to generate new IP); Torres has begun this process by demonstrating that an isomer of oxine, 2-hydroxyquinoline, is superior to oxine in complexing 89Zr and incorporating it into liposomes (Figure 2, unpublished).

![Figure 2. Structure of 2-hydroxyquinoline (top) and 8-hydroxyquinoline (oxine, bottom)](image)

Using fundamental principles of coordination chemistry, the student will design new candidate ligands for Zr-89. At Imperial College, within the Long group, he/she will synthesise “non-radioactive or cold” zirconium complexes with the aim of characterising their coordination and physicochemical properties (solubility, stability). The student will gain skills in synthetic inorganic chemistry and characterisation (X-ray, MS, IR, etc)

The student will then synthesise and characterise the complexes using Zr-89, available at the labs at St. Thomas’ and test their properties (stability, lipophilicity), gaining skills in radiochemistry under the supervision of Torres and Blower. Further evaluation will be conducted of the retention/efflux of radioactivity in labelled cells in a variety of...
human cell types including neutrophils, eosinophils, lymphocytes (including engineered T-cells), dendritic cells, stem cells and other transplantable cells, and liposomal drug carriers provided by our collaborator Prof. A. Gabizon (Jerusalem). He is a renowned scientist and clinician in the field of nanomedicine and cancer therapy and co-inventor of Doxil®, a successful liposomal anticancer drug; and a full evaluation of the effects of radiolabelling on survival and differentiation/phenotype in vitro and in vivo behaviour of the cells, to ensure that the trafficking observed by PET truly reflects the behaviour of the cells. This will provide the requisite data for selecting the first human evaluation of the Zr-89 PET cell tracking method, which will be planned first in patient cohorts currently imaged by SPECT-isotope (Tc-99m, In-111) labelled cells in order to confirm that the new method matches gold standard technology, then in new more demanding settings such as atherosclerosis where the smaller size of lesions and smaller cell numbers severely limit the utility of current SPECT methods and highlighting the advantages of PET (resolution, sensitivity, quantification).

References: