The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL:

PROJECT DETAILS	
Project Title:	Novel probes for investigating the tumour microenvironment and tissue targeting.
SUPERVISORY TEAM	
Primary Supervisor(s):	Dr Gurdip Bhalay
Associate Supervisor(s):	Professor Nicola Valeri; Dr Gabriela Kramer-Marek
Backup Supervisor:	Dr Swen Hoelder
Lead contact person for the project:	Dr Gurdip Bhalay
DIVISIONAL AFFILIATION	
Primary Division:	Cancer Therapeutics Unit
Primary Team:	Medicinal Chemistry Team 1 (Bhalay)
Other Division (if applicable):	Molecular Pathology, Radiotherapy and Imaging
Other Team (if applicable)):	GI Cancer Biology (Valeri); Radiotherapy and imaging (Kramer-Marek)
PROJECT PROPOSAL	

BACKGROUND TO THE PROJECT

The tumour microenvironment (TME) [1] is the cellular environment in which the tumour exists and plays an important role in cancer progression and metastasis. Specific probes that allow us to visualise different tumour cell processes amd cellular features are useful tools for basic biology research as well as for the diagnosis and characterisation of therapy response [2]. An ideal imaging probe must be sensitive and selective towards a biological effector molecule (analyte) as well as being capable of providing a spatially resolved response in order to monitor dynamic processes within a relevant timescale.

This proposal aims to explore cell-trapping strategies for increasing the half-life of the imaging agent through covalent adduct formation or by lowering passive permeability. Our prototype RBPs (Figure 1) are designed to react chemoselectively with the analytes H_2O_2 [3], H_2S [4] or CYP-reductase [5] that are present in the TME. We will investigate selective release, reaction rates and retention of the probes for the application in basic biology research as well as in the clinic for visualising tumours where their longer residency time should provide an improved image contrast and their biased tissue distribution would benefit the patient by lowering exposure of healthy tissue to radiation.

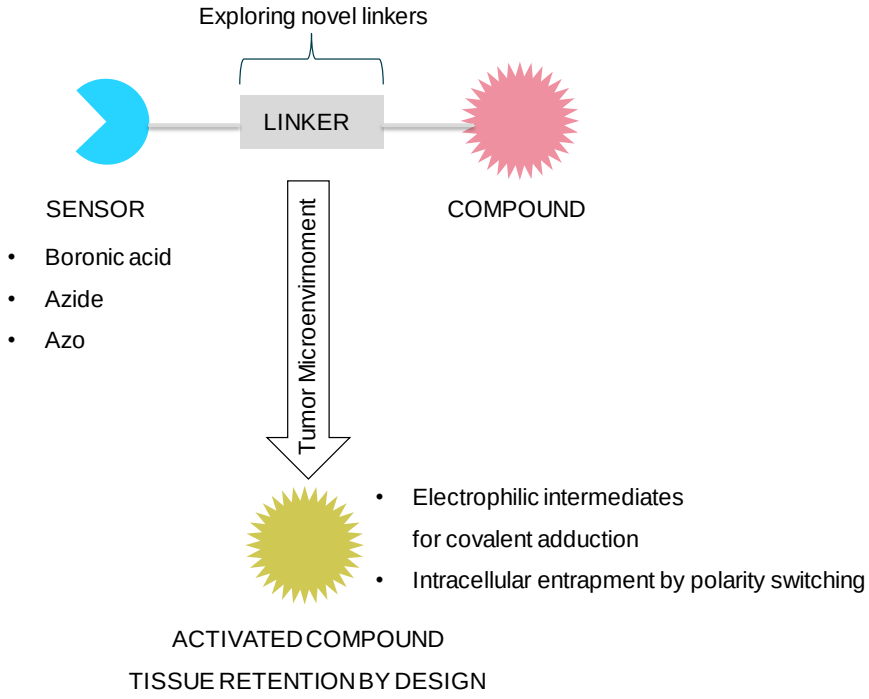

Figure 1: Activation of a reaction-based probe (RBP) in the tumor microenvironment (TME).

Prospective probes for H_2O_2, H_2S and CYP-reductase imaging will be evaluated in cell-based models through collaboration with researchers at the ICR. The proof-of-concept studies will be performed in CHO cells and afterwards we will move to colorectal and breast cancer cell lines. Finally we will test RBPs in 3D organoid mono and co-culture derived from multiple metastatic deposits of patients with metastatic bowel and gastroesophageal cancers. We will modulate culture conditions (normoxia/hypoxia), growth factors and co-culture cytotypes (cancer associated fibroblasts and endothelial cells) in order to model different TME conditions in different metastatic sites and PBPs uptake and retention.

Conventional detection and uptake of these probes will be through both confocal microscopy and flow cytometry (optical probes) and using a Wallac Wizard gamma counter (radioligands). We will study the biological applications these probes for monitoring intracellular H_2O_2, H_2S and CYP-reductase changes in living cells under different conditions.

PROJECT AIMS

- Design and synthesise novel probes that sense H_2O_2, H_2S and CYP-reductase. Then perform proof-of-mechanism studies in cell-free and *in vitro* cell-based systems.
- Identify putative reactive intermediates using nucleophilic trapping agents or fluorescence labelling.
- Explore if sensitivity of the RBP can be tuned through steric/electronic effects.
- Bioimaging in living cells and testing the probes in 3D organoids mono and co-cultures.
- Investigate potential of boro-RBPs as prodrugs targeting the tumour microenvironment.

RESEARCH PROPOSAL (max. 1000 words) Please provide information on the approaches to be used and the expected outcomes**.**

A series of prototype RBPs have been designed that feature boronic acid, azide and azo functionality as the analyte sensing head groups. The student will directly contribute to the design of the RBPs as well as devising synthetic routes and then applying them to practice. Initially our work will focus on the boronic acid based sensors and a decision to progress towards azide and azo systems will be contingent upon progress.

Task 1. Hydrogen peroxide (H_2O_2) detection. Many diseases associated with human aging including cancer have a strong oxidative stress component as a result of unregulated production of reactive oxygen species (ROS). In particular, hydrogen peroxide is a major ROS byproduct in living organisms and a common marker for oxidative stress. Evidence supports a role for H_2O_2 as a second messenger in normal cellular signal transduction [6, 7, 8]. Peroxide bursts in response to cell receptor stimulation can affect several classes of essential signalling proteins that control cell proliferation and/or cell death. Included are kinases such as the mitogen-activated protein (MAP) [9] kinase family, transcription factors such as nuclear factor κB (NF-κB) [10] and activating protein 1 (AP-1), as well as various protein tyrosine phosphatases (PTPs), ion channels and G-proteins. Additionally, it has been proposed that cancer cells behave as metabolic parasites [11], as they use targeted oxidative stress as a "weapon" to extract recycled nutrients from adjacent stromal cells. Oxidative stress in cancer-associated fibroblasts triggers autophagy and mitophagy, resulting in compartmentalized cellular catabolism, loss of mitochondrial function, and the onset of aerobic glycolysis, in the tumour stroma. As such molecular probes sensitive towards H_2O_2 could be useful tools to unravel complex biology.

The first stage of this study will examine the concept of entrapping a fluorescent probe within the cell cytosol by increasing the charge of the molecule once inside the cell [12]. In principle this should prevent passive diffusion of the probe out through the cell membrane and the subsequent accumulation result in a more intense image with reduced background signal. Our test case will start by exploring simple coumarin derivatives such as **3**; chemistry will begin with the synthesis of RBP **1** (Figure 2) as well as compound **4** as a non-releasing mechanistic control compound of the immolative step. These compounds will be reacted with H_2O_2 to establish if the expected conversion of **1** to phenol **2** followed by formation of coumarin **3** and CO_2 is occurring. The reference compound **4** will be subjected to the same experimental conditions to demonstrate that **4** is converted to its corresponding phenol and that **3** is not released. A successful outcome will attempt to repeat this in a cell based system initially using a CHO host pre-treated with physiologically relevant concentrations of H_2O_2 to provide a proof-of-principle before progressing to disease relevant cells. The student will work with Dr Konstantinos Drosopoulos to learn how to apply confocal microscopy and flow cytometry with input from Professor Spiros Linardopoulos, Professor Nicola Valeri and Dr Gabriela Kramer-Marek on selection of commercially available cell lines (e.g. colorectal and breast cancers) as well as 3D models of primary human tumour cell lines which better reflect the heterogeneity of these cancers and are amenable to preclinical testing of novel therapeutic strategies. Further work on probe design will seek to establish if the reactivity of RBPs like **1** are can be manipulated by steric and electronic changes at the A-ring (Figure 2). In summary, the expected outcomes are: synthesis of RBPs, proof-of-principle in cell based systems and for the student to have learned how to use confocal microscopy.

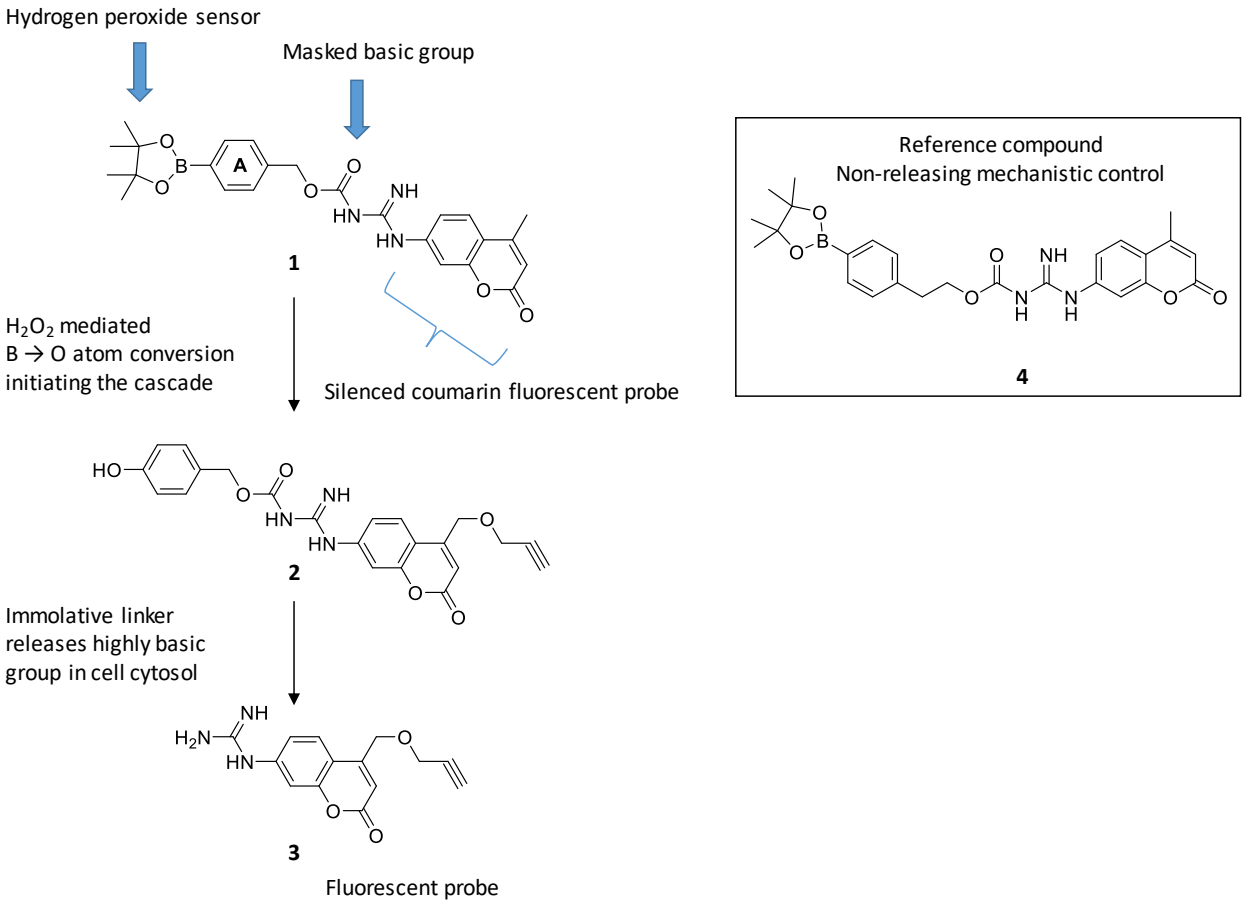

Figure 2: RBP sensing H_2O_2 detected through accumulation of coumarin and fluorescence intensity.

Task 2. Hydrogen sulfide (H_2S) detection. Emerging data indicate that H_2S plays an important role in the regulation of tumour cell biology and it was recently shown that H_2S induces DNA damage and alters the cell cycle in various mammalian cells [13]. However, the effects of H_2S on cancer are controversial and remain unclear. Endogenously produced H_2S has a role in the accumulation or proliferation of cells and the potential biological and clinical significance of H_2S has been the subject of intense debate in recent years. In colon cancer cells over production of cystathionone β-synthase [14] derived H_2S has a pro-tumourigenic effect by both promoting angiogenesis and acting as an alternative energy source for cancer cell metabolism. Despite considerable progress in our understanding about H_2S, much still needs to be learned about its production at the site of tissue injury and its downstream signalling pathways on cell growth. We want to develop H_2S-sensing histochemical probes that operate *via* activity-based sensing to generate electrophilic species capable of covalently binding to local tissue which can be used for histochemical analysis of tissue [15].

To begin the student will prepare RBP **6a** (Figure 3) and treat it with a solution of H_2S to monitor the formation of indole **8** *via* reduction of azide **6a** to amine **6b** and subsequent immolation. Compound **5** (R=H) will be prepared as a non-releasable control compound to eliminating the possibility that **8** is being produced through an alternative mechanism.

At this point we will explore the potential of **7** to be utilised as an electrophilic species for tissue pre-targeting by establishing if the reactivity of **7** can be controlled through steric/electronic changes at the A-ring of **5**. Thus a series of analogues of **5** will be made incorporating these design elements and the

RBPs subjected to H_2S treatment in the presence of electrophile trapping agents such as cysteine. An ideal outcome will generate evidence that it is possible to extend the half-life of methide imino species derivatives of **7**. The prioritisation of further work against other opportunities will be decided at this junction. After this we will move to the synthesis of azide-RBP **9** and investigate if H_2S can trigger the generation of **10**. With convincing evidence gained from electrophile trapping experiments the study will proceed to the CHO cell (pretreated with H_2S) paradigm. The trans cyclooctene click tag on **10** will provide a means to visualise the 'marked' cells through fluorescence imaging.

Next we will test RPBs in 3D organoids obtained from multiple metastatic sites of patients with gastro-oesophageal and colorectal cancers. Using a combination of mono- and co-culture we will replicate different TME scenarios modulating the concentration of growth factors, oxygen levels and presence/absence of other cytotypes such as cancer associated fibroblasts and endothelial cells. We will monitor RPBs and the uptake of the activated compound under different conditions and from different metastases from the same patient testing in vitro whether cancer heterogeneity contributes to different uptake and/or activation of RBPs.

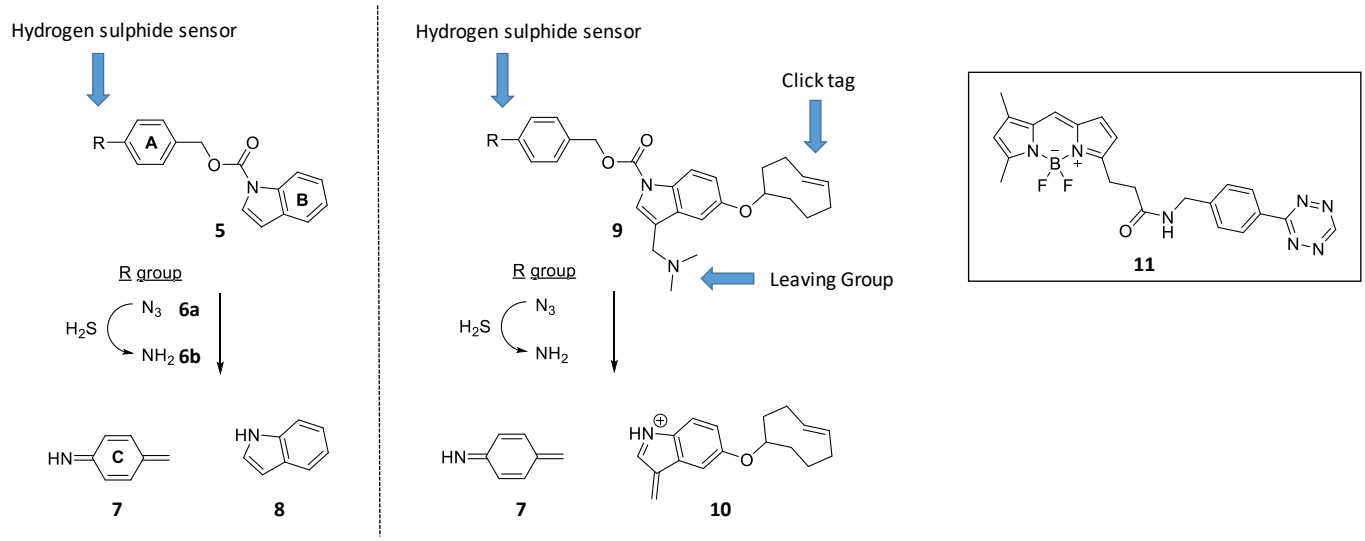

Figure 3: RBPs for histochemical labelling based upon H_2S activity.

Task 3. Azo bond sensors. This body of work will be started depending upon success with task 1 and 2 and isn't described due to restrictions of space. To note we have access to an external expert Professor Stuart Conway (Oxford University) who is familiar in the application of azo bond containing probes and using hypoxia chambers to characterise them. Professor Conway will be at the ICR as part of his secondment for part of the duration of this project and will be a valuable source of experience.

Overall, the student has an opportunity to apply themselves on a multidisciplinary project to become a skilful synthetic chemist who is experienced in working with cell based assays, confocal microscopy and associated chemical biology techniques.

LITERATURE REFERENCES

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CANDIDATE PROFILE

Note: the ICR's standard minimum entry requirement is a relevant undergraduate Honours degree (First or 2:1)

Pre-requisite qualifications of applicants: e.g. BSc or equivalent in specific subject area(s)	Chemistry BSc (hon) degree or equivalent or pharmacy degree (MPharm)
Intended learning outcomes: Please provide a bullet point list (maximum of seven) of the knowledge and skills you expect the student to have attained on completion of the project.	• Organic synthesis. • Using NMR, mass spectrometry and other analytical instruments plus interpreting data generated. • Medicinal chemistry principles. • Use of confocal microscopy and flow cytometry. • Working with in vitro cell cultures.

ADVERTISING DETAILS

Project suitable for a student with a background in:	☐ Biological Sciences

	☐ Physics or Engineering √ Chemistry ☐ Maths, Statistics or Epidemiology ☐ Computer Science ☐ Other (provide details)
Keywords:	**1. Chemical biology**
	2. Imaging
	3. Synthesis
	4. Probes
	5. Medicinal chemistry
	6. Cancer