Better together: Engineering fluorescent proteins to monitor dynamic protein-protein interactions in situ

Main supervisor: Dr D. Dafydd Jones: jonesdd@cardiff.ac.uk
Second supervisor: Dr Oliver Castell: castello@cardiff.ac.uk

Proposed start date: 26th July 2021 (flexible with earlier or later start dates available)
Length: 6 weeks

Project description: The majority of cellular proteins associate to form an oligomeric quaternary structure. Oligomerisation enables and controls activity, thus is pivotal to understanding the molecular events that underpin life. As such there has been a lot of effort to develop approaches to monitor protein oligomerisation in the cell. The most common is through the use of fluorescent proteins as they can be genetically tagged onto target proteins, and interactions measured and followed in situ by processes such as FRET (Förster resonance energy transfer). There is one major problem: FRET relies on the use of at least two different fluorescent proteins so only complexes between different proteins can be monitored. This means that homo-oligomerisation – the self-oligomerisation of the same protein AND the most common form of oligomerisation in biology – cannot be monitored in situ. Your project addresses this need through the design of fluorescent proteins that functionally respond to homo-oligomerisation. Building on recent work in the Jones lab (DOI: 10.1038/s42004-019-0185-5) you will generate fluorescent proteins that switch their fluorescent properties on conversion from a monomer to a dimer. Several designs based on green and yellow fluorescent proteins have be generated and show the required changes in fluorescence on homo-dimerisation. You will test these designs by attaching the new fluorescent proteins to a naturally homo-dimeric protein: dimerisation of the natural protein will promote fluorescent protein association so change the fluorescent output. During the course of the project will be learn a range of techniques including protein engineering, biophysical analysis and bioimaging.

If needed due to COVID restrictions, the project can be adapted by switching from a wet lab project to an in silico project. The student can use computational approaches developed in the Jones group to refine the original fluorescent protein variant design using recently determined 3D structures of homo-dimer fluorescent proteins, in silico mutagenesis, molecular docking and molecular dynamics. The student can also use the in silico approaches to investigate adaption/engineering of red fluorescent to achieve a similar aim to that currently being investigated with green and yellow FPs.

Do time-of-day-dependent B cell rhythms alter the intestinal bacteria composition?

Main supervisor: Dr James Alexander Pearson: pearsonj1@cardiff.ac.uk
Second supervisor: Prof. F. Susan Wong: wongfs@cardiff.ac.uk

Proposed start date: 21st June 2021 (flexible)
Length: 6 weeks

Project description: Circadian rhythms, referring to time-of-day-dependent changes, control many aspects of our daily life, including immune responses (e.g. antibody responses) and intestinal bacteria composition; however, it is unknown what time-of-day-dependent immune rhythms occur specifically in response to the intestinal bacteria.

We hypothesise that the time-of-day strongly influences intestinal B cell-mediated immune responses. Thus, the aim of this research is to identify how intestinal B cell responses to bacteria in the gut of in vivo models are altered at different times-of-day.

To investigate this aim, using batched samples already collected and available for study:

1. Extract and analyse bacterial DNA from intestinal samples for 16s rRNA sequencing to identify the bacteria present in the intestine at different times-of-day.
2. Quantify intestinal antibody and associated cytokine concentrations by ELISA and antibody-bound bacteria by flow cytometry (readouts of how the B cells are responding to bacteria).
3. Extract RNA from intestinal B cells/tissue to perform quantitative PCR to evaluate time-of-day-dependent gene expression from genes associated with immune responses to bacteria.
4. Analyse the data for rhythmicity to determine if the data is significantly altered at different times-of-day using DiscoRhythm.

Understanding time-of-day-dependent intestinal immune responses may help us identify when immune responses naturally occur and what role individual immune subsets e.g. B cells have in this regard. Understanding B cell responses to bacteria will greatly advance our knowledge of how these important interactions shape immune function.

All samples are already available for study, enabling the student to start work at the earliest convenience. If necessary due to COVID restrictions, experiments will be conducted by members of the lab with the student virtually observing the experiments by video call. The background to these experiments and data analysis will be discussed in virtual meetings with the PI, which will take place at least twice a week. The student will be able to conduct all analysis using freely available software from home. In addition to this, the student will attend virtual weekly laboratory meetings and journal clubs with the Diabetes Research Group to further enhance their learning. The student will also present their data (virtually) in the final week to the group.

Evaluating adaptive immune responses induced by adenoviral vectored vaccines encoding novel, broadly reactive SARS-CoV-2 immunogens

Main supervisor: Prof Alan Parker: parkeral@cardiff.ac.uk
Second supervisor: Dr Carly Bliss: blissc@cardiff.ac.uk

Proposed start date: 1st July 2021 (flexible)
Length: 6 weeks

Project description: All leading COVID-19 vaccines target immune responses against the SARS-CoV-2 spike structural protein. Mutations within the spike protein are common, leading to immune escape and reduced vaccine efficacy. Spike escape mutant evolution will also be driven by such vaccines targeting only the spike. Our laboratory has identified highly conserved non-structural antigens, with potential to induce broadly reactive immunity against SARS-CoV-2 and its variants. These non-structural antigens are predicted to be immunogenic, however preliminary evaluation is required to assess their tolerability in vivo.

Specifically, one of the identified SARS-CoV-2 non-structural antigens has an elevated degree of sequence similarity to the mouse homologue, therefore antigen tolerance will be closely monitored for signs of autoreactivity following vectored delivery in mice. Leucocytes will be isolation from blood and tissue samples extracted from mice by the Postdoctoral Fellow overseeing the project, and flow cytometry with intracellular cytokine staining performed to enumerate antigen-specific T cells. Cross-reactivity of these cellular responses against self-antigen sequences will specifically be investigated, to monitor for self-antigen responses.

The project will inform whether these novel, non-structural SARS-CoV-2 targets are viable as antigens to be taken forward into pre-clinical vaccine studies. All licensure is in place for in vivo testing. The student will assist in experimental design and set up, and will learn all required laboratory-based and analytical techniques, as well as participating in discussions regarding the downstream applications of their data.

Pandemic mitigation strategies will see the project's Postdoctoral Fellow perform a suitably scaled down version of experiments and generate datasets for the student to analyse. The student would be trained via virtual platforms on the application of multiple software programmes (e.g. FlowJo, GraphPad Prism, Microsoft Excel) to analyse titration data, in addition to the self/non-self immunogenicity data generated by the Postdoctoral Fellow. This would include polyfunctional analyses and statistical testing, and the write up of findings for publication. Understanding these complex analyses alone would strengthen a student's CV, in addition to giving a thorough taster into the depth of analytical processing a career as an immunologist requires.

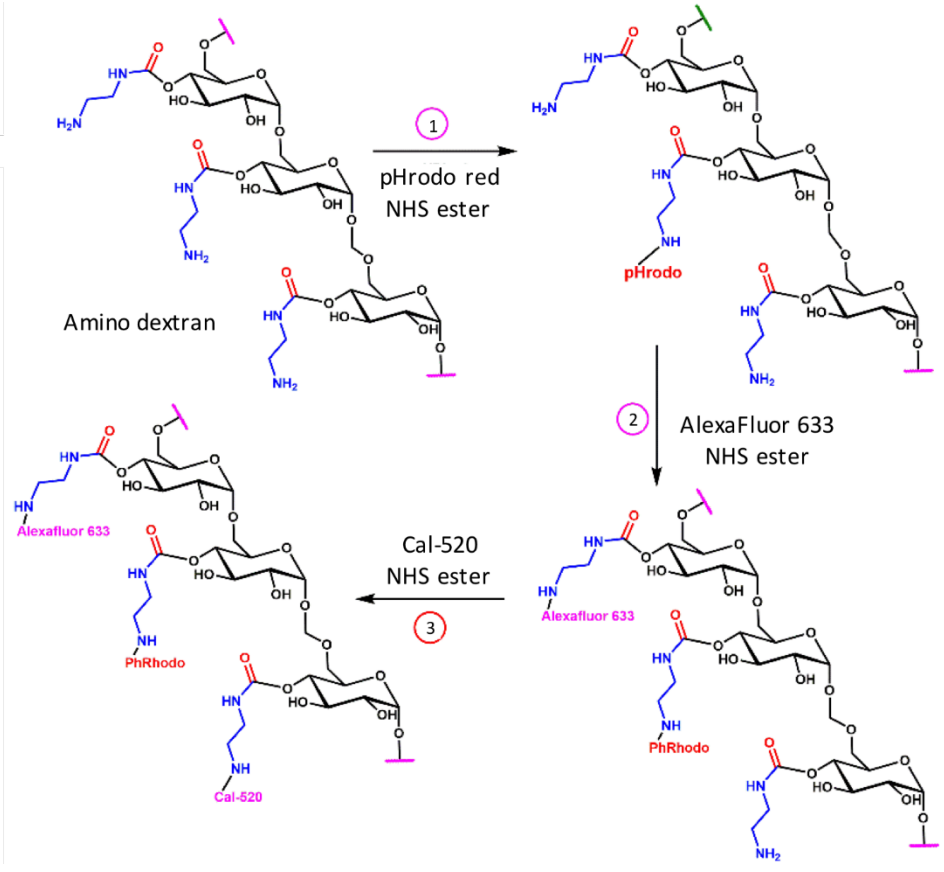
Generating a novel dextran conjugated fluorescent cellular Ca2+ sensor

Main supervisor: Dr Emyr Lloyd-Evans: lloyd-evanse@cardiff.ac.uk
Second supervisor: Dr Fabrizio Pertusatti: pertusatif1@cardiff.ac.uk, Dr. Helen Waller-Evans: waller-evansh@cardiff.ac.uk

Proposed start date: July 2021 (flexible)
Length: 6-8 weeks (flexible)

Project description: Our aim with this project is to utilise the sugar chemistry expertise of our colleague Dr Pertusatti to generate a simple dextran conjugate of the Ca2+ sensor Cal520 (cadaverine) alongside the pH probe amino-pHrodo (both already in house). This relatively simple chemistry will povide the first dual pH and Ca2+ sensor for lysosomes. Following successful conjugation of the probe it will be tested in cells from lysosomal disease patients which have known changes in lysosomal pH and Ca2+ in order to validate the effectiveness of the new probe. The student will gain skills in simple organic chemistry and cell biology techniques that are routine in our labs including, if there is time, testing the probe in a high content imaging system. This entirely novel probe will also be used as preliminary data to support our resubmission of a grant to BBSRC where the development of this probe has been requested.

There is the possibility to run the project either in the ELE lab (where there is sufficient space in the FRG) or at the MDI (HWE lab), if needed due to COVID restrictions. The chemistry is simple enough that it can be done in Biosi, MDI or Pharmacy. Should all lab work be restricted then we have older data from other Ca2+ binding dextran probes that can be analysed.



Pattern recognition in differentiating bone mesenchymal stem cells – microscopy big data image analytics

Main supervisor: Prof Rachel J Errington: erringtonrj@cardiff.ac.uk
Second supervisor: Dr Dimitris Parthimos: parthimos@cardiff.ac.uk

Proposed start date: Start date end of May onwards
Length: 6-7 weeks

Project description: The project focuses on the image-based characterization of stem cell differentiation in bone, in response to tissue heterogeneity, nanoparticle dosing, and the multi-scalar effects of therapeutic perturbation on tissue dynamics. The student will gain experience in optical microscopy, design-of-experiment and developing a 2D and 3D bone niche model; alongside cell and tissue phenotyping with dyes and fluorescent nano-particles.

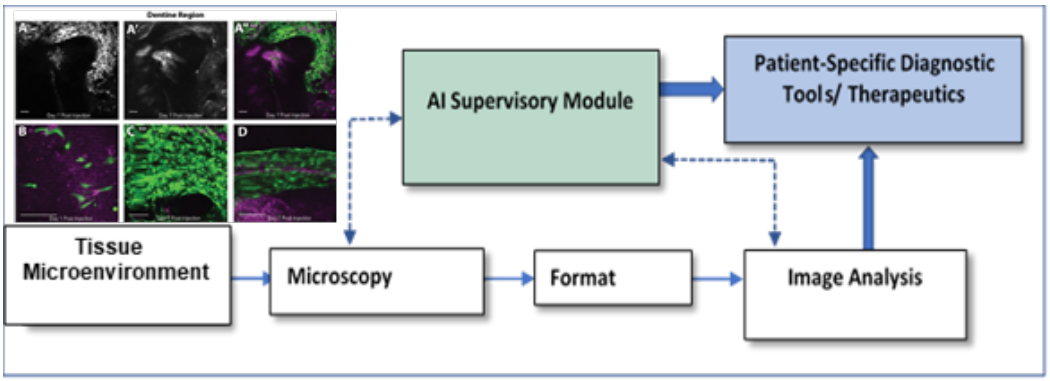


Image processing and data analysis (Figure) will be employed to establish a fully multidisciplinary framework. The key challenge is how to transform raw images to quantitative actionable data using big-data computational workflows, and quantify uncertainties in the work flow. We are working closely with the US National Institute of Standards and Technology (NIST) to ensure we develop robust pipelines that eventually allow for FDA approval. Artificial Intelligence technology will be employed for image characterisation, an important step towards precision biology and medicine.

Defining the problem – Cell characterisation and provenance of analytical pipelines

- Phenotypic characterisation of cells in a bone niche environment to identify the stem cell-like properties of specific lineages under treatment.
- The underlying biology is complex and heterogeneous therefore very large data sets are required to achieve statistical significance. In this context, reproducibility and repeatability of the data is essential for decision making.
- Measurement methodologies and pipelines are critical, as there is strong interdependence of sample preparation, instrumentation and data analytics on the measurement output.

The student will have a unique experience working at the biology-computational interdisciplinary interface, providing a rich experience in biophysics, bone biology and AI analytics.

To account for the ongoing Covid-19 uncertainty the project is built around a strong computational component. It is our aim that the student will gain lab exposure and experimental procedures experience. Our labs have remained open throughout the pandemic by following rigorous distancing procedures and shift patterns. However, if required, it will be easy to shift he project focus onto the analytics component by employing legacy data, without limiting the scope of the work or knowledge acquired.