Title of Project:	A degron-nanobody fusion platform to decode kinase–regulated cellular pathways
Cell Mechanism Supervisor Name	Malcolm Wlakinshaw
Quantitative Supervisor Name	Alison Hulme

Summary of project

The proposed PhD project will design (Walkinshaw) and engineer (Hulme) labelled nanobodies against *Pediculus humanus corporis* (*Ph*)PINK1 bound to ubiquitin in the 'C-terminally retracted' (Ub-CR) conformation (PDB: 6EQI); PINK1 phosphorylates ubiquitin and regulates mitophagy and this has impacts in both cancer and neurodegeneration. A series of RING-domains (degrons) linked to PINK1-Ub specific nanobody fusion proteins will be designed that can function as ubiquitin ligases resulting in degradation of the PINK1 kinase. This will allow identification of novel phosphor- substrates of the kinase and provide the basis for a prototype degron-nanobody fusion platform that allows the creation of virtually any type of synthetic nanobody-encoded construct. Related nanobody-encoded 'post-code' constructs specific to other protein kinases can direct the target kinase to other compartments in the cell such as the nucleus, polysome, ER, or plasma membrane to re-wire signal transduction and develop novel disease models. These nanobodies will be covalently tagged with novel Raman-active tags designed in the Hulme lab. Raman tagging of nanobodies with spectroscopically bioorthogonal tags offers a significant advantage over fluorescent based methods due to the absence of cellular background and the ability to multiplex due to the narrow frequency range of Raman vibrational motifs. Each epitope-specific nanobody will be uniquely labelled, allowing their simultaneous imaging, e.g. on different cancer cell types.

This is a highly interdisciplinary project involving computational chemistry (to design the nanobody), molecular biology (to express and characterise the nanobodies and targets) and cell biology to examine the distributions of the tagged nanobodies providing novel mechanistic insights into signal transduction.

What quantitative skills will the student acquire or develop during their PhD project?

Specific skills will include:

Cell biology:
Working in collaboration with Professors Ted Hupp and Val Brunton at ECRC, the student will learn how to:
- handle different cancer cell types and understand PINK1-mediated signal transduction pathways
- acquire images with a bespoke stimulated Raman scattering (SRS) microscope
- analyse images using packages such as ImageJ (2D) and Matlab (3D reconstruction)

Quantitative skills:
- run molecular dynamics simulations and model protein-protein interactions
- express, purify and characterise protein
- carry out surface plasmon resonance measurements to measure binding constants
- apply chemical methods to vary the tag, linker and conjugation chemistry
- use spectroscopic techniques to characterise the tag and tagged nanobodies (NMR, MS)