Principal Supervisor: Professor Richard Napier, School of Life Sciences, University of Warwick

Other Supervisor(s)/department: Dr Charo del Genio, Applied Mathematics Research Centre, University of Coventry.

Where will you be based: University of Warwick, School of Life Sciences, Gibbet Hill

Duration: 3-4 years

Project Title: Evolving molecular switches

Project Description:
You will study two ligand-activated molecular switches in detail. Crystal structures for the related F-box proteins TIR1 and COI1 are available. Each protein binds a small molecule in a deep pocket and this pair creates a transcription factor (TF) binding site. Once bound, the TF is ubiquitinated and broken down. The ligands and TF degron domains have evolved from a common ancestor. By understanding this ancestry you will gain insights into F-box protein diversification and you will develop omic-led approaches to help identify other possible ligands and associated TFs for the 700+ F-box proteins waiting for functional annotation. Alternatively, you may redesign TIR1 to respond specifically to a ligand of your choice and/or redesign the TF degron to make a synthetic molecular switch. Precision control using benign molecules could open a new era of safer strategies for food production.

Work Plan:
We routinely purify TIR1 and its homologues using baculovirus expression and affinity chromatography. We have virus lines for COI1 and for some ancestral receptors. The associated TFs are available from E. coli expression. We routinely assay binding using Biacore technology. You will establish quantitative structure activity relationships using our extensive ligand analogue libraries. You will learn appropriate software routines both for summarising the binding data and for predicting novel ligands. This includes use of our bespoke tomological docking software. You will design sets of site-specific mutants for each degron family and examine how specificity is conferred and lost in each case, quantifying the 'pharmacological costs' of affinity vs specificity.
It is expected that the work will identify which critical residues in and around the binding pocket confer specificity for ligand and degron respectively, as well as permitted latitude in degron sequence and presentation. The results will form the basis of potential new synthetic molecular switches.

Key experimental skills involved:
Cloning and tissue culture; Protein expression, purification, solubilisation
Biophysics (surface plasmon resonance, isothermal titration calorimetry, dynamic light scattering, thermal shift assays etc)
Functional assays (e.g. structure-activity relationship assays)
Depending on interest, molecular dynamics, computational chemistry

References:

- Lee et al. 2014. Defining binding efficiency and specificity of auxins for SCF(TIR1/AFB)-Aux/IAA co-receptor complex formation. ACS Chem Biol. Mar 21;9(3):673-82. doi: 10.1021/cb400618m.
- Hoyerova K, Hosek P, Quareshy M, Li J, Klima P, Kubes M, Yemm AA, Neve P, Tripathi A, Bennett MJ, **Napier RM.** (2017) Auxin molecular field maps define AUX1 selectivity: many auxin herbicides are not substrates. *New Phytologist* 217(4):1625-1639, doi 10.1111/nph.14950

- Geuten K., Irish V. 2010. Hidden Variability of Floral Homeotic B Genes in Solanaceae Provides a Molecular Basis for the Evolution of Novel Functions. 22(8):2562-78. doi: 10.1105/tpc.110.076026