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**Other Supervisor(s):**

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

**Duration:** 3-4 years

**Project Title:** Gathering cargo for endocytosis using TPLATEs

### **Project Description**

The formation of clathrin-coated vesicles at the plasma membrane of plant cells proceeds via either an adaptor protein 2 (AP2) complex or the early TPLATE complex (Zhang et al., 2015) which may later recruit AP2. The TPLATE complex is plant kingdom specific (Gadeyne et al., 2014), although some protists such as *Dictyostelium* spp. retain an ancestral version (Hirst et al, 2014). TPLATE is a mixed octamer and downregulation of any of its subunits blocks endocytosis, and mutants give rise to defective plant growth and development. It is clearly a fundamentally important complex.

When discovered, the TPLATE complex was shown to include some cargo proteins, including PIN1 and PIN2 (Gadeyne et al., 2014). PIN proteins are auxin transport proteins, and they are involved in establishing and maintaining morphogenic hormone gradients (Bennett, 2015).

We will prepare membrane-enriched samples using differential centrifugation and then optimise the use of SMALPs to solubilise intact and functional complexes in collaboration with Prof Tim Dafforn, Birmingham (Lee et al., 2016). TPLATE complexes will be purified using pull-down experiments. We will use proteomics to identify which proteins co-purify and gel to grid methods to collect TPLATE complexes ready for cryo-electron microscopy and structural determination. SMALP solubilisation is preferred over detergent-based solubilisation methods because it preserves a shell of native lipids around the target complex. The project will not only define structures for the important TPLATE complex, but it will identify the interactions sites between TPLATE and PINs to help us understand how cargo proteins are selected for clathrin-mediated endocytosis.

### **Key experimental skills involved:**

Membrane protein techniques, including SMALP technologies, centrifugation, solubilisation.

Protein expression, purification, native gel methods,

Cryo-electron microscopy techniques, data handling and structural biology techniques.

Cell biology

### **References:**

Bennett, T. (2015) PIN proteins and the evolution of plant development. Trends Plant Sci. 20(8):498-507. doi: 10.1016/j.tplants.2015.05.005

Gadeyne A et al., (2014) The TPLATE adaptor complex drives clathrin-mediated endocytosis in plants. Cell 156(4):691-704. doi: 10.1016

Heard et al (2015) Identification of Regulatory and Cargo Proteins of Endosomal and Secretory Pathways in Arabidopsis thaliana by Proteomic Dissection. Mol Cell Proteomics 1796-813.

Hirst et al., (2015) TSET, an ancient and widespread trafficking complex. eLife 3: e02866

Lee SC et al., (2016) A method for detergent-free isolation of membrane proteins in their local lipid environment. *Nat Protocols* 11(7):1149-62. doi: 10.1038

Zhang et al., (2014) Change your TPLATE, change your fate: plant CME and beyond. *Trends in Plant Science* 20: 41-48.