Designing lipid nanoparticles for highly efficient CRISPR/Cas9 ribonucleoprotein delivery and gene editing in pest insects

Supervisory team:
Rothamsted supervisor: Dr Thomas G. Emyr Davies (Rothamsted Research)
Academic supervisor: Prof Paul Verkade (University of Bristol)
Dr Jana Friedrich (Rothamsted Research), Dr Aimee L. Boyle (University of Bristol)

Host institution: Rothamsted Research (Harpenden)
Submit applications to this project to University of Bristol

Project description:
Scarless gene editing holds enormous potential for agricultural pest management as it allows pest species-specific genome modification without impacting beneficial insects, pollinators or crops. Despite its potential advantages, the main obstacle to apply CRISPR/Cas9 technology applications in the field is the delivery and efficient transport of material into the insect and to target cells owing to various biological hurdles. Transporting ribonucleoprotein complexes (RNPs) containing the Cas9 protein and guide RNA is the simplest method for genome editing. This method has various advantages over plasmids and other RNA delivery forms, including fast action and low off-target editing rates. However, RNPs are complex in composition and charge characteristics making it challenging for them to cross the insects cell membrane. RNPs encapsulated in nanocarriers can be internalized into cells through endocytosis, and following endosomal escape they are able to enter the nucleus to carry out CRISPR/Cas9-mediated genome editing. As such, biocompatible lipid nanoparticles (LNPs) can serve as nanocarriers. However, the challenge arises because conventional LNPs currently available are unlikely to deliver RNP complexes effectively, due to the large size and cationic nature of the Cas9 endonuclease. That means, novel and optimized LNPs are necessary to achieve the most effective transfer state and guarantee high genome editing efficiency. Furthermore, optimal formulation parameters for in vivo gene editing might differ between insect species or targeting cell type. Particle properties can be improved for example by fine tuning the organic excipients of LNPs, microfluidic mixing parameters and pH, or modifying and functionalising the particle surface by attaching moieties (e.g. using cell-penetrating or coiled-coil peptides). The absence of data for LNP-mediated delivery of the CRISPR/Cas9 gene editing system in insects makes this project challenging but also exciting, likely to yield impactful output for the Agri-industry and excellent publication material to advance the field and to find sustainable solutions for insect pest management. This PhD will be deploying methods in bionanotechnology, molecular biology, biochemistry, bioimaging and computational modelling, aimed to design LNPs that will be screened in vitro in insect cells as well as ex and in vivo in pest insects. The project is supported by Rothamsted's expertise in CRISPR/Cas9 gene editing and entomology alongside Bristol's expertise in lipid nanoparticles, peptide design, bioimaging, endocytosis and cellular trafficking. The student will further benefit from Rothamsted's state-of-the art gene editing and cell culture laboratories equipped with automated instruments for the precise and controlled generation and analysis of LNPs.

Our aim as the SWBio DTP is to support students from a range of backgrounds and circumstances. Where needed, we will work with you to take into consideration reasonable project adaptations (for example to support caring responsibilities, disabilities, other significant personal circumstances) as well as flexible working and part-time study requests, to enable greater access to a PhD. All our supervisors support us with this aim, so please feel comfortable in discussing further with the listed PhD project supervisor to see what is feasible.