The structural-functional basis for long-lived DNA diffusion by a helicase-nuclease

Supervisory team:
Main supervisor: Prof Mark Szczelkun (University of Bristol)
Second supervisor: Dr Alan Cheung (University of Bristol)

Collaborators: Dr Ralf Seidel (University of Leipzig)

Host institution: University of Bristol

Project description:
Helicases are vital enzymes with a wide range of roles in DNA and RNA metabolism. Although the separation of two strands of a double-helical polynucleotide is the typical helicase role, a range of unconventional properties have also been discovered for Superfamily 2 helicases such as remodelling of chromatin or RNA sensing. A model example is the Type III Restriction-Modification (RM) enzymes that protect bacteria and archaea against infections by mobile genetic elements such as phages. Type III enzymes utilise their helicase domains to establish a long-lived conformation state that can communicate along kilobase pair DNA distances by thermally-driven 1-dimenstional DNA diffusion, aka "DNA sliding". We have recently established that the Type III helicase uses dsDNA translocation along ~15 bp to initiate sliding, but the nature of the sliding conformation is completely unknown. Using AI-driven protein structural prediction, we have proposed that the nuclease domain has a "clamp-like" structure that can wrap around DNA as the sliding interface. The nature of the sliding assembly and why it is so long-lived has general relevance for other DNA-interacting proteins that slide, such as DNA repair proteins.

In this collaborative project between the Szczelkun and Cheung labs at the University of Bristol, you will employ a combination of biophysical methods and structural determination to test the clamp hypothesis. You will firstly modify the nuclease domain to mutate putative structural features and to introduce fluorescent labels. You will then use millisecond time-resolution stopped flow fluorescence to measure enzyme activity and conformational changes (e.g.' by FRET) and a single-molecule combined optical trap and confocal scanning fluorescent microscope (C-trap) to follow the effects on DNA sliding. In parallel, you will design DNA substrates to trap the stable sliding state and then use these for structural analysis by cryo electron microscopy. You will be based in the Szczelkun lab which is supported by funding from the BBSRC and ERC, and is part of the thriving and collegiate School of Biochemistry at the University of Bristol. There will be close collaboration with the Cheung lab throughout the project. For students who might want to study part-time, during periods of protein purification a full-time effort will be required.

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.