Protein Choreography of CRISPR/Cas9: Dynamics, Mechanism, and Next-Generation Genome Editing

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Essential background: Genome editing enzymes, first characterized as molecular defences in bacteria, now underpin transformative research and applied advances. Their ability to introduce precise and programmable changes to genetic material enables applications in biological research, biotechnology and medicine. However, the gap in our understanding of their catalytic mechanisms imposes key limitations, in particular off-target cleavage remains a major barrier to clinical translation. Addressing these deficits is essential to fully unlock the potential of genome editing systems and to design next-generation tools with enhanced specificity, efficiency, and safety.

Aims: The goal of this highly interdisciplinary and timely PhD project is to identify the structural pathways of catalysis by CRISPR/Cas9 genome editing enzymes. We want to learn how these large proteins move in order to function and how these movements can go wrong and lead to off-target effects – impacting specificity and efficiency. We will learn the precise 'molecular joints' that are critically required to move and explore their function by re-engineering them – ultimately aiming to develop enzymes with more efficient, more precise function as a result.

Research activities: The student will create a coupled loop between structural proteomics experiments and mathematical modelling. The core experiments will be to observe functioning Cas9 enzymes by non-equilibrium hydrogen/deuterium-exchange mass spectrometry (HDX-MS), measuring their structural dynamics with per amino acid per millisecond spatiotemporal resolution. This will use our prototype robotics and the fabrication of custom microfluidics devices. Dynamical mathematical models will be made, optimised and trained on the experimental data. This combined interdisciplinary approach will be used to simulate data-driven atomistic 'molecular movies' of CRISPR/Cas9 as it functions. This will identify dynamic features to be targeted for mutation, exploring their dynamics:function relationship with the goal to optimise characteristics, such as specificity and efficiency.

Training: The student will learn and become expert at cutting edge structural proteomics, microfluidics, dynamical mathematical modelling and generative AI tools (e.g. BioEmu-1). They will also gain highly transferrable skills, such as molecular biology and recombinant protein production and excellence in oral and written scientific communication, that will develop their autonomy during and after the PhD.

More information: Please contact Dr Jonathan Phillips (jj.phillips@exeter.ac.uk) and see The Protein Choreography Group website (https://lsi.exeter.ac.uk/groups/phillips-group) for more information.