Single-molecule analysis of the CRISPR-associated transposons (CAST)

Supervisory team:
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Project description:
A key challenge in the development of programmable genome engineering tools has been the reliable insertion of large DNA sequences into genomes, for example to rescue a mutated gene with a functional copy. A recent potential breakthrough has been the characterisation by the Zhang and Sternberg groups of CRISPR-associated transposons (CAST). Classical transposons, often termed "jumping genes", can insert into random DNA sites and occasionally into specific locations. However, it has proved difficult to re-engineer the transposase enzymes to target user-defined sites. CAST direct the integration of a DNA cargo using type I or type V CRISPR effectors that are guided by readily re-programmed crRNA. The efficiency of CAST have been demonstrated in vivo in bacterial cells, but little is known about the enzyme mechanism. This project aims to use single-molecule microscopy to directly follow this process in real time.

In a collaboration between the Szczelkun and Berger labs, you will establish single molecule assays to measure each step in the integration process. You will first use magnetic tweezers to measure the dynamics of R-loop formation by the CRISPR effectors, and to determine the effect of DNA mismatches. This information is important in understanding off-target binding. The assay can be adapted in the presence of the transposase proteins to follow the subsequent DNA cleavage and integration steps. By fluorescently-labelling the proteins and/or DNA cargo, you will use optical tweezers to follow CAST complexes as they search along DNA for a specific sequence. This process can then be challenged by binding protein roadblocks, such as histones, mimicking packaged DNA found in a cell. In addition to single-molecule techniques, you will also gain experience in protein expression and engineering, and in biochemical enzyme assays. You will be based in the Szczelkun lab which is supported by funding from the BBSRC and ERC.