Title of Project:	Single-molecule microscopy to interrogate binding of the Cas9 enzyme in different chromatin contexts.
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Summary of project

The bacterial nuclease Cas9 with single-guide RNAs (sgRNAs) is a powerful tool for site-specific genome editing. Catalytically inactive dCas9 is used to interrogate/manipulate chromatin states by transcriptional activator/repressor recruitment to select genomic loci or as a molecular hook to generate proteomic profiles of repetitive regions such as telomeres.

The molecular interactions between Cas9, sgRNA, and template DNA are well understood through in vitro studies. However, different chromatin environments are likely to influence Cas9 binding in vivo. Detailed kinetic information of how particular chromatin states interfere with Cas9 binding is necessary for the efficient and reliable use of this methodology in genome editing.

We use fission yeast *Schizosaccharomyces pombe* to study specialized chromatin environments and have generated tools with which specific chromosomal loci can be engineered, using synthetic approaches, to exhibit distinct chromatin states, i.e. euchromatin or heterochromatin. Because the fission yeast genome is only 13.8 Mb, analysis of dCas9 binding will be less prone to artefacts induced by off-target binding.

The project aim is to visualize and interrogate dCas9 binding in distinct well-defined chromatin contexts. Chromatin immunoprecipitation (ChIP) will be performed to assay enrichment of dCas9 at several target loci assembled in distinct chromatin states. To determine the dynamics of the dCas9 search process and its residence time on different chromatin types we will use single molecule microscopy combined with single-particle tracking (SPT). Fusion of dCas9 molecules to several fluorescent proteins as well as tags allowing the use of different organic fluorophore conjugates will be tested to efficiently visualize dCas9 at the single molecule level in vivo. SPT trajectories will be analysed using custom made MATLAB scripts to extract diffusion coefficients for different populations of dCas9 (diffusive, bound to DNA in distinct chromatin states).

Integration of data generated from ChIP and SPT experiments will allow characterization of the dCas9 target search process and binding parameters in distinct chromatin states. The resulting parameters will inform further rational engineering of high-fidelity Cas9 nucleases suitable for genome editing in different chromatin contexts. Furthermore, this study will establish conditions for single-molecule microscopy in fission yeast that can be applied to other proteins of interest in future studies.

What quantitative skills will the student acquire or develop during their PhD project?

ChIP-qPCR and ChIP-seq experiments (and analysis thereof) to measure the enrichment of dCas9 at specific loci in different chromatin contexts.

Fluorescence microscopy and single-particle tracking to visualize the dCas9 molecule and determine kinetic parameters in a cellular environment.

Programming in MATLAB and statistical analysis including the use of machine learning algorithms to classify trajectories.