Project outline

1. Project outline describing the scientific rationale of the project (max 4,000 characters incl. spaces and returns)

The control of gene transcription is one of the main regulatory steps for cellular gene regulation. Transcriptional activation is triggered by signal-dependent assembly of transcription factor (TF) complexes on enhancers to initiate epigenetic modification and remodeling of chromatin and recruitment of the RNA polymerase II preinitiation complex (PIC). Recently great progress has been made, using cryoEM, in our understanding of the PIC structure but how cellular signaling controls transcriptional activation is not understood at a mechanistic level.

One of the best-understood model systems for metazoan gene regulation is found in the innate immune system, which is crucial to limit pathogen infections. In the innate immune system, several groups of pattern recognition receptors induce different signalling pathways leading to production of a variety of antiviral molecules including type I interferons and proinflammatory cytokines. Previous work from the laboratory has revealed mechanistic and structural insights into how the interplay between cellular signalling, TF activation and coassembly on transcriptional enhancers controls gene expression.

This model system is particularly suitable for structural studies due to its naturally compact organization and the proximity of the enhancer DNA elements to the +1 transcription start site. Recent developments in cryoEM and advances in the structural understanding of the constituent components of the PIC make it realistic to study the entire process from enhancer to PIC assembly. The overall focus of the proposal is to use, this well-defined natural and compact promoter to gain mechanistic insights into the multicomponent molecular machinery that controls transcriptional activation. Numerous TFs are classic oncogenes and tumor suppressors that are difficult to target with small molecule drugs. Understanding the detailed mode of how enhancers control transcriptional activation will aid in the development of new pharmacological approaches in cancer and other critical human diseases.

The first major aim of this project is to determine by cryoEM the structure of the fully assembled enhancer comprising activated TFs bound to the transcriptional coactivator CBP/p300 on a DNA template containing the +1 nucleosome. CBP/p300
are closely related modular proteins that contain several well-defined domains. Through these domains, CBP/p300 interact with more than 400 TFs including important oncoproteins and tumour suppressors such as Fos/Jun, MYC, NFkB, p53 and BRACA1 as well as oncogenic fusion proteins such as BRD4-NUT. Interaction of p300 with BRD4-NUT results in an aggressive subtype of lung and head and neck cancer, called NUT midline carcinoma. BRD4-NUT binds to and activates p300 resulting in chromatin hyperacylation and dysregulation of genome expression. Direct pharmacological targeting of such TFs remains an elusive challenge.

The second major aim is to assemble the enhancer complexes and PIC on the promoter and to determine the structure by cryoEM. We will produce the general transcription factors (GTFs) and PIC components TBP, TFIIA, TFIIIB, TFIIE, TFIIIF and TFIIIS recombinantly from Ecoli. Polymerase II, TFIID and TFIIH will be epitope tagged using CRISPR/Cas9 and purified from nuclear extracts using the extensive expertise shared between the Panne and Revyakin labs. Recent data from the Revyakin group, indicate that GTFs interact with DNA promiscuously, at time scales of approximately, 1s. We will use a stepwise assembly of the PIC by sequential incubation on a DNA template with the desired GTFs and enhancer components. Complexes will be purified and imaged by cryoEM and single particle reconstruction. Having access to a biochemically well-defined model system will allow visualization, in real-time, the dynamics of enhancer complex assembly and control of the PIC machinery during transcription initiation.

Techniques that will be undertaken during the project

Structural biochemistry, protein expression in various expression systems including Ecoli, Insect cells and Mammalian cell culture. Complexes will be reconstituted and imaged in the Midlands Regional cryoEM facility or at the eBIC facility at the Diamond Light Source. The material developed will also be used for single-molecule imaging with the goal to reveal insights into the dynamic nature of the transcriptional machinery.