Genetic Circuits and Multicellular Systems with CRISPR/dCas9

Natural genetic circuits enable cells to make sophisticated digital decisions. Building equally complex synthetic circuits in eukaryotes remains difficult, however, because commonly used components leak transcriptionally, do not arbitrarily interconnect or do not have digital responses. Here, I describe the use of CRISPR dCas9 as the main component of a programmable, reconfigurable eukaryotic transcription factor. We have developed these components for cell-cell communication in yeast, for developmental control in plants, and for building large scale genetic circuits and cascades. In particular, we designed *dCas9-Mxi1*-based NOR gates in *Saccharomyces cerevisiae* that allow arbitrary connectivity and large genetic circuits. Because we used the chromatin remodeller *Mxi1*, our gates showed minimal leak and digital responses. We built a combinatorial library of NOR gates that directly convert guide RNA (gRNA) inputs into gRNA outputs, enabling the gates to be 'wired' together. We constructed logic circuits with up to seven gRNAs, including repression cascades with up to seven layers. Modelling predicted the NOR gates have effectively zero transcriptional leak explaining the limited signal degradation in the circuits. This talk will describe our results with dCas9 and a larger framework for understanding computation in genetic circuits and multicellular systems.