Using strand displacement in RNA-based gene circuits

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Nucleic acid strand displacement reactions are key processes of dynamic DNA nanotechnology. They enable the removal of nucleic acid strands from duplexes or the invasion of secondary structures formed by self-complementary nucleic acid sequences. This in turn allows the realization of nucleic acid based molecular devices that are driven by hybridization/strand displacement cycles. A promising area of application for strand displacement processes is found in the context of RNA-based gene regulation processes such as transcriptional and translational riboregulators, or CRISPR/Cas mechanisms [1].
In this talk we will discuss several of such applications: We will show how RNA-based strand displacement can be used to switch the conformation of guide RNAs for the CRIPSR associated nuclease Cas12a (formerly known as Cpf1). Such strand displacement gRNAs (SD gRNAs) can be used to activate the action of Cas12a via appropriate trigger RNAs. It is also possible to create multi-input AND gates for such SD gRNAs, and also to operate them in the context of bacterial gene expression (using the catalytically inactive dCas12a) [2].
Another class of RNA regulators are toehold switch riboregulators, previously developed by Green et al. [3], which are based on the sequestration of the ribosome binding site of an mRNA inside of a hairpin structure, which can be broken via toehold-mediated strand invasion by trigger RNA molecules. We demonstrate how the action of toehold switches can be further modulated via strand displacement processes using antisense trigger RNAs, which can be used, e.g., to implement a genetic XOR gate in bacteria.

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