tRNA mimicking structures to control and monitor biological processes

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Chapter 7

Summary
Synthetic biology is focused on the rational construction of biological systems based on engineering principles. At the early stage of development in the field of synthetic biology, significant progress has been made in designing biological parts and assembling them into complex genetic switches to achieve basic functionalities. These switches have been used to construct proof-of-principle systems with promising results in industrial and medical applications. But most of the current bacterial switches are cis encoded, which lack the versatility with respect to input signals which can be processed. The incorporation of cis encoded switches at the leader mRNA may interfere with folding of the downstream located mRNA and will require genetic modification upstream of the target gene. Due to the genetic modification at the upstream mRNA, bacteria might lose some regulatory genetic elements present in the upstream region of the mRNA. To overcome this problem, in chapter 2, we present versatile and modular RNA switches that are trans encoded and based on tRNA-mimicking structures (TMSs). These switches provide a high degree of freedom for reengineering and can thus be designed to accept a wide range of inputs, including RNA, small molecules, and proteins. Being able to be produced from a trans region of an RNA, these switches do not require alteration upstream of genetic sequences and thus help to mitigate the problem of losing necessary regulatory elements. This powerful approach enables control over the translation of protein expression from plasmid and genome DNA.

After realizing the strong potential of TMS switches in controlling gene expression by versatile stimuli, we advanced our system to implement the RNA based TMS switch in a more controllable fashion. In this regard, we considered light as a pivotal tool that can be used as an input signal to finely tune the output from the TMS switch. Controlling gene expression by light with spatiotemporal resolution not only allows understanding and manipulating fundamental biological processes, but also fuels the development of novel therapeutic strategies. In complement to exploiting optogenetic tools, photochemical strategies mostly rely on the incorporation of photo-responsive small molecules into the corresponding bio-macromolecular scaffolds. Therefore, generally large synthetic effort is required and the reversible switching of gene expression within a single system remains a challenge. To overcome these problems, in chapter 3, we engineered the tRNA mimicking structure (TMS) under control of small photo-switchable signalling molecules. The signalling molecules consist of two amino glycoside molecules that are connected via an azobenzene unit. The light responsiveness of our system originates from the photo-switchable noncovalent
interactions between the signalling molecule and the TMS switch, leading to the demonstration of photochemically controlled expression of two different genes. This modular design will provide a powerful platform for controlling the expression of other functional proteins with high spatiotemporal resolution employing light as a stimulus.

So far, we have achieved to manipulate the expression of a target gene with an RNA-based TMS switch, after which we asked ourselves if we could fabricate the DNA counterpart of our RNA-based switch. The notion that drives our motivation to engineer a ssDNA switch is that the most reported bacterial switches are RNA structures, which cannot respond to inputs that solely bind to ssDNA. In chapter 4, we have introduced a trans encoded ssDNA switch by simply converting the RNA based TMS switch into its deoxynucleotide analogue. Expanding the structure of the switch to DNA allows exploiting the vast number of DNA-based aptamers which allows their use as recognition units in the ssDNA-switch. To the best of our knowledge, this is the first report on intracellular ssDNA switches for controlling gene expression capable of responding to versatile stimuli. The ssDNA switch was constructed using the Ec86 retron that produces ssDNA by reverse transcriptase (RT). This ssDNA switch is highly efficient with up to 150-fold change in the gene expression levels between the ON and OFF states. Like the RNA based TMS switch, the ssDNA switch accommodates different type of inputs (RNA and small molecules) with multiple arms in the structure providing flexibility to encode different functional parts.

Apart from controlling gene expression, we also focused to implement our RNA based tool to explore other biological functions. Fluorescence-based probes provide a unique insight into the otherwise obscure inner machinery of the living cell. However, the design of probes selective for novel analytes is notoriously challenging, especially where substrate-binding proteins are not available. In chapter 5, we present an entirely genetically encoded RNA-based sensor with a modular design feature that allows straightforward repurposing towards new analytes. We obtained a novel aptamer for GFP by a SELEX-type evolution directly in Escherichia coli, which we conjugated to a second aptamer to yield a genetically encoded sensor. The sensor does not require exogenous ligands and is instead based on turn-on fluorescence as analyte-binding releases GFP. We validated the method versus biologically relevant small molecules and proteins in
*E. coli* by simple RNA-aptamer replacement. This method has the potential to be a standard tool for analyte detection in cells.