Design and application of transcription factor based metabolite sensors in *Escherichia coli*

Identification of enzymes with the activity of interest is one of the major bottlenecks in enzyme and metabolic engineering. We have established a versatile ultra-high-throughput screening system for NADPH-dependent enzymes. It is based on the [2Fe-2S] cluster-containing transcriptional regulator SoxR that activates expression of soxS in the absence of NADPH in *Escherichia coli*. We coupled the response to the expression of an auto fluorescent protein and the specific fluorescence of sensor containing cells correlated with enzyme activity of an NADPH-dependent alcohol dehydrogenase from *Lactobacillus brevis* (*LbADH*). This property enabled sorting of single cells harboring wild-type *LbAdh* from those with lowered or without *LbAdh* activity by fluorescence-activated cell sorting (FACS). In a proof-of-principle application, the system was used successfully to screen a mutant *LbAdh* library for variants showing improved activity with the substrate 4-methyl-2-pentanone. To demonstrate the broad range of biosensors applications in *E. coli* we additionally describe the construction of two flavonoid responsive biosensors. The transcriptional activator FdeR from *Herbaspirillum seropedicae SmR1* responds to naringenin, while the repressor QdoR from *Bacillus subtilis* is inactivated by quercetin and kaempferol. The QdoR-biosensor was successfully applied for the detection of kaempferol production in vivo at the single cell level by FACS. Furthermore, the amount of produced kaempferol highly correlated with the specific fluorescence of *E. coli* cells containing flavonol synthase from Arabidopsis thaliana (fls1). These results show the potential of biosensors to minimize the construction time in bacterial cell engineering.