Tailor-made Transcriptional Biosensors

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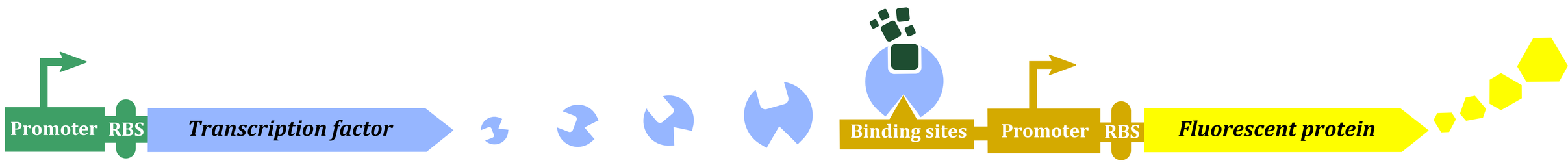
Introduction

"If you cannot measure it, you cannot improve it." (Lord Kelvin, 19th century). Measurement of key pathway metabolites is essential in metabolic engineering to ensure the development of efficient and optimal microbial production strains. Current methods for analysis of intracellular metabolites are cumbersome and demand ex vivo, destructive techniques such as HPLC, GC and LC-MS. As an elegant solution, transcriptional biosensors, consisting of transcriptional regulatory circuits found in nature, can be used. These biosensors enable in vivo, non-destructive, proportional, high-throughput and even simultaneous measurement of specific metabolites of interest.

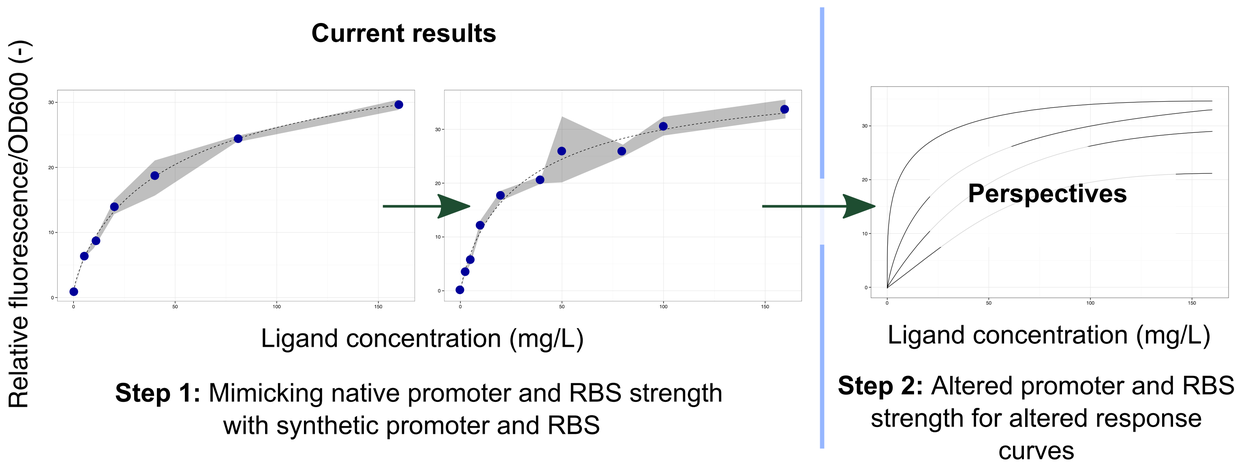
Tailor-made characteristic response curve

Engineering the native biosensor parts enables the development of unique biosensors with characteristic response curves customized to the researcher's needs. Transcriptional biosensor architecture can be divided into three basic levels of control: **(1)** the transcription factor operon, **(2)** the reporter operon with the transcription factor binding sites and **(3)** the (plasmid) copy number of the complete biosensor.



Transcription factor

The ratio of transcription factor expression versus the copy number of the binding sites and the intracellular concentration of the specific ligand plays a key role in defining the biosensor response curve. By altering the promoter and RBS strength of the transcription factor operon, the response curve can be altered.

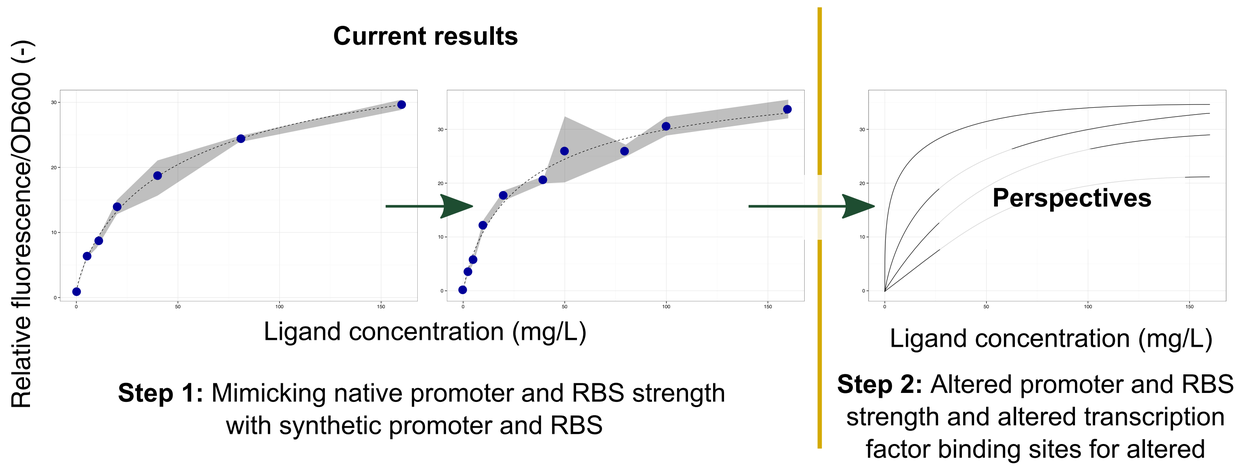


Step 1: Mimicking native promoter and RBS strength with synthetic promoter and RBS

Step 2: Altered promoter and RBS strength for altered response curves

Transcription factor binding sites

The ratio of fluorescent protein expression versus the copy number of the binding sites and the expression of transcription factor plays a key role in defining the biosensor response curve. By altering the promoter and RBS strength of the reporter operon and the position and copy number of the transcription factor binding sites, the response curve can be altered.

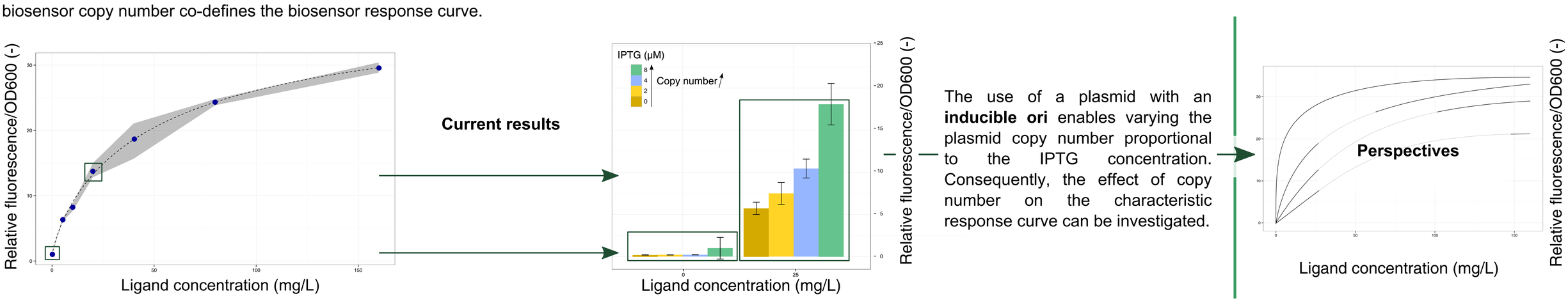


Step 1: Mimicking native promoter and RBS strength with synthetic promoter and RBS

Step 2: Altered promoter and RBS strength and altered transcription factor binding sites for altered response curves

Biosensor copy number

The (plasmid) copy number of the biosensor directly effects the total expression of both transcription factor and fluorescent reporter protein and the copy number of the transcription factor binding sites. Consequently, the biosensor copy number co-defines the biosensor response curve.



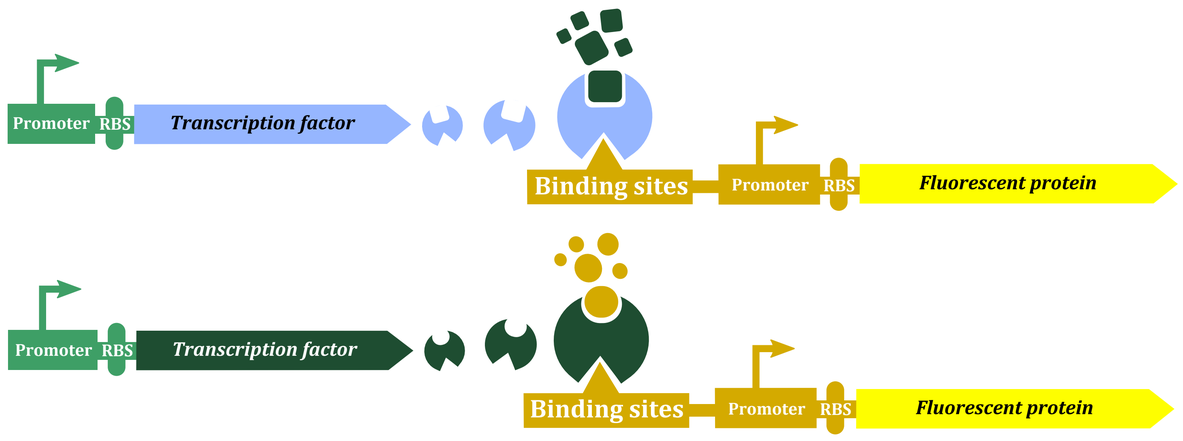
The use of a plasmid with an **inducible ori** enables varying the plasmid copy number proportional to the IPTG concentration. Consequently, the effect of copy number on the characteristic response curve can be investigated.

Tailor-made substrate specificity

Engineering transcription factors enables the development of biosensors with unique molecule-specificities customized to the researcher's needs. Within the same transcription factor family, highly conserved DNA- and ligand-binding domains are distinguishable. Additionally, the corresponding transcription factor binding sites also show high conservation. Therefore, complete transcription factors or even specific ligand-binding protein domains are interchanged with a (part of) the transcription factor of a charaterized biosensor to create chimeric biosensors with other ligand specificities but predictable response curves.

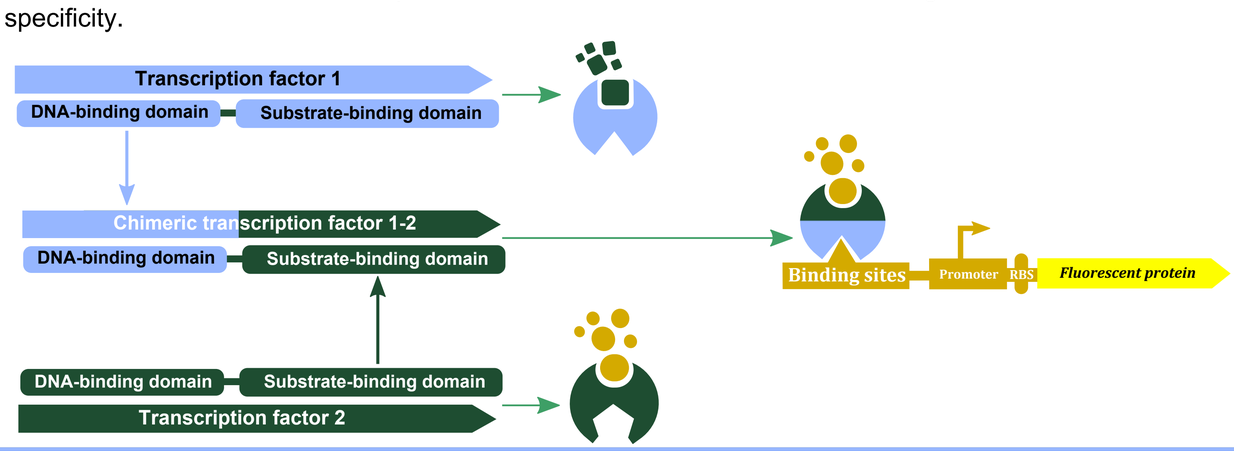
Chimeric transcriptional regulation pairs

Due to the high conservation of DNA-binding sites across different species, combining a ligand specific transcription factor from one species with DNA-binding sites from a characterized biosensor, can create a functional chimeric bioesensor with different ligand specificity. These chimeric biosensors only differ in their transcription factor, thus enhancing construction efficiency and response curve predictability.



Chimeric transcription factors

Transcription factors with conserved and distinct DNA- and ligand-binding protein domains and with different ligand specificities are used to construct chimeric transcription factors. Therefore, a characterized biosensor with a defined response curve is used as a chassis for constructing a predictable chimeric biosensor with a chimeric transcription factor that contains the substrate binding domain with the desired specificity.



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