DESIGNER BIOSENSORS FOR ENGINEERED METABOLIC PATHWAY OPTIMIZATION

Mohamed Nasr, Centre for Applied Synthetic Biology, Dept. of Biology, Concordia University, Canada
mohamed.nasr@concordia.ca
David Kwan, Centre for Applied Synthetic Biology, Dept. of Biology, Concordia University, Canada
Vincent Martin, Centre for Applied Synthetic Biology, Dept. of Biology, Concordia University, Canada

Key Words: Biosensors, Transcription Factors, Semi-Rational Engineering, Adipic Acid

Synthetic biology techniques aimed at constructing artificial metabolic pathways in genetically modified microorganisms are emerging as important, sustainable methods to produce biofuels, pharmaceuticals and value-added chemicals. To reach industrially relevant scales, challenges related to pathway bottlenecks and system optimization must be addressed. Since these pathways are usually built of multiple enzymes, improving pathway efficiency by processes such as enzyme directed evolution offers a solution to these limitations. However, screening methods for the majority of products of these enzymatic pathways are laborious and inefficient. The purpose of this work is to utilize transcriptional repressor-based biosensors, predominantly from the TetR family, to develop fast and high-throughput detection methods of artificial metabolic products.

Transcriptional repressors bind specific effectors or effector families, which limits their usability as biosensors in many engineered pathways. This project aims at expanding the toolbox of repressors available by engineering their effector-binding domains to respond to alternative effector molecules. As a proof of principle, using a semi-rational approach, we will engineer repressors to respond to intermediates of an engineered metabolic pathway to adipic acid that has been derived from the shikimate pathway. Adipic acid is a precursor of nylon and plastics and is currently produced unsustainably from petrochemicals, with worldwide annual demands of over 2 million tonnes. Our “designer” biosensors will be utilized to improve yields of an adipic acid-producing yeast strain. Particularly, they will be used as genetic circuits within engineered strains for pathway dynamic control, which is a method for in vivo real-time control of gene expression. As well, these biosensors will be used as screening tools for the directed evolution of pathway enzymes.