GENETIC BIOSENSOR ENABLES IN VIVO GLYCOSYLTRANSFERASE SCREENING

Evan K. Chaberski, The Novo Nordisk Foundation Center for Biosustainability, Denmark
Evacha@biosustain.dtu.dk
Michael K. Jensen, The Novo Nordisk Foundation Center for Biosustainability, Denmark
Ditte H. Welner, The Novo Nordisk Foundation Center for Biosustainability, Denmark

Key Words: Biosensor, Glycosyltransferase, High-throughput screening, Enzyme discovery

Glycosylation of natural products can alter their solubility and bioavailability, among other properties, which makes glycosyltransferases useful tools for increasing the production and/or generating novel compounds in microbial cell factories. However, the discovery and screening of new enzymes and engineered variants is often a low-throughput endeavor due to the need for over-expression and purification prior to *in vitro* experiments, which do not necessarily represent the *in vivo* activities of the enzyme. Therefore, a genetic biosensor controlling GFP expression was developed based on the flavonoid responsive transcriptional-repressor QdoR and expressed in *E. coli*. Due to the induced fluorescent response upon feeding the flavonoids Quercetin and Kaempferol, but not to their glucosides, the activity of UDP-dependent glycosyltransferases (UGTs) could be screened *in vivo*. Furthermore, a variant of QdoR was generated by directed evolution that showed greater dose-responsiveness and proved to allow greater discrimination of cellular populations and was thus more useful for *in vivo* UGT screening. The designed biosensor-based method will greatly increase the throughput of glycosyltransferase discovery and engineering.

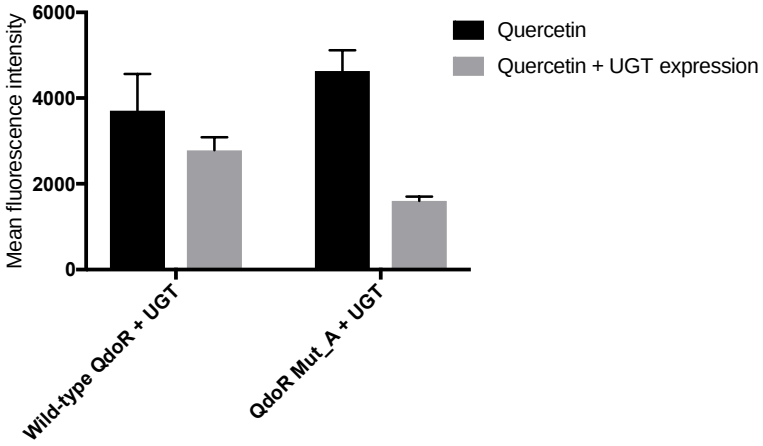

Figure 1 – Expression of flavonoid UGT results in significant decrease of fluorescence due to glycosylation of the flavonoid Quercetin.