ABSTRACT

RESEARCH PAPER: Development and Application of a High Throughput Assay System for the Detection of Rieske Dioxygenase Activity

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COLLEGE: Science and Humanities

DATE: May 2021

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The chemical industry currently accounts for 14% of all greenhouse gas emissions and is projected to become the largest global consumer of oil by 2030. In the Ball State Laboratory for Biocatalysis Research, we strive to address this problem through the development and application of novel enzymatic catalysts. Enzymatic catalysts are environmentally benign because they can be used in aqueous solutions and are fully biodegradable. As such, they can play a role in eliminating the need for petroleum-based solvents and toxic heavy-metal catalysts in the production of fine chemicals.

Rieske dioxygenases (RDO) are a class of enzymes known for their ability to perform the *cis*-dihydroxylation of aromatic compounds. They have been widely applied in synthetic chemistry due to their ability to provide enantiopure metabolites that can be used in the synthesis of high-value compounds. Yet, the utility of these enzymes has been limited by their substrate scope and strict selectivity.

Here, we seek to develop novel RDO catalysts with improved or expanded reactivity through directed evolution. In order to detect the relative *cis*-dihydroxylation activity of engineered Rieske dioxygenase variants, a novel high throughput assay system for the detection of their *cis*-

diol metabolites was developed. Here, the *cis*-diol metabolites produced by active dioxygenases in aqueous fermentation broths are oxidized using sodium (*meta*)periodate to produce diene-dialdehydes. These dialdehydes are then conjugated with a reactive fluorescent probe to give a strong, concentration-dependent fluorescent signal. In this way, this metaperiodate fluorescein *cis*-diol assay (MPFCD) detects the relative activity of RDOs with different aromatic substrates. This novel high throughput assay system has allowed for the activity of rationally engineered Rieske dioxygenase variants, produced through saturation mutagenesis, to be determined, and for the identification of variants that demonstrate significantly altered reactivity profiles in comparison to the parent enzyme.