ENGINEERING HYDROXYLASE AND KETOREDUCTASE ACTIVITY, SELECTIVITY, AND STABILITY FOR A SCALABLE CONCISE SYNTHESIS OF BELZUTIFAN

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Exquisite stereo- and regioselectivity is a key advantage of biocatalytic reactions. In this talk, we present the identification and development of two enzymes, an α-ketoglutarate-dependent dioxygenase and a ketoreductase, that together enable the installation of three adjacent stereocenters in the oncology treatment belzutifan. Biocatalytic oxidations a powerful tool for selective C-H bond activation. However, industrial use of enzymes catalyzing aerobic hydroxylation is presently limited by their substrate scope and stability under practical conditions. We report the engineering and application of an Fe/αKG-dependent dioxygenase for the direct stereo- and regio-selective hydroxylation of a non-native fluoroindanone *en route* to belzutifan, replacing a five-step chemical synthesis with a direct enantioselective hydroxylation. Desired product formation was found to be limited by enzyme stability and product overoxidation, and these properties were subsequently improved by directed evolution, yielding a biocatalyst capable of >15,000 total turnovers. We further report the identification and directed evolution of a ketoreductase (KRED) to perform a dynamic kinetic resolution in tandem with chemical fluorination with 50% organic co-solvent. Highlighting the industrial utility of these biocatalysts, the high-yielding, green, and efficient oxidation and dynamic kinetic resolution were demonstrated at kilogram scale for the synthesis of belzutifan.