RIESKE NON-HEME DIOXYGENASES: VERSATILE BIOCATALYSTS FOR THE GENERATION OF VICINAL *CIS*-DIOLS

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Key Words: Rieske non-heme dioxygenases – *cis*-Dihydroxylation – DOs – Arene substrates.

Vicinal *cis*-diols are a common motif in many biologically active natural products and important intermediates in the flavor and fragrance as well as pharmaceutical industries. Most of the chemical approaches for *cis*-dihydroxylations are based on transition-metal catalysts like osmium and manganese, which are expensive and toxic. Hence, the enzyme-catalyzed asymmetric dihydroxylation is a powerful tool to overcome the obstacles encountered using such chemical approaches. With the Rieske non-heme dioxygenases (ROs) a promising biocatalytic alternative to generate very selectively vicinal *cis*-diols is at our disposal. Also known as the non-heme analog to P450 monooxygenases[1], these enzymes are multicomponent systems, which only need molecular oxygen for the vicinal *cis*-dihydroxylation (s. Figure 1).

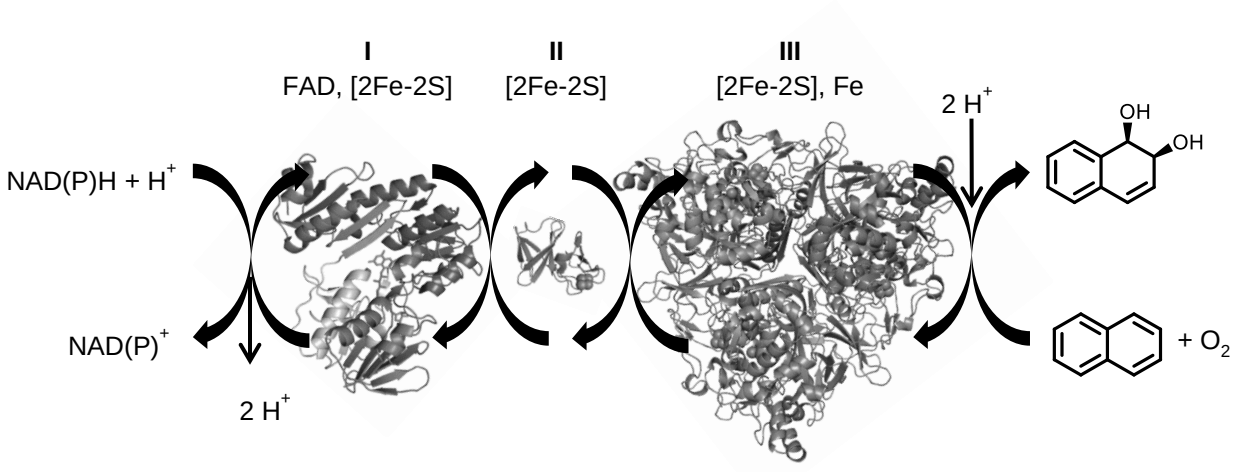

*Figure 1 – Schematic representation of dihydroxylation of naphthalene by multicomponent system Rieske non-heme dioxygenase (**I**: FAD dependent reductase, **II**: Ferredoxin, **III**: Hexameric oxygenase)*

The high potential and good mutability of these enzymes were already shown in previous studies of our group. By introducing a single point mutation in the active site of the cumene dioxygenase from *Pseudomonas fluorescens* we could achieve improved selectivities (≥95 %) and conversions (>99 %) towards alkenes.[2] Herein, we report a comprehensive mutagenesis study of the active site of naphthalene dioxygenase from *Pseudomonas* NCIB 9816-4 comprising 62 variants in order to discover important structure-function relationships between the positioning of the substrates and the geometry of the active site. Introducing a single point mutation led to drastic shifts in chemo-, regio- and stereoselectivity (≥ 90%) while the residual activity towards the natural substrate remained (up to 100%). Besides allylic mono- and dihydroxylation, *O*-dealkylation and desaturation reactions were observed. With a substrate walk using differently substituted arenes, important residues in the active site were identified and conclusions about the structure-function relationship were drawn.

[1] Blank, L. M., Ebert, B. E., Bühler, K., and Bühler, B., Antioxid. Redox Signaling (2010), p. 349–394.
[2] Gally, C., Nestl, B. M. and Hauer, B., Angew. Chem. (2015), p. 12952–12956.