ENGINEERING THE SUBSTRATE SCOPE OF THE FE(II) DEPENDENT HALOGENASE WELO15

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Selective halogenation is an important reaction for late-stage functionalisation of drug-like molecules. Performing halogenations under mild conditions using sodium chloride as the chlorine source has great potential for sustainable catalysis. The discovery of non-heme iron (NHI) and 2-oxoglutarate dependent halogenases, acting directly on a small organic molecule and not on acyl-carrier bound substrates,[1,2] has eliminated a major drawback of know NHI-halogenases. Hence, these enzymes represent attractive starting points for developing biocatalytic routs for selective, aliphatic chlorination, a paramount challenge in organic synthesis. The wild-types have a narrow natural substrate-scope and are unexplored for biocatalytic applications.[3] After solving the crystal structure of WelO15 from *Westiella intricata*, we used directed evolution to redesign the active site using a small-but-smart amino acid alphabet, thereby limiting the screening effort to a HPLC compatible throughput. New variants were found, able to chlorinate novel synthesized non-natural substrates. This study represents a first step towards milder, selective chlorination using biocatalysis.

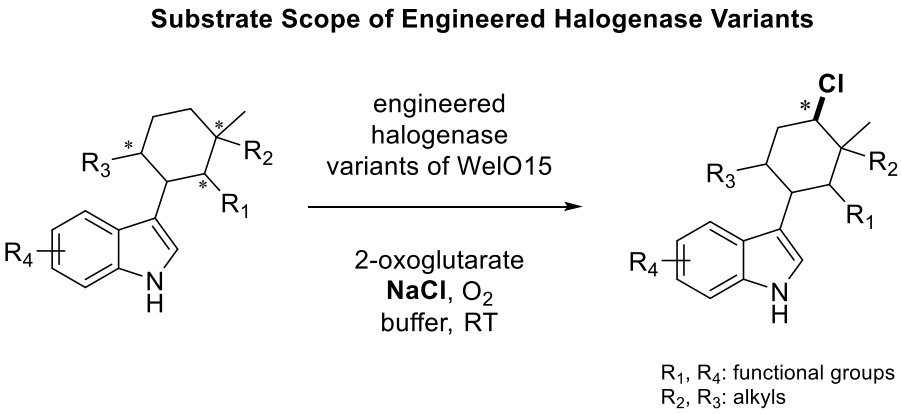

Figure 1. Chlorination of non-natural substrates with Fe(II), 2-oxoglutarate and oxygen dependent halogenase WelO15 after engineering the substrate scope via directed evolution.

[1] M. L. Hillwig, X. Liu, Nat. Chem. Biol. 2014, 10, 921–923.
[2] M. L. Micallef, D. Sharma, B. M. Bunn, L. Gerwick, R. Viswanathan, M. C. Moffitt, BMC Microbiology 2014, 14, 213.
[3] M. L. Hillwig, Q. Zhu, K. Ittiamornkul, X. Liu, Angew. Chem. Int. Ed. 2016, 55, 5780–5784.