Engineering Fe(II)/α-Ketoglutarate-Dependent Halogenases and Desaturases

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Molecular scaffolds offers an elegant route to create novel biologically active entities. In discovery chemistry, inactivated C-H bonds in particular are regarded as promising, if challenging, points of diversification as they allow to create new analogs without resorting to de novo synthesis. As a prerequisite for this approach, however, the reaction procedures must be compatible with already existing functional groups in the lead structural scaffold – a task still challenging most chemical methodologies. In this context, iron/α-ketoglutarate dioxygenases, enzymes which are capable of halogenating and hydroxylating sp3 carbons with high stereo- and regiocontrol under benign conditions, have attracted particular attention. This enzyme family's reported substrate scope, however, is often limited to natural substrates and their close analogues. By employing a combination of smart library design and machine learning assisted directed evolution, we engineered several iron/α-ketoglutarate dependent dioxygenases for the late-stage functionalization of molecules of pharmaceutical interest, ranging from non-natural amino acids to bulky macrolides, hitherto not accepted substrates. Notably, our enzyme engineering approach allowed us to rapidly identify more active enzyme variants increasing the apparent kcat and the turnover number of the enzymes by orders of magnitude. In addition, in case of the halogenases, we could precisely predict and consequently modulate the regioselectivity of halogenation allowing the targeted analysis of the small molecule's structure-function activity in biological assays.