IDENTIFICATION AND ENGINEERING OF A DYE-DECOLORIZING PEROXIDASE (DYP) FOR C—C-BOND FORMING CARBENE-TRANSFER REACTIONS

Martin J. Weissenborn, Leibniz-Institut für Pflanzenbiochemie & MLU Halle Wittenberg, Germany
martin.weissenborn@ipb-halle.de

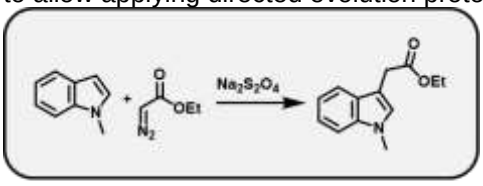
Key Words: Directed evolution, ISM, C-C bond formation, carbene-transfer, Golden Gate

A new enzymatic reaction-type of carbene-transfer reactions has been shown by the seminal work of Arnold and coworkers in 2013.[1] An impressive set of reactions was demonstrated since then predominantly employing the enzymes $P450_{BM3}$, Myoglobin and cytochrome C.[2]

In 2016, we identified the dye-decolorizing peroxidase (DyP) YfeX from *E. coli* to catalyse carbene-transfer reactions. YfeX is an iron-heme enzyme with histidine as proximal ligand and remarkable expression as well as stability levels. Using this DyP, we could show carbonyl olefinating activities in the presence and absence of phosphines.[3]

Recently, we were able to demonstrate the activity of YfeX on C—H functionalisations of indole using the standard carbene-precursor ethyl diazoacetate (EDA) in collaboration with the Koenigs lab (RWTH Aachen).[4] In this study, we could additionally show and investigate the carbene-transfer reaction of diazoacetonitrile (DAN) onto indole. This reaction is of high interest as it provides direct access to important precursors for the synthesis of tryptamines such as serotonin. We could improve both reactions by an initial alanine-scan of the active site residues and deuterium-labelling experiments providing insights into the mechanism, which revealed a different reaction mechanism of the two diazo-compounds.

To further improve these carbene-transfer reactions, we set out to develop several high throughput techniques to allow applying directed evolution protocols. We developed a novel Golden Gate-based mutagenesis method, which allows the saturation of up to five positions simultaneously within one day.[5] Aided by the freely available online tool for the primer design as well as a graphical analysis of the sequencing—accessible at https://msbi.ipb-halle.de/GoldenMutagenesisWeb/ —this Golden Mutagenesis technique provides rapid and facile access to small and large mutant libraries.



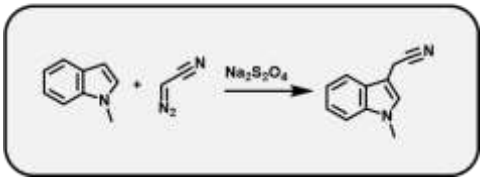


Figure 1 – The carben-transfer reactions with N-methyl-indoles

We used this technique and others to perform five rounds of directed evolution on the carbene-transferring reaction to indole. We applied a combination of single-codon saturation mutagenesis (SCSM)[6] and iterative saturation mutagenesis[7] that resulted in reshaping the active site.

The evolved enzyme shall be applied in microproduction units in the framework of the Leibniz Research Cluster.

[1] P. S. Coelho, E. M. Brustad, A. Kannan, F. H. Arnold, Science 2013, 339, 307-310.
[2] O. F. Brandenberg, R. Fasan, F. H. Arnold, Curr. Opin. Biotechnol. 2017, 47, 102-111.
[3] M. J. Weissenborn, S. A. Low, N. Borlinghaus, M. Kuhn, S. Kummer, F. Rami, B. Plietker, B. Hauer, ChemCatChem 2016, 8, 1636-1640.
[4] K. J. Hock, A. Knorrscheidt, R. Hommelsheim, J. Ho, M. J. Weissenborn, R. M. Koenigs, Angew Chem Int Ed Engl 2019, 58, 3630-3634.
[5] P. Puellmann, C. Ulpinnis, S. Marillonnet, R. Gruetzner, S. Neumann, M. J. Weissenborn, bioRxiv 2018.
[6] Z. Sun, R. Lonsdale, X. D. Kong, J. H. Xu, J. Zhou, M. T. Reetz, Angew. Chem. Int. Ed. Engl. 2015, 54, 12410-12415.
[7] M. T. Reetz, S. Prasad, J. D. Carballeira, Y. Gumulya, M. Bocola, J. Am. Chem. Soc. 2010, 132, 9144-9152.