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A “Broad Spectrum” Carbene Transferase for Synthesis of Chiral α -Trifluoromethylated Organoborons

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Directed evolution generated an enzyme for the enantioselective synthesis of α -trifluoromethylated organoborons—potentially attractive synthons for fluorinated compounds.

The realization that biocatalysis is one of the important elements for a more sustainable approach to chemical synthesis has resulted in a drive for finding enzymes for the catalysis of abiological reactions. The group of recent Nobel laureate Frances Arnold has pioneered the application of heme-containing enzymes for the catalysis of carbene transfer reactions. Key to this development was recognition that these reactions are mechanistically similar to the oxygen transfer reactions catalyzed by heme-containing enzymes such as cytochromes P450.^{1,2} This has led to a range of “carbene transferases”: engineered heme-containing enzymes for carbene transfer reactions such as cyclopropanations and X–H insertion reactions, including abiological Si–H and B–H insertions.^{3,4} In this issue of *ACS Central Science*, Houk, Arnold, and colleagues report an enzymatic platform for the enantioselective synthesis of a broad range of α -trifluoromethylated (α -CF₃) organoborons, which are potentially attractive synthetic building blocks (synthons) for fluorinated compounds.⁵ The reaction involves the insertion of a carbene into the B–H bond of the *N*-heterocyclic carbene borane synthon **1** (Scheme 1), using diazotrifluoroalkanes of general structure **2**, as a carbene precursor.

The carbene transferases presented in this paper are derived from bacterial protein cytochrome *c* (denoted as *Rma cyt c*), which also served as the basis for the previously reported Si–H and B–H insertion enzymes. The enzyme was used in whole *Escherichia coli* cells where it was directed to the periplasm, and demonstrated better results in catalysis compared to the isolated proteins.⁴

As expected, the wild-type protein showed no appreciable catalytic activity in the organofluorine synthesis. Therefore, site

saturation mutagenesis of residues that could shape the binding pocket was undertaken, with a focus on residues that contribute to the binding of the carbene intermediate and borane substrate as well as residues in the surface loop (Figure 1). The rationale for this was that in previous work it was shown that mutations in this loop make it more dynamic.^{4,6} This approach was predicted to give rise to more open conformations and contribute to the formation of an active site where the CF₃ group would be pointed into the heme pocket, and the bulkier alkyl substituent would be directed toward the solvent. The screening efforts resulted in the identification of the mutant enzyme, designated BOR-CF₃, which gave rise to 2460 turnovers and an enantiomeric ratio of 97.5:2.5 in the reaction of **1** with **2a**. Consistent with the hypothesis, BOR-CF₃ indeed proved capable of converting a variety of diazoalkane substrates containing significantly different R groups, such as **2b**, with 500–2900 turnovers and high enantioselectivities. A substrate with a smaller alkyl chain such as **2c** was not converted.

The effect of the mutations and the origins of the stereocontrol was investigated by computational studies. To this aim, the substrate and the carbene intermediate were embedded into BOR-CF₃ and subjected to large-scale molecular dynamics (MD) simulations. The results suggest that the CF₃ group of the substrate and the carbene intermediate is nicely accommodated at the inner side of the cavity, due to the structural rearrangements promoted by a mutation. Additionally, residues located in the front loop and at the entrance of the cavity are suggested to stabilize the phenyl group of the substrate, which appears pointing toward the solvent. In this orientation, the pro-*R* face of the substrate is the only exposed toward the free volume of the cavity, which explains the observed preference for the *R* enantiomer. Furthermore, the enhanced dynamics of the front loop by mutations likely facilitates the entrance of the borane substrate **1**, as well as generates an appropriate binding

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