STEREODIVERGENT CYCLOPROPANATION OF UNACTIVATED ALKENES WITH HEME PROTEINS

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Cyclopropyl motifs are present in a variety of compounds important to pharmaceutical, agrochemical, and fragrance industries. The asymmetric synthesis of cyclopropanes is often performed under harsh conditions with toxic, precious metal chiral catalysts. In 2013, the first example of biocatalytic alkene cyclopropanation was reported, using an engineered cytochrome P450 enzyme [1]. Since then, several heme proteins were reported to cyclopropanate a variety of styrenyl alkenes [2], but none have been shown to asymmetrically cyclopropanate more challenging substrates such as unactivated, aliphatic alkenes using the native iron-heme cofactor. Here we report that heme proteins can cyclopropanate unactivated alkenes and that stereoselectivity and activity can be tuned by directed evolution. A few rounds of site-saturation mutagenesis and screening yielded four protein variants with high enantio- and diastereoselectivity for complementary isomers, enabling stereodivergent synthesis of aliphatic cyclopropanes. These iron-porphyrin proteins are fully genetically encoded, and the reactions can be performed under mild, aqueous conditions with whole cells or purified protein. The protein enhances the activity of the native iron-heme cofactor, giving access to a broad array of cyclopropanated products. This example showcases the ability to quickly and efficiently engineer proteins for non-natural biocatalytic function.

[1] P.S. Coelho, E.M. Brustad, A. Kannan, F.H. Arnold, Olefin cyclopropanation via carbene transfer catalyzed by engineered cytochrome P450 enzymes., Science. 339 (2013) 307–10.
[2] O.F. Brandenberg, R. Fasan, F.H. Arnold, Exploiting and engineering hemoproteins for abiological carbene and nitrene transfer reactions, Curr. Opin. Biotechnol. 38 (2017) in press.