A152 · De novo design of hydroxylation enzymes

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Hydroxylation of carbon-hydrogen bonds is one of the quintessential chemical transformations in biology and synthetic chemistry, underpinning metabolism, feedstock valorization, and the precision synthesis of complex molecules. Enzymes are uniquely powerful catalysts for this reaction: by precisely controlling substrate binding orientation, proteins can achieve exceptional enantio- and site-selectivity unmatched by small-molecule catalysts. Yet drug discovery continues to demand hydroxylation catalysts with ever-expanding scope, rapidly outpacing what protein mutagenesis and metagenomic mining can supply. Here, we use de novo protein design to show that biocatalysts can instead be built from scratch and optimized directly for the selective hydroxylation of any molecule of interest. Using deep learning-based design tools, beginning with RFdiffusion3, we constructed entirely new proteins organized around a heme cofactor, engineered to bind a substrate precisely adjacent to the reactive center. Working first with model systems, we established design principles for balancing the delicate reactivity of this chemistry under conditions of high oxidative stress. These principles enabled us to extend the approach to structurally complex substrates presenting significant enantio- and site-selectivity challenges, which we met successfully. Together, this work establishes a general strategy for the on-demand creation of biocatalysts capable of complex natural and abiological chemistries, offering a path beyond the limitations of natural enzyme discovery and directed evolution.

De novo design of hydroxylation enzymes

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