

Asymmetric Enzymatic Hydrophosphorylation through O_2 Activation

Background/Objective

Phosphorus-containing compounds are widely used in pharmaceuticals and agrochemicals. However, nature's enzymatic C–P bond formation is extremely rare. Existing chemical methods often require harsh oxidants or activated phosphorus precursors that limit chemoselectivity and rarely achieve high enantioselectivity (ee). Enzymes could overcome these limitations by precisely guiding highly reactive radical intermediates, but they have not been reported for P-centered radical generation. A biocatalytic method for direct addition of common phosphorus compounds to alkenes would provide a mild and selective route to diverse products.

Approach

Molecular oxygen is an abundant oxidant, but its partial reduction generates reactive oxygen species (ROS) that can damage enzymes and disrupt radical reactions. We reasoned that an engineered flavin enzyme could harness ROS to generate phosphorus-centered radicals from P–H donors while controlling enantioselectivity through enzymatic hydrogen atom transfer (HAT). Through semi-rational and machine learning-guided directed evolution and mechanistic studies, we developed a biocatalytic platform for asymmetric synthesis of chiral organophosphorus compounds.

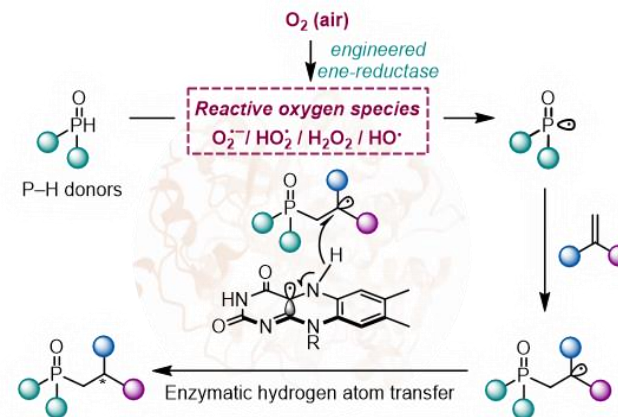
Results

We successfully engineered flavin-dependent enzyme OYE1 variants to catalyze asymmetric enzymatic hydrophosphorylation via oxygen activation under both light and dark conditions. Mechanistic studies revealed the roles of the individual ROS generated in situ and how enzymatic HAT controls enantioselectivity. The top three evolved enzymes accepted diverse P–H donors and enabled the biosynthesis of valuable phosphorus-containing scaffolds, including the broad-spectrum herbicide *L*-glufosinate-ammonium at 63% yield and >99% ee.

Significance/Impacts

This work expands biocatalysis to programmable C–P bond formation and establishes a new paradigm for channeling oxygen reactivity in enzymes.

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Asymmetric enzymatic hydrophosphorylation enabled by reactive oxygen species.