

## AN ARTIFICIAL METALLOENZYME FOR A BIMOLECULAR DIELS–ALDER REACTION

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**Key Words:** Diels–Alder metalloenzyme, computational design, directed evolution, enzyme catalysis, chemo- and stereoselectivity

The Diels–Alder reaction, one of the most important in organic chemistry, forms functionalized six-membered cycloadducts in a single step. While widely used to construct complex biologically active molecules in the laboratory, [4+2] cycloadditions are rarely employed for natural product biosynthesis in cells owing to the lack of appropriate enzymes. Creating artificial metalloenzymes able to exploit Lewis acid catalysis for substrate activation could change this situation. Embedding a metal ion in a chiral protein binding pocket potentially combines the best aspects of two worlds – transition metal and enzymatic catalysis – to achieve both high activity and selectivity. Here we report the transformation of a zinc-binding helical bundle into an artificial metalloenzyme that efficiently catalyzes a hetero-Diels–Alder reaction between 3-vinyl indole and an azachalcone derivative by a process of design and laboratory evolution. The best enzyme, DA7, performed >15,000 turnovers per active site and produced only a single product stereoisomer (>99% *ee*). Detailed kinetic analysis showed that this catalyst is more than two orders of magnitude more proficient than other known Diels–Alderases, including many designed catalysts and natural enzymes involved in polyketide natural products biosynthesis. The remarkable activity of DA7 can be ascribed to the Zn(II) ion, which activates the heterodiene for reaction, and a shape complementary binding pocket that preorganized the reactants for efficient reaction and exacting control over chemo-, diastereo-, and enantioselectivity. These results establish the feasibility of combining design and evolution to harness the structural and functional properties of metal ions to produce remarkably active enzymes for an important abiological reaction. Extending this approach to metal ions other than zinc, and to scaffolds beyond helical bundles, can be expected to produce proficient custom-metalloenzymes for a wide spectrum of unnatural chemical transformations.