acib Project Proposal

Synthesis of aldehyde and ketones by enzymatic alkene cleavage

SUMMARY
The perfect catalyst for biocatalysis: (i) is easily available in high amounts from expression in *E. coli*, (ii) is solvent and thermostable (iii) reaches high conversion rates (iv) displays high selectivity (v) forms no/few side products and (vi) can be easily optimized by protein engineering for specific target substrates. Cupin TM1459 combines all these requirements.

Background
Aldehydes and ketones are accessible by many synthetic routes, one important tool being the oxidative cleavage of alkenes. Chemical approaches such as ozonolysis or metal-based methods display several drawbacks, including explosive character of the intermediates generated or low yield and poor chemoselectivity. Several enzymes are known to catalyze oxidative alkene cleavage reactions to different extent - in many cases as minor (undesired) side reaction. In most cases selectivity is lacking and many other products are observed.

acib-Technology
The cupin TM1459 from *Thermotoga maritima* is a small thermo- and solvent stable β-barrel protein, which catalyzes the oxidative alkene cleavage reaction in the presence of molecular oxygen and tertiary butyl hydroperoxide at ambient temperature. We developed and optimized two high-throughput assays for the detection of product aldehydes or ketones and applied it during protein engineering of TM1459 (Steiner et al., Front Microbiol, 2016, 7:1511). The currently best variant catalyzes the formation of acetophenone from α-methylstyrene with 97% conversion and shows a broad substrate scope.

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Under protection of a CDA we offer to discuss with you all available options. If a project looks feasible, we offer evaluation in a small PoC-study if an alkene, which will result in the target aldehyde or ketone, is easily available from natural resources or can be easily synthesized. The currently available enzyme variants, including but not limited to Cupin TM1459, will be tested for the specific alkene substrate and a strategy will be developed for the optimization of the conversion of the target alkene including reaction and protein engineering. Once we have confirmed that the target product can be synthesized, we will offer a comprehensive project plan for the reaction and protein engineering strategy.

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