Characterization of engineered new-to-nature aldolases and their application in cascade synthesis

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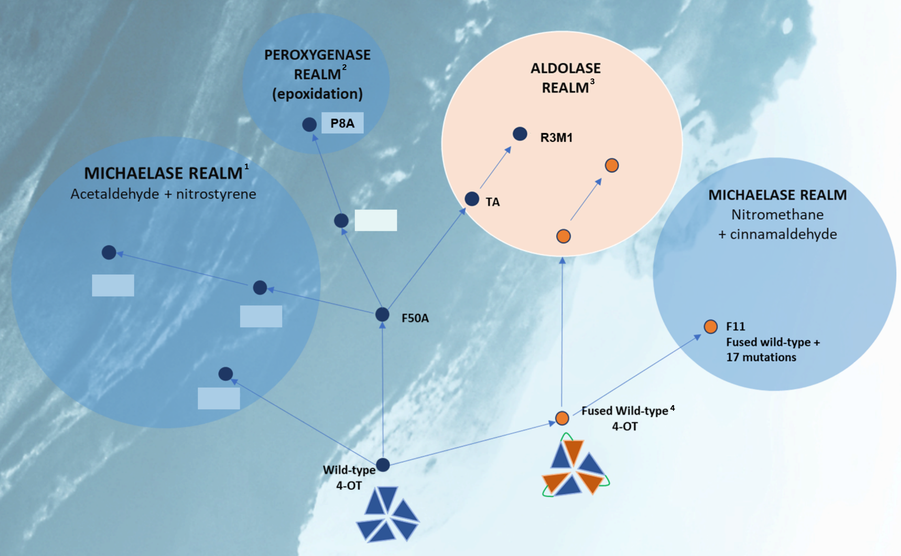
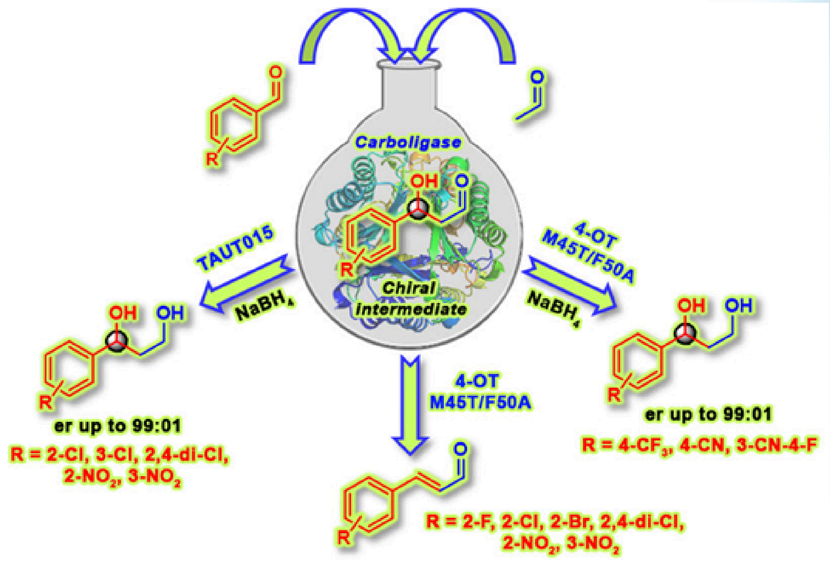
4-OT: a promiscuous biocatalyst

4-OT can be engineered for a variety of reactions



This study focuses on aldolase activity

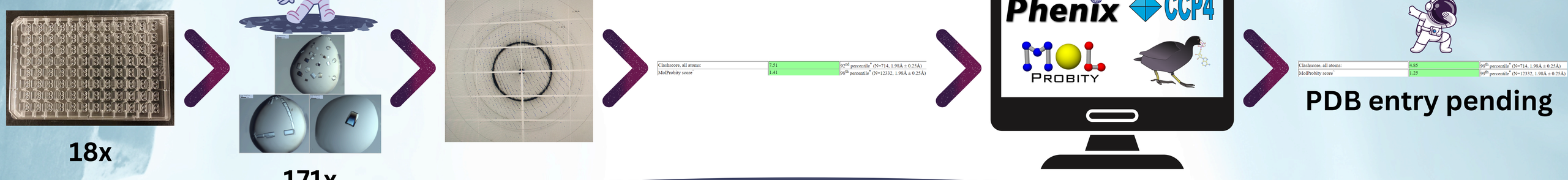


Products obtained by both aldol addition and condensation from various aromatic aldehydes observed using 4-OT [5]

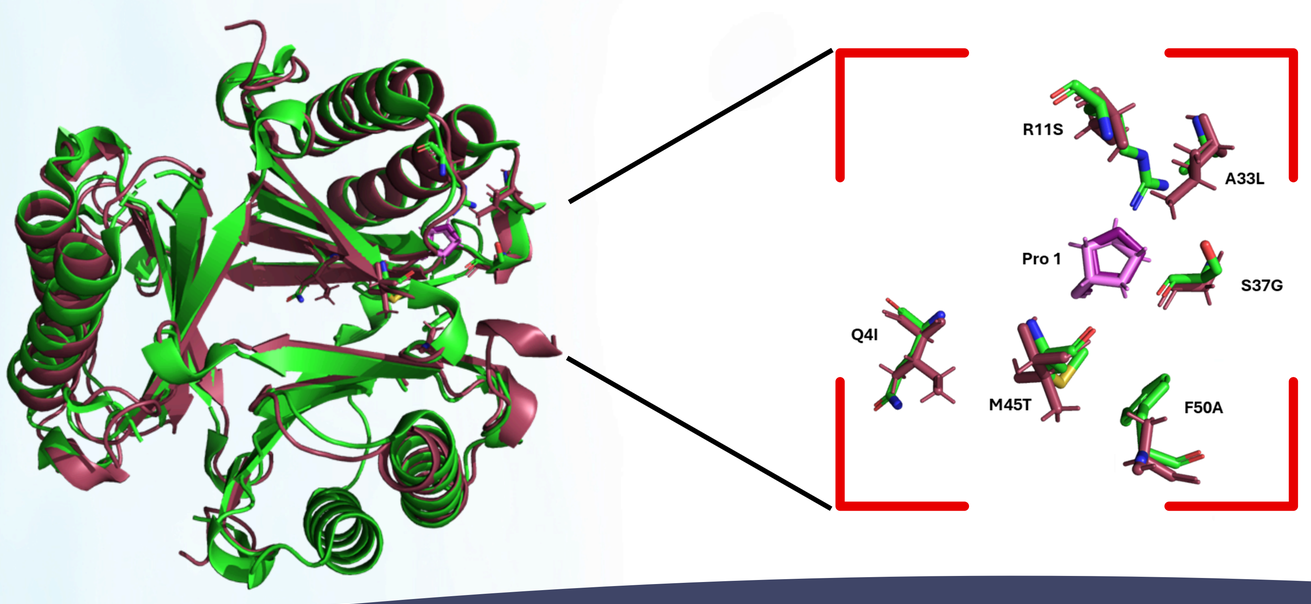
AIMS:

- Structural characterization of new to nature aldolases
- Structure-inspired optimization of these aldolases
- Application in cascade synthesis

Crystallizing engineered 4-OT



3 rounds of directed evolution, 160 fold observed increase in aldolase activity from WT [6]



- Mutations concentrated around the active site (except Q4I)
- Bulky residues replaced to enlarge active site pocket

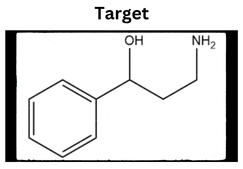
Application in an enzymatic cascade (in progress)

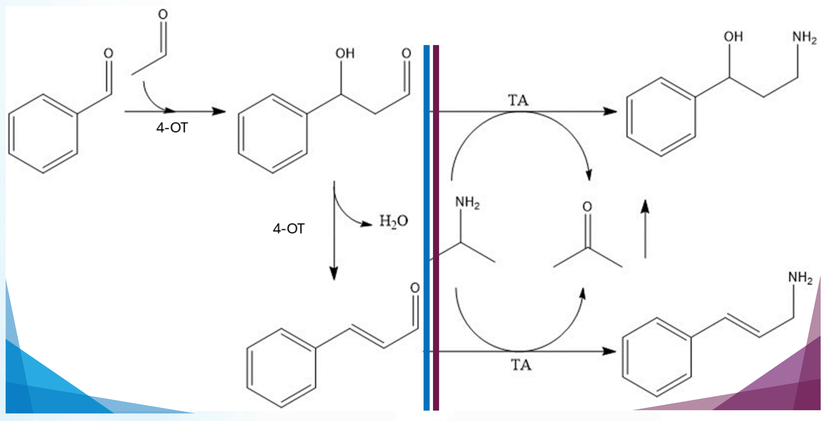
First biocatalyst from directed evolution

Prescreening aided by colorimetric probe [7]

First step:
4-OT variant R3M1 catalyses the aldol addition of benzaldehyde and acetaldehyde to form the aldol intermediate, which subsequently undergoes enzymatic dehydration to form cinnamaldehyde

Target





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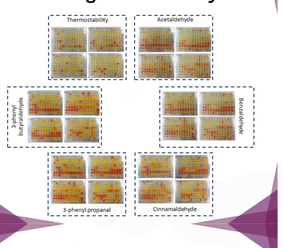
Second biocatalyst from metagenomic library



Second step:
A transaminase transfers the amino group from isopropylamine (amine donor) to the formed aldol intermediate and to the cinnamaldehyde byproduct to form 3-hydroxy-3-phenylpropanamine and cinnamylamine respectively

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