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Methods to Restore Biosynthetic Enzyme Activity for Improved Pathway Flux and Bioproduction

Researchers at Stanford have identified why engineered pathways that convert formate into multi-carbon products chronically underperform, and have developed an enzymatic repair strategy that recovers the inactivated enzymes, increasing pathway flux and extending reaction longevity for bioproduction applications.

Formate produced by electrochemical reduction of CO_2 is among the most attractive feedstocks for sustainable biomanufacturing, because its supply scales with renewable electricity and is independent of arable land. A decade of engineering has produced numerous new-to-nature pathways for assimilating formate into multi-carbon metabolites, yet these designs consistently deliver only a small fraction of the performance their component enzyme activities predict, limiting commercial utility. The Stanford inventors traced this shortfall to a previously unrecognized chemical liability: the activated intermediates that these pathways rely on spontaneously and non-enzymatically modify the pathway's own enzymes, inactivating them. The effect is pathway-wide rather than specific to any single design, and it is not caused by the intermediate the field has long assumed to be the principal culprit.

Building on this insight, the inventors developed an approach that reverses the modification enzymatically while limiting accumulation of the damaging intermediate, restoring productive flux. In a reconstituted pathway, the combined strategy increased product formation approximately 16-fold. The inventors have demonstrated improved production of acetyl-CoA and related thioesters from formate. Acetyl-CoA is an entry molecule for the production of numerous bioproducts, including therapeutics, isoprenoids, sustainable aviation fuels, chemicals, and other products. More broadly, the technology provides both a repair

strategy and a design rule for addressing enzyme inactivation, a key limitation in synthetic pathway performance.

Stage of Development:
Demonstrated in lab: functional rescue demonstrated in a reconstituted multi-enzyme pathway in vitro

Applications

- Bioproduction of acetyl-CoA and other CoA thioesters from formate
- Carbon-negative manufacturing of chemicals, fuels, and materials
- Production of acetyl-CoA-derived therapeutics
- Chemical biomanufacturing
- Improved performance and longevity of new-to-nature synthetic biosynthetic pathways
- Cell-free and in vitro biocatalytic manufacturing
- Production of isoprenoids
- Sustainable aviation fuels
- Enzyme engineering, biocatalyst stabilization, and pathway diagnostics

Advantages

- Recovers inactive biosynthetic pathway enzymes rather than replacing or redesigning them
- Increases biosynthetic pathway flux and extends reaction longevity
- Addresses a bottleneck common to an entire class of pathway designs, not a single enzyme
- May enable more sustained production of valuable acetyl-CoA-derived products

Publications

- Manuscript in progress

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