

# Comparative genomics of *Aspergillus nidulans* and section *Nidulantes*

## Background/Objective

- *Aspergillus nidulans* is an important model organism for eukaryotic biology and the reference for the section *Nidulantes* in comparative studies.

## Approach

- We de novo sequenced the genomes of 25 species of this section at JGI. Whole-genome phylogeny of 34 *Aspergillus* species and *Penicillium chrysogenum* clarifies the position of clades inside section *Nidulantes*.

## Results

- Categorized 2118 secondary metabolite gene clusters (SMGC) into 603 families across *Aspergilli*.
- There is remarkable diversity of organization of MAT-loci both within and between the different clades.

## Significance/Impacts

- Proteins that have been investigated in *A. nidulans* as well as its SMGC families are generally present in the section *Nidulantes*, supporting its role as model organism.

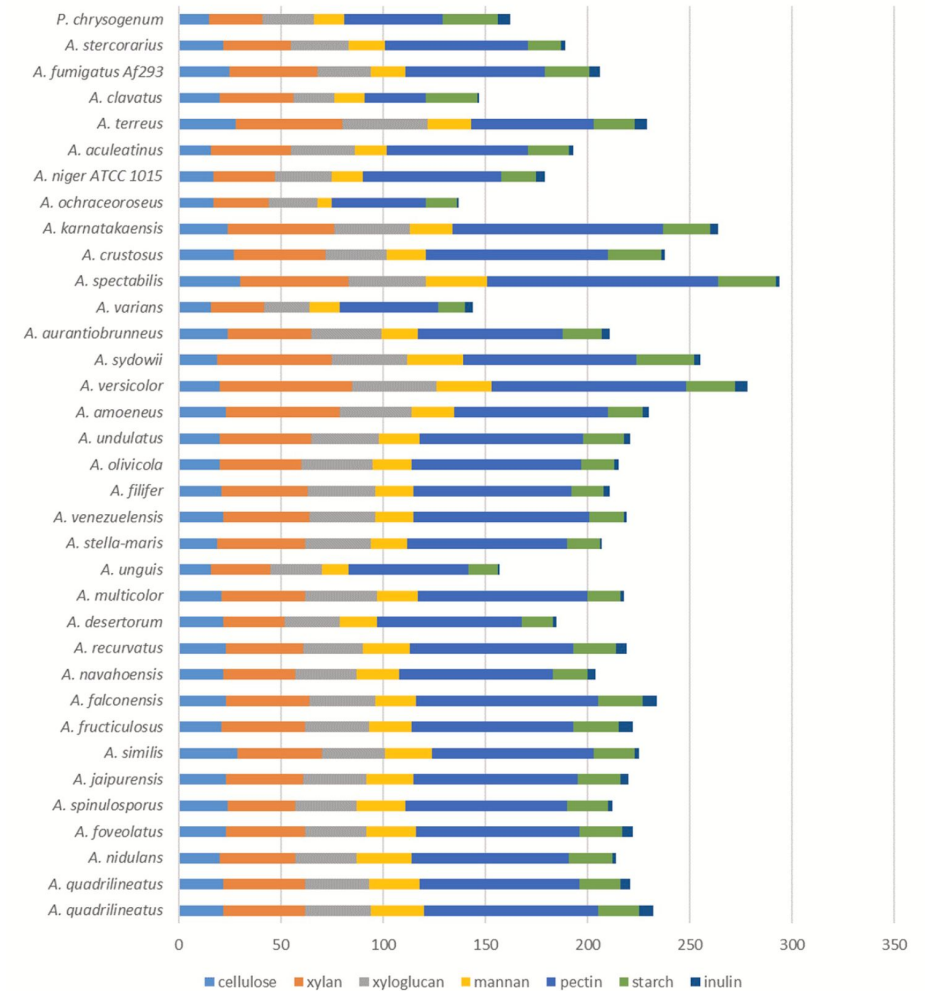


Figure: Comparative analysis of CAZymes in section *Nidulantes* and reference species. Comparison of the number of genes related to degradation of different plant polysaccharides.

# Characterization of Kariya (*Hildegardia barteri* (Mast.) Kosterm) Seed Oil Fatty Acid Methyl Ester Prepared from Basic Catalytic Transesterification

## Background/Objective

- There is growing demand for biodiesel. Biomass-derived biodiesel, especially from lesser-known oil sources, is a compelling alternative to conventional sources.

## Approach

- This research focuses on analyzing the fatty acid methyl esters (FAMES) derived from Kariya (*Hildegardia barteri* (Mast.) Kosterm) seed oil through basic catalytic transesterification and assessing its potential as a biodiesel feedstock.

## Results

- Significant differences in FAME composition were observed among the samples, with hexane–ethanol Kariya oil biodiesel (HE-KOB) showing the highest FAME content (76.1%).

## Significance/Impacts

- These findings highlight the feasibility of utilizing Kariya seed oil as a biodiesel feedstock, emphasizing the importance of solvent selection and transesterification conditions in optimizing FAME.

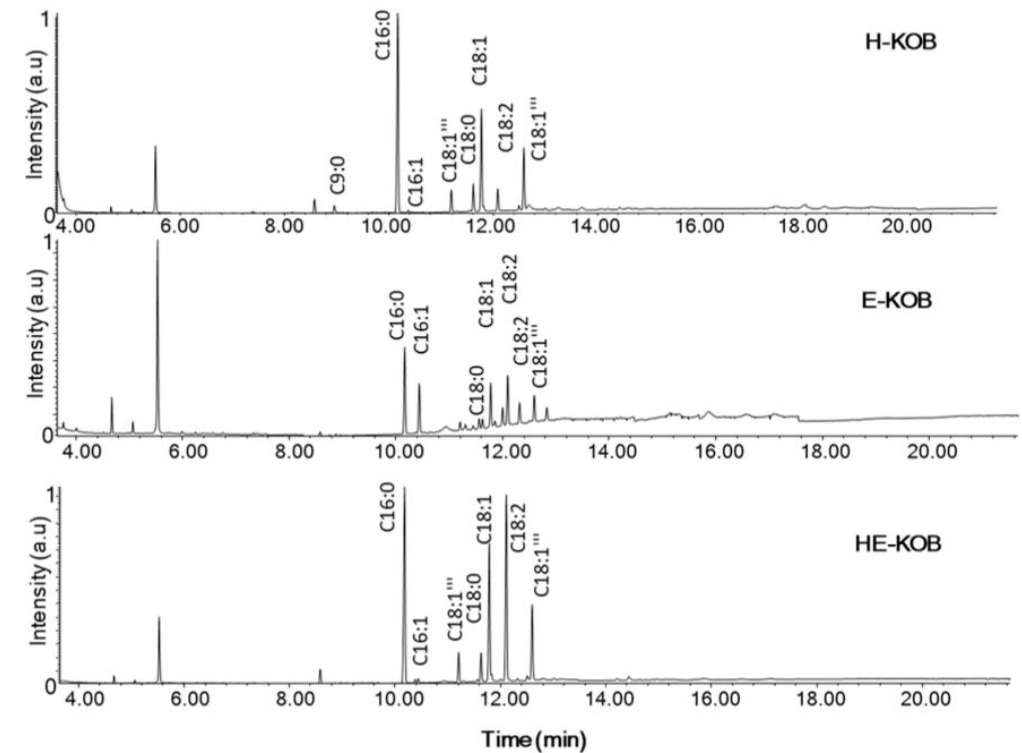


Figure: Chromatographs from GC-MS analysis of the three biodiesel samples obtained from Kariya seed oil ('''marked on some peaks indicates that the compound contained terminal triple bond).

# Separation of life stages within anaerobic fungi (Neocallimastigomycota) highlights differences in global transcription and metabolism

## Background/Objective

- Anaerobic fungi encode carbohydrate-active enzymes.
- Objective: perform first transcriptomic profile of the young gut fungal life stage (zoospores).

## Approach

- Filter cultured zoospores from mature cells for high-quality RNA extraction and RNA-Seq.
- Analyze differential gene expression (zoospores vs. mats).

## Results

- Zoospores upregulate catabolic carbohydrate-active enzymes, transcriptionally priming to encounter plant matter, despite being grown on glucose.

## Significance/Impacts

- Foundational transcriptomic dataset to generate hypotheses for future experimentation.
- Inform gene targets for genetic engineering.

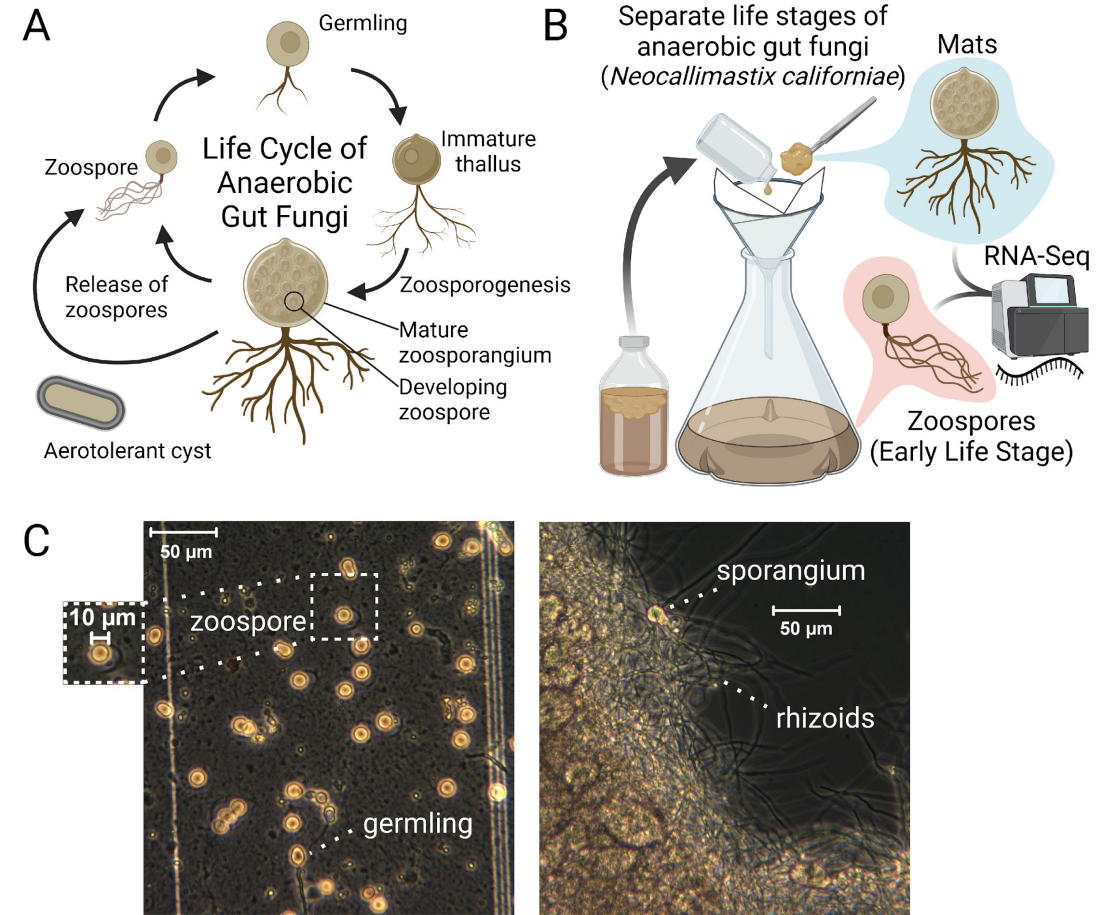


Figure: (A) Anaerobic gut fungi possess an unusual, zoosporic life cycle. (B) A method was developed to enrich samples for zoospores and extract high-quality RNA for sequencing. (C) Zoospore-enriched samples were distinguishable from mature cells.

## Background/Objective

- This study highlights the challenge of using designs that inactivate reactions that encoded by under-characterized proteins, in genome-scale designs with multi-gene edits.

## Approach

- We performed promoter variation, gene and metabolite complementation and growth rate analyses.
- Context-specific models were generated using proteomics data.

## Results

- The sensitivity analysis of the context-specific models showed how a single gene intervention and the resulting limiting enzymatic reaction can impact both growth and production.

## Significance/Impacts

- Learnings will improve genome-scale design fidelity for model and non-model hosts, for a range of carbon sources and final products.

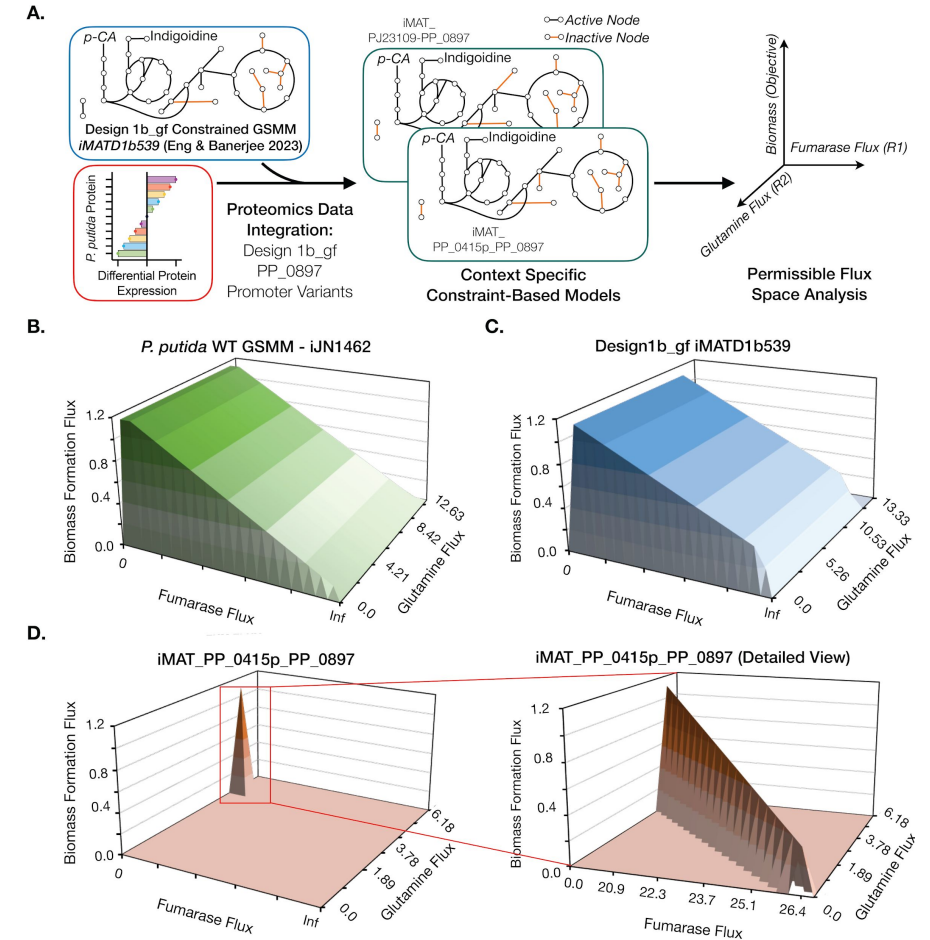


Figure: Proteomics-constrained context-specific metabolic models reveal narrow permissible fumarase flux space for growth-coupled production.

# Technoeconomic analysis for near-term scale-up of bioprocesses

## Background/Objective

- TEAs aim to predict the long-term economic viability and often use nth plant cost and performance parameters.
- This article explores TEA methods for early-stage scale-up, critically evaluates their applicability to biofuels and bioproducts.

## Approach

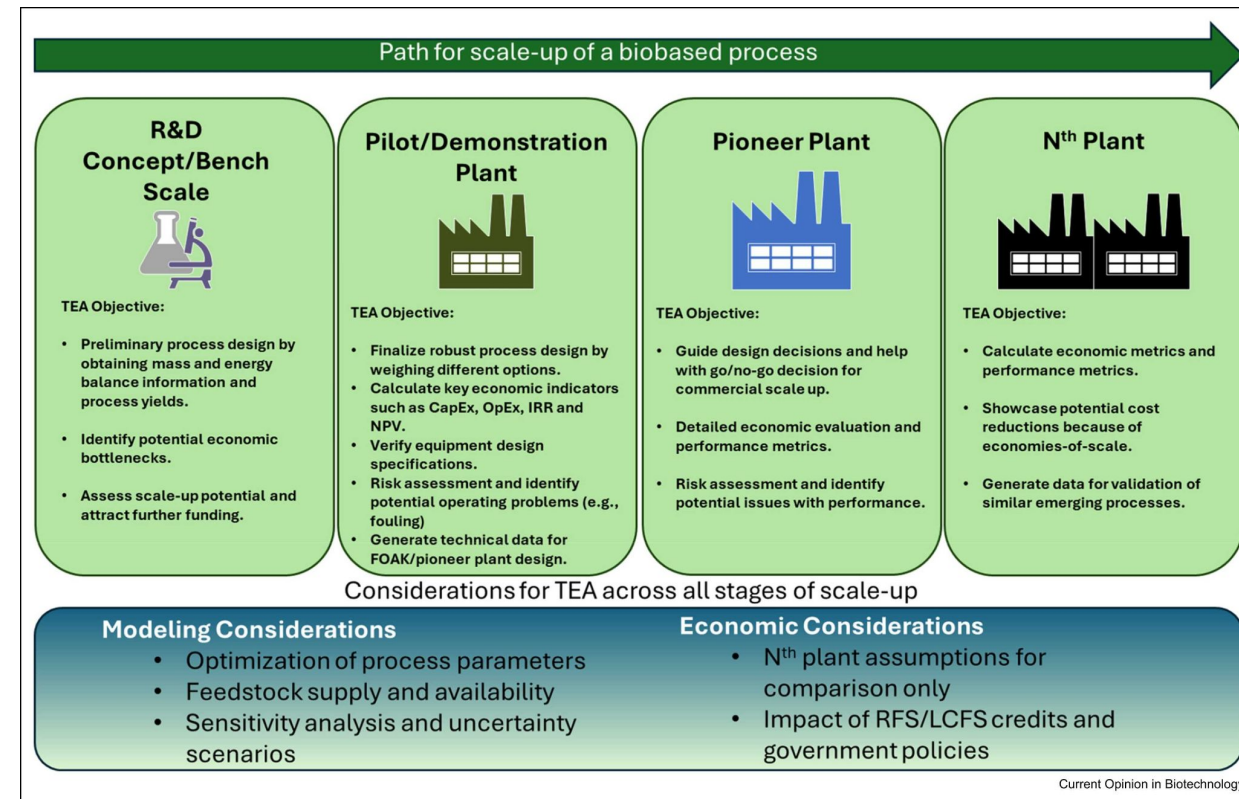
- Lays out and critiques methods for conducting first-of-a-kind/pioneer plant cost analyses.
- Recommends improvements.

## Results

- Different economic metrics are more relevant for FOAK.
- Need update to RAND method with bioprocess specific data.

## Significance/Impacts

- TEA can guide research to overcoming valley of death, rather than focusing only on long-term viability.
- May lead to different technological focus for R&D.



# Prenol production in a microbial host via the ‘Repass’ Pathways

## Background/Objective

- Prenol and isoprenol are promising advanced biofuels and serve as biosynthetic precursors for industrially relevant compounds.
- Achieving high titer prenol has remained elusive.

## Approach

- The expression of specific isopentenyl monophosphate kinases with model-guided enzyme substitution of the isomerases and phosphatases enabled selective cycling of monophosphates and diphosphates.

## Results

- Pairing with the canonical MVA pathway resulted in 300 mg/L prenol at a 30:1 ratio with isoprenol, and pairing with the “IPP-Bypass” pathway resulted in 526 mg/L prenol at a 72:1 ratio with isoprenol.
- Coexpressing the prenyltransferase acPT1 yielded 48.3 mg/L of the potential therapeutic precursor drupanin from p-coumarate.

## Significance/Impacts

- The repass pathways establish a unique strategy for tuning diphosphate precursors to drive isoprenoid biosynthesis and prenylation reactions.

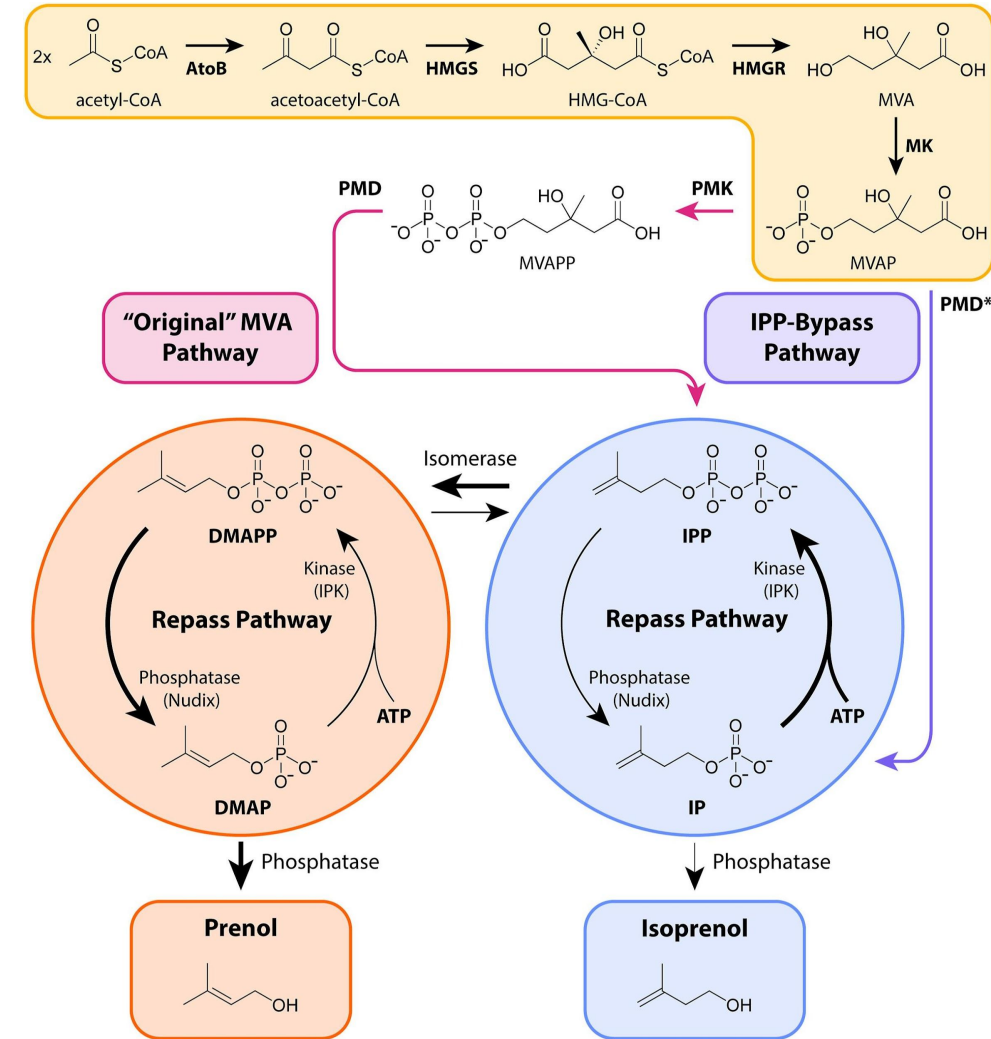


Figure: An overview of the repass pathways to produce prenol and isoprenol.

# ENABLED PUBLICATIONS

# A map of the rubisco biochemical landscape

## Background/Objective

- Rubisco is the primary CO<sub>2</sub>-fixing enzyme of the biosphere<sup>1</sup>, yet it has slow kinetics. The roles of evolution and chemical mechanism in constraining its biochemical function remain debated.

## Approach

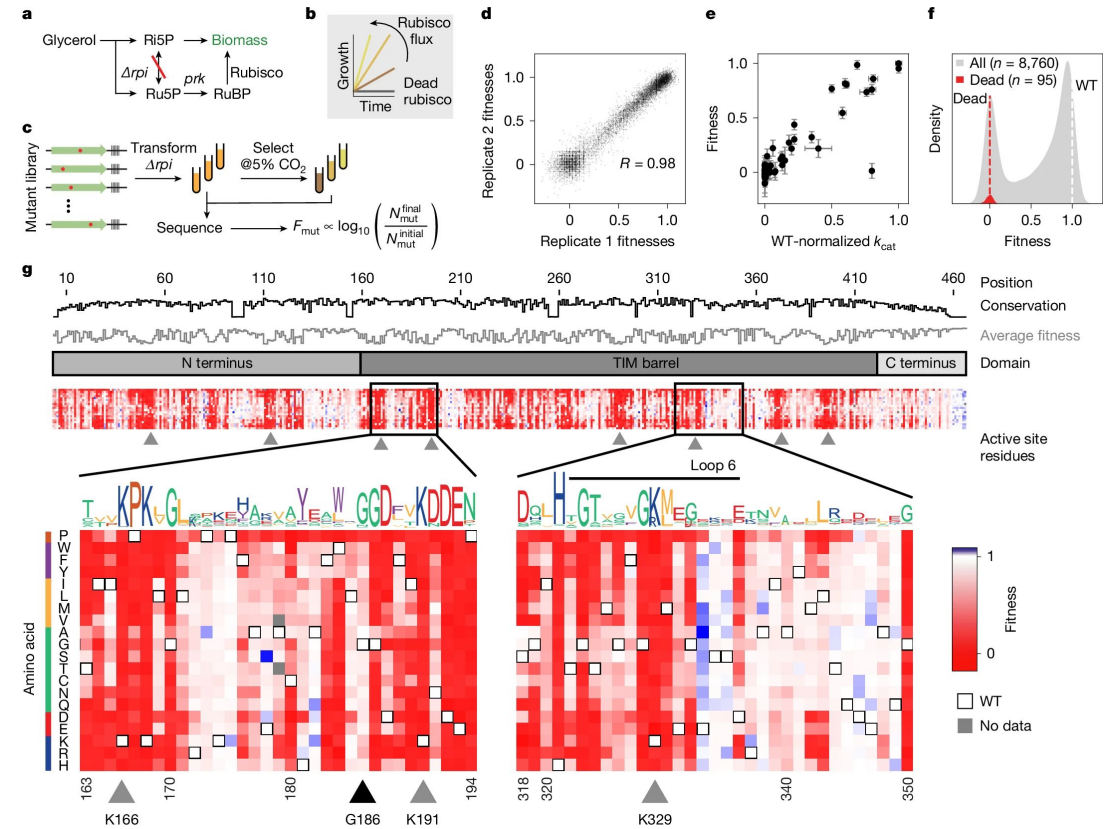
- Here we developed a massively parallel assay, using an engineered *Escherichia coli* in which enzyme activity is coupled to growth, to systematically map the sequence–function landscape of rubisco.

## Results

- This approach identified many highly conserved positions that tolerate mutation and rare mutations that improve CO<sub>2</sub> affinity.

## Significance/Impacts

- These data lays down the groundwork for further enzyme engineering efforts.



# Circularity in polydiketoenamine thermoplastics via control over reactive chain conformation

## Background/Objective

- Encoding circularity in polymers is important for enabling and perfecting the chemical recycling of plastic waste.

## Approach

- Investigated how the proximity of polar functionality near cleavage sites in bio-based polydiketoenamine thermoplastics affects hydrolysis rates in aqueous media.

## Results

- Oxy-functionalization of amine and triketone monomers at specific sites near acid-cleavable diketoenamine (DKE) bonds results in polydiketoenamine (PDK) deconstruction rates that are fast and tunable over three orders of magnitude.

## Significance/Impacts

- Enabled the creation of high performance circular PDK thermoplastics and recyclable adhesives therefrom.

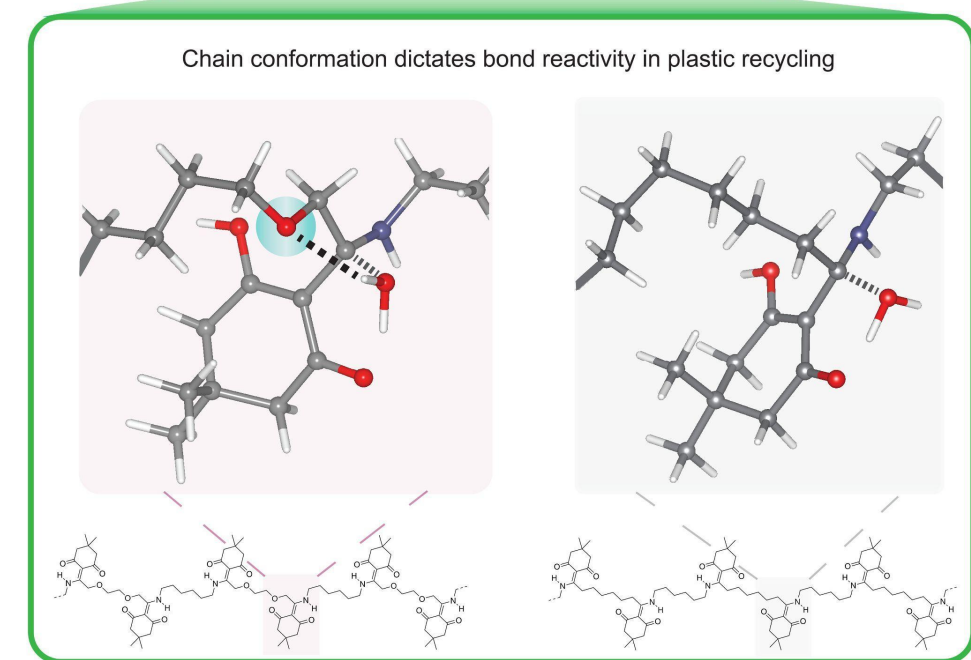
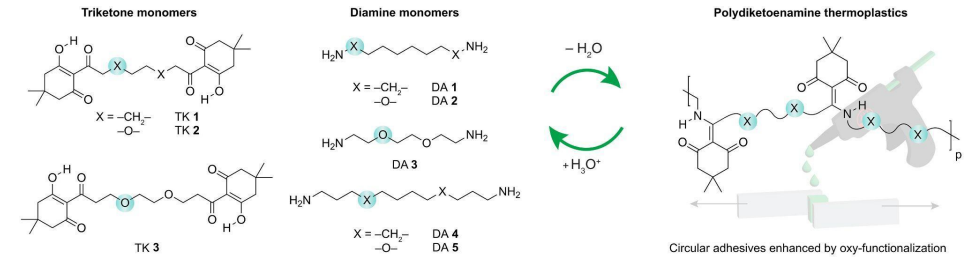


Figure: The efficiency by which linear polydiketoenamines (PDK) are deconstructed to triketone and amine monomers in acidolytic media is both tunable and fast by controlling reactive chain conformation in oxy-functionalized PDK materials.