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Diversity-driven Biochemical Survey Reveals Widespread Dimerization Throughout the Rubisco Superfamily

Background/Objective

- Rubisco fixes nearly all organic carbon, yet biochemical study has been limited to a few form I and II subclades.
- Survey structural and functional diversity of rubisco across deep-branching, sparsely sampled clades of the tree.

Approach

- Selected 104 phylogenetically diverse rubiscos from 1,859 sequence clusters; expressed and purified them in E. coli.
- Determined oligomeric state of 18 soluble variants by SEC-SAXS and solved a P. syntrophicum crystal structure.

Results

- 15 of 18 characterized rubiscos were dimeric, supporting a dimeric ancestral state for the superfamily.
- Measured melting points from 57.5 to 95.2 °C and found an unusually large 557-residue deep-branching rubisco.

Significance/Impacts

- Advances BER goals in microbial systems biology and the evolution of carbon-fixation enzymes across the tree of life.
- Expands JBEI's rubisco engineering toolkit for improving carbon capture in bioenergy feedstocks and hosts.

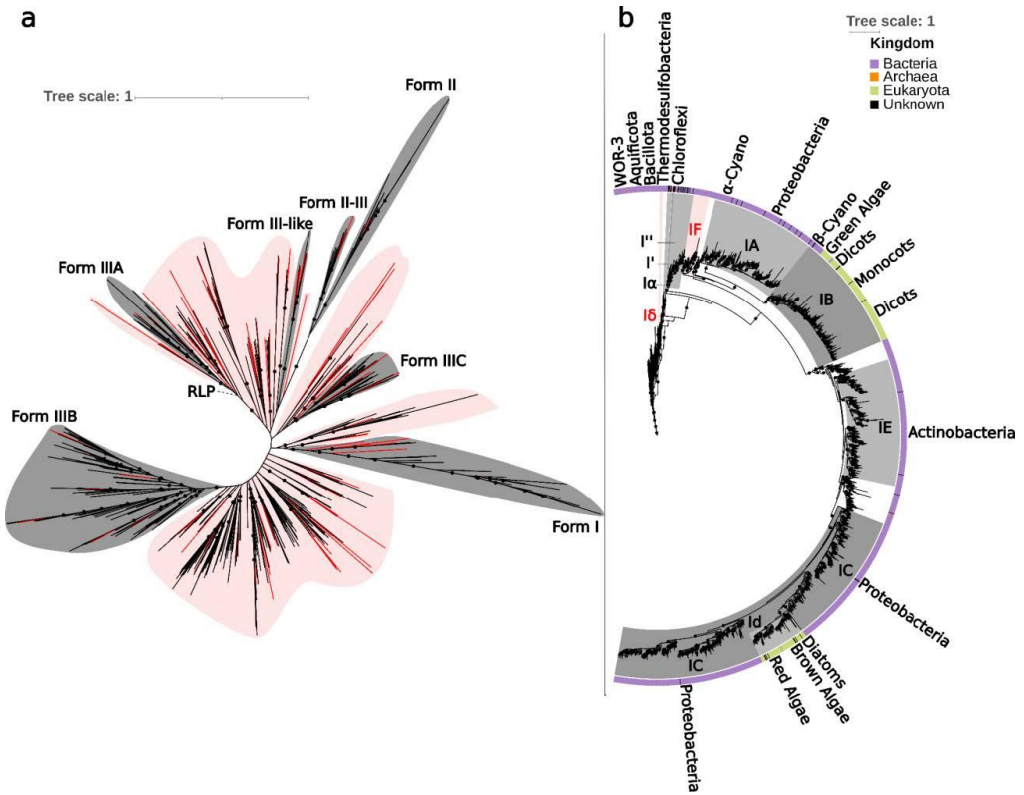

Figure caption: Our tree topology of the rubisco superfamily depicts *bona fide* (*i.e.*, referred to as forms I through III) rubiscos forming a monophyletic clade apart from RLP **(Figure 1a)**, consistent with previous phylogenetic analyses 1,13,14. Similarly, we observed that the forms I and II— the only two rubisco forms currently experimentally associated with CBB Cycle— are separated by several short but well-supported internal branches, and do not resolve as sibling clades **(Figure 1a)**. Within the form I rubiscos, numerous subforms with different taxonomic, structural, and kinetic attributes are currently recognized1. Here, we retain the nomenclature for the previously recognized IA, IB, IC, ID and IE subforms **(Figure 1b)**.

Kehl, A. J., et.al. Nature Communications. doi: 10.1038/s41467-026-73982-5 (JBEI #1321)

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Assembly and Reactions of Artificial Metalloenzymes in *Streptomyces Albus*

Background/Objective

- Artificial metalloenzymes enable new-to-nature reactions, but in-cell assembly had been limited to E. coli hosts.
- Assemble an iridium artificial metalloenzyme in *Streptomyces albus,* a chassis for complex natural-product pathways.

Approach

- Integrated CYP119 variant genes into *S. albus* J1074 and supplied the Ir(Me)MPIX cofactor without added transporters.
- Ran whole-cell cyclopropanation of carvone and styrene with ethyl diazoacetate; tracked Ir uptake by ICP-MS.

Results

- *S. albus* natively imported the cofactor, incorporating ~37%; extra CYP119 copies raised selectivity to 72%.
- Reached ~5,700 turnovers per Ir atom, ~2.5x the engineered E. coli value and ~20x the purified holoenzyme.

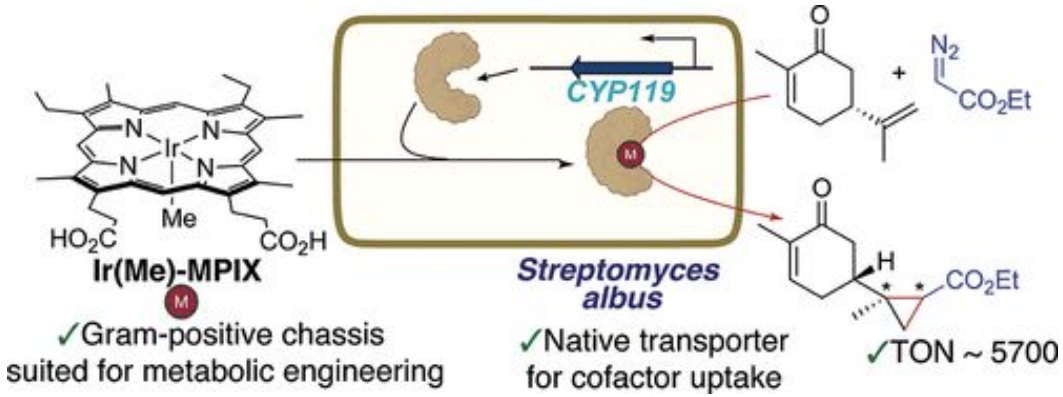

Figure caption: Schematic assembly and biocatalytic reaction of an iridium-containing artificial metalloenzyme (Ir-ArM) in *Streptomyces albus*. The iridium cofactor (Ir(Me)MPIX) is taken up into the cytoplasm of the engineered *S. albus* host to assemble the Ir-ArM (utilizing CYP119). Once assembled *in vivo*, the metalloenzyme Mukhopadhyay catalyzes an abiological carbene transfer to the unactivated, disubstituted double bond of an exogenously added terpene, achieving exceptionally high turnover numbers (TON ~5700).

Significance/Impacts

- Supports BER synthetic-biology aims by extending biocatalysis into Gram-positive natural-product chassis.
- Broadens JBEI host range for integrating abiological chemistry into bioproduct and bioenergy pathways.

Chakraborty, S., et.al. Journal of the American Chemical Society. doi: 10.1021/jacs.6c00752 (JBEI #1322)

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Learning from Nature's Plant Engineers: Hijacking Metabolism and Development Beyond Genetics

Background/Objective

- Plant synthetic biology is largely synonymous with genetic engineering, which is slow and often genotype-limited.
- Survey how microbes and insects reprogram plant metabolism non-genetically to inspire new engineering routes.

Approach

- Reviewed natural systems: Xanthomonas TALEs, phytoplasma effectors (SAP05/SAP11), Ustilago maydis, and insect galls.
- Organized strategies into a control-layer framework spanning transcription, hormones, and resource allocation.

Results

- Identified a shared principle: large outcomes arise from targeting regulatory hubs, not rebuilding pathways de novo.
- Mapped effectors and peptides into reusable primitives for on-demand organ, tissue, and metabolic-state control.

Significance/Impacts

- Aligns with BER aims by reframing programmable, non-genetic control of plant metabolism and development.
- Informs JBEI feedstock engineering for tailored cell walls, biomaterials, and higher-value bioenergy crops.

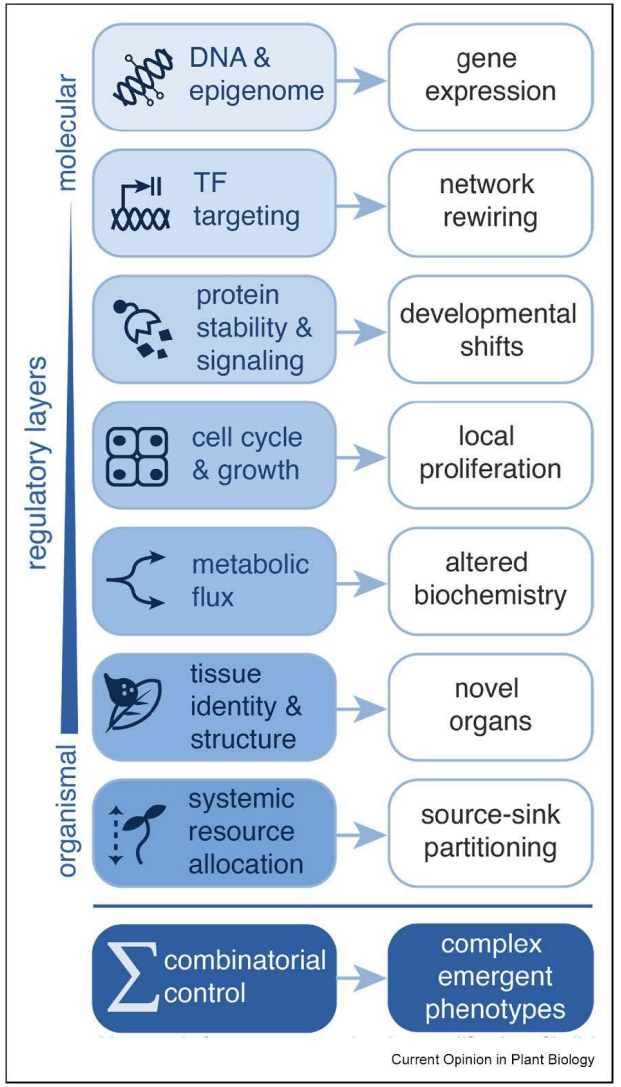

Figure caption: Control layer framework for programming plant state. Plant-associated organisms manipulate hierarchical regulatory layers—spanning from molecular signaling to organism-wide resource allocation—to reprogram host phenotypes. This framework reframes plant engineering as the composable control of system states rather than individual gene modifications.

Sarkiss, A. E., et.al. Current Opinion in Plant Biology. doi: 10.1016/j.pbi.2026.102905 (JBEI #1323)

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Tyrosine-sulfated Peptide-Induced Flavonol Biosynthesis Controls Elongation and Differentiation in Arabidopsis Primary Root

Background/Objective

- Root zonation balances cell growth and maturation, but how flavonol metabolism is spatially controlled is unclear.
- Tested whether the sulfated peptide PSY1 reshapes root zonation by regulating flavonol biosynthesis in Arabidopsis.

Approach

- Used ProPSY1:GFP reporters, psy1, tpst-1 and psyr1,2,3 mutants, and synthetic PSY1 in Arabidopsis Col-0 roots.
- Paired RNA-seq of PSY1-treated root tips with DPBA flavonol staining and LC-MS/MS metabolomics of root exudates.

Results

- PSY1 treatment shifted 253 genes; flavonol biosynthesis genes (CHS, FLS1, MYB12) rose in the differentiation zone.
- psy1 mutants had shorter mature cells; flavonol-deficient tt4-11 lost the PSY1 growth response and H2O2 reduction.

Significance/Impacts

- Advances BER understanding of peptide signaling and specialized metabolism governing plant root development.
- Builds JBEI expertise in plant cell-wall and metabolite pathways underpinning bioenergy feedstock improvement.

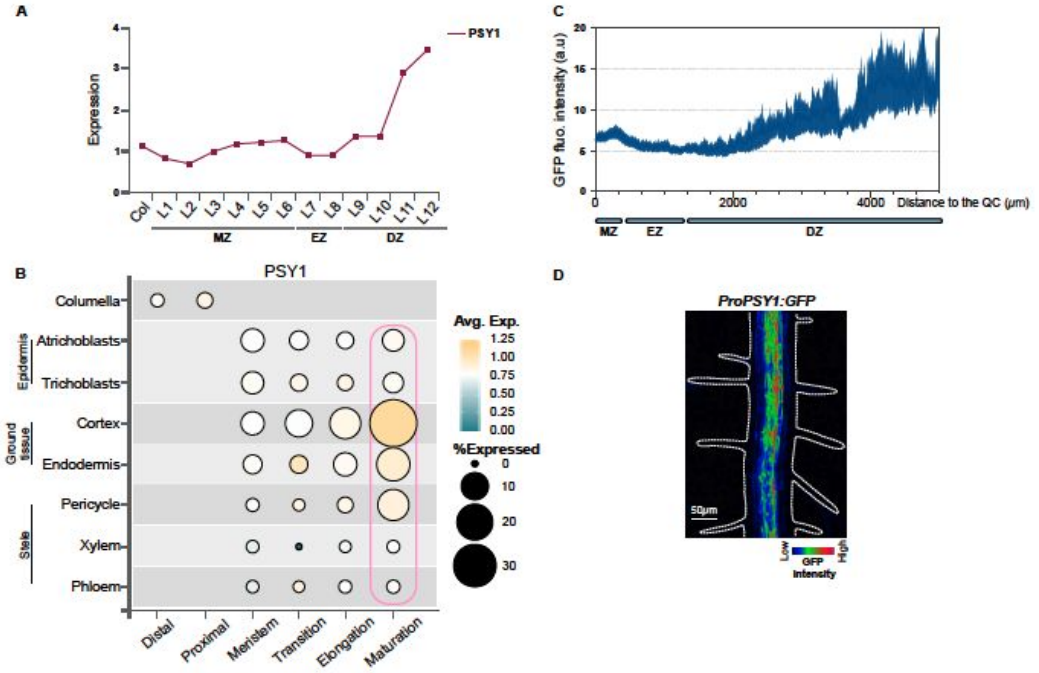

Figure caption: Supplementary Figure S1. PSY1 expression in Arabidopsis roots. (A, B) PSY1 longitudinal expression profiles from the RootMap database (Brady et al. 2007) and the single-cell root atlas (Shahan et al. 2022). In (B), dot size represents % expressed cells, and color indicates mean normalized expression across developmental stages (Distal, Proximal, MZ, TZ, EZ, DZ). The pink square marks tissues imaged in (D). (C) Mean fluorescence intensity (± s.e.m., n=10) in 6-day-old ProPSY1:GFP seedlings. (D) Maximum intensity projection of ProPSY1:GFP in the differentiation zone (4500µm from QC). RGB-rainbow scale indicates intensity; white dashed lines denote organ boundaries. (MZ=meristematic zone, EZ=elongation zone, DZ=differentiation zone). Supports Figure 1.

Ercoli, M. F., et.al. The Plant Cell. doi: 10.1093/plcell/koag180 (JBEI #1324)

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NMR of Fully and Partially 13C-Enriched Biomass Enhances Pendent Group Structural Characterization

Background/Objective

- Fully 13C-labeled biomass promised NMR sensitivity gains but 13C-13C coupling degrades standard HSQC and HMBC.
- Sought NMR experiments that exploit, rather than suffer from, full 13C enrichment in lignin and cell-wall samples.

Approach

- Applied constant-time HSQC, CT-HSQC-TOCSY, and a relayed C-C-FLOPSY experiment to 13C-enriched lignins and walls.
- Used maize, sorghum, and poplar enzyme lignins and whole cell walls, with synthesized model compounds as anchors.

Results

- C-C-FLOPSY mapped full carbon networks, assigning elusive quaternary carbons that HMBC fails to reach on walls.
- Spectra resolved tricin, cis/trans-p-coumarates, p-hydroxybenzoates, and ferulates as free-phenolic pendent units.

Significance/Impacts

- Supports BER plant cell-wall science by enabling rigorous structural characterization of lignin from 13C biomass.
- Strengthens JBEI lignin valorization by clarifying pendent-group chemistry relevant to feedstock deconstruction.

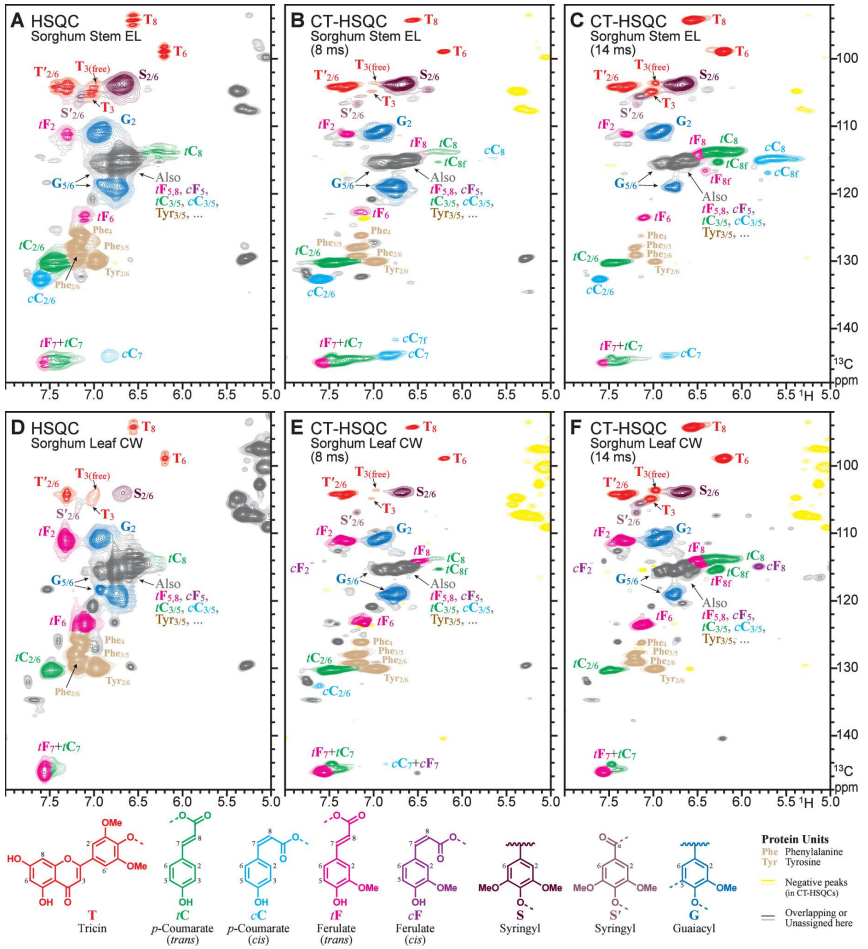

Figure 1. Sorghum 1H–13C correlation spectra. (A) The aromatic region of a normal HSQC spectrum from 13C-labeled sorghum stem enzyme lignin (EL) shows broadening due to 13C–13C coupling. (B–C) Constant-time HSQC (CT-HSQC) spectra with (B) 8 ms and (C) 14 ms CT periods; the latter shows improved sensitivity for tricin T peaks versus syringyl lignin S2/6. (D–F) Corresponding plots for sorghum leaf cell wall (CW). Peaks are colored by structure; gray indicates overlapping signals (e.g., at ~6.7/115 ppm, including G5/6, C3/5, F5, Tyr3/5, and F8). Lighter contours represent 2-fold amplified data to reveal minor peaks; yellow denotes negative peaks in CT-HSQC data.

Ralph, J., et.al. Analytical Chemistry. doi: 10.1021/acs.analchem.5c08043 (JBEI #1325)

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Engineering an Extremely Hybrid PKS for Adipic Acid Production

Background/Objective

- Adipic acid for nylon is made by N2O-emitting petrochemistry; microbial routes lack modular PKS flexibility.
- Aimed to build the first in vivo PKS pathway to adipic acid using a carboxyl-retaining loading module.

Approach

- Identified EtnB, a succinyl-CoA-loading module, and joined seven PKS modules across six non-native junctions.
- Used an engineered borL communication linker; expressed the hybrid PKS in *E. coli* and *P. putida*.

Results

- The linker raised adipic acid titers; malonate supplementation increased production by roughly 10-fold.
- EtnB selectively loaded succinyl-CoA in vivo; the pathway ran in both *E. coli* and *P. putida* chassis.

Significance/Impacts

- Advances BER synthetic-biology goals of engineering microbes for sustainable production of industrial chemicals.
- Extends JBEI PKS and bioconversion toolkits toward renewable diacids for the emerging bioeconomy.

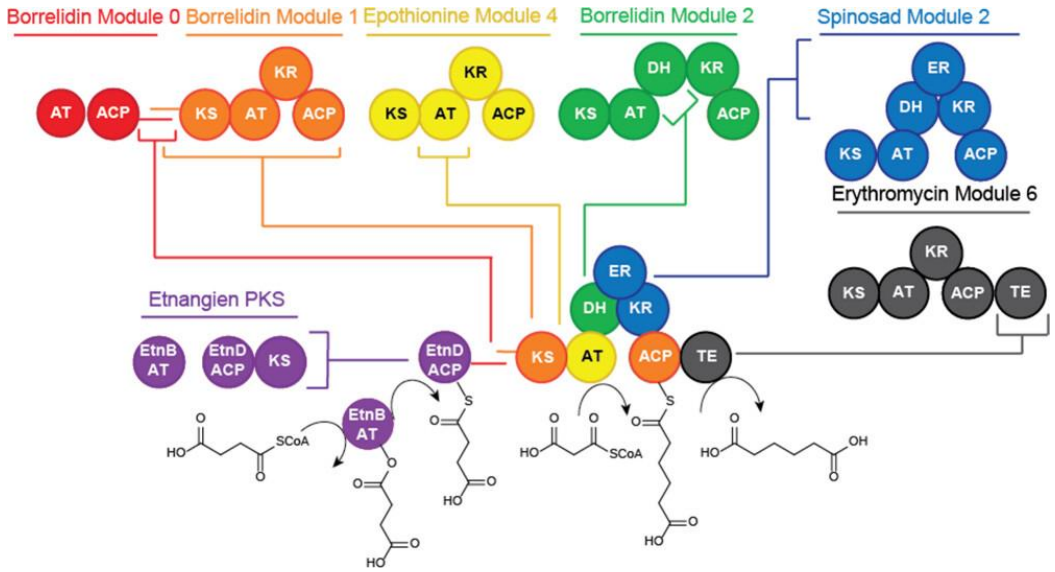

Figure caption: Retrobiosynthetic design and architecture of an extremely hybrid PKS platform for adipic acid production. This schematic illustrates a first-of-its-kind polyketide synthase (PKS) pathway engineered for the *in vivo* production of the industrial nylon monomer adipic acid (AA). The platform utilizes EtnB, a noncanonical succinyl-CoA-loading module that uniquely retains its terminal carboxyl group to enable dicarboxylic polyketide biosynthesis. To ensure efficient chain translocation, EtnB is coupled to a downstream fully reducing extension module via an engineered communication linker designed to optimize ACP–KS domain interactions and enhance titers. This highly chimeric assembly integrates genetic components from five distinct organisms, combining domains from seven PKS modules across six non-natural junctions into a functional biocatalyst robustly expressed in both *Escherichia coli* and *Pseudomonas putida*.

Klass, S. H., et.al. ACS Synthetic Biology. doi: 10.1021/acssynbio.5c00972 (JBEI #1326)

Enabled Publications

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How should reproducibility be approached in plastic recycling?

Background/Objective

- Many of the same methods used for biomass conversion apply to valorization of waste plastics
- Across the growing body of literature, there is a lack of consistency and reproducibility

Approach

- The authors commented on how to improve studies that use chemical and/or biological methods to deconstruct and valorize plastic wastes
- The JBEI team commented on the application TEA and LCA, and the range of assumptions and methods that make cross-comparison challenging

Results

- The field needs to coalesce around a set of commonly accepted assumptions and move toward common, machine-readable formats
- Contamination can reduce yields, add energy-intensive preprocessing steps, or reduce equipment lifetimes.

Significance/Impacts

- This article brings together leads in the field to offer a set of authoritative recommendations
- The suggested methods draw from, and have implications for, biomass conversion to fuels and products

Figure caption: With the growing importance of developing new and improved methodologies for plastic recycling, conducting reproducible research and ensuring that results are transferable across labs are increasingly important. This Voices article reflects on how academia and industry view the path forward for strengthening reproducibility to advance science and enable a circular plastics economy.

Baur, M., et.al. Chem Circularity. doi: 10.1016/j.checir.2026.100046 (JBEI #137)