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Vision and Development of a Design, Implementation, and Verification Automation (DIVA) Software Platform for DNA Construction

Background/Objective

- DNA construction is a prerequisite for biological research but diverts researchers from primary objectives.
- Our need is software enabling one person to execute batched DNA construction for an entire research institute.

Approach

- Built DIVA, a platform to enable design of DNA constructs, submit designs for construction to dedicated staff, and track DNA construction as it progresses
- Integrated with ICE parts repositories, BOOST, BLiSS, j5, and OpenVectorEditor for combinatorial design.

Results

- DIVA has served JBEI since 2013 and the public since 2018, with 523+ registered users and 4,000 designs.
- Design and Build workflows captured timestamps, labor, reagent, and instrument costs to expose bottlenecks.

Significance/Impacts

- Advances BER goals for scalable, reproducible DNA construction underpinning microbial systems biology.
- Accelerates JBEI strain engineering for biofuels and bioproducts by automating the design-build cycle.

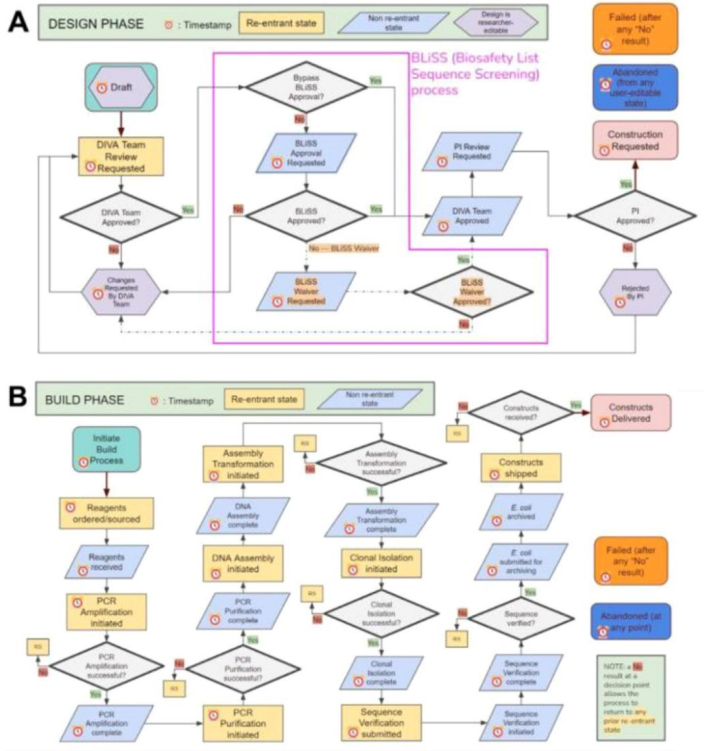

Figure caption: (A) Design workflow begins at the state "Draft", and conditionally progresses through other states, including "DIVA Team Review Requested", "Changes Requested by DIVA Team", "BLiSS Approval Requested" (biosecurity approval), "BLiSS Waiver Requested", "DIVA Team Approved", "PI Review Requested" (budgetary approval), "Rejected by PI", and "Construction Requested". (B) Build workflow begins at the state "Initiate Build Process" and conditionally progresses through other states, including "Reagents Ordered/Sourced", "Reagents Received", "PCR Amplification Initiated", "PCR Amplification Complete", "PCR Purification Initiated", "PCR Purification Complete", "DNA Assembly Initiated", "DNA Assembly Complete", "Assembly Transformation Initiated", "Assembly Transformation Complete", "Clonal Isolation Initiated", "Clonal Isolation Complete", "Sequence Verification Submitted", "Sequence Verification Initiated", "Sequence Verification Complete", "E. coli Submitted for Archiving", "E. coli Archived", "Constructs Shipped", and "Constructs Delivered".

Plahar, H., et.al. ACS Synthetic Biology. doi: 10.1021/acssynbio.6c00197 (JBEI #1329)

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Microbial catalysts unlock vitamin bioproduction

Background/Objective

- Microbial vitamin E production has been limited to tocotrienols, with tocopherol blocked by enzyme gaps.
- Assesses how Rhodobacter sphaeroides can serve as a chassis for scalable de novo tocopherol production.

Approach

- Reviews engineering of R. sphaeroides using homogentisate phytyltransferase and tocopherol cyclase modules.
- Highlights mutational studies and molecular dynamics mapping UbiE n transfer to C-1 or C-2 sites.

Results

- Endogenous UbiE showed promiscuous methyltransferase activity, converting δ-tocopherol to α-tocopherol.
- Engineered strains reached 508.2 mg/L in shake flasks and 3.51 g/L total tocopherols in fed-batch culture.

Significance/Impacts

- Supports BER interest in microbial metabolic capacity and non-classical enzymatic catalysts in bacteria.
- Extends JBEI isoprenoid pathway expertise toward lignocellulosic feedstocks for bioeconomy products.

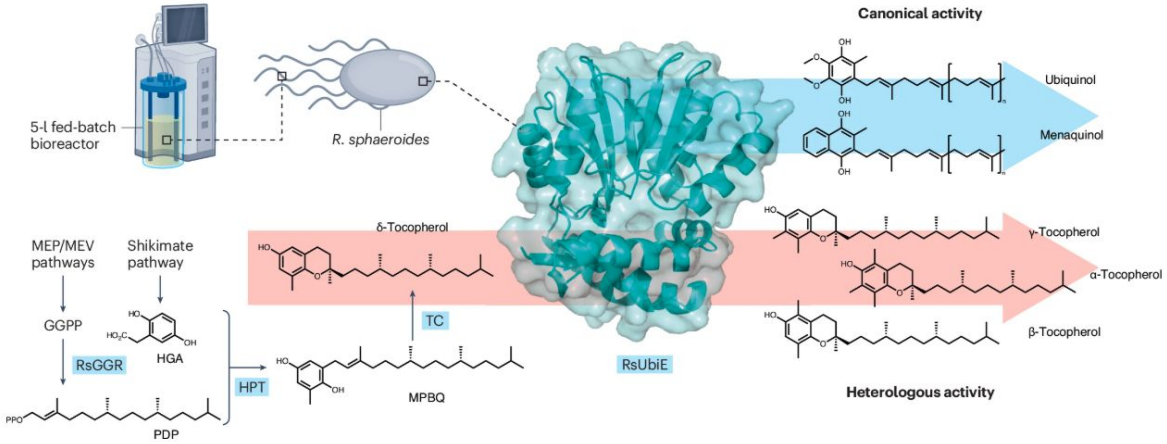

Figure caption: Promiscuous activity of endogenous UbiE facilitates non-classical tocopherol diversification in *R. sphaeroides*. Schematic representation of the engineered biosynthetic pathway for α-tocopherol in *Rhodobacter sphaeroides*, showing the condensation of shikimate-derived homogentisic acid (HGA) and phytyl diphosphate (PDP)—generated from geranylgeranyl pyrophosphate (GGPP) via geranylgeranyl diphosphate reductase (RsGGR)—by homogentisate phytyltransferase (HPT) to yield 2-methyl-6-phytyl-benzoquinone (MPBQ), which is subsequently cyclized by tocopherol cyclase (TC) to form δ-tocopherol; whereas endogenous UbiE natively functions in bacterial ubiquinone and menaquinone biosynthesis (canonical activity), its substrate promiscuity allows it to methylate the tocopherol chromanol ring, converting δ-tocopherol into β- or γ-tocopherol and ultimately into fully methylated α-tocopherol, thereby bypassing the requirement for dedicated heterologous plant methyltransferases (MEP/MEV, methylerythritol phosphate and mevalonate pathways).

Ramirez, A. I., et.al. Nature Metabolism. doi: 10.1038/s42255-026-01584-y (JBEI #1328)

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Engineered polyketide synthases enable a microbial chassis for recyclable plastics with tunable properties

Background/Objective

- Crosslinked thermosets resist recycling, and PDK monomers such as dimedone are petrochemical and costly.
- Engineer PKSs to biosynthesize diverse chiral β-keto-δ-lactone monomers for tunable recyclable plastics.

Approach

- Computationally screened 144 BKDLs, identifying solvation free energy as the driver of depolymerization.
- Built hybrid type I PKSs in E. coli K207-3 and Streptomyces albus using ClusterCAD-guided module swaps.

Results

- Bioreactor cultivation of engineered E. coli reached 1.84 g/L BKDL 4 on glucose within 24 hours.
- Resulting PDKs showed tunable Tg of 53–98 °C and depolymerization from 0–60 °C with 93–96% monomer recovery.

Significance/Impacts

- Advances BER goals in microbial systems biology and biosynthetic conversion of renewable carbon.
- Shows corn-stover BKDLs beat petrochemical dimedone on cost and GHG, supporting JBEI bioeconomy aims.

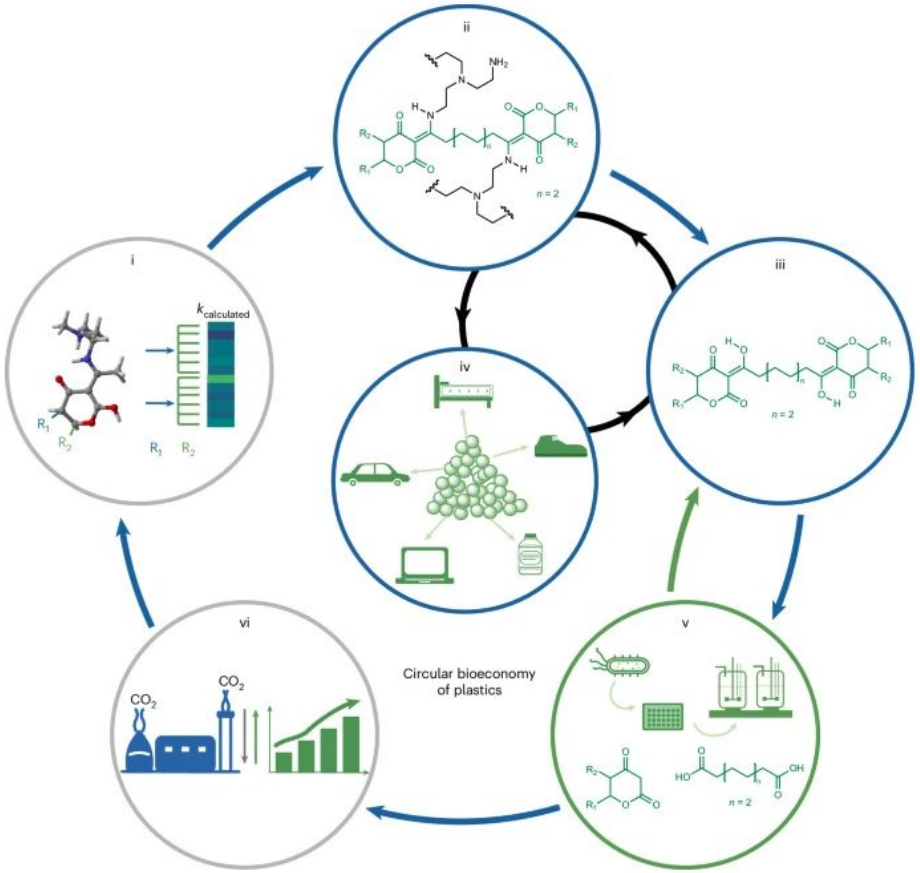

Figure caption: Circular lifecycle of BKDL-PDK plastics. Computational modeling guides monomer selection (i–iii) produced via engineered microbial fermentation (v). Synthesized PDK plastics (iv) undergo closed-loop chemical depolymerization to recover pure monomers (iv → iii), supported by TEA and LCA evaluations (vi).

Wang, Z., et.al. Nature Biotechnology. doi: 10.1038/s41587-026-03229-7 (JBEI #1329)

Enabled Publications

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Biocatalytic C3 β-O-Glycosylation of Triterpenes and Sterols to Synthesize Natural and Unnatural Saponins

Background/Objective

- Saponin production is limited by chemical synthesis complexity and the stringent specificity of most GT1s.
- Assess whether GuUGT73F15 can glycosylate varied triterpenes and sterols at the C3 hydroxyl position.

Approach

- Expressed MBP-tagged GuUGT73F15 in E. coli and ran in vitro glycosylation with 22 triterpenes and sterols.
- Screened 12 UDP-sugar donors by LC-UV/MS and modeled substrate binding poses with Boltz-2x predictions.

Results

- GuUGT73F15 produced 130 unique monoglycosylated saponins, over 100 of them not reported in nature.
- Enzyme enabled an antibody–saponin conjugate and in vitro synthesis of the QS-21 intermediate QA-TriX.

Significance/Impacts

- Supports BER interest in enzyme substrate permissivity and the structural basis of microbial biosynthesis.
- Uses JBEI glycosyltransferase and yeast engineering tools to broaden high-value bioproduct pipelines.

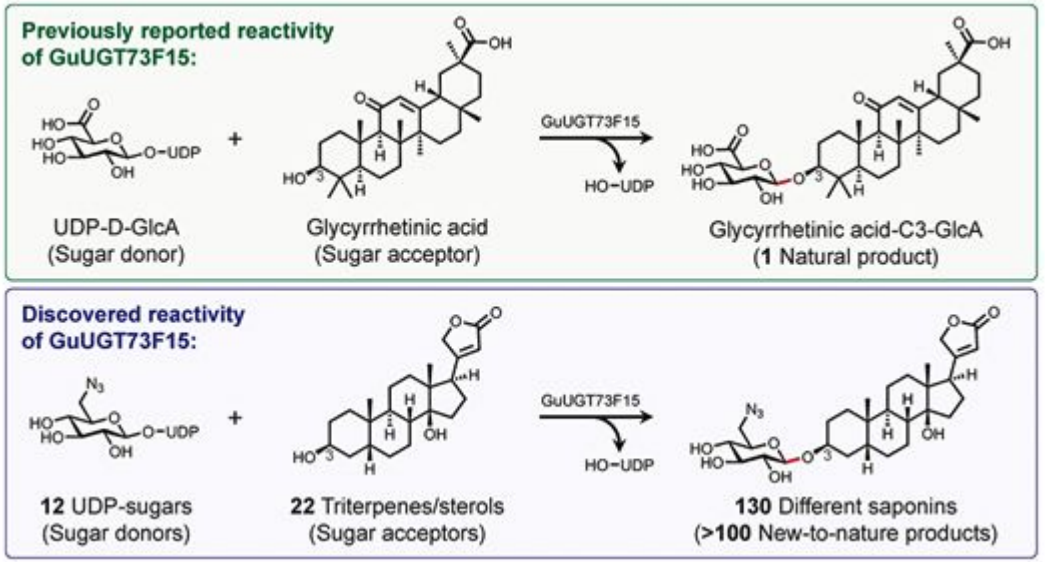

Figure caption: Previously limited to producing 1 natural saponin from a single donor-acceptor pair, the enzyme GuUGT73F15 was shown to accept 12 UDP-sugar donors and 22 triterpene/sterol acceptors to synthesize 130 unique saponins, including over 100 new-to-nature products.

Winegar, P. H., et.al. Journal of the American Chemical Society. doi: 10.1021/jacs.6c07628 (JBEI #138)

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Air Emissions Impacts of Fuel Substitution in the United States Cement Industry

Background/Objective

- Cement kilns require high heat provided by fuel combustion.
- Alternative fuels for industrial processes, including biomass, can help the 88 U.S. integrated cement plants be competitive in international markets.

Approach

- Modeled 16 alternative fuels using the BioSiting Tool assessed fuel availability within 80 km of each cement plant in the U.S.
- Built facility-level emission factors by kiln type and control technology, then applied InMAP modeling.

Results

- Alternative fuel share could rise from 17% to 73% with available resources.
- Aggressive adoption of alternative fuels can also improve human health around U.S. cement plants after accounting for sulfur and nitrogen releases.

Significance/Impacts

- Highlights the importance of alternative fuels for U.S. heavy industry.
- Advances JBEI goals by identifying new markets for bio-based fuels and quantifying the benefits to U.S. industry.

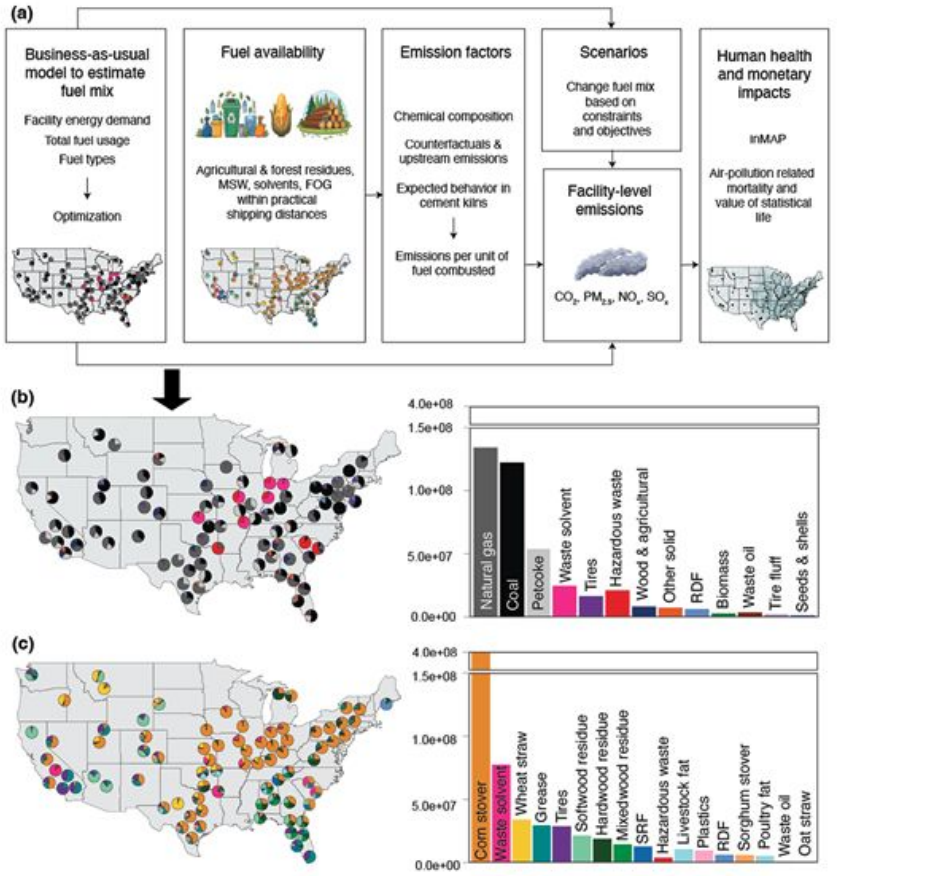

Figure caption: Shows a schematic representation of the methodology. To quantify emissions associated with fuel combustion in cement kilns, we first simulated business-as-usual (BAU) fuel usage at the facility level. Data on industry-wide fuel consumption and types of fuels used by each facility were obtained from the American Cement Association (ACA), formerly Portland Cement Association (PCA), which also breaks down total fuel consumption by categories of kiln, year modernized, capacity, and region.

Ostroski, A., et.al. Environmental Science & Technology. doi: 10.1021/acs.est.5c18471 (JBEI #139)