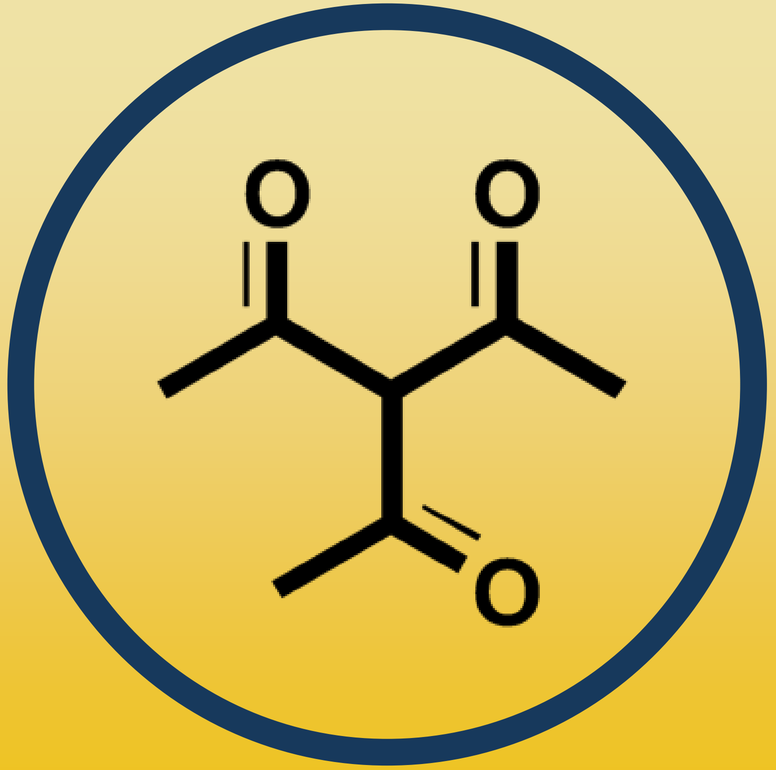
DOE Bioenergy Technologies Office (BETO) 2021 Project Peer Review

Design and Development of Bio-Advantaged Vitrimers as Closed-Loop Bioproducts



Mar 10, 2021
Technology Area Session

UC Berkeley: Jay Keasling & Kristin Persson

Lawrence Berkeley National Lab: Brett Helms, Tom Russell, Corinne Scown

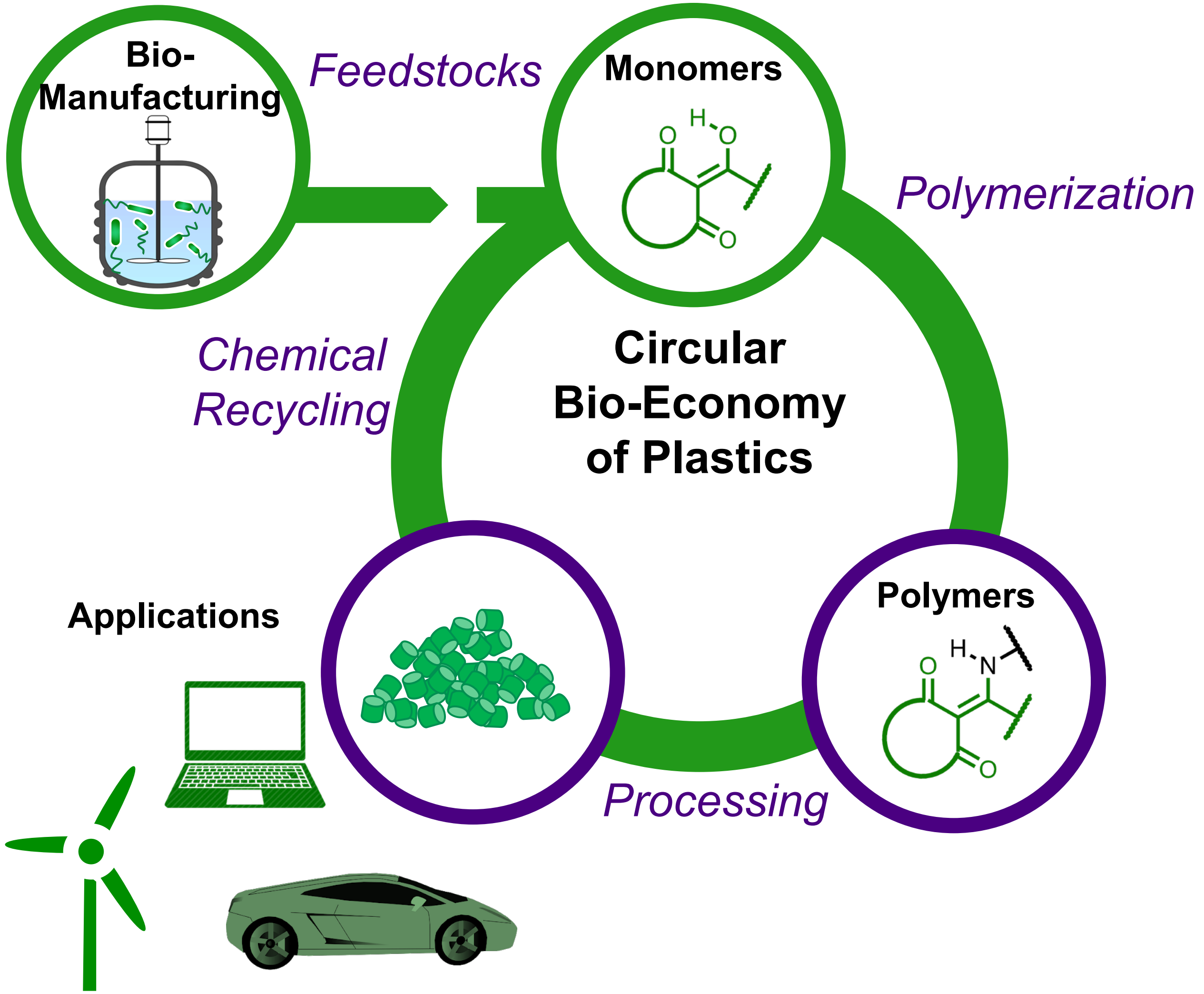
Berkeley UNIVERSITY OF CALIFORNIA

BERKELEY LAB

Project Overview

BETO review

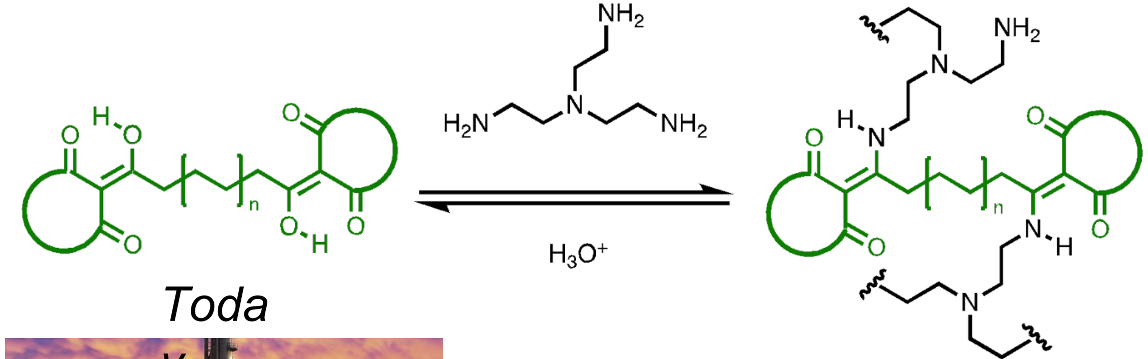
Vision



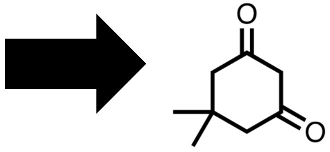
Advancing Beyond State-of-the-Art

Triketone Monomers

Infinitely Recyclable Polydiketoenamines

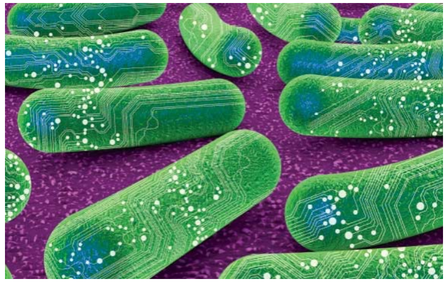


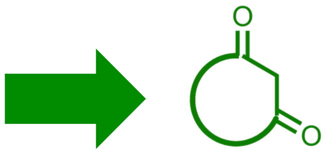
Today



Limited Functionality

Future

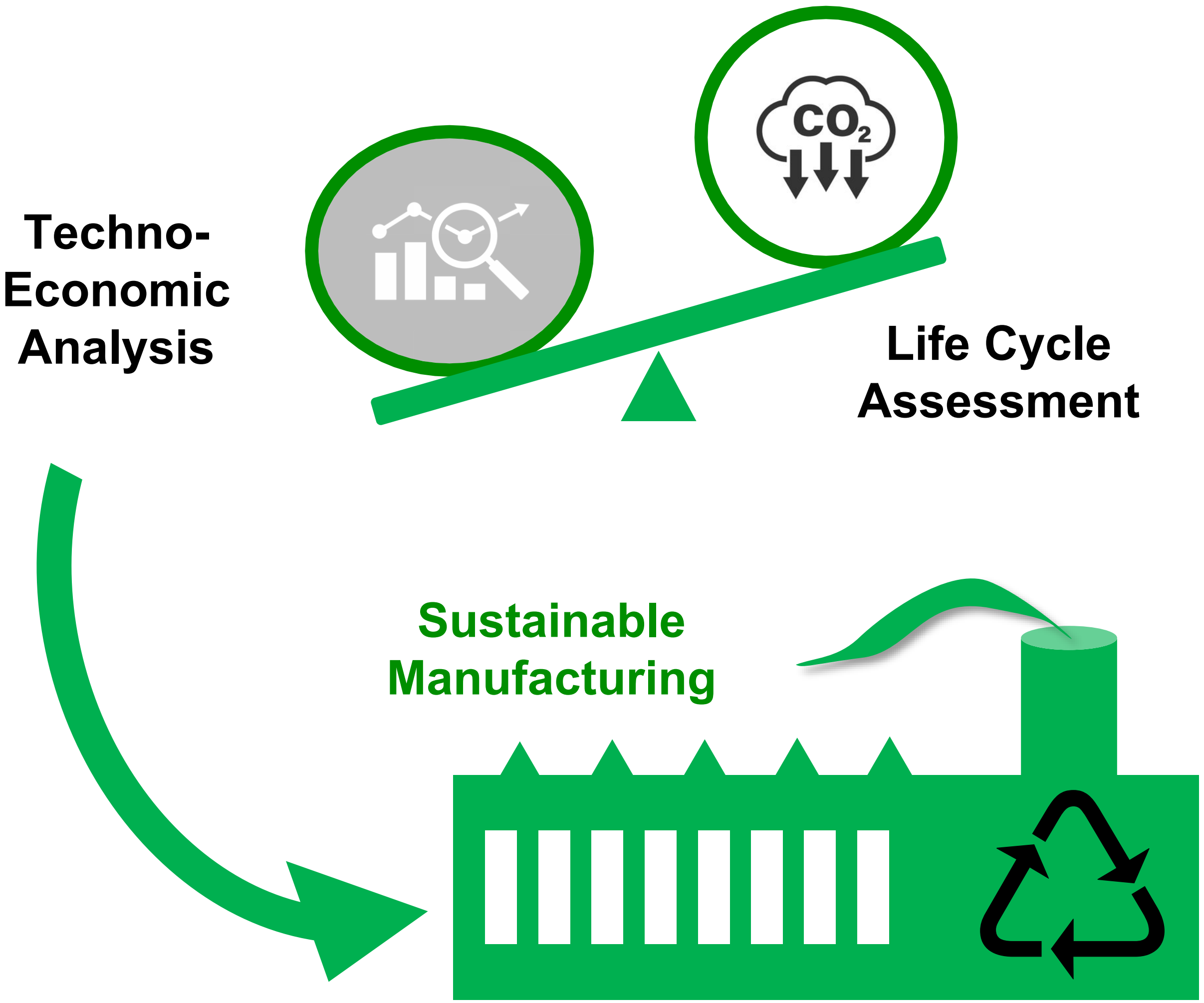




Bio-Advantaged Functionality

Project Overview

Potential Impacts



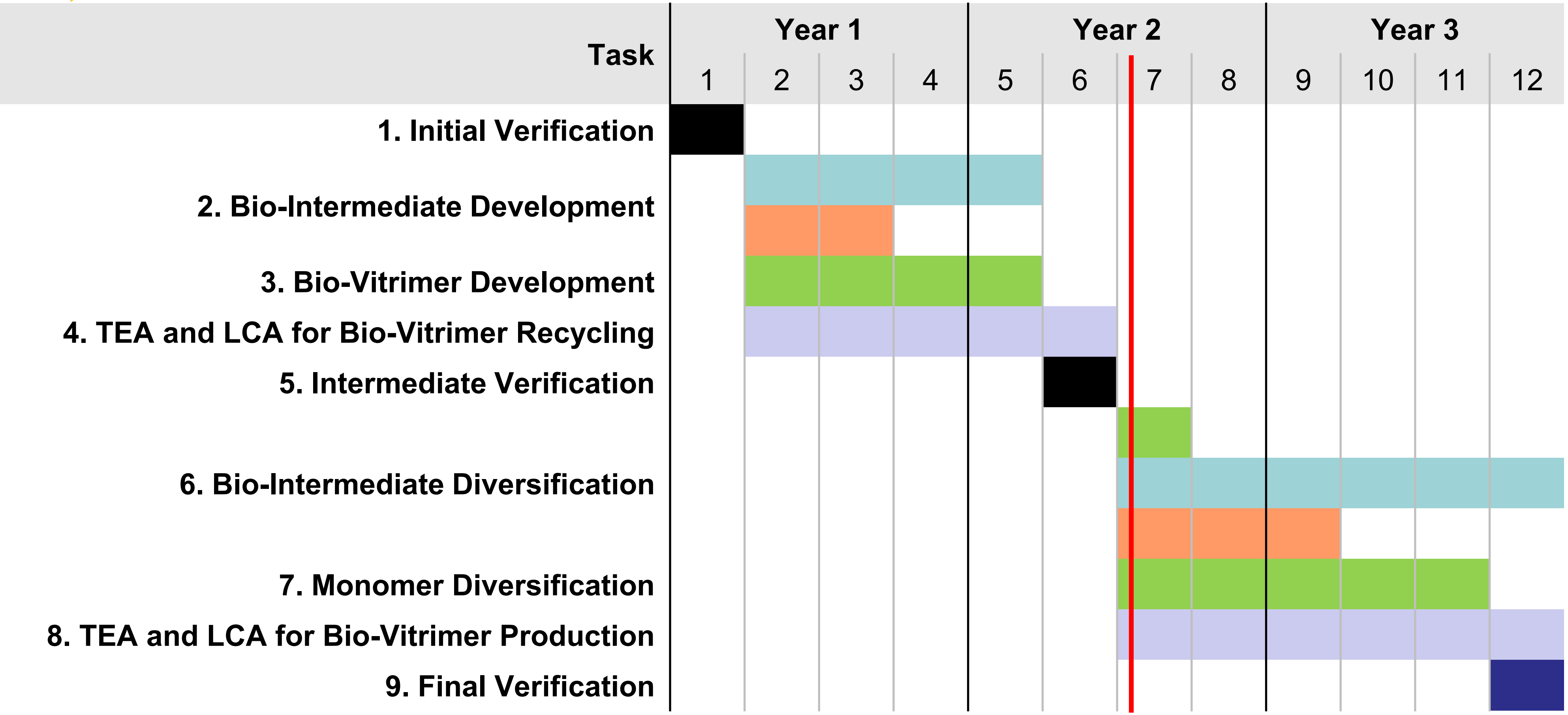
Potential Risks

Poor scalability of either bio- or chemical synthesis processes

Bio-Monomers fail to deliver market-differentiating performance advantages

Minimum selling price too high for widespread adoption in the market

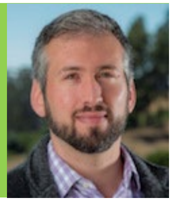
1 - Management



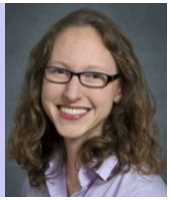
Keasling Group
Biology
UCB

Persson Group
Computational
UCB

Helms Group
Chemistry
LBNL



Scown Group
Energy/ Environmental
LBNL

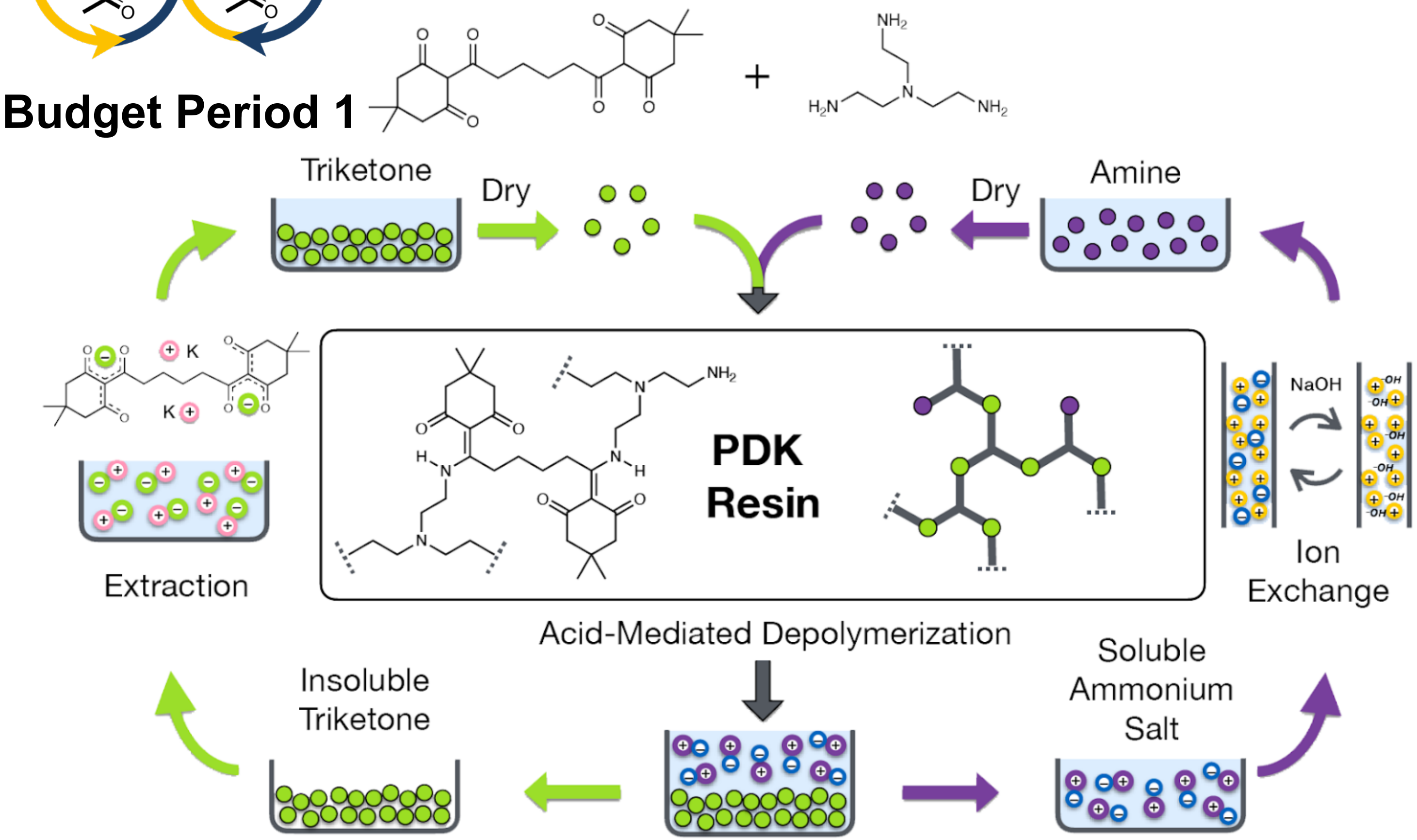


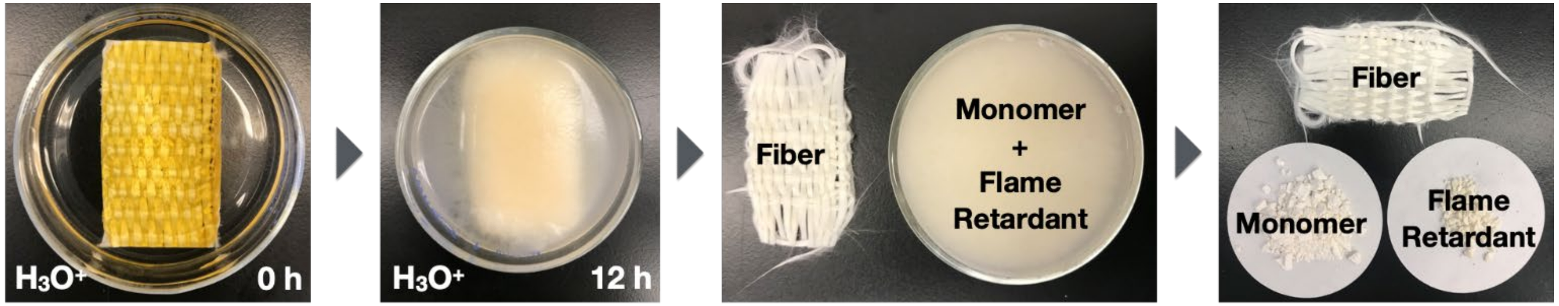
Risk	Mitigation
Scalability of chemically recyclable polymer bio-products.	Techno-economic analysis and life-cycle assessment of key processes.
Market adoption as a performance-advantaged and sustainable bio-product.	Work with industry to tailor performance for specific uses. Demonstrate biosynthetic route to key feedstocks and minimize losses in recycling.

2 - Approach

BETO review

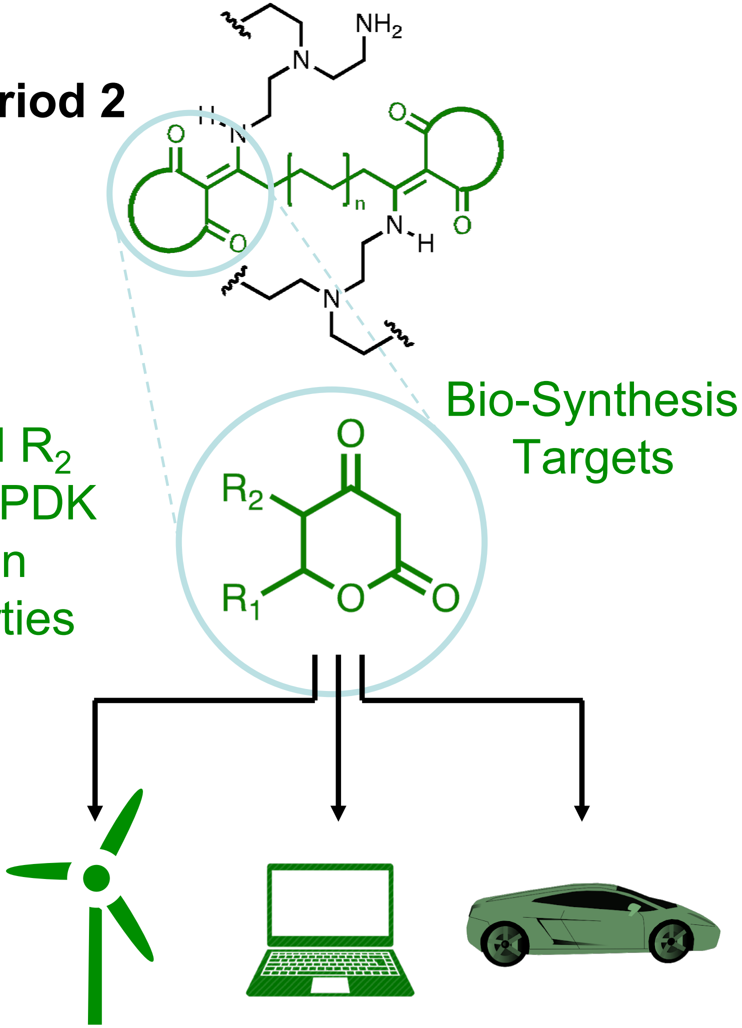
Budget Period 1





Helms et al. Nat. Chem. **11**, 442 (2019)

Budget Period 2

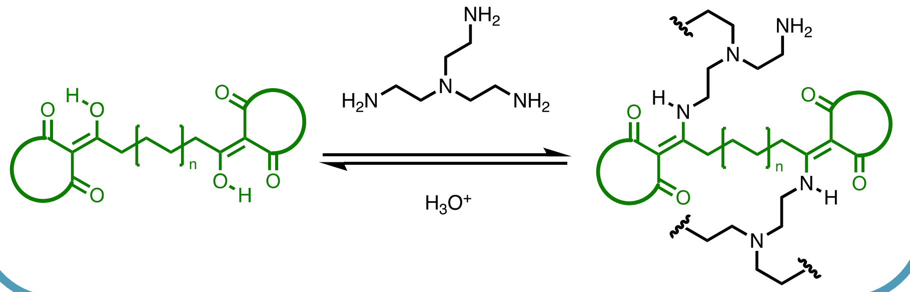


R_1 and R_2 Dictate PDK Resin Properties

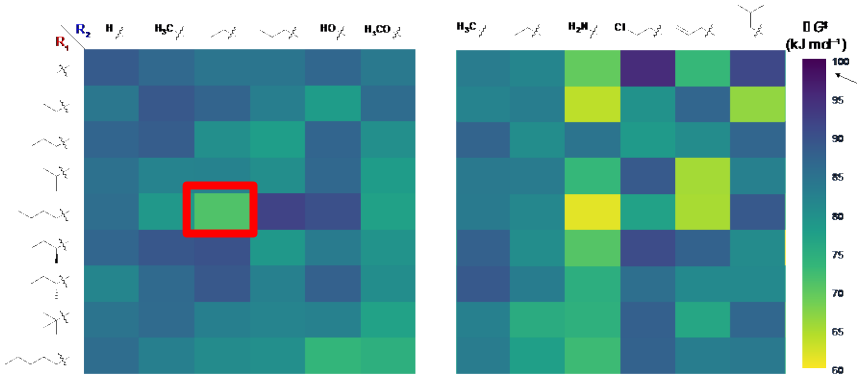
Objective: Co-Design Bio-PDK Resins for Properties AND Chemical Recycling

2 – Approach

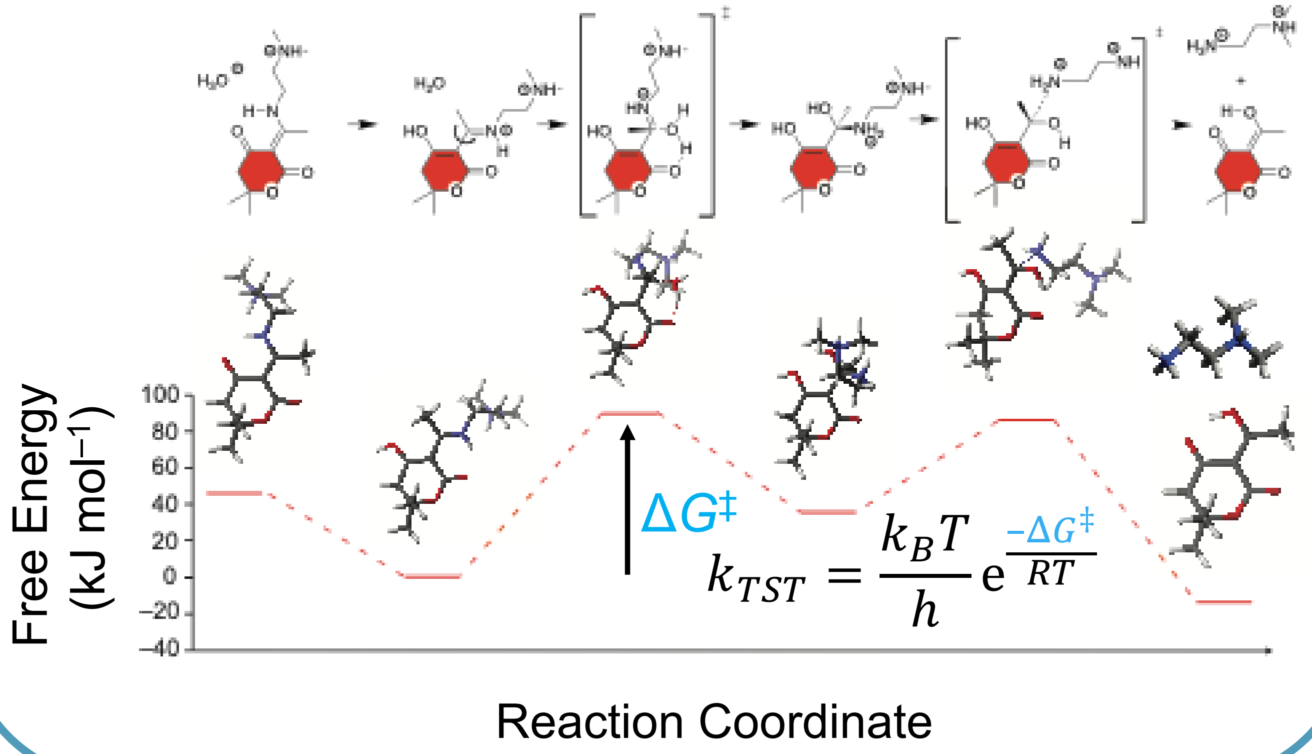
Diketoenamine Hydrolysis in Acid Unlocks Chemical Recycling



HT Screens Predict Variants with Most Favorable Recycling Rates



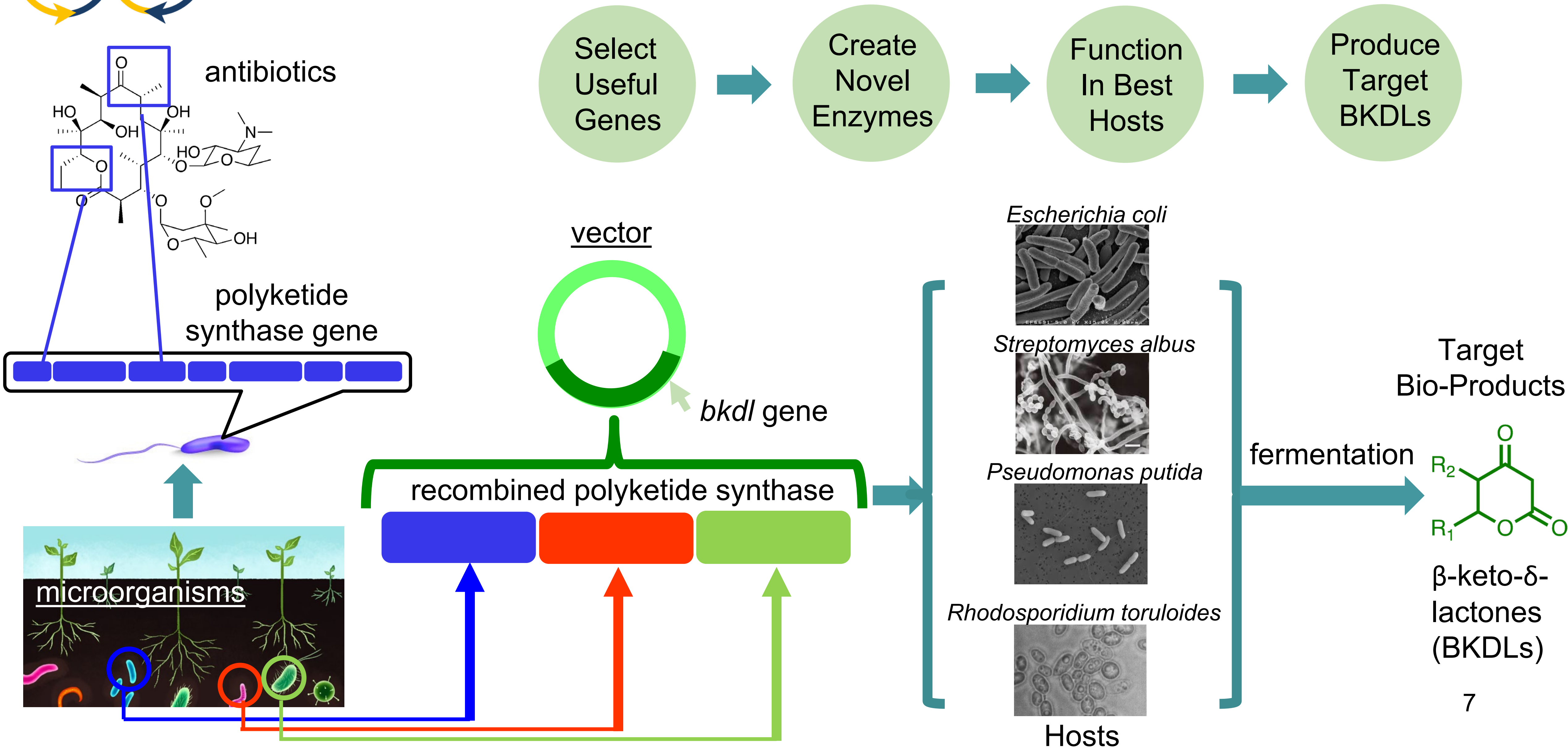
Compute Energetics for Hydrolysis



Validate with Experiment

Recommend Specific BKDLs for Bio

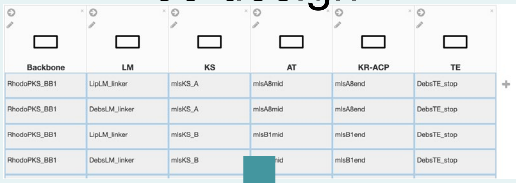
2 Approach


Select Useful Genes
Create Novel Enzymes
Function In Best Hosts
Produce Target BKDLs
antibiotics
polyketide synthase gene
microorganisms
vector
bkdl gene
recombined polyketide synthase
Escherichia coli
Streptomyces albus
Pseudomonas putida
Rhodosporidium toruloides
Hosts
fermentation
Target Bio-Products
β-keto-δ-lactones (BKDLs)

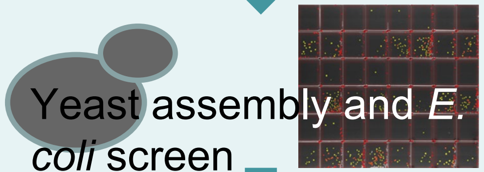
2 – Approach

Design and Build

J5 design



PCR & ZAG analysis

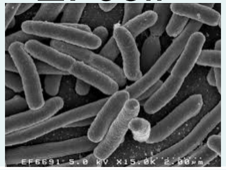


Yeast assembly and *E. coli* screen

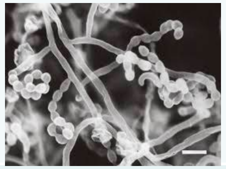
DIVA NGS sequencing

Host Development

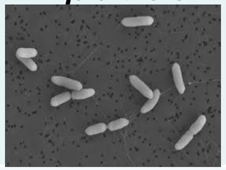
E. coli



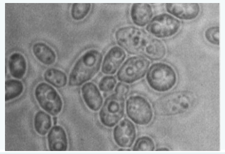
S. albus



P. putida



R. toruloides



Fermentation

1~5 mL

10~50 mL

1~5 L

100~300 L

Test

- LC-MS
- GC-MS
- Metabolomics
  Go/No-Go: > 1 g/L
- Proteomics

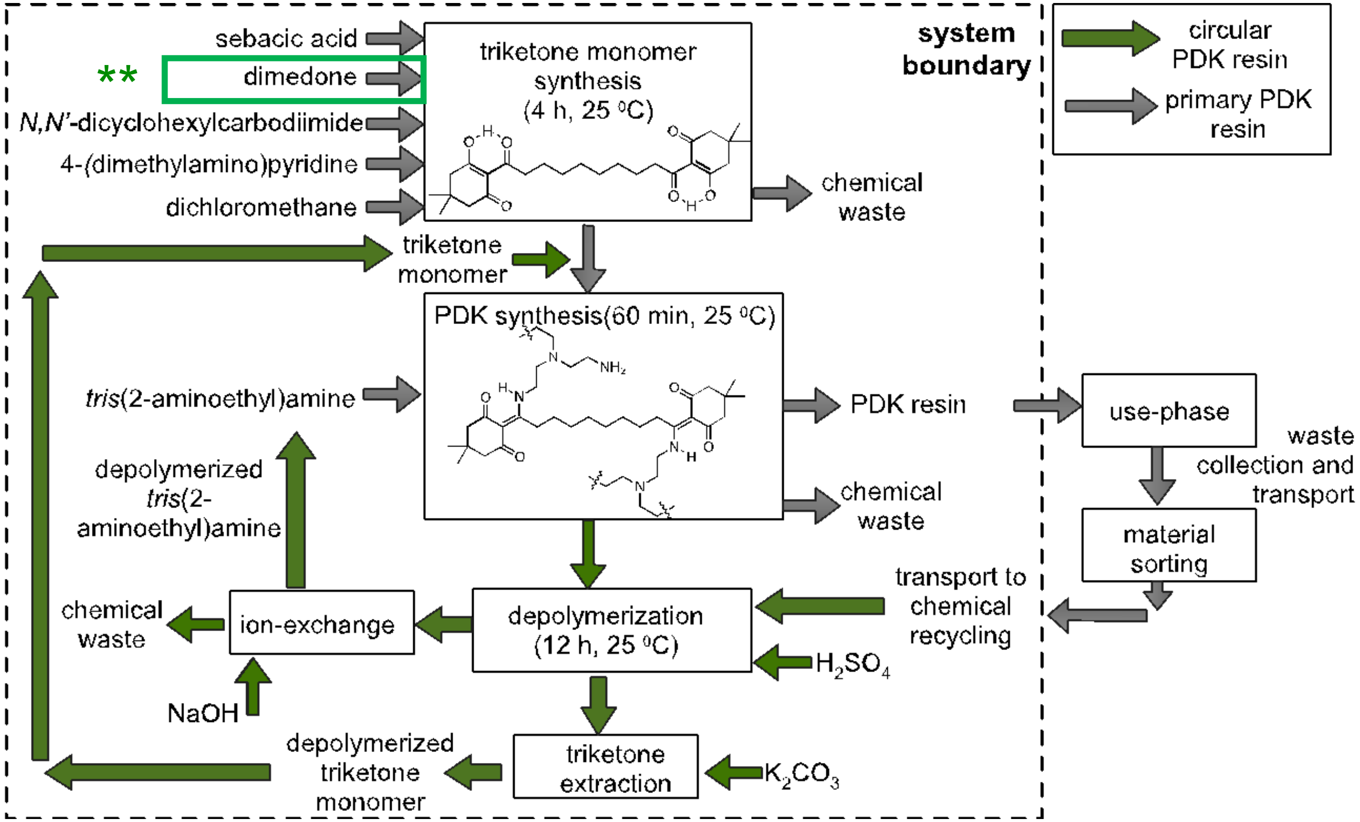
Learn

- Substrate Specificities
- Functional Fusion Boundaries
- Product Toxicity
- Protein-Protein Interactions

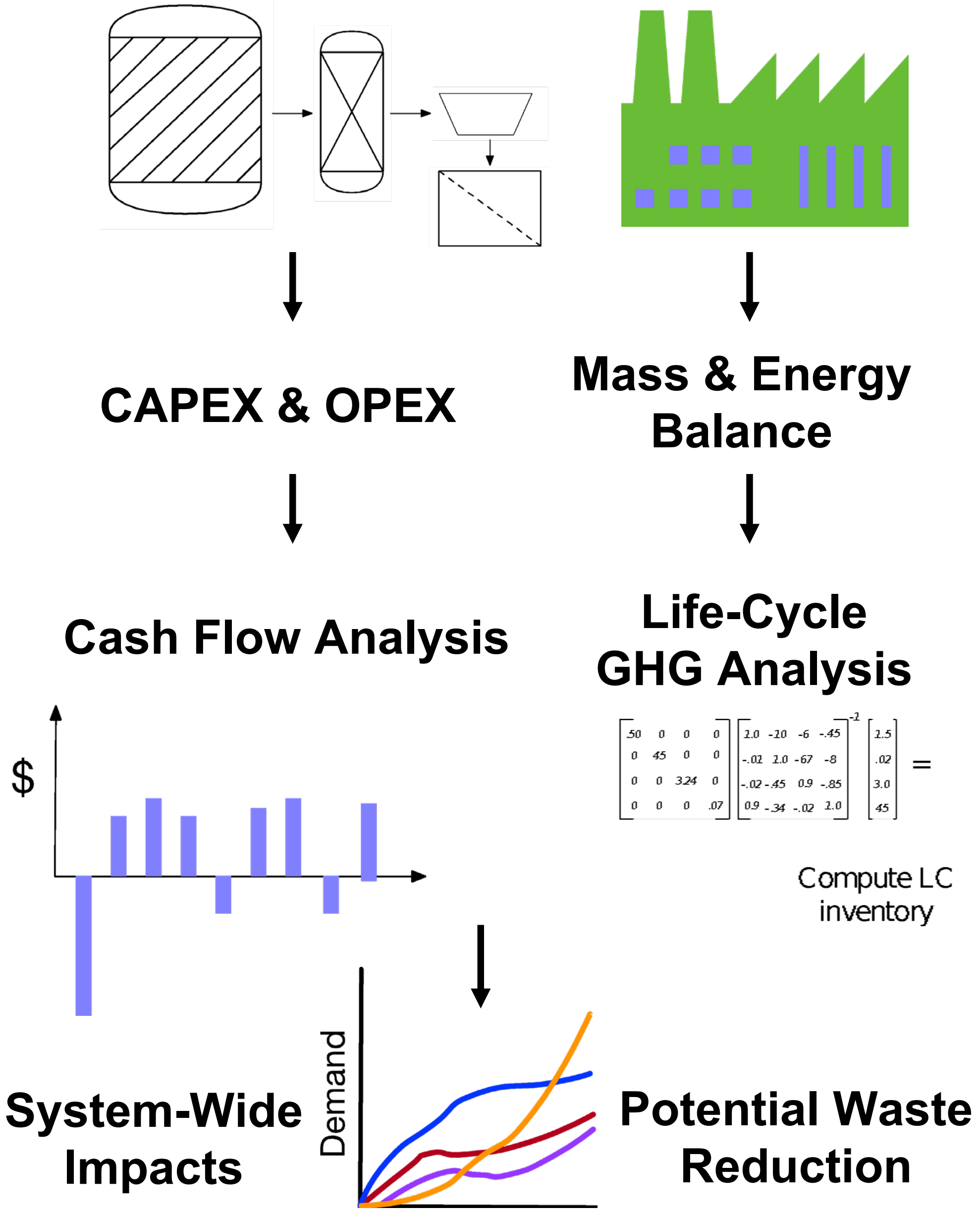
Automation / High-throughput platform

2 – Approach

Baseline Chemistry for PDK Synthesis



** Bio-Based BKDLs are the focus of Budget Period 3

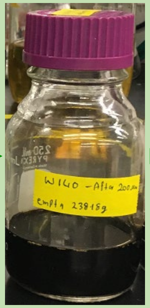
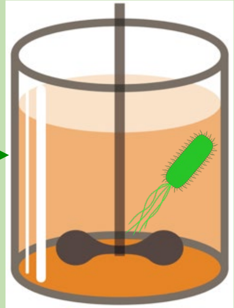
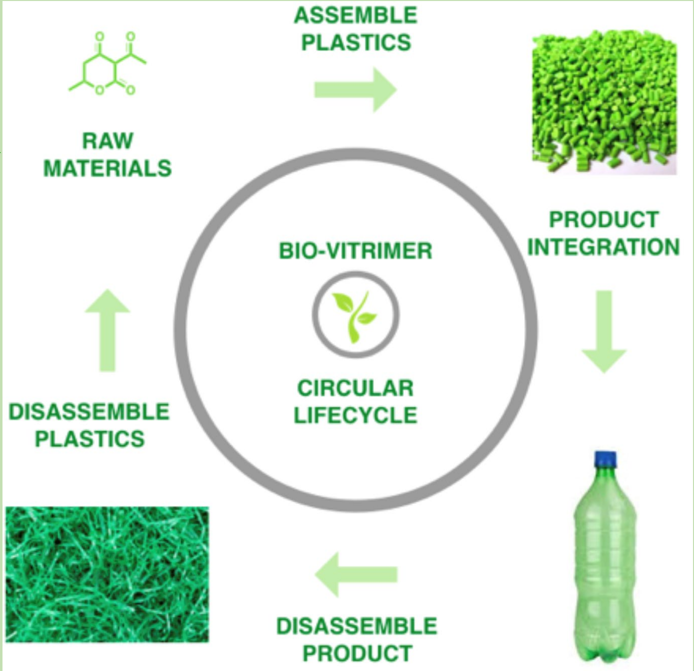


Source: Vora et al. *in revision.*

3- Impact

Need	Impact of Chemistry Development
Low intensity processes for chemically recycling polymers to monomer	Depolymerization of PDK resins at ambient temperature in strong acid, as we have demonstrated, provides significant energy savings by comparison to polymer deconstruction by pyrolysis. Lifecycle GHG emissions for circular PDKs, as we have demonstrated, are orders of magnitude lower than primary resin production, highlighting value of circularity in sustainable manufacturing.
Low loss processes for refining monomers	Lossless recovery of monomers, as we have demonstrated is possible with PDKs, is atypical by comparison to commodity polymers.
High bio-content in circular polymer resin	Responds to market pull for bio-based sustainable polymers
Bio-Advantaged performance	Showcases unique and high value for bio-products over conventional feedstocks
Scalable and low cost processes for monomer and resin production	De-risks commercialization prospects for the platform to meet market demand for industrial end uses

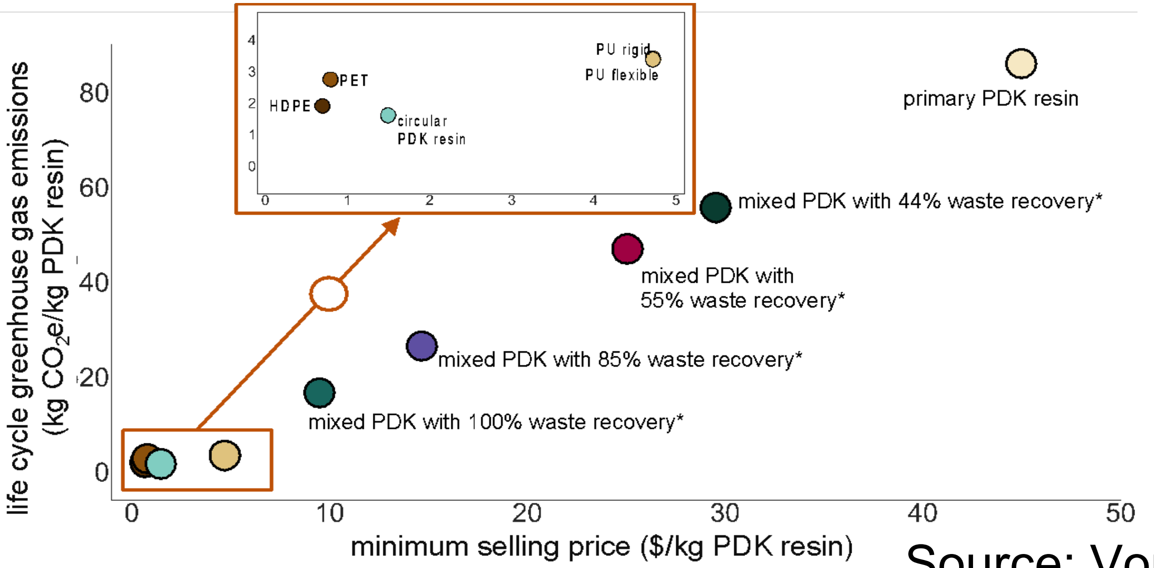
3- Impact: Bio-Advantaged Products

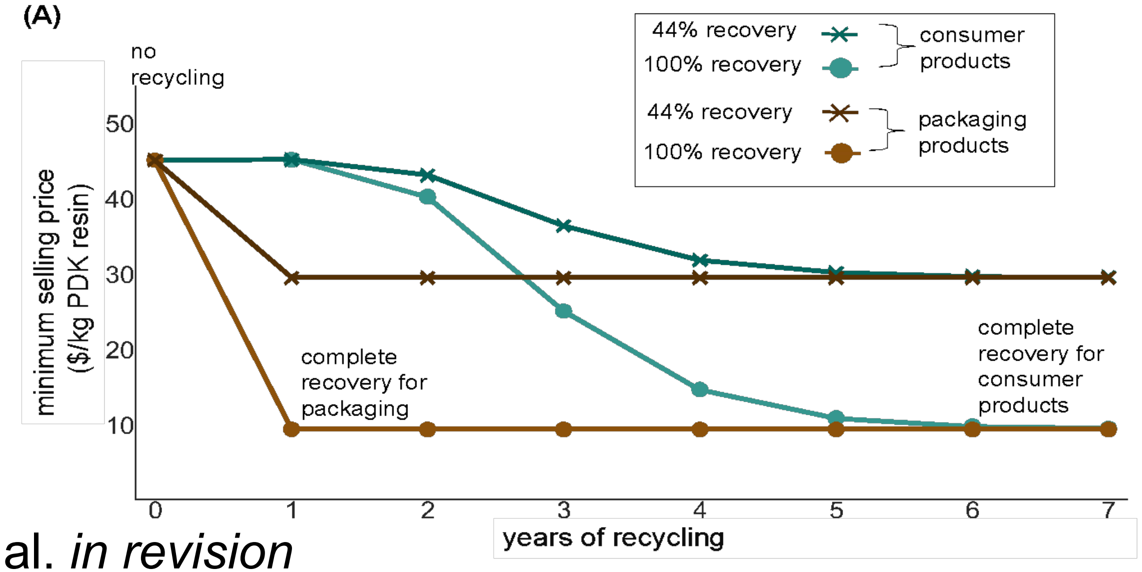
Biosynthesis lowers intensity of feedstock production and refinement and enables resilience in manufacturing supply chains	 Corn Stover  Hydrolysate  Fermentation     Enable biosynthesis of future bio-products
High-throughput DNA assembly	A platform for design and building *bkdl* genes & testing in high-throughput.
Proper hosts for biosynthesis	Host optimization for biosynthesis of diverse polyketide products

3- Impact: Bio-Advantaged Products

BETO review

Need	Impact of TEA & LCA
Prioritize process development research	Identify key solvents, reagents, catalysts, and processes with high costs or GHG emissions, as well as byproducts with hazardous waste implications
Infrastructure needs	Determine system-wide recovery rates necessary to hit cost & GHG targets
Selection of use cases	Identify use-cases with sufficient recovery potential
Prioritization of PDK properties	Identify resins and composites that meet target product specs, but also minimize losses in recycling for resource recovery (monomers, fillers, etc.)





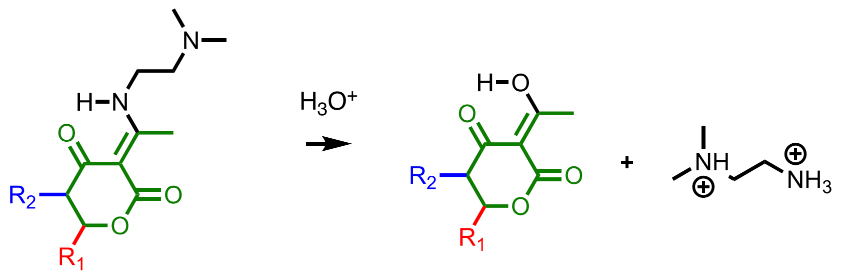
Source: Vora et al. *in revision*

4- Progress and Outcomes

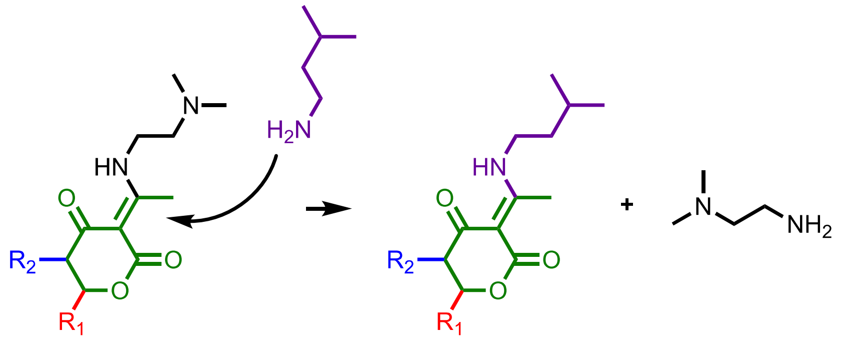
BETO review

Sub-Task Progress	Outcome
Budget Period 2: Screen >100 γ, δ substituted BKDLs for hydrolysis energy barrier	• Screened 108 BKDLs varying in R_1 and R_2 • Predicted a **strong** effect on the hydrolysis energy barrier, up to 40 kJ mol^{-1} • **Significance:** Recycling rates can be controlled by choice of R_1 and R_2
(Ahead of) Budget Period 3: Screen >100 γ, δ substituted BKDLs for amine-bond exchange energy barrier	• Screened 16 BKDLs substituted at R_1 and R_2 • Predicted a **negligible** effect on the amine-bond exchange energy barrier, < 5 kJ mol^{-1} • **Significance:** Energetics of re-processing PDKs is low and not strongly dictated by R_1 and R_2

Screens for Post-Consumer Chemical Recycling to Monomer



Screens for Post-Industrial Recycling via Scrap Recovery

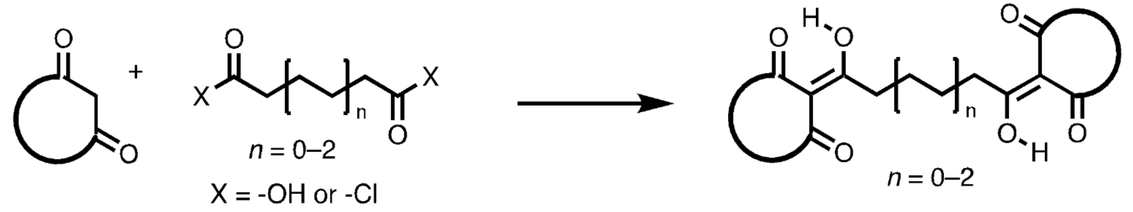


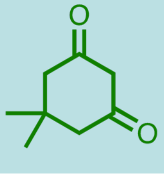
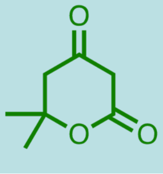
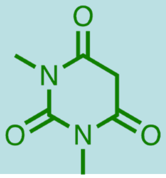
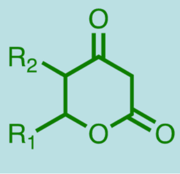
4- Progress and Outcomes

BETO review

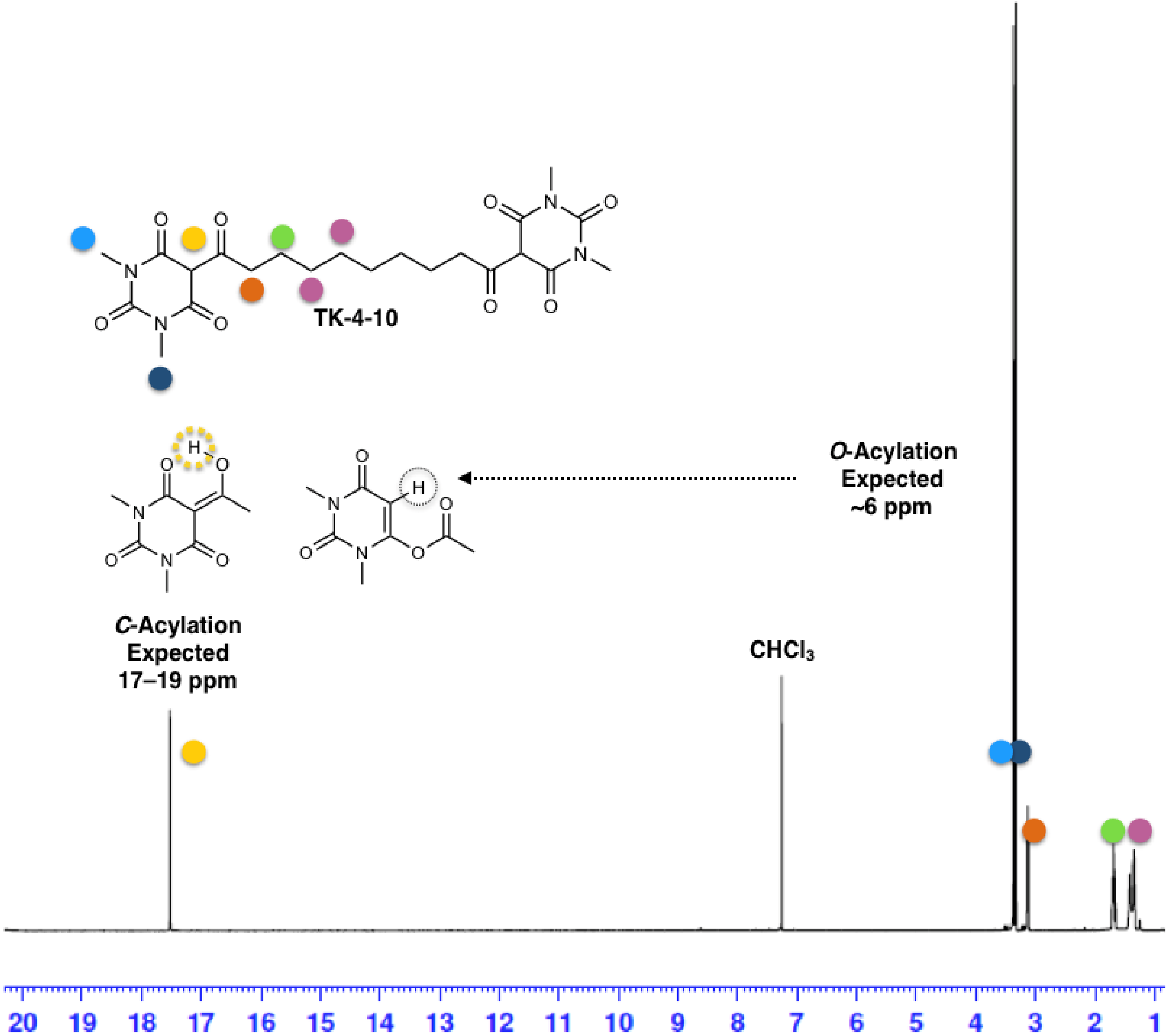
Milestone 3.1.1: Demonstrate 50-g batch of triketone biomonomers: 100% C- vs. O-acylation

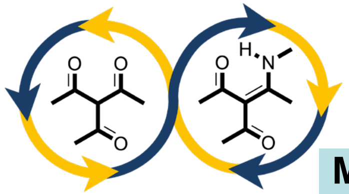
LBNL condenses 2 TKs or BKDLs with ≥3 diacids, must be regiospecific by NMR



				
Adipic Acid (n=0)	90% (<20-g scale; discovery chemistry)	N.D.	91% (<20-g scale; **LCA-informed chemistry**)	N.D.
Suberic Acid (n=1)	93% (<20-g scale; discovery chemistry)	N.D.	90% (<20-g scale; **LCA-informed chemistry**)	N.D.
Sebacic Acid (n=2)	91% (**50-g** scale; discovery chemistry)	84% (<20-g scale; discovery chemistry)	85% (**150-g** scale; **LCA-informed chemistry**)	65%* (<20-g scale; discovery chemistry)

* unoptimized yields



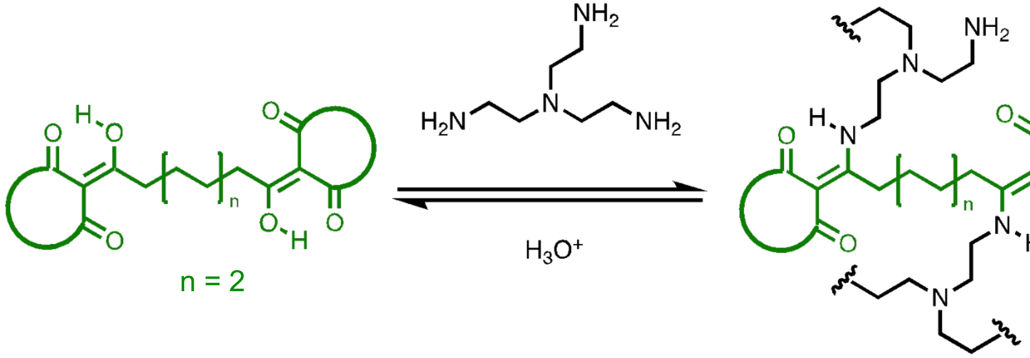


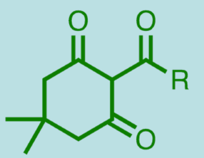
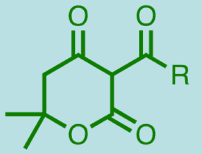
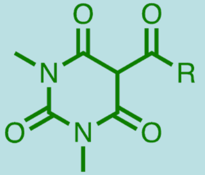
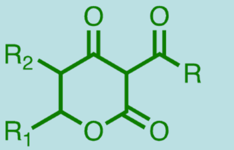
4- Progress and Outcomes

BETO review

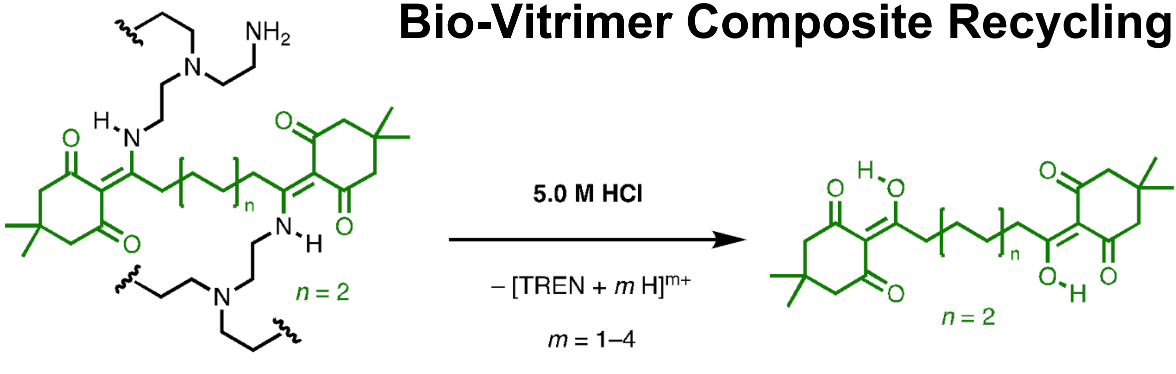
Milestone 3.2.1: Demonstrate a 25-g vitrimer batch size with >25% biomass content and <3% VOC content	Polymerization of biomass-derived triketone and amine monomers at LBNL, show <3% mass loss at 150 ˚C by TGA
Milestone 3.4.1: Demonstrate chemical depolymerization molded vitrimer substrates ≥1 g	LBNL chemically recycles >10 vitrimers with 0–30% w/w filler, >90% TK recovery in >90% purity by NMR

Bio-Vitrimer Synthesis and Recycling



				
Synthesis	Scale: 25 g VOC: 0.61% Bio-content: 81%	Scale: 2 g VOC: 0.03% Bio-content: 81%	Scale: 25 g VOC: 0.01% Bio-content: 82%	Scale: 2 g VOC: 0.5% Bio-content: 83%
Recycling	Scale: 5 g TK Yield: 92% Purity: 100%	Scale: 2 g TK Yield: 99% Purity: 96%	Scale: 5 g TK Yield: 88% Purity: 100%	Scale: 2 g TK Yield: 96% Purity: 96%

Bio-Vitrimer Composite Recycling



Carbon Black 			**Silica** 		
10% w/w	20% w/w	30% w/w	10% w/w	20% w/w	30% w/w
Scale: 1 g TK Yield: 72% Purity: 100%	Scale: 1 g TK Yield: 87% Purity: 100%	Scale: 1 g TK Yield: 90% Purity: 100%	Scale: 1 g TK Yield: 90% Purity: 100%	Scale: 1 g TK Yield: 83% Purity: 100%	Scale: 1 g TK Yield: 90% Purity: 100%

4- Progress and Outcomes

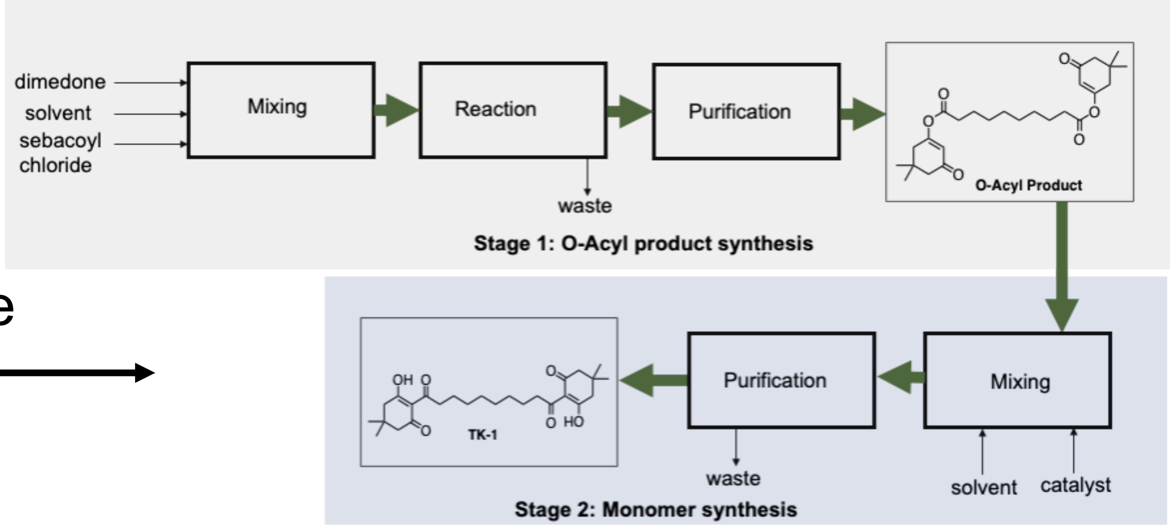
BETO review

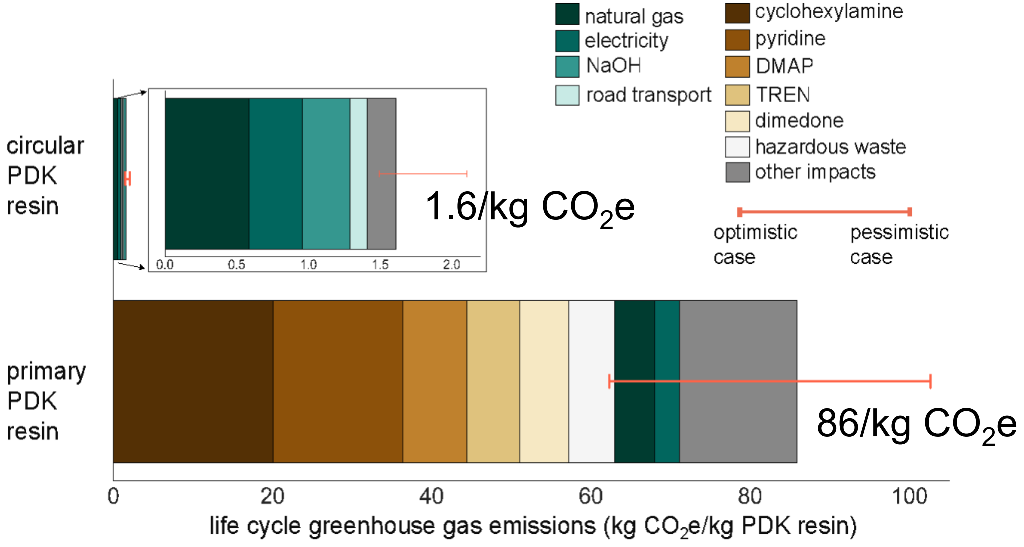
Q4	M2.4.1	UCB reports titer, rate, and yield (TRY) from 50 mL shake flasks in three hosts. Report the best host based on these parameters.	√
BP2	Go/No-Go	Demonstrate a titer of > 1 g/L of β-keto-δ-lactones (BKDLs) in ~~an optimized strain at > 1 L scale in fed-batch fermenter.~~	√

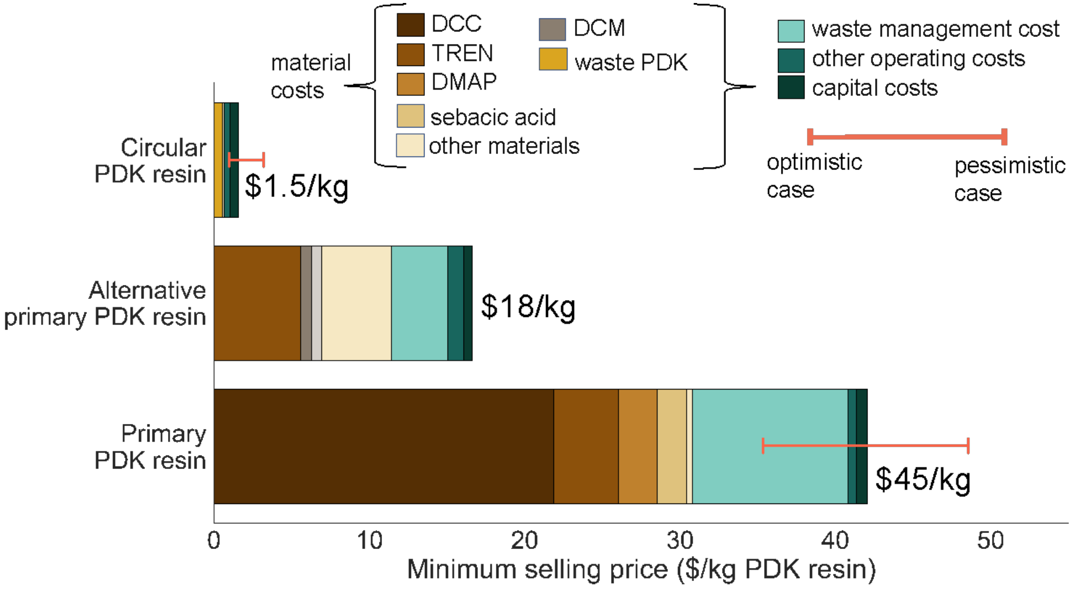
		E. coli	*S. albus*	*P. putida*	*R. toruloides*
Task 2.1: DNA Design And Assembly ➢ ~ 45 % success rate in DNA assembly	Introduce *bkdl* genes	√	√	×	√
Task 2.4: Host Selection And Strain Development ➢ ~ 50 % success rate in *bkdl* integrations	Screen different species	√	√	×	×
	Integrate *sfp* for polyketide synthase function	√	×	√	√
	Integrate precursor pathways for providing building blocks	√	×	√	×
	Knock-out degradation pathways for products accumulation	√	×	×	×
Task 2.5: Host Selection And Strain Development ➢ 4 g/L BKDL production with cellulosic sugars ➢ 0.09 g / g cellulosic glucose	Titer of BKDLs production	0.77 g/L √	78 mg/L √	NA	4.27 g/L √

4- Progress and Outcomes

- Technoeconomic analysis & life-cycle GHG assessment of baseline PDK synthesis
- TEA/LCA-informed development of alternative DCC and DMAP-free chemistry
- Preliminary analysis of BKDL production from cellulosic sugars







Source: Vora et al. *in revision*

Summary

1. Management: PI Keasling manages the project. Keasling Group, Persson Group, Helms Group and Scown Group take specific responsibilities in Tasks and Milestones. Risks on commercialization can be mitigated by bioproduction and circularity of vitrimers.

2. Approach:
- Identify BKDL targets via screens of hydrolysis activation barrier for using DFT and MD
- Close the loop in Design–Build–Test–Learn for BKDL production with high-throughput platform.
- Integrate BDKLs into Bio-Based PDK resins and validate predictions for performance and recyclability.
- Model of process chemistry and assess impact of bio-products on sustainability targets for circularity

3. Impact: Vitrimers can be synthesized from sustainable resources with a reduced environmental impact. Vitrimers can be predicted and designed to be recyclable and non-toxic. Techno-economic analysis and life-cycle assessment informs best path to commercialization.

4. Progress and Outcomes:
- Demonstrated PDK vitrimer production with >80 % bio-content and >95% resource recovery.
- Demonstrated the engineering of microorganisms to produce 4.27 g/L BKDL feedstocks.
- Built model for baseline vitrimer synthesis and preliminary analysis of BKDL production.
- Built model for prediction of polymerization and depolymerization of PDK vitrimers from BKDLs.

Quad Chart Overview

Timeline
- 07/01/2019
- 06/30/2022

	FY20 Costed	Total Award
DOE Funding	$351,839	$1,017,861
Project Cost Share	$64,658	$499,466.00

Project Partners*
Lawrence Berkeley National Laboratory

Project Goal
Design and develop infinitely recyclable and therefore closed-loop polymeric bio-based materials, specifically focusing on a new class of polymers called vitrimers that combine the processing and recycling ease of thermoplastics with the performance advantages of thermosets.

End of Project Milestone
- Demonstrate 1g/L of C6 diacid in fed-batch fermenter.
- Demonstrate PDK vitrimer platform technology readiness wrt formulation and circularity: both chemical recyclability and scrap recovery for 10-g vitrimer samples with >75% biomass content, <1% VOC content, 0–30% w/w filler.

Funding Mechanism
DE-FOA-0001916, Topic 3a. Performance Advantaged Bioproduct Identification

*Only fill out if applicable.

Additional Slides