DOE Bioenergy Technologies Office (BETO) 2023 Project Peer Review

FERMENTATIVE PRODUCTION OF TULIPALIN A: A NEXT-GENERATION, SUSTAINABLE MONOMER THAT DRASTICALLY IMPROVES THE PERFORMANCE OF pMMA

April 7th 2023
Biochemical Conversion and Lignin

Aaron Korkegian, PhD
ARZEDA

Project Overview

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THE ENVIRONMENTAL IMPACT OF CHEMICALS AND PLASTICS IS A MAJOR CHALLENGE

The Solution is the Development of Sustainable Green Alternatives That Improve Performance

SUSTAINABILITY & HIGHER VALUE PRODUCTS FOR BIOREFINING

Biological Production of Acrylate from Lignocellulosics

Petroleum: non-sustainable non-renewable resource

CO_2

Sequestered Carbon Release

Petrochemical Plant

Lignocellulosics: sustainable, renewable

CO_2

Captured Carbon

Fermentation

Acrylate Plastics

TULIPALIN A

A Plant-Based Sustainable Acrylate

Unknown metabolic pathway

Biosynthesi
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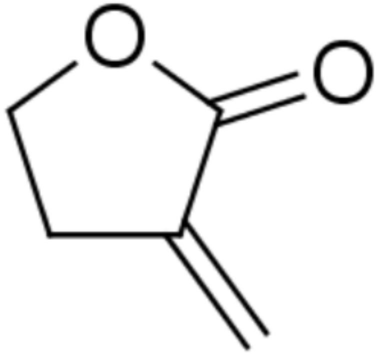

Tulipalin A
(a-Methylene Butyrolactone, MBL)

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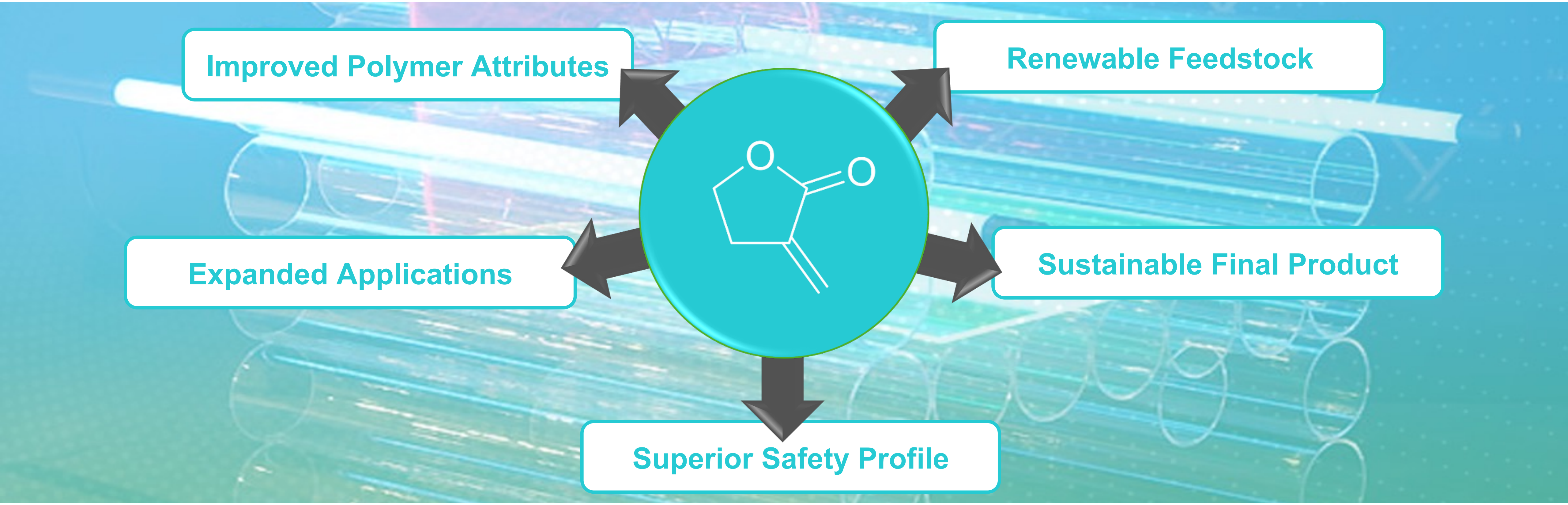
TULIPALIN A: A PERFORMANCE IMPROVING SUSTAINABLE ACRYLATE FOR MATERIALS

Replacement for Petro-derived methyl-methacrylate (MMA)



As a monomer at scale, Tulipalin A is:

- Performance advantaged to MMA
- Price competitive to MMA
- Process compatible for polymerization with MMA

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HOW IS TULIPALIN MADE TODAY?

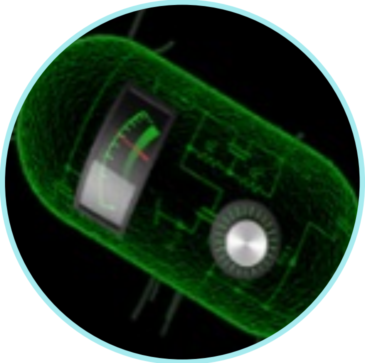
Current Issues and limits

Current commercial production routes are limited…

- Extracted from tulips (Economically infeasible)
- Chemical process (Economically not viable and carbon intense)

…and suffer from cost-of-manufacturing and volume issues

- Price: **>$1000/kg**
- Volume: **>1kg** unavailable commercially



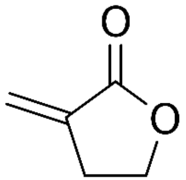
No commercially viable biological production process

- The way that tulips make Tulipalin A is unknown
- No known microorganism produce Tulipalin A naturally

ARZEDA PLATFORM FULLY SUPPORT DEVELOPMENT CYCLE

Pathway and Protein Design, Protein Expression, Strain Engineering, Bioprocess Development

Target Product



CLOUD-BASED DESIGN

PATHWAY DESIGN [SCYLAX™]

Evaluates Millions of Potential Pathways

PROTEIN DESIGN [ARCHYTAS™]

5k

CPUs

HIGH-THROUGHPUT PROTEIN TESTING

DNA BUILD

TWIST

teselagen

LABCYTE

PROTEIN ASSAY

5,000

proteins/enzymes expressed + assayed

MANUFACTURING PROCESS DEVELOPMENT

EXPRESSION STRAIN ENGINEERING

16x 0.5L
8x 1L
5x 10L
fermenters

DOWNSTREAM PROCESSING

Kg Scale

CMO Partner*

*proven engagement at 1000L scale production of Arzeda products

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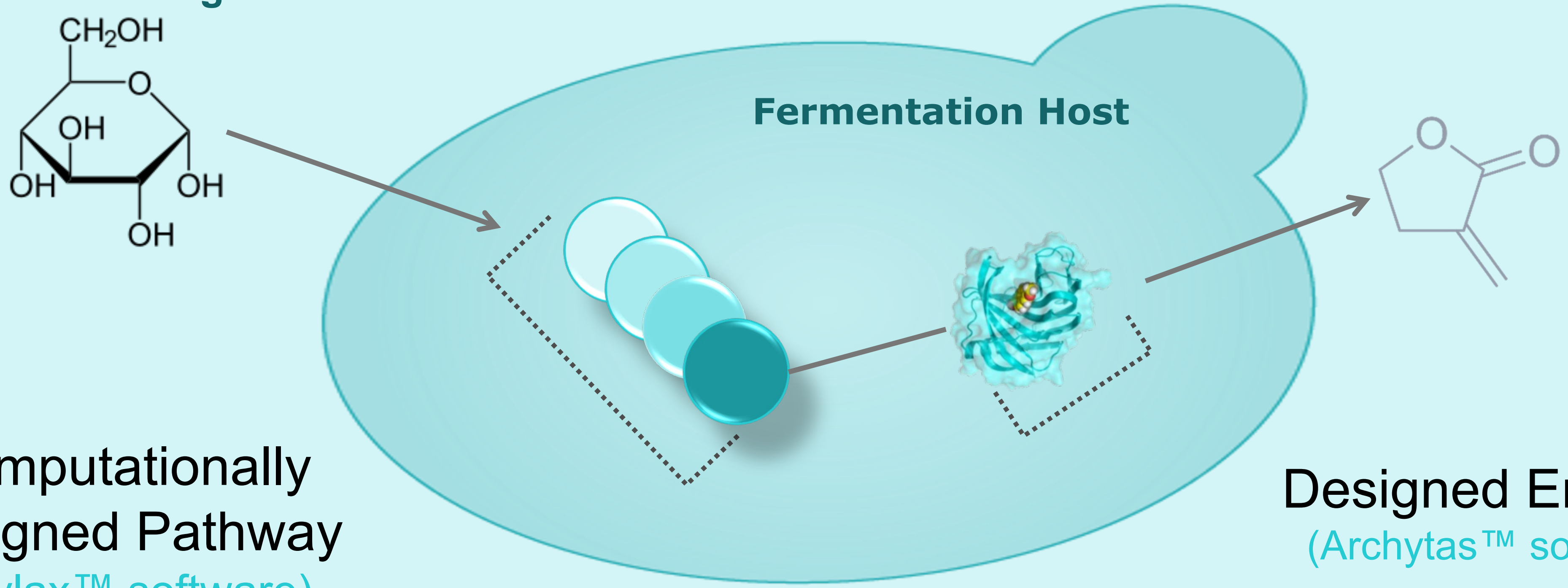
PROJECT OBJECTIVE

Use Synthetic Biology To Create A Microbial Strain Fermenting Lignocellulosics to Tulipalin A

Bio-derived sugars



Tulipalin A

Computationally Designed Pathway
(Scylax™ software)

Designed Enzyme
(Archytas™ software)

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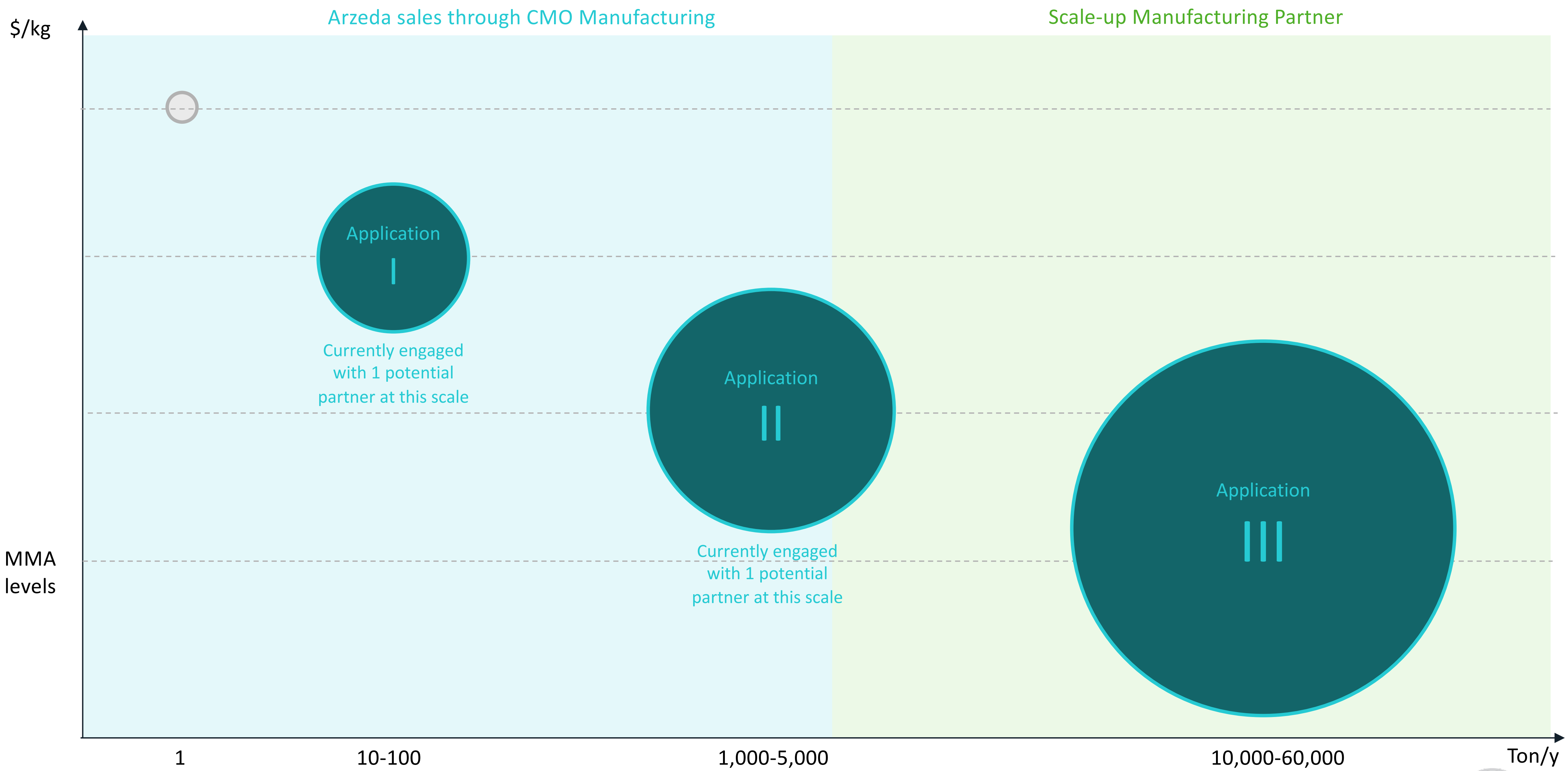
ENABLING TULIPALIN FERMENTATION



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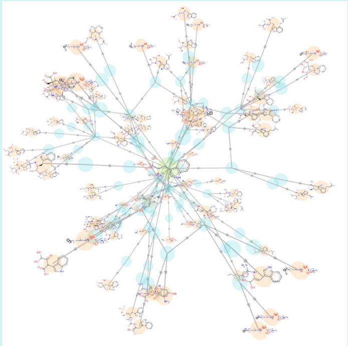
RISKS

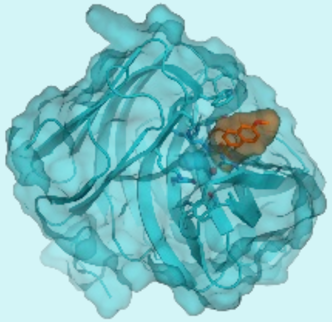
Enumeration of Project Challenges

Pathway Design



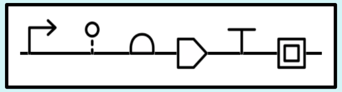
Can A Pathway Be Identified For Production of Product from A Biological Intermediate

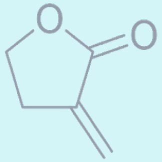
Enzyme Design Activity and Specificity



Can Sufficient Enzyme Activity/Specificity be Engineered

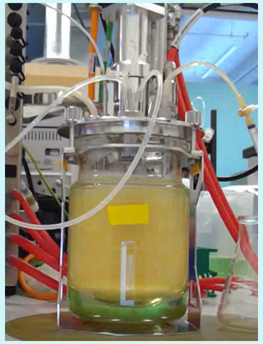
Proof-of-Concept Strain





Can Product Be Produced in Strain

Production Strain & Fermentation Process

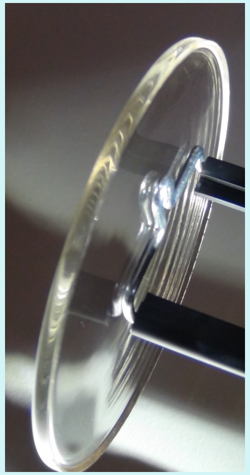


Can Sufficient Titers be Reached for Product Purification/Testing

Downstream Process Development

Can Sufficient Product Purity Be Reached From Processing Fermentation Broth

Market Risk



Does Final Product Have Desirable/Marketable Properties

Project Approach, Progress and Outcomes

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PROJECT STRUCTURE - PROTEIN DESIGN & SYNBIO FOR ORGANISM ENGINEERING

Integrating Computational Pathway Design, Protein Design, Strain Construction & Process Development

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- Pathway Design
- Enzyme Design and Optimization
- Strain Engineering
- Fermentation Process & DSP
- Polymerization

PNNL (Leo Fiefield)

Pacific Northwest NATIONAL LABORATORY

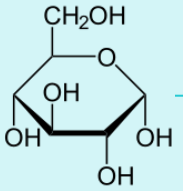
- Commercial MMA Monomer and PMMA Properties Recapitulation
- Arzeda MBL Monomer and PMBL Properties Testing

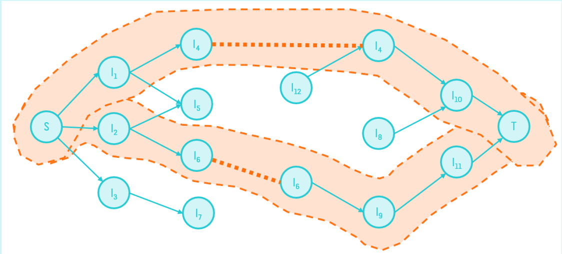
APPROACH: VALIDATING AND IMPROVING ENZYME ACTIVITY/SPECIFICITY

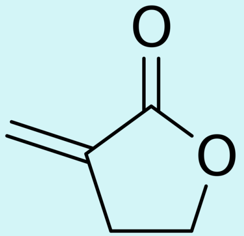
Design, Build, Test for de novo Enzymatic Pathway and Desired Enzyme Properties

Pathway Design (Scylax™)

Bio-derived sugars



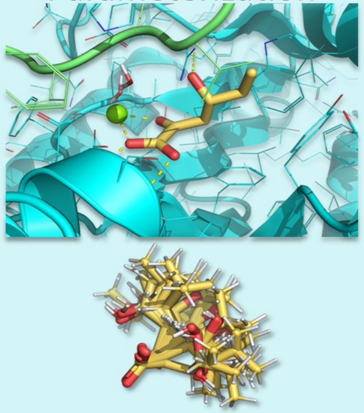




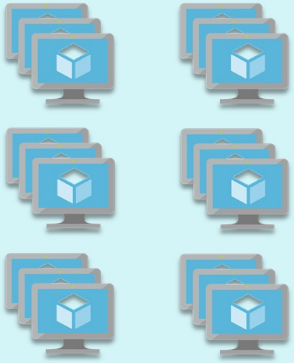
Product

Enzyme Design (Archytas™)

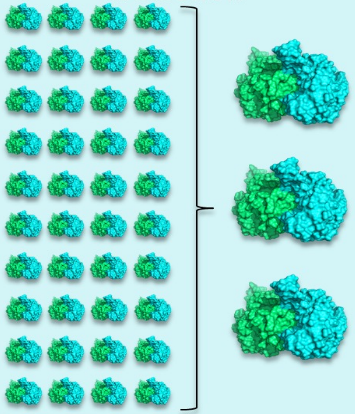
Parameterization



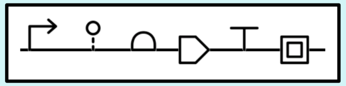
Computation

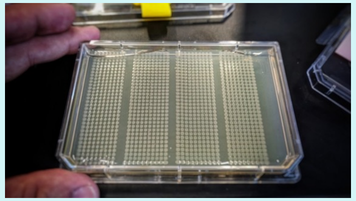


Selection

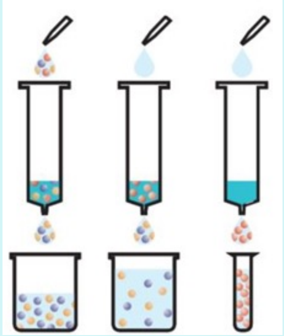


Build





Test



Produce and test enzymes *in vitro*

Milestone: Produce Product from Pathway in vitro for at least 2 Distinct Pathways

Milestone: Enzymes in pathway reach sufficient specificity/productivity defined by production goals

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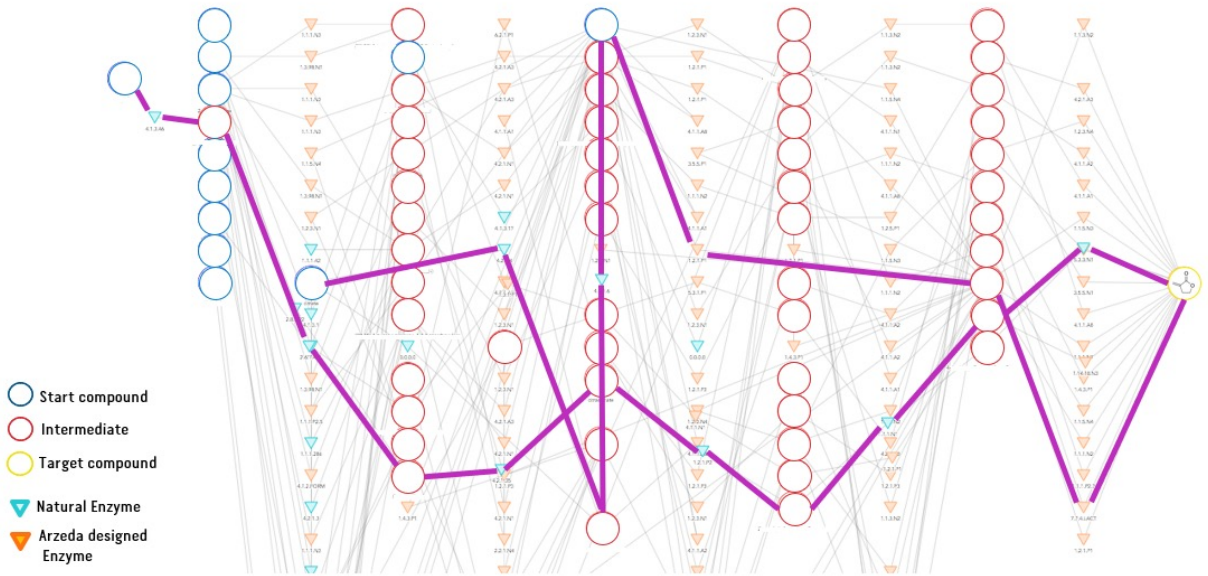
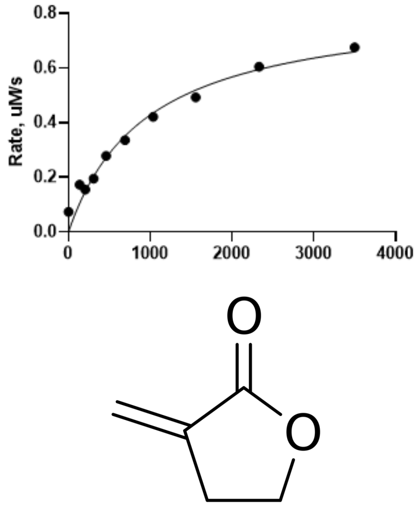
OUTCOME: VALIDATING AND IMPROVING ENZYME ACTIVITY/SPECIFICITY

Design, Build, Test for de novo Enzymatic Pathway and Desired Enzyme Properties



Pathways Ranked by Feasibility Assessment

10 Potential valid pathways identified



Demonstrated full conversion of starting metabolite to product using purified enzymes *in vitro* for 2 pathways

Produce and test enzymes *in vitro*

Milestone: Produce Product from Pathway in vitro for at least 2 Distinct Pathways: ***Achieved***

Milestone: Enzymes in pathway reach sufficient specificity/productivity defined by production goals: ***Achieved***

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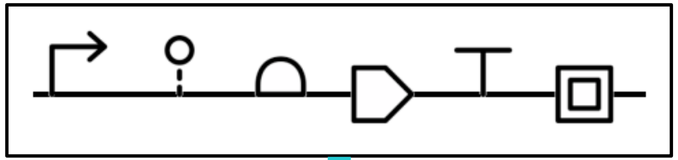
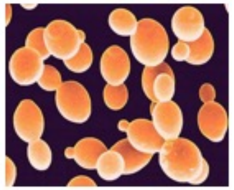
DEVELOP PROOF OF CONCEPT STRAIN

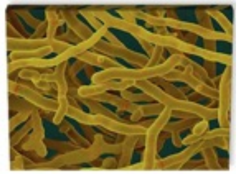
Design, Build, Test for de novo Enzymatic Pathway and Desired Enzyme Properties



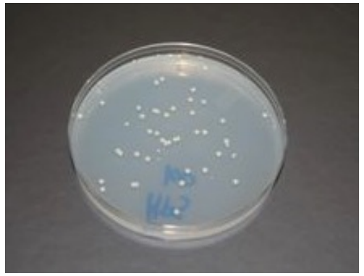
Bacteria

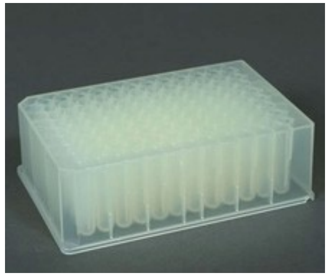


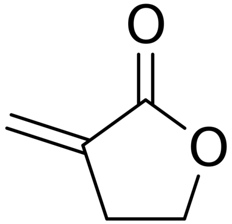
Yeast



Filamentous Fungi







Strain Produces Detectable Amount of Product

Milestone: Strain Produces Sufficient Quantities of Intermediate Metabolite

Milestone: Strain Produces Detectable Product from 2 Distinct Pathways

Milestone: Strain Produces Detectable Product from Lignocellulosic Hydrolysate

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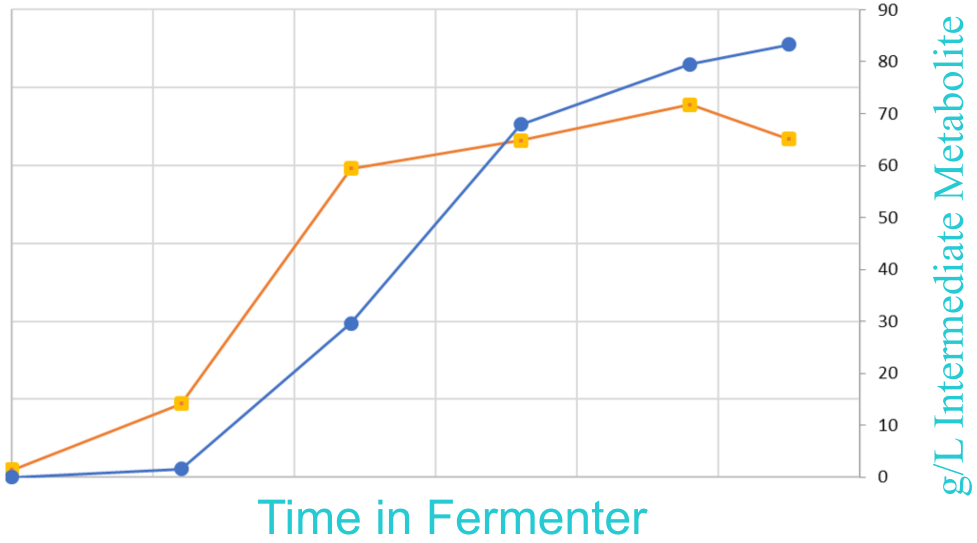
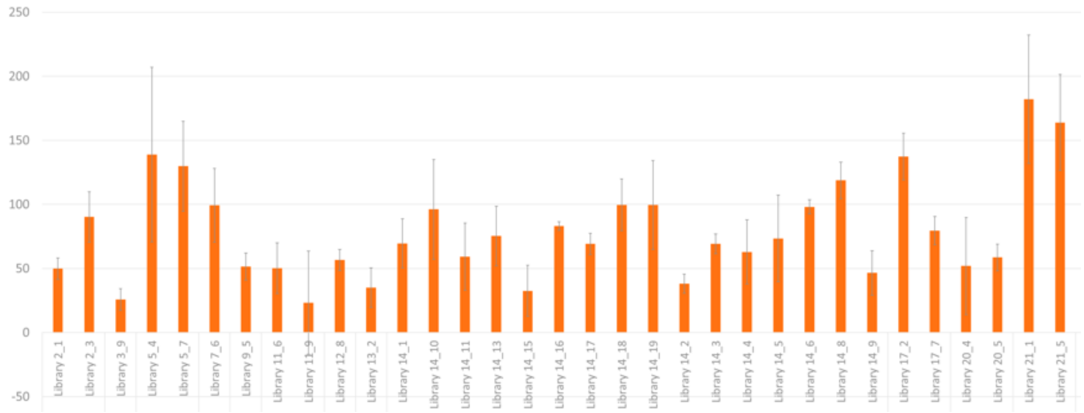
OUTCOME: DEVELOP PROOF OF CONCEPT STRAIN

Design, Build, Test for de novo Enzymatic Pathway and Desired Enzyme Properties



Engineered strain able to produce up to **80 g/L of Intermediate metabolite** in fermentation



Initial strains developed based on two pathways both showed low level but significant production of product over the base strain.

After some refinement Pathway 5 produced **150 mg/L of product**

Strain Produces Detectable Amount of Product

Milestone: Strain Produces Sufficient Quantities of Intermediate Metabolite: ***Achieved***

Milestone: Strain Produces Detectable Product from 2 Distinct Pathways: ***Achieved***

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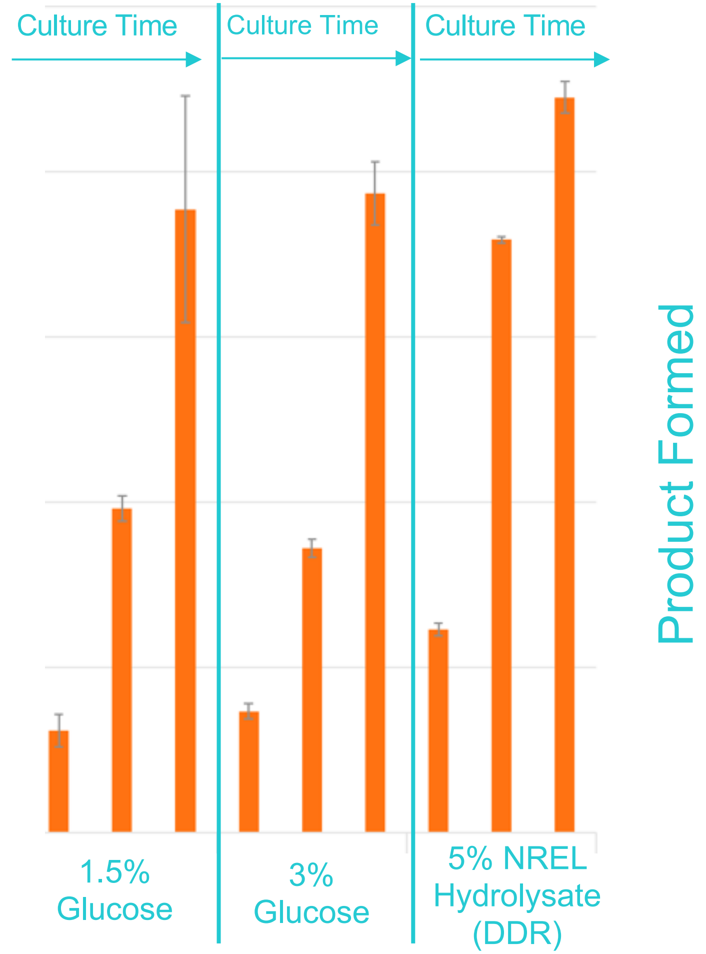
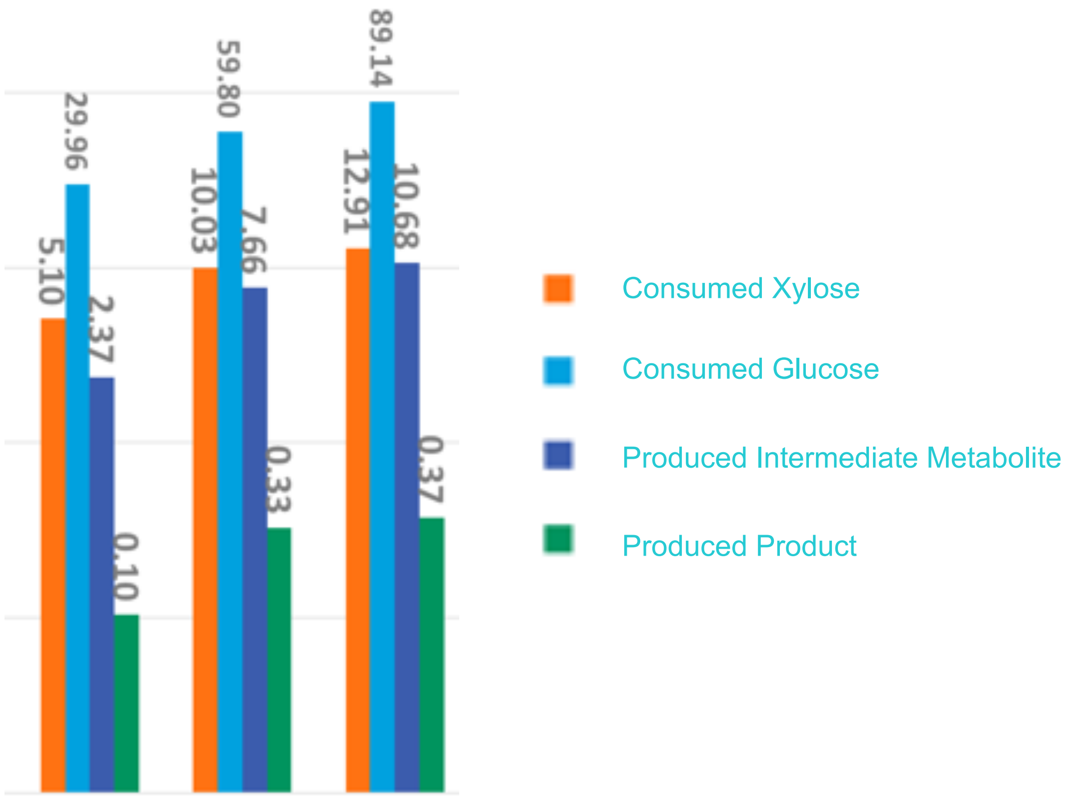
OUTCOME: DEVELOP PROOF OF CONCEPT STRAIN

Strain Produces Product Utilizing Lignocellulosic Hydrolysate as Feedstock





Strain Produces Detectable Amount of Product

Milestone: Strain Produces Detectable Product from Lignocellulosic Hydrolysate: ***Achieved***

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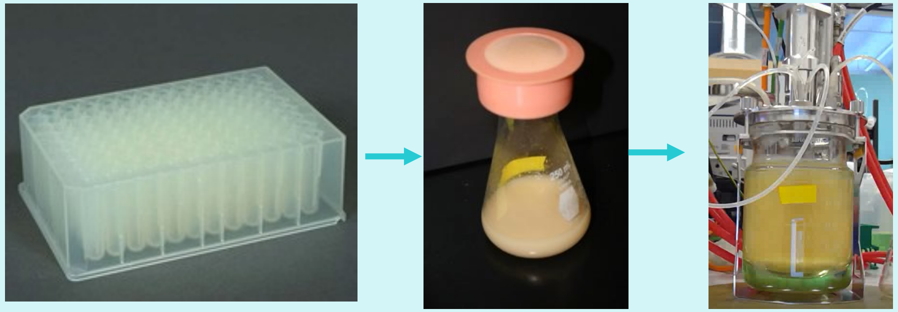
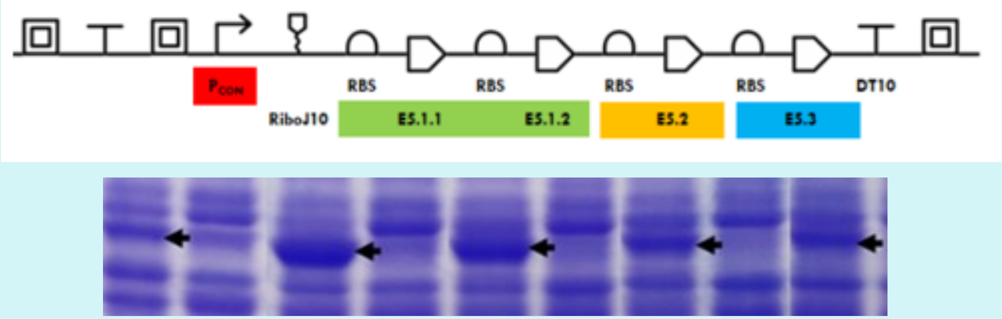
APPROACH: STRAIN/ENZYME OPTIMIZATION

Improve Titers and Reach Production Titers Sufficient for Scaling to Kg Production

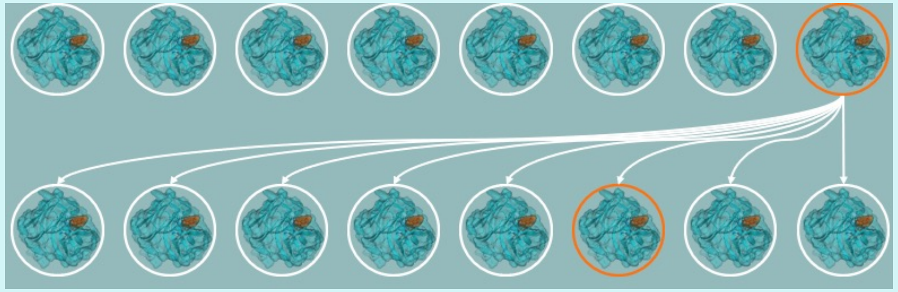
Culture Scaling



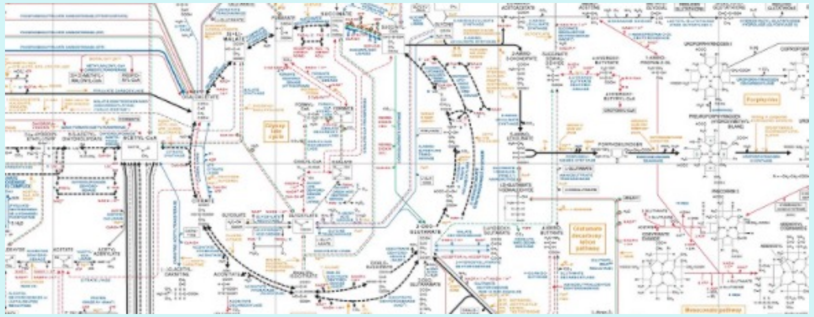
Enzyme Expression Refinement



Enzyme Iterative Improvement



Metabolic Engineering



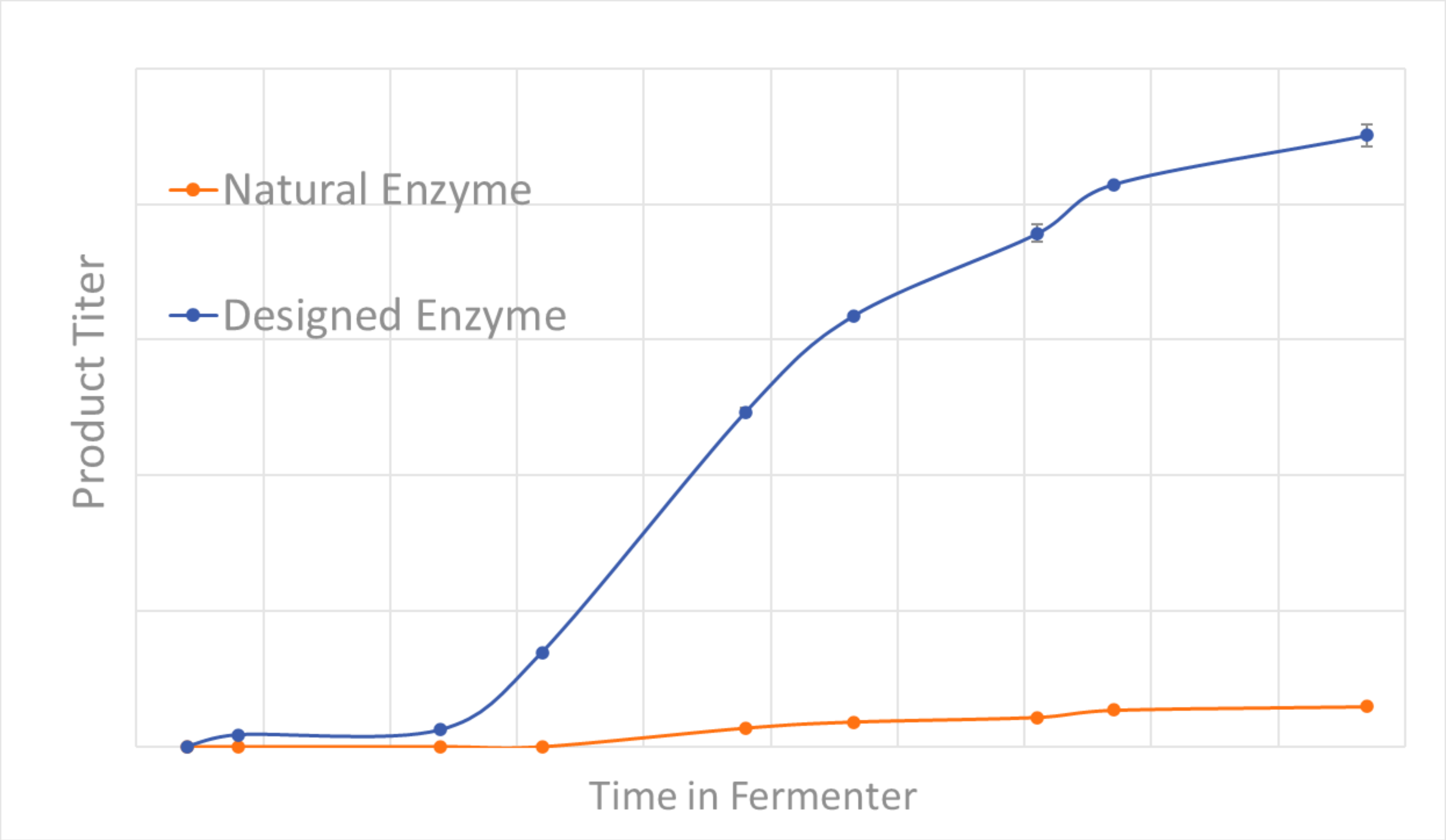
Strain Produces Detectable Amount of Product

Milestone: Strain Demonstrates Improved titers reaching Intermediate Production Goal

Go/No-Go Decision: Strain Reaches Titer sufficient for Kg scale production

PROGRESS: STRAIN/ENZYME OPTIMIZATION

Improvement to Titers through Enzyme Design



Strain Produces Detectable Amount of Product

Milestone: Strain Demonstrates Improved titers reaching Intermediate Production Goal

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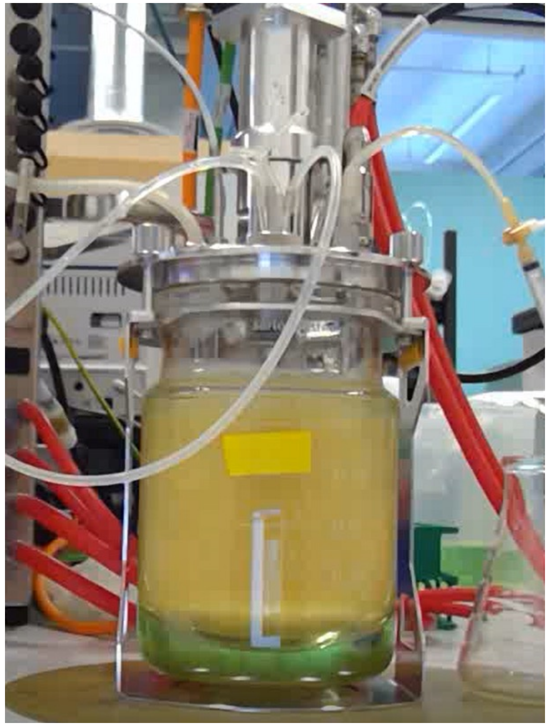
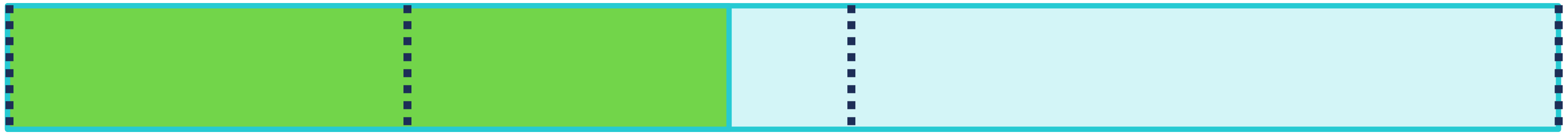
PROGRESS: STRAIN/ENZYME OPTIMIZATION

Reaching Titers of 5 g/L



- Introduction of pathway into host organism
- Current strain capable of production titers of 5 g/L
- Working on iterative refinement of enzyme properties and expression to improve yield and increase ratio of product to intermediate metabolite



Detectable Product

1 g/L intermediate production milestone

8 g/L Kg production possible

20 g/L Initial Market Viability

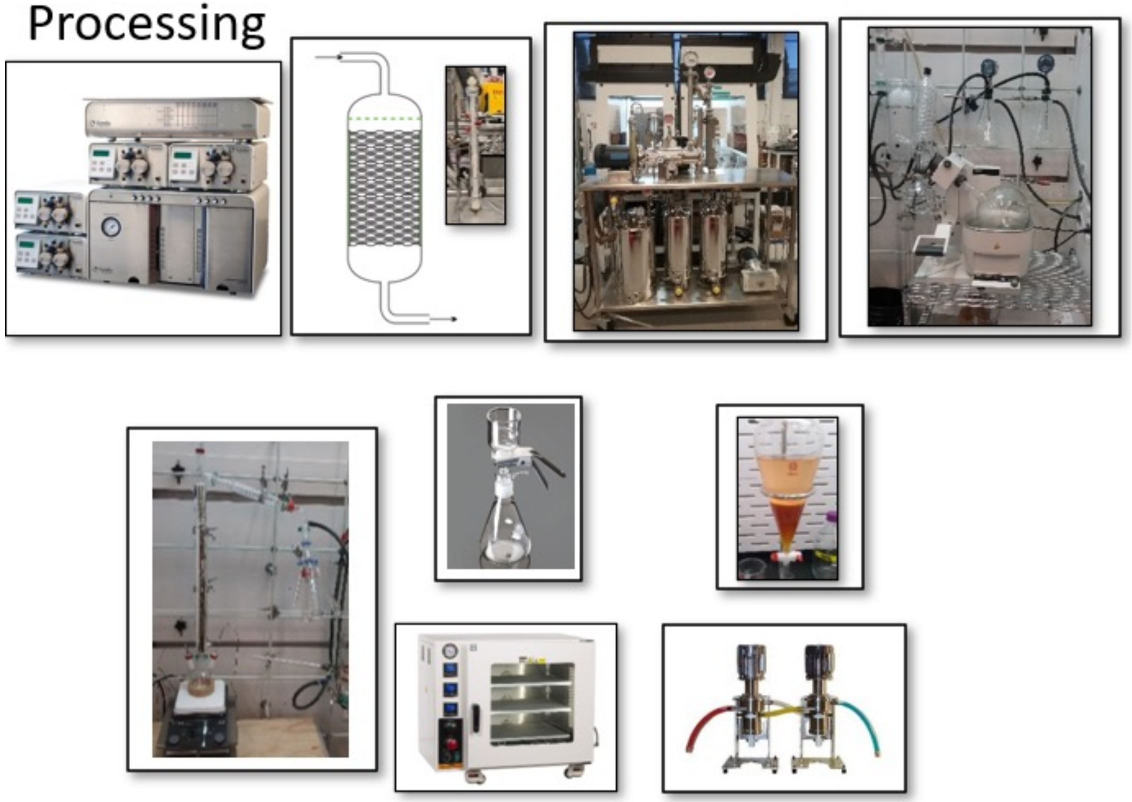
Achieved** intermediate production target milestone for **BP2 > BP3 Go Decision
Have not encountered insurmountable issue with production
*Titers and Ratios Required for Economic Kg Production: **In Progress***

APPROACH: DOWNSTREAM PROCESSING AND PROPERTIES TESTING

Demonstrate Ability to Produce High Grade MBL and Polymer from Mock Fermentation Broth

Crude Broth

Processing



Analytics

Agilent Infinity 1260 LC II
UV & RI Detection

Shimadzu GCMS QP-2010
MS & FID Detection

Mettler Toledo UV-5
Spectrophotometer

Produce MBL at gram and then Kg scale from Mock Fermentation Broth

Milestone: Produce at least 100g of polymer grade (>99% pure) MBL from mock fermentation broth

Milestone: Demonstrate Desirable Properties

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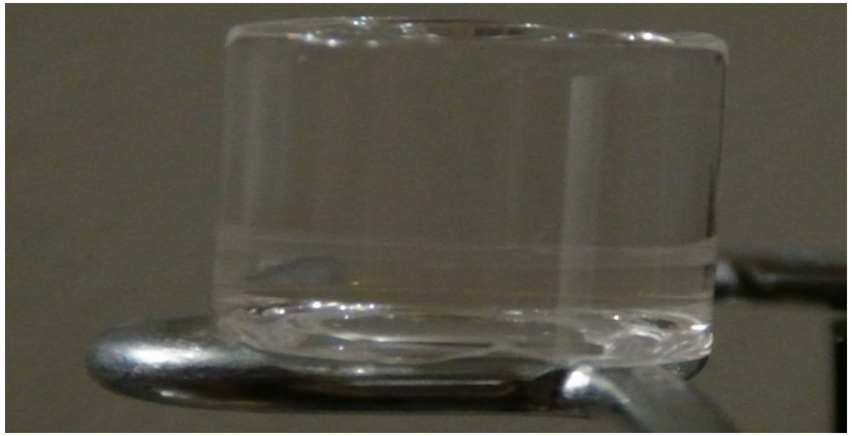
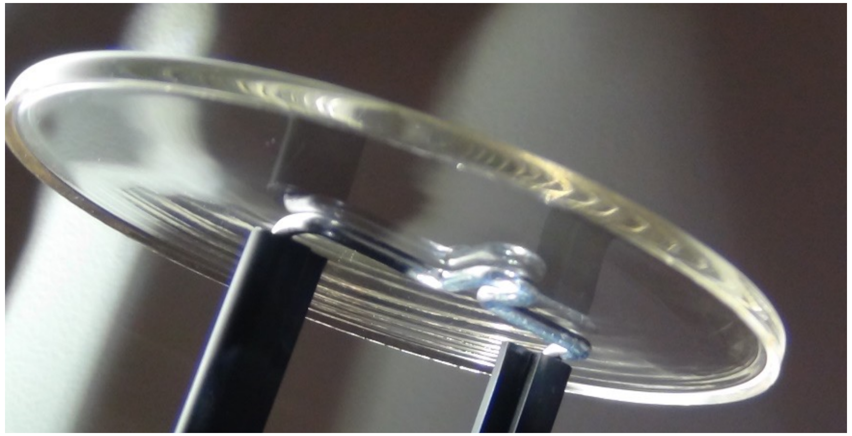
OUTCOME: DOWNSTREAM PROCESSING AND PROPERTIES TESTING

Demonstrate DSP and Polymerization





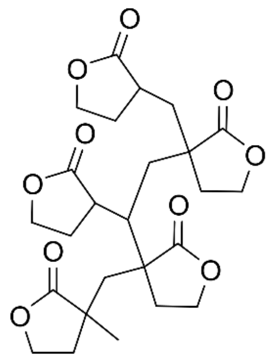
Produce MBL at gram and then Kg scale from Mock Fermentation Broth

Milestone: Produce at least 100g of polymer grade (>99% pure) MBL from mock fermentation broth:
Achieved

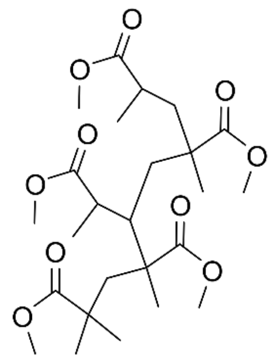
OUTCOME: DOWNSTREAM PROCESSING AND PROPERTIES TESTING

Value Proposition as A Performance Improving Monomer/Additive for Polymers

Property	Measure	Literature PMBL	Arzeda PMBL	PMMA
Thermal	Glass Transition Point T_g (°C)	194°C/195°C	195°C	105°C
Mechanical	Modulus of Elasticity (MPa)	1999/3439	5972	2855
	Tensile Strength (MPa)	36.7/62.7	72.7	70
	Elongation at Break	1.3%/6.5%	1.3%	2.5%
Optical	Light Transmission	N/A	> 88%	92%
Solvent Resistance	Toluene, 30 Day Immersion at 20°C	N/A	Pass	Fail



PMBL



PMMA

Produce MBL at gram and then Kg scale from Mock Fermentation Broth

Milestone: Demonstrate Desirable Properties: ***Achieved***

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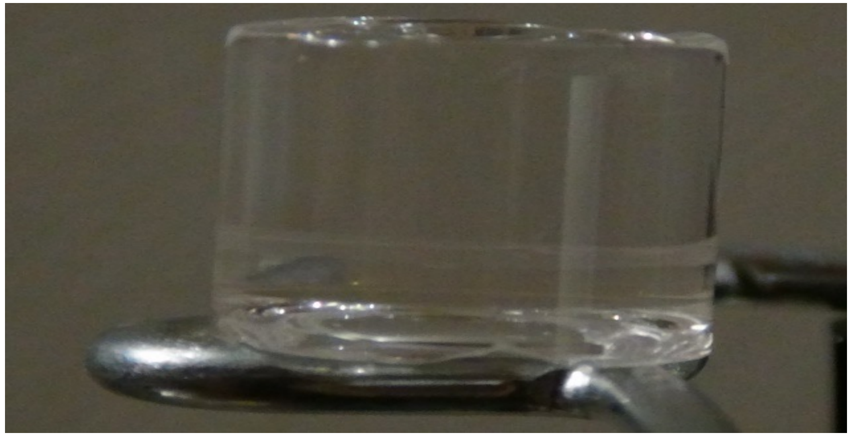
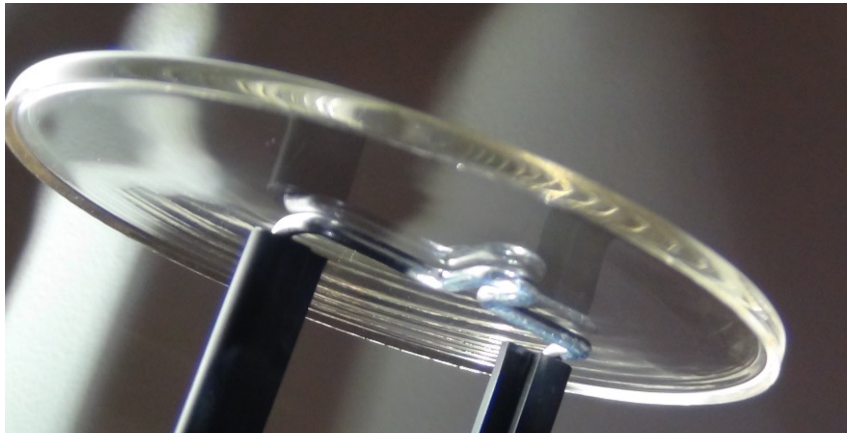
END OF PROJECT GOAL

Bio-Based MBL Produced From Lignocellulosics





End of Project Goal

Bio-based MBL Produced by Strain, Processed and Polymerized into at least 1 KG of Acrylate Polymer with Confirmed Desirable Properties: ***In Process***

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Impact

DISSEMINATION OF RESULTS

CONFERENCES PRESENTATIONS:

GRC Enzymes & Metabolic Pathways 2019
American Chemical Society, Spring Meeting 2019 and 2021
SIM Fuels & Chemicals Symposium 2019
University Stuttgart, Germany 2020
American Society of Biochemistry and Molecular Biology, Discover BMB 2023

PATENTS

Patent Applications Filing in Process

PUBLIC OUTREACH

Professor Dave Explains Public Science Outreach Channel (Youtube - 2.4M subscribers):
30-minute Two Part Video on Our Work – Synthetic Biology and Materials Science Part 1 and 2

COMMERCIALIZATION

Over 2Kg of Tulipalin was shipped to 15 chemicals and materials companies for testing
LOI for manufacturing and market development partnerships in negotiation

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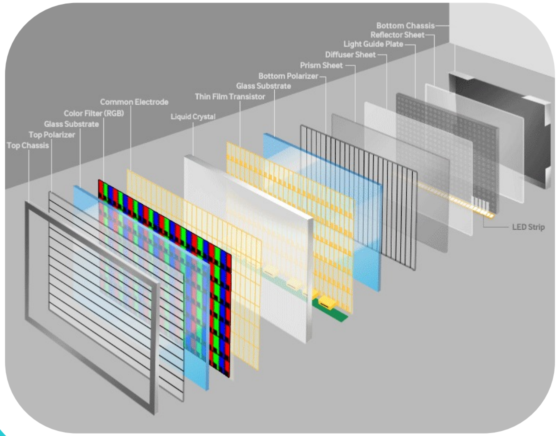
TULIPALIN A: PERFORMANCE IMPROVEMENT TO DISPLACE PMMA AND POLYCARBONATES

Business Development Efforts Have Uncovered 5 Different Areas for MBL Applications for Materials

TRANSPARENT PLASTIC CASTS (Application 2/3)

- Improved weathering and decreased discoloration
- Decreased scratch and marring
- Higher solvent resistance
- Automotive “lightweighting” by replacing glass or poly-carbonate

DISPLAYS (Application 2 / 3)



- Excellent light transmission
- Thinner light guides, diffusers, etc. due to higher refractive index
- Far greater heat resistance

LENSES (Application 2 / 3)

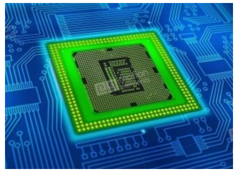
- Thinner and lighter lenses due to higher refractive index
- Less discoloration
- Higher scratch resistance

Cosmetics (Application 2)

- Biobased Acrylate
- Acrylate rheology modified

PHOTORESIST (Application 1)

- Increased Etch Resistance



Arzeda.

Summary

Arzeda.

SUMMARY

Enzymatic Pathways Designed and Validated Capable of Producing Product *in vitro*

Strain Implementing Designed Pathways Capable of Producing Product

Strain Improvements have Increased Titer to 5 g/L

Scalable Downstream Process Can Achieve Polymer Grade >99.9% MBL

MBL Polymer Demonstrate Significant Improvement Over Petro-derived MMA

Potential Industrial Partners Have Tested and Expressed Interest In Product Properties

End of Deck

Arzeda.

Quad Chart Overview (Competitive Project)

Timeline
- Start Oct 1st 2018
- End Sept 30th 2023

	Total Award	Total Spent
DOE Funding	$1.7 million	*$1.2 million*
Project Cost Share	$502k	*$1.6 million*

Project Partners
- ARZEDA
- PNNL

Project Goal
The goal of this project is to develop a strain capable of fermentative production of Tulipalin A from lignocellulosics at titers that are viable for kilogram production

End of Project Milestone
bio-based MBL produced by Escherichia coli fermentation of lignocellulosics and processed with DSP 2.0 is incorporated into at least 1KG of acrylate polymer material and improves Tg by at least 50%

Funding Mechanism
FOA-001916 DOE BEEPS Performance Advantaged Bioproducts 2018