DOE Bioenergy Technologies Office (BETO)
2021 Project Peer Review

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

March 10th 2021
Performance Advantaged BioProducts Review Panel

Alexandre Zanghellini, PhD
ARZEDA

Project Overview

THE ENVIRONMENTAL IMPACT OF CHEMICALS AND PLASTICS IS A MAJOR CHALLENGE

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

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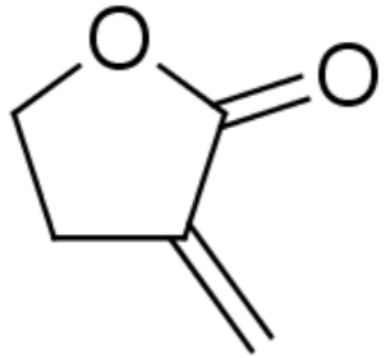
TULIPALIN A

A Plant-Based Sustainable Acrylate

Unknown metabolic pathway

Biosynthesized



Tulipalin A
(a-Methylene Butyrolactone, MBL)

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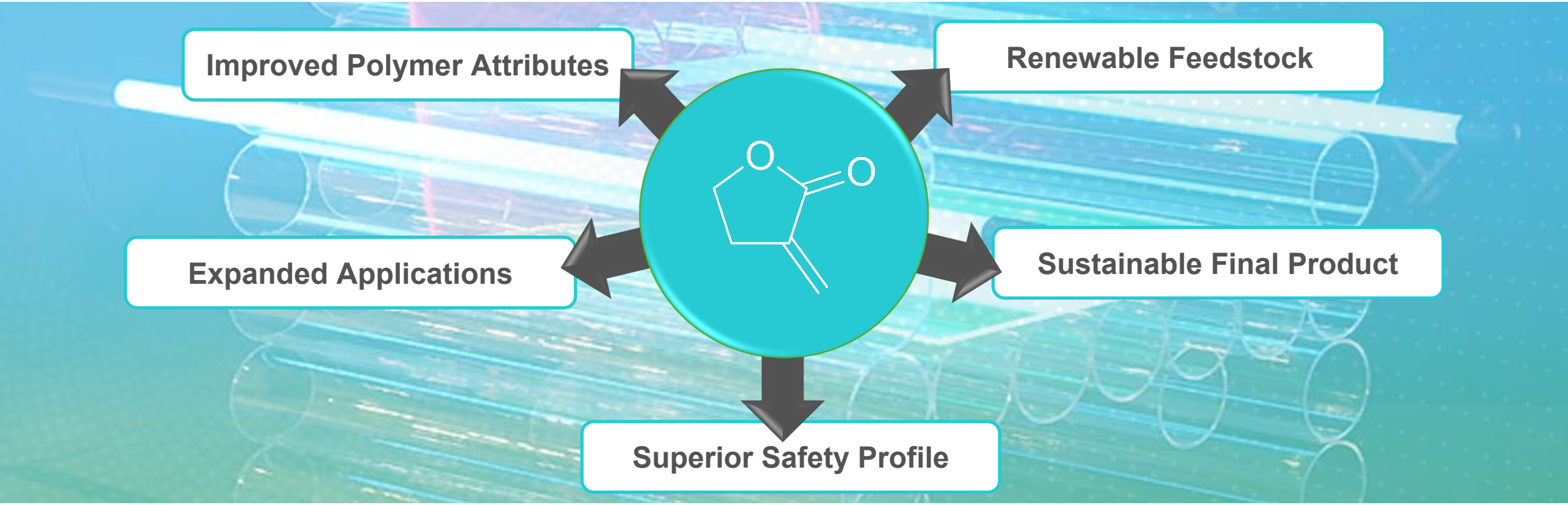
TULIPALIN A: A PERFORMANCE IMPROVING SUSTAINABLE ACRYLATE FOR MATERIALS

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?

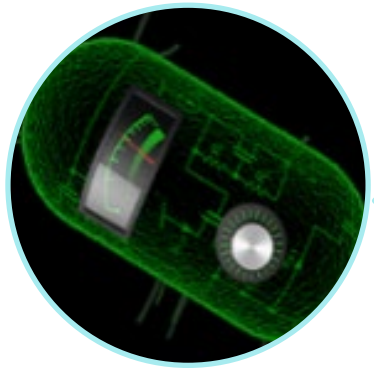
Current Issues and limits

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

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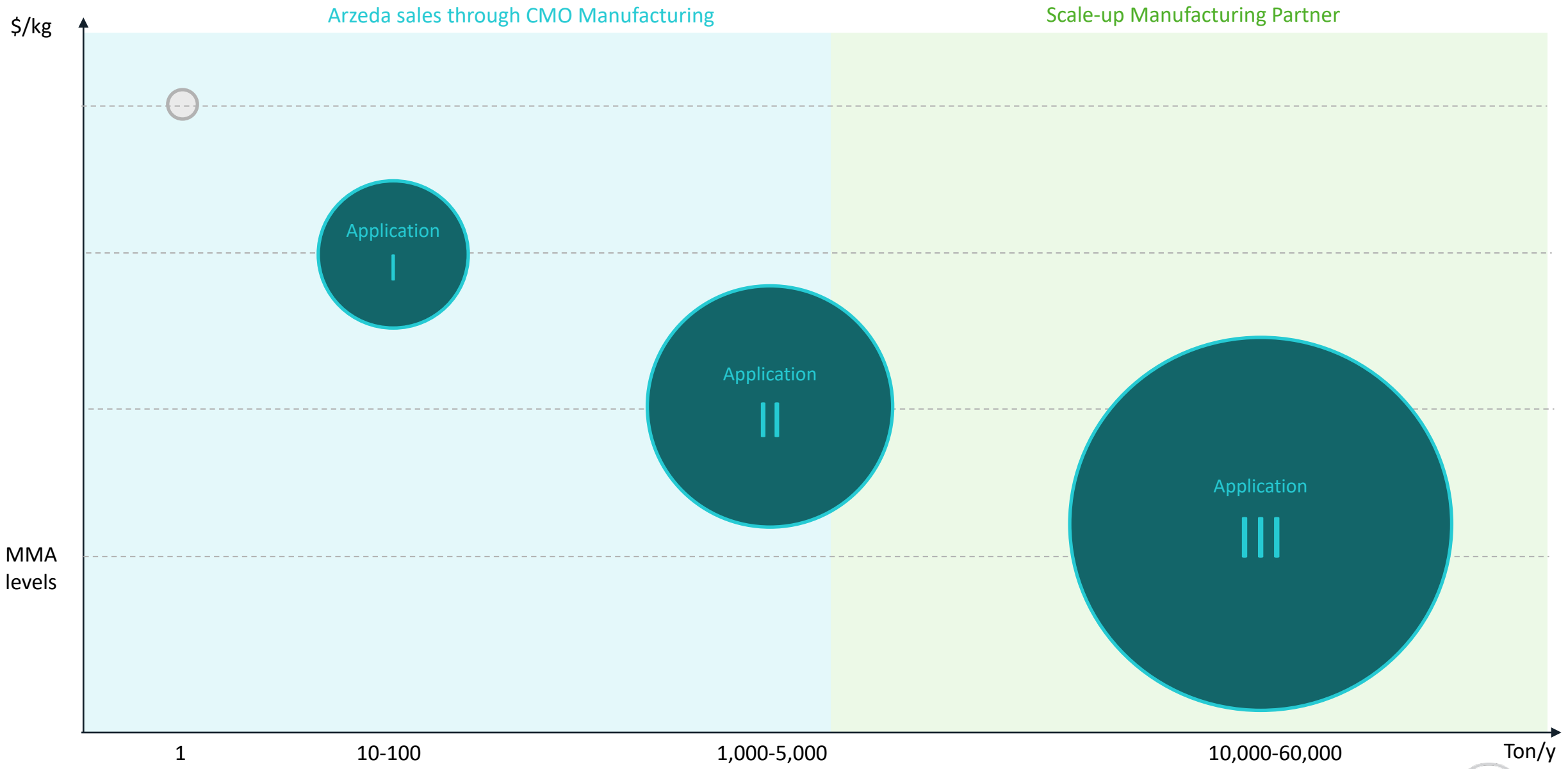
ENABLING TULIPALIN FERMENTATION



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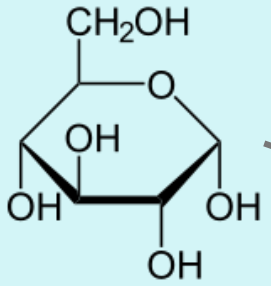
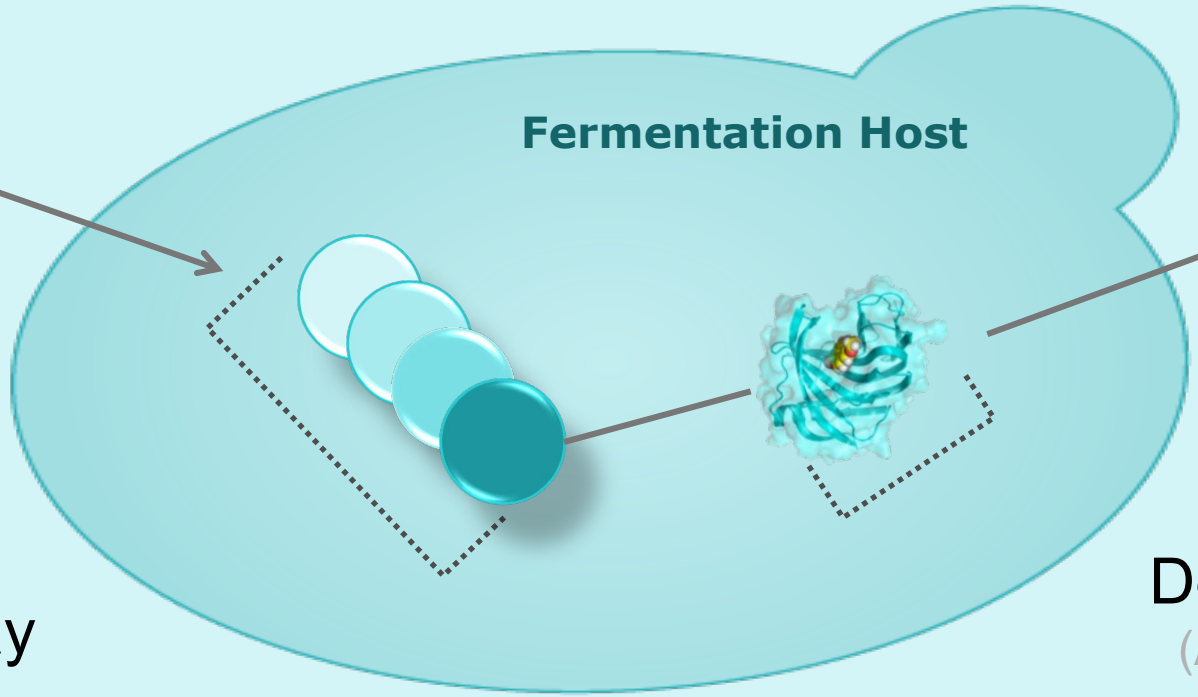
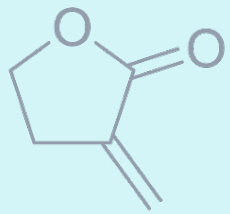
PROJECT OBJECTIVE

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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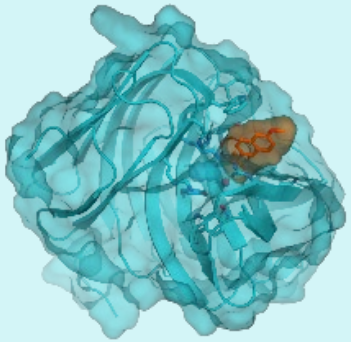
RISKS

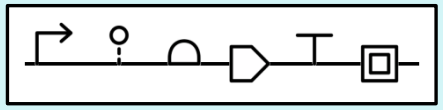
Enumeration of Project Challenges

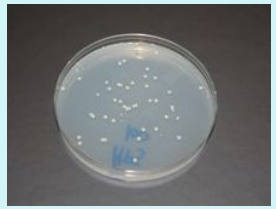
Enzyme Design Activity and Specificity

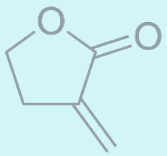


Can Sufficient Enzyme Activity/Specificity be Engineered

Proof-of-Concept Strain

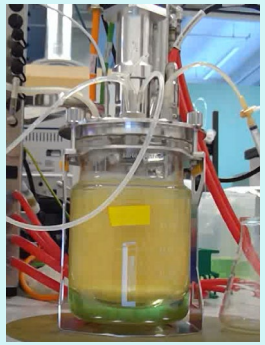






Can Product Be Produced in Strain

Production Strain & Fermentation Process

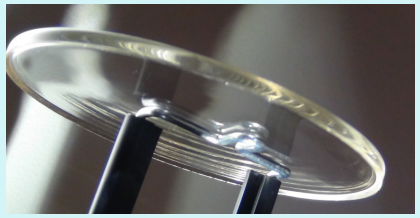


Can Sufficient Titers be Reached for Product Purification/Testing

Downstream Process Development

Can Sufficient Product Purity Be Reached From Processing Fermentation Broth

Market Risk



Does Final Product Have Desirable/Marketable Properties

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Project Management

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)

Commercial MMA Monomer and PMMA Properties Recapitulation

Arzeda MBL Monomer and PMBL Properties Testing

DOE BEEPS PROJECT MANAGEMENT

Communication

MILESTONES

- *Each task has milestones with defined deliverables meant to address risks for verification*
- *Data provided to DOE for accomplished milestones and milestone completion to be confirmed*

QUARTERLY UPDATES

- *Update presentations and reports are provided quarterly to communicate progress towards milestones and objectives*

ANNUAL VERIFICATION

- *Each Budget Period ends in a verification where associated data for each milestone and deliverable is presented and/or demonstrated*

GO NO/GO DECISIONS

- *Each annual Budget Period has defined Go No/Go Decision points which decide progress to the next budget period*

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Project Approach

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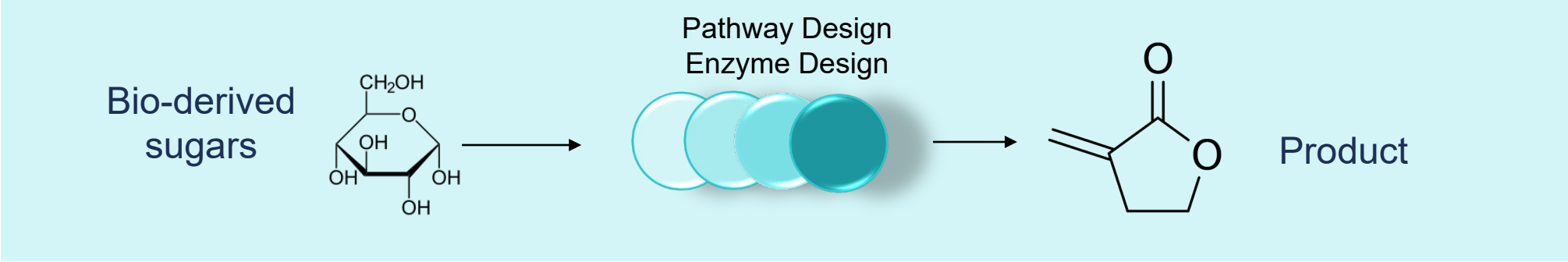
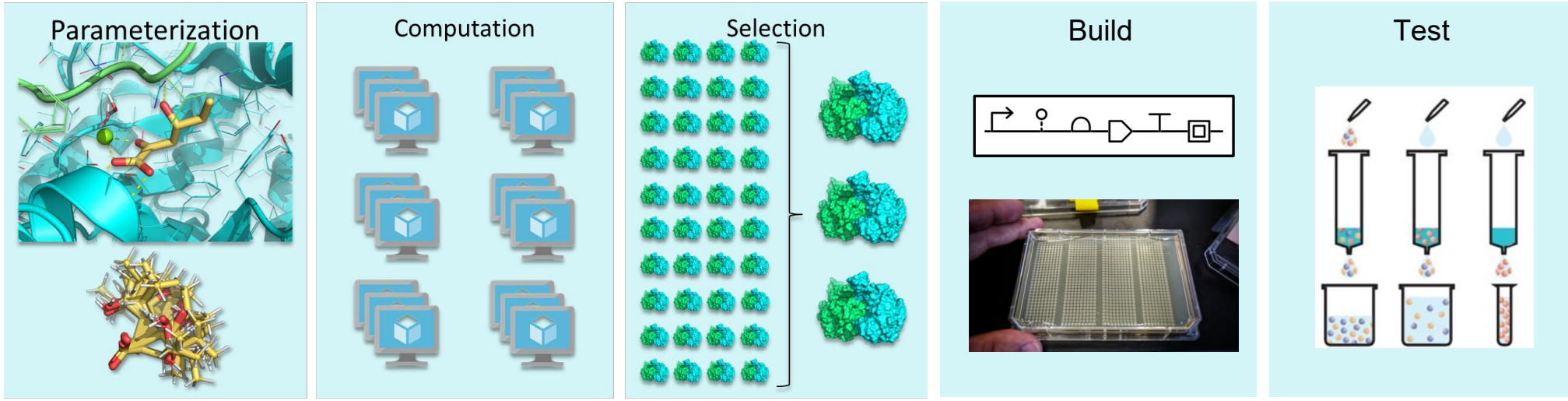
VALIDATING AND IMPROVING ENZYME ACTIVITY/SPECIFICITY

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





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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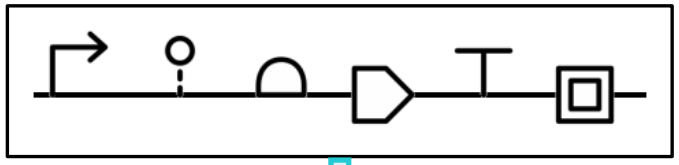
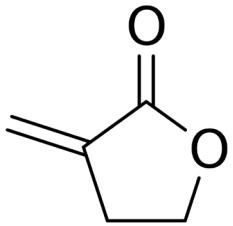
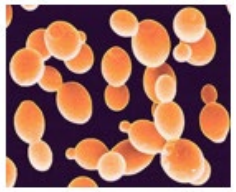
DEVELOP PROOF OF CONCEPT STRAIN

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



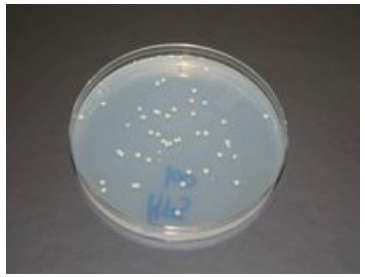


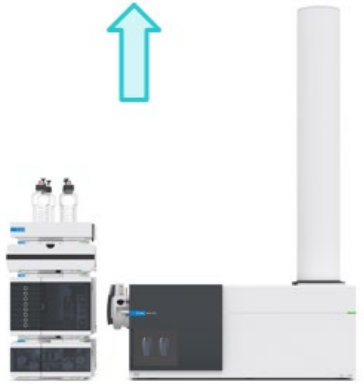
Bacteria



Yeast

Filamentous Fungi





Strain Produces Detectable Amount of Product

Milestone: Strain Produces Sufficient Quantities of Starting Metabolite

Milestone: Strain Produces Detectable Product from 2 Distinct Pathways

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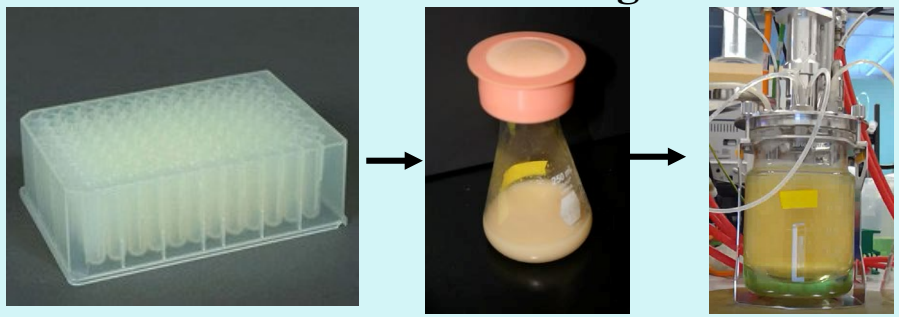
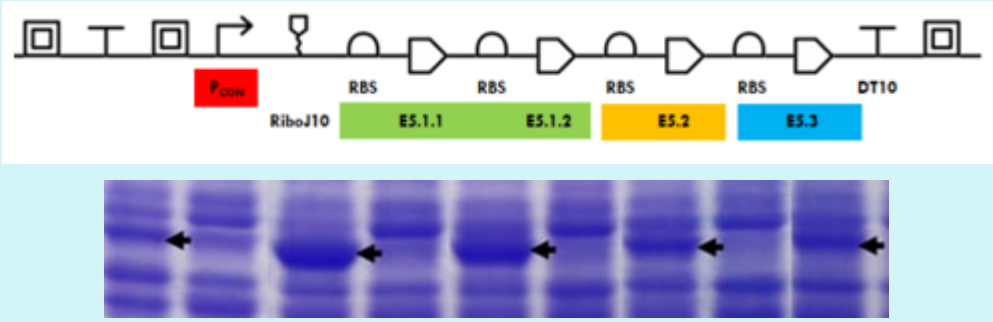
STRAIN OPTIMIZATION

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

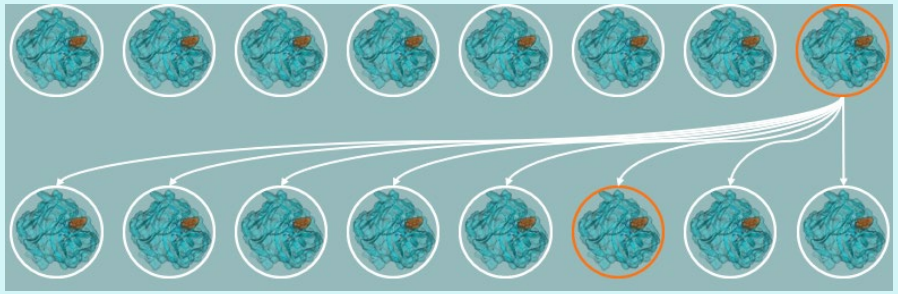
Culture Scaling



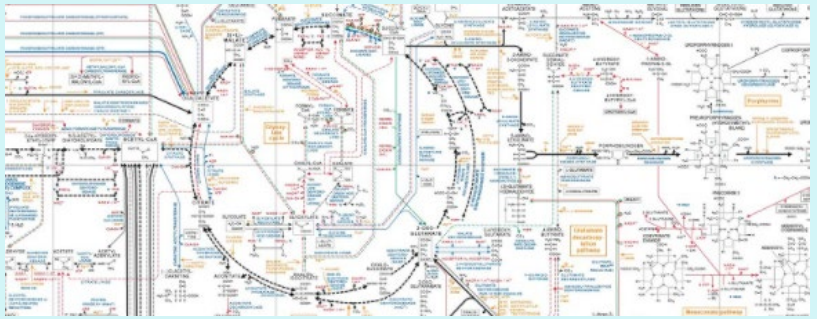
Enzyme Expression Refinement



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

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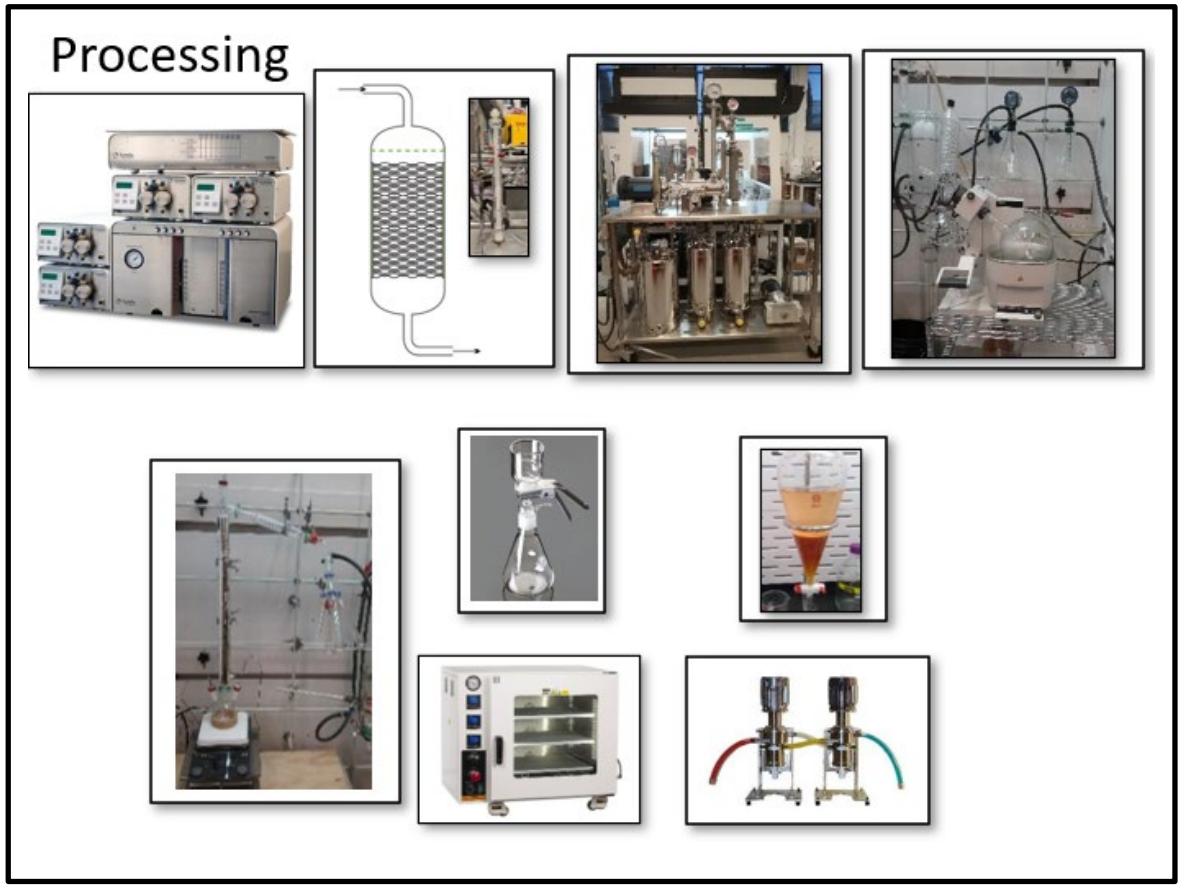
DOWNSTREAM PROCESSING

Demonstrate Ability to Produce High Grade MBL from Mock Fermentation Broth

Crude Broth



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 100gr of polymer grade MBL from mock fermentation broth

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POLYMERIZATION AND PROPERTIES TESTING

Demonstrate Process and Confirm Marketable Properties

Successfully Polymerize MBL to pMBL and Confirm Desirable Properties

Milestone: Physical comparison between pMBL and pMMA recapitulates/updates public disclosures for improved properties

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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

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IMPACT

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TULIPALIN A: 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

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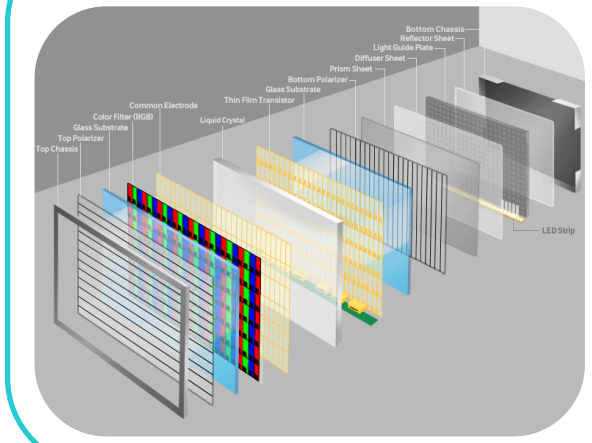
TULIPALIN A: PERFORMANCE IMPROVEMENT TO DISPLACE PMMA AND POLYCARBONATES

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

TRANSPARENT PLASTIC CASTS

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

DISPLAYS



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

LENSES

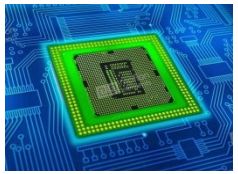
- Thinner and lighter lenses due to higher refractive index
- Less discoloration
- Higher scratch resistance

OPTIC FIBERS

- Improved heat resistance
- Increased refractive index for increased critical angle

PHOTORESIST

- Increased Etch Resistance



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DISSEMINATION OF RESULTS

CONFERENCES PRESENTATIONS:

GRC Enzymes & Metabolic Pathways 2019
American Chemical Society, Spring Meeting 2019
SIM Fuels & Chemicals Symposium 2019
University Stuttgart, Germany 2020

PATENTS

Provisional Applications Filing On-going

COMMERCIALIZATION

A total of 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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Progress and Outcomes

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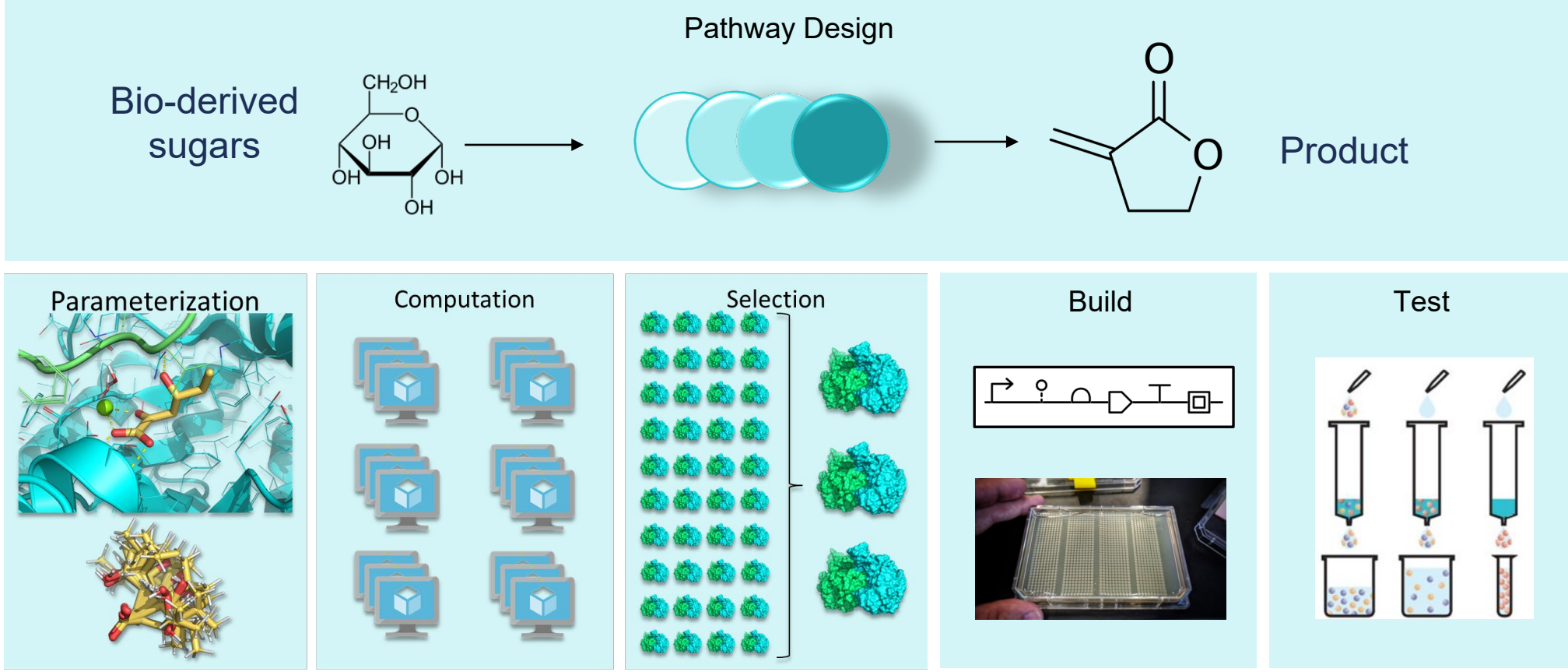
PATHWAY VALIDATED IN VITRO

Target Enzyme properties achieved



- Designed Ten Potential Pathways to Product From Producible Metabolic Intermediate
- Identified Three Pathways That Produce Product *In Vitro*
- Two Pathways met desired specificity/productivity targets needed to advance

Milestones Met

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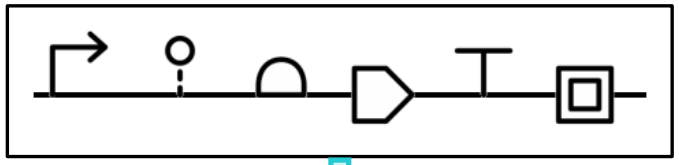
PROOF OF CONCEPT STRAIN PRODUCES DETECTABLE PRODUCT

Production Confirmed for Two Independent Pathways



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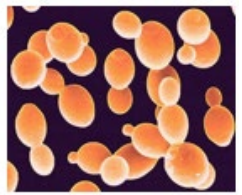

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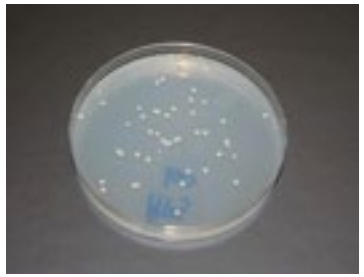

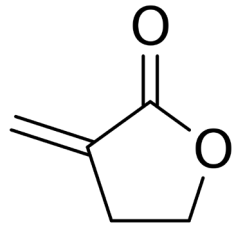

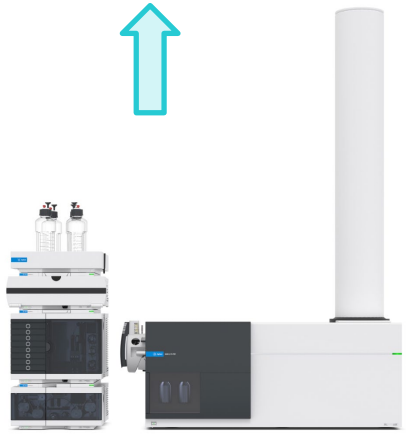

Strain Capable of Producing Needed Starting Metabolite at High Titer and Yield (up to 80 g/L)
Demonstrated successful production of Detectable Levels of Product for Two Pathways
Milestones Met

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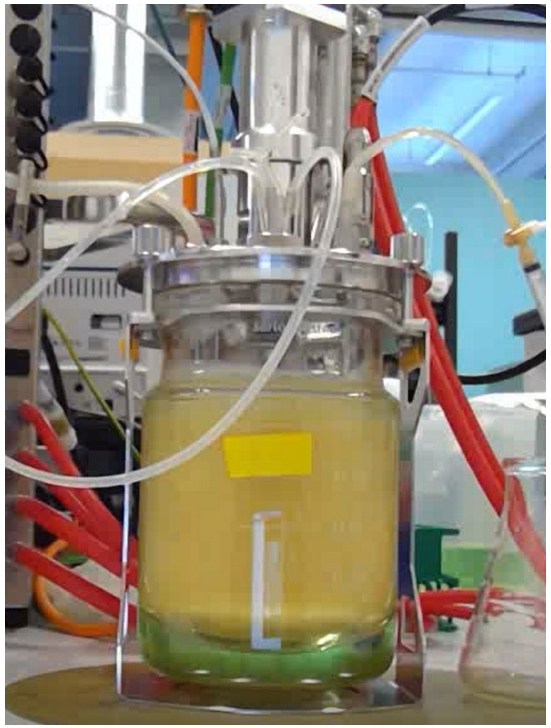
FERMENTATION STRAIN TITERS FOR PRODUCTION OF TULIPALIN A SIGNIFICANTLY IMPROVED

Reaching Titers of 0.25 g/L from Lignocellulosic Hydrolysate



- Introduction of pathway into host organism
- Current strain capable of production titers of 0.25 g/L
- Working on iterative refinement of enzyme properties and expression

Detectable Product

1 g/L intermediate production milestone

8 g/L Kg production possible

20 g/L Initial Market Viability

Close to achieving intermediate production target milestone
Have not encountered insurmountable issue with production
In Progress

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PRODUCED SAMPLES OF TULIPALIN A (BIOMBL™) FOR TESTING

Downstream Processing for Samples Shipped for Customer Testing

- Up to 1Kg samples produced at >99.9% purity demonstrated using Arzeda's downstream process
- Samples provided to potential commercial partners to validate value and performance of Arzeda's BioMBL
- Several potential customers have validated purity of product and improved properties of polymer

Purity and Quantity Metrics for Polymer Grade MBL Exceeded
>100g of >99.9% MBL Produced from Product Purified From Mock Fermentation Broth
Milestone Met

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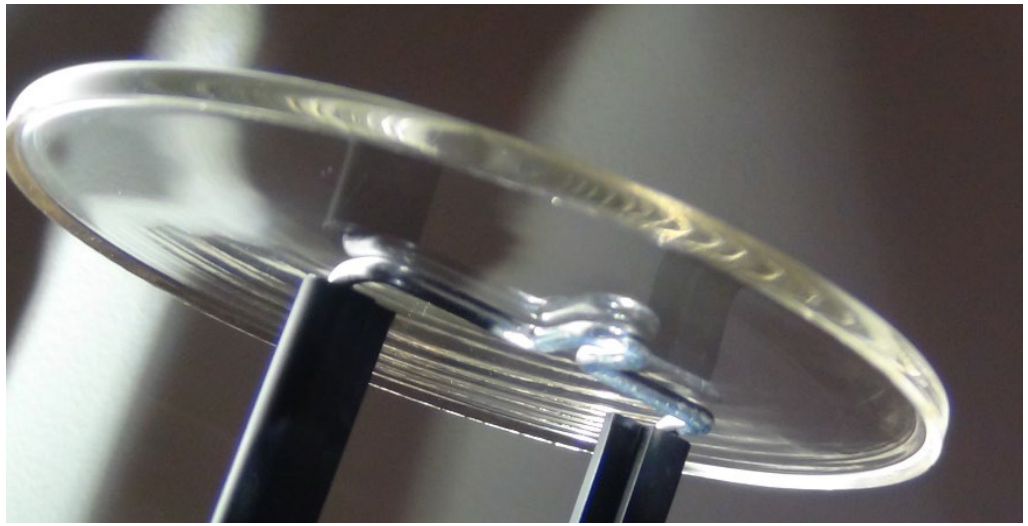
ARZEDA HAS PRODUCED MBL POLYMER AND CONFIRMED HIGH-VALUE PROPERTIES

Arzeda Internal Polymerization and Materials Properties Evaluation



100% biobased disks
Made by Arzeda

100% biobased plugs
Superior optical properties
Made by Arzeda

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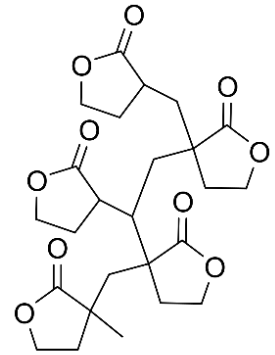
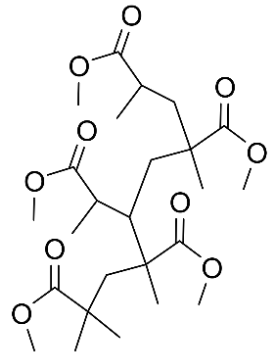
TULIPALIN A : PERFORMANCE IMPROVED OVER PMMA

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 Elascticity (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

Desirable Property Metrics Met or Exceeded
Properties Assessed Not Present in Literature
Milestone Met

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Summary

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 0.25 g/L

Scalable Downstream Process Can Achieve Polymer Grade >99.9% MBL

MBL Polymer Demonstrate 100% Improvement Over Petro-derived MMA

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End of Deck

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Quad Chart Overview (Competitive Project)

Timeline

- Oct 1st 2018

	FY20 Costed	Total Award
DOE Funding	-	$1.6 million
Project Cost Share	-	$502k

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

*Only fill out if applicable.