DOE Bioenergy Technologies Office (BETO) 2019 Project Peer Review

Biochemical Process Modeling and Simulation (BPMS 2.5.1.100)

March 4-5, 2019
Biochemical Conversion

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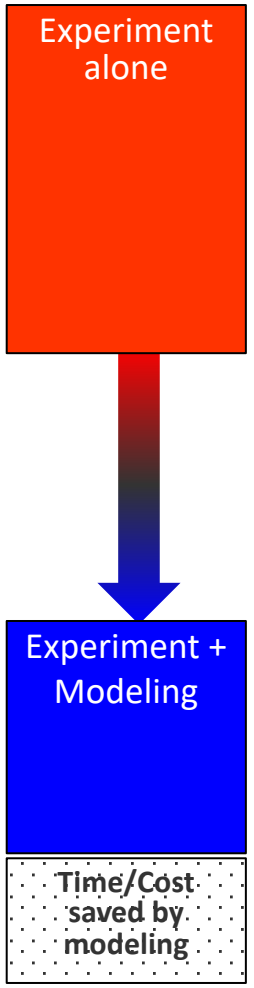
NREL

Goal Statement

Reduce research time and cost, **increasing efficiency,** using **theory, modeling, and simulation** to examine experimentally inaccessible solution space.

Provide **actionable guidance** to experimental, engineering and TEA research based on mechanistic predictions and design principles:

- Down select of targets, methods, and catalysts
- Mutations for enzymes
- Metabolic target products
- Chemical formulations for polymers
- Metabolic knockouts and insertions
- Reactor optimizations



Outcomes:

- Greater productivity in fuels and products leading to reaching the 2022 $3 gasoline gallon equivalent (GGE) target
- Redox-balanced, thermodynamically feasible metabolic pathways that produce energy-dense product from variable biomass (Ct-L)
- Accurate techno-economic analysis models for aerobic reactors
- Better carbon efficiency in conversion and higher-valued coproducts.

Relevance: Accelerates research, provides complementary insights and broadens research space

Modeling Relevance

All science follows the process of

- **Observing** how a system works
- **Making assumptions** about that system
- **Testing those assumptions** and iteratively refining them

Engineering and Design uses those assumptions to solve problems

How does Modeling improve Experiment and Engingeering?

Modeling Relevance

Mathematical modeling

→ forces us to be explicit about our assumptions

→ by converting them into mathematical relationships

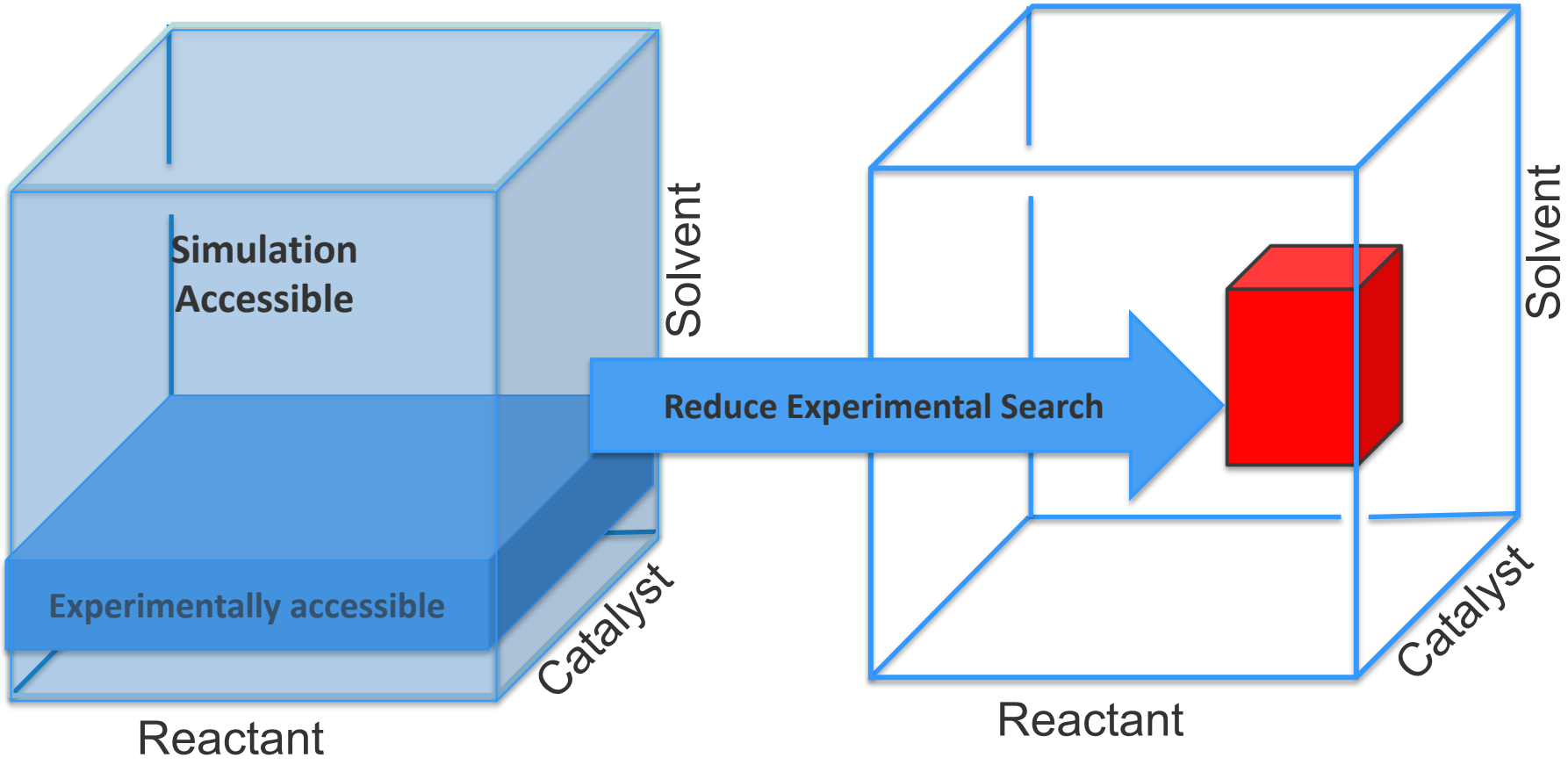
Then rigorously explore **consequences of assumptions**

- **Demonstrate** that our understanding of the process is mathematically consistent (or not consistent)
- **Perform** 'what if' analyses
- **Optimize** for a given goal
- Where models fail, **quantify limits** of our understanding

This defines our Goal, Objectives, and Relevance

Modeling Relevance

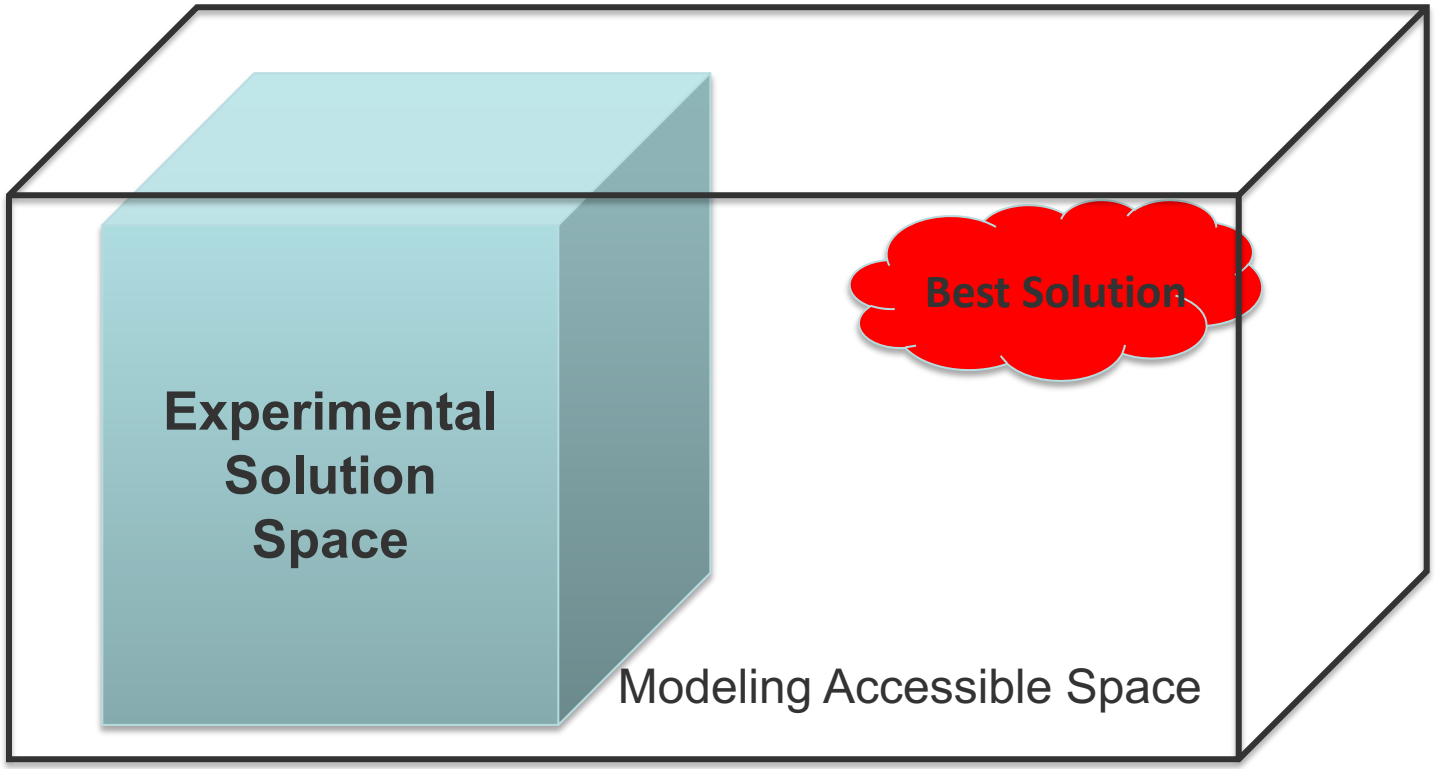
Modeling reduces experimental work and time.
Solution space is too big for experiment but accessible by modeling.



Example: Determine the right aliphatic compound for conversion from sugars in microbes based on ease of extraction before experimental efforts are put into implementing the conversion in microbes.

Modeling Relevance

Modeling can find solutions unavailable to standard experimental search.



Examples: Risk too high

- Mutations/knockouts believed to be fatal to microbe
- Testing reactor designs at Industrial scale
- Exploring triple mutants.

Quad Chart Overview

Timeline

- Project start date: October 1, 2018
- Project end date: September 30, 2021
- Percent complete: 17%

Budget	Total Pre FY17	FY 17 Costs	FY 18 Costs	FY 19- Project End Date
DOE Funded	$1.5M/yr	$1.2M	$0.7M	$3.15M (1.05/yr)

Partnering

2.5.1.307 CCPC Consortium Comp Chem and Phys

CCPC www.cpcbiomass.org

2.3.4.100 Lignin Utilization (LU)
2.5.3.105 Agile BioFoundry
2.5.4.100 Enzyme Engineering and Optimiz (EEO)
2.3.2.105 Biological Upgrading of Sugars (BUS)
2.4.3.102 Targeted Microbial Developmnt (TMD)
2.2.3.100 Low Temp Advanced Deconstruction
2.1.0.100 Biochemical Platform Analysis

NREL_Computer Center

NSF XSEDE (Computers)
U. Kentucky
ORNL
U Portsmouth, UK
U. CO Boulder
U. CO Denver
U. South Florida
U. Michigan
Northeastern

Barriers addressed

Ct-N Multiscale computational framework accelerating technology

Ct-C Process Development for Conversion of Lignin

Ct-F Increasing the yield from catalytic processes

Ct-G Decreasing the cost to developing novel ind. relevant catalysts

Ct-K Developing methods for Co-product Production

Ct-L Decreasing devel. time for ind. relevant microorgansims

Ct-M Current reactors are not designed to handle many harsh conditions

Objective

Provide **actionable guidance** to experiment, engineers and TEA from mechanistic predictions and design principles:

- Mutations for enzymes
- Metabolic target products
- Chemical formulations for polymers
- Metabolic knockouts and insertions
- Reactor optimizations

Reduce research time and cost, **increasing efficiency,** using **theory, modeling, and simulation** to examine experimentally inaccessible solution space.

End of Project Goal

Deliver complementary metabolic modeling and CFD methodologies (set of predicted metabolic pathway modifications, sugar feeding rates, oxygen sparging rates, reactor designs) leading to at least 20% increase in 2,3 BDO titer or 20% increase in yield of 2,3 BDO.

1 – Project Overview

- **Identify bottlenecks where theory and modeling are appropriate**
- **Improve hydrolytic and metabolic enzymes** through enzyme design
- **Formulate design principles** via the understanding the effect of structure on function
- **Engineer and modify metabolic pathways;** increase yield, titer, and productivity
- **Determine best fermentation conditions**, media, gas sparging for microbes
- Predict best configurations and conditions for **industrial-scale reactors**
- Provide **reliable models for TEA** analysis where data or models are inadequate.

Three Tasks: ***1*** *Molecular Modeling*
2 *Metabolic Modeling and Pathway Engineering*
3 *Mechanistic Process Modeling*

This project grew over the past 4 years from enzyme modeling to include homogeneous catalysis, metabolic modeling, and reactor and process design. Began in 2010 as subtask to Enzyme Design Project.

www.cpcbiomass.org

Joined CCPC (Consortium for Computational Physics and Chemistry) in FY18

14 collaborations finished in FY17-FY18 (see Addendum 3)
8 collaborative research projects in progress (see Addendum 3)

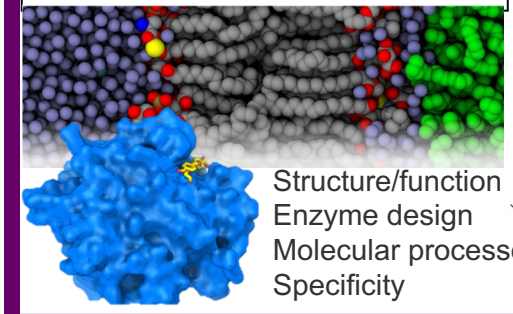
2 – Approach

2 – Approach (Management)

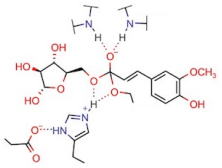
Project: Biochemical Process Modeling and Simulation
M. Crowley

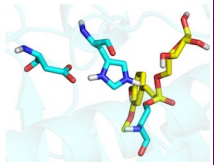
1 - Molecular Modeling - Brandon Knott

Molecular dynamics
Quantum mechanics



QM and QM/MM approaches to upgrading chemistry and catalysis

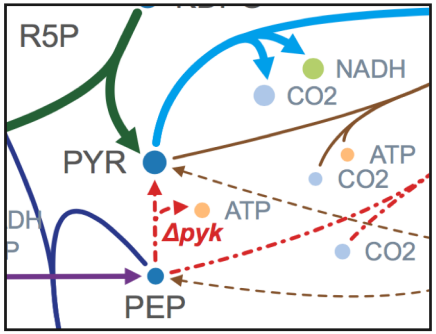




Structure/function
Enzyme design
Molecular processes
Specificity

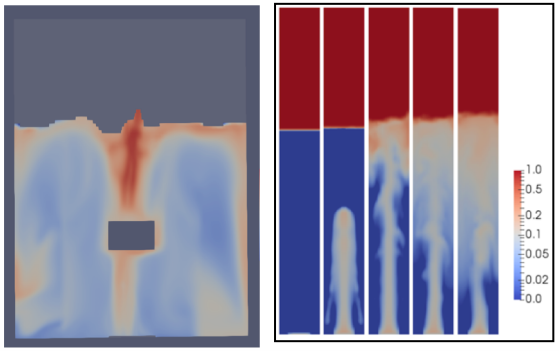
2 - Metabolic Modeling and Pathway Engineering - Yannick Bomble

- Metabolic models
- Metabolic pathway flux analysis
- Kinetic modeling
- DBTL **Learn** efforts and omics analyses



3-Mechanistic Process Modeling - James Lischeske

Coupled CFD/Rxn-diffusion
Multi-scale modeling



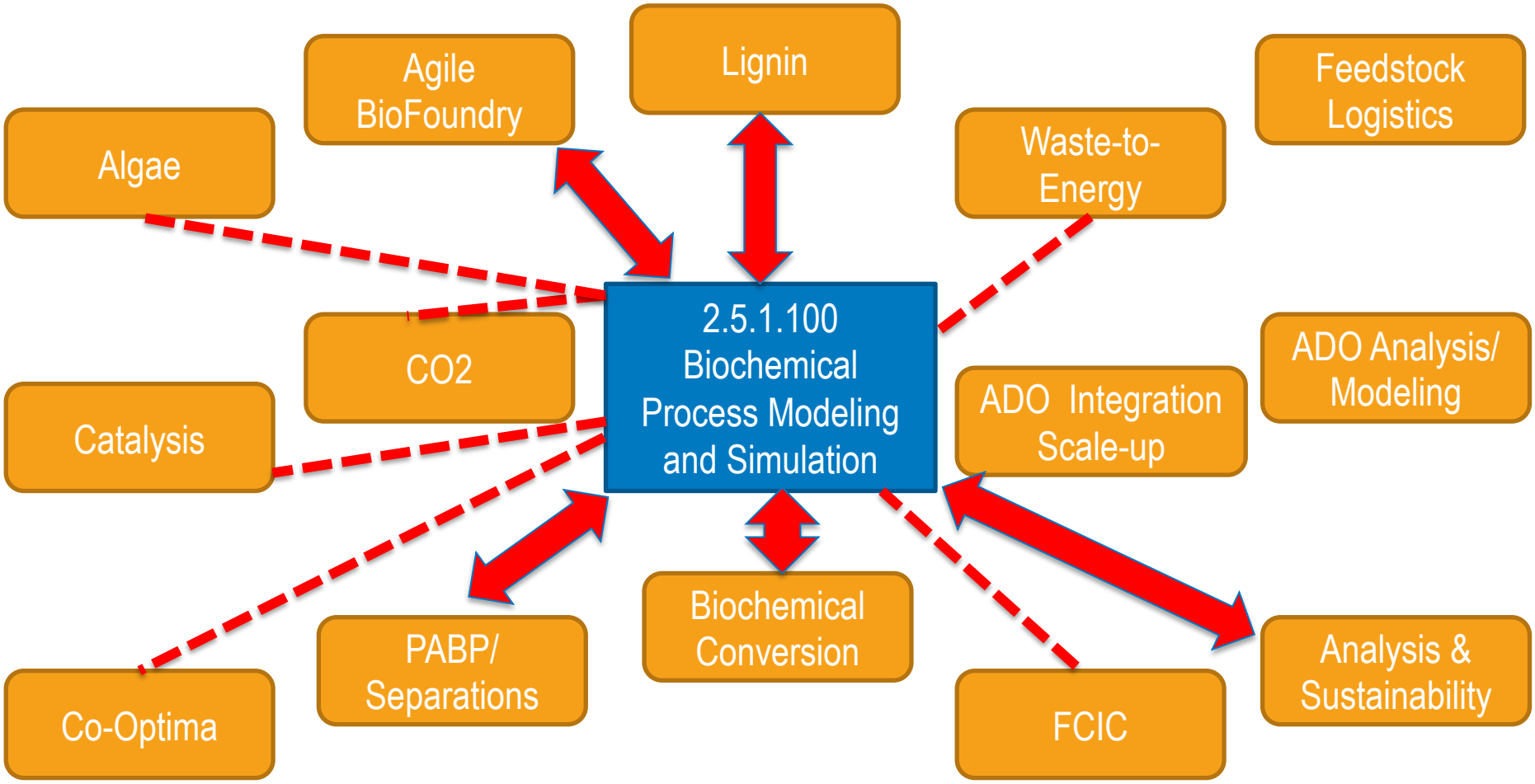
Project split into tasks by modeling type, managed by person with appropriate expertise

Task Managers responsible for:

- Relevance
- AOP, Milestones, quarterly reporting according to the guidance of BETO
- Communication with other projects
- Tracking go/no-go activities
- Budget management

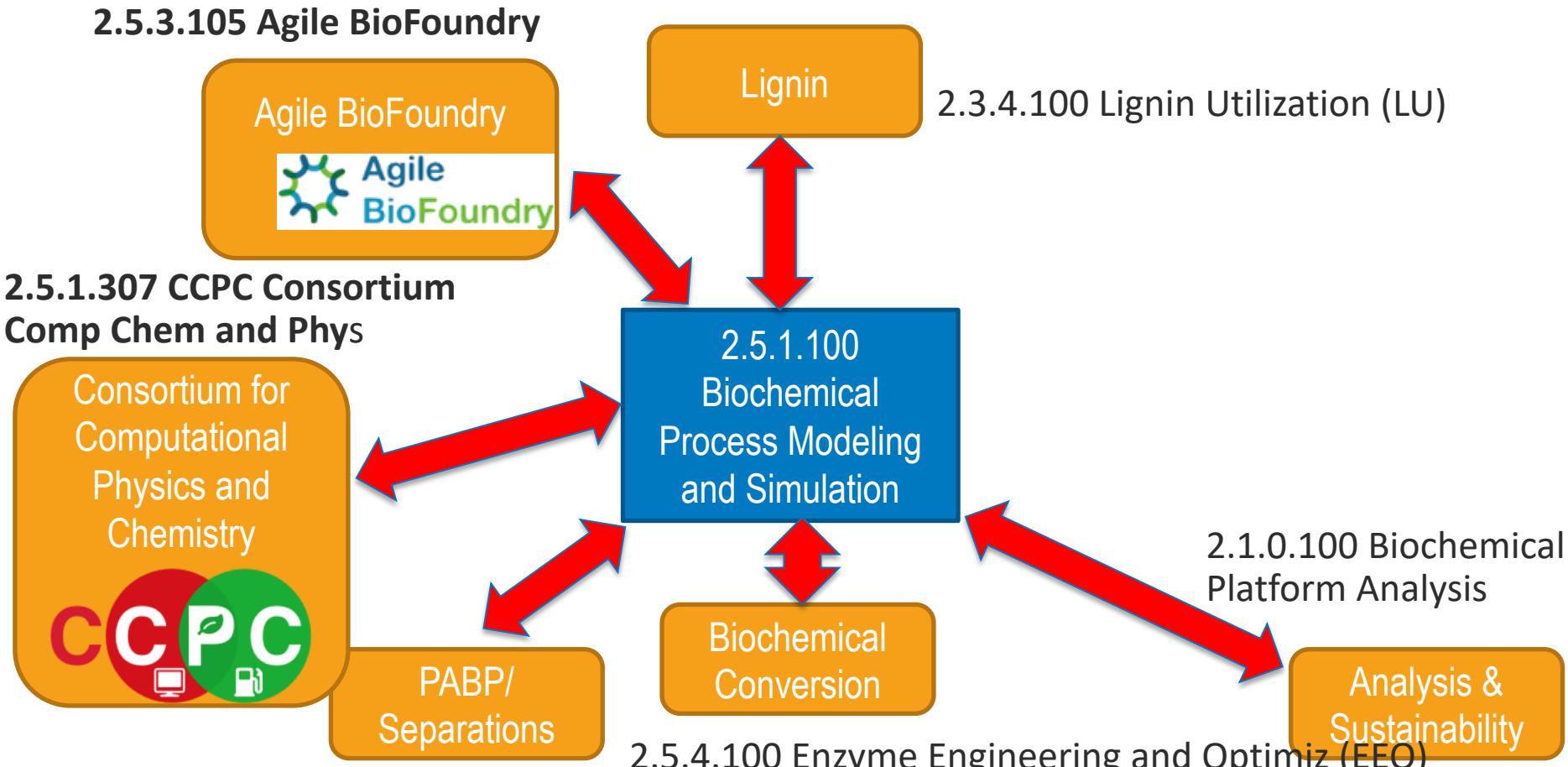
2 – Approach (Management)

Project: Biochemical Process Modeling and Simulation



2 – Approach (Management)

Project: Biochemical Process Modeling and Simulation



2 – Approach (Technical)

- **Objective:**
  - Gain **insight**, discover new approaches and solutions
  - **Guide and stimulate design, experiment, and engineering;** select most promising directions
  - **Increase research efficiency**
- **Approach:**
  - **Bolster experiment and design** with **theory, simulation, and modeling**
  - Use MultiScale Approach:
    Molecular (Task 1), Metabolic/Cellular (Task 2), and Macroscopic (Task 3) simulation
  - Leverage EERE computer resource: **Peregrine/Eagle (NREL)**
  - Leverage **CCPC** (Consortium for Computational Physics and Chemistry) collaborations using all theory and modeling expertise **across laboratories**
  - **Strong and regular communication** between experimental and modeling efforts
  - Target most **relevant bottlenecks and barriers** in **most BETO-relevant processes**
  - **Go/no-go decisions** to stop ineffective approaches, replace with new approaches that will deliver needed insight in time for 2022 and 2030 targets
- **Success Factors:**
  - Insights achieved, solutions found, unproductive efforts avoided
  - Reduced time to solution: increasing titer, efficiency, speed, performance
  - New routes to advanced fuels and co-products
- **Challenges:**
  - Software and methods need to be developed to meet the questions and necessary speed for timely answers (MD, CFD, QM/MM, FE, analysis)
  - Local computer hardware needs to stay at state-of-the-art

3 – Technical Accomplishments
Progress
Results

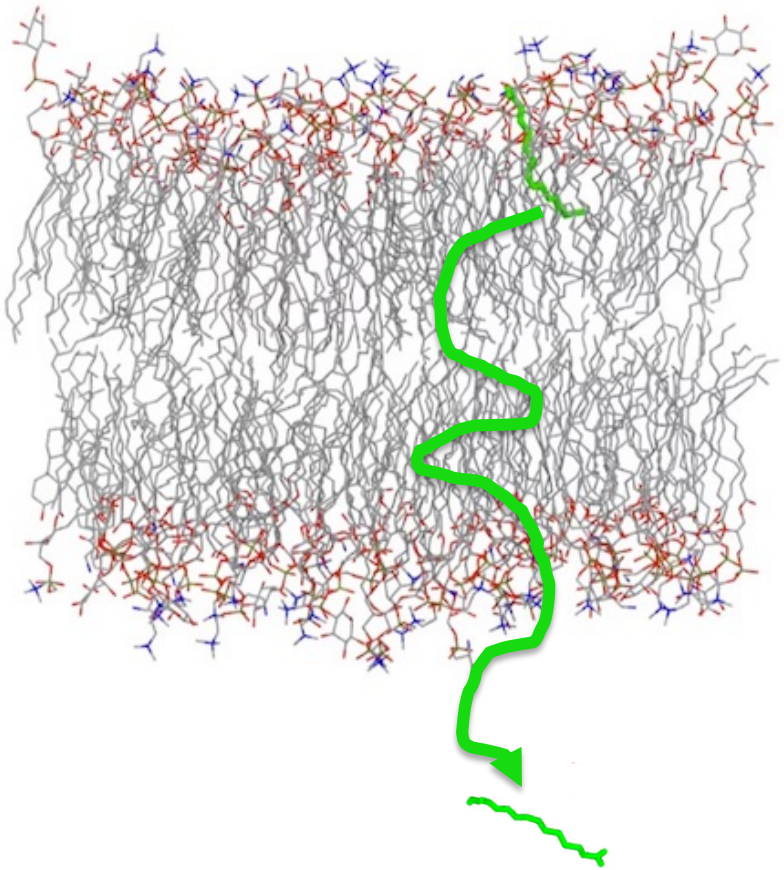
3 – Progress – Membrane Transport (molecular)

Process design via deep understanding of substrate and product membrane permeability

RELEVANCE: Crossing of multiple substrates and products **spurs the engineering of enhanced biological hosts** for upgrading to fuels and chemicals

- For biological funneling, the *very heterogeneous* slate of lignin degradation monomers **must get into the cell** *and*...
- For biological upgrading, products must then **get out of the cell**
- Membrane permeability dictates product egress from host cells and toxicity of product molecules
- Developed **high-throughput method** for determination of mechanism and rate of membrane crossing
- We can then answer:
  - **Active vs. passive transport** – are transporter proteins needed?
  - **Which target molecules** diffuse readily out of cells? Should products be functionalized?

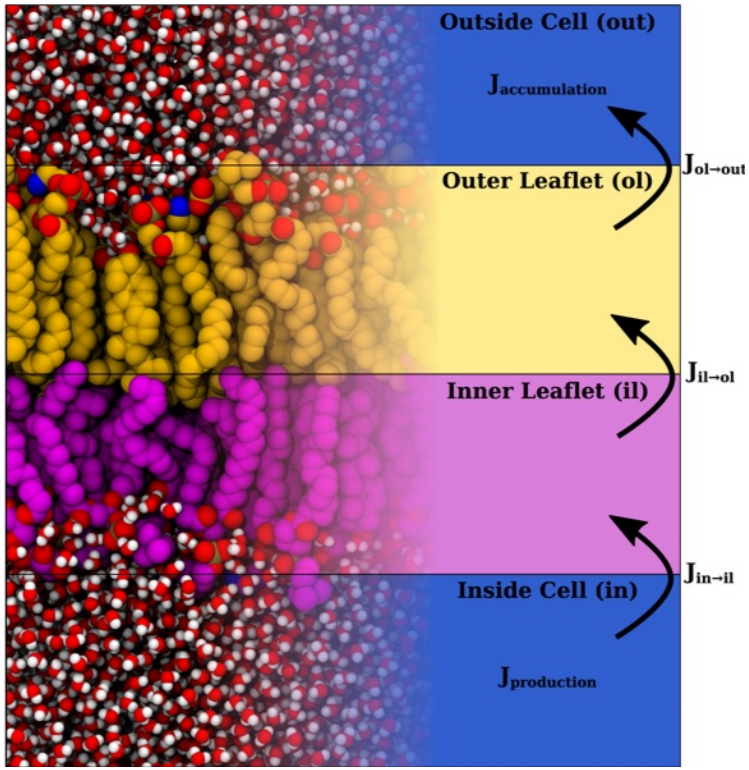
Video Fatty acid transport across lipid bilayer

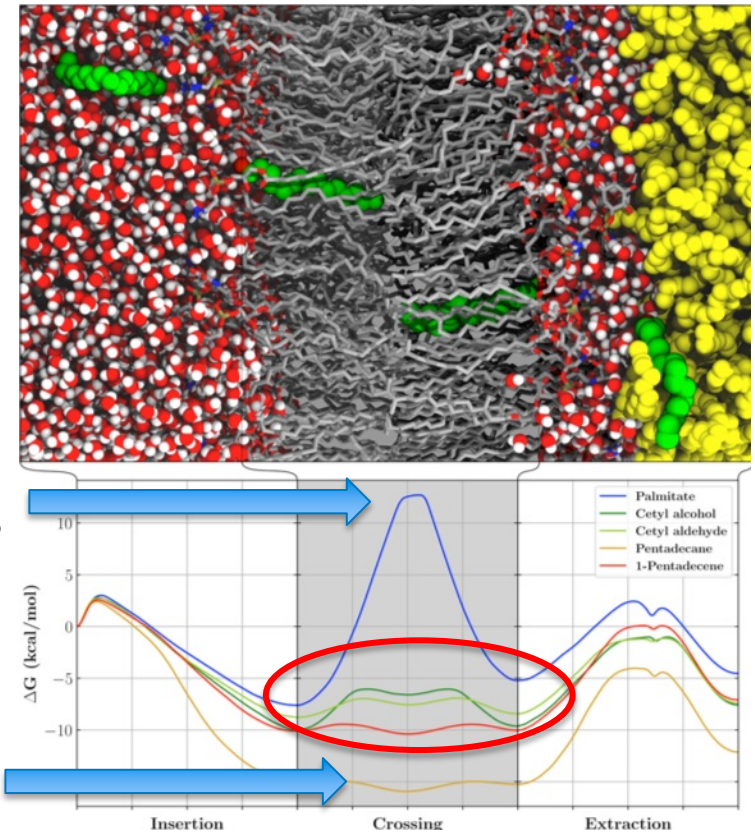


3 – Progress – Membrane Transport (molecular)

- We determined the permeability of **fatty acids** and terpenoids across biological membranes

$$P^{-1} = \int_{\xi_l}^{\xi_u} \frac{\exp\left(\Delta G\left(\xi\right)\beta\right)}{D\left(\xi\right)} d\xi$$





Barrier to fatty acids

Alkyls collect

Functionalization? Fatty alcohols demonstrate superior passive transport rates relative to other fatty acid products

3 – Progress – Membrane Transport (molecular)

- We determined the permeability of fatty acids and **terpenoids** across biological membranes

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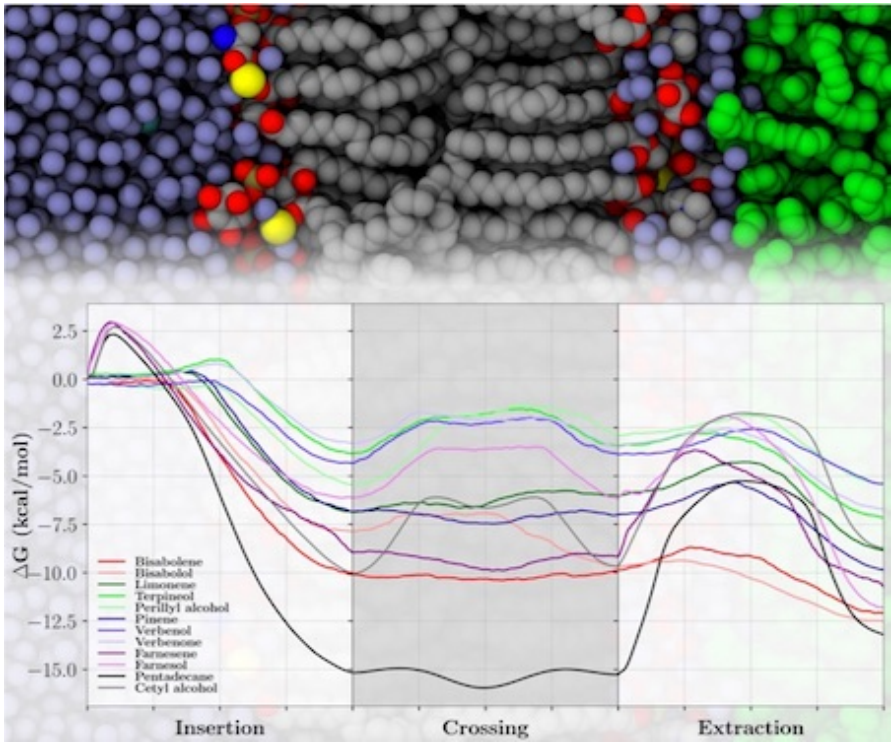
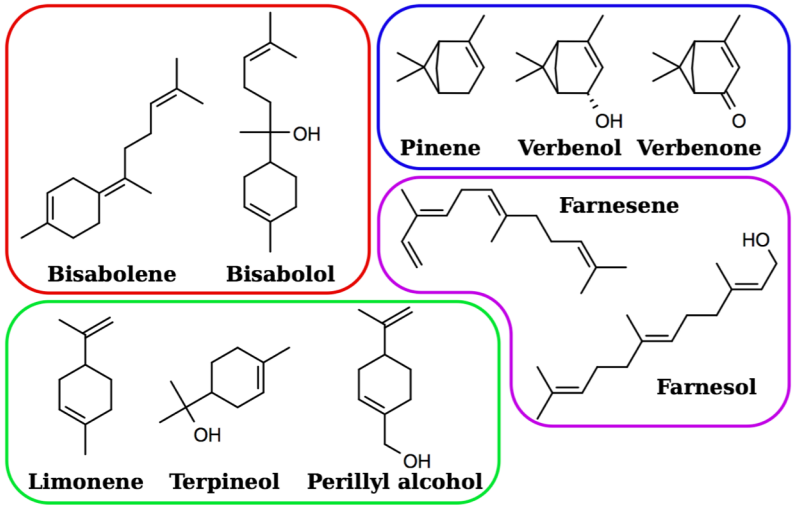




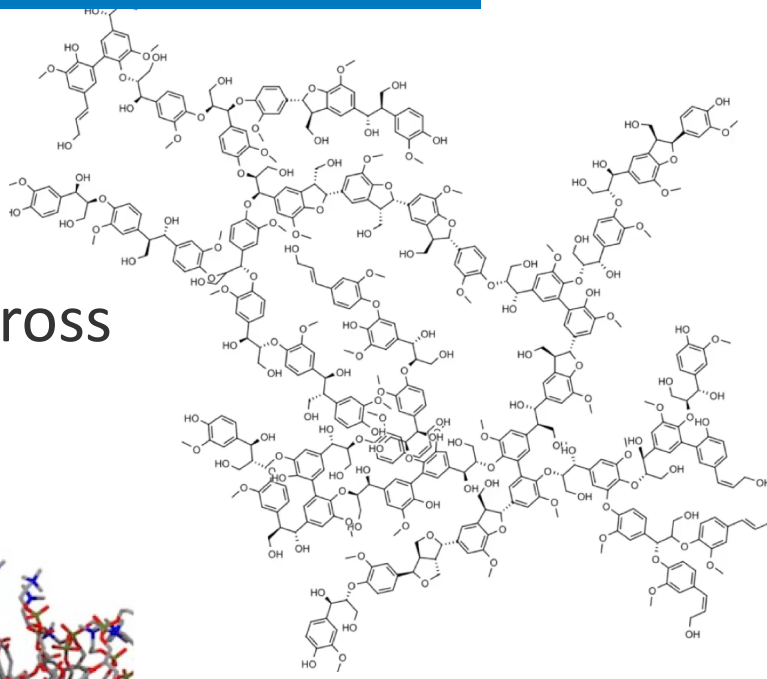
Transporters? We predict transporter proteins are **not needed** for terpenoid products

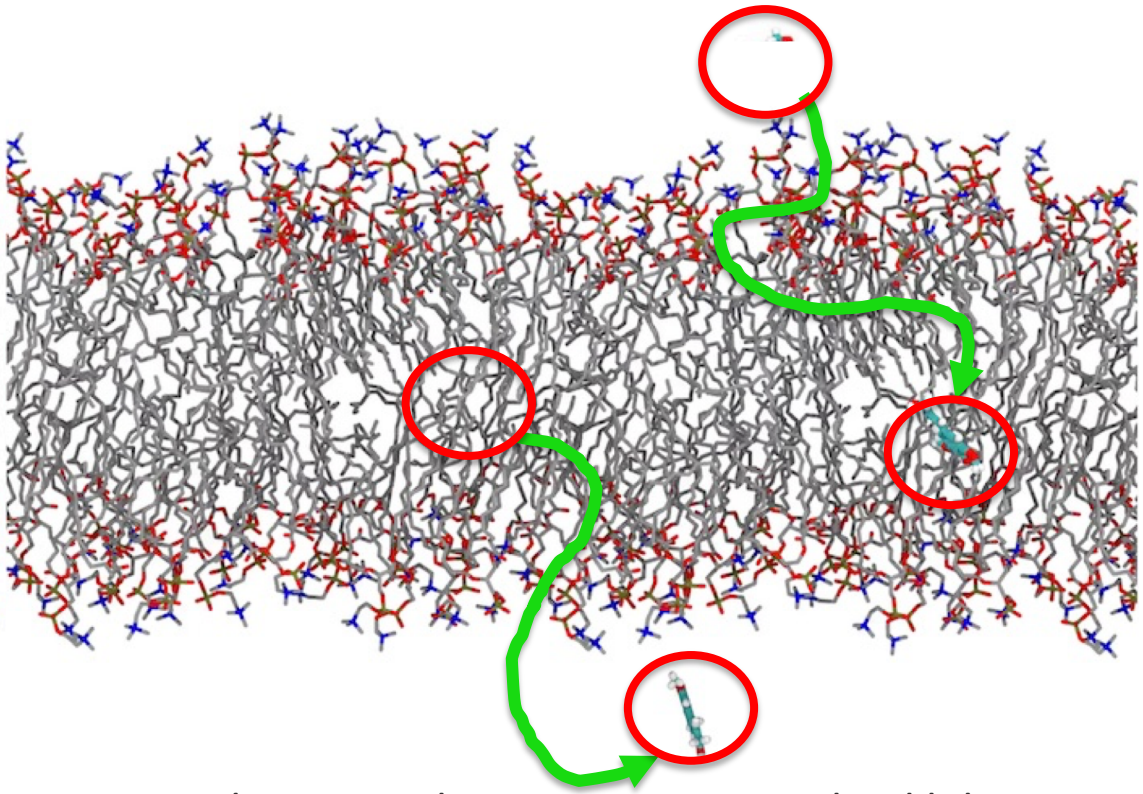
Functionalization?

Oxidizing terpenoids **would not improve** membrane transit

3 – Progress – Membrane Transport (molecular)

- Context: Lignin valorization
- Only possible lignin force field developed in this project
- Lignin breakdown products must cross the cell membrane





Video Syringyl monomer crossing lipid bilayer

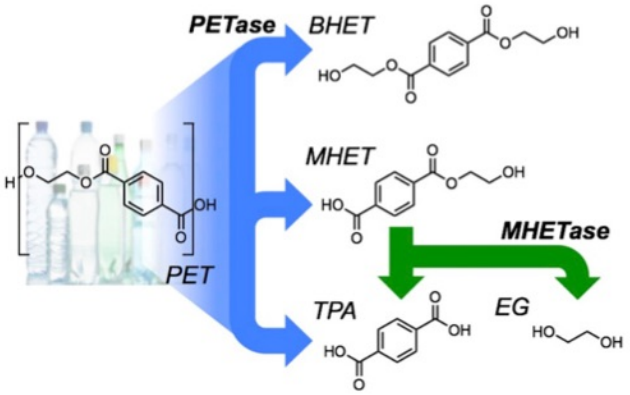
This work enabled by breakthroughs in the molecular modeling of…

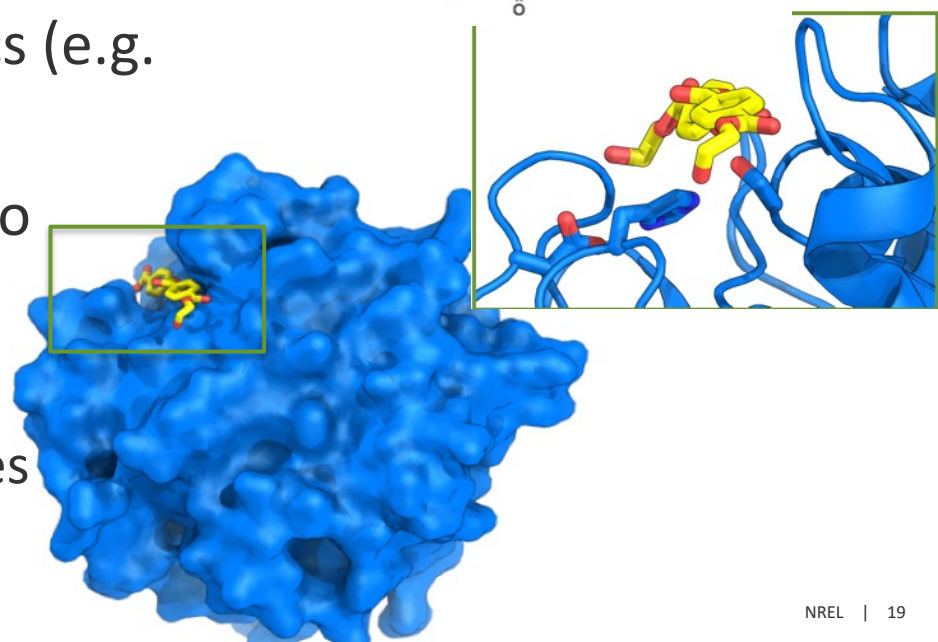
3 – Progress – Enzymatic plastics recycling (molecular)

Guiding enzyme engineering for enhanced plastics recycling

RELEVANCE: Plastics (a major MSW component) represent a carbon waste product and major environmental problem. We are enabling the Circular Economy for plastics.

- Leveraging BETO investment in biomass recalcitrance, metabolic engineering, and process development to drive enzyme engineering towards the cost-efficier upcycling of commodity plastics (e.g. PET)
- Docking – how does PET bind to PETase?
  - Provides engineering targets
  - First step in mechanism studies



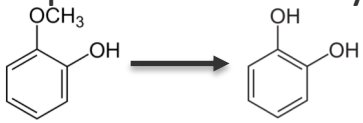


3 – Progress – Broadening lignin substrate utilization

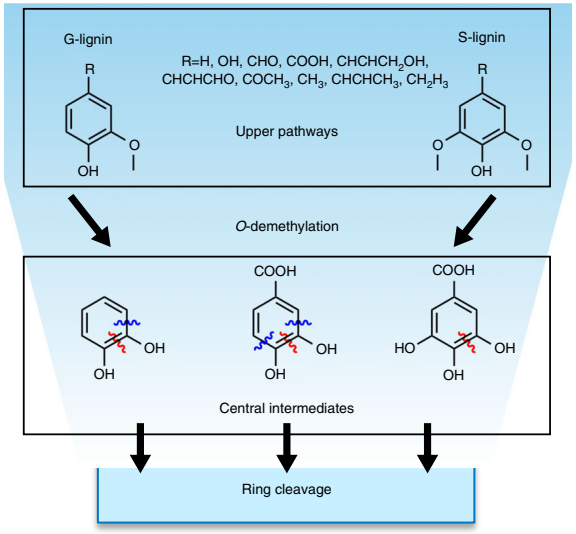
Biological funneling for lignin valorization

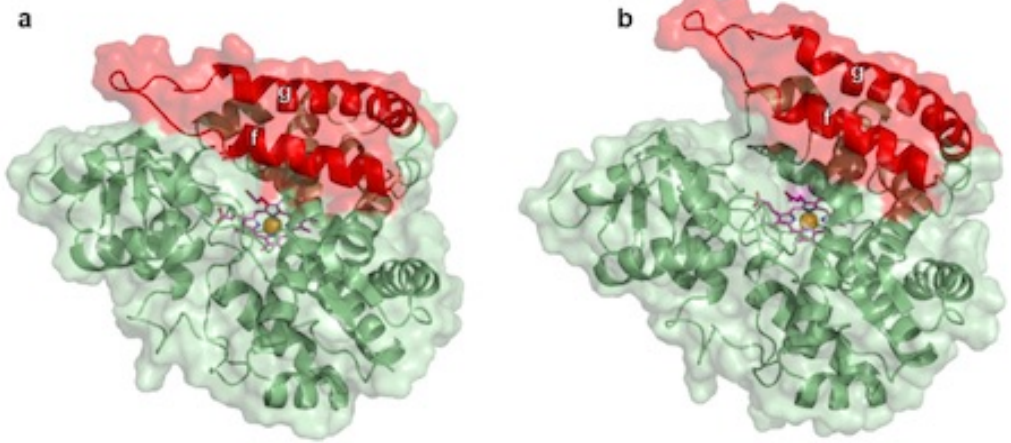
RELEVANCE: Driving the engineering of bottleneck enzymes for the upgrading of lignin degradation products to biofuels and products

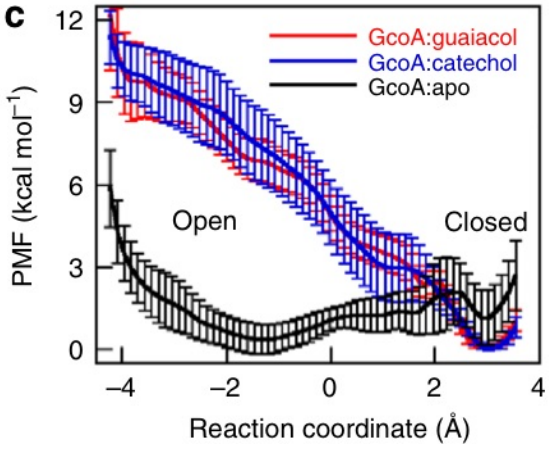
- Engineering microbes to convert a broad slate of lignin degradation products is a promising strategy for lignin valorization
- GcoA is a P450 protein capable of natively demethylating guaiacol to catechol



- MD simulations revealed that the substrate access "lid" opens spontaneously on the microsec time scale with empty active site, but is an activated process when substrate/product are bound
  - Also, MD reveals critical roles for active site hydrophobic residues that subsequently become engineering targets…





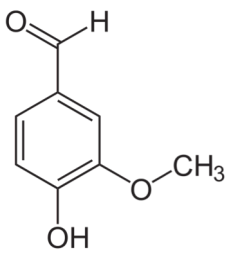


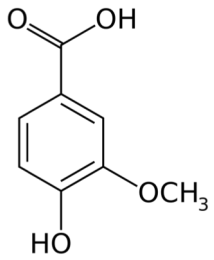
3 – Progress – Broadening lignin substrate utilization

- A single point mutation makes GcoA active on syringol (simplest monomer of S lignin) and improves its activity on native guaiacol (simplest monomer of G lignin)
- Simulations reveal the link between the mutation (sequence / structure) and the enhanced activity on non-native substrates (function)

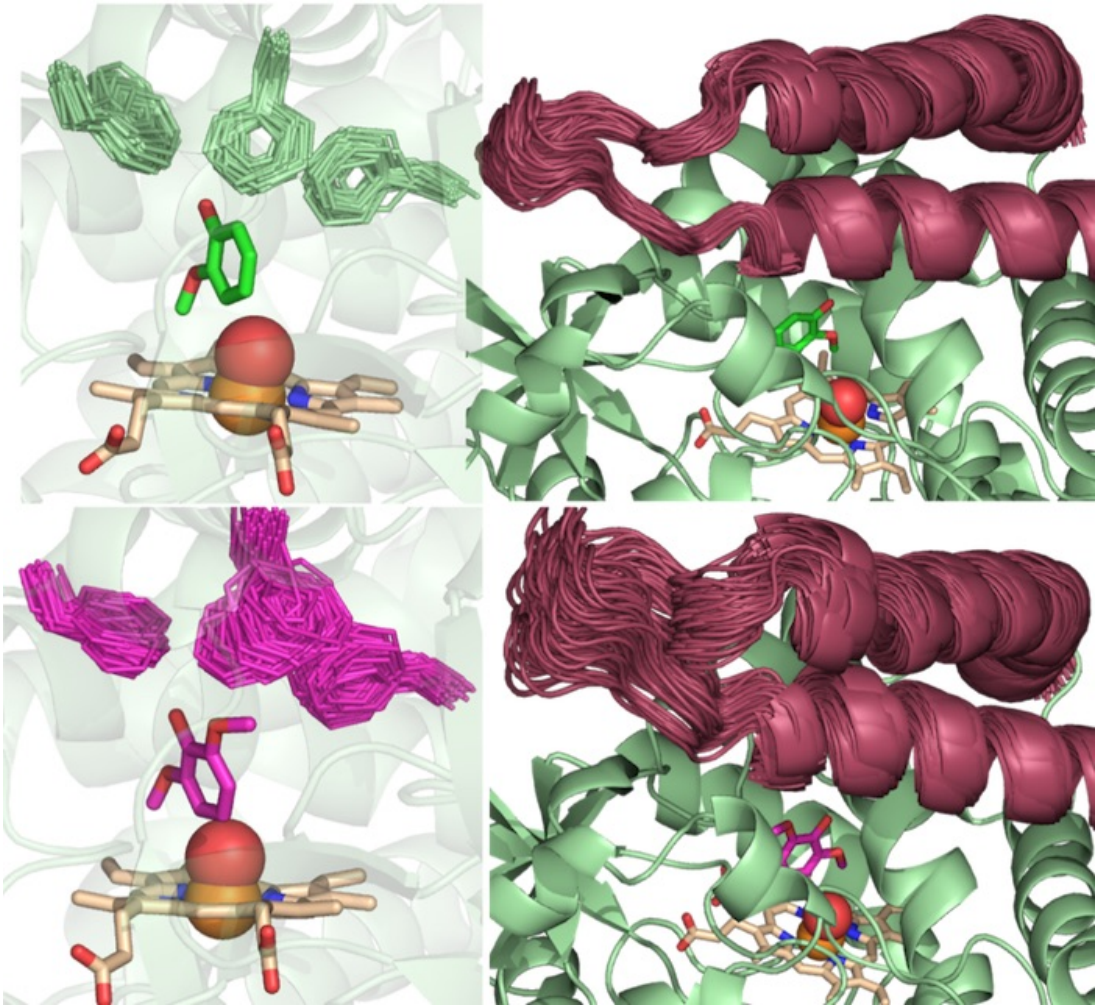
Next up

Vanillin and vanillate





Guaiacol in WT GcoA



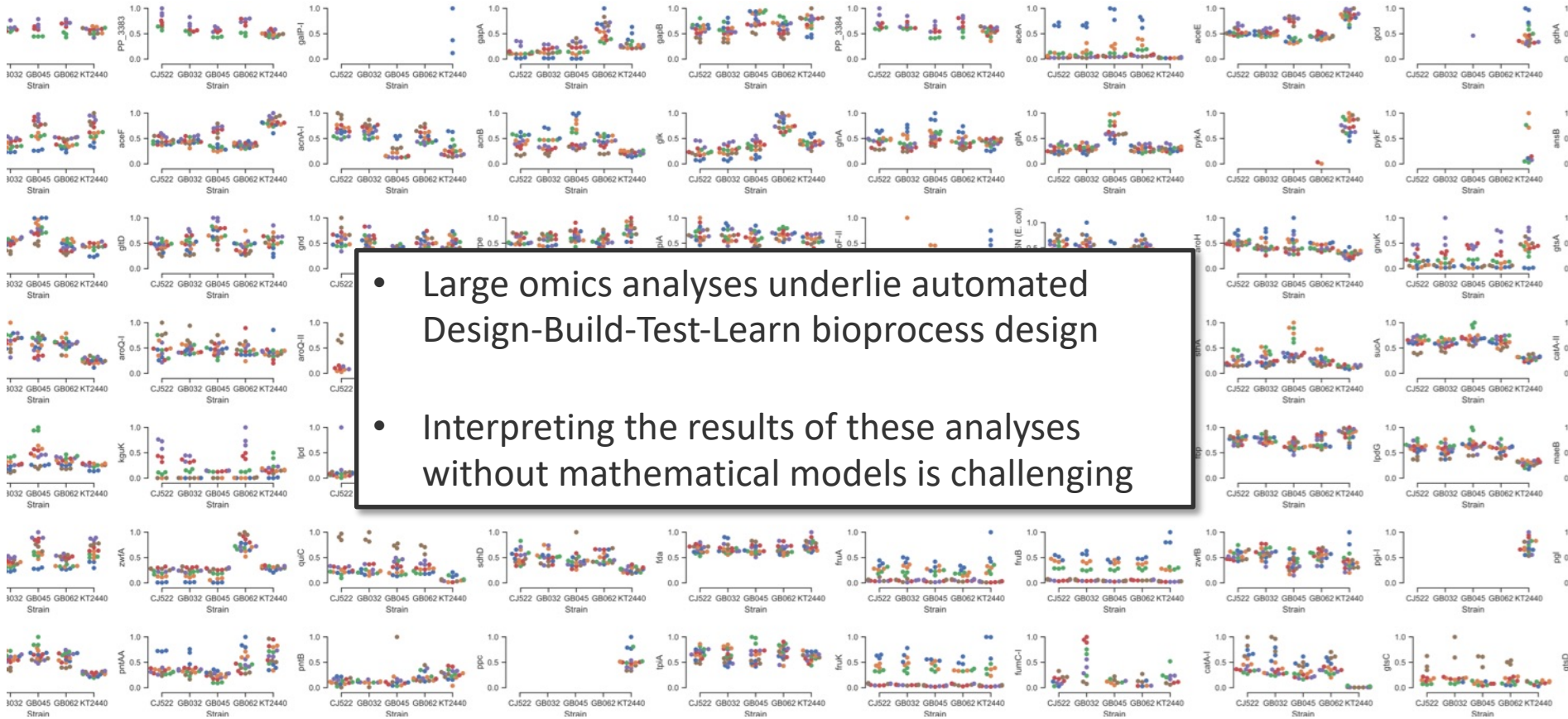
Syringol in WT GcoA

3 – Progress DBTL (step 1)

“Learn”-ing from omics data

RELEVANCE:

- **Convert ‘Omics data from high-throughput experiments into actionable strategies to improve processes in industrially-relevant hosts.**
- **Collaboration with Agile Biofoundry**



- Large omics analyses underlie automated Design-Build-Test-Learn bioprocess design
- Interpreting the results of these analyses without mathematical models is challenging

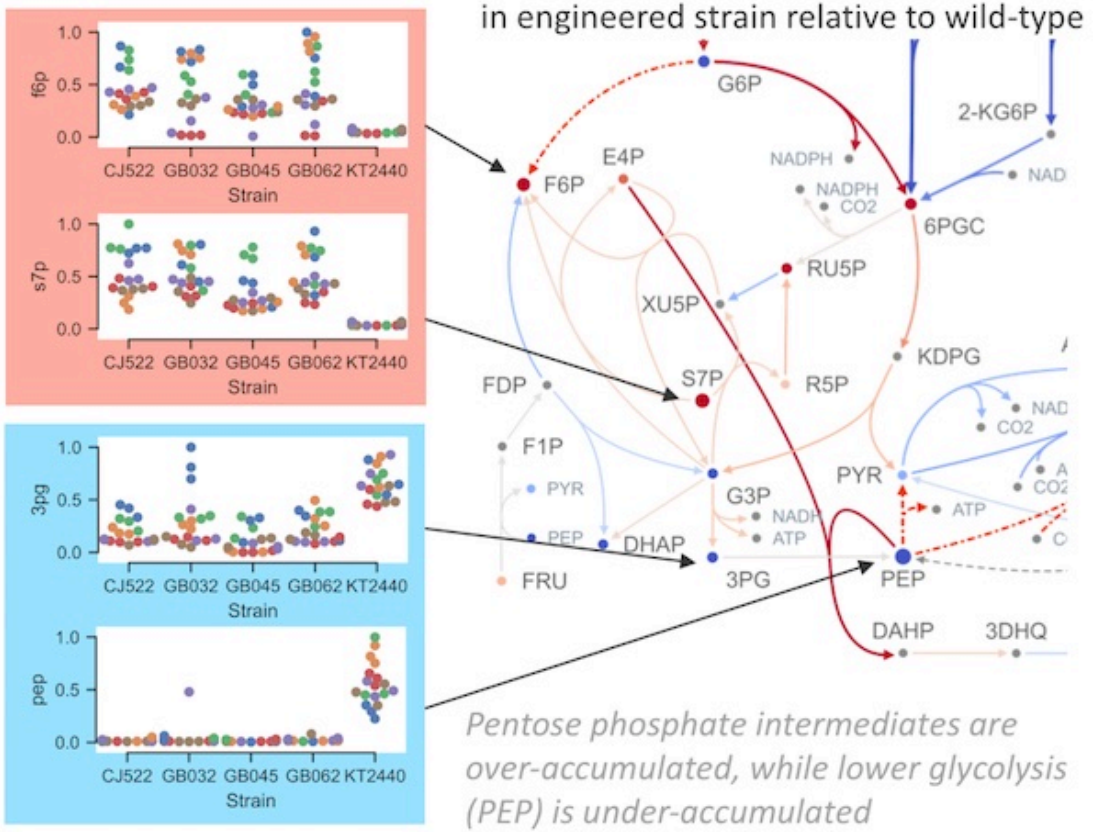
3 – Progress DBTL (step 2)

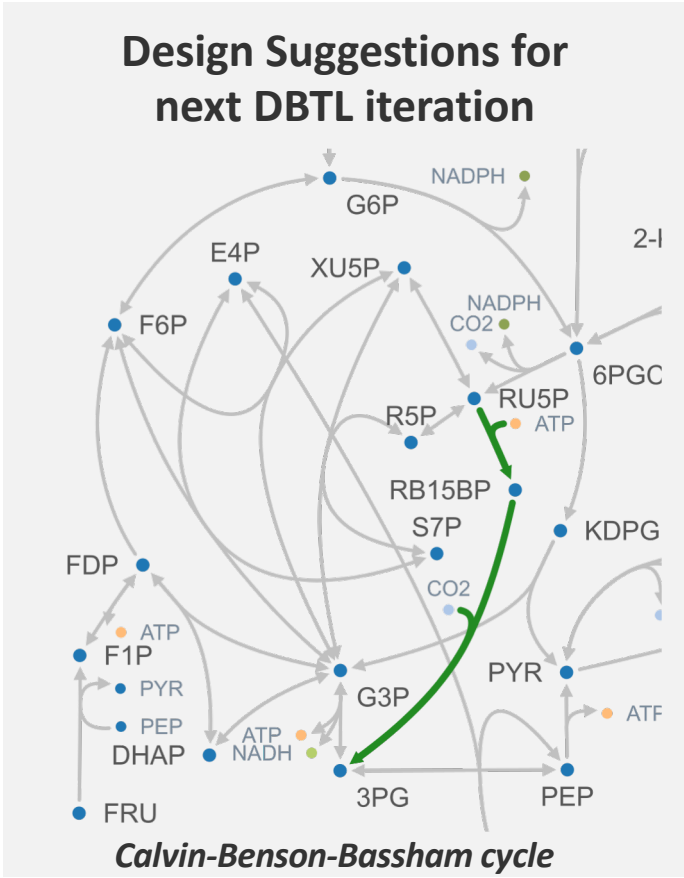
“Learn”-ing from omics data

- Models provide framework for rational exploration of modifications to improve strain performance

RELEVANCE:

- **Convert ‘Omics data from high-throughput experiments into actionable strategies to improve processes in industrially-relevant hosts.**
- **Collaboration with Agile Biofoundry**



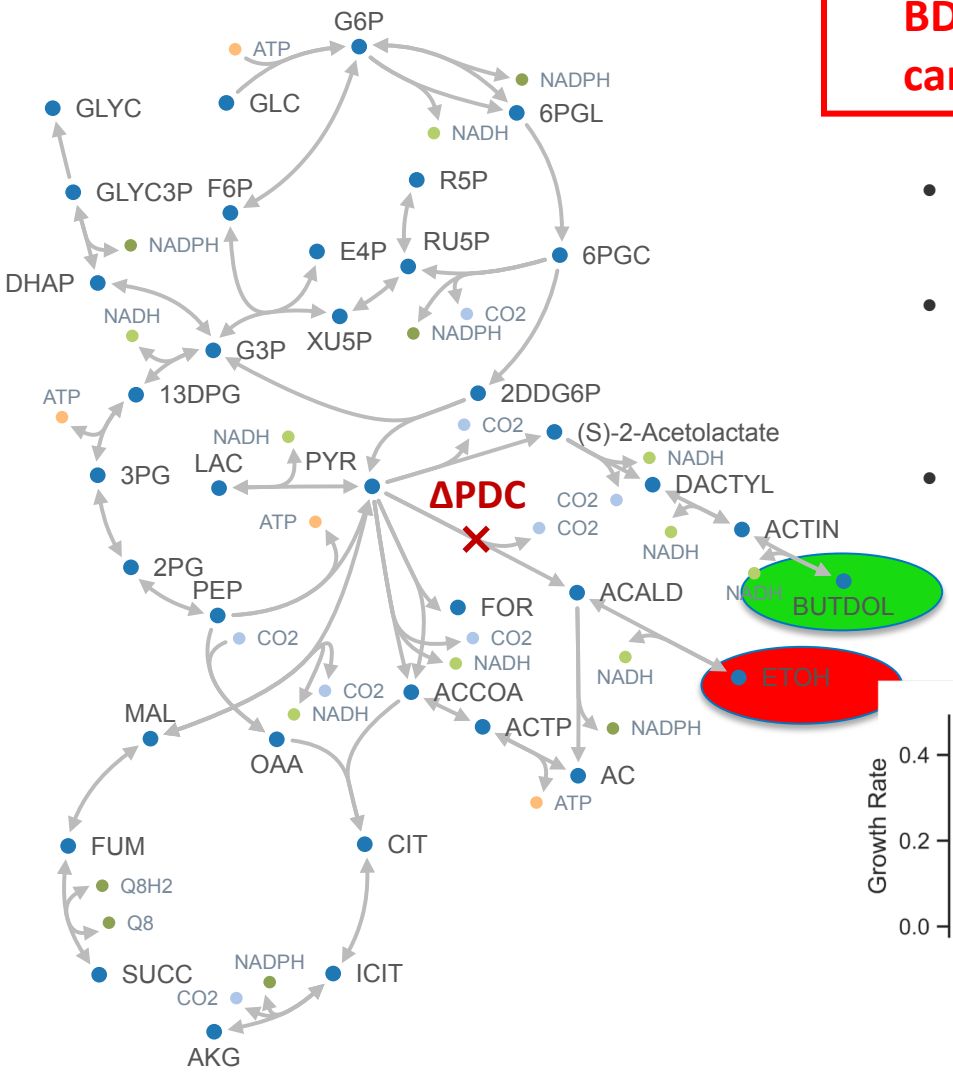


3 – Progress 2,3 BDO Upgrading

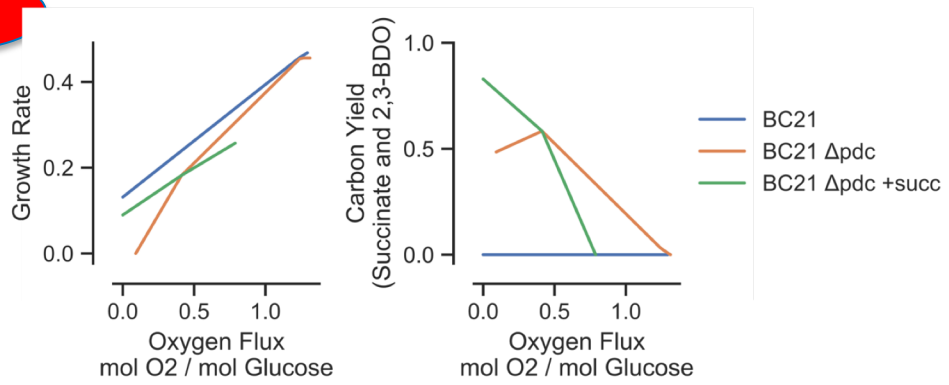
O_2 Requirements of 2,3-BDO

RELEVANCE:

- **Found feasible pathways for anaerobic 2,3-BDO production, quantified maximum carbon yield and growth rate**



- 2,3 BDO fermentation requires an external electron acceptor, i.e., oxygen
- Succinic acid can be catalytically converted to 1,4-BDO, and can serve as an electron acceptor
- Modeled 2,3-BDO (with optional succinate production) as a function of external O_2 concentration

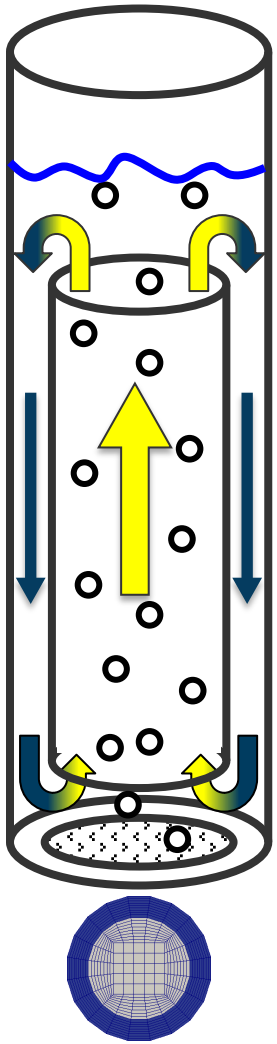


3 – Progress – Aerobic Bioreactor

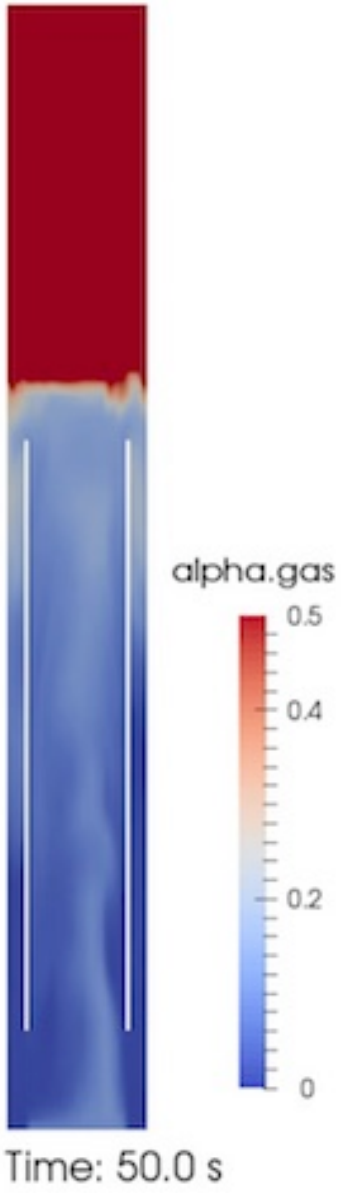
Aerobic Bioreactor CFD

RELEVANCE: Enable (micro-)aerobic pathways and inform techno-economic analysis

- **Adapted OpenFOAM two-phase solver**
  - Gas-liquid mass transfer
  - Oxygen depletion in the liquid (mimicking microorganism metabolism)
- **Tested bubble-column reactor types**
  - Central inlet reactor
  - Draft-tube air-lift reactor
- **Simulations of aeration rates** needed to achieve specific oxygen-transfer rates—important to determine reactor costs
  - Commercial-scale reactors have improved OTR because higher head pressures increase O_2 saturation
  - CFD results confirmed empirical-engineering calculations for costs.

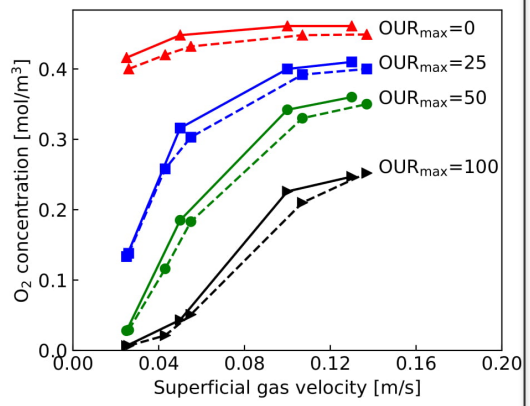


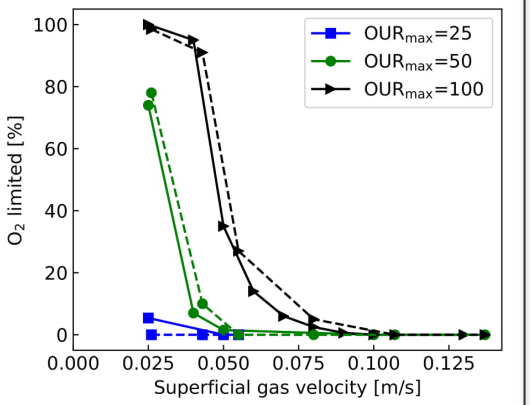
Draft-tube air-lift reactor



3 – Progress – Aerobic Reactor Design

Bubble and Airlift





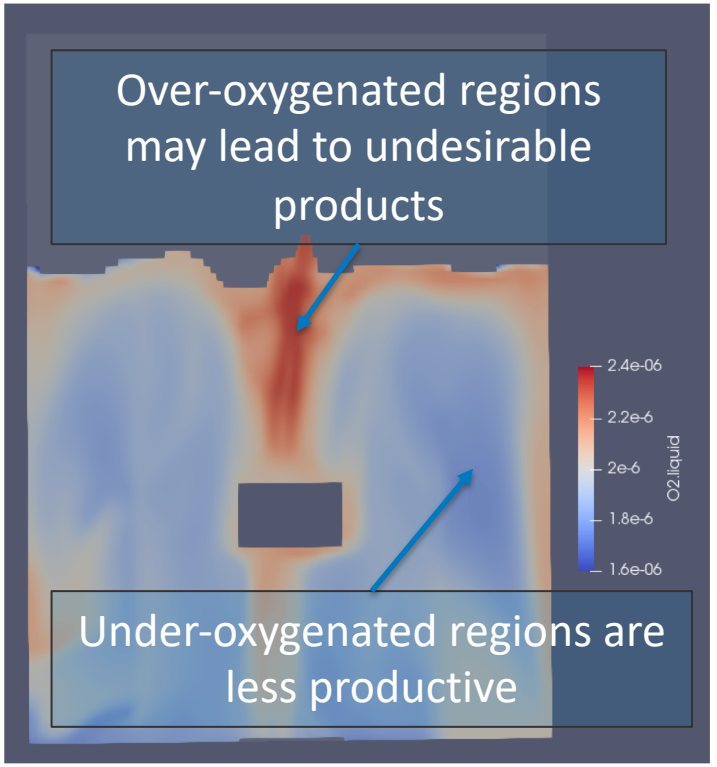
Oxygen concentration (top) and oxygen-limited volume fraction (bottom) v superficial velocity for large bubble column reactor (solid) and airlift reactor (dashed) at different oxygen uptake rates.

CSTR

Continuing development of dynamic model in more reactor geometries, focused on scale-up of micro-aerobic fermentation pathways

Implemented dynamic model in a rotational geometry to simulate a 1000L CSTR with a Rushton impeller.

The product distribution of BDO-producing organisms is highly sensitive to aerobic environment. Homogeneity across the reactor is important for translating high-titers at the bench to productivity at scale.



3 – Progress – Completed

14 collaborations finished in FY17-FY18

8 collaborative research projects in progress

Listed in Addendum 3

4 – Relevance

4 – Relevance

Reduce Cost of Research and Time-to-Solution

- Membrane transport modeling and screening for **sugar and lignin upgrading**
- Membrane composition screening and design for **determining and removing toxicity** of reactants and products
- **Lignin-upgrading** enzymes designed for more substrates, increasing carbon efficiency
- **Plastic-degrading enzymes** designed for circular economy
- **New Polymers** co-designed with degrading pathways in mind
- Explain mechanism of action for enhancing deconstruction enzymes, **reducing cost of hydrolysis** steps
- **New Omics Methods** developed for DBTL cycles – more accurate, more efficient
- Reactor models predict outcomes outside of experiment, **lowering uncertainties of TEA**
- Reactor modeling to quantify **viability of current micro-aerobic pathways**

4 – Relevance

Discover NEW solutions to bottlenecks

- Discovered possible solutions for BDO production redox imbalance
- Determined optimal fatty-acid derivatives for production in continuous production mode

Provide NEW insights

- **TEA** enhanced by accurate models; can now accurately include many reactor design variables at full industrial scale
- Knockouts considered lethal provide **higher productivity**
- **Lignin demethylation** step enzyme active site essentials uncovered → increase range of effectiveness, **increase carbon utilization**

Technology Transfer

- Lignin Force Field and Builder publicly available for all lignin-related molecular modeling
- Omics methods to be released for public use
- Reactor models are publicly available for industrial use
- Record of Invention, publications

5 – Future Work

5 – Future Work

- Automate learn-and-design methodology using ensemble-based kinetic models and derive optimal changes to enzyme expression from metabolomics, proteomics, and fluxomics measurements in *P. putida*
- Lignin-derived polymers
- Enzymatic degradation of plastics – broadening substrate specificity
- Molecular interactions of lignin with biomass polysaccharides
- Design of chemically recyclable polymers
- Engineering catabolic enzymes for upgrading of lignin degradation products
- De-risk scale-up of micro-aerobic fermentation through reactor design and simulation

5 – Future Work

GO/NOGO Q2 FY20

BDO titer and yield increases through prediction and modeling.

Develop complementary metabolic modeling and CFD methodologies (set of predicted metabolic pathway modifications, sugar feeding rates, oxygen sparging rates, reactor designs) A GO decision indicates continuation as planned. A NOGO decision can take different directions, pursuant to discussion with DOE. One direction could be to reevaluate the methodologies used and modify as needed another direction could be refocus efforts elsewhere where metabolic modeling and CFT could have a greater impact.

There are three major directions of research toward predictions for improvement:

1) Metabolic modeling leading to prediction of pathways to reduce the need for sparging.

2) Dynamic process modeling to develop timing sequences for oxygen sparging and other process parameters

3) Reactor design for maximum sparging control leading to specific aeration rates that produce maximum yield and titer.

Criteria: Produces at least a 10% increase in 2,3 BDO titer or 10% increase in yield of 2,3 BDO over current strains from either glucose, xylose, arabinose, or all of these C5/C6 sugars. This project will improve yield through a combination of metabolic pathway, dynamic modeling for sparging rates and timing, and sparging reactor design.

6 – Summary

Summary

Overview: Solve Bottlnecks where theory and modeling are appropriate in enzyme design, metabolic pathways, industrial-scale reactors, and TEA analysis

Approach: Use Quantum, Molecular, and Finite-Element methods to model, understand, and predict solutions for bottlenecks in **close collaboration and iteration with experimental and engineering BETO projects**

Progress/Accomplishments

Membrane permeability of upgrading reactants and products	Machine-learning approaches for microbe design
Lignin model for complete biomass modeling	Solutions for BDO production
Plastic-degrading enzyme design	Aerobic bioreactor conditions optimized
increased enzymatic processing of lignin: Ferulic acid esterase mechanism	Metabolic model of low-pH yeast to design optimal C5 and C6 sugars for acids and esters
GH5 cellulase engineering for thermostability	Mechanistic enzymatic hydrolysis model explaining rate slowdown
GH45 cellulase mechanism (FY18 Q4 milestone met)	Low-order phenomenological model for implementation in CFD and process models

Summary

Relevance: Reduced cost of research and time-to-solution **reduces cost and time-to-market for bioproducts**. Codesign of plastics and enzymes enabling circular bio-economy. Decrease toxicity of bioprocess intermediates and products drives cost down in upgrading

Future Work: Automate learn-and-design methodology, derive optimal changes to enzyme expression, design lignin-derived polymers, broaden enzyme specificity in plastics degradation and lignin upgrading, design bio-derived chemically recyclable polymers, de-risk scale-up of micro-aerobic fermentation

Thank You

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This work was authored by the National Renewable Energy Laboratory, operated by Alliance for Sustainable Energy, LLC, for the U.S. Department of Energy (DOE) under Contract No. DE-AC36-08GO28308. Funding provided by U.S. Department of Energy Office of Energy Efficiency and BioEnergy Technologies Office. The views expressed in the article do not necessarily represent the views of the DOE or the U.S. Government. The U.S. Government retains and the publisher, by accepting the article for publication, acknowledges that the U.S. Government retains a nonexclusive, paid-up, irrevocable, worldwide license to publish or reproduce the published form of this work, or allow others to do so, for U.S. Government purposes.

Additional Slides

Responses to Previous Reviewers' Comments

- If your project is an on-going project that was reviewed previously, address 1-3 significant questions/criticisms from the previous reviewers' comments (refer to the 2017 Peer Review Report, see notes section below)
- Also provide highlights from any Go/No-Go Reviews

Note: This slide is for the use of the Peer Reviewers only – it is not to be presented as part of your oral presentation. These Additional Slides will be included in the copy of your presentation that will be made available to the Reviewers.

Addendum 3 – Progress – Completed

- Task1: Membrane design via small molecule permeability
  - Fatty acids, terpenoids, lignin derivatives
- Task1: Parameterization of lignin forcefield
- Task1: Lignin model builder
- Task1: Enzymatic recycling of plastics
- Task1: Enzymatic upgrading of lignin degradation products
- Task2: Analyzed omics data to suggest strain designs for improved muconate production
- Task2: Designed pathways for 2,3-BDO production under limited O_2 environments
- Task3: Built a validated platform for interrogating aerobic bioreactors at scale with multi-phase transport and reaction

Addendum 3 – Progress – Work in Progress

- Molecular mechanism of (rate-limiting) dissociation of processive cellulases
- Lignin solvation dependence on molecular structure
- Membrane permeability of lignin monomers
- Binding of GcoA to biological membranes
- Ion diffusion within biomass
- Cis/trans lipid isomerization – impact on membrane structure and permeability
- ReaxFF
- Application of bioreactor models to novel reactors for micro-aerobic fermentation

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- Brandon Knott, Josh Vermaas, Jerry Ståhlberg, Gregg Beckham, Mike Crowley, Computational Insights into the Catalytic Function of Processive Cellulases. Invited Talk. American Institute of Chemical Engineers Annual Meeting, Pittsburgh, PA. October 2018.

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