JGI JOINT GENOME INSTITUTE
A DOE OFFICE OF SCIENCE USER FACILITY

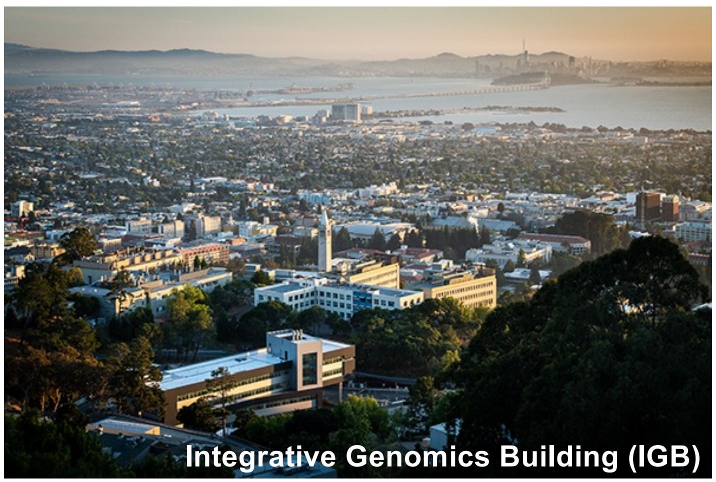
# Opportunities for Facility-Enabled Science at the DOE Joint Genome Institute (JGI)

**Yasuo Yoshikuni, Ph.D.**
**Head, DNA Synthesis Science Program**
**12/20/2024**

国立大学法人
東京農工大学
Tokyo University of Agriculture and Technology

# Overview of Research Focus Areas

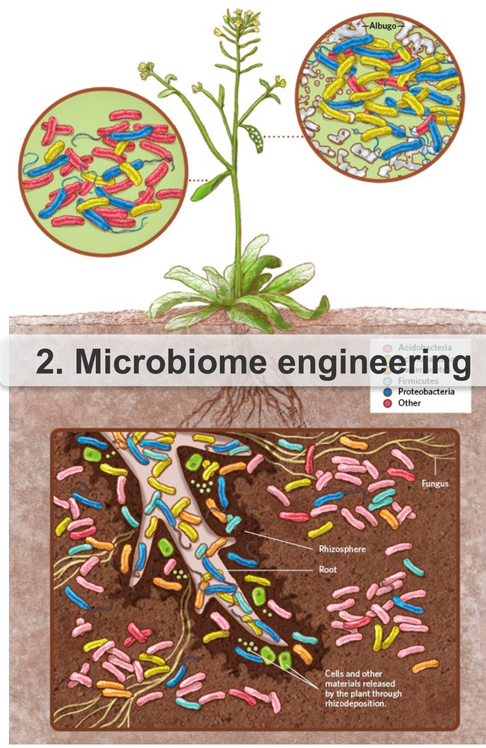
## DNA Synthesis Science Program




## Yoshikuni Research Group

### Biomass-side

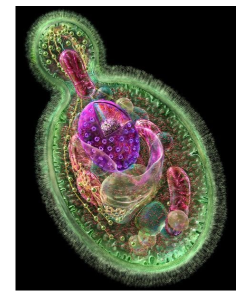
1. Plant N-utilization



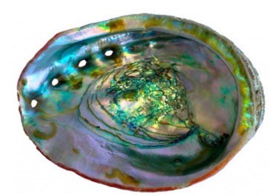

2. Microbiome engineering

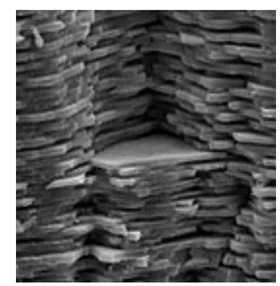
### Product-side

3. Biofuel and Bioproducts



4. Hybrid Biomaterials





# Overview of Research Focus Areas

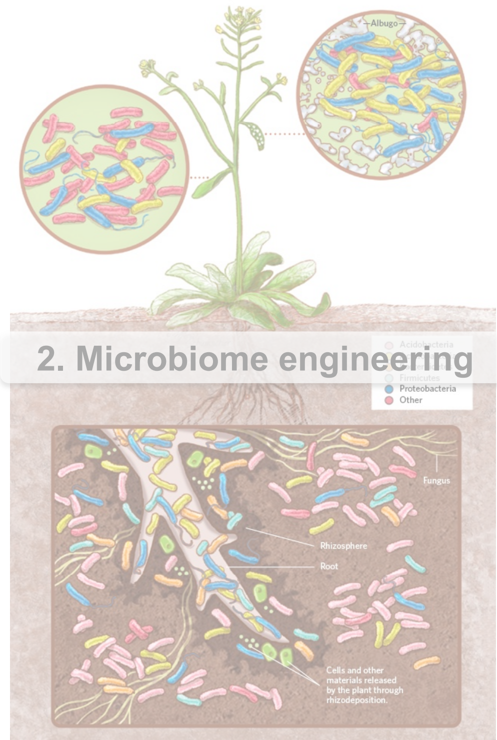
## DNA Synthesis Science Program

JOINT GENOME INSTITUTE
DEPARTMENT OF ENERGY


## Yoshikuni Research Group

### Biomass-side

1. Plant N-utilization

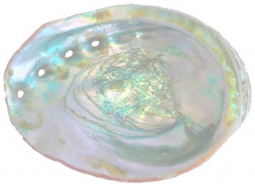


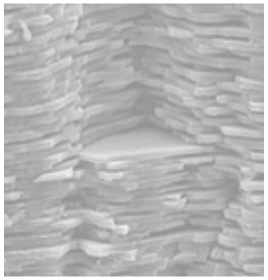

2. Microbiome engineering

### Product-side

3. Biofuel and Bioproducts

4. Hybrid Biomaterials





# The DOE Joint Genome Institute


**JGI MISSION:**

To provide the global research community with ***free access*** to the most advanced integrative genome science capabilities in support of the DOE energy & environmental research mission

**U.S. Department of Energy Office of Science User Facility**

- **JGI established in 1997, User facility from 2004**
- **Located at Lawrence Berkeley National Laboratory**
- **~285 staff; $91.6 M annual funding**
- **2,038 Global Primary Users; >10,000 Data Users**

# DNA Synthesis Science Program

JGI JOINT GENOME INSTITUTE

## Mission Statement

**To harness the power of DNA synthesis, strain engineering, and biosystems design for DOE mission relevant discovery and applications**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Our Science | Our Projects | Data & Tools | User Programs | News & Publications
Calls for User Proposals | Special Initiatives & Programs | Product Offerings | User Support | Submit a Proposal

User Programs
Home › User Programs › User Program Info › CSP Overview › CSP Functional Genomics

Calls for User Proposals
CSP Overview
CSP Annual Call
CSP New Investigator
CSP Functional Genomics
FICUS Overview
Closed Calls
Review Process and Scoring Criteria
DOE Mission Relevance
FAQ

More topics:
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News
Science Highlights
Blog
Webinars
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Featured Profiles

CSP Functional Genomics

About This Call

SUBMIT

The CSP Functional Genomics call is to enable users to perform state-of-the-art functional genomics research and to help them translate genomic information into biological function. The emphasis is on projects leveraging JGI capabilities to enhance understanding of gene and genome function, particularly those not readily achievable without the capabilities and expertise available at the JGI.

Proposals to this call may be submitted to the JGI at any time using a simple web-based form (begin a new proposal document at proposals.jgi.doe.gov to see the proposal format) and are reviewed twice a year. All proposals will undergo an internal pre-review to ensure technical feasibility and alignment with JGI and DOE missions. Screened proposals will be reviewed for scientific merit, DOE relevance, feasibility and impact. Applicants are encouraged to review the Synthetic Biology Internal Review Process guidelines, as insufficient information will delay or potentially defer approval for the proposal.

For questions about whether your project is appropriate, or for program specifics or technical guidance, please contact Yasuo Yoshikuni (DNA synthesis program head), Trent Northen (metabolomics group lead), Tanja Woyke, Interim Deputy for User Programs, or Miranda Harmon-Smith (project manager). For questions about the application process, please contact Miranda Harmon-Smith.

JGI Engagement: Accessing Functional Genomics Capabilities Webinar

Watch the JGI Engagement: Accessing Functional Genomics Capabilities Webinar hosted by the DNA Synthesis Science group. The webinar invites researchers to submit white papers to the Community Science Program's Functional Genomics call for proposals.

Current Call (OPEN)

1/30/2025 (DEADLINE)

## Large-scale synbio projects

- 500 kbp DNA synthesis, construct assembly
- Fast-track metabolic engineering
- sgRNA library
- Strain engineering

etc.…

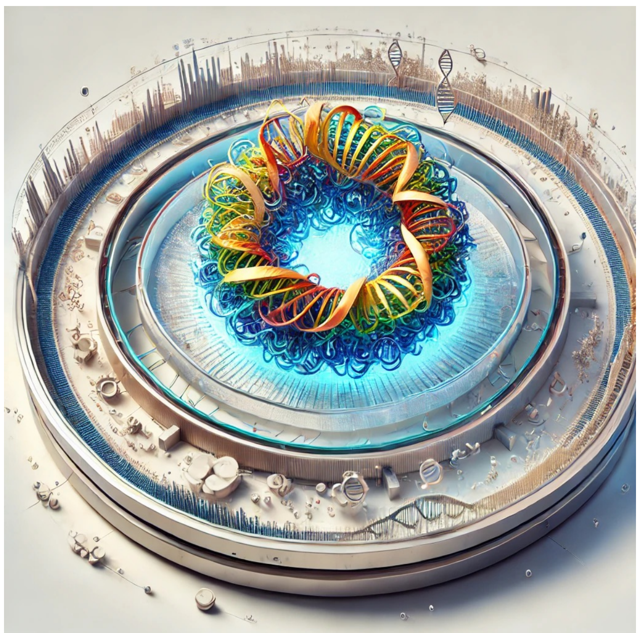
## Key achievements

- > 300 projects
- > 200 publications

*Including Nature, Science, Nature Sister Journals*

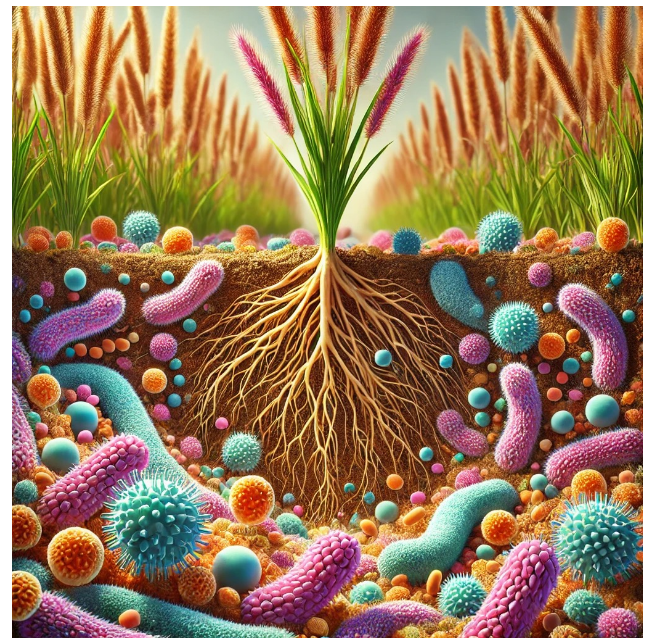
# Strategic Focus Areas – Implementation of 2018 Plan

### *1. Genomes to structure and function*



### *2. High-throughput functional genomics*

### *3. Microbe-microbe and plant-microbe interactions*



Science Portfolio 1

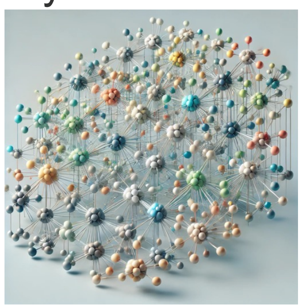
# Genomes to Structure and Function

The program oversees scientific communications with Users and facilitate capability integration

User-Driven

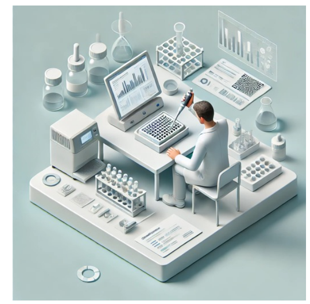
Data Mining


Sequence Data Analysis & Selection



DNA Synthesis

Functional Characterization



BER Structural Biology & Bioimaging

JGI IMG/MER INTEGRATED MICROBIAL GENOMES & MICROBIOMES

Natalia Ivanova

Simon Roux

Platform

Ian Blaby

Metabolomics

Trent Northen

Katherine Louie

SLAC NATIONAL ACCELERATOR LABORATORY

ALS ADVANCED LIGHT SOURCE

Argonne NATIONAL LABORATORY

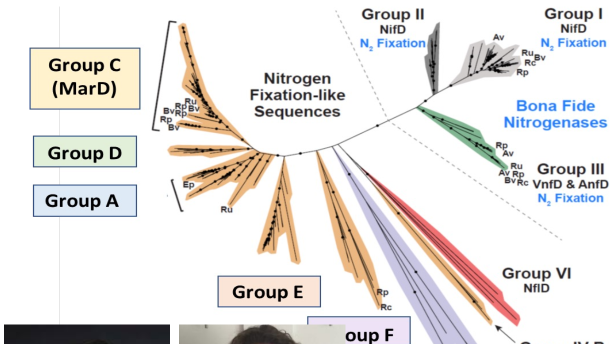
Brookhaven National Laboratory

EMSL

OAK RIDGE National Laboratory

Science Portfolio 1

# Discovery of Ethylene Enzymes for Bioplastics

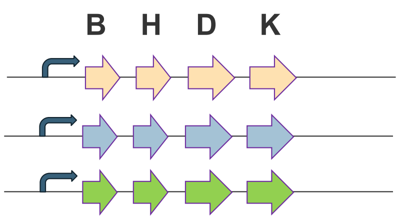





Kelly Wrighton

Emiley Eloe-Fadrosh

## Gene Cluster Assembly



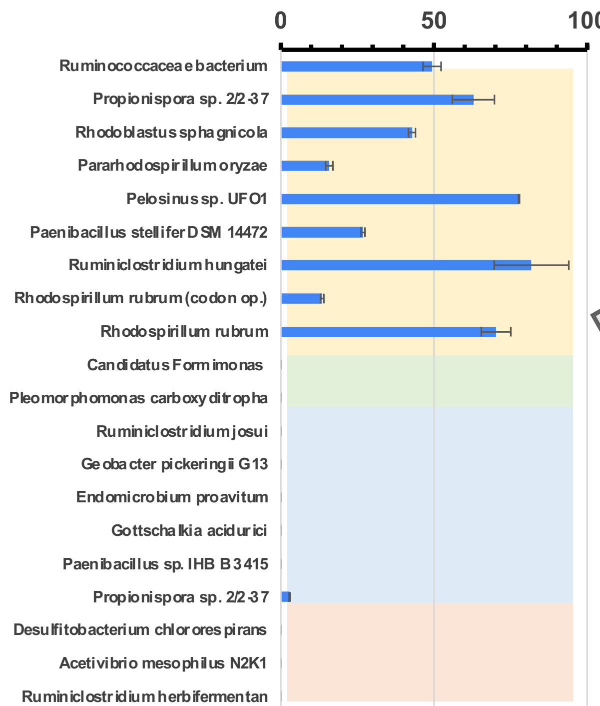


Ian Blaby

## Screen for functional Ethylene Enzymes

Justin North

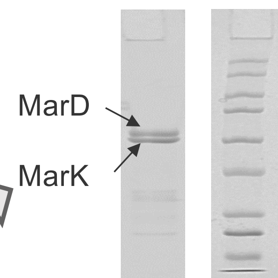

Ethylene Group

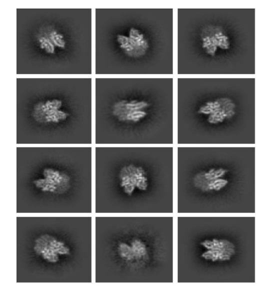



## Structure Determination

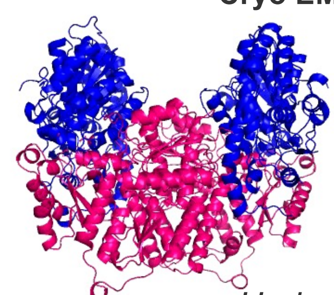
Sean McSweeney

Dale Kreitler



Cryo EM



*Under review*

# Other Examples

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**Challenge:** Integration of capabilities across facilities

**Results:** Demonstrated the power of capability integration

ACS central science

**Intersubunit Coupling Enables Fast $CO_2$-Fixation by Reductive Carboxylases**

Hasan DeMirci, Yashas Rao, Gabriele M. Stoffel, Bastian Vögeli, Kristina Schell, Aharon Gomez, Alexander Batyuk, Cornelius Gati, Raymond G. Sierra, Mark S. Hunter, E. Han Dao, Halil I. Ciftci, Brandon Hayes, Fredric Poitevin, Po-Nan Li, Manat Kaur, Kensuke Tono, David Adrian Saez, Samuel Deutsch, Yasuo Yoshikuni, Helmut Grubmüller, Tobias J. Erb,* Esteban Vöhringer-Martinez,* and Soichi Wakatsuki*

ACS central science

**A Synthetic Gene Library Yields a Previously Unknown Glycoside Phosphorylase That Degrades and Assembles Poly-β-1,3-GlcNAc, Completing the Suite of β-Linked GlcNAc Polysaccharides**

Spencer S. Macdonald, Jose H. Pereira, Feng Liu, Gregor Tegl, Andy DeGiovanni, Jacob F. Wardman, Samuel Deutsch, Yasuo Yoshikuni, Paul D. Adams, and Stephen G. Withers*

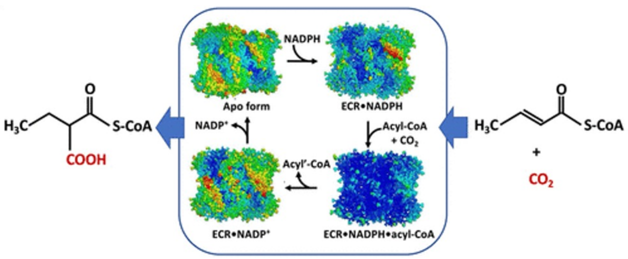
communications biology

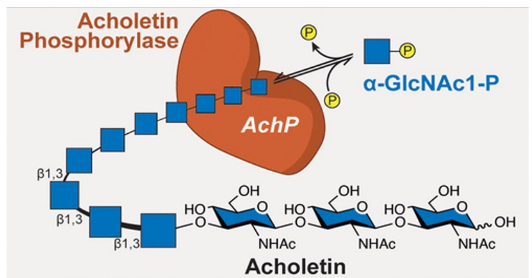
https://doi.org/10.1038/s42004-024-01242-y

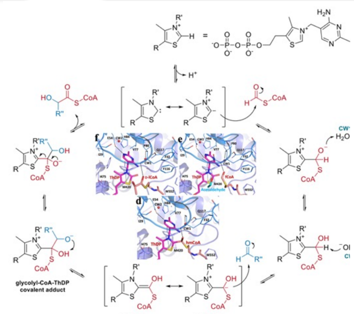
**Revealing reaction intermediates in one-carbon elongation by thiamine diphosphate/CoA-dependent enzyme family**

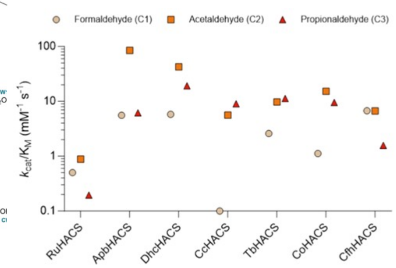
Check for updates

Youngchang Kim[1,2], Seung Hwan Lee[3], Priyanka Gade[2], Maren Nattermann[4], Natalia Maltseva[2], Michael Endres[1], Jing Chen[3], Philipp Wichmann[4], Yang Hu[3], Daniel G. Marchal[4], Yasuo Yoshikuni[5], Tobias J. Erb[4,6], Ramon Gonzalez[3], Karolina Michalska[1,2] & Andrzej Joachimiak[1,2,7]







Soichi Wakatsuki
SLAC


Tobias Erb
MAX PLANCK GESELLSCHAFT

The world fastest $CO_2$ fixation enzymes
CSP 1755

**FICUS PILOT**


Steve Withers
UBC


Paul Adams
ALS

Novel glycoside phosphorylase: CSP 2572

**FICUS PILOT**

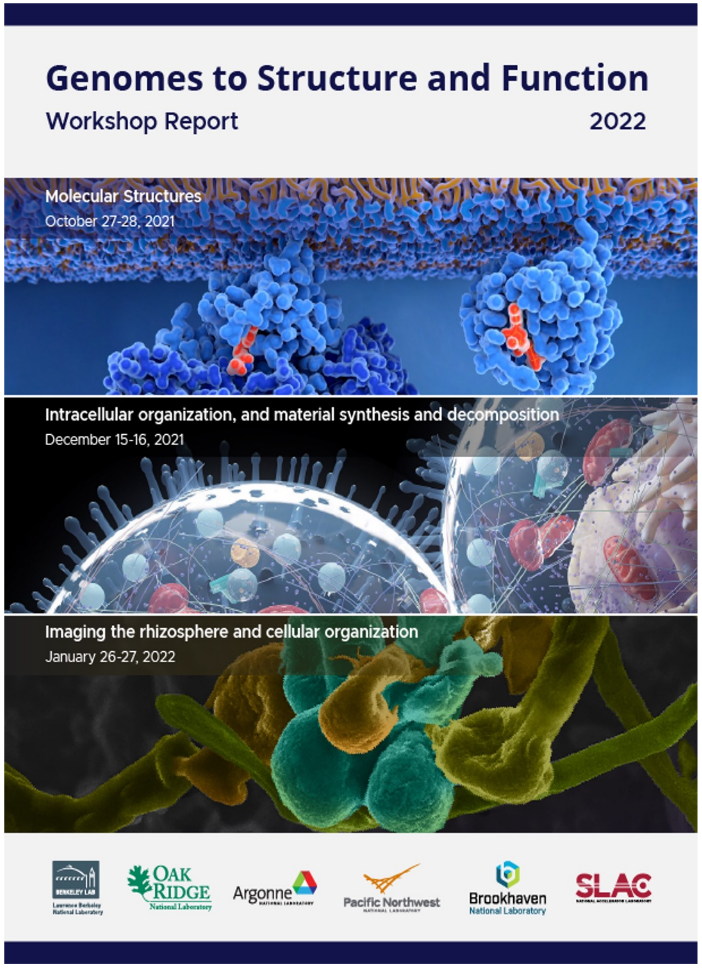

Andrzej Joachimiak
Argonne NATIONAL LABORATORY


Ramon Gonzalez
UNIVERSITY of SOUTH FLORIDA

Synthetic C1-trophy Pathway
CSP 505301

**FICUS PILOT**

# Genomes to Structure and Function Workshop



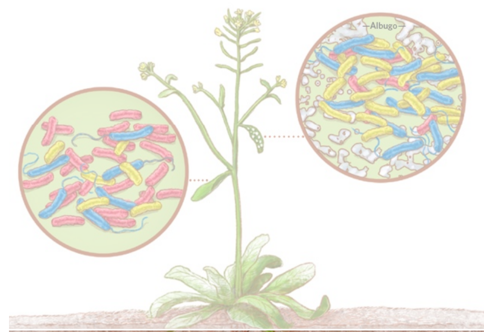

**Challenges and opportunities identified through the workshop**

1. Science
2. Technology development
3. User Facility Integration

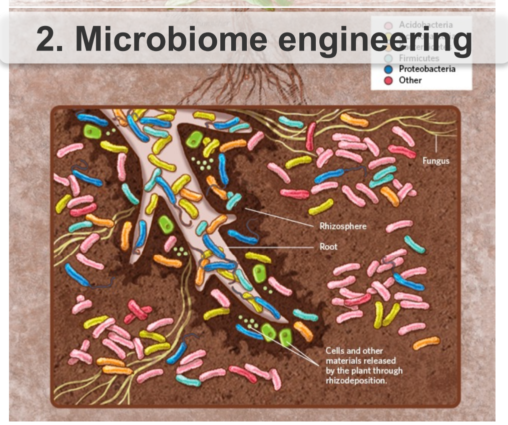
# Overview of Research Focus Areas


## Yoshikuni group

### 1. Plant N-utilization

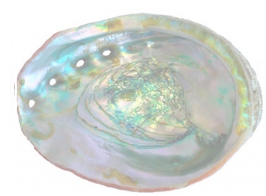


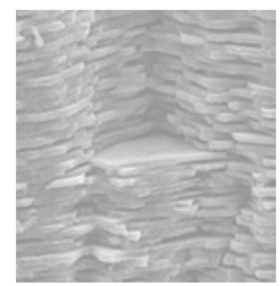

### 2. Microbiome engineering



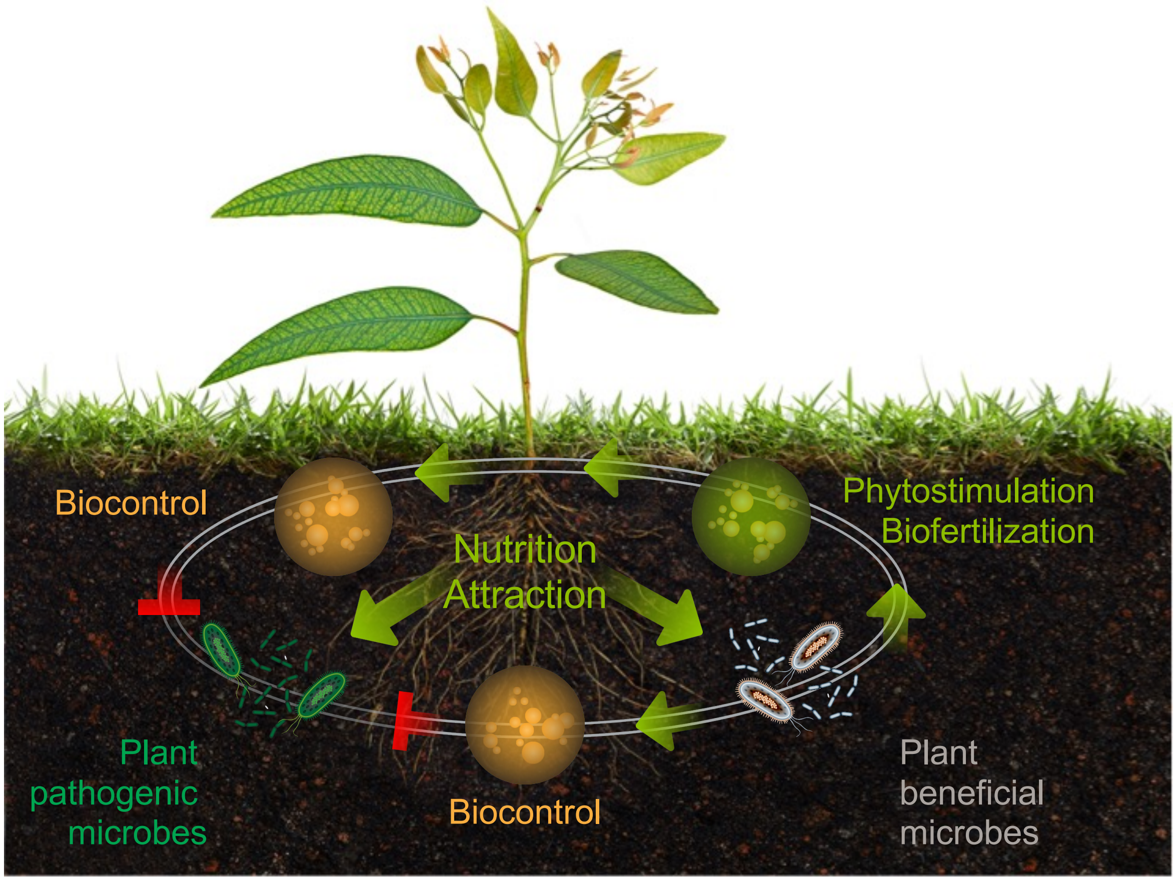

### 3. Biofuel and Bioproducts

### 4. Hybrid Biomaterials





# Plant-Microbe Interactions in Sustainable Agriculture



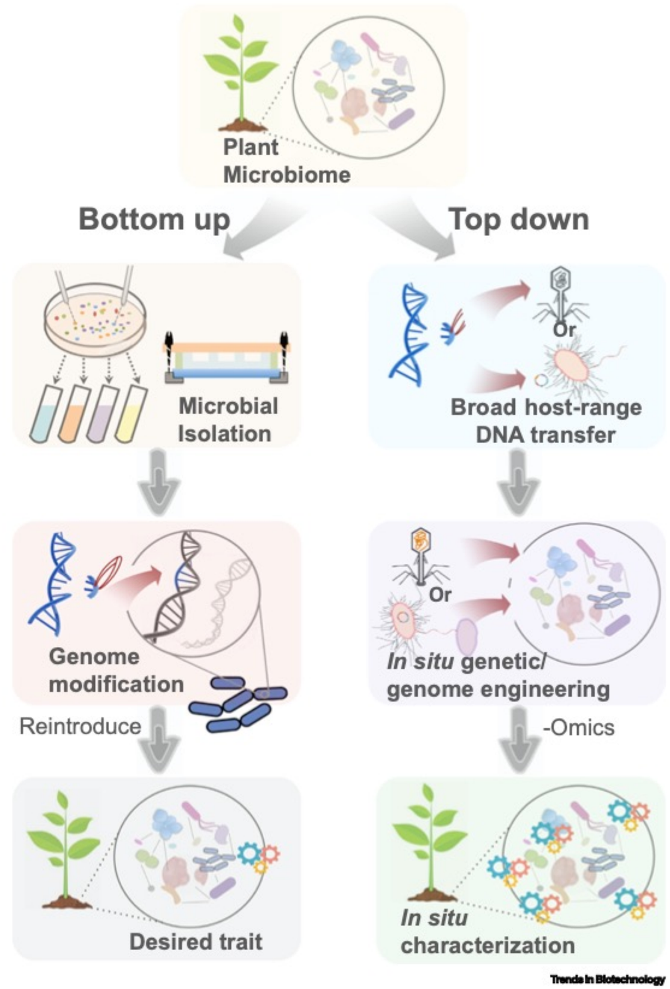
# Microbiome Engineering in Agriculture

Trends in Biotechnology (2021)

Review

## Microbiome Engineering: Synthetic Biology of Plant-Associated Microbiomes in Sustainable Agriculture

Jing Ke,[1,6] Bing Wang,[1,6] and Yasuo Yoshikuni[1,2,3,4,5,*]



# 1. Tool Development for Microbiome Engineering

# Development Of Chassis Independent Recombinase-assisted Genome Engineering (CRAGE)

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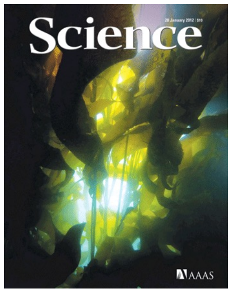



## An Engineered Microbial Platform for Direct Biofuel Production from Brown Macroalgae

Adam J. Wargacki,[1*] Effendi Leonard,[1*] Maung Nyan Win,[1*] Drew D. Regitsky,[1] Christine Nicole S. Santos,[1] Peter B. Kim,[1] Susan R. Cooper,[1] Ryan M. Raisner,[1] Asael Herman,[1†] Alicia B. Sivitz,[1‡] Arun Lakshmanaswamy,[1] Yuki Kashiyama,[1,2,3] David Baker,[4] Yasuo Yoshikuni[1§]

ACS Synthetic Biology
pubs.acs.org/synthbio
Technical Note

## CRAGE-Duet Facilitates Modular Assembly of Biological Systems for Studying Plant–Microbe Interactions

Bing Wang,[¶] Zhiying Zhao,[¶] Lauren K. Jabusch, Dawn M. Chiniquy, Koyo Ono, Jonathan M. Conway, Zheyun Zhang, Gaoyan Wang, David Robinson, Jan-Fang Cheng, Jeffery L. Dangl, Trent R. Northen, and Yasuo Yoshikuni*

Cite This: ACS Synth. Biol. 2020, 9, 2610–2615
Read Online


nature communications

ARTICLE

Received 24 Feb 2013 | Accepted 23 Aug 2013 | Published 23 Sep 2013 DOI: 10.1038/ncomms3503

## Implementation of stable and complex biological systems through recombinase-assisted genome engineering

Christine Nicole S. Santos[1,†], Drew D. Regitsky[1,†] & Yasuo Yoshikuni[1]

nature protocols

PROTOCOL

## Engineering complex biological systems in bacteria through recombinase-assisted genome engineering

Christine Nicole S Santos[1,4] & Yasuo Yoshikuni[1–3]

[1]Bio Architecture Lab, Inc., Berkeley, California, USA. [2]BALChile S.A., Santiago, Chile. [3]BAL Biofuels S.A., Santiago, Chile. [4]Present address: Manus Biosynthesis, Inc., Cambridge, Massachusetts, USA. Correspondence should be addressed to Y.Y. (yoshikuni@ba-lab.com).

PLOS ONE

RESEARCH ARTICLE

## Bacterial genome editing by coupling Cre-*lox* and CRISPR-Cas9 systems

Hualan Liu[1,2☯], David S. Robinson[1☯], Zong-Yen Wu[1,3], Rita Kuo[1], Yasuo Yoshikuni[1,2,4], Ian K. Blaby[1,2], Jan-Fang Cheng[1,2*]

1 US Department of Energy Joint Genome Institute, Berkeley, California, United States of America, 2 Environmental Genomics and Systems Biology Division, Lawrence Berkeley National Laboratory, Berkeley, California, United States of America, 3 Department of Veterinary Medicine, National Chung Hsing University, Taichung, Taiwan, ROC, 4 Biological Systems and Engineering Division, Lawrence Berkeley National Laboratory, Berkeley, California, United States of America

ELSEVIER
Contents lists available at ScienceDirect
Metabolic Engineering
journal homepage: www.elsevier.com/locate/meteng

## Development of platforms for functional characterization and production of phenazines using a multi-chassis approach via CRAGE

Jing Ke[a], Zhiying Zhao[a], Cameron R. Coates[a,b], Michalis Hadjithomas[a], Andrea Kuftin[a], Katherine Louie[a], David Weller[c,d], Linda Thomashow[c,d], Nigel J. Mouncey[a,e], Trent R. Northen[a,e], Yasuo Yoshikuni[a,b,e,f,g,*]

Cell Chemical Biology CellPress

Resource

## CRAGE-CRISPR facilitates rapid activation of secondary metabolite biosynthetic gene clusters in bacteria

Jing Ke,[1,6] David Robinson,[1,6] Zong-Yen Wu,[1] Andrea Kuftin,[1] Katherine Louie,[1] Suzanne Kosina,[1] Trent Northen,[1,2] Jan-Fang Cheng,[1,2,*] and Yasuo Yoshikuni[1,2,3,4,5,7,*]

ARTICLES
https://doi.org/10.1038/s41564-019-0573-8
nature microbiology

## CRAGE enables rapid activation of biosynthetic gene clusters in undomesticated bacteria

Gaoyan Wang[1,10], Zhiying Zhao[1,10], Jing Ke[1,10], Yvonne Engel[2,10], Yi-Ming Shi[2,10], David Robinson[1], Kerem Bingol[3], Zheyun Zhang[1], Benjamin Bowen[1,4], Katherine Louie[1], Bing Wang[1], Robert Evans[1], Yu Miyamoto[1], Kelly Cheng[1], Suzanne Kosina[4], Markus De Raad[4], Leslie Silva[1], Alicia Luhrs[5], Andrea Lubbe[5], David W. Hoyt[3], Charles Francavilla[5], Hiroshi Otani[1,4], Samuel Deutsch[1,4,6], Nancy M. Washton[3], Edward M. Rubin[1], Nigel J. Mouncey[1,4], Axel Visel[1,4], Trent Northen[1,4], Jan-Fang Cheng[1,4], Helge B. Bode[1,7*] and Yasuo Yoshikuni[1,4,6,8,9*]

SYNTHETIC BIOLOGY

Synthetic Biology, 2020, 5(1): ysaa015
doi: 10.1093/synbio/ysaa015
Advance Access Publication Date: 3 September 2020
Research Article

## CRAGE-mediated insertion of fluorescent chromosomal markers for accurate and scalable measurement of co-culture dynamics in *Escherichia coli*

Avery J.C. Noonan[1], Yilin Qiu[1], Joe C.H. Ho[2], Jewel Ocampo[2], K.A. Vreugdenhil[1], R. Alexander Marr[1], Zhiying Zhao[3], Yasuo Yoshikuni[3,4,5,6,7], and Steven J. Hallam[1,2,8,9,10,11,*]

AMERICAN SOCIETY FOR MICROBIOLOGY Applied and Environmental Microbiology®

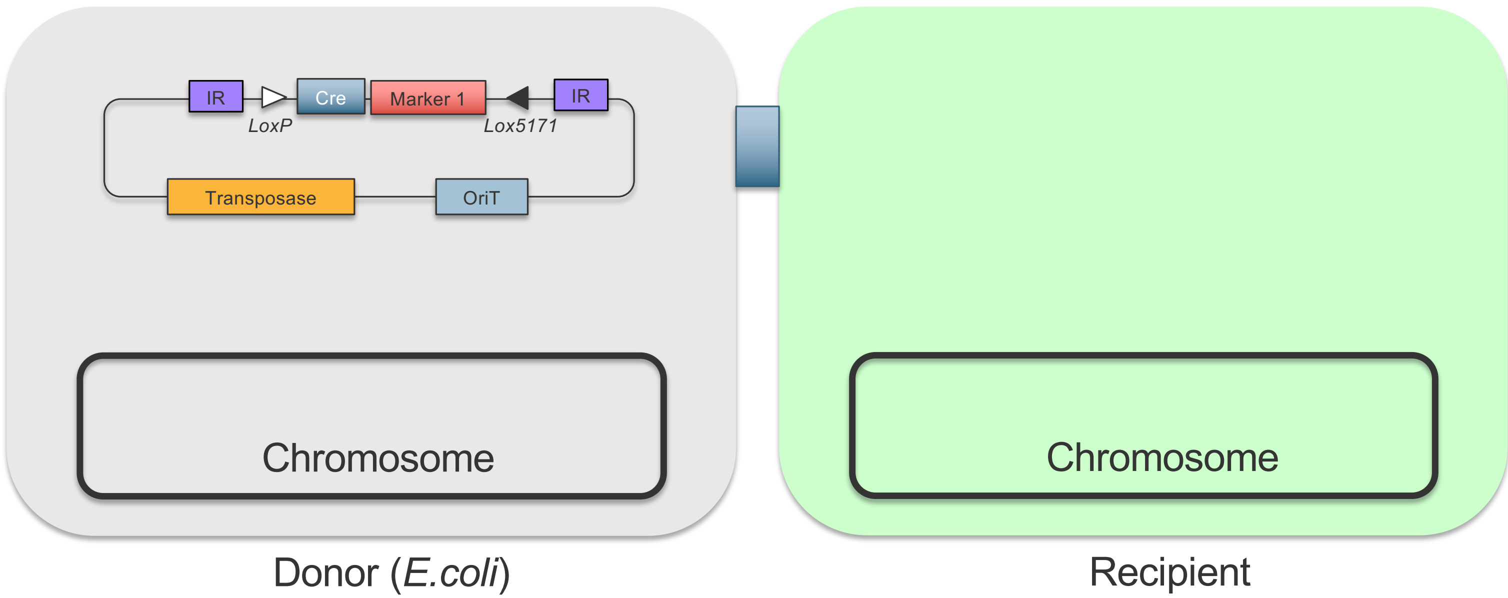
BIOTECHNOLOGY
September 2019 Volume 85 Issue 18 e01210-19
https://doi.org/10.1128/AEM.01210-19

## Engineered Root Bacteria Release Plant-Available Phosphate from Phytate

Christine N. Shulse, Mansi Chovatia, Carolyn Agosto, Gaoyan Wang, Matthew Hamilton, Samuel Deutsch, Yasuo Yoshikuni, Matthew J. Blow

# Step1: Inserting Landing Pad in Recipient Microbes

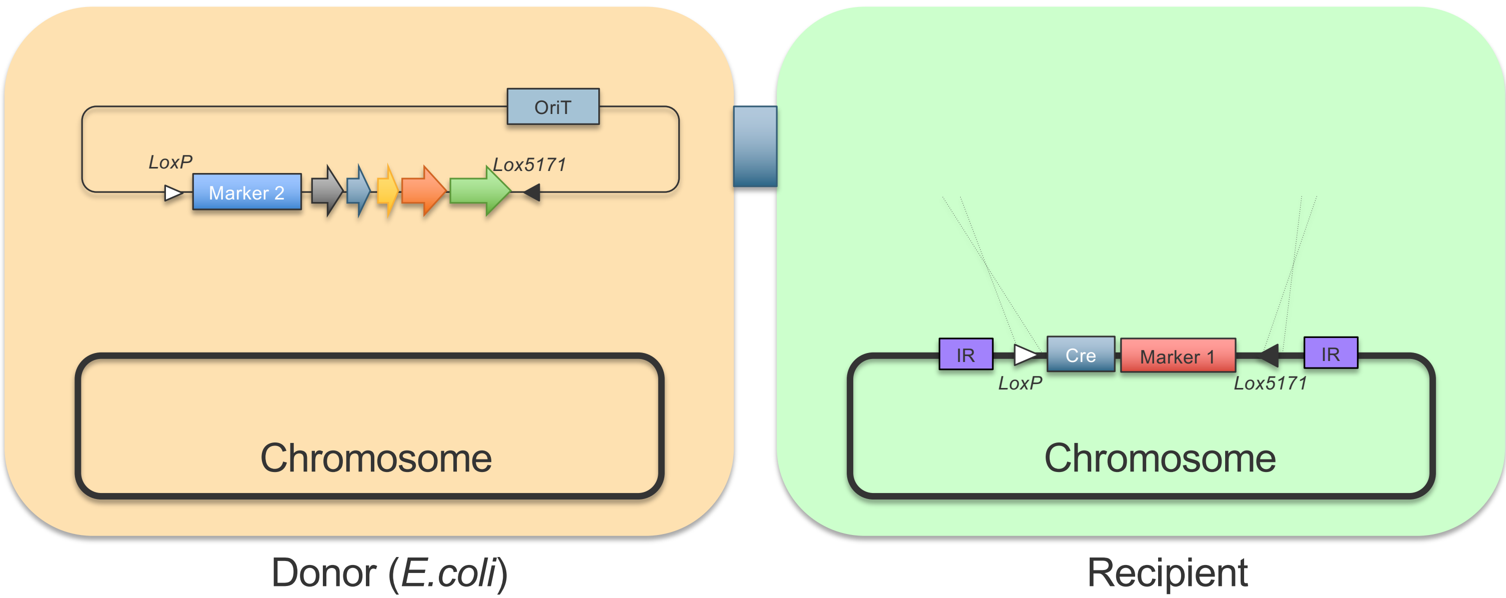
## Step 1. Landing pad insertion into the genome




Santos et al., *Nature Communications* (2013) & *Nature protocols* (2014), Wang et al. *Nature Microbiology* (2019)

# Step 2: Gene Insertion via Cre-loxP

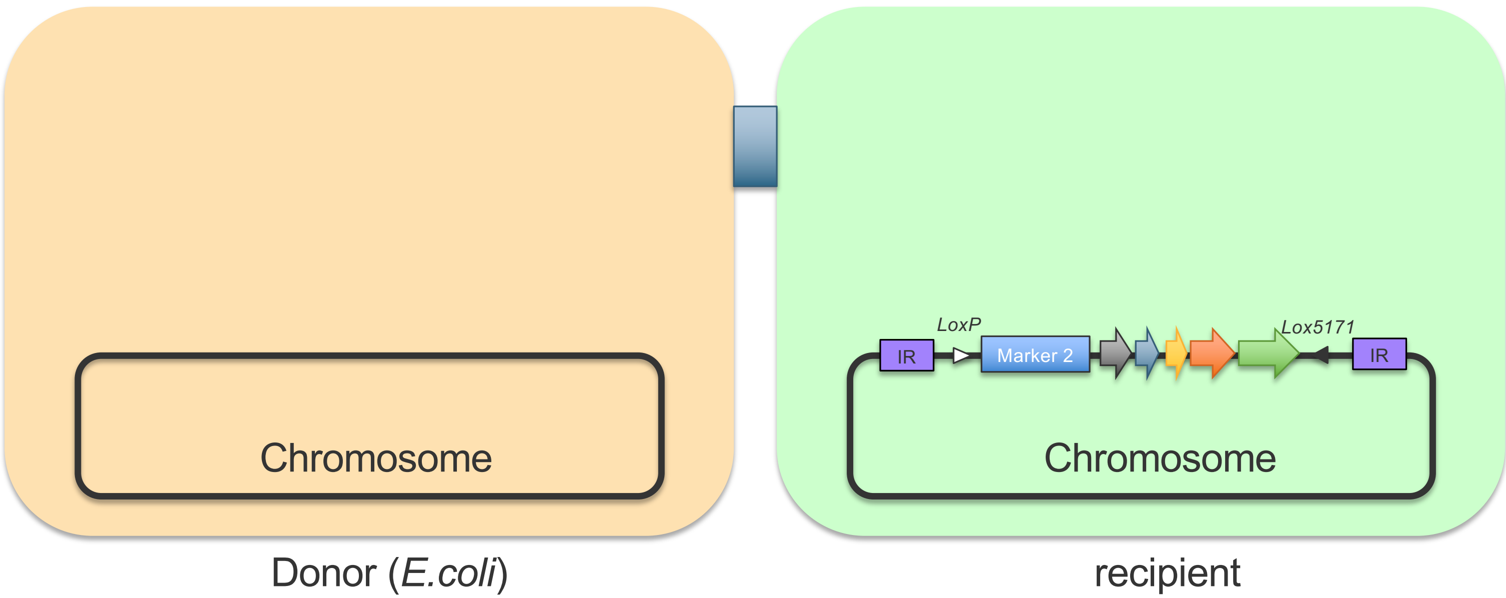
## Step 2. Gene/pathway insertion




Santos et al., *Nature Communications* (2013) & *Nature protocols* (2014), Wang et al. *Nature Microbiology* (2019)

# Step 2: Gene Insertion via Cre-loxP

### Step 2. Gene/pathway insertion

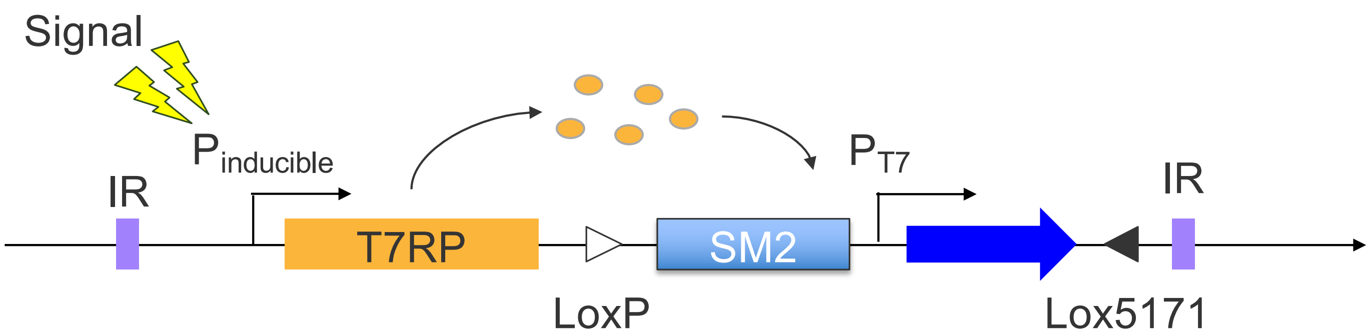



Santos et al., *Nature Communications* (2013) & *Nature protocols* (2014), Wang et al. *Nature Microbiology* (2019)

# Step 3: Expression of Inserted Gene/pathway

A design principle can be standardized

**Step 3. Activation of inserted genes**

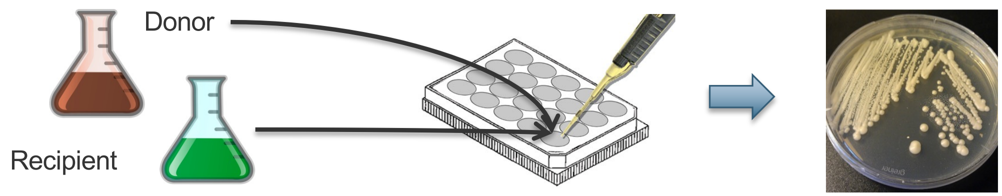





Santos et al., *Nature Communications* (2013) & *Nature protocols* (2014), Wang et al. *Nature Microbiology* (2019)

# CRAGE: Versatile Genome Engineering Tool

- Single-step pathway (~60 kbp) integration
- Transformation is automated and scaled



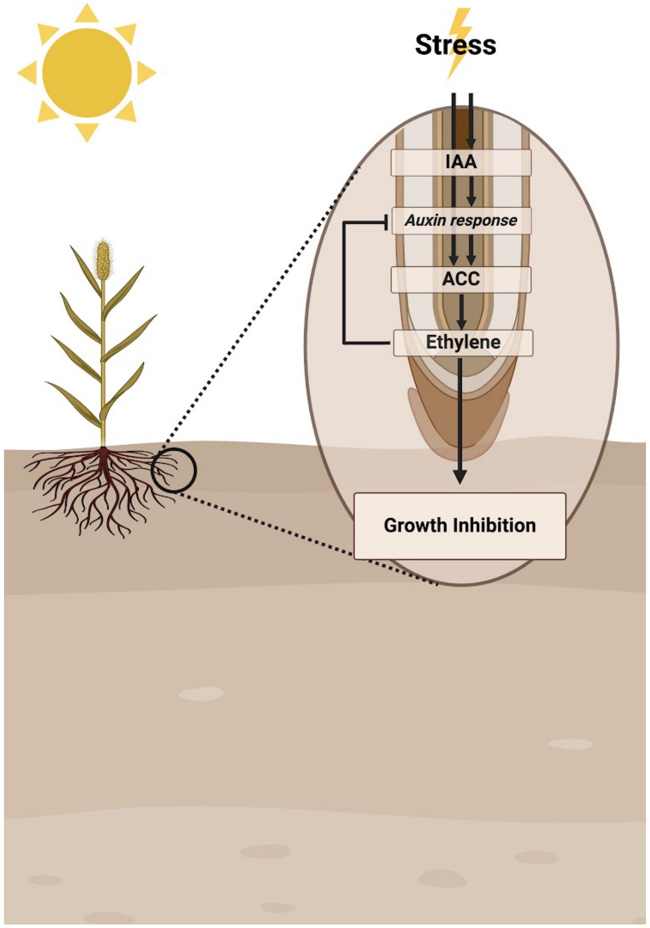

- Standardized design principle (a single shuttle system)
- Dual integration is feasible
- Engineering of 60+ bacterial species
  - α, β, and γ-Proteobacteria
  - Actinobacteria
  - Firmicute
  - Cyanobacteria
  - Bacteroidetes

# Drought Is One Of The Most Critical Issues

- **Drought impacts**
  - 1.5 billion people affected by drought in 2017
  - Loss of US $125 billion globally
  - Estimated impact on 75% of global population by 2050 (UNCCD, 2023)
  - 23 million people severely food insecured (WFP,2023).

UNCCD website

# Microbiome Engineering Has Potential To Reduce The Negative Effect Of Drought

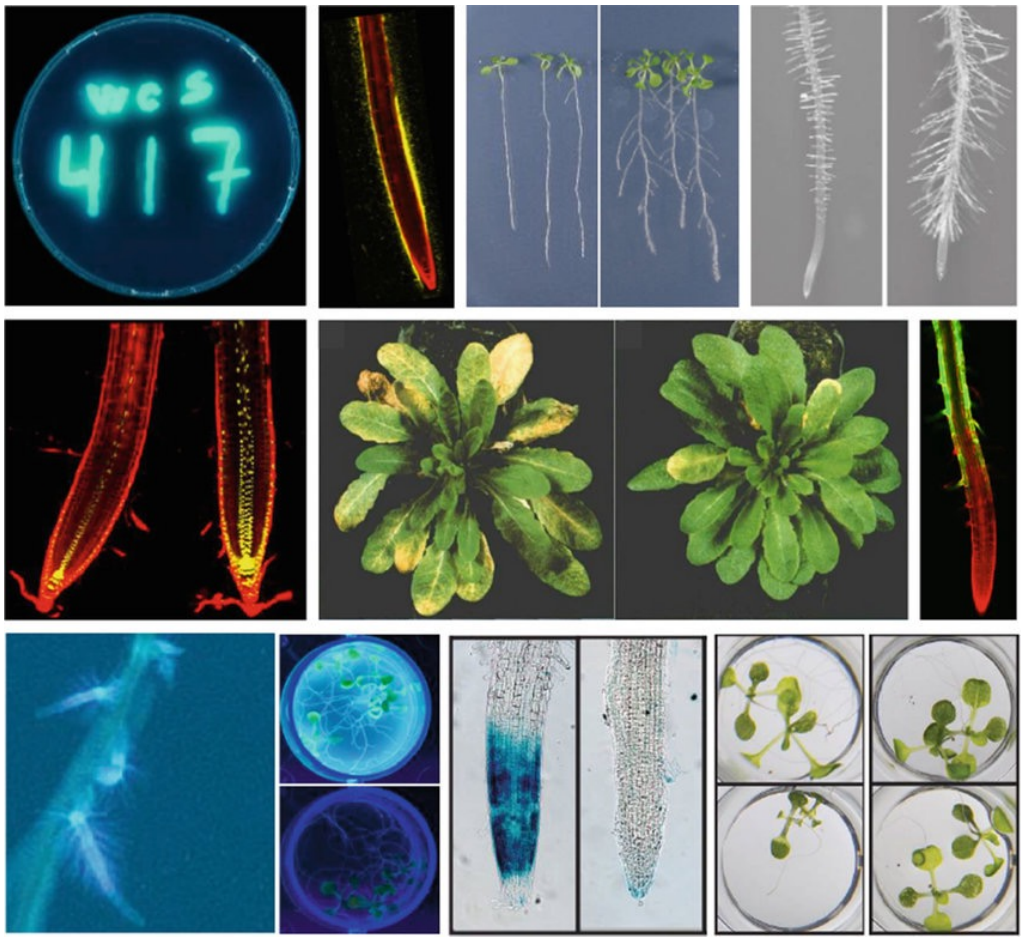


# Design Principles: IAA- and AcdS-producing Syn PGPR

**Chassis Selection**

1. **Persistent colonizer capable of colonizing diverse plant species**

# *Pseudomonas simiae* WCS417 is a Model PGPR

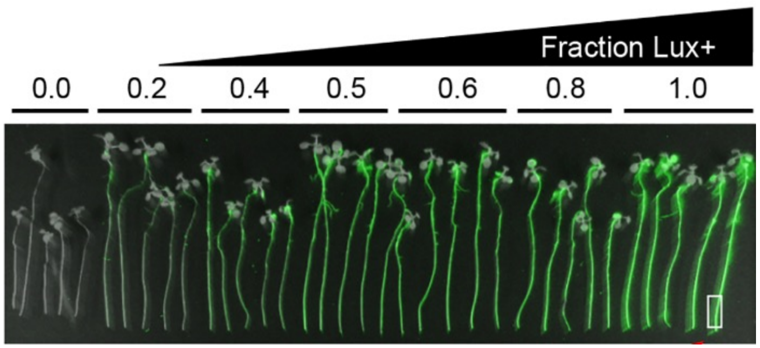



- Originally isolated from wheat fields
- Colonizes diverse plant species
- Controls soil borne diseases
  - Fungi, nematode, bacteria

Pieterse et al., Plant and Soil (2020) 10.1007/s11104-020-04786-9

# *P. simiae* WCS417 Is A Persistent Colonizer Across The Entire Root Systems

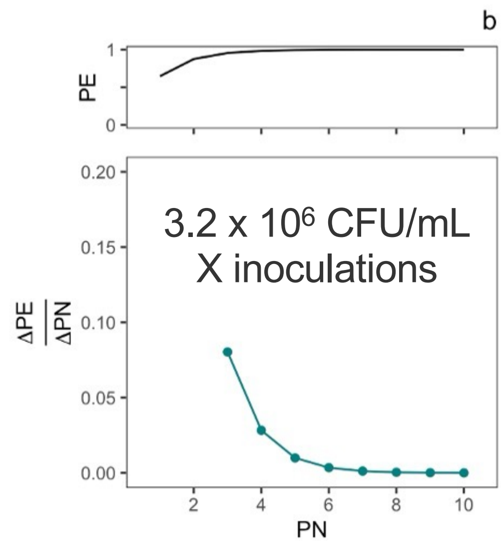
*P. simiae* WCS417 can colonize across the entire root systems




Root colonization of

Cole et al.
PLoS Biiology (2019)

*P. simiae* WCS417 can persists in soil and plant roots




Klimasmith et al. Frontiers in Microobiology (in press)

# Design Principles: IAA- And AcdS-producing Syn PGPR

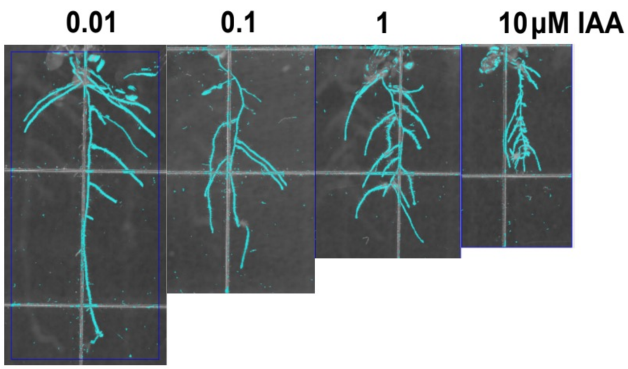
JGI JOINT GENOME INSTITUTE

**Chassis Selection**

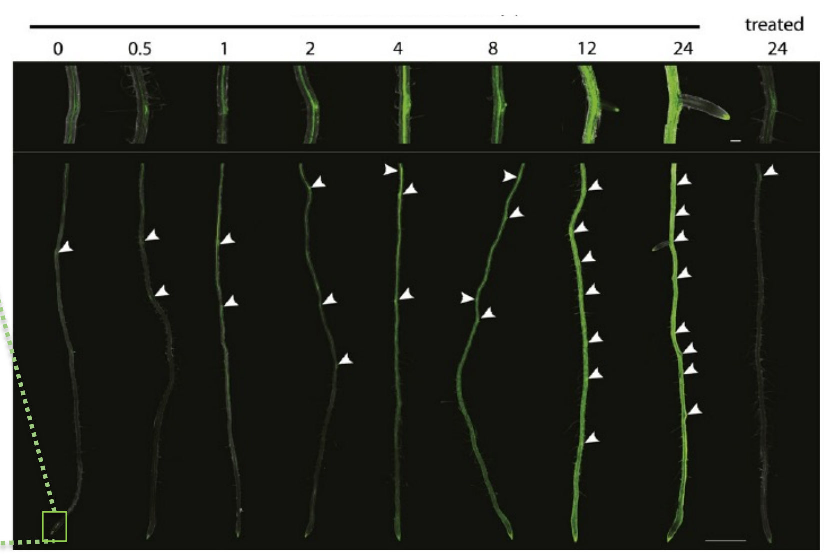
1. Persistent colonizer capable of colonizing diverse plant species
2. Capable of producing IAA and AcdS only around the root tips

# IAA Gradient Is Important For Maintaining The Plant Physiology

- Plant roots are sensitive to IAA
- IAA is concentrated at root tips
- Entire roots are saturated with auxin signal if treated with IAA



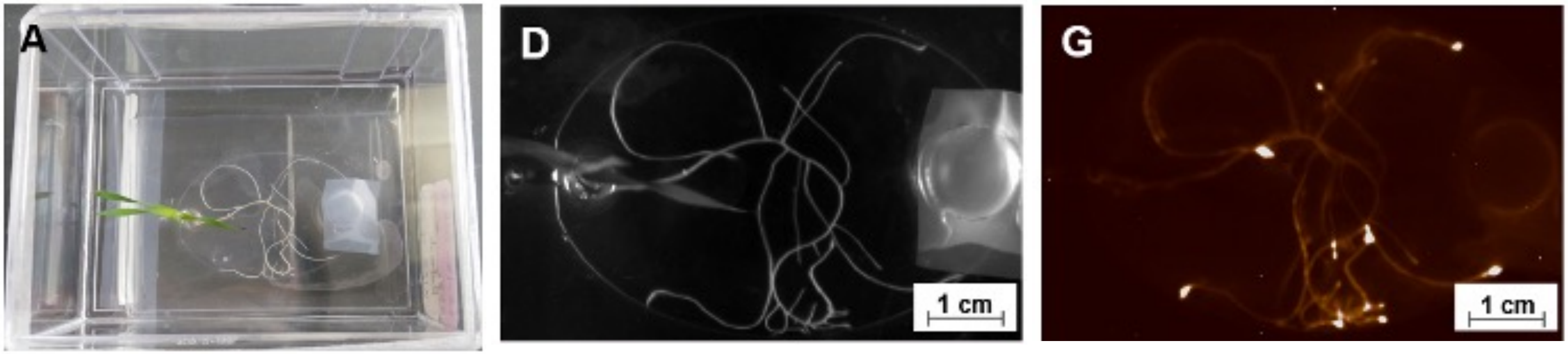

Auxin sensor DR5::GFP



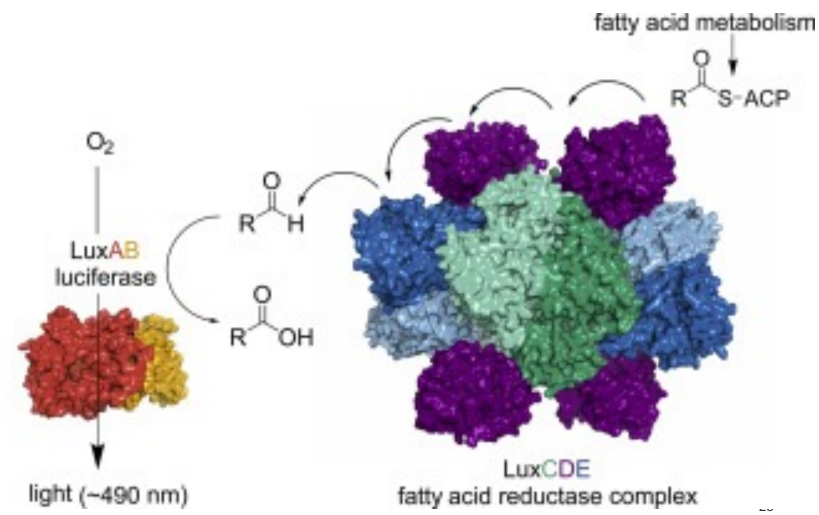

Lewis et al., The Plant Cell (2013) 10.1105/tpc.113.114868

# *P. Simiae* WCS417 May Be Able To Provide IAA Activity Mainly To The Plant Root Tips

JGI JOINT GENOME INSTITUTE




*P. simiae* WCS417 can colonize the root systems, but the only cells around the root tips are physiologically active




**Gao et al., JoVE Journal (2018)**
**10.3791/57170**

# Design Principles: IAA- and AcdS-producing Syn PGPR

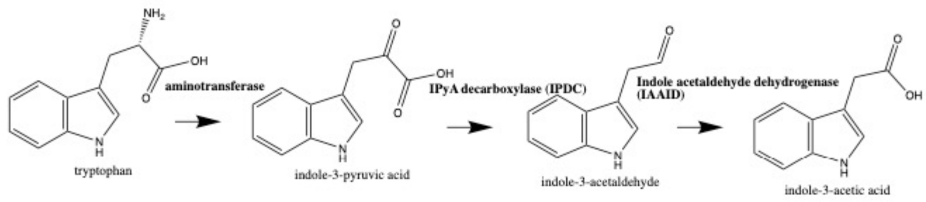
## Chassis Selection

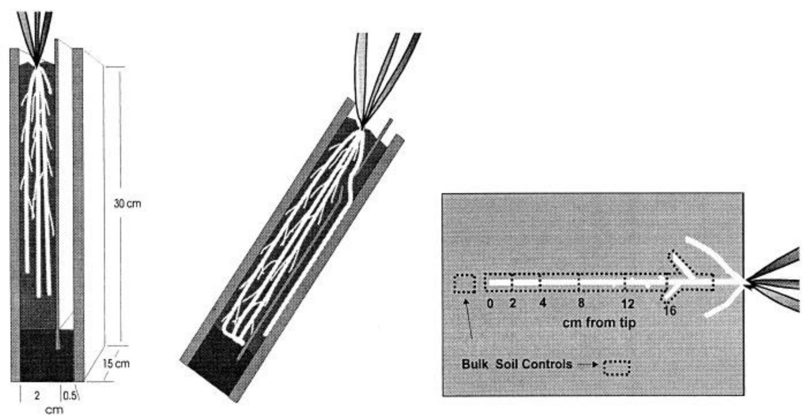
1. Persistent colonizer capable of colonizing diverse plant species
2. Capable of producing IAA and AcdS only around the root tips

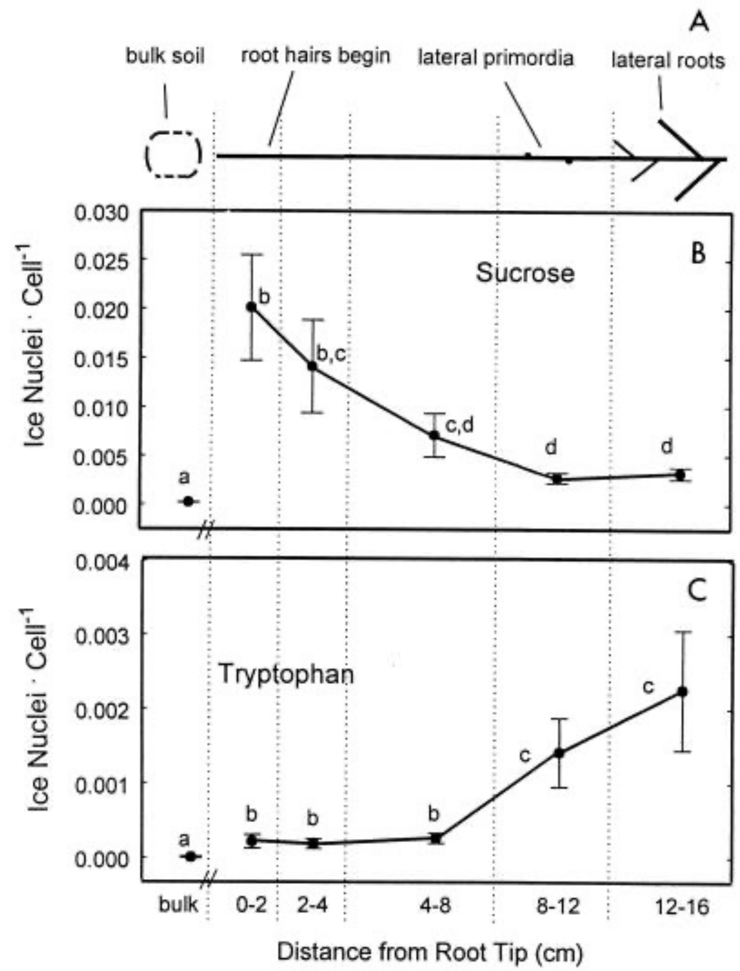
## Pathway selection

1. Robust IAA producer independent of Trp in plant exudates

# Plants Produce Trp More Abundantly From The Parts Closer To Shoot



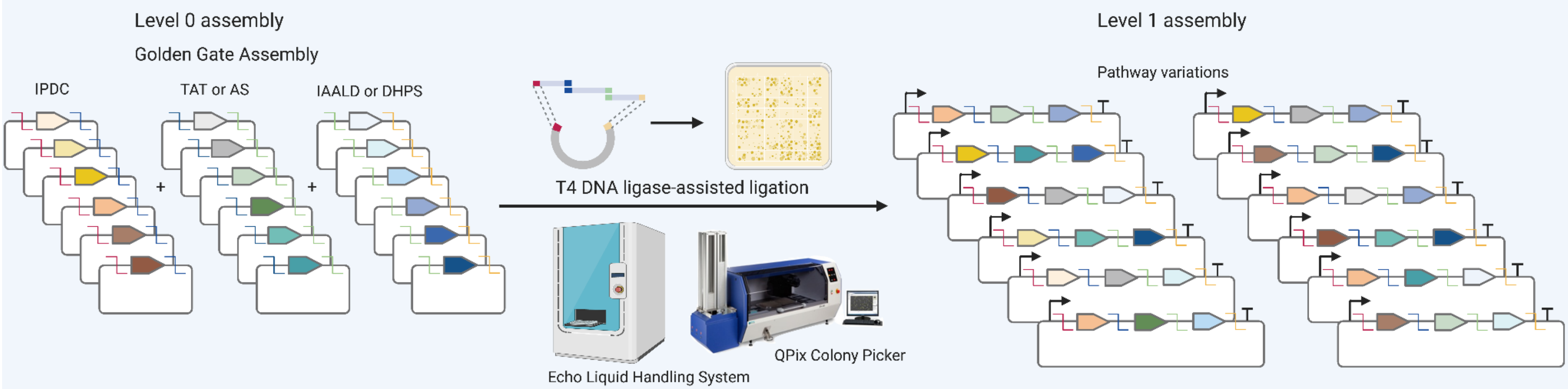



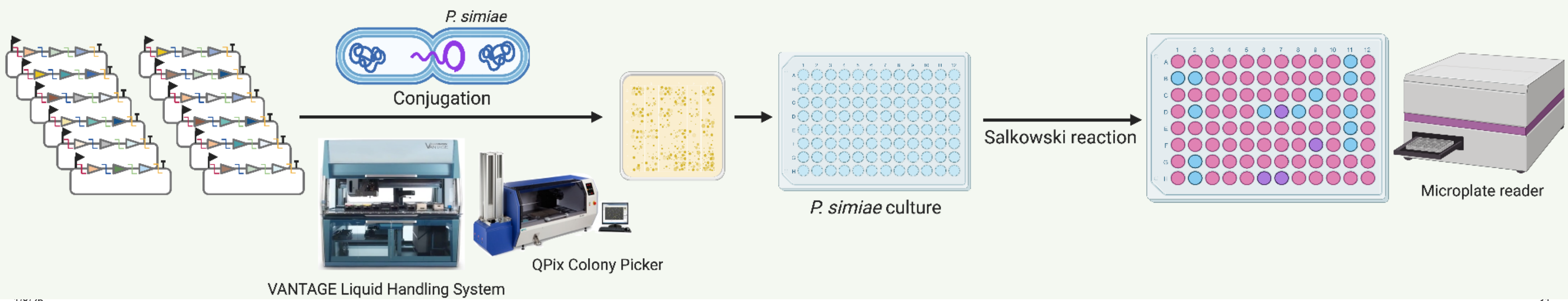

Jaeger III et al., AEM (1999) 10.1128/aem.65.6.2685-2690.1999

# Development Of an Efficient IAA Production Strain

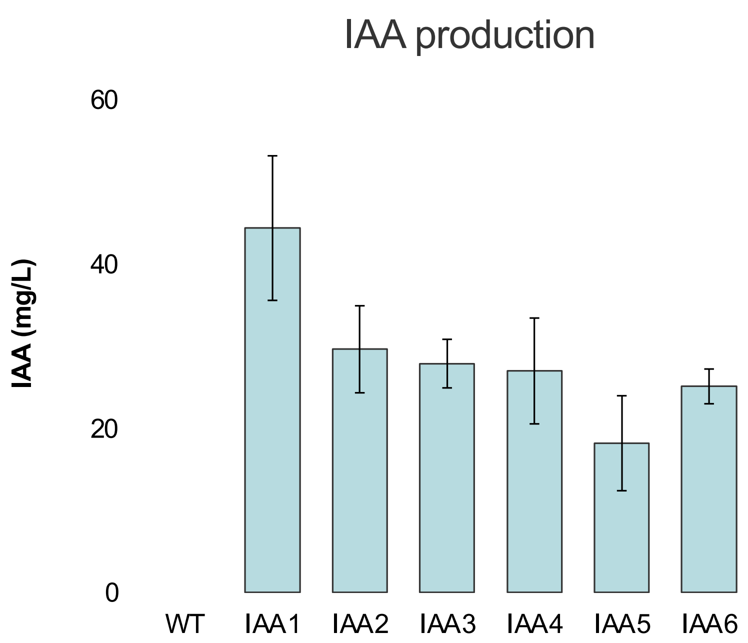
## IAA biosynthesis pathway assembly

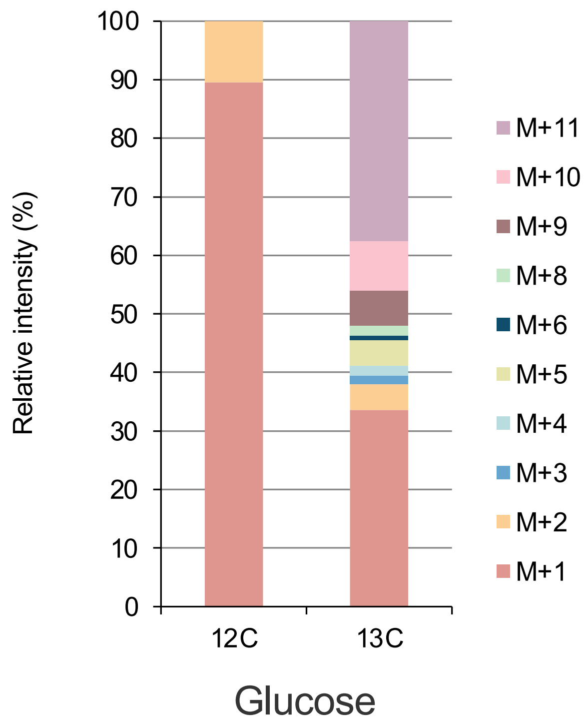



## IAA biosynthesis pathway integration and IAA production measurement



# Development Of An Efficient IAA Production Strain



# Design Principles: IAA- And AcdS-producing Syn PGPR

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## Pathway selection

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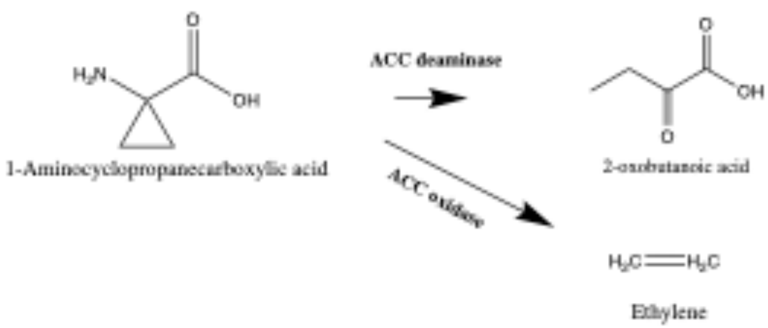
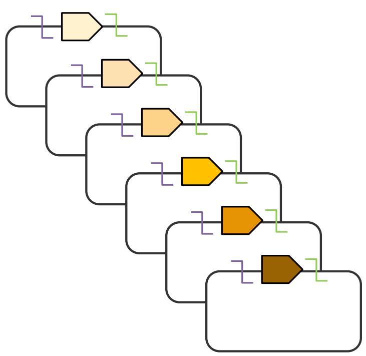
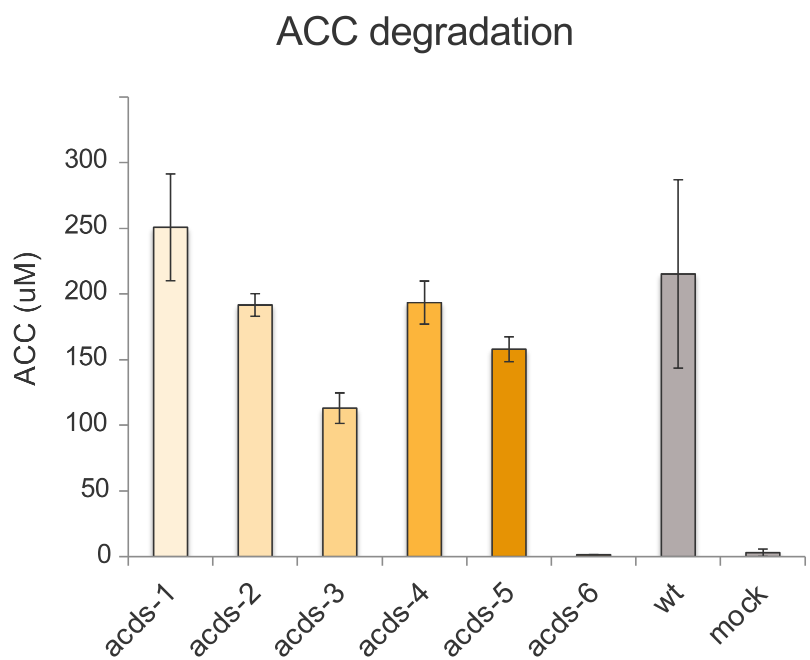
2. Robust AcdS producer

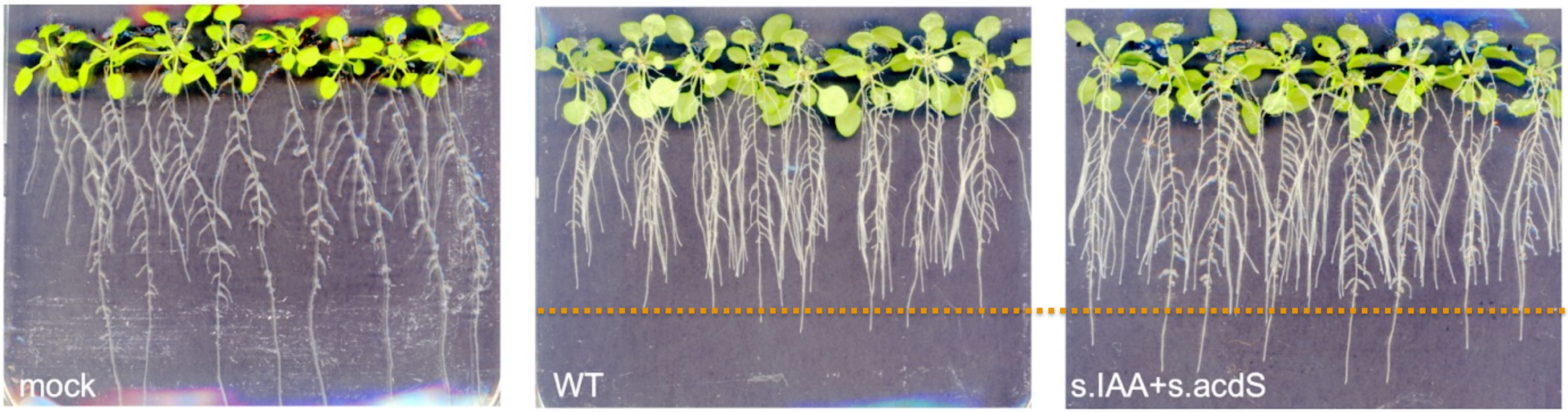
# Development Of An Efficient ACC Deaminating Strain



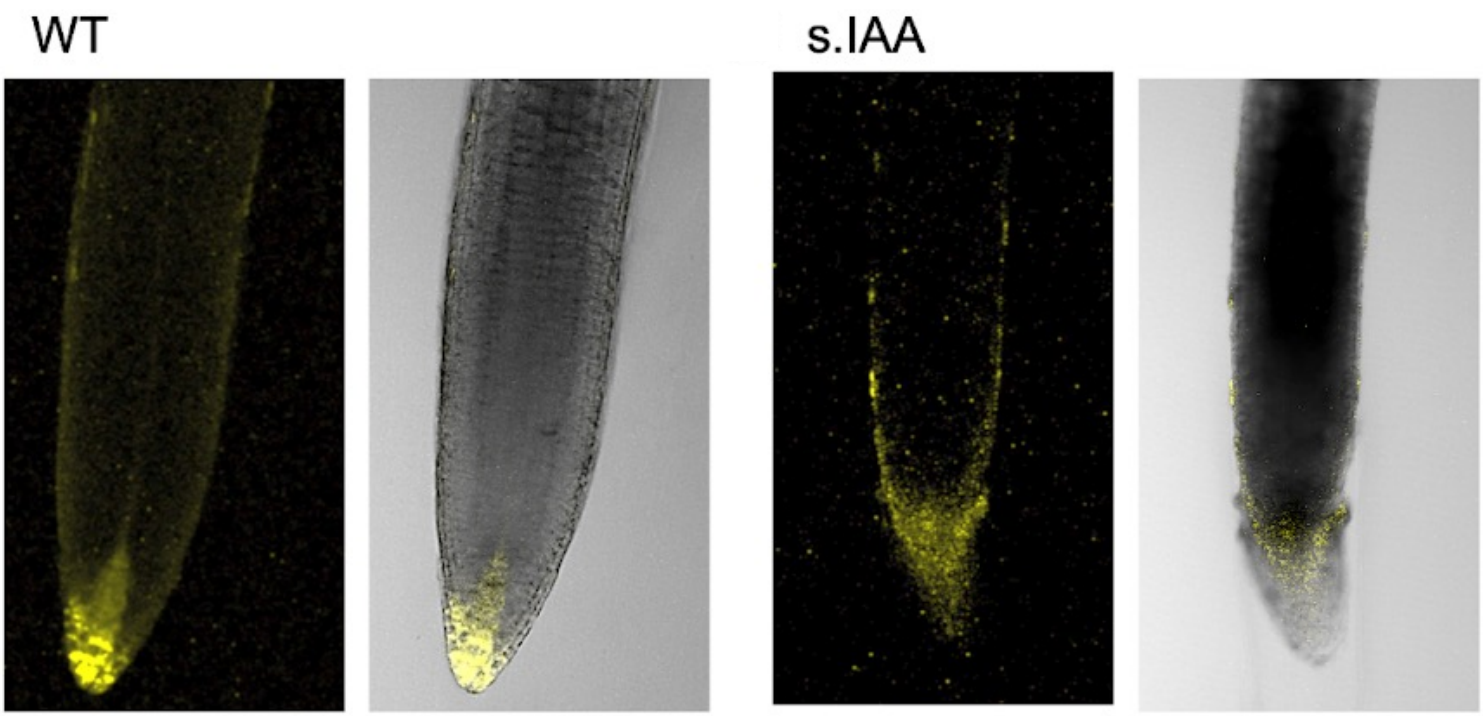





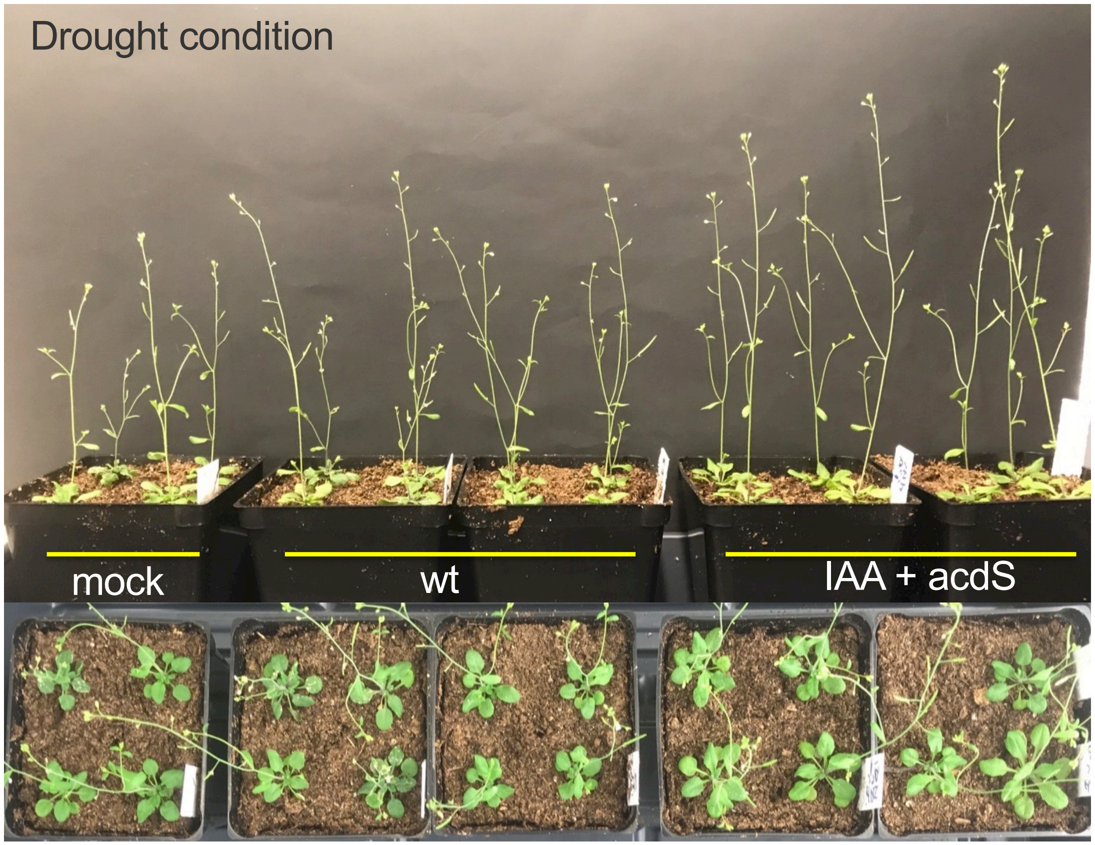
# IAA- and AcdS-producing SynPGPR Can Promote The Root Growth

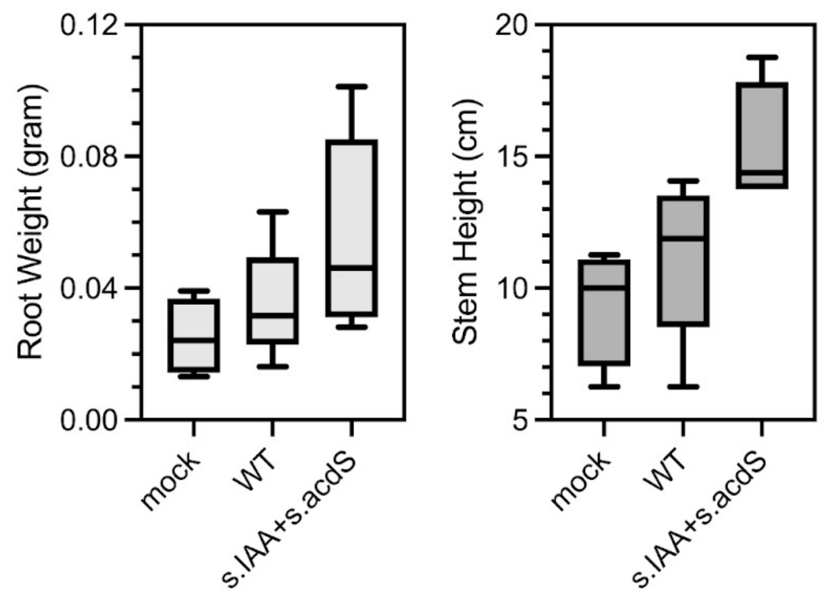


# Our IAA Production Strain Can Maintain The IAA Gradient



# Our SynPGPR Can Promote The Growth Of *Arabidopsis*



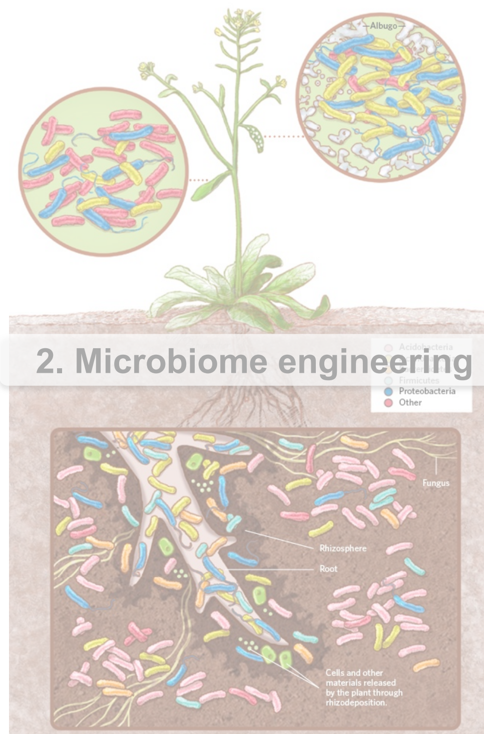
# Summary

- CRAGE is a powerful strain engineering technology for microbial engineering
- Engineered microbes can tremendously increase plant biomass yield under the normal and drought conditions

# Overview of Research Focus Areas


## Yoshikuni group

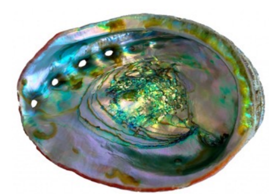
1. Plant N-utilization

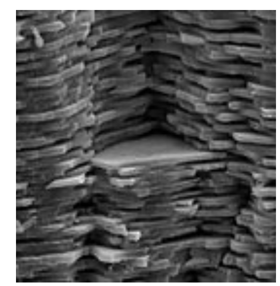



2. Microbiome engineering

3. Biofuel and Bioproducts

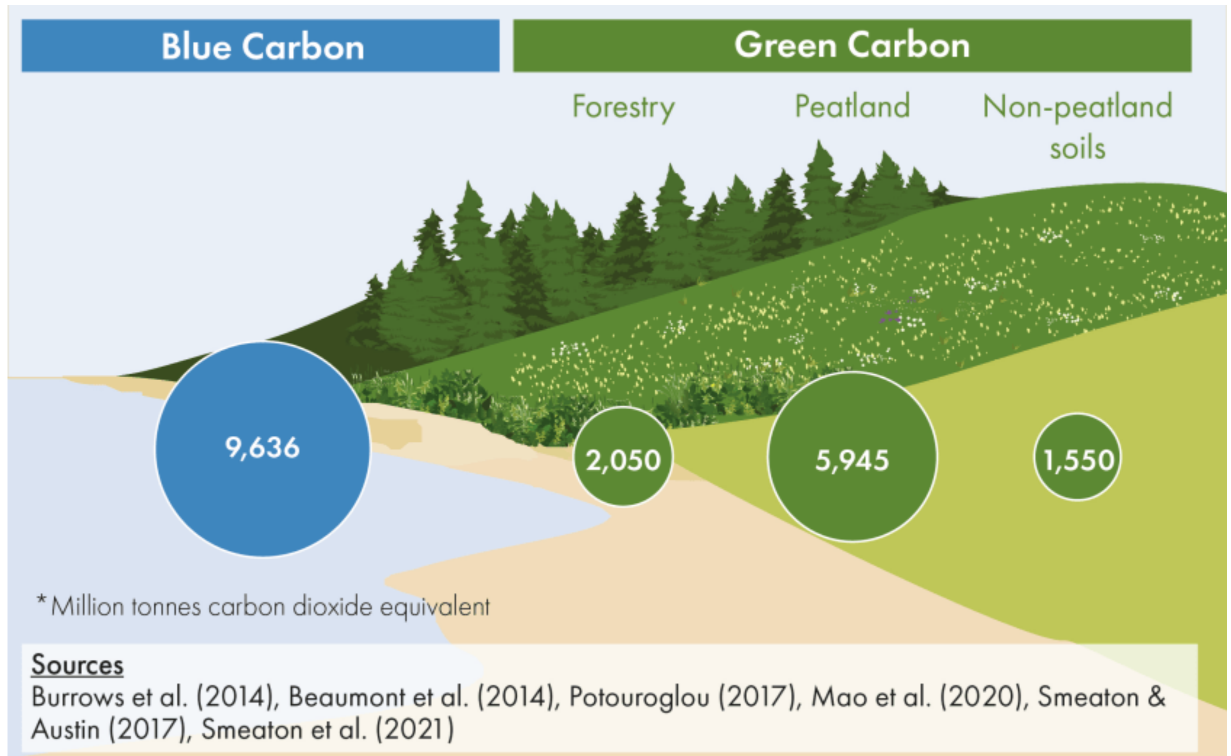
4. Hybrid Biomaterials





# Carbons with Different Colors

**BLACK CARBON**
Carbon particles given off by hot fires, like coal plants, forest fires, and combustion from cars



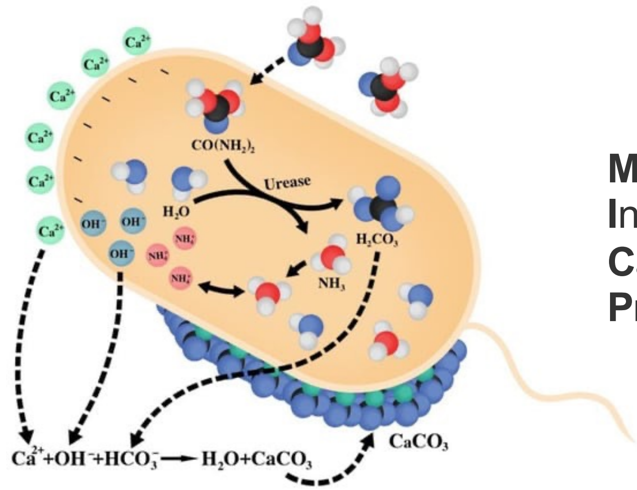

https://upscwithnikhil.com/article/environment/four-types-of-carbon

# A new carbon with different color – yellow?

- N, P, and K rather than C
  - 2.8 kg-$CO_{2eq}$/kg-$NH_3$
  - 1.9 kg-$CO_{2eq}$/kg-$P_2O_5$
  - 0.5 kg-$CO_{2eq}$/kg-$K_2O$
- Urine composes only 1% of total wastewater, but it contains roughly 70-90% of nitrogen (N) and 50-65% of phosphorus (P) in waste streams
- **Urine diversion process** - If we can fully utilize urine, 21%, 12%, and 20% of global N-, P-, and K-fertilizer demands are met
- Problem: low price of fertilizers

# What chemicals should we make to subsidize urine diversion system

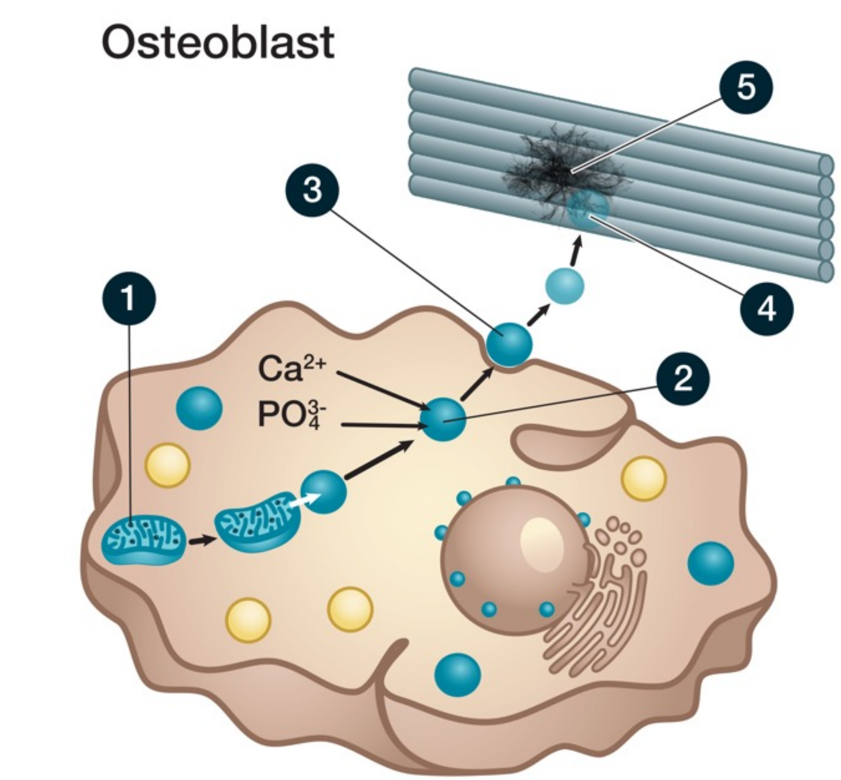
| Chemical | Concentration in g/100 ml urine |
|---|---|
| Water | 95 |
| Urea | 2 |
| Sodium | 0.6 |
| Chloride | 0.6 |
| Sulfate | 0.18 |
| Potassium | 0.15 |
| Phosphate | 0.12 |
| Creatinine | 0.1 |
| Ammonia | 0.05 |
| Uric acid | 0.03 |
| Calcium | 0.015 |
| Magnesium | 0.01 |
| Protein | -- |
| Glucose | -- |



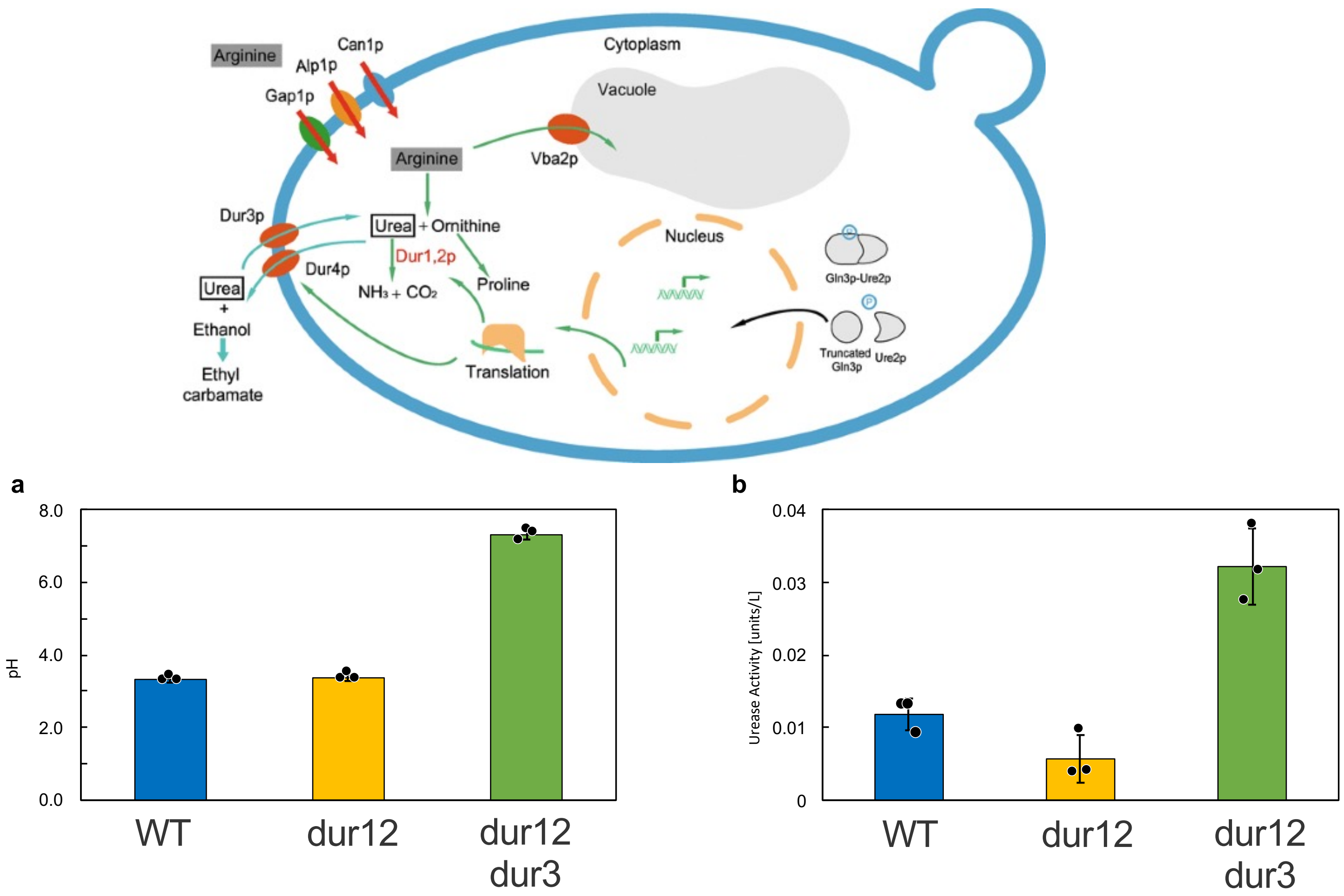

**M**icrobial
**I**nduced
**C**alcite
**P**recipitation

- **Hydroxyapatite might be a very interesting material to make**
  - High price point: $80/kg - $10,000/kg
  - $3.1B market size
  - Orthopedic, dental care products, plastic surgery, food, and pharmaceutical applications
  - Filter, Insulation, replacement of plastics, construction materials

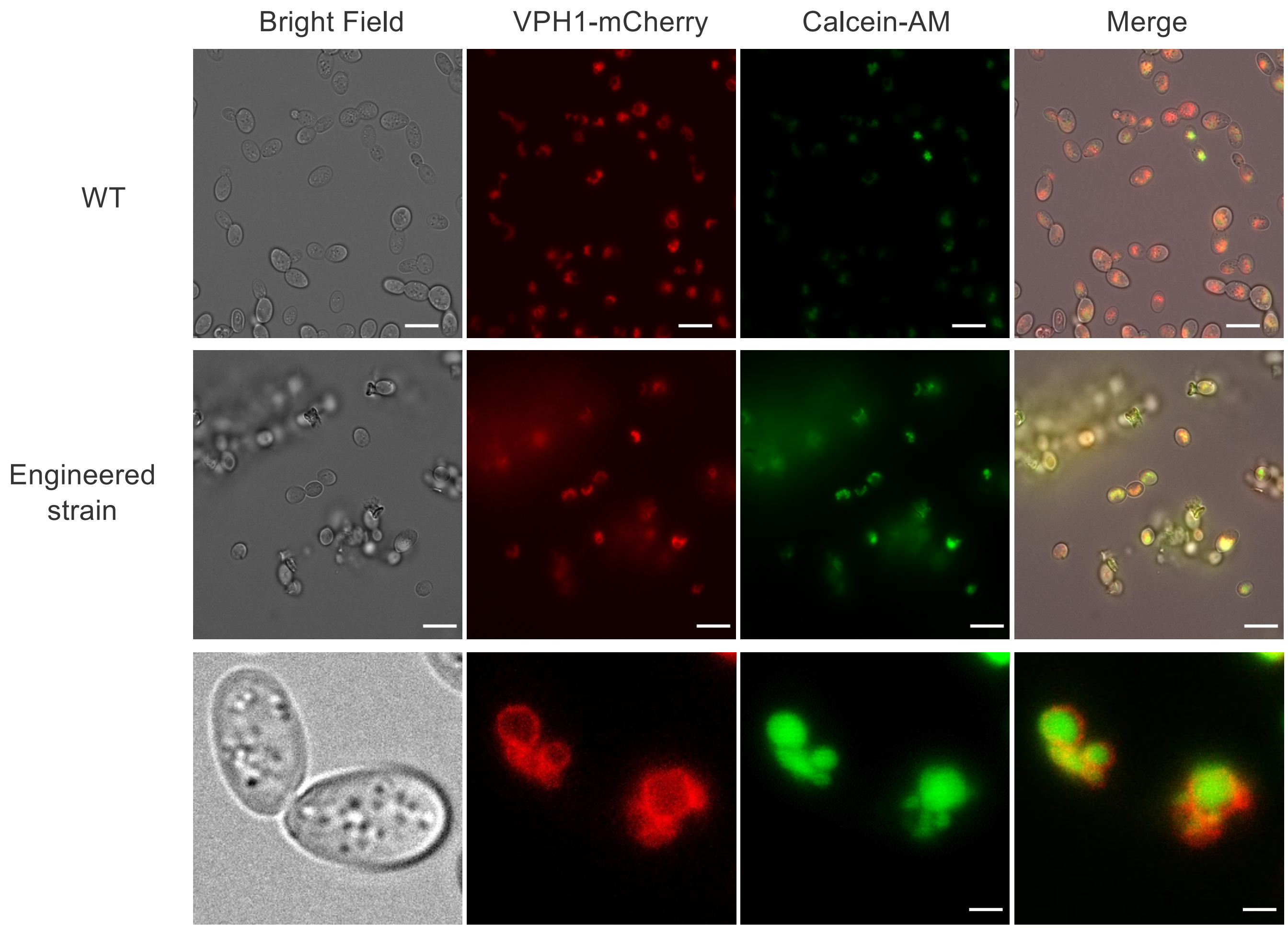
# Design principles of osteoyeast platform

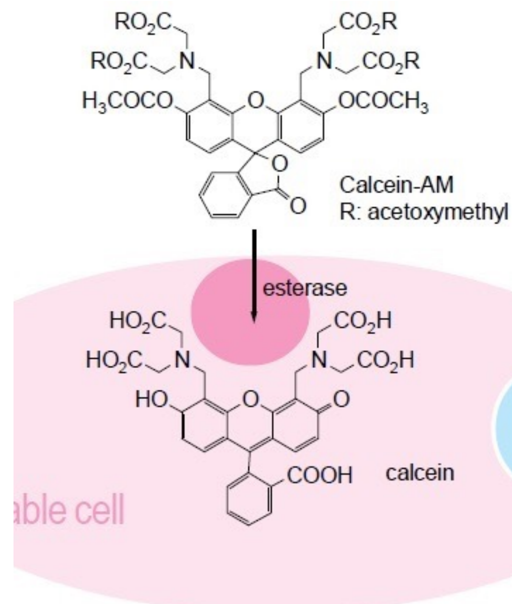


# Overexpression of urea degrading enzymes

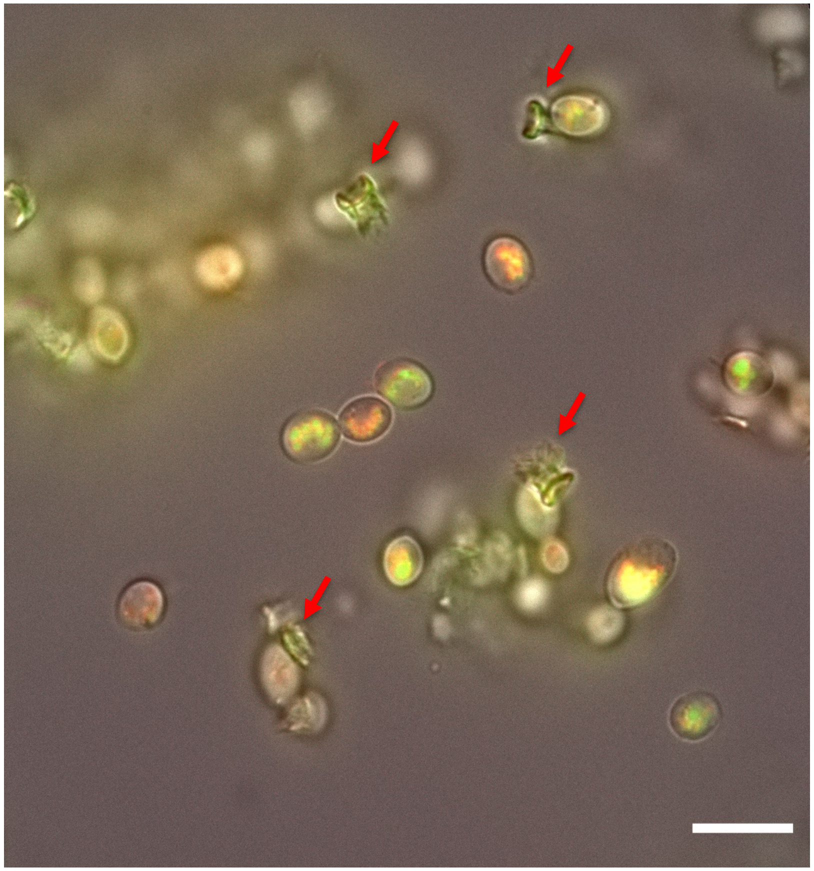


# Engineered yeast increased accumulation of calcium in vacuoles

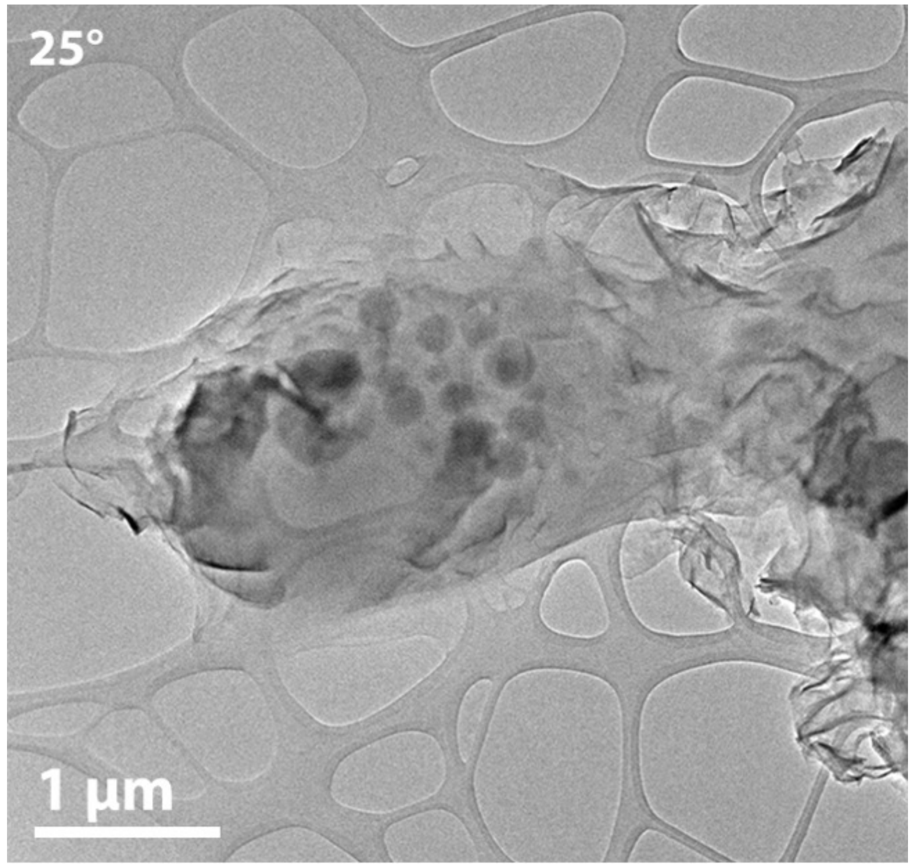


# Engineered yeast produced crystals



# Hydroxyapatite synthesis mediated by the osteoyeast platform analyzed using TEM



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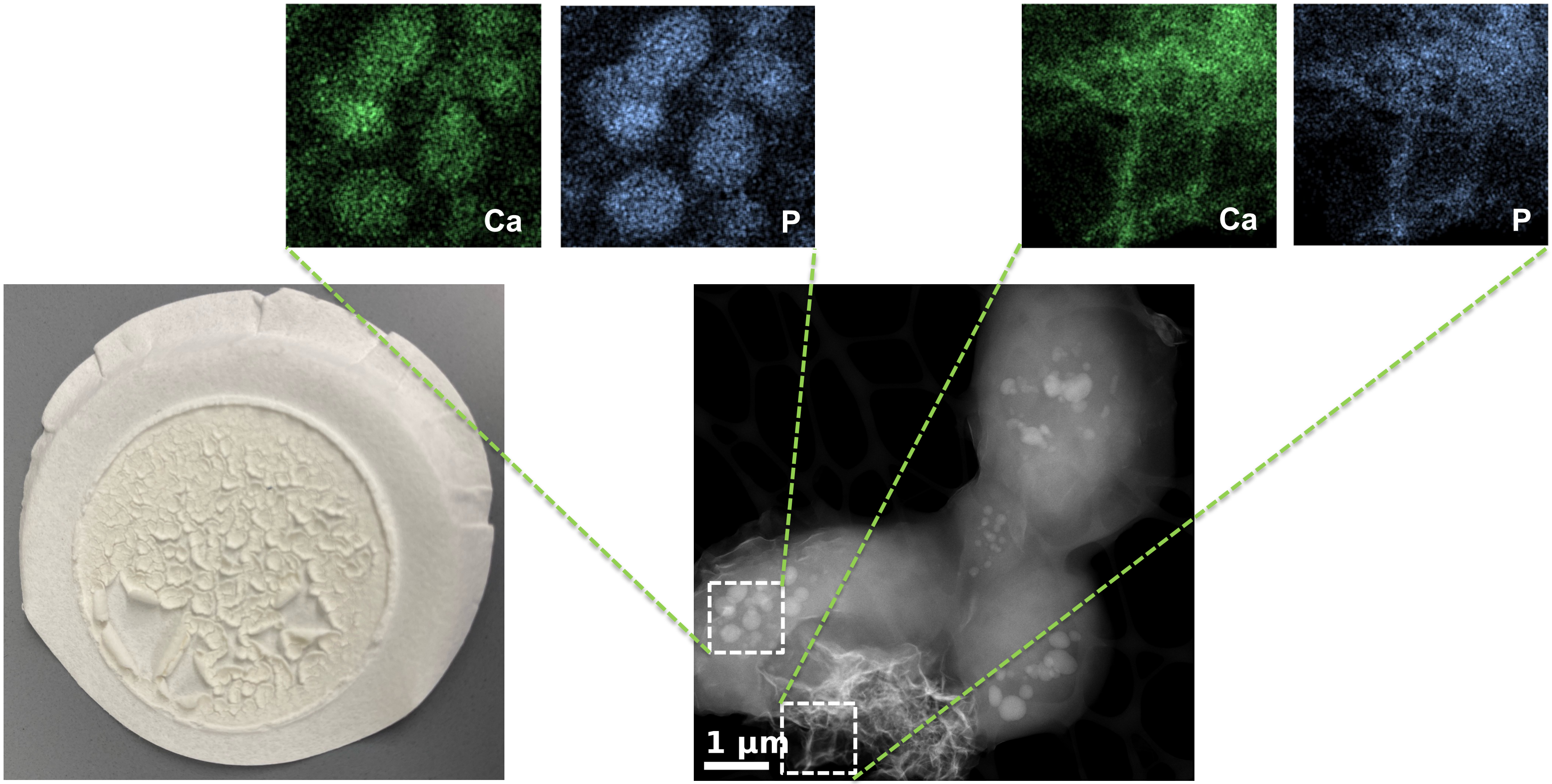
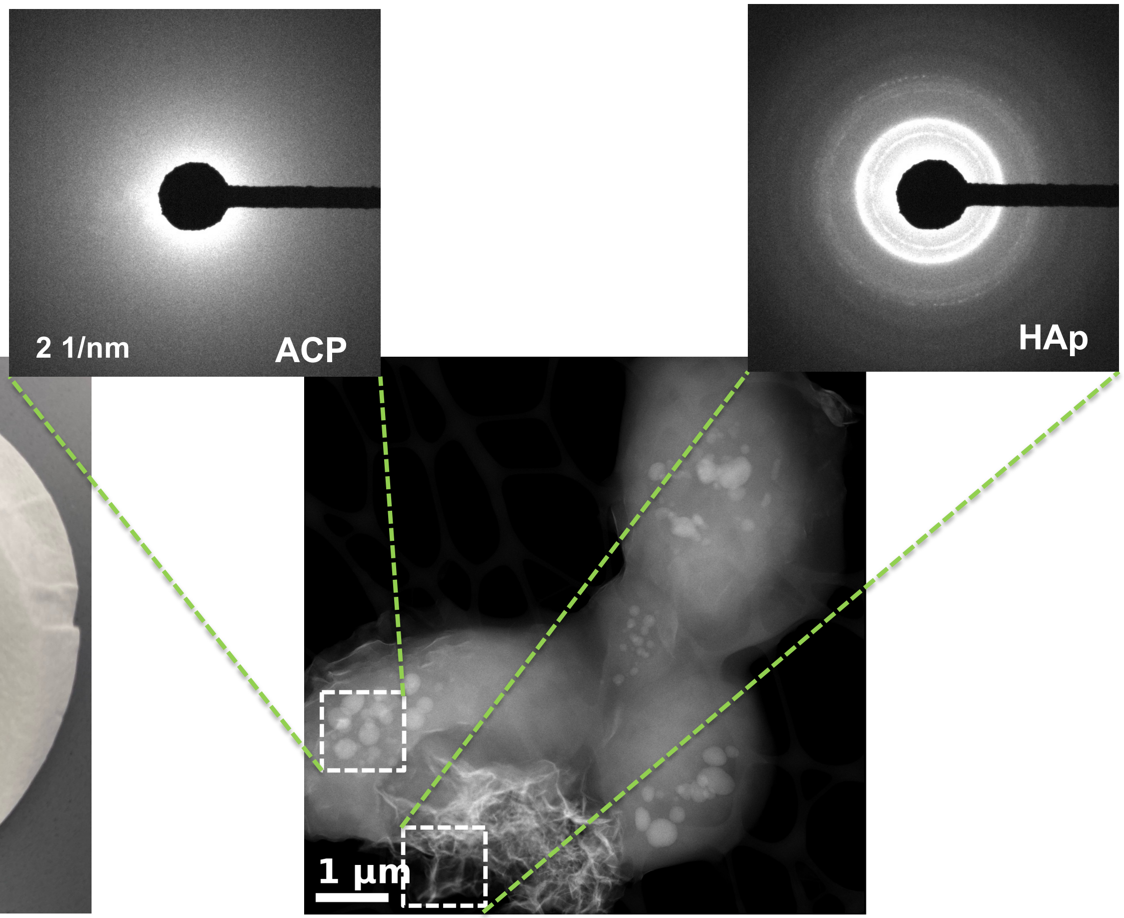


# Hydroxyapatite (HAp) synthesis mediated by the osteoyeast platform analyzed using TEM



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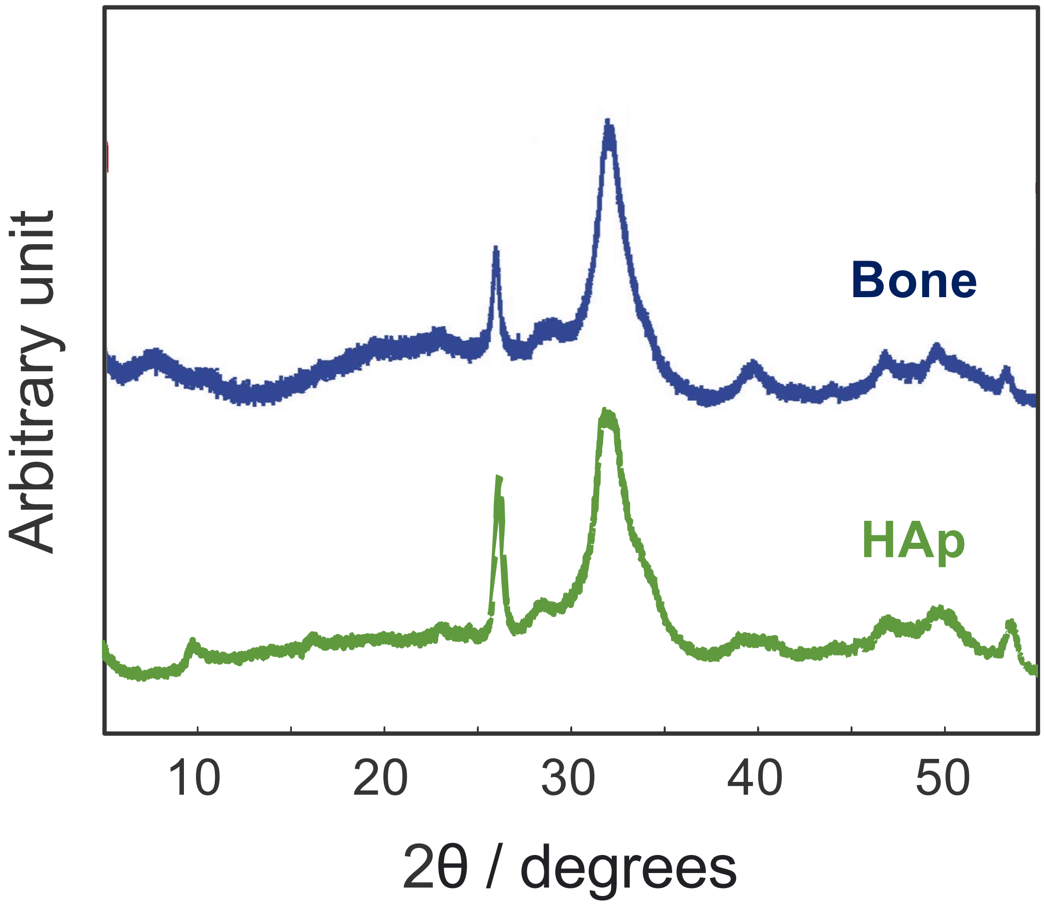
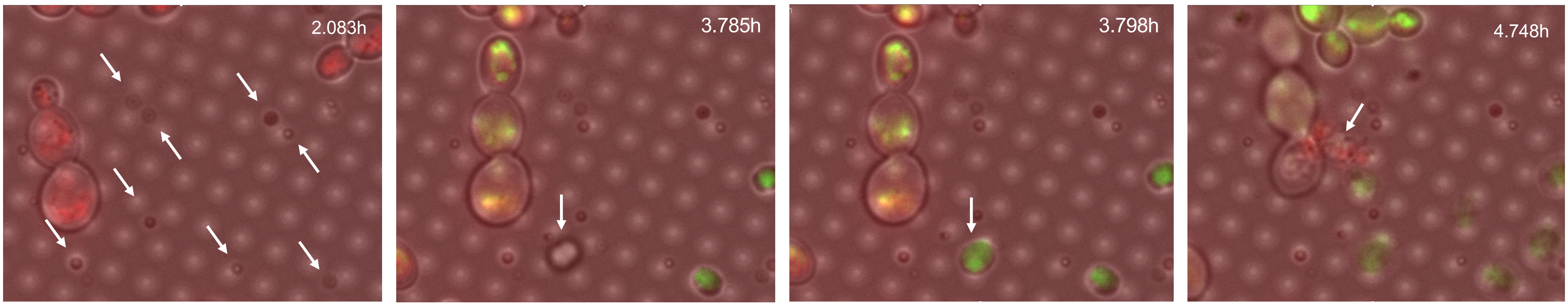


[redacted]

# HAp synthesis mediated by the osteoyeast platform using correlative imaging analyses



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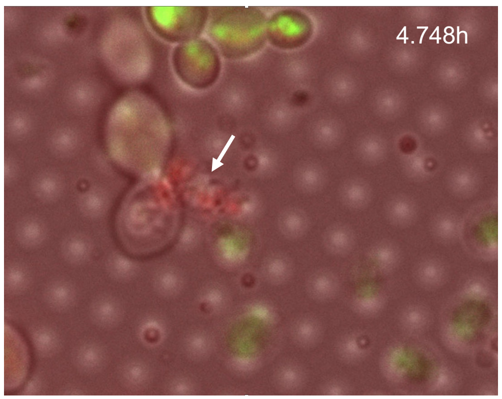


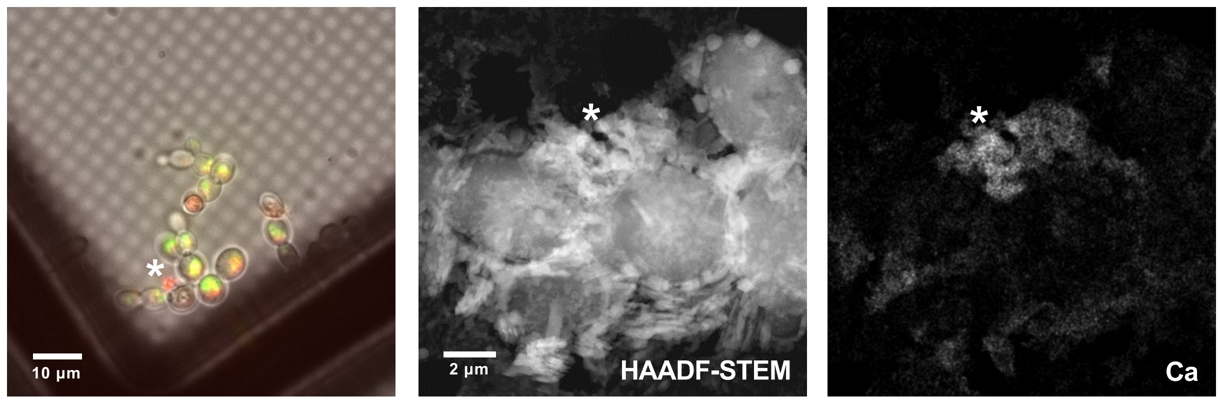

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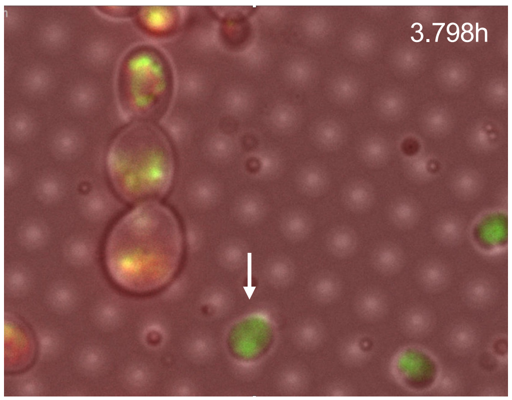


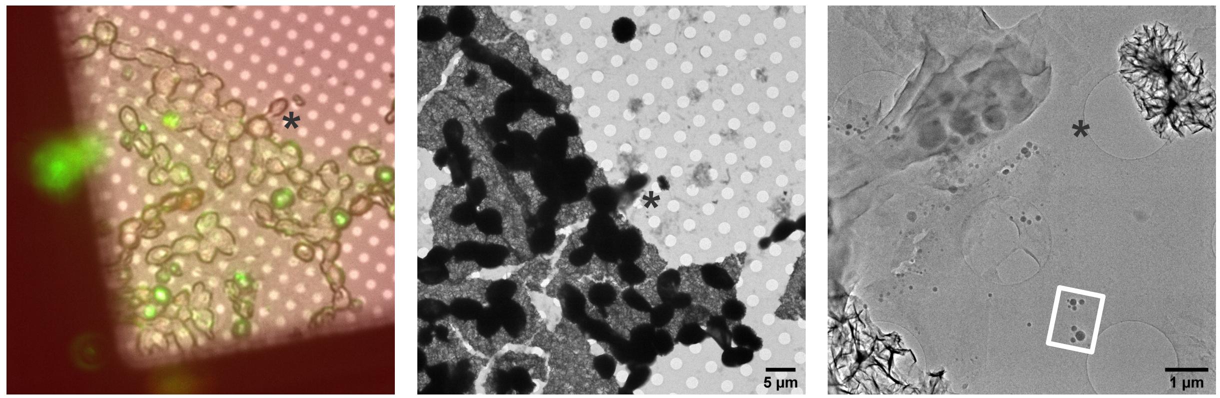

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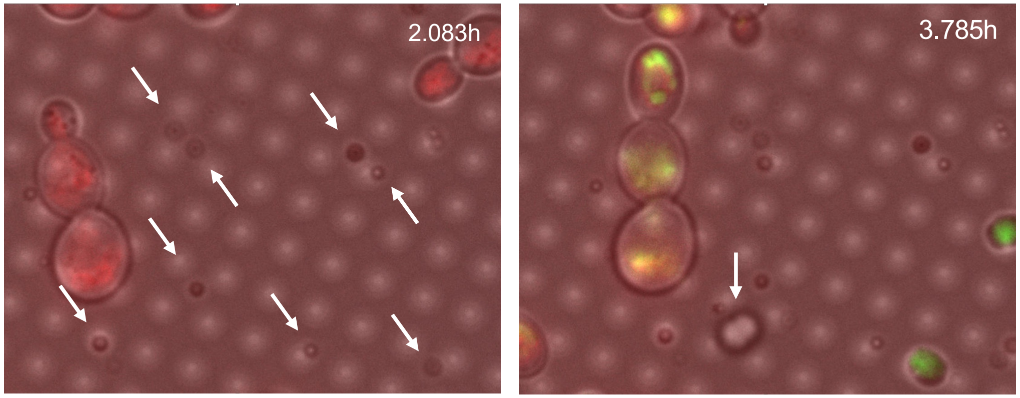
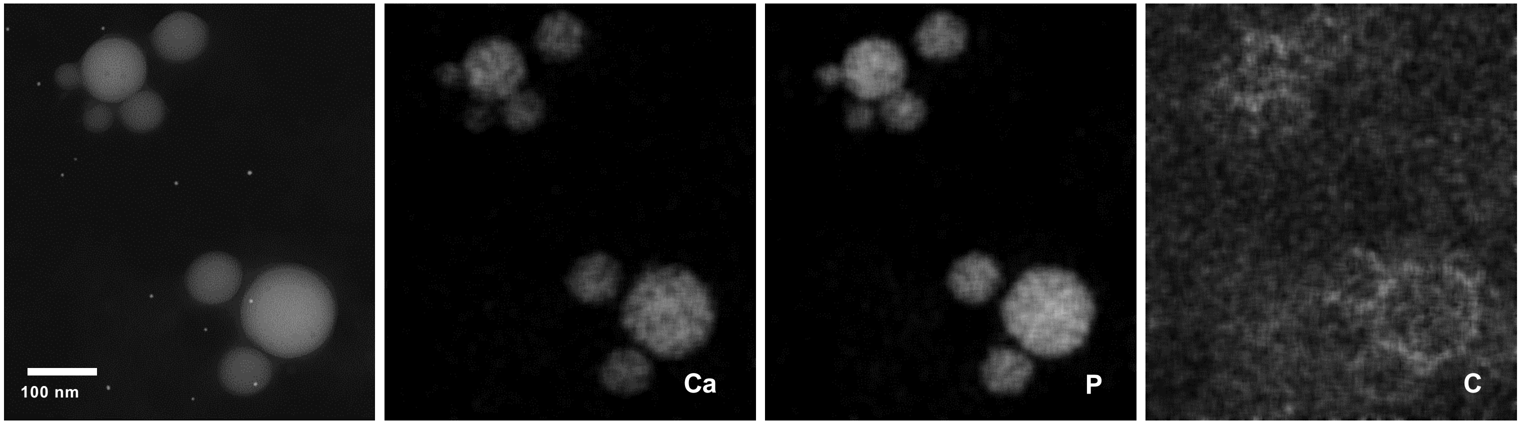
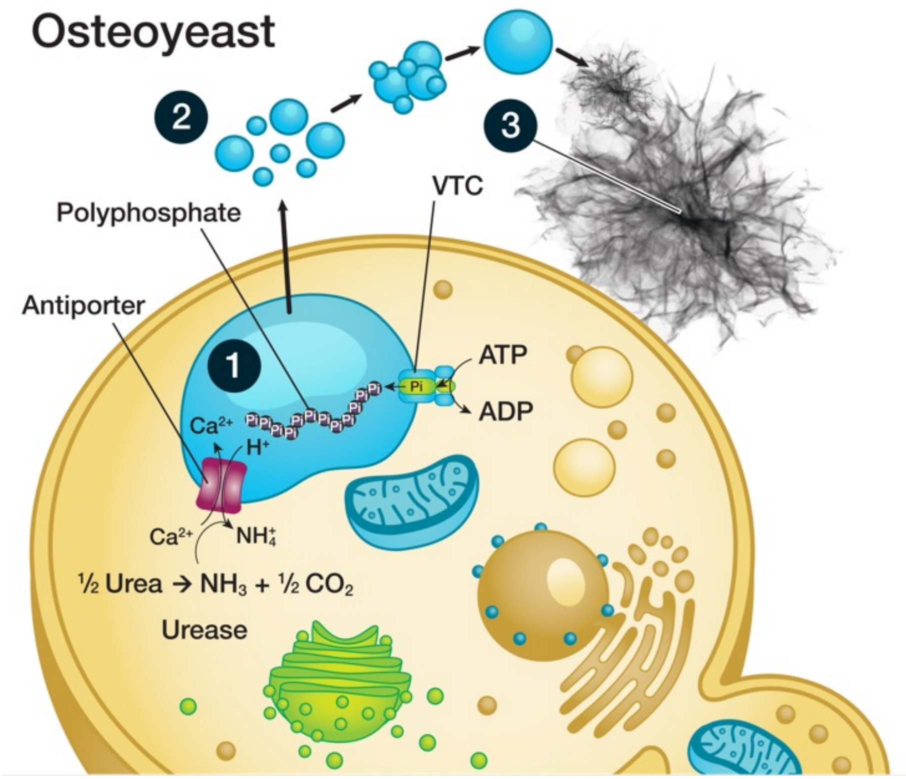
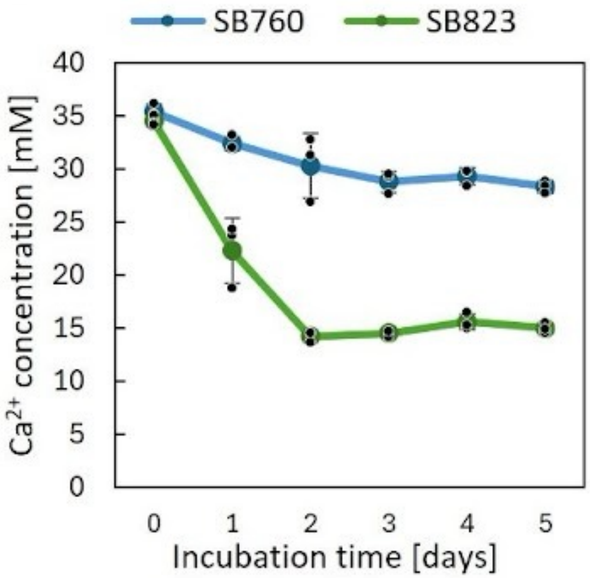


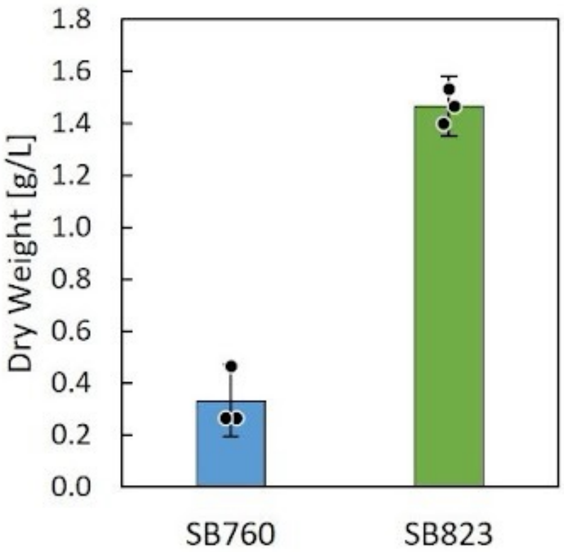



# Mechanism for osteoyeast to produce HAp



# HAp synthesis directly from urine



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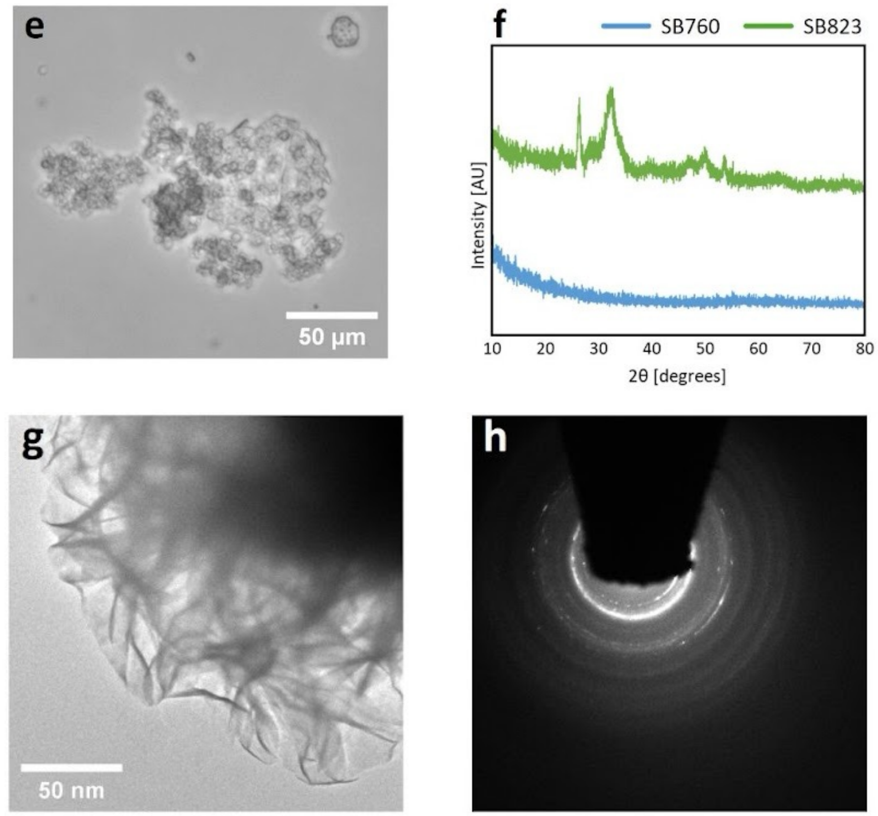
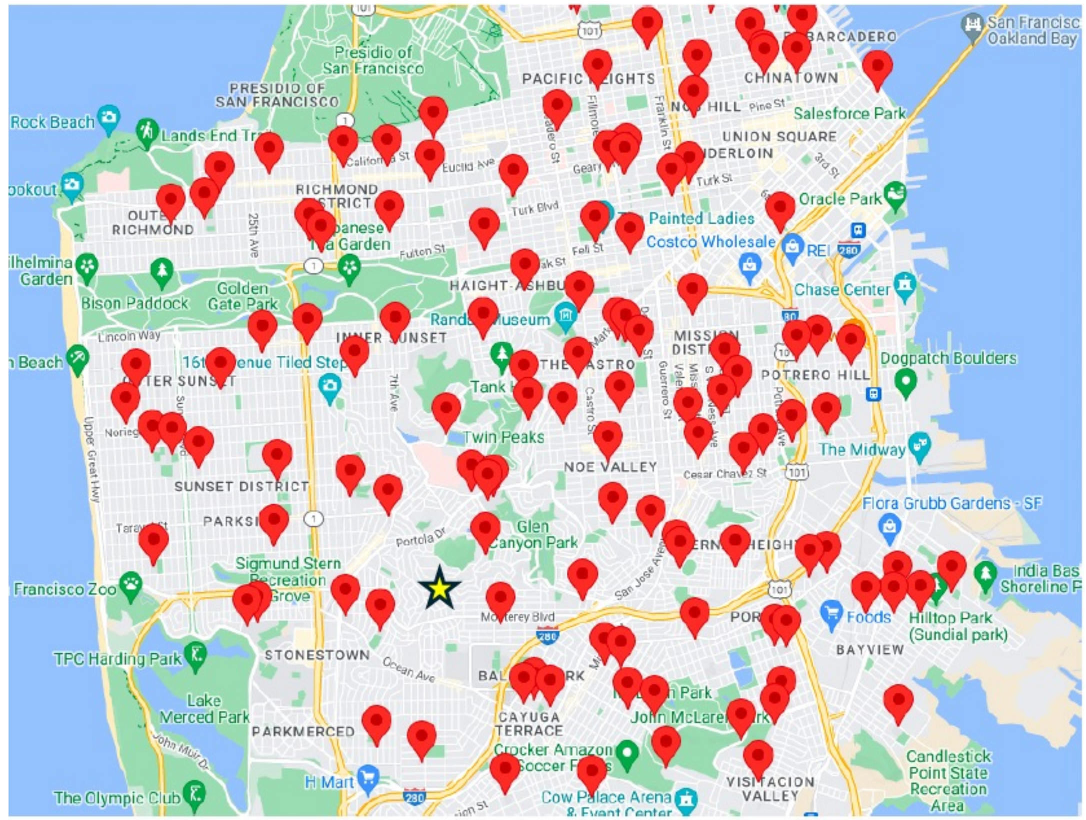


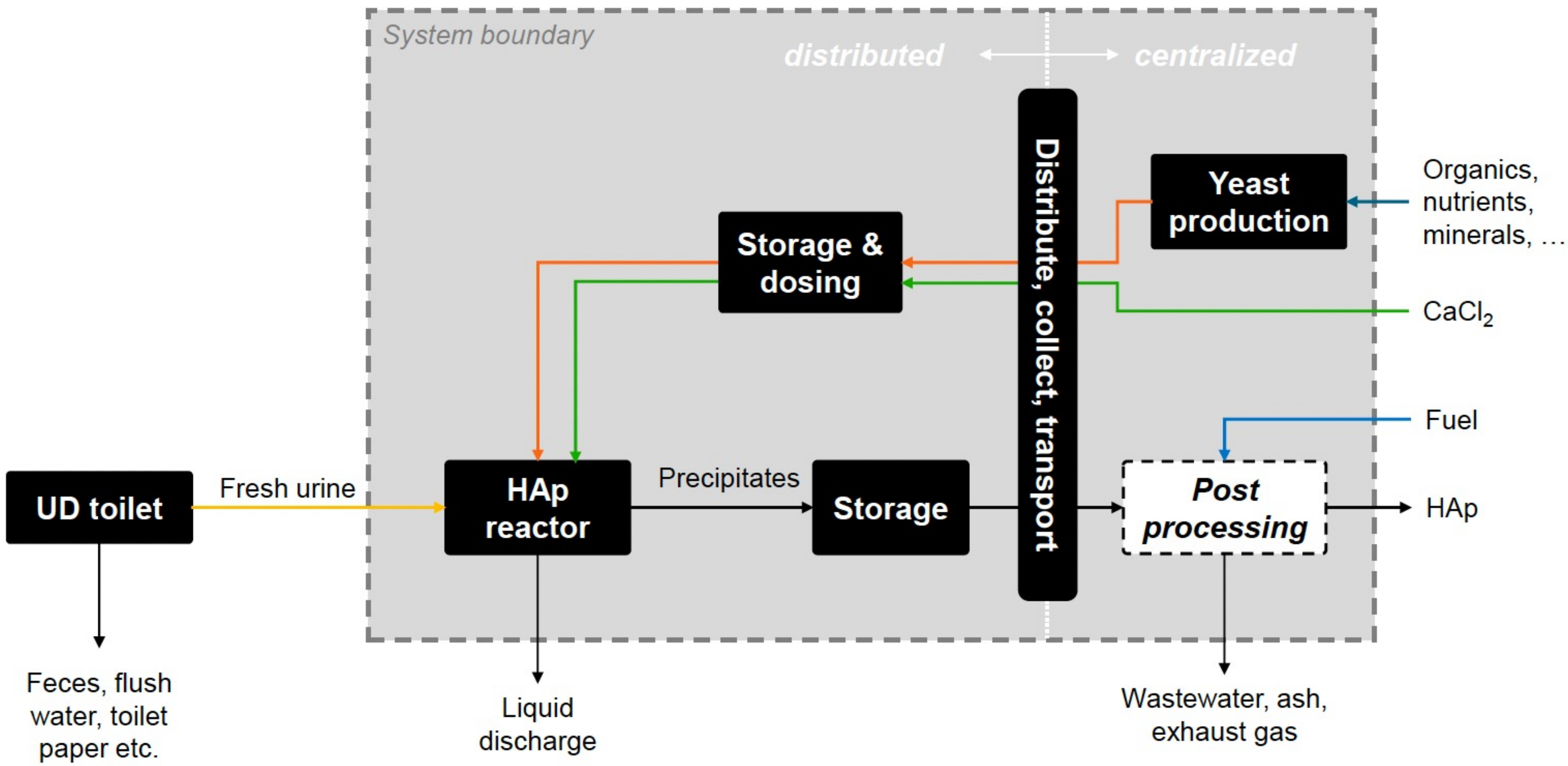
# Technoeconomic assessment of HAp synthesis from Urine

- HAp production systems are deployed across a densely populated city, e.g., San Francisco
- Potential locations ( ) for deployment have consistently large fluxes of urine, e.g., schools, shopping malls
- Osteoyeasts are produced at a central location ( ) and supplied to the distributed HAp reactors regularly.
- Precipitates will be collected regularly and further processed at the same central location ( ).
- Toilets capable of urine diversion are assumed to be readily available at the deployed locations.




- *San Francisco Unified School District*

# Technoeconomic assessment of HAp synthesis from Urine



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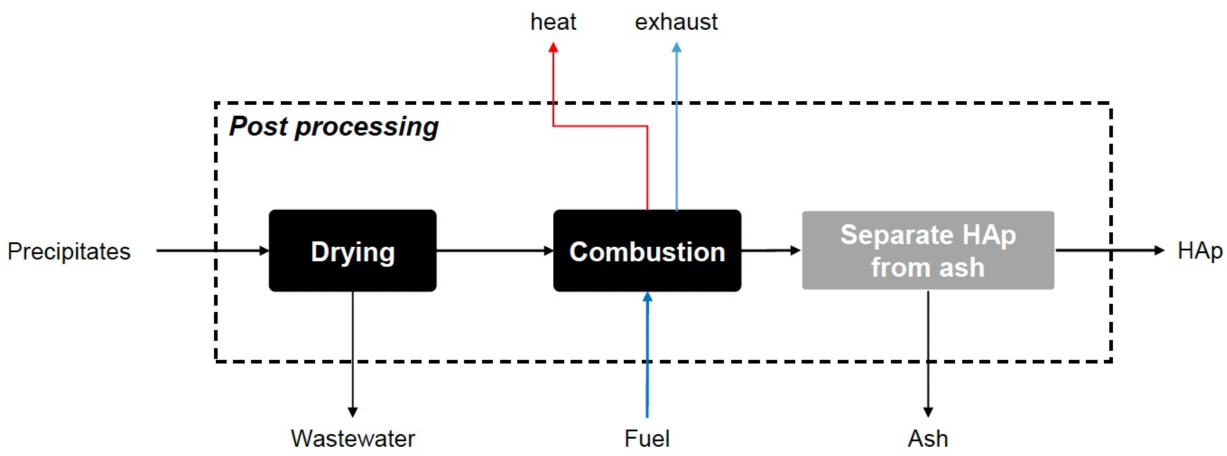
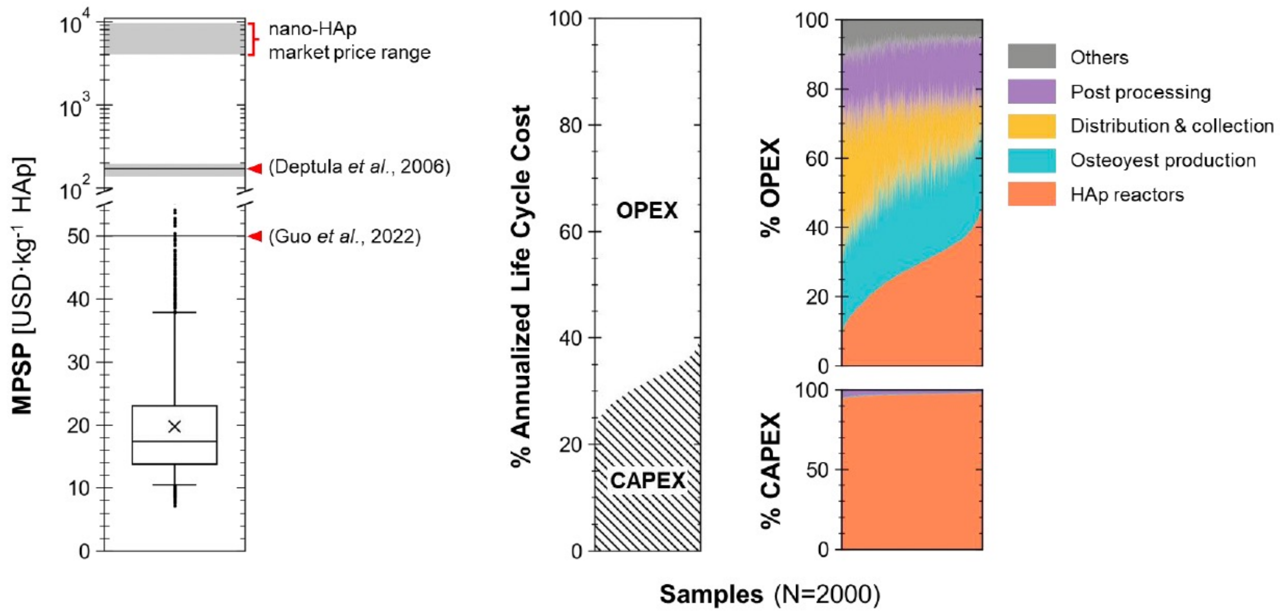


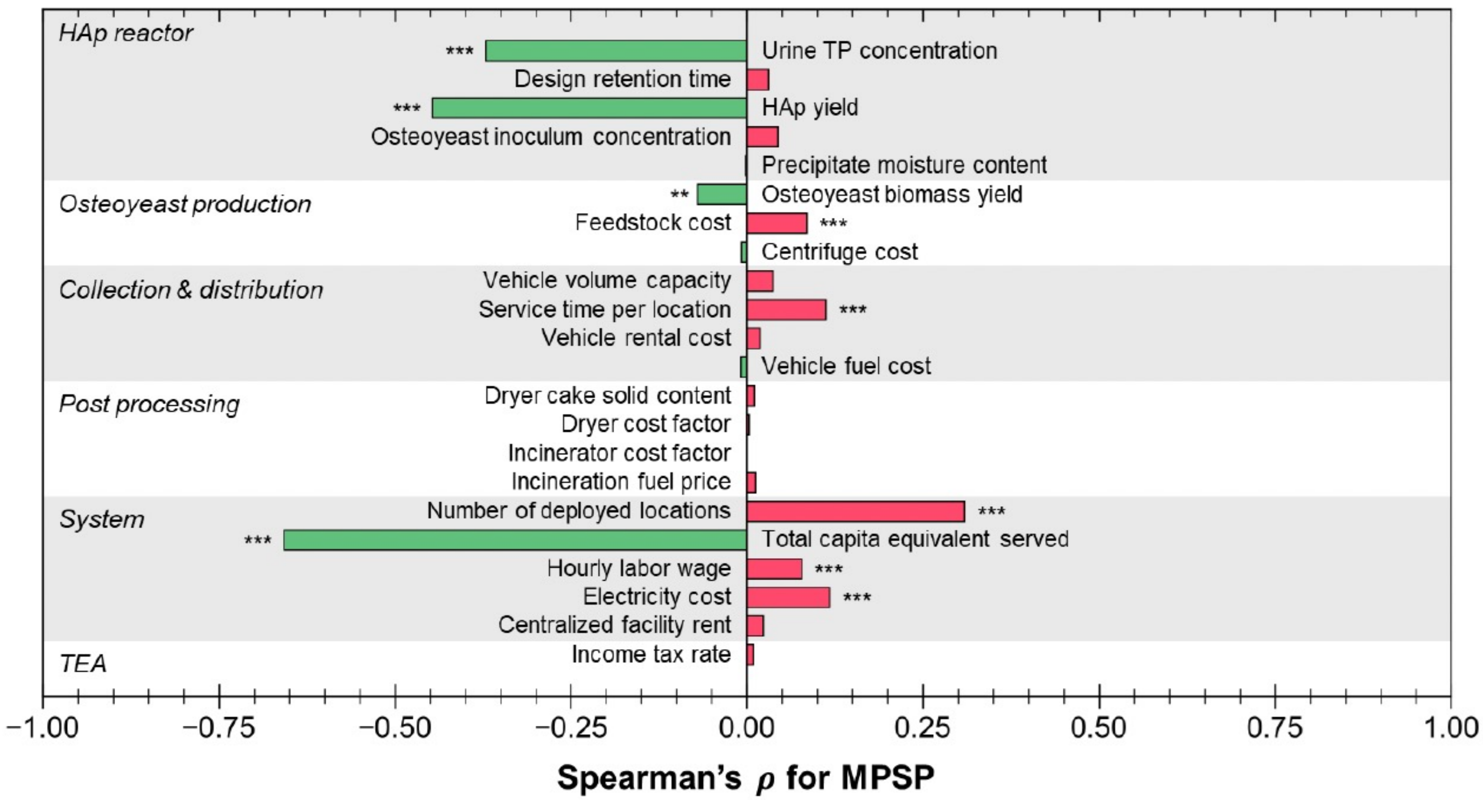
# Technoeconomic assessment of HAp synthesis from Urine

- Other TEA assumptions
  - Project lifetime = 10 years
  - Discount rate = 0.05
- TEA indicators
  - Minimum product selling price to break even (**MPSP**, in USD/kg HAp)
  - Annualized life cycle cost
  - **CAPEX** (capital expenditure)
  - **OPEX** (operation expenditure)



# Technoeconomic assessment of HAp synthesis from Urine

**MPSP** (Minimum Product Selling Price, in USD/kg HAp)



# Summary

- Introduced a new yellow carbon concept
- The osteoyeast platform designed based on osteoblast can produce Hap directly from urine with a high yield
- Osteoyeast-based HAp production makes the urine diversion process profitable

# Acknowledgements

## Synthetic Biology Group
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- Dr. Jing Ke
- Mr. Koyo Ono
- Mr. Yu Miyamoto
- Dr. Isaak Muller
- Mr. David Robinson
- Dr. Yusuke Sasaki
- Dr. Sang-Woo Han
- Dr. Ping-Hung Hsieh
- Dr. Bing Wang
- Dr. Gaoyan Wang
- Dr. Zong-Yen Wu
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- Ms. Leslie Silva

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- Dr. Axel Visel

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- Dr. Matt Blow

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- Dr. Chuck Francavilla

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- Dr. Kerem Bingol
- Dr. David W. Hoyt

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- Yi-Ming Shi

Scripps Institution of Oceanography UCSD
- Prof. Paul Jensen

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Dr. Jeff Dangl

U.S. Department of Energy


**Please contact me if you are interested in a Post-Doc position**