

iGEM
2019  Calgary

y  O I L

An all-encompassing solution to the green seed
problem

Canada is the world's largest producer and
exporter of canola oil

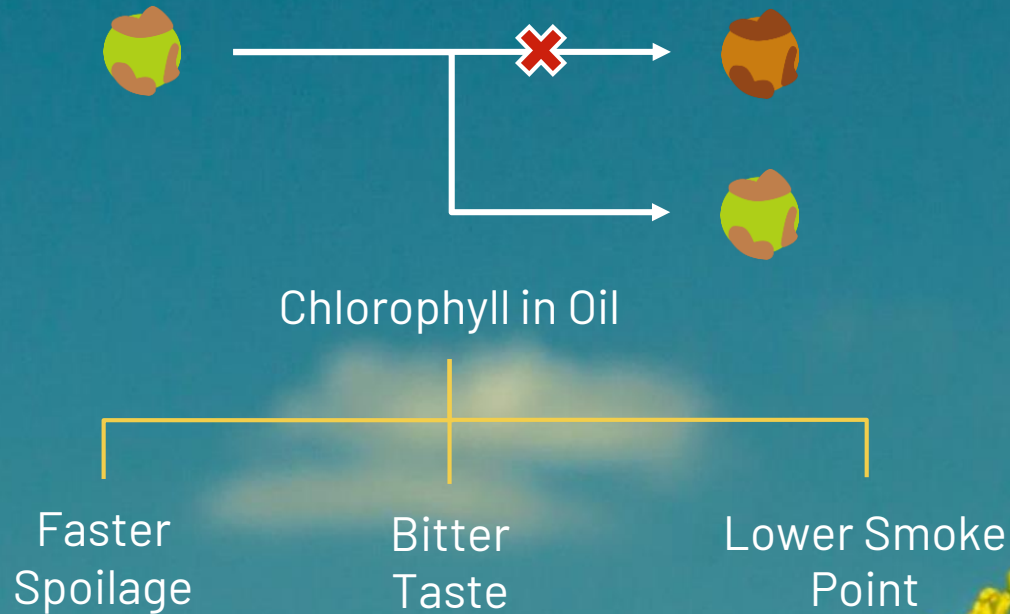


A photograph of yellow flowers, likely rapeseed, against a clear blue sky with a few wispy clouds. The flowers are in the foreground, and the sky is the background.

We are losing **\$150 million dollars** every year

Because our oil is green

The Green Seed Problem

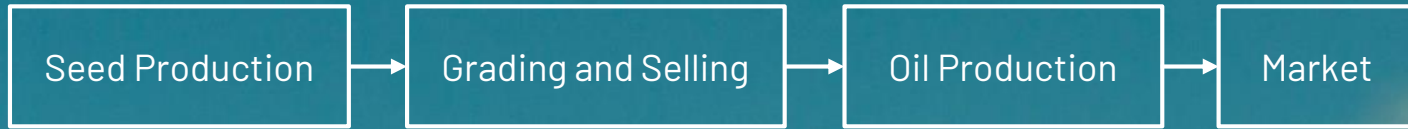


The logo for 'yOIL' is centered on the page. The letter 'y' is a solid yellow color. The letter 'O' is white with a cluster of yellow flowers and green stems inside it. The letters 'I', 'L', and 'L' are solid white. The background is a clear blue sky with some light clouds and yellow flowers in the foreground.

yOIL

An all-encompassing solution to the green seed
problem

The Green Seed Problem



The Green Seed Problem

Oil Production

Oil Producers

Extra Processing: Acid Activated Clay



Green
oil

Clay
method

Oil Production

**Up to 20% of Oil
Lost**



**Negative
Environmental
Consequences**

“Clay is the most **expensive** input cost”

-Dallas Gade
Project Manager, Richardson Oilseed

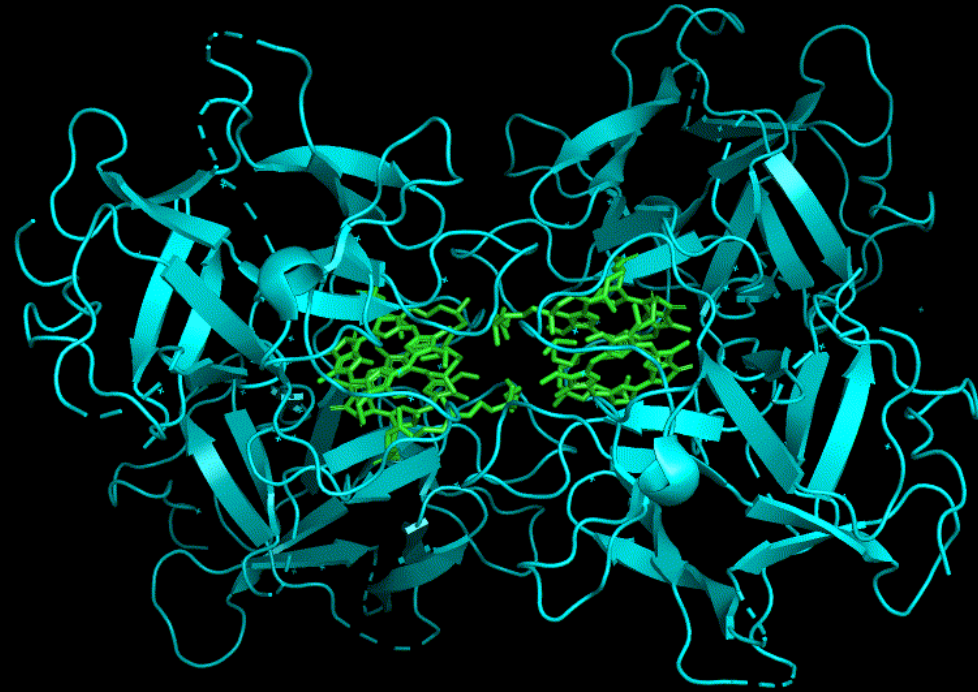


Synthetic biology can improve green oil processing

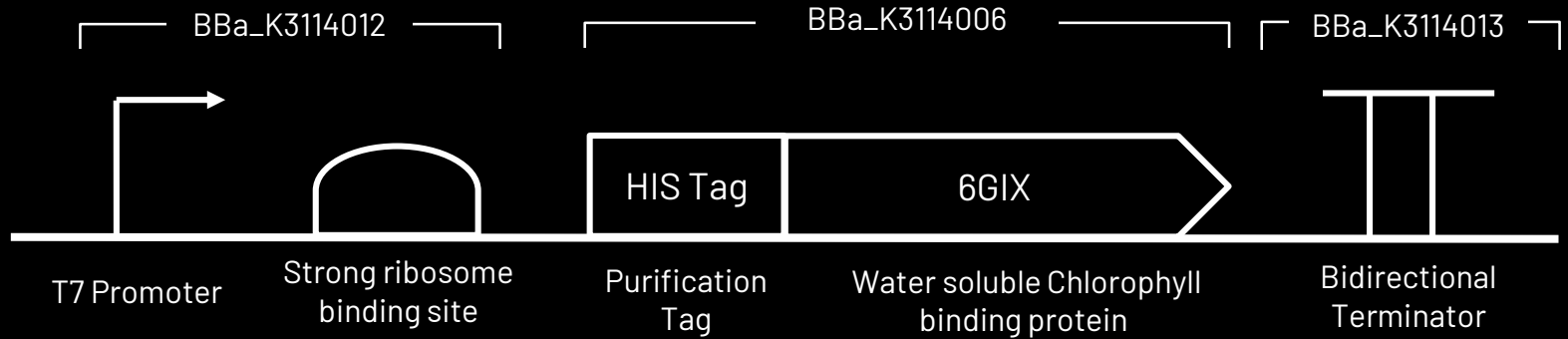
Our Solution

6GIX: Water Soluble Chlorophyll
Binding Protein

- 1) Binds four chlorophyll molecules
- 2) Protein-chlorophyll complex removed
- 3) Pure yellow oil

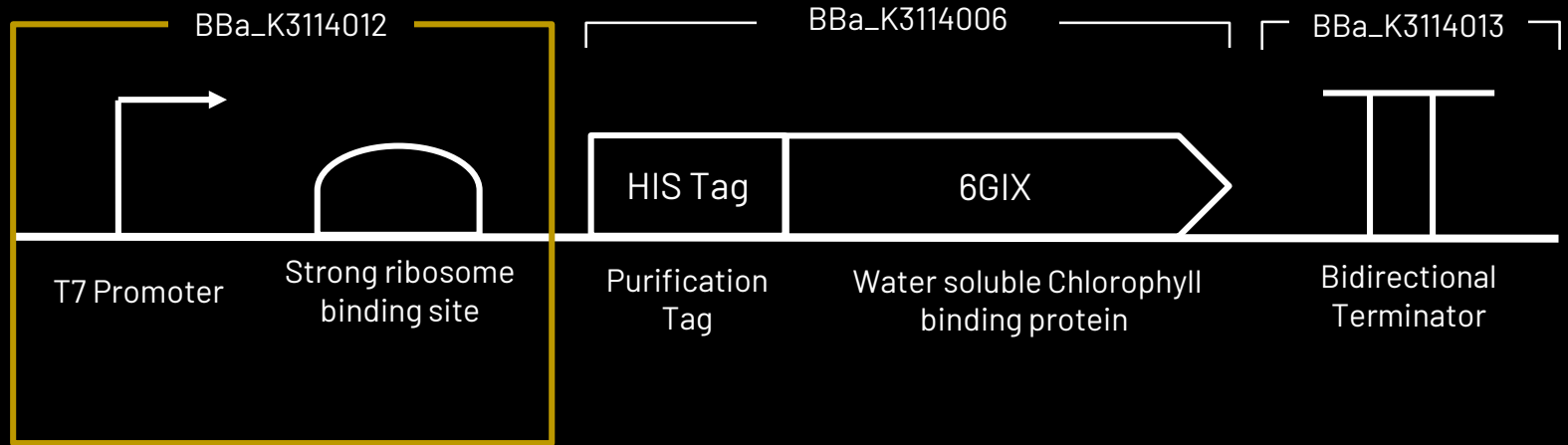


Synbio for Green Seed Processing

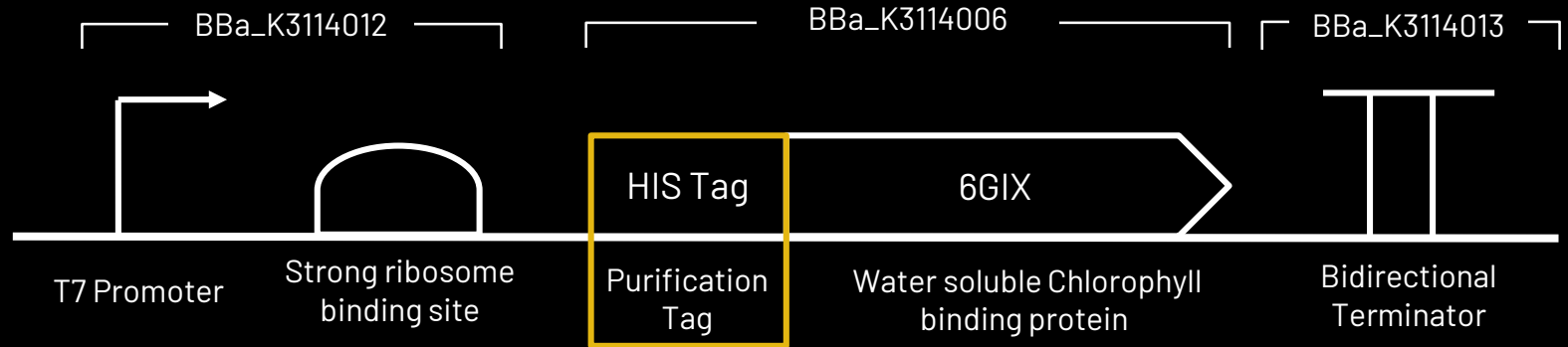


BBa_K3114015 allows golden gate cloning
(Improved BBa_K1467400)

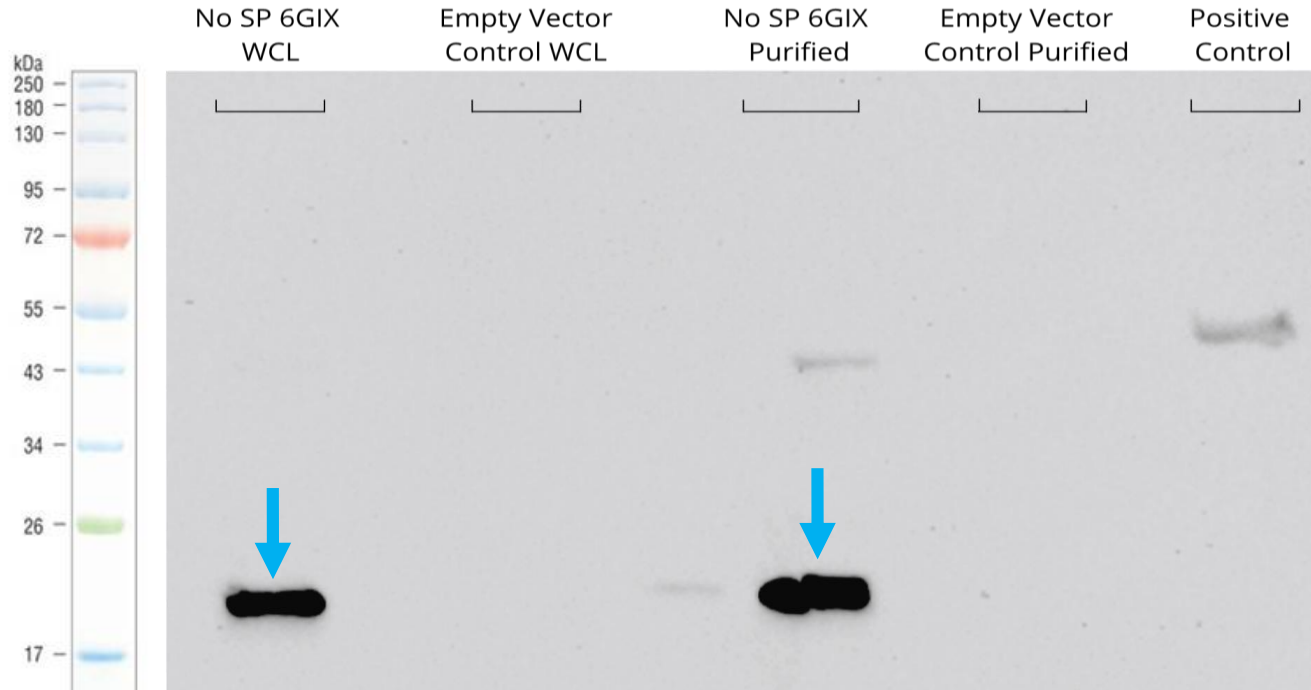
Synbio for Green Seed Processing



Synbio for Green Seed Processing



Purification of 6GIX



We SUCCESSFULLY expressed and purified 6GIX from the Cell Lysate

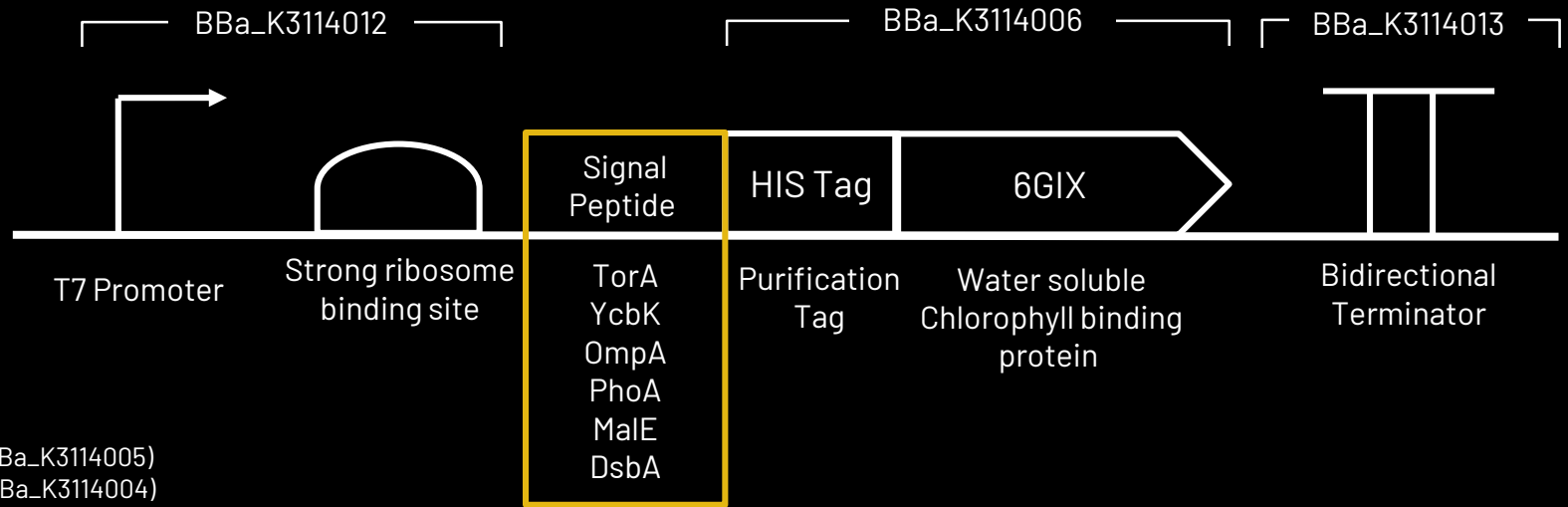
How can we **minimize costs** spent on purification?



Dr. Raymond Turner

University of Calgary
Biochemistry Faculty
Professor

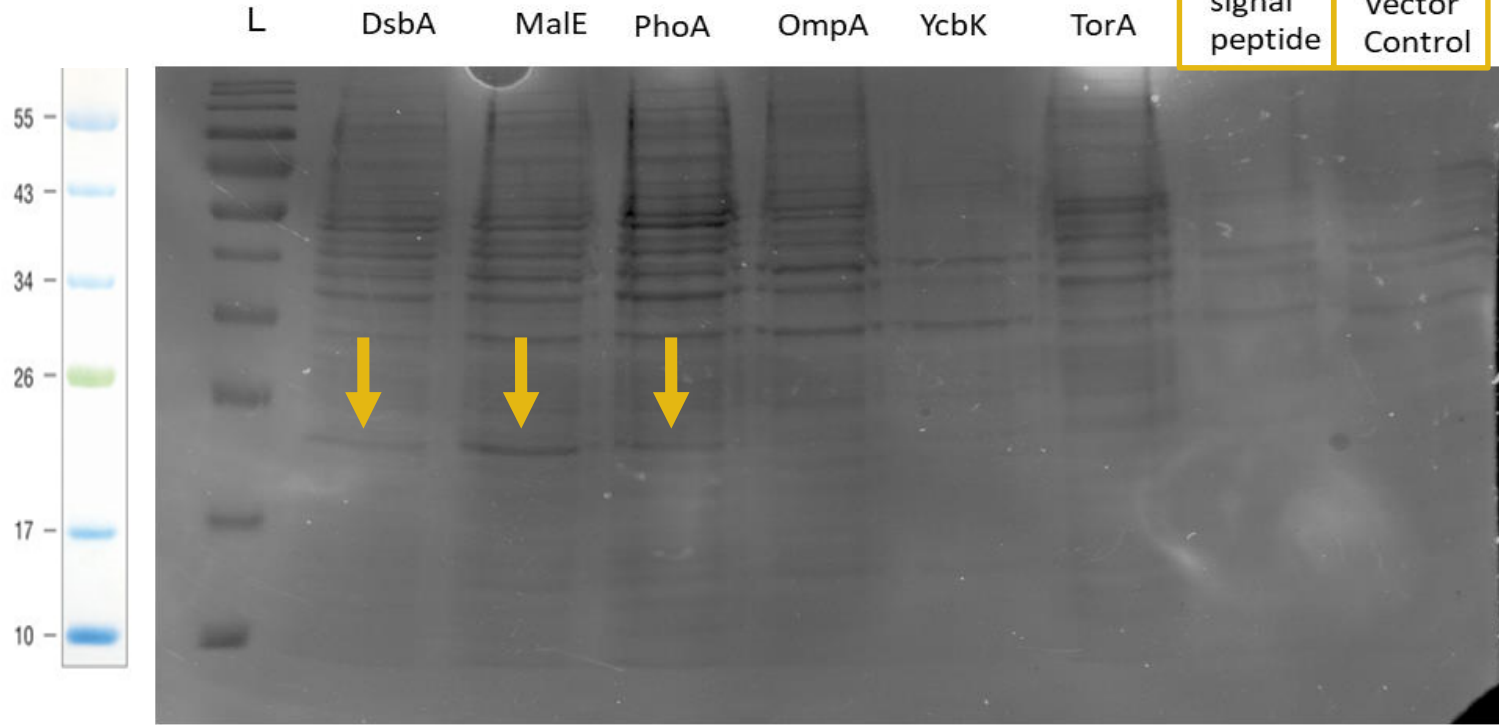
Synbio for Green Seed Processing



TorA: (BBa_K3114005)
YcbK: (BBa_K3114004)
OmpA: (BBa_K3114002)
PhoA: (BBa_K3114003)
MalE: (BBa_3114001)
DsbA: (BBa_K3114000)

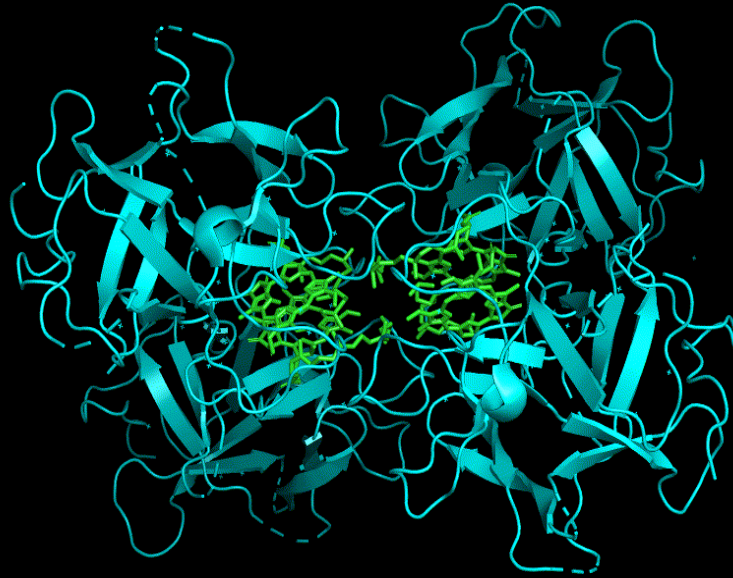
Signal Peptide Results

Periplasmic extraction gel



PhoA, MalE, and DsbA signal peptides SECRETE 6GIX into periplasm

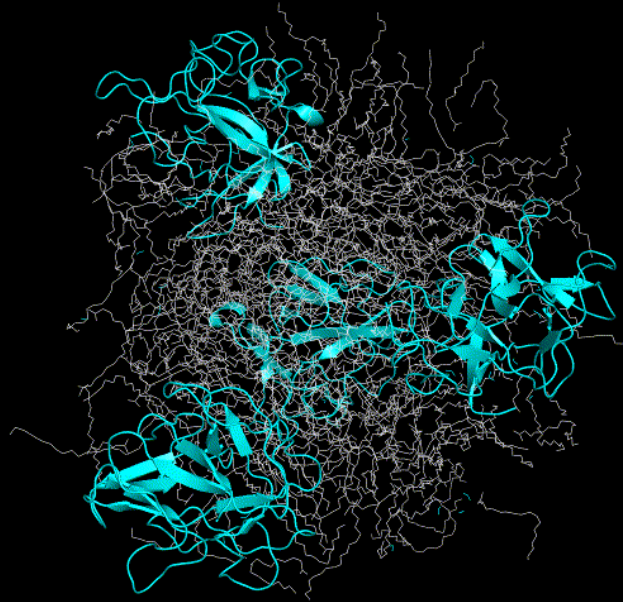
Hydrophobic Effect



6GIX normal structure

Hydrophobic Effect

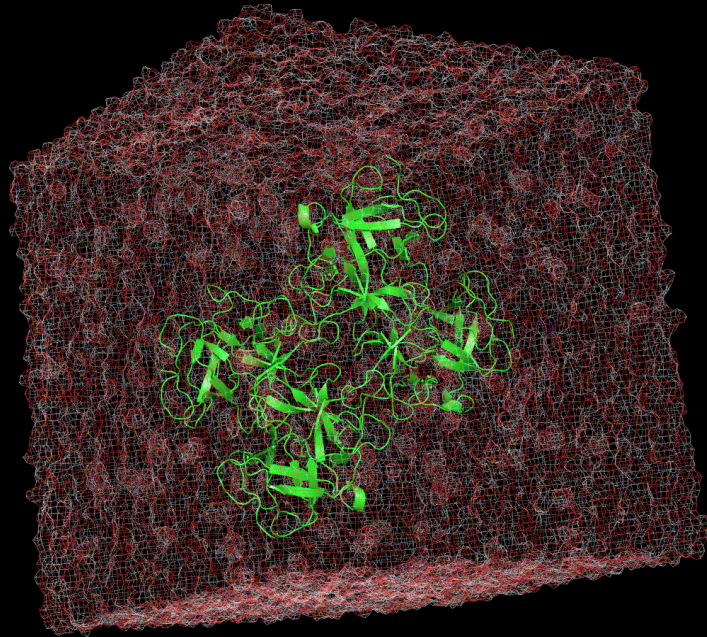
Water soluble proteins don't work in oil!!!!!!



6GIX denatured structure

Our Solution

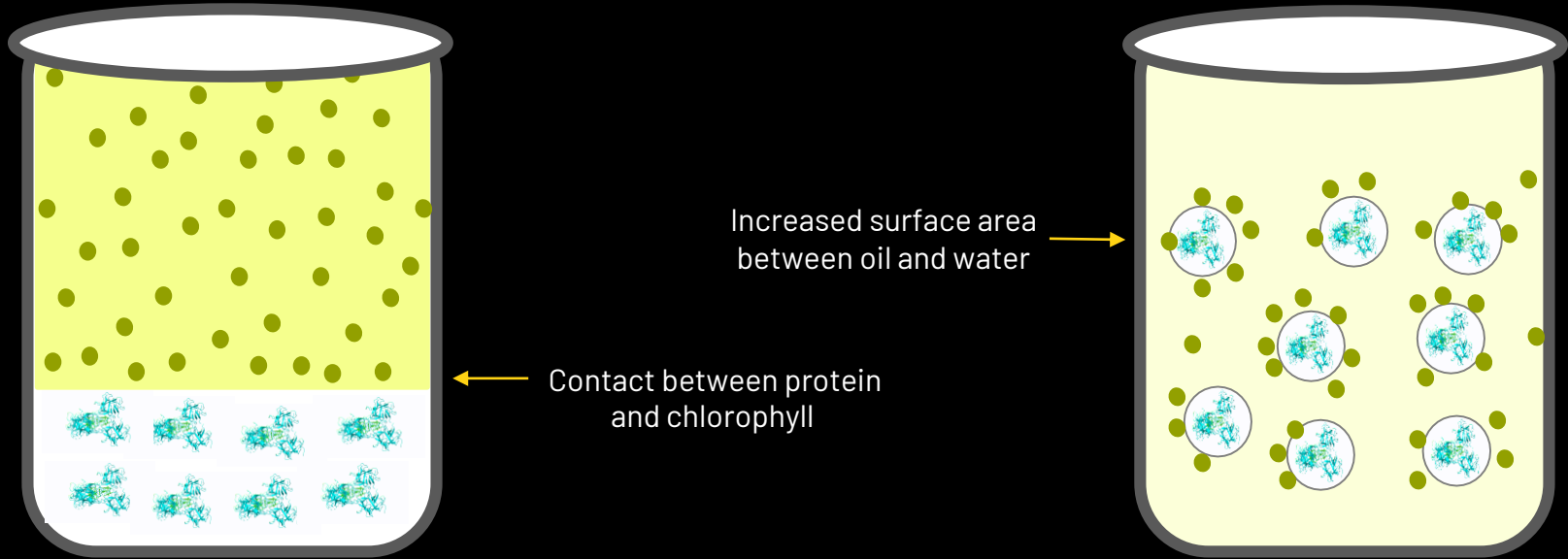
6GIX in Aqueous Phase



6GIX in water droplet

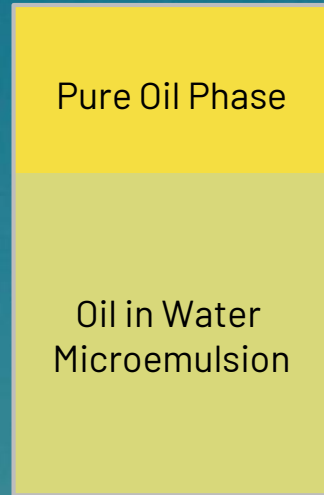
Our Solution

Oil-in-Water Emulsion



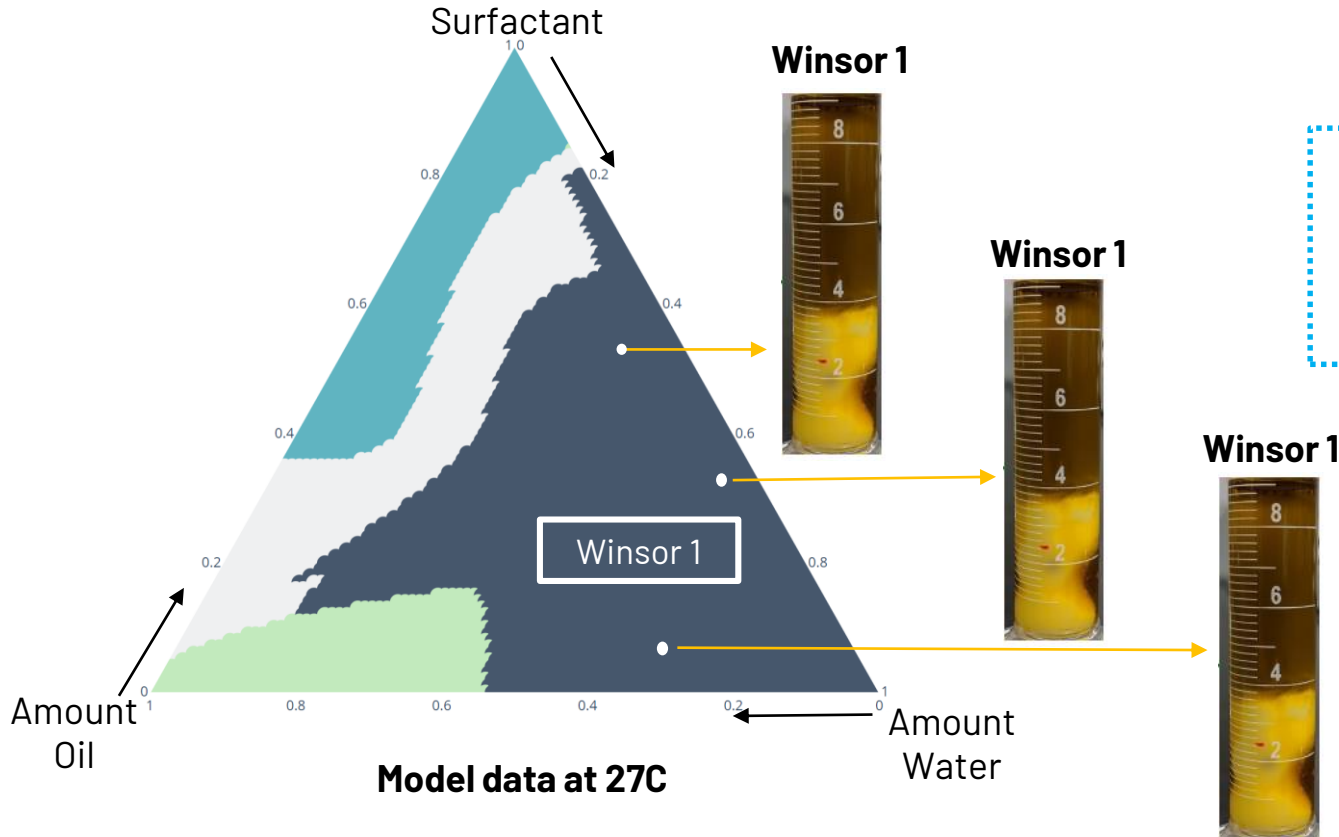
Emulsion Types

Different combinations of phases give different emulsions



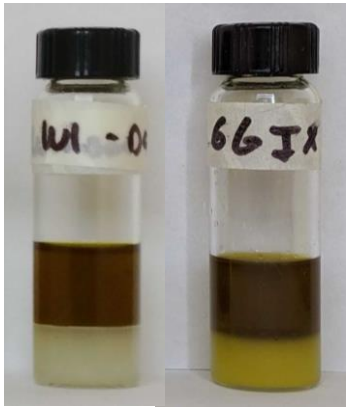
Winsor 1

Phase Diagram Models



6GIX Emulsion Results

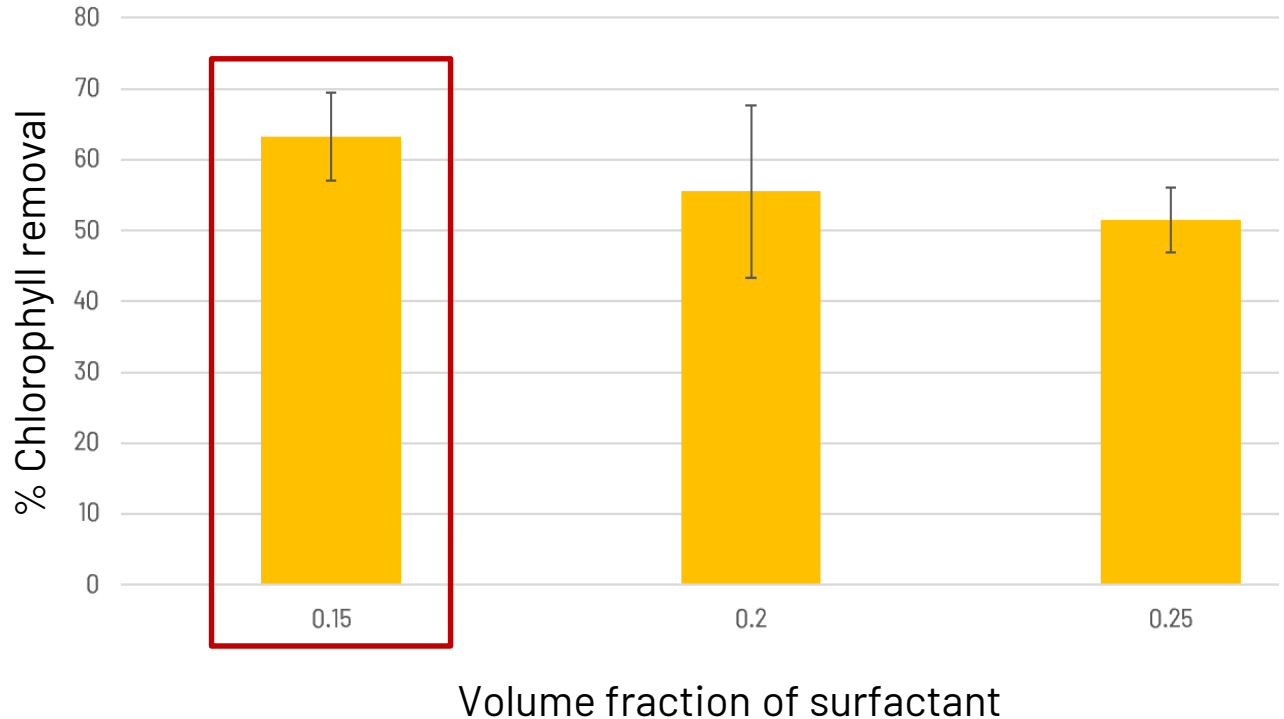
Three different emulsion compositions



No protein
Emulsion

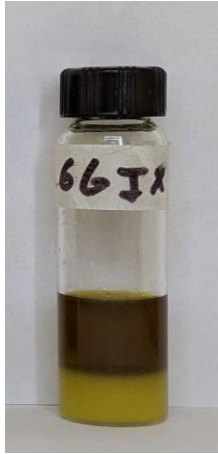
6GIX
Emulsion

6GIX Concentration= ~5uM

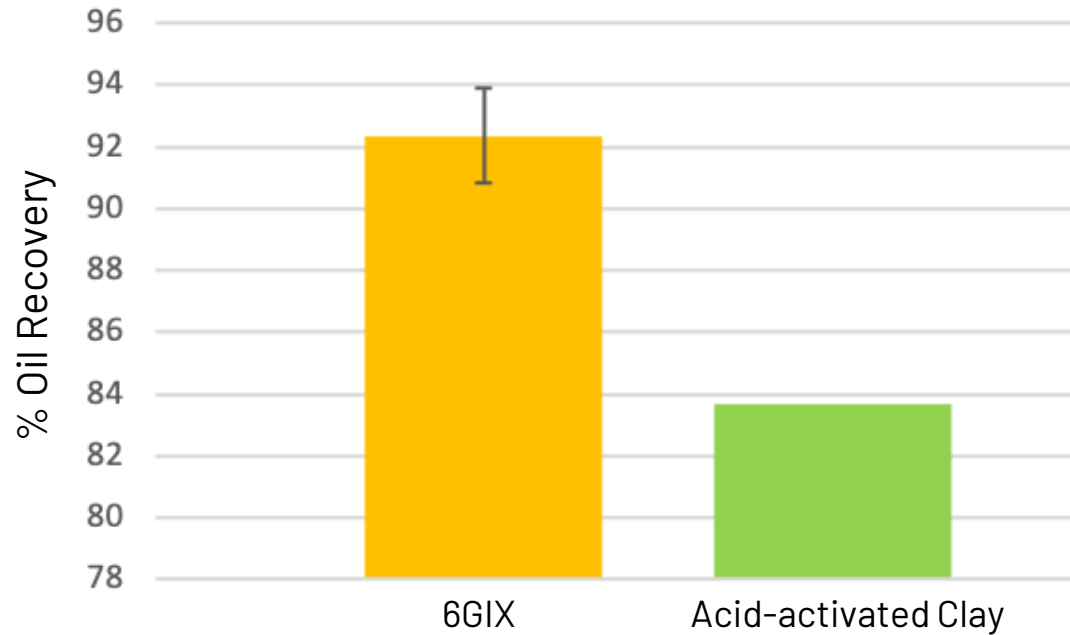


Oil Recovery

How much oil is lost through processing

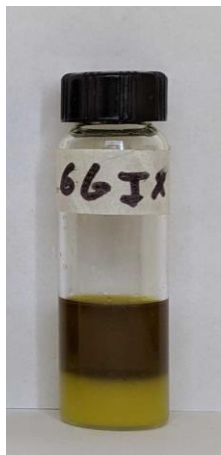


6GIX
Emulsion

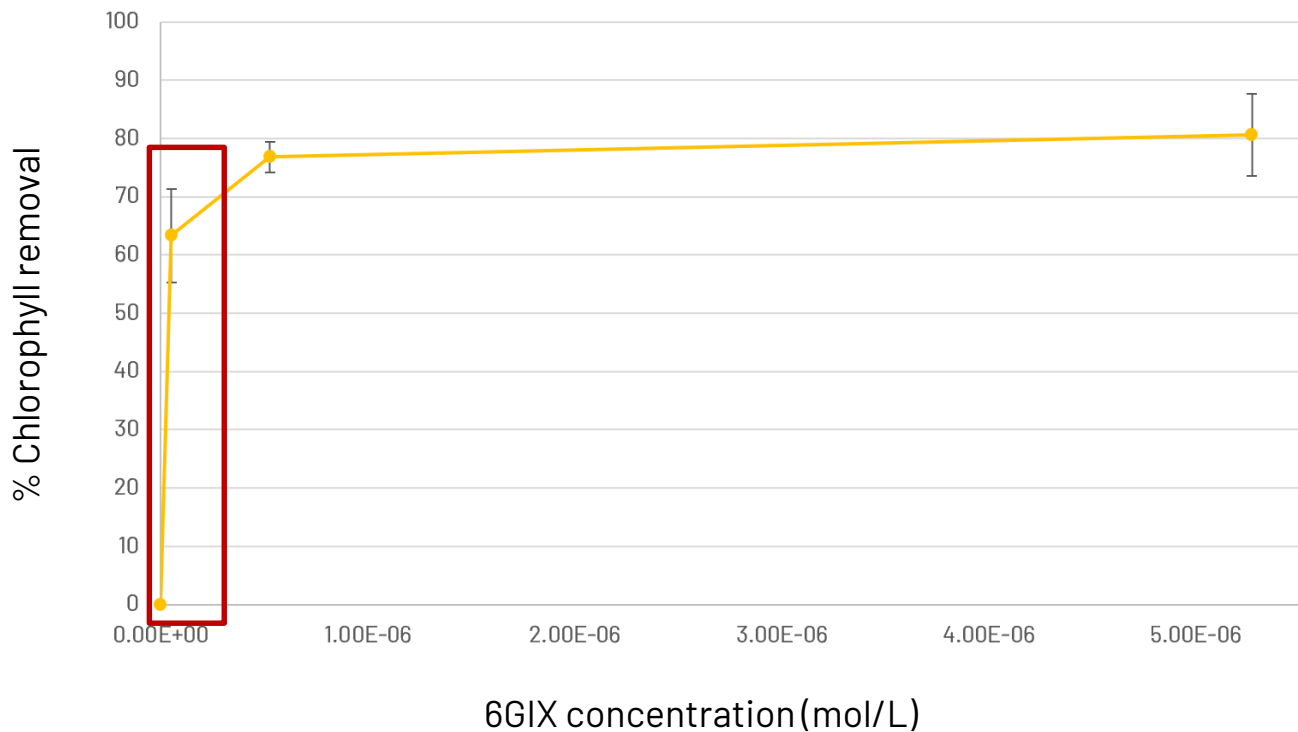


6GIX Emulsions Results

Protein concentration vs. Chlorophyll removal



Aqueous phase with 6GIX



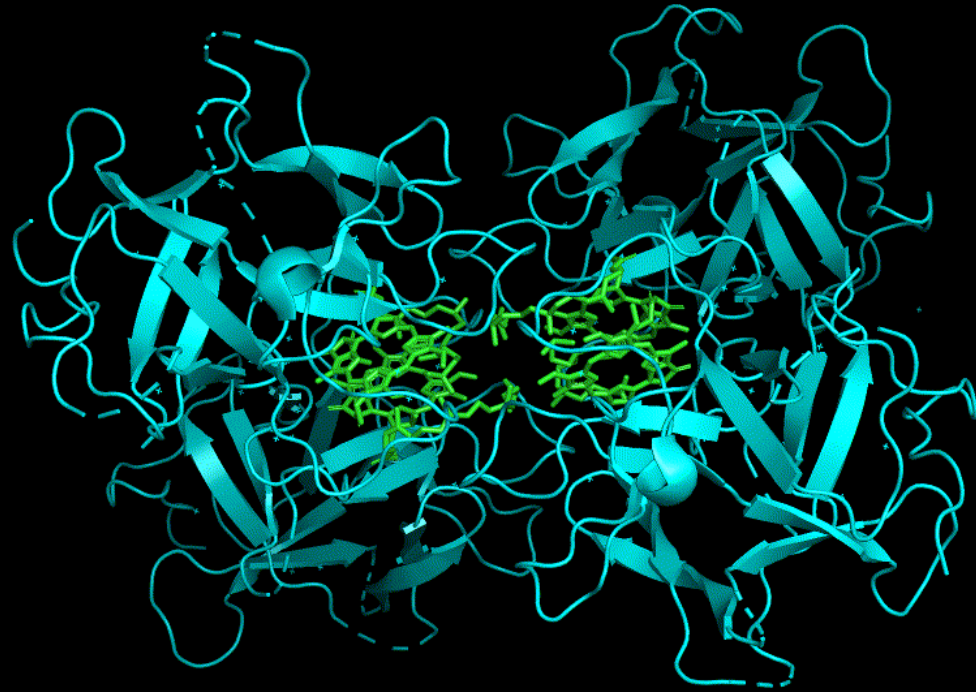
6GIX Instability

How to improve function and stability

12 amino acids cause variance

Denaturing at interface

iGAM – Genetic algorithm and PCA



MODGIX

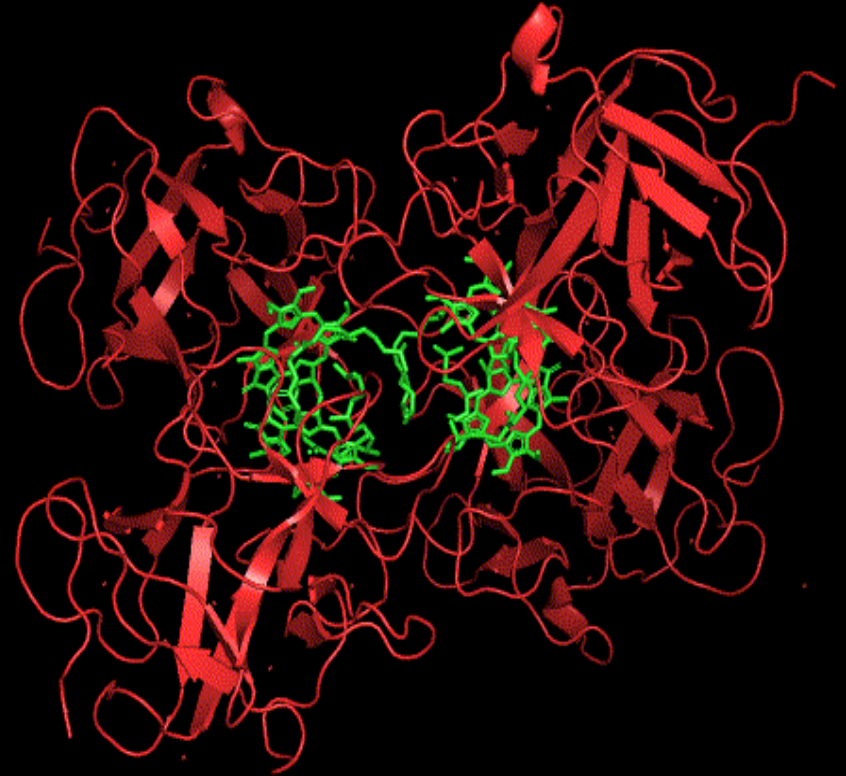
Modified 6GIX Protein

Modified for enhanced Chlorophyll Binding

Designed for stability

Higher binding potential

Less stabilizing agents required



Accomplishments

✓ Produce 6GIX

✓ Secrete 6GIX

✓ Purify 6GIX

✓ Determine best emulsion compositions

✓ Prove 6GIX's function in purifying green oil

→ Produce 6GIX on industrial level

→ Optimize for industry

Canola Oil Industry Pipeline

Oil Production



Chlorophyll
Extraction

Canola Oil Industry Pipeline





Craig Shand



Ward Toma



John Mayko

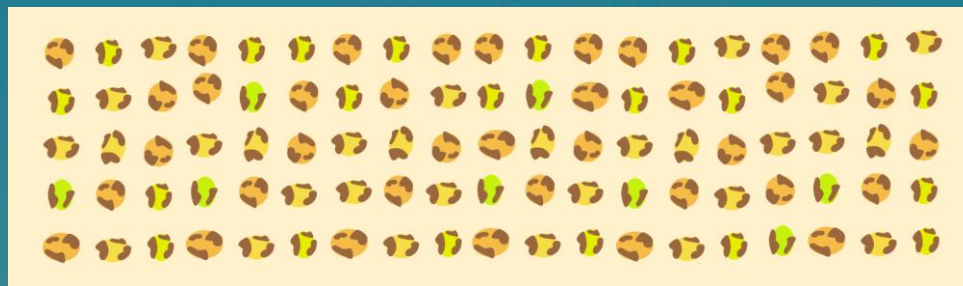
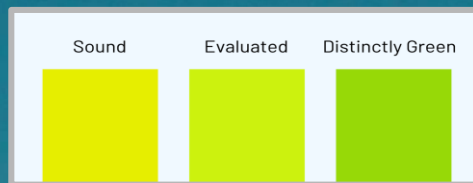


“Green is the difference between **profit** and **loss.**”

-Craig Shand
Co-Owner/Operator, Shand Farms Ltd.

Current Grading System

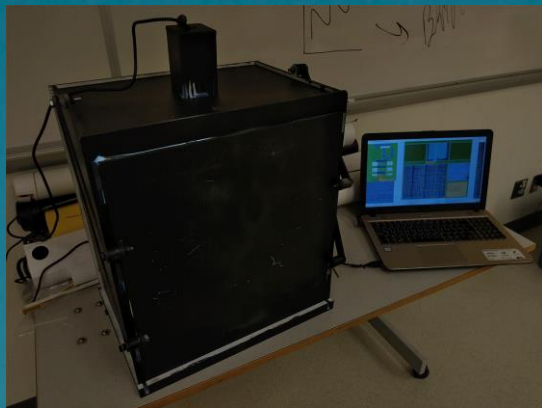
Seed Sample Assessed Against Colour
Guide



Canadian Grain
Commission



Mean Green Machine



Consistent Lighting



Accurate Capture



Colour Determination
Algorithm

Can we empower farmers with the ability to produce **better quality seeds**?



Angela Brackenreed
Agronomist



Autumn Barnes
Agronomist

Sunny Days

A **Precise Predictive Algorithm** to **Inform Effective Agronomy**



Within 2.5 degrees on average

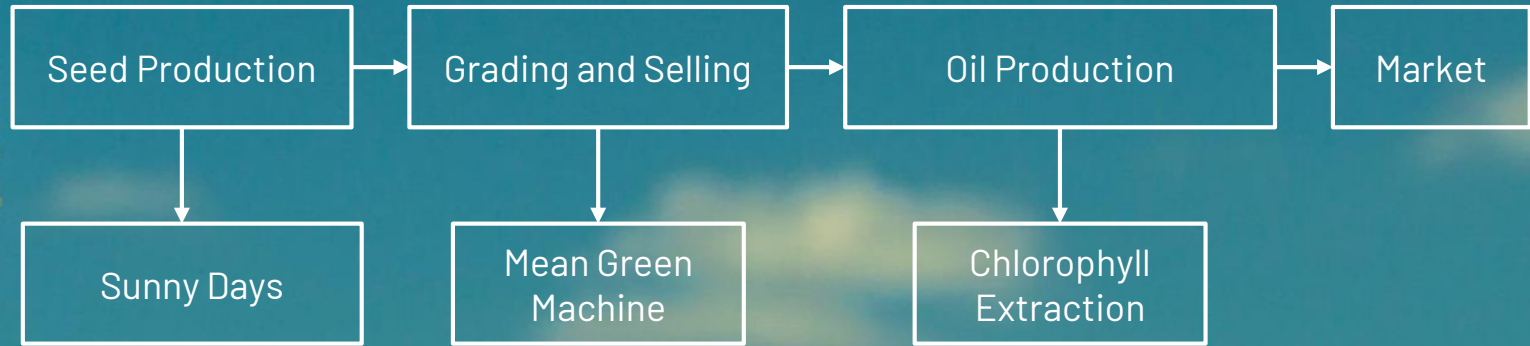


Prediction 5 months in advance



Informed Management

Canola Oil Industry Pipeline



Check out our poster and wiki!



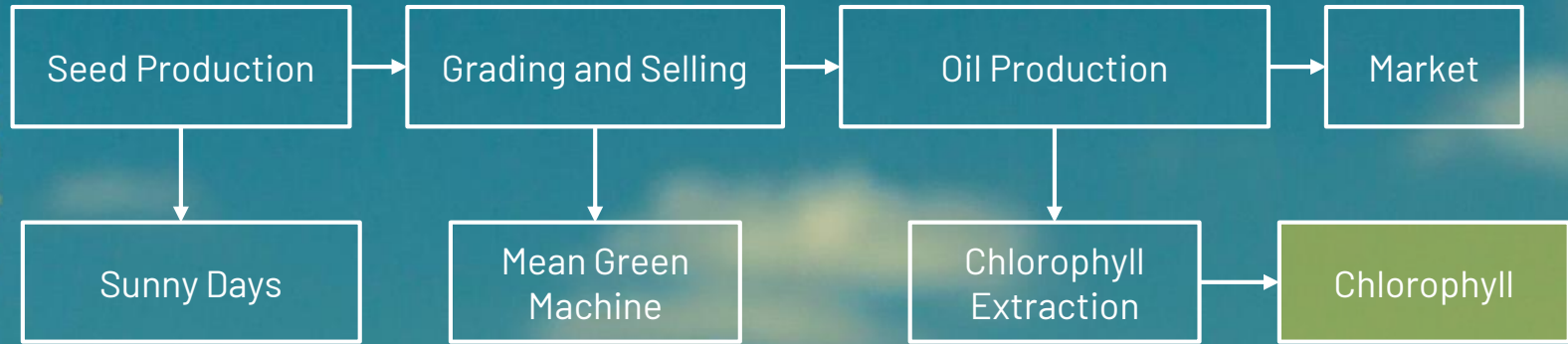
canolaPALOOZA





Green seed is a **financial problem.**

Canola Oil Industry Pipeline



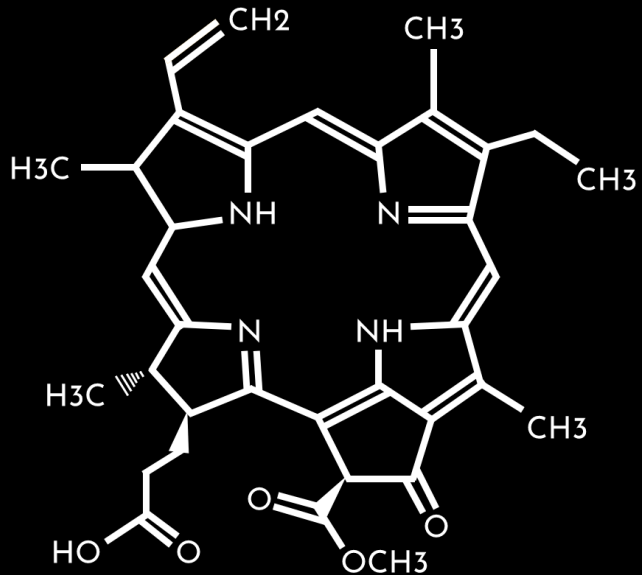


“Byproducts are valuable if they **offset** production costs”

- Dallas Gade

General Manager, Richardson Oilseed

Pheophorbide a



High value

Research: cancer & anti-fungal
treatment

Canola suffers from fungal
diseases

Sclerotinia

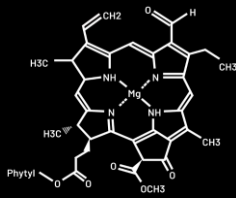


"Anybody growing canola in Alberta will have to deal with [fungus]."

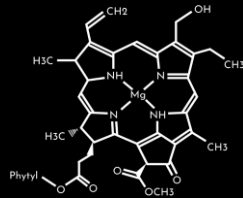
John Mayko

Farmer, The Alberta Canola Producers Commission

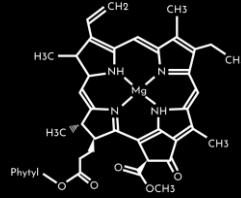
Chlorophyll to Pheophorbide: Steps



Chlorophyll b



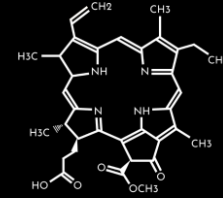
7HCA



Chlorophyll a



Pheophytin a



Pheophorbide a

Chlorophyll b
Reductase
(CBR)

7-hydroxymethyl
chlorophyll a reductase
(7-HCAR)

Magnesium
Dechelatase
(SGR)

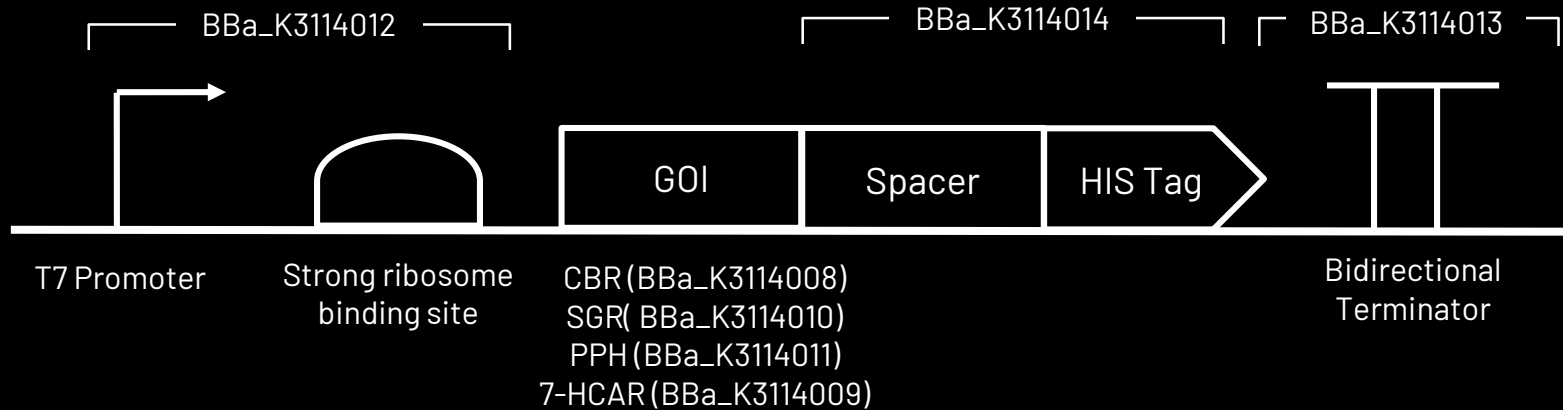
Pheophytinase
(PPH)

Stage One

Stage Two

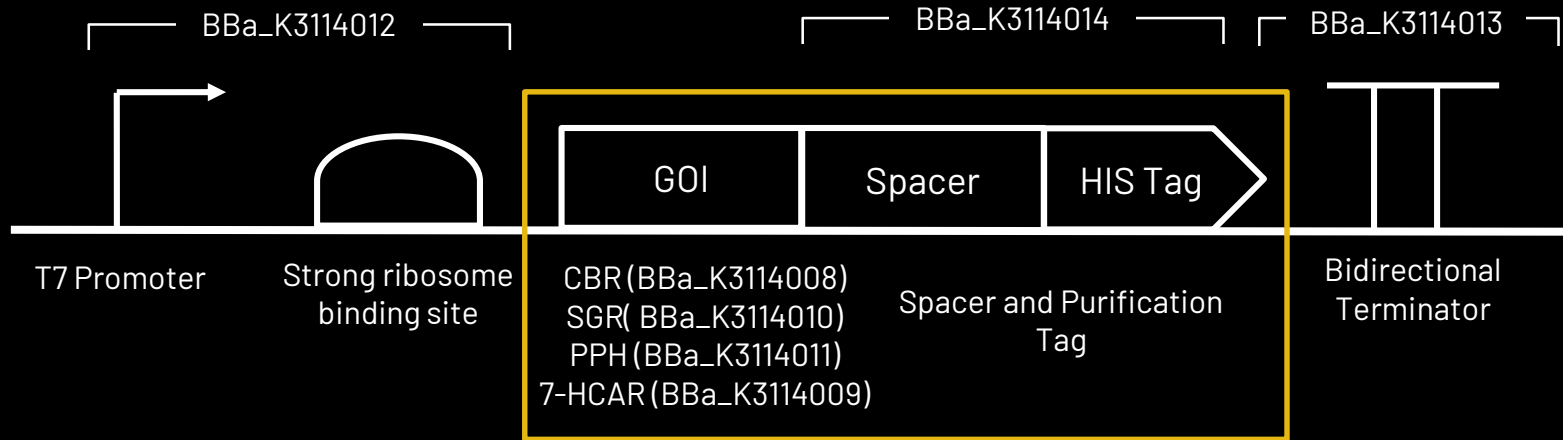
Synbio for Green Seed

Chlorophyll Degradation Proteins



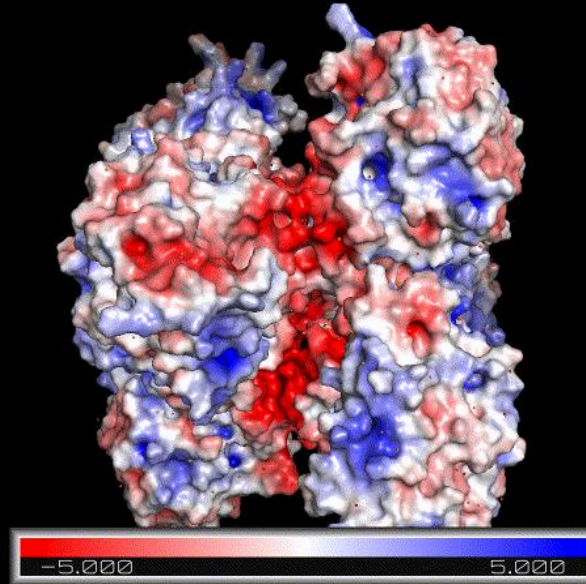
Synbio for Green Seed

Chlorophyll Degradation Proteins



Synbio for Green Seed

Protein-Spacer-His Tag

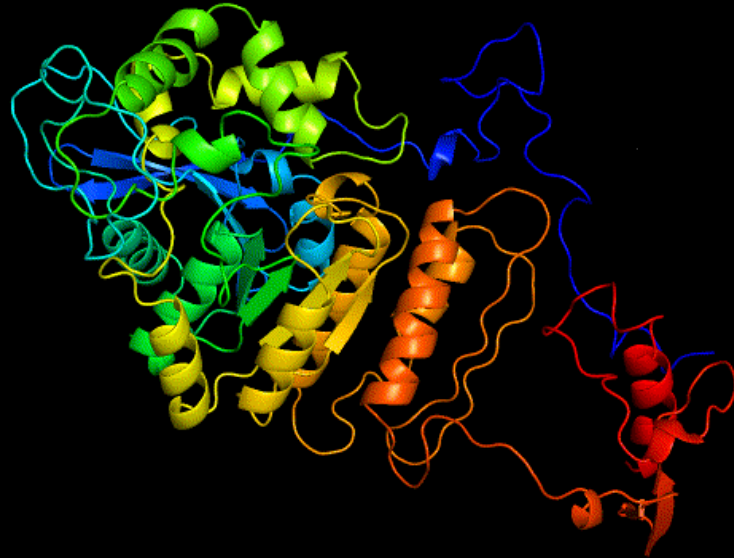


(Meguro et al., 2011)

7-HCAR

Synbio for Green Seed

Protein-Spacer-His Tag

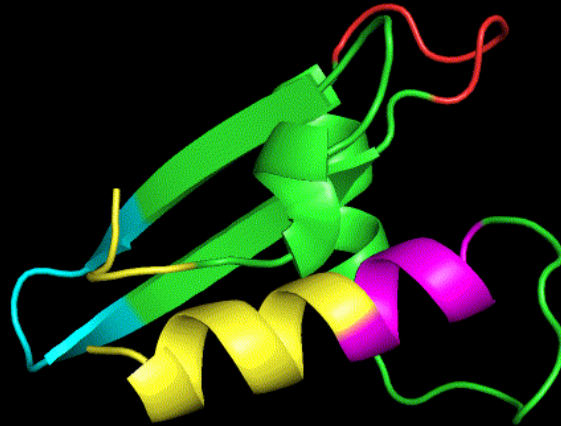


(Guyer, Salinger, Krügel,
& Hörtensteiner, 2017)

PPH (Predictive Homology Model)

ICARUS Universal Spacer

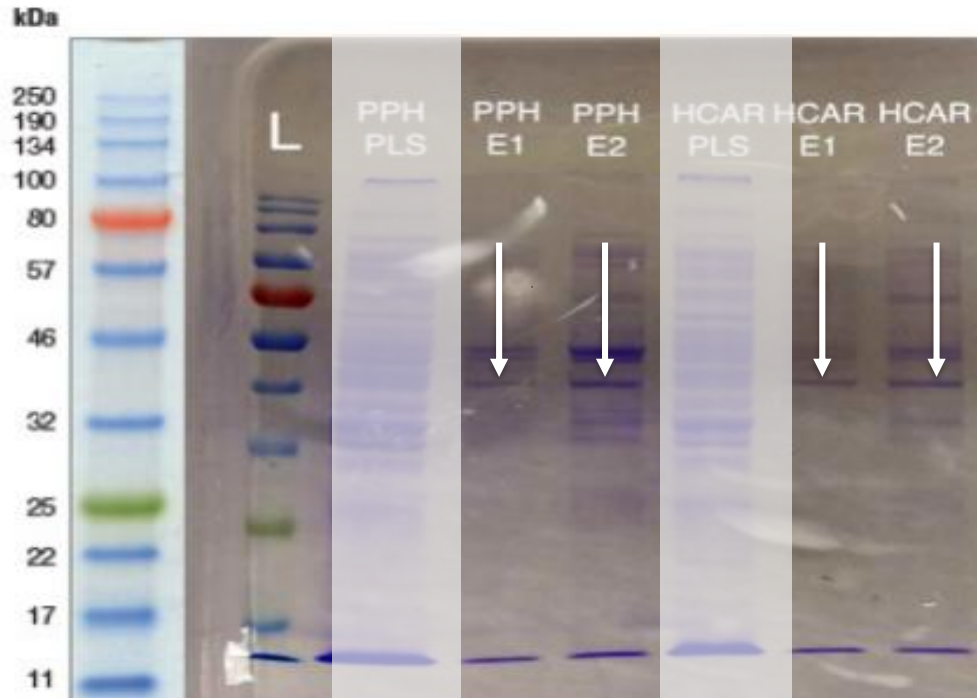
BBa_K3114014



ICARUS (Predictive Homology Model)

Protein Purification

Protein-Spacer-His Tag



Both HCAR and PPH SUCCESSFULLY purified.

Pheophytin



Pheophytin + Pheophorbide



Pheophytin + PPH = Pheophorbide



Pheophytin - Red
Pheophorbide - Pink

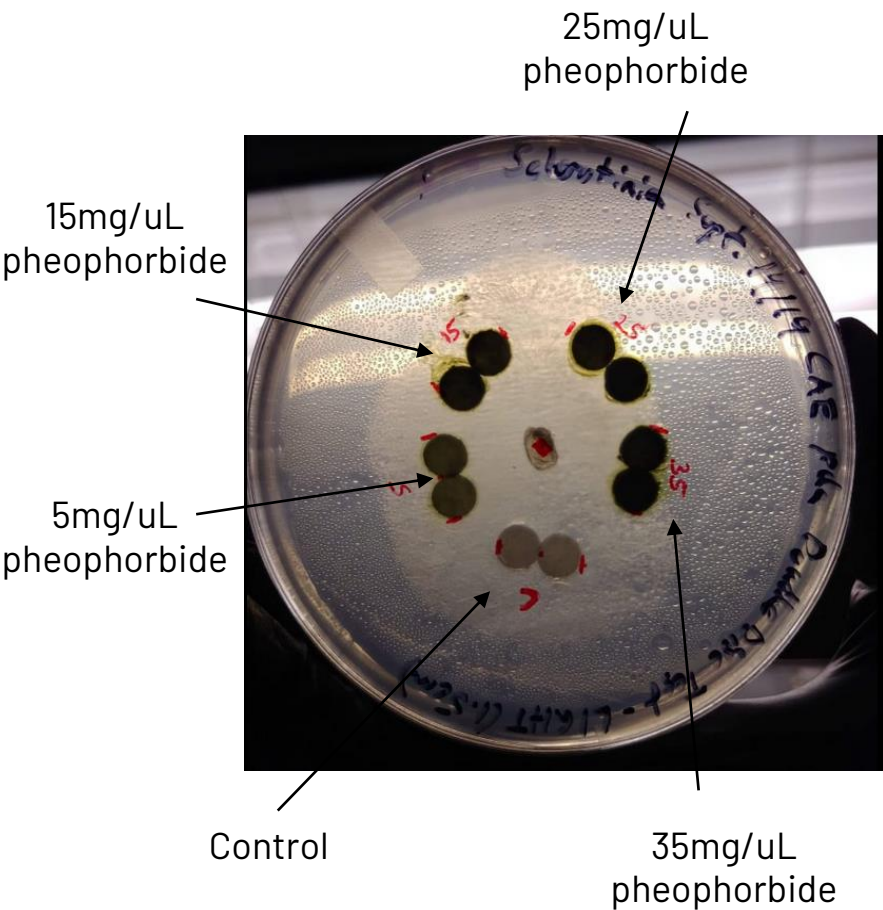
Thin Layer Chromatography

Showing PPH Function

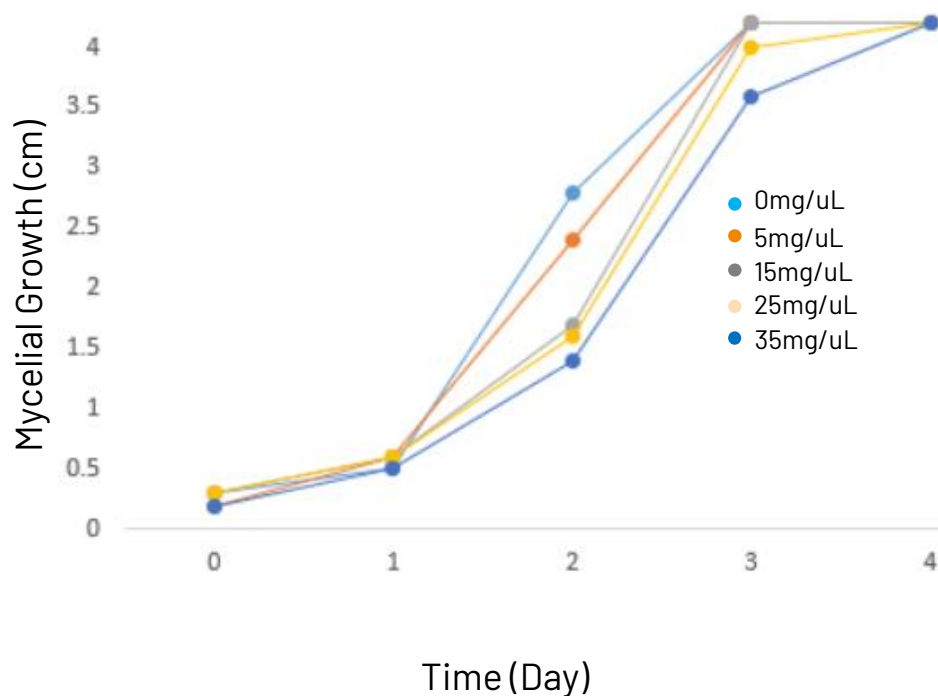
Pheophytinase (PPH) converts Pheophytin a into Pheophorbide

Pheophorbide Testing

Disc Test



Mycelial growth in pheophorbide a presence



Accomplishments



Produced PPH and HCAR



Purified PPH and HCAR with ICARUS



Showed Pheophorbide Inhibited sclerotinia

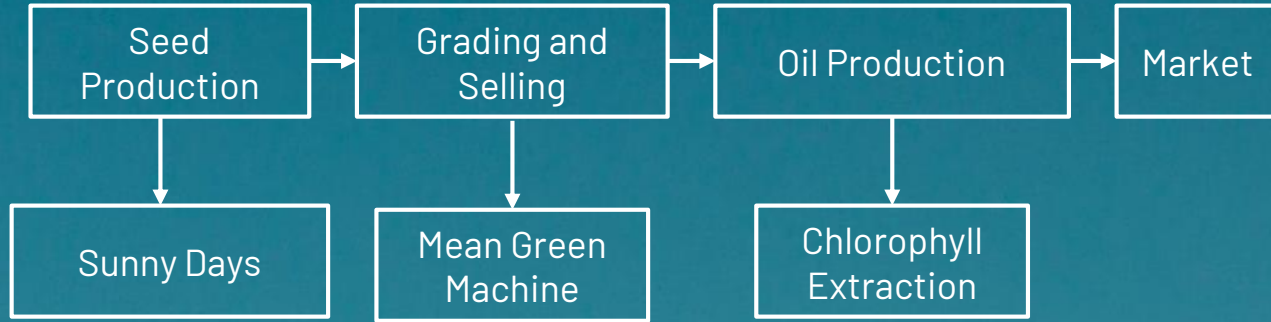


Produce chlorophyll degradation enzymes

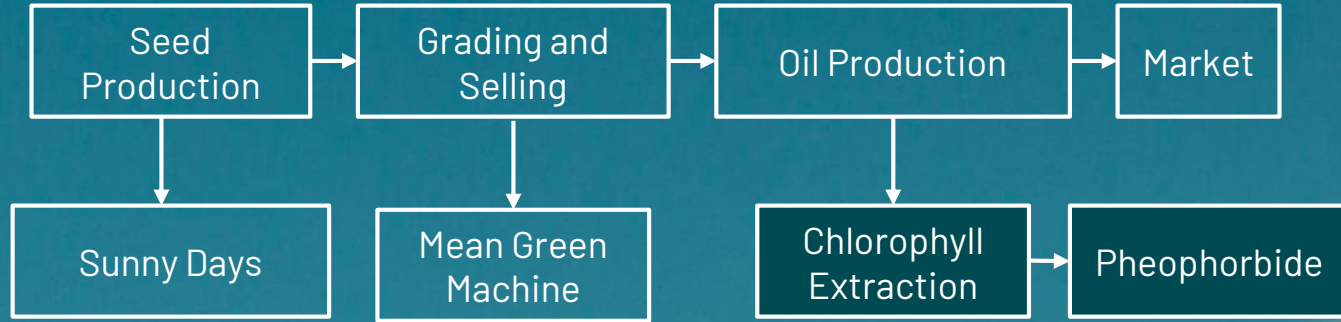


Field test Pheophorbide

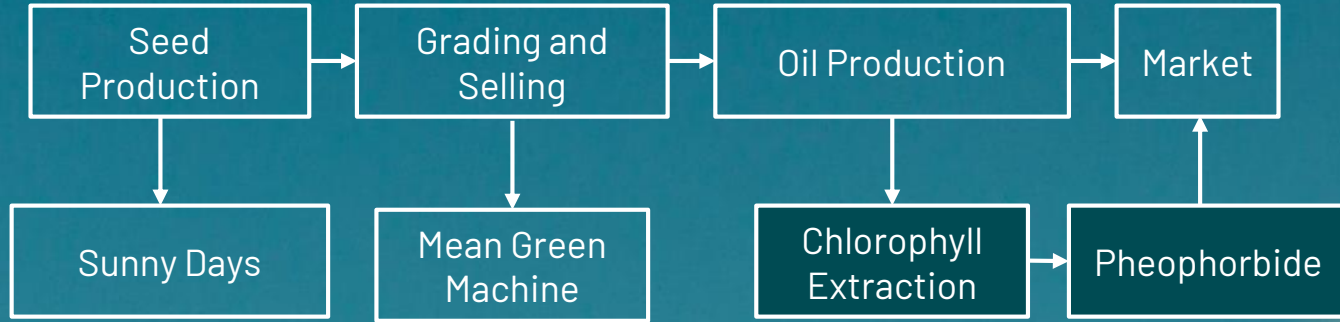
Canola Oil Industry Pipeline



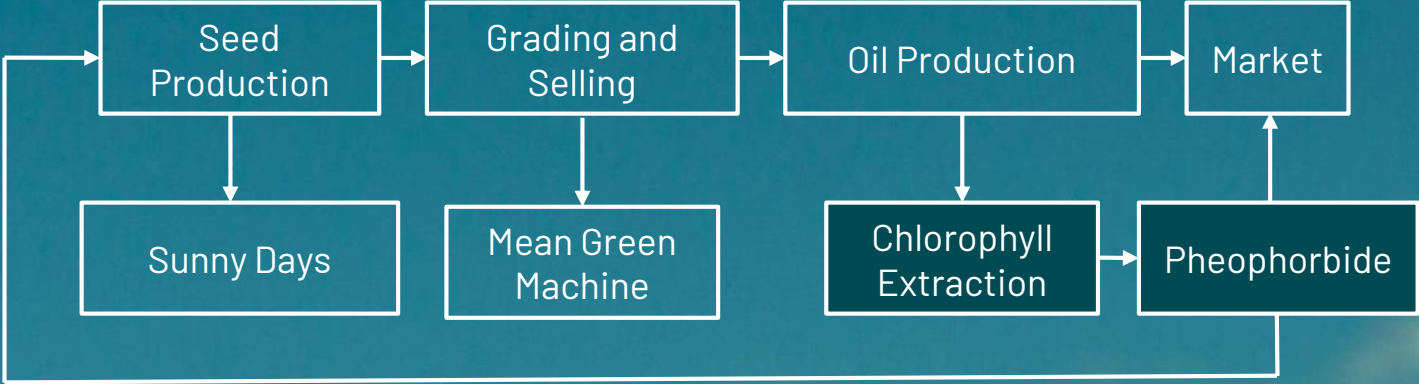
Canola Oil Industry Pipeline



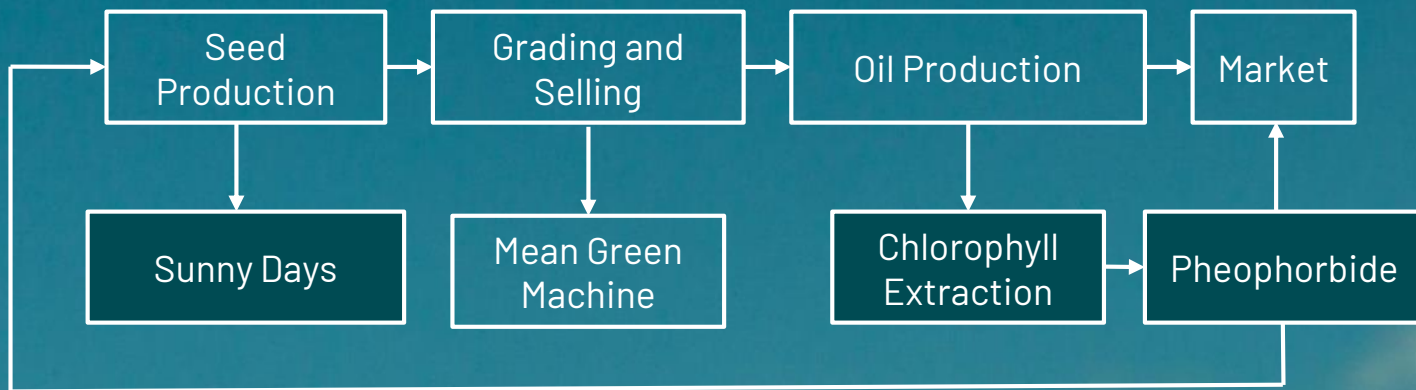
Canola Oil Industry Pipeline



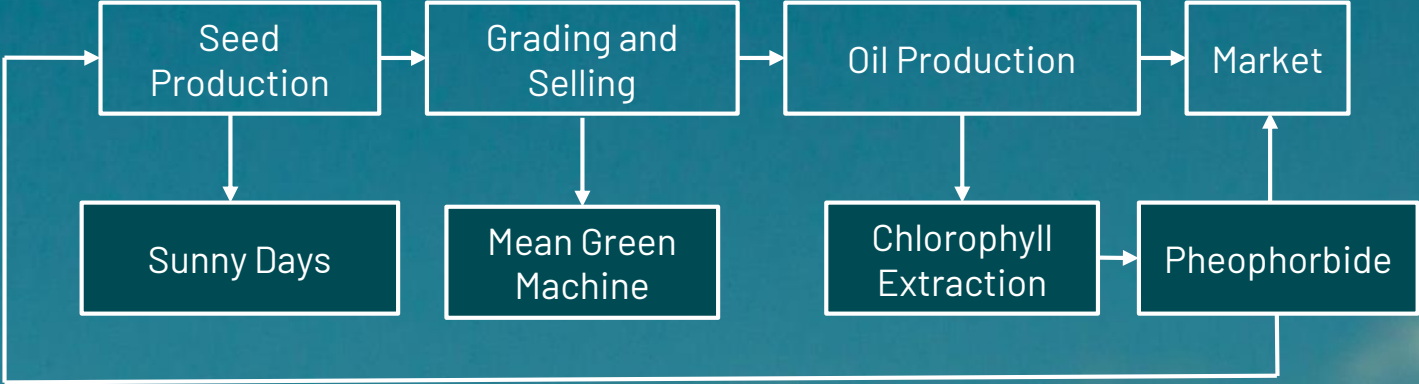
Canola Oil Industry Pipeline



Canola Oil Industry Pipeline



Canola Oil Industry Pipeline



Building
Partnerships



IP Protection

The background of the slide is a clear blue sky with a few wispy white clouds. In the foreground, there are several yellow flowers on green stems, some in focus and some blurred. The flowers are positioned on the left and right sides of the frame.

28 New Parts

7 Models that Informed Project Design

37 Stakeholder Meetings

Unquantifiable Number of Hours in the Lab

yOIL

An all-encompassing solution to the green seed problem

Sunny Days

Standardized
Grading

Chlorophyll
Extraction

Pheophorbide

General Support:

David Bailey
Anita Ludwar
Patrick Wu
Dr. Gijs van Rooijen
Emily Hicks
Robert Mayall
Dr. Peter Facchini
Swapan Kakumanu
Dr. John Baker
Dr. Gavin Cameron
Deirdre Lobb

General Wet Lab Support:

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Dr. Vanina Zaremburg
Dr. Isabelle-Barette-Ng
Daniel Ziemianowicz
Margaret Redaud-Young
Dr. Dae-Kyun Ro

Chlorophyll Extraction:

Dr. Raymond Turner
Dr. Marie Elizabeth Fraser
Dr. Marcus Samuel
Dr. Ian Lewis
Dr. Gordon Chua
Dr. Joe Harrison

Emulsions:

Dr. Nashaat Nassar
Dr. Giovannantonio Natale
Dr. Hector Siegler
Dr. Jianxun He
Dr. Kazi Sumon

Chlorophyll Repurposing:

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Dr. Heather Addy
Fran Cusack
John Mayko
Dr. Kelly Turkington

HP:

Craig Shand
Randall Weselake
Dr. Veronique Barthez
Angela Brackenreed
Autumn Barnes
Pleasant Valley Oil Mills
Dallas Gade
Laurence Parslow
James Rae
Ward Toma
Dr. Brian Beres

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John Pye
Bogusia Gierus
Erin Kelly

Dry Lab General:

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Dr. David Anderson
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Sean Mason
Dr. Usman Alim
Jeff Danielson
Dr. Thierry Chekouo
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Dr. Laura De Castro

Entrepreneurship:

Elisa Park
Noren Howg
Erin Kulhawy

UNIVERSITY OF
CALGARY



CUMMING SCHOOL
OF MEDICINE

UNIVERSITY OF
CALGARY



Vice President
Research Office

SCHULICH

School of Engineering



geekStarter program
and funders



ALBERTA INNOVATES



APRIL 25, 2019



Shell Experiential Energy
Learning Program

UNIVERSITY OF
CALGARY



FACULTY OF
SCIENCE

UNIVERSITY OF
CALGARY



PROVOST
OFFICE

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CALGARY



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and funders



UCalgary
GivingDay
APRIL 25, 2019

ALBERTA INNOVATES



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CALGARY



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UNIVERSITY OF
CALGARY



PROVOST
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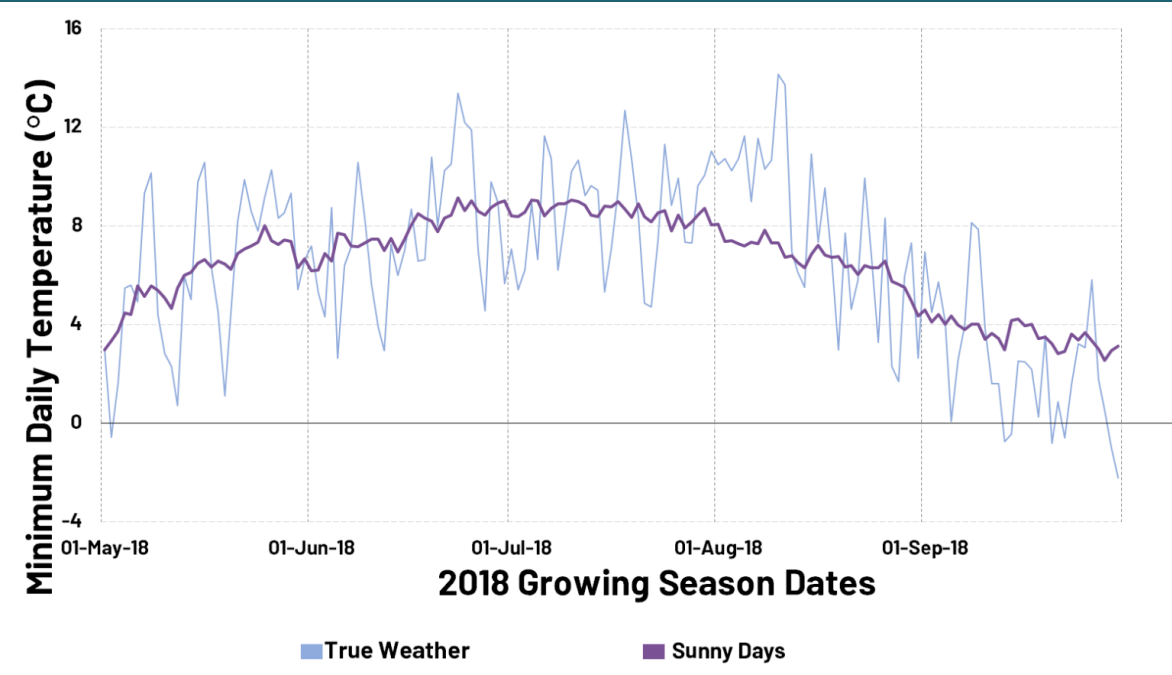
Chlorophyll
Extraction

Pheophorbide

Sunny
Days

Sunny Days

A Precise Predictive Algorithm to Inform Timing of Seeding



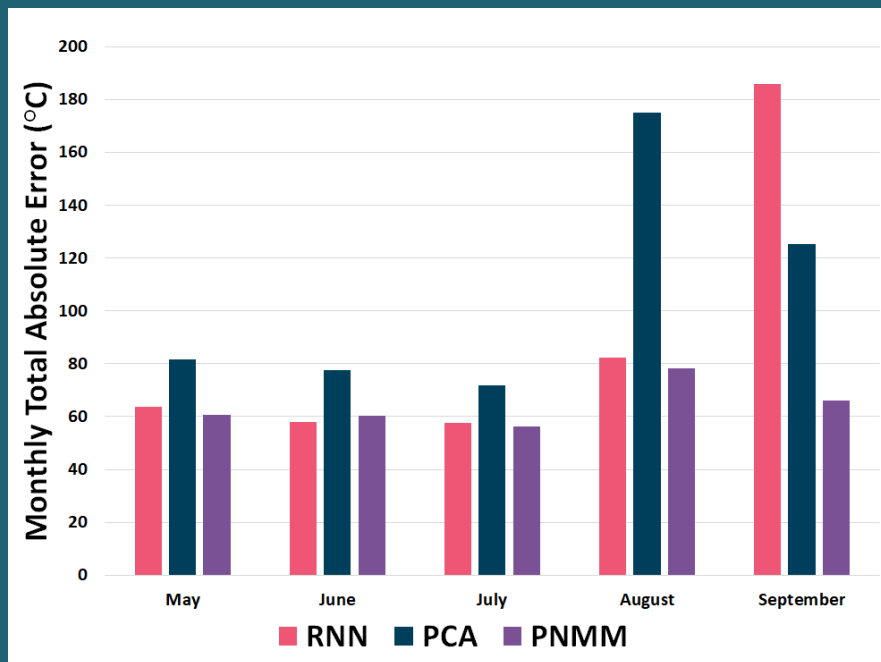
Prediction 5 months in advance

Within 2.1 degrees on average

In Peer Review at Alberta Academic Review

Sunny Days

A Precise Predictive Algorithm to Inform Timing of Seeding



Prediction 5 months
in advance

Within 2.1 degrees on
average

In Peer Review at Alberta
Academic Review

Air temperature forecasts' accuracy of selected short-term and long-term numerical weather prediction models over Poland

Sebastian Kendzierski¹, Bartosz Czernecki¹, Leszek Kolendowicz¹ and Adam Jaczewski²

¹Department of Climatology, Adam Mickiewicz University, Poznań, Poland

²Institute of Meteorology and Water Management – National Research Institute, Warszawa, Poland

Sunny Days

Comparison With Contemporary Methods

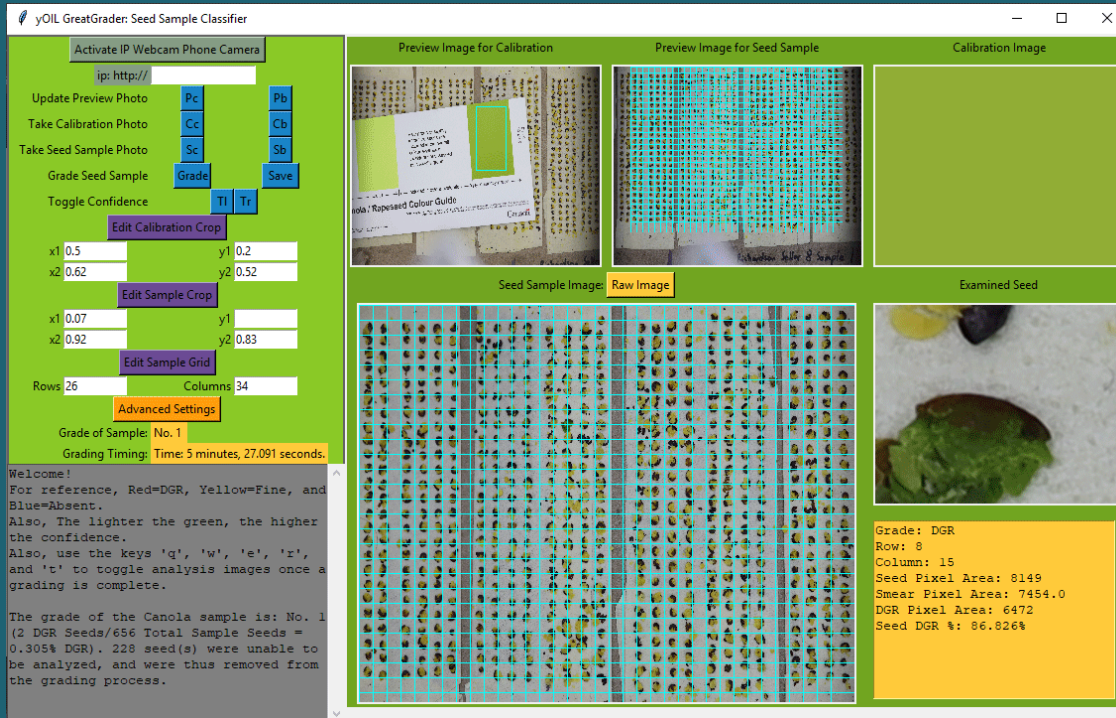
Sunny Days accomplished estimates for 180 days with a Mean Absolute Error of 2.109 C

Table 3. Statistical results of long-term forecast in different time horizons.

MODEL	<i>t</i> (hs)	ME	MAE	RMSE	MSE	BIAS	<i>r</i>
GFS	0–48	−0.09	1.63	2.24	5.00	0.97	0.94
	49–96	−0.07	1.91	2.56	6.53	0.96	0.95
	97–144	−0.17	2.38	3.17	10.02	0.93	0.93
	145–192	−0.23	2.93	3.87	14.94	0.90	0.93
	193–240	−0.25	3.52	4.60	21.85	0.86	0.92
HIRLAM	0–48	0.11	1.53	2.06	4.25	0.97	0.98
	49–96	0.09	1.82	2.42	5.84	0.96	0.98
	97–144	−0.04	2.31	3.07	9.42	0.94	0.97
	145–192	−0.18	2.97	3.92	15.34	0.90	0.95
	193–240	−0.15	3.40	4.46	19.91	0.87	0.94

Standardized Seed Grading

GreatGrader



A software tool for standardizing seed grading:

- Calibrate the system using a colour chip
- Take a seed sample picture
- Crop and divide seeds
- Grade
- Examine individual seeds
- See confidence

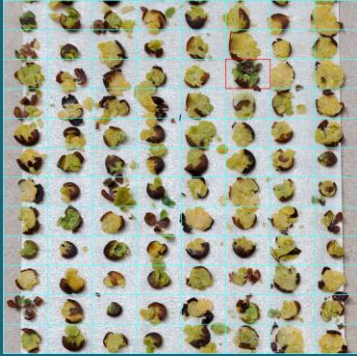
GitHub links:

Python Script Version:

<https://github.com/iGEMCalgary/DGRClassification>

Standalone Version for Windows 10 64-bit: <https://github.com/iGEMCalgary/GreatGrader>

GreatGrader Grading Process



1) Seed Sample



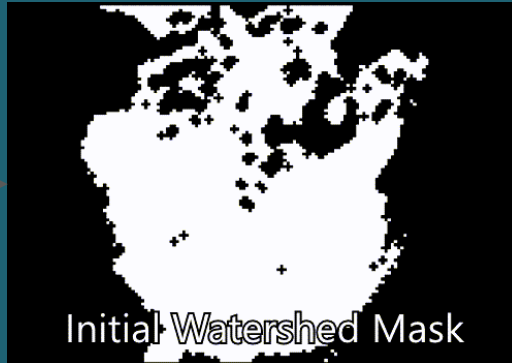
2) Individual Seed



3) Watershed Marking



4) Watershed Mask

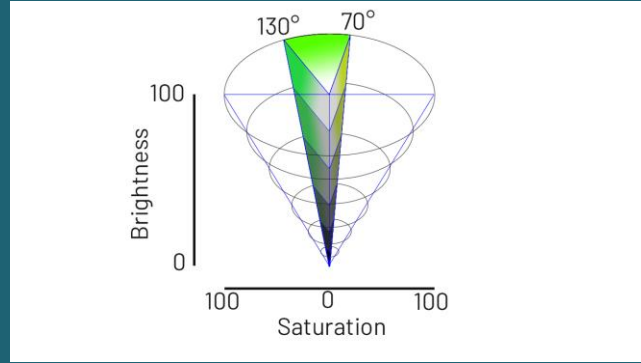


5) Modified Flood Fill



6) Final Seed Mask

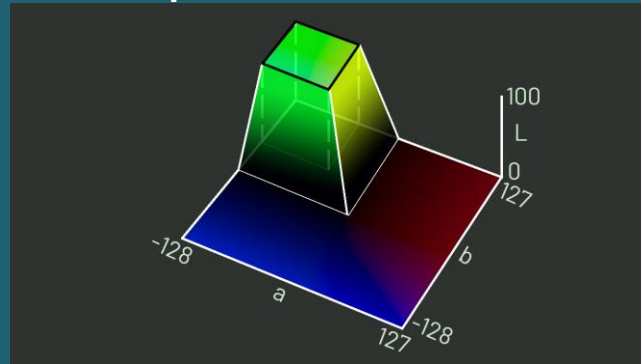
GreatGrader Grading Process



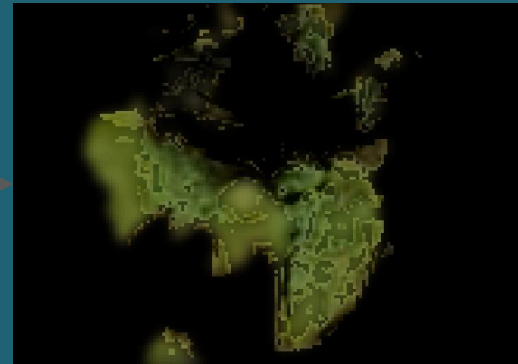
HSV colour space distance calculations used



7) Smear Parse



Lab colour space distance calculations used



8) DGR pixel grading

GreatGrader Performance

Sample	Human Grading DGR%	MGM & GG DGR%	Absolute Error (DGR%)	Time to Grade (seconds)
CGC #1	6.400%	7.444%	1.044%	164.287
CGC #2	7.000%	7.739%	0.739%	189.261
Grainger	4.143%	5.734%	1.591%	289.408
Richardson	0.308%	0.305%	0.003%	267.353

GreatGrader Performance

CGC Sample 1 Overall (GreatGrader):

145/1948 = 7.444% Distinctly Green Seed

1

$$\begin{aligned} 68/(612-105) &= \\ 68/507 &= \\ 13.4\% \end{aligned}$$

2

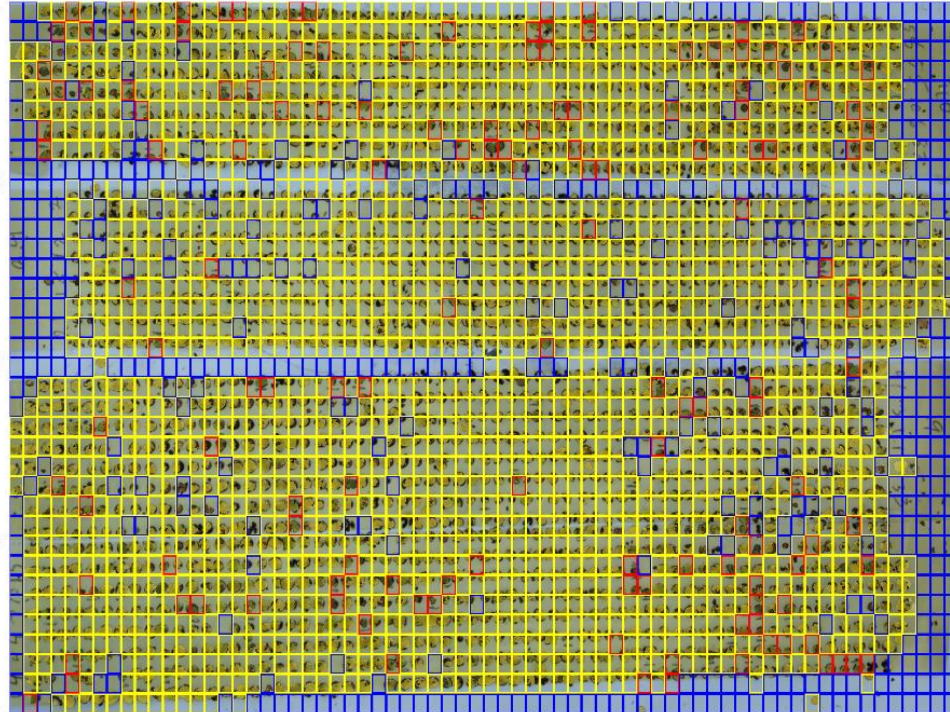
$$\begin{aligned} 11/(612-135) &= \\ 11/477 &= \\ 2.3\% \end{aligned}$$

3

$$\begin{aligned} 22/(612-137) &= \\ 22/475 &= \\ 4.6\% \end{aligned}$$

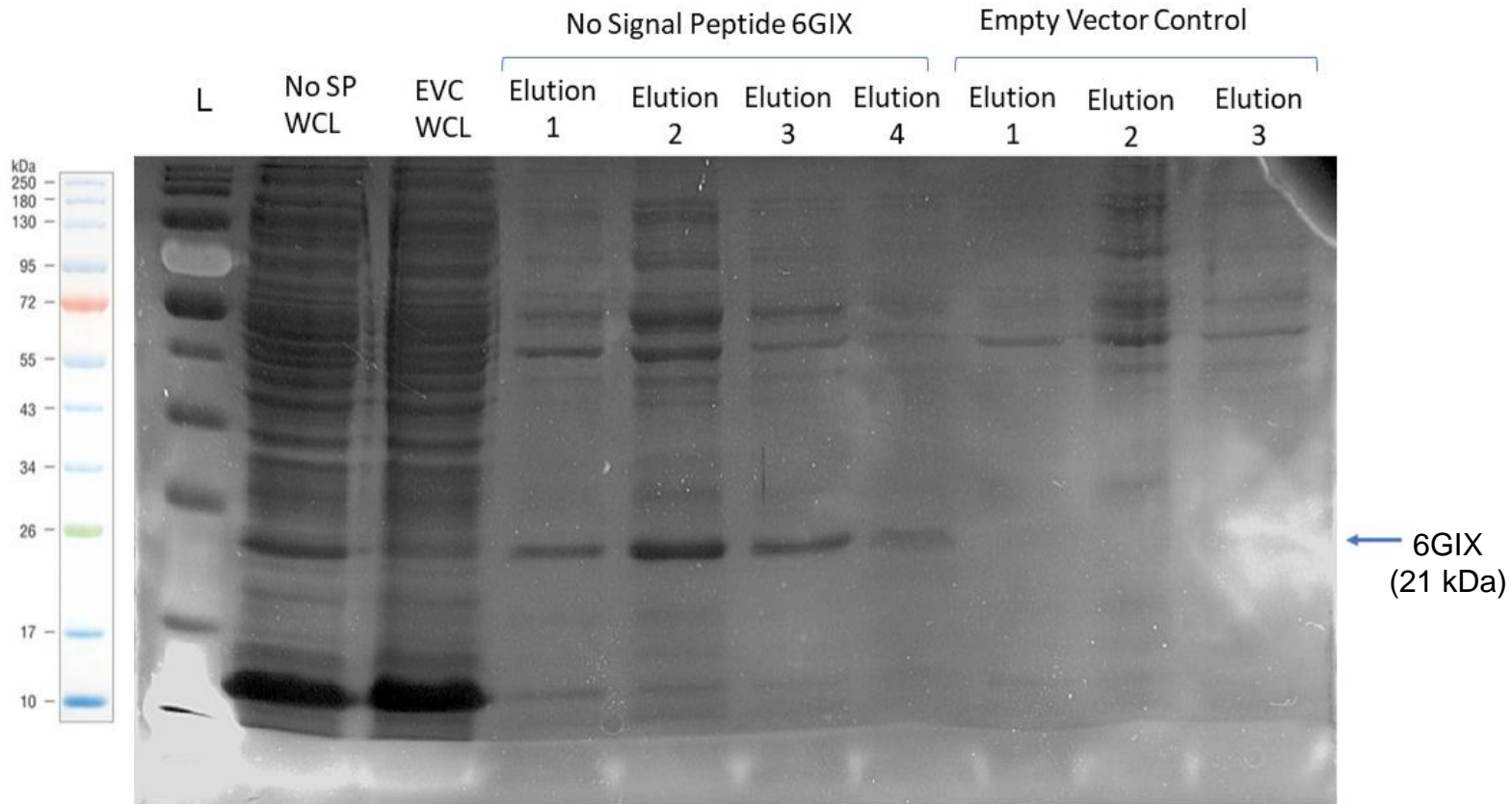
4

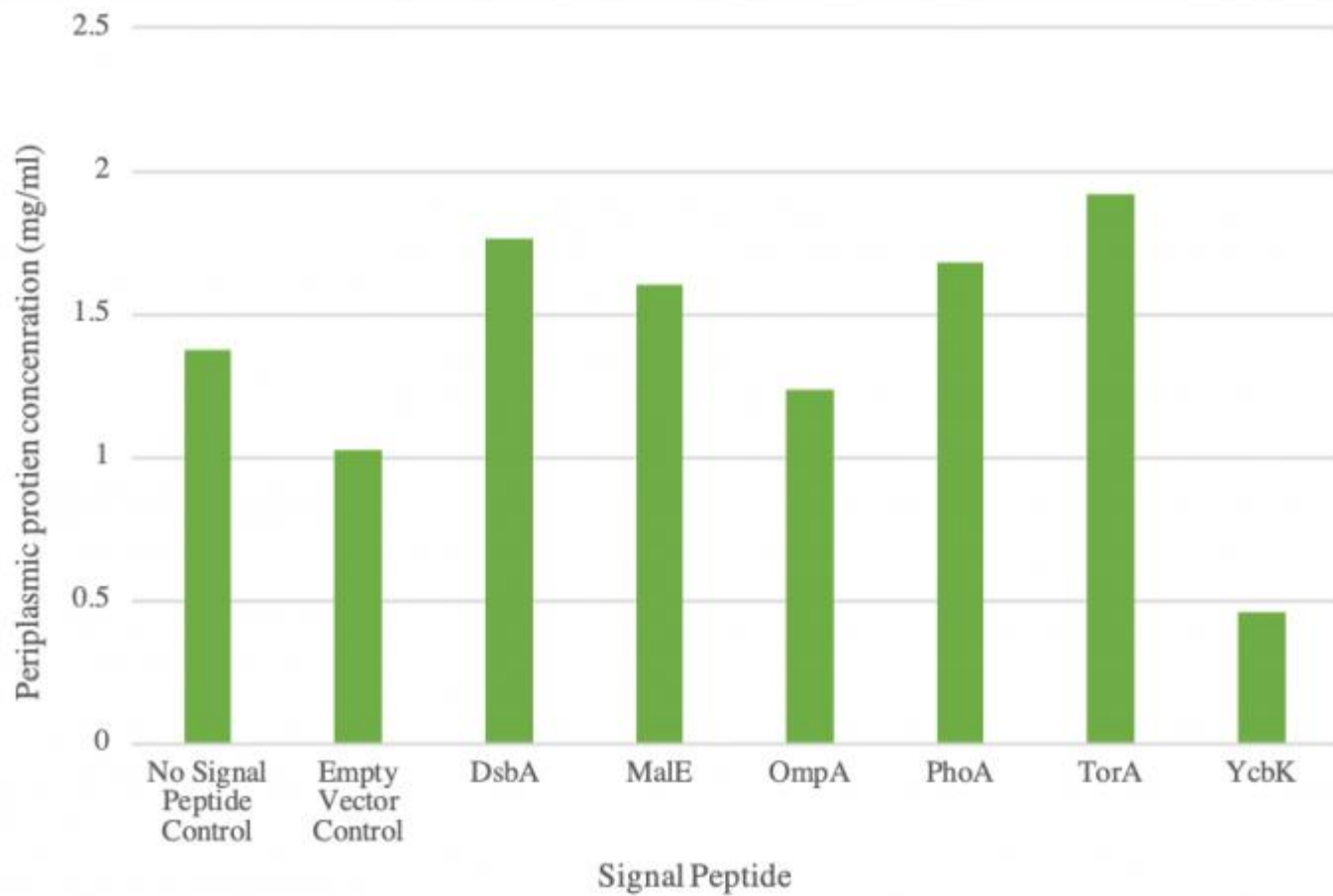
$$\begin{aligned} 44/(612-127) &= \\ 44/485 &= \\ 9.1\% \end{aligned}$$



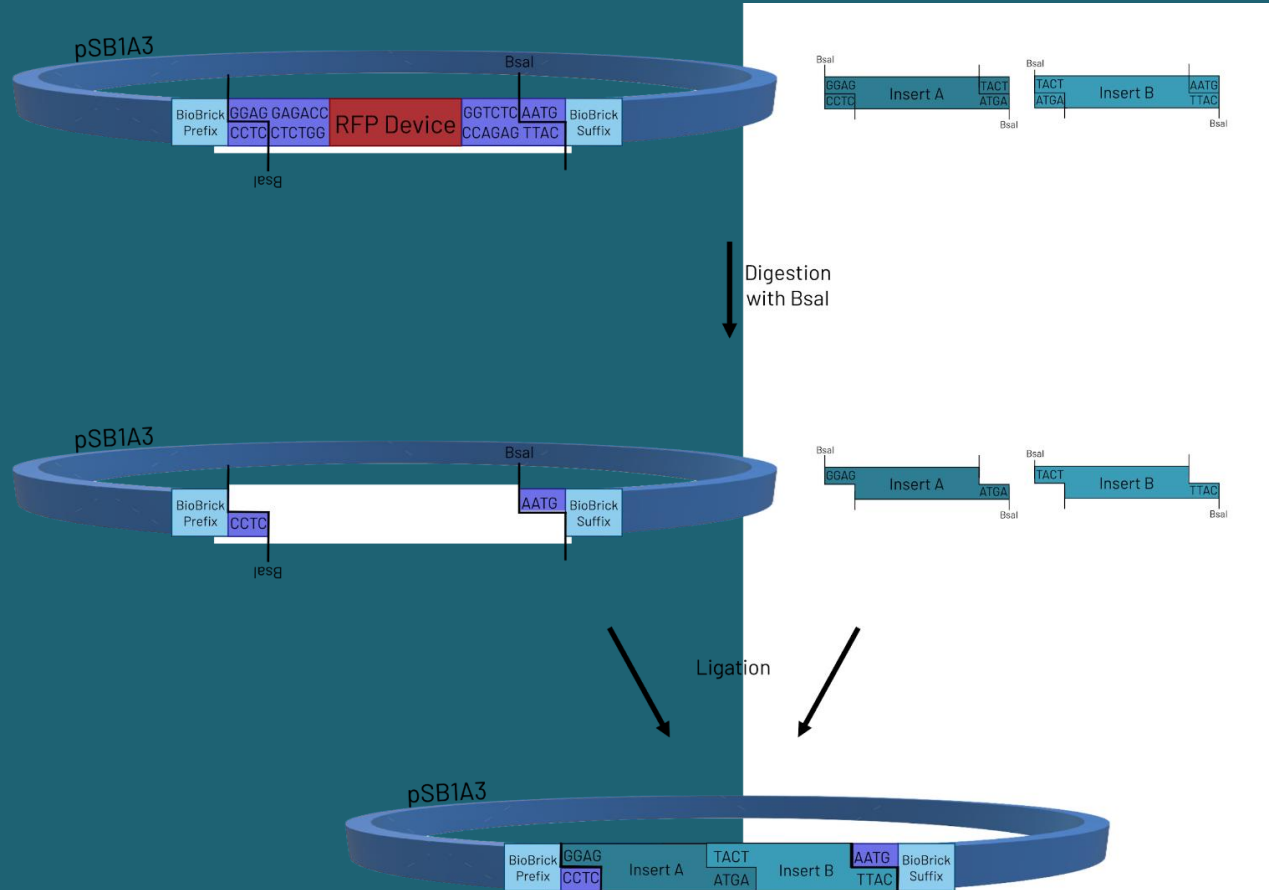
6GIX

Production





RFP Flipper Devices for Golden Gate Assembly



Emulsions

Green oil
(unprocessed)

Acid activated clay
(AAC)

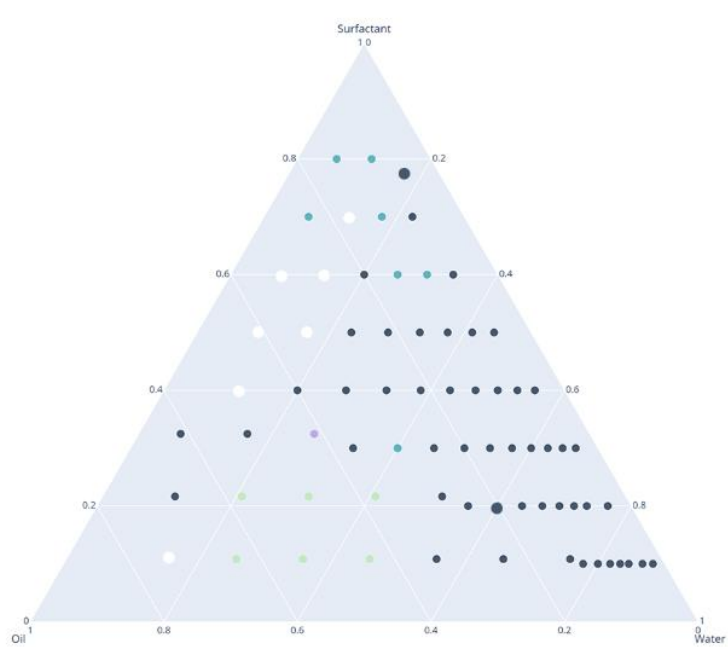
BSA emulsion

6GIX emulsion

Control emulsion
(buffer only)

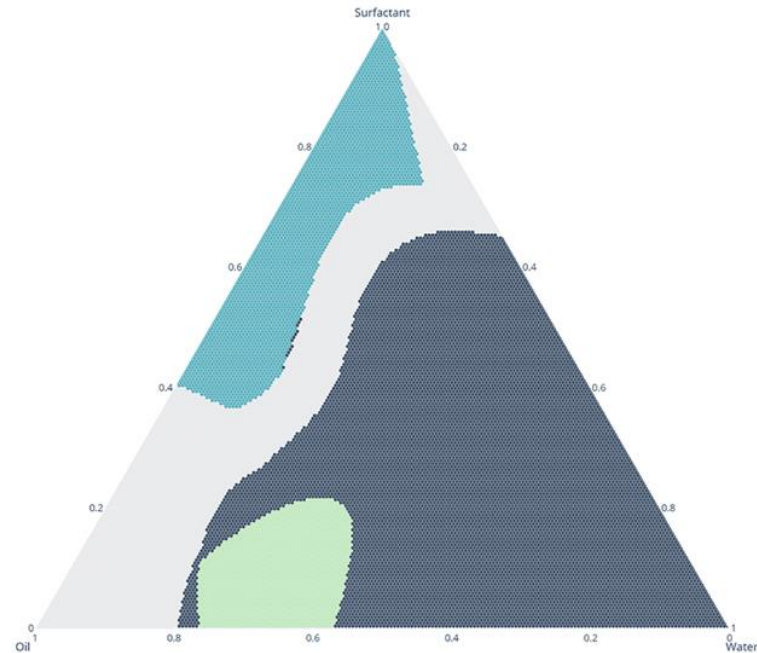
Yellow oil (fully
processed)





Collected Lab Data

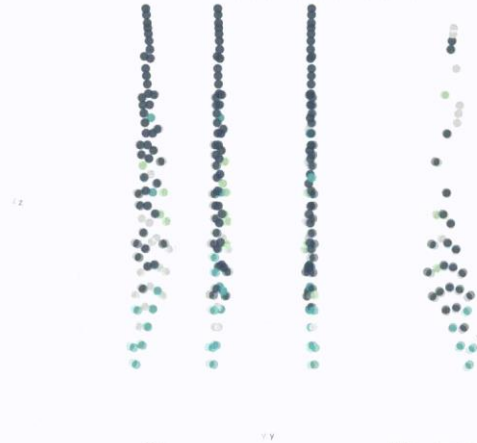
SVM



Classification Model

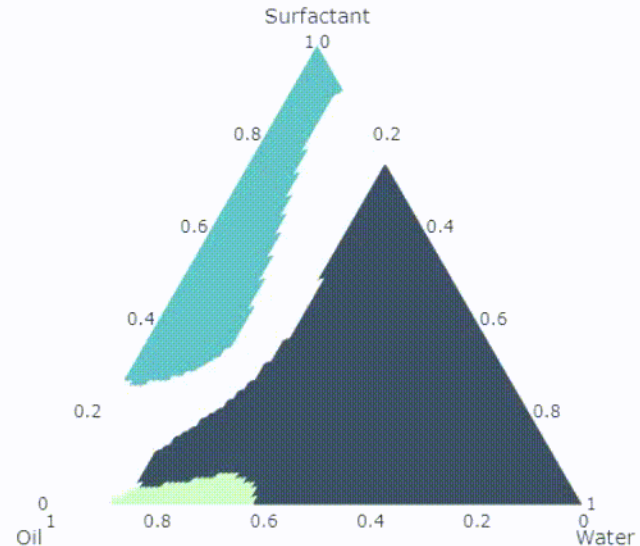
Phase Modelling Process

1. Experimental Data
2. SVM with RBF Kernel models phase data for the experimental temperatures
3. MLP models phase data for any temperature



Experimental Data

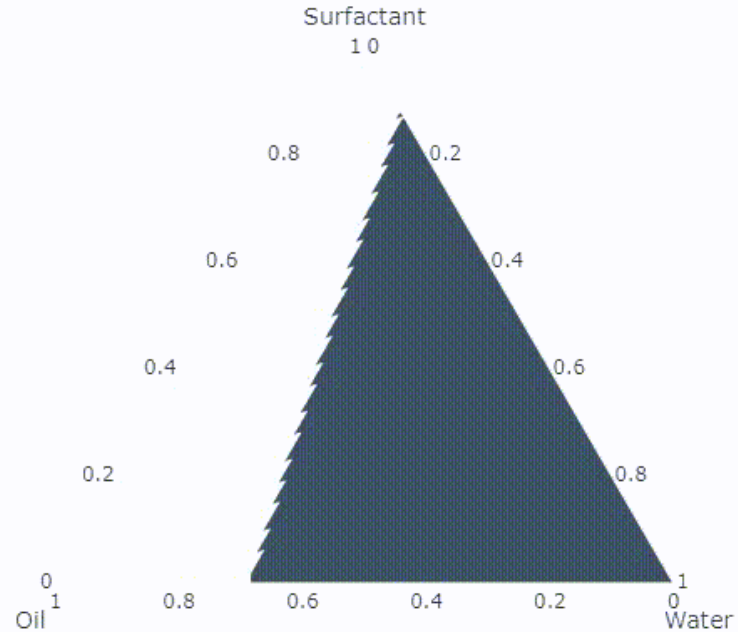
Ternary Scatter Plot, T = 295.15K



Validation using Confidence Map

High confidence areas do **not change** in **predicted phase** for underfit to overfit models

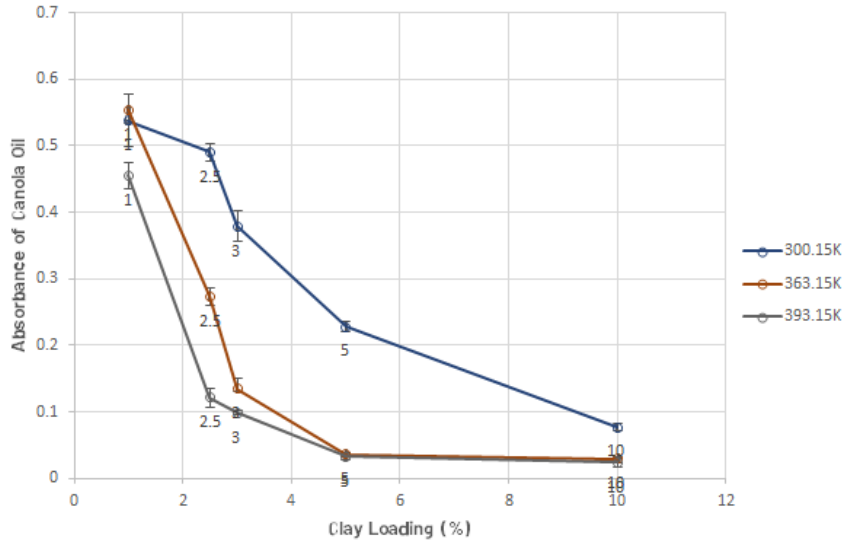
Ternary Scatter Plot, $T = 300.15\text{K}$



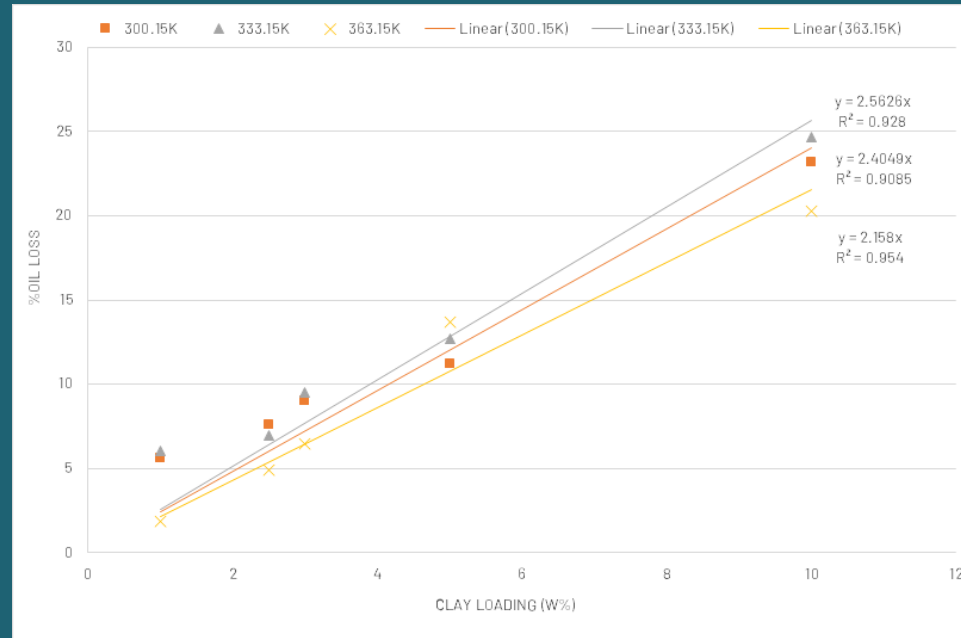
Acid-Activated Clay Performance

Chlorophyll Removal and Oil Loss

Chlorophyll Removal with Acid-Activated Clay

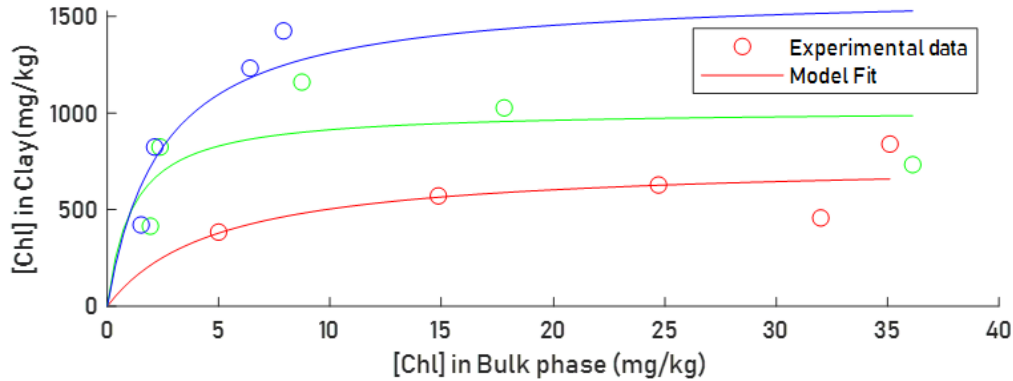


Chlorophyll Removal Experimental Results



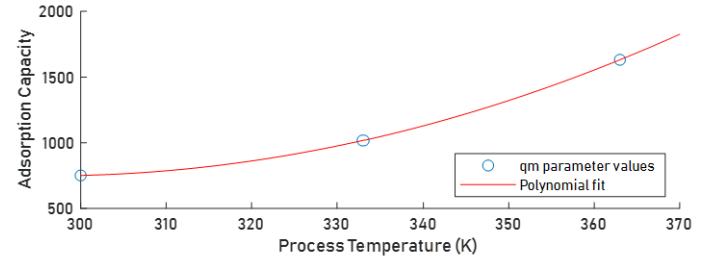
Oil Loss Experimental Results

Acid-Activated Clay Performance Chlorophyll Removal and Oil Loss



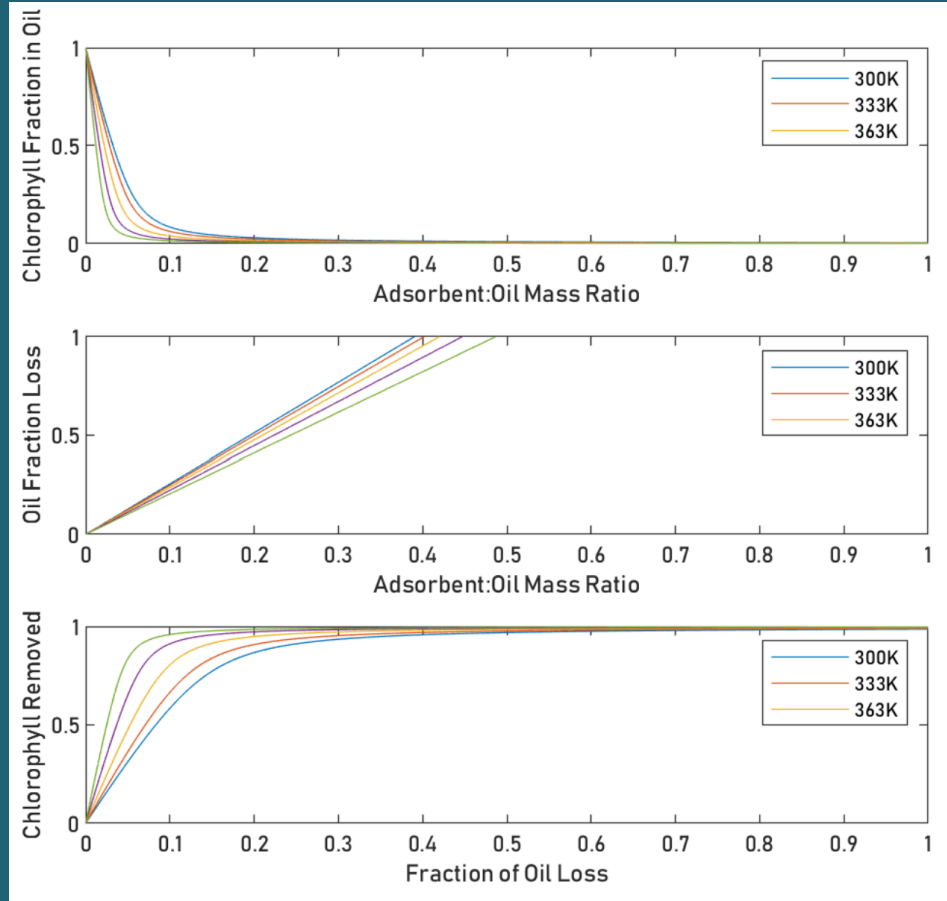
Langmuir Isotherm Model Fitting

R =

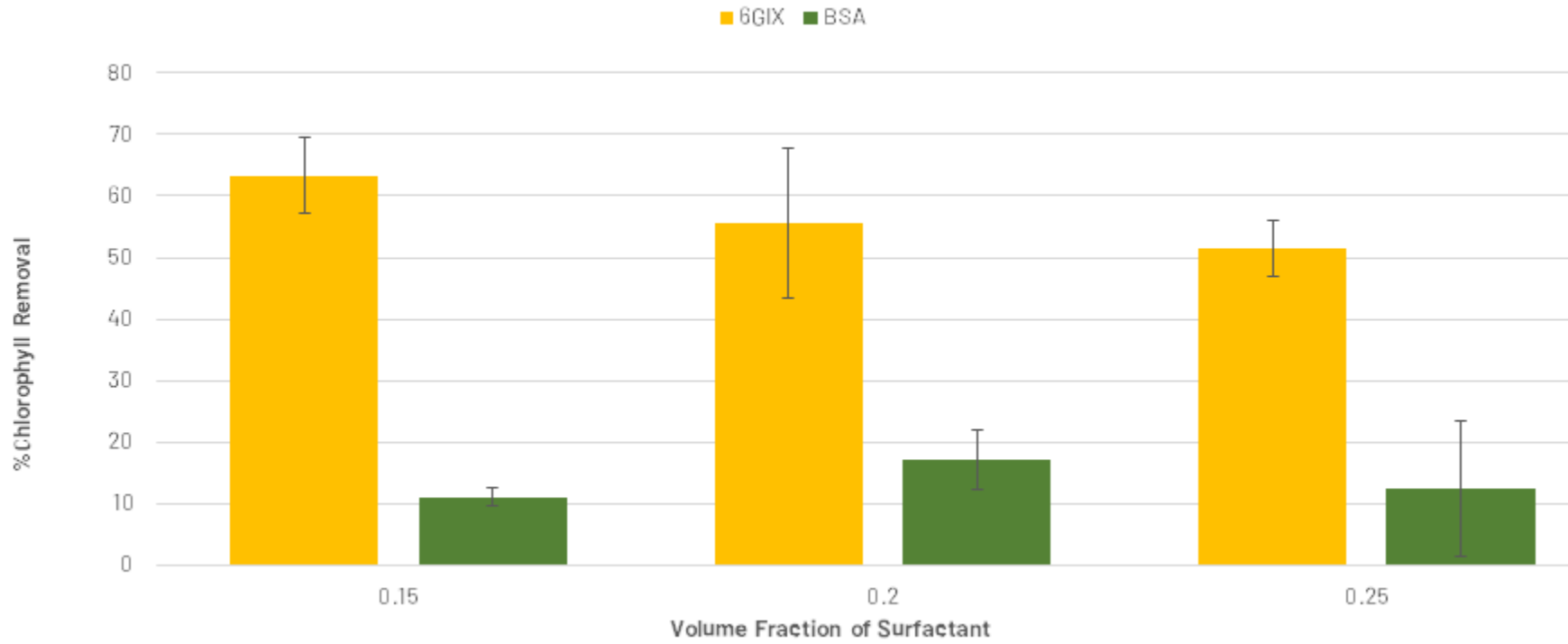


Temperature Dependence Fitting

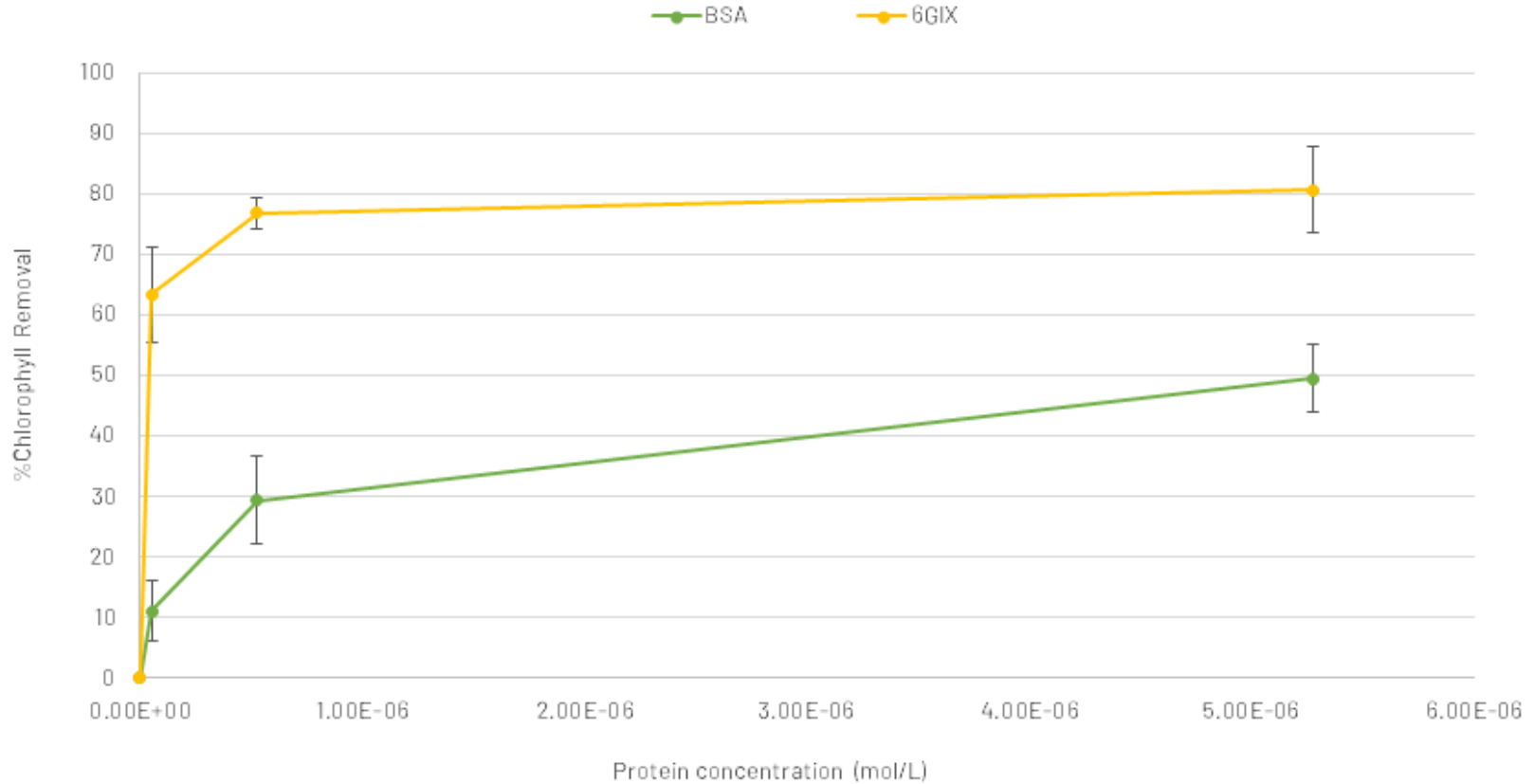
Acid Activated Clay Performance Model



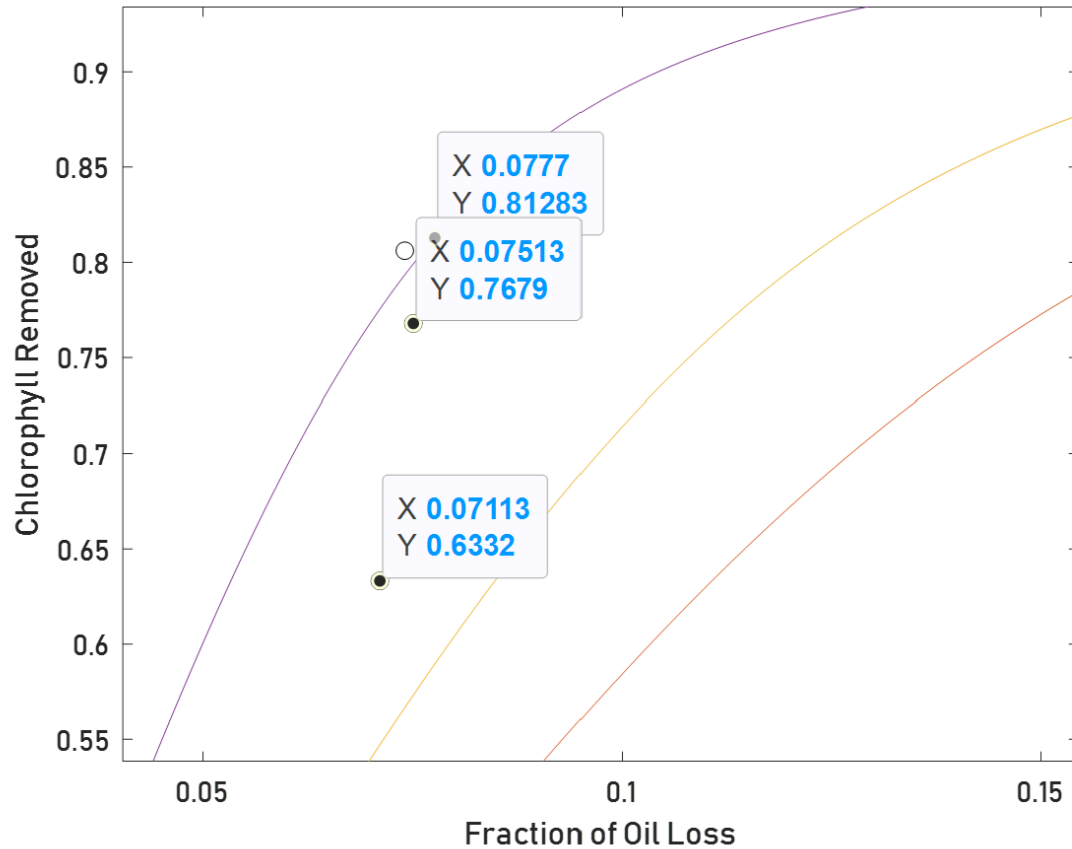
Surfactant Composition Experiments



Emulsified-Protein Chlorophyll Removal



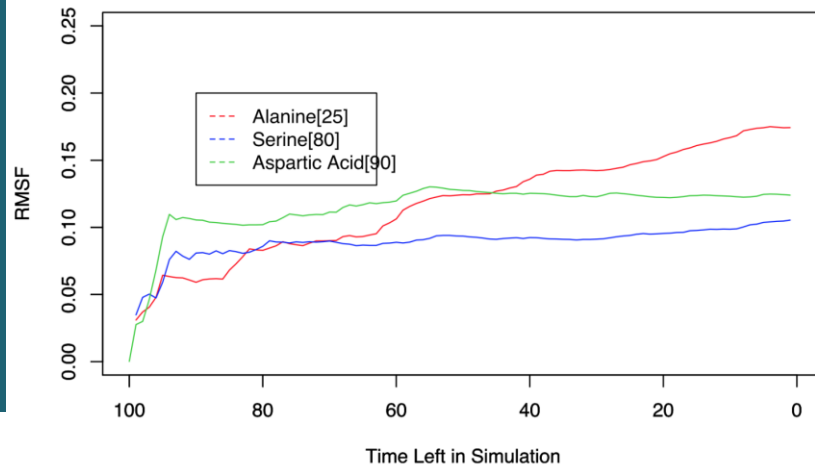
Performance Comparison



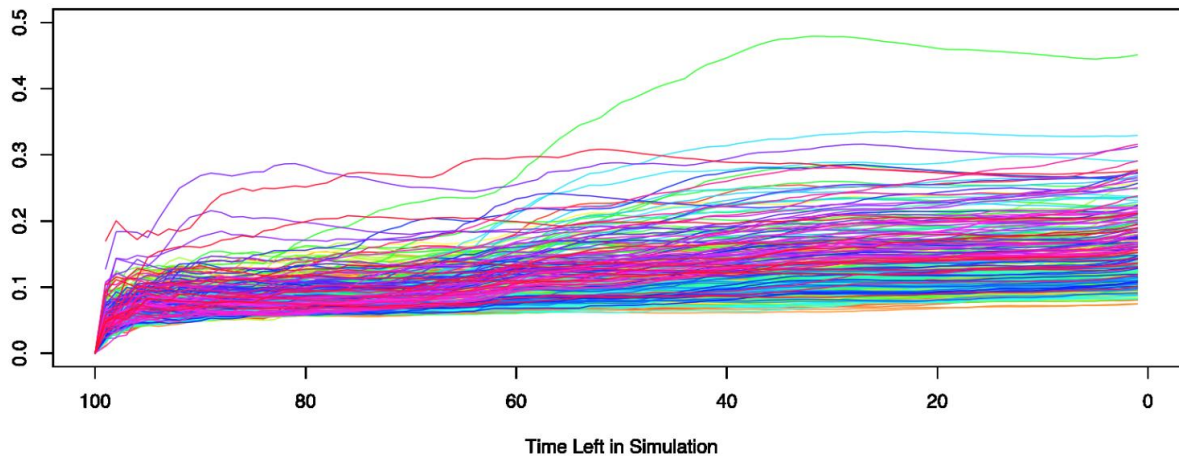
MODGIX

Measurement

RMSF For Specific Amino Acids



RMSF Curves Generated For All ModGIX Amino Acids



Pheophorbide Production

CHLOROPHYLL REPURPOSING ACHIEVEMENTS

1. **Universal spacer (ICARUS)** for large proteins with a binding pocket that has strong electrostatic interactions

First to:

2. Test pheophorbide a on *Sclerotinia sclerotiorum*

3. Test pheophorbide a on *Pestalotiopsis microspora*

4. Execute pheophorbide a **disc test**

5. Purify **Pheophytinase**

6. Submit these proteins to the registry, characterized.

TO BE

1. Pheophorbide **minimum inhibitory concentration**
1. First **in-vitro system** to degrade chlorophyll a and b → pheophorbide a enzymatically
1. **Quantitative characterization** of pheophytinase

Chlorophyll Repurposing Achievements

1. Recombinant PPH converted pheophytin into pheophorbide, our desired product, not seen in prior literature.

ICARUS

1. ICARUS, a novel contribution, functionally allowed for purification of HCAR and PPH using a 6xHis-tag, despite problematic electrostatic interactions

Anti-Fungal

1. The harmful canola pathogen *S. sclerotiorum*'s mycelium was inhibited by pheophorbide a treatment, not seen in prior literature
2. Comparative analysis of *P. microspora* suggests pheophorbide a specificity toward *S. sclerotiorum* inhibition

TO BE

1. Pheophorbide **minimum inhibitory concentration**
1. First **in-vitro system** to degrade chlorophyll a and b → pheophorbide a enzymatically
1. **Quantitative characterization** of pheophytinase

PHEOPHYTINASE PURIFICATION

Journal of Experimental Botany, Vol. 69, No. 4 pp. 879–889, 2018
doi:10.1093/jxb/erx326 Advance Access publication 23 September 2017
This paper is available online free of all access charges (see http://jxb.oxfordjournals.org/open_access.html for further details)



RESEARCH PAPER

Catalytic and structural properties of pheophytinase, the phytol esterase involved in chlorophyll breakdown

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Institute of Plant and Microbial Biology, University of Zurich, Zollikerstrasse 107, CH-8008 Zurich, Switzerland

* Correspondence: shorten@botinst.uzh.ch

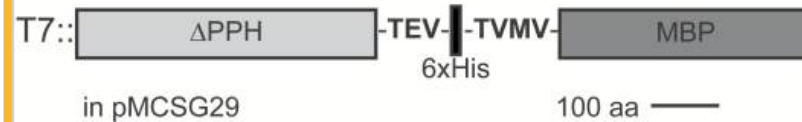
Received 14 July 2017; Editorial decision 21 August 2017; Accepted 21 August 2017

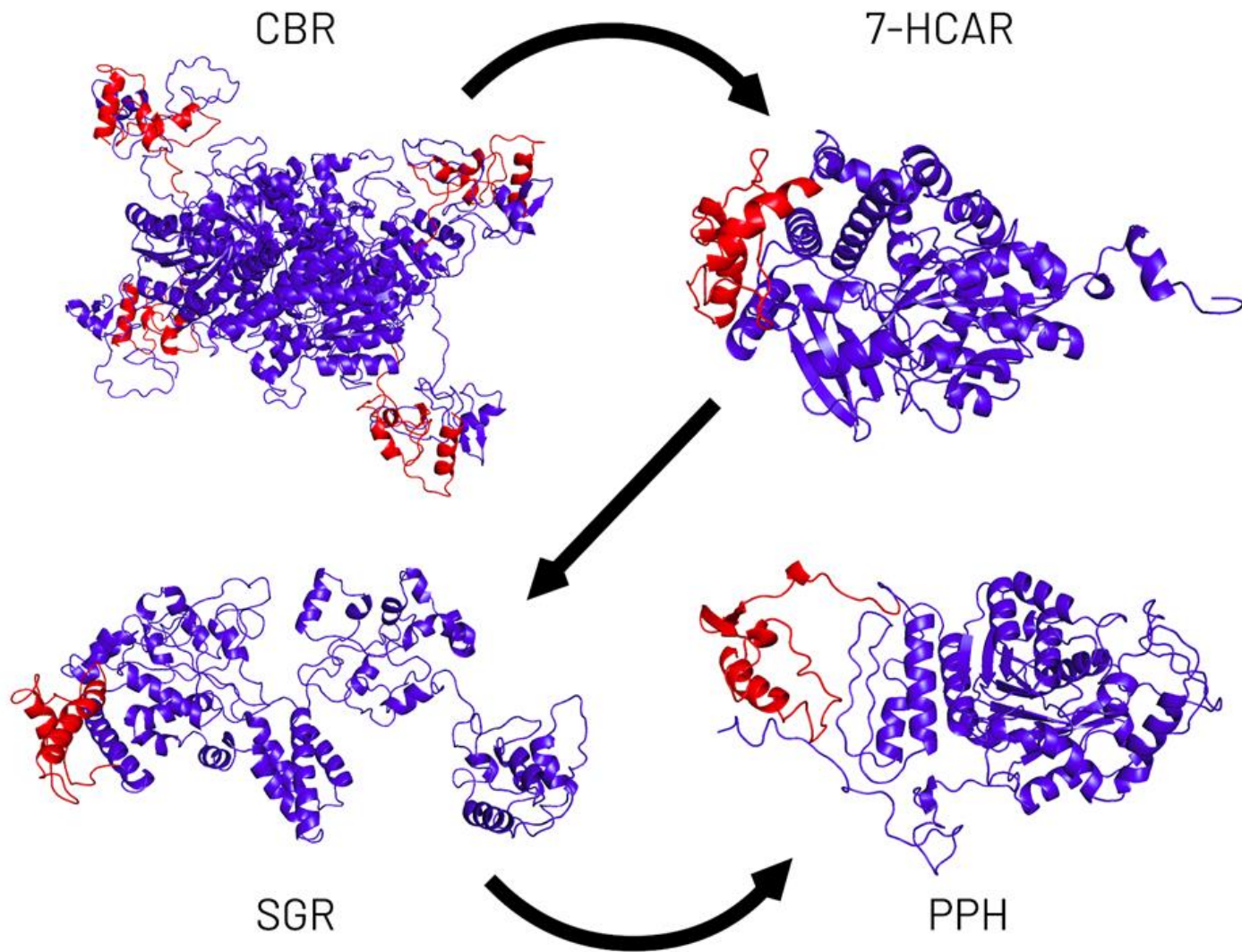
Editor: Christine Foyer, Leeds University, UK

Abstract

During leaf senescence and fruit ripening, chlorophyll is degraded in a multistep pathway into linear tetrapyrroles called phyllobilins. A key feature of chlorophyll breakdown is the removal of the hydrophobic phytol chain that renders phyllobilins water soluble, an important prerequisite for their ultimate storage in the vacuole of senescent cells. Chlorophyllases had been considered for more than a century to catalyze dephytylation *in vivo*; however, this was recently refuted. Instead, pheophytinase was discovered as a genuine *in vivo* phytol hydrolase. While chlorophyllase acts rather unspecifically towards different porphyrin substrates, pheophytinase was shown to specifically dephytylate pheophytin, namely Mg-free chlorophyll. The aim of this work was to elucidate in detail the biochemical and structural properties of pheophytinase. By testing different porphyrin substrates with recombinant pheophytinase from *Arabidopsis thaliana* we show that pheophytinase has high specificity for the acid moiety of the ester bond, namely the porphyrin ring, while the nature of the alcohol, namely the phytol chain in pheophytin, is irrelevant. *In silico* modelling of the 3-dimensional structure of pheophytinase and subsequent analysis of site-directed pheophytinase mutant forms allowed the identification of the serine, histidine, and aspartic acid residues that compose the catalytic triad, a classical feature of serine-type hydrolases to which both pheophytinase and chlorophyllase belong. Based on substantial structural differences in the models of *Arabidopsis* pheophytinase and chlorophyllase 1, we discuss potential differences in the catalytic properties of these two phytol hydrolases.

Despite intensive attempts to purify and crystallize recombinant PPH, we were unsuccessful. Instead, we modelled the 3D structure of PPH based on publicly available structures of other α/β fold hydrolases. This model (Fig. 5) allowed the identifica-





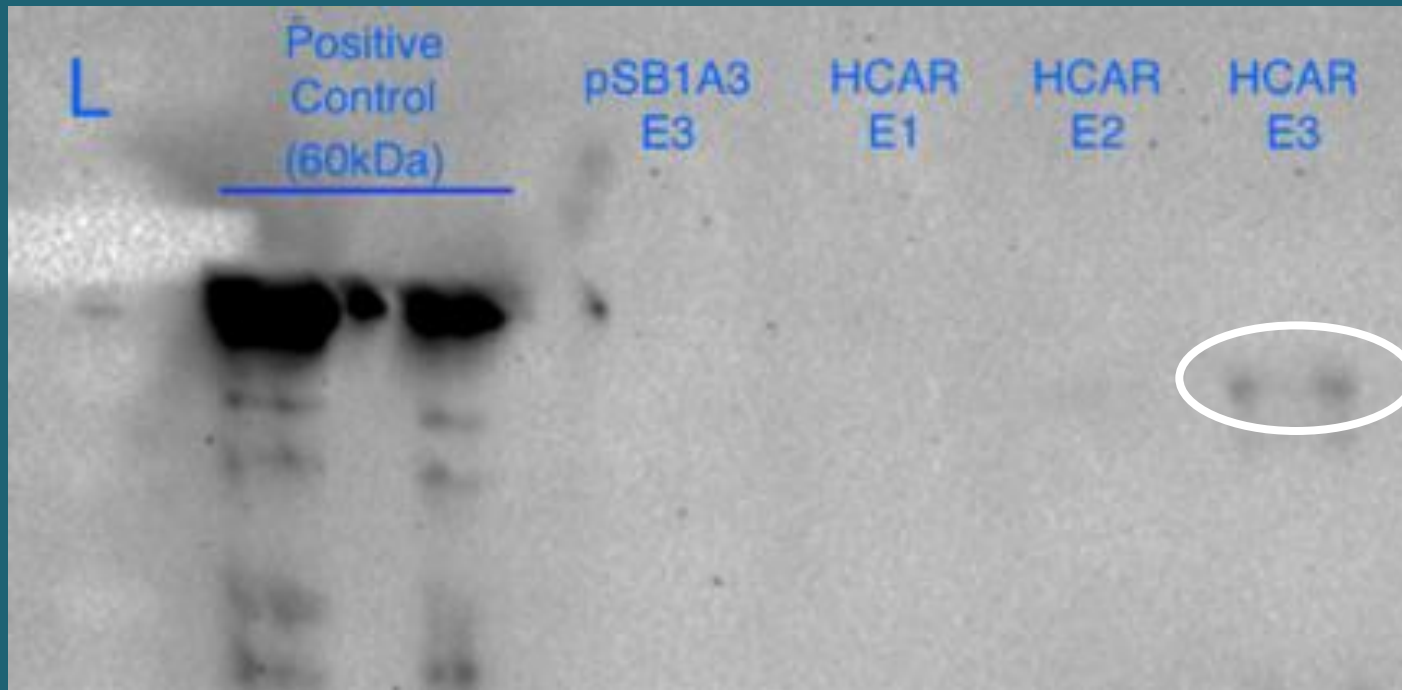
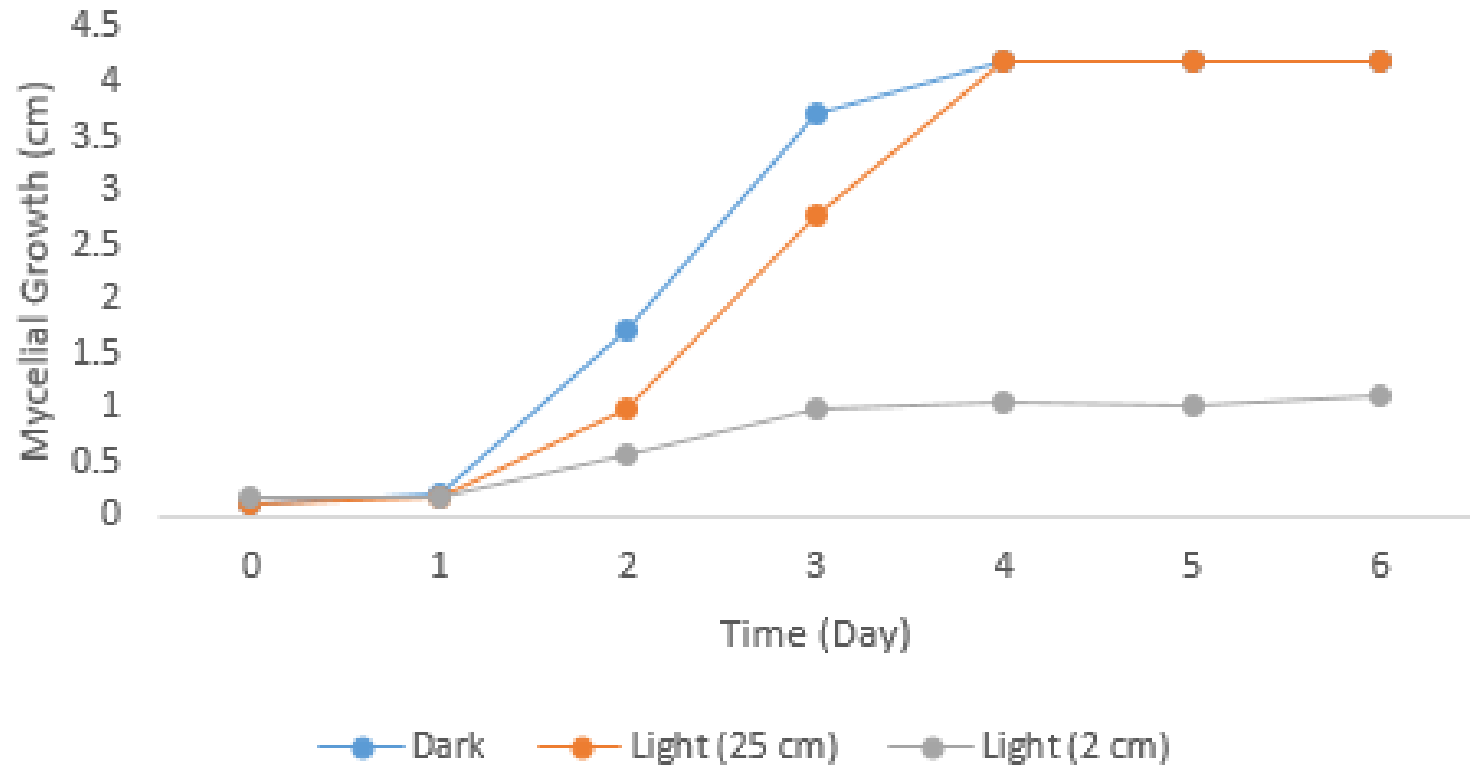


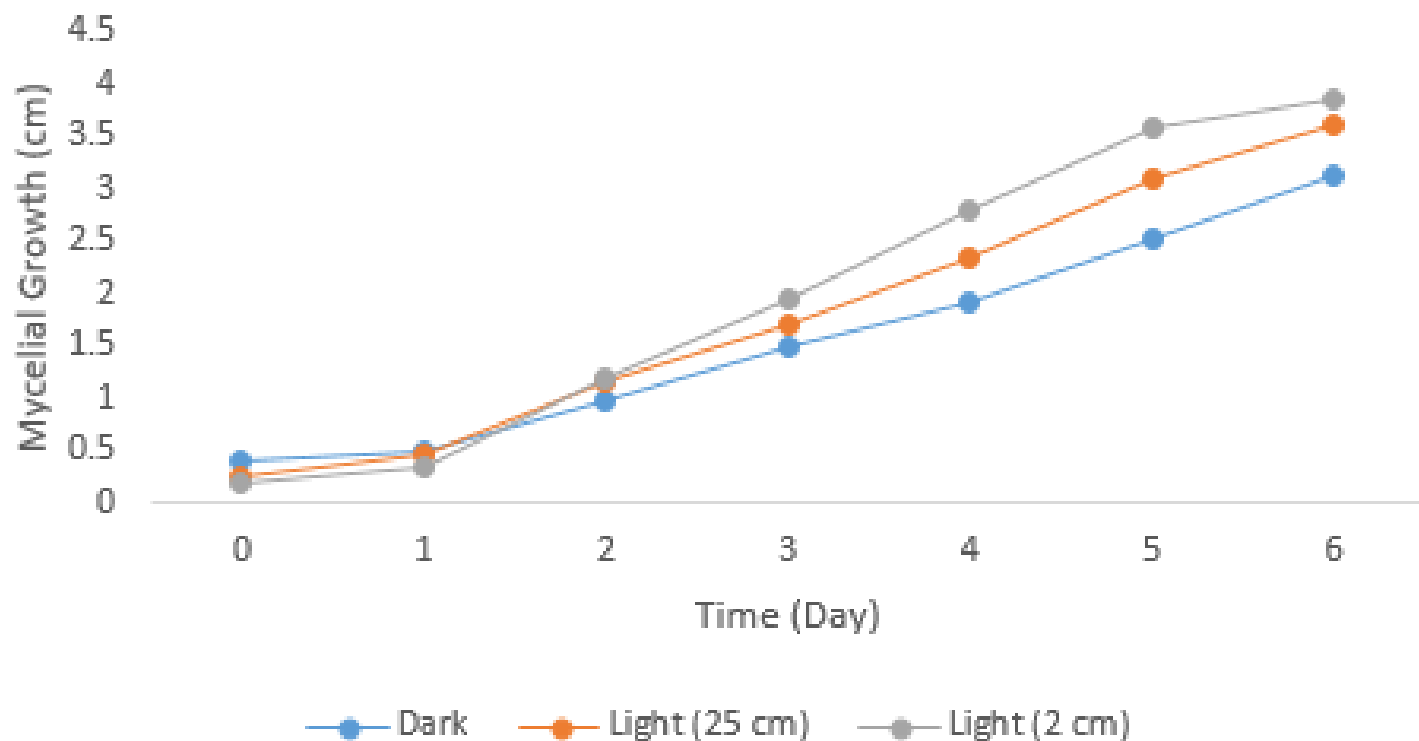
Figure 6. **Western Blot HCAR Purification Confirmation.** 10% SDS-PAGE was run and transferred to a PVDF membrane. An "anti-his-tag MAb" primary antibody was used with an "anti-mouse IgG conjugated with HRP" secondary antibody. ECL was used to visualise. Lanes from left to right on the SDS-PAGE were ladder (Color Prestained Protein Standard, Broad Range (11–245 kDa) (NEB)), ~60 kDa protein (positive control) in two lanes, pSB1A3 (plasmid control in BL21) - elution fraction 3, HCAR - elution fraction 1, HCAR - elution fraction 2, HCAR - elution fraction 3. HCAR is 58 kDa.



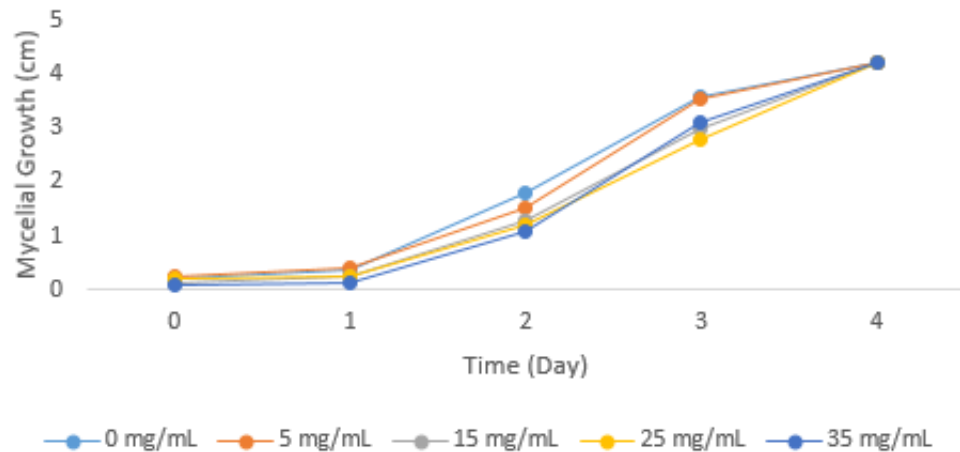
Sclerotinia sclerotiorum Growth Controls



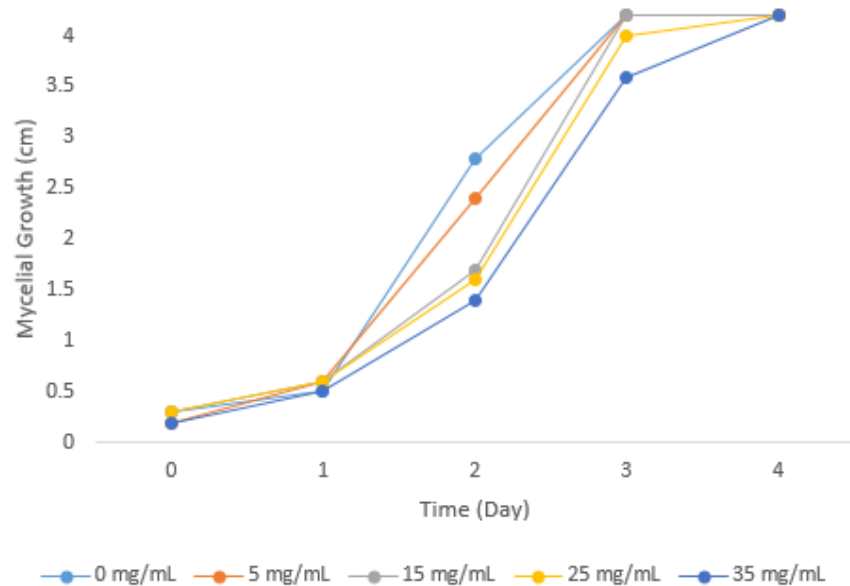
Pestalotiopsis microspora Growth Controls



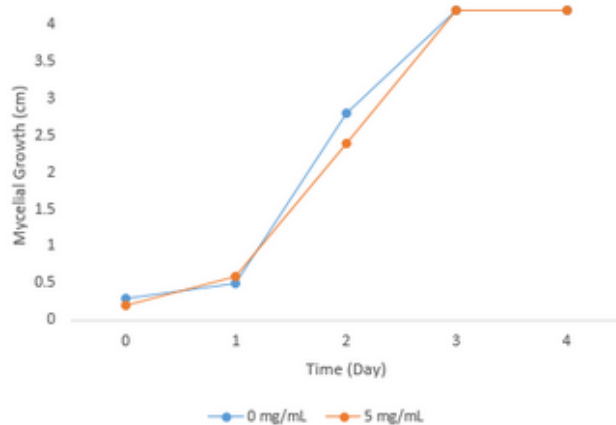
Sclerotinia sclerotiorum Mycelial Growth with Pheophorbide a in Dark



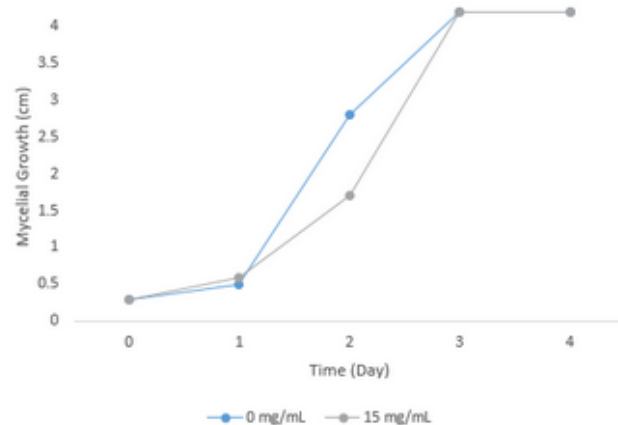
Sclerotinia sclerotiorum Mycelial Growth with Pheophorbide a in Light



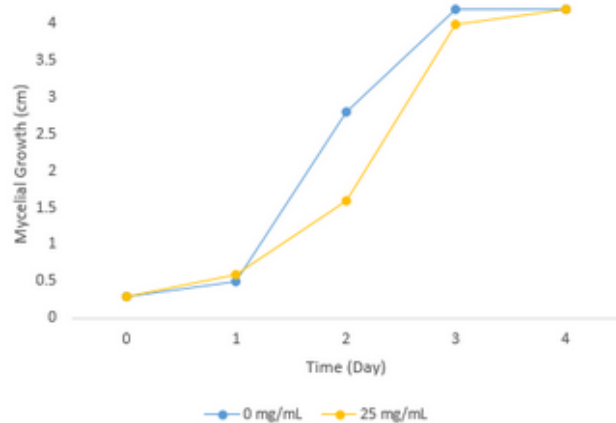
Sclerotinia sclerotiorum Mycelial Growth with Pheophorbide a in Light



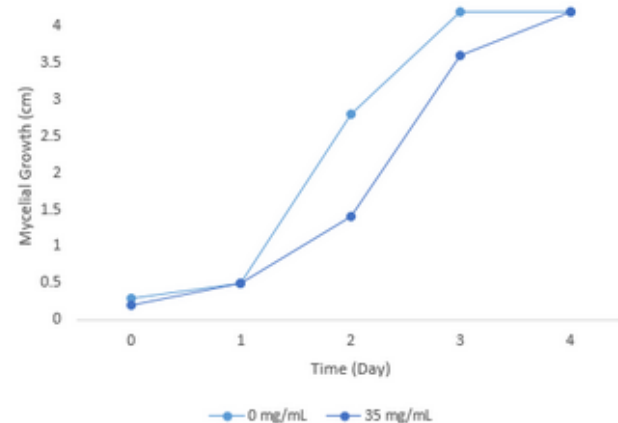
Sclerotinia sclerotiorum Mycelial Growth with Pheophorbide a in Light



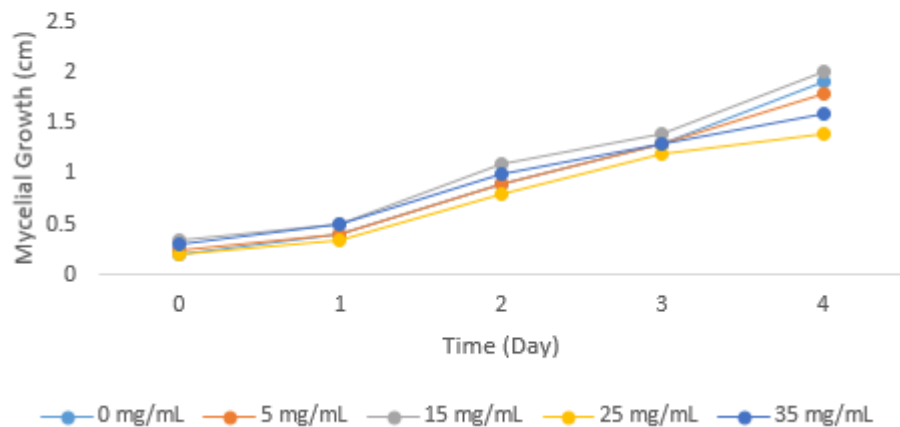
Sclerotinia sclerotiorum Mycelial Growth with Pheophorbide a in Light



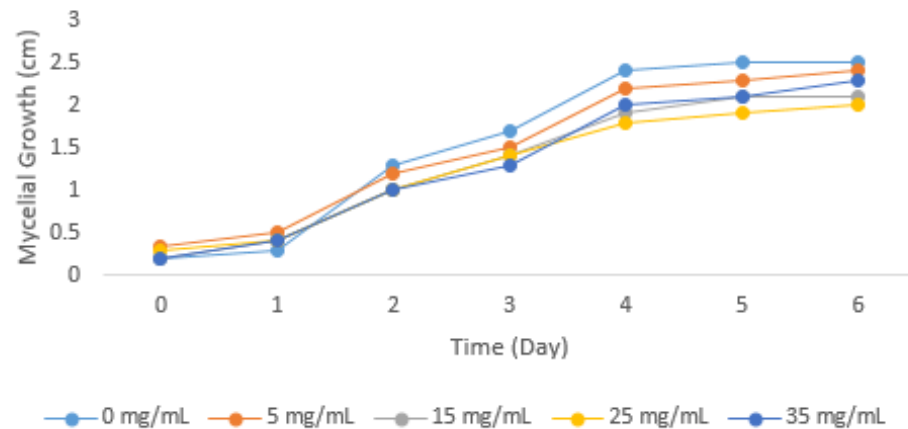
Sclerotinia sclerotiorum Mycelial Growth with Pheophorbide a in Light



Pestalotiopsis microspora Mycelial Growth with Pheophorbide a in Dark



Pestalotiopsis microspora Mycelial Growth with Pheophorbide a in Light



Education & Outreach



In the Community



IGEM Calgary and The Biological Students' Association Present

THE GOOD, THE BAD, AND THE BIOLOGICAL

Wednesday, September 25, 2019
5PM — 7PM @ The Hunter Hub in MacHall
(Food and refreshments will be provided)

A discussion on why we need to innovate with controversial biotechnology and how we can stop it from destroying us.

Education and Outreach

MDSC 507: Introduction to Synthetic Biology

Lab Skills Workshop (Lethbridge)

Webber Academy (1 and 2)

Canmore Mindfuel Event

CanolaPALOOZA

Faculty Talk

JulyGEM

Telus SPARK

Pacific Northwest Meetup

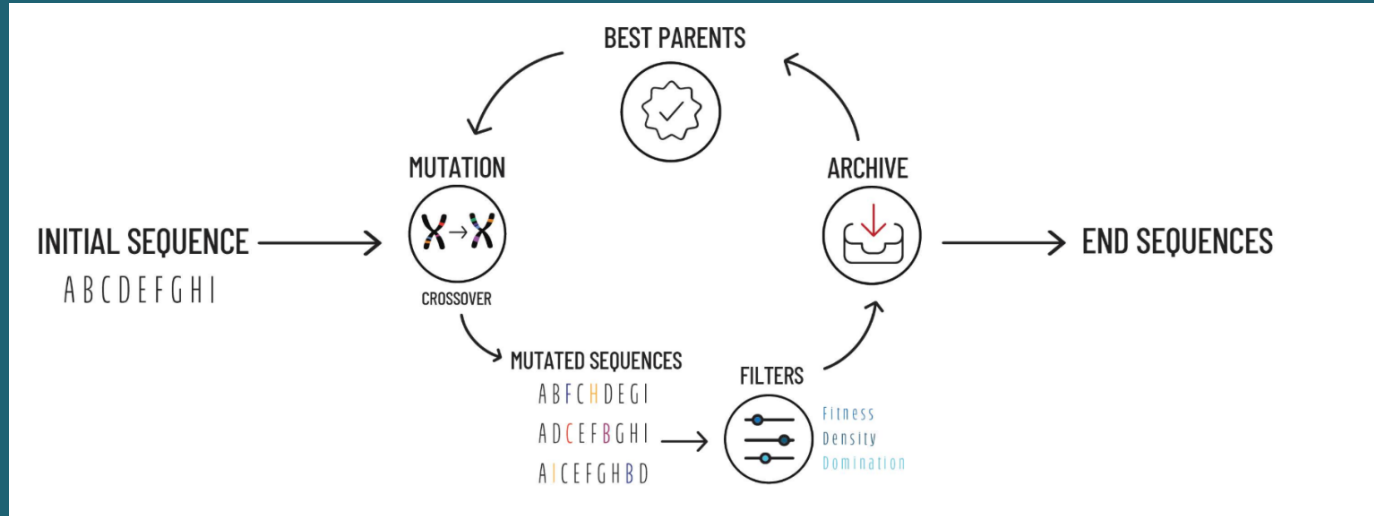
Philosophical Discussion

Bacteria Night

Functionality

Based on the work of Brian Weitzner/codon-harmony

repeats,
gc-richness,
and hairpins.



Interface

Settings

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Gc richness max

Gc richness chunk size

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