Saffron in a Kan:
A Biosynthetic Alternative to Saffron

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Saffron

- Most expensive spice in the world (up to $11,000/kilo)
- 90% from Iran
- Under clinical investigation
  - Anticarcinogenic
  - Antidepressant
What Makes Saffron, Saffron?

- **Crocin**
  - Color
- **Picrocin**
  - Flavor
- **Safranal**
  - Aroma
The Plan

Phase I
- Optimize zeaxanthin production in *E. coli*
- Model zeaxanthin and crocin production in *Synechocystis*

Phase II
- Overexpress ZCD and UGTCs2 in *E. coli*, and characterize expression
- Express ZCD and UGTCs2 in *Synechocystis*

Phase III
- Co-express ZCD and UGTCs2
- Characterize their products produced
Characterization of BBa_395704
Characterization of cell growth under induced expression of BBa_395704

OD 600 vs Time (hrs)
Characterization of amount of carotenoid production per cell

OD450/OD600 at 14hrs

2%

0.142

0.132

0.20%

0.148

0.152

0.02%

0.196

0.222

0%

0.100

0.147

1mM IPTG
0mM IPTG
Flux Balance Analysis

- Performed modeling in MATLAB using COBRA Toolbox
- Used previous genome-scale model of Synechocystis sp PCC6803 from Knoop et al.

1. Genome-scale metabolic reconstruction
2. Mathematically represent metabolic reactions and constraints
3. Mass balance defines a system of linear equations
4. Define objective function
   \[ Z = c_1^T v_1 + c_2^T v_2 + \ldots \]
   To predict growth, \[ Z = v_{\text{biomass}} \]
5. Calculate fluxes that maximize \( Z \)
Pathway Addition using COBRA Toolbox

- Added isoprenoid, carotenoid, and saffron synthesizing pathways to Knoop model
- Optimized for zeaxanthin, crocin, and picrocrocin production, and growth
Optimization of zeaxanthin production in Synechocystis

Figure 1: The predicted zeaxanthin production as a function of light intensity and carbon dioxide uptake.
Phase II: The Original design

- Modularity built-in
- PsbA2 Promoter (BBa_K390050) with mutation
Phase II: Limiting Inclusion Bodies

- Inducible Promoter
- Fusion Protein
- His-tag

pBAD

Fusion Protein → ZCD → Hisx6 → Stop

BBa_K906102
Phase II: Characterization

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Youth Learning Center (YLC)

- Central West End of St. Louis
- Strong relationship with WashU students
- Focused on students in grades 6 - 8
- Three parts - lesson, brainstorming and experiment
Lesson

Genetic Engineering
Washington University in St. Louis
IGEM

Similarities?

How similar do you think the DNA is in your body to a fruit fly?
A. 22% the same
B. 38% the same
C. 60% the same → VS.
D. 72% the same
E. 99% the same

What is DNA made up of?

• There 4 bases – A, C, G, T
• They form a double helix like a spiral staircase.
• The differences in order and number define how the body works.

Our Project-Background
• Saffron

Current Genetic Engineering Research
• Business is booming
• Efficiency
• Crops, domestic farm animals, cutting CO₂ in atmosphere, drug development, and more!
• Have you guys heard of any genetic engineering projects?

Genetic Engineering
• refers to the direct manipulation of DNA by humans
• the insertion of DNA from other organisms or even synthetic DNA into an organism’s genome
• The modified plants or animals are called Genetically Engineered Organisms (GMOs)
Brainstorming
Brainstorming (cont.)

- Cheetah + Electric eel
- Frog + Cow
- Human + Shark + Eagle
- Fly + Boy
- Cat + Man

- Speed of a cheetah for a human.
- Electric eel that fly
- Make a Dolphins fly
- A digging frog

- A Cow that swims and jumps out of the water. Nyla
Experiment

- Creating plates of *E. coli* with GFP or RFP
- Used two biobricks: BBa_I13522 (GFP) and BBa_I13521 (RFP)
Young Scientists Program (YSP)

- YSP is a program run by graduate students at the WashU School of Medicine
  - Teaching teams
  - Mentoring
  - Teaching kits

- Collaboration with YLC will guarantee that iGEM stays involved in the local community
Thank you

Faculty Advisors
Joe Jez
Dantas Gauntam

Graduate Student Advisors
Bert Berla
Larry Page
Arul Varman

Student Advisors
Brian Landry
Jonathan Herman
Questions?