

Cyanobacteriochromes as a Viable Natural Alternative to Synthetic Food Dyes

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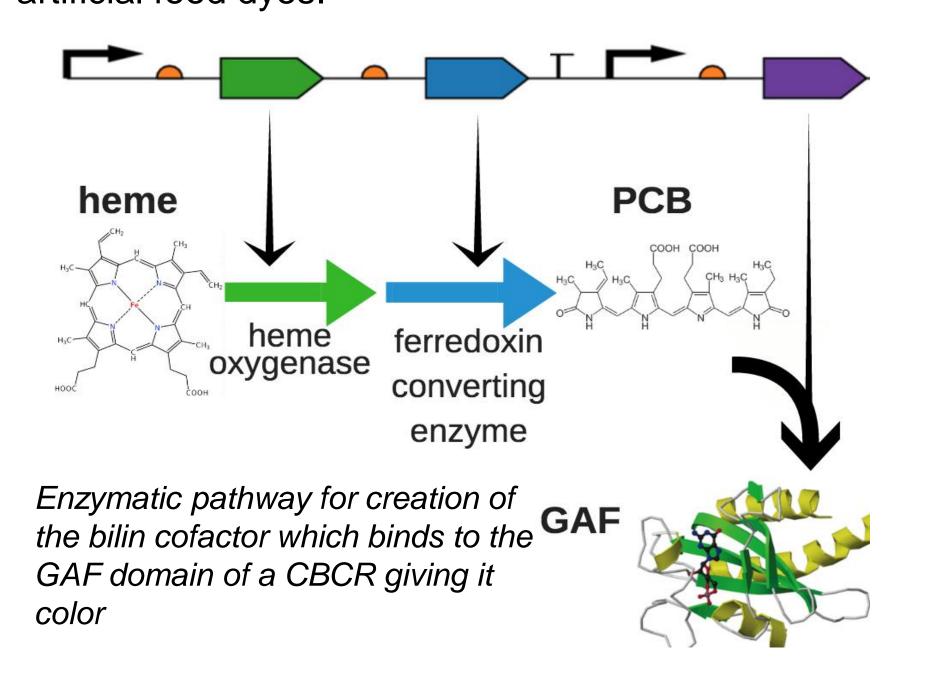
Motivation

Color is innate in food perception and consumers expect vivid colors -- beyond those already naturally present in food. However, due to backlash against artificial colorants, some large food companies have pledged to exclusively use natural food colorings.



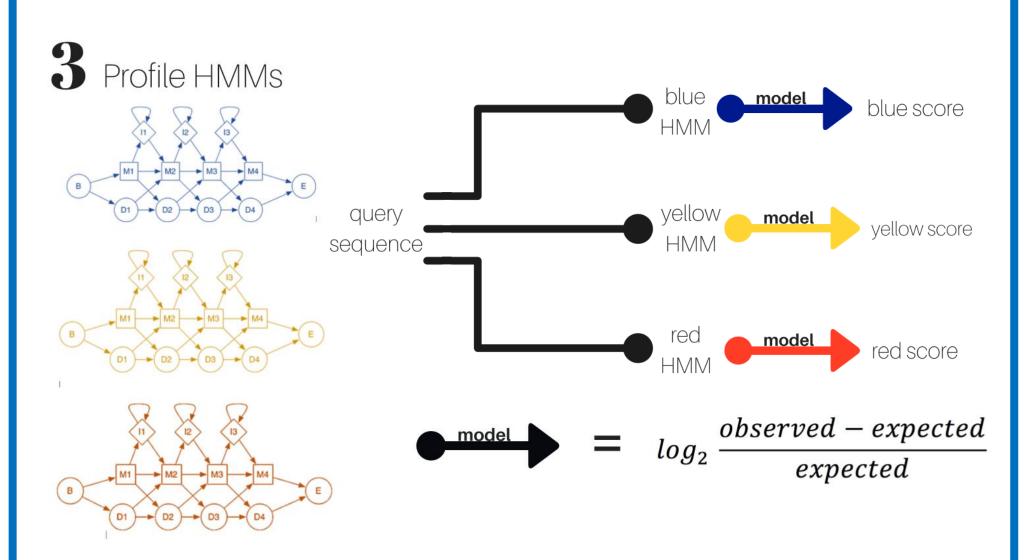
Natural substitutes for blue dye #1 are thought to be the hardest to find.

In this project we demonstrate that cyanobacteriochrome (CBCR) proteins – like those found in spirulina - are a viable natural alternative to artificial food dyes.



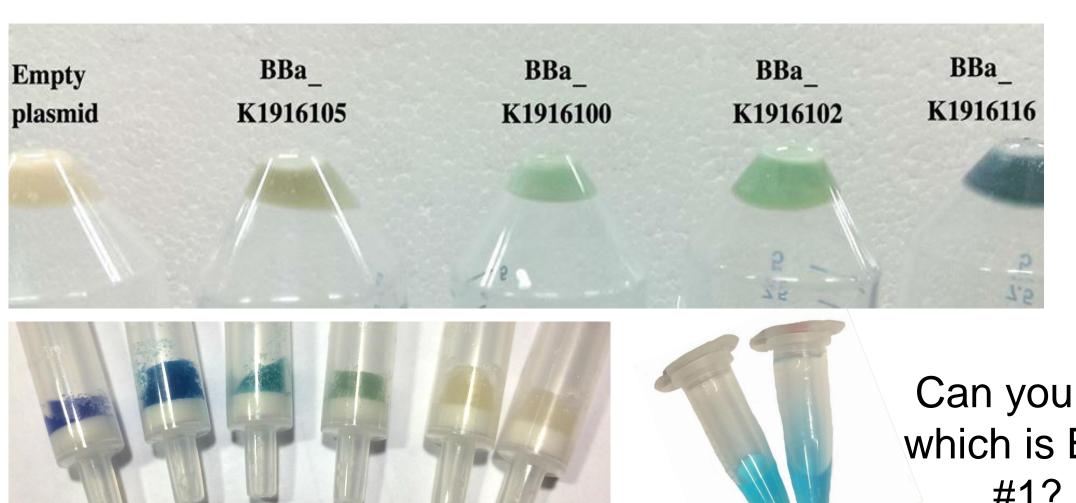
Finding New Colors

To expand the color spectrum of natural food dyes, we used mined metagenomic data to find novel CBCR proteins with varying absorption spectra. We developed a program that can predict whether the CBCRs will be primarily blue, red, or yellow based on their sequence.



Identifies patterns in CBCR protein sequences based on their intrinsic GAF domain; this model provides users with predictions on what color the CBCR pigmented protein is likely to reflect. Try it out at ucdigem.pythonanywhere.com

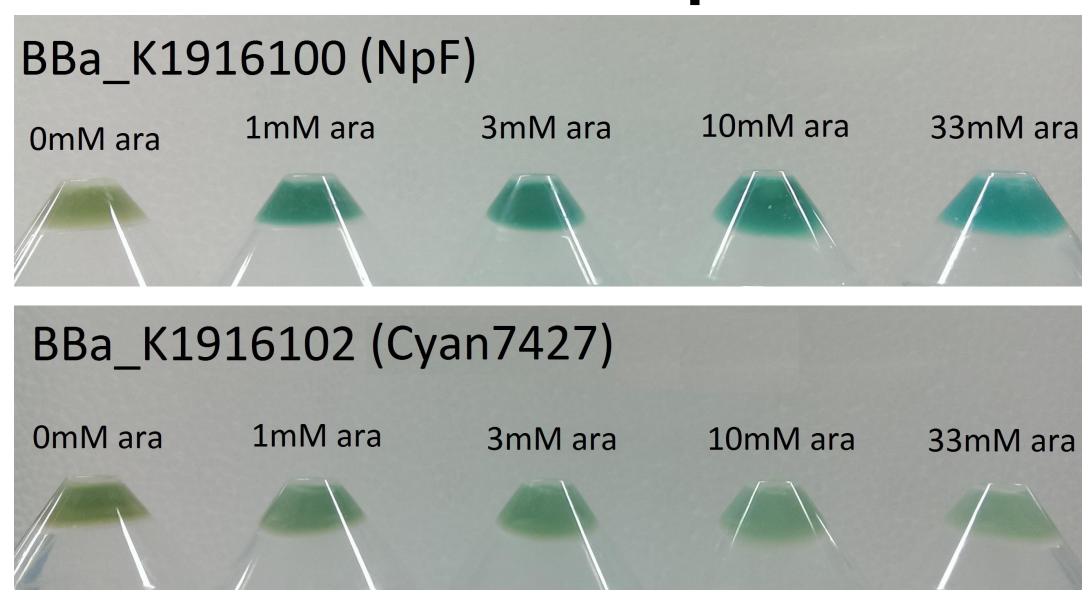
Characterization of Novel GAF Proteins

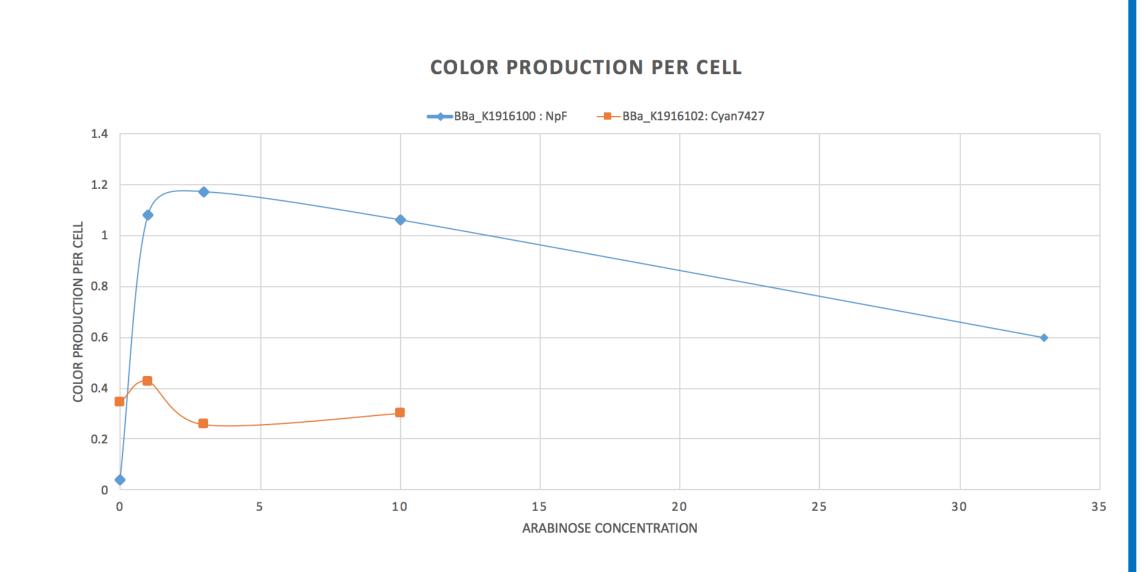


Can you tell which is Blue #1?

Metagenomic mining was used to discover 13 protein sequences three were transformed into *E.coli* and subsequent spectrophotometric analysis were performed to find that our model correctly predicted all three GAF sequences would reflect above 600 nm. The spectra shown here as compared to Blue #1.

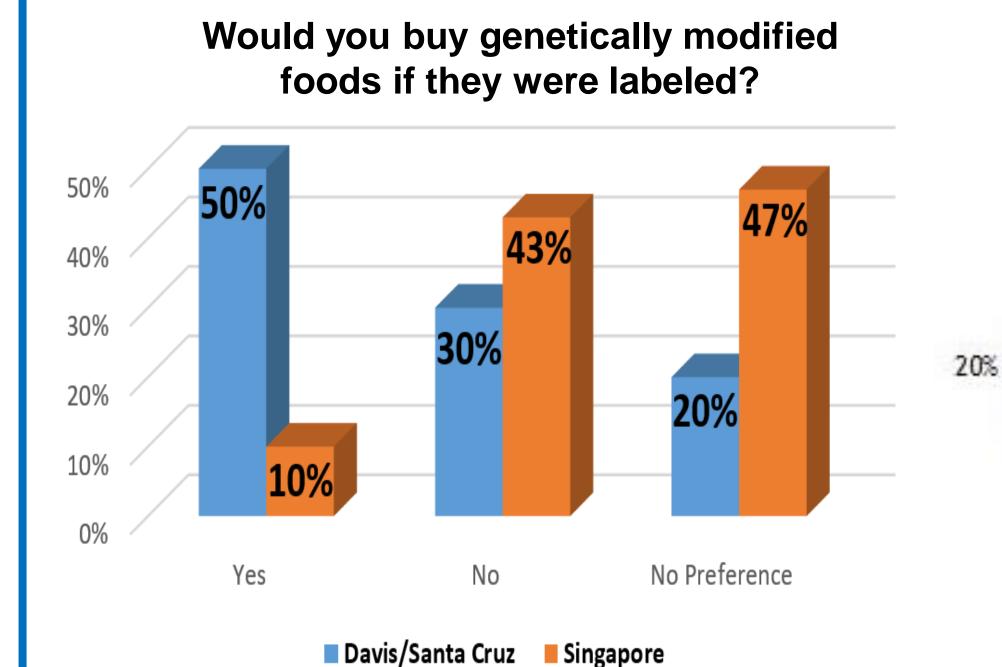
Protein Production Optimization





We induced cultures with different concentrations of arabinose, and after harvesting we assessed pellet volume and color vibrancy to see if there was an optimal inducer concentration.

Consumer Acceptance of Product



synthesis, or no preference?

5% 2% GMO

No Preference
Chem Synthesized
Other Response
I don't know

66%

66%

Which would you prefer? Dye from

an altered organism, chemical

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INSTITUTIONAL REVIEW BOARD ADMINISTRATION

We created a guide to be used as a tool for future iGEM teams conducting surveys to ensure that the data produced does not violate human experimentation rights and can be used in future research

We propose incremental and exponential movements toward a wealth of labels that allow accurate information to consumers and open a realistic dialogue about the current human food system.

Collaboration Forum: iGEMmatch

iGEMmatch.org promotes collaborations between iGEM teams. iGEM Match enables users to identify other teams with compatible projects and provides a communication modality to facilitate team-team collaboration.



Work in progress

In order to use our CBCRs for food applications and industrial production, our next step is to adapt our research in *E. coli* for expression in *Bacillus subtilis*, which has been designated by the FDA as a generally regarded as safe (GRAS) organism.

Genetic Circuit for CBCR Expression in B. Subtilis



This circuit architecture allows for the high-level constitutive production of PCB cofactor (pVEG) and the independent tuning of of CBCR synthesis.

Expression Chassis to Explore

- **B.** Subtilis KO7 (BGSC Accession # 1A1134): Seven protease deletions, no major secreted proteases, free of antibiotic resistance genes and integrated plasmids
- B. Subtilis PY79 (BGSC Accession # 1A747): Wildtype strain, containing normal secreted and wall-associated proteases
- B. Subtilis spo0A3 (BGSC Accession # 1S1): Does not produce alkaline or neutral proteases, non-sporulating

Acknowledgements

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