Our Members: Joe Alexander, Christopher Clifford, Justin Emlen, Jasmine Jiang, Michelle Ngo, Dafne Ordonez, Jeremiah Pavelka, Cody Sarcinella, Ronald Truong
* Over half the world depends on rice as a source of calories
* Most people prefer white, polished rice
What is white rice?

*Milling & Polishing:
* removes the outer hull
* removes the bran and germ layers
* leaves the rice shiny with an extended shelf life

*However, the bran contains most of the nutrition*
## Proximate B vitamin content of brown and white rice

<table>
<thead>
<tr>
<th>Item</th>
<th>Brown Rice</th>
<th>White Rice</th>
</tr>
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<tbody>
<tr>
<td>Thiamine, mg/100g</td>
<td>0.29-0.61</td>
<td>0.02-0.11</td>
</tr>
<tr>
<td>Riboflavin, mg/100g</td>
<td>0.04-0.14</td>
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</tr>
<tr>
<td>Cobalamin, mg/100g</td>
<td>0.00</td>
<td>0.00</td>
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<tr>
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<td>3.5-5.3</td>
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Many foods can be enriched with vitamins and minerals.

Fortification is used to overcome nutrient deficiencies.

What is fortification?
The Problem with Rice Fortification

* Rice fortification using the traditional approach has had limited success
* Many cultures wash rice before cooking
* Added vitamins are leached away
Golden Rice
Genetically engineered to produce beta-carotene
Horizontal gene transfer
Many countries don’t accept it due to public perception and culture
Our Alternative Approach
What is iRICE?

* A modular fusion protein that can be added to rice
* A starch binding domain is connected to a nutrient-related domain of choice
Advantages of iRICE

* No alteration of the rice genome

* Easily adaptable to multiple cultivars of rice

* Can control the amount of nutrients added to rice

* Starch-binding domain prevents loss of vitamins during washing
Glucoamylase in *Rhizopus oryzae* is a two domain enzyme.

The N-terminal domain binds starch (pictured).

A construct connecting our Starch Binding Protein to RFP was created. This allows for visual confirmation of effectiveness of binding.
Proof of Concept

* Protein was sprayed onto uncooked rice and allowed to dry

* The rice was photographed after washing and rinsing under the tap
Our Other Fusion Proteins

* Vitamin B12 Binding Protein
* Thiamine Binding Protein
* Lysine Rich Protein
Vitamin B12

* B12 deficiency is an under-recognized issue

* Vegetarians and people whose diets are heavily dependent on rice

* Severe deficiency can lead to permanent brain damage
Vitamin B12 Binding Protein

* ABC Transporters in *E. coli*

* B12BP is a periplasmic protein associated with the transport of vitamins into the cell.
A fusion of SBP and B12BP was created with an inducible promoter.

Results indicate that it is working in both its starch binding capabilities and B12 binding capabilities.
Starch-B12 Binding Protein was isolated using the amylose column

* 42 kDa
B12 Binding Assay
Results of binding assay show concentration of B12-HRP bound to our protein.

* Created Scatchard Plot based on binding assay.

* Calculated $K_d$ to be 52.3 pM.
Future Research

* Further characterization of binding properties

* Competitive binding studies

* Temperature studies to determine effects of cooking

* pH studies to determine effects of digestion
Further Applications of iRICE

* iRICE serves as a prototype for the delivery of even more vitamins and minerals

* Add essential amino acids

* Other applications such as edible vaccines
*The importance of education*
Bill Nye Science Fair
Vitamin Catching Game
Valley Road Field Day
Survey

iGEM Survey 2012

Knowledge of Golden Rice?
Would you eat Golden Rice?
Would you eat GMO?
Would you eat iRICE if proven safe?
Which augmentation do you prefer?

1 - Supplementation added later
2 - No Preference
3 - GMO Rice
Experiment GMO Labeling

Contains Genetically Modified Ingredients

Non-Genetically Modified
“If people were more educated about what GM was, they wouldn’t be bothered by the idea of eating GM foods.”

“The fear is the fear of not knowing, not a fear of the food itself. People just don’t have enough knowledge about it.”
Helping Other Teams

* CU Boulder
* India
Achievements

Submitted working BioBricks to the Registry of Standard Parts

<table>
<thead>
<tr>
<th>Name</th>
<th>Type</th>
<th>Description</th>
<th>Designer</th>
<th>Length</th>
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<tbody>
<tr>
<td>BBA_K931002</td>
<td>Composite</td>
<td>A Red Fluorescent Protein - Starch Binding Protein Fusion</td>
<td>Joe Alexander</td>
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<tr>
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<td>Composite</td>
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<td>Justin Emlen</td>
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<td>BBA_K931000</td>
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<td>SBP - TBP</td>
<td>Chris Clifford</td>
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<tr>
<td>BBA_K931006</td>
<td>Coding</td>
<td>Lysine Rich Protein (LRP)</td>
<td>Michelle Ngo</td>
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<tr>
<td>BBA_K931007</td>
<td>Intermediate</td>
<td>SBP - B12BP</td>
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Achievements

- Submitted working BioBricks to the Registry of Standard Parts
- Characterized a BioBrick
- Contributed to public education and awareness (Human Practice)
- Helped Team CU-Boulder
Contributions

* Our Advisors: Dr. Chong Tang, Dr. Christie Howard, Dr. David Shintani

* Our Sponsors:
Thank you for your time and consideration!
Western Blot of Starch B12 Binding Protein
The 25 kDa lysine-rich protein was purified using a Ni-NTA spin column. Lane 1 contains protein standard, lane 2 crude *E. coli* extract, lanes 3-6 pH 6.3 column washes, lane 6 pH 4.5 elution of his-tag proteins.
Western analysis from Ni column purification was conducted to detect the 35 kDa lysine-rich starch binding protein using His-tag antibodies. Instead, a 60 kDa unknown protein was detected in the pH 4.5 elution, possibly due to a cloning error. Lane 1 contains protein standard, lanes 2-8 pH 6.3 washes, lane 9 pH 4.5 elution of his-tag proteins, lane 10 crude extract.
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