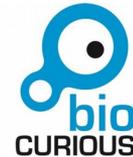


Real Vegan Cheese

Ben Rupert 14-08-15



What is Real Vegan Cheese?



flickr: gee01

We are engineering baker's yeast to produce casein, which is the milk protein which is responsible for the unique properties of cheese. This will allow us to make cheese which is identical to animal derived cheese but which is vegan.

But Why?

Many reasons for this project:

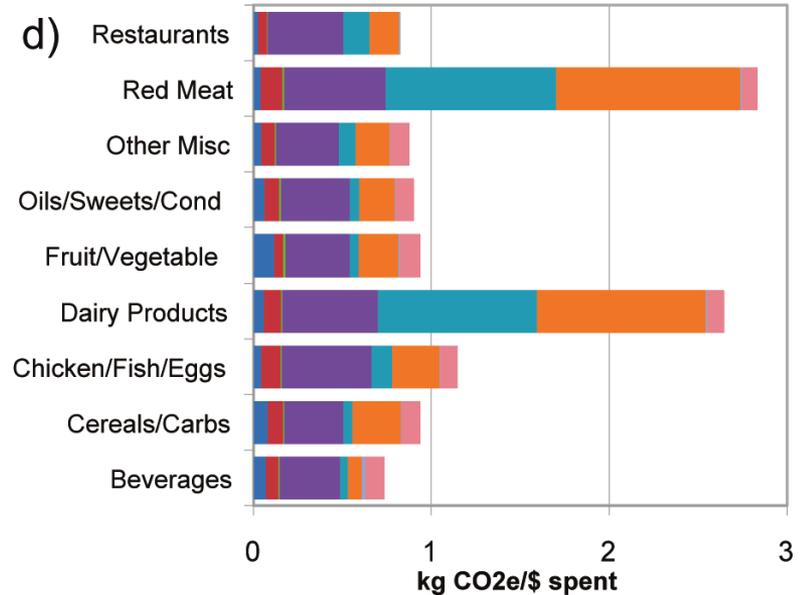
- Give people who are interested in biotech an alternative pathway to learn the basic science and practical skills needed.
- Show that community labs can produce work on par with academic and corporate labs.
- Change public perception of genetically modified organisms.
- Reduce the environmental impact of agriculture.
- End the use of animals for milk, and eventually all agriculture.

From my personal perspective, this isn't about making better cheese for vegans. It's about making acceptable vegan cheese for everyone else.

Impact of Dairy

A few facts:

- 270 million dairy cows
- Veal industry dependent on dairy industry
- 4% of global GHG emissions from dairy according to UN [1]
- ~10x more water to produce dairy milk vs. Almond milk [2]
- Runoff from dairy farms pollutes local waterways



[1] Greenhouse Gas Emissions from the Dairy Sector - UN report

[2] Slate article "The Thirsty West" by Eric Holthaus

[3] Graph: Weber, CL and Matthews HS, *Environ. Sci. Technol.*, 2008, 3508-3513.

Impact of Animal Agriculture

A few facts:

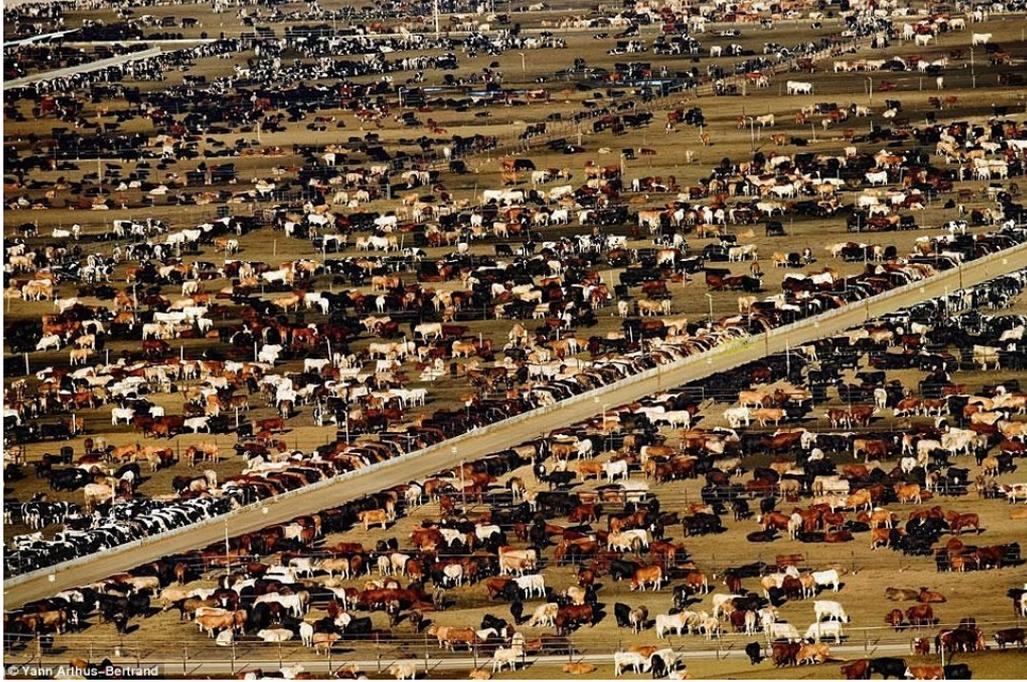
- 56% of the water used in the US goes to growing feed for animals. [1]
- Animal agriculture is responsible for 18% of all greenhouse gas emissions, more than all transportation combined. [2]
- Livestock uses 1/3 of the earth's ice-free land. [3]

[1] Jacobson, Michael F. "More and Cleaner Water."

[2] Fao.org. Spotlight: Livestock impacts on the environment.

[3] FAO. "Livestock a major threat to environment"

Impact of Animal Agriculture



© Yann Arthus-Bertrand



Who

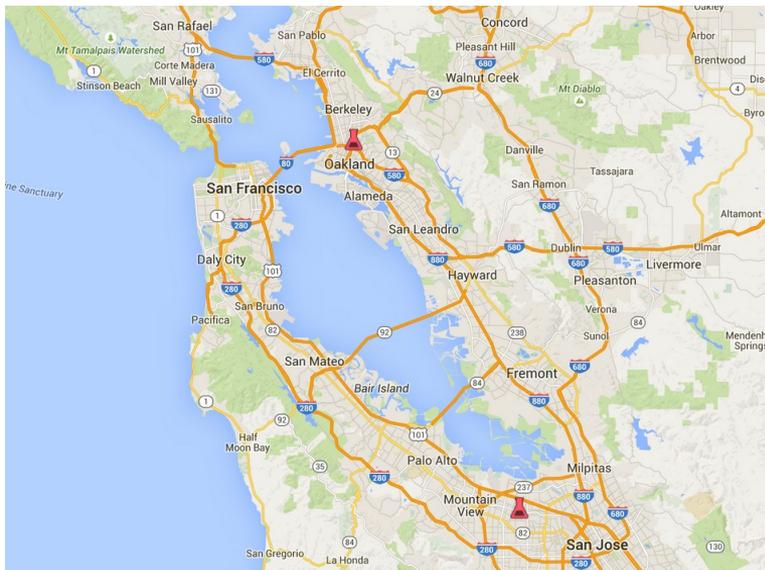


We have members spanning a broad range of backgrounds:

- PhD and Masters level biologists working in medicine and computational biology
- Computer programmers with little previous lab science experience
- High school students
- All volunteer, no one is paid and we work nights and weekends

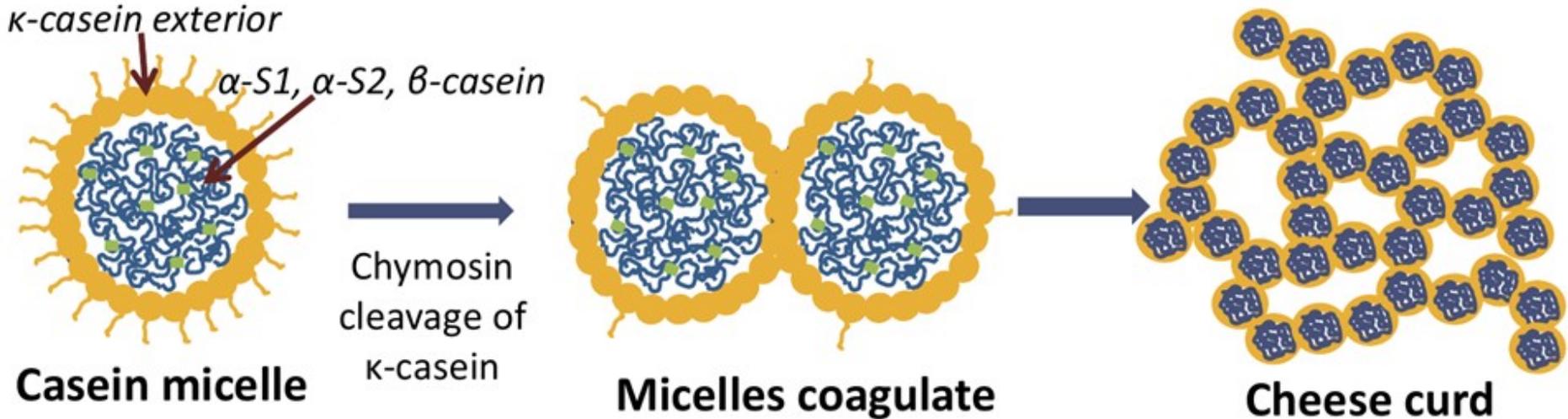
Where

We are a collaboration between two labs:



Counter Culture Labs and Biocurious

What's Special About Casein?



- Casein is a complex of four proteins
- Inside casein micelle is hydrophobic and wants to separate from water
- Calcium clusters and protein bound phosphate groups hold together proteins inside structure
- The end of the k-casein on the surface of the micelle is hydrophilic
- The k-casein hydrophilic end is coated in sugars (although functional w/o sugar)
- The sugars and phosphate groups are added to the proteins by enzymes after the proteins are expressed

Other Casein(ish) Sources?

- Young animals need relatively large amounts of nutrients compared to adult animals.
- Mammals have solved this problem with the unique solution of milk.
- Casein specifically allows milk to carry more protein, fat and calcium than an aqueous solution would normally permit.
- No other organisms but mammals are known to make anything like casein.
- Processing of plant proteins may still get there, but GM yeast is a more direct approach

If we want casein, we need to genetically modify = insert animal genes into microorganism

Our Process

Search for genes of interest in genome
We can make cheese from *any* sequenced mammal!

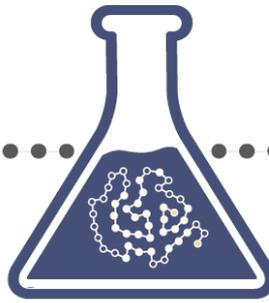
Synthesize DNA
No animals involved!

Insert casein genes into yeast
Using strawberry DNA as carrier

Express & Purify casein proteins
Secreted into supernatant

Make vegan "milk"
Casein micelles + sugars + fats

Make Cheese!



Search Animal Genome
for Milk Protein DNA

Synthesize Yeast DNA
Based on Those Sequences

Put DNA into Yeast

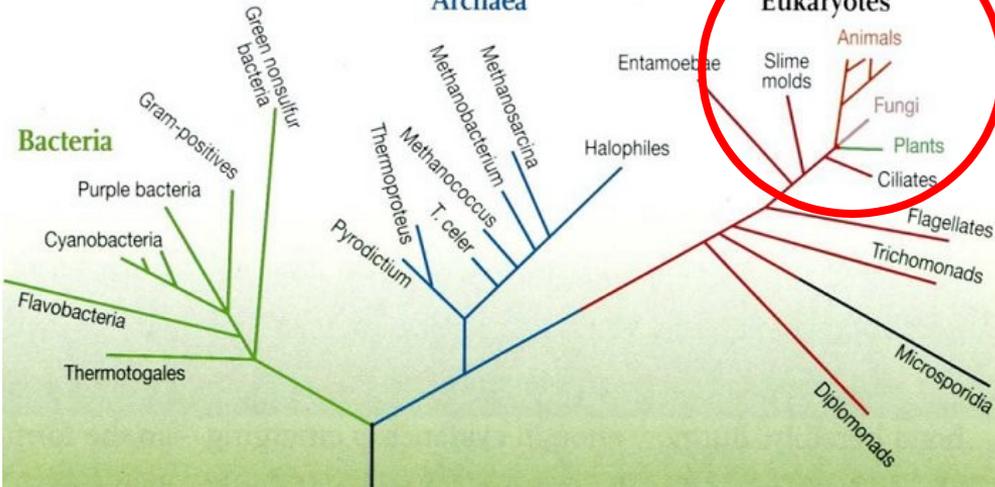
Collect Milk Proteins from Yeast

Make Vegan Milk

Make Real Vegan Cheese

Why Yeast

THE TREE OF LIFE



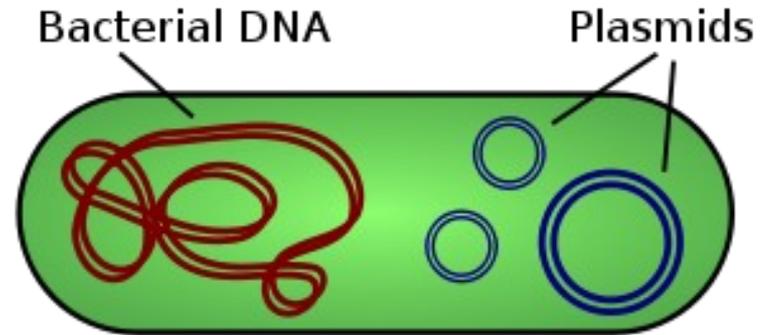
- Yeast are close to animals on the evolutionary tree.
- Yeast glycosylate proteins, bacteria do not.
- Native glycosylation in yeast may be similar enough to mammals that we do not need to engineer in the mammal enzyme.

How Do We Get the Data In?

```
biohacker@lab:~$ sftp DNA@yeast
sftp> put casein.gene
```

- Not quite this easy, but not as hard as many people think
- We can use a plasmid

A plasmid is a small DNA molecule within a cell that is physically separated from a chromosomal DNA and can replicate independently.



Production and Reconstruction



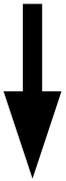
κ -casein



β -casein

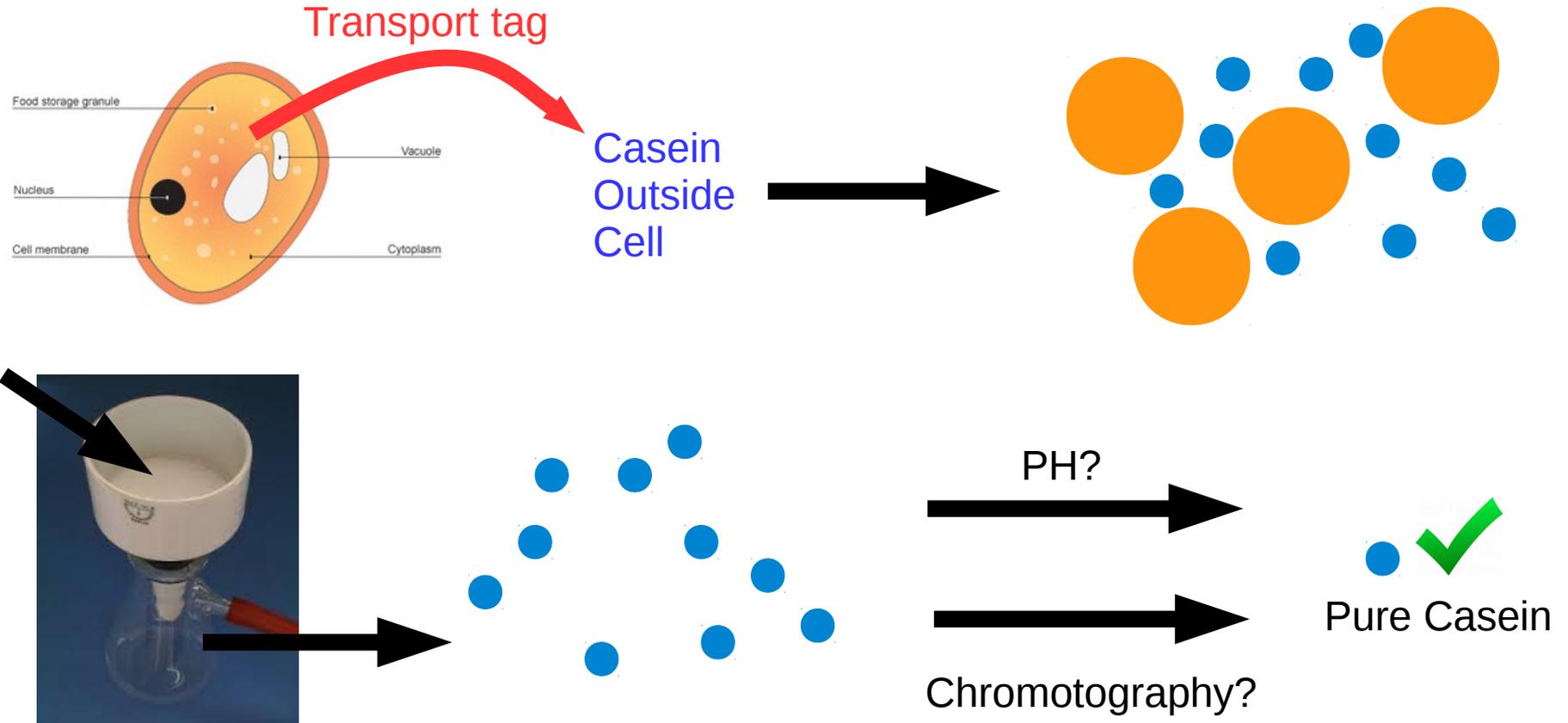


α -S1 casein

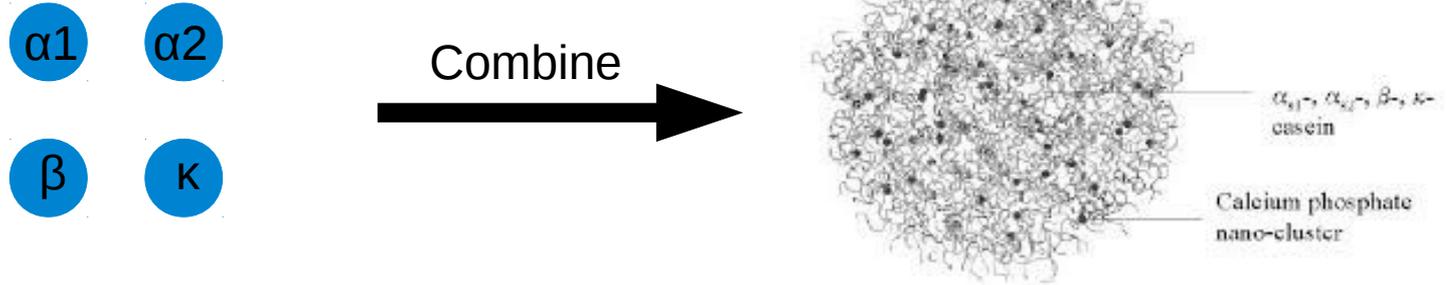


α -S2 casein

Production and Reconstruction



Production and Reconstruction



+ Sugars
+ Fats



But Aren't GMOs Bad?



GMO = Monsanto = Evil?

Problems real and perceived:

Farmers can be sued for saving GM seeds to replant next year



- Conventionally Bred
- EPO upheld patents



(Thanks Willow!)

Glyphosate resistant AKA Roundup Ready



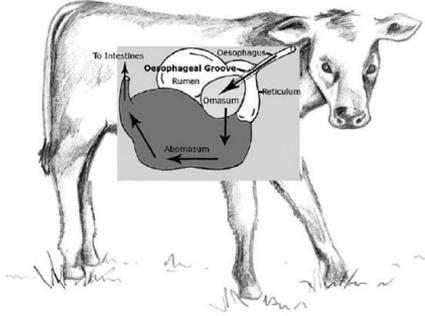
Can be dangerous to people applying Roundup

Not dangerous to consumers

Crop yields are increased

A Few Good GMOs

Chymosin, the most important element in rennet, is used in cheese production.



Slaughtered calf:

- ✗ Supply
- ✗ Reliability of product



80% of the rennet is now made in e. coli

If you eat cheese you eat this

Insulin was extracted from animal pancreas before genetic engineering



Pig Pancreas:

- ✗ Supply
- ✗ Reliability of product
- ✗ Sometimes poisoned people



GMO:
Abundant
Reliable
Modifiable

Any Sequenced Mammal?

Cow

- This is what everyone expects
- Cheese will be most like currently available cow milk cheese

Human

- Some people are allergic to cow casein
- Human casein should have the least incidence of allergy

Narwhal

- Because why not?
- Will likely be the first narwhal cheese ever made



Real vegan narwhal cheese? Well, I'm sure synthetic biology can get weirder — but this is a great start.

-Mike Loukides, radar.oreilly.com

Progress So Far

Organizational/Outreach

- Functional non-hierarchical organization, non-profit
- Incorporated a non-profit to carry on the project
- Engaged broad community, doing relevant work with a fraction of normal resources
- Successful Indiegogo campaign.

FDA

- Discussions with US Food and Drug Administration. They are generally comfortable with our work but against human proteins.

Cheese making

- Making reconstituted milk from isolated cow casein, along with vegan fats and sugars and taking it on to cheese.
- When actual materials are ready this formulation step will already be done.

Progress So Far

Lab

- Designed 11 new plasmid constructs with bovine and human casein proteins and kinases (phosphorylation enzyme).
- Successfully transformed in e. coli to amplify plasmids = sufficient plasmid.
- Confirmed sequence of 10/11 plasmids (11th toxic to e. coli, can do without).
- Transformed into a uracil deficient yeast.
 - Control plates w/o uracil still growing, something wrong here...
- Several other uracil deficient strains were donated.
 - New strain behave as expected.
- We are still unsure if we are producing k-casein, which is the most important.
 - Yeast with this plasmid aren't growing well, which may indicate that this protein is toxic to yeast.
 - This is obviously a problem, but we may be able to solve it, with science!

iGEM Competition



Beyond iGEM

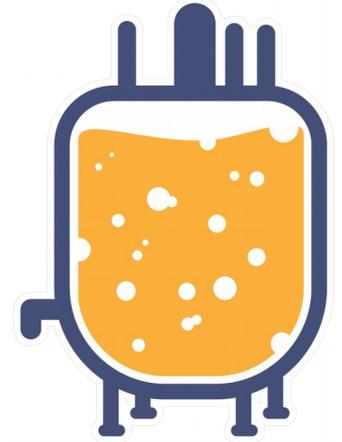
Next steps for bovine casein

- Keep working on k-casein.
- Figure out post translational modification.
- Scale up to a few grams protein.
- Figure out reconstitution of micelle.
- Make cheese.

Move on to narwhal and repeat steps

Purchase large bioreactor for next scale up

- We have \$10K from Indiegogo campaign
- We should be able to buy a 50L reactor with this.



Press

We have been featured in over **100 articles** & a **Reddit AMA!**



Prospects for Scale Up

- This will be the first attempt to make a bulk food from recombinant protein
- Based on scientific literature we believe we can get 2.5 g/L protein.
- At that output we estimate that we could make 5g protein for about the cost of 1L of craft brew beer
- That price places it as a specialty product for vegans.
- Possible further optimization in *Saccharomyces cerevisie*.
- Other yeast strains may get higher yield, *Pichia pastoris*?
- Other fungi? *Aspergillus oryzae* (used in Sake production) has been suggested to us as a high yielding alternative.

This is a long term project, still in early stages but we are keeping momentum

Impact of Success

- Cut GHG emissions by $\frac{1}{2}$ at the 2.5 g/L yield, and better with higher yield per liter.
- Dairy is water inefficient, our system will be better but we don't have numbers yet.
- If we never get the price down, we may still encourage people to go vegan.
- All open source, which allows many people to take this to commercialization.
 - Maximizes chances that someone will figure out cost minimization.
- If we become price competitive with dairy cheese we may be able to end the use of cows for dairy.
- Home vegan cheese factories?

Other High Tech Vegan Food



Muufri

- Very similar to Real Vegan Cheese
- Venture funded
- Reproduce protein, fat and sugar from milk
- Traditional patent strategy
- Current state of project?



- Producing molecules responsible for meat flavor from veggies
- Have an ingredient called “plant blood” from legumes
- Started by Pat Brown from Stanford



Hampton Creek

- Non-GMO
- Egg replacement from pea protein
- Highly successful as a business
- Searched for nearest evolutionary cousin of egg proteins

Many More!

Get Involved

Real Vegan Cheese organizational meeting:

- CCL-igem on Google Groups.
- In the SF Bay area you can participate at our two labs.
- From other places in the world, get in contact. We will find a way to work together.

Do this on your own:

- All of our work is open source.
- Work on this project at your own hackerspace with or without us.

Contact me:

benjamin.lee.rupert@gmail

->please include RVC in subject

Meowdip on twitter