Synthetic Biology and Light-Dependent Systems

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Designing Biological Systems for Programed Interface with the Environment

- The synthetic biology platform
- Microbes that process sunlight and CO2
- Photosynthetic ecosystems and organisms

Can we make Biology easier to engineer?

- Rapid, inexpensive DNA synthesis
- Sequenced genomes for raw materials
- Information explosion via the internet
- Worldview from computer chip design
  - Appreciation of biological modularity
    eg promoters, genes, proteins

Biological Modularity

What does Nature have to offer?

- **Examples of modularity:**

  Genes (promoters, ORFs, introns, enhancers)

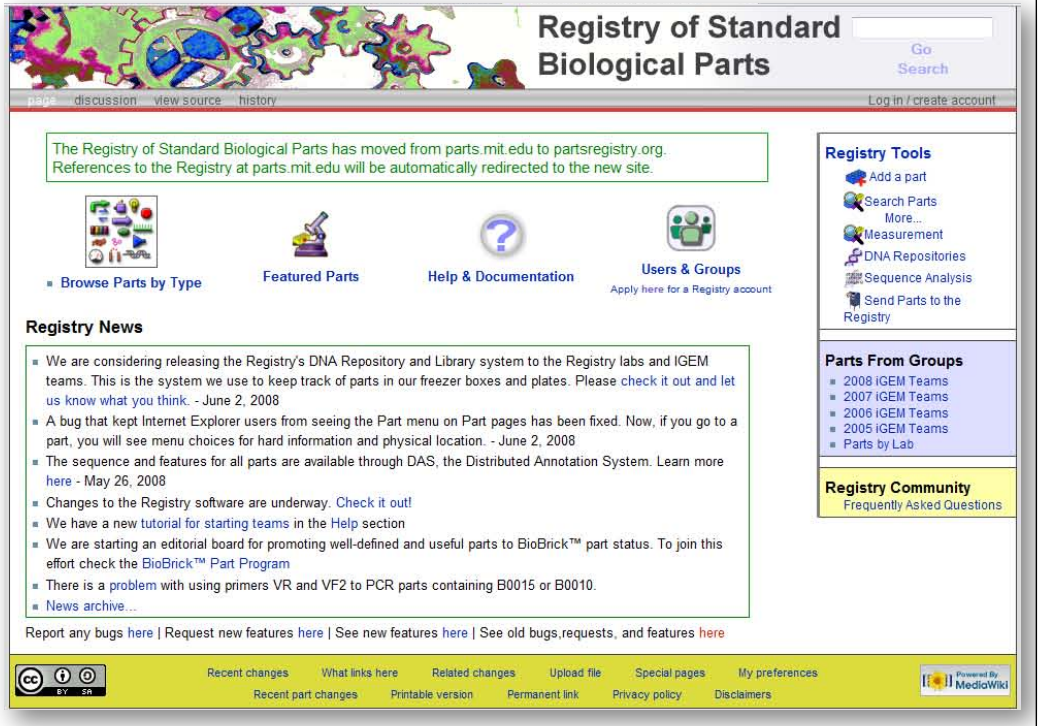
  RNA (Translation, stability, export, localization)

  Proteins (Targeting, DNA binding, dimerization, degradation)

  Pathways (Signaling, metabolism)

- **Biological design can test the limits of modularity**

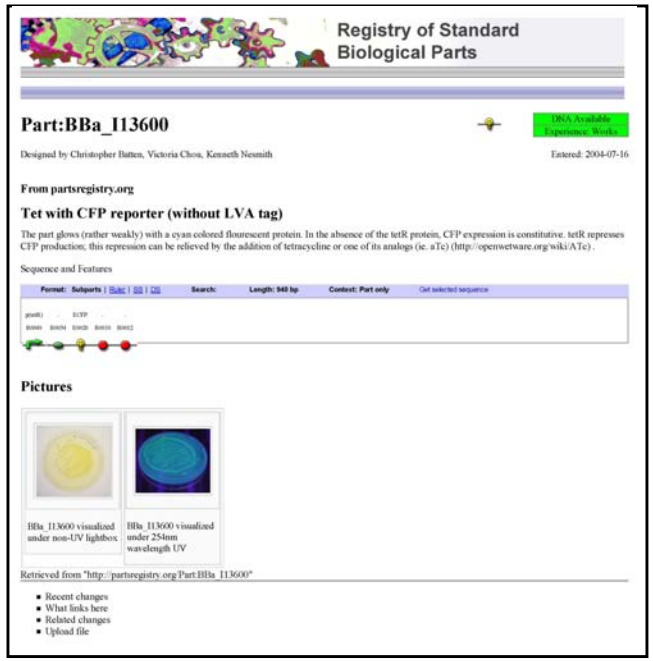
Bacterial Devices - Abstraction



partsregistry.org

Example Part

igem.org



Registry of Standard Biological Parts

Part:BBa_I13600

DNA Available
Experience Works

Designed by Christopher Batten, Victoria Chou, Kenneth Neumith

Entered: 2004-07-16

From partsregistry.org

Tet with CFP reporter (without LVA tag)

The part glows (rather weakly) with a cyan colored flourescent protein. In the absence of the tetR protein, CFP expression is constitutive. tetR represses CFP production; this repression can be relieved by the addition of tetracycline or one of its analogs (ie. aTc) (http://openwetware.org/wiki/ATc) .

Sequence and Features

Format: **Subparts** | Ruler | SS | DS **Search:** **Length: 940 bp** **Context: Part only** Get selected sequence

Pictures

BBa_I13600 visualized under non-UV lightbox

BBa_I13600 visualized under 254nm wavelength UV

Retrieved from "http://partsregistry.org/Part:BBa_I13600"

- Recent changes
- What links here
- Related changes
- Upload file

Can we make biological design predictable?

- Standardized parts
- Measurements of behavior
- Mathematical models
- Gene to genome synthesis - when does the ‘experiment’ start?

  - Consider the future synthetic biologist.....

Designing Light-Dependent BioSystems

Engineering microorganisms for energy production

Conclusions from the JASON report:

Boosting efficiency of fuel formation form microorganisms is THE major technological application of Synthetic Biology

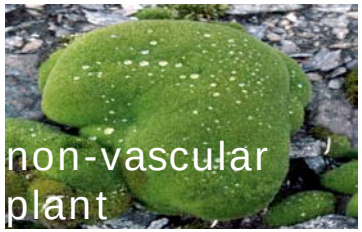
Engineering fuel production from microbes is a SYSTEMS problem

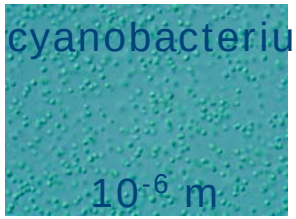
Successful engineering requires a basic understanding of the system to be engineered (multiple feedback loops, etc)

Study Leader Mike Brenner; 6/23/06

Photosynthetic organisms come in all shapes and sizes

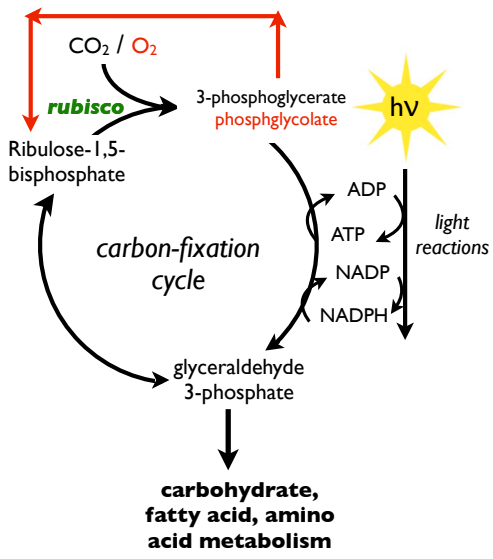
10^2 m



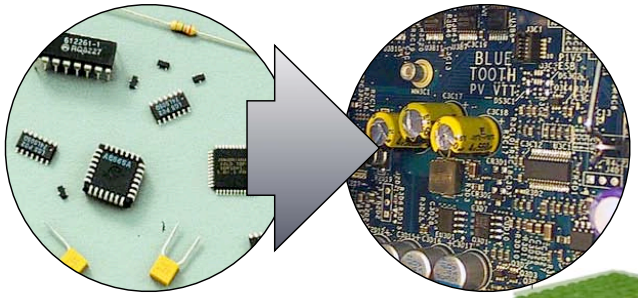


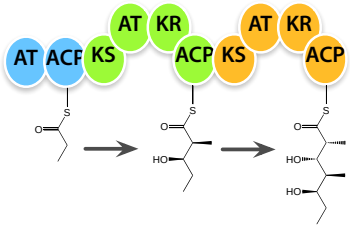
Cyanobacteria are responsible for ~50% of all photosynthesis on earth!

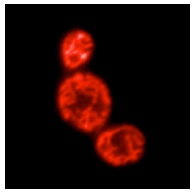
Reaction

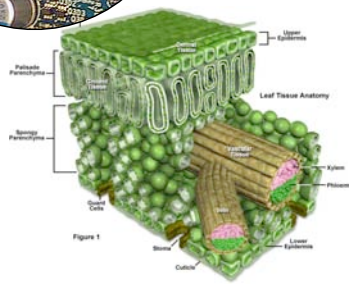

CO_2 / O_2
rubisco
3-phosphoglycerate
phosphglycolate
hν
Ribulose-1,5-
bisphosphate
ADP
ATP
NADP
NADPH
light
reactions
carbon-fixation
cycle
glyceraldehyde
3-phosphate
carbohydrate,
fatty acid, amino
acid metabolism

spatial organization is important for engineering and biology

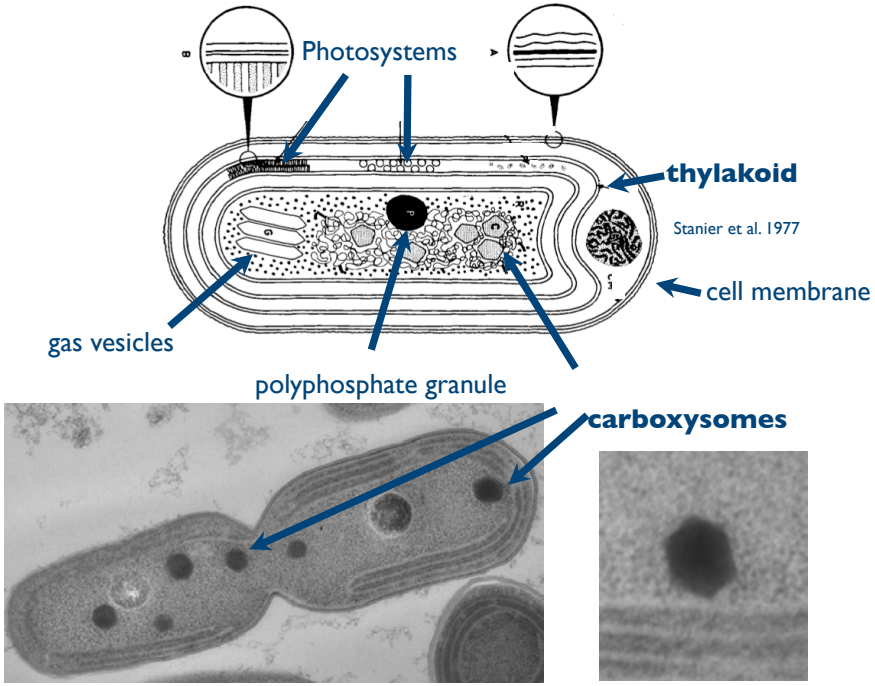








Cyanobacteria contain cytoplasmic structures



Carboxysomes are a large organized unit

- Concentrate Rubisco+ Carbonic Anhydrase+ CO_2
- Solution to rubisco's inefficiency!
- Resemble viral capsid

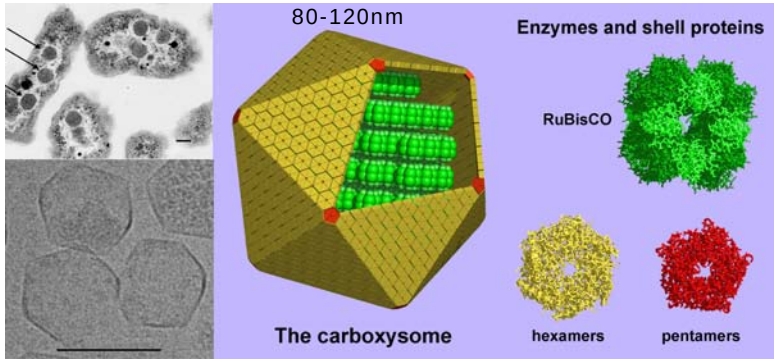
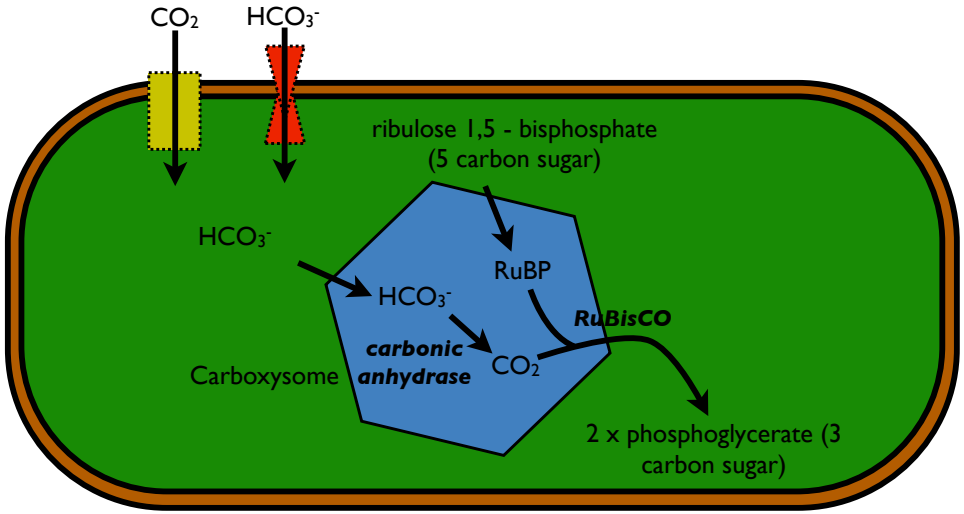


Figure from http://upload.wikimedia.org/wikipedia/commons/5/54/Carboxysome_3_images.png

The enzyme carbonic anhydrase, which catalyzes the production of CO_2 from bicarbonate

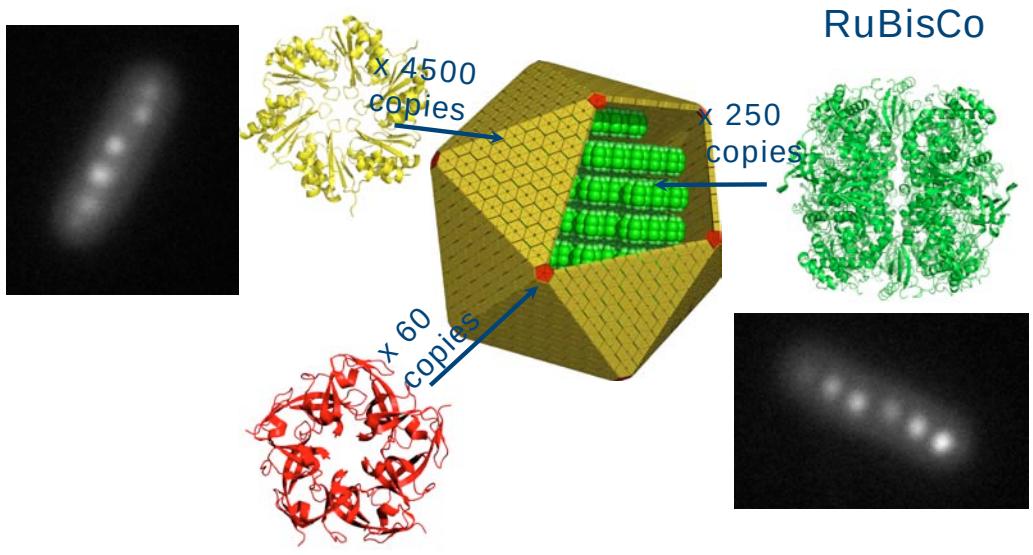
The carboxysome is part of a carbon concentrating mechanism

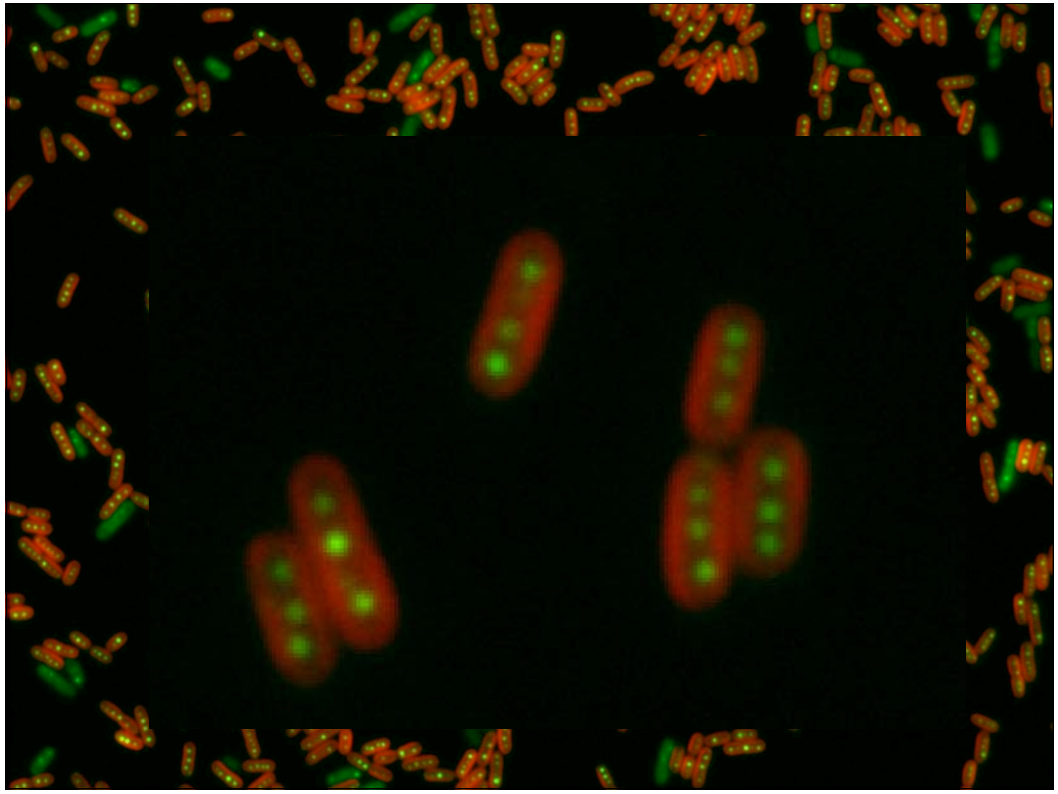


This system increases the cell's affinity for CO_2 1000x!

Dave S.

Carboxysomes can be labeled with fluorescent proteins





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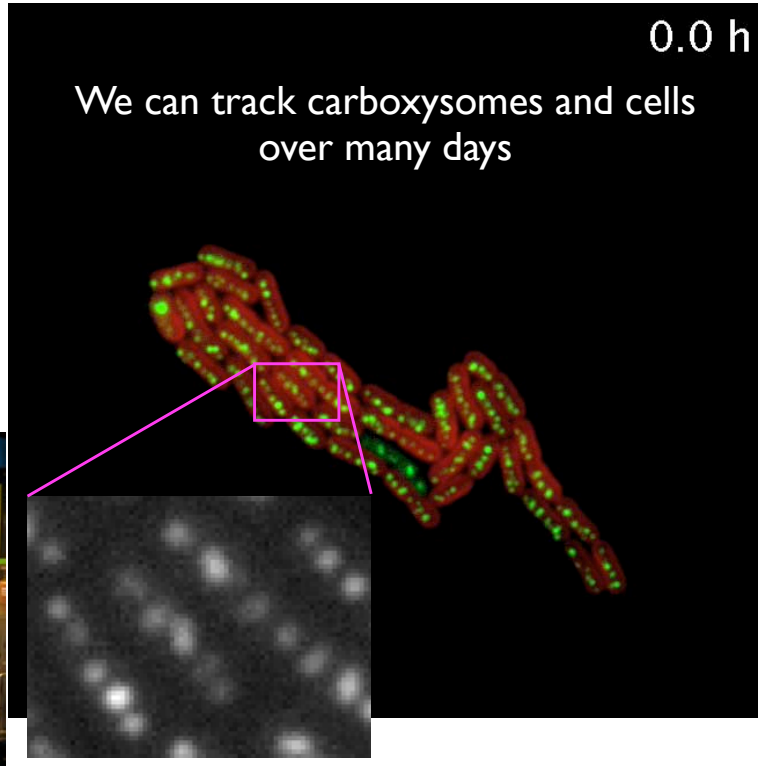
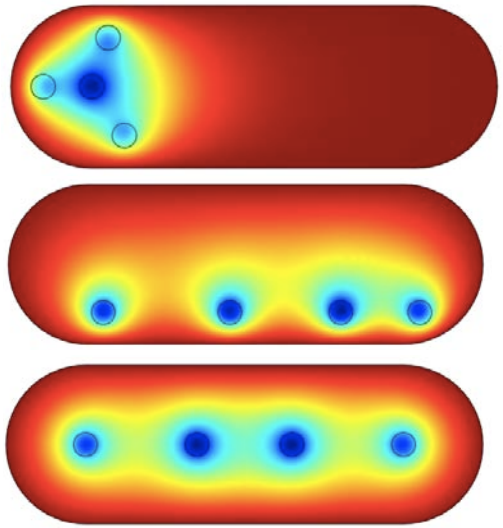
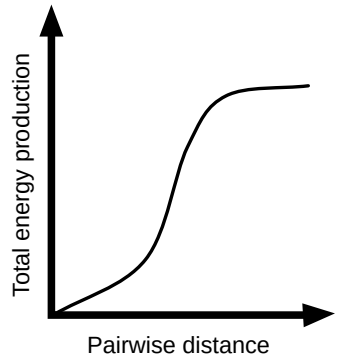


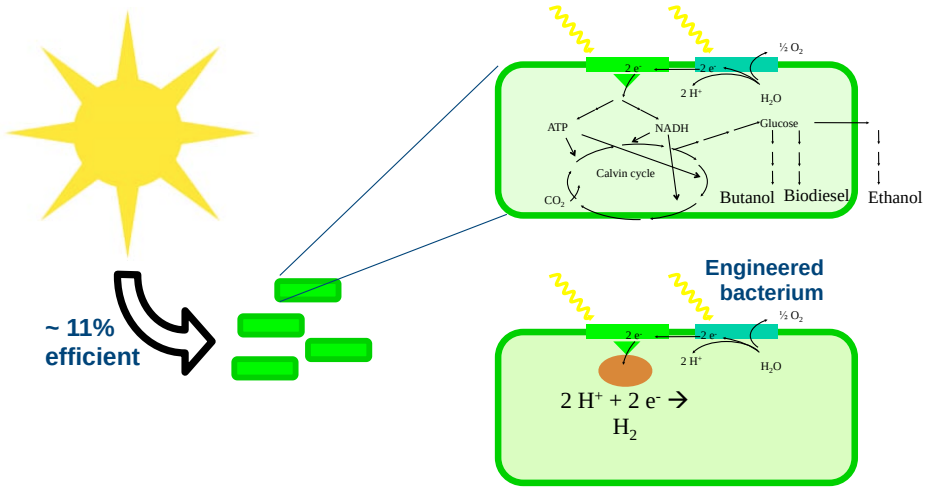
Even spatial distribution can optimize metabolism

- A diffusion-limited reaction will be susceptible to spatial arrangements



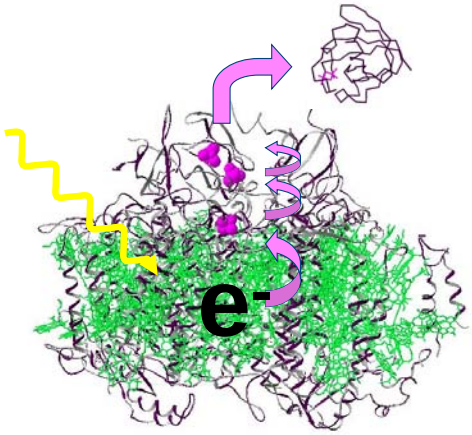


Harnessing Bacterial Photosynthesis

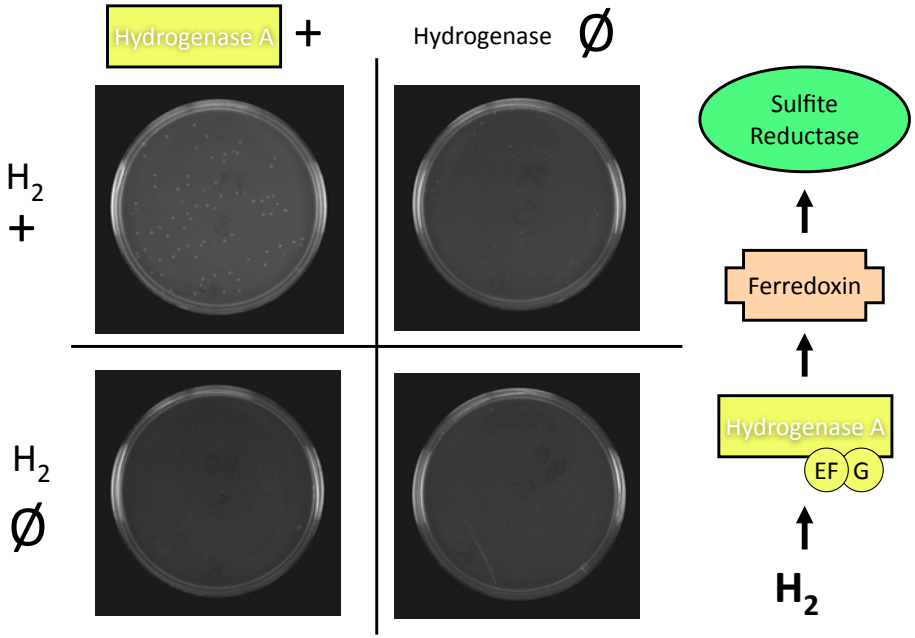


FeS clusters: Biology's Nanowires

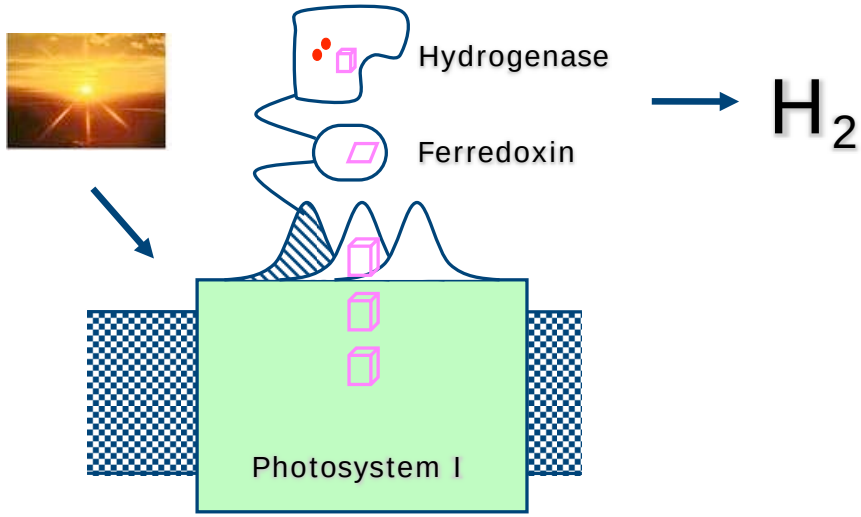
- A photon lands on Photosystem I
- The electron is excited to enter the iron-sulfur clusters
- The electron exits by tunneling into ferredoxin



Hydrogenase Rescues Growth on Minimal Media

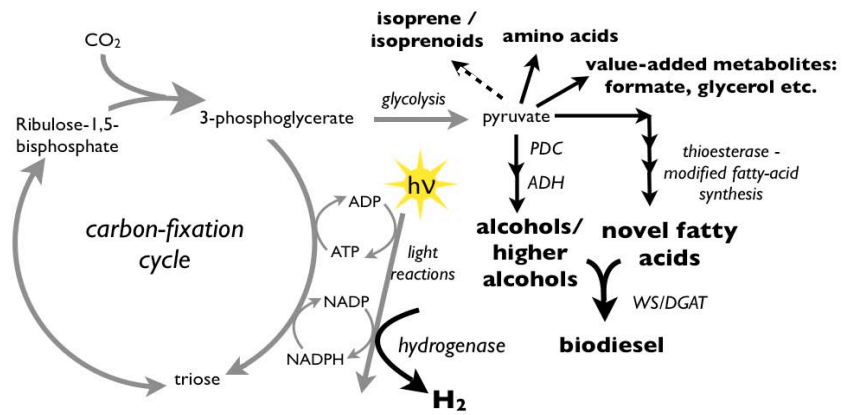


Grand Challenge: Can we Efficiently Connect Photosynthesis to Hydrogen Production



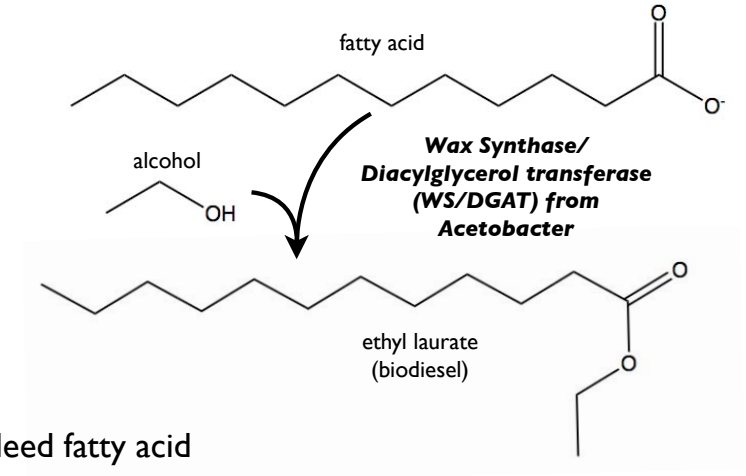
H_2

Platform for chemical synthesis



Enormous advantage for biomass-independent chemical synthesis

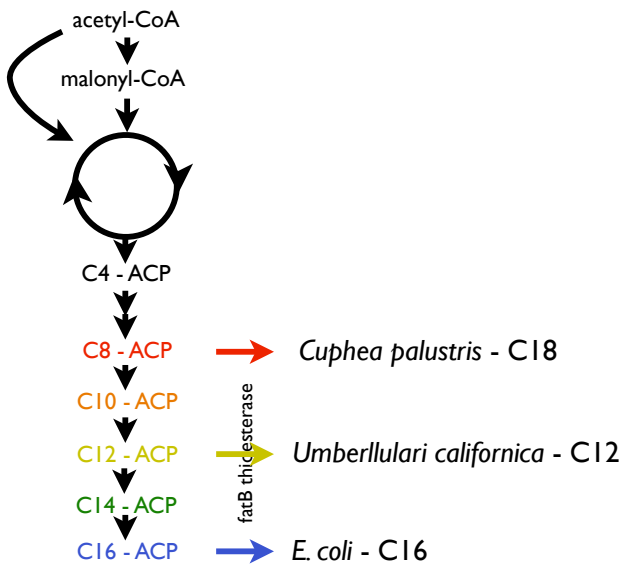
Wax Synthase can synthesize biodiesel *in vivo*



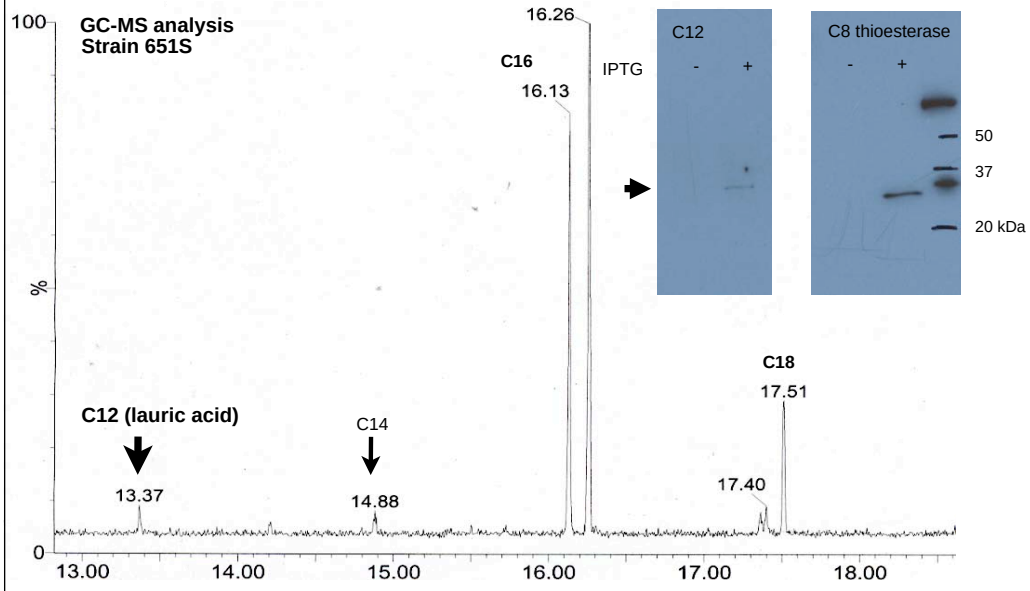
1. Need fatty acid
2. Need alcohol

Kalscheuer et al. 2006

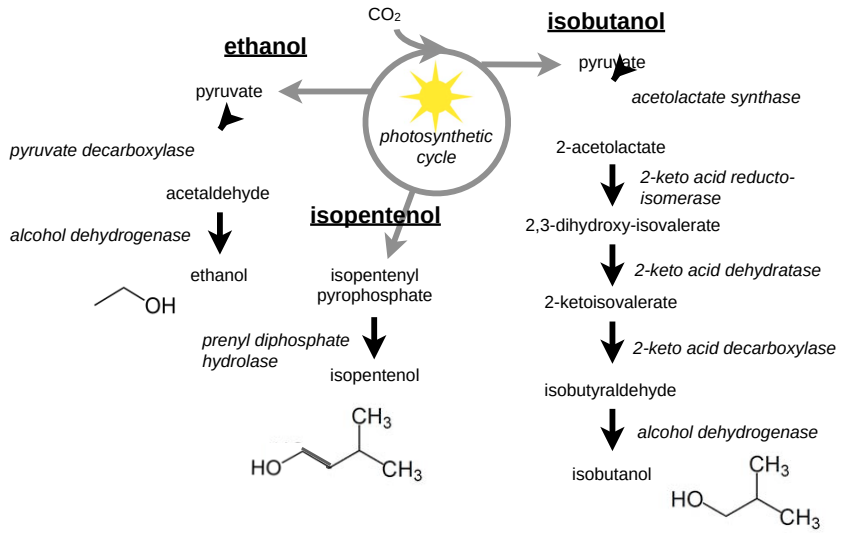
Thioesterase determines fatty acid chain length



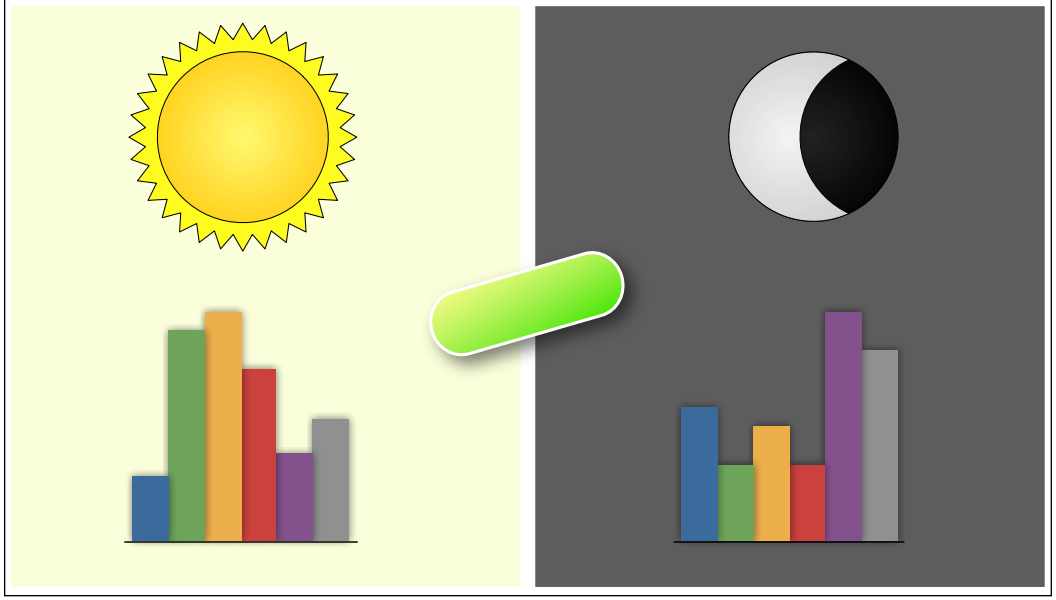
Thioesterases are expressed and functional in *Synechococcus*

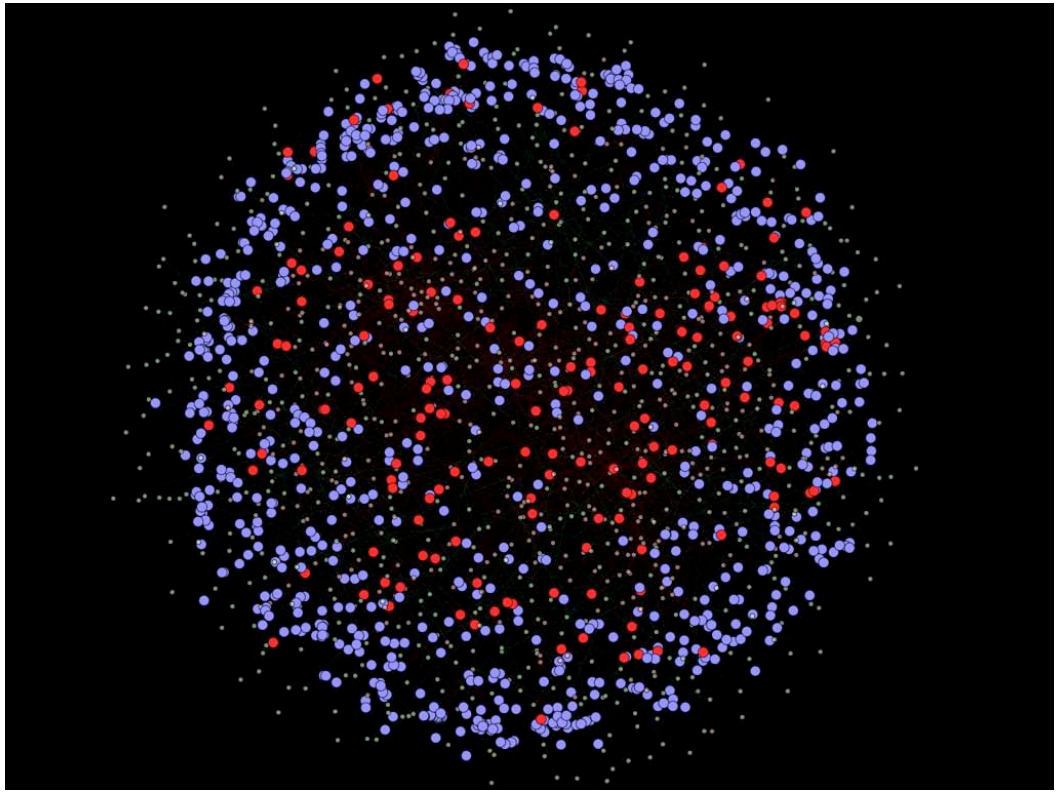


We are testing three alcohol synthesis pathways

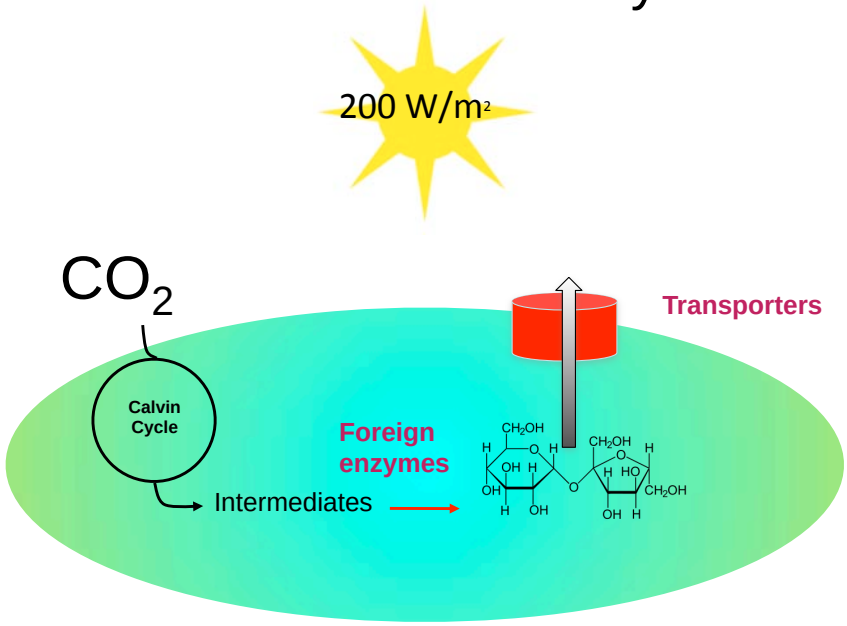


how does metabolism vary with the circadian rhythm?



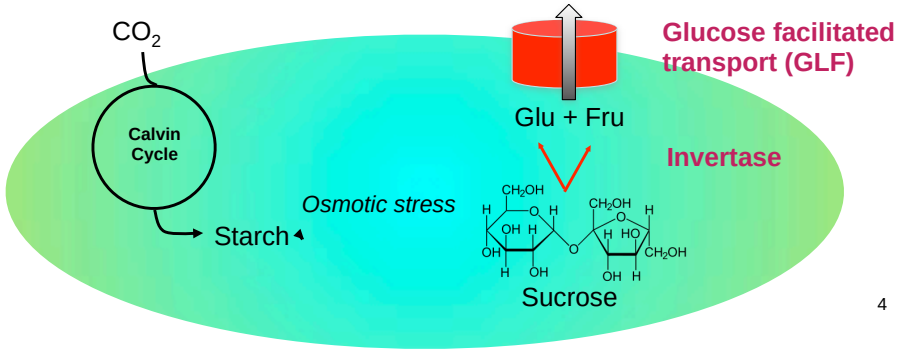


Platform for chemical synthesis



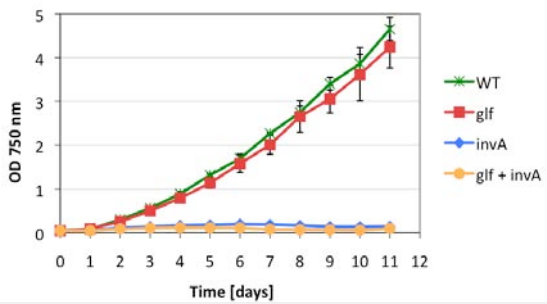
Sugar production in cyanobacteria

- Sugars = feedstock for all metabolic engineering
- Glucose + fructose mixture = ‘high-fructose corn syrup’
- Engineering concept:
  - Induce sucrose production by osmotic stress (natural process)
  - Invertase = enzyme that cleaves sucrose
  - GLF = facilitated diffusion transporter for glucose, fructose



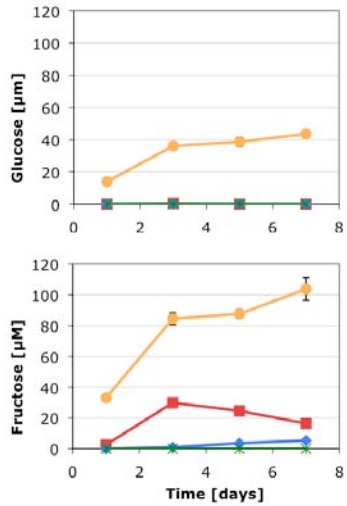
Expression of *glf* and *invA* in *Synechococcus* to produce glucose and fructose

Cell growth (IPTG + 300 mM NaCl)

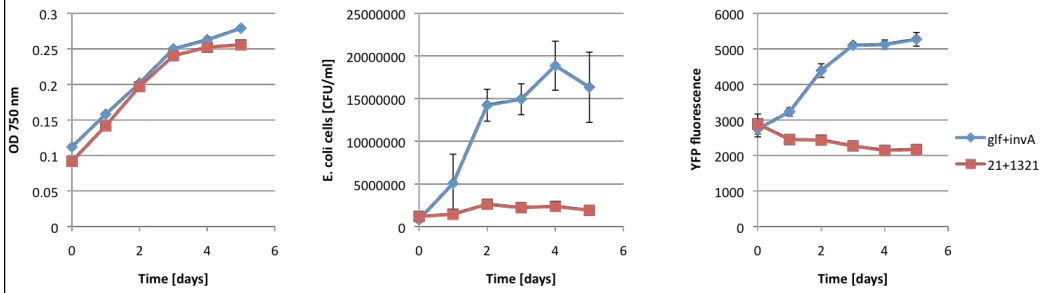


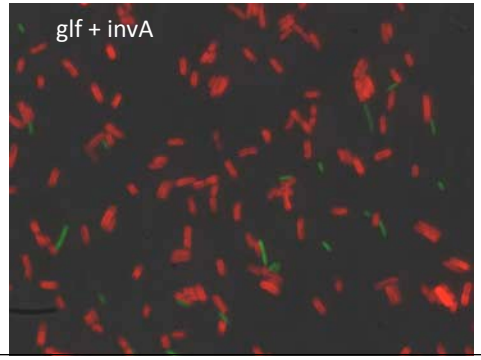
Total sugar produced by day 3 = 40% of dry weight of cells

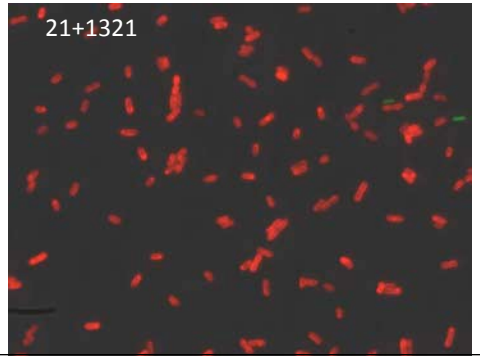
sugar in the medium:



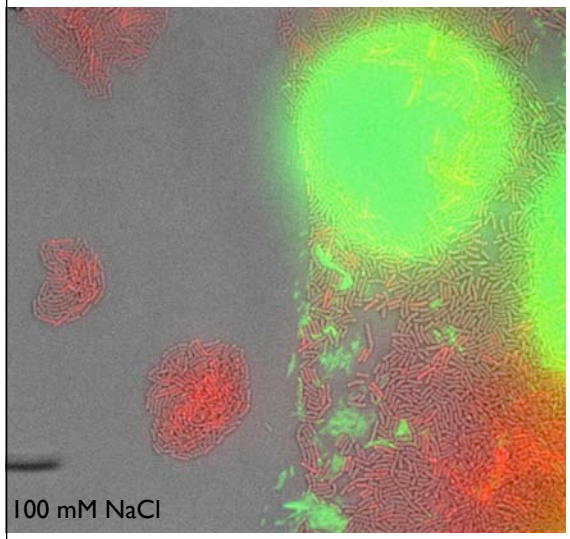
Synechococcus and E. coli coculture

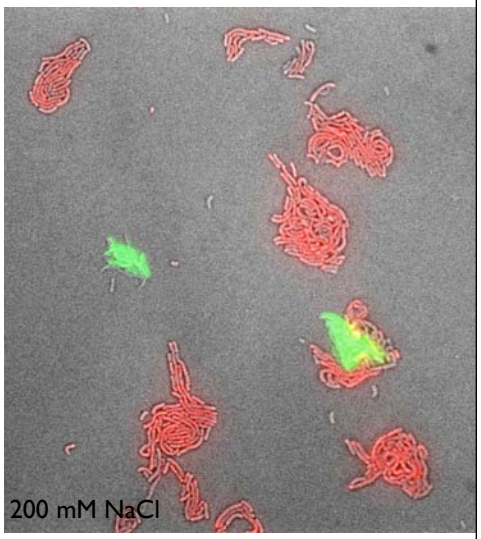




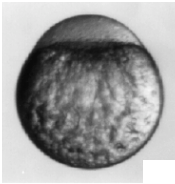


Feeding *E. coli* with light (on plates)

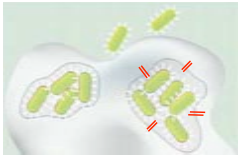




How can we get bacteria into animal cells?



1. Inject into zygote



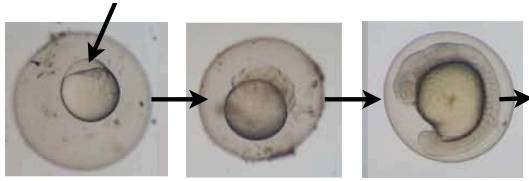
2. Express Invasin and Listriolysin

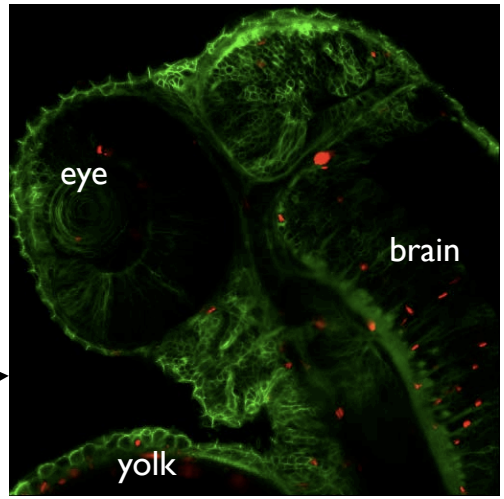
3. Phagocytosis

Making Photosynthetic Fish?

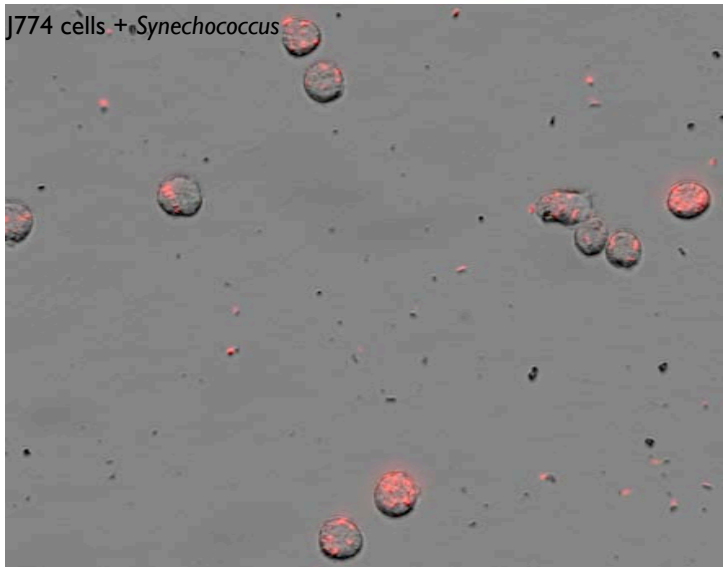
glf + *invA*

microinject engineered *Synechococcus* into zebrafish zygote

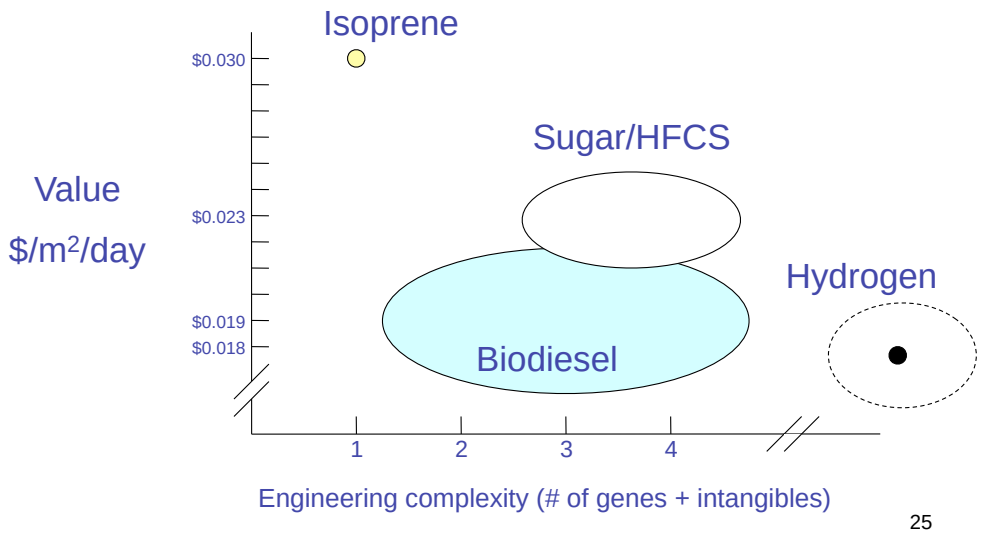




Synechococcus is taken up by macrophages



Mix of commercial potential and scientific feasibility



Getting to a bio-solar energy economy: Thoughts and guesses

Technical step	Genetic engineering Large-scale production	2-3 years 3-5 years (pilot-scale)
Major challenges	Rational basis for engineering Light distribution in scale-up Business model to move to fuel production Excessive top-down control	Need basic research Need DOE support Production of higher-value compounds first (or subsidies) Fund multiple competing approaches
Prognostication	?	

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