Washington University’s first iGEM team
Food and Energy Track

sunglasses for sphaeroides
optimizing R. sphaeroides for bioenergy applications
Introduction
Life in a Photobioreactor

Large Light Harvesting Antenna  Small Light Harvesting Antenna
Life in a Photobioreactor

- Photosynthetic Productivity
- Photon Flux Density (µmol m⁻² s⁻¹)
- Energy Produced
- Energy Wasted Through NPQ
- Theoretical Energy Production
- Photosynthetic Saturation Curve
ENERGY

PRODUCED

WASTED
Leaf Size in the Eastern Black Oak

High Branch  Middle Branch  Lower Branch

David Sibley- “The Sibley Guide to Trees
The Project
The Organism

- New chassis for synthetic biology
- *Rhodobacter sphaeroides* is a purple Alphaproteobacteria.
- Metabolically flexible:
  - aerobic and anaerobic respiration
  - Phototrophic under anaerobic conditions with light.
- *R. sphaeroides* is one of the best understood photosynthetic organisms.
  - photosystem is located in intracytoplasmic membrane invaginations
  - Light Haresting Complex 2 (LH2)
  - Light Harvesting Complex 1 (LH1)
  - Reaction Center (RC).
  - These pigment-protein complexes non-covalently bind bacteriochlorophylls and carotenoids.
Light Harvesting Antenna 2

• LH2 absorbs photons maximally at the wavelengths of 850 and 800 nm

• Funnels its energy to LH1 and the reaction center for photochemistry.

• The two subunits of LH2 are coded for by the pucB/A genes

• Naturally promoted by the puc promoter.
Wild Type

High Oxygen

- Puc promoter downregulated
- No expression of pucB/A and thus LH2

Low Oxygen

- Transcription from the puc promoter is high
- pucB/A expressed, high LH2 expression (big antenna complex)
Synthetic Regulation of pucB/A
Under Low light intensities Cph8 active and OmpR phosphorylated, leading to puc B/A and LHII expression

• Keep under low oxygen tension
• Cph8 light sensor under control of puc promoter, and puc B/A genes behind OmpC promoter
• High light intensities repress OmpR phosphorylation and puc B/A expression

• LH2 Expression is inversely correlated to light Intensity
Submitted 10 parts to the registry

Plan to submit 2 more in the near future

10 total *R. sphaeroides* specific parts

Constructed 4 other parts that aren’t compatible with Registry Standards
### pucB/A as a reporter

- LH2 absorption at 800 and 850nm is absent in LH2 deficient mutant DBCΩ
- Indicates its efficacy as a reporter.
- Expression is higher from genomic DNA than on pRKCBC3
- Indicates that can use pRKCBC3 + pucPromoter and pucB/A as truncated antenna

<table>
<thead>
<tr>
<th>Part/Accession #Component</th>
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<tbody>
<tr>
<td>BBa_K27001</td>
<td>Cph8 (planned resubmission)</td>
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<tr>
<td>BBa_K27004</td>
<td>puc A</td>
</tr>
<tr>
<td>BBa_K27005</td>
<td>puc B</td>
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<tr>
<td>BBa_K27006</td>
<td>puc BA</td>
</tr>
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<td>BBa_K27007</td>
<td>puc promoter</td>
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<td>BBa_K27009</td>
<td>PucPromotor+GFP</td>
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<td>BBa_K27011</td>
<td>RBS34+OmpR(sph)+Term (synthesized)</td>
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<td>BBa_K27012</td>
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<td>BBa_K27013</td>
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<td>BBa_K27015</td>
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Strength of the puc Promoter

- The puc promoter is down-regulated at high oxygen tension
- Nearly constituent at low oxygen tension
Tissue Flask Experiment

- Designed this experiment to examine:
  - how available light influences growth on a series of bioreactors
  - the effect of Non-Photochemical Quenching and photodamage
- The first group to measure these parameters
- Light that passes through this flask is the only source of light for those flasks behind it
- Conducted experiment on *R. sphaeroides 2.4.1* and *R. sphaeroides DBCΩ* (LH2 Knockout)
- Measured Growth Rates (OD 600) using a spectrophotometer
- Measured the absolute irradiance of light incident on the flasks using a spectroradiometer
Absolute Irradiance on Flask 2

Wild Type

DBCΩ
Absolute Irradiance on Flask 3

Wild Type

DBCΩ
Absolute Irradiance on Flask 4

Wild Type

DBCΩ
Absolute Irradiance on Flask 5

Wild Type

DBCΩ
Flask 1 Growth

**Wild Type**

**DBCΩ**

Growth over 5 days at OD 600 for Flask 1

- WT absorbs LH2 wavelength light at 800 and 850 nm
- Yet WT grows the same amount as DBCΩ

- As such, it can be reasoned that NPQ is occurring as the photons absorbed by LH2 don’t appear to affect growth
Flask 2 Growth

Wild Type

DBCΩ

Growth over 5 days at OD 600 for Flask 2

• WT second flask grew extremely well
• Appears that photodamage also occurred in WT flask 1 as it grew less than flask 2

• DBCΩ flask 2 grew less than flask 1
• Likely due to attenuated light at LH1 870 nm wavelength from first flask
Growth over 5 days at OD 600 for Flask 3

- WT growth is at the same rate as DBCΩ
- Light available at LH2 wavelengths is depleted
- Does not contribute to growth

- DBCΩ flask 3 grew less than flask 1 and 2
- Also due to attenuated light at LH1 870 nm wavelength
Flask 4 Growth

**Wild Type**

![Graph showing growth of WT over 5 days with OD 600 measurement.]

**DBCΩ**

![Graph showing growth of DBCΩ over 5 days with OD 600 measurement.]

**Dark Growth (Heterotrophic)**

<table>
<thead>
<tr>
<th></th>
<th>OD Day 5</th>
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<tbody>
<tr>
<td>WT</td>
<td>.122</td>
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<tr>
<td>DBCΩ</td>
<td>.151</td>
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</table>

Growth over 5 days at OD 600 for Flask 4

- WT is at heterotrophic growth levels
- DBCΩ is still growing photosynthetically
Flask 5 Growth

**Wild Type**

**DBCΩ**

**Dark Growth** (Heterotrophic)

<table>
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<th>OD Day 5</th>
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<tbody>
<tr>
<td>2.4.1</td>
<td>.122</td>
</tr>
<tr>
<td>DBCΩ</td>
<td>.151</td>
</tr>
</tbody>
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Growth over 5 days at OD 600 for Flask 5

- WT is at heterotrophic growth levels
- DBCΩ is still growing photosynthetically
Cumulative Growth

The Cumulative Growth of Wild Type Tissue Flasks

- Cumulative growth of the tissue flasks as measured by the sum of the optical densities of the respective cell type's tissue flasks at a given day.
- The contribution of a given tissue flask to cumulative growth is displayed.

The Cumulative Growth of DBCΩ Tissue Flasks
Conclusions of Tissue Flask Experiment

- Photodamage occurred in WT Flask 1
- NPQ quenching occurred in WT Flask 1
- LH2 Wavelength light in the WT was depleted after Flask 2
- All photosynthetic growth in the WT flasks was absent after Flask 3
- All DBCΩ flasks grew photosynthetically and the amount of growth was inversely correlated to depth in the bioreactor
- Cumulative WT flask growth is greater than DBCΩ

The size of the Light Harvesting Antenna (LH2) is inversely correlated to light intensity. As such, these effects should not be observed and growth should be greater than the wild type.
Modeling our Mutant vs. the WT
In a bioreactor, cells at the surface absorb more than enough light to saturate their photosynthetic apparatus, transmitting less energy to deeper layers.

For wild type cells, the saturation curve is approximately the same for all cells, regardless of their incident light intensity.

Wild type: $C_k = C_{wt}$ for all $k$

\[ A(i) = C_{wt}(1 - e^{-si}) \]

\[ s = 0.1115 \]
\[ C_{wt} = 2.1182 \]
\[ i_o = 64 \frac{\mu W}{cm^2 \cdot nm} \]
- For our mutant cells, this curve scales as a function of light intensity, due to negative regulation of LH2 complex production.

\[
\text{Wild type: } C_k = C_{\text{wt}} \text{ for all } k
\]

\[
\text{Mutant: } C_k = (C_{\text{max}} - C_{\text{min}}) e^{(\frac{-1}{s})\left(\frac{i_k}{i_o}\right)} + C_{\text{min}}
\]

- \[C_{\text{max}} = 1.5 C_{\text{wt}}\]
- \[C_{\text{min}} = 0.25 C_{\text{wt}}\]
- \[s = 0.1115\]
- \[C_{\text{wt}} = 2.1182\]
- \[i_o = 64 \frac{\mu W}{\text{cm}^2 \cdot \text{nm}}\]

- For our mutant cells, this curve scales as a function of light intensity, due to negative regulation of LH2 complex production.
Assumptions

• Saturation curve: Absorbance as a function of incident light intensity. The coefficient changes with intensity in the mutant only.

\[ A(i) = c(i_k)[1 - e^{-si}] \]
Revisions based on empirical data

• Background LH1 absorbance

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
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<tbody>
<tr>
<td>DBComega</td>
<td>0.011</td>
<td>0.065</td>
<td>0.178</td>
<td>0.292</td>
<td>0.406</td>
<td>0.512</td>
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<tr>
<td>2.4.1</td>
<td>0.011</td>
<td>0.067</td>
<td>0.51</td>
<td>1.508</td>
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<td>DBComega/(WT)</td>
<td>1</td>
<td>0.97014925</td>
<td>0.34901961</td>
<td>0.19363395</td>
<td>0.18761553</td>
<td>0.2048</td>
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</table>

• Divide Mutant by correction factor (1 - 0.2)

• Photoinhibition

Growth over 5 days at OD 600 for Flask 1

• Limit first flask absorbance to 1
Layer 3

Wild Type Reactor

Mutant Reactor

Total Absorbance

Optical Density

LH2 Absorbance

Incident Light (μW/cm²/nm)

Wild Type

Mutant

Incident Light Intensity
Layer 5

**Table:**

<table>
<thead>
<tr>
<th>Absorbance</th>
<th>Wild Type</th>
<th>Mutant</th>
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<tbody>
<tr>
<td>Mean</td>
<td>0.389</td>
<td>0.476</td>
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<tr>
<td>Std. Dev.</td>
<td>0.480</td>
<td>0.375</td>
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Conclusions and Future Work

Achievements
• Able to characterize 2 parts and submit 10 new parts to the Registry of Standard Biological Parts
• Built our complete genetic construct
• The results of this experiment on WT and DBComega match the assumptions that we had laid out at the beginning of the project
• Able to use empirical data to model our mutant’s growth under the same conditions

Future Work
• Complete tissue flask experiment for DBCΩ with pRKCBC3 (truncated antenna because lower expression levels from plasmid)
• Experimental measurements with mutant
• Characterize the puc promoter under various light conditions and additional oxygen tensions
• Find time-domain characteristics for our system
• Apply this system to a more complex organism, such as a cyanobacterial or algal species

Conclusion
• Observed how light availability at certain wavelengths changes through a bioreactor and the influence of NPQ on light availability and cell growth
• Demonstrated the potential for a synthetically regulated light harvesting antenna to improve photosynthetic productivity for a series of photobioreactors.
  • Proportional to the gain in yield of a desired metabolic product such as Chemicals, Biofuels, or Drugs
• This work is applicable to all groups that seek to produce biofuels or other chemicals with photosynthetic microbes
The Team

- Students
  - Biology: Jacob Rubens, Jaffre Athman, Stephanie Chang, Jacob Cecil, Colin Foley
  - Biomedical Engineering: Brendan Cummings, Alice Meng, Thomas Stevens, James Kugler
  - EECE: Jeff Knudsen
- Advisors: Barb Honchak, Aaron Collins, Larry Page, Joseph Tang
- Faculty:
  - Dr. Robert Blanksnehip
  - Dr. Chris Kirmaier
  - Dr. Yinjie Tang
Acknowledgements

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  – The Washington University Career Center
  – The Energy, Environmental and Chemical Engineering Department
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• The Washington University Undergraduate Research Office for their incredible ongoing support
• Dr. Robert Kranz, Dr. Debbie Hanson, Dr. Neil Hunter, and Dr. Chris Voigt for advise and strains/plasmids
• iGEM, Randy Rettberg, Megan Lizarazo, our Judges!
System Modeling Equations

Assumptions:
- CphB and OmpR expression/degradation in dark has reached steady state
- [CphB] = [OmpR] since they lie downstream of same promoter

\[
\frac{dCphB^*}{dt} = \frac{\beta_{CphB} (1 - L)(R_p)}{K_{R_p} + R_p} - \alpha_{CphB} CphB^* \quad \text{OmpR phosphorylation}
\]

\[
\frac{dU_m}{dt} = \frac{\beta_{OmpC} (R_p)^{\gamma}}{K_{R_p}^{\gamma} + (R_p)^{\gamma}} + \beta_{OmpC} - \alpha_{OmpC} U_m \quad \text{OmpC binding; pucB/A gene transcription}
\]

\[
\frac{dU_p}{dt} = \beta_{OmpC} U_m - \alpha_{OmpC} U_p \quad \text{pucB/A gene translation}
\]

Time evolution of pucB/A expression with light switch

![Diagram showing the time evolution of pucB/A expression with a light switch.](image)