Transcriptional Sensor of Voltage Exposure
Outline

- Our Team
- Our Idea
  (meet Gabe the Grad Student)
- Instrumentation
- Experimentation
- Our Accomplishments
The Team

Department of Chemical and Biomolecular Engineering

DEPARTMENT OF BIOPHYSICS

DEPARTMENT OF BIOLOGY
Concept / Machine-Cell Interface

- Voltage Signal Sensitivity in *S. cerevisiae*
T.G.I.F!
TO: INCUBATOR
MSG
GROWTH
STOP
Concept / Previous Work

Valencia 2009
- Aequorin, Ca$^{++}$ sensitive enzyme
  - Fast
  - Not extensible

JHU 2010
- Transcriptional control
- More diverse application
- Can be a component for other biobrick systems
Concept / Our Plan

Crz1p

Calcineurin

Ca^{2+}

Strongly Activated Promoter

GFP

Weakly Activated Promoter

RFP

-P_{i}

Ca^{2+}

ATP → ADP + P_{i}
Concept / The CDRE

- Calcineurin-Dependent Response Element (Stathopoulos 1997)
- As small as 7bp (Yoshimoto 2002)
Experiments / DIY Instrumentation

- Homemade aluminum electrodes
  - Successful
  - Imprecise electric field
  - Low throughput
- Gold-plated coaxial 8 well electroporator
  - High throughput
  - Linear electric field lines

Goal: be here
Instrumentation / DIY μFluidics

- Microfluidics
  - Low voltages
  - Uniform electric field
  - Parts integration
  - Details of fabrication proved challenging
Experiments / Instrumentation
Experiments / Visualizing Crz1

- **Target**: Crz1
- **Tag**: GFP
- **Apparatus**: homemade electrodes, confocal microscope
- **Input**: voltage stimulus
- **Output**: GFP moves into the nucleus

1) Calcineurin de-phosphorylates Crz1p  
2) Crz1 is free to localize in the nucleus  
3) Crz1 oscillates in and out in waves with a temporal period on the scale of minutes  
4) This oscillation is damped, the Crz1 population is eventually returned to the cytosol
Modeling / Signal to Expression

Crz1 oscillates into and out of the nucleus

Transcriptional response continues for days
Experiments / FKS2 Optimization

- **Motivation:** establish ranges for electrostimulation voltage and duration
- **Target:** 4xCDRE from FKS2 promoter
- **Tag:** RFP
- **Input:**
  - voltage stimulus ranging from 2-10V
  - Exposure time of 0-80sec (log intervals)
- **Output:** Minima of exposure time and voltage established

6 Volts of electrostimulation  
10 Volts of electrostimulation
Experiments / FKS2 Honing

- **Motivation:** establish optima for electrostimulation voltage and duration
- **Target:** 4xCDRE from FKS2 promoter
- **Tag:** RFP
- **Input:** 8V for 30-130 seconds (10s intervals)
- **Output:** Threshold for response specified in voltage, stimulus duration dimensions
Experiments / Summary

What we have

• Voltage – 4-8V, 8V works best

• Duration – 30-130 seconds works well, 1 minute is a good rule of thumb

• Delay – 8 hours
Experiments / FKS2 with Pumps

- **Motivation:** cells were dying from the voltage, so we let them keep their vesicular Ca\(^{2+}\) pumps.
- **Target:** CDRE from FKS2 promoter
- **Tag:** RFP
- **Apparatus:** electroporator, fluorometer, OD reader
- **Input:** voltage stimulus of 8V for 40-140 seconds
- **Output:** Linear regime of transcription-duration relation

Fluorescence from FKS2 + Vesicular Ca\(^{2+}\) Pumps
Experiments / PMC1, New Promoter

- **Motivation:** characterize another CDRE
- **Target:** The PMC1 gene
- **Tag:** YFP
- **Input:**
  - Voltage stimulus of 8V
  - Duration 40-140 seconds
- **Output:** Linear regime of transcription-duration relation
Results / Characterizations

Our characterization consists of...

• Establishing the effects of...
  – Voltage input amplitude
  – Stimulus duration
  – Vesicular pump presence

• On...
  – Normal fluorescent protein output

• Using a...
  – High-throughput experimentation method
Our Accomplishments

• Extended previous work on voltage sensitivity (Valencia 2009), bringing the response from the biochemical domain into the transcriptional.
• Constructed a library of 7 voltage-activated yeast upstream activation sequences.
• Characterized 2 full voltage-activated promoters for yeast.
• Developed a mathematical model of our system.
• Leveraged post-translational modification machinery to initiate transcription.
• Fabricated a microfluidic prototype device.
Acknowledgements

Advice, Funding, and Training, and Workspace:

• Build-A-Genome Lab (JHU Bio-ChemBE-BME)
  – Jef Boeke
  – Jessica Dymond
  – Yizhi Cai
• Cunningham Lab (JHU Biology)
  – Kyle Cunningham
  – Adam Kim
• Ostermeier Lab
  – Marc Ostermeier
• Integrated Imaging Center
  – Richard McCarty
  – Erin Pryce
• Microfabrication lab
  – Huy Vo
• JHU Biology, esp. Dr. Beverly Wendland
• JHU Biomedical Engineering, esp. Dr. Timothy McVeigh and Gail Spence
• JHU Chemical and Biomolecular Engineering
• JHU Alumni Foundation
• JHU Provosts Undergraduate Research Award
• JHU Undergraduate Admissions, esp. Maggie Kennedy