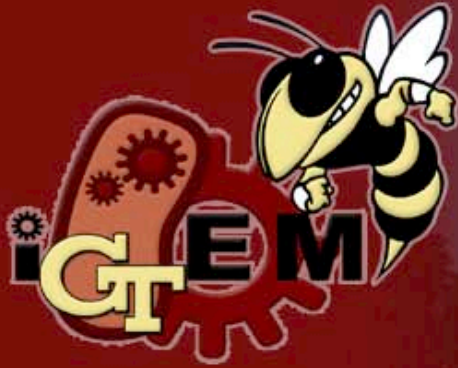


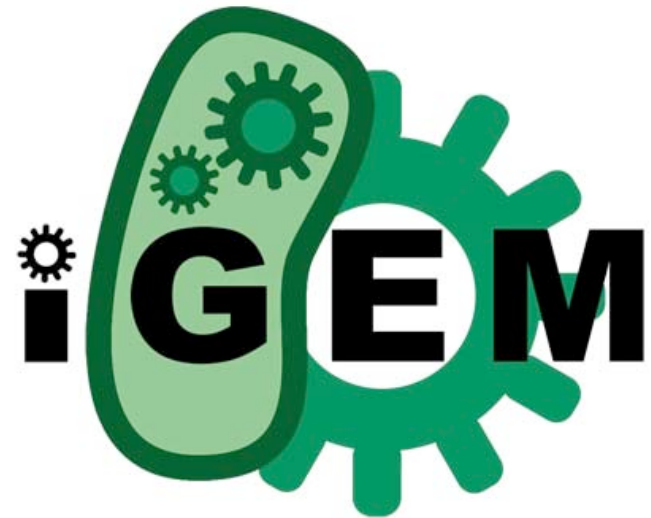


iGEM Team Georgia Tech 2010

**Inducing a
thermogenic
response to cold
shock in**

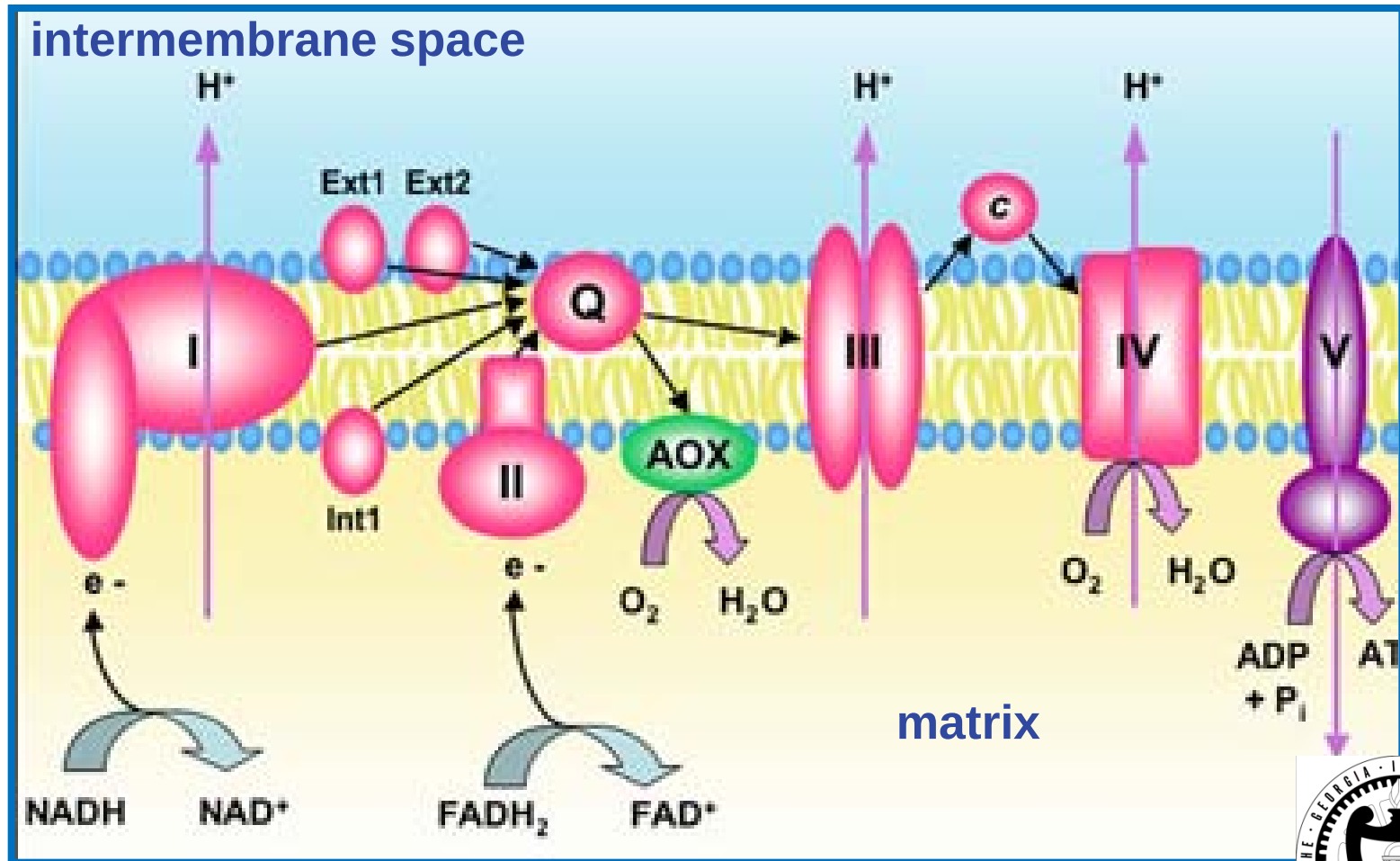
Escherichia coli

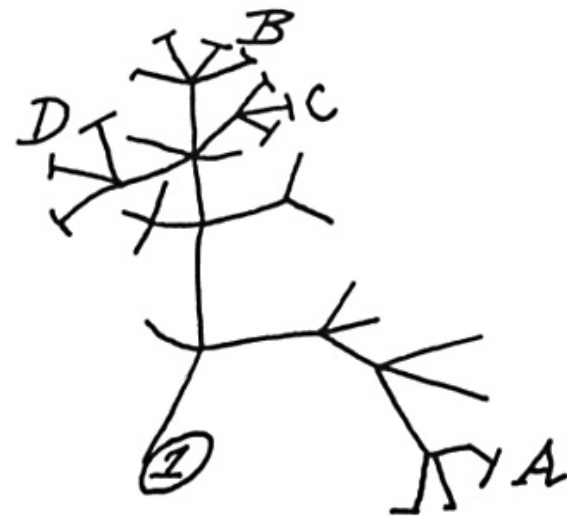
**Georgia Institute of Technology
November 18, 2010**

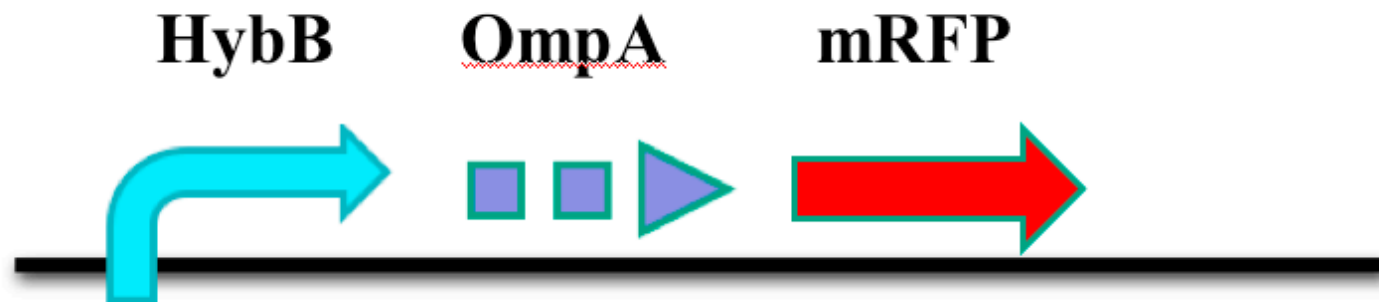
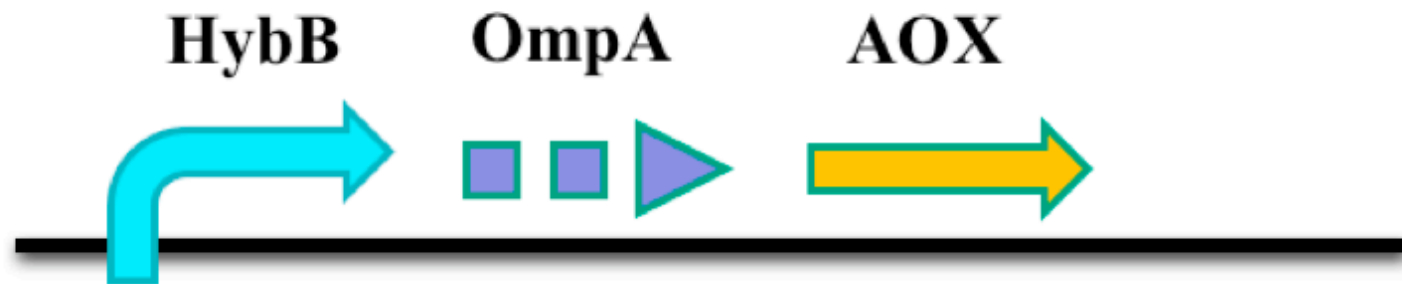


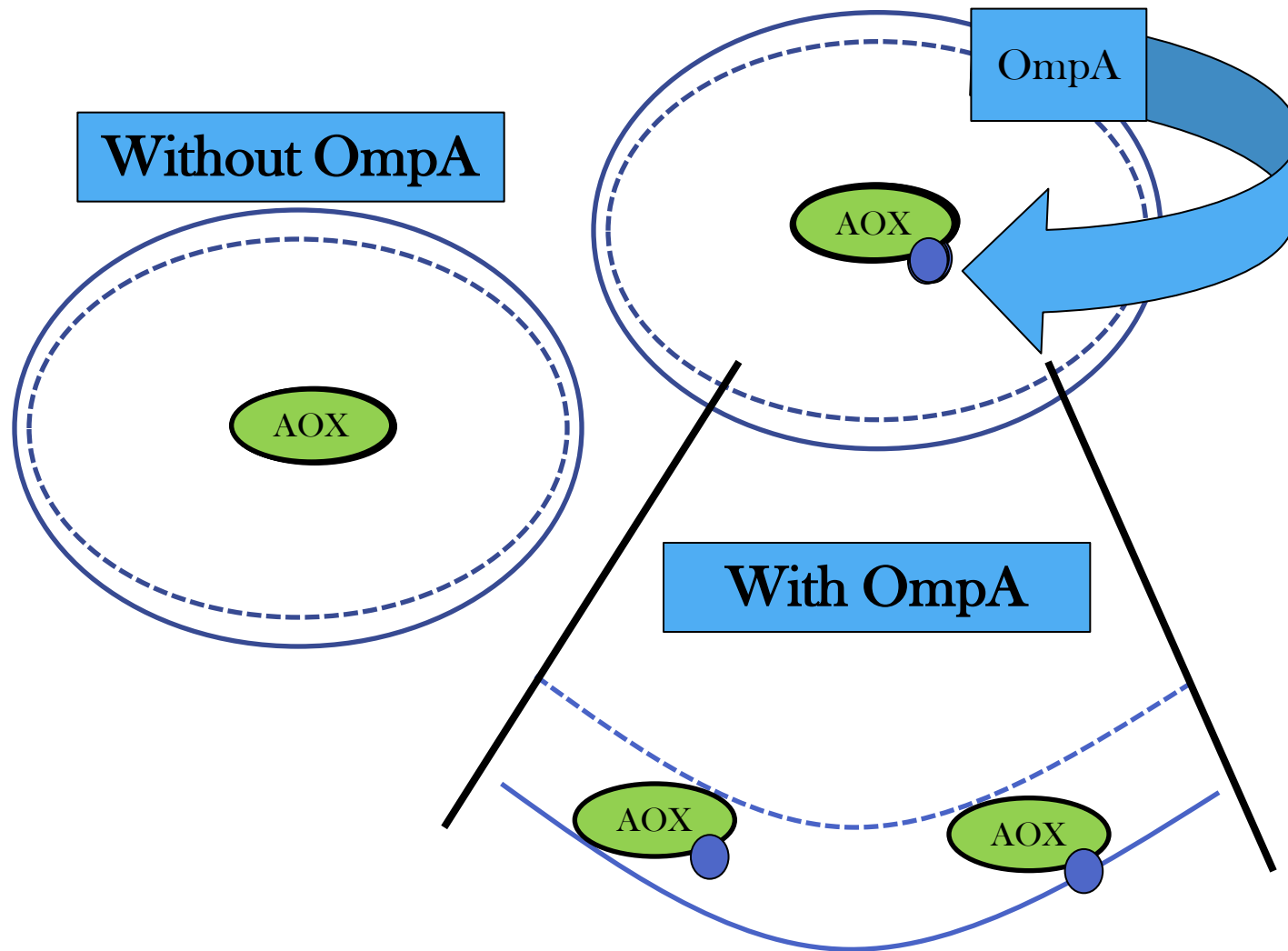


Heat generating gene: AOX

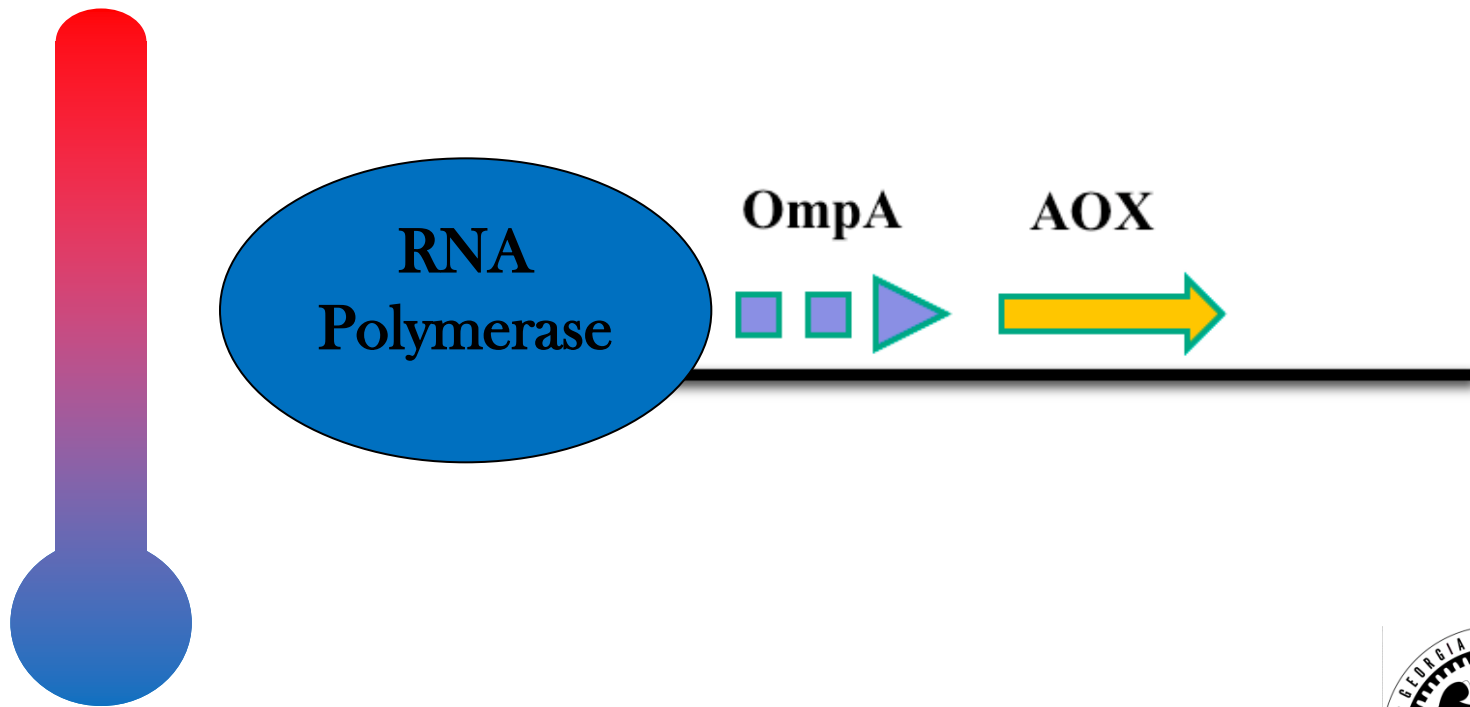








Cold Shock Response

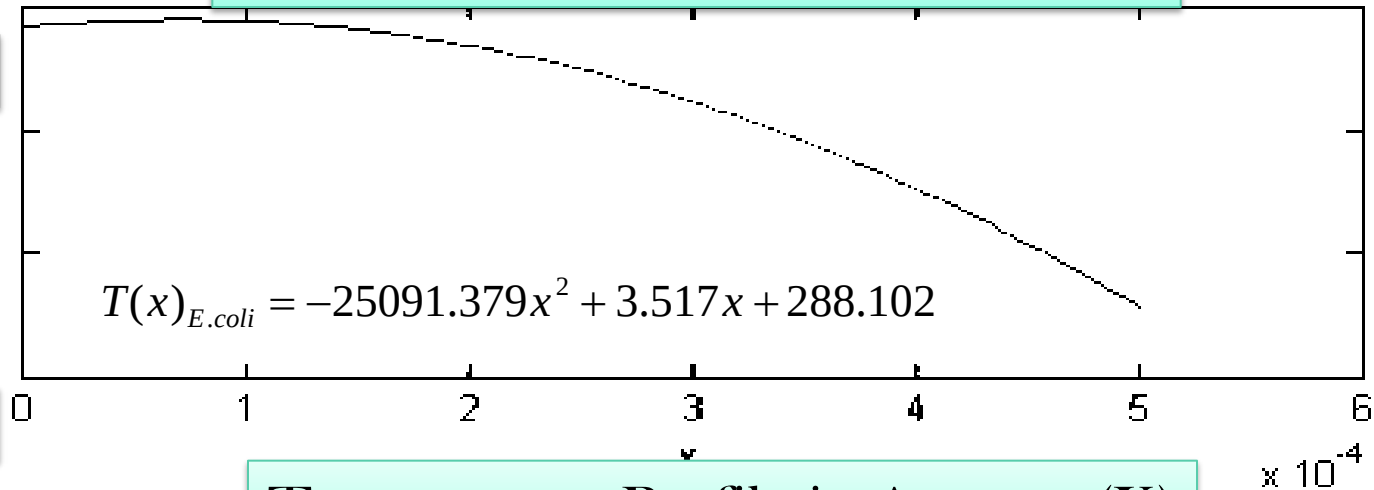


Temperature Profile In E. coli (K)

288.102

288.096

$$T(x)_{E.coli} = -25091.379x^2 + 3.517x + 288.102$$

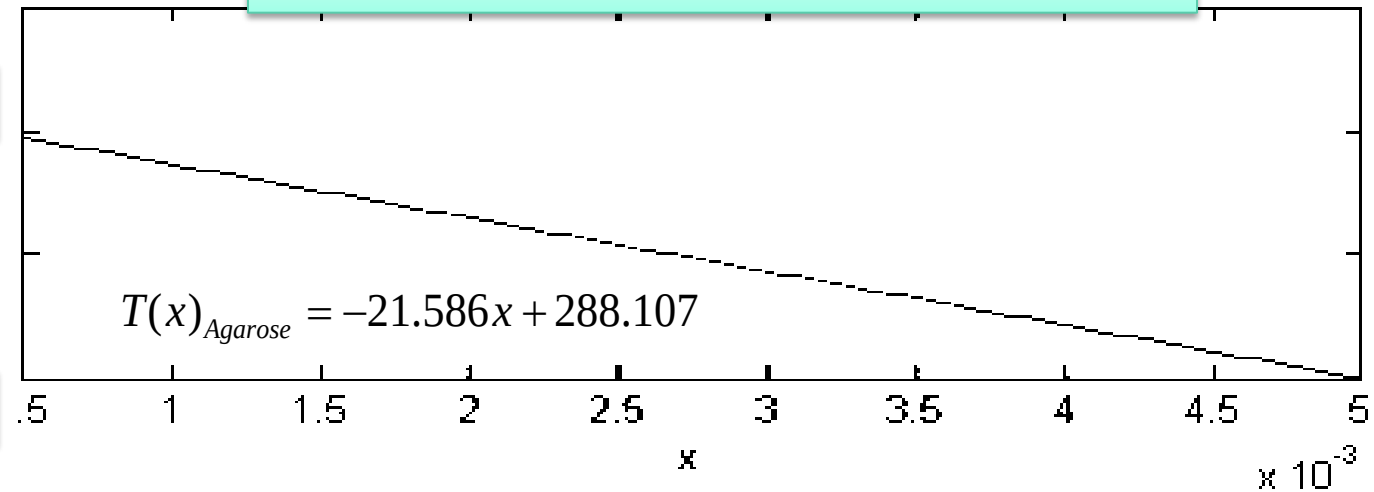


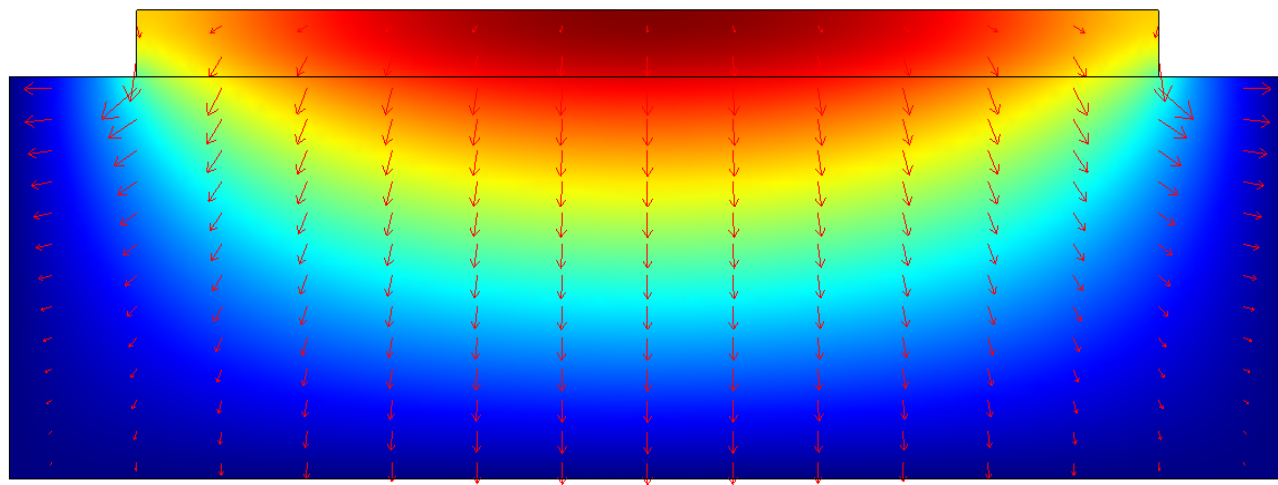
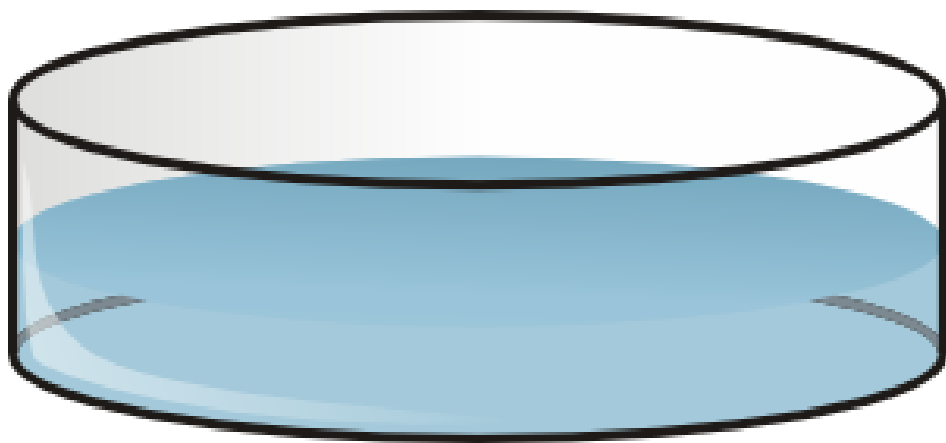
Temperature Profile in Agarose (K)

288.150

288.000

$$T(x)_{Agarose} = -21.586x + 288.107$$

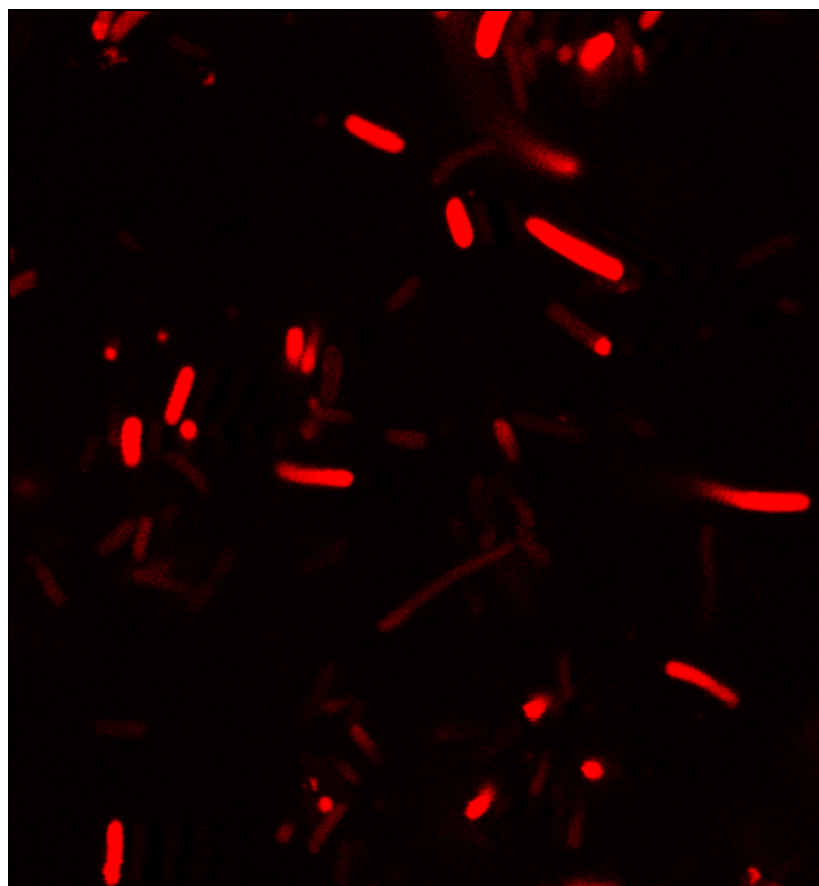




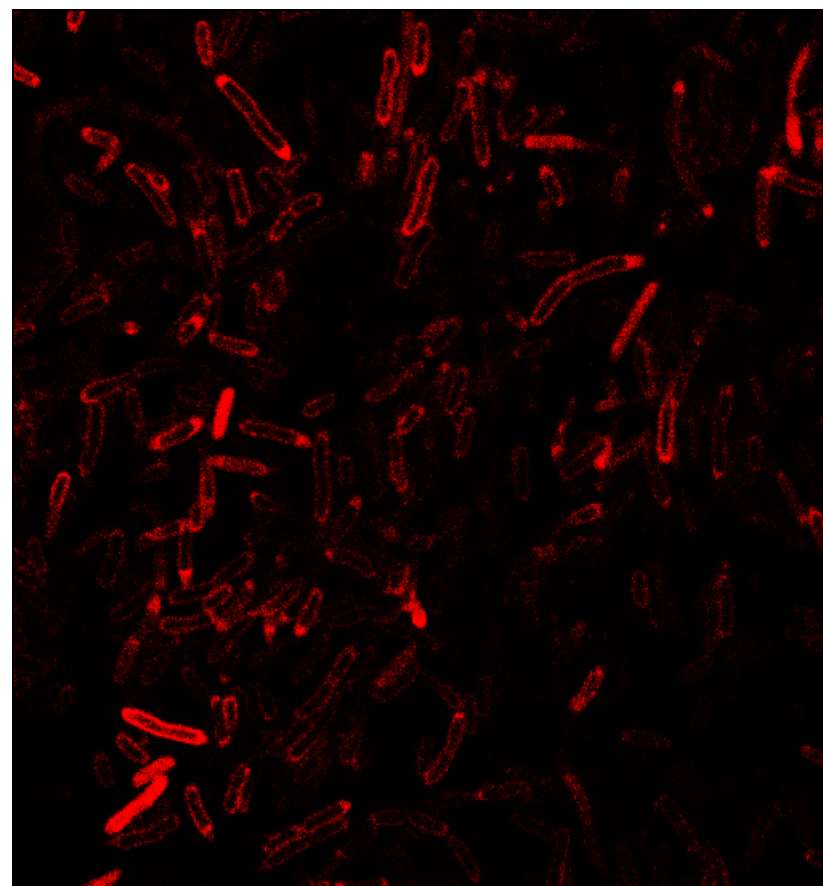
288.065

288.000



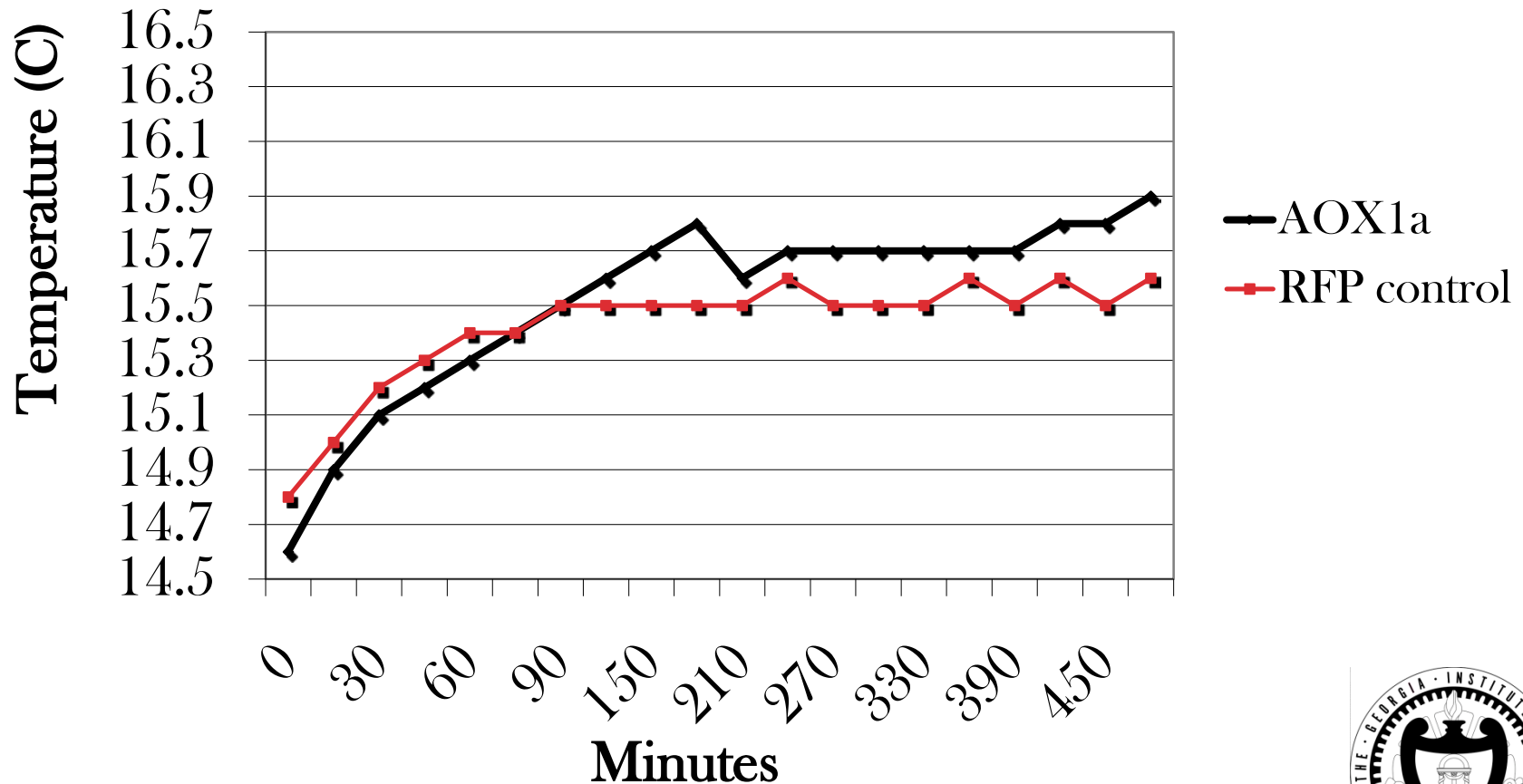


HybB-mRFP

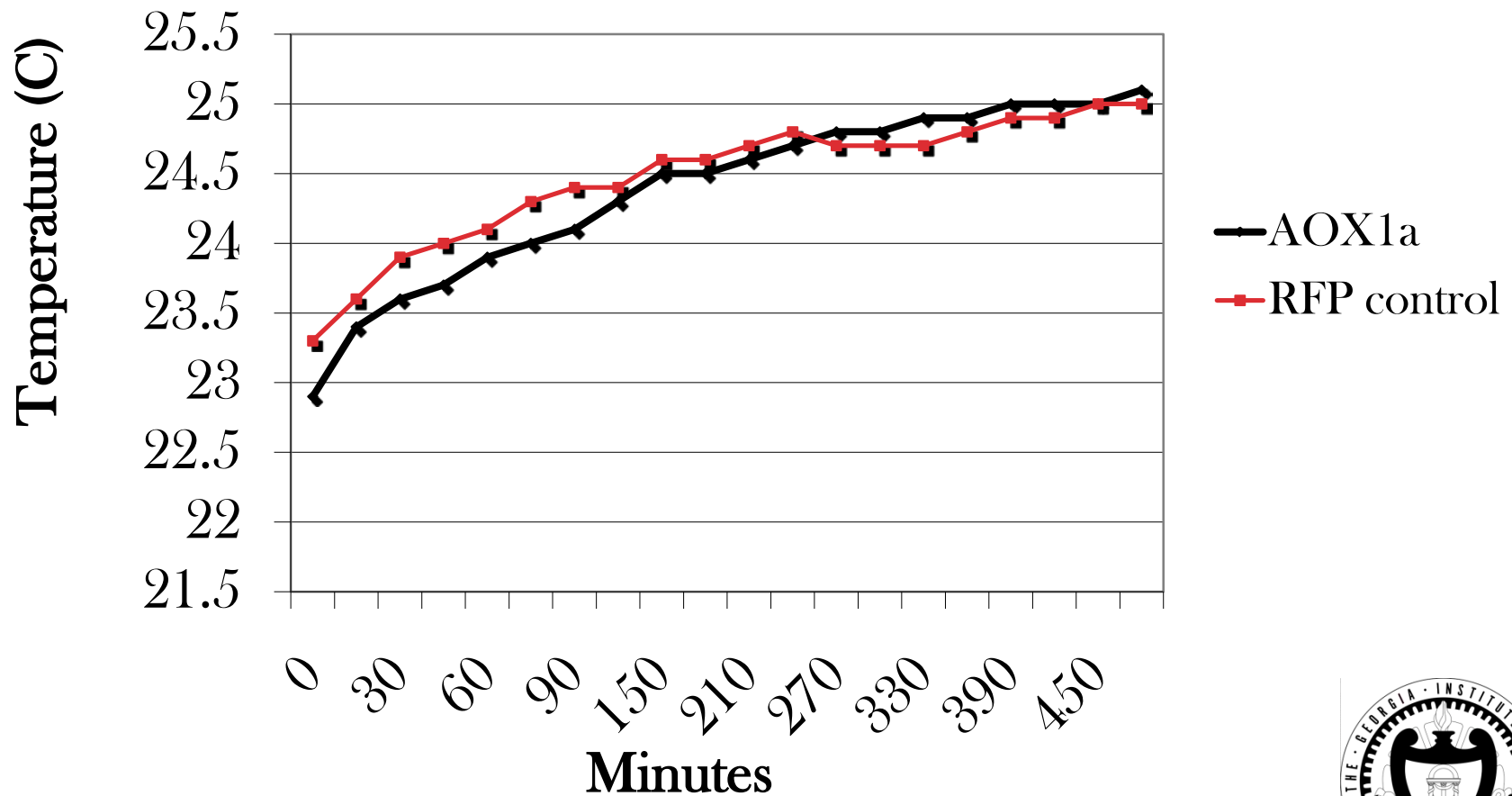


HybB-OmpA-mRFP

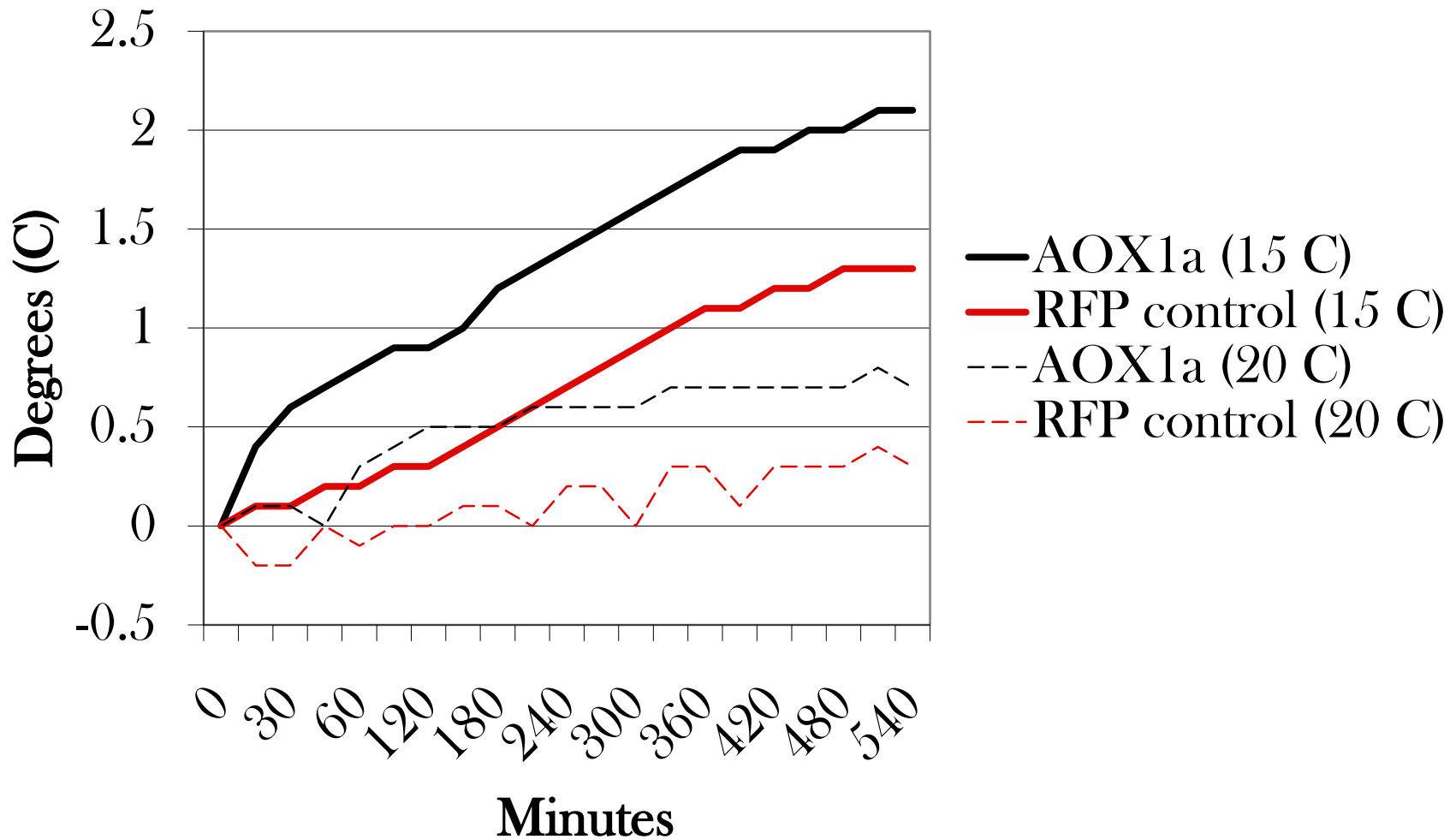
Absolute Temperature by Strain at 15 ° C



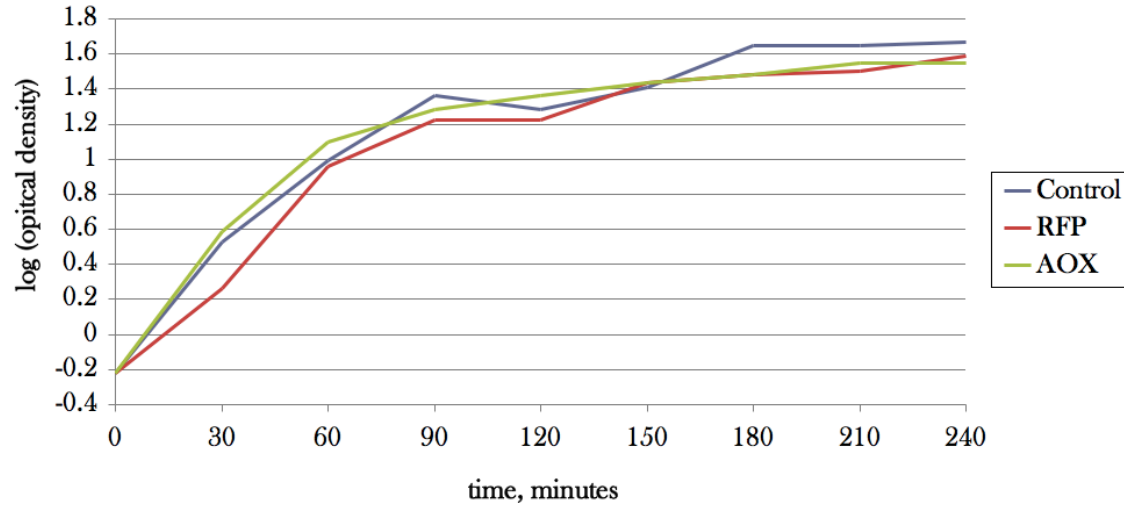
Absolute Temperature by Strain at 25 ° C



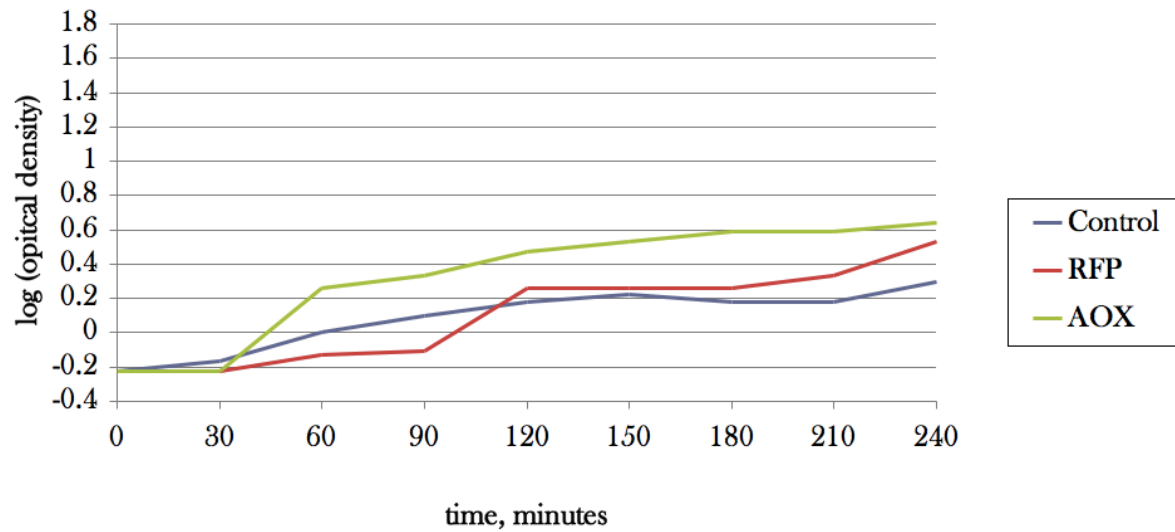
Heat Production Above Baseline After Cold Shock

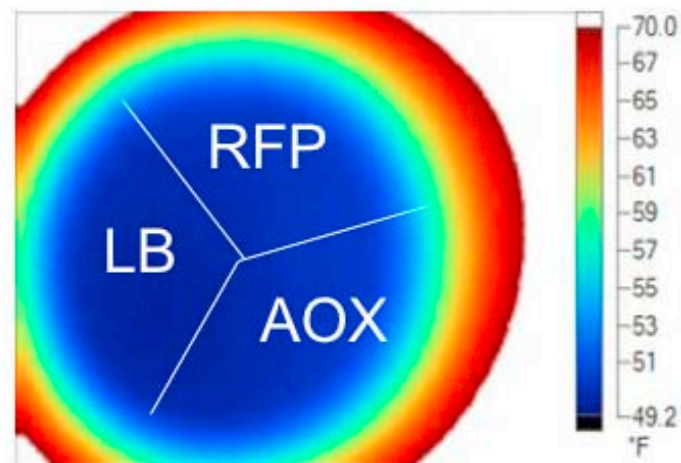
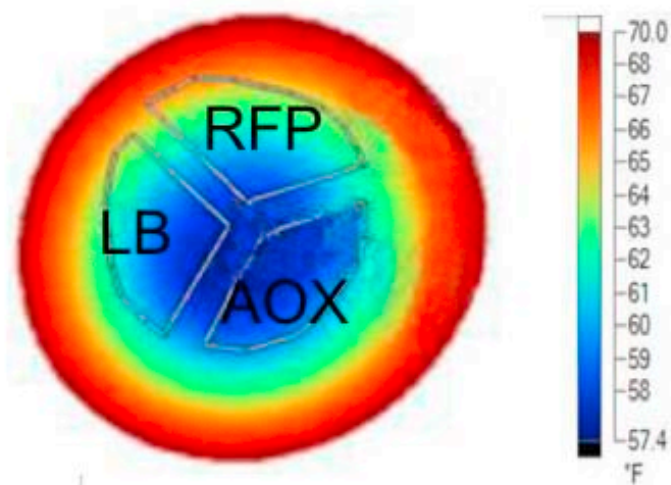
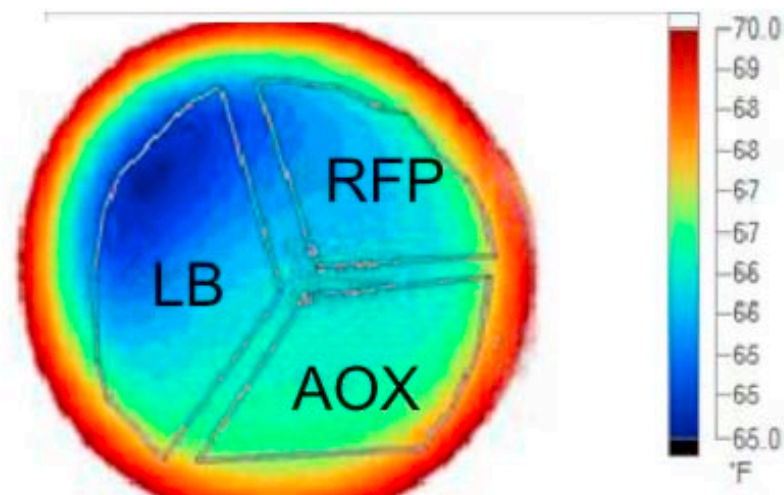
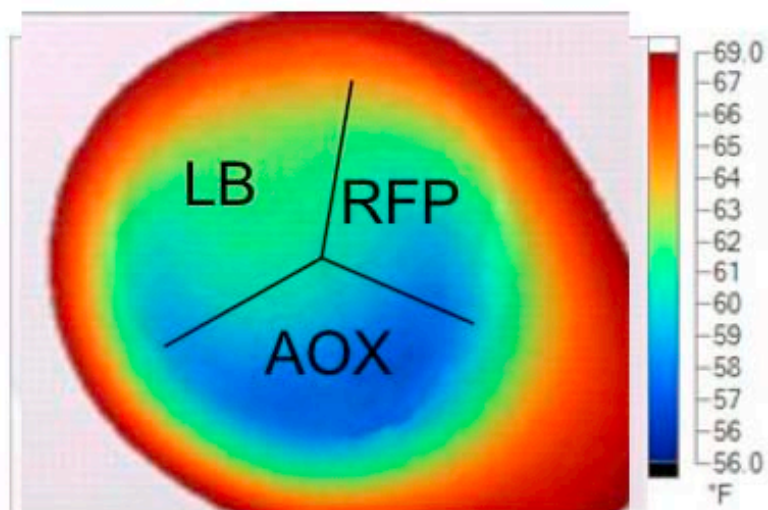


Bacterial Growth at 37° C

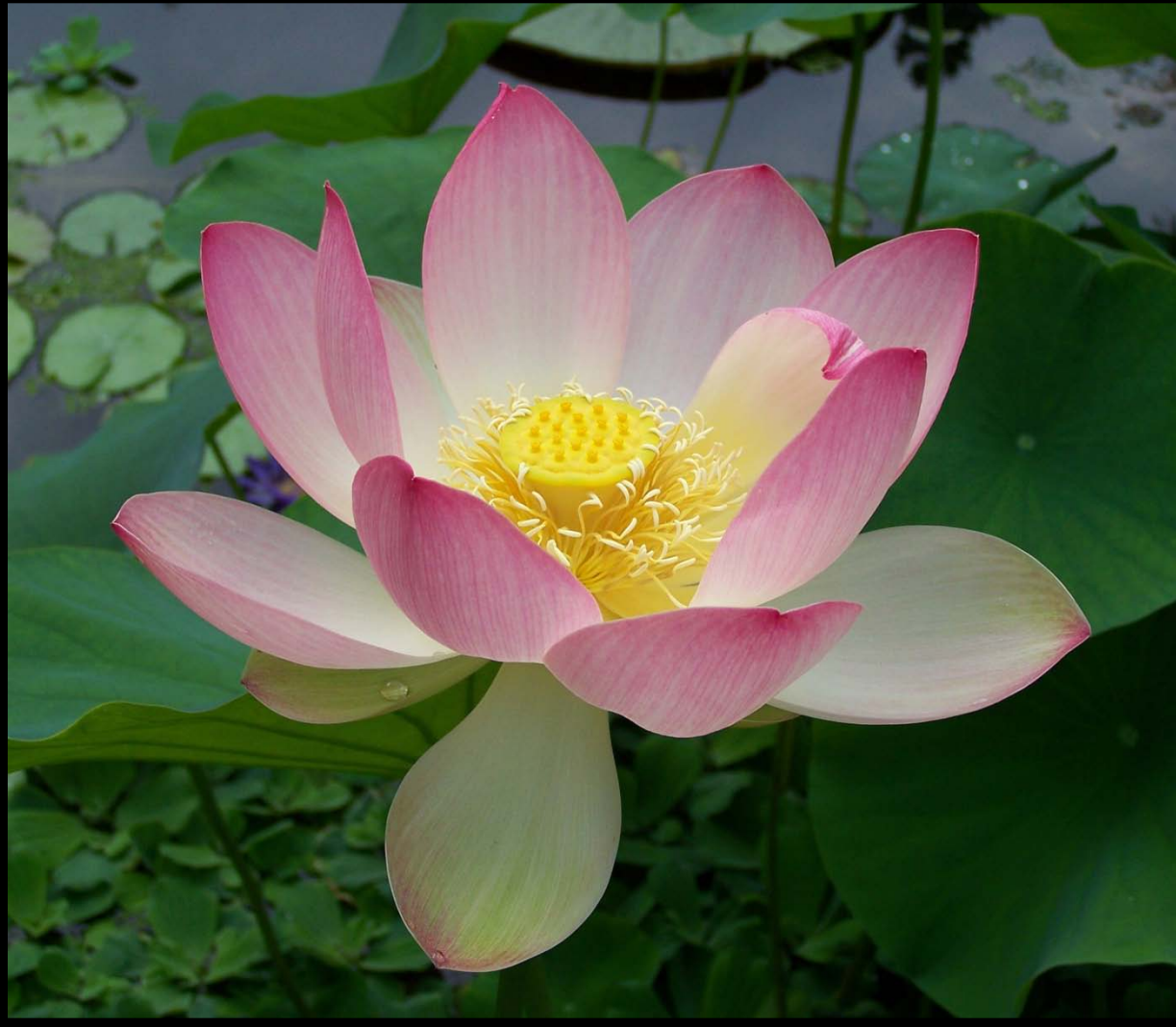


Bacterial Growth at 15° C



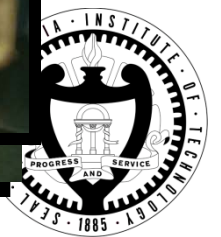


Conclusion



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Inducing a Thermogenic Response to Cold Shock in *E. coli*

Mitesh Agrawal, Margo Clark, Robert Fee, Christina Graves, Alta Hassan, Scott Holmes, Gita Mahmoudabadi, Christian Mandrycky, Debika Mitra, Amy Schwartz, Shadeah Suleiman and Siddharth Tandia

Georgia Institute of Technology, Atlanta, GA, USA



ABSTRACT

Alternative Oxidase (AOX) is known to rewire the electron transport chain (ETC) in thermogenic plants and transfer electrons to exothermic redox reactions. The AOX gene of the same plant was cloned into *E. coli*. The AOX gene was then expressed in *E. coli* and the resulting cells were grown in a liquid culture medium. The AOX gene was then expressed in *E. coli* and the resulting cells were grown in a liquid culture medium. The AOX gene was then expressed in *E. coli* and the resulting cells were grown in a liquid culture medium.

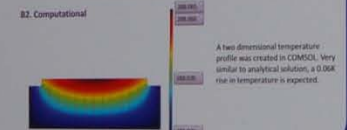
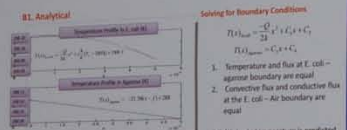
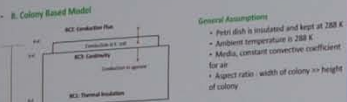
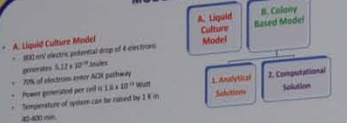
BACKGROUND

The synthesis of ATP during normal respiration is coupled with the flow of electrons through the electron transport chain to a terminal electron acceptor. In addition to this pathway, higher plants contain a pathway resistant to inhibition by rotenone, which transfers electrons from the ETC to oxygen, which is then reduced to water. This pathway is used to generate ATP, free energy in alternative oxidase (AOX) as heat.

METHODOLOGY



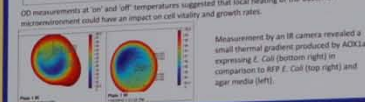
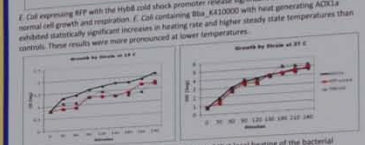
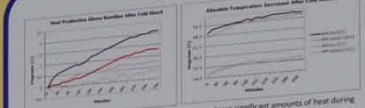
MODELING



TARGETING SIGNAL VERIFICATION



RESULTS



DISCUSSION

The AOX1 gene was successfully cloned into *E. coli*, expressed at low temperatures via the hybrid cold shock promoter, and routed to the bacterial periplasm using a OmpA targeting sequence. Repeated experiments demonstrated faster rates of heating and higher steady state temperatures of 0.2 K over control strains. These effects were more pronounced in colder environments. Increases in temperature agreed closely with derived analytical models for heat output and heat transfer in liquid and solid media.

FUTURE WORK

Growth conditions and cold shock activity in *E. coli* containing AOX could be explored and further optimized to increase heat generation. The post-translational metabolic regulation of AOX in thermogenic plants could be studied to improve understanding of how heating could be maintained for extended periods.

REFERENCES

1. Mitesh, M. et al. (2019) 'Heat Up or Not? That is the Question: Alternative Oxidase in the Thermogenic Plant *Saxifraga hypnifolia*'. *Plant Physiology*, 182, 180-190.
2. Mitesh, M. et al. (2019) 'The alternative oxidase (AOX) is a mitochondrial enzyme involved in plant cell metabolism. The AOX gene was cloned into *E. coli* and the resulting cells were grown in a liquid culture medium. The AOX gene was then expressed in *E. coli* and the resulting cells were grown in a liquid culture medium. The AOX gene was then expressed in *E. coli* and the resulting cells were grown in a liquid culture medium.'

ACKNOWLEDGEMENTS

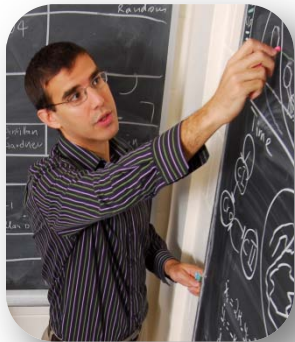
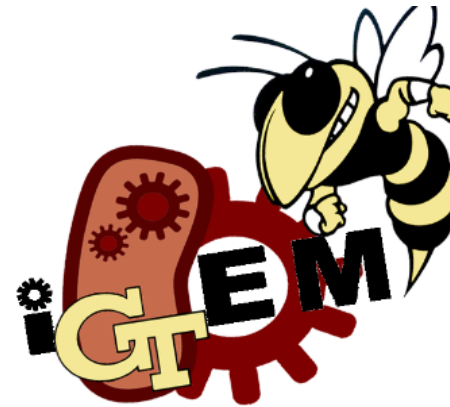
We would like to thank our advisors Dr. Eric Schacht, Dr. Joshua Nink, Dr. Mark McDonald, and our advisors Megan Cole, Michael D. and John, and Ryan Smith.



Dr. Eric Gaucher



Dr. Mark Styczynski

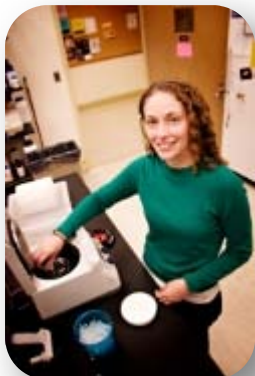


Dr. Joshua Weitz



Ryan Randall

Below: Richard Joh, Scott Holmes, Christian Mandrycky, Sid Tantia, Mitesh Agrawal, Rob Fee, Dr. Mark Styczynski, Shadeah Suleiman, Amy Schwartz, Debika Mitra, Atta Hassan, Monica Huynh, Margo Clark, Christina Graves, Gita Mahmoudabadi [not pictured]



Dr. Megan Cole



Richard Joh

