



NORTHWESTERN UNIVERSITY

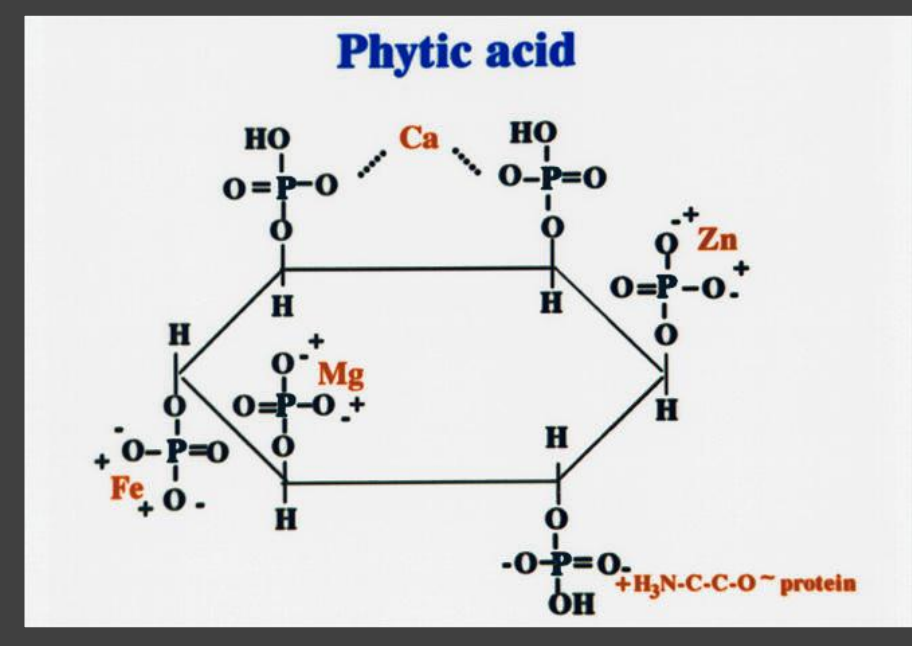
THE *Phytastic* PROBIOTIC: INCREASING THE BIOAVAILABILITY OF NUTRIENTS IN THE DIGESTIVE SYSTEM

Sarah Hartman, Michael Kenton, Grant Nicholas, Lajja Patel, Tae Seong, Brian Tang, Yuan Tao
Advisors: Dr. Michael Jewett, Dr. Joshua Leonard, Dr. John Mordacq, Dr. Keith Tyo, Jennifer Schoborg



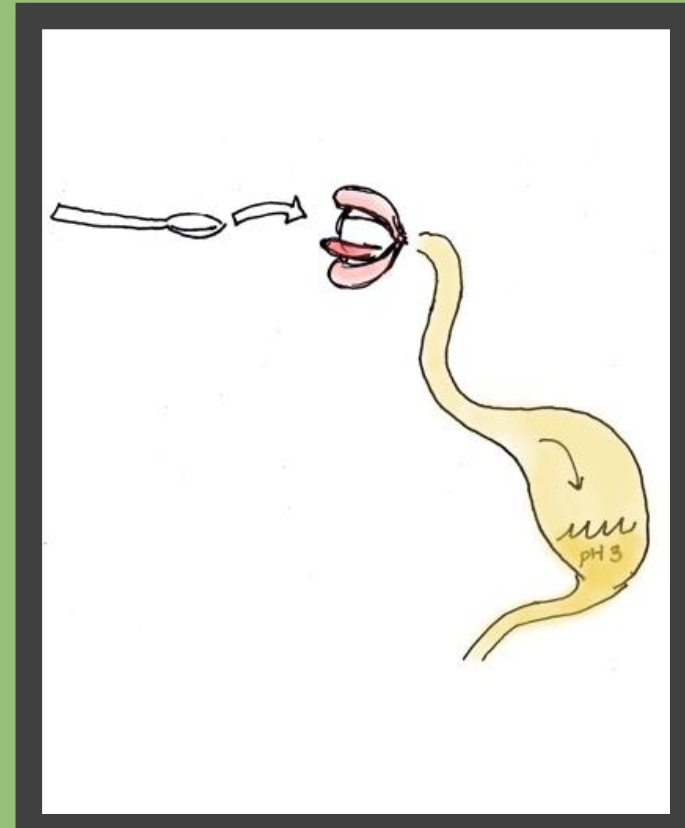
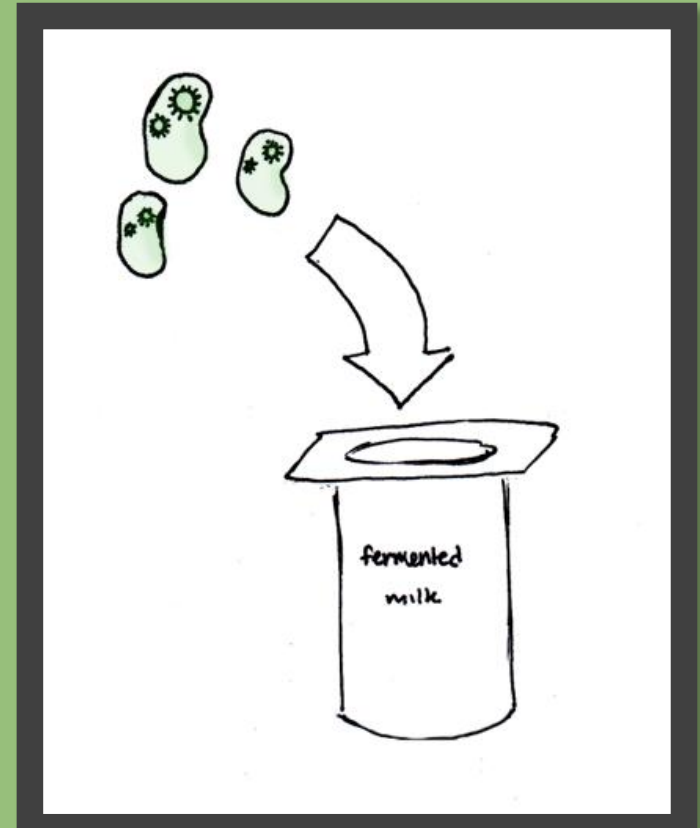
PROBLEM

- Iron deficiency affects 2 billion people, or over 30% of the world's population.
- Deficient populations may not lack in intake of iron, but rather lack in the intake of *bioavailable* iron.
- Phytic acid is a prevalent chelator of iron and other nutrients in food, rendering them unavailable for absorption.



IMPLEMENTATION

- Hydrolyze phytic acid with phytase, releasing chelated nutrients.
- Develop novel phytase delivery system utilizing fermented milk products native to the culture.



PHYTASE SYNTHESIS

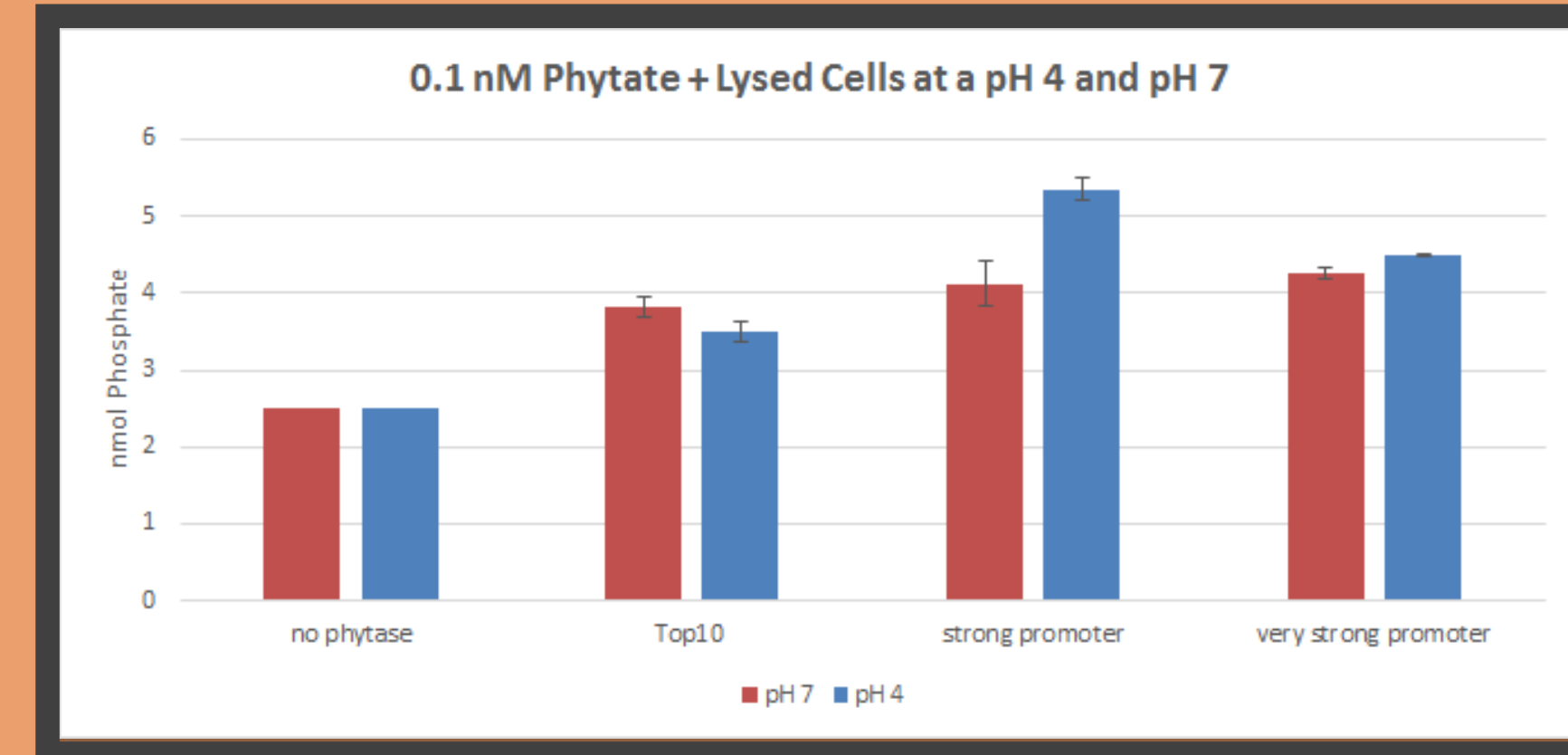
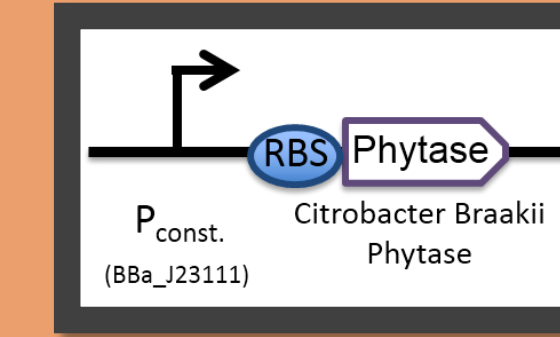
- Clone four different types of phytase from four different organisms: *Escherichia coli*, *Aspergillus niger*, *Bacillus subtilis*, and *Citrobacter braakii*.

PROTEIN RELEASE

- Engineer a probiotic that lyses and releases pre-synthesized phytase only when the probiotic is exposed to the acidic environment of the stomach (pH ~1.35-3.5)

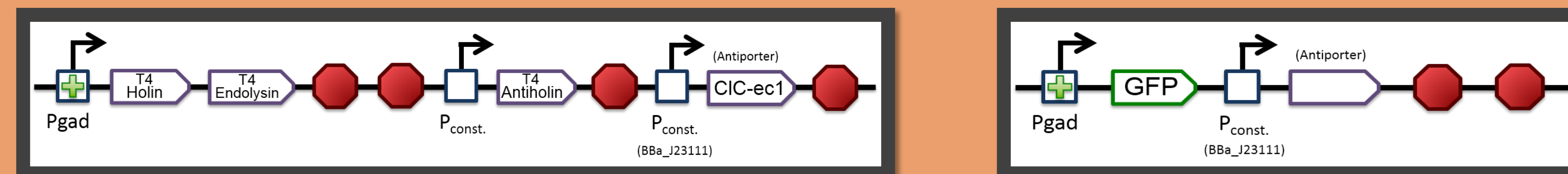
PHYTASE SYSTEM

- Citrobacter* phytase grown in *E. coli* liberated phosphate from the phytic acid solution.

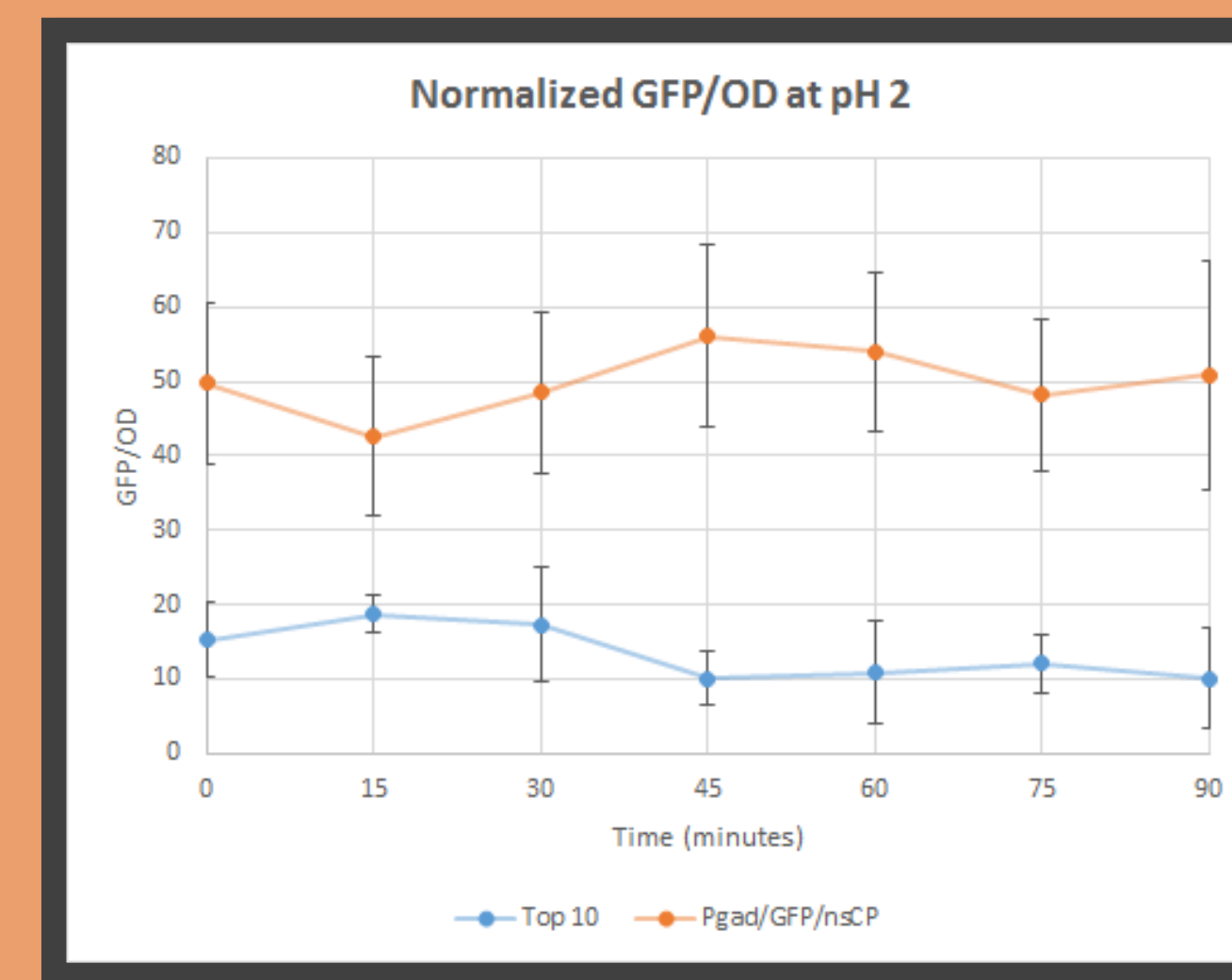
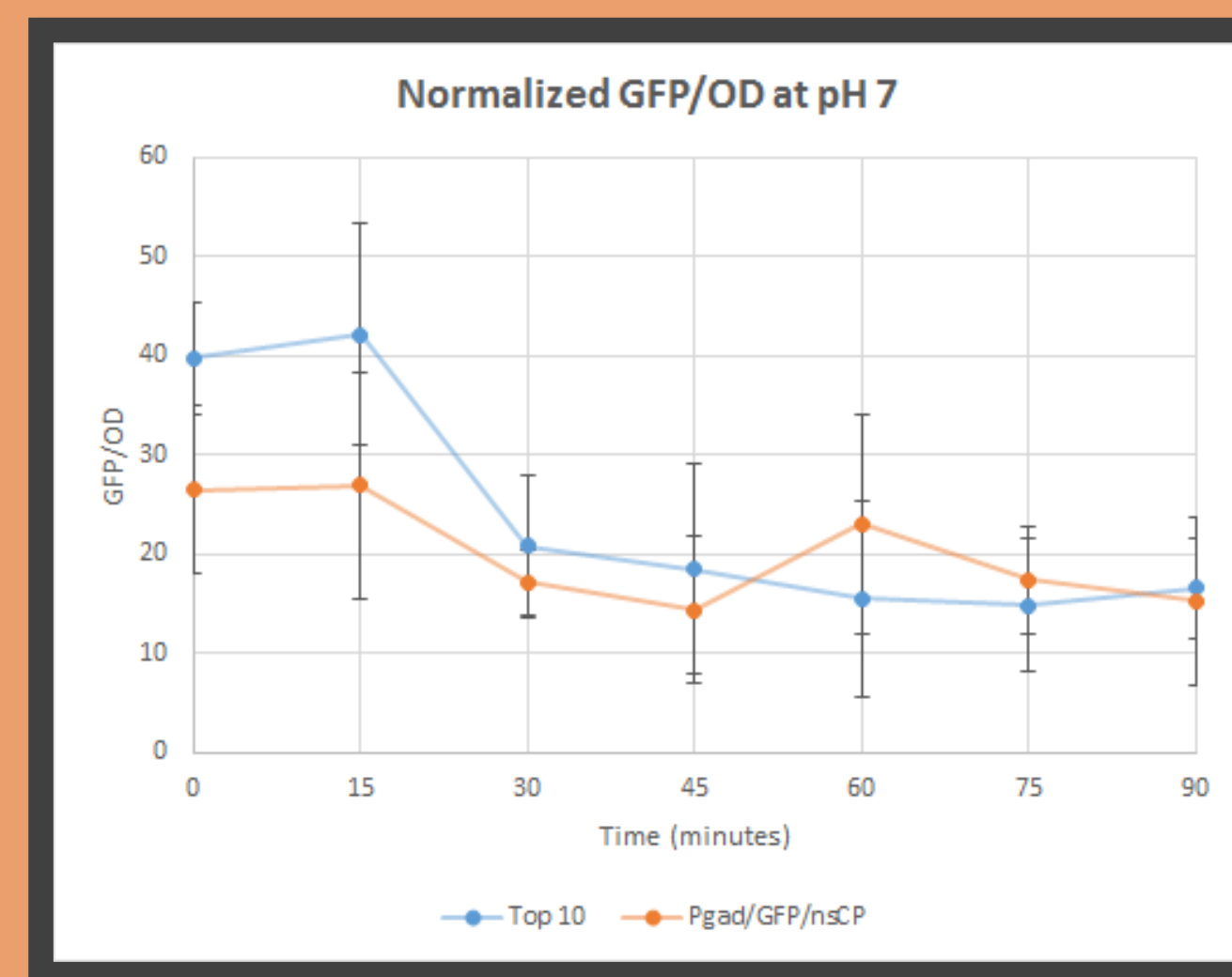


0.1 nM phytic acid was added to the sonicated cell lysate at both a pH of 7 and a pH of 4.5. At pH 4.5, lysed cells with phytase system demonstrate a higher phytase activity due to higher amount of free phosphate cut from the phytate.

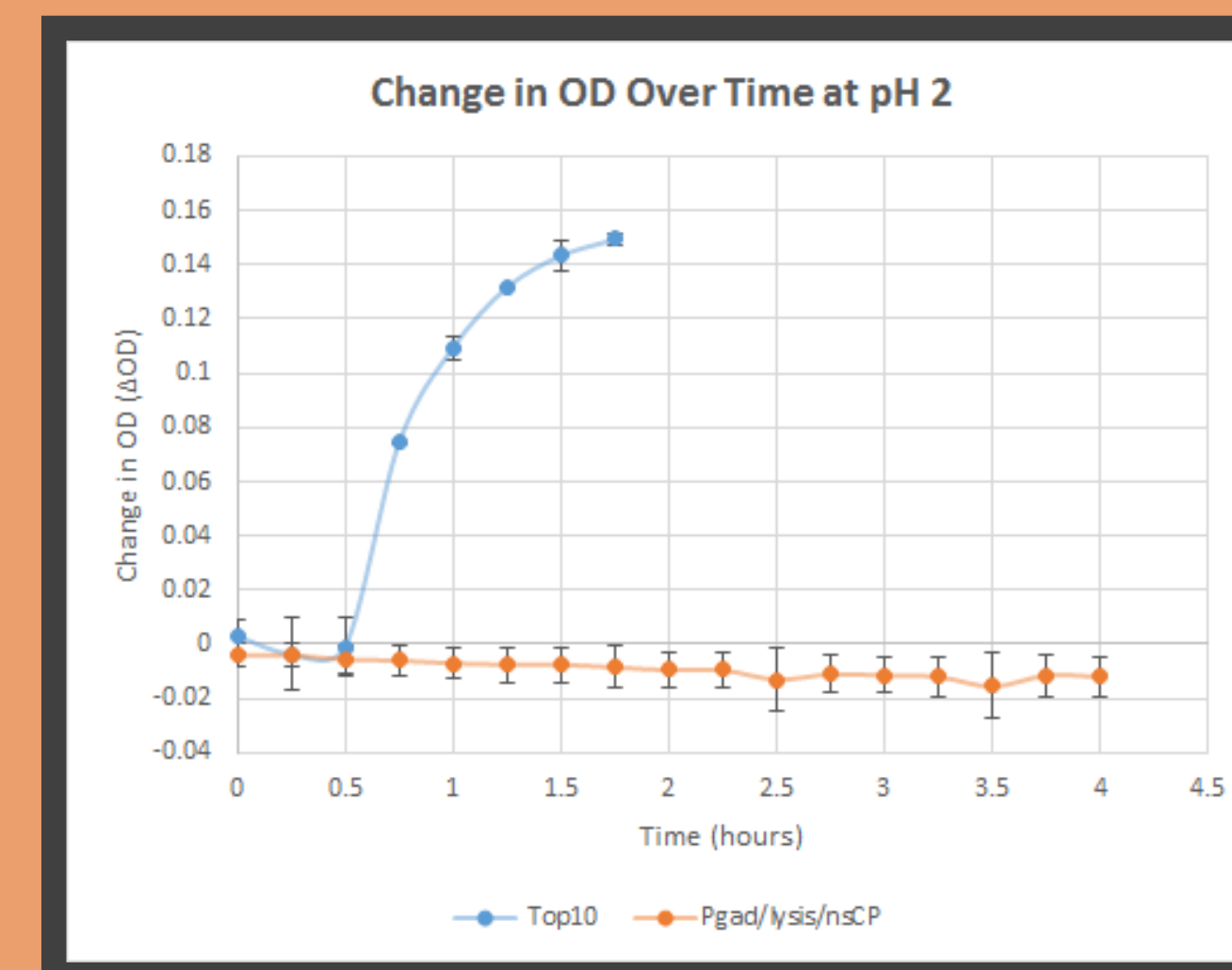
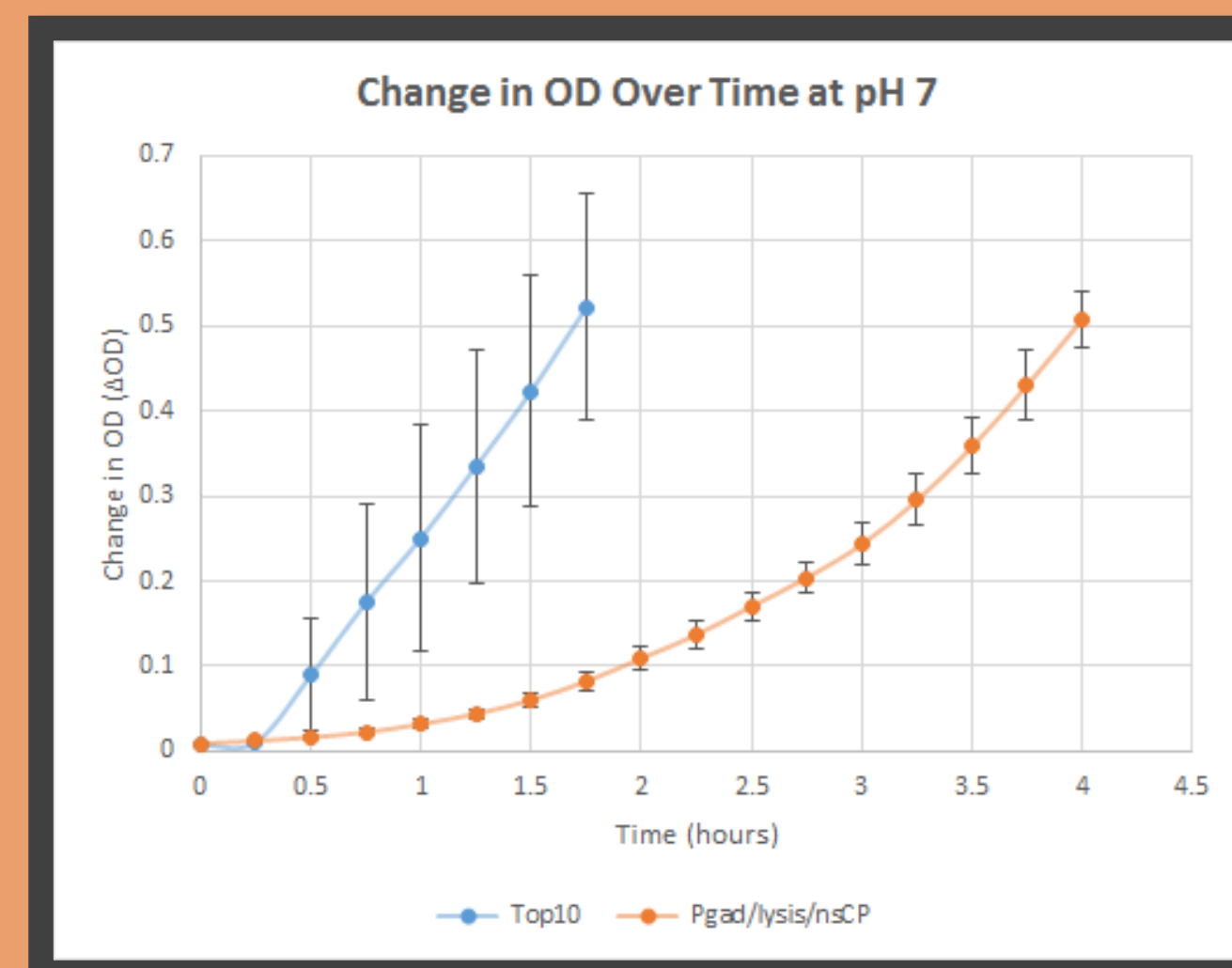
pH SYSTEM



- CIC antiporter imports chloride ions in exchange for protons.
- P_gad promoter is induced when chloride concentration rises.



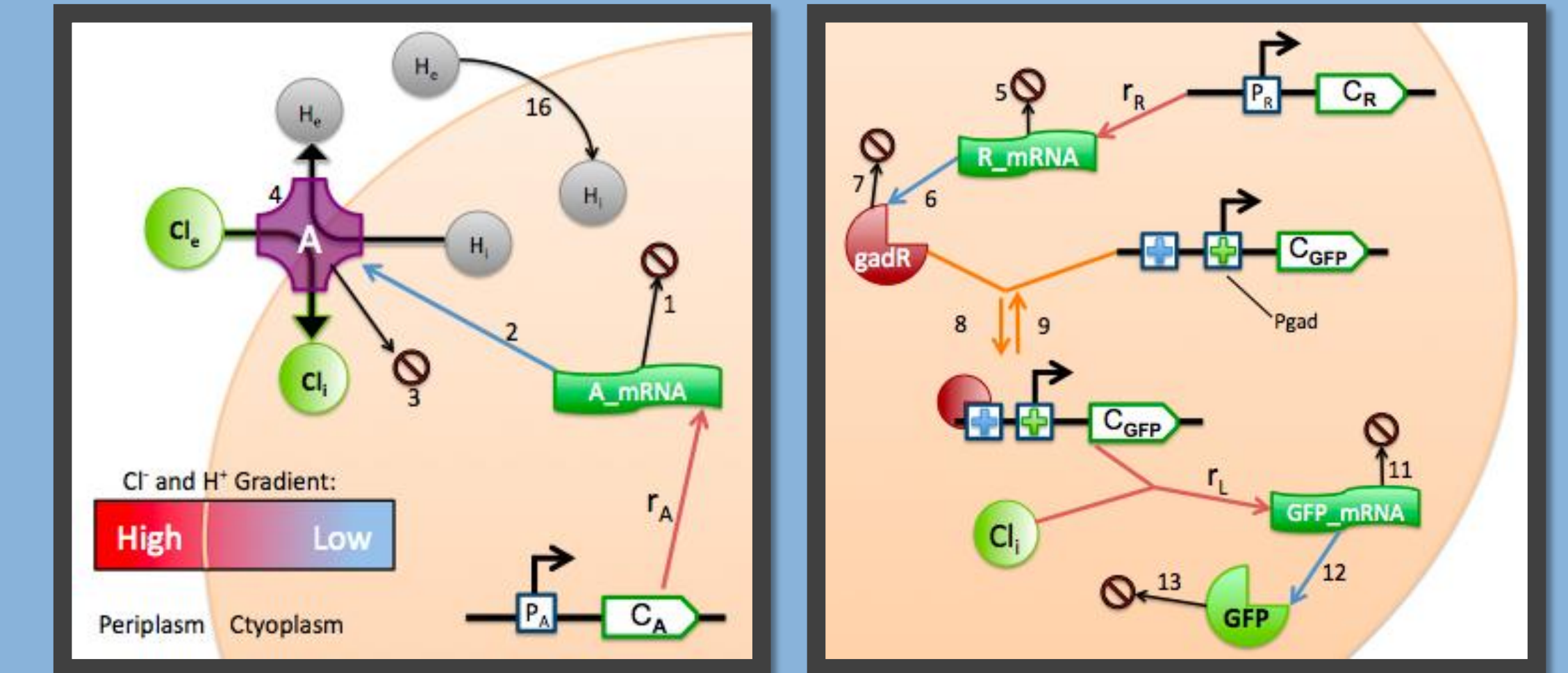
At pH 7, cells with the pH-inducible system have the same fluorescence as top10 cells (control). At pH 2, the cells have a higher fluorescence than the control.



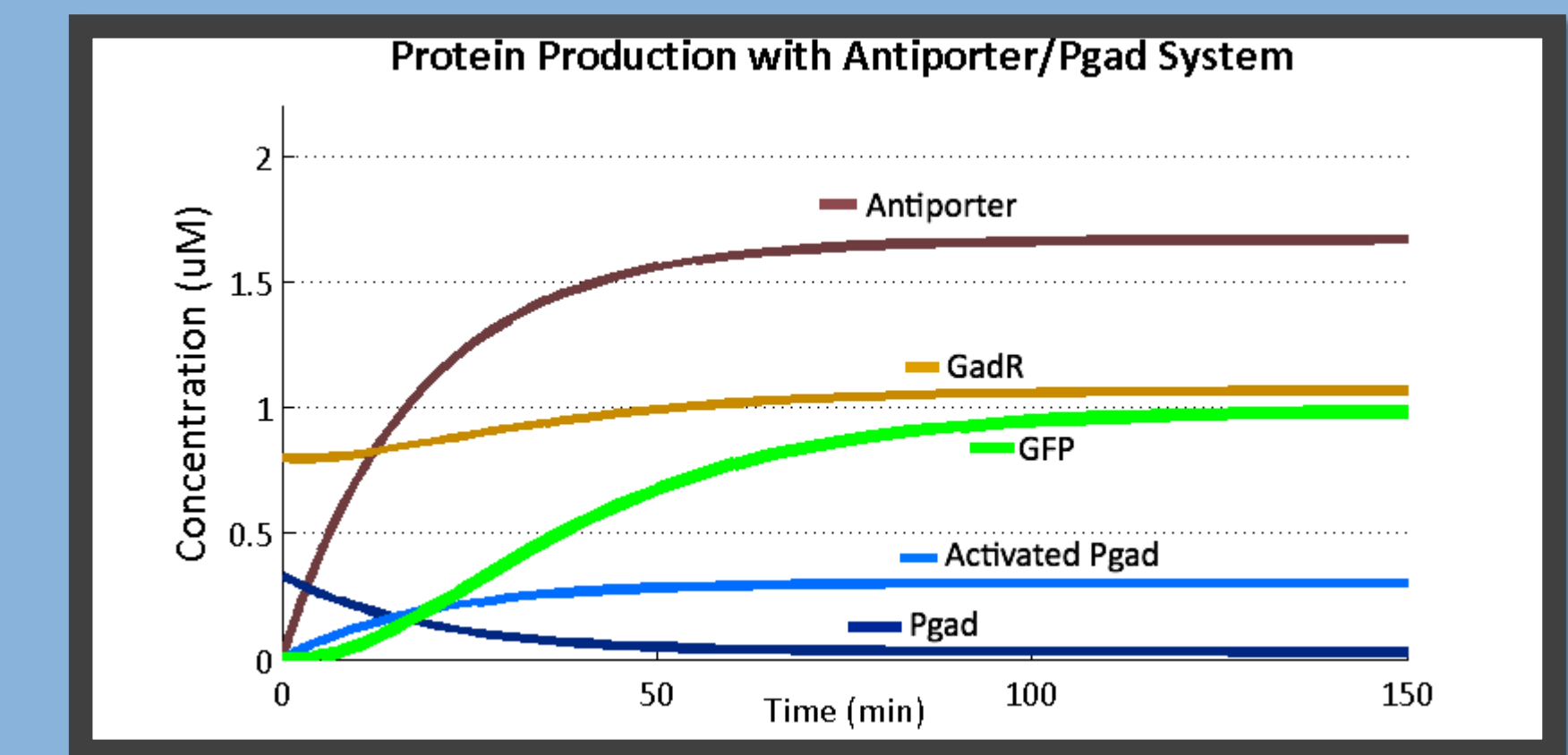
At pH 7, cells with the pH-inducible system grow considerably slower than control. At pH 2, the cell population noticeably decreases in comparison to the control.

MODELING

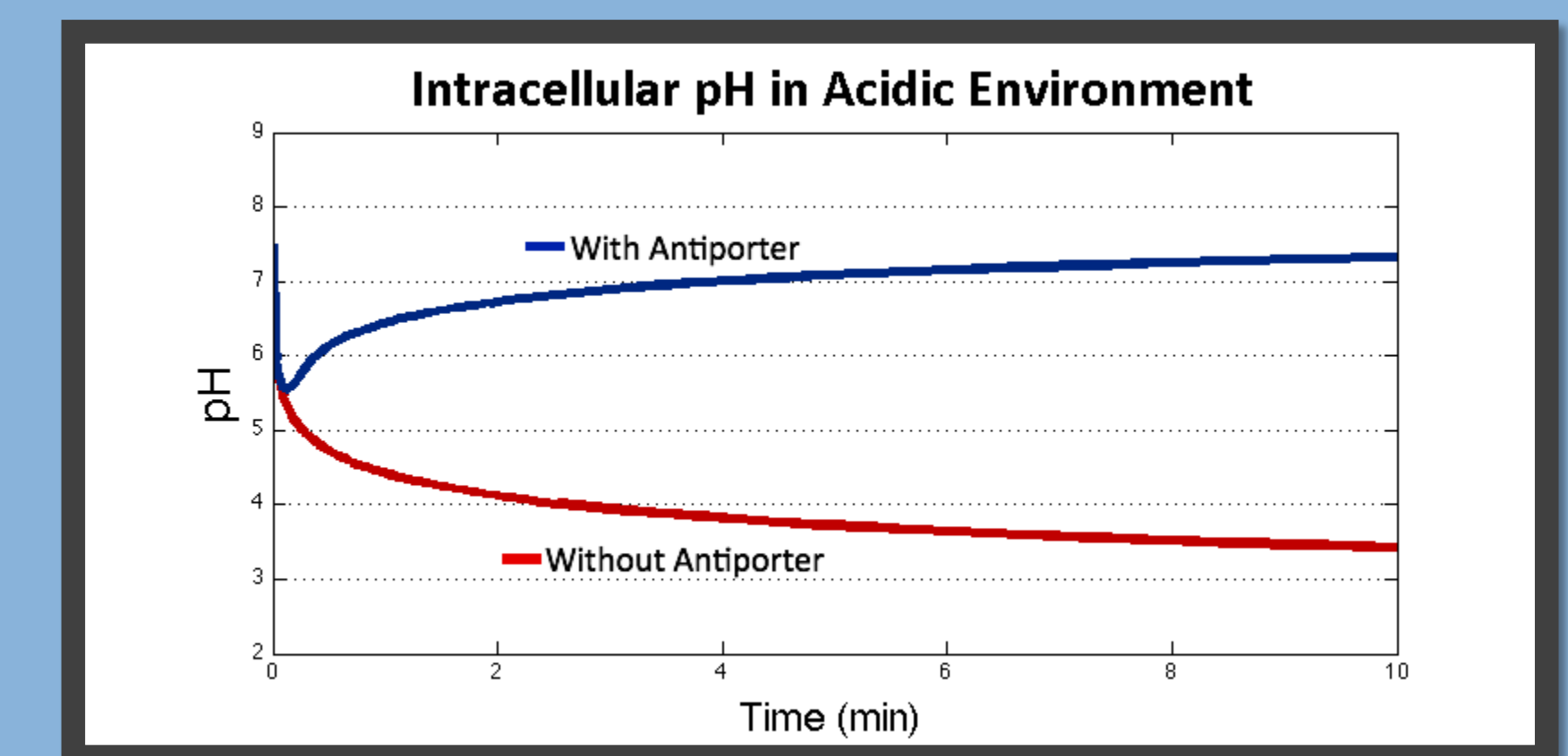
- Designed a model that simulates *Phytastic* cells entering the stomach to examine the plausibility of using the system for nutritional purposes.



System of ODEs was created using species from the antiporter (left) and P_gad/GFP (right) components.



GFP concentration rises as chloride levels increase and P_gad becomes activated. GFP reaches target concentration (1 μM) within 2.5 hours.



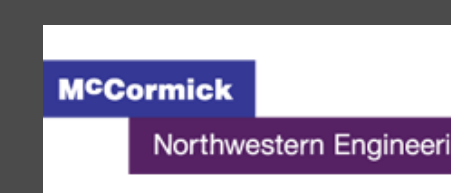
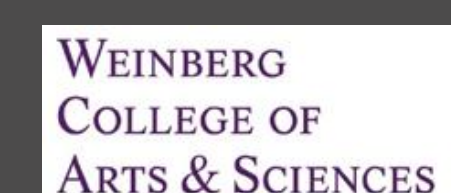
Intracellular pH is affected by H⁺ influx and its transport out of the cytoplasm by the antiporter. The model shows cells quickly responding to dropping intracellular pH. Stability is restored within minutes.

CONCLUSION

- Phytase system produces phytase within the cell.
- Releasing the phytase demonstrates significant phytase activity.
- pH-inducible system demonstrates strong induction of both GFP and lysing of cells when introduced to a low-pH environment

ACKNOWLEDGEMENTS

Special thanks to the Department of Biological Sciences for lab space and materials, as well as the graduate students of the Jewett Lab for their constant assistance and willingness to take time to address our questions and concerns. Finally, thanks to the 2011 Hong Kong-CUHK team for taking time to send us a part they characterized that was not available in the Parts Registry!



CITATIONS

Phytic acid chelation image: Syed S. Sohail and David A. Roland, Sr., Fabulous Phytase: Phytase Enzyme Proving Helpful to Poultry Producers and Environment. Highlights of Agricultural Research, 1999 46.
Kefir image: Fermented Milk. Discover the Incredible Health Benefits of Kefir. 11 October 2012. <http://products.mercola.com/body-ecology/kefir.htm>