



# A System for the Expression and Control of Appetite Regulation Protein in *E.coli*

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## Abstract

Appetite can shape a person's life, from body composition to mood, yet the means for controlling appetite are very limited...

The 2010 Citadel-Charleston iGEM team invites you to join us as we explore a novel healthcare solution to obesity and weight-related ailments: influencing the desire to eat through an engineered strain of intestinal microflora. Our team has focused research and development on Peptide Tyrosine Tyrosine (PYY), a peptide which could permit microorganisms native to the gastrointestinal tract to interact with the central nervous system and manipulate the perception of hunger. The Citadel-Charleston Team has worked to express the PYY<sub>3-36</sub> peptide in *E.coli* and to ensure that its expression is strictly controlled by means of a cellular population limit.

## PYY<sub>3-36</sub>

PYY<sub>3-36</sub> is an endogenous hormone of the ileum and colon released in proportion to the calories consumed during a meal. The peptide can be absorbed into the circulatory system and pass the blood-brain barrier where it acts as a primary contributor to energy homeostasis. By exerting agonist action on the NPY Y2-receptor in the arcuate nucleus of the hypothalamus, PYY<sub>3-36</sub> causes an inhibition of gastric motility and a substantial reduction in appetite.

The goal of The Citadel iGEM team was to construct a RFC10-complaint Biobrick part that encoded the protein PYY<sub>3-36</sub> and could be successfully integrated into genetic circuits in the chassis *E.coli*. Furthermore, we hoped to show that systems incorporating Biobrick protein coding sequences such as PYY<sub>3-36</sub> could be designed for *E.coli* that would have application potential as novel, safe, and potent means for influencing neurological processes such as appetite from across the brain-gut axis.

The Citadel team has succeeded in formulating a system for the expression and regulation of PYY<sub>3-36</sub> in *E.coli*. We have designed a Biobrick part (K373000) that encodes the protein of interest. This part will be made available in the Registry of Standard Biological Parts.

## Population Control Circuit

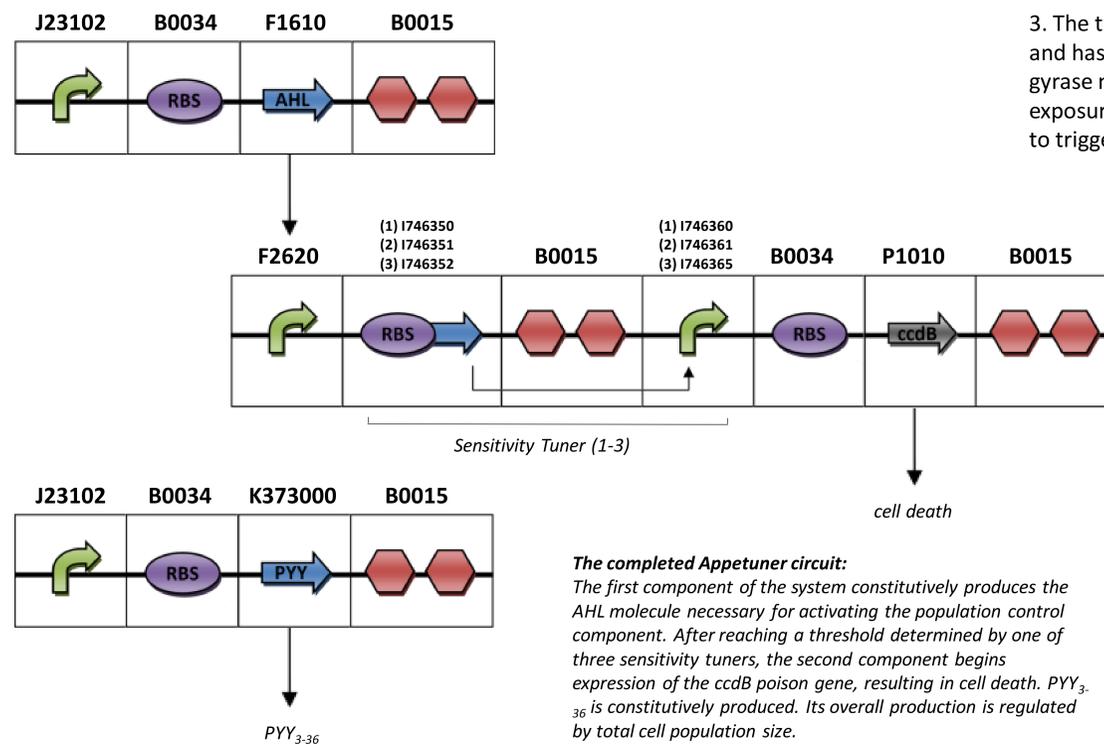
For PYY<sub>3-36</sub> to be an effective candidate for microbial supplementation and use in a brain-gut appetite control system it would need to be under strict regulation. Overproduction of the peptide should be prevented by a secondary control module. This new module would work to maintain a specific, desirable level of PYY<sub>3-36</sub> production. Our control module is based on the programmed population control model developed by You *et al.* (2). We designed a poison-generating module that becomes activated only once a colony of cells has reached such a density that additional PYY<sub>3-36</sub> production is not desired.

Three key features of the control module make a population limit possible:

1. The first feature is inter-cellular communication, or quorum sensing. The N-Acyl Homoserine Lactone (AHL) family of molecules is produced constitutively by all cells in the colony. These molecules accumulate within the cell and diffuse into the extracellular space. As the colony grows, the concentration gradient is reversed, and uptake of AHL into the cells dominates. In this way, AHL acts as a broadcasting system for the density of the colony as a whole.
2. The second feature is a sensitivity tuner. Thanks to work done previously by past Cambridge iGEM teams, the Registry now boasts a set of parts designed to fine-tune the level at which transcription of a given downstream sequence can begin. When coupled with the AHL inter-cellular communication system described above, these parts allow the designer to construct a device which sets a precise threshold marker for population size, the attainment of which triggers the transcription of downstream sequences in the circuit.
3. The third feature is a cell poison. The CcdB gene was selected for this purpose as it is lethal when expressed in *E.coli* and has been shown to be effective at population control in current research. The peptide operates by rendering DNA gyrase nonfunctional. Once the translation of CcdB has begun, the colony size begins to decrease as cells die from exposure to the poison. The cell population continues to drop until it is below the density level producing enough AHL to trigger transcription of the poison downstream of the sensitivity tuner.

The whole system, consisting of the primary and control modules, allows the population to cycle. The colony grows until it passes the threshold, then decreases in density until poison production is cut off. This oscillatory period has been shown in previous research to dampen as the cycles continue, leveling out along the "switch point" where CcdB is expressed.

Due to time constraints and the natural hurdles encountered by a group of undergraduate students and professors new to biological engineering and the iGEM competition, The Citadel team has not completed construction of the target circuit. We hope to continue this work in the future or to inspire others to extend research with PYY<sub>3-36</sub>.



## References

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## Acknowledgements

This project was supported by The Citadel's Office of the Provost and Department of Biology.