SPEED

Sejal Dhawan, Theresa Gibney, Ethan Jones, Christine Li, Wukun Liu, Alyssa Luz-Ricca, Callan Monette, Cedar Ren, CiCi Zheng, Gregory D. Conradi Smith*, Margret Saha**

Department of Applied Science*, Department of Biology**, College of William & Mary, Williamsburg, VA

INTRODUCTION

In order to provide a launching pad for future work on dynamic circuits, we created a **simple, modular and predictable** degradation based system for the control of gene expression speed using an E. coli orthogonal Lon protease and associated protein degradation tags (pdt).

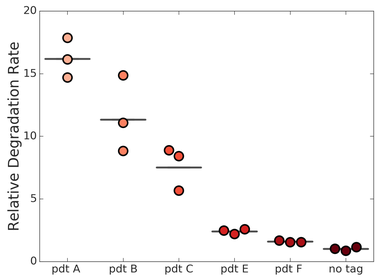
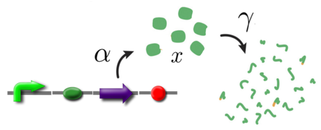


Fig. 1: Observed relative degradation rates of our pdt tagged inducible constructs (BBa_K2333427-33) at steady state (ss). Each data point represents the geometric mean of at least 10,000 single cell measurements taken by FACS

SIMPLE KINETIC MODEL

Consider a model where: X is an inducible gene which, once activated, produces **protein x** at a rate α and degrades protein *x* at a rate γ.

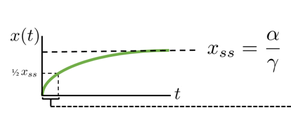


$$\frac{dx}{dt} = \alpha - \gamma x$$

Then the following differential equation represents [x] at a given time *t:*

$$x(t) = \frac{\alpha}{\gamma}\left(1 - \frac{1}{e^{\gamma t}}\right)$$

We can then determine that [x] at steady state will be α / γ, and that the time it takes for [x] to reach half of that value ($\tau_{1/2}$) is $\ln(2)/\gamma$, and thus determined by γ alone.



$$x_{ss} = \frac{\alpha}{\gamma}$$

$$\tau_{1/2} = \frac{\ln(2)}{\gamma}$$

SPEED

We increased the speed of our characterization constructs (Fig. 2), and found that the speed increase relative to degradation rate aligned with our predictions (Fig. 3)

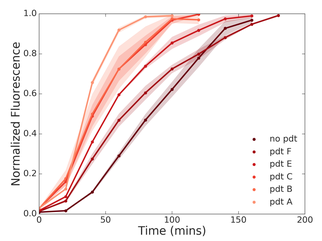


Fig 2: Time course FACS measurements of inducible constructs (as Fig 1.). Data points are the geometric mean of three biological replicates normalized to steady state (max fluorescence). Time points after steady state not shown. Shading represents geometric std deviation

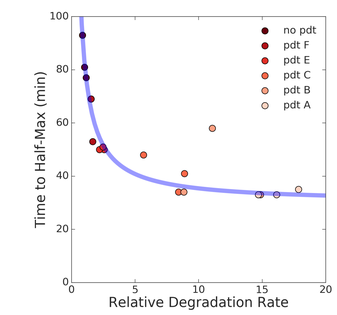


Fig 3. A comparison between the relative degradation rates at steady state and $\tau_{1/2}$ (from Fig 2)

READJUSTMENT

Since $x_{ss} = \frac{\alpha}{\gamma}$, then we should be able to increase α to maintain x_{ss} without changing speed.

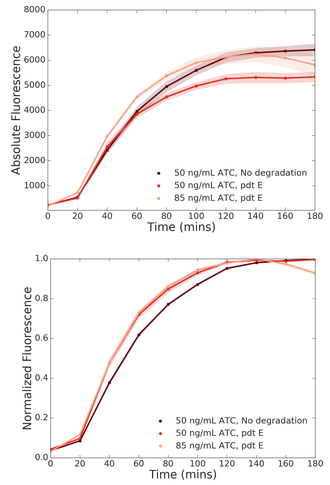


Fig 4: Raw (top) and ss normalized (bottom) fluorescence measurements of our characterization constructs at original (50ng/mL aTc) and increased production parameters (85ng/mL aTc). Data collected as (Fig 1 and 2).

PROOF OF CONCEPT

We collaborated with UMaryland iGEM to increase the response speed of their copper detector

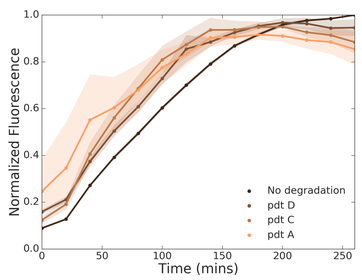
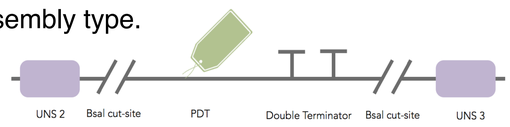


Fig 6: Time course measurements of modified copper sensing parts (Bba_K2333437-42). Parts were created by adding different strength pdts onto the existing circuit, just as any future team using this system would. Data collected and displayed as Fig 1. and 2

CLONING PARTS

To help future teams, we created *E. coli* codon-optimized cloning-ready protein degradation tags that can be used for one-step cloning with any assembly type.



DYNAMIC CONTROL

To demonstrate that our system could create dynamic circuits, we constructed and tuned an incoherent feed forward loop (IFFL), which generates a pulsatile output.

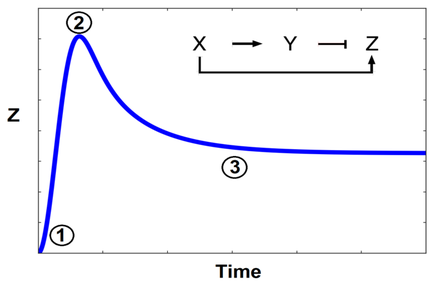


Fig 7: Schematic of the architecture of an IFFL. An IFFL's pulsatile output depends on the activation of Z by X (1) before a sufficient amount of protein Y is generated to have a repressive effect (2), at which point the inhibition and activation of Z balance out to a steady state (3).

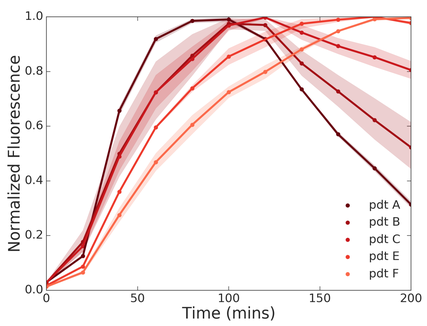


Fig 8: Creating and tuning the sharpness of our IFFL's pulse behavior. Time course FACS measurements of inducible constructs in an IFFL regime. Methods and constructs as Fig. 2.

MODELING

To enable multi protein use, we developed a model that accounts for protease loading and saturation effects and fit it to our data by using Bayesian parameter estimation with MCMC.

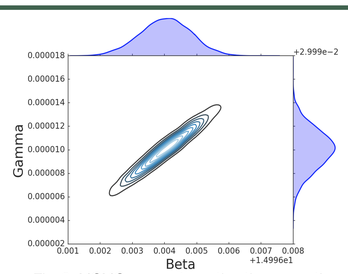
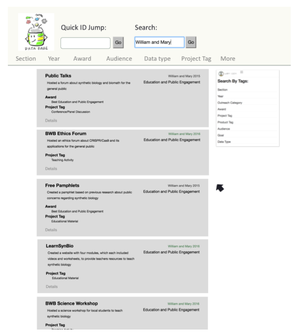


Fig. 9: MCMC parameter estimation correctly estimates simulated values for both beta and gamma (15 and .03)., and identifies a strong positive correlation between beta and gamma.

OUTREACH



We created a searchable outreach database with the projects of gold medal teams from the past two years. Each of the 1,439 entries is standardized for ease of use, and organized by a number of parameters for search-ability.

Attributions

All experiments, cloning, planning, design and other work was done by student members of the team unless explicitly indicated otherwise
We'd like to thank all of our advisors and everyone else who supported us along the way, including:

Dr. Margaret Saha, PI: For her general support and her endless dedication and enthusiasm to our team
Dr. Gregory Smith, Co-PI: For invaluable math modeling guidance
John Marken, PhD student, and Andy Halleran, PhD student: For their help with data analysis and visualization, and invaluable insights
Dr. Eric Bradley: For his tireless assistance with lab facilities and management
Dr. Dennis Manos, Vice Provost of Research: For providing us with necessary financial and intellectual support

Financial Support

Dr. Dennis Manos, Vice Provost of Research
Dean Kate Conley, Dean of the Faculty, Arts and Sciences
Howard Hughes Medical Institute Science Education Grant to the College of William and Mary
GenScript
Epoch Life Science Inc.
Integrated DNA Technologies

References:

Introduction and pdt system:
- Eyal Gur and Robert T Sauer. Evolution of the ssra degradation tag in mycoplasma: specificity switch to a different protease. Proceedings of the National Academy of Sciences, 105(42):16113– 16118, 2008.
- D Ewen Cameron and James J Collins. Tunable protein degradation in bacteria. Nature biotechnology, 32(12):1276–1281, 20
Modeling and circuit design:
- Shmoolik Mangan and Uri Alon. Structure and function of the feed-forward loop network motif. Proceedings of the National Academy of Sciences, 100(21):11980–11985, 2003.
- Uri Alon. An introduction to systems biology: design principles of biological circuits. CRC press, 2006
- Gene images adapted from Newcastle iGEM 2010 and Cameron and Collins (above)