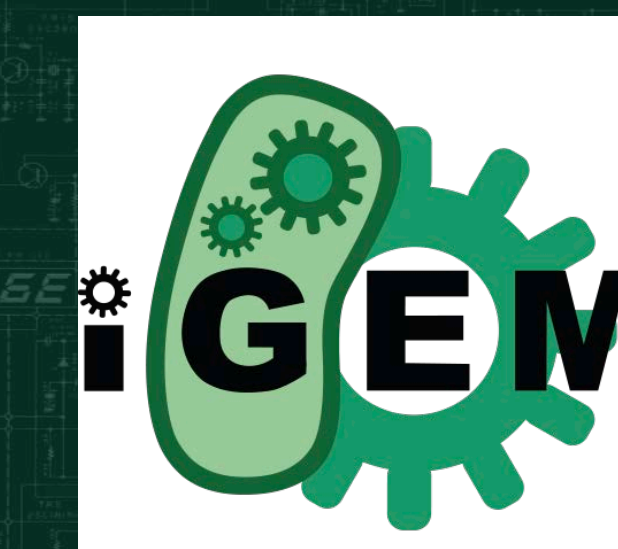




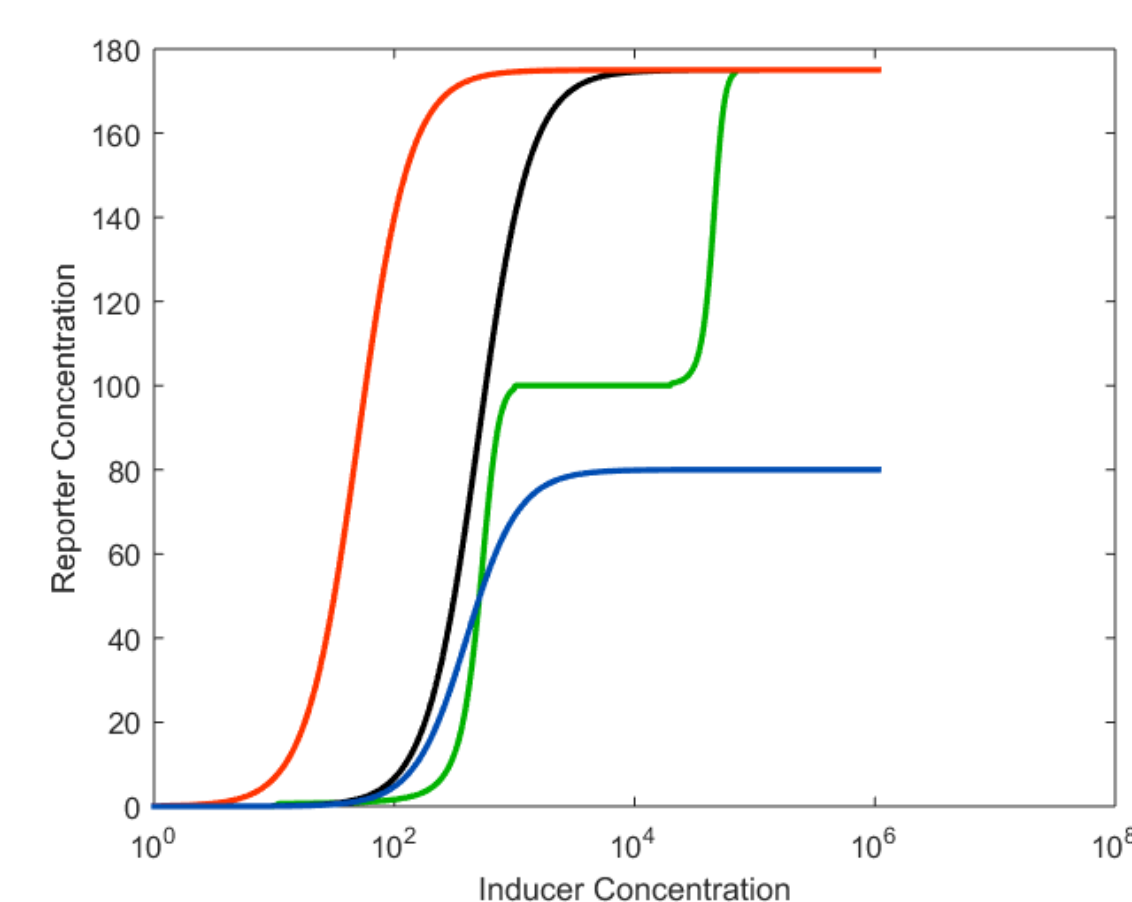
# The Circuit Control Toolbox

Kalen Clifton, Christine Gao, Ethan Jones, Likhitha Kolla, Joseph Maniaci, John Marken, John Mitchell, Callan Monette, Adam Reiss, Andrew Halleran, Gregory D. Smith\*, Margaret S. Saha\*\*

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



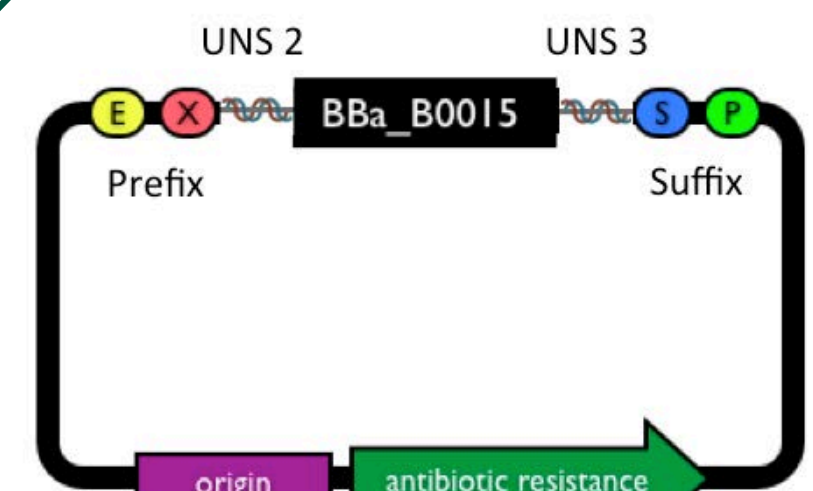
## Circuit Control Toolbox



- RiboJ: Genetic Insulation
- Synthetic Enhancer: Multistate Expression
- Decoy Binding Array: Threshold Tuning
- RBS Library: Magnitude Scaling

A central problem of synthetic biology is tuning a circuit's ultimate output without disrupting its underlying function. Our Circuit Control Toolbox provides teams with a suite of orthogonal parts which can be added to the end of an arbitrary circuit to induce modular and additive effects on its transfer function, without altering the original circuit architecture.

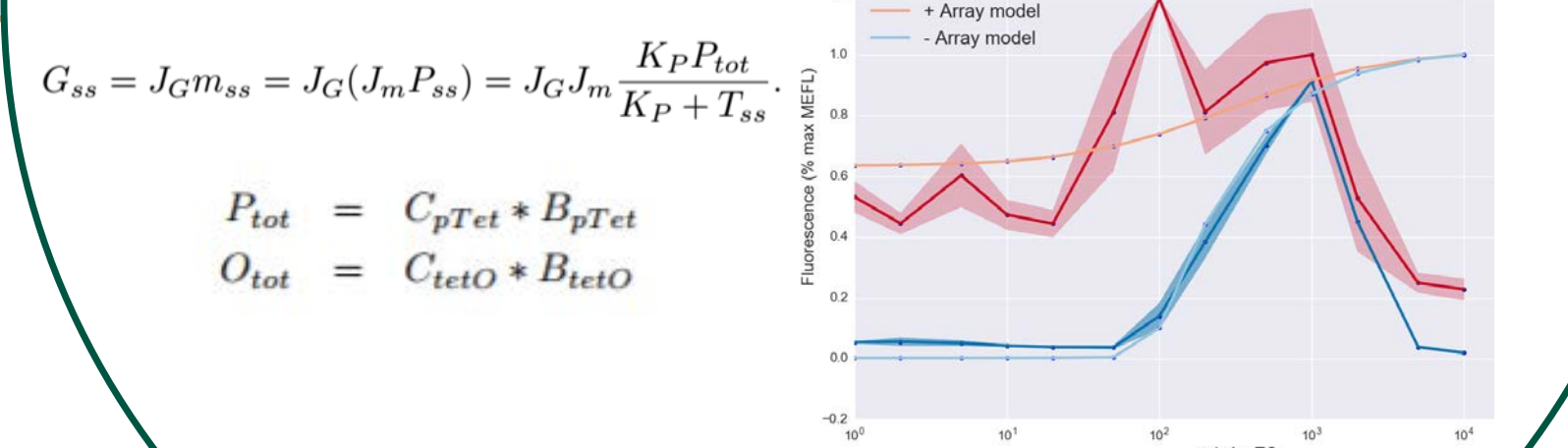
## UNS



A new iGEM backbone standard designed for Gibson Assembly

## Modeling

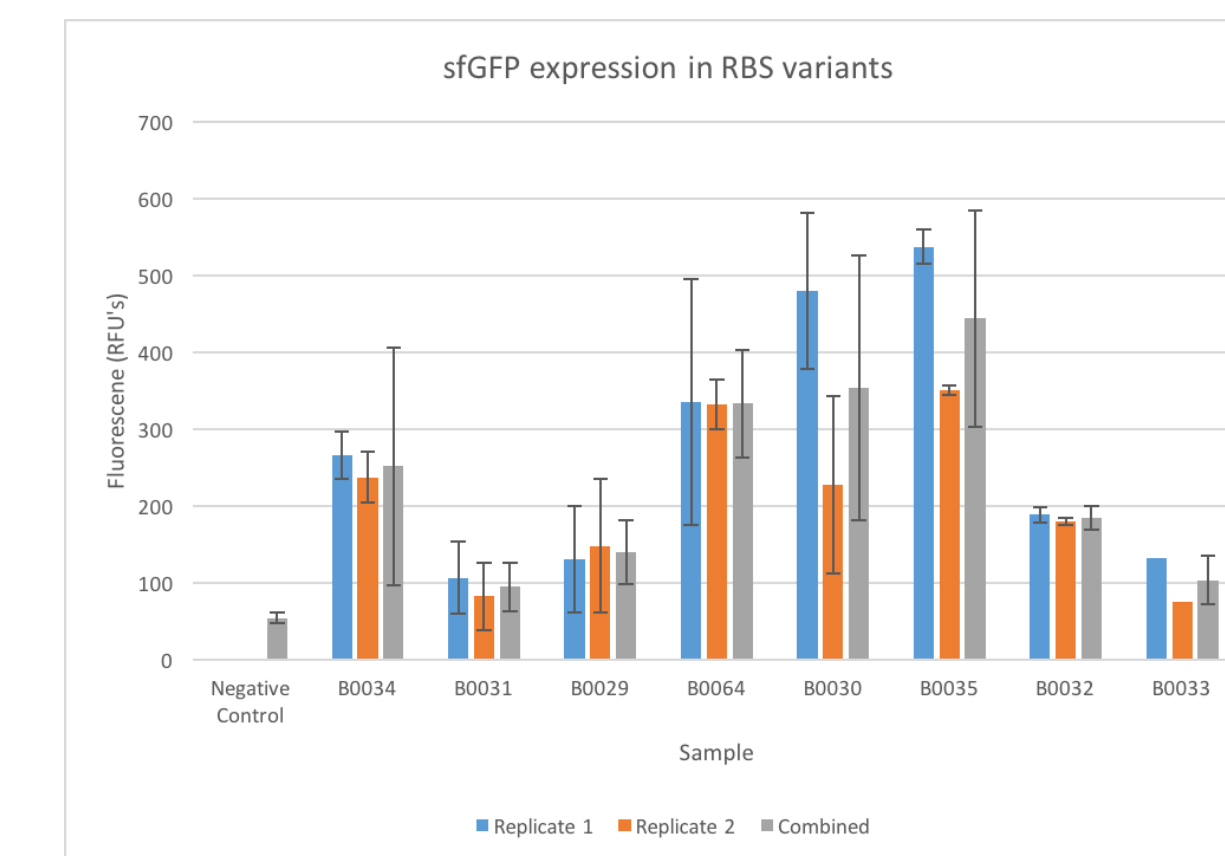
We developed a mathematical model parameterized by physiological values for Toolbox parts. Our kinetic ODE model of a basic GFP-expression system includes constitutively expressed TetR inhibiting GFP expression. Model accounts for the presence of decoy TetO arrays, and activity of aTC, which disables TetR's DNA-binding ability when bound.



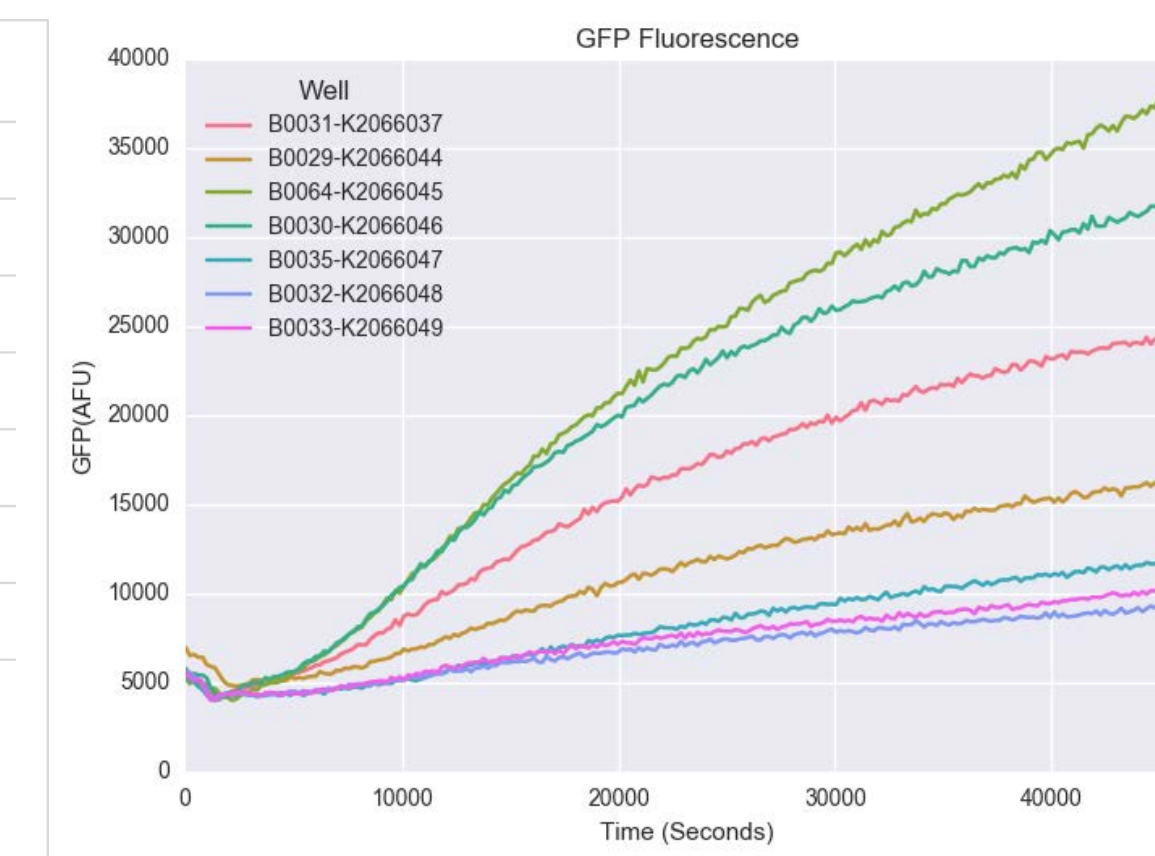
Binding arrays are constrained to low copy models as a high copy introduces intrinsic metabolic strain.

## Collaborations

We collaborated with UPitt and Alverno's iGEM teams to characterize RBS strength in cell-free systems. We sent blinded versions of our RBS characterization devices to University of Pittsburgh and Alverno High School's iGEM team, who characterized the devices in S30 and TX-TL, respectively. Both teams' results were generally consistent with ours and previous characterization efforts, but the B0064 RBS was found to be stronger in both cell-free systems.

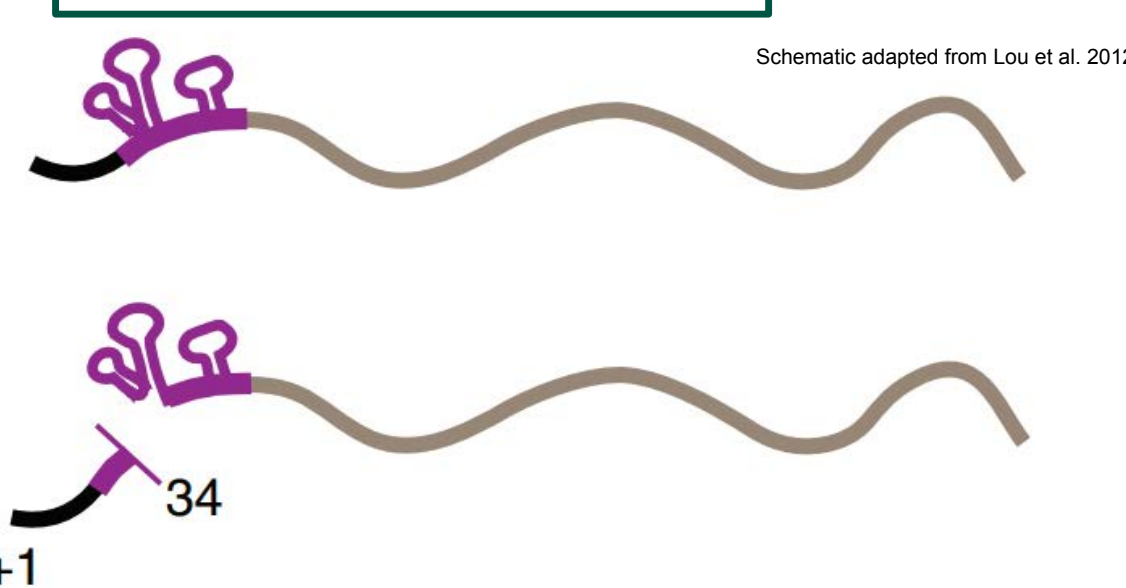


RBS Characterization UPitt iGEM-S30 system

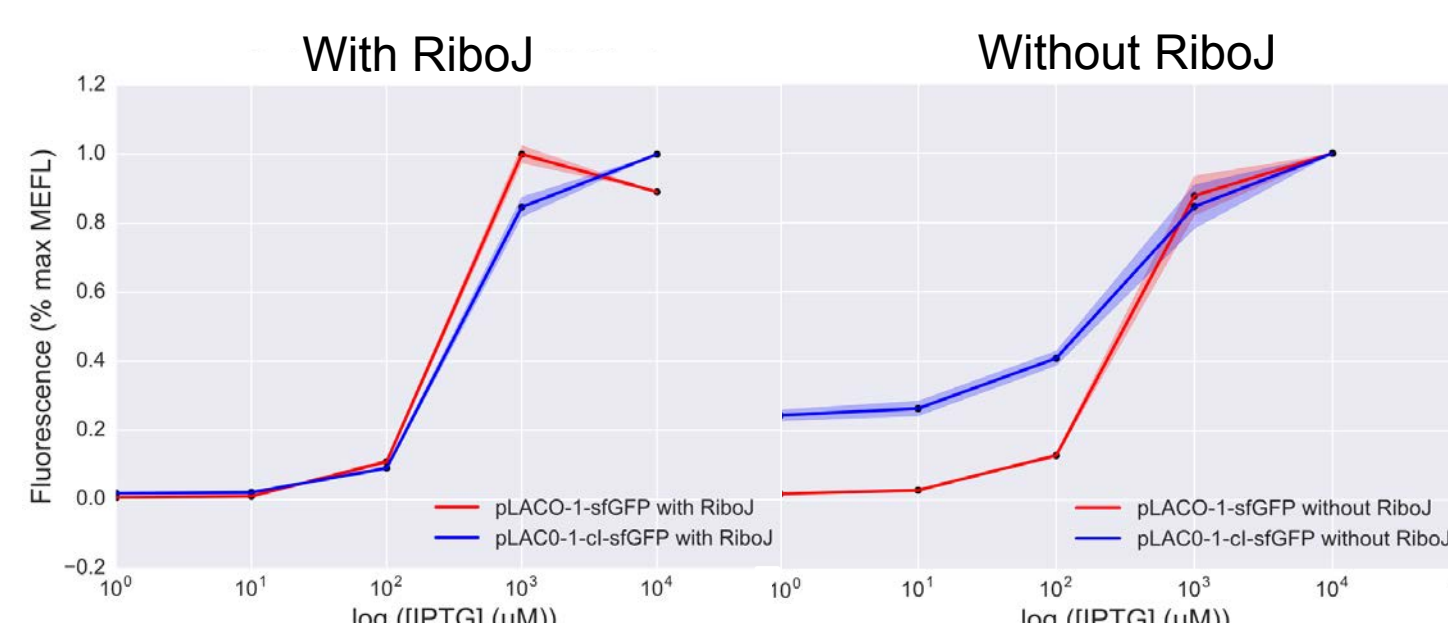


Alverno High School iGEM-TX-TL

## RiboJ

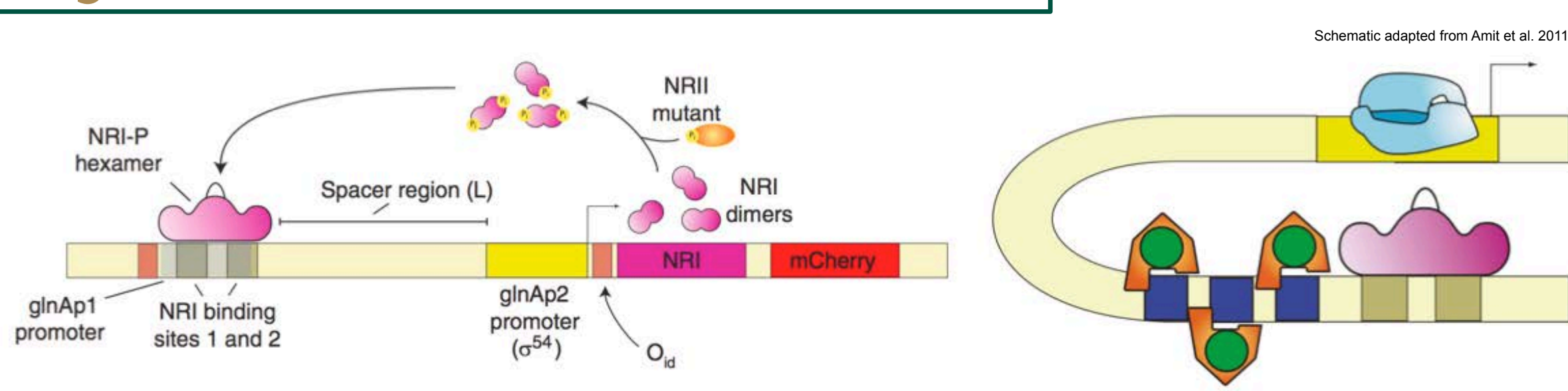


The self cleaving ribozyme RiboJ standardizes the 5' UTR of the transcript, causing the normalized transfer functions of circuit components to be invariant to the choice of protein coding sequence<sup>4</sup>. This insulation ensures that our Toolbox and its modifications are applicable to arbitrary genetic circuits, regardless of their choice of output protein.

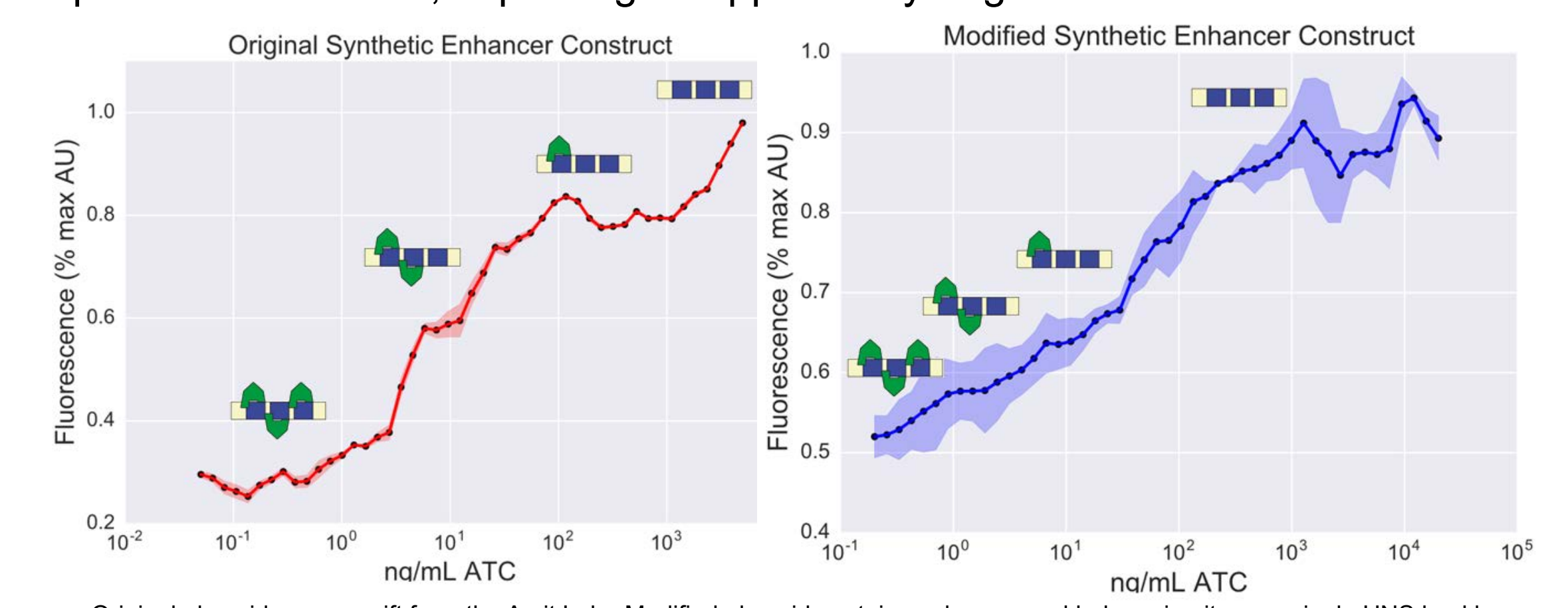


Use of RiboJ collapses relative expression curves

## Synthetic Enhancer

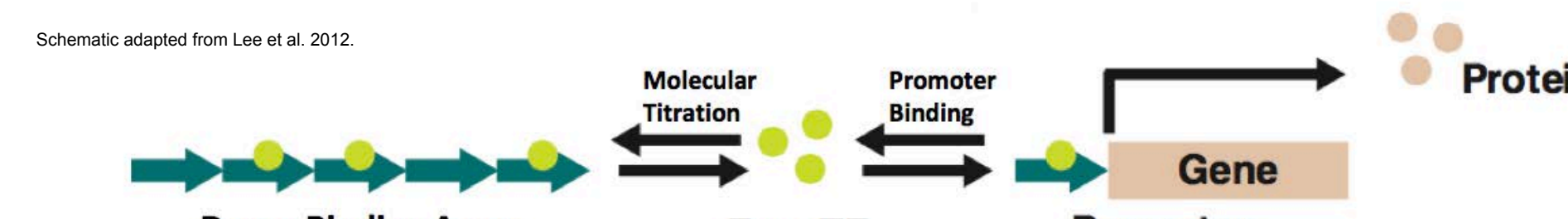


The synthetic enhancer construct allows genes to exhibit multi-state transfer functions where each state corresponds to a distinct number of proteins bound to a binding cassette in the spacer region. We improved on the synthetic enhancer construct by combining its essential components into one plasmid and removing its dependence on IPTG, improving its applicability to genetic circuit modification.

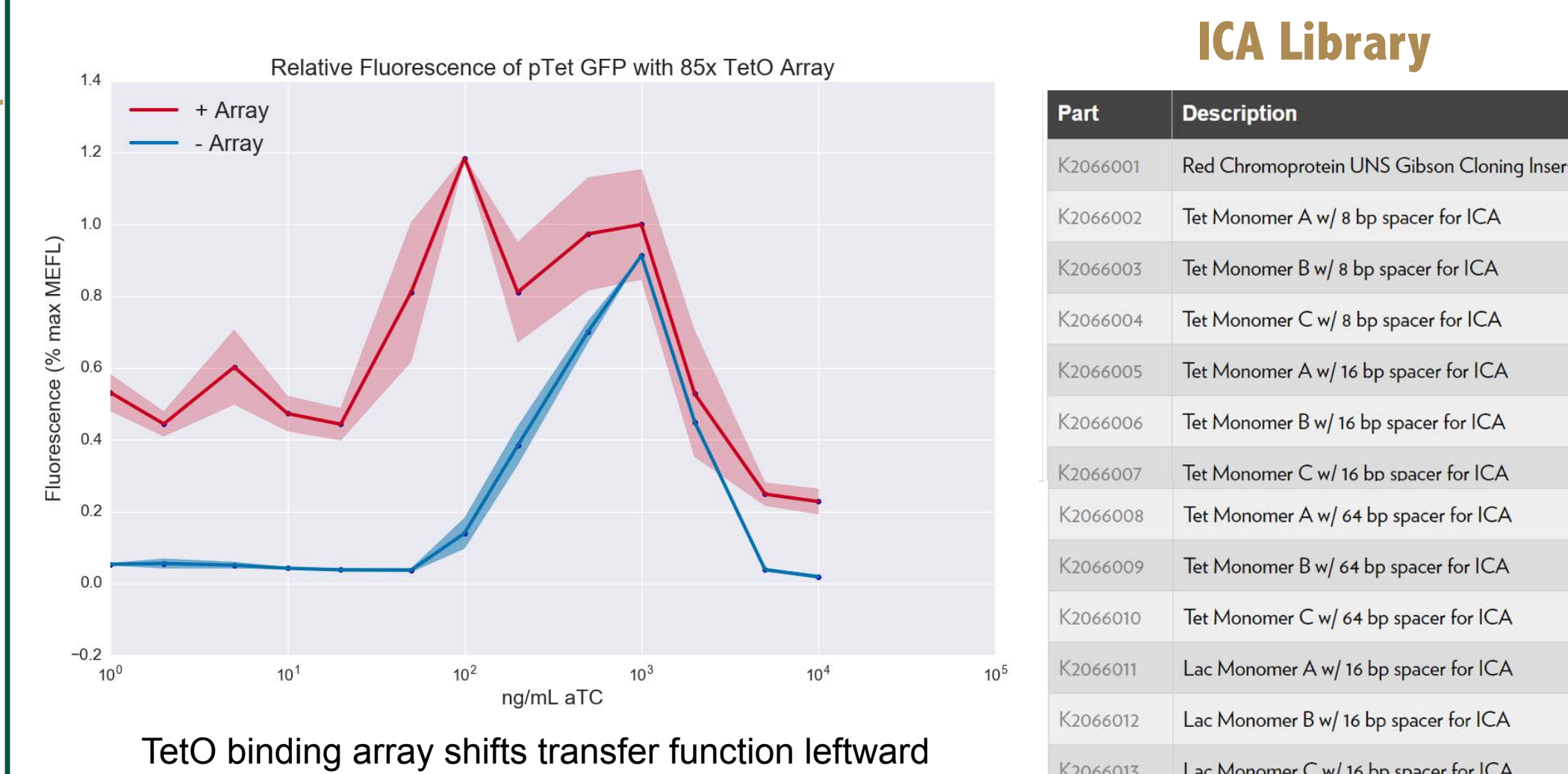


Original plasmids were a gift from the Amit Lab. Modified plasmid contains enhancer and helper circuits on a single UNS backbone

## Decoy Binding Array

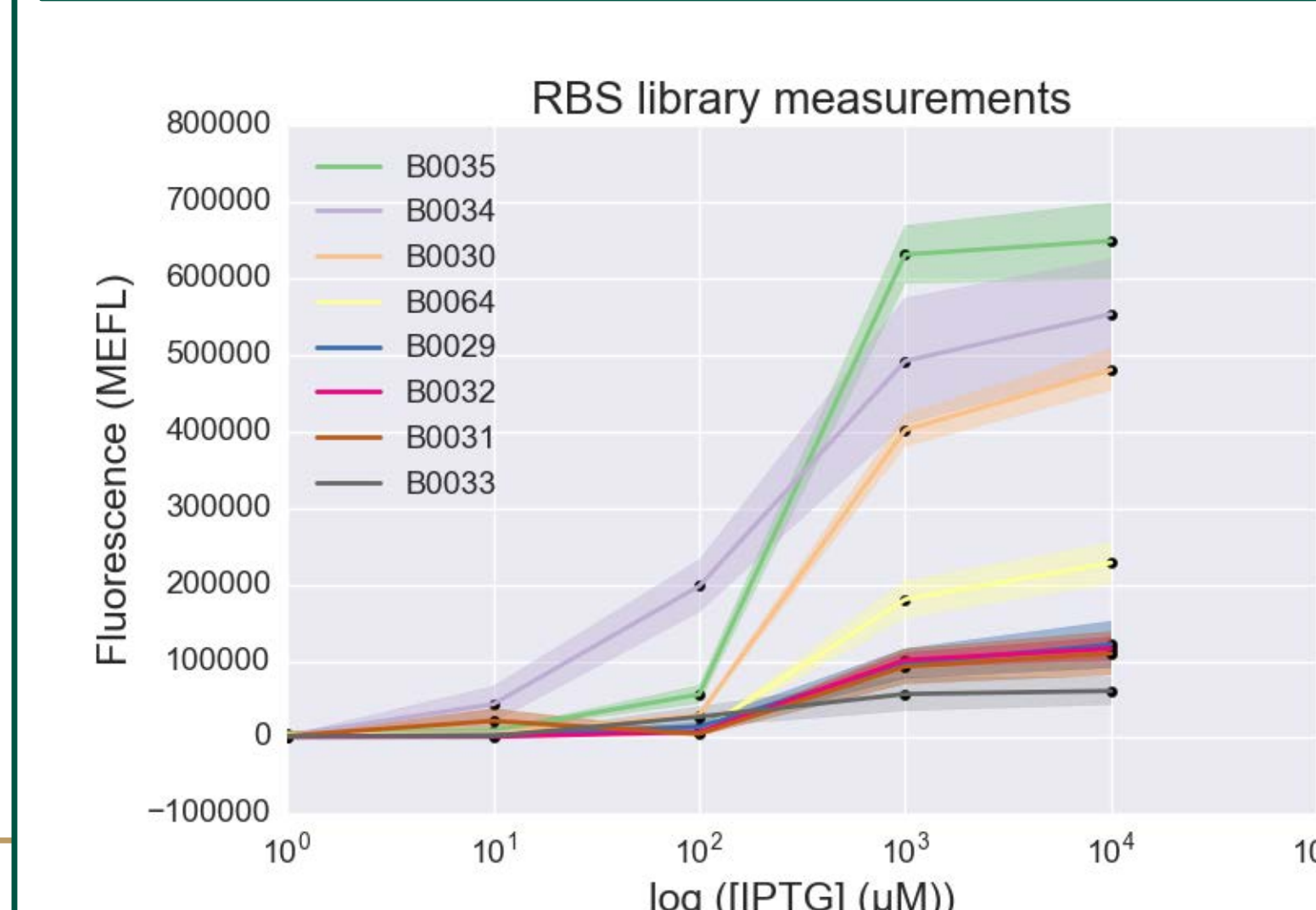


Transcription Factors can be titrated out using Decoy Binding Arrays. This titration shifts the threshold of a given transfer function, allowing for tuning of the sensitivity of a given circuit to an arbitrary transcription factor. The magnitude of the shift is determined by the number of binding sites, and our ICA library allows for the construction of arbitrary length arrays.



TetO binding array shifts transfer function leftward

## RBS Characterization



We characterized the community library of RBSs using a standardized reporter construct that is driven by an inducible promoter and contains RiboJ upstream of the RBS sequence. This device allows for characterization of RBS strength over a dynamic range of transcriptional activity while also standardizing the 5' UTR of the transcript through RiboJ insulation to remove the influence of promoter choice on the results of the characterization.

## Outreach



### Building With Biology Workshop

We held a science workshop targeted at local children and their parents, where we performed scientific activities and discussed our questions and concerns with GMOs



### CRISPR/Cas9 Forum

We invited the Williamsburg community to discuss the implications and ethics of genome editing in bioremediation, medicine, agriculture, and biotechnology.

### LearnSynBio.org

We built a website to host our instructional videos and educational materials to introduce high school students to the ideas in Synthetic Biology, with a focus on integrating mathematical modeling. We collaborated with high school teachers to ensure the easy integration of our materials into the classroom.

## Achievements

- Built and characterized the Circuit Control Toolbox, which allows future teams to have precise, modular control over their circuits' behavior with targeted, orthogonal modifications that are applicable to arbitrary circuits.
- The Toolbox contains 118 parts on the UNS backbone standard, including:
  - A library of monomers to build binding arrays of arbitrary length.
  - A standardized RBS characterization device for rigorous measurement of RBS effects over dynamic gene expression levels.
  - An improved synthetic enhancer construct designed for compatibility with gene circuit integration.
  - Created a website, LearnSynBio.org, designed to promote and teach synthetic biology to high school students with an explicit focus on integrating mathematical modeling.

## Citations

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