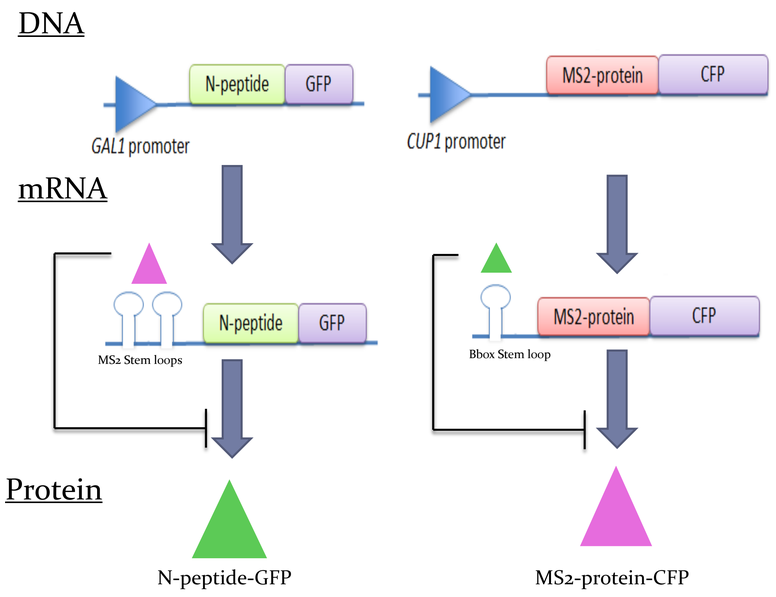
# AYESWITCH

Krystal Joan Annand  Brychan Cromwell  Lisa Dryburgh  Joseph Hoare  Justyna Kucia  Stephen Lam  Christina McLeman  Ben Porter  Margaret-Ann Seger  Liz Threlkeld

## Our System:

The "*AYE*SWITCH" is a genetic toggle switch engineered in yeast that allows for mutual inhibition of protein expression at the translational level. Our purpose was to create a system that allowed switching between two states and mathematical model was used to help guide the biology to achieve this goal.

Construction of the switch involved two DNA constructs, GAL1p-[Npeptide-GFP] and CUP1p-[MS2-CFP]. These encode for a fusion of RNA binding and fluorescent proteins. When co-expressed in yeast, these constructs mutually inhibit one another by binding to RNA stem loops, forming a biological 'Toggle Switch'.




### Transcription

$$\frac{d[M_1]}{dt} = \frac{\lambda_1 [GAL]^2}{{K_1}^2 + [GAL]^2} - (\mu_1 + T)[M_1]$$

$$\frac{d[M_2]}{dt} = \frac{\lambda_3}{1 + \left(\frac{[METH]}{K_3}\right)^4} - (\mu_3 + T)[M_2]$$

### Translation

$$\frac{d[GFP]}{dt} = \frac{\lambda_2 [M_1]}{1 + \left(\frac{[CFP]}{K_2}\right)^{n2}} - (\mu_2 + T)[GFP]$$

$$\frac{d[CFP]}{dt} = \frac{\lambda_4 [M_2]}{1 + \left(\frac{[GFP]}{K_4}\right)^1} - (\mu_4 + T)[CFP]$$

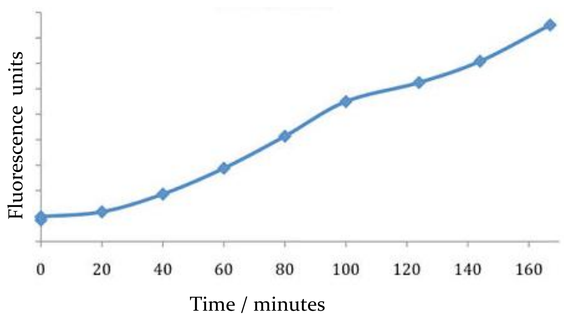
These equations model the rate of change of the mRNA concentrations depending on galactose and methionine concentrations.

These equations model the rate of change of GFP and CFP protein concentrations translated from their respective mRNAs.

For all the above equations, the three terms represent production, degradation, and dilution.

## Successful Fusion of GFP with a Regulatory Protein

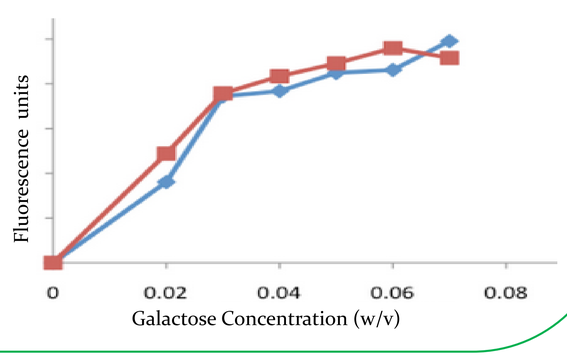
### Timed Induction of GAL1




**Left**: Characterisation experiments using GAL1p-[Npeptide-GFP] showed that translational fusion of GFP to a regulatory protein (N-peptide) was successful. This is shown by an increased GFP expression with evolving timed induction.

**Right**: This graph shows the results of dose response experiments using GAL1p-[Npeptide-GFP]. Only low concentrations of galactose are required to induce expression. This confirms that the GAL1 promoter can be regulated and further confirms the successful gene expression.

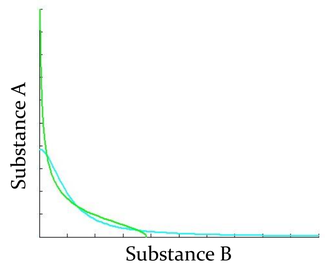
### Dose Response of GAL1




## Bistability Analysis

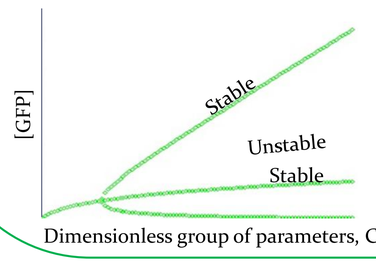
Dynamic equilibrium points occur when the production rate is perfectly balanced by degradation and dilution. In our system, this can occur at up to three different concentration states. Two of these are stable (bistability), and correspond to the expression of green- and cyan- fluorescent proteins, respectively. If our system is bistable then a toggle switch can be constructed.

Nullclines graph the protein concentrations for a system at equilibrium. Intersection of the nullclines in three or more places indicates that our system is bistable.




**Left**: This shows a generic scenario with the concentrations of substances A and B required to achieve steady state protein concentration (see blue and green curves). The nullclines (i.e. the protein concentrations) intersect at three equilibrium points, giving an 'ideal' bistability plot.

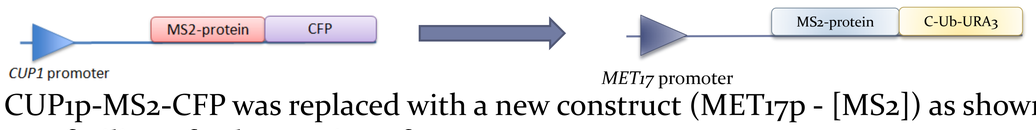
Our goal was to determine the range of parameter values that give rise to bistability.




**Left:** Our bifurcation diagram of the system ranges over the parameter space of GFP and C. The fixed points (i.e. nullcline intersections) of the system at these parameter values are plotted. Bistabilty exists in regions with three fixed points.

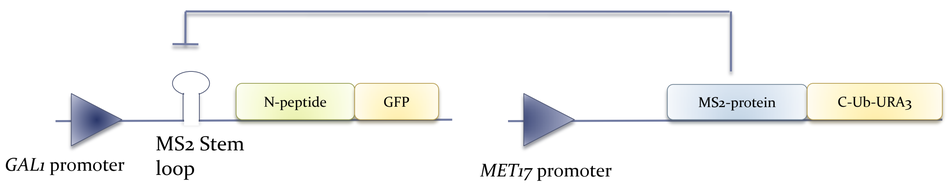
## CUP1p–[MS2-CFP] Troubleshooting

The CUP1p-[MS2-CFP] did not express MS2-CFP as expected. Troubleshooting experiments were carried out to test the DNA sequence of the promoter and CFP. In addition gene cassette replacement experiments to test the functionality of regulatory components were performed. These showed that both promoter and CFP sequences functioned as expected. Therefore, non-expression could be caused by Bbox stem loop blocking translation or fusion of MS2 to CFP resulting in non-functioning protein. These experiments suggest that replacing these elements in our CUP1p-[MS2-CFP] construct would create a viable switch.

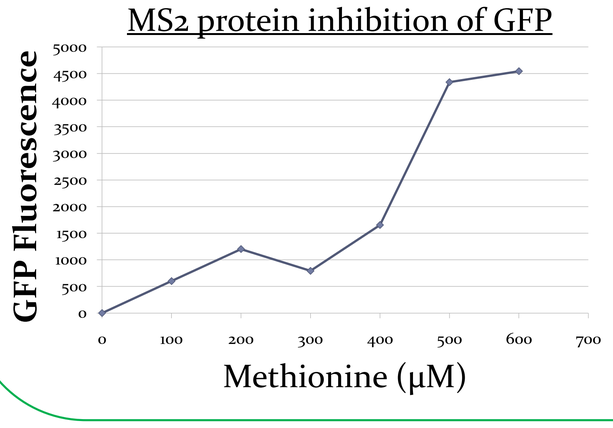



The CUP1p-MS2-CFP was replaced with a new construct (MET17p - [MS2]) as shown above to facilitate further testing of our system.

## Characterising the Translational Repression




### MS2 protein inhibition of GFP

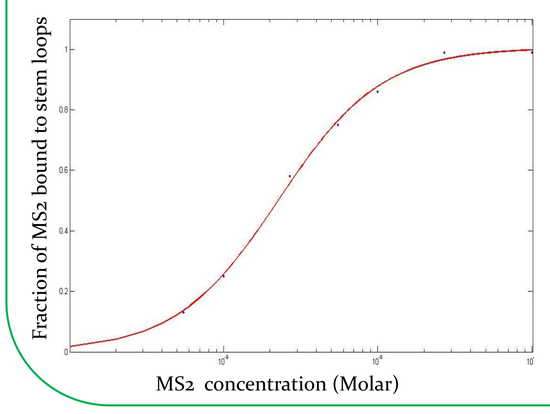



The translational repression of GAL1p-[Npeptide-GFP] was characterised by the trans-expression of the MS2 protein using MET17p - [MS2].

Results showed translational inhibition of GFP expression via binding of MS2 coat protein to stem loop is possible. This indicates our system is a viable design.

## Determination of Hill Coefficient $n_2$

The value of Hill coefficient n2, which is important for determining switching behaviour, was the only parameter not available from the literature. The number of MS2 stem loops in the DNA strands indicated that $n_2$ = 2 or 3. We determined the actual value of $n_2$ to be 2.6 by fitting our data to an MS2 binding curve from a paper by Witherell *et al.*[1]



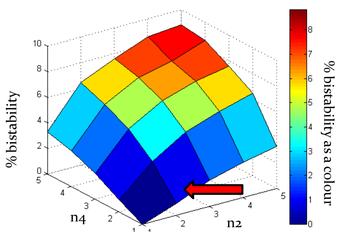

**Left**: This is the reconstruction of the MS2 binding curves from Witherell *et al.*[1] in MATLAB. After fitting the below function to the data points ($R^2$ = 0.998), we determined $n_2$ to be 2.6 ± 0.3.

$$f([MS2]) = \frac{\beta [MS2]^{n_2}}{{K_2}^{n_2} + [MS2]^{n_2}}$$

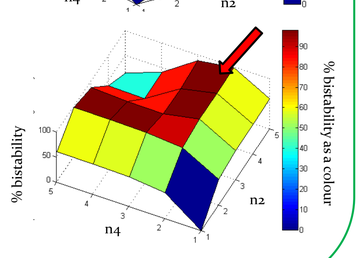
[1] Witherell, G.W., *et al.* (1990), 'Cooperative Binding of R17 Coat Protein to RNA', *Biochemistry*, Vol. 29, pp. 11051-11057

## Parameter Space Analysis

We selected values for $\lambda_i$, $K_i$ and $\mu_i$ from a range of two orders of magnitude around the average literature values. The large range was due to the uncertainty in the parameter values. The goal here was to determine which parameter combinations resulted in bistability.




Our conclusion: 2% of the parameter combinations tested for the original parameters and $n_2$=2.6 and $n_4$=1, resulted in bistability (top right picture). An ideal parameter set for the average values was found that gave 98% of the parameter combinations yielding bistability (bottom right picture). Experimental modifications could have been implemented accordingly with more time.




## Conclusion

We designed, constructed and tested a novel genetic toggle switch which was regulated at the translational level. We demonstrated that the individual constructs expressed functional fluorescent proteins as expected. A mathematical model was also used to predict the probability of successful switching. Further theoretical analysis established an ideal set of parameters for the switch to function optimally. We submitted four successfully-categorised BioBricks and characterised existing GAL1 and mOrange BioBricks from the Registry of Parts.