**Introduction:**

Biological switches are fundamental to cellular memory. Responses to changes in regulating enzymes has different responses on various cellular pathways. In this case, a positive feedback transcriptional pathway can be affected by activator regulation. Perturbating positive feedback can create distinct states, which could be described as “tunable dynamics” in the picture.

[A close up of text on a white background

Description automatically generated](https://www.researchgate.net/publication/305040236_Allosteric_proteins_as_logarithmic_sensors)

Different signal levels, or perturbations in the cellular environment can create various responses in activity. In this study, sequestrating the activator signal level demonstrates different ranges of sensitivity. Bistable switches can be described as ultrasensitivity where cells are either in one state or the other. In other words, activity is very sensitive at certain ranges of sequestration or perturbation. *However, the requirements for cellular memory aren’t completely understood*. Specifically, no one has definitively shown that sequestration and positive feedback is enough to build a bistable switch, which is the focus of the paper, “Sequestration-based bistabilty enables tuning of the switching boundaries and design of a latch”. In this study, bistable switches were implemented by an activator exhibiting positive feedback and a sequestering anti-activating molecule. An outline of the model, mad in [Biorender](https://biorender.com/) is shown below:

A close up of a map

Description automatically generated

Their sequestration-based switch was modeled in E. coli using plasmids derived from Bacillus subtilis which encoded the anti-activator and activator molecules, rsiW and sigW respectively. Changes in these levels were reported by fluorescent proteins contained within these plasmids.

The researchers set out to verify that sequestration and positive feedback is sufficient to build a bistable switch. This contrasts previously described models in which cooperativity has been studied as the main source of ultrasensitivity. To test the “tunabilitiy” of their model switch, they systematically perturbed Anhydrotetracycline (aTC) and Arabinose (Ara) levels to induce “on” and “off” states.

**Modelling Approach:**

The modeling details of the researchers’ simulated memory unit was described as shown below. *The equation represents the rate of change of total sigma factor concentration in terms of anti-sigma factor and several parameters*. The authors reduced their analytical model by making the **following assumptions**:

1. the rate of transcription must be slower than protein binding. This is much like the quasi-state approximation where the rate of formation and dissociation of enzyme binding is slower than product formation upon substrate binding.
2. Gene expression is controlled only by the transcriptional activator and anti-activator sequestration.
3. Molecule degradation occurs at the same rate amongst all well-mixed molecules.
4. Molecule numbers are continuous, and the system is deterministic rather than stochastic as it would be in reality.

|  |  |
| --- | --- |
| *Base production of sigma factor* | *Basal* |
| *Rate of maximum protein production* | *V* |
| *Michaelis Menten constant* | *Km* |
| *Rate of production and dilution* |  |

The dissociation constant represents the binding affinity of the sigma and anti-sigma factor. Combining the definitions of the dissociation constant with the previous equation yielded their reduced model that they implemented in MATLAB. This yields the second equation when combined with the original ODE:

In this final reduced equation alpha represents the production rate of aTc-inducible promoter while beta is the maximal rate of sigma factor production and kappa is the half-maximal concentration for production. For numerical analysisthese parameters were set to the following values:

Using these parameters, I modeled the reduced differential equation, which produced apparent ultrasensitivity with small initial concentration. This can be observed by the slope within the outlined region. This was expected since their model exhibited bistability:

A close up of a map

Description automatically generated

(sigma\_integration.py)

Another important aspect of their method was generation of bifurcation and hysteresis diagrams from their model. The researchers wished to observe a change in bistability boundaries upon changes in total activator and anti-activator. The bistable region occurs at specific concentrations of aTc and Ara shown between the two lines. In this case, there is a stochastic switch that allows seemingly instantaneous switch between “on” and “off” states. In the hysteresis plot, bistability can be observed by vertical nullclines, that go through both bistable segments as well as an unstable saddle point.

A screenshot of a social media post

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Nullclines

Monostable

Bistable

Monostable

Monostable

Bistable region

Monostable

(bifurcation.py, hysteresis.py)

The last plot corresponds to a model that the researchers implemented themselves to measure stochastic switching in E. coli cell populations. Stochastic switching between phenotypes with different growth rates shows the instability that occurs in the hysteresis diagram in cases where there are three steady states. Understanding this model gives insight into the cellular behaviors between seemingly binary states.

A screen shot of a computer

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(stochastic\_switch.py)

**Hypothesis**: I postulated that changing the overall rate of decay would change the boundaries in which the system demonstrates bistability. The parameter was kept constant in the researchers’ simulations. If overall decay rate affects the hysteresis boundaries, it’s likely that it could be a further source of regulation in vivo.

To test this, I changed the parameter slightly to see if there was a change in the hysteresis plot. Vertical nullclines can be drawn to see what ranges they will pass through three points, which corresponds to bistability. In addition, I investigated the changes in bifurcation analysis to see if a change in stability boundary states were observed.

**Results:**

I graphed the stable regions and the boundaries at which stable behavior was seen. The point at which the line changes trajectories horizontally indicated a change to instability and also bistability. This describes a saddle node, where perturbations can diverge two different ways along a certain path. Determining the stability of the stochastic model that the researchers implemented gave me intuition into the MATLAB implementations. In the code, saddle points were determined to construct the bifurcation lines separating monostable and bistable states. I found that the stochastic model supported this:

*(saddle node)*

When changing the parameter I relied on the model reduction definition where . In my python implementation, I modified my simulation of their hysteresis plot in python to change such that . represents the original parameter and represents that value that accounts for a change in overall decay of the anti-activator and activator.

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Description automatically generated

(varying\_decay.py)

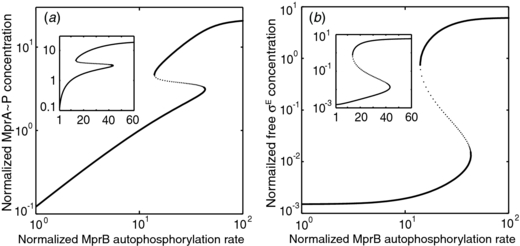
was changed from 1 to 0.9. Changing aTc and Ara levels also have a different effect on bistability due to decay as seen with the bifurcation diagram. By increasing decay, the bistable region becomes smaller. This supports my hypothesis that overall decay of the activator and anti-activator factors are important for sequestration based- bistability. The same goes for dilution, since also represents dilution in the researcher’s model.A screenshot of a cell phone

Description automatically generated

(Bifurcation\_expounded.py)

**Discussion/ New Findings:**

the researchers verified that bistabilty can be generated without relying on cooperative transcription factors. Alternatively, nonlinearity in growth rate combined with positive feedback could result in bistabilty. One example of a future area of interest from the paper was a model of the MprA/MprB system in mycobacteria as another example of similar sequestration based-bistability. In the image bellow, this system is measured against post-translational modification which is yet another contributor to bistability.

[](https://iopscience.iop.org/article/10.1088/1478-3975/7/3/036005)

Both the researchers’ models represent bistability and demonstrate that it can be achieved under different cellular environments. This could range from cooperativity, sequestration or disease related cellular processes that affect these processes, such as degradation. If there is a take-home lesson from this research, it’s that there are multiple ways to build a bistable switch. This may be complicated by multiple layers of regulation but understanding the requirements for cellular memory may yield information about how to interpret highly regulated cell metabolism.

**References:**

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