Systems Biology Project

"A positive-feedback-based bistable 'memory module' that governs a cell fate decision"

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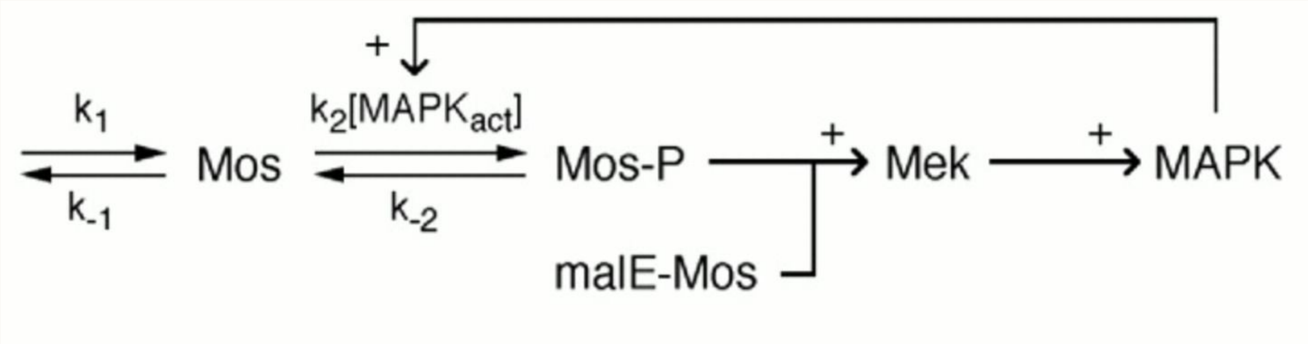
Introduction & Abstract of the paper

- The maturation of Xenopus oocytes can be thought of as a process of **cell fate induction**, with the **immature oocyte** representing the **default fate** and the **mature oocyte** representing the **induced fate.**
- Crucial mediators of Xenopus oocyte maturation, including the **p42 mitogen-activated protein kinase (MAPK)** and the **cell-division cycle protein kinase Cdc2**, are known to be organized into positive feedback loops.
- Such positive feedback loops could produce an actively maintained **'memory'** of a transient inductive stimulus and could explain the irreversibility of maturation.
- Basically shows how a group of intrinsically **reversible signal transducers** can generate an **irreversible response** at a systems level, and how cell fate can be maintained by a **self-sustaining** pattern of **protein kinase** activation.

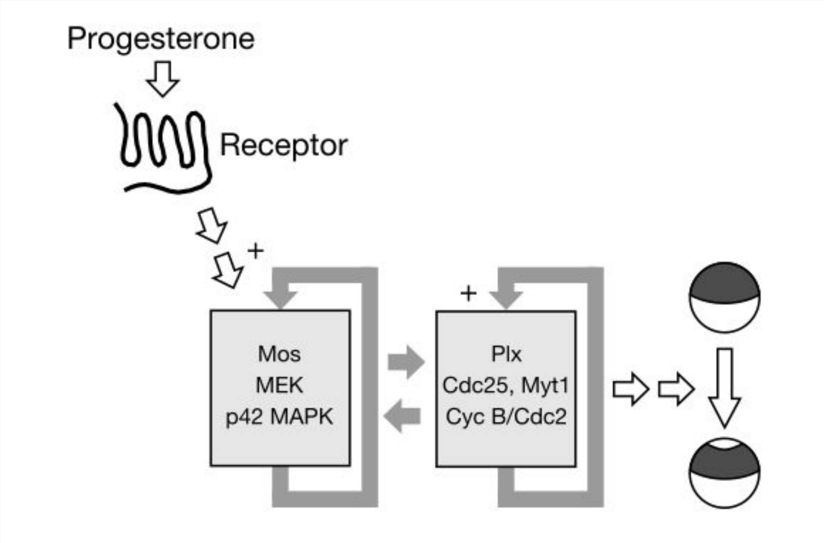
Background

- Mos **activates** p42 MAPK through the intermediacy of **MEK**, and active p42 MAPK feeds back to **promote** the accumulation of **Mos**.
- This **protein–synthesis–dependent positive feedback loop** is strong enough to allow p42 MAPK to show an **all–or–none, bistable response** to **progesterone or microinjected Mos**.



Background

- Cdc2 promotes **activation** of the Cdc2 activator **Cdc25**, and promotes **inactivation** of the Cdc2 inhibitor **Myt1**.
- p42 MAPK acts positively on Cdc2 by promoting the **inactivation** of **Myt1**, and Cdc2 acts positively on p42 MAPK by **promoting** the **accumulation** of **Mos.**



Hypothesis & Methods

- A system whose positive feedback is strong enough to produce bistability should show either hysteresis or, if the feedback is particularly strong, irreversibility.
- **Induction Phase:** Incubated immature oocytes with different concentrations of progesterone, waited until oocyte maturation had reached a plateau, and then measured the **percentage of GVBD (%Germinal Vesicle Breakdown), p42 MAPK phosphorylation, Cdc2 activation and progesterone binding.**
- Progesterone-treated oocytes showed dose-dependent increases in all of these responses, with **maximal kinase activation and %GVBD** obtained with **600 nM progesterone**.
- **Maintenance Phase: I**ncubated oocytes with 600 nM progesterone, waited until GVBD had reached a plateau, washed the oocytes for 10 h to remove progesterone
- Oocytes were found to maintain maximal p42 MAPK and Cdc2 activities after being washed free of progesterone.
- using a **chimaeric protein of Raf and the oestrogen receptor (DRaf:ER)**, and the fact that **oestradiol** can introduce a stimulus into the MAPK cascade, we confirm our findings.

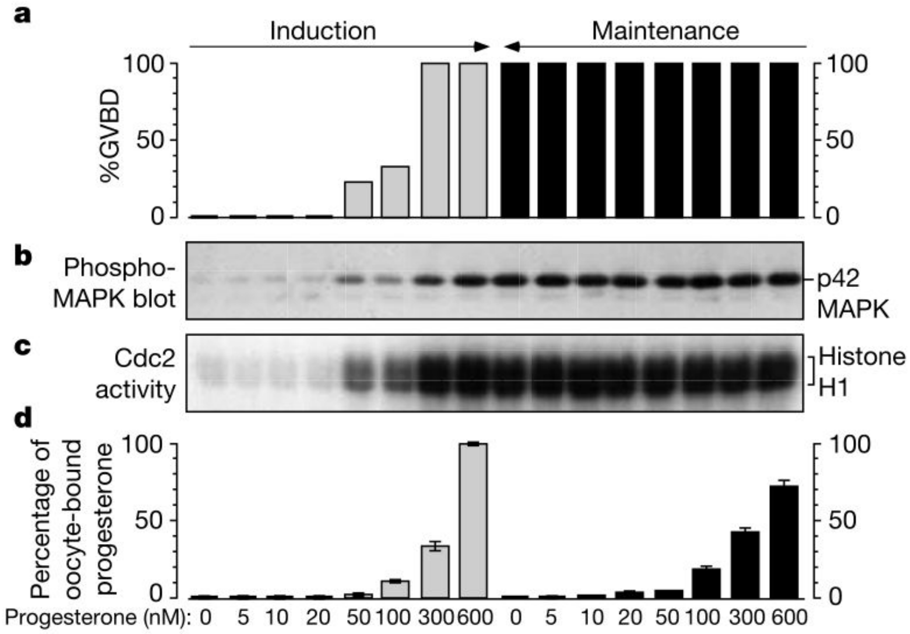
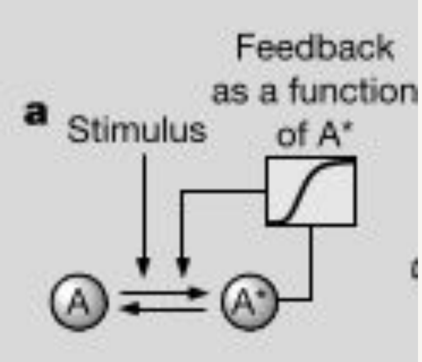


Figure 2 Irreversibility in the biochemical responses of oocytes to progesterone. GVBD (**a**), p42 MAPK phosphorylation (**b**), Cdc2 H1 kinase activity (**c**) and progesterone binding (**d**) were assessed at the end of the induction period and the end of the maintenance period. GVBD, MAPK phosphorylation and Cdc2 activity data are from one of three similar experiments. Progesterone binding data (shown as means ± s.d.) are from a separate experiment.

Further solidification

- To determine whether positive feedback is essential for maintaining this memory, we made use of three ways of blocking feedback from p42 MAPK to Mos:
    - feedback inhibitor: **cycloheximide**
        - In the **absence** of cycloheximide, oestradiol again caused marked **increases** in the steady-state activities of DRaf:ER, p42 MAPK and Cdc2, and these responses were undiminished after washing.
        - In the **presence** of cycloheximide, the responses of DRaf:ER and p42 MAPK to oestradiol were **blunted**, and the response of Cdc2 was markedly diminished. In addition, the responses were no longer irreversible
    - feedback inhibitor: **morpholino Mos antisense oligonucleotide (Mos-AS)**
        - blocks **progesterone-induced Mos synthesis**
        - Mos synthesis is required for the observed irreversibility.
    - feedback inhibitor: **PD98059 (MEK inhibitor)**
        - blocks feedback effects that depend on MEK activation

Simple Positive Feedback Loop

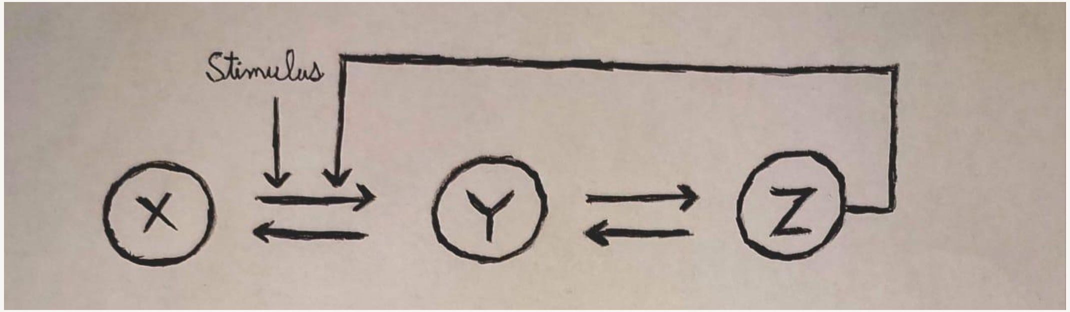


$$\frac{d[A^*]}{dt} = \{\text{stimulus} \times ([A_{tot}] - [A^*])\} + f\frac{[A^*]^n}{K^n + [A^*]^n}([A_{tot}] - [A^*]) - k_{inact}[A^*]$$

n denotes the Hill coefficient, K is the effector concentration for half-maximum response and f represents the strength of the feedback.

Setting $\frac{d[A^*]}{dt} = 0$, it follows that stimulus $= \frac{f \times [A^*]^n[A_{tot}] - k_{inact}K^n[A^*] - (f + k_{inact})[A^*]^{n+1}}{([A^*] - [A_{tot}])([A^*]^n + K^n)}$

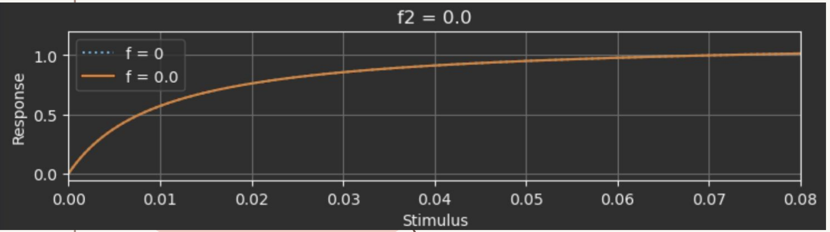
Multi-Component Positive Feedback Loop

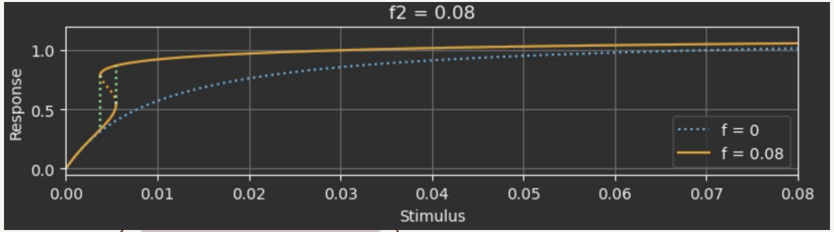


$$\frac{d[Y]}{dt} = \left\{ stimulus \times ([X_{tot}]-[Y]-[Z]) \right\} + f\,\frac{[Z]^n}{K^n+[Z]^n}\,([X_{tot}]-[Y]-[Z]) - k_{Y1}[Y]$$

$$\frac{d[Z]}{dt} = k_{Y2}[Y] - k_z[Z]$$

Results - Model Simulation for Different Feedback Strengths



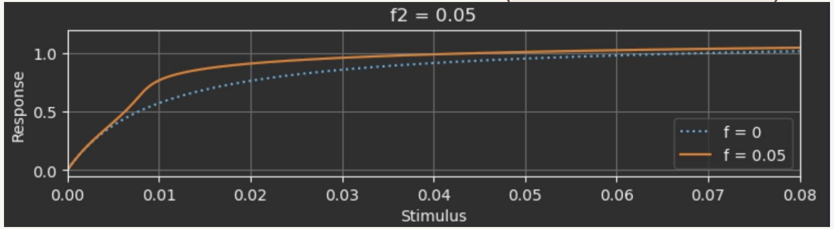


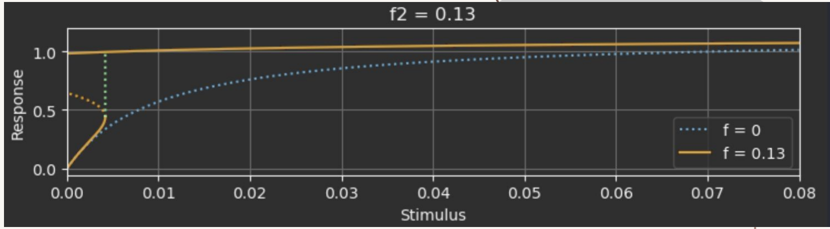
The curve starts getting a sigmoidal shape

Irreversibility of response, after threshold crossed

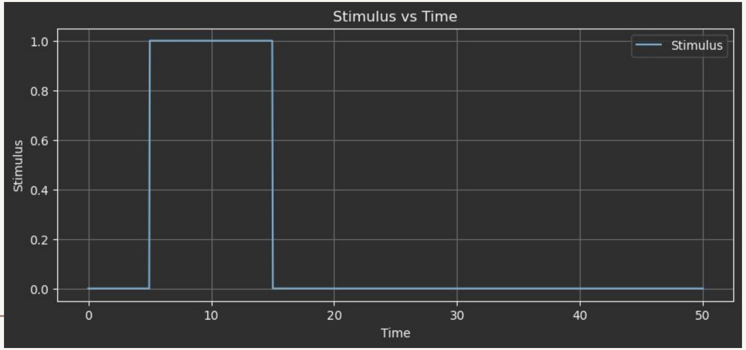
The curve behaves like simple regulation

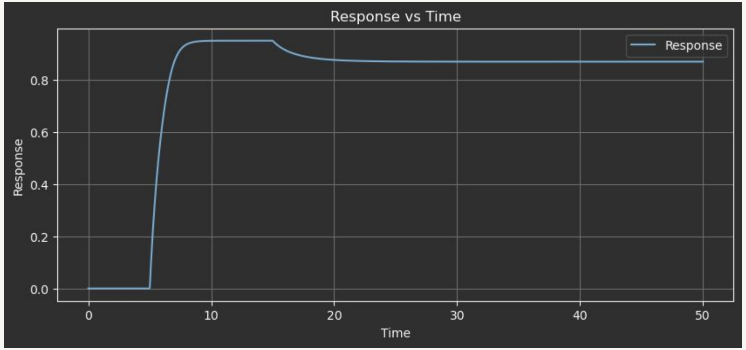
We see the presence of hysteresis





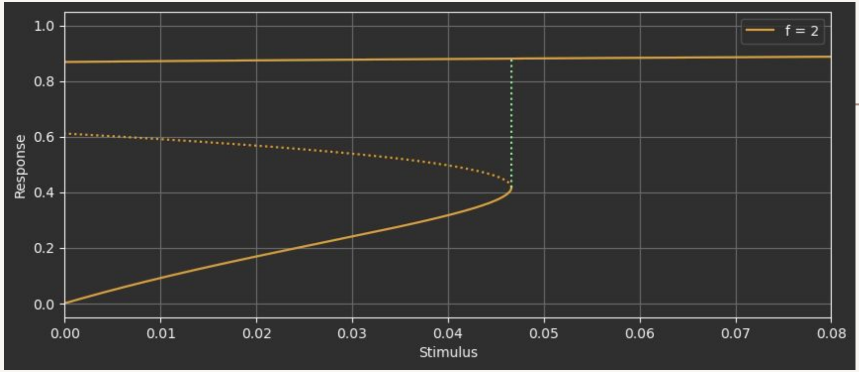
Results - Irreversibility and Memory





1

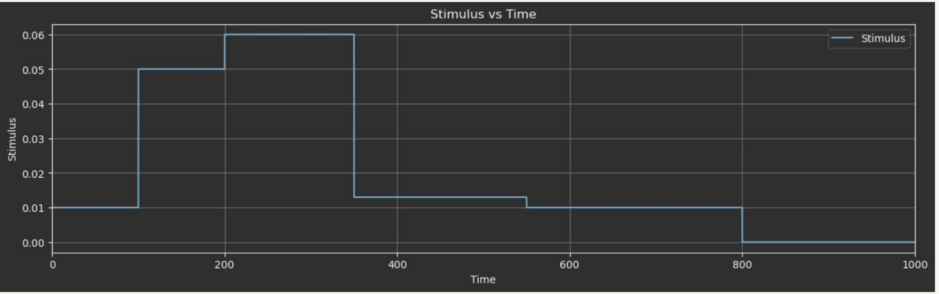
We can see that once Response reaches the steady state of around 0.88, it cannot return to 0. It can only follow the curve in the top portion

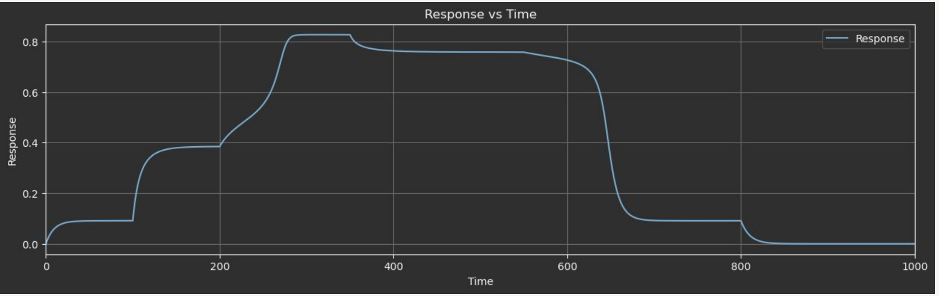


2

When we give an input pulse as Stimulus, the Response reaches at maximum and upon removing the Stimulus, it does not decrease a lot and stabilizes. This shows how it continues to retain memory.

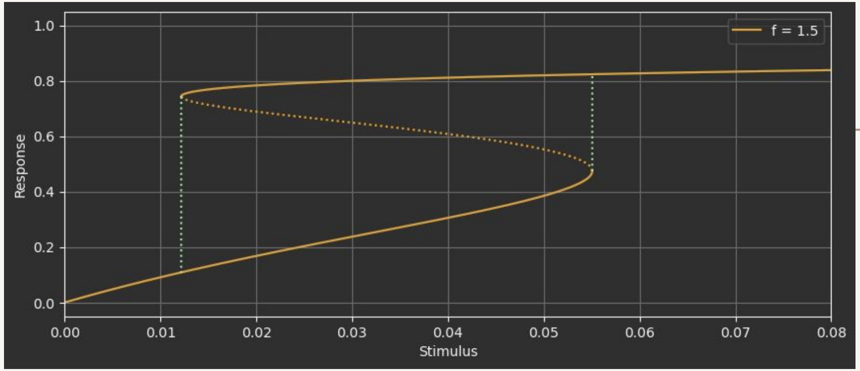
Results - Hysteresis





1

We can observe a very nice simulation of hysteresis that is achieved by this model. The response jumps from one stable steady state to the other upon crossing the respective thresholds



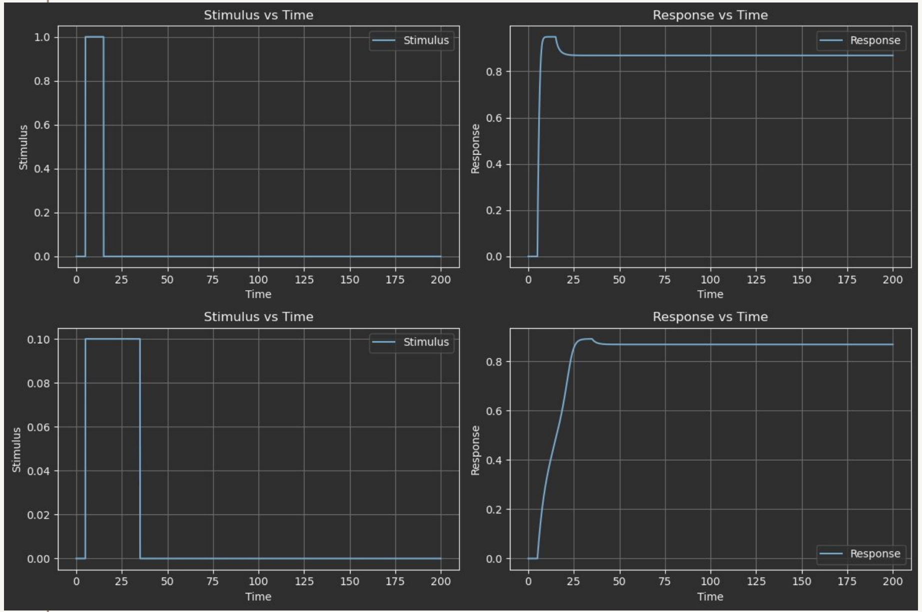
2

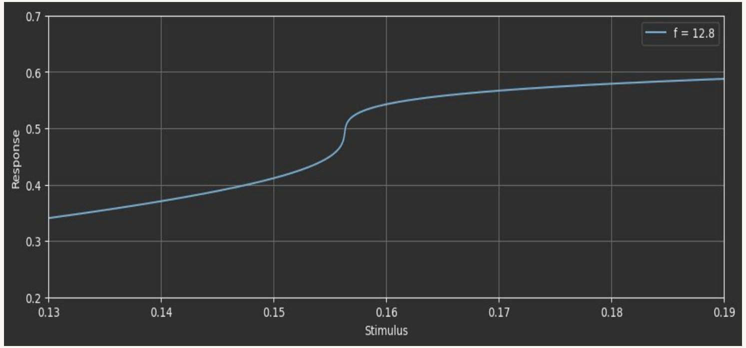
We try to get the response to stabilize for different values of stimulus and through the plot we see that the response does not trace back the same path it took initially.

Results - Response Time and Switch-like Behaviour

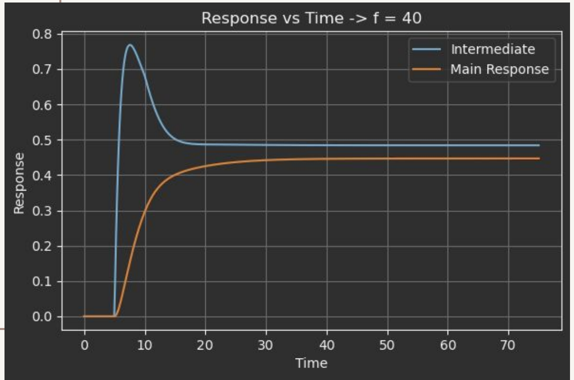
We can observe that a smaller concentration of stimulus requires a larger time to stabilize the system

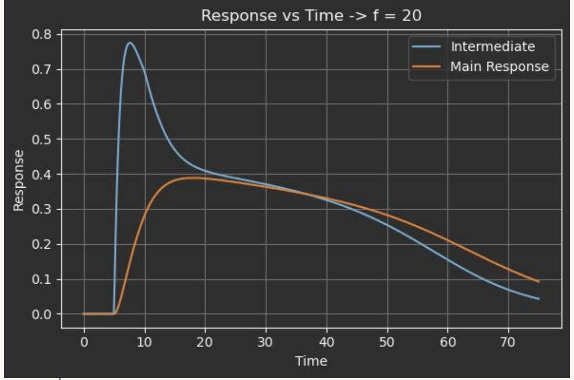
Taking a certain value for the feedback strength, we see that our curve is sigmoidal in nature. It can behave like a switch and is sensitive in the center region





Results - Multi Component Positive Feedback





1

We see that even with multi components, a stronger feedback leads to irreversibility

2

Similarly, when the feedback is weak, we see that the response dies down after removal of stimulus

3

It also takes longer to reach steady state. Hence there is a concept of delay.

4

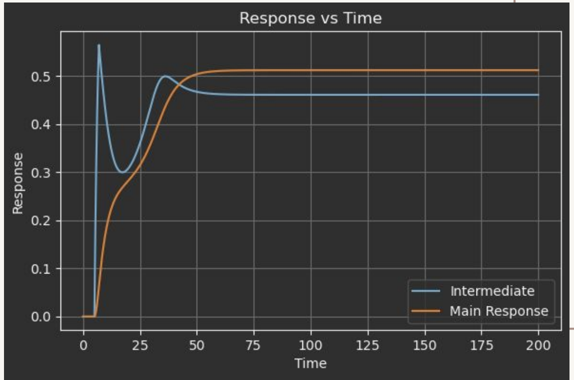
We give a very short stimulus pulse, with a very low magnitude as well

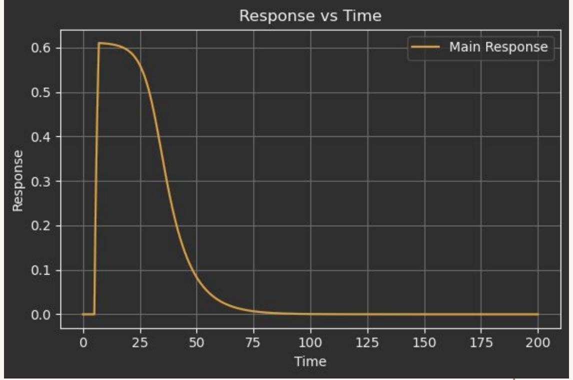
5

The multi component model is still able to reach the irreversible state. The signal is amplified due to the intermediate.

6

However, our initial simple model's response dies out. It needs a stronger signal.





Conclusion

- **Maturation inertia**: Once the oocytes have matured, they continue to remain in that irreversible state.
- Experimental evidence that in a physiological process of cell fate induction, Xenopus oocyte maturation, a bistable signalling system converts a transient stimulus into a **reliable, self-sustaining, effectively irreversible** pattern of protein activities.
- Studies of artificial bistable gene expression systems in **Escherichia coli and Saccharomyces cerevisiae** have shown that bistable systems can in fact function as **memory modules**.
- We have seen how important positive feedback loops are for phenomenon like hysteresis and irreversibility
- Depending on the various **degree of strength** of the feedback, as well as the values of other parameters, we can slowly move from **simple regulation** to **hysteresis** and eventually **irreversibility** and **memory**.
- We observe how various factors such as the amount of stimulus, its duration etc also affect the overall process.
- Lastly we also observe similar phenomenon in our more complex model and show how having multiple components can allow to further add features such as delay and amplification into the system as well, making it more versatile.

THANK YOU

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