Excitability in Gene Regulatory Networks

Linan Shi’s Undergraduate Thesis (Jun 2, 2022)

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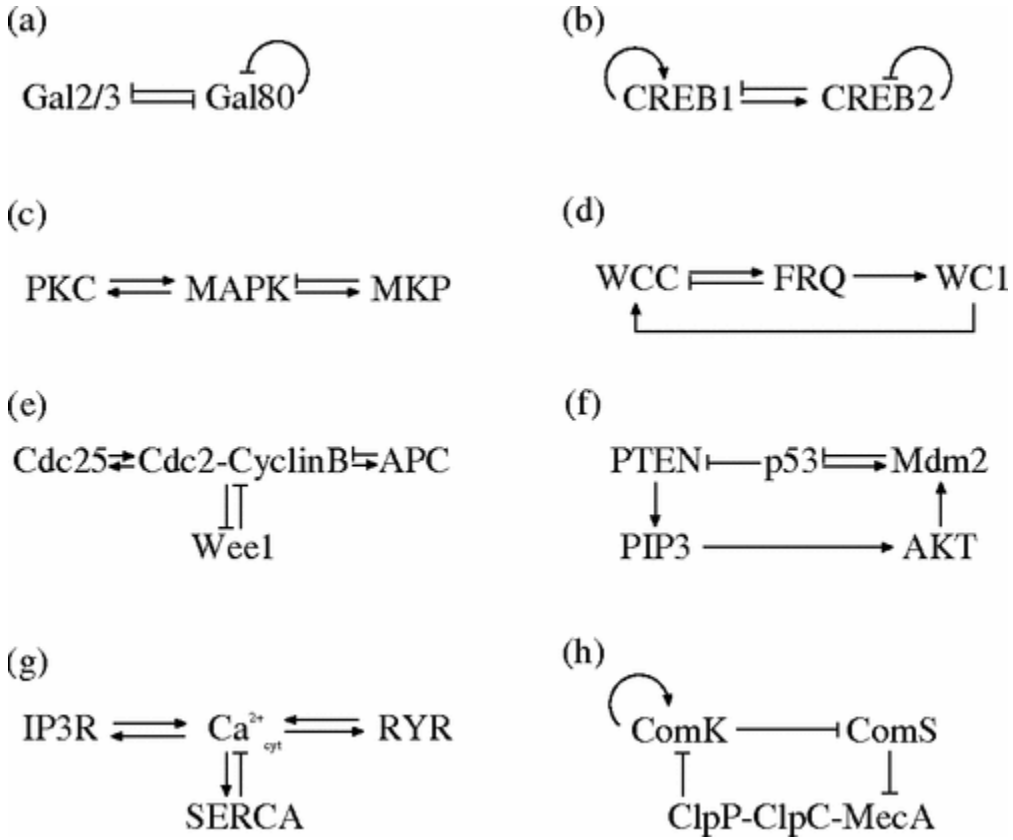
Background: Interlinked Positive and Negative Feedback Loops and Excitability

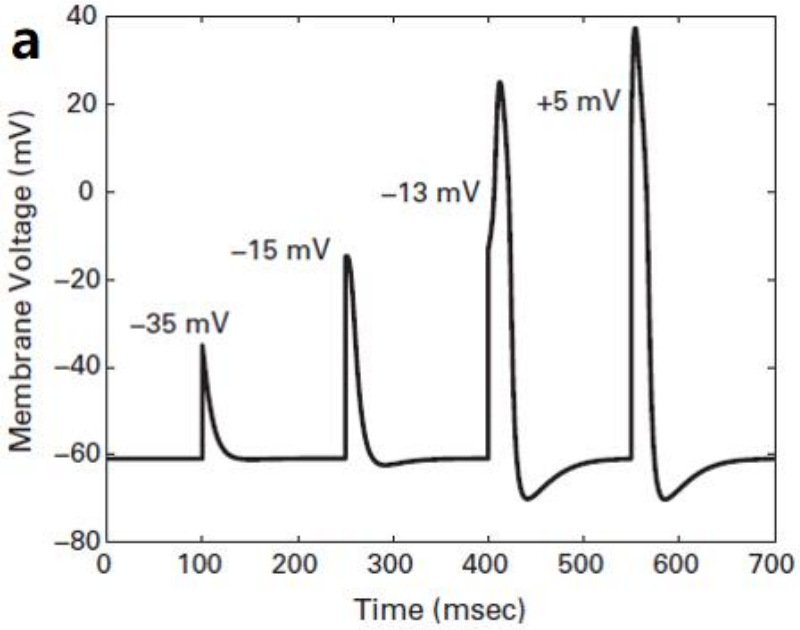


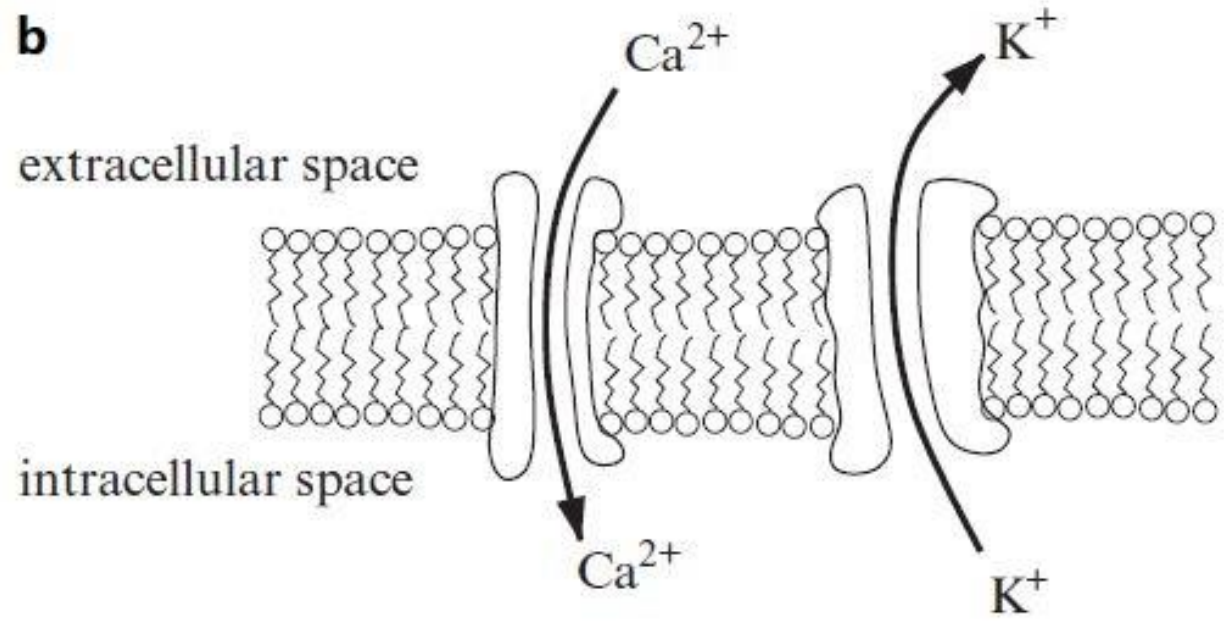
TIAN X J, ZHANG X P, LIU F, et al. Physical Review E, 2009, 80(1): 011926.

- Interlinked positive and negative feedback loops are universal in biological systems. Different kinds of behaviors can be achieved, such as mono-stability, bi-stability, oscillation and **excitability**.
- Excitability is the ability of systems to enter an excited state quickly in response to a stimulus and to return to the initial state when the stimulus is withdrawn. Two examples: the neuron's action potential and the Bacillus subtilis competence.

Example: Neuron's Action Potential

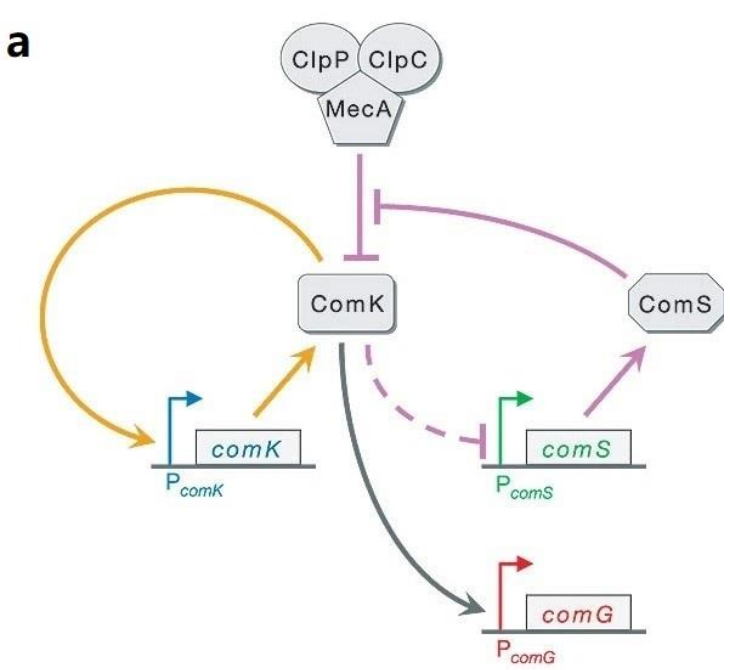
Herris-Lecar model

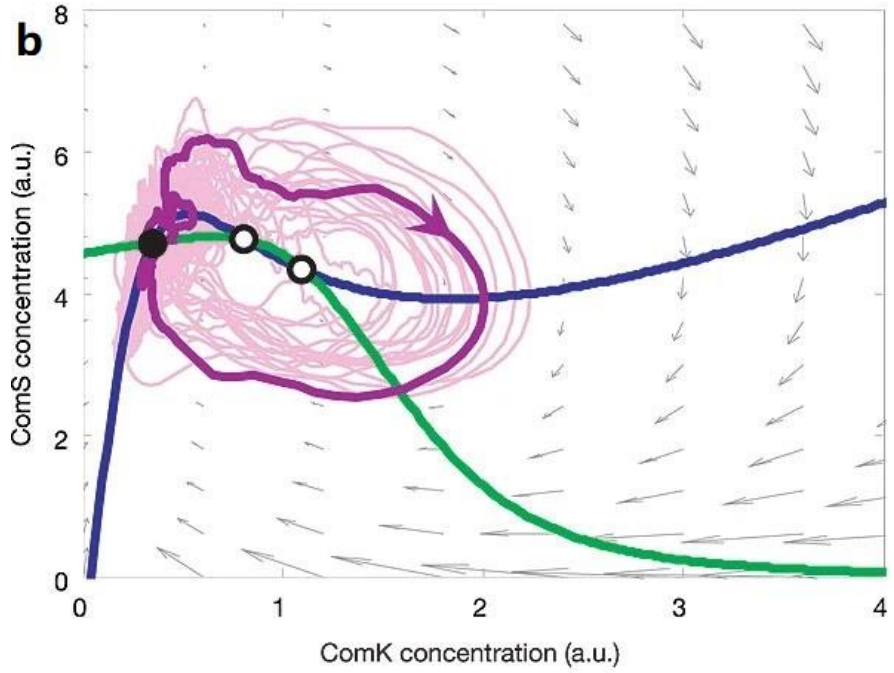




INGALLS B P. MIT press, 2013.

Example: Bacillus Subtilis Competence





SÜEL G M, GARCIA-OJALVO J, LIBERMAN L M, et al. Nature, 2006, 440(7083): 545-550.

Question: How to achieve excitability in a gene regulatory network with 3 nodes?

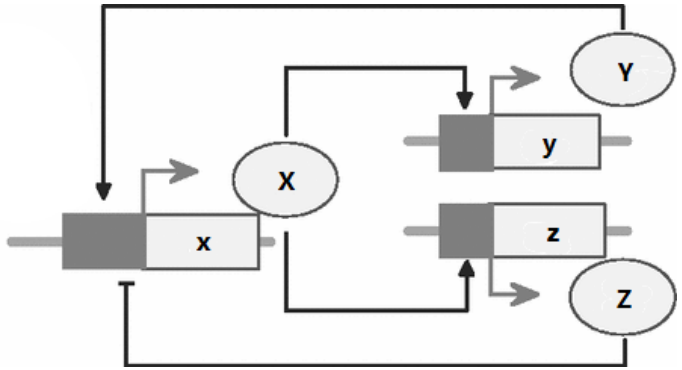
Mathematical Model

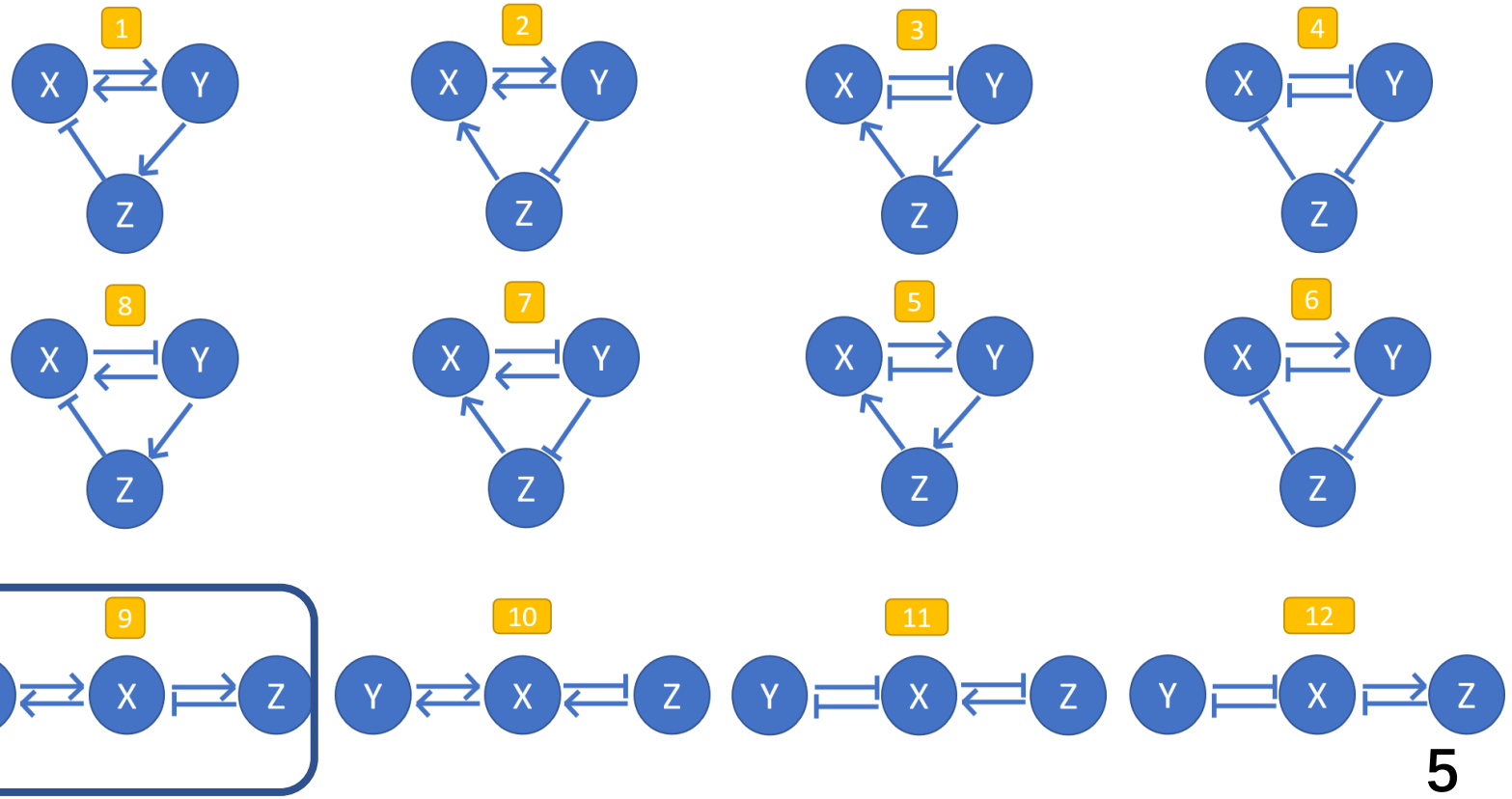
- The **transcription** process is regulated. Regulatory proteins' binding to DNA is considered quasi-static, thus we use **Hill-function** to describe the binding, and thus the rate of production of protein.
- The mRNA was treated quasi-statically as the usual mRNA kinetic time scale is much smaller than its corresponding protein kinetic time scale.

$$\frac{dx}{d\tau} = \eta \frac{y^{n_1}}{1 + y^{n_1} + z^{n_2}} - x + \beta_x$$

$$\theta_y \frac{dy}{d\tau} = \kappa \frac{x^{n_3}}{1 + x^{n_3}} - y + \beta_y$$

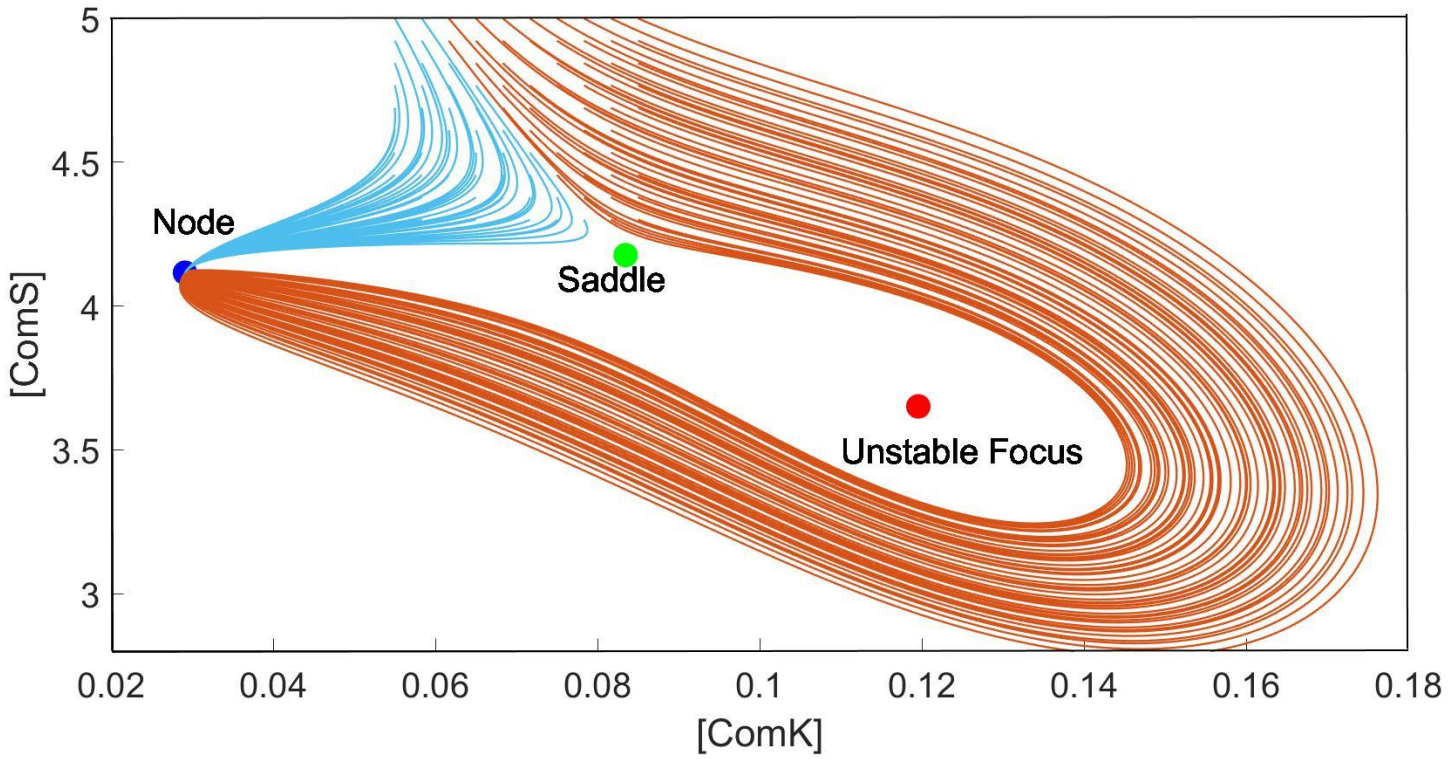
$$\theta_z \frac{dz}{d\tau} = \alpha \frac{x^{n_4}}{1 + x^{n_4}} - z + \beta_z$$





Excitability and Fixed Points

- Fixed Points analysis: 3 fixed points – a stable node/focus (rest), a saddle and an unstable focus.
- Excitement can be activated by a transient perturbation: the trajectory will cross the stable manifold of the saddle, then cross the unstable focus, and finally returns to the stable fixed point.



Background from: SÜEL G M, GARCIA-OJALVO J, LIBERMAN L M, et al. Nature, 2006, 440(7083): 545-550.

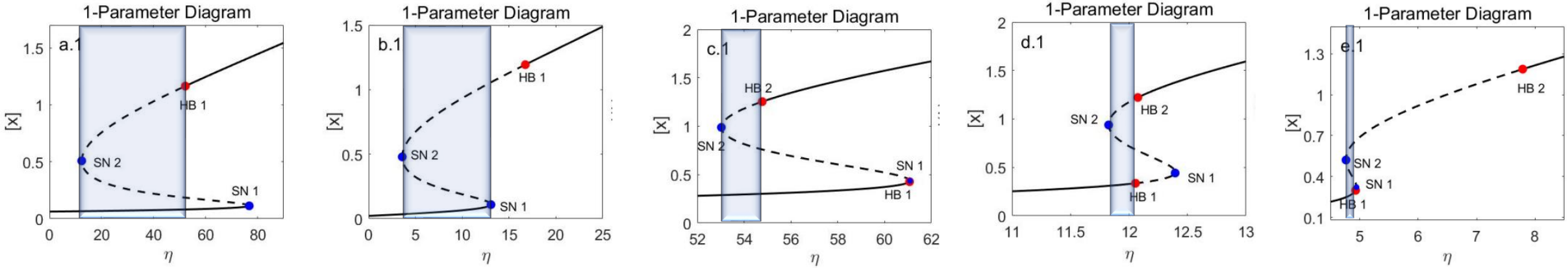
Excitability and 1-Parameter Diagram of Bifurcations

$$\frac{dx}{d\tau} = \eta \frac{y^{n1}}{1+y^{n1}+z^{n2}} - x + \beta_x$$

$$\theta_y \frac{dy}{d\tau} = K \frac{x^{n3}}{1+x^{n3}} - y + \beta_y$$

$$\theta_z \frac{dz}{d\tau} = \alpha \frac{(x/K)^{n4}}{1+(x/K)^{n4}} - z + \beta_z$$

- Choose η to draw 1-Parameter Diagram, choose other parameters as random numbers (0.01-100, logspace). (For each network structure among 12, 3 trials, each 100,000 set of random numbers.)
- If the relationship of bifurcations on the diagrams looks like the 5 below, it indicates that within a range of η, the system has 3 fixed points: a stable node/focus, a saddle and an unstable focus.



Steady-State Analysis is applied to identify bifurcations and draw these diagrams.

Steady-State Analysis with Jacobian Matrix Near the Fixed Points

$$\frac{dx}{d\tau} = F(x,y,z) \qquad \begin{bmatrix} \frac{\partial F}{\partial x} & \frac{\partial F}{\partial y} & \frac{\partial F}{\partial z} \\ \frac{\partial G}{\partial x} & \frac{\partial G}{\partial y} & \frac{\partial G}{\partial z} \\ \frac{\partial H}{\partial x} & \frac{\partial H}{\partial y} & \frac{\partial H}{\partial z} \end{bmatrix} \qquad \begin{bmatrix} a & b & c \\ d & e & f \\ g & h & i \end{bmatrix}$$

$$\frac{dy}{d\tau} = G(x,y,z)$$

$$\frac{dz}{d\tau} = H(x,y,z)$$

$$\lambda^3 + A_C\lambda^2 + B_C\lambda + C_C = 0$$

$$A_C = -(a+e+i)$$

$$B_C = ae + ai + ei - (cg + bd + fh)$$

$$C_C = afh + bdu + ceg - (aei + bfg + cdh)$$

Saddle-node bifurcation: $\lambda=0 \leftrightarrow C_C=0$

Hopf bifurcation: $\lambda=0\pm i\omega \leftarrow A_C\, B_C - C_C=0$

For x in range [0,100]

- A_C, B_C, and C_C can be denoted as functions of x (steady), to identify the bifurcations
- η can be denoted as a function of x, draw the 1-Parameter Diagram

$$\frac{dx}{d\tau} = \eta\frac{y^{n_1}}{1+y^{n_1}+z^{n_2}} - x + \beta_x$$

$$\theta_y\frac{dy}{d\tau} = \kappa\frac{x^{n_3}}{1+x^{n_3}} - y + \beta_y$$

$$\theta_z\frac{dz}{d\tau} = \alpha\frac{x^{n_4}}{1+x^{n_4}} - z + \beta_z$$

$$b = \frac{\eta n y^{n-1}(1+z^n)}{(1+y^n+z^n)^2}$$

$$c = -\frac{\eta n z^{n-1}(y^n)}{(1+y^n+z^n)^2}$$

$$d = \frac{\kappa n x^{n-1}}{\theta_y(1+x^n)^2}$$

$$g = \frac{\alpha n (x/K)^{n-1}}{K\theta_z[1+(x/K)^n]^2}$$

$$A_C = 1 + \frac{1}{\theta_y} + \frac{1}{\theta_z}$$

$$B_C = \frac{1}{\theta_y} + \frac{1}{\theta_z} + \frac{1}{\theta_y\theta_z} - cg - bd$$

$$C_C = \frac{1}{\theta_y\theta_z} - \frac{1}{\theta_y}cg - \frac{1}{\theta_z}bd$$

$$\eta = (x-\beta_x)\frac{1+y^n+z^n}{y^n}$$

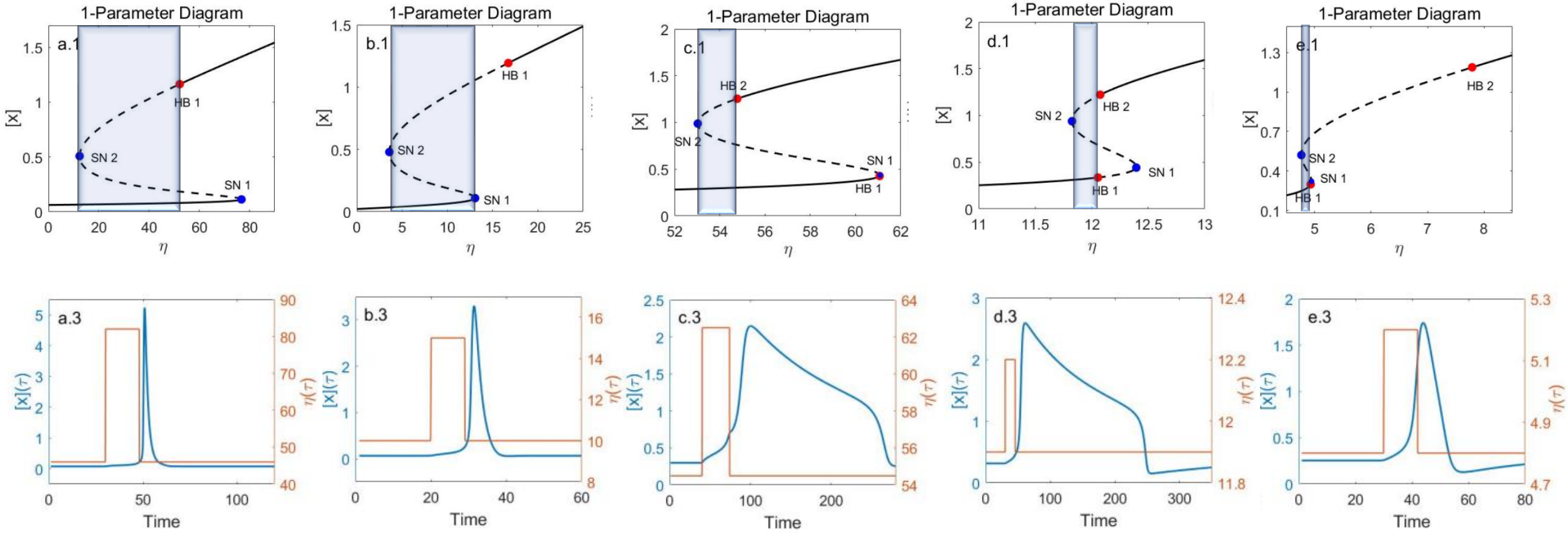
$$b = (x-\beta_x)\frac{n(1+z^n)}{y(1+y^n+z^n)}$$

$$c = -(x-\beta_x)\frac{nz^{n-1}y^n}{1+y^n+z^n}$$

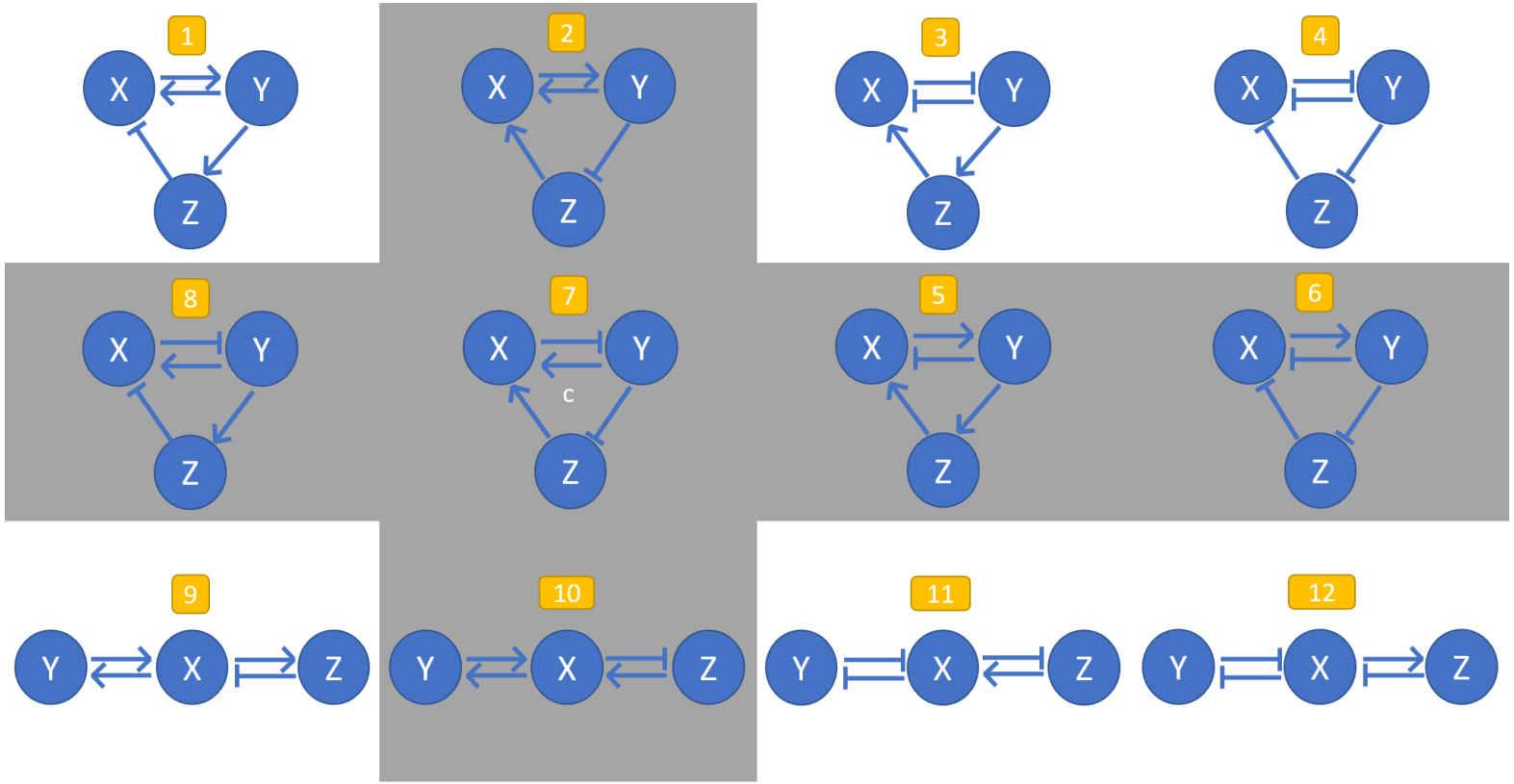
$$y = \kappa\frac{x^n}{1+x^n} + \beta_y$$

$$z = \alpha\frac{(x/K)^n}{1+(x/K)^n} + \beta_x$$

Excitability and 1-Parameter Diagram of Bifurcations



Network structures and excitability



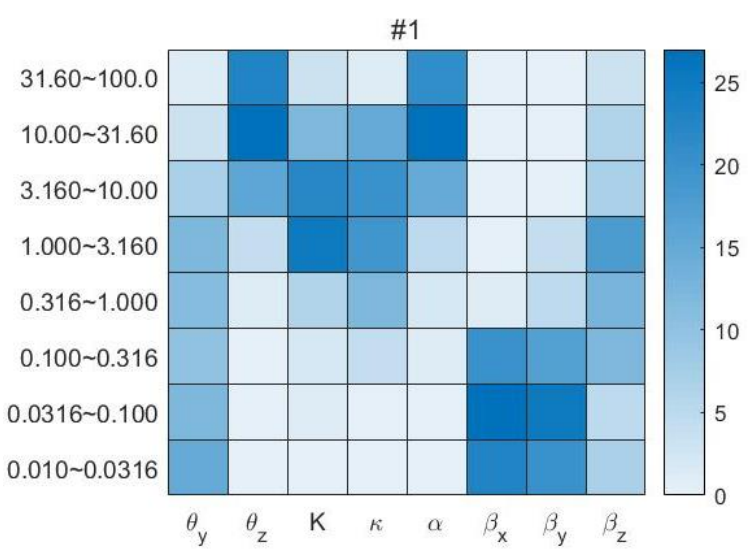
Conclusion:

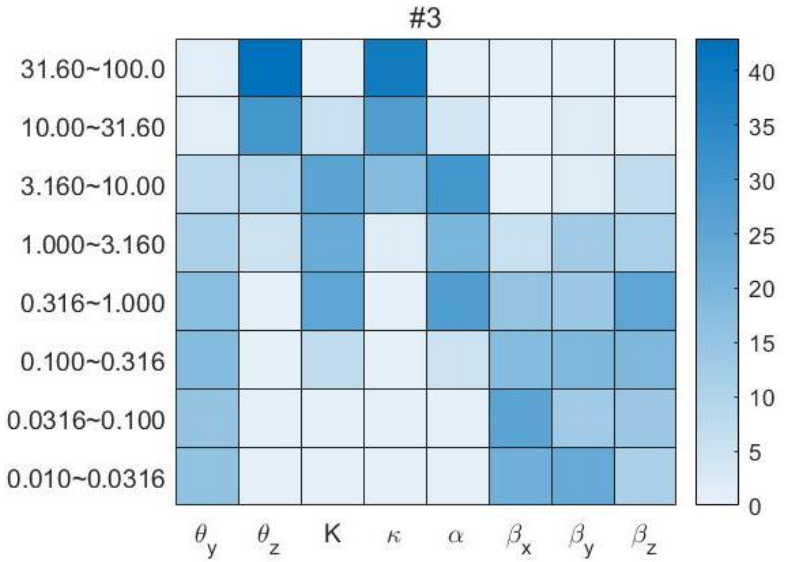
Six network structures with

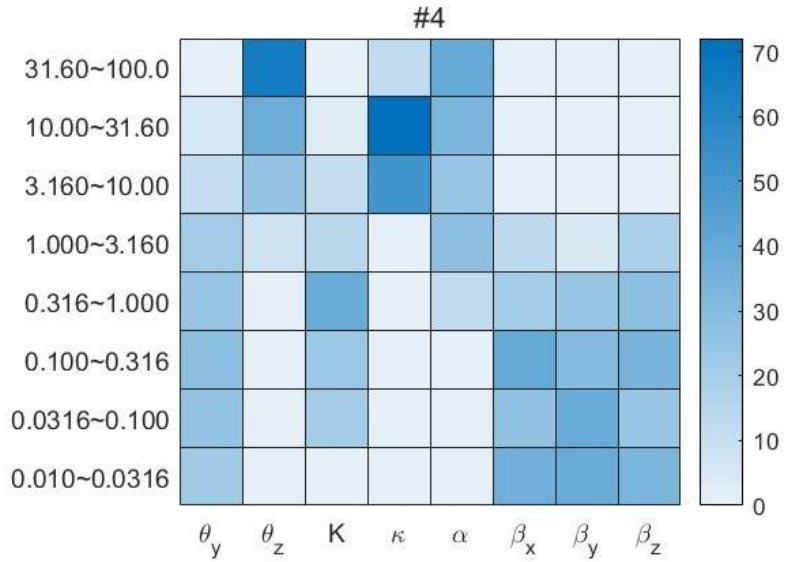
- (5-8) **positive feedback involving three nodes** or
- (2,7,10) **two nodes simultaneously promoting another node**

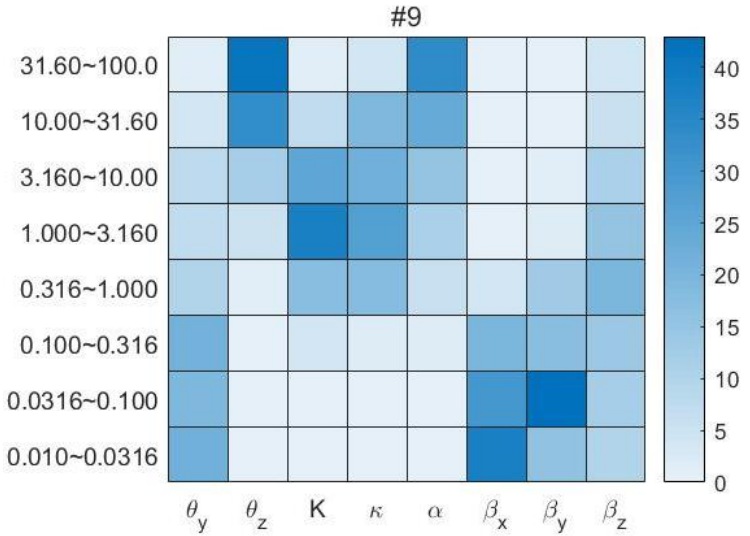
are difficult to achieve excitability, while the others can allow for excitability.

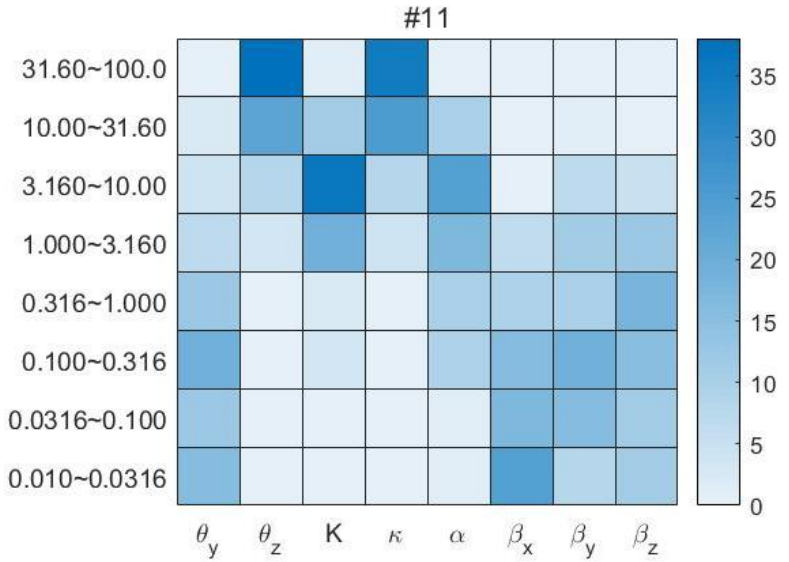
Parameter distribution of Excitable Networks

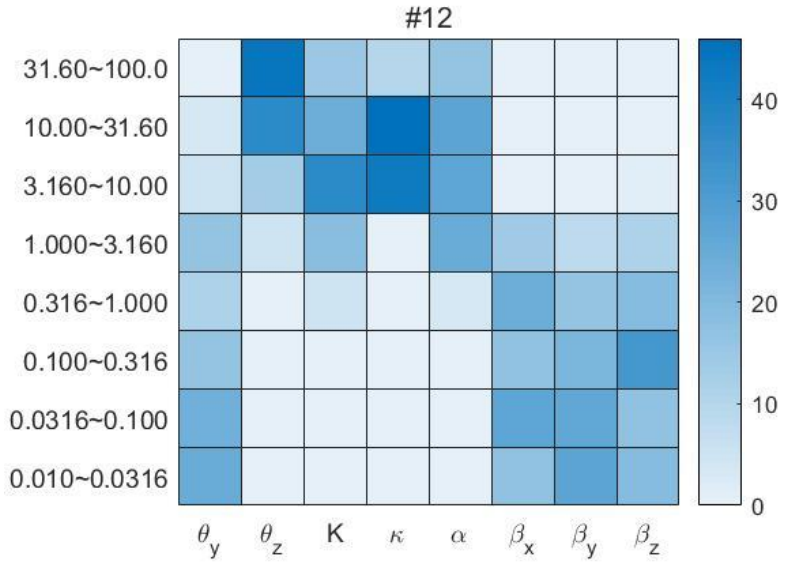




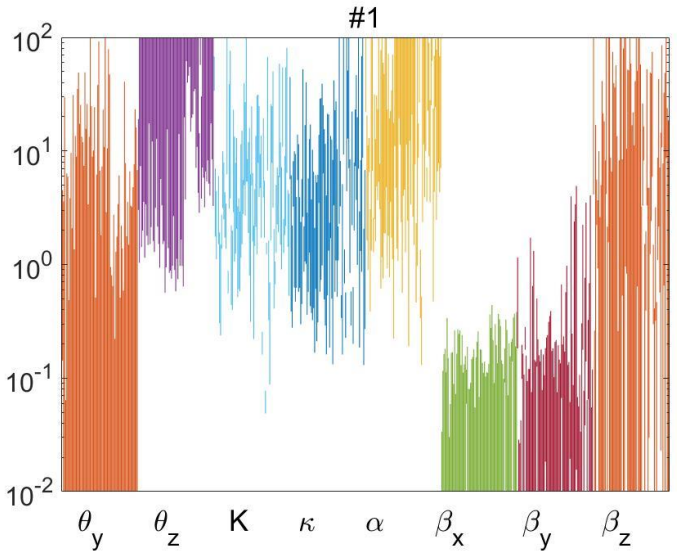


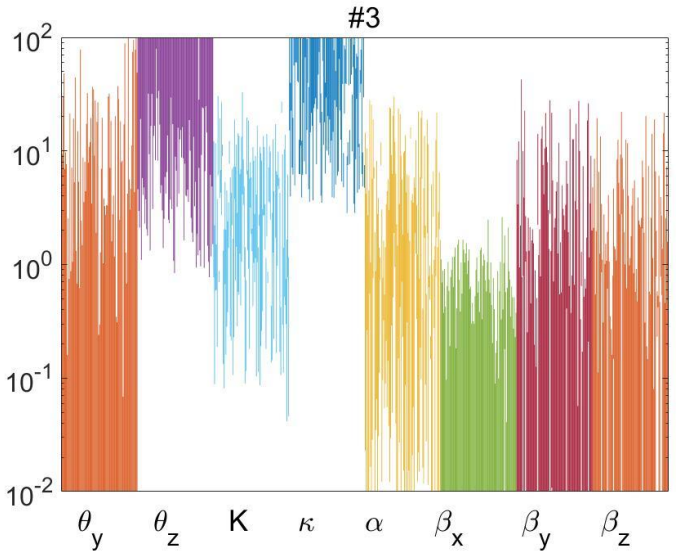


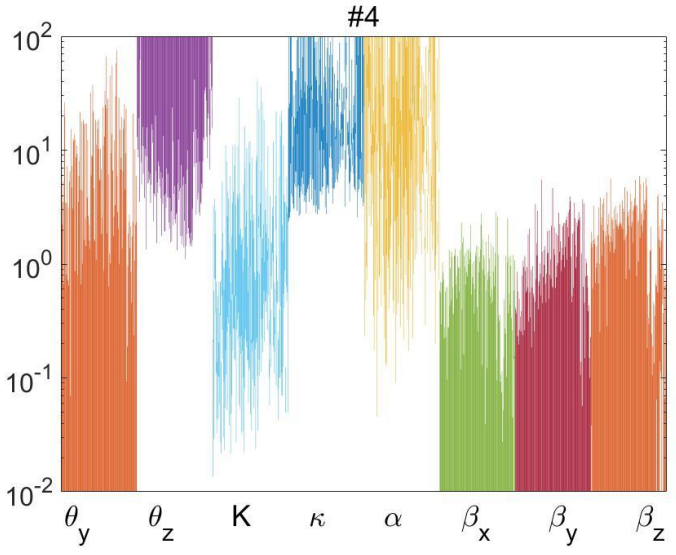


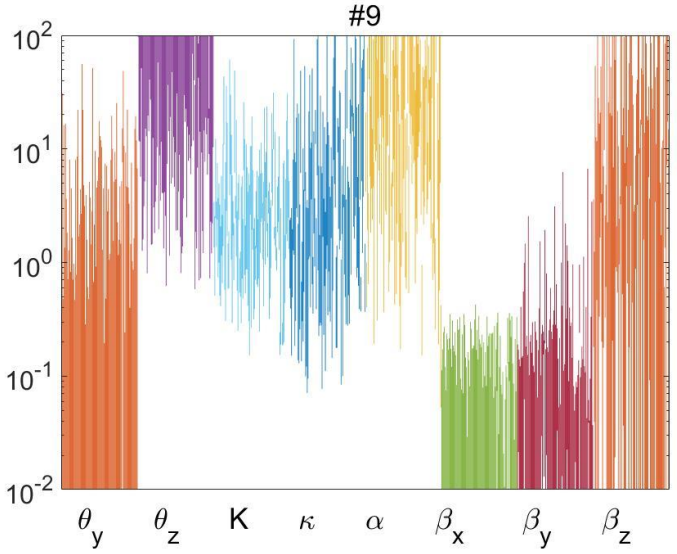


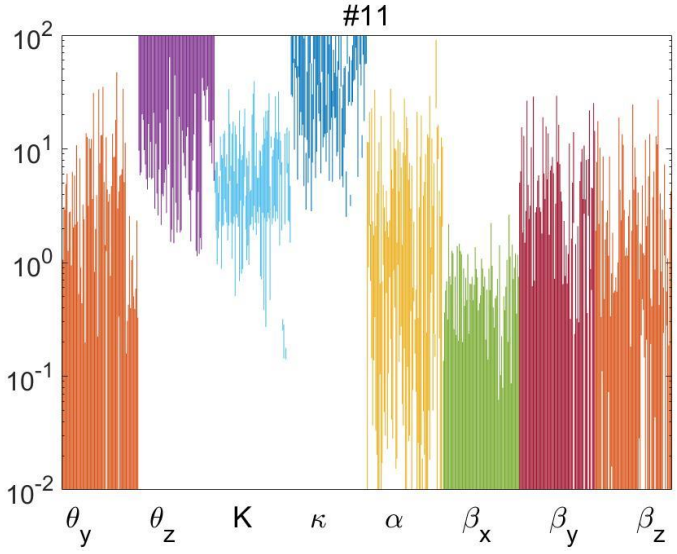
Parameter Sensitivity of Excitable Networks

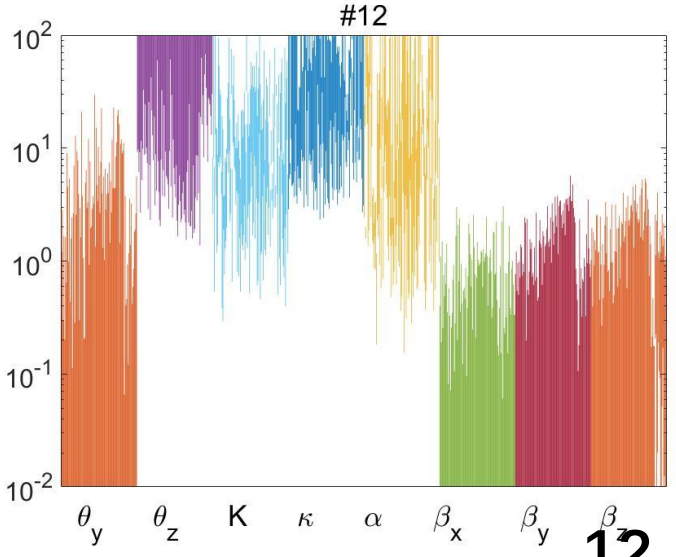






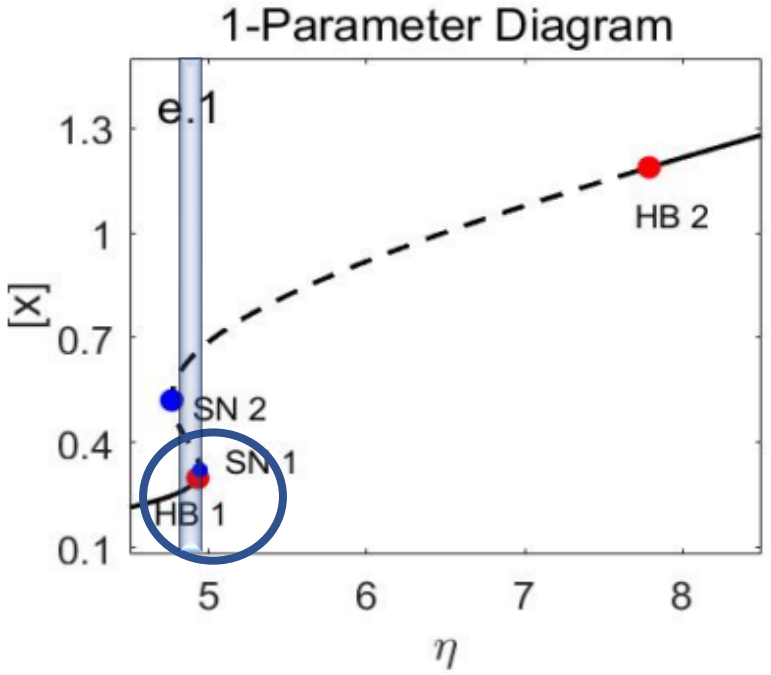


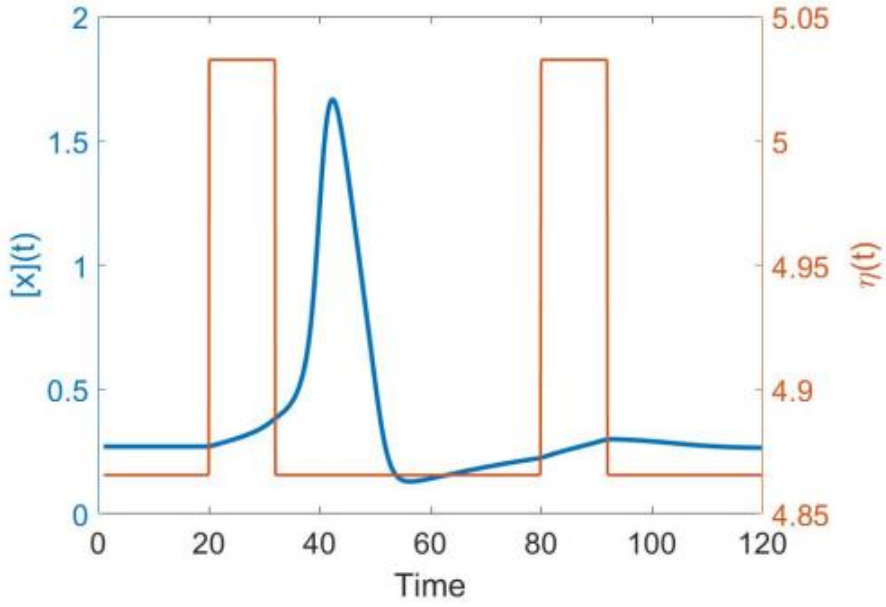




Hyperpolarization and Refractory Period

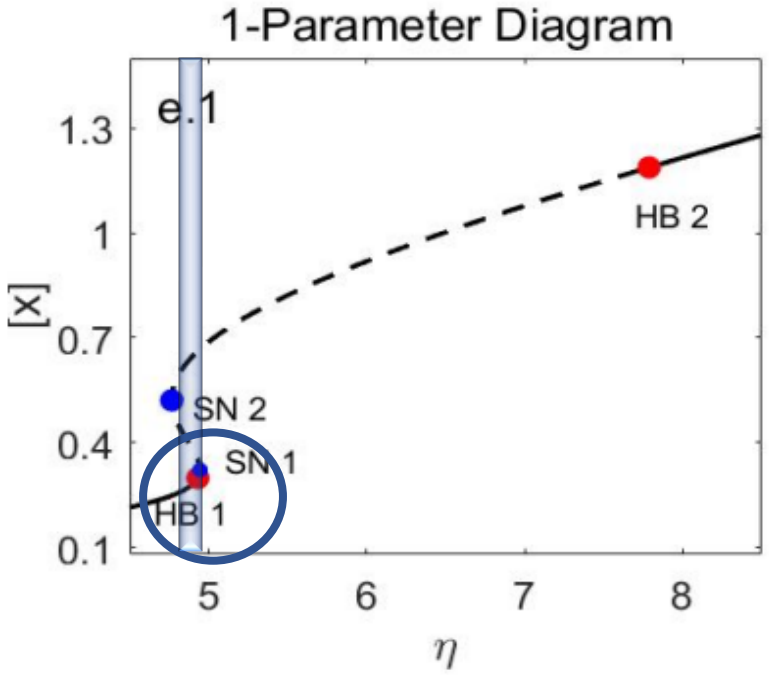
- Stable fixed point: stable node or stable focus
- **Stable node**: eigenvalues of Jacobian matrix are negative real numbers
- **Stable focus**: eigenvalues of Jacobian matrix include conjugate complex roots, and real parts of all eigenvalues are negative

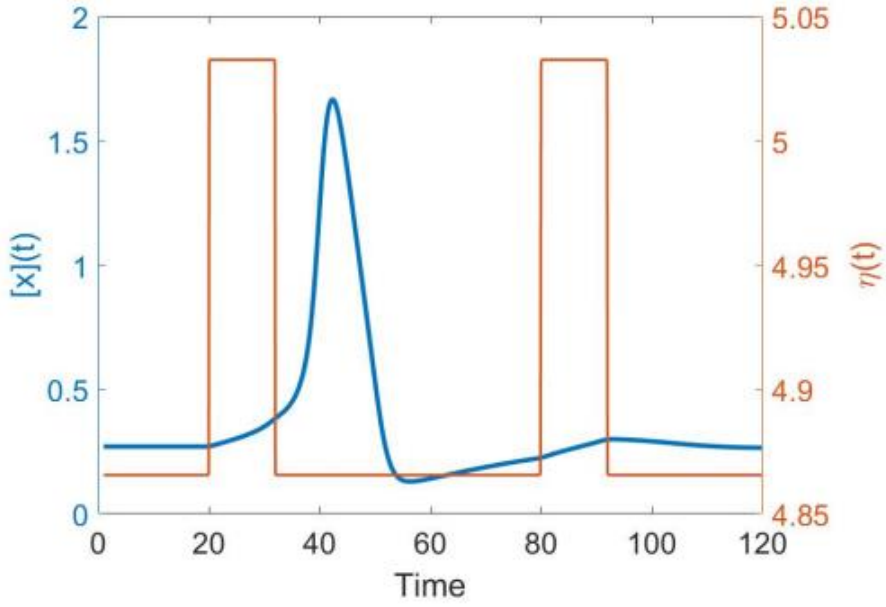




Hyperpolarization and Refractory Period

- If stable fixed point is a focus but not a node, vector filed circles around the stable fixed point, **hyperpolarization (draw on the black board)**
- During hyperpolarization, **refractory period**, restrict the frequency of excitement (e.g. **neuron's action potential**)





Excitability under Intrinsic Noise

- In biological systems, the intrinsic noise due to factors such as transcription can be ignored because the number of expressed proteins is relatively large and the dynamical properties of many networks are mainly regulated by the expression mean. But in some situations, we cannot ignore the effect of intrinsic noise…
- To analyse intrinsic noise, we cannot treat mRNA as quasi-static any more, based on the fact that **stochasticity of transcription** plays a much more important role than stochasticity of translation.
- Regulatory proteins' binding to DNA is still treated as quasi-static.
- Use **Gillespie algorithm** to simulate **all 15 reactions of transcription and translation** in a 3-node network.

Excitability under intrinsic noise

$$\frac{dx}{d\tau} = \eta \frac{y^{n1}}{1+y^{n1}+z^{n2}} - x + \beta_x$$
$$\theta_y \frac{dy}{d\tau} = \kappa \frac{x^{n3}}{1+x^{n3}} - y + \beta_y$$
$$\theta_z \frac{dz}{d\tau} = \alpha \frac{(x/K)^{n4}}{1+(x/K)^{n4}} - z + \beta_z$$

New parameters to decide (former parameters only gives the relationship between those new parameters). I refer to a paper of my reference to choose them.

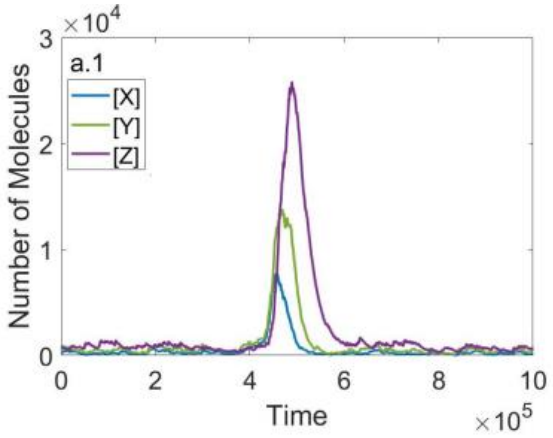
$$\frac{dm_x}{dt} = \Omega v_{mx} \frac{(\frac{Y}{K_{yx}})^n}{\Omega^n + (\frac{Y}{K_{yx}})^n + (\frac{Z}{K_{zx}})^n} - d_{mx} m_x + \Omega b_{mx}$$
$$\frac{dm_y}{dt} = \Omega v_{my} \frac{(\frac{X}{K_{xy}})^n}{\Omega^n + (\frac{X}{K_{xy}})^n} - d_{my} m_y + \Omega b_{my}$$
$$\frac{dm_z}{dt} = \Omega v_{mz} \frac{(\frac{[Y]}{K_{yz}})^n}{1 + (\frac{[Y]}{K_{yz}})^n} - d_{mz} m_z + b_{mz}$$
$$\frac{dX}{dt} = v_{px} m_x - d_{px} X$$
$$\frac{dY}{dt} = v_{py} m_y - d_{py} Y$$
$$\frac{dZ}{dt} = v_{pz} m_z - d_{pz} Z$$

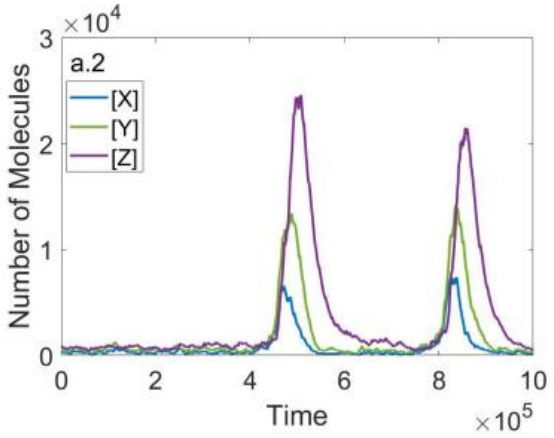
$$f(\Omega, v_{mx}, K_{yx}, K_{zx}, n) = \Omega v_{mx} \frac{(\frac{Y}{K_{yx}})^n}{\Omega^n + (\frac{Y}{K_{yx}})^n + (\frac{Z}{K_{zx}})^n}$$
$$g(\Omega, v_{my}, K_{xy}, n) = \Omega v_{my} \frac{(\frac{X}{K_{xy}})^n}{\Omega^n + (\frac{X}{K_{xy}})^n}$$
$$h(\Omega, v_{mz}, K_{yz}, n) = \Omega v_{mz} \frac{(\frac{[Y]}{K_{yz}})^n}{1 + (\frac{[Y]}{K_{yz}})^n}$$

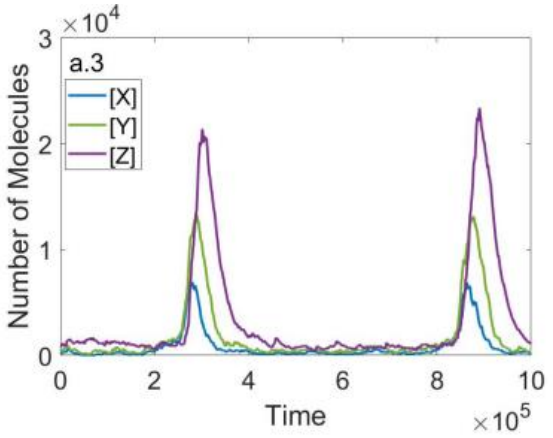
$$p_x^{const} \xrightarrow{\Omega b_{mx}} p_x^{const} + mRNA_X$$
$$p_x \xrightarrow{f(\Omega, v_{mx}, K_{yx}, K_{zx}, n)} p_x + mRNA_X$$
$$mRNA_X \xrightarrow{d_{mx}} \varnothing$$
$$p_y^{const} \xrightarrow{\Omega b_{my}} p_y^{const} + mRNA_Y$$
$$p_y \xrightarrow{g(\Omega, v_{my}, K_{xy}, n)} p_y + mRNA_Y$$
$$mRNA_Y \xrightarrow{d_{my}} \varnothing$$
$$p_z^{const} \xrightarrow{\Omega b_{mz}} p_z^{const} + mRNA_Z$$
$$p_z \xrightarrow{h(\Omega, v_{mz}, K_{yz}, n)} p_z + mRNA_Z$$
$$mRNA_Z \xrightarrow{d_{mz}} \varnothing$$
$$mRNA_X \xrightarrow{v_{px}} mRNA_X + X$$
$$X \xrightarrow{d_{px}} \varnothing$$
$$mRNA_Y \xrightarrow{v_{py}} mRNA_Y + Y$$
$$Y \xrightarrow{d_{py}} \varnothing$$
$$mRNA_Z \xrightarrow{v_{pz}} mRNA_Z + Z$$
$$Z \xrightarrow{d_{pz}} \varnothing$$

Fixed Points and Activation of auto-excitement

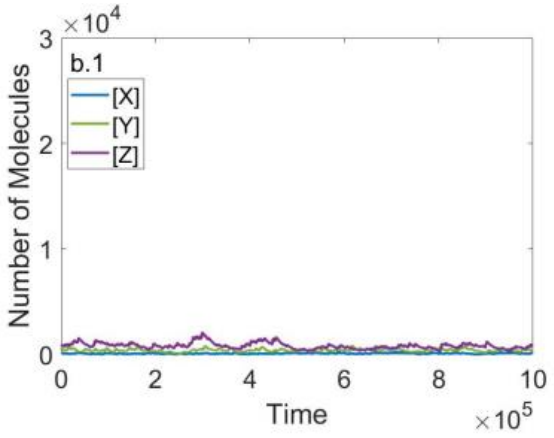
v_{mx} = 0.07 (η=14): Stable node near "excited" side of the saddle, easier for auto-excitement

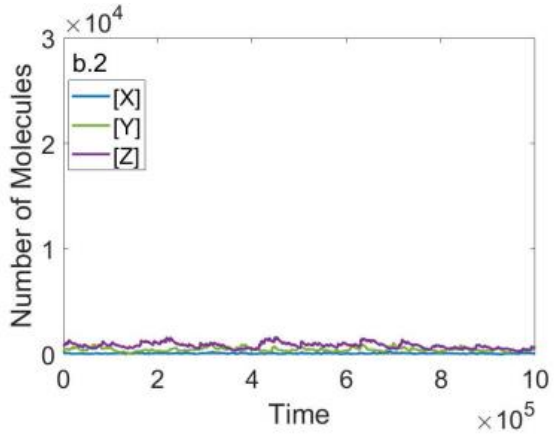


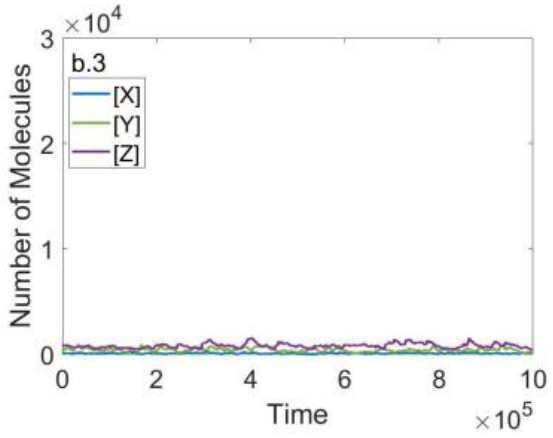


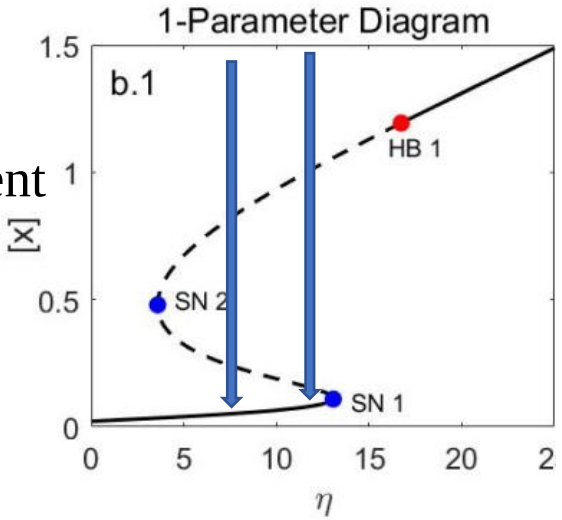


v_{mx} = 0.037 (η=7.5): Stable node far from "excited" side of the saddle, no auto-excitement

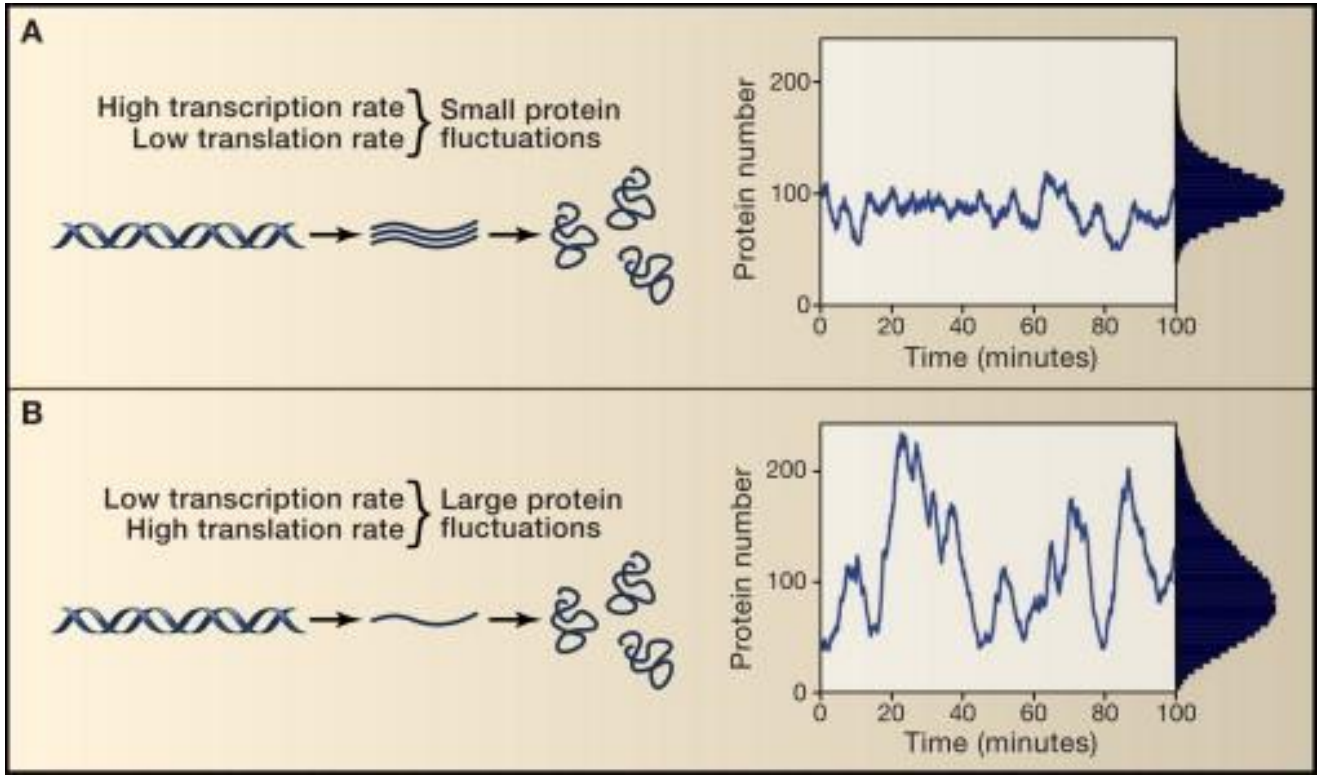








Rates of transcription and translation and intrinsic noise



$$\frac{dm_x}{dt} = \Omega v_{mx} \frac{(\frac{Y}{K_{yx}})^n}{\Omega^n + (\frac{Y}{K_{yx}})^n + (\frac{Z}{K_{zx}})^n} - d_{mx} m_x + \Omega b_{mx}$$

$$\frac{dm_y}{dt} = \Omega v_{my} \frac{(\frac{X}{K_{xy}})^n}{\Omega^n + (\frac{X}{K_{xy}})^n} - d_{my} m_y + \Omega b_{my}$$

$$\frac{dm_z}{dt} = \Omega v_{mz} \frac{(\frac{[Y]}{K_{yz}})^n}{1 + (\frac{[Y]}{K_{yz}})^n} - d_{mz} m_z + b_{mz}$$

$$\frac{dX}{dt} = v_{px} m_x - d_{px} X$$

$$\frac{dY}{dt} = v_{py} m_y - d_{py} Y$$

$$\frac{dZ}{dt} = v_{pz} m_z - d_{pz} Z$$

×m

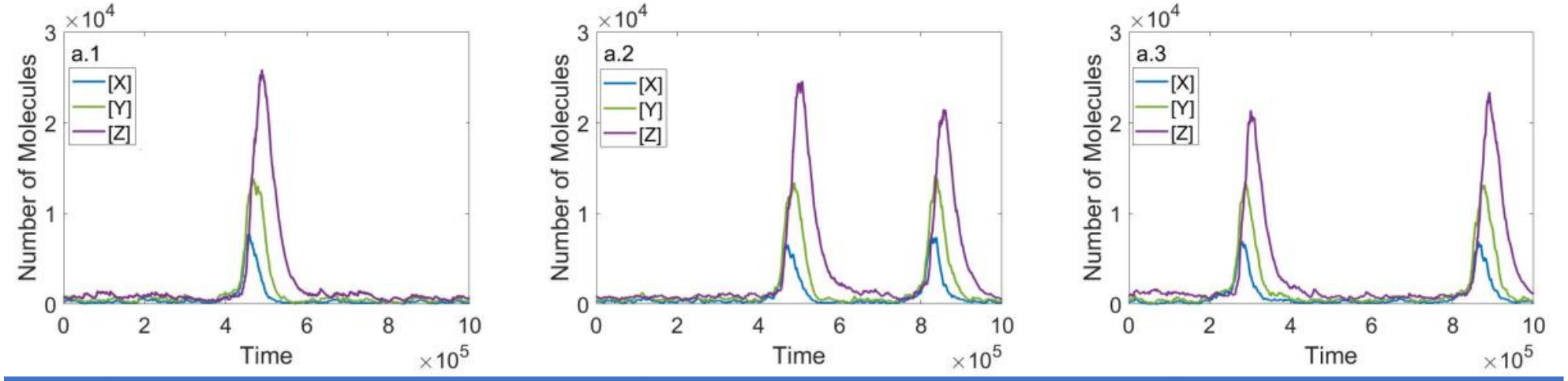
Same Mean

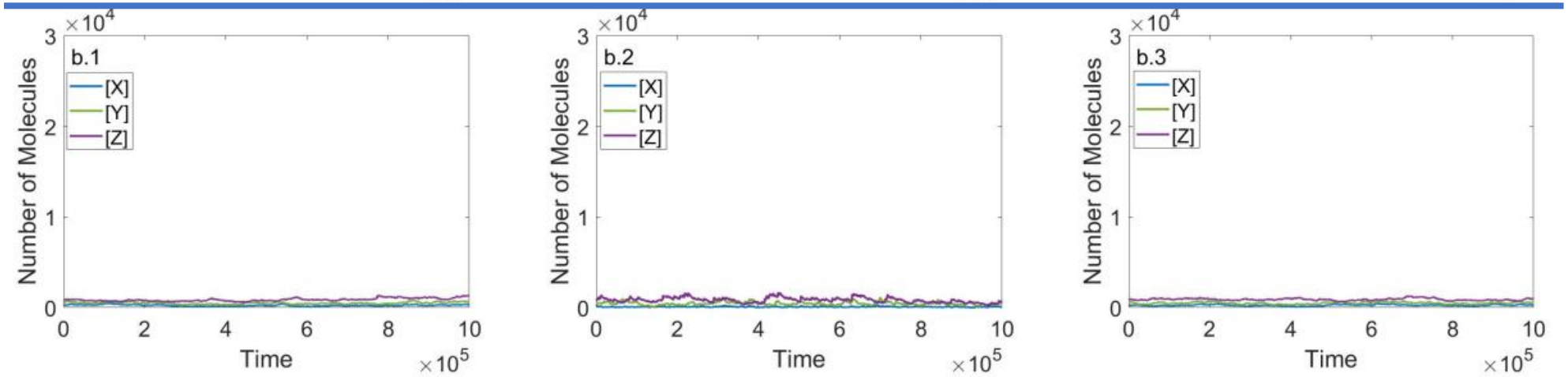
×n

When the transcription rate is high, variability in protein levels is low (A), but when the transcription rate is lowered and the translation rate is raised, gene expression is far noisier (B), even at the **same mean** (Ozbudak et al. (2002))

Rates of transcription and translation and intrinsic noise

Transcription: ×0.5 Translation: ×2, easier for auto-excitement

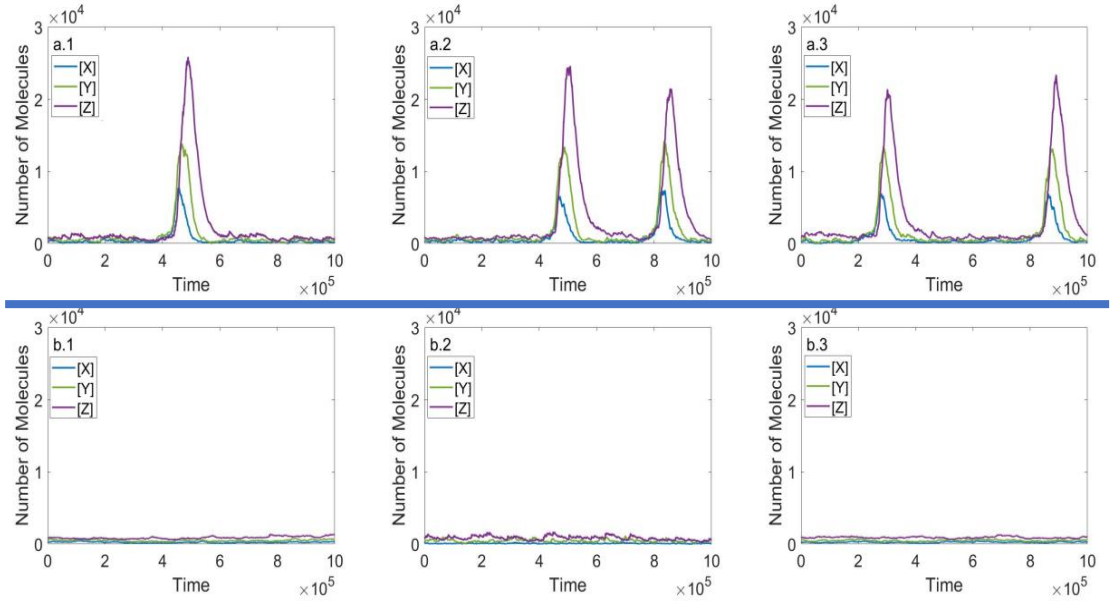




Transcription: ×1 Translation: ×1, no auto-excitement

Rates of transcription and translation and intrinsic noise

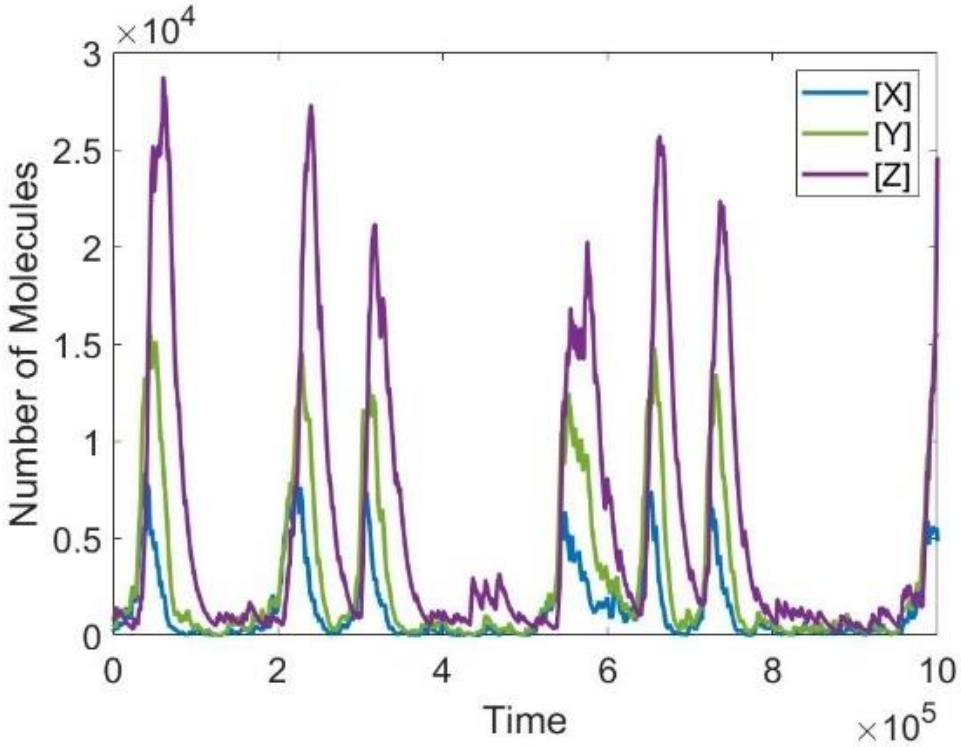
Transcription: ×0.5 Translation: ×2



Transcription: ×1 Translation: ×1

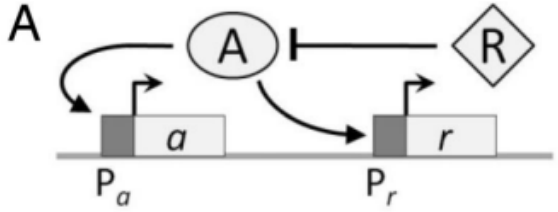
Transcription: ×0.25 Translation: ×4

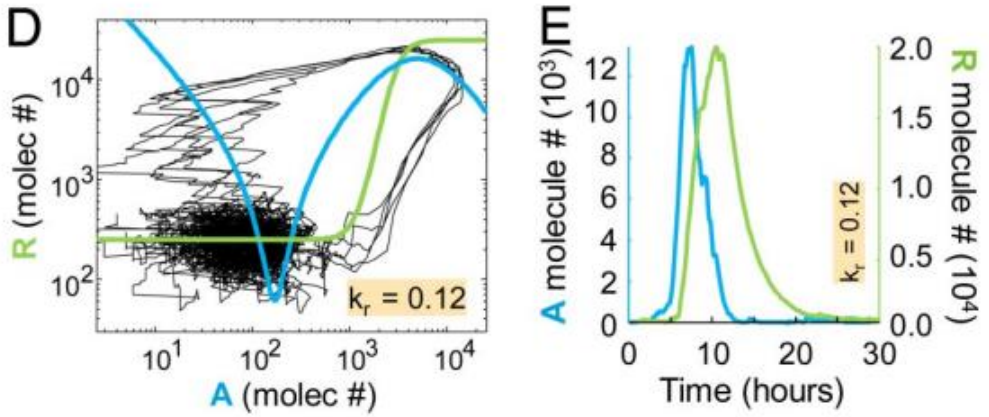
A lot of auto-excitement

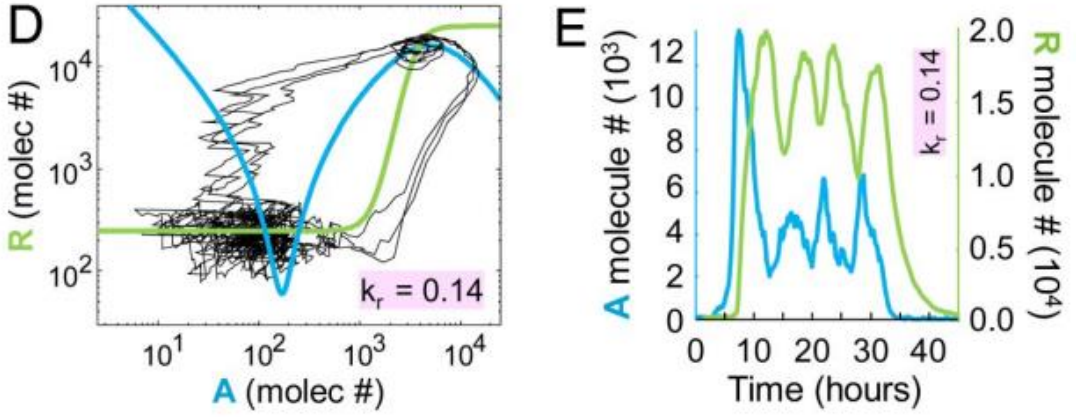


A genetic timer induced by intrinsic noise

- Marc Turcotte et al. constructed **a genetic timer** with network interlinked PFL & NFL. (The timer behaviour is achieved by intrinsic noise, and can't be achieved in deterministic models!)
- The unstable state is stabilized by **intrinsic noise**: Normally trajectories cross the unstable focus and heads to the rest state directly, but under certain conditions, the trajectories may **circle around the unstable focus for several times**.
- Similar time spend for each circle, **polymodal distribution of duration time**.
- This new behaviour may be an inspiration in synthetic biology.







A genetic timer induced by intrinsic noise

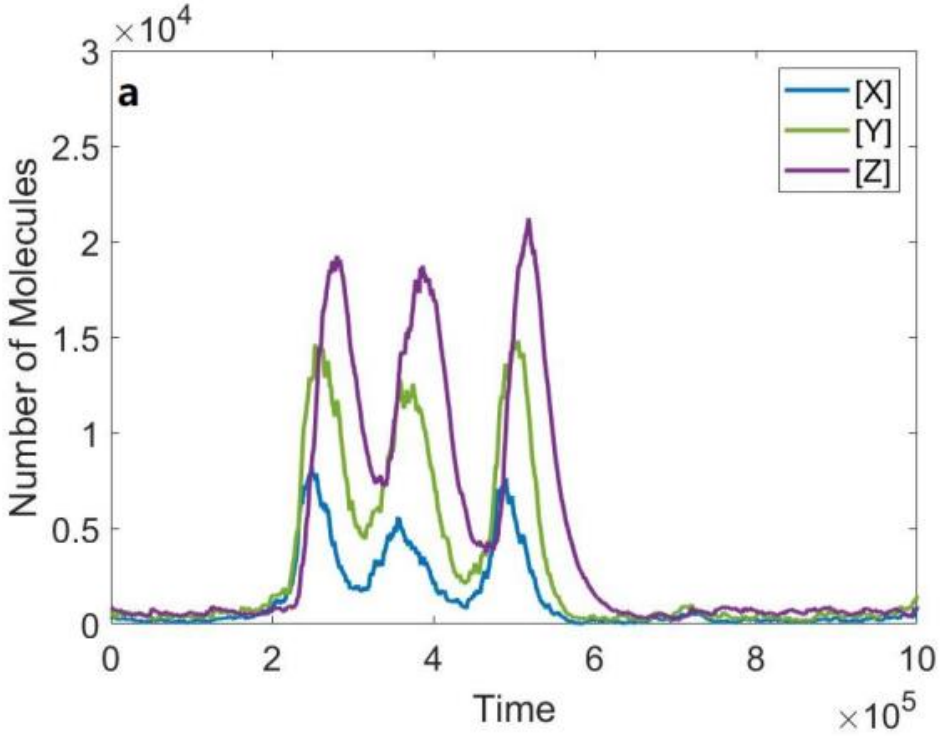
Mathematical essence for a genetic timer:

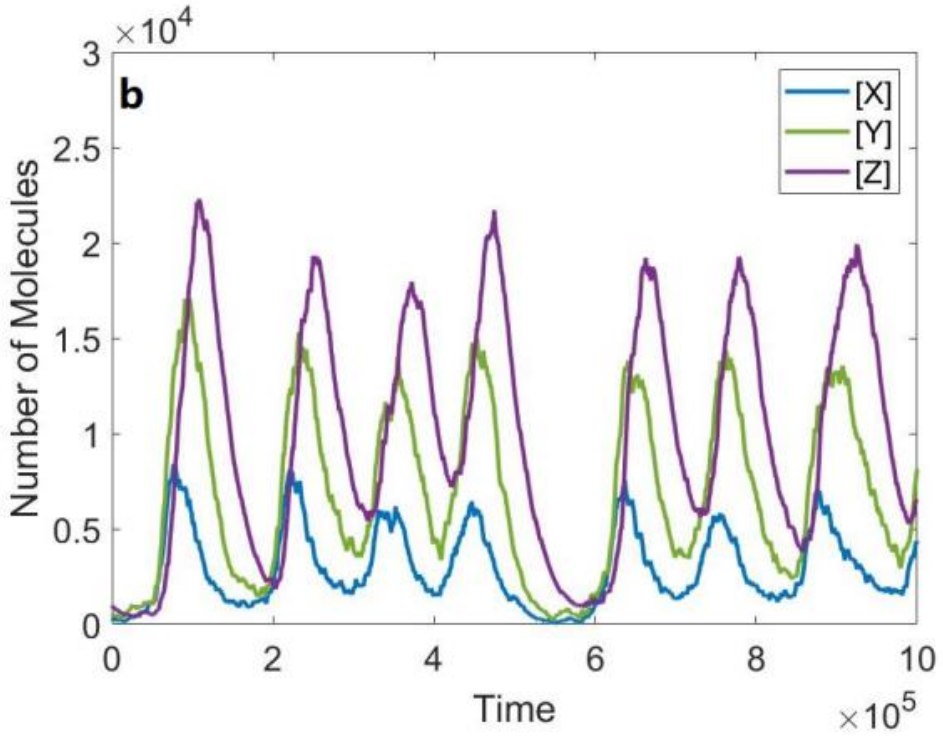
- Able to trigger auto-excitement: appropriate relative rates for transcription and translation, “excited site” of the saddle sufficiently near the stable state.
- **Small absolute value** for the conjugate eigenvalues of the **unstable focus**: more likely to “circle around”

With this information, I can also build a genetic timer in my 3-node network by simple steps of adjusting parameters.

Explain an example on the blackboard.

A genetic timer induced by intrinsic noise





Summary

- Constructed mathematical model of 3-node genetic networks of interlinked PFL and NFL (Regulated on transcription level)
- Fixed points and excitability.
- Among 12 structures, only 6 structures are excitable.
- Parameter distribution of excitable networks.
- Stable fixed point: node or focus. Focus: hyperpolarization and refractory period.
- Intrinsic noise and auto-excitement: relationship between fix points.
- Intrinsic noise and auto-excitement: relationship between rates of transcription and translation.
- Intrinsic noise to achieve a “genetic timer”.

thanks