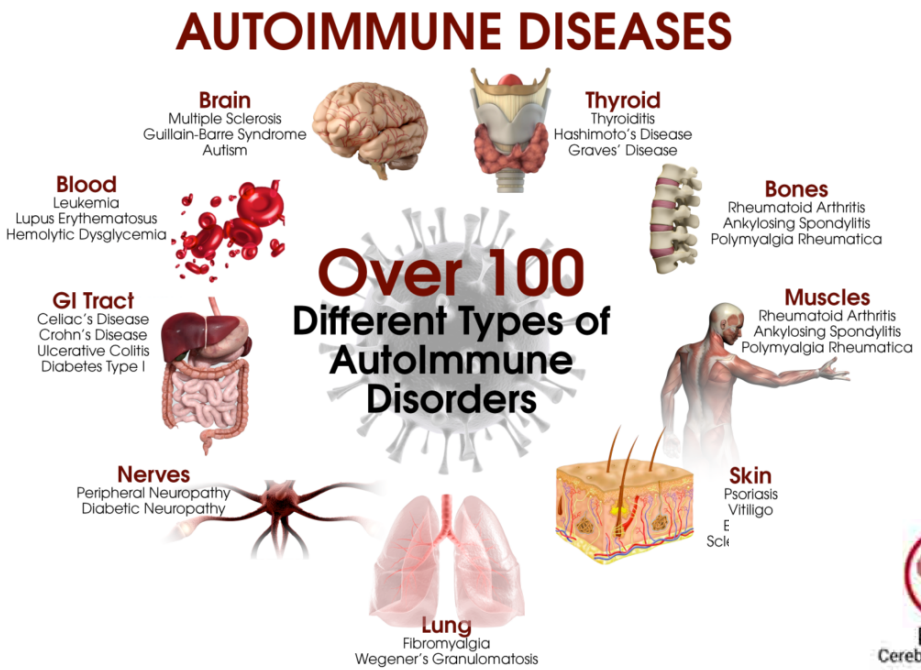
Unraveling the sensitivity of two motif structures under random perturbation

Samrat Chatterjee
Drug Discovery Research Centre,
Translational Health Science and Technology Institute,
NCR biotech science cluster,
Faridabad-121001, India.

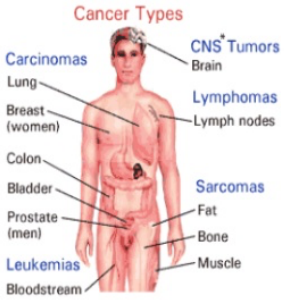
With
Mr. Suvankar Halder and Dr. Nandadulal Bairagi

Diseases caused by cell signaling error

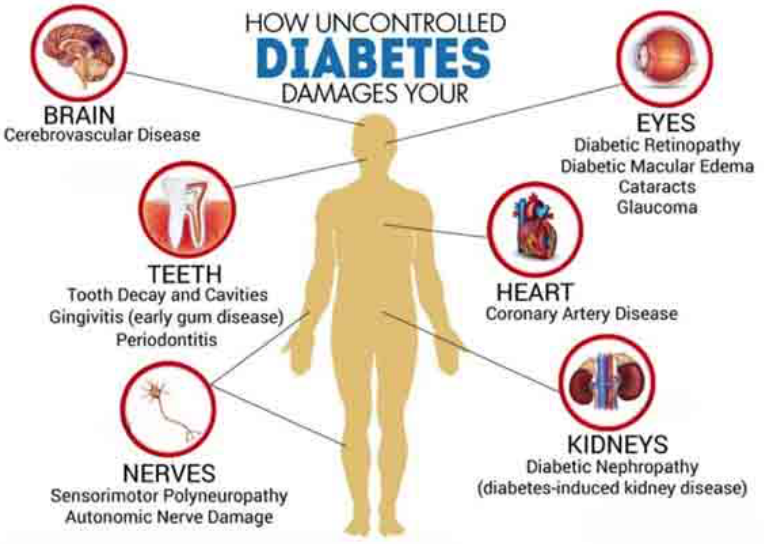


Different Types of Cancer:

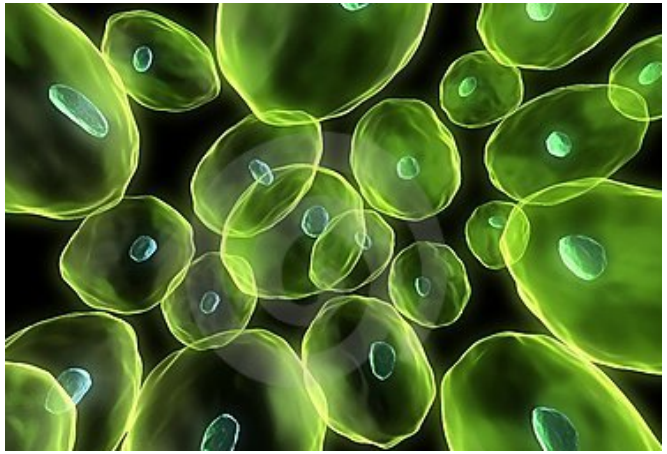
- Bone cancer
- Breast cancer
- Eye cancer
- Kidney cancer
- Leukemia cancer
- Lung cancer
- Neuroblastoma
- Skin cancer
- Prostate

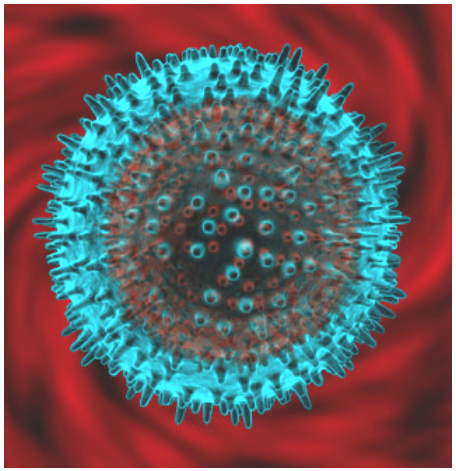


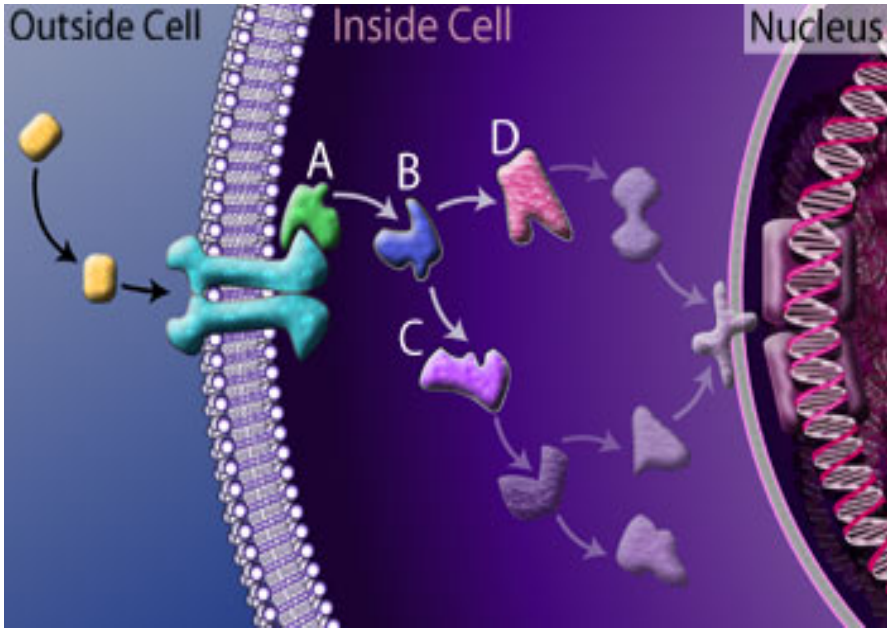
3



Cell signaling is part of a complex system of **communication** that **governs basic cellular activities** and coordinates cell actions.







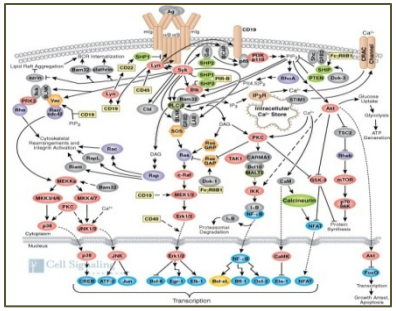
The ability of cells to perceive and correctly respond to their micro-environment is the basis of **development, tissue repair**, and **immunity** as well as **normal tissue homeostasis**.

Errors in cellular information processing are responsible for diseases such as **cancer, autoimmunity**, and **diabetes.**

By understanding cell signaling, diseases may be treated effectively.

Background work: Unraveling the Design Principle for Motif Organization in Signaling Networks *

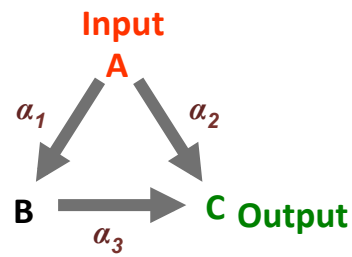
Aim: To understand the motif organization in filtering noise in the signal.



REF: Ma W, Trusina A, El-Samad H, Lim WA, Tang C (2009) .Cell 138: 760-773.

Motif Type	Feature
Coherent I	a1>0, a3>0, a2>0
Incoherent I	a1>0, a3<0, a2>0
Coherent II	a1<0, a3>0, a2<0
Incoherent II	a1<0, a3<0, a2<0
Coherent III	a1>0, a3<0, a2<0
Incoherent III	a1>0, a3>0, a2<0
Coherent IV	a1<0, a3<0, a2>0
Incoherent IV	a1<0, a3>0, a2>0

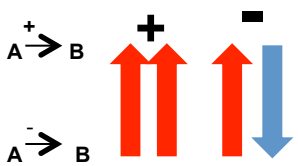
Feed-forward loop (FFL)



A+B (Inactive) → B(active)

B (Active form) ⇄ (1-B) (Inactive form)

B(active) + B(Inactive) = B(tot) = 1



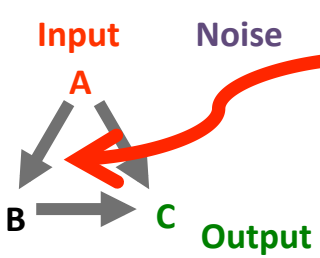
$$\frac{dA}{dt} = \alpha A\left(1-\frac{A}{\beta}\right) \equiv F_1(A,B,C),$$

$$\frac{dB}{dt} = k_1 - \delta_1 B + a_1 A(1-B) \equiv F_2(A,B,C),$$

$$\frac{dC}{dt} = k_2 - \delta_2 C + a_2 A(1-C) + a_3 B(1-C) \equiv F_3(A,B,C),$$

Stability result:

Very robust and unconditionally stable.

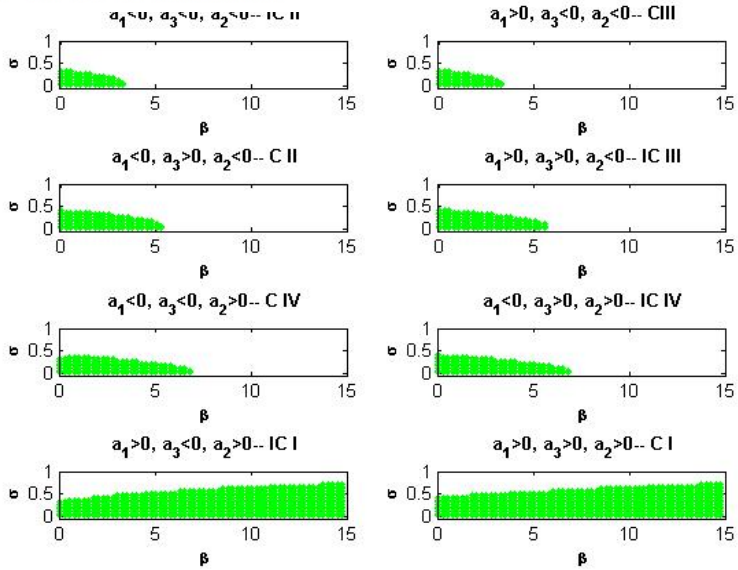


Generation of **inappropriate and non-specific r responses** that may potentially be induced through either noise in the external milieu, or through stochastic perturbations of the intracellular components (e.g. **mutations, alterations in protein turn-over rates** etc). May cause Disease like **cancer, autoimmunity** etc

$$dA = F_1(A,B,C)dt + \sigma_1(A - A^*)d\xi_t^1$$
$$dB = F_2(A,B,C)dt + \sigma_2(B - B^*)d\xi_t^2$$
$$dC = F_3(A,B,C)dt + \sigma_3(C - C^*)d\xi_t^3$$

*** Chatterjee and Kumar. (2011) Plos One (6(12): e28606)**

Ranked structure based on stability area (2D parameter space)



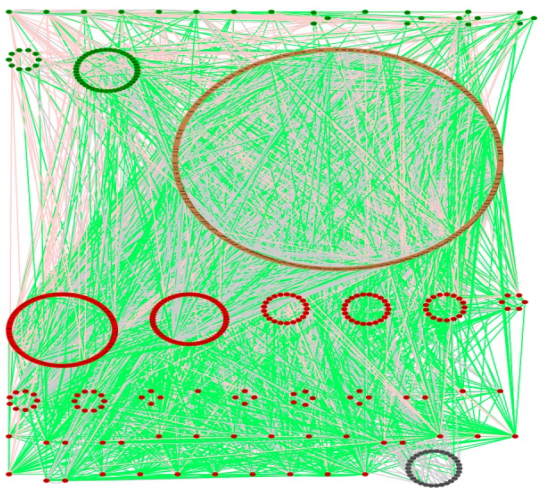
Stable region in sigma-beta plane

Larger the stability region, more robust the structure

Rank 1	Rank2	Rank 3	Rank 4	Rank 5	Rank 6	Rank 7	Rank 8
-4	-3	-2	-1	1	2	3	4

Scoring of signaling intermediates for their vulnerability to noise in a human cancer signaling network*

*Cui Q, Ma Y, Jaramillo M, Bari H, Awan A, et al. (2007) A map of human cancer signaling. Mol Syst Biol 3: 152.



Human Signaling Network
(1634 nodes and 5089 interactions)

↓

Search for Frequency of nodes
in various 3 node FFLs

↓

Cumulative Score for
Individual nodes
(Based on ranks of FFLs in Table)

↓

Dividing the network node as
robust category or **vulnerable category**
(depending on their scores)

Example of scoring:

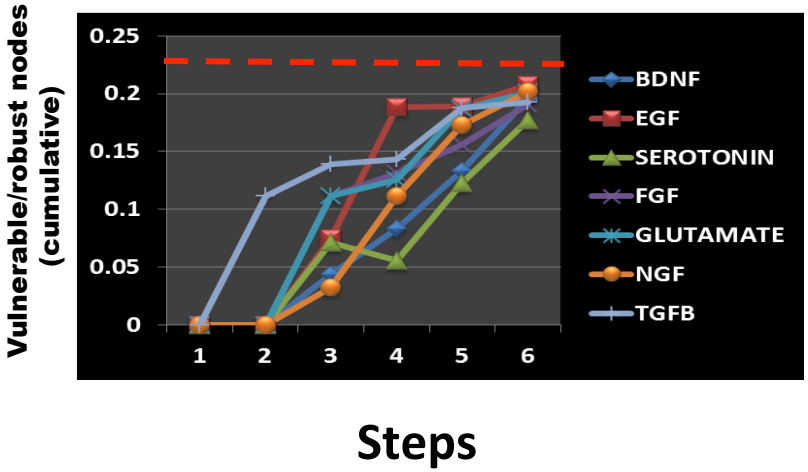
Node A---occur in motif of rank 8 with frequency 2+ motif of rank 2 with frequency 3, then

Total rank= 2*r(8)+3*r(5) **(>4: robust, <- 4: vulnerable)**

Background Mathematics

➢Observations (from the cancer signaling network)

1.



Starting with different receptor molecules and going 6 steps downstream we observed that early steps of the network are significantly enriched with the robust nodes.

2.

Node Function	Robust Nodes (%)	Vulnerable Nodes (%)
Kinase	28.08	8
Transcription Factor	12.35	6.67
Receptor	9.87	8
Adaptor	6.79	4
Phosphatases	1.54	12
Apoptosis	3.08	22.67

Robust

Vulnerable

3. The genes whose mutations are associated with cancer **(e.g. ABL1, BRCA1, SRC, ATM, BRAF, PTEN, TGFBR2, EGFR, RB, p53, SMAD4 etc)** belongs to the robust category.

Limitations and future expansion:

Limitations:

During our study we restricted our work **to three node FFLs**, but other categories of motifs, like **Feed Back Loops, Four-node FFLs, and Bifans** are also present in the signaling network.
Consequently, an extension of the analysis described here to **incorporate all such motifs can clearly be expected to provide many additional insights** into the regulatory aspects of signal transduction.

Moreover just to understand the system we simplify the model assuming white noise added to the deterministic system. There are **many other ways to incorporate noise and to extend the model depending on the system**.

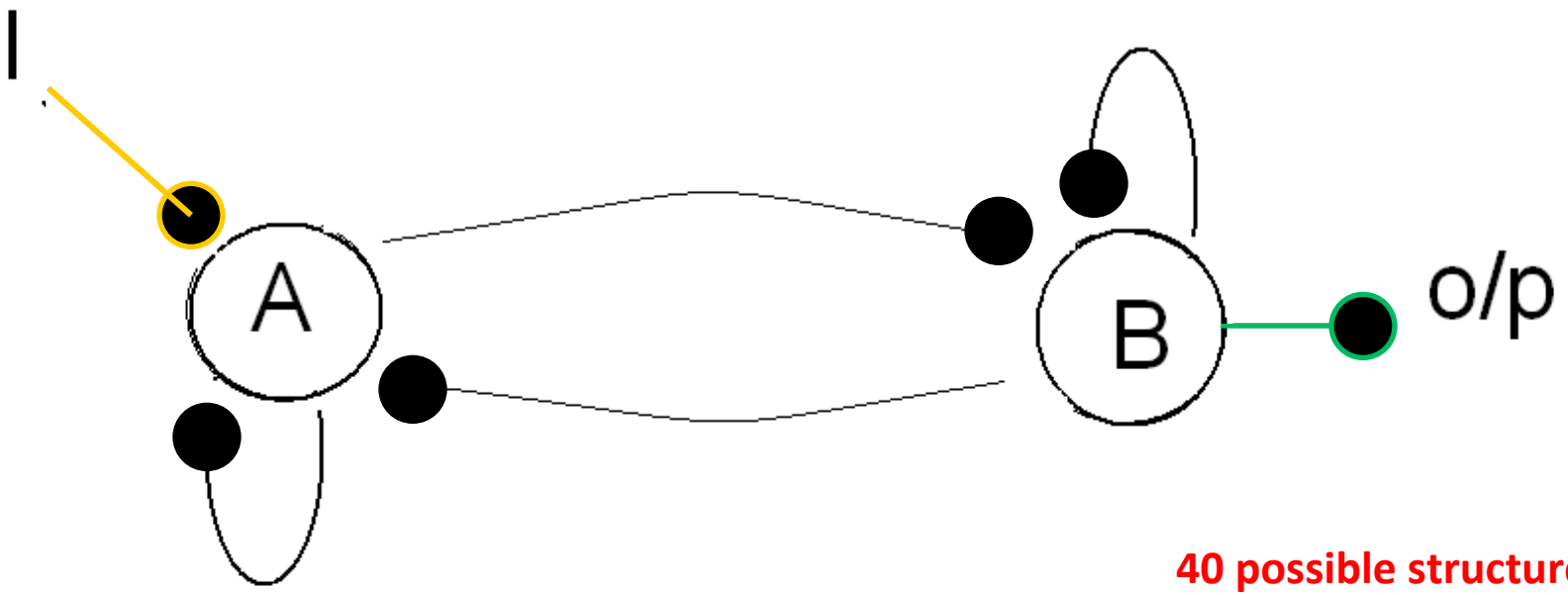
Future expansion:

Extensive study on all possible two node motif structure is done and the most dynamically enriched structure is identified.

Those **dynamically enriched structure will start with all possible two-nodes and then be expanded to two nodes** and then to four nodes.

Finally stochastic perturbations will be studied on them to identify the sensitive node among a more general structure.

Two node general structure

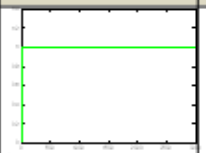
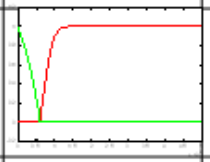
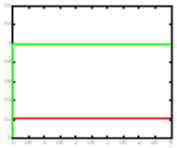
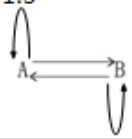
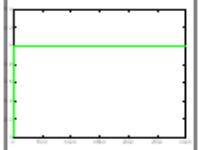
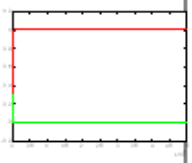
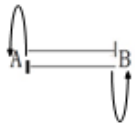


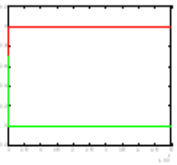
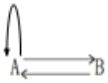
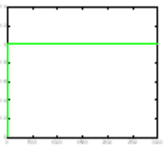
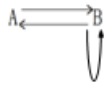
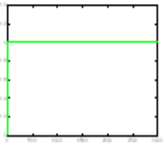
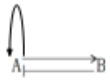
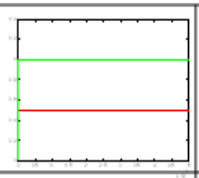
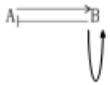
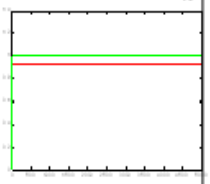
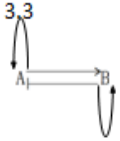
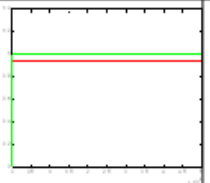
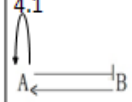
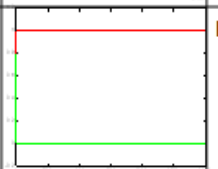
40 possible structures

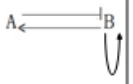
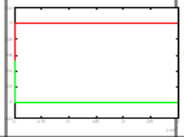
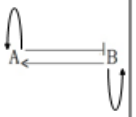
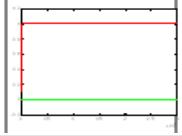
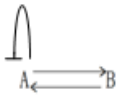
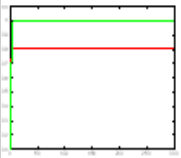
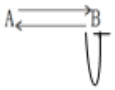
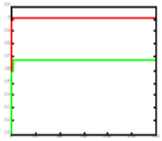
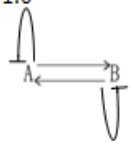
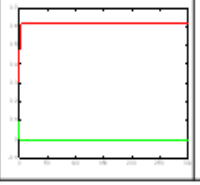
$$\frac{dA_P}{dt} = \frac{k_1.i.(1-A_P)}{k_{m1}+(1-A_P)} + \frac{k_2.B_P.(1-A_P)}{k_{m2}+(1-A_P)} + \frac{k_5.A_P.(1-A_P)}{k_{m5}+(1-A_P)}$$

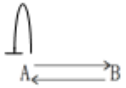
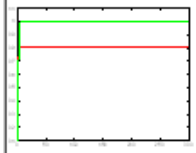
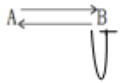
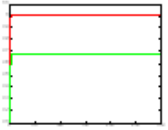
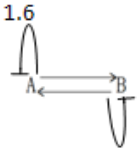
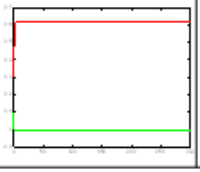
$$\frac{dB_P}{dt} = \frac{k_3.A_P.(1-B_P)}{k_{m3}+(1-B_P)} + \frac{k_4.B_P.(1-B_P)}{k_{m4}+(1-B_P)}$$

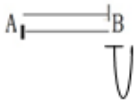
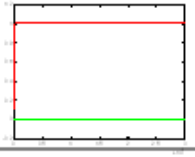
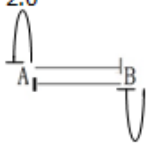
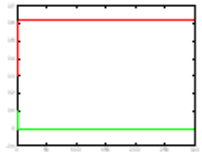
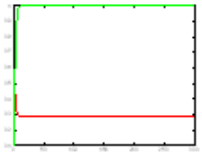
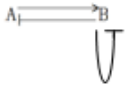
thsti ट्रांसलेशनल स्वास्थ्य विज्ञान एवं प्रौद्योगिकी संस्थान TRANSLATIONAL HEALTH SCIENCE AND TECHNOLOGY INSTITUTE

Structure of Model (input I at each A)	Fixed points	Nature of polynomial	B_p=f(I) ; nature of roots (ouptut)	Figure(s) from numerical simulations	Parameters
1.	$A_p = 1$ $B_p = 1$	--------	For any +ve value of I ; Bp = 1		Default set (all k's=1 and km's=0.5;i=5)
2.	Ap=1 Bp=0	--------	For any +ve value of I; Bp=0		Initial Bp=1 Initial Ap=0.000006 i=0.00005
3.	Bp=1 Ap=roots of eqn	Quadratic eqn in Ap	For any +ve value of I; Bp=1		Default with I = 0.5; Two +ve roots out of which one feasible.
1.3 	Ap=1 Bp=1		For any +ve value of I; Bp=1		Default set
2.1	Ap=1 Bp=0		For any +ve value of I; Bp=0		Default set
2.2			Unknown nature (		
2.3 			Unknown nature		

4.	Ap=1 Bp=0		For any +ve value of I; Bp=0		Default set
1.1 	Ap=1 Bp=1		For any +ve value of I; Bp=1		Default set
1.2 	Ap=1 Bp=1		For any +ve value of I; Bp=1		Default set
3.1 	Bp=1 Ap= roots of polynomial	Fourth order polynomial in Ap	For any +ve value of I; Bp=1		Default set
3.2 	Bp=1 Ap= roots of polynomial	Second order polynomial in Ap(atleast one +ve root exists)	For any +ve value of I; Bp=1		Default set
3.3 	Bp=1 Ap= roots of polynomial	Fourth order polynomial in Ap	For any +ve value of I; Bp=1		Default set
4.1 	Bp=0 Ap=1		For any +ve value of I; Bp=0		Default set

4.2 	Ap=1,Bp=0 And Ap=1,Bp= roots of second order equation devoid of I	Quadratic in Bp with coefficients containing only parameters and not I.	Steady state value of Bp depends on parameters and independent of i. **OR** it is 0		←Default set (quad eqn gives imaginary roots in this case)
4.3 	Ap=1,Bp=0 And Ap=1,Bp= roots of second order equation devoid of I	Quadratic in Bp with coefficients containing only parameters and not I.	Steady state value of Bp depends on parameters and independent of i. **OR** it is 0		←Default set
1.4 	Bp=1 Ap=roots of 4th order eqn	4th order polynomial in Ap	Bp=1 for any I		Default set with i=0.8
1.5 	Bp=0.68 Ap=1	Cubic eqn in Bp	Bp independent of I (depends on just parameters)		Default set with i=0.8
1.6 			Unknown nature!		
2.4	Bp=0 Ap=roots of cubic	Cubic in Ap	Bp=0 for any I		Default set with i=0.8

1.4 	Bp=1 Ap=roots of 4th order eqn	4th order polynomial in Ap	Bp=1 for any I		Default set with i=0.8
1.5 	Bp=0.68 Ap=1	Cubic eqn in Bp	Bp independent of I (depends on just parameters)		Default set with i=0.8
1.6 			Unknown nature!		
2.4	Bp=0 Ap=roots of cubic	Cubic in Ap	Bp=0 for any I		Default set with i=0.8

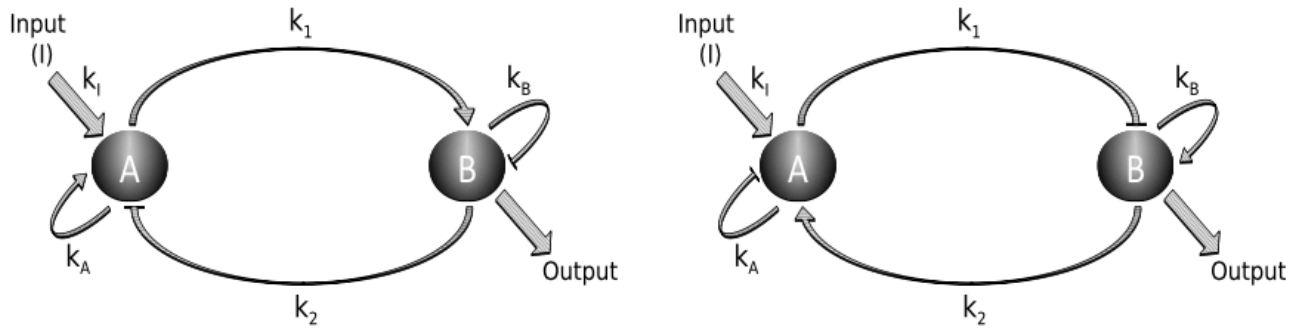
2.5 	Bp=0 Ap=1		Bp=0 for any I		Default set with i=0.8
2.6 	Bp=0 Ap= roots of cubic	Cubic in Ap	Bp=0 for any I		Default set with i=0.8
3.4	Bp=1 Ap=roots of 4th order eqn	4th order in Ap	Bp=1 for any I		Default set with i=0.8
3.5 			Unknown nature!		

3.6			Unknown nature		
4.4	Bp=0 Ap=0.62		Bp=0 for any I		Default set With i=0.8
4.5	Bp=0 Ap=1		Bp=0 for any I		Default set
4.6	Bp=0 Ap=0.63		Bp=0 for any I		Default set With i=0.8

4.8	Bp=0		Bistability observed		
1.7	Bp=0.68 Ap=1 always	Cubic eqn in Bp (independ-ent of I)	Bp=solution of the cubic equation (independent of I)		Default set With i=0.8
1.8	Bp=1 Ap=0.81		Bp=1 for any I		Default set with i=0.8
2.7	Bp=0 Ap=1		Bp=0 for any I		Default set With i=0.8

2.8			Unknown nature		
3.7	Bp=		Bistablity observed		
3.8	Bp=1 Ap=0		Bp=1 for any I		Default set with i=0.8
4.7	Bp=0 Ap=1		Bp=0 for any I		Default set

Two frequently observed topologies



(a) (b)

Figure 1. Schematic diagram for two-node motif showing all possible interactions between two nodes. The Fig. (a) depicts the structure 1 and The Fig. (b) depicts the structure 2. Details of the structures are given in the text.

Structure 1

$$\frac{dA}{dt} = \frac{k_I I(1-A)}{k_{mI}+(1-A)} + \frac{k_A A(1-A)}{k_{mA}+(1-A)} - \frac{k_2 BA}{k_{m2}+A},$$
$$\frac{dB}{dt} = \frac{k_1 A(1-B)}{k_{m1}+(1-B)} - \frac{k_B B^2}{k_{mB}+B}, \qquad (1)$$

Structure 2

$$\frac{dA}{dt} = \frac{k_I I(1-A)}{k_{mI}+(1-A)} + \frac{k_2 B(1-A)}{k_{m2}+(1-A)} - \frac{k_A A^2}{k_{mA}+A},$$
$$\frac{dB}{dt} = \frac{k_B B(1-B)}{k_{mB}+(1-B)} - \frac{k_1 AB}{k_{m1}+B}. \qquad (2)$$

Structure 1

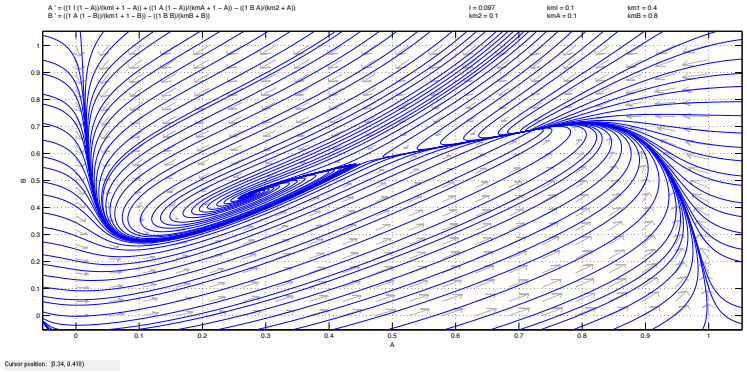


Figure. Phase portrait showing bistability of the structure 1.

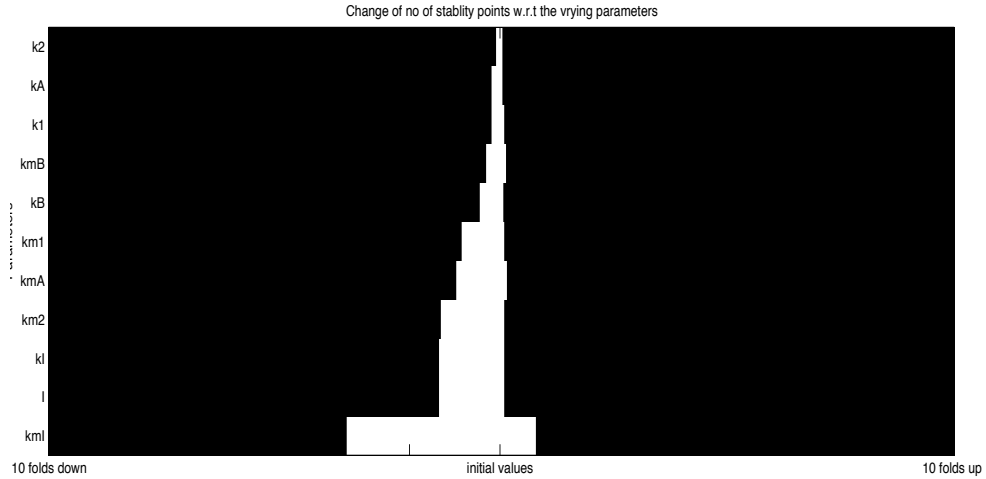


Figure. Parameter ranges for which the number of equilibrium point(s) changes. Here, the black colour shows the range of each parameter for which the system has only one stable point and the white colour shows the range of each parameter for which the system is bistable.

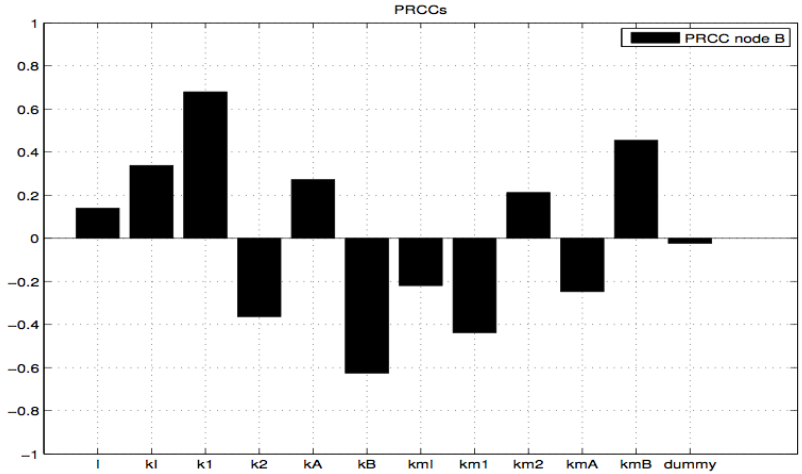


Figure. Global sensitivity analysis (GSA) of model parameters of node B using Latin Hypercube Sampling (LHS) method.

Structure 2

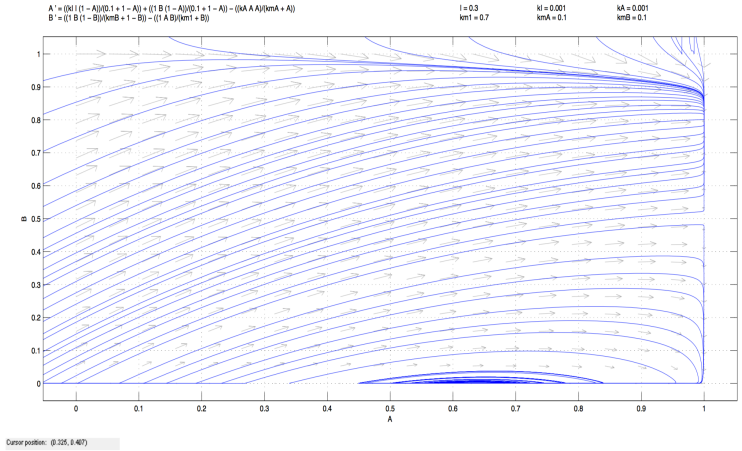


Figure. Phase portrait showing bistability of the structure 2.

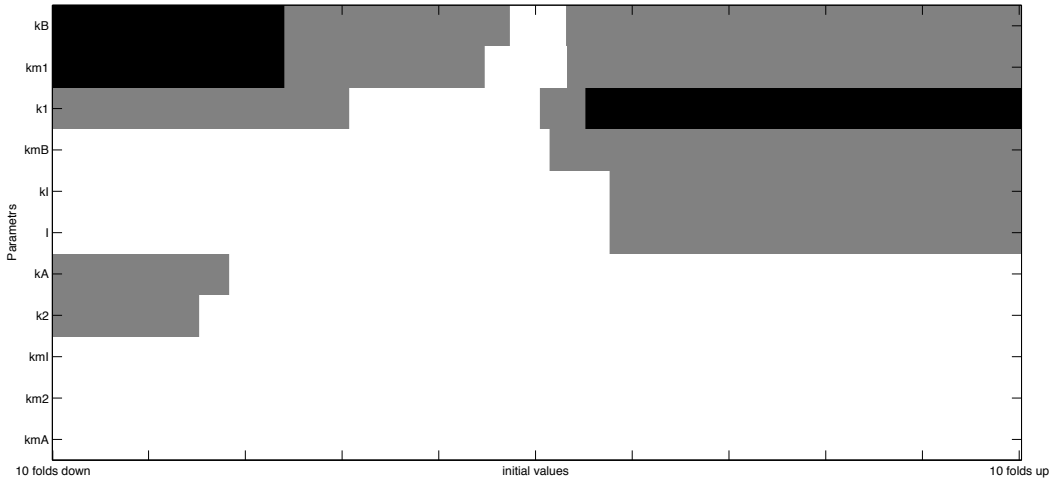


Figure. Parameter ranges for which the number of equilibrium point(s) changes. Here, the black colour shows the range of each parameter for which the system has no stable point, grey colour shows the range of each parameter for which the system has only one stable equilibrium point and the white colour shows the range of each parameter for which the system is bistable.

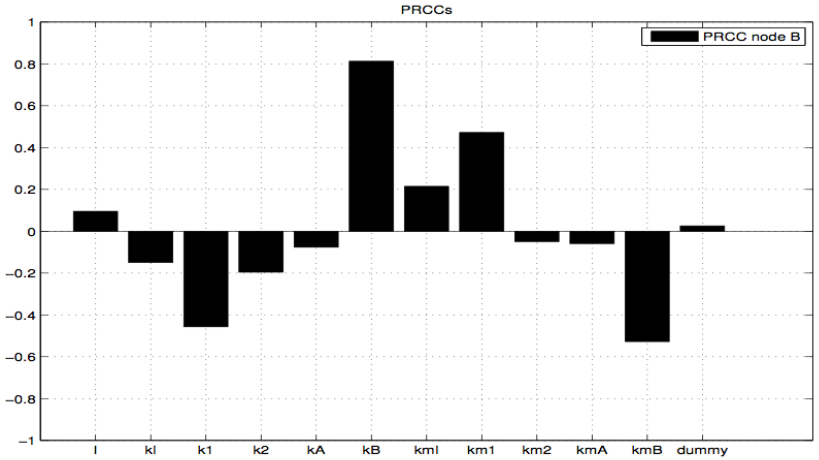


Figure. Global sensitivity analysis (GSA) of model parameters of node B using Latin Hypercube Sampling (LHS) method.

Stochastic model

$$dA = F_1(A,B)dt + \sigma_1(A - A^*)d\xi_t^1 \ ,$$
$$dB = F_2(A,B)dt + \sigma_2(B - B^*)d\xi_t^2 \ . \tag{18}$$

where σ_1 and σ_2 are real constants and known as the intensity of the fluctuations , $\xi_t^i = \xi_i(t)$, $i = 1, 2$ are standard Wiener processes, independent of each other, and F_1, F_2 are defined in the Eq. (3). We consider Eq. (18) as an Ito stochastic differential system of the type

$$dX_t = F(t, X_t)dt + G(t, X_t)d\xi_t \tag{19}$$

where the solution (X_t,$t > 0$) is an Ito process, 'F' is the drift coefficient, 'G' is the diffusion coefficient and ξ_t is a two dimensional stochastic process having scaler Wiener process components with increments $\triangle\xi_t^j = \xi_j(t + \triangle t) - \xi_j(t)$ are independent Gaussian random variables $\mathrm{N}(0, \triangle t)$. In the case of system (18),

$$X_t = \begin{bmatrix} A \\ B \end{bmatrix}, \xi_t = \begin{bmatrix} \xi_t^1 \\ \xi_t^2 \end{bmatrix}, \tag{20}$$

$$F = \begin{bmatrix} F_1(A,B) \\ F_2(A,B) \end{bmatrix}, G = \begin{bmatrix} \sigma_1(A - A^*) & 0 \\ 0 & \sigma_2(B - B^*) \end{bmatrix} \tag{21}$$

Bistability under stochastic perturbation

Structure 1

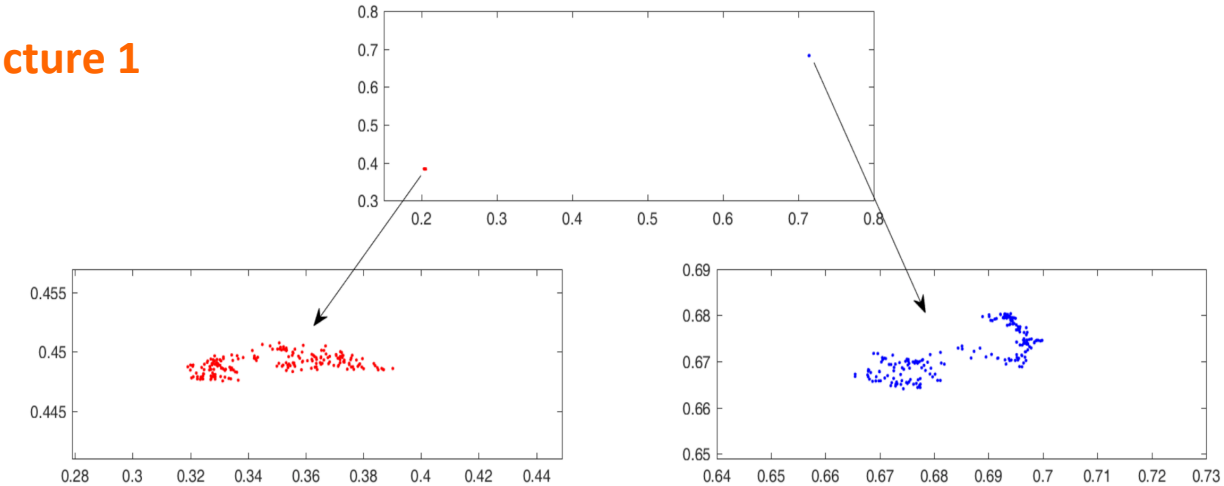


Figure. Phase plane diagram for structure 1. Top figure shows stable nature of E* for low value of $\sigma_{1,2}$ = 0.1 and the bottom figures shows the probability clouds for $\sigma_{1,2}$ = 1.3, above the threshold value.

Structure 2

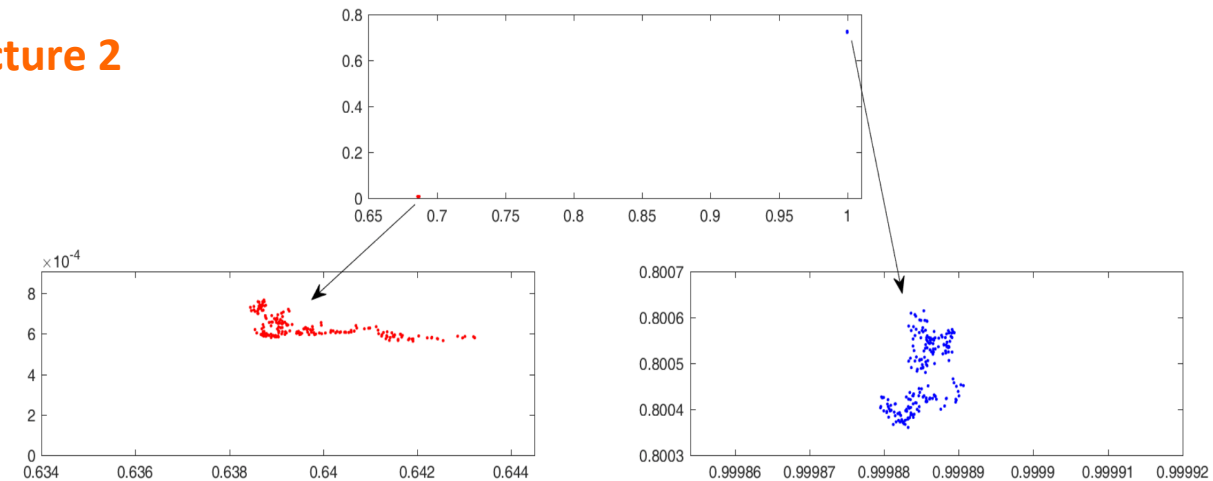


Figure. Phase plane diagram for structure 2. Top figure shows stable nature of E* for low value of $\sigma_{1,2}$ = 0.1 and the bottom figures shows the probability clouds for $\sigma_{1,2}$ = 1.2, above the threshold value.

Observations

- We studied **two well observed motif structures which shows bistability**, i.e., depending upon the initial conditions the final outcome can take any of the two steady state values.

- We observed that the **range of output signal depends on the structure but the sensitivity of the parameter is independent of the structure.**

- In both the structures, it is the **downstream node which is more sensitive** in the outcome of output signal.

- We also observed that under **random perturbation with high noise intensity, the systems looses its stability** and the bistable points scattered leading to undesirable output signal.

➢Studied so far……..

- A possible **design principle** is proposed, for higher-order organization of motifs into larger networks in order to **achieve specific biological output**.

- Using our ranks in a human cancer network, we observed that the **molecules are so arranged** inside the cell **to reduce the effect of 'any noise'** on the cell signaling to minimum.

The proposed ranks could be use to get an initial idea of the important molecules based on their motif structure(s) and will accelerate screening experiments.

The information can be use to filter noise from the signal. This could be a potential therapeutic strategy in case of diseases like auto-immunity.

➢Ongoing study…..

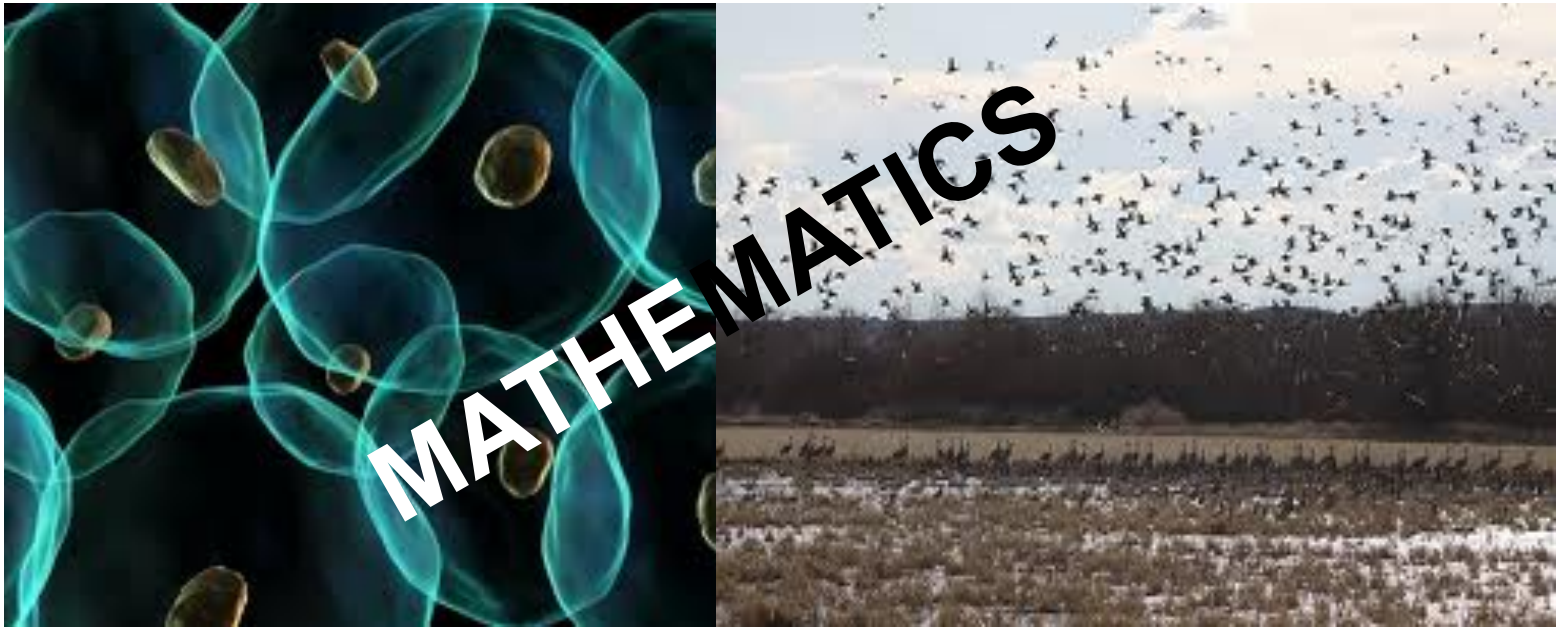
The ranking is refining using all possible structure to increase robustness of the result.

This is a small study focusing on only two specific structures, but it shows the importance of the structure and the noise in the signalling mechanism. In future we will extend our study on other structures and on higher dimension with three and possibly four nodes.

Acknowledgement:

This work is supported by DBT (Govt. of India), grant no. BT/ PR13086/ BRB/ 10/ 1380/ 2015.

THANK YOU



Mathematics behind the analysis

Back

Equilibrium point and stability analysis

The interior equilibrium point,
(final steady state)

$$E^* \longrightarrow \quad A^* = \beta, \quad B^* = \frac{k_1 + a_1\beta}{\delta_1 + a_1\beta}, \; C^* = \frac{k_2 + a_1\beta + a_3 B^*}{\delta_2 + a_1\beta + a_3 B^*}$$

$$\begin{pmatrix} -\alpha & 0 & 0 \\ a_1(1-B^*) & -\delta_1 - a_1\beta & 0 \\ a_2(1-C^*) & a_3(1-C^*) & -\delta_2 - a_2\beta - a_3 B^* \end{pmatrix}$$

The eigenvalues associated with the matrix are negative real numbers, and so the interior equilibrium point **is always stable.**

Back

Def: Stochastic stability

$$\begin{aligned} dx_1 &= F_1(x_1, x_2 \ldots x_m)dt + \sigma_1(x_1 - x_1^*)d\xi_t^1 \\ dx_2 &= F_2(x_1, x_2 \ldots x_m)dt + \sigma_2(x_2 - x_2^*)d\xi_t^2 \\ &\vdots \\ dx_m &= F_m(x_1, x_2 \ldots x_m)dt + \sigma_m(x_m - x_m^*)d\xi_t^m, \end{aligned} \quad (1)$$

where σ_i, $i = 1, 2, ..m$ are real constants and known as intensity of environmental fluctuation, $\xi_t^i = \xi_i(t)$, $i = 1, 2, ..., m$ are standard Wiener processes independent from each other [3] and F_i, $i = 1, 2, ..m$ are the corresponding deterministic part of the variables $(x_1, x_2, ..., x_m)$.

Stochastic stability [from Mao (1997)]:
The trivial solution of equation (1) is said to be stochastic stable or stable in probability if for every pair of $\epsilon \in (0, 1)$ and $r > 0$, there exists a $\delta = \delta(\epsilon, r, t_0) > 0$ such that

$$P\{|x(t; t_0, x_0)| < r \quad for\ all \quad t \geq t_0\} \geq 1 - \epsilon,$$

whenever $|x_0| < \delta$. Otherwise, it is said to be unstable.

The trivial solution is said to be stochastically asymptotically stable if it is stochastically stable and, moreover, for every $\epsilon \in (0, 1)$, there exists a $\delta_0 = \delta_0(\epsilon, t_0) > 0$ such that

$$P\{|x(t; t_0, x_0)| = 0\} \geq 1 - \epsilon,$$

whenever $|x_0| < \delta_0$.

Method:

Stochastic model:

$$\begin{aligned} dA &= F_1(A,B,C) + \sigma_1(A - A^*)d\xi_t^1, \\ dB &= F_2(A,B,C) + \sigma_2(B - B^*)d\xi_t^2, \quad (1) \\ dC &= F_3(A,B,C) + \sigma_3(C - C^*)d\xi_t^3, \end{aligned}$$

Where, $\sigma_i, i = 1,2,3,$ are real constants and known as the intensities of fluctuations, $\xi_t^i = \xi_i(t), i = 1,2,3,$ are standard Wiener processes, independent of each other. We consider (1) as an Ito stochastic differential system of type

$$dX_t = \chi(t, X_t)dt + g(t, X_t)d\xi_t, \; X_{t0} = X_0. \quad (2)$$

In the equation,

$$X_t = (A,B,C)^T, \; \xi_t = (\xi_t^1, \xi_t^2, \xi_t^3)^T, \; \chi = (F_1, F_2, F_3),$$

$$g = \begin{pmatrix} \sigma_1(A - A^*) & 0 & 0 \\ 0 & \sigma_2(B - B^*) & 0 \\ 0 & 0 & \sigma_3(C - C^*) \end{pmatrix}$$

The linearized version of (2) at E^* is given by

$$du(t) = H(u(t))dt + g(u(t))d\xi(t),$$

Where,

$$H(u(t)) = \left(-\alpha u_1, a_1(1 - B^*)u_1 - (\delta_1 + a_1\beta)u_2, a_2(1 - C^*)u_1 + a_3(1 - C^*)u_2 - (\delta_2 + a_2\beta + a_3B^*)u_3\right)$$

$$g(u(t)) = \begin{pmatrix} \sigma_1 u_1 & 0 & 0 \\ 0 & \sigma_2 u_2 & 0 \\ 0 & 0 & \sigma_3 u_3 \end{pmatrix}$$

Back

e that in the equilibrium E^* corresponds to the origin in the new coordinates $(u_1, u_2, u_3) = (0, 0, 0)$. Let us define the set $\Gamma = \{(t \geq t_0) \times R^3, t_0 \in R^+\}$. Let $Z \in C^3(\Gamma)$ be a continuously differentiable function of time t and twice continuously differentiable function of u. Let us introduce the following operator acting on Z defined by

$$LZ(t,u) = \frac{\partial Z(t,u(t))}{\partial t} + H^T(u(t))\frac{\partial Z(t,u)}{\partial u} + \frac{1}{2}\mathrm{tr}\left[g^T(u(t))\frac{\partial^2 Z(t,u)}{\partial u^2}g(u(t))\right],$$

where $\frac{\partial Z}{\partial u} = \left(\frac{\partial Z}{\partial u_1}, \frac{\partial Z}{\partial u_2}, \frac{\partial Z}{\partial u_3}\right)^T$, $\frac{\partial^2 Z(t,u)}{\partial u^2} = \left(\frac{\partial^2 Z}{\partial u_j \partial u_i}\right)$ for $i, j = 1, 2, 3$ and $\mathrm{tr}[A]$ denotes the trace of the matrix A.

To analyze the stability, we need a result (Afanas'ev (1996)) which we write in a particular case as follows

Proposition 1. Suppose for $i = 1, 2, 3$ for suitable $K_i > 0$ the function $Z(t,u) \in C^3(\Gamma)$ satisfies the inequalities

$$K_1|u|^2 \leq Z(t,u) \leq K_2|u|^2, \quad LZ(t,u) \leq -K_3|u|^2.$$

Then the trivial solution is globally asymptotically stable in probability.

Theorem: Assuming that $\det(Q) > 0$, where Q is given below, it is observed that the zero solution of system is asymptotically stable if

$$\sigma_1 = \sqrt{\frac{\alpha}{2}} \equiv \sigma_{1c}, \quad \sigma_2 = \sqrt{\frac{\delta_1 + a_1\beta}{2}} \equiv \sigma_{2c}, \quad \sigma_3 = \sqrt{\frac{\delta_1 + a_2\beta + a_3 B^*}{2}} \equiv \sigma_{3c},$$

$$\text{and, } Q = \begin{bmatrix} \frac{\alpha}{2} - \sigma_1^2 & -\frac{1}{4}a_1(1-B^*) & -\frac{1}{4}a_2(1-C^*) \\ -\frac{1}{4}a_1(1-B^*) & \frac{\delta_1 + a_1\beta}{2} - \sigma_2^2 & -\frac{1}{4}a_3(1-C^*) \\ -\frac{1}{4}a_2(1-C^*) & -\frac{1}{4}a_3(1-C^*) & \frac{\delta_1 + a_2\beta + a_3 B^*}{2} - \sigma_3^2 \end{bmatrix}$$

Back

Formula defining the rank

We devised an indigenous method to estimate the cumulative vulnerability score for each of the nodes in a signaling network. Suppose N denotes the node number and K_i denotes the number of times the Nth node occurs in the ith rank. Let S_i be the score given to the ith rank such that $s(i) = s_i = \begin{cases} (i-1) - \frac{n}{2}, for \quad i \le n/2 \\ i - \frac{n}{2}, for \quad i > n/2 \end{cases}$

where n denotes the total number of motif types (eight in the present study).

Then the cumulative score given for N^{th} node is

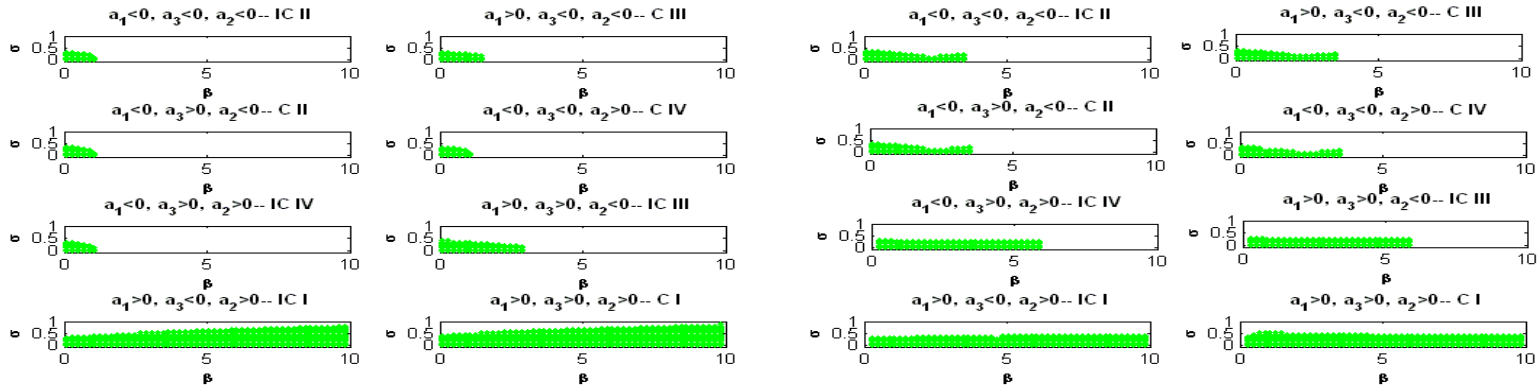
$$N = \sum_{i=1}^{n} K_i S_i$$

Nodes with the cumulative score greater than $n/2$ was then identified as robust nodes while those with less than $-n/2$ were classified as vulnerable nodes.

Back

Supplementary Figure S1

A



Kinetic law 1 (Simple Mass-action)

$$\frac{dA}{dt} = \alpha A\left(1 - \frac{A}{\beta}\right),$$

$$\frac{dB}{dt} = k_1 - \delta_1 B + a_1 A(1-B),$$

$$\frac{dC}{dt} = k_2 - \delta_2 C + a_2 A(1-C) + a_3 B(1-C),$$

Kinetic law 2 (Michaelis-Menten)

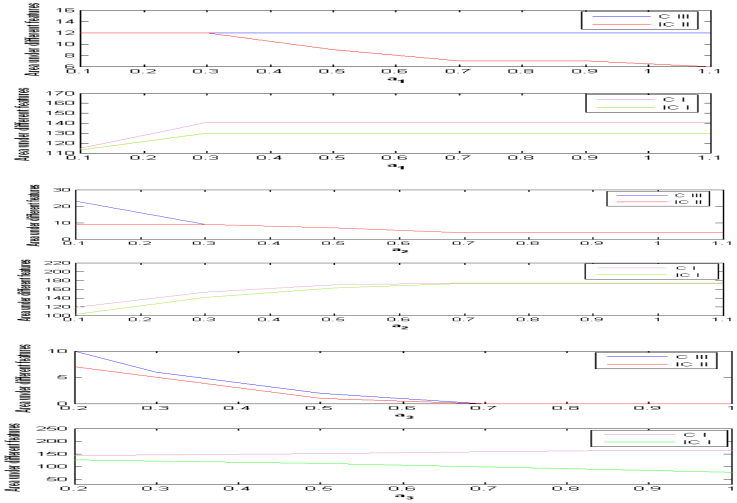
$$\frac{dA}{dt} = \alpha A\left(1 - \frac{A}{\beta}\right),$$

$$\frac{dB}{dt} = k_1 - \delta_1 B + \frac{a_1 A(1-B)}{b + (1-B)},$$

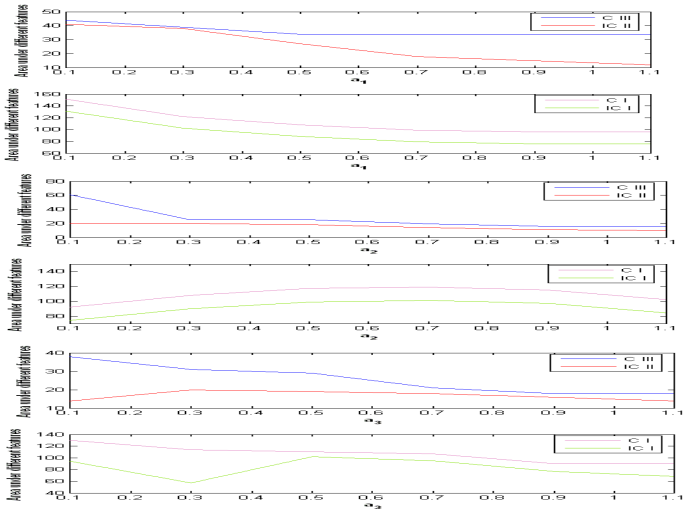
$$\frac{dC}{dt} = k_2 - \delta_2 C + \frac{a_2 A(1-C)}{c_1 + (1-C)} + \frac{a_3 B(1-C)}{c_2 + (1-C)},$$

B

Model with Simple Mass-action law



Model with Michaelis-Menten



Back