Quantification of Signaling Networks

P. K. Vinod[1] *AND K. V. Venkatesh*[1,2]

Abstract | Studies in living system in the past several decades have generated qualitative understanding of the molecular interactions resulting in large networks. These networks were essentially deciphered by breaking the components of a cell through a reductionist approach. Biological networks comprising of interactions between genes, proteins and metabolites co-ordinate in the regulation of cellular processes. However, understanding the cellular function also requires quantitative information including network dynamics, which results due to an inherent design principle embedded in the network. Interactions within the network are well organized to form a definite regulatory structure, which in turn exhibits different emergent properties. The property of the network helps the cell to achieve the desired phenotypic state in a controlled manner. The dynamics of the network or the relationship between network structure and cellular behavior cannot be understood intuitively from the interaction map of the network. Computational methods can now be employed to study these networks at system level. The field of systems biology looks at integrating the interaction maps obtained through molecular biological approach. Various studies at the system level have been reported for pathways namely chemotactic response in bacteria, cell cycle and osmotic signaling in yeast, growth factor stimulated signaling pathways in mammals. This review focuses on understanding signaling networks with the help of mathematical models.

[1]*School of Biosciences and Bioengineering, Indian Institute of Technology, Bombay, Powai, Mumbai 400076, India*
[2]*Department of Chemical Engineering, Indian Institute of Technology, Bombay, Powai, Mumbai 400076, India*
venks@che.iitb.ac.in

Keywords: Feedback regulation, simulation and modeling, systemic properties, biological networks

1. Introduction

Understanding biological responses at a system level by incorporating information from genetic, signaling and metabolic networks represent a great challenge in modern biology. With the availability of high throughput genomic and proteomic data, it has become possible to map the cellular networks involved in a phenotypic response.[1–4] Such mapping of the network strongly depends on computational modeling techniques to interpret the data and define the connectivity in a network.[5–8] Biological networks are organized into modules of protein–protein, protein ligand and protein DNA interactions embedded with feedback and feedforward loops to form signaling and gene regulatory networks. The interactions within the network affect one or more components and are non-linear in nature.[9,10] Cells respond appropriately to diverse signals by coordinating complex network of protein and gene interactions. The dynamics of these interactions determine the cellular behavior to varying extracellular conditions, which are not best understood from just the wiring diagrams of the biological networks. Therefore, there is a growing need to understand the relationship between the network structure and the resulting cellular behavior.[11–15] A system level analysis is required to study the emergent properties of the network such as robustness, bistability and oscillations, which enables the cell to respond in a controlled and specified fashion.[16–19] Feedback regulation prevalent in the biological networks is a key mechanism that give rise to the functional properties of the network.[20,21]

Recent studies have shown that biological networks have imbedded in them the design and control strategies which are also utilized in typical engineering systems.[12,22–24] Understanding the design principles of biological network can help in modifying and constructing biological networks with desired properties.[25,26] This essentially means a paradigm shift from evaluating interaction map of a network to quantifying the network interactions for gaining operational insights into the cellular regulation. Quantification of biological network requires construction of mathematical models to best describe the operation of the network.[27–30] Modeling and simulation of signaling networks are becoming increasingly popular with models developed based on the quantitative experimental information of individual components such as time course data, dose response curves, protein concentrations and binding constants.[31–36] Mathematical models provide a window to study the dynamics, control and design principles of a system, which eventually helps the system to achieve the desired state. Models developed can be effectively used to predict the network behavior, which can be subjected to experimental verification and further refinement. Such comprehensive understanding based on quantitative experiments and computational modeling to gain insights into the physiology of a cellular process is termed as systems biology.[37–41] In this review, we provide an in depth understanding needed to study and quantify signaling networks with examples taken from well-defined signaling pathways in yeast and mammalian systems. We draw attention to basic building blocks present in the pathways, various emergent properties of the network, modeling techniques and analysis methods employed in pathway modeling.

2. Building blocks of the signaling network

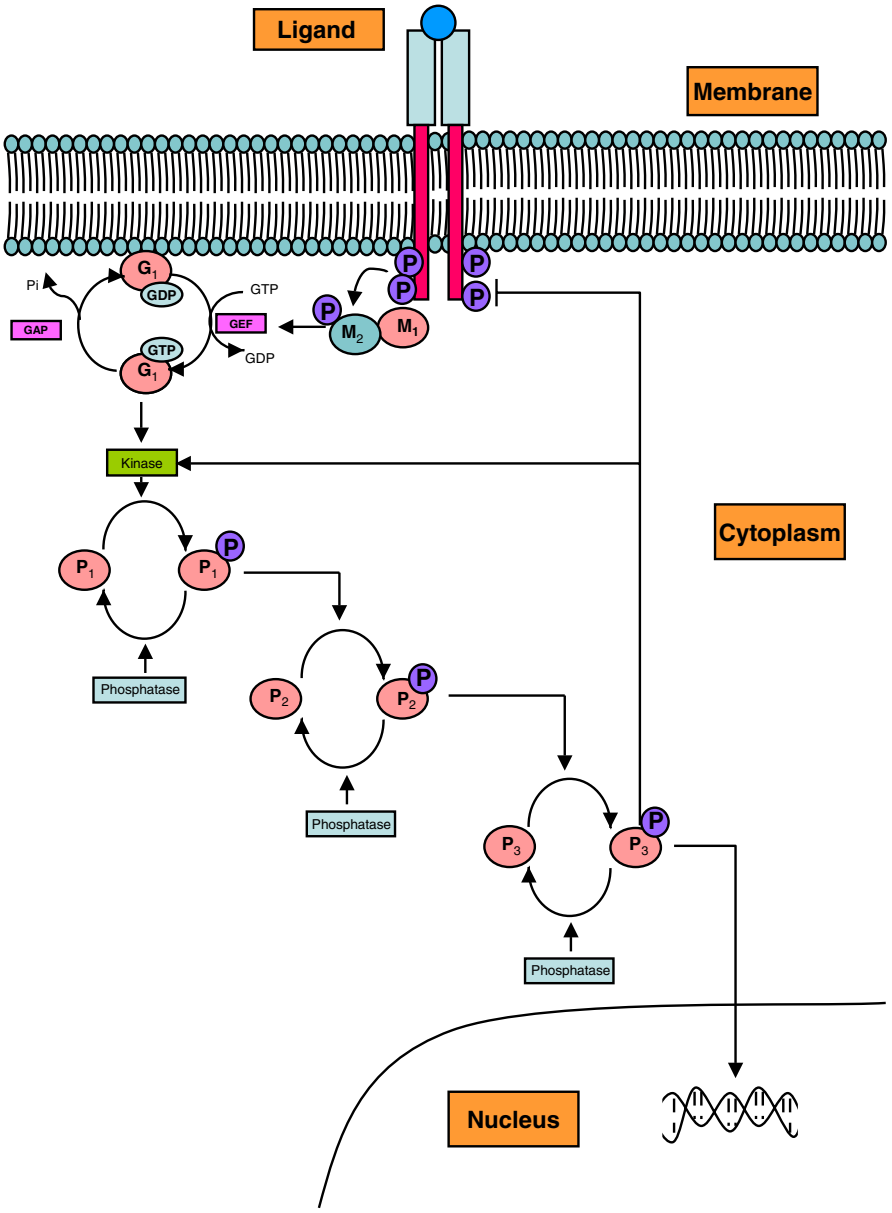
Signaling network is an integrated system of multiple signaling pathways, which co-ordinate together in the regulation of cellular behavior. Signaling network sense the input changes in the environment and transmit the signal into the nucleus to regulate gene expression. A signaling pathway comprises of membrane receptors, GTP binding proteins, allosteric interactions, protein–protein interactions, cycles of covalent modification of proteins, such as phosphorylation–dephosphorylation cycles and translocation of proteins across the nuclear membrane (Fig. 1).[42,43] Membrane receptors function upstream to sense the environmental cues and also to generate the signals required for the activation of cascade of proteins in the cytoplasm. This helps to relay the signal and to activate gene expression inside the nucleus. Receptors are transmembrane proteins which undergo conformational changes or dimerization or autophosphorylation upon ligand binding to propagate the signal into the cell. The cytoplasmic domain of the receptor functions to signal downstream through GTP binding proteins. This receptor domain also serves as a phosphorylation site for the downstream kinase, which typically constitutes a feedback. G proteins are transformed from GDP to GTP bound state to activate the signaling cascades. This is typically composed of cycles of covalent modification of proteins and allosteric interactions, which relay the signal to the downstream target, mostly a transcriptional activator to drive the expression of specific genes.

Covalent modification of a protein is catalyzed by enzymes, which receives the input for turning on or off a cascade (Fig. 1). For example, a phosphorylation cycle coordinated by a kinase and phosphatase forms a part of majority of signaling pathways. Furthermore, the downstream information is fed back to the upstream for controlling the flow of information either through activation (positive feedback) or inhibition (negative feedback). In addition, signaling pathways gets additional inputs from other pathways resulting in cross-activation or cross-inhibition for integrating and processing multiple/parallel input information.[44] The downstream protein kinase initiates the nuclear events involving transport of the kinase from the cytoplasm to nucleus, which depends on the specific import and export factors. The transported protein can function as a transcriptional activator or as an inhibitor or can activate other proteins for regulating the expression of genes, which represents the final outcome of the network.

3. Steady state and dynamic properties of signaling network

The output characteristic of signaling network in response to an input stimulus depends on the network structure, which is capable of exhibiting different emergent properties. The input–output characteristic of the network provides insights into the network properties that are well understood either as function of time or independent of time. In response to an input, the network behavior can shift from one state to a new state, which remains invariant with time or can exhibit a transient behavior before returning to the original state. A state which is invariant with time is termed as steady state, but can vary depending upon the strength of the input stimuli. The transient behavior is termed as a dynamic response of the network with respect to an input stimulus. In the following section, we discuss the different network properties and the regulatory structures, which are capable of exhibiting these properties.

Figure 1: *A simple schematic representation of a signaling pathway.* Binding of ligand (such as growth factor/nutrients) to the membrane receptor activates the receptor to undergo autophoshorylation. Receptor activates G-proteins, 'G_1' (such as Ras2) through exchange of GTP from GDP via phosphorylation of adapter protein complex, M1-M2 (such as Grb2-Shc). This event triggers the sequential activation of protein cascades 'P1', 'P2' and 'P3' (such as MAPKKK, MAPKK and MAPK) through phosphorylation. Phosphatase deactivates the cascade through dephosphorylation. The activated protein 'P3' translocate into the nucleus to activate the gene expression. In addition, P3 exerts a positive feedback by activation of upstream kinase and a negative feedback by preventing the phosphorylation of the receptor.



3.1. *Ultrasensitivity*

Cells are subjected to constant change in environmental cues and have to respond by triggering appropriate levels of signaling response based on the strength of the stimulus. Thus, for a given input signal, an output response can be determined at steady state. This relationship between input–output responses can be obtained either through experiments or through simulations. For example, a typical Michaelis Menten response

indicates that for a continuous change in the input signal, the output response increases and later saturates in a hyperbolic fashion. However, signaling pathways can yield input–ouput response where there is threshold to activate and a steep rise beyond the threshold.[45] Such an input–output response converts a continuously varying (graded) input stimulus into a binary (switch like) response, which depends upon the regulatory structure of the network. Ultrasensitivity is a term used to describe such a sensitive response.[46,47] Hills equation is typically used to quantify the sensitivity of the signaling pathway based on the stimulus dose response curve. The equation was originally used to empirically describe allosteric activation/inhibition of proteins.[48] The Hills equation is defined as,

$$f = \left(\frac{I^{nH}}{K_{0.5}^{nH} + I^{nH}} \right) \tag{1}$$

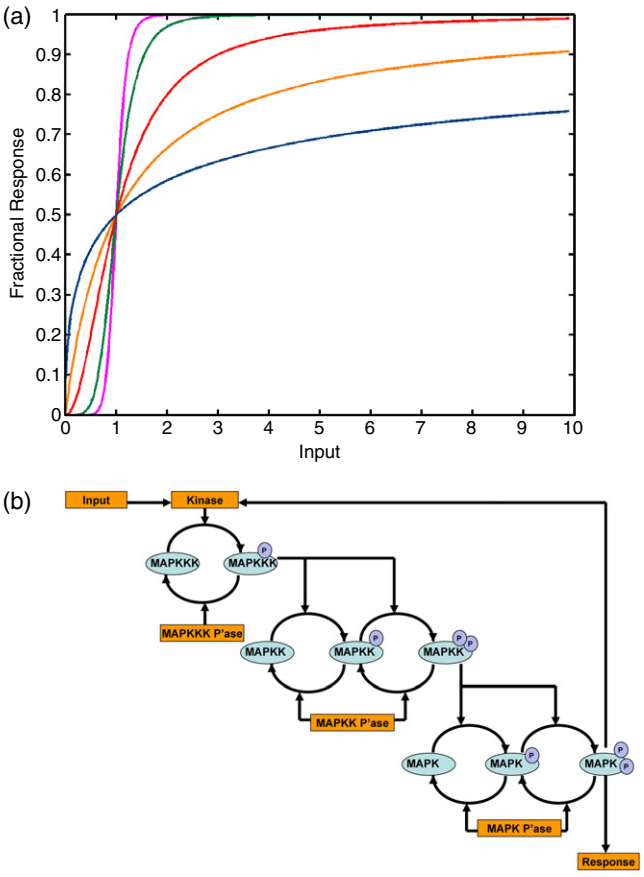
where 'f' represents the fractional activation of output response with varying input 'I' and nH represents Hills coefficient. The input signal required for 50% activation of the network is termed as the half saturation constant ($K_{0.5}$). Depending upon the value of Hills coefficient, the shape of the stimulus response curve changes from hyperbolic to sigmodial, indicating the measure of steepness of the curve. The Hill coefficient is computed based on the fold change in input stimuli required to take a response from 10% activation to 90% activation

$$nH = \frac{\log(81)}{\log(\frac{I_{90}}{I_{10}})}. \tag{2}$$

For a response with a Hills coefficient equal to 1, 81 fold change in input is required to reach 90% of maximum response, which represents a typical hyperbolic Michaelis Menten response. With Hills coefficient greater than 1, response tend to become sigmodial, indicating the sensitive nature of the dose response, which is defined as ultrasensitivity. The response curves that are less steeper than Michaelis Menten curve are termed as subsensitive responses (Fig. 2(a)). The input–output response can be analyzed for activating a pathway by increasing the input concentration (switching on) or for deactivating a pathway by decreasing the input concentration (switching off). A system that yields the same dose–response curves, irrespective of whether the system is switched on or off, is termed to be monostable. Mathematically, this implies that for a given input signal, the system has only one stable steady state that can be attained (Fig. 2(a)). Different sources in signaling pathways are shown to produce ultrasensitive response. Positive cooperativity of allosteric protein is shown to be ultrasensitive. Similarly, multistep effect when the same effector acts at more than one step in a pathway is also shown to be a source of ultrasensitivity.[45,49] Goldbeater and Koshland in a landmark paper have examined the steady state behavior of a simple covalent modification system and demonstrated the existence of zero order ultrasensitivity.[46] This is defined as steep change in the modified protein concentration, when modifying enzymes are saturated by their protein substrate. Furthermore, multistep effect can combine with zero order effect to enhance the sensitivity of biochemical systems controlled by covalent modifications.[50]

Earliest effort to gain system level understanding of ultrasensitivity in signaling pathways was demonstrated by Ferrel and co workers.[51,52] It has been demonstrated that the ultrasensitivity in mitogen-activated protein kinase (MAPK) cascade (Fig. 2(b)) in *Xenopus* oocytes can convert graded stimuli into all or none response.[51] MAPKKK was shown to exhibit hyperbolic behavior, whereas the MAPKK and MAPK were shown to exhibit sigmodial behavior with Hills coefficient of 1.7, and 4.9, respectively. Zero order effect and dual phosphorylation of both MAPK and MAPKK make MAPK pathway highly sensitive. Furthermore, analysis of individual oocytes maturation in response to progesterone demonstrated a response with Hills coefficient of 35, indicating that the MAPK cascade was highly ultrasensitive.[53] This marked increase in the intrinsic sensitivity of MAPK cascade was due to the presence of positive feedback loop embedded in the cascade, which increased the abruptness of the response. Ultrasensitivity in MAPK cascade can function to filter out noise and as well help the cells to switch between discrete states without occupying an intermediate state. Recently, mathematical simulation of interlinked positive feedbacks revealed that linking slow to fast positive feedback creates a "dual time" switch, which is rapidly inducible and also provides resistance to noise in the upstream components of the signaling pathway.[54] In general, ultrasensitivity offer cells with many advantages, which primarily depends on the different sources capable of introducing non-linearity into the system. It defines a threshold for 'switching on' or 'switching off' of a response. This does not mean that the cells always convert the graded input into switch like output. Sources such as the presence of negative feedback can counteract and convert the non-linear system into linear to make the response graded. Further, cells are also shown to respond both in a binary and graded fashion.[55,56]

Figure 2: Ultrasensitivity in signaling pathways. (a) The response curves of the network with respect to the input, which shows different sensitivities ranging from a subsensitive response with nH = 0.5 (blue), hyperbolic response with nH = 1 (dark yellow) and ultrasensitive response with nH = 2 (red), nH = 4 (green) and nH = 6 (pink). (b) Schematic representation of MAPK pathway, which consist of MAPKKK, MAPKK and MAPK. Input to the cascade involves activation of kinase of MAPKKK, which undergo single phosphorylation to activate MAPKK through dual phosphorylation. MAPKK in turn dual phoshorylate MAPK to activate the signaling response. MAPK exerts a positive feedback through activation of kinase of MAPKKK. The magnitude of activation of MAPKKK, MAPKK and MAPK is also under the control of respective phosphatase (P'Pase).



3.2. *Amplification*

Signaling pathways have to sense and respond to small changes in the extracellular conditions. Networks are known to respond even under weak input stimulus. Such capability to respond in principle arises from the ability of signaling pathway to amplify a weak stimulus. Signaling pathway amplify the initial stimulus received by the signaling receptor in the course of signal transduction. Signal amplification increases along the cascade and the amplification at particular step depends upon the signal amplitude of the preceding step.[30] Amplification in a phosphorylation–dephosphorylation cycle of a signaling cascade also depends on the ratio of counteracting kinase to phosphatase reaction rates. The reaction rate of phosphatase should to be lesser than kinase to bring about signal amplification. A dose response plot for activation at each step of cascade with respect to input stimulus shows a shift in the curve to the left as the signal is relayed from the receptor to the most downstream effector (Fig. 3). The half saturation constant ($K_{0.5}$) is used to quantify amplification. A shift towards right is indicative of signal deamplification. Ratio of half saturation constant of the dose response curves can give a measure of fold change in amplification, which is given by equation (3).

$$\text{Fold change in amplification} = \frac{K_{0.5}\ (\text{upstream})}{K_{0.5}\ (\text{downstream})} \quad (3)$$

where $K_{0.5}$(upstream) and $K_{0.5}$(downstream) are half saturation constants with respect to upstream

and downstream components of a signaling pathway, respectively. The activation of MAPK pathway in *Xenopus* oocytes demonstrates a 30 fold amplification in MAPKK and 100 fold amplification in MAPK with respect to the upstream kinase, MAPKKK.[51] Cascading of signal through multiple steps as seen in MAPK pathway not only increases the sensitivity of the signal, but also helps in amplifying the weak signal. The fold change in amplification is relevant only for the weak input signals, which starts to decrease with increasing strength of stimulus. At higher value of the stimulus, the upstream gets saturated implying that there is a threshold beyond which the amplification is lost.[30] For example, in Fig. 3, the upstream gets saturated (more than 90%) at higher input value. Under this input value, the downstream is also at saturation indicating no role for amplification. Amplification helps the cells to respond to the smallest variation in the environment, thereby helps the cell to sense and respond to a broad range of stimulus strength. However, such a property of signaling pathway can also lead to amplification of noise .[57]

3.3. *Bistability*

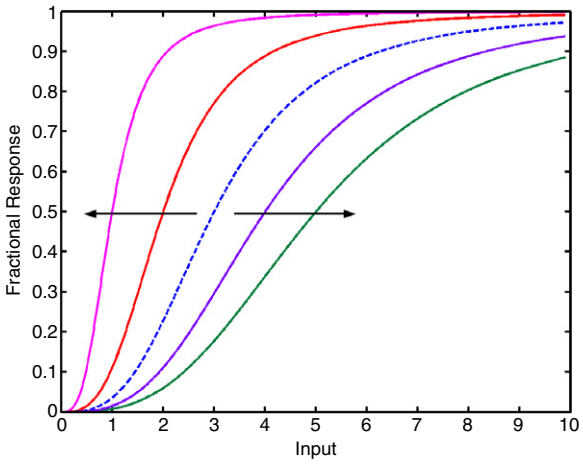
The dose response curves for certain pathways demonstrate distinct curves for "switching on" and "switching off" of the system, unlike a monostable response. Such responses yield two stable steady states for a given input concentration. The nature of such responses is discontinuous all or none type, which depends on the feedback regulation prevailing in a signaling pathway. A system that can toggle between two alternative stable steady-states but cannot rest in an intermediate unstable state is said to be bistable.[58] Cell signaling systems containing positive feedback loops or double negative feedback loops are shown to exhibit bistable behavior (Figs 4(a) and 4(b)).[21] The positive feedback should be ultrasensitive to produce two thresholds, one for switching on and other for switching off. Furthermore, a bistable system also exhibits some degree of hysteresis, where the system will remain switched on even below the threshold value of stimulus required for switching on (Fig. 4(d), red color). However, the system switches off at very lower stimulus value. Therefore, a bistable system has the potential to remember the stimulus long after it has been removed.[59] Outside the stimulus range over which bistable state exists, the system become monostable and the point at which bistability disappears is termed as bifurcation point. Early examples of prokaryotic systems which were shown to exhibit bistability were λ phage lysis/lysogeny decision and *Escherichia coli lac* operon.[60] Bistable response of λ-phage is primarily due to two mutually repressing proteins (double negative feedback), CI and Cro, which maintain the lysogeny and lytic states of λ-phage, respectively. Positive feedback regulation in *lac* operon is shown to exhibit bistable behavior.[58]

Furthermore, *Xenopus* oocytes in addition to all or none type response of oocytes maturation to progesterone, also shows irreversibility on removal of the signal, progesterone. With the removal of the input signal the feedback itself can sustain the response of the network.[61,62] The regulatory circuit of *Xenopus* oocytes consisting of Mos, MEK, and p42 MAPK with positive feedback of p42 MAPK on Mos accumulation exhibits a bistable response, which becomes irreversible with increase in the strength of positive feedback (Fig. 4(d), pink color). Similarly, the positive feedback regulation of Jun N-terminal kinase (JNK) cascade in response to progesterone is also shown to exhibit bistability.[63] Such positive feedback based memory module function typically in cell fate decision. Furthermore, Kholodenko and co-workers have demonstrated bistability at single stage of MAPK pathway through regulatory structure involving multisite phosphorylation catalyzed by different kinases and dephosphorylation reactions catalyzed by same phosphatase (Fig. 4(c)).[64] This feature can exhibit bistability in MAPK cascade even in the absence of positive feedback loop. Thus, it can be seen with gradual increase in strength of the feedback, the system switches from hyperbolic to sigmodial (ultrasensitive) to bistable to hysteresis to irreversible response.[58,59]

3.4. *Oscillations*

Periodic oscillations represent the most prominent type of rhythm encountered in biological system. Non-linear nature of signaling can make the system unstable and thus system tend to oscillate with an amplitude and duration away from the steady state. Such change in dynamic behavior is termed as Hopf Bifurcation.[65–67] Negative feedback regulation forms the major source of oscillation in signaling cascades (Fig. 5(a)).[19,20] Usually, a negative feedback plays a role in homeostasis by turning off the signal after activation (transient behavior), which is termed as desensitization effect or adaptation.[32,68] Under perfect adaptation, increasing the strength of the stimulus increases the magnitude of the response but decreases the rise time and duration of signaling before resetting to the prestimulus condition with the influence of negative feedback (Fig. 5(b)). The height of the peak measures the signal amplitude, width of the peak measures the signal duration and the rise time measures the time required to attain a maximum response. However, the

Figure 3: *Amplification in signaling pathways.* The response of the network with respect to the activation at different levels in a cascade (input). Dotted curve represents the response curve with respect to the activation of the upstream component in the cascade. Shift in response curves towards the left (red, pink) of the dotted curve represent the increase in fold change in amplification from upstream to downstream of a cascade. Similarly, a shift towards right (violet, green) of the dotted curve indicates deamplification, a decrease in fold change in amplification from upstream to downstream of a cascade.



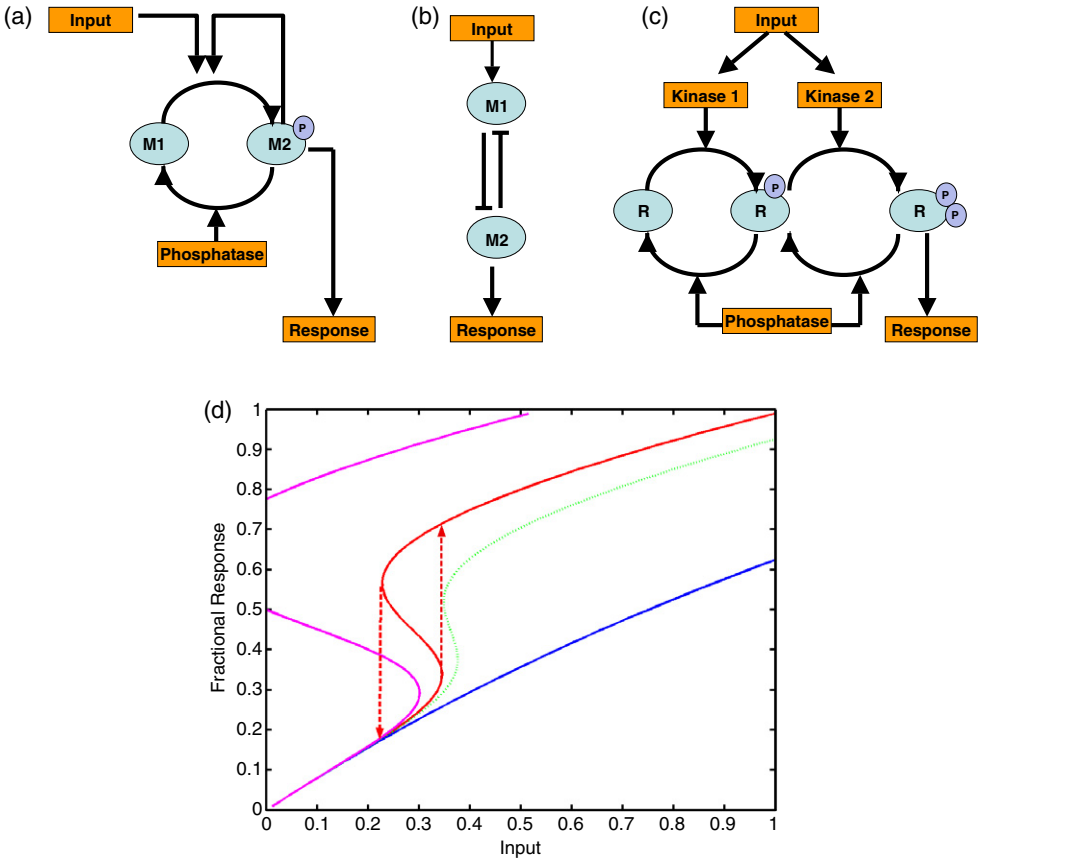
oscillation arises primarily with introduction of time delay in negative feedback, with larger time delay producing sustained oscillation (Fig. 5(c)), while shorter time delay producing damped oscillation. (Fig. 5(d)). Such kind of oscillators is termed as negative feedback oscillators.[69,70] Moreover, both positive and negative feedbacks are shown to exhibit oscillatory behavior called relaxation oscillators (Fig. 6).[70] Positive-feedback makes a dynamic system bistable, which act as a threshold and a delayed process involving negative feedback forces the system to switch between alternate states, thereby generating oscillations. The property of oscillation is shown to be crucial in the regulation of circadian clock, cytosolic free calcium (Ca^{2+}), cyclic AMP production and cell cycle regulation.[65,66,69] There are excellent reviews by Tyson *et al.* (2003) and Kholodenko (2006) illustrating different oscillators.[19,71]

Furthermore, Kholodenko has demonstrated that the combination of a negative feedback and ultrasensitivity in MAPK cascade can bring about sustained biochemical oscillations.[20,72] In mammalian cells, extracellular signal-regulated kinase Erk (MAPK) activates phospholipase-A2 and phosphorylates MAPKKK/SOS, creating positive and negative feedback circuits, respectively in MAPK pathway.[20,72] Bhalla and co-workers have demonstrated that the MAPK cascade can operate with one (monostable) or two (bistable) stable states in growth factor-stimulated signaling network.[17,72] With addition of negative feedback loops, such as MAPK phosphorylation of MAPKKK and MAPK activation of MAP kinase phosphatase (MKP-1), the authors were able to generate the oscillatory behavior only under extreme non-physiological parameter values.[73] In fact, it was demonstrated that positive and negative feedback in MAPK cascade provide bistability and flexibility in switching between bistable and monostable states.[72] Recent work has demonstrated that even in the absence of negative feedback, a multistage MAPK cascade with single stage capable of producing bistability can produce oscillatory behavior.[74] Interestingly, experimental study demonstrate that Erk MAPK activation demonstrates damped oscillation, which could possibly be due to the negative feedback involving MAPK activation of phosphatase, MKP-1.[75] Such damped oscillations with system resetting to prestimulus level can be interpreted as adaptation. In fact, negative feedback regulation in MAPK pathway is shown to generate signal adaptation.[76] Thus, it can be observed that the negative feedback regulation of signaling pathways is capable of bringing about adaptative and oscillatory behavior.

3.5. *Robustness*

Robustness is a fundamental property of the biological system required to maintain its function in the face of perturbation and uncertainty.[77–79] It is also an inherent feature of evolvable complex system. Moreover, robustness is only linked to maintain the cellular function under perturbation rather than to maintain a steady state as in the

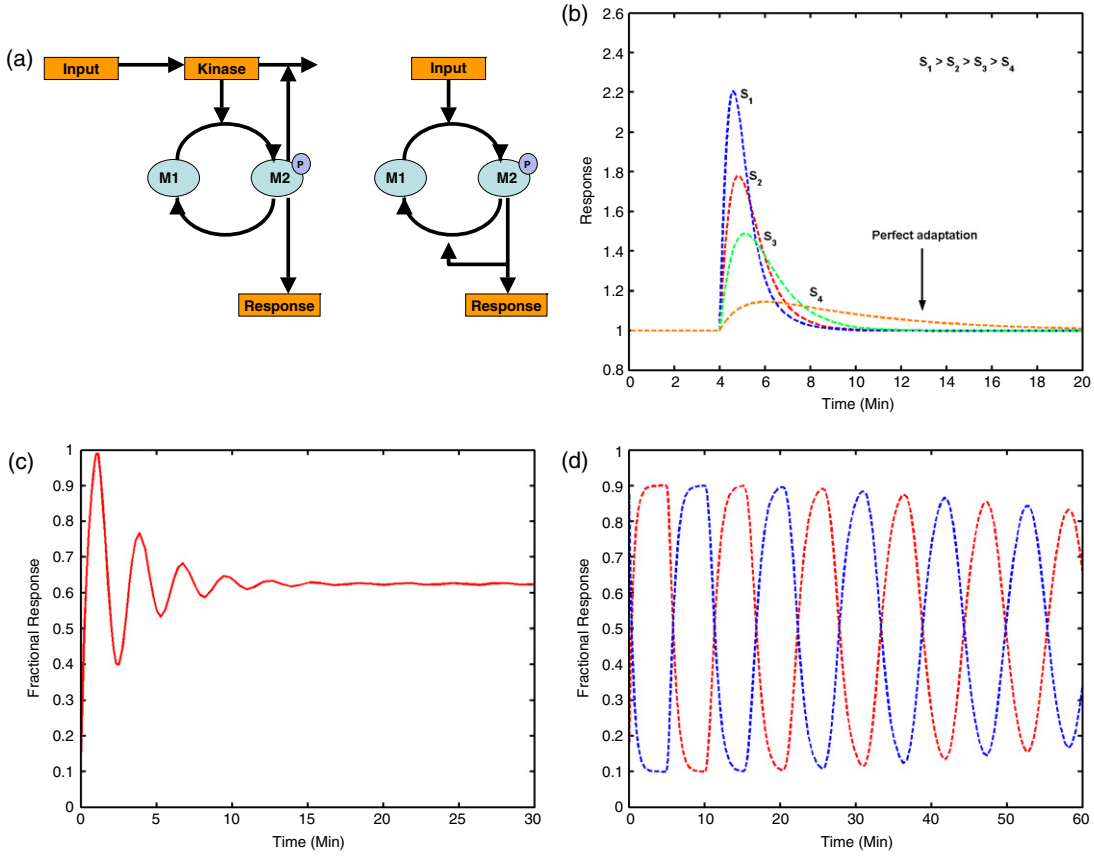
Figure 4: Bistable response of signaling pathways. Different regulatory structures capable of eliciting bistable response are (a) positive feedback (b) Double negative feedback (c) dual phosphorylation with different kinase and same phosphatase. (f) Response curve with respect to input under varying strength of positive feedback. With increase in strength of positive feedback the response changes from subsensitive (blue) to bistable (green and red) to irreversibility (pink). The degree of hysteresis, which is the difference in the threshold for switching on and off vary between the bistable curves. Green curve show smaller degree of hysteresis than red curve (dotted arrow).



case of homeostasis. However, robustness and homeostasis becomes identical if maintaining a steady state is required for a particular cellular function. Thus, robustness can manifest in moving the system to a prestimulus steady state or to a new steady state to maintain the cellular functions irrespective of perturbations. This indicates that the compensatory mechanism present in the network design make the required adjustments to overcome perturbations. Major source of system perturbation include environment and allelic variability, which influence multiple parameters in the network. Robustness is achieved through different means such as feedback control, modularity, and redundancy. Control circuits involving positive and negative feedback play a principle role in maintaining the function in face of uncertainties. Positive feedback is needed for producing bistable or sensitive state, which can clearly distinguish the function from the non-stimulated state. This is required to maintain the robust cellular decision in wake of noise and fluctuations in the input stimuli. Negative feedback function to reduce the differences between the response output and set point, thereby help to dampen the noise and insulate against perturbation.[77,78]

In a landmark paper by Barkai and Leibler (1997) on bacterial chemotaxis, the authors have demonstrated that property of adaptation is robust to variations in the biochemical parameters of the network (Fig. 7(a)).[16] A bacterium is able to modify the tumbling frequency under wide range of attractant concentration, to move along the gradients. This adaptative feature is shown biochemically as a result of change in the methylation state of a receptor, which functions to compensate the effect of variation of chemoattractants concentration on tumbling

Figure 5: *Oscillatory behavior of the signaling pathways.* (a) Simple regulatory motifs comprising of negative feedback, which are capable of exhibiting different network behaviors. (b) Adaptation is the transient behavior of the network, with response always resetting to the prestimulus level under different strengths of stimulus (S1, S2, and S3). (c) Oscillation with amplitude decreasing with time to reach a steady state is referred to as damped oscillation. (d) Oscillation with uniform period and amplitude is referred to as sustained oscillation.



frequency (Fig. 7(b)). Quantitative model developed based on chemotaxis network showed that the precision of adaptation is maintained under different perturbation, which is a direct consequence of the network architecture involving negative feedback at the level of receptor methylation.[80] A control analysis on this model suggested the involvement of integral feedback control. The time integral of the difference between actual output and set point is fed back into the system via the methylation state of the receptor.[81] Moreover, systems having multiple feedbacks are also demonstrated to function towards maintaining robust response. Multiple feedback comprising of three distinct negative feedbacks in tryptophan regulatory network of *Escherichia coli* offer robust adaptation to variation in system parameters while maintaining rapid response to achieve homeostasis.[82]

Further, modularity of system, which involves encapsulation of function in modules, helps to maintain the function in event of damage to one module. Similarly, redundancy helps to protect against the failure of specific component through alternate mechanism or back up strategy. Robustness is shown to play a role in the performance of the regulatory circuit of lambda phage decision, regulation of glutamine synthetase (GS) in *E. coli* and also with tricyclic enzymes cascades, as seen in MAPK pathway.[83–85] Although, robustness helps the system to maintain specific function to wide range of perturbations, it also becomes a setback in case the system needs to adapt differently under unexpected perturbations.[86] Such associated trade-offs also needed to be addressed in the study for robustness.

3.6. *Crosstalk*

Signaling pathways, specific to a phenotypic response rather than being discrete and separate units are wired to interact with each other,

Figure 6: *Relaxation oscillator. Positive feedback is coupled to slow negative feedback to yield relaxation oscillator.* The activated (phosphorylated) protein 'M2' activates the activator kinase yielding a positive feedback and also inhibits the synthesis of the activator kinase yielding a negative feedback. Positive feedback produces a threshold, whereas negative feedback force the system to oscillate.

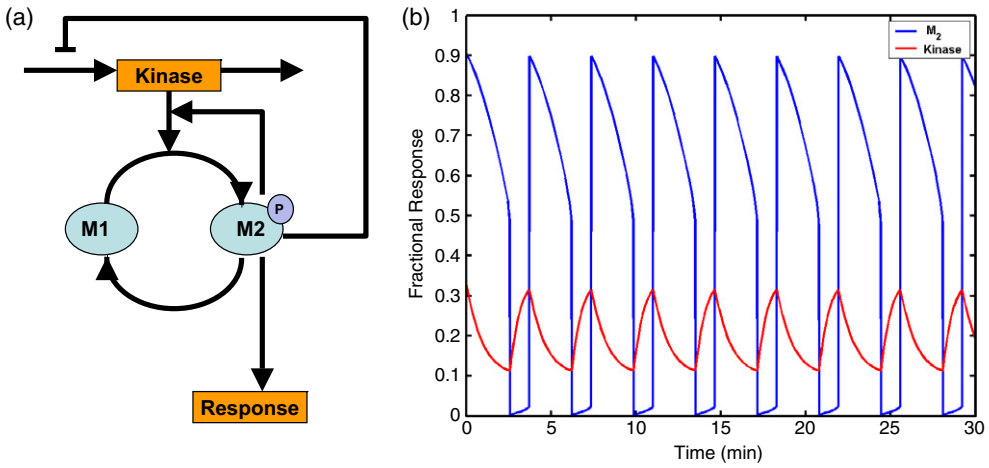
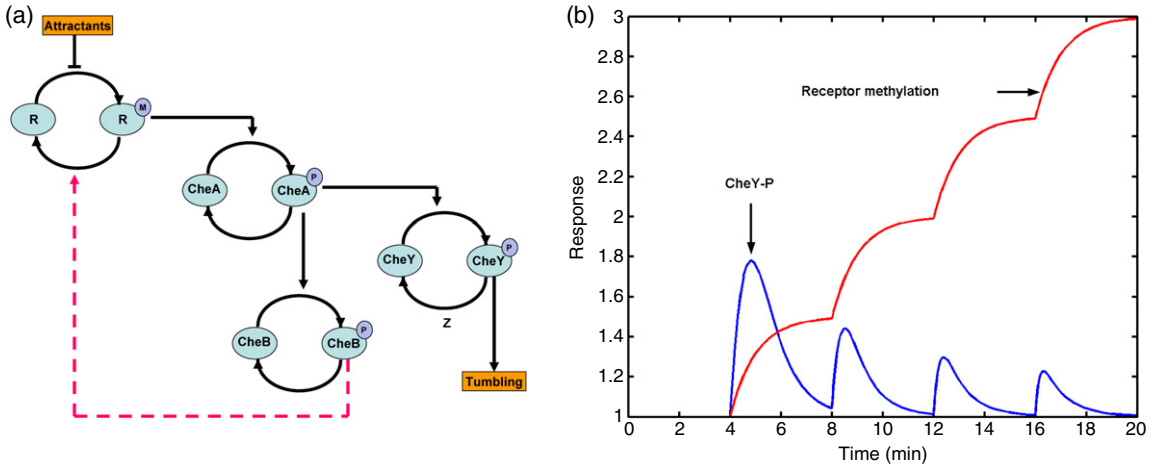


Figure 7: *Robustness of signaling network.* In Chemotaxis pathway binding of attractants to chemoreceptors decreases the methylation of the receptor, which is required to activate the kinase CheA through autophosphorylation. CheA activates CheY, which interacts with the flagellar motors to help in tumbling. CheZ inactivates CheY through dephosphorylation. In addition, CheA activates CheB, which demethylate the receptor thereby constituting a negative feedback. (b) The behavior of the network with step input of chemoattractants at 4 min, 8 min, 12 min and 16 min. The methylation of the receptor increase continuously with step input yielding a robust adaptive response involving CheY activation under wide range of attractant concentration. However, the extent of adaptation depends on the saturation limit of receptor.



which is termed as crosstalk. This helps the cell to process wide range of inputs and to trigger appropriate response using limited and shared components inside the cell. However, such integrated network also has the potential to produce undesirable response. Cross inhibitory mechanism function to insulate the pathways from non-specific activation.[87] In addition, signaling pathways are capable of preventing spillage of signals to other pathways through other insulating mechanisms such as combinatorial signaling and compartmentalization, which are unique to a pathway.[88,89] Combinatorial signaling requires more than one input to activate the output response. Compartmentalization prevents the shared component from activating the pathway localized in different compartments of the cell. Similarly, sequestering and activation function of

scaffold, can limit cross activation. On other hand, cross activation can also help to amplify a weak signal, provided multiple signals are required to activate a single phenotypic response. However, this property becomes insignificant in presence of a strong stimulus. Quantification of degree of crosstalk can elucidate its role in establishing a phenotypic response. Crosstalk is best understood as a ratio of activation of component by intrinsic (input) stimulus to extrinsic (crosstalk) stimulus with respect to the final output response of the pathway, which is given by equation (4).[90]

$$\text{Measure of crosstalk, } C = \frac{\text{Ri}}{\text{Re}} \tag{4}$$

'Ri' and 'Re' represents the intrinsic and extrinsic stimulus, respectively. If the activation of output response of one pathway by another pathway's input is more than its own signal input ($C < 1$), then the crosstalk is strong. While $C > 1$ indicates absence of crosstalk and thus, the pathway is perfectly insulated. Such a measure of crosstalk is termed as fidelity of the pathway.[88] Another measure of crosstalk is specificity, which is defined as the ratio of desirable output to non-desirable output. If the pathway is activated by its own signal and but does not affect the output of other pathway, then the specificity is infinite or complete. However, with some cross activation the specificity will be finite. For measure of specificity less than one, input signal to one pathway cross activate the output response of other pathway more than its own output response. In general different insulating mechanisms work towards increasing the specificity and fidelity of a signaling pathway.[88]

The above section on emergent properties of the network co-relates the network properties with the different regulatory structures present in the network. Such relationship can be best understood through quantification of the network, which requires construction of mathematical models. In next section, we discuss the different quantification methods and analysis, which can be employed to study signaling networks.

4. Quantification methods and analysis

The objective of the mathematical modeling varies from predicting the dynamics of the system in response to a stimulus to understanding the emergent properties of the network. The choice of the quantification methods depends on the availability of the information that can be used to build a model. Due to scarcity of quantitative data from experiments, mathematical models of the biological systems have to depend on the qualitative/semi-quantitative data such as western blot data. Input–output relationship of the network can be measured experimentally and the mathematical model can be constrained to predict system parameters to match experimental observation. The choice of some parameter values depends on the experimental data obtained from wild type and mutants, while the rest has to be fixed based on parametric sensitivity analysis (see below). Models provide an ideal platform to test the effect of concentration and operating parameters, to study the effect of network perturbation (in-silico mutation), to analyze the roles and contributions of different interactions, to predict the emergent properties of the network and to identify the missing information. In general, a mathematical model developed should be able to generate valid hypothesis which can drive future experiments. Considering the complexity of biological system, it will be better to build models for smaller modules of the network before integrating the modules. This helps to study the role and contribution of individual modules towards the network response. Moreover, building a complicated model such as the stochastic models is tedious and hence can be attempted only after considering the continuum approximations of the system.

Quantification of signaling network involves representing the set of elementary reactions of allosteric interaction, covalent modification, feedback interactions and transport of proteins as a system of chemical reactions. Chemical kinetic rate equations are used to describe elementary reactions. Each rate equation represents ordinary differential equation (ODE), which specify variation of component concentration with time. In a well mixed or homogeneous system, the solution of ODE with concentration as continuous variable forms the basis of deterministic modeling.[36]

$$\frac{\mathrm{d}C}{\mathrm{d}t} = V_G - V_c. \tag{5}$$

The concentration of component C depends on the generation (V_G) and consumption (V_C) rate of the component, which in turn depends on the stoichiometry and kinetics of the reaction. Reaction can be zero order (synthesis), first order (degradation) or non linear, typically second order reactions or Michaelis Menten type kinetics. The kinetics selected should be appropriate for the type of biochemical reactions, in most cases the mass-action kinetics are appropriate (equation (6)).

$$A + B \Leftrightarrow C$$
$$V_a = k_b \times C - k_f \times A \times B. \tag{6}$$

However, in enzyme catalyzed reaction with substrate concentration much higher than enzyme concentration (saturation) Michaelis Menten kinetics should be applied.

$$V = V_{\max}\left(\frac{S^n}{K_m^n + S^n}\right) \tag{7}$$

'V_{max}' represent the maximum reaction rate, 'S' represents the substrate concentration, 'Km' represents Michaelis Menten constant for the enzyme and 'n' indicates the degree of non-linearity. Dynamic behavior of signaling pathways can be studied by solving a set of coupled non-linear ordinary differential equations (ODEs) representing the individual components of the system, which is given by

$$\frac{\mathrm{d}C}{\mathrm{d}t} = N.V \tag{8}$$

where component concentrations of the network is represented as $C = [c_1 c_2 c_3 \ldots c_n]^T$ and reaction rates of the network (flux distribution) is represented as $V = [v_1 v_2 v_3 \ldots v_m]^T$. Individual reaction rates depends on the kinetic description of the reaction involving regulation of enzymes and kinetic parameters. Further, N represents the stochiometric matrix, where rows of the matrix correspond to network components and columns of the matrix correspond to reactions with each element of the matrix is the stochiometric coefficient of a component in the associated reaction. N is invariant against time, kinetics and concentrations. Dot product of N and V results in Jacobian matrix, which can be solved numerically. Such formulation of the network is termed as kinetic modeling of signaling pathways. Kinetic modeling of biochemical reactions can be simplified considerably if the overall reaction is studied with the aid of the quasi-steady-state or equilibrium approximations. Steady state behavior of the system can be obtained by setting the derivatives of all concentrations zero and solving a set of non linear algebraic equations simultaneously.

$$\frac{\mathrm{d}C}{\mathrm{d}t} = 0 \tag{9}$$

Steady state modeling approach provides insights into the emergent properties of the network and also helps to identify the role and contribution of individual regulatory structures.

In deterministic approach, the dynamics of system is studied only with variation in time but not space. The localization of components in different compartments influence the response characteristics, hence a compartmentalized ODE needs to be formalized. The same component in different compartments is treated as separate species and transport across the compartments are modeled as fluxes. Compartmental ODE modeling can capture the dynamics of spatially restricted reactions, however with assumptions that the transport rate across the compartment is at slower rate and the compartment is well mixed. However, such assumption does not hold good for non-homogenous systems, where there is explicit dependence of variable on spatial distributed processes such as diffusion reaction. The dynamics of signaling pathway in relation to variation in space and time can be best described using a partial diffusion equation (PDE).[19] The concentration of the components in a compartment depends on the independent variables, such as diffusion, and biochemical reactions, which are described by diffusion-reaction equation.

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} + v \tag{10}$$

where D represents the diffusion coefficient, C is the concentration of the component, t is the time, x is the spatial variable and v represents the rate of generation and consumption of the component. Nonlinear PDE requires more parameters as compared to ODE and also need to specify the boundary conditions in addition to the initial conditions. Moreover, solving PDE requires more computation and also time as compared to ODE.

Furthermore, in a deterministic approach the system is considered to be macroscopic, well mixed and the reaction is continuous. This is a simplification of the chemical reaction, which actually involve discrete, random collision between molecules. Moreover, the biochemical reaction occurs inside the cell, where the volume is small. Chemical reaction becomes deterministic in nature, if the reaction occurred at numerous times per generation, which average out the randomness. However, many biological reactions occur infrequently leading to fluctuations, which can be attributed to the stochastic nature of the reaction. Lesser numbers of molecules and limited diffusion due to the structural organization of the cell contributes towards the fluctuation of biochemical reaction. Stochastic modeling approach involves predicting the probability of collision between molecules resulting in reactions at discrete time intervals. The discrete probability distribution of reaction as function of time is described by chemical master equation (CME).[91] There are numerous stochastic approaches for modeling reactions, but they are difficult to implement analytically and researchers resort to

numerical studies. One key simulation technique is the stochastic simulation approach to chemical reactions developed by Gillespie (1977) through the stochastic simulation algorithm (SSA).[91] However, stochastic representations are complicated in nature and hence the system can be modeled as continuous reactions before attempting using stochastic simulations.

Other methods based on qualitative discrete framework is Boolean networks, where variables are quantified as binary output, i.e. on or off and the value of the one variable is functionally related via a logical rule to the values of other variable.[92] Dynamics of the system is generated by updating the Boolean function, which causes system transition in accordance with logical rules. In a logical regulatory map, each node represents a protein or gene and arc (directed edges) with signs (positive/negative) representing the interaction. Each interaction is characterized by a source and target which is labeled by an integer (a threshold). This indicates the specific condition under which the interaction takes place. If the value of the source variable is equal to threshold, the interaction is said to be functional and their actions are described by logical parameters, which defines the activation of target. The dynamics of the system is represented by the state transition graph, where nodes represent the states of the system and arc represents the transition between the states.

Mathematical model can also be constructed only based on the stoichiometry of the biochemical reaction called as stochiometric model. This model is useful when mechanistic details and kinetic parameters are not available. Topological structure of the reaction mechanism indicating which species are linked by reactions forms the basis of stochiometric modeling. Such an analysis is widely used in analyzing the metabolic networks, which essentially function along with the signaling network to convert the nutritional input into cellular response. The stochiometric modeling approach is also demonstrated to be useful for analyzing the signaling network from the perspective of input–output relationship, crosstalk, measure of redundancy, contribution of individual reactions in signaling pathways and evaluation of co-related reaction rates.[93] Such properties essentially depend upon the network structure. This approach effectively involves formulating stochiometric matrix of the signaling network based on the reactions of the network involving allosteric binding, dimerization and phosphorylation reactions. In stoichiometric modeling approach, quasi steady state approximation is employed (equation (11)).

$$0 = N.V. \tag{11}$$

Stoichiometry matrix N captures the structural relationship between the network components. This results in system of linear equations, which can be solved using linear optimization techniques. However, this often results in infinite number of solutions. To obtain appropriate solution it is necessary to constrain the optimization based on objective functions. Signaling network is subjected to mass balance and thermodynamic constraints to generate a set of systemic pathways that can fully characterize a network.[93] However, major drawback of stoichiometric models is the limited predictive power due to lack of regulatory information, which can only be included in the formulation of a kinetic model.

The models formulated based on the above stated methodologies can be analyzed by subjecting the model to perturbation in order to understand the influence of component concentrations and model parameter values on the overall response of the network. Such an approach is termed as parametric sensitivity analysis. Systemic behavior is evaluated with respect to variation in single parameter or multiple parameters, to evaluate the key parameters in the network and to study robustness of the network. Variation of multiple parameters results in multidimensional analysis with sensitivity of network behavior varying in space. Such an analysis provides a global view of the network behavior with operating zones based on the minimization of objective function. The solution space is represented by hills and valleys, with valleys representing the less sensitivity region in response to variation in parameters. Primary goal will be to find global minima of the objective function using global optimization techniques such as monto carlo simulation.[94] Such optimization techniques can also used to estimate the parameters from experimental results. Moreover, the reference parameter set of the network can be defined and test parameter sets can be generated by random variation of parameters. The sensitivity of the global response can be plotted against the distance between the reference and test parameter set to get a scatter plot, which gives the measure of robustness of the network.

Dynamic analysis methods such as stability and bifurcation analysis are often used to identify the qualitative changes occurring in a non-linear dynamical system with respect to parameter variation.[67,95] The dynamics of the system changes qualitatively either with system returning to original steady state called stable steady state or become unstable or shift to new steady state under perturbation. Such qualitative change in the location and stability of steady states is determined by the parameter values of the system with different

possibilities emerging in different parameter ranges. Bifurcation analysis trace down the qualitative changes which occurs at points in the parameter space called bifurcation points. The most common bifurcations in biochemical networks is saddle node bifurcation and hopf bifurcation, which usually leads to bistability and limit cycle oscillations, respectively. The set of non-linear differential equation is solved at steady state and the stability of steady state is determined based on the eigenvalues of Jacobian matrix.

4.1. *Modeling tools and environment*

Simulation of biological system depends on the powerful numerical analysis methods to retrieve the solution of set of non-linear mathematical equations (ODEs, PDEs, stochastic, algebraic equations). Biological analysis software is publicly available for deterministic and stochastic simulations and for model analysis such as parameter estimation, parameter sensitivity analysis and bifurcation analysis. The details of the software are available in `www.sbml.org`. Majority of them are graphical user interface (GUI) based modeling environment and provides an opportunity for user with limited computational and mathematical background to simulate the biological systems. Biological models can also be analyzed using general mathematical programming environment such as MATAB and MATHEMATICA.[96,97] The modeling environments also provide a provision to translate the developed mathematical models into Systems Biology Markup Language (SBML), which facilitates the exchange of models among the modeling community.

5. Yeast Systems biology

Yeast *Saccharomyces cerevisiae* offers an excellent eukaryotic model system to understand the design principles involved in control of different physiological response. Increasing wealth of experimental and computational data and the ease with which the genetic manipulation can be made makes it an attractive model organism to quantify and test hypothesis. The most well studied signaling pathway in yeast are MAPK pathway, which consist of three protein kinases MAPKKK, MAPKK and MAPK acting sequentially. There are functionally multiple MAPK pathways involved in regulating mating, filamentous growth, cell integrity, and osmotic stress.[98] These MAPK pathways share components and co-ordinate/crosstalk with other pathways in regulating the phenotype. Several experimental and theoretical works have addressed how MAPK pathway achieves specificity towards particular phenotype.[87,99] However, little is known about the relevance of the crosstalk of MAPK pathway with other pathways involved in a phenotype. Furthermore, MAPK pathways involved in mating, osmotic and filamentous growth are well characterized and are subjected to mathematical modeling. In this section, we will be discussing in brief the modeling of yeast MAPK pathways and the insights gained into the principle of operation.

5.1. *Mating pheromone pathway*

Yeast MAPK pathway is experimentally shown to demonstrate a graded response in the presence of pheromone.[100] This is in contrast to the behavior of MAPK pathway in *Xenopus* oocytes, which demonstrated a all or none type response in the presence of progesterone. This demonstrates how homologous signaling pathways have different signaling characteristics. In yeast mating pathway, the mating signal, α factor pheromone bind and activates receptor Ste2, which in turn activates the release of hetrodimer G protein, G$b\gamma$ through removal of GDP from Gα. These events trigger the MAPK cascade, which are bound to a scaffold protein Ste5. The MAPK cascade includes Ste11 (MAPKKK), Ste7 (MAPKK) and Fus3 (MAPK). Phosphorylated Fus3 signals to the downstream effectors for induction of gene expression necessary for mating, cell cycle arrest and polarized growth in the direction of pheromone (Fig. 8(a)).[98,101] The transcriptional response to pheromone signal is mediated by Ste12, which binds to pheromone response element present in the target genes. MAPK pathway is subjected to feedback regulation involving multiple positive and negative feedbacks, the significance of which is slowly being addressed through mathematical modeling. The pathway design, in general ensures the downregulation of the pathway after successful activation of target processes.[101] Negative feedback regulation of pheromone pathway exist at multiple levels, which includes (1) binding of pheromone leading to receptor ubiquitination and degradation; (2) GTP hydrolysis by Sst2, which in turn is activated by Fus3; (3) Dephosphorylation of Fus3 by protein phosphatases Ptp2, Ptp3, and Msg5; (4) α factor pheromone degradation facilitated by Bar1, which is under the transcriptional control of Ste12; (5) Expression of inhibitors Msg5 and Sst2 is also under the control of Ste12 and (6) Ste11 (MAPKKK) undergoes ubiquitination and MAPK dependent degradation. Similarly, positive feedbacks are shown to be functional such as (1) synthesis of receptor Ste2 and Fus3 controlled by Ste12 and (2) autoregulation of Fus3 and Ste12. In general, transcription by Ste12 affords multiple feedback loops (Fig. 8(a)). The dynamic model of pheromone pathway was

developed by Kofahl and Klipp (2004), which best describe the time course of phosphorylation of different proteins, complex formation, phenotype of different mutants, time scales in which different feedback proceeds and have also predicted the duration of several signals.[102] Furthermore, prolong stimulation by pheromones have shown to decrease the activity of the pheromone pathway, which indicates the desensitization effect. This property helps the cell to resume vegetative growth after growth arrest in the presence of mating signal. Computational model also indicated the important negative feedback loops required to desensitize the mating signal, thereby helping in having a perfect adaptation. Furthermore, the quantification of hetrodimeric G protein activation and de-activation using in vivo experiments and mathematical model provided a comprehensive understanding of G protein coupled receptor (GPCR) regulation in pheromone signaling.[103]

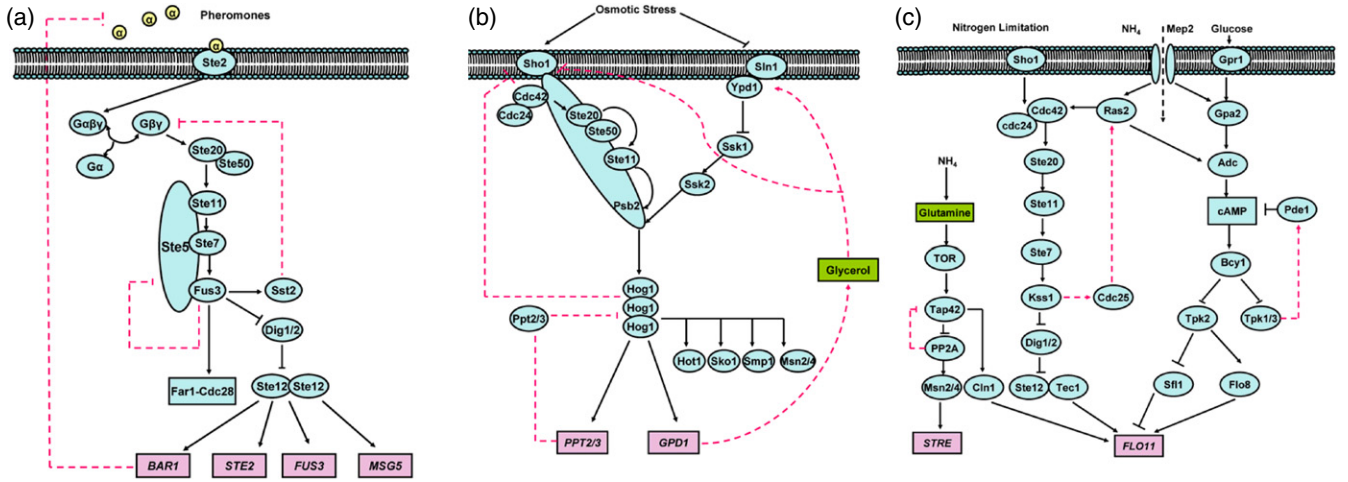
5.2. *Effect of scaffolding*

The pheromone pathway is highly dependent on the scaffold protein Ste5, which tethers Ste11 (MAPKKK), Ste7 (MAPKK), and Fus3 (MAPK) to form a complex. This helps to keep the kinases and their substrates in close proximity, as well as to prevent the influence of phosphatase. Such a function of Ste5 also prevents the crosstalk with other MAPK pathways.[99] Phosphorylation on the scaffold is shown to occur in a processive manner, whereas in the solution phosphorylation occurs through distributive fashion.[104] Processive mechanism involves only one collision for multiple phosphorylation, whereas distributive mechanism involves multiple collision with same kinase with each step contributing towards single phosphorylation. Interestingly, in model of *Xenopus* MAPK pathway, dual phosphorylation of MAPKK and MAPK were considered to occur in a distributive fashion.[51] However, with the assumption of processive mechanism for phosphorylation reduced the predicted Hill coefficient from 1.7 to 1.3 for MAPKK and from 4.9 to 1.5 for MAPK.[51] This indicates that the steepness of the dose response depends upon the mechanism of phosphorylation. Hence, scaffold can function to reduce the Hills coefficient of signaling pathways. However, this need not be the case as the dephosphorylation in scaffold are precluded due to sterical obstruction of the phosphatase, which can make the response sensitive. Furthermore, increased concentration of kinases and scaffold together produce a zero order effect leading to a sensitive response. Thus, concentration of the scaffold, the substrate, and the phosphatase concentration play an important role in determining the Hill coefficients of the MAPK pathway. Most importantly, presence of a negative feedback can make the dose response less sensitive, thereby transmitting the mating signal in a linear (graded) fashion in addition to its role in signal desensitization.[105] It is also possible that the presence of scaffold protein in yeast may account in part for the dramatic differences in the performance of *Saccharomyces* and *Xenopus* MAP kinase cascades. Adding to this debate is a recent study which shows that the yeast pheromone pathway exhibits a bimodal gene expression in a specific range of pheromone concentrations, resulting from the combination of bistability in pheromone induced gene expression and stochastic noise.[106] MAPK mediated activation of Ste12 upregulates the expression of mating-induced genes forming positive feedback loops to exhibit bistable behavior.

5.3. *Osmotic pathway*

Microorganisms in their natural environment are often subjected to osmotic stress (change in the activity of water), which makes them to employ molecular mechanism required for osmoadaptation. Signal transduction pathways involved in osmoadaptation sense the osmotic changes and transmit the signal to trigger appropriate gene expression. During hyperosmotic shock, decrease in activity of water leads to dehydration of cells. This is overcome by physiological response involving accumulation of osmolytes such as glycerol, which helps to regain the cell volume and turgor pressure.[68] Osmoadaptation in yeast is shown to involve a well characterized MAPK pathway called HOG pathway, which controls the accumulation of glycerol. Hog1 (MAPK) is shown to activate the expression of *GPD1* and *GPP1*, whose products are enzymes involved in glycerol synthesis. Closing of glycerol regulated Fps1 channel also controls its accumulation inside the cell. Sln1 and Sho1 are the upstream sensors and they function as independent branches in the control of activation of HOG pathway (Fig. 8(b)).[68,107] These two branches have redundant function and are shown to have different sensitivity to osmotic stimulation, which is postulated to help the cells to respond over wide range of osmotic stress. Sln1 branch is shown to be sensitive to osmotic changes and responds in a linear fashion, whereas Sho1 branch respond in all or non fashion operating with a threshold for activation.[68] Sln1 and Sho1 regulate the activation of Psb2, which functions as MAPKK towards the activation of Hog1. Sho1 binding to Psb2, brings together the Ste20 and Ste11 (MAPKKK), which leads to the activation of Ste11

Figure 8: *Yeast systems biology.* (a) Pheromone signaling involving α factor mediated activation of MAPK cascade with different feedback regulations. (b) Osmotic signaling involving two branches, Sho1 and Sln1 (phosphorelay module) with multiple negative feedback regulation through phosphatase activation and desensitization of receptor. (c) Filamentous growth network involving multiple pathways cAMP-PKA, MAPK and TOR pathways co-ordinate together in the regulation of *FLO11* under nitrogen limitation. TOR pathway controls the general stress response (STRE) and translational regulation of G1 cyclins through Tap42. Double negative feedback look in TOR pathway leads to bistability in filamentous growth network.



and Psb2. Other than MAPKK function, Psb2 also functions as a scaffold for Sho1 branch. Interestingly, this raises the question whether scaffold dependent activation of Sho1 branch can lead to graded response instead of all or none response. Sln1 is a negative regulator of the HOG pathway, which is activated under hypo osmotic condition and inactivated during hyperosmotic condition. Sln1 branch contain phosphorelay module of Sln1-Ypd1-Ssk1. Inactivation of Sln1 leads to dephoshorylation of Ssk1 via Ypd1. This leads to the activation of MAPKKKs Ssk2/22, which in turn activate Psb2. Moreover, Hog1 mediated effects are transient due to the effect of multiple negative feedbacks loop, which offers a perfect adaptation (Fig. 8(b)).[68] Osmoadaptation in yeast offer an interesting network to systematical analyze through mathematical modeling to gain further insights into the network operation involving multiple feedbacks.

A comprehensive dynamic model of osmotic signaling was developed by Klipp et al, which serves as a tool for studying osmoadaptation.[108] The model is able to predict the dynamic operation of the several processes such as (1) crucial role of closing of Fps1 channel leading to initial increase in glycerol accumulation using basal level synthesis of glycerol (2) higher glycerol concentration depends on the glycerol production with increase in glycerol synthesizing enzyme Gpd1, which leads to long term adaptation and (3) phosphorelay module of three components Sln1, Ypd1, Ssk1 demonstrate a switch like response, which depends on a number of components. Furthermore, simulations and experiments demonstrated that the accumulation of glycerol plays a major role in the downregulation of the HOG pathway by way of biophysical effects such as cell swelling and increase in turgor pressure, which have the effect on Sln1 activation and Fps1 opening. Less significant effect of phosphatase Ppt2 concentration was observed on the downregulation of HOG pathway through deactivation of Hog1. However, the phosphatase is required for reversing the system quickly with downregulation of HOG pathway to sensitize the system for any further increases in osmotic shock. The major drawback of the model was that the effect of Sho1 branch was not included and the need for redundant pathways in osmotic signaling was not analyzed.[108] Moreover, Sho1 branch was recently confirmed to have negative feedback regulation directly through inactivation of Sho1 by Hog1, however the relevance and need of this negative feedback with respect to the negative feedback exerted by turgor pressure requires investigation.[109] Both pheromone and osmotic MAPK pathways bring about transient response due to the presence of multiple negative feedback loops. However, in osmotic signaling the feedback does not desensitize the sensor for further activation by osmotic signal, whereas the negative feedback in pheromone signal desensitize the receptor to prevent further activation.[101] This stem from the fact that the Sln1 sensor can switch

between activated and deactivated state depending upon the turgor pressure to adapt perfectly.[68] However, in pheromone signaling the receptor gets degraded by feedback mechanism and re-synthesis depends on the removal of pheromone. This difference in adaptive principle is indicative of the relative importance of the signaling pathways towards cell's survival.

5.4. *Filamentous growth network*

Diploid yeast *Saccharomyces cerevisiae* respond to nitrogen starvation by invoking filamentous growth, which helps them to forage for nutrient and reach an environment conducive for growth. This adaptive mechanism is under the control of complex signaling network of multiple signaling pathways, cAMP-PKA, MAPK and TOR pathways, which are global regulators involved in multiple function inside the cell (Fig. 8(c)).[110,111] These pathways integrate to control the expression of *FLO11*, a flocculin gene involved in filamentous growth through multiple transcriptional activators Flo8p, Ste12, Tec1 and Mss11. However, little is known in terms of how these pathways integrate the environmental signal into gene expression. Experimental and steady state modeling approach have helped to unravel the operating principle governing the conversion of nutrient signal into *FLO11* expression.

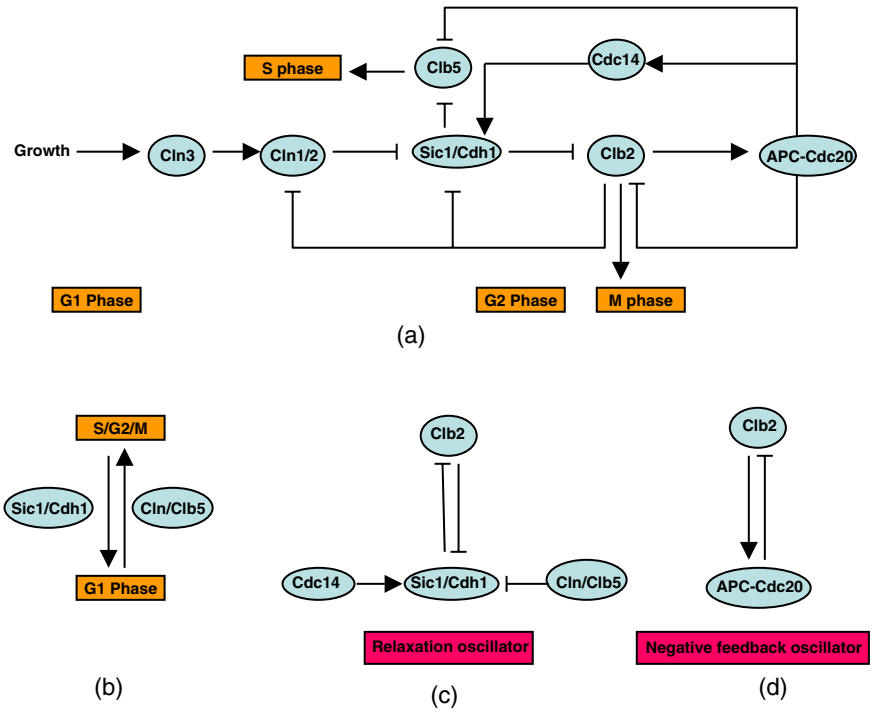
Mathematical model of cAMP and MAPK pathways have been constructed to study their role and contribution towards *FLO11* expression.[112] Further, the effect of crosstalk on the signaling pathway and expression of *FLO11* were also analyzed. *FLO11* expression demonstrated a highly sensitive response with respect to cAMP-PKA pathway activation, whereas a subsensitive response to MAPK pathway activation. The sensitive nature of *FLO11* expression is attributed to an inhibitor of *FLO11*, Sfl1 inactivated by cAMP-PKA pathway, which brings about a switch like behavior to *FLO11* expression. Experimental evidence from Fink's group suggests that Sfl1 also plays a role in bringing heterogeneity in *FLO11* expression.[113] Furthermore, crosstalk from MAPK pathway helps in amplifying the cAMP-PKA at upstream of the network and also functions at the gene level to amplify gene expression through helping in cooperative binding of Flo8. Experiments have demonstrated that the steady state expression of *FLO11* was bistable over a range of inducing ammonium sulphate concentration based on the preculturing condition.[114] Further, yeast switched from *FLO11* expression to accumulation of trehalose, a response dependent on the expression of stress-responsive element (STRE) genes controlled by transcriptional activator Msn2/4, with decrease in the inducing concentration to complete starvation. The analysis included a steady state modeling of the integrated network including the ammonium sulphate transport and sensing functions. Mep2, an ammonium sensor functions upstream of cAMP and MAPK pathway to sense and transport ammonium sulphate. Similarly, TOR pathway functions as intracellular sensor for nitrogen and has functions to maintain the vegetative growth of the cell. The double negative feedback loop in the TOR pathway is shown to elicit a bistable response, which differentiate between vegetative growth, filamentous growth and STRE response.[114] Negative feedback on TOR pathway functions to restrict the expression of *FLO11* under nitrogen starved condition and also on re-addition of nitrogen to starved cells (Fig. 8(c)). In general, these global signaling pathways respond with specific sensitivity to regulate the expression of *FLO11* under nitrogen limitation. Such differences in sensitivities of different pathways help to differentiate between multiple phenotypes using global signaling pathways.

5.5. *Budding cell cycle model*

Molecular mechanism of cell cycle control in budding yeast is well characterized. Cell cycle involves processes such as DNA synthesis, bud emergence, spindle formation, nuclear division and cell separation. Progression through cell cycle involves temporal gaps G1 and G2 (growing and preparation) inserted between S phase (DNA synthesis) and M phase (mitosis). There are multiple control mechanisms which monitor the progress through cell cycle called checkpoints. Cyclin (Cln1-3 and Clb1-6) synthesis and degradation play a principal role in coordinating cell cycle regulation.[115,116] Other main components are cyclin-dependent protein kinase (Cdk) which form complex with cyclins and phosphorylate specific protein targets to induce both S and M phase depending on the type of cyclin it binds (Fig. 9(a)). Cdk/cyclin complexes can be downregulated by inhibitory phosphorylation of the Cdk subunit and by binding to a stoichiometric inhibitor (cyclin-dependent kinase inhibitor (CKI)). The cell cycle regulation has been subjected to extensive mathematical modeling to gain insight into the dynamics of cell division.[116–118] An integrated model developed by Tyson and co-workers is available in the budding cell cycle homepage (`http://mpf.biol.vt.edu/research/budding_yeast_model/pp/index.php`).[118]

Cell cycle network structure has all the ingredients to exhibit multiple properties such as bistability, oscillation, irreversibility and robustness, which provides sharp transition between different

Figure 9: *Budding yeast cell cycle regulation.* (a) Cell cycle progression through G1, S, G2 and M phase are coordinated through synthesis and degradation of cyclins. G1 phase is controlled by cyclins Cln1/2/3, with Cln3 regulated by critical size of the cell and Cln1/2 synthesis regulated by Cln3. The transition from G1 to S phase is governed by activation of cyclin Clb5, through inactivation of its inhibitor Sic1(CK1)/Cdh1 by Cln1/2. The transition from S to M phase occurs with accumulation of Clb2, which also occurs with removal of its inhibitor Sic1/Cdh1, however with time delay. Clb2 decreases the synthesis of Cln1/2 and Clb5. The exit from the mitosis is governed by activation of negative feedback through APC/Cdc20, which brings about the degradation of Clb2. Cdc20 also activates Sic/Cdh1 through Cdc14 to inactivate Clb2. APC/Cdc20 also degrades the Clb5 cyclin. (b) A bistable motif representing the switch between G1 and S/M controlled by Cln/Clb5 and Sic1|I/Cdh1, respectively. (c) A relaxation oscillator involving alternate high and low Clb2. (d) Negative feedback oscillator involving negative feedback regulation of Clb2 through activation of APC/Cdc20.



phases and also to maintain specific phase. Cell cycle regulation exhibits a closed loop behavior of switching the activity of mitotic Clb-Cdk1 from low to high and maintaining the activity before switching it back to low for the next round of cell division.[116] The basis for cell cycle regulation is mutual antagonism between Clb-Cdk and the inhibitors Sic1/Cdh1, which can yield multiple steady state of G1 and S/M. Transition between these states depends on the control system which alter the strength of the feedback (Fig. 9(b)).[116,118] Transition from G1 to S/M depends on the synthesis of G1 cyclins, which can antagonize the inhibitors Sic1/Cdh1, leading to activation of Clb-Cdk1. G1 cyclins synthesis gets inhibited by Clb-Cdk1, which also antagonizes Sic/Cdh1. Similarly, the transition from S/M to G1 depends on the negative feedback loop activated through APC-Cdc20 by Clb-Cdk1 leading to its inactivation and activation of Sic1/Cdh1. Checkpoints help in maintaining G1 and M phase. G1 phase is maintained until the inhibition of synthesis of G1 cyclins by cell's critical size condition and M phase is maintained with delay in the activation of negative feedback. Cross (2003) have shown that the cell cycle regulation embeds both a negative feedback oscillator involving negative feedback regulation of Clb-Cdk1 through APC-Cdc20 and a relaxation oscillator involving alternating high and low Clb (Figs 9(b) and (c)).[69] However, both these oscillators are redundant in their function to help in exiting mitosis (M/G1) and can function independently. Such redundancy can contribute towards the robustness of the cell cycle. Interestingly, evidence from *Xenopus laevis* also suggested that G2/M can function like toggle switch, where Clb2-Cdk1 is involved in mutual activation with Cdc25 (phosphatase) and mutual inhibition with Wee1 (kinase) (Fig. 10). Bistability in this module help to maintain a discrete M phase.[70,119] This is interesting considering the fact

that the module of Clb-Cdk1 can exhibit bistability, a steady state property and oscillation, a dynamic property. Ferrel and co-workers have analyzed this relationship using a simple computational model and demonstrated that the module functions as relaxation oscillator with positive feedback essential for obtaining sustained oscillation, which was also verified experimentally.[120] Hence, negative feedback loop can convert the bistability into a limit cycle behavior with checkpoints serving as bifurcation point, which makes cell-cycle network extremely stable and robust for its function.

6. Mammalian signaling pathways

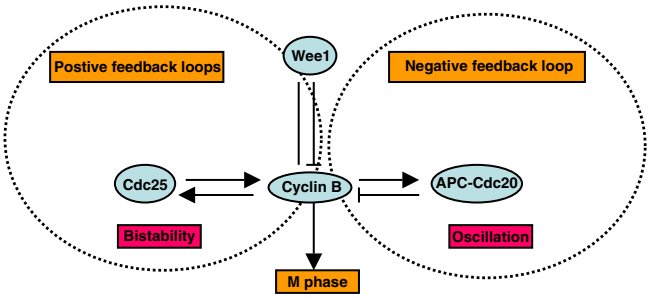
Extensive studies using mammalian cells have deciphered signaling pathways involved in the regulation of cell growth, survival, proliferation, metabolism and apoptosis.[121,122] Quantification of pathways in the mammalian cells can be exploited to understand pathogenesis of a disease state and in identification of drug targets. Signaling through receptor tyrosine kinases (RTKs) plays a central role in the control of cellular processes. Malfunctioning of RTK signaling functions leads to various disease conditions such as cancer, chronic inflammatory syndrome and diabetics.[123,124] Epidermal growth factor receptor (EGFR) and insulin receptor belong to the family of RTKs, which upon stimulation activates multiple interacting pathways such as phospholipase C-γ (PLCγ), phosphatidylinositol 3-kinase (PI3K)-AKT/protein kinase B (PKB) and extracellular signal-regulated kinase (ERK)/MAPK pathways.[121,122] Activated EGFR interacts with adapter protein, growth factor-receptor-bound protein 2 (Grb2) directly or through Shc, which helps to couple the signal from EGFR with Ras. Grb2 releases the guanine nucleotide-releasing factor, Son-of-sevenless (SOS), which catalyses the conversion of GDP to GTP on Ras, resulting in Ras activation. This in turn results in the activation of MAPK cascade, namely Raf1, Mek1 and Erk (Fig. 11).

Several computational models of the EGFR-MAPK signaling have been developed to understand its dynamics.[125,126] Earlier models were primarily focused on the dynamics of the receptor trafficking and ligand induced endocytosis, which predicted the properties such as the robust proportional control behavior of EGFR. Model describing the signaling from receptor to the Ras GTPase was developed by Kholodenko (1999) using experimental information. Models of MAPK signaling were developed by Bhalla (1999, 2002) and Asthagiri and Lauffenburger (2001) demonstrating bistability (positive feedback) and adaptive (negative feedback) behavior, respectively.[17,72,76] Brightman and Fell (2001) showed through a quantitative model that differential feedback regulation can bring about transient and sustained activation of MAPK in response to epidermal growth factor (EGF) and nerve growth factor (NGF), respectively.[127] Interestingly, recent study confirmed this hypothesis to show that the activation of negative feedback by EGF leads to transient response and activation of positive feedback by NGF leads to bistable response.[128] The first detailed large-scale mathematical model (`http://web.mit.edu/dllaz/egf_pap/`) of the basic EGFR signaling was developed by Schoeberl *et al.* (2002), which described the effect of receptor internalization on MAPK activation.[129] Authors showed that the amplitude of MAPK activation demonstrates a non-linear dependence on EGFR. A realistic multi compartmental model demonstrated the differential kinetics of EGFR activation by EGF and transforming growth factor-alpha (TGF-alpha), which was also verified experimentally.[130] Mathematical models were also developed addressing the role of crosstalk between MAPK and AKT/PKB pathways and how MAPK output characteristics (time delay, signal amplitude, duration of output) depended on the component concentrations and parameter values.[131,132]

Another growth factor stimulated pathway other than EGFR is the insulin pathway. Binding of Insulin to its receptor activates PI3K/AKT pathway in addition to MAPK pathway, to control cell differentiation, protein synthesis and glucose metabolism involving uptake of glucose and glycogen/lipid synthesis.[124] On insulin binding, the receptor undergo autophosphorylation and enhanced tyrosine activity to activate insulin receptor protein (IRS1), which serves as a docking site for the regulatory subunit of PI3K. This leads to the activation of PI3K, which in turn phosphorylate the phosphatidylinositol 3,4,5-trisphosphates (PIP3) to activate 3-phosphoinositide-dependent protein kinase (PDK)-1. Further, PDK1 and PIP3 activate the downstream kinases Akt and protein kinase C (PKC), which facilitate the translocation of glucose transporter GLUT4 from intracellular compartment of the cell to plasma membrane (Fig. 11).[124,133] PTP1-B is a phosphatase that negatively regulates the insulin signaling by dephosphorylating the insulin receptor and IRS1. Insulin pathway is also subjected to multiple feedback regulation involving phosphorylation of PTP1B (positive feedback) and IRS-1 (negative feedback) controlled by Akt and PKC. Furthermore, Akt activates nutrient mTOR signaling, which exerts a negative feedback on insulin signaling.

Quon and co-workers have carried out extensive mathematical modeling of insulin signaling starting

Figure 10: Integration of bistability and oscillation to yield a relaxation oscillator in G2/M phase in *Xenopus laevis*. Bistability arises due to mutual activation and inhibition of cyclin Clb2 with Cdc25 (phosphatase) and Wee1 (kinase), respectively. Oscillation depends upon the negative feedback regulation of Clb2 through activation of APC/Cdc20.



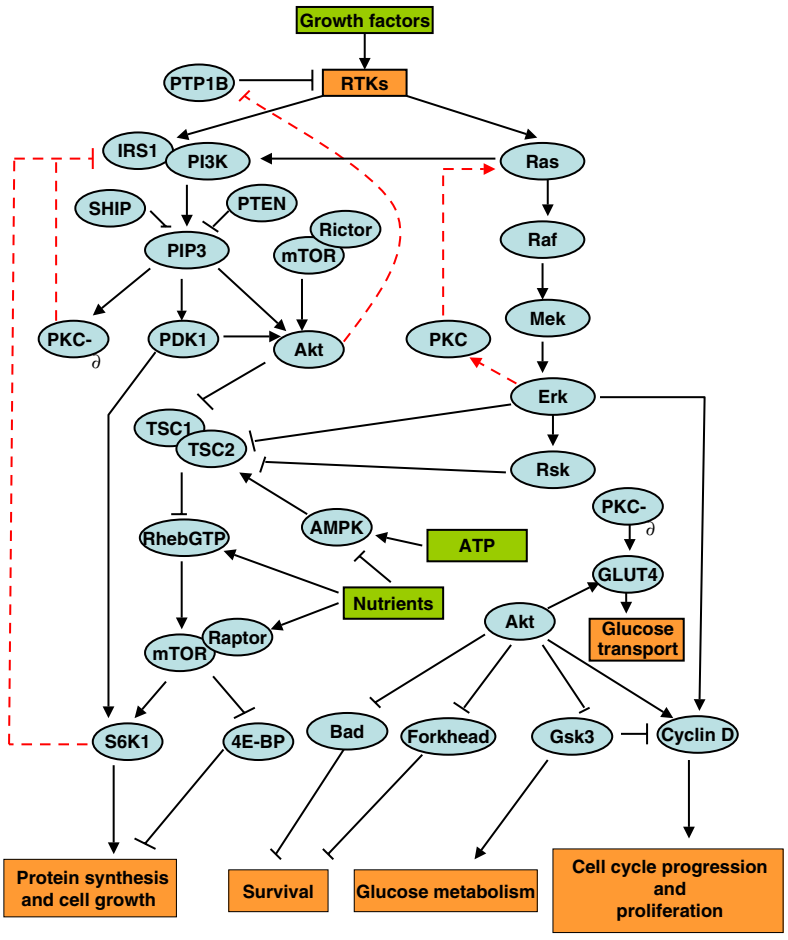
with subsystem models such as insulin receptor binding kinetics, receptor recycling and GLUT4 translocation.[134–136] An integrated model was developed by including these subsystems and also post receptor signaling, which now serve as a tool for studying insulin signaling.[137] Model simulations matched well with the experimental data and also demonstrated the effects of feedbacks. The study demonstrated that in the absence of feedbacks the half saturation constant for GLUT4 and PI3K increase slightly without much change in the sensitivity of the response compared to the response in the presence of feedback. Similarly, without feedback the biphasic behavior of PKC activation was lost. However, the role and contribution of individual feedback was not analyzed. Recently, a steady state model of the insulin pathway demonstrated that GLUT4 translocation can operate as a bistable switch with respect to insulin concentration.[138] The study demonstrated the effect of component concentration and parameters of insulin pathway on GLUT4 translocation, which provided insights into the pathological conditions such as insulin resistance. On other hand, negative feedback regulation of insulin signaling can be expected to give rise to an oscillatory behavior. Such positive and negative feedbacks can function together to make bistable or oscillatory behavior of GLUT4 robust. Any modification to robust setting either due to overactivation of the positive feedback or negative feedback can result in a disease state.

Furthermore, Akt is involved in crosstalk with tumor suppressor protein, p53, which control the cell's decision between survival and death. Akt and p53 form a mutually antagonist circuit. p53 activates tumor suppressor gene PTEN (phosphatase and tensin homolog), which inhibits PIP3 of Akt signaling. On other hand Akt activates another oncogene Mdm2, which inhibits p53. Interestingly, p53 also activates Mdm2 (Fig. 12).[139,140] This forms the simple network of cancer relevant genes. Computational model developed showed that the network can exhibit bistable behavior producing robust survival-death switch.[141] Moreover, the model predicted the apoptosis threshold and network perturbation due to DNA damage and Akt inhibition which can be experimentally tested. Interesting, a simple mathematical model suggests that the negative feedback between p53 and Mdm2 can bring about oscillation in p53 protein levels in response to stress. This feature is proposed to help the cells to repair the damaged DNA without triggering apoptosis due to excessive activation of p53.[142,143] Different models discussed so far have regulatory structures capable of eliciting both oscillatory and bistable response, which can offer the cell an advantage in terms of achieving the desired phenotype. These properties can be expected to be coupled (as seen in the cell cycle regulation) to offer robust cellular response. The other signaling pathways for which mathematical model have been developed is Jak–Stat pathway, WNT/β-catenin pathway, calcium–calmodulin network, integrated signaling network in neurons and cell death pathways.[17,144–146]

7. Conclusion and perspectives

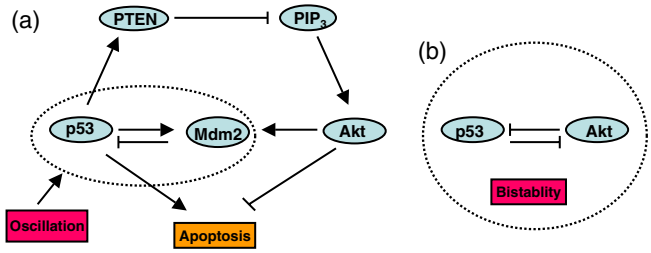
Quantification of signaling pathways brings together different qualitative and quantitative information of individual proteins to gain a system level understanding of cellular processes, which is not evident from the interaction map. In the current scenario, there is a vast pool of qualitative information and limited amount of quantitative information that are available to model biological systems. Mathematical models are constrained by the lack of quantitative data and have to depend upon the assumption of parameters derived from

Figure 11: *Receptor tyrosine kinase (RTK) Signaling.* Binding of growth factor (insulin) to insulin receptor activates (PI3K)-AKT/protein kinase B (PKB) and extracellular signal-regulated kinase (ERK)/MAPK pathways. PI3K-AKT signaling controls the translocation of GLUT4, a glucose transporter required for uptake of glucose, protein synthesis through activation of mTOR signaling, glucose metabolism, cell survival and proliferation. Insulin signaling is subjected to positive feedback through inactivation of phosphatase PTP1B, which inactivates the signaling by dephosphorylating the insulin receptor and insulin receptor substrate (IRS1). Negative feedback regulation involves activation of S6K1 and PKC-ζ, which phosphorylate serine residues in IRS1 to prevent tyrosine phosphorylation of IRS1. S6K1 is activated by mTOR pathway, which is controlled by insulin signaling pathway through inactivation of tuberous sclerosis complex, TSC1/2. mTOR pathway is also controlled by nutrients and ATP independent of growth factor signaling. ERK signaling involves activation of MAPK cascade, which controls cell cycle progression and proliferation. MAPK cascade is subjected to positive feedback regulation through activation of PKC and negative feedback regulation through phosphorylation of MAPKKK/SOS. In addition ERK pathway crosstalk with PI3K-AKT signaling through activation of PI3K and inactivation of TSC1/2.



biological intuition. With the help of method such as parameter sensitivity analysis it has become possible to explore the entire spectrum of parameter space under which the network behavior changes qualitatively. A mathematical model, such as for the budding cell cycle comprising of greater than 100 unknown parameters, was successfully able to capture the dynamics of cell cycle using qualitative information of different mutants. The different examples in yeast and mammalian system demonstrated that the system design offer the cells to respond either in transient or sustained manner. Signaling network exhibit emergent properties, which help the cells to achieve the desired phenotypic state in a controlled and robust manner. The regulation of biological systems through feedback control can offer the system with different properties such as ultrasensitivity, bistability, hysteresis, irreversibility, adaptation, oscillation and robustness. However, the biological control involving feedback regulation can also make the system vulnerable under non-physiological

Figure 12: *Crosstalk between Akt and p53, central regulator of cells's survival and death.* Regulatory circuit of Akt-p53 provide oscillatory behavior through negative feedback regulation of p53 by oncogene Mdm2 and bistable behavior through mutually antagonist circuit involving Akt and p53



conditions. For example, hyperactivation of positive feedback makes reversible system irreversible and hyperactivation of negative feedback can make responsive system less sensitive. This is of bigger significance, especially in understanding disease states such as cancer and diabetes, a manifestation also resulting from the hyperactivation of positive and negative feedbacks in growth factor signaling pathway, respectively.

Mathematical model which are discussed here essentially demonstrated how different regulatory structures in the signaling network function and contributed towards the survival and growth of cells under different environmental conditions. It should be noted that the different regulatory structures of the network only constitute the sub modules of the network and need not necessarily influence the network properties as a whole. Building a mathematical model of the cellular process allows the researcher to test these possibilities through in silico mutation, which is experimentally cumbersome. Model predictions narrow down the targets and identify the presence of components, which biologist can test or decipher in limited time. The model validation through experiments helps to refine the model and to increase the predicting capability of the model. Such a process involving experimental and modeling approach should operate in a cyclic fashion to contribute towards better understanding of cellular processes.

Molecular biology has and will continue to decipher various interactions to yield a molecular map through reductionist approach. It is becoming clear that a cellular response cannot be completely understood by just knowing the molecular map of signaling networks. Quantification of these networks at a system level is essential to understand cellular functions. This implies that the future experiments should strive at obtaining quantitative data at the systems level. Theoretical methodologies must also be developed to handle large number of interactions between components and a rational approach in analyzing system parameters. A integrative approach of model development in hand with experimental techniques will help in characterizing a cellular function, which further can provide insights into disease states and drug discovery. Future aim in quantitative biology will work towards integrating networks at genetic, signaling and metabolic levels to obtain a cell level perspective.

Acknowledgements

K.V.Venkatesh acknowledges financial support from the Swarnajayanti fellowship, Department of Science and Technology, India.

Received 29 March 2008; revised 15 April 2008.

References

1. M. Vidal, "A biological atlas of functional maps", *Cell,* Vol. 104(3), 333–339, 2001.
2. G. MacBeath, "Protein microarrays and proteomics", *Nature Genetics,* Vol. 32, 526–532, 2002.
3. H. Zhu, and M. Snyder, "'Omic' approaches for unraveling signaling networks", *Current Opinion in Cell Biology,* Vol. 14(2), 173–179, 2002.
4. G.D. Bader, A. Heilbut, B. Andrews, M. Tyers, T. Hughes, and C. Boone, "Functional genomics and proteomics: charting a multidimensional map of the yeast cell", *Trends in Cell Biology,* Vol. 13(7), 344–356, 2003.
5. M.B. Eisen, P.T. Spellman, P.O. Brown, and D. Botstein, "Cluster analysis and display of genome-wide expression patterns", *Proceedings of the National Academy of Sciences of the United States of America,* Vol. 95(25), 14863–14868, 1998.
6. Z. Bar-Joseph, G.K. Gerber, T.I. Lee, N.J. Rinaldi, J.Y. Yoo, F. Robert, D.B. Gordon, E. Fraenkel, T.S. Jaakkola, R.A. Young, and D.K. Gifford, "Computational discovery of gene modules and regulatory networks", *Nature Biotechnology,*Vol. 21(11), 1337–1342, 2003.
7. N. Friedman, "Inferring cellular networks using probabilistic graphical models", *Science,* Vol. 303(5659), 799–805, 2004.
8. G.W. Carter, "Inferring network interactions within a cell", *Briefings in Bioinformatics,* Vol. 6(4), 380–389, 2005.
9. J.D. Murray, " *Mathematical Biology: An Introduction*", Springer, New York, 2002.
10. H. Qian, and T.C. Reluga, "Nonequilibrium thermodynamics and nonlinear kinetics in a cellular signaling switch", *Physical Review Letters,* Vol. 94(2), 2005.
11. D. Butler, "Computing 2010: from black holes to biology", *Nature,* Vol. 402(6761), C67–C70, 1999.

12. L.H. Hartwell, J.J. Hopfield, S. Leibler, and A.W. Murray, "From molecular to modular cell biology", *Nature,* Vol. 402(6761), C47–C52, 1999.
13. J.J. Tyson, K. Chen, and B. Novak, "Network dynamics and cell physiology", *Nature Reviews Molecular Cell Biology,* Vol. 2(12), 908–916, 2001.
14. H. Ge, A.J.M. Walhout, and M. Vidal, "Integrating 'omic' information: a bridge between genomics and systems biology", *Trends in Genetics,* Vol. 19(10), 551–560, 2003.
15. D.M. Wolf, and A.P. Arkin, "Motifs, modules and games in bacteria", *Current Opinion in Microbiology,* Vol. 6(2), 125–134, 2003.
16. N. Barkai, and S. Leibler, "Robustness in simple biochemical networks", *Nature,* Vol. 387(6636), 913–917, 1997.
17. U.S. Bhalla, and R. Iyengar, "Emergent properties of networks of biological signaling pathways", *Science,* Vol. 283(5400), 381–387, 1999.
18. J.A. Papin, T. Hunter, B.O. Palsson, and S. Subramaniam, "Reconstruction of cellular signalling networks and analysis of their properties", *Nature Reviews Molecular Cell Biology,* Vol. 6(2), 99–111, 2005.
19. B.N. Kholodenko, "Cell-signalling dynamics in time and space", *Nature Reviews Molecular Cell Biology,* Vol. 7(3), 165–176, 2006.
20. B.N. Kholodenko, "Negative feedback and ultrasensitivity can bring about oscillations in the mitogen-activated protein kinase cascades", *European Journal of Biochemistry,* Vol. 267(6), 1583–1588, 2000.
21. J.E. Ferrell, "Self-perpetuating states in signal transduction: positive feedback, double-negative feedback and bistability", *Current Opinion in Cell Biology,* Vol. 14(2), 140–148, 2002.
22. C.V. Rao, and A.P. Arkin, "Control motifs for intracellular regulatory networks", *Annual Review of Biomedical Engineering,* Vol. 3, 391–419, 2001.
23. M.E. Csete, and J.C. Doyle, "Reverse engineering of biological complexity", *Science,* Vol. 295(5560), 1664–1669, 2002.
24. U. Alon, "Biological networks: The tinkerer as an engineer", *Science,* Vol. 301(5641), 1866–1867, 2003.
25. A.P. Arkin, "Synthetic cell biology", *Current Opinion in Biotechnology,* Vol. 12(6), 638–644, 2001.
26. J. Hasty, D. McMillen, and J.J. Collins, "Engineered gene circuits", *Nature,* Vol. 420(6912), 224–230, 2002.
27. M. Schauer, and R. Heinrich, "Quasi-steady-state approximation in the mathematical-modeling of biochemical reaction networks", *Mathematical Biosciences,* Vol. 65(2), 155–170, 1983.
28. J.E. Bailey, "Mathematical modeling and analysis in biochemical engineering: Past accomplishments and future opportunities", *Biotechnology Progress,* Vol. 14(1), 8–20, 1998.
29. D.E. Koshland, "The era of pathway quantification", *Science,* Vol. 280(5365), 852–853, 1998.
30. R. Heinrich, B.G. Neel, and T.A. Rapoport, "Mathematical models of protein kinase signal transduction", *Molecular Cell,* Vol. 9(5), 957–970, 2002.
31. B.N. Kholodenko, O.V. Demin, G. Moehren, and J.B. Hoek, "Quantification of signaling by epidermal growth factor receptor", *Biophysical Journal,* Vol. 76(1), A226–A226, 1999.
32. A.R. Asthagiri, and D.A. Lauffenburger, "Bioengineering models of cell signaling", *Annual Review of Biomedical Engineering,* Vol. 2, 31–53, 2000.
33. U.S. Bhalla, "Understanding complex signaling networks through models and metaphors", *Progress in Biophysics & Molecular Biology,* Vol. 81(1), 45–65, 2003.
34. N.J. Eungdamrong, and R. Iyengar, "Computational approaches for modeling regulatory cellular networks", *Trends in Cell Biology,* Vol. 14(12), 661–669, 2004.
35. H.M. Sauro, and B.N. Kholodenko, "Quantitative analysis of signaling networks", *Progress in Biophysics & Molecular Biology,* Vol. 86(1), 5–43, 2004.
36. B.B. Aldridge, J.M. Burke, D.A. Lauffenburger, and P.K. Sorger, "Physicochemical modelling of cell signalling pathways", *Nature Cell Biology,* Vol. 8(11), 1195–1203, 2006.
37. T. Ideker, T. Galitski, and L. Hood, "A new approach to decoding life: Systems biology", *Annual Review of Genomics and Human Genetics,* Vol. 2, 343–372, 2001.
38. H. Kitano, "Perspectives on systems biology", *New Generation Computing,* Vol. 18(3), 199–216, 2000.
39. H. Kitano, "Systems biology: A brief overview", *Science,* Vol. 295(5560), 1662–1664, 2002.
40. H. Kitano, "Computational systems biology", *Nature,* Vol. 420(6912), 206–210, 2002.
41. T. Ideker, "Systems biology 101 — what you need to know", *Nature Biotechnology,* Vol. 22(4), 473–475, 2004.
42. G.Z. Weng, U.S. Bhalla, and R. Iyengar, "Complexity in biological signaling systems", *Science,* Vol. 284(5411), 92–96, 1999.
43. J. Downward, "The ins and outs of signaling", *Nature,* Vol. 411(6839), 759–762, 2001.
44. J.D. Jordan, E.M. Landau, and R. Iyengar, "Signaling networks: The origins of cellular multitasking", *Cell,* Vol. 103(2), 193–200, 2000.
45. D.E. Koshland, "Switches, thresholds and ultrasensitivity", *Trends in Biochemical Sciences,* Vol. 12(6), 225–229, 1987.
46. A. Goldbeter, and D.E. Koshland, "An amplified sensitivity arising from covalent modification in biological systems", *Proceedings of the National Academy of Sciences of the United States of America,* Vol. 78(11), 6840–6844, 1981.
47. D.E. Koshland, A. Goldbeter, and J.B. Stock, "Amplification and adaptation in regulatory and sensory systems", *Science,* Vol. 217, 220–225, 1982.
48. A.V. Hill, "The combinations of haemoglobin with oxygen and carbon monoxide", *Biochemical Journal,* Vol. 7, 471–480, 1913.
49. A. Goldbeter, and G. Dupont, "Allosteric regulation, cooperativity, and biochemical oscillations", *Biophysical Chemistry,* Vol. 37(1–3), 341–353, 1990.
50. A. Goldbeter, and D.E. Koshland, "Ultrasensitivity in biochemical systems controlled by covalent modification — interplay between zero-order and multistep effects", *Journal of Biological Chemistry,* Vol. 259(23), 14441–14447, 1984.
51. C.Y.F. Huang, and J.E. Ferrell, "Ultrasensitivity in the mitogen-activated protein kinase cascade", *Proceedings of the National Academy of Sciences of the United States of America,* Vol. 93(19), 10078–10083, 1996.
52. J.E. Ferrell, "Tripping the switch fantastic: How a protein kinase cascade can convert graded inputs into switch-like outputs", *Trends in Biochemical Sciences,* Vol. 21(12), 460–466, 1996.
53. J.E. Ferrell, and E.M. Machleder, "The biochemical basis of an all-or-none cell fate switch in Xenopus oocytes", *Science,* Vol. 280(5365), 895–898, 1998.
54. O. Brandman, J.E. Ferrett, R. Li, and T. Meyer, "Interlinked fast and slow positive feedback loops drive reliable cell decisions", *Science,* Vol. 310(5747), 496–498, 2005.
55. S.R. Biggar, and G.R. Crabtree, "Cell signaling can direct either binary or graded transcriptional responses", *Embo Journal,* Vol. 20(12), 3167–3176, 2001.
56. E.M. Ozbudak, M. Thattai, H.N. Lim, B.I. Shraiman, and A. van Oudenaarden, "Multistability in the lactose utilization network of *Escherichia coli*", *Nature,* Vol. 427(6976), 737–740, 2004.
57. T. Shibata, and K. Fujimoto, "Noisy signal amplification in ultrasensitive signal transduction", *Proceedings of the National Academy of Sciences of the United States of America,* Vol. 102(2), 331–336, 2005.
58. M. Laurent, and N. Kellershohn, "Multistability: a major means of differentiation and evolution in biological systems", *Trends in Biochemical Sciences,* Vol. 24(11), 418–422, 1999.
59. J.E. Ferrell, and W. Xiong, "Bistability in cell signaling: How

to make continuous processes discontinuous, and reversible processes irreversible", *Chaos,* Vol. 11(1), 227–236, 2001.
60. T.H. Tian, and K. Burrage, "Bistability and switching in the lysis/lysogeny genetic regulatory network of bacteriophage lambda", *Journal of Theoretical Biology,* Vol. 227(2), 229–237, 2004.
61. W. Xiong, and J.E. Ferrell, "A positive-feedback-based bistable 'memory module' that governs a cell fate decision", *Nature,* Vol. 426(6965), 460–465, 2003.
62. J.E. Ferrell, "Xenopus oocyte maturation: new lessons from a good egg", *Bioessays,* Vol. 21(10), 833–842, 1999.
63. C.P. Bagowski, and J.E. Ferrell, "Bistability in the JNK cascade", *Current Biology,* Vol. 11(15), 1176–1182, 2001.
64. N.I. Markevich, J.B. Hoek, and B.N. Kholodenko, "Signaling switches and bistability arising from multisite phosphorylation in protein kinase cascades", *Journal of Cell Biology,* Vol. 164(3), 353–359, 2004.
65. A. Goldbeter, " *Biochemical Oscillations and Cellular Rhythms: The Molecular Bases of Periodic and Chaotic Behaviour*" Cambridge University Press, 1997.
66. A. Goldbeter, "Computational approaches to cellular rhythms", *Nature,* Vol. 420(6912), 238–245, 2002.
67. V. Chickarmane, B.N. Kholodenko, and H.M. Sauro, "Oscillatory dynamics arising from competitive inhibition and multisite phosphorylation", *Journal of Theoretical Biology,* Vol. 244(1), 68–76, 2007.
68. S. Hohmann, "Osmotic stress signaling and osmoadaptation in Yeasts", *Microbiology and Molecular Biology Reviews,* Vol. 66(2), 300–372, 2002.
69. F.R. Cross, "Two redundant oscillatory mechanisms in the yeast cell cycle", *Developmental Cell,* Vol. 4(5), 741–752, 2003.
70. J.R. Pomerening, E.D. Sontag, and J.E. Ferrell, "Building a cell cycle oscillator: hysteresis and bistability in the activation of Cdc2", *Nature Cell Biology,* Vol. 5(4), 346–351, 2003.
71. J.J. Tyson, K.C. Chen, and B. Novak, "Sniffers, buzzers, toggles and blinkers: dynamics of regulatory and signaling pathways in the cell", *Current Opinion in Cell Biology,* Vol. 15(2), 221–231, 2003.
72. U.S. Bhalla, P.T. Ram, and R. Iyengar, "MAP kinase phosphatase as a locus of flexibility in a mitogen-activated protein kinase signaling network", *Science,* Vol. 297(5583), 1018–1023, 2002.
73. U.S. Bhalla, and R. Iyengar, "Robustness of the bistable behavior of a biological signaling feedback loop", *Chaos,* Vol. 11(1), 221–226, 2001.
74. L. Qiao, R.B. Nachbar, I.G. Kevrekidis, and S.Y. Shvartsman, "Bistability and oscillations in the Huang–Ferrell model of MAPK signaling", *Plos Computational Biology,* Vol. 3(9), 1819–1826, 2007.
75. J.J. Hornberg, M.R. Tijssen, and J. Lankelma, "Synergistic activation of signalling to extracellular signal-regulated kinases 1 and 2 by epidermal growth factor and 4 beta-phorbol 12-myristate 13-acetate", *European Journal of Biochemistry,* Vol. 271(19), 3905–3913, 2004.
76. A.R. Asthagiri, and D.A. Lauffenburger, "A computational study of feedback effects on signal dynamics in a mitogen-activated protein kinase (MAPK) pathway model", *Biotechnology Progress,* Vol. 17(2), 227–239, 2001.
77. H. Kitano, "Biological robustness", *Nature Reviews Genetics,* Vol. 5(11), 826–837, 2004.
78. J. Stelling, U. Sauer, Z. Szallasi, F.J. Doyle, and J. Doyle, "Robustness of cellular functions", *Cell,* Vol. 118(6), 675–685, 2004.
79. H. Kitano, "Towards a theory of biological robustness", *Molecular Systems Biology,* Vol. 3, 137, 2007.
80. U. Alon, M.G. Surette, N. Barkai, and S. Leibler, "Robustness in bacterial chemotaxis", *Nature,* Vol. 397(6715), 168–171, 1999.
81. T.M. Yi, Y. Huang, M.I. Simon, and J. Doyle, "Robust perfect adaptation in bacterial chemotaxis through integral feedback control", *Proceedings of the National Academy of Sciences of the United States of America,* Vol. 97(9), 4649–4653, 2000.
82. S. Bhartiya, N. Chaudhary, K.V. Venkatesh, and F.J. Doyle, "Multiple feedback loop design in the tryptophan regulatory network of *Escherichia coli* suggests a paradigm for robust regulation of processes in series", *Journal of the Royal Society Interface,* Vol. 3(8), 383–391, 2006.
83. J.W. Little, D.P. Shepley, and D.W. Wert, "Robustness of a gene regulatory circuit", *Embo Journal,* Vol. 18(15), 4299–4307, 1999.
84. V.K. Mutalik, P. Shah, and K.V. Venkatesh, "Allosteric interactions and bifunctionality make the response of glutamine synthetase cascade system of Escherichia coli robust and ultrasensitive", *Journal of Biological Chemistry,* Vol. 278(29), 26327–26332, 2003.
85. V.K. Mutalik, A.P. Singh, J.S. Edwards, and K.V. Venkatesh, "Robust global sensitivity in multiple enzyme cascade system explains how the downstream cascade structure may remain unaffected by cross-talk (vol 558, pg 79, 2004)", *Febs Letters,* Vol. 579(1), 292–293, 2005.
86. H. Kitano, K. Oda, T. Kimura, Y. Matsuoka, M. Csete, J. Doyle, and M. Muramatsu, "Metabolic syndrome and robustness tradeoffs", *Diabetes,* Vol. 53, S6–S15, 2004.
87. M.N. McClean, A. Mody, J.R. Broach, and S. Ramanathan, "Cross-talk and decision making in MAP kinase pathways", *Nature Genetics,* Vol. 39(3), 409–414, 2007.
88. N.L. Komarova, X.F. Zou, Q. Nie, and L. Bardwell, "A theoretical framework for specificity in cell signaling", *Molecular Systems Biology,*1:2005.0023, 2005.
89. L. Bardwell, X.F. Zou, Q. Nie, and N.L. Komarova, "Mathematical models of specifcity in cell signaling", *Biophysical Journal,* Vol. 92(10), 3425–3441, 2007.
90. J. Schaber, B. Kofahl, A. Kowald, and E. Klipp, "A modelling approach to quantify dynamic crosstalk between the pheromone and the starvation pathway in baker's yeast", *Febs Journal,* Vol. 273(15), 3520–3533, 2006.
91. D.T. Gillespie, "Exact stochastic simulation of coupled chemical reactions". *Journal of Physical Chemistry,* Vol. 81, 2340–2361, 1977.
92. A. Faure, A. Naldi, C. Chaouiya, and D. Thieffry, "Dynamical analysis of a generic Boolean model for the control of the mammalian cell cycle", *Bioinformatics,* Vol. 22(14), E124–E131, 2006.
93. J.A. Papin, and B.O. Palsson, "Topological analysis of mass-balanced signaling networks: a framework to obtain network properties including crosstalk", *Journal of Theoretical Biology,* Vol. 227(2), 283–297, 2004.
94. C.G. Moles, P. Mendes, and J.R. Banga, "Parameter estimation in biochemical pathways: A comparison of global optimization methods", *Genome Research,* Vol. 13(11), 2467–2474, 2003.
95. E.J. Doedel, H.B. Keller, and J.P. Kernevez, "Numerical analysis and control of bifurcation problems (I) bifurcation in finite dimensions." *International Journal of Bifurcation and Chaos,* Vol. 1(3), 493–520, 1991a.
96. M. Ullah, H. Schmidt, K.H. Cho, and O. Wolkenhauer, "Deterministic modelling and stochastic simulation of biochemical pathways using MATLAB", *IEE Proceedings Systems Biology,* Vol. 153(2), 53–60, 2006.
97. H. Schmidt, G. Drews, J. Vera, and O. Wolkenhauer, "SBML export interface for the systems biology toolbox for MATLAB", *Bioinformatics,* Vol. 23(10), 1297–1298, 2007.
98. M.C. Gustin, J. Albertyn, M. Alexander, and K. Davenport, "MAP kinase pathways in the yeast Saccharomyces cerevisiae", *Microbiology and Molecular Biology Reviews,* Vol. 62(4), 1264–1300, 1998.
99. M.A. Schwartz, and H.D. Madhani, "Principles of map kinase signaling specificity in *Saccharomyces cerevisiae*", *Annual Review of Genetics,* Vol. 38, 725–748, 2004.

100. M.A. Portiz, S. Malmstrom, M.K.H. Kim, P.J. Rossmeissl, and A. Kamb, "Graded mode of transcriptional induction in yeast pheromone signalling revealed by single-cell analysis", *Yeast,* Vol. 18(14), 1331–1338, 2001.
101. L. Bardwell, "A walk-through of the yeast mating pheromone response pathway", *Peptides,* Vol. 25(9), 1465–1476, 2004.
102. B. Kofahl, and E. Klipp, "Modelling the dynamics of the yeast pheromone pathway", *Yeast,* Vol. 21(10), 831–850, 2004.
103. T.M. Yi, H. Kitano, and M.I. Simon, "A quantitative characterization of the yeast heterotrimeric G protein cycle", *Proceedings of the National Academy of Sciences of the United States of America,* Vol. 100(19), 10764–10769, 2003.
104. A. Levchenko, J. Bruck, and P.W. Sternberg, "Scaffold proteins may biphasically affect the levels of mitogen-activated protein kinase signaling and reduce its threshold properties", *Proceedings of the National Academy of Sciences of the United States of America,* Vol. 97(11), 5818–5823, 2000.
105. D.Y. Shao, W. Zheng, W.J. Qiu, O.Y. Qi, and C. Tang, "Dynamic studies of scaffold-dependent mating pathway in yeast", *Biophysical Journal,* Vol. 91(11), 3986–4001, 2006.
106. S. Paliwal, P.A. Iglesias, K. Campbell, Z. Hilioti, A. Groisman, and A. Levchenko, "MAPK-mediated bimodal gene expression and adaptive gradient sensing in yeast", *Nature,* Vol. 446(7131), 46–51, 2007.
107. H. Saito, and K. Tatebayashi, "Regulation of the osmoregulatory HOG MAPK cascade in yeast", *Journal of Biochemistry,* Vol. 136(3), 267–272, 2004.
108. E. Klipp, B. Nordlander, R. Kruger, P. Gennemark, and S. Hohmann, "Integrative model of the response of yeast to osmotic shock", *Nature Biotechnology,* Vol. 23(8), 975–982, 2005.
109. N. Hao, M. Behar, S.C. Parnell, M.P. Torres, C.H. Borchers, T.C. Elston, and H.G. Dohlman, "A systems-biology analysis of feedback inhibition in the Sho1 osmotic-stress-response pathway", *Current Biology,* Vol. 17(8), 659–667, 2007.
110. J.M. Gancedo, "Control of pseudohyphae formation in Saccharomyces cerevisiae", *Fems Microbiology Reviews,* Vol. 25(1), 107–123, 2001.
111. M. Gagiano, F.F. Bauer, and I.S. Pretorius, "The sensing of nutritional status and the relationship to filamentous growth in Saccharomyces cerevisiae", *Fems Yeast Research,* Vol. 2(4), 433–470, 2002.
112. N. Sengupta, P.K. Vinod, and K.V. Venkatesh, "Crosstalk between cAMP-PKA and MAP kinase pathways is a key regulatory design necessary to regulate FLO11 expression", *Biophysical Chemistry,* Vol. 125(1), 59–71, 2007.
113. A. Halme, S. Bumgarner, C. Styles, and G.R. Fink, "Genetic and epigenetic regulation of the FLO gene family generates cell-surface variation in yeast", *Cell,* Vol. 116(3), 405–415, 2004.
114. P. K. Vinod, N. Sengupta, P.J. Bhat, and K.V. Venkatesh, "Integration of Global Signaling Pathways, cAMP-PKA, MAPK and TOR in the Regulation of FLO11", *PLoS ONE,* Vol. 3(2), e1663, 2008.
115. K. Nasmyth, "At the heart of the budding yeast cell cycle", *Trends in Genetics,* Vol. 12(10), 405–412, 1996.
116. K.C. Chen, A. Csikasz-Nagy, B. Gyorffy, J. Val, B. Novak, and J.J. Tyson, "Kinetic analysis of a molecular model of the budding yeast cell cycle", *Molecular Biology of the Cell,* Vol. 11(1), 369–391, 2000.
117. A. Ciliberto, B. Novak, and J.J. Tyson, "Mathematical model of the morphogenesis checkpoint in budding yeast", *Journal of Cell Biology,* Vol. 163(6), 1243–1254, 2003.
118. K.C. Chen, L. Calzone, A. Csikasz-Nagy, F.R. Cross, B. Novak, and J.J. Tyson, "Integrative analysis of cell cycle control in budding yeast", *Molecular Biology of the Cell,* Vol. 15(8), 3841–3862, 2004.
119. W. Sha, J. Moore, K. Chen, A.D. Lassaletta, C.S. Yi, J.J. Tyson, and J.C. Sible, "Hysteresis drives cell-cycle transitions in Xenopus laevis egg extracts", *Proceedings of the National Academy of Sciences of the United States of America,* Vol. 100(3), 975–980, 2003.
120. J.R. Pomerening, S.Y. Kim, and J.E. Ferrell, "Systems-level dissection of the cell-cycle oscillator: Bypassing positive feedback produces damped oscillations", *Cell,* Vol. 122(4), 565–578, 2005.
121. J. Schlessinger, "Cell signaling by receptor tyrosine kinases", *Cell,* Vol. 103(2), 211–225, 2000.
122. T. Zhu, E.L.K. Goh, R. Graichen, L. Ling, and P.E. Lobie, "Signal transduction via the growth hormone receptor", *Cellular Signalling,* Vol. 13(9), 599–616, 2001.
123. Y. Yarden, and M.X. Sliwkowski, "Untangling the ErbB signalling network", *Nature Reviews Molecular Cell Biology,* Vol. 2(2), 127–137, 2001.
124. A.R. Saltiel, and C.R. Kahn, "Insulin signalling and the regulation of glucose and lipid metabolism", *Nature,* Vol. 414(6865), 799–806, 2001.
125. H.S. Wiley, S.Y. Shvartsman, and D.A. Lauffenburger, "Computational modeling of the EGF-receptor system: a paradigm for systems biology", *Trends in Cell Biology,* Vol. 13(1), 43–50, 2003.
126. S.J. Vayttaden, S.M. Ajay, and U.S. Bhalla, "A spectrum of models of signaling pathways", *Chembiochem,* Vol. 5(10), 1365–1374, 2004.
127. F.A. Brightman, and D.A. Fell, "Differential feedback regulation of the MAPK cascade underlies the quantitative differences in EGF and NGF signalling in PC12 cells", *Febs Letters,* Vol. 482(3), 169–174, 2000.
128. S.D.M. Santos, P.J. Verveer, and P.I.H. Bastiaens, "Growth factor-induced MAPK network topology shapes Erk response determining PC-12 cell fate", *Nature Cell Biology,* Vol. 9(3), 324–U139, 2007.
129. B. Schoeberl, C. Eichler-Jonsson, E.D. Gilles, and G. Muller, "Computational modeling of the dynamics of the MAP kinase cascade activated by surface and internalized EGF receptors", *Nature Biotechnology,* Vol. 20(4), 370–375, 2002.
130. H. Resat, J.A. Ewald, D.A. Dixon, and H.S. Wiley, "An integrated model of epidermal growth factor receptor trafficking and signal transduction", *Biophysical Journal,* Vol. 85(2), 730–743, 2003.
131. K. Moelling, K. Schad, M. Bosse, S. Zimmermann, and M. Schweneker, "Regulation of Raf-Akt cross-talk", *Journal of Biological Chemistry,* Vol. 277(34), 31099–31106, 2002.
132. K. Mayawala, C.A. Gelmi, and J.S. Edwards, "MAPK cascade possesses decoupled controllability of signal amplification and duration", *Biophysical Journal,* Vol. 87(5), L1–L2, 2004.
133. E.V. Obberghen, "Signaling through the insulin receptor and the insulin-like growth factor-1 receptor", *Diabetologia,* Vol. 27, S125—S134, 1994.
134. M.J. Quon, and L.A. Campfield, "A mathematical-model and computer-simulation study of insulin-receptor regulation", *Journal of Theoretical Biology,* Vol. 150(1), 59–72, 1991.
135. M.J. Quon, "Advances in kinetic-analysis of insulin-stimulated Glut-4 translocation in Adipose-Cells", *American Journal of Physiology,* Vol. 266(1), E144–E150, 1994.
136. S. Wanant, and M.J. Quon, "Insulin receptor binding kinetics: Modeling and simulation studies", *Journal of Theoretical Biology,* Vol. 205(3), 355–364, 2000.
137. A.R. Sedaghat, A. Sherman, and M.J. Quon, "A mathematical model of metabolic insulin signaling pathways", *American Journal of Physiology–Endocrinology and Metabolism,* Vol. 283(5), E1084–E1101, 2002.
138. L. Giri, V.K. Mutalik, and K.V. Venkatesh, "A steady state analysis indicates that negative feedback regulation of PTP1B by Akt elicits bistability in insulin-stimulated GLUT4 translocation", *Theoretical Biology and Medical Modelling,* Vol. 1(2), 2004.
139. T.M. Gottlieb, J.F.M. Leal, R. Seger, Y. Taya, and M. Oren, "Cross-talk between Akt, p53 and Mdm2: possible implications for the regulation of apoptosis", *Oncogene,*

Vol. 21(8), 1299–1303, 2002.
140. L.D. Mayo, and D.B. Donner, "The PTEN, Mdm2, p53 tumor suppressor-oncoprotein network", *Trends in Biochemical Sciences,* Vol. 27(9), 462–467, 2002.
141. K.B. Wee, and B.D. Aguda, "Akt versus p53 in a network of oncogenes and tumor suppressor genes regulating cell survival and death", *Biophysical Journal,* Vol. 91(3), 857–865, 2006.
142. R.L. Bar-Or, R. Maya, L.A. Segel, U. Alon, A.J. Levine, and M. Oren, "Generation of oscillations by the p53-Mdm2 feedback loop: A theoretical and experimental study", *Proceedings of the National Academy of Sciences of the United States of America,* Vol. 97(21), 11250–11255, 2000.
143. G. Lahav, N. Rosenfeld, A. Sigal, N. Geva-Zatorsky, A.J. Levine, M.B. Elowitz, and U. Alon, "Dynamics of the p53-Mdm2 feedback loop in individual cells", *Nature Genetics,* Vol. 36(2), 147–150, 2004.
144. A. Goldbeter, G. Dupont, and M.J. Berridge, "Minimal model for signal-induced Ca-2+ oscillations and for their frequency encoding through protein-phosphorylation", *Proceedings of the National Academy of Sciences of the United States of America,* Vol. 87(4), 1461–1465, 1990.
145. E. Lee, A. Salic, R. Kruger, R. Heinrich, and M.W. Kirschner, "The roles of APC and axin derived from experimental and theoretical analysis of the Wnt pathway", *Plos Biology,* Vol. 2(3), 405–406, 2004.
146. J.A. Papin, and B.O. Palsson, "The JAK-STAT signaling network in the human B-cell: An extreme signaling pathway analysis", *Biophysical Journal,* Vol. 87(1), 37–46, 2004.

P. K. Vinod is a research student in the School of Biosciences and Bioengineering, Indian Institute of Technology, Bombay. His area of research includes experimental and theoretical quantification of biological systems.

K. V. Venkatesh is a Professor in the Department of Chemical Engineering and School of Biosciences and Bioengineering, Indian Institute of Technology, Bombay. His research involves interfacing engineering with biology and medicine. His research group has applied engineering design principles to biological systems to obtain insights into its evolution, operation and regulation. Venkatesh's group has developed novel theoretical methodologies to analyze biological networks at genetics, signaling and metabolic levels.