Signaling networks in *Leishmania* macrophages deciphered through integrated systems biology: a mathematical modeling approach

Milsee Mol · Milind S Patole · Shailza Singh

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Abstract Network of signaling proteins and functional interaction between the infected cell and the leishmanial parasite, though are not well understood, may be deciphered computationally by reconstructing the immune signaling network. As we all know signaling pathways are well-known abstractions that explain the mechanisms whereby cells respond to signals, collections of pathways form networks, and interactions between pathways in a network, known as cross-talk, enables further complex signaling behaviours. In silico perturbations can help identify sensitive crosstalk points in the network which can be pharmacologically tested. In this study, we have developed a model for immune signaling cascade in leishmaniasis and based upon the interaction analysis obtained through simulation, we have developed a model network, between four signaling pathways i.e., CD14, epidermal growth factor (EGF), tumor necrotic factor (TNF) and PI3 K mediated signaling. Principal component analysis of the signaling network showed that EGF and TNF pathways can be potent pharmacological targets to curb leishmaniasis. The approach is illustrated with a proposed workable model of epidermal growth factor receptor (EGFR) that modulates the immune response. EGFR signaling represents a critical junction between inflammation related signal and potent cell regulation machinery that modulates the expression of cytokines.

Keywords *Leishmania* · Mathematical modeling · Principal component analysis · Systems biology · Signaling dynamics

Abbreviations

AP-1	Activator protein 1
cAMP	Cyclic adenosine monophosphate
CD	Cluster determinant
COPASI	Complex pathway simulator
DC	Dendritic cell
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
Erk1/2	Extracellular signal-regulated kinases
GAP	GTPase-activating protein
GTP	Guanosine triphosphate
IFN γ	Interferon
IKK	I κ B kinase
IL	Interleukin
INOH	Integrating network objects hierarchies
IRAK1/4	IL1 receptor associated kinase
JAK2	Janus kinase
KEGG	Kyoto encyclopedia of genes and genomes
KTIM	Kinase tyrosyl-based inhibitory motif
LMR	<i>Leishmania major</i> response
LPG	Lipophosphoglycan
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinase
MyD88	Myeloid differentiating factor
NF- κ B	Nuclear factor κ B
NK	Natural killer cells
NO	Nitric oxide
ODE	Ordinary differential equation
PAK	P21-activated kinase
PCA	Principal component analysis
PfEMP1	Erythrocyte membrane protein 1

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M. Mol · M. S. Patole · S. Singh (✉)
National Centre for Cell Science, NCCS Complex, Ganeshkhind,
Pune University Campus, Pune, India
e-mail: shailza_iitd@yahoo.com; singhs@nccs.res.in

PI3 K	Phosphatidylinositol 3-kinase
PKA/C	Protein kinase A/C
PRRs	Pattern-recognition receptors
SBML	System biology markup language
SHP1	Protein-tyrosine phosphatase
STAT-1	Signal transducer and activator of transcription
TAB 1/2	TAK1 activating binding protein
TAK 1	TGF- β activated kinase 1
TLR	Toll-like receptors
TNF α	Tumor necrotic factor alpha
TRAF	Tumor necrotic factor (TNF) receptor activating factor

Introduction

Systems biologists view the signaling cascade as, a step-by-step biochemical reaction leading to the construction of the whole pathway. Interconnecting the pathways through crosstalk points, leads to a signaling network of interacting signaling substrates receiving inputs and generating outputs. A model on a signaling network is built on the basis of topological representation of its components and their links, which describes the biological systems' dynamic behavior equipping the model with predictive power (Muller and Ram 2010). In time, the purpose of computational modeling is to enhance the understanding of highly complex bio-signaling systems through the identification of important components and their interactions, leading to valuable predictions. These may be predictions regarding effects of particular targeted drugs or which components in the system needs to be perturbed to achieve a desired system response.

Keeping this purview in mind, a mathematical model can be used to generate emergent network insights, identify gaps in biological knowledge and make testable hypothesis. Different methodologies for dynamic signal transduction modeling exist, ranging from discrete approaches, such as state charts, Petri nets, logic-based descriptions such as Boolean networks, through differential equations to stochastic and rule-based methods (Golightly and Wilkinson 2006; Heiner et al. 2004; Hlavacek et al. 2006; Kestler et al. 2008; Sadot et al. 2008). Qualitative modeling can be used to map out many interactions and interconnectivities between network components and quantitative modeling, to study the parameters that govern the kinetics of signaling network.

It is well established fact that, signal transduction networks are the bridge between the extracellular environment and a cautiously orchestrated cellular response. The integration of these signaling events results in the activation or inactivation of transcription factors, which govern the

expression of several genes. Thus they play a very vital role in translating environmental cues to cellular behaviors; malfunctioning signaling networks can lead to a variety of pathologies (Hughey et al. 2009).

Pattern-recognition receptors (PRRs) initiate innate immunity through pathogen recognition. Signaling pathways downstream of PRRs and their cross talk control the immune response in an effective manner, eliminating the recognized pathogen (Lee and Kim 2007). In the arms race of host—microbe co-evolution, microbial pathogens have evolved ingenious ways to evade the host immune responses, by manipulating the signaling crosstalk between the receptors of the immune system thereby down regulating the immune responses ensuring their survival inside the host (Hajishengallis and Lambris 2011). Several clinically important human pathogens like *Mycobacterium tuberculosis*, *Helicobacter pylori* and HIV-1 induce crosstalk between toll like receptors (TLRs) and the C-type lectin DC-SIGN leading to high levels of interleukin (IL)-10 production by dendritic cell (DC)s (Gringhuis et al. 2009). Similarly, *Plasmodium falciparum* selectively inhibits IL-12 production and suppresses DC maturation and T cell activation through interactions with the scavenger receptor CD36 and TLR2 through the erythrocyte membrane protein 1 (PfEMP1), expressed on infected erythrocytes (Patel et al. 2007). *Leishmania major*, an intracellular parasite of macrophages, seems to benefit from complement activation and C5aR-induced inhibition of Th1 cell-mediated immunity (Hawlish et al. 2005). Many bacterial pathogens use effector proteins to target the kinase signaling cascades like the nuclear factor κ B (NF- κ B), mitogen-activated protein kinase MAPK, phosphatidylinositol 3-kinase (PI3 K)/Akt, and p21-activated kinase (PAK) pathways and manipulating the cross talk points (Krachler et al. 2011). cAMP signaling pathways have been described in *Trypanosoma cruzi* and *Trypanosoma brucei*, that regulates PKA with striking effects on cell survival and cell proliferation, through the inhibition of DNA, RNA and protein synthesis (McDonough and Rodriguez 2012).

Leishmania have evolved numerous strategies to subvert host immune responses so they can coexist with their host species. Macrophages act as the principal host cells for *Leishmania*, playing a central role in the development of the immune response to these parasites via antigen presentation, secretion of microbicidal products, and production of inflammatory mediators. The early events during host cell-parasite interaction determine the outcome of infection.

Toll-like receptors provide a link between innate and adaptive immunity, allowing recognition of a wide range of microbial products, including those of parasitic pathogens. Down the TLR signaling cascade, are many adaptor proteins like MyD88, TRAF6, IRAK1/4, TAK 1 and TAB 1/2, which can be regulated by intracellular negative regulators,

for protection against uncontrolled inflammatory response. *Leishmania* is thought to interact with TLR 2, 3, 4 and 9 on the macrophage surface and subvert the cellular response for intracellular survival (Gallego et al. 2011; Ben-Othman et al. 2009; Tuon et al. 2008).

Protein-tyrosine phosphatase (SHP1) is said to interact with IRAK1 by binding to an evolutionarily conserved kinase motif KTIM and completely inactivating its intrinsic kinase activity (Abu-Dayyeh et al. 2008). SHP-1 has been also shown to directly inactivate JAK2 and Erk1/2, and to play a role in the negative regulation of several transcription factors involved in macrophage activation such as: NF- κ B, STAT-1, and AP-1. Erk1/2 is also inhibited by parasite derived ceramide and cysteine proteinases (Shio et al. 2012).

Leishmania donovani exploits the host deubiquitinating enzyme A20, a negative regulator of TLR signaling, to subvert the host immune response. A20 is induced by the parasite, which deubiquitinates TRAF6 and blocks the IKK-NF κ B mediated downstream signaling, leading to the inhibition of the host defensive cytokine response (Supriya et al. 2012). *L. major*, LPG, a phosphoglycan belonging to a family of unique *Leishmania* glycol-conjugates, up regulates both mRNA and the membrane expression of TLR-2 in NK cells. Additionally, IFN γ and TNF α production and nuclear translocation of NF κ B are enhanced (Becker et al. 2003). LPG also blocks the production of NO and oxidative burst by binding to the regulatory domain of PKC (Shadab and Ali 2011). Recently, it was shown that in macrophages infected with *Leishmania*, Notch 1 and 2 receptors are over expressed leading to the over expression of Hes 1 and Hes 5 gene, which results in the production of anti-inflammatory cytokine IL10 (Zhang et al. 2012). It has been demonstrated that *L. major* counteracts the IFN- γ response in macrophages on a large scale, by modulating expression of genes involved in the innate immune response, cell adhesion, proteasomal degradation, TLR expression, a variety of signaling molecules, and matrix metallo-proteinases, for its own survival and replication purposes (Dogra et al. 2007).

PI3 K is activated after triggering of many TLR members like TLR2, TLR4 etc., suggesting the presence of 'shared' signaling pathways for TLR-mediated activation of PI3 K. MyD88, IRAK and TRAF6 are involved in such 'shared' signaling pathway or pathways. Recent studies have also shown that PI3 K is an endogenous suppressor of IL-12 production triggered by TLR signaling and limits excessive Th1 polarization. It is possible that crosstalk occurs between these three negative regulatory signaling pathways in TLR signaling (Troutman et al. 2012; Ni et al. 2012). Despite these strong immune responses, *Leishmania* persist in a latent form, which suggests a dynamic relationship between the host immune system and *Leishmania* that results in a balance between host survival and its control.

Genetic mapping of susceptibility to *L. major* has revealed the presence of gene loci (Imr 3–5, 10, 13–15) involved in symptoms associated with leishmaniasis, like skin lesions, hepatomegaly, splenomegaly etc. Such studies have given an insight into complex interactions between parasite and host's genes controlling the outcome of infection, and their influence on different components of the immune response (Havelkova et al. 2006).

Integration of simulation and experimental information will dictate the design of explanatory and predictive models for biological systems. Since building a complex biological system entails bringing together many aspects of biological processes and incorporating approximations to fill in any gaps in knowledge, hypotheses formed need to be iteratively verified through experimentation and continually-refined model simulations (Pham et al. 2008). In this study, we have developed a model for immune signaling cascade in leishmaniasis and based upon the interaction analysis obtained through simulation, we have developed a model network, between four signaling pathways i.e., CD14, EGF, TNF and PI3 K mediated signaling.

Networking results in several emergent properties that the individual pathways do not have, like (1) Extended signal duration (2) Definition of threshold stimulation for biological affects (3) Multiple signal outputs and (4) Activation of feedback loops (Bhalla and Iyengar 1999). Since, signaling pathways can be pharmacologically manipulated, a deeper network level understanding of their inter-relations, control and the mechanisms whereby they regulate hosts' immune response to pathogenic infection, should permit the development of rational therapies to curb neglected infectious agents, such as *Leishmania*. Moreover, simulation of gene knock-out models based on sensitivity analysis can allow development of experimentally testable hypothesis.

Methodology

The schematics of the work flow for the study is represented in Fig. 1.

Reconstruction of signaling pathways and model definition

Reconstruction of the signaling pathway has been done to find the missing links and to get deeper insight into the interactions between the signaling proteins building larger networks, it is desirable to explain the complexity of biological system at higher level. Quantitative modeling of these interactions will play an important role in understanding fundamental intra- and inter-cellular processes. In particular, quantitative modeling of the dynamics of

biological pathways has drawn much attention recently, helps in understanding the progression of disease.

Decomposition approach was used by us because it is having a key advantage, that it exploits the structure of a large pathway model to break it into smaller components, whose parameter estimation problem can then be solved independently. This leads to significant improvements in computational efficiency due to the reduction in the dimensionality of the search space (Koh et al. 2006).

Mitogen-activated protein kinase pathway was decomposed into 4 major pathways viz CD14, PI3K, TNF and EGF. The interactome for the signaling cascade mechanism considered for the study was collected by literature survey and from signal cascade databases like the KEGG, INOH Pathway Database (release 4.0), Pathway Interaction Database and Science Signaling Database. These databases have experimentally validated physical interactions among human proteins. A total of 65 nodes (interacting proteins) and 76 edges (physical interactions) were identified in the reconstructed signaling network. The edges were assigned with a total of 132 kinetic parameters governed by the Law of Mass Action (for association and dissociation reactions) and Michaelis–Menten kinetics (phosphorylation/dephosphorylation/ubiquitination) and initial concentrations of all the participating nodes. The reconstruction of the chosen signaling network was done in MATLABs' SimBiology tool box (7.11.1.866) (The MathWorks Inc.) package which uses the systems biology markup language (SBML).

Network simulation using ordinary differential equation (ODE)

Dynamics of the network was studied by simulating the system in SimBiology toolbox. MATLAB provides a wide range of solvers for simulating stochastic and deterministic systems.

One can specify the solver type, simulation time, absolute and relative tolerances, controls for dimensional analysis and unit conversion in simulation settings, depending on the type of model to be simulated. Simulation plots can be generated for the visualization of simulation results.

With the knowledge of initial concentration and parameters, numerical simulations were performed using Stiff Deterministic ODE15 s type solver. Complex biological systems, such as the signaling network can be viewed as networks of chemical reactions that can be analyzed mathematically using ODEs, which is the most common simulation approach used in computational systems biology. ODE helps in determining time-dependent changes of the concentrations of the signaling proteins and protein complexes. This solver type deals with the models with both fast and slow changing variables.

Ordinary differential equation for MAPK pathway at defined initial concentration and kinetic parameters is generated in the following form:

$$\frac{d([CD14] \cdot V_{cytoplasm})}{dt} = -V_{cytoplasm} \cdot (0.67 \cdot [CD14]) \quad (1)$$

$$\frac{d([TLR4] \cdot V_{cytoplasm})}{dt} = +V_{cytoplasm} \cdot (0.67 \cdot [CD14]) - V_{cytoplasm} \cdot (.33667[TLR4]) \quad (2)$$

$$\frac{d([TNF] \cdot V_{cytoplasm})}{dt} = -V_{cytoplasm} \cdot (0.46 \cdot [TNF]) \quad (3)$$

$$\frac{d([EGF] \cdot V_{cytoplasm})}{dt} = -V_{cytoplasm} \cdot (0.62667 \cdot [EGF]) \quad (4)$$

$$\begin{aligned} \frac{d([NF - \text{kappaB}\{cytoplasm\}] \cdot V_{cytoplasm})}{dt} &= +V_{cytoplasm} \cdot (.6 \cdot [IkB/NF - \text{kappaB}]) \\ &- V_{cytoplasm} \cdot (.09 \cdot [NF - \text{kappaB}\{cytoplasm\}] \cdot V_{cytoplasm}). \end{aligned} \quad (5)$$

Fig. 1 Schematic representation of workflow

Simulation of the ODE model helps in studying the dynamics of the signaling protein, that how they are acting on changing parameters and how the change in parameter of one species affects the other species. Interaction or crosstalk between the signaling proteins can be inferred with the help of simulations.

Parameter estimation

In the process of model calibration, following a computational approach ultimately assigns representative values to the parameters that characterize the model equations (rate constants, kinetic orders, etc.) in such a manner that the model reproduces the desired behavior of the system, as is characterized by available experimental data (Fig. 2). The underlying strategy is to determine those values for the model parameters that make the differences between the actual measurements and the values produced by the corresponding model simulation as small as possible. Manual training of the parameter set, including the fine-tuning of values until the model is able to simulate the desired graphs with sufficient accuracy and algorithmic data fitting is performed due to lack of experimental evidences.

In the current study, we designed two synthetic experiments to illustrate these concepts. The first computational experiment resulted in an artificial time series, where the concentrations of the metabolites were measured at different time points after a transient stimulation of the system with an input signal. In fact, these measurements are simulation results, which are artificially corrupted with noise. For the model calibration, we assume not to know the parameter values used in the simulation. Optimization methods were used to tune the value of the model parameters until model simulations achieve a satisfactory reproduction of the data. The quality and quantity of available data are inadequate to allow sufficient estimation of the

Fig. 2 Flowchart for parameter estimation

underlying parameters values so the parameters were estimated using computational approach. There was increase in the number of parameters with the increase in model size and details of models, as a result the problem of dimensionality hinders with the estimation algorithm. To deal with such problem, constraints were imposed on the model which reduces the dimensionality of the parameter space to a smaller feasible region and make parameter estimation computationally easier.

Validation

Many sources of uncertainty including noise of experimental data, absence of parameter information and poor understanding of the biological mechanism impose a limitation in our confidence in the model, so validation of model is required. Validation of the model has been done by comparing the model performance with the available experimental data. Further validation was done by performing various analyses like statistical analysis, sensitivity analysis and PCA.

Network statistics

Network statistics like clustering coefficient, diameter, radius, average paths, mean path length, number of nodes and edges, isolated nodes and self-loops were calculated.

Shortest path and diameter

Adjacency matrix was constructed to determine the shortest path and diameter of the signaling pathway (Fig. 3). Adjacency matrix is a binary, square matrix representing the edges (connection between two signaling proteins)

Fig. 3 Steps for calculating shortest path length and pathway diameter

between nodes (signaling proteins). Matrix was constructed using MATLAB.

Time series data and flux analysis

Time series data and flux were calculated by using COP-ASI software in which SBML format file was taken as the input. With the help of time series data flux of the reactions were calculated.

Sensitivity analysis

Sensitivity analysis calculates the time-dependent sensitivities of all the species states with respect to species initial conditions and parameter values in the model. Thus, if a model has a species x and two parameters y , and z , the time-dependent sensitivities of x with respect to each parameter value are the time-dependent derivatives,

$$\frac{\partial x}{\partial y}, \frac{\partial x}{\partial z}$$

where, the numerator is the sensitivity output and the denominators are the sensitivity inputs to sensitivity analysis. In SimBiology tool sensitivity analysis is supported only by the ODE solvers. Function “SensitivityAnalysis” helps in calculating the time-dependent sensitivities of all the species states. Sensitivity of each signaling protein in the signaling network was calculated with respect to other signaling proteins.

Principal component analysis (PCA)

PCA generates a new set of variables (principal component coefficient, score), called principal components. Each principal component is a linear combination of the original variables. All the principal components are orthogonal to each other, so there is no redundant information. The principal components as a whole form an orthogonal basis for the space of the data. PCA was done using MATLAB and the function for PCA is “princomp”.

Syntax used for calculating principal component is

$$[\text{COEFF}, \text{SCORE}] = \text{princomp}(\text{X})$$

where, ‘X’ = n -by- p matrix of sensitivity coefficients of the reactions in signaling network. This command returns principal component coefficient and score of the sensitivity coefficient matrix.

Model reduction

Dimensionality reduction was done by combining the PCA with flux analysis and PCA with sensitivity and their respective graphs were plotted using Sigma Plot software a

3-D mesh plot was plotted between concentration, sensitivity and flux for the dimensionality reduction.

Results and discussion

Crosstalk points

Comparative analysis of simulation graphs between individual pathways and entire network shows the effect of species dynamism when acting in a concert.

Lipopolysaccharide is a potent activator of the immune system, which combines with CD14, a GPI- anchored protein lacking a cytoplasmic domain. LPS and CD14 complex combine with TLR4, a transmembrane protein molecule consisting of extracellular leucine rich repeats and an intracellular signaling domain. Together, these molecules have the function to transmit the LPS signal across the plasma membrane. CD14 and TLR-4 complex transduces a signal detected by MyD88 and is passed down stream by a cascade of IRAK’s, TRAF-6 and NIK resulting in the activation of NF- κ B. Henceforth it can be said that CD14 and TLR-4 gene expression is markedly increased in a time –dependent manner. These finding suggest that LPS could upregulate the expression of CD14 and TLR gene in leishmaniasis which can further increase the biological effect of the LPS. Thus gene polymorphism of the CD14 receptor involved in the LPS recognition could be associated with the chronic form of the disease in *Leishmania*. The precise connection between RIP-TRAF2 or IRAK-TRAF6 complexes and NF- κ B are less well established but are thought to involve the MAPK kinase family members MEKK-1 or NF- κ B-inducing kinase (NIK). Both of these protein kinases can directly phosphorylate and activate I κ B kinase (IKK) which is a multi-protein enzyme complex consisting of IKK-1, IKK-2 and IKK- γ subunits.

TLR-4 triggers key intracellular pathways controlled by the transcription factor NF κ B leading to production of cytokines like TNF α , IL-12, IL-1 β , and TGF β which mediates and amplify the inflammatory responses. Our study showed that LPS stimulation could markedly up regulate the expression of CD14, TLR-4 genes compared with the increase in TNF α and IL-12. gp63 as a strong inhibitor for NF κ B is capable of lowering the expression of cytokines or the expression of CD14 and TLR4 genes which is quite obvious in the predicted graphs.

Previous studies implied that TNF α and EGF might share a common signal transduction pathway. TNF α and EGF could enhance c-fos gene expression when PKC is down-regulated. NF κ B binding activity is dramatically activated by TNF α but not by EGF. The binding of transcription factors AP-1, c-Jun, c-fos was

enhanced by EGF and TNF α . TNF α induced activation of c-fos gene partially through a common signal with EGF by EGFR.

Shortest path and diameter

The shortest path lengths were calculated for each species in individual pathways. The maximum of the shortest path lengths give the diameter of pathway. CD14, TNF and EGF showed the maximum average paths among all four pathways and hence can be said that these have more interacting species in the network and therefore are robust.

Network statistics

A total of 65 nodes and 76 edges are present in the MAPK network. No self-loops or any isolated nodes were observed. Low clustering coefficient value shows the presence of a linear network i.e. clustering cannot be done in this network.

Flux analysis

Flux Balance Analysis is essential to regulate the fluxes involved in the metabolic pathway under different conditions. Using the time series data, fluxes for each of the reaction involved in the signaling network were calculated until a steady state condition was achieved where the metabolic flux becomes zero. Among all the reactions in the network CD14, TNF and EGF had the highest values for flux and contribute to the network dynamics.

Sensitivity analysis

Sensitivity analysis is the study of the effect of variation in the critical outcomes of a given biochemical system can be categorized and assigned, qualitatively or quantitatively, to different sources of variation in the system. In the context of a mathematical model, the uncertainty associated with the input parameters provided for the model can be studied using the sensitivity analysis which identifies the sensitive parameters in the system whose variation significantly affect the critical response of the system. These parameters could then serve as potential candidates for drug-mediated modulation. Parameters like individual reaction rate constants (K_m , V_{max} , K_f and K_b), system variables like individual species concentrations (C_i) were included in the sensitivity analysis. Sensitivity analysis was used to identify the critical receptors in the family related to the activation of NF- κ B, an important protein downstream of the signaling system. We found that gp63, LPG, LPS, SHP-1, a member of the EGFR receptor family, have a dominant role in this activation process and is a promising target for drug inhibition. This

can later be tested as a new potential drug that targets the receptor by inhibiting its phosphorylation.

Flux balance analysis is appropriate for understanding steady state processes but they are not well suited for transient processes. Biochemical systems theory can be applied to steady-state problem but estimation of kinetic orders and rate constants under unsteady state conditions becomes challenging. Correlation analysis overcomes this limitation, but it is more difficult to interpret. To complement these approaches, it is suggested in the study that sensitivity analysis along with PCA may provide a simple, systematic and powerful methodology for analysis of biological networks. Such analysis can be applied to study the characteristics of non-steady-state phenomena such as those which are typical during signal transduction.

Fig. 4 Principal Component Analysis. Filled circle CD14, open circle TRAF-6, filled inverted triangle PIP2, open triangle Raf, filled square MEKK1, open square MKK3/6, filled diamond MKK4/7, open diamond NF- κ B, filled triangle TNF, open inverted triangle EGF

Fig. 5 Sensitivity and flux analysis. Filled circle CD14, open circle NF- κ B, filled inverted triangle EGF, open triangle TNF

Fig. 6 3D plot for **a** TNF **b** n.NF-kappaB **c** TRAF-6 **d** MKK3/6 **e** MKK4/7 **f** PIP2 **g** Raf

Fig. 8 Proposed experimental model of infection and modulation of immune response

Flux analysis alone does not capture the critical rate controlling features of the reaction network. Based on model reduction it was observed that the least effect on the signal output is by the reactions corresponding to the internalization of the receptor and its degradation. EGF receptor along with phosphorylated Grb2 and SOS are the intermediates that regulate the system output via equations 30–38. The concentrations of these intermediates were found to control the rate of Ras-GTP formation via equations 40 and 41. Internalization of these intermediates also forms an important part of the reduced model. Hence for the overall model reduction flux analysis has to be combined with PCA to yield a better model.

Figure 6 captures the essential features of the EGF pathway for the range of concentrations and time scales depicted in the other multi-panel plots.

Among the cytoplasmic reactions, the sensitivity coefficient of the EGF binding reaction (equation 30) and Raf de-phosphorylation reaction (equation 42) are augmented at lower doses, suggesting that these are important steps regulating EGF signaling as represented in Fig. 6. It was observed that some reactions, in particular the reactions regulating the binding of GAP with the autophosphorylated and dimerized EGFR (equation 31) and the reaction controlling Ras-GTP formation (equation 41) exhibited a higher fluxes at an intermediate concentration as evident from Fig. 7.

Further based on the datasets generated we formulate a workable model of EGFR that modulates the immune response. EGFR signaling represents a critical junction

between inflammation related signal and potent cell regulation machinery that modulates the expression of cytokines (Fig. 8).

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