Modeling biomolecular site dynamics in immunoreceptor signaling systems

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Abstract

The immune system plays a central role in human health. The activities of immune cells, whether defending an organism from disease or triggering a pathological condition such as autoimmunity, are driven by systems of molecular machinery. To understand this machinery, we are interested in a systems biology approach that parallels approaches used to engineer manmade machines, one that involves computational modeling based on empirical mechanistic knowledge and integrated experimental efforts to test model predictions and to quantify key parameters of system behavior. Decades of experimentation have elucidated many of the biomolecules and interactions involved in immune signaling. Recently developed technologies are providing new quantitative information about these processes and inviting us to explore a new horizon of systems-level understanding, such as the dynamical aspects of biomolecular interaction networks responsible for information processing and decision-making in immune cells. It is important for modeling methods to keep pace with experimental advances. In this chapter, we focus on the dynamic, site-specific, and context-dependent nature of interactions in immunoreceptor signaling (i.e., the biomolecular site dynamics of immunoreceptor signaling), the challenges associated with capturing these details in computational models, and how these challenges have been met through use of a rule-based modeling approach. Focusing on models for signaling processes that involve the B-cell antigen receptor (BCR) and the linker for activation of T cells (LAT), we discuss how the rule-based modeling approach can capture multi-site phosphorylation and multivalent binding interactions responsible for formation of signaling complexes. We propose that novel questions about biomolecular site dynamics can now be asked because of newly developed rule-based modeling capabilities and complementary experimental capabilities.

Introduction

Immune cells must process information about their changing environment to respond to signs of damage and infection. These cells possess surface receptors that bind extracellular ligands and initiate intracellular signaling, with information propagating through complex networks of molecular interactions. Dysregulation of these networks, and resulting dysregulation of immune responses, can lead to pathological conditions such as allergies, asthma, and autoimmunity. Thus, an understanding of signaling in immune cells is needed for improved understanding and treatment of disease. The complexity of cell signaling challenges intuition, but computational modeling offers the possibility of expanding our reasoning capabilities to obtain a predictive understanding of how immune cells respond to stimuli (Germain et al., 2011; Chakraborty and Das, 2010).

Intracellular signals are propagated through enzyme-catalyzed reactions and noncovalent interactions, which are mediated by specific sites within biomolecules, which tend to each contain multiple functional components or sites. Examples of biomolecular sites involved in cell signaling include tyrosine residues that undergo phosphorylation, SH3 and proline-rich motifs that interact with one another, and PH domains that bind phospholipids. The outcomes of biomolecular interactions are changes in population levels of chemical species (e.g., multimolecular complexes and protein phosphoforms). The biomolecular site dynamics of cell signaling are governed by the same laws of physics and chemistry that govern chemical reaction kinetics (Kholodenko, 2006). Chemical reaction kinetics have long been modeled through the formalism of ordinary differential equations (ODEs). Use of ODE models is widespread in systems biology (Hucka et al., 2003; Le Novere et al., 2005; 2006) and has yielded useful insights (Kreeger and Lauffenburger, 2010). However, formulation of an ODE model depends on availability of a reaction network, for which

one must enumerate all species that can be populated, and make definite statements about how these species are connected and influence each other. In the case of cell signaling systems, this requirement can become a significant obstacle to model specification. This difficulty arises from the multisite structures of biomolecules.

As an example, let us consider the T-cell receptor (TCR)/CD3 complex. This receptor contains 10 immunoreceptor tyrosine-based activation motifs (ITAMs) (Cambier, 1995), each of which contains two tyrosine residues. Each of the 20 ITAM tyrosine residues has two possible states: phosphorylated or unphosphorylated. As a result, the TCR/CD3 complex has accessible to it $2^{20} \approx 1$ million possible phosphorylation states. Without quantitative characterization of phosphorylation kinetics, the exact phosphoform of a given receptor at a given time cannot be narrowed through logical reasoning alone to less than 1 million possible phosphoforms. A reaction network capturing the full spectrum of TCR phosphoforms would contain 1 million (chemical species) nodes, corresponding to 1 million ODEs, which would be impractical to specify. Thus, despite a wealth of information available about this receptor and proteins associated with it, specifying a reaction network that fully captures the possible consequences of known protein interactions and modifications is a challenge. Even if the populated chemical species and active chemical reactions could be identified within an experimental system to narrow the scope of modeling , any changes of the system, such as protein copy number variations, could alter the populated chemical species and active chemical reactions. Thus, traditional modeling approaches are problematic when one is interested in the site-specific dynamics of a biomolecular interaction network, i.e., biomolecular site dynamics.

The problem is not that biomolecular site dynamics cannot be modeled but rather that available mechanistic knowledge is difficult to translate into a traditional model form. Arising

more naturally from available mechanistic knowledge of cell signaling is a view that leverages the modular, multisite nature of proteins and other biomolecules. This view is the rule-based perspective, in which interactions between sites of binding partners are taken to be modular, meaning somewhat independent of molecular context. An interaction is modular if a rule can be specified to represent the interaction (i.e., to define when the interaction occurs and with what rate) and the rule does not completely define the reactants. For example, if ligand-receptor binding is independent of receptor phosphorylation, then the interaction is modular. A rule can be specified for ligand-receptor binding that is agnostic with respect to receptor phosphorylation status. Rule-based modeling allows the translation of mechanistic knowledge into computational models consistent with chemical reaction kinetics. Because an interaction can be represented without complete knowledge of the participating reactants, there is no need to specify a reaction network, which eliminates a major barrier to modeling of biomolecular site dynamics.

A rule concisely describes the necessary and sufficient conditions required of reactants for a reaction to occur. A rule-based model captures the same chemical kinetics as an ODE-based model (up to assumptions of modularity, which may be relaxed as needed to accommodate empirical observations), while permitting simulation of chemical kinetics without pre-generation of a reaction network. This method has been reviewed in detail elsewhere (Hlavacek et al., 2006; Hlavacek, 2011; Chylek et al., 2012) and comprehensive guides to rule-based modeling software tools are available (Faeder et al., 2009; Smith et al., 2012). Here, rather than reviewing the method, we review how this approach has been used to study biomolecular site dynamics of immunoreceptor signaling systems

These systems are characterized by at least two mechanisms that, as we will discuss, the rule-based approach is well-suited to capture: aggregation (or multivalent binding) and multi-site

phosphorylation. Aggregation of receptors is induced by interactions with multivalent ligands, and serves to initiate signaling (Metzger, 1992; Dintzis et al., 1976; 1983). Following aggregation of the IgE receptor (FcεRI), for example, the first biochemically detectable event in intracellular signaling is multi-site phosphorylation of receptor ITAMs. Each of these motifs contains two canonical tyrosine residues that, when phosphorylated, serve as docking sites for SH2 domains of Src- and Syk-family kinases, which trigger subsequent signaling events. Aggregation reemerges in the cytoplasm, as signaling complexes assemble through multivalent interactions of scaffold/adaptor/linker proteins (Houtman et al., 2006). Aspects of these complex processes have been formalized, simulated, and analyzed in the example models that we will discuss in this review.

Comparison of modeling assumptions

An ODE model is specified by making statements about how concentrations of chemical species change with time. Thus, a modeler is steered towards making assumptions about which species can be populated. These assumptions must sometimes be *ad hoc*, and may be at odds with available data. For example, the number of phosphorylation states of a TCR/CD3 complex could be reduced through a "virtual phosphorylation site" assumption, as it has been called in a study of ErbB receptor signaling by Birtwistle et al. (2007). Under this assumption, multiple ITAM tyrosine residues would be treated as a single site. This assumption would be serviceable for some purposes, such as a model that aims to elucidate how overall features of TCR signaling are affected by ligand-receptor binding kinetics (Lipniacki et al., 2008). However, if a modeler aimed to investigate the dependence of signaling events on the number or identity of specific ITAMs, a question that has been investigated experimentally (Holst et al., 2008), a virtual phosphorylation

site assumption would be limiting. A virtual phosphorylation site could also be problematic in cases where different phosphotyrosines interact with different binding partners, as is the case for the linker for activation of T cells (LAT), because if multiple sites are treated as one, a false competition between binding partners may arise.

In contrast, a rule-based model is specified by making statements about site-specific requirements that must be met by reactants for a reaction to occur. A modeler is then steered towards making assumptions about the modularity or cooperativity of interactions. Which set of assumptions is preferable depends on what type of information is available. Given that signaling proteins are generally composed of modular domains (Pawson and Nash, 2003), specification of a model in the form of rules is often more efficient than specification in the form of ODEs. However, the two approaches are complementary, mirroring alternate modeling formalisms that are found in other fields (e.g., use of Lagrangian and Eulerian coordinates in fluid dynamics).

Summary of recent modeling work

The rule-based modeling approach has been used to investigate a number of biological systems. In Table 1 we summarize recent applications aimed at understanding immune signaling, and in Table 2, we summarize applications aimed at understanding other types of cell signaling systems, as well as general mechanisms of cell signaling. We consider only applications from 2007 to present; earlier applications have been reviewed elsewhere (Hlavacek et al., 2006). It is worth noting that the rule-based approach, although developed for and most commonly used for modeling of cell signaling systems, has been used to model other processes, such as metabolism (Mu et al., 2007; Faulon, 2010), viral capsid assembly (Jamalyaria et al., 2005; Zhang et al., 2005), and labor market dynamics (Khün and Hillmann, 2010). A number of software tools for rule-based

modeling are available (Faeder et al., 2009; Moraru et al., 2008; Mallavarapu et al., 2009; Lok et al., 2005; Lis et al., 2009; Colvin et al., 2009; Colvin et al., 2010; Sneddon et al., 2011; Xu et al., 2011; Clarke et al., 2008; Ollivier et al., 2010, Gruenert et al., 2010; Smith et al., 2012, Meier-Schellersheim et al., 2006; Angermann et al., 2012; Feret and Krivine, 2012; Andrews et al., 2010). In addition, a number of rule-based models have been developed as demonstrations in methodological work. For example, to demonstrate use of a model visualization tool, a rule-based model of the MAP kinase signaling network in yeast was developed, with all parameter values set to 1 (Tiger et al., 2012).

[Table 1 here]

[Table 2 here]

Modeling of LAT aggregation

In Tables 1 and 2, we list several examples of applications of rule-based modeling. We will now discuss one topic in detail: interactions of the linker protein LAT. This protein is subject to multi-site phosphorylation and forms aggregates with other signaling proteins through multivalent interactions, exemplifying two processes commonly found in immunoreceptor signaling. We will discuss a series of recently developed models for investigation of LAT aggregation. These models were obtained through traditional modeling methods and rule-based modeling, which provides an opportunity to highlight differences in model specification and the type of information that can be gained from the different approaches.

LAT is a transmembrane protein that undergoes multi-site phosphorylation following stimulation of immunoreceptor signaling. Its four distal tyrosine residues have well-characterized roles as binding sites for SH2 domains of other signaling proteins. One of these proteins is Grb2, whose SH2 domain can bind phosphorylated tyrosines 171, 191 and 226 in LAT. Grb2 also

contains a pair of SH3 domains, which interact with proline-rich sequences in SOS1. The presence of at least four proline-rich sequences in SOS1 allow it to cross-link two Grb2 molecules. In this way, aggregates of LAT-Grb2-SOS1 can form. LAT aggregation has been observed following stimulation of T cells (Bunnell et al., 2002) and mast cells (Wilson et al., 2001), and the Grb2 binding sites in LAT are required for this process (Houtman et al., 2006). Expression of a SOS1 proline-rich region that can bind only one Grb2 molecule inhibits LAT aggregation and attenuates downstream signaling events, including calcium mobilization (Houtman et al., 2006). These results indicate that LAT's capacity to aggregate is relevant for its physiological function.

The valency of LAT for Grb2 can vary from zero to three, and depends on how many LAT tyrosine residues are phosphorylated. Thus, aggregation of LAT is influenced by its phosphorylation state. This dependence has been explored quantitatively by Goldstein and co-workers, using a combination of equilibrium theory borrowed from polymer chemistry, and rule-based modeling.

[Fig 1 here]

Nag et al. (2009) formulated an equilibrium continuum model to compare aggregation of bivalent LAT vs. trivalent LAT. The molecules and interactions considered in the model are illustrated in Fig. 1. The equilibrium model predicted that an increase in valency from two to three leads to a dramatic increase in average LAT aggregate size. For a homogenous population of trivalent LAT, a sol-gel coexistence region is predicted if concentrations of LAT, Grb2, and SOS1 are within certain ranges. The following equation was derived for the fraction of LAT molecules in the gel, which is a super aggregate, containing a significant fraction of all LAT present in equilibrium with unclustered LAT and small LAT aggregates:

$$f_g = 1 - \frac{2(1+\beta)^2}{\alpha\sigma\chi\mu\theta g^2 s} \qquad (1)$$

where s is the fractional concentration of free SOS1 and σ is a negative cooperativity factor. The parameter β is given by the following equation:

$$\beta = K_{GL}G + 2K_{GL}K_{GS}GS + 2\sigma K_{GL}K_{GS}^2 G^2 S \qquad (2)$$

where K_{GL} is the solution equilibrium constant for Grb2 binding to LAT, K_{GS} is the solution equilibrium constant for Grb2 binding to free SOS1, G is the cytosolic concentration of Grb2 free of SOS1, and S is the cytosolic concentration of SOS1 free of Grb2. The nondimensional parameters α, χ, μ, and θ are defined as follows:

$$\alpha = 3\overline{K}_{GL}L_t; \quad \chi = K_{GL}G_T \qquad (3)$$

$$\mu = 2K_{GS}S_T; \quad \theta = K_{GS}G_T \qquad (4)$$

where $\overline{K}_{GL}$ is the surface equilibrium cross-linking constant for membrane-associated Grb2 binding to LAT at the end of a chain, and G_T, L_T, and S_T are the total concentrations of Grb2, LAT, and SOS1, respectively.

The equilibrium relations given above were found by enumerating all possible complexes of LAT, Grb2, and SOS1, with the exception of cyclic aggregates. Enumeration of these complexes requires insights from the field of branching processes, because binding of trivalent LAT to three Grb2 molecules can form linear chains and tree-like networks. A statistical weight (relative concentration) is assigned to each complex. These weights enter into a partition function, which is a convergent infinite sum that can be reduced to an algebraic expression. The partition function can be used to obtain conservations laws for each molecule in the system, from which one can calculate the free concentrations of these molecules as well as concentrations of other species. The terms of the partition function are obtained by assuming detailed balance, which holds under

equilibrium conditions. Thus, the model is silent about reaction kinetics. An ODE model for the chemical kinetics of this system cannot be feasibly specified because of the large number of distinct chemical species that can be populated (Yang et al., 2008). The equilibrium model is analogous to an earlier model for binding of a trivalent ligand to a bivalent cell-surface receptor (Goldstein and Perelson, 1984), and can be thought of as a model for binding of a soluble cytosolic bivalent ligand (Grb2-SOS1-Grb2) to a trivalent membrane receptor (LAT).

The equilibrium model of Nag et al. (2009) is exact in the continuum limit, i.e., for a system of infinite size. However, a cell is of finite size. To evaluate the effect of finite system size, a rule-based model was developed and simulated (to steady state), and its predictions were compared to those of the equilibrium model. The rules of the model are presented in Fig. 2, and the model was simulated using a network-free simulation method (Yang et al., 2008). At the time at which the model was developed, problem-specific code was required to perform network-free simulation. General-purpose network-free simulators have since become available (Colvin et al., 2009; Colvin et al., 2010; Sneddon et al., 2011). Steady-state results obtained from the rule-based model were found to be consistent with the equilibrium model. Information equivalent to Eq. 1 can be obtained by simulating the rule-based model to steady state and calculating the fraction of LAT molecules in the largest aggregate. The rule-based model is analogous to other rule-based models for ligand-induced receptor aggregation that have recently been studied (Yang et al., 2008; Monine et al., 2010).

Although Nag et al. (2009) focused on equilibrium behavior, their rule-based model enables study of the kinetics of aggregation, which are not captured in their equilibrium model. Kinetics of aggregation of multivalent binding partners have been modeled using traditional modeling methods (e.g., ODEs) by restricting the scope of the model to consideration of, for

example, only ligand states (Perelson and DeLisi, 1980); consideration of the full range of possible complexes requires a rule-based approach. In addition, a rule-based approach allows cyclic aggregates to be considered, as demonstrated by Monine et al. (2010). The approach of Perelson and DeLisi (1988) becomes unwieldy when cyclic aggregates, or rings, can form (Posner et al., 1995).

[Fig 2 here]

A second set of models related to LAT aggregation has recently been used by Nag et al. (2012) to evaluate the robustness of their earlier predictions. Rather than assuming a homogenous population of trivalent or bivalent LAT, a mixed population of trivalent, bivalent, and monovalent LAT was assumed. Monovalent LAT blocks aggregate growth, and bivalent LAT prevents branching. It was found that the presence of monovalent and bivalent LAT reduced the size of the sol-gel coexistence region in parameter space. Consideration of varying valency is important, because a distribution of LAT phosphoforms is likely to be found in cells. This distribution could shift as signaling progresses because of different kinetics of phosphorylation at different sites (Houtman et al., 2005), which we discuss in more detail below.

Modeling early events in BCR signaling

The rule-based approach has also been applied to study of early events in BCR signaling (Barua et al., 2012). A rule-based model was used to study the roles of two related but distinct Src-family kinases: Lyn and Fyn. These kinases are involved in interlocked positive and negative feedback loops. Positive feedback arises as receptor ITAMs are phosphorylated, generating binding sites for Lyn, Fyn, and Syk. Negative feedback arises as the adaptor protein PAG is phosphorylated. Phosphotyrosines in PAG recruit Csk, a kinase that phosphorylates Lyn and Fyn

at negative regulatory sites. By incorporating available mechanistic knowledge into a rule-based model capturing the interactions of the receptor (BCR), Lyn, Fyn, Syk, Csk, and PAG, the site dynamics of this system were explored, and the effects of perturbations (e.g., protein knockdown and overexpression) were evaluated. It was found that oscillations in Syk activity could arise for certain ranges of the stimulatory signal, and for certain expression levels of Lyn and Fyn. It was also found that bistability could arise in cells lacking Lyn or Csk. These results represent model predictions that are experimentally testable.

Integration with experimentation

Development of detailed computational models is justified by availability of detailed experimental data. Emerging technologies have enabled examination of site-specific aspects of cell signaling, in some cases at the single-molecule level. Insights and questions derived from these studies motivate modeling efforts to capture a comparable level of detail.

In the case of LAT, site-specific antibodies have been used to monitor phosphorylation kinetics of tyrosine residues 132 and 191 following TCR stimulation (Houtman et al., 2005). It was found that tyrosine 191 becomes phosphorylated significantly faster than tyrosine 132. This difference could have noteworthy consequences for regulation of downstream signaling, because these sites have distinct binding partners: phosphorylated tyrosine 132 binds phospholipase Cγ1, whereas phosphorylated tyrosine 191 binds Gads and related adaptor proteins. These types of site-specific interactions are captured naturally in a rule-based model.

Another area in which site-specific phosphorylation has proven significant is partial phosphorylation of ITAMs. The consensus view is that, when doubly phosphorylated, ITAMs recruit the tandem SH2 domains of Syk-family kinases. However, it is now clear that

phosphorylation of a single ITAM tyrosine residue may lead to recruitment of a different set of signaling proteins, with distinct consequences for downstream signaling. By using mono-SH2 and dual-SH2 domain recombinant proteins as probes for singly- and doubly-phosphorylated ITAMs, it has been found that ITAM monophosphorylation is associated with anergy and activation of the negative regulators Dok-1 and SHIP-1 in BCR signaling (O'Neill et al., 2011). Activation of this inhibitory circuit may be linked to recruitment of the kinase Lyn to singly-phosphorylated ITAMs of the Igα/β subunits of the BCR.

Development of useful models will depend on suitable data sets for model parameterization. A potentially rich source of data is likely to come from quantitative high-resolution mass spectrometry (MS). MS can be used to detect phosphorylation of specific residues in an unbiased manner (Cox and Mann, 2011), enabling discovery of previously uncharacterized phosphorylation sites in receptor subunits and their downstream targets (Nguyen et al., 2009; Brockmeyer et al., 2011). MS can also be used to quantitatively track changes in phosphorylation levels of known phosphorylation sites as signaling progresses, including changes that occur on short time scales (Dengjel et al., 2007). In the near future, novel methods for single-molecule MS may even offer the possibility of characterizing the phosphoforms of individual proteins (Naik et al., 2009). We propose that rule-based modeling is well-suited for mechanistic interpretation of large-scale MS datasets, because the rule-based formalism entails the same site-specific resolution (Creamer et al., 2012).

Other advances in quantitative measurements will also provide modelers with systematic measurements of binding constants for protein domains involved in immunoreceptor signaling. High-throughput platforms have been developed and used to measure the affinities of SH2 domains of human proteins for specific phosphotyrosine peptides (Kaushansky et al., 2008; Hause

et al., 2012). Such tools can potentially be used to determine the parameters needed for mechanistic modeling.

Lastly, super-resolution imaging techniques have enabled observation of signaling complexes on the nanoscale (Huang et al., 2010). These studies have elucidated the spatial reorganization of proteins that occurs during immunoreceptor signaling, including aggregation of LAT following TCR stimulation (Sherman et al., 2011). The ability to image aggregation at high resolution, such that individual molecules can be distinguished, means that predictions from rule-based models about aggregation, such as a predicted aggregate size distribution, could potentially be tested in a direct manner.

Conclusions & Future directions

A great deal of knowledge about immunoreceptor signaling has been accumulated. In addition, modern experimental methodologies allow us to obtain data that pertains to the site-specific details of molecular interactions. We believe that these aspects can be integrated to form a more complete, and more predictive, picture of how immune cells sense and respond to their environment. Our approach to piecing together this picture is to translate biological knowledge into chemical kinetics, using a formalism that naturally captures the way that biological knowledge is often characterized informally in scientific discourse. This formalism, in which rules are used to represent interactions and their contextual dependencies, allows us to capture molecular site dynamics (e.g., site-specific details of protein-protein interactions), more comprehensively simulate the reaction networks that mediate cell signaling, and manipulate specific features of cell signaling systems *in silico*. Until recently, only a subset of rule-based models could be simulated. With the development of network-free simulation methods (Danos et

al., 2007; Yang et al., 2008; Colvin et al., 2009; Colvin et al., 2010; Sneddon et al., 2011; Yang and Hlavacek, 2011), simulation of a much wider array of models is now possible.

Mechanistic models have value as hypothesis generators and as vehicles of understanding (Lander, 2010). A number of interesting biological questions can now be addressed via rule-based modeling, in part because this approach facilitates consideration of the full spectrum of possible phosphoforms for a protein of interest, which could be especially valuable for the study of ITAMs and related motifs that can have opposing regulatory functions that depend on phosphoform (Blank et al., 2009). We believe that the rule-based modeling approach is needed to address these and other questions that will emerge as the intricate machinery of immune signaling is explored further.

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Table 1: Recent studies of immune signaling that have employed rule-based modeling

Reference	Topic of study	Software used
Lipniacki et al. (2008)	Feedbacks in T-cell receptor signaling	BioNetGen
Nag et al. (2009)	LAT aggregation	Problem-specific code
An and Faeder (2009)	Toll-like receptor signaling	BioNetGen
Nag et al. (2009)	Serial engagement of FcεRI	BioNetGen
Monine et al. (2010)	Steric effects on aggregation of FcεRI	Problem-specific code
Nag et al. (2010)	Syk activation in mast cells	BioNetGen
Artymov et al. (2010)	Co-receptors in T-cell receptor signaling	SSC
Nag et al. (2012)	LAT aggregation with varying valency	Problem-specific code
Barua et al. (2012)	Interlocked feedbacks in B-cell antigen receptor signaling	BioNetGen

Table 2: Other recent studies of cell signaling that have employed rule-based modeling

Reference	Topic of study	Software used
Barua et al. (2007)	Interactions of Shp-2	BioNetGen
Barua et al. (2008)	Interactions of tandem SH2 domains	BioNetGen
Barua et al. (2009)	Jak kinase activation	BioNetGen
Gong et al. (2010)	HMGB1 signaling in cancer	BioNetGen
Ray and Igoshin (2010)	Transcriptional feedback in bacteria	BioNetGen, Mathematica
Malleshaiah et al. (2010) (see supplemental material of this paper)	Scaffold proteins and switch-like behavior	Facile, ANC, Matlab
Dushek et al. (2011)	Multi-site phosphorylation	Smoldyn
Selivanov et al. (2011)	Mitochondrial respiration	Problem-specific code
Sorokina et al. (2011)	The post-synaptic proteome of the neuronal synapse	RuleStudio, jsim, R
Thomson et al. (2011)	Yeast pheromone signaling	BioNetGen, MATLAB
Geier et al. (2011)	Integrin activation	BioNetGen
Ghosh et al. (2011)	Iron homeostasis in tuberculosis	KaSim, Cytoscape
Abel et al. (2012)	Influence of the membrane environment on bistability	SSC
Deeds et al. (2012)	Combinatorial complexity in protein interaction networks	KaSim
Kocienewski et al. (2012)	Dual phosphorylation in the MAP kinase cascade	BioNetGen
Michalski and Loew (2012)	Activation of CaMKII	BioNetGen, VCell

Figure 1: A model of interactions among LAT, Grb2, and SOS1 (Nag et al., 2009; 2012), represented as an extended contact map (Chylek et al., 2011). Boxes represent proteins, domains, and motifs. Double-headed arrows represent non-covalent interactions, and arrows are numbered to correspond to rules in Fig. 2. Post-translational modifications are designated by small squares connected to flags that indicate the site and type of modification (e.g., "pY171" refers to phosphorylation of tyrosine 171). LAT contains three tyrosine residues that can be phosphorylated and serve as binding sites for the SH2 domain of Grb2. Grb2 also contains a pair of SH3 domains, which are taken to be a single site in the model. Four proline-rich sequences in SOS1 are taken to be a pair of sites that can bind Grb2. Thus, Grb2 can bind SOS1 to form a 1:1 complex, which can be bound by a second Grb2 molecule to form a Grb2-SOS1-Grb2 complex. This ternary complex can cross-link two LAT molecules. An example of a molecule type definition is `LAT(PY,PY,PY)`, which represents LAT containing three phosphorylated tyrosine residues, which by being assigned the same name are taken to be indistinguishable by convention. An example of a rule is `LAT(PY) + GRB2(SH2,SH3) <-> LAT(PY!1).GRB2(SH2!1,SH3).` This rule indicates that free Grb2 can reversibly bind a free phosphosite in LAT via its SH2 domain. It is assumed that the other two phosphotyrosines in LAT do not influence the interaction, and are omitted from the rule. For a more complete model specification, see Fig. 2.

Figure 2: Abbreviated BioNetGen input file for a model of LAT, Grb2, and SOS1 interactions. The molecule types block specifies the molecules included in the model, and the components that each molecule contains. The reaction rules block contains rules that represent interactions that can occur in the model. Rules are numbered to correspond to arrows in Fig. 1. Note that multiple rules correspond to each arrow. An arrow represents an interaction; the rules corresponding to a given arrow each represents the kinetics of the interaction in a unique molecular context. The simulation command at the bottom calls a network-free simulator. Not shown are the parameters block, in which parameters are assigned values; the seed species block, in which initial conditions are set; and the observables block, in which model outputs are specified. For more information about the contents of a BioNetGen input file, see Faeder et al. (2009).

Figure 1

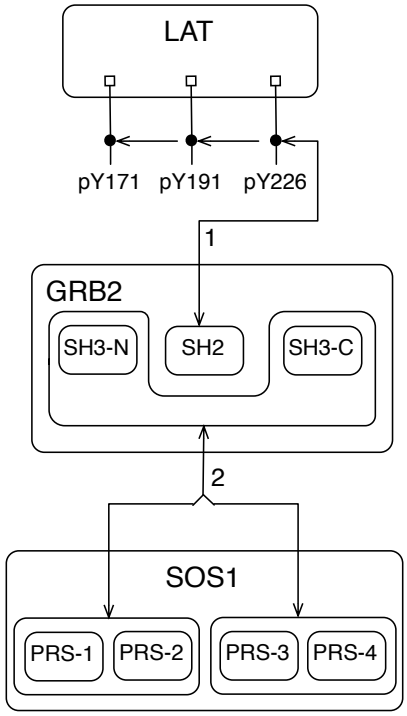
LAT
pY171
pY191
pY226
1
GRB2
SH3-N
SH2
SH3-C
2
SOS1
PRS-1
PRS-2
PRS-3
PRS-4

Figure 2

```
begin molecule types
LAT(PY,PY,PY)
GRB2(SH2,SH3)
SOS1(PRS,PRS)
end molecule types

begin reaction rules

# 1a: Free GRB2 binds free SOS1
GRB2(SH2,SH3)+SOS1(PRS,PRS) <->
GRB2(SH3!1,SH2).SOS1(PRS!1,PRS) kp1,km1

# 1b: Free GRB2 binds SOS1 bound to GRB2
GRB2(SH2,SH3)+SOS1(PRS,PRS!1).GRB2(SH3!1,SH2) <->
GRB2(SH3!2,SH2).SOS1(PRS!1,PRS!2).GRB2(SH3!1,SH2) s*kp1,km1

# 1c: Membrane-associated GRB2 binds free SOS1
GRB2(SH3,SH2!+)+SOS1(PRS,PRS) <->
GRB2(SH3!1,SH2!+).SOS1(PRS!1,PRS) kp1,km1

# 1d: Membrane-associated GRB2 binds SOS1 bounds to GRB2
GRB2(SH3,SH2!+)+SOS1(PRS,PRS!1).GRB2(SH3!1,SH2) <->
GRB2(SH3!2,SH2!+).SOS1(PRS!2,PRS!1).GRB2(SH3!1,SH2)
s*kp1,km1

# 1e: Free GRB2 binds membrane-associated SOS1
GRB2(SH3,SH2)+SOS1(PRS,PRS!1).GRB2(SH3!1,SH2!+) <->
GRB2(SH3!2,SH2).SOS1(PRS!2,PRS!1).GRB2(SH3!1,SH2!+)s*kp1,km1

# 1f: Membrane-associated GRB2 binds membrane-associated SOS1
GRB2(SH3,SH2!+)+SOS1(PRS,PRS!1).GRB2(SH3!1,SH2!+) <->
GRB2(SH3!2,SH2!+).SOS1(PRS!2,PRS!1).GRB2(SH3!1,SH2!+) kps,km1

# 2a: LAT binds free GRB2
LAT(PY)+GRB2(SH2,SH3) <->
LAT(PY!1).GRB2(SH2!1,SH3) kp2,km2

# 2b: LAT binds GRB2 bound to SOS1
LAT(PY)+GRB2(SH2,SH3!1).SOS1(PRS!1,PRS) <->
LAT(PY!2).GRB2(SH2!2,SH3!1).SOS1(PRS!1,PRS) kp2,km2

# 2c: LAT binds membrane-associated GRB2
LAT(PY)+GRB2(SH2,SH3!1).
SOS1(PRS!1,PRS!2).GRB2(SH2,SH3!2) <->
LAT(PY!3).GRB2(SH2!3,SH3!1).
SOS1(PRS!1,PRS!2).GRB2(SH2,SH3!2) kp2,km2

# 2d: LAT binds membrane-associated GRB2
LAT(PY)+GRB2(SH2,SH3!1).SOS1(PRS!1,PRS!2).
GRB2(SH2!3,SH3!2).LAT(PY!3) <->
LAT(PY!4).GRB2(SH2!4,SH3!1).SOS1(PRS!1,PRS!2).
GRB2(SH2!3,SH3!2).LAT(PY!3) kp2,km2

end reaction rules

# Call network-free simulator
simulate_nf({t_end=>1000,n_steps=>100});
```