Chapter 30
Leveraging Modeling Approaches: Reaction Networks and Rules

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Abstract We have witnessed an explosive growth in research involving mathematical models and computer simulations of intracellular molecular interactions, ranging from metabolic pathways to signaling and gene regulatory networks. Many software tools have been developed to aid in the study of such biological systems, some of which have a wealth of features for model building and visualization, and powerful capabilities for simulation and data analysis. Novel high-resolution and/or high-throughput experimental techniques have led to an abundance of qualitative and quantitative data related to the spatiotemporal distribution of molecules and complexes, their interactions kinetics, and functional modifications. Based on this information, computational biology researchers are attempting to build larger and more detailed models. However, this has proved to be a major challenge. Traditionally, modeling tools require the explicit specification of all molecular species and interactions in a model, which can quickly become a major limitation in the case of complex networks – the number of ways biomolecules can combine to form multimolecular complexes can be combinatorially large. Recently, a new breed of software tools has been created to address the problems faced when building models marked by combinatorial complexity. These have a different approach for model specification, using reaction rules and species patterns. Here we compare the traditional modeling approach with the new rule-based methods. We make a case for combining the capabilities of conventional simulation software with the unique features and flexibility of a rule-based approach in a single software platform for building models of molecular interaction networks.

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1 Models of Reaction Networks

Modelers usually create a model of cellular processes by explicit specification of a reaction network consisting of molecular species and reactions. This is currently the most common paradigm implemented in model building and simulation software such as VCell ([1, 2], http://vcell.org), CellDesigner ([3], http://celldesigner.org), Copasi ([4], http://copasi.org), ECell (http://e-vell.org), MCell (http://www.mcell.cnl.salk.edu), and others. Each species has to be created, named, and reactions specified and assigned to the appropriate compartment within the cell. For each interaction described in the model, a user chooses the appropriate kinetic formalism and inputs relevant parameter values. A model usually includes molecular species corresponding to experimentally identified or hypothesized events, such as ligand–receptor binding, phosphorylation events, etc. Such models can fit experimental data and provide useful predictions. After a reaction network is specified, it can be simulated in order to identify time-courses for species.

Figure 30.1a illustrates a simplified version of seminal model of signaling by epidermal growth factor (EGF) receptor (EGFR) developed by Kholodenko et al. [5]. The major assumptions and elements of reaction network used in this model were later reused is some other modeling studies [6,7]. This reaction network (which is typical to many studies of receptor-initiated signal transduction) is comprised of reactions for binding of extracellular ligand (L) to cell-surface receptor (R), ligand-induced (LR) dimerization of receptors (D), phosphorylation of cytoplasmic

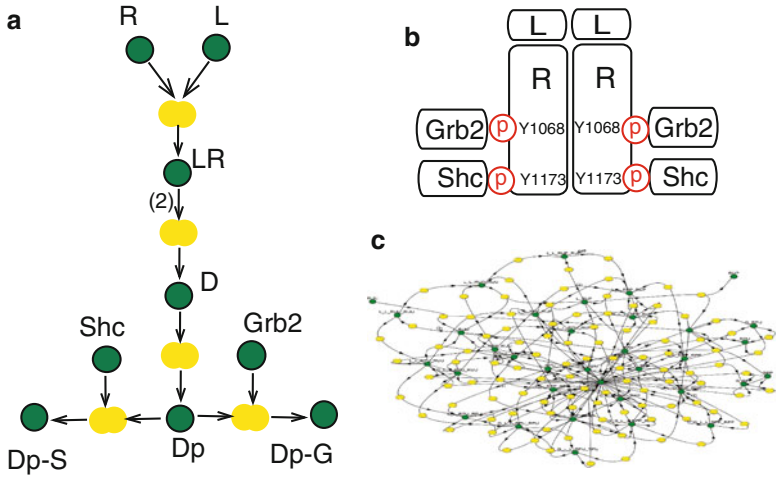

Fig. 30.1 (**a**) A bipartite graph (VCell notations: *green nodes* correspond to species, *yellow* nodes – to reactions) representing a model of initial events in EGFR signaling. (**b**) The potential protein complex arising during EGFR dimer signaling. (**c**) The Vcell model corresponding to the model describing interactions among all protein complexes looks like a maze consisting of *green* and *yellow dots*

receptor tyrosines (Dp) by the intrinsic protein tyrosine kinase, and competitive binding of adapter proteins Grb2 and Shc to a dimer (at most one protein per dimer).

The main paradigm in the reaction network approach is that every species represents a *pool*, a set of "things" that are indistinguishable from the standpoint of the processes (reactions) in which they participate. It provides a convenient way to visualize the molecular interactions as a graph where each species is a node. It also provides a unique and convenient mapping of the model to a mathematical description, where concentration of each species is a variable in time, which can be used to simulate the evolution of the system over time.

1.1 Limitations of the Reaction Network Approach

This approach of describing in detail all elements of a reaction network has several obvious limitations. Whether or not the reaction network is specified manually by the modeler, or through some computer-aided process (such as automated import from a pathway database [8]), it may include only a limited number of species and reactions. Thus, the model is usually based on mechanistic assumptions that limit the size of the reaction network.

Consider, for example, the EGFR signaling network described above. It includes many simplifications, such as omitting events like: ligand binding to cytosolically modified (phosphorylated at some combination of residues) receptor, ligand dissociation from the receptor in a dimer, dissociation of phosphorylated receptors in a dimer, multiple proteins bound to distinct phosphorylated receptors residues at the same time, etc. Additionally, lumping tyrosines of both receptor molecules in a dimer means that in the model they are phosphorylated and dephosphorylated simultaneously, and excludes the possibility that modification of individual tyrosines during signaling may affect the signaling outcome.

One might surmise that molecular complexes are often not considered in detail simply because of Occam's razor concept. As long as the model predictions match experimental observations, one can use simplifying assumptions and omit many details deemed unnecessary. However, these simplifications are often not motivated by experimental considerations. In [9], we reviewed the evidences contradicting some of these assumptions. Individual tyrosines of EGFR may have distinct temporal patterns of phosphorylation, Grb2 and Shc may be simultaneously associated with a single copy of EGFR, which is consistent with the observed nucleation of large heterogeneous protein complexes in other systems, and receptor monomers may be responsible for the spatial spread of receptor phosphorylation observed in response to localized EGF stimulation and therefore involved in signaling. Thus, it is conceivable that distinct combinations of phosphorylated receptor complexes may have distinct functions. Despite this evidence, simplified assumptions preventing formation of receptor dimers with multiple adaptor proteins bound to both receptors and receptor monomers have been used in most modeling studies of EGF receptor signaling.

The main reason to include simplifying assumptions is that without them, many more molecular species and reactions must be considered. For example, if we allow adapter proteins to simultaneously bind to distinct phosphorylated binding sites on receptors and allow receptors in dimers to dissociate, we need to include into the model all multiple forms of monomeric and dimeric EGFR complexes (like the one shown in Fig. 30.1b). Thus, we need to account for 93 species (12 monomeric and 78 dimeric receptor complexes, and proteins EGF, Grb2, and Shc). One can see (Fig. 30.1c) that such model is not easily tractable in a regular reaction editor (here shown in VCell). Furthermore, note that the number of species corresponding to multiple phosphoforms of receptor stacks up explosively: to track phosphorylation of the nine tyrosines of EGFR, one needs to account for the $2^9 = 512$ different phosphorylation states of an individual receptor and the 131,328 distinct combinations of phosphorylation states of receptors in a dimer. Of course, the true scope of such complexity is uncertain and may lie well below these theoretical maximal numbers due to various constraints, such as steric clashes, that might play a role in limiting the combinatoric possibilities in signal-transduction systems. But one would need to have the capability to handle very large number of species and reactions in order for models to be able to capture critical features of variability in signaling [10].

Another practical problem is that such models based on simplified assumptions usually involve species that lump together entities that correspond to specific experimental measurements, and a new model may be required to describe each new set of measurements. A related issue is the fact that even if one would like to include all molecular details of protein complexes in the model, there often is a lack of knowledge of the required detailed mechanisms of interactions and related kinetic parameters. However, new high-throughput flow cytometry and mass spectrometry measurements provide a wealth of information about interactions and activities of proteins [11], which now can be (and should be) included in some models.

2 Rule-Based Models

An alternative to the conventional modeling approach, that has been gaining increasing acceptance, is to attempt to create a model description that is capable of accounting for all the potential molecular complexes and interactions among them that can be generated during a response to a signal. A feasible strategy to implement this is a rule-based approach [12]. In this approach, protein–protein interactions and their effects are represented in the form of reaction rules that serve as generators of chemical species and reactions. This method, discussed in more detail in [12–14], provides an opportunity to consider the whole nomenclature of potential protein complexes, including their phosphoforms, modifications, and interactions that can potentially be generated during the response to signaling. Moreover, this approach also allows exclusion of those species and reactions that cannot be realized, e.g., because of cooperativity and steric clashes.

The rule-based approach has been initially developed based on the modularity of protein domains [15]. A model is specified as a set of reaction rules, which are associated with specific rate laws. Given a set of species, a reaction rule identifies those species that have the features required to undergo the transformation from reactants to products specified in the reaction rule. Interactions represented in a reaction rule do not depend on features not explicitly indicated. Thus, multiple species may qualify as reactants in a type of reaction defined by a reaction rule.

The modeler can define which components and modifications of a molecule or molecular assembly affect a particular chemical transformation, and which do not. Furthermore, the modeler has the ability to account for steric clashes, cooperativity, and any other factors that might influence the rate of a reaction. A reaction rule can state, for example, that "any cell-surface monomeric receptor having an available extracellular binding site and any free extracellular ligand can interact and form a ligand–receptor complex; the probability of this interaction depends only on the total numbers of cell-surface monomeric receptors and extracellular ligands and does not depend on the specific state of the receptor cytosolic portion." In this example, we assume that the cytoplasmic state of a receptor does not affect ligand–receptor binding, which implies that to parameterize all reactions specified by the ligand–receptor interaction rule, we need just two rate constants: on and off rates. Biophysicists would argue that any cytosolic modification will definitely affect all portions of a receptor, and hence must affect these rates. However, as we have shown [16] the model read-outs are relatively robust to parameter variations within the same reaction rule. Thus, in practice, the number of reaction rules (and rate constants) that the user must provide to specify a model is comparable to the number of assumptions about interactions among molecular domains considered in the model, which is usually much less than the total number of actual reactions. Moreover, the number of rate constants that the model is built upon can be limited to those that come directly from experiments, such as in vitro binding affinities for multiple SH2/PTB domains and tyrosines.

Moreover, when the user changes a model to include new assumptions about mechanisms of molecular interactions (such as replacing competitive binding of proteins to a scaffold with cooperative binding), these rate constants remain unchanged. This is in a contrast with a reaction network model where variables representing lumped entities often require adjusted kinetic laws.

2.1 Simulation Methods for Rule-Based Models

A model where the reaction network is explicitly specified always allows one to directly derive a unique mathematical formulation as a set of differential equations in variables corresponding to species concentrations/population numbers. The same is not true for rule-based models – they do not immediately provide the formalism required for simulation and they need pre-processing. In some cases, the rule-based model can be expanded into an explicit reaction network, such as by using

an iterative algorithm for processing reaction rules [13, 14] (e.g., the algorithm implemented in the BioNetGen software – which is, in fact, the origin of the name: Biological Network Generator). The iterations of rule application are halted when specified termination conditions (like reaching a predefined size of an oligomer) are satisfied or all possible reactions have been generated. The exact size of the generated reaction network depends, in general, on the entire set of reaction rules and also on the set of species to which reaction rules are initially applied.

Sometimes a reaction network can be of potentially unlimited size, such as when reaction rules provide a way for infinite elongation of molecular chains, e.g., while specifying actin filaments. In this scenario, "on-the-fly" network generation can be used. Reaction rule evaluation is embedded in a discrete-event Monte Carlo simulation of reaction kinetics, and reactions are generated only when a species is first populated during a simulation [14, 17].

However, the on-the-fly method still requires network generation: a product of a reaction generated by a reaction rule has to be identified either as a new species or as the species that was previously generated and already in the reaction network. This becomes a serious computational problem when the generated reaction network contains species that have complicated topological structures, such as species representing branched actin filaments. To deal with it, a "network free simulation" approach [18–20] was recently introduced. In this method, a model does not have to have its reaction network generated prior to and/or during simulation steps. Individual instances of possible species and interactions are accounted for and reaction rules are evaluated directly during the simulation. Depending on the size and complexity of the system, network-free simulation can be much more effective from a computational standpoint, although it is limited to using discrete simulation algorithms.

2.2 Graphical Representations for Rule-Based Models

One obstacle to the acceptance of rule-based modeling is the unusual way such models are being specified. Modelers tend to think of a model as a pathway or a reaction sequence, where the product of one reaction is used as a reactant (or a catalyst) in another reaction. Reaction rules are intrinsically disconnected, because not all of the multiple species that are products of a certain reaction rule participate in another reaction. Products and reactants of the reaction rule are no more pools of identical things, like species nodes in reaction networks. For example, let Rule 1 be the ligand binding to a receptor with three intracellular binding sites. Let Rules 2–4 be the independent binding of different adapter proteins to each of the three receptor binding sites. Thus, all product species of a ligand–receptor binding rule are divided into three intersecting subsets that can participate in three reaction rules of protein binding (Fig. 30.2).

To describe rule-based models, several non-network-based representations are used. One way of model specification is using a specially designed scripting

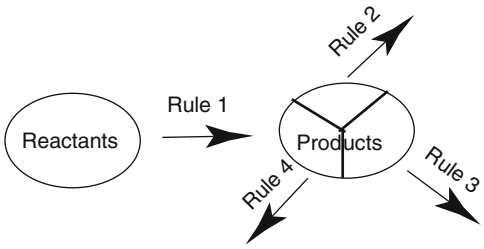

Fig. 30.2 Visualization of rule-based modeling as a reaction network is difficult. Each node is not a pool of identical things but corresponds to a set of species, each of which can participate in different rules

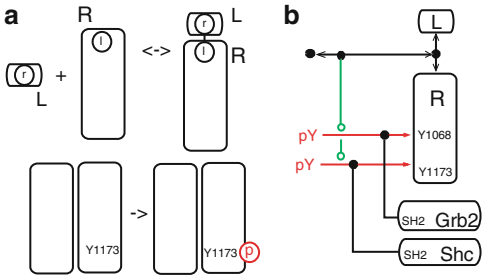

Fig. 30.3 Graphical representations of rule-based models. (**a**) Two rules are represented as cartoons that show only relevant features for each interaction. In the first rule, a ligand L can bind any receptor R provided binding sites l and r or R and L, respectively, are unbound. The second rule says that tyrosine Y1173 can be phosphorylated provided there is another receptor in proximity. (**b**) Molecular interaction map (MIM) representation of all interactions in Fig. 30.1a. Although compact, it might be ambiguous and the temporal order of interactions is difficult to infer

language, such as BioNetGen language (BNGL, [21]) or Kappa language [22] This approach requires intimate knowledge of such specialized languages, and thus is typically used only by advanced users.

Another approach is to use a graphical specification of all reaction rules following certain conventions, as described in [23, 24]. In this approach each molecular entity (protein, receptor, DNA, etc.) in a model is specified as a box containing components that denote features of a molecule. Several boxes can be joined to form a species or species pattern by connecting components. Each reaction rule is specified as a separate cartoon describing reactants and products (Fig. 30.3a).

Yet another approach to specify rule-based models is using cartoons representing interactions among molecular entities and their components, such as entity relationship diagrams in SBGN [25], molecular interaction maps [26] (Fig. 30.3b), or extended contact maps [27]. However, specification of rule-based models in this way is often ambiguous as the temporal order of interactions is difficult to infer [28]. This approach is used mostly to supplement the model-building process using a scripting language [29].

As a conclusion, all current approaches for specification of rule-based models are distinct from the usual model specification as a reaction network and thus require special training in rule-based modeling in order to be used. However, with all these

shortcomings, and despite being relatively new, the rule-based modeling approach has been used to develop a wide range of models [9, 30–34]. Several software tools with some rule-based modeling capabilities have been developed in recent years, including BioNetGen [13, 21], STOCHSIM [35], Moleculizer [17], K-factory [22], and Simmune [36].

3 Merging Reaction Network and Rule-Based Models

The reaction network and rule-based modeling approaches are complementary and have distinct representation schemas. The reaction network approach has the advantage of often being in one-to-one correspondence with cartoons representing signaling pathways and a defined mathematical representation. However, these advantages fade away as more and more details are included into the model, as very large reaction networks become cluttered and difficult to deal with. The rule-based approach has the advantage of being able to account for all details of molecular activities and interactions, but the complete overview of the biological system evolution may not be apparent until network generation or model simulation is performed.

It would be of enormous advantage to the modeling community if these two approaches would seamlessly work together. We have recently developed several prototype methodologies to use rule-based modeling alongside reaction network modeling, and to implement these two techniques into a common modeling and simulation interface.

3.1 Extending a Reaction Network by Adding Species Features

There are several classes of use cases where reaction rules can be organically used to extend existing models. Consider, for example, a model of a reaction network where the user wants to add a fluorescent tag to some features. For a minimal reaction network like $A + B \rightarrow C$, $C + D \rightarrow E$, if species A is fluorescently labeled, then the result of interaction of A with B, species C, will be fluorescently labeled as well. The fluorescence will be then passed to species E. If we now want to model a mixture of fluorescent and non-fluorescent species, we will need to double the size of original reaction network by adding extra reactions $Af + B \rightarrow Cf$, $Cf + D \rightarrow Ef$ (Fig. 30.4). This information makes the reaction network more cluttered and does not provide any new information, since often the kinetic behavior of fluorescent and non-fluorescent species is the same. Such an extension of the model can be easily described by introducing a "fluorescence" feature of molecules A, C, and E, and specifying that the value of this feature (fluorescent or non-fluorescent) is preserved when participating in reactions. Now each former reaction node becomes a reaction rule node, as it describes the same interaction for two different species: fluorescent

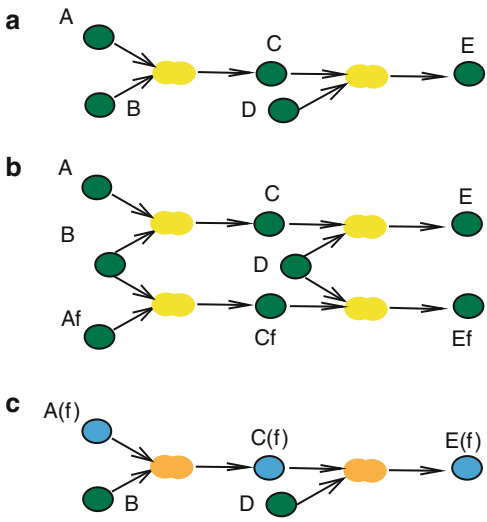

Fig. 30.4 An example of reaction network being extended by introducing fluorescent labeling. (**a**) The simple reaction network. (**b**) The network with species A, C, and E being fluorescently labeled. (**c**) The same reaction network displayed with two types of nodes – *green nodes* for individual species and *blue nodes* for species templates that have modifiable feature f (fluorescence). Reaction nodes become rule nodes and change color to *orange*

and non-fluorescent. Thus, the reactions become now reaction rules. This is a natural way to introduce rule-based modeling into a regular network.

Using this approach, a rule-based model can be build atop of a regular reaction network by converting some species and reactions into species patterns and reaction rules. In this scenario, each species can be extended into a species type by adding a set of features (attributes) and specifying allowable and default values of these features. Thus, a species is converted to a species template that defines a set of species. A second step is the conversion of a reaction to a reaction rule. A reaction where some of reactants or products are species templates becomes a reaction rule, as it is now applied to a set of species selected by a species template.

3.2 Extending a Reaction Network by Specifying Multimolecular Species

A more complicated case is to extend a reaction network that includes multimolecular species – for example the complex depicted in Fig. 30.1b where a transmembrane receptor R can bind extracellular ligand L, associate with another transmembrane receptor R, and bind two intracellular proteins Grb2 and Shc. To extend a reaction network into a rule-based model, the user can start from extending species L and R into species types. A ligand L becomes a species type by introducing a single feature "binding to R." A receptor R becomes a species type by introducing features "binding site for L," "phosphosite Y1," and "phosphosite Y2." Each feature may have several possible values, e.g., phosphosites can have values of "phosphorylated" or "unphosphorylated." Note that in BNGL species types are called molecules, and

features are called components. When all features are uniquely specified (for example, values of phosphosites are set to "unphosphorylated"), a species type includes "things" of the same kind, e.g., it represents a species. Thus, species type is more than species, in this example it includes at least four species representing different phosphoforms (those are species with sites Y1 and Y2 being unphosphorylated, phosphorylated, and pairwise different).

When the user follows a reaction network graph and sees a reaction arrow starting at two species extended into species type, the user has to convert the reaction into a reaction rule, and a product into a species template. Indeed, the species LR becomes a species template containing two species types L and R. To proceed, the user must specify a bond between components of L and R, by setting values of "binding site for R" to "bound to R" and "binding site for L" to "bound to L".

By traversing the reaction graph, each species node in a reaction diagram can be converted into a species template. While doing that, new features are introduced for each species type. For example, when converting dimerization reaction into a reaction rule, the user must specify how two ligand–receptor complexes are connected into the larger dimeric complex. To do it, a new feature "binding to R" must be specified for the species type R. Now each species type in the reaction of ligand–receptor binding becomes a species template, as receptor species type now has two features ("binding to L" and "binding to R"), and the second feature was not specified (ligand could potentially bind to a receptor connected to another receptor). As we noted in Fig. 30.2, the reaction network graph with nodes representing species templates does not represent a reaction network, as nodes do not represent identical "things" anymore. However, we still can use the reaction network graph with composite nodes in place of species templates. These nodes contain all species types used in a species template (Fig. 30.5). Here we follow the notations of [21] where the *center* and *content* of a reaction rule were introduced. The rule center contains all species types that have features changed during the interaction (for example, a binding site becomes bound or a phosphosite becomes phosphorylated), while content contains molecular entities that affect the interaction but remain unmodified. For example, in a dimer transphosphorylation rule, one receptor subject to phosphorylation belongs to a rule center, and another receptor that acts as a kinase and thus remains unmodified belongs to content. In a graphical presentation, a composite node for a reactant contains only reaction center.

Note that the reaction rule is valid only if it can be uniquely converted into a set of reactions. This is often not trivial. Consider the case where there is one reactant species template and one product species template, but each has a different number of unspecified features. The number of reactant species defined by the reactant species template will be different from the number of product species generated by the product species template. This makes one-to-one mapping impossible. Thus, conversion of a reaction to a reaction rule must be done with caution: selected reactant and product species must be converted to species templates, and a mapping between reactant species template and product species template has to be established.

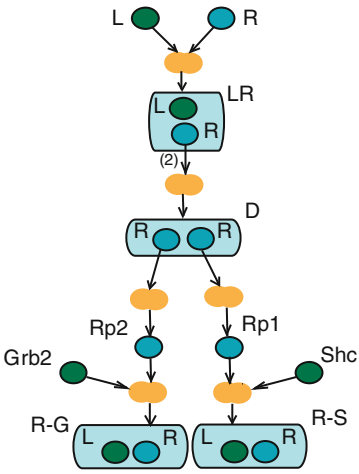

Fig. 30.5 The combination of reaction network view with rule-based modeling. *Green nodes* correspond to species, *blue nodes* to species templates. *Orange nodes* correspond to rules. *Arrows* connect molecular entities that are modified during interactions (rule centers)

3.3 Prototype for a Unified Modeling Interface

We are implementing the approach discussed above into the VCell modeling and simulation framework [1, 2]. By combining a reaction network specified in the VCell editor (explicitly specifying individual species and reactions) with multicomponent species and rules of interactions, a user should be able to use rule-based specifications within the familiar "look and feel" environment of the physiology editor. The new VCell interface introduced in version 5.0 (public beta release as of this writing) includes dual views for a reaction network: a set of tables describing all model elements, and a bipartite graph with species and reaction nodes. Both views can be extended to support rule-based features.

The tabular view is a good interface for extending an existing reaction networks into a rule-based model, or for creating a new rule-based model. The table can be used to specify features and possible feature states for species types, and drop-down menus are a good way to select feature states for species templates and specify bonds. Reaction rules can be specified in two tables that represent reactant and product parts of a rule.

The reaction network view can be used for visualizing a composite rule-based and network model. A mix of species and species patterns, reactions and rules, is illustrated in Figs. 30.4 and 30.5. The reaction network can be also "flattened," when all species and reactions are generated. Flattening is possible and provides essential information only when network generation is possible. In the flattened view, regular species and species generated from reaction rules are displayed as a usual bipartite graph, where each species carries all the features inherited from reaction rule specification. Thus, many modes of model visualization are possible – collapsing all species corresponding to certain reaction rule, displaying sub-network consisting only of species with a certain feature (like fluorescence), etc.

We aim to provide an expert system guiding users in building a rule-based model, providing suggestions on what features have to be introduced for each molecular entity and each interaction. Significant efforts are still required to introduce a convenient way to mix rule-based and network models.

4 Conclusions

Quantitative modeling studies have rapidly spread across many domains of biology in recent years and the scientific community has been putting a great deal of efforts into standardization. These efforts are crucial for more efficient and accurate transmission of biological knowledge between different communities in research, education, publishing, and more. Standards like Systems Biology Markup Language (SBML, [37]), Systems Biology Graphical Notations (SBGN, [25]), and Biological PAthway eXchange (BioPAX, [38]) are already widely used to provide exchange of models, visualization schemas, and pathway data, respectively. The goal is to provide interoperability between various methods and tools, as it has become clear that there is no single strategy and platform that can cover all needs.

Initially, all of these standards have been developed based on the conventional approach to model building. However, the rule-based modeling approach is gaining momentum, and the old paradigm that every species in a model, every node in a graph description and every entity in a database consist of identical "things" is phasing out. This has been recognized by the community, and each of the standards mentioned above has some capabilities to describe "generic" entities and elements of rule-based modeling. SBML has a package ("L3 multi") under development to enable description of multistate and multicomponent species and rules of interactions among them. SBGN and BioPAX have proposals to introduce generic entities that describe sets of species that may participate in multiple interactions.

The VCell simulation and modeling framework always strives to be on the leading edge of new technologies, be user-friendly, and be compatible with the community standards. It is often not easy, as in the case of rule-based modeling. However, adoption of the new standards will hopefully facilitate the development of more tools with mixed capabilities, just like our prototype of VCell-BioNetGen integration.

References

1. Slepchenko BM, Schaff JC, Macara I, Loew LM (2003) Quantitative cell biology with the virtual cell. Trends Cell Biol 13(11):570–576
2. Moraru II, Schaff JC, Slepchenko BM et al (2008) Virtual cell modelling and simulation software environment. IET Syst Biol 2(5):352–362

3. Funahashi A (2003) The ERATO systems biology workbench and systems biology markup language: an integrated environment and standardization for systems biology. Tanpakushitsu Kakusan Koso 48(7):810–816
4. Hoops S, Sahle S, Gauges R et al (2006) COPASI – a COmplex PAthway SImulator. Bioinformatics 22(24):3067–3074
5. Kholodenko BN, Demin OV, Moehren G, Hoek JB (1999) Quantification of short term signaling by the epidermal growth factor receptor. J Biol Chem 274(42):30169–30181
6. Hatakeyama M, Kimura S, Naka T et al (2003) A computational model on the modulation of mitogen-activated protein kinase (MAPK) and Akt pathways in heregulin-induced ErbB signalling. Biochem J 373(Pt 2):451–463
7. Schoeberl B, Eichler-Jonsson C, Gilles ED, Muller G (2002) Computational modeling of the dynamics of the MAP kinase cascade activated by surface and internalized EGF receptors. Nat Biotechnol 20(4):370–375
8. Blinov ML, Ruebenacker O, Schaff JC, Moraru II (2010) Modeling without borders: creating and annotating VCell models using the web. Lecture Notes Bionform 6053:3–17
9. Blinov ML, Faeder JR, Goldstein B, Hlavacek WS (2006) A network model of early events in epidermal growth factor receptor signaling that accounts for combinatorial complexity. Biosystems 83(2–3):136–151
10. Mayer B, Blinov M, Loew L (2009) Molecular machines or pleiomorphic ensembles: signaling complexes revisited. J Biol 8(9):81
11. Schulze WX, Deng L, Mann M (2005) Phosphotyrosine interactome of the ErbB-receptor kinase family. Mol Syst Biol 1:2005.0008
12. Hlavacek WS, Faeder JR, Blinov ML, Posner RG, Hucka M, Fontana W (2006) Rules for modeling signal-transduction systems. Sci STKE 2006(344):re6
13. Blinov ML, Faeder JR, Goldstein B, Hlavacek WS (2004) BioNetGen: software for rule-based modeling of signal transduction based on the interactions of molecular domains. Bioinformatics 20(17):3289–3291
14. Faeder JR, Blinov ML, Goldstein B, Hlavacek WS (2005) Rule-based modeling of biochemical networks. Complexity 10:22–41
15. Pawson T, Nash P (2003) Assembly of cell regulatory systems through protein interaction domains. Science 300(5618):445–452
16. Faeder JR, Blinov ML, Goldstein B, Hlavacek WS (2005) Combinatorial complexity and dynamical restriction of network flows in signal transduction. Syst Biol 2(1):5–15
17. Lok L, Brent R (2005) Automatic generation of cellular reaction networks with Moleculizer 1.0. Nat Biotechnol 23(1):131–136
18. Yang J, Monine MI, Faeder JR, Hlavacek WS (2008) Kinetic Monte Carlo method for rule-based modeling of biochemical networks. Phys Rev E: Stat Nonlinear Soft Matter Phys 78(3 Pt 1):031910
19. Colvin J, Monine MI, Faeder JR, Hlavacek WS, Von Hoff DD, Posner RG (2009) Simulation of large-scale rule-based models. Bioinformatics 25(7):910–917
20. Colvin J, Monine MI, Gutenkunst RN, Hlavacek WS, Von Hoff DD, Posner RG (2010) RuleMonkey: software for stochastic simulation of rule-based models. BMC Bioinform 11:404
21. Faeder JR, Blinov ML, Hlavacek WS (2009) Rule-based modeling of biochemical systems with BioNetGen. Meth Mol Biol 500:113–167
22. Danos V, Feret J, Fontana W, Krivine J (2007) Scalable simulation of cellular signaling networks. Lect Notes Comput Sci 4807:139–157
23. Blinov ML, Yang J, Faeder JR, Hlavacek WS (2006) Graph theory for rule-based modeling of biochemical networks. Trans Comput Syst Biol Vii 4230:89–106
24. Blinov ML, Yang J, Faeder JR, Hlavacek WS (2006) Depicting signaling cascades. Nat Biotechnol 24(2):137–138
25. Le Novere N, Hucka M, Mi H et al (2009) The systems biology graphical notation. Nat Biotechnol 27(8):735–741
26. Kohn KW (2001) Molecular interaction maps as information organizers and simulation guides. Chaos 11(1):84–97

27. Chylek LA, Hu B, Blinov ML et al (2011) Guidelines for visualizing and annotating rule-based models. Mol Biosyst 7(10):2779–2795
28. Kohn KW, Aladjem MI, Kim S, Weinstein JN, Pommier Y (2006) Depicting combinatorial complexity with the molecular interaction map notation. Mol Syst Biol 2:51
29. Xu W, Smith AM, Faeder JR, Marai GE (2011) RuleBender: a visual interface for rule-based modeling. Bioinformatics 27(12):1721–1722
30. An GC, Faeder JR (2009) Detailed qualitative dynamic knowledge representation using a BioNetGen model of TLR-4 signaling and preconditioning. Math Biosci 217(1):53–63
31. Faeder JR, Hlavacek WS, Reischl I, Blinov ML, Metzger H, Redondo A, Wofsy C, Goldstein B (2003) Investigation of early events in Fc epsilon RI-mediated signaling using a detailed mathematical model. J Immunol 170(7):3769–3781
32. Lipniacki T, Hat B, Faeder JR, Hlavacek WS (2008) Stochastic effects and bistability in T cell receptor signaling. J Theor Biol 254(1):110–122
33. Mu F, Williams RF, Unkefer CJ, Unkefer PJ, Faeder JR, Hlavacek WS (2007) Carbon-fate maps for metabolic reactions. Bioinformatics 23(23):3193–3199
34. Nag A, Monine MI, Blinov ML, Goldstein B (2010) A detailed mathematical model predicts that serial engagement of IgE-FcepsilonRI complexes can enhance Syk activation in mast cells. J Immunol 185(6):3268–3276
35. Le Novere N, Shimizu TS (2001) STOCHSIM: modelling of stochastic biomolecular processes. Bioinformatics 17(6):575–576
36. Meier-Schellersheim M, Klauschen F, Angermann B (2009) Computational modeling of signaling networks for eukaryotic chemosensing. Meth Mol Biol 571:507–526
37. Hucka M, Finney A, Sauro HM et al (2003) The systems biology markup language (SBML): a medium for representation and exchange of biochemical network models. Bioinformatics 19(4):524–531
38. Demir E, Cary MP, Paley S et al (2010) The BioPAX community standard for pathway data sharing. Nat Biotechnol 28(9):935–942