Toward predictive modeling of large and complex biological signaling networks

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The main goal of this article is to identify critical issues that arise when building *in silico* models that can predict the behavior of complex biological signaling networks. We discuss practical and effective approaches to overcome, circumvent, or moderate these issues. Here we focus on modeling spatially homogenous systems, such as those consisting of cells of the same type receiving the same input. Proper modeling of spatially heterogeneous systems requires additional model features and added modeling scales, beyond those required for spatially homogenous systems. These different modeling scales are defined here as different layers in the hierarchical relationships in a network. For example, protein molecules and cells that contain the protein molecules belong to different scales.

What is a biological signaling network?

A network is a flexible framework that describes a group of interconnected things, both the components of the group and their relationships[1]. A network is graphically represented by nodes, which represent components of the network, and directed edges between any two nodes, which represent an influence from the source node to the target node. In a biological signaling network, a node typically represents a gene, protein, or compound that mediates signals; a directed edge represents signal flows. Signal convergence and/or divergence may occur at network nodes.

A model reflecting a network's underlying mechanisms is required for type II predictions

First, we clarify the different types of predictions that can be made by an *in silico* model. The requirements for models that predict network behavior are completely different for predictions made in data-rich versus data-poor realms (type I and type II predictions, respectively). Data-rich and -poor are relative terms: while the extremes are clearly different, the boundary between these realms has no general definition. Figure 1 illustrates the difference between these

realms using a simple case. A single output (y -axis) of a network is produced by one network input (x -axis). The data (blue points) cluster around the true mean (black curve) of the input-output relationship, with some random noise. When a model determined by an arbitrary function (in Figure 1, $y = ax^2$) is fit to the data (red dashed curve), this model can well predict the output values given input values within the data-rich realm, which is indicated by the fact that the true and model functions are close to each other in the realm. Thus, an arbitrary function enables type I prediction. In contrast, the model prediction accuracy of an arbitrary function could deteriorate rapidly in any data-poor realm. When challenged with type II prediction, the arbitrary function used in Figure 1 does not do well: the true and model functions lie far apart in the data-poor realm. It has often been pointed out that good models of biological phenomena must make testable, accurate predictions [2, 3]. We wish to refine the logic of this consensus: there are two classes of model predictions, type I and type II. The utility of models that can make accurate type II predictions is much broader than that of models that can only make accurate type I predictions. Merely assessing type I predictions of an *in silico* model could make a model appear more effective than it actually is in explaining network behavior. In this perspective, we are interested in guidelines for building models that can make reasonable type II predictions.

If a network's mechanistic details, such as the network structure and its associated parameters, are unknown, machine-learning approaches, such as neural networks, can be used to build a network model [4, 5]. Such approaches are the equivalent of fitting an arbitrary function to the network data (as in Figure 1), but with multiple inputs instead of a single input (so that the function is fit in a higher-dimensional space). Models relying purely on machine-learning techniques can make reasonable type I predictions, but they may not perform as well at type II prediction. In addition, a practical problem arises from machine-learning approaches: when there are many different inputs to a model, the space of possible network behaviors grows exponentially

with the number of inputs. Collecting sufficient data to assemble a truly data-rich realm in this high-dimensional space may be costly, time-intensive, and at worst impractical.

Another complicating issue in machine-learning modeling is overfitting. Overfitting arises when the model complexity (e.g., the complexity of the arbitrary function) surpasses the complexity of the true relationships between network inputs and outputs. For example, when a needlessly complex function is fit to the data, the fitted curve can wind back and forth merely picking up the variation in data caused by random noise (Figure 1, green curve). Although this complex function fits the sample data better than a simpler arbitrary function (red dashed curve), it has a lower predictive power even within a data-rich realm: the green curve deviates from the black curve. In data-rich realms, this overfitting can be controlled by iterative resampling-based methods. In one such method, cross-validation [6], the data is randomly re-sampled, and a model is fit to partial training data. The model is then used to predict the left-out test data. Iteratively performing this process numerous times allows reliable quantification of the model's prediction accuracy. When comparing multiple models with different levels of complexity to determine which model is the best, overall prediction accuracies among the models are compared. In summary, we can build models accurate in type I prediction without knowing the underlying network mechanisms as long as we can collect sufficient data to constitute a data-rich realm and we control for overfitting.

A modeling approach diametrically opposite of pure machine-learning approaches is to capture every detailed mechanism in a network: a completely bottom-up, molecular mechanistic modeling approach. If we knew every single component and every quantitative rule that uniquely determines the source node effects on each target node, a completely bottom-up, mechanistic model could be built. Further, with complete knowledge of the initial state of every node and knowledge of external input values, the behavior of a deterministic network can be numerically simulated with an *in silico* model. Since this model would be an exact virtual copy of the network of interest, it would be able to make accurate type II predictions as well as accurate

type I predictions. The drawback of this modeling approach is that it requires almost perfect knowledge of the components, rules, and the initial states. The feasibility of this modeling approach rapidly collapses as system knowledge falls short of perfect (Figure 2). This is because if many parameter values, such as reaction rates of chemical reactions in the network, are unknown, diverse parameter value sets may all yield similarly good fits to the model. More troubling, in such an unconstrained case, the predicted parameter value sets for the best fit model may be highly sensitive to small uncertainties in data and in known parameter values. Worse, even if the parameters are stable for a fixed network structure, different network structures with different parameters may fit equally well [7, 8]. The best-fitting model may not be the right one in a highly unconstrained modeling situation. Unfortunately, obtaining almost complete knowledge of a large network is currently unrealistic. Data complexity is often a strongly limiting factor, and successful implementation of molecular mechanistic modeling has been mostly limited to relatively small networks [9-11]. Nevertheless, to make reasonable type II predictions, a model needs to reflect the mechanisms underlying the network, i.e., the model has to be to some extent a mechanistic model. For example, if we know the true input-output relationship in Figure 1 is sigmoidal, a sigmoid function, rather than an arbitrary function, can be used as the model. The model fitted to the data can make reasonable type II predictions (orange dashed curve). Thus, incorporation of mechanistic information in a model is an important theme to make an *in silico* model that can make reasonable type II predictions.

Summarization of a network

It usually is not feasible to find the detailed molecular mechanisms of a complex biological network. How then can we build an *in silico* model that can make reasonable type II predictions for a large network? We need to summarize the network down to a tractable size, which is often called network reduction. We should recognize that whenever mechanistic models are produced, one scale, or a few consecutive scales, are chosen to define the components of the

model. For example, when modeling a biological signaling network, no one argues that the components of the model should be quarks and leptons, although quarks and leptons are the fundamental building blocks of everything. Such a model would span an absurd number of scales. A biological signaling network operates at a much higher scale than the laws governing quarks and leptons. Inclusion of lower scale(s) vastly increases the number of components and, consequently, the complexity of the model structure. Thus, a model that spans multiple scales is much more difficult to handle than a model that spans very few scales. A realistic model consists of components that summarize lower scale components and their relationships. Therefore, models of biological signaling networks are typically built on the scale of genes or molecules, including proteins [9-11].

Despite consensus among biologists that genes or molecules are the elemental units of a signaling network, are these at the right scale for building an *in silico* model of a large complex network, a model that can make decent type II predictions of the network's behavior? A large signaling network could contain a large number of molecular components. In such a situation, a molecular mechanistic modeling approach is unrealistic. We propose that effective modeling of large complex biological networks requires further summarization of molecular network components into a relatively small number of functional units. We can efficiently and cost-effectively build a mechanistic model with the summarized components. We should not be tightly tied to the historically "fundamental" gene- or molecule-scales for a network model.

To identify more summarized network components, we need principles for condensing detailed networks into simpler networks with summarized network components as nodes. Let's start with a tree-like network (Figure 3a), where each node represents a protein involved in the network. Signal flow in a tree-like signal transduction network is straightforward, propagated down linear tree limbs, possibly branching into parallel pathways. If a node (protein) is removed, for example by mutating the gene encoding the protein, the change in the network behavior is easily predicted. The network loses everything "downstream" from that node (Figure

3b). However, if the network is modified by adding an edge to that creates a single signal convergence point, where two branches merge into one (Figure 3c), the behavior of the now web-like network cannot be easily deduced. Signal convergence makes the network behavior complex. The network structure alone no longer predicts the network response when a node is removed upstream from the convergence point (Figure 3d). We also need to know the convergence rule where the signals merge, and probably the strength of the individual signals before they connect, to compute the node output at the convergence point. For example, this rule might be the maximum value of two input signals (Figures 3c and d). Types of signal convergence include feedback and feed-forward loops, and signal cross-talk.

Conventional genetics logic holds that the function of a gene can be deduced from the phenotype of a loss-of-function mutant. Specifically, a gene's function is held to be the opposite of the mutant phenotype relative to the wild-type (the intact network's) phenotype. This logic presupposes a tree-like network; it does not necessarily apply in a web-like network. The phenotype observed at output O3 when comparing the mutant (Figure 3d) to wild type (Figure 3c) does not reveal the function of the removed node. Rather, it reflects the characteristics of the remaining signal "pathway," which is completely invisible without the mutation. We need a new approach, different from the traditional genetics approach, if we aim to gather accurate, quantitative, mechanistic information for a web-like network. We now discuss one such approach.

To summarize a web-like network, we make one assumption: each node in our network only has a monotonous relationship between each input and each output. That is, when a node has multiple inputs, the other inputs are held to arbitrary constant values. Then a collection of nodes in an unbranching network segment—without signal convergence or divergence points—can be condensed into a single node, without strongly influencing the behavior of the entire network. Only nodes where signals diverge or converge remain in a summarized network, as well as input and output terminal nodes. For example, the starting networks in Figures 3a and 3c can be

reduced to those in Figures 3e and 3f, respectively. Using this procedure for network summarization, knowledge of only the rules and inputs at signal convergence nodes is sufficient for building an *in silico* model that simulates network behavior well. Knowing all the components in summarizable “pathway” parts is not as important.

Modeling with a summarized network in practice

There are two practical challenges in modeling with a summarized network. First, how can we discover the summarized network structure when we lack a detailed starting network structure? Second, how can we discover the rules where edges converge? Major network hubs provide a practical solution to the first challenge of network summarization. Hubs are by definition structurally major convergence nodes [12]. They have major impacts on the network response when they are removed. We introduce another assumption: signal convergence events that induce large network response effects occur predominantly at network hubs. We define network response comprehensively, as the change in the states of network components, not merely the effect on final network output. Thus, we can make a minimal network using major network hubs as summarized nodes. Together, the products of the genes that produce strong network response phenotypes when mutated become the nodes of a good summarized network.

The second challenge is to discover rules governing signal convergence for the summarized network. Such rules need to be experimentally learned since there are no fundamental laws from which to derive them. Desirable features in such experiments are: (1) the activity of each node in the summarized network should be measured along a time series, since the input is dynamic in a signaling network; (2) the strength of the inputs to each summarized node should be changed in various combinations across wide ranges. To discover general mechanisms, experiments should sample a full swath of network behavior, instead of generating data from a narrow subset of possible network responses.

The node activity level is defined as the output level of a node. By assumption, this activity has a monotonous relationship with the input to a downstream node targeted by this node. To discover the convergence rule at a node, information is required for both input and output levels at a node. It is ideal if a node output level can be measured directly, such as via a protein or hormone concentration, or the concentration of an activated protein kinase. A convenient alternative is the mRNA concentration of an appropriate marker gene that follows the node's activity. If marker gene(s) are not known, using transcriptome data to select good marker genes is a practical option. Care must be taken, however, to find genes uniquely responsive to a signal of interest. When network components are summarized, a substantial time delay may occur in signaling from one node to another. This is because a single edge connecting two nodes in a summarized network may actually represent multiple molecular events. These events may include time-consuming processes, such as transcription, protein maturation, and vesicle trafficking. Sufficient time resolution is needed along a time-series of node activity measurements to determine kinetic parameters, such as time delays.

Biological signaling networks are often robust against perturbations internal or external to the network[13]. In a robust network, it is difficult to induce large changes in node input levels for many input combinations at every summarized node. The effects of internal or external perturbations could rapidly be compensated in the network. If inputs to each node can be externally controlled, such as by ectopic applications of a hormone molecule, it may be possible to drastically change the node inputs. However, such a possibility is usually limited to a small number of node inputs. Thus, to drastically change the node input levels throughout the network, deep network perturbations, such as simultaneous perturbations at multiple nodes, are often required. To discover the signal convergence rule at a node, node inputs must be decoupled from each other by both independently and jointly varying input values across a wide range of levels. Exhaustively combining individual node perturbations achieves this purpose. Profiling a combinatorially complete set of perturbations of all major network nodes is a powerful

way to learn *de novo* the mechanisms underlying the network response, as detailed below. Further, we emphasize how critical it is to identify a comprehensive set of network hubs which control the response being studied.

The plant immune signaling network is a complex network.

In the following, we discuss our work of modeling a plant immune signaling network as a case study in applying the modeling principles discussed above. Plants recognize pathogen-derived signals and induce a battery of defense responses, which are regulated by a signaling network[14, 15]. A pathogen adapted to a plant host delivers effectors into the plant cell. These effectors can interfere with components of the plant immune signaling network[14, 16]. Since microbes can evolve much faster than plants[17], the plant immune signaling network needs to withstand perturbations from pathogen effectors without depending heavily on evolutionary adaptations. The plant immune signaling network must therefore rely on its complexity[18]. The network output must be highly robust against perturbations internal to the network caused by pathogen effectors. Simultaneously the plant immune signaling network needs to have its output tuned according to its input. Various molecules can be recognized as pathogen attack signals and stimulate immunity[14, 19]. The reliability of each molecule as an attack signal varies: some molecules are only carried by pathogens, and others are shared with non-pathogens. Since the immune response is expensive to plants[20], misfiring a strong immune response based on a pathogen attack signal of low reliability would have substantial plant fitness costs[21, 22]. Furthermore, different defense responses have different efficacies against different pathogens[23, 24]. The plant immune signaling network has inevitably evolved to be very complex to fulfill these simultaneous requirements of robustness and tunability.

Step-wise reconstitution of a network enables mechanistic interpretation of network behavior.

To discover the mechanisms underlying the plant immune signaling network, practicality demanded that we simplify a network with many hundreds of components into a summarized network with a handful of components[25]. For our summarized network, we chose four components: the jasmonate (JA), ethylene (ET), salicylate (SA), and PAD4 signaling sectors. Targeted removal of each of these sectors had strong effects on network responses without producing any strong morphological phenotypes from the embryo stage up to the adult rosette leaf stage. We used the latter stage for data collection. Removal of each signaling sector was achieved by null mutations in the *DDE2*, *EIN2*, *SID2*, and *PAD4* genes[26-30], which are hub genes for the JA, ET, SA and PAD4 sectors respectively. We found that simultaneous removal of the four signaling sectors mostly abolished pattern-triggered immunity (PTI) elicited by a bacterial molecular pattern, flg22 (flg22-PTI), and effector-triggered immunity (ETI) initiated by a bacterial effector, AvrRpt2 (AvrRpt2-ETI)[25]. Thus, these four signaling sectors together approximately compose a bottleneck of the signaling network that mediates flg22-PTI and AvrRpt2-ETI. Simultaneous removal of the four signaling sectors fits our definition of a deep perturbation of these signaling networks. To facilitate *de novo* discovery of signal convergence rules, a combinatorially complete set of plants, with respect to the four sectors, were constructed to isolate sectors and sector interactions[25]. Each plant carried a different subset of mutant alleles for the four hub genes. Plants represented a full range of network perturbation states, from intact (the wild type) to abolished (the quadruple mutant).

In conventional genetics, a mutant phenotype is compared to the wild-type phenotype to infer the true function of the wild-type allele of a mutated gene. The function assigned to the gene is the opposite of the mutant phenotype. However, as we showed in Figure 3d, this logic generally does not work in a network with significant signal converging points. Is it then impossible to learn the mechanisms underlying a complex network? No, but this is where deep

perturbation of a network makes all the difference. We need a deep perturbation that mostly (ideally, completely) disrupts the biological function of interest. From this “ground state”, a network reconstitution strategy can be taken [31, 32]. This approach is the reverse of the conventional genetics approach: a mutant phenotype is compared not to the wild-type phenotype but rather the phenotype of the (almost) completely abolished network. This network reconstitution strategy is required to disentangle the independent effects of network sectors from the collective effects that rely on or compensate for the effects of other network sectors. In a triple mutant, only one sector remains intact, so the effect of this sector on induced immunity was estimated by comparing the phenotype of the triple mutant to that of the quadruple mutant, where the network was almost completely removed. For example, the direct effect of SA on either flg22-PTI or AvrRpt2-ETI was estimated by comparing immunity in a triple mutant with an intact SA sector, but with the JA, ET and PAD4 sectors removed, to immunity in the quadruple mutant. In this manner, the individual effect of each sector on immunity was estimated. The effect of having two sectors present was determined by including double mutant phenotypes in this analysis. For example, using the double mutant with the JA and ET sectors removed, but the PAD4 and SA sectors intact, the effect of the PAD4 and SA sectors together was estimated. This joint effect was described as the individual additive effects of the PAD4 and SA sectors, together with the interaction between the PAD4 and SA sectors ($\sim \text{PAD4} + \text{SA} + \text{PAD4:SA}$). The interaction is the deviation of the network output from the combined additive effects of the two separate sectors. If the separate effects are positive and the interaction is positive, the two sectors interact in a synergistic manner. If the separate effects are positive and the interaction is negative, the two sectors interact in a compensatory manner. All two-sector interactions were estimated using the relevant double mutant phenotypes. By continuing this stepwise reconstitution procedure, the three-sector interactions were estimated by including the single mutant data and the four-sector interaction was estimated by including the wild-type data.

This procedure of estimating the individual sector effects and their interactions based on stepwise network reconstitution was formerly called “signaling allocation analysis”[25].

The signaling machinery for flg22-PTI and AvrRpt2-ETI highly overlaps, since the four-sector network is responsible for about 80 percent of the phenotype in both cases. But, using a stepwise network reconstitution approach, we demonstrated that flg22-PTI and AvrRpt2-ETI use the same network quite differently. Some flg22-PTI signaling interactions were synergistic and others were compensatory, but those for AvrRpt2-ETI were predominantly compensatory[25]. This contrast explains well the general observations that pathogen effectors suppress PTI by strongly interfering with PTI signaling, while pathogens typically evade recognition rather than suppress ETI by interfering with immune network signaling[14, 16]. Since in ETI the signaling sectors strongly compensate one another, it is very difficult for a pathogen to inhibit one point in the network and thereby strongly reduce immunity[25, 32]. Thus, signaling allocation based on stepwise network reconstitution produced mechanistic descriptions of signaling sector effects and interactions and provided meaningful interpretation of a long-standing experimental observation.

Dynamic modeling of the network

Signaling allocation analysis provided an accurate mechanistic description of the sector roles in immune responses of interest. However, it did not reveal deeper mechanisms causal to the described characteristics. For example, are the differences in the sector contributions and sector interactions between ETI and PTI caused by a different wiring of the network structure in each case or by differing signal flow rates in the same network? We next investigated whether signal flows in a structurally invariant signaling network can explain the difference among PTI triggered by different MAMPs[33]. Induced immunity is a highly-summarized phenotype, abridging multiple signaling events into a single value. Measurements of induced immunity (i.e., network output) alone cannot provide much information to elucidate what happens inside the

network even with a stepwise network reconstitution strategy. This is because knowing a signal convergence node's output does not uniquely determine the input levels and the converging rule; our system knowledge is underconstrained. The sector activity levels must be directly measured in tandem with the stepwise network reconstitution. As discussed above, there are likely delays in the signal flows in a summarized network. Furthermore, in a dynamic network response, key signal flows may be transient. In light of these considerations, we collected the data at two time points after MAMP treatment; two is the minimum requirement to assess signal flow dynamics. These two timepoints were chosen with the aid of our earlier studies[34].

The sector activities were measured by their gene-expression proxies in all our 16 combinatorial genotypes at our two selected timepoints, in response to four different network stimulations: 3 MAMPs, and mock treatment[33]. To rigorously test our hypothesis of a conserved network structure, we needed to externally perturb the network in very different ways. The chosen MAMPs represented signatures from both bacterial and fungal pathogens, which presumably require different network responses[14, 19]. From this multivariate set of sector activity data, we built a regularized multiple regression model that can explain the dynamics of the sector activities as well as the immunity level triggered by the MAMP treatments[33]. Predictive power was assessed both by cross-validation and with additional experimental data. We found that a network model invariant for different MAMPs had a predictive power as good as or better than models with MAMP-specific structures. Thus, we concluded that changes to the network structure are negligible during PTI triggered by the different MAMPs. Moreover, our modeling demonstrated that the contributions to PTI from each sector and their interactions were caused by different MAMP-specific signal input patterns entering the four-sector network. This mechanism explains the tunability of the network output: the network transduces different input patterns into different response patterns. Our model also provided mechanistic insight regarding network robustness against internal perturbations: among all network features, ET inhibition of the JA sector was central to robustness against internal perturbations. Thus, this model, built on

semi-dynamic profiling of a step-wise reconstituted network revealed the mechanistic basis of the network's robustness and tunability[33].

Furthermore, this model revealed unexpected mechanistic relationships among the four sectors. Notably, we found the JA sector was required for activation of the SA sector in the absence of the PAD4 sector. Moreover, with regard to SA sector activation, the JA sector and the PAD4 sectors compensate for each other (i.e. at least one of them negatively influences the SA activating function of the other)[33]. Numerous prior observations exist that removal of the JA sector led to higher SA sector activation, compared to the wild-type genotype (e.g., [35-38]). This has been interpreted as an SA sector inhibition by the JA sector. However, we found that this accepted mechanistic interpretation is probably incorrect. A more likely mechanism is: removing the JA sector removes the negative interaction between the JA and PAD4 sectors on SA sector activation. This is a good example to demonstrate that in a network with significant convergence points, phenotype comparison of a shallowly perturbed network state, such as a single mutant, to the intact network, can be misleading. Deeper perturbations are needed to elucidate the network's signaling mechanisms.

We also used cross-validation to demonstrate substantial type II prediction power in our semi-dynamic modeling scheme. A model obtained by fitting the same network model structure to the data from only two MAMPs and the mock data predicted well the data from the remaining MAMP treatment. The signal flows in the network were very similar for elf18 and chitosan, whereas flg22 signal flows were very different from elf18 or chitosan. That is, the flg22 data realm was clearly distinguishable from the realm of the elf18 and chitosan data. Therefore, it is particularly remarkable that the model fitted to the elf18, chitosan, and mock data was able to predict the flg22 data well[33]. The model was able to make decent type II predictions, which suggests that the model captured the correct underlying mechanisms.

Learning dynamic rules of the network

Our dynamic network model explained how signal flows in the network produced salient properties of the network, such as robustness and tunability. However, the model can be improved. A model that employs time-independent rules is more likely to fully explain fundamental mechanisms. Our semi-dynamic model applied time-dependent rules at the nodes: nodes connected at different timepoints were allowed different coefficients[33]. In addition, an ideal model would also capture nonlinearity in the network produced at signal convergence points. Limited by the statistical power in our data, we assumed a linear model structure. The next goal is to discover this more ideal model. Data for this next modeling iteration should be collected along detailed timecourses that span the complex network response in addition to varying data parameters included in the former data. Once such a model with time-independent rules is built, the network responses, including the network output, can be computed when the input patterns along the timecourse are specified, i.e., the model will be self-sufficient. The ability of the model to make type II predictions can be broadly tested: the model will be used to predict the network response to input ranges and patterns very different from those used in model fitting.

Concluding remarks

We have laid out a roadmap to building *in silico* network models that can make decent type II predictions. The keys are: a summarized network, deep and combinatorial perturbations, measurements of the activity of each component of the summarized network, time-varying inputs, and time-series measurements of each sector's output.

Deep perturbation is not a practical strategy in every biological network. It would be difficult to apply this strategy to biological networks that control biological processes essential for the organism of interest. Furthermore, the importance of the deep perturbation strategy decreases if the core of the network has tree-like network structure and properties. As discussed, the properties of the plant immune signaling network are expected to be more complex than non-

immune plant signaling networks. The key in modeling signaling networks is to identify the salient biological properties of the network of interest and then to generate data with the power to enable a modeling endeavor that elucidates the mechanisms driving those network properties.

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Figure legends

Figure 1. Predictions in data-rich and data-poor realms (a conceptual diagram). Black curve shows the true mean of the input-output relationship. Blue points are data points. If a model based on an arbitrary function (here, $y = ax^2$) is fit (red dashed curve) to the data, this model yields good predictions within the data-rich realm: the red dashed curve closely follows the black curve. In the data-poor realm, however, the red dashed curve strongly deviates from the black curve, indicating loss of predictive power. If an overly complex arbitrary function is used as a model (green curve), predictive power is lost even within the data-rich realm; this is called overfitting. If we have some mechanistic information about the input-output relationship, such as that the relationships must be sigmoidal, we can obtain a model that can make reasonable predictions in both data-rich and data-poor realms, here by fitting a sigmoidal curve to the data (orange dashed curve).

Figure 2. The predictive power of a fully detailed mechanistic model collapses when the modeler's knowledge slightly decreases from perfect (a conceptual diagram). Red curve, a full mechanistic model; blue curve, an ideal modeling method. We would like our ability to predict system behavior to grow well, when supplied with system knowledge. That is, we want to glean system insight from necessarily incomplete information.

Figure 3. Network properties vary between tree-like and web-like signaling networks (conceptual diagrams). a. A tree-like network. b. In a tree-like network, removal of a node leads predictably to loss of any signals downstream from the perturbed node. c. A web-like network. The network from (a) now includes a single edge that introduces a signal converging point. d. When a node upstream from the signal convergence node is removed, the output of the convergence node cannot be predicted from the network structure alone. If the signal convergence rule chooses the maximum of the two inputs, the output O3 does not become 0, as it does in the tree-like network in (b). Black circles indicate nodes. Blue arrows indicate signal flows between nodes, and their width represents the strength of signal flow. The color intensity of the output nodes O1-O4 shows the output amount. The red 'no' symbol indicates node removal. Red dashed arrows indicate signal flows lost by the node removal. e. A network summarized from (a). f. A network summarized from (c).

Fig 1

Figure 2, a half column width
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Figure 3, a half column width
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