Published in final edited form as:

Clin Pharmacol Ther. 2013 December ; 94(6): 651–658. doi:10.1038/clpt.2013.176.

Network-Based Approaches in Drug Discovery and Early Development

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Abstract

Identification of novel targets is a critical first step in the drug discovery and development process. Most diseases such as cancer, metabolic disorders, and neurological disorders are complex, and their pathogenesis involves multiple genetic and environmental factors. Finding a viable drug target–drug combination with high potential for yielding clinical success within the efficacy–toxicity spectrum is extremely challenging. Many examples are now available in which network-based approaches show potential for the identification of novel targets and for the repositioning of established targets. The objective of this article is to highlight network approaches for identifying novel targets with greater chances of gaining approved drugs with maximal efficacy and minimal side effects. Further enhancement of these approaches may emerge from effectively integrating computational systems biology with pharmacodynamic systems analysis. Coupling genomics, proteomics, and metabolomics databases with systems pharmacology modeling may aid in the development of disease-specific networks that can be further used to build confidence in target identification.

Target identification is a critical first step in drug discovery and development given that substantial resource investments are needed to enable the search for lead compounds, structure optimization, and subsequent clinical development of candidates capable of modulating the selected drug target(s). The cost of false positives in particular can be very high, especially if they meet efficacy expectations but fail owing to toxicity late in clinical development. Many drug candidates fail during phase II and phase III trials, largely due to unexpected toxicity and/or lack of efficacy.¹ Unfavorable pharmacokinetic (PK) properties responsible for inadequate or suboptimal systemic drug exposure, such as poor bioavailability and rapid clearance, are now seldom problematic. However, the breakdown of ensuring drug efficacy might often be attributable to the reductionist view that there is a specific genetic or protein target that can be modulated to ameliorate the disease state.² Similarly, adverse effects can result from an inability to predict interactions with off-targets and downstream processes and tissues. Clearly, new approaches are needed to identify single and sets of biologically plausible targets within the context of systemic pharmacodynamic properties.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

Significant insights and improved understanding of drug action have been realized from recognizing that both drugs and pathophysiological processes give rise to complex and dynamic clinical phenotypes by altering natural interconnected biochemical networks.³ Examples are becoming available in which network-based approaches are used not only to identify novel targets but also to reposition targets to overcome resistance to current therapy or improve treatment outcomes by using multitargeted drugs or combination regimens.^{4–7} Csermely *et al.*⁸ have published a comprehensive review of the development and application of molecular networks in drug discovery. They provide an in-depth overview of the types of networks, available computational tools, and basic strategies for network-based drug targeting. The major types of networks are listed in Table 1, and most applications fall within the horizontal slices shown in Figure 1, which represent networks at different spatiotemporal scales of biological organization. Each scale can be characterized based on the currently available and relevant biochemical and physiological knowledge, which by nature can be quantitative (e.g., material flows and binding affinity) or qualitative (e.g., trends in gene and protein expression or activities). Multiscale models are needed to identify critical interscale relationships for understanding and predicting vertical information flow and ultimately linking drug interactions at the molecular level to therapeutic responses and adverse events.

At the conceptual level, a systems-based approach can be applied to identify targets for any given disease; however, there are specific pathologies for which this methodology will likely be more useful. Drug targeting for diseases that involve extensive interactions between multiple systems and those with complex etiology will most likely benefit from a network-based approach. Appreciation of the underlying pathophysiological mechanisms may influence the approach to identifying druggable targets. Csermely *et al.*⁸ propose the so-called “central hit” strategy for targeting diseases characterized by flexible networks (e.g., cancer), whereas more rigid systems (e.g., type 2 diabetes mellitus) may need a “network influence” approach. For cancer, the targeting of critical network nodes would seek to disrupt the network and induce cell death in malignant tissues, whereas such an approach for metabolic disorders would likely generate adverse effects or drug toxicity. By contrast, the network influence strategy seeks to identify nodes and edges of multitissue biochemical pathways for blocking specific lines of communication and essentially redirecting information flow.⁹

The purpose of this review is to highlight approaches for evaluating drug and pharmacophore effects on regulatory networks, for modeling the interactions between such networks, and for analyzing the connectivity as a means to rationally identify and prioritize drug targets. A particular focus will be on signal transduction. Quantitative systems pharmacology is an emerging discipline that aims to integrate methods of systems biology and physiologically based PK/pharmacodynamic concepts to advance small-molecule and macromolecule drug discovery, development, and therapeutics.¹⁰ We and others contend that systems pharmacology may have considerable potential in addressing aspects of the multiscale modeling challenge in understanding drug action and adding confidence in target selection.^{11,12} Coupling network and PK/pharmacodynamic systems-based approaches provides a mathematical formalism for exploring the dynamics and influence of

interconnected elements and motifs. This may eventually improve the specificity of target selection, predict or help mitigate off-target effects, and achieve precision medicine through an enhanced understanding of interindividual patient variability.

HORIZONTAL FLOW OF INFORMATION AND MATERIAL

The layers in Figure 1 each represent one or more systems existing within a specific temporal and spatial scale. To characterize the interactions at each level, some proxy must be developed that can incorporate available qualitative and quantitative information. Mathematical models may serve as surrogates for physical systems, and at the very least, should be descriptive of experimental data. The ultimate goal is to develop a model that is capable of predicting how a system will behave under conditions different from those in which the model was developed. Model performance and the ability of a model to generalize and predict future events are directly influenced by the level and quality of the data incorporated from experimental systems.¹³ In the case of drug target identification, this implies a decrease in confidence for predicted responses when a model is further removed from directly accounting for the underlying relevant physiology and pharmacology.

The schematic shown in Figure 2a depicts a hypothetical system in which the upregulation of some state A leads to the eventual upregulation of downstream state D. The states B and C represent intermediate steps involved in this process. The states can represent any given biological event, such as cellular signal transduction,¹⁴ paracrine and autocrine signaling in a microenvironment,¹⁵ the transfer of information between tissues and organ systems within the body,¹⁶ and many others. Although this is a small theoretical network, most relevant pharmacological models can be regarded similarly as “directed graphs” in which the arrows indicate reactions, pathways, or influences. For example, physiologically based PK models¹⁷ can be considered directed graphs at the organ level, in which nodes are tissue spaces and arrows (or edges) reflect blood flow or drug clearance processes. When the elements of Figure 2a are continuous processes, and rich data are available, the trafficking of materials and information can be characterized by a set of coupled ordinary differential equations. Disease state, environmental covariates, and other interindividual covariates can be incorporated into the model via appropriate modifications to the model structure and/or parameters of the system. Such models often require large amounts of data derived from tightly controlled experiments that seek to elucidate dynamic relationships among individual elements. Perturbations in the system, such as specific chemical probes, genetically altered cells or animals, and small interfering RNA inhibition, are typically used to identify the connectivity of underlying networks and properties of the cellular microenvironment. By characterizing each process, or group of processes, predictions can be generated for responses to drugs or other perturbations (Figure 2b); however, achieving the level of detail required to develop a dynamical ordinary differential equation model can be expensive and time consuming.

Although rich dynamical models may be desirable, their structures cannot be readily extended to integrate and explicitly leverage less quantitative data characteristic of high-throughput molecular biology platforms (e.g., metabolic fluxes and cell signaling). Various network models, such as gene regulatory networks, have been developed to represent this

type of information and characterize processes ranging from transcription and activation of proteins to movement of materials.¹⁸ Gene regulatory networks can be identified from information derived from high-throughput experimentation such as the use of microarrays via Bayesian inference.^{19,20} Model structures can then be further refined through simulations tested by direct experimentation. A simple representation of how these networks might cascade and interact is shown in Figure 2c, with drug interacting with cell membrane receptors or targets, squares representing genomic information, triangles depicting components of the interactome (molecular interactions within the cell),²¹ and circles identifying the elements of metabolic flux networks.²²

Many network models are static representations of a system, in which the nodes and edges are fixed or time invariant. In addition, changes in a particular state might be considered quantal (i.e., all-or-none) in nature (Figure 2d, with changes in state D directly reflecting a change in state A). Although this approach may be adequate for the simple system shown in Figure 2a, most pharmacological systems are more complicated in terms of the number of states, redundancy through feedback and feedforward loops, and intrinsic network robustness. Therefore, integrating more components reflective of the overall system, even though qualitative in nature, may be required for purposes of target identification. Discrete dynamic modeling is one approach that simultaneously leverages the dynamic nature of continuous ordinary differential equation–based models and the static connectivity information that is generated through the development of different network structures.^{23,24} The connectivity of a network model is enumerated through a set of Boolean relationships, with one state equation for each node in the network—the value of the node at any given time step denoted by “*” in Figure 2e. The system is initialized at some known state, where the values of the nodes are set to “true” (=1) if they are in their active state or “false” (=0) if they are in their inactive state. The impact of modulating one or more nodes from their nominal value(s) (e.g., fixing the right-hand side of the update equations) can then be elucidated by stepping forward in time and updating the nodes according to the Boolean relationships.

The update order in discrete dynamic modeling, whether nodes should be updated with regard to the previous or current state of the inputs (synchronous vs. asynchronous), needs assessment in each situation. Adopting a rule whereby nodes are always updated with regard to the input states at the previous time step (e.g., the value of D in the current time step would be determined by the values of B and C at the previous time step) results in a deterministic system. The relative dynamics of the nodes can be explored by simulating a Boolean system forward in time repeatedly, allowing the update order to be randomly selected, and averaging the results at each time step.²⁵ For our simple model in Figure 2a, the value of B at a given time step may be determined by A at the previous or current time step, depending on the randomly selected update order. The time is discrete and dimensionless, but simulations can sometimes be calibrated to experimental data to establish temporal inferences. For example, assuming that a change in node A results in an upregulation of D at a later time (Figure 2a), then the time point in the discrete model at which this change occurs can be potentially mapped to an experimentally determined time (dashed line in Figure 2e).²⁵ The conditions under which temporal bridging between discrete dynamic modeling and continuous systems is appropriate need study for each system.

DATA RESOURCES

The construction of mechanism-based network models requires detailed information regarding biochemical and physiological processes for the relevant species and pathological conditions.^{26,27} Although links between regulatory networks and other system information may be available in the literature, collecting these data, ascertaining the reliability of any given connection in the network, and assembling them into a form amenable for analysis can be a daunting task.²⁸ Extensive online databases and network analysis programs are available to facilitate the integration of large amounts of biological data (see Tables 1–9 in Csermely *et al.*⁸ for a thorough listing). For example, the Kyoto Encyclopedia of Genes and Genomes provides a repository of information and tools to enable pathway analyses based on genomic studies.^{28,29} The Kyoto Encyclopedia of Genes and Genomes databases include metabolic and signal transduction information for several species. In addition, the MetaCyc databases offer comparable functionality in terms of reference pathways found in the Kyoto Encyclopedia of Genes and Genomes, and the BioCyc databases provide species-specific information.³⁰ Both MetaCyc and BioCyc exclude proprietary information and attribute the pathway information provided to sources. Other databases include the University of Minnesota Biocatalysis/Biodegradation Database³¹ and the BRAunschweigENzyme Database.³² Although separate databases can be useful in and of themselves, their utility is substantially enhanced by comparing and integrating their information. BioWarehouse is a tool that pulls data from multiple sources and places them into either an Oracle or a MySQL database that can then be queried.³³ Pathway Tools is a popular software that facilitates data and pathway visualization and making computational inferences (e.g., identifying possible metabolic pathways).³⁴ It is also possible to access the Pathway/Genome Databases generated by Pathway Tools directly through available programming interfaces (Lisp, Java, or Perl) and in exported form using any language capable of parsing text files.

The ability to identify drug targets from complex pharmacological networks would be enhanced significantly by further combining complementary data sets across analytical platforms. For example, folding in high-throughput data for chemical structure and activity would facilitate identification of pharmacologically relevant compounds relative to identified targets.³⁵ Because many drug-candidate failures result from the inability to anticipate toxic effects, a database of adverse drug response information could be incorporated to help predict off-target or downstream effects.^{36,37}

NETWORK ANALYSIS METHODOLOGIES

Modeling techniques can leverage experimental data, network structures, and kinetic parameters in order to predict system behavior under new conditions. For drug target identification, this can involve assuming drug modulation or elimination of a network node but more likely involves changing a combination of nodes or edges^{2,38–41} to achieve some pharmacological response. Metrics are needed that integrate these details simultaneously to identify optimal network perturbations with a high likelihood of eliciting the desired therapeutic response and limiting adverse effects. Graph theory provides a means of analysis that ranks the importance or centrality of nodes within a network.⁴² The importance of a node is highly dependent on how the overall network is structured relative to the studied

outcomes. A node within a network might seem important in a particular region but have little impact on overall outcome owing to nonobvious system redundancies. Therefore, different types of centrality should be considered in order to classify the relative importance of nodes and edges.^{43–45}

The simplest measure of importance, degree centrality, measures the net sum of total node connections. This is a tempting metric because it is straightforward in terms of calculation and easy to understand: nodes with many connections, or hubs, are likely to be important. However, degree centrality is a localized measure of importance, and it ignores the influence of the larger network structure.⁴⁶ Broader measures of nodal or edge importance are available to better reflect their role in signal transduction from an overall network perspective. Biochemical and genomic networks are influenced by natural selection, suggesting that signal transduction through such networks should be relatively efficient.⁴⁷ A propagating signal will likely take the shortest path through a network,⁴⁸ and two metrics that consider the increased likelihood of signals taking the shortest path through a network are closeness centrality and betweenness centrality.⁴³ As the name implies, closeness centrality is a measure of how close a node is to other nodes, thereby expanding the local measure of degree centrality to a more regional level of importance by assuming that the closer a node is to other nodes, the more likely it is to be included in shortest paths. Betweenness centrality ranks the number of shortest paths that pass through each node of the network. The more shortest paths that any given node is involved in is therefore used as an indicator of importance.⁴³

Betweenness centrality is the metric most likely to identify important nodes for random networks. However, evolutionary biological processes produce complex systems with semi-autonomous modular units.⁴⁹ Therefore, metrics that incorporate the local importance of degree centrality, the regional significance of closeness centrality, and the global importance of betweenness centrality may provide improved measures of importance. The metric bridging centrality attempts to identify nodes within a network that act as bridges between modular subnetworks and the global network.⁵⁰ Nodes with high bridging centrality can more effectively disrupt information flow within a network with lesser loss of structural integrity.

Many techniques for optimizing networks originate from the field of operations research. Fundamentally, these methods attempt to maximize or minimize some measure of efficiency by manipulating aspects of the system in a constrained fashion. Determining the shortest path between two nodes in a network is an example of this type of optimization, with the objective of minimizing the steps from the first node to the last while adhering to the connectivity constraints of the network.⁵¹ Another more application-oriented example is the use of optimization theory to identify connectivity and redundancy in metabolic networks.⁵² A central theme is to consider the model equations of the system as just another set of constraints on the system. In addition, constraints can be incorporated into the cost function being optimized. This converts an absolute or hard constraint into a soft constraint. In physiological terms, although some level of side effects might be tolerable, cost functions can be modified to penalize such side effects. In type 2 diabetes, for example, the objective function might reflect efficacy biomarkers, such as glycosylated hemoglobin A1c

concentrations, as well as those associated with adverse cardiac events. Constraints could then be added to represent the underlying genomic, interactomic, and metabolic networks; specify the number of targets to identify; and include any hard constraints that should not be violated.^{52,53} The main challenge is the large degrees of freedom within the system. However, constrained optimization techniques can actually produce better results with more constraints because each constraint can limit the search space and consequently the number of possible solutions.

CHALLENGES AND OPPORTUNITIES

In addition to achieving sufficient drug exposure at the site of action, a full understanding of the biological network in which the drug target exists is needed to fully exploit network-based methods for drug discovery. Cellular signaling networks are the result of an evolutionary process that favors redundancy and robustness in response to environmental challenges;⁴⁷ such networks are limited by whether critical nodes are included in the network and the quality of the data. Another concern is that most network models contain well-defined edges, and important temporal changes in nodal connectivity can be ignored. Finally, vertical integration and multiscale modeling in the presence of multiplatform data continue to be major hurdles in systems pharmacology, and improvements are needed to add confidence to targets selected from network-based methods.

Disease progression

Pathophysiological processes are not time invariant; pharmacological effects may change in time, and optimal windows of opportunity to effectively treat disease can open and close with such progression. The heterogeneity and dynamic behavior of most cancers are good examples of such treatment challenges.^{54,55} Imatinib, a tyrosine kinase inhibitor used to treat patients with chronic myelogenous leukemia, acts by binding to the tyrosine kinase domain of bcl-abl and inactivating transcription.⁵⁶ However, a single point mutation can permanently activate this domain and render imatinib ineffective.⁵⁷ This mutation can be detected by assay, but such direct measures are not always available. Network analysis may facilitate assessment of the impact of specific mutations on drug efficacy. In particular, modeling methods that consider the temporal evolution of network structure⁵⁸ could be used to evaluate network connectivity, the likelihood of signals to traverse different branches of a network, and the variations in connectivity caused by disease progression and pharmacological intervention. Drawing parallels from resistors in electrical networks to linking nodes in biological systems might also broaden the definition of such links and influence model performance.⁴⁶

Biological system robustness

Biological systems are complex and frequently involve compensatory and feedback and feedforward mechanisms. The need to understand the impact of redundancy and robustness on drug targets is perhaps exemplified in the development of antimicrobial agents. For example, many of the enzymes thought to be critical for the proliferation and pathogenesis of *Salmonella enterica* were found to be nonessential.⁵⁹ This was attributed to the

robustness of the cellular network and the interaction between host and pathogens. The resilient nature of interconnected systems can be difficult to predict.⁶⁰

Polytropic nature of biological systems

The interrelationships among complex physiological control systems can further complicate the direct translation of pharmacological targets. The relatively rigid nature of metabolic networks resulting in type 2 diabetes mellitus is a classic example. Thiazolidinediones activate peroxisome proliferator-activated receptors, which increases insulin sensitivity and normalizes glucose uptake (measured by a decrease in hemoglobin A1c);⁶¹ however, control mechanisms associated with glucose regulation are also implicated or closely associated with other physiological systems such as regulation of bone mineral density^{62,63} and cardiovascular hemodynamics.^{64,65} Therefore, the glucose-lowering effects of compounds such as rosiglitazone need to be balanced against a potential increase in the risk of heart failure.^{66,67} Such examples directly support a “network influence” targeting concept.⁸ The multitissue, genome-scale metabolic network of Bordbar *et al.*⁶⁸ is a promising modeling approach to this problem. Two metabolic cycles (the alanine and Cori cycles) and the absorptive state of metabolism were integrated into tissue-specific spaces (hepatocytes, myocytes, and adipocytes) and linked through the blood. The model predicted differentially expressed reactions between obese and diabetic obese subjects that were not obvious from transcription data and that could also be used to explain macroscopic observations such as increased free fatty acid and lactate concentrations, increased oxidative tissue damage, and decreased taurine concentrations in diabetic subjects.

Interpatient variability

The impact and analysis of biological networks are further complicated by patient variability. A combination of genetic, epigenetic, and environmental factors and disease status can contribute to variability at any spatiotemporal scale, from PK to gene and protein expression levels and activity, and inclusion of this information is important when identifying targets. As an example, interpatient variability was recently used to guide the study of the serotonin reuptake inhibitor citalopram for the treatment of depression.⁶⁹ In an effort to identify genetic factors associated with sensitivity to treatment and indicators of remittance, logistic regression was performed using these factors against single-nucleotide polymorphisms. This analysis identified potential pathways to investigate and better understand the clinical response to citalopram. However, the genomic information helped explain a particular phenomenon rather than predict response (the objective of target identification).

Vertical integration of spatiotemporal scales

Modeling the effective communication between the horizontal systems shown in Figure 1 poses a formidable collective challenge in developing a quantitative systems pharmacology framework. Multiscale and multiplatform modeling will require further pooling of available data and increasing accessibility, combined with the development and sharing of tools and models to comprehensively analyze this information. In the following perspective, we suggest that as disease-specific networks and drug-targeting algorithms continue to evolve,

quantitative systems pharmacology modeling can play a major role in vertically integrating critical nodes and motifs and streamlining the assessment of potential drug targets in early discovery.

PERSPECTIVE

A conceptualization of the drug-target identification problem is shown in Figure 3. Collated biological data (e.g., genomic, proteomic, and metabolomic data) represent a substantial canon of knowledge, even though it may be underdeveloped at any point in time. Exploratory computational platforms will need to be flexible enough to leverage prior information and incorporate new relationships as they are revealed. Disease-specific networks over several scales (genomic, interactomic, and metabolic) could be used to establish an individual canonical network or to sample from the overall species, potentially focusing the scope of the analysis.⁷⁰ After accounting for natural and pathological progression, tissue-specific subnetworks and constituent processes can be constructed. In Figure 3, the target is identified in specific tissues (the liver in this example) along with a marker of the phenotypic response to treatment. Portions of the network in the target tissue are also present in a companion tissue (in this instance, the kidney). Network analysis of the connectivity in the target tissues can be used to suggest possible targets, biomarkers, and local undesired effects. Finally, with the incorporation of chemoinformatics (e.g., chemical structure and/or activity assay data), it may be possible to prospectively narrow the selection of lead chemical structures and identify off-target downstream effects.

Of course, this is an idealized approach to drug target identification, which will require substantial efforts and innovations to realize. The initial suppositions include the existence of meaningful measurements of efficacy and toxicity, as well as underlying knowledge of the networks that exist and function in the applicable tissues. Of note, systems pharmacology models should be developed to generate hypotheses to be followed up with iterative *in vivo* and *in vitro* testing. Ideally, multiscale models should connect the time course of drug exposure at sites of action, drug-target interactions, pharmacological signal transduction, and any relevant macroscale physiological control systems. In due course a well-constrained network will arise that is more amenable to analysis through constrained optimization. Examples are emerging in which small systems models effectively link PK, drug-binding, signal transduction, and phenotypic effects to explore mechanisms of drug action and combination regimens in cancer systems.^{71,72} Methods based on principles of graph theory have been advanced to identify the systemic importance of nodes within networks, potentially providing guidance for selecting essential components of such multiscale models.⁷³ The eventual clinical challenge will be to develop so-called enhanced pharmacodynamic models that essentially integrate mechanistic biochemical models with genomic/proteomic information, which could serve as a basis for further target validation and precision medicine.⁷⁴

CONCLUSIONS

Toxicity and lack of efficacy are key determinants of late-stage failures in drug development. The biological basis for these failures is largely a result of inadequate

understanding of the interconnectedness of biological systems active at multiple scales of organization. Although it is possible to achieve accurate measurements of the intensity and time course of physiological responses at the macroscopic level, much of the data that are being generated to resolve the foundational basis for these macroscopic responses are largely static, qualitative, or semiquantitative. Network-based analysis of curated experimental data is yielding new insights into mechanisms of biological systems and potential drug target(s). Targets may be further qualified in the preclinical phase using multiscale and translational systems pharmacology models, which may ultimately provide a quantitative framework for proposing novel drug combinations, projecting interindividual variability, and achieving individualized pharmacotherapy. New innovations in experimental and computational systems are needed to realize the ideal of predicting the therapeutic and adverse effects of new chemical entities from first principles.

Acknowledgments

This work was funded, in part, by National Institutes of Health grant 59780 (to D.E.M.) and the University at Buffalo Center for Protein Therapeutics. Support from the National Multiple Sclerosis Society (grants RG3743 and RG4836-A-5) and the Department of Defense Multiple Sclerosis Program (grant MS090122) to the Ramanathan laboratory is gratefully acknowledged. The authors thank Prof William J. Jusko and Vaishali Chudasama (University at Buffalo, State University of New York) for their assistance in preparing the manuscript.

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Figure 1.

Horizontal and vertical integration. The complexities faced in target identification result from the interactions of spatiotemporal scales, and the vertical integration of these layers is the key to identifying drug targets that can overcome the current shortcomings associated with unpredicted toxicities and failures due to lack of efficacy.

Figure 2.

Discrete and continuous dynamic modeling of directed graphs. **(a)** Illustration of predicted output for different modeling methodologies for a theoretical network with a given connectivity. **(b)** Changes in output D in response to upregulation of A for a continuous system. **(c)** Layers of information networks and their interactions constitute the challenges of drug-target identification. Squares represent genomic information characterized by gene regulatory networks, and triangles represent proteins, which constitute the bulk of the interactomic network. Ultimately, these systems give rise to changes in metabolic flux, represented by circles. As an example of signal transduction, **(d)** the quantal change in state A results in downstream signaling and upregulation of state D, **(e)** which can be recapitulated with a discrete dynamic model (DDM). The linkage between time steps of DDMs and continuous time to event in hypothetical continuous systems is shown by the shaded region linked by a dashed line. The Boolean network for the model in panel **a** is also listed in panel **e**.

Figure 3.

The canonical network topology of the species represents all of the possible connections (e.g., genomic, proteomic, and metabolic) that are possible within that species. Individual patients or groups of patients can be represented as a sample from the species canonical network to produce the individual canonical network—all of the possible connections within this sample. Through differentiation, aging in the patient, and disease progression, components of the individual canonical network will be active in different tissues within the body.

Table 1

Major types of molecular networks

Chemical structure and reaction networks
Protein structure networks
Protein–protein interaction networks
Signal transduction networks
Genetic interaction networks
Metabolic networks

Based on Csermely *et al.*⁸

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