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Biochemical network-based drug-target prediction

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The use of networks to aid the drug discovery process is a rather new but booming endeavor. A vast variety of different types of networks are being constructed and analyzed for various different tasks in drug discovery. The analysis may be at the level of establishing connectivity, topology, and graphs, or may go to a more quantitative level. We discuss here how computational systems biology approaches can aid the quantitative analysis of biochemical networks for drug-target prediction. We focus on networks and pathways in which the components are related by physical interactions or biochemical processes. We particularly discuss the potential of mathematical modeling to aid the analysis of proteins for druggability.

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Introduction

Systems biology has changed the paradigm for drug-target identification from considering the roles of individual genes or proteins in diseases to studying the structure, properties, and behavior of various types of biochemical networks and their changes in perturbed states such as cancer or apoptosis. This new approach opens the way to predicting the effects of targeting distinct genes or proteins not only based on their individual properties, for example, binding constants and binding specificity profiles, but also based on their position and function in one or more networks. Such networks may comprise disease-relevant compounds or may cover all similar components of the cell such as all proteins or all genes, or may be heterogeneous as regards compound/molecule type. New experimental technologies, data analysis strategies, and modeling approaches are being developed for the prediction of medically relevant properties of these networks, most notably useful and effective drug targets. Here, we will focus on computational approaches to the prediction of drug targets using network information (Figure 1). We first give a brief overview of some of the key advances in using network analysis for drug-target identification. We then discuss the application of 'classical' systems biology mathematical modeling approaches to study signaling and metabolic networks for drug-target prediction. These approaches to the computational prediction of drug targets are increasingly supported by domain-specific computational tools (see Box 1) and databases (see Box 2).

Target prediction using homogenous and heterogeneous protein interaction networks

Recent experimental advances in high-throughput proteomics have led to a wealth of data on protein–protein interactions. Despite the many inconsistencies in these data [16], there is great interest in mining it for drug-target identification. Analyses show that known human drug targets tend to occur at middle-degree to low-degree nodes, that is, less connected nodes [17•]. Targeting these can be expected to result in drugs with fewer side effects (and greater synergetic efficacy in a drug made of several compounds). Thus, ranking of proteins by the topological properties of the human protein–protein interaction network is one strategy for drug-target identification [18]. Another approach is to characterize the interaction properties in protein–protein complexes, for example, by identifying the domains involved in binding or by analyzing the 3D structure. Comparison of domain–domain interactions and interfaces across an interactome can guide the identification of selective drug targets or drugs targeting multiple proteins (to block parallel pathways in a network) [19]. Structural analysis can be carried out to identify pockets where drugs could bind and compare their properties with binding pockets on other proteins in the network [20].

Combining protein interaction data with other data in heterogeneous networks will provide a stronger basis for drug-target prediction. Yildirim *et al.* [21] built a bipartite graph linking drugs and proteins and identifying topological features, for example, hubs in this network are target proteins to which many drugs bind, and differences in the graph for etiological and palliative drugs. Mestres *et al.* [22•] combined data from 7 databases to assemble a drug-target network with 4767 unique interactions between 802 drugs and 480 targets (each drug has on average 6 targets).

Such drug-target networks will increasingly be complemented by and integrated with networks containing other biological and medical data (see review [23]), as well as

Figure 1

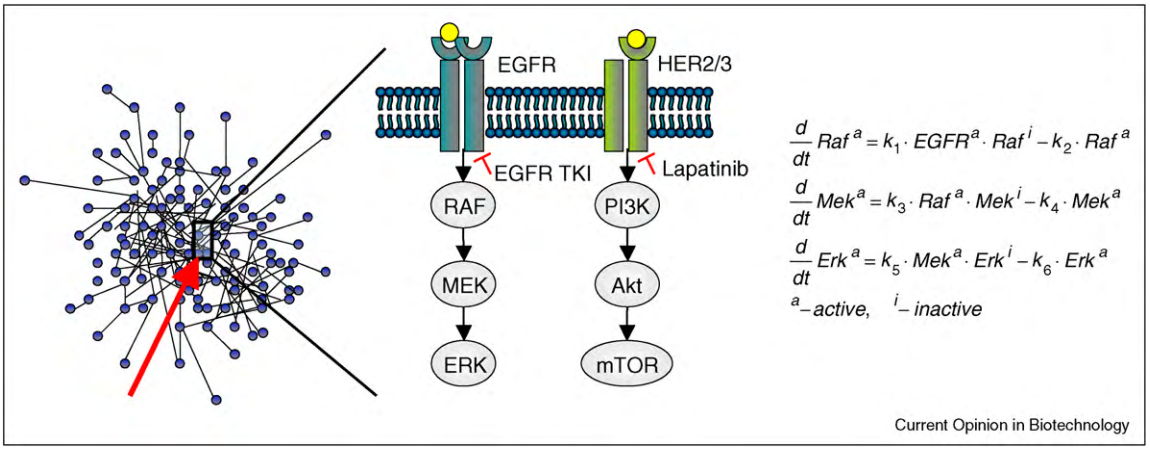

Illustration of different levels of computational network approaches. Protein–protein interaction networks (left) represent physical or other types of interactions of virtually all proteins in a cell and provide means to detect candidate targets, for example hubs. Signaling networks (middle) are specific types of protein networks involved in the transmission of information, for example, about external growth factors. Their dynamics can be described by differential equations (right), which can be used to compute suitable targets.

drug–drug networks. The latter can be built according to chemical similarity [22•,24] or from phenotypic side-effect similarities [25] and can help to identify new therapeutic applications for known drugs.

Pathway analysis is an approach to convert network data into models (and defining local networks) and its application in drug discovery is described, for example, in Yuryev [26].

Target prediction in signaling networks

Cells receive information about growth factors, nutrients, toxic compounds, and other external changes via so-called signaling pathways. The activated membrane-located receptor induces a cascade of protein interactions and modifications, which eventually not only regulates transcription factors and, hence, gene expression, but may also have side effects on cell cycle and metabolism. Within signaling pathways, information is not only linearly transmitted, but also processed through signal integration, cross-activation, positive, or negative feedback. Experimental and computational systems biology have intensively studied a number of human signaling pathways, among them the EGFR [27], Wnt [28•], Jak/STAT [29], TGFβ [30,31], and NFκB pathways [32].

The models are either formulated in a qualitative way describing how activity states of upstream compounds influence activities of downstream compounds, or as sets of ordinary differential equations (ODEs) describing the temporal evolution of the involved components and their activity. Models are usually formulated to solve a question that cannot be answered by gathering experimental data alone. They help to understand the network architecture and the observed dynamics and to rationalize the interdependence of separately measured data such as external stimulation, phosphorylation states, localization, protein interactions, and transcriptional activation, for example [33]. Signaling pathway models have been used to explain dynamic features such as the effect of positive or negative feedback, for example [34] or crosstalk between different pathways [35]. On top, the modeling provides a framework for hypothesis generation and for prediction of the effects of intervention.

Drug-target prediction by in silico systems biology needs sufficiently well described networks including the network structure and compound interaction properties. Then, mathematical models can be useful to predict targets for treatment and test the outcome of different target positions, treatment strengths, target combinations, or temporal combination scenarios [7,36]. Having a sound mathematical model at hand, one can also address a number of relevant questions through simulation, such as the effect of parameter variability (mimicking biological variability) on the prediction quality, the impact of parallel pathways bypassing the target, or saturation of the target.

With respect to successful application, this field is only in its early phase since there is still a lack of sufficiently well described networks. The major obstacle is – despite massive gathering of data in all omics – to obtain data of the right type, amount, coverage, and quality that covers the pathway dynamics and that is sufficient to determine the model parameters. For example, high-throughput techniques such as microarrays or deep sequencing are not of much help, comprehensive phosphoproteomic data are, if we want to understand the protein modification dynamics in the first minutes or hours after addition of growth factor.

Box 1 Computational tools for systems biology and target prediction

CellDesigner: http://www.celldesigner.org, [1]

CellDesigner is a structured diagram editor for drawing gene-regulatory and biochemical networks. Networks are drawn based on the process diagram, with graphical notation system proposed by Kitano, and are stored using the Systems Biology Markup Language (SBML), a standard for representing models of biochemical and gene-regulatory networks [2•].

COPASI: COmplex PAthway SImulator

http://www.copasi.org, [3]

COPASI is a software application for the simulation and analysis of biochemical networks and their dynamics. COPASI is a stand-alone program that supports models in the SBML standard format and can simulate their behavior using ODEs or Gillespie's stochastic simulation algorithm; arbitrary discrete events can be included in such simulations. The software carries out several analyses of the network and its dynamics and has extensive support for parameter estimation and optimization. COPASI provides the means to visualize data in customizable plots, histograms, and animations of network diagrams.

iPATH: Interactive Pathways Explorer

http://pathways.embl.de/index.html, [4]

Web-based tool for displaying and analyzing metabolic pathways. The pathways in specific organisms can be viewed and analyzed in the context of a global pathways map.

SABIO-RK: System for the Analysis of Biochemical Pathways - Reaction Kinetics

http://sabio.h-its.org, [5]

SABIO-RK is a web-based application employing the SABIO relational database that contains information about biochemical reactions, their kinetic equations with their parameters, and the experimental conditions under which these parameters were measured. It is able to export SBML format files of selected reactions sets together with kinetic information.

SYCAMORE: SYstems biology Computational Analysis and MOdeling Research Environment

http://sycamore.h-its.org, [6]

SYCAMORE is a browser-based application that facilitates construction, simulation, and analysis of kinetic models in systems biology projects. It provides tools for database supported modeling, basic model checking, and the estimation of unknown kinetic parameters based on protein structures. In addition, it offers some guidance to allow non-expert users to perform basic computational modeling tasks.

TIde: Target Identification

http://sysbio.molgen.mpg.de/tide, [7]

TIde is a tool for the automatic identification of optimal drug targets in kinetic models of biochemical networks based on ordinary differential equations (ODEs). Given a model in the standard Systems Biology Markup Language (SBML) format, it will identify promising drug targets for different effective modifier concentrations. Target combinations are also featured.

Box 2 Drug-target databases

Drugbank: http://www.drugbank.ca/, [8,9]

Includes manually annotated main mode of action of drugs for over 4000 drug-target pairs. Combines detailed drug data (e.g. chemical, pharmacological, pharmaceutical) with comprehensive target information (e.g. on sequence, structure, pathway). It can be searched by pathway.

Matador: Manually Annotated Targets and Drugs Online Resource

http://matador.embl.de, [10]

Includes direct and indirect modes of action of drugs with targets and can be queried with drug or target protein. The manually annotated list of direct (binding) and indirect interactions between proteins and chemicals was assembled by automated text-mining followed by manual curation.

Pdtd: Potential Drug Target Database

http://www.dddc.ac.cn/pdtd/, [11]

Database providing potential drug targets of known 3D structure annotated by therapeutic area and associated diseases. Structures are from the Protein Data Bank (PDB).

STITCH: Chemical-Protein Interactions

http://stitch.embl.de/, [12,13]

A resource to explore known and predicted interactions of chemicals and proteins. Chemicals are linked to other chemicals and proteins by evidence derived from experiments, databases and the literature. STITCH contains interactions for over 74,000 small molecules and over 2.5 million proteins in 630 organisms. Results are provided with an annotated graphical view of the protein–protein, protein–chemical and chemical–chemical interaction network, including display of known molecular 3D structures.

SuperTarget: http://bioinf-tomcat.charite.de/supertarget/, [10]

Allows searching of a database of over 7000 drug-target pairs with querying by drug, target, pathway, ontology, or cytochrome P450.

TDR: Targets Database

http://tdrtargets.org/, [14]

Identification and ranking of targets against neglected tropical diseases, part of a WHO project. Searches a genome to rank potential targets according to about 10 different criteria relevant to druggability. It is aimed at facilitating the identification and prioritization of drug targets in pathogens causing neglected diseases.

TTD: Therapeutic Targets Database

http://xin.cz3.nus.edu.sg/group/cjttd/ttd.asp, [15]

A database that provides information about known therapeutic protein and nucleic acid targets, the targeted disease, pathway information, and the corresponding drugs directed at each of these targets. It can be searched by giving the name of a biochemical pathway of interest and then will provide information on all known drug targets in the query pathway.

However, there are already very promising examples among signaling pathway models such as the study of the ErbB network with sensitivity analysis [37•], which identified ErbB3 as a key node in response to ligands. The relative effectiveness of eight ErbB ligands with respect to their ability to stimulate phosphorylation of certain downstream proteins was tested and for the most effective ligands the early signaling dynamics as function of time and ligand concentration were compared with respect to target phosphorylation. The efficacy was tested

in mice *in vivo*. Another example is Logical modeling, which assigns activation or inactivation properties to protein interactions, which has led to comprehensive description EGFR/ErbB signaling network enabling the prediction of the effects of various ligand stimulations [27].

Boolean modeling (assigning values of 1 or 0 to active or inactive protein, respectively, which are updated according to the states of their modifiers in temporal fashion) of the ErbB receptor regulated G1/S transition has revealed potential new and alternative targets in case of *de novo* trastuzumab resistance in breast cancer [38].

Chronobiology studies various rhythms followed by living beings, such as cell cycle, circadian, or annual rhythms. Cell cycle models can be used like signaling pathway models to predict drug effects, but also to predict the effect of, for example, apoptosis and DNA damage [39]. Also circadian rhythms are investigated, their components identified [40] and their dynamics described with mathematical models [41]. Chronotherapy applies the finding that cancer treatment has different effects if supplied at different times of the day, both shown experimentally and with mathematical models [42•,43].

Although a major aim of systems biology is to cover comprehensive networks there are limits to this concept when it comes to manageable, predictive dynamic models. Such a model must naturally have boundaries discriminating between processes considered relevant for the investigated problem and 'the rest' of the cell. Although whole cell models are envisaged for the future, they are currently not at hand owing to limited data availability and owing to lack of methods for combining all knowledge about cellular life into one approach. Consequently, effects that are out of the scope of that model cannot be predicted (e.g. a pure cell cycle model will not predict metabolic effects of a protein kinase inhibitor, even though they might exist).

Target prediction in metabolic networks

Metabolism, the breaking down and synthesis of compounds, has long been investigated by computational approaches. This is probably owing to the fact that very early on in biochemical research, biotechnological as well as pharmaceutical interests were pursued by trying to influence the metabolism of microorganisms and different eukaryotic cell types. In systems biology, these approaches have been intensified. The complexity of metabolic networks in living cells asks for computational models that support the analysis and understanding of the metabolic behavior of these cells. Different techniques are employed for this purpose and are also specifically used to aid drug-target identification:

In the absence of kinetic parameters, so-called stoichiometric network analyses can be used. These are either aiming at computing subsystems able to exhibit a steady state and thus represent a potentially viable system (e.g. extreme currents, elementary modes) or they use some objective function to compute the fluxes in order to achieve an optimal outcome with respect to these boundary conditions. The latter is called flux balance analysis and has been employed in drug-target identification [44].

With the knowledge of kinetic parameters and equations, more detailed and powerful kinetic models can be set up. As in the case of signaling systems, these are mostly formulated as ordinary differential equations and used to evaluate the temporal behavior of the system of interest. Examples are the model of Prostaglandin H Synthase-1 (COX-1) developed to predict inhibition effects of nonsteroidal anti-inflammatory drugs [45] or a model for bacterial methicillin-resistance [46].

Such models can be used to, for example, analyze which points in the metabolism of a pathogen are most vulnerable and thus, probably good drug targets. Thus, a specialized framework for the modeling of Mycobacterium tuberculosis [47] has this aim. A systematic strategy to scan for vulnerable points is the performance of a sensitivity analysis. In general, such an analysis investigates how strong the influence of specific parameters is on an observable, for example, how reaction rate of each enzyme in a pathway affects the steady state concentration of a metabolite. A specialized kind of sensitivity analysis is the so-called metabolic control analysis. This has been employed in investigating vulnerable points in Trypanosoma [48].

One problem for sensitivity analyses is the fact that these are local methods, which means the result will be different if several parameters in the system are changed. In the absence of complete knowledge about kinetic parameters, this calls for more global strategies. One such strategy, which can also be used for the purpose of drug-target identification, has been suggested recently [49]. It employs optimization strategies to find the global maximum or minimum of sensitivity in the complete parameter space.

All in all, as stated above, despite promising results, for example, for the development of drugs against certain parasites/pathogens, there are still not too many success stories of systems biology in drug-target identification in metabolism. Again, this is mostly owing to the fact that the necessary data for the development of quantitative models are often lacking and new methods have to be developed that deal with systems with a lot of unknown parameters.

Summary and outlook

Taken together, the combination of experimental and computational approaches in systems biology has already revealed a number of promising examples of rational and

network-based drug-target prediction. Further progress will be based on precise and reproducible data and mathematical descriptions to create predictive and helpful models. Increasingly, it will be possible to go beyond a static view of networks to consider their dynamics and aim for temporally structured drug administration to first change the sensitivity pattern in the network and then attack the most vulnerable points.

The necessity for a network-based approach to drug-target prediction is clear when one considers that many compounds are abandoned during drug development because they act on a target other than the intended target(s). Moreover, successful drug-target prediction necessitates considering not only individual cells but also effects in the human body. Therefore, future studies must include cell-type specific modeling and also integrate pharmacokinetics and pharmacodynamics. With the increasing speed of high-throughput analyses and rising computer power, it will become possible to integrate network-oriented research with specific, individualized information and thereby bring the dream of personalized medicine within reach [50].

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