Network-Based Strategies Can Help Mono- and Poly-pharmacology Drug Discovery: A Systems Biology View

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Abstract: The cellular network and its environment govern cell and organism behavior and are fundamental to the comprehension of function, misfunction and drug discovery. Over the last few years, drugs were observed to often bind to more than one target; thus, poly-pharmacology approaches can be advantageous, complementing the “one drug - one target” strategy. Targeting drug discovery from the systems biology standpoint can help in studies of network effects of mono- and poly-pharmacology. In this mini-review, we provide an overview of the usefulness of network description and tools for mono- and poly-pharmacology, and the ways through which protein interactions can help single- and multi-target drug discovery efforts. We further describe how, when combined with experimental data, modeled structural networks which can predict which proteins interact and provide the structures of their interfaces, can model the cellular pathways, and suggest which specific pathways are likely to be affected. Such structural networks may facilitate structure-based drug design; forecast side effects of drugs; and suggest how the effects of drug binding can propagate in multi-molecular complexes and pathways.

Keywords: Poly-pharmacology, network pharmacology, protein-protein interaction inhibitors, systems biology, protein-protein interfaces, modeling.

INTRODUCTION

Early drug discovery sought “magic bullets”; i.e., maximally selective molecules for specific targets related to specific diseases. About 90 percent of the drugs designed following this paradigm fail in the late and most costly stages of clinical trials [1]. Developing a new drug takes up to 15-20 years [2], which highlights the need to broaden the current strategies. Drug discovery is challenging due to structural and sequence similarity among members of the same protein family which typically lead to active site conservation, and thus possible drug promiscuity; system diseases, which may relate to gene associations, redundancy in biological networks, and cross-talks between pathways, may make it even more complex. The cause of many common diseases (such as cancer, cardiovascular diseases and mental disorders) is not complications related to a single anomaly, but rather is related to multiple molecular deviations [3]. Drugs designed with a “one-to-one” strategy may block the functions of their primary targets; however, at the same time they may lead to secondary reactions [4]. The increasing awareness of the high probability of such drug promiscuity has led to searches for new, enhanced strategies in drug discovery.

Target-centered drug discovery approaches are handicapped in foreseeing the outcome of drug regimen. The disturbance caused by a drug on an organism is not limited to only its targets; for instance, when human cancer cells were subject to drug treatment, the 1000 monitored (different) proteins in individual cells were all observed to be altered [5], illustrating the inherent difficulties in prediction of the outcome of drug-target interaction [6]. System-wide drug action may be better-understood using network analyses. There are approximately 25,000 genes in the human genome and 1,800 of them are known to be linked with various diseases, and this is likely to be an underestimation. The FDA approved drugs target around 400 gene products [7]. In the last decade, although the number of new

drugs increased, the number of drug targets did not rise as much. Marketed drugs generally interact with old targets [8]; thus, we would expect that more targets will be discovered as we delve into the human protein-protein interaction (PPI) network.

Below, we first provide an overview of poly-pharmacology followed by the cellular protein-protein interaction network, and its usefulness in drug discovery. We then describe how the modeling of the structural protein interaction networks, which predicts which proteins interact and how they interact, and uses this information together with experimental data to construct the cellular pathways can help drug discovery. Modeling the protein interactions and the structural pathways can help mono- and poly-pharmacology, an important area which currently attracts much interest.

POLY-PHARMACOLOGY

Poly-pharmacology searches for lead compounds that bind to multiple targets, and introduces a new concept of network pharmacology, which enlarges the ‘drugome’ [9]. It builds upon systems biology and drug discovery [10] aiming to treat diseases through multiple targets, which can be both a drug with several targets or a number of drugs with distinct targets. Poly-pharmacology describes and advocates consideration of a “many-to-many” relation between a ligand-protein couple, in contrast to a dominant “one drug-one target” drug design paradigm [11]. The novel computational approaches to poly-pharmacology have been reviewed by Xie *et al.* [12] recently.

PPI TARGETING DRUGS

Biological functions depend on signal transduction cascades [13]; and a large class of therapeutics target signaling [14]. Signaling typically takes place through PPIs. Blocking the information flow requires the design of (often) small molecules which inhibit these key interactions. A well-known example is heterotrimeric G-protein subunit; designing inhibitors for G-protein-effectors or receptors can modulate GPCR signaling pathway.

Design of drugs that disrupt PPIs is known to be notoriously difficult. This is for two reasons: protein-protein interfaces have a

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more flat surface when compared to enzymes and usually do not have grooves which can serve as binding pockets [15]. The pockets on protein-protein interfaces are typically smaller than those in protein-ligand interactions [16] and difficult to drug. However, now it is becoming increasingly possible to overcome these handicaps, and PPI inhibitors are gaining importance as a class of drug targets [17]. One of the most important findings was the fact that interface regions usually contain clusters of residues, which are key contributors to the binding energy. These smaller regions of the interaction surface constitute “hot spots” [18-20]. Different studies showed that small molecules target hotspots on the protein-protein interfaces [20, 21]. Hot spots on an interface may be predicted via HotPoint [22], HSPred [23], KFC [24], and additional servers. Moreover, currently allosteric drugs that bind elsewhere and lead to conformational changes in the interface appear increasingly feasible. Nonetheless, although designing drugs for disrupting protein-protein interactions has surged, some such drugs have long been in existence. Protease inhibitors are well-known marketed examples of this drug class [25]. The number of PPI targeting drugs is rising; examples include inhibitors targeting IL-2 [26], MDM2 [27, 28], BCL-2/BCL-XL[29], XIAP [30] and VLA-4 [30]. There are a number of reviews [13, 15, 25, 31-35], which investigate PPI inhibiting drugs, and these provide a more extensive list.

Pockets at the active sites of enzymes are typically stable, with high population times. Recently attempts to target PPI have also focused on detection and targeting of transient dynamic pockets, which may be stabilized upon drug binding. Such grooves can be found in PPIs [36] and elsewhere on protein surfaces and can serve as orthosteric and allosteric binding sites. Transient pockets occur often [37]; the question is their size and population time. Furthermore, the surface of interacting proteins is flexible and some disordered proteins can only be solved upon interaction with their partners [38]. Their flexibility implicates formation of transient pockets, which are very useful for inhibitor design [39]. A number of drugs have been reported to stick to these transient pockets on the surface of protein interfaces [40]. Metz *et al.* [41] proposed a tool that locates transient pockets of PPIs on the basis of geometry, and molecular dynamics simulation protocols are also being developed toward this aim. Further validation of the presence of at least small pockets comes from a computational analysis of crystal structures. This study observed that among 18 protein-protein complexes, 16 contain pre-existing pockets in their unbound structures [42].

A number of clinical therapies are based on humanized monoclonal antibodies which disrupt PPI. These therapies have high specificity and low toxicity; however, they also have some deficiencies such as lack of cell/blood-brain barrier permeability, and poor oral bioavailability. Thus, humanized monoclonal antibodies therapies may not be broadly applicable to PPI inhibitor design [13] in the near future.

The data on PPI inhibitors has been compiled in databases and literature. One of these, 2P2I [43], is a database which provides structural data for a collection of protein-protein interfaces with known inhibitors. TIMBAL [44] is also a database where one can find small molecules disrupting PPIs. Furthermore, Sali *et al.* spotted “bi-functional positions” of proteins (overlapping ligand and protein binding sites) by aligning homologous proteins. They pointed out to the significant number of proteins that have such bi-functional positions and released the collection of structurally characterized modulators of protein interactions at <http://pibase.janelia.org> [45].

DRUGS TARGETING MULTIPLE PROTEINS

Side effects are one of the main reasons for drug failure [46]. In the last 10 years nearly 20 drugs have been banned from the market for causing severe side effects [47]. Adverse effects can be caused by the inherent mechanism of action of a drug, by toxic metabolites following drug degradation and by unpredictable side effects due to

“off-targets” of drug interactions. A number of studies highlight the promiscuity as a common attribute of drugs. Yildirim *et al.* [8] constructed a drug-target network from 4252 drugs targeting 394 human proteins. They found out that among 890 drugs 788 had at least one common target and in that network the average number of target proteins was 1.8 per drug. This result revealed the fact that new drugs generally target known druggable proteins and that the number of drugs targeting others is low in the market. However a more recent study by Mestres *et al.* [48], updated the average number of target proteins per drug as 6.3, which points to the high tendency of drugs to be multi-targeted. Paolini *et al.* [49] searched for the extent of promiscuity in the global pharmacological space and they also observed that among 276,122 active drug compounds, 35% hit multiple targets. The data compiled in the drug-target databases also indicates this many-to-many behavior. Some of these databases are listed in the Table 1. Among the multi-target drug examples, there are a number of kinase inhibitors, which operate by affecting multiple targets [50, 51] and the anticancer drug lenalidomide [12]. Several multi-target drugs were also discovered by chance [52].

There are examples suggesting that targeting multiple proteins simultaneously may be successful, such as non-steroidal anti-inflammatory drugs (NSAIDs); antidepressants; multi-target kinase inhibitors and anticancer drugs [53]. Drug combinations (‘cocktails’) may bind at different sites on the same protein; or to multiple different proteins. Examples include the three drugs combination used to treat HIV infection, which is composed of reverse-transcriptase and protease inhibitors [6] and the drug combinations known as “CHOP”, which is used in the treatment of non-Hodgkin’s lymphoma [54]. Another synergistic drug combination example is Cytarabine and Aplidin (Fig. 1), used for enhancing their antitumor activities in leukaemia and lymphoma models [55]. Cytarabine is an anticancer drug used in the treatment of patients with leukemia [56] and Aplidin is another, which activates EGFR, Src, JNK and p38MAPK [57] and inhibits VEGF [58]. When system-wide effect of Aplidin is investigated, it is observed to activate death receptor of Fas ligands [59]. This outcome of Aplidin may be due to the activation of JNK/p38 MAPK pathway [60]. In turn, Fas ligand activates the receptor-mediated extrinsic cascade of apoptosis [61]. In addition, Cytarabine increases cellular stress by inhibiting DNA repair and RNA synthesis and drops the MCL1 level which leads to activation of CASPs [62]. Finally CASPs triggers the apoptosis via the mitochondrial intrinsic cascade [61].

Due to drug combinations’ condensed side effects and enhanced treatment capability, understanding the underlying mechanisms is an important and interesting task, as well as detecting new applicable drug cocktails. There is an increasing number of publications in this area; such as the “drug cocktail network” built in order to investigate existing drug cocktails and identifying new ones, in a recent work [63]. They mentioned that drugs in a cocktail tend to interact with same partners and share common therapeutic effects. Another example is the computational method Zhao *et al.* [64] used for inferring new drug combinations. They combined molecular and pharmacological properties of drugs for this purpose and looked for feature patterns enriched in drug combinations. Predictions of their method were 69 % supported by literature. Moreover, they have also found important clues about the working principles of combinatorial therapies. “Combinatorial Drug Assessment”[65] is an alternative tool for combinatorial drug discovery, which uses gene expression profiling and multiple signaling pathways. Lastly Wang *et al.* [66] considered drug combinations in the perspective of genetic interaction network and associated human pathways. They found that drug combinations alter functionally correlated pathways and have a smaller influence range in the genetic interaction networks.

A SYSTEMS BIOLOGY VIEW

Biological systems are governed by physical and functional interactions. Systems biology simulates and orchestrates the mole-

Table 1. List of some drug-target databases.

Server / Database	Explanation	Web Site
DrugBank [102]	Detailed drug data with comprehensive drug target information	http://www.drugbank.ca/
TTD [103]	Therapeutic Target Database	http://bidd.nus.edu.sg/group/ttd/
Stitch [104]	Chemical-Proteins Interactions database	http://stitch.embl.de/
PDSP Ki [105]	Capabilities of drugs binding to molecular targets	http://pdsp.med.unc.edu/kidb.php
PDTD [106]	Potential Drug Target Database	http://www.dddc.ac.cn/pdtd/
Wombat-PK [107]	Clinical Pharmacokinetics and Drug Target Information	http://www.sunsetmolecular.com/index.php?option=com_content&view=article&id=16&Itemid=11
BindingDB [108]	measured binding affinities of protein targets and small molecules	http://www.bindingdb.org/bind/index.jsp
PDBBind [109]	a collection of experimentally measured binding affinity data	http://sw16.im.med.umich.edu/databases/pdbbind/index.jsp
KeggDrug [110]	Drug information resource of approved drugs	http://www.genome.jp/kegg/drug/
PubChem [111]	Biological activities of small molecules	http://pubchem.ncbi.nlm.nih.gov/
PharmGKB [112]	pharmacogenomics knowledge resource	http://www.pharmgkb.org/
DART [113]	Drug adverse reaction and target database	http://xin.cz3.nus.edu.sg/group/drt/dart.asp
SuperTarget [114]	A resource for analyzing drug-target interactions	http://insilico.charite.de/supertarget/
Promiscuous [115]	A resource of protein-protein and protein-drug interactions	http://bioinformatics.charite.de/promiscuous/
CTD [116]	Comparative Toxicogenomics Database	http://ctdbase.org/
sc-PDB [117]	Database of Druggable Binding Sites from the Protein Data Bank	http://cheminfo.u-strasbg.fr:8080/scPDB/2011/db_search/accueil.jsp?uid=4540503983234320384

cules to optimally adapt the organism response to its environment. Diseases disturb the network; ‘good’ drugs restore the network to its ‘proper’ desired state [67]. Network descriptions and analyses are important tools in systems biology; they are powerful in abstracting the complex relationships inside cells and between them, and they often provide clues for drug discovery [68]. While data are incomplete, and the approaches may not have matured, network descriptions and tools are gradually becoming common place [69].

The human protein-protein interaction (PPI) network is huge with approximately 130,000 binary interactions between proteins [70], and is expected to be far larger, with around 650,000 PPIs [71]. Protein-protein interaction networks are of vast importance in medicine [72, 73]. From the drug development standpoint, we would expect it to have critical components, which would make enticing drug targets. Network topology may help, because drug targets are usually not arbitrarily located on the protein interaction networks [74]. Drugs that perturb topologically critical nodes (such as highly connected nodes) have increased risk of causing lethality [75], while blocking the targeted function. That’s why marketed drugs do not generally target high degree nodes [76]. An ‘ideal’ drug target would have fewer neighbors while being located at some strategic point of the human disease network [6]. Such a target may be a non-vital bridging node [77]. It may disturb the information flow and the disease process while not causing serious side

effects. For complex diseases like cardiovascular disease, central nervous system disorders, cancer, Alzheimer and aging, a network perspective is critically important. These require consideration of the global map of protein interactions and estimation of the expected outcome on the multiple, inter-connected pathways. As we describe below, in some diseases that are resistant to drug therapy [78], network-based strategy, where another protein in the same pathway is targeted may suggest alternative targets that may lead to the sought outcome [79, 80].

When enriched by high-throughput data, networks may obtain possible responses to a drug or optimum combination of drugs for reaching a desired outcome. On the other hand, because such data is derived from population of cells, its accuracy for specific environments and physiological states may be compromised. Networks may be analyzed using mathematical models such as Flux Balance Analysis [43, 81-83], differential equations [84], Petri Nets [85], Integer Linear Programming [86] and Boolean logic gates [87].

Another use of networks is its topological properties such as hubs, betweenness, modules, etc.. Network topology determines the information flow. Information flow and robustness analyses are used to locate essential components. These algorithms are utilized to find perturbed proteins by hypothetical drugs [88] or for locating optimum drug targets that have little influence on other functions,

Fig. (1). Synergistic drug combination of Cytarabine and Aplidin enhances antitumor activities.

apart from the intended one [89-92]. A key question is how to choose an efficient combination of multiple drug targets; especially those that while not key players in central pathways, ensure information flow among the network elements [77].

THE ADVANTAGES AND HANDICAPS OF MODELED PROTEIN-PROTEIN INTERACTIONS IN MONO- AND POLY-PHARMACOLOGY

Key requirements in drug discovery are the availability of protein structures and their interactions; the pathways in which they are located and the pathway cross-talk; and how similar are the binding sites to which they bind to those of other proteins in the cell. Modeling protein interactions can help by predicting which proteins interact, which permits the construction of more complete pathways and the cellular network. These may allow prediction of how targeting a specific protein can affect the entire system. The PRISM server [93, 94] is one of the tools which predicts the interacting protein couples and their interface structures. Further, because the modeled interactions provide also the information on how the proteins interact, they allow prediction of which partners interact through the same binding site. Thus, if a particular protein is targeted, this may abolish the competitive binding at the same shared site, driving the system in a certain direction, which the structural network may forecast. Such predictions may be particularly powerful for multi-molecular complexes, which are prone to toxic side effects. If the drugs target certain PPIs, the structural network may suggest the other PPI which share similar motifs [95]; and as such, may also be affected by the drug, which may also lead to toxic side effects.

Networks may be used to explain side effects of multi-target drugs. Xie *et al.* [96] studied the side effects of torcetrapib, which is an inhibitor of cholesteryl ester transfer protein (CETP). Torcetrapib was a proposed treatment for cardiovascular disease and was in clinical trials. The authors compared all ligand-binding sites in all available protein structures, with the pockets on torcetrapib and

created an off-target binding network. They combined their study with biological pathways and found the likely reasons for the effects of torcetrapib on blood pressure.

Propagation of the effects of drugs in the network may be observed for orthosteric and allosteric drugs [97, 98]. In the case of orthosteric drugs, which block the protein active site, the protein is impaired and its function is abolished; in the case of allosteric drugs, the modulating effects of drugs propagate through the protein and, through the protein-protein interactions, across the pathways. However, the effects are likely to be strongest in proteins sharing the same complex.

A key handicap of modeled structural networks is that they provide a static view of cell, and of the proteins. Yet, the cellular network is highly dynamic; proteins associate and dissociate. This is challenging to model, because the affinities of their interactions which are typically measured in solution, do not necessarily reflect the *in vivo* environment, where the affinities at one binding site are affected by prior allosteric events at different sites, for example, binding of other partners or post-translational modifications. They also may not account for the presence of co-factors; and fluctuations in the environment. An additional challenging problem is protein dynamics; protein structures fluctuate, and the distributions of their conformational ensembles change dynamically, which affects the binding site conformations and drug binding [97]. Accounting for dynamics in the proteins and across the pathways and the network is an extremely challenging problem. This is because it both necessitates detailed experimental data and highly demanding computational requirements. To date, modeling on the network scale is not able to fully address these problems [68]. However, for specific proteins, on the local scale, Nuclear Magnetic Resonance (NMR) and molecular dynamic simulations may be able to provide some clues.

Despite these shortcomings, single- and multi-drug pharmacology can benefit from the modeled structural proteome. Predictions

are able to provide leads and hypotheses, which can then be validated by experiment.

CONCLUSIONS

The network-like structure established by the interactions of proteins has been discovered already some years ago [99, 100]; and although there have been many PPI curation studies and considerable research, the human PPI network is still believed to be lacking important interactions [70]. The success of network-based methods is tightly connected to the completeness of data; and as pointed out in a recent review [101] databases are far from being complete. As more protein interactions are discovered, systems biology will be able to simulate the mechanism inside and between the cells more effectively. Structural data of single proteins and their complexes is also expected to increase. As a consequence, complete models of systems biology and structural pathways will hopefully pave a path to tailored patient-specific treatments.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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