Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Chemical Biology

Rational approaches to targeted polypharmacology: creating and navigating protein–ligand interaction networks

James T Metz and Philip J Hajduk

Many successful drugs bind to and modulate multiple targets *in vivo*. Successfully navigating protein–ligand polypharmacology will be a crucial and increasingly utilized component of pharmaceutical research. As publicly available databases of ligand activity values continue to grow in size and quality, infrastructure is needed to enable scientists to create and interact with these networks to fuel hypothesis-driven science. While most of the individual tools for creating this infrastructure exist, effectively connecting the data to the network to the scientist is very much a work in progress. Standards for publishing network data are also important to facilitate the analysis and comparison of networks from different research groups using different methods.

Address
Pharmaceutical Discovery Division, Abbott Laboratories, Abbott Park, IL 60064-3500, United States

Corresponding author: Hajduk, Philip J (philip.hajduk@abbott.com)

Current Opinion in Chemical Biology 2010, **14**:498–504

This review comes from a themed issue on
Next Generation Therapeutics
Edited by Michael Gelb and Adrian Whitty

Available online 6th July 2010

1367-5931/$ – see front matter

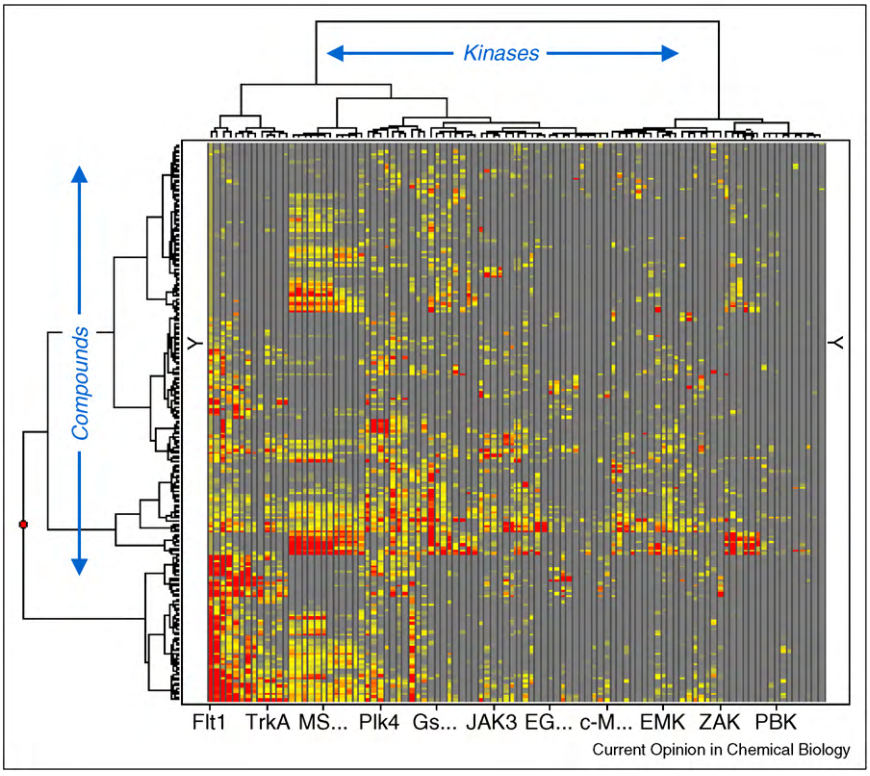
DOI 10.1016/j.cbpa.2010.06.166

Introduction

Over the past two decades, the vast majority of pharmaceutical discovery research could be classified as a 'reductionist' or target-centric approach, wherein a single molecular entity thought to be involved in disease onset or progression was targeted for therapeutic intervention. While this strategy has certainly led to the discovery of several novel drugs (e.g. HIV protease inhibitors and Gleevec®, among others), there has been significant criticism that, overall, the return on investment has been rather disappointing [1,2]. This intentional shift toward 'magic bullets' targeting individual 'disease-causing genes' [3,4] largely ignores earlier (and arguably equally successful) drug discovery efforts that by necessity targeted entire organisms, often in the absence of knowledge about the target or mechanism. This 'holistic' or systems-centric approach was the basis for the discovery of many highly successful drugs (e.g. valproic acid and clozapine, among others) whose putative molecular target(s) are only recently coming to light. Surprisingly, interrogation of these older drugs using panels of conventional biochemical assays indicate that many of them are promiscuous and exhibit activity against a wide range of molecular targets. In fact, it is now commonly accepted that the polypharmacology of these drugs (i.e. their ability to modulate the activity of multiple protein targets) is at least partly responsible for their efficacy, such that they can be viewed as 'magic shotguns' [5]. It is also significant that even drugs designed to target one specific molecular entity can demonstrate unexpected efficacy that can be linked to activity against additional targets, as in the case of Gleevec and the PDGF receptor [6]. Mestres *et al.* analyzed a drug–target network consisting of 4767 unique interactions and 802 drugs leading to the conclusion that, on average, a drug interacts with 6 targets [7]. It is interesting to note that the thousand year old traditional folk medicine of several cultures (e.g. Ayurvedic medicine) has implicitly embraced polypharmacology for activity [8]. These observations have led to a new wave of pharmaceutical research that has variously been termed 'systems chemical biology,' [9] 'system-based' discovery, 'network-based' discovery, 'multi-targeted' drug design, or 'targeted polypharmacology' [10••]. In essence, all of these designations recognize what may be obvious in hindsight: that trying to treat complex, heterogeneous diseases that tend to result from multiple molecular abnormalities (such as cancer, cardiovascular, and psychiatric disorders) with a highly specific drug that targets a single molecular entity will have, at best, a very low probability of success. Instead, these diseases must be addressed with a more integrated methodology, and the complexity of the disease must be equally matched by the complexity of our approach. The problem is, given the inherent difficulties in targeting even a single molecular entity, how do we rationally approach targeting two, three, or even more protein targets? Which targets are most likely to be modulated in concert by a single drug? Or, conversely, which drugs or drug scaffolds are most amenable to multi-targeted drug design? And how do we avoid the likely increased risk of toxicity resulting from *unwanted* polypharmacology? Another complication is that off-target activities of medicines may be due to the connectivity of a signal transduction network affected by a compound and not necessarily the promiscuity or polypharmacology of the compound itself [11].

While complete answers to these questions are well outside of the scope of this review, an explosion of papers is appearing in the literature that gives the drug discovery scientist an important first step: the means to map the

Figure 1



Heat map analysis of kinase activity data. Activity data for 200 compounds (*y*-axis) against 100 kinases (*x*-axis) is shown, where the compounds and kinases have been clustered (using the hierarchical clustering algorithm within Spotfire) according to their activity profile. Activity data ranges from less than or equal to 10 nM (red) to 1 μM (yellow). Values greater than 1 μM are in gray.

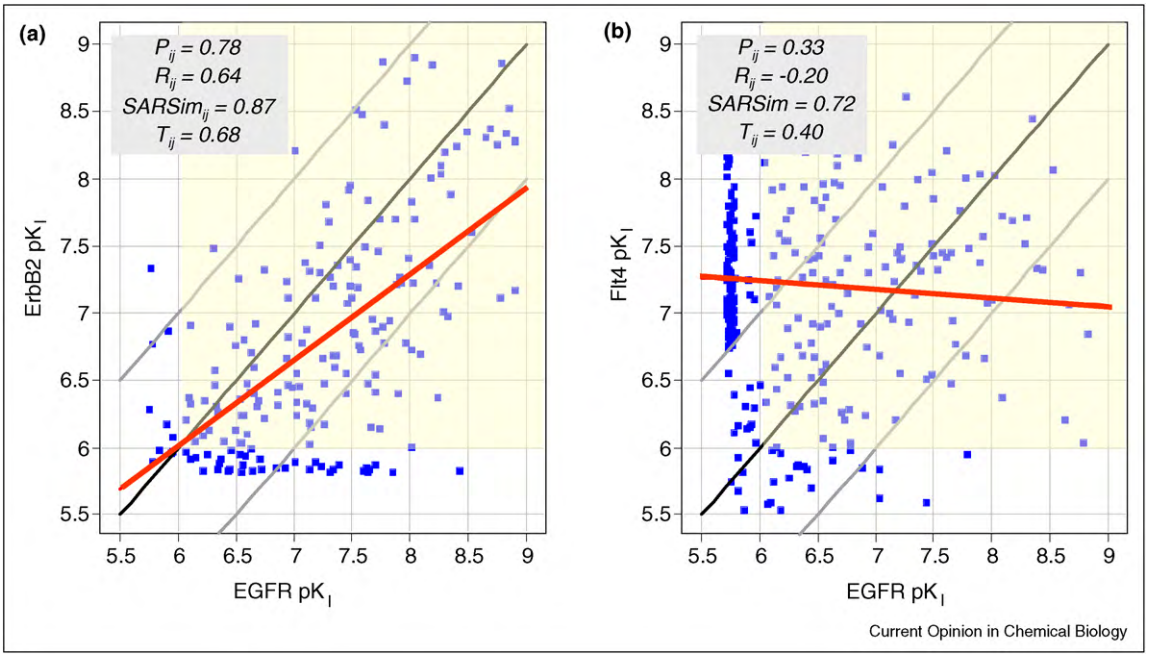
connections between targets not in sequence space but in pharmacological space. A pharmacological relationship between two proteins is defined as their ability to bind with similar affinities to a range of chemical entities, regardless of their underlying sequence similarity. For example, it has long been known that the protein kinases GSK3β and CDK4 exhibit very similar structure–activity relationships, despite sharing only 28% sequence identity [12•]. While this unexpected pharmacological similarity between pairs or triplets of proteins has been long known, our ability to interrogate these relationships on a proteome-wide scale and to quantitatively assess their importance has only recently emerged. This review will discuss methods for establishing pharmacological relationships between proteins and how these values can be used to construct interaction networks that can guide the design of multi-targeted drugs.

Quantifying pharmacological relationships

The conventional approach to assessing the degree of similarity between two protein targets I and J is to evaluate their sequence identity or homology, with higher identity (or homology) indicating a closer relationship between the two proteins, and a concomitant increase in the expectation that a compound with high affinity for Target I may also exhibit affinity toward Target J. At least in the realm of protein kinases, a general rule of thumb is that any two kinases with >60% sequence identity are likely to exhibit similar affinities for the same chemical inhibitors [13]. However, as illustrated above for GSK3β and CDK4, the converse is not as generalizable as even very distantly related kinases (from a gene sequence perspective) can exhibit surprisingly high pharmacological similarity. These pharmacological relationships must be empirically determined by testing large numbers of compounds in common against an array of protein targets. The similarity in the resulting inhibition or ligand-binding profiles then yields insight into the degree of relatedness.

The most common approach to understanding the relationship between proteins based on their ligand-binding profiles is simply to directly examine the profile itself, and employ any of a variety of clustering algorithms to place similar profiles in similar clusters. Some of the earliest work in this area was described by Weinstein in 1997 [14•]. An example of a profile analysis is given in Figure 1 for in-house kinomics data, where a set of 200 compounds was tested against a panel of 100 different protein kinases. In this example, compounds that exhibit

Figure 2



Deriving pharmacology metrics. Examples of activity data (given in pK_i units) and resulting pharmacological assessments for EGFR against (A) ErbB2 and (B) Flt4. The Pharmacology Interaction strength (P_{ij}) is essentially the fraction of compounds contained within 1-log unit of unity (denoted by the black and gray solid lines). The Pearson R_{ij} is derived from a linear fit of the data, shown in red. The Tanimoto T_{ij} is the fraction of compounds that exhibit sub-μM potency against both enzymes, denoted by the compounds in the yellow shaded area.

similar inhibition patterns across kinases are grouped together by row, while kinases that exhibit similar patterns across compounds are grouped by column. While such an analysis can be exceptionally insightful when trying to interrogate the most pharmacologically related proteins to your target of interest, it suffers from several disadvantages. First, proteins are grouped by profile 'similarity,' which is somewhat arbitrary and yields no real insight into whether the proximity in the resulting topology is biologically relevant. Second, these analyses are only applicable to proteins against which this specific set of compounds has been tested. This, it has no transferability to other systems or datasets. Historically, this has limited the investigation of pharmacological similarity to very local analyses on limited datasets.

Several approaches have been put forward to overcome these problems and quantify the pharmacological similarity of two proteins based on their ability to bind with similar affinities to different compounds. In an early study, a novel set of fingerprints (Similog keys) were used to identify both active ligands binding to the same target as well as active ligands to similar targets [15]. Latter approaches have extended these concepts and permit the pharmacological grouping of targets that may have little or no structural or sequence similarity. A number of metrics have been put forward to quantify these relationships, as listed in Table 1. One of most basic is the familiar Pearson correlation coefficient, R_{ij}, where the potencies of a set of compounds against Target I and Target J are compared for dependence. An example of this is shown in Figure 2 for highly related (Figure 2A) and unrelated (Figure 2B) protein kinases. Unfortunately, the Pearson correlation coefficient can be highly sensitive to outliers (e.g. compounds very potent against Target I but inactive against Target J), which is especially true for small datasets. Vieth *et al.* [12•,13] developed the 'SAR Similarity' measure, which is essentially the mean absolute deviation of pIC_{50} values (negative base-10 logarithm of the IC_{50}) between the two kinases (normalized by the pIC_{50} range). Other related measures for assessing 'profile similarity' are the profile Tanimoto

Table 1

Various parameters for assessing pharmacological relationships based on ligand-binding profiles

Parameter	Symbol
Sequence identity	S_{ij}
Pearson correlation coefficient	R_{ij}
Pharmacology interaction strength [18••]	P_{ij}
SAR similarity [12•]	$SARSim_{ij}$
Tanimoto similarity[a] [16•]	T_{ij}
Jaccard distance [17]	J_{ij}

[a] The Tanimoto similarity T_{ij} is simply 1 minus the Jaccard distance ($1 - J_{ij}$).

Table 2

Correlation matrix for six pharmacology similarity parameters based on in-house kinomics data

	S_{ij}	P_{ij}	R_{ij}	$SARSim_{ij}$	T_{ij}	J_{ij}
S_{ij}	1.0	0.30	0.35	0.29	0.31	0.31
P_{ij}	0.30	1.0	0.38	0.82	0.45	0.45
R_{ij}	0.35	0.38	1.0	0.28	0.25	0.25
$SARSim_{ij}$	0.29	0.82	0.28	1.0	0.30	0.30
T_{ij}	0.31	0.45	0.25	0.30	1.0	1.0
J_{ij}	0.31	0.45	0.25	0.30	1.0	1.0

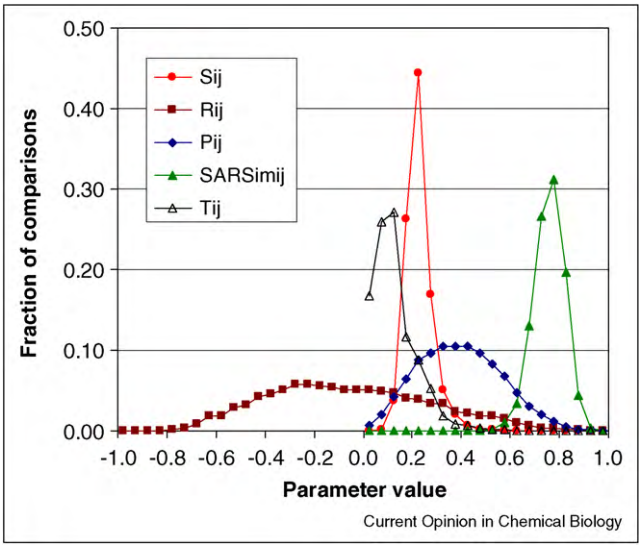
coefficient (T_{ij}) [16•] or Jaccard distance (J_{ij}) [17], which designate compounds as either 'active' or 'inactive' (based on a user-defined activity level) to reduce the profile to a sequence of bits. Yet another measure was introduced by Paolini *et al.*, termed the Pharmacology Interaction strength (P_{ij}) that measures the fraction of compounds tested against two proteins that exhibit comparable affinity [18••]. Examples of all of these parameters are given in Figure 2. It is also important to note that several of these measures are highly correlated (see Table 2), such that one does not need to calculate all of them in order to infer a pharmacological link between two targets. In our lab, we have relied on S_{ij} (sequence identity), R_{ij} (the Pearson correlation), and P_{ij} (Hopkins' interaction strength) as three relatively uncorrelated views of ligand polypharmacology.

All the pharmacology measures discussed above require that activity data be available for some subset of compounds against each pair of targets that are to be assessed. This precludes the utilization of the vast majority of publicly (or even privately) available activity databases, as most compounds are only tested against a very small number (usually one) of protein targets. Recent work from Shoichet's group has paved the way for using ligand *similarity* rather than *activity* comparisons to derive pharmacology relationships between different proteins [19,20•,21••]. Thus, one only needs a set of actives for Target I and a completely independent set of actives for Target J in order to assess pharmacological similarity.

Interpreting pharmacological relationships

In deriving pharmacology relationships based on ligand-binding, once a set of compounds with associated activity has been identified, a number can be calculated. However, great care must be taken in interpreting these values and postulating whether or not a pharmacological relationship exists between two targets. First, all of these parameters span a range of values (see Figure 3 for ranges of these values for in-house kinomics data), and there is no *a priori* guidance as to what value constitutes a significant pharmacological link. For example, Paolini *et al.* [18••] used a P_{ij} value cut-off of 0.1 to derive genome-wide interaction networks (e.g. over all protein families). However, as can be observed from Figure 3, the vast majority (~97%) of

Figure 3



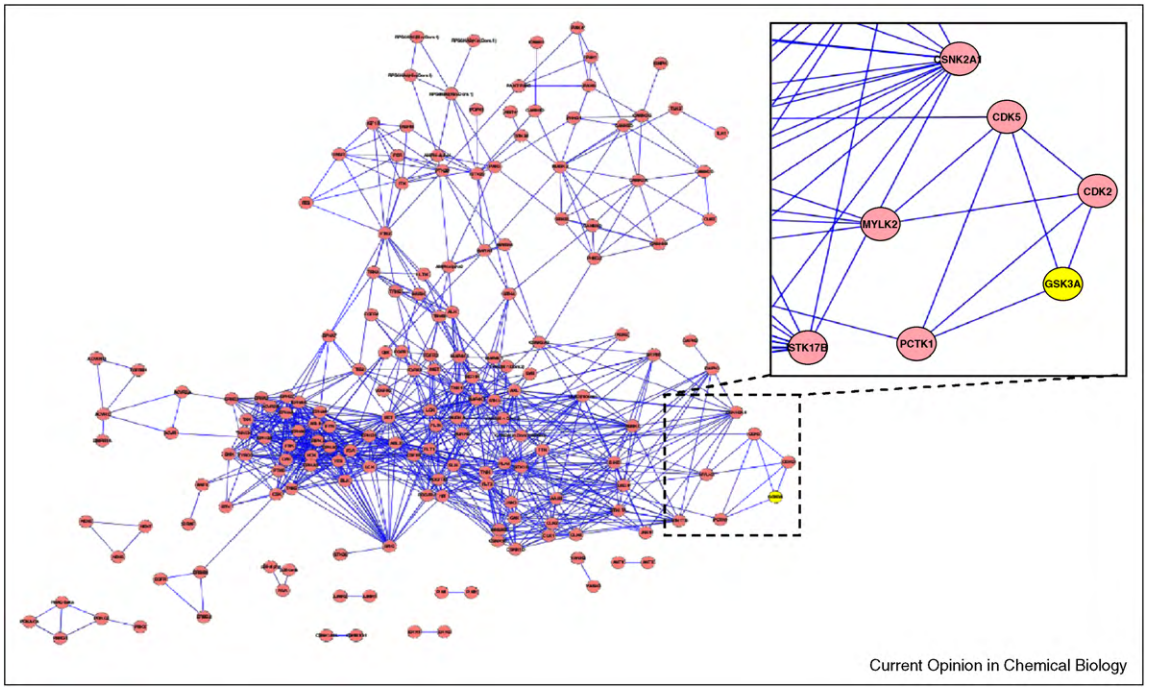
Distribution of values for a series of pharmacology parameters. The range of values for various pharmacology metrics varies widely, and care must be taken to define 'significant' values for the purpose of deriving pharmacology networks.

kinase pairs exceed this threshold and are pharmacologically 'related' at this level of discrimination. Another significant precaution in interpreting pharmacology values is that, unlike sequence identity, interactions can strengthen or weaken as new data becomes available [22]. As a result, interactions proposed with one set of data may completely disappear upon re-analysis with a different or larger dataset. This will be especially true when interaction networks are proposed based on only a small number of compounds (for example, some of the pharmacology 'connections' reported in the literature are based on a single active in common between two proteins). The converse is also true, in that relationships might not be inferred simply owing to lack of data, and not on the basis of data suggesting that no link exists. Impact of this 'data completeness' problem on commonly utilized public databases has been the topic of several recent publications [22]. Vogt and Mestres have also shown that for the case of target-only networks (where the relationships between targets is derived from many compound observations, as is the case for the metrics listed in Table 1), there is information loss of more detailed relationships of compound subsets and targets [23]. Finally, it should be recognized that simply observing a correlation of potency values between two targets does not in fact establish that a true causative pharmacological relationship exists, as is well documented in the study of causality [24•].

Leveraging pharmacological relationships

Despite these cautions, researchers have been able to utilize ligand-based pharmacology data to construct sequence-independent interaction networks that yield

Figure 4



Sequence-independent kinome-inhibitor interaction map derived from publicly available data [16•]. In this view of 100 kinases (requiring a minimum T_{ij} value of 0.3), the majority of kinases are contained in a dense, highly interconnected network where achieving selectivity is unlikely. A smaller number of kinases exist as disconnected 'islands.' The pharmacological link between the GSK and CDK families of kinases (which exhibit low sequence similarity) is shown in the inset to the right.

unprecedented insight into polypharmacology. Although networks have been studied by academics since the 18th century, it is only recently that they have gained much wider interest and application [25]. Once a pharmacology parameter has been identified and a cut-off for significance established, visualizations that allow a researcher to navigate the connections between targets can be generated using a variety of software packages (a partial listing is given in Table 3). Cytoscape is free software that can take as input a simple text file and generate a number of network representations, as illustrated in Figure 4 for reported kinomics data [16•]. This gives the researcher an immediate feel for the 'connectedness' of the network as a whole and more specifically for the target under consideration. Although networks may in some cases appear complicated, the information in a network is similar to a set of interconnected 'hotspots' in a heat map [26]. As a result, networks may better convey a sense of the pharmacological connectedness than a heat map. Connections between different targets indicate a significantly higher than random probability that a compound with activity against one target will also inhibit the connected targets. This, of course, can represent either an opportunity (targeted polypharmacology) or a liability (an off-target to be avoided). As examples, such sequence-independent pharmacology networks have been constructed for a set of 200 kinases using kinome profiling data [16•], a set of 480 drug targets using publicly available activity data [7], and 700 targets using a combination of internal and external activity data [18••].

Table 3

Software programs for creating and navigating protein–ligand interaction networks

Software	Website or Reference
Cytoscape	http://cytoscape.org/
GCG Growtree	Devereux (1984) [28]
TreeDyn	Chevenet (2006) [29]
PHYLIP	http://evolution.genetics.washington.edu/phylip.html
SARANEA	Lounkine (2010) [30]
SNAVI	Ma'ayan (2009) [31]

It is important to emphasize that any of these connections ultimately correspond to *probabilities* of targeting multiple proteins. Thus, the presence of a connection does not guarantee polypharmacology, and the lack of a connection does not suggest that it is not possible to simultaneously modulate both proteins with a single compound—only that it has either not yet been observed or will be less common. Connections made with the P_{ij} or T_{ij} values are especially intuitive here, as the cut-off used corresponds to the likelihood of hitting both targets with a single

compound (e.g. a P_{ij} value of 0.6 for a protein pair means that 60% of the compounds tested were equipotent against both targets, while a T_{ij} of 0.6 indicates that 60% of the compounds tested were below a defined potency threshold against both targets).

Summary

Successfully navigating protein–ligand polypharmacology will be a crucial and increasingly utilized component of pharmaceutical research. As publicly available databases of ligand activity values continue to grow in size and quality, the infrastructure will need to be developed to enable scientists to create and interact with these networks in order to fuel hypothesis-driven science. While most of the individual tools for creating this infrastructure exist, effectively connecting the data to the network to the scientist is very much a work in progress. Although there are no current standards for publishing networks at the present time, it is strongly suggested that authors include network information in the form of an easily readable, delimited text file. This will greatly facilitate the analysis and comparison of networks from various research groups and from various methods. Software tools to compare networks are available [27]. Other tools, algorithms, and network metrics are in-progress and will be described in future publications.

References and recommended reading

Papers of particular interest, published within the annual period of review, has been highlighted as:

- • of special interest
- •• of outstanding interest

1. Betz UA, Farquhar R, Ziegelbauer K: **Genomics: success or failure to deliver drug targets?** *Curr Opin Chem Biol* 2005, **9(4)**:387-391.
2. Betz UA: **How many genomics targets can a portfolio afford?** *Drug Discov Today* 2005, **10(15)**:1057-1063.
3. Sams-Dodd F: **Optimizing the discovery organization for innovation**. *Drug Discov Today* 2005, **10(15)**:1049-1056.
4. Sams-Dodd F: **Target-based drug discovery: is something wrong?** *Drug Discov Today* 2005, **10(2)**:139-147.
5. Roth BL, Sheffler DJ, Kroeze WK: **Magic shotguns versus magic bullets: selectively non-selective drugs for mood disorders and schizophrenia**. *Nat Rev Drug Discov* 2004, **3(4)**:353-359.
6. Frantz S: **Drug discovery: playing dirty**. *Nature* 2005, **437(7061)**:942-943.
7. Mestres J, Gregori-Puigjane E, Valverde S, Sole RV: **The topology of drug–target interaction networks: implicit dependence on drug properties and target families**. *Mol Biosyst* 2009, **5(9)**:1051-1057.
8. Burris TP, Montrose C, Houck KA, Osborne HE, Bocchinfuso WP, Yaden BC, Cheng CC, Zink RW, Barr RJ, Hepler CD: **The hypolipidemic natural product guggulsterone is a promiscuous steroid receptor ligand**. *Mol Pharmacol* 2005, **67(3)**:948-954.
9. Oprea TI, Tropsha A, Faulon JL, Rintoul MD: **Systems chemical biology**. *Nat Chem Biol* 2007, **3(8)**:447-450.
10. •• Morphy R: **Selectively nonselective kinase inhibition: striking the right balance**. *J Med Chem* 2010, **53(4)**:1413-1437.

An excellent overview of the challenges and opportunities associated with targeted polypharmacology, specifically in the realm of protein kinase inhibitors.

11. Fliri AF, Loging WT, Volkmann RA: **Drug effects viewed from a signal transduction network perspective**. *J Med Chem* 2009, **52(24)**:8038-8046.
12. • Vieth M, Higgs RE, Robertson DH, Shapiro M, Gragg EA, Hemmerle H: **Kinomics-structural biology and chemogenomics of kinase inhibitors and targets**. *Biochim Biophys Acta* 2004, **1697(1–2)**:243-257.

One of the earliest reports to devise a target similarity metric based on activity data ($SARSim_{ij}$) for compounds tested in common against pairs of related targets (i.e. kinases). Dendrograms of the kinases were then generated using sequence similarity and $SARSim_{ij}$ values, and were compared. It was found that highly homologous kinases (sequence identity $>$ 60%) are inhibited comparably by small molecules. As a consequence, achieving ligand selectivity for highly homologous kinases is expected to be difficult.

13. Vieth M, Sutherland JJ, Robertson DH, Campbell RM: **Kinomics: characterizing the therapeutically validated kinase space**. *Drug Discov Today* 2005, **10(12)**:839-846.
14. • Weinstein JN, Myers TG, O'Connor PM, Friend SH, Fornace AJ Jr, Kohn KW, Fojo T, Bates SE, Rubinstein LV, Anderson NL *et al.*: **An information-intensive approach to the molecular pharmacology of cancer**. *Science* 1997, **275(5298)**:343-349.

One of the first attempts to analyze a large group of compounds ($N \sim$ 460,000 cpds) and activity data from 60 human cancer cell lines. A heatmap was generated by clustering in both compound and target activity dimensions. Although the results were not interpreted using a network format, it should be recognized that heatmaps and networks are closely related data representations. Results from the analysis were then used to guide the search for candidate anticancer drugs that are not dependent on intact p53 suppressor gene function.

15. Schuffenhauer A, Floersheim P, Acklin P, Jacoby E: **Similarity metrics for ligands reflecting the similarity of the target proteins**. *J Chem Inf Comput Sci* 2003, **43(2)**:391-405.
16. • Bamborough P, Drewry D, Harper G, Smith GK, Schneider K: **Assessment of chemical coverage of kinome space and its implications for kinase drug discovery**. *J Med Chem* 2008, **51(24)**:7898-7914.

A large-scale analysis of kinome polypharmacology with associated networks and underlying data.

17. Bernasconi P, Chen M, Galasinski S, Popa-Burke I, Bobasheva A, Coudurier L, Birkos S, Hallam R, Janzen WP: **A chemogenomic analysis of the human proteome: application to enzyme families**. *J Biomol Screen* 2007, **12(7)**:972-982.
18. •• Paolini GV, Shapland RH, van Hoorn WP, Mason JS, Hopkins AL: **Global mapping of pharmacological space**. *Nat Biotechnol* 2006, **24(7)**:805-815.

This publication ushered in the recent strong interest in network pharmacology or global pharmacological similarity owing to the creation and utilization of a target similarity metric, P_{ij}, which went beyond similarity for a related group of proteins. This research thus demonstrated the practical feasibility of computing and analyzing pharmacological similarity in a much larger, global context.

19. Hert J, Keiser MJ, Irwin JJ, Oprea TI, Shoichet BK: **Quantifying the relationships among drug classes**. *J Chem Inf Model* 2008, **48(4)**:755-765.
20. • Keiser MJ, Roth BL, Armbruster BN, Ernsberger P, Irwin JJ, Shoichet BK: **Relating protein pharmacology by ligand chemistry**. *Nat Biotechnol* 2007, **25(2)**:197-206.

Shoichet's group developed an interesting approach (termed 'SEA' for Similarity Ensemble Approach) that employs ligand similarity, novel statistical techniques, and careful analysis of probability distributions to permit the prediction of ligand activity against a database of targets. Hence, it is no longer necessary that a common set of ligands be tested against a pair of targets. The predicted activities are then used to construct network connections between targets.

21. •• Keiser MJ, Setola V, Irwin JJ, Laggner C, Abbas AI, Hufeisen SJ, Jensen NH, Kuijer MB, Matos RC, Tran TB *et al.*: **Predicting new molecular targets for known drugs**. *Nature* 2009, **462(7270)**:175-181.

This follow-up article from Shoichet's group highlights the power of SEA. More than 3000 US FDA-approved drugs were analyzed against hundreds of targets resulting in thousands of unanticipated associations. Thirty were tested resulting in 23 confirmed, new drug–target associations.

22. Mestres J, Gregori-Puigjane E, Valverde S, Sole RV: **Data completeness—the Achilles heel of drug–target networks**. *Nat Biotechnol* 2008, **26(9)**:983-984.

23. Vogt I, Mestres J: **Drug–target networks**. *Mol Inf* 2010, **29**:10-14.

24. • Pearl J: **Causal inference in statistics: an overview**. *Stat Surv* 2009, **3**:96-146.
This extensive review article written by an expert on causality is fundamental to key questions concerning cause and effect relationships that pharmacological networks and systems biology will inevitably generate.

25. Newman M: **The physics of networks**. *Phys Today* 2008, **61**:33-38.

26. Merico D, Gfeller D, Bader GD: **How to visually interpret biological data using networks**. *Nat Biotechnol* 2009, **27(10)**:921-924.

27. Assenov Y, Ramirez F, Schelhorn SE, Lengauer T, Albrecht M: **Computing topological parameters of biological networks**. *Bioinformatics* 2008, **24(2)**:282-284.

28. Devereux J, Haeberli P, Smithies O: **A comprehensive set of sequence analysis programs for the VAX**. *Nucleic Acids Res* 1984, **12(1 Pt 1)**:387-395.

29. Chevenet F, Brun C, Banuls AL, Jacq B, Christen R: **TreeDyn: towards dynamic graphics and annotations for analyses of trees**. *BMC Bioinform* 2006, **7**:439.

30. Lounkine E, Wawer M, Wassermann AM, Bajorath J: **SARANEA: a freely available program to mine structure–activity and structure–selectivity relationship information in compound data sets**. *J Chem Inf Model* 2010, **50(1)**:68-78.

31. Ma'ayan A, Jenkins SL, Webb RL, Berger SI, Purushothaman SP, Abul-Husn NS, Posner JM, Flores T, Iyengar R: **SNAVI: desktop application for analysis and visualization of large-scale signaling networks**. *BMC Syst Biol* 2009, **3**:10.