

the wild-type complexes that, on burial from solvent, pay an electrostatic desolvation penalty that is uncompensated by the interactions that they make in the complex. Such a distinction is something to which the physics-based electrostatic model deployed in the calculations is well suited. Thus, most successful predictions involved the substitution of polar with apolar residues, which will be less penalized for desolvation on complex formation. Where new polar interactions were also introduced, for instance, by replacing a wild-type threonine with an aspartate, these occurred on the periphery of the complex where detailed complementarity is less important. A pragmatic lesson may be that one can often improve affinity more by reducing desolvation penalties than by optimizing interaction energies.

By the same standard, the lack of correlation between the full energy function and the experimental interaction energies makes clear that this work is not a general solution to the problem of calculating ligand-protein binding affinities. As the authors acknowledge, the geometries of the complexes were refined by calculation using the full energy function, which was then discarded in favor of the electrostatic terms alone. This cannot be right, and may be expected to fail in many cases. Indeed, whereas single-site substitutions were well predicted in this study, calculations on multi-site substitutions correlated

poorly with experiment, as did predictions on the D1.3 antibody-lysozyme complex.

Notwithstanding these caveats, the results reported by Lippow *et al.* are encouraging. Their predictions derive from a general physics-based model that should be widely applicable to the electrostatic component of binding affinity, and the calculations were rapid enough to guide an experimental program of affinity maturation. The problems encountered here may be addressable by greater sampling of the states and configurations open to proteins and their ligands; methods to do so have been developed, although they remain slow enough to prohibit application to a large number of possible ligands. The calculations in Lippow *et al.* balance physical rigor with practicality and may be useful for optimizing other protein-protein complexes.

1. Lippow, S.M., Wittrup, K.D. & Tidor, B. *Nat. Biotechnol.* **25**, 1171–1176 (2007).
2. Chang, C.E. & Gilson, M.K. *J. Am. Chem. Soc.* **126**, 13156–13164 (2004).
3. Woo, H.J. & Roux, B. *Proc. Natl. Acad. Sci. USA* **102**, 6825–6830 (2005).
4. Mobley, D.L. *et al.* *J. Mol. Biol.* **371**, 1118–1134 (2007).
5. Tirado-Rives, J. & Jorgensen, W.L. *J. Med. Chem.* **49**, 5880–5884 (2006).
6. van Gunsteren, W.F. & Berendsen, H.J.C. *Angew. Chem. Int. Ed. Engl.* **29**, 992–1023 (1990).
7. Matthews, B.W. *Annu. Rev. Biochem.* **62**, 139–160 (1993).
8. Li, S. *et al.* *Cancer cell* **7**, 301–311 (2005).

including sunitinib (Sutent) and imatinib (Gleevec), have demonstrated that their anti-cancer effects are most likely due to their action on multiple signaling kinases. Chemogenomics and large-scale ligand profiling have also provided evidence that many drugs act on multiple targets.

Polypharmacology has traditionally been viewed by drug designers as an undesirable property that needs to be removed or reduced to produce ‘clean’ drugs that act on single targets. The assumption of ‘one drug for one target for one disease’ has influenced many aspects of drug-discovery strategy, including disease classification, target identification, screening, drug design and clinical-trial design. However, advances in systems biology suggest that complex diseases may not be effectively treatable by interventions at single nodes.

Large-scale gene knock-out experiments in model organisms have shown that biological systems are remarkably resilient to attack and perturbation. The robust phenotypes of biological systems often result from compensatory signaling routes that bypass the inhibition of individual proteins. Network biology theory predicts that modulating multiple nodes simultaneously is often required for modifying phenotypes⁴. Taken together, observations of phenotypic robustness after gene deletion and network biology theory indicate that in several instances exquisitely selective compounds may exhibit a lower-than-desired efficacy for the treatment of disease. Thus, compounds that selectively act on two or more targets of interest in theory should be more efficacious than single-target agents.

Yıldırım *et al.* applied network analysis to drugs and drug targets. Integrating publicly available drug data with genetic-disease associations, gene-expression information and protein-protein interaction data, they investigated the relationships between approved drugs. If drugs acted selectively on single targets, we should not expect a network but rather isolated, bipartite nodes. Although their analysis was limited by the relatively scarce amount of public drug-screening data⁵ compared with proprietary screening and literature databases, and by the incomplete mapping of the human protein interactome, the authors nevertheless observed a rich network of polypharmacology interactions between drugs and their targets. Indeed, drugs acting on single targets appear to be the exception.

Yıldırım *et al.*'s findings add to our growing understanding of the role of polypharmacology in drug action. Using network analysis of integrated data sets and a network distance metric, they were able to distinguish between palliative drugs, which relieve symptoms,

Network pharmacology

Andrew L Hopkins

Network biology illuminates our understanding of drug action.

The common analogy of drug action is that of a lock and key, with a drug acting as a selective ‘key’ that fits into the ‘lock’ of a specific drug target. Over the past two decades, the concept of designing exquisitely selective ligands to avoid unwanted side effects has become the predominant paradigm in drug discovery. However, a growing body of post-genomic biology is revealing a far more complex picture of drug action. An elegant new study by Yıldırım *et al.*¹ in this issue illustrates not only that there are many keys for each lock but also that it is far more

common than expected for a single key to fit multiple locks. Viewing drug action through the lens of network biology may provide insights into how we can improve drug discovery for complex diseases.

In recent years it has been appreciated that many effective drugs in therapeutic areas as diverse as oncology, psychiatry and anti-infectives act on multiple rather than single targets²—a phenomenon known as ‘polypharmacology’³. For example, the antibacterial action of β -lactams depends on inhibition of at least two of the multiple penicillin-binding proteins to induce cell death. Similarly, fluoroquinolone antibiotics inhibit both ParC and GyrA. Anti-psychotic drugs commonly exhibit a wide spectrum of activities across entire families of serotonin and dopamine receptors². Extensive studies on several new protein kinase inhibitors,

Andrew L. Hopkins is in the Division of Biological Chemistry and Drug Discovery, College of Life Sciences, University of Dundee, Dow Street, Dundee, DD1 5EH, United Kingdom.
e-mail: a.hopkins@dundee.ac.uk

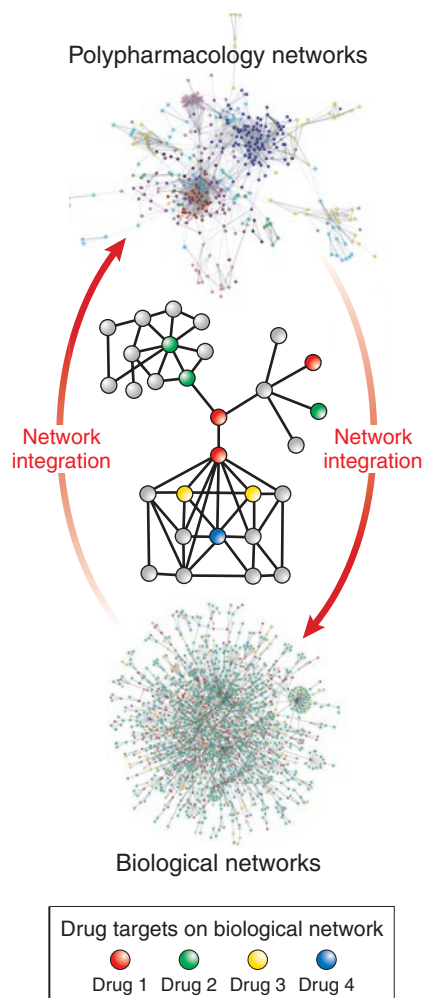


Figure 1 Network pharmacology. A network-centric view of drug action is built by mapping drug-target (polypharmacology) networks (left) onto biological networks (right). The network in the center is a part of the biological network in which nodes (proteins) targeted by the same drug are represented in the same color. Drug efficacy and toxicity can be understood by action at specific nodes and hubs.

sibility that statistical network analysis could be a useful tool for prioritization of potential drug targets a priori.

Mapping the polypharmacology network onto the human disease-gene network revealed not only that drugs commonly act on multiple targets but also that drug targets are often involved with multiple diseases. Over 40% of drug targets that map with disease genes mapped to more than one disease. In another study, a network analysis of the OMIM database of genetic associations showed that the genetic origins of most diseases are shared with other diseases: of 1,284 disorders catalogued in OMIM, 867 share at least one gene with another disorder⁶. This finding provides motivation to the growing interest in recent years in drug repurposing or indication-discovery strategies.

Traditionally, medicinal chemists have approached the design of ligands with multiple activities with trepidation, fearing complex structure-activity relationships or conju-

gated ligands with high molecular weights^{7,8}. However, the polypharmacology of approved drugs shows that a more opportunistic approach exploiting multi-target activity may be more attractive. Combining chemogenomics with network biology may enable a new network-pharmacology approach to drug discovery^{3,8,9} (Fig. 1). Developing methods to aid polypharmacology design⁹ can help to both improve efficacy and predict unwanted off-target effects. The recognition, informed by systems biology, that drugs for many disease states may require multiple activities to be efficacious, together with the observed promiscuity of old, small-molecule drugs, may indeed hold the clues to designing a new generation of drugs that perturb biological networks rather than individual targets.

1. Yıldırım, M.A., Goh, K.-I., Cusick, M.E., Barabási, A.-L. & Vidal, M. *Nat. Biotechnol.* **25**, 1119–1126 (2007).
2. Roth, B.L., Sheffler, D.J. & Kroeze, W.K. *Nat. Rev. Drug Discov.* **3**, 353–359 (2004).
3. Paolini, G.V., Shapland, R.H., van Hoorn, W.P., Mason, J.S. & Hopkins, A.L. *Nat. Biotechnol.* **24**, 805–815 (2006).
4. Barabási, A.L. & Oltvai, Z.N. *Nat. Rev. Genet.* **5**, 101–113 (2004).
5. Wishart, D. *et al. Nucleic Acids Res.* **34**, D668–D672 (2006).
6. Goh, K.-I. *et al. Proc. Natl. Acad. Sci. USA* **104**, 8685–8690 (2007).
7. Hopkins, A.L., Mason, J.S. & Overington, J.P. *Curr. Opin. Struct. Biol.* **16**, 127–136 (2006).
8. Morphy, R. & Rankovic, Z. *Drug Discov. Today* **12**, 156–60 (2007).
9. Keiser, M.J. *et al. Nat. Biotechnol.* **25**, 197–206 (2007).

and drugs that act directly on disease genes. Interestingly, the network distance metric highlights the trend in recent years towards an increase in drugs that target the genes associated with disease.

Network biology may also play a role in drug-target identification. Is it possible to identify drug targets from their position in a biological network? When the authors mapped drug targets onto human protein interaction data, they found that drug targets tend to have more interactions than average proteins but fewer interactions than essential proteins to a statistically significant degree. These data suggest that drug targets tend to be nodes positioned in a ‘goldilocks’ region of biological networks lying between the essential hubs and redundant peripheral nodes. If drug targets were positioned at nodes that are too highly connected, they would likely be essential proteins, whose perturbation may lead to toxicity. On the other hand, if drug targets were positioned at nodes at the periphery, they would likely be redundant, with little effect on disease phenotype if perturbed. The observation that drug targets, in general, tend to be highly connected but not essential opens up the pos-

AAV vectors and tumorigenicity

Mark A Kay

Chromosomal integration of rAAV vectors may induce hepatocellular carcinoma in neonatally treated mice.

Among the many viral vectors under development for gene therapy, recombinant adeno-associated viral (rAAV) vectors have shown special promise because of their efficacy and outstanding safety record in animals. Indeed, this vector system has been used to cure several diseases in animal models and is gaining in popularity for clinical gene-therapy trials. However, a recent study by Donsante *et al.*¹, published in *Science*, has revived a debate on the safety of rAAV vectors². The

Mark A. Kay is in the Departments of Pediatrics and Genetics, Rm G305, Stanford University, Stanford, California 94305, USA.
e-mail: Markay@stanford.edu

authors found that neonatal mice treated with a high dose of an rAAV vector showed an increased incidence of hepatocellular carcinoma. Moreover, they discovered a common vector-integration site near a microRNA (miRNA) cluster in 4/4 independent tumors, suggesting a mechanism based on insertional mutagenesis.

Several lines of evidence indicated that the rAAV vectors were contributing to malignancy. rAAV vector fragments were not detected in surrounding normal liver tissue. Genes close to the rAAV vector proviruses were overexpressed. About 10% of the ~400 known mouse microRNA sequences as well as some of the small nucleolar RNA genes are