Chapter 4
Systems Pharmacology: An Overview

Marc R. Birtwistle, Jens Hansen, James M. Gallo, Sreeharish Muppirisetty, Peter Man-Un Ung, Ravi Iyengar and Avner Schlessinger

Abstract Systems pharmacology has evolved from a discipline that focuses on drug action at the organ level to a discipline that combines traditional pharmacokinetic and pharmacodynamic modeling with recent systems biology approaches. The integration of high-throughput data technologies with computational data analysis and modeling offers new opportunities to overcome the one disease, one target, one drug approach. Whole genomic or transcriptomic sequencing and proteomics allow qualitative, and sometimes quantitative, snapshots of the cellular state at any given condition (e.g., during disease or after drug treatment) that can be the basis for the development of whole cell models to predict drug responses. Networks of protein–protein interactions that were confirmed by experimental and computational analysis of the structure of the interaction partners can be combined with graph theory to identify modules regulating the cellular state. Dynamic modeling and sensitivity analysis allow the identification of robust and fragile nodes within these modules to identify putative drug targets for single or combinatorial drug treatment. Traditional pharmacokinetic and pharmacodynamic modeling complements these approaches by predicting drug concentration and target perturbation at the site of drug action. Such general systems pharmacology models are a great leap forward towards the development of patient-specific drug response models as a main component of precision medicine.

Keywords Networks-based · Personalized medicine · Bioinformatics · Pharmacogenomics · Genbank · Cytochrome P450 · Protein–protein interaction (PPI) · Interactome · Druggability · Genomic variation · Nodes

M.R. Birtwistle · J. Hansen · J.M. Gallo · S. Muppirisetty · P.M.-U. Ung · R. Iyengar (✉) · A. Schlessinger
Department of Pharmacology and Systems Therapeutics, Systems Biology Center New York, Icahn School of Medicine at Mount Sinai, Box 1215 One Gustave Levy Place, New York, NY 10029, USA
e-mail: ravi.iyengar@mssm.edu

D.E. Mager and H.H.C. Kimko (eds.), *Systems Pharmacology and Pharmacodynamics*, AAPS Advances in the Pharmaceutical Sciences Series 23,
DOI 10.1007/978-3-319-44534-2_4

4.1 Introduction

Over the past two decades, as our understanding of molecular and cellular characteristics of biological organisms has grown, it has become clear that to understand how components come together to form functional units, such as the actin cytoskeleton for cell movement or the secretory machinery for hormone secretion, requires us to study biological processes in a holistic manner. By holistic, we mean that we keep track of both the components that make up the functional units and also how they interact to give rise to function (Sabathie et al. 1975; Weng et al. 1999). This field of study is called systems biology. The development of high throughput experimental technologies in genomics that allow us to sequence all the genes in a genome simultaneously (Shendure and Lieberman Aiden 2012) and measure genome-wide patterns of expression and gene modification (such as DNA methylation) provide a comprehensive basis for understanding the genetic and genomic underpinnings of cellular, tissue/organ, and organismal functions. Similarly, development of proteomics using mass spectrometry (Picotti and Aebersold 2012) has contributed to our knowledge of protein and protein state (e.g., phosphorylation) profiles within cells and tissues. The large data sets produced by these omics technologies can seldom be intuitively understood and require statistical and computational analyses for the data to be converted into knowledge. Typically a variety of statistical tools, Bayesian models, as well as network analyses based on graph theory are used to analyze large data sets. Bioinformatics is the field that studies the organization and storage of large data sets and their subsequent statistical and computational analysis. Although the pictures produced by such analyses are reasonably comprehensive, they are often qualitative snapshots in time and seldom or incompletely provide information about the dynamic or quantitative capabilities of systems. Yet, critical for drug action and pharmacology approaches is knowledge of precisely such characteristics—dose and timing. What is needed in this regard is a different class of experiments that are not yet high-throughput, as well as dynamical models based on differential equations to understand systems dynamics. Irrespective of the approaches used, it should be appreciated that systems biology uses an integrated approach wherein experiments and computational models are combined to provide insight into how the systems are organized and how this organization leads to function. The approach of integrating experiments and computation is not new in biomedical sciences. It has been used for over 50 years in biochemistry, physiology, and pharmacology. In the past, however, combinations of experiments and models dealt with functions at a single scale. Typically biochemistry focused on the atomic and molecular scale, whereas physiology and pharmacology focused on tissue/organ level functions. A distinctive characteristic of current systems biology approaches is that it is multiscale—often both in levels of organization as well as across time scales. Such analyses have the potential to help us understand how molecules and interactions can give rise to functions manifested at the cellular, tissue/organ, and organismal levels.

4.2 Systems Pharmacology: A Network-Based View of Drug Action

Traditionally, systems pharmacology has been used to describe studies of drug action at the level of organ systems (Brunton et al. 2011). A set of two workshops at the National Institutes of Health in 2008 and 2011 led to a white paper that provides an expansive view of quantitative and systems pharmacology in current times (Sorger et al. 2011). Currently, systems pharmacology in academia describes a research area that combines both high- and low-throughput experimental approaches of systems biology as well as a range of computational approaches including network analyses for drug discovery and studying drug action (Berger and Iyengar 2009; Zhao and Iyengar 2012).

The concepts from graph theory, the branch of mathematics focused on the study of networks, has been enormously useful in understanding regulatory features of cell and tissues/organs. Components of cells interact both directly and indirectly, and most components have multiple interactions. Networks capture these interactions and provide frameworks for understanding of how regulation arises from the interactions between cellular components. Feedback inhibition, which in network parlance is called a negative feedback loop, has been long known in biochemistry (Lehninger et al. 1992), but knowing how such loops can work in the context of other regulatory features provides insight into the regulatory capability of the system. The systematic description of regulatory units called network motifs (Milo et al. 2002), and the ability to identify such motifs within large regulatory networks (Wendell and Cianci 1992), provides mechanistic understanding of how the organization of systems contributes to regulatory capability. Since dysregulation is often a key feature of the pathophysiological state, understanding physiological and pathophysiological systems as networks is useful for both drug discovery and studying drug action. These studies are generally based on molecular interactions between the drug and its targets when these targets interact with and regulate other cellular components or the network biology of drug action (Zhao and Iyengar 2012). These network-based approaches have been useful in understanding the basis for cancer combination therapy (Boran and Iyengar 2010a, b), devising treatment regimens for optimal efficacy (Boran and Iyengar 2010a), origins of drug induced adverse events such as arrhythmias (Berger et al. 2010), and how multi-drug combinations can mitigate serious adverse events (Zhao et al. 2013). These successes indicate the value of network-based reasoning in the study of drug action.

Systems pharmacology is often thought of as extension of pharmacokinetics and pharmacodynamics that have been rooted in compartmental and physiologically based pharmacokinetic models and biomarker-based pharmacodynamic models. Each perspective—traditional and systems-based—serves a valuable role and represents the different facets of systems pharmacology that are schematically shown in Fig. 4.1. In this overview we briefly describe the components of systems pharmacology in the second decade of the 21st century that highlight an evolution in

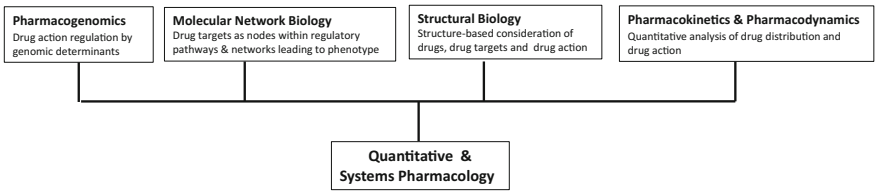

Fig. 4.1 Quantitative and systems pharmacology is a multidisciplinary field. The different areas of research that are integrated in quantitative and systems pharmacology are depicted as a flow diagram

going from structural biology-based drug discovery to a more integrated mechanistic pharmacokinetic and pharmacodynamic regime that accounts for genomic and epigenomic control of drug action. For this we start with a brief description of bioinformatics for drug discovery, drug action, and pharmacogenomics.

4.3 Systems Pharmacology: Relationships to Personalized and Precision Medicine

For some diseases, genomic determinants are useful predictors of drug efficacy as well as drug toxicity. Coding region SNPs have been very useful in identifying the ability of cytochrome P450 variants to metabolize warfarin. This relationship has allowed for the development of treatment regimens that enable the titrating of drug dosage to obtain maximal efficacy in reducing clot formation while minimizing the risk for internal bleeding (Aithal et al. 1999). The presence or absence of certain cancer mutations may determine whether a certain receptor antagonist would be an effective drug. For example, blockers of receptor tyrosine kinase activity are unlikely to be effective if there are downstream mutations in Ras that would likely change signal flux and drug efficacy. Of note, the Food and Drug Administration recommends genetic testing to ascertain that K-Ras is not mutated before the receptor tyrosine kinase inhibitor cetuximab is used for treatment of colorectal cancer (De Roock et al. 2013). Tailoring the use of medication or adjusting its dosage according to individual genetic makeup is called personalized medicine. This terminology is fairly synonymous with the term pharmacogenomics, which is described in a section below and is well known in clinical pharmacology. More recently a new term called “precision medicine” has been introduced (Toward Precision Medicine: Building a Knowledge Network for Biomedical Research and a New Taxonomy of Disease 2011) that the National Academies have defined as disease classification based on molecular characteristics. It is easy to see that the terms personalized and precision medicine are conflated to imply that genetic characteristics are the predominant characteristics to consider in disease classification and drug therapy. Whereas genetic characteristics are very important, there is

likely to be more to precision medicine in fact, to make it precise. What seems to be missing is a significant appreciation that the underlying principles of drug action are quantitative dynamic processes embodied in pharmacokinetics and pharmacodynamics. It is these disciplines that account for drug interactions—both pharmacokinetic and pharmacodynamic—and allow drug dosing schedules to be tailored to patients. In addition, depending on the extent of the models, drug toxicity can be predicted and used as a valuable guide to design therapeutic regimens. Although these dynamic processes can be analyzed semi-empirically in pharmacokinetic and pharmacodynamics studies, such analyses are not necessarily constrained by molecular details, and with the advent of systems-based approaches and its merging with pharmacokinetic/pharmacodynamic analyses, systems pharmacology has gained traction. Systems pharmacology will likely grow in importance given the interest and emphasis on precision medicine and the appeal to tailor therapy in the context of genomic and epigenomic determinants, as well as regulatory networks. Thus it is likely that systems pharmacology and precision medicine will develop in a naturally coordinated manner in the future.

4.4 Bioinformatics for Systems Pharmacology

The ability to construct large networks that can be used to understand drug action and drug discovery depends on the availability of large data sets. Among the largest and best characterized ones are those that contain gene related information, including gene variants that are related to drug action. These include the NCBI databases on various types of genomic information. Among the oldest is GenBank, which contains an annotated collection of all DNA sequences. Currently there are 19 databases under the heading DNA and RNA and another 17 databases under Genes and Expression. There may be some overlapping information, but overall these numbers provide a view of the vastness of the genomic data that is publicly available. A few databases that are directly relevant to systems pharmacology are listed in Table 4.1. In addition to genomic sequence information, there are also databases for gene and protein interactions, along with ontologies that associate genes with pathways and function. Gene Ontology is a widely used ontology database. There also exist a number of drug related databases such as Connectivity Map, DrugBank, PubChem and FAERS (Table 4.1). Each of these databases is important for systems pharmacology as these enable the construction of different types of networks to understand both drug action and to discover new drug targets for complex diseases.

Table 4.1 Representative list of databases used in systems pharmacology

Gene sequence and annotation	
NCBI gene_info	Contains gene related information such as taxonomy ids (i.e., organism), ENSEMBL gene identifiers, Human Protein Reference Database Identifiers (HPRD), official gene symbols, gene ids, gene synonym names and full gene names
NCBI gene2refseq	Contains gene related information such as taxonomy ids, gene ids, refSeq status, RNA nucleotide and protein accession identifiers and official gene symbols
NCBI homoloGene	Contains information about gene homologs between different organisms
MGI vertebrate homology	Contains information about gene homologs between mouse, human, rat and other organisms
NCBI dbSNP	Contains single nucleotide polymorphisms (SNPs), insertions and deletions, microsatellites and non-polymorphic variants
Gene or protein interactions	
TRANSFAC PWM	Associates transcription factors with their target genes
ChipX enrichment analysis (ChEA) background database	Associates transcription factors with their target genes, based on experimental results obtained from ChIP-chip and ChIP-seq studies
Stitch	Bork lab database of predicted protein-drug interactions using various approaches http://stitch.embl.de/
Kinase enrichment analysis (KEA) background database	Associates protein kinases with their protein phosphorylation targets
Ontologies: association of genes with biological processes or pathways	
Gene ontology (GO)	Probably the most extensive ontology available, categorizes its terms into three namespaces: biological process, cellular component and molecular function
Kyoto encyclopedia of genes and genomes (KEGG)	A smaller ontology with a focus on metabolic pathways. KEGG also offers metabolic pathway maps on their website
WikiPathways	Further ontologies that associate genes with biological pathways
Reactome pathway	
Online Mendelian Inheritance in Man (OMIM)	Associates human genes with genetic phenotypes
Mouse genome informatics (MGI) mammalian phenotype	A large ontology that associates genes with mammalian phenotypes
Drug related expression changes	
Connectivity map (CMPA)	Contains genome-wide transcriptional expression data from human cells that have been treated with various bioactive small molecules. It can be used to identify a small molecule that up- or down-regulated a certain gene set of interest

(continued)

Table 4.1 (continued)

Drug related databases	
Drug bank	Contains detailed drug-related information, such as an extensive list of drug target genes, drug-drug interactions, drug metabolizing enzymes and drug indication
PubChem	Database of small molecules and their activities in different biological assays that were obtained by high-throughput screening assays
ZINC	Largest dataset of purchasable small molecules for virtual screening http://zinc.docking.org/
ChEMBL	Database with small molecules and their activities against biological assays
FAERS FDA—adverse events reporting system	Contains information about adverse events documented by healthcare professionals during the treatment with a drug or drug combinations

4.4.1 Pharmacogenomics

As mentioned above, pharmacogenomics connects drug action in an individual to specific characteristics of the individual's genome. Variations in the genome are thought to account for a significant part of inter-individual variability in drug action, and genomic characteristics are used for determining dosing regimens as well as predictions for responsiveness to therapy. The best known example for prediction of dosing regimens based on genomic variations is the use of warfarin to regulate blood coagulation and thrombosis. Cytochrome P450 isoform CYP2C9 regulates the metabolism of warfarin. CYP2C9 has two polymorphisms that reduce the level of enzyme activity towards warfarin; consequently, increased warfarin concentrations in the blood result in an increased risk of bleeding. So if a patient has these CYP2C9 polymorphisms, the dosage of warfarin can be titrated to optimize therapy while reducing the risk of bleeding. Testing for warfarin metabolism has become a common approach to titrating warfarin dosage in clinical practice.

In treatment of cancers, genomic status, most often defined as presence or absence of oncogenic mutations, can be used to predict responsiveness to certain drugs. *KIT* oncogene mutations reduce the responsiveness of gastrointestinal stromal tumors to imatinib (Aithal et al. 1999); *k-RAS* oncogene mutations in colorectal cancer reduce responsiveness to cetuximab (De Roock et al. 2010); and epidermal growth factor receptor mutations in non-small-cell lung cancer alter responsiveness to gefitinib or erlotinib (Toward Precision Medicine: Building a Knowledge Network for Biomedical Research and a New Taxonomy of Disease 2011). In most of these cases, mechanisms underlying the genotype to drug response relationships are not fully understood, and one of the major goals of pharmacology is to provide mechanistic understanding of such multiscale relationships.

4.4.2 *Structural Reasoning in Systems Pharmacology*

Structural biology has long been an area in which experimental data and computational modeling have been integrated to obtain biological knowledge. Going from X-ray diffraction patterns to protein structures have always required building models, and even more so in modern structure determination via X-ray crystallography or NMR. Thus melding structural reasoning into systems pharmacology approaches for drug discovery involves integrating models to scale across multiple levels of organization. Several themes that form the basis for integrating models for multiscale understanding are described here.

4.4.2.1 Protein Structure Informs Network Biology

Since biological networks are defined by the physical interactions between their components, description of the precise molecular interactions between proteins, nucleic acids, and small molecules is a prerequisite for understanding the dynamics of lower resolution interaction networks and ultimately for designing drugs that perturb these networks. For instance, a highly connected node in a protein–protein interaction (PPI) network can be a protein that interacts with multiple partners through the same surface region at different times in different pathways, or a protein that interacts with multiple partners simultaneously using distinct surface regions (Kim et al. 2006). Currently, there are over 100,000 experimentally determined protein structures available in the Protein Data Bank (Rose et al. 2013); however, only a small fraction of these structures correspond to protein complexes within the interactome, making it difficult to map protein structure onto networks that can be easily generated with high-throughput approaches. Multiple computational approaches have been developed to predict the three-dimensional structures of protein complexes in various network types, relying on homology modeling, in which a target complex structure is based on experimentally determined structure of a related protein or protein complex (Davis et al. 2006; Sali and Blundell 1993). Mosca et al. developed Interactome3D, which is an automated homology modeling pipeline and visualization tool, to map structural information onto any PPI dataset provided by the user (Mosca et al. 2013). Homology modeling-based approaches can also be used to annotate previously unknown physical interactions. PrePPI is a Bayesian-based approach that combines protein structure information with co-expression and functional similarity data to predict protein–protein interactions (Zhang et al. 2012). PrePPI identified more protein–protein interactions in the yeast proteome than those identified with high-throughput experimental approaches and with higher accuracy, as well as covered a different fraction of the interactome (Zhang et al. 2012).

In addition, many key cellular functions are executed by large complexes or assemblies, such as the proteasome and ribosome. Experimental determination of the atomic structures of such assemblies can be technically challenging and costly,

and their modeling via homology is difficult because of limited structural coverage of their components. Integrative modeling approaches such as the integrative modeling platform (IMP), which generates three-dimensional structures or models of large complexes using restraints that are derived from various low-resolution data types (e.g., protein–protein interactions), can identify the domains and residues that are involved in specific interactions (Russel et al. 2012). The yeast nuclear pore complex structure, which includes 456 proteins, was modeled with IMP using low-resolution data from diverse sources, such as affinity purification of protein sub-complexes, sedimentation analysis, and electron microscopy (Alber et al. 2007).

4.4.2.2 Structural Considerations Guide Drug Discovery

Efficacy of a drug is strongly influenced by the extent that the target controls the phenotype and the strength of binding between the drug and target(s). The former is related to the system as a whole, and is typically analyzed by sensitivity analysis of models that link the target to phenotype (Birtwistle et al. 2007; Schoeberl et al. 2009; Zhang et al. 2014). The latter is related to target 'druggability', that is, particular structural features, such as binding pockets of suitable size, shape, and electrostatic properties to accommodate drug-like molecules with optimal bioavailability properties (e.g., the Lipinski rule-of-five (Lipinski et al. 2001)). Hopkins and Groom introduced the term "druggable genome" and estimated that about 10 % of the genes in the human genome are druggable, but only about half of these genes are both druggable and relevant to disease (Hopkins and Groom 2002). These druggable targets are primarily GPCRs, protein kinases, ion channels, and membrane transporters (Hopkins and Groom 2002).

Druggability of a putative target is typically analyzed with protein structural methods focused on physicochemical properties such as the presence of a surface hydrophobic pocket (Cheng et al. 2007; Kozakov et al. 2011; Perot et al. 2010). Recent efforts attempt to extend the druggable genome by targeting protein–protein interactions (Wells and McClendon 2007), developing covalent probes (Singh et al. 2011), as well as by targeting allosteric or cryptic binding sites that cannot always be observed in a static X-ray structure doi:10.1002/bip.22742 (Ung et al) (Ostrem et al. 2013). For example, for many years the Ras protein has been considered "undruggable". Ostrem et al. (2013) used a tethering technique to screen compounds against the cancer-associated Ras G12C variant. The X-ray structure of Ras G12C bound to a novel tethered inhibitor revealed a newly exposed pocket, thus providing a framework for developing more potent and bioavailable inhibitors.

Rational or structure-based drug discovery includes a wide range of approaches aimed at developing small organic molecules that bind druggable pockets and rely on concepts from medicinal chemistry and protein structure. This approach has been used to successfully develop at least ten marketed drugs, including the antihypertensive drug aliskiren (Rahuel et al. 2000), the antiviral telapravir (Lin et al. 2006), and the anticancer drug vemurafenib (Bollag et al. 2012). Computational

approaches are particularly useful for identifying novel chemical scaffolds and for optimizing lead molecules or known drugs against particular targets. For example, in structure-based virtual screening ('molecular docking') a large compound library is computationally screened, in which each small molecule is sampled in many configurations and scored based on its complementarity to the target structure (Shoichet 2004). This quick and inexpensive method—based on protein structure rather than the chemical structure of known small molecule ligands—is a powerful and validated tool for identifying novel chemical entities. For a protein target without an experimentally determined structure, virtual screening can be performed against homology models (Baker and Sali 2001; Jacobson and Sali 2004; Schlessinger et al. 2013). Furthermore, characterizing various conformational states of target proteins (e.g., with homology modeling or molecular dynamics simulations) enables researchers to identify conformation-specific modulators, thereby further increasing the pharmacological space (Durrant and McCammon 2013).

An emerging paradigm in modern drug discovery is one type of polypharmacology, in which a drug interacts with multiple targets with significant affinity to obtain effective therapy (Keiser et al. 2009; Roth et al. 2004; Xie et al. 2011). This is different from traditional drug discovery approaches, where a highly potent and selective drug is optimized toward a single specific biological target. Polypharmacology is observed in the treatment of various multigenic diseases such as central nervous system disorders and cancer. For example, the cancer drug sorafenib is a kinase inhibitor that binds to multiple targets such as BRAF, KDR and p38α in their inactive conformations (Fig. 4.2). Because polypharmacological modulators often have lower binding affinity to multiple targets rather than potently binding one single target (Xie et al. 2012), previously "undruggable" sites, such as those involving protein–protein interfaces, can also be targeted by such drugs. In rational polypharmacology, a drug or a cocktail of drugs is/are designed against multiple targets simultaneously, taking into account both drug and target structure. Dar et al. combined medicinal chemistry, biochemical assays, and fly genetics to systematically identify five distinct functional targets and 'anti-targets' for the treatment of Ret-MEN2B cancers (Dar et al. 2012). Anti-targets are proteins that when inhibited by drug, have undesirable effects such as increased toxicity or reduced efficacy, and thus should be avoided. They then developed two novel compounds that optimally interact with those targets/anti-targets to produce strongly efficacious compounds now in clinical trials. It can readily be seen that multiple targets could be part of a functional network, so we may in the future design single or multiple drugs that exert their effects by modulating the behavior of functional cellular networks rather than by modulating the activity of a single target. Identification of such targets and anti-targets clearly interfaces tightly with network-based thinking as described above.

Although protein structure-based approaches can be useful for rationalizing side effect and efficacy of polypharmacological drugs (Geier et al. 2013; Schlessinger et al. 2011), small molecule-based approaches are significantly more efficient in capturing unintended 'off-targets' that predict adverse drug reactions. The chemical similarity ensemble approach (SEA) relates proteins based on the chemical

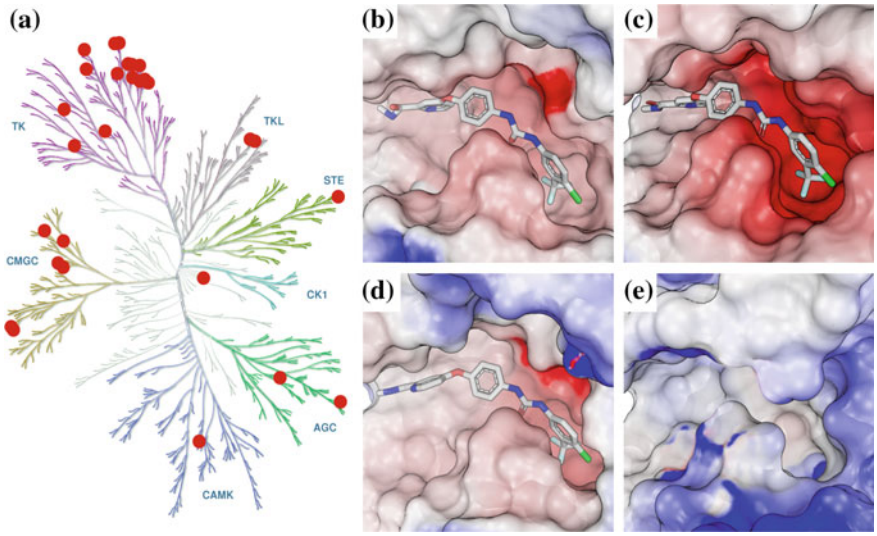

Fig. 4.2 Polypharmacology of the cancer drug sorafenib. **a** Phylogenetic tree of the human kinome (illustration reproduced courtesy of Cell Signaling Technology, Inc. (www.cellsignal.com), generated with Kinome Render (Chartier et al. 2013)), with sorafenib targets *highlighted in red circles*. The binding site structure of various protein kinases is shown in surface representation. **b** BRAF (PMID: 15035987) (Wan et al), **c** KDR (McTigue et al. 2012), and **d** p38α (Simard et al. 2009) are targets of sorafenib and their corresponding binding sites have negative electrostatic potential (*red*); **e** ERK is not a target of sorafenib and its modeled binding site exhibits lower negative electrostatic potential (*blue*) (PMID: 25420233; doi:10.1021/cb500696t) (Ung et al)

similarity among their ligands to build cross-target similarity networks that accurately identify previously unknown protein-drug interactions (Keiser et al. 2009).

4.4.2.3 Genomic Variation Imposes Constraints on Networks Through Protein and Drug Structure

Identifying which regions of a protein are responsible for different interactions is a key step toward understanding how protein function and interactions will be affected by genomic variation, including point mutations, deletions, and other mutation types. Nonsynonymous single nucleotide polymorphism (nsSNP) is a genetic variation that involves amino acid substitutions that can have a dramatic effect on stability, hydrogen-bond network, conformational dynamics, interaction, and many other physiologically important properties of proteins. Such properties can be critical for interacting with their partners including proteins, nucleic acids, and small molecule drugs (Wang and Moult 2001). For example, nsSNPs alter kinetic parameters of signaling pathways (e.g., E542K, E545K and H1047R on p110α for PI-3K catalytic activity). Protein structure reasoning can be applied to design "personalized drugs" that interact with variants associated with specific

diseases. Using X-ray crystallography and fragment-based screening, vemurafenib was specifically designed to target the BRAF V600E mutant for treating metastatic melanoma (Bollag et al. 2012).

Predicting how mutations affect protein structure, function, and druggability is therefore critical for modern drug discovery and personalized medicine. Various approaches aim at predicting mutation effect on functions such as destabilization of the native structure or interference with the binding of other proteins or small molecules (AlQuraishi et al. 2014; Kumar et al. 2009; Ramensky et al. 2002). Examples include (i) machine learning-based methods that are trained on sequence and biophysical features, such as solvent accessibility, flexibility, packing, and conservation of residues (Bromberg and Rost 2007; Kumar et al. 2009), and (ii) physics-based methods that compute the folding free energy to quantify the magnitude of a mutational effect on stability (Schymkowitz et al. 2005). Notably, deleterious point mutations can occur in binding interfaces and affect protein–protein interactions and lead to network rewiring and new targets. Interestingly, only a small fraction of the total residues in the binding interface contribute to most of the energy that is associated with binding (Wells and McClendon 2007). These interaction-stabilizing residues, which are often dubbed "hot spots", can also be predicted from structure using different approaches, such as computational alanine scanning, which computes the free energy effect of mutation to alanine for each of the binding interface residues, and other methods that consider evolutionary conservation (Kortemme and Baker 2002; Zhao et al. 2014). Analyzing the structural consequences of mutations in the context of networks and pathways can provide a mechanistic description for disease states and predict phenotypic effect. Kiel and Serrano performed a systematic analysis of 956 RASopathy and cancer mutations based on structures and energy predictions and showed that for the same gene, the type of mutation determines the diseases state (Kiel and Serrano 2014). Energy changes are higher for cancer mutations compared to RASopathy mutations, and RASopathy mutations are likely to cause only minor pathway dysregulation. These studies highlight how computational approaches allow the identification of relationships from structural variants to phenotypes to understand drug action. It is expected as such approaches get integrated into the drug discovery process, more personalized efficacious treatment for complex diseases such as type-2 diabetes, cancer, and heart failure will be forthcoming.

4.5 Network Dynamics in Systems Pharmacology

Network representations allow us to describe how molecules within the cell are connected through chemical reactions to one another. The organization within networks is often called topology, and network topology allows one to trace how a perturbation such as that evoked by a drug may be connected to important phenotypic outcomes such as cell fates or physiological responses. These networks can be analyzed to detect hubs, highly connected molecules that may be effective drug

targets. An ubiquitous network that regulates cell survival and proliferation is made up by the MAP-kinase and PI-3 kinase pathways as regulated by growth factor receptors (Fig. 4.3; von Kriegsheim et al. 2009). Such networks show topological features such as feedback and feed-forward loops. As described above, when information from network topology is combined with atomic structural information of the biological molecules within these networks, one can further identify what kinds of small molecule drugs may bind selectively to potential targets. Furthermore, at least in some cases, one can infer how this binding may depend on genomic variation that changes amino acid sequences, or understand how epigenetic information dictates connections between a perturbation and outcome in a context-dependent manner. This kind of reasoning can give tremendous insight into generating hypotheses for pharmacological targeting of disease-related networks. However there are limitations. One major limitation is the lack of consideration of quantitative and dynamic relationships between network nodes. If a certain network

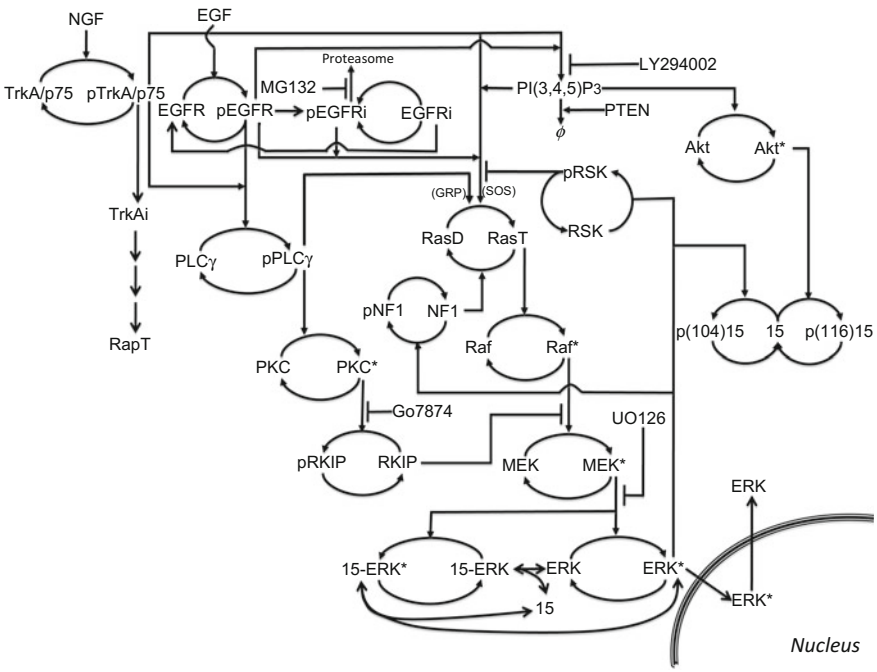

Fig. 4.3 Schematic of EGF and NGF signaling to ERK and Akt in PC-12 cells. Adapted from (von Kriegsheim et al. 2009). Epidermal growth factor (EGF) and nerve growth factor (NGF), stimulate activation of the EGF receptor (EGFR) and the TrkA receptor (NGFR), respectively, which can be internalized to different degrees (denoted by *i*). These active receptors lead to recruitment of activators and inhibitors of the Ras and PI-3K pathways, including a spatial regulator PEA-15 (denoted by 15). These pathways interact to form a complex network that regulates activity of ERK and Akt kinases, which in turn regulate multiple phenotypic outputs, depending on their temporal and spatial patterns. Multiple pharmacological compounds target nodes in this network

node is perturbed, will the perturbation be strong enough to propagate significantly to affect cell fate, or in the case of drug action, alter pathophysiological behaviors?

4.5.1 Fragile and Robust Nodes

Network and structural information give insight into a traditional definition of druggability by identifying a drug target and whether it is likely one can find a small molecule to bind this target. Although these are necessary criteria for defining drug targets, they are not sufficient. The question posed above evaluates druggability using the criterion of fragility or robustness of the target; only the former attribute is sought for a potential drug target. Fragility or robustness of a node is a systems-level feature of the biological network that defines how strong or weak a perturbation of that node affects an outcome of interest, and it is inherently quantitative. A fragile node mediates a large change in response to a small perturbation, and a robust node the opposite. We posit that an effective drug needs not only to be both structurally compatible with the target as is currently a usual focus of drug development, but also that the target must be "fragile", meaning that at therapeutic concentrations of the drug, it binds the target with adequate selectivity and avidity such that concentrations of the drug-target complex are sufficient for this perturbation to be propagated with significant strength to the functional effector of the network to evoke a therapeutic physiological response. In contrast, robust nodes would not sufficiently alter their activity upon binding the drug and hence do not induce change in response. Static, qualitative network models that are focused on topology give us a limited ability to evaluate such fragility.

4.5.2 Sensitivity Analysis to Assess Fragility

A common way to assess such systems-level druggability depends on first casting the biological network in terms of the elementary biochemical reactions that comprise it. This usually gives rise to ordinary, partial, or stochastic differential equation models that describe how these networks propagate signals and respond to drugs over space and time in a dose-dependent manner. Importantly, such models can give insight into this fragility or robustness question through a variety of systems engineering-inspired approaches including sensitivity analysis (Csete and Doyle 2002; Stelling et al. 2004; Kitano 2007). Sensitivity analysis is a collection of many methods that share the basic property of perturbing quantities in a mathematical model of a system, and then observing the change in an output(s) of interest. The magnitude of the sensitivity coefficient is related to fragility—the larger the sensitivity the more fragile the target. However, not all types of sensitivity analysis are appropriate for such biological networks. Because these networks are usually incompletely understood and non-linear, global sensitivity analyses, which

account for uncertainties in models and are not affected by non-linearities, are better suited for making such inferences of fragility (Kim et al. 2010; Zhang et al. 2014), and have even been used to make successful predictions for drug development, which surprisingly involved a kinase dead receptor ErbB3 (Schoeberl et al. 2009, 2010).

Predicting fragility of a single node is useful for understanding diseases that can be treated by a single drug or arise from defects in a single gene. However, many non-communicable diseases that are progressive are multi-factorial and would benefit from combination therapy. Additionally, an otherwise efficacious drug might have unacceptable toxicity that can be mitigated by a second drug (Zhao et al. 2013). High-throughput screening technologies can quite effectively explore responses of cells to single drugs and even dose responses of those drugs. When one is considering drug combinations; however, the number of potential experiments explodes into a number that is experimentally infeasible. Mathematical models of the system of interest can be useful to providing leads on potentially good drug combinations. Because they are based only on simulation, they are almost always quicker to evaluate than a typical high-throughput screening assay, as well as less expensive. In order for model analysis to yield useful predictions, those predictions must be precise, meaning that the variance in the prediction of drug response when model uncertainty is taken into account is relatively small. Small, here, is defined as that level which allows a robust decision to be made as to whether the drug combination is suitable for testing or not, and as such may be quite different depending on the particular scenario. Most of these models have a significant amount of uncertainty in them, both in the connections between biological molecules (network topology) and in the values of parameters that describe these interactions (e.g., binding affinities). How much experimental data does one need to build a model that makes precise and usable predictions for drug discovery as well as drug action? A gold standard would be a dataset that gives so-called full observability of the system, meaning that based on the measurements made, one can uniquely calculate the values of every unique chemical species in the model. However, one needn't know every parameter in the model precisely to make useful predictions of how model variables respond to perturbations; most if not all models of biological signaling networks exhibit a property called sloppiness in which time trajectories of key model variables are quite invariant to changes in most model parameters (Gutenkunst et al. 2007a, b). From a biological perspective this is not surprising, since this implies that key model variables are robust to most perturbations, which should be the case due to evolutionary pressures for these networks to function in the face of multiple forms of uncertainty and noise.

In any case, this understanding of sloppiness still does not answer the question of data requirements for the model. Just how well do we need to know the parameter values to make predictions of responses to single drugs and drug combinations that are of sufficient precision? To gain insight into this question, we performed simulations with a model of a mitogen activated protein kinase cascade (MAPK) (Huang and Ferrell 1996) (Fig. 4.4a) which controls many important cellular functions from yeast to mammals (Kholodenko and Birtwistle 2009) and is thus the

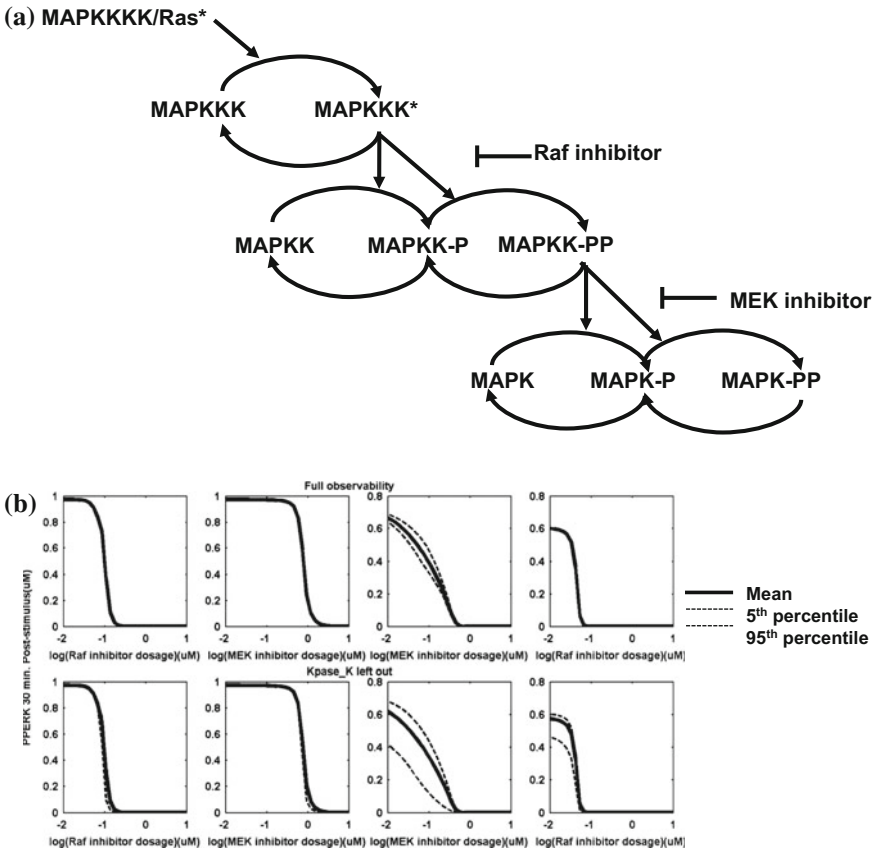

Fig. 4.4 Drug Action in the MAP—kinase pathway. Simulations with the Huang-Ferrell MAPK model using MAPKKK (Raf) and MAPKK (MEK) inhibitors. **a** Simplified model schematic. **b** Drug dosage responses computed as function of steady-state active MAPK/ERK (PPERK) concentration after drug administration for Raf, MEK, and Raf and MEK combined inhibitions. At least 25 drug dose response curves make up each plot, which correspond to different good fitting parameter sets, and are summarized by their mean, 5th and 95th percentiles. The *top panel* is simulations when the model is fit to simulated data that gives full system observability, and the *bottom panel* corresponds to when just a single observable, the MAPK-MAPK phosphatase complex, is removed from estimation. The *first two columns* are drug dosage responses for Raf and MEK inhibitors alone, and the *last two columns* correspond to drug combinations

target of many drugs, particularly anti-cancer drugs. We simulated time-course data (with added noise) from this system in response to an endogenous activation of the pathway, and ensured that the considered data were both experimentally feasible and gave full system observability. Next, different sets of kinetic parameters with the same goodness of fit were estimated based on minimizing the error between model predictions and simulated data using at least 25 different starting guesses to evaluate parametric uncertainty. These fitted parameter sets were then used to

predict MAPK (ERK) steady-state responses to a MAPKKK (Raf) drug, a MAPKK (MEK) drug, and a combination of the two that produced a range of predictions for drug responses. The gold standard dataset that allows full observability of this system (15 different quantities measured over time), not surprisingly, yielded very precise predictions of responses to single drugs and the drug combinations (Fig. 4.4b). However, if a single observable is left out during the parameter estimation, for example a somewhat non-obviously important one, such as the total level of MAPK/MAPK phosphatase complex (which actually has small sensitivity), single drug dose response predictions remain quite precise, whereas drug combination response predictions lose a significant amount of precision (Fig. 4.4b). This result holds for several such leave-one-out simulation experiments. Thus, it seems that making precise and therefore useful model-based predictions for drug combination responses requires a higher fidelity of parametric certainty, and therefore more experimental data than is often considered. More research into this important topic is needed if such models are to be useful in informing drug development and choices for potentially effective drug combinations to test further in experimental studies.

4.5.3 Sensitivity Analysis for Discovery of Targets for Combination Therapy

The previous line of thought is based on evaluating a pre-existing hypothesis for a drug combination; will the combination of two selected drugs be effective? How can we use quantitative biological network models to suggest co-fragile nodes, and therefore potential drug combinations a priori, before drugs are selected? This is an area where global sensitivity analysis is useful. While there are a variety of methods for global sensitivity analysis, we have had success in applying the method of Sobol to such differential equation models of biological pathways (Zhang et al. 2014; Sobol 2001; Saltelli 2008). One advantage of Sobol sensitivity analysis is that it allows rigorous calculation of uncertainty on the sensitivity coefficients, and each sensitivity coefficient is bounded between 0 and 1, with certain sums constrained to add to unity. Thus, the absolute value of Sobol sensitivity coefficients is directly meaningful and its statistical significance can be evaluated. Although Sobol sensitivity analysis can require a significant number of model simulations, it is a parallel problem, so utilizing high performance computing resources to implement it is quite straightforward and easily scaled. Based on Sobol sensitivity analysis, one can estimate both the impact of single parameters on an important output of interest (1st or total order terms), and the simultaneous impact that two parameters have on the output of interest (so-called 2nd order interaction terms). Single parameter sensitivity coefficients can be useful to identify drug combinations when simulations are performed with a first drug already selected and present in the model. The 2nd order parameter sensitivity coefficients that quantify interactions between

parameters give direct insight into potentially useful drug combinations without having to first consider a single drug.

Such simulations to suggest drug combinations of course do not give any insight into dosing strategies or regimens. For these purposes one must couple biological network dynamics that regulate drug action with pharmacokinetic models of the drugs.

4.6 Networks to Pharmacodynamics

Most textbooks of biochemistry contain detailed diagrams of biochemical processes; from glycolysis pathways to the TCA cycle to signaling pathways and networks. These descriptions of interconnected biochemical reactions (networks) provide a blueprint of normal physiology and also how they can be disrupted in various diseases. The level of detail and complexity is impressive and each reaction subset or single reactions that detail molecular conversions and enzyme kinetics can be further scrutinized and elaborated. Although such description of biochemical pathways and networks have existed for some time and are continually revised and expanded, they (the most part) have had little influence on drug discovery or studies of drug action. The modus operandi in drug discovery had been rational drug design for specific targets. Whether that drug design process involved protein structure and computer docking or more simply chemical modifications of lead compounds, the focus was the drug-target interaction defined by K_i and IC_{50} values. Even as drug discovery evolved into high-throughput screening techniques, the one drug-one target approach has been commonly used. It is, with few exceptions, fair to say that the worlds of detailed biochemistry of pathways and networks and the drug discovery and development were silos on different farms.

Now the drug discovery enterprise is changing; its deconstruction is not so much to do with an appreciation of the complexity of drug action but rather a realization that drug development expenditures are escalating faster than the return on investment; new blockbuster drugs are hard to find. Within this milieu of reanalysis, new strategies are being considered; from a growing emphasis on moving modeling and simulation, a standard practice in pharmaceutical companies, to more “radical” systems-based approaches (Benson and van der Graaf 2014; Milligan et al. 2013; Birtwistle et al. 2013). Such systems-based drug development is evolving from the juxtaposition of an appreciation of complex drug action and the revolution of bioinformatics heralded by the technological advances in genomics, including broad-based microarrays and next generation sequencing. The mindset that drug action is complex—not one drug-one target—is a natural evolution that also includes drug toxicity, and now, terms like “off-targets” and “repurposing” are common and have spawned new industries (Hurle et al. 2013; McCarthy et al. 2013). Informatics serves many functions from identifying prognostic risk factors such as individual genes and their variants, to gene sets or signatures as biomarkers for disease progression, to serving as a template for personalized medicine. It is the

latter use that may impact preclinical drug discovery and development with a central question, "which patients will benefit from our drug"? Superimposing complex drug action and a gene regulatory network on a single canvas paints a new picture that may be referred to as systems pharmacology. The gene network can be used to develop protein networks, or these protein networks can be developed independently using proteomic tools. A systems pharmacological view of drug action, whether for therapeutic efficacy or toxicity, can be cast as a subnetwork of drug targets within a larger regulatory network comprised of cell signaling pathways (Fig. 4.3). Pharmacodynamics (Levy 1966) predates this systems view and had more or less adopted the early view of one drug:one target where the measured response was either the direct drug target—target engagement—or a tell-tale downstream biomarker, for example phosphorylated Erk is often a measured biomarker for EGFR inhibitors (Wang et al. 2008). Not dissimilar to the black box compartmental modeling that is done in pharmacokinetics, pharmacodynamic models have also largely been a black box, the input is a drug concentration and the output of the box a measured response; mostly relative to control or pre-dose. Of course the limitation of black box pharmacodynamics is that without a mechanistic framework, patient responses may not correlate to the black box output, and even if they do, there are sufficient variations of the inner working of the black box that interpretation of the biomarker is fortuitous rather than causal. A good example is the work done by Iyengar et al. (2012) in which an EGFR biochemical network containing up to four genetic alterations (i.e., overexpression, SNP, miRNA expression, methylation status of promoter) exposed to the same degree of EGFR inhibition produced uniquely different responses based on tumor size. Measurement of a single biomarker—here tumor size or extent of EGFR inhibition—provides no insight as to why tumor size varied in these virtual patients or why the same degree of EGFR inhibition (80 % in this case) did not produce the same tumor size. The EGFR model was referred to as an enhanced pharmacodynamic model (ePD) to denote the shift from traditional pharmacodynamic models. Others have also appreciated complex drug action and put forth pharmacodynamic models that could double as a biochemical scheme. For example, a pharmacodynamic model for methotrexate depicted its multiple enzyme targets as well as interconnected metabolites (Panetta et al. 2010). The potential value of ePD models is clear. Whether a drug has only one or multiple targets, the influence of measured or predicted changes in the interconnecting proteins on signal flux and the final drug response can be determined. Thus, each patient may have a unique pharmacodynamic network or model—cast as a set of ODEs—that provides a simulation tool of their drug response (i.e., precision medicine). The individual ePD model outputs can be used directly to tailor therapy or analyzed as a population of patients to address questions like "is there a subset of patients that favorably respond to the drug and what are the biochemical characteristics"? By addressing the latter questions early in the drug development process, perhaps with a virtual population, an efficient means to decide on the future development strategy could be attained.

4.7 Systems-Based PK/PD Models

Pharmacodynamic models rarely exist as isolated entities and are linked to pharmacokinetic models to increase their usefulness by providing a total pharmacological package (Fig. 4.5). Historically, PK models were multi-compartment models that consisted of a set of black boxes to represent the various tissue regions that behaved kinetically quite similar. A link to the drug target organ via an effect compartment sufficed as a combined PK/PD model, wherein the effect compartment was often represented as the Sigmoid E_{max} model (Sheiner et al. 1979). In many cases, rather than the effect compartment formulation, the plasma drug concentration—again generated from the classic compartmental modeling approach—provided the link to the pharmacodynamic model. Even with the seminal advance of indirect response pharmacodynamic models made by Jusko and coworkers, plasma drug concentrations most often provided the link between PK and PD models (Dayneka et al. 1993). The simplicity of plasma drug concentrations was also utilized in conjunction with a VEGFR ePD model used to design novel multidrug regimens (Zhang et al. 2014). Although plasma drug concentrations have merit in

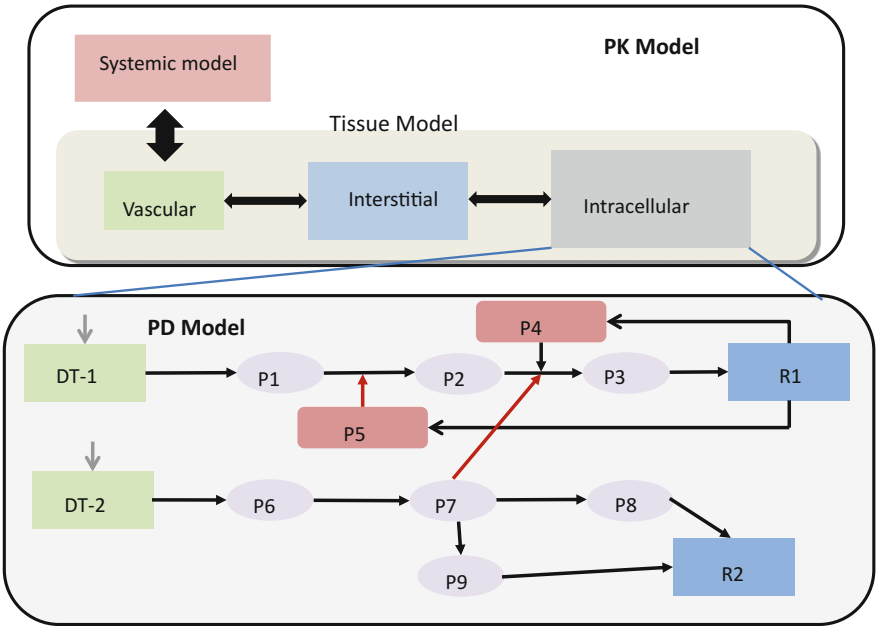

Fig. 4.5 Idealized physiologically-based pharmacokinetic/enhanced pharmacodynamic model. The ability to predict intracellular drug concentrations provides a natural link to mechanistic ePD models. *Thick black arrows* in PK model represent drug transport, DT-1 and DT-2 are 2 different drug targets, P1–P9 are different proteins with interconnecting arrows representing enzymatic reactions, R1 and R2 are two different drug responses, and *red arrows* represent negative feedback reactions

the clinical application of PK/PD models, it is known that drug concentrations at cellular receptors in tissues drive responses and may not necessarily mirror plasma drug concentrations. An alternative pharmacokinetic modeling approach—physiologically-based (PB) PK models—had been devised based on the prediction of tissue drug concentrations that was somewhat obscure until it was rediscovered during the upheaval in the drug discovery industry (Rowland et al. 2011). Since the original intent of PBPK models was to predict drug concentrations based on species- and drug-dependent parameters, it may be casted as an in silico tool to evaluate candidate drug pharmacokinetics expeditiously and without extensive data. During this same period, others appreciated the tissue-based assessment of drug disposition, its mechanistic potential, and scalability to patients (Gallo et al. 2004; Laplanche et al. 2007; Zhou et al. 2007). Now adjoining PBPK and enhanced pharmacodynamic models was a natural fit (Gallo 2013), and highlighted by an early investigation of the anticancer drug geldanamycin by the D'Argenio group (87). PBPK models can be used to estimate intracellular drug concentration (Fig. 4.5) and this capability was extended to a new paradigm referred to as cell-type specific (CTS) PBPK/ePD models (Ballesta et al. 2014). The full power of CTS PBPK/ePD models has yet to be realized; however, it provides a foundation to contrast drug efficacy and toxicity in their appropriate cell types, and further to account for heterogeneity and personalized therapy (Ballesta et al. 2014). At the same time, due to the potential size and number of model parameters in PBPK/ePD models, the same considerations of model construction and parameter estimation (sloppiness) faced by network biochemical models as mentioned above also confronts PBPK/ePD models (Gutenkunst et al. 2007a). Nonetheless, given their potential to enhance mechanistic models, drug development, and novel therapeutic strategies, it is believed that the field of system pharmacology will embrace these challenges and through innovation move the field forward.

4.8 Future of Systems Pharmacology

The majority of diseases for which we lack effective therapeutics are complex and progressive in nature. These include cardiovascular diseases such as heart failure, type-2 diabetes, and kidney disease among others. Both the progression of the disease and response to current therapy show considerable variability among patients. These differences have highlighted the need for understanding the individual patient in terms of her/his genomic and proteomic characteristics. Such understanding of disease mechanisms in individual patients has been largely focused on using genetic characteristics of the individuals. This approach has been successful in limited cases in which singular genetic (and protein) abnormalities determine drug response, such as for warfarin therapy. However, for complex diseases, we are likely to have multiple genes as well as postgenomic events regulating the disease state. Hence a systems biology-based approach that uses network topology to describe disease states is likely to be a very useful step in

developing dynamical models for disease progression. These models in turn should enable the definition of mechanism-based rules to not only understand why a drug may be effective but can further be applied to identify combinations of drug targets (Fig. 4.6, blue boxes). Some of these targets may already have drugs that bind and modulate their activity. In such cases, these drugs can be repurposed to treat other diseases distinct from the one they were originally intended to treat. In other cases, we may need to develop new drugs for particularly novel targets, but these efforts can be made more efficient when systems level models are available.

Beyond the general approach of considering disease networks in population of patients, there is an urgent need to understand variability, as disease progression differs among patients (?) and the same drugs show variable efficacy in different individuals. Here the development of disease state models that are personalized for each patient using the genomic characteristics guided by protein structure constraints, as well as dynamical features that are patient specific, can set the stage for ePD models that lead to precise individualized dosing regimens (Fig. 4.6, orange boxes). The integration of systems biology with pharmacology has given rise to the new field of systems pharmacology that provides an intellectual framework to consider complex physiology, pathophysiology, and drug action in a systematic

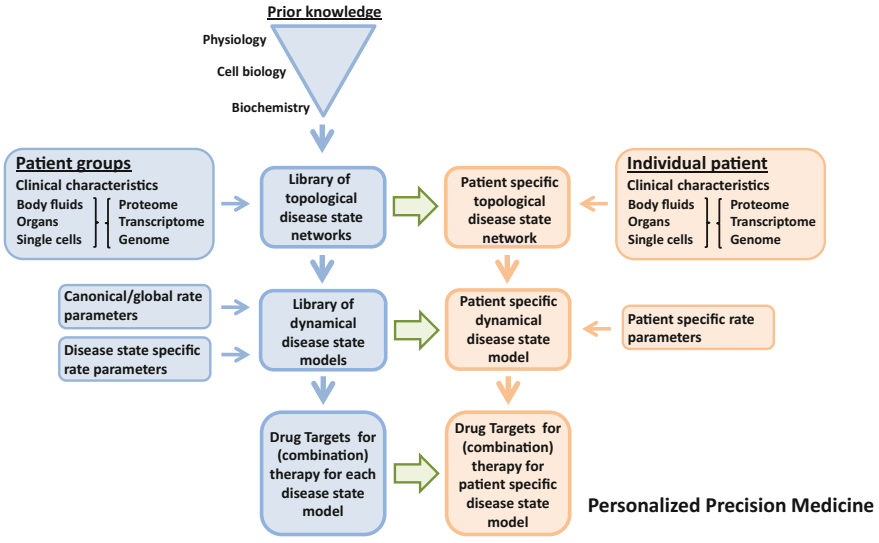

Fig. 4.6 Disease state specific models for systems pharmacology. The process diagrams for workflow in systems pharmacology for drug discovery for combination therapy and for personalized precision medicine. Typically data from groups of patients are combined to construct canonical disease state networks that can be used to construct dynamical models and ideal combinations of targets that are predicted to be efficacious. The topological disease state networks can be personalized for an individual patient using genomic, transcriptomic, or proteomic determinants. Such networks can serve as the basis of dynamical models that are personalized using patient specific parameters that can be used to predict dosing regimen and therapeutic strategies for the specific patient

manner to drive both drug discovery and therapeutics at an individual level. Thus, the impact of systems pharmacology on personalized and precision medicine is likely to be substantial.

References

Aithal GP, Day CP, Kesteven PJ, Daly AK (1999) Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement and risk of bleeding complications. Lancet 353(9154):717–719. doi:10.1016/S0140-6736(98)04474-2

Alber F, Dokudovskaya S, Veenhoff LM, Zhang W, Kipper J, Devos D, Suprapto A, Karni-Schmidt O, Williams R, Chait BT, Sali A, Rout MP (2007) The molecular architecture of the nuclear pore complex. Nature 450(7170):695–701. doi:10.1038/nature06405

AlQuraishi M, Koytiger G, Jenney A, MacBeath G, Sorger PK (2014) A multiscale statistical mechanical framework integrates biophysical and genomic data to assemble cancer networks. Nat Genet 46(12):1363–1371. doi:10.1038/ng.3138

Baker D, Sali A (2001) Protein structure prediction and structural genomics. Science 294 (5540):93–96. doi:10.1126/science.1065659

Ballesta A., Zhou Q, Zhang X, Lv H, Gallo JM (2014) Multiscale design of cell-type-specific pharmacokinetic/pharmacodynamic models for personalized medicine: application to temozolomide in brain tumors. CPT Pharmacometrics Syst Pharmacol 3:e112

Benson N, van der Graaf PH (2014) The rise of systems pharmacology in drug discovery and development. Future Med Chem 6(16):1731–1734. doi:10.4155/fmc.14.66

Berger SI, Iyengar R (2009) Network analyses in systems pharmacology. Bioinformatics 25 (19):2466–2472. doi:10.1093/bioinformatics/btp465

Berger SI, Ma'ayan A, Iyengar R (2010) Systems pharmacology of arrhythmias. Sci Signal 3(118): ra30. doi:10.1126/scisignal.2000723

Birtwistle MR, Hatakeyama M, Yumoto N, Ogunnaike BA, Hoek JB, Kholodenko BN (2007) Ligand-dependent responses of the ErbB signaling network: experimental and modeling analyses. Mol Syst Biol 3:144. doi:10.1038/msb4100188

Birtwistle MR, Mager DE, Gallo JM (2013) Mechanistic vs. empirical networks models of drug action. CPT Pharmacometrics Syst Pharmacol 2(9):1–3

Bollag G, Tsai J, Zhang J, Zhang C, Ibrahim P, Nolop K, Hirth P (2012) Vemurafenib: the first drug approved for BRAF-mutant cancer. Nat Rev Drug Discov 11(11):873–886. doi:10.1038/nrd3847

Boran AD, Iyengar R (2010a) Systems approaches to polypharmacology and drug discovery. Curr Opin Drug Discov Devel 13(3):297–309

Boran AD, Iyengar R (2010b) Systems pharmacology. Mt Sinai J Med 77(4):333–344. doi:10.1002/msj.20191

Bromberg Y, Rost B (2007) SNAP: predict effect of non-synonymous polymorphisms on function. Nucleic Acids Res 35(11):3823–3835. doi:10.1093/nar/gkm238

Brunton L, Chabner B, Knollman B (2011) The pharmacological basis of therapeutics, 12th edn. McGrawHill Medical, London

Chartier M, Chenard T, Barker J, Najmanovich R (2013) Kinome Render: a stand-alone and web-accessible tool to annotate the human protein kinome tree. PeerJ 1:e126. doi:10.7717/peerj.126

Cheng AC, Coleman RG, Smyth KT, Cao Q, Soulard P, Caffrey DR, Salzberg AC, Huang ES (2007) Structure-based maximal affinity model predicts small-molecule druggability. Nat Biotechnol 25(1):71–75. doi:10.1038/nbt1273

Csete ME, Doyle JC (2002) Reverse engineering of biological complexity. Science 295 (5560):1664–1669. doi:10.1126/science.1069981

Dar AC, Das TK, Shokat KM, Cagan RL (2012) Chemical genetic discovery of targets and anti-targets for cancer polypharmacology. Nature 486(7401):80–84. doi:10.1038/nature11127

Davis FP, Braberg H, Shen MY, Pieper U, Sali A, Madhusudhan MS (2006) Protein complex compositions predicted by structural similarity. Nucleic Acids Res 34(10):2943–2952. doi:10.1093/nar/gkl353

Dayneka NL, Garg V, Jusko WJ (1993) Comparison of four basic models of indirect pharmacodynamic responses. J Pharmacokinet Biopharm 21(4):457–478

De Roock W, Jonker DJ, Di Nicolantonio F, Sartore-Bianchi A, Tu D, Siena S, Lamba S, Arena S, Frattini M, Piessevaux H, Van Cutsem E, O'Callaghan CJ, Khambata-Ford S, Zalcberg JR, Simes J, Karapetis CS, Bardelli A, Tejpar S (2010) Association of KRAS p.G13D mutation with outcome in patients with chemotherapy-refractory metastatic colorectal cancer treated with cetuximab. JAMA 304(16):1812–1820. doi:10.1001/jama.2010.1535

De Roock W, Jonker DJ, Di Nicolantonio F, Sartore-Bianchi A, Tu D, Siena S, Lamba S, Arena S, Frattini M, Piessevaux H, Van Cutsem E, O'Callaghan CJ, Khambata-Ford S, Zalcberg JR, Simes J, Karapetis CS, Bardelli A, Tejpar S (2013) Association of KRAS p.G13D mutation with outcome in patients with chemotherapy-refractory metastatic colorectal cancer treated with cetuximab. JAMA 304(16):1812–1820. doi:10.1001/jama.2010.1535

Durrant JD, McCammon JA (2013) Molecular dynamics simulations and drug discovery. BMC Biol 9:71. doi:10.1186/1741-7007-9-71

Gallo JM (2013) Physiologically based pharmacokinetic models of tyrosine kinase inhibitors: a systems pharmacological approach to drug disposition. Clin Pharmacol Ther 93(3):236–238. doi:10.1038/clpt.2012.244

Gallo JM, Vicini P, Orlansky A, Li S, Zhou F, Ma J, Pulfer S, Bookman MA, Guo P (2004) Pharmacokinetic model-predicted anticancer drug concentrations in human tumors. Clin Cancer Res 10(23):8048–8058. doi:10.1158/1078-0432.CCR-04-0822

Geier EG, Schlessinger A, Fan H, Gable JE, Irwin JJ, Sali A, Giacomini KM (2013) Structure-based ligand discovery for the Large-neutral Amino Acid Transporter 1, LAT-1. Proc Natl Acad Sci USA 110(14):5480–5485. doi:10.1073/pnas.1218165110

Gutenkunst RN, Waterfall JJ, Casey FP, Brown KS, Myers CR, Sethna JP (2007a) Universally sloppy parameter sensitivities in systems biology models. PLoS Comput Biol 3(10):1871–1878

Gutenkunst RN, Casey FP, Waterfall JJ, Myers CR, Sethna JP (2007b) Extracting falsifiable predictions from sloppy models. Ann NY Acad Sci 1115:203–211. doi:10.1196/annals.1407.003

Hopkins AL, Groom CR (2002) The druggable genome. Nat Rev Drug Discov 1(9):727–730. doi:10.1038/nrd892

Huang CY, Ferrell JE Jr (1996) Ultrasensitivity in the mitogen-activated protein kinase cascade. Proc Natl Acad Sci USA 93(19):10078–10083

Hurle MR, Yang L, Xie Q, Rajpal DK, Sanseau P, Agarwal P (2013) Computational drug repositioning: from data to therapeutics. Clin Pharmacol Ther 93(4):335–341. doi:10.1038/clpt.2013.1

Iyengar R, Zhao S, Chung SW, Mager DE, Gallo JM (2012) Merging systems biology with pharmacodynamics. Sci Transl Med 4(126):126ps127. doi:10.1126/scitranslmed.3003563

Jacobson M, Sali A (2004) Comparative protein structure modeling and its applications to drug discovery. Annual Reports in Medicinal Chemistry Inpharmatica Ltd., London, pp 259–276

Keiser MJ, Setola V, Irwin JJ, Laggner C, Abbas AI, Hufeisen SJ, Jensen NH, Kuijer MB, Matos RC, Tran TB, Whaley R, Glennon RA, Hert J, Thomas KL, Edwards DD, Shoichet BK, Roth BL (2009) Predicting new molecular targets for known drugs. Nature 462(7270):175–181. doi:10.1038/nature08506

Kholodenko BN, Birtwistle MR (2009) Four-dimensional dynamics of MAPK information processing systems. Wiley Interdiscip Rev Syst Biol Med 1(1):28–44. doi:10.1002/wsbm.16

Kiel C, Serrano L (2014) Structure-energy-based predictions and network modelling of RASopathy and cancer missense mutations. Mol Syst Biol 10:727

Kim PM, Lu LJ, Xia Y, Gerstein MB (2006) Relating three-dimensional structures to protein networks provides evolutionary insights. Science 314(5807):1938–1941. doi:10.1126/science.1136174

Kim KA, Spencer SL, Albeck JG, Burke JM, Sorger PK, Gaudet S, Kim do H (2010) Systematic calibration of a cell signaling network model. BMC Bioinf 11:202. doi:10.1186/1471-2105-11-202

Kitano H (2007) A robustness-based approach to systems-oriented drug design. Nat Rev Drug Discov 6(3):202–210. doi:10.1038/nrd2195

Kortemme T, Baker D (2002) A simple physical model for binding energy hot spots in protein-protein complexes. Proc Natl Acad Sci USA 99(22):14116–14121. doi:10.1073/pnas.202485799

Kozakov D, Hall DR, Chuang GY, Cencic R, Brenke R, Grove LE, Beglov D, Pelletier J, Whitty A, Vajda S (2011) Structural conservation of druggable hot spots in protein-protein interfaces. Proc Natl Acad Sci USA 108(33):13528–13533. doi:10.1073/pnas.1101835108

Kumar P, Henikoff S, Ng PC (2009) Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc 4(7):1073–1081. doi:10.1038/nprot.2009.86

Laplanche R, Meno-Tetang GM, Kawai R (2007) Physiologically based pharmacokinetic (PBPK) modeling of everolimus (RAD001) in rats involving non-linear tissue uptake. J Pharmacokinet Pharmacodyn 34(3):373–400. doi:10.1007/s10928-007-9051-7

Lehninger A, Nelson D, Cox M (1992) Principles of biochemistry, 2nd edn. Worth Publishers Inc, New York

Levy G (1966) Kinetics of pharmacologic effects. Clin Pharmacol Ther 7(3):362–372

Lin C, Kwong AD, Perni RB (2006) Discovery and development of VX-950, a novel, covalent, and reversible inhibitor of hepatitis C virus NS3.4A serine protease. Infect Disord Drug Targets 6(1):3–16

Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 46(1–3):3–26. doi:10.1016/S0169-409X(00)00129-0

McCarthy JJ, McLeod HL, Ginsburg GS (2013) Genomic medicine: a decade of successes, challenges, and opportunities. Sci Transl Med 5(189):189sr184. doi:10.1126/scitranslmed.3005785

McTigue M, Murray BW, Chen JH, Deng YL, Solowiej J, Kania RS (2012) Molecular conformations, interactions, and properties associated with drug efficiency and clinical performance among VEGFR TK inhibitors. Proc Natl Acad Sci USA 109(45):18281–18289. doi:10.1073/pnas.1207759109

Milligan PA, Brown MJ, Marchant B, Martin SW, van der Graaf PH, Benson N, Nucci G, Nichols DJ, Boyd RA, Mandema JW, Krishnaswami S, Zwillich S, Gruben D, Anziano RJ, Stock TC, Lalonde RL (2013) Model-based drug development: a rational approach to efficiently accelerate drug development. Clin Pharmacol Ther 93(6):502–514. doi:10.1038/clpt.2013.54

Milo R, Shen-Orr S, Itzkovitz S, Kashtan N, Chklovskii D, Alon U (2002) Network motifs: simple building blocks of complex networks. Science 298(5594):824–827. doi:10.1126/science.298.5594.824

Mosca R, Ceol A, Aloy P (2013) Interactome3D: adding structural details to protein networks. Nat Methods 10(1):47–53. doi:10.1038/nmeth.2289

Ostrem JM, Peters U, Sos ML, Wells JA, Shokat KM (2013) K-Ras(G12C) inhibitors allosterically control GTP affinity and effector interactions. Nature 503(7477):548–551. doi:10.1038/nature12796

Panetta JC, Sparreboom A, Pui CH, Relling MV, Evans WE (2010) Modeling mechanisms of in vivo variability in methotrexate accumulation and folate pathway inhibition in acute lymphoblastic leukemia cells. PLoS Comput Biol 6(12):e1001019. doi:10.1371/journal.pcbi.1001019

Perot S, Sperandio O, Miteva MA, Camproux AC, Villoutreix BO (2010) Druggable pockets and binding site centric chemical space: a paradigm shift in drug discovery. Drug Discov Today 15(15–16):656–667. doi:10.1016/j.drudis.2010.05.015

Picotti P, Aebersold R (2012) Selected reaction monitoring-based proteomics: workflows, potential, pitfalls and future directions. Nat Methods 9(6):555–566. doi:10.1038/nmeth.2015

Rahuel J, Rasetti V, Maibaum J, Rueger H, Goschke R, Cohen NC, Stutz S, Cumin F, Fuhrer W, Wood JM, Grutter MG (2000) Structure-based drug design: the discovery of novel nonpeptide orally active inhibitors of human renin. Chem Biol 7(7):493–504. doi:10.1016/S1074-5521(00)00134-4

Ramensky V, Bork P, Sunyaev S (2002) Human non-synonymous SNPs: server and survey. Nucleic Acids Res 30(17):3894–3900

Rose PW, Bi C, Bluhm WF, Christie CH, Dimitropoulos D, Dutta S, Green RK, Goodsell DS, Prlic A, Quesada M, Quinn GB, Ramos AG, Westbrook JD, Young J, Zardecki C, Berman HM, Bourne PE (2013) The RCSB Protein Data Bank: new resources for research and education. Nucleic Acids Res 41(Database issue):D475–D482. doi:10.1093/nar/gks1200

Roth BL, Sheffler DJ, Kroeze WK (2004) Magic shotguns versus magic bullets: selectively non-selective drugs for mood disorders and schizophrenia. Nat Rev Drug Discov 3(4):353–359. doi:10.1038/nrd1346

Rowland M, Peck C, Tucker G (2011) Physiologically-based pharmacokinetics in drug development and regulatory science. Annu Rev Pharmacol Toxicol 51:45–73. doi:10.1146/annurev-pharmtox-010510-100540

Russel D, Lasker K, Webb B, Velazquez-Muriel J, Tjioe E, Schneidman-Duhovny D, Peterson B, Sali A (2012) Putting the pieces together: integrative modeling platform software for structure determination of macromolecular assemblies. PLoS Biol 10(1):e1001244. doi:10.1371/journal.pbio.1001244

Sabathie M, de Coninck L, Fabre P, Michel G (1975) Use of diarginine α-ketoglutarate following abdominal surgery. Apropos of 30 cases. Sem Hop Ther 51(9):457–458

Sali A, Blundell TL (1993) Comparative protein modelling by satisfaction of spatial restraints. J Mol Biol 234(3):779–815. doi:10.1006/jmbi.1993.1626

Saltelli A (2008) Global sensitivity analysis: the primer. Wiley, Chichester

Schlessinger A, Geier E, Fan H, Irwin JJ, Shoichet BK, Giacomini KM, Sali A (2011) Structure-based discovery of prescription drugs that interact with the norepinephrine transporter, NET. Proc Natl Acad Sci USA 108(38):15810–15815. doi:10.1073/pnas.1106030108

Schlessinger A, Khuri N, Giacomini KM, Sali A (2013) Molecular modeling and ligand docking for solute carrier (SLC) transporters. Curr Top Med Chem 13(7):843–856. doi:10.1016/CTMC-EPUB-20130411-7

Schoeberl B, Pace EA, Fitzgerald JB, Harms BD, Xu L, Nie L, Linggi B, Kalra A, Paragas V, Bukhalid R, Grantcharova V, Kohli N, West KA, Leszczyniecka M, Feldhaus MJ, Kudla AJ, Nielsen UB (2009) Therapeutically targeting ErbB3: a key node in ligand-induced activation of the ErbB receptor-PI3K axis. Sci Signal 2(77):ra31. doi:10.1126/scisignal.2000352

Schoeberl B, Faber AC, Li D, Liang MC, Crosby K, Onsum M, Burenkova O, Pace E, Walton Z, Nie L, Fulgham A, Song Y, Nielsen UB, Engelman JA, Wong KK (2010) An ErbB3 antibody, MM-121, is active in cancers with ligand-dependent activation. Cancer Res 70(6):2485–2494. doi:10.1158/0008-5472.CAN-09-3145

Schymkowitz J, Borg J, Stricher F, Nys R, Rousseau F, Serrano L (2005) The FoldX web server: an online force field. Nucleic Acids Res 33(Web Server Issue):W382–W388. doi:10.1093/nar/gki387

Sheiner LB, Stanski DR, Vozeh S, Miller RD, Ham J (1979) Simultaneous modeling of pharmacokinetics and pharmacodynamics: application to d-tubocurarine. Clin Pharmacol Ther 25(3):358–371

Shendure J, Lieberman Aiden E (2012) The expanding scope of DNA sequencing. Nat Biotechnol 30(11):1084–1094. doi:10.1038/nbt.2421

Shoichet BK (2004) Virtual screening of chemical libraries. Nature 432(7019):862–865. doi:10.1038/nature03197

Simard JR, Getlik M, Grutter C, Pawar V, Wulfert S, Rabiller M, Rauh D (2009) Development of a fluorescent-tagged kinase assay system for the detection and characterization of allosteric kinase inhibitors. J Am Chem Soc 131(37):13286–13296. doi:10.1021/ja902010p

Singh J, Petter RC, Baillie TA, Whitty A (2011) The resurgence of covalent drugs. Nat Rev Drug Discov 10(4):307–317. doi:10.1038/nrd3410

Sobol IM (2001) Global sensitivity indices for nonlinear mathematical models and their Monte Carlo estimates. Math Comput Simulat 55(1–3):271–280. doi:10.1016/S0378-4754(00)00270-6

Sorger PK, Allerheiligen SRB, Abernethy DR, Altman RB, Brouwer KLR, Califano A, D'Argenio DZ, Iyengar R, Jusko WJ, Lalonde R, Lauffenburger DA, Shoichet B, Stevens JL, Subramaniam S, Van der Graaf P, Vicini P (2011) Quantitative and systems pharmacology in the post-genomic era: new approaches to discovering drugs and understanding therapeutic mechanisms. Paper presented at the QSP Workshop Group

Stelling J, Sauer U, Szallasi Z, Doyle FJ III, Doyle J (2004) Robustness of cellular functions. Cell 118(6):675–685. doi:10.1016/j.cell.2004.09.008

Toward Precision Medicine: Building a Knowledge Network for Biomedical Research and a New Taxonomy of Disease (2011) Committee on a framework for development a new taxonomy of disease, board on life sciences. Division on Earth and Life Studies, National Research Council

von Kriegsheim A, Baiocchi D, Birtwistle M, Sumpton D, Bienvenut W, Morrice N, Yamada K, Lamond A, Kalna G, Orton R, Gilbert D, Kolch W (2009) Cell fate decisions are specified by the dynamic ERK interactome. Nat Cell Biol 11(12):1458–1464. doi:10.1038/ncb1994

Wang Z, Moult J (2001) SNPs, protein structure, and disease. Hum Mutat 17(4):263–270. doi:10.1002/humu.22

Wang S, Guo P, Wang X, Zhou Q, Gallo JM (2008) Preclinical pharmacokinetic /pharmacodynamic models of gefitinib and the design of equivalent dosing regimens in EGFR wild-type and mutant tumor models. Mol Cancer Ther 7(2):407–417. doi:10.1158/1535-7163.MCT-07-2070

Wells JA, McClendon CL (2007) Reaching for high-hanging fruit in drug discovery at protein-protein interfaces. Nature 450(7172):1001–1009. doi:10.1038/nature06526

Wendell A, Cianci JP (1992) Factors affecting distribution of catheter-injected local anesthetic. Anesthesiology 77(1):211–212 (author reply 213)

Weng G, Bhalla US, Iyengar R (1999) Complexity in biological signaling systems. Science 284 (5411):92–96

Xie L, Evangelidis T, Bourne PE (2011) Drug discovery using chemical systems biology: weak inhibition of multiple kinases may contribute to the anti-cancer effect of nelfinavir. PLoS Comput Biol 7(4):e1002037. doi:10.1371/journal.pcbi.1002037

Xie L, Kinnings SL, Bourne PE (2012) Novel computational approaches to polypharmacology as a means to define responses to individual drugs. Annu Rev Pharmacol Toxicol 52:361–379. doi:10.1146/annurev-pharmtox-010611-134630

Zhang QC, Petrey D, Deng L, Qiang L, Shi Y, Thu CA, Bisikirska B, Lefebvre C, Accili D, Hunter T, Maniatis T, Califano A, Honig B (2012) Structure-based prediction of protein-protein interactions on a genome-wide scale. Nature 490(7421):556–560. doi:10.1038/nature11503

Zhang XY, Birtwistle MR, Gallo JM (2014) A general network pharmacodynamic model-based design pipeline for customized cancer therapy applied to the VEGFR pathway. CPT Pharmacometrics Syst Pharmacol 3:e92. doi:10.1038/psp.2013.65

Zhao S, Iyengar R (2012) Systems pharmacology: network analysis to identify multiscale mechanisms of drug action. Annu Rev Pharmacol Toxicol 52:505–521. doi:10.1146/annurev-pharmtox-010611-134520

Zhao S, Nishimura T, Chen Y, Azeloglu EU, Gottesman O, Giannarelli C, Zafar MU, Benard L, Badimon JJ, Hajjar RJ, Goldfarb J, Iyengar R (2013) Systems pharmacology of adverse event mitigation by drug combinations. Sci Transl Med 5:206ra140. doi:10.1126/scitranslmed.3006548

Zhao N, Han JG, Shyu CR, Korkin D (2014) Determining effects of non-synonymous SNPs on protein-protein interactions using supervised and semi-supervised learning. PLoS Comput Biol 10(5):e1003592. doi:10.1371/journal.pcbi.1003592

Zhou Q, Guo P, Kruh GD, Vicini P, Wang X, Gallo JM (2007) Predicting human tumor drug concentrations from a preclinical pharmacokinetic model of temozolomide brain disposition. Clin Cancer Res 13(14):4271–4279. doi:10.1158/1078-0432.CCR-07-0658