JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
Perspective
pubs.acs.org/JACS

Systems Biology Brings New Dimensions for Structure-Based Drug Design

Jianfeng Pei,[†] Ning Yin,[†] Xiaomin Ma,[†] and Luhua Lai*[,†,‡,§]

[†]Center for Quantitative Biology, Academy for Advanced Interdisciplinary Studies, Peking University, Beijing 100871, China
[‡]Beijing National Laboratory for Molecular Science, State Key Laboratory for Structural Chemistry of Unstable and Stable Species, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China
[§]Peking-Tsinghua Center for Life Sciences, Peking University, Beijing 100871, China

ABSTRACT: In this Perspective, we focus on new, systems-centric views of structure-based drug design (SBDD) that we believe will impact future drug discovery research and development. We will first discuss new ways to identify drug targets based on systems intervention analysis, and then we will introduce emerging SBDD methods driven by advancements in systems biology.

1. INTRODUCTION

Structure-based drug design (SBDD) methods have rapidly progressed in parallel with advances in molecular biology, structural biology, computational chemistry and biology, and computer science. SBDD techniques provide powerful tools for identifying hit molecules as starting points for medicinal chemistry. In particular, molecular docking methods based on three-dimensional biological macromolecule structures and compound libraries (target-based virtual screening) have played a major role in the development of therapeutically important small molecules. SBDD methodologies and progress have been summarized in many reviews.[1−6] Briefly, it is well recognized that SBDD can reduce drug research and development (R&D) time and cost.[6,7] Though issues such as the high false positive rate of virtual screening, the difficulty of considering target flexibility in docking, and the inaccuracy of scoring functions for estimating target−ligand binding free energy still represent major challenges to current SBDD methodologies, SBDD is already an indispensable tool that has advanced drug R&D. Many of the drugs on market or in clinical trials were influenced, at least partially, by SBDD.[8,9]

Entering the 21st century, with the rapid development of "omics" techniques, many potential drug targets were identified, and a golden era of target-based drug discovery was expected to occur. Unfortunately, the cost of drug R&D has steadily increased, while investment returns have decreased. The annual number of FDA-approved drugs has not increased, and the number of new molecular entities (NMEs) is on the decline.[10,11] Most potential compounds fail in clinical trials due to a lack of efficacy or adverse side effects.[12] It appears that the modern pharmaceutical industry is bottlenecked by NME production. Diseases and drug action mechanisms are far more complex than previously assumed. Systems-based drug discovery approaches provide a possible solution to break this bottleneck. Systems biology focuses on the systems-level study of biological molecules and their interactions.[13] Utilizing tools such as biological network modeling and large-scale data analysis, systems biology has provided deep insights into how biological functions emerge from the complex interactomes.[14,15] Moreover, systems biology enables rationalization and prediction of drug effects and side effects; thus it holds promise as a next-generation drug discovery approach.[16−18] Successful systems-level regulation for disease intervention requires new methods for biological network simulations and control strategy predictions, which further requires new developments in SBDD.

In this Perspective, we provide a brief overview of SBDD directions in a systems-centric view that we believe to be important for future drug R&D. The first part evaluates the identification of new targets to broaden drug discovery scope, with an emphasis on systems-centric intervention. The second part covers new directions for SBDD methods that are driven by systems biology. Figure 1 shows some of the directions we believe important for SBDD in the systems biology era and their relationships with systems-based drug discovery.

2. SYSTEMS BIOLOGY AND DRUG TARGET IDENTIFICATION

Complex diseases such as cancer, diabetes, and cardiovascular diseases present significant challenges for drug discovery. Diseases of complex etiology are difficult to treat effectively by targeting a single site, because of the divergent or redundant structures in underlying disease networks.[19] Drugs with high specificity toward a particular target may still have unexpected adverse effects, as modulation of the target activity can have repercussions on distant parts of the biological network. On the other hand, highly selective drugs may show lower efficacy due to the inherent robustness of biological systems. For anticancer agents (as well as antibiotics and antiviral agents), similarities between host and "pathogen" biochemistry, as well as the tendency of pathogens to generate resistant mutations, can also be a limitation. Systems biology (systems)-based drug discovery provides new hope to address these problems.[20−22]

The complex interactions of biological macromolecules are exemplified by the increasingly refined mapping of broad biological networks including protein−protein interaction (PPI),[23] metabolic,[24] and signaling[25] networks. Systems-based drug design can take advantage of these networks. Considering a drug whose binding induces structural changes in its target as

Received: May 14, 2014
Published: July 25, 2014

 © 2014 American Chemical Society dx.doi.org/10.1021/ja504810z | J. Am. Chem. Soc. 2014, 136, 11556−11565

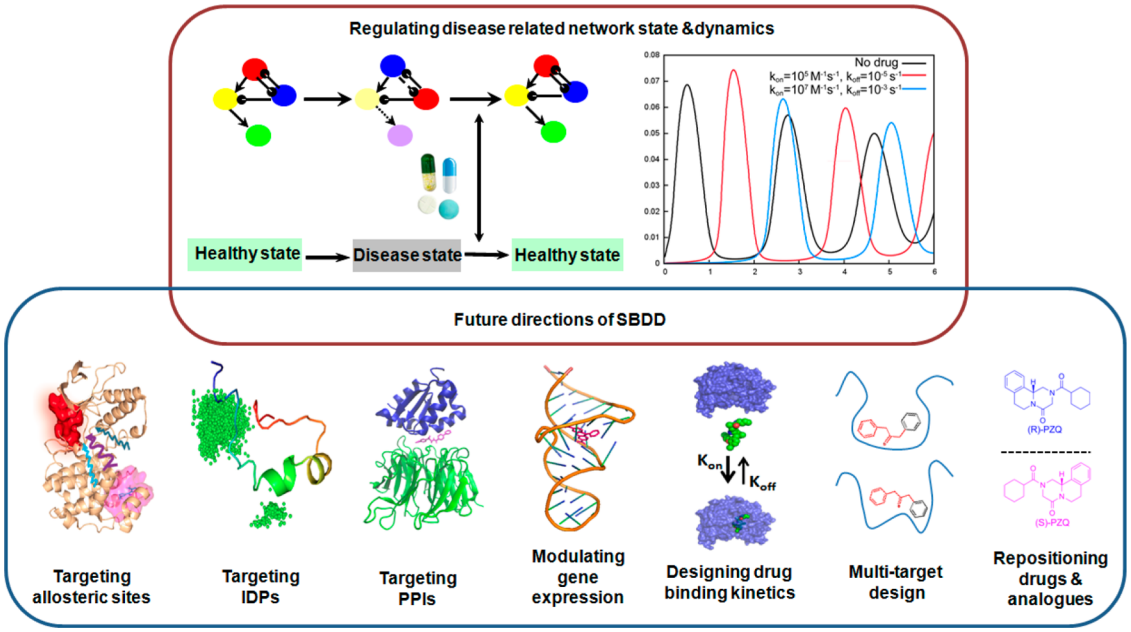

Figure 1. Systems-based drug intervention and new directions for SBDD. Top: illustration of disease intervention at the systems level to regulate disease-related network state and dynamics. Bottom: list of important new directions for SBDD. Abbreviations used: IDPs, intrinsically disordered proteins; PPIs, protein−protein interactions.

well as functional changes, this structural change could spread past the immediate binding partners of the target, through the complex PPI interactome in human cells, and exhibit long-range effects and side effects. Drugs utilizing this kind of behavior are named allo-network drugs.[26−28]

In the following sections, we shall discuss two key areas of systems-based target identification (section 2.1). The first is network state changes with respect to diseases. Using the human inflammatory arachidonic acid metabolic network as an example, we look at diseases as an undesirable network state and reframe the goal of drug therapy toward shifting the network back to a normal state.[29,30] The second area is signaling dynamics and disease. Typical signal transduction pathways, such as p53 and NFκB, output signals with different dynamic patterns that may lead to diseases under certain circumstances.

Next we will discuss new directions for SBDD and the development of systems-based drug discovery studies. From a network point of view, up-regulating target activity is equally important as down-regulating its activity, which may be best realized using allosteric regulators. Compared to normal orthosteric drugs, allosteric drugs provide many benefits (section 2.2). Alteration of network dynamics can also be achieved by disturbing expression levels of related targets (section 2.3). Lastly, in addition to drug binding affinity, binding kinetics are also important for determining drug efficacy (section in 2.4).

2.1. Systems-Based Drug Discovery. The central issue of network-based drug discovery is the selection of drug targets. Broadly speaking, this requires a network-level characterization of disease and a clear understanding of normal and diseased network states. These networks are dynamic models that respond to perturbations through series of regulation and feedback. Understanding these connections and dynamics forms the foundation of systems-based drug design.

2.1.1. Disease as a Network State. To identify optimal intervention sites as possible drug targets in disease-related

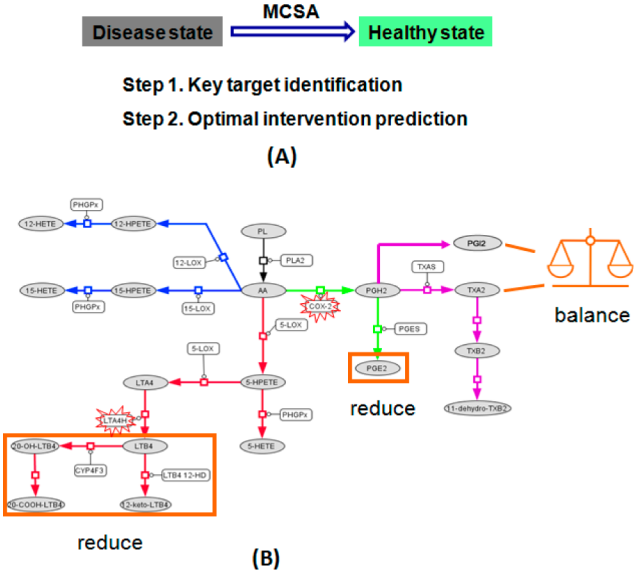

Figure 2. MTOI algorithm and its application in the human arachidonic acid metabolic network. (A) Illustration of MTOI, which uses a Monte Carlo simulated annealing-based search algorithm to identify key targets and optimal intervention solutions. (B) A simplified human arachidonic acid metabolic network. Concentrations of species in orange rectangles need to be reduced, while those of PGI2 and TXA2 need to be balanced to reduce side effects. One solution found by MTOI is the simultaneous inhibition of COX-2/COX-1 and LTA4H. Adapted from ref 29.

networks, a search algorithm is needed. For example, our laboratory developed an algorithm to investigate anti-inflammatory drugs targeting arachidonic acid metabolic pathways.[29] Based on the established ordinary differential equation model, we proposed a Monte Carlo simulated annealing-based search algorithm, MTOI ("Multiple Target Optimal Intervention"), for target identification. Using this algorithm, we predicted potential target combinations with high

dx.doi.org/10.1021/ja504810z | J. Am. Chem. Soc. 2014, 136, 11556−11565

efficacy and fewer side effects (Figure 2).[30] Due to biological redundancy in the networks, multiple intervention sites are often required. This demonstrates the advantage of a systems-based drug discovery approach, in which the effects of drug combinations could be predicted and rationalized. By modeling drug combination outcomes in all the possible three-node enzymatic networks, we concluded that synergistic or antagonistic drug combinations depend on network topology.[31] These findings provide useful hints for developing new combination drugs.

Conversely, drug combinations can serve as a powerful perturbation approach that can be used to elucidate biological interaction networks.[32,33] The study of drug combinations within a network context in both directions will certainly contribute to our understanding of biological complexity and help identify optimal intervention strategies.

2.1.2. Modulating Network Dynamics. Biological networks exhibit interesting dynamic behaviors, such as bistability and oscillation, through carefully tuned regulatory structures.[34] Many of these dynamic behaviors are essential for biological functions. For example, the regulatory network that governs the DNA damage-induced apoptotic pathway contains a bifurcation point where the cells will either arrest growth or proceed into apoptosis. Proteins associated with these bifurcation points were found to correspond with high-frequency oncogenic mutations.[35] Disrupting the normal oscillatory dynamics of circadian clocks can cause circadian disorders. It has been hypothesized that changes in the bistable signal−response dynamics could be related to tumorigenesis.[36] With increasing network dynamic details being uncovered, rational design of dynamics-modulating drugs may be feasible. Using drugs to modulate network dynamic properties provides an attractive solution to potentially treat complex diseases like cancer or circadian disorders.

A particularly interesting property of signaling networks is their ability to respond to different signals with different signaling molecule dynamics. In mammalian systems, the transcription factors p53 and NF-κB are two notable examples of proteins with different dynamic behavior responses.[37] They are both important signaling molecules related to diseases like cancer, and they are attractive drug targets.[38,39] In a notable study by Behar et al., signaling dynamics, rather than signaling molecules, were theorized to be a potential target for pharmaceutical intervention.[40] The researchers theorized that modulating the signaling dynamics would provide a way to selectively block unwanted responses to specific signals while leaving responses to other signals intact. This hypothesis was experimentally tested using the TNF-signaling pathway by selectively targeting one signal−response relationship, proving that this therapeutic paradigm was possible.

Network-based drug discovery presents challenges and opportunities for next-generation drug discovery. A number of pharmaceutical companies have been founded on the basis of exploiting this opportunity and have produced interesting, novel solutions to complex diseases such as Charcot-Marie-Tooth disease type 1A.[41] The key to successful network-based drug design lies in large-scale quantitative studies of biological systems that provide a basis for measuring and predicting drug-induced responses. Advances in high-throughput phenotyping techniques and large-scale computational modeling have opened the door for a network era of drug discovery.

2.2. Allosteric Binding Sites as Drug Targets. Compared to conventional orthosteric drugs for specific protein targets, allosteric drugs have several advantages,[42] including fewer or reduced side effects and different regulation mechanisms. Figure 3 shows how allosteric drugs work. Notably, allosteric drugs can increase activity of the target,[43] such as enzyme catalysis rate or substrate binding strength, which is difficult to achieve with orthosteric drugs. For cases where orthosteric sites are not suitable for small-molecule drug design, such as large PPI interfaces,[44] allosteric drugs can be developed as a better alternative. One successful example is allosteric inhibitors of lymphocyte function-associated antigen-1 (LFA-1).[44] The natural compound naringenin was shown to inhibit the TGF-β ligand−receptor interaction. Molecular dynamics simulations revealed the allosteric mechanism of how naringenin disrupts the PPI.[45]

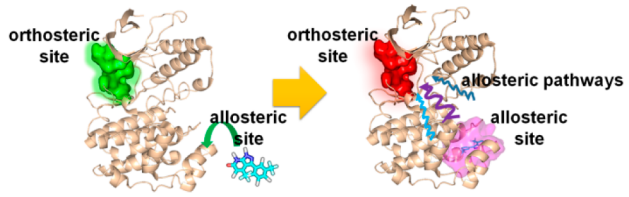

Figure 3. Schematic diagram of how allosteric drugs work. When drugs bind to the allosteric sites, the effects spread to orthosteric sites through structural or dynamic changes, resulting in activity modulation.

Unfortunately, in most cases, we cannot identify where potential allosteric sites might be located or how they might regulate the target activity. Most known allosteric-regulating molecules were discovered by high-throughput experimental screening.[46,47] The first step for rational allosteric drug design is to identify allosteric binding sites. While all non-fibrous proteins have potential allosteric sites,[48] only 907 allosteric site-modulator structural complexes have been collected in the AlloSteric Database.[49] The MutInf method[50] and support-vector machine (SVM) predictions[51] have been developed to identify allosteric hot spots in proteins. The purpose of allosteric site prediction is to identify pockets that are suitable for small-molecule binding. A number of programs can be used for this purpose, including CAVITY.[52] Using this strategy, Miao et al. performed long-time-scale, accelerated molecular dynamics simulations on human M2 muscarinic receptor, an important class A (rhodopsin-like) GPCR, and identified seven potential allosteric sites[53] based on FTMAP[54] analysis. As molecular dynamic simulations are expensive and may not capture all conformational changes within relatively short-time-scale, more efficient coarse-grained models can be used. We developed an allosteric site prediction method using a two-state Gō model.[55] Based on the concept that allostery is a conformational shift process,[48,56] we constructed a two-state conformational ensemble biased for a single protein state and then added perturbations to potential allosteric binding sites predicted by CAVITY.[52] If these perturbations caused population redistribution, the site was designated a potential allosteric site. Allosteric inhibitors and activators for *Escherichia coli* D-3-phosphoglycerate dehydrogenase were successfully discovered on the basis of the predicted sites.[57] Normal-mode analysis is another widely used coarse-grained method that has been applied to predict allosteric sites. Panjkovich et al. compiled a non-redundant test set of proteins with known allosteric sites and performed normal-mode analysis on them. They observed significant changes in protein flexibility upon allosteric ligand binding in 70% of sites.[58] In another study they

used LIGSITEcsc[59] to detect potential binding sites and then added an octahedron to represent a simplified ligand into each site. If the protein flexibility changed significantly, this site was designated a potential allosteric site. The PARS web server was built on the basis of this method.[60] Huang et al. developed another Web server, Allosite,[61] which uses the FPocket algorithm[62] and an SVM algorithm to predict allosteric sites.

Allosteric site prediction methods that can efficiently consider both long-distance effects and local detailed interactions are needed. As mentioned before, drug binding induces structural and dynamic property changes in its target. This effect could spread beyond the primary target binding partners and throughout the complex interactome, exhibiting long-range effects and producing side effects. Utilizing this effect could provide new solutions for designing drugs directed at targets without suitable orthosteric drug binding sites or at proteins that are networked to targets lacking structural information.

Another promising drug binding target is intrinsically disordered proteins (IDPs). IDPs exist as dynamic conformational ensembles. This important and common proteins class is found in all kingdoms of life. A large number of cell signaling proteins and transcription factors are IDPs or possess IDP domains.[63] Many IDPs are associated with human diseases[64] and could serve as potential drug design targets. From a network perspective, IDPs are often central players in PPI networks.[65,66] Acting as hubs that have multiple binding partners in interactomes, they could serve as promising drug targets for diseases like cancer.[14,67] IDPs are also suitable for use as allosteric proteins,[68] and some have shown allosteric effects on signaling.[68] Drug design targeting IDPs is difficult, and few examples have been reported. One example is the development of inhibitors that target the oncoprotein c-Myc IDP domain, discovered by experimental screening.[69,70] Although it is difficult to design drugs targeting IDPs due to limited structural information, the study by Jin et al. provided insights into targeting c-Myc$_{370-409}$ that may be extrapolated to rationally design allosteric drugs targeting IDPs in the future.[71]

2.3. Modulating Drug Target Gene Expression. Target-based drug design has been the dominant paradigm for modern drug discovery. The first hint of a potential target may come from RNA and/or protein expression studies on target tissues or from comparison of diseased versus healthy tissues.[72] Target-based approaches can effectively develop novel treatments for validated targets in a rational way. Drugs are developed to interact with drug targets, directly modulating their activities or functions. However, drugs can also be developed to act upon drug target expression and modulate their activity by influencing the target molecule quantity in the cell. These drugs are referred to as target expression modulators (TEMs) in this Perspective. TEMs provide a more direct and effective way to treat diseases by influencing the amount of target protein rather than modulating its activity after it has been expressed, particularly for up-regulation. Considering that many proteins lack suitable binding sites for drug-like molecules and that expanding computational drug discovery to PPI disruption has proven to be a formidable challenge,[73] TEMs present a prominent advantage by providing a means to indirectly target a protein through targeting biomolecules that influence transcription or post-transcriptional processes.

Compounds that could directly affect transcription or post-transcriptional processes include, but are not limited to, the following classes: (1) small molecules that directly bind cis-regulatory elements on or near gene promoter sequences;[74−76] (2) small molecules that interfere with the transcription apparatus, such as transcription factors or transcription products, by affecting mRNA stability;[77−79] (3) small molecules that epigenetically regulate gene expression by influencing DNA methylation, histones modification, and micro-RNA transcription;[80,81] and (4) small molecules that target the proteostasis system to increase or decrease the total protein concentration.[82,83] Some small molecules, such as genistein and vanillin, have been shown to inhibit Polo-like kinase 1 (Plk1) expression,[84] but their detailed mechanisms remain unknown. It has been theorized that they might down-regulate Plk1 expression by influencing DNA damage repair systems. Developing SBDD methods to design TEMs is a promising direction for future drug research, as gene expression and protein degradation are important parts of biological networks.

2.4. The Influence of Drug Binding Kinetics. Drug binding kinetics plays a major role in determining drug efficacy in many systems.[85−89] The protein−ligand binding energy landscape may be influenced by binding kinetics.[90] Particularly, a drug's residence time on its target is frequently found to correlate with efficacy, stressing the importance of designing slow-dissociating or even covalently binding drugs.[85,86,91] On the other hand, association kinetics are also important for drug action.[87,92] These results advocate the consideration of binding kinetics as an important factor in the drug design process.

We performed a comprehensive computational analysis of drug binding kinetics on various pharmacological situations including enzyme inhibition, receptor binding, multi-target drug targeting, signal transduction pathways, and metabolic networks.[93] We demonstrated that fast-associating drugs show better enzyme inhibitory effects, earlier and higher receptor occupancy peaks, and better multi-target performances, while slow-dissociating drugs show prolonged receptor occupancy, consistent with the literature.[85,86,91] Different situations produce slightly different kinetics−efficacy relationships, and each must be considered separately. On the systems level, binding kinetics can change the overall effect of drugs and affect signaling dynamics (Figure 4). The drug binding kinetic effects also depend on network topology and where the target is located in the network. For successful drug discovery, both molecular binding kinetics and systems-level requirements should be considered.

As the importance of the kinetic parameters on drug efficacy became more apparent, the push toward rational design of binding kinetics gained momentum. Experimental and computational studies have linked the relationship between target structure and binding kinetics in several systems.[94−97] Bai et al. computationally characterized the binding free energy landscape of Huperzine A to acetylcholinesterase and accurately predicted the binding kinetic constants between the two.[94] Schneider et al. conducted a structure−kinetic relationship study on the CDK8/CycC system and identified key binding kinetic structural determinants in a series of compounds.[97] PPI kinetics have also been studied[98] due to their importance in network states and dynamics. These studies serve as a basis for structure-based binding kinetics design, which may greatly enhance our ability to specifically tune network-level responses of drugs.

dx.doi.org/10.1021/ja504810z | J. Am. Chem. Soc. 2014, 136, 11556−11565

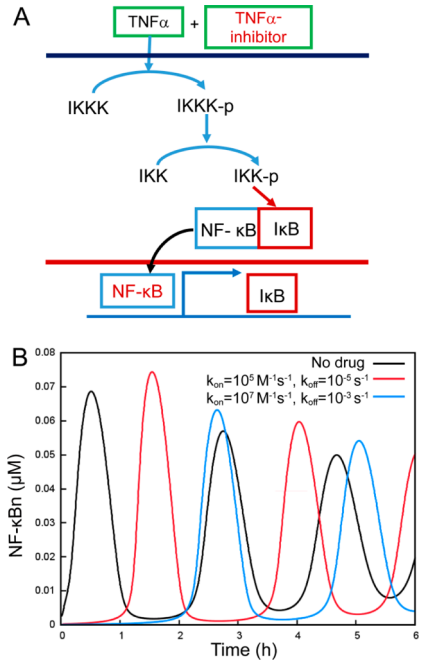

Figure 4. Drugs with different binding kinetics can induce different modulations in signaling dynamics. (A) The TNFα-induced NF-κB signaling pathway. When TNFα is used to stimulate the cells, NF-κB is shuttled into and out of the nucleus, resulting in oscillatory nuclear NF-κB concentrations. (B) Different TNFα inhibitors demonstrate different NF-κB nuclear shuttling dynamics based on their drug kinetics. Adapted from ref 93. Abbreviations used: TNFα, tumor necrosis α; IKKK, IκB kinase kinase; IKKK-p, phosphorylated IKKK; IKK, IκB kinase; IKK-p, phosphorylated IKK; IκB, inhibitor of κB; NF-κB, nuclear factor κ-light-chain-enhancer of activated B cells.

3. NEW DIRECTIONS FOR SBDD IN THE SYSTEMS BIOLOGY ERA

As mentioned in the Introduction, systems-based drug discovery provides potential solutions for treating complex diseases and developing NME drugs. Systems-level regulation of disease calls for new developments in SBDD. Here we discuss several advancements that we believe will be important for future drug discovery.

3.1. *De Novo* Drug Design. The application of docking-based virtual screening (VS) using a protein−ligand docking method has boomed in the past two decades. A notable weakness of VS is that the identified hits are limited to available compounds that cover only a fraction of chemical and drug space. Compared to VS, computational *de novo* drug design can develop novel molecular entities without structural limitations and highly efficient scaffolds with the required pharmacological profiles. Considering the immensity of drug-like chemical space, *de novo* design is theoretically ideal for designing drug candidates, especially to meet the potential larger drug space requirement for systems-based drug design. A number of *de novo* drug design programs have been developed in the past two decades. The most frequently used programs include AlleGrow, BOMB, LeapFrog, Ligbuilder, Ludi, MCSS, and SPROUT.[99]

Despite the advantages, *de novo* drug design methods have not been widely adopted by medicinal chemists in routine drug discovery. Only a few hits have been discovered using *de novo* drug design programs compared with the success of VS programs. According to Kutchukian and Shakhnovich[99] and our own survey, the average number of experimental validation studies using *de novo* design programs between 2005 and 2013 was less than 10 annually. The lack of popularity may be related to the fact that *de novo* design is not a high-throughput approach like high-throughput screening (HTS) or VS. Synthesis of *de novo* designed compounds is often labor-intensive and time-consuming due to the involvement of numerous scaffolds. Usually, *de novo* designed molecules are difficult to synthesize unless chemical synthesis methods are considered in the design process. Therefore, a practical *de novo* drug design program has to design drug candidates with high synthesis accessibility and high success rates (i.e., fewer false positives). This is more demanding than VS, as the compounds selected can normally be purchased in bulk for downstream testing.

We developed a multi-purpose program called LigBuilder[100,101] based on our previously developed program RASSE[102] for structure-based *de novo* drug design. In the current release, version 2.0, the synthesizability of designed compounds can be analyzed in real time with an embedded chemical reaction database and a retrosynthesis analyzer. A cavity detection procedure is implemented to detect and shape potential ligand binding sites in protein targets and estimate their ligandability and druggability. Multiple evaluation criteria were implemented to improve the design success rate. One representative example is the design and optimization of Cyclophilin A inhibitors using LigBuilder 2.0. Using a single design round, a novel cyclosporin A inhibitor was designed and shown experimentally to have greater potency than the positive control.[103] This successful example and others[104−108] have verified the effectiveness of LigBuilder.

De novo drug design methods can be applied to optimize hits from fragment-based screening.[109−112] Fragment screening focuses on small moiety-like compounds that bind to various regions inside the target binding site; a scaffold is then developed to connect these multiple, independent fragments into a single compound. One of the primary advantages of fragment-based screening is that fragment hits generally exhibit strong binding with respect to their size, and their subsequent optimization should lead to compounds with better pharmacokinetic properties compared with HTS or VS hits.[112] In summary, *de novo* drug design methodology is an ideal and important branch of SBDD; however, it needs further improvement before it can be practically and popularly applied. The main directions for the improvement include designing compounds with high probability for chemical synthesis and designing compounds with high success rates to overcome low-throughput issues.

3.2. Multi-target Drug Design. Single-target drugs are often less effective in controlling complex diseases with multiple pathogenic factors, such as diabetes, inflammation, cancer, and central nervous system disorders.[113−115] Biological network analysis generally provides multiple-target control solutions, and single-target solutions are rare. Combination therapy or multi-target therapy is necessary to effectively treat these diseases.

Combination drugs, defined as a concerted pharmacological intervention using several compounds that interact with multiple targets, have increasingly been used to treat many diseases, including cancer, inflammation, type 2 diabetes, and AIDS.[19,116−118] Multicomponent mixtures extracted from natural products have historically been used in traditional medicine, referred to as "ethnopharmacology". Network simulations and network pharmacology have been used

dx.doi.org/10.1021/ja504810z | J. Am. Chem. Soc. 2014, 136, 11556−11565

successfully to understand the effects of traditional Chinese medicine.[119−121] Combination drugs present several potential problems, including possible drug−drug interactions, poor patient compliance, especially in treating asymptomatic diseases like hypertension, and different pharmacokinetics/pharmacodynamics properties for each component, that make the drug combination outcome hard to control.[122]

Multi-target drugs, which are able to interact with several drug targets simultaneously, lead to new and more effective medications for a variety of complex diseases, despite some having relatively weaker activities for their respective targets. Although the discovery process for multi-target drugs is more complicated in the SBDD design and optimization stages due to the increased constraints of multiple targets, the risks and costs for clinic trials are similar to those of traditional single-target drug development; as only one drug is used, problems caused by drug combination can be avoided. Dual-function inhibitors have been found to remain active across broader concentration ranges than combination drugs.[29] In recent years, methods for multi-target ligand discovery such as linker strategy and framework combination have been developed.[122,123] Cross (sequential) virtual screening is also a commonly used method.[124] We have used sequential screening to find dual-function inhibitors of 5-LOX and mPGES-1,[125] and a framework combination strategy to design novel dual-function inhibitors of COX-2 and LTA_4H.[126] We also developed a common pharmacophore model-based cross screening method and successfully used it to find highly potent dual-target inhibitors for LTA_4H and PLA2.[127]

The framework approach is based on the integration of multiple compounds through the fusion of common or similar sub-structures.[122,123] The combined molecules resulting from this approach are usually much smaller than two distinct structures directly linked with a flexible chain; however, their ligand efficiencies are usually lower than those of general preclinical compounds, which may lead to poor oral pharmacokinetics. For cross screening, the chance of success is generally low, especially for unrelated targets with distinct binding sites. Therefore, it is critical for multi-target compounds to be "highly integrated" in order to make the most of each component group between both targets. A general strategy for multi-target rational drug design against dissimilar targets needs to be developed. We recently expanded our *de novo* drug design program LigBuilder into a multi-target design program, LigBuilder 3. We enabled the *de novo* design and molecular optimization algorithm to handle multiple targets (to be published). We designed a multi-target inhibitor from scratch, considering multiple interactions for each component group. This *de novo* design approach is expected to produce high ligand efficiency, which is critical for multi-target drugs. Multi-target lead optimization is also implemented in LigBuilder 3, which could help researchers to find potential multi-target optimization solutions. Besides the multi-target growing strategy (Figure 5), an "ensemble linking" strategy is implemented to promote "fragment linking" algorithm efficiency in both fragment-based and multi-target drug design. This is particularly helpful for high-efficiency recombination of known inhibitors and framework combinations. The LigBuilder 3 program was experimentally validated by designing dual-target inhibitors for COX-2 and LTA4H,[128] providing a solution for rational design and optimization of highly integrated multi-target drugs, especially for proteins with dissimilar binding pockets (Figure 6).

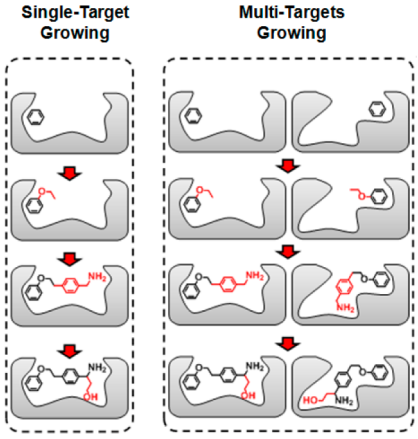

Figure 5. Schematic diagram of the Ligbuilder 3 single-target (left) and multi-target (right) growing algorithm. The fragments grown in each step are colored in red.

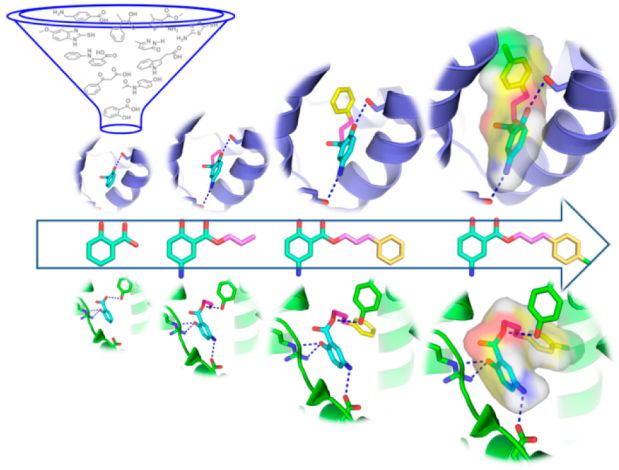

Figure 6. A general multi-target rational drug design strategy for dissimilar targets. In the first step, the multi-target seeds were experimentally identified by focused fragment library screening. Subsequently, multi-target *de novo* ligand design with an iterative fragment-growing strategy was used to evolve the fragment seeds. As a proof-of-concept study, a promising cyclooxygenase-2 and leukotriene A4 hydrolase dual-target inhibitor was developed. Adapted from ref 128.

As the available chemical space for multi-target ligands is much smaller than for single-target ligands, *de novo* design for multi-target drugs might be a better choice. Optimization of multi-target leads is far more complicated than that of single-target leads because the "optimization landscape" is no longer a simple stepwise "group-activity" profile. The requirement for binding affinity balance across multiple binding targets will significantly reduce the available chemical space in the lead structure. As a result, stepwise optimization in multi-target design frequently refines to local, rather than global, minima. The increased dimensions of the "optimization landscape" make the stepwise strategy less efficient, and more extensive global structure sampling is necessary. This is difficult to achieve manually but is performed automatically by Ligbuilder 3. A possible drawback of multi-target drugs is that developing drugs for multiple targets is intrinsically more challenging than developing them for single targets, which will in most cases further diminish the rate of optimized NME production.

dx.doi.org/10.1021/ja504810z | J. Am. Chem. Soc. 2014, 136, 11556−11565

3.3. Repositioning Drug Analogues. Drug repositioning is an attractive strategy for developing new therapeutic purposes for existing drugs. This is supported theoretically because drugs often interact with multiple targets.[129−131] Since the repositioned drug has already passed a significant number of toxicity and other clinical tests, its safety is known and the risk of failure due to adverse toxicology is reduced. Repurposed drugs can bypass much of the early R&D costs and time needed before bringing the drug to market. Finding new uses for existing drugs is a proven shortcut between the laboratory and the clinic.

The idea of drug repositioning is not new. It has been used since the early 1990s, mostly as a serendipitous process. In recent years, as the value of drug repositioning has become evident and with the advancements of systems biology, researchers have developed new pharmacological and computational tools to make the process more systematic and to maximize the drug's potential.[132−135] A number of successful applications of drug repositioning have been reported.[134]

While the advantages of drug repositioning are apparent, it faces some challenges due to intellectual property issues surrounding the original drug. These issues can be complex, and it may not always make sense to take a repositioned drug to market from a commercial point of view. About 9000 off-patent drugs are suitable for repositioning, but only 40% of them are available to researchers.[136] Although a comprehensive clinical drug library screen could be established, the yield of repositioned drugs would be limited due to drug availability.

Here, we propose the concept of "repositioning drug analogues" by finding new potential indications for drug analogues, such as drug precursors or optical isomers, targeted at alternate diseases. Since drug analogues have structures very similar to those of marketed drugs, they have high potential to become drugs themselves. The number of drug analogues is much larger than that of existing drugs, making them an abundant resource for developing new leads in different targets. Drug analogues are often ignored because they show no or reduced potency against the original disease, but this may not hold true for new diseases.

3.4. Designing Drugs To Target Protein−Protein Interactions (PPIs). PPIs form signaling nodes and hubs that transmit pathophysiological cues along molecular networks to achieve an integrated biological output, thereby promoting pathogenesis and/or disease progression. Pathway perturbation, through the disruption of PPIs, offers a novel and effective strategy for curtailing the transmission of pathogenic signals.[137] Inhibition of PPIs with small molecules has emerged as a new way to modulate the activity of proteins and generate potential new drugs against this tremendous reservoir of potential targets.[44] Successes—especially the marketed cardiovascular drug Titrobifan, a glycoprotein IIb/IIIa inhibitor, and anti-HIV drug Maraviroc, an inhibitor of the CCR5−gp120 interaction—highlighted the potential of the PPI targeting approaches.[137]

The remarkable differences between the traditional ligand−protein format and PPI make the discovery and design of PPI inhibitors particularly challenging.[138] Lack of appropriate technologies to tackle the shallow protein−protein interface has hampered SBDD drug discovery research in this area. Computational strategies to address these challenges include three major avenues: (a) Use the structures of known PPI inhibitors to predict future PPI inhibitors.[138] (b) Use SBDD to identify a fragment, usually a peptide, that is critical for PPI, providing a basis for further rational optimization. Many potent inhibitors have been discovered by engineering small molecules or peptides based on or mimicking the natural structure of binding peptides.[137] (c) Identify "hot spots" on PPI surfaces that could bind small fragments, and design linkers to connect the fragments.[139] Fragment-based or *de novo* design methods are useful in this case. These approaches inevitably generate ligands of high molecular weight and lipophilicity. Recent surveys of the existing PPI inhibitors concluded that the PPI chemical space is non-Ro5-compliant. Furthermore, PPI inhibitors have lower ligand efficiency than the traditional inhibitors.[138] Several existing potential lead molecules present poor binding efficiency index (BEI) and surface efficiency index (SEI) values. Associated with these poor physicochemical properties are high attrition rate of PPI compounds in preclinical discovery research and the lack of adequate pharmacokinetic and safety profiles in clinical development. However, despite having an unsatisfactory BEI/SEI value, Navitoclax presents a pharmacokinetics profile for oral dosing and does well in phase IIa trials. This case indicates that new standards are required to evaluate the delivery properties of PPI inhibitors, and a new delivery system is also needed.[140]

As orthosteric sites of PPIs are often found not suitable for small-molecule drug design, allosteric modulation is then expected to be more effective and specific for PPI targets. Successful examples are allosteric inhibitors of LFA-1,[141] nitric oxide synthase enzymes,[142] and nerve-growth factor.[143] Methods mentioned in section 2.2 can be used to predict the possible allosteric binding site of PPIs for SBDD. PPIs usually have dynamic structures. Thermal motions may make transient pockets suitable for small-molecule binding. Only using static structures of PPIs may not be enough to capture the dynamic binding sites. Though some pocket-finding software predicted the pockets well on both static and free structures,[144] molecular dynamics simulation is useful in revealing the potential druggable allosteric pockets.

4. SUMMARY

In summary, we have given a brief overview of the progress of some current structure-based drug design methods and offered our outlook on new and developing SBDD directions that may help to circumvent the current bottleneck in drug research. The most promising directions are related to systems-centric views of drug design, which are critically important for treating complex diseases. Systems-based drug design transcends single-target drug design, which is often hampered by adverse side effects and low *in vivo* efficacy. Systems-based drug design itself faces challenges, including how to develop reliable system analysis methods that currently lack network integrity and quantitative data,[145,146] how to define normal and diseased network states, and how to combine human and pathogen or parasite networks. The development of systems-based drug design also creates a demand for novel SBDD methods, including multi-target drug design, binding kinetics design, allosteric site targeting, IDP targeting, target gene expression modulation, and drug or drug analogue repositioning. These new dimensions for SBDD are now in their early stages, and significant attention should be paid to the progress of their theories and applications.

In this Perspective we focused on methods for designing small-molecule drugs; however, alternative ways to regulate network states or dynamics include designing PPI-regulating proteins,[147−150] and more attention should be paid to nucleic acid-based therapeutics (antisense, RNAi, microRNA, and gene

repair-based strategies). Additionally, the SDBB methods discussed here can be used in chemical biology to investigate macromolecule−small molecule interaction mechanisms[151] and in synthetic biology to design artificial molecular parts for functional modules.[152−154]

AUTHOR INFORMATION

Corresponding Author

lhlai@pku.edu.cn

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Supported in part by the Ministry of Science and Technology of China (grant nos. 2012AA020308, 2012AA020301) and the National Natural Science Foundation of China (grant nos. 81273436, 11021463, 20873003). The authors thank Dr. Yaxia Yuan and Dr. Erchang Shang for their help in preparing some of the figures and all the former and present members in the Lai laboratory for their contributions to the work cited in this perspective.

REFERENCES

(1) Kitchen, D. B.; Decornez, H.; Furr, J. R.; Bajorath, J. *Nat. Rev. Drug Discovery* **2004**, *3*, 935.
(2) Andricopulo, A. D.; Salum, L. B.; Abraham, D. J. *Curr. Top. Med. Chem.* **2009**, *9*, 771.
(3) Chen, L.; Morrow, J. K.; Tran, H. T.; Phatak, S. S.; Du-Cuny, L.; Zhang, S. *Curr. Pharm. Des.* **2012**, *18*, 1217.
(4) Maddaford, S. P. *Methods Mol. Biol.* **2012**, *841*, 351.
(5) Zheng, M.; Liu, X.; Xu, Y.; Li, H.; Luo, C.; Jiang, H. *Trends Pharmacol. Sci.* **2013**, *34*, 549.
(6) Kalyaanamoorthy, S.; Chen, Y. P. *Drug Discovery Today* **2011**, *16*, 831.
(7) Tan, J. J.; Cong, X. J.; Hu, L. M.; Wang, C. X.; Jia, L.; Liang, X. J. *Drug Discovery Today* **2010**, *15*, 186.
(8) Traxler, P.; Bold, G.; Buchdunger, E.; Caravatti, G.; Furet, P.; Manley, P.; O'Reilly, T.; Wood, J.; Zimmermann, J. *Med. Res. Rev.* **2001**, *21*, 499.
(9) Jorgensen, W. L. *Science* **2004**, *303*, 1813.
(10) Mullard, A. *Nat. Rev. Drug Discovery* **2014**, *13*, 85.
(11) Mullard, A. *Nat. Rev. Drug Discovery* **2013**, *12*, 87.
(12) Khanna, I. *Drug Discovery Today* **2012**, *17*, 1088.
(13) Kitano, H. *Science* **2002**, *295*, 1662.
(14) Barabasi, A. L.; Oltvai, Z. N. *Nat. Rev. Genet.* **2004**, *5*, 101.
(15) Kitano, H. *Nature* **2002**, *420*, 206.
(16) Berg, E. L. *Drug Discovery Today* **2014**, *19*, 113.
(17) Iskar, M.; Zeller, G.; Zhao, X. M.; van Noort, V.; Bork, P. *Curr. Opin. Biotechnol.* **2012**, *23*, 609.
(18) Butcher, E. C.; Berg, E. L.; Kunkel, E. J. *Nat. Biotechnol.* **2004**, *22*, 1253.
(19) Keith, C. T.; Borisy, A. A.; Stockwell, B. R. *Nat. Rev. Drug Discovery* **2005**, *4*, 71.
(20) Butcher, E. C. *Nat. Rev. Drug Discovery* **2005**, *4*, 461.
(21) Barabasi, A. L.; Gulbahce, N.; Loscalzo, J. *Nat. Rev. Genet.* **2011**, *12*, 56.
(22) Loscalzo, J.; Barabasi, A. L. *Wiley Interdiscip. Rev. Syst. Biol. Med.* **2011**, *3*, 619.
(23) Franceschini, A.; Szklarczyk, D.; Frankild, S.; Kuhn, M.; Simonovic, M.; Roth, A.; Lin, J.; Minguez, P.; Bork, P.; von Mering, C.; Jensen, L. J. *Nucleic Acids Res.* **2013**, *41*, D808.
(24) Kanehisa, M.; Goto, S.; Sato, Y.; Furumichi, M.; Tanabe, M. *Nucleic Acids Res.* **2012**, *40*, D109.
(25) Schaefer, C. F.; Anthony, K.; Krupa, S.; Buchoff, J.; Day, M.; Hannay, T.; Buetow, K. H. *Nucleic Acids Res.* **2009**, *37*, D674.
(26) Szilagyi, A.; Nussinov, R.; Csermely, P. *Curr. Top. Med. Chem.* **2013**, *13*, 64.
(27) Csermely, P.; Nussinov, R.; Szilagyi, A. *Curr. Top. Med. Chem.* **2013**, *13*, 2.
(28) Nussinov, R.; Tsai, C. J.; Csermely, P. *Trends Pharmacol. Sci.* **2011**, *32*, 686.
(29) Yang, K.; Ma, W.; Liang, H.; Ouyang, Q.; Tang, C.; Lai, L. *PLoS Comput. Biol.* **2007**, *3*, e55.
(30) Yang, K.; Bai, H.; Ouyang, Q.; Lai, L.; Tang, C. *Mol. Syst. Biol.* **2008**, *4*, 228.
(31) Yin, N.; Ma, W.; Pei, J.; Ouyang, Q.; Tang, C.; Lai, L. *PLoS One* **2014**, *9*, e93960.
(32) Lehar, J.; Zimmermann, G. R.; Krueger, A. S.; Molnar, R. A.; Ledell, J. T.; Heilbut, A. M.; Short, G. F., III; Giusti, L. C.; Nolan, G. P.; Magid, O. A.; Lee, M. S.; Borisy, A. A.; Stockwell, B. R.; Keith, C. T. *Mol. Syst. Biol.* **2007**, *3*, 80.
(33) Garmaroudi, F. S.; Marchant, D.; Si, X.; Khalili, A.; Bashashati, A.; Wong, B. W.; Tabet, A.; Ng, R. T.; Murphy, K.; Luo, H.; Janes, K. A.; McManus, B. M. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 17053.
(34) Tyson, J. J.; Chen, K. C.; Novak, B. *Curr. Opin. Cell Biol.* **2003**, *15*, 221.
(35) Chen, J.; Yue, H.; Ouyang, Q. *PLoS Comput. Biol.* **2014**, *10*, e1003451.
(36) Araujo, R. P.; Liotta, L. A.; Petricoin, E. F. *Nat. Rev. Drug Discovery* **2007**, *6*, 871.
(37) Purvis, J. E.; Lahav, G. *Cell* **2013**, *152*, 945.
(38) Wang, Z.; Sun, Y. *Transl. Oncol.* **2010**, *3*, 1.
(39) Baud, V.; Karin, M. *Nat. Rev. Drug Discovery* **2009**, *8*, 33.
(40) Behar, M.; Barken, D.; Werner, S. L.; Hoffmann, A. *Cell* **2013**, *155*, 448.
(41) Ainsworth, C. *Nat. Med.* **2011**, *17*, 1166.
(42) Peracchi, A.; Mozzarelli, A. *Biochim. Biophys. Acta* **2011**, *1814*, 922.
(43) Tsai, C. J.; Del Sol, A.; Nussinov, R. *Mol. BioSyst.* **2009**, *5*, 207.
(44) Arkin, M. R.; Wells, J. A. *Nat. Rev. Drug Discovery* **2004**, *3*, 301.
(45) Yang, Y.; Xu, Y.; Xia, T.; Chen, F.; Zhang, C.; Liang, W.; Lai, L.; Fang, X. *Chem. Commun.* **2011**, *47*, 5440.
(46) Datta, D.; Scheer, J. M.; Romanowski, M. J.; Wells, J. A. *J. Mol. Biol.* **2008**, *381*, 1157.
(47) Hardy, J. A.; Lam, J.; Nguyen, J. T.; O'Brien, T.; Wells, J. A. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 12461.
(48) Gunasekaran, K.; Ma, B.; Nussinov, R. *Proteins* **2004**, *57*, 433.
(49) Huang, Z.; Mou, L.; Shen, Q.; Lu, S.; Li, C.; Liu, X.; Wang, G.; Li, S.; Geng, L.; Liu, Y.; Wu, J.; Chen, G.; Zhang, J. *Nucleic Acids Res.* **2014**, *42*, D510.
(50) McClendon, C. L.; Friedland, G.; Mobley, D. L.; Amirkhani, H.; Jacobson, M. P. *J. Chem. Theory Comput.* **2009**, *5*, 2486.
(51) Demerdash, O. N.; Daily, M. D.; Mitchell, J. C. *PLoS Comput. Biol.* **2009**, *5*, e1000531.
(52) Yuan, Y.; Pei, J.; Lai, L. *Curr. Pharm. Des.* **2013**, *19*, 2326.
(53) Miao, Y.; Nichols, S. E.; McCammon, J. A. *Chem. Biol. Drug Des.* **2014**, *83*, 237.
(54) Ngan, C. H.; Bohnuud, T.; Mottarella, S. E.; Beglov, D.; Villar, E. A.; Hall, D. R.; Kozakov, D.; Vajda, S. *Nucleic Acids Res.* **2012**, *40*, W271.
(55) Qi, Y. F.; Wang, Q.; Tang, B.; Lai, L. H. *J. Chem. Theory Comput.* **2012**, *8*, 2962.
(56) Swain, J. F.; Gierasch, L. M. *Curr. Opin. Struct. Biol.* **2006**, *16*, 102.
(57) Wang, Q.; Qi, Y.; Yin, N.; Lai, L. *PLoS One* **2014**, *9*, e94829.
(58) Panjkovich, A.; Daura, X. *BMC Bioinformatics* **2012**, *13*, 273.
(59) Huang, B.; Schroeder, M. *BMC Struct. Biol.* **2006**, *6*, 19.
(60) Panjkovich, A.; Daura, X. *Bioinformatics* **2014**, *30*, 1314.
(61) Huang, W.; Lu, S.; Huang, Z.; Liu, X.; Mou, L.; Luo, Y.; Zhao, Y.; Liu, Y.; Chen, Z.; Hou, T.; Zhang, J. *Bioinformatics* **2013**, *29*, 2357.
(62) Le Guilloux, V.; Schmidtke, P.; Tuffery, P. *BMC Bioinformatics* **2009**, *10*, 168.
(63) Liu, J.; Perumal, N. B.; Oldfield, C. J.; Su, E. W.; Uversky, V. N.; Dunker, A. K. *Biochemistry* **2006**, *45*, 6873.

(64) Uversky, V. N.; Oldfield, C. J.; Dunker, A. K. *Annu. Rev. Biophys.* **2008**, *37*, 215.
(65) Cumberworth, A.; Lamour, G.; Babu, M. M.; Gsponer, J. *Biochem. J.* **2013**, *454*, 361.
(66) Liu, Z.; Huang, Y. *Protein Sci.* **2014**, *23*, 539.
(67) Ekman, D.; Light, S.; Bjorklund, A. K.; Elofsson, A. *Genome Biol.* **2006**, *7*, R45.
(68) Motlagh, H. N.; Wrabl, J. O.; Li, J.; Hilser, V. J. *Nature* **2014**, *508*, 331.
(69) Hammoudeh, D. I.; Follis, A. V.; Prochownik, E. V.; Metallo, S. J. *J. Am. Chem. Soc.* **2009**, *131*, 7390.
(70) Yin, X.; Giap, C.; Lazo, J. S.; Prochownik, E. V. *Oncogene* **2003**, *22*, 6151.
(71) Jin, F.; Yu, C.; Lai, L.; Liu, Z. *PLoS Comput. Biol.* **2013**, *9*, e1003249.
(72) Gashaw, I.; Ellinghaus, P.; Sommer, A.; Asadullah, K. *Drug Discovery Today* **2011**, *16*, 1037.
(73) Thomas, J. R.; Hergenrother, P. J. *Chem. Rev.* **2008**, *108*, 1171.
(74) Damsma, G. E.; Alt, A.; Brueckner, F.; Carell, T.; Cramer, P. *Nat. Struct. Mol. Biol.* **2007**, *14*, 1127.
(75) Wang, X. D.; Ou, T. M.; Lu, Y. J.; Li, Z.; Xu, Z.; Xi, C.; Tan, J. H.; Huang, S. L.; An, L. K.; Li, D.; Gu, L. Q.; Huang, Z. S. *J. Med. Chem.* **2010**, *53*, 4390.
(76) Balasubramanian, S.; Hurley, L. H.; Neidle, S. *Nat. Rev. Drug Discovery* **2011**, *10*, 261.
(77) Shaw, K. T.; Utsuki, T.; Rogers, J.; Yu, Q. S.; Sambamurti, K.; Brossi, A.; Ge, Y. W.; Lahiri, D. K.; Greig, N. H. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 7605.
(78) Michael, T. H.; Reyes-Irisarri, E.; Hüll, M.; Kummer, M. P. *Curr. Neuropharm.* **2011**, *9*, 643.
(79) Ramya Kapadia, J. H.; R, V. *Front. Biosci.* **2009**, 1813.
(80) Sharma, S.; Kelly, T. K.; Jones, P. A. *Carcinogenesis* **2010**, *31*, 27.
(81) Saito, Y.; Liang, G.; Egger, G.; Friedman, J. M.; Chuang, J. C.; Coetzee, G. A.; Jones, P. A. *Cancer Cell* **2006**, *9*, 435.
(82) Nicoll, A. J.; Trevitt, C. R.; Tattum, M. H.; Risse, E.; Quarterman, E.; Ibarra, A. A.; Wright, C.; Jackson, G. S.; Sessions, R. B.; Farrow, M.; Walthod, J. P.; Clarke, A. R.; Collingea, J. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 17610.
(83) Van Goor, F.; Hadida, S.; Grootenhuis, P. D.; Burton, B.; Stack, J. H.; Straley, K. S.; Decker, C. J.; Miller, M.; McCartney, J.; Olson, E. R.; Wine, J. J.; Frizzell, R. A.; Ashlock, M.; Negulescu, P. A. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 18843.
(84) Schmit, T. L.; Ledesma, M. C.; Ahmad, N. *Pharm. Res.* **2010**, *27*, 989.
(85) Copeland, R. A.; Pompliano, D. L.; Meek, T. D. *Nat. Rev. Drug Discovery* **2006**, *5*, 730.
(86) Lu, H.; Tonge, P. J. *Curr. Opin. Chem. Biol.* **2010**, *14*, 467.
(87) Ohlson, S. *Drug Discovery Today* **2008**, *13*, 433.
(88) Swinney, D. C.; Anthony, J. *Nat. Rev. Drug Discovery* **2011**, *10*, 507.
(89) Vauquelin, G.; Charlton, S. J. *Br. J. Pharmacol.* **2010**, *161*, 488.
(90) Wei, D.; Zheng, H.; Su, N.; Deng, M.; Lai, L. *J. Chem. Inf. Model.* **2010**, *50*, 1855.
(91) Copeland, R. A. *Future Med. Chem.* **2011**, *3*, 1491.
(92) Bairy, S.; Wong, C. F. *Proteins* **2011**, *79*, 2491.
(93) Yin, N.; Pei, J.; Lai, L. *Mol. BioSyst.* **2013**, *9*, 1381.
(94) Bai, F.; Xu, Y.; Chen, J.; Liu, Q.; Gu, J.; Wang, X.; Ma, J.; Li, H.; Onuchic, J. N.; Jiang, H. *Proc. Natl. Acad. Sci. U.S.A.* **2013**, *110*, 4273.
(95) Carroll, M. J.; Mauldin, R. V.; Gromova, A. V.; Singleton, S. F.; Collins, E. J.; Lee, A. L. *Nat. Chem. Biol.* **2012**, *8*, 246.
(96) Schmidtke, P.; Luque, F. J.; Murray, J. B.; Barril, X. *J. Am. Chem. Soc.* **2011**, *133*, 18903.
(97) Schneider, E. V.; Bottcher, J.; Huber, R.; Maskos, K.; Neumann, L. *Proc. Natl. Acad. Sci. U.S.A.* **2013**, *110*, 8081.
(98) Bai, H.; Yang, K.; Yu, D.; Zhang, C.; Chen, F.; Lai, L. *Proteins* **2011**, *79*, 720.
(99) Kutchukian, P. S.; Shakhnovich, E. I. *Expert Opin. Drug Discovery* **2010**, *5*, 789.
(100) Yuan, Y.; Pei, J.; Lai, L. *J. Chem. Inf. Model.* **2011**, *51*, 1083.
(101) Wang, R.; Gao, Y.; Lai, L. *J. Mol. Model.* **2000**, *6*, 498.
(102) Luo, Z.; Wang, R.; Lai, L. *J. Chem. Inf. Comput. Sci.* **1996**, *36*, 1187.
(103) Ni, S.; Yuan, Y.; Huang, J.; Mao, X.; Lv, M.; Zhu, J.; Shen, X.; Pei, J.; Lai, L.; Jiang, H.; Li, J. *J. Med. Chem.* **2009**, *52*, 5295.
(104) Goldberg, D. R.; Hao, M. H.; Qian, K. C.; Swinamer, A. D.; Gao, D. A.; Xiong, Z.; Sarko, C.; Berry, A.; Lord, J.; Magolda, R. L.; Fadra, T.; Kroe, R. R.; Kukulka, A.; Madwed, J. B.; Martin, L.; Pargellis, C.; Skow, D.; Song, J. J.; Tan, Z.; Torcellini, C. A.; Zimmitti, C. S.; Yee, N. K.; Moss, N. *J. Med. Chem.* **2007**, *50*, 4016.
(105) Cogan, D. A.; Aungst, R.; Breinlinger, E. C.; Fadra, T.; Goldberg, D. R.; Hao, M. H.; Kroe, R.; Moss, N.; Pargellis, C.; Qian, K. C.; Swinamer, A. D. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3251.
(106) Kandil, S.; Biondaro, S.; Vlachakis, D.; Cummins, A. C.; Coluccia, A.; Berry, C.; Leyssen, P.; Neyts, J.; Brancale, A. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2935.
(107) Park, H.; Bahn, Y. J.; Ryu, S. E. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4330.
(108) Bhayye, S. S.; Roy, K.; Saha, A. *Med. Chem. Res.* **2014**, 1.
(109) Congreve, M.; Chessari, G.; Tisi, D.; Woodhead, A. J. *J. Med. Chem.* **2008**, *51*, 3661.
(110) Chessari, G.; Woodhead, A. J. *Drug Discovery Today* **2009**, *14*, 668.
(111) Sheng, C.; Zhang, W. *Med. Res. Rev.* **2013**, *33*, 554.
(112) Hoffer, L.; Renaud, J. P.; Horvath, D. *Comb. Chem. High Throughput Screen.* **2011**, *14*, 500.
(113) Brown, D.; Superti-Furga, G. *Drug Discovery Today* **2003**, *8*, 1067.
(114) Kamb, A.; Wee, S.; Lengauer, C. *Nat. Rev. Drug Discovery* **2007**, *6*, 115.
(115) Cavalli, A.; Bolognesi, M. L.; Minarini, A.; Rosini, M.; Tumiatti, V.; Recanatini, M.; Melchiorre, C. *J. Med. Chem.* **2008**, *51*, 347.
(116) Feala, J. D.; Cortes, J.; Duxbury, P. M.; Piermarocchi, C.; McCulloch, A. D.; Paternostro, G. *Wiley Interdiscip. Rev. Syst. Biol. Med.* **2010**, *2*, 181.
(117) Fitzgerald, J. B.; Schoeberl, B.; Nielsen, U. B.; Sorger, P. K. *Nat. Chem. Biol.* **2006**, *2*, 458.
(118) Perelson, A. S.; Essunger, P.; Cao, Y.; Vesanen, M.; Hurley, A.; Saksela, K.; Markowitz, M.; Ho, D. D. *Nature* **1997**, *387*, 188.
(119) Gu, S.; Yin, N.; Pei, J.; Lai, L. *Mol. BioSyst.* **2013**, *9*, 1931.
(120) Gu, S.; Yin, N.; Pei, J.; Lai, L. *Mol. BioSyst.* **2013**, *9*, 2696.
(121) Liang, X.; Li, H.; Li, S. *Mol. BioSyst.* **2014**, *10*, 1014.
(122) Morphy, R.; Rankovic, Z. *J. Med. Chem.* **2005**, *48*, 6523.
(123) Morphy, R.; Kay, C.; Rankovic, Z. *Drug Discovery Today* **2004**, *9*, 641.
(124) Knox, A. J.; Price, T.; Pawlak, M.; Golfis, G.; Flood, C. T.; Fayne, D.; Williams, D. C.; Meegan, M. J.; Lloyd, D. G. *J. Med. Chem.* **2009**, *52*, 2177.
(125) Wu, Y.; He, C.; Gao, Y.; He, S.; Liu, Y.; Lai, L. *J. Med. Chem.* **2012**, *55*, 2597.
(126) Chen, Z.; Wu, Y.; Liu, Y.; Yang, S.; Chen, Y.; Lai, L. *J. Med. Chem.* **2011**, *54*, 3650.
(127) Wei, D.; Jiang, X.; Zhou, L.; Chen, J.; Chen, Z.; He, C.; Yang, K.; Liu, Y.; Pei, J.; Lai, L. *J. Med. Chem.* **2008**, *51*, 7882.
(128) Shang, E.; Yuan, Y.; Chen, X.; Liu, Y.; Pei, J.; Lai, L. *J. Chem. Inf. Model.* **2014**, *54*, 1235.
(129) Besnard, J.; Ruda, G. F.; Setola, V.; Abecassis, K.; Rodriguiz, R. M.; Huang, X. P.; Norval, S.; Sassano, M. F.; Shin, A. I.; Webster, L. A.; Simeons, F. R.; Stojanovski, L.; Prat, A.; Seidah, N. G.; Constam, D. B.; Bickerton, G. R.; Read, K. D.; Wetsel, W. C.; Gilbert, I. H.; Roth, B. L.; Hopkins, A. L. *Nature* **2012**, *492*, 215.
(130) Lounkine, E.; Keiser, M. J.; Whitebread, S.; Mikhailov, D.; Hamon, J.; Jenkins, J. L.; Lavan, P.; Weber, E.; Doak, A. K.; Cote, S.; Shoichet, B. K.; Urban, L. *Nature* **2012**, *486*, 361.
(131) Ma'ayan, A.; Jenkins, S. L.; Goldfarb, J.; Iyengar, R. *Mt. Sinai J. Med.* **2007**, *74*, 27.
(132) Li, Y. Y.; Jones, S. J. *Genome Med.* **2012**, *4*, 27.
(133) Lussier, Y. A.; Chen, J. L. *Sci. Transl. Med.* **2011**, *3*, 96ps35.

(134) Ashburn, T. T.; Thor, K. B. *Nat. Rev. Drug Discovery* **2004**, *3*, 673.
(135) Sirota, M.; Dudley, J. T.; Kim, J.; Chiang, A. P.; Morgan, A. A.; Sweet-Cordero, A.; Sage, J.; Butte, A. J. *Sci. Transl. Med.* **2011**, *3*, 96ra77.
(136) Chong, C. R.; Sullivan, D. J., Jr. *Nature* **2007**, *448*, 645.
(137) Ivanov, A. A.; Khuri, F. R.; Fu, H. *Trends Pharmacol. Sci.* **2013**, *34*, 393.
(138) Sperandio, O.; Reynes, C. H.; Camproux, A. C.; Villoutreix, B. O. *Drug Discovery Today* **2010**, *15*, 220.
(139) London, N.; Raveh, B.; Schueler-Furman, O. *Curr. Opin. Chem. Biol.* **2013**, *17*, 952.
(140) Morelli, X.; Bourgeas, R.; Roche, P. *Curr. Opin. Chem. Biol.* **2011**, *15*, 475.
(141) Kallen, J.; Welzenbach, K.; Ramage, P.; Geyl, D.; Kriwacki, R.; Legge, G.; Cottens, S.; Weitz-Schmidt, G.; Hommel, U. *J. Mol. Biol.* **1999**, *292*, 1.
(142) McMillan, K.; Adler, M.; Auld, D. S.; Baldwin, J. J.; Blasko, E.; Browne, L. J.; Chelsky, D.; Davey, D.; Dolle, R. E.; Eagen, K. A.; Erickson, S.; Feldman, R. I.; Glaser, C. B.; Mallari, C.; Morrissey, M. M.; Ohlmeyer, M. H.; Pan, G.; Parkinson, J. F.; Phillips, G. B.; Polokoff, M. A.; Sigal, N. H.; Vergona, R.; Whitlow, M.; Young, T. A.; Devlin, J. J. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 1506.
(143) Niederhauser, O.; Mangold, M.; Schubenel, R.; Kusznir, E. A.; Schmidt, D.; Hertel, C. *J. Neurosci. Res.* **2000**, *61*, 263.
(144) Fuller, J. C.; Burgoyne, N. J.; Jackson, R. M. *Drug Discovery Today* **2009**, *14*, 155.
(145) Dai, Z.; Lai, L. *Mol. BioSyst.* **2014**, *10*, 1385.
(146) He, C.; Wu, Y.; Lai, Y.; Cai, Z.; Liu, Y.; Lai, L. *Mol. BioSyst.* **2012**, *8*, 1585.
(147) Zhang, C.; Shen, Q.; Tang, B.; Lai, L. *Angew. Chem., Int. Ed.* **2013**, *52*, 11059.
(148) Zhang, C.; Lai, L. *Proteins* **2012**, *80*, 1078.
(149) Zhang, C.; Lai, L. *Biochem. Soc. Trans.* **2011**, *39*, 1382.
(150) Zhang, C.; Lai, L. *J. Comput. Chem.* **2011**, *32*, 2598.
(151) Bi, S.; Yu, D.; Si, G.; Luo, C.; Li, T.; Ouyang, Q.; Jakovljevic, V.; Sourjik, V.; Tu, Y.; Lai, L. *Proc. Natl. Acad. Sci. U.S.A.* **2013**, *110*, 16814.
(152) Ouyang, Q.; Lai, L.; Tang, C. *ACS Synth. Biol.* **2012**, *1*, 254.
(153) Bayer, T. S.; Smolke, C. D. *Nat. Biotechnol.* **2005**, *23*, 337.
(154) Peisajovich, S. G.; Garbarino, J. E.; Wei, P.; Lim, W. A. *Science* **2010**, *328*, 368.