Chemistry & Biology
Perspective

How Chemoproteomics Can Enable Drug Discovery and Development

Raymond E. Moellering[1] and Benjamin F. Cravatt[1,*]
[1]The Skaggs Institute for Chemical Biology and Department of Chemical Physiology, The Scripps Research Institute, La Jolla, CA 92037, USA
*Correspondence: cravatt@scripps.edu
DOI 10.1016/j.chembiol.2012.01.001

Creating first-in-class medications to treat human disease is an extremely challenging endeavor. While genome sequencing and genetics are making direct connections between mutations and human disorders at an unprecedented rate, matching molecular targets with a suitable therapeutic indication must ultimately be achieved by pharmacology. Here, we discuss how the integration of chemical proteomic platforms (such as activity-based protein profiling) into the earliest stages of the drug discovery process has the potential to greatly expand the scope of proteins that can be pharmacologically evaluated in living systems, and, through doing so, promote the identification and prioritization of new therapeutic targets.

Introduction

Paradoxes abound in the modern world of drug discovery. Genome sequences have provided a complete parts list describing all of the proteins in the human body, and high-throughput screening technologies offer platforms for exposing these proteins to millions of small molecules. Yet as has been well documented by others (Munos and Chin, 2011; Bunnage, 2011), such informational and technical advances have not yet yielded a corresponding increase in new first-in-class medicines. While the reasons for this are complex and multifold, we will take the stance in this Perspective that at least part of the problem with drug discovery today is that, for the critical step of early stage target characterization in both academia and industry, pharmacology has been largely displaced by molecular biology and genetics. This has created a methodological disconnect between the early (genetically driven) and late (pharmacologically driven) stages of the drug development process that, for the reasons outlined below, can impede and even prevent the progression of potentially interesting therapeutic targets. We argue that recent advances in chemical proteomic ("chemoproteomic") methods should inspire a reintegration of pharmacology into the earliest stages of target characterization, such that it serves as a driver for, rather than responder to, biological discovery. Establishing a renewed commitment to pharmacology that is guided by modern chemoproteomic technologies has the potential to create a much more efficient path to mine the proteome for new drug targets.

Decades ago, pharmacology and the chemical probes that it provides were integral to the process of biological discovery, often providing the first insights into new protein targets and biochemical pathways that affect mammalian physiology and disease (Harding et al., 1989; Miller et al., 1990; Thornberry et al., 1992; Swinney and Anthony, 2011). Many of these pharmacological tools represented valuable "proof-of-relevance" probes, which we define for the purposes of this Perspective as meeting the minimalist definition of compounds that can block (or agonize) a protein of interest with good potency and selectivity in both cell and animal models. Some of these pharmacologically driven discoveries led to groundbreaking medicines that are still used to treat human disorders today (Swinney and Anthony, 2011). However, with the emergence of advanced molecular biology methods in the late 1980s through the 1990s, pharmacology somehow lost its sheen for early stage target characterization, perhaps appearing as a somewhat cumbersome approach that lacked the technical simplicity and specificity of genetic methods. In the ensuing decades, molecular biology maintained and arguably even extended its dominion over pharmacology, which was relegated to a much later step in the target characterization pipeline that was only initiated once substantial biological understanding of the protein target had been achieved (usually through years of molecular biology-driven research). This order of events brings us to the here and now, where we are entering the second decade of research since the first report of complete mouse and human genome sequences, and yet we still lack proof-of-relevance small-molecule probes for the vast majority of mammalian proteins. Should this deficiency be attributed to the degree of difficulty in developing such probes or a lack of firm commitment to do so? We believe, perhaps not surprisingly, that the answer is a combination of both, but also that the second issue is more of a contributory factor than generally realized.

No doubt about it—pharmacology is more difficult than genetics. Each protein target, owing to its distinct structure and function, presents a special set of challenges for chemical probe development, and many have argued that only a small fraction of the human proteome is even, in principle, "druggable" (Russ and Lampel, 2005; Overington et al., 2006). In contrast, genetic methods have few if any target boundaries. The technical ease with which genes can be selectively knocked down or out of cell and animal models is seductive, and rodent and human genetics can further provide some of the most convincing validation for target relevance to disease. However, genetic disruption of a protein, which often results in the loss of protein expression throughout life and is impractical for numerous protein classes (e.g., developmentally essential genes), may not mirror the effects of pharmacologically blocking its activity in a mature organism. Several elegant inducible and tissue-specific knockout/overexpression genetic systems have been introduced in recent years to allow for more specific spatial

and temporal control of gene expression; however, even these systems do not fully recapitulate the diverse ways that pharmacological probes can modulate protein function. Many drugs, for instance, produce their biological and medical effects, as well as avoid toxicity, by partial blockade (or activation) of a protein or by targeting proteins in a subset of tissues. Drugs can also affect multiple protein targets in vivo to produce therapeutic effects. Modeling such partial target modulation or polypharmacology by genetics is problematic.

Extrapolating from our knowledge of successful drugs and their targets and mechanisms of action, one could argue that pharmacology, no matter how challenging it may be, should be placed front and center in any serious attempt to mine the proteome for new drug targets. Ideally, one would like to generate a proof-of-relevance small-molecule probe for every protein in the mammalian proteome. The big question then becomes: How can we best pursue this ambitious goal, especially in today's research environment, where the pharmaceutical industry, a historical juggernaut for developing first-in-class pharmacological probes, is rapidly moving away from early stage target discovery and validation (Munos and Chin, 2011; Bunnage, 2011)? As will be elaborated on below, we believe that this change presents a tremendous opportunity for the academic research community to create a new and more target-inclusive approach to mammalian pharmacology. Emerging chemoproteomic methods offer ways to develop proof-of-relevance probes for proteins that span the full spectrum of annotation to include those with established activities and proteins that lack functional annotation. Success could usher in a ''back to the future'' era of scientific research where pharmacology once again serves as a principal driving force for early stage biological discoveries that, when coupled with insights into mechanism-of-action provided by chemoproteomics, propel our understanding of small-molecule effects on protein function in living systems. This knowledge can then be used to prioritize new targets and, perhaps more accurately, new drug-target pairs for clinical development.

Genome Sequences as a Foundation for Modern Pharmacology

One cannot overstate the importance of complete genome sequences for modern approaches to pharmacology. We now understand the full complement of proteins encoded by the human genome (splice variants and posttranslationally modified proteins excepted), and many human proteins can be grouped into structurally and mechanistically related families based on sequence homology. These complete protein families provide a valuable starting point for asking an interesting set of pharmacological questions. Across how many protein families do drugged targets distribute? Within these druggable families, how many members have proof-of-relevance probes? For probes that target multiple members of a given protein family, is this polypharmacology reflected in simple sequence-relatedness among the shared protein targets? Are protein families that lack drugged members more difficult to target with chemical probes, or do they simply represent portions of the proteome that have not yet been experimentally investigated? Chemoproteomics is well-suited to address some if not all of these important questions.

Chemoproteomics for Targeting Druggable but as yet Undrugged Proteins

Several reviews and perspectives have discussed the topic of the ''druggability'' of the human proteome (Russ and Lampel, 2005; Overington et al., 2006), often making the point that the typical protein families considered most amenable to small-molecule pharmacology (such as enzymes, channels, and receptors) represent only a modest fraction of all human proteins. What is rarely mentioned in these perspectives, however, is what fraction of the druggable proteome has already been drugged? This is an important issue considering that the sum of all enzymes, channels, and receptors in the human proteome likely totals well over 2,000 proteins, a healthy number of candidate drug targets by any account. To better grasp the current state of our pharmacological coverage of the druggable proteome, we consider two strategies that aim to discover chemical probes for new protein targets: (1) chemoproteomics of large enzyme families and (2) chemoproteomics combined with phenotypic screening. Together, these approaches have generated the first proof-of-relevance chemical probes for many enzymes, receptors, and channels, while at the same time underscoring that such probes are still lacking for a substantial fraction of the druggable proteome. We discuss how chemoproteomic methods can facilitate the completion of pharmacological maps for such portions of the druggable but as yet undrugged proteome.

Chemoproteomics of Large Enzyme Families

Several of the largest protein families in humans, including kinases, hydrolases, and oxidoreductases, are enzymes that are related by a common mechanism and/or structure. The chemoproteomic method activity-based protein profiling (ABPP) targets the shared mechanistic and structural features of large enzyme classes using active site-directed chemical probes to create a versatile platform for enzyme and inhibitor discovery (Cravatt et al., 2008). Here, we review how ABPP is being used to develop proof-of-relevance inhibitors for enzymes using the serine hydrolases as a case study.

Competitive ABPP to Develop Serine Hydrolase Inhibitors

Serine hydrolases are one of the largest and most diverse enzyme classes in nature, with more than 200 predicted members in humans (Simon and Cravatt, 2010). Serine hydrolases are bound together, not by sequence or structure, but by a common catalytic mechanism that uses an activated serine nucleophile to hydrolyze ester, thioester, or amide bonds in small-molecule, peptide, and protein substrates. Individual serine hydrolases have been found to perform vital biological functions in both prokaryotic and eukaryotic organisms, including involvement in bacterial cell wall biosynthesis, viral protein processing, blood clotting, lipid metabolism, and termination of neurotransmitter and hormone signaling (Long and Cravatt, 2011). Selective inhibitors have played prominent roles in the functional characterization of serine hydrolases and, in several instances, been developed into drugs to treat human disorders such as diabetes (Thornberry and Weber, 2007), obesity (Nelson and Miles, 2005), Alzheimer's disease (Racchi et al., 2004), and bacterial (Kluge and Petter, 2010) and viral (Vermehren and Sarrazin, 2011) infections. Based on these translational successes, one might presume that pharmacology tools are available for a large

fraction of serine hydrolases; however, this is not the case. Indeed, we estimate that proof-of-relevance inhibitors have been developed for less than 10% of the >200 human serine hydrolases. This percentage does not appreciably increase if one restricts the analysis to the more druggable ~120 metabolic serine hydrolases (i.e., removing from consideration the ~120 trypsin/chymotryspin proteases, which some may consider difficult to drug) (Figure 1A).

Given the biological importance and clinical relevance of serine hydrolases, it is worth asking: Why do most enzymes from this class still lack proof-of-relevance inhibitors? One potential explanation is that the physiological functions for the majority of mammalian serine hydrolases remain poorly characterized (Simon and Cravatt, 2010; Long and Cravatt, 2011), which could limit interest in targeting these enzymes for inhibitor development. This is a classic "chicken and egg" problem, where investment in pharmacology awaits deeper biological knowledge, but acquiring this knowledge could itself depend on the availability of pharmacological tools. Indeed, others have noted that, for protein families such as the nuclear hormone receptors, there is a direct relationship between the extent of biological understanding of a protein (as estimated by publications on the protein) and whether a chemical probe exists for this protein (Edwards et al., 2011). This relationship certainly appears to hold true for serine hydrolases (Figure 1B), which has motivated us to consider how to place pharmacology ahead of biology for these enzymes, such that proof-of-relevance inhibitors can be developed to study the entire enzyme class, including members that lack functional annotation.

Developing inhibitors for enzymes typically requires knowledge of their substrates for assay configuration. This criterion has historically hindered efforts to create full pharmacological maps for large enzyme classes, such as the serine hydrolases, that possess many uncharacterized members. In recent years, however, chemoproteomic methods, such as ABPP, have emerged to address this challenge. We have shown that reporter-tagged fluorophosphonates (FPs) (Liu et al., 1999) serve as activity-based probes for the vast majority of mammalian serine hydrolases (Bachovchin et al., 2010). Competitive ABPP can thus form the basis for a near-universal assay format for serine hydrolase inhibitor discovery and optimization, where small molecules are evaluated for their ability to block FP probe labeling in a range of biological systems (Bachovchin et al., 2010; Leung et al., 2003) (Figure 2A). Such competitive ABPP programs might initiate with a high-throughput screen, where the reaction between fluorophore-tagged FP probes and purified serine hydrolases is measured by fluorescence polarization (fluopol, Figure 2A) (Bachovchin et al., 2009, 2011). Hits from these screens are then immediately screened for activity and selectivity in proteomes using gel-based or mass spectrometry (MS)-based platforms for competitive ABPP (Figure 2B), which collectively assay >50 serine hydrolases in parallel (Bachovchin et al., 2011). An interesting output of these proteomic assays is not only the rank-ordering of hits for the screened enzyme target, but also the discovery of lead inhibitors for other enzymes that were part of the counterscreen (Leung et al., 2003). Lead compounds that show promising potency and selectivity can be then be optimized through an iterative process of medicinal chemistry and competitive ABPP, culminating in candidate proof-of-relevance inhibitors that are ready for in vivo testing.

Confirming inhibitor activity and selectivity in vivo can be challenging, especially for enzymes that lack known substrates or product biomarkers. Here, we have found that competitive ABPP can also serve an important purpose, where the technology is used to evaluate serine hydrolase activities in cells and tissues from inhibitor-treated animals (Bachovchin et al., 2011; Adibekian et al., 2011; Chang et al., 2011; Long et al., 2009). Competitive ABPP thus enables the enzyme target to serve as its own biomarker for inhibitor activity in vivo, allowing for a direct and quantitative assessment of the extent and duration of target occupancy at a given dose of inhibitor. To be fair, the ex vivo assessment of inhibitor activity by competitive ABPP is technically more straightforward to perform with irreversible enzyme inhibitors (due to the stability of the covalent enzyme-inhibitor interaction), but should also be applicable to tight-binding reversible inhibitors that display slow off-rates.

Following the general competitive ABPP workflow outlined above, proof-of-relevance inhibitors have been developed for several serine hydrolases (Figure 1), and at least one of these compounds has even progressed into human clinical trials (the fatty acid amide hydrolase inhibitor PF-04457845 [Johnson et al., 2011]). The targeted serine hydrolases belong to diverse branches of the enzyme class and include both characterized and uncharacterized members (Figure 1). Lead inhibitors have also been identified for many additional serine hydrolases by competitive ABPP (Bachovchin et al., 2010; Adibekian et al., 2011), creating the first semblance of a pharmacological map for this large enzyme class. Although still incomplete, this map possesses multiple features that are informative for family-wide inhibitor development programs. First, sequence relatedness proves to be a rather poor predictor of shared pharmacology among serine hydrolases (Bachovchin et al., 2010), indicating that traditional strategies that involve counterscreening against the nearest sequence-neighbor enzymes may fail to uncover important inhibitor cross-reactivity across large enzyme classes. Second, competitive ABPP has identified several mechanism-based chemotypes, such as the 1,2,3-triazole urea (Adibekian et al., 2011) and aza-beta-lactam (Bachovchin et al., 2011), that have not yet been extensively explored for serine hydrolase inhibition. These chemotypes offer exciting starting points for targeted libraries to improve pharmacological coverage of the serine hydrolase class. Third, because competitive ABPP provides a unifying assay for initial library screening, hit optimization, and verification of inhibitor activity in vivo, inhibitor development programs have yielded proof-of-relevance probes at an excellent pace (usually within 1-2 years of program inception). The iterative feedback from competitive ABPP assays in proteomes also expands the scope of probe development across the serine hydrolase class by identifying lead inhibitors for additional family members. In this way, many inhibitor optimization programs can be launched and progressed in parallel.

Chemproteomics Applied to Other Enzyme Classes

Competitive ABPP and additional chemoproteomic methods have been used to assess inhibitor activity for many other enzyme classes, including cysteine proteases, kinases, histone deacetylases, and cytochrome P450s.

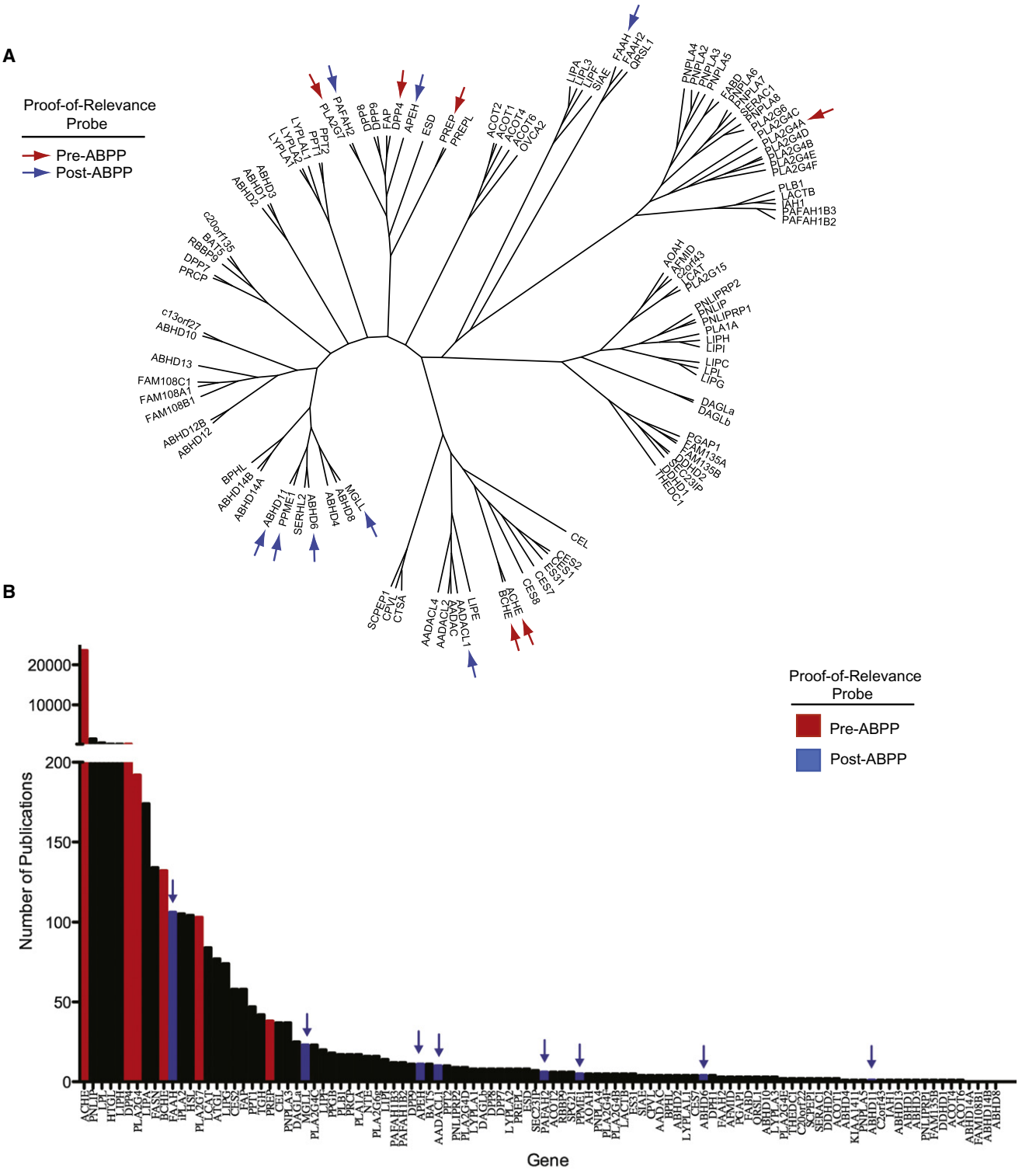

Figure 1. The Metabolic Serine Hydrolases as a Case Study for the Druggable, but Not Yet-Drugged Proteome

(A) Tree diagram showing the ~115 human metabolic serine hydrolases. Enzymes possessing proof-of-relevance chemical probes developed prior to the application of ABPP to serine hydrolases are marked with red arrows. Enzymes with proof-of-relevance probes discovered with ABPP platforms are marked with blue arrows.

(B) Chart displaying metabolic serine hydrolases as a function of number of scientific publications, where enzymes possessing proof-of-relevance chemical probes developed prior to or with the use of ABPP are marked in red and blue, respectively. Blue arrows are used to mark poorly characterized enzymes with low publication number for which ABPP has generated proof-of-relevance probes.

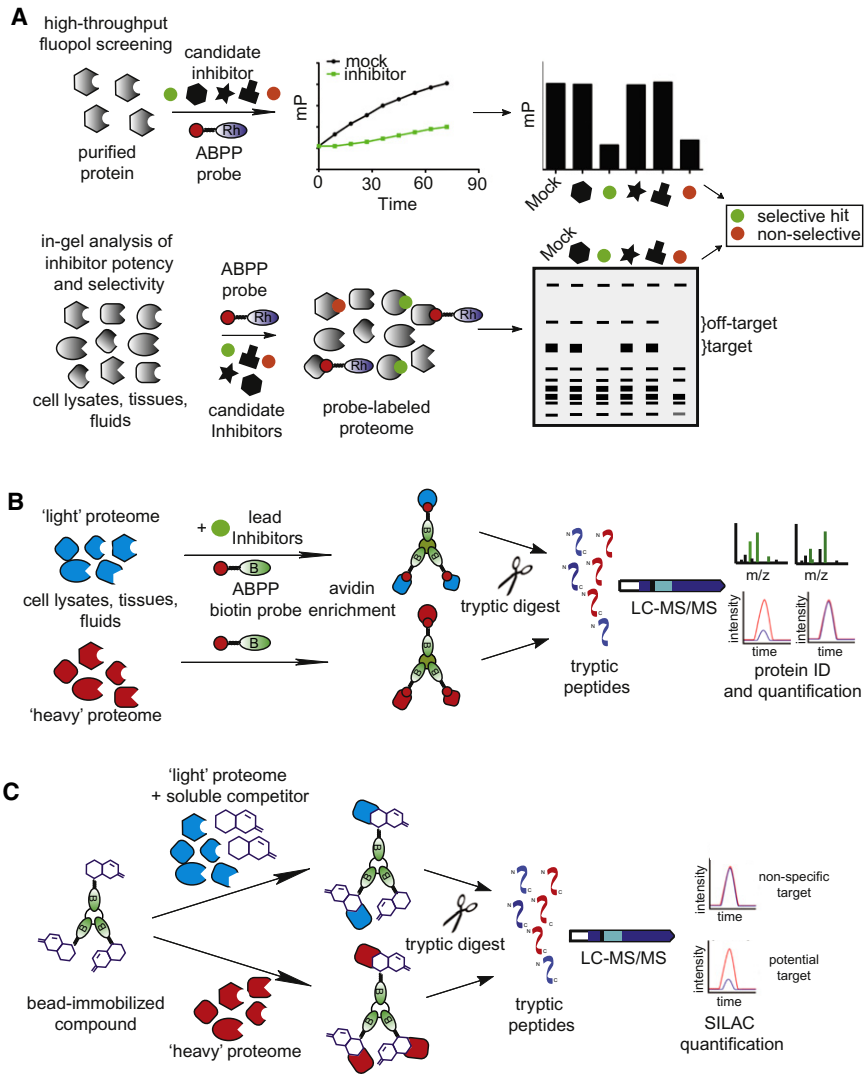

Figure 2. Representative Chemoproteomic Platforms for Drug Discovery and Development

(A) Competitive ABPP for high-throughput screening of small-molecule libraries using fluorescence polarization (fluopol) for hit discovery and gel-based selectivity profiling in proteomes for hit prioritization.

(B) SILAC-ABPP for quantitative assessment of inhibitor selectivity in proteomes.

(C) Affinity enrichment combined with SILAC to quantify small-molecule-interacting proteins from native proteomes.

Bogyo and colleagues have employed competitive ABPP to develop selective inhibitors for the malarial cysteine proteases falcipain-1 (Greenbaum et al., 2002), PfSU B1 (Arastu-Kapur et al., 2008), DPAP1 (Deu et al., 2010), and DPAP3 (Arastu-Kapur et al., 2008) and have shown that pharmacological blockade of these enzymes impairs the malaria parasite's life cycle.

Kinases, like serine hydrolases, are an extremely large and diverse enzyme class with several hundred members in humans. Several kinases, especially those with genetic ties to cancer (Zhang et al., 2009), have been the focus of intense drug development programs in the pharmaceutical industry, but in aggregate, these only account for a modest fraction of all human kinases. Academic and biotechnology researchers have begun to fill in the pharmacological gaps in the kinome, often using chemoproteomic methods to optimize the target selectivity of lead inhibitors (Goldstein et al., 2008). Multiple strategies have been introduced for this purpose, virtually all of which exploit the conserved ATP binding pocket to create general assay platforms. Examples of successful kinase inhibitor profiling platforms include (1) phage-display screening of compounds for competitive blockade of interactions between kinases and immobilized non-selective inhibitors (Fabian et al., 2005; Davis et al., 2011), (2) MS screening of compounds for competitive disruption of interactions between kinases and immobilized broad-spectrum inhibitors (Bantscheff et al., 2007; Ong et al., 2009), and (3) ABPP of compounds for competitive disruption of interactions between kinases and acylphosphate-ATP probes (Patricelli et al., 2007, 2011) (Figure 2B). The latter two approaches, which use MS to identify and quantify affinity-enriched kinases, are distinguished by their applicability to native proteomes, which facilitates matching endogenous kinase selectivity profiles with the cellular activity for inhibitors. Analogous to the aforementioned advances made with serine hydrolases, academic and biotechnology efforts have already begun to deliver valuable proof-of-relevance inhibitors for poorly characterized kinases, including RSK1 (Cohen et al., 2005, 2007), RIP1 (Degterev et al., 2008), TORC1/TORC2 (Feldman et al., 2009), MPS1 (Kwiatkowski et al., 2010), BKM1 (Yang et al., 2010), LRRK2 (Deng et al., 2011a), ERK5 (Deng et al., 2011b), Ack1 (Miduturu et al., 2011), HIPK (Miduturu et al., 2011), NEK2 (Henise and Taunton, 2011), and isoform-selective PI3K inhibitors (Knight et al., 2006). Interestingly, some of these inhibitors irreversibly inactivate kinases by modifying noncatalytic active-site cysteine residues (Cohen et al., 2005; Henise and Taunton, 2011), which has enabled their conversion into activity-based probes (Cohen et al., 2007).

Multiple chemoproteomic strategies for profiling HDAC inhibitors have also been introduced, including the creation of activity-based probes (Salisbury and Cravatt, 2007, 2008; Fischer et al., 2011) and immobilized broad-spectrum inhibitors (Bantscheff et al., 2011). Clickable, photoreactive activity-based probes have been shown to label HDACs in living cells, facilitating the discovery of HDAC activities that were impaired upon cell lysis (Salisbury and Cravatt, 2007, 2008). These ABPP studies also provided some of the first evidence that the HDAC inhibitor SAHA, historically considered a pan-class I and

II HDAC inhibitor, shows selectivity for a subset of HDACs (1, 2, 3, and 6) (Salisbury and Cravatt, 2007), a finding that has been confirmed with advanced substrate assays (Bradner et al., 2010). Chemoproteomic enrichment of inhibitor-interacting proteins has also identified unexpected specificity within HDAC protein complexes and additional potential targets for SAHA that are outside of the HDAC family (Fischer et al., 2011; Bantscheff et al., 2011).

Activity-based probes for CYP450s, which have been synthesized in clickable form to enable in vivo profiling (Wright and Cravatt, 2007), have uncovered instances where clinically approved drugs stimulate probe-labeling of individual CYP450s (Wright et al., 2009). This ''activation'' may reflect heterotropic cooperativity, a special feature of CYP450s, which can possess large active sites capable of simultaneously binding multiple small molecules.

Chemoproteomics for Target Discovery in Phenotypic Screens

Cell-based phenotypic screening offers another powerful means for developing proof-of-relevance chemical probes for proteins (Swinney and Anthony, 2011). One potential advantage of cell-based screening compared to the ''protein family-centric'' approaches mentioned above is that it can identify small-molecule probes for a protein, or collection of proteins, that lack robust biochemical assays. Connecting bioactive small molecules emerging from cell-based screens with their protein targets remains, however, a technically challenging endeavor (Kasper et al., 2009). In recent years, chemoproteomic methods have been introduced that are substantially improving the success rate of target identification in small-molecule phenotypic screens. Affinity chromatography, wherein small molecules are covalently attached to a solid support and used to ''fish out'' interacting proteins from cell lysates, is a well-established method that has succeeded in identifying the protein targets of many bioactive compounds, including natural products (Harding et al., 1989; Taunton et al., 1996), enzyme inhibitors (Thornberry et al., 1992; Cravatt et al., 1996), and hits from cellular screens (Chen et al., 2006; Yagoda et al., 2007). However, historically, these approaches have been limited by sensitivity and a lack of quantitation. Chemoproteomic solutions have been introduced to address these problems. First, modern MS methods provide a tremendous boost in sensitivity such that even low-abundance protein targets of small molecules can be identified. MS methods can also be used to quantify the extent of target enrichment by affinity chromatography, as elegantly demonstrated by Ong et al. (2009) in their use of stable isotope labeling with amino acids in cell culture (SILAC) to map the targets of several small molecules (Figure 2C), including the kinase inhibitor K252a and the anticancer agent piperlongumine (Raj et al., 2011). In both cases, multiple protein targets were identified (kinases and a set of oxidative stress enzymes, respectively). ABPP methods have also offered a chemoproteomic means to detect, enrich, and identify the protein targets of bioactive small molecules (Evans et al., 2005; Hall et al., 2011).

The use of chemoproteomic strategies for target characterization in small-molecule phenotypic screens has yielded some intriguing findings, including the realization that many bioactive compounds appear to produce their pharmacological effects through modulating multiple protein targets (Chen et al., 2006; Raj et al., 2011). That these protein targets can be unrelated by sequence, structure, or function underscores the importance of chemoproteomic methods that broadly survey the proteins that bind to bioactive small molecules. It is also interesting to note that, while many of the small molecules discovered in cell-based screens represent the first proof-of-relevance probes for their respective protein targets, the targets themselves typically belong to druggable classes of proteins (enzymes, channels, and receptors). In numerous cases where phenotypic screens were employed to identify compounds capable of perturbing targets or pathways perceived to be ''undruggable,'' such as Ras (Yagoda et al., 2007), Wnt (Huang et al., 2009), mutant p53 (Raj et al., 2011), and Sox2-mediated induced pluripotent stem cell induction (Ichida et al., 2009), the identified compounds were shown to target enzymes or channels that would be considered quite tractable by conventional drug discovery efforts. Each of these efforts thus discovered new pharmacological control points for historically challenging biological pathways, while at the same time underscoring the considerable gaps that remain in our coverage of the druggable proteome.

Summary: Reflecting Back and Projecting Forward

The contributions made to date by chemoproteomics to early stage drug discovery can be grouped into a few general categories: (1) providing platforms for assaying small-molecule libraries against poorly characterized members of large protein classes; (2) optimizing the selectivity of lead probes by screening against many proteins in parallel, often directly in native proteomes and sometimes even in vivo; and (3) identifying the protein targets for bioactive small molecules discovered in phenotypic screens (Figure 3). The value of chemoproteomics can also extend to later stages in the drug development process by providing, for instance, a way to understand unanticipated drug toxicities, as well as in vivo-biomarker assays that report on target engagement in humans (Arastu-Kapur et al., 2011) (Figure 3). There are certain portions of the proteome, such as the hydrolases and kinases, where chemoproteomic approaches have become integral components of the drug development process and are helping to create proof-of-relevance probes that span the full membership of these large enzyme classes. This integration reflects, at least in part, the advanced state of chemoproteomic technologies available to profile these enzymes. Chemoproteomics has also become a preferred method for addressing the dreaded ''target identification problem'' in phenotypic screening programs, and can even be used to more carefully reassess the proteins that interact with drugs of purportedly known mechanisms of action. Bearing such thoughts in mind, we propose a handful of directions where chemoproteomics could be taken to further advance drug discovery.

Completing the Pharmacological Map of the Druggable Proteome

An emerging theme from the family-centric and phenotypic screening strategies discussed herein is the impact that chemoproteomics can have on developing first-in-class, proof-of-relevance probes for the large number of proteins that fall into

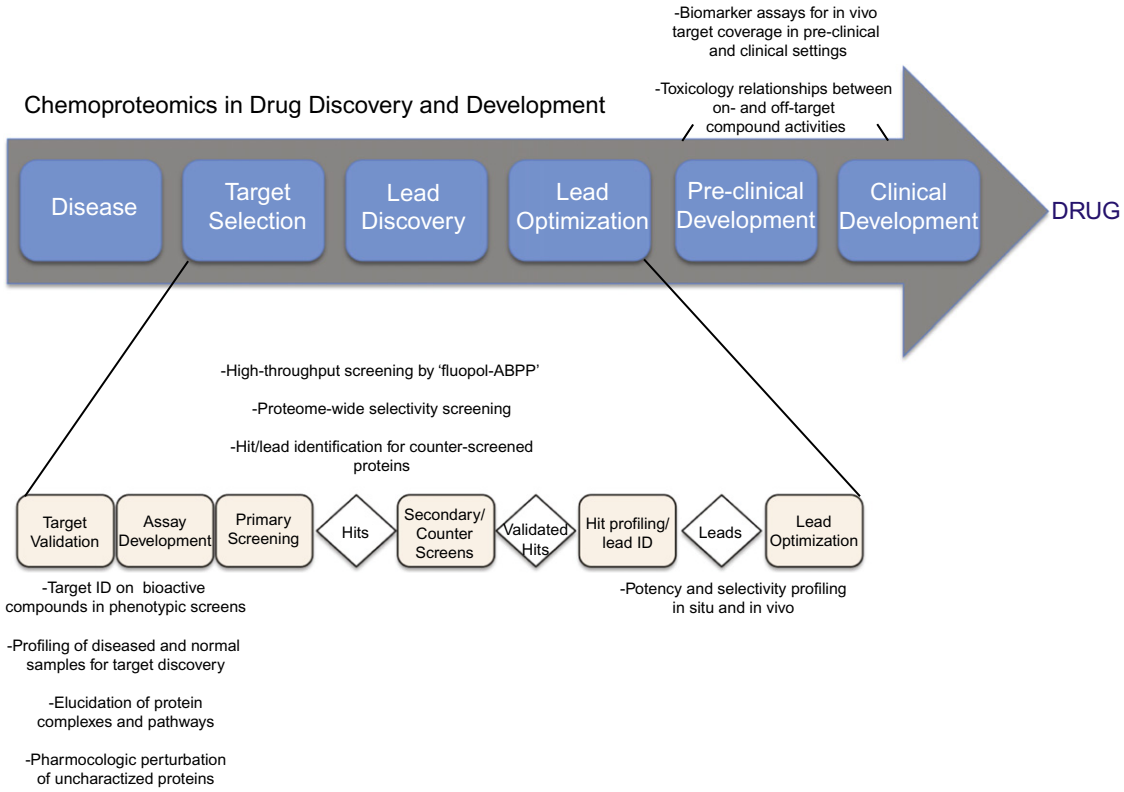

Figure 3. How Chemoproteomics Can Enable Drug Discovery and Development from the Earliest Stages of Target and Lead Compound Discovery through Lead Optimization and Biomarker Assays in Preclinical and Clinical Development
Shown are ways that chemoproteomics can assist the drug discovery and development process from early stage target and lead compound discovery through lead optimization and biomarker analysis in preclinical and clinical development.

the category of druggable but not yet drugged. Philosophically, it is worth asking: Why were these druggable proteins, until recently, lacking chemical probes? One possibility is that many of these proteins were only discovered upon sequencing the human genome and thus could not have been the focus of medicinal chemistry efforts that predated this landmark event. However, we do not believe that this explanation is satisfactory. Take, for instance, the enzymes involved in glycolysis, which were first characterized many decades ago. Most of the pharmacological tools used to perturb glycolytic enzymes consist of low-affinity, broadly reactive chemotypes (e.g., alpha-keto-halogens, arsenate salts) appended onto simplified substrate-like scaffolds that are unlikely to exhibit suitable selectivity and pharmacokinetic properties for in vivo biological studies (Table S1). That specific glycolytic enzymes such as PKM2 (Christofk et al., 2008) and PGAM1 (Evans et al., 2005; Vander Heiden et al., 2010) have now moved to the forefront as potential anticancer targets only serves to underscore the frustrating gap in our pharmacological toolbox to assess this classical metabolic pathway. Fortunately, efforts are now underway to develop proof-of-relevance probes for glycolytic enzymes (Walsh et al., 2011), but we should be self-critical enough to ask retrospectively: Why has it taken so long for these programs to kick into action? We believe, as stated at the outset of this Perspective, that the answer to the question is at least partly related to the displacement of pharmacology by molecular biology and genetics as modern approaches for perturbing protein function. By pushing pharmacology back to the tail end of biological discovery, we unnecessarily delay the creation of chemical probes that could serve as valuable tools for assessing protein function and translating this information into new medicines.

We recognize that there have been historical challenges associated with the de novo discovery of useful small-molecule probes in academic settings, but with the availability of public screening centers and advancement of chemoproteomic methods for target discovery and inhibitor optimization, these issues have now largely been addressed. We therefore posit that the infrastructure and technologies are in place to complete our pharmacological map of the druggable proteome. Returning, for instance, to glycolytic enzymes as an example. Many of these enzymes possess aberrantly reactive nucleophilic residues (Weerapana et al., 2010) and use cofactor-binding sites (e.g., ATP, NADH) that could be exploited for designing activity-based probes to assist in assaying the activity and selectivity of inhibitors. Lead inhibitors could also be evaluated for their selectivity using the other chemoproteomic methods described in this Perspective.

Discovery Biology through Pharmacology

As already demonstrated for kinases and hydrolases, the integration of small-molecule screening with chemoproteomics can be extended to develop proof-of-relevance probes for

proteins that lack known functions. One might ask: Why bother developing small-molecule probes for uncharacterized proteins? The answer is at least 2-fold. First, these inhibitors, when applied to cell and organism phenotypic assays, offer powerful tools to discover the functions of proteins, as we (Chiang et al., 2006) and others (Arastu-Kapur et al., 2008; Yang et al., 2010; Deng et al., 2011a) have demonstrated. These functional assignments can include elucidating the biochemical pathways regulated by proteins (Chiang et al., 2006), as well as the identification of specific diseases where probe-target pairs might be progressed for drug development. Indeed, one only need consider some of the most successful recent drug targets, such as dipeptidylpeptidase IV (DPPIV) (Thornberry and Weber, 2007), for which proof-of-relevance inhibitors combined with a simple glucose-tolerance test in rodents would have designated this enzyme as potential type 2 diabetes drug target. Subsequent "peptidomic" studies could then be used to discover endogenous substrates of DPPIV (Tagore et al., 2009), which might not only help to explain the pharmacological effects of disrupting this enzyme, but also serve as pharmacodynamic biomarkers for assessing its inhibition in human clinical studies. DPPIV is also an interesting example because it represents a validated drug target that, to our knowledge, lacks any direct human genetic ties to diabetes. Thus, while human genetics can certainly guide us to new drug targets, it should not, in our opinion, be used blindly as a filter for this purpose, especially at the risk of de-prioritizing druggable proteins from direct pharmacological investigation in relevant disease models.

Even if initial phenotypic screens with a chemical probe fail to reveal the biological activity of a protein, having such probes in hand should enable rapid pharmacological confirmation of functional assignments determined by other, complementary approaches. Mouse and human genetic studies, for instance, are linking missense, nonsense, and activating mutations in protein-coding genes to disease at an increasing rate, but determining the mechanistic basis and translational relevance for genotype-phenotype connections still depends on the availability of pharmacological probes. From a drug development perspective, having proof-of-relevance chemical probes for poorly characterized proteins thus sets the stage for "anticipatory pharmacology," wherein a genetic finding can be rapidly exploited for new medicines. An excellent example of anticipatory pharmacology is crizotinib (Gadgeel and Bepler, 2011), an ALK inhibitor that was recently approved for treating non-small cell lung cancer (NSCLC). Crizotinib was originally developed as an inhibitor of another kinase, c-Met (Zou et al., 2007); however, upon the discovery that activating mutations in ALK are causative for a substantive fraction of NSCLCs (Soda et al., 2007), crizotinib was quickly repurposed for treating this disease. In this case, it was fortunate that crizotinib happened to inhibit not only its originally intended target c-Met, but also ALK. As a contrast, consider another enzyme, isocitrate dehydrogenase-1 (IDH1), for which activating mutations have also recently been linked to cancer (Parsons et al., 2008; Dang et al., 2009). Despite being a well-studied metabolic enzyme (first molecularly characterized over a decade ago [Nekrutenko et al., 1998]) and a putatively druggable protein, no inhibitors were available for IDH1 at the time of its discovery as a cancer-relevant protein. Progress toward validating IDH as a drug target must thus now await the development of selective and in vivo-active inhibitors for this enzyme. Considering that IDH1 belongs to a large family of NADPH-dependent dehydrogenases, we anticipate that chemoproteomics methods could play an important role in inhibitor optimization for this enzyme. More generally, ALK and IDH1 serve as interesting case studies to advocate for more systematic efforts to create proof-of-relevance chemical probes for the entire druggable proteome, such that biological discoveries linking these proteins to human disease can be rapidly translated into new medicines.

Chemoproteomics for Purposive Polypharmacology

Crizotinib is one of several examples of multitarget kinase inhibitors that have been approved as therapeutics. In some cases, it appears that the polypharmacological mechanism of action is important for drug efficacy (Knight et al., 2010). The realization that drugs often produce their biological activity through affecting multiple protein targets has inspired consideration of purposeful polypharmacology as a way to develop new medicines (Parsons et al., 2008; Knight et al., 2010; Boran and Iyengar, 2010). Converting polypharmacology into a predictable science, however, will require methods to fully assess the target profile of drugs in complex biological systems, and, here, chemoproteomics stands out as a particularly powerful approach. There are many recent examples where chemoproteomics has helped to define the set of proteins that are responsible for mediating the cellular effects of bioactive small molecules. In some cases, the compendium of targets belong to the same family of proteins (e.g., the JQ1 and I-BET151 inhibitors of bromodomains (Delmore et al., 2011; Dawson et al., 2011); the piperlongumine inhibitor of glutathione S-transferases and related oxidative stress response enzymes [Raj et al., 2011]), while in others, they show little or no mechanistic or structural homology (e.g., the SC1 agent that promotes embryonic stem cell self-renewal through targeting both RasGAP and ERK1 proteins [Chen et al., 2006]). Chemoproteomics is particularly well-suited for uncovering such unanticipated cases of shared pharmacology that span unrelated protein families. Once relevant target sets are defined, ensuing medicinal chemistry can focus on coordinately optimizing compounds to maintain the desired target profile for drug action.

Chemproteomics for Mapping Protein Complexes and Pathways

In addition to facilitating the characterization of direct targets of bioactive small molecules, chemoproteomics can also lend insights into the endogenous protein complexes that contain these protein targets. This information can contribute to drug discovery and target validation in several ways, including providing a more complete understanding of the composition of complexes and pathways in which a protein of interest resides and uncovering unanticipated specificity that chemical probes might display for subcomplexes. Moulick et al. (2011), for instance, employed affinity chromatography using small-molecule probes and HSP90-specific antibodies to enrich HSP90-containing protein complexes. This approach identified a known HSP90 "client" oncoprotein in chronic myelogenous leukemia cells, Bcr-Abl, as a preferentially associated protein in probe-enriched but not immunoenriched HSP90 complexes. Furthermore, the abundance of HSP90-associated Bcr-Abl in a cell

line was shown to correlate with sensitivity to HSP90 inhibition. As HSP90 is known to associate with a variety of oncogenic proteins (Trepel et al., 2010), chemoproteomic enrichment of this chaperone in diverse cancers could be used as a method to identify active oncogenic pathways and suitable therapeutic options. A similar approach has been employed to identify the protein targets and associated complexes of diverse classes of HDAC inhibitors (Bantscheff et al., 2011). When coupled with quantitative mass spectrometry, these studies revealed compound specificity for not only the intended HDAC targets but also HDAC-associated protein complexes, thus highlighting the importance of assessing compound activity within endogenous complexes, rather than in reconstituted in vitro systems. Finally, a multifaceted, quantitative chemoproteomics approach was recently used to identify protein-binding partners for several bromodomain and extraterminal (BET) proteins in MLL fusion leukemic cells (Dawson et al., 2011). Affinity-capture of BET proteins with acetylated-histone peptides, BET-specific antibodies, and BET-binding small molecules provided a quantitative profile of BET-associated protein complexes, which included a novel MLL fusion protein-containing transcriptional complex. This insight provided a rationale to study the effects of BET inhibitors in MLL fusion-positive cancer cells, which proved highly sensitive to BET inhibition in vitro and in vivo. Each of these examples highlights how chemoproteomics can enrich our biological understanding of emerging drug targets by mapping their connectivity to disease-relevant protein complexes and pathways.

Chemoproteomics for Targeting Undruggable Proteins

Much of this Perspective has focused on the role that chemoproteomics can play in completing our pharmacological map of the druggable proteome. We also believe, however, that the described technologies can impact future efforts to develop chemical probes for the undruggable proteome. New chemistries, such as stapled peptides (Henchey et al., 2008) and small-molecule mimetics of protein secondary structures (Berg et al., 2002; Shahian et al., 2009), are emerging that enable pharmacological perturbation of historically challenging protein classes, such as transcription factor complexes (Berg et al., 2002; Moellering et al., 2009; Emami et al., 2004), GTPases (Patgiri et al., 2011), and apoptotic effectors (Walensky et al., 2004; Bernal et al., 2007). Confirming target interactions and selectivity for such agents in complex biological systems, however, remains a difficult task. Many of the chemoproteomic methods described herein should be amenable to addressing this problem. Given the modular synthesis of peptides one could consider, for instance, embedding within a stapled peptide a photoreactive, clickable unit to enable crosslinking to protein targets in living cells. Indeed, a similar approach was recently employed to map the interaction surfaces of photoreactive, stapled BH3 domain peptides in vitro (Braun et al., 2010). Likewise, the full complement of cellular proteins that interact with a protein-protein interaction disrupter could be mapped using affinity enrichment coupled with SILAC proteomics. The information acquired in such chemoproteomic experiments would serve to guide the optimization of drug activity and selectivity across the proteome to accelerate the translation of emerging chemical probes that target undruggable proteins into new medicines. Furthermore, enrichment of protein complexes that contain these targets could in principle identify alternative, more classically druggable sites for pharmacological intervention, as discussed above.

Conclusions

We began this Perspective with a stated goal of making an argument for the reintegration of pharmacology into early stage target discovery programs. We believe that chemoproteomic methods have matured to the point where they can efficiently guide the development of proof-of-relevance chemical probes for a substantial fraction of the human proteome. These probes possess many important advantages over genetic approaches for target perturbation, including the potential to disrupt protein function in a temporally controlled manner and without affecting protein expression, to partially activate or inhibit protein targets, and to affect multiple protein targets in parallel. It is also instructive to recognize that protein classes, like hydrolases and kinases, which have been a focal point of chemoproteomic investigations to date, also harbor some of the most rapidly expanding suites of chemical probes. The unbiased nature of chemoproteomic methods has even facilitated the development of probes for uncharacterized members of these enzyme classes (Bachovchin et al., 2010; Miduturu et al., 2011; Li et al., 2007). We find this contribution of chemoproteomics to modern pharmacology to be particularly exciting in that it is delivering versatile chemical probes that can be used to assign functions to proteins in virtually any biological model or system. Nonetheless, the majority of human proteins, even those that reside within the druggable proteome, still lack proof-of-relevance probes and addressing this problem will require a more systematic approach to integrate chemoproteomics, as well as other screening platforms that evaluate small-molecule activity and specificity (Entzeroth et al., 2000), into the basic fabric of pharmacology. We are skeptical that this integration can occur exclusively within the domain of the pharmaceutical industry, which has been steadily moving away from early stage drug discovery. Rather, we believe the challenge (or opportunity, as we would prefer to view it) will also need to be met by the academic research community. Undoubtedly, continued collaboration between academic and pharmaceutical institutions to match identified compound-target pairs with relevant therapeutic indications will be necessary to rapidly translate early-stage discovery efforts into new medicines. By embracing the development of proof-of-relevance chemical probes, and the continued advancement and implementation of chemoproteomic methods to ensure probe quality, we should enjoy an era of pharmacology-driven biological discovery that enhances the efficiency of converting basic knowledge on protein and pathway function into new first-in-class medicines to treat human disease.

SUPPLEMENTAL INFORMATION

Supplemental Information includes one table and can be found with this article online at doi:10.1016/j.chembiol.2012.01.001.

ACKNOWLEDGMENTS

We thank G. Simon for assistance with figures and helpful discussions. We are grateful for the support of the National Institutes of Health (grants CA087660,

CA132630, and DA025285), the Damon Runyon Cancer Research Foundation (Howard Hughes Medical Institute postdoctoral fellowship to R.E.M.), and the Skaggs Institute for Chemical Biology.

REFERENCES

Adibekian, A., Martin, B.R., Wang, C., Hsu, K.L., Bachovchin, D.A., Niessen, S., Hoover, H., and Cravatt, B.F. (2011). Click-generated triazole ureas as ultrapotent in vivo-active serine hydrolase inhibitors. Nat. Chem. Biol. *7*, 469–478.

Arastu-Kapur, S., Ponder, E.L., Fonović, U.P., Yeoh, S., Yuan, F., Fonović, M., Grainger, M., Phillips, C.I., Powers, J.C., and Bogyo, M. (2008). Identification of proteases that regulate erythrocyte rupture by the malaria parasite Plasmodium falciparum. Nat. Chem. Biol. *4*, 203–213.

Arastu-Kapur, S., Anderl, J.L., Kraus, M., Parlati, F., Shenk, K.D., Lee, S.J., Muchamuel, T., Bennett, M.K., Driessen, C., Ball, A.J., and Kirk, C.J. (2011). Nonproteasomal targets of the proteasome inhibitors bortezomib and carfilzomib: a link to clinical adverse events. Clin. Cancer Res. *17*, 2734–2743.

Bachovchin, D.A., Brown, S.J., Rosen, H., and Cravatt, B.F. (2009). Identification of selective inhibitors of uncharacterized enzymes by high-throughput screening with fluorescent activity-based probes. Nat. Biotechnol. *27*, 387–394.

Bachovchin, D.A., Ji, T., Li, W., Simon, G.M., Blankman, J.L., Adibekian, A., Hoover, H., Niessen, S., and Cravatt, B.F. (2010). Superfamily-wide portrait of serine hydrolase inhibition achieved by library-versus-library screening. Proc. Natl. Acad. Sci. USA *107*, 20941–20946.

Bachovchin, D.A., Mohr, J.T., Speers, A.E., Wang, C., Berlin, J.M., Spicer, T.P., Fernandez-Vega, V., Chase, P., Hodder, P.S., Schürer, S.C., et al. (2011). Academic cross-fertilization by public screening yields a remarkable class of protein phosphatase methylesterase-1 inhibitors. Proc. Natl. Acad. Sci. USA *108*, 6811–6816.

Bantscheff, M., Eberhard, D., Abraham, Y., Bastuck, S., Boesche, M., Hobson, S., Mathieson, T., Perrin, J., Raida, M., Rau, C., et al. (2007). Quantitative chemical proteomics reveals mechanisms of action of clinical ABL kinase inhibitors. Nat Biotechnol. *25*, 1035–1044.

Bantscheff, M., Hopf, C., Savitski, M.M., Dittmann, A., Grandi, P., Michon, A.M., Schlegl, J., Abraham, Y., Becher, I., Bergamini, G., et al. (2011). Chemoproteomics profiling of HDAC inhibitors reveals selective targeting of HDAC complexes. Nat. Biotechnol. *29*, 255–265.

Berg, T., Cohen, S.B., Desharnais, J., Sonderegger, C., Maslyar, D.J., Goldberg, J., Boger, D.L., and Vogt, P.K. (2002). Small-molecule antagonists of Myc/Max dimerization inhibit Myc-induced transformation of chicken embryo fibroblasts. Proc. Natl. Acad. Sci. USA *99*, 3830–3835.

Bernal, F., Tyler, A.F., Korsmeyer, S.J., Walensky, L.D., and Verdine, G.L. (2007). Reactivation of the p53 tumor suppressor pathway by a stapled p53 peptide. J. Am. Chem. Soc. *129*, 2456–2457.

Boran, A.D., and Iyengar, R. (2010). Systems approaches to polypharmacology and drug discovery. Curr. Opin. Drug Discov. Devel. *13*, 297–309.

Bradner, J.E., West, N., Grachan, M.L., Greenberg, E.F., Haggarty, S.J., Warnow, T., and Mazitschek, R. (2010). Chemical phylogenetics of histone deacetylases. Nat. Chem. Biol. *6*, 238–243.

Braun, C.R., Mintseris, J., Gavathiotis, E., Bird, G.H., Gygi, S.P., and Walensky, L.D. (2010). Photoreactive stapled BH3 peptides to dissect the BCL-2 family interactome. Chem. Biol. *17*, 1325–1333.

Bunnage, M.E. (2011). Getting pharmaceutical R&D back on target. Nat. Chem. Biol. *7*, 335–339.

Chang, J.W., Nomura, D.K., and Cravatt, B.F. (2011). A potent and selective inhibitor of KIAA1363/AADACL1 that impairs prostate cancer pathogenesis. Chem. Biol. *18*, 476–484.

Chen, S., Do, J.T., Zhang, Q., Yao, S., Yan, F., Peters, E.C., Schöler, H.R., Schultz, P.G., and Ding, S. (2006). Self-renewal of embryonic stem cells by a small molecule. Proc. Natl. Acad. Sci. USA *103*, 17266–17271.

Chiang, K.P., Niessen, S., Saghatelian, A., and Cravatt, B.F. (2006). An enzyme that regulates ether lipid signaling pathways in cancer annotated by multidimensional profiling. Chem. Biol. *13*, 1041–1050.

Christofk, H.R., Vander Heiden, M.G., Harris, M.H., Ramanathan, A., Gerszten, R.E., Wei, R., Fleming, M.D., Schreiber, S.L., and Cantley, L.C. (2008). The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. Nature *452*, 230–233.

Cravatt, B.F., Wright, A.T., and Kozarich, J.W. (2008). Activity-based protein profiling: from enzyme chemistry to proteomic chemistry. Annu Rev Biochem. *77*, 383–414.

Cohen, M.S., Zhang, C., Shokat, K.M., and Taunton, J. (2005). Structural bioinformatics-based design of selective, irreversible kinase inhibitors. Science *308*, 1318–1321.

Cohen, M.S., Hadjivassiliou, H., and Taunton, J. (2007). A clickable inhibitor reveals context-dependent autoactivation of p90 RSK. Nat. Chem. Biol. *3*, 156–160.

Cravatt, B.F., Giang, D.K., Mayfield, S.P., Boger, D.L., Lerner, R.A., and Gilula, N.B. (1996). Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. Nature *384*, 83–87.

Dang, L., White, D.W., Gross, S., Bennett, B.D., Bittinger, M.A., Driggers, E.M., Fantin, V.R., Jang, H.G., Jin, S., Keenan, M.C., et al. (2009). Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. Nature *462*, 739–744.

Davis, M.I., Hunt, J.P., Herrgard, S., Ciceri, P., Wodicka, L.M., Pallares, G., Hocker, M., Treiber, D.K., and Zarrinkar, P.P. (2011). Comprehensive analysis of kinase inhibitor selectivity. Nat. Biotechnol. *29*, 1046–1051.

Dawson, M.A., Prinjha, R.K., Dittmann, A., Giotopoulos, G., Bantscheff, M., Chan, W.I., Robson, S.C., Chung, C.W., Hopf, C., Savitski, M.M., et al. (2011). Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia. Nature *478*, 529–533.

Degterev, A., Hitomi, J., Germscheid, M., Ch'en, I.L., Korkina, O., Teng, X., Abbott, D., Cuny, G.D., Yuan, C., Wagner, G., et al. (2008). Identification of RIP1 kinase as a specific cellular target of necrostatins. Nat. Chem. Biol. *4*, 313–321.

Delmore, J.E., Issa, G.C., Lemieux, M.E., Rahl, P.B., Shi, J., Jacobs, H.M., Kastritis, E., Gilpatrick, T., Paranal, R.M., Qi, J., et al. (2011). BET bromodomain inhibition as a therapeutic strategy to target c-Myc. Cell *146*, 904–917.

Deng, X., Dzamko, N., Prescott, A., Davies, P., Liu, Q., Yang, Q., Lee, J.D., Patricelli, M.P., Nomanbhoy, T.K., Alessi, D.R., and Gray, N.S. (2011a). Characterization of a selective inhibitor of the Parkinson's disease kinase LRRK2. Nat. Chem. Biol. *7*, 203–205.

Deng, X., Yang, Q., Kwiatkowski, N., Sim, T., McDermott, U., Settleman, J.E., Lee, J.D., and Gray, N.S. (2011b). Discovery of a benzo[e]pyrimido-[5,4-b][1,4]diazepin-6(11H)-one as a potent and selective inhibitor of big MAP kinase 1. ACS Med Chem Lett *2*, 195–200.

Deu, E., Leyva, M.J., Albrow, V.E., Rice, M.J., Ellman, J.A., and Bogyo, M. (2010). Functional studies of Plasmodium falciparum dipeptidyl aminopeptidase I using small molecule inhibitors and active site probes. Chem. Biol. *17*, 808–819.

Edwards, A.M., Isserlin, R., Bader, G.D., Frye, S.V., Willson, T.M., and Yu, F.H. (2011). Too many roads not taken. Nature *470*, 163–165.

Emami, K.H., Nguyen, C., Ma, H., Kim, D.H., Jeong, K.W., Eguchi, M., Moon, R.T., Teo, J.L., Kim, H.Y., Moon, S.H., et al. (2004). A small molecule inhibitor of beta-catenin/CREB-binding protein transcription [corrected]. Proc. Natl. Acad. Sci. USA *101*, 12682–12687.

Entzeroth, M., Chapelain, B., Guilbert, J., and Hamon, V. (2000). High throughput drug profiling. J. Autom. Methods Manag. Chem. *22*, 171–173.

Evans, M.J., Saghatelian, A., Sorensen, E.J., and Cravatt, B.F. (2005). Target discovery in small-molecule cell-based screens by in situ proteome reactivity profiling. Nat. Biotechnol. *23*, 1303–1307.

Fabian, M.A., Biggs, W.H., 3rd, Treiber, D.K., Atteridge, C.E., Azimioara, M.D., Benedetti, M.G., Carter, T.A., Ciceri, P., Edeen, P.T., Floyd, M., et al. (2005). A small molecule-kinase interaction map for clinical kinase inhibitors. Nat. Biotechnol. *23*, 329–336.

Feldman, M.E., Apsel, B., Uotila, A., Loewith, R., Knight, Z.A., Ruggero, D., and Shokat, K.M. (2009). Active-site inhibitors of mTOR target rapamycin-resistant outputs of mTORC1 and mTORC2. PLoS Biol. *7*, e38.

Fischer, J.J., Michaelis, S., Schrey, A.K., Diehl, A., Graebner, O.Y., Ungewiss, J., Horzowski, S., Glinski, M., Kroll, F., Dreger, M., and Koester, H. (2011). SAHA Capture Compound—a novel tool for the profiling of histone deacetylases and the identification of additional vorinostat binders. Proteomics *11*, 4096–4104.

Gadgeel, S.M., and Bepler, G. (2011). Crizotinib: an anaplastic lymphoma kinase inhibitor. Future Oncol. *7*, 947–953.

Goldstein, D.M., Gray, N.S., and Zarrinkar, P.P. (2008). High-throughput kinase profiling as a platform for drug discovery. Nat. Rev. Drug Discov. *7*, 391–397.

Greenbaum, D.C., Baruch, A., Grainger, M., Bozdech, Z., Medzihradszky, K.F., Engel, J., DeRisi, J., Holder, A.A., and Bogyo, M. (2002). A role for the protease falcipain 1 in host cell invasion by the human malaria parasite. Science *298*, 2002–2006.

Hall, C.I., Reese, M.L., Weerapana, E., Child, M.A., Bowyer, P.W., Albrow, V.E., Haraldsen, J.D., Phillips, M.R., Sandoval, E.D., Ward, G.E., et al. (2011). Chemical genetic screen identifies Toxoplasma DJ-1 as a regulator of parasite secretion, attachment, and invasion. Proc. Natl. Acad. Sci. USA *108*, 10568–10573.

Harding, M.W., Galat, A., Uehling, D.E., and Schreiber, S.L. (1989). A receptor for the immunosuppressant FK506 is a cis-trans peptidyl-prolyl isomerase. Nature *341*, 758–760.

Henchey, L.K., Jochim, A.L., and Arora, P.S. (2008). Contemporary strategies for the stabilization of peptides in the α-helical conformation. Curr. Opin. Chem. Biol. *12*, 692–697.

Henise, J.C., and Taunton, J. (2011). Irreversible Nek2 kinase inhibitors with cellular activity. J. Med. Chem. *54*, 4133–4146.

Huang, S.M., Mishina, Y.M., Liu, S., Cheung, A., Stegmeier, F., Michaud, G.A., Charlat, O., Wiellette, E., Zhang, Y., Wiessner, S., et al. (2009). Tankyrase inhibition stabilizes axin and antagonizes Wnt signalling. Nature *461*, 614–620.

Ichida, J.K., Blanchard, J., Lam, K., Son, E.Y., Chung, J.E., Egli, D., Loh, K.M., Carter, A.C., Di Giorgio, F.P., Koszka, K., et al. (2009). A small-molecule inhibitor of tgf-Beta signaling replaces sox2 in reprogramming by inducing nanog. Cell Stem Cell *5*, 491–503.

Johnson, D.S., Stiff, C., Lazerwith, S.E., Kesten, S.R., Fay, L.K., Morris, M., Beidler, D., Liimatta, M.B., Smith, S.E., Dudley, D.T., et al. (2011). Discovery of PF-04457845: a highly potent, orally bioavailable, and selective urea FAAH inhibitor. ACS Med Chem Lett *2*, 91–96.

Kasper, A.C., Baker, J.B., Kim, H., and Hong, J. (2009). The end game of chemical genetics: target identification. Future Med Chem *1*, 727–736.

Kluge, A.F., and Petter, R.C. (2010). Acylating drugs: redesigning natural covalent inhibitors. Curr. Opin. Chem. Biol. *14*, 421–427.

Knight, Z.A., Gonzalez, B., Feldman, M.E., Zunder, E.R., Goldenberg, D.D., Williams, O., Loewith, R., Stokoe, D., Balla, A., Toth, B., et al. (2006). A pharmacological map of the PI3-K family defines a role for p110alpha in insulin signaling. Cell *125*, 733–747.

Knight, Z.A., Lin, H., and Shokat, K.M. (2010). Targeting the cancer kinome through polypharmacology. Nat. Rev. Cancer *10*, 130–137.

Kwiatkowski, N., Jelluma, N., Filippakopoulos, P., Soundararajan, M., Manak, M.S., Kwon, M., Choi, H.G., Sim, T., Deveraux, Q.L., Rottmann, S., et al. (2010). Small-molecule kinase inhibitors provide insight into Mps1 cell cycle function. Nat. Chem. Biol. *6*, 359–368.

Leung, D., Hardouin, C., Boger, D.L., and Cravatt, B.F. (2003). Discovering potent and selective reversible inhibitors of enzymes in complex proteomes. Nat. Biotechnol. *21*, 687–691.

Li, W., Blankman, J.L., and Cravatt, B.F. (2007). A functional proteomic strategy to discover inhibitors for uncharacterized hydrolases. J. Am. Chem. Soc. *129*, 9594–9595.

Liu, Y., Patricelli, M.P., and Cravatt, B.F. (1999). Activity-based protein profiling: the serine hydrolases. Proc. Natl. Acad. Sci. USA *96*, 14694–14699.

Long, J.Z., and Cravatt, B.F. (2011). The metabolic serine hydrolases and their functions in mammalian physiology and disease. Chem. Rev. *111*, 6022–6063.

Long, J.Z., Li, W., Booker, L., Burston, J.J., Kinsey, S.G., Schlosburg, J.E., Pavón, F.J., Serrano, A.M., Selley, D.E., Parsons, L.H., et al. (2009). Selective blockade of 2-arachidonoylglycerol hydrolysis produces cannabinoid behavioral effects. Nat. Chem. Biol. *5*, 37–44.

Miduturu, C.V., Deng, X., Kwiatkowski, N., Yang, W., Brault, L., Filippakopoulos, P., Chung, E., Yang, Q., Schwaller, J., Knapp, S., et al. (2011). High-throughput kinase profiling: a more efficient approach toward the discovery of new kinase inhibitors. Chem. Biol. *18*, 868–879.

Miller, D.K., Gillard, J.W., Vickers, P.J., Sadowski, S., Léveillé, C., Mancini, J.A., Charleson, P., Dixon, R.A., Ford-Hutchinson, A.W., Fortin, R., et al. (1990). Identification and isolation of a membrane protein necessary for leukotriene production. Nature *343*, 278–281.

Moellering, R.E., Cornejo, M., Davis, T.N., Del Bianco, C., Aster, J.C., Blacklow, S.C., Kung, A.L., Gilliland, D.G., Verdine, G.L., and Bradner, J.E. (2009). Direct inhibition of the NOTCH transcription factor complex. Nature *462*, 182–188.

Moulick, K., Ahn, J.H., Zong, H., Rodina, A., Cerchietti, L., Gomes DaGama, E.M., Caldas-Lopes, E., Beebe, K., Perna, F., Hatzi, K., et al. (2011). Affinity-based proteomics reveal cancer-specific networks coordinated by Hsp90. Nat. Chem. Biol. *7*, 818–826.

Munos, B.H., and Chin, W.W. (2011). How to revive breakthrough innovation in the pharmaceutical industry. Sci. Transl. Med. *3*, 89cm16.

Nekrutenko, A., Hillis, D.M., Patton, J.C., Bradley, R.D., and Baker, R.J. (1998). Cytosolic isocitrate dehydrogenase in humans, mice, and voles and phylogenetic analysis of the enzyme family. Mol. Biol. Evol. *15*, 1674–1684.

Nelson, R.H., and Miles, J.M. (2005). The use of orlistat in the treatment of obesity, dyslipidaemia and type 2 diabetes. Expert Opin. Pharmacother. *6*, 2483–2491.

Ong, S.E., Schenone, M., Margolin, A.A., Li, X., Do, K., Doud, M.K., Mani, D.R., Kuai, L., Wang, X., Wood, J.L., et al. (2009). Identifying the proteins to which small-molecule probes and drugs bind in cells. Proc. Natl. Acad. Sci. USA *106*, 4617–4622.

Overington, J.P., Al-Lazikani, B., and Hopkins, A.L. (2006). How many drug targets are there? Nat. Rev. Drug Discov. *5*, 993–996.

Parsons, D.W., Jones, S., Zhang, X., Lin, J.C., Leary, R.J., Angenendt, P., Mankoo, P., Carter, H., Siu, I.M., Gallia, G.L., et al. (2008). An integrated genomic analysis of human glioblastoma multiforme. Science *321*, 1807–1812.

Patgiri, A., Yadav, K.K., Arora, P.S., and Bar-Sagi, D. (2011). An orthosteric inhibitor of the Ras-Sos interaction. Nat. Chem. Biol. *7*, 585–587.

Patricelli, M.P., Szardenings, A.K., Liyanage, M., Nomanbhoy, T.K., Wu, M., Weissig, H., Aban, A., Chun, D., Tanner, S., and Kozarich, J.W. (2007). Functional interrogation of the kinome using nucleotide acyl phosphates. Biochemistry *46*, 350–358.

Patricelli, M.P., Nomanbhoy, T.K., Wu, J., Brown, H., Zhou, D., Zhang, J., Jagannathan, S., Aban, A., Okerberg, E., Herring, C., et al. (2011). In situ kinase profiling reveals functionally relevant properties of native kinases. Chem. Biol. *18*, 699–710.

Racchi, M., Mazzucchelli, M., Porrello, E., Lanni, C., and Govoni, S. (2004). Acetylcholinesterase inhibitors: novel activities of old molecules. Pharmacol. Res. *50*, 441–451.

Raj, L., Ide, T., Gurkar, A.U., Foley, M., Schenone, M., Li, X., Tolliday, N.J., Golub, T.R., Carr, S.A., Shamji, A.F., et al. (2011). Selective killing of cancer cells by a small molecule targeting the stress response to ROS. Nature *475*, 231–234.

Russ, A.P., and Lampel, S. (2005). The druggable genome: an update. Drug Discov. Today *10*, 1607–1610.

Salisbury, C.M., and Cravatt, B.F. (2007). Activity-based probes for proteomic profiling of histone deacetylase complexes. Proc. Natl. Acad. Sci. USA *104*, 1171–1176.

Salisbury, C.M., and Cravatt, B.F. (2008). Optimization of activity-based probes for proteomic profiling of histone deacetylase complexes. J. Am. Chem. Soc. *130*, 2184–2194.

Shahian, T., Lee, G.M., Lazic, A., Arnold, L.A., Velusamy, P., Roels, C.M., Guy, R.K., and Craik, C.S. (2009). Inhibition of a viral enzyme by a small-molecule dimer disruptor. Nat. Chem. Biol. *5*, 640–646.

Simon, G.M., and Cravatt, B.F. (2010). Activity-based proteomics of enzyme superfamilies: serine hydrolases as a case study. J. Biol. Chem. *285*, 11051–11055.

Soda, M., Choi, Y.L., Enomoto, M., Takada, S., Yamashita, Y., Ishikawa, S., Fujiwara, S., Watanabe, H., Kurashina, K., Hatanaka, H., et al. (2007). Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature *448*, 561–566.

Swinney, D.C., and Anthony, J. (2011). How were new medicines discovered? Nat. Rev. Drug Discov. *10*, 507–519.

Tagore, D.M., Nolte, W.M., Neveu, J.M., Rangel, R., Guzman-Rojas, L., Pasqualini, R., Arap, W., Lane, W.S., and Saghatelian, A. (2009). Peptidase substrates via global peptide profiling. Nat. Chem. Biol. *5*, 23–25.

Taunton, J., Hassig, C.A., and Schreiber, S.L. (1996). A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p. Science *272*, 408–411.

Thornberry, N.A., and Weber, A.E. (2007). Discovery of JANUVIA (Sitagliptin), a selective dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. Curr. Top. Med. Chem. *7*, 557–568.

Thornberry, N.A., Bull, H.G., Calaycay, J.R., Chapman, K.T., Howard, A.D., Kostura, M.J., Miller, D.K., Molineaux, S.M., Weidner, J.R., Aunins, J., et al. (1992). A novel heterodimeric cysteine protease is required for interleukin-1 beta processing in monocytes. Nature *356*, 768–774.

Trepel, J., Mollapour, M., Giaccone, G., and Neckers, L. (2010). Targeting the dynamic HSP90 complex in cancer. Nat. Rev. Cancer *10*, 537–549.

Vander Heiden, M.G., Locasale, J.W., Swanson, K.D., Sharfi, H., Heffron, G.J., Amador-Noguez, D., Christofk, H.R., Wagner, G., Rabinowitz, J.D., Asara, J.M., and Cantley, L.C. (2010). Evidence for an alternative glycolytic pathway in rapidly proliferating cells. Science *329*, 1492–1499.

Vermehren, J., and Sarrazin, C. (2011). New hepatitis C therapies in clinical development. Eur. J. Med. Res. *16*, 303–314.

Walensky, L.D., Kung, A.L., Escher, I., Malia, T.J., Barbuto, S., Wright, R.D., Wagner, G., Verdine, G.L., and Korsmeyer, S.J. (2004). Activation of apoptosis in vivo by a hydrocarbon-stapled BH3 helix. Science *305*, 1466–1470.

Walsh, M.J., Brimacombe, K.R., Veith, H., Bougie, J.M., Daniel, T., Leister, W., Cantley, L.C., Israelsen, W.J., Vander Heiden, M.G., Shen, M., et al. (2011). 2-Oxo-N-aryl-1,2,3,4-tetrahydroquinoline-6-sulfonamides as activators of the tumor cell specific M2 isoform of pyruvate kinase. Bioorg. Med. Chem. Lett. *21*, 6322–6327.

Weerapana, E., Wang, C., Simon, G.M., Richter, F., Khare, S., Dillon, M.B., Bachovchin, D.A., Mowen, K., Baker, D., and Cravatt, B.F. (2010). Quantitative reactivity profiling predicts functional cysteines in proteomes. Nature *468*, 790–795.

Wright, A.T., and Cravatt, B.F. (2007). Chemical proteomic probes for profiling cytochrome p450 activities and drug interactions in vivo. Chem. Biol. *14*, 1043–1051.

Wright, A.T., Song, J.D., and Cravatt, B.F. (2009). A suite of activity-based probes for human cytochrome P450 enzymes. J. Am. Chem. Soc. *131*, 10692–10700.

Yagoda, N., von Rechenberg, M., Zaganjor, E., Bauer, A.J., Yang, W.S., Fridman, D.J., Wolpaw, A.J., Smukste, I., Peltier, J.M., Boniface, J.J., et al. (2007). RAS-RAF-MEK-dependent oxidative cell death involving voltage-dependent anion channels. Nature *447*, 864–868.

Yang, Q., Deng, X., Lu, B., Cameron, M., Fearns, C., Patricelli, M.P., Yates, J.R., 3rd, Gray, N.S., and Lee, J.D. (2010). Pharmacological inhibition of BMK1 suppresses tumor growth through promyelocytic leukemia protein. Cancer Cell *18*, 258–267.

Zhang, J., Yang, P.L., and Gray, N.S. (2009). Targeting cancer with small molecule kinase inhibitors. Nat. Rev. Cancer *9*, 28–39.

Zou, H.Y., Li, Q., Lee, J.H., Arango, M.E., McDonnell, S.R., Yamazaki, S., Koudriakova, T.B., Alton, G., Cui, J.J., Kung, P.P., et al. (2007). An orally available small-molecule inhibitor of c-Met, PF-2341066, exhibits cytoreductive antitumor efficacy through antiproliferative and antiangiogenic mechanisms. Cancer Res. *67*, 4408–4417.

