Title
Activity-based proteomic and metabolomic approaches for understanding metabolism

Authors
Hunerdosse, Devon
Nomura, Daniel K

Publication Date
2014-08-01

DOI
10.1016/j.copbio.2014.02.001

Peer reviewed

Published in final edited form as:
Curr Opin Biotechnol. 2014 August ; 0: 116–126. doi:10.1016/j.copbio.2014.02.001.

Activity-Based Proteomic and Metabolomic Approaches for Understanding Metabolism

Devon Hunerdosse[1] and **Daniel K. Nomura**[1,*]

[1]Program in Metabolic Biology, University of California, Berkeley, Berkeley, CA 94720

Abstract

There are an increasing number of human pathologies that have been associated with altered metabolism, including obesity, diabetes, atherosclerosis, cancer, and neurodegenerative diseases. Most attention on metabolism has been focused on well-understood metabolic pathways and has largely ignored most of the biochemical pathways that operate in (patho)physiological settings, in part because of the vast landscape of uncharacterized and undiscovered metabolic pathways. One technology that has arisen to meet this challenge is activity-based protein profiling (ABPP) that uses activity-based chemical probes to broadly assess the functional states of both characterized and uncharacterized enzymes. This review will focus on how ABPP, coupled with inhibitor discovery platforms and functional metabolomic technologies, have led to discoveries that have expanded our knowledge of metabolism in health and disease.

Introduction

In the post-genomic era, scientists are faced with the daunting task of deciphering the biochemical and (patho)physiological functions of the vast landscape of poorly understood or uncharacterized enzymes (1, 2). Understanding the biological functions of these uncharacterized enzymes will undoubtedly lead to an expansion of our knowledge of metabolic pathways and to novel therapeutic targets that can be manipulated to treat metabolic diseases. Indeed, a large number of complex human pathologies are now associated with dysregulated metabolism that now includes obesity, diabetes, cancer, and inflammatory diseases, but most research has focused on well-established biochemical or regulatory pathways, largely ignoring the majority of poorly understood or uncharacterized networks in metabolism (3). Being able to identify key nodal metabolic pathways, not only in the well-characterized metabolic realm but also in the undiscovered biochemical networks, will undoubtedly lead to new therapeutic strategies for combatting diseases associated with metabolism. This review will focus on one technology, activity-based protein profiling (ABPP) that has emerged as a powerful platform, when coupled with functional metabolomic approaches, to characterize novel functions of previously

*dnomura@berkeley.edu.

characterized enzymes or uncovering the functions of uncharacterized enzymes in complex (patho)physiological settings and develop potent and selective small-molecule inhibitors for these enzymes.

Broad profiling of enzyme activities

While traditional genomic and proteomic profiling approaches have yielded tremendous amounts of information, these technologies do not provide information on the functional state of enzymes in complex living systems, which can be regulated by post-translational modifications or inhibition. The last decade has seen the emergence of powerful chemical proteomic (chemoproteomic) and mass-spectrometry-based approaches that facilitate the assessment of enzyme activities or protein hyper-reactivities *en masse*.

One such chemoproteomic platform is called activity-based protein profiling (ABPP), which uses active-site directed chemical probes to assess enzyme activities in complex biological samples (4-6). An activity-based probe consists of a chemical reactive group that covalently reacts with the active sites of enzymes, coupled to an analytical handle to read-out enzyme activities by SDS/PAGE and fluorescence (e.g. probes coupled to rhodamine) (gel-based ABPP) or enrichment and mass spectrometry-based proteomic platforms (e.g. probes coupled to biotin) (ABPP-Multidimensional Protein Identification Technology (ABPP-MudPIT)) (Figure 1) (5, 6). Thus, these probes facilitate the detection and enrichment of entire families of enzymes that are united by common catalytic mechanisms (e.g., kinases, phosphatases, proteases, histone deacetylases, and hydrolases) (Table 1) (4, 5). Unique to ABPP platforms is the ability of these probes to assess the functional state of uncharacterized enzymes in the proteome, since the chemical probes react with the active sites based on reactivity and not on the state of functional annotation. ABPP also enables the detection of changes in enzyme activities that occur without changes in abundance at the mRNA or protein level and facilitates the functional assessment of very low abundance enzymes, which can be enriched with activity-based probes for subsequent proteomic analysis(7).

ABPP has been previously used to identify many dysregulated enzyme activities that underlie human diseases or enzyme activities that can be used for industrial applications. There are numerous successful examples of ABPP platforms used to identify unique and novel metabolic enzymes that drive cancer pathogenesis that may represent promising targets for cancer therapy. Using the serine hydrolase-directed fluorophosphonate activity-based probe, Cravatt, Nomura, and colleagues have shown enzyme activities such as KIAA1363 and monoacylglycerol lipase (MAGL) as upregulated in aggressive human cancer cells and primary human tumors and were critical nodal enzymes in driving malignant and tumorigenic features of cancer (8, 9). These probes have also been used to identify the enzymes urokinase (uPA) and tissue plasminogen activator (tPA), as highly secreted enzymes in aggressive human breast cancer cells (10, 11). Quigley and colleagues showed that active extracellular uPA, but not total uPA levels, were upregulated in high-intravasating variants of human fibrosacroma HT-1080 cells and that blocking uPA inhibited invasion *in vitro* and intravasation and metastasis *in vivo (12).* Using the serine hydrolase probe, Cheresh and colleagues profiled primary human ductal adenocarcinomas and

identified retinoblastoma-binding protein 9 (RBBP9) as a tumor-associated serine hydrolase that promotes anchorage-independent growth *in vitro* as well as pancreatic carcinogenesis *in vivo* through overcoming TGF-β-mediated antiproliferative signaling by reducing Smad2/3 phosphorylation (13).

ABPP has also been used to identify nodal or dysregulated enzyme activities in bacteria or in viral infections. Pezacki used ABPP to identify carboxylesterase 1 (CES1) as an upregulated enzyme activity in hepatitis C virus (HCV)-infected hepatoma cells that was also critical in maintaining viral replication (14). The same group used a non-directed phenyl sulfonate ester probe to target a broad range of enzyme families and showed that HCV infection led to dysregulation of several protein activities that may be relevant to HCV replication (15). Wright and colleagues recently used a cysteine-reactive sulfonate ester probe and the serine hydrolase probe to identify several dysregulated enzyme activities in *Aspergillus fumigatus*, the primary pathogen causing the devastating pulmonary disease Invasive Aspergillosis (16). The same group also developed activity-based probes for cellulose degrading enzymes in *Clostridium thermocellum* which may have applications in biofuel development (17).

ABPP has also been used to identify important enzymes involved in the development of insulin resistance and the metabolic syndrome. Wright and colleagues developed a chemical probe for ATP-binding proteins by incorporating reactive acyl phosphate moieties that directly acylate the lysine ε-amino residues of ATP-binding proteins such as ATPases, kinases, and nucleotide-binding proteins. This probe facilitated the identification of altered citric acid cycle enzymes, oxidative phosphorylation, and lipid metabolism enzymes in mitochondria isolated from the skeletal muscle of high-fat diet fed mice (18). Cravatt and Barglow used an α-chloroacetamide dipeptide probe library and serine hydrolase probes to profile enzyme activities in obese *ob/ob* mice and identified multiple dysregulated metabolic activities including fatty acid synthase, hydroxypyruvate reductase, MAGL, malic enzyme, and liver carboxylesterase (19).

ABPP platforms have also been successfully used as imaging agents for imaging dysregulated metabolism in cancer cells. Bogyo and colleagues have developed a suite of chemical probes for cysteine proteases and caspases and have successfully used these probes for *in vivo* imaging of tumors whose formation, growth, and invasiveness are promoted by activation of cathepsins *(20-26)*. These probes can potentially be used in the clinic to define tumor margins, diagnose tumor grade, assess drug-target occupancy, and monitor tumor apoptosis *in vivo*. Cravatt and colleagues also developed an imaging probe for the cancer-associated serine hydrolase KIAA1363 to provide temporal and spatial tracking of KIAA1363 in aggressive human cancer cells (27).

There have also been pioneering efforts to perform high-throughput screening (HTS) of enzyme activity assays to facilitate the identification and characterization of enzymes with desired enzyme activities or for inhibitor discovery efforts (to be covered below). ABPP is amenable to HTS strategies using fluorescent-tagged activity-based probes and fluorescence polarization (fluopol) screening (28). Siuzdak and Northen have also developed innovative HTS enzyme activity assays based on a Nanostructure-Initiator Mass Spectrometry (NIMS).

NIMS offers superior resolution and sensitivity to MALDI and allows for spatially defined mass analysis of peptide microarrays, single cells, or even tissues (Northen et al., 2007). The NIMS-based enzymatic (Nimzyme) assay immobilizes enzyme substrates on a “soft” (noncovalent) mass spectrometry surface by fluorous-phase interactions and enzyme products are then detected by desorption/ionization. This technique allows surface washing steps to reduce signal suppression in complex biological samples, is sensitive to very low abundance enzymes (500 femptograms), and works with a wide range of pHs and temperatures (29). While there are natural or artificial substrate assays that can be performed to assess the activities of enzymes by color or by indirectly measuring product formation by a coupled assay or biosensor, these assays are only applicable to a narrow range of biochemical transformations for which methods have been developed. Mass spectrometry-based assays are more universal but often require lengthy chromatographic separations, reducing throughput. Northen and colleagues have further advanced this platform by combining the NIMs technology with acoustic printing to speed up the liquid-liquid handling process to make this approach even faster (30). *Nimzyme* is a NIMS-based analytical method that detects enzyme activities in complex biological mixtures, circumvents time-intensive chromatographic separations by in situ fluorous affinity purification. In combination with acoustic sample deposition, *Nimzyme* assays are amenable to HTS approaches for optimizing conditions for enzyme activities (e.g. temperature, time, pH, buffer conditions) or testing a library of mutated or evolved enzymes for new functionalities.

Using this approach Suizdak, Northen, and colleagues were able to identify and directly characterize β-1,4-galactosidase activity directly from complex proteomes from a thermophilic microbial community lysate (29). They also applied the *Ninzyme* technology coupled with acoustic printing to characterize glycosyl hydrolases (30). Recently, Cheng et al used the NIMs assay in the thermophilic cellulolytic actinomycete *Thermobispora bispora* to identify optimal growth conditions to maximize β-glucosidase production towards discovering and characterizing enzymes from environmental microbes for industrial and biofuel applications.

Collectively, approaches like ABPP and NIMS are modern technologies that expand our ability to identify and characterize important enzyme activities on a much broader or faster scale to identify important metabolic enzymes in diseases or in industrial applications.

Chemoprotomics for Developing Selective Small-Molecule Inhibitors for Metabolic Enzymes

The development of chemical tools to interrogate metabolic enzymes of interest is invaluable for both further investigating their underlying biology and developing small-molecules for drug development. Important to the generation of chemical tools is the ability to validate the selectivity and efficacy of the small-molecule to not only make certain that the follow-up biology is due to on-target effects, but also to ensure safety of the molecule for follow-up clinical development.

ABPP has emerged as a powerful platform for developing potent and selective small-molecule inhibitors for both characterized and uncharacterized enzymes, which have in-turn been used to better understand metabolic pathways in living systems (6, 31) (Figure 2A). Because activity-based probes bind to the active sites of enzymes, inhibitors can be competed against probe binding, facilitating a competitive platform for inhibitor discovery (6, 32, 33). Furthermore, because the activity-based probes assess enzyme activities of large numbers of enzymes, the selectivity of the small-molecules can be tested across entire enzyme class(es). Thus, this competitive ABPP approach can be utilized to develop potent and selective inhibitors for any enzyme, regardless of its state of annotation, if there is a cognate activity-based probe for the enzyme of interest. Selectivity of covalent inhibitors can be further tested across the entire proteome by developing a small-molecule mimic of the lead compound that incorporates a bioorthogonal handle (e.g. alkyne and azide) (34-36). This probe can then be reacted with complex proteomes, subjected to click chemistry to append an analytical handle (e.g. biotin or fluorophore) and analyzed by mass-spectrometry or in-gel fluorescence to identify on-target engagement as well as any off-targets.

This competitive ABPP platform can be employed in a medium-throughput gel-based format with fluorescent activity-based probes (competitive gel-based ABPP), a lower throughput but more in-depth mass-spectrometry-based proteomics format with biotin-tagged activity-based probes (competitive ABPP-MudPIT), or an HTS format using fluorescence polarization (competitive fluopol-ABPP) (Figure 2B) and fluorescent activity-based probes against large compound libraries (6, 31).

Competitive ABPP screening platforms have lead to the discovery and development of many potent and selective enzyme inhibitors that have been used for in-depth biological characterization of enzymes (patho)physiological settings as well as to ascertain the potential of these enzymes and their inhibitors as therapeutic targets and therapeutics (34, 36-40).

Competitive ABPP platforms have particularly benefited the pharmacological targeting of the endogenous cannabinoid (“endocannabinoid”) system (41, 42). The endocannabinoid system consists of two endogenous signaling lipids, 2-arachidonoylglycerol (2-AG) and anandamide. which bind to cannabinoid receptors to modulate responses in pain, inflammation, and mood (42-44). Targeting endocannabinoid degradation and synthesis have been put forth as promising therapeutic strategies for combatting a variety of pathologies. Many of these enzymes, such as the 2-AG hydrolyzing enzyme MAGL, anandamide-hydrolyzing enzyme fatty acid amide hydrolase (FAAH), and the 2-AG biosynthetic enzyme diacylglycerol lipase (DAGL), all belong to the serine hydrolase enzyme class (42). Inhibitor discovery for the serine hydrolase superfamily of enzymes has benefitted from chemical libraries of electrophilic carbamate and triazole urea scaffolds that specifically target the nucleophilic catalytic mechanism of serine hydrolases (32, 33, 45). Screening of serinehydrolase directed chemical libraries, coupled with traditional medinical chemistry efforts, has facilitated the discovery of potent, selective, and *in vivo*-active inhibitors for many potential therapeutic serine hydrolase targets (41).

MAGL inhibitors found through a competitive ABPP screen of a structurally diverse carbamate library and subsequent medicinal chemistry efforts generated the carbamate JZL184 as the first potent, selective, and *in vivo* active MAGL inhibitor (37). JZL184 has been used extensively to characterize the biochemical function of MAGL using metabolomic technologies, and to implicate this enzyme as a therapeutic target for cancer, inflammation and inflammatory diseases, neurodegenerative diseases such as Alzheimer's and Parkinson's diseases, anxiety, and pain (9, 37, 43, 46-48). Many generations of inhibitors for FAAH have been developed and tested for their selectivity using competitive ABPP platforms to elucidate the role of FAAH as the primary degrading enzyme for the endogenous cannabinoid signaling lipid anandamide and the utility of FAAH inhibitors in combatting pain and inflammation through heightening anandamide signaling (39, 49). FAAH inhibitors are now in clinical trials for treatment of pain and inflammation. DAGL inhibitors were also developed using competitive ABPP platforms and used to show that the DAGL pathway is an important pathway for generation of arachidonic acid precursor pools for eicosanoid synthesis in macrophages to modulate inflammatory responses (50). Gel-based and MudPIT-based competitive ABPP have been used to develop many more inhibitors for various characterized and uncharacterized enzymes that may eventually have therapeutic potential, including the cancer-associated serine hydrolase KIAA1363, acyl peptide hydrolase, alpha/beta hydrolase domain-containing protein 11 (ABHD11), and platelet activating factor acetylhydrolase 2 (PAFAH2) (40, 45). Fluopol-ABPP has been used several times to identify inhibitors for many other metabolic enzymes that have therapeutic potential including the potential anti-cancer targets protein methyl esterase 1 (PME1) and RBBP9 and the anti-inflammatory target protein arginine deaminase 4 (PAD4) (28, 34, 51).

Thus, competitive ABPP platforms are powerful approaches for developing small-molecule inhibitors for both characterized and uncharacterized metabolic enzymes, which can be used for expanding our knowledge of metabolism in (patho)physiology, but also to develop chemical tools for subsequent translational development. While NIMS has not yet been used for HTS discovery of enzyme inhibitors, NIMS would also be an attractive strategy for small-molecule inhibitor development using mass-spectrometry as the read-out, instead of fluorescence polarization.

Metabolomic Approaches to Define and Map Biochemical Pathways

Modern technologies, such as ABPP and NIMS to assay the activities of enzymes and develop small-molecule enzyme inhibitors are powerful strategies that allow us to more broadly assess metabolism beyond well-understood and characterized biochemical pathways. These technologies can then be combined with advanced targeted and untargeted mass spectrometry-based metabolomic approaches to define the endogenous substrate/product relationships as well as the larger metabolic networks controlled by metabolic enzymes. Targeted metabolomics approaches consist of targeting for specific masses and associated parent and fragment ion mass-to-charge ratios (m/z) using mass-spectrometry allowing for the quantification of several hundred known metabolites. However, the metabolome is highly physicochemically diverse and likely consists of many metabolites whose structures are yet unknown. Thus, untargeted metabolomic profiling platforms, such

as discovery metabolite profiling (DMP), have arisen to capture a much wider metabolomic landscape (52, 53) (Figure 3). While untargeted metabolomics likely still does not capture the entirety of the metabolome, this approach broadly scans detectable ions across a large m/z range using mass-spectrometry platforms and the resulting large datasets are processed by bioinformatic tools to align, integrate, and compare all m/z ion intensities between different biological samples and identify differentially changing ions.

There are several examples of how targeted and DMP-based metabolomics have been successfully applied to discover novel functions of previously characterized enzymes or uncovering the role of completely uncharacterized enzymes, towards understanding the roles of these enzymes in normal physiology and disease. Using these platforms to profile differentially changed metabolites in FAAH-deficient mice, FAAH was found to not only regulate the levels of N-acylethanolamine (NAE), but also N-acyltaurine (NAT) lipid species. While it was known that FAAH regulated arachidonoyl NAE (anandamide) and its action upon cannabinoid receptors and cannabinoid-mediated antinociceptive phenotypes, DMP led to the discovery that FAAH also regulates NAT levels, which activate TRP ion channels giving rise to unique physiological actions mediated by FAAH (54).

Chiang et al. had found that the uncharacterized enzyme KIAA1363 was upregulated across multiple types of aggressive human cancer cells. However, the role of this enzyme was completely unknown. Using DMP, Chiang et al. discovered that this enzyme deacetylates the ether lipid 2-acetyl monoalkylglycerol ether (2-acetyl MAGE) to produce MAGE and subsequently the tumor-promoting lipid lysophosphatidic acid-ether (LPAe) to fuel aggressive features of cancer cells (55). Selective KIAA1363 inhibitors, developed through competitive ABPP platforms, have been used to lower MAGE and LPAe, to suppress cancer cell motility and tumorigenesis.

In another example, targeted metabolomics and DMP were also essential in establishing MAGL as a nodal enzyme that not only controls 2-AG and other monoacylglycerols, but also the arachidonic acid pool that generates pro-inflammatory prostaglandins in certain tissues such as brain, liver, and lung (46). While this enzyme was known to regulate monoacylglycerols, metabolomics led to the unique discovery that this enzyme feeds into the pathway that generates arachidonic acid for the synthesis of pro-inflammatory eicosanoids. This biochemical understanding of MAGL function has in-turn led to the discovery that MAGL inhibitors show potent anti-inflammatory effects and neuroprotection against Parkinson’s and Alzheimer’s disease as well as inflammatory tissue injury in liver and lung (46, 47, 56, 57).

Metabolomics also led to the discovery that MAGL plays a distinct and unique role in aggressive cancer cells in regulating fatty acid levels and a fatty acid network enriched in protumorigenic signaling lipids that drive cancer pathogenicity (8). These findings were unexpected since MAGL does not play a major role in regulating cancer cell free fatty acid levels, and represents a retasked (patho)physiological function of this enzyme in malignant cancer cells. These findings led to understanding the mechanism of action behind MAGL inhibitors and their anti-tumorigenic and anti-pathogenic function in cancer (8, 58).

Blankman et al utilized both targeted and DMP-based methods to establish the previously uncharacterized enzyme ABHD12 as a lysophasphatidylserine (LPS) hydrolase and showed that ABHD12-deficiency leads to elevations in brain levels of LPS, which subsequently stimulates toll-like receptor 2 (TLR2) and causes neuroinflammation and auditory and motor deficiencies, recapitulating the human neurodegenerative condition polyneuropathy, hearing loss, ataxia, retinitis pigmentosa, and cataract (PHARC) associated with a loss-of-function ABHD12 mutation (59).

Perhaps one of the most provocative examples of how metabolomics has been used to assign a completely altered and unique function to a well-characterized enzyme in cancer cells has been in elucidating the role of a mutant form of isocitrate dehydrogenase 1 (IDH1) in cancers. IDH1 catalyzes the oxidative decarboxylation of isocitrate to α-ketoglutarate with concomitant reduction of $NADP^+$ to NADPH. Multiple genome-wide analyses of glioma and acute myeloid leukemia patients had identified an arginine 132 to histidine mutation in the active site of IDH1 (60, 61). Metabolomic profiling revealed that this IDH1 R132 mutant led to the generation of a novel oncometabolite 2-hydroxyglurarate (2-HG) (62). Surprisingly, the authors discovered that the R132 mutant IDH1 *consumed* NADPH and reduced α-ketoglutarate to 2-HG (62). These studies provided the first evidence for a mutated enzyme in cancer conferring a neomorphic function to yield an unforeseen metabolite. Subsequent studies have shown that 2-HG has also functions as an epigenetic regulator in cancer, primarily through an increase in CpG island methylation (63) through acting as a competitive inhibitor of α-ketoglutarate dependent demethylases (64).

In yet another example of how cancer cells rewire their metabolism to fuel their pathogenicity, Ulanovskaya et al. discovered that nicotinamide *N*-methyltransferase (NNMT), which catalyzes the transfer of the methyl group of *S*-adenosyl-methionine (SAM) to nicotinamide, was overexpressed in a variety of tumors. Using metabolomics, the authors showed that NNMT overexpression led to a build up of the stable metabolic product 1-methylnicotinamide, revealing a mechanism by which cancer cells consume methyl units from SAM and ultimately alter the epigenetic potential of the cell. This included both hypomethylation of histones and cancer related proteins and increased expression of protumorigenic genes (65). SAM metabolism was also found to be coupled to threonine, which provides a large fraction of cellular glycine and acetylcoenzyme A needed for SAM synthesis. Depletion of threonine from the culture medium of mouse embryonic stem cells decreased SAM accumulation resulting in decreased histone 3 lysine 4 methylation and ultimately slowed growth and increased differentiation (66).

Metabolomic technologies have also been used to study the metabolic effects of viral infection of host cells to provide energy for viral replication. Viral infections are not typically considered metabolic diseases, however viral replication requires massive metabolic demands from the host cell (67). Therefore, infection of adult humans with viruses like herpes simplex virus-1 (HSV-1) or human cytomegalovirus (HCMV) can have profound effects on host cell metabolism and are major causes of human diseases. Metabolomic analysis of HSV-1 infected fibroblasts revealed a shift in central carbon metabolism toward the production of pyrimidine nucleotide metabolites. HCMV infected cells showed enhanced glycolytic flux and TCA cycle to fuel fatty acid biosynthesis (68).

Furthermore, reducing the expression of a single metabolic enzyme, argininosuccinate synthetase (AS1), was sufficient to mimic these HSV-1 induced metabolomic changes to improve viral replication (69). These metabolomic approaches can point to potential new sites for antiviral therapy (70).

In summary, targeted and untargeted metabolomic platforms have been successfully and repeatedly used to identify novel functions to previously well-characterized enzymes or to uncharacterized enzymes, which has led to understanding how these enzymes function in regulating metabolism in normal physiology or dysregulated metabolism in diseases such as cancer, neurodegenerative diseases, tissue injury, and infection.

Conclusions

Collectively, we have reviewed how chemical proteomic strategies such as ABPP and NIMs and metabolomic platforms have arisen to undertake the daunting task of demystifying the undiscovered and uncharacterized aspects of metabolism in (patho)physiological settings, towards identifying unique and nodal metabolic pathways that can be targeted for disease therapy or other commercial applications. We also show how the ABPP platform has been used to develop potent and selective small-molecule inhibitors for even previously uncharacterized enzymes, giving rise to chemical tools to further interrogate enzyme function as well as translational development of enzyme inhibitors for disease therapy. The integration of these technologies, such as ABPP, NIMS, and metabolomic profiling platforms with traditional sequencing and quantitative proteomics approaches will be critical moving forward towards gaining a more complete understanding of how altered enzymatic pathways cause alterations in metabolites which, in turn, may regulate protein function, signaling pathways, or other aspects of metabolism to fuel disease pathogenesis. While these technological platforms can certainly be advanced and improved to increase throughput, improve sensitivity, increase metabolic coverage, and quicken the process of uncovering novel metabolite and PTM structures, it is no longer necessary for scientists to remain focused on well-understood metabolic pathways. With the increasing sophistication of these modern approaches, we should actively and systematically mine the largely uncharacterized metabolic landscape for unique and novel metabolic networks that can be effectively targeted to treat human diseases.

Acknowledgments

We thank the members of the Nomura Research Group for critical reading of the manuscript. This work was supported by grants from the National Institutes of Health (R21CA170317, R01CA172667, R00DA030908) and the Searle Scholar Foundation.

References

1. Simon GM, Cravatt BF. Challenges for the ‘chemical-systems’ biologist. Nature chemical biology. 2008; 4:639–642.
2. Simon GM, Cravatt BF. Activity-based proteomics of enzyme superfamilies: serine hydrolases as a case study. The Journal of biological chemistry. 2010; 285:11051–11055. [PubMed: 20147750]
3. Edwards AM, Isserlin R, Bader GD, Frye SV, Willson TM, Yu FH. Too many roads not taken. Nature. 2011; 470:163–165. [PubMed: 21307913]

4. Evans MJ, Cravatt BF. Mechanism-based profiling of enzyme families. Chemical reviews. 2006; 106:3279–3301. [PubMed: 16895328]
5. Nomura DK, Dix MM, Cravatt BF. Activity-based protein profiling for biochemical pathway discovery in cancer. Nature reviews. Cancer. 2010; 10:630–638.
6. Moellering RE, Cravatt BF. How chemoproteomics can enable drug discovery and development. Chemistry & biology. 2012; 19:11–22. [PubMed: 22284350]
7. Jessani N, Niessen S, Wei BQ, Nicolau M, Humphrey M, Ji Y, Han W, Noh DY, Yates JR 3rd, Jeffrey SS, Cravatt BF. A streamlined platform for high-content functional proteomics of primary human specimens. Nature methods. 2005; 2:691–697. [PubMed: 16118640]
8. Nomura DK, Long JZ, Niessen S, Hoover HS, Ng SW, Cravatt BF. Monoacylglycerol lipase regulates a fatty acid network that promotes cancer pathogenesis. Cell. 2010; 140:49–61. [PubMed: 20079333]
9. Nomura DK, Lombardi DP, Chang JW, Niessen S, Ward AM, Long JZ, Hoover HH, Cravatt BF. Monoacylglycerol lipase exerts dual control over endocannabinoid and fatty acid pathways to support prostate cancer. Chemistry & biology. 2011; 18:846–856. [PubMed: 21802006]
10. Jessani N, Liu Y, Humphrey M, Cravatt BF. Enzyme activity profiles of the secreted and membrane proteome that depict cancer cell invasiveness. Proceedings of the National Academy of Sciences of the United States of America. 2002; 99:10335–10340. [PubMed: 12149457]
11. Jessani N, Humphrey M, McDonald WH, Niessen S, Masuda K, Gangadharan B, Yates JR 3rd, Mueller BM, Cravatt BF. Carcinoma and stromal enzyme activity profiles associated with breast tumor growth in vivo. Proceedings of the National Academy of Sciences of the United States of America. 2004; 101:13756–13761. [PubMed: 15356343]
12. Madsen MA, Deryugina EI, Niessen S, Cravatt BF, Quigley JP. Activity-based protein profiling implicates urokinase activation as a key step in human fibrosarcoma intravasation. The Journal of biological chemistry. 2006; 281:15997–16005. [PubMed: 16611636]
13. Shields DJ, Niessen S, Murphy EA, Mielgo A, Desgrosellier JS, Lau SK, Barnes LA, Lesperance J, Bouvet M, Tarin D, Cravatt BF, Cheresh DA. RBBP9: a tumor-associated serine hydrolase activity required for pancreatic neoplasia. Proceedings of the National Academy of Sciences of the United States of America. 2010; 107:2189–2194. [PubMed: 20080647]
14. Blais DR, Lyn RK, Joyce MA, Rouleau Y, Steenbergen R, Barsby N, Zhu LF, Pegoraro AF, Stolow A, Tyrrell DL, Pezacki JP. Activity-based Protein Profiling Identifies a Host Enzyme, Carboxylesterase 1, Which Is Differentially Active during Hepatitis C Virus Replication. Journal of Biological Chemistry. 2010; 285:25602–25612. [PubMed: 20530478]
15. Singaravelu R, Blais DR, McKay CS, Pezacki JP. Activity-based protein profiling of the hepatitis C virus replication in Huh-7 hepatoma cells using a non-directed active site probe. Proteome science. 2010; 8:5. [PubMed: 20181094]
16. Wiedner SD, Ansong C, Webb-Robertson BJ, Pederson LM, Fortuin S, Hofstad BA, Shukla AK, Panisko EA, Smith RD, Wright AT. Disparate proteome responses of pathogenic and nonpathogenic aspergilli to human serum measured by activity-based protein profiling (ABPP). Molecular & cellular proteomics: MCP. 2013; 12:1791–1805. [PubMed: 23599423]
17. Chauvigne-Hines LM, Anderson LN, Weaver HM, Brown JN, Koech PK, Nicora CD, Hofstad BA, Smith RD, Wilkins MJ, Callister SJ, Wright AT. Suite of activity-based probes for cellulose-degrading enzymes. Journal of the American Chemical Society. 2012; 134:20521–20532. [PubMed: 23176123]
18. Sadler NC, Angel TE, Lewis MP, Pederson LM, Chauvigne-Hines LM, Wiedner SD, Zink EM, Smith RD, Wright AT. Activity-based protein profiling reveals mitochondrial oxidative enzyme impairment and restoration in diet-induced obese mice. PloS one. 2012; 7:e47996. [PubMed: 23110155]
19. Barglow KT, Cravatt BF. Discovering disease-associated enzymes by proteome reactivity profiling. Chemistry & biology. 2004; 11:1523–1531. [PubMed: 15556003]
20. Berger AB, Vitorino PM, Bogyo M. Activity-based protein profiling: applications to biomarker discovery, in vivo imaging and drug discovery. American journal of pharmacogenomics: genomics-related research in drug development and clinical practice. 2004; 4:371–381. [PubMed: 15651898]

21. Blum G, von Degenfeld G, Merchant MJ, Blau HM, Bogyo M. Noninvasive optical imaging of cysteine protease activity using fluorescently quenched activity-based probes. Nature chemical biology. 2007; 3:668–677.
22. Edgington LE, Berger AB, Blum G, Albrow VE, Paulick MG, Lineberry N, Bogyo M. Noninvasive optical imaging of apoptosis by caspase-targeted activity-based probes. Nature medicine. 2009; 15:967–973.
23. Edgington LE, Verdoes M, Bogyo M. Functional imaging of proteases: recent advances in the design and application of substrate-based and activity-based probes. Current opinion in chemical biology. 2011; 15:798–805. [PubMed: 22098719]
24. Edgington LE, Verdoes M, Ortega A, Withana NP, Lee J, Syed S, Bachmann MH, Blum G, Bogyo M. Functional imaging of legumain in cancer using a new quenched activity-based probe. Journal of the American Chemical Society. 2013; 135:174–182. [PubMed: 23215039]
25. Kato D, Boatright KM, Berger AB, Nazif T, Blum G, Ryan C, Chehade KA, Salvesen GS, Bogyo M. Activity-based probes that target diverse cysteine protease families. Nature chemical biology. 2005; 1:33–38.
26. Lee J, Bogyo M. Development of near-infrared fluorophore (NIRF)-labeled activity-based probes for in vivo imaging of legumain. ACS chemical biology. 2010; 5:233–243. [PubMed: 20017516]
27. Chang JW, Moellering RE, Cravatt BF. An activity-based imaging probe for the integral membrane hydrolase KIAA1363. Angewandte Chemie. 2012; 51:966–970. [PubMed: 22162147]
28. Bachovchin DA, Brown SJ, Rosen H, Cravatt BF. Identification of selective inhibitors of uncharacterized enzymes by high-throughput screening with fluorescent activity-based probes. Nature biotechnology. 2009; 27:387–394.
29. Northen TR, Lee JC, Hoang L, Raymond J, Hwang DR, Yannone SM, Wong CH, Siuzdak G. A nanostructure-initiator mass spectrometry-based enzyme activity assay. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105:3678–3683. [PubMed: 18319341]
30. Greving M, Cheng X, Reindl W, Bowen B, Deng K, Louie K, Nyman M, Cohen J, Singh A, Simmons B, Adams P, Siuzdak G, Northen T. Acoustic deposition with NIMS as a high-throughput enzyme activity assay. Analytical and bioanalytical chemistry. 2012; 403:707–711. [PubMed: 22407334] ** This paper describes the coupling of acoustic printing on chip with NIMS for high-throughput enzyme activity assays.
31. Medina-Cleghorn D, Nomura DK. Chemical approaches to study metabolic networks. Pflugers Archiv: European journal of physiology. 2013; 465:427–440. [PubMed: 23296751]
32. Li W, Blankman JL, Cravatt BF. A functional proteomic strategy to discover inhibitors for uncharacterized hydrolases. Journal of the American Chemical Society. 2007; 129:9594–9595. [PubMed: 17629278]
33. Bachovchin DA, Ji T, Li W, Simon GM, Blankman JL, Adibekian A, Hoover H, Niessen S, Cravatt BF. Superfamily-wide portrait of serine hydrolase inhibition achieved by library-versus-library screening. Proceedings of the National Academy of Sciences of the United States of America. 2010; 107:20941–20946. [PubMed: 21084632]
34. Bachovchin DA, Mohr JT, Speers AE, Wang C, Berlin JM, Spicer TP, Fernandez-Vega V, Chase P, Hodder PS, Schurer SC, Nomura DK, Rosen H, Fu GC, Cravatt BF. Academic cross-fertilization by public screening yields a remarkable class of protein phosphatase methylesterase-1 inhibitors. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108:6811–6816. [PubMed: 21398589] ** This paper describes how fluopol-ABPP was used to identify a highly potent and selective PME1 inhibitor directly from the screening library. Subsequently, the inhibitor was turned into a bioorthogonal probe to assess its selectivity on a proteome-wide scale.
35. Johnson DS, Stiff C, Lazerwith SE, Kesten SR, Fay LK, Morris M, Beidler D, Liimatta MB, Smith SE, Dudley DT, Sadagopan N, Bhattachar SN, Kesten SJ, Nomanbhoy TK, Cravatt BF, Ahn K. Discovery of PF-04457845: A Highly Potent, Orally Bioavailable, and Selective Urea FAAH Inhibitor. ACS medicinal chemistry letters. 2011; 2:91–96. [PubMed: 21666860]
36. Ahn K, Smith SE, Liimatta MB, Beidler D, Sadagopan N, Dudley DT, Young T, Wren P, Zhang Y, Swaney S, Van Becelaere K, Blankman JL, Nomura DK, Bhattachar SN, Stiff C, Nomanbhoy TK, Weerapana E, Johnson DS, Cravatt BF. Mechanistic and pharmacological characterization of

PF-04457845: a highly potent and selective fatty acid amide hydrolase inhibitor that reduces inflammatory and noninflammatory pain. The Journal of pharmacology and experimental therapeutics. 2011; 338:114–124. [PubMed: 21505060]

37. Long JZ, Li W, Booker L, Burston JJ, Kinsey SG, Schlosburg JE, Pavon FJ, Serrano AM, Selley DE, Parsons LH, Lichtman AH, Cravatt BF. Selective blockade of 2-arachidonoylglycerol hydrolysis produces cannabinoid behavioral effects. Nature chemical biology. 2009; 5:37–44.
38. Long JZ, Nomura DK, Vann RE, Walentiny DM, Booker L, Jin X, Burston JJ, Sim-Selley LJ, Lichtman AH, Wiley JL, Cravatt BF. Dual blockade of FAAH and MAGL identifies behavioral processes regulated by endocannabinoid crosstalk in vivo. Proceedings of the National Academy of Sciences of the United States of America. 2009; 106:20270–20275. [PubMed: 19918051]
39. Ahn K, Johnson DS, Mileni M, Beidler D, Long JZ, McKinney MK, Weerapana E, Sadagopan N, Liimatta M, Smith SE, Lazerwith S, Stiff C, Kamtekar S, Bhattacharya K, Zhang Y, Swaney S, Van Becelaere K, Stevens RC, Cravatt BF. Discovery and characterization of a highly selective FAAH inhibitor that reduces inflammatory pain. Chemistry & biology. 2009; 16:411–420. [PubMed: 19389627]
40. Chang JW, Nomura DK, Cravatt BF. A potent and selective inhibitor of KIAA1363/AADACL1 that impairs prostate cancer pathogenesis. Chemistry & biology. 2011; 18:476–484. [PubMed: 21513884]
41. Bachovchin DA, Cravatt BF. The pharmacological landscape and therapeutic potential of serine hydrolases. Nature reviews. Drug discovery. 2012; 11:52–68.
42. Blankman JL, Cravatt BF. Chemical probes of endocannabinoid metabolism. Pharmacological reviews. 2013; 65:849–871. [PubMed: 23512546]
43. Mulvihill MM, Nomura DK. Therapeutic potential of monoacylglycerol lipase inhibitors. Life sciences. 2013; 92:492–497. [PubMed: 23142242]
44. Ahn K, McKinney MK, Cravatt BF. Enzymatic pathways that regulate endocannabinoid signaling in the nervous system. Chemical reviews. 2008; 108:1687–1707. [PubMed: 18429637]
45. Adibekian A, Martin BR, Wang C, Hsu KL, Bachovchin DA, Niessen S, Hoover H, Cravatt BF. Click-generated triazole ureas as ultrapotent in vivo-active serine hydrolase inhibitors. Nature chemical biology. 2011; 7:469–478.
46. Nomura DK, Morrison BE, Blankman JL, Long JZ, Kinsey SG, Marcondes MC, Ward AM, Hahn YK, Lichtman AH, Conti B, Cravatt BF. Endocannabinoid hydrolysis generates brain prostaglandins that promote neuroinflammation. Science. 2011; 334:809–813. [PubMed: 22021672] ** Nomura et al. discovered, using metabolomic profiling, that 2-AG hydrolysis generates the primary arachidonic acid pool for pro-inflammatory eicosanoid biosynthesis. The authors showed that pharmacological blockade of MAGL, by an inhibitor developed through competitive ABPP, led to neuroprotection in a Parkinson's disease mouse model through lowering eicosanoids and suppressing neuroinflammation.
47. Piro JR, Benjamin DI, Duerr JM, Pi Y, Gonzales C, Wood KM, Schwartz JW, Nomura DK, Samad TA. A dysregulated endocannabinoid-eicosanoid network supports pathogenesis in a mouse model of Alzheimer's disease. Cell reports. 2012; 1:617–623. [PubMed: 22813736]
48. Kinsey SG, Long JZ, O'Neal ST, Abdullah RA, Poklis JL, Boger DL, Cravatt BF, Lichtman AH. Blockade of endocannabinoid-degrading enzymes attenuates neuropathic pain. The Journal of pharmacology and experimental therapeutics. 2009; 330:902–910. [PubMed: 19502530]
49. Long JZ, LaCava M, Jin X, Cravatt BF. An anatomical and temporal portrait of physiological substrates for fatty acid amide hydrolase. Journal of lipid research. 2011; 52:337–344. [PubMed: 21097653]
50. Hsu, KL.; Tsuboi, K.; Speers, AE.; Brown, SJ.; Spicer, T.; Fernandez-Vega, V.; Ferguson, J.; Cravatt, BF.; Hodder, P.; Rosen, H. Probe Reports from the NIH Molecular Libraries Program. Bethesda (MD): 2010. Optimization and characterization of a triazole urea inhibitor for diacylglycerol lipase beta (DAGL-beta).
51. Knuckley B, Jones JE, Bachovchin DA, Slack J, Causey CP, Brown SJ, Rosen H, Cravatt BF, Thompson PR. A fluopol-ABPP HTS assay to identify PAD inhibitors. Chemical communications. 2010; 46:7175–7177. [PubMed: 20740228]

52. Saghatelian A, Cravatt BF. Discovery metabolite profiling--forging functional connections between the proteome and metabolome. Life sciences. 2005; 77:1759–1766. [PubMed: 15964030]
53. Vinayavekhin N, Homan EA, Saghatelian A. Exploring disease through metabolomics. ACS chemical biology. 2010; 5:91–103. [PubMed: 20020774]
54. Saghatelian A, McKinney MK, Bandell M, Patapoutian A, Cravatt BF. A FAAH-regulated class of N-acyl taurines that activates TRP ion channels. Biochemistry. 2006; 45:9007–9015. [PubMed: 16866345]
55. Chiang KP, Niessen S, Saghatelian A, Cravatt BF. An enzyme that regulates ether lipid signaling pathways in cancer annotated by multidimensional profiling. Chemistry & biology. 2006; 13:1041–1050. [PubMed: 17052608]
56. Cao Z, Mulvihill MM, Mukhopadhyay P, Xu H, Erdelyi K, Hao E, Holovac E, Hasko G, Cravatt BF, Nomura DK, Pacher P. Monoacylglycerol lipase controls endocannabinoid and eicosanoid signaling and hepatic injury in mice. Gastroenterology. 2013; 144:808–817. e815. [PubMed: 23295443]
57. Costola-de-Souza C, Ribeiro A, Ferraz-de-Paula V, Calefi AS, Aloia TP, Gimenes-Junior JA, de Almeida VI, Pinheiro ML, Palermo-Neto J. Monoacylglycerol Lipase (MAGL) Inhibition Attenuates Acute Lung Injury in Mice. PloS one. 2013; 8:e77706. [PubMed: 24204926]
58. Kinsey SG, Nomura DK, O'Neal ST, Long JZ, Mahadevan A, Cravatt BF, Grider JR, Lichtman AH. Inhibition of monoacylglycerol lipase attenuates nonsteroidal anti-inflammatory drug-induced gastric hemorrhages in mice. The Journal of pharmacology and experimental therapeutics. 2011; 338:795–802. [PubMed: 21659471]
59. Blankman JL, Long JZ, Trauger SA, Siuzdak G, Cravatt BF. ABHD12 controls brain lysophosphatidylserine pathways that are deregulated in a murine model of the neurodegenerative disease PHARC. Proceedings of the National Academy of Sciences of the United States of America. 2013; 110:1500–1505. [PubMed: 23297193] ** Untargeted metabolomics was used to discover the biochemical function of a previously uncharacterized enzyme ABHD12 as a lysophophatidyl serine hydrolase. The authors found that accumulation of this lipid from loss-of-function of ABHD12 leads to neuroinflammation and recapitulates a degenerative disease, PHARC.
60. Parsons DW, Jones S, Zhang XS, Lin JCH, Leary RJ, Angenendt P, Mankoo P, Carter H, Siu IM, Gallia GL, Olivi A, McLendon R, Rasheed BA, Keir S, Nikolskaya T, Nikolsky Y, Busam DA, Tekleab H, Diaz LA, Hartigan J, Smith DR, Strausberg RL, Marie SKN, Shinjo SMO, Yan H, Riggins GJ, Bigner DD, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW. An integrated genomic analysis of human glioblastoma Multiforme. Science. 2008; 321:1807–1812. [PubMed: 18772396]
61. Mardis ER, Ding L, Dooling DJ, Larson DE, McLellan MD, Chen K, Koboldt DC, Fulton RS, Delehaunty KD, McGrath SD, Fulton LA, Locke DP, Magrini VJ, Abbott RM, Vickery TL, Reed JS, Robinson JS, Wylie T, Smith SM, Carmichael L, Eldred JM, Harris CC, Walker J, Peck JB, Du FY, Dukes AF, Sanderson GE, Brummett AM, Clark E, McMichael JF, Meyer RJ, Schindler JK, Pohl CS, Wallis JW, Shi XQ, Lin L, Schmidt H, Tang YZ, Haipek C, Wiechert ME, Ivy JV, Kalicki J, Elliott G, Ries RE, Payton JE, Westervelt P, Tomasson MH, Watson MA, Baty J, Heath S, Shannon WD, Nagarajan R, Link DC, Walter MJ, Graubert TA, DiPersio JF, Wilson RK, Ley TJ. Recurring Mutations Found by Sequencing an Acute Myeloid Leukemia Genome. New Engl J Med. 2009; 361:1058–1066. [PubMed: 19657110]
62. Dang L, White DW, Gross S, Bennett BD, Bittinger MA, Driggers EM, Fantin VR, Jang HG, Jin S, Keenan MC, Marks KM, Prins RM, Ward PS, Yen KE, Liau LM, Rabinowitz JD, Cantley LC, Thompson CB, Heiden MGV, Su SM. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. Nature. 2009; 462:739–U752. [PubMed: 19935646]
63. Figueroa ME, Abdel-Wahab O, Lu C, Ward PS, Patel J, Shih A, Li YS, Bhagwat N, Vasanthakumar A, Fernandez HF, Tallman MS, Sun ZX, Wolniak K, Peeters JK, Liu W, Choe SE, Fantin VR, Paietta E, Lowenberg B, Licht JD, Godley LA, Delwel R, Valk PJM, Thompson CB, Levine RL, Melnick A. Leukemic IDH1 and IDH2 Mutations Result in a Hypermethylation Phenotype, Disrupt TET2 Function, and Impair Hematopoietic Differentiation. Cancer Cell. 2010; 18:553–567. [PubMed: 21130701]

64. Xu W, Yang H, Liu Y, Yang Y, Wang P, Kim SH, Ito S, Yang C, Wang P, Xiao MT, Liu LX, Jiang WQ, Liu J, Zhang JY, Wang B, Frye S, Zhang Y, Xu YH, Lei QY, Guan KL, Zhao SM, Xiong Y. Oncometabolite 2-Hydroxyglutarate Is a Competitive Inhibitor of alpha-Ketoglutarate-Dependent Dioxygenases. Cancer Cell. 2011; 19:17–30. [PubMed: 21251613]

65. Ulanovskaya OA, Zuhl AM, Cravatt BF. NNMT promotes epigenetic remodeling in cancer by creating a metabolic methylation sink. Nature chemical biology. 2013; 9:300–306. ** This paper describes NNMT as an upregulated enzyme in aggressive human cancer cells that heightens the methylation of nicotinamide to siphon methyl sources away from methylation of histones, thereby altering the epigentic and transcriptional landscape of cancer cells to drive their pathogenic features. These discoveries were uncovered through metabolomic profiling.

66. Shyh-Chang N, Locasale JW, Lyssiotis CA, Zheng Y, Teo RY, Ratanasirintrawoot S, Zhang J, Onder T, Unternaehrer JJ, Zhu H, Asara JM, Daley GQ, Cantley LC. Influence of threonine metabolism on S-adenosylmethionine and histone methylation. Science. 2013; 339:222–226. [PubMed: 23118012] **Metabolomic profiling led to the discovery that abundance of SAM appears to influence trimethylation of histone H3 lysine 4 (H3K4me3), providing a possible mechanism by which modulation of a metabolic pathways might influence stem cell fate.

67. Munger J, Bajad SU, Coller HA, Shenk T, Rabinowitz JD. Dynamics of the cellular metabolome during human cytomegalovirus infection. PLoS pathogens. 2006; 2:e132. [PubMed: 17173481]

68. Vastag L, Koyuncu E, Grady SL, Shenk TE, Rabinowitz JD. Divergent effects of human cytomegalovirus and herpes simplex virus-1 on cellular metabolism. PLoS pathogens. 2011; 7:e1002124. [PubMed: 21779165] ** Using metabolic flux analysis, Vastag et al. discovered that the human cytomegalovirus (HCMV) increased glycolytic flux, increased TCA compound levels and flux into fatty acid synthesis, whereas herpes simplex virus type-1 (HSV-1) increased anapleurotic influx into TCA cycle feeding into pyrimidine biosynthesis.

69. Grady SL, Purdy JG, Rabinowitz JD, Shenk T. Argininosuccinate synthetase 1 depletion produces a metabolic state conducive to herpes simplex virus 1 infection. Proceedings of the National Academy of Sciences of the United States of America. 2013; 110:E5006–5015. [PubMed: 24297925]

70. Rabinowitz JD, Purdy JG, Vastag L, Shenk T, Koyuncu E. Metabolomics in drug target discovery. Cold Spring Harbor symposia on quantitative biology. 2011; 76:235–246.

Highlights

1. There are many enzymatic pathways that remain uncharacterized in the human genome.
2. Activity-based proteomics globally assesses enzyme activities
3. Activity-based proteomics can be used for inhibitor discovery of enzymes
4. Metabolomics can dissect enzyme function in complex living systems.

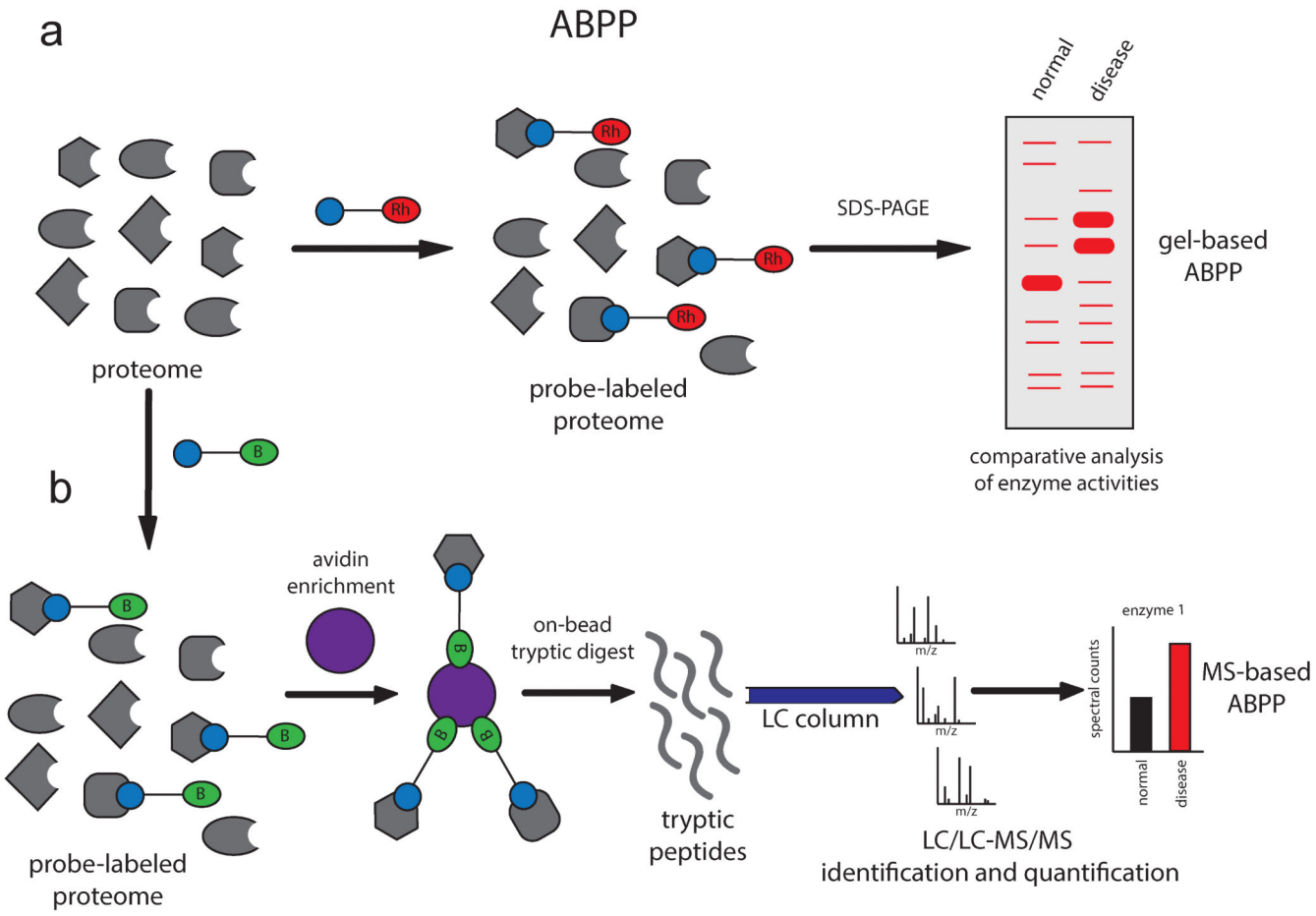


Figure 1. Activity-based protein profiling (ABPP)

ABPP uses active site-directed chemical probes to broadly assess the functional state of enzymes across enzyme families. These probes consist of a reactive group and a detection handle, most commonly rhodamine (Rh) or biotin (B). In gel-based ABPP, native proteomes are reacted with the probe and proteins are separated by SDS-PAGE and visualized by fluorescent scanning. MS-based ABPP facilitates the identification and quantification of enzyme activities following avidin enrichment, on-bead tryptic digest, and resolution by Multidimensional Protein Identification Technology (MudPIT).

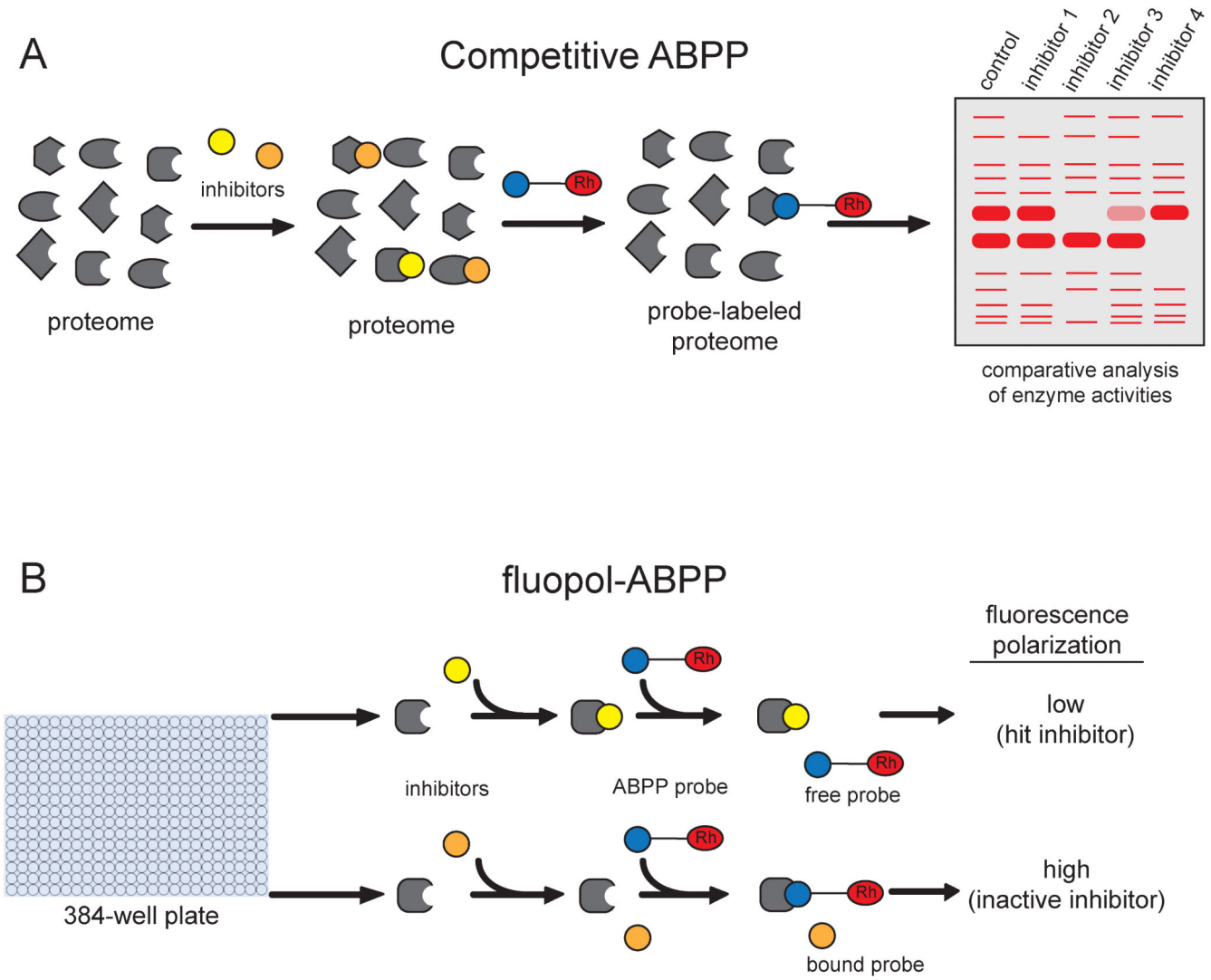


Figure 2. Competitive ABPP and fluopol ABPP for the discovery of enzyme inhibitors

(A) Competitive ABPP assesses the potency and selectivity of small molecule inhibitors in native proteomes by competing with the ability of the probe to bind. Enzyme inhibition is indicated by a loss of fluorescent intensity by gel or by a loss of spectral counts by MS. (B) Fluopol ABPP is a HTS version of competitive ABPP conducted with pure or recombinant protein. Fluorescence polarization is high if enzyme activity is high (inactive inhibitor) and low if enzyme activity is low (active inhibitor).

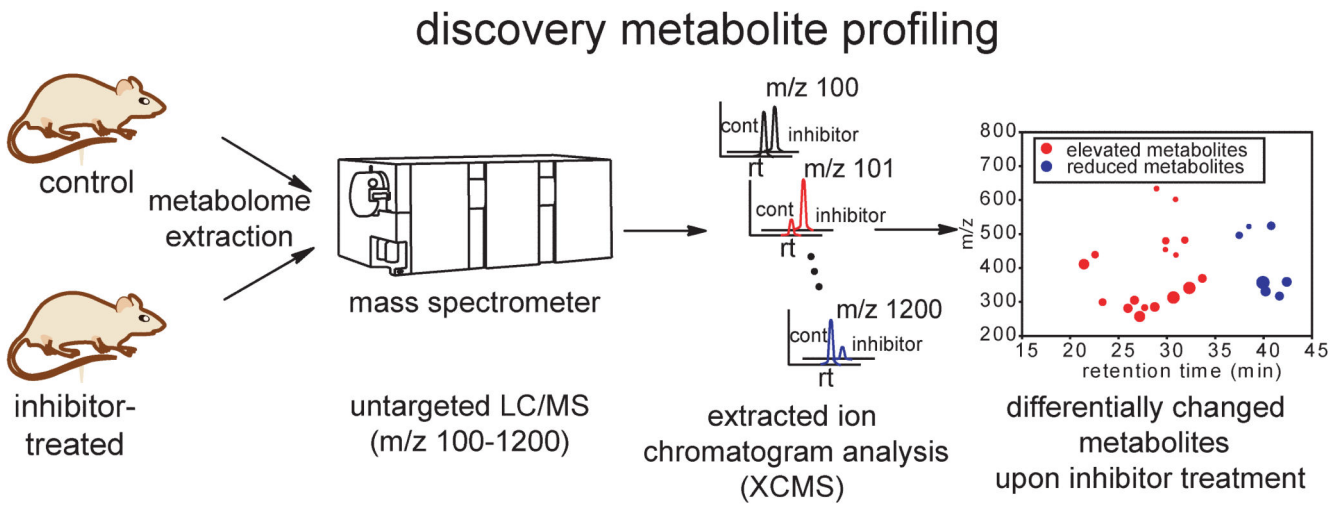
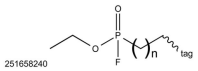
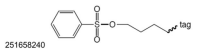
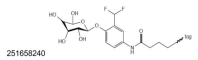
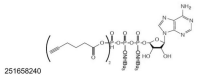
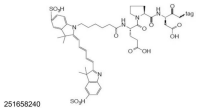
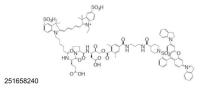


Figure 3. Discovery Metabolite Profiling

In untargeted metabolomics, the mass spectrometer scans a large mass range (m/z 100-1,200) for known and unknown metabolites. Datasets are then analyzed by bioinformatics platforms which align, quantify, and identify metabolites that are significantly altered between treatment groups.

Table 1

Probe target	Structure	Applications
Serine hydrolases		Identified upregulated KIAA1363 and MAGL activities (8, 9) and increased secreted uPA and tPA in aggressive cancer (10, 11). Identified increased RBBP9 activity in pancreatic carcinomas (13).
Phenyl sulfonate esters		Targeted several enzyme families and showed that infection by hepatitis C leads to dysregulation of several protein activities (15).
Glycoside hydrolase enzymes		Used to profile functional cellulose-degrading enzyme activity in bacteria applicable to the development of biofuels (17).
ATP-binding enzymes		Identification of altered TCA cycle, oxidative phosphorylation, and lipid metabolism enzymes in skeletal muscle mitochondria of high fat-fed mice (18).
Caspases		Visualization of apoptosis in colon tumors of mice treated with Apomab (22).
Cysteine proteases		Used to identify highly upregulated legumain activity in activated macrophages and for in vivo imaging of tumor formation (24).