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Activity-based proteomic and metabolomic approaches for understanding metabolism

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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.

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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 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.

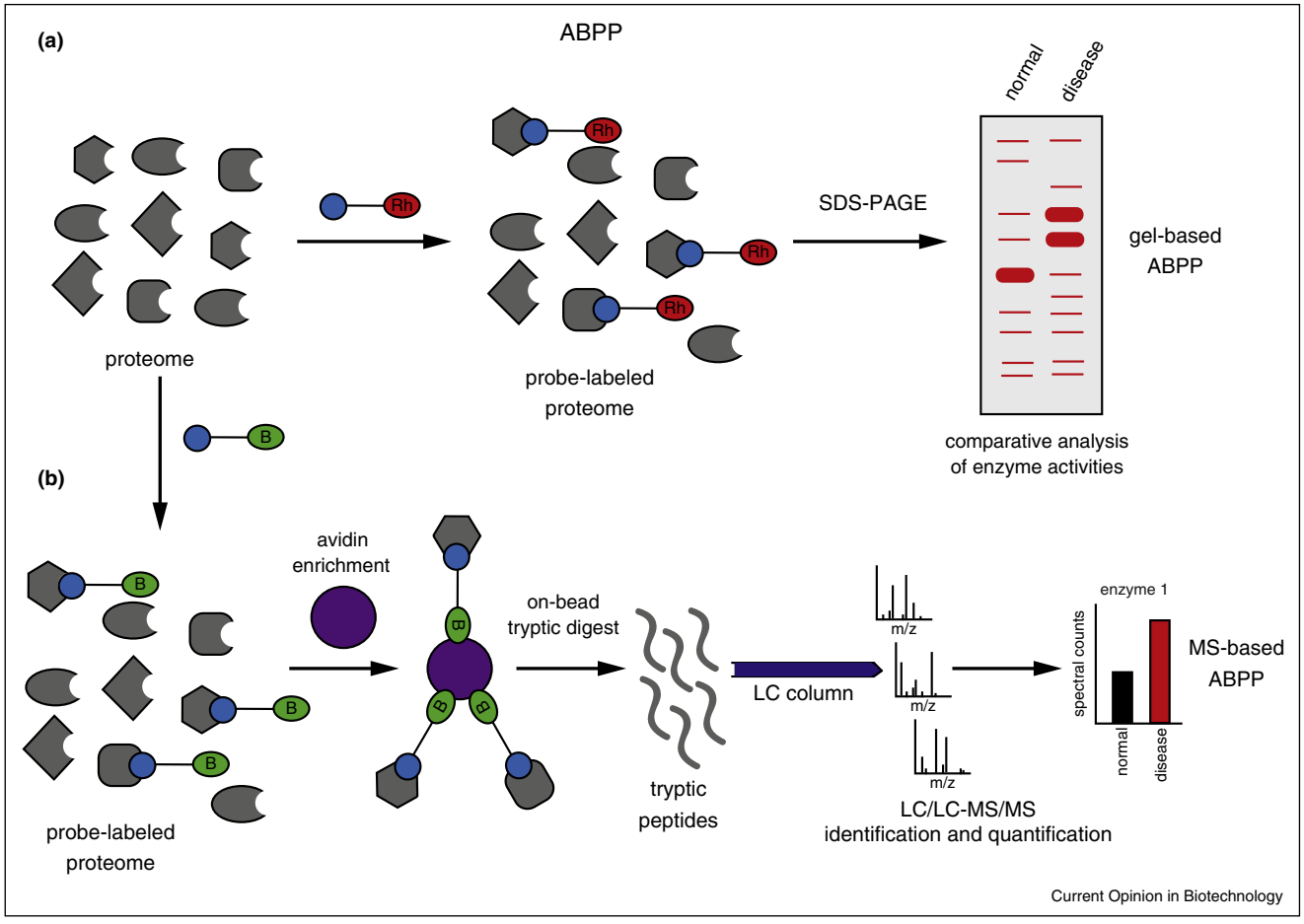
Broad profiling of enzyme activities

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

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).

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 fibrosarcoma 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