Review

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Advances in Activity-Based Protein Profiling of Functional Tyrosines in Proteomes

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Dedicated to Professor Benjamin F. Cravatt III to celebrate his 2022 Wolf Prize in Chemistry

Abstract: Activity-based protein profiling (ABPP) is a chemical proteomic method for investigating functional states of proteins in native biological settings. By quantifying changes in probe binding states of active and regulatory protein sites, ABPP reveals functional information on protein regulation and can be configured in competitive settings to determine global selectivity profiles of tool compounds and drugs in lysates, cells, and animals. Chemical probes used for ABPP analyses can target protein families with conserved enzymatic or structural features or can broadly profile the proteome using electrophiles with reactivity towards functional groups on amino acid side chains. The latter approach has provided insights to protein sites involved in allosteric regulation and non-enzymatic functions. This review introduces quantitative ABPP workflows and discusses electrophilic groups used for ABPP profiling of functional sites in the proteome with an emphasis on tyrosine residues.

Introduction

Chemical tools offer a complementary approach to genetic methods (CRISPR-Cas9 knockouts and RNAi knockdowns[1]), and the former has distinct advantages; the effects are rapid, reversible, and can be used in almost any cell type, disease model, patient-derived samples, or *in vivo*.[1–2] Importantly, these tools can be used in a native system, allowing for the investigation of endogenous proteins. This capability provides insights into biological function and aids in identifying off-targets and potential toxicity, which contribute to the development of safer and more selective agents. The acute pharmacological modulation of proteins can also minimize network-wide compensatory effects that can occur with 'chronic' blockade of proteins using genetic methods.

A fraction (~22–40%) of the human proteome is considered "druggable" and an estimated 11% of the proteome has a reported ligand.[3] A smaller fraction of the human proteome (1–4%) can be investigated using chemical probes with sufficient potency (<100 nM on-target activity), selectivity (10-fold selectivity against tested off-targets), and permeability (active in cells at <10 μM).[3] The development of quality chemical probes for diverse protein classes is needed and has prompted large-scale projects including Target 2035,[4] Chemical Probes Portal[5] and the NIH-funded project – Molecular Libraries Program.[6] Such community-wide efforts aim to expand the portfolio of validated small molecule probes for basic and translational efforts against difficult protein targets (often deemed "undruggable"). For example, an objective of the Target 2035 project is to develop a suite of chemical tools to modulate every protein in the proteome by 2035.[4]

In this review, we discuss the role of activity-based protein profiling (ABPP) in advancing discovery of chemical probes to study protein function. The field of ABPP and chemoproteomics in general has introduced powerful approaches to assist in the global analysis and assignment of function to uncharacterized proteins in the mammalian proteome.[7] In some instances, the basic discoveries enabled subsequent drug discovery efforts for developing therapeutic agents in neurological disorders[8] and cancer.[9] ABPP methods for investigating catalytic serine/threonine or cysteine residues have advanced considerably and are now widely implemented in an increasing number of academic and drug discovery programs. Expanding the types of amino acid residues targetable by ABPP is important for advancing protein function and chemical probe discovery. In this review, we highlight recent progress in development of ABPP

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strategies to investigate functional tyrosine residues in the proteome.

Activity-Based Protein Profiling

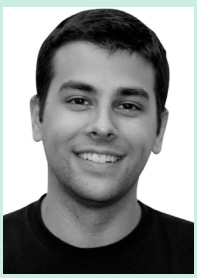
Cells respond to environmental changes, in part, by rapid alterations in protein activity and localization through post-translational modifications.[10] These changes in response to a stimulus typically occur at a time scale much faster than changes in protein expression, which highlights that activity, not protein level, is an essential parameter in understanding biological regulation. Traditionally, enzyme activity is monitored by measuring substrate turnover. However, in complex systems (e.g., lysates and cells), multiple enzymes can compete for substrate, which confounds the ability to measure specific changes in activity of individual enzymes. Activity-based protein profiling (ABPP) utilizes small molecule probes that covalently bind to active site residues to monitor functional states of proteins.[7] ABPP can report on protein activity that is dynamically regulated through post-translational modifications,[11] allosteric modulation,[12] localization[13] and substrate competition.[14]

The covalent modification of targets within the proteome can be measured by various biochemical and analytical methods such as sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) or high-content, tandem liquid chromatography-mass spectrometry (LC–MS/MS). With these two strategies, ABPP is an enabling tool for the study of proteins when the substrate is unknown or when traditional substrate-enzyme assays are not available or cannot be performed in complex systems. ABPP offers distinct advantages relative to traditional biochemical assays because it provides a readout of the protein's activity by monitoring endogenous proteins in their native biological context. ABPP can provide further insights into protein function and interaction partners when compared with analysis of purified protein activity.

Comparative ABPP for target discovery relies on the comparison of multiple proteomes in order to determine differing levels of protein activity.[7a] These proteomes are prepared from cell samples with different biological properties (healthy vs. disease) to assess differences in enzyme activities that can be ascribed to cellular phenotypes.[7a] Comparative ABPP provides multiple advantages for target discovery when compared with traditional expression-based methods. Protein expression does not necessarily correlate to activity that is driving the observed phenotype in a living system because of PTMs, protein-protein interactions, and subcellular localization of proteins. These activity changes, however, can be directly measured by activity-based probes (ABPs).

ABPP can be configured in a competitive format to discover covalent inhibitors[15] and activators[16] (Figure 1). Inhibitors are evaluated by their ability to block probe labeling of enzymes. Competitive ABPP provides several advantages over conventional screening methods. First, enzymes are tested within their native proteome, which alleviates the need to optimize conditions for recombinant expression and protein purification. Second, an ABP can act as a surrogate for substrates, which is useful when the substrate is unknown or in cases where a biochemical assay is not available. Finally, because ABPs target several members of a protein class, the potency and selectivity of the compounds can be tested in parallel in a single experiment[15] (Figure 1). Reversible inhibitors can be screened in this manner by carefully controlling the kinetic conditions of probe labeling.[17]

In a gel-based ABPP experiment, fluorophore conjugated ABPs are used for direct detection, or an azido-tagged



Jeff Brulet received his B.S. from the University of New Hampshire. He pursued doctoral studies in development and application of SuTEx chemistry for chemical biology under the supervision of Prof. Ku-Lung Hsu at the University of Virginia and obtained his PhD degree in 2020. Dr. Brulet is currently a Senior Scientist at Scorpion Therapeutics.

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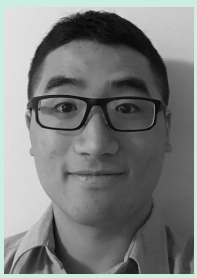

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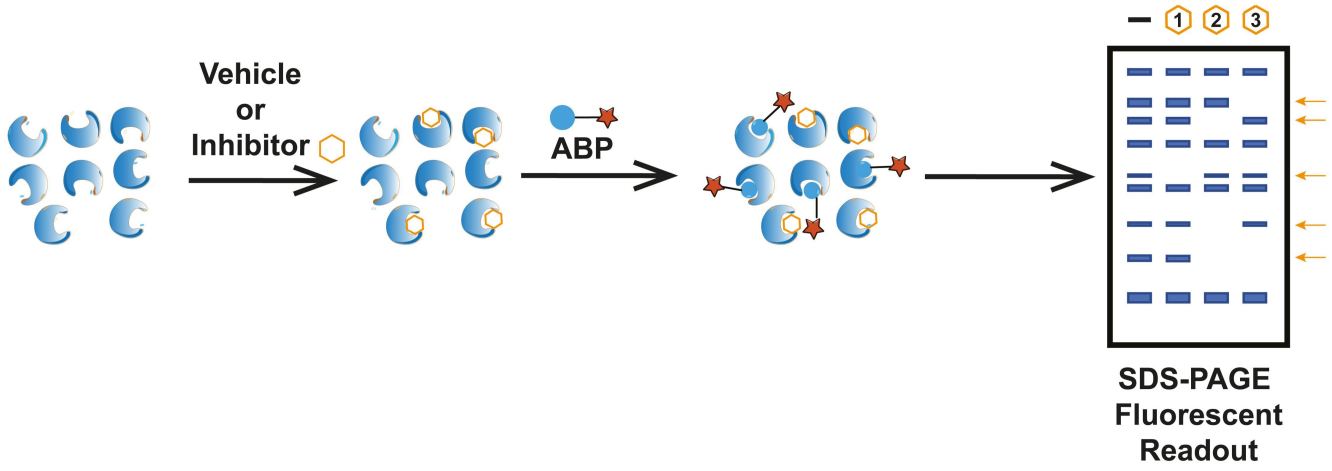

Figure 1. General workflow for gel-based, competitive activity-based protein profiling. The general design of an activity-based probe (ABP) is shown as the electrophilic group (blue circle), linker (black line), and reporter tag (red star). Pretreatment with compounds of interest block probe labeling that can be measured as reduced fluorescent ABP labeling (highlighted by orange arrows) to assess inhibitor potency and selectivity across the proteome.

fluorophore can be conjugated to alkyne-ABP-modified proteomes using copper(I)-catalyzed azide-alkyne [3 + 2] cycloaddition[18] (CuAAC, "click chemistry") to visualize protein activity by in-gel fluorescence scanning. After completion of SDS-PAGE, probe-labeled proteins can be visualized by in-gel fluorescence scanning to identify changes in native (or recombinant proteins if using overexpressed systems) protein activity. Proteins which have been labeled by an ABP will be identified as a fluorescent band due to the covalent attachment of a fluorophore. Changes in probe labeling can be detected and quantified to study differences in protein activity. In a competitive gel-based ABPP experiment, the sample of interest (live cell or lysate) is first treated with an inhibitor compound before ABP labeling. Samples can then undergo CuAAC to attach a fluorophore if using alkyne-modified ABPs, or directly processed for SDS-PAGE and in-gel fluorescence scanning. Reductions in probe labeling, which can be indicative of occupancy of inhibitor in the corresponding binding site, is used to evaluate potency of inhibitor against a protein of interest and potential off-target activity across the proteome (Figure 1).

LC-MS ABPP

In tandem LC-MS/MS-based ABPP experiments, an affinity enrichment tag such as biotin or releasable desthiobiotin (either directly modified or CuAAC conjugated after alkyne-ABP labeling) is used for on-bead trypsin digestion of enriched proteins for protein identification or enrichment of probe-modified peptides for site of binding identification. The reduction in enriched peptide abundances can be used to quantify ligand competition of protein and binding sites. As described below, competitive ABPP can be coupled with quantitative proteomic methods such as stable isotope labeling with amino acids in cell culture (SILAC)[19] and tandem mass tag (TMT) labeling[20] to increase quantitation capabilities (Figure 2).

ABPP analyses are typically performed using a "bottom-up" proteomics approach that measures peptide fragments generated from proteolysis to infer the identity of probe-bound protein.[21] In most bottom-up proteomic workflows the proteins are solubilized and denatured. Disulfide bonds are reduced, and cysteine thiol groups are carbamidomethylated using iodoacetamide (IAA). Next, proteins are digested into peptides using sequence-specific proteases (e.g., trypsin, chymotrypsin) and the resulting peptides (including probe-modified peptides) are analyzed by LC-MS/MS.[22] The experimental peptide fragmentation spectra are matched to predicted peptide fragmentation generated *in silico* from protein-coding genes of genomes.[23] Statistical analyses are performed using decoy databases to gain confidence in peptide identification to infer proteins detected in samples.[24]

Ions formed from ESI-MS experiments can behave in a concentration sensitive manner and are mostly independent of flow rate and injection volumes.[25] The ability for linearity between the sample concentrations and ion signal forms the basis of quantitation in mass spectrometry-based proteomics. After the sample preparation described above, the peptides generated are separated by liquid chromatography and ionized by electrospray ionization (ESI) followed by injection into the mass spectrometer for MS1 and MS2 (MS/MS) analysis. The initial analysis on the mass analyzer measures the m/z of the peptide molecular ions (MS1). Next, the sequential dissociation of peptide molecular ions and analysis of the peptide fragment ions (MS2) enables the identification of the primary amino acid sequences of the peptides as well as the site of probe modification.[23] Quantitation of the abundances of probe-modified peptides provides information on the functional state of inferred proteins and when performed

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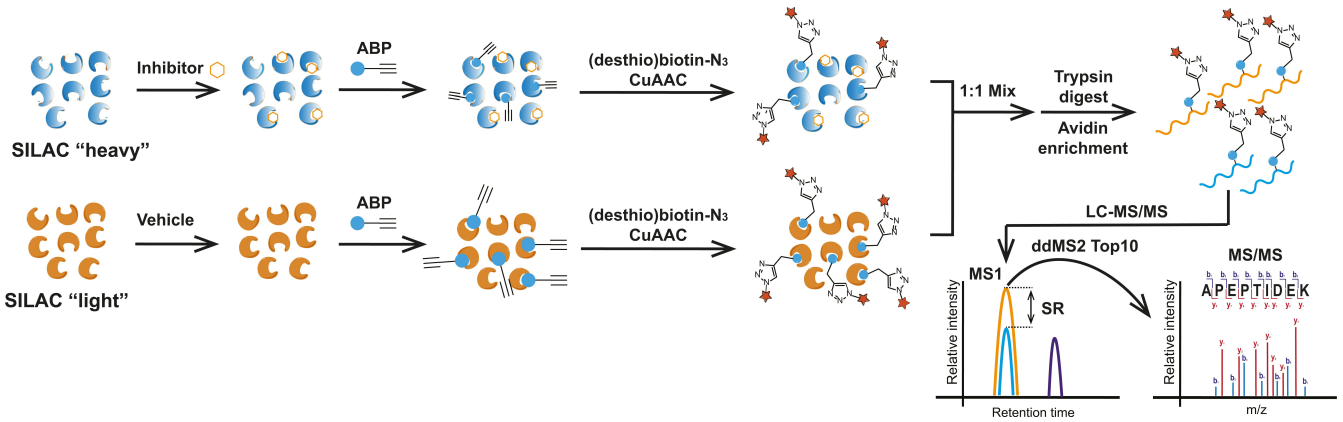

Figure 2. General workflow for LC-MS ABPP for quantifying (SILAC labeling) inhibitor activity against proteins and binding sites across the proteome. The workflow depicts a general LC-MS/MS-based quantitative ABPP workflow including sample preparation and analysis. The SILAC light and heavy cells or cell lysates are differentially treated with either DMSO or inhibitor followed by labeling with the activity-based probe (ABP). Cells are harvested, washed, and fractionated (typically to soluble and membrane fractions) to yield the proteome. The proteome subsequently undergoes CuAAC with a (desthio)biotin-PEG-azide tag. The tagged proteins are digested with protease (e.g., trypsin), enriched by avidin affinity chromatography, eluted, and analyzed by LC-MS/MS to identify probe-modified sites that are labeled by ABP. Binding sites that are competed by inhibitor pretreatment are identified by reduced MS1 peptide area under the curve (AUC) and quantified by the altered SILAC ratio (SR).

across the proteome can be used to evaluate ligand/inhibitor binding potency and selectivity.[21]

Quantification in LC-MS ABPP analyses can be achieved through different approaches. Label-free quantification (LFQ), as the name suggests, is based on the comparison of ion intensities or MS/MS sequencing events (i.e., spectral counting) for selected proteolytic peptides without modification to the peptides.[26] The relative quantitative information for the peptides of interest in experimental conditions is generated by comparing to that of their counterparts in the control conditions. The advantages of LFQ include flexibility in the sample input amount, the sample preparation is straightforward (no peptide labeling steps or metabolic incorporation into cells), and quantitation is cost effective (no additional reagents are required). However, the quantitation accuracy is difficult for low abundance proteins and can require significant computing resources to measure large numbers of mass spectral features.

Isobaric tags containing stable isotopes (^{13}C,^{15}N) have been used to label proteins. These labels can be incorporated chemically (e.g., isobaric tags for relative and absolute quantification, iTRAQ; tandem mass tag, TMT; isotope-coded affinity tag, ICAT[27]) or metabolically (e.g., SILAC[19]). SILAC has been used extensively for ABPP analyses using various activity-based probes to investigate a diversity of protein classes.[15,28] Tandem mass tags (TMT) as a chemically induced quantitation reagent consist of N-hydroxysuccinimide-ester (NHS)-based isobaric reagents.[29]

TMT is an emerging method for quantitative labeling in ABPP analyses because it provides higher multiplex capabilities compared with SILAC.[30] It is designed to perform highly multiplexed analysis of many samples in the same analytical run. The NHS reactive group modifies the primary amine on the lysine side chain and the N-terminus of peptides (Fig-ure 3A). Each TMT reagent can label differentially treated samples and create a set of peptides labeled with a different TMT reporter group but share the same overall molecular weight (with minor variances) by a combination of isotope distribution between the mass balancer and reporter (Figure 3B). The different TMT reporter groups provide measurable mass differences between peptides generated from each individual treatment condition.[29]

In a TMT proteomics workflow, TMT-labeled peptide analytes from different treatments pass through the mass analyzer simultaneously with the same molecular ion m/z. Fragmentation of the peptide yields the different TMT reporter tags associated with each ion, corresponding to the peptides from each treatment condition. Differences in abundance between the reporter ions provide quantitative information (Figure 3C). The overall adduct mass for TMT (all current variants) is less than 400 Da, which minimizes the relative mass increase of the analytes. The m/z of the reporter ions, which are between 100–200 Da, fall within a "silent region" of the tandem mass spectra where there are relatively few background ions (peptide fragments, detergent, buffer, denaturant) that would interfere with the resolution of reporter ion abundances. TMT has found widespread application in the field of quantitative proteomics due to its universal applicability without the need to introduce heavy isotope-labeled amino acids by metabolic uptake in cell culture or tissues,[31] and increased capacity for multiplexed concurrent sample analysis (e.g., up to 18 samples can be multiplexed[32]).

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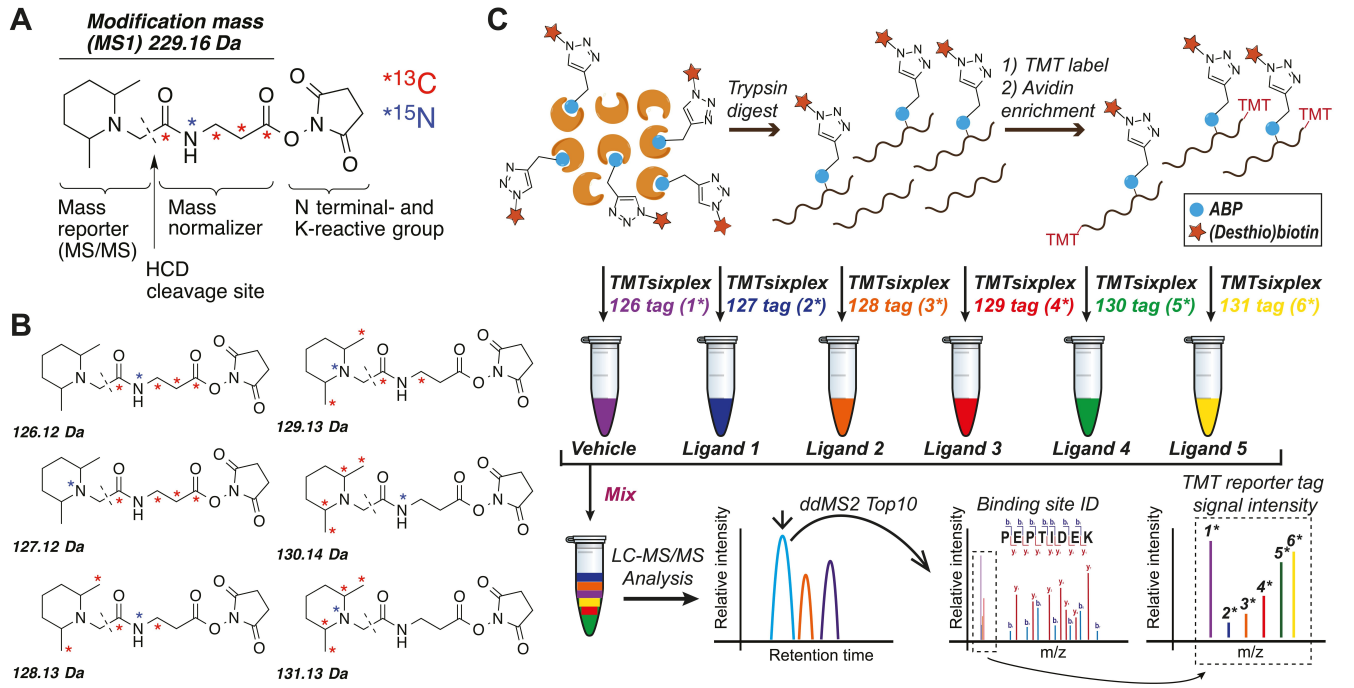

Figure 3. Quantitative proteomics using tandem mass tag (TMT) labeling. TMT 6-plex is shown as an example. (A) Tandem mass tag (TMT) reagents are N-hydroxysuccinimide-ester based isobaric tags designed to multiplex samples and analyze concurrent samples within the same analytical run. The asterisk depicts either a ^{13}C (red) or ^{15}N atom (blue). (B) Structures and stable heavy isotope distribution of TMTsixplex reagents. (C) In a TMT-ABPP proteomics workflow, differentially treated samples are combined for a single analysis and TMT-labeled, probe-modified peptides from different experimental conditions pass through the mass analyzer simultaneously with the same molecular ion m/z. The sample treatment denotation and quantitation information are revealed upon dissociation of the peptide that yields the different TMT reporter tags denoting each ion.

Design and Development of Activity-Based Probes

Chemical probes used for ABPP studies range from more selective agents that target a protein family based on conserved active site features to reactive electrophiles that modify amino acid sites across broad protein classes. An example of the former probe class include fluorophosphonates for targeting catalytic serines on serine hydrolases[33] or ATP-acyl phosphate probes for ATP utilizing proteins including kinases.[34] There are also examples of nucleophilic probes and ligands for ABPP investigations of electrophilic functionalities on proteins.[35]

ABPs have been used to identify ligandable sites on proteins, develop potent and selective inhibitors, and study post- translational modifications including crotonylation, methylation, deamination, and phosphorylation to understand their roles in human physiology and disease.[30c,g,36] An ABP consists of 3 principal components: (1) a reactive group that utilizes a range of electrophilic groups to covalently modify amino acid sites; (2) a linker that separates the reactive group from the reporter tag; and (3) a reporter tag that enables the visualization or enrichment of probe-modified proteins or peptides (Figure 1 and 2). The linker could be a simple alkyl chain or elements to increase the solubility (e.g., polyethylene glycol (PEG)[37]) or enzymatic recognition (e.g., a polypeptide to resemble an enzymatic substrate[38]) of the probe. There are variations of chemical- (e.g., disulfide[39] and diazo[40] containing) and protease-cleavable (e.g., ENLYFQG sequence from tobacco etch virus protease[41]) linkers that can undergo cleavage with mild chemical or enzymatic conditions.

The tag is used for the visualization or affinity enrichment of probe-modified proteins or peptides. Historically, radioisotope label (i.e., ^{125}I) tags were used as a visualization tag in activity-based proteomics.[42] Fluorescent tags are now widely adopted in ABPP studies and include the use of rhodamine and fluorescein[43] as well as cell-permeable BODIPY (dipyrromethene boron difluoride) for live cell studies.[44] Affinity tags are used for enrichment and identification of the target proteins of ABPs. The tagged proteins or peptides can be enriched through affinity chromatography followed by identification and quantification via LC–MS/MS. Biotin-avidin-based interaction is widely-used for affinity chromatography for ABPP. The proteins or peptides labeled by the (desthio)biotin-containing ABPs can be enriched with immobilized avidin beads and eluted (in the case of desthiobiotin-tagged ABPs) for ABPP analysis.[21]

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The fluorescent and affinity tags are integral parts of ABPs but could also be appended to the probe-modified proteins via biorthogonal conjugation (e.g., CuAAC) if the ABP contains a latent reporter handle such as a terminal alkyne group. The incorporation of a small and largely inert alkyne group into the design of ABPs reduces binding effects from the reporter tag and can directly identify the targets of elaborated drug compounds through clickable analogs.[15]

The reactive group is composed of an electrophilic moiety that facilitates covalent reaction of the ABP with amino acid residues located in binding sites on the target proteins. With the advancement of covalent chemistry development, several nucleophilic amino acids are pharmacologically accessible by ABPP methods including cysteine,[30d,45] lysine,[34a,36a,46] tyrosine,[36f,46c,47] serine,[28,48] threonine,[49] histidine,[50] methionine,[51] and aspartate/glutamate.[52] Examples of reactive groups used for ABPP analyses and their corresponding nucleophilic amino acid targets are shown in Figure 4.

Development of Chemical Probes for ABPP Analysis of Tyrosine Sites

The phenol side chain of tyrosines can function as a nucleophilic site for protein modification by covalent compounds.[53] The amphiphilic nature of the phenol group[54] facilitates location of tyrosines in diverse protein domains involved in catalysis and recognition (e.g. RNA binding[55]) that can be further regulated through phosphorylation[56] and post-translational modifications.[57] These features of tyrosines, combined with the natural low abundance (~2–3%[58]) of this residue, affords opportunities to perturb catalytic and non-catalytic protein function using covalent small molecules. Importantly, protein tyrosine kinase dysfunction (resulting in abberant phosphorylation on tyrosine sites) is prominent in human disease including cancer and has served as an important target for development of therapeutic agents.[59]

Electrophilic groups for covalent binding to tyrosines have been reported.[36f,46c,47a–f] This review will focus on sulfone-based electrophiles, which have emerged as important reactive groups for ABPP investigations[47b,60] and drug discovery.[61] Sulfonyl fluorides, for example, have been shown to covalently modify a wide range of nucleophilic residues[50b,62] including tyrosines.[47c,63] The unique properties imparted by the use of a fluoride leaving group for sulfonyl-fluoride reaction with nucleophiles has prompted further exploration of this electrophile class as a new type of 'click chemistry' reaction through sulfur (VI) fluoride exchange (SuFEx) chemistry. A comprehensive description of SuFEx chemistry, which also encompasses fluorosulfates[64] and sulfuramidimidoyl fluorides,[65] is beyond the scope of this review and we instead point the reader to a previous review on this topic (Ref. [66]).

The development of sulfur-triazole exchange (SuTEx) chemistry permitted functional group modifications to the leaving group of sulfone-based electrophiles used for ABPP investigations.[47b] SuTEx probes are composed of a sulfonyl-triazole core that can be functionalized on both the sulfone adduct- (portion that remains bound to protein after covalent reaction) and triazole leaving group (presumably departing as a triazolide[47b]). Modifications to the adduct and leaving group can affect reactivity and molecular recognition in covalent binding of SuTEx compounds to protein sites in lysates and cells.[47a,67] Importantly, modifications to the triazole group can influence the chemoselectivity of SuTEx probes for tyrosine versus lysine residues (Figure 5); this feature was important for developing the global tyrosine-directed SuTEx probe HHS-482, which bears a para-methoxyphenyl substituent on the 3-

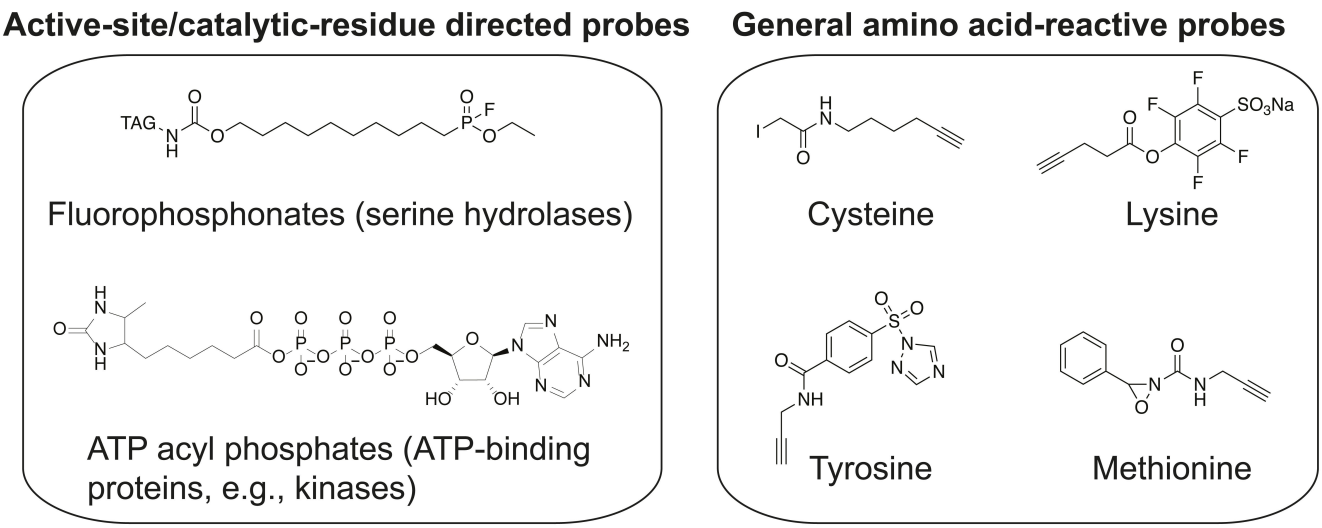

Figure 4. Examples of active-site/catalytic-residue directed probes and electrophiles that generally modify amino acid sites on proteins for ABPP investigations.

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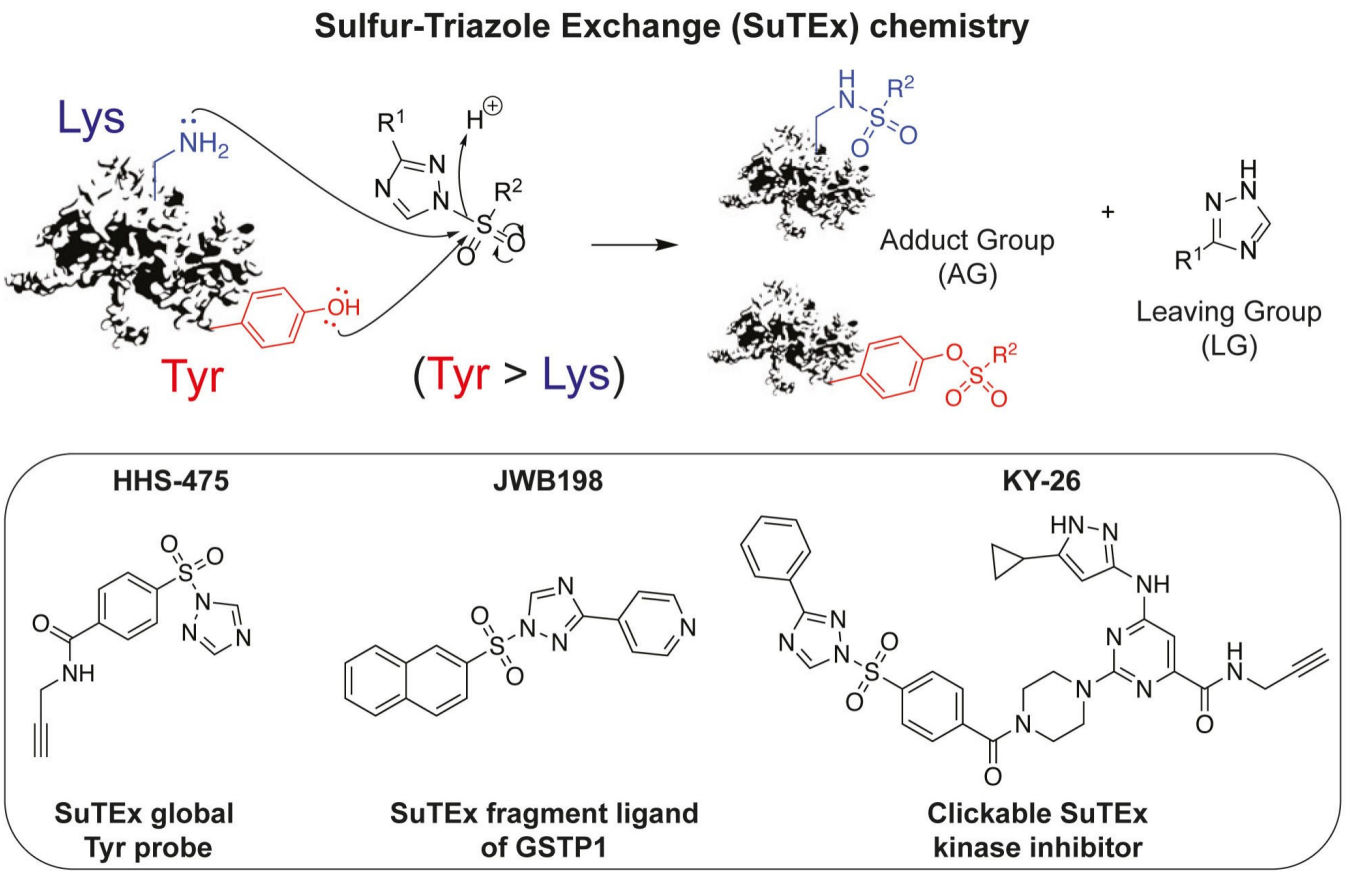

Figure 5. Development of SuTEx for ABPP investigation of functional and ligandable tyrosine sites on proteins.

position of the 1,2,4-triazole group (>70% of probe-modified residues assigned to tyrosines in ABPP analyses[36f]).

The 'harder' electrophilic character of the sulfonyl sulfur[68] is a potential explanation for the preference of SuTEx and other sulfone-based ABPs to covalently bind tyrosine and lysine over cysteine residues. The enhanced tyrosine versus lysine binding of reported SuTEx analogs (e.g., HHS-482[36f]) is an important feature of this electrophilic class and the mechanistic basis for this preference is currently being investigated. Initial solution reactivity studies by HPLC demonstrated that the triazole group appears to increase the phenol reactivity of sulfone-based electrophiles.[36f]

The SuTEx electrophile has been deployed in several formats for ABPP investigations. Sulfonyl-triazoles bearing an alkyne reporter tag can serve as detection probes for chemical proteomic profiling of hundreds to thousands of tyrosine (and lysine to a lesser extent) sites on proteins[36f] (e.g., HHS-475, Figure 5). Non-alkyne analogs permit functional modifications to the adduct- and leaving group end of sulfonyl-triazoles for developing fragment-like electrophiles to assess ligandability of tyrosine sites in proteomes[47a] and cells[67] using competitive ABPP formats (e.g., GSTP1 inhibitor JWB198, Figure 5). The SuTEx scaffold can also be functionalized with more elaborated binding elements to direct binding activity towards specific protein classes including kinases[69] (e.g., KY-26, Figure 5).

The development of SuTEx chemoproteomics has also led to advancements in understanding tyrosine biochemistry. Akin to discovery of cysteines with enhanced nucleophilic character (i.e., hyper-reactive cysteines[45a]), SuTEx probes facilitated the identification of hyper-reactive tyrosines in proteomes. In these studies, cell lysates were treated with 1X or 10X concentrations of SuTEx probe ([probe]) followed by LC-MS/MS and bioinformatics analyses to bin tyrosine sites into groups that displayed saturated probe binding at high and low [probe] (assigned as hyper-reactive) compared with tyrosines that showed concentration-dependent probe labeling (non-hyper-reactive sites). From this experiment, approximately 5% of quantified sites were found to be hyper-reactive. Subsequent domain enrichment analyses revealed that these residues were enriched for enzymatic function and were less likely to phosphorylated.[36f] This prioritization strategy was key for identifying a hyper-reactive Y475 site in the poorly characterized YjeF_N domain of the processing body (PB) protein, enhancer of mRNA-decapping protein 3[70] (EDC3). Subsequent functional studies demonstrated the importance of EDC3 Y475 in regulating the PB response of stressed cells through regulation of EDC3 phosphorylation and protein-protein interaction state.[71]

SuTEx ABPP analysis was also used to identify tyrosine probe binding events that were sensitive to pervanadate, a global inhibitor of tyrosine phosphatases.[72] These studies tested whether accumulation of phosphorylation at PTP-regulated tyrosine sites could be detected by ABPP via blockade of SuTEx probe labeling. These studies identified and validated Y705 and Y228 as pervanadate-sensitive, phosphotyrosine sites on signal transducer and activator of transcription 3 (STAT3) and catenin delta-1 (CTNND1), respectively. This work provided important proof-of-concept that SuTEx probe labeling can be used as a complementary method to study tyrosine PTMs, such as phosphorylation.[36f]

Summary and Outlook

ABPP is a powerful technique for direct measurement of native protein activities in lysates, cells, and *in vivo* to gain functional information that is not easily achieved with gene- and protein-expression analyses alone. Post-translational modifications, which includes amino acid-specific modifications (e.g., phosphorylation and acetylation) and proteolytic activation of zymogens, can rapidly alter the proteomic composition of cells and demands technologies like ABPP that can capture these activity-dependent responses to stimuli. The facile integration of ABPs into modern proteomic workflows is enabling broader access and adoption of this methodology into academic and drug discovery programs. The ability to globally assess selectivity of compounds using competitive ABPP is accelerating the lead optimization process for developing covalent inhibitors and drugs.[15]

To expand the protein classes addressable by ABPP, new reactive groups are being developed to investigate protein sites beyond active site residues (e.g., catalytic serines) and intrinsically nucleophilic residues (e.g., cysteines).[21] Electrophilic groups for ABPP investigations of protein sites containing a tyrosine residue are emerging and enabling biochemical and cell biological investigations.[36f,46c,47a–f] The SuTEx electrophile, for example, has advanced ABPP analyses of tyrosines by (i) facilitating the global discovery of ligandable tyrosines in the human proteome,[36f] (ii) established a prioritization strategy to identify functional tyrosine sites based on reactive/structural features,[36f] (iii) demonstrated capabilities for tuning the reactivity and selectivity of sulfonyl-triazoles for a tyrosine site of interest using medicinal chemistry,[47a] (iv) provided a facile means for target identification in cell lysate and phenotypic screening formats,[67] and (v) established proof-of-concept for late stage functionalization for developing targeted covalent inhibitors with an embedded SuTEx reactive group.[69]

Sulfone-based electrophiles, which includes SuFEx[66] and SuTEx,[47b] are effective covalent binders to tyrosine sites but do exhibit secondary binding activity to other nucleophilic residues (e.g., serine,[73] histidine,[50b] and lysine[74]). The ability to optimize sulfone-based electrophiles for enhanced binding of tyrosine versus other nucleophilic amino acid sites on proteins is an important future endeavor to further advance this electrophilic class for ABPP studies. Functional modifications to the triazole group of SuTEx compounds has provided initial evidence that chemoselectivity towards tyrosines is possible with sulfone-based electrophiles.[36f] Exploration of additional leaving groups including the use of alternative heterocycles is likely to provide important and interesting insights to the tunability of the sulfone electrophile for tyrosine ABPP profiling.

Competing Interests

K.-L.H. is scientific founder and advisor to Umbra Therapeutics.

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Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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