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Chemoproteomics in Medicinal Chemistry: A Comprehensive Review on Mapping the Druggable Proteome

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ABSTRACT

Chemoproteomics has emerged as a powerful platform in medicinal chemistry, enabling systematic exploration of the druggable proteome. By integrating covalent probes with quantitative mass spectrometry allows residue-level mapping of reactive amino acids and provides insights into protein function, ligandability, and drug-target interactions. This approach has expanded the boundaries of drug discovery by identifying novel covalent inhibitors, cryptic binding pockets, and previously inaccessible protein classes. Furthermore, chemoproteomics has facilitated the development of targeted protein degraders by uncovering covalent E3 ligase recruiters, thereby advancing selective proteome editing strategies. Despite its rapid growth, several limitations remain, including restricted proteome coverage, functional annotation gaps, and challenges in translating in vitro data to clinical applications. Advances in probe design, integration with computational tools, and adaptation to patient-derived systems are expected to overcome these barriers. Looking forward, chemoproteomics is positioned to play a central role in precision medicine by tailoring therapeutic interventions to patient-specific proteomic landscapes.

KEYWORDS: Chemoproteomics; Covalent Inhibitors; Drug Discovery; Druggable Proteome; Mass Spectrometry; Medicinal Chemistry; Targeted Protein Degradation.

1. Introduction

Drug discovery has historically relied on serendipitous findings, followed by rational design approaches driven by structural biology and high-throughput screening. Despite these advances, most of the human proteome remains **"undruggable"** with conventional methods. Chemoproteomics, an interdisciplinary field that combines chemical biology with proteomics, has emerged as a transformative platform to interrogate protein function, ligandability, and target engagement at the proteome-wide scale. One of the pioneering demonstrations of this strategy was the development of activity-based protein profiling (ABPP), which employed covalent chemical probes to monitor the functional state of enzyme families directly in complex proteomes [1].

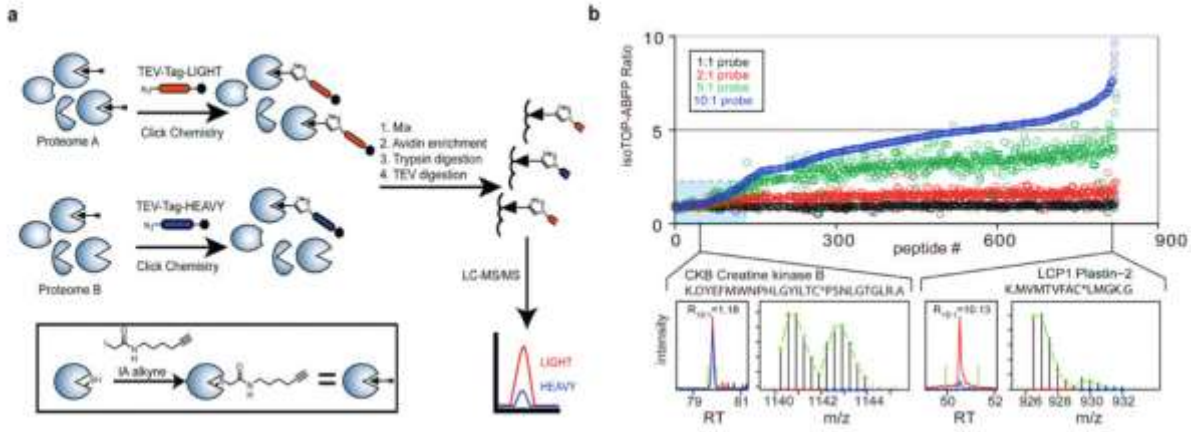

Figure 1. A quantitative approach to globally profile cysteine reactivity in proteomes [1].

Subsequent advances introduced quantitative reactivity profiling, enabling the systematic prediction of functional and ligandable cysteines across the human proteome [2]. These early chemoproteomic studies established that chemical reactivity patterns could be exploited to expand druggable space beyond classical binding sites. The integration of thermal proteome profiling (TPP) further broadened the applicability of chemoproteomics by allowing unbiased measurement of drug-protein interactions in living cells [3]. Together, these innovations created the foundation for mapping the "druggable proteome" proteins and sites that can be modulated with small molecules and provided medicinal chemists with a powerful framework for identifying targets, discovering ligands, and predicting off-target effects. Chemoproteomics has now advanced into an essential technology in modern drug discovery pipelines, guiding the development of covalent inhibitors, uncovering allosteric sites, and enabling proteome-wide profiling of pharmacological interventions. In this review, we examine the chemoproteomic methodologies most relevant to medicinal chemistry, highlight their applications in expanding the druggable proteome, and discuss challenges and opportunities for future integration into precision medicine.

2. Chemoproteomic Methodologies

Chemoproteomics encompasses a diverse set of experimental platforms designed to interrogate protein reactivity, ligandability, and drug-target engagement directly in complex biological systems. Each methodology provides complementary insights into the druggable proteome, and collectively, they have reshaped strategies in medicinal chemistry.

2.1 Activity-Based Protein Profiling (ABPP)

ABPP was among the earliest chemoproteomic platforms, enabling the use of covalent probes to monitor the functional state of enzymes in their native environments. By incorporating reactive electrophiles tethered to reporter handles, ABPP can capture active enzymes and assess inhibitor selectivity. Early studies using competitive ABPP revealed broad maps of lipid-binding proteins and their ligandability in mammalian cells [4]. The subsequent development of enantiomeric probe pairs further allowed the dissection of stereoselective binding events across thousands of proteins [5].

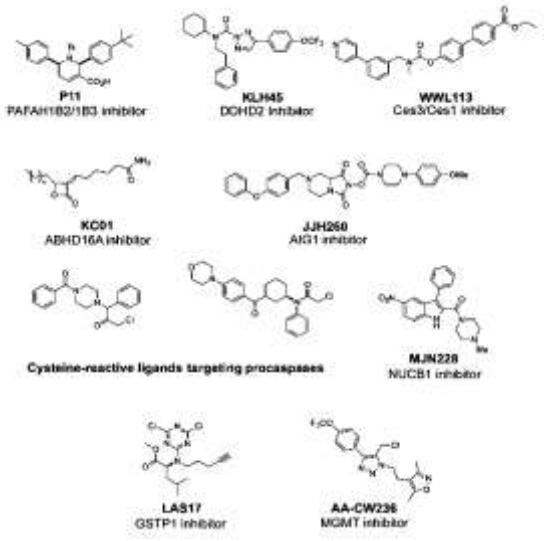

Figure 2. Examples of small-molecule inhibitors developed using competitive ABPP platforms [5].

2.2 Cysteine-Reactivity Profiling and Electrophile Scopes

Residue-centric ABPP approaches have been particularly impactful for cysteine profiling, given its nucleophilicity and central role in catalysis and regulation. The introduction of isotopically labelled isoDTB tags provided a robust method for proteome-wide cysteine quantification [6]. High-coverage workflows, such as SP3-FAIMS chemoproteomics, further enhanced sensitivity and coverage of the human cysteinome [7].

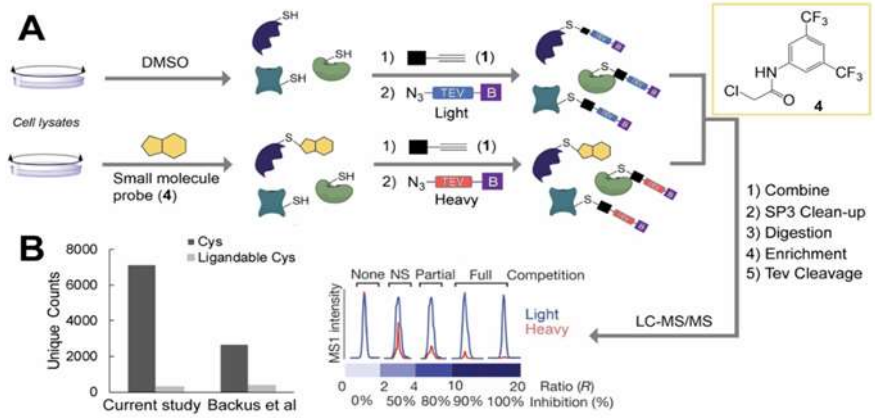

Figure 3. Chemoproteomic analysis of ligandable cysteines with SP3. A) Schematic workflow of competitive isoTOP-ABPP with SP3. B) Comparison of ligandable cysteines and total cysteines identified with isoTOP-ABPP treated with compound 4 [7].

2.3 Thermal Proteome Profiling (TPP) and CETSA

Beyond covalent chemistry, drug–target interactions can be probed by monitoring protein thermal stability shifts in living cells. The cellular thermal shift assay (CETSA) established that ligand binding often stabilizes proteins against denaturation, thereby enabling drug engagement readouts in intact systems [8]. This principle was extended in thermal proteome profiling (TPP), where proteome-wide monitoring of stability changes provided unbiased maps of drug-target interactions and off-targets [9].

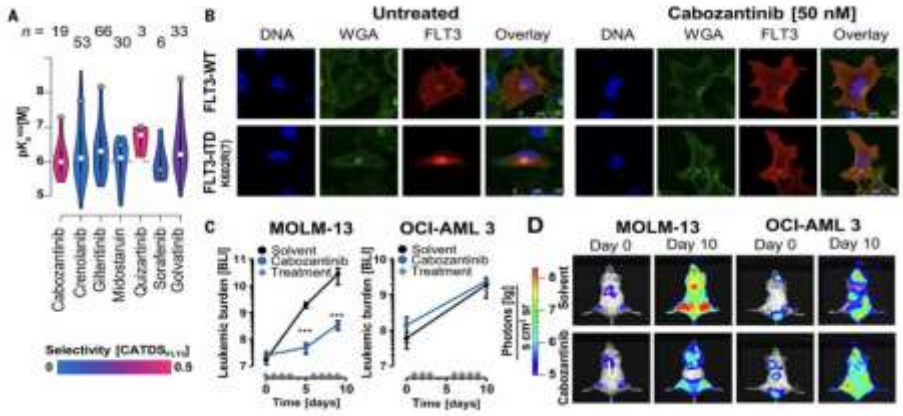

Figure 4. Repurposing of Cabozantinib for the treatment of FLT3-ITD-positive acute myeloid [9].

2.4 Proximity Labelling Approaches

Chemoproteomic platforms have also expanded to include non-covalent, proximity-based strategies. The BioID system, which employs a promiscuous mutant biotin ligase, enabled the mapping of protein interaction networks by covalent tagging of proximal partners [10].

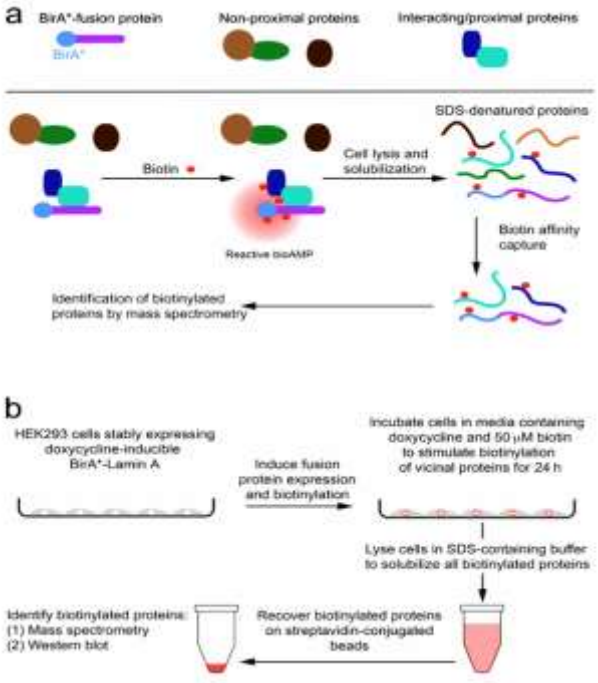

Figure 5. Model for application of the BioID method [10].

Similarly, APEX peroxidase labelling permitted high-resolution spatial proteomics, allowing the mapping of subcellular proteomes with temporal precision [11]. While initially developed for protein-protein interaction studies, these methods are increasingly being adapted to probe drug-modulated protein networks in medicinal chemistry.

3. Residue-Resolved Ligandability and Warhead Scope

The ability to interrogate individual amino acid side chains within complex proteomes has transformed the understanding of protein ligandability. By developing residue-selective chemistries and coupling them with mass spectrometry-based readouts, chemoproteomics enables medicinal chemists to define and exploit previously hidden druggable hotspots.

3.1 Cysteine Reactivity Mapping

Cysteine residues, due to their nucleophilicity and redox sensitivity, have been the primary focus of residue-resolved chemoproteomics. Quantitative cysteine reactivity profiling demonstrated that highly reactive cysteines often overlap with functional or druggable sites. Similarly, chemoproteomic mapping of cysteine oxidation and phosphorylation status revealed dynamic regulation of reactivity, underscoring their importance in signalling and draggability [12].

3.2 Electrophiles Targeting Cysteine-Modified Proteins

Beyond profiling intrinsic cysteine reactivity, tailored electrophiles have been applied to map ligandable cysteines across entire proteomes. Lipid-derived electrophiles were shown to covalently modify reactive cysteines, providing insight into stress-responsive protein regulation [13]. These studies established a framework for designing electrophilic warheads for covalent drug discovery.

3.3 Lysine-Targeting Electrophiles

While cysteine has dominated covalent inhibitor design, lysine residues are abundant and offer complementary targeting opportunities. Global chemoproteomic surveys identified lysine-reactive electrophiles, expanding the ligandable proteome to new functional residues [14]. These findings highlight lysine as a promising frontier for medicinal chemistry efforts in covalent inhibitor design.

3.4 Methionine Bioconjugation Strategies

Methionine residues, though less reactive, represent underexplored nucleophilic sites. The development of redox-activated chemical tags (ReACT) provided selective labelling strategies for methionine across proteomes [15]. More recently, copper(I)-nitrene platforms enabled direct methionine labelling, further expanding residue-specific chemical biology tools [16].

3.5 Targeting Acidic Residues

In addition to nucleophilic amino acids, acidic residues such as aspartate and glutamate can be covalently targeted. Parker and colleagues introduced electrophiles capable of mapping reactive acidic side chains proteome-wide, revealing novel druggable hotspots previously inaccessible to traditional covalent chemistry [17].

3.6 Advances in Quantitative Site-Identification

The integration of chemoproteomics with isobaric tandem mass tag (TMT) chemistry has streamlined the identification and quantification of ligandable sites. Functionalized TMT-alkyne reagents improved click-based workflows, allowing residue-resolved profiling of diverse electrophile reactions with higher throughput [18]. Such quantitative innovations are critical for systematically expanding the amino acid scope of ligandable proteomes.

4. High-Throughput Discovery and Community Resources

The evolution of chemoproteomic methodologies has enabled the systematic exploration of druggable sites at an unprecedented scale. Large-scale studies combining covalent probe libraries with quantitative mass spectrometry have generated comprehensive reactivity maps, fuelling medicinal chemistry by identifying ligandable hotspots across thousands of proteins.

4.1 Proteome-Wide Ligandability Maps

One of the earliest large-scale efforts to define the **"ligandable proteome"** employed isotopically labelled probes and competition assays, revealing thousands of reactive cysteines across human proteomes [19]. These data provided medicinal chemists with functional annotations for reactive sites, many of which were previously unrecognized as druggable. Building on this, quantitative profiling of electrophile fragments systematically assessed their ability to covalently engage proteins, creating the foundation for covalent fragment-based screening [20].

4.2 Fragment-Based Covalent Screening

Fragment-based approaches have been particularly powerful in expanding covalent drug discovery. Screening libraries of electrophilic fragments directly in human cells identified a broad landscape of covalently ligandable proteins [21]. This approach not only mapped ligandability but also revealed cryptic binding pockets that would be invisible to conventional screening, thus providing a chemical biology toolkit for medicinal chemistry.

4.3 Data Integration and Publicly Available Resources

The accumulation of chemoproteomic datasets necessitated centralized resources for data accessibility and integration. The Cysteinome database was developed to collate cysteine chemoproteomic datasets, providing a community-accessible atlas of cysteine reactivity and ligandability [22]. More recently, ProteomeXchange repositories have hosted raw and processed datasets from multiple chemoproteomic studies, allowing systematic reanalysis and cross-comparison across labs [23]. These platforms are instrumental in bridging experimental chemoproteomics with computational predictions and machine learning applications in drug discovery.

4.4 Implications for Medicinal Chemistry

By democratizing access to chemoproteomic datasets and probe libraries, high-throughput and community-driven efforts have accelerated drug discovery pipelines. Medicinal chemists can now integrate residue-resolved ligandability maps with structural and pharmacological data, guiding the rational design of covalent inhibitors, allosteric modulators, and degraders. These resources effectively lower the barrier to entry for covalent chemistry in both academic and pharmaceutical settings.

5. Chemoproteomics in Drug Discovery Applications

Chemoproteomics has moved beyond method development to deliver concrete medicinal-chemistry outcomes. By providing residue-level maps of ligandability and direct measures of target engagement in cells and tissues, chemoproteomic approaches have catalyzed the discovery and optimization of covalent inhibitors, revealed cryptic allosteric sites, guided degrader (PROTAC/electrophile-based) design, and illuminated polypharmacology relevant to drug safety and immunomodulation.

5.1 Covalent Inhibitor Discovery and Optimization

Covalent inhibition, once avoided for safety concerns, has resurged through chemoproteomic validation of target engagement and off-target profiles. Early quantitative chemical-proteomics demonstrated that kinase inhibitors and other small molecules often engage numerous unintended targets, highlighting the need for proteome-wide profiling during lead optimization [24]. Chemoproteomic fragment screens and competitive ABPP have since been used to discover covalent fragments that selectively modify functional residues, enabling the structure-guided elaboration of covalent leads with improved selectivity and cellular activity [25,26]. These workflows accelerate the progression from fragment hits to optimized covalent inhibitors by revealing both productive engagement and off-target liabilities early in the campaign.

5.2 Identification of Allosteric and Cryptic Binding Sites

One powerful contribution of chemoproteomics is the discovery of allosteric or cryptic pockets that are not apparent from static structural snapshots. Chemoproteomic mapping of ligandable residues and fragment engagement across a proteome frequently uncovers sites distal to canonical active sites, which can be exploited for allosteric modulation. Such discoveries have provided new avenues to modulate "difficult" targets and to develop compounds with novel mechanisms of action, thereby broadening the scope of medicinal chemistry beyond orthosteric active sites [26].

5.3 Chemoproteomics-Guided Targeted Protein Degradation

The chemoproteomic identification of covalent ligandable handles on E3 ligases and other degradation machinery has directly enabled the design of targeted protein degraders. Electrophile-guided discovery approaches uncovered nucleophilic residues on particular E3 ligases that could be engaged by small electrophilic ligands to recruit ubiquitin machinery, enabling nuclear-restricted or selective degradation of targets via electrophilic PROTAC-like constructs [27]. Follow-up studies expanded the toolbox of covalent handles and validated multiple E3 ligases as recruitable degradation hubs, establishing chemoproteomics as a route to identify new ligase-ligand pairs for degrader design [28,29]. These advances demonstrate how proteome-wide reactivity maps can translate into modular chemical strategies for targeted protein removal.

5.4 Polypharmacology, Safety Deconvolution, and Immunomodulatory Chemistry

Chemoproteomics has also been critical in dissecting adverse or unexpected pharmacology. Activity-based approaches revealed off-target proteins responsible for the neurotoxic effects observed in a clinical candidate, enabling retrospective mechanistic clarification and informing safer lead design. In parallel, chemoproteomic profiling of immune cells has identified electrophile-sensitive proteins and pathways that modulate immune function, suggesting routes to design small molecules that tune immune responses through covalent engagement of key regulators [30]. Together, these examples underscore how chemoproteomics not only finds new targets but also refines safety and immunomodulatory profiles during drug development.

6. Challenges and Limitations of Chemoproteomics

Despite remarkable advances, chemoproteomics faces important technical and conceptual limitations that constrain its translation into broader medicinal chemistry pipelines. These challenges span **coverage, quantitative accuracy, functional interpretation, and clinical applicability**.

6.1 Proteome Coverage and Residue Bias

Chemoproteomics heavily relies on reactive probes (e.g., iodoacetamide- or sulfonyl-fluoride-based) that preferentially label nucleophilic amino acids, most prominently cysteine and lysine. As a result, large swaths of the proteome, including acidic and hydrophobic residues, remain underexplored [31]. Although emerging probes expand coverage to methionine, tyrosine, and histidine, universal coverage of the proteome remains elusive.

6.2 Quantitative and Contextual Limitations

Quantitative profiling is sensitive to experimental parameters such as probe concentration, cell type, and proteomic depth. Small variations can yield inconsistent engagement data, complicating reproducibility across laboratories [32]. Moreover, in-cell conditions differ markedly from in vivo pharmacology, where drug distribution, metabolism, and competing biomolecules may alter reactivity.

6.3 Functional Annotation Gap

Even when ligandable sites are robustly identified, their functional significance is often unclear. Not all covalently modified residues translate to pharmacologically actionable sites. Linking residue-level engagement with biological consequences remains a central bottleneck [33].

6.4 Off-Target and Safety Considerations

The promiscuous nature of electrophilic fragments and covalent inhibitors raises safety concerns. Several clinical failures, such as those linked to unanticipated off-target liabilities, highlight the need for rigorous off-target deconvolution during early drug discovery [34]. Chemoproteomics provides powerful tools for such profiling, but interpretation of polypharmacology data is complex.

6.5 Translation to Clinical Pharmacology

Most chemoproteomic studies are performed in cell lines or ex vivo systems, with limited translation into clinical settings. Technical barriers include low-abundance protein detection, sample preparation from patient material, and the complexity of capturing transient drug–protein interactions *in-vivo* [35]. Developing streamlined, clinically adaptable workflows remains a future priority.

7. Conclusion

Chemoproteomics has emerged as a transformative approach in medicinal chemistry, providing residue-level insights into the druggable proteome and enabling the discovery of covalent inhibitors, electrophilic degraders, and novel ligandable sites. By combining chemical biology with advanced proteomics, this field has expanded the therapeutic landscape beyond traditional enzyme targets, offering new opportunities in oncology, immunology, and neurodegeneration. Despite its success, challenges remain, including limited coverage across amino acid classes, variability in quantitative profiling, and the gap between in vitro findings and clinical translation. Addressing these barriers requires continued innovation in probe design, integration with computational methods, and adaptation of workflows for patient-derived samples. Looking forward, chemoproteomics is expected to converge with personalized medicine, providing patient-specific druggability maps that guide tailored therapeutic strategies. Ultimately, chemoproteomics represents not only a powerful discovery tool but also a cornerstone technology for the next generation of drug development.

8. Acknowledgment

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9. Disclosure of conflict of interest

The authors declare no conflict of interest.

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