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Reimagining Druggability Using Chemoproteomic Platforms

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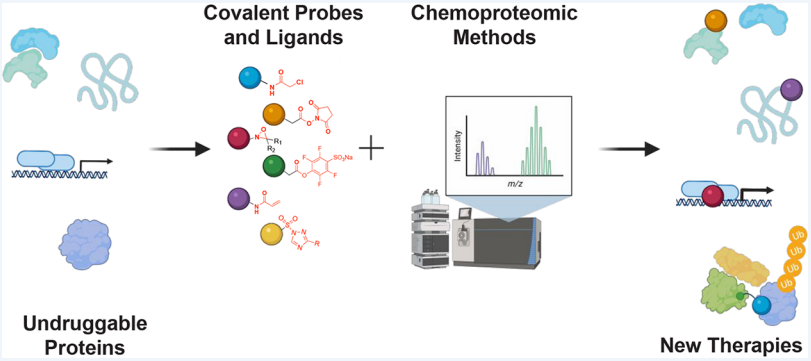

CONSPECTUS: One of the biggest bottlenecks in modern drug discovery efforts is in tackling the undruggable proteome. Currently, over 85% of the proteome is still considered undruggable because most proteins lack well-defined binding pockets that can be functionally targeted with small molecules. Tackling the undruggable proteome necessitates innovative approaches for ligand discovery against undruggable proteins as well as the development of new therapeutic modalities to functionally manipulate proteins of interest. Chemoproteomic platforms, in particular activity-based protein profiling (ABPP), have arisen to tackle the undruggable proteome by using reactivity-based chemical probes and advanced quantitative mass spectrometry-based proteomic approaches to enable the discovery of "ligandable hotspots" or proteome-wide sites that can be targeted with small-molecule ligands. These sites can subsequently be pharmacologically targeted with covalent ligands to rapidly discover functional or nonfunctional binders against therapeutic proteins of interest. Chemoproteomic approaches have also revealed unique insights into ligandability such as the discovery of unique allosteric sites or intrinsically disordered regions of proteins that can be pharmacologically and selectively targeted for biological modulation and therapeutic benefit. Chemoproteomic platforms have also expanded the scope of emerging therapeutic modalities for targeted protein degradation and proteolysis-targeting chimeras (PROTACs) through the discovery of several new covalent E3 ligase recruiters. Looking into the future, chemoproteomic approaches will unquestionably have a major impact in further expansion of existing efforts toward proteome-wide ligandability mapping, targeted ligand discovery efforts against high-value undruggable therapeutic targets, further expansion of the scope of targeted protein degradation platforms, the discovery of new molecular glue scaffolds that enable unique modulation of protein function, and perhaps most excitingly the development of next-generation small-molecule induced-proximity-based therapeutic modalities that go beyond degradation. Exciting days lie ahead in this field as chemical biology becomes an increasingly major driver in drug discovery, and chemoproteomic approaches are sure to be a mainstay in developing next-generation therapeutics.

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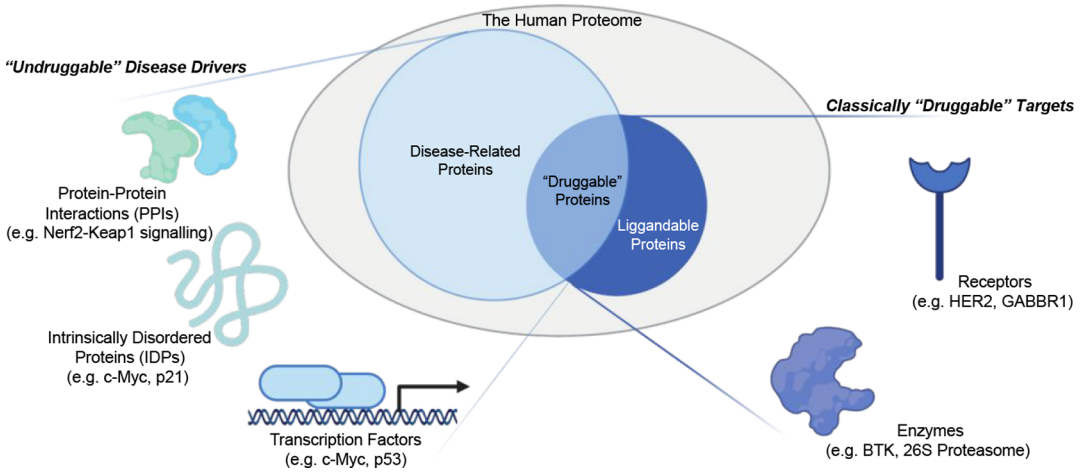
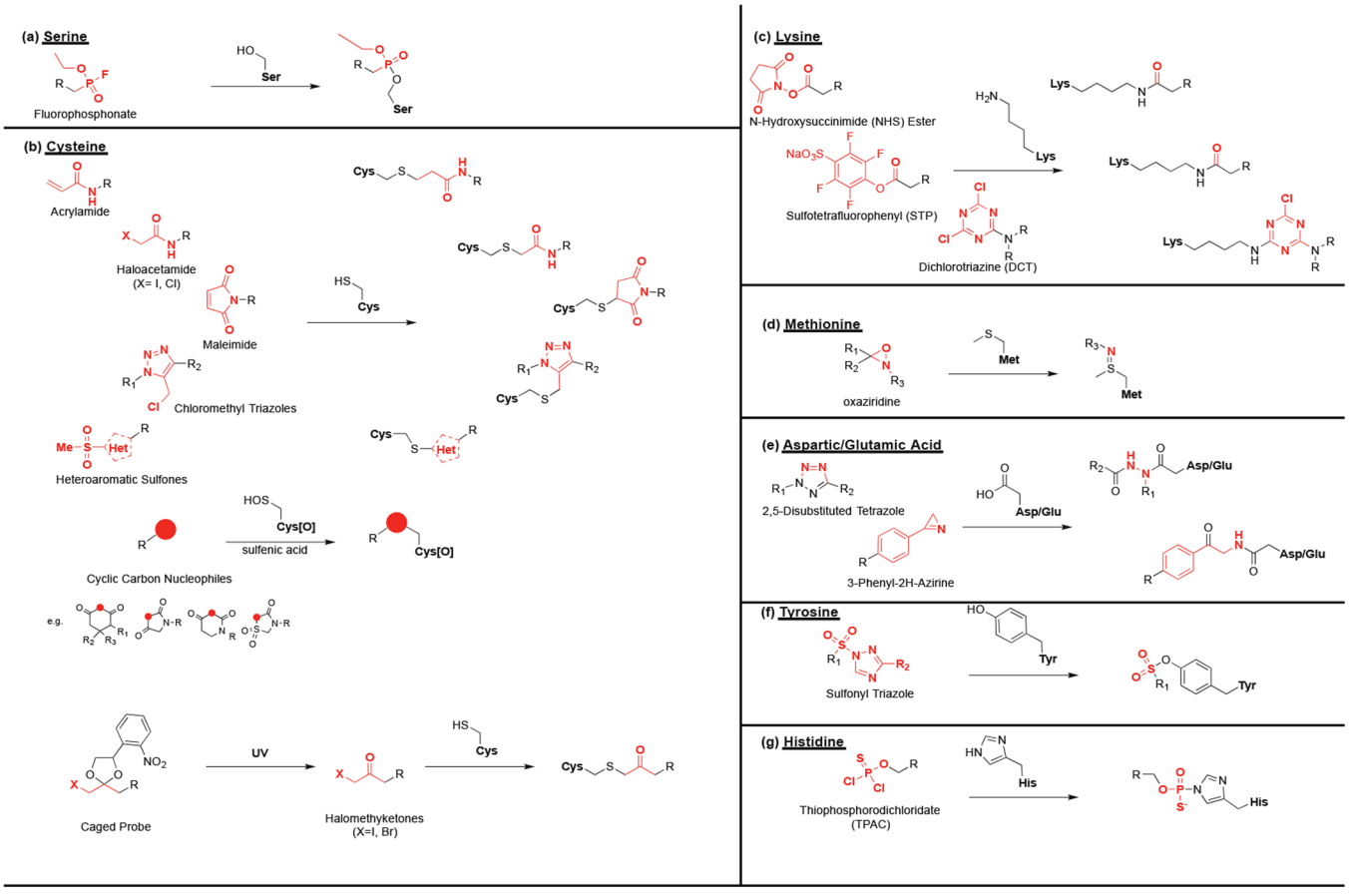


Figure 1. The undruggable proteome. More than 85% of the human proteome remains intractable toward ligand development, including key disease-related targets.

Table 1. Overview of Chemoproteomic Probes



paper describes the use of chemoproteomic approaches to discover that the natural product nimbolide targets an intrinsically disordered C8 in the E3 ubiquitin ligase RNF114 and that nimbolide can be used as a novel E3 ligase recruiter for targeted protein degradation applications.

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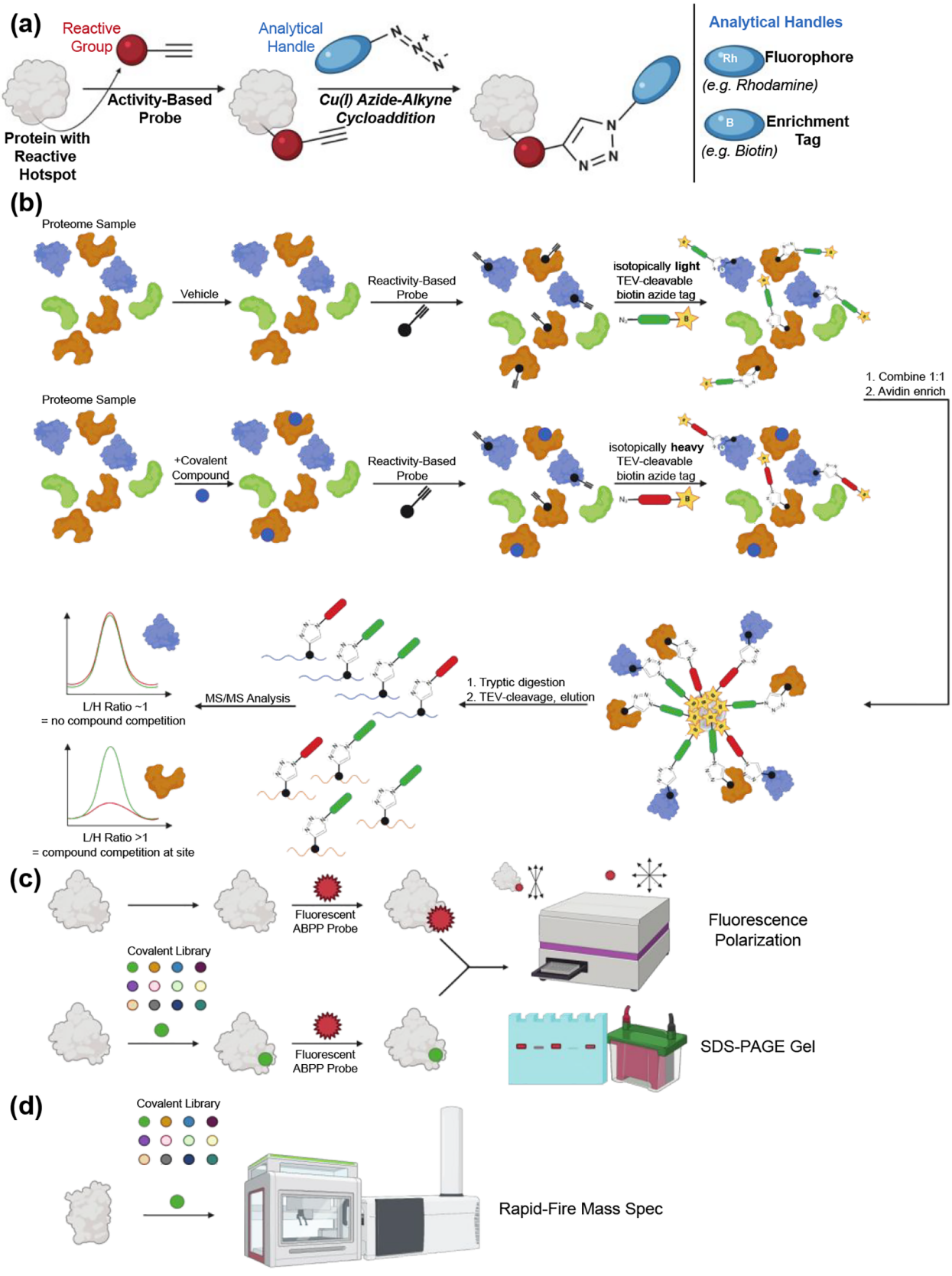

Figure 2. Activity-based protein profiling (ABPP) probes and uses in chemoproteomics. (a) ABPP probes consist of a covalent warhead and an alkyne handle for attachment of a reporter or enrichment tag. (b) Example workflow for use of ABPP probes in isoTOP-ABPP chemoproteomic experiments. (c) Fluorophore-modified probes can be competed against libraries of covalent fragments to identify ligands to proteins of interest. (d) Binding of covalent fragments can be read out directly using rapid-fire mass spectrometry readouts.

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1. INTRODUCTION

Thousands of gene and protein drivers of human diseases have been discovered that represent potential therapeutic targets for drug discovery. Despite the discovery of these therapeutic targets, most of these proteins have remained untranslated. A major challenge in developing therapeutics against these targets is that most proteins in the human proteome (>85%) are considered "undruggable", or intractable to traditional drug discovery efforts, because most proteins lack easily identifiable binding pockets or catalytic active sites that can be functionally and selectively targeted with a small-molecule, antibody, or other therapeutic modality[5,6] (Figure 1). Proteins in the undruggable category include protein complexes that are involved in scaffolding non-enzymatic functions and may not possess deep binding pockets that can be targeted to disrupt or stabilize protein interactions. Additionally, identifying binding pockets that can be pharmacologically targeted with small molecules within intrinsically disordered proteins remains challenging. There are many known, and likely even more yet to be discovered, therapeutic targets that reside in the 85% of the proteome that is currently intractable to classical drug discovery approaches.

Tackling the undruggable proteome with small molecules necessitates approaches for discovering ligandable sites and corresponding ligands within these proteins as well as the development of new therapeutic modalities to functionally manipulate the protein of interest. This Account specifically focuses on how chemoproteomic approaches enable ligand discovery against undruggable proteins, how these technologies can expand the scope of emerging induced-proximity-based therapeutic modalities (e.g., proteolysis-targeting chimeras (PROTACs) and molecular glues), and how these approaches can facilitate the development of novel therapeutic paradigms.

2. CHEMOPROTEOMIC PLATFORMS FOR MAPPING LIGANDABLE HOTSPOTS

Among chemoproteomic approaches, activity-based protein profiling (ABPP) has become one of the most versatile methods to discover both ligandable sites and corresponding ligands directly from complex biological samples. Originally pioneered by Cravatt and Bogyo, ABPP originated with active-site directed chemical probes for various enzyme classes, including hydrolases, proteases, kinases, and others.[7−10] These activity-based probes were able to read out the functional state of large numbers of active enzymes directly in complex biological systems, regardless of the state of functional characterization of the particular target (Table 1a). Using this strategy, many groups have identified dysregulated enzyme activities in disease and have developed potent and selective inhibitors against many of these targets. These studies have been previously covered in reviews and will not be covered here.[8,11]

ABPP in its modern form utilizes broadly reactive chemical probes that react with amino acid hotspots within proteins to map proteome-wide reactive and ligandable sites directly in complex proteomes or biological systems (Figure 2a).[12−16] These chemical probes exploit inherent differences in side-chain reactivity from local protein microenvironments that cause certain nucleophilic amino acid residues to be more reactive than others. Reactivity-based probes possess a reactive warhead that covalently reacts with nucleophilic amino acid hotspots (e.g., cysteines, lysines, serines, tyrosines) that are often enriched in catalytic sites, post-translational modification sites, protein−protein interaction sites, metal binding sites, allosteric binding pockets, or other binding modes. Probe binding to these sites can be read out with a reporter handle such as a fluorophore, biotin, or alkyne handle—the latter of which can be coupled with subsequent copper-mediated alkyne−azide cycloaddition (CuAAC) to append fluorescent or enrichment handles for downstream fluorescence detection or enrichment and elution for proteomic identification of probe-labeled proteins and peptides.[12,15,17−20]

Evaluating probe-modified peptides identified in chemoproteomic experiments of many laboratories in the chemical biology field reveals tens of thousands of nucleophilic sites that have been modified by the array of existing reactivity-based probes, with the potential to capture hundreds of thousands of sites in the future.[13−16,21−23] While not all of these sites represent binding pockets, there are likely thousands of sites within these data that represent potential ligandable hotspots that can be interrogated by a suitable electrophile, making chemoproteomic approaches a powerful entry point for discovery of ligandable sites and new covalent small-molecule binders against targets of interest. Discovery of new ligandable sites is especially significant in light of the new wave of bispecific therapies, which can convert noninhibitory ligands into consequential binders by recruiting effector proteins. Multispecific induced-proximity therapies such as bifunctional small-molecule protein degraders, also known as PROTACs, are transforming modern drug discovery efforts,[24] and as we will discuss, discovery and characterization of novel binding sites using chemoproteomics is one strategy for rapidly advancing induced-proximity-based therapeutic modalities.

Perhaps the best-established use of ABPP in recent years is for mapping proteome-wide cysteine hotspots because of the nucleophilic thiol group that makes them particularly favorable targets. Quantitative profiling of reactive cysteines in the proteome was initially enabled by a quantitative ABPP chemoproteomic platform termed isotopic tandem orthogonal proteolysis ABPP (isoTOP-ABPP) by Weerapana et al. to assess the extent of irreversible protein reactivity via cysteine-specific iodoacetamide alkyne (IA-alkyne) probes (Figure 2b).[12] This methodology was first used to quantify cysteine hyper-reactivity but has since been applied more generally to identify a wide variety of small-molecule cysteine binding sites and to assess covalent molecule selectivity, which will be discussed later. In addition to traditional haloacetamides and maleimide cysteine probes, many other ABPP-compatible cysteine-reactive probes have been developed (Table 1b). These include chloromethyltriazoles (CMTs) and heteroaromatic sulfones as readily accessible, tunable cysteine-reactive warheads,[25,26] cyclic C-nucleophiles to probe the sulfenic acid oxidation state of cysteine,[27] and an iodomethyl ketone electrophile paired with a UV-activated caging group for optimal sensitivity to cysteine reactivity in living cells.[28] The ABPP method also continues to evolve with advances in sample preparation, separation of peptides, mass spectrometry capabilities, and bioinformatics. A recent study by Yan et al. combines optimized biotin conjugation methods and solid-phase SP3 extraction methods

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with field asymmetric ion mobility mass spectrometry (FAIMS) and the Thermo trihybrid Eclipse mass spectrometer to identify >15 000 unique cysteines, greatly increasing coverage of the cysteine proteome.[21]

The next most widely exploited amino acid for ligandability outside of cysteine has been lysine (Table 1c). Weerapana developed dichlorotriazine-based chemoproteomic probes,[29] Ward et al. used a commercially available NHS-ester probe,[13] and Hacker et al. identified an amine-reactive pentynoic acid sulfotetrafluorophenyl ester probe to cumulatively profile proteome-wide lysine reactivity and to identify potential ligandable lysine hotspots.[14] Newer reactivity-based probes continue to be discovered against other amino acids, including oxaziridine-based reagents that target methionine,[30] 3-phenyl-2*H*-azirines that target glutamic and aspartic acids under ambient conditions,[31] and 2,5-disubstituted tetrazoles that target these acids under light activation[32] as well as sulfur−triazole exchange chemistry (SuTEX) that targets tyrosines (Table 1d−f).[33] The development of additional chemoproteomically compatible reactivity-based probes will undoubtedly expand the identification of further ligandable sites that can be pharmacologically interrogated with small molecules.

3. CHEMOPROTEOMICS-ENABLED COVALENT LIGAND SCREENING TO TACKLE THE UNDRUGGABLE PROTEOME

To access the potential ligandable sites identified with reactivity-based probes and ABPP, covalent ligand screening approaches that are enabled by chemoproteomic approaches have arisen as a powerful strategy for ligand discovery against undruggable proteins and to enhance the scope of induced-proximity-based therapeutic modalities. To profile covalently acting small molecules, the compound of interest can be competed against probe binding to facilitate target identification directly in complex living systems using quantitative ABPP chemoproteomic approaches, including competitive isoTOP-ABPP and other derivatives of this approach (Figure 2b).[12,14,16,22,23,34−36] The original isoTOP-ABPP method consists of treating animals in vivo, cells in situ, or proteomes in vitro with vehicle or covalent ligand, followed by in vitro labeling of the resulting proteomes or in situ labeling of cells or animals with a reactivity-based probe, wherein sites that are bound by the covalent ligand are not accessible for reactivity-based probe binding compared with vehicle-treated controls. Probe-modified proteins are subsequently appended with a cleavable and isotopically encoded biotin−azide tag by CuAAC, after which control and treated proteomes are combined in a 1:1 ratio for subsequent avidin enrichment, tryptic digestion of enriched probe-modified proteins, and elution of probe-modified tryptic peptides for mass-spectrometry-based analysis of isotopic peptide ratios to map the proteome-wide sites targeted by the covalent ligands. Various derivatives of this strategy have been developed, including noncleavable, acid-cleavable, or photocleavable isotopically encoded biotin or desthiobiotin tags or incorporation of quantitative mass tags at the final eluted peptide stage using tandem mass tagging (TMT) for downstream quantitative proteomic analysis.[15,18−20,37] Extensions of this ABPP platform for target identification of covalent ligands have also been developed by the Marto group with their covalent inhibitor site identification (CITe-Id) approach that enables site-specific identification of covalent ligands through special chromatography conditions and monitoring of specific gas-phase fragmentation pathways for peptides covalently modified by irreversible inhibitors.[38]

Collectively, the aforementioned quantitative chemoproteomic approaches can be used for a variety of applications, including (1) generating ligandability maps of covalent ligand−target pairs directly in living cells toward generating a small-molecule ligand for every protein in the proteome; (2) determining the proteome-wide selectivity and targets of covalent ligands to enable subsequent medicinal chemistry efforts to generate more selective lead compounds and to help derisk covalent compounds; and (3) identifying ligandable hotspots and proteins targeted by hit covalent ligands arising from phenotypic or biochemical covalent ligand screens to understand mechanisms of action. Target-based screens can also be performed to identify covalent ligands against targets of interest using moderate- to high-throughput screening (HTS) methods, including fluorescence polarization- and gel-based ABPP approaches wherein covalent ligands are competed against the binding of a fluorophore-conjugated reactivity-based probe to recombinant protein (Figure 2c).[22,39,40] Alternatively, HTS using rapid injection and intact mass spectrometry screening for covalent mass adducts on recombinant proteins is another versatile approach to covalent ligand discovery against pure protein targets of interest (Figure 2d).[41]

Extensive work across the chemoproteomics field has showcased the potential of pairing cysteine-reactive covalent ligand screening with chemoproteomics for rapid discovery of new ligandable hotspot and ligand pairs. Notably, a 2016 paper by Backus et al. used isoTOP-ABPP to profile the proteome-wide cysteine reactivity of cysteine-reactive fragments and found >700 cysteines that could be targeted by these covalent ligands, including across many targets considered undruggable.[42] Using the same type of approach, Vinogradova et al. mapped the interactions of small-molecule electrophiles with cysteines in human T cells.[43] Using chemoproteomic methods, this study enabled the exploration of changes in cysteine reactivity upon T cell activation, deployed promiscuous scout ligands to broadly profile reactivity, and identified electrophilic T cell modulators that showed the promise of this approach to yield novel therapeutics.[43] Recently Kuljanin et al. used a streamlined TMT-based multiplexed quantitative ABPP approach to rapidly profile 285 cysteine-reactive electrophiles against >8000 cysteines to develop a ligandability map of covalent ligands against proteome-wide cysteine hotspots.[15]

Beyond successes of ABPP-based chemoproteomic approaches in ligand discovery against cysteine hotspots, considerable progress has also been made in targeting lysine-reactive hotspots as well.[14] Hacker et al. identified an amine-reactive pentynoic acid sulfotetrafluorophenyl ester probe that was successful in covalently modifying over 9000 lysine residues in the human proteome.[14] This led to the discovery of functional lysines that could be targeted by covalent ligands, thus expanding the scope of the ligandable proteome. A separate effort from Ward et al. instead used a commercially available NHS-ester probe to profile lysine as the primary target of the probe.[13] By the use of NHS-ester-based fragments, selectivity could be conferred toward specific nucleophilic sites of interest, showing the possibility of using this scaffold in lysine targeting. Brulet et al. showcased how chemoproteomic profiling of fragment libraries based on SuTEx chemistry could enable covalent targeting of specific tyrosine sites on proteins.[44] The authors identified a large fraction of tyrosine sites (~30%) on proteins that could be liganded across >1500 probe-modified

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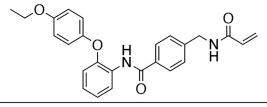
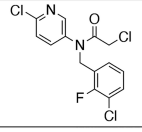
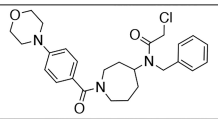
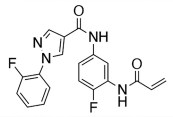
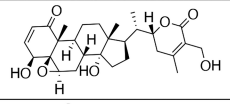
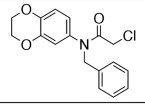
Table 2. Examples of Covalent Ligand−Protein Pairs Discovered through Chemoproteomic Methods

Compound Name	Structure	Protein Target
(a) EN4		c-Myc (C171)
(b) BPK-26		NR0B1 (C274)
(c) BPK-29		
(d) EN6		ATP6V1A (C277)
(e) Withaferin A		PPP2R1A (C277)
(f) JNS 1-40		

sites quantified by chemoproteomics. They further demonstrated selective and functional targeting of specific tyrosines on proteins such as DPP3 and GSTP1.[44]

Chemoproteomics-enabled covalent ligand discovery platforms have also been successfully used in both target-based and phenotypic screening paradigms to rapidly discover covalent ligand−target pairs against classically undruggable or novel protein targets. While we cannot review all of the success stories here, we highlight a few examples below. Boike, Cioffi, and co-workers performed a chemoproteomics-enabled target-based and cellular screen with cysteine-reactive covalent ligand libraries to discover EN4 (Table 2a), which selectively targeted an intrinsically disordered ligandable cysteine, C171, against the intractable oncogenic transcription factor c-MYC, leading to inhibition of MYC/MAX binding to its E-box consensus DNA sequence, inhibition of MYC transcriptional activity, and impaired cancer pathogenicity.[45]

Bar-Peled et al. used isoTOP-ABPP to profile ligandable cysteines within proteins that are selectively expressed in KEAP1 mutant nonsmall cell lung cancer cells, wherein they identified NR0B1 as a nuclear receptor that engages in a protein complex to regulate the transcriptional output of KEAP1 mutant lung cancer cells.[46] The authors then performed a target-based covalent ligand screen against a ligandable C274 within an NR0B1 protein interaction domain and identified compounds BPK-26 and BPK-29 (Table 2b,c) that disrupted the NR0B1 complex to impair the anchorage-independent growth of KEAP1 mutant cancer cells.

In a rational structure-guided approach using a peptide substrate, the Gray lab developed a covalent inhibitor against an active-site C113 of the peptidyl−prolyl cis/trans isomerase NIMA-interacting 1 (Pin 1), which is commonly overexpressed in human cancers. Using a biotin-derivatized analogue of their compound and CITe-Id, the authors demonstrated the proteome-wide selectivity of their compound in cells.[47] In another example, Resnick et al. used high-throughput intact mass spectrometry screening using covalent binding as a readout to rapidly identify covalent ligand hits against seven targets using a cysteine-reactive acrylamide and chloroacetamide fragment library.[41]

There have also been many studies showcasing the utility of ABPP-based chemoproteomic platforms in identifying targets and mechanisms of covalent ligands that arise from phenotypic screens. In work by Chung et al., cellular screening of a cysteine- and lysine-reactive covalent ligand library for activation of autophagy yielded a cysteine-reactive acrylamide hit, EN6 (Table 2d), that was found to selectively target a unique ligandable cysteine C277 in the catalytic subunit of the lysosomal v-ATPase, ATP6 V1A, leading to simultaneous inhibition of mTORC1 recruitment to the lysosome as well as activation of the v-ATPase, induction of autophagy, and improved cellular clearance of toxic TDP-43 protein aggregates.[23]

Chemoproteomics has also been useful in investigating the mode of action of bioactive and covalently acting natural products and exploiting natural product mechanisms with fully synthetic compounds. Using isoTOP-ABPP, Grossman, Ward, and co-workers discovered that the anticancer natural product withaferin A (Table 2e) covalently modified C277 at the protein interaction interface of the regulatory subunit of the tumor suppressor protein phosphatase 2A (PP2A) complex PPP2R1A, leading to activation of PP2A activity and impaired cancer cell pathogenicity.[22] Uncovering this ligandable site targeted by this natural product enabled a target-directed covalent ligand screen leading to the potent, selective, and in vivo-active fully synthetic cysteine-reactive covalent ligand JNS 1-40 (Table 2f) that mimicked the action of withaferin A in targeting C277 of PPP2R1A to activate PP2A activity and impair breast cancer cell proliferation and tumorigenesis.[22]

Beyond covalent ligand interactions with amino acid hotspots, chemoproteomic profiling using fully functionalized fragments, consisting of a variable small-molecule reversibly acting

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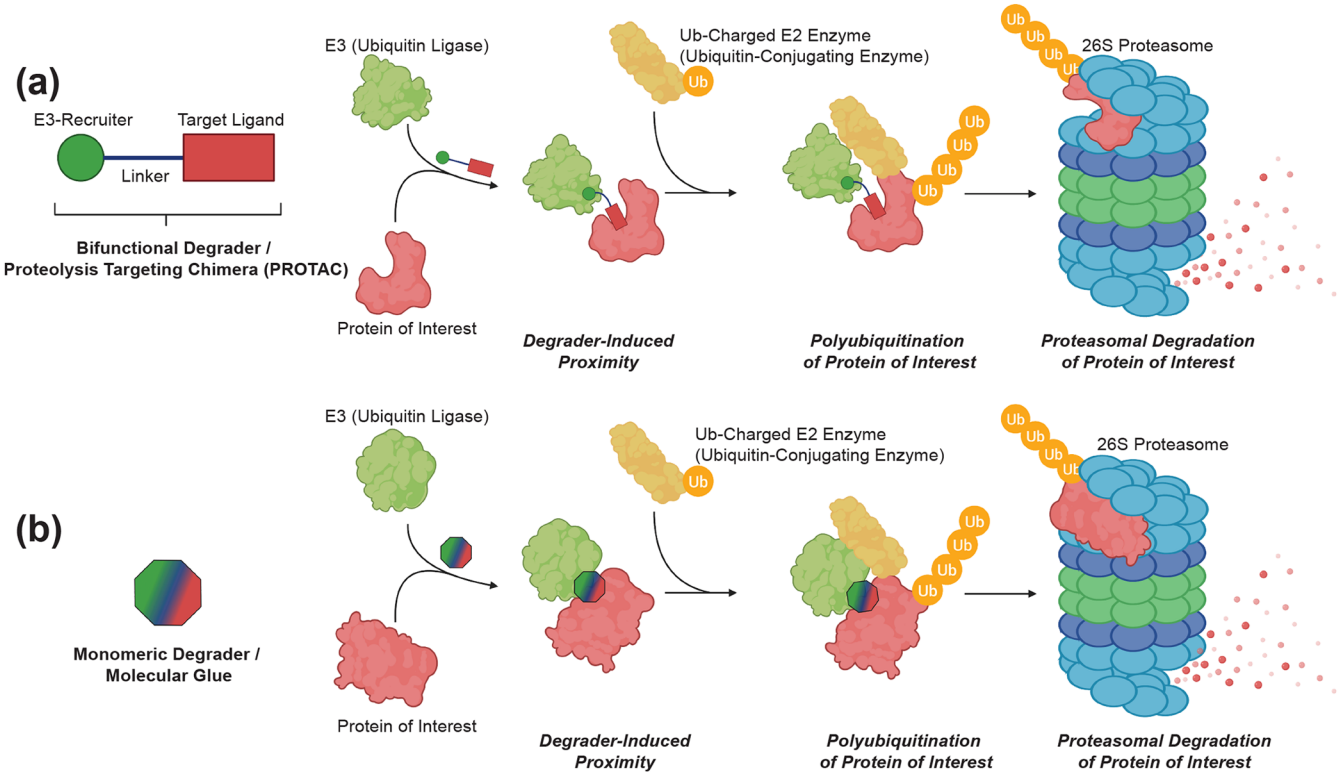

Figure 3. Targeted protein degradation (TPD) strategies. TPD hijacks endogenous protein degradation pathways for non-native protein substrates. (a) Heterobifunctional protein degraders or PROTACs consist of an E3 ligase recruiter linked to a protein-targeting ligand. (b) Molecular glue degraders are monovalent molecules that induce the proximity of an E3 ligase with a neo-substrate protein.

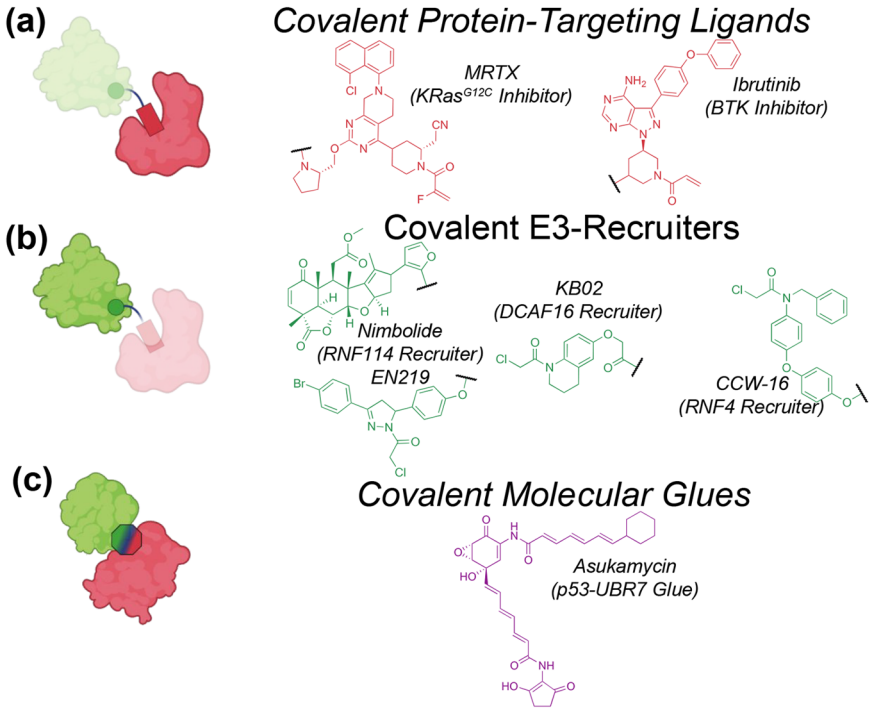

Figure 4. Chemoproteomic contributions to TDP. (a) Covalent protein-targeting ligands in PROTACs. (b) Covalent E3 ligase recruiters for TPD applications. (c) Covalent molecular glues discovered using chemoproteomics.

fragment conjugated to a constant tag bearing an alkyne and photoactivatable diazirine handle, has also arisen as a powerful strategy for mapping the ligandable proteome beyond electrophilic fragment and nucleophilic amino acid hotspot interactions. Parker et al. performed competitive chemoproteomic profiling of a library of fragments against a library of fully

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functionalized fragments to identify thousands of reversible small-molecule−protein interactions directly in human cells wherein they were able to advance selective and functional ligands against proteins such as PTGR2 and SLC25A20 for which there were no prior ligands.[48] Wang et al. expanded on this initial work with next-generation fully functionalized enantiomeric probe pairs that enabled the identification of stereoselective protein−fragment interactions in cells.[49] Grant et al. also employed a fully functionalized fragment screen to covalently capture fragment−protein interactions using recombinant protein against targets such as myoglobin, lysozyme, KRas4B(G12D), BRD4-BD1, and BCL6 and identified fragment ligands against each of these targets.[50]

4. CHEMOPROTEOMICS TO ENABLE SMALL-MOLECULE INDUCED-PROXIMITY PARADIGMS

4.1. Targeted Protein Degradation

Targeted protein degradation (TPD) is a powerful therapeutic paradigm that has arisen to tackle the undruggable proteome. TPD uses small molecules to induce artificial proximity of an E3 ubiquitin ligase or other adapter proteins that mediate protein degradation with a neo-substrate protein to induce the ubiquitination and degradation of specific target proteins (Figure 3).[24] These degrader molecules can take the form of heterobifunctional degraders or PROTACs, in which an E3 ligase-recruiting ligand is linked to a protein-targeting ligand (Figure 3a), or can be molecular glue degraders, which are linker-free monomeric compounds that induce the proximity of an E3 ligase to a neo-substrate (Figure 3b). TPD is an attractive approach to tackle the undruggable proteome, since a protein degrader (1) does not necessitate an inhibitor against a protein, but a mere protein binder, to facilitate the development of a PROTAC; (2) eliminates not only the catalytic function of a protein but also any associated scaffolding function; and (3) induces the catalytic degradation of its targets.[24] Despite the tremendous promise of TPD approaches, major bottlenecks still exist, including (1) ligand discovery against undruggable disease-causing proteins and (2) a dearth of available E3 ligase recruiters that can be exploited for TPD applications, as there are recruiters for only nine E3 ligases, with most of the published degraders using ligands for VHL and cereblon, despite the existence of >600 E3 ligases in the human genome.[51] Chemoproteomic approaches can overcome both of these challenges (Figure 4).

Chemoproteomic approaches using covalent ligand libraries or fully functionalized fragment libraries are powerful strategies for ligand discovery against intractable disease targets and complement other approaches such as DNA-encoded libraries. While covalent targeting of the protein of interest will likely prevent the catalytic degradation of targets, covalent PROTACs still enable degradation of their target proteins in cells, as has been shown with degraders against the kinases BTK and BLK (Figure 4a).[52,53] Covalent warheads on protein-targeting ligands can be converted into covalent reversible warheads to restore the catalytic degradation properties of PROTACs, as has been demonstrated with covalent versus covalent reversible BTK degraders.[53]

Chemoproteomics-enabled covalent ligand discovery approaches have proven to be powerful in expanding the arsenal of novel E3 ligase recruiters. The use of covalent E3 recruiters for TPD applications is particularly attractive because these degraders still possess catalytic degradation properties since the covalently modified E3 ligase can be recovered to engage and degrade another target protein. Having a diversity of E3 ligase recruiters will undoubtedly expand the types of targets that can be degraded and improve the selectivity of degradation. The importance of E3 ligase selection was underscored by cumulative work demonstrating that only VHL and not CRBN was capable of degrading endogenous, untagged $KRas^{G12C}$.[54,55] A recent massive profiling effort to assess the effect of kinase inhibitors, linkers, and E3 ligase recruiters in mapping the scope of the degradable kinome also demonstrated the importance of recruiter choices in degrading specific kinases.[56] Chemoproteomics approaches have led to the recent discovery of four covalent recruiters against three E3 ligases: RNF114, RNF4, and DCAF16 (Figure 4b).

Ward et al. performed a target-based ABPP screen against RNF4 by competing a cysteine-reactive covalent ligand library against the binding of a cysteine-reactive fluorescent probe to recombinant RNF4.[57] The authors discovered CCW16, which nonfunctionally and covalently targeted zinc-coordinating cysteines in RNF4, as a novel RNF4 recruiter for TPD applications, wherein the authors demonstrated RNF4-dependent degradation of BRD4 with a degrader linking CCW16 to the BRD4 inhibitor JQ1.[57]

In a second example, in deciphering the targets and mechanism of action of the covalently acting anticancer natural product nimbolide using isoTOP-ABPP, Spradlin et al. discovered that nimbolide selectively targeted intrinsically disordered cysteine C8 on the E3 ligase RNF114 at a substrate recognition site and that nimbolide could be used as a novel RNF114 recruiter for PROTAC applications.[58] The authors demonstrated that linking nimbolide to JQ1 led to the selective degradation of BRD4 in a proteasome- and RNF114-dependent manner.[58] Subsequent studies showed the broader utility of nimbolide-based degraders. Tong, Spradlin, and co-workers demonstrated that nimbolide linked to the BCR-ABL kinase inhibitor dasatinib led to selective degradation of the fusion oncogene BCR-ABL over c-ABL, whereas the opposite selectivity was observed with CRBN and VHL recruiters.[59] Luo, Spradlin, and co-workers subsequently performed a target-based ABPP screen with a cysteine-reactive covalent ligand library to discover a fully synthetic and more synthetically tractable RNF114 covalent ligand, EN219, that mimicked the action of nimbolide and could be similarly used in TPD applications, wherein degraders linking EN219 to JQ1 showed selective degradation of BRD4 in an RNF114-dependent manner[60] (Figure 4b).

In another chemoproteomics-enabled approach to discover a covalent E3 recruiter, Zhang et al. demonstrated the use of covalent scout fragments in bifunctional degraders to screen for degraders of FKBP12.[61] After ligands that enabled degradation were identified, chemoproteomics enabled deconvolution of the engaged E3. The authors found that their degrader worked through recruitment of the Cullin E3 ligase DCAF16.[61]

Taken together, these papers demonstrate the success of covalent recruiter development using chemoproteomic platforms for TPD applications.

4.2. Chemoproteomics Enables Molecular Glue Discovery

Beyond enabling heterobifunctional PROTACs, chemoproteomic platforms can also facilitate the discovery of new molecular glues—monomeric small molecules that induce the proximity of a protein with a neo-substrate protein through the formation of a

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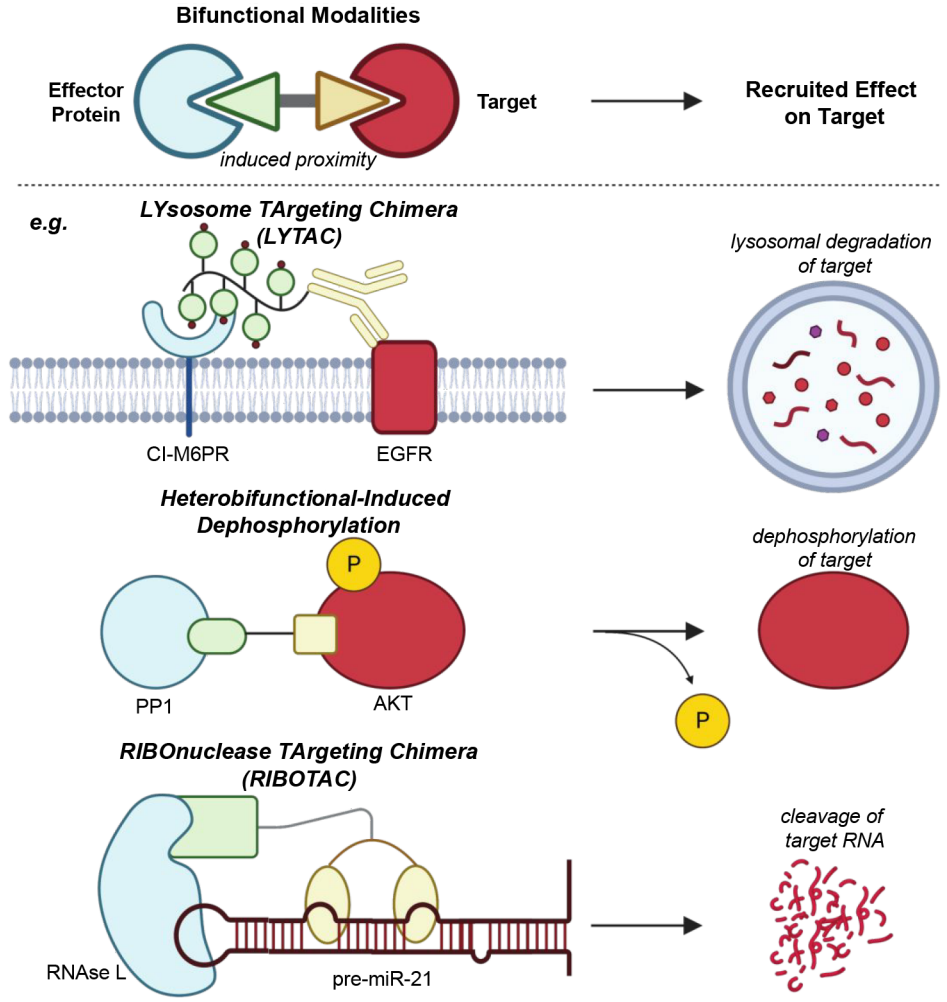

Figure 5. Bifunctional induced-proximity modalities. Examples of the use of heterobifunctional molecules to modulate protein functions beyond ubiquitination are shown.

ternary complex, leading to a neomorphic function.[62] Most molecular glues have been discovered fortuitously, including rapamycin, which binds to FKBP12, leading to the recruitment and partial inhibition of mTORC1; the cotylenin diterpenes, which form interfaces between 14-3-3 protein families and various proteins; and thalidomide and other immunomodulatory imide drug (IMiD) analogues that bind to the E3 ligase cereblon to recruit neo-substrates such as SALL4 or Ikaros to ubiquitinate and degrade these targets.[63] Chemoproteomics has also enabled the discovery of new molecular glue scaffolds. In a recent study, Isobe et al. used chemoproteomics to discover that the manumycin polyketide natural product asukamycin, which bears multiple electrophilic sites, selectively targeted C374 on UBR7 in cancer cells and formed a multicovalent ternary complex with the tumor suppressor TP53, leading to thermal stabilization of TP53, increased TP53 DNA binding, activation of TP53 transcriptional activity, and cell death (Figure 4c).[64]

Given the sporadic and fortuitous discovery of molecular glues to date, there has been a rise of interest in rational and targeted molecular glue discovery. Mayor-Ruiz et al. recently demonstrated that phenotypic screening for cytotoxic compounds paired with a counterscreen in hyponeddylated cells to identify neddylation-dependent toxicity can uncover cullin-dependent molecular glues.[65] This type of strategic phenotypic screen could also be performed with covalent ligand libraries to identify novel molecular glues and subsequently coupled with chemoproteomic and quantitative proteomic approaches to rapidly deconvolute the molecular glue ternary complex partners.

4.3. Novel Induced Proximity-Based Therapeutic Modalities

The potential of heterobifunctional therapies is not limited to TPD.[66] The use of heterobifunctional molecules has been expanded to targeted lysosomal degradation (LYTACs), targeted autophagy (AUTACs), targeted dephosphorylation using phosphatase recruiters, targeted protein phosphorylation with kinase recruiters (PHICS), and redirection of ribonucleases to destroy malignant RNAs, among others (Figure 5).[67−69] These emerging induced-proximity-based therapeutic modalities are just the tip of the iceberg of what is possible. A critical step for enabling the next wave of induced-proximity paradigms using heterobifunctional compounds will be the discovery of recruiters for various protein modules that can be recruited to target proteins to enable targeted and precise modulation of protein functions. Given the potential power of chemoproteomic strategies to rapidly uncover unique ligandable sites and corresponding ligands, chemoproteomic approaches will likely be critical players, alongside DNA-encoded libraries, small-molecule microarrays, and other screening platforms, to

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enable recruiter discovery against any protein of interest to enable next-generation induced-proximity paradigms.

5. CONCLUSION

Overcoming the challenge of the undruggable proteome necessitates the development and advancement of innovative technologies for ligand discovery and the development of novel therapeutic modalities to functionally manipulate the undruggable proteome for therapeutic benefit. Chemoproteomic approaches have arisen as a powerful strategy for both ligand discovery and expanding the scope of small-molecule induced-proximity paradigms. Chemoproteomic approaches have also revealed unique insights into ligandability, such as the discovery of unique allosteric sites or intrinsically disordered regions of proteins that can be pharmacologically and selectively targeted for biological modulation and therapeutic benefit.

The future of chemoproteomic platforms in drug discovery will be further enabled by expansion of (1) new reactivity-based probes for more amino acids, amino acid modifications, and even DNA/RNA space; (2) better and more sophisticated covalent ligand and fully functionalized fragment libraries; (3) better photoaffinity labeling approaches for advancing fully functionalized fragment screening approaches; and (4) advancements in peptide extraction, separation, mass-spectrometry-based analysis, and bioinformatic approaches. Looking into the future, chemoproteomic approaches will unquestionably have major impact in (1) further expansion of existing efforts toward proteome-wide ligandability mapping; (2) targeted ligand discovery efforts against high-value undruggable therapeutic targets; (3) expansion of the scope of targeted protein degradation; (4) the discovery of new molecular glue scaffolds that enable unique modulation of protein function; and perhaps most excitingly (5) the development of next-generation small-molecule induced-proximity-based therapeutic modalities that go beyond degradation. Exciting days are ahead in this field as chemical biology becomes an increasingly major driver in drug discovery, and chemoproteomic approaches are sure to be a mainstay in developing next-generation therapeutics.

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Biographies

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Erika Zhang is currently an undergraduate student at the University of California, Berkeley, majoring in Chemistry. She has been a research assistant under the guidance of Professor Daniel Nomura. Her research interests include using chemical biology approaches to enable drug discovery.

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