



Cite this: *Org. Biomol. Chem.*, 2025, **23**, 10262

Received 30th May 2025,  
Accepted 21st October 2025

DOI: 10.1039/d5ob00905g  
rsc.li/obc

## A perspective on cysteine-reactive activity-based probes

Constantin M. Nuber,<sup>a</sup> Max A. Schwab<sup>b,c</sup> and David B. Konrad  <sup>\*a,b,c</sup>

Activity-based protein profiling (ABPP) is a powerful method to investigate protein function, identify protein targets and determine the mechanisms-of-action of chemical probes as well as small molecule drugs. Our perspective provides an overview of the design of covalent cysteine-targeted activity-based probes (ABPs) that rely on reactive elements ('chemotypes' or 'warheads') as well as functional handles and describes their use in mass spectrometry-based chemoproteomics analysis. These segments are followed by strategies for the development of advanced ABPs that are employed in live cells for chemoproteomics analyses and to install post-translational modifications. Our discussion extends to their potential, limitations and opportunities, followed by perspectives on advancing the reach of ABPP and its implications for the pharmaceutical industry.

### Introduction

#### Activity-based protein profiling (ABPP)

In the ongoing quest to identify highly specific drugs that target disease-related isoforms and post-translational states of proteins, advanced technologies are developed as key drivers to gain an in-depth understanding of disease mechanisms. In this context, understanding protein localization and function is an essential foundation to enable combating many human illnesses including cancer.<sup>1</sup> While proteomic methods such as shotgun liquid chromatography (LC)-mass spectrometry (MS) platforms, protein microarrays and yeast two-hybrid screenings have amplified the understanding of *in vitro* protein functions, interactions and expression patterns, they are still limited in addressing proteins in their diverse, native states.<sup>2–6</sup> Due to the pioneering development of the chemoproteomic technology ABPP (Fig. 1), enzyme functions derived from chemical reactivity can be analyzed within complex biological systems.<sup>7,8</sup> By using covalently binding small molecules, referred to as activity-based probes (ABPs), it is possible to determine enzyme activity, identify ligandable sites on proteins and uncover the mechanism-of-action of drugs.<sup>9</sup> These probes have been developed to target a fraction of the proteome, ranging from a few to thousands of proteins with shared reactivity and/or catalytic features, which, depending

on their properties, can be employed in cell lysates or in the native proteome within live cells.<sup>10</sup> To analyze the interactions between ABPs and proteins, the most commonly used methods include gel-based visualization techniques like sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and MS.

#### Cysteine as a target of ABPs

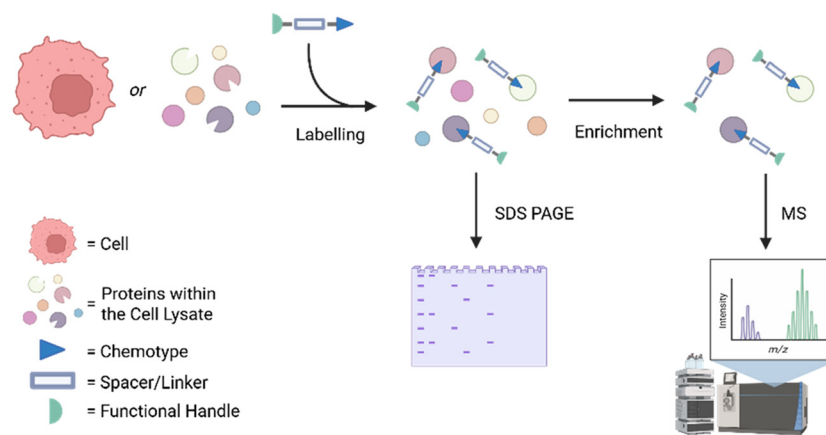
A large fraction of the available ABPs engage cysteine residues. Cysteine is unique among the 20 proteogenic amino acids due to its elevated nucleophilicity and redox activity, which form the foundation for its highly conserved tasks within several classes of proteins.<sup>11,12</sup> The high nucleophilicity of the thiol side chain stems from the large radius of the sulfur atom, which enables a better stabilization of negative charges compared to, for instance, the smaller oxygen. This feature imparts cysteine with a  $pK_A \leq 8.2$ , which is influenced by the surrounding protein microenvironment.<sup>13–15</sup> Characteristics that can enhance the ionization of thiols include proximal positively charged amino acids, hydrogen bonding and a localization at the N-terminal end of an  $\alpha$ -helix.<sup>16</sup> Cysteines with a low  $pK_A$  that facilitates their ionization to the highly nucleophilic thiolate anion at physiological pH are referred to as "hyper-reactive".<sup>13,17</sup> Although cysteine has a low overall abundance in the proteome, it is present in 97% of all human proteins which, combined with its high nucleophilicity, makes it an ideal target for certain electrophiles and thus the development of covalent chemical probes.<sup>18</sup> Depending on its location within a protein, the function of a cysteine can range from a nucleophile in enzyme active sites to stabilizing secondary/tertiary protein structures by forming disulfide bonds or having a regulatory function as a redox-active anchor for post-translational modifications (PTMs). As regulatory

<sup>a</sup>Department of Pharmacy, Ludwig-Maximilians-Universität München, Butenandtstr. 5-13, 81377 Munich, Germany. E-mail: david.konrad@cup.lmu.de

<sup>b</sup>Department of Pharmaceutical Sciences, Universität Wien, Josef-Holaubek-Platz 2, 1090 Vienna, Austria. E-mail: david.benjamin.konrad@univie.ac.at

<sup>c</sup>Vienna Doctoral School of Pharmaceutical, Nutritional and Sport Sciences (PhaNuSpo), Universität Wien, Josef-Holaubek-Platz 2, 1090 Vienna, Austria





**Fig. 1** Schematic depiction of a classical ABPP workflow: First, cells or cell lysates are treated with an ABP. Next, the identification of the proteins is pursued through e.g. in-gel fluorescence using SDS-PAGE or enrichment of the probe-labelled proteins using an appropriate tag followed by MS analysis.

sites, they are involved in relaying signals into biological responses through their site-specific chemical modification.<sup>19</sup> The thiol (SH) residue on cysteine works as e.g. a sensor for redox signaling processes due to the ability of the sulfur atom to assume a wide variety of oxidation states.<sup>16</sup> These oxidative PTMs (oxPTMs) include sulfenic acid (–SOH), sulfinic acid (–SO<sub>2</sub>H), sulfenyl amide (–SNH<sub>2</sub>), S-nitrosothiol (–SNO) and disulfide (–S–S–). The large number of redox-active proteins and the wide array of modifications which function as modulators in the interaction profiles, subcellular localization and signaling events highlight the importance of cysteine as a regulatory site and the impact of oxPTMs, which are comparable in their reach to phosphorylation.<sup>16</sup> Uncovering the functions of a specific cysteine residue within the proteome, however, remains a challenge with conventional methods. Harnessing ABPP in combination with tailored ABPs mitigates this challenge by allowing investigating e.g. the reactivity of a cysteine,<sup>17</sup> its potential as an anchor for covalent small molecules<sup>20</sup> and its ability to host oxPTMs.<sup>21</sup>

## Activity-based probes

### Activity-based probe design

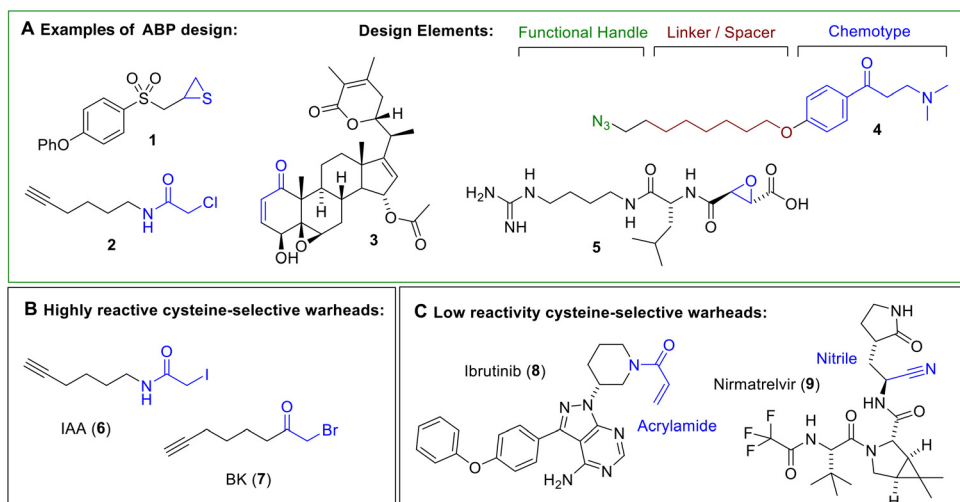
In a typical ABPP experiment, cells or cell lysates are incubated with an ABP which covalently labels a certain amino acid on specific proteins, such as a subset of enzymes (Fig. 1).<sup>22</sup> Classical ABPs contain three elements: (1) a reactive group that covalently binds to the target protein(s), a so-called chemotype or warhead; (2) a spacer/linker or ligand structure. The latter is used to non-covalently guide the ABP to specific active sites or binding pockets; and (3) a functional handle suitable for e.g. bioorthogonal modification, visualization or enrichment of the labelled proteins.<sup>8</sup> Several analytical platforms can be employed by using suitable tags, typically involving fluorophore handles in combination with SDS-PAGE or (desthio)biotin for protein enrichment coupled to MS methods. Thereby, a global view of the interaction of the ABP with the proteome is obtained.

### Warhead reactivity

Since the inception of the concept in 1999, numerous different probes for ABPP experiments were developed.<sup>23</sup> In general, the ABPs are designed to follow classical medicinal chemistry requirements related to size, solubility in aqueous systems and stability in a native protein environment.<sup>24</sup> The chemotype, however, plays the leading role to ensure an effective modification of the desired protein subset. By optimizing the specific reactivity of warheads, the coverage can be enhanced and cross-reactivities minimized. Using probes which are too reactive can lead to the labelling of undesired residues which lie outside the scope of the envisioned application.<sup>25,26</sup> Consequently, if the chemotype reactivity is too low, a fraction of the desired protein sites may not be engaged which decreases the coverage of the ABP. Commonly used chemical probes are based on reactive groups that are found in e.g. mechanism-based inhibitors,<sup>27,28</sup> natural products,<sup>29,30</sup> classical electrophiles<sup>31–33</sup> and prodrugs<sup>34</sup> (for selected examples see 1–5, Fig. 2A).

ABPs, in general, can be divided into non-directed and directed variants.<sup>35</sup> The latter are either directed by a ligand structure<sup>20</sup> or a specific reactivity of a chemotype, which can be subdivided into the following: (1) a chemotype that targets a mechanistically related family of enzymes. Here, in-depth knowledge about the structure, mechanism and affinity of the proteins is essential.<sup>39</sup> These chemotype-directed ABPs are based on a warhead that engages the well-defined reactivity of an enzyme active site, such as the labelling of serine hydrolases with fluoro-phosphonates;<sup>36</sup> (2) a chemotype that is highly reactive but selectively targets a specific amino acid side chain such as iodoacetamide alkyne (IAA, **6**)<sup>17</sup> and bromomethyl ketone (BK, **7**)<sup>37</sup> (Fig. 2B);<sup>38</sup> and (3) ligand-directed ABPs such as covalent inhibitors, that harbor a low-reactivity chemotype which requires the ligand structure to bind to a proximal protein binding pocket to engage the target amino acid.<sup>39</sup> Ibrutinib (**8**),<sup>40</sup> for instance, hosts an acrylamide while nirmatrelvir<sup>41</sup> (**9**) hosts a nitrile (Fig. 2C).<sup>42</sup> In contrast, non-directed ABPs often incorporate





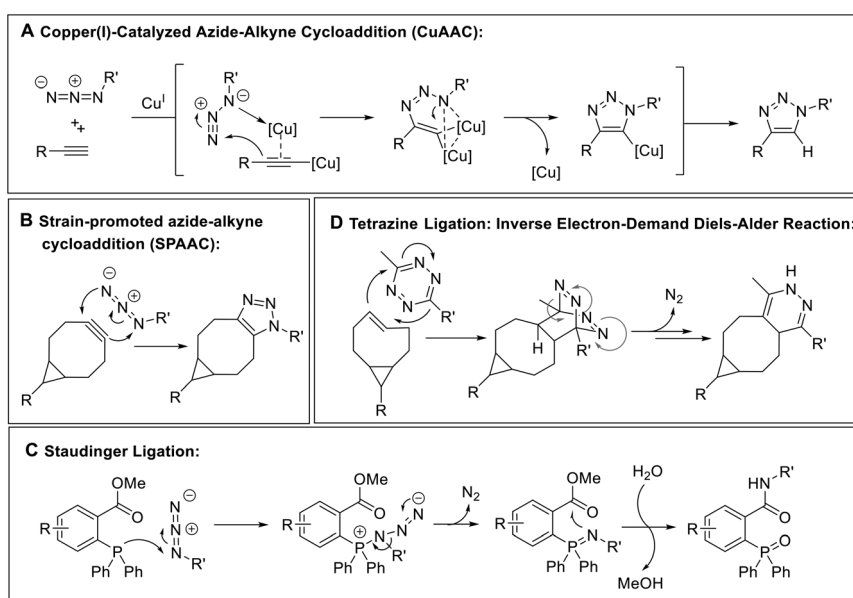
**Fig. 2** (A) Selected examples of covalent chemical probes based on a mechanism-based inhibitor (1), classical electrophile (2), enzyme-activated prodrug (4) and natural products (3 and 5). (B) Examples of high-reactivity warheads that target cysteine. (C) Examples of chemical probes that host low-reactivity warheads targeting cysteine.

higher-reactivity electrophilic, nucleophilic or photoreactive chemotypes which can bind to a broader set of proteins.<sup>43</sup> Their design requires no knowledge about the protein, which is often used to extend ABPP methods.<sup>44</sup>

### Functional handles and bioorthogonal labelling

In addition to the chemotype, the functional handle is subject to consistent optimizations. To avoid non-specific labelling due to the size and structure of a fluorophore or enrichment tag and thereby ensure broad applicability of the probe, most ABPs contain a bioorthogonally reacting functional group as a ‘click-

able’ handle. Using alkyne or azide residues allows the specific coupling with a complementary reaction partner *via* a copper(i)-catalyzed azide–alkyne [3 + 2] cycloaddition (CuAAC) (Fig. 3A) to install the desired tag on the probe-labelled protein.<sup>27</sup> Recent studies have uncovered that the amount of the reducing agent tris(2-carboxyethyl)phosphine (TCEP) used in the CuAAC reaction is important to avoid a three-component copper(i)-catalyzed azide–alkyne–thiol background reaction that decreases the coverage in chemical proteomics studies.<sup>28</sup> Copper-free variants were developed to avoid the toxicity of metals in living cells, such as the strain-promoted azide–alkyne [3 + 2] cycloaddition (SPAAC) (Fig. 3B).<sup>29,30</sup> Moreover, alternative ‘click’ reactions, such as the



**Fig. 3** Reaction mechanisms for the (A) CuAAC; (B) SPAAC; (C) Staudinger ligation; and (D) tetrazine ligation.



Staudinger ligation (Fig. 3C) or the tetrazine ligation, which is based on an inverse electron-demand Diels–Alder reaction (Fig. 3D), were developed for the bioorthogonal coupling of functional tags.<sup>31–33</sup> Common examples of ‘clickable’ tags are tetramethylrhodamine (TAMRA) azide for a visualization *via* SDS-PAGE<sup>34</sup> or biotin/desthiobiotin (DTB)-based azides for an enrichment in chemoproteomics workflows.<sup>35,36</sup>

## Mass spectrometry-based chemoproteomics analysis

### Bifunctional enrichment tags

The introduction of bioorthogonal chemistry into ABPP experiments<sup>45,46</sup> has provided the opportunity to harness advanced tags with multiple features. One example is bifunctional molecules which harbor a proteolytic cleavage site along with an enrichment handle that are employed for so-called tandem orthogonal proteolysis (TOP)–ABPP experiments.<sup>33,47,48</sup> This method relies on tags that contain a biotin handle for streptavidin pull-down experiments connected *via* a tobacco etch virus (TEV) protease cleavage site to a bioorthogonally reacting azide group, which can be ‘clicked’ onto ABP-labelled proteins. After the enrichment of proteins and a protein digestion with *e.g.* trypsin, the tag can be cleaved using TEV protease to specifically elute the labelled peptides from the streptavidin beads. This approach allows the determination of exact modification sites on the tagged proteins using MS analysis. TOP–ABPP furthermore enables proteome-wide analysis of amino acid specificity of electrophilic compounds and, thereby, uncovering the selectivity of haloacetamides, such as IAA (6, Fig. 2), towards cysteine side chains.<sup>33</sup>

### Isotopically labelled enrichment tags

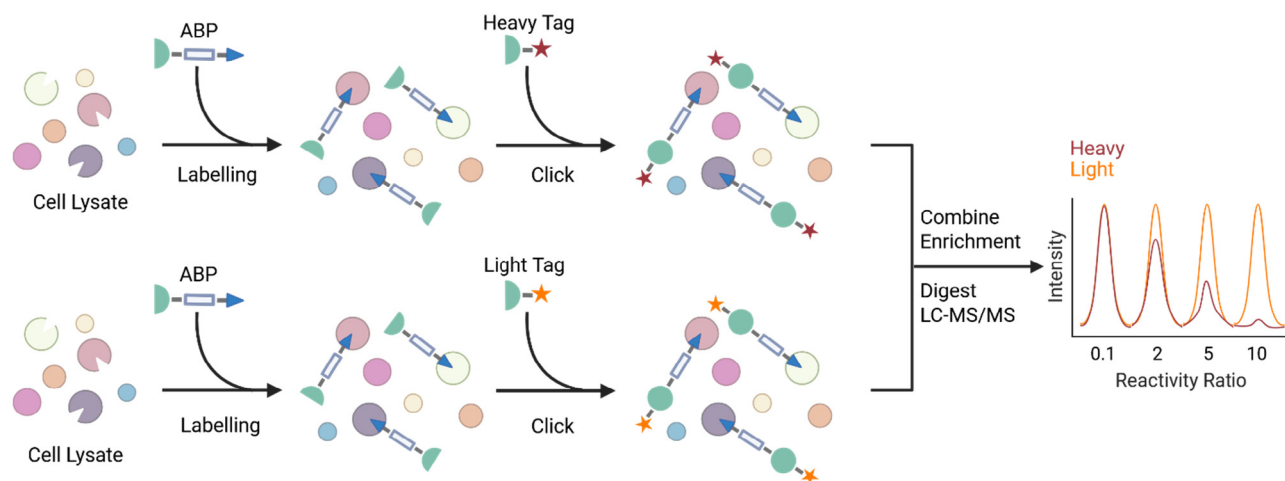
Further developments of the methodology harnessed isotopically labelled enrichment tags. These tags enable the splitting

of a sample proteome into two channels (Fig. 4). The channels can be differentially treated with *e.g.* two concentrations of an ABP (see section ‘‘Reactivity profiling’’), two treatment times or complementary treatment regimens such as a covalent inhibitor along with DMSO as a control (see section ‘‘Ligandability profiling’’). As part of the workflow, the two channels are eventually recombined into one sample after appending the light and heavy tags, respectively, onto the probe-labelled proteins/peptides and analyzed by mass spectrometry (LC-MS/MS). The different masses that stem from the isotopic labels allow determining the fraction of peptides that originate from each channel during MS analysis which enables a direct, quantitative comparison. As such, the isotopically labelled TEV-cleavable enrichment tag (TEV tag, 10) (Fig. 5) enables a multiplexed MS-based analysis referred to as isotopic TOP (isoTOP)–ABPP (Fig. 4).<sup>17,20,49</sup>

This method was improved by using isotopically labelled DTB azide (isoDTB, 11) tags (Fig. 5).<sup>50,51</sup> The isoDTB tags have the advantage that they can be eluted from streptavidin beads *via* specific solvent mixtures and do not require a proteolytic step, which shortens the chemoproteomics workflow. Due to their lower chemical complexity, they are straightforward to synthesize and have recently been commercialized which provides access to a broader spectrum of researchers.

### Reactivity profiling

A combination of the isoTOP–ABPP method and the chemical probe IAA (6) is used to quantitatively determine the reactivity of functional cysteines within the proteome. IAA is one of the most commonly used high-reactivity cysteine-selective ABPs that provides the ability to determine the inherent nucleophilicity of the thiol side chain on target amino acids, which is imparted by the local protein microenvironment. These reactivity profiling experiments are performed by subjecting cell lysates to high (*e.g.*, 100  $\mu\text{M}$ ) and low (*e.g.*, 10  $\mu\text{M}$ ) concentrations of IAA.<sup>17</sup> The



**Fig. 4** Schematic depiction of an isoTOP–ABPP workflow: Labelling of a proteome with two sets of conditions, followed by appending a heavy or light isoDTB tag, respectively. After combination, enrichment, and digestion steps, LC-MS/MS analysis allows the identification of the exact modification sites for the ABP.



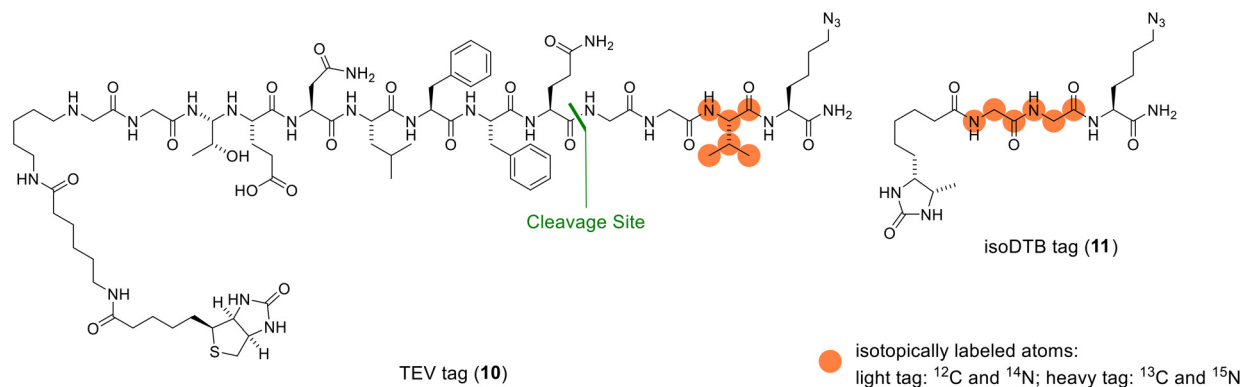


Fig. 5 Chemical structures of isotopically labelled TEV and isoDTB tags.

differently treated proteomes ('channels') are labelled with heavy or light TEV tags, respectively, which allows their combination into one sample and their parallel analysis by LC-MS/MS. A cysteine residue is considered highly reactive ('hyper-reactive') if it is labelled to a similar extent by a low concentration of the probe compared to a high concentration. Profiling the reactivity of cysteines by using IAA aids the deduction of their functions. Cysteines with high reactivity typically correspond to active site nucleophiles, regulatory and redox-active sites as well as binders of metal ligands, whereas low reactivity is associated with *e.g.* structural disulfide-forming residues. It was furthermore demonstrated that the reactivity of cysteines in differently treated proteomes can result from changes in the PTM state of a protein. A comparison between asynchronous HeLa cells and HeLa cells that were synchronized in early mitosis, which is characterized by a high stoichiometry in protein phosphorylation, showed bidirectional changes in cysteine reactivity often occurring in proximity to serine/threonine phosphorylation sites.<sup>52</sup> In this regard, it is reasonable to assume that the reactivity of a cysteine is changed in case (1) it is the site of modification; (2) the PTM is installed in proximity; or (3) a distant modification leads to an altered conformation – all of which affect the cysteine microenvironment (Fig. 6).

### Ligandability profiling

A more advanced variant of ABPP, often used in early drug discovery, includes competition experiments.<sup>53,54</sup> This method, named ligandability profiling, can be used for screening ligandable sites and for the identification of binding pockets of small molecules in the native cellular environment. In a competitive ABPP experiment, cells are pretreated with a covalent small molecule that binds irreversibly to proteins (Fig. 7), which is followed by cell lysis and incubation with a broadly reactive ABP.<sup>55</sup> Consequently, only proteins which were not modified by the small molecule ligand are labelled with the probe. The analytical tag will be exclusively conjugated to the ABP–protein adducts. By comparing two channels, with and without pretreatment, the binding site along with the extent of protein labelling by the covalent small molecule can be determined. Competitive ABPP has been used to map ligandable cysteines and identify novel

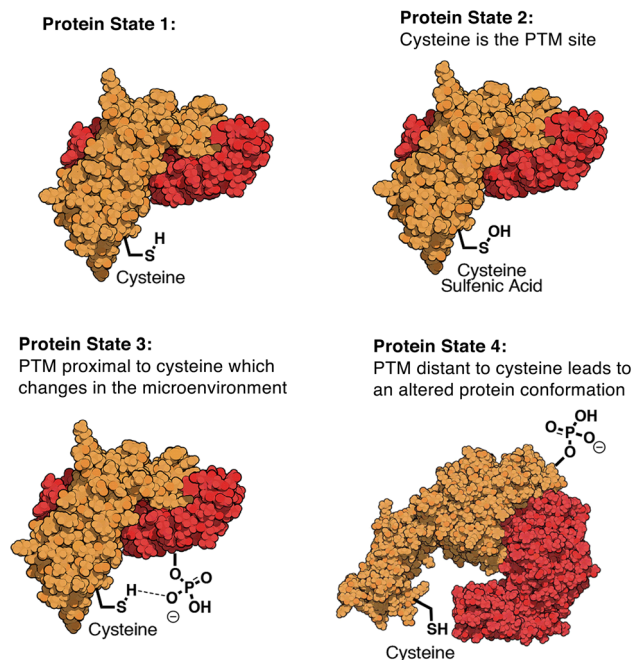


Fig. 6 Schematic depiction of the underlying mechanisms that influence the cysteine reactivity in response to altered PTM states.

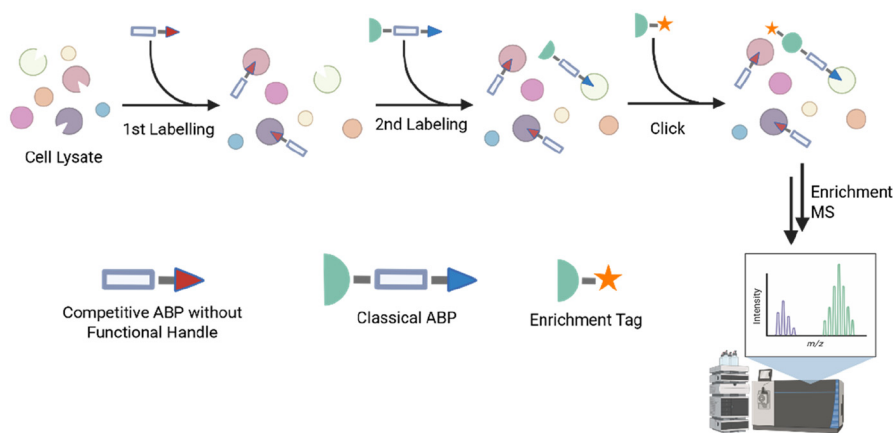
small molecule modulators both in bacteria<sup>50</sup> and in human cells.<sup>56,57</sup> The competitors that were employed in these experiments range from ABPs with highly reactive warheads, such as IAA (6),<sup>20</sup> which direct towards more reactive cysteines, to ABPs with low-reactivity warheads combined with promiscuous ligand structures, such as scout fragments<sup>58</sup> or broadly kinase domain-targeted ligands like XO44.<sup>42</sup>

## Activity-based protein profiling in live cells

### Disadvantages of conventional workflows

As described above, ABPP is a powerful chemoproteomics technology that aids in uncovering the function of specific





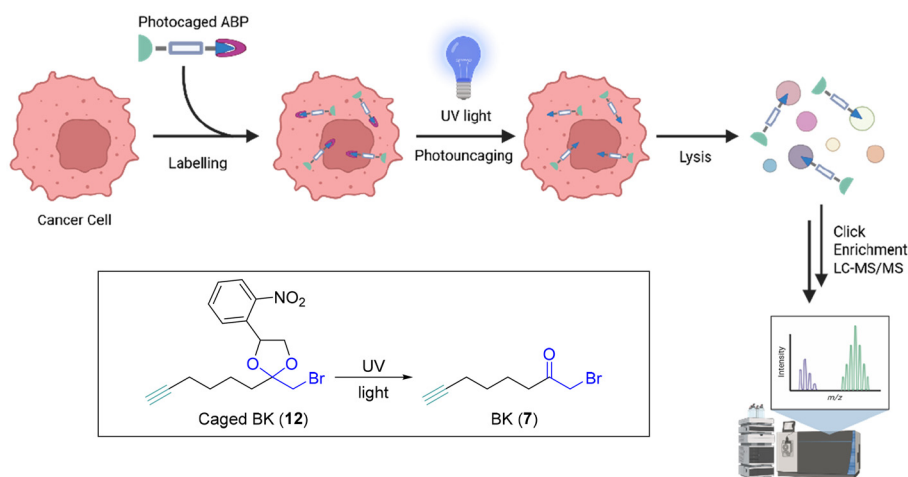
**Fig. 7** Simplified schematic depiction of a competitive ABPP workflow: Labelling with a covalently binding small molecule ligand followed by labelling with a 'clickable' ABP. Identification of the small molecule-labelled sites by a decrease in ABP labelling compared to a control channel.<sup>55</sup>

cysteine residues, drug development and PTM-based protein states. The conventional workflows, however, necessitate incubating cell lysates with ABPs which does not allow retaining the native cellular environment of proteins. This may lead to changes in the protein states and interaction networks which are, for instance, dependent on oxPTMs on cysteine that are installed based on proximal redox signaling pockets and other intracellular microenvironments which perturb the investigated protein activity and reactivity.<sup>59,60</sup> To overcome this issue, it has been demonstrated that IAA-based probes can be used in intact bacteria to assess redox-modified cysteines.<sup>61</sup> However, directly applying reactive IAA (**6**) derivatives to intact cells is not particularly feasible for applications that rely on subtle changes in cysteine reactivity, such as reactivity profiling in *e.g.* human cells, since a concentration gradient of the added ABP is established that spans from the plasma membrane to the intracellular compartments through a gradual permeation of the compound through the cell. This feature

will perturb reactivity measurements in favor of proteins that reside in 'probe hotspots', *i.e.*, microenvironments where the probe can establish its application concentration at a faster pace compared to other intracellular structures during the dilution process. This is compounded by the high toxicity of the probe, which may lead to adapted signaling cascades that originate from probe hotspots and influence the native cellular function during ABP distribution, which hinders recording meaningful changes in protein engagement.<sup>37</sup>

#### Photoactivatable ABPs

To circumvent these difficulties, a photocaged haloketone chemical probe was developed which remains inactive unless it is irradiated with light (Fig. 8).<sup>37,62</sup> This allows incubation of the cells with the compound before activating the cysteine-reactive chemotype through an external stimulus, which prevents the formation of a concentration gradient and facilitates temporal control over covalent binding. Remarkably, a combi-



**Fig. 8** Simplified schematic depiction of an ABPP workflow in living cells with a photoactivatable ABP: Incubation of the cells with non-reactive ABP followed by photocaging of the reactive chemotype.



nation of AlphaFold2-predicted side chain accessibility with a meta-analysis of cysteine profiling datasets described that live-cell labelling with caged- $\alpha$ -bromomethyl ketone (caged BK, **12**) has a bias for exposed residues, while haloketone or haloamide-based probes, such as BK (**7**), in lysates show an increased bias against buried cysteine residues.<sup>63</sup> This data suggests drastic changes in cysteine reactivity attributable to the native labelling in cellular environments. A drawback of the presented approach, however, is the need to use UV light to activate the warhead reactivity, which is toxic to cells and might perturb labelling profiles.

## Harnessing advanced ABPs in live cells: protein-specific installation of PTMs

### Combining genetic engineering with chemical reactivity

To facilitate the precise labelling of specific proteins in live cells with alkynylated analogues of PTMs, the vinyl aldehyde warhead of the covalent chemical probe 4-hydroxynonenal (HNE)-alkyne (**13**) was caged with a light-responsive anthraquinone residue through an ether linkage (Fig. 9A).<sup>64</sup> This photoactivatable residue hosted a terminal alkyl chloride group, a warhead that specifically labels HaloTags. The HaloTag is a self-labelling protein tag that can be linked to a protein-of-interest (POI) through genetic engineering, thereby allowing protein-specific labelling with ABPs. This approach was demonstrated for *e.g.* Keap1 and PTEN. Here, cells are incubated with the chemical probe PreHNE-alkyne (HtPHA, **14**), which can be specifically activated with UV light (365 nm) to release the electrophilic probe in stoichiometric amounts with regard to the POI.<sup>65,66</sup> This enables the spatiotemporal control of protein labelling and minimizes off-target engagement due to a proximal release mechanism. Therefore, it is a powerful tool to investigate the consequences of specific and time-dependent signaling events in living cells.<sup>67</sup> The downside of this platform are the fact that it relies on genetic engineering of the POI, which might perturb its protein interaction networks and localization within live cells, and the use of UV light which is toxic to cells.

### Inhibitor-directed cargo release

One approach for circumventing these limitations harnessed methacrylamide warheads that provide the ability to release a

cargo upon binding to a cysteine.<sup>68</sup> This chemotype was installed on the Bruton's tyrosine kinase (BTK) inhibitor ibrutinib (**8**) in place of the acrylamide and the O<sup>2</sup>-protected di-azaniumdiolate (NONOate) was appended as a leaving group to the  $\alpha$ -methyl moiety which yielded TSNO1 (**15**) (Fig. 9B).<sup>69</sup> NONOates offer a controllable NO-release capability and the binding of the TSNO1 construct to C481 of BTK triggered S-nitrosation at Cys527. Harnessing this approach enhanced phosphorylation inhibition at Y551 compared to ibrutinib and thus the effect of covalent inhibition. This strategy was extended to FGFR4 and HER2.

## Addressing the limitations of advanced ABPs

Our discussion highlighted the potential, limitations and opportunities of contemporary approaches along with the design of advanced ABPs that can be employed in live cells. The latter harnessed the use of photoactivatable chemical probes which allowed performing reactivity profiling in the native proteome. However, a drawback of the presented approaches is the need to use UV light to activate the warhead reactivity, which is toxic to cells and might perturb labelling profiles and/or cellular responses. To advance this technology, the use of visible light-responsive photocages would enable avoiding the use of hazardous UV irradiation to retain native biological functions during the labelling process.

### Subcellular microenvironment profiling

A conceivable advancement in ABP development would entail extending their reach in chemoproteomics analysis from whole live cells to focused labelling events within certain protein microenvironments. In cells, proteins are localized in defined subcellular environments and compartments.<sup>70</sup> These microenvironments are essential for protein signaling and function.<sup>71</sup> Thus, determining the protein composition within compartments and microenvironments could offer fundamental biological insights, which could play a critical role in the development of therapeutics.<sup>72</sup> The combination of ABPs and protecting groups that mask the warhead reactivity and can be removed in response to certain stimuli provides an intriguing foundation for this approach. To date, the spatiotemporal subcellular mapping is often controlled by irradiation with light.<sup>73</sup> To increase the selectivity of compartment-specific ABP label-

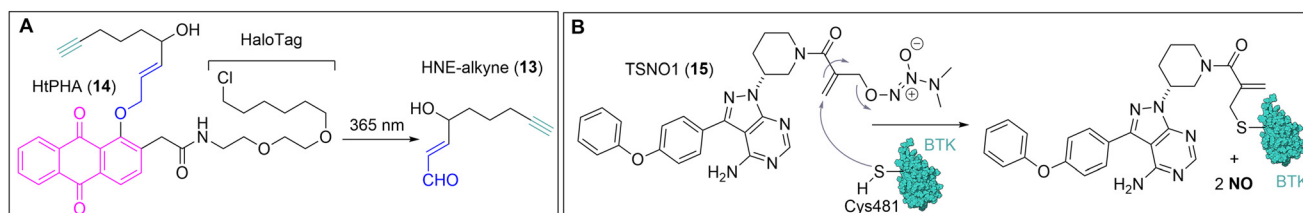


Fig. 9 (A) Photouncaging reaction of HtPHA (**14**) to HNE-alkyne (**13**). (B) Reaction of TSNO1 (**15**) with C481 of BTK.



ling, for instance, functional groups could be implemented which dictate the transport to specific organelles, such as the use of triphenylphosphonium species to localize compounds to mitochondria, on the photocage.<sup>74</sup> In addition, protecting groups could be installed to temporarily neutralize the warhead reactivity which are, in turn, removed by second messengers. With this strategy, the ABPPs stay inactive unless triggered by a desired stimulus (Fig. 10). This approach could aid in uncovering the protein microenvironments that these signaling molecules are released into. An example of a second messenger is hydrogen peroxide, which belongs to the class of reactive oxygen species (ROS) that propagate redox signals *via* the installation of oxPTMs on cysteine residues and plays a key role in aging cells and cancer progression.<sup>75</sup> Its concentration strongly varies in different cellular organelles and microenvironments.<sup>76,77</sup> In excess, H<sub>2</sub>O<sub>2</sub> is responsible for oxidative stress and, if left unregulated, can lead to cell death through *e.g.* ferroptosis. Identifying proteins that reside in sub-cellular microenvironments with high ROS concentrations could thus aid in pinpointing suitable drug targets.

### Advancing the installation of PTMs with ABPPs

A pioneering approach that combines genetic engineering with chemical reactivity facilitated the spatiotemporal control of the protein-specific installation of PTMs on cysteine.<sup>67</sup> This was achieved with minimal off-target engagement due to a proximal release mechanism which is a powerful tool to investigate the consequences of specific and time-dependent signaling events in living cells.<sup>67</sup> The downside of this platform is the fact that it relies on the use of hazardous UV light, which could be addressed by harnessing visible light-inducible photocages. In addition, the genetic engineering of the POI might perturb its protein interaction networks and localization within live cells. This limitation was addressed by an alternative approach that relied on an inhibitor-directed cargo release mechanism harnessing methacrylamide warheads on the BTK inhibitor ibrutinib (8) in place of the acrylamide to generate TSNO1.<sup>69</sup> TSNO1 facilitated the controllable release of NO which performed an *S*-nitrosation at Cys527 with its binding to C481 of BTK. A challenge for this approach is that the installation of a PTM cannot be facilitated without secondary label-

ling with a covalent chemical probe. To circumvent this limitation, a traceless ligand-directed installation of a PTM could be conceived, which was demonstrated for the directed acylation of lysine.<sup>78</sup> Alternatively, for cysteine-targeted ligands, an advanced version of the methacrylate chemotype could be developed that functions through a reversible covalent mechanism<sup>79,80</sup> and could detach from the protein once the cargo is released.

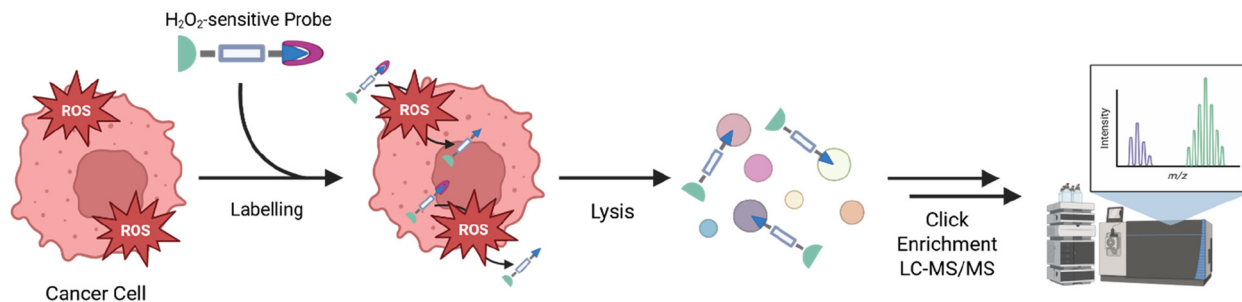
## Perspective: advancing the reach of ABPP

### ABPP databases

Due to its unique reactivity which promoted the establishment of ABPPs based on a wide range of thiol-reactive functional groups, cysteine is the most widely studied amino acid with ABPP to date. This is highlighted by the publication of large databases for human cysteine chemoproteomics data. For example, CysDB is a comprehensive repository which hosts records for the labelling of 24% of the cysteinome (62 888 residues).<sup>81</sup> The Ligandable Cysteine Database specializes in cysteines that can be labelled using small molecule fragments and describes the engagement of >20 000 cysteines within >6000 proteins.<sup>82</sup> A quantitative pan-cancer analysis with a focus on cell line-dependent changes in ligandability across 400+ cancer cell lines yielded >78 000 quantified cysteines along with >5900 ligandable sites which are hosted by the DrugMap database.<sup>83</sup> The latter showcases that changes in mutational states and the PTM landscape can impart small molecules with state-dependent selectivity for cysteines on certain proteins. Combined, these databases provide a foundation for the targeted development of new cysteine-reactive chemical probes and drug candidates.

### Expanding the reach of ABPP through chemical innovation

Contemporary research has afforded many reactive motifs that allow the engagement of amino acids beyond cysteine with high selectivity.<sup>51</sup> Nucleophilic amino acids are among the most common targets. While lysine has received considerable attention,<sup>38,78,78</sup> warheads for tyrosine,<sup>84,85</sup> methionine,<sup>86</sup>



**Fig. 10** Simplified schematic depiction of a potential H<sub>2</sub>O<sub>2</sub>-sensitive microenvironment-dependent cysteine profiling workflow: Treatment of living cells with a chemical probe that is activated by the second messenger leads to the labelling of proteins in environments with high concentrations of H<sub>2</sub>O<sub>2</sub>. Lysis of the cells is then followed by a typical ABPP workflow.



tryptophan,<sup>87,88</sup> glutamate/aspartate,<sup>89,90</sup> and arginine,<sup>91</sup> among others, have been established.<sup>51,79,80</sup> Intriguingly, chemotypes that target serine/threonine residues beyond their highly nucleophilic versions in enzyme active sites<sup>92</sup> remain scarce and while histidine is a common off-target of ABPs directed at lysine and cysteine, highly selective warheads for proteome-wide studies remain elusive.<sup>93</sup> It is worth noting that the reactivity of an amino acid is strongly influenced by the local protein microenvironments, which may hinder its labelling with available chemotypes. Cysteine, lysine, histidine, aspartate, glutamate and arginine, for instance, can be present as protonated or deprotonated versions depending on the local pH value. To account for this variability, it is necessary to develop microenvironment-specific, amino acid-selective warheads. The need for additional research in this area is compounded by the demand for broadly reactive amino acid-selective chemotypes for proteome-wide labelling and low-reactivity warheads for the development of protein-selective ABPs and drug candidates. For the latter, the literature shows that the availability of binding modes beyond irreversible covalent bonds to reversible covalent mechanisms<sup>94</sup> and the implementation of covalent ligand-directed release chemistry<sup>68</sup> are highly desirable. Based on the function of an amino acid as an anchor for a PTM, warheads can be developed that specifically engage the modified residues, which has been shown for cysteine sulfenic acid.<sup>21</sup>

## Perspective on the pharmaceutical industry

### Assessing on/off-target engagement

Although many historically important drugs, such as aspirin, penicillin, esomeprazole and clopidogrel, are covalent inhibitors, this modality was considered as a risk factor in the pharmaceutical industry for decades based on the view that chemical reactivity drives off-target engagement.<sup>95,96</sup> In this regard, ABPP is a powerful method that mitigates the risk of non-specific binding by determining the protein engagement of small molecules within the proteome. A key advantage of this approach is that the drug candidates can be subjected to live cells, which accounts for interactions that might not be found using *in vitro* assays based on the native cellular environment of proteins that facilitates, among other factors, the installation of PTMs, protein-protein interactions (PPIs) and compartmentalization. As such, this method allows the confirmation of target engagement and uncovering undesired off-target reactivities under physiological conditions. To showcase the potential of ABPP, the off-target binding of the fatty acid amide hydrolase (FAAH) inhibitor BIA 10-2474, which led to the death of one volunteer and produced mild-to-severe neurological symptoms in four others during phase I clinical trials, was analyzed in an effort to identify the cause of the clinical neurotoxicity.<sup>97</sup> As a foundation for the analysis, the labelling of PF04457845, an FAAH inhibitor developed by Pfizer that did not produce neurotoxic effects in phase 1 clinical

trials, was compared to that of BIA 10-2474. This experiment revealed that BIA 10-2474 targets several lipases that are not targeted by PF04457845 – a highly selective FAAH inhibitor – which suggests that the toxicity arises from the promiscuity of the lipase inhibitor from Bial Pharmaceuticals. Based on this precedent, ABPP was used to assess the off-target reactivity of many covalent inhibitors, including those of EGFR T790M proteins.<sup>98</sup>

### Identification of unrecognized binding sites

A strong signal for the importance of ABPP for the pharmaceutical industry was the acquisition of Vividion Therapeutics, a company that is founded on a strong chemoproteomics platform, by the Bayer AG for \$1.5 billion. Vividion specializes in harnessing cysteine-reactive small molecules to identify unrecognized binding sites on historically undruggable proteins. Their approach has yielded an inhibitor that specifically blocks the interaction between RAS and PI3K $\alpha$  which has entered phase 1 clinical trials<sup>99</sup> along with novel covalent allosteric inhibitors of *e.g.* the WRN helicase<sup>100</sup> and JAK1.<sup>101</sup> Jnana Therapeutics<sup>102</sup> and Belhara Therapeutics<sup>103</sup> harness photoaffinity labelling to identify binding sites for the development of reversibly binding drugs which has empowered Jnana Therapeutics<sup>102</sup> to discover an SLC6A19 amino acid transporter inhibitor currently being evaluated for its ability to treat phenylketonuria in phase 3 clinical trials.<sup>99</sup> Frontier Medicines and Matchpoint Therapeutics combine chemoproteomics with machine learning methods.<sup>99</sup>

## Summary and conclusion

The combination of ABPs with chemoproteomics ABPP approaches has yielded a transformative platform to investigate protein function, identify protein targets and elucidate the mechanisms-of-action of bioactive small molecules. Cysteine-targeted activity-based probes have emerged as powerful tools that leverage the unique reactivity of cysteines to enable covalent labelling across the proteome. ABPs consist of an optimized reactive warhead that balances reactivity, coverage and selectivity, along with a linker or ligand structure and a bioorthogonal functional handle, which are essential to enable the visualization and enrichment of probe-bound proteins. Advances in chemoproteomics techniques such as bifunctional enrichment tags, isotopic labelling and quantitative reactivity as well as ligandability profiling have allowed pinpointing functional cysteines and unrecognized binding pockets. Recent developments extend to the ability to profile cysteine reactivity in live cells, achieve spatiotemporal control of cysteine labelling with PTMs and identify druggable protein states with ABPP. Future directions in probe design may entail directing probes to specific organelles and mapping protein microenvironments. Beyond academic research, ABPP has significant implications for drug discovery within the pharmaceutical industry, providing means to confirm target engagement and assess off-target labelling under physiological con-



ditions which built the foundation for the resurgence of covalent drugs. The ability to uncover unrecognized binding sites has led to the development of new drug candidates which are currently being evaluated in clinical trials. A new avenue that is pursued by recently founded companies is the integration of large datasets obtained by ABPP with machine learning.

## Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

## Conflicts of interest

The authors declare no competing financial interest.

## Data availability

The data that is referred to in this perspective is available within the cited references.

## Acknowledgements

The authors thank the Fonds der Chemischen Industrie for a Liebig fellowship to DBK and a PhD fellowship to CMN. MAS holds a PhD fellowship of the Vienna Doctoral School of Pharmaceutical, Nutritional and Sport Sciences (PhaNUspo) of the University of Vienna. This work was supported by the Deutsche Forschungsgemeinschaft (DFG) Priority Program SPP 2306 (KO 5903/2-1, no. 461576077). The figures were, in part, prepared using Biorender.

## References

- 1 M.-C. Hung and W. Link, Protein Localization in Disease and Therapy, *J. Cell Sci.*, 2011, **124**(20), 3381–3392, DOI: [10.1242/jcs.089110](https://doi.org/10.1242/jcs.089110).
- 2 H. Zhu, M. Bilgin and M. Snyder, Proteomics, *Annu. Rev. Biochem.*, 2003, **72**(1), 783–812, DOI: [10.1146/annurev.biochem.72.121801.161511](https://doi.org/10.1146/annurev.biochem.72.121801.161511).
- 3 G. MacBeath, Protein Microarrays and Proteomics, *Nat. Genet.*, 2002, **32**(S4), 526–532, DOI: [10.1038/ng1037](https://doi.org/10.1038/ng1037).
- 4 M. P. Washburn, D. Wolters and J. R. Yates, Large-Scale Analysis of the Yeast Proteome by Multidimensional Protein Identification Technology, *Nat. Biotechnol.*, 2001, **19**(3), 242–247, DOI: [10.1038/85686](https://doi.org/10.1038/85686).
- 5 S. P. Gygi, B. Rist, S. A. Gerber, F. Turecek, M. H. Gelb and R. Aebersold, Quantitative Analysis of Complex Protein Mixtures Using Isotope-Coded Affinity Tags, *Nat. Biotechnol.*, 1999, **17**(10), 994–999, DOI: [10.1038/13690](https://doi.org/10.1038/13690).
- 6 T. Ito, K. Ota, H. Kubota, Y. Yamaguchi, T. Chiba, K. Sakuraba and M. Yoshida, Roles for the Two-Hybrid System in Exploration of the Yeast Protein Interactome, *Mol. Cell. Proteomics*, 2002, **1**(8), 561–566, DOI: [10.1074/mcp.R200005-MCP200](https://doi.org/10.1074/mcp.R200005-MCP200).
- 7 N. Jessani and B. F. Cravatt, The Development and Application of Methods for Activity-Based Protein Profiling, *Curr. Opin. Chem. Biol.*, 2004, **8**(1), 54–59, DOI: [10.1016/j.cbpa.2003.11.004](https://doi.org/10.1016/j.cbpa.2003.11.004).
- 8 B. F. Cravatt, A. T. Wright and J. W. Kozarich, Activity-Based Protein Profiling: From Enzyme Chemistry to Proteomic Chemistry, *Annu. Rev. Biochem.*, 2008, **77**(1), 383–414, DOI: [10.1146/annurev.biochem.75.101304.124125](https://doi.org/10.1146/annurev.biochem.75.101304.124125).
- 9 A. E. Speers and B. F. Cravatt, Activity-Based Protein Profiling (ABPP) and Click Chemistry (CC)–ABPP by MudPIT Mass Spectrometry, *Curr. Protoc. Chem. Biol.*, 2009, **1**(1), 29–41, DOI: [10.1002/9780470559277.ch090138](https://doi.org/10.1002/9780470559277.ch090138).
- 10 K. T. Barglow and B. F. Cravatt, Activity-Based Protein Profiling for the Functional Annotation of Enzymes, *Nat. Methods*, 2007, **4**(10), 822–827, DOI: [10.1038/nmeth1092](https://doi.org/10.1038/nmeth1092).
- 11 J. Yang, K. S. Carroll and D. C. Liebler, The Expanding Landscape of the Thiol Redox Proteome, *Mol. Cell. Proteomics*, 2016, **15**(1), 1–11, DOI: [10.1074/mcp.O115.056051](https://doi.org/10.1074/mcp.O115.056051).
- 12 D. W. Bak, T. J. Bechtel, J. A. Falco and E. Weerapana, Cysteine Reactivity across the Subcellular Universe, *Curr. Opin. Chem. Biol.*, 2019, **48**, 96–105, DOI: [10.1016/j.cbpa.2018.11.002](https://doi.org/10.1016/j.cbpa.2018.11.002).
- 13 G. Bulaj, T. Kortemme and D. P. Goldenberg, Ionization-Reactivity Relationships for Cysteine Thiols in Polypeptides, *Biochemistry*, 1998, **37**(25), 8965–8972, DOI: [10.1021/bi973101r](https://doi.org/10.1021/bi973101r).
- 14 N. M. Giles, A. B. Watts, G. I. Giles, F. H. Fry, J. A. Littlechild and C. Jacob, Metal and Redox Modulation of Cysteine Protein Function, *Chem. Biol.*, 2003, **10**(8), 677–693, DOI: [10.1016/S1074-5521\(03\)00174-1](https://doi.org/10.1016/S1074-5521(03)00174-1).
- 15 J. Clayden, N. Greeves and S. Warren, in *Organic Chemistry*, Oxford university press, Oxford, 2nd edn, 2012.
- 16 C. E. Paulsen and K. S. Carroll, Cysteine-Mediated Redox Signaling: Chemistry, Biology, and Tools for Discovery, *Chem. Rev.*, 2013, **113**(7), 4633–4679, DOI: [10.1021/cr300163e](https://doi.org/10.1021/cr300163e).
- 17 E. Weerapana, C. Wang, G. M. Simon, F. Richter, S. Khare, M. B. D. Dillon, D. A. Bachovchin, K. Mowen, D. Baker and B. F. Cravatt, Quantitative Reactivity Profiling Predicts Functional Cysteines in Proteomes, *Nature*, 2010, **468**(7325), 790–795, DOI: [10.1038/nature09472](https://doi.org/10.1038/nature09472).
- 18 D. G. Hoch, D. Abegg and A. Adibekian, Cysteine-Reactive Probes and Their Use in Chemical Proteomics, *Chem. Commun.*, 2018, **54**(36), 4501–4512, DOI: [10.1039/C8CC01485J](https://doi.org/10.1039/C8CC01485J).
- 19 C. Klomsiri, P. A. Karplus and L. B. Poole, Cysteine-Based Redox Switches in Enzymes, *Antioxid. Redox Signaling*, 2011, **14**(6), 1065–1077, DOI: [10.1089/ars.2010.3376](https://doi.org/10.1089/ars.2010.3376).
- 20 K. M. Backus, B. E. Correia, K. M. Lum, S. Forli, B. D. Horning, G. E. González-Páez, S. Chatterjee,



- B. R. Lanning, J. R. Teijaro, A. J. Olson, D. W. Wolan and B. F. Cravatt, Proteome-Wide Covalent Ligand Discovery in Native Biological Systems, *Nature*, 2016, **534**(7608), 570–574, DOI: [10.1038/nature18002](https://doi.org/10.1038/nature18002).
- 21 L. Fu, Y. Jung, C. Tian, R. B. Ferreira, R. Cheng, F. He, J. Yang and K. S. Carroll, Nucleophilic Covalent Ligand Discovery for the Cysteine Redoxome, *Nat. Chem. Biol.*, 2023, **19**, 1309–1319, DOI: [10.1038/s41589-023-01330-5](https://doi.org/10.1038/s41589-023-01330-5).
- 22 A. B. Berger, P. M. Vitorino and M. Bogyo, Activity-Based Protein Profiling: Applications to Biomarker Discovery, In *Vivo* Imaging and Drug Discovery, *Am. J. Pharmacogenomics*, 2004, **4**(6), 371–381, DOI: [10.2165/00129785-200404060-00004](https://doi.org/10.2165/00129785-200404060-00004).
- 23 H. Fang, B. Peng, S. Y. Ong, Q. Wu, L. Li and S. Q. Yao, Recent Advances in Activity-Based Probes (ABPs) and Affinity-Based Probes (ABPs) for Profiling of Enzymes, *Chem. Sci.*, 2021, **12**(24), 8288–8310, DOI: [10.1039/D1SC01359A](https://doi.org/10.1039/D1SC01359A).
- 24 C. Bissantz, B. Kuhn and M. Stahl, A Medicinal Chemist's Guide to Molecular Interactions, *J. Med. Chem.*, 2010, **53**(14), 5061–5084, DOI: [10.1021/jm100112j](https://doi.org/10.1021/jm100112j).
- 25 A. Scaloni, P. Ferranti, G. De Simone, G. Mamone, N. Sannolo and A. Malorni, Probing the Reactivity of Nucleophile Residues in Human 2,3-diphosphoglycerate/Deoxy-hemoglobin Complex by Aspecific Chemical Modifications, *FEBS Lett.*, 1999, **452**(3), 190–194, DOI: [10.1016/S0014-5793\(99\)00601-8](https://doi.org/10.1016/S0014-5793(99)00601-8).
- 26 M. K. Dennehy, K. A. M. Richards, G. R. Wernke, Y. Shyr and D. C. Liebler, Cytosolic and Nuclear Protein Targets of Thiol-Reactive Electrophiles, *Chem. Res. Toxicol.*, 2006, **19**(1), 20–29, DOI: [10.1021/tx050312l](https://doi.org/10.1021/tx050312l).
- 27 R. R. Rando, Chapter 24 Mechanism-Based Irreversible Enzyme Inhibitors, in *Annual Reports in Medicinal Chemistry*, Elsevier, 1974, vol. 9, pp. 234–243. DOI: [10.1016/S0065-7743\(08\)61446-3](https://doi.org/10.1016/S0065-7743(08)61446-3).
- 28 M. Ikejiri, M. M. Bernardo, R. D. Bonfil, M. Toth, M. Chang, R. Fridman and S. Mobashery, Potent Mechanism-Based Inhibitors for Matrix Metalloproteinases, *J. Biol. Chem.*, 2005, **280**(40), 33992–34002, DOI: [10.1074/jbc.M504303200](https://doi.org/10.1074/jbc.M504303200).
- 29 T. Böttcher, M. Pitscheider and S. A. Sieber, Natural Products and Their Biological Targets: Proteomic and Metabolomic Labeling Strategies, *Angew. Chem., Int. Ed.*, 2010, **49**(15), 2680–2698, DOI: [10.1002/anie.200905352](https://doi.org/10.1002/anie.200905352).
- 30 C. Drahl, B. F. Cravatt and E. J. Sorensen, Protein-Reactive Natural Products, *Angew. Chem., Int. Ed.*, 2005, **44**(36), 5788–5809, DOI: [10.1002/anie.200500900](https://doi.org/10.1002/anie.200500900).
- 31 M. Pitscheider and S. A. Sieber, Cinnamic Aldehyde Derived Probes for the Active Site Labelling of Pathogenesis Associated Enzymes, *Chem. Commun.*, 2009, **25**, 3741, DOI: [10.1039/b905527d](https://doi.org/10.1039/b905527d).
- 32 M. J. Evans, A. Saghatelian, E. J. Sorensen and B. F. Cravatt, Target Discovery in Small-Molecule Cell-Based Screens by in Situ Proteome Reactivity Profiling, *Nat. Biotechnol.*, 2005, **23**(10), 1303–1307, DOI: [10.1038/nbt1149](https://doi.org/10.1038/nbt1149).
- 33 E. Weerapana, G. M. Simon and B. F. Cravatt, Disparate Proteome Reactivity Profiles of Carbon Electrophiles, *Nat. Chem. Biol.*, 2008, **4**(7), 405–407, DOI: [10.1038/nchembio.91](https://doi.org/10.1038/nchembio.91).
- 34 S. T. A. Koenders, E. J. Van Rooden, H. Van Den Elst, B. I. Florea, H. S. Overkleeft and M. Van Der Stelt, STA-55, an Easily Accessible, Broad-Spectrum, Activity-Based Aldehyde Dehydrogenase Probe, *ChemBioChem*, 2020, **21**(13), 1911–1917, DOI: [10.1002/cbic.201900771](https://doi.org/10.1002/cbic.201900771).
- 35 M. J. Evans and B. F. Cravatt, Mechanism-Based Profiling of Enzyme Families, *Chem. Rev.*, 2006, **106**(8), 3279–3301, DOI: [10.1021/cr050288g](https://doi.org/10.1021/cr050288g).
- 36 Y. Liu, M. P. Patricelli and B. F. Cravatt, Activity-Based Protein Profiling: The Serine Hydrolases, *Proc. Natl. Acad. Sci. U. S. A.*, 1999, **96**(26), 14694–14699, DOI: [10.1073/pnas.96.26.14694](https://doi.org/10.1073/pnas.96.26.14694).
- 37 M. Abo and E. Weerapana, A Caged Electrophilic Probe for Global Analysis of Cysteine Reactivity in Living Cells, *J. Am. Chem. Soc.*, 2015, **137**(22), 7087–7090, DOI: [10.1021/jacs.5b04350](https://doi.org/10.1021/jacs.5b04350).
- 38 S. M. Hacker, K. M. Backus, M. R. Lazear, S. Forli, B. E. Correia and B. F. Cravatt, Global Profiling of Lysine Reactivity and Ligandability in the Human Proteome, *Nat. Chem.*, 2017, **9**(12), 1181–1190, DOI: [10.1038/nchem.2826](https://doi.org/10.1038/nchem.2826).
- 39 B. F. Cravatt, Activity-Based Protein Profiling – Finding General Solutions to Specific Problems, *Isr. J. Chem.*, 2023, **63**(3–4), e202300029, DOI: [10.1002/ijch.202300029](https://doi.org/10.1002/ijch.202300029).
- 40 A. T. Voice, G. Tresadern, R. M. Twidale, H. Van Vlijmen and A. J. Mulholland, Mechanism of Covalent Binding of Ibrutinib to Bruton's Tyrosine Kinase Revealed by QM/MM Calculations, *Chem. Sci.*, 2021, **12**(15), 5511–5516, DOI: [10.1039/D0SC06122K](https://doi.org/10.1039/D0SC06122K).
- 41 J. B. Tuttle, C. Allais, C. M. N. Allerton, A. S. Anderson, J. T. Arcari, L. M. Aschenbrenner, M. Avery, J. Bellenger, S. Berritt, B. Boras, B. P. Boscoe, L. M. Buzon, R. D. Cardin, A. A. Carlo, K. J. Coffman, A. Dantonio, L. Di, H. Eng, K. A. Farley, R. A. Ferre, K. S. Gajiwala, S. A. Gibson, S. E. Greasley, B. L. Hurst, E. P. Kadar, A. S. Kalgutkar, E. A. Lachapelle, L. F. Lanyon, J. Lee, J. Lee, Y. Lian, W. Liu, L. A. Martínez-Alsina, S. W. Mason, S. Noell, J. Novak, R. S. Obach, K. Ogilvie, S. V. O'Neil, G. Ostner, D. R. Owen, N. C. Patel, M. Pettersson, R. S. Singh, D. K. Rai, M. R. Reese, S. Sakata, M. F. Sammons, J. G. Sathish, R. Sharma, C. M. Steppan, A. Stewart, L. Updyke, P. R. Verhoest, L. Wei, S. W. Wright, E. Yang, Q. Yang and Y. Zhu, Discovery of Nirmatrelvir (PF-07321332): A Potent, Orally Active Inhibitor of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS CoV-2) Main Protease, *J. Med. Chem.*, 2025, **68**(7), 7003–7030, DOI: [10.1021/acs.jmedchem.4c02561](https://doi.org/10.1021/acs.jmedchem.4c02561).
- 42 Q. Zhao, X. Ouyang, X. Wan, K. S. Gajiwala, J. C. Kath, L. H. Jones, A. L. Burlingame and J. Taunton, Broad-Spectrum Kinase Profiling in Live Cells with Lysine-Targeted Sulfonyl Fluoride Probes, *J. Am. Chem. Soc.*, 2017, **139**(2), 680–685, DOI: [10.1021/jacs.6b08536](https://doi.org/10.1021/jacs.6b08536).
- 43 S. Liu, B. Zhou, H. Yang, Y. He, Z.-X. Jiang, S. Kumar, L. Wu and Z.-Y. Zhang, Aryl Vinyl Sulfonates and Sulfones as Active Site-Directed and Mechanism-Based Probes for Protein Tyrosine Phosphatases, *J. Am. Chem. Soc.*, 2008, **130**(26), 8251–8260, DOI: [10.1021/ja711125p](https://doi.org/10.1021/ja711125p).



- 44 G. C. Adam, E. J. Sorensen and B. F. Cravatt, Proteomic Profiling of Mechanistically Distinct Enzyme Classes Using a Common Chemotype, *Nat. Biotechnol.*, 2002, **20**(8), 805–809, DOI: [10.1038/nbt714](https://doi.org/10.1038/nbt714).
- 45 H. C. Kolb, M. G. Finn and K. B. Sharpless, Click Chemistry: Diverse Chemical Function from a Few Good Reactions, *Angew. Chem., Int. Ed.*, 2001, **40**(11), 2004–2021, DOI: [10.1002/1521-3773\(20010601\)40:11%253C2004::AID-ANIE2004%253E3.0.CO;2-5](https://doi.org/10.1002/1521-3773(20010601)40:11%253C2004::AID-ANIE2004%253E3.0.CO;2-5).
- 46 A. E. Speers and B. F. Cravatt, Profiling Enzyme Activities In Vivo Using Click Chemistry Methods, *Chem. Biol.*, 2004, **11**(4), 535–546, DOI: [10.1016/j.chembiol.2004.03.012](https://doi.org/10.1016/j.chembiol.2004.03.012).
- 47 A. E. Speers and B. F. Cravatt, A Tandem Orthogonal Proteolysis Strategy for High-Content Chemical Proteomics, *J. Am. Chem. Soc.*, 2005, **127**(28), 10018–10019, DOI: [10.1021/ja0532842](https://doi.org/10.1021/ja0532842).
- 48 E. Weerapana, A. E. Speers and B. F. Cravatt, Tandem Orthogonal Proteolysis-Activity-Based Protein Profiling (TOP-ABPP)—a General Method for Mapping Sites of Probe Modification in Proteomes, *Nat. Protoc.*, 2007, **2**(6), 1414–1425, DOI: [10.1038/nprot.2007.194](https://doi.org/10.1038/nprot.2007.194).
- 49 C. Wang, E. Weerapana, M. M. Blewett and B. F. Cravatt, A Chemoproteomic Platform to Quantitatively Map Targets of Lipid-Derived Electrophiles, *Nat. Methods*, 2014, **11**(1), 79–85, DOI: [10.1038/nmeth.2759](https://doi.org/10.1038/nmeth.2759).
- 50 P. R. A. Zanon, L. Lewald and S. M. Hacker, Isotopically Labeled Desthiobiotin Azide (isoDTB) Tags Enable Global Profiling of the Bacterial Cysteinome, *Angew. Chem., Int. Ed.*, 2020, **59**(7), 2829–2836, DOI: [10.1002/anie.201912075](https://doi.org/10.1002/anie.201912075).
- 51 P. R. A. Zanon, F. Yu, P. Musacchio, L. Lewald, M. Zollo, K. Krauskopf, D. Mrdović, P. Raunft, T. E. Maher, M. Cigler, C. Chang, K. Lang, F. D. Toste, A. I. Nesvizhskii and S. M. Hacker, Profiling the Proteome-Wide Selectivity of Diverse Electrophiles, *ChemRxiv*, 2021, DOI: [10.26434/chemrxiv-2021-w7rss-v2](https://doi.org/10.26434/chemrxiv-2021-w7rss-v2).
- 52 E. K. Kemper, Y. Zhang, M. M. Dix and B. F. Cravatt, Global Profiling of Phosphorylation-Dependent Changes in Cysteine Reactivity, *Nat. Methods*, 2022, **19**(3), 341–352, DOI: [10.1038/s41592-022-01398-2](https://doi.org/10.1038/s41592-022-01398-2).
- 53 M. J. Niphakis and B. F. Cravatt, Enzyme Inhibitor Discovery by Activity-Based Protein Profiling, *Annu. Rev. Biochem.*, 2014, **83**(1), 341–377, DOI: [10.1146/annurev-biochem-060713-035708](https://doi.org/10.1146/annurev-biochem-060713-035708).
- 54 G. M. Simon, M. J. Niphakis and B. F. Cravatt, Determining Target Engagement in Living Systems, *Nat. Chem. Biol.*, 2013, **9**(4), 200–205, DOI: [10.1038/nchembio.1211](https://doi.org/10.1038/nchembio.1211).
- 55 M. P. Baggelaar and M. Stelt, Competitive ABPP of Serine Hydrolases, *Methods Mol. Biol.*, 2017, **1491**, 161, DOI: [10.1007/978-1-4939-6439-0\\_12](https://doi.org/10.1007/978-1-4939-6439-0_12).
- 56 E. V. Vinogradova, X. Zhang, D. Remillard, D. C. Lazar, R. M. Suci, Y. Wang, G. Bianco, Y. Yamashita, V. M. Crowley, M. A. Schafroth, M. Yokoyama, D. B. Konrad, K. M. Lum, G. M. Simon, E. K. Kemper, M. R. Lazear, S. Yin, M. M. Blewett, M. M. Dix, N. Nguyen, M. N. Shokhirev, E. N. Chin, L. L. Lairson, B. Melillo, S. L. Schreiber, S. Forli, J. R. Teijaro and B. F. Cravatt, An Activity-Guided Map of Electrophile-Cysteine Interactions in Primary Human T Cells, *Cell*, 2020, **182**(4), 1009–1026, DOI: [10.1016/j.cell.2020.07.001](https://doi.org/10.1016/j.cell.2020.07.001).
- 57 M. Takahashi, H. B. Chong, S. Zhang, T.-Y. Yang, M. J. Lazarov, S. Harry, M. Maynard, B. Hilbert, R. D. White, H. E. Murrey, C.-C. Tsou, K. Vordermark, J. Assaad, M. Gohar, B. R. Dürr, M. Richter, H. Patel, G. Kryukov, N. Brooijmans, A. S. O. Alghali, K. Rubio, A. Villanueva, J. Zhang, M. Ge, F. Makram, H. Griesshaber, D. Harrison, A.-S. Koglin, S. Ojeda, B. Karakyriakou, A. Healy, G. Popoola, I. Rachmin, N. Khandelwal, J. R. Neil, P.-C. Tien, N. Chen, T. Hosp, S. Van Den Ouweland, T. Hara, L. Bussema, R. Dong, L. Shi, M. Q. Rasmussen, A. C. Domingues, A. Lawless, J. Fang, S. Yoda, L. P. Nguyen, S. M. Reeves, F. N. Wakefield, A. Acker, S. E. Clark, T. Dubash, J. Kastanos, E. Oh, D. E. Fisher, S. Maheswaran, D. A. Haber, G. M. Boland, M. Sade-Feldman, R. W. Jenkins, A. N. Hata, N. M. Bardeesy, M. L. Suvà, B. R. Martin, B. B. Liau, C. J. Ott, M. N. Rivera, M. S. Lawrence and L. Bar-Peled, DrugMap: A Quantitative Pan-Cancer Analysis of Cysteine Ligandability, *Cell*, 2024, **187**(10), 2536–2556, DOI: [10.1016/j.cell.2024.03.027](https://doi.org/10.1016/j.cell.2024.03.027).
- 58 V. M. Crowley, M. Thielert and B. F. Cravatt, Functionalized Scout Fragments for Site-Specific Covalent Ligand Discovery and Optimization, *ACS Cent. Sci.*, 2021, **7**(4), 613–623, DOI: [10.1021/acscentsci.0c01336](https://doi.org/10.1021/acscentsci.0c01336).
- 59 S. M. Couvertier, Y. Zhou and E. Weerapana, Chemical-Proteomic Strategies to Investigate Cysteine Posttranslational Modifications, *Biochim. Biophys. Acta, Proteins Proteomics*, 2014, **1844**(12), 2315–2330, DOI: [10.1016/j.bbapap.2014.09.024](https://doi.org/10.1016/j.bbapap.2014.09.024).
- 60 S.-I. Bibli and I. Fleming, Oxidative Post-Translational Modifications: A Focus on Cysteine S-Sulfhydration and the Regulation of Endothelial Fitness, *Antioxid. Redox Signaling*, 2021, **35**(18), 1494–1514, DOI: [10.1089/ars.2021.0162](https://doi.org/10.1089/ars.2021.0162).
- 61 N. C. Sadler, M. R. Melnicki, M. H. Serres, E. D. Merkley, W. B. Chrisler, E. A. Hill, M. F. Romine, S. Kim, E. M. Zink, S. Datta, R. D. Smith, A. S. Beliaev, A. Konopka and A. T. Wright, Live Cell Chemical Profiling of Temporal Redox Dynamics in a Photoautotrophic Cyanobacterium, *ACS Chem. Biol.*, 2014, **9**(1), 291–300, DOI: [10.1021/cb400769v](https://doi.org/10.1021/cb400769v).
- 62 M. Abo, D. W. Bak and E. Weerapana, Optimization of Caged Electrophiles for Improved Monitoring of Cysteine Reactivity in Living Cells, *ChemBioChem*, 2017, **18**(1), 81–84, DOI: [10.1002/cbic.201600524](https://doi.org/10.1002/cbic.201600524).
- 63 M. E. H. White, J. Gil and E. W. Tate, Proteome-Wide Structural Analysis Identifies Warhead- and Coverage-Specific Biases in Cysteine-Focused Chemoproteomics, *Cell Chem. Biol.*, 2023, **30**(7), 828–838, DOI: [10.1016/j.chembiol.2023.06.021](https://doi.org/10.1016/j.chembiol.2023.06.021).
- 64 X. Fang, Y. Fu, M. J. C. Long, J. A. Haegele, E. J. Ge, S. Parvez and Y. Aye, Temporally Controlled Targeting of 4-Hydroxynonenal to Specific Proteins in Living Cells, *J. Am. Chem. Soc.*, 2013, **135**(39), 14496–14499, DOI: [10.1021/ja405400k](https://doi.org/10.1021/ja405400k).



- 65 H.-Y. Lin, J. A. Haegele, M. T. Disare, Q. Lin and Y. Aye, A Generalizable Platform for Interrogating Target- and Signal-Specific Consequences of Electrophilic Modifications in Redox-Dependent Cell Signaling, *J. Am. Chem. Soc.*, 2015, **137**(19), 6232–6244, DOI: [10.1021/ja5132648](https://doi.org/10.1021/ja5132648).
- 66 S. Parvez, Y. Fu, J. Li, M. J. C. Long, H.-Y. Lin, D. K. Lee, G. S. Hu and Y. Aye, Substoichiometric Hydroxynonenylation of a Single Protein Recapitulates Whole-Cell-Stimulated Antioxidant Response, *J. Am. Chem. Soc.*, 2015, **137**(1), 10–13, DOI: [10.1021/ja5084249](https://doi.org/10.1021/ja5084249).
- 67 S. Parvez, M. J. C. Long, H.-Y. Lin, Y. Zhao, J. A. Haegele, V. N. Pham, D. K. Lee and Y. Aye, T-REX, on-Demand Redox Targeting in Live Cells, *Nat. Protoc.*, 2016, **11**(12), 2328–2356, DOI: [10.1038/nprot.2016.114](https://doi.org/10.1038/nprot.2016.114).
- 68 R. N. Reddi, E. Resnick, A. Rogel, B. V. Rao, R. Gabizon, K. Goldenberg, N. Gurwicz, D. Zaidman, A. Plotnikov, H. Barr, Z. Shulman and N. London, Tunable Methacrylamides for Covalent Ligand Directed Release Chemistry, *J. Am. Chem. Soc.*, 2021, **143**(13), 4979–4992, DOI: [10.1021/jacs.0c10644](https://doi.org/10.1021/jacs.0c10644).
- 69 C. Zhang, H. Ye, D. Ji, T. Xiong, J. Yu, Z. Qian, C. Li, H. Li, Z. Liang, Y. Zhang, J. Wu and Z. Huang, Warhead Strategy for Targeted Protein S-Nitrosation, *J. Am. Chem. Soc.*, 2025, **147**(30), 26726–26738, DOI: [10.1021/jacs.5c07409](https://doi.org/10.1021/jacs.5c07409).
- 70 E. Lundberg and G. H. H. Borner, Spatial Proteomics: A Powerful Discovery Tool for Cell Biology, *Nat. Rev. Mol. Cell Biol.*, 2019, **20**(5), 285–302, DOI: [10.1038/s41580-018-0094-y](https://doi.org/10.1038/s41580-018-0094-y).
- 71 N. C. Bauer, P. W. Doetsch and A. H. Corbett, Mechanisms Regulating Protein Localization, *Traffic*, 2015, **16**(10), 1039–1061, DOI: [10.1111/tra.12310](https://doi.org/10.1111/tra.12310).
- 72 B. Z. Stanton, E. J. Chory and G. R. Crabtree, Chemically Induced Proximity in Biology and Medicine, *Science*, 2018, **359**(6380), eaao5902, DOI: [10.1126/science.aao5902](https://doi.org/10.1126/science.aao5902).
- 73 J. B. Geri, J. V. Oakley, T. Reyes-Robles, T. Wang, S. J. McCarver, C. H. White, F. P. Rodriguez-Rivera, D. L. Parker, E. C. Hett, O. O. Fadeyi, R. C. Oslund and D. W. C. MacMillan, Microenvironment Mapping via Dexter Energy Transfer on Immune Cells, *Science*, 2020, **367**(6482), 1091–1097, DOI: [10.1126/science.aay4106](https://doi.org/10.1126/science.aay4106).
- 74 C. A. Kulkarni, B. D. Fink, B. E. Gibbs, P. R. Chheda, M. Wu, W. I. Sivitz and R. J. Kerns, A Novel Triphenylphosphonium Carrier to Target Mitochondria without Uncoupling Oxidative Phosphorylation, *J. Med. Chem.*, 2021, **64**(1), 662–676, DOI: [10.1021/acs.jmedchem.0c01671](https://doi.org/10.1021/acs.jmedchem.0c01671).
- 75 A. V. Milton and D. B. Konrad, Epithelial-Mesenchymal Transition and H<sub>2</sub>O<sub>2</sub> Signaling – a Driver of Disease Progression and a Vulnerability in Cancers, *Biol. Chem.*, 2022, **403**(4), 377–390, DOI: [10.1515/hsz-2021-0341](https://doi.org/10.1515/hsz-2021-0341).
- 76 *Reactive Oxygen Species and Oxidative Damage in Plants Under Stress*, 2015, ed. D. K. Gupta, J. M. Palma and F. J. Corpas, SpringerLink Bücher, Springer, Cham, 1st edn, 2015. DOI: [10.1007/978-3-319-20421-5](https://doi.org/10.1007/978-3-319-20421-5).
- 77 D. B. Zorov, M. Juhaszova and S. J. Sollott, Mitochondrial Reactive Oxygen Species (ROS) and ROS-Induced ROS Release, *Physiol. Rev.*, 2014, **94**(3), 909–950, DOI: [10.1152/physrev.00026.2013](https://doi.org/10.1152/physrev.00026.2013).
- 78 E. M. Ryan, M. A. Norinskiy, A. K. Bracken, E. E. Lueders, X. Chen, Q. Fu, E. T. Anderson, S. Zhang and M. E. Abbasov, Activity-Based Acylome Profiling with *N*-(Cyanomethyl)-*N*-(Phenylsulfonyl)Amides for Targeted Lysine Acylation and Post-Translational Control of Protein Function in Cells, *J. Am. Chem. Soc.*, 2024, **146**(40), 27622–27643, DOI: [10.1021/jacs.4c09073](https://doi.org/10.1021/jacs.4c09073).
- 79 M. Gehringer and S. A. Laufer, Emerging and Re-Emerging Warheads for Targeted Covalent Inhibitors: Applications in Medicinal Chemistry and Chemical Biology, *J. Med. Chem.*, 2019, **62**(12), 5673–5724, DOI: [10.1021/acs.jmedchem.8b01153](https://doi.org/10.1021/acs.jmedchem.8b01153).
- 80 L. Hillebrand, X. J. Liang, R. A. M. Serafim and M. Gehringer, Emerging and Re-Emerging Warheads for Targeted Covalent Inhibitors: An Update, *J. Med. Chem.*, 2024, **67**(10), 7668–7758, DOI: [10.1021/acs.jmedchem.3c01825](https://doi.org/10.1021/acs.jmedchem.3c01825).
- 81 L. M. Boatner, M. F. Palafox, D. K. Schweppe and K. M. Backus, CysDB: A Human Cysteine Database Based on Experimental Quantitative Chemoproteomics, *Cell Chem. Biol.*, 2023, **30**(6), 683–698, DOI: [10.1016/j.chembiol.2023.04.004](https://doi.org/10.1016/j.chembiol.2023.04.004).
- 82 M. Kuljanin, D. C. Mitchell, D. K. Schweppe, A. S. Gikandi, D. P. Nusinow, N. J. Bulloch, E. V. Vinogradova, D. L. Wilson, E. T. Kool, J. D. Mancias, B. F. Cravatt and S. P. Gygi, Reimagining High-Throughput Profiling of Reactive Cysteines for Cell-Based Screening of Large Electrophile Libraries, *Nat. Biotechnol.*, 2021, **39**(5), 630–641, DOI: [10.1038/s41587-020-00778-3](https://doi.org/10.1038/s41587-020-00778-3).
- 83 M. Takahashi, H. B. Chong, S. Zhang, T.-Y. Yang, M. J. Lazarov, S. Harry, M. Maynard, B. Hilbert, R. D. White, H. E. Murrey, C.-C. Tsou, K. Vordermark, J. Assaad, M. Gohar, B. R. Dürr, M. Richter, H. Patel, G. Kryukov, N. Brooijmans, A. S. O. Alghali, K. Rubio, A. Villanueva, J. Zhang, M. Ge, F. Makram, H. Griesshaber, D. Harrison, A.-S. Koglin, S. Ojeda, B. Karakyriakou, A. Healy, G. Popoola, I. Rachmin, N. Khandelwal, J. R. Neil, P.-C. Tien, N. Chen, T. Hosp, S. Van Den Ouweland, T. Hara, L. Bussema, R. Dong, L. Shi, M. Q. Rasmussen, A. C. Domingues, A. Lawless, J. Fang, S. Yoda, L. P. Nguyen, S. M. Reeves, F. N. Wakefield, A. Acker, S. E. Clark, T. Dubash, J. Kastanos, E. Oh, D. E. Fisher, S. Maheswaran, D. A. Haber, G. M. Boland, M. Sade-Feldman, R. W. Jenkins, A. N. Hata, N. M. Bardeesy, M. L. Suvà, B. R. Martin, B. B. Liau, C. J. Ott, M. N. Rivera, M. S. Lawrence and L. Bar-Peled, DrugMap: A Quantitative Pan-Cancer Analysis of Cysteine Ligandability, *Cell*, 2024, **187**(10), 2536–2556, DOI: [10.1016/j.cell.2024.03.027](https://doi.org/10.1016/j.cell.2024.03.027).
- 84 J. W. Brulet, A. M. Ciancone, K. Yuan and K. Hsu, Advances in Activity-Based Protein Profiling of Functional Tyrosines in Proteomes, *Isr. J. Chem.*, 2023, **63**(3–4), e202300001, DOI: [10.1002/ijch.202300001](https://doi.org/10.1002/ijch.202300001).
- 85 H. S. Hahm, E. K. Toroitich, A. L. Borne, J. W. Brulet, A. H. Libby, K. Yuan, T. B. Ware, R. L. McCloud, A. M. Ciancone and K.-L. Hsu, Global Targeting of Functional Tyrosines Using Sulfur-Triazole Exchange



- Chemistry, *Nat. Chem. Biol.*, 2020, **16**(2), 150–159, DOI: [10.1038/s41589-019-0404-5](https://doi.org/10.1038/s41589-019-0404-5).
- 86 S. Lin, X. Yang, S. Jia, A. M. Weeks, M. Hornsby, P. S. Lee, R. V. Nichiporuk, A. T. Iavarone, J. A. Wells, F. D. Toste and C. J. Chang, Redox-Based Reagents for Chemoselective Methionine Bioconjugation, *Science*, 2017, **355**(6325), 597–602, DOI: [10.1126/science.aal3316](https://doi.org/10.1126/science.aal3316).
- 87 X. Xie, P. J. Moon, S. W. M. Crossley, A. J. Bischoff, D. He, G. Li, N. Dao, A. Gonzalez-Valero, A. G. Reeves, J. M. McKenna, S. K. Elledge, J. A. Wells, F. D. Toste and C. J. Chang, Oxidative Cyclization Reagents Reveal Tryptophan Cation- $\pi$  Interactions, *Nature*, 2024, **627**(8004), 680–687, DOI: [10.1038/s41586-024-07140-6](https://doi.org/10.1038/s41586-024-07140-6).
- 88 S. J. Tower, W. J. Hetcher, T. E. Myers, N. J. Kuehl and M. T. Taylor, Selective Modification of Tryptophan Residues in Peptides and Proteins Using a Biomimetic Electron Transfer Process, *J. Am. Chem. Soc.*, 2020, **142**(20), 9112–9118, DOI: [10.1021/jacs.0c03039](https://doi.org/10.1021/jacs.0c03039).
- 89 A. V. West, G. Muncipinto, H.-Y. Wu, A. C. Huang, M. T. Labenski, L. H. Jones and C. M. Woo, Labeling Preferences of Diazirines with Protein Biomolecules, *J. Am. Chem. Soc.*, 2021, **143**(17), 6691–6700, DOI: [10.1021/jacs.1c02509](https://doi.org/10.1021/jacs.1c02509).
- 90 K. Bach, B. L. H. Beerkens, P. R. A. Zanon and S. M. Hacker, Light-Activatable, 2,5-Disubstituted Tetrazoles for the Proteome-Wide Profiling of Aspartates and Glutamates in Living Bacteria, *ACS Cent. Sci.*, 2020, **6**(4), 546–554, DOI: [10.1021/acscentsci.9b01268](https://doi.org/10.1021/acscentsci.9b01268).
- 91 A. X. Jones, Y. Cao, Y.-L. Tang, J.-H. Wang, Y.-H. Ding, H. Tan, Z.-L. Chen, R.-Q. Fang, J. Yin, R.-C. Chen, X. Zhu, Y. She, N. Huang, F. Shao, K. Ye, R.-X. Sun, S.-M. He, X. Lei and M.-Q. Dong, Improving Mass Spectrometry Analysis of Protein Structures with Arginine-Selective Chemical Cross-Linkers, *Nat. Commun.*, 2019, **10**(1), 3911, DOI: [10.1038/s41467-019-11917-z](https://doi.org/10.1038/s41467-019-11917-z).
- 92 B. Racioppo, N. Qiu and A. Adibekian, Serine Hydrolase Activity-Based Probes for Use in Chemical Proteomics, *Isr. J. Chem.*, 2023, **63**(3–4), e202300016, DOI: [10.1002/ijch.202300016](https://doi.org/10.1002/ijch.202300016).
- 93 P. N. Joshi and V. Rai, Single-Site Labeling of Histidine in Proteins, on-Demand Reversibility, and Traceless Metal-Free Protein Purification, *Chem. Commun.*, 2019, **55**(8), 1100–1103, DOI: [10.1039/C8CC08733D](https://doi.org/10.1039/C8CC08733D).
- 94 Faridoun, R. Ng, G. Zhang and J. J. Li, An Update on the Discovery and Development of Reversible Covalent Inhibitors, *Med. Chem. Res.*, 2023, **32**(6), 1039–1062, DOI: [10.1007/s00044-023-03065-3](https://doi.org/10.1007/s00044-023-03065-3).
- 95 R. A. Bauer, Covalent Inhibitors in Drug Discovery: From Accidental Discoveries to Avoided Liabilities and Designed Therapies, *Drug Discovery Today*, 2015, **20**(9), 1061–1073, DOI: [10.1016/j.drudis.2015.05.005](https://doi.org/10.1016/j.drudis.2015.05.005).
- 96 J. Singh, R. C. Petter, T. A. Baillie and A. Whitty, The Resurgence of Covalent Drugs, *Nat. Rev. Drug Discovery*, 2011, **10**(4), 307–317, DOI: [10.1038/nrd3410](https://doi.org/10.1038/nrd3410).
- 97 A. C. M. Van Esbroeck, A. P. A. Janssen, A. B. Cognetta, D. Ogasawara, G. Shpak, M. Van Der Kroeg, V. Kantae, M. P. Baggelaar, F. M. S. De Vrij, H. Deng, M. Allarà, F. Fezza, Z. Lin, T. Van Der Wel, M. Soethoudt, E. D. Mock, H. Den Dulk, I. L. Baak, B. I. Florea, G. Hendriks, L. De Petrocellis, H. S. Overkleeft, T. Hankemeier, C. I. De Zeeuw, V. Di Marzo, M. Maccarrone, B. F. Cravatt, S. A. Kushner and M. Van Der Stelt, Activity-Based Protein Profiling Reveals off-Target Proteins of the FAAH Inhibitor BIA 10-2474, *Science*, 2017, **356**(6342), 1084–1087, DOI: [10.1126/science.aaf7497](https://doi.org/10.1126/science.aaf7497).
- 98 S. Niessen, M. M. Dix, S. Barbas, Z. E. Potter, S. Lu, O. Brodsky, S. Planken, D. Behenna, C. Almaden, K. S. Gajiwala, K. Ryan, R. Ferre, M. R. Lazear, M. M. Hayward, J. C. Kath and B. F. Cravatt, Proteome-Wide Map of Targets of T790M-EGFR-Directed Covalent Inhibitors, *Cell Chem. Biol.*, 2017, **24**(11), 1388–1400, DOI: [10.1016/j.chembiol.2017.08.017](https://doi.org/10.1016/j.chembiol.2017.08.017).
- 99 K. Garber, Probing the Proteome, *Nat. Biotechnol.*, 2025, **43**(8), 1216–1220, DOI: [10.1038/s41587-025-02737-2](https://doi.org/10.1038/s41587-025-02737-2).
- 100 K. A. Baltgalvis, K. N. Lamb, K. T. Symons, C.-C. Wu, M. A. Hoffman, A. N. Snead, X. Song, T. Glaza, S. Kikuchi, J. C. Green, D. C. Rogness, B. Lam, M. E. Rodriguez-Aguirre, D. R. Woody, C. L. Eissler, S. Rodiles, S. M. Negron, S. M. Bernard, E. Tran, J. Pollock, A. Tabatabaei, V. Contreras, H. N. Williams, M. K. Pastuszka, J. J. Sigler, P. Pettazzoni, M. G. Rudolph, M. Classen, D. Brugger, C. Claiborne, J.-M. Plancher, I. Cuartas, J. Seoane, L. E. Burgess, R. T. Abraham, D. S. Weinstein, G. M. Simon, M. P. Patricelli and T. M. Kinsella, Chemoproteomic Discovery of a Covalent Allosteric Inhibitor of WRN Helicase, *Nature*, 2024, **629**(8011), 435–442, DOI: [10.1038/s41586-024-07318-y](https://doi.org/10.1038/s41586-024-07318-y).
- 101 M. E. Kavanagh, B. D. Horning, R. Khattry, N. Roy, J. P. Lu, L. R. Whitby, E. Ye, J. C. Brannon, A. Parker, J. M. Chick, C. L. Eissler, A. J. Wong, J. L. Rodriguez, S. Rodiles, K. Masuda, J. R. Teijaro, G. M. Simon, M. P. Patricelli and B. F. Cravatt, Selective Inhibitors of JAK1 Targeting an Isoform-Restricted Allosteric Cysteine, *Nat. Chem. Biol.*, 2022, **18**(12), 1388–1398, DOI: [10.1038/s41589-022-01098-0](https://doi.org/10.1038/s41589-022-01098-0).
- 102 H. J. Wobst, A. Viader, G. Muncipinto, R. Hollibaugh, D. Van Kalken, C. T. Burkhardt, S. M. Cantin, R. M. Bates, Y. Regimbald-Dumas, L. Gross, M. T. Antalek, J. E. Zweig, F. Wu, T. J. Rettenmaier, M. T. Labenski, N. Pullen, H. S. Blanchette, J. L. Henderson, H. H. Weng, T. A. Vaughn, D. G. Brown, J. P. Throup and J. C. Barrish, SLC6A19 Inhibition Facilitates Urinary Neutral Amino Acid Excretion and Lowers Plasma Phenylalanine, *JCI Insight*, 2024, **9**(21), e182876, DOI: [10.1172/jci.insight.182876](https://doi.org/10.1172/jci.insight.182876).
- 103 L. P. Conway, A. M. Jadhav, R. A. Homan, W. Li, J. S. Rubiano, R. Hawkins, R. M. Lawrence and C. G. Parker, Evaluation of Fully-Functionalized Diazirine Tags for Chemical Proteomic Applications, *Chem. Sci.*, 2021, **12**(22), 7839–7847, DOI: [10.1039/D1SC01360B](https://doi.org/10.1039/D1SC01360B).

