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Current Opinion in Chemical Biology

# Activity- and reactivity-based proteomics: Recent technological advances and applications in drug discovery

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**Abstract**

Activity-based protein profiling (ABPP) is recognized as a powerful and versatile chemoproteomic technology in drug discovery. Central to ABPP is the use of activity-based probes to report the activity of specific enzymes or reactivity of amino acid types in complex biological systems. Over the last two decades, ABPP has facilitated the identification of new drug targets and discovery of lead compounds in human and infectious disease. Furthermore, as part of a sustained global effort to illuminate the druggable proteome, the repertoire of target classes addressable with activity-based probes has vastly expanded in recent years. Here, we provide an overview of ABPP and summarise the major technological advances with an emphasis on probe development.

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**Current Opinion in Chemical Biology** 2021, **60**:20–29

This reviews comes from a themed issue on **Omics**

Edited by **Nichollas Scott** and **Laura Edgington-Mitchell**

For a complete overview see the Issue and the Editorial

https://doi.org/10.1016/j.cbpa.2020.06.011



**Keywords**

Activity-based protein profiling, Reactivity-based protein profiling, Activity-based probes, Chemical proteomics, Drug discovery, Target identification and validation.

## Background

Recent genetic evidence reveals a significant proportion of understudied proteins contribute to human disease [1]. Despite this, biomedical researchers typically focus on a narrow set of well-characterised targets [2], resulting in only ~10% of the human proteome being accessible to pharmacological modulation by antibodies or chemical tools [3]. There is an ongoing need to develop new tools and technologies to support identification of new drug targets and conception of novel therapeutic strategies.

Activity-based protein profiling (ABPP) is a technique for monitoring the activity of enzymes in complex proteomes based on chemoproteomics — the combined application of chemical probes with quantitative proteomics. Since the 1990s, ABPP has been successfully used to characterise protein function, supporting the discovery of druggable targets and small molecule inhibitors [4]. Central to ABPP is the use of activity-based probes (ABPs), which are small molecules functionalised with; (1) an electrophilic warhead for covalent binding of specific amino acids on target proteins; (2) a linker that tunes the specificity of the ABP and/or minimises undesirable interactions between warhead and tag and; (3) a fluorescent and/or affinity tag for detection or purification of labelled targets (Figure 1a). Following labelling, proteins can be visualised by in-gel fluorescence scanning of proteins separated by SDS-PAGE, or enriched (typically via avidin-based enrichment of biotin-tagged targets) for downstream identification by liquid chromatography-tandem mass spectrometry (LC-MS/MS; Figure 1b) [4]. For MS-based analyses, enzyme activity can be quantitatively assessed in two or more samples using label-free quantification or isotopic labelling. In the latter approach, unique isotopic signatures are typically introduced into proteins or peptides through the use of isotopically differentiated biotin tags, tandem mass tags (TMTs), or by Stable Isotope Labeling by Amino acids in Cell culture (SILAC) [5]. This is commonly applied to identify aberrant enzyme activity in disease models (Figure 1ci) or assess target engagement of small molecule inhibitors (Figure 1cii).

Over the past two decades, ABPs have been developed for profiling the situational activity of a diverse range of enzymes classes [4,6]. These probes target conserved residues within the active site of their target proteins, with specificity driven by the mechanistic differences between enzyme families. Early examples of class-specific probes include the fluorophosphonates for

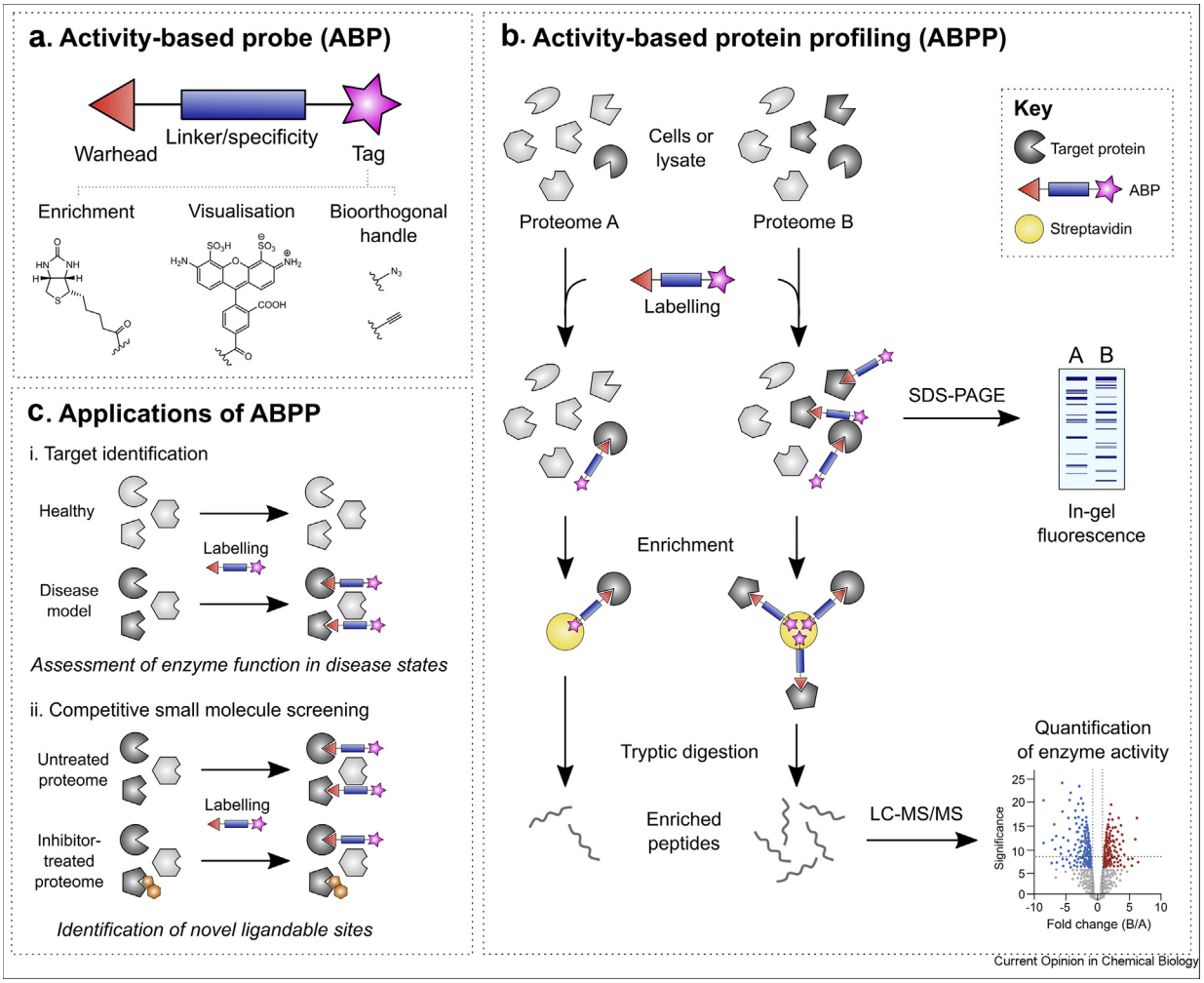
labelling active-site of serine hydrolases and acyloxymethyl ketone–based probes for papain-like cysteine proteases [7,8]. Since these initial developments, probes have been developed for chemoselective profiling of diverse enzyme classes including cathepsins [9], legumains [10], caspases [11], proteasomal proteases [12], arginine methyltransferases [13], kinases [14], tyrosine and serine/threonine phosphatases [15–17], glycosidases [18] and deubiquitinases (DUBs) [19]. Together, their applications have provided new insights into the functions of proteins in a variety of disease contexts [20–22], uncovering new biology and facilitating the identification of drugs and drug targets [23].

This review aims to provide an update on the technological advances in ABPP over the last two years. Here, we focus on the development of probes for new enzyme classes and amino acid types, highlighting the key insights in drug discovery that have been enabled through their application. We apologise to colleagues whose work may not have been mentioned due to space limitations.

## Class-specific probes

Recent advances in class-specific profiling have focused on expanding the repertoire of enzymes targetable with ABPs. This includes proteins that mediate the addition ('writers') and removal ('erasers') of post-translational

**Figure 1**




**Overview of activity-based protein profiling (ABPP). (a)** Generic structure of an activity-based probe (ABP). ABPs may be functionalised with affinity tags (e.g. biotin, depicted) and/or fluorophores (e.g. Alexa Fluor 488) for enrichment and visualisation of labelled species, respectively. Latent handles (e.g. azide or alkyne groups) may be incorporated to enable bioorthogonal ligation of tags via copper(I)-catalysed azide–alkyne cycloaddition (CuAAC) [24]. **(b)** General workflow of an ABPP experiment. Typically, proteomes are labelled in cells or *in vitro* with an ABP directed toward a particular enzyme class or amino acid type. If a fluorophore tag is used, labelled proteins are separated on by SDS-PAGE and directly visualised via fluorescence scanning. By contrast, biotinylated proteins are enriched on a streptavidin-immobilised resin and digested, generating probe-modified peptides for analysis by liquid chromatography-mass spectrometry (LC-MS/MS). Differences in the extent of protein labelling between two independent samples (e.g. healthy and diseased tissue) can be quantified or visualised, enabling the identification of new drug targets or sites for inhibitor development. (**c**) Common applications of ABPP for target identification and in small-molecule screens.

modifications (PTMs), and whose dysregulation is associated with the onset of a diverse range of human diseases [25]. For instance, Nemmara et al. [26] developed a series of benzimadzole-alkyne probes for selective in-cell labelling of protein arginine deaminases, which catalyse protein citrullination and are implicated in a range of malignant cancers and autoimmune conditions such as rheumatoid arthritis and Alzheimer's disease. Furthermore, ABPs have been developed for various components of the ubiquitin (Ub) protein degradation system (E1, E2, E3 enzymes and DUBs), whose aberrant activity can lead to neurodegeneration, developmental disorder or cancer [27]. Typically, these probes feature one or more full-length Ub moieties that serve as a common recognition element for Ub-conjugating and deconjugating enzymes, and/or a C-terminal electrophile that binds to a catalytic cysteine residue within the active site of their respective target(s) (comprehensively reviewed in a study by Hewings et al. [28]). More recent work has involved tuning the specificity of probes toward distinct subclasses of enzymes. For instance, E2-Ub conjugates have been engineered to incorporate unnatural acids for selective labelling of RING and HECT family E3 ligases [29,30]). To overcome issues associated with cell impermeability of large Ub-based probes, peptide- and small-molecule–based ABPs have been developed for profiling DUB activity in live cells [31–33]. In a recent study, Nattawadee et al. [33] report the first selective cyanopyrrolidine probe for Ubiquitin Carboxy-Terminal Hydrolase L1 (UCHL1), and demonstrate its use in identifying potent inhibitors for UCHL1–dependent pulmonary fibrosis. In addition, small-molecule ABPs have been used to determine the selectivity profiles of two putative covalent USP14/UCH-37 inhibitors, b-AP15 and VLX1570 (a phase I/II clinical trial candidate) [34]. Here, competitive ABPP experiments using alkyne-tagged analogues revealed that these compounds interact with many additional proteins beyond their reported targets, leading to the formation of large protein aggregates and cell death. While providing a molecular explanation for the cytotoxic chemotype of these inhibitors, this study emphasised the importance of using validated chemical tools for probing DUB activity.

Metabolic enzymes have also received increased attention as targets for probe development. For example, Hoegl et al. [35] describe the development of clickable pyridoxal analogues for profiling the activity of pyridoxal phosphate–dependent enzymes (PLP-DEs), which are widely recognised as important drug targets in infectious and human disease [36]. Here, proteomic profiling of PLP-DE activity in the bacterial pathogen *Staphylococcus aureus* revealed several potential targets for PLP-based drug development and was later successfully applied to screen off-target activity of the antituberculosis drug, D-cycloserine. However, one inherent limitation of using pyridoxal mimetics in prokaryotic cells is the requirement to ablate the biosynthesis of native PLP substrates, which otherwise compete with the probe for active site of PLP-DEs. As highlighted by the authors, this precludes full proteomic coverage of PLP-DEs in these systems and potentially misses important therapeutic targets. More recently, a broad-spectrum ABP, STA-55, was reported for the aldehyde dehydrogenase (ALDH) family [37], which has roles in diverse metabolic pathways and are associated with a range of metabolic diseases including type II hyperprolinaemia and Sjögren–Larson syndrome [38]. In this study, the authors applied STA-55 to screen the selectivity profiles of published ALDH inhibitors in A549 lung cancer cells, highlighting their use in validating target engagement against this enzyme class. This revealed that putative ALDH inhibitors.

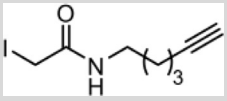
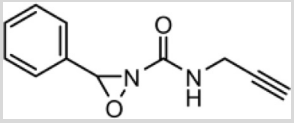
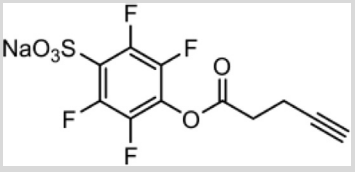
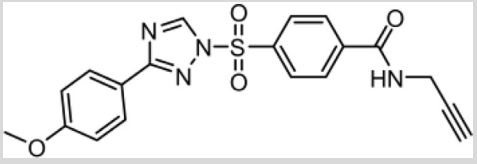
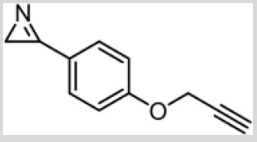
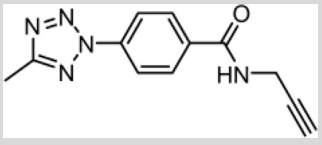
## Broad-spectrum reactivity probes

Nucleophilic amino acids facilitate diverse biochemical functions in proteins, ranging from enzyme catalysis to serving as sites for PTM. Moreover, electrophilic moieties that engage amino acid–associated nucleophiles are being increasingly incorporated into covalent ligands and drugs to enhance their selectivity, potency and/or pharmacokinetic properties [39,40]. For instance, targeting of noncatalytic cysteines in oncogenic kinases has emerged as a successful strategy for enhancing the selectivity of tyrosine kinase inhibitors, culminating in U.S. Food and Drug Administration (FDA) approval of several covalent drugs for cancer treatment [41].

The recent success of covalent inhibitors in the clinic has stimulated the development of chemical proteomic technologies to identify new nucleophilic sites for covalent targeting. One key advance has been 'reactivity-based profiling' (RBP), which is typically based on the isotopic tandem orthogonal proteolysis ABPP (isoTOP-ABPP) platform [42]. IsoTOP-ABPP and related methods are reliant upon 'broad-spectrum reactivity probes' which possess an electrophilic warhead with preferential reactivity for a specific amino acid type(s) (Table 1). These probes do not typically possess any of the aforementioned structural elements (linkers, detection moieties). This increases cell permeability and minimises any possible structural contributions to probe-target binding energies, effectively eliminating any 'specificity' elements not related to the chemistry of the warhead. For isoTOP-ABPP, a proteome is treated with two concentrations (e.g. 1 × and 10×) of an alkyne-linked broad-spectrum reactive probe. These two independently labelled proteomes are then click-conjugated to biotin tags, combined, enriched, digested and analysed by LC-MS/MS. Because peptides modified by the probe are directly detected, both the target protein and probe-modified residue(s) are identified. The underlying rationale for the method is that by comparing the extent of labelling between the two

**Table 1**

**Published reactivity probes and their proteomic applications.**

| Probe | Specificity | Structure | Year | Applications[a] |
|---|---|---|---|---|
| IA-alkyne | Cysteine |  | 2010 | RP, LD, PSI |
| O×4 | Methionine |  | 2017 | RP |
| STP-alkyne | Lysine |  | 2017 | RP. LD |
| HHS-482 | Tyrosine |  | 2020 | RP, LD. PSI |
| AZ-9 | Aspartate, Glutamate |  | 2020 | RP |
| Probe 2 | Aspartate, Glutamate |  | 2020 | LD |

[a] RP = reactivity profiling; LD = ligand discovery; PSI = PTM site identification; PTMs = post-translational modifications; STP = sulphotetrafluorophenyl.

treatments, the reactivity of an individual labelled residue can be quantified; 'hyperreactive' sites label equally at high and low concentrations, whereas less reactive sites are only identified when the probe is present at saturating levels. Initially applied to rank cysteine reactivity in the human proteome using an iodoacetamide-alkyne (IA-alkyne) probe (Table 1) [42], hyperreactivity was found to be a predictor of cysteine functionality. This identified a key application for broad-spectrum RBP in identifying functional sites with potential druggability.

Following its initial development, isoTOP-ABPP has since been adapted for competitive screening of endogenous and exogenous small molecules. Here, proteomes are pretreated with a cysteine-reactive electrophile prior to probe labelling, and ligand binding at a given residue is assessed *via* a reduction in labelling relative to a vehicle-treated control. This has enabled the identification of cysteines that are sensitive to oxidative PTMs [43,44], as well as those targeted by natural products and FDA-approved inhibitors [45,46]. Furthermore, competitive isoTOP-ABPP has been applied for proteome-wide screening of cysteine-reactive covalent fragments, facilitating the identification of reactive sites that can be pursued as targets in traditional site-directed ligand discovery [47].

To further investigate proteins targetable with covalent inhibitors, researchers have expanded this technology to other amino acid types. To date, alkyne probes have been developed for chemoselective profiling of methionine [48], lysine [49], tyrosine [50], aspartate and glutamate (Table 1) [51,52], which together have facilitated the identification of new targets in a variety of disease contexts. For instance, Hacker et al. [49] developed a lysine-directed sulphotetrafluorophenyl probe (STP-alkyne; Table 1) for competitive screening of amine-reactive fragments against the proteomes of three cancer cell lines. These studies revealed several hyperreactive and ligandable lysines in proteins considered to be 'undruggable' and/or lacking small-molecule probes. Furthermore, ligand binding was demonstrated to inhibit the activity of several metabolic enzymes through the disruption of active, allosteric and protein−protein interaction sites. More recently, sulfur-triazole exchange (SuTEx) chemistry was introduced to facilitate the development of phenol-reactive probes for functional profiling of reactive tyrosines [50]. Originally applied to monitor the activation of phosphotyrosine sites in cancer cells via global inhibition of tyrosine phosphatase activity, the sulfur-triazole exchange platform was subsequently adapted for fragment-based ligand discovery [53]. Here, competitive screening of phenol-reactive 1,2,4-sulfonyl triazoles identified ligandable sites in several oncogenes that affected enzyme activity. This work highlighted tyrosine residues as viable therapeutic targets in ovarian and squamous cell lung carcinomas. Supporting the rapidly increasing applications of this ABPP approach, Bach et al. [51] recently developed a series of photoactivatable 2,5-disubstituted tetrazoles for site-specific labelling of aspartates and glutamates (Table 1). Applied to the bacterial pathogen *S. aureus* for competitive screening of carboxylic acid−directed ligands, this study highlighted hydrazonyl chlorides as promising electrophiles for the development of covalent inhibitors targeting these amino acids.

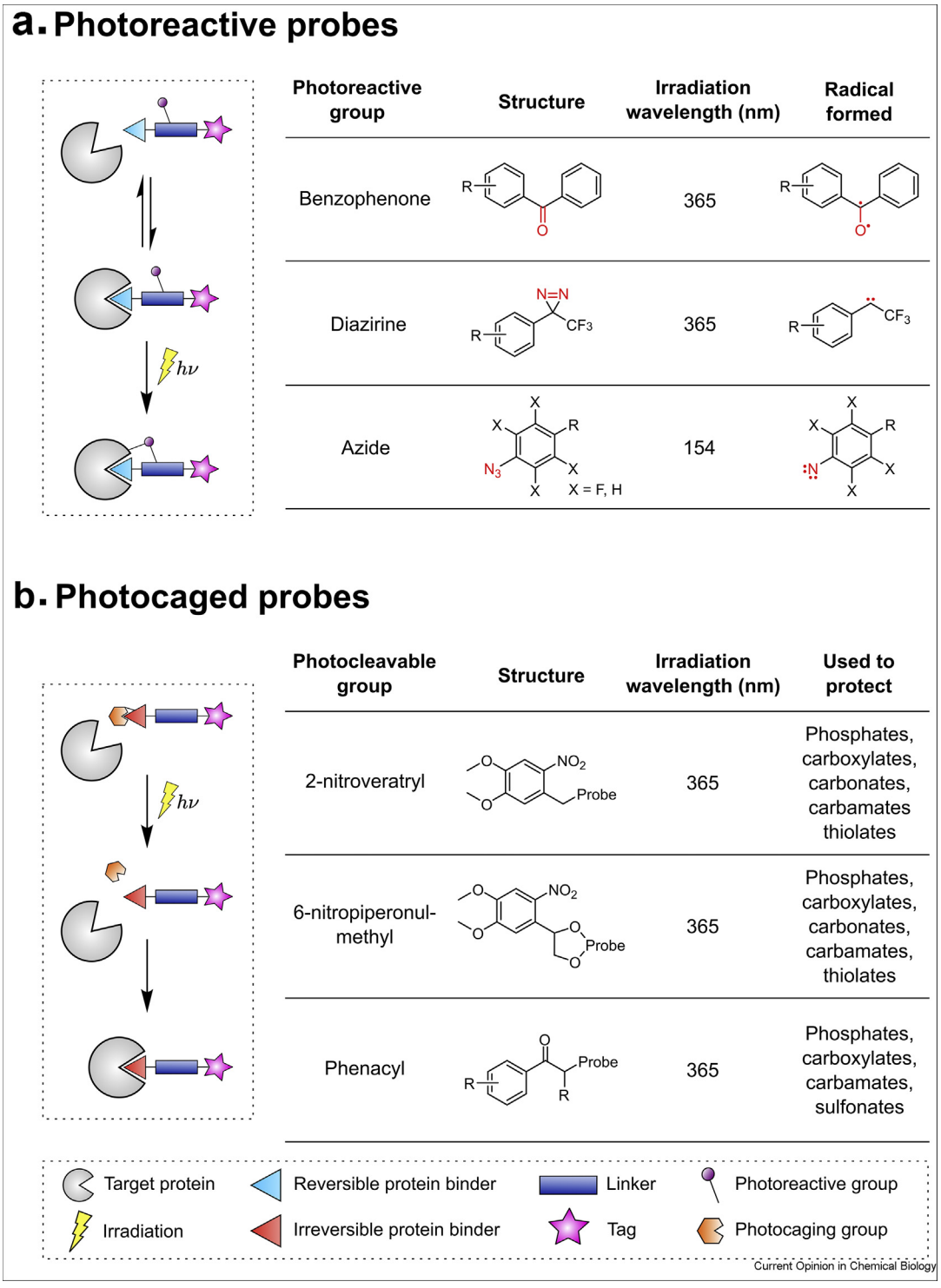
Future developments in RBP may involve the application to amino acids beyond those highlighted in this review. For example, while yet to be applied at the proteome level, chemistries have been developed for site-specific labelling of histidine [54] and tryptophan [55] residues in recombinant proteins. However, the identification and accurate quantification of probe-labelled sites remains a major challenge in residue-centric proteomics. For instance, MS1-based quantification (e.g. Stable Isotope Labelingby Amino acids in Cell culture) is often performed using complex bespoke data analysis pipelines (e.g. CIMAGE) [42], which may not be easily implemented in nonspecialist labs. Furthermore, these commonly used methods are limited in their multiplexing capacity, with only two samples typically being analysed in a single MS run. The recent integration of isobaric tagging (e.g. TMT) into ABPP workflows has enabled analysis of 10 samples in parallel [56] and may increase the accessibility of multiplexed peptide/protein quantification. However, these MS2 labelling approaches require advanced triple-stage MS (MS3) methods to remove 'ratio compression' [57], a phenomenon in which coeluting peptides distort the differences in TMT reporter ion abundance between samples.

## Photoactivatable probes

A recent advance in ABP technology is the development of photoactivatable probes, which fall into two main categories; photoreactive and photocaged. As depicted in Figure 1a, the typical workflow of ABPP relies upon covalent binding of an ABP to its target protein(s). This has limited the application of this technology to target classes where covalent binding mechanisms are chemically accessible. However, profiling targets of reversible, noncovalent, small molecules is often required. Photoreactive probes incorporate an additional moiety, which on irradiation with ultraviolet (UV) light form highly reactive, radical species. This radical then drives the formation of a covalent bond between the probe and reversibly bound proteins (Figure 2a). The reactivity and instability of the radical typically helps ensure that only direct targets of the probe are covalently modified [58,59]. Common photoreactive groups include benzophenones [60], diazirines [61] and aryl azides [62] (Figure 2a). Recently, these have been successfully incorporated into ABPs to facilitate the identification of targets of diaminoquinazolines and truncated myosin A peptides in the malaria parasite, *Plasmodium falciparum* [63,64], and the engagement of inhibitors for cannabinoid receptors [65] and poly (ADP-ribose) polymerases (PARPs) [61].

Prioritisation of potential targets identified by ABPP is one of the fundamental challenges the field faces.

**Figure 2**




**Mechanisms of photoreactive and photocaged probes.** (**a**) Photoreactive probes incorporate a reversible protein binder and a photoreactive group which, on irradiation with suitable wavelengths of UV light, form a highly reactive radical which irreversibly binds the protein of interest facilitating the downstream workflow of ABPP. Photocaged probes include a light-sensitive protecting group on the electrophilic warhead which, on irradiation with UV light, is removed, exposing the warhead which can bind the protein of interest. (**b)** Examples of commonly used photoreactive groups, the wavelength of UV light required to form the reactive radical and the radical formed [18,59,68]. (**c**) Examples of potential photocaging moieties, including wavelength required for cleavage and groups they can be used to protect, demonstrating the potential breadth of use [69,70]. UV, ultraviolet; ABPP, activity-based protein profiling.

Although efforts are made to enhance specificity, reactive electrophiles will invariably bind to a multitude of proteins. In addition to facilitating the profiling of proteins which cannot be targeted by covalent small molecules, photoreactive probes were used in a recent study into the stereoselectivity of protein targets, and how this may be used to aid in target prioritisation. Eight pairs of ABPs were synthesised, all of which incorporated photoreactive diazirine moieties and differed only in absolute stereochemistry, and screened in cell-based assays. More than 150 of the identified proteins exhibited stereo-specific interactions, with only one stereoisomer of the enantioprobe pair engaging the target. Where target enrichment was seen with several enantioprobes, stereo-specific interactions were exhibited with only one probe pair. Protein targets identified spanned wide functional and structural classes, and several were previously unidentified. Known ligands were shown to inhibit enantioprobe binding, validating probe engagement of functional targets.

Enantioprobes can be used to prioritise targets, with only probes exhibiting stereoselective interactions taken forward for further medicinal chemistry studies. This novel ABPP technique not only enhances field-wide knowledge of the druggable proteome but may be useful in future medicinal chemistry studies to develop high-affinity and suitably selective inhibitors [56]. It is however worth noting that this study did not rule out the possibility that stereo-specific interactions were as a result of preferential proximity of the photoreactive group to the protein in one enantiomer, leading to enhanced binding affinity.

Photocaged probes incorporate a protecting group on the probe's electrophilic warhead, which is cleaved upon exposure to specific wavelengths of UV light. In effect the probe's reactivity is masked until the electrophile is uncaged, enabling cell concentration of the probe to equilibrate before labelling and preventing probe interactions with membrane thiols and reactive lipids. This increases spatial and temporal control over target engagement, which can be restricted to a specific cellular region, or a specific time point during treatment [66] (Figure 2b). Photocaging strategies rely on validated protecting groups (Figure 2b) and have only recently been applied to ABPP, with their use to-date limited to profiling the reactivity of cysteines [66,67]. In this study, the authors demonstrated that photocaging of an iodoacetamide probe limited broad-spectrum reactivity and mass off-target engagement, providing notable benefits including reduced cytotoxicity and decreased background readings [67]. As implementation of this technique remains limited, effects of the caging group on probe-protein binding affinity and effective cellular distribution are yet to be ruled out as potential drawbacks.

## Future directions of ABPP

The classes of enzyme and amino acid now addressable with ABPs have expanded tremendously over recent years. Collectively, the application of these probes to a diverse range of biological systems has facilitated the discovery of novel drug targets and inhibitors in a variety of disease contexts, which serve as a strong foundation for the rational design of next-generation therapeutics. Considering persistent global health challenges such as antimicrobial resistance, there remains a continual need to expand the druggable proteome and develop new mechanistic classes of drugs. With new advances in synthetic chemistry and the advent of exciting global initiatives such as Target 2035 (which aims to develop pharmacological tools to study the entire human proteome by 2035) [1], it can be anticipated that the extent of protein classes targetable by ABPP will rise exponentially in the near future. Future developments could also involve expanding the ABPP workflow to target other biomolecules, such as nucleic acids. By coupling traditional enrichment methods with a next-generation sequencing read-out, it may be possible to quantitatively profile reactive sites within clinically relevant DNA or RNA (e.g. regulatory or ribosomal RNAs), aiding the development new therapeutic strategies.

Recent developments in stereospecific profiling of protein-fragment interactions has provided powerful means to prioritise ligandable sites as prospective targets. Despite these advances, the selection of identified targets for downstream investigation remains biased and is typically based on the availability of assays and/or functional information for the associated protein. As a result, uncharacterised targets with potential therapeutic value are often overlooked. Moreover, there currently exists no method for expedient prioritisation of enzymes, reactive or ligandable amino acids as targets following their proteomic identification. Current genetic approaches for functional characterisation of proteins and/or specific amino acid residues involve time-consuming complementation and mutagenesis strategies. Hence, there is a clear need to develop technologies that enable functional prioritisation of reactive sites and the full realisation of ABPP's remarkable potential. With the advent of genome editing technologies such as CRISPR/Cas9, the functional characterisation of proteins and reactive sites is now possible at a systems level. Thus, integration of such technologies with ABPP into systematic 'multiomics' platforms for target prioritisation may address a bottleneck in the early drug discovery pipeline and facilitate the expansion of the druggable proteome. Indeed, CRISPR-based saturated mutagenesis screens have been developed to assess the functionality of amino acids within individual genes and at multiple loci across genomes [71–73]. However, to our knowledge, these approaches have yet been applied

in a residue-specific manner to interrogate reactive sites identified in ABPP experiments.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

This work was supported by grants BB/M011178/1 from the BBSRC (to HJB, EWT and MAC), NC/S001239/1 from the NC3Rs (to CJW and MAC), and 202553/Z/16/Z from the Wellcome Trust & Royal Society (to MAC).

## References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. ** Carter AJ, Kraemer O, Zwick M, Mueller-Fahrnow A, Arrowsmith CH, Edwards AM: **Target 2035: probing the human proteome**. *Drug Discov Today* 2019, **24**:2111–2115.
Highlights the importance of expanding the druggable proteome. This article introduces Target 2035, a collaborative initiative that aims to generate pharmacological modulators for the majority of human proteome within the next 15 years

2. Nelson MR, Tipney H, Painter JL, Shen J, Nicoletti P, Shen Y, Floratos A, Sham PC, Li MJ, Wang J, *et al.*: **The support of human genetic evidence for approved drug indications**. *Nat Genet* 2015, **47**:850–860.

3. Oprea TI, Bologa CG, Brunak S, Campbell A, Gan GN, Gaulton A, Gomez SM, Guha R, Hersey A, Holmes J, *et al.*: **Unexplored therapeutic opportunities in the human genome**. *Nat Rev Drug Discov* 2018, **17**:317–332.

4. Cravatt BF, Wright AT, Kozarich JW: **Activity-based protein profiling: from enzyme chemistry to proteomic chemistry**. *Annu Rev Biochem* 2008:383–414.

5. Chen X, Wong YK, Wang J, Zhang J, Lee YM, Shen HM, Lin Q, Hua ZC: **Target identification with quantitative activity based protein profiling (ABPP)**. *Proteomics* 2017, **17**.

6. Yang P, Liu K: **Activity-based protein profiling: recent advances in probe development and applications**. *ChemBioChem* 2015, **16**:712–724.

7. Liu Y, Patricelli MP, Cravatt BF: **Activity-based protein profiling: the serine hydrolases**. *Proc Natl Acad Sci USA* 1999, **96**:14694–14699.

8. Greenbaum D, Baruch A, Hayrapetian L, Darula Z, Burlingame A, Medzihradszky KF, Bogyo M: **Chemical approaches for functionally probing the proteome**. *Mol Cell Proteomics : MCP* 2002, **1**:60–68.

9. Bogyo M, Verhelst S, Bellingard-Dubouchaud V, Toba S, Greenbaum D: **Selective targeting of lysosomal cysteine proteases with radiolabeled electrophilic substrate analogs**. *Chem Biol* 2000, **7**:27–38.

10. Sexton KB, Witte MD, Blum G, Bogyo M: **Design of cell-permeable, fluorescent activity-based probes for the lysosomal cysteine protease asparaginyl endopeptidase (AEP)/legumain**. *Bioorg Med Chem Lett* 2007, **17**:649–653.

11. Sexton KB, Kato D, Berger AB, Fonovic M, Verhelst SHL, Bogyo M: **Specificity of aza-peptide electrophile activity-based probes of caspases**. *Cell Death Differ* 2007, **14**:727–732.

12. Hewings DS, Flygare JA, Wertz IE, Bogyo M: **Activity-based probes for the multicatalytic proteasome**. *FEBS J* 2017, **284**:1540–1554.

13. Obianyo O, Causey CP, Osborne TC, Jones JE, Lee YH, Stallcup MR, Thompson PR: **A chloroacetamidine-based inactivator of protein arginine methyltransferase 1: design, synthesis, and in vitro and in vivo evaluation**. *ChemBioChem* 2010, **11**:1219–1223.

14. Patricelli MP, Szardenings AK, Liyanage M, Nomanbhoy TK, Wu M, Weissig H, Aban A, Chun D, Tanner S, Kozarich JW: **Functional interrogation of the kinome using nucleotide acyl phosphates**. *Biochemistry* 2007, **46**:350–358.

15. Kumar S, Zhou B, Liang F, Wang WQ, Huang Z, Zhang ZY: **Activity-based probes for protein tyrosine phosphatases**. *Proc Natl Acad Sci USA* 2004, **101**:7943–7948.

16. Kalesh KA, Tan LP, Lu K, Gao L, Wang J, Yao SQ: **Peptide-based activity-based probes (ABPs) for target-specific profiling of protein tyrosine phosphatases (PTPs)**. *Chem Commun* 2010, **46**:589–591.

17. Shreder KR, Liu Y, Nomanhboy T, Fuller SR, Wong MS, Gai WZ, Wu J, Leventhal PS, Lill JR, Corral S: **Design and synthesis of AX7574: a microcystin-derived, fluorescent probe for serine/threonine phosphatases**. *Bioconjugate Chem* 2004, **15**:790–798.

18. Vocadlo DJ, Bertozzi CR: **A strategy for functional proteomic analysis of glycosidase activity from cell lysates**. *Angew Chem Int Ed* 2004, **43**:5338–5342.

19. Conole D, Mondal M, Majmudar JD, Tate EW: **Recent developments in cell permeable deubiquitinating enzyme activity-based probes**. *Front Chem* 2019, **7**:876.

20. Benns HJ, Tate EW, Child MA: **Activity-based protein profiling for the study of parasite biology**. *Curr Top Microbiol Immunol* 2018, **420**:155–174.

21. Nomura DK, Dix MM, Cravatt BF: **Activity-based protein profiling for biochemical pathway discovery in cancer**. *Nat Rev Cancer* 2010, **10**:630–638.

22. Keller LJ, Babin BM, Lakemeyer M, Bogyo M: **Activity-based protein profiling in bacteria: applications for identification of therapeutic targets and characterization of microbial communities**. *Curr Opin Chem Biol* 2020, **54**:45–53.

23. Moellering RE, Cravatt BF: **How chemoproteomics can enable drug discovery and development**. *Chem Biol* 2012, **19**:11–22.

24. Kolb HC, Finn MG, Sharpless KB: **Click chemistry: diverse chemical function from a few good reactions**. *Angew Chem Int Ed Engl* 2001, **40**:2004–2021.

25. Karve TM, Cheema AK: **Small changes huge impact: the role of protein posttranslational modifications in cellular homeostasis and disease**. *J Amino Acids* 2011, **2011**:207691.

26. Nemmara VV, Subramanian V, Muth A, Mondal S, Salinger AJ, Maurais AJ, Tilvawala R, Weerapana E, Thompson PR: **The development of benzimidazole-based clickable probes for the efficient labeling of cellular protein arginine deiminases (PADs)**. *ACS Chem Biol* 2018, **13**:712–722.

27. Rape M: **Ubiquitylation at the crossroads of development and disease**. *Nat Rev Mol Cell Biol* 2018, **19**:59–70.

28. Hewings DS, Flygare JA, Bogyo M, Wertz IE: **Activity-based probes for the ubiquitin conjugation-deconjugation machinery: new chemistries, new tools, and new insights**. *FEBS J* 2017, **284**:1555–1576.

29. Mathur S, Fletcher AJ, Branigan E, Hay RT, Virdee S: **Photo-crosslinking activity-based probes for ubiquitin RING E3 ligases**. *Cell Chem Biol* 2020, **27**:74–82 e76.

30. Xu L, Fan J, Wang Y, Zhang Z, Fu Y, Li YM, Shi J: **An activity-based probe developed by a sequential dehydroalanine formation strategy targets HECT E3 ubiquitin ligases**. *Chem Commun (Camb)* 2019, **55**:7109–7112.

31. Gui W, Ott CA, Yang K, Chung JS, Shen S, Zhuang Z: **Cell-Permeable activity-based ubiquitin probes enable intracellular profiling of human deubiquitinases**. *J Am Chem Soc* 2018, **140**:12424–12433.

32. Geurink PP, Kooij R, Sapmaz A, Liu S, Xin B-T, Janssen GMC, van Veelen PA, Dijke Pt, Ovaa H: **A small-molecule activity-based probe for monitoring ubiquitin C-terminal hydrolase L1 (UCHL1) activity in live cells and Zebrafish Embryos**. *bioRxiv* 2019.

33. * Panyain N, Godinat A, Lanyon-Hogg T, Lachiondo-Ortega S, Will EJ, Soudy C, Mondal M, Mason K, Elkhalifa S, Smith LM, *et al.*: **Discovery of a potent and selective covalent inhibitor and activity-based probe for the deubiquitylating enzyme UCHL1, with antifibrotic activity**. *J Am Chem Soc* 2020, **142**: 12020–12026.
Reports the first small-molecule ABP for selective profiling Ubiquitin Carboxyl-Terminal Hydrolase L1(UCHL1) activity in live cells. Using this probe, the authors identify new potent inhibitors of UCHL1-dependent pulmonary fibrosis, and reassesses compounds previously reported as UCHL1 inhibitors.

34. Ward JA, Pinto-Fernandez A, Cornelissen L, Bonham S, Diaz-Saez L, Riant O, Huber KVM, Kessler BM, Feron O, Tate EW: **Re-evaluating the mechanism of action of alpha,beta-unsaturated carbonyl DUB inhibitors b-AP15 and VLX1570: a paradigmatic example of unspecific protein cross-linking with michael acceptor motif-containing drugs**. *J Med Chem* 2020, **63**:3756–3762.

35. * Hoegl A, Nodwell MB, Kirsch VC, Bach NC, Pfanzelt M, Stahl M, Schneider S, Sieber SA: **Mining the cellular inventory of pyridoxal phosphate-dependent enzymes with functionalized cofactor mimics**. *Nat Chem* 2018, **10**:1234–1245.
Describes the development of pyridoxal substrate mimetics to profile pyridoxal phosphate-dependent enzyme (PLP-DEs) activity. Applied to the bactierial pathogen *Staphylococcus aureus*, the authors identify novel PLP-DEs with potential druggability, and successfully screen the selectivity of the antituberculosis inhibitor, D-cycloserine.

36. Amadasi A, Bertoldi M, Contestabile R, Bettati S, Cellini B, di Salvo ML, Borri-Voltattorni C, Bossa F, Mozzarelli A: **Pyridoxal 5'-phosphate enzymes as targets for therapeutic agents**. *Curr Med Chem* 2007, **14**:1291–1324.

37. Koenders STA, van Rooden EJ, van den Elst H, Florea BI, Overkleeft HS, van der Stelt M: **STA-55, an easily accessible, broad-spectrum, activity-based aldehyde dehydrogenase probe**. *Chembiochem* 2020, **21**:1911–1917.

38. Vasiliou V, Thompson DC, Smith C, Fujita M, Chen Y: **Aldehyde dehydrogenases: from eye crystallins to metabolic disease and cancer stem cells**. *Chem Biol Interact* 2013, **202**:2–10.

39. Singh J, Petter RC, Baillie TA, Whitty A: **The resurgence of covalent drugs**. *Nat Rev Drug Discov* 2011, **10**:307–317.

40. Visscher M, Arkin MR, Dansen TB: **Covalent targeting of acquired cysteines in cancer**. *Curr Opin Chem Biol* 2016, **30**: 61–67.

41. Carmi C, Mor M, Petronini PG, Alfieri RR: **Clinical perspectives for irreversible tyrosine kinase inhibitors in cancer**. *Biochem Pharmacol* 2012, **84**:1388–1399.

42. Weerapana E, Wang C, Simon GM, Richter F, Khare S, Dillon MB, Bachovchin DA, Mowen K, Baker D, Cravatt BF: **Quantitative reactivity profiling predicts functional cysteines in proteomes**. *Nature* 2010, **468**:790–795.

43. Deng X, Weerapana E, Ulanovskaya O, Sun F, Liang H, Ji Q, Ye Y, Fu Y, Zhou L, Li J, *et al.*: **Proteome-wide quantification and characterization of oxidation-sensitive cysteines in pathogenic bacteria**. *Cell Host Microbe* 2013, **13**:358–370.

44. Zhou Y, Wynia-Smith SL, Couvertier SM, Kalous KS, Marletta MA, Smith BC, Weerapana E: **Chemoproteomic strategy to quantitatively monitor transnitrosation uncovers functionally relevant S-nitrosation sites on cathepsin D and HADH2**. *Cell Chem Biol* 2016, **23**:727–737.

45. Blewett MM, Xie J, Zaro BW, Backus KM, Altman A, Teijaro JR, Cravatt BF: **Chemical proteomic map of dimethyl fumarate-sensitive cysteines in primary human T cells**. *Sci Signal* 2016, **9**:rs10.

46. Guo CJ, Chang FY, Wyche TP, Backus KM, Acker TM, Funabashi M, Taketani M, Donia MS, Nayfach S, Pollard KS, *et al.*: **Discovery of reactive microbiota-derived metabolites that inhibit host proteases**. *Cell* 2017, **168**:517–526 e518.

47. Backus KM, Correia BE, Lum KM, Forli S, Horning BD, Gonzalez-Paez GE, Chatterjee S, Lanning BR, Teijaro JR, Olson AJ, *et al.*: **Proteome-wide covalent ligand discovery in native biological systems**. *Nature* 2016, **534**:570–574.

48. Lin S, Yang X, Jia S, Weeks AM, Hornsby M, Lee PS, Nichiporuk RV, Iavarone AT, Wells JA, Toste FD, *et al.*: **Redox-based reagents for chemoselective methionine bioconjugation**. *Science* 2017, **355**:597–602.

49. * Hacker SM, Backus KM, Lazear MR, Forli S, Correia BE, Cravatt BF: **Global profiling of lysine reactivity and ligandability in the human proteome**. *Nat Chem* 2017, **9**:1181–1190.
Describes the initial development and application of a sulphotetrafluorophenyl (STP) probe for profiling reactive and ligandable lysines in the human proteome. Here the authors identify amine fragment-lysine interactions that inhibit the enzymatic activity of several oncogenes, highlighting lysine targeting as a viable therapeutic strategy in cancer drug discovery.

50. ** Hahm HS, Toroitich EK, Borne AL, Brulet JW, Libby AH, Yuan K, Ware TB, McCloud RL, Ciancone AM, Hsu KL: **Global targeting of functional tyrosines using sulfur-triazole exchange chemistry**. *Nat Chem Biol* 2020, **16**:150–159.
Introduces sulphur-triazole exchange (SuTEx) chemistry for the development of phenol-reactive covalent probes. Using a series of probes, tyrosine reactivity and phosphorylation was profiled across cancer cell proteomes, identifying several attractive sites for tyrosine-directed ligand discovery.

51. Bach K, Beerkens BLH, Zanon PRA, Hacker SM: **Light-activatable, 2,5-disubstituted tetrazoles for the proteome-wide profiling of aspartates and glutamates in living bacteria**. *ACS Cent Sci* 2020, **6**:546–554.

52. Ma N, Hu J, Zhang ZM, Liu W, Huang M, Fan Y, Yin X, Wang J, Ding K, Ye W, *et al.*: **2H-Azirine-Based reagents for chemoselective bioconjugation at Carboxyl residues inside live cells**. *J Am Chem Soc* 2020, **142**:6051–6059.

53. Brulet JW, Borne AL, Yuan K, Libby AH, Hsu KL: **Liganding functional tyrosine sites on proteins using sulfur-triazole exchange chemistry**. *J Am Chem Soc* 2020, **142**:8270–8280.

54. Jia S, He D, Chang CJ: **Bioinspired thiophosphorodichloridate reagents for chemoselective histidine bioconjugation**. *J Am Chem Soc* 2019, **141**:7294–7301.

55. Seki Y, Ishiyama T, Sasaki D, Abe J, Sohma Y, Oisaki K, Kanai M: **Transition metal-free tryptophan-selective bioconjugation of proteins**. *J Am Chem Soc* 2016, **138**: 10798–10801.

56. ** Wang Y, Dix MM, Bianco G, Remsberg JR, Lee HY, Kalocsay M, Gygi SP, Forli S, Vite G, Lawrence RM, *et al.*: **Expedited mapping of the ligandable proteome using fully functionalized enantiomeric probe pairs**. *Nat Chem* 2019, **11**:1113–1123.
Demonstrates the use of enantiomeric probe pairs to map stereospecific protein-ligand interactions, and how these can be used to expedite target prioritisation.

57. Ting L, Rad R, Gygi SP, Haas W: **MS3 eliminates ratio distortion in isobaric multiplexed quantitative proteomics**. *Nat Methods* 2011, **8**:937–940.

58. * Deng H, Lei Q, Wu Y, He Y, Li W: **Activity-based protein profiling: recent advances in medicinal chemistry**. *Eur J Med Chem* 2020, **1**:112–151.
Covers key advances in the use of photo-reactive groups for labelling of proteins that are otherwise not amenable to covalent targeting.

59. Salisbury CM, Cravatt BF: **Activity-based probes for proteomic profiling of histone deacetylase complexes**. *Proc Natl Acad Sci USA* 2007, **104**:1171–1176.

60. Dugan A, Majmudar CY, Pricer R, Niessen S, Lancia JK, Fung HYH, Cravatt BF, Mapp AK: **Discovery of enzymatic targets of transcriptional activators via in vivo covalent chemical capture**. *J Am Chem Soc* 2016, **138**:12629–12635.

61. Howard RT, Hemsley P, Petteruti P, Saunders CN, Molina Bermejo JA, Scott JS, Johannes JW, Tate EW: **Structure-guided design and in-cell target profiling of a cell-active target engagement probe for PARP inhibitors**. *ACS Chem Biol* 2020, **15**:325–333.

62. Wright MH, Sieber SA: **Chemical proteomics approaches for identifying the cellular targets of natural products**. *Nat Prod Rep* 2016, **33**:681–708.

63. Lubin AS, Rueda-Zubiaurre A, Matthews H, Baumann H, Fisher FR, Morales-Sanfrutos J, Hadavizadeh KS, Nardella F, Tate EW, Baum J, *et al.*: **Development of a photo-cross-linkable diaminoquinazoline inhibitor for target identification in Plasmodium falciparum**. *ACS Infect Dis* 2018:523–530.

64. Saunders CN, Cota E, Baum J, Tate EW: **Peptide probes for Plasmodium falciparum MyoA tail interacting protein (MTIP): exploring the druggability of the malaria parasite motor complex**. *ACS Chem Biol* 2020.

65. Soethoudt M, Stolze SC, Westphal MV, Van Stralen L, Martella A, Van Rooden EJ, Guba W, Varga ZV, Deng H, Van Kasteren SI, *et al.*: **Selective photoaffinity probe that enables assessment of cannabinoid CB2 receptor expression and ligand engagement in human cells**. *J Am Chem Soc* 2018, **140**: 6067–6075.

66. Abo M, Weerapana E: **A caged electrophilic probe for global analysis of cysteine reactivity in living cells**. *J Am Chem Soc* 2015, **137**:7087–7090.

67. * Abo M, Bak DW, Weerapana E: **Optimization of caged electrophiles for improved monitoring of cysteine reactivity in living cells**. *ChemBioChem* 2017, **18**:81–84.
Describes the optimisation and application of photoactivatable thiol-reactive probes for profiling cysteine reactivity in live cells. This study highlights the benefits of using photocaged probes to reduce cytotoxicity and enhance spatial and temporal control of protein labelling.

68. Dubinsky L, Krom BP, Meijler MM: **Diazirine based photoaffinity labeling**. *Bioorg Med Chem* 2012, **20**:554–570.

69. Karas JA, Scanlon DB, Forbes BE, Vetter I, Lewis RJ, Gardiner J, Separovic F, Wade JD, Hossain MA: **2-nitroveratryl as a photocleavable thiol-protecting group for directed disulfide bond formation in the chemical synthesis of insulin**. *Chemistry* 2014, **20**:9549–9552.

70. Klán P, Šolomek T, Bochet CG, Blanc A, Givens R, Rubina M, Popik V, Kostikov A, Wirz J: **Photoremovable protecting groups in chemistry and biology: reaction mechanisms and efficacy**. *Chem Rev* 2013, **113**:119–191.

71. Findlay GM, Daza RM, Martin B, Zhang MD, Leith AP, Gasperini M, Janizek JD, Huang X, Starita LM, Shendure J: **Accurate classification of BRCA1 variants with saturation genome editing**. *Nature* 2018, **562**:217–222.

72. Choudhury A, Fenster JA, Fankhauser RG, Kaar JL, Tenaillon O, Gill RT: **CRISPR/Cas9 recombineering-mediated deep mutational scanning of essential genes in Escherichia coli**. *Mol Syst Biol* 2020, **16**:e9265.

73. Garst AD, Bassalo MC, Pines G, Lynch SA, Halweg-Edwards AL, Liu R, Liang L, Wang Z, Zeitoun R, Alexander WG, *et al.*: **Genome-wide mapping of mutations at single-nucleotide resolution for protein, metabolic and genome engineering**. *Nat Biotechnol* 2017, **35**:48–55.