Seeing small molecules in action with bioorthogonal chemistry

Anuradha S. Raghavan and Howard C. Hang

The Laboratory of Chemical Biology and Microbial Pathogenesis, The Rockefeller University, 1230 York Avenue Box 250, New York, NY 10065, United States

Chemical probes that target specific protein families offer powerful tools to accelerate drug discovery. Small molecules modified with uniquely reactive functional groups and detection tags provide novel tools to characterize complex proteomes functionally and also to help determine the specificity of small molecule inhibitors toward various enzyme/protein classes. This review highlights the application of bioorthogonal chemistries in combination with chemical probes, which together are offering unprecedented opportunities to dissect the functions of enzyme/protein families *in vivo* and enabling more precise target identification of small molecules. Advances in chemical probes and bioorthogonal reactions are poised to reveal new therapeutic targets and to facilitate the discovery and characterization of small molecules aimed at disease.

Introduction

The postgenomic era has afforded an unprecedented opportunity to address human disease. The remarkable advances in gene sequencing suggest the possibility of identifying all genetic mutations associated with major diseases and implementation of personalized medicine [1]. While the global analyses of gene and protein expression as well as metabolite levels in normal and disease tissues are beginning to reveal new biological pathways for therapeutic intervention, more precise methods are still needed to functionally characterize proteomes and identify specific druggable targets. On the basis of known drugs and their protein targets, it has been suggested that only a fraction of a proteome might be targeted for therapeutic intervention by small molecule drugs [2]. The fraction of the proteome that is actually 'druggable' is still unclear, because new mechanisms of action for small molecules are continuing to emerge. To realize the promise of personalized medicine, new experimental methods are needed to characterize the activity of genes and small molecules *in vivo*.

Activity-based protein profiling (ABPP)

Chemical probes appended with detection tags are providing new opportunities to analyze complex proteomes and characterize small molecule inhibitor specificities (Fig. 1) [3–5]. The attachment of detection tags (radioactive isotope, affinity tag or fluorophore) onto covalent inhibitors or photoaffinity agents has enabled the analysis of discrete protein families in complex mixtures [3–6]. To target specific protein families selectively, chemical probes typically contain a reactive functional group for covalent labeling of proteins and a unique chemical scaffold to confer binding affinity to subsets of proteins (Fig. 1). The reactive functional groups can be electrophiles that target nucleophilic amino acid residues or photocrosslinkers that allow covalent labeling of proteins upon UV-irradiation. Following the incubation of chemical probes with tissue or cell lysates, specifically labeled enzymes/proteins can be visualized after gel-electrophoresis by autoradiography, immunoblot or in-gel fluorescence scanning depending on the detection tag. Furthermore, the use of affinity tags allows the enrichment of specifically labeled polypeptides for protein identification using modern mass-spectrometry-based proteomic methods (Fig. 1). The development of specific chemical probes has benefited from numerous studies on the mechanism-based inhibitors of various enzyme families [7,8]. These studies have yielded chemical probes for serine hydrolases, proteasome, cysteine proteases, metalloproteases, aspartyl proteases, glycosidases, phosphatases, kinases and other enzyme families [3–5,9–11]. These chemical probes often selectively target active enzyme populations in complex mixtures and have been described as activity-based probes (ABPs) or mechanism-based probes (MBPs) [3–6]. The application of chemical probes to survey globally the

Corresponding author: Hang, H.C. (hhang@mail.rockefeller.edu)

1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2008.09.014

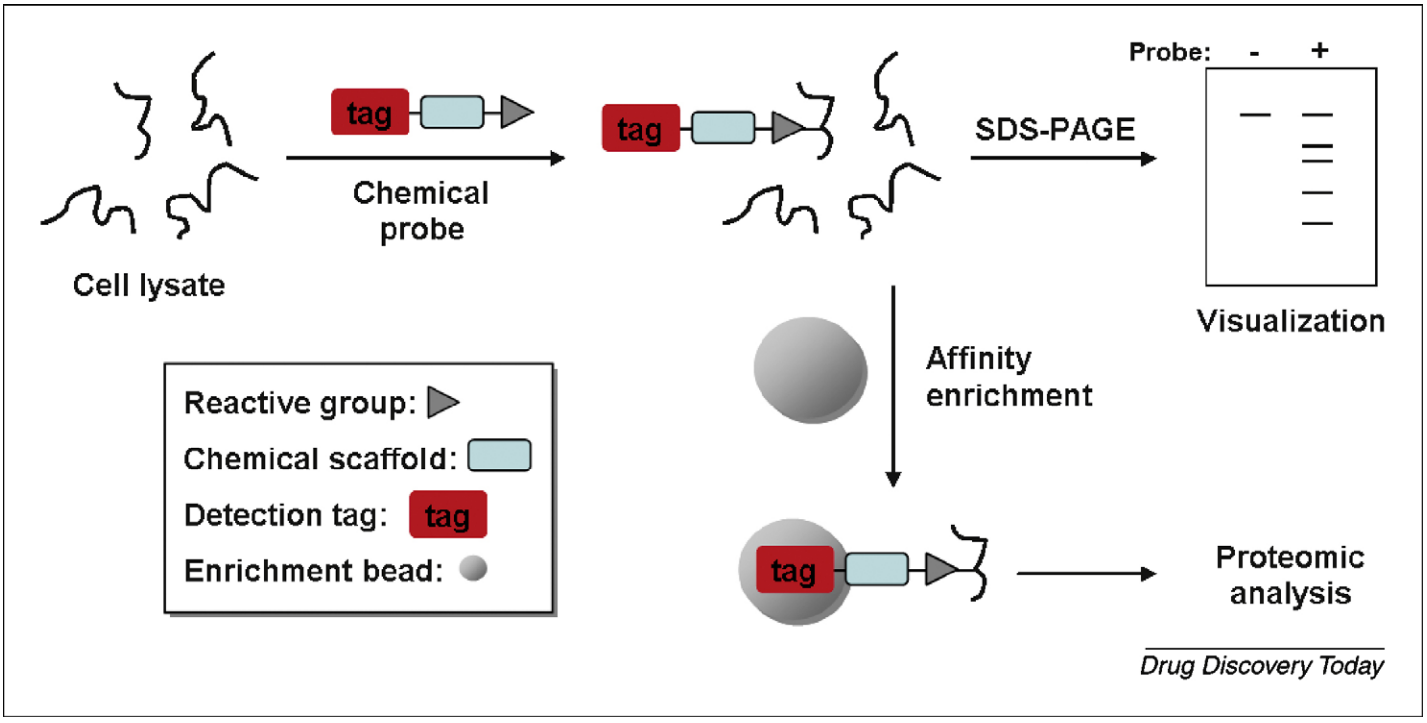


FIGURE 1

The analysis of small molecule–protein interactions with chemical probes. Small molecules modified with reactive electrophiles or photocrosslinkers enable covalent labeling of specific protein targets depending on the chemical scaffolds of the probes. Detection tags such as fluorophores or biotin allow the visualization of probe-labeled proteins by in-gel fluorescence scanning or immunoblot. Alternatively, the probe-labeled proteins can be identified by mass spectrometry-based proteomics after affinity enrichment.

activity/expression of discrete protein families in tissue or cell lysates has been termed activity-based protein profiling (ABPP) [3,4,6,12].

Use of ABPP in biological systems

While bioinformatic methods enable the classification of gene and protein functions based on sequence comparisons, the activities of many genes and proteins remained to be established and identified. The ability to evaluate the activity/expression of specific protein families in complex mixtures with chemical probes provides a powerful means to identify unpredicted activities and to characterize proteomes functionally. For example, the development of chemical probes that target deubiquitinating enzymes has enabled the characterization of these proteases in many organisms [12,13]. Remarkably, the application of these deubiquitinating enzyme probes has revealed previously unappreciated protease activities in microbial pathogens such as viruses [14,15] and bacteria [16]. Alternatively, ABPP using a fluorophosphonate-rhodamine (FP-rhodamine) probe and semi-quantitative in-gel fluorescence scanning revealed unique profiles of serine hydrolases associated with the invasiveness of cancer cell lines [17]. In addition to *in vitro* studies, the design of quenched chemical probes that become fluorescent after protein labeling has allowed the noninvasive imaging of cathepsin cysteine proteases in mouse models of tumorigenesis [18]. These studies highlight the utility of chemical probes for the identification of novel protein activities, comparative analysis of discrete enzyme families associated with disease and visualization of specific protein activities in living animals, all of which will be invaluable for determining novel therapeutic targets.

The discovery and characterization of small molecules for drug discovery is challenging. High-throughput screening of chemical libraries may reveal new leads for drug development, but the specificities and mechanisms of action for many small molecules are difficult to determine and can hinder their development into bona fide drugs. Chemical probes provide excellent tools to determine small molecule–protein interactions and afford a powerful means to determine the specificity of small molecules toward discrete protein families and identify new lead compounds for further development. For example, a chemical probe such as FP-rhodamine can be used to evaluate the potency and selectivity of small molecules that inhibit serine hydrolases by measuring the ability of potential inhibitors that block the labeling of diverse enzymes in cell lysates [19]. Indeed, this approach revealed potent trifluoromethyl ketone-containing compounds that selectively inhibit various serine hydrolases [19]. An important application of chemical probes is the ability to evaluate inhibitor potency and selectivity simultaneously in complex mixtures, enabling the rapid discrimination of promiscuous compounds from promising leads for drug development.

Bioorthogonal techniques used to explore small molecule–protein interactions

The direct visualization of small molecule–protein interactions with chemical probes has provided new tools to accelerate drug discovery. Certain small molecule–protein interactions might require factors only present in living systems that are not preserved in cell lysates. Detection tags such as biotin or fluorophores, however, can influence the specificity of chemical probes and also limit their utility in living cells. The emergence of two chemoselective reactions that function under physiological environments,

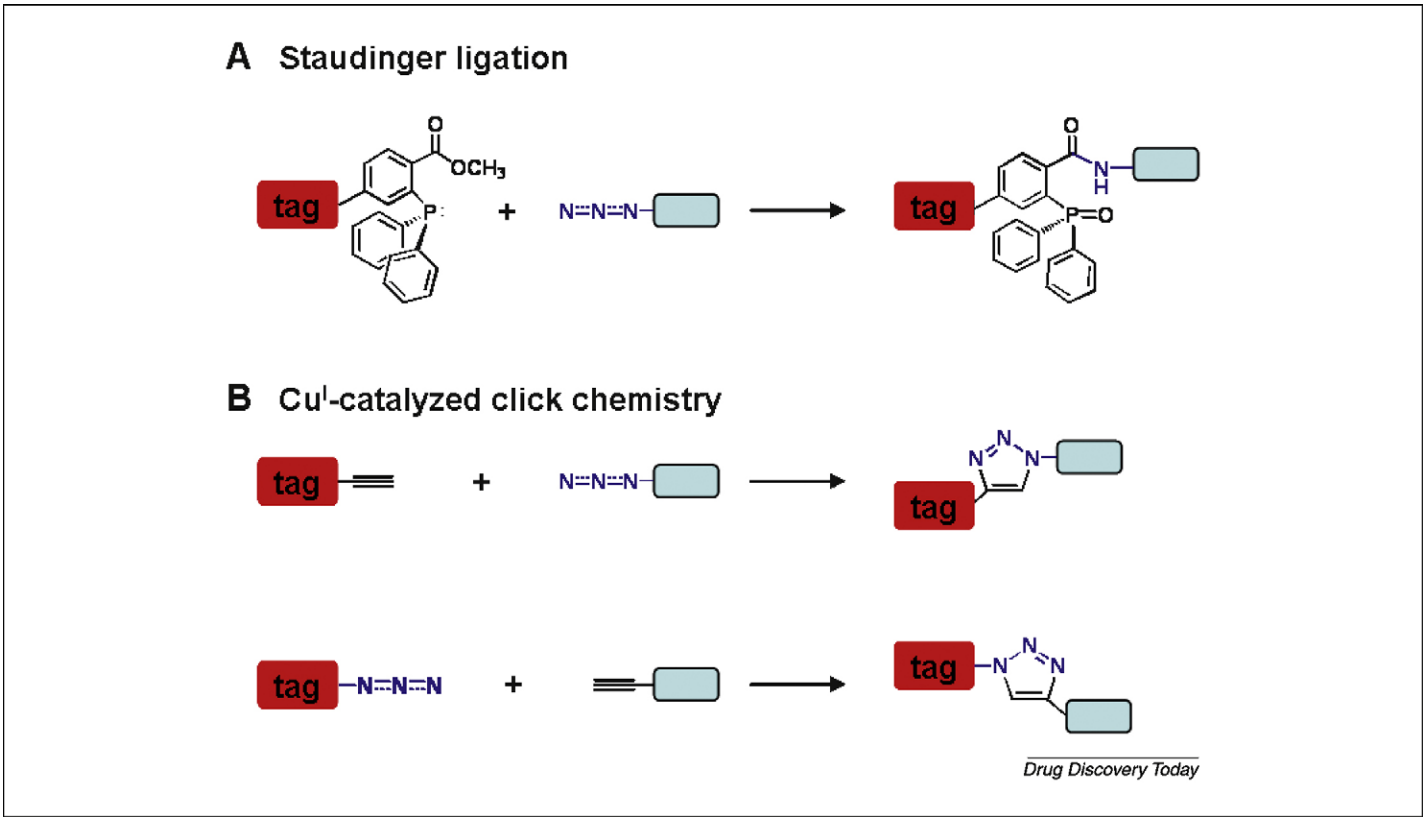


FIGURE 2

Bioorthogonal labeling methods. **(A)** Staudinger ligation. **(B)** Cu^{I}-catalyzed Huisgen [3 + 2] cycloaddition or click chemistry.

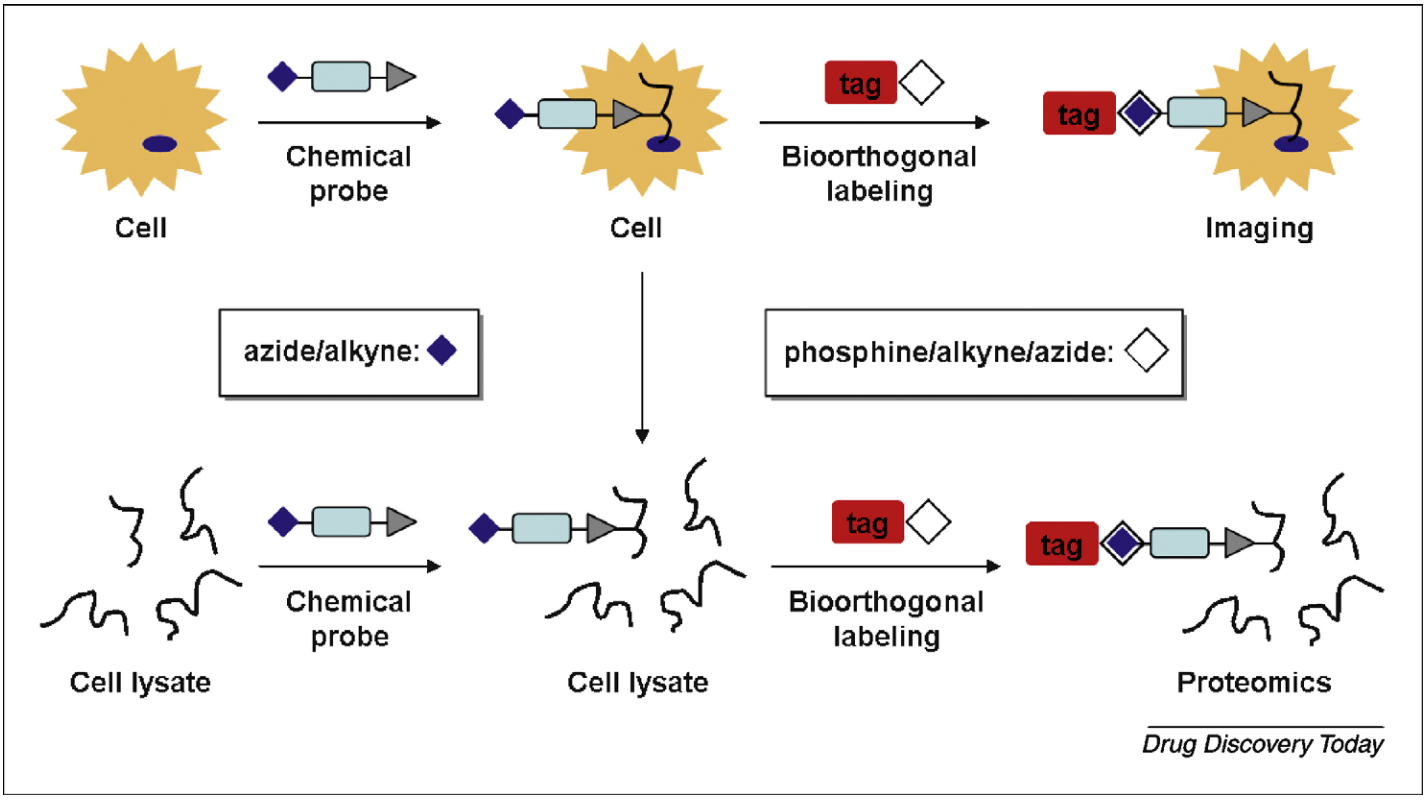


FIGURE 3

A two-step labeling approach enables the application of chemical probes to living cells and the generation of chemical probes with unique specificity. Application of bioorthogonal labeling methods allows the visualization and identification of probe-labeled proteins.

the Staudinger ligation [20] and the Huisgen [3 + 2] cycloaddition or 'click-chemistry' [21–23] (Fig. 2), has enabled the installation of detection tags after chemical probes have reacted with target proteins. The Staudinger ligation involves the reaction between alkyl/aryl azides with methylester-modified triphenylphosphines, which results in the formation of a covalent amide bond between the two reactants (Fig. 2A) [20]. Alternatively, alkyl/aryl azides can undergo Cu(I)-catalyzed [3 + 2] cycloadditions with terminal alkynes to yield triazole products (Fig. 2B) [21,22]. Importantly, both of these reactions proceed efficiently in aqueous environments and exhibit relatively low reactivity with chemical functionality in biomolecules (nucleic acids, proteins and metabolites)

TABLE 1

Survey of chemical probes used for targeting various protein classes in conjunction with bioorthogonal labeling.

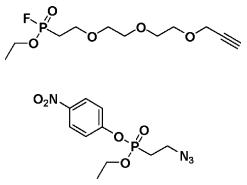
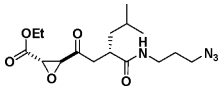
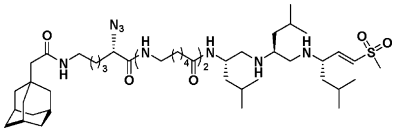
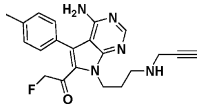
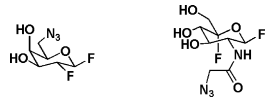
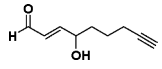
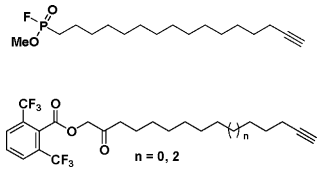
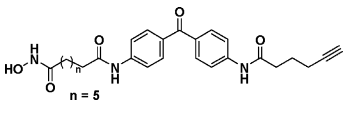
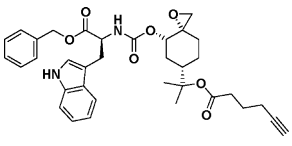
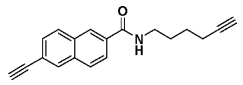
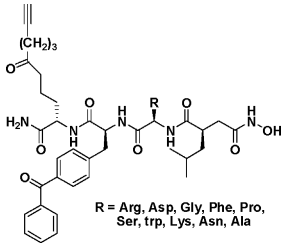
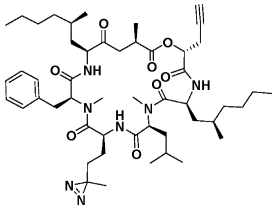
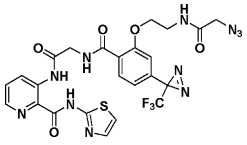
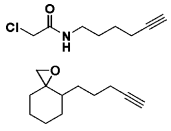
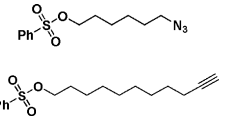
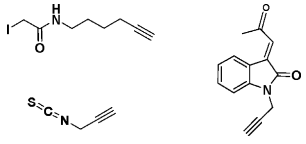
Chemical probe	Protein targets	Bioorthogonal chemistry	Refs
Directed: electrophile			
	Serine hydrolases	Click chemistry	[38,44]
	Serine and cysteine hydrolases	Click chemistry	[37]
	Cysteine proteases (cathepsins)	Staudinger ligation	[28]
	Proteosome	Staudinger ligation	[27]
	Kinases	Click chemistry	[35]
	Glycosidases	Click chemistry Staudinger ligation	[29,30]
	Oxidoreductases	Click chemistry Staudinger ligation	[39]
	Fatty acid associated proteins	Click chemistry	[55]
	Histone deacetylases	Click chemistry	[32,33]
	Glycolytic enzyme: phosphoglycerate mutase-1	Click chemistry	[42]

TABLE 1 (*Continued*)

Chemical probe	Protein targets	Bioorthogonal chemistry	Refs
	Cytochrome P450 monooxygenases	Click chemistry	[41]
Directed: photocrosslinker			
	Metalloproteases	Click chemistry	[31]
	Protein transporter	Click chemistry	[43]
	Methionine aminopeptidase	Click chemistry	[34]
Broad reactivity			
	Disparate proteome reactivity	Click chemistry	[51]
	Multiple enzymes: transferases, dehydrogenases, hydratases	Click chemistry	[25,26]
	Cysteine residues	Click chemistry	[40]

and have consequently been termed 'bioorthogonal' [24]. The invention of these bioorthogonal reactions enables the use of alkyl azides and alkynes as small chemical reporters on metabolites or probes that can be subsequently converted into detection tags (Fig. 3) [24]. Herein, we survey the union of chemical probes with bioorthogonal labeling methods, which has yielded new chemical probes with enhanced selectivity and improved pharmacological properties for selective labeling of protein targets in living cells.

Although the Staudinger ligation [20] and click chemistry [21–23] were not originally developed for applications using chemical probes, researchers quickly realized the utility of bioorthogonal chemistry and have applied this two-step protein labeling procedure to several chemical probes for *in vitro* and cellular labeling studies (Fig. 3 and Table 1). Early examples of combining chemical probes with bioorthogonal labeling methods were demonstrated with ABPs that target serine hydrolases [25,26] and proteasome subunits [27]. Since then, several azide/alkyne-modified chemical probes have been developed to target cysteine proteases[28], glycosidases [29,30], metalloproteases [31], histone deacetylases [32,33], methionine aminopeptidases [34], kinases [35] as well as a variety of other protein classes [36–44] (Table 1). Of note, the decoupling of detection tags from chemical probes alters and often improves the specificity of protein labeling compared to directly modified chemical probes, which is readily apparent with

cysteine proteases [28,45], metalloproteases [11,31], kinases [35,46] and methionine aminopeptidases [34]. The diversity of azide/alkyne-modified chemical probes listed above highlights the utility of this two-step labeling approach for targeting various protein classes.

The application of bioorthogonal labeling methods with chemical probes to living systems should provide us with a deeper understanding of cellular processes that are not apparent in cell lysates and that might only occur in the context of living cells. For example, the MBP azido-E-64 enabled the selective labeling of the endocytic cysteine protease cathepsin (Cat) B in living cells (Table 1, entry 3) [28]. Subsequent bioorthogonal labeling with phosphine reagents via the Staudinger ligation allowed the biochemical and proteomic analysis of labeled polypeptides as well as the visualization of active Cat B within endocytic compartments of individual cells by fluorescence microscopy [28]. Application of azido-E-64 and bioorthogonal labeling methods to *Salmonella typhimurium*-infected macrophages demonstrated that wild-type virulent *S. typhimurium* resides in endocytic compartments that are devoid of active Cat B [28]. By contrast, nonpathogenic *Escherichia coli* or heat-killed *S. typhimurium* localized to endocytic compartments of macrophages containing active Cat B [28]. These data suggest that one mechanism of *S. typhimurium* survival in macrophages involves the exclusion of degradative enzymes within bacteria-containing vacuoles [28].

Cell-permeable chemical probes also provide excellent tools to dissect signal transduction. The development of fmk-pa an alkyne-modified chemical probe targeted at the C-terminal kinase domain (CTD) of p90 ribosomal protein S6 kinases (RSKs), afforded a potent and cell-permeable inhibitor of CTD RSKs in cells (Table 1, entry 5) [35]. Intracellular labeling of RSKs with fmk-pa followed by click chemistry and in-gel fluorescence scanning analysis of the corresponding cell lysates afforded a robust means to quantify the extent of CTD RSK inhibition. Interestingly, the saturating levels of RSK1 and RSK2 CTD kinase inhibition with fmk-pa selectively blocked cellular signaling in response to phorbol ester activation but did not perturb the activation of RSKs by lipopolysaccharide stimulation, suggesting discrete functions for the CTD of RSKs in signal transduction [35]. These studies demonstrate how chemical probes and bioorthogonal labeling methods can be used to dissect cellular pathways.

Target identification of active small molecules from high-throughput cell-based screening efforts is a major challenge for chemical genetics and drug discovery [47,48]. Surveying libraries of chemical probes in cell-based assays offers a means to correlate phenotypes with protein labeling, which may afford unique insight into small molecule protein targets and their mechanisms of action in cells. To establish a platform for correlating small molecule–protein interactions with cellular phenotypes, a small chemical probe library inspired by natural products was synthesized [49]. This chemical probe library contained spiroepoxides as reactive electrophiles and alkynes as bioorthogonal reporters [49]. Survey of this spiroepoxide chemical probe library for antiproliferative effects on breast cancer cell lines identified a spiroepoxide compound, MJE3, with low micromolar activity (Table 1, entry 10) [49]. A comparative analysis of proteins selectively labeled by MJE3 versus inactive spiroepoxide chemical probes using click chemistry and mass-spectrometry-based proteomics revealed phosphoglycerate mutase 1 as a glycolytic enzyme that may be important for cancer cell proliferation [49]. The analytical platforms for characterizing proteins targeted by chemical probes have been essential for ABPP studies and the dissection of specific cellular pathways using small molecule probes. In this regard, the development of a tandem orthogonal proteolysis (TOP) strategy for click chemistry labeling, affinity enrichment, selective elution with tobacco etch virus (TEV) protease and proteomic analysis of probe-labeled proteins has been instrumental [50,51]. In addition to the selective elution of enriched proteins with TEV protease [50,51], the diazobenzene cleavable linker provides a complementary selective elution strategy for proteomic analysis of probe-labeled proteins [52–54]. These studies provide a proof-of-concept for screening cellular phenotypes with chemical probe libraries and determining possible small molecule mechanisms of action using bioorthogonal labeling methods and proteomics.

Concluding remarks

The integration of chemical probes with bioorthogonal labeling methods has unearthed new possibilities for investigating complex biological pathways with small molecules for basic science and drug discovery. The functional profiling of proteomes with chemical probes is likely to identify additional novel protein activities in various cellular contexts and diseases that will afford new targets for drug discovery. At the same time, chemical probes are providing powerful tools for characterizing the specificity of compounds for drug development. Importantly, the implementation of bioorthogonal labeling methods has enabled the transition of chemical probes from cell lysates into living cells, which has opened up new ways of dissecting cellular processes and discovering unique pathways associated with cellular phenotypes. This chemical platform will not only shed light into fundamental signaling pathways but also affords a more precise means of understanding the mechanisms of action for drugs. The integration of chemical probes with other genomic, proteomic and metabolomic methods therefore provides exciting possibilities for addressing human diseases in the postgenomic era.

Acknowledgements

H.C.H. thanks The Rockefeller University and Irma T. Hirschl/Monique Weill-Caulier Trust for financial support. A.R. thanks the New York Community Trust-Heiser Grant for a postdoctoral fellowship. The authors thank Paul Dossa and Jacob Yount for helpful comments on this manuscript.

References

1 van't Veer, L.J. and Bernards, R. (2008) Enabling personalized cancer medicine through analysis of gene-expression patterns. *Nature* 452, 564–570

2 Overington, J.P. *et al.* (2006) How many drug targets are there? *Nat. Rev. Drug Discov.* 5, 993–996

3 Cravatt, B.F. *et al.* (2008) Activity-based protein profiling: from enzyme chemistry to proteomic chemistry. *Annu. Rev. Biochem.* 77, 383–414

4 Sadaghiani, A.M. *et al.* (2007) Tagging and detection strategies for activity-based proteomics. *Curr. Opin. Chem. Biol.* 11, 20–28

5 Saghatelian, A. and Cravatt, B.F. (2005) Assignment of protein function in the postgenomic era. *Nat. Chem. Biol.* 1, 130–142
6 Uttamchandani, M. *et al.* (2008) Activity-based protein profiling: new developments and directions in functional proteomics. *Chembiochem* 9, 667–675
7 Powers, J.C. *et al.* (2002) Irreversible inhibitors of serine, cysteine, and threonine proteases. *Chem. Rev.* 102, 4639–4750
8 Walsh, C.T. (1984) Suicide substrates, mechanism-based enzyme inactivators: recent developments. *Annu. Rev. Biochem.* 53, 493–535
9 Hagenstein, M.C. *et al.* (2003) Affinity-based tagging of protein families with reversible inhibitors: a concept for functional proteomics. *Angew Chem. Int. Ed. Engl.* 42, 5635–5638
10 Chan, E.W. *et al.* (2004) Developing photoactive affinity probes for proteomic profiling: hydroxamate-based probes for metalloproteases. *J. Am. Chem. Soc.* 126, 14435–14446
11 Saghatelian, A. *et al.* (2004) Activity-based probes for the proteomic profiling of metalloproteases. *Proc. Natl. Acad. Sci. U. S. A.* 101, 10000–10005
12 Borodovsky, A. *et al.* (2005) Small-molecule inhibitors and probes for ubiquitin- and ubiquitin-like-specific proteases. *Chembiochem* 6, 287–291
13 Love, K.R. *et al.* (2007) Mechanisms, biology and inhibitors of deubiquitinating enzymes. *Nat. Chem. Biol.* 3, 697–705
14 Kattenhorn, L.M. *et al.* (2005) A deubiquitinating enzyme encoded by HSV-1 belongs to a family of cysteine proteases that is conserved across the family Herpesviridae. *Mol. Cell* 19, 547–557
15 Schlieker, C. *et al.* (2005) A deubiquitinating activity is conserved in the large tegument protein of the herpesviridae. *J. Virol.* 79, 15582–15585
16 Misaghi, S. *et al.* (2006) Chlamydia trachomatis-derived deubiquitinating enzymes in mammalian cells during infection. *Mol. Microbiol.* 61, 142–150
17 Jessani, N. *et al.* (2002) Enzyme activity profiles of the secreted and membrane proteome that depict cancer cell invasiveness. *Proc. Natl. Acad. Sci. U. S. A.* 99, 10335–10340
18 Blum, G. *et al.* (2007) Noninvasive optical imaging of cysteine protease activity using fluorescently quenched activity-based probes. *Nat. Chem. Biol.* 3, 668–677
19 Leung, D. *et al.* (2003) Discovering potent and selective reversible inhibitors of enzymes in complex proteomes. *Nat. Biotechnol.* 21, 687–691
20 Saxon, E. and Bertozzi, C.R. (2000) Cell surface engineering by a modified Staudinger reaction. *Science* 287, 2007–2010
21 Rostovtsev, V.V. *et al.* (2002) A stepwise huisgen cycloaddition process: copper(I)-catalyzed regioselective ''ligation'' of azides and terminal alkynes. *Angew Chem. Int. Ed. Engl.* 41, 2596–2599
22 Tornoe, C.W. *et al.* (2002) Peptidotriazoles on solid phase: [1,2,3]-triazoles by regiospecific copper(i)-catalyzed 1,3-dipolar cycloadditions of terminal alkynes to azides. *J. Org. Chem.* 67, 3057–3064
23 Wang, Q. *et al.* (2003) Bioconjugation by copper(I)-catalyzed azide-alkyne [3 + 2] cycloaddition. *J. Am. Chem. Soc.* 125, 3192–3193
24 Prescher, J.A. and Bertozzi, C.R. (2005) Chemistry in living systems. *Nat. Chem. Biol.* 1, 13–21
25 Speers, A.E. *et al.* (2003) Activity-based protein profiling in vivo using a copper(i)-catalyzed azide-alkyne [3 + 2] cycloaddition. *J. Am. Chem. Soc.* 125, 4686–4687
26 Speers, A.E. and Cravatt, B.F. (2004) Profiling enzyme activities in vivo using click chemistry methods. *Chem. Biol.* 11, 535–546
27 Ovaa, H. *et al.* (2003) Chemistry in living cells: detection of active proteasomes by a two-step labeling strategy. *Angew Chem. Int. Ed. Engl.* 42, 3626–3629
28 Hang, H.C. *et al.* (2006) Mechanism-based probe for the analysis of cathepsin cysteine proteases in living cells. *ACS Chem. Biol.* 1, 713–723
29 Vocadlo, D.J. and Bertozzi, C.R. (2004) A strategy for functional proteomic analysis of glycosidase activity from cell lysates. *Angew Chem. Int. Ed. Engl.* 43, 5338–5342
30 Stubbs, K.A. *et al.* (2008) Synthesis and use of mechanism-based protein-profiling probes for retaining beta-D-glucosaminidases facilitate identification of *Pseudomonas aeruginosa* NagZ. *J. Am. Chem. Soc.* 130, 327–335
31 Sieber, S.A. *et al.* (2006) Proteomic profiling of metalloprotease activities with cocktails of active-site probes. *Nat. Chem. Biol.* 2, 274–281
32 Salisbury, C.M. and Cravatt, B.F. (2008) Optimization of activity-based probes for proteomic profiling of histone deacetylase complexes. *J. Am. Chem. Soc.* 130, 2184–2194
33 Salisbury, C.M. and Cravatt, B.F. (2007) Activity-based probes for proteomic profiling of histone deacetylase complexes. *Proc. Natl. Acad. Sci. U. S. A.* 104, 1171–1176
34 Qiu, W.W. *et al.* (2007) Activity-based protein profiling for type I methionine aminopeptidase by using photo-affinity trimodular probes. *Chembiochem* 8, 1351–1358
35 Cohen, M.S. *et al.* (2007) A clickable inhibitor reveals context-dependent autoactivation of p90 RSK. *Nat. Chem. Biol.* 3, 156–160
36 Weerapana, E. *et al.* (2008) Disparate proteome reactivity profiles of carbon electrophiles. *Nat. Chem. Biol.* 4, 405–407
37 Bottcher, T. and Sieber, S.A. (2008) Beta-lactones as privileged structures for the active-site labeling of versatile bacterial enzyme classes. *Angew Chem. Int. Ed. Engl.* 47, 4600–4603
38 Gillet, L.C. *et al.* (2008) In-cell selectivity profiling of serine protease inhibitors by activity-based proteomics. *Mol. Cell. Proteomics* 7, 1241–1253
39 Vila, A. *et al.* (2008) Identification of protein targets of 4-hydroxynonenal using click chemistry for ex vivo biotinylation of azido and alkynyl derivatives. *Chem. Res. Toxicol.* 21, 432–444
40 Macpherson, L.J. *et al.* (2007) Noxious compounds activate TRPA1 ion channels through covalent modification of cysteines. *Nature* 445, 541–545
41 Wright, A.T. and Cravatt, B.F. (2007) Chemical proteomic probes for profiling cytochrome p450 activities and drug interactions in vivo. *Chem. Biol.* 14, 1043–1051
42 Evans, M.J. *et al.* (2005) Target discovery in small-molecule cell-based screens by in situ proteome reactivity profiling. *Nat. Biotechnol.* 23, 1303–1307
43 MacKinnon, A.L. *et al.* (2007) Photo-leucine incorporation reveals the target of a cyclodepsipeptide inhibitor of cotranslational translocation. *J. Am. Chem. Soc.* 129, 14560–14561
44 Dijkstra, H.P. *et al.* (2008) Selective and diagnostic labelling of serine hydrolases with reactive phosphonate inhibitors. *Org. Biomol. Chem.* 6, 523–531
45 Greenbaum, D. *et al.* (2000) Epoxide electrophiles as activity-dependent cysteine protease profiling and discovery tools. *Chem. Biol.* 7, 569–581
46 Cohen, M.S. *et al.* (2005) Structural bioinformatics-based design of selective, irreversible kinase inhibitors. *Science* 308, 1318–1321
47 Burdine, L. and Kodadek, T. (2004) Target identification in chemical genetics: the (often) missing link. *Chem. Biol.* 11, 593–597
48 Berg, T. (2005) Cellular profiling of small-molecule bioactivities: an alternative tool for chemical biology. *Angew Chem. Int. Ed. Engl.* 44, 5008–5011
49 Evans, M.J. *et al.* (2007) Mechanistic and structural requirements for active site labeling of phosphoglycerate mutase by spiroepoxides. *Mol. Biosyst.* 3, 495–506
50 Speers, A.E. and Cravatt, B.F. (2005) A tandem orthogonal proteolysis strategy for high-content chemical proteomics. *J. Am. Chem. Soc.* 127, 10018–10019
51 Weerapana, E. *et al.* (2007) Tandem orthogonal proteolysis-activity-based protein profiling (TOP-ABPP) – a general method for mapping sites of probe modification in proteomes. *Nat. Protoc.* 2, 1414–1425
52 Jaffe, C.L. *et al.* (1980) New clevable photoreactive heterobifunctional cross-linking reagents for studying membrane organization. *Biochemistry* 19, 4423–4429
53 Verhelst, S.H. *et al.* (2007) A mild chemically cleavable linker system for functional proteomic applications. *Angew Chem. Int. Ed. Engl.* 46, 1284–1286
54 Fonovic, M. *et al.* (2007) Proteomics evaluation of chemically cleavable activity-based probes. *Mol. Cell. Proteomics* 6, 1761–1770
55 Raghavan, A.S. *et al.* (2008) Chemical probes for profiling fatty acid-associated proteins in living cells. *Bioorg. Med. Chem. Lett.* In press