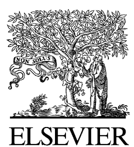

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Activity-based protein profiling of microbes

Natalie C Sadler and Aaron T Wright

Activity-based protein profiling (ABPP) in conjunction with multimodal characterization techniques has yielded impactful findings in microbiology, particularly in pathogen, bioenergy, drug discovery, and environmental research. Using small molecule chemical probes that react irreversibly with specific proteins or protein families in complex systems has provided insights in enzyme functions in central metabolic pathways, drug–protein interactions, and regulatory protein redox, for systems ranging from photoautotrophic cyanobacteria to mycobacteria, and combining live cell or cell extract ABPP with proteomics, molecular biology, modeling, and other techniques has greatly expanded our understanding of these systems. New opportunities for application of ABPP to microbial systems can enhance protein annotation, characterize protein activities in myriad environments, and reveal signal transduction and regulatory mechanisms in microbial systems.

Addresses
Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA 99352, USA

Corresponding author: Wright, Aaron T (Aaron.Wright@PNNL.gov)

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Introduction

Activity-based protein profiling (ABPP) is a powerful strategy for dissecting the function of microbial systems relevant to human health, bioenergy production, and the environment. Characterization of enzymes, transporters, protein localization, regulatory dynamics, and new drug discoveries has been accomplished using chemical probes with advanced proteomic techniques. ABPP encompasses the application of an increasingly broad collection of probe motifs that vary in both protein reactivity and mode of binding in diverse biological samples. Chemical probes have been intuitively applied to cell extracts and living systems with characterization by gel electrophoresis, fluorescence microscopy, and/or mass spectrometry. To date, ABPP in microbial research have only begun to be applied, but great promise remains in research such as uncovering the mechanisms by which energy-relevant microbes and communities and microbial pathogens function and respond to external stimuli. Here, we discuss the basis of ABPP and its application to microbial systems.

ABPP — probe design strategies

We broadly apply the term ABPP to a diverse collection of probes with multiple modes of reactivity *in vitro* and in living organisms (Table 1): first, mechanism-based probes report on function by reactions dependent upon the catalytic mechanism of the target enzymes; second, probes that mimic small-molecules/metabolites for identifying specific protein interactions, for example, drugs or small molecule probes with electrophilic or photoreactive moieties; and third, probes that report on protein regulatory modifications. Each probe type consists of three moieties: a reactive group that forms an irreversible covalent bond with a target protein, a binding or spacer group (e.g., protein substrate or metabolite) that directs a probe toward a protein or protein family and may also impart cell permeability, and a reporter tag for rapid and sensitive measurement of labeled enzymes by fluorescence or mass spectrometry-based proteomics (Figure 1). Probes are often designed 'tag-free' with an alkyne or azide compatible with the bioorthogonal Huisgen-Sharpless [3 + 2] cycloaddition reaction ('click chemistry') for multimodal reporter tag attachment [1]. This also decreases probe size, thereby minimizing undesirable impacts on reactivity with target proteins, and increasing cell permeability [2–5].

ABPP with antibiotic derived probes

Activity-based strategies are well suited for characterizing the mode of action by which drugs inhibit or activate an enzyme, enzyme family, or metabolic pathway, and for unraveling non-canonical drug metabolism pathways. Several ABPP studies have focused on antibiotics and antibiotic resistance. In 2008, Staub *et al.* synthesized natural and synthetic β-lactam probes for application in antibiotic resistant *Bacillus licheniformis*, *Listeria welshimeri*, and *Pseudomonas putida* [6]. The β-lactam probes labeled and inhibited a subset of the penicillin binding proteins including the virulence-associated enzyme ClpP and a resistance associated β-lactamase. These results indicate potential highly selective targets for fine-tuned antibiotic discovery. A similar ABPP approach was utilized to characterize the effects of the nucleoside antibiotic showdomycin. A tag-free showdomyocin probe was applied to *Staphylococcus aureus* and inhibited enzymes involved in cell wall biosynthesis, notably the essential MurA1 and MurA2 [7].

In 2011, Eirich *et al.* sought to profile the targets of the antibiotic vancomycin. Despite an established mode of

Table 1

Representative activity-based probes and their microbial applications

Probe target	Structure	Applications
Antibiotic reactive proteins		Showdomycin derived probe was applied to *S. aureus* to identify the antibiotic's targets. Cell wall biosynthesis enzymes MurA1 and MurA2 were identified [7]
Serine proteases		Studies to identify virulence mechanisms in *A. fumigatus* [12,13]. Characterized Glpg in *E. coli* [14]
ATPases	X = $NHBu_3^+$	Studies performed to determine pathogenicity and features of latency in *M. tuberculosis* [16–18]. PknB was identified as a critical regulator and replication switch [18]
Fatty acid synthases		Pantetheinyl probes crossed the cell membrane and were transformed into CoA analogues in *E. coli*, *S. oneidensis*, and *B. brevis* [21–23]
Glycoside hydrolases	R=H or Ac	Used to profile active cellulose degrading enzymes in *C. thermocellum* [24] and *T. reesei* [25] for biofuel application
Redox reactive proteins		H_2O_2 sensitive proteins were profiled in *P. aeruginosa* and *S. aureus*; LasR, ExaC, ArcA, and GAPDH were among the highlighted targets [29]. Redox reactive proteins were profiled *in vivo* during various nutrient levels in *Synechococcus* [32,33]

action, questions remain about the drug. These questions prompted researchers to develop a series of vancomycin probes enabled with a photoreactive moiety for binding target proteins and were then employed to identify non-canonical vancomycin targets in live *S. aureus* and *Enterococcus faecalis*. Two uncharacterized targets were identified with likely roles in vancomycin's efficacy: autolysin in *S. aureus* and an ABC transporter in *E. faecalis* [8]. In a similar approach, derivatives of the β-lactam-containing antibiotic cephalosporin were used to identify active penicillin-binding proteins (PBPs) in both *Bacillus subtilis* PY79 and an unencapsulated derivative of D39 *Streptococcus pneumonia*. The probes were applied *in vivo* to explore cell wall PBPs during cell division. Fluorescence microscopy indicated cephalosporin C-based probes as more selective than a penicillin V analogue, which labels all PBPs [9]. Similar approaches may lead to rational design of drug variants with improved selectivity and potency, and for identifying potential routes of microbial drug resistance.

Recently, an ABPP approach was employed with 4-hydroxyderricin, an isolate from the plant *Angelica keiskei* which features antibiotic potency against several strains of bacteria including *S. aureus*. The 4-hydroxyderricin probe bound and inhibited seryl-tRNA synthetases (STS), enzymes crucial for the biosynthesis of proteins. Sample alkylating pretreatments resulted in reduced probe labeling suggesting that the probe was binding to one or more Cys residues in the protein. Mutation studies were then performed to selectively replace each of the five cysteines with alanine; interestingly all five mutant enzymes displayed no catalytic activity emphasizing that a perturbation of just one Cys is sufficient to interfere with the amino acylation of tRNAs by STS. The results suggest that 4-hydroxyderricin inhibits STS activity by the alkylation of essential Cys residues within the enzyme and may induce apoptosis by halting protein biosynthesis. These results point toward a previously unrecognized role in regulation by this enzyme and illuminate it as a novel drug target [10•].

ABPP of enzyme function in microbial pathogens

Serine proteases

Serine proteases are a widespread group of proteolytic enzymes crucial to numerous physiological processes and implicated in microbial virulence mechanisms. Several probes to broadly characterize the serine hydrolase family of enzymes via a fluorophosphonate (FP) reactive group have been developed following initial work by Cravatt and coworkers [11]. While these probes are widely

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