Published in final edited form as:

Curr Opin Chem Biol. 2020 February ; 54: 45–53. doi:10.1016/j.cbpa.2019.10.007.

Activity-based protein profiling in bacteria: Applications for identification of therapeutic targets and characterization of microbial communities

Laura J Keller¹, Brett M Babin², Markus Lakemeyer², Matthew Bogyo^{2,3,*}

¹Departments of Chemical & Systems Biology, Stanford University School of Medicine, 300 Pasteur Drive, Stanford, CA 94305, USA

²Departments of Pathology, Stanford University School of Medicine, 300 Pasteur Drive, Stanford, CA 94305, USA

³Departments of Microbiology and Immunology, Stanford University School of Medicine, 300 Pasteur Drive, Stanford, CA 94305, USA

Abstract

Activity-based protein profiling (ABPP) is a robust chemoproteomic technique that uses activity-based probes to globally measure endogenous enzymatic activity in complex proteomes. It has been utilized extensively to characterize human disease states and identify druggable targets in diverse disease conditions. ABPP has also recently found applications in microbiology. This includes using activity-based probes (ABPs) for functional studies of pathogenic bacteria as well as complex communities within a microbiome. This review will focus on recent advances in the use of ABPs to profile enzyme activity in disease models, screen for selective inhibitors of key enzymes, and develop imaging tools to better understand the host-bacterial interface.

Keywords

Activity-based protein profiling; bacteria; microbiome

Introduction

Bacteria have profound impacts on human health, ranging from life-threatening infections by pathogenic bacteria to commensal bacteria living in symbiosis to maintain the healthy state of a host. ‘Omics’-based techniques have been invaluable for mapping interactions between pathogenic bacteria and the host (reviewed in [1]) as well as dynamic changes in the composition and functional output of bacterial communities in response to diet [2,3] or

*Corresponding author: Bogyo, Matthew (mbogyo@stanford.edu).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflict of Interest Statement

The authors declare no competing financial interest.

disease [4,5]. While these studies are able to capture snapshots of complex functional profiles of host-bacteria and bacteria-bacteria interactions, unbiased 'omics'-based approaches alone do not inherently provide ways to prioritize key regulators of a given state. The application of chemical tools has become invaluable for the study of bacterial systems. Of note, activity-based protein profiling (ABPP) has been applied to overcome some of the limitation of unbiased 'omics'. This approach uses chemical probes that provide a direct readout of enzyme activity, thus enabling enrichment of relevant enzymatic targets in complex and dynamically regulated systems. Furthermore, because ABPP identifies enzymes via chemical modification of their active sites, it focuses on inherently druggable targets that have the potential for modulation by small molecule therapeutics [6]. In this review, we will highlight the recent applications of ABPP to studies of both pathogenic and commensal bacteria, including early advances in the use of ABPP to characterize gut microbiome function.

Activity-based protein profiling (ABPP)

ABPP measures enzyme activity using an activity-based probe (ABP) that covalently modifies active-site nucleophiles in an activity-dependent manner. ABPs comprise a warhead for covalent bond formation, a linker that confers specificity, and a tag for the visualization or enrichment of the labeled enzymes (Figure 1a) [7]. An ABP-labeled proteome can either be separated by gel electrophoresis and visualized via a fluorophore tag or be enriched by affinity purification using an affinity tag, digested, so that targets can be identified by LC-MS/MS (Figure 1b) [8,9]. Additionally, some ABPs have an alkyne-based tag such that an azide-containing fluorophore or biotin can be added via copper-catalyzed or copper-free alkyne-azide cycloaddition (CuAAC) [10] after probe labeling.

One of the most significant benefits of ABPP is that it allows dynamic profiling of multiple enzymes in their endogenous environments [8,11]. Furthermore, it selects for enzymes that are amenable to modulation by small molecules and provides a platform to screen for selective inhibitors, even for enzymes with unknown function and unknown substrates, through competitive ABPP (Figure 1c) [7,12]. There are a number of excellent reviews that cover the application of ABPP to bacterial pathogenesis [13,14], host-microbe interactions [15,16], and host-associated and environmental microbial communities [17]. In addition, there have been many studies using affinity-based protein profiling (reviewed in [18]), which utilizes a photoreactive group to covalently label a target with the probe rather than relying on the reactivity of the catalytic residues of a target. Here, we will discuss recent examples of diverse applications of ABPP, including profiling enzymatic activity *in vitro*, identifying enzymes involved in the host-bacteria interface, and developing inhibitors and imaging probes for important bacterial enzyme targets.

ABPP as a profiling approach for bacteria in culture

As a first step toward understanding which proteins are important for host-microbe interactions, ABPP can be used to identify and classify enzymes from bacteria grown *in vitro*. While these studies are limited in that they cannot accurately replicate the complex environment inside a host, certain aspects of the pathogenic state of bacteria can be

mimicked *in vitro*, and the ability to precisely manipulate experimental conditions allows for well-controlled comparative studies of how enzyme activities changes.

Mycobacterium tuberculosis, the causative agent of tuberculosis, infects almost a quarter of the world's population in a dormant state [19], which is especially hard to treat because of its reduced metabolism [20]. To understand which enzymes remain active in dormancy, Ortega and co-workers used a serine-reactive fluorophosphonate-based ABP **1** containing a polyethylene glycol (PEG)-based linker to profile serine hydrolase activity in a hypoxic model of *M. tuberculosis* dormancy [21]. Of the 75 enzymes annotated as hydrolases or hypothetical proteins that were identified under exponential growth conditions, approximately half were detected under non-replicative hypoxic conditions, albeit with reduced activity. The others were not detected at all, consistent with reduced metabolism during dormancy. Furthermore, the authors were able to define the hydrolytic function of several identified unannotated enzymes [21], highlighting the shortcomings of bioinformatics-based annotation of highly divergent bacteria and the power of ABPP to experimentally annotate and confirm biochemical functions of enzymes. Tallman and co-workers performed a similar study with fluorophosphonate-based ABP **2**, which had a saturated alkyl chain linker to specifically target lipid-metabolizing esterases [22]. This study identified five esterases active in both normoxic and hypoxic states that were also identified by Ortega and co-workers [21], suggesting that these enzymes are promising targets for drugs to treat both active and latent tuberculosis. In fact, several drugs that have anti-mycobacterial effects, including the anti-diabetes drug tetrahydrolipstatin (compound **3**) [23] and analogs of the natural product cyclipostin P (compound **4**) [24], have been shown via ABPP to act by covalently inhibiting the activity of multiple lipid-metabolizing enzymes. While neither of the studies by Ortega and co-workers [21] and Tallman and co-workers [22] went beyond profiling the enzymes active in hypoxic *M. tuberculosis*, both demonstrate the value of ABPP in poorly annotated bacterial genomes.

Chemoproteomic approaches have also been developed to profile reactive cysteine [25] and lysine [26] residues in mammalian proteomes, using iodoacetamide alkyne (ABP **5**) and sulfotetrafluorophenyl ester alkyne (ABP **6**), respectively. However, these probes label active site residues as well as cysteines and lysines that play other roles in protein function. Cysteine residues, for example, can serve as metal ligands or redox-active disulfides [27]. Deng and co-workers used the general cysteine-reactive ABP **5** to identify oxidation-sensitive cysteines in *Pseudomonas aeruginosa* and *Staphylococcus aureus*, mapping out pathogen response to oxidative stress induced by hydrogen peroxide [28]. The host uses oxidative stress as one tactic to combat infections, so understanding how bacteria respond to this defense mechanism can help predict behavior in an actual infection. As these probes and others [29,30] can target both catalytic and non-catalytic sites, they are also valuable tools for identifying novel druggable targets across the bacterial proteome.

ABPP as a tool for characterizing host-bacteria interactions *in vivo*

Beyond using ABPs to profile enzyme activity in individual species of pathogenic bacteria, their application is especially powerful in analyzing complex host-bacteria interactions.

There have been a number of recent examples of ABPs used to profile enzyme activity in *in vivo* models of infection and in the gut microbiome.

Using the fluorophosphonate-based ABP **7**, Hatzios and co-workers profiled active serine hydrolases in the cecal fluid of *Vibrio cholerae*-infected infant rabbits and in human choleric stool [31]. This ABP-based enrichment identified four *V. cholerae* serine proteases that were not detected in a total proteomic analysis. One of these proteases, IvaP, modulates the activity of both host and *V. cholerae* serine hydrolases, and all four serine proteases together decreased the abundance of several host proteins, including one that enhances macrophage phagocytosis of bacteria [32]. Consequently, the identification of *V. cholerae* enzymes active in infection pinpointed several bacterial proteases that manipulate the host response and potentially allow for bacterial evasion of the innate immune system [31].

Unlike infections by a single pathogenic bacterial species, in the gut there exists a rich community of commensal bacteria whose makeup changes in response to many different external stimuli and disease states. With this added complexity, enrichment for low abundance proteins with ABPs is especially advantageous. A recent effort by Mayers and co-workers applied ABPs to the gut microbiome in a mouse model of colitis [33]. A metaproteomic analysis of both host- and microbial-derived proteins from fecal pellets found that mice with colitis had elevated host inflammatory proteins, host serine protease inhibitors, and general microbial metabolic proteins. Interestingly, the authors did not detect bacterial proteases, which are known to play an important role in the pathogenesis of colitis (reviewed in [34]). By enriching with the cysteine-reactive ABP **8**, elevated activity of microbial Cys-type peptidases and dehydrogenases was detected in animals with colitis [33]. While this study was not able to single out a key enzyme from a particular bacterial species with this broadly reactive ABP, the authors demonstrated that ABPs can successfully enrich for relevant yet low-abundant enzymes from complex samples.

More recent work applying ABPs to the microbiome has approached this complexity by focusing on a specific class of enzymes. Microbial β -glucuronidases, for example, remove glucuronic acid, which host enzymes conjugate to drugs to help their clearance. As a result, high expression of these microbial enzymes has detrimental effects on drug clearance [35]. To understand which of the 250+ identified bacterial β -glucuronidases [36] are active *in vivo*, Whidbey and co-workers developed the substrate-based ABP **9** that is processed by β -glucuronidases to produce a reactive intermediate that labels nearby nucleophilic residues [37]. The authors labeled bacteria isolated from the mouse gut with fluorophore-conjugated ABP **9** and used fluorescence-activated cell sorting (FACS) to isolate the bacteria positive for β -glucuronidase activity. This workflow allowed both the quantification of total β -glucuronidase activity and the identification of bacterial species with active β -glucuronidases via 16S rRNA sequencing. For example, perturbation by vancomycin treatment globally decreased β -glucuronidase activity, although the bacterial species that had active β -glucuronidases differed across litters of mice [37]. Despite the small size of their study, the authors showed that the strategy of combining ABPs and FACS in the gut microbiome will be very useful in connecting molecular-level mechanistic results with systems-level bacterial community studies.

An ABP has also been developed for bile salt hydrolases [38], a class of gut bacterial enzymes that catalyze the first step of microbial metabolism of host-secreted conjugated bile acids. The downstream secondary bile acids affect host metabolism [39–41] and are dysregulated in inflammatory bowel diseases [42]. Although a photoreactive probe based on the structure of bile acids was developed and used to identify bile acid-interacting proteins in mammalian cells [43], the bile salt hydrolases in bacteria that are active *in vivo* had not been well-characterized. To address this gap in knowledge, Parasar and co-workers treated bacteria isolated from mouse fecal pellets with the cholic acid-based covalent ABP **10** [38]. In a mouse model of colitis, global activity of bile salt hydrolases was elevated, although the level of activity of individual bile salt hydrolases across several species in the phylum Bacteroidetes varied from mouse to mouse. Similar to the work by Whidbey and co-workers [37], only a small cohort of mice was used, so the biological relevance of this inter-mouse variability is not well-understood. However, future studies with larger sample sizes and additional ABPs will further the understanding of host and bacterial enzyme function in the gut microbiome, the contribution of individual bacteria to this overall function, and the alterations that occur upon disease progression.

ABPP as a method for developing inhibitors

Beyond simply identifying enzymes whose activities are relevant and druggable in a pathogenic context, ABPP can also directly enable the development of selective inhibitors for enzymes of interest. By measuring enzyme activity in its endogenous environment, inhibitors can be tested without expressing recombinant protein or even knowing the native substrates of the enzymes. Selective inhibitors can then be used to control enzyme activity temporally, even in currently genetically intractable species, and to visualize bacterial subcellular enzyme activity in complex environments. There are two primary approaches for identifying new classes of inhibitors, either screening a compound library via competitive ABPP (Figure 1c) or developing electrophile-containing molecules inspired by the endogenous substrate of an enzyme, both of which will be explored in this section.

Staphylococcus aureus, a leading cause of nosocomial infections, can form biofilms that are resistant to antibiotic treatment [44]. To identify serine hydrolases that are active in this state, ABPP was performed using the fluorophosphonate-based ABP **11** [45]. This chemoproteomic study resulted in the identification of 12 α,β -hydrolase domain-containing serine hydrolases active in live bacteria during biofilm growth. Within this newly discovered enzyme family named fluorophosphonate binding hydrolases (Fphs), only two enzymes had been previously studied and the majority lacked even basic annotation as hydrolases in the Uniprot database (<https://www.uniprot.org/>). Because the targets were identified using an ABP, it was possible to immediately screen a focused library of covalent serine-reactive electrophiles to identify selective inhibitors of individual members of this family of hydrolases. Chloroisocoumarin **12**, for example, showed potent and selective inhibition of FphB. Pretreatment of bacteria with compound **12** decreased the colonization efficiency of wild-type *S. aureus* in the liver during a systemic infection mouse model, suggesting this enzyme may be an important virulence factor that is required for early colonization of host tissues [45]. In a follow-up study, competitive ABPP was used to screen a library of serine-reactive 1,2,3-triazole ureas to identify additional specific inhibitors of Fph enzymes [46].

One of the most promising hits from the library screen was converted to the fluorescent ABP **13**, and its use as a selective imaging probe highlighted the heterogeneity of FphE enzyme activity across a population of isogenic bacteria and identified compensatory functions within this network of Fph enzymes [46].

Inspired by natural products, the Sieber lab and others have used β -lactone-containing molecules to design a number of inhibitors [47,48]. One such compound, compound **14**, inhibited *M. tuberculosis* growth at low doses [49]. Using an alkyne handle-containing analog of compound **14**, Lehmann and co-workers identified two targets of this molecule, antigen 85 and polyketide synthase 13, both of which are serine hydrolases instrumental in mycolic acid biosynthesis. They confirmed these ABPP results *in vitro* and demonstrated that compound **14** was capable of decreasing the pool of newly synthesized mycolate α -chains. Furthermore, compound **14** had synergistic killing effects with other approved antibiotics such as rifampicin and vancomycin, showing that compounds that mimic crucial bacterial metabolites, in this case the β -hydroxy motif of mycolic acid, can be used as novel antibiotics [49].

Conclusions and perspectives

To truly understand how bacteria, both good and bad, can affect human health, understanding of the context of host-bacteria and bacteria-bacteria interactions is essential. As demonstrated in this review, small molecule ABPs are valuable tools for capturing enzyme activity in the native environment of bacteria. They have applications ranging from profiling enzymes active in disease to developing small molecule inhibitors against identified enzymes. The ability of ABPs to enrich for active enzymes is especially powerful in complex systems such as the gut microbiome.

Moving forward, simpler models of gut microbial communities, ranging from gnotobiotic mice with defined microbial communities to mono- or co-culturing commensal bacterial *in vitro*, can provide insight into the activity of relevant enzymes in a well-controlled manner. In particular, we think these models can help narrow the focus specifically onto pathobiont, or contextually pathogenic, bacteria. Many diseases, such as inflammatory bowel diseases or colon cancer, are characterized by a shift in gut microbial communities to establish a state of dysbiosis that favors the growth and dominance of pathobiont bacteria [50–53]. We believe that, by using approaches such as ABPP to identify relevant bacterial enzymes that are essential for maintaining a disease state and then developing selective inhibitors for those enzymes (Figure 2), it will be possible to shift the composition of a microbiome back to a healthy state in order to treat diverse human diseases.

Acknowledgements

This work was supported by National Institutes of Health grants R01 EB026332 and R01 EB026285 (M.B.). L.J.K. was supported by Stanford ChEM-H Chemistry/Biology Interface Predoctoral Training Program, Stanford Molecular Pharmacology Training Grant, and Stanford Graduate Fellowship. B.M.B. was supported by the A.P. Giannini Foundation.

References and recommended reading

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

* of special interest

** of outstanding interest

1. Khan MM, Ernst O, Manes NP, Oyler BL, Fraser IDC, Goodlett DR, Nita-Lazar A: Multi-Omics Strategies Uncover Host–Pathogen Interactions. *ACS Infect Dis* 2019, 5:493–505. [PubMed: 30857388]
2. Saffouri GB, Shields-Cutler RR, Chen J, Yang Y, Lekatz HR, Hale VL, Cho JM, Battaglioli EJ, Bhattarai Y, Thompson KJ, et al.: Small intestinal microbial dysbiosis underlies symptoms associated with functional gastrointestinal disorders. *Nat Commun* 2019, 10:2012. [PubMed: 31043597]
3. Gehrig JL, Venkatesh S, Chang H-W, Hibberd MC, Kung VL, Cheng J, Chen RY, Subramanian S, Cowardin CA, Meier MF, et al.: Effects of microbiota-directed foods in gnotobiotic animals and undernourished children. *Science* 2019, 365:eaau4732. [PubMed: 31296738]
4. Franzosa EA, Sirota-Madi A, Avila-Pacheco J, Fornelos N, Haiser HJ, Reinker S, Vatanen T, Hall AB, Mallick H, McIver LJ, et al.: Gut microbiome structure and metabolic activity in inflammatory bowel disease. *Nat Microbiol* 2018, doi:10.1038/s41564-018-0306-4.
5. Lloyd-Price J, Arze C, Ananthakrishnan AN, Schirmer M, Avila-Pacheco J, Poon TW, Andrews E, Ajami NJ, Bonham KS, Brislawn CJ, et al.: Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. *Nature* 2019, 569:655–662. [PubMed: 31142855]
6. Bachovchin DA, Cravatt BF: The pharmacological landscape and therapeutic potential of serine hydrolases. *Nat Rev Drug Discov* 2012, 11:52–68. [PubMed: 22212679]
7. Jeffery DA, Bogoy M: Chemical proteomics and its application to drug discovery. *Curr Opin Biotechnol* 2003, 14:87–95. [PubMed: 12566007]
8. Jessani N, Niessen S, Wei BQ, Nicolau M, Humphrey M, Ji Y, Han W, Noh D-Y, Yates JR, Jeffrey SS, et al.: A streamlined platform for high-content functional proteomics of primary human specimens. *Nat Methods* 2005, 2:691–697. [PubMed: 16118640]
9. Sadaghiani AM, Verhelst SH, Bogoy M: Tagging and detection strategies for activity-based proteomics. *Curr Opin Chem Biol* 2007, 11:20–28. [PubMed: 17174138]
10. Rostovtsev VV, Green LG, Fokin VV, Sharpless KB: A Stepwise Huisgen Cycloaddition Process: Copper(I)-Catalyzed Regioselective “Ligation” of Azides and Terminal Alkynes. *Angew Chemie Int Ed* 2002, 41:2596–2599.
11. Bachovchin DA, Ji T, Li W, Simon GM, Blankman JL, Adibekian A, Hoover H, Niessen S, Cravatt BF: Superfamily-wide portrait of serine hydrolase inhibition achieved by library-versus-library screening. *Proc Natl Acad Sci U S A* 2010, 107:20941–6. [PubMed: 21084632]
12. Niphakis MJ, Cravatt BF: Enzyme Inhibitor Discovery by Activity-Based Protein Profiling. *Annu Rev Biochem* 2014, 83:341–377. [PubMed: 24905785]
13. Puri AW, Bogoy M: Applications of small molecule probes in dissecting mechanisms of bacterial virulence and host responses. *Biochemistry* 2013, 52:5985–96. [PubMed: 23937332]
14. Sadler NC, Wright AT: Activity-based protein profiling of microbes. *Curr Opin Chem Biol* 2015, 24:139–144. [PubMed: 25531039]
15. Wright MH: Chemical Proteomics of Host-Microbe Interactions. *Proteomics* 2018, 18:1700333.
16. Gregor R, David S, Meijler MM: Chemical strategies to unravel bacterial–eukaryotic signaling. *Chem Soc Rev* 2018, 47:1761–1772. [PubMed: 29260158]
17. Whidbey C, Wright AT: Activity-Based Protein Profiling-Enabling Multimodal Functional Studies of Microbial Communities. *Curr Top Microbiol Immunol* 2019, 420:1–21. [PubMed: 30406866]
18. Wright MH, Sieber SA: Chemical proteomics approaches for identifying the cellular targets of natural products. *Nat Prod Rep* 2016, 33:681–708. [PubMed: 27098809]
19. WHO | Global tuberculosis report 2018. WHO 2019,

20. Caño-Muñoz S, Anthony R, Niemann S, Alffenaar J-WC: New Approaches and Therapeutic Options for Mycobacterium tuberculosis in a Dormant State. *Clin Microbiol Rev* 2018, 31.
21. Ortega C, Anderson LN, Frando A, Sadler NC, Brown RW, Smith RD, Wright AT, Grundner C: Systematic Survey of Serine Hydrolase Activity in Mycobacterium tuberculosis Defines Changes Associated with Persistence. *Cell Chem Biol* 2016, 23:290–298. [PubMed: 26853625]
22. Tallman KR, Levine SR, Beatty KE: Small-Molecule Probes Reveal Esterases with Persistent Activity in Dormant and Reactivating Mycobacterium tuberculosis. *ACS Infect Dis* 2016, 2:936–944. [PubMed: 27690385]
23. Ravindran MS, Rao SPS, Cheng X, Shukla A, Cazenave-Gassiot A, Yao SQ, Wenk MR: Targeting lipid esterases in mycobacteria grown under different physiological conditions using activity-based profiling with tetrahydrolipstatin (THL). *Mol Cell Proteomics* 2014, 13:435–48. [PubMed: 24345785]
24. Nguyen PC, Delorme V, Bénarouche A, Martin BP, Paudel R, Gnawali GR, Madani A, Puppo R, Landry V, Kremer L, et al.: Cyclopostins and Cyclophostin analogs as promising compounds in the fight against tuberculosis. *Sci Rep* 2017, 7:11751. [PubMed: 28924204]
25. Weerapana E, Wang C, Simon GM, Richter F, Khare S, Dillon MBD, Bachovchin DA, Mowen K, Baker D, Cravatt BF: Quantitative reactivity profiling predicts functional cysteines in proteomes. *Nature* 2010, 468:790–795. [PubMed: 21085121]
26. 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. [PubMed: 29168484]
27. Bak DW, Bechtel TJ, Falco JA, Weerapana E: Cysteine reactivity across the subcellular universe. *Curr Opin Chem Biol* 2019, 48:96–105. [PubMed: 30508703]
28. 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. [PubMed: 23498960]
29. 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. [PubMed: 26020833]
30. Ward CC, Kleinman JI, Nomura DK: NHS-Esters As Versatile Reactivity-Based Probes for Mapping Proteome-Wide Ligandable Hotspots. *ACS Chem Biol* 2017, 12:1478–1483. [PubMed: 28445029]
31. Hatzios SK, Abel S, Martell J, Hubbard T, Sasabe J, Munera D, Clark L, Bachovchin DA, Qadri F, Ryan ET, et al.: Chemoproteomic profiling of host and pathogen enzymes active in cholera. *Nat Chem Biol* 2016, 12:268–274. [PubMed: 26900865]
32. Tsuji S, Yamashita M, Hoffman DR, Nishiyama A, Shinohara T, Ohtsu T, Shibata Y: Capture of heat-killed Mycobacterium bovis bacillus Calmette-Guérin by intelectin-1 deposited on cell surfaces. *Glycobiology* 2009, 19:518–26. [PubMed: 19179460]
33. Mayers MD, Moon C, Stupp GS, Su AI, Wolan DW: Quantitative Metaproteomics and Activity-Based Probe Enrichment Reveals Significant Alterations in Protein Expression from a Mouse Model of Inflammatory Bowel Disease. *J Proteome Res* 2017, 16:1014–1026. [PubMed: 28052195] **Application of activity-based probes in the gut microbiome that measured an upregulation of microbial peptidase activity in colitis, which was not detected at the proteomic level.
34. Carroll IM, Maharshak N: Enteric bacterial proteases in inflammatory bowel disease-pathophysiology and clinical implications. *World J Gastroenterol* 2013, 19:7531. [PubMed: 24431894]
35. Pellock SJ, Redinbo MR: Glucuronides in the gut: Sugar-driven symbioses between microbe and host. *J Biol Chem* 2017, 292:8569–8576. [PubMed: 28389557]
36. Pollet RM, D'Agostino EH, Walton WG, Xu Y, Little MS, Biernat KA, Pellock SJ, Patterson LM, Creekmore BC, Isenberg HN, et al.: An Atlas of β -Glucuronidases in the Human Intestinal Microbiome. *Structure* 2017, 25:967–977.e5. [PubMed: 28578872]
37. Whidbey C, Sadler NC, Nair RN, Volk RF, DeLeon AJ, Bramer LM, Fansler SJ, Hansen JR, Shukla AK, Jansson JK, et al.: A Probe-Enabled Approach for the Selective Isolation and Characterization of Functionally Active Subpopulations in the Gut Microbiome. *J Am Chem Soc*

- 2019, 141:42–47. [PubMed: 30541282] **Coupling activity-based probes and fluorescence activated cell sorting enabled the enrichment of bacteria with specific enzymatic activity from a complex environment and the identification of the bacterial species.
38. Parasar B, Zhou H, Xiao X, Shi Q, Brito IL, Chang PV.: Chemoproteomic Profiling of Gut Microbiota-Associated Bile Salt Hydrolase Activity. *ACS Cent Sci* 2019, 5:867. [PubMed: 31139722] *Use of a substrate-based activity-based probe for the identification of specific bacterial bile salt hydrolases from the fecal pellets of conventionally-raised mice. Activity of individual enzymes varied from mouse to mouse but global activity across the entire microbiome shifted upon disease.
39. Fiorucci S, Distrutti E: Bile Acid-Activated Receptors, Intestinal Microbiota, and the Treatment of Metabolic Disorders. *Trends Mol Med* 2015, 21:702–714. [PubMed: 26481828]
40. Devlin AS, Fischbach MA: A biosynthetic pathway for a prominent class of microbiota-derived bile acids. *Nat Chem Biol* 2015, 11:685–90. [PubMed: 26192599]
41. Yao L, Seaton SC, Ndousse-Fetter S, Adhikari AA, DiBenedetto N, Mina AI, Banks AS, Bry L, Devlin AS: A selective gut bacterial bile salt hydrolase alters host metabolism. *Elife* 2018, 7.
42. Duboc H, Rajca S, Rainteau D, Benarous D, Maubert M-A, Quervain E, Thomas G, Barbu V, Humbert L, Despras G, et al.: Connecting dysbiosis, bile-acid dysmetabolism and gut inflammation in inflammatory bowel diseases. *Gut* 2013, 62:531–9. [PubMed: 22993202]
43. Zhuang S, Li Q, Cai L, Wang C, Lei X: Chemoproteomic Profiling of Bile Acid Interacting Proteins. *ACS Cent Sci* 2017, 3:501–509. [PubMed: 28573213]
44. McCarthy H, Rudkin JK, Black NS, Gallagher L, O'Neill E, O'Gara JP: Methicillin resistance and the biofilm phenotype in *Staphylococcus aureus*. *Front Cell Infect Microbiol* 2015, 5:1. [PubMed: 25674541]
45. Lentz CS, Sheldon JR, Crawford LA, Cooper R, Garland M, Amieva MR, Weerapana E, Skaar EP, Bogoy M: Identification of a *S. aureus* virulence factor by activity-based protein profiling (ABPP). *Nat Chem Biol* 2018, 14:609–617. [PubMed: 29769740] **Activity-based protein profiling identified a new family of *S. aureus* bacterial serine hydrolases including a context-dependent virulence factor whose activity could be selectively inhibited by a small molecule resulting in reduced infection of specific tissues in a mouse model of infection.
46. Chen L, Keller LJ, Cordasco E, Bogoy M, Lentz CS: Fluorescent Triazole Urea Activity-Based Probes for the Single-Cell Phenotypic Characterization of *Staphylococcus aureus*. *Angew Chemie Int Ed* 2019, 58:5643–5647. *Converting a selective inhibitor to a fluorescent imaging tool enabled the measurement of the subcellular localization of enzyme activity and overall heterogeneity within a population.
47. Böttcher T, Sieber SA: β -Lactones as Specific Inhibitors of ClpP Attenuate the Production of Extracellular Virulence Factors of *Staphylococcus aureus*. *J Am Chem Soc* 2008, 130:14400–14401. [PubMed: 18847196]
48. Sharifzadeh S, Boersma MJ, Kocaoglu O, Shokri A, Brown CL, Shirley JD, Winkler ME, Carlson EE: Novel Electrophilic Scaffold for Imaging of Essential Penicillin-Binding Proteins in *Streptococcus pneumoniae*. *ACS Chem Biol* 2017, 12:2849–2857. [PubMed: 28990753]
49. Lehmann J, Cheng T-Y, Aggarwal A, Park AS, Zeiler E, Raju RM, Akopian T, Kandrö O, Sacchettini JC, Moody DB, et al.: An Antibacterial β -Lactone Kills *Mycobacterium tuberculosis* by Disrupting Mycolic Acid Biosynthesis. *Angew Chemie Int Ed* 2018, 57:348–353. *A small molecule mimicking a vital biosynthetic pathway can serve as a potent enzymatic inhibitor and its effects can be confirmed by metabolomic analyses of its products.
50. Shin N-R, Whon TW, Bae J-W: Proteobacteria: microbial signature of dysbiosis in gut microbiota. *Trends Biotechnol* 2015, 33:496–503. [PubMed: 26210164]
51. Zhou Y, Chen H, He H, Du Y, Hu J, Li Y, Li Y, Zhou Y, Wang H, Chen Y, et al.: Increased *Enterococcus faecalis* infection is associated with clinically active Crohn disease. *Medicine (Baltimore)* 2016, 95:e5019. [PubMed: 27684872]
52. Pötgens SA, Brossel H, Sboarina M, Catry E, Cani PD, Neyrinck AM, Delzenne NM, Bindels LB: *Klebsiella oxytoca* expands in cancer cachexia and acts as a gut pathobiont contributing to intestinal dysfunction. *Sci Rep* 2018, 8:12321. [PubMed: 30120320]

53. Chassaing B, Gewirtz AT: Mice harboring pathobiont-free microbiota do not develop intestinal inflammation that normally results from an innate immune deficiency. PLoS One 2018, 13:e0195310. [PubMed: 29617463]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 1. Activity-based protein profiling (ABPP) applications.

(a) Schematic structure of an activity-based probe containing a generally reactive warhead for covalent binding, a linker conferring specificity, and a tag. (b) The general workflow for ABPP. The preparation of the proteome will vary based on the sample, but most studies highlighted in this review use a combination of gel-based and LC/MS-MS-based ABPP. (c) The general workflow for competitive ABPP, utilized in Lentz *et al.* [45] and Chen *et al.* [46]. Intact bacteria are treated with compounds to bind target enzymes and residual enzyme activity measured by labeling with a broad-spectrum ABP. Engagement of the target by the inhibitor is measured as a loss of signal from the ABP. The approach allows confirmation of target engagement as well as a readout of overall target selectivity within a class of related enzymes.

Figure 2. Future applications for ABPP for treating diseases mediated by microbiome dysbiosis. (a) Schematic diagram of the gut microbial communities of healthy mice and diseased mice with a bloom of a pathobiont bacteria. Representative gel-based ABPP profiling are shown for the healthy and disease-state microbiomes, which enables identification of enzymes that are specific to the diseased microbiome. Competitive ABPP can then be used to screen for inhibitors that selectively target the disease-specific microbial enzyme leading to decreased viability of the pathobiont bacteria in the community. (b) Treating the diseased mouse with a pathobiont-specific inhibitor selectively kills the pathobiont bacteria and allows the microbial community to return to a healthy state.

Table 1.

Activity-based probes and compounds referenced in this review, including the name of the molecule in the original paper, its targets, and the biological sample(s) in which it was tested. For each molecule, the warhead is colored red and the tag is colored blue.

	Structure	Compound Name	Enzyme Class	Biological Sample	Top Targets of Interest	Reference
1		FP-ABP	Serine hydrolases	<i>Mycobacterium tuberculosis</i>	78 serine hydrolases or hypothetical proteins; Rv2224c (CaeA/Hip1), Rv2284 (LipM), Rv2970c (LipN), Rv0183, Rv1683	Ortega et al., <i>Cell Chem Biol.</i> , 2016
2		Desthiobiotin-FP	Serine hydrolases	<i>Mycobacterium tuberculosis</i>	52 serine hydrolases; Rv2224c (CaeA/Hip1), Rv2284 (LipM), Rv2970c (LipN), Rv0183, Rv1683	Tallman et al., <i>ACS Infect Dis.</i> , 2016
3		THL	Serine lipid esterases	<i>Mycobacterium bovis</i> BCG	14 serine hydrolases; LipH, TesA	Ravindran et al., <i>Mol Cell Proteomics</i> , 2014
4		CyC ₁₈	Serine/cysteine nucleophiles	<i>Mycobacterium tuberculosis</i> H37Rv	23 enzymes; HsaD	Nguyen et al., <i>Sci Rep.</i> , 2017
5		IA probe	Reactive cysteine residues	<i>Pseudomonas aeruginosa</i> , <i>Staphylococcus aureus</i>	<i>P. aeruginosa</i> : 82 oxidation-sensitive Cys residues, LasR, ExaC, ArcA, GAPDH. <i>S. aureus</i> : 113 oxidation-sensitive Cys residues, GAPDH	Weerapana et al., <i>Nature</i> , 2010; Deng et al., <i>Cell Host Microbe</i> , 2013
6		STP alkyne 1	Reactive lysine residues	--	--	Hacker et al., <i>Nat Chem.</i> , 2017
7		FP-biotin	Serine hydrolases	<i>Vibrio cholerae</i> -infected rabbit cecal fluid, human choleric stool	233 rabbit proteins, 71 human proteins, 14 <i>V. cholerae</i> proteins; IvaP, VCA0812, VCA0803, VC1200	Hatzios et al., <i>Nat Chem Biol.</i> , 2016
8		BioGlyCMK	Reactive cysteine residues	Mouse fecal pellets	Peptidases, alcohol dehydrogenases, acetaldehyde dehydrogenases	Mayers et al., <i>J Proteome Res.</i> , 2017
9		GlcA-ABP	β -glucuronidases	Mouse gastrointestinal microbes	Lachnospiraceae, Erysipelotrichaceae, Hungateiclostridiaceae, Lactobacillaceae, Paenibacillaceae	Whidbey et al., <i>J Am Chem Soc.</i> , 2019

	Structure	Compound Name	Enzyme Class	Biological Sample	Top Targets of Interest	Reference
10		Ch-AOMK	Bile salt hydrolases	Mouse fecal pellets	<i>Bacteroides</i> sp., <i>Bacteroidales bacterium</i> , <i>Muribaculaceae bacterium</i>	Parasar et al., <i>ACS Cent Sci.</i> , 2019
11		FP-TMR	Serine hydrolases	<i>Staphylococcus aureus</i>	12 serine hydrolases; FphB	Lentz et al., <i>Nat Chem Biol.</i> , 2018
12		JCP251	FphB	<i>Staphylococcus aureus</i>	--	Lentz et al., <i>Nat Chem Biol.</i> , 2018
13		9a	FphE	<i>Staphylococcus aureus</i>	--	Chen et al., <i>Angew Chem Int Ed.</i> , 2019
14		EZ120	Mycolic acid-binding proteins	<i>Mycobacterium tuberculosis</i>	Ag85, Pks13	Lehmann et al., <i>Angew Chem Int Ed.</i> , 2018