REVIEW

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Monitoring host–pathogen interactions using chemical proteomics

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With the rapid emergence and the dissemination of microbial resistance to conventional chemotherapy, the shortage of novel antimicrobial drugs has raised a global health threat. As molecular interactions between microbial pathogens and their mammalian hosts are crucial to establish virulence, pathogenicity, and infectivity, a detailed understanding of these interactions has the potential to reveal novel therapeutic targets and treatment strategies. Bidirectional molecular communication between microbes and eukaryotes is essential for both pathogenic and commensal organisms to colonise their host. In particular, several devastating pathogens exploit host signalling to adjust the expression of energetically costly virulent behaviours. Chemical proteomics has emerged as a powerful tool to interrogate the protein interaction partners of small molecules and has been successfully applied to advance host–pathogen communication studies. Here, we present recent significant progress made by this approach and provide a perspective for future studies.

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1. Introduction

The evolution and spread of antimicrobial resistance have made drug-resistant bacterial infections one of the most serious global threats to human health in the 21st century.[1] Classical antibiotics target essential processes in bacteria and thus exert selection pressure, which drives antimicrobial resistance formation.[2] This is further aggravated by the dissemination and maintenance of resistance factors among pathogens, *e.g.* by widespread horizontal gene transfer. In contrast to this,

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the development of novel antibiotics is extremely sluggish[3] and no new antimicrobial drug has been developed by traditional methods in the last 30 years.[4] Therefore, the speed of microbial resistance development and the difficulties in the antibiotic discovery have culminated in a serious global threat which urgently necessitates the development of novel therapeutic strategies with unprecedented modes of action beyond the conventional mechanisms.[2] Innovative novel approaches have already exhibited promising antibacterial effects in in vitro and/or in vivo experiments such as phage therapy,[5] phage endolysins,[6,7] CRISPR-Cas systems,[8] efflux pump inhibitors,[9] combinatorial therapy,[10] antibacterial monoclonal antibodies,[11] and virulence inhibition,[12] among others.[2] However, additional antimicrobial strategies are still urgently needed.

While conventional antimicrobial research mainly focuses on bactericidal or bacteriostatic compounds, the inhibition of bacterial communication has been put forward as a novel therapeutic strategy.[13–15] Although this approach may not kill bacteria, limiting their ability to propagate an infection is an attractive way to block pathogenicity (pathoblocker) and help the immune system to take over their elimination. Microbial pathogens have lived and evolved for millions of years in close association with their hosts and their mutual interaction has become important for their life cycle and infectivity.[16] Pathogens have developed elaborate strategies to circumvent multiple eukaryotic defence lines and can thus pose severe threats to human health. Often, these pathogenicity mechanisms rely on the interactions with the host environment, therefore, deciphering the molecular processes at the interface of eukaryotic hosts and bacterial pathogens could help to conceive unprecedented antimicrobial therapeutic strategies and to reveal novel antibiotic targets against infectious diseases.[17] Chemical proteomics is a valuable technique to interrogate this molecular communication since it can be applied to track the interaction of proteins with small molecules involved in interkingdom signalling. Here, we illustrate popular chemical proteomics techniques and summarise recent advances by this approach in the field of host–pathogen communication.[18–20] This review focusses on studies employing chemical probes to elucidate biological processes involved in the interplay of animal hosts with pathogenic bacteria/mycobacteria and how this communication can contribute to virulence. Host interactions with the microbiome,[21–25] viruses,[26–28] or protozoans,[29,30] have been reviewed elsewhere. Moreover, recent reviews have covered related topics such as chemical proteomics to elucidate bacterial biology,[31] host biology during infection,[32] or cell–cell communication.[33]

2. Chemical proteomics tools

Chemical proteomics is a multidisciplinary approach which utilises small molecule probes to label and detect specific proteins or subsets of the proteome. Probes may be used for the identification of protein targets of small molecules or to label proteins using chemical analogues of enzymatic substrates or of precursors of post-translational modifications (PTMs) via metabolic incorporation by endogenous cellular biosynthesis machineries.[34] Activity-based protein profiling (ABPP)/affinity-based protein profiling (AfBPP) is a strategy to identify protein targets of chemical compounds and requires the derivatisation of the bioactive molecules with chemical handles.[35,36] These probes can be used for target identification, searching for druggable targets as well as for functional studies of proteins.[35,36] It is noteworthy that this technique is based on the activity or affinity between small molecule probes and their targets regardless of protein abundance.[37–46] Central to ABPP and AfBPP is the design and synthesis of suitable probes closely mimicking the native bioactive small molecules. It is essential that the probes maintain all structural elements necessary to retain the bioactivity of the parent compound, often requiring an assessment of the structure activity relationship. The activity-based probes (ABPs) typically consist of functionally different features: (1) a bioactive small molecule-based binding group to direct the engagement of the probe to its targets. In the case of affinity-based reversible binding, photoreactive groups such as diazirines, benzophenones, or aryl azides are incorporated into the probe. (2) A reporter tag which enables subsequent visualisation or enrichment after bioorthogonal ligation. (3) Optionally, a linker can connect the ligand and the reporter tag to provide spatial separation as well as to fine-tune physicochemical properties such as solubility.

Metabolic labelling is commonly used to tag biomolecules by incorporating a reporter group via the endogenous enzymatic machinery of a cell. This is achieved by feeding a chemically modified building block (e.g., lipid, amino acid, nucleotide, carbohydrate) to a biological system such as cells or animals.[47–49] As many components are distinct in microbial pathogens from eukaryotic cells, substantial progress in host–pathogen interaction studies has been made using metabolic labelling.[50–58]

In both approaches, chemical probes may directly be modified with a reporter group such as a fluorophore or an affinity

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handle (*e.g.*, biotin) to enable the analysis of target proteins. Often, however, it is more convenient to use smaller, latent tags which perturb biological functions to a lesser extent, and which can be chemically derivatised at a later point. This two-step approach typically applies azides or terminal alkynes as tags which can be derivatised by bioorthogonal chemistries such as copper(I)-catalysed azide–alkyne cycloaddition (CuAAC),[59,60] the strain-promoted azide–alkyne cycloaddition (SPAAC)[61–63] or the Staudinger ligation.[61,64,65] A typical chemoproteomics workflow includes the following steps: the probes are incubated with live cells to allow the ligand bind to its targets or facilitate metabolic incorporation. In case of labelling with reversible binders, the affinity-based probe should be irradiated with UV light after incubation to trigger covalent bond formation between its photoreactive group and its binding partners. This covalent bond assures a robust complex that remains stable in the following steps. Next, the reporter tag is appended most typically by "click chemistry". This can be a fluorophore for the visualisation of target proteins of the bioactive small molecules, or a biotin tag which allows the purification of the covalently bound targets on avidin beads followed by tryptic digestion and liquid chromatography tandem mass spectrometry analysis (LC-MS/MS). Current mass spectrometry technologies combined with label-free or isotopic labelling enable global and comprehensive profiling of protein targets of small molecules by the comparison of probe-treated proteome to control samples (Fig. 1(a) and (b)).[66] Alternative chemoproteomic methods have been developed which circumvent the need for chemically modified probes. Proteomic strategies such as drug affinity responsive target stability (DARTS),[67] cellular thermal shift assay (CETSA),[68] thermal proteome profiling (TPP),[69,70] or limited proteolysis coupled with MS (LiP-MS)[71–73] employ native bioactive molecules to elucidate shifts in protein stability upon small molecule binding.

3. Host–microbe interactions *via* signalling molecules

Bacteria have lived in close association with eukaryotic hosts and, as a consequence, they have evolved specific receptors for small molecules such as eukaryotic hormones (Fig. 2(a)).[74] Opportunistic bacterial pathogens can even exploit these signalling systems to adjust their expression of virulence genes to the host's stress status.[75] For instance, dynorphin A, a mammalian peptide hormone that is released during host stress enhances the production of the toxin pyocyanin by the opportunistic pathogen *Pseudomonas aeruginosa*.[76] To identify protein targets of dynorphin A in *P. aeruginosa* PAO1, Wright *et al.* devised a set of peptide photoprobes **1–5**. They mainly consisted of the dynorphin A 1–13 sequence and carried a terminal alkyne as well as a benzophenone in **1** and **2**, an aryl azide in **3**, or a diazirine in **4** and **5**.[77] Probes **3** and **4** unravelled the histidine kinase ParS as target which was confirmed by dynorphin A mediated upregulation of a ParS-dependent bacterial defence response against antimicrobial peptides on the proteome level (Fig. 2(b)).

The histidine kinase ParS forms a two-component system with its cognate response regulator ParR. In two-component systems, the most widespread type of bacterial signalling sensors,[78] the histidine kinase ultimately transfers a phosphate group onto an aspartyl residue in the response regulator when it senses a signal in the periplasm, thereby activating the transcription of its target genes. Phosphoaspartates consist of a mixed carboxylic acid–phosphoric acid anhydride (acyl phosphate), which are highly prone to nucleophilic attack, and therefore, hydrolysis.[79] Due to the short lifetime of phosphoaspartate modifications and their transient nature, it is hardly possible to directly detect them by common MS-based (phosphoproteomics) methods. In an approach termed reversed-polarity ABPP (RP-ABPP), Chang *et al.* reported a nucleophilic
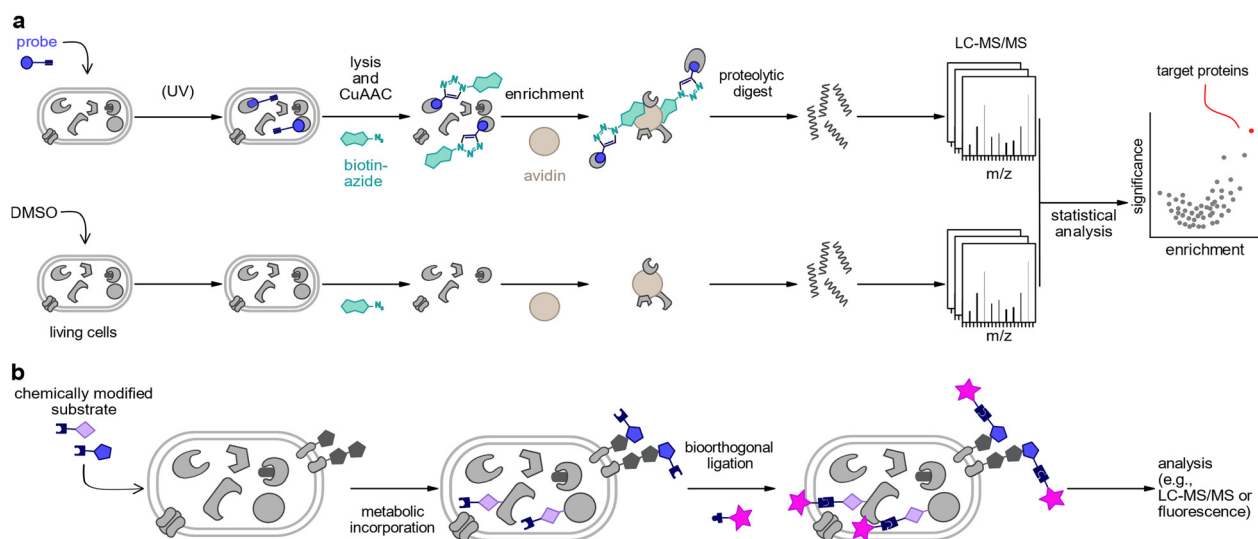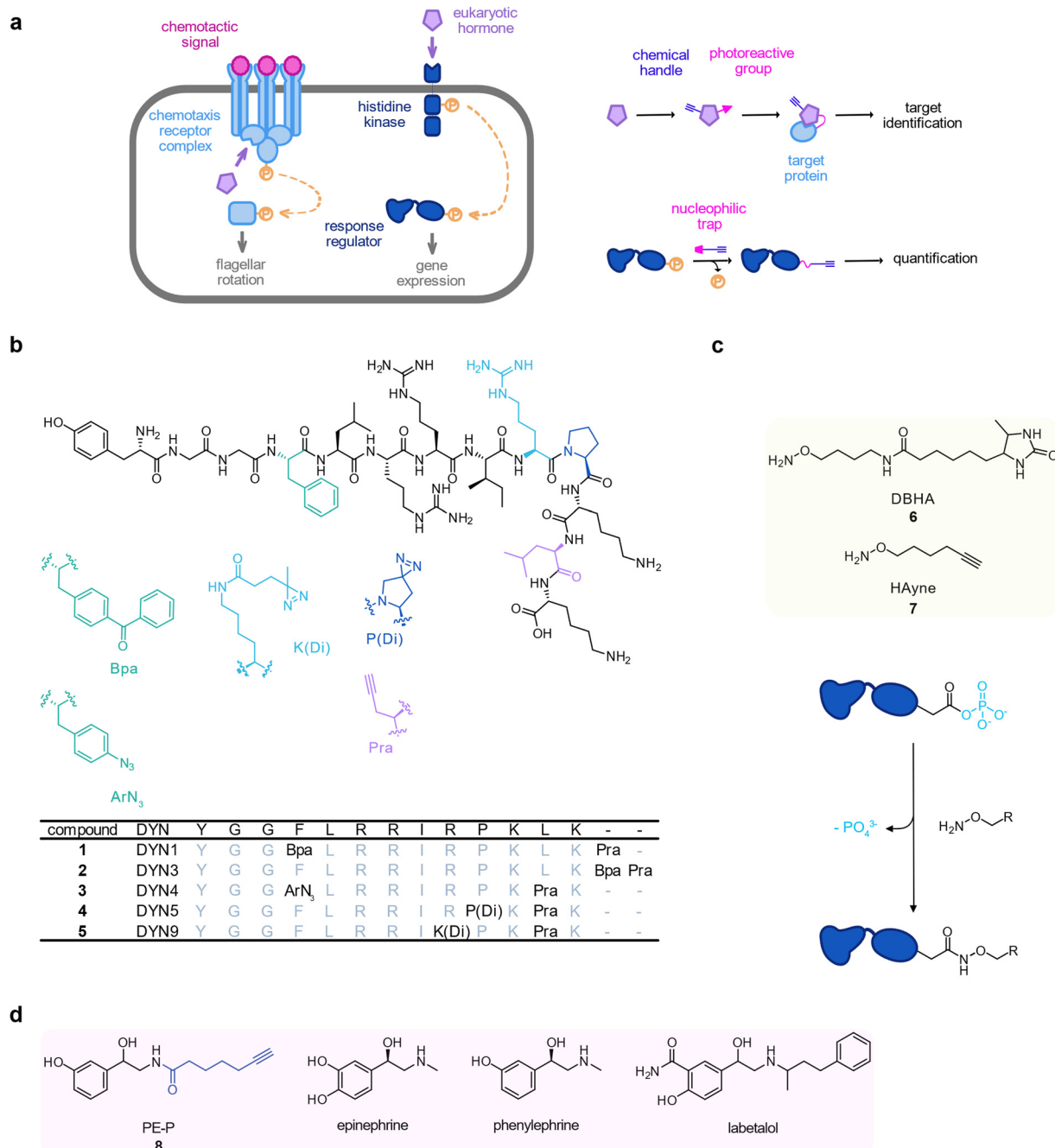

Fig. 1 Chemical proteomics strategies for the investigation of host–pathogen interactions. (a) Basic concept of activity-based protein profiling (ABPP)/affinity-based protein profiling (AfBPP). CuAAC: copper(I)-catalysed azide alkyne cycloaddition. (b) General scheme for metabolic labelling.

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Fig. 2 Investigation of hormone-based interkingdom signalling. (a) Schematic representation of bacterial sensing of eukaryotic hormones by the chemotaxis signalling complex (left) or a two-component system (right) and probes for target identification (top) or monitoring of two-component system activation (bottom). (b) Dynorphin A probes **1–5**. Amino acids in the dynorphin A sequence were replaced by unnatural building blocks as indicated by the colours. (c) Hydroxylamine probes **6** (DHBA) and **7** (HAyne), (d) Catecholamine hormones and probe **8** (PE-P).

hydroxylamine probe **6** (desthiobiotin-containing O-alkyl hydroxylamine probe, DBHA) equipped with a desthiobiotin handle to capture phosphoaspartate modifications by converting them into stable hydroxamates.[80] Allihn et al. used a refined, minimal clickable hydroxylamine alkyne probe **7** (HAyne) to trap response regulators phosphorylated in the presence of dynorphin.[81] **7** was applied in live *P. aeruginosa* challenged with dynorphin and labelled proteins were ligated to isotopically
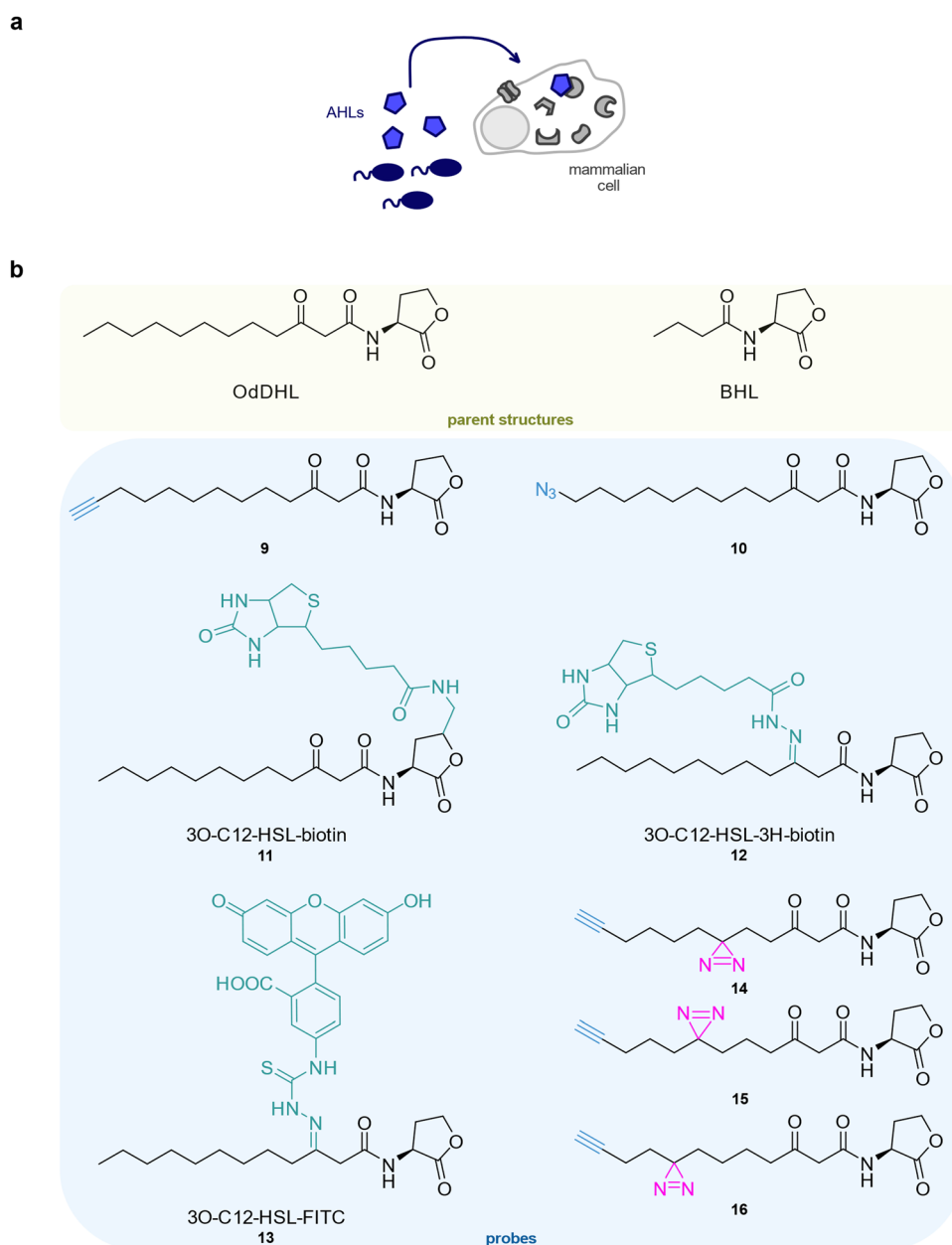
labelled desthiobiotin azide (isoDTB) tags for peptide enrichment and quantification.[82] As expected, ParR was phosphorylated in the presence of dynorphin, corroborating the results of the previous study. Interestingly, this approach revealed that another response regulator, CprR, responded to dynorphin treatment in an even stronger fashion. Similar to the ParRS system, CprR is part of a two-component system involved in the antimicrobial peptide defence system (Fig. 2(c)).

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The catecholamine stress hormones epinephrine and norepinephrine (also known as adrenaline and noradrenaline) modulate a variety of virulence associated phenotypes in prokaryotes including enhanced motility of *Vibrio campbelli*.[83–86] The bacterial adrenergic sensor of these hormones was recently revealed using photoaffinity labelling in a study by Weigert Muñoz *et al.*[87] The catechol group is a widespread motif in bacterial siderophores to bind and scavenge iron. Catecholamines are also exploited by bacteria as so-called xenosiderophores to acquire iron from the host.[86] To decipher the cellular receptors of these hormones, a probe was designed based on the adrenergic agonist phenylephrine, which, due to the lack of an intact catechol group, cannot act as a xenosiderophore. The probe still retained the enhanced motility phenotype but did not bind to iron-dependent proteins allowing to focus on the receptor targets. This inherently photoreactive probe, **8** (PE-P), enriched the chemotaxis protein CheW in live *V. campbellii* (Fig. 2(d)). Interestingly, binding of **8** at CheW was outcompeted not only by the parent compounds phenylephrine and epinephrine but also by the clinically applied adrenergic antagonist labetalol, corroborating that CheW is indeed an adrenergic receptor. Consistent with this, epinephrine and phenylephrine interfered with the chemotaxis of *V. campbellii* towards glucose.

Fig. 3 Investigation of autoinducer-mediated interkingdom signalling. (a) Targets of bacterial AHLs were investigated in human cells using different chemical probes. (b) Structures of AHL probes. BHL: *N*-butyryl homoserine lactone, OdDHL: *N*-(3-oxododecanoyl)-homoserine.

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RSC Chem. Biol., 2024, **5**, 73–89 | 77

Vice versa, bacterial signalling molecules can affect eukaryotic cell physiology (Fig. 3(a)). Quorum sensing denotes bacterial communication *via* diffusible small molecule signals, termed autoinducers, in order to coordinate cellular processes within their community or across species. Beyond that, autoinducers are known to influence eukaryotic cell physiology and to promote chronic inflammation and bacterial survival within the host.[88–90] *N*-acyl homoserine lactones (AHLs) are common signal molecules of Gram-negative bacteria,[91] which induce the phosphorylation of MAPK p38 and eIF2α in mammalian cells and compromise the production of inflammation mediators.[90] They furthermore attenuate TLR4-dependent innate immune responses to lipopolysaccharides (LPS) by disrupting NF-κB signalling in mammalian cells.[89]

An initial study from the Janda group reported a set of clickable AHL probes based on *N*-(3-oxododecanoyl)-homoserine lactone (OdDHL) from *P. aeruginosa* which were equipped with a terminal alkyne **9** or an azide **10** but lacked a photocrosslinker.[92] Interestingly, **9** showed good activity in a *P. aeruginosa* autoinducer assay but induced only weak phosphorylation of MAPK p38 and eIF2α phosphorylation in mammalian cells. Interestingly, **10** was especially active in the latter system. The D-homoserine lactone enantiomer of **10** was inactive in both systems.[90,92] At that stage, probes **9** and **10** were not used for target identification experiments. Another study from the Vikström lab used biotin-coupled OdDHL probes **11** and **12** to detect the IQ-motif containing GTPase-activating protein (IQGAP1) in Caco-2 cells as a putative protein target of OdHL.[93] Interestingly, IQGAP1 could only be enriched by **12** but not by **11** in which the homoserine ring was modified. A fluorescein-coupled OdDHL probe **13** revealed the co-localisation of OdDHL and IQGAP1. By targeting IQGAP1, *P. aeruginosa* could induce remodelling of the host actin cytoskeleton to aid the infection process. Furthermore, the Meijler group designed three OdDHL diazirine probes **14–16** to enable the identification of protein targets *via* chemical proteomics.[94–96] These probes bound to their native bacterial receptor LasR, and also elicited phosphorylation of the eukaryotic initiation factor 2a (eIF2a) and protein kinase p38.[94,95] Although **14** and **15** induced the strongest responses in a *P. aeruginosa* QS reporter strain, **15** was most active in human cells, illustrating the context-dependence of biological activity. Finally, **14** was applied to identify OdDHL targets in human cells using stable isotope labelling by amino acids in cell culture (SILAC) labelling.[96] The major vault protein (MVP) was identified as a target of OdDHL and the binding sites of **14** were within regions that could be involved in the interaction of MVP with lipid rafts. The translocation of MVP into lipid rafts was indeed promoted by OdDHL and it was proposed that these newly functionalised membranes can gauge apoptotic signalling in human cells. A recent study from the Meijler group reports chemical probes based on another *P. aeruginosa* signal, the short-chain *N*-butyryl homoserine lactone (BHL), which were applied for the identification of protein targets in *P. aeruginosa*.[97] However, these probes have not yet been employed in an interkingdom signalling set-up (Fig. 3(b)).

4. Post-translational modifications between host and pathogens

4.1 AMPylation

Some pathogenic bacteria hijack human cellular processes by introducing effector proteins which post-translationally modify host proteins to alter their function, often targeting pathways that ultimately facilitate bacterial infection and replication in the host.[98] Bacterial effector proteins that transfer an adenosine 5′-monophosphate (AMP) group onto a hydroxy group of host proteins have been discovered in important human pathogens such as VopS from *Vibrio parahaemolyticus*,[99] IbpA from *Histophilus somni*,[100] and DrrA from *Legionella pneumophila*.[101] These so-called AMPylators target proteins from the family of Rho GTPases (among others), which typically can no longer interact with downstream effectors as a consequence.[99–101] These processes can promote bacterial invasion by interfering with the host's actin skeleton dynamics (VopS, IbpA)[99,100] or facilitate bacterial survival and replication inside the host cell (DrrA).[102,103] Chemical AMP probes have enabled the identification of low abundance targets after enrichment in a global and unbiased fashion. Grammel *et al.* devised an alkynylated AMPylation probe, N^6-propargyl adenosine-5′-triphosphate **17** (N^6pATP),[104] which was accepted by VopS, Fic2, and DrrA, and applicable in HeLa lysates, where the selective labelling of known targets such as Cdc42 was achieved. Follow-up studies employed **17** (N^6pATP) in a cell-free protein array to detect novel substrates of bacterial AMPylators,[105–107] or the human AMPylator FICD protein in cell lysate using mass spectrometric analysis of enriched proteins.[108] The more sterically demanding N^6-(6-amino)hexyl-ATP-5-carboxyl-fluorescein **18** (Fl-ATP) was applied to reveal VopS targets in whole cell extracts which were pulled down by an α-fluorescein antibody and analysed with mass spectrometry.[109] However, these probes could not be used in live cells as the highly negatively charged triphosphate moiety impeded cell permeability.[110,111] Kielkowski *et al.* devised two cell permeable pro-nucleotide probes, **19** (pro-N^6pA) and **20** (pro-N^6azA), where the negative charge of the phosphate was masked by a phosphoramidate.[112,113] Metabolomics experiments showed that pro-N^6pA is metabolised to the corresponding **17** (N^6pATP) in the cell.[113] A recent study by Rauh *et al.* applied **19** (pro-N^6pA) to directly monitor AMPylation of human cells by the *V. parahaemolyticus* effector VopS after infection in live cells.[114] This facilitated the tracking of AMPylation over time, identification of AMPylation sites, and the identification of both known and previously unknown Rho GTPase targeted by VopS. Infection with pathogens like *P. aeruginosa* PAO1 or *E. coli* CFT073, both of which carry Fic domain-containing proteins with only low similarity to the Fic domains of virulence effectors, did not result in **19** (pro-N^6pA) labelling. This study therefore illustrates how only certain pathogens apply this infection strategy. Gulen *et al.* introduced co-substrate-linked enzymes as a novel sort of macromolecular ABPP-like probe for proteome profiling of AMPylated proteins.[115] The bacterial nucleotide transferases IbpA and BepA were engineered to contain a cysteine in the ATP binding pocket at a
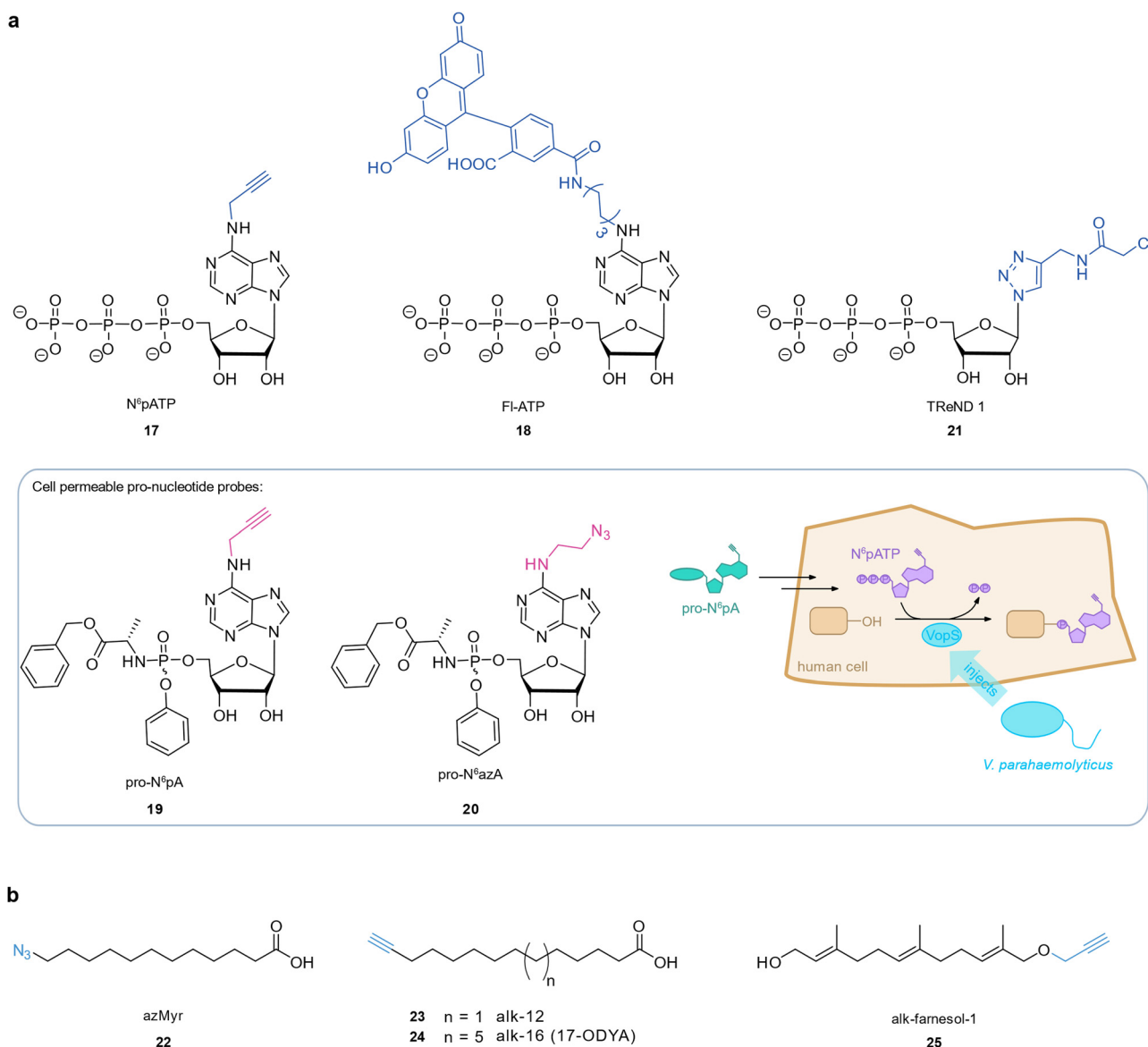
position that enabled covalent binding to an electrophilic ATP analogue, termed thiol-reactive nucleotide derivatives (TreNDs). A probe **21** (TreND 1) tethered to IbpA was introduced into human cell lysate and the resulting ternary complexes were identified by affinity enrichment and mass spectrometry which revealed a set of hitherto unknown AMPylation targets (Fig. 4(a)).

4.2 Lipidation

Bacterial pathogens also target protein lipidation, a post-translational modification that serves as a membrane anchor.[116,117] Bioorthogonal probes have emerged as powerful tools to study protein lipidation in an unbiased approach[118] and have widely been used in the context of host–pathogen interactions.

The *Shigella flexneri* effector protein IpaJ proteolytically cleaves *N*-myristoyl glycine off mammalian proteins.[119] Metabolic labelling with myristic acid azide **22** (azMyr) in human

cells transfected with IpaJ revealed a broad target spectrum of the bacterial protease.[119] These targets could be identified in a later study, in which the authors used the corresponding myristic acid alkynyl-analogue **23** (alk-12),[120] for metabolic labelling in human cells infected with *S. flexneri*.[121] Proteomics analyses by mass spectrometry revealed that a suite of Golgi-associated ARF/ARL GTPases is targeted by the pathogen, which is in line with the Golgi destabilisation observed during *S. flexneri* infection.[121] The *Vibrio cholerae* toxin RID acts as an N^ϵ-fatty acyltransferase on human Rho GTPases with a strong preference for Rac1, a regulator of the actin cytoskeleton, disrupting related pathways.[122] This was shown in cells metabolically labelled with **22** (alk-16) and transfected with RID from *V. cholerae* or infected with the bacteria to monitor the acylation of Rac1.[122] Similarly, Liu *et al.* performed metabolic labelling of HeLa cells with **24** (alk-16) followed by transfection

Fig. 4 Investigation of host–pathogen interactions through post-translational protein modifications. (a) AMPylation probes. (b) Lipidation probes.

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RSC Chem. Biol., 2024, **5**, 73–89 | **79**

with the *S. flexneri* virulence factor IcsB or by infection with the pathogen. Labelled proteins were enriched and identified by mass spectrometry.[123] IcsB was shown to acylate a great number of small GTPases of the Ras superfamily to disturb bacterial autophagy and facilitate its survival.[123] These examples show how pathogens can modify the lipidation of host proteins. On the other hand, bacteria may exploit host lipidation enzymes to acylate their effector proteins for functional activation, stabilisation, and sub-cellular localisation. An alkynyl-farnesol reporter **25** (alk-farnesol-1)[124] was applied in live HeLa cells transfected with *Legionella pneumophila* effector proteins to reveal their post-translational modification by human prenyltransferases.[125] Prenylation was monitored by in-gel fluorescence scanning following immunoprecipitation of the *Legionella* proteins and ligation to a fluorescent dye. This approach revealed that multiple *Legionella pneumophila* effector proteins are prenylated by the host cell and, as a consequence, targeted to the membranes of host organelles.[125] **24** has also served as tool to monitor protein palmitoylation.[126] *Salmonella typhimurium* effector proteins were shown to be palmitoylated by host proteins after being delivered into the host cell.[127] In addition, mammalian cells transiently expressing the bacterial effectors as well as the putative palmitoylators were metabolically labelled with **24**[120] and modifications of bacterial effectors were read out as performed with probe **25**.[127] This study revealed palmitoylation as a critical trait for effector activity and localisation.[127] Similarly, palmitoylation of the *Legionella pneumophila* effector proteins GobX and LpdA was shown by metabolic labelling of human cells transfected with the effectors.[128,129] The *Brucella* effector PrpA was shown to be palmitoylated in an analogous approach (Fig. 4(b)).[130]

5. Interactions of the pathogen envelope with the host

The bacterial envelope is the interface between bacteria and their surroundings and is implicated in many interactions with the host.[131] As many bacterial components are absent in human cells, metabolic labelling using probes mimicking bacterial cell wall building blocks can be performed within infected host cells.[132,133] Furthermore, the bacterial cell wall is a major target of antibiotics and therefore of large scientific interest.[2] The basic peptidoglycan structure consists of a conserved disaccharide backbone formed by the two aminosugars *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNAc).[133,134] MurNAc is connected to a tetra- or pentapeptide typically containing a D-ala-D-ala motif at its fourth and fifth position. As bacterial D-alanine metabolism readily tolerates unnatural substrates, D-alanine derivatives serve as chemical reporters to directly track peptidoglycan in a selective manner.[135,136]

A study from the Bertozzi lab applied D-ala analogues modified with an alkyne or azide bioorthogonal handle, *R*-propargylglycine **26** (alkDala) and *R*-2-amino-3-azidopropanoic acid **27** (azDala), to label newly synthesised peptidoglycan

in replicating bacteria inside macrophages.[136] *Listeria monocytogenes* is an intracellular pathogen that uses a tail of polymerised host actin for motility after infection.[137] Labelling studies with **26** revealed that the presence of host actin skewed bacterial division cycles to increase the proportion of freshly divided cells, which favours the formation of actin tails on *L. monocytogenes*. Thus, peptidoglycan labelling allowed to monitor how bacteria adjust their growth and division in a complex environment such as the host cell.
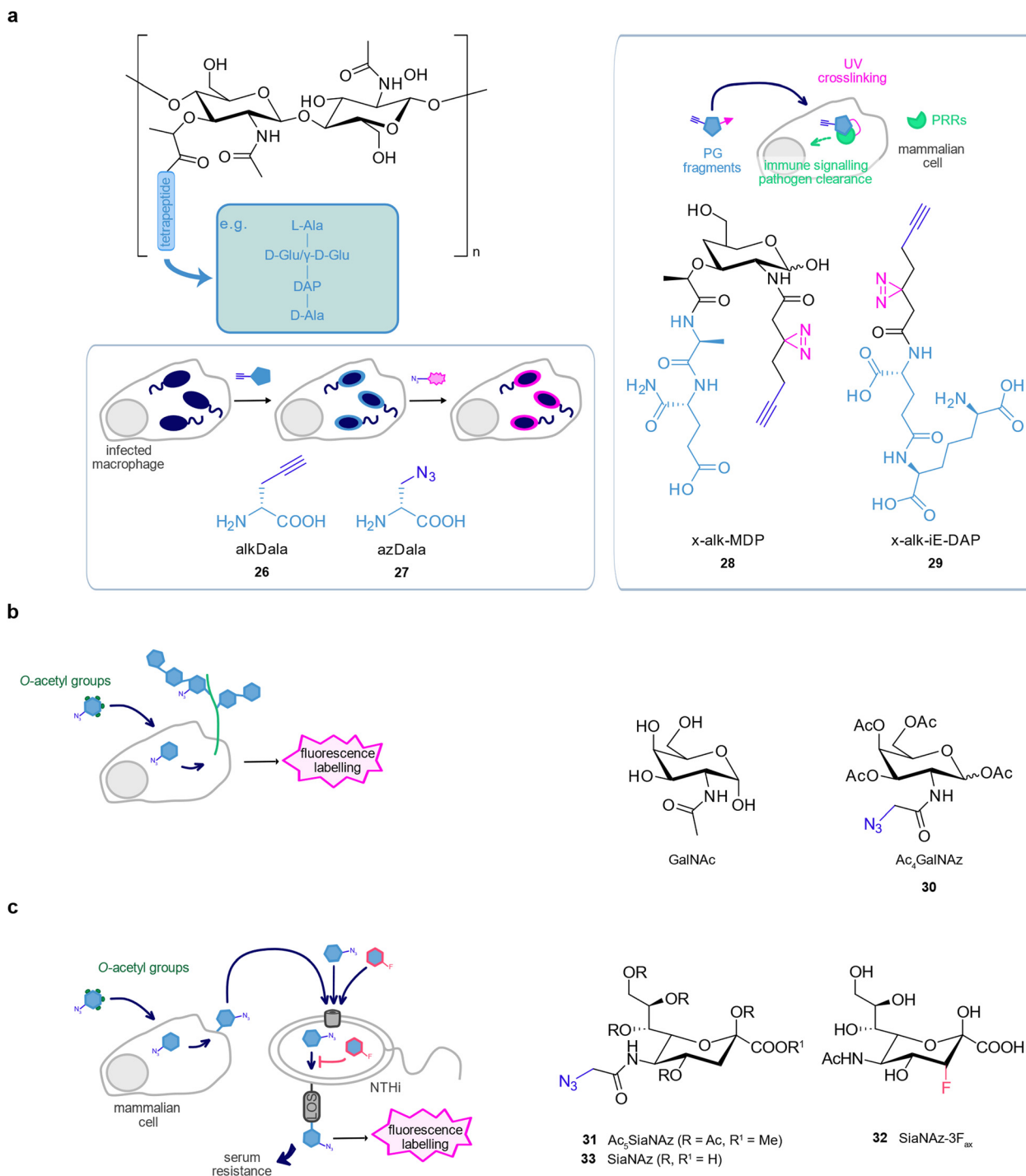
Wang *et al.* derivatised the peptidoglycan fragments γ-D-glutamyl-*meso*-diaminopimelic acid (iE-DAP) and muramyl-dipeptide (MDP) to generate photoaffinity probes, **28** (x-alk-MDP) and **29** (x-alk-iE-DAP), to detect their direct interaction with the mammalian intracellular immune receptors NOD1 and NOD2, respectively. **28** revealed the formation of a transient ternary complex between MDP, Arf6, and NOD2, which was no longer formed with a loss-of-function NOD2 mutant associated with Crohn's disease, suggesting this complex plays a role in the disease (Fig. 5(a)).[138]

A clickable sugar analogue, tetra-acetylated *N*-azidoacetyl-galactosamine **30** (Ac$_4$GalNAz), has been applied to monitor bacteria–host interactions by labelling host glycoproteins. Mucins are heavily glycosylated proteins and a key component of the intestinal mucus layer, where they form a barrier against invading pathogens. **30** carried acetylated hydroxy groups to facilitate cellular uptake that are removed by intracellular esterases and successfully labelled mucin-type O-linked glycoproteins.[139] **30** has been used to follow the fate of mucins in live mice at different stages of infection with *Helicobacter pylori*, the causative agent of intestinal tract ulcers.[140] Interestingly, *H. pylori* slowed the secretion of mucins to the cell surface, likely to impair pathogen clearing.[140] In another study, labelling of transmembrane mucins at the surface of murine cornea with **30** revealed that *P. aeruginosa* preferentially colocalised with GalNAz-labelled regions (Fig. 5(b)).[141]

Human cells are covered by a glycocalyx, a dense layer of sugars attached to surface proteins and lipids. Pathogens use sialic acids – abundant constituents of the glycocalyx – to recognise and bind to their host.[142] Beyond that, many bacteria remove sialic acid from human cells to incorporate it into their own cell surface. This molecular mimicry facilitates evasion of the immune response and promotes survival within the host.[143] Scavenging of host sialic acid by non-typeable *Haemophilus influenzae* (NTHi) could be monitored by metabolic labelling in a coculture model with primary human bronchial epithelial cells (PHBEC).[144,145] Mammalian cells only take up peracetylated sialic acids by passive diffusion whilst bacteria rely on active uptake of the unprotected sugar. Therefore, PHBEC cells were selectively labelled by feeding an acetylated azide sialic acid probe **31** (Ac$_5$SiaNAz) which was then removed by a sialidase enzyme and incorporated by NTHi. The incorporation was monitored by CuAAC to biotin followed by staining with fluorescent streptavidin and flow cytometry.[145] Interestingly, an analogous alkyne probe was resistant to removal from the mammalian glycans. Noteworthy, this pathway has been studied for its potential as an antiinfective target. The chemical

Fig. 5   Sugar-based probes. (a) Peptidoglycan probes to label the bacterial cell wall. (b) GalNAc probes to label host glycoproteins (c) Sialic acid probes to monitor incorporation of host sialic acids by bacteria.

analogue 3-fluorosialic acid **32** (SiaNAz-3F$_{ax}$), for instance, proved to selectively inhibit NTHi LOS sialylation and abolish serum resistance.[145] In another study, an unprotected azide sialic acid probe **33** (SiaNaz) was used to study a suite of potential inhibitors of LOS sialylation by quantifying the competition of labelling by **33** in NTHi.[146] This is an example of how chemical biology methods can elucidate a potential antivirulence treatment (Fig. 5(c)).

Mycobacteria possess a unique membrane structure that is crucial for their interaction with the host. One key constituent is the non-mammalian disaccharide trehalose, which is as a precursor for essential cell wall glycolipids and other metabolites in mycobacteria. Backus *et al.* first developed a fluorescein-containing trehalose probe **34** (FITC-trehalose) that successfully labelled *M. tuberculosis* within macrophages (Fig. 6(a)).[147] The Bertozzi group published azide-modified trehalose probes

35–38 that were incorporated into the mycomembrane of different mycobacteria[148] as well as a solvatochromic trehalose probe **39** (DMN-Tre) whose fluorescence is activated upon being metabolically incorporated into the hydrophobic environment of the mycomembrane.[149] DMN-Tre labelling proved superior compared to a non-fluorogenic analogue **40** (6-FlTre) as it
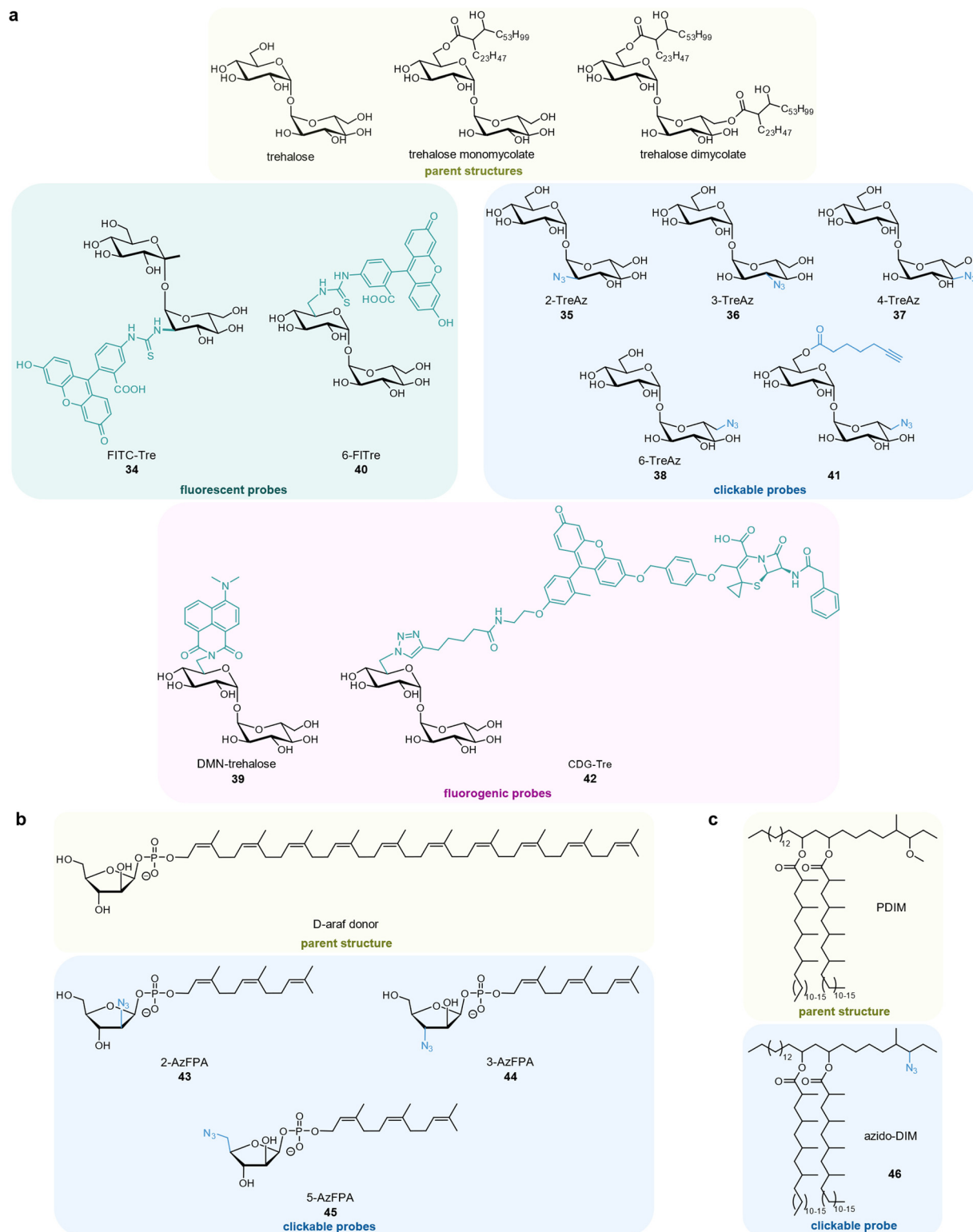

Fig. 6   Mycomembrane components and corresponding probe derivatives. (a) Trehalose-based probes. (b) Arabinose-based probes. (c) Phthiocerol dimycocerosate-based probe.

produced significantly less background.[149] A bifunctional trehalose probe **41** comprising both a terminal alkyne and azide group was designed by Pohane *et al.* It labels trehalose dimycolate (TDM) and arabinogalactan-linked mycolate (AGM) with its alkyne group under antigen 85 (Ag85) mycoloyltransferases catalysis while the resulting free trehalose azide probe labels trehalose monomycolate (TMM). Monitoring mycomembrane biosynthesis and trehalose recycling this way revealed that trehalose recycling efficiency increased upon carbon starvation with a concomitant increase in LpqY-SugABC expression.[150] Dai *et al.* developed a cephalosphorinase-dependent green trehalose **42** (CDG-Tre) fluorogenic probe whose fluorescence is activated by the β-lactamase BlaC, which is uniquely expressed by *Mycobacterium tuberculosis*.[151] As expected, the probe was modified with mycolic acid by mycobacterial enzymes and incorporated into the cell wall and therefore enabled selective fluorescence imaging of mycobacteria inside live macrophages.[151] Altogether, trehalose-based probes constitute a very promising approach to monitor glycolipid dynamics, also in infection models, as trehalose is metabolically orthogonal to eukaryotic hosts[152–155] and several derivatives of it have been published.[152–155]

D-Arabinofuranose (D-Araf), is another essential component of the mycobacterial membrane that is absent in human cells. Mycobacteria enzymatically incorporate arabinose from arabinose-phospholipid donors by integral membrane glycosyltransferases rather than from free arabinose. The Kiessling group disclosed clickable azide derivatives 2-AzFPA **43**, 3-AzFPA **44**, and 5-AzFPA **45** mimicking the phospholipid precursor (Fig. 6(b)).[156] 2-AzFPA **43** was selectively incorporated into arabinofuranose-containing glycans in the cell surface of Msmeg and strain-promoted azide–alkyne cycloaddition (SPAAC) ligation to fluorescent dyes enabled its detection within infected macrophages.[156]

Another study from the Bertozzi lab used an azido-derivative of the mycobacterial virulence lipid phthiocerol dimycocerosate (PDIM) **46** (azido-DIM), to directly track it in an *in vivo* infection model of *Mycobacterium marinum* in live zebrafish (Fig. 6(c)).[157] After ligating the azide handle to a fluorescent dye, it was observed that PDIM spread into the epithelial membranes of the host and that this process was dependent on host cholesterol. The spread of PDIM into host epithelial membranes was crucial for mycobacterial survival as it caused a suppression of the innate immune response. Treating the zebrafish with statins (inhibitors of cholesterol biosynthesis) significantly decreased mycobacterial infectivity.

6. Enzyme-mediated host–pathogen interactions monitored by fluorophosphonates

Fluorophosphonate probes have been widely used as tools to study the activity of serine proteases in the context of disease,[158–161] however, there are only few reports of their use in infection models. Hatzios *et al.* used activity-based fluorophosphonate probes **47** and **48** to identify proteases that were active in animals or humans infected with *Vibrio cholerae* (Fig. 7(a) and (b)).[162] They revealed that the serine protease secreted by *V. cholerae*, IvaP, reduced bacterial surface binding of intelectin, a host protein likely involved in microbial recognition. A follow-up study reported the cleavage of intelectin by IvaP.[163] Similarly, Lentz *et al.* used a fluorophosphonate
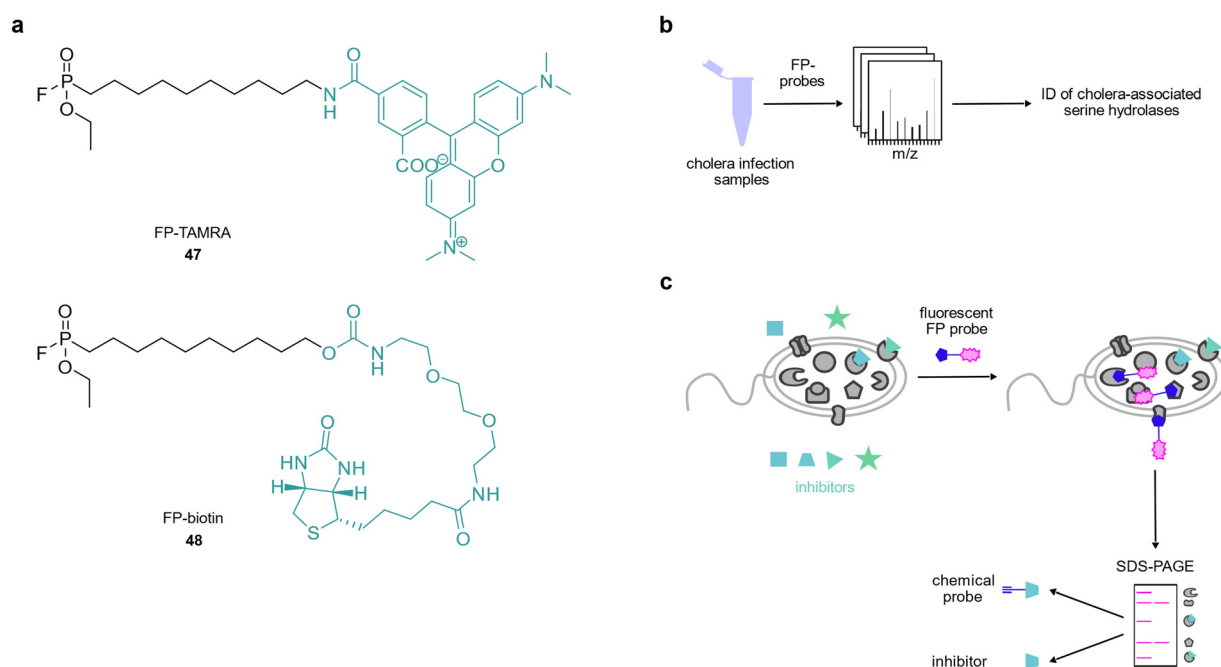

Fig. 7 Investigation of hydrolases in the context of bacterial infection. (a) Fluorophosphonate probes. (b) Fluorophosphonate probes were applied to identify hydrolases in cholera infection samples and to (c) discover novel inhibitors of virulence-related hydrolases.

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RSC Chem. Biol., 2024, **5**, 73–89 | 83

tetramethylrhodamine probe[164] in *S. aureus* to show that the serine hydrolase FphB was activated by components of eukaryotic cell membranes and serum and that this enzyme was required for full *S. aureus* infectivity.[165] Hence, this enzyme class provides promising targets for the development of selective inhibitors and the authors performed competitive labelling using a fluorescent fluorophosphonate probe to identify a selective inhibitor of FphB which reduced *S. aureus* infectivity *in vivo* (Fig. 7(c)).[165]

7. Outlook

Chemical proteomics is a powerful tool to study the interaction of chemical compounds with proteins in a global and unbiased way which has helped elucidate the mode of action of a number of biologically active small molecules.[37] However, there are limitations that necessitate special attention in the experimental design. One major challenge is that chemical proteomics studies often generate a suite of background hits which requires thorough control experiments to work out genuine targets that can be linked to the biological activity. This is especially important when photoreactive probes are used as they typically have high target promiscuity. Moreover, the incorporation of reactive groups or chemical handles into the probes may in some cases affect the biological activity and alter the target spectrum of the probes. This risk can be minimised by the application of multiple, chemically diverse probes and by performing careful structure–activity-relationship studies. Finally, although there are recent examples of small molecules targeting non-protein targets such as nucleic acids,[166,167] chemical proteomics primarily focusses on proteins and might therefore miss targets such as nucleic acids or lipids.[168] Nevertheless, chemical proteomics is an invaluable approach for determining interactions between small molecules and proteins as no prior knowledge on the identity of potential targets is required. Provided the probes are cell-permeable, their engagement with protein targets can be studied within live cells where proteins have native activity, expression levels, and localisation.

Here, we have highlighted recent progress in the study of host–pathogen interactions achieved *via* chemical proteomics. This includes not only how bacteria sense host hormones such as dynorphin A and how the host responds to bacterial quorum sensing molecules, but also eukaryotic post-translational protein modifications exploited by microbes such as AMPylation, and lipidation, among others. The application of unnatural building blocks such as D-Ala analogues expands our understanding of the bacterial envelope which constitutes the interface of bacteria and their surroundings. These techniques have greatly benefitted from the development of bioorthogonal chemistries,[169] the commercial availability of noncanonical biological building blocks,[170] and advances in mass spectrometry techniques.[171–174] Nevertheless, more efforts are still needed to resolve remaining questions. First, most reported studies represent a snapshot of host–pathogen interactions at certain infection time points but the interplay between host and pathogen is more complex and dynamic and may vary across different stages of infection.[175,176] Therefore, monitoring these interactions in a time-resolved manner would provide a more complete view. In a pioneering study, Tao *et al.* reported a photoactivatable multifunctional chemical probe to crosslink bacterial and host proteins during *Salmonella* internalisation into macrophages.[177] The photoactivatability allowed for time-resolved profiling of pathogen entry and revealed protein–protein interactions at different time points.[177] Indeed, proteins involved in host actin polymerisation, which is important for pathogen internalisation, interacted with microbial proteins during early infection stages, while more nuclear proteins were identified in the late endocytosis process.[177] Second, the majority of host–pathogen interaction studies has employed cellular systems rather than animal models. However, *in vitro* studies are not always suitable to reflect *in vivo* situations and animal studies would dramatically improve the physiological relevance of the results.[178–180] In an intriguing approach, Waldor *et al.* have analysed microbe–host interactions at the bacterial envelope by biotinylation of proteins associated with the surface of *V. cholerae* isolated from an infant rabbit model.[181] This approach revealed that numerous host proteins associate with *V. cholerae* and that their abundance on the pathogen surface is driven by the presence of cholera toxin. Some of the identified host proteins (AnxA1, LPO and ZAG) could also recognise distinct taxa of the murine intestinal microbiota. This suggests that the host factors may play a role in intestinal homeostasis – a discovery that would be elusive to *in vitro* studies.[181] A challenge for *in vivo* studies is to detect small amounts of pathogen proteins. However, with the rapid technical development of mass spectrometry, the sensitivity of proteomic studies has significantly increased.[171,182] For example, more than 1000 proteins can now be identified and quantified in single mammalian cells *via* single cell proteomics with elaborate isolation techniques, sample processing methods, and mass spectrometry instrumentation.[183,184] These novel techniques may allow to minimise the required bacterial cell number in proteomic analysis for host–pathogen interaction studies, although bacteria possess much fewer proteins compared to mamalian cells.[185] Finally, chemical proteomics approaches often rely on small molecules or small cellular building blocks since they are convenient to be modified. However, host–pathogen interactions are often mediated by macromolecules, an area of research that is currently underexplored. For example, some bacterial pathogens are capable of directly sensing and responding to host cytokines to facilitate their survival and transmission.[186,187] IL-1β is reported to accelerate the growth of virulent *E. coli*,[188] and human IFN-γ can activate a virulent phenotype of *P. aeruginosa*.[189] Since the incorporation of unnatural amino acids into proteins or larger peptides has become more accessible by genetic code expansion or site-specific modification, the elucidation of protein-based host–pathogen interaction is now more easily accessible.[190–195] Studying macromolecule-based communication could greatly benefit from chemical proteomics and would further elucidate another

aspect of host–pathogen interactions from both bacterial and host perspectives.[196]

The antimicrobial resistance crisis requires novel approaches to treating bacterial infections. Targeting virulence pathways has been put forward as a promising strategy as it is expected to exert less selective pressure on pathogens compared to classical therapies. We have highlighted studies in which chemical probes have already been employed to identify chemical inhibitors, e.g., for the *S. aureus* hydrolase FphB or for sialylation of NTHi LOS[146,165] but overall, to this date, there are not many reports of chemical inhibitors targeting host–pathogen interactions. This could be due to the fact that interkingdom communication is a very complex process and still not very well understood. We have summarised a number of studies that employed metabolic labelling to monitor, for instance, PTMs introduced on host proteins by bacterial pathogens. These approaches could be used in future studies to characterise potential antagonists of bacterial effector proteins involved in AMPylation or lipidation of host enzymes, for example. The interference with QS has already been investigated for some time as a potential antivirulence approach and several studies have successfully identified compounds that reduced virulence in important human pathogens by blocking interbacterial communication at different points in the signalling cascade.[197–203] Interfering with interkingdom signalling has been explored in a study from the Sperandio group which reports LED209, an inhibitor targeting the two-component system QseBC.[14] QseBC acts as a sensor for both the QS signal AI3 and the eukaryotic hormone epinephrine and its inhibition resulted in reduced bacterial virulence in animal studies.[14] In general, two-component systems provide intriguing antivirulence targets as they are critical to many bacterial survival and pathogenicity mechanisms and are furthermore absent in mammalian cells. Indeed, chemical inhibition of two-component systems has been shown to result in reduced virulence in both *in vitro* and *in vivo* studies.[204,205] Our review has delineated the potential of chemical proteomics to uncover novel targets involved in host–pathogen interactions and to elucidate underlying biological pathways.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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RSC Chem. Biol., 2024, **5**, 73–89 | 89