ABPP and Host–Virus Interactions

Geneviève F. Desrochers and John Paul Pezacki

Contents

Abstract Successful viral infection, as well as any resultant antiviral response, relies on numerous sequential interactions between host and viral factors. These interactions can take the form of affinity-based interactions between viral and host macromolecules or active, enzyme-based interactions, consisting both of direct enzyme activity performed by viral enzymes and indirect modulation of the activity of the host cell's enzymes via viral interference. This activity has the potential to transform the local microenvironment to the benefit or detriment of both the virus and the host, favouring either the continuation of the viral life cycle or the host's antiviral response. Comprehensive characterisation of enzymatic activity during viral infection is therefore necessary for the understanding of virally induced diseases. Activity-based protein profiling techniques have been established as effective and practicable tools with which to interrogate the regulation of enzymes' catalytic

G. F. Desrochers · J. P. Pezacki (✉)
Department of Chemistry and Biomolecular Sciences, University of Ottawa,
10 Marie Curie Pvt., Ottawa, ON K1N 6N5, Canada
e-mail: john.pezacki@uottawa.ca

activity and the roles played by these enzymes in various cell processes. This paper will review the contributions of these techniques in characterising the roles of both host and viral enzymes during viral infection in humans.

1   Introduction

Viruses are infectious biological agents which lack the ability to replicate independently; specifically, they lack translational and metabolic machinery, and they are therefore obligate intracellular parasites. Viral particles consist of either an RNA or DNA genome surrounded by a protein capsid which protects the genetic material. Certain viruses are additionally enveloped by a lipid bilayer derived from host membranes. In order to propagate, viruses must use host cell systems to assemble new RNA, DNA, proteins and lipid envelopes. Though details of viral life cycles vary from virus to virus, there are five general stages: cell entry, translation of viral proteins, replication of the genome, assembly of the viral particle and egress from the cell (Fig. 1). Activity-based profiling has been used broadly to characterise
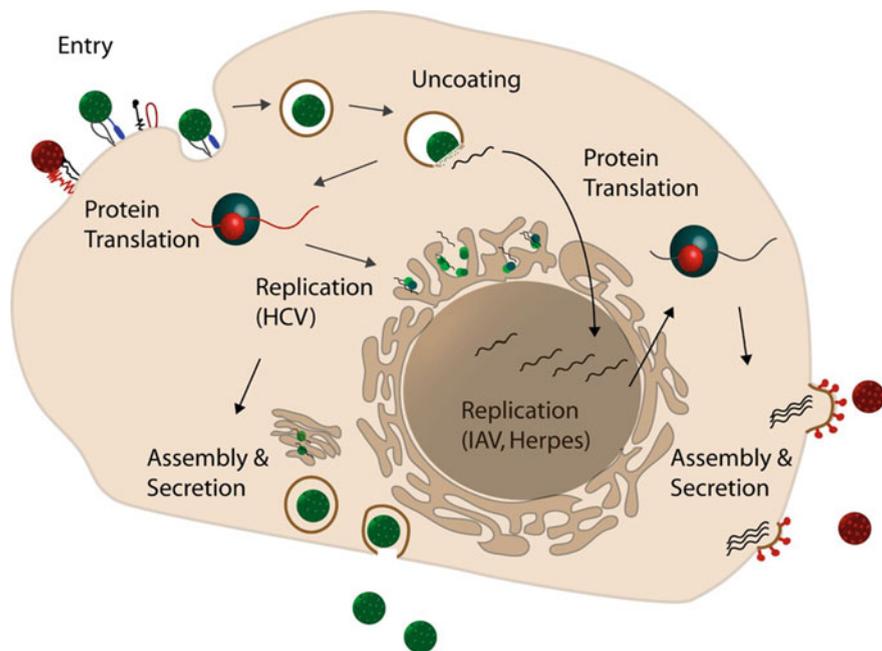
Fig. 1 Viruses utilise multiple host systems throughout their life cycle. Life cycles differ between viruses. Positive-strand RNA viruses (ex: HCV) follow the path illustrated on the left: receptor binding, internalisation, protein translation, replication within membranous structures in the cytosol, assembly and secretion. Viruses such as influenza A and herpesviruses follow the path on the right: replication within the nucleus, export to the cytoplasm, protein translation, assembly and budding from the cell

host–virus interactions. The approaches used and the discoveries of these techniques accorded will be highlighted herein.

The molecular processes involved in virus propagation require the diversion of energy, molecular building blocks and other essential resources. These need to be diverted from the host cell's systems and into pathways and processes needed by the virus. It is important to note that metabolic energy is consumed not only by the synthesis of new viral particles, but also by the remodelling of the cellular environment to meet the demands of the virus. Viruses require specific and often tightly regulated conditions to propagate efficiently. A good example lies in the replication of positive-strand RNA viruses, which occurs within specialised regions called replication organelles (RO) (Neufeldt et al. 2018). These organelles create an optimal environment for replication, allowing the concentration of the required host and viral factors and shielding viral RNA from the innate immune response (Neufeldt et al. 2018). The morphology of these organelles differs from virus to virus: they can be spherical or tubular, originate from the ER, Golgi body, endosome or lysosome, and be composed of single, double or multi-membrane vesicles (Novoa et al. 2005; Neufeldt et al. 2018). These membranes furthermore possess specific lipid profiles unique to each virus. The Rubella virus RO requires elevated saturated fatty acid levels, while West Nile virus, hepatitis C virus and enteroviruses require high cholesterol (Harak and Lohmann 2015; Paul and Bartenschlager 2015). The hepatitis C RO also requires elevated levels of sphingolipids and phosphory-lated phosphoinosides (PIPs) (Hsu et al. 2010; Harak and Lohmann 2015; Paul and Bartenschlager 2015). The large amount of metabolic energy required to change the lipid profile of a cell in this manner necessitates significant perturbation of normal lipid homeostasis.

The diversion of metabolic energy and the remodelling of host cell architecture are initiated by viral enzymes; however, direct modification of cellular architecture by viral enzymes is not always feasible due to the small range of functionalities encoded by viruses. Changes in host cell function, through changes in abundance and activity of key enzymes, can be induced by the virus as homeostatic responses to virus-induced changes as well as immunological responses to infection. Viral genomes can be extremely small: the hepatitis B virus, for example, has a genome 3.2 kb long (Liang 2009). As a result, viruses utilise the machinery of the cells they infect to replicate their own genome and assemble new viral particles for release and subsequent reinfection of new host cells.

Functional changes that are induced either directly or indirectly often are the result of changes in enzyme catalytic activity of both viral and host-derived enzymes. Interrogating changes to enzymatic activity during viral infection is therefore essential to form a complete understanding of the mechanisms of virus infection. Activity-based protein profiling (ABPP) techniques (Barglow and Cravatt 2007; Cravatt et al. 2008) are ideally suited to answering questions on the perturbation of enzyme function by host–virus interactions. These techniques use small molecule probes to covalently label active enzymes, while inactive enzymes remain unmodified. By including reporter tags in these probes, it is possible to quantitatively report differences in levels of active enzyme within complex proteomic samples.
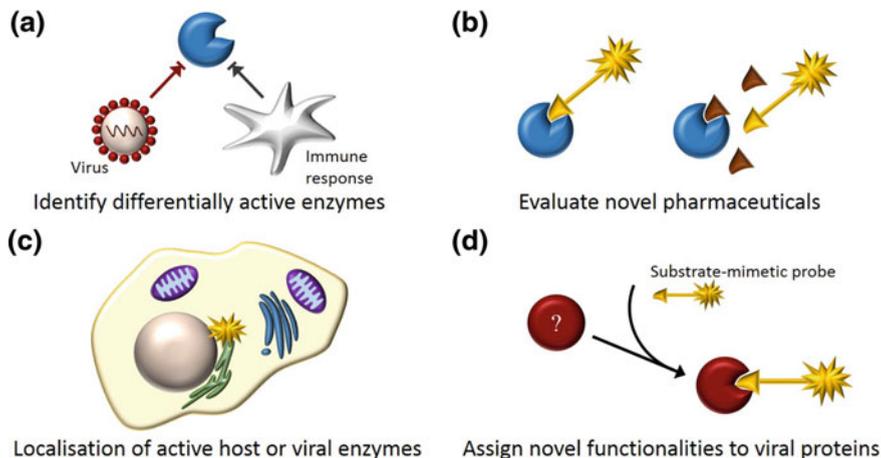
Fig. 2 Contributions of ABPP to the understanding of host–virus interactions. **a** Activity-based profiling identifies and quantifies changes to enzymatic activity caused by the virus or by the host's antiviral defence mechanisms. **b** Competitive activity-based labelling evaluates the effectiveness of novel antiviral compounds. **c** Fluorophore conjugated activity-based probes allow imaging of active enzyme localisation. **d** Activity-based labelling with substrate-mimetic activity-based probes identifies new targets for viral enzymes

This chapter will describe the contributions made by ABPP to the understanding of host–virus interactions. ABPP has supplied a diverse toolbox of activity-based probes which have been applied to study both viral and host enzymes: identifying host enzymes dysregulated during different stages of viral infection, determining how the virus effects these changes and characterizing viral enzymes' functionalities, structure, and catalytic and inhibitory mechanisms (Fig. 2).

2 Viral Entry

For most viruses, the entry process consists of recognition of their target cell, attachment to the cell's membrane, internalisation of the virus into the cell, usually by an endocytic pathway, and the release of viral genetic material into the cytosol of the cell. In the case of enveloped viruses, this requires the fusion of the viral envelope with the endosomal membrane to form a pore through which the genetic material passes. The mechanism of formation of this pore varies between viruses, many of which require significantly different host cell conditions or factors to induce fusion.

A major focus in efforts to design novel pharmaceuticals has been the development of cell entry inhibitors. Targeting cell entry prevents the spread of infection as well as the irreversible damage to tissues caused by infection. Furthermore, as

host factors and not viral factors are usually targeted, it can impede the emergence of drug-resistant strains. Antiviral pharmaceuticals targeting cell entry have been developed against hepatitis B virus, human immunodeficiency virus (HIV) and herpesviruses (De Clercq and Li 2016; Sun et al. 2018; de Castro and Camarasa 2018), and entry inhibitors against other viruses such as HCV are currently being developed (Xiao et al. 2015; Qian et al. 2016).

Viruses such as the SARS coronavirus have been shown to require the activity of lysosomal cathepsins, such as cathepsin L, in order to enter the cytosol (Bosch et al. 2008). Cathepsin L cleaves the SARS-CoV spike protein, found on the viral envelope, thereby activating it and allowing membrane fusion to proceed (Bosch et al. 2008). It has furthermore been demonstrated to play an accessory role in Ebola infection, cleaving the GP1 protein in conjunction with cathepsin B and triggering membrane fusion (Chandran et al. 2005). The epoxide-based peptide-mimetic probe DCG-04 was developed to label cysteine proteases, a class which includes cathepsins (Greenbaum et al. 2000).

Shah et al. applied this probe to study novel SARS and Ebola virus entry inhibitors by competitive ABPP (Shah et al. 2010). Cells treated with their novel inhibitors demonstrated significantly reduced labelling by DCG-04, indicating that they were able to target cathepsin L in live cells and suggesting that they inhibited viral entry by targeting cathepsin L (Shah et al. 2010). This study demonstrates how an ABP can be applied in the development of novel pharmaceuticals targeting cell entry.

3 Viral Replication

Members of the *flaviviridae* family significantly remodel their host cell architecture to form an environment favourable for replication. The hepatitis C virus, one of the most studied viruses from this family, induces the formation of double-membrane vesicles inside a membranous web derived from the endoplasmic reticulum (Neufeldt et al. 2018). The formation of the membranous web during HCV infection necessitates significant alteration of the local lipid profile, which results from viral hijacking of lipid metabolic enzymes (Neufeldt et al. 2018). Activity-based protein profiling has been used to identify which enzymes are targeted by the virus and to determine how their regulation is modulated.

3.1 Role of Triglycerides

One of the most pronounced HCV-induced changes to cell architecture is the appearance of larger and more numerous lipid droplets near sites of viral replication (Neufeldt et al. 2018). This is the result of both increases in fatty acid synthesis and decreases in fatty acid oxidation and secretion (Syed et al. 2010).

Fatty acid synthase (FASN), the enzyme responsible for de novo synthesis of fatty acids, has been associated with HCV replication as early as 2002; however, the nature of the interactions between HCV and FASN was not completely characterised (Su et al. 2002). A probe based on the β-lactone inhibitor orlistat, orlistat-alkyne, was shown to strongly and selectively label active FASN (Yang et al. 2010). This probe was used to investigate how HCV altered FASN activity. By comparing changes in protein expression and probe labelling, Nasheri et al. determined that FASN activity was significantly increased during HCV replication by increases both in protein expression and post-translational activation (Nasheri et al. 2013). Imaging of active FASN using the orlistat-alkyne probe demonstrated that HCV does not alter the localisation of FASN, indicating that the post-translational regulation of FASN occurs by another mechanism (Nasheri et al. 2013). Increased activity of FASN during HCV replication was further confirmed using probes containing a β-lactam warhead (Nasheri et al. 2014).

HCV-mediated upregulation of intracellular lipids has been shown to be regulated via numerous mechanisms: in addition to increases in lipid synthesis, decreases in lipolysis and lipid secretion have also been demonstrated to play a significant role (Syed et al. 2010). Singaravelu et al. used a novel activity-based probe with a phenyl sulfonate ester warhead (PS) to identify enzymes displaying differential activity during HCV replication (Singaravelu et al. 2010). The PS probe was shown to label an enzyme involved in the β-oxidation of fatty acids, the electron transfer flavoprotein subunit alpha (ETFA). Comparison of labelling in HCV-replicating and naïve cells shows a decrease in ETFA activity during HCV replication, suggesting that targeting of ETFA may be a mechanism by which HCV reduces lipid oxidation and increases intracellular lipid levels (Singaravelu et al. 2010).

In a more recent study, Nourbakhsh et al. used a fluorescently labelled fluorophosphonate probe (Fig. 3a shows desthiobiotin-labelled variant) to profile serine hydrolase activity in infected vs. naïve hepatoma cells. They identified one enzyme, arylacetamide deacetylase (AADAC), which was significantly down-regulated during HCV infection (Nourbakhsh et al. 2013). It was subsequently demonstrated that AADAC induces lipolysis and decreases VLDL secretion of lipids, thereby decreasing the levels of intracellular triglycerides (Nourbakhsh et al. 2013). Altogether, this study demonstrated that HCV decreases the activity of the serine hydrolase AADAC in order to increase intracellular lipid levels via the inhibition of lipolysis and lipid secretion.

3.2 Role of Phosphatidylinositides

Phosphatidylinositides (PIs) are minor components of the intracellular membranes which play important roles in establishing organelle identity and in propagating cell signalling, and certain species have additionally been shown to be able to induce high membrane curvature (McMahon and Gallop 2005). A phosphorylated species
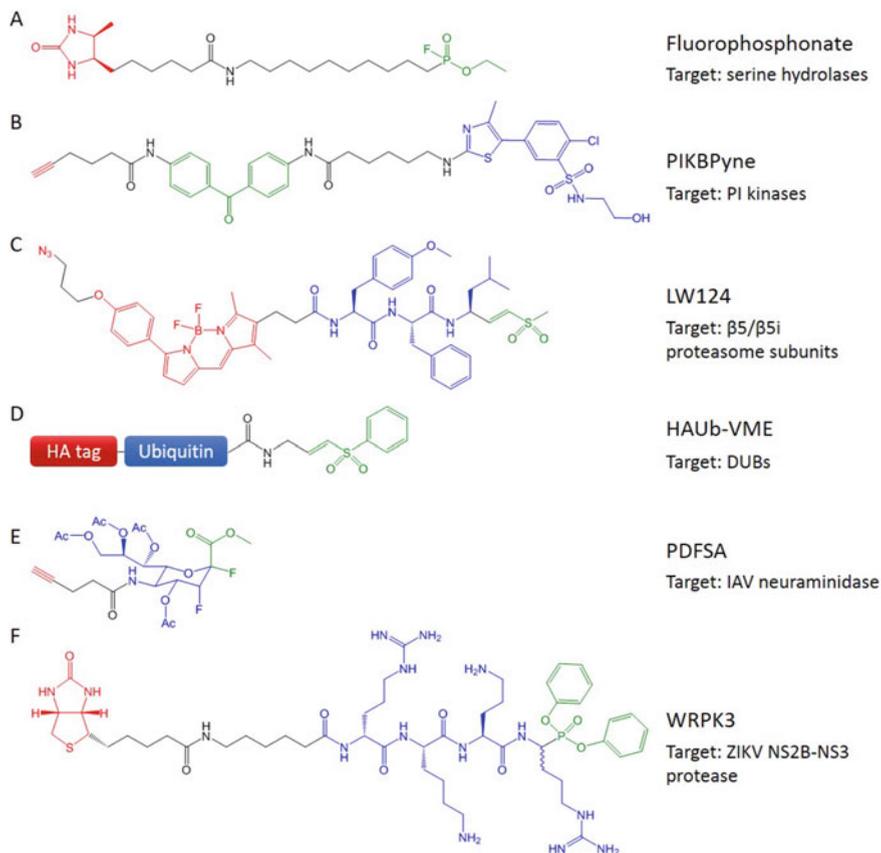
Fig. 3 Examples of types of activity-based probes used to interrogate host–virus interactions. Targeting groups shown in blue, reactive groups shown in green, and reporter tags or handles for attachment of reporter tags shown in red. **a** Inhibitor-based fluorophosphonate conjugated to desthiobiotin possesses a broad specificity towards host cell serine hydrolases. **b** Inhibitor-based PIKBPyne labels specific host cell lipid kinases, phosphatidylinositol kinases type III. **c** Peptide-mimetic LW124 selectively labels proteasomal subunit β5 and immunoproteasomal subunit β5i. The BODIPY group allows LW124 to be used as a fluorescent probe, while the azide handle allows the attachment of affinity tags for purification of labelled targets. **d** Substrate-mimetic HAUb-MVE targets deubiquitinating enzymes from both viruses and host cells. The HA tag allows detection of labelling and purification of labelled enzymes. **e** Substrate-derived PDFSA targets neuraminidases on the envelope of the influenza A virus. **f** Peptide-based WRPK3 contains unnatural amino acids and specifically labels Zika virus NS2B-NS3 protease

of PI, PI4P, has been shown to be upregulated in the membranous web; it has been hypothesised that this it plays a role in establishing the morphology of the membranous web (Hsu et al. 2010; Bishé et al. 2012). PI4P is synthesised from PI by four PI-kinases (PIKs) which are differentially localised within the cell (Bishé et al. 2012). The identity of the kinases which contribute to HCV-mediated upregulation of PI4P levels within the replication complex has been a subject of significant interest (Delang et al. 2012; Bishé et al. 2012). While PIKs such as PIKA are well established as HCV host factors, the role of other PIKs, such as PIKB, during infection remained ambiguous (Delang et al. 2012).

In order to investigate the activity of PI4Ks during HCV infection, Sherratt et al. synthesised a novel activity-based probe, PIKBPyne, derived from the well-known reversible PIK active-site inhibitor PIK93 (Fig. 3b) (Sherratt et al. 2014). The warhead of this probe contained a PIK93 group to enable selective interaction with active PIKs, and a UV-inducible benzophenone crosslinker to form a covalent bond between the probe and its protein target. Using this probe, it was shown that PI4KB activity, but not expression, is upregulated by HCV infection (Sherratt et al. 2014). This finding illustrates the important role ABPP can play in characterising the dysregulation of host factors by viruses.

As the increase in PI4KB activity did not correlate with increased protein expression, activity was hypothesised to be regulated post-translationally. PI4KB activity is known to be regulated by stable protein–protein interactions which recruit PI4KB to membranes and stabilise an activating phosphorylation at Ser294 (Hausser et al. 2005, 2006; Balla 2013). To investigate the role of these interactions in the post-translational regulation of PI4KB activity, Desrochers et al. synthesised new PIKBPyne probes containing flexible linkers of varying length between the targeting PIK93 moiety and the crosslinking benzophenone (Desrochers et al. 2018). These flexible-linker PIKBPyne probes were shown to be able to label proteins interacting with active PI4KB. Labelling with PIKBPyne demonstrated an HCV-induced decrease in the interaction between PI4KB and the Golgi-resident protein ACBD3 (Desrochers et al. 2018). It had previously been reported that HCV induced a change in the localisation of PI4KB from the Golgi towards sites of viral replication (Zhang et al. 2012). This finding suggests that the upregulation of PI4P levels in the membranous web during HCV replication may occur partially through the disruption PI4KB–ACBD3 interaction, allowing the relocalisation of PI4KB.

3.3 Role of Cholesterol

The double-membrane vesicles of the HCV replication sites have been shown to contain elevated levels of cholesterol (Neufeldt et al. 2018). Activity-based probes have been applied to characterise the processes by which cholesterol metabolism is hijacked during HCV replication.

Fluorophosphonate-based probes were used to profile serine hydrolase activity during HCV replication, in order to identify enzymes differentially regulated by the

virus (Blais et al. 2010a, b). Carboxylesterase 1 (CES1), a liver-abundant serine hydrolase which displays cholesteryl ester hydrolase activity, was identified as a target of activity-based labelling by both fluorophosphonate-based and peptide-based probes containing either a serine or a threonine moiety (Ross et al. 2010; Blais et al. 2010a, b). CES1 activity has been shown to play a role in decreasing the levels of intracellular cholesteryl ester, as well as the secretion of intracellular triglycerides by the VLDL pathway (Zhao et al. 2007; Ross et al. 2010). It has furthermore been demonstrated that CES1 activity is not always correlated to its expression, suggesting that the regulation of CES1 function relies on post-translational modifications and interactions (Ross et al. 2010). Activity-based labelling is therefore ideally suited for interrogating the function of CES1. Both fluorophosphonate and the peptide-based probes demonstrated significantly increased activity during HCV replication, suggesting that CES1 is activated as part of the host cell's antiviral strategy to counteract the virally induced increases in cholesterol and triglycerides (Blais et al. 2010a, b).

β-lactam warhead probes, described above, have also been used to investigate cholesterol metabolic enzymes. These probes have been shown to label hydroxymethylglutaryl-CoA synthase 1 (HMGCS1), an enzyme which catalyses an early step in cholesterol synthesis (Mazein et al. 2013; Nasheri et al. 2014). Labelling with the β-lactam probe showed an increases HMGCS1 activity during HCV replication, suggesting that differential activation of this enzyme is a mechanism by which HCV induces the formation of a favourable replication environment (Nasheri et al. 2014). This finding provided further confirmation of the suggested role of HMGCS1 in HCV infection (Kapadia and Chisari 2005).

Many of the changes in the activity of lipid metabolic enzymes are the result of alterations to their post-translational mechanisms which regulate them. Changes to the activity of signalling pathways which control this post-translational regulation are therefore of great interest. Many signalling pathways rely on kinase activity, and profiling kinase activity therefore has the potential to provide information on the regulation of multiple cell systems and pathways. The probe wortmannin-yne, based on the irreversible kinase inhibitor wortmannin, was designed to be able to broadly profile kinase activity (Desrochers et al. 2015). Comparison of labelling by wortmannin-yne in cells replicating HCV and in naïve cells identified multiple regulatory kinases whose catalytic activity was altered by HCV. Kinases in the MAPK pathway, in particular, displayed significant decreases (Desrochers et al. 2015). This study confirmed previous reports of HCV-mediated decreases in MAPK signalling and demonstrates how ABPs can be used to profile kinase activity.

This section has described ABPs which target lipid metabolic enzymes and how they have been used to profile changes to the regulation of lipids during viral infection. Hijacking of lipid homeostasis is a major contributor to the diversion of metabolic energy during infection. However, other metabolic pathways are also perturbed during infection: glycolysis in particular is targeted by multiple diverse viruses, including HCV, influenza A virus, and several herpesviruses, though the mechanisms by which glycolysis is mis-regulated are not fully understood

(Goodwin et al. 2015). The development of novel probes targeting glucose metabolic enzymes represents an opportunity to increase our understanding of metabolic flux during viral infection.

4 Programmed Cell Death

A fundamental aspect of the innate immune response to infection lies in the activation of apoptotic and cell death pathways upon viral invasion of the cell in order to restrict the spread of infection throughout the host (Barber 2001). Commercial peptide-based probes, FLICA[TM], have been established as chemical-based tools to study apoptosis (Furman et al. 2009). These probes are designed to report on the activity of caspaces, enzymes essential to the progression of apoptosis (Furman et al. 2009). Using these probes, Furman et al. determined that norovirus infection induces apoptosis, as demonstrated by the significant increase in caspace activity. Mass spectrometry analysis of enzymes labelled by these probes revealed that they are equally capable of reporting on the activity of the cysteine protease cathepsin B. It was shown that cathepsin B is activated during norovirus infection and acts as an upstream activator of the apoptotic pathway (Furman et al. 2009).

Cathepsin D is a lysosomal aspartic peptidase which has also been shown to induce apoptosis (Benes et al. 2008). Using a peptide-based probe containing a phenylalanine targeting group, Blais et al. demonstrated that HCV significantly down-regulates the activity of cathepsin D, suggesting that HCV decreases cathepsin D activity in order to avoid apoptosis of infected cells (Blais et al. 2010a). Activation of anti-apoptotic genes was also demonstrated during infection of Dengue virus, a close relative of HCV. Novel ATP-based probes containing acyl phosphate warheads were used to profile the activity of kinases during Dengue infection (Vetter et al. 2012). The activity of DNA-dependant protein kinase (DNA-PK), an anti-apoptotic enzyme which recognises and repairs DNA double-stranded breaks, was shown to be significantly upregulated by Dengue infection (Vetter et al. 2012). Altogether, ABPP has shown that both HCV and Dengue virus act to counteract the activation of the apoptotic pathway.

5 Viral Evasion of the Immune Response

Cathepsins also play a role in regulating inflammation and the recruitment of the adaptive immune response during infection. Cathepsin G, for example, has been shown to participate in the degradation of proteins for presentation by the major histocompatibility complex (Burster et al. 2010). It has additionally been shown to negatively regulate STAT5 transcriptional activation (Schuster et al. 2007), as well as inflammation (Burster et al. 2010). Zou et al. reported a novel peptide-based probe, Mars116, specific for cathepsin G (Zou et al. 2012). Labelling of cathepsin G

by Mars116 was significantly decreased 12 days after Epstein–Barr virus (EBV) infection (Zou et al. 2012). Labelling of four other cathepsins, cathepsins X, B and S, by DAP22c also showed a significant decrease during EBV infection. This decrease in the activities of immune-linked cathepsins is suggestive of an immune escape strategy by EBV (Zou et al. 2012).

5.1 *Viral Alterations to Proteasome Activity*

The proteasome is a multi-subunit structure responsible for the degradation of damaged or excess proteins within the cell and plays an essential role in protein quality control, cell signalling, immune responses and apoptosis (Kammerl and Meiners 2016). A specialised form of the proteasome, the immunoproteasome, consists of many of the same regulatory subunits in addition to three variant catalytic subunits found only in the immunoproteasome (Ferrington and Gregerson 2012). This specialised form of the proteasome plays a role in directing the activity of the adaptive immune system. It is induced during infection and generates peptides with a hydrophobic C-terminus for cell-surface display in MHC class I molecules. CD8 T cells surveil these peptides and propagate an immune response upon recognition of foreign material (Ferrington and Gregerson 2012). The existence of mixed proteasomes, containing both the constitutively expressed and immunoproteasome subunits, has also been demonstrated, indicating that both categories of catalytic subunits could be implicated in immune response (Kammerl and Meiners 2016).

The catalytically active proteasomal subunits, though not serine hydrolases, containing a nucleophilic threonine as the catalytic residue (Marques et al. 2009; Nasheri et al. 2015), have been shown to be labelled by the activity-based probe fluorophosphonate (Fig. 3a) (Shahiduzzaman et al. 2014). Using a fluorophosphonate-based probe, Shahiduzzaman et al. have shown that the activity of several proteasomal subunits, PSMA2, PSMA3, PSMA6, PSMB3, increased during influenza A infection, possibly due to increases in immunoproteasome activation (Shahiduzzaman et al. 2014). Though fluorophosphate was able to interrogate the activity of a few proteasomal subunits, several other subunits were not detected.

Probes able to specifically and quantitatively assess the activities of all the possible catalytic subunits of the proteasome have been developed by various groups over the past decade. The first to be developed, MV151, is a peptide-mimetic probe conjugated to a BODIPY fluorophore, and labels all catalytically active proteasome subunits (Verdoes et al. 2006). Other more specific probes were later developed: LW124, a probe specific for the β1 subunit and LMP2, its immunoproteasome analogue, and MVB127, a probe specific for β5 and its analogue LMP7 (Fig. 3c) (Li et al. 2013; Keller et al. 2015).

These probes were applied by Keller et al. to investigate perturbation to the proteasome system during viral infection. Proteasome activity was shown to be

increased during murine gamma-herpes virus infection using the general-use MV151 probe. LW124 and MVB127 were used to assess the changes to catalytic activity of the proteasomal subunits versus the activity of their immunoproteasomal analogues during infection. The identity of the labelled subunits was determined based on their reactivity and their molecular weight, as determined by in-gel fluorescent imaging. It was demonstrated that while the activity of the proteasomal subunits remained relatively unchanged, the activity of the immunoproteasome subunits increased significantly during the early stages of infection, before decreasing as the infection progressed (Keller et al. 2015). While these probes had been previously demonstrated to be specific proteasome probes (Verdoes et al. 2006; Li et al. 2013), Keller et al. were the first to demonstrate how they can be used to specifically assess regulatory changes to the activity of the immunoproteasome in the context of viral infection (Keller et al. 2015).

These increases in proteasome activity represent the part of the host's response to viral infection wherein proteasomal degradation of proteins is increased in order to generate antigenic peptides for the recruitment of the adaptive immune response. Viral targeting of proteasomal activity could therefore be a potential immune evasion strategy. Recently, Nasheri et al. used a β-lactone-based activity-based probe, orlistat-alkyne, to label active proteasome subunits and thereby interrogate proteasome activity during hepatitis C virus infection (Nasheri et al. 2015). It was shown that the activity of multiple members of the proteasome system displayed decreased activity in HCV infected cells. PSMB5, which displays broad specificity and chymotrypsin-like hydrolase activity (Marques et al. 2009), PSMC6, an ATPase which regulates proteasome activity (Coux et al. 1996), and two immunoproteasomal subunits, PSME1, and PSME2 (de Graaf et al. 2011) were down-regulated between 40 and 80% (Nasheri et al. 2015). Interestingly, changes to protein expression did not match the observed changes in enzymatic activity (Nasheri et al. 2015). Altogether, this suggests that HCV targets proteasomal degradation by altering both protein expression and post-translational regulation as part of its efforts to evade the immune response. Furthermore, this study highlights the advantages of activity-based protein profiling in addition to traditional proteomics to study virally induced perturbation of host cell systems.

5.2   NFκB Signalling During Herpesvirus Infection

Nuclear factor kappa B (NFκB) is a transcription factor, activated during viral infection, which induces the expression of genes involved in the innate immune response (Oeckinghaus and Ghosh 2009). The signalling cascade which is responsible for the activation of NFκB during infection relies in great part on the ubiquitination of key signal transducers (Wertz and Dixit 2010). The auto-ubiquitination of the ubiquitin ligase TRAF6 promotes the activity of the TAK1 kinase (Wertz and Dixit 2010). The IκB kinase (IKK) complex is then

activated via TAK1-mediated phosphorylation and TRAF6-mediated ubiquitination (Wertz and Dixit 2010; van Gent et al. 2014). IKK phosphorylation of IκB results in its ubiquitination and subsequent degradation (Oeckinghaus and Ghosh 2009). NFκB is sequestered in the cytoplasm by IκB; upon its degradation, NFκB translocates to the nucleus and induces the expression of antiviral, pro-inflammatory and pro-apoptotic genes (Oeckinghaus and Ghosh 2009).

The activity of enzymes responsible for the regulation of ubiquitination is therefore of interest when investigating host–virus interactions due to the importance of ubiquitination to the immune response to infection. Deubiquitinating enzymes (DUBs) are enzymes responsible for removing ubiquitin from ubiquitinated proteins. As they are cysteine proteases, they are excellent candidates for suicide-based activity probes (Borodovsky et al. 2002).

HAUb probes are based on modified ubiquitin proteins, and mimic the natural substrate of DUBs, allowing them to specifically target DUBs. Ubiquitin is recombinantly expressed with the haemagglutinin tag HA, which functions as a reporter of enzymes labelling. A thiol-reactive group functioning as the warhead is subsequently added to the C-terminus via intein-based chemical ligation. Several variants were produced, containing several different warheads: chloroethylamine (Cl), bromoethylamine (Br2), bromopropylamine (Br3), glycine vinylmethylsulfone (VS), glycine vinyl methylester (VME), glycine vinyl phenylsulfone (VSPh), and glycine vinylcyanide (VCN) (Borodovsky et al. 2002). Immunoblotting and MS/MS profiling of labelled DUBs demonstrated a slight but significant difference in the labelling patterns of the HAUb probes (Borodovsky et al. 2002; Ovaa et al. 2004). The probe containing a vinyl methylester warhead (HAUb-VME, Fig. 3d) displayed the broadest labelling capacity and was used in most subsequent studies (Borodovsky et al. 2002; Ovaa et al. 2004).

After developing these probes, Ovaa et al. applied them to interrogate the activity of host cell deubiquitinating proteins during infection by the herpesvirus Epstein–Barr virus (EBV) (Ovaa et al. 2004). Herpesviruses are large double-stranded DNA viruses and have been shown to modulate the immune response via multiple different mechanisms (Cruz-Muñoz and Fuentes-Pananá 2017). Using the HAUb-VME probe, Ovaa et al. labelled active host cell deubiquitinating enzymes and identified multiple DUBs whose activity was increased during infection: UCH-L3, USP-15, UCH-L1, UCH37, USP7 and USP9X. Several of these enzymes have been shown to regulate the NFκB pathway. USP15 negatively regulates NFκB activation by deubiquitinating IκB (Harhaj and Dixit 2011), while USP7 decreases NFkB signalling by deubiquitinating TRAF6 and IKK (Nanduri et al. 2013). On the contrary, USP37 has been shown to be required for IκB-α degradation and therefore NFkB activity (Mazumdar et al. 2010). Altogether, Ovaa et al. demonstrated that the herpesvirus EBV differentially regulates DUBs implicated in the regulation of the innate immune response, suggesting that this may be a pro-viral immune evasion mechanism.

5.3 Virally Encoded Deubiquitinases

Kattenhorn et al. applied the HAUb-VME probes designed by Borodovsky et al. to study the perturbation of ubiquitination-regulating enzymes during infection of Herpes simplex virus 1 (HHV-1), a herpesvirus related to EBV. MS/MS analysis of labelled proteins identified an enzyme which did not correspond to any known DUB, and whose sequence was found to correspond to a viral protein with unknown function, the large tegument protein deneddylase (UL36) (Kattenhorn et al. 2005).

Perturbation of host cell processes by viruses is usually indirect as the limited size of viral genomes means the virus cannot itself encode the functionalities it requires. Herpesviruses, however, are large, double-stranded DNA viruses and possess larger and more complex genomes than the simpler RNA viruses discussed elsewhere in this chapter. HHV-1, for example, encodes approximately 80 proteins (Macdonald et al. 2012).

UL36 does not share significant sequence homology with any other DUB known at the time (Kattenhorn et al. 2005); tradition bioinformatics could therefore not have predicted its function. This discovery highlights one of the unique advantages of activity-based protein profiling: it reports directly on the functional output of enzymes and allows the identification of novel targets. UL36 has recently been shown to deubiquitinate IκBα, preventing its degradation and promoting the sequestration of NFκB outside of the nucleus and thereby interfering with the activation of the innate immune response (Ye et al. 2017).

Following this discovery, several research groups postulated the existence of homologous deubiquitinating enzymes in closely related viruses whose function had remained undiscovered due to their lack of homology to previously charac-terised DUBs. Having been established as an effective probe of herpesvirus deu-biquitinating enzymes, HAUb-VME was used to interrogate the activity of viral proteins from several different viruses.

Schlieker et al. identified an Epstein–Barr virus protein, BPLF1, and a murine cytomegalovirus protein (MCMV), M48, which possessed significant sequence homology to the HHV-1 UL36 (Schlieker et al. 2005). They subsequently demonstrated that both BPLF1 and M48 were labelled by the HAUb-VME probe, indicating that these proteins were enzymes which targeted and hydrolysed ubiq-uitinated proteins (Schlieker et al. 2005). It was later shown that BPFL1 acts similarly to HHV-1's UL36, inhibiting the activation of NFκB by catalysing the removal of ubiquitin from the sequestering protein IκBα (van Gent et al. 2014). It has furthermore been demonstrated that BPLF1 exerts additional control over the NFκB pathway, deubiquitinating both TRAF6 and subunits of IKK, and thereby inhibiting the propagation of TLR signalling and the resultant activation of NFκB (Saito et al. 2013; van Gent et al. 2014). Taken together, this demonstrates that EBV uses the virally encoded DUB functions to inhibit the activation of the antiviral innate immune response.

Another herpesvirus, human cytomegalovirus (HCMV), has also been shown to encode a deubiquitinating enzyme (Wang et al. 2006). HAUb-VME labelled a high molecular weight enzyme, pUL48, whose role in infection had previously been unknown. In the absence of pUL48 deubiquitinating activity, virion production was significantly inhibited, though not entirely blocked, indicating that pUL48 deubiquitinating activity contributes to, but is not required for, virion production. HAUb-VME has also been used to identify similar deubiquitinating proteins in murine gamma herpesvirus 68 (MHV-68) and Marek's disease virus (MDV) (Gredmark et al. 2007; Jarosinski et al. 2007).

Assigning novel functionalities is one of the more well-known uses of ABPP, and an essential primary step in characterising poorly understood enzymes which opens up further avenues of investigation. Contextualisation of the reactivity of an enzyme enables the determination of its specific targets. The ubiquitin chains targeted by the DUBs discussed in this section are linked to each other by one of the seven different linkage sub-types, corresponding to the location of the lysine residue to which the next ubiquitin is bound: Lys^6, Lys^{11}, Lys^{27}, Lys^{29}, Lys^{33}, Lys^{48} and Lys^{63}. New diUb-VME probes were developed containing two ubiquitin moieties conjugated by one of the seven possible linkage types, thereby allowing the determination of DUB's linkage specificity (Mulder et al. 2014). These probes were used to interrogate the substrate specificity of the SARS coronavirus papain-like protease (PLPro), a deubiquitinating enzyme, thought to play a role in innate immune evasion (Békés et al. 2016). The structural basis of PLPro's specificity was determined by co-crystalising it in conjunction with a diUb-VME probe. Co-crystalisation of enzymes with their substrates is often challenging, as the enzyme will catalyse the substrate and release the products. Using a substrate-mimetic ABP ensures that it is retained within the active site, preserving the interactions which give rise to specificity as well as the active conformation of the enzyme. This showed that PLpro interacts with the two ubiquitin moieties, but has few interactions with the interface between the two (Békés et al. 2016). This suggests that specificity originates from the orientation of the ubiquitin proteins in the S1 and S2 pockets, and not from recognition of the linkage itself. Screening with a panel of Ub-VME probes containing mono-Ub or di-Ub and possessing different linkage types demonstrated that PLpro possessed a similarly low labelling efficiency for mono-Ub and most di-Ub probes (Békés et al. 2016). This lends further support to the idea that interactions with a properly oriented S2 ubiquitin stabilise the enzyme-substrate complex and contribute to the specificity of SARS PLpro.

6 Viral Assembly and Egress

The last stage of the viral life cycle consists of the assembly of the viral particle and its release into the extracellular space. The majority of the contributions made by ABPP towards the understanding of virus infection have focused on viral entry and

replication. In some cases, this has been due to limitations in cell culture models of infection: these models could replicate the genome but were unable to efficiently assemble infectious particles (Lohmann and Bartenschlager 2014). As viral assembly and egress do rely on host factors to translocate to the plasma membrane and exit the cell, profiling enzymatic activity during these processes is of significant interest.

Hijacking of cellular trafficking plays a significant role in the packaging and secretion of viruses. The COPI system is essential for correct localisation of IAV particles, while HCV utilises the COPII system to travel from the ER to the Golgi, at which point it can be trafficked through the VLDL secretory pathway (Pohl et al. 2016; Syed et al. 2017). These systems are regulated in part by Rab GTPases, host cell enzymes which play an important role in trafficking throughout the cell (Hutagalung and Novick 2011) and which have been implicated in the egress of multiple viruses (Hogue et al. 2014; Pohl et al. 2016; Takacs et al. 2017). Rab11 has been shown to be essential for the trafficking of IAV RNA from the perinuclear region to the plasma membrane (Pohl et al. 2016). Another Rab protein, Rab1, has been proposed to mediate transport of nascent HCV virions from the ER to the Golgi (Takacs et al. 2017). Rab proteins have also been shown to play a role in DNA virus egress in the case of herpesviruses (Hogue et al. 2014). Altogether, this suggests that Rab GTPase activity could be an interesting target for activity-based profiling during viral infection.

IAV budding has been shown to involve the activity of a class of ATPases, the mitochondrial F1FO-ATPases. These enzymes localise to the plasma membrane and are necessary for efficient budding of new virions, though the precise nature of their contribution has yet to be fully understood (Pohl et al. 2016). ATP-based probes have previously been used to profile host enzyme activity during Dengue infection (Vetter et al. 2012) and applying them to profile the changes in activity of various ATPases during IAV infection could yield new information with regards to how enzymes are recruited and utilised during viral egress.

Lipid regulatory systems, which play an important role in earlier stages of viral life cycles, continue to play an important role in viral egress. HCV provides a good example of this, as its egress relies on the very-low-density-lipoparticle (VLDL) secretory pathway to release the mature virus particle into the bloodstream (Neufeldt et al. 2018). Certain VLDL-associated enzymes, such as the triglyceride-recruiting enzyme ABHD5 or the phospholipid synthesis enzyme LPCAT1, have been identified as pro- or antiviral HCV host factors. However, the interactions between these enzymes and HCV are not fully understood (Vieyres et al. 2016; Beilstein et al. 2017). Profiling enzymes which regulate this pathway could therefore reveal novel information clarifying how it is hijacked by HCV. Even viruses such as influenza, which do not rely on a lipid secretory pathway, still alter the cell's lipid profile in order to exit into the bloodstream (Pohl et al. 2016). Influenza virus egress occurs by budding from the plasma membrane, a process requiring an altered membrane lipid composition (Pohl et al. 2016). Profiling changes to lipid regulatory networks during the assembly and egress of virions

could therefore yield new information and improve our understanding of host–virus interactions during late stages of infection.

In general, activity-based profiling on these later stages of viral infection has the potential to yield novel host factors requirements for productive infections. Targeting the secretion of new virions could play an important role within combinatorial antiviral strategies to limit the spread of viral infections.

7 Selective Labelling of Individual Viral Enzymes

Selective labelling of viral enzymes enables the measurement of their activity within a complex proteome. While viral enzymes can be labelled by broadly reactive probes such as fluorophosphonate or HAUb-VME, as discussed in the preceding sections, it can be difficult to detect them amongst the more numerous host cell enzymes which are labelled simultaneously (Blais et al. 2010a). For this reason, specific and selective probes have been developed against multiple viral enzymes.

The infectious particle of the influenza A virus (IAV), the most common cause of the flu, incorporates two viral enzymes on its surface, haemagglutinin (HA) and neuraminidase (NA) (Wohlbold and Krammer 2014). NA is known to play many roles; the most well-established being its sialidase activity which cleaves sialic acid from the nascent virions following virion secretion (Wohlbold and Krammer 2014). NA enzymatic activity has also been suggested to play a role in viral entry, though the mechanism by which this could occur has not been well established (Su et al. 2009; Wohlbold and Krammer 2014). The NA active site is highly conserved between strains of IAV strains, making NA an attractive target for chemical probes as well as therapeutic drugs (Wohlbold and Krammer 2014).

In 2005, Lu et al. reported the synthesis of a mechanism-based neuraminidase probe, consisting of a sialic acid targeting moiety and an *ortho*-difluoromethyl phenyl warhead attached to a biotin reporter tag (Lu et al. 2005). They demonstrated that this probe was able to specifically label neuraminidases and could be used to detect neuraminidase activity. Of particular interest in this study was a demonstration of a more uncommon application of activity-based probes for isolation of viral particles. Immobilised probe bound to NA enzymes present on the particle surface, thereby enabling the isolation of the entire virion (Lu et al. 2005). While this probe was able a covalently label IAV NA, there were several limitations. Its large size and multiple rings necessitated millimolar concentrations to label even purified protein. The probe was also unable to penetrate cell membranes, which precluded the application of this probe in complex systems. Lastly, though the sialic acid did convey some selectivity, the difluoromethyl phenyl warhead nevertheless reacted non-specifically with multiple targets in a complex environment (Tsai et al. 2013).

A more streamlined probe was reported by Tsai et al. Instead of the inclusion of a separate warhead group in addition to the sialic acid moiety, the sialic acid sugar

was modified to include fluorine at C2 and C3, displacing a hydroxyl group and a hydrogen, respectively. The electron-withdrawing property of the fluorines transforms the probe into an irreversible mechanism-based inhibitor. Two probes were made: DFSA and an ester-protected probe, PDFSA, both containing the sialic acid warhead and an alkyne handle (Fig. 3e) (Tsai et al. 2013). PDFSA is able to label NA in situ at micromolar concentrations, allowing imaging of sub-cellular localisation of the viral enzyme during infection (Tsai et al. 2013). PDFSA was able to label wild-type NA as well as mutant NA resistant to active-site antivirals, indicating that this probe could be applied to screen for the presence of drug-resistant strains by competitive ABPP (Tsai et al. 2013).

The Zika virus has recently been the subject of international attention following the South American outbreak which caused a dramatic increase in the rates of microencephaly in newborn infants (Lei et al. 2016). The Zika virus, like most positive-strand RNA viruses, encodes a serine protease, NS3, which cleaves the precursor polyprotein into the mature viral proteins. As activity of the NS3 protease is essential to the establishment of a productive infection, the development of tools capable of characterising its activity and regulation is of great interest.

Proteases are capable of displaying high selectivity for the specific amino acid sequences they cleave. While it is possible to design activity-based probes based on these sequences, these probes risk lacking specificity to individual proteases due to the overlapping substrate specificity of closely related enzymes. Recently, this problem was addressed by using unnatural amino acids to expand the chemical space of their peptides' building blocks. The Hybrid Combinatorial Substrate Library (HyCoSuL) uses 102 unnatural amino acids in addition to the twenty canonical ones to build optimal substrates containing a variable 4-amino acid sequence targeting the S1–S4 pockets of the protease active site (Kasperkiewicz et al. 2014). To allow specific activity-based labelling, a fluorogenic coumarin and a diphenyl phosphate warhead are added (Kasperkiewicz et al. 2014). This library has previously been used to design probes targeting human proteases (Kasperkiewicz et al. 2015; Poreba et al. 2016, 2018). Recently, the HyCoSuL methodology was applied to design a probe, WRPK3, which rapidly labels Zika's NS3 protease at low nanomolar concentrations (Fig. 3f) (Rut et al. 2017). This probe has the potential to be applied to interrogate NS3 activity during Zika infection. These studies demonstrate a useful application of unnatural amino acids which has the potential to be used to design activity-based probes against other viral proteases.

8 Future Objectives

The majority of the activity-based profiling reported in this chapter was performed either in vitro on proteomes isolated from animal models or cultured cells, or in situ on live cell monocultures. While this has been able to provide information on targets of viral mediation of host cell systems, the physiological relevance of this information has been subject to some doubt. Cell lysis and protein isolation

procedures used in in vitro labelling have the potential to destroy enzyme-cofactor interactions which may result in loss of information about the enzyme's true activity in vivo. Labelling cells in situ preserves the regulation of catalytic activity present in living cells; however, they do not always accurately represent the effects of infection on a live organism. As such, the development of probes which could be administered in vivo would provide the opportunity of characterise changes to enzyme activity in a more complex and physiologically relevant manner.

9 Conclusions

Over the past decade, activity-based protein profiling has proven to be a useful chemical proteomics tool for the interrogation of the role enzymes play in viral infection, providing information not otherwise accessible by traditional genomic and proteomic methods. ABPP has been used to quantify enzymatic activity, detect changes in the localisation of active enzymes, identify protein–protein interactions regulating activity and assign novel functionalities to proteins playing important roles in viral infection. When considered as a part of a larger body of research, they have greatly contributed to our understanding of viral infection and host response.

References

Balla T (2013) Phosphoinositides: tiny lipids with giant impact on cell regulation. Physiol Rev 93:1019–1137

Barber GN (2001) Host defense, viruses and apoptosis. Cell Death Differ 8:113–126. https://doi.org/10.1038/sj.cdd.4400823

Barglow KT, Cravatt BF (2007) Activity-based protein profiling for the functional annotation of enzymes. Nat Methods 4:822–827. https://doi.org/10.1038/nmeth1092

Beilstein F, Lemasson M, Pène V et al (2017) Lysophosphatidylcholine acyltransferase 1 is downregulated by hepatitis C virus: impact on production of lipo-viro-particles. Gut 66:2160–2169. https://doi.org/10.1136/gutjnl-2016-311508

Békés M, van der Heden van Noort GJ, Ekkebus R et al (2016) Recognition of Lys48-Linked Di-ubiquitin and Deubiquitinating Activities of the SARS Coronavirus Papain-like Protease. Mol Cell 62:572–585. https://doi.org/10.1016/j.molcel.2016.04.016

Benes P, Vetvicka V, Fusek M (2008) Cathepsin D—many functions of one aspartic protease. Crit Rev Oncol Hematol 68:12–28. https://doi.org/10.1016/j.critrevonc.2008.02.008

Bishé B, Syed G, Siddiqui A (2012) Phosphoinositides in the hepatitis C virus life cycle. Viruses 4:2340–2358. https://doi.org/10.3390/v4102340

Blais DR, Brûlotte M, Qian Y et al (2010a) Activity-based proteome profiling of hepatoma cells during hepatitis C virus replication using protease substrate probes. J Proteome Res 9:912–923. https://doi.org/10.1021/pr900788a

Blais DR, Lyn RK, Joyce MA et al (2010b) Activity-based protein profiling identifies a host enzyme, carboxylesterase 1, which is differentially active during hepatitis C virus replication. J Biol Chem 285:25602–25612. https://doi.org/10.1074/jbc.M110.135483

Borodovsky A, Ovaa H, Kolli N et al (2002) Chemistry-based functional proteomics reveals novel members of the deubiquitinating enzyme family. Chem Biol 9:1149–1159

Bosch BJ, Bartelink W, Rottier PJM (2008) Cathepsin L functionally cleaves the severe acute respiratory syndrome coronavirus class I fusion protein upstream of rather than adjacent to the fusion peptide. J Virol 82:8887–8890. https://doi.org/10.1128/JVI.00415-08

Burster T, Macmillan H, Hou T et al (2010) Cathepsin G: roles in antigen presentation and beyond. Mol Immunol 47:658–665. https://doi.org/10.1016/j.molimm.2009.10.003

Chandran K, Sullivan NJ, Felbor U et al (2005) Endosomal proteolysis of the Ebola virus glycoprotein is necessary for infection. Science 308:1643–1645. https://doi.org/10.1126/science.1110656

Coux O, Tanaka K, Goldberg AL (1996) Structure and functions of the 20S and 26S proteasomes. Annu Rev Biochem 65:801–847. https://doi.org/10.1146/annurev.bi.65.070196.004101

Cravatt BF, Wright AT, Kozarich JW (2008) Activity-based protein profiling: from enzyme chemistry to proteomic chemistry. Annu Rev Biochem 77:383–414. https://doi.org/10.1146/annurev.biochem.75.101304.124125

Cruz-Muñoz ME, Fuentes-Pananá EM (2017) Beta and gamma human herpesviruses: agonistic and antagonistic interactions with the host immune system. Front Microbiol 8:2521. https://doi.org/10.3389/fmicb.2017.02521

de Castro S, Camarasa M-J (2018) Polypharmacology in HIV inhibition: can a drug with simultaneous action against two relevant targets be an alternative to combination therapy? Eur J Med Chem 150:206–227. https://doi.org/10.1016/J.EJMECH.2018.03.007

De Clercq E, Li G (2016) Approved antiviral drugs over the past 50 years. Clin Microbiol Rev 29:695–747. https://doi.org/10.1128/CMR.00102-15

de Graaf N, van Helden MJG, Textoris-Taube K et al (2011) PA28 and the proteasome immunosubunits play a central and independent role in the production of MHC class I-binding peptides in vivo. Eur J Immunol 41:926–935. https://doi.org/10.1002/eji.201041040

Delang L, Paeshuyse J, Neyts J (2012) The role of phosphatidylinositol 4-kinases and phosphatidylinositol 4-phosphate during viral replication. Biochem Pharmacol 84:1400–1408. https://doi.org/10.1016/j.bcp.2012.07.034

Desrochers GF, Cornacchia C, McKay CS, Pezacki JP (2018) Activity-based phosphatidylinositol kinase probes detect changes to protein–protein interactions during hepatitis C virus replication. ACS Infect Dis. https://doi.org/10.1021/acsinfecdis.8b00047

Desrochers GF, Sherratt AR, Blais DR et al (2015) Profiling kinase activity during hepatitis C virus replication using a wortmannin probe. ACS Infect Dis 1:443–452. https://doi.org/10.1021/acsinfecdis.5b00083

Ferrington DA, Gregerson DS (2012) Immunoproteasomes. In: Progress in molecular biology and translational science, pp 75–112

Furman LM, Maaty WS, Petersen LK et al (2009) Cysteine protease activation and apoptosis in Murine norovirus infection. Virol J 6:139. https://doi.org/10.1186/1743-422X-6-139

Goodwin CM, Xu S, Munger J (2015) Stealing the keys to the kitchen: viral manipulation of the host cell metabolic network. Trends Microbiol 23:789–798. https://doi.org/10.1016/J.TIM.2015.08.007

Gredmark S, Schlieker C, Quesada V et al (2007) A functional ubiquitin-specific protease embedded in the large tegument protein (ORF64) of murine gammaherpesvirus 68 is active during the course of infection. J Virol 81:10300–10309. https://doi.org/10.1128/JVI.01149-07

Greenbaum D, Medzihradszky KF, Burlingame A, Bogyo M (2000) Epoxide electrophiles as activity-dependent cysteine protease profiling and discovery tools. Chem Biol 7:569–581

Harak C, Lohmann V (2015) Ultrastructure of the replication sites of positive-strand RNA viruses. Virology 479–480:418–433. https://doi.org/10.1016/J.VIROL.2015.02.029

Harhaj EW, Dixit VM (2011) Deubiquitinases in the regulation of NF-κB signaling. Cell Res 21:22–39. https://doi.org/10.1038/cr.2010.166

Hausser A, Link G, Hoene M et al (2006) Phospho-specific binding of 14-3-3 proteins to phosphatidylinositol 4-kinase III β protects from dephosphorylation and stabilizes lipid kinase activity. J Cell Sci 119:3613–3621

Hausser A, Storz P, Märtens S et al (2005) Protein kinase D regulates vesicular transport by phosphorylating and activating phosphatidylinositol-4 kinase III β at the Golgi complex. Nat Cell Biol 7:880–887. https://doi.org/10.1038/ncb1289

Hogue IB, Bosse JB, Hu J-R et al (2014) Cellular mechanisms of alpha herpesvirus egress: live cell fluorescence microscopy of pseudorabies virus exocytosis. PLoS Pathog 10:e1004535. https://doi.org/10.1371/journal.ppat.1004535

Hsu N-Y, Ilnytska O, Belov G et al (2010) Viral reorganization of the secretory pathway generates distinct organelles for RNA replication. Cell 141:799–811. https://doi.org/10.1016/j.cell.2010.03.050

Hutagalung AH, Novick PJ (2011) Role of Rab GTPases in membrane traffic and cell physiology. Physiol Rev 91:119–149. https://doi.org/10.1152/physrev.00059.2009

Jarosinski K, Kattenhorn L, Kaufer B et al (2007) A herpesvirus ubiquitin-specific protease is critical for efficient T cell lymphoma formation. Proc Natl Acad Sci 104:20025–20030. https://doi.org/10.1073/pnas.0706295104

Kammerl IE, Meiners S (2016) Proteasome function shapes innate and adaptive immune responses. Am J Physiol Lung Cell Mol Physiol 311:L328–L336. https://doi.org/10.1152/ajplung.00156.2016

Kapadia SB, Chisari FV (2005) Hepatitis C virus RNA replication is regulated by host geranylgeranylation and fatty acids. Proc Natl Acad Sci U S A 102:2561–2566. https://doi.org/10.1073/pnas.0409834102

Kasperkiewicz P, Poreba M, Snipas SJ et al (2014) Design of ultrasensitive probes for human neutrophil elastase through hybrid combinatorial substrate library profiling. Proc Natl Acad Sci 111:2518–2523. https://doi.org/10.1073/pnas.1318548111

Kasperkiewicz P, Poreba M, Snipas SJ et al (2015) Design of a selective substrate and activity based probe for human neutrophil serine protease 4. PLoS ONE 10:e0132818. https://doi.org/10.1371/journal.pone.0132818

Kattenhorn LM, Korbel GA, Kessler BM et al (2005) A deubiquitinating enzyme encoded by HSV-1 belongs to a family of cysteine proteases that is conserved across the family herpesviridae. Mol Cell 19:547–557. https://doi.org/10.1016/j.molcel.2005.07.003

Keller IE, Vosyka O, Takenaka S et al (2015) Regulation of immunoproteasome function in the lung. Sci Rep 5:10230. https://doi.org/10.1038/srep10230

Lei J, Hansen G, Nitsche C et al (2016) Crystal structure of Zika virus NS2B-NS3 protease in complex with a boronate inhibitor. Science 353:503–505. https://doi.org/10.1126/science.aag2419

Li N, Kuo C-L, Paniagua G et al (2013) Relative quantification of proteasome activity by activity-based protein profiling and LC-MS/MS. Nat Protoc 8:1155–1168. https://doi.org/10.1038/nprot.2013.065

Liang TJ (2009) Hepatitis B: the virus and disease. Hepatology 49:S13–S21. https://doi.org/10.1002/hep.22881

Lohmann V, Bartenschlager R (2014) On the history of hepatitis C virus cell culture systems. J Med Chem 57:1627–1642. https://doi.org/10.1021/jm401401n

Lu C-P, Ren C-T, Lai Y-N et al (2005) Design of a mechanism-based probe for neuraminidase to capture influenza viruses. Angew Chemie Int Ed 44:6888–6892. https://doi.org/10.1002/anie.200501738

Macdonald SJ, Mostafa HH, Morrison LA, Davido DJ (2012) Genome sequence of herpes simplex virus 1 strain KOS. J Virol 86:6371–6372. https://doi.org/10.1128/JVI.00646-12

Marques AJ, Palanimurugan R, Matias AC et al (2009) Catalytic mechanism and assembly of the proteasome. Chem Rev 109:1509–1536. https://doi.org/10.1021/cr8004857

Mazein A, Watterson S, Hsieh W-Y et al (2013) A comprehensive machine-readable view of the mammalian cholesterol biosynthesis pathway. Biochem Pharmacol 86:56–66. https://doi.org/10.1016/J.BCP.2013.03.021

Mazumdar T, Gorgun FM, Sha Y et al (2010) Regulation of NF-kappaB activity and inducible nitric oxide synthase by regulatory particle non-ATPase subunit 13 (Rpn13). Proc Natl Acad Sci U S A 107:13854–13859. https://doi.org/10.1073/pnas.0913495107

McMahon HT, Gallop JL (2005) Membrane curvature and mechanisms of dynamic cell membrane remodelling. Nature 438:590–596. https://doi.org/10.1038/nature04396

Mulder MPC, El Oualid F, ter Beek J, Ovaa H (2014) A native chemical ligation handle that enables the synthesis of advanced activity-based probes: diubiquitin as a case study. ChemBioChem 15:946–949. https://doi.org/10.1002/cbic.201402012

Nanduri B, Suvarnapunya AE, Venkatesan M, Edelmann MJ (2013) Deubiquitinating enzymes as promising drug targets for infectious diseases. Curr Pharm Des 19:3234–3247

Nasheri N, Joyce M, Rouleau Y et al (2013) Modulation of fatty acid synthase enzyme activity and expression during hepatitis C virus replication. Chem Biol 20:570–582. https://doi.org/10.1016/j.chembiol.2013.03.014

Nasheri N, McKay CS, Fulton K et al (2014) Hydrophobic triaryl-substituted β-lactams as activity-based probes for profiling eukaryotic enzymes and host-pathogen interactions. ChemBioChem 15:2195–2200. https://doi.org/10.1002/cbic.201402097

Nasheri N, Ning Z, Figeys D et al (2015) Activity-based profiling of the proteasome pathway during hepatitis C virus infection. Proteomics 15:3815–3825. https://doi.org/10.1002/pmic.201500169

Neufeldt CJ, Cortese M, Acosta EG, Bartenschlager R (2018) Rewiring cellular networks by members of the flaviviridae family. Nat Rev Microbiol 16:125–142. https://doi.org/10.1038/nrmicro.2017.170

Nourbakhsh M, Douglas DN, Pu CH et al (2013) Arylacetamide deacetylase: a novel host factor with important roles in the lipolysis of cellular triacylglycerol stores, VLDL assembly and HCV production. J Hepatol 59:336–343. https://doi.org/10.1016/j.jhep.2013.03.022

Novoa RR, Calderita G, Arranz R et al (2005) Virus factories: associations of cell organelles for viral replication and morphogenesis. Biol Cell 97:147–172. https://doi.org/10.1042/BC20040058

Oeckinghaus A, Ghosh S (2009) The NF-κB family of transcription factors and its regulation. Cold Spring Harb Perspect Biol 1:a000034–a000034. https://doi.org/10.1101/cshperspect.a000034

Ovaa H, Kessler BM, Rolen U et al (2004) Activity-based ubiquitin-specific protease (USP) profiling of virus-infected and malignant human cells. Proc Natl Acad Sci 101:2253–2258. https://doi.org/10.1073/pnas.0308411100

Paul D, Bartenschlager R (2015) Flaviviridae replication organelles: oh, what a tangled web we weave. Annu Rev Virol 2:289–310. https://doi.org/10.1146/annurev-virology-100114-055007

Pohl MO, Lanz C, Stertz S (2016) Late stages of the influenza a virus replication cycle—a tight interplay between virus and host. J Gen Virol 97:2058–2072. https://doi.org/10.1099/jgv.0.000562

Poreba M, Rut W, Vizovisek M et al (2018) Selective imaging of cathepsin L in breast cancer by fluorescent activity-based probes. Chem Sci 9:2113–2129. https://doi.org/10.1039/C7SC04303A

Poreba M, Solberg R, Rut W et al (2016) Counter selection substrate library strategy for developing specific protease substrates and probes. Cell Chem Biol 23:1023–1035. https://doi.org/10.1016/j.chembiol.2016.05.020

Qian X-J, Zhu Y-Z, Zhao P, Qi Z-T (2016) Entry inhibitors: new advances in HCV treatment. Emerg Microbes Infect 5:e3. https://doi.org/10.1038/emi.2016.3

Ross MK, Streit TM, Herring KL (2010) Carboxylesterases: dual roles in lipid and pesticide metabolism. J Pestic Sci 35:257–264. https://doi.org/10.1584/jpestics.R10-07

Rut W, Zhang L, Kasperkiewicz P et al (2017) Extended substrate specificity and first potent irreversible inhibitor/activity-based probe design for Zika virus NS2B-NS3 protease. Antiviral Res 139:88–94. https://doi.org/10.1016/j.antiviral.2016.12.018

Saito S, Murata T, Kanda T et al (2013) Epstein-barr virus deubiquitinase downregulates TRAF6-mediated NF-κB signaling during productive replication. J Virol 87:4060–4070. https://doi.org/10.1128/JVI.02020-12

Schlieker C, Korbel GA, Kattenhorn LM, Ploegh HL (2005) A deubiquitinating activity is conserved in the large tegument protein of the herpesviridae. J Virol 79:15582–15585. https://doi.org/10.1128/JVI.79.24.15582-15585.2005

Schuster B, Hendry L, Byers H et al (2007) Purification and identification of the STAT5 protease in myeloid cells. Biochem J 404:81–87. https://doi.org/10.1042/BJ20061877

Shah PP, Wang T, Kaletsky RL et al (2010) A small-molecule oxocarbazate inhibitor of human cathepsin L blocks severe acute respiratory syndrome and ebola pseudotype virus infection into human embryonic kidney 293T cells. Mol Pharmacol 78:319–324. https://doi.org/10.1124/mol.110.064261

Shahiduzzaman M, Ezatti P, Xin G, Coombs KM (2014) Proteasomal serine hydrolases are up-regulated by and required for influenza virus infection. J Proteome Res 13:2223–2238. https://doi.org/10.1021/pr5001779

Sherratt AR, Nasheri N, McKay CS et al (2014) A new chemical probe for phosphatidylinositol kinase activity. ChemBioChem 15:1253–1256. https://doi.org/10.1002/cbic.201402155

Singaravelu R, Blais DR, McKay CS, Pezacki J (2010) Activity-based protein profiling of the hepatitis C virus replication in Huh-7 hepatoma cells using a non-directed active site probe. Proteome Sci 8:5. https://doi.org/10.1186/1477-5956-8-5

Su AI, Pezacki JP, Wodicka L et al (2002) Nonlinear partial differential equations and applications: genomic analysis of the host response to hepatitis C virus infection. Proc Natl Acad Sci 99:15669–15674. https://doi.org/10.1073/pnas.202608199

Su B, Wurtzer S, Rameix-Welti M-A et al (2009) Enhancement of the influenza A hemagglutinin (HA)-mediated cell-cell fusion and virus entry by the viral neuraminidase (NA). PLoS ONE 4:e8495. https://doi.org/10.1371/journal.pone.0008495

Sun D, Zhu L, Yao D et al (2018) Recent progress in potential anti-hepatitis B virus agents: structural and pharmacological perspectives. Eur J Med Chem 147:205–217. https://doi.org/10.1016/j.ejmech.2018.02.001

Syed GH, Amako Y, Siddiqui A (2010) Hepatitis C virus hijacks host lipid metabolism. Trends Endocrinol Metab 21:33–40. https://doi.org/10.1016/j.tem.2009.07.005

Syed GH, Khan M, Yang S, Siddiqui A (2017) Hepatitis C virus lipoviroparticles assemble in the endoplasmic reticulum (ER) and bud off from the ER to the golgi compartment in COPII vesicles. J Virol 91:e00499–17. https://doi.org/10.1128/JVI.00499-17

Takacs CN, Andreo U, Dao Thi VL et al (2017) Differential regulation of lipoprotein and hepatitis C virus secretion by Rab1b. Cell Rep 21:431–441. https://doi.org/10.1016/J.CELREP.2017.09.053

Tsai C-S, Yen H-Y, Lin M-I et al (2013) Cell-permeable probe for identification and imaging of sialidases. Proc Natl Acad Sci U S A 110:2466–2471. https://doi.org/10.1073/pnas.1222183110

van Gent M, Braem SGE, de Jong A et al (2014) Epstein-barr virus large tegument protein BPLF1 contributes to innate immune evasion through interference with toll-like receptor signaling. PLoS Pathog 10:e1003960. https://doi.org/10.1371/journal.ppat.1003960

Verdoes M, Florea BI, Menendez-Benito V et al (2006) A fluorescent broad-spectrum proteasome inhibitor for labeling proteasomes in vitro and in vivo. Chem Biol 13:1217–1226. https://doi.org/10.1016/J.CHEMBIOL.2006.09.013

Vetter ML, Rodgers MA, Patricelli MP, Yang PL (2012) chemoproteomic profiling identifies changes in DNA-PK as markers of early dengue virus infection. ACS Chem Biol 7:2019–2026. https://doi.org/10.1021/cb300420z

Vieyres G, Welsch K, Gerold G et al (2016) ABHD5/CGI-58, the chanarin-dorfman syndrome protein, mobilises lipid stores for hepatitis C virus production. PLoS Pathog 12:e1005568. https://doi.org/10.1371/journal.ppat.1005568

Wang J, Loveland AN, Kattenhorn LM et al (2006) High-molecular-weight protein (pUL48) of human cytomegalovirus is a competent deubiquitinating protease: mutant viruses altered in its active-site cysteine or histidine are viable. J Virol 80:6003–6012. https://doi.org/10.1128/JVI.00401-06

Wertz IE, Dixit VM (2010) Signaling to NF-κB: regulation by ubiquitination. Cold Spring Harb Perspect Biol 2:a003350–a003350. https://doi.org/10.1101/cshperspect.a003350

Wohlbold T, Krammer F (2014) In the shadow of hemagglutinin: a growing interest in influenza viral neuraminidase and its role as a vaccine antigen. Viruses 6:2465–2494. https://doi.org/10.3390/v6062465

Xiao F, Fofana I, Thumann C et al (2015) Synergy of entry inhibitors with direct-acting antivirals uncovers novel combinations for prevention and treatment of hepatitis C. Gut 64:483–494. https://doi.org/10.1136/gutjnl-2013-306155

Yang P-Y, Liu K, Ngai MH et al (2010) Activity-based proteome profiling of potential cellular targets of orlistat—an FDA-approved drug with anti-tumor activities. J Am Chem Soc 132:656–666. https://doi.org/10.1021/ja907716f

Ye R, Su C, Xu H, Zheng C (2017) Herpes simplex virus 1 ubiquitin-specific protease UL36 abrogates NF-κB activation in DNA sensing signal pathway. J Virol 91:e02417–16. https://doi.org/10.1128/JVI.02417-16

Zhang L, Hong Z, Lin W et al (2012) ARF1 and GBF1 generate a PI4P-enriched environment supportive of hepatitis C virus replication. PLoS ONE 7:e32135. https://doi.org/10.1371/journal.pone.0032135

Zhao B, Song J, St. Clair RW, Ghosh S (2007) Stable overexpression of human macrophage cholesteryl ester hydrolase results in enhanced free cholesterol efflux from human THP1 macrophages. Am J Physiol Physiol 292:C405–C412. https://doi.org/10.1152/ajpcell.00306.2006

Zou F, Schmon M, Sienczyk M et al (2012) Application of a novel highly sensitive activity-based probe for detection of cathepsin G. Anal Biochem 421:667–672. https://doi.org/10.1016/j.ab.2011.11.016