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Review

# Retroviral proteases and their roles in virion maturation

Jan Konvalinka [a,b], Hans-Georg Kräusslich [c,d,*], Barbara Müller [c,d]

[a] Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Gilead Sciences and IOCB Research Center, Flemingovo n. 2, 166 10 Prague 6, Czech Republic
[b] Department of Biochemistry, Faculty of Science, Charles University in Prague, Hlavova 8, 128 43 Prague 2, Czech Republic
[c] Department of Infectious Diseases, Virology, University Hospital Heidelberg, Im Neuenheimer Feld 324, 69120 Heidelberg, Germany
[d] Molecular Medicine Partnership Unit, Heidelberg, Germany

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ABSTRACT

Proteolytic processing of viral polyproteins is essential for retrovirus infectivity. Retroviral proteases (PR) become activated during or after assembly of the immature, non-infectious virion. They cleave viral polyproteins at specific sites, inducing major structural rearrangements termed maturation. Maturation converts retroviral enzymes into their functional form, transforms the immature shell into a metastable state primed for early replication events, and enhances viral entry competence. Not only cleavage at all PR recognition sites, but also an ordered sequence of cleavages is crucial. Proteolysis is tightly regulated, but the triggering mechanisms and kinetics and pathway of morphological transitions remain enigmatic.

Here, we outline PR structures and substrate specificities focusing on HIV PR as a therapeutic target. We discuss design and clinical success of HIV PR inhibitors, as well as resistance development towards these drugs. Finally, we summarize data elucidating the role of proteolysis in maturation and highlight unsolved questions regarding retroviral maturation.



**Contents**



## Introduction: proteases are essential retroviral enzymes

With genome sizes in the range of 10 kilobases, retroviruses have to rely on strategies of genetic economy in order to encode all proteins required for particle formation, replication and spread.

* Corresponding author at: Department of Infectious Diseases, Virology, University Hospital Heidelberg, Im Neuenheimer Feld 324, 69120 Heidelberg, Germany. Tel.: +49 6221 565001.
*E-mail address:* hans-georg.kraeusslich@med.uni-heidelberg.de (H.-G. Kräusslich).

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A possible way to solve this problem, employed by many different virus groups, is the expression of polyproteins that are subsequently proteolytically processed into functional subunits. Beyond saving space on the genome by using a single set of transcriptional and translational control elements, this approach provides the option to alter the functionality of a particular protein in a controlled manner. Furthermore, it ensures the production of multiple proteins in defined stoichiometry and their targeting to specific sites using a single targeting signal. Retroviruses make use of this strategy by translating their major structural proteins and the viral replication enzymes as part of the group specific antigen (Gag) or polymerase (Pol) polyprotein entity, respectively. Together with the envelope (*env*) open reading frame (ORF), *gag* and *pol* constitute hallmark ORF of all retroviruses (Vogt, 1997).

In a retrovirus-producing cell, viral components assemble into particles in a process mainly orchestrated by the Gag polyprotein, which forms spherical or semi-spherical shells encasing the viral genome. The resulting immature particles are non-infectious, however. Proteolysis of the polyproteins, accompanied by drastic rearrangement of virus architecture, is essential to convert these immature virions into mature, infectious virus (Swanstrom and Wills, 1997). Early biochemical studies on murine and avian retroviruses provided evidence for the synthesis and processing of viral polyproteins (Vogt and Eisenman, 1973; Vogt et al., 1975) and revealed that the corresponding protease is encoded by the virus itself (Dittmar and Moelling, 1978; Vogt et al., 1979; von der Helm, 1977; Yoshinaka and Luftig, 1977). Today, we know that the virus encoded protease (PR) represents an essential enzyme of all retroviruses.

PR itself is encoded as part of a polyprotein whose production often requires a ribosomal frameshift event or read-through of a stop codon. The exact arrangement of the *gag*, *pro* and *pol* ORFs varies between different retroviruses ((Vogt, 1997); Fig. 1). Depending on genome structure, PR is encoded either as part of *gag* (e.g. Rous sarcoma virus (RSV), a member of the avian sarcoma and leucosis viruses, ASLV), as part of *pol* (e.g. human immunodeficiency virus, HIV-1; murine leukemia virus, MLV), or as a separate ORF (e.g. Mason-Pfizer monkey virus, M-PMV). As a consequence, PR is either synthesized in equimolar amounts to Gag, in equimolar amounts to reverse transcriptase (RT) and integrase (IN), or in lower amounts than the structural proteins, but higher than the other enzymes.

Retroviral proteases (correctly denominated"retropepsins" (Barrett et al., 2012)) and their inhibition have been intensively studied for several decades, and specific aspects are covered in several excellent recent reviews, some of which are cited below. We refer the reader to these reviews for further information and sincerely apologize to all colleagues whose excellent work could not be discussed and cited here due to space limitations.

## Structure and function of retroviral proteases

Retropepsins undoubtedly represent the most thoroughly studied proteolytic enzymes in the history of science (Barrett et al., 2012). The last three decades have witnessed an amazing concentration of resources and intellectual potential on the study of these comparatively simple hydrolytic enzymes, leading to an unprecedented accumulation of information regarding their structure, enzymology and inhibition. These efforts have been mostly fueled by the need to develop effective inhibitors of HIV-1 PR as antiviral drugs. Accordingly, HIV-1 PR is by far the most studied of all retropepsins. Other enzymes that have been studied in some detail include the PR of RSV, the prototype alpharetrovirus, of MLV, the prototype gammaretrovirus, and the PRs of HIV-2 and human T-cell leukemia virus (HTLV) due to the pathogenicity of the corresponding viruses. In addition, M-PMV PR has been studied primarily because of its unusual expression (as a separate ORF between *gag* and *pol*) and because of the unique maturation pathway of this betaretrovirus (see below). According to its dominance in the scientific literature, we will focus our review mostly on the description of the structure, substrate specificity and mechanism of action of HIV-1 PR and will discuss specific and divergent features of other retropepsins.

Retropepsins are relatively small aspartic proteases comprised of two identical monomers of 10–15 kDa each (Fig. 2). As other enzymes of this class, they display optimal activity on peptide substrates in vitro under acidic conditions and are poorly active on model peptides at neutral or higher pH (e.g. Billich et al., 1988; Darke et al., 1989; Hyland et al., 1991). Three amino acids in the active site (Asp, Thr/Ser and Gly) form a catalytic triad common to all aspartic proteases. While the cellular aspartic proteases such as pepsin or cathepsin D carry two catalytic aspartates within a single

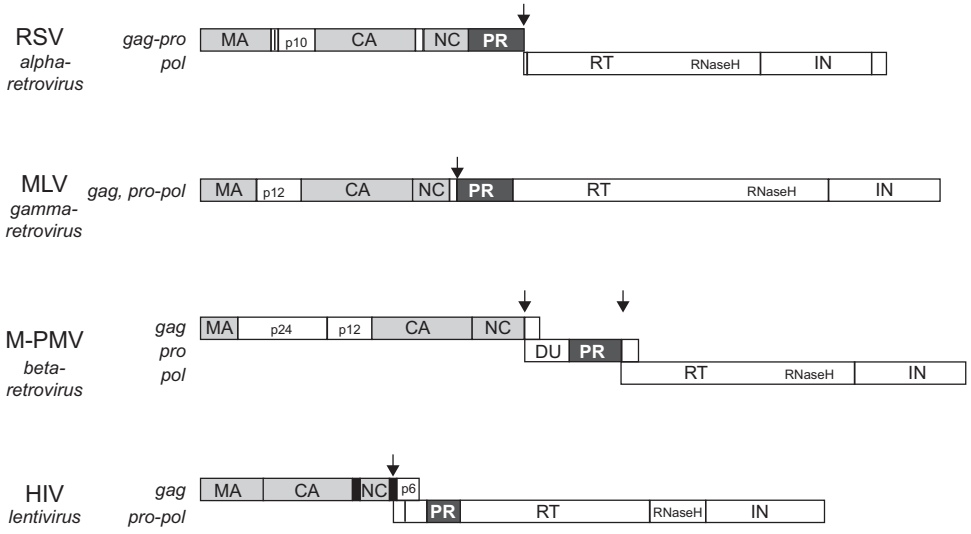


**Fig. 1.** PR is expressed as part of retroviral polyproteins. The schemes show the genomic localization of the PR encoding region (dark gray box) in different exemplary retroviruses. Vertical lines represent cleavage sites of the respective PR; arrows indicate sites of translational read-through (MLV) or frameshift (other viruses) resulting in expression of Gag–Pro or Gag–Pro–Pol polyproteins, respectively. Canonical Gag domains (MA, CA, NC) are colored light gray. Spacer peptide 1 (SP1) and 2 (SP2) (black boxes) are localized between the CA and NC domains and between the NC and p6 domains of HIV-1 Gag, respectively. RSV, Rous sarcoma virus, MLV, murine leukemia virus; M-PMV, Mason-Pfizer monkey virus; HIV-1, human immunodeficiency virus. DU, dUTPase

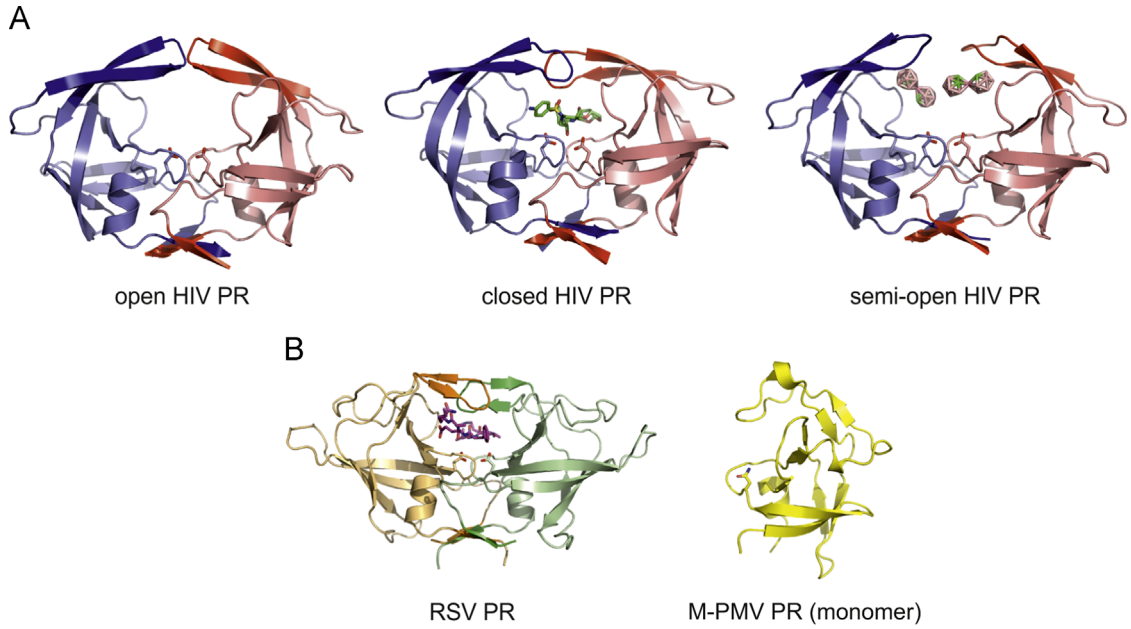


**Fig. 2.** Three-dimensional structures of selected retroviral PRs. (A) Crystal structures of HIV-1 PR in three conformations: the apo-form of the HIV-1 PR with flaps in open conformation (PDB code 3PHV (Lapatto et al., 1989)), HIV-1 PR with flaps in closed conformation (PDB code 4LL3 (Kozisek et al., 2014)) with inhibitor (DRV) bound in the enzyme active site shown in sticks (with carbon atoms colored green, oxygen atoms red, nitrogens blue and sulfur yellow), HIV-1 PR with flaps in semi-open conformation (PDB code 1ZTZ (Cigler et al., 2005). Inhibitor bound in the enzyme active site (metallacarborane) is shown in sticks (with carbon atoms colored yellow, boron atoms cyan, and cobalt atom maroon). In all panels, the flaps (residues 43-58) and dimerization zone (residues 1–4 and 96–99) are highlighted in darker color and active site aspartates are highlighted in sticks. The figure was generated using program PyMol (*www.pymol.org*). **(B)** Structures of other retroviral PRs: RSV PR with bound inhibitor (PDB code 1BAI), monomeric M-PMV PR (PDB code 3SQF (Khatib et al., 2011)). Figure courtesy of Pavlína Řezáčová.

polypeptide, one catalytic aspartate is provided by each monomeric subunit in the case of retropepsins (Pearl and Blundell, 1984). In the vicinity of the active site, a hydrogen bond network called the "fireman's grip" (Pearl and Blundell, 1984) supports the relative rigidity of the core of these enzymes. A glycine rich "flap" region forms a dynamic "roof" covering the active cleft of all retropepsins and plays an important role in substrate recognition (Fig. 2A). This flexible region consisting of two β-hairpins undergoes significant conformational alterations, changing its topology between the open, semi-open and closed conformation (Fig. 2A), depending on the binding of a substrate or inhibitor. PR dimerization is mainly mediated by its N- and C-termini (residues 1–4 and 96–99 in the case of HIV-1 PR) intertwining into a four-stranded antiparallel β-sheet (Fig. 2). The requirement for dimerization to achieve catalytic competence is an important regulatory feature contributing to the tight control of proteolytic processing in the viral replication cycle (see below).

The monomer of RSV PR (124 amino acids; Fig. 2B) is larger than that of HIV-1, mostly because of two larger surface loops including the "flap". These loops are highly dynamic and not well defined in most X-ray structures of RSV PR. The presence of Asp–Ser–Gly instead of Asp–Thr–Gly in the active site triplet has been suggested to lead to a looser folding of the "fireman's grip" structure and thus to lower dimer stability of RSV PR (Konvalinka et al., 1995). This lower dimer stability translates into lower enzymatic activity of RSV PR compared to HIV-1 PR, which may compensate for the higher PR production since the enzyme is produced as a component of the Gag polyprotein in this case (Fig. 1). Exchanging Ser to Thr in the active site of RSV PR resulted in increased specific activity of the enzyme and aberrant virion morphology (Konvalinka et al., 1992; Sedlacek et al., 1993).

M-PMV PR (Fig. 2B) differs in several aspects from the PR of other retroviruses. It was shown to exist in three forms, differing in the length of the C-terminus (17 kDa, 13 kDa and 12 kDa), with both 17 and 13 kDa forms found in viral particles (Pichova, 2013). The enzyme crystallizes as a monomer rather than in its active, dimeric form, impeding refinement of its structure by molecular replacement using either models or known NMR structures (Veverka et al., 2001). Finally, the structure of the monomer was solved with the help of players of a multi-level computer game "Foldit" (Khatib et al., 2011), who generated models useful for the solution of a crystal structure. Interestingly, the M-PMV PR structure lacks the dominant interfacial beta-sheet that stabilizes the dimers of other retropepsins (Fig. 2B, right).

The substrate specificity of retropepsins is complex. Sequencing of mature retroviral proteins provided initial information on the substrate specificity of the respective PR. Much of the early information has been obtained by the diligent work of Oroszlan and co-workers (reviewed e.g. in (Oroszlan and Luftig, 1990)). Subsequent experiments involving cleavage of peptide libraries and panels of synthetic peptides by retroviral PRs and the identification of cellular protein substrates of these enzymes provided a refined picture of the substrate specificity of these enzymes, again revealing a relaxed specificity (e.g. Cameron et al., 1992; Grinde et al., 1992; Konvalinka et al., 1990; Kotler et al., 1988). This result was consistent with early studies indicating promiscuous cleavage of denatured protein substrates (Dittmar and Moelling, 1978), while the respective folded protein was much more resistant, indicating a contribution of structural accessibility to PR specificity.

A comprehensive picture of the substrate specificity of proteolytic enzymes can be provided by proteomic identification of cleavage sites (PICS). This method, introduced by Overall and co-workers, enables the simultaneous characterization of the cleavage sites of an endopeptidase by LC-MS analysis of the newly created N-termini in trypsin-treated cell lysates offered as substrates (Schilling et al., 2011). Fig. 3 shows results for HIV-1 PR using a peptide library comprised of 1156 hydrolyzed peptides from the HEK293T proteome (Tykvart et al., 2015). The figure shows favored residues in the region from P5 to P5′ represented as a heat map (Fig. 3A) or sequence logo representation (Fig. 3B). The major substrate specificity signature features of HIV PR involve (i) a preference for Glu in the P2′ position; (ii) a certain preference for large aliphatic and

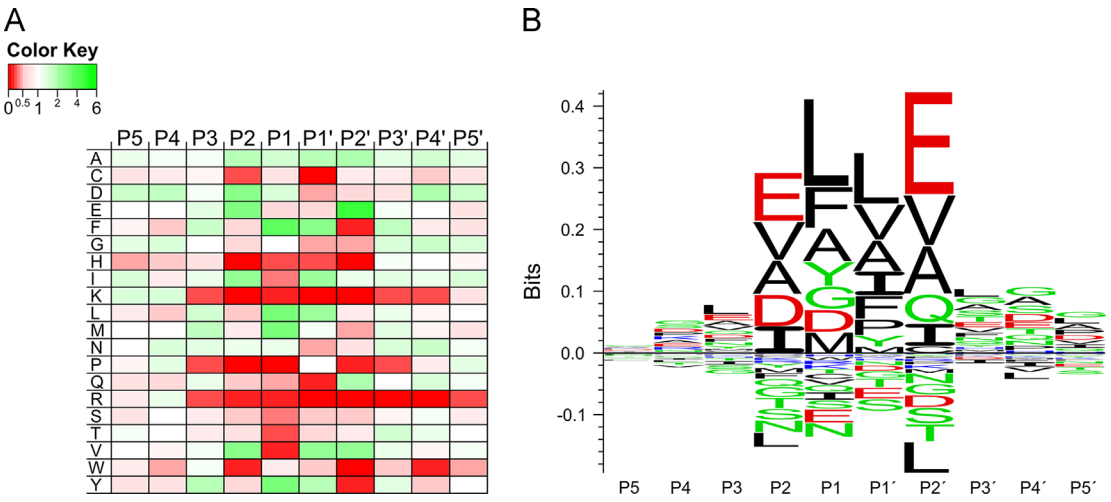


**Fig. 3.** Heat map (A) and Kullback–Leibler sequence logo representation (B) of substrate specificity of HIV PR obtained from a PICS experiment (Tykvart et al., 2015). A tryptic library from HEK293T cell lysates was cleaved by recombinant HIV PR. As a control, the same tryptic library was treated by a catalytically inactive mutant of HIV PR (D25N). (A) The heat-map illustrates ratios of individual amino acid occurrence at given position compared to overall probability of given amino acid occurrence in the human proteome. Ratios above 1 (preferred) are shown in green while ratios below 1 (rejected) are shown in red with shading intensity corresponding to the ratio. (B) A Kullback–Leiber sequence logo was generated from cleaved sequences using the Seq2Logo tool (Thomsen and Nielsen, 2012). Amino acids enriched at a given position are shown on the positive *y*-axis and depleted amino acids on the negative *y*-axis. Height of each letter is proportional to its probability of occurrence at the given position. Amino acids are color coded to highlight acidic (red), basic (blue) and hydrophobic (black) residues. Five positions each on both sides of the cleavage site (P5′–P5) are shown. Figure courtesy of Michal Svoboda.

aromatic residues in the P1 and P1′ positions; and (iii) the rather unusual preference for Pro in the P1′ position. Beta-branched amino acids are largely excluded from the P1 position, and this has been used to generate inactivating cleavage site mutations in various positions of Gag (e.g. Coren et al., 2007; Pettit et al., 2002, 1991; Wiegers est al., 1998).

Numerous other analyses on the substrate specificity of HIV-1 and other retroviral PRs revealed a similar picture: there is no clear pattern in the primary sequence explaining the molecular recognition of the processing sites or peptide substrates, but a certain preference for aromatic or large aliphatic amino acid residues in P1–P1′, for Pro in P1′ and for large and hydrophobic amino acids in the P2 and P2′ subsites has been generally observed ((Griffiths et al., 1992; Richards et al., 1990); compare Fig. 3). Interestingly, most retropepsins efficiently cleave an X-Pro bond (where X is a large, aromatic amino acid such as Tyr or Phe), a feature rarely seen in other endopeptidases. It is important to point out that the substrate preferences in individual pockets of the enzyme are not independent, and that the nature of the amino acid residues in the P1–P1′ positions influences the preference of the enzyme for the residues in neighboring substrate specificity pockets (Griffiths et al., 1992; Tozser et al., 1997).

The apparent discrepancy between the rather promiscuous sequence specificity without clear signature patterns on the one hand, and the fairly strict requirement of the virus for appropriately ordered processing of individual proteins (see below) may be resolved by the notion that the enzyme seems to preferentially cleave unfolded linker regions between individual folded domains while recognizing a common 3D motif shared by all cognate substrates (Chellappan et al., 2007; Prabu-Jeyabalan et al., 2002).

## PR as a target for therapeutic intervention

### *HIV-1 PR as a prime target for antiretroviral chemotherapy*

The importance of HIV-1 as a human pathogen has spurred the development of specific small molecule compounds that block replication by inhibiting various steps in viral replication. Since the discovery of HIV-1 in 1983, about 30 different antiretroviral drugs have been introduced into clinical practice. Since inactivation of HIV-1 PR renders the virus non-infectious (Kohl et al., 1988) and even partial inhibition of proteolysis severely affects virus replication (Kaplan et al., 1993), specific inhibitors of HIV-1 PR are highly effective antiviral agents. The introduction of PR inhibitors (PIs) in 1995 as part of highly active anti-retroviral therapy (HAART) was a critical step for decreasing mortality and prolonging life expectancy of HIV-positive patients under treatment. At present, there are nine HIV PIs approved as antiviral agents by the US Food and Drug Administration (FDA).

The literature on HIV PIs is very large (the Web of Science returns over 60,000 search results for “HIV Protease Inhibitor”) and has been the subject of a number of excellent and thorough reviews (e.g. Adamson, 2012; Anderson et al., 2009; De Clercq, 2005; Pokorna et al., 2009; Wlodawer and Erickson, 1993). Within the frame of this article, we will only briefly introduce the currently approved PIs and will expand this to some less orthodox approaches towards PI inhibition that have not been recently reviewed.

All FDA-approved HIV PIs are competitive inhibitors binding to the enzyme active site. The first generation drugs have been designed as peptidomimetics of the respective processing substrate (Fig. 4). Most of these compounds suffer from poor bioavailability and unwanted side effects. PIs often interfere with lipid metabolism and trafficking pathways (Barbaro and Iacobellis, 2009; Duvivier et al., 2009; Mallewa et al., 2008). These side effects are not only medically relevant per se, but can also decrease the adherance to treatment regimes and thus contribute indirectly to the evolution of resistance (see below).

Pharmacokinetic properties of PIs can be improved by co-administration of an inhibitor of cytochrome P450 3A4, the microsomal enzyme responsible for the metabolism of PI and other xenobiotics. Interestingly, one of the first clinically approved HIV PIs, ritonavir (RTV), was soon recognized as a potent inhibitor of cytochrome P450 3A4 and is currently used almost exclusively as pharmacokinetic boosting agent increasing the plasma concentration of other PIs (Moyle, 2001). In fact, all other clinically used PIs with the exception of nelfinavir (NFV) are routinely co-administered with a low dose of RTV as a pharmacokinetic booster.

The first generation compounds saquinavir, ritonavir, indinavir and amprenavir (SQV, RTV, IDV, APV; Fig. 4) were designed to bind

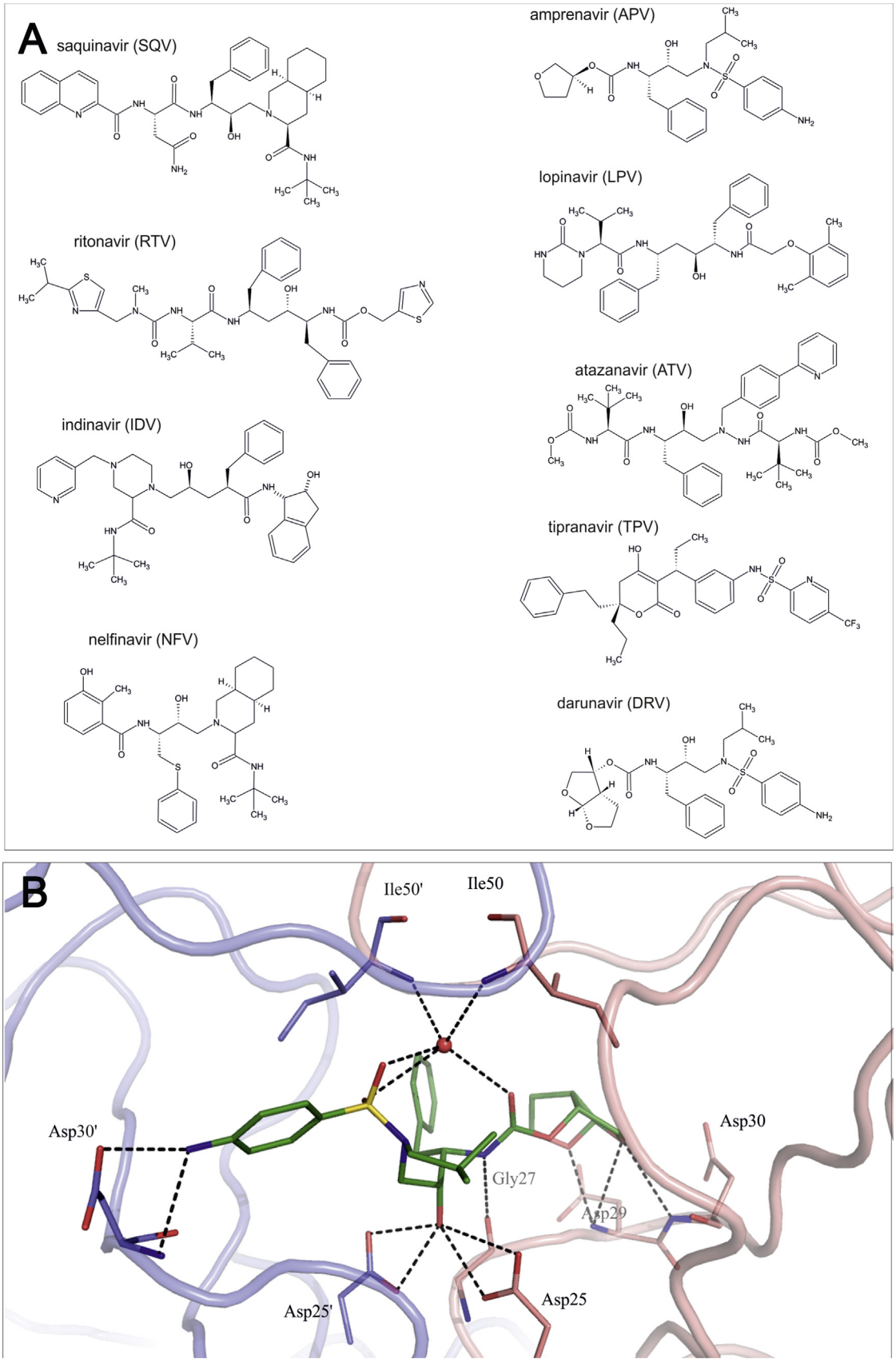


**Fig. 4.** Inhibitors of HIV PR (A) Chemical structures of FDA approved HIV PI. (B) Binding of DRV to the active site of HIV-1 PR. Hydrogen bonding interactions between DRV and PR are represented as dotted lines. The figure was generated using the PDB code 3GGU (Kozisek et al., 2014) and program PyMol (*www.pymol.org*). Figure courtesy of Pavlína Řezáčová.

wild-type HIV-1 PR. Their binding modes share common features: tight binding to the enzyme is achieved by non-polar van der Waals interactions of the inhibitor's side-chains. These interactions can be easily disrupted by conservative amino acid mutations in the vicinity of the binding cleft, thereby promoting rapid resistance development. Thus, further drug development aimed at PIs active against HIV-1 PR variants resistant to first generation PIs, as well as at compounds with less side effects and/or increased oral availability. Examples of second generation PIs are lopinavir (LPV; (Sham et al., 1998)) and the most recently approved drug darunavir (DRV; formerly TMC-114, UIC-94017 (Koh et al., 2003)). An important feature of the latter compound is the bis-tetrahydrofuranyl moiety by which it differs from the structurally related PI APV (Fig. 4). This moiety forms crucial hydrogen bond interactions with the main chain of Asp29 and Asp30 in the PR binding cleft, mimicking the hydrogen bond network of the polyprotein substrate of HIV PR (Prabu-Jeyabalan et al., 2000). The compound fits to the "substrate envelope" within the binding cleft of HIV-1 PR as proposed by Schiffer et al. (Chellappan et al., 2007; Prabu-Jeyabalan et al., 2002). This binding mode results in broad specificity of the inhibitor towards a variety of highly mutated, resistant PR species. Several lines of indirect evidence further suggest that DRV might bind not only to the active site, but also to a surface pocket in the flaps of the enzyme (Kovalevsky et al., 2008, 2006), and may thus act by a dual mechanism (i.e. involving dimerization inhibition in addition to targeting the active site (Koh et al., 2007)). However, analyses of DRV resistant virus identified in patients under prolonged DRV treatment did not reveal an additional cluster of mutations that would support such an alternative mode of action (Saskova et al., 2009).

*Antiretroviral resistance development*

The extremely high replication rate of HIV-1, together with a lack of proofreading activity of RT, results in rapid accumulation of random mutations in the viral genome. It has been estimated that as many as $10^{10}$ new virus particles are generated every day in an untreated patient (Perelson et al., 1996). This high replication rate together with the low fidelity of the viral RT predicts that every possible change occurs every day at every position of the viral genome in a patient with active viral replication (Coffin, 1995). The high population dynamics result in mixed virus populations ('quasispecies') carrying highly diverse PR coding sequences, and some PI resistance mutations have been observed in the absence of any previous use of PIs (e.g. (Kozal et al., 1996)). As a consequence, development of inhibitors requiring multiple resistance mutations and combination therapy is crucial in order to raise the genetic barrier to resistance and prevent the rapid selection of resistant virus variants under therapy. The emergence of resistance against HIV-1 PIs represents an impressive example of microevolution in the test tube and of evolutionary plasticity in vivo: this enzyme of 99 amino acids can tolerate the exchange of 22 residues (i.e. more than one fifth of its sequence), while still retaining the ability to correctly process its substrate and support viral replication (Menendez-Arias, 2013; Rhee et al., 2010).

Resistance can develop against every PI (or any other antiviral drug) in clinical use, and cross resistance against multiple PIs can also occur (for a more detailed report on this topic see (Anderson et al., 2009; Fun et al., 2012; Menendez-Arias, 2013; Rhee et al., 2010) and references therein). Mutations conferring viral resistance to PIs are divided into three groups: major (primary) mutations, minor (secondary, compensatory) mutations, and (tertiary) mutations in the processing sites of the Gag and Gag–Pro–Pol polyproteins (Fig. 5). The major mutations directly affect the binding cleft of the enzyme and result in decreased binding affinity of the PI; this often correlates with decreased affinity of the polyprotein substrate, and these mutations therefore generally come at a cost for viral replication fitness. Secondary and tertiary mutations restore fitness by enhancing substrate affinity or turnover rates; this can be achieved by alteration of PR (secondary mutations) or by adaptation of its substrate (tertiary mutations).

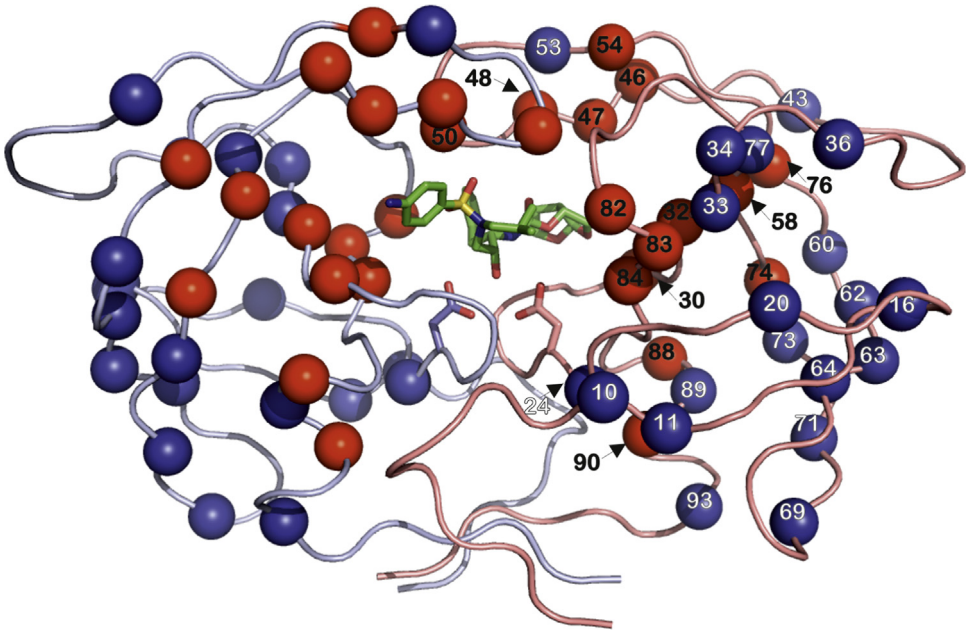


**Fig. 5.** Sites of known primary and secondary resistance-associated mutations in HIV-1 PR. Three dimensional model of the HIV-1 PR structure depicting mutations associated with resistance to clinically used PI. Backbones of the two monomeric subunits are represented as light blue and red lines, respectively; active site aspartates and the inhibitor DRV (green) bound to the active site are represented as stick models. Mutated residues found in resistant PR variants are indicated with their Cα atoms (spheres) and colored red and blue for major (primary) and minor (secondary) mutations, respectively; numbers refer to amino acid positions. The figure was generated using the structure of a highly mutated patient derived HIV-1 PR (PDB code 3GGU (Saskova et al., 2009)) and program PyMol (*www.pymol.org*). Tertiary resistance associated mutations, i.e. exchanges at PR recognition sites in the substrates, are not included in the figure. Figure courtesy of Pavlína Řezáčová.

At least 50 residues of HIV-1 PR have been reported to be prone to primary or secondary resistance mutations (Menendez-Arias, 2013; Rhee et al., 2010). In addition to amino acid substitutions, insertions of one to six residues in HIV-1 PR have been reported that also decrease PI sensitivity (Kozisek et al., 2008b).

Tertiary resistance mutations do not affect PR itself, but alter amino acid sequences at polyprotein processing sites. These mutations adapt the substrate to the modified requirements of the mutated enzyme's binding cleft (Doyon et al., 1996; Mammano et al., 1998; Zhang et al., 1997). Nijhuis et al. observed that mutations in the vicinity of the processing sites can accumulate even before the occurrence of primary mutations in the PR coding region. This phenomenon may be important for early resistance development and may explain HIV-1 therapy failures in the presence of wild type PR (Nijhuis et al., 2007).

Resistance development is delayed for PIs with a higher genetic barrier to resistance, i.e. compounds that require multiple mutations in PR for resistance development. An example is DRV, which has been designed to fit the 3D-space in the binding cleft of HIV-1 PR that represents the minimal volume for the binding of natural substrates (see above) and to form backbone-backbone hydrogen bonds difficult to modify by amino acid exchange. A highly resistant HIV-1 variant recently isolated from a patient under DRV treatment harbored 22 mutations in the PR coding region (Kozisek et al., 2012). This PR variant retained only 1.4% of wild-type enzymatic activity (as quantified by in vitro analysis using recombinant PR), but was still able to support viral replication. If the in vitro processing kinetics correctly reflects the situation in the budding virus, this number would suggest a threshold for PR activity required for HIV-1 infectivity. One must consider, however, that alterations in Km may not be relevant at the very high concentrations (mM range) of enzyme and substrate inside the budding virion (Konvalinka et al., 1995).

*Novel compounds*

Development of PI resistance can be overcome by either increasing blood plasma concentration of the drug or by administering a compound with a different binding mode to the enzyme and thus a different resistance profile (Pokorna et al., 2009). Therefore, academia and pharmaceutical industry continue to search for novel, highly active compounds with improved resistance profiles, better pharmacokinetic properties, and fewer adverse effects. This can be achieved by identifying chemical scaffolds with different binding modes. One strategy is to design irreversible inhibitors of HIV-1 PR, which react with the active site aspartate residues and yield an inactive covalent adduct. The only mutation that could plausibly cause resistance against such a small irreversible inhibitor would be a mutation of the active site Asp, which in turn would inactivate the enzyme and thereby block viral replication. Thus, irreversible inhibitors would be expected to be less sensitive to PR mutations that decrease the binding affinity of the inhibitor. Many attempts have been made to develop an irreversible inhibitor that covalently binds to the catalytic aspartates. However, there has been little progress since the identification of the initial irreversible peptidomimetic inhibitors ((Ro et al., 1998); reviewed in (Schimer and Konvalinka, 2014)). Irreversible inhibitors available to date are characterized by insufficient selectivity and specificity for HIV-1 PR and a short half-life in vivo.

Blocking dimerization of PR is another potential target for alternative PIs, since this process is indispensable for proteolytic activity. Indeed, small molecules interfering with HIV-1 PR dimerization display inhibitory potential (Zhang et al., 1991), and were reported to be active against PR variants bearing resistance-causing mutations (Shultz et al., 2004). Short peptides that mimic the C- and N-terminal parts of PR yielded PIs with micromolar potency (Zhang et al., 1991). These peptide sequences have been connected with various non-peptidic linkers (Zutshi and Chmielewski, 2000), increasing antiviral potency (Davis et al., 2006; Vidu et al., 2010). The active site-directed compound DRV (see above) has been suggested to also disrupt PR dimerization, and thus to target the same enzyme by two independent approaches (Koh et al., 2011', 2007).

Apart from the dimerization domain, there are other structural features that could be a target for drug development, including the movable "flaps". When the enzyme switches from the open state to the closed (substrate-bound) state (Fig. 2), the flap hinge region (residues 37–42) presents a small hydrophobic binding site. This site could be targeted by small molecules, thereby preserving the closed state and preventing PR from binding its substrate. Small chemical building blocks such as 2-methylcyclohexanol or indole-6-carboxylic acid have shown some promise and may provide a starting scaffold for further development (Perryman et al., 2010). Other compounds, such as beta-lactams, bind to areas of the flap region closer to the active site (Sperka et al., 2005). Cigler et al (2005) targeted the flap region with metallocarboranes. These compounds retained high inhibitory activity even against highly mutated PR that displayed low susceptibility to currently used PIs (Kozisek et al., 2008a; Rezacova et al., 2009). Metallocarboranes, with their unique binding mode and a good cross-resistance profile, are an interesting scaffold that already proved its potential for the design of inhibitors of other biologically relevant enzymes (carbonic anhydrase IX, (Brynda et al., 2013)).

Finally, there is also surprising structural and chemical diversity among the more "conventional" compounds targeting the active site of HIV PR. The PR substrate binding cleft can accommodate fullerenes (Friedman et al., 1998), large ruthenium clusters (Wong et al., 2006), seven-membered cyclic ureas (Lam et al., 1994), compounds with peptide bonds replaced by pyrrolinones (reviewed in (Smith et al., 2011)), β-hydroxy γ-lactams (Wu et al., 2012), sulfoximines (Lu and Vince, 2007) or dibenzodiazepine derivatives (Schimer et al., 2012). Clearly, HIV-1 PR will continue to be an exciting target for antiviral drug development.

## Role of PR in the retroviral replication cycle

*Immature and mature retroviral particles: same, but different*

Retroviral Gag and Gag–Pro–Pol polyproteins are processed into functional subdomains during maturation, accompanied by dramatic structural rearrangements within the virion. Canonical Gag domains are from N- to C-terminus, MA (matrix) lining the viral envelope, CA (capsid) forming the mature capsid structure and NC (nucleocapsid) packaging and condensing the viral genomic RNA ((Vogt, 1997); see Fig. 1). In addition, all retroviral Gag polyproteins carry so-called "late motifs" recruiting the cellular endosomal sorting complex required for transport (ESCRT) machinery and thus facilitating virus release (Bieniasz, 2009; Votteler and Sundquist, 2013). Besides that, Gag polyproteins from different retroviruses may contain further subunits and in some cases short spacer peptides that are important for the regulation of the maturation process (see below), but without known function in the mature virus ((Vogt, 1997), see Fig. 1). The morphology of mature virions varies between different retroviruses, but immature and mature particles can be clearly distinguished by their appearance in electron micrographs for every retrovirus (Fig. 6; reviewed in (Swanstrom and Wills, 1997)).

Immature particles are characterized by a spherical or semi-spherical shell of Gag polyproteins, in which MA lines the lipid envelope, CA forms important protein–protein lattice contacts and NC, bound to the viral genome, extends towards the center of the

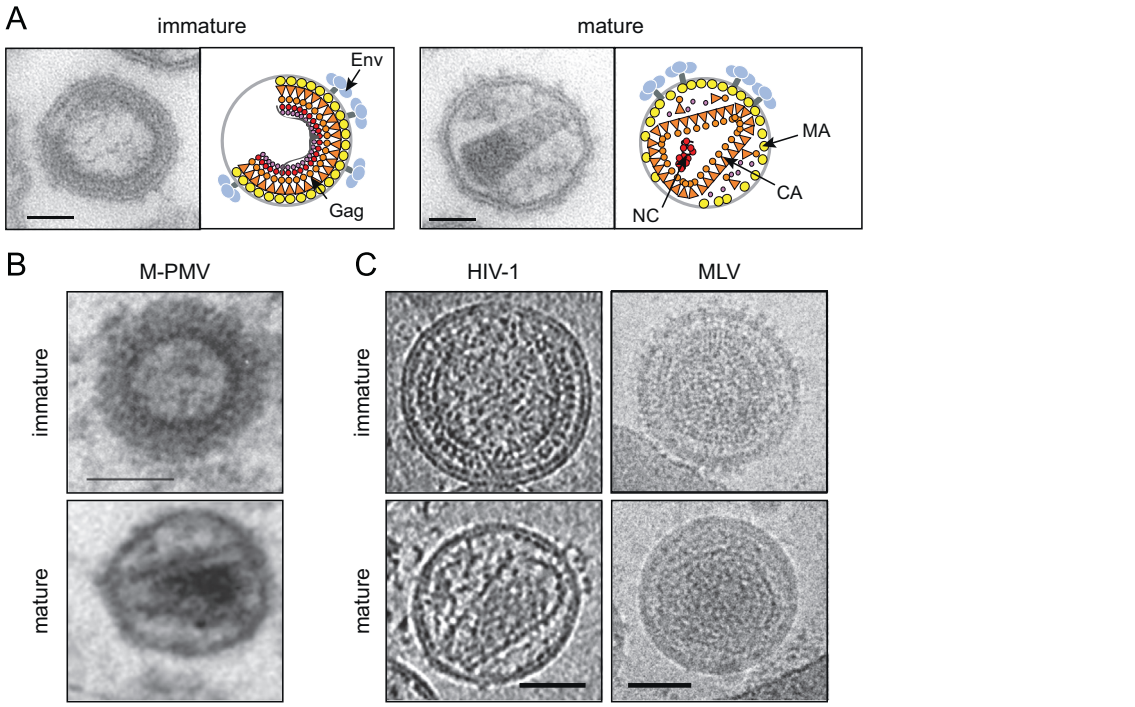


**Fig. 6.** Proteolytic maturation of retroviruses induces morphological changes. (A) Rearrangements of Gag domains accompanying HIV-1 maturation. The figure shows thin-section electron micrographs of immature and mature HIV-1 and sketches indicating the position of Gag domains. (B, C) Morphologies of exemplary immature and mature retrovirus particles visualized by thin-section EM (B; M-PMV) or cryo-electron tomography (C; HIV-1, MLV). Images courtesy of Michaela Rumlova (M-PMV), John Briggs (HIV-1; panel C) and Alan Rein (MLV). Scale bars: 50 nm.

particle. Mature particles, in contrast, contain a condensed nucleoprotein core surrounded by a spherical, tubular or conical capsid (Swanstrom and Wills, 1997). CA domain hexamers are the basic elements of both the immature and the mature lattice. Nevertheless, the architecture of the immature and mature lattices of a given retrovirus differ significantly; this is characterized in the highest structural detail for HIV-1, but also holds true for other retroviruses. In the nascent HIV-1 bud, curvature is attained by small irregular defects in the Gag lattice. The released immature Gag shell comprises a large gap (Carlson et al., 2008; Wright et al., 2007), presumably derived from the action of ESCRT components closing the viral bud and facilitating virus release. In contrast, mature retroviral capsids are closed fullerene-type structures where curvature is accomplished by the introduction of CA pentamers into the hexameric lattice (Ganser et al., 1999; Li et al., 2000). The structure of the immature and mature hexamer itself also differs substantially, and there is little overlap between CA intermolecular contact surfaces used for immature and mature hexamer formation (Bharat et al., 2012). Recent high resolution cryo electron-tomography studies revealed further differences between retroviruses in the immature lattice: M-PMV and HIV-1 both exhibit a similar lattice architecture and almost identical position of the CA C-terminal domain, while the CA N-terminal domains show significantly different contacts, attesting to the enormous plasticity of the CA protein (Bharat et al., 2012; Schur et al., 2014).

*Regulation of proteolytic maturation: it is all about control*

It is evident that the complex process of retrovirus maturation has to be tightly regulated in space and time. Initiation and progress of polyprotein cleavage have to be closely coordinated with virus assembly and budding. Furthermore, individual structural components should be released in a defined sequence to allow for complex structural rearrangements within the confined environment of a ~125 nm particle. How this precise control and regulation is accomplished is largely unclear, however. Important open questions concern the nature of the triggering event and its timing with respect to virus formation. Likewise, the kinetics of polyprotein processing in the context of the assembled virus is unknown, and the dynamics and pathway of structural rearrangements have yet to be analyzed.

For retroviruses assembling at the plasma membrane, proteolysis should ideally be delayed until the structural polyproteins reach the assembly site and are confined in the nascent particle. Several lines of evidence suggest that proteolysis indeed only occurs concomitant with or shortly after virion release (i) morphologically immature particles are detected in the vicinity of virus-producing cells in electron micrographs; (ii) nascent HIV-1 buds at the plasma membrane display an immature Gag lattice structure (Carlson et al., 2008); (iii) polyprotein subunits appear to be present in particles in constant, roughly equimolar amounts. It is currently not known, how the delay of polyprotein processing is accomplished. In most retroviruses, Gag:PR stoichiometry is regulated by expression through stop-codon suppression or translational frameshift from the Gag reading frame (see Fig. 1). Since these events occur only in a fraction of translation events, approximately 20 molecules of Gag are synthesized per PR monomer. Lower expression of PR compared to Gag is not an absolute requirement, however: genomes of ASLVs encode PR as a domain of Gag and thus synthesize the enzyme in equimolar amounts with the polyprotein, indicating that mechanisms other than intracellular concentration of PR subdomains can be important to prevent premature proteolysis.

An important regulatory mechanism used by all retroviruses is the expression of a catalytically inactive PR monomer; PR domains within the initially translated precursors thus need to dimerize to form an active enzyme. In the context of the polyprotein, dimers of

the HIV-1 PR region are less stable and adopt an inactive conformation for most of the time (Agniswamy et al., 2012; Huang et al., 2011; Louis et al., 1999; Tang et al., 2008), As a consequence, they display low enzymatic activity and significantly decreased sensitivity towards PIs targeting the mature enzyme (Davis et al., 2012; Pettit et al., 2004). Initial processing appears to occur upstream of the N-terminus of PR (SP1-NC and TF-p6pol cleavage sites) by an intramolecular mechanism, resulting in enhancement of enzymatic activity (Pettit et al., 2004', 2003', 2005). Due to its dependence on precursor dimerization, PR activity is controlled by intracellular polyprotein concentration, and PR may thus be activated by concentrating PR-monomer containing polyproteins upon virus assembly. Particular spatial arrangements induced by lattice formation, as well as interaction with lipids or nucleic acids may also play a role in PR activation (see below). Accordingly, retroviruses engineered to encode two monomers of PR joined by a flexible linker display enhanced, premature Gag processing and loss of virus formation and infectivity (Burstein et al., 1991; Krausslich, 1991). Premature processing can also be induced by overexpression of PR or Gag–Pro–Pol (e.g.(Mergener et al., 1992; Park and Morrow, 1991)), by activating mutations in PR (e.g. (Xiang et al., 1997)) or – in the case of HIV-1 – by addition of certain non-nucleosidic RT inhibitors, which promote Gag–Pro–Pol dimerization by stabilizing intermolecular interactions between RT domains (Figueiredo et al., 2006).

Dimer formation is likely not the only trigger mechanism, however. Some HIV-1 variants de-targeted from the plasma membrane by large deletions in the MA domain assemble into apparently intact particles at intracellular membranes, but these particles remain morphologically immature (Facke et al., 1993; Gallina et al., 1994; Reil et al., 1998). An important role for the site of retrovirus assembly on maturation can be deduced from experiments with murine intracisternal A-type particles (IAP), a group of endogenous retroviruses that normally bud at the endoplasmatic reticulum. Only immature particles are observed in IAP-producing cells, although the IAP PR is catalytically competent (Strisovsky et al., 2002). Re-targeting IAP assembly to the plasma membrane by modification of targeting signals within Gag led to efficient Gag processing by IAP PR (Welker et al., 1997). These observations suggest that specific feature(s) encountered at the plasma membrane (or a subset of cellular membranes) may serve as additional trigger to initiate proteolysis. This hypothesis is further strengthened by the maturation pathway of D-type retroviruses like M-PMV, which assemble complete immature capsids in the cytoplasm, whereas polyprotein processing and morphological maturation are delayed until their budding from the plasma membrane.

The clear distinction between immature assembly and maturation in M-PMV could be exploited to investigate potential triggering mechanisms. Parker and Hunter (2001) reported that proteolysis of cell-free immature M-PMV capsids is induced upon addition of reducing agents, suggesting that a specific intracellular redox environment might serve as a trigger for maturation. Similarly, treatment with a mild oxidizing agent has been shown to result in reversible inhibition of MLV PR (Campbell et al., 2002). Reversible oxidation of cysteine residues in the PR dimer interface has also been proposed to regulate HIV-1 PR activity (Davis et al., 1999), in particular with respect to the initial intramolecular cleavage event in Gag–Pro–Pol (Daniels et al., 2010). Whether redox regulation of PR indeed represents a key maturation trigger in the retroviral life cycle and/or whether other factors e.g. specific cellular proteins or lipids may play a role remains to be determined. Since retroviral PRs display maximal activity at lower than neutral pH it is intuitive to propose that a (transient) decrease in local pH may also contribute to PR activation. It should be noted in this respect, however, that the optimal conditions for full Gag and Gag–Pro–Pol processing in the natural context are not known. Whereas the pH optimum of PR using synthetic peptide substrates or denatured proteins in vitro is in the acidic range (e.g. (Billich et al., 1988; Darke et al., 1989; Hyland et al., 1991), the exact pH-activity profile varies for different peptide substrates and conditions. The presentation of cleavage sites in the context of a folded protein further influences the pH-sensitivity of the reaction. While cleavage at the CA-SP1 processing site in HIV-1 Gag was found to be significantly enhanced by acidification (Pettit et al., 1994), cleavage at MA–CA using either full length Gag (Pettit et al., 1994) or an engineered protein substrate (Lee et al., 2012) occurred with similar efficiency at pH 6.0 or 7.0. How alterations in pH would affect initiation and completion of proteolytic processing in the full viral context is thus difficult to predict. To date, experimental evidence for a role of the local and potentially transient pH environment in PR activation is lacking. The detection of punctuate HIV-1 Gag assemblies upon labeling of the protein with the pH-sensitive fluorophore pHluorin (Jouvenet et al., 2008) indicates a local pH of 6.0 or higher at many membrane-associated Gag assemblies in steady-state.

### *Role of individual cleavage sites and the functional contribution of spacer peptides*

As outlined above, CA hexamers form the central core of the immature and the mature lattice, but contact surfaces involved differ greatly. The inter-hexamer distance is smaller in the immature than in the mature lattice, indicating that the immature lattice is more tightly packed (reviewed in (Briggs and Krausslich, 2011; Ganser-Pornillos et al., 2012)). Furthermore, only about 50% of CA molecules packaged into the virus as part of the polyproteins are required for formation of the mature cone (Lanman et al., 2004). While the pathway of morphological maturation is currently unknown, these findings have been taken as evidence that maturation does not involve condensation from a spherical shell to a conical core, but rather involves dissociation of the immature lattice followed by reassembly of the mature capsid. Such major rearrangements occurring within the confined space of a virus particle, with Gag subunits present at mM concentration, obviously require precise organization.

Many lines of evidence from biochemical and virological studies indicate that retroviral proteolysis is indeed tightly regulated, and that this regulation occurs through sequential cleavage at individual sites within the Gag polyprotein (Fig. 7). Amino acid sequences at the different PR recognition sites vary, and kinetic parameters determined in vitro using PR and synthetic peptides differ greatly between individual sites (reviewed in (Tozser and Oroszlan, 2003)). These differences are reflected in different cleavage rates at individual sites when Gag or Gag–Pro–Pol are used as substrates in vitro (Pettit et al., 2004', 2002', 2005', 1994). A distinct sequence of cleavages is also followed in the viral context, as indicated by characteristic patterns of intermediate cleavage products detected in cell lysates, and in virus particles upon partial inhibition of PR with suboptimal PI concentrations (Kaplan et al., 1993). Consistently, blocking cleavage at a particular site in Gag by mutagenesis can also affect processing at another site.

Mutational studies from many labs showed that complete cleavage of retroviral Gag at multiple sites is essential for successful maturation. In most cases, blocking processing by mutagenesis at a single site severely reduced or abolished infectivity (e.g. (Coren et al., 2007; Oshima et al., 2004; Wiegers et al., 1998)). Furthermore, not only complete inhibition, but also reduction of cleavage efficiency at an individual site can be detrimental. In the case of HIV-1 it has been demonstrated that partially processed intermediates, in particular the slowly cleaved late intermediate CA-SP1, can exert a strong dominant-negative effect on infectivity (Checkley et al., 2010; Lee et al., 2009; Muller et al., 2009). This is

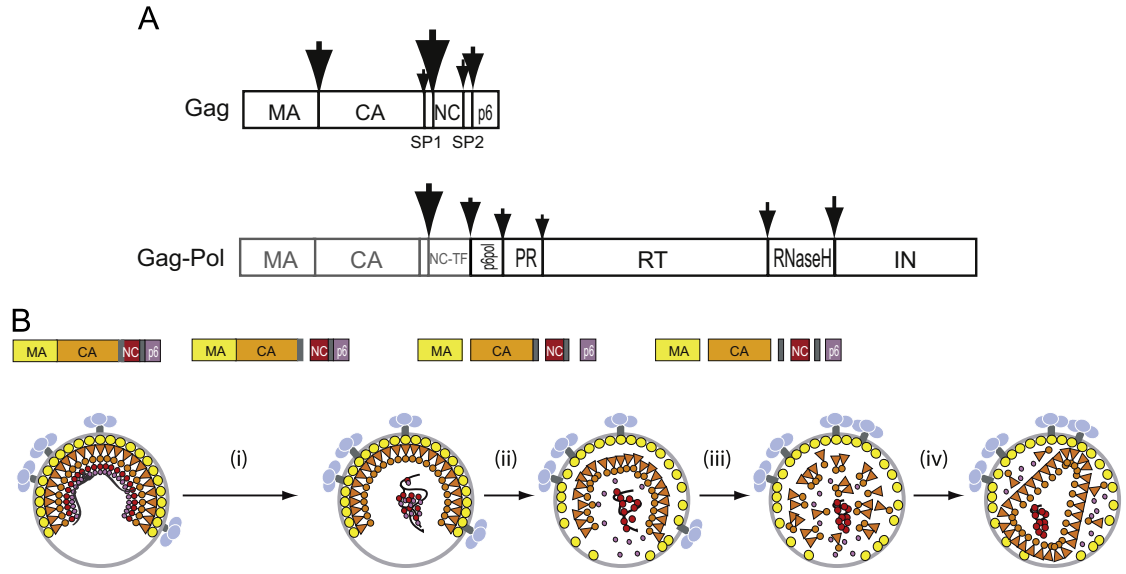


**Fig. 7.** Sequential steps of HIV-1 maturation. (A) PR cleavage sites in HIV-1 Gag and Pol,. Sizes of arrowheads indicate relative rates of cleavage determined in vitro. TF, transframe peptide. (B) Model for HIV-1 morphological maturation mediated by sequential cleavage of Gag. (i) Initial processing separates NC-SP2-p6 from the MA–CA lattice and allows initial nucleoprotein condensation. (ii) Cleavage between NC-SP2 and p6 promotes further nucleoprotein condensation. Processing at the MA–CA cleavage site releases CA from the membrane bound MA layer and induces resolution of the ordered immature lattice. (iii) Cleavage of the spacer peptides from CA and NC results in full nucleocapsid condensation and dissociation of the CA layer. (iv) Fully processed CA protein forms a mature capsid surrounding the condensed nucleoprotein complex.

in accordance with the fact that PI concentrations yielding only a modest inhibition of Gag cleavage affect HIV-1 morphogenesis and result in significant inhibition of viral infectivity (Kaplan et al., 1993).

In the case of HIV-1 Gag, data from numerous biochemical, mutational and structural studies can be summarized into a model of the maturation process (Fig. 7B). Based on the information on processing kinetics at different sites, cleavages within Gag can be divided into three categories: (i) initial cleavage at the SP1-NC boundary separates the RNA-bound NC region from the MA–CA lattice; (ii) subsequent cleavages separate the CA-lattice from the membrane associated MA layer and free the NC region from the p6 domain; (iii) the final processing steps cleave the spacer peptides SP1 and SP2 from their N-terminal fusion partners CA and NC, respectively. These late events, in particular cleavage of the SP1 peptide, play a crucial regulatory role in morphological maturation. In the case of the genetically and morphologically distinct viruses HIV-1, RSV and M-PMV, the C-terminal region of CA and the adjacent spacer peptide or a spacer-like region of CA, respectively, represent a keystone of the immature hexameric lattice; mutations or deletions in this region affect immature particle formation (e.g. Accola et al., 1998; Keller et al., 2008; Krausslich et al., 1995). Early cryo-EM analyses of immature HIV-1 (Briggs et al., 2009; Wright et al., 2007) suggested that this region forms a 6-helix bundle, supporting the previous proposition that this region exhibits helical propensity (Accola et al., 1998). Consistent with the helix bundle model, a synthetic peptide covering SP1 and adjacent amino acids can fold into an alpha-helix at high concentrations similar to those present within the virion (Datta et al., 2011). A similar structure has also been suggested for RSV and M-PMV (Bush et al., 2014; de Marco et al., 2010a). While MLV does not encode a spacer peptide directly downstream of CA, a sequence at the C-terminus of MLV CA was shown to be important for particle assembly and has been proposed to form a 'charged assembly helix' (Cheslock et al., 2003; Oshima et al., 2004). A very recent high resolution cryo-EM model of immature HIV-1 particles, while still not allowing unequivocal assignment of side chains in the SP1 region, indicates that the respective regions from six Gag molecules form a hollow bundle transcending from the CA hexamer to the interior nucleocapsid layer (Schur et al., 2014).

Cleavage both upstream and downstream of the CA-SP1 module is required to initiate resolution of the immature lattice (de Marco et al., 2010b). However, inhibition of subsequent processing between CA and SP1 prevents dissociation of the CA layer (Wiegers et al., 1998) and apparently locks the lattice in an unproductive, partially disordered state (de Marco et al., 2010b; Keller et al., 2013), lending further support to the disassembly-assembly model of capsid formation. This phenotype is not only observed upon mutation of the CA-SP1 processing site, but is also the explanation for the anti-HIV-1 effect of betulinic acid derivatives (e.g. bevirimat; (Li et al., 2003)) that specifically block PR cleavage at this site through interaction with the Gag substrate (Adamson, 2012', 2009).

The role of the second spacer peptide in HIV-1 Gag is less clearly defined. Mutational analyses in the Gag C-terminal region revealed that the NC-SP2 processing step is dispensable for efficient virus replication in tissue culture, while inhibiting both cleavages in the NC-SP2-p6 region severely impaired virus viability (Coren et al., 2007). More detailed structural analyses of virus mutants indicated that not the presence of the spacer peptide itself, but possibly the proper kinetics of cleavages in this region is relevant (de Marco et al., 2012). This interpretation is in agreement with the observation that mutations in the NC-SP2 region are characteristically observed as tertiary resistance mutations restoring fitness to HIV-1 variants carrying resistance mutations in PR, which affect the catalytic activity of the enzyme (reviewed in (Clavel and Mammano, 2010; Fun et al., 2012)). Furthermore, NC-SP2-p6, NC-SP2 and NC display different abilities to condense nucleic acid ((Mirambeau et al., 2010) and references therein) suggesting that successive processing steps in this region regulate the dynamics of nucleocapsid core formation. Thus, in the case of HIV-1, the spacer peptides may be proposed to control the process of morphological maturation by coordinating nucleocapsid condensation with assembly of the surrounding conical capsid.

### *Dynamics and pathway of proteolytic maturation*

HIV-1 proteolytic maturation presumably involves only the viral PR and its substrates Gag and Gag–Pro–Pol in the relatively defined environment of the virus particle. Nevertheless, the

reaction entails at least 66 distinct substrates, intermediates or products, and numerous competing intermolecular interactions occurring simultaneously (Konnyu et al., 2013). Furthermore, the arrangement of the substrates in a tightly packed multimeric lattice is likely to impose additional constraints that cannot be mimicked using purified proteins in vitro. Dynamics of retroviral processing are difficult to investigate due to the fact that virus formation in tissue culture occurs in an asynchronous manner. Individual cells in a population infected at the same time will enter the state of virus production at different time points, and individual HIV-1 particles at the plasma membrane of a single cell assemble in an asynchronous manner over a time of several hours (Ivanchenko et al., 2009; Jouvenet et al., 2008). Accordingly, biochemical ensemble measurements or electron micrographs capture a mixture of particles at various stages of assembly and maturation, precluding the analysis of individual stages.

Therefore, the simple question 'how long does retroviral maturation take?' is still unanswered. Dale et al. (2011) proposed that HIV-1 Gag proteolysis, in the context of virus transmission between T-cells through 'virological synapses', occurs only after endosomal uptake of the virus into the new host cell and proceeds slowly over a period of several hours. However, this interpretation is inconsistent with the fact that morphologically mature particles, but not recognizable maturation intermediates, are easily detected by EM in the vicinity of virus producing cells. Furthermore, mature virions are even observed in the narrow space between two synapse-forming T-cells (e.g. Jolly et al., 2011; Martin et al., 2010).

In the case of M-PMV, the clear distinction between assembly and maturation allowed studying the kinetics of intravirion Gag processing in vitro. This analysis yielded a half time of roughly 15 min for initial cleavage, but incomplete generation of mature products (Parker and Hunter, 2001). While this approach is not feasible for other types of retroviruses, triggering HIV-1 maturation in vitro can be achieved by preparation of virions in the presence of a PI followed by rapid inhibitor removal ("inhibitor wash-out"). Wash-out of PIs with high off-rates ($k_{off}$, i.e. high velocity constants of the dissociation of the PR–PI complex) yields virions carrying nearly fully cleaved Gag (Mattei et al., 2014). Processing is still limited by the inhibitor off-rate even under optimized conditions, however. Consequently, infectivity cannot be recovered and particles displayed disturbed Pol processing and aberrant morphology (Mattei et al., 2014). To address this problem, we have recently developed a photocleavable HIV-1 PR inhibitor that allows rapid activation of the enzyme by UV illumination (Schimer et al., 2015). Use of this novel tool resulted in more rapid in situ Gag processing: we observed a half-time of ~20–30 min for mature CA formation, roughly consistent with a mathematical model for HIV-1 maturation (Konnyu et al., 2013). At the current state this represents only an upper limit of the time frame, however, and more detailed analyses will be required to unravel the dynamics of HIV-1 maturation.

Asynchronicity of virus formation also impedes the characterization of the nature and timing of structural changes involved in morphological maturation. It is generally assumed that Gag proteolysis and morphological maturation coincide, but this is not formally proven. As outlined above, the architecture of immature and mature HIV-1 particles suggest that maturation involves dissociation of the immature lattice followed by assembly of the mature core. Models for the stepwise process relied mainly on the investigation of viruses partially inhibited for processing by mutation at individual PR recognition sites or inhibitor treatment (e.g. de Marco et al., 2010a, 2012, 2010b; Keller et al., 2011, 2013)), whereas structural information on *bona fide* maturation intermediates is lacking. Structural data on HIV-1 particles with unclosed capsid structures (Yu et al., 2013) and of particles showing neither mature nor immature morphology that were detected in the vicinity of virus-producing cells (Woodward et al., 2015) have been taken as evidence that mature capsid formation occurs through growth of curled CA sheets. Unambiguous interpretation of the latter analyses is hampered by the fact that the history and future of individual particles cannot be deduced from still images, however, preventing clear distinction between productive maturation intermediates and dead end products.

Recently, an alternative model for morphological maturation was derived from analysis of assemblies observed in fortuitously occurring large membranous structures comprising Gag layers and multiple capsid-like structures (Frank et al., 2015). Based on these structures, the authors proposed that morphological maturation does not involve dissociation, but rather a gradual transition of the immature CA layer to a sheet evolving into a conical capsid. How the analyzed assemblies relate to intermediates of productive maturation of individual particles is unclear, however.

### *Role of proteolysis for viral infectivity*

Proteolytic processing of retroviral polyproteins is critical for infectivity for several reasons. First, the viral enzymes PR, RT and IN are synthesized as part of polyprotein precursors and need to be converted into the mature form for full enzymatic activity. Second, the change in virus architecture is required to transform the comparatively stable Gag shell into a metastable state ready for controlled release of the genomic information in a newly infected target cell. For several retroviruses, the immature Gag shell has been shown to be resistant towards removal of the lipid envelope by detergent treatment whereas the mature capsid of many retroviruses, including HIV-1, rapidly dissociates under these conditions (e.g. Oshima et al., 2004; Park and Morrow, 1993; Stewart et al., 1990). These observations are in agreement with the assignment of the two structures as stable 'production mode' and metastable 'infection mode'. Besides being ready for uncoating, the mature retroviral capsid also seems to serve important functions in the early replication phase, and recent studies have indicated that the HIV-1 capsid or a capsid-derived structure may remain intact during reverse transcription and possibly even during transport of the preintegration complex into the host cell nucleus, at least in some host cell types (Arhel, 2010 and references therein; Peng et al., 2014). These findings are consistent with mutations in the CA domain of Gag exhibiting a phenotype in early replication and with the observation that various host factors affecting early HIV-1 replication exert their function by binding to the capsid or a capsid-derived structure ((Hilditch and Towers, 2014; Matreyek and Engelman, 2013) and references therein).

Finally, proteolytic maturation can increase retrovirus entry efficiency. In the case of MLV and M-PMV, this effect is accomplished by PR-catalyzed removal of a C-terminal peptide from the transmembrane glycoproteins in the viral envelope. Occurring late in virus formation, this processing step enhances the fusogenicity of the respective Env protein (Brody et al., 1994; Rein et al., 1994). Correlation between maturation and entry efficiency was also observed for HIV-1 (Murakami et al., 2004; Wyma et al., 2004), although the Env protein of this virus is not a substrate for PR. Two – not mutually exclusive – explanations have been put forward based on virological, biophysical and imaging analyses. First, immature HIV-1 particles display significantly higher mechanical stiffness than mature particles as determined by atomic force microscopy (Kol et al., 2007), consistent with the tightly packed Gag lattice underlying the viral envelope being converted into a more loosely connected matrix layer. The increased flexibility of the mature particle may result in a lower energy barrier for lipid envelope deformations that need to occur during the membrane fusion process. Second, Gag processing status appears to be

correlated to the viral Env protein. Truncation of the long Env cytoplasmatic tail (CT) relieves the fusion defect of immature HIV-1 particles, suggesting that immature Gag-EnvCT interactions hinder the fusion process (Murakami et al., 2004; Wyma et al., 2004).

Recently developed super-resolution fluorescence microscopy (SR-FM) methods provided an opportunity to study the distribution of Env molecules on a large number of individual particles and correlate this to virus maturation status and infectivity (Chojnacki et al., 2012). Whereas other enveloped viruses often carry a densely packed array of viral glycoproteins on their surface, only a sparse number of Env trimer spikes have been detected on the surface of HIV-1 (Chertova et al., 2006; Zhu et al., 2006). SR-FM analyses revealed that Env molecules on the surface of most immature particles are distributed into multiple clusters, whereas mature viruses are characterized by a single Env cluster, and this was dependent on the Env CT (Chojnacki et al., 2012). These observations support a model proposing constrained lateral movement of Env molecules embedded in a tightly packed Gag lattice. Resolution of this lattice by Gag cleavage would allow Env molecules to diffuse and associate based on interactions in the CT region, which in turn may result in enhanced fusion competence (Chojnacki et al., 2012). An 'inside-out signaling' mechanism translating proteolytic maturation of the inner virus structure to changes on the viral surface could ensure that only those viruses whose cores have converted to 'infection mode' will actually enter the host cell cytoplasm.

## Conclusion: unsolved mysteries

About forty years of intense research have gone by since retroviral polyproteins were first identified (Vogt and Eisenman, 1973) and virus-encoded proteases were found to be key replication enzymes of retroviruses (von der Helm, 1977). Nevertheless, a number of very basic questions regarding the viral maturation process remain unanswered to date. As outlined above, these questions concern mainly the nature and timing of the triggering event for PR activation and the dynamics and pathway of morphological maturation.

Since the main obstacle for answering these questions is the asynchronous process of virus formation in tissue culture, addressing these issues will require (i) experimental systems allowing direct visualization of PR activation or Gag proteolysis at individual assembly sites or within individual virions and/or (ii) methods for a precisely triggered onset of proteolysis in bulk samples. Precise triggering of the onset of maturation, e.g. by use of a photolabile HIV-1 PR inhibitor, would not only allow to investigate the precise time course of polyprotein processing. In conjunction with a – not yet available – live-cell readout for Gag processing, this might eventually also enable the investigation of the temporal correlation between the already well characterized Gag assembly process (Baumgartel et al., 2012; Jouvenet et al., 2011) and proteolysis. Such systems would further enable us to investigate the nature and role of potential triggers for PR activation. Finally, combining rapid induction of proteolysis with new developments in SR-FM permitting the resolution of transitions in virion architecture, and with advanced cryo-electron tomography providing sub-nanometer resolution, may eventually clearly define structural intermediates and elucidate the pathway of HIV-1 maturation.

Experimental systems for an inducible activation of PRs in situ and the continuous development of fluorescent labeling and readout methods, as well as impressive progress in the fields of imaging and image analysis, promise to provide us with suitable tools for visualization of events within individual virions or at individual assembly sites in the near future. Ultimately, this might allow us to directly observe the complex and fascinating process of infectious retrovirus formation.

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## References

Accola, M.A., Hoglund, S., Gottlinger, H.G., 1998. A putative alpha-helical structure which overlaps the capsid-p2 boundary in the human immunodeficiency virus type 1 Gag precursor is crucial for viral particle assembly. J. Virol. 72, 2072–2078.

Adamson, C.S., 2012. Protease-mediated maturation of HIV: inhibitors of protease and the maturation process. Mol. Biol. Int. 2012, 604261.

Adamson, C.S., Salzwedel, K., Freed, E.O., 2009. Virus maturation as a new HIV-1 therapeutic target. Exp. Opin. Ther. Targets 13, 895–908.

Agniswamy, J., Sayer, J.M., Weber, I.T., Louis, J.M., 2012. Terminal interface conformations modulate dimer stability prior to amino terminal autoprocessing of HIV-1 protease. Biochem. 51, 1041–1050.

Anderson, J., Schiffer, C., Lee, S.K., Swanstrom, R., 2009. Viral protease inhibitors. Handb. Exp. Pharmacol. 189, 85–110.

Arhel, N., 2010. Revisiting HIV-1 uncoating. Retrovirology 7, 96.

Barbaro, G., Iacobellis, G., 2009. Metabolic syndrome associated with HIV and highly active antiretroviral therapy. Curr. Diab. Rep. 9, 37–42.

Barrett, A.J., Rawlings, N.D., Woessner, F.J., 2012. Handbook of Proteolytic Enzymes. Academic Press Inc., London.

Baumgartel, V., Muller, B., Lamb, D.C., 2012. Quantitative live-cell imaging of human immunodeficiency virus (HIV-1) assembly. Viruses 4, 777–799.

Bharat, T.A., Davey, N.E., Ulbrich, P., Riches, J.D., de Marco, A., Rumlova, M., Sachse, C., Ruml, T., Briggs, J.A., 2012. Structure of the immature retroviral capsid at 8A resolution by cryo-electron microscopy. Nat. 487, 385–389.

Bieniasz, P.D., 2009. The cell biology of HIV-1 virion genesis. Cell Host Microbe 5, 550–558.

Billich, S., Knoop, M.T., Hansen, J., Strop, P., Sedlacek, J., Mertz, R., Moelling, K., 1988. Synthetic peptides as substrates and inhibitors of human immune deficiency virus-1 protease. J. Biol. Chem. 263, 17905–17908.

Briggs, J.A., Krausslich, H.G., 2011. The molecular architecture of HIV. J. Mol. Biol. 410, 491–500.

Briggs, J.A., Riches, J.D., Glass, B., Bartonova, V., Zanetti, G., Krausslich, H.G., 2009. Structure and assembly of immature HIV. Proc. Natl. Acad. Sci. USA 106, 11090–11095.

Brody, B.A., Rhee, S.S., Hunter, E., 1994. Postassembly cleavage of a retroviral glycoprotein cytoplasmic domain removes a necessary incorporation signal and activates fusion activity. J. Virol. 68, 4620–4627.

Brynda, J., Mader, P., Sicha, V., Fabry, M., Poncova, K., Bakardiev, M., Gruner, B., Cigler, P., Rezacova, P., 2013. Carborane-based carbonic anhydrase inhibitors. Angew. Chem. 52, 13760–13763.

Burstein, H., Bizub, D., Skalka, A.M., 1991. Assembly and processing of avian retroviral gag polyproteins containing linked protease dimers. J. Virol. 65, 6165–6172.

Bush, D.L., Monroe, E.B., Bedwell, G.J., Prevelige Jr., P.E., Phillips, J.M., Vogt, V.M., 2014. Higher-order structure of the Rous sarcoma virus SP assembly domain. J. Virol. 88, 5617–5629.

Cameron, C.E., Grinde, B., Jentoft, J., Leis, J., Weber, I.T., Copeland, T.D., Wlodawer, A., 1992. Mechanism of inhibition of the retroviral protease by a Rous sarcoma virus peptide substrate representing the cleavage site between the gag p2 and p10 proteins. J. Biol. Chem. 267, 23735–23741.

Campbell, S., Oshima, M., Mirro, J., Nagashima, K., Rein, A., 2002. Reversal by dithiothreitol treatment of the block in murine leukemia virus maturation induced by disulfide cross-linking. J. Virol. 76, 10050–10055.

Carlson, L.A., Briggs, J.A., Glass, B., Riches, J.D., Simon, M.N., Johnson, M.C., Muller, B., Grunewald, K., Krausslich, H.G., 2008. Three-dimensional analysis of budding

sites and released virus suggests a revised model for HIV-1 morphogenesis. Cell Host Microbe 4, 592–599.
Checkley, M.A., Luttge, B.G., Soheilian, F., Nagashima, K., Freed, E.O., 2010. The capsid-spacer peptide 1 Gag processing intermediate is a dominant-negative inhibitor of HIV-1 maturation. Virology 400, 137–144.
Chellappan, S., Kairys, V., Fernandes, M.X., Schiffer, C., Gilson, M.K., 2007. Evaluation of the substrate envelope hypothesis for inhibitors of HIV-1 protease. Proteins 68, 561–567.
Chertova, E., Chertov, O., Coren, L.V., Roser, J.D., Trubey, C.M., Bess Jr., J.W., Sowder 2nd, R.C., Barsov, E., Hood, B.L., Fisher, R.J., Nagashima, K., Conrads, T. P., Veenstra, T.D., Lifson, J.D., Ott, D.E., 2006. Proteomic and biochemical analysis of purified human immunodeficiency virus type 1 produced from infected monocyte-derived macrophages. J. Virol. 80, 9039–9052.
Cheslock, S.R., Poon, D.T., Fu, W., Rhodes, T.D., Henderson, L.E., Nagashima, K., McGrath, C.F., Hu, W.S., 2003. Charged assembly helix motif in murine leukemia virus capsid: an important region for virus assembly and particle size determination. J. Virol. 77, 7058–7066.
Chojnacki, J., Staudt, T., Glass, B., Bingen, P., Engelhardt, J., Anders, M., Schneider, J., Muller, B., Hell, S.W., Krausslich, H.G., 2012. Maturation-dependent HIV-1 surface protein redistribution revealed by fluorescence nanoscopy. Science 338, 524–528.
Cigler, P., Kozisek, M., Rezacova, P., Brynda, J., Otwinowski, Z., Pokorna, J., Plesek, J., Gruner, B., Doleckova-Maresova, L., Masa, M., Sedlacek, J., Bodem, J., Krausslich, H.G., Kral, V., Konvalinka, J., 2005. From nonpeptide toward noncarbon protease inhibitors: metallacarboranes as specific and potent inhibitors of HIV protease. Proc. Natl. Acad. Sci. USA 102, 15394–15399.
Clavel, F., Mammano, F., 2010. Role of Gag in HIV resistance to protease inhibitors. Viruses 2, 1411–1426.
Coffin, J.M., 1995. HIV population dynamics in vivo: implications for genetic variation, pathogenesis, and therapy. Science 267, 483–489.
Coren, L.V., Thomas, J.A., Chertova, E., Sowder 2nd, R.C., Gagliardi, T.D., Gorelick, R.J., Ott, D.E., 2007. Mutational analysis of the C-terminal gag cleavage sites in human immunodeficiency virus type 1. J. Virol. 81, 10047–10054.
Dale, B., McNerney, G.P., Thompson, D.L., Hubner, W., de Los Reyes, K., Chuang, F.Y, Huser, T., Chen, B.K., 2011. Cell-to-cell transfer of HIV-1 via virological synapses leads to endosomal virion maturation that activates viral membrane fusion. Cell Host Microbe 10, 551–562.
Daniels, S.I., Davis, D.A., Soule, E.E., Stahl, S.J., Tebbs, I.R., Wingfield, P., Yarchoan, R., 2010. The initial step in human immunodeficiency virus type 1 GagProPol processing can be regulated by reversible oxidation. PloS One 5, e13595.
Darke, P.L., Leu, C.T., Davis, L.J., Heimbach, J.C., Diehl, R.E., Hill, W.S., Dixon, R.A., Sigal, I.S., 1989. Human immunodeficiency virus protease. Bacterial expression and characterization of the purified aspartic protease. J. Biol. Chem. 264, 2307–2312.
Datta, S.A., Temeselew, L.G., Crist, R.M., Soheilian, F., Kamata, A., Mirro, J., Harvin, D., Nagashima, K., Cachau, R.E., Rein, A., 2011. On the role of the SP1 domain in HIV-1 particle assembly: a molecular switch? J. Virol. 85, 4111–4121.
Davis, D.A., Brown, C.A., Singer, K.E., Wang, V., Kaufman, J., Stahl, S.J., Wingfield, P., Maeda, K., Harada, S., Yoshimura, K., Kosalaraksa, P., Mitsuya, H., Yarchoan, R., 2006. Inhibition of HIV-1 replication by a peptide dimerization inhibitor of HIV-1 protease. Antivir. Res. 72, 89–99.
Davis, D.A., Soule, E.E., Davidoff, K.S., Daniels, S.I., Naiman, N.E., Yarchoan, R., 2012. Activity of human immunodeficiency virus type 1 protease inhibitors against the initial autocleavage in Gag-Pol polyprotein processing. Antimicrob. Agents Chemother. 56, 3620–3628.
Davis, D.A., Yusa, K., Gillim, L.A., Newcomb, F.M., Mitsuya, H., Yarchoan, R., 1999. Conserved cysteines of the human immunodeficiency virus type 1 protease are involved in regulation of polyprotein processing and viral maturation of immature virions. J. Virol. 73, 1156–1164.
De Clercq, E., 2005. New approaches toward anti-HIV chemotherapy. J. Med. Chem. 48, 1297–1313.
de Marco, A., Davey, N.E., Ulbrich, P., Phillips, J.M., Lux, V., Riches, J.D., Fuzik, T., Ruml, T., Krausslich, H.G., Vogt, V.M., Briggs, J.A., 2010a. Conserved and variable features of Gag structure and arrangement in immature retrovirus particles. J. Virol. 84, 11729–11736.
de Marco, A., Heuser, A.M., Glass, B., Krausslich, H.G., Muller, B., Briggs, J.A., 2012. Role of the SP2 domain and its proteolytic cleavage in HIV-1 structural maturation and infectivity. J. Virol. 86, 13708–13716.
de Marco, A., Muller, B., Glass, B., Riches, J.D., Krausslich, H.G., Briggs, J.A., 2010b. Structural analysis of HIV-1 maturation using cryo-electron tomography. PLoS Pathog. 6, e1001215.
Dittmar, K.J., Moelling, K., 1978. Biochemical properties of p15-associated protease in an avian RNA tumor virus. J. Virol. 28, 106–118.
Doyon, L., Croteau, G., Thibeault, D., Poulin, F., Pilote, L., Lamarre, D., 1996. Second locus involved in human immunodeficiency virus type 1 resistance to protease inhibitors. J. Virol. 70, 3763–3769.
Duvivier, C., Kolta, S., Assoumou, L., Ghosn, J., Rozenberg, S., Murphy, R.L., Katlama, C., Costagliola, D., group, A.H.s., 2009. Greater decrease in bone mineral density with protease inhibitor regimens compared with nonnucleoside reverse transcriptase inhibitor regimens in HIV-1 infected naive patients. AIDS 23, 817–824.
Facke, M., Janetzko, A., Shoeman, R.L., Krausslich, H.G., 1993. A large deletion in the matrix domain of the human immunodeficiency virus gag gene redirects virus particle assembly from the plasma membrane to the endoplasmic reticulum. J. Virol. 67, 4972–4980.
Figueiredo, A., Moore, K.L., Mak, J., Sluis-Cremer, N., de Bethune, M.P., Tachedjian, G., 2006. Potent nonnucleoside reverse transcriptase inhibitors target HIV-1 Gag–Pol. PLoS Pathog. 2, e119.
Frank, G.A., Narayan, K., Bess Jr., J.W., Del Prete, G.Q., Wu, X., Moran, A., Hartnell, L.M., Earl, L.A., Lifson, J.D., Subramaniam, S., 2015. Maturation of the HIV-1 core by a non-diffusional phase transition. Nat. Commun. 6, 5854.
Friedman, S.H., Ganapathi, P.S., Rubin, Y., Kenyon, G.L., 1998. Optimizing the binding of fullerene inhibitors of the HIV-1 protease through predicted increases in hydrophobic desolvation. J. Med. Chem. 41, 2424–2429.
Fun, A., Wensing, A.M., Verheyen, J., Nijhuis, M., 2012. Human Immunodeficiency Virus Gag and protease: partners in resistance. Retrovirology 9, 63.
Gallina, A., Mantoan, G., Rindi, G., Milanesi, G., 1994. Influence of MA internal sequences, but not of the myristylated N-terminus sequence, on the budding site of HIV-1 Gag protein. Biochem. Biophys. Res. Commun. 204, 1031–1038.
Ganser-Pornillos, B.K., Yeager, M., Pornillos, O., 2012. Assembly and architecture of HIV. Adv. Exp. Med. Biol. 726, 441–465.
Ganser, B.K., Li, S., Klishko, V.Y., Finch, J.T., Sundquist, W.I., 1999. Assembly and analysis of conical models for the HIV-1 core. Science 283, 80–83.
Griffiths, J.T., Phylip, L.H., Konvalinka, J., Strop, P., Gustchina, A., Wlodawer, A., Davenport, R.J., Briggs, R., Dunn, B.M., Kay, J., 1992. Different requirements for productive interaction between the active site of HIV-1 proteinase and substrates containing -hydrophobic*hydrophobic- or -aromatic*pro- cleavage sites. Biochemistry 31, 5193–5200.
Grinde, B., Cameron, C.E., Leis, J., Weber, I.T., Wlodawer, A., Burstein, H., Skalka, A.M., 1992. Analysis of substrate interactions of the Rous sarcoma virus wild type and mutant proteases and human immunodeficiency virus-1 protease using a set of systematically altered peptide substrates. J. Biol. Chem. 267, 9491–9498.
Hilditch, L., Towers, G.J., 2014. A model for cofactor use during HIV-1 reverse transcription and nuclear entry. Curr. Opin. Virol. 4, 32–36.
Huang, L., Li, Y., Chen, C., 2011. Flexible catalytic site conformations implicated in modulation of HIV-1 protease autoprocessing reactions. Retrovirology 8, 79.
Hyland, L.J., Tomaszek Jr., T.A., Meek, T.D., 1991. Human immunodeficiency virus-1 protease. 2. Use of pH rate studies and solvent kinetic isotope effects to elucidate details of chemical mechanism. Biochemistry 30, 8454–8463.
Ivanchenko, S., Godinez, W.J., Lampe, M., Krausslich, H.G., Eils, R., Rohr, K., Brauchle, C., Muller, B., Lamb, D.C., 2009. Dynamics of HIV-1 assembly and release. PLoS Pathog. 5, e1000652.
Jolly, C., Welsch, S., Michor, S., Sattentau, Q.J., 2011. The regulated secretory pathway in CD4(+) T cells contributes to human immunodeficiency virus type-1 cell-to-cell spread at the virological synapse. PLoS Pathog. 7, e1002226.
Jouvenet, N., Bieniasz, P.D., Simon, S.M., 2008. Imaging the biogenesis of individual HIV-1 virions in live cells. Nature 454, 236–240.
Jouvenet, N., Simon, S.M., Bieniasz, P.D., 2011. Visualizing HIV-1 assembly. J. Mol. Biol. 410, 501–511.
Kaplan, A.H., Zack, J.A., Knigge, M., Paul, D.A., Kempf, D.J., Norbeck, D.W., Swanstrom, R., 1993. Partial inhibition of the human immunodeficiency virus type 1 protease results in aberrant virus assembly and the formation of noninfectious particles. J. Virol. 67, 4050–4055.
Keller, P.W., Adamson, C.S., Heymann, J.B., Freed, E.O., Steven, A.C., 2011. HIV-1 maturation inhibitor bevirimat stabilizes the immature Gag lattice. J. Virol. 85, 1420–1428.
Keller, P.W., Huang, R.K., England, M.R., Waki, K., Cheng, N., Heymann, J.B., Craven, R.C., Freed, E.O., Steven, A.C., 2013. A two-pronged structural analysis of retroviral maturation indicates that core formation proceeds by a disassembly-reassembly pathway rather than a displacive transition. J. Virol. 87, 13655–13664.
Keller, P.W., Johnson, M.C., Vogt, V.M., 2008. Mutations in the spacer peptide and adjoining sequences in Rous sarcoma virus Gag lead to tubular budding. J. Virol. 82, 6788–6797.
Khatib, F., DiMaio, F., Foldit Contenders, G., Foldit Void Crushers, G., Cooper, S., Kazmierczyk, M., Gilski, M., Krzywda, S., Zabranska, H., Pichova, I., Thompson, J., Popovic, Z., Jaskolski, M., Baker, D., 2011. Crystal structure of a monomeric retroviral protease solved by protein folding game players. Nat. Struct. Mol. Biol. 18, 1175–1177.
Koh, Y., Aoki, M., Danish, M.L., Aoki-Ogata, H., Amano, M., Das, D., Shafer, R.W., Ghosh, A.K., Mitsuya, H., 2011. Loss of protease dimerization inhibition activity of darunavir is associated with the acquisition of resistance to darunavir by HIV-1. J. Virol. 85, 10079–10089.
Koh, Y., Matsumi, S., Das, D., Amano, M., Davis, D.A., Li, J., Leschenko, S., Baldridge, A., Shioda, T., Yarchoan, R., Ghosh, A.K., Mitsuya, H., 2007. Potent inhibition of HIV-1 replication by novel non-peptidyl small molecule inhibitors of protease dimerization. J. Biol. Chem. 282, 28709–28720.
Koh, Y., Nakata, H., Maeda, K., Ogata, H., Bilcer, G., Devasamudram, T., Kincaid, J.F., Boross, P., Wang, Y.F., Tie, Y., Volarath, P., Gaddis, L., Harrison, R.W., Weber, I.T., Ghosh, A.K., Mitsuya, H., 2003. Novel bis-tetrahydrofuranylurethane-containing nonpeptidic protease inhibitor (PI) UIC-94017 (TMC114) with potent activity against multi-PI-resistant human immunodeficiency virus in vitro. Antimicrob. Agents Chemother. 47, 3123–3129.
Kohl, N.E., Emini, E.A., Schleif, W.A., Davis, L.J., Heimbach, J.C., Dixon, R.A., Scolnick, E.M., Sigal, I.S., 1988. Active human immunodeficiency virus protease is required for viral infectivity. Proc. Natl. Acad. Sci. USA 85, 4686–4690.
Kol, N., Shi, Y., Tsvitov, M., Barlam, D., Shneck, R.Z., Kay, M.S., Rousso, I., 2007. A stiffness switch in human immunodeficiency virus. Biophys. J. 92, 1777–1783.
Konnyu, B., Sadiq, S.K., Turanyi, T., Hirmondo, R., Muller, B., Krausslich, H.G., Coveney, P.V., Muller, V., 2013. Ga–Pol processing during HIV-1 virion maturation: a systems biology approach. PLoS Comput. Biol. 9, e1003103.
Konvalinka, J., Horejsi, M., Andreansky, M., Novek, P., Pichova, I., Blaha, I., Fabry, M., Sedlacek, J., Foundling, S., Strop, P., 1992. An engineered retroviral proteinase from myeloblastosis associated virus acquires pH dependence and substrate specificity of the HIV-1 proteinase. EMBO J. 11, 1141–1144.

Konvalinka, J., Litterst, M.A., Welker, R., Kottler, H., Rippmann, F., Heuser, A.M., Krausslich, H.G., 1995. An active-site mutation in the human immunodeficiency virus type 1 proteinase (PR) causes reduced PR activity and loss of PR-mediated cytotoxicity without apparent effect on virus maturation and infectivity. J. Virol. 69, 7180–7186.
Konvalinka, J., Strop, P., Velek, J., Cerna, V., Kostka, V., Phylip, L.H., Richards, A.D., Dunn, B.M., Kay, J., 1990. Sub-site preferences of the aspartic proteinase from the human immunodeficiency virus, HIV-1. FEBS Lett. 268, 35–38.
Kotler, M., Katz, R.A., Danho, W., Leis, J., Skalka, A.M., 1988. Synthetic peptides as substrates and inhibitors of a retroviral protease. Proc. Natl. Acad. Sci. USA 85, 4185–4189.
Kovalevsky, A.Y., Ghosh, A.K., Weber, I.T., 2008. Solution kinetics measurements suggest HIV-1 protease has two binding sites for darunavir and amprenavir. J. Med. Chem. 51, 6599–6603.
Kovalevsky, A.Y., Liu, F., Leshchenko, S., Ghosh, A.K., Louis, J.M., Harrison, R.W., Weber, I.T., 2006. Ultra-high resolution crystal structure of HIV-1 protease mutant reveals two binding sites for clinical inhibitor TMC114. J. Mol. Biol. 363, 161–173.
Kozal, M.J., Shah, N., Shen, N., Yang, R., Fucini, R., Merigan, T.C., Richman, D.D., Morris, D., Hubbell, E., Chee, M., Gingeras, T.R., 1996. Extensive polymorphisms observed in HIV-1 clade B protease gene using high-density oligonucleotide arrays. Nat. Med. 2, 753–759.
Kozisek, M., Cigler, P., Lepsik, M., Fanfrlik, J., Rezacova, P., Brynda, J., Pokorna, J., Plesek, J., Gruner, B., Grantz Saskova, K., Vaclavikova, J., Kral, V., Konvalinka, J., 2008a. Inorganic polyhedral metallacarborane inhibitors of HIV protease: a new approach to overcoming antiviral resistance. J. Med. Chem. 51, 4839–4843.
Kozisek, M., Henke, S., Saskova, K.G., Jacobs, G.B., Schuch, A., Buchholz, B., Muller, V., Krausslich, H.G., Rezacova, P., Konvalinka, J., Bodem, J., 2012. Mutations in HIV-1 gag and pol compensate for the loss of viral fitness caused by a highly mutated protease. Antimicrob. Agents Chemother. 56, 4320–4330.
Kozisek, M., Lepsik, M., Grantz Saskova, K., Brynda, J., Konvalinka, J., Rezacova, P., 2014. Thermodynamic and structural analysis of HIV protease resistance to darunavir – analysis of heavily mutated patient-derived HIV-1 proteases. FEBS J. 281, 1834–1847.
Kozisek, M., Saskova, K.G., Rezacova, P., Brynda, J., van Maarseveen, N.M., De Jong, D., Boucher, C.A., Kagan, R.M., Nijhuis, M., Konvalinka, J., 2008b. Ninety-nine is not enough: molecular characterization of inhibitor-resistant human immunodeficiency virus type 1 protease mutants with insertions in the flap region. J. Virol. 82, 5869–5878.
Krausslich, H.G., 1991. Human immunodeficiency virus proteinase dimer as component of the viral polyprotein prevents particle assembly and viral infectivity. Proc. Natl. Acad. Sci. USA 88, 3213–3217.
Krausslich, H.G., Facke, M., Heuser, A.M., Konvalinka, J., Zentgraf, H., 1995. The spacer peptide between human immunodeficiency virus capsid and nucleocapsid proteins is essential for ordered assembly and viral infectivity. J. Virol. 69, 3407–3419.
Lam, P.Y., Jadhav, P.K., Eyermann, C.J., Hodge, C.N., Ru, Y., Bacheler, L.T., Meek, J.L., Otto, M.J., Rayner, M.M., Wong, Y.N., et al., 1994. Rational design of potent, bioavailable, nonpeptide cyclic ureas as HIV protease inhibitors. Science 263, 380–384.
Lanman, J., Lam, T.T., Emmett, M.R., Marshall, A.G., Sakalian, M., Prevelige Jr., P.E., 2004. Key interactions in HIV-1 maturation identified by hydrogen–deuterium exchange. Nat. Struct. Mol. Biol. 11, 676–677.
Lapatto, R., Blundell, T., Hemmings, A., Overington, J., Wilderspin, A., Wood, S., Merson, J.R., Whittle, P.J., Danley, D.E., Geoghegan, K.F., et al., 1989. X-ray analysis of HIV-1 proteinase at 2.7A resolution confirms structural homology among retroviral enzymes. Nature 342, 299–302.
Lee, S.K., Harris, J., Swanstrom, R., 2009. A strongly transdominant mutation in the human immunodeficiency virus type 1 gag gene defines an Achilles heel in the virus life cycle. J. Virol. 83, 8536–8543.
Lee, S.K., Potempa, M., Kolli, M., Ozen, A., Schiffer, C.A., Swanstrom, R., 2012. Context surrounding processing sites is crucial in determining cleavage rate of a subset of processing sites in HIV-1 Gag and Gag–Pro–Pol polyprotein precursors by viral protease. J. Biol. Chem. 287, 13279–13290.
Li, F., Goila-Gaur, R., Salzwedel, K., Kilgore, N.R., Reddick, M., Matallana, C., Castillo, A., Zoumplis, D., Martin, D.E., Orenstein, J.M., Allaway, G.P., Freed, E.O., Wild, C.T., 2003. PA-457: a potent HIV inhibitor that disrupts core condensation by targeting a late step in Gag processing. Proc. Natl. Acad. Sci. USA 100, 13555–13560.
Li, S., Hill, C.P., Sundquist, W.I., Finch, J.T., 2000. Image reconstructions of helical assemblies of the HIV-1 CA protein. Nature 407, 409–413.
Louis, J.M., Clore, G.M., Gronenborn, A.M., 1999. Autoprocessing of HIV-1 protease is tightly coupled to protein folding. Nat. Struct. Biol. 6, 868–875.
Lu, D., Vince, R., 2007. Discovery of potent HIV-1 protease inhibitors incorporating sulfoximine functionality. Bioorg. Med. Chem. Lett. 17, 5614–5619.
Mallewa, J.E., Wilkins, E., Vilar, J., Mallewa, M., Doran, D., Back, D., Pirmohamed, M., 2008. HIV-associated lipodystrophy: a review of underlying mechanisms and therapeutic options. J. Antimicrob. Chemother. 62, 648–660.
Mammano, F., Petit, C., Clavel, F., 1998. Resistance-associated loss of viral fitness in human immunodeficiency virus type 1: phenotypic analysis of protease and gag coevolution in protease inhibitor-treated patients. J. Virol. 72, 7632–7637.
Martin, N., Welsch, S., Jolly, C., Briggs, J.A., Vaux, D., Sattentau, Q.J., 2010. Virological synapse-mediated spread of human immunodeficiency virus type 1 between T cells is sensitive to entry inhibition. J. Virol. 84, 3516–3527.
Matreyek, K.A., Engelman, A., 2013. Viral and cellular requirements for the nuclear entry of retroviral preintegration nucleoprotein complexes. Viruses 5, 2483–2511.
Mattei, S., Anders, M., Konvalinka, J., Krausslich, H.G., Briggs, J.A., Muller, B., 2014. Induced maturation of human immunodeficiency virus. J. Virol. 88, 13722–13731.
Menendez-Arias, L., 2013. Molecular basis of human immunodeficiency virus type 1 drug resistance: overview and recent developments. Antivir. Res. 98, 93–120.
Mergener, K., Facke, M., Welker, R., Brinkmann, V., Gelderblom, H.R., Krausslich, H.G., 1992. Analysis of HIV particle formation using transient expression of subviral constructs in mammalian cells. Virology 186, 25–39.
Mirambeau, G., Lyonnais, S., Gorelick, R.J., 2010. Features, processing states, and heterologous protein interactions in the modulation of the retroviral nucleocapsid protein function. RNA Biol. 7, 724–734.
Moyle, G., 2001. Use of HIV protease inhibitors as pharmacoenhancers. AIDS Read. 11, 87–98, quiz 107–108.
Muller, B., Anders, M., Akiyama, H., Welsch, S., Glass, B., Nikovics, K., Clavel, F., Tervo, H.M., Keppler, O.T., Krausslich, H.G., 2009. HIV-1 Gag processing intermediates trans-dominantly interfere with HIV-1 infectivity. J. Biol. Chem. 284, 29692–29703.
Murakami, T., Ablan, S., Freed, E.O., Tanaka, Y., 2004. Regulation of human immunodeficiency virus type 1 Env-mediated membrane fusion by viral protease activity. J. Virol. 78, 1026–1031.
Nijhuis, M., van Maarseveen, N.M., Lastere, S., Schipper, P., Coakley, E., Glass, B., Rovenska, M., de Jong, D., Chappey, C., Goedegebuure, I.W., Heilek-Snyder, G., Dulude, D., Cammack, N., Brakier-Gingras, L., Konvalinka, J., Parkin, N., Krausslich, H.G., Brun-Vezinet, F., Boucher, C.A., 2007. A novel substrate-based HIV-1 protease inhibitor drug resistance mechanism. PLoS Med. 4, e36.
Oroszlan, S., Luftig, R.B., 1990. Retroviral proteinases. Curr. Top. Microbiol. Immunol. 157, 153–185.
Oshima, M., Muriaux, D., Mirro, J., Nagashima, K., Dryden, K., Yeager, M., Rein, A., 2004. Effects of blocking individual maturation cleavages in murine leukemia virus gag. J. Virol. 78, 1411–1420.
Park, J., Morrow, C.D., 1991. Overexpression of the gag–pol precursor from human immunodeficiency virus type 1 proviral genomes results in efficient proteolytic processing in the absence of virion production. J. Virol. 65, 5111–5117.
Park, J., Morrow, C.D., 1993. Mutations in the protease gene of human immunodeficiency virus type 1 affect release and stability of virus particles. Virology 194, 843–850.
Parker, S.D., Hunter, E., 2001. Activation of the Mason-Pfizer monkey virus protease within immature capsids in vitro. Proc. Natl. Acad. Sci. USA 98, 14631–14636.
Pearl, L., Blundell, T., 1984. The active site of aspartic proteinases. FEBS Lett. 174, 96–101.
Peng, K., Muranyi, W., Glass, B., Laketa, V., Yant, S.R., Tsai, L., Cihlar, T., Muller, B., Krausslich, H.G., 2014. Quantitative microscopy of functional HIV post-entry complexes reveals association of replication with the viral capsid. eLife 3, 1–21.
Perelson, A.S., Neumann, A.U., Markowitz, M., Leonard, J.M., Ho, D.D., 1996. HIV-1 dynamics in vivo: virion clearance rate, infected cell life-span, and viral generation time. Science 271, 1582–1586.
Perryman, A.L., Zhang, Q., Soutter, H.H., Rosenfeld, R., McRee, D.E., Olson, A.J., Elder, J.E., Stout, C.D., 2010. Fragment-based screen against HIV protease. Chem. Biol. Drug Des. 75, 257–268.
Pettit, S.C., Everitt, L.E., Choudhury, S., Dunn, B.M., Kaplan, A.H., 2004. Initial cleavage of the human immunodeficiency virus type 1 GagPol precursor by its activated protease occurs by an intramolecular mechanism. J. Virol. 78, 8477–8485.
Pettit, S.C., Gulnik, S., Everitt, L., Kaplan, A.H., 2003. The dimer interfaces of protease and extra-protease domains influence the activation of protease and the specificity of GagPol cleavage. J. Virol. 77, 366–374.
Pettit, S.C., Henderson, G.J., Schiffer, C.A., Swanstrom, R., 2002. Replacement of the P1 amino acid of human immunodeficiency virus type 1 Gag processing sites can inhibit or enhance the rate of cleavage by the viral protease. J. Virol. 76, 10226–10233.
Pettit, S.C., Lindquist, J.N., Kaplan, A.H., Swanstrom, R., 2005. Processing sites in the human immunodeficiency virus type 1 (HIV-1) Gag–Pro–Pol precursor are cleaved by the viral protease at different rates. Retrovirology 2, 66.
Pettit, S.C., Moody, M.D., Wehbie, R.S., Kaplan, A.H., Nantermet, P.V., Klein, C.A., Swanstrom, R., 1994. The p2 domain of human immunodeficiency virus type 1 Gag regulates sequential proteolytic processing and is required to produce fully infectious virions. J. Virol. 68, 8017–8027.
Pettit, S.C., Simsic, J., Loeb, D.D., Everitt, L., Hutchison 3rd, C.A., Swanstrom, R., 1991. Analysis of retroviral protease cleavage sites reveals two types of cleavage sites and the structural requirements of the P1 amino acid. J. Biol. Chem. 266, 14539–14547.
Pichova, I., 2013. Mason-Pfizer mokey virus retropepsin. In: Rwalings, N., Salvesen, G. (Eds.), Handbook of Proteolytic Enzymes. Academic Press Inc., London.
Pokorna, J., Machala, L., Rezacova, P., Konvalinka, J., 2009. Current and novel inhibitors of HIV protease. Viruses 1, 1209–1239.
Prabu-Jeyabalan, M., Nalivaika, E., Schiffer, C.A., 2000. How does a symmetric dimer recognize an asymmetric substrate? A substrate complex of HIV-1 protease. J. Mol. Biol. 301, 1207–1220.
Prabu-Jeyabalan, M., Nalivaika, E., Schiffer, C.A., 2002. Substrate shape determines specificity of recognition for HIV-1 protease: analysis of crystal structures of six substrate complexes. Structure 10, 369–381.
Reil, H., Bukovsky, A.A., Gelderblom, H.R., Gottlinger, H.G., 1998. Efficient HIV-1 replication can occur in the absence of the viral matrix protein. EMBO J. 17, 2699–2708.
Rein, A., Mirro, J., Haynes, J.G., Ernst, S.M., Nagashima, K., 1994. Function of the cytoplasmic domain of a retroviral transmembrane protein: p15E-p2E cleavage

activates the membrane fusion capability of the murine leukemia virus Env protein. J. Virol. 68, 1773–1781.
Rezacova, P., Pokorna, J., Brynda, J., Kozisek, M., Cigler, P., Lepsik, M., Fanfrlik, J., Rezac, J., Grantz Saskova, K., Sieglova, I., Plesek, J., Sicha, V., Gruner, B., Oberwinkler, H., Sedlacek, J., Krausslich, H.G., Hobza, P., Kral, V., Konvalinka, J., 2009. Design of HIV protease inhibitors based on inorganic polyhedral metallacarboranes. J. Med. Chem. 52, 7132–7141.
Rhee, S.Y., Taylor, J., Fessel, W.J., Kaufman, D., Towner, W., Troia, P., Ruane, P., Hellinger, J., Shirvani, V., Zolopa, A., Shafer, R.W., 2010. HIV-1 protease mutations and protease inhibitor cross-resistance. Antimicrob. Agents Chemother. 54, 4253–4261.
Richards, A.D., Phylip, L.H., Farmerie, W.G., Scarborough, P.E., Alvarez, A., Dunn, B.M., Hirel, P.H., Konvalinka, J., Strop, P., Pavlickova, L., et al., 1990. Sensitive, soluble chromogenic substrates for HIV-1 proteinase. J. Biol. Chem. 265, 7733–7736.
Ro, S., Baek, S.G., Lee, B., Park, C., Choy, N., Lee, C.S., Son, Y.C., Choi, H., Koh, J.S., Yoon, H., Kim, S.C., Ok, J.H., 1998. NMR and topochemical studies of peptidomimetic HIV-I protease inhibitors containing a cis-epoxide amide isostere. Bioorg. Med. Chem. Lett. 8, 2423–2426.
Saskova, K.G., Kozisek, M., Rezacova, P., Brynda, J., Yashina, T., Kagan, R.M., Konvalinka, J., 2009. Molecular characterization of clinical isolates of human immunodeficiency virus resistant to the protease inhibitor darunavir. J. Virol. 83, 8810–8818.
Schilling, O., auf dem Keller, U., Overall, C.M., 2011. Protease specificity profiling by tandem mass spectrometry using proteome-derived peptide libraries. Methods Mol. Biol. 753, 257–272.
Schimer, J., Cigler, P., Vesely, J., Grantz Saskova, K., Lepsik, M., Brynda, J., Rezacova, P., Kozisek, M., Cisarova, I., Oberwinkler, H., Kraeusslich, H.G., Konvalinka, J., 2012. Structure-aided design of novel inhibitors of HIV protease based on a benzodiazepine scaffold. J. Med. Chem. 55, 10130–10135.
Schimer, J., Konvalinka, J., 2014. Unorthodox inhibitors of HIV protease: looking beyond active-site-directed peptidomimetics. Curr. Pharm. Des. 20, 3389–3397.
Schimer, J., Pavova, M., Anders, M., Pachl, P., Sácha, P., Cigler, P., Weber, J., Majer, P., Rezácová, P., Kräusslich, H., Müller, B., Konvalinka, J., 2015. Triggering HIV polyprotein processing inside virions by rapid photodegradation of a tight-binding photodestructable protease inhibitor. Nature Commun. 6, 1-8. http://dx.doi.org/10.1038/ncomms7461 (Article no. 6461).
Schur, F.K., Hagen, W.J., Rumlova, M., Ruml, T., Muller, B., Krausslich, H.G., Briggs, J.A., 2014. Structure of the immature HIV-1 capsid in intact virus particles at 8.8A resolution. Nature.
Sedlacek, J., Fabry, M., Coward, J.E., Horejsi, M., Strop, P., Luftig, R.B., 1993. Myeloblastosis associated virus (MAV) proteinase site-mutated to be HIV-like has a higher activity and allows production of infectious but morphologically altered virus. Virology 192, 667–672.
Sham, H.L., Kempf, D.J., Molla, A., Marsh, K.C., Kumar, G.N., Chen, C.M., Kati, W., Stewart, K., Lal, R., Hsu, A., Betebenner, D., Korneyeva, M., Vasavanonda, S., McDonald, E., Saldivar, A., Wideburg, N., Chen, X., Niu, P., Park, C., Jayanti, V., Grabowski, B., Granneman, G.R., Sun, E., Japour, A.J., Leonard, J.M., Plattner, J.J., Norbeck, D.W., 1998. ABT-378, a highly potent inhibitor of the human immunodeficiency virus protease. Antimicrob. Agents Chemother. 42, 3218–3224.
Shultz, M.D., Ham, Y.W., Lee, S.G., Davis, D.A., Brown, C., Chmielewski, J., 2004. Small-molecule dimerization inhibitors of wild-type and mutant HIV protease: a focused library approach. J. Am. Chem. Soc. 126, 9886–9887.
Smith 3rd, A.B., Charnley, A.K., Hirschmann, R., 2011. Pyrrolinone-based peptidomimetics. “Let the enzyme or receptor be the judge”. Acc. Chem. Res. 44, 180–193.
Sperka, T., Pitlik, J., Bagossi, P., Tozser, J., 2005. Beta-lactam compounds as apparently uncompetitive inhibitors of HIV-1 protease. Bioorg. Med. Chem. Lett. 15, 3086–3090.
Stewart, L., Schatz, G., Vogt, V.M., 1990. Properties of avian retrovirus particles defective in viral protease. J. Virol. 64, 5076–5092.
Strisovsky, K., Smrz, D., Fehrmann, F., Krausslich, H.G., Konvalinka, J., 2002. The murine endogenous retrovirus MIA14 encodes an active aspartic proteinase that is functionally similar to proteinases from D-type retroviruses. Arch. Biochem. Biophys. 398, 261–268.
Swanstrom, R., Wills, J.W., 1997. Synthesis, assembly, and processing of viral proteins. In: Coffin, J.M., Hughes, S.H., Varmus, H.E. (Eds.), Retroviruses. Cold Spring Harbor, NY.
Tang, C., Louis, J.M., Aniana, A., Suh, J.Y., Clore, G.M., 2008. Visualizing transient events in amino-terminal autoprocessing of HIV-1 protease. Nature 455, 693–696.
Thomsen, M.C., Nielsen, M., 2012. Seq2Logo: a method for construction and visualization of amino acid binding motifs and sequence profiles including sequence weighting, pseudo counts and two-sided representation of amino acid enrichment and depletion. Nucleic Acids Res. 40, W281–287.
Tozser, J., Bagossi, P., Weber, I.T., Louis, J.M., Copeland, T.D., Oroszlan, S., 1997. Studies on the symmetry and sequence context dependence of the HIV-1 proteinase specificity. J. Biol. Chem. 272, 16807–16814.
Tozser, J., Oroszlan, S., 2003. Proteolytic events of HIV-1 replication as targets for therapeutic intervention. Curr. Pharm. Des. 9, 1803–1815.
Tykvart, J., Barinka, C., Svoboda, M., Navrátil, V., Soucek, R., Hubálek, M., Hradilek, M., Sácha, P., Lubkowski, J., Konvalinka, J., Structural and biochemical characterization of a novel aminopeptidase from human intestine. J. Biol. Chem., 2015, http://dx.doi.org/10.1074/jbc.M114.628149.
Veverka, V., Bauerova, H., Zabransky, A., Pichova, I., Hrabal, R., 2001. Backbone resonance assignment of protease from Mason-Pfizer monkey virus. J. Biomol. NMR 20, 291–292.
Vidu, A., Dufau, L., Bannwarth, L., Soulier, J.L., Sicsic, S., Piarulli, U., Reboud-Ravaux, M., Ongeri, S., 2010. Toward the first nonpeptidic molecular tong inhibitor of wild-type and mutated HIV-1 protease dimerization. ChemMedChem 5, 1899–1906.
Vogt, V.M., 1997. Retroviral Virions and Genomes. In: Coffin, J.M., Hughes, S.H., Varmus, H.E. (Eds.), Retroviruses. Cold Spring Harbor, NY.
Vogt, V.M., Eisenman, R., 1973. Identification of a large polypeptide precursor of avian oncornavirus proteins. Proc. Natl. Acad. Sci. USA 70, 1734–1738.
Vogt, V.M., Eisenman, R., Diggelmann, H., 1975. Generation of avian myeloblastosis virus structural proteins by proteolytic cleavage of a precursor polypeptide. J. Mol. Biol. 96, 471–493.
Vogt, V.M., Wight, A., Eisenman, R., 1979. In vitro cleavage of avian retrovirus gag proteins by viral protease p15. Virology 98, 154–167.
von der Helm, K., 1977. Cleavage of Rous sarcoma viral polypeptide precursor into internal structural proteins in vitro involves viral protein p15. Proc. Natl. Acad. Sci. USA 74, 911–915.
Votteler, J., Sundquist, W.I., 2013. Virus budding and the ESCRT pathway. Cell Host Microbe 14, 232–241.
Welker, R., Janetzko, A., Krausslich, H.G., 1997. Plasma membrane targeting of chimeric intracisternal A-type particle polyproteins leads to particle release and specific activation of the viral proteinase. J. Virol. 71, 5209–5217.
Wiegers, K., Rutter, G., Kottler, H., Tessmer, U., Hohenberg, H., Krausslich, H.G., 1998. Sequential steps in human immunodeficiency virus particle maturation revealed by alterations of individual Gag polyprotein cleavage sites. J. Virol. 72, 2846–2854.
Wlodawer, A., Erickson, J.W., 1993. Structure-based inhibitors of HIV-1 protease. Annu. Rev. Biochem. 62, 543–585.
Wong, E.L., Sun, R.W., Chung, N.P., Lin, C.L., Zhu, N., Che, C.M., 2006. A mixed-valent ruthenium-oxo oxalato cluster Na7[Ru4(mu3-O)4(C2O4)6] with potent anti-HIV activities. J. Am. Chem. Soc. 128, 4938–4939.
Woodward, C.L., Cheng, S.N., Jensen, G.J., 2015. Electron cryotomography studies of maturing HIV-1 particles reveal the assembly pathway of the viral core. J. Virol. 89, 1267–1277.
Wright, E.R., Schooler, J.B., Ding, H.J., Kieffer, C., Fillmore, C., Sundquist, W.I., Jensen, G.J., 2007. Electron cryotomography of immature HIV-1 virions reveals the structure of the CA and SP1 Gag shells. EMBO J. 26, 2218–2226.
Wu, X., Ohrngren, P., Joshi, A.A., Trejos, A., Persson, M., Arvela, R.K., Wallberg, H., Vrang, L., Rosenquist, A., Samuelsson, B.B., Unge, J., Larhed, M., 2012. Synthesis, X-ray analysis, and biological evaluation of a new class of stereopure lactam-based HIV-1 protease inhibitors. J. Med. Chem. 55, 2724–2736.
Wyma, D.J., Jiang, J., Shi, J., Zhou, J., Lineberger, J.E., Miller, M.D., Aiken, C., 2004. Coupling of human immunodeficiency virus type 1 fusion to virion maturation: a novel role of the gp41 cytoplasmic tail. J. Virol. 78, 3429–3435.
Xiang, Y., Ridky, T.W., Krishna, N.K., Leis, J., 1997. Altered Rous sarcoma virus Gag polyprotein processing and its effects on particle formation. J. Virol. 71, 2083–2091.
Yoshinaka, Y., Luftig, R.B., 1977. Properties of a P70 proteolytic factor of murine leukemia viruses. Cell 12, 709–719.
Yu, Z., Dobro, M.J., Woodward, C.L., Levandovsky, A., Danielson, C.M., Sandrin, V., Shi, J., Aiken, C., Zandi, R., Hope, T.J., Jensen, G.J., 2013. Unclosed HIV-1 capsids suggest a curled sheet model of assembly. J. Mol. Biol. 425, 112–123.
Zhang, Y.M., Imamichi, H., Imamichi, T., Lane, H.C., Falloon, J., Vasudevachari, M.B., Salzman, N.P., 1997. Drug resistance during indinavir therapy is caused by mutations in the protease gene and in its Gag substrate cleavage sites. J. Virol. 71, 6662–6670.
Zhang, Z.Y., Poorman, R.A., Maggiora, L.L., Heinrikson, R.L., Kezdy, F.J., 1991. Dissociative inhibition of dimeric enzymes. Kinetic characterization of the inhibition of HIV-1 protease by its COOH-terminal tetrapeptide. J. Biol. Chem. 266, 15591–15594.
Zhu, P., Liu, J., Bess Jr., J., Chertova, E., Lifson, J.D., Grise, H., Ofek, G.A., Taylor, K.A., Roux, K.H., 2006. Distribution and three-dimensional structure of AIDS virus envelope spikes. Nature 441, 847–852.
Zutshi, R., Chmielewski, J., 2000. Targeting the dimerization interface for irreversible inhibition of HIV-1 protease. Bioorg. Med. Chem. Lett. 10, 1901–1903.