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The structural biology of HIV-1: mechanistic and therapeutic insights

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Abstract

Three-dimensional molecular structures can provide detailed information on biological mechanisms and, in cases where molecular function impacts on human health, significantly aid in the development of therapeutic interventions. Over the past 23 years, key components of the lentivirus HIV-1, including its envelope glycoproteins and capsid, and the replication enzymes reverse transcriptase, integrase and protease, have accordingly been scrutinized to near atomic scale resolution. Structural analyses of the interactions between viral and host cell components have moreover yielded key insights into the mechanisms of virus entry, chromosomal integration, transcription and egress from cells. Here, we review recent advances in HIV-1 structural biology, focusing on the impact these results have had on our understanding of virus replication and the development of new therapeutics.

HIV-1 arose through several independent zoonotic transmissions of simian immunodeficiency viruses during the last century [1–3]. Today, HIV-1, along with its less widespread cousin HIV-2, infects over 30 million people worldwide. Both viruses belong to the *Retroviridae*, a viral family that has left numerous scars of ancient infections in mammalian genomes, with derelict retroviral sequences comprising as much as 8% of our "own" DNA [4]. The evolutionary success of this family is contrasted by its deceptive simplicity: encoding only 16 proteins, HIV-1 can persistently infect humans, subverting the innate and adaptive immune systems. Viral replication at the cellular level proceeds through a series of steps that start when a virus productively engages cell surface receptors and ends when nascent particles mature into infectious virions (Fig. 1). During this process, HIV-1 exploits a myriad of cellular factors to accomplish specific tasks at the same time as host restriction factors fight to suppress replication [5,6]. The mainstream highly active anti-retroviral therapy (HAART) drug cocktails that are primarily used to target the reverse transcriptase (RT) and protease (PR) enzymes potently suppress viral loads and transmission rates, yet complications can arise from compound toxicity and the emergence of resistant strains (Box 1). Advances in structural biology can aid the development of next-generation compounds that are active against previously exploited targets, help to define new drug targets, and boost the effectiveness of vaccination strategies. This review proceeds stepwise through the HIV-1 replication cycle, highlighting the impact that major structural biology advances have had on our understanding of virus growth and the development of new anti-retroviral therapies.

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Box 1

Highly active anti-retroviral therapy

Approximately thirty different drugs targeting four different steps in the HIV-1 replication cycle are currently approved for administration to HIV-positive individuals in the US (see http://www.aidsmeds.com/list.shtml). Nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) target the reverse transcription step that converts the viral genomic RNA into linear double stranded DNA whereas protease inhibitors (PIs) inhibit protease enzyme activity that is critical for the maturation of viral particles that bud out from infected cells. Two different inhibitors can block the entry of the virus into new target cells by thwarting either the interaction between the viral envelope glycoprotein gp120 and the CCR5 co-receptor (maraviroc) or the formation of the gp41 six-helix bundle that drives the fusion between the viral and cellular membranes (Fuzeon). The sole integrase strand transfer inhibitor (INSTI), raltegravir, blocks integrase's strand transfer activity that is required to insert viral DNA into a host cell chromosome. Highly active anti-retroviral therapy or HAART routinely prescribes an NRTI, NNRTI and PI as a single pill or in various pill combinations. The combinatorial approach to drug treatment significantly suppresses the probability for the selection and resulting outgrowth of resistant HIV-1 strains that quickly arise during monotherapy.

Virus entry

The HIV-1 envelope spikes, which comprise trimers of non-covalently linked heterodimers of the surface gp120 and transmembrane gp41 glycoproteins [7–9], initiate a cascade of conformational changes that culminates in fusion between the viral and host cell membranes and the release of the viral core into the cytoplasm. HIV-1 primarily infects CD4-positive T lymphocytes and macrophage cells. An initial interaction between gp120 and the surface receptor CD4 induces the formation of a bridging sheet between the inner and outer domains of the gp120 monomer, exposing the binding site for a second cell surface molecule, typically the chemokine receptor CCR5 [10–12] (Fig. 1, step 1). Co-receptor engagement leads to insertion of the fusion peptide located at the N-terminus of gp41 into the cell membrane, which in turn triggers significant rearrangements between trimerized N- and C-terminal heptad repeat sequences within gp41, the formation of a six helical hairpin structure, and the apposition and fusion of the viral and host cell membranes [13–15](Fig. 1, step 2).

Initial cryo-electron tomography studies provided crucial glimpses of the HIV-1 envelope and its associated conformational flexibility [7,8], although the low-resolution models that were generated left many key aspects of the native structure unresolved [9,16,17]. Higher-resolution crystallographic studies using engineered HIV-1 glycoprotein constructs have been instrumental in developing entry inhibitors and elucidating the mechanistic basis of virus neutralization by antibodies. Recent studies have highlighted the striking flexibility of the core gp120 structure, which allows extreme conformational changes upon CD4 engagement without destabilizing the interaction with gp41 [12,18]. CD4 binds gp120 at a depression formed between the inner and outer domains, where the CD4 residue Phe43 partially fills a hydrophobic cavity (Fig. 2a) [10]. Small molecules designed to bind to and extend further into this pocket display antiviral activity, and increasing the gp120 binding affinity might lead to the development of clinically useful inhibitors [19].

Most antibodies directed against gp120 tend to be strain-specific and moreover fail to neutralize the virus. Several groups recently described patient-derived gp120-reactive antibodies with broad HIV-1 neutralization activity [20–24]. Wu and colleagues [21,22] took a

structure-based approach to stabilize the CD4-bound conformation of gp120 using disulfide bonds and redesign its surface to mask positions exterior to the CD4 binding site. Using one such construct as bait, patient B cell clones producing antibodies with remarkably broad neutralizing activity were recovered. Structural characterization of these antibodies revealed that in binding to gp120, the heavy chains of the immunoglobulins mimic CD4 (Fig. 2a, b), with their epitopes almost precisely overlapping the primary CD4-binding site on gp120 [22,25]. These results define the structural basis for HIV-1 neutralization by antibodies that engage the CD4 binding site. Interestingly, immunoglobulins isolated from the sera of different donors using the resurfaced gp120 construct were derived from the same precursor heavy chain gene (IGHV1-2*02) that had subsequently undergone extensive affinity maturation [21,22,25]. The requirement for extensive somatic mutation to achieve virus neutralization [21,22] might pose a challenge for the experimental elicitation of such antibodies. However, the recent discovery of highly potent gp120-binding antibodies with alternative modes of action suggests there are multiple genetic pathways to achieve cross-clade HIV-1 neutralization [20,23,24]. These results should encourage attempts to design immunogens to elicit humoralimmunity for vaccination purposes.

Peptides derived from gp41 N-terminal [26] or C-terminal [27] sequences, which disrupt the six-helix bundle formation and hence membrane fusion, possess potent antiviral activity. A peptide based on the C-terminal sequence was licensed as Fuzeon in 2003, although the requirement for twice-daily injections and the relative ease through which drug mutations arise have limited its utility. D-peptides that target a pocket at the base of the N-terminal gp41 helical structure are also potent antivirals, and may overcome some of the limitations associated with Fuzeon use [28].

Post-entry events: uncoating to integration

The HIV core, which houses the replication enzymes RT and integrase (IN) as well as the viral genomic RNA, is encased by a cone-shaped shell [29] composed of the viral capsid (CA) protein. Recent work has highlighted interactions among composite CA molecules that underlie the structural integrity and functionalityof the protective shell [30–32].

Uncoating

Partial CA shell dissolution, which is required for reverse transcription [33,34], is a recently verified therapeutic target [35] (Fig. 1, step 3). Moreover, the underlying features of the assembled shell seem to determine its propensity to uncoat [32]. CA protein, which comprises independently folded N-terminal and C-terminal domains (NTD and CTD) connected by a flexible linker [36,37], can assemble into ring structures containing five or six protomers [31,32] (Fig. 3a, b). The rings further congregate to form a fullerene cone composed predominantly of hexamers; seven pentamers at the wide end and five at the narrow end allow for shape declinations [32,38] (Fig. 3c), and the flexibility of intramolecular NTD–CTD and intermolecular CTD–CTD interactions further contribute to the curvature of the shell lattice [30,32] (Fig. 3a, b). The relatively high concentration of penton declinations expected at the narrow end of the cone may furthermore serve to initiate uncoating [32].

TRIM5α, a potent HIV-1 restriction factor isolated from rhesus macaques [39], recognizes the assembled CA structure to accelerate uncoating [40] and activate innate immune signalling pathways [41]. A replacement of the N-terminal RING domain of rhesus TRIM5α with that from the related human TRIM21 protein yielded a chimera that is amenable to recombinant techniques [42]. The hybrid construct forms 2D hexameric crystalline arrays in the presence of a higher-order six-fold lattice of HIV-1 CA [43]. Such CA-templated multimerisation may underlie functional HIV-1 restrictionby rhesus TRIM5 α through a pattern recognition mechanism that is common to other components of the innate immune system [41].

Stimulation of premature uncoating could moreover be a useful therapeutic approach; for example, PF-3450074, a small molecule inhibitor of HIV-1 replication that binds to a pocket within the NTD of CA (Fig. 3d), may work by triggering premature uncoating through destabilization of CA–CA interactions [35,44].

Viral DNA synthesis

Reverse transcription and integration of the resultant linear viral DNA molecule into a host cell chromosome occurs within the context of the nucleoprotein complex structures that are derived from the viral core (Fig. 1, steps 4–6). High-resolution HIV-1 RT structures have been available for a number of years, with initial drug-and nucleic acid template -bound crystal structures reported nearly 2 decades ago [45,46].

HIV-1 RT is a heterodimer composed of p66 and p51 subunits, with p66 harbouring two functional active sites: an N-terminal RNA- and DNA-dependent DNA polymerase and a C-terminal RNase H that digests the RNA component of RNA/DNA hybrids. The polymerase domain resembles a right hand with four subdomains: fingers, thumb, palm and connection (Fig. 4a) [45–48]. During DNA polymerization, the catalytic residues Asp110, Asp185 and Asp186 within the palm subdomain activate the DNA primer 3′ hydroxyl and stabilize the hypothetical pentavalent α-phosphorous intermediate state within the substrate dNTP, incorporating the nucleotide into the growing DNA chain and liberating free pyrophosphate (Fig. 4b) [48].

Two classes of antiviral drugs, nucleoside and non-nucleoside RT inhibitors (NRTI and NNRTI, respectively), inhibit DNA polymerization and are core components of HAART (Box 1). Upon phosphorylation in infected cells, NRTIs mimic natural nucleoside triphosphates and are incorporated into the viral DNA by RT. Lacking the 3′-OH group needed for incorporation of the subsequent nucleotide, NRTIs act as chain terminators, and viral resistance to some of these small molecules accordingly occurs through drug exclusion mechanisms. For instance, mutations of Met184 (to Val or Ile) selectively preclude the binding of oxathiolane-containing inhibitors like 3TC over dNTPs with normal deoxyribose rings (Fig. 4b) [48,49]. However, resistance to azidothymidine (AZT) and other thymidine analogues puzzled researchers for some time: inexplicably, the mutant RT from AZT-resistant virus strains efficiently incorporated AZT monophosphate into the viral DNA [50]. Instead of preventing incorporation, the mutant enzyme developed the ability to excise the incorporated drug from the primer strand. Remarkably, RT accomplishes this by utilizing ATP as a pyrophosphate donor to excise the incorporated drug in the form of an AZT-adenosine tetraphosphate adduct, regenerating an active 3′-OH primer terminus in a reaction that is mechanistically equivalent to the reversal of the polymerization step [51,52]. Recent structural analyses revealed that the AZT resistance mutations K70R, T215Y and K219Q create an optimal ATP-binding site between the fingers and palm subdomains of RT to promote the excision reaction [53](Fig. 4c).

NNRTIs are allosteric inhibitors that induce the formation of a flexible binding pocket through relatively large conformational changes involving Tyr181, Tyr188 and the primer grip (residues 227–235 within the palm subdomain) [45,54,55] (Fig. 4d). The mechanistic basis of inhibition may be due to displacement of the primer grip [56] or the 3-stranded β-sheet that contains the catalytic triad [55,57]. Stacking interactions between the aromatic side-chains of Tyr181 and Tyr188 and first-generation NNRTIs like nevirapine contribute significantly to drug binding [45], and the associated mutations accordingly conferred resistance due to loss of aromatic character [58]. K103N is also fairly widely associated with NNRTI resistance, and the Asn103-Tyr188 interaction in the mutant RT appears to restrict the movement of Tyr188 that is required for drug binding [59,60]. The more recently developed diarylpyrimidine NNRTIs TMC-125 and TMC-278 retain potency in the face of first-generation NNRTI

resistance mutations, with inherent drug flexibility contributing significantly to high affinity compound binding to the mutant RT [61](Fig. 4d).

Reverse transcription is inhibited by the cellular restriction factor APOBEC3G, a virion-incorporated cytidine deaminase that both impedes elongation [62,63] and converts nascent cytidines in viral cDNA to uracils [64–66]. HIV-1 accordingly deploys a countermeasure, the Vif protein, which antagonizes the incorporation of APOBEC3G by binding and inducing its degradation in virus producer cells [67,68]. Such observations highlight the importance of the Vif–APOBEC3G nexus for antiviral drug development, and small molecules that limit the ability of Vif to degrade APOBEC3G and, accordingly, inhibit HIV-1 infection have been described [69,70].

APOBEC3G harbours two cytidine deaminase domains: the NTD mediates virion incorporation whereas the CTD is a functional deaminase [71–73]. Several NMR [74–76] and X-ray crystal [77,78] structures of the CTD revealed a 5-stranded β sheet intermixed with 5 helices, with conserved elements of the catalytic zinc-coordination motif (H/C-X-E-$X_{23–28}$-PC-X_2-C) contributed by a pair of α helices. These results afford important glimpses into the mechanism of HIV deamination, although additional structures that incorporate the NTD and especially the single-stranded DNA substrate will reveal a more complete picture of catalysis. Structures that include Vif should further aid the development of novel antiviral compounds.

Integration

IN possesses two catalytic activities, 3′ processing and DNA strand transfer. Each end of the HIV-1 DNA long terminal repeat (LTR) is cleaved adjacent to the invariant dinucleotide sequence CA, unveiling recessed 3′ termini. IN then uses the 3′ hydroxyls to cut chromosomal DNA strands across a major groove, at the same time joining the viral DNA ends to the target DNA 5′-phosphates. Host enzymes complete the integration process by repairing the single strand gaps abutting the unjoined viral DNA 5′ ends, resulting in establishment of a stable provirus (Fig. 1, step 6). IN-mediated reversal of integration is impossible, although rare instances of cell-mediated homologous recombination across the LTRs can excise proviral DNA [79]. Site-specific recombinases can be engineered to similarly excise the HIV-1 provirus *ex vivo* [80], although such approaches would appear to be far from clinical application.

Although crystal and NMR structures of various fragments of HIV-1 IN were reported over several years [81], detailed views of the functional IN-viral DNA nucleoprotein complex, or intasome, were lacking until recently. Given that clinically useful HIV-1 IN inhibitors selectively interact with the intasome rather than free IN [82], this dearth of structural information limited drug development. Recent successes are owed to the tractability of the intasome derived from the related prototype foamy virus (PFV), a member of the *Spumavirus* retroviral genus, by X-ray crystallography [83,84]. An overview of these advances is given here; for in-depth reviews see refs [85,86].

The intasome contains a dimer-of-dimers of IN, with only one subunit of each dimer binding a viral DNA end [83] (Fig. 5a, b). Thus, akin to RT, functional IN active sites are delegated to a subset of protein molecules within the multimeric complex. The intasome accommodates the target DNA within a cleft between the functional active sites in a severely bent conformation (Fig. 5b, c). The contortion in target DNA allows the intasome active sites (which are separated from one another by as much as 26.5 Å) to access their target scissile phosphodiester bonds [84]. The residues of the catalytic D, D-35-E motif coordinate two divalent metal ions, revealing roles in viral DNA 3′-OH nucleophile activation and scissile phosphodiester bond destabilization during DNA strand transfer [83,84] (Fig. 5c). The reversal

of the reaction appears to be restricted by a conformational change, which causes a 2.3-Å displacement of the newly formed viral-target DNA phosphodiester bond from the IN active site following transesterification [84].

The clinically approved HIV-1 IN inhibitor raltegravir and similar small molecules that are in development preferentially inhibit DNA strand transfer activity, and IN strand transfer inhibitors (INSTIs) fortuitously harbour broad anti-retroviral activity [87–89]. Results based on PFV intasome-INSTI co-crystal structures have been accordingly illuminating. INSTIs harbour two common moieties: co-planar heteroatoms (typically three oxygen atoms) that chelate the active site metal ions [90] and halogenated benzyl groups, whose function until recently was largely speculative. INSTIs engage the bound metal ions, only slightly influencing their positions within the IN active site. Primarily through interactions with the penultimate viral DNA G·C base pair and a 3_{10} helix (Pro145-Gln146 in HIV-1 IN), INSTI halogenated benzyl groups assume the position of the terminal adenine ring, ejecting the viral 3′-deoxyadenosine with its associated 3′-OH nucleophile from the active site [83,88]. This displacement of the DNA strand transfer nucleophile forms the mechanistic basis of INSTI action. In addition, INSTIs sterically preclude target DNA binding, explaining the competition between target DNA and the small molecules [82,84]. The PFV model has provided important clues about the mechanism of drug resistance associated with HIV-1 IN mutations selected in the presence of raltegravir [88].

Analogous to RT, there is precedence that a second region of HIV-1 IN, in this case distal from the active site, affords an opportune location for allosteric inhibitor binding. Lentiviruses such as HIV-1 favour integration within active genes due to an interaction between IN and the chromatin binding protein LEDGF/p75 (reviewed in [91]). The IN binding domain (IBD) of LEDGF/p75 is a pseudo HEAT repeat analogous topology domain that consists of two units of a helix-hairpin-helix repeat [92], and the LEDGF/p75 hotspot residues Ile365 and Asp366 at the tip of the N-terminal hairpin nestle into a cleft at the HIV-1 IN CCD dimer interface [93]. In a remarkable example of structure-based drug design, Debyser and colleagues discovered a novel class of HIV-1 IN inhibitors capable of suppressing viral replication. These small molecules, termed LEDGINs, mimic the LEDGF/p75-IN interaction *in silico* and inhibit protein-protein binding *in vitro* [94]. Given the highly conserved nature of INSTI binding at the active site [88,95] and the likelihood of considerable cross-resistance among INSTIs [96], the development of such allosteric HIV-1 IN inhibitors is highly desirable.

Viral mRNA biogenesis and transport

Integration marks the transition from the early to late phase of HIV-1 replication, in which the focus shifts to viral gene expression followed by the assembly and egress of nascent viral particles. Transcription, which initiates from the U3 promoter within the upstream LTR (Fig. 1, step 7), requires the viral Tat transactivator protein for efficient elongation. Viral mRNAs are produced as a variety of alternatively spliced species. The smaller messages are exported readily from the nucleus, whereas the unspliced and singly spliced mRNAs require the action of Rev. This small viral protein acts as an adaptor, binding to the Rev-response element (RRE) located within the mRNA *env* coding region and the nuclear export factor CRM1 (step 8 in Fig. 1). Recent structural biology advances yield insight into the mechanisms of Tat transactivation [97] and Rev-dependent mRNA export [98,99].

Transcriptional elongation

Tat recruits the cellular positive transcription elongation factor P-TEFb, comprising the Cdk9 kinase and cyclin T1 (CycT1) subunits, to the viral trans-activation response (TAR) element present in stalled transcripts [100,101]. Subsequent phosphorylation of the heptad

repeat residues Ser2 and Ser5 in the CTD of the large subunit of RNA polymerase II by activated Cdk9 stimulates transcriptional elongation.

Tat is largely unstructured in the absence of binding ligands [102]. TAR binding occurs primarily via an α-helical Arg-rich motif (ARM), which inserts into the RNA major groove within the stem-loop structure [103]. The N-terminal activation domain of Tat, which contains acidic/Pro-rich, zinc binding motifs and core subdomains, assumes an ordered structure upon P-TEFb binding [97]. Within the complex, Tat primarily interacts with the CycT1 subunit, also contacting the T loop region of Cdk9 (Fig. 6a). Tat binding stimulates phosphorylation of RNA polymerase II CTD Ser5 heptad repeat residues by Cdk9 [104] and reciprocal conformation changes in the kinase accordingly alter the substrate-binding surface of P-TEFb. Crucially, the fact that Tat induces conformational changes in P-TEFb suggests that it may be possible to develop anti-HIV agents directed against P-TEFb with limited sideeffects on its normal cellular functions [97].

mRNA export

Rev binds to the RRE in a highly cooperative manner, forming an RNA-dependent dimer en route to a higher order Rev-RNA multimer [105,106]. The structural basis for Rev multimerisation was recently elucidated by two complementary crystallographic studies [98,99]. Rev adopts an amphipathic helical hairpin, which multimerizes via face-to-face and back-to-back symmetric interfaces stabilized by conserved hydrophobic interactions (Fig. 6b). Collectively, the crystal structures [98,99] describe both types of interface and allow modelling of a Rev hexamer, which projects pairs of ARMs on one side and C-terminal nuclear export signals for latching onto the cellular CRM1 nuclear export factor on the other (Fig. 6b). The relative orientations of the ARMs in the context of the oligomer are thought to dictate the selectivity of the viral protein for the RRE structure and sequence. The model also accounts for the cooperativity of RNA binding by Rev, although a more complete structure including the RRE will be required to explain the details of protein-RNA recognition.

Viral egress and maturation

The retroviral structural proteins CA, matrix (MA) and NC are synthesized as parts of the Gag precursor polypeptide, and HIV-1 Gag is sufficient to assemble virus-like particles at the plasma membrane and bud from cells [107] (Fig. 1, steps 10 and 11). MA, through an N-terminal myristic acid [108,109] and conserved basic amino acid residues [110–112], contributes to Gag membrane association. The differential exposure of the myristate through a process known as the myristyl switch [113] allows Gag to associate preferentially with the plasma membrane rather than intracellular membranes. The switch can be activated by phosphatidylinositol 4,5-bisphosphate [114], a phospholipid that is concentrated in the inner leaflet of the plasma membrane and interacts directly with MA [115]. Several steps along the pathway of HIV-1 assembly and particle release from cells have been targeted for antiviral drug development.

Viral late domains and the cellular ESCRT machinery

Retroviral budding is orchestrated by interactions between Pro-rich motifs in Gag that are known as late (L) domains and cellular class E vacuolar protein sorting (Vps) proteins, the actions of which are required to form the nascent particle and sever it from the plasma membrane. The intended functions of Vps proteins are in the formation of multi-vesicular bodies (MVBs), a reaction that is topologically identical to virus budding as in each case a membrane-coated vesicle leaves the cytoplasm, and in abscission during cell division [116,117]. Most class E Vps proteins function as subunits of endosomal sorting

complexes required for transport (ESCRT), which come in four varieties (ESCRT-0, ESCRT-I, ESCRT-II, ESCRT-III). ESCRT-I and ESCRT-II function during membrane budding, whereas ESCRT-III is important for membrane scission. Recent advances have yielded structures of several class E proteins as well as the class E protein–L domain interactions that are crucial for virus budding from infected cells (see [118,119] for in -depth reviews).

The C-terminal HIV-1 Gag cleavage product p6 harbours two L domains: P(T/S)AP and $LYPx_{1–3}L$ [120,121]. The TSG101 component of ESCRT-I engages P(T/S)AP whereas ALIX, itself not formally an ESCRT protein, binds $LYPx_{1–3}L$ [121,122]. ALIX contains three domains, an N-terminal Bro1 domain, an interior V domain and a C-terminal proline-rich domain (PRD). The boomerang-shaped Bro1 domain interacts with different isoforms of the ESCRT-III protein CHMP4, whereas $LYPx_{1–3}L$ interacts with arm 2 of the α helical V domain [123–126]. The PRD within ALIX in turn interacts with TSG101 [127], accounting for the direct link that ALIX provides between ESCRT-I and ESCRT-III [121,128]. Highlighting one potential target for the development of inhibitors of HIV-1 budding, the P(T/S)AP domain inserts into a cleft on the N-terminal UEV domain of TSG101 (Fig. 7) [129,130].

Restriction of viral egress

The type II trans-membrane (TM) protein CD317/BST2/tetherin inhibits the release of budding particles by retaining them on the plasma membrane of the virus producer cell [131,132] (step 12 in Fig. 1). Tetherin consists of a short N-terminal cytoplasmic tail followed by a TM region, an approximate 110-residue ectodomain ending on an amphipathic sequence that reconnects the protein to the plasma membrane [133]. The hydrophobic C-terminal peptide of tetherin, initially thought to be a signal for glycosyl phosphatidylinositol modification, may in fact function as a second TM domain [134]. Thisunusual dual membrane -bound topology of tetherin led to several models, involving extended or laterally arranged parallel or anti-parallel protein dimers at the cell surface, to explain virus tethering [131], and a number of recent X-ray crystal structures revealed that the ectodomain indeed forms a parallel dimeric α helical coiled coil [135–137]. In addition, the tetherin dimers can further assemble head-to-head into tetramers via formation of a four-helix bundle [136,137]. However, mutations designed to ablate tetramer formation did not eliminate tetherin function, indicating that tetramerization is not essential for HIV-1 restriction [137]. These data highlight the extended ectodomain coiled coil dimer as the likely virus tethering unit. Ectodomain residues Ala88 and Gly109, which disfavoured coiled coil packing, probably impart some flexibility to the structure, perhaps facilitating terminal anchor insertion into the viral membrane [136].

HIV-1 Vpu, also a TM protein, counteracts the restriction by tetherin [131,132] through a mechanism that depends on a direct interaction between the viral and host proteins [138,139]. Previously elucidated structures of Vpu fragments yielded limited insight into the mechanism of the Vpu–tetherin interaction, though a recent NMR analysis of lipid membrane-embedded TM peptides indicates a likely anti-parallel helix-helix binding interface [140].

Protease and virus maturation

The final step of the viral lifecycle, which is mediated by PR and occurs concomitant with or soon after budding, converts immature particles to infectious virions via the proteolysis of Gag and Gag-Pol precursor polypeptides to yield the structural components MA, CA and NC, and the PR, RT and IN enzymes [141] (Fig. 1, step 13). Cryo-electron tomography revealed Gag structural rearrangements that occur within immature particles during proteolysis and maturation [142,143] and characterized cellular sites of HIV-1 budding [144].

Following cleavage of the MA/CA bond, a novel β hairpin formed by a salt-bridge between the liberated Pro1 N-terminus and Asp51 in CA triggers core shell assembly [145]. Recent evidence indicates that the morphological transitions occuring during HIV-1 particle assembly and maturation represent druggable targets. A 12-mer peptide, selected in a phage display screen for binding to the HIV-1 CA CTD, potently restricted CA assembly *in vitro* [146]. Bevirimat, a betulinic acid derivative of herbal origin, inhibited HIV-1 replication by specifically blocking PR cleavage of the CA-SP1 junction [147]. Exposure to bevirimat leads to stabilization of the immature CA lattice in HIV-1 virions [148]. CAP1 is another small molecule reported to elicit abnormal HIV-1 core morphologies [149]. Binding of CAP1 to the CA NTD involves formation of a deep hydrophobic pocket, which serves as a ligand binding site [150]. The binding mode of CAP1 is therefore very different from that of PF-3450074, which engages a pre-formed pocket on the CA NTD surface (Fig. 3d) [35]. It seems likely that the distortion in CA structure associated with CAP1 binding interferes with CA hexamer assembly.

Unlike the previously discussed viral enzymes, the structure of full-length PR [151–153] preceded the approval of the initial clinical inhibitor that targeted the enzyme by several years [154]. Accordingly, the development of PR inhibitors (PIs) has benefited more from structure-based design efforts than other anti-retroviral drugs, and readers are directed to refs [155 and 156] for historical accounts of the interplay between PR structure and the development of PIs and resistance mechanisms.

The nine different peptide bonds within Gag and Gag-Pol that are cleaved by PR display limited primary sequence homology. Co-crystallization of six peptide substrates with PR defined a common substrate volume or envelope, indicating that substrate shape rather than primary sequence is a key predictor of functionality [157]. The approved PIs are competitive inhibitors that bind to the enzyme active site, and overlays of PR–PI co-crystal structures identified regions of the so-called PI envelope that protruded from the substrate envelope and contacted amino acid residues that, when changed, confer drug resistance [158]. These findings led to the hypothesis that PIs designed to fit more snugly within the substrate envelope would display favourable genetic resistance barriers, and some novel amprenavir-based compounds displayed marginally improved binding profiles to drug resistant PR as compared to the wild-type enzyme *in vitro* [159]. Because compounds with enhanced binding affinities for wild-type PR bound drug-resistant enzymes relatively less well than amprenavir, additional work is required to determine whether substrate envelope-based PIs will display beneficial profiles against drug resistant strains in the clinic.

Conclusions and perspectives

HIV-1 has been analyzed by structural biology techniques more so than any other virus, with partial or complete structures known for all 16 of its protein components and additional structures determined for substrate- and host factor-bound complexes. Structural biology will continue to have a significant impact on HIV/AIDS research moving forward by providing high-resolution glimpses of target protein–drug complexes and viral–host interactions, such as CA–TRIM5α, Vif–APOBEC3G or Vpu–tetherin, which will reveal novel druggable sites. Despite decades of research, the interactions between HIV-1 and host proteins that underlie some steps in the viral life cycle, for example the import of the preintegration complex into the nucleus (Fig. 1, step 5), are only now being illuminated. The simian immunodeficiency virus Vpx protein was moreover recently shown to counteract the SAMHD1 restriction factor that inhibits HIV-1 reverse transcription and infection of monocytic cells [160,161], indicating that these protein complexes could define new pathways for antiviral drug developmentas well.

Notwithstanding the ongoing work with PIs, it will be interesting to see if structure-based substrate/inhibitor envelope hypotheses will apply to the development of other HIV-1 inhibitors. Because NNRTIs form induced fit binding pockets, they would appear to be poor candidates for this technique. The relatively tight overlay of multiple bound drugs at the IN active site and similarities in drug positions with the ejected terminal adenosine base [88] hints that INSTIs could be another drug class to benefit from such approaches. 3D structures of new drug targets as well as inhibitor or antibody-bound targets will predictably increase the pace of antiviral development and help guide vaccine development efforts [162,163]. The development of new technologies and improvements in existing methods will also significantly influence structural virology moving forward. Single-particle electron cryo-microscopy has recently yielded near-atomic resolution structures of a number of so-called naked viruses that, unlike HIV-1, lack an exterior envelope lipid bilayer [164]. Although the icosahedral symmetry underlying these structures greatly facilitated their determination, ongoing developments in instrumentation and computational science may very well yield similar resolution structures for particles that possess less inherent symmetry.

The development of HAART has dramatically changed the face of the HIV/AIDS epidemic since the disease was first recognized 30 years ago. Considered virtually a death sentence prior to the advent of anti-retroviral drugs, HIV-1 infection is now a manageable chronic disease. Yet, despite these remarkable advances, there remains significant room for improvement. Some of the drugs, in particular the PIs, exert toxic side-effects. More tolerable antiviral regimens could strengthen patient compliance and consequently reduce the emergence of resistant strains. Although the recently approved INSTI raltegravir is apparently non-toxic, the relative ease by which it selects for drug resistant strains highlights the need for second-generation INSTIs with more favorable genetic barriers to the resistance. The development of compounds that inhibit functions of less explored drug targets, in particular of the accessory HIV-1 proteins and host factors, would be of obvious benefit as well. The availability and efficacy of the current arsenal of anti-retroviral drugs should not be taken for granted. It is important to bear in mind, that the majority of the HIV-infected population do not have access to the advanced treatment options. Short of an effective vaccination strategy, the ongoing race against drug resistance can best be won by sustained effort to develop novel ever more potent and tolerable antiviral approaches.

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Biographies

Alan Engelman

Alan Engelman received his Ph.D. in Molecular Biology and Microbiology from Tufts University School of Medicine in Boston, Massachusetts in 1990 and focused on the mechanism of HIV DNA integration as a postdoctoral fellow at the National Institute of Diabetes and Digestive and Kidney Diseases in Bethesda, Maryland. He joined the faculty of the Dana-Farber Cancer Institute and Harvard Medical School in Boston in 1995, where he studies the molecular virology of HIV-host interactions.

Peter Cherepanov

Peter Cherepanov obtained his Ph.D. in Medical Sciences from the University of Leuven, Belgium in 2000 and did postdoctoral work at the University of Leuven and Dana-Farber Cancer Institute on HIV integration. He was a lecturer and then professor at Imperial College London from 2005 to 2011. Presently, he is a group leader at Clare Hall Laboratories, Cancer Research UK. Research in his laboratory is focusing on structural biology of chromatin and retroviral integration.

At a glance

- HIV-1 replication relies on the proper functioning of specific viral proteins and three of these, protease, integrase, and reverse transcriptase with associated RNase H activity, are enzymes. Antiviral drugs that inhibit protease, integrase and reverse transcriptase DNA polymerase activities are approved for treating AIDS patients. The highly active anti-retroviral therapy or HAART regimens utilize cocktails of three inhibitors to suppress HIV-1 replication and the outgrowth of drug resistant viral strains.
- HIV-1 replication depends on a plethora of functional interactions between its proteins and those of the host. Other cellular proteins, which are referred to as restriction factors, work to counteract virus growth. TRIM5α, APOBEC3G, tetherin and SAMHD1 are examples of such restriction factors.
- In addition to enzyme active sites, critical viral-host protein interactions define targets for therapeutic intervention. Drugs might block interactions between the virus and host proteins needed for replication, as is the case for the approved entry inhibitor maraviroc, or enhance the effects of cell restriction factors.
- Neutralization of the viral envelope glycoprotein gp120 by the adaptive immune system underscores AIDS vaccine development strategies.
- Structural biology studies yield three-dimensional glimpses of protein function at near atomic resolution. Such results form the cornerstones of modern antiviral drug and vaccine development efforts.

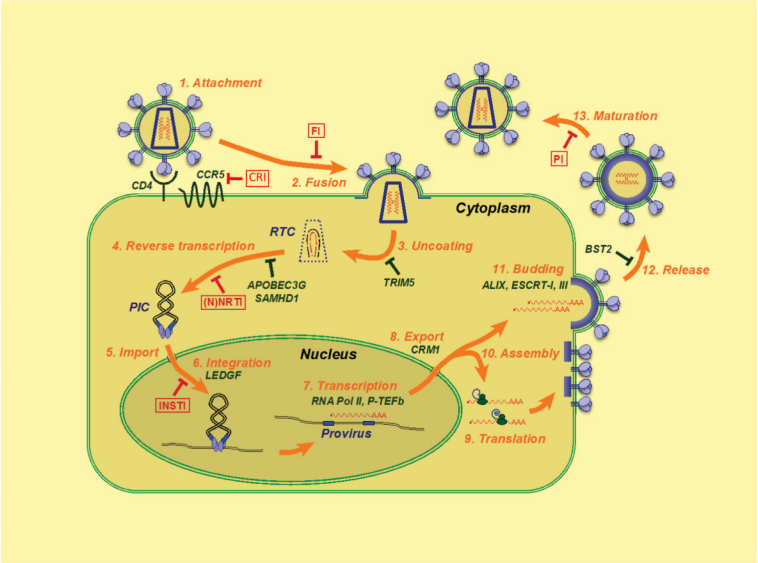

Figure 1.

Schematic overview of the HIV-1 replication cycle. The infection begins when envelope glycoprotein spikes engage the CD4 receptor and the membrane spanning co-receptor (step 1; cell proteins discussed in text are indicated in green type), which leads to viral–cell membrane fusion and entry of the virus particle into the cell (step 2). Partial core shell uncoating (step 3) facilitates reverse transcription (step 4), which in turn yields the preintegration complex (PIC). Following import into the cell nucleus (step 5), PIC-associated IN orchestrates formation of the integrated provirus (step 6). Proviral transcription (step 7) yields different sizes of viral mRNAs (not shown), the larger of which require energy-dependent export to leave the nucleus (step 8). Genome-length mRNAs serve as a template for protein production (step 9) or viral particle assembly with protein components (step 10). ESCRT-mediated viral particle budding (step 11) and release (step 12) from the cell is accompanied or followed shortly thereafter by PR-mediated maturation (step 13) to create an infectious viral particle. Each step in the HIV-1 lifecycle is a potential target for antiviral intervention [165]; the sites of action of clinical inhibitors (boxed) and cellular restriction factors are indicated with red and green block signs, respectively. CRI, CCR5 inhibitor; FI, fusion inhibitor; RTC, reverse transcription complex.

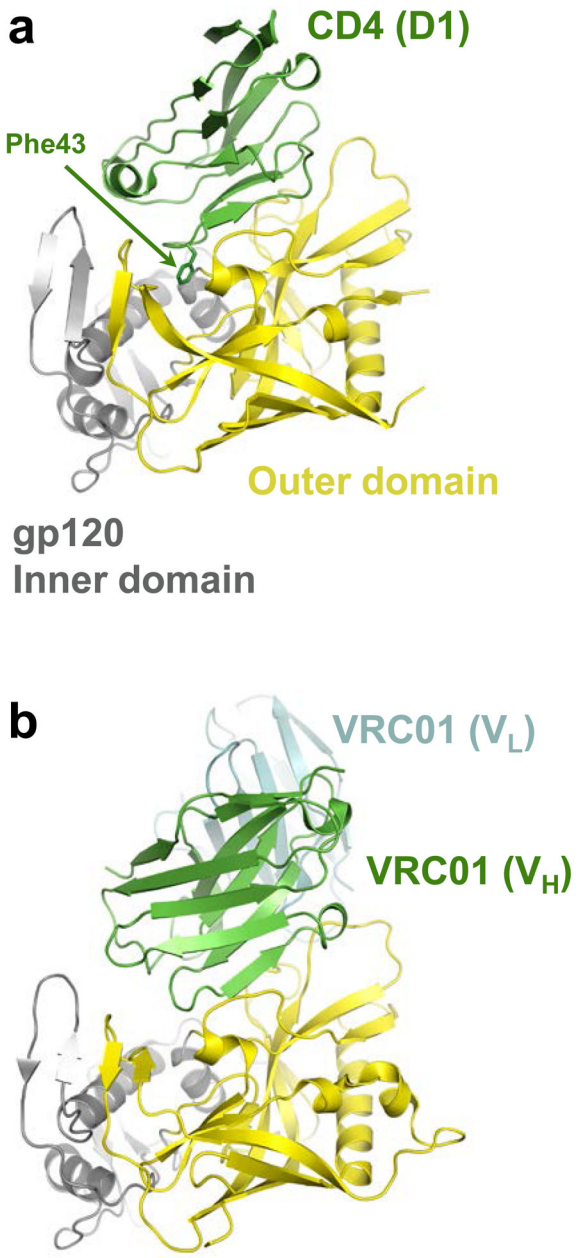

Figure 2.

CD4 and CD4-mimicking antibody binding to the gp120 core. (a) Structure of HIV-1 gp120 (outer domain is shown in yellow and inner domain in gray) in complex with CD4 (green; pdb code 3JWD). Only immunoglobulin-like domain 1 (D1) of CD4 is shown; the Phe43 side-chain is depicted as sticks. (b) VRC01 antibody–gp120 co-crystal structure (pdb code 3NGB, heavy chain shown in green and light chain in cyan) oriented as in panel a. Only the variable domains of the heavy (VH) and light (VL) chains of the antibody are shown.

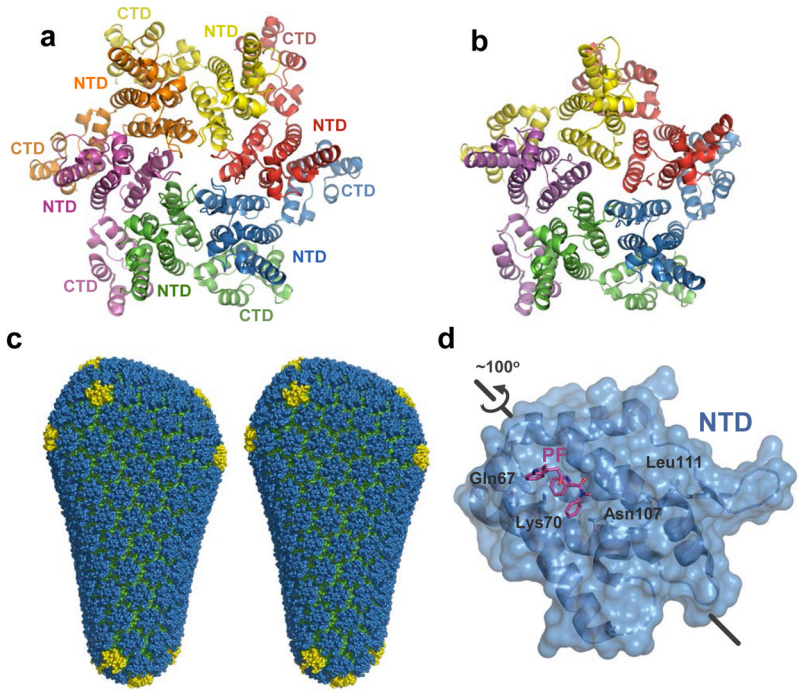

Figure 3.

HIV-1 capsid structures. Crystal structures of the hexameric (a, pdb code 3H47) and pentameric (b, pdb code 3P05) full-length HIV-1 CA assemblies. Individual subunits are coloured by chain. (c) The model for the complete HIV-1 capsid, based on the crystal structures [32]. NTDs of the hexameric and pentameric CA units are shown in blue and yellow, respectively; CTDs are green. (d) HIV-1 CA NTD in complex with PF-3450074 (PF, pdb code 2XDE). The orientation is related to that of the blue NTD in panel a by an ~100° rotation, as shown. Residues critical forPF -3450074 binding as revealed by resistance mutations [44] are indicated.

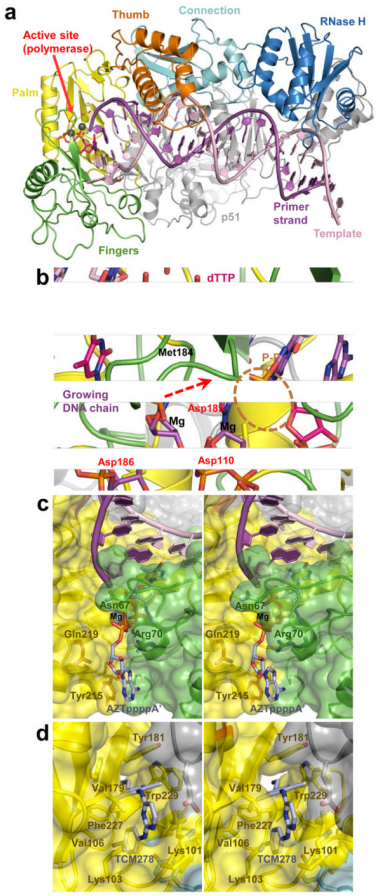

Figure 4.

Structural analyses of HIV-1 RT function and its inhibition by small molecules. (a) Overview of the HIV-1 RT-template-primer complex (pdb code 1RTD). The protein and DNA chains are shown as cartoons. The subdomains of the active RT subunit are indicated and colour-coded; the inactive (p51) subunit is shown in gray. The structure contains a bound molecule of dTTP in the active site (pink). Grey spheres are Mg atoms. (b) Close-up of the polymerase active site (pdb code 1RTD) and DNA polymerization. The 3′-hydroxyl, absent in the original structure [48], is added for illustration purposes. The direction of nucleophilic attack is indicated by a dashed arrow. The catalytic residues, Met184 and the leaving pyrophosphate group (P–P) are shown as sticks and indicated. RT chain colours are conserved from panel a. (c) Stereo view of ATP-binding pocket in AZT-resistant HIV-1 RT (pdb code 3KLE). The excision product (AZTpppp A′) is shown as sticks with carbon atoms in light blue. Protein chains are shown as cartoons with semitransparent surfaces (colouring as in panel a); residues implicated in AZT resistance are indicated. (d) TCM278 bound to HIV-1 RT (pdb code 2ZD1). RTresidues forming the NNRTI-binding pocket are indicated.

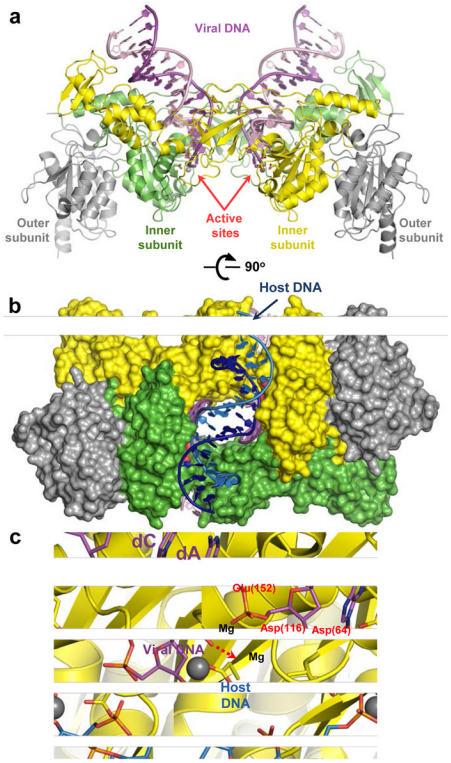

Figure 5.

Retroviral intasome structures and mechanism of IN catalysis. (a) Overview of the PFV intasome structure (pdb code 3OY9). The active (inner) IN chains are shown as green and yellow cartoons; catalytically inactive (outer) chains are gray. The transferred and non-transferred viral DNA strands are shown in dark and light magenta, respectively. Active site carboxylates are shown as sticks and divalent metal ions as gray spheres. (b) The PFV intasome in complex with a host DNA mimic (light and dark blue; pdb code 3OS2). IN chains are shown in space-fill mode conserving colours from panel a. (c) DNA strand transfer. The model is based on structures of the Mn^{2+}-bound intasome and target capture complex (see [84] for details). IN is shown as cartoons with D, D-35-E active site residues as sticks. DNA is shown as sticks; the invariant viral dA and dC nucleotides are indicated. Colours are conserved from panel a. Residue numbering corresponds to the HIV-1 IN sequence. Direction of nucleophilic attack is indicated by a red dashed arrow.

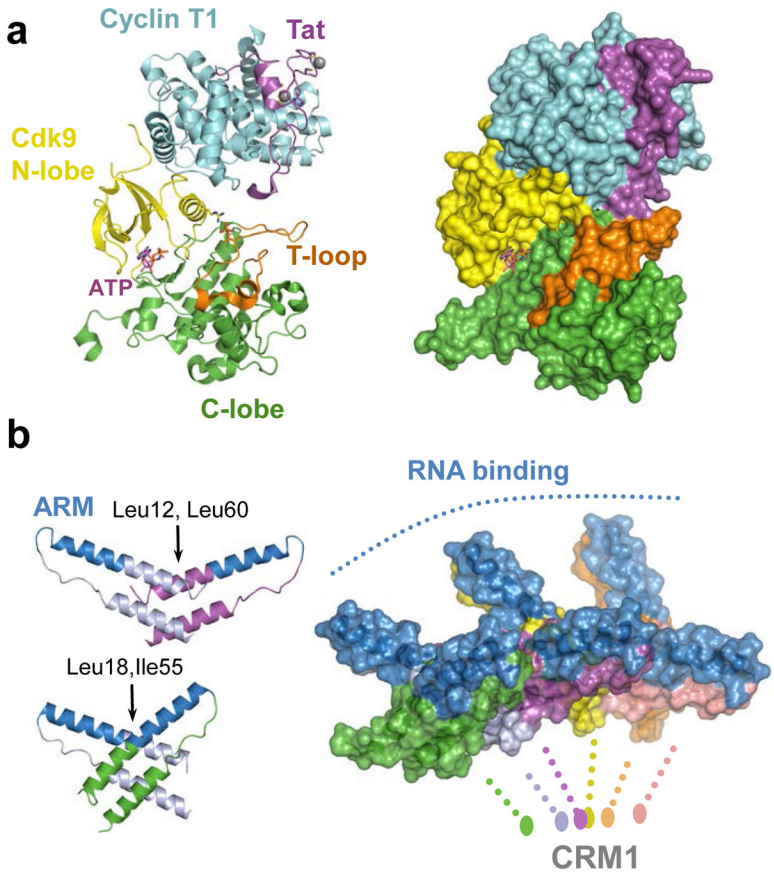

Figure 6.

Higher-order Tat and Rev structures. (a) Crystal structure of HIV-1 Tat in complex with ATP-bound P-TEFb (pdb code 3MIA). The protein chains are shown as cartoons (left) or in space-fill mode (right). The N-lobe of Cdk9 is shown in yellow, C-lobe in green, and the T-loop in orange; Cyclin T1 is in blue, and HIV-1 Tat is shown in magenta. ATP bound to the active site of Cdk9 is shown as sticks and indicated. Gray spheres are Zn atoms. (b) Left: dimeric assemblies of HIV-1 Rev core observed in crystals (pdb codes 2X7L and 3LPH). Rev monomers are shown as cartoons and colored by chain, expect for the ARM motifs, which are blue. The crystal structures elucidate two types of Rev-Rev hydrophobic interfaces, one involving Leu12 and Leu60 and the other Leu18 and Ile55. Right: model of the Rev hexamer based on the dimeric structures, shown in space-fill mode. The oligomer projects RNA-binding ARM domains (blue) on one side, with CRM1-binding nuclear export signals (not resolved in the current structures) emanating from the other side.

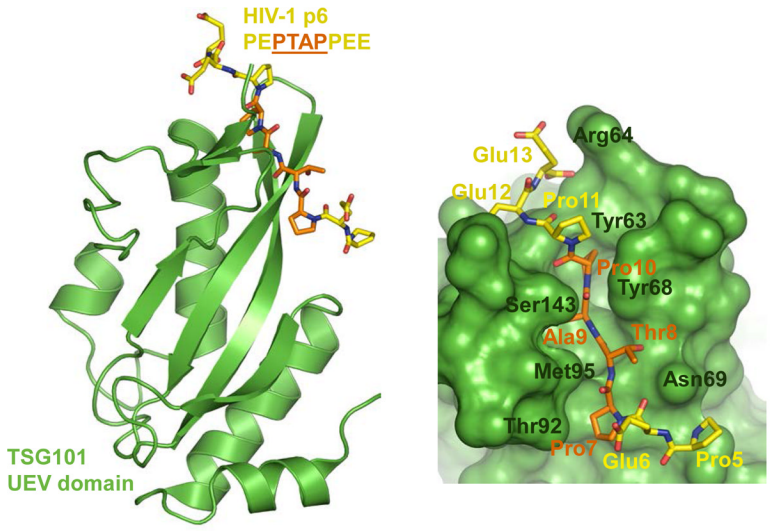

Figure 7.

Virus-cell interactions and HIV-1 budding. The structure of the UEV domain of TSG101 bound to the PT(S)AP domain of HIV-1 p6 protein (pdb code 3OBU). TSG101 is shown as green ribbon (left) or space-fill (right) cartoons. P6 (residues 5–12; PEPTAPPEE) is shown as sticks; the carbon atoms of the core L domain PTAP and the flanking regions are orange and yellow, respectively. Some of the key TSG101 residues involved in the interaction are indicated on the right panel.