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1. Introduction

Molecular recognition is a fundamental phenomenon observed in all biological systems organisation – proteins, nucleic acids and their complexes, cells and tissues. Molecular recognition is governed by specific attractive interactions between two or more partner molecules through non-covalent bonding such as hydrogen bonds, metal coordination, electrostatic effects, hydrophobic and van der Waals interactions. The partners – receptors and substrate(s) or ligands – involved in molecular recognition, exhibit molecular complementarity that can be adjusted over the recognition process. Competition and cooperation, the two opposite natural effects contributing to selective and specific recognition between participating partners, are the basic principles of substrate/ligand/inhibitor or protein binding to its targets.

The tertiary structures of biological objects (proteins and nucleic acids) are formed mainly by hydrogen bonds (enthalpic contributions) and by hydrophobic contacts (mostly entropic contributions). With a few exceptions, (e.g. ligand binding to the Ah receptor), the organisation of ligand-protein complexes depends primarily on hydrogen bonding.

In the process of a ligand binding to its target the hydrogen bonds contribute to (i) the orientation of the substrates/ligands/inhibitors by a receptor, frequently associated with a conformational/structural adjustment of the interacting agents; (ii) the specific recognition of substrates/ligands/inhibitors and selectivity between sterically or structurally similar but biochemically different species; (iii) the affinity of ligands/inhibitors – the most decisive factor in drug design.

To describe the pharmacological properties of a given ligand or inhibitor, the knowledge of the site where the inhibitor is to bind with the target and of which interaction(s) control the specific recognition of the inhibitor by its target(s), represents a corner stone factor. Only a limited number of target-ligand molecular complexes have been characterized experimen-
tally at the atomic level (X-ray or NMR analysis) [1]. Part of them describes the binding mode of therapeutically relevant ligands to biologically non-relevant and non-pertinent targets (e.g., the HIV-1 integrase specific inhibitor RAL was published as a ligand fixed to the PFV intasome [2,3]). Consequently, a large quantity of reliable information on target-ligand binding is based on molecular docking methods which generate insights into the interactions of ligands with the amino acid residues in the binding pockets of the targets, and also predict the corresponding binding affinities of ligands [4]. The first step of a docking calculation consists of the choice or generation/construction of the therapeutically appropriate target. Frequently the target modeling is a hard computational task which requires the application of sophisticated theoretical methods and constitutes a fascinating creative process.

Therefore, theoretical studies contribute first, to establish biologically valid models of the targets; second, through the use of these models, to the understanding of the protein functional properties; and finally to apply this data to rational drug design.

Here we compile and review the data on the molecular structure, properties and interactions of the HIV-1 integrase representing from one side, a characteristic example of a poly-functional and complex biological object interacting with different viral and cellular partners and from another side, an attractive therapeutical target. We attempt to extract key messages of practical value and complement references with our own research of this viral enzyme. We characterized the structural and conformational features of Raltegravir (RAL), the first integrase specific inhibitor approved for the treatment of HIV/AIDS, and we analyzed the factors contributing to RAL recognition by the viral targets.

2. The HIV-1 integrase and integrase-viral DNA pre-integration complex

2.1. Activities

The HIV-1 integrase (IN) is a key enzyme in the replication mechanism of retroviruses, catalyzing the covalent insertion of the reverse-transcribed DNA into the chromosomes of the infected cells [5]. Once integrated, the provirus persists in the host cell and serves as a template for the transcription of viral genes and replication of the viral genome, leading to production of new viruses (Figure 1a). A two-step reaction is required for covalent integration of viral DNA (vDNA) into host DNA (hDNA). First, IN binds to a short sequence located at either end of the long terminal repeat (LTR) of the viral DNA and catalyzes an endo-nucleotide cleavage. This process is known as 3'-processing reaction (3'-P), resulting in the removal of two nucleotides from each of the 3'-ends of the LTR and the delivery of hydroxyl groups for nucleophilic attacks (Figure 1 b).

The cleaved (pre-processed) DNA is then used as a substrate for the strand transfer (ST) reaction, leading to the covalent insertion of the vDNA into genome of the infected cell [5,7]. The ST reaction occurs at both ends of the vDNA simultaneously, with an offset of precisely five base pairs between the two distant points of insertion. The integration process is accomplished by the removal of unpaired dinucleotides from the 5'-ends of the vDNA, the filling
in of the single-strand gaps between viral and target DNA molecules and ligation of the 3'-ends of the vDNA to the 5'-ends of the hDNA (Figure 1 b). These two reactions are spatially and temporally separated and energetically independent: the 3'-processing takes place in the cytoplasm of the infected cells, whereas strand transfer occurs in the nuclei. They are catalyzed by the enzyme in different conformational and oligomerisation states: dimerization is required for the 3'-processing step [8,9], while tetrameric IN is believed to be required for strand transfer [10-12].

Figure 1. The HIV-1 replication cycle (a) and catalytic steps involved in the insertion of viral DNA into the human genome (b) [6].

2.2. Structural data

The HIV-1 IN is a 288 amino acids enzyme (32 kDa) that consists in three structurally distinct domains: (i) the N-terminal domain (NTD, INN) with a non-conventional HHCC zinc-finger motif, promoting protein multimerization; (ii) the central core domain (CCD, INČČ) containing a canonical D,D,E motif performing catalysis and involved in DNA substrate recognition [35]; (iii) the C-terminal domain (CTD, INČČ), which non-specifically binds DNA and helps to stabilize the IN•vDNA complex [13]. Both integration steps, 3'-P and ST, involve the active site and the active site flexible loop formed by ten residues, INČČ. Neither the structure of isolated full-length IN from HIV-1 nor that of IN complex with its DNA substrate has been determined. Nevertheless, the structures of the isolated HIV-1 domains or two domains were characterized by X-ray crystallography (34 structures) and NMR analysis (9 structures) [1]. NTD presented by 6 NMR structure solutions (1WJA, 1WJB, 1WJC, 1WJE, and 1WGF) [14-16] was classified by SCOP as the ‘all alpha helix’ structure and consists of four helices stabilized by a Zn²⁺ cation coordinated with the HHCC motif (His12, His16, Cys40 and Cys43); the sequence from 43 to 49 residue are disordered (Figure 2). Structure of CTD was also characterized by NMR (3 deposited solutions (1IHV, 1IHW and 1QMC) [17,18]. According to the SCOP classification it presents the ‘all beta strand’ structure and consists of five anti-parallel β-strands forming a β-barrel and adopting an SH3-like fold (Figure 2).
The human IN CCD characterized by X-ray analysis has been reported as 14 different crystal structures (1HYV, 1HYZ, 1EXQ, 1QS4, 1B92, 1B9D, 1BHL, 1BH4, 1BIS, 1BIU, 1BIZ, 1BL3, 1ITG and 2ITG). The wild-type IN was resolved with a poor precision (1ITG) [19], the other structures represent engineered mutants, either single (F185K/H) [20-23], double (W131E and F185K; G149A and F185K or C56S and F185K) [24-26] or multiple (C56S, W131D, F139D and F185K) [27] mutants which were designed to overcome the poor solubility of the protein. The core domain has a mixed $\alpha/\beta$ structure, with five $\beta$-sheets and six $\alpha$-helices (Figure 2).
flexible loop comprising residues 140–149, in which conformational changes are required for 3'-P and ST reactions. These activities require the presence of a metallic cofactor(s), the Mg$^{2+}$, which binds to the catalytic residues D64, D116 and E152. The number of Mg$^{2+}$ cations is different for the distinct enzymatic reactions and consequently, for the different IN states: a single Mg$^{2+}$ cation in non-processed IN, and two in processed IN. The structures of avian sarcoma virus (ASV) IN [21] and the Tn5 transposase[30] have provided evidence of a two-metal active site structure, which has been used to build metal-containing IN models [31-33].

Crystallographic structures of IN$^{1-212}$ and IN$^{50-288}$ two-domain constructs have also been obtained for W131D/F139D/F185K and C56S/W131D/F139D/F185K/C180S mutants, respectively (Figure 2) [34,35]. In the first of these structures, there is an asymmetric unit containing four molecules forming pairs of dimers connected by a non-crystallographic two fold axis, in which the catalytic core and N-terminal domains are well resolved, their structures closely matching those found with isolated IN$^{1-41}$ and IN$^{50-212}$ domains, and connected by a highly disordered linking region (47-55 amino acids). The X-ray structure of the other two-domain construct, IN$^{50-288}$, showed there was a two-fold symmetric dimer in the crystal. The catalytic core and C-terminal domains were connected by a perfect helix formed by residues 195–221. The local structure of each domain was similar to the structure of the isolated domains. The dimer core domain interface was found to be similar to the isolated core domain, whereas the dimer C-terminal interface differed from that obtained by NMR.

2.3. Theoretical models

All these structural data characterising the HIV-1 IN single or two-domains allow the generation of biologically relevant models, representing either the unbound dimeric enzyme or IN complexed with the viral or/and host DNA [29].

IN acts as a multimer [36]. Dimerization is required for the 3'-processing step, with tetrameric IN catalyzing the ST reaction [37,38]. Dimeric models were built to reproduce the specific contacts between IN and the LTR terminal CA/TG nucleotides identified in vitro [39,40]. However, most models include a tetrameric IN alone or IN complex with either vDNA alone or vDNA/hDNA, recapitulating the simultaneous binding of IN to both DNAs required for strand transfer (Figure 3 b–d).

These models were either based on the partial crystal structure of IN [32,44] or constructed by analogy with a synaptic Tn5 transposase complex described in previous studies [42,45,46].

Most models include an Mg$^{2+}$ cationic cofactor and take into account both structural data and biologically significant constraints (Figure 2 b–d). In particular, HIV-1 IN synaptic complexes (IN•vDNA•hDNA) have been constructed taken into account the different enzymatic states occurring during the integration process (Figure 3 d) [41,42]. Such complexes have also been characterized by electron microscopy (EM) and single-particle imaging at a resolution of 27 Å [47]. Recently the X-ray complete structure of the Primate Foamy Virus (PFV)
integrase in complex with the substrate DNA and Raltegravir or Elvitegravir has also recently been reported (Figure 3 e) [2].

Figure 3. Integrase architecture and organization. Theoretical models: (a) dimeric model of the full-length IN•vDNA-complex [39]; (b) tetramer models of the IN•vDNA [27]; (c and d) synaptic complexes IN4•vDNA•hDNA [41,42]; (e) X-ray structure of the PFV IN•vDNAintasome [2]; and (f) EM maps reconstitution of IN•vDNA•hDNA complex with LEGDF [43]. Protein and DNA structures are presented as cartoon with colour coded nucleotides and Zn$^{2+}$ and Mg$^{2+}$ cations shown as balls. The active site contains two Mg$^{2+}$ cations in (a) and one in (b–d).

In this complex, the retroviral intasome consists of an IN tetramer tightly associated with a pair of viral DNA ends. The overall shape of the complex is consistent with a low-resolution structure obtained by electron microscopy and single-particle reconstruction for HIV-1 IN complex with its cellular cofactor, the lens epithelium-derived growth factor (LEDGF) (Figure 3 d) [43].

2.5. Targets models representing the HIV-1 integrase before and after 3'-processing

Recently new HIV-1 IN models were generated by homology modeling. They represent with a certain level of reliability two different enzymatic states of the HIV-1 IN that can be explored as the biological relevant targets for design of the HIV-1 integrase inhibitors (Figure 4). The generated models are based on the experimental data characterising either the partial structures of IN from HIV-1 or full-length IN from PFV. The models of the separated full-length HIV-1 integrase represent the unbound homodimers of IN (IN1-270) containing either one or two Mg$^{2+}$ cations in the active site – a plausible enzymatic state before the 3’
processing. The catalytic site loop encompassing ten residues forms the boundary of the active site. This loop shows either a coiled structure [20,22,24] or contains an Ω-shaped hairpin [28,48].

Figure 4. Structural models of the HIV-1 integrase. (a) Model of unbound IN representing the homodimeric enzyme before the 3' processing; (b) Model of the simplified (dimeric form) IN•DNA pre-integration complex; (c) Superimposition of monomeric subunits from two models in which catalytic site loop residues 140-149 are shown by colours (red and green). The proteins are shown as cartoons, Mg⁡⁺ ions as spheres (in magenta). (d) Schematic representation of the HIV and PVF active site loop secondary structure prediction, according to consensus 1 and consensus 2.

It will be useful to note that we evidenced a high flexibility of the functional domains in unbound IN by using the Normal Modes Analysis (NMA) [49,50]. Particularly, CTD is characterized by a large scissors-like movement (Figure 5 a). We established that the catalytic site loop in unbound IN with two Mg⁡⁺ cations in the active site is more rigid due to the stabilising role of the coordination of the Mg⁡⁺ cations by three active site residues, D₆₄, D₁₁₆ and E₁₅₂, whereas the catalytic site loop flexibility increases significantly (Figure 5 b, c).

Figure 5. Normal modes illustrating fragments movement in unbound IN. A scissors-like movement in CTD (a); the catalytic site loop displacement in unbound IN with one and two Mg⁡⁺ cation(s) in the active site (b) and (c) respectively (S. Abdel-Azeim, personal communication).
The simplified model of the HIV-1 IN•vDNA pre-integration complex represents the homodimer of integrase non-covalently attached to the two double strands of the viral DNA with two removed nucleotides GT at each 3’-end (Figure 4 b), and likely depicts the biologically active unit of the IN•vDNA strand transfer intasome. The IN•vDNA model was generated from the X-ray structure of the PFV intasome [2]. Despite the very low sequence identity (22%) between the HIV-1 and PFV INs, the structure-based alignment of the two proteins demonstrates high conservation of key secondary structural elements and the three PFV IN domains shared with HIV-1 IN have essentially the same structure as the isolated IN domains from HIV-1 [51]. Moreover, the structure of the PFV intasome displays a distance between the reactive 3’ ends of vDNA that corresponds to the expected distance between the integration sites of HIV-1 IN target DNA (4 base pairs). Consequently, we suggested that the PFV IN X-ray structure represents an acceptable template for the HIV-1 IN model generation [52].

Two models of different states of the HIV-1 IN show a strong dissimilarity of their structure evidenced by divergent relative spatial positions of their structural domains, NTD, CCD and CTD (Figure 4 c). These tertiary structural modifications altered the contacts between IN domains and the structure and conformation of the linker regions. Particularly, the NTD-CCD interface exhibits substantial changes: in the unbound form the NTD-CCD interface belongs to the same monomer subunit whereas in the vDNA-bound form the interface is composed of residues from the two different subunits. Moreover, IN undergoes important structural transformation leading to structural re-organisation of the catalytic site loop; the coiled portion of the loop reduces from ten residues in the unbound form to five residues in the vDNA-bound form. Such effect may be induced either by the vDNA binding or it can derive as an artefact produced from the use of structural data of the PFV IN as a template for the model generation. Prediction of IN sequence secondary structure elements indicates a more significant predisposition of IN from HIV-1 to be folded as two helices linked by a coiled loop than the IN from PFV (Figure 4 d). Prediction results obtained with high reliability (>75%) correlate perfectly with the X-ray data characterising the WT HIV-1 integrase (1B3L) [22] and its double mutant G140A/G149A (1B9F) [26]. The helix elongation accompanied by loop shortening may be easily induced by the enzyme conformational/structural transition between the two integration steps prompted by substrate binding.

This structure can be used to generate reliable HIV-1 IN models for Integrase Strand Transfer Inhibitors (INSTIs) design. However, the active site loop adopts a five-residue coil structure, rather than the ten-residue extended loop observed in HIV-1 IN. This difference may be due to a difference in the sequence of the two enzymes or an effect induced by DNA binding, and caution is therefore required in the use of this structure as a template for modelling biologically relevant conformations of HIV-1 IN [2,45].

2.6. Transition pathway between two IN states and the allosteric binding sites

Two different states of the HIV-1 IN represent the enzyme structures before and after 3’-processing. Under integration process, IN as many other proteins undergo large conformational transitions that are essential for its functions (Figure 6) [53-55]. Tertiary structural
changes precede and accompany these quaternary transitions in the HIV-1 IN as was evidenced by Targeted Molecular Dynamics (TMD) [56] and Meta Dynamics (MD) [57] (Figure 6 c, d).

![Figure 6. Transition states ensemble between A and B structures (a) (A. Blondel, personal communication). A series of conformations visited by the HIV-1 IN over transition from unbound IN to IN•vDNA complex before (red) and after (blue) 3’-processing (b) obtained by Targeted Molecular Dynamics (TMD) (c) and Meta Dynamics (MD) simulations (d) (S. Abdel-Azeim, personal communication).](image)

Our results, first, provide a description of structure-dynamics-function relationships which in turn supplies a plausible understanding of the IN 3’-processing at the atomic level. Second, the calculated intermediate conformations along the trajectories were scanned for molecular pockets - a means of exploring putative allosteric binding sites, particularly positioned on the IN C-terminal domain (CTD), which is responsible for the vDNA recognition (Figure 7).

3. Raltegravir

The integrase inhibitors were developed to block either the 3’-processing or the strand transfer reaction [58-60]. Raltegravir (RAL), the first IN inhibitor approved for AIDS treatment [61] specifically inhibits the ST activity and was confirmed as an integrase ST inhibitor (IN-STI), whereas the 3’-P activity was inhibited only up to a certain concentration [62,63]. The potency of RAL has been described at the level of half-maximal inhibitory concentration (IC50 values) in cellular antiviral and recombinant enzyme assays, kinetic analysis and slow-binding inhibition of IN-catalyzed ST reaction [62-68]. Particularly, it has an IC50 of 2 to 7nM for the inhibition of recombinant IN-mediated ST in vitro and an IC95 of 19 and 31 nM in 10% FBS (fetal bovine serum) and 50% NHS (normal human serum), respectively. This
drug has been reported to be approximately 100-fold less specific for the inhibition of 3’-processing activity compared to strand transfer. The dissociation rate of RAL with IN•vDNA complex was slow, with $k_{\text{off}}$ values of $(22 \pm 2) \times 10^{-6} \text{ s}^{-1}$. The dissociative half-life value measured for RAL with the wild type IN•vDNA complex was 7.3 h and 11.0 h obtained at 37°C and at 25°C respectively.

Figure 7. Pockets detected on the surface of the HIV-1 Integrase intermediate conformations obtained by Targeted Molecular Dynamics (TMD) simulations. (S. Abdel-Azeim, personal communication).

Like other antiretroviral inhibitors, RAL develops/induces a resistance effect. Resistance to RAL was associated with amino acids substitutions following three distinct genetic pathways that involve either N155H, either Q148R/K/H or Y143R primary mutation [69,70]. The last mutation was reported as rare [71]. It was supposed that the integrase active site mutation N155H causes resistance to raltegravir primarily by perturbing the arrangement of the active site $\text{Mg}^{2+}$ ions and not by affecting the affinity of the metals or the direct contacts of the inhibitor with the enzyme [72].

G140S has been shown to enhance the RAL resistance associated with Q148R/K/H [73]. The kinetic gating and/or induced fit effect have been reported as possible mechanisms for RAL
resistance of the G140S/Q148H mutant [74]. A third pathway involving the Y143R/C/H mutation and conferring a large decrease in susceptibility to RAL has been described [75].

3.1. Structure and conformational flexibility

No experimental data characterizing RAL unbound structure or RAL binding mode to the HIV-1 IN has been reported. In this regard, the characterization of RAL conformational preferences and the study of its binding to the HIV-1 IN represent an important task for determining the molecular factors that contribute to the pharmacological action of this drug. Crystallographic data describing the separate domains of the HIV-1 IN and the full-length PFV IN with its cognate DNA deposited in the PDB, provide useful experimental starting guide for the theoretical modeling of the structurally unstudied objects, IN and IN•vDNA complex of HIV-1 as the RAL targets.

RAL, incorporating two pharmacophores, is a multipotent agent capable to hit more than one target in HIV-1, the unbound IN, the viral DNA or IN•vDNA complex. RAL shows the configurational E/Z isomerism and a high conformational flexibility due to eight aliphatic single bonds. Two pharmacophores, (1) 1,3,4-oxadiazole-2-carboxamide and (2) carbonylamino-1-N-alkyl-5-hydroxypyrimidinone, possessing structural versatility through the orientation of carboxamide fragments respective to the aromatic rings, show E-, Z-configuration states characterizing the relative position of the vicinal 1–4 and 1–5 oxygen atoms [48] (Chart 1). The molecule has a set of multiple H-bond donor and acceptor centres. These molecular features together with high structural flexibility provide an abundance of alternative mono- and bi-dentate binding sites in a given RAL conformation.

![Chart 1. RAL structure. The E- and Z-isomers of 1,3,4-oxadiazole-2-carboxamide (1) and carbonylamino-1-N-alkyl-5-hydroxypyrimidinone (2) pharmacophores are stabilized by intramolecular H-bonds.](image)

The chelating properties of protonated or deprotonated RAL are also determined by the E- or Z- configuration (Chart 2). Consequently, RAL can contribute in the recognition and binding of different partners – H-donor, H-acceptors, charged non-metal atoms and metal cations – in topologically distinct regions of IN by applying the richness of its molecular and
structural properties. For instance, RAL as a bioisoster of adenine can block IN interaction with DNA [48] or sequester metal cofactor ions [76].

Chart 2. Metal chelating properties of 1, 3, 4-oxadiazole-2-carboxamide (1) and carbonylamino-1-N-alkyl-5-hydroxy-pyrimidinone (2) moieties.

The conformational preferences of RAL were examined in the gas phase (conformational analysis), in water solution (molecular dynamics, MD, in explicit solvent) and in the solid state (the fragment-based analysis using the crystallographic data from Cambridge Structural Database, CSD [77]). Conformational analysis of the different isomeric states of RAL in the gas phase indicates a small difference between the energy profiles of the Z-ŗ/Z-Ř and E-ŗ/Z-Ř isomers suggesting a relatively low energetical barrier between these two inhibitor states (Figure Ş). A slight preference for the Z-configuration of carbonylamino-hydroxypyrimidinonepharmacophore in the gas phase was observed, in coherence with the established predisposition of β-ketoenols – a principle corner stone of this pharmacophore – to adopt the Z-isomer in the solid state (Figure 9 b) [78-80]. The preference of aliphatic β-ketoenols to form energetically favorable Z-configuratrition has been predicted early by ab initio studies at the B3LYP/3-G** level of theory [81].

The Cambridge Structural Databank search (CSD) [77] based on molecular fragments mimicking the RAL pharmacophores statistically demonstrates the preferential E-configuration of oxadiazolecarboxamide-like molecules and the Z-configuration of carbonylamino-hydroxypyrimidinone-like molecules in the solid state (Figure 9 a and b respectively). The halogenated aromatic rings, widely used pharmacophores, show a great level of conformational flexibility (Figure 9, c), allowing to contribute to a better inhibitor affinity in the binding site.
Figure 8. RAL conformations in the gas phase. Free energy profiles obtained by relaxed scans around the single bonds of RAL from 0 to 360° with an increment step of 30°, considering the four RAL isomers: (a) Z-1/Z-2, (b) Z-1/E-2, (c) E-1/Z-2 and (d) E-1/E-2. The curves representing the rotations around torsion angles τ1, τ2, τ3 and τ4 are shown in blue, red, green and violet colours. The values of τ1, τ2, τ3 and τ4 observed in RAL crystal structure 3OYA are indicated by asterisks.

3.2. Raltegravir-metal recognition

Synthesized as a metal cations chelating ligand, RAL can bind the metal by both pharmacophores in different isomerisation states. Probing the RAL chelating features with relevant cations, K, Mg and Mn, we evidenced that in the majority of metal complexes, the carbonylaminoxydipyrimidinone-like fragments are observed in the Z configuration in the solid state (Figure 10).
Figure 9. RAL conformations in the solid state. CSD fragment-based analysis of the RAL subunits indicates the E- (blue triangles) and Z- (red squares) conformations of oxadiazolecarboxamide–like molecules (a) and the Z-configuration of carbonylamino-hydroxypyrimidinone-like molecules (b). The halogenated phenyl ring conformation RAL geometry in PFV complex is shown in (c and d respectively). The RAL crystal structure parameters are indicated by asterisks. The alternative configurations of the carbonylamino-hydroxypyrimidinone derivatives are demonstrated by structure of RAL precursor molecules, GACMUT, MEADAP and POPYOJ, and RAL inhibitor (d-g).

The oxadiazolecarboxamide-like pharmacophore is observed in the metal complexes as two isomers and demonstrates a strong selectivity to the metal type: the Z isomer binds K and Mg while the E isomer binds mainly Mn. The higher probability of Mg\(^{2+}\) cation coordination by the Z-isomer of both pharmacophores indicates that the presence of two Mg\(^{2+}\) cations at the integrase binding site may be a decisive factor for stabilisation of the Z/Z configuration of RAL which is observed in the PFV intasome complex [2,3].

Therapeutically used RAL is in deprotonated state neutralised by K cation. Such drug formula corresponds to the optimal condition allowing efficient cations replacement in cells. The significantly higher affinity of both pharmacophores to Mg relatively to K permits a positive competition between these cations, resulting in the change of RAL composition from a pharmaceutically acceptable potassium (K) salt to a biologically relevant Mg complex.
Figure 10. Probing of ligand interactions with Mg, Mn and K by CSD fragment-based search for the metal-ligand complexes (Chart 2, and scatterplots (a-d). Metal complexes are indicated by bull symbols: red squares (Mg), blue circles (Mn) and orange triangles (K). The RAL crystal structure is shown (f) and the RAL parameters are indicated by asterisks in (a and c).
3.3. Raltegravir recognition by the HIV-1 targets

The published docking studies report located within the active site of either unbound IN or IN•vDNA complex. Distinct poses of RAL representing different RAL configuration and modes of Mg\(^{2+}\) cations chelation were observed [74,82-84].

Our docking calculations of RAL onto each model evidenced that (i) the large binding pocket delimited by the active site and the extended catalytic site loop in the unbound IN can accommodate RAL in distinct configurational/conformational states showing a lack of interaction specificity between inhibitor and target; (ii) the well defined cavity formed by the active site, vDNA and shortened catalytic site loop provides a more optimised RAL binding site where the inhibitor is stabilised by coordination bonds with Mg\(^{2+}\) cations in the Z/Z-configuration (Figure 11).

Additional stabilisation of RAL is provided by non-covalent interactions with the environing residues of IN and the viral DNA bases. Based on our computing data we suggested earlier the stabilizing role of the vDNA in the inhibitors recognition by IN•vDNA pre-integration complex [51]. It was experimentally evidenced that RAL potently binds only when IN is in a binary complex with vDNA [85], possibly binding to a transient intermediate along the integration pathway [86]. Terminal bases of the viral DNA play a role in both catalytic efficiency [87,88] and inhibitor binding [89-91].

It was reported recently that unprocessed viral DNA could be the primary target of RAL [92]. This study is based on the PFV DNA and several oligonucleotides mimicking the HIV-1 DNA probed by experimental and computing techniques.

To explore the role of the HIV-1 viral DNA in RAL recognition we docked RAL onto the non-cleaved and cleaved DNA (the terminal GT nucleotides were removed) [79]. We found that RAL docked onto the non-cleaved vDNA is positioned in the minor groove of the substrate. No stabilising interactions between the partners, RAL and vDNA, were observed. In contrast, in the processed (cleaved) vDNA the Z/Z isomer of RAL takes the place of the remote GT based and is stabilised by strong and specific H-bonds with the unpaired cytosine. These H-bonds characterize the high affinity and specific recognition between RAL and the unpaired cytosine similarly to those observed in the DNA bases pair G-C.

Based on the docking results we suggested that the inhibition process may include as a first step the RAL recognition by the processed viral DNA bound to a transient intermediate IN state. RAL coupled to vDNA shows an outside orientation of all oxygen atoms, excellent putative chelating agents of Mg\(^{2+}\) cations, which could facilitate the insertion of RAL into the active site. The conformational flexibility of RAL further allows the accommodation/adaptation of the inhibitor in a relatively large binding pocket of IN•vDNA pre-integration complex thus producing various RAL docked conformation. We believe that such variety of RAL conformations contributing to the alternative enzyme residue recognition may impact the selection of the clinically observed alternative resistance pathways to the drug [29] and references herein.
4. Conclusions and perspectives

The HIV-1 Integrase is an essential retroviral enzyme that covalently binds both ends of linear viral DNA and inserts them into a cellular chromosome. The functions of this enzyme are based on the existence of specific attractive interactions between partner molecules or cofactors – IN, viral DNA and Mg$^{2+}$ cations. Structure-based drug development seeks to identify and use such interactions to design and optimize the competitive and specific modulator of such functional interactions. Drug design and optimisation process require knowledge about interaction geometries and binding affinity contributing to molecular recognition that can be gleaned from crystallographic and modeling data.
We have resumed the available structural information related to the retroviral integrase. We used this data to generate biologically relevant HIV-1 targets – the unbound IN, the viral DNA (vDNA) and the IN•vDNA complex – which represent with a certain level of reliability, two different enzymatic states of the HIV-1 over the retroviral integration process.

We have characterised the RAL binding, a very flexible molecule displaying the E/Z isomerism, to the active site of its HIV-1 targets which mimic the integrase states before and after the 3'-processing. The docked conformations represent a spectrum of possible conformational/configurational states. The best docking scores and poses confirm that the generated model representing the IN•vDNA complex is the biologically relevant target of RAL, the strand transfer inhibitor. This finding is consistent with well-documented and commonly accepted inhibition mechanism of RAL, based on integral biological, biochemical and structural data.

RAL docking onto the IN•vDNA complex systematically generated the RAL chelated to Mg$^{2+}$ cations at the active site by the pharmacophore oxygen atoms. The identification of IN residues specifically interacting with RAL is likely a very difficult task and the exact modes of binding of this inhibitor remain a matter of debate. Most probably the flexible nature of RAL results in different conformations and the mode of binding may differ in terms of the interacting residues of the target, which trigger the alternative resistance phenomenon.

The identified RAL binding to the processed viral DNA shed light on a putative, even plausible, step of the RAL inhibition mechanism.

We have implemented dynamic properties to the HIV-1 targets characterisation, particularly, the internal protein collective motions and the global conformational transition. Such transitions play an essential role in the function of many proteins, but experiments do not provide the atomic details on the path followed in going from one end structure to the other. For the dimeric IN, the transition pathway between the unbound and bound to vDNA is not known, which limits information of the cooperative mechanism in this typical allosteric system, where both tertiary and quaternary changes are involved. Description of the IN intermediate conformations open a way to localise the allosteric pockets, which in turn can be selected as the putative binding sites for small molecules in a virtual screening protocol.

Novel drugs, targeted the HIV-1 Integrase, outcome mainly due to the rapid emergence of RAL analogues (for example, GS-9137 or elvitegravir, MK-2048 and S/GSK 1349572, currently under clinical trials [93]). The clinical trials of several RAL analogues (BMS-707035, GSK-364735) were suspended. All these molecules specifically suppress the IN ST reaction. We conceive that the future HIV-1 integrase drug development will be mainly oriented to design of inhibitors with a mechanism of action that differs from that of RAL and its analogues. Distinct conceptions are potentially conceivable: (i) Design of the allosteric inhibitors, able to recognize specifically the binding sites that differ from the IN active site. Inhibitor V-165, belonging to such type inhibitors, prevents IN binding with the viral DNA such blocking 3'-processing reaction [94]. (ii) Design of the protein-protein inhibitors (PPIs) acting on interaction interface between either viral components (the IN monomers upon multimerization process or sub-units of the IN•vDNA complex) [95,96], or between viral
and cellular proteins (IN/LEDGF) [97,98]. These alternative strategies represent rational and prospective directions in the HIV-1 integrase drug development.

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