Ab initio molecular dynamics studies on HIV-1 reverse transcriptase triphosphate binding site: Implications for nucleoside–analog drug resistance

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Abstract

Quantum-chemical methods are used to shed light on the functional role of residues involved in the resistance of HIV-1 reverse transcriptase against nucleoside-analog drugs. Ab initio molecular dynamics simulations are carried out for models representing the adduct between the triphosphate substrate and the nucleoside binding site. The triphosphate is considered either deprotonated or protonated at the γ -position. Although the protonated form already experiences large rearrangements in the ps time scale, the fully deprotonated state exhibits a previously unrecognized low-barrier hydrogen bond between Lys65 and γ -phosphate. Absence of this interaction in Lys65→Arg HIV-1 RT might play a prominent role in the resistance of this mutant for nucleoside analogs (Gu Z et al., 1994b, *Antimicrob Agents Chemother* 38:275–281; Zhang D et al., 1994, *Antimicrob Agents Chemother* 38:282–287). Water molecules present in the active site, not detected in the X-ray structure, form a complex H-bond network. Among these waters, one may be crucial for substrate recognition as it bridges Gln151 and Arg72 with the β -phosphate. Absence of this stabilizing interaction in Gln151→Met HIV-1 RT mutant may be a key factor for the known drug resistance of this mutant toward dideoxy-type drugs and AZT (Shirasaka T et al., 1995, *Proc Natl Acad Sci USA* 92:2398–2402; Iversen AK et al., 1996, *J Virol* 70:1086–1090).

Keywords: ab initio molecular dynamics; Car-Parrinello; HIV reverse transcriptase; low-barrier hydrogen-bond; mutations; resistance

Reverse transcriptase from the human immunodeficiency virus type 1 (HIV-1 RT) is a major target for anti-AIDS therapy (Larder et al., 1987; Frankel & Young, 1998). This enzyme catalyzes the transcription of the single-stranded RNA viral genome into double-stranded DNA, which subsequently becomes integrated into host cell chromosomes (Frankel & Young, 1998; Turner & Summers, 1999). HIV-1 RT is a complex machinery, acting both as RNA/DNA dependent DNA-polymerase and as a ribonuclease H (Frankel & Young, 1998; Huang et al., 1998; Turner & Summers, 1999).

The large substrate diversity of HIV-1 RT (Tantillo et al., 1994), in comparison to the cellular isoenzymes (Cheng et al., 1987),

represents the basis of the successful therapeutic use of nucleotide-analog drugs (NRTIs) (Fig. 1). The triphosphorylated form of the NRTIs are believed to inhibit HIV-1 RT activity by both competitive inhibition as well as chain termination (Quan et al., 1998). In the latter case, the viral enzyme is able to incorporate into the growing DNA strand the triphosphorylated form of several analogs whose 3'-OH group is missing or replaced with different functions, such as the azide group in AZT (Fig. 1). The viral DNA elongation is then blocked thereby inhibiting replication of the virus particles (Taylor et al., 1990; Goody et al., 1991; Herdewijn, 1992; Schinazi, 1993).

Drug benefit is severely limited by the capability of the virus to develop mutations, which ultimately lead to drug resistance (Larder, 1993; Richman, 1993; De Clercq, 1994; Sarafianos et al., 1999). The spectrum of alterations is rather broad, as evinced by genetic and biochemical studies performed either in the laboratory or in clinical trials (Boyer et al., 1994; Tantillo et al., 1994; Larder & Stammers, 1999). Single mutations effective against drug action are usually accompanied sequentially by three to four additional mutations so that several highly resistant mutation patterns are observed. Resistant strains against NRTIs include mutations at amino acids: (1) Gln151→Met, Ala62→Val, Val75→Ile,

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Abbreviations: AIDS, acquired human immunodeficiency syndrome; AZT, 3'-azido-deoxythymidine; DFT, density functional theory; ddC, 2',3'-dideoxycytidine; ddI, 2',3'-dideoxyinosine; dTTP, deoxythymidine-triphosphate; ELF, electron localization function; HbF, hydrogen bond frequency; HIV-1, human immunodeficiency virus type 1; LBHB, low-barrier hydrogen bond; MD, molecular dynamics; NRTI, nucleoside-analog reverse transcriptase inhibitor; RT, reverse transcriptase; tP, triphosphate; VAC, velocity autocorrelation function; WT, wild-type; 3TC, 2'-dideoxy-3'-thiacytidine.

Fig. 1. Chemical formulas of selected nucleotide analogs binding to HIV-1 RT currently in clinical use for anti-AIDS therapy.

Phe77→Leu, Phe116→Tyr (AZT, ddI, and ddC multi-drug resistance; Shirasaka et al., 1995; Iversen et al., 1996); (2) Met41→Leu, Asp67→Asn, Lys70→Arg, Thr215→Tyr/Phe, Lys219→Gln (AZT resistant; Larder & Kemp, 1989; Kellam et al., 1992; Carroll et al.,

1994; Krebs et al., 1997); (3) Lys65→Arg (ddI, ddC, and 3TC cross-resistant; Gu et al., 1994b; Zhang et al., 1994; Arion et al., 1996); (4) Met184→Val/Leu (3TC resistant; Boucher et al., 1993; Gao et al., 1993; Tisdale et al., 1993).

Drug suppression is governed by diverse factors, which range from changes of DNA processivity (resistance against ddI, ddC: Arion et al., 1996; and 3TC: Boyer & Hughes, 1995), and of drug/substrate molecular recognition (resistance against ddI, AZT: Martin et al., 1993; and ddI, ddC: Gu et al., 1994a) to removal of chain-terminating residues from blocked primers (resistance against AZT: Meyer et al., 1999).

The recent determination of the crystal structure of a ternary catalytic complex of HIV-1 RT with a substrate (dTTP) and the DNA-primer and template (Huang et al., 1998) (Fig. 2A) has provided the structural basis of drug resistance. Several mutations causing resistance to NRTIs turn out to be located close to the nucleoside binding site (Fig. 2B), indicating that this type of resistance is related to a modification of the molecular recognition between phosphate and/or nucleobase-sugar moieties (Zhang et al., 1994) and the enzyme.

First principles quantum mechanics offers powerful techniques to study biochemical problems. Detailed quantum mechanical treatments of molecular and electronic structure are emerging as tools for the prediction of molecular geometry, reaction paths of enzymatic transformations, electrostatic effects, and interpretations of spectroscopic probes of biomolecular environments at very accurate levels. Ab initio calculations are also increasingly being applied to systems of pharmaceutical relevance, helping to shed light on intricate and subtle aspects of substrate- and drug-enzyme intermolecular interaction (Cheney et al., 1988; Beveridge & Heywood, 1993; Perakyla & Pakkanen, 1995, 1996; Beveridge, 1996; Alber et al., 1998; De Santis & Carloni, 1999; Piana & Carloni, 2000).

Fig. 2. **A:** dTTP–DNA–primer/template–HIV-1 RT p66 ternary complex (Huang et al., 1998). dTTP and residues important for triphosphate binding are represented as balls and sticks, DNA strands as thin lines. The p66 unit is 560 residues long, with its DNA polymerase domain (blue, right) and RNase H functional domain (red, left), respectively. **B:** In the dTTP binding site (P H-bonds to Lys65, Arg72 side chains, and Asp113, Ala114 backbone amides). Figures were made with MOLSCRIPT (Kraulis, 1991).

Here, we use ab initio quantum chemical methods to study the nature of the intermolecular interactions between HIV-1 RT and nucleosidic phosphate, which plays a major role in drug resistance (Zhang et al., 1994) for the AZT and dideoxy-type of drugs (ddI, ddC). Our aim is to gain insights into the functional role of key amino acids essential for nucleoside binding, as well as to provide information on the stability and protonation state of the triphosphate moiety.

Our calculations are carried out within the Car–Parrinello ab initio molecular dynamics scheme (Car & Parrinello, 1985). This approach appears favorable in several respects. Being based on the density functional theory with gradient corrections, it allows a rather accurate description of intermolecular forces (such as H-bonding) and of charge distribution in relatively large systems, as required here. Furthermore, it fully incorporates temperature effects, which are crucial for biological systems (Brooks et al., 1988). Finally, it has already shown to reproduce accurately structural and dynamical properties of phosphate moieties (Alber et al., 1999a, 1999b) and of enzyme active sites (Alber et al., 1998; De Santis & Carloni, 1999; Piana & Carloni, 2000).

Computational procedure

Structural models

Our starting model was the catalytic HIV-1 RT complex, whose crystal structure has been solved at 3.2 Å resolution (Huang et al., 1998) (Protein Data Bank (PDB) entry code: 1RTD) (Fig. 2A), immersed in water.

The initial positions of water molecules were constructed as follows: the ternary complex of HIV-1 RT p66 with substrate (dTTP) and the DNA-primer and template (Huang et al., 1998) was immersed in a 10 Å thick shell of waters. Sodium counterions were added to ensure electroneutrality. Water molecules and protein polar hydrogens were equilibrated by performing 100 ps of classical MD simulation using the GROMOS96 suite of programs (Van Gunsteren et al., 1996) at 300 K. (All the heavy atoms of the protein/DNA complex were kept constrained at their X-ray position.) The united atom GROMOS-A force field was used. Constant temperature simulation was achieved by coupling the system with a Berendsen's thermal bath (Berendsen et al., 1984) with a relaxation time of 0.2 ps.

Water molecules interacting to the biomolecule exhibited residence times of few tens ps, which is a much longer timescale than that of our Car–Parrinello calculations. All water molecules, which were within a distance of 5 Å to the triphosphate, at the last MD snapshot, were included in our quantum chemical model. We may expect that adding second-shell water molecules to the system will not change qualitatively our results.

At the present stage, it is impossible to perform ab initio simulations on the entire protein. Thus, our investigation was limited to a portion of the triphosphate (tP)-binding site (Fig. 2B), namely to complexes I and II (Fig. 3A). This choice appears to capture all the crucial interactions with the triphosphate moiety (the focus of the present work), yet it is still manageable from a computational point of view. The effect of the environment is then estimated by including the protein electrostatic field in the calculations.

Complexes I and II were made up of several ingredients (Fig. 3A).

The sugar-triphosphate moiety

The protonation state of tP is not known. In aqueous solution, the primary phosphate ionization state (one negative charge per

Fig. 3. **A:** Model complexes I, II used in the quantum-mechanical calculations. They include: (1) dTTP sugar–triphosphate moiety; (2) the two Mg²⁺ ions; (3) Asp110, Asp185 side chains (modeled as formate anions), Lys65 (modeled as methylammonium cation) and Arg72 (modeled as guanidinium cation) side chains; (4) Val111, Gly112, Asp113 protein backbones; (5) Ala 114; (6) selected water molecules; (7) primer 3'-hydroxy group modeled as methanol. For the sake of clarity, H atoms (except those of Asp110 and Asp185) are not shown. **B:** Atom labeling of tP binding site. Mg²⁺ ion–donor atom coordination bonds are represented in dashed lines. For the sake of clarity, only H-bonds formed by tP and waters WAT1, WAT3, and WAT7 are shown (dotted lines). It is fully deprotonated; II is protonated on the OC3 atom.

phosphate unit) prevails up to a pH of around 7, where the last proton is removed from the terminal phosphate group (Saenger, 1983). Thus, both the fully deprotonated (I) and the mono-protonated (II) forms could exist in the protein at physiological pH. The proton in II was located on the OC3(tP) atom of the γ -phosphate

group (Fig. 3B) as (1) the latter has higher pK_a ($pK_a = 7.5$) than the α - and β -moieties (Saenger, 1983); (2) protonation of γ -phosphate-OC1 and γ -phosphate-OC2 atoms partially disrupts H-bond patterns and Mg(II) coordination, respectively.

Two Mg(II) coordination polyhedra

These are composed of water molecules and the essential Asp110 and Asp185 residues (Kaushik et al., 1996), modeled as formate anions (Fig. 3A) and the primer-hydroxy group. The latter, which was not present in the X-ray structure, was added according to standard bond lengths and angles. [Asp186, the third member of the "Asp triade" in the active site (Kaushik et al., 1996) was not included, as it neither binds to the Mg^{2+} ions nor to the substrate (Larder et al., 1987; Kaushik et al., 1996).]

Groups interacting with the triphosphate anion

Arg72 and Lys65 side chains (modeled as guanidinium and methylammonium cations, respectively); the entire Ala114 residue; Val111 carbonyl group and Gly112, Asp113, Tyr115 backbone units.

Selected (16) water molecules

These are present in the active site channel from the classical simulation and located close to the tP (see above).

Charges of I and II were 0 and +1, respectively.

Calculations

Quantum-chemical calculations were performed within the framework of the density functional theory (DFT). Gradient corrected exchange and correlation functionals with the Becke (1988) and Lee-Yang-Parr (Lee et al., 1988) parameterizations were used. The basis set consisted of a plane-wave expansion up to an energy cutoff of 70 Ry. Only the Γ -point of the Brillouin zone was included. Ionic cores and valence electrons interactions were described by Troullier–Martins type (Troullier & Martins, 1991) pseudopotentials. Isolated system conditions were used as in Barnett and Landman (1993).

Car–Parrinello DFT-based MD simulations (Car & Parrinello, 1985) of complexes I and II were performed for 1.5 ps at 298 K. A time step of 0.121 fs and a fictitious electron mass of 1,000 a.u. were used. Constant temperature simulations were achieved by coupling the systems with a Nosé thermostat (Nosé, 1984) at 300 K with a frequency of 500 cm^{-1} .

In the first 0.26 ps, the Mg^{2+} ions, hydrogen atoms, and all water molecules were relaxed, keeping the protein heavy atoms constrained at their X-ray crystallographic positions. For the rest of the simulations, constraints were imposed only on the $C\alpha$ atoms of the residues G112–A114 and those H-atoms representing the connecting atoms of the residues (H_C in Fig. 3B), the primer carbon and the C1',C2' atoms of the ribose (Fig. 3B). Similar approaches have shown to describe accurately the structural and dynamical properties of other enzymatic systems (Alber et al., 1998; De Santis & Carloni, 1999; Piana & Carloni, 2000).

Geometry optimization was carried out also for model I, using the direct inversion of the iterative subspace (DIIS) method (Pulay, 1980; Hutter et al., 1994) for the electronic and ionic degrees of freedom. For geometry optimized complex I, some calculations include the external electrostatic potential of the HIV-1 RT p66-DNA complex (Huang et al., 1998). Protein–DNA hydrogen atoms were added with the amber suite of programs. Sodium counterions were added to neutralize the DNA.

The protein–DNA external potential $\Phi(r)$ at point r is evaluated according to:

$$\Phi_{prot}(r) = \sum_i \frac{q_i}{|r_i - r|}$$

where q_i represents the RESP (Cornell et al., 1993) atomic point charges of atom i with coordinates r_i , following the procedure of our previous work (De Santis & Carloni, 1999; Piana & Carloni, 2000). Charges on protein residues that are included in our quantum model have been set to zero. The protein/DNA-electrostatic potential is included in the electron/nucleus attraction integral of the Kohn–Sham Hamiltonian. The calculation with the external potential is carried out for a single-point calculation of a specific conformation of I. This conformation was obtained by geometry optimization in vacuo.

All calculations, performed with the parallel version of the Car–Parrinello (Car & Parrinello, 1985) code CPMD V3.0h (Hutter et al., 1995), required 500 h on the 64-node Cray T3E supercomputer.

Calculated properties

The electronic structure was characterized in terms of centers of maximally localized Wannier orbitals (WOC) (Silvestrelli et al., 1998; Silvestrelli & Parrinello, 1999a; Mazari & Vanderbilt, 1997), recently implemented in the Car–Parrinello scheme (Silvestrelli & Parrinello, 1999a). The relative position of these centers have been shown to be related to change in Pauling electronegativity of two atoms forming a bond and thus provide an estimate for the bond polarity (Alber et al., 1999b). Bond polarity indices (BI) were calculated following a procedure of Alber et al. (1999b).

The WOCs were also used to calculate molecular electric dipole moments. Following the procedure of Silvestrelli and Parrinello (1999b), we assume that the electronic charge distribution is concentrated in point charges located at the WOCs. This allows separation of the charge distribution in contributions from distinct elements. Changes of dipole moments can be a measure of polarization effects due to the molecular interactions in our complexes.

Velocity autocorrelation functions (VAC) were calculated for the atoms of selected bonds (OH bonds in the water molecules, the PO and OH bond of the γ -phosphate and the N–H bonds in K65) as in Alber et al. (1999a) and Allen and Tildesley (1986), where $t_{max} = 0.6$ ps. The corresponding power spectrum was obtained as Fourier transform of the VAC. The frequency interval of the transformation was chosen as 10 cm^{-1} . The VAC was calculated over the trajectory of the last 1.2 ps. The dependence of frequency on sampling was investigated by calculating the VAC in the (0.3–0.9) and (0.9–1.5) ps time ranges. The maximum difference among all calculated frequencies turned out to be as small as 20 cm^{-1} .

H-bond frequency (HbF) is calculated as the number of H-bonds for each atom normalized to the total number of trajectory steps; that is, $HbF = 1$ implies that a H-bond is always maintained during the dynamics. Hydrogen bonds are assumed if $d(\text{hydrogen} - \text{H-bond acceptor}) \leq 2.5 \text{ \AA}$ and $120 \leq \angle(\text{H-bond donor} - \text{hydrogen H-bond acceptor}) \leq 180$.

Results

In this section, we describe the structural and dynamical properties of complexes representing the triphosphate binding site in HIV-1 RT in different protonation states, the fully deprotonated (I) and

the monoprotonated (II) forms, in the absence or in the presence of the protein electrostatic field.

Fully deprotonated form (complex I)

Triphosphate (tP) conformational stability and H-bond network at the active site

The relative orientation of phosphate to the ribose is conveniently described in terms of the angle ξ between atoms OC3'(ribose), PA(tP), and PB(tP), and distance D between atoms OC3'(ribose) and PC(tP) (see Fig. 4A). After 0.5 ps ξ and D fluctuates around average values (Fig. 4B; Table 1). Most intermolecular enzyme-tP contacts are maintained during the dynamics: Table 2 shows that tP keeps its H-bonds with Arg72, Lys65, Asp113, and Ala114 backbone.

Our simulation also allows to establish the interactions of the ribose-3'-hydroxy group. Table 1 indicates that a stable intramolecular hydrogen bond to the β -phosphate unit is formed during the entire length of the MD simulation (Table 2). The rotational fluctuations around the 3'-OH in the ribose 3'-hydroxy group allows for H-bonding either to OB1 or OB2.

tP also interacts extensively with water molecules, forming and breaking several H-bonds during the dynamics (Table 2). Table 3 compares selected properties of the strongly bound water molecules (namely WAT1, WAT3, and WAT7, with time averaged H-acceptor distances less the 2 Å) with those obtained from simulations of water in the bulk (Silvestrelli & Parrinello, 1999b) and in the gas phase (Silvestrelli & Parrinello, 1999b). The MD averaged O—H bonds of these waters are significantly longer than those in vacuo and slightly longer than those in bulk, presumably as a result of stronger H-bond interactions (Jeffrey & Saenger, 1991). Consistently, these ordered waters also cause a decrease of the O—H stretching frequency with respect to water in bulk (Table 3).

A good indication of polarization effects on the water molecules, and thus a qualitative estimate on its electrostatic binding contribution, is given by the size of the molecular dipole moment of the waters. Therefore, we have calculated the molecular dipole moments of all waters derived by the maximally localized Wannier functions (see Computational procedure section) (Silvestrelli & Parrinello, 1999b). For our analysis we have used the geometry optimized structure of a MD snapshot.

The averaged dipole moment of all water molecules in our model turns out to be smaller than for water in bulk ($\mu_{av} = 2.6$ Deybe, in comparison to a recent investigations on water in bulk $\mu_{bulk} = 3.0$ Deybe). This small value arises from contributions of some of the waters relatively far from the biomolecule, whose H-bond valences are partially unsaturated ($\mu_{av} = 2.5$ Debye). In contrast, waters coordinated to Mg^{2+} and/or binding to phosphate show relatively large polarization ($\mu_{av} = 3.0$ Deybe). Waters that contribute significantly to tP stabilization (namely WAT1, WAT3, and WAT7) are more polarized in comparison to the averaged water dipole moment. In particular WAT3 reveals a dipole moment as large as $\mu_{WAT3} = 3.2$ Deybe, due to the strong electric field of Arg72 side chain and β -phosphate charged groups. As a result, WAT3 is very well ordered, and it fluctuates around an average position. Due to its electrostatic properties and well-ordered structure, we would expect significant contribution to tP recognition.

However, in the crystal structure, no water molecules are resolved at the present resolution. Inspection of the X-ray structure

Fig. 4. A: Definition of angle ξ and distance D . B: plot of ξ , D as a function of time in complexes I (blue) and II (red). The yellow lines denote the corresponding values of the X-ray structure. C: Conformation of the tP moiety in the X-ray structure (yellow) and the models I (blue) and II (red) taken from the last MD snapshots.

(Huang et al., 1998) suggests that a water at the favorable WAT3 position would be accommodated in a small cavity formed by tP and the side chains of Arg72, Lys65, Gln151, and Phe116.

Table 1. Selected structural properties time averaged for the last 1.2 ps^a

	I	II
D[NeHa(Lys65)···OC1(tP)]	1.2(0.1)	4.5(0.7)
D[OC1(tP)–Nε(Lys65)]	2.6(0.1)[–0.1]	4.8(0.5)[2.3]
D[NeHb(Lys65)···OBC(tP)]	3.4(0.3)	4.2(0.5)
D[OBC(tP)–Nε(Lys65)]	3.5(0.3)[0.1]	3.3(0.3)[–0.1]
D[η1(Arg72)···Nε(Lys65)]	4.5(0.2)[0]	4.9(0.3)[0.4]
D[Nη1Ha(Arg72)···OA1(tP)]	2.1(0.2)	2.6(0.4)
D[Nη1(Arg72)···OA1(tP)]	3.0(0.2)[0]	3.2(0.3)[0.2]
D[Nη1Hb(Arg72)···OB1(tP)]	2.2(0.4)	3.5(0.6)
D[Nη1(Arg72)···OB1(tP)]	3.2(0.4)[–1.3]	4.3(0.5)[–0.2]
D[Nη1Ha(Arg72)···OAB(tP)]	2.4(0.2)	2.6(0.3)
D[Nη1(Arg72)···OAB(tP)]	3.0(0.1)[0.3]	3.3(0.3)[0.6]
D[NH(Asp113)···OBC(tP)]	2.5(0.1)	3.7(0.4)
D[N(Asp113)···OBC(tP)]	3.4(0.2)[0.3]	4.6(0.4)[1.5]
D[NH(Ala114)···OB2(tP)]	2.1(0.2)	2.9(0.1)
D[N(Ala114)···OB2(tP)]	3.1(0.2)[–0.1]	3.9(0.2)[0.7]
D[C3'–OH···OB1(tP)]	2.8(0.6)	3.9(0.3)
D[C3'–O···OB1(tP)]	3.6(0.5)[0.6]	4.7(0.3)[1.7]
D[C3'–OH···OB2(tP)]	2.7(0.5)	4.0(0.3)
D[C3'–O···OB2(tP)]	3.5(0.3)[–0.6]	4.7(0.2)[0.6]
D[Mg(1)···OA2(tP)]	2.3(0.3)[–0.7]	2.1(0.1)[–0.9]
D[C3'(ribose)–PC(tP)]	7.1(0.1)[0.4]	7.9(0.1)[1.2]
D[H(WAT1)···OA1(tP)]	1.7(0.2)	3.0(0.5)
D[H(WAT2)···OA1(tP)]	2.6(0.3)	4.4(0.7)
D[H(WAT2)···OA2(tP)]	3.0(0.3)	3.9(0.4)
D[H(WAT3)···OB1(tP)]	1.8(0.2)	1.9(0.3)
D[H(WAT5)···OC1(tP)]	2.1(0.3)	1.8(0.1)
D[H(WAT6)···OC2(tP)]	2.6(0.5)	4.1(0.4)
D[H(WAT7)···OC3(tP)]	1.9(0.4)	3.9(1.0)
δ[Mg(1)]	0.8(0.2)	0.9(0.3)
δ[Mg(2)]	0.4(0.2)	1.1(0.4)
∠[Nε(Lys65)···Ha···OC1(tP)]	167(6)	100(14)
∠[C3'(ribose)···PA(tP)···PB(tP)]	66(2)[4]	80(3)[18]

^aDistances (d), deviations (δ), and angles (∠) from the starting and X-ray structure are reported for complexes I and II. Distances and deviations are in Å, angles in °. Standard deviations and deviations from the X-ray structure (Huang et al., 1998) are reported in round and square parenthesis, respectively.

Presumably the Gln151 residue is positioned to further hydrogen bond with this water molecule (Fig. 5).

Lys65-tP interactions

The Lys65-γ-phosphate salt bridge is well maintained during the dynamics (Tables 1, 2). The salt bridge is characterized by very short Lys-tP distances (Table 1); interestingly, proton hopping between OC1(tP) and ε(Lys65) occurs several times in the ps time-scale (Fig. 6A). Each proton transfer event is correlated to local minima in the (OC1(tP)-Nε(Lys65) distance.

This type of interaction, characterized by fast proton hopping between H-bond donor and acceptor, is described as “low-barrier hydrogen bond” (LBHB). Theoretical work (Piana & Carloni, 2000) and suggestions from experimental studies (Rodriguez et al., 1993) have pointed to the existence of a LBHB in the HIV-protease cleavage site, for the biological function of serine proteases (Lin et al., 1998; De Santis & Carloni, 1999) and in enzyme model substrates (Schiott et al., 1998).

Table 2. Protein- and water-phosphate hydrogen bonding patterns in complex I and II^a

H-bond pair	I HbF	II HbF
OA1(tP)-HN(Arg72)	0.97 (0.98)	0.58 (0.69)
OA1(tP)-H(WAT2)	0.31 (0.62)	0.02 (0.16)
OA1(tP)-H(WAT1)	1.00 (1.00)	0.49 (0.54)
OAB(tP)-HN(Arg72)	0.29 (0.31)	0.26 (0.39)
OA2(tP)-H(WAT2)	0.01 (0.01)	0.00 (0.00)
OB1(tP)-HN(Arg72)	0.67 (0.77)	0.06 (0.17)
OB1(tP)-H(C3'OH)	0.45 (0.66)	0.00 (0.00)
OB1(tP)-H(WAT3)	1.00 (1.00)	0.86 (1.00)
OB1(tP)-H _a (Lys65)	0.00 (0.00)	0.71 (0.77)
OB2(tP)-HN(Asp113)	0.03 (0.17)	0.22 (0.79)
OB2(tP)-HN(Ala114)	1.00 (1.00)	0.18 (0.67)
OB2(tP)-H(C3'OH)	0.33 (0.56)	0.00 (0.07)
OBC(tP)-HN(Asp113)	0.57 (0.99)	0.11 (0.23)
OBC(tP)-H _a (Lys65)	0.01 (0.01)	0.43 (0.52)
OC1(tP)-H _a (Lys65)	1.00 (1.00)	0.20 (0.22)
OC1(tP)-H(WAT5)	0.97 (1.00)	1.00 (1.00)
OC2(tP)-H(WAT6)	0.38 (0.47)	0.00 (0.08)
OC3(tP)-H(WAT7)	0.89 (1.00)	0.24 (0.35) ^b

^aH-bond frequency (HbF) is calculated as the number of H-bonds for each atom normalized to the total number of trajectory steps. Hydrogen bonds are assumed if $d(\text{hydrogen} - \text{H-bond acceptor}) \leq 2.5 \text{ \AA}$ and $120^\circ \leq \angle(\text{H-bond donor} - \text{hydrogen} - \text{H-bond acceptor}) \leq 180^\circ$. HbF is one when an H-bond is always maintained during the dynamics. In parentheses the HbF with a H-bond criteria of $d(\text{hydrogen} - \text{H-bond acceptor}) \leq 3.0 \text{ \AA}$ and $120^\circ \leq \angle(\text{H-bond donor} - \text{hydrogen} - \text{H-bond acceptor}) \leq 180^\circ$ is also indicated.

^bWAT7 oxygen as H-bond acceptor.

Fig. 5. Position of WAT3 and tP (ball and stick) of the last MD snapshot, fitted to the corresponding positions in the HIV-1 RT X-ray structure (as lines). Possible hydrogen bonds of WAT3 are indicated with dotted lines.

Table 3. Comparison of selected properties of WAT1, WAT3, WAT7 in model I with isolated and liquid water

	Ab initio MD (WAT1, WAT3, WAT7) This work	Optimized structure, isolated water (Silvestrelli & Parrinello, 1999b)	Ab initio MD liquid water (Silvestrelli & Parrinello, 1999b)
D(O-H)	1.00(0)	0.97	0.99
ν_{OH}	2,700–3,000	—	3,300 ^b

^aDistances are reported in Å; vibrational frequencies in cm⁻¹.^bEstimated from the D₂O vibrational spectrum (Silvestrelli et al., 1997). **Fig. 6.** tP-Lys65 interactions. NeH₃(Lys65)···OC1(tP) distances as a function of time in (A) I and (B) II. (C–E) Electron localization function (ELF) projected on the OC1···H_a···Ne plane (C) before, (D) during, and (E) after the proton transfer in the LBHB (complex I). The ELF ranges from blue (0.20) to red (0.98). For the sake of clarity only the Lys65 and γ -phosphate atoms are shown. (F) Three-centered molecular orbital in the transition state involving the proton shared by OC1(tP) and Ne(Lys65). Isodensity contour level corresponds to -5.5 a.u. (yellow) and 5.5 a.u. (green).

proton transfer. Red areas indicate spatial regions of strong electron localization (ELF close to 1), in which the Pauli exclusion principle has little influence on the electron distribution. In the initial state (Fig. 6C), high values of ELF on atom Ne(Lys65) indicate the electron lone pair, the red area between OC1(tP) and H_a specify the covalent chemical bond. In the “transition state” (Fig. 6D), chemical bonding changes dramatically. The significant increase of ELF between atoms H_a and Ne(Lys65) denotes the formation of a new covalent bond, while ELF is still significant (and, hence, the bonding highly covalent) along the OC1(tP)···H_a bond. Calculation of the electronic structure in terms of Kohn–Sham one-electron levels provides a consistent and an independent evidence of a Ne(Lys65)···H_a···OC1(tP) covalent interaction. A covalent σ -type molecular orbital stabilizes the transition state (Fig. 6F), confirming the ideas of Gilli and Cleland (Cleland, 1992; Schiott et al., 1998).

Environment effects

Our MD simulations do not take into account the presence of the outer protein electrostatic field. To estimate its effect, we have analyzed the electronic structure of a geometry optimized MD snapshot in terms of maximally localized Wannier centers (WOC) (see Computational procedure section). We compare the geometrical variations of the WOC along a chemical bond as a result of the presence of the protein/DNA electrostatic potential. This analysis has been shown to be a precise indicative of environmental effects on the electronic structure and can be related to changes in bond polarity (Alber et al., 1999b). We focus here on changes of electronic structure of the LBHB and of WAT3.

Figure 7 compares the positions of the WOCs with and without the inclusion of the protein electrostatic field. We observe only a minimal shift of the WOC along the Ne(Lys65)–H_a bond that corresponds to only a small variation in bond polarity due to the effect of the electric field (Fig. 7). We calculate the difference in bond polarity to be as small as $\Delta B_{\text{NH}_a}(\text{N}) = 0.002$, which corresponds to only a very small change in Pauling electronegativity (~ 0.02 units) (Alber et al., 1999b). In comparison, the difference

Fig. 7. Centers of maximally localized Wannier orbitals (WOC) calculated for a geometry optimized MD snapshot of complex I (see Computational procedure section). Positions of WOCs are depicted as yellow spheres in vacuo and green spheres with the inclusion of the protein electric field.

in BI within all three Lys65–NH bonds is as much as $\Delta B_{\text{NH}}(\text{N}) = 0.114$. Thus, inclusion of the protein electric field appears not to affect the electronic properties in the Ne(Lys65)···H_a···OC1(tP) interactions.

We note also only minor changes in electronic structure of WAT3, and consequently, the size of the molecular dipole moment is not affected by the inclusion of the protein electrostatic field.

Magnesium coordination

After 0.3 ps, both magnesium(II) ions are stable octahedral coordinated. Whereas the complete coordination shell of Mg(2) is already known from the X-ray structure, the exact position and coordination of Mg(1) are unknown, as the primer 3'-OH terminus, which is expected to be coordinated to Mg(1) is included in the model and it is not present in the X-ray structure (Huang et al., 1998).

Therefore, significant structural arrangements in the Mg(1) position take place, as demonstrated by the large difference of the time-averaged position in comparison to X-ray data (Table 1).

The Mg(1)–OA2(tP) coordination distance is reduced during our simulation up to a value comparable to the other Mg(1)–oxygen distances [average 2.2(0.1) Å] in the same complex.

The coordination shell includes OD1B(Asp110), OD2A(Asp185), OA2(tP), two water molecules (WAT2, WAT9), and the primer 3'-OH terminus (Fig. 8A). WAT2 hydrogen bonds also to the α -phosphate and could play a role in phosphate binding (Fig. 8A). During the dynamics Mg(2) is coordinated to OA2(tP), OB2(tP), OC2(tP), OV(Val111), OD2B(Asp185), and OD1B(Asp110) (Fig. 8B), as already shown in the X-ray structure.

Vibrational frequencies can probe the binding of metal ions (Wang et al., 1998). The non-ester P–O(tP) stretching frequencies are calculated to decrease by $\approx 200 \text{ cm}^{-1}$ upon binding to Mg^{2+} , in agreement with experimental data (Wang et al., 1998).

Monoprotonated form (complex II)

Protonation of the γ -phosphate in model II has a dramatic effect on the stability of the complex, already in the very short time scale investigated.

H-bond pattern and conformational flexibility

The interactions between the protein and tP are highly affected by the protonation of the γ -phosphate. H-bonds are partially or totally lost between the β -phosphate and the enzyme backbone Ala114 and Asp113 and residue Arg72 as well as between the β -phosphate and the C3'-OH group (Tables 1, 2).

As a consequence, the D and ξ geometric parameters are significantly larger than those of model I, and the tP conformation differs largely from the X-ray structural template (Fig. 4B; Table 1).

Solvent structuring is similar to I. Several waters bind to tP, among which only WAT3 is particularly well ordered in both complexes, fluctuating around an average position during the dynamics. This is due presumably to strong interactions with Arg72 and tP charged groups.

Lys65–tP interactions

No proton exchange takes place between Lys65 and the γ -phosphate during the timescale investigated (Fig. 6B); instead, Lys65 turns out to interact preferentially with the β -phosphate

Fig. 8. Mg^{2+} coordination shell of (A) Mg(1) and (B) Mg(2) for model I and (C) Mg(1) and (D) Mg(2) of model II, taken from the corresponding last MD snapshot.

H-bond acceptors OBC(tP) and OB1(tP) (Tables 1, 2), which causes large structural rearrangements.

Overall, the structural differences to the X-ray structure are significantly larger than for I. The distance between the Arg72 and Lys65 side chain is rather large (Table 1) compared with that of the X-ray structure and the MD-averaged distance in I. This is presumably a consequence of the unfavorable electrostatic Lys65–Arg72 interactions.

Magnesium coordination

The Mg(1) averaged position is rather similar to that in complex I (Table 1). However, after 0.7 ps of simulation, we observe some rearrangements in its coordination sphere, which results in the loss of a water ligand (WAT9) (Fig. 8C). This rearrangement is initiated by structural variations of the Asp185 group that compensates for the octahedral coordination of Mg(1). Presumably, this is a consequence of the larger conformational fluctuations in tP in model II, which affects also the positions of the charged Asp residues. The coordination polyhedra for Mg(2) is stable and similar to complex I (Fig. 8D), whereas its averaged position deviates significantly from the X-ray position (Table 1) as a result of the large structural arrangement in tP conformation.

In conclusion, our calculations suggest that the triphosphate–enzyme pocket complex in its X-ray structural conformation is

much more flexible if the γ -phosphate is protonated, and that intermolecular interactions provide less stabilization of the adduct in terms of H-bonding.

Discussion

Our quantum-mechanical calculations, carried out for the fully deprotonated form (complex I) and the monoprotonated form (in the γ position) (complex II), provide information on chemical bonding and intermolecular interactions of the substrate–triphosphate moiety at the HIV-1 RT nucleoside binding site.

Within the limitations of the relatively small model (such as the limited representation of hydration) of the short timescale explored, we find a high sensitivity of tP conformational properties on changes in the chemical environment of the γ -phosphate.

The fully deprotonated form of tP turns out to be the more stable and is in good agreement to the HIV-1 RT X-ray structure. The conformational stability of the triphosphate (tP) anion could be achieved not only—as expected—by strong electrostatic interactions with the positively charged enzyme pocket, but also by means of previously unrecognized fast proton exchange with the highly conserved Lys65 residue (Barber et al., 1990). Proton hopping between the OC1(tP) and N ϵ (Lys65) atoms occurs in the sub-ps timescale and is characterized by a very short heteroatomic

distance (Table 1). Analysis of chemical bonding, in terms of electron localization functions (Fig. 6C–E) (Becke & Edgecombe, 1990) and Kohn–Sham one-electron levels (Fig. 6F), indicates that the LBHB is covalent (and thus highly directional) in nature. Similar findings have recently been reported for other enzyme models (Schiott et al., 1998; De Santis & Carloni, 1999), confirming the results obtained with an atoms in molecule analysis (Schiott et al., 1998). However, we have to mention that a quantitative description of the proton motion in the LBHB would require a quantum treatment of the proton (Marx et al., 1999), an explicit treatment of environment effects and a longer timescale than that of the present study.

Inclusion of the external protein electrostatic field appears not to affect the electronic properties in the Lys65–phosphate interactions. As the description of the motion of the protons (as well as of all the other atoms of the system) are classical, the quantum nature of proton dynamics is expected to modify the picture quantitatively but not qualitatively.

The energetic contribution of the LBHB in binding is difficult to assess. The increased covalent bonding contribution between Lys65 and the γ -phosphate is accompanied by partial charge neutralization, and thus could be easily counterbalanced by the expected loss of electrostatic stabilization in the binding pocket. However, a large degree of covalency is expected to be strongly directional and thus may be an important factor in stabilizing the bioactive conformation.

A crucial point regards the reliability of our computational approach in describing the LBHB. To the best of our knowledge, these are the first calculations pointing to a LBHB between a lysine and a triphosphate group, characterized by rather different pK_a . [pK_a (Lys) = 10.4; pK_a (tP) = 7.5, pK_a (His) = 6.4, pK_a (Asp) = 4.0 from Saenger, 1983; Fersht, 1997.] The presence of this interaction cannot be firmly established by these calculations only, as DFT–BLYP is known to underestimate proton transfer barriers (Sirois et al., 1997). Further investigations through experiments [such as NMR experiments (Perrin & Nielson, 1997)] and different high-level theoretical methods (such as MP2/H-F) are required to put this result on more fundamental grounds.

The possible presence of the LBHB may have implications for drug resistance. Lys65 is a key residue for the emergence of resistance; the mutation Lys65→Arg causes resistance toward dideoxy type drugs (Gu et al., 1994b; Sluis-Cremer et al., 2000) (ddI, ddC). At the speculative level, the very large pK_a difference between Arg and tP might hamper the fast proton exchange. The loss of a covalent and directional phosphate stabilization could affect those substrates to a higher extent, which lack the 3' hydroxy group of the natural substrate (namely, ddI, ddC), as this hydroxy group stabilizes further the tP bioactive conformation with an intramolecular H-bond.

Thus, the directionality to the covalent Lys65–tP interaction (Table 1) could play a prominent role in stabilizing the bioactive phosphate conformation.

Protonation of γ -phosphate largely affects the conformational properties of tP and Lys65 (complex II in Table 1), as a consequence of the loss of the covalent LBHB and of the decrease of electrostatic interactions between tP and the protein active site positively charged groups. The deprotonated form turns out to be the most stable in the enzymatic pocket of HIV-1 RT and, therefore, our calculations point to the presence of this form in the active site of HIV-1 RT. Notice that although the fraction of the unprotonated form at physiological pH in aqueous solution is only

~30% (Saenger, 1983), in the protein the pK_a of phosphate might increase because of the strong interactions with a variety of positively charged groups (Mg²⁺, Lys65, Arg72).

Water molecules, not detected in the X-ray structure, form a complex H-bond network at the active site. A well-ordered water (WAT3 in Fig. 5), highly polarized by the environment, appears to play a role in recognition of tP, by bridging the latter with Arg72 and presumably Gln151. Mutation of the latter with nonpolar residues could affect the WAT3 H-bond network and may be a factor in the well-known resistance of mutation Gln151→Met toward dideoxy type drugs (ddI, ddC, and 3TC) and AZT (Shirasaka et al., 1995).

In conclusion, our quantum chemical calculations reveal a variety of functionally important characteristics of tP/HIV-1 RT interactions, which cannot be discerned by visual inspection of the molecular structure. Several ingredients, such as the polarization forces, the treatment of bond-forming/breaking processes in the LBHB and temperature effects might play a critical role for substrate binding and possibly for drug-resistance mechanisms.

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References

- Alber F, Folkers G, Carloni P. 1999a. Conformational analysis of dimethyl phosphate in aqueous solution: A density-functional theory-based molecular dynamics study. *J Mol Struct (Theochim)* 489:237–245.
- Alber F, Folkers G, Carloni P. 1999b. Dimethyl phosphate: Stereoelectronic versus environmental effects. *J Phys Chem B* 103:6121–6126.
- Alber F, Kuonen O, Scapozza L, Folkers G, Carloni P. 1998. Density functional studies on herpes simplex virus type 1 thymidine kinase-substrate interactions: The role of Tyr-172 and Met-128 in thymine fixation. *Proteins* 31:453–459.
- Allen MP, Tildesley DJ. 1986. *Computer simulation of liquids*. New York: Oxford Science Publications.
- Arion D, Borkow G, Gu Z, Wainberg MA, Parniak MA. 1996. The K65R mutation confers increased DNA polymerase processivity to HIV-1 reverse transcriptase. *J Biol Chem* 271:19860–19864.
- Barber A, Hizi A, Maizel JV, Hughes SH. 1990. HIV-1 reverse transcriptase: Structure predictions for the polymerase domain. *AIDS Res Hum Retroviruses* 6:1061–1072.
- Barnett RN, Landman U. 1993. Born-Oppenheimer molecular dynamics simulations of finite systems: Structure and dynamics. *Phys Rev B* 48:2081–2097.
- Becke A. 1988. Density-functional exchange-energy approximation with correct asymptotic behaviour. *Phys Rev A* 38:3098–3100.
- Becke AD, Edgecombe KE. 1990. A simple measure of electron localization in atomic and molecular systems. *J Phys Chem* 92:5397–5403.
- Berendsen HJ, Postma JPM, Van Gunsteren WF, Di Nola A, Haak JR. 1984. Molecular dynamics with coupling to an external bath. *J Phys Chem* 88:3684–3690.
- Beveridge A. 1996. A theoretical study of torsional flexibility in the active site of aspartic proteinases: Implications for catalysis. *Proteins* 24:322–334.
- Beveridge AJ, Heywood GC. 1993. A quantum mechanical study of the active site of aspartic proteinases. *Biochemistry* 32:3325–3333.
- Boucher CA, Cammack N, Schipper P, Schuurman R, Rouse P, Wainberg MA, Cameron JM. 1993. High-level resistance to (–) enantiomeric 2'-deoxy-3'-thiacytidine in vitro is due to one amino acid substitution in the catalytic site of human immunodeficiency virus type 1 reverse transcriptase. *Antimicrob Agents Chemother* 37:2231–2234.
- Boyer PL, Ferris AL, Clark P, Whitmer J, Frank P, Tantillo C, Arnold E, Hughes SH. 1994. Mutational analysis of the fingers and palm subdomain of human immunodeficiency virus type-1 (HIV-1) reverse transcriptase. *J Mol Biol* 243:472–483.
- Boyer PL, Hughes SH. 1995. Analysis of mutations at position 184 in reverse transcriptase of human immunodeficiency virus type 1. *Antimicrob Agents Chemother* 39:1624–1628.

- Brooks CL III, Karplus M, Pettit BM. 1988. *Proteins: A theoretical perspective of dynamics, structure, and thermodynamics*. New York: Wiley.
- Car R, Parrinello M. 1985. Unified approach for molecular dynamics and density-functional theory. *Phys Rev Lett* 55:2471–2474.
- Carroll SS, Geib J, Olsen DB, Stahlhut M, Shafer JA, Kuo LC. 1994. Sensitivity of HIV-1 reverse transcriptase and its mutants to inhibition by azidothymidine triphosphate. *Biochemistry* 33:2113–2120.
- Cheney J, Cheney BV, Richards WG. 1988. Calculation of NH...pi hydrogen bond energies in basic pancreatic trypsin inhibitor. *Biochim Biophys Acta* 954:137–139.
- Cheng YC, Dutschman GE, Bastow KF, Sarngadharan MG, Ting RYC. 1987. Human immunodeficiency virus reverse transcriptase. General properties and its interactions with nucleoside triphosphate analogs. *J Biol Chem* 262:2187–2189.
- Cleland WW. 1992. Low-barrier hydrogen bonds and low fractionation factor bases in enzymatic reactions. *Biochemistry* 31:317–319.
- Cornell WL, Cieplak P, Bayly CI, Kollman PA. 1993. Application of RESP charges to calculate conformational energies, hydrogen bond energies and free energies of solvation. *J Am Chem Soc* 115:9620–9630.
- De Clercq E. 1994. HIV resistance to reverse transcriptase inhibitors. *Biochem Pharmacol* 47:155–169.
- De Santis L, Carloni P. 1999. Serine proteases: An ab initio molecular dynamics study. *Proteins* 37:611–618.
- Fersht A. 1997. *Enzyme structure and mechanism*. New York: W.H. Freeman and Company.
- Frankel AD, Young JA. 1998. HIV-1: Fifteen proteins and an RNA. *Annu Rev Biochem* 67:1–25.
- Gao Q, Gu Z, Parniak MA, Cameron J, Cammack N, Boucher C, Wainberg MA. 1993. The same mutation that encodes low-level human immunodeficiency virus type 1 resistance to 2',3'-dideoxyinosine and 2',3'-dideoxycytidine confers high-level resistance to the (–) enantiomer of 2',3'-dideoxy-3'-thiacytidine. *Antimicrob Agents Chemother* 37:1390–1392.
- Goody RS, Mueller B, Restle T. 1991. Factors contributing to the inhibition of HIV-reverse transcriptase by chain-terminating nucleotides in vitro and in vivo. *FEBS Lett* 291:1–5.
- Gu Z, Fletcher RS, Arts EJ, Wainberg MA, Parniak MA. 1994a. The K65R mutant reverse transcriptase of HIV-1 cross-resistant to 2',3'-dideoxycytidine, 2',3'-dideoxy-3'-thiacytidine, and 2',3'-dideoxyinosine shows reduced sensitivity to specific dideoxynucleoside triphosphate inhibitors in vitro. *J Biol Chem* 269:28118–28122.
- Gu Z, Gao Q, Fang H, Salomon H, Parniak MA, Goldberg E, Cameron J, Wainberg MA. 1994b. Identification of a mutation at codon 65 in the IKKK motif of reverse transcriptase that encodes human immunodeficiency virus resistance to 2',3'-dideoxycytidine and 2',3'-dideoxy-3'-thiacytidine. *Antimicrob Agents Chemother* 38:275–281.
- Herdewijn PA. 1992. Novel nucleoside strategies for anti-HIV and anti-HSV therapy. *Antiviral Res* 19:1–14.
- Huang H, Chopra R, Verdine GL, Harrison SC. 1998. Structure of a covalently trapped catalytic complex of HIV-1 reverse transcriptase: Implications for drug resistance. *Science* 282:1669–1675.
- Hutter J, Ballone P, Bernasconi M, Focher P, Fois E, Godecker S, Parrinello M, Tuckerman M. 1995. *CPMD version 3.0h*; 1995–96. MPI für Festkörperforschung, Stuttgart, and IBM Zurich Research Laboratory.
- Hutter J, Lüthi HP, Parrinello M. 1994. Electronic structure optimization in plane-wave-based density functional calculations by direct inversion in the iterative subspace. *Comput Mater Sci* 2:244–248.
- Iversen AK, Shafer RW, Wehrly K, Winters MA, Mullins JJ, Chesebro B, Merigan TC. 1996. Multidrug-resistant human immunodeficiency virus type 1 strains resulting from combination antiretroviral therapy. *J Virol* 70:1086–1090.
- Jeffrey GA, Saenger W. 1991. *Hydrogen bonding in biological structures*. New York: Springer-Verlag.
- Kaushik N, Rege N, Yadav PN, Sarafianos SG, Modak MJ, Pandey VN. 1996. Biochemical analysis of catalytically crucial aspartate mutants of human immunodeficiency virus type 1 reverse transcriptase. *Biochemistry* 35:11536–11546.
- Kellam P, Boucher CA, Larder BA. 1992. Fifth mutation in human immunodeficiency virus type 1 reverse transcriptase contributes to the development of high-level resistance to zidovudine. *Proc Natl Acad Sci USA* 89:1934–1938.
- Kraulis PJ. 1991. MOLSCRIPT: A program to produce both detailed and schematic plots of protein structures. *J Appl Crystallogr* 24:946–950.
- Krebs R, Immendorfer U, Thrall SH, Wohrl BM, Goody RS. 1997. Single-step kinetics of HIV-1 reverse transcriptase mutants responsible for virus resistance to nucleoside inhibitors zidovudine and 3-TC. *Biochemistry* 36:10292–10300.
- Larder BA. 1993. Inhibitors of HIV reverse transcriptase as antiviral agents and drug resistance. In: Skalka AM, Goff SP, eds. *Reverse transcriptase*. Cold Spring, New York: Cold Spring Harbor Laboratory Press. pp 205–222.
- Larder BA, Kemp SD. 1989. Multiple mutations in HIV-1 reverse transcriptase confer high-level resistance to zidovudine (AZT). *Science* 246:1155–1158.
- Larder BA, Purifoy DJ, Powell KL, Darby G. 1987. Site-specific mutagenesis of AIDS virus reverse transcriptase. *Nature* 327:716–717.
- Larder BA, Stammers DK. 1999. Closing in on HIV drug resistance. *Nat Struct Biol* 6:103–106.
- Lee C, Yang W, Parr RG. 1988. Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. *Phys Rev B* 37:785–789.
- Lin J, Westler WM, Cleland WW, Markley JL, Frey PA. 1998. Fractionation factors and activation energies for exchange of the low barrier hydrogen bonding proton in peptidyl trifluoromethyl ketone complexes of chymotrypsin. *Proc Natl Acad Sci USA* 95:14664–14668.
- Martin JL, Wilson JE, Haynes RL, Furman PA. 1993. Mechanism of resistance of human immunodeficiency virus type 1 to 2',3'-dideoxyinosine. *Proc Natl Acad Sci USA* 90:6135–6139.
- Marx D, Tuckerman ME, Hutter J, Parrinello M. 1999. The nature of the hydrated excess proton in water. *Nature* 397:601–604.
- Mazari N, Vanderbilt D. 1997. Maximally localized generalized Wannier functions for composite energy bands. *Phys Rev B* 56:12847–12864.
- Meyer PR, Matsuura SE, Mian AM, So AG, Scott WA. 1999. A mechanism of AZT resistance: An increase in nucleotide-dependent primer unblocking by mutant HIV-1 reverse transcriptase. *Mol Cell* 4:35–43.
- Nosé S. 1984. A unified formulation of the constant temperature molecular dynamics method. *J Chem Phys* 81:511–519.
- Perakyla M, Pakkanen TA. 1995. Model assembly study of the ligand binding by p-hydroxybenzoate hydroxylase: Correlation between the calculated binding energies and the experimental dissociation constants. *Proteins* 21:22–29.
- Perakyla M, Pakkanen TA. 1996. Ab initio models for receptor-ligand interactions in proteins. 4. Model assembly study of the catalytic mechanism of triosephosphate isomerase. *Proteins* 25:225–236.
- Perrin CL, Nielson JB. 1997. “Strong” hydrogen bonds in chemistry and biology. *Annu Rev Phys Chem* 48:511–543.
- Piana S, Carloni P. 2000. Conformational flexibility of the catalytic Asp dyad in HIV-1 protease: An ab initio study on the free enzyme. *Proteins* 39:26–36.
- Pulay P. 1980. Convergence acceleration of iterative sequences. The case of SCF iteration. *Chem Phys Lett* 73:393–398.
- Quan Y, Gu Z, Li X, Liang C, Parniak MA, Wainberg MA. 1998. Endogenous reverse transcriptase assays reveal synergy between combinations of the M184V and other drug resistance-conferring mutations in interactions with nucleoside analog triphosphates. *J Mol Biol* 277:237–247.
- Richman DD. 1993. HIV drug resistance. *Annu Rev Pharmacol Toxicol* 33:149–164.
- Rodriguez EJ, Angeles TS, Meek TD. 1993. Use of nitrogen-15 kinetic isotope effects to elucidate details of the chemical mechanism of human immunodeficiency virus 1 protease. *Biochemistry* 46:12380–12385.
- Saenger W. 1983. *Principles of nucleic acid structure*. New York: Springer-Verlag.
- Sarafianos SG, Das K, Ding J, Boyer PL, Hughes SH, Arnold E. 1999. Touching the heart of HIV-1 drug resistance: The fingers close down on the dNTP at the polymerase active site. *Chem Biol* 6:R137–R148.
- Schinzari RF. 1993. Competitive inhibitors of human immunodeficiency virus reverse transcriptase. *Perspect Drug Discovery Design* 1:151–169.
- Schiott B, Iversen BB, Madsen GKH, Larsen FK, Bruice TC. 1998. On the electronic nature of low-barrier hydrogen bonds in enzymatic reactions. *Proc Natl Acad Sci USA* 95:12799–12802.
- Shirasaka T, Kavlick MF, Ueno T, Gao WY, Kojima E, Alcaide ML, Chokekijchai S, Roy BM, Arnold E, Yarchoan R. 1995. Emergence of human immunodeficiency virus type 1 variants with resistance to multiple dideoxynucleosides in patients receiving therapy with dideoxynucleosides. *Proc Natl Acad Sci USA* 92:2398–2402.
- Silvestrelli PL, Bernasconi M, Parrinello M. 1997. Ab initio infrared spectrum of liquid water. *Chem Phys Lett* 277:478–482.
- Silvestrelli PL, Marzari N, Vanderbilt D, Parrinello M. 1998. Maximally-localized Wannier functions for disordered systems: Application to amorphous silicon. *Solid State Commun* 107:7–11.
- Silvestrelli PL, Parrinello M. 1999a. Water molecule dipole in the gas and in the liquid phase. *Phys Rev Lett* 82:3308–3311.
- Silvestrelli PL, Parrinello M. 1999b. Structural, electronic, and bonding properties of liquid water from first principles. *J Chem Phys* 111:3573–3580.
- Silvi B, Savin A. 1994. A classification of chemical bonds based on topological analysis of electron localization functions. *Nature* 371:683–686.
- Sirois S, Proynov EI, Nguyen DT, Salahub DR. 1997. Hydrogen-bonding in glycine and malonaldehyde: Performance of the Lap1 correlation functional. *J Chem Phys* 107:6770–6781.
- Sluis-Cremer N, Arion D, Kaushik N, Lim H, Parniak MA. 2000. Mutational analysis of Lys65 of HIV-1 reverse transcriptase. *Biochem J* 348:77–82.
- Tantillo C, Ding J, Jacobo-Molina A, Nanni RG, Boyer PL, Hughes SH, Pau-

- wels R, Andries K, Janssen PA, Arnold E. 1994. Locations of anti-AIDS drug binding sites and resistance mutations in the three-dimensional structure of HIV-1 reverse transcriptase. Implications for mechanisms of drug inhibition and resistance. *J Mol Biol* 243:369–387.
- Taylor EW, van Roey P, Schinazi RF, Chu CK. 1990. A stereochemical rationale for the activity of the anti-HIV nucleosides. *Antiviral Chem Chemother* 1:163–173.
- Tisdale M, Kemp SD, Parry NR, Larder BA. 1993. Rapid in vitro selection of human immunodeficiency virus type 1 resistant to 3'-thiacytidine inhibitors due to a mutation in the YMDD region of reverse transcriptase. *Proc Natl Acad Sci USA* 90:5653–5656.
- Troullier N, Martins JL. 1991. Efficient pseudopotentials for plane-wave calculations. *Phys Rev B* 43:1993–2006.
- Turner BG, Summers MF. 1999. Structural Biology of HIV. *J Mol Biol* 285:1–32.
- Van Gunsteren WF, Billeter SR, Eising AA, Hunenberger PH, Kruger P, Mark AE, Scott WR, Berendsen HJ. 1996. *GROMOS96, BIOMOS, biomolecular software b v*. Zürich: Laboratory of Physical Chemistry, ETH.
- Wang JH, Xiao DG, Deng H, Webb MR, Callender R. 1998. Raman difference studies of GDP and GTP binding to c-Harvey ras. *Biochemistry* 37:11106–11116.
- Zhang D, Caliendo AM, Eron JJ, DeVore KM, Kaplan JC, Hirsch MS, D'Aquila RT. 1994. Resistance to 2',3'-dideoxycytidine conferred by a mutation in codon 65 of the human immunodeficiency virus type 1 reverse transcriptase. *Antimicrob Agents Chemother* 38:282–287.