Binding of Phosphinates and Phosphonates inhibitors to Aspartic Proteases: a first principle study.

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SUPPLEMENTARY INFORMATION

Classical MD simulations on the HIV(-) model have been performed to provide an equilibrated structure for the QM/MM calculations. Calculations were performed with the GROMACS program[1;2]. The AMBER[3] and TIP3P[4] force fields were used for the protein frame and for water, respectively. The parameters of the ligands and the Asp dyad were derived as follows. Point charges were derived according to the RESP procedure[5]. The electrostatic potential has been calculated at the HF/6-31G* level of theory with the Gaussian program package[6] for the neutral and ionized species. During the fitting step, constraints were imposed to force the same point charges on atoms not described at the QM level in QM/MM simulations. Van der Waals and bonded interactions were accounted by the standard AMBER force field[3]. During the simulation, the active site atoms have been constrained at their initial position. Bonds involving hydrogen atoms were constrained using the LINCS algorithm[7]. A timestep of 1.0 fs was used during the dynamics. Periodic boundary conditions have been applied. A full evaluation of the electrostatics has been taken into account[8]. A 12 Å cutoff for the real part of electrostatic interaction and for van der Waals interactions was used. Constant temperature (300 K) and pressure (1 bar) conditions were achieved by coupling the systems with a Nose-Hoover thermostat[9;10] and a Parrinello-Rahman barostat[11], respectively. The solvent has been equilibrated for ~400 ps, while keeping the complex fixed at the crystal conformation. Then, 2 ns MD were carried out. The final MD structure was used as initial model for the QM/MM simulations.

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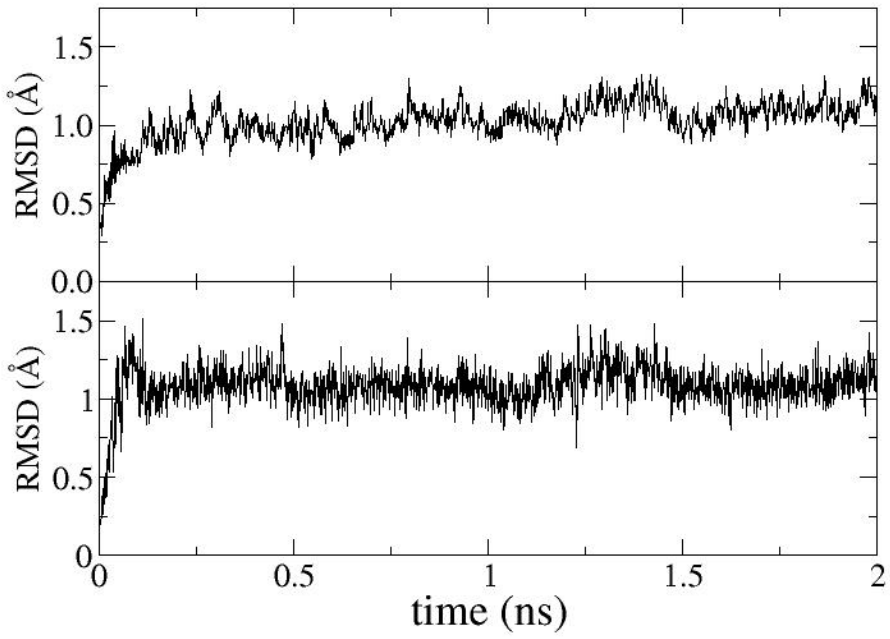


Figure 1S. Root mean square deviation (RMSD) from the crystal structure during the classical MD simulations for the HIV-1 protease complex. Top: minimum RMSD for the protein backbone. Bottom: RMSD for the inhibitor after fitting the protein backbone.

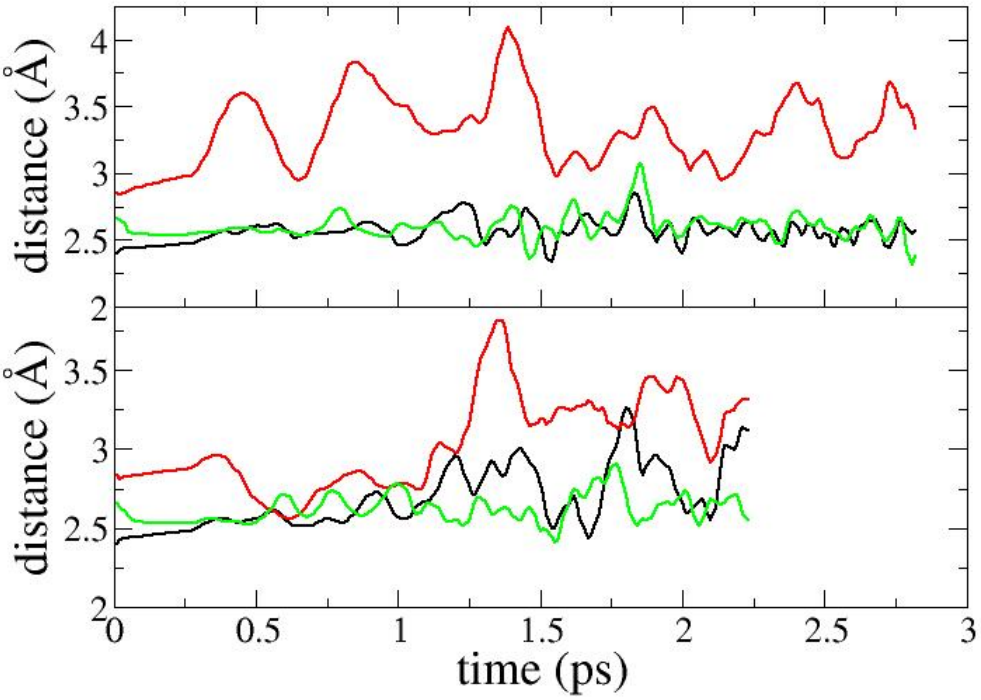


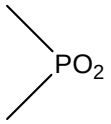
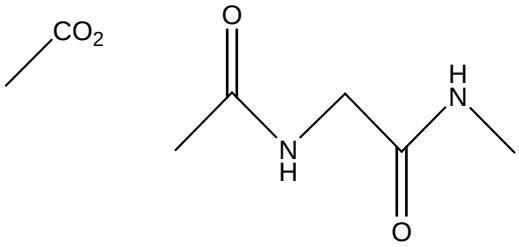
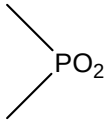
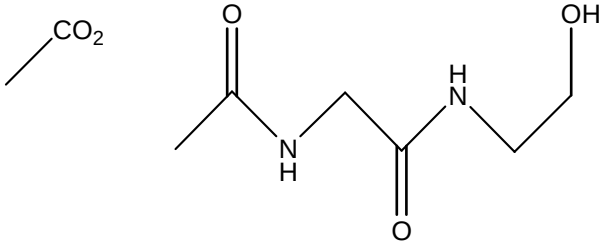
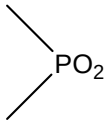
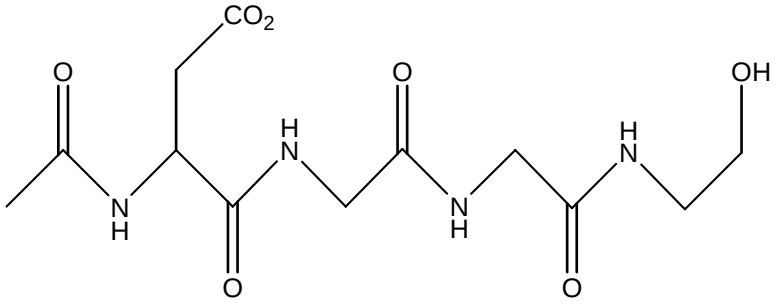
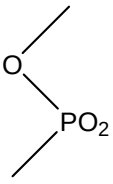
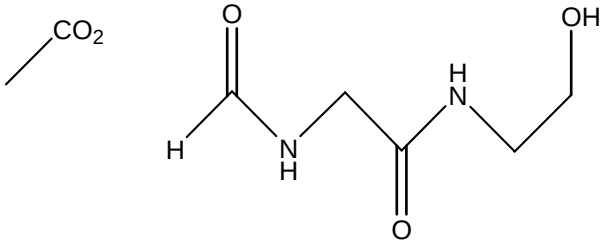
Figure 2S. QM/MM MD: active site distances between oxygen atoms during the simulation of **HIV(-) (**top) and **HIV(0)** (bottom) active site forms. Red: O1@Asp25-O1@Asp25'; black: O2@Asp25'-OP1; green: O2@Asp25-OP2. Atom names as in Figure 1.

Table 1S. HIV-1 AP**:** RMSD of the final QM/MM MD conformation with respect to the x-ray structure after least square fit to the protein backbone.

Model	**RMSD backbone (Å)**	**RMSD active site (Å)**
HIV(-)	1.3	1.5
HIV(0)	1.2	2.0

Table 2S. Average H-bond distances from QM/MM MD simulations of models **HIV(-)** and **HIV(-)B**. H-bond angles are very similar in both models (165º ± 10º) and are not reported. Atom names as in Figure 1.

distances (Å)	**model HIV(-)**	**model HIV(-)B**
OP2-O2@Asp25	2.56 ± 0.09	2.60 ± 0.10
OP1-O2@Asp25'	2.55 ± 0.08	2.57 ± 0.10
O1@Asp25-O1@Asp25'	3.27 ± 0.19	3.05 ± 0.20
O2@Asp25-H2	1.05 ± 0.04	1.03 ± 0.04
OP2-H2	1.53 ± 0.11	1.60 ± 0.11
O2@Asp25'-H1	1.05 ± 0.03	1.04 ± 0.04
OP1-H1	1.51 ± 0.09	1.55 ± 0.12

I. endothiapepsin complex	inhibitor	protein
A		 ASP35/219 THR36/220 GLY37/221 SER38/THR222
B		 ASP35/219 THR36/220 GLY37/221 SER38/THR222
C		 PHE34/ALA218 ASP35/219 THR36/220 GLY37/221 SER38/THR222
II. penicillopepsin complex		
B		 ASP33/213 THR34/214 GLY35/215 SER36/THR216

Scheme 1. Chemical structures of the fragments in the models used in the DFT calculations of the euraryotic aspartyl proteases.