

# Pressure induced conformational dynamics of HIV-1 protease: A Molecular Dynamics simulation study.

B. R. Meher\*, M. V. Satish Kumar and Kausik Sen

Computational Biology Research Laboratory, Department of Biotechnology,  
Indian Institute of Technology, Guwahati, Assam-781039, India

## Abstract

*The conformational dynamics of HIV-1 protease (HIV-pr) is known to be essential for ligand binding and determination of cavity size, which changes with several common physiological parameters like temperature, pressure, pH conditions and of course the protein backbone mutations. In this work, the effect of pressure on the conformation and dynamics of HIV-pr was studied in silico at 1 bar (0.987 atm) and 3 Kbar pressure conditions. It can be seen from the literature that protein containing significant number of hydrophobic residues would expose its hydrophobic groups to the solvent exposed area under high pressure conditions, which eventually changes the dynamics and hence conformation of the protein. From our observations, the dynamics studies showed that, although the collective dynamics is restricted under pressure this is not true for some specific residues. From the secondary structure analysis it was observed that turns and bends are favored under high pressure at the expense of  $\alpha$ -helices and  $\beta$ -sheets resulting in the reduction of structural variability. Solvent accessible surface (SAS) area of both the low and high pressure simulations showed significant differences. It was also observed that with the elevation in pressure, the hydrophobic effect is decreased. All these conformational changes at high pressure condition may have a special impact on the binding affinity of drugs to the active site region, which may have a direct/indirect effect on the drug resistance behavior of HIV-pr.*

**Keywords:** High pressure, molecular dynamics simulation, protein conformation, AMBER force field, Solvent accessible surface (SAS)

\* Corresponding author: brmeher@gmail.com

## 1. Introduction

HIV-1 protease, an indispensable enzyme for HIV replication, is an important target for drug design strategies to combat AIDS. It acts at the late stage of infection by cleaving the Gag and Gag-Pol polyproteins to yield mature infectious virions. The enzyme is a

member of the aspartic protease family and is a homodimer of 99 residues in each chain. The active site region of the protein is covered by two glycine rich, antiparallel  $\beta$ -hairpins referred as flaps. The volume of the active site and the accession of ligand to the active site are controlled by the dynamics of the two flaps [1-2]. Conformation of the proteins directs the protein functions. The conformational dynamics of HIV-pr is known to be essential for the ligand binding and determination of cavity size, that changes with several common physiological parameters like temperature, pressure, pH conditions [3] and of course the protein backbone mutations [4]. The change in the conformation of the protein leads to the decreased binding affinity of the drugs and eventually escort to the drug resistance phenomenon.

Pressure plays an important role in the conformation of the proteins *in vivo*. Pressure-induced structural changes have been reported for deoxymyoglobin [5], lysozyme [6-9], BPTI [10], myoglobin [11], apomyoglobin [12], ubiquitin [13], Arc repressor [14], HPr [15], and various other proteins [16-18]. HIV-pr is being studied under normal temperature and pressure conditions *in vitro* and *in silico* as well. In comparison, no report has been filed investigating the dependence of protein dynamics and conformation on high pressure for HIV-pr. It has been shown that, under high pressure the hydrophobic core of the protein bulges out to the solvent exposed area, which eventually changes the dynamics and hence conformation of the protein [19]. HIV-pr has several hydrophobic residues in its most important regions; i.e. flap and active site region. So to illustrate the pressure induced conformational changes of HIV-1 protease and hence the emergence of drug resistance, we performed two simulations in the normal 1bar pressure and in a high 3Kbar pressure. We observed that the volume of the protein shrunk w.r.t. the increase in pressure and the secondary structures have substantial alteration at high pressure w.r.t the simulation time. As expected in accordance to the previous studies, we didn't find considerable changes in the hydrophobic core region rather the hydrophobicity first increased and eventually decreased at high pressure. Interestingly the rate of

change of hydrophobic SAS and total SAS seems to be more in the case of high pressure 3 Kbar than in 1 bar system.

## 2. Computational details:

MD simulations were started using a crystal structure of HIV-pr (pdb ID: 1HHP) [20] of resolution 2.7 Å with semi-open conformation. The Leap module of the AMBER program package [21] was used to prepare the system for simulation. The *ff99SB* [22] force field with TIP3P [23] water models was used for both the 1bar (1 atm = 1.01325 bar = 101.325 kPa) and 3Kbar simulations. The system was immersed in a water box of size 83.5 x 62.5 x 68.9 Å<sup>3</sup> containing >8000 water molecules. The net positive charge on the system was neutralized through the addition of chloride ions. The charged-states of both the catalytic Asp-25 and Asp-124 were kept deprotonated in the simulations. The electrostatic interactions were calculated with the particle mesh ewald (PME) method [24]. Constant temperature and pressure conditions in the simulation were achieved by coupling the system to a Berendsen's thermostat and barostat [25]. Bonds involving the hydrogen atoms were kept rigid using SHAKE. The *g\_sas* [26] and DSSP [27] programs were used to analyze the solvent accessible surface (SAS) area of each residue and the secondary structure changes during the MD simulations.

The system was minimized in two phases. In the first phase, the system was minimized giving restraints (30kcal/mol/Å<sup>2</sup>) to protein and crystallographic waters for 500 steps with subsequent second phase minimization of the whole system. Then the system was heated to 300K over 25 ps with a 1 fs time step. The protein atoms were restrained with force constant of 30 kcal/mol/Å<sup>2</sup> at the NVT ensemble. After that the force constant was reduced by 10 kcal/mol/Å<sup>2</sup> in each step to reach the unrestrained structure in three steps of 10 ps each. The system was then switched over to the NPT ensemble and equilibrated without any restraints for 180 ps. The system was equilibrated in total of 235 ps in 1 bar.

Now the system divided in to two. The first set was slowly submitted to increasing the pressure, whilst the second was kept at atmospheric pressure. The pressure in the first system was elevated in 22 concatenated runs during a total 2.2 ns as follows: 1–10 bar for 100 ps; 10–50 bar for 100 ps; 50–100 bar for 100 ps; 100–1000 bar (100 bar increase during 100 ps for each run); 1000–3000 bar (200 bar increase during 100 ps for each run). The second system was allowed to run for an equivalent 2.2 ns MD simulation period at 1 bar. Being achieved this point both the systems were allowed to evolve during one ns each, in order to produce comparably equilibrated starting points for the low and high pressure systems. After this

both the 1 bar and 3Kbar trajectories were run for 30 ns. The time step for MD production run was 2 fs.

## 3. Results and Discussions:

### 3.1 Mobility Analysis:

To diagnose the relative mobility of the protein at both normal and high pressure conditions we have analyzed the root mean square fluctuations (RMSF) of  $\alpha$ -carbon atoms of all frames recorded for 30 ns. (Fig-1). It was found that with increase in pressure the average residue mobility is significantly decreased. The blue and red straight lines represent the average RMSF for the 1 bar and 3 Kbar pressure conditions respectively. The average C $\alpha$  mobility at 1 bar is about 1.078 times higher than the corresponding average at 3 Kbar. This is mostly true for all  $\alpha$ -carbons with the noteworthy exceptions of residues like 9, 25, 26, 29, 31, 37, 39, 61, 67, 111-113, 140-142, 152,154, 158 and 171-173. On the other hand, the most notorious reduction in mobility is observed for the Thr-80 & 179 in the active site wall region. This can be further discussed under secondary structure analysis.

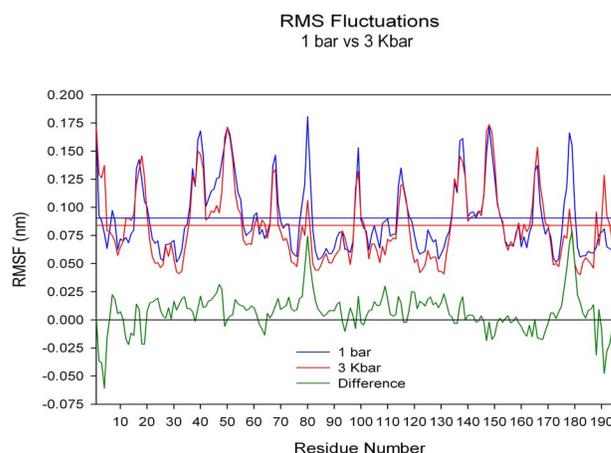


Figure 1. RMSF of  $\alpha$ -carbon atoms at 1 bar (Blue) and 3 Kbar (Red) and their difference (Green).

### 3.2 RMSD:

To analyze possible structural similarities and differences during simulations we have computed the RMSD. The equilibrated structure at 3.435 ns was used as reference structure for the calculation of the  $\alpha$ -carbon RMSD for both low and high pressure simulation. Figure 2 shows the RMSD for both the 1 bar and 3 Kbar systems. It can be seen that the 1 bar system has reached a point of reasonable stability around the value of 1.35 sigma units. Whereas the high pressure 3 Kbar system shows an unstable behavior and appears to be more fluctuating as compare to 1 bar system. As a whole we can conclude

that 1bar simulation has reached a reasonably stable conformation but structures at 3 Kbar do not tend to repeat themselves.

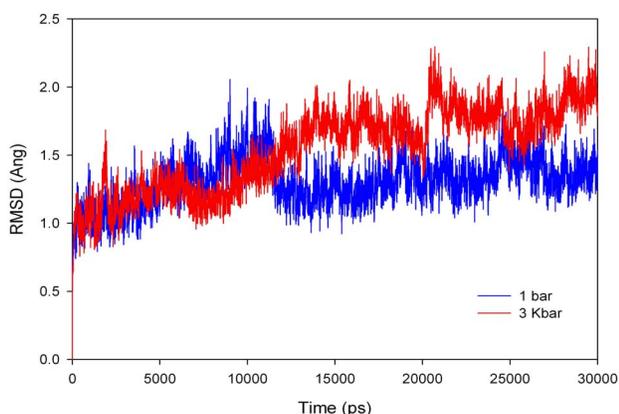


Figure 2. RMSD of  $\alpha$ -carbon atoms at 1 bar (Blue) and 3 Kbar (Red).

### 3.3 Secondary Structure analysis:

Comparative analysis of the secondary structures from 30 ns for both the normal and high pressure conditions, it is observed that the global changes in the protein conformation remains unchanged. Marked specific local changes seen with the expense of  $\beta$ -sheets and  $\alpha$ -helices to turns and bends, which are summarized below:

Res 49-140 region: This part of the protein shows distinct differences between 1 bar and 3 Kbar.

Bends, turns and coils are formed in the expense of  $\beta$ -sheets and are more prominent around 20-25 ns for 1 bar. But in 3 Kbar the changes in structure seems throughout the simulation period indicating the structural instability under high pressure correlating the RMSD shown in fig-2.

Chain-B helix (Res 185-191): The most notable difference seen here by the occupancy of turns over helices from around 10-25 ns where the  $\alpha$ -helix is being disrupted under high pressure.

### 3.4 Solvent accessed surface (SAS) analysis:

In order to supplement our observations and conclusions extracted under RMSD and secondary structure analysis and discussion, we now proceed to analyze the protein solvent accessed surface. Figure 3 (a) and 3 (b) shows the solvent accessed surface for hydrophilic, hydrophobic and total residues of the protein at 1 bar and 3 Kbar respectively. It was found that 1 bar system fluctuates reasonably around apparently stable SAS values. Whereas 3 Kbar system shows a somewhat stable behavior for the

hydrophilic exposed surface, whilst the hydrophobic and total SAS shows increasing behavior first and then substantially decreasing behaviour. But in no case, the 3 Kbar system never reaches above the 1 bar average. This may be accounted on the basis of restricted flap dynamics with high pressure. In addition to this, the rate of change of hydrophobic SAS and total SAS seems to be more in the case of high pressure 3 Kbar system than in 1 bar system.

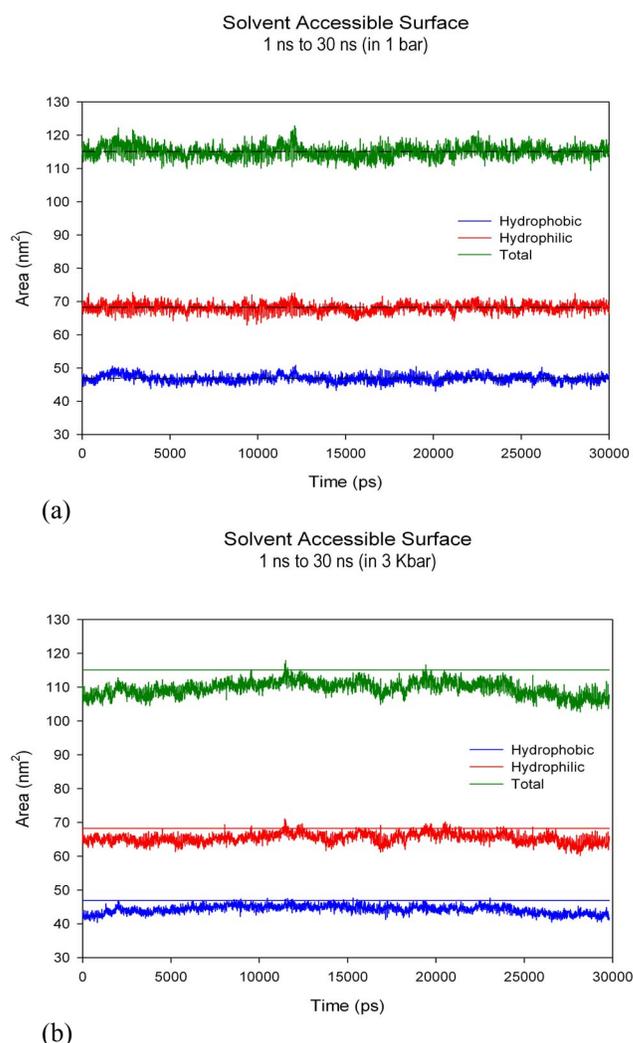


Figure 3. SAS for the whole 30 ns simulation time at (a) 1 bar and (b) 3 Kbar. The dashed black line shows the average SAS for each type in (a) and the solid line in (b) shows the average SAS for each type from 1 bar, not from 3 Kbar.

### 3.5 Radius of gyration ( $R_g$ ) analysis:

To measure the protein's volume under high pressure the  $R_g$  was studied and is presented in the Fig-4. As expected there occurs decrease in protein volume with increase in

pressure as seen in the figure. This is due to the fact that the structure becomes compact with increase in pressure.

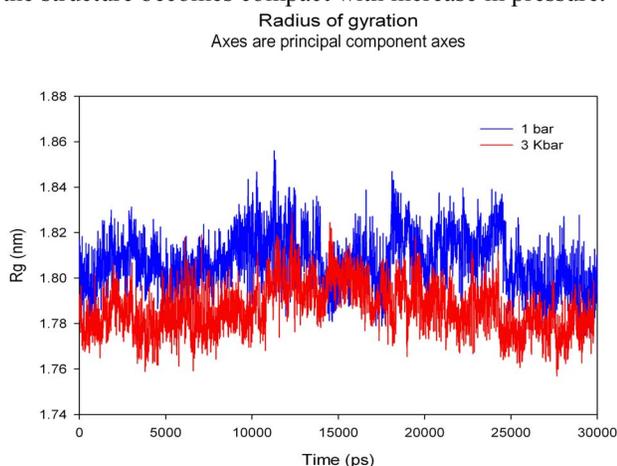


Figure 4. Radius of gyration of  $\alpha$ -carbon atoms at 1 bar (Blue) and 3 Kbar (Red).

Fig 5 shows the average structure of protein at 1 bar and 3 Kbar conditions. It is clearly seen that with increase in pressure from 1 bar to 3 Kbar the volume is decreased and the structure becomes more compact leading to deformed conformation.

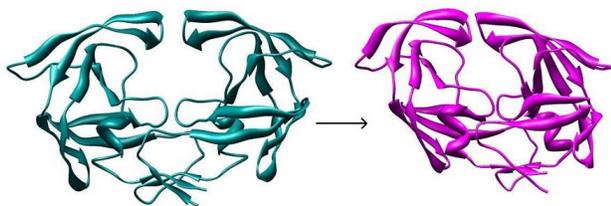


Figure 5. Comparison of the averaged structure of 1 bar (Cyan) and 3 Kbar (Pink) over 30 ns simulation

#### 4. Conclusion:

Comparative study of the HIV-pr at 1 bar and 3 Kbar pressure, gives a clear picture of the conformational changes induced through high pressure. A short simulation time is not good enough to have a concrete idea upon the effect of pressure on the dynamics of protein. Nevertheless some interesting facts can be extracted from the partially evolved protein structure. Our present study reproduces not only the global behavior of HIV-pr, but also is able to account for anomalous behavior of regions in and around flaps and active site. Our results also demonstrate the increase in compactness of structure under high pressure that affects the secondary structure of the protein with reasonable changes in  $\alpha$ -helix and  $\beta$ -sheets to turns and bends. Regarding the protein mobility study the results confirms the general decrease in the structural degrees of freedom of this protein under

high pressure. The difference in SAS with pressure increase shows that pressure acts as an unique conformer selector consequently reducing the structural variability. We can also see that the active site region of the protein was shrunk to a greater extent with pressure. This may affect considerably the binding affinity of drugs to the active site region. Further investigation is required to throw light on this.

#### Acknowledgement:

The authors thank IIT Guwahati for providing the computational facility and financial support.

#### References:

- [1] S. Piana, P. Carloni, and M. Parrinello, Role of conformational fluctuations in the enzymatic reaction of HIV-1 protease. *J. Mol. Biol.*, 2002, 319, 567–583.
- [2] S. Piana, P. Carloni, and U. Rothlisberger. Drug resistance in HIV-1 protease: Flexibility-assisted mechanism of compensatory mutations. *Protein Sci.* 2002, 11, 2393–2402.
- [3] D. Kovalesky, V. Dubyna, A. E. Mark, and A. Kornelyuk. A molecular dynamics study of the structural stability of HIV-1 protease under physiological conditions: The role of Na<sup>+</sup> ions in stabilizing the active site. *Proteins*, 2005, 58, 450-458.
- [4] P. Bandyopadhyay, and B. R. Meher. Drug resistance of HIV-1 protease against JE-2147: I47V mutation investigated by molecular dynamics simulation. *Chem. Bio. Drug. Des.* 2006, 67, 155-161.
- [5] T. Yamato, J. Higo, Y. Seno, and N. Gō, Conformational deformation in deoxymyoglobin by hydrostatic pressure. *Proteins*, 1993, 16, 327-340.
- [6] A. N. McCarthy, and J. R. Grigera, Effect of pressure on the conformation of proteins. A molecular dynamics simulation of lysozyme. *J. Mol. Gr. Mod.* 2006, 24, 254–261.
- [7] E. Paci and M. Marchi, Intrinsic compressibility and volume compression in solvated proteins by molecular dynamics simulation at high pressure *Proc. Natl. Acad. Sci.* 1996, 93, 11 609-11614.
- [8] K. Akasaka, T. Tezuka, and H. Yamada, Pressure-induced changes in the folded structure of lysozyme *J. Mol. Biol.* 1997, 271, 671-678.
- [9] M. Refaee, T. Tezuka, K. Akasaka, and M. P. Williamson, Pressure-dependent changes in the solution structure of Hen Egg-white Lysozyme. *J. Mol. Biol.* 2003, 327, 857-865.
- [10] B. Wroblowski, J. F. Diaz, K. Heremans, and Y. Engelborghs, Molecular mechanisms of pressure induced conformational changes in BPTI. *Proteins*, 1996, 25, 446-455.

- [11] P. Urayama, G. N. Phillips, Jr., and S. M. Gruner, Probing substates in sperm whale myoglobin using high-pressure crystallography. *Structure* 2002, 10, 51-60.
- [12] A. N. McCarthy, and J. R. Grigera, Pressure denaturation of apomyoglobin: A molecular dynamics simulation study. *Biochim. Biophys. Acta* 2006, 1764, 506-515.
- [13] R. Kitahara, S. Yokoyama, and K. Akasaka, NMR Snapshots of a Fluctuating Protein Structure: Ubiquitin at 30 bar-3 Kbar *J. Mol. Biol.* 2005, 347, 277-285.
- [14] D. Trzesniak, R. D. Lins, and W. F. van Gunsteren. Protein under pressure: Molecular Dynamics simulation of the Arc repressor. *Proteins.* 2006, 65, 136-144.
- [15] M. Canalia, and T. E. Malliavin. Molecular Dynamics simulation of HPr under hydrostatic pressure. *Biopolymers*, 2004, 74, 377-388.
- [16] K. Heremans and L. Smeller, Protein structure and dynamics at high pressure. *Biochim. Biophys. Acta* 1998, 1386, 353-370.
- [17] E. Paci, High pressure simulations of biomolecules. *Biochim. Biophys. Acta* 2002, 1595, 185-200.
- [18] S. M. Gruner. Soft Materials and Biomaterials under Pressure, *High-Pressure Crystallography*, edited by A. Katrusiak and P. McMillan, Kluwer Academic Publisher, Netherlands, 2004.
- [19] G. Hummer, S. Garde, A. E. García, M. E. Paulaitis, and L. R. Pratt. The pressure dependence of hydrophobic interactions is consistent with the observed pressure denaturation of proteins *Proc. Natl. Acad. Sci.* 1998, 95, 1552-1555.
- [20] S. Spinelli, Q.Z. Liu, P.M. Alzari, P.H. Hirel, and R.J. Poljak, The three-dimensional structure of the aspartyl protease from the HIV-1 isolate BRU. *Biochimie* 1991, 73, 1391-1396.
- [21] D. A. Case, *et al.* AMBER 8; University of California: San Francisco, CA, 2004.
- [22] V. Hornak, R. Abel, A. Okur, B. Strockbine, A. Roitberg and C. Simmerling, Comparison of multiple Amber force fields and development of improved protein backbone parameters. *Proteins*, 2006, 65, 712-725.
- [23] W.L. Jorgensen, J. Chandrasekhar, J. Madura, M.L. Klein. Comparison of simple potential functions for simulating liquid water. *J. Chem. Phys.* 1983, 79, 926-935.
- [24] U. Essmann, L. Perea, M.L. Berkowitz, and T. Darden, A smooth particle mesh ewald method. *J Chem Phys.* 1995, 103, 8577- 8593.
- [25] H.J.C. Berendsen, J.P.M. Postma, W.F. van Gunsteren, A. Dinola,; J.R. Haak, Molecular dynamics with coupling to an external bath. *J Chem Phys.* 1984, 81, 3684-3690.
- [26] D. van der Spoel, E. Lindahl, B. Hess, A.R. van Buuren, E. Apol, P.J. Meulenhoff, D.P. Tieleman, A.L.T.M. Sijbers, K.A. Feenstra, R. van Drunen and H.J.C. Berendsen, Gromacs User Manual version 3.2, 2004.
- [27] W. Kabsch, and C. Sander, Dictionary of protein secondary structure: pattern recognition of hydrogen-bonded and geometrical features. *Biopolymers.* 1983, 22, 2577-637.