

Pressure Stability of HSP90 N-terminal Domain: Insights from Molecular Dynamics Simulations

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Introduction

Protein structural characterization is one of the key elements in understanding its mode of action, function, stability, physical and chemical composition and role in the organism. Researchers use variety of tools to investigate the properties of the proteins, however only molecular simulations provide information about protein structure dynamics at the atomic scale. It is well known that high pressure unfolds the proteins and volumetric properties could be analyzed by applying high pressure on the molecule. Protein response to pressure is slow, requiring simulations on the elongated time scale thus increased computational resources are necessary for pressure effects to arise. Several dynamic simulations each 100 ns in length were run at different pressures to investigate the volumetric and hydration parameters of the 90 kDa Heat Shock Protein N-terminal domain.

Objective

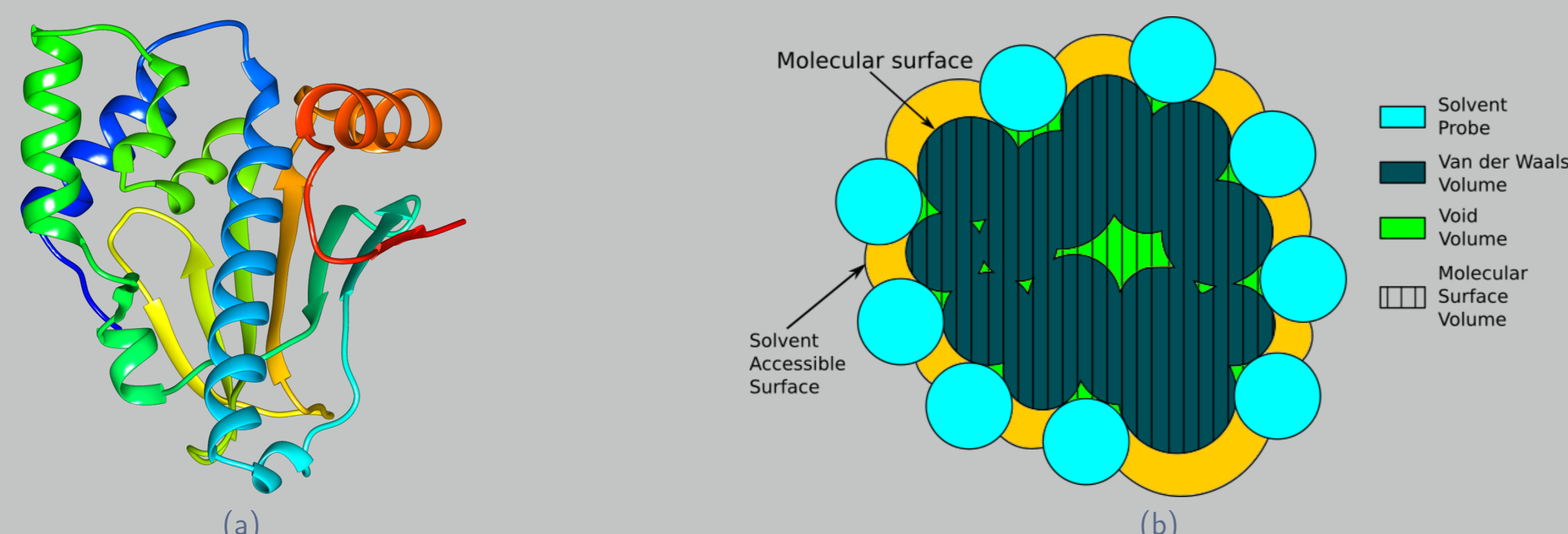


Figure: a - Hsp90 NTD structure, b - protein surface and volume definition.

Key research questions

- ▶ What is Hsp90 NTD hydration shell dynamics under different pressures?
- ▶ How Hsp90 NTD responds to pressure in terms of protein stability and compressibility?

Methods

- ▶ **Preparation of structure:** one HSP90 NTD structure (PDB ID - 1UYL) was cut and transformed using Modeller software.
- ▶ **Molecular dynamics simulation:** gromacs software was used for simulations. Structures were energetically minimized for 3 ns, 100 ns simulations were run at 1 bar, 1000 bar and 6000 bar pressures at constant 273 K temperature.
- ▶ **Calculation of volumetric properties of a protein:** protein molecular volumes were calculated using ProteinVolume program.
- ▶ **Determination of structural parameters:** RMSD and SASA were calculated using MDTraj Python library.
- ▶ **Estimation of the hydration shell around protein:** distance between oxygen atoms of the water molecule and all non hydrogen atoms of the protein were calculated using MDTraj. Water distribution as a function of distance was estimated in different pressures.
- ▶ **Isothermal compressibility:** isothermal compressibility is a measure of the relative volume change as a response to a pressure change. The equation used for determining isothermal compressibility:

$$\beta_T = -\frac{1}{V} \frac{\partial V}{\partial P}$$

Results: Volume

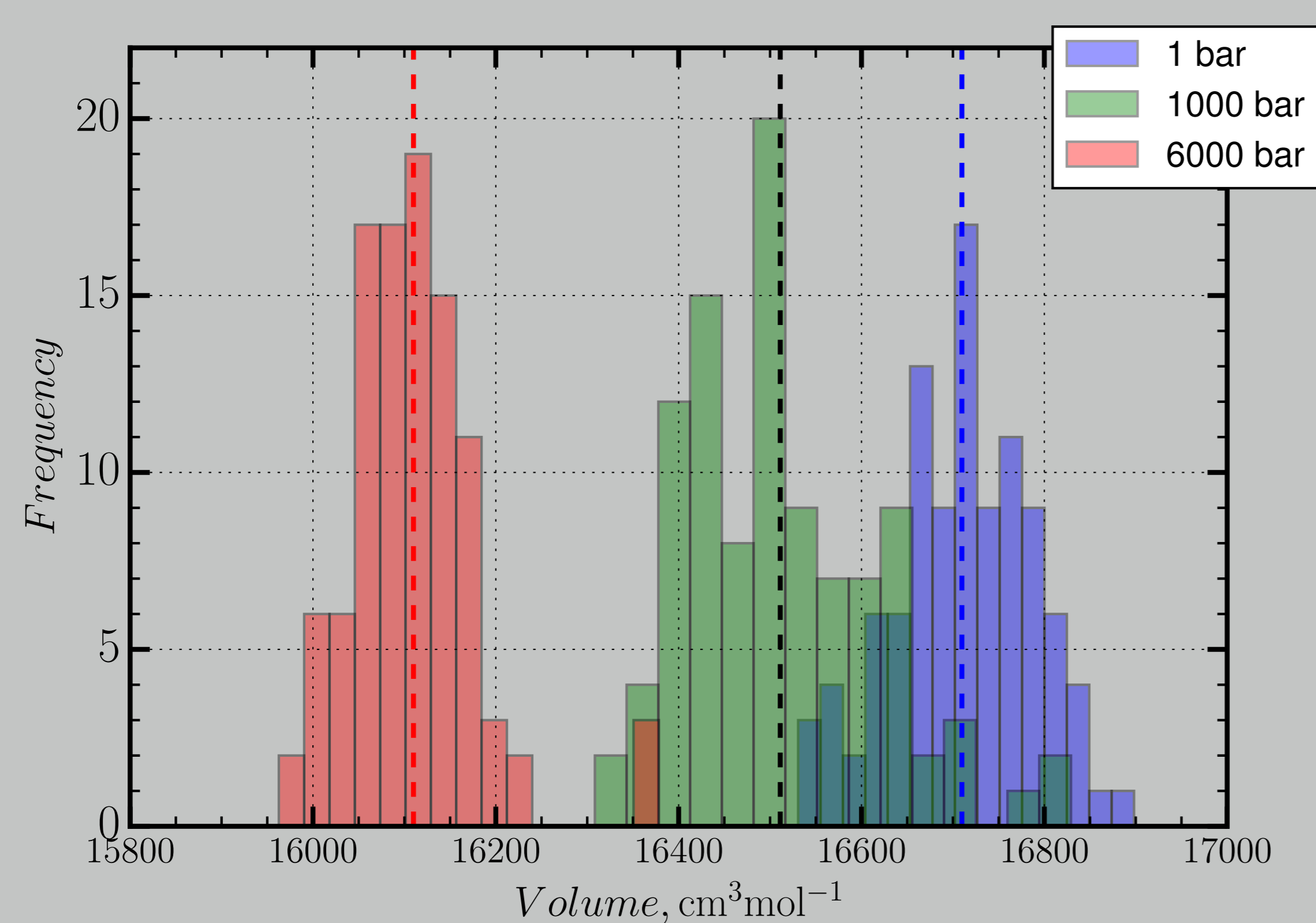


Figure: Protein volume distribution during molecular dynamics simulation in constant 1 bar, 1000 bar and 6000 bar pressures.

Results: SASA

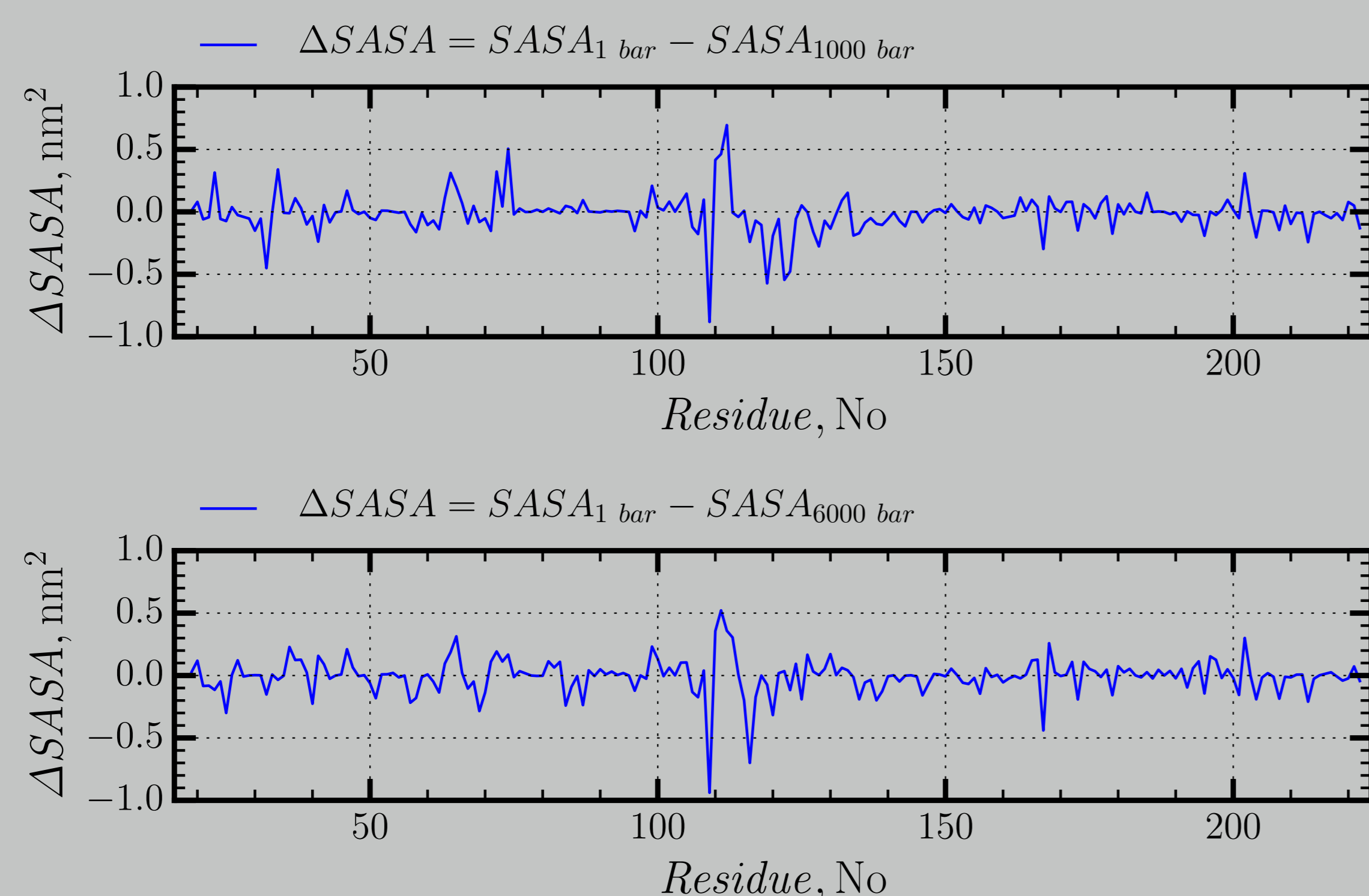


Figure: Average change in surface accessible solvent area between structures in 6000 bar and 1000 bar.

Results: RMSD

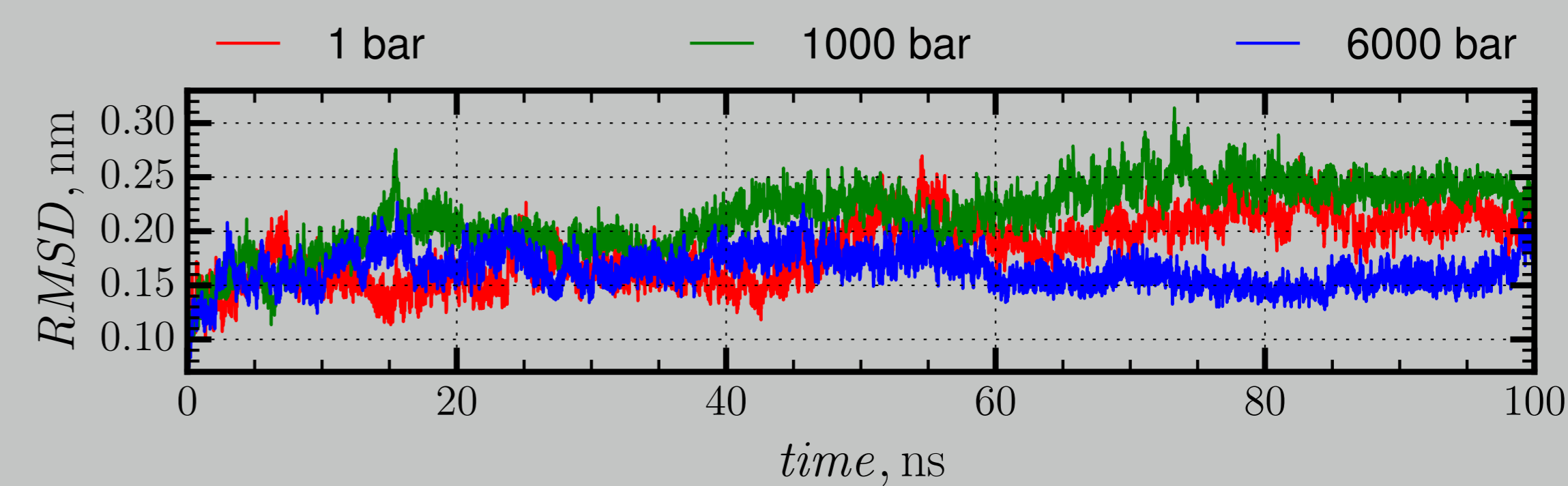


Figure: Root mean square deviation (RMSD) of the structure during simulation in different pressures.

Results: Hydration Shell

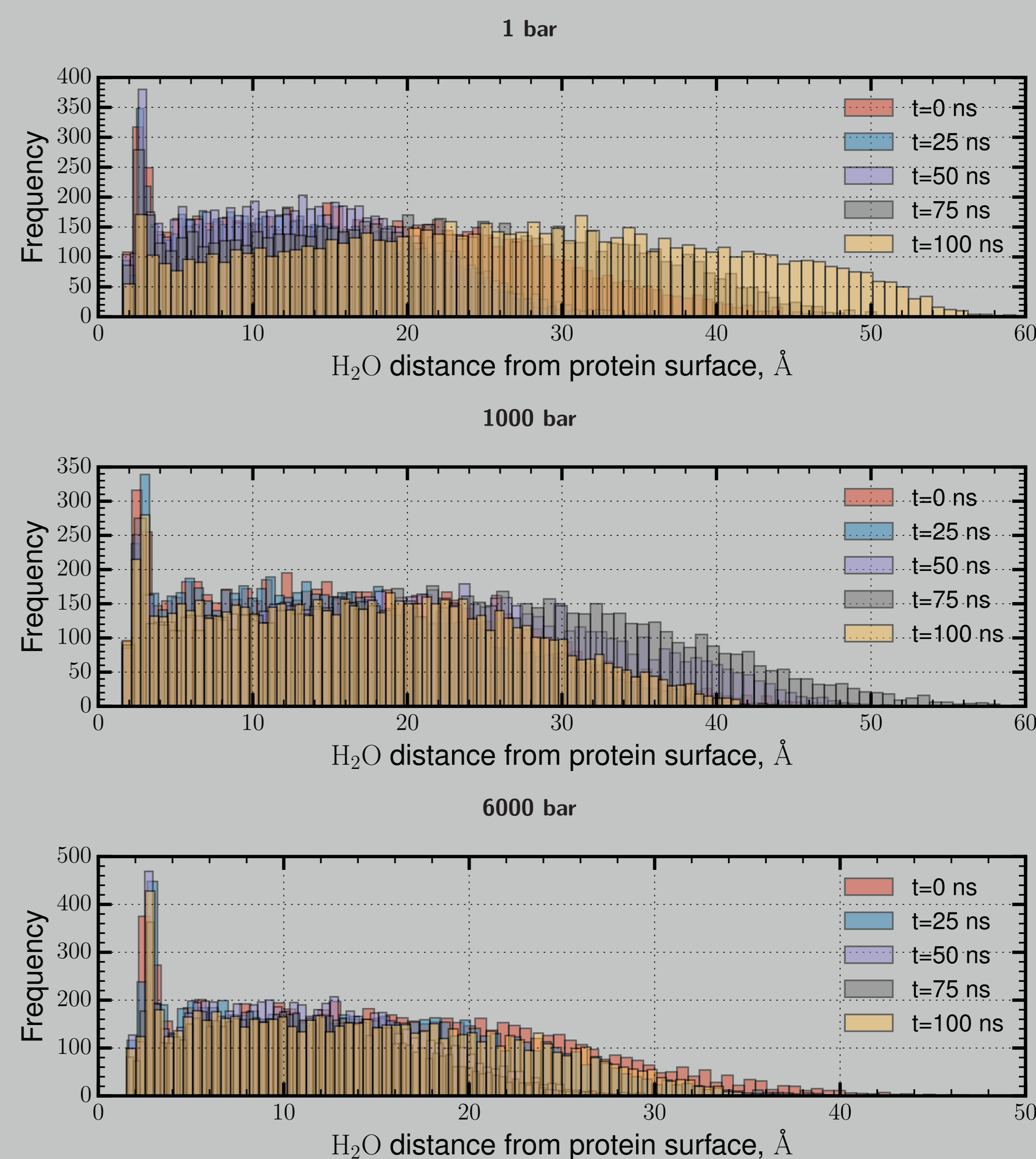


Figure: Water distribution around the protein surface at different pressures. Each graph provides information about water density at five moments in simulation: 0 ns, 25 ns, 50 ns, 75 ns and 100 ns.

Results: Table

Table: Mean volume and isothermal compressibility of Hsp90 N-terminal domain (PDB ID: 1UYL) in 1 bar, 1000 bar and 6000 bar pressures.

Parameter	Value
Mean Volume, $\text{cm}^3 \text{mol}^{-1}$, $P = 1 \text{ bar}$	16710 ± 77
Mean Volume, $\text{cm}^3 \text{mol}^{-1}$, $P = 1000 \text{ bar}$	16511 ± 104
Mean Volume, $\text{cm}^3 \text{mol}^{-1}$, $P = 6000 \text{ bar}$	16109 ± 70
Isothermal Compressibility (mean), bar^{-1}	$7.8 \times 10^{-6} \pm 3.8 \times 10^{-6}$

Discussion

Three molecular dynamics simulations of 100 ns length in 1 bar, 1000 bar and 6000 bar constant pressures were produced. The resulting trajectories showed that structure was stable and did not unfold even at 6000 bar pressure as could be seen in RMSD graph. The volume of the protein changed at different pressures and this change was used to calculate isothermal compressibility, which showed the value of $7.8 \times 10^{-6} \pm 3.8 \times 10^{-6} \text{ bar}^{-1}$. Not all parts of the protein are equally compressible, so the change in solvent accessible surface area (SASA) and hydration shell around protein were investigated. It was found that SASA vastly fluctuates in the lid segment (108 - 125 amino acid residue) of the protein, therefore this region is less rigid than the rest part of the protein. Moreover, the hydration shell becomes about 25 % more dense and distinct from the bulk solvent as the pressure increases from 1 bar to 6000 bar, thus water plays an important role in protein response to pressure. Also this result shows that water density tends to accumulate near the surface of the protein molecule and plays an important role to its unfolding at higher pressures, possibly because of water insertion into the internal voids. The further steps in analysing pressure impact on protein should include the effect of internal voids volume changes and their filling with water.

Conclusions

- ▶ Hsp90 N-terminal domain lid segment is most susceptible to pressure effects.
- ▶ Water density around the surface of the protein increases around 25 % at 6000 bar compared with water density at 1 bar.

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