Assignment 8
Report on MD simulation of **Trp-cage** Protine

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March 24, 2025

1 Process 1: Simulation on GROMACS

I have used Gromacs for Molecular Dynamics (MD) simulations of Trpcage, a tiny mini-protein.
All the files of this simulation are in the directory **'/home/smahato/MD25/MDassign8'** of **MAXWELL2**.

1.1 Visualize the protein in VMD.

The Trp-cage mini-protein consists of an α-helix and some flexible loop regions. α-helix and tail part make an 'U' type folding structure.
Then i have generated the topology files (topol.top) of this conformation using Gromacs for CHARMM27 forcefield using gmx pdb2gmx tool.

Figure 1: The initial configuration of Trp-cage mini-protein

1.2 Solvate the system

The protein is not stable without solvent. So before begning i have solvated the system in 3.5 nm cubic box.The protein is now immersed in a box of water molecules.
In addition we got extra ions in the system so now the system has net charge. If the protein carries an overall net charge, it will repel or attract ions in a non-physical way during the simulation. To

correct this, we added a few chloride (Cl^-) ions to balance the charge.The system is now neutral and physically realistic.

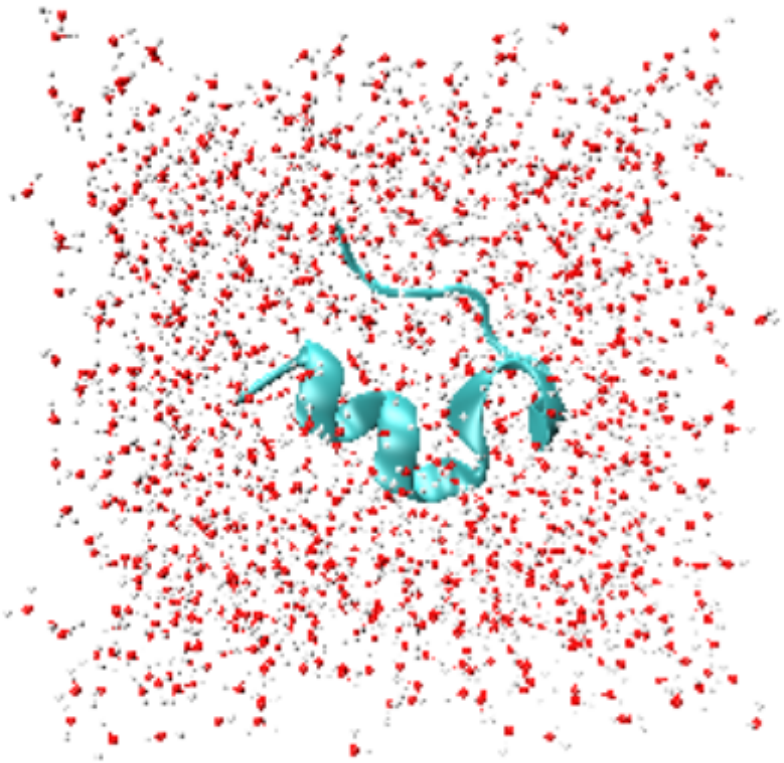

Figure 2: Protein structure with water molecule.

1.3 Now energy-minimize the system

Although we are almost ready to go. But before real simulation, to get physical observable we minimize the system to remove unphysical very high force.After minimization the maximum force on minimized system is **98.269 KJ/mlo** and the Total potential energy $\mathbf{-6.38 \times 10^4}$ **KJ**.

Below i have shown the change of potential energy in the **Figure 3**.

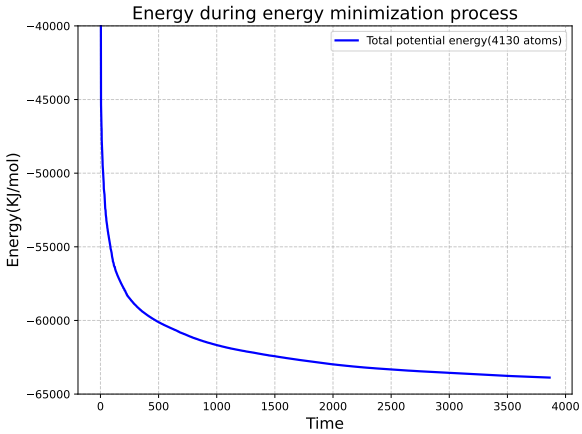

Figure 3: Total potential energy of the system during energy minimization process

We have done run the simulation and save the trajectory in a file.The performance is **521 ns/day**.

1.4 post-process the trajectory

After the simulation, i did post-process the trajectory by centering protein at the box and removing periodic image related artifact using **gmx trjconv**. Also visualize the post-processed trajectory in VMD.

Below i have compared initial and final configuration of the Protein in **Figure 4 and Figure 5**.

1.4.1 Observations:

The protein, which initially looked free-floating, is now more dynamic. The $\alpha - helix$ remains stable, but some of the loop regions appear more structured or may have slightly rearranged. Water molecules continuously exchange positions, forming and breaking hydrogen bonds around the protein.

Figure 4: *Initial configuration*

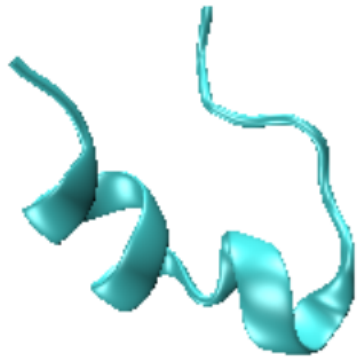

Figure 5: *Final configuration*

1.5 Trajectory analysis:

I have calculated and ploted...
i) RMSD of protein relative to reference structure using gmx rms tool, see **Figure 6**.
ii) Root mean squared fluctuation of each residue (RMSF) of the protein structure using gmx rmsf tool, see **Figure 7**.
iii) The size of protein by calculating Radius of gyration using gmx gyrate tool, see see **Figure 8**.
iv) Number of hydrogen bonds present within protein as a function of time using gmx hbond, see **Figure 9**.
v) The $C_\alpha - C_\alpha$ radial distribution function, see **Figure 10**.

1.5.1 Observations about the trajectory:

In the **Figure 6** below we see RMSD gradually increasing which indicate slow structural rearrangements.
The Root Mean Square Fluctuation (RMSF) measures the flexibility of each residue in the protein over time. At the beginning we see high value of RMSF that it was very flexible then get decreases over time and again increases. When it decreases we see at that time Radius of gyration(**Figure 8**) also become smaller which indicate a stable and compare to smaller structure.

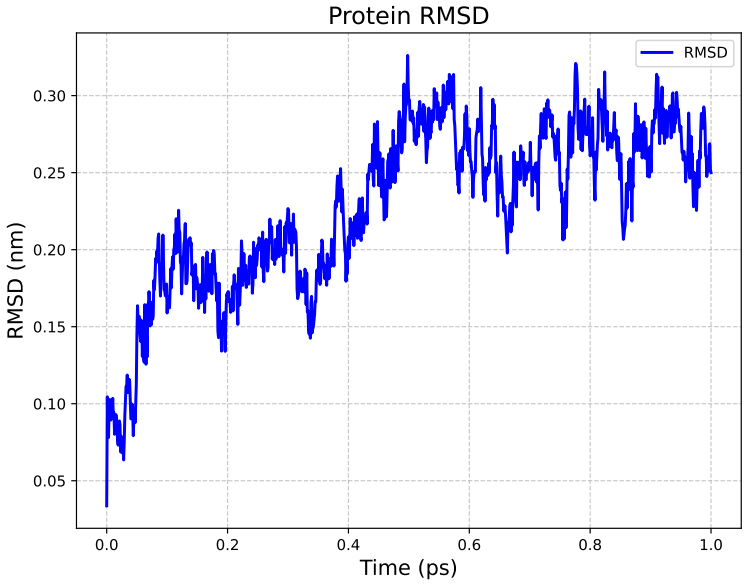

Figure 6: *RMSD of protein relative to reference structure.*

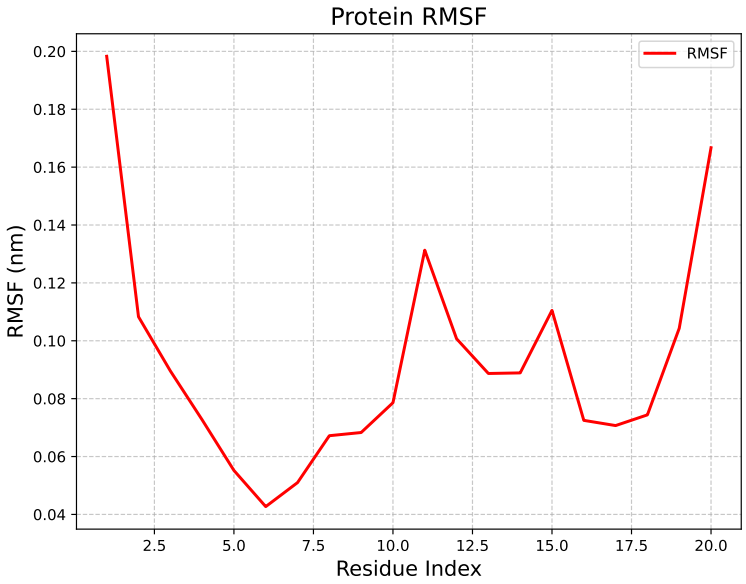

Figure 7: *Root mean squared fluctuation.*

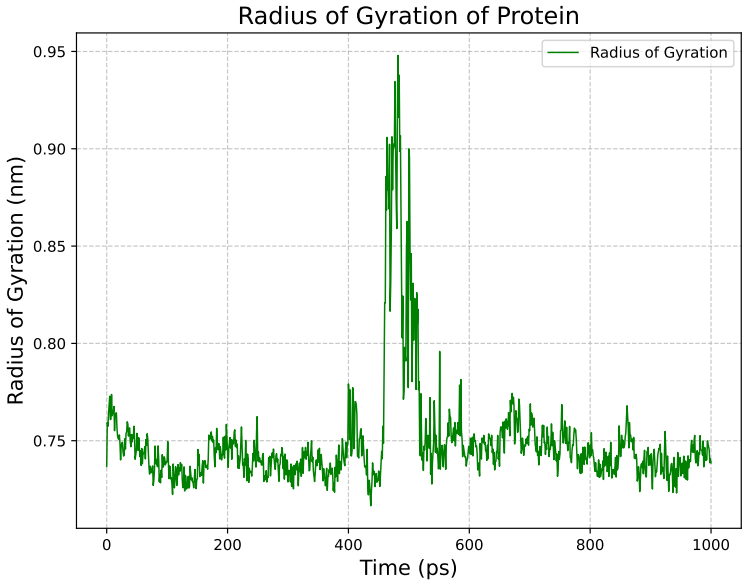

Figure 8: *Radius of gyration of the protein during simulation.*

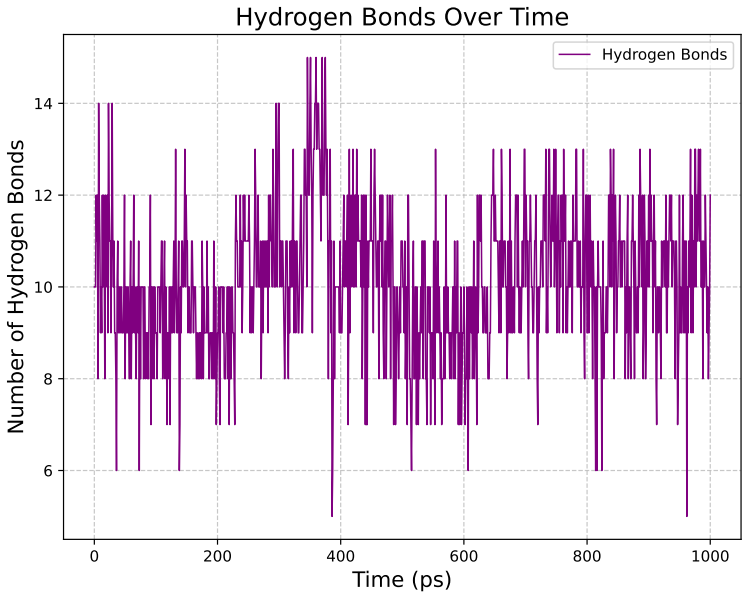

Figure 9: *Number of hydrogen bonds present within protein with time*

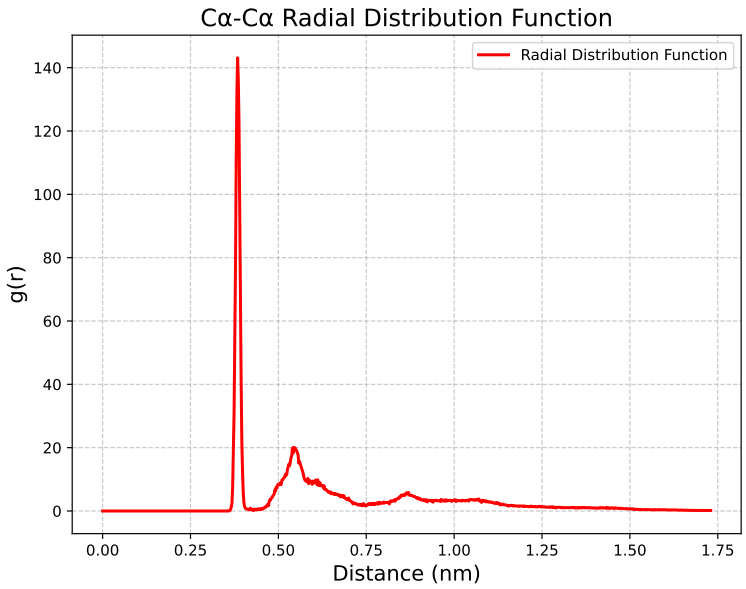

Figure 10: *The $C_\alpha - C_\alpha$ radial distribution function*

2 Process 2: Using CHARMM-GUI

I have done all the process in CHARMM-GUI useing Charmm36 forcefield there and the plots are given below.
All the files of this simulation are in the directory **'/home/smahato/MD25/MDassign8/CHARMM/charmm-gui-3DMX/charmm-gui-4099355955/gromacs'** of **MAXWELL2**.

All the observation are almost similar but has little difference because we have used different force field here.Description about the plot in the figure are given in the caption of the figures.

2.1 Initial configuration

This is the initial configuration of Trp-cage mini-protein in CHARMM-GUI.

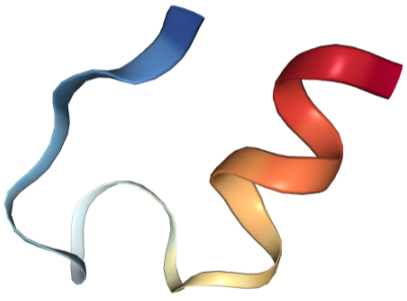

Figure 11: *Initial configuration*

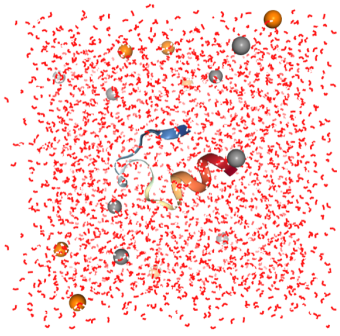

Figure 12: *Initial configuration with water and ions.*

2.2 Analysis

Below i have shown plots of following quantaties i)RMSD ii)RMSF iii)Radius of gyration iv)Hydrogen bonds v)$C_\alpha - C_\alpha$ radial distribution function.

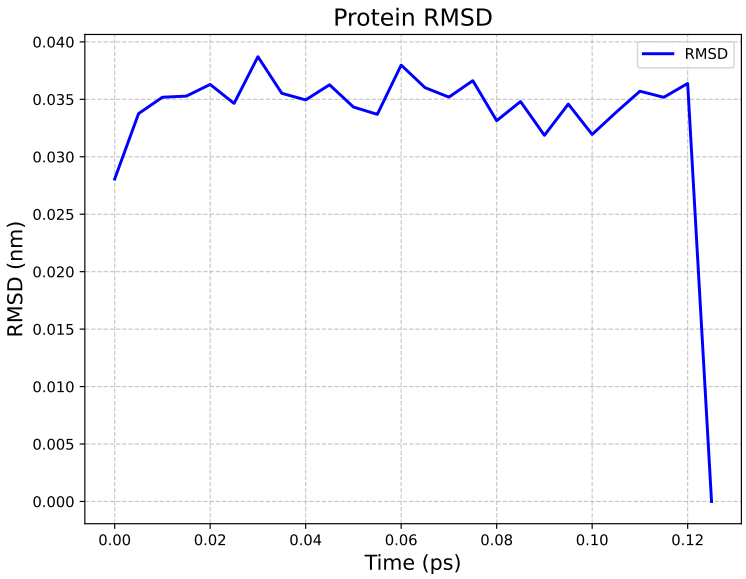

Figure 13: *RMSD of protein relative to reference structure.*

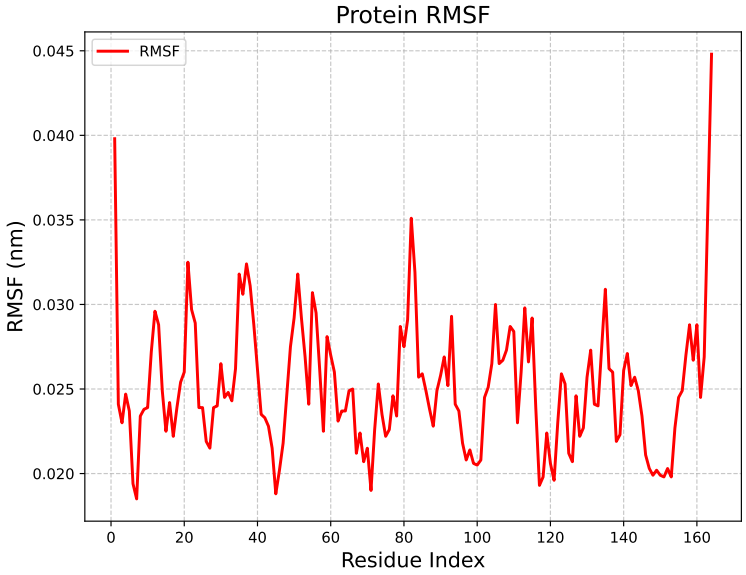

Figure 14: *Root mean squared fluctuation.*

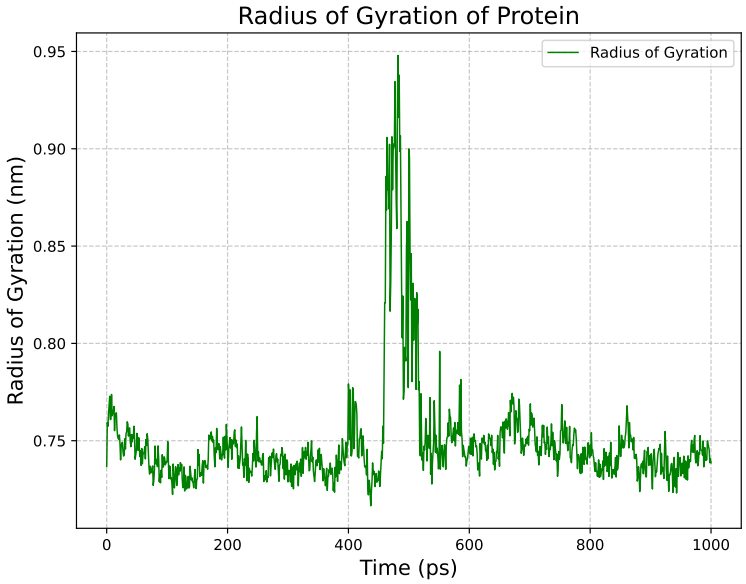

Figure 15: *Radius of gyration of the protein during simulation.*

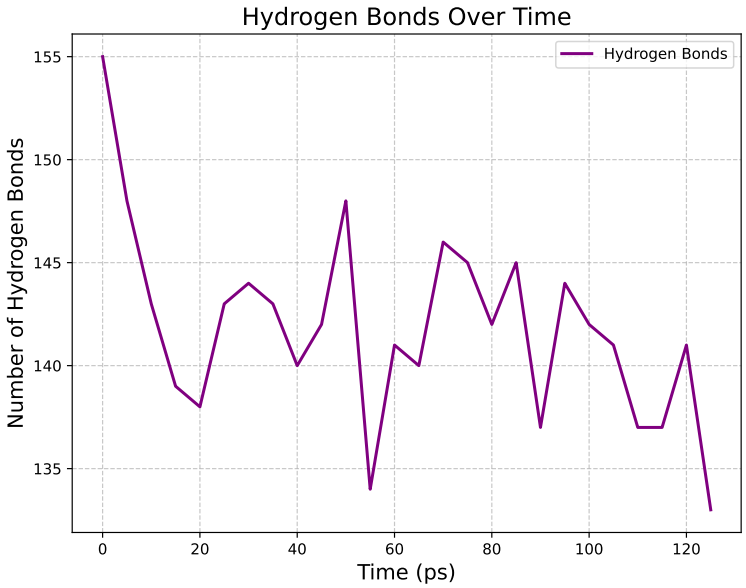

Figure 16: *Number of hydrogen bonds present within protein with time*

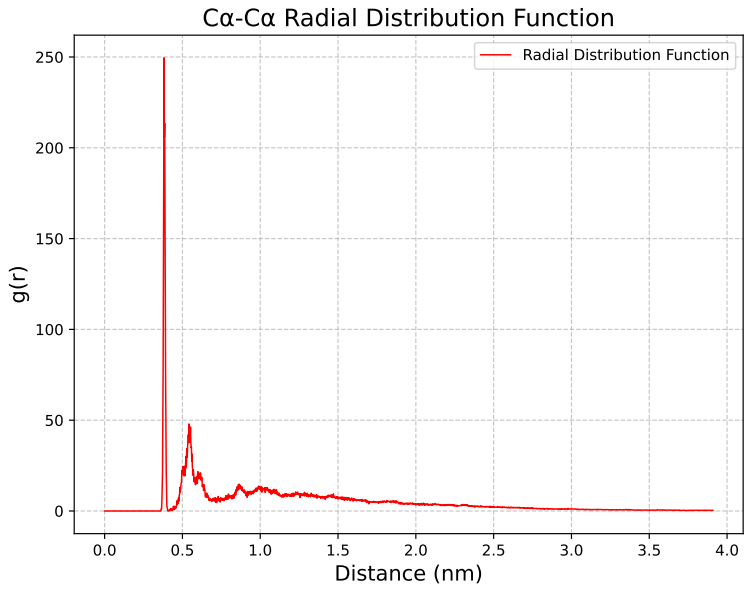

Figure 17: *The $C_\alpha - C_\alpha$ radial distribution function*

3 Conclusion

We have done Molecular Dynamics (MD) simulation of the Trp-cage mini-protein using GROMACS with the CHARMM27 force field, unraveling its structural evolution and dynamic behavior in an aqueous environment. Beginning with the initial configuration, we solvated and neutralized the system. Did energy minimization,and run for a short but insightful 500 ps time.
We have also performed Molecular Dynamics (MD) simulation of the Trp-cage mini-protein using CHARMM-GUI's Solution Builder with the CHARMM36 force field. By directly obtaining the 1L2Y PDB structure, we easily generated the necessary input files, including topology and parameter files, and then run in GROMACS which give us similar results.

References

[1] N. H. Andersen, R. M. Fesinmeyer, J. W. Neidigh, and B. Barua. The trp-cage: A notably stable mini-protein fold. In J. Martinez and J.-A. Fehrentz, editors, *Peptides 2000: Proceedings of the 26th European Peptide Symposium*, pages 45–46, Paris, France, 2001. EDK.

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[3] M. J. Gething and J. Sambrook. Protein folding in the cell. *Nature*, 355(6355):33–45, 1992.

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[7] Ruhong Zhou. Trp-cage: Folding free energy landscape in explicit water. *Proc. Natl. Acad. Sci.*, 100:13280–13285, 2003.