
Molecular Dynamics Simulation of Cholera Toxin A1 Polypeptide

Presented By:

SYED LAL BADSHAH

Institute:

NCEPC, UINIVERSITY OF PESHAWAR, PAKISTAN.

ARIZONA STATE UNIVERSITY, AZ, USA.

INTRODUCTION

Cholera Toxin----- AB5 Toxin Family

B subunit

103 amino acid residues

Arranged as two α -helices and ten β -strands

Binding Domain----- GM1 of Plasma
membrane of lumen cell of intestine.

A-Subunit

Enzymatic domain

Mr ~ 27,400.

240 amino acid residues

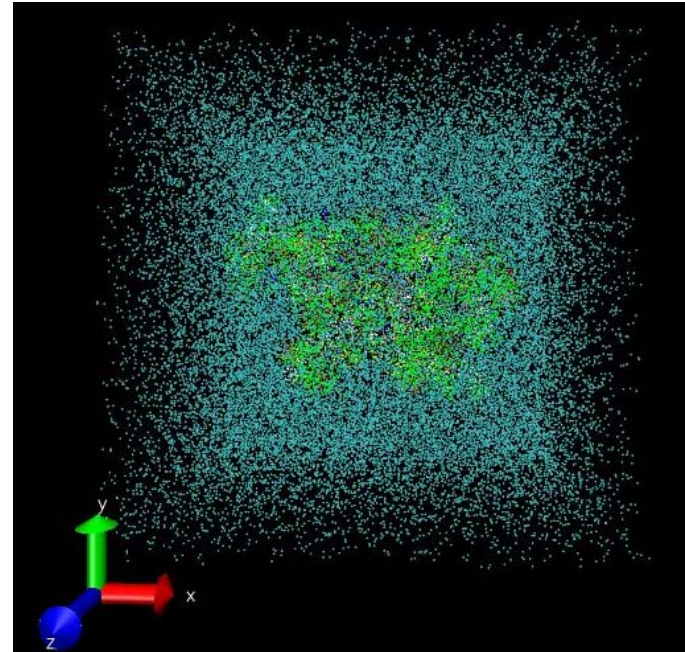
Mixture of α -helices and β -strands

Catalytic site at surface

Retro-translocation mechanism

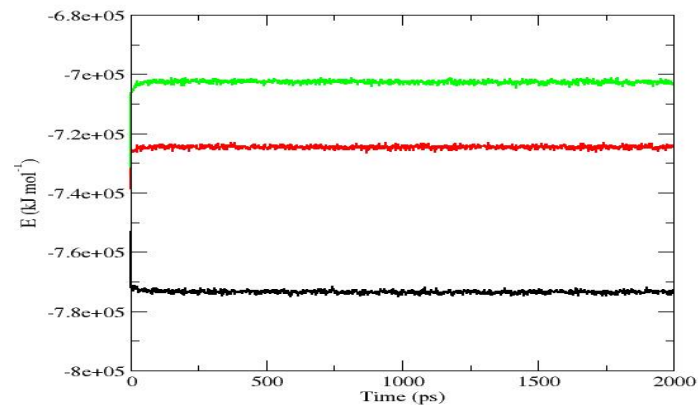
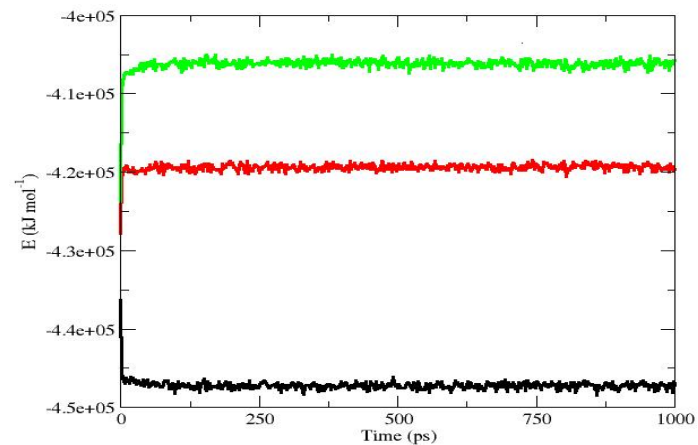
Computational Work

1. Generation of topology file
2. Generation of geometrical box
3. Addition of solvent
4. Adding counter ions
5. Energy minimization
6. Equilibration
7. Molecular dynamics simulation production



Total Energy: Thermodynamically stable.
CTA1-ARF6 more stable than CTA1.

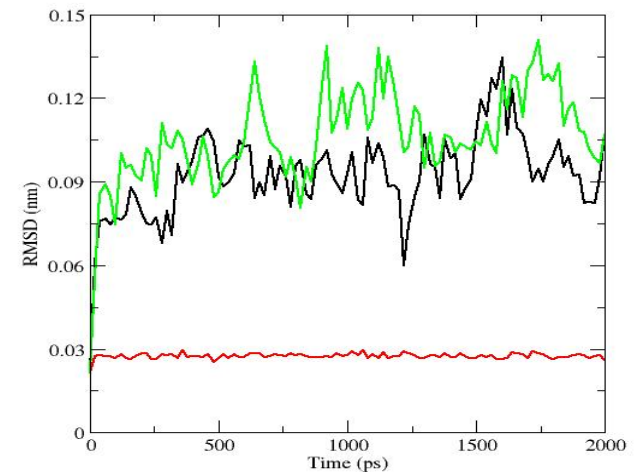
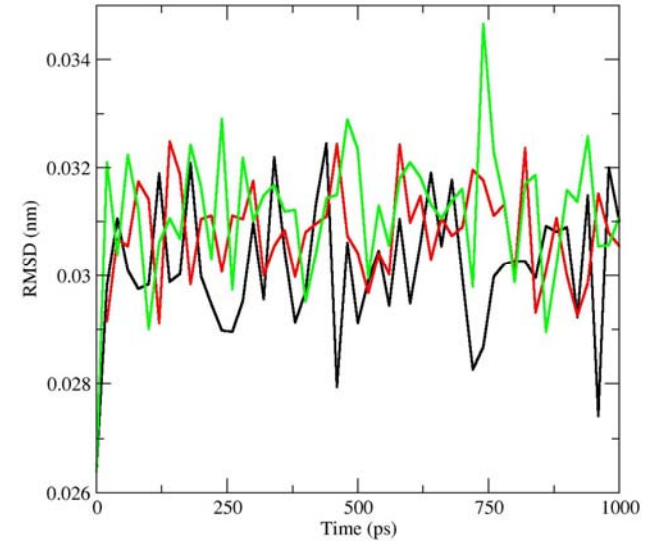
Temperature (K)	Mean total energy of CTA1 ($\times 10^5$ kJ mol ⁻¹)	Mean total energy of CTA1-ARF6 Complex ($\times 10^5$ kJ mol ⁻¹)
283	-4.48	-7.73
310	-4.195	-7.25
323	-4.07	-7.03



Root mean square deviation

Temperature (K)	Mean C α RMSD of CTA1 (nm)	Mean C α RMSD of complex (nm)
283	0.0307	0.084
310	0.0310	0.027
323	0.0310	0.120

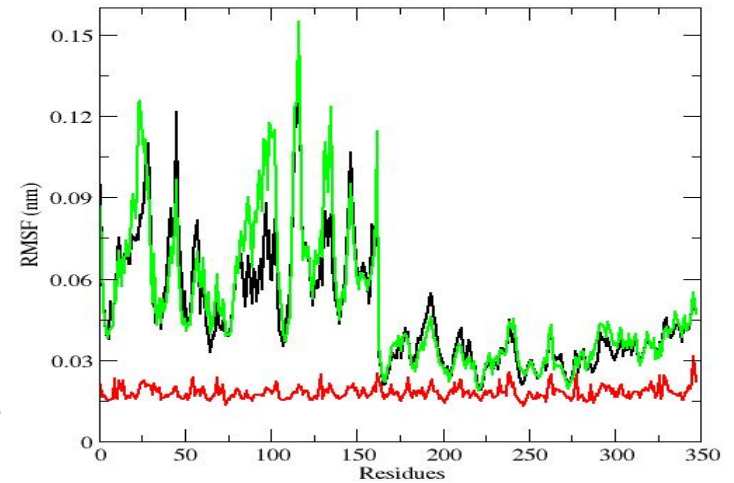
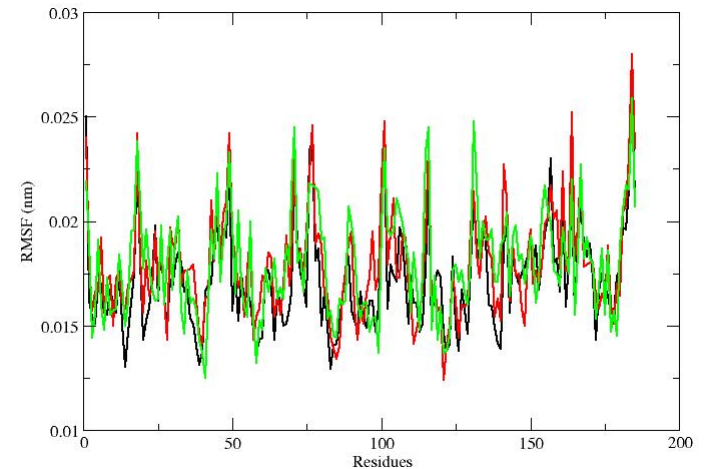
ARF6 not only acts as a activator of the toxin but also stabilize its structure. Results were in agreement with NMR data of earlier workers.



Root mean square fluctuation of amino acid residues of cholera toxin

Nonflexible residues	C α RMSF (nm)	Highly flexible residues	C α RMSF (nm)
Glutamine 121	0.0124	Glycine 184	0.0280
Valine 85	0.0134	Glycine 164	0.0252
Tyrosine 40	0.0141	Alanine 101	0.0248

Targeting both rigid and flexible residues of the catalytically active and other sites of the toxin.

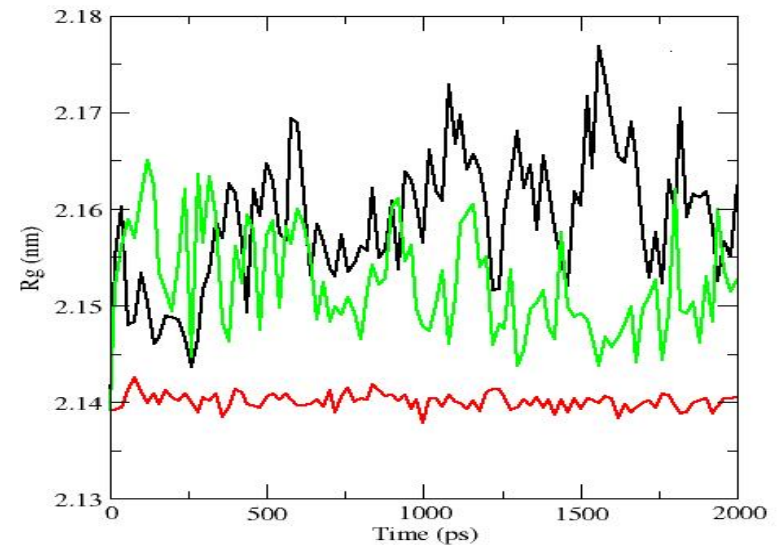
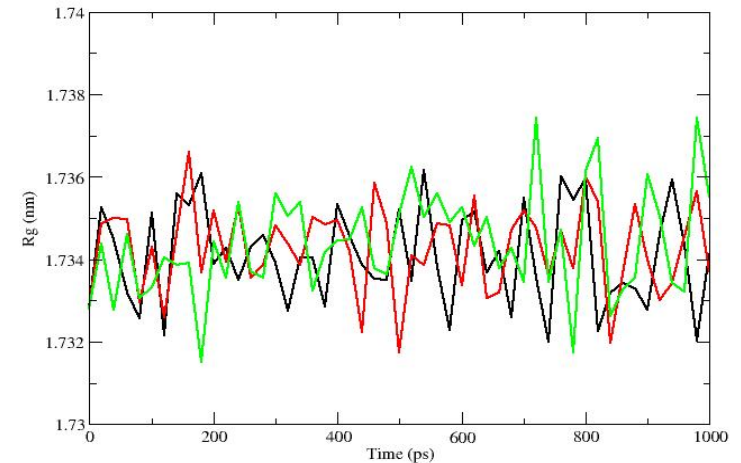


Radius of gyration of CTA1 and its complex with ARF6

Temperature (K)	Minimum Rg (nm)	Maximum Rg (nm)	Mean Rg (nm)
283	1.732	1.736	1.734
310	1.732	1.736	1.735
323	1.732	1.737	1.734

Temperature (K)	Minimum Rg (nm)	Maximum Rg (nm)	Mean Rg (nm)
283	2.145	2.175	2.16
310	2.138	2.1425	2.14
323	2.145	2.165	2.155

Continuous unfolding and refolding of the toxin through out simulation time period

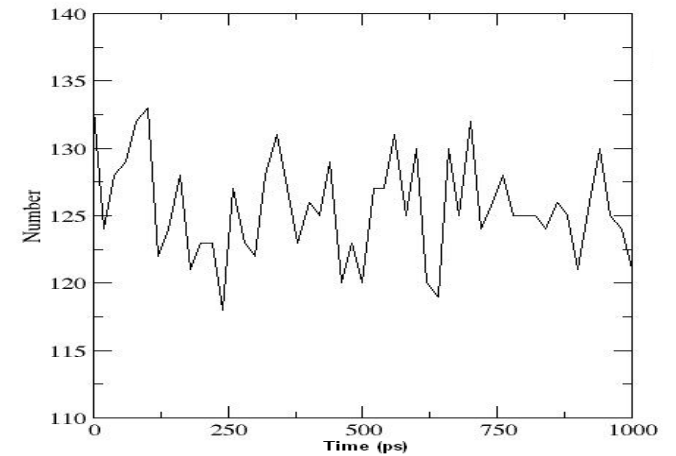


Hydrogen Bonds Analysis: conformational transition, toxin stability and to know about the toxin interactions with the surrounding solvent molecules

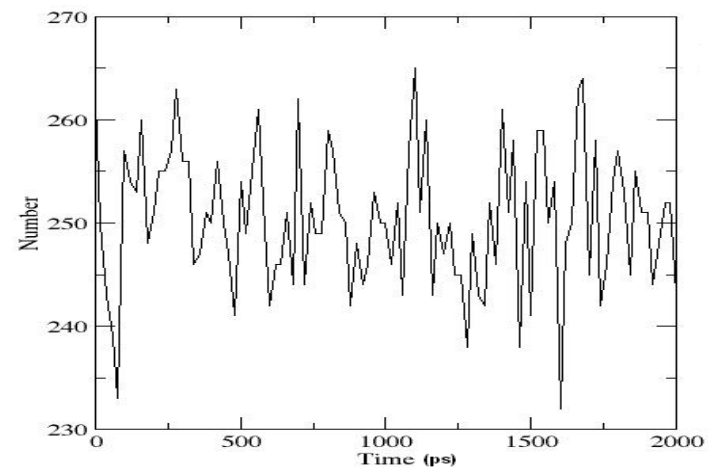
Temperature (K)	Protein-protein hydrogen bonds	Protein-solvent hydrogen bonds
283	125	433
310	123	420
323	125	415

Temperature (K)	Protein-protein hydrogen bonds	Protein-solvent hydrogen bonds
283	247	770
310	252	742
323	246	735

Hydrogen Bonds



Hydrogen Bonds



Solvent accessible surface area:

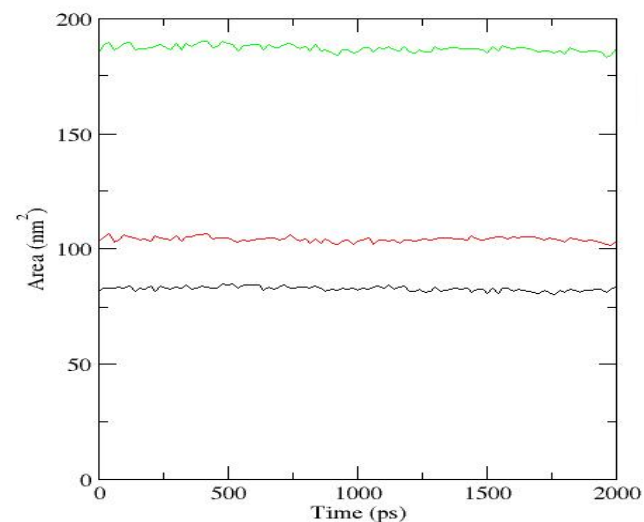
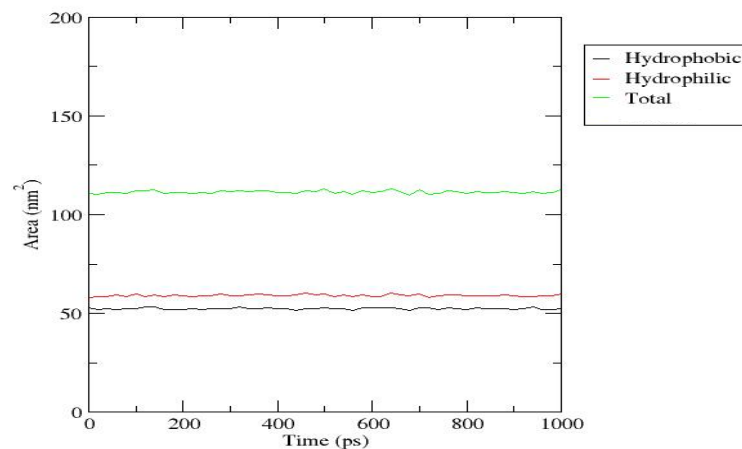
Hydrophilic nature

Easily transfer in polar solvents.

Temperature (K)	Hydrophilic SASA (nm ²)	Hydrophobic SASA (nm ²)	Total SASA (nm ²)
283	58.5	52.5	111
310	58.5	52.5	111
323	58.5	52.5	111

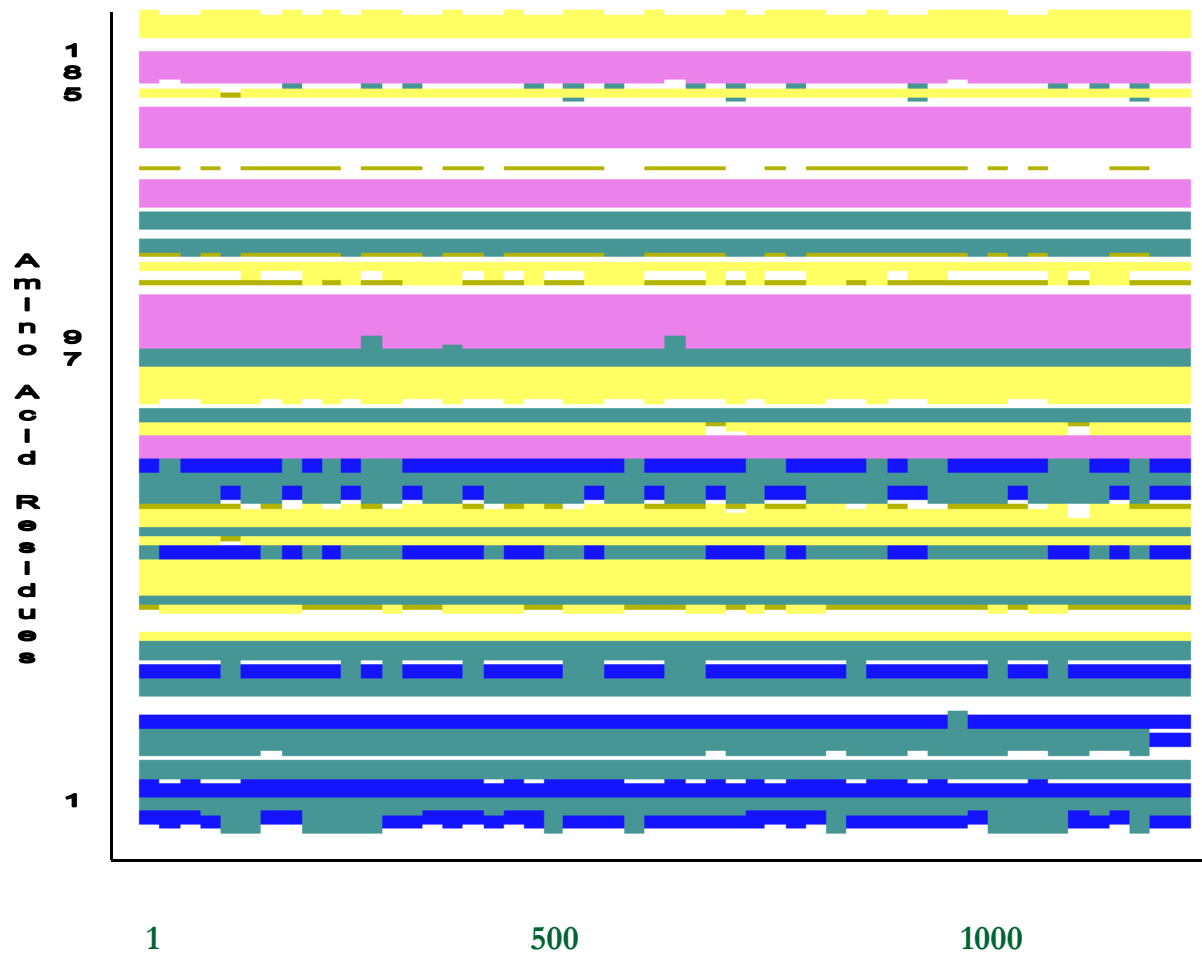
Temperature (K)	Hydrophilic SASA (nm ²)	HydrophobicS ASA (nm ²)	Total SASA (nm ²)
283	104.0	83.0	187.0
310	102.5	81.57	184.2
323	104.5	84.5	189.0

Solvent Accessible Surface



Secondary Structure

- Calculated through Timeline plugin module of VMD having structural identification (STRIDE) of protein algorithm.
 - Calculates secondary structure of protein as a function of time based on the changes in H- bond analysis, main chain dihedral angles and H-bond distances of amino acid residues.
 - Regular conformation units : α -helices, 3-10 helices, β -sheets and β -turns
 - Irregular conformations: loops or coils
 - Results: Transformation of conformational units during the MD Simulation.
-



Secondary structure variations as a function of time of CTA1 at 323 K. Blue denotes 3-10 helix, cyan denotes turn, magenta denotes α -helix, tan denotes bridge, white denotes coil and yellow denotes β -sheet.

Conclusions

- Partially disordered secondary structure, which makes its safe passage through endoplasmic reticulum.
 - CTA in both forms of proteins possess hydrophilic character and is a lethal biochemical weapon as it transfers easily through water reservoirs.
 - It is imperative to design and synthesized inhibitory drugs for cholera toxin, to make it dysfunctional and inactive inside the human body.
-