Residue 229

Computational Analysis of 1XTC and Mutant 1XTC (D229E)

Karim Matta, Ashley Cruz, John W. Craft, Jr.
Department of Biology and Biochemistry, University of Houston Houston, TX 77204-5001

Abstract

Cholera is an infectious and sometimes fatal bacterial disease that causes severe diarrhea and dehydration. It can be contracted by food or drinking water contaminated with the bacterium Vibrio cholerae. The bacterium Vibrio cholerae secretes Cholera toxin (CT), a six protein AB5 structure. Subunit A is subdivided into two domains: A1, an enzymatic active site and A2, an α–helix tail linker. The B pentamer is composed of five chains that form a ring around the central pore in the structure. This study aims to use Molecular Dynamics Simulations and NMR as a tool to analyze the CT mechanisms that make it more pathogenic than that of the heat-labile enterotoxin of Escherichia coli (LT). These two forms share a 88% similarity; therefore, it would be fundamental to obtain these differences. Through computational data, we can obtain the impact the structure has over changes in specific amino acids in the A2 tail. These impacts may explain the experimental data of CT's higher toxicity relative to LT. This study is posed to compare the structure of a wild type 1xtc CT structure obtained from the PDB to a 1xtc mutant with a point mutation at position 229 in the PDB structure. We suspect that the A2-B pentamer interactions due to the mutation will cause a shift in the linker region of the A1 and the A2 subunit domains.

Methodology

Through the use of Computational Molecular dynamics (MD) simulation using Gromacs and GRACE on the UH Sabine cluster, we are able to analyze the interactions between the residues of 1xtc and the mutant 1xtc D229E. Specifically, we are analyzing the Arginines in the beta pentamer interactions with residue 229 in CT. We expect this to be vital to the physiological effect produced by the Cholera toxin. We used the programs listed below to get pictures and generate the data seen in the presentation.

- PyMOL
- UCSF Chimera
- RCDC Unix Cluster
- Gromacs
- Grace

Results and Discussion

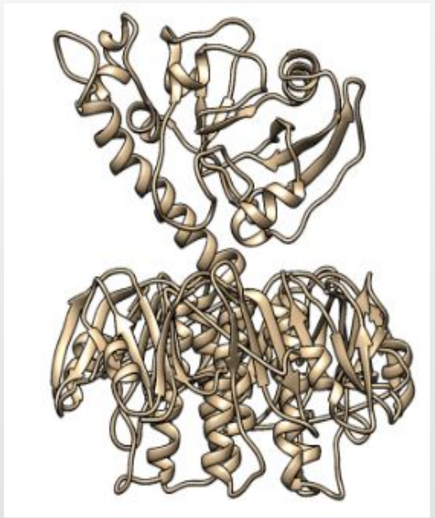
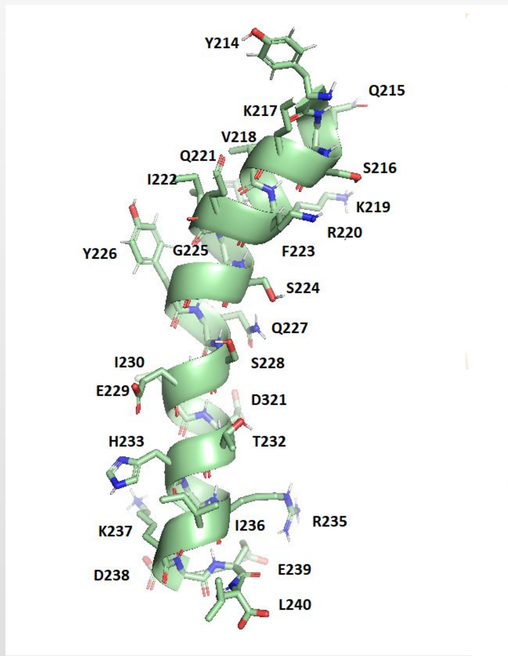


Figure 1. UCSF Chimera Representation of the wild type 1XTC Cholera.

Figure 2. Labeled 1xtc D229E A2 tail.



1xtc vs. 1xtc mutant

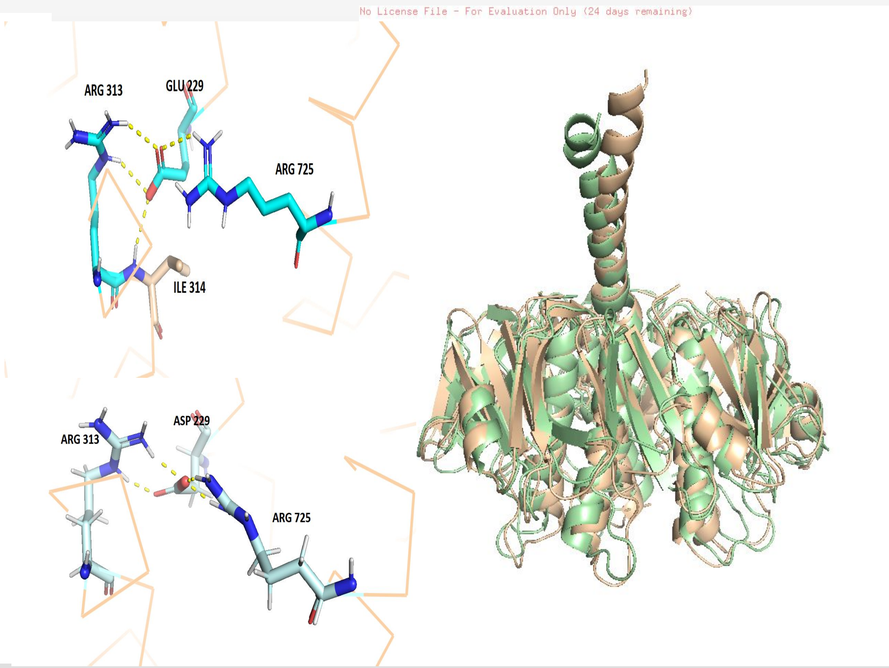
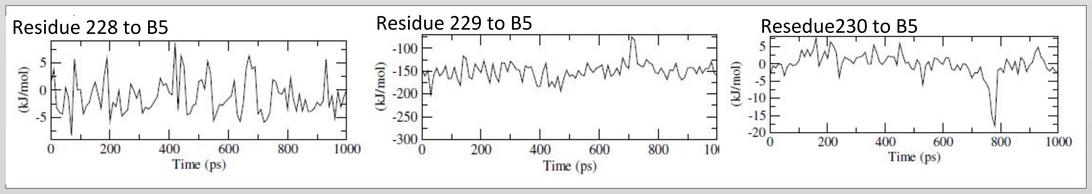


Figure 4 & 5. Comparison of the 1xtc mutant (top) and the wildtype (bottom)

Figure 6. Alignment of the mutant (Green) structure and the wildtype (Wheat)



Mutant 1xtc Interactions with Arginine

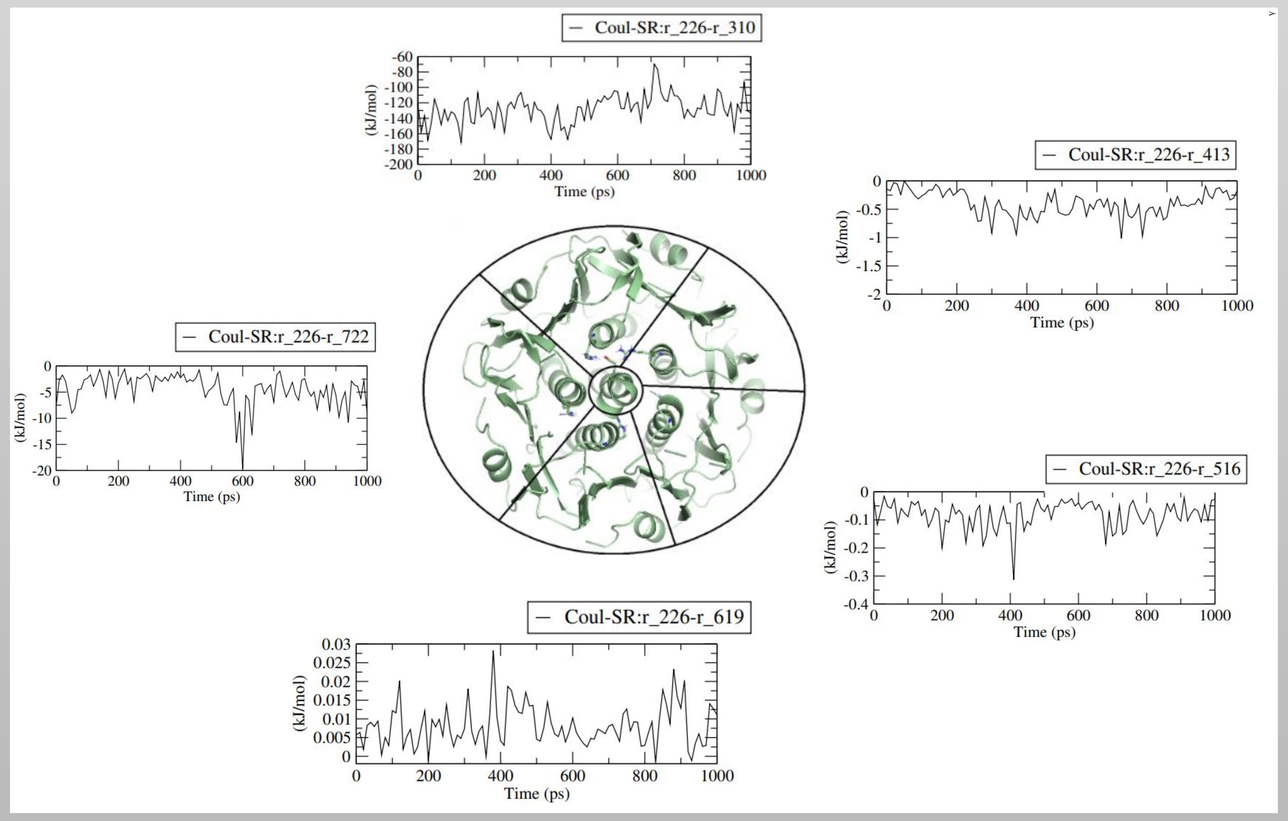


Figure 3. Displays the top view of the mutant's beta pentamer with the 5 Arginines overview. Gromac Energy Plots accompany each arginine. They demonstrate the energy interactions between the arginine and the single point mutation D229E

Conclusion

- The D229E mutant of 1xtc was generated using PyMOL Using gromacs on the sabine cluster after 10 ms of molecular dynamics simulations and energy minimizations. Final mutant trajectories are recorded in .gro structure files and show significant changes between the wild-type 1xtc.

Future Work

- In the future we would like to quantify the shift seen in the A2 linker region and compare it to the wild type 1xtc and other similar structures like the heat-labile 1lts.

References

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Taylor M., Burress H., Banerjee T., Ray s., Curtis D., Tatulian S., Teter K., 2014. "Substrate-Induced Unfolding of Protein Disulfide Isomerase Displaces the Cholera Toxin A1 Subunit from Its Holotoxin" by Public Library of Science.

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