Probing Mechanical and Thermodynamic Properties of *Vibrio Cholerae* Toxt Binding to DNA by Molecular Dynamics Simulations

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Synopsis

The main objective of this research is to investigate DNA binding mechanisms to ToxT, a transcriptional activator of genes coding Cholera Toxin (CT) and Toxin Co-regulated Pilus (TCP). Determining the molecular mechanism of DNA-ToxT interaction is a milestone towards full understanding of the acute intestinal infection caused by the bacterium V. cholerae. ToxT binds to 13-bp sequences called toxboxes, which are located upstream of the genes whose transcription is activated by ToxT. In the present study, all-atom Molecular Dynamics Simulations (MD) of the toxbox system in the NPT ensemble were used to obtain thermodynamic properties of the toxbox system by calculating energies using the MD implementation of the generalized Born/surface area (GB/SA) implicit solvation method, and configurational entropies by quasi-harmonic approximation (QH) combined with the minimally coupled subspace approach (MCSA). The effects of sequence-dependent DNA conformation (DNA crookedness) on mechanical properties of the toxbox system was studied by performing MD on toxboxes subject to constant stretching forces.

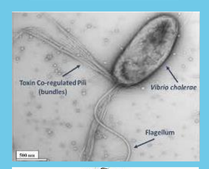
Background

Cholera – A Major Epidemic Disease

- Caused by bacterium *Vibrio cholerae*
- Central role in infectious disease research
- World Acute, water-borne infectious disease
- ~ 4 million cases each year in the developing word

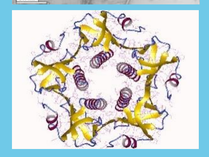
Major Virulence Factors

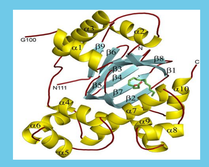
- Cholera toxin (CT), a protein that causes watery diarrhea.
- Toxin-coregulated pilus (TCP): flexible appendage on the surface of bacterial cells required for aggregation of *V. cholerae* within the intestine.

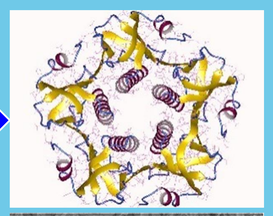


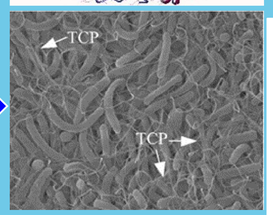
ToxT Protein

- Transcription of the genes encoding CT and TCP are regulated by ToxT protein.
- Binding of ToxT to bacterial DNA is required to activate transcription.
- ToxT binds to specific 13-bp DNA sequences called toxboxes.









Objectives

Long Term Objective

- Calculate and compare free energies of ToxT-DNA complex and individual units (ToxT, DNA) using experimental data of the structure of the complex.

Short Term Objective

- Perform equilibrated simulations (NPT ensemble) of toxbox.
- Perform Steered MD of toxbox under constant force.

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- Calculate mechanical properties (stretch modulus of DNA sequence)
- Calculate absolute entropy from equilibrated simulation
- Calculate entropy change between equilibrium structure and structure under constant force

Methods

Molecular Dynamic Simulation (MDS)

- 27 bp nucleotide sequence including ("GATTTTTGATTTT"–toxbox 1) required for the ToxT activation of PctxAB was chosen for MD.
- Nucleic Acid Builder (NAB) programming language was used to model the toxbox with Right-Handed B-DNA (Arnott) structure.
- Amber 16.0 is used as the primary simulation package.
- MD timescales are limited to ~1μs

Methods

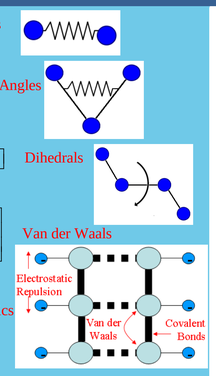
$$U_{total} = \sum_{Bonds} K_r (r - r_0)^2 \quad \text{Bonds}$$

$$+ \sum_{Angles} K_\theta (\theta - \theta_0)^2 \quad \text{Polar Angles}$$

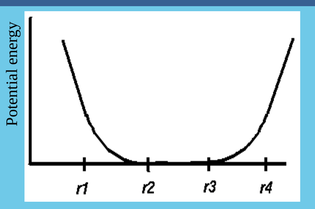
$$+ \sum_{Dihedrals} \frac{V}{2}\left[1 + \cos(n\phi - \gamma)\right] \quad \text{Dihedrals}$$

$$+ \sum_{Atoms} \varepsilon_{ij}\left[\left(\frac{R_{ij}}{r_{ij}}\right)^{12} - \left(\frac{R_{ij}}{r_{ij}}\right)^{6}\right] \quad \text{Van der Waals}$$

$$+ \sum_{Charges} \frac{q_i q_j}{\varepsilon r_{ij}} \quad \text{Electrostatics}$$



Form of the Hamiltonian employed in a typical MDS



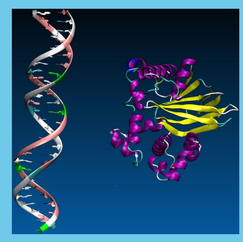
Distance between two COM

$$E_{res}(R) = \begin{cases} A_1 R + B_1 & \text{if } R \le r_1 \\ rk_2(R - r_2)^2 & \text{if } r_1 < R \le r_2 \\ 0 & \text{if } r_2 < R \le r_3 \\ rk_3(R - r_3)^2 & \text{if } r_3 < R \le r_4 \\ A_2 R + B_2 & \text{if } R > r_4 \end{cases}$$

Form of the piece-wise defined function

Results & Conclusion

Our preliminary data are very encouraging and confirm that the outcome of this research is expected to have significant experimental and theoretical impact, because the DNA-ToxT complex is a paradigmatic disease model for transcriptional activators. Furthermore, the insight obtained in this project will be equally applicable to unravel the molecular mechanisms driving host-pathogen interactions.



27 bps (toxbox1+T+toxbox2) DNA sequence and ToxT protein

References

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