University of New Hampshire
College of Engineering and Physical Sciences

CRISPR/Cas9 Enzyme Structure and Mechanism

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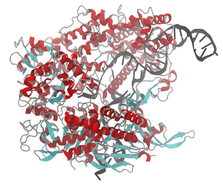

PDB Code: 4ZT9

ABSTRACT

The project objective was to study the CRISPR/Cas9 system, and the mechanism it utilizes to cleave DNA strands. This method utilizes clustered regularly interspaced short palindromic repeats (CRISPRs), an associated bacterial endonuclease (Cas9), and a short guide RNA strand to target a genomic area of interest [2]. The CRISPR/Cas9 system acts as a pair of molecular scissors that cuts DNA strands, allowing for modification or correction. Computational simulations were created and studied to look at specific and important portions of the enzyme and to place the enzyme in surroundings similar to the human body. Viral DNA was compared to CRISPR RNA to show the replication and extraction.

BACKGROUND

Problem:

- Bacteria and viruses compete in the same atmosphere for survival
  - ➢ CRISPR is as a defense mechanism by storing viral DNA into bacteria cells similar to an immune system
- Millions of people suffer from diseases caused by genetic mutations
  - ➢ CRISPR potentially could be used to edit the human genome to cure these types of disease.

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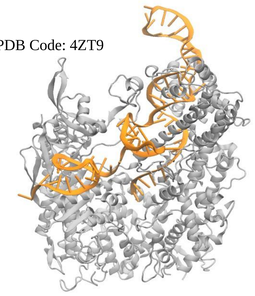


Figure 1: CRISPR/Cas9 enzyme

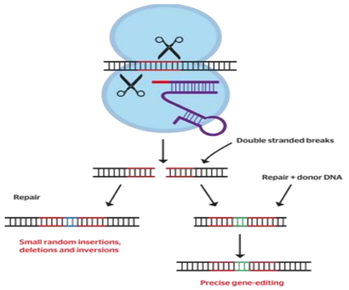


Figure 2: General mechanism

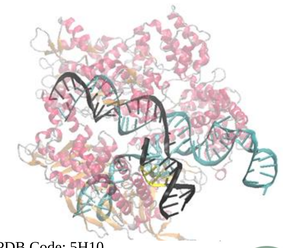
PROJECT OBJECTIVE AND METHODS

- Computational Simulation Analysis with VMD/NAMD
  - ➢ Create images of CRISPR/Cas9 enzyme in order to study specific parts of the system such as PAM, inactive state vs active, replication of viral DNA with PDBs
  - ➢ Successfully put the CRISPR enzyme in the simulated surroundings of the human genome including temperature and ions with a 10,000 step generic water box simulation consisting of 180,000 atoms with stability
  - ➢ Root Mean Squared Difference of the molecule in the water box
  - ➢ Generic buried surface area TCL scripting to show crRNA embedded into the protein

RESULTS

- Large portion of overlap
- Loop folds into beta sheet

Figure 3: CRISPR/Cas9 enzyme with PAM highlighted



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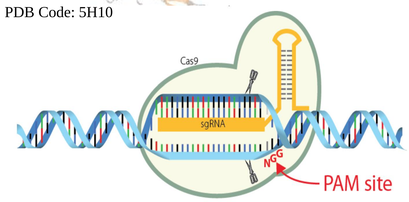


Figure 5: Mechanism of CRISPR including PAM

Active PDB Code: 4ZT9

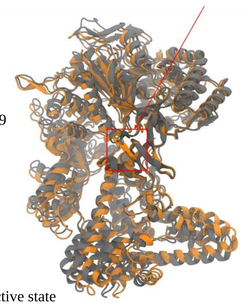


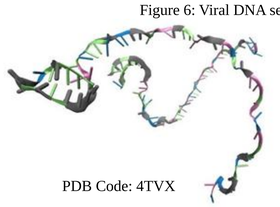
Figure 4:Inactive state vs. Active state

Inactive PDB Code: 4ZT0

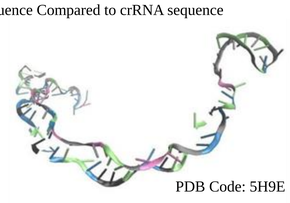
- PAM site highlighted
- Cas9 enzyme target
- Black invading DNA
- Cyan guide RNA
- Protein is shown in red and orange

Figure 6: Viral DNA sequence Compared to crRNA sequence

- Left Viral DNA
- Right *E.coli* crRNA
- 20 base pairs long



PDB Code: 4TVX



PDB Code: 5H9E

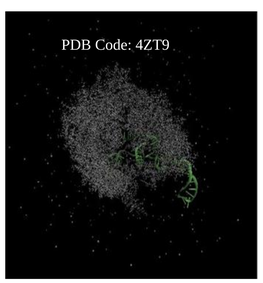


Figure 7: Water box molecule with only ions present

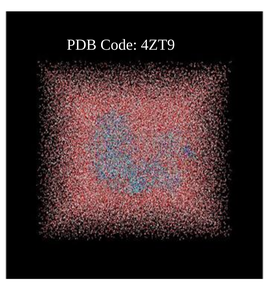


Figure 8: Molecule in water box and ions

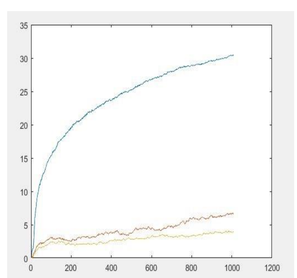
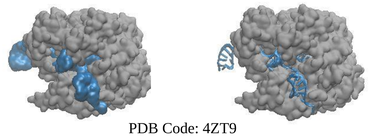


Figure 9:RMSD plot of water box

- Water box simulation with and without H_2O molecules and only ions
- RMSD plot of molecule in water box simulation showing the stability of different parts of CRISPR in the human genome surroundings

- Buried surface area
  - ➢ Comparing the difference between protein, nucleic and both together.



PDB Code: 4ZT9

Figure 9: CRISPR/Cas9 with surface area emphasized

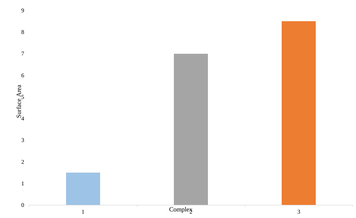


Figure 10: Bar graph relating surface areas

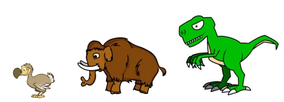
CONCLUSION

Computational Analysis

- CRISPR in *E.coli* successfully extracted viral DNA and placed it within the system to use as a defense mechanism
- An active state of CRISPR is more stable than an inactive
- PAM site where CRISPR attacks the viral DNA was able to found and highlighted
- CRISPR/Cas9 enzyme would be able to live and survive in the surroundings of the human genome

Future Direction

CRISPR is being investigated for applications including cancer treatment, lime disease treatment, antibiotics replacement, editing of the human genome, reconstructing genomes of extinct species, improved food and fuel development and assisted genetic surgeries.



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[3] S. Harris and J. Doudna (2017). Waking up with Sam Harris #106- Humanity 2.0 (with Jennifer A. Doudna) [online] Waking up: Sam Harris Available at: https://www.youtube.com/watch?v=NbujQ1-A9-A. [Accessed 5 Dec. 2017].

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ACKNOWLEDGEMENTS

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