Molecular Dynamic Simulations of CRISPR and HIV

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Abstract

In the United States, there were 37,600 new HIV infections in 2014, with an estimated 1.1 million people living with the disease in 2015, according to the CDC. HIV targets the cell receptor CD4 and chemocine coreceptors CCR5 or CXCR5. Some individuals possess a mutation within CCR5 that causes a resistance to HIV-1. One HIV+ patient in Berlin, Timothy Brown, developed an immunity to the virus after a bone marrow transplant from a donor who possessed this CCR5 mutation. After this coincidence, researchers attempted a variety of gene therapies, such as zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and Cas9. CRISPR/Cas9 is a promising new tool that scientists have been using to do direct editing of genomes with more ease and specificity than ever before. CRISPR stands for cluster of regularly interspaced palindromic repeats, which are segments of RNA that are found in many prokaryotes to defend the organism against viral infections and unwanted gene transfers. Cas is a gene cluster that mediates the response to the RNA encoded in the CRISPR segments. Cas are designated by the protein complex responsible for interference. Together, these molecules identify a segment of target RNA, extract it, and replace it with another segment. They make up part of the adaptive immune system of eukaryotic cells. This research hopes to create a predictive model by analyzing the existing gene therapy data from previous studies, and using numerical molecular dynamic simulation software to glean more information about those results. Previously published studies discuss how gene therapies such as ZFN and CRISPR are used to modify either the host genome or the viral genome[3], and then experiments are performed to determine whether this therapy is effective at preventing viral infection. By analyzing the bonding characteristics of different strands of RNA, it may be possible to predict which RNA segments make the best candidates for gene therapies that will confer resistance to HIV infection.

1. Method

1.1 Control – Dynamics of 2xlk

The PDB file 2xlk represents the crystal structure of Csy4 in complex with a segment of RNA[1]. As a control, chains A and C of 2xlk was simulated for 20.1 ns. By comparing the characteristics of these molecules with other guide RNA strands, the success of the simulation can be gleaned.

1.2 gRNA types 1, 6, and 7

Hou and team[2] used CRISPR with different mutations of characteristic RNA strands to remove the CXCR4 gene, which is one of the pathways that HIV uses to infect human CD_4^+ T cells. Different RNA strands had different success rates in preventing HIV infection.

The crux of this research was to determine if there was a difference in the bonding characteristics of the different RNA strands that caused the differences in viral resistance, using molecular dynamic simulations.

1.3 Molecular Dynamics

- In addition to the control, three simulations were set up for each of the RNA types in complex (right) with the endoribonuclease Csy4 (left).
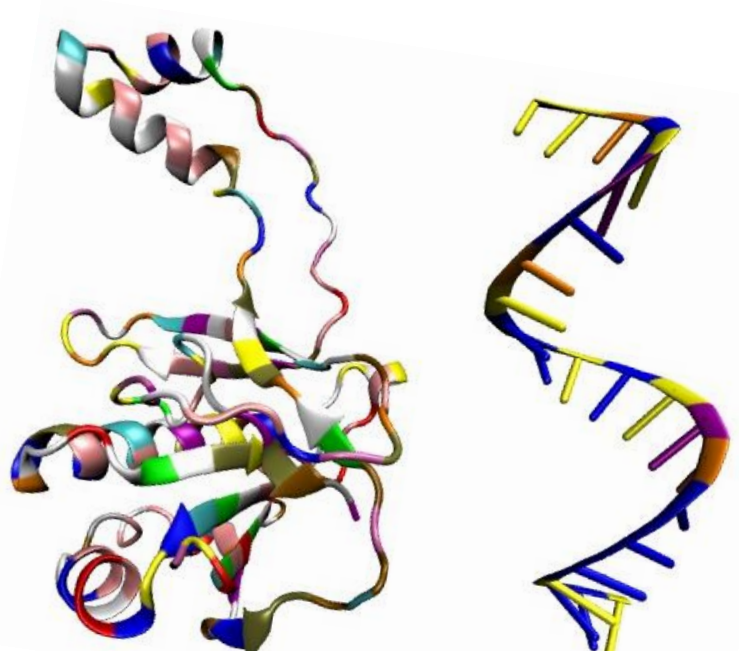
- All simulations were carried out for at least 100 ns.
- All simulations used CHARMM force field.
- Periodic boundary conditions were assumed using a constant temperature of 300K and a pressure of 1atm.
- At least 10,000 steps energy minimization was performed first to stabilize the system, with both fixed and unfixed atoms (but movable solvents).
- The control RNA strand, #1, was simulated for 175 ns. Strand #6 was simulated for 140 ns.
- RMSD, electrostatic interactions, etc. were used to compare the simulations of the RNA types.
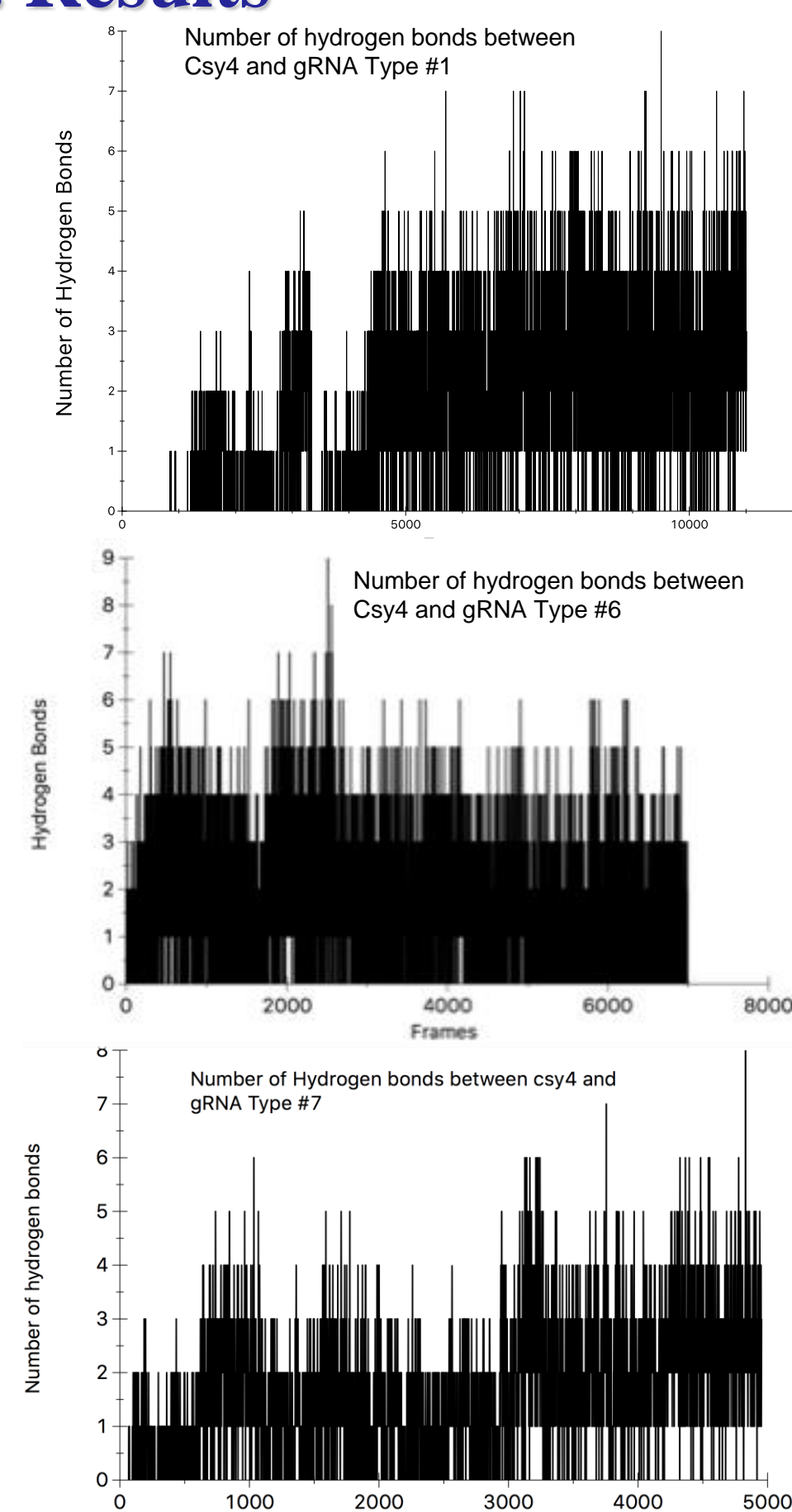
2. Results

Figure 1a, 1b, 1c: *Comparison of the hydrogen bonds between RNA strands and Csy4 in the control (top, type #1), type #6 (middle), and type #7 (bottom).*
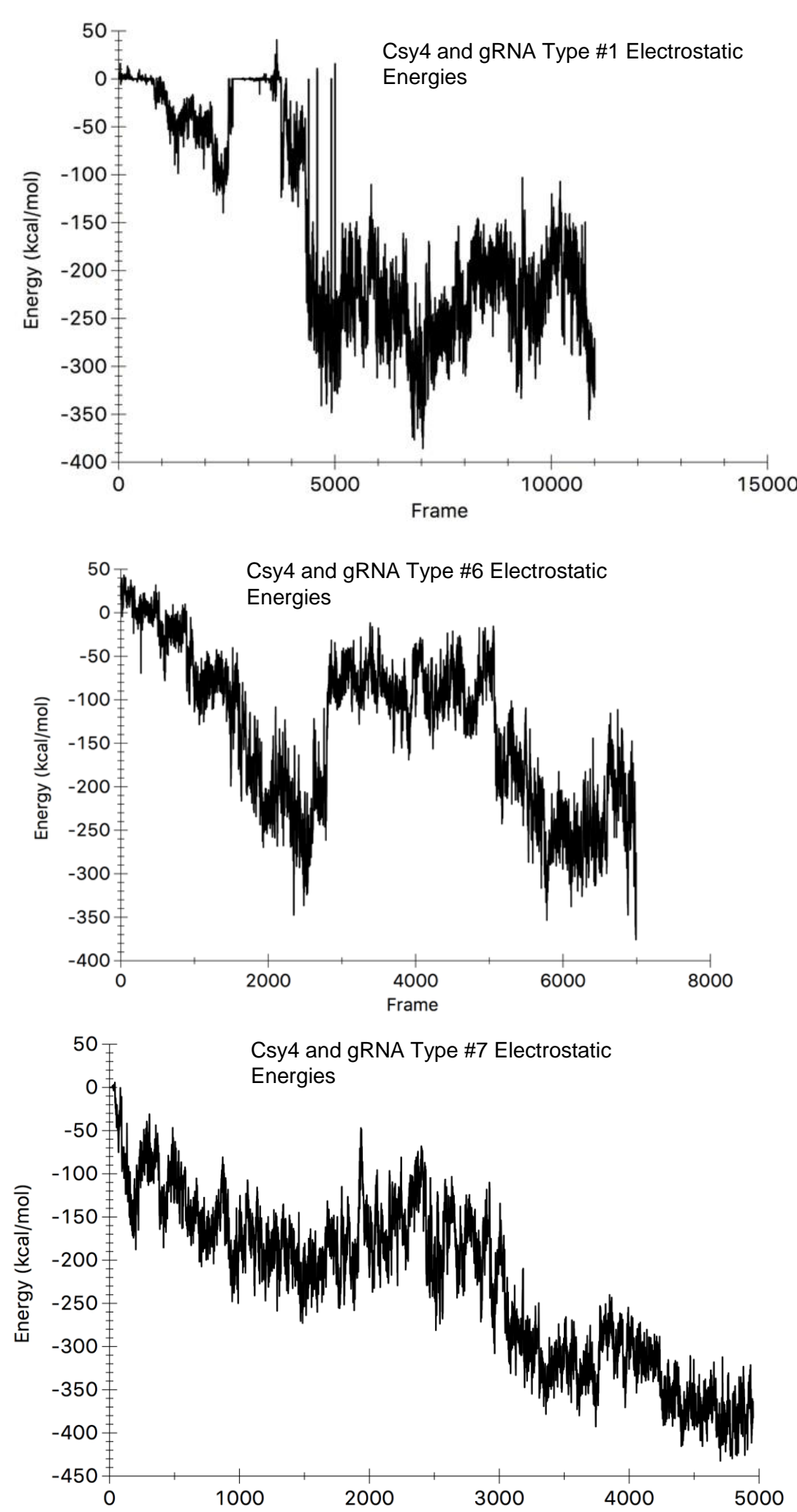

Figure 2a, 2b, 2c: *Comparison of electrostatic energies of wild type RNA #1 (top) and mutated RNA type #6 (middle) and type #7 (bottom).*
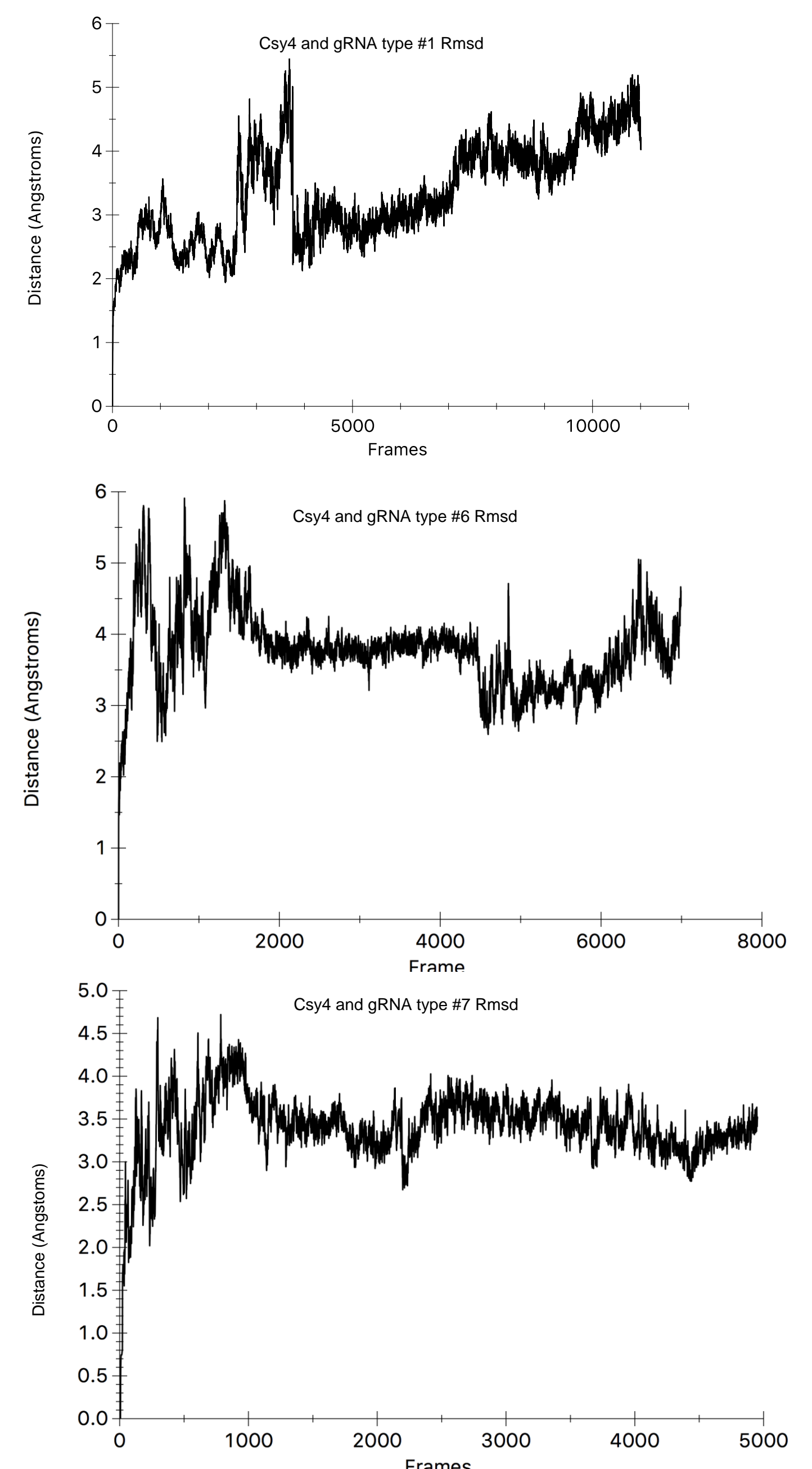

Figure 3: *Rmsd plots of RNA wild type #1 (top), mutated RNA #6 (middle), mutated RNA type #7.*

3. Conclusion

Conclusions are still very much preliminary. Looking at the Rmsd of the control PDB file, 2xlk_AC (not shown), it seems that this has already equilibrated. Since this is an x-ray of a molecule in vitro, this stands to reason.

The differences between the control RNA type #1, and the mutated type #6 and type #7 are apparent in the data collected by Hou et al[2], but more simulations need to be done before their performance as gene modifiers can be predicted using VMD simulations.

4. References

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2. Hou, Panpan, Shuliang Chen, Shilei Wang, Xiao Yu, Yu Chen, Meng Jiang, Ke Zhuang, et al. "Genome Editing of CXCR4 by CRISPR/Cas9 Confers Cells Resistant to HIV-1 Infection." *Scientific Reports* 5 (October 20, 2015). https://doi.org/10.1038/srep15577.

3. Price, Aryn A., Arash Grakoui, and David S. Weiss. "Harnessing the Prokaryotic Adaptive Immune System as a Eukaryotic Antiviral Defense." *Trends in Microbiology* 24, no. 4 (April 2016): 294–306. https://doi.org/10.1016/j.tim.2016.01.005.