Ribonuclease H (RNase H) is a non-sequence-specific endonuclease present in all branches of life. It degrades the RNA strand in DNA: RNA hybrid molecules during DNA replication and a myriad of other biological processes; retroviral infection is one such process that has clinical significance[1]. The retroviruses Murine Leukemia Virus (MLV) and HIV contain essential RNase H domains in their multi-domain reverse transcriptase proteins that are necessary for viral replication. The "handle region", an extended loop in RNase H, is a prime target to explore this necessity as it is critical for substrate recognition[2-4]. A breadth of information exists on this region's dynamics, but important gaps remain unfilled; gaps that may potentially lead to creating effective drugs to treat the above-mentioned viruses. Comparing homologous proteins from organisms that live in different thermal environments can tell us a lot about a protein's dynamics[5-12].

The proteins used for this project are E. coli RNase H (ecRNH), a mesophilic protein active at ambient temperature, and Thermus thermophilus RNase H (ttRNH) which is only active at elevated temperature. NMR Spectroscopy and Molecular Dynamic (MD) simulations indicate that ecRNH mainly populates an open state at room temperature, while ttRNH populates a closed state (Figure 1); as temperature increases, the populations trend towards equality, as expected from the Boltzmann equation[7,11,12]. MD simulations and computational analysis of these proteins uncovered a mutation, V98A, which raises the Michaelis constant for ecRNH, thus giving it ttRNH-like properties[7]. It is suspected that the alanine mutation at position 98 disallows a neighboring valine, V101, from interacting with it in a way that stabilizes the "closed" conformation. The disappearance and shift of V101 rotamer population distributions in V98A ecRNH MD trajectories compared to Wt trajectories support this claim (Figure 2).

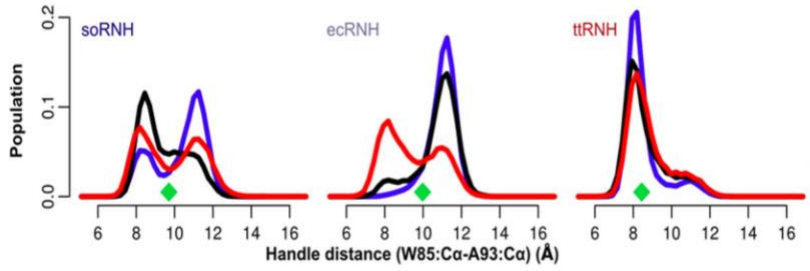

Figure 1. Conformational population distribution of RNase H from three organisms. From left to right the organisms represented are a psychrotroph (soRNH- optimally grows at low temperatures), mesophile (ecRNH- optimally grows at moderate temperatures) and a thermophile (ttRNH- optimally grows at high temperatures). The blue, black and red plots respectively represent population distributions at 0°C, 37°C, and 67°C. Smaller handle distances correspond to a "closed" state and larger distances an "open" state. *Figure taken from reference 7.

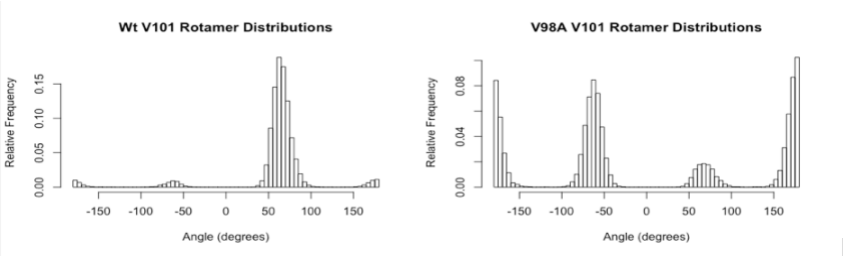

Figure 2. V101 Rotamer population distribution of Wt. and Mt. V98A RNaseH. Valine rotamers include Trans (+/- 180^0), Gauche + (60^0) and Gauche – (-60^0). A 100 ns Wt. MD trajectory produced V101 rotamers (left) that predominantly occupied the gauche + state. Conversely, V98A MD trajectory produced V101 rotamers with gauche + in the minority.

References

1. Crouch, R. J. (1990). Ribonuclease H: from discovery to 3D structure. *New Biol.* 2, 771–777.
2. Yang, W., Hendrickson, W. A., Crouch, R. J. & Satow, Y. (1990). Structure of ribonuclease H phased at 2 A˚ resolution by MAD analysis of the selenomethionyl protein. *Science*, 249, 1398–1405.
3. Mandel, A. M., Akke, M., & Palmer, I. A. (1995). Backbone Dynamics of Escherichia coli Ribonuclease HI: Correlations with Structure and Function in an Active Enzyme. *Journal of Molecular Biology*, 246(1), 144-163.
4. Keck, J. L., & Marqusee, S. (1996). The Putative Substrate Recognition Loop of Escherichia coli Ribonuclease H Is Not Essential for Activity. *Journal of Biological Chemistry*, 271(33), 19883-19887.
5. Butterwick JA, Palmer AG (2006) An inserted Gly residue fine tunes dynamics between mesophilic and thermophilic ribonucleases H. *Protein Science* 15, 2697– 2707
6. Butterwick JA, Patrick Loria J, Astrof NS, Kroenke CD, Cole R, et al. (2004) Multiple time scale backbone dynamics of homologous thermophilic and mesophilic ribonuclease HI enzymes. *J Mol Biol* 339: 855–871
7. Stafford, K. A., Robustelli, P., & Palmer, A. G. (2013). Thermal Adaptation of Conformational Dynamics in Ribonuclease H. *PLoS Computational Biology*, 9(10), 1-10.
8. Hollien, J., & Marqusee, S. (1999). Structural distribution of stability in a thermophilic enzyme. *Proceedings of the National Academy of Sciences*, 96(24), 13674-13678.
9. Hollien, J., & Marqusee, S. (1999). A Thermodynamic Comparison of Mesophilic and Thermophilic Ribonucleases H †. *Biochemistry*, 38(12), 3831-3836.
10. Robic, S., Berger, J. M., & Marqusee, S. (2001). Contributions of folding cores to the thermostabilities of two ribonucleases H. *Protein Science*, 11(2), 381-389.
11. Stafford, K. A., Trbovic, N., Butterwick, J. A., Abel, R., Friesner, R. A., & Palmer, A. G. (2015). Conformational Preferences Underlying Reduced Activity of a Thermophilic Ribonuclease H. *Journal of Molecular Biology*, 427(4), 853-866.
12. Zeiske, T., Stafford, K. A., & Palmer, A. G. (2016). Thermostability of Enzymes from Molecular Dynamics Simulations. *Journal of Chemical Theory and Computation*, 12(6), 2489-2492.