ASSESSMENT OF CHAO- VERSUS KOSMOTROPIC PROPERTIES OF BRINE SOLUTIONS THROUGH THEIR EFFECTS ON ANCIENT PROTEIN STRUCTURE. S. D. Osborne[1], M. D. Molayousefi[2], M. Moradi[2], and V. F. Chevrier[3]; [1]University of Arkansas, Environmental Dynamics, Fayetteville, Arkansas, sdo002@uark.edu, [2]University of Arkansas, Department of Chemistry and Biochemistry, Fayetteville, Arkansas, [3]University of Arkansas, AR Center for Space and Planetary Sciences, Fayetteville, Arkansas

Introduction: The formation, stability, and precipitation of proteins that emerge in naturally occurring brine solutions (water and salt) across the solar system may have significantly aided in providing the essential environmental conditions for organic interactions necessary in subsequent life formation [1,2,3]. The relationship between water and salts in brine solutions (chao/kosmotropic behavior) has a substantial influence on the structural soundness, precipitation, and bioavailability of proteins [4], and may provide insight into the components and interactions that were required during life's earliest developmental stages. Life on Earth began in salt water; so here, we propose that the nature of the salt directly influences the structural changes of biomacromolecules (in this case an ancient, ubiquitous proteins) and corresponds to the chao/kosmotropic behavior of the salt.

Solutes in water are defined as kosmotropic (order-maintaining) if they contribute to the overall structural stability of hydrogen bond interactions, and therefore also contribute to the overall structural stability of biomacromolecules. Kosmotropes cause the hydrogen bonds in water molecules to favorably interact, which in effect stabilizes intramolecular interactions in macromolecules. On the other hand, chaotropicity (order-destroying) describes the consequent disordering of protein structures when dissolved in salts and water.

Various cations and anions have been ordered in terms of their degree of chao- or kosmotropicity on a scale called the Hoffmeister Series, and some work has been attempted to quantify this property [5]. However, more work needs to be done in order to properly quantify chao/kosmotropic responses in protein stability.

Universally significant thermodynamic parameters, such as temperature and water activity, also exist within specific boundaries for life. Therefore, the relationship between primordial biochemistry and water-salt composition is difficult to quantify. Directly relating protein structural responses to chao- or kosmotropic behavior remains difficult, especially when these behaviors are also influenced by other thermodynamic properties, perhaps simultaneously. In addition to this, how exactly these early proteins react in the presence of these brines across the solar system is largely unknown. Some observations of these conditions have accumulated due to a large variety of brines potentially present on icy moons or terrestrial bodies, like Mars and early Earth.

The scope of this research pertains to ancestral amino acid sequences required for the beginning stages of life, with a focus on their structural changes when exposed to brines that exhibit varying degrees of kosmo- or chaotropicity. The selected brines for this study consist of 0.15 and 0.75 M of NaCl, $MgCl_2$, and $(NH_4)SO_4$ with $MgCl_2$ being the most chaotropic analog, and $(NH_4)SO_4$ being the most kosmotropic analog [5]. NaCl was used as a neutral analog.

This abstract presents the preliminary results of molecular dynamics simulations exposing the prokaryotic ubiquitin-like protein (Pup) to varying concentrations of NaCl and $MgCl_2$. See Figure 1 for the AlphaFold structure of Pup derived from CHARMM and VMD analysis:

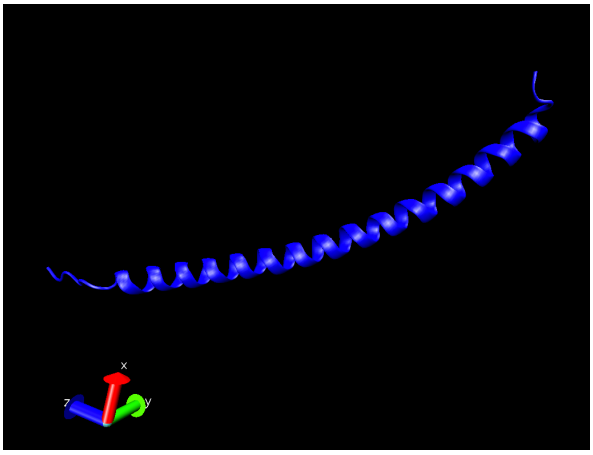

Figure 1: AlphaFold structure of prokaryotic ubiquitin-like protein (Pup) derived from CHARMM and VMD analysis [6,7]

Methods: The ancestral sequence of Pup has been recently decoded with impressive certainty [6,7]. Using the Pup structure provided by AlphaFold: A0A2P2CDG8-F1 [6,7] we utilized all-atom molecular dynamics (MD) to investigate the differential structural dynamics of the Pup protein in different salts at different concentrations. All simulations were performed using the NAMD 3.0 simulation package and the CHARMM36m all-atom additive force field.

Our Pup was virtually solvated in a box of TIP3P waters using the CHARMM protein software and the VMD simulator, containing either 0.15 M NaCl, 0.75 M NaCl, or 0.15 M $MgCl_2$. Systems were composed of approximately 60,000 atoms with a simulation box dimension of ~(84 x 84 x 84) $Å^3$. Each simulation ran for 100 nano- seconds. Simulations were carried out using a

2-fs time step at 298 K using a Langevin integrator with a damping coefficient of = 0.5 ps^{-1} at 1 atm of pressure.

Results: The results of the MD simulations of Pup's brine exposure outcomes are featured in Figure 2 (a-c):

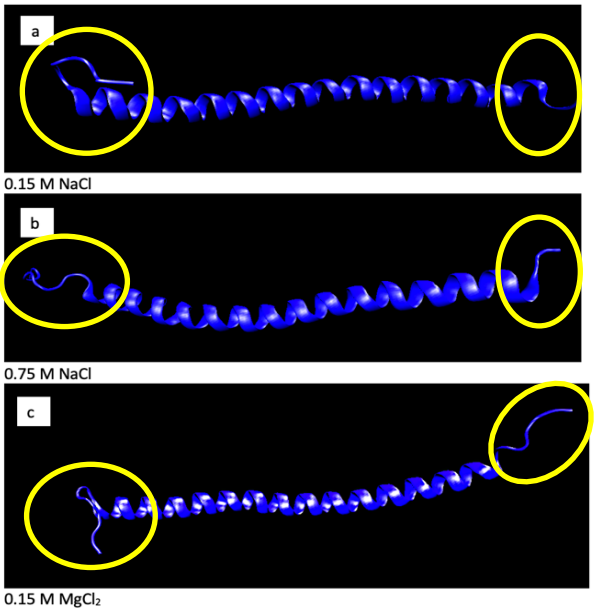

Figure 2 (a-c): CHARMM outcomes of Pup protein brine exposure represented by VMD; terminal end changes circled in yellow. a: Original protein; b: after exposure to 0.15 M NaCl and c: after exposure to 0.75 $MgCl_2$.

Visually, we observe varying degrees of unfolding for each salt at Pup's terminal amino acid strings. These visually noticeable changes in protein structure represent the chao- and kosmotropic response of the protein when exposed to the saltwater solution.

We also performed an RMSD analysis (root mean square function) to compare the differences between numerical measurements related to protein structural change [8]. Figure 3 demonstrates the results of the RMSD analysis comparing degree of protein structure change during 100 ns:

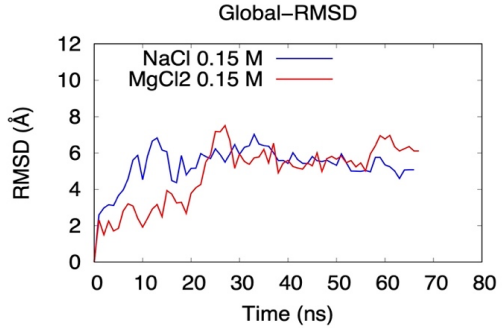

Figure 3: RMSD analysis comparing degree of protein structure change; obtained using VMD

The RMSD results show a larger change in the first 20 ns for the protein in NaCl compared to $MgCl_2$. However, the RMSD converges to a similar value above 20 ns, suggesting similar change is elicited between the two environments. Since neither NaCl or $MgCl_2$ represent extreme cases of chao- and kosmortopicity, this comparable end result is not entirely surprising.

According to the Hoffmeister Series scale, $MgCl_2$ is a more chaotropic salt than NaCl, [5]. However, neither salt exhibits an extreme trend towards chaotropicity, especially since both NaCl and $MgCl_2$ have the same anion. This explains why these two different salts with two only create slightly different responses within the protein's structure in each particular brine. This observation speaks to the subtle (but potentially significant) changes resulting from chao-kosmo influences at the molecular level.

Conclusion: The degree of terminal unfolding does vary depending on salt and concentration. Each new terminal unfolding pattern becomes unique and distinguished from the rest, including becoming different from the unexposed protein in Figure 1.

Unlike ubiquitin, Pup is intrinsically disordered in its free state or when covalently attached to substrate proteins [8]. Pupylation and proteasomal degradation have been shown to ensure survival of bacteria under challenging conditions, like nitrogen-starvation or DNA-damaging conditions including UV radiation [8]. A protein's ability to interact with its environment depends on its ability to maintain its primary structure. The results of this study demonstrate that when in the presence of salts, chao- and kosmotropicity play an important role in understanding how a protein will interact with, and change according to, its briny environment.

References: [1] Guida, Samuela. Chemical Engineering Journal: 427. 2022. [2] Barat, J.M. Journal of Food Science: 5. 2002. [3] Szymczak, Mariusz. Institute of Food Science and Technology: 52, 154-160. 2017. [4] Yano, Yohko. Langmuir: 32, 9892-9898. 2016. [5] Cray, Johnathan. Environmental Microbiology: 15, 1. 2012. [6] Jumper, J et al. Highly accurate protein structure prediction with AlphaFold. Nature. 2021. [7] Varadi, M et al. AlphaFold Protein Structure Database: massively expanding the structural coverage of protein-sequence space with high-accuracy models. Nucleic Acids Research. 2021. [8] BioChemCoRe. RMSD/RMSF Analysis. 2018.