The Cold Denaturation of Proteins

Nada Nazzal

nada.nazzal.234@gmail.com

Supervisor: Judith Peters

jpeters@ill.fr

1. Introduction and Objectives

- Proteins are vital to all biological functions.
- Protein denaturation involves the breakdown of tertiary and secondary structures.
- Environmental changes (pH, ionic strength, temperature, pressure, solutes) can lead to denaturation.
- This project focuses on cold denaturation of proteins, using techniques like UV-VIS spectroscopy, DSC, FT-IR, densitometry, Circular Dichroism, and neutron scattering.

Objective: To clarify the cold denaturation process, which is usually reversible but not well understood—key for improving nutrient and pharmaceutical preservation.

2.Methods

Hen egg white lysozyme is used as the model protein under various solvent conditions ($H_2O, D_2O, H_2O + urea, D_2O + urea$).

- **UV/Visible Spectroscopy** is used for its potential of detecting secondary structure shifts during thermal transitions by monitoring absorbance changes as the protein is heated.
- **Densitometry and DSC** are used to study protein transitions quantitatively. Through these techniques a thermodynamic description of protein transition can be achieved.
  Hawley's equation: $\Delta G = \Delta G_o + \frac{\Delta\kappa'}{2}(P - P_o)^2 + \Delta\alpha'(T - T_o)(P - P_o) + \Delta V_o(P - P_o) - \Delta C_p[T(\ln(\frac{T}{T_o}) - 1) + T_o] - \Delta S_o(T - T_o)$.
  These techniques allow us to derive thermodynamic parameters from Hawley's equation and generate the protein's stability curve.

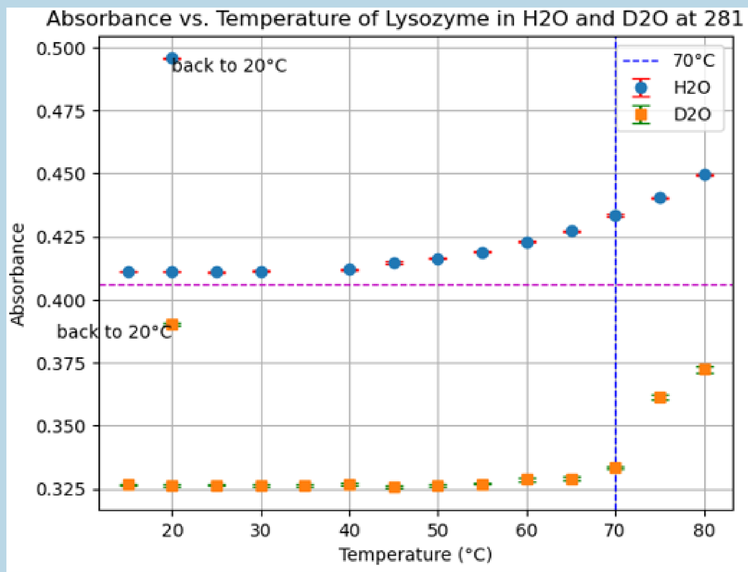
4.Conclusion

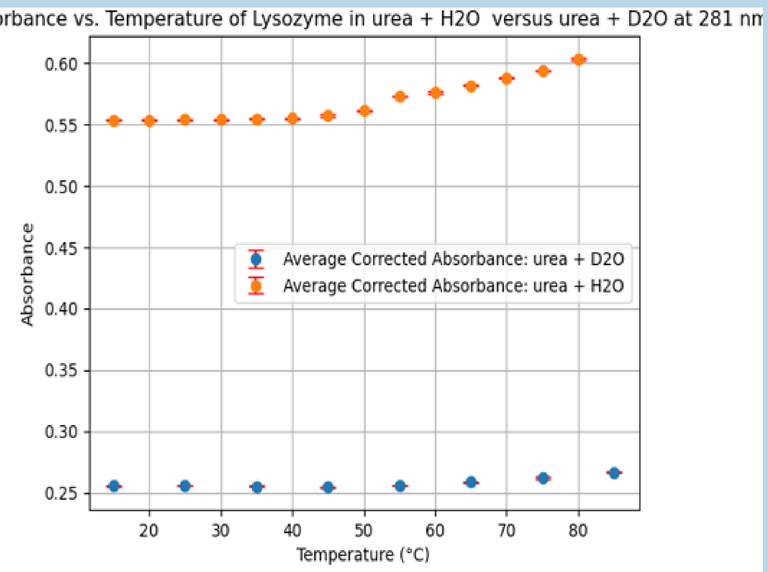
UV-Vis spectroscopy showed minimal protein dynamics in D_2O, indicating reduced activity. Preliminary results suggest lysozyme transitions are sensitive to environmental factors but need further validation. Densitometry and DSC provided thermodynamic parameters for Hawley's equation, allowing us to generate the stability curve.Despite some discrepancies with the predicted cold denaturation point, the ellipsoidal free energy landscape aligns well with both predictions and experimental data. The plot successfully predicts the hot denaturation point, and the maximum observed pressure is within a reasonable range.

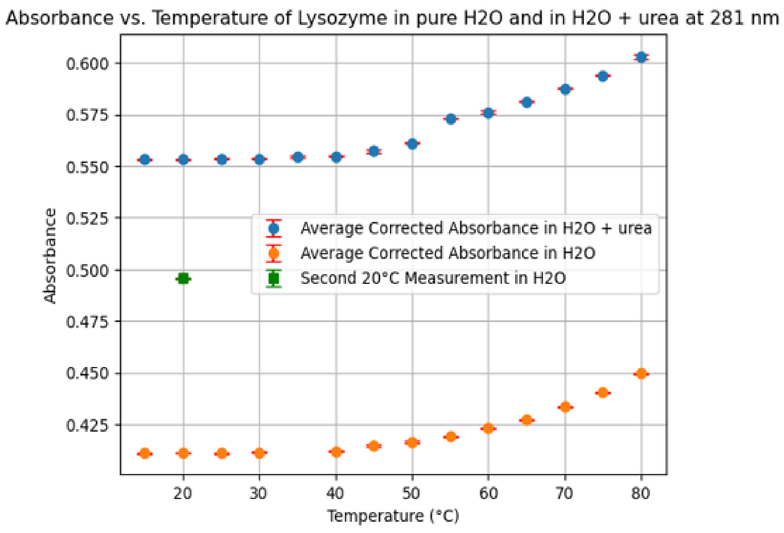
Thanks to SoftNano Graduate School for the internship and ESONN School for the opportunity to participate in this event, which broadened my knowledge in nanosciences.

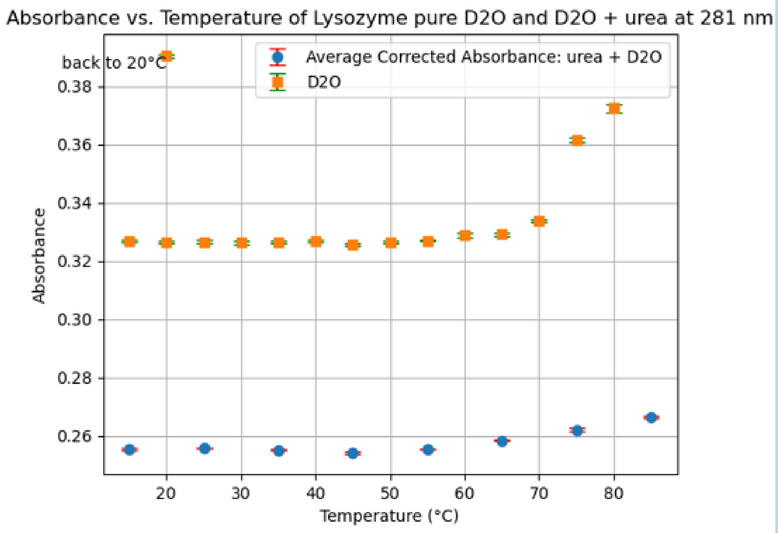
3.Results

A) UV results The profiles show a sigmoidal trend, consistent with previous findings. Lysozyme's absorbance differs between H_2O and D_2O , indicating that D_2O stabilizes the protein structure. Urea's destabilizing effect is evident from increased absorbance, though surprisingly, absorbance in D_2O was lower with urea than without.

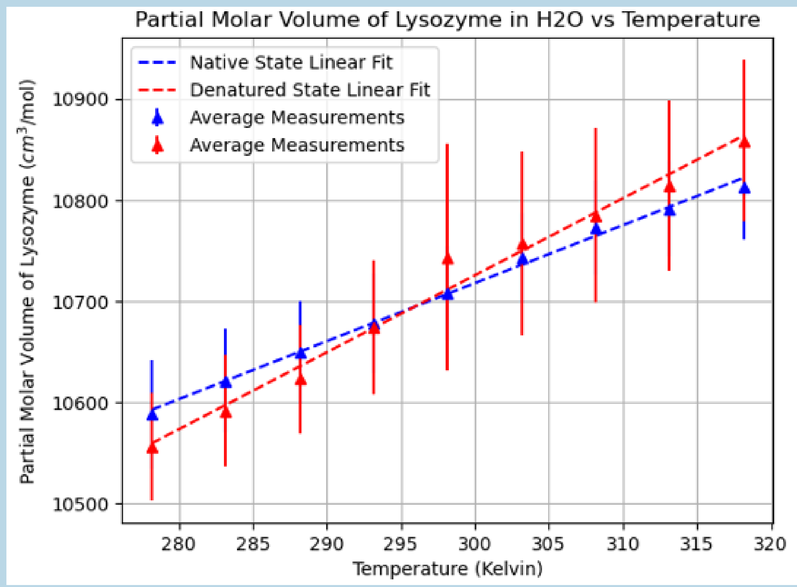


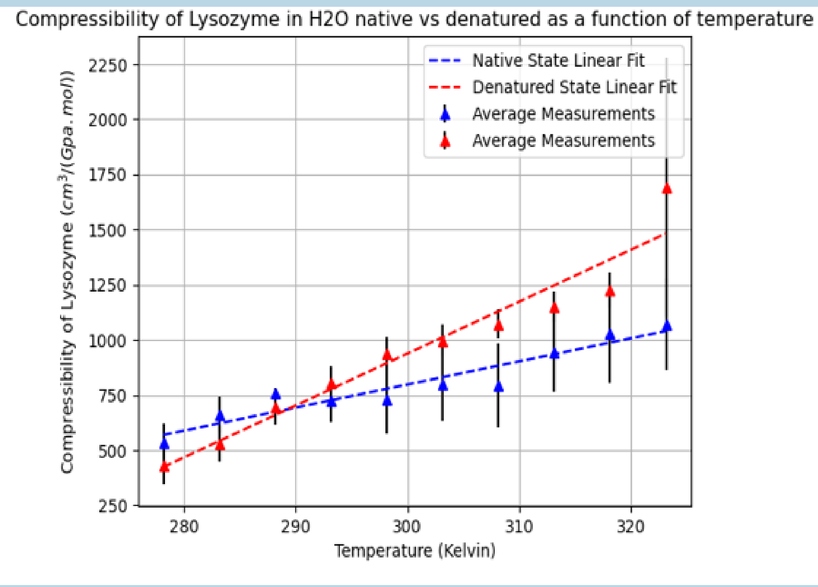




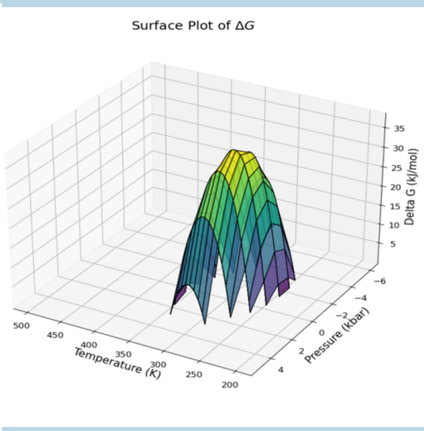


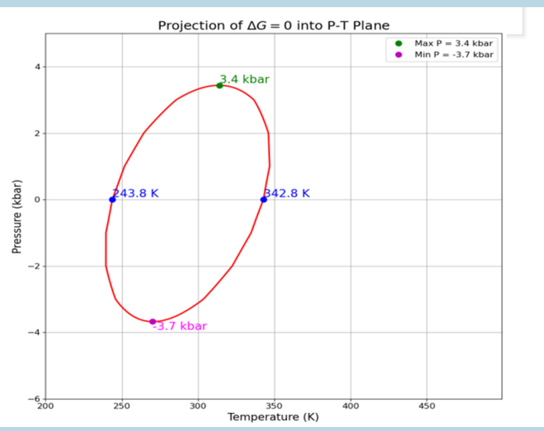
B) Densitometry and DSC: Densitometry provided temperature-dependent plots of partial molar volume ($\bar{V}$) and compressibility ($\bar{\kappa}$) for lysozyme. As expected, $\bar{V}$ and $\bar{\kappa}$ increase with temperature due to enhanced protein mobility. Typically, these values are higher in the denatured state than in the native state, but this difference was not observed at low temperatures.





Symbol	Value	Unit
ΔV_o	88.1332	cm^3/mol
$\Delta\kappa'$	699.3982	$cm^3/GPa \cdot mol$
$\Delta\alpha'$	1.8761	$cm^3/mol \cdot K$
ΔS_o	1.4474	$kJ/mol \cdot K$
ΔG_o	0	kJ/mol
ΔC_p	2.448	$kJ/mol \cdot K$





5.References

[1] Simon A Hawley. Reversible pressure-temperature denaturation of chymotrypsinogen. Biochemistry, 10(13):2436–2442, 1971.

[2] David Whitford. Proteins: structure and function. John Wiley & Sons, 2013.

[3] Python Software Foundation. Python: Programming language, 2024.

[4] Revanur Ravindra and Roland Winter. On the temperature–pressure free-energy landscape of proteins. ChemPhysChem, 4(4):359–365, 2003.