TRANSITIONAL WATER DYNAMICS ON THE TRP PROTEIN

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INTRODUCTION TO THE PROJECT
THE TRP PROTEIN (A MODEL PROTEIN)

• We are trying to study the water dynamics of the Trp protein at seven different sites in the protein.

• Trp is a small protein. Its only 20 amino acids in length.
THE RESEARCH

• We want to measure the water density (how many surrounding water molecules) at seven trp protein sites

• Also, we want to measure the water diffusivity rate at various temperatures

• This will show us how strongly water interacts at each site.
BIG IDEA #1

• Water is an integral component to protein function.
• Without water, a protein is not functional.
• For example, it is believed that water dynamics are necessary for a ligand to reach the active site of a protein.
BIG IDEA #2 (CONTRIBUTION TO SCIENTIFIC KNOWLEDGE)

- We are gathering experimental data on the water dynamics of trp to try to confirm that the computational results gathered by Kim et al. are reliable.

- Basically, we are trying to see if the data correlates between the experimental results and computational results.

- Implications: If the data does correlate, it helps validate the computational methods of water dynamics on protein.
EXPERIMENTAL METHODS

WATER DYNAMICS STUDY ON THE TRP MODEL PROTEIN
PREPARING SAMPLES

• We take our seven different samples and attach our spin-label called methyl methanesulfonothioate (MTSL) to the cysteine side chain.
  • Each protein sample has been mutated to only have one cysteine at the specific site
  • I.e. Y3C means that amino acid 3, tyrosine, has been replaced by cysteine.
  • Cysteine is essential to form a disulfide bond with our spin label MTSL
DO THE ALIQUOTS ACTUALLY HAVE PROTEIN?

- Run sample from each aliquot through the continuous-wave electron paramagnetic resonance (CW-EPR) to determine if it actually contains SL-protein.
- Two sharp peaks and one dull wide peak demonstrates that there is SL-protein present (vs 3 sharp peaks which means only MTSL).
- Most samples contained the most protein in the first collected aliquot.
determines structure...
ELECTRON SPIN ECHO ENVELOPE MODULATION (ESEEM)

• ESEEM is used to measure the water density at each peptide site
• Solvent is prepared with 30% glycerol and 70% D20
• Sample is frozen quickly with liquid nitrogen to ensure water is in a "glassy-state"
OVERHAUSER DYNAMIC NUCLEAR POLARIZATION (ODNP)

It is used to measure water diffusivity and therefore how strongly water interacts at each site.

CliRavinath Kausik and Songi Han
J. Am. Chem. Soc.; (Article), 2009, 131 (51), 18254-18256; DOI: 10.1021/ja9060849
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RESULTS AND ANALYSIS
Measuring the Dynamic Transition

Han Research Group UCSB
**T₂ is Suited to Probe the PDT**

Realize the same ~15 K temperature shift

**MD Simulation**

\( P = \frac{g_{\text{H}_2\text{O}}}{g_{\text{Protein}}} \)


**T₂ Experiment**

Han Research Group UCSB
Connecting PDT Behavior to Hydration Water Properties
The $T_2^*$ probes the PDT site specifically.

Protein fluctuations correlate to hydration water fluctuations.
PRELIMINARY CURRICULUM IDEAS
PROTEIN & WATER STRUCTURE AND FUNCTION: BEYOND THE QUATERNARY STRUCTURE

• Possibly make two separate units (one for chemistry and one for biology)

• Big idea: demonstrate that water plays an integral role in creating protein structure and protein function
  • Based on research conclusions that water density and diffusivity changed with protein motion.
CHEMISTRY—INTERMOLECULAR FORCES

• Students can look at the side chain of an amino acid to determine whether it is polar or nonpolar
  • Students would have to understand the electronegativity differences at each bond

• How would these side chains interact with water molecules?
  • Polar bonds and hydrogen bonds

• How many water molecules will bond here?
  • Water density at site
CHEMISTRY-- KINETICS

• Water helps proteins function and function more effectively

• Demos or labs:
  • Track the rate of the reaction that shows that water functions as a catalyst
  • Ex: magnesium + silver nitrate
• No specific example yet
• In research project, we saw at which temperature the protein-water dynamics became "active"
  • Enthalpy and heat capacity
BIOLOGY IDEAS

• Hydrophilic versus hydrophobic regions of a protein

• Role of water on protein structure
  • How water helps in folding (tertiary) & how water helps in finding other subunits (quaternary)

• Case study on aquaporins?
  • Integral membrane Protein that helps in influx and outflux of water
BIOLOGY IDEAS

• Water increases the surface area of protein to increase substrate binding & quaternary structure formation
• Water is an integral component to protein function.
• Without water, a protein is not functional.
WHAT I LEARNED (& LIKED) THIS SUMMER

• 1. I definitely belong in the classroom
• 2. I am still capable of learning
• 3. Sometimes learning science is HARD (and know I feel a little more empathy toward my students)
• 4. Don’t let Ryan cut the copper wire on your probe coil prematurely
• 5. … and I really hope I can create some great curriculum from all that I learned
THANK YOU!!!

RYAN BARNES

FRANK KINNAMAN

HAN GROUP
BIG IDEA #2 (CONTRIBUTION TO SCIENTIFIC KNOWLEDGE)

• Sang Beom Kim et al. already published a scientific report on the temperature-dependence of water dynamics on the trp protein titled: "Computational investigations of dynamical transitions in Trp-cage protein"

• Kim's research was based on computational biochemistry simulations.

• So why are we doing this research?
Hydration Water Motion Correlates to Protein Motion
Raw ESEEM Data
COLLECT ALIQUOTS OF SPIN-LABELED PROTEIN

• 1mg of protein + MTSL is mixed in 800uL of MidiQ H2O.
• For each spin-labeled (SL) protein type, we collect three 250uL aliquots of sample after it is passed through column chromatography.
EPR Spectra C: exp_ata emx60621 Trp Purification

Absorption Spectrum

file: auto_figures/Absorption_Q5C_1conc_measurement.pdf

Estimated Spin Concentration
Verifying Sample Composition

Unlabeled : Labeled

- 20:1
- 50:1
- 100:1

PDT temperature is unchanged

Han Research Group UCSB
After cutting and zero filling

Peaks, zoomed in to integration region
\[ T_1 = 2.042 \pm 0.025 \text{ s} \]

**DNP parameters:**