TRANSITIONAL WATER DYNAMICS ON THE TRP PROTEIN

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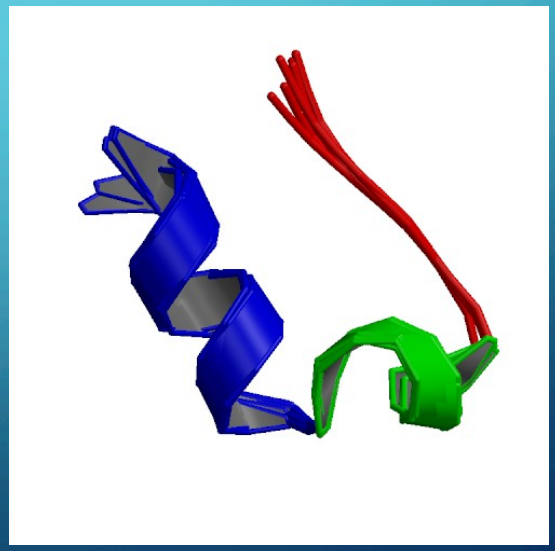
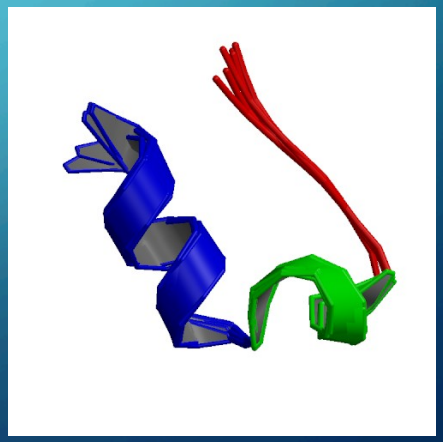


INTRODUCTION TO THE PROJECT

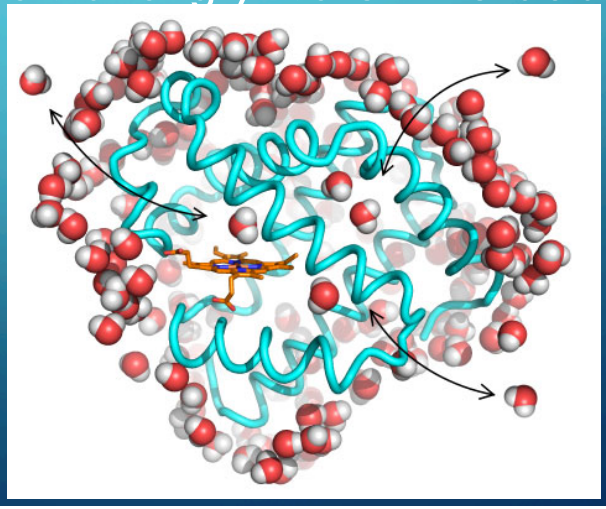
THE TRP PROTEIN (A MODEL PROTEIN)

- 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.



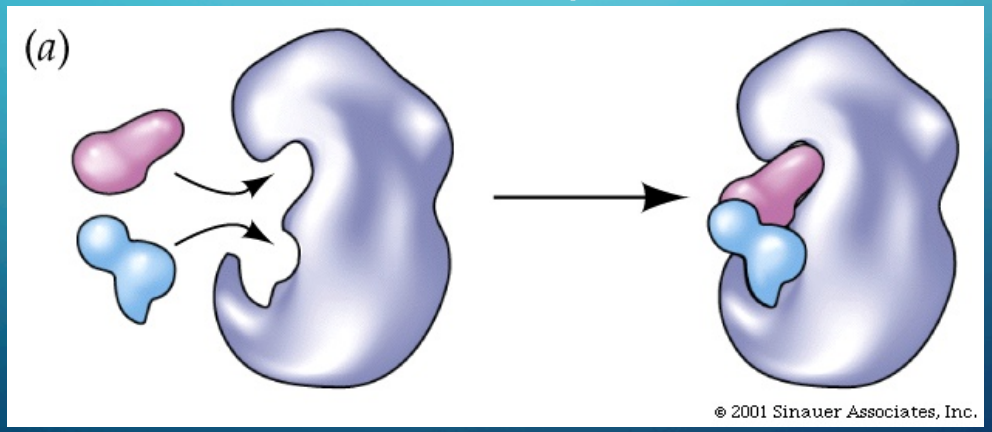
THE RESEARCH

- 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)

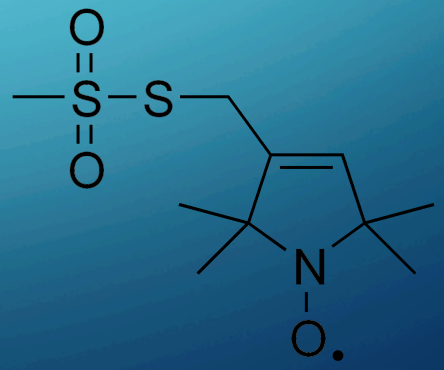
- We are gathering *experimental* data on the water dynamics of trp to try to confirm that the computational results gathered by Kim et al. are reliable.
- Basically, we are trying to see if the data correlates between the experimental results and computational results.
- Implications: If the data does correlate, it helps validate the computional methods of water dynamics on protein.

EXPERIMENTAL METHODS

WATER DYNAMICS STUDY ON THE TRP MODEL PROTEIN

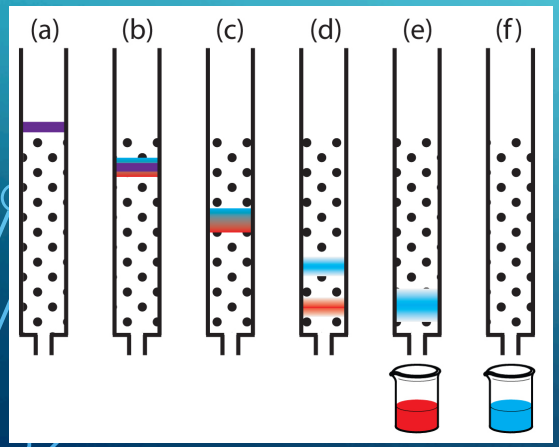
PREPARING SAMPLES

- 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.

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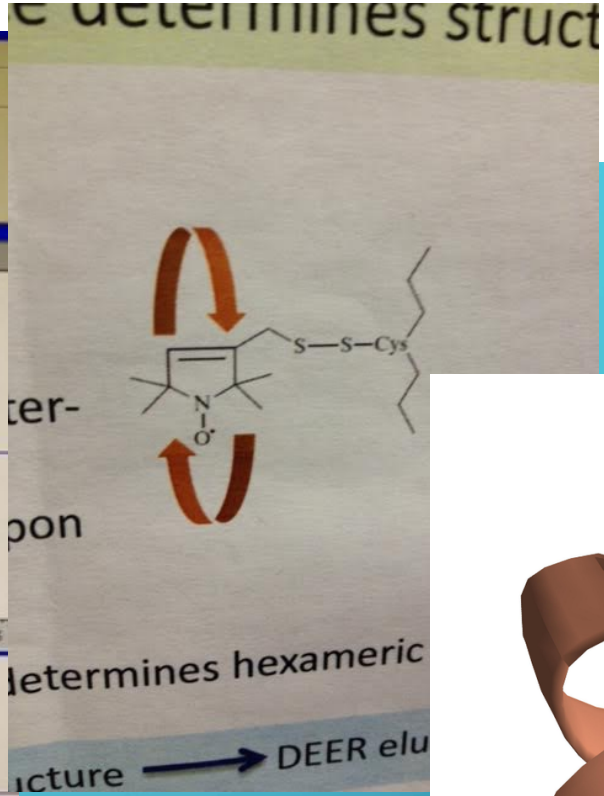
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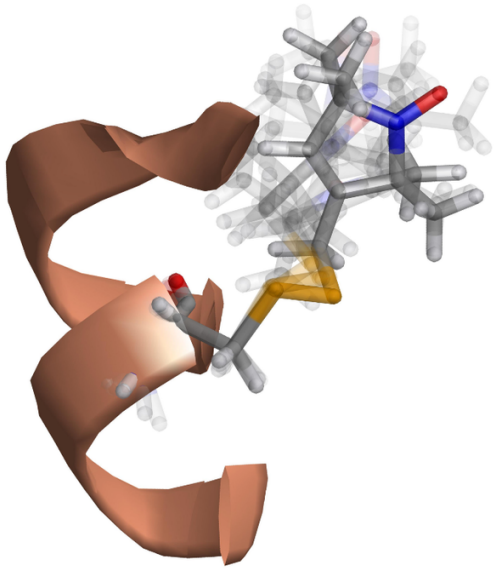
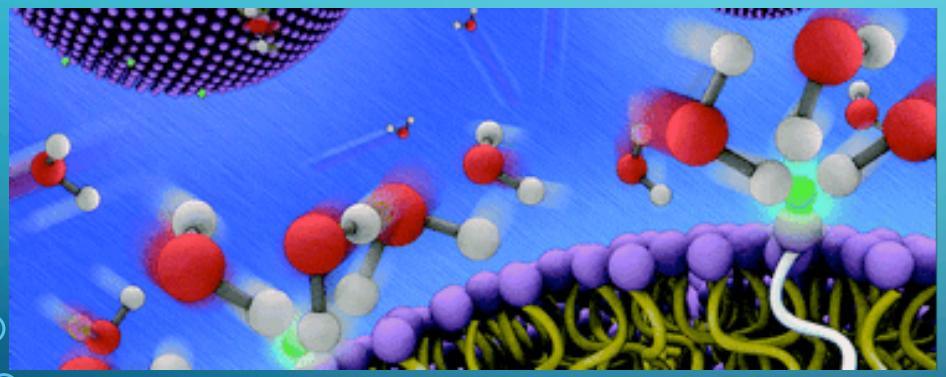


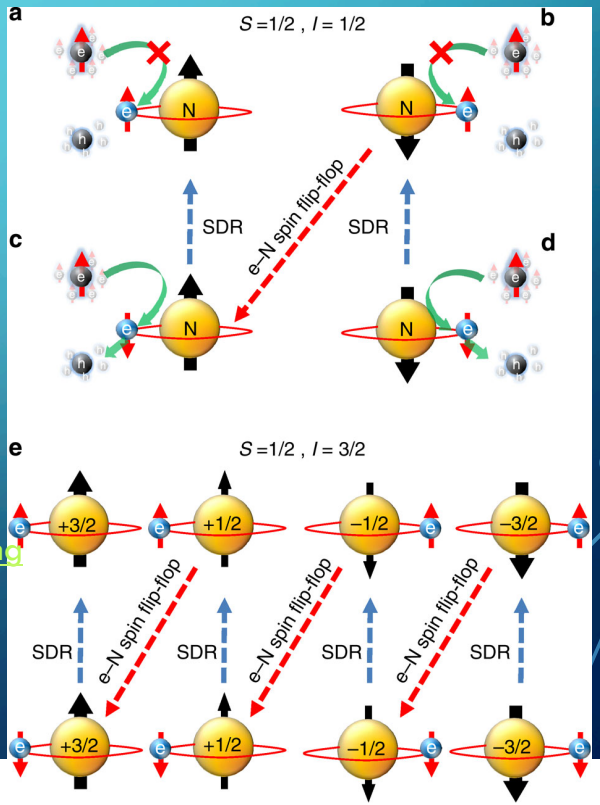
ELECTRON SPIN ECHO ENVELOPE MODULATION (ESEEM)

- 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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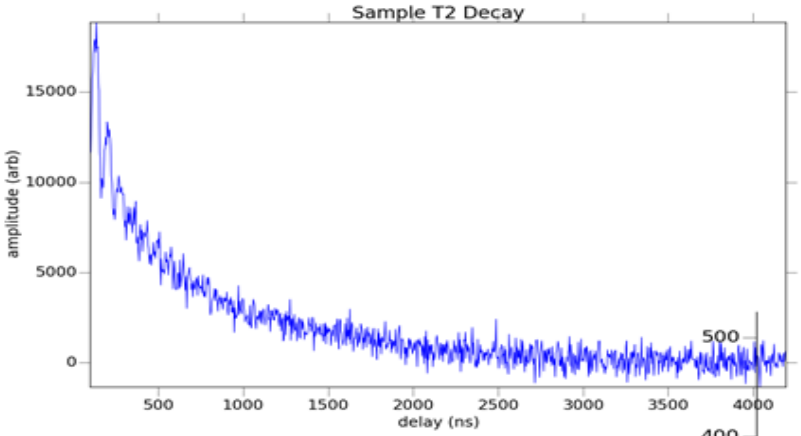
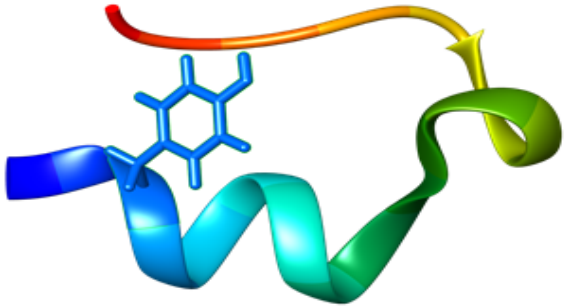
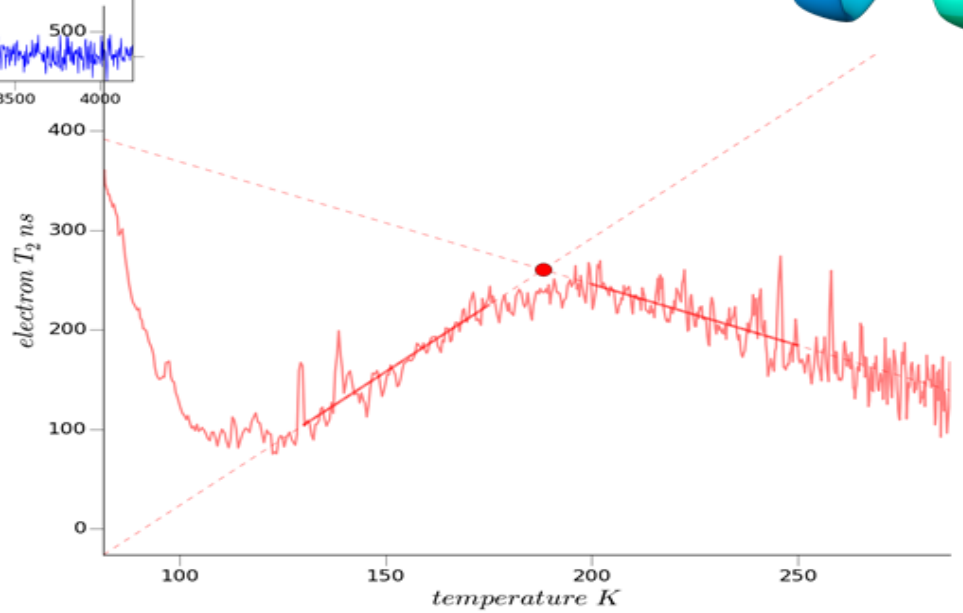
- X.J. Wang,
- I.A. Buyanova,
- L. Geelhaar,
- H. Riechert,
- A.J. Ptak
- C.W. Tu
- W.M. Chen

RESULTS AND ANALYSIS

Measuring the Dynamic Transition



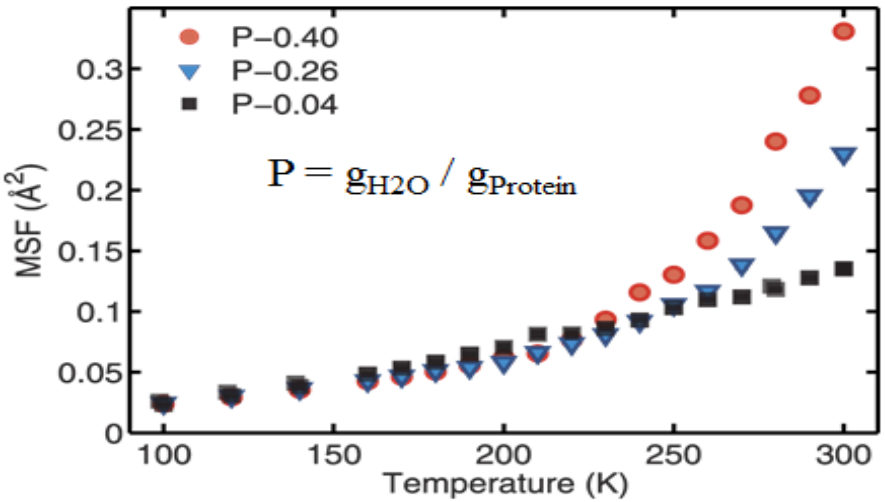




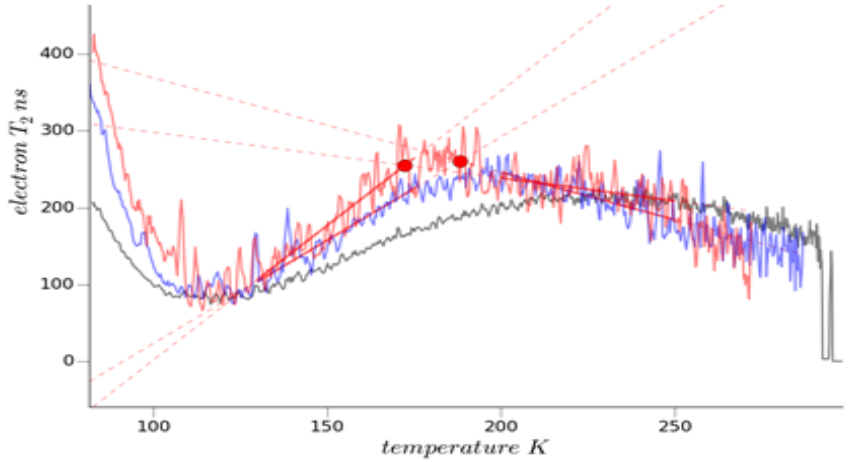
T_2 is Suited to Probe the PDT

Realize the same ~15 K temperature shift

MD Simulation



T_2 Experiment



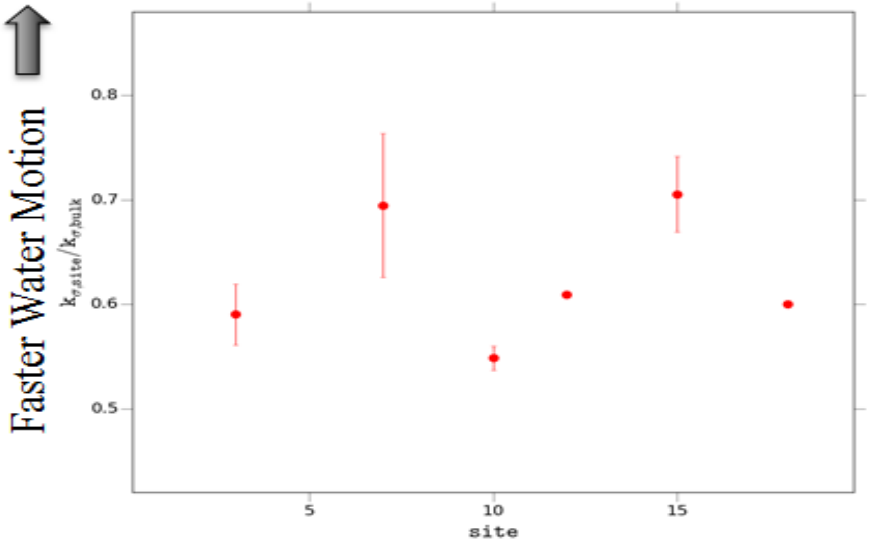
Kim, S. B., Gupta, D. R. & Debenedetti, P. G. Scientific Reports 6, 25612 (2016).

Connecting PDT Behavior to Hydration Water Properties

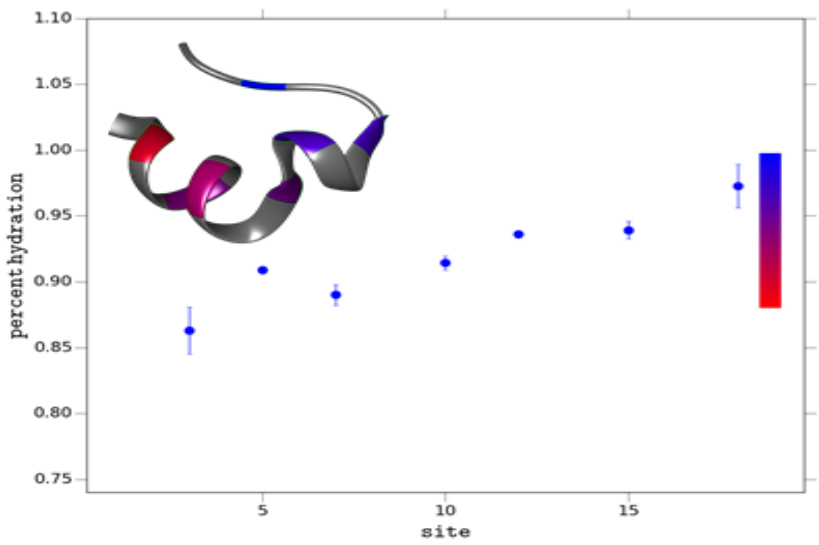
Overhauser DNP

ESEEM

Hydration Water Motion

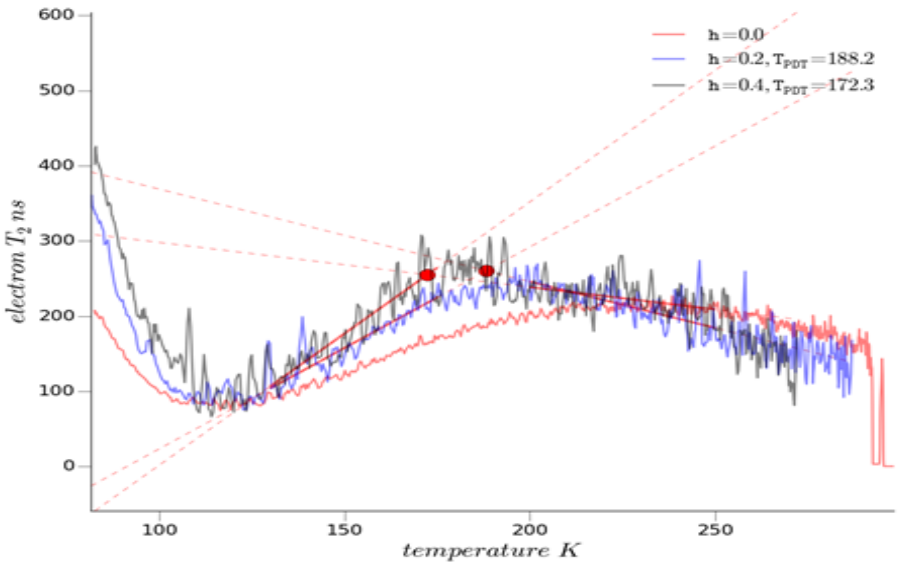


Hydration Water Density

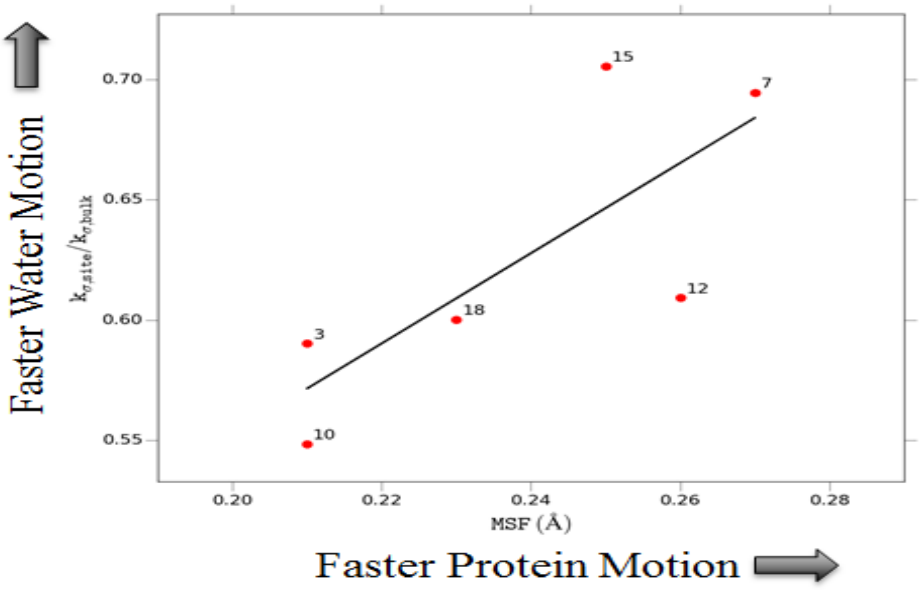


Conclusion

The T_2^e probes the PDT site specifically



Protein fluctuations correlate to hydration water fluctuations



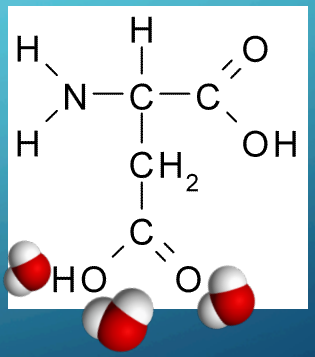
PRELIMINARY CURRICULUM IDEAS

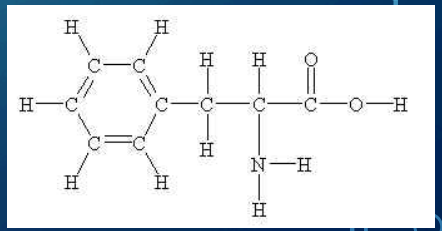
PROTEIN & WATER STRUCTURE AND FUNCTION: BEYOND THE QUATERNARY STRUCTURE

- 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



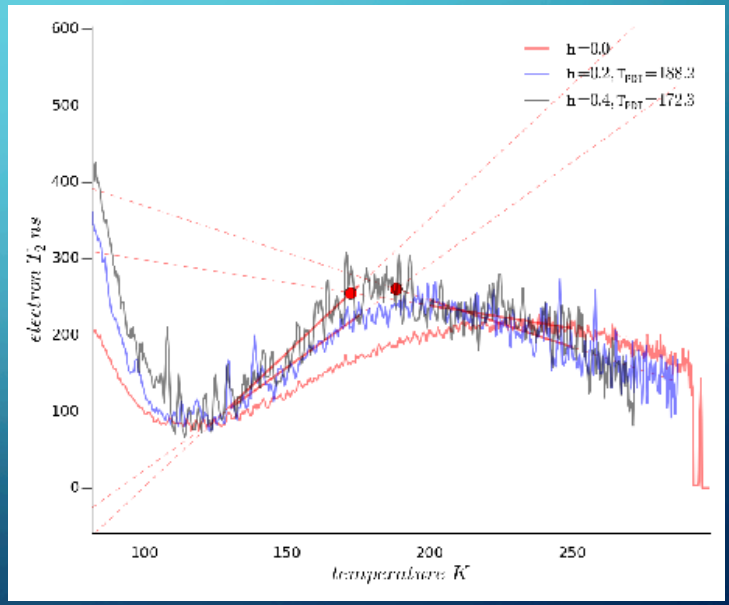


CHEMISTRY-- KINETICS

- 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

CHEMISTRY-- THERMODYNAMICS

- No specific example yet
- In research project, we saw at which temperature the protein-water dynamics became "active"
  - Enthalpy and heat capacity



BIOLOGY IDEAS

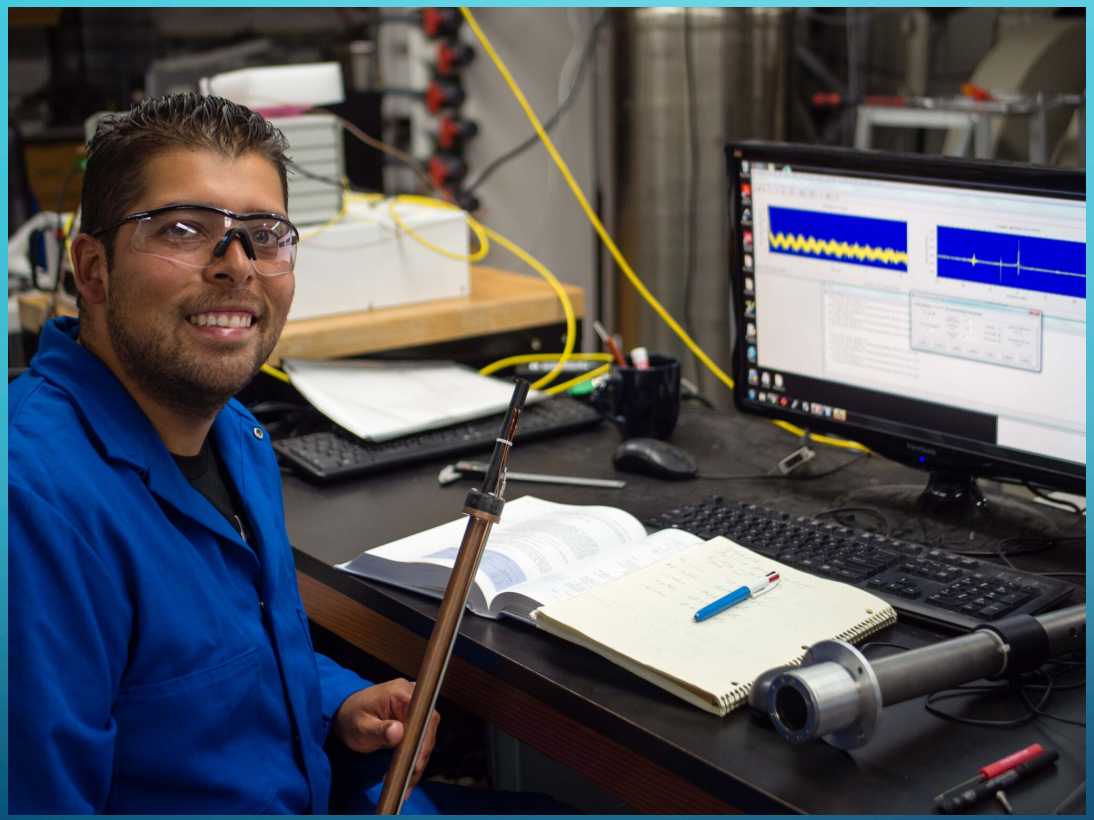
- Hydrophilic versus hydrophobic regions of a protein
- Role of water on protein structure
  - How water helps in folding (tertiary) & how water helps in finding other subunits (quaternary)
- Case study on aquaporins?
  - Integral membrane Protein that helps in influx and outflux of water

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

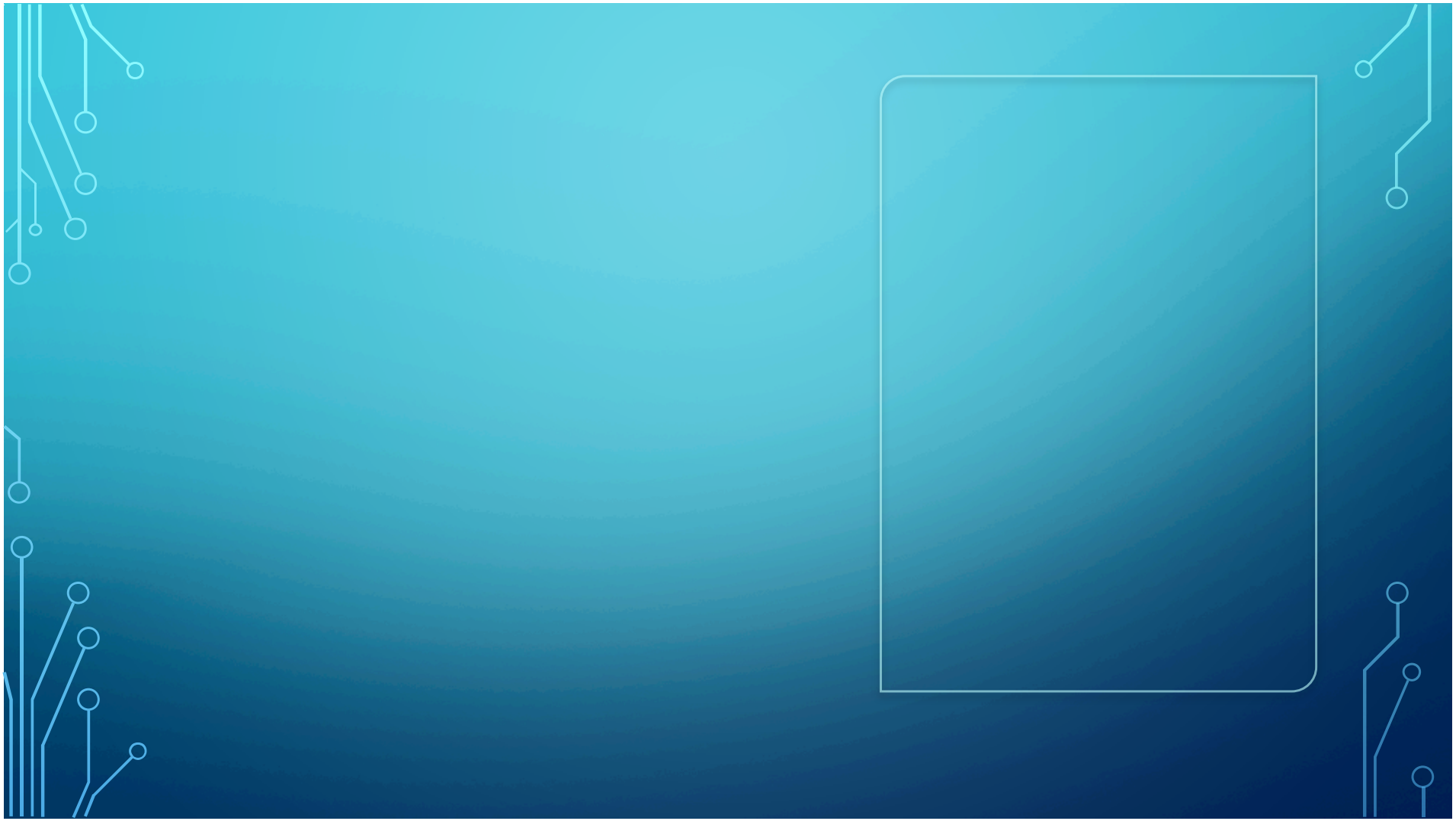


THANK YOU!!!

RYAN BARNES

FRANK KINNAMAN

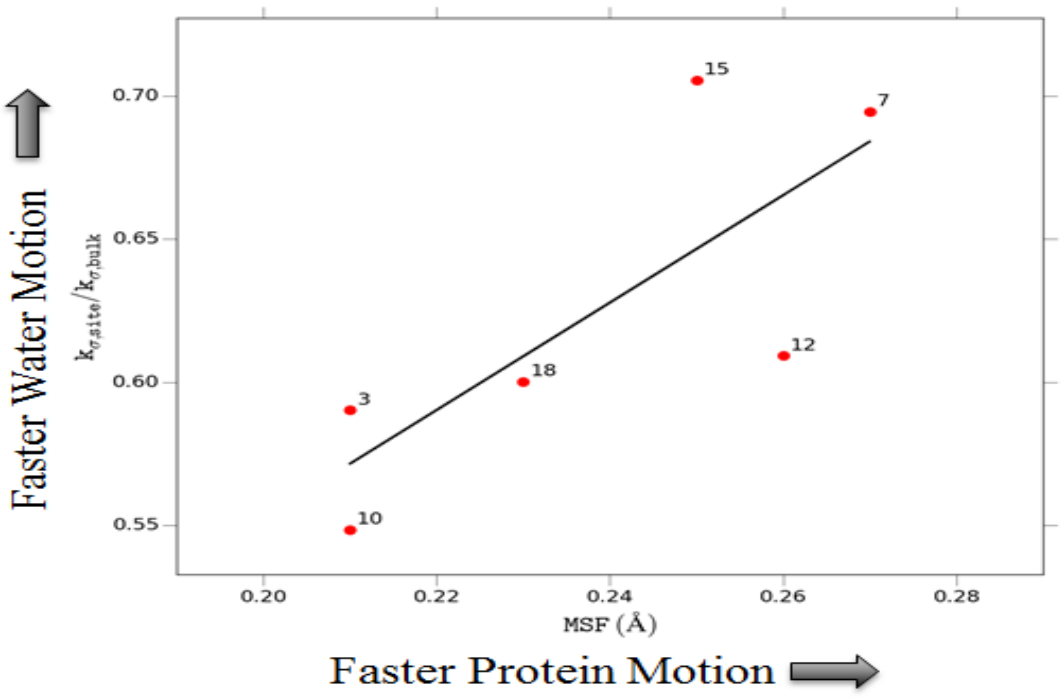
HAN GROUP



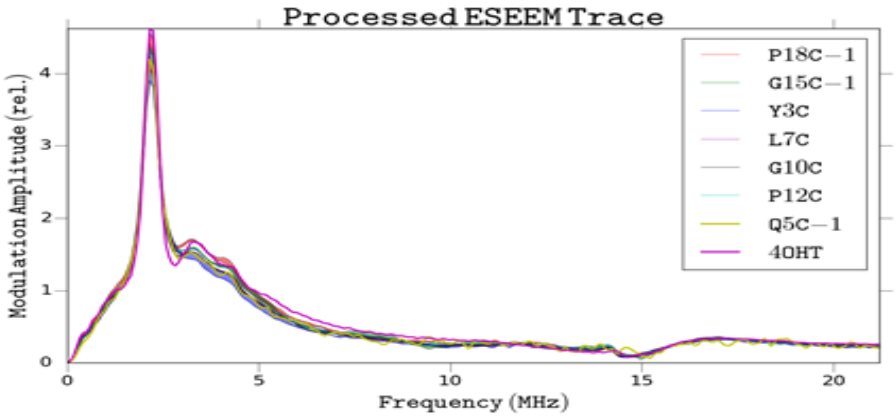
BIG IDEA #2 (CONTRIBUTION TO SCIENTIFIC KNOWLEDGE)

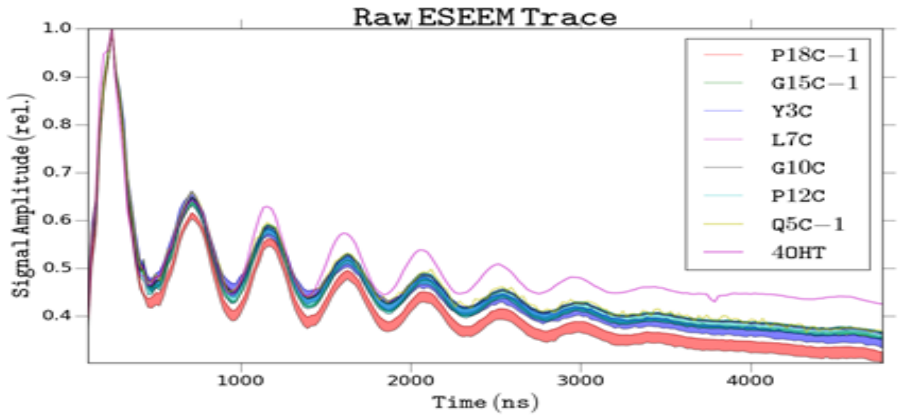
- Sang Beom Kim *et al.* already published a scientific report on the temperature-dependence of water dynamics on the trp protein titled: "Computational investigations of dynamical transitions in Trp-cage protein"
- Kim's research was based on computational biochemistry simulations.
- So why are we doing this research?

Hydration Water Motion Correlates to Protein Motion



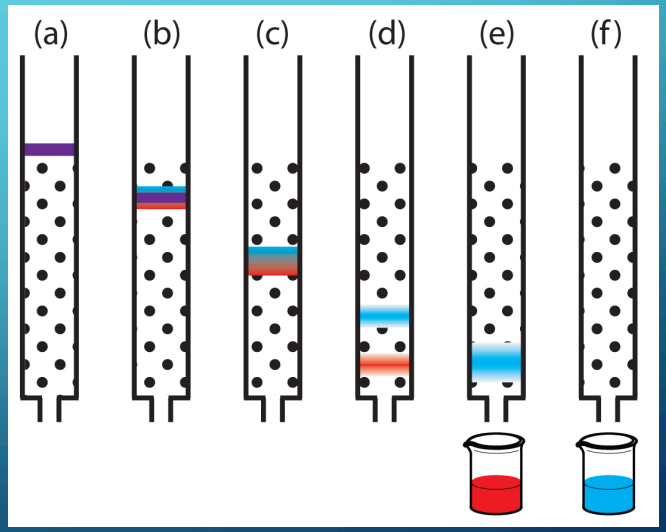
Raw ESEEM Data



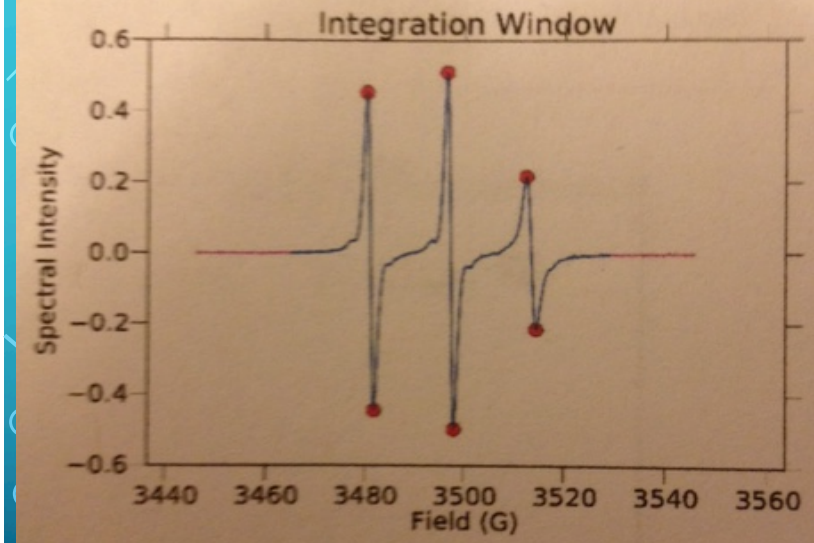


COLLECT ALIQUOTS OF SPIN-LABELED PROTEIN

- 1mg of protein + MTSL is mixed in 800uL of MidiQ H20.
- For each spin-labeled (SL) protein type, we collect three 250uL aliquots of sample after it is passed through column chromatography.



EPR Spectra C:$\exp_d ata_e mx60621_T rpPu$



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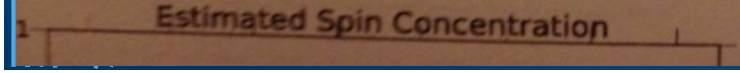
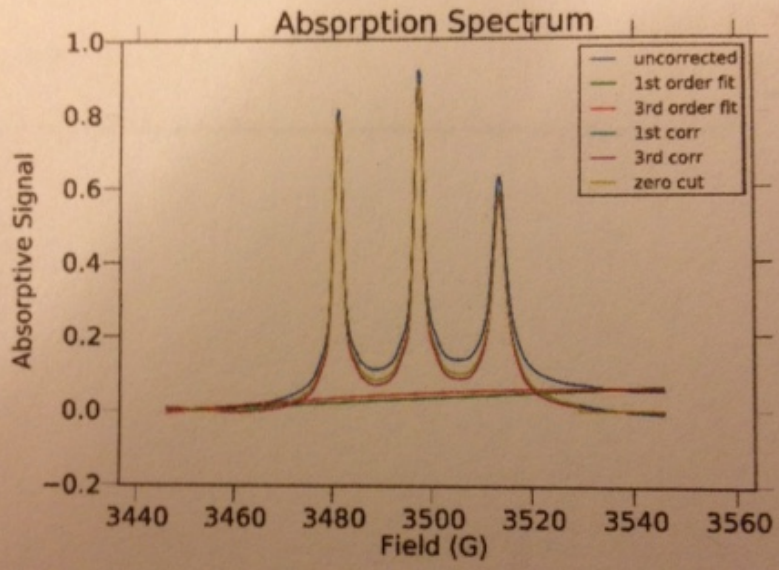


R. Barnes Notebook

$urification¶18C - 1(concmeasurement)$:



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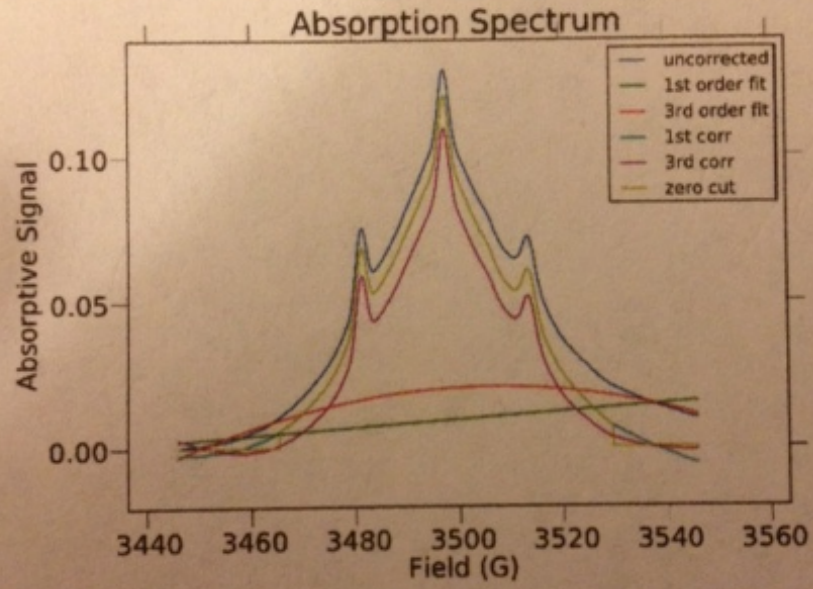

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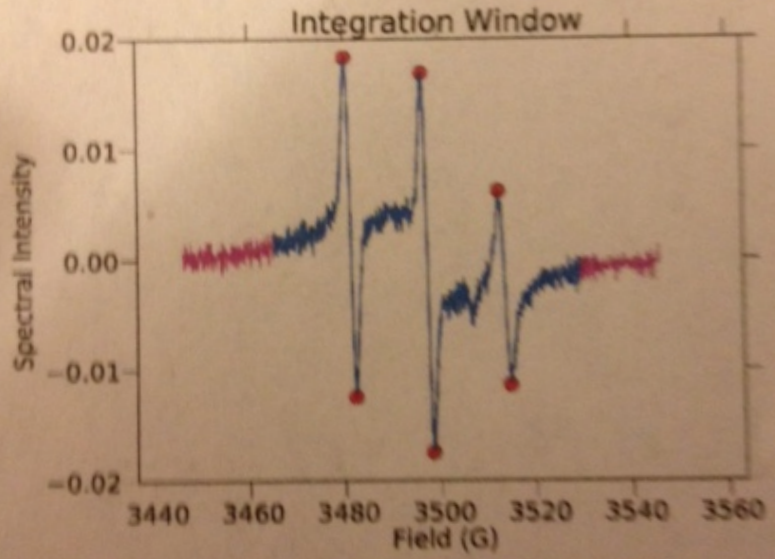
EPR Spectra C: $\exp_d ata_e mx60621_T rpPuri$



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Estimated Spin Concentration

Verifying Sample Composition

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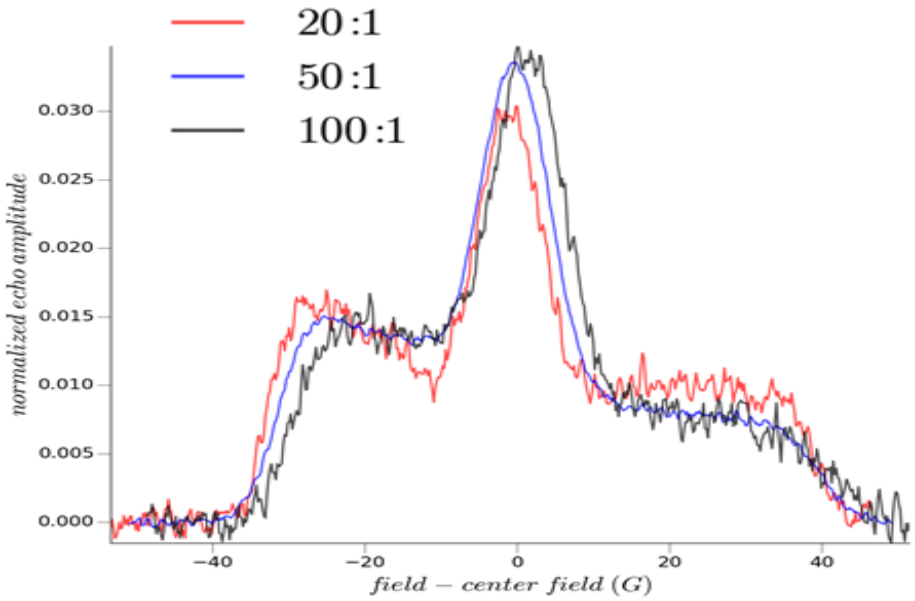
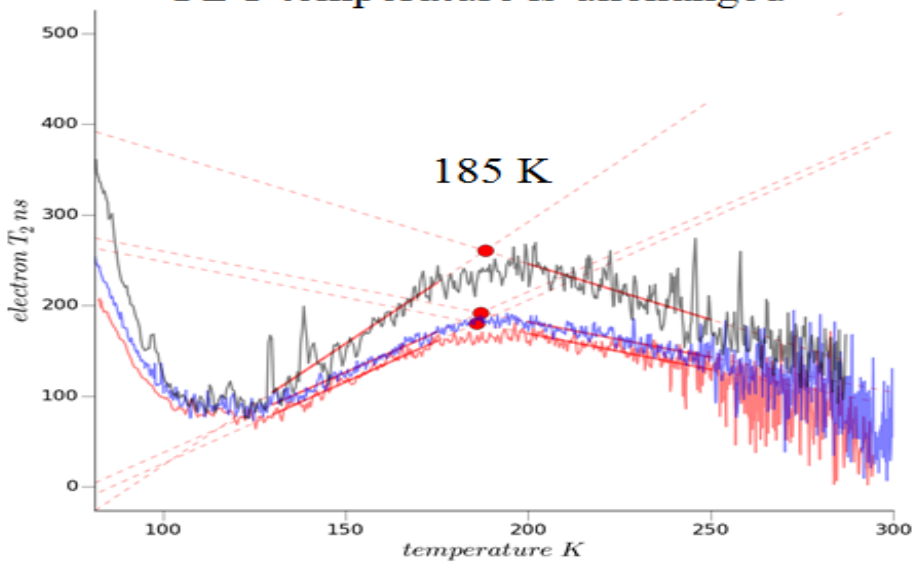
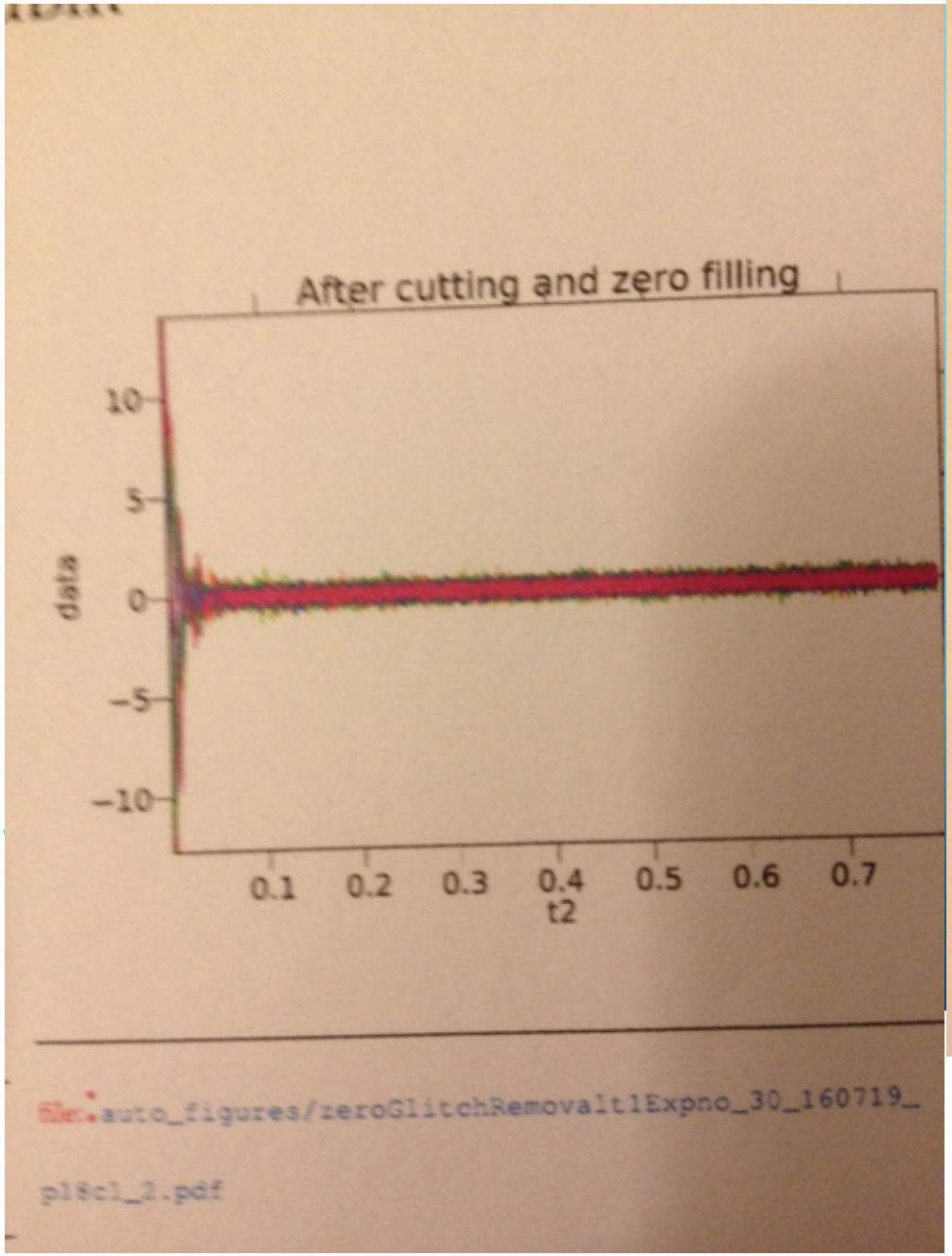


PDT temperature is unchanged

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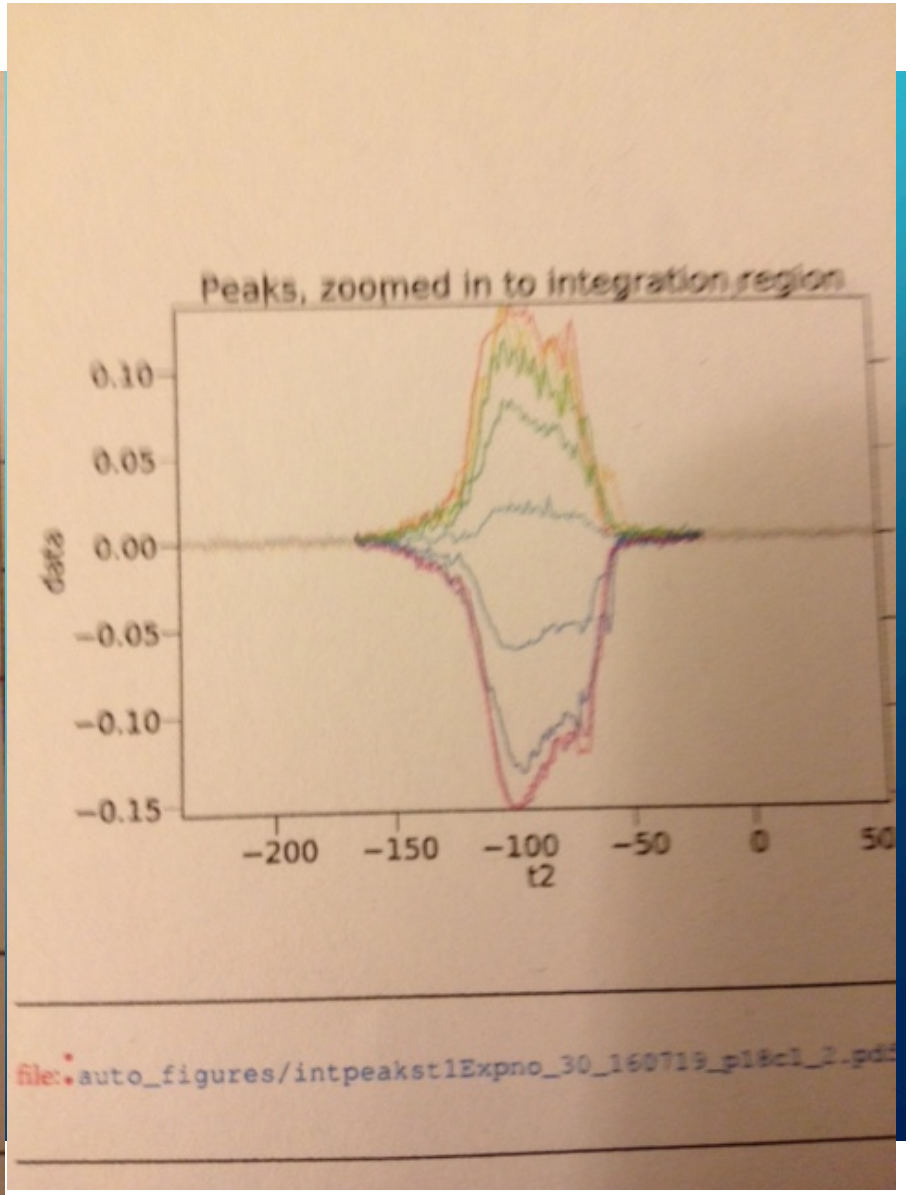
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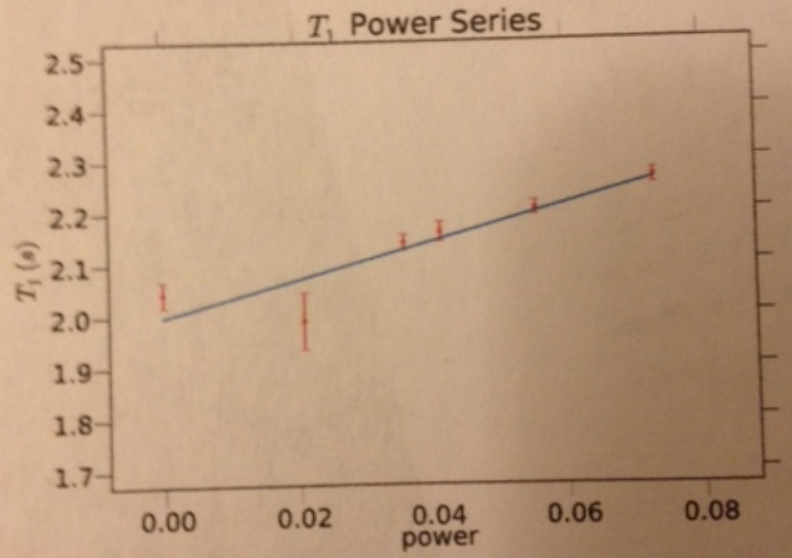

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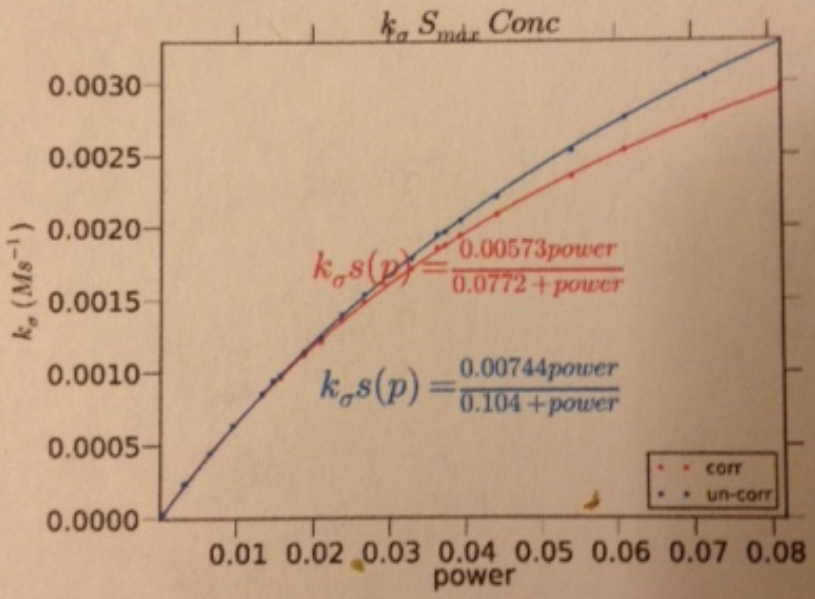
DNP parameters:



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