

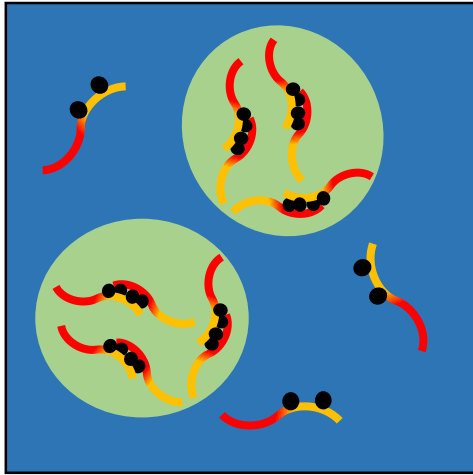


# Solvation in Biomolecular Condensates – THz Spectroscopy and the Role of TELBE

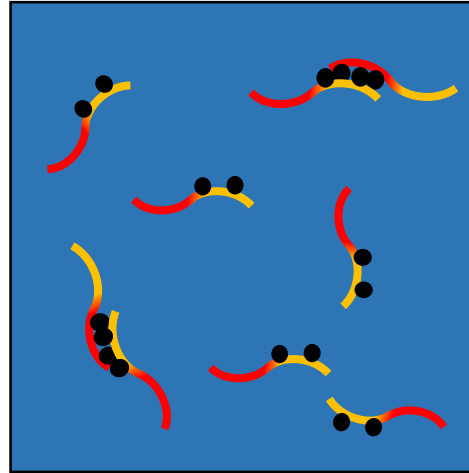
Ellen Adams

June 2<sup>nd</sup>, 2022, Helmholtz Zentrum Dresden Rossendorf

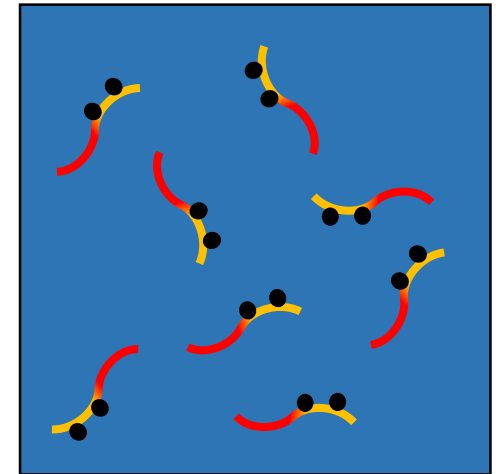
# Biomolecular Condensates



Membrane-less  
Liquid-like droplets

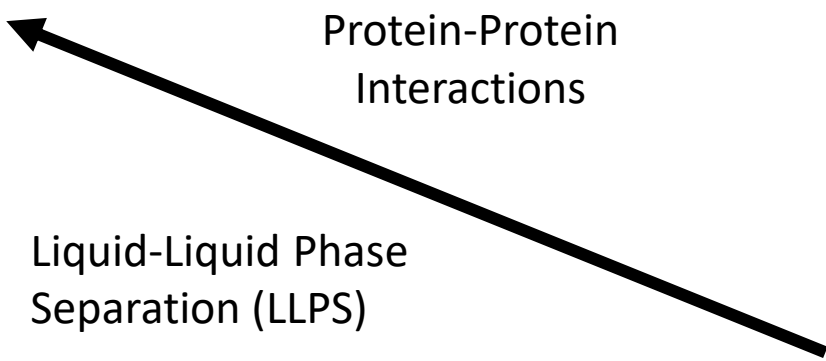


Protein-Protein  
Interactions

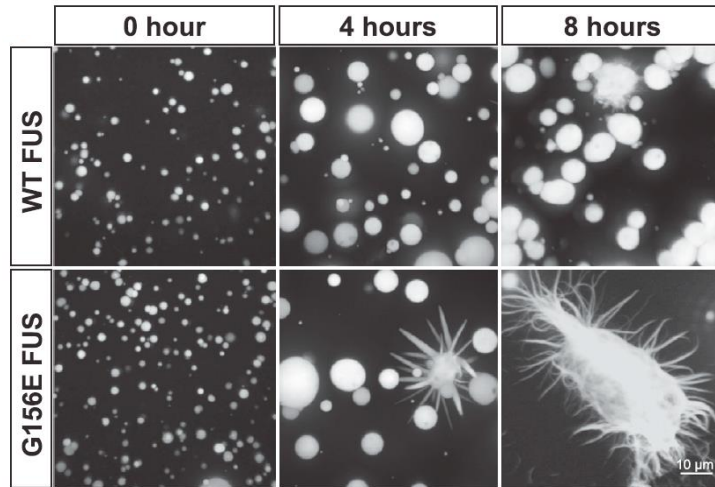


Intrinsically  
disordered proteins

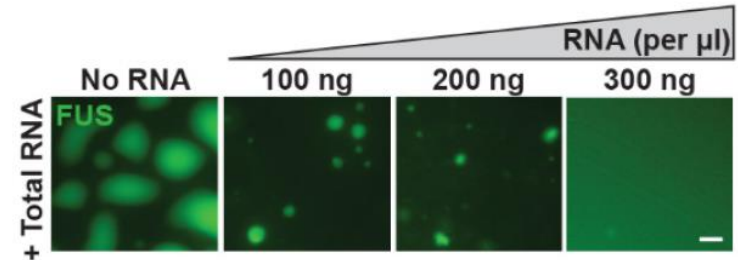
Liquid-Liquid Phase  
Separation (LLPS)



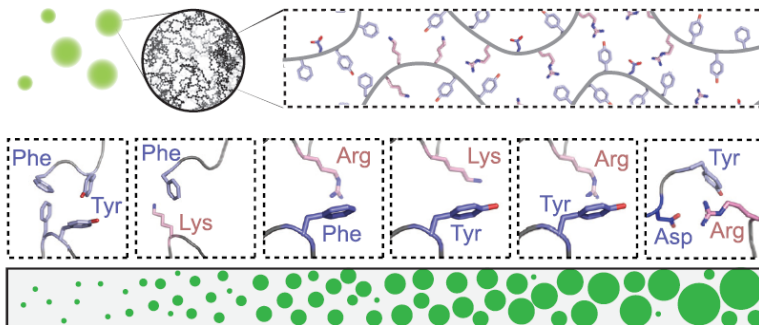
# LLPS – Neurotoxic Fibrils



Gene mutations induce aggregation



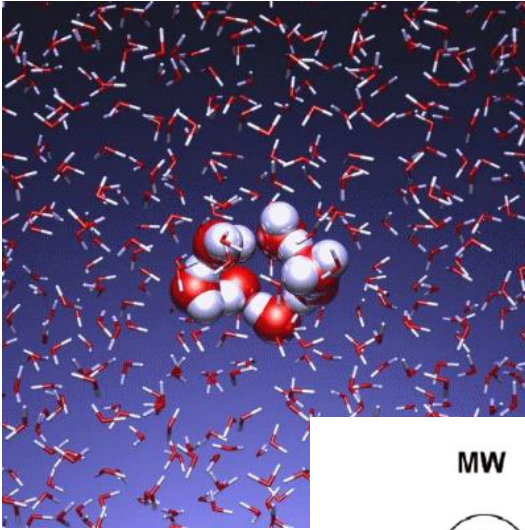
Presence of co-solutes can inhibit LLPS



What is the role of solvent in LLPS?

Cation- $\pi$  interactions are molecular driving force

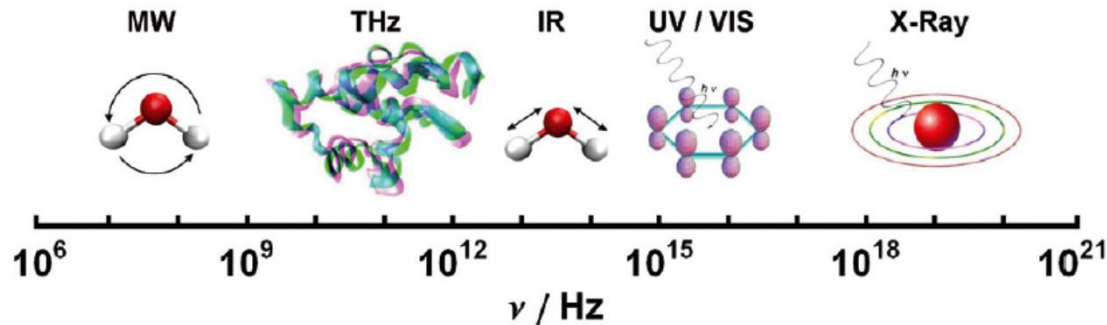
# TeraHertz (THz) Spectroscopy of Aqueous Solutions



Animation  
provided by  
Matthias  
Heyden

Water is a unique liquid

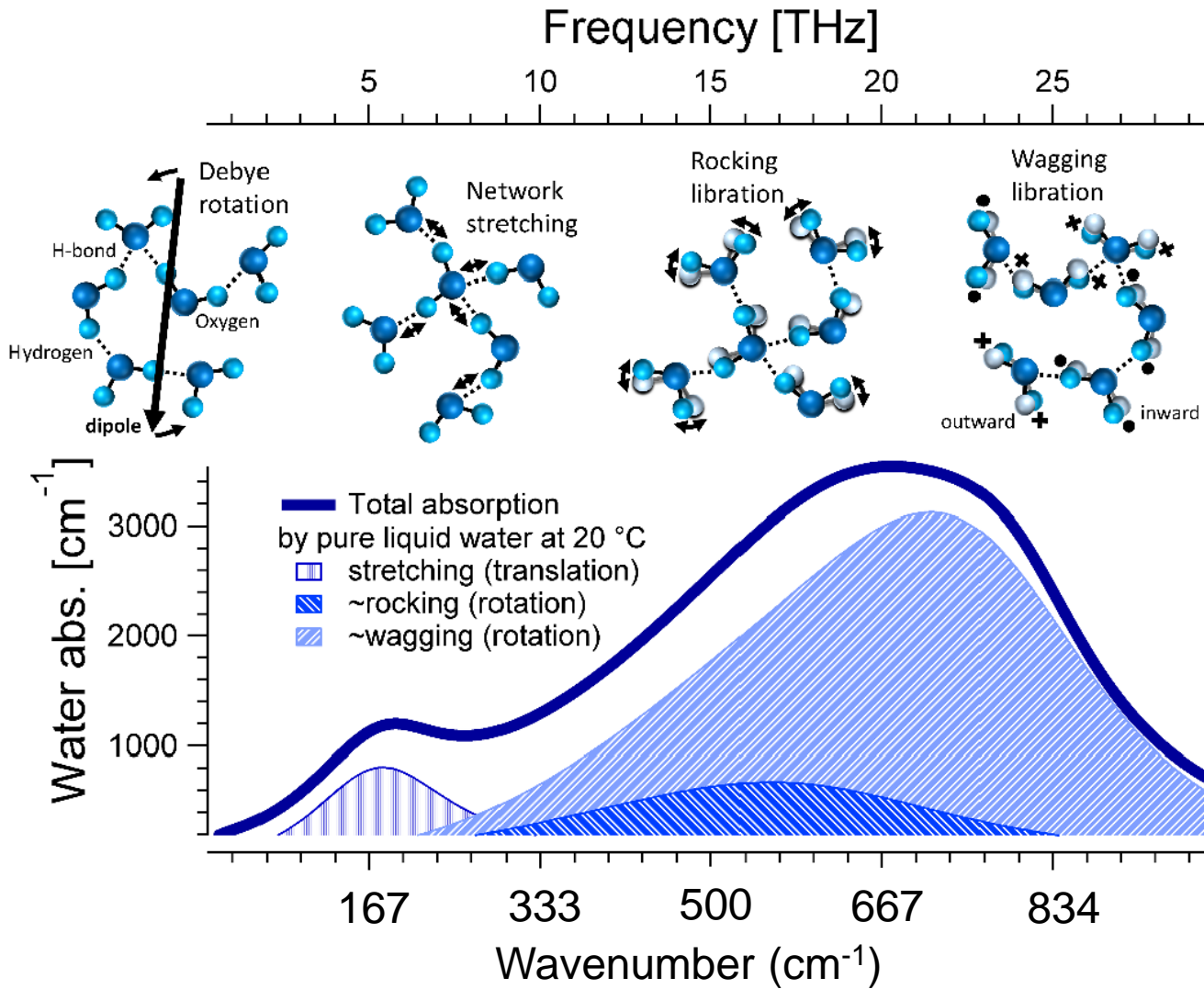
- Extended hydrogen bonding network
- Picosecond dynamics



THz spectroscopy directly probes sub-ps dynamics of extended hydrogen bonding network

- Intermolecular vibrational and rotational motions: hydration modes

# Water THz Absorption Spectra



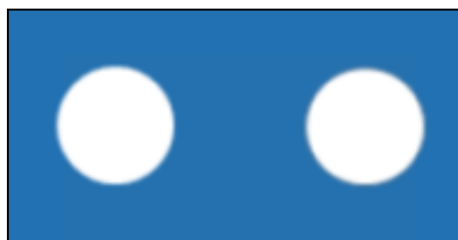
Novelli, et al. *Materials*, 2020, 13 (6), 1311\_1-1331\_15

# Spectral Signature of Hydration Water

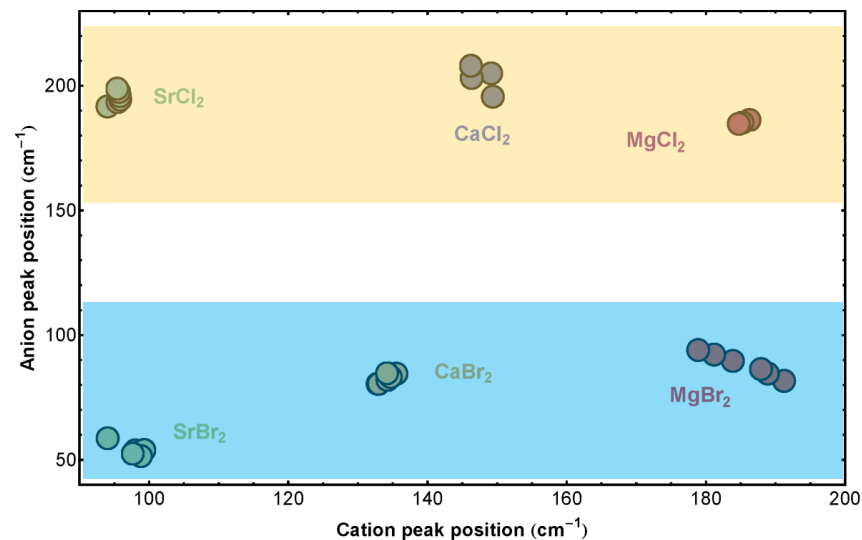
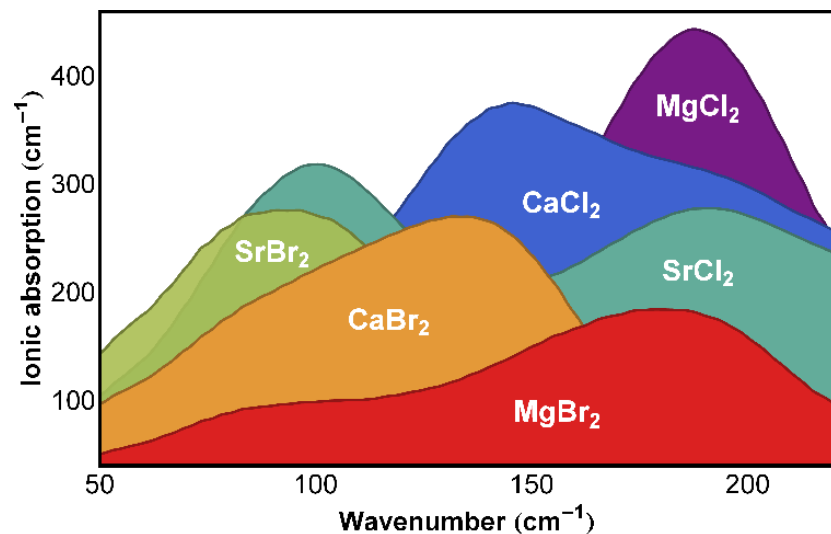
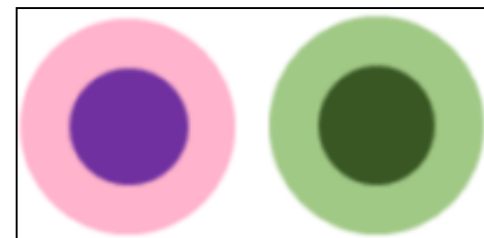
Absorption Solution



Absorption Bulk Water



Absorption Hydration Water



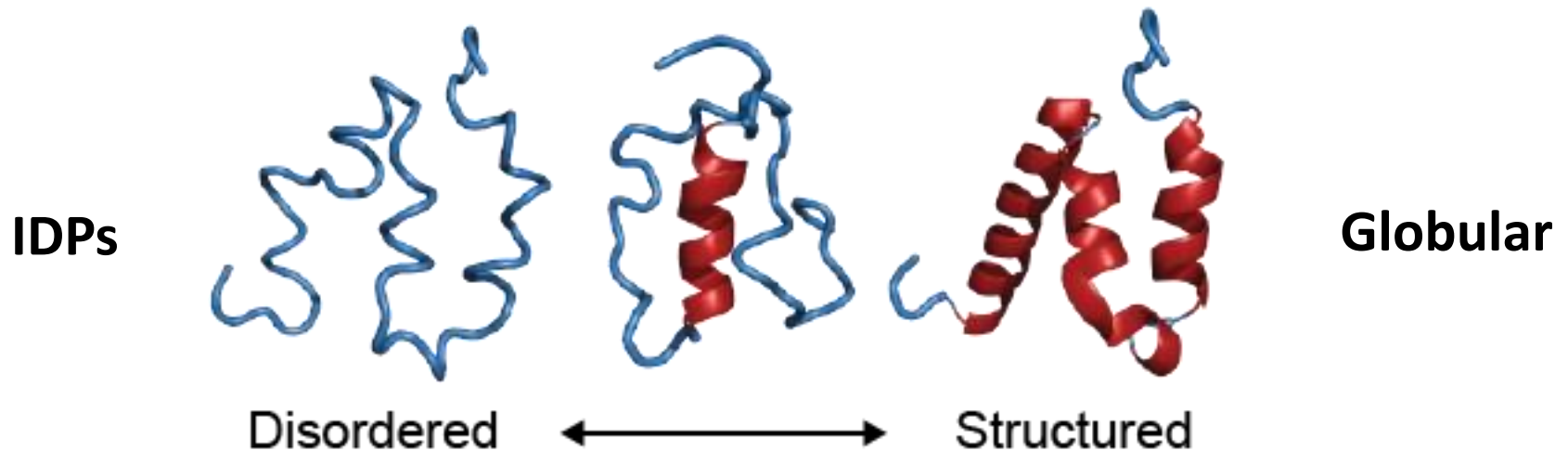
Clearly separable hydration peaks for specific solutes

Funkner, et al. *Materials*, **2012**, 134, 1030; Schwaab, et al, *Angew. Chem. Int. Ed.* **2019**, 58, 3000-3013



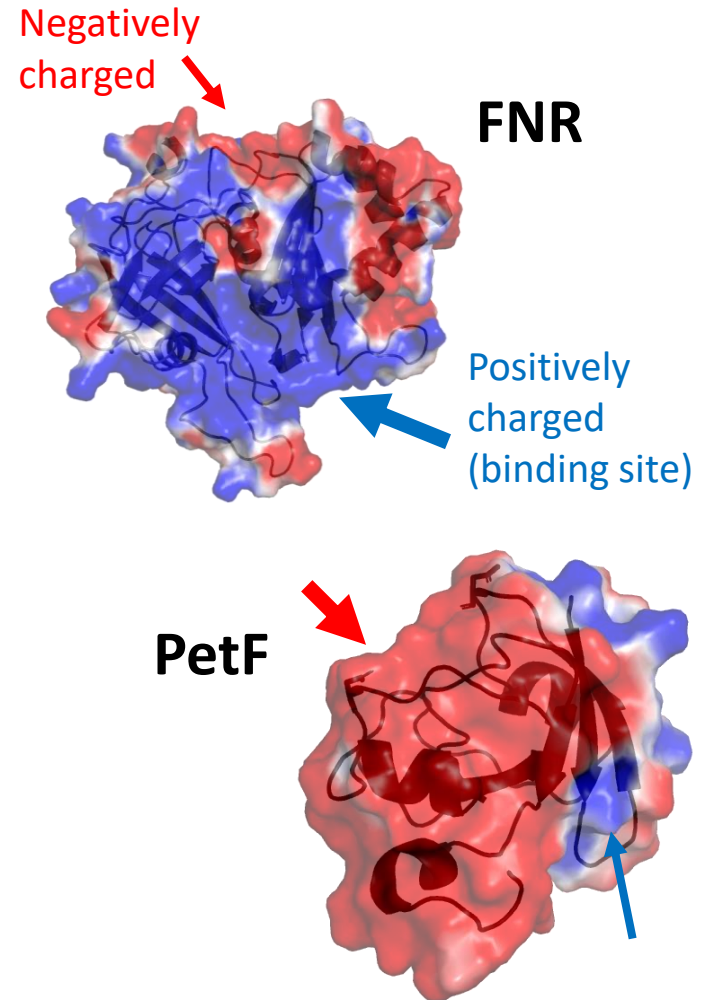
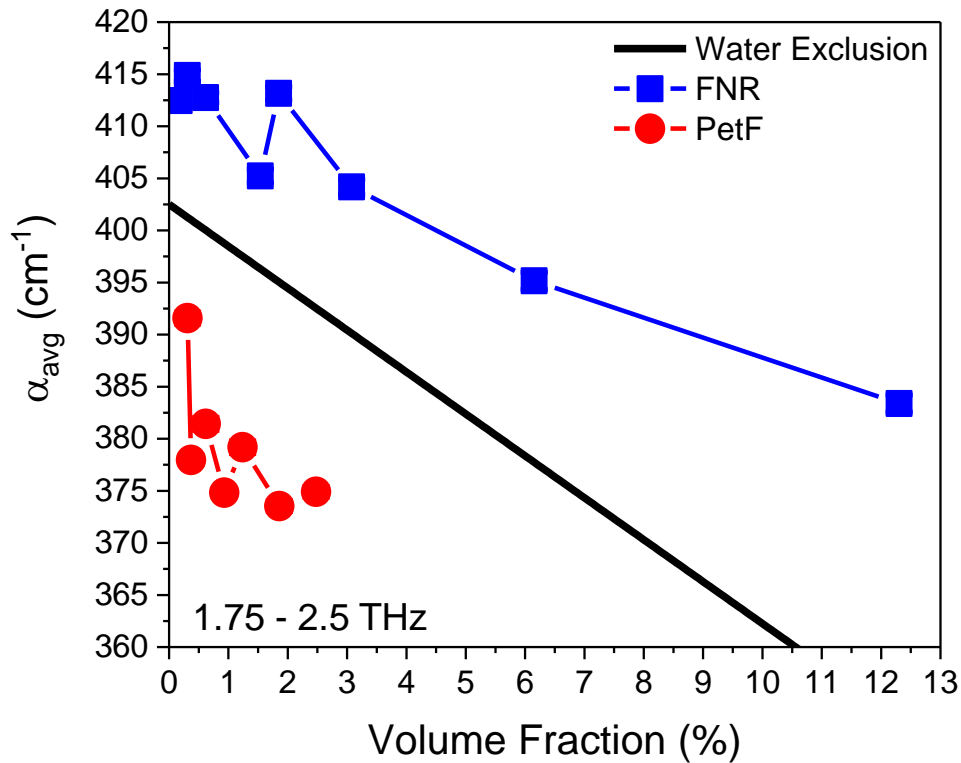
# Spectral Signature of Proteins

## The protein disorder continuum



## Complex Surfaces & Complex Shapes

# THz Absorption Ferredoxins

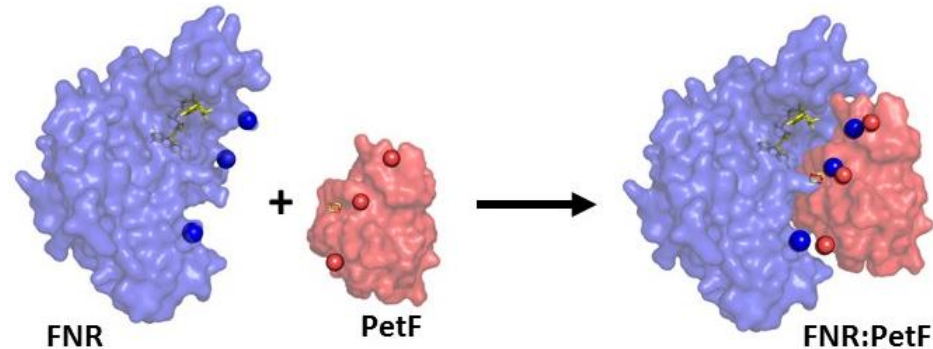
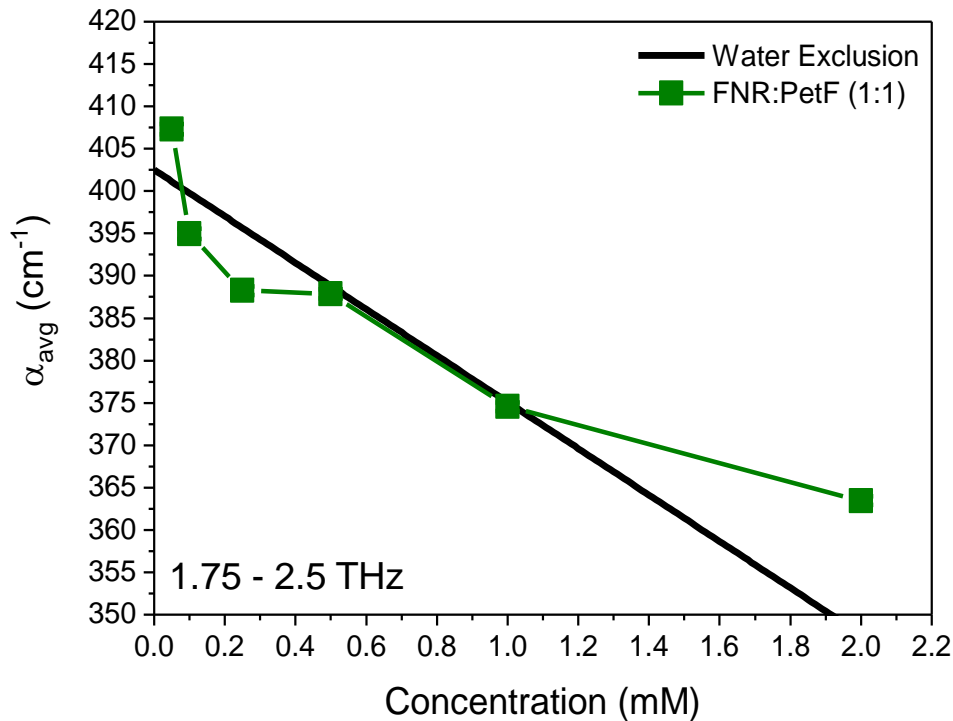


Diakonova, et al. *Phys. Biol.* **2016**, 13, 056004

Adams, et al. *PCCP*, **2020**, 22, 7451



# Transient Complex FNR:PetF



THz response correlates to the surface electrostatic potential of ferredoxin surface

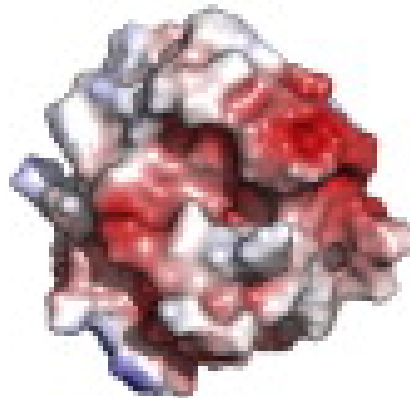
Adams, et al. *PCCP*, **2020**, 22, 7451

# Matrix Metalloproteinase (MMP) Surface Properties

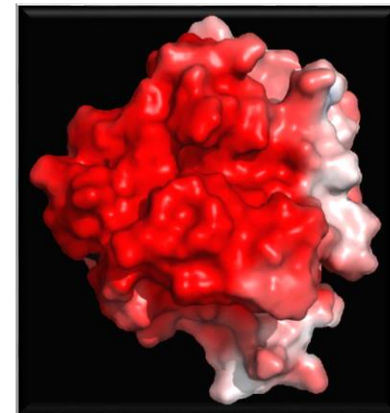
**MMP9-WT**



**MMP14-WT**



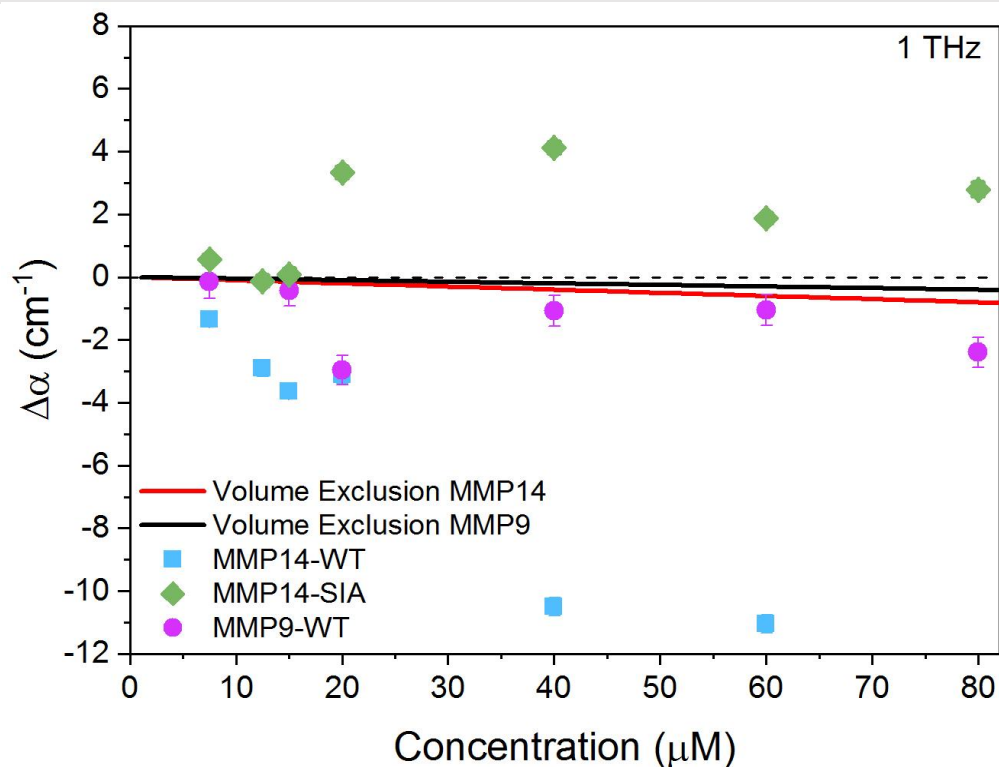
**MMP14-SIA**



**Stabilized  
Inactive  
Mutant**

Adams, et al. *JACS Au* **2021**, 1, 1076–1085

# THz Absorption of MMPs



THz Absorption 
-
+

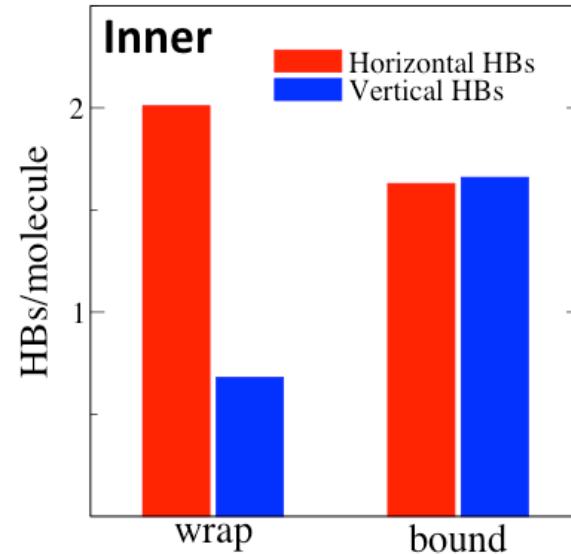
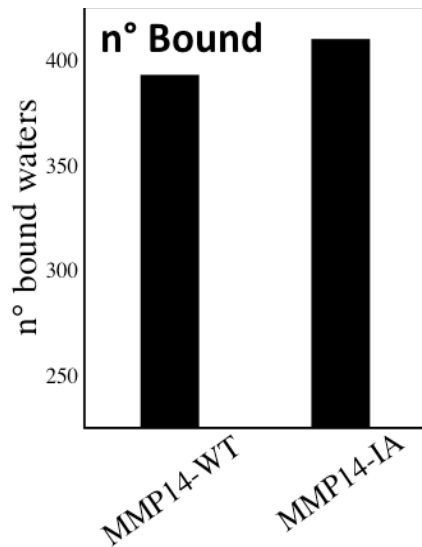
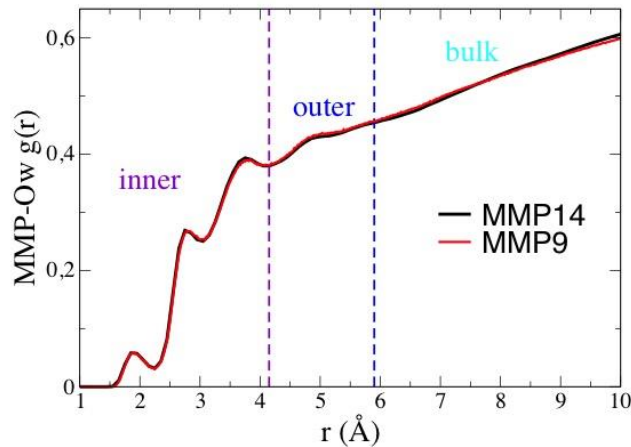
MMP14-WT < MMP9-WT < MMP14-SIA

-
+

Surface Electrostatic Potential

Adams, et al. *JACS Au* **2021**, 1, 1076–1085

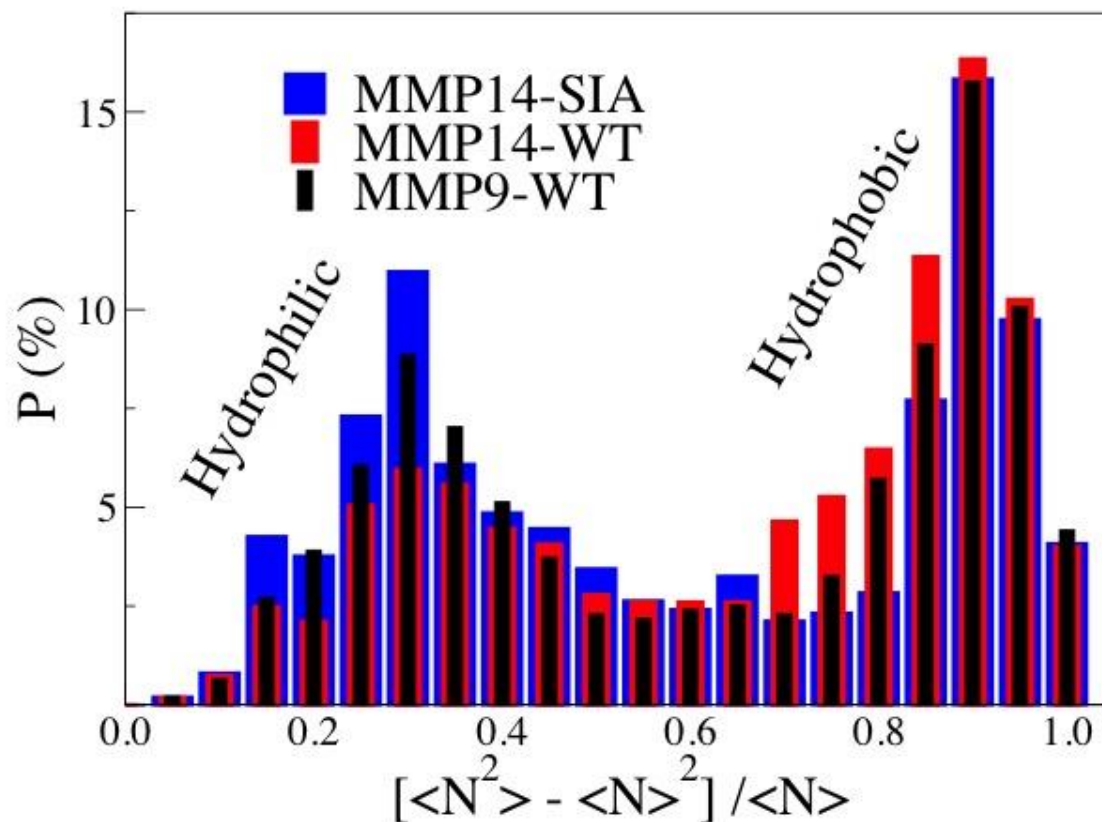
# Water Populations in Inner Hydration Shell



Water near hydrophobic moieties form more hydrogen bonds

Increased number of bound waters in hydration shell of MMP14-SIA

# Water Density Fluctuations at MMP Surface



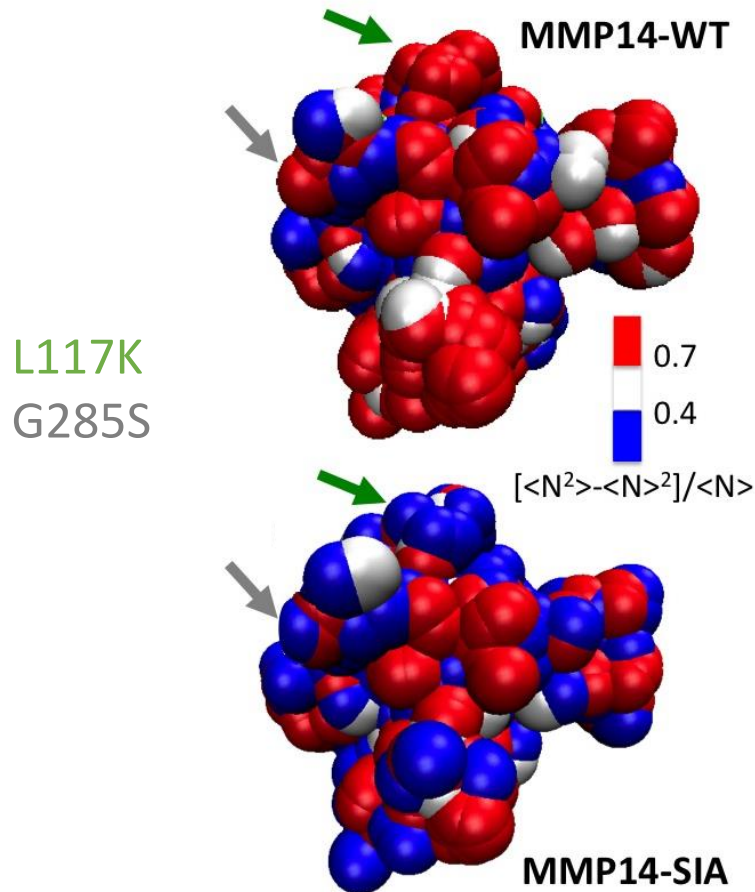
MMP14-WT < MMP9-WT < MMP14-SIA

Hydrophobic



Hydrophilic

# Impact of Mutations on Extended Hydrophobicity/Hydrophilicity



Local topological and morphological context matter:

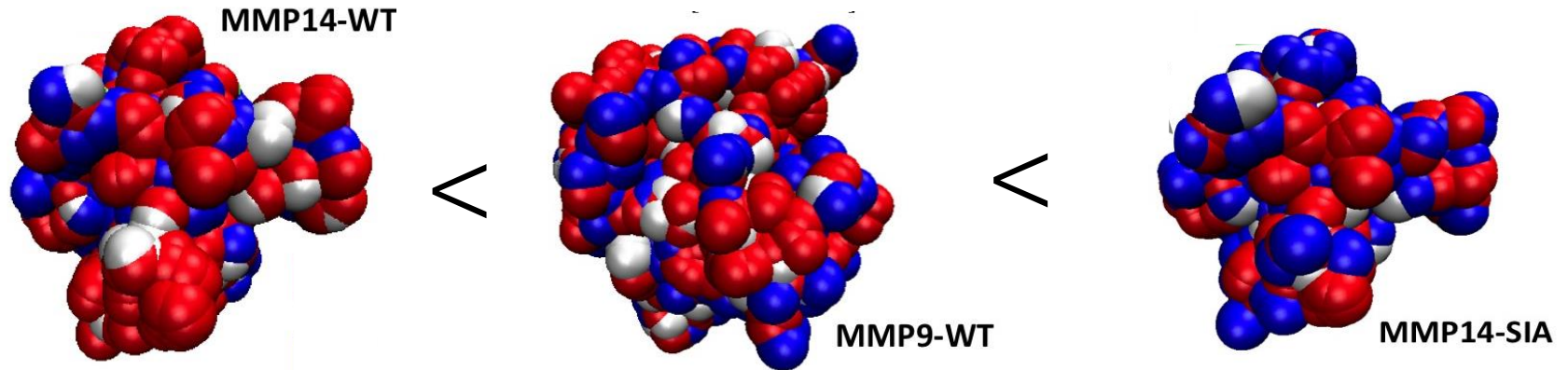
Mutation of isolated hydrophobic residue to hydrophilic converts an entire “patch” to hydrophilic

Adams, et al. *JACS Au* **2021**, 1, 1076–1085



# Surface Mutations Impact Hydration Shell

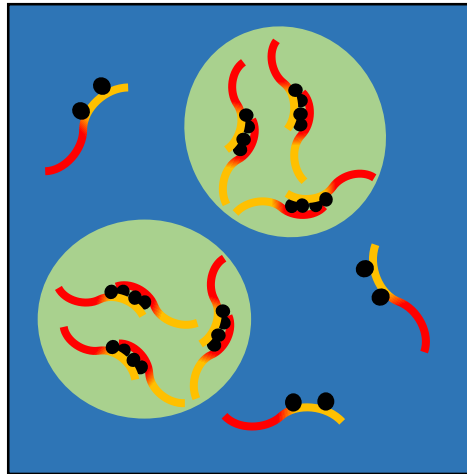
- THz absorption of the hydration shell of MMPs correlates to surface properties
  - Increased absorption for more hydrophilic/positively charged



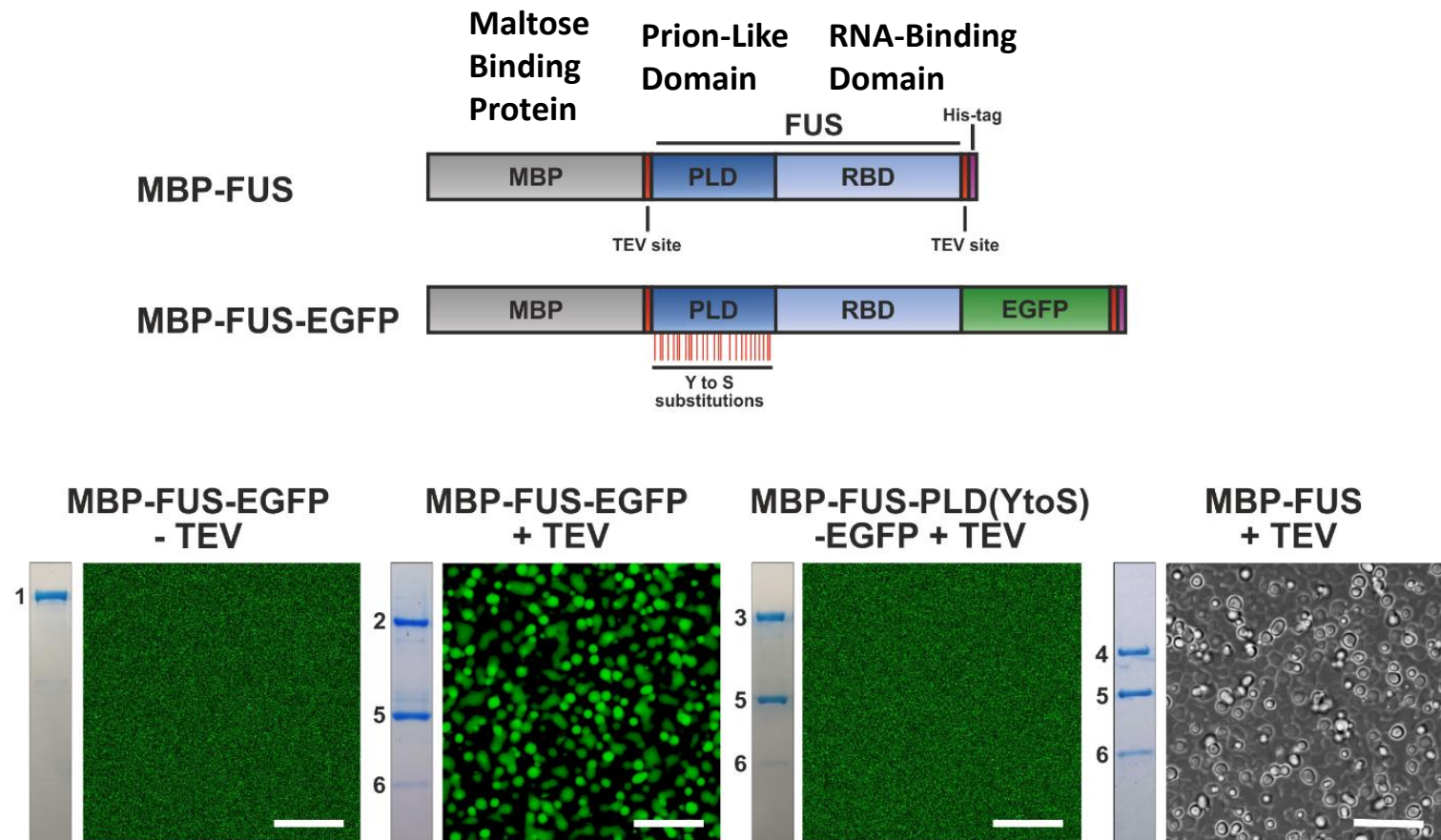
- Surface site mutations have the ability to change the hydration environment of an extended region depending on local topography

Adams, et al. *JACS Au* **2021**, 1, 1076–1085

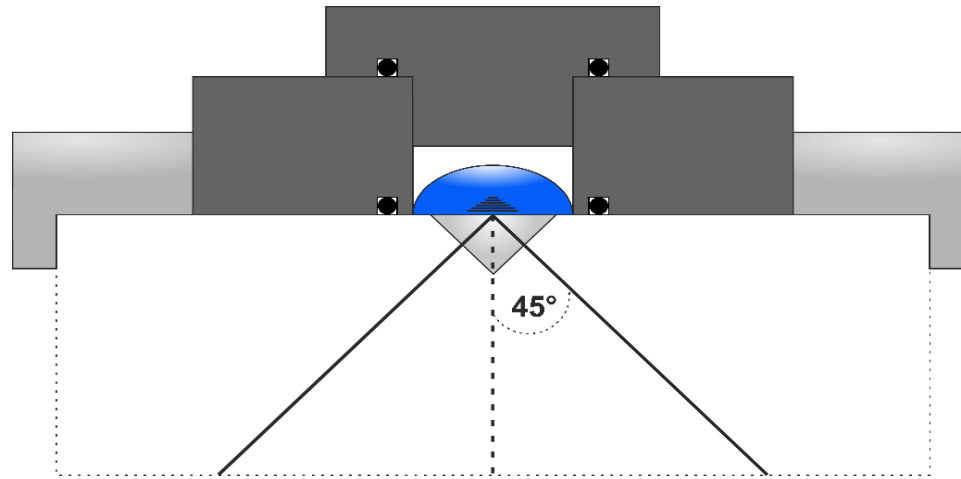
# Intrinsically Disordered Protein Solvation



# TEV Induced LLPS of FUS (Fused in Sarcoma)



# Attenuated Total Reflection (ATR) THz Spectroscopy

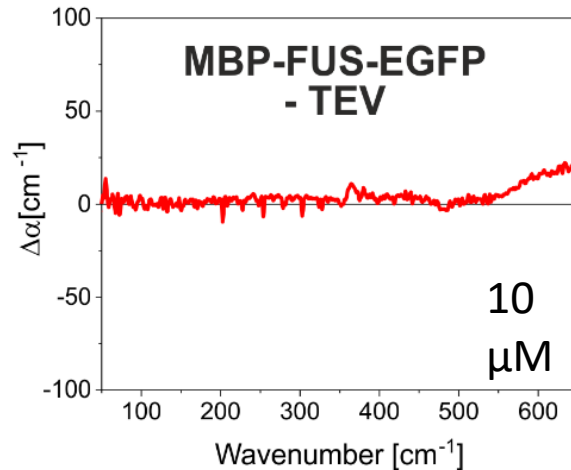


Probe depth  
between 2-11  $\mu\text{m}$

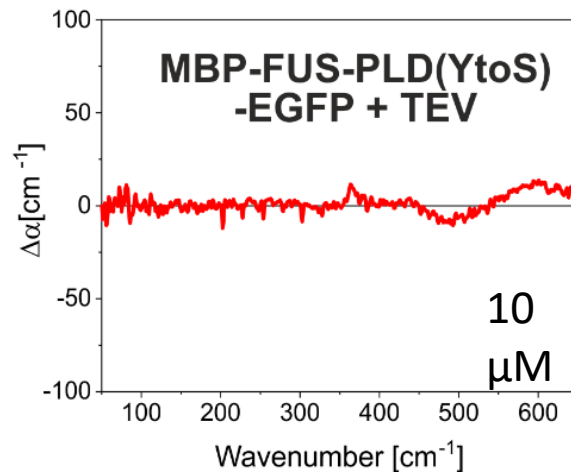
FUS sample was allowed to digest for 30 minutes, then placed on crystal surface

Spectra were collected for a period one hour

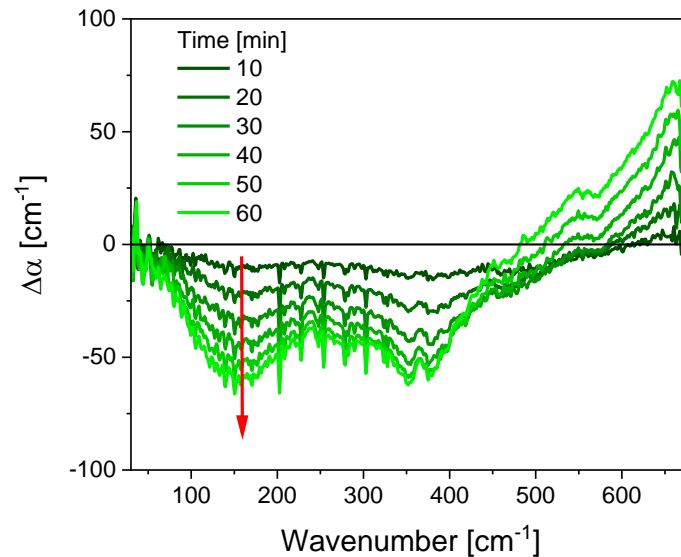
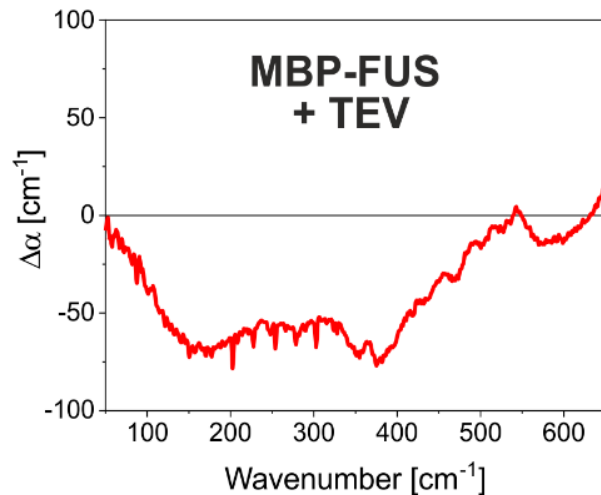
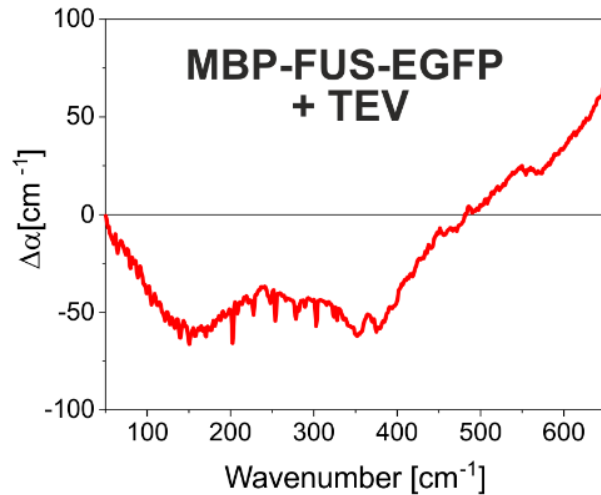
# Non-LLPS systems



No change in  $\Delta\alpha$  observed  
for non-phase separated  
systems



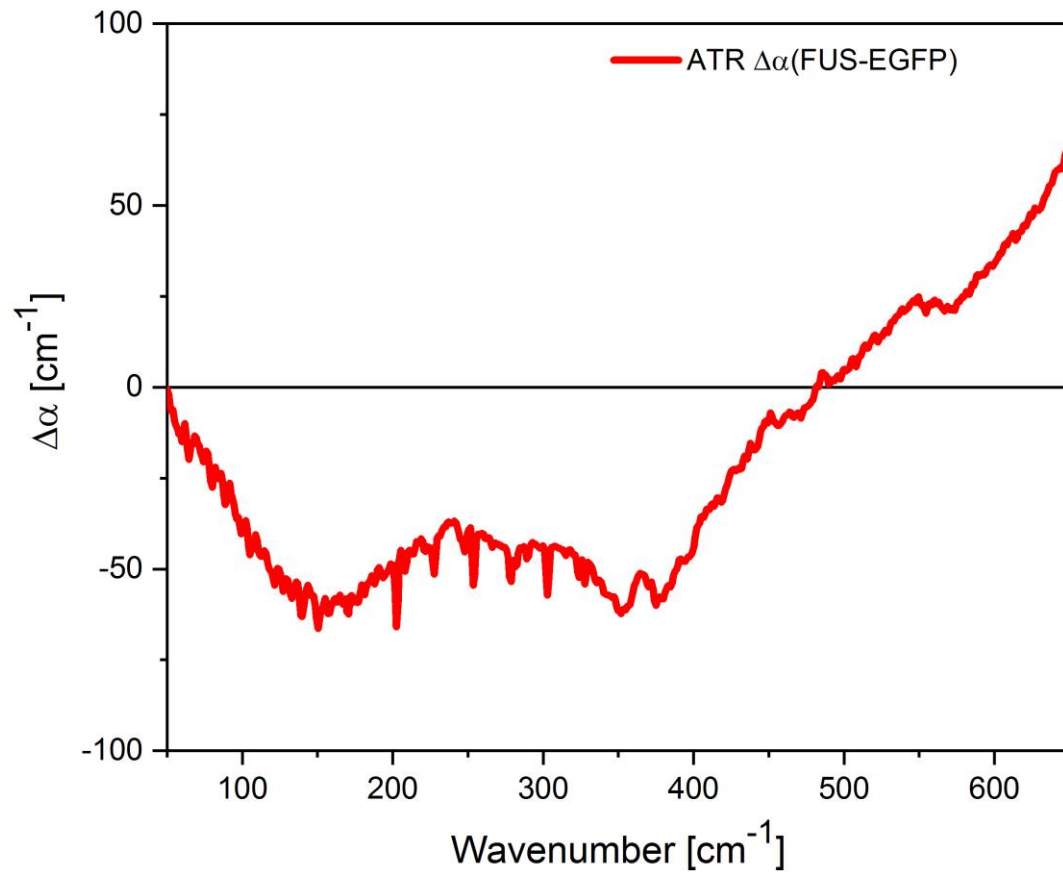
# LLPS FUS Droplets Spectral Signature



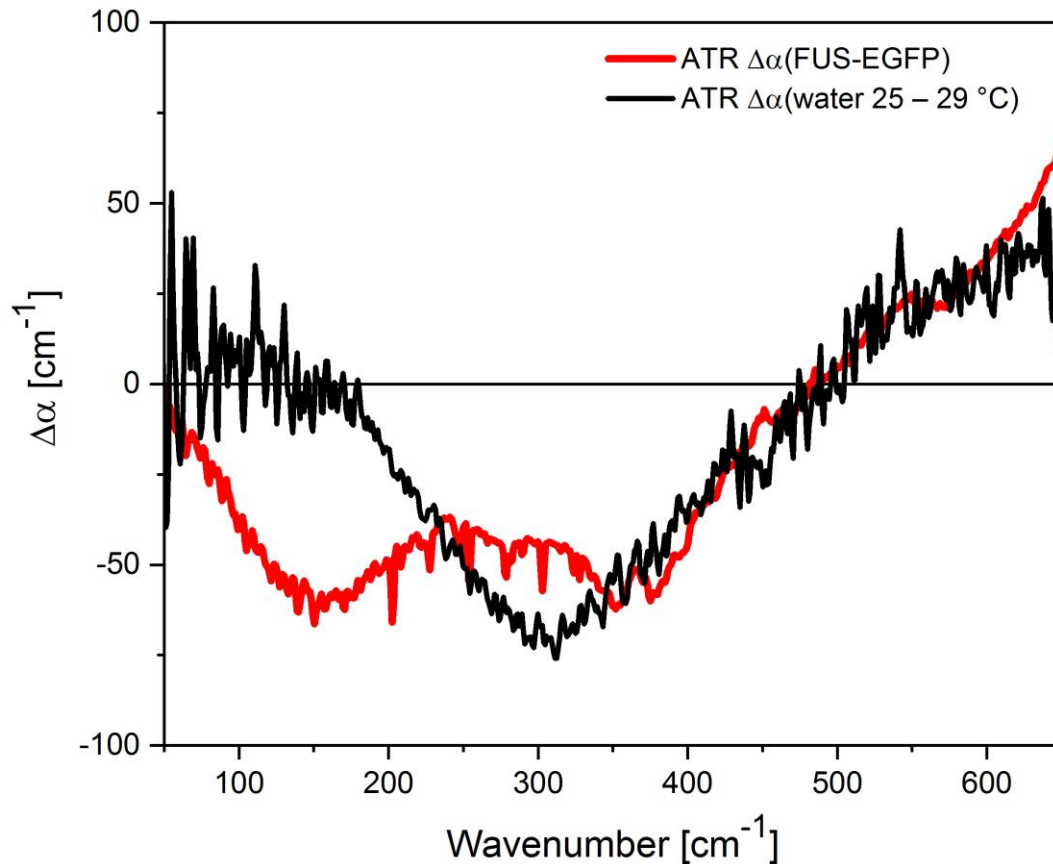
- Two spectral features observed at 155 and 360  $\text{cm}^{-1}$
- Spectral intensity increases with time, but bands do not change



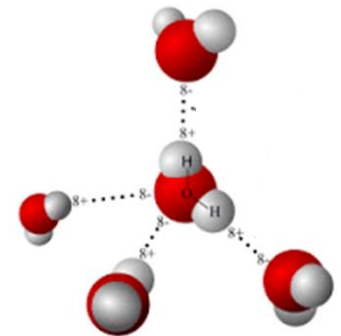
# Molecular Changes in Water Network



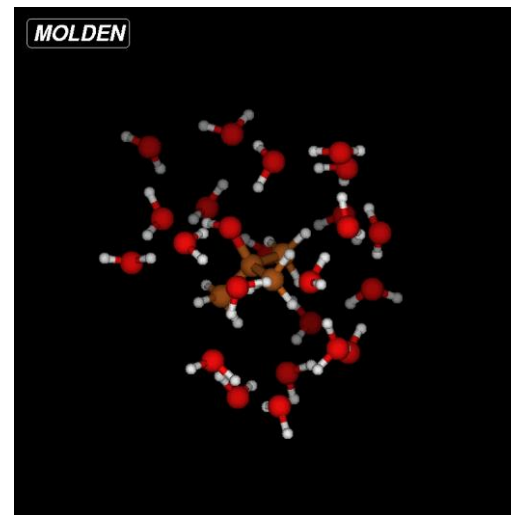
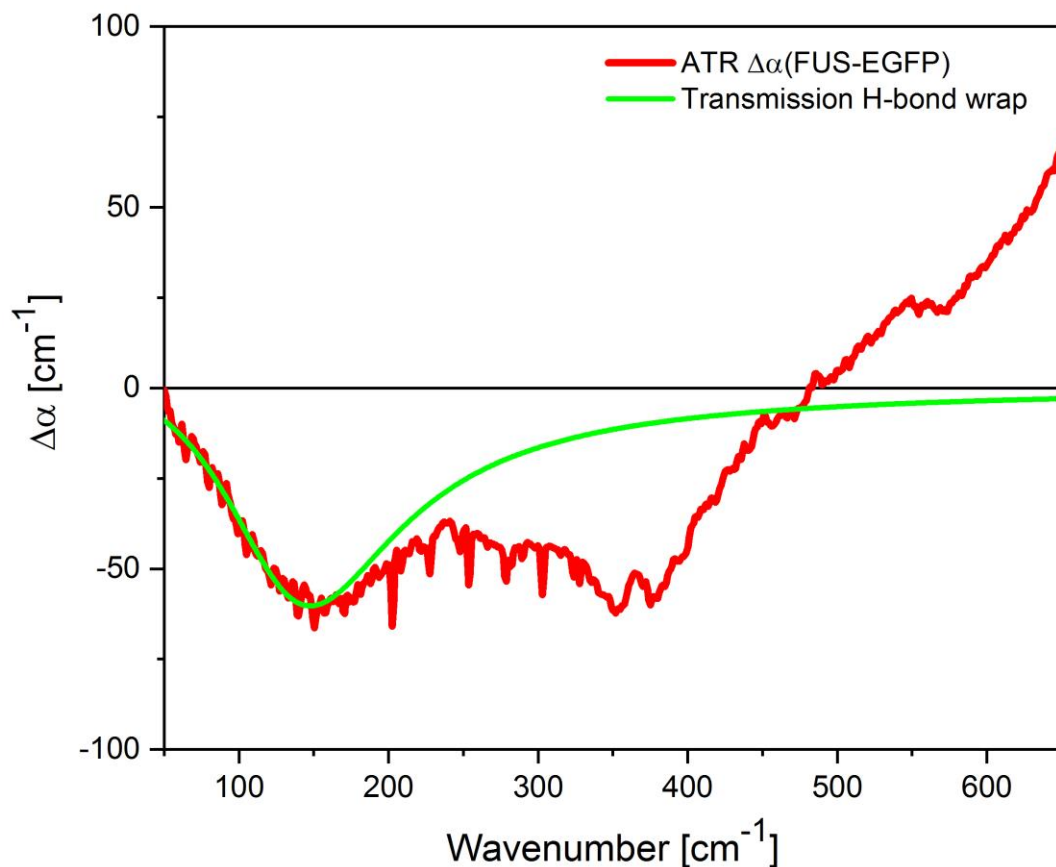
# Molecular Changes in Water Network



- High frequency band (360  $\text{cm}^{-1}$ )
- More tetrahedrally coordinated or stiffer water network



# Molecular Changes in Water Network

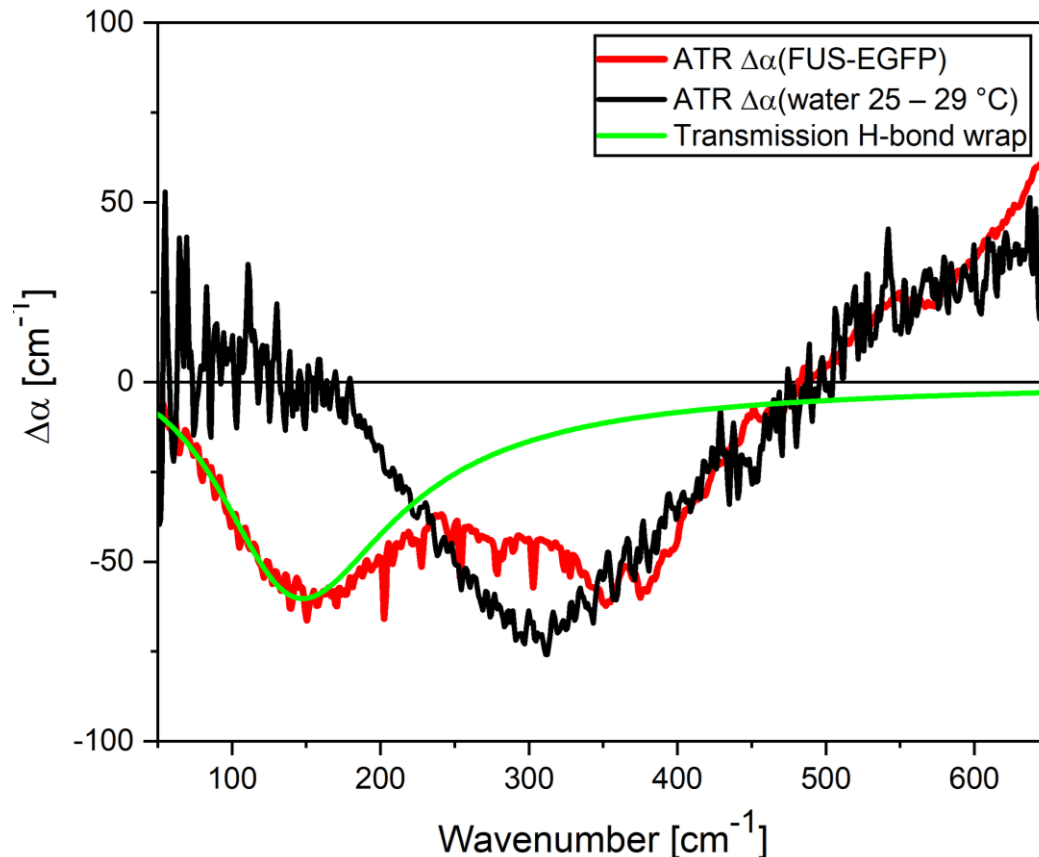


Animation  
provided  
by Simone  
Pezzotti

- Low frequency band (155  $\text{cm}^{-1}$ )
- Hydration water, 2-D water network near hydrophobic surface

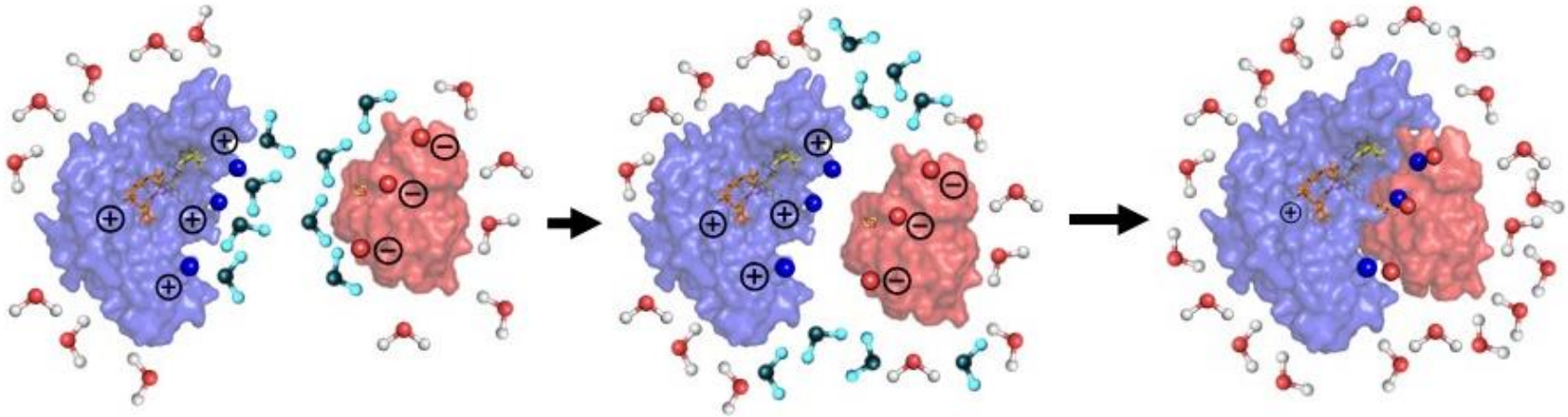
Conti Nibali, et al. *J. Phys. Chem. Lett.* **2020**, 11 (12), 4809-4816

# Molecular Changes in Water Network



- High frequency band (360 cm<sup>-1</sup>)
  - More tetrahedrally coordinated or stiffer water network
- Low frequency band (155 cm<sup>-1</sup>)
  - Hydration water, 2-D water network near hydrophobic surface

# Solvent Contribution in Biomolecular Recognition



**Free energy:**  $\Delta G = \Delta H - T\Delta S$

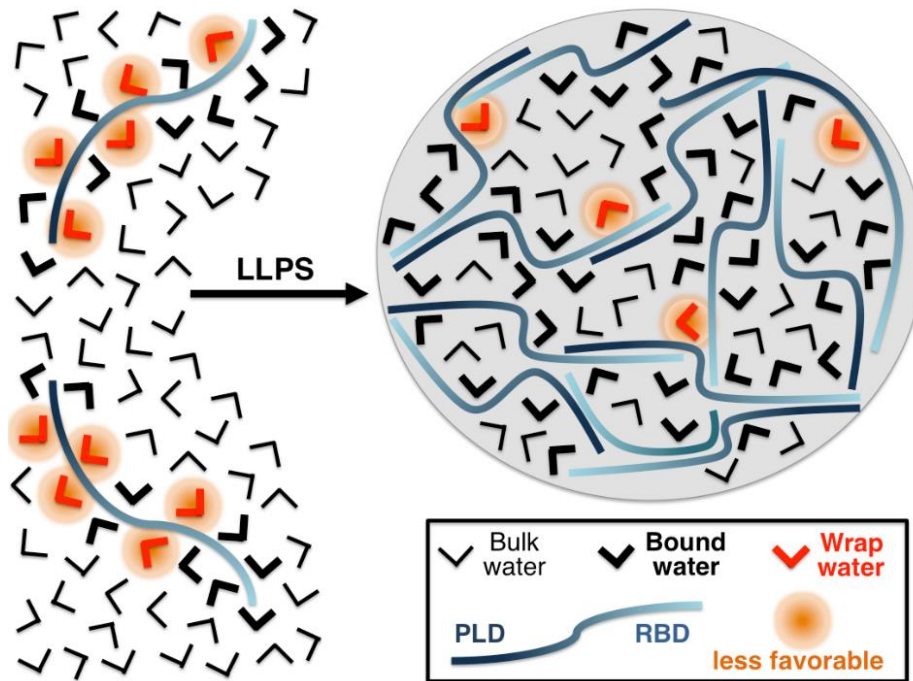
**Maximize  $\Delta H$**  : Optimize binding energy

**Minimize  $\Delta S$**  : Release of retarded water molecules into the bulk

Hydration water molecules act as an entropic reservoir --> molecular driving force

Adams, et al. *PCCP*, **2020**, 22, 7451

# Summary and Conclusions

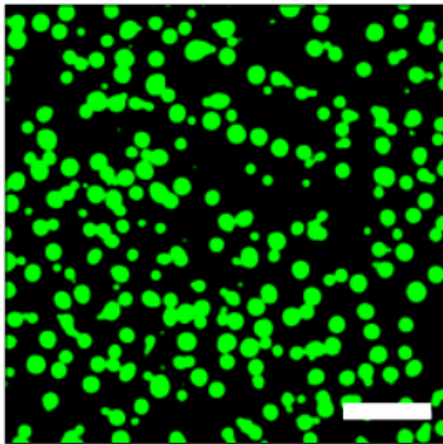


- LLPS water network spectral fingerprint observed
  - Loss of FUS hydration water
  - Increased constrained water molecule
- Two concerted molecular driving forces for LLPS
  - Entropic: loss of less favorable hydration water (protein-water interaction)
  - Enthalpic: cation- $\pi$  interaction (protein-protein interaction)



# Future Directions

## Solvent Properties of Biomolecular Condensates



### IDP Hydration

- Protein-protein driving forces
- Mutations
- Aggregates

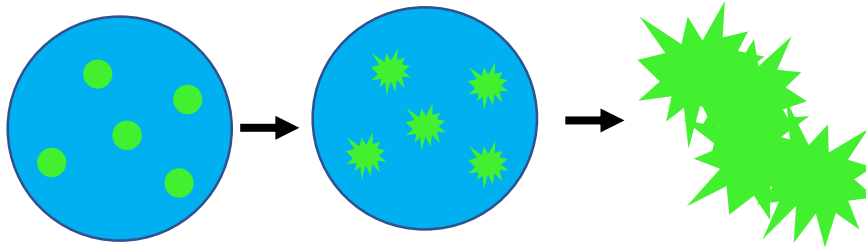
### Solvent Dynamics

- Condensate aging

### Interfacial Properties

- Interfacial water
- Chemical speciation

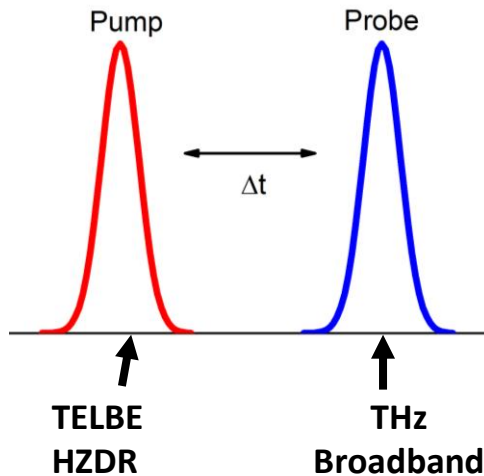
# TELBE – Solvent Dynamics - Condensate Aging



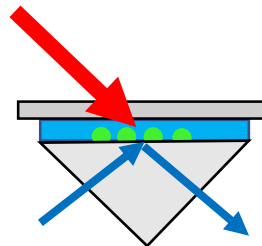
**Solvent Dynamics?**

Develop TELBE pump-THz  
broadband probe  
to investigate solvent dynamics of  
LLPS droplets

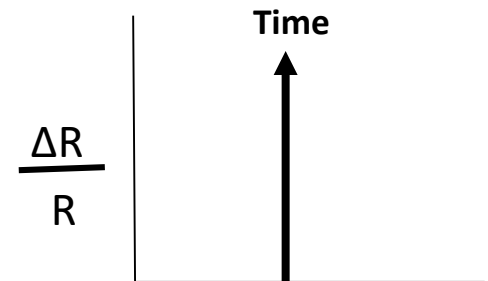
**Nonlinear THz Spectroscopy**



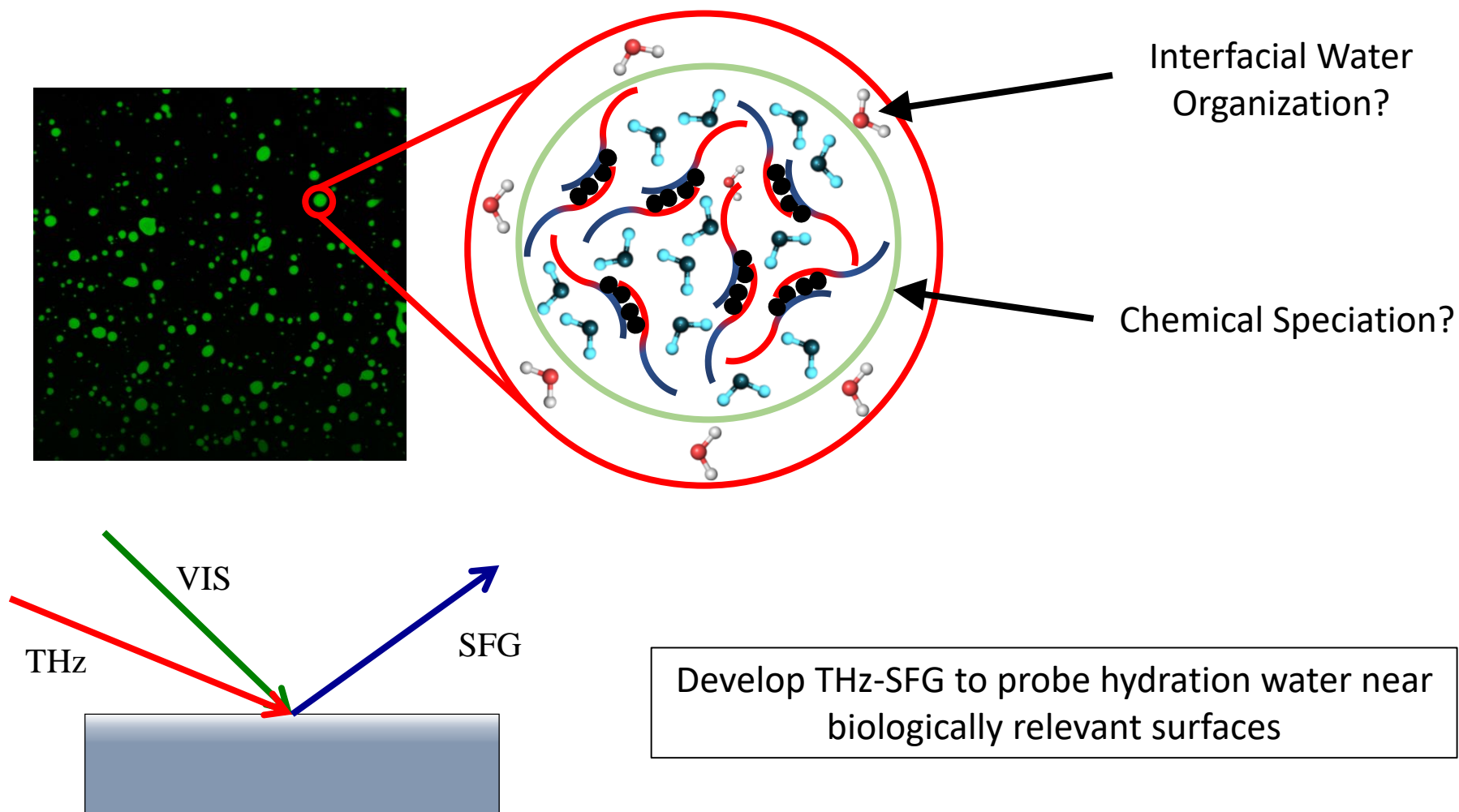
TELBE  
1 THz



Broadband  
0.3-6 THz



# FELBE – Interfacial Properties



# Acknowledgements

Prof. Martina Havenith – Ruhr Uni Bochum  
Dr. Simone Pezzotti – Ruhr Uni Bochum  
Jonas Ahlers – Ruhr Uni Bochum  
Prof. Konstanze Winklhofer – Ruhr Uni Bochum  
Prof. Jörg Tatzelt – Ruhr Uni Bochum  
Dr. Verian Bader – Ruhr Uni Bochum



Lehrstuhl für Physikalische Chemie II

