



# Effects of Kinetic and Thermodynamic Transitions of Confined Water on the Structures of Isolated and Cytoplasmic Proteins

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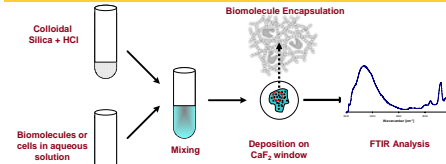
## Introduction

Biological materials (such as enzymes, proteins and antibodies) can be confined in nanoporous matrices, stabilized, and stored for extended periods of time without loss of activity.<sup>1</sup> Similarly, a handful of studies conducted with bacteria, plant, and mammalian cells showed that these complex microorganisms also have increased biostability, and function when they are encapsulated.<sup>2</sup> In these studies, the microorganisms were encapsulated in rigid mesopores (which prevented their mobility, and proliferation) surrounded by a high permeability nanoporous biomaterial, which enabled diffusion of nutrients and bi-products.

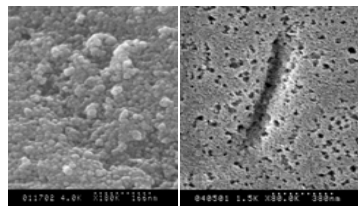
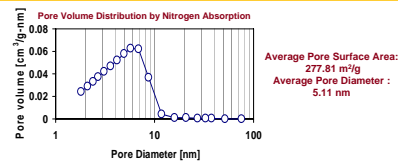
Similarities between confined water in close proximity to surfaces, and hydration waters of macromolecules and membranes are profound. We are focused on understanding the effects of confinement on water dynamics, and its influence on the dynamics of biomacromolecules. We have utilized infrared spectroscopy to identify the different stable and metastable states, and phase transitions of nanopore confined water at cryogenic temperatures. We have extended our analysis to quantify the structural changes in the nanopore confined isolated proteins (lysozyme) and the cellular proteins of intact organisms (*Geobacter sulfurreducens* and LNCAp cells).

## Methods

### Silica Gel Encapsulation



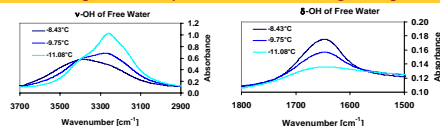
### Characterization of the Silica Gel



Images of LNCAp Cells Encapsulated in Silica Gel

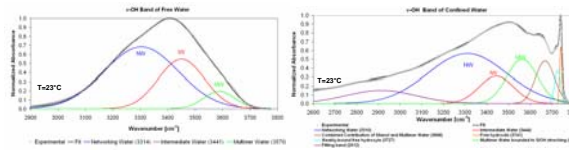
## Results

### Changes in the IR Spectra of Free Water During Cooling

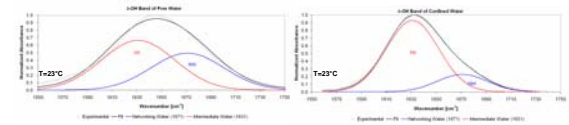


When cooled, free water v-OH band peak shifts to lower wavenumbers, and the δ-OH band peak shifts to higher wavenumbers until it disappears upon freezing.

### Deconvolution of the v-OH Band for Free and Confined Water



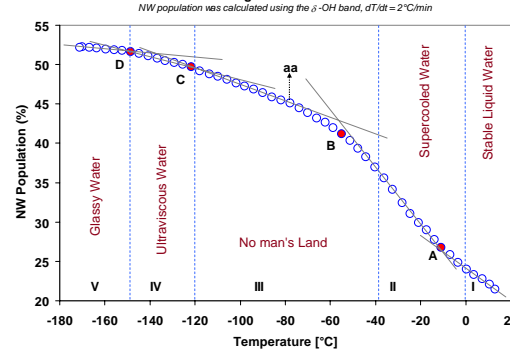
### Deconvolution of the δ-OH Band for Free and Confined Water



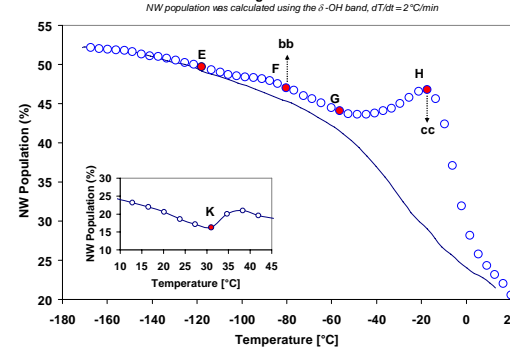
Networking Water (NW) has a coordination number of 4, Intermediate Water (IW) has a coordination number of 3, and Multimer Water (MW) has a coordination number of 1 or 2.<sup>3</sup>

### Transitions of the Silica-Confined Water

#### Cooling of Confined Water

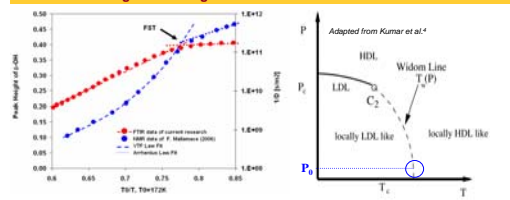


#### Warming of Confined Water

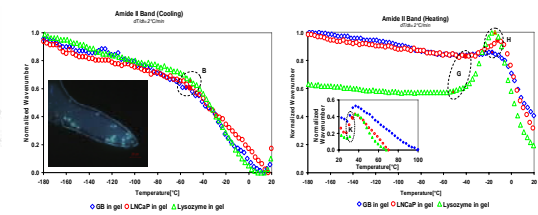


Point A: Crystallization of water in a small fraction of the larger pores.  
Point B: Fragile-to-Strong (FST) transition of supercooled water  
Point C: Onset of ultraviscous region  
Point D: Vitrification  
Point E: I<sub>c</sub> formation  
Point G: I<sub>c</sub> to I<sub>h</sub> transition  
Point H: I<sub>h</sub> melting  
Point K: C<sub>p</sub> minimum

### Fragile-to-Strong Transition of the Silica-Confined Water

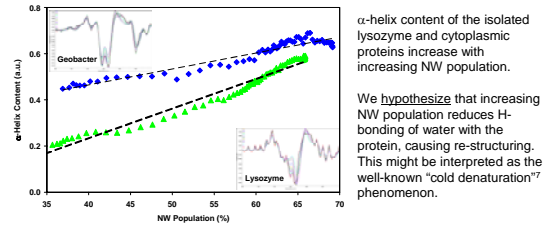


### Transitions of Confined Macromolecules



Amide I band is used to quantify the changes in the secondary structures of isolated and cellular proteins. It is important to note that the inflection point (Point B) located at -51°C is identical to the FST temperature of supercooled water. This temperature also corresponds to the "glass transition temperature" for proteins. Evidently, FST of the water molecules that were bound to the amino groups triggered this transition.<sup>5, 6</sup> Similarly, protein structural dynamics follow water at Points C and H (I<sub>c</sub> to I<sub>h</sub> transition, and I<sub>h</sub> melting, respectively).

### Protein Compaction Upon Supercooling?



## Conclusions

- Nanopore confined water in silica gels did not crystallize down to -170°C, even at very slow cooling rates (2°C/min).
- Nanopore Confined water experienced Fragile-to-Strong transition (FST) at ~ -51°C (222 K). Ultraviscous and glassy state transitions were also identified during cooling. During warming, crystallization of glassy water into I<sub>c</sub> and I<sub>h</sub> occurred at -109°C and -48°C, respectively.
- High Density Water (HDL): NW has tetrahedrally coordinated hydrogen bond structure, which is **not** fully developed. Low Density Water (LDW): NW has an "ice-like" hydrogen bond network structure, which is fully developed.
- For isolated, and cytoplasmic proteins, the secondary structures, and therefore the activities of the biomolecules are dictated by the thermodynamic and kinetic transitions of their hydration water.
- Protein glass transition is at the same temperature as FST of the confined water, which is in close proximity to C<sub>p</sub> maximum of water (~ -45°C).
- The direct correlation between the alpha-helix content of the protein and NW population increase suggests that the stability of the protein increases due to compaction and increased organization. However, this causes a deviation from the native structure. This change in structure can be correlated to "cold denaturation". It is known that cold-denaturation is a fully reversible process, and the structure of the protein is very different from that of the heat- and chemical-denatured states.

## References

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## Acknowledgements

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