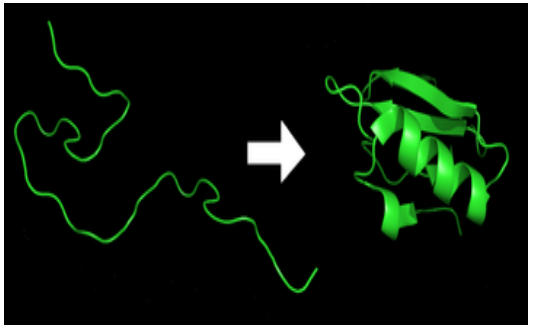
Observing solvation free energy changes in biological processes with THz spectroscopy

Simone Pezzotti
Ruhr University Bochum

Solvation in biology: importance & challenges

Protein folding

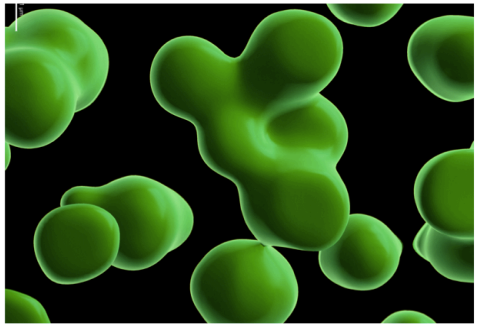


D. M. Huang et al. **PNAS**, 97, 8324 (2000).

Hydration

entropy & enthalpy

Liquid-liquid phase separation



S. F. Banani et al. **Nat. Rev. Mol. Cell Biol.** 18, 285 (2017).

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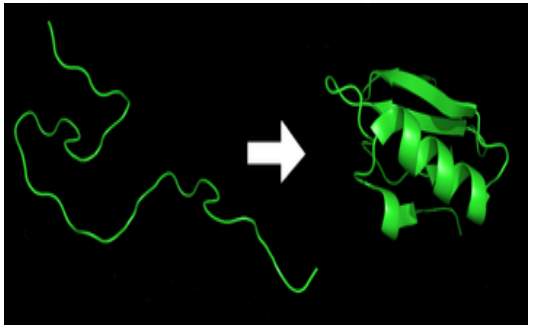

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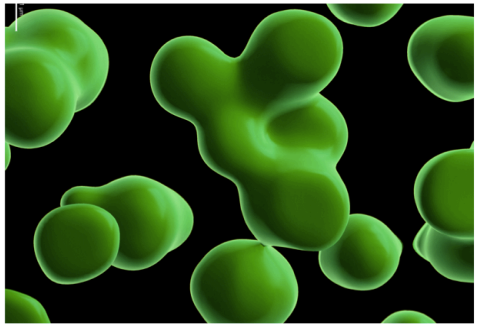
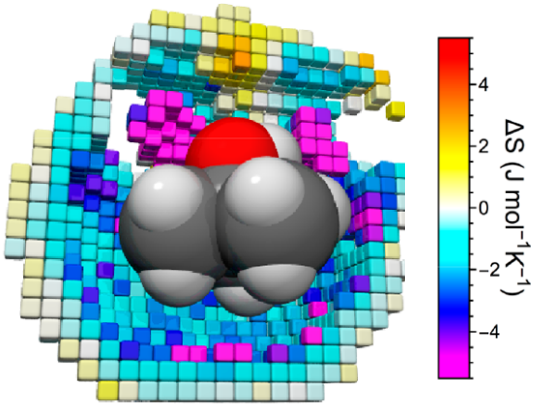
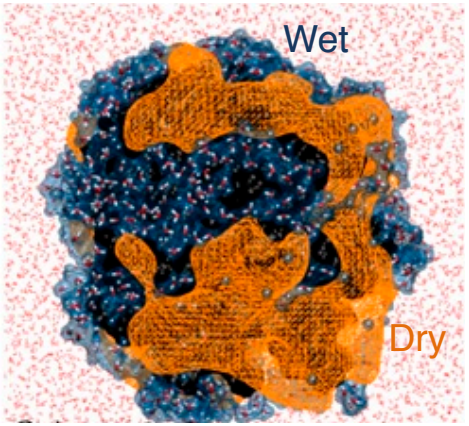


Challenges

- Map local solvation contributions
- Improve time resolution



V. C. Nibali et al. **JPCL**,11, 4809 (2020)



N. B. Rego et al. **PNAS**, 118, e2018234118 (2021)

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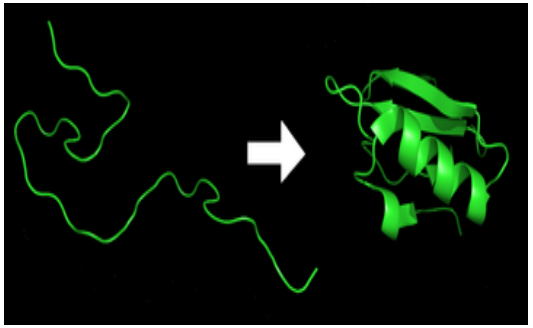

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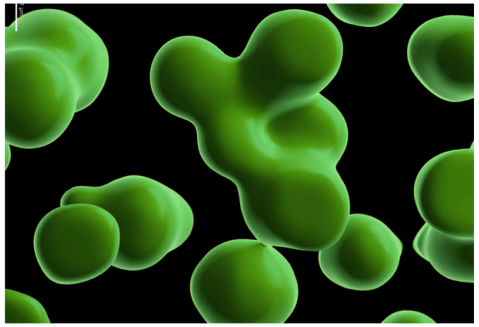

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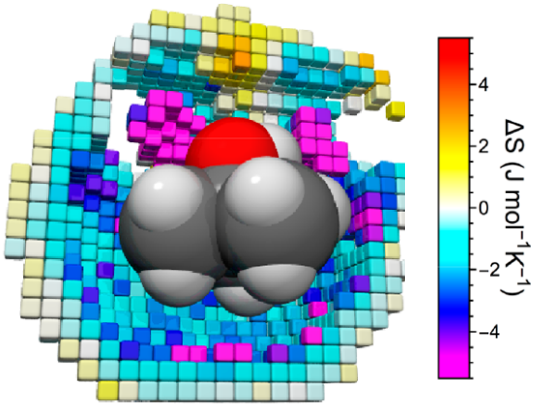
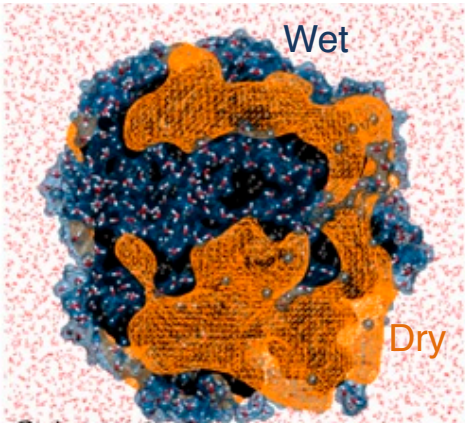


V. C. Nibali et al. **JPCL**,11, 4809 (2020)

THz-calorimetry



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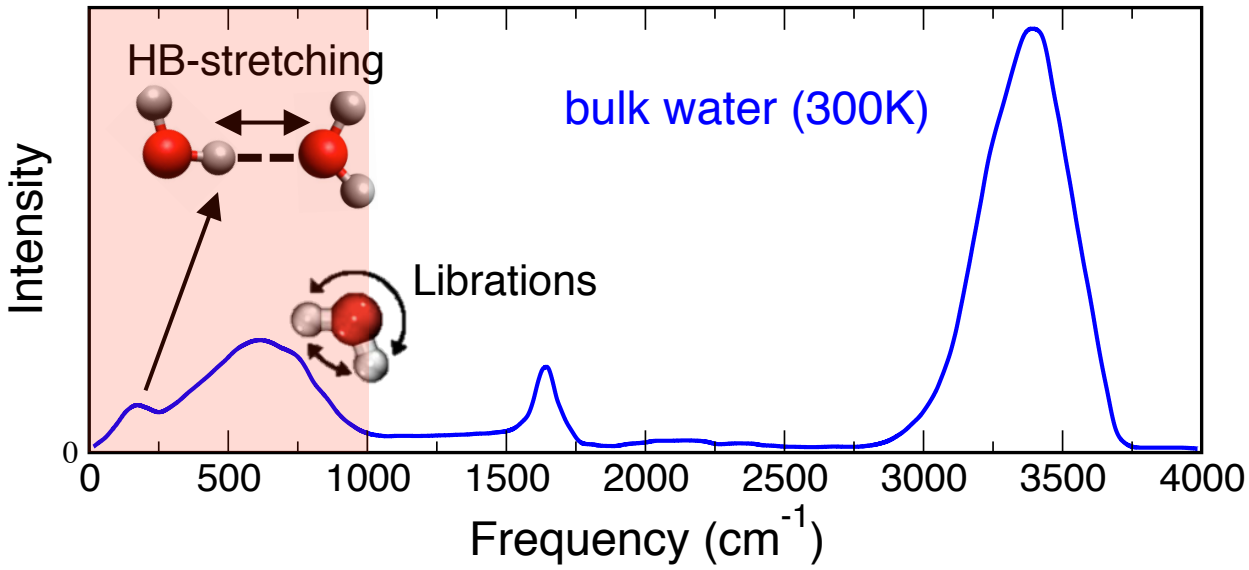
THz spectral range is ideal to look at hydration



Advantages of THz:

✓ Hydration shell motions

✓ Distinguish water/solute

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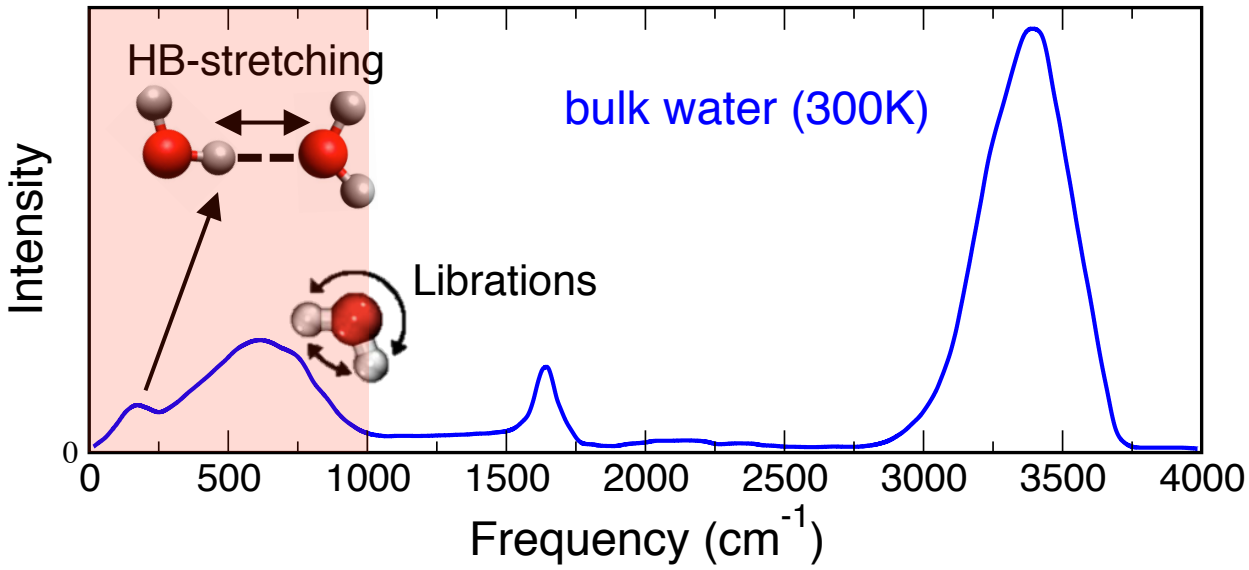

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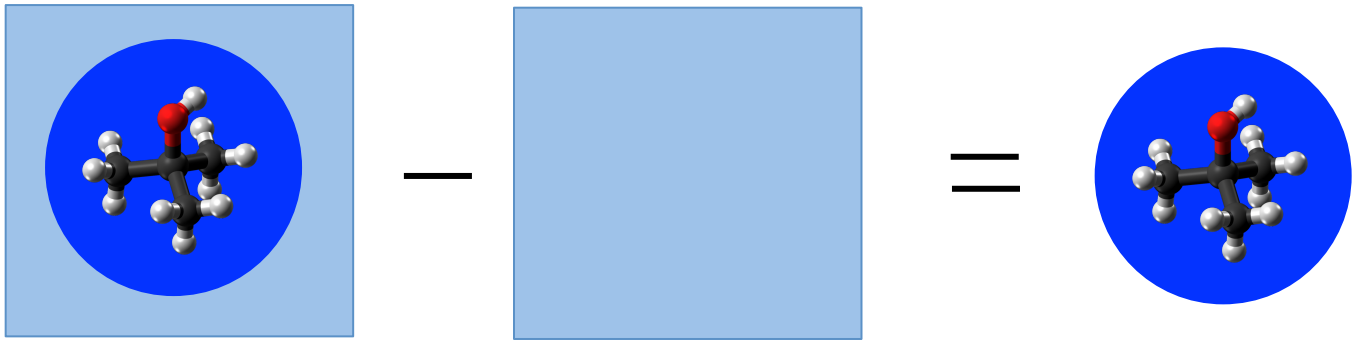


THz spectrum of the solution

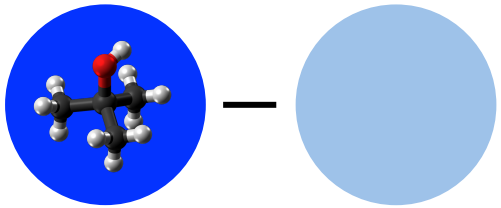
THz spectrum of bulk water

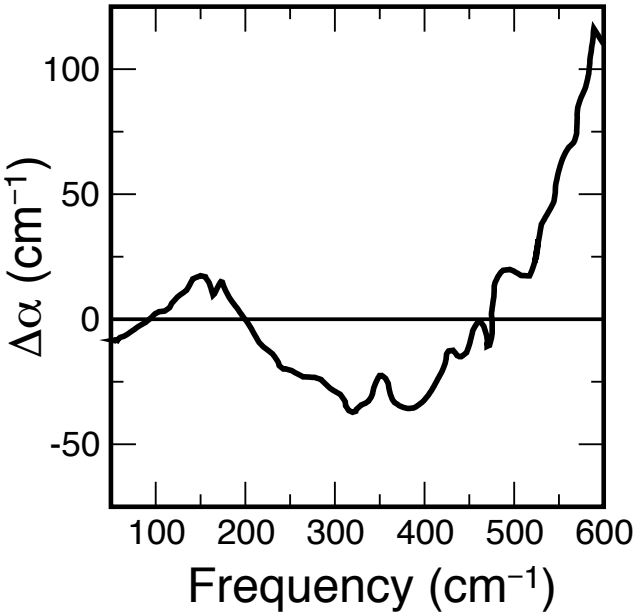
✓ Solvation-shell specific

F. Bohm et al. **Angewandte** 56, 9981 (2017)

4 good reasons why using THz to map hydration S and H

Difference THz spectrum





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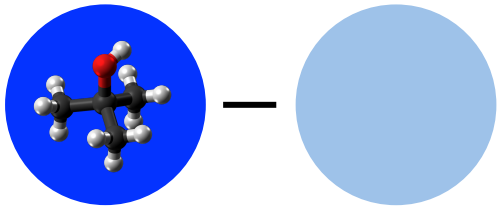

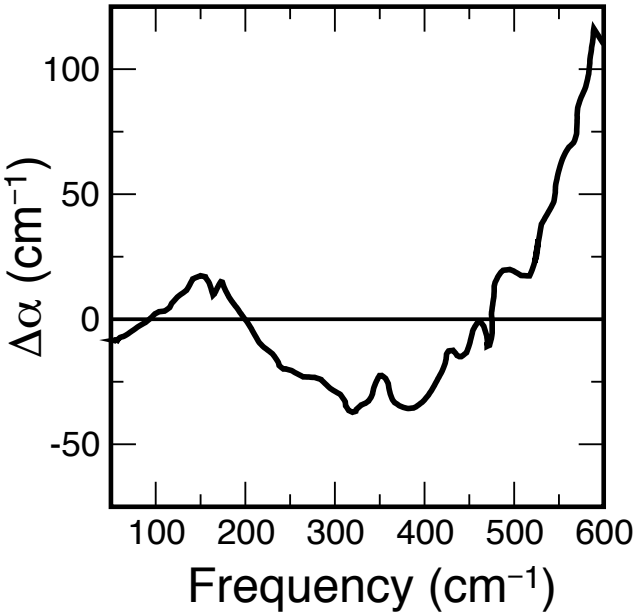

✓ Low frequency density of state: > 95% of the entropy of water

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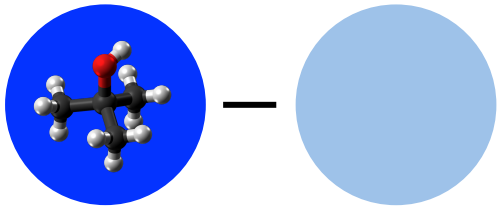

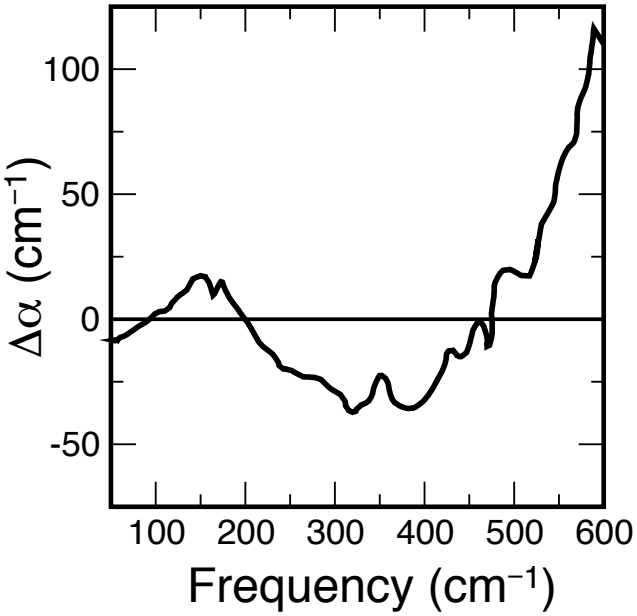

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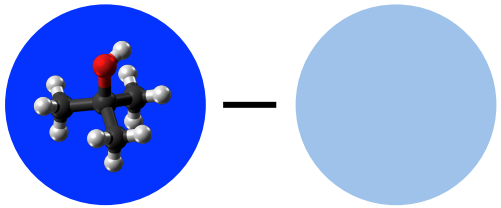

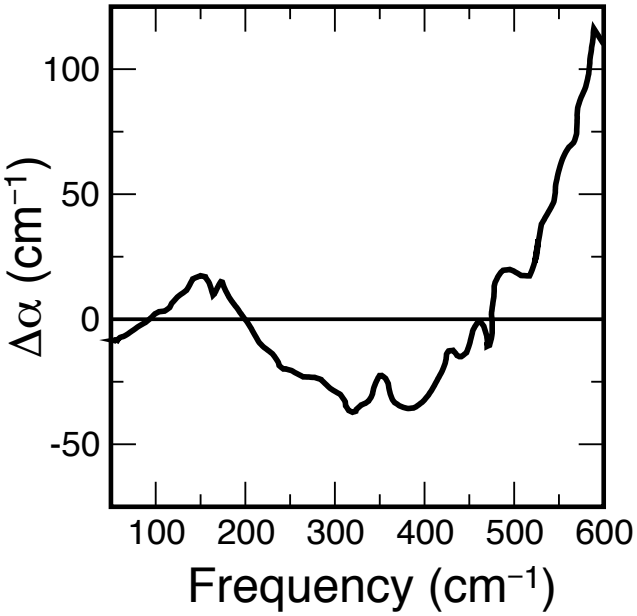

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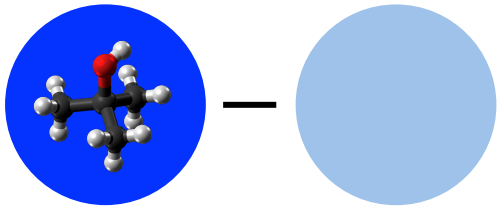

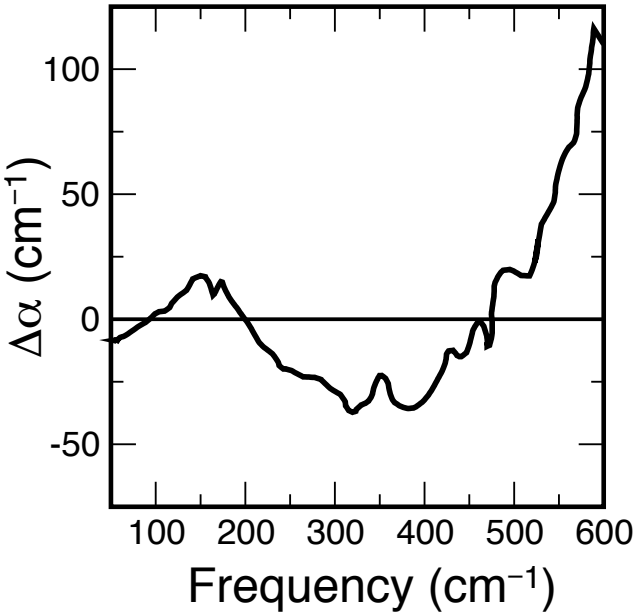

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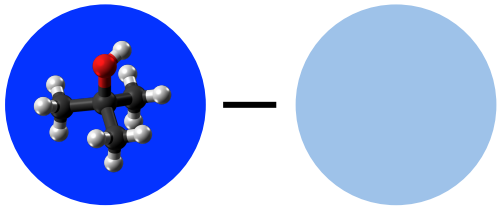

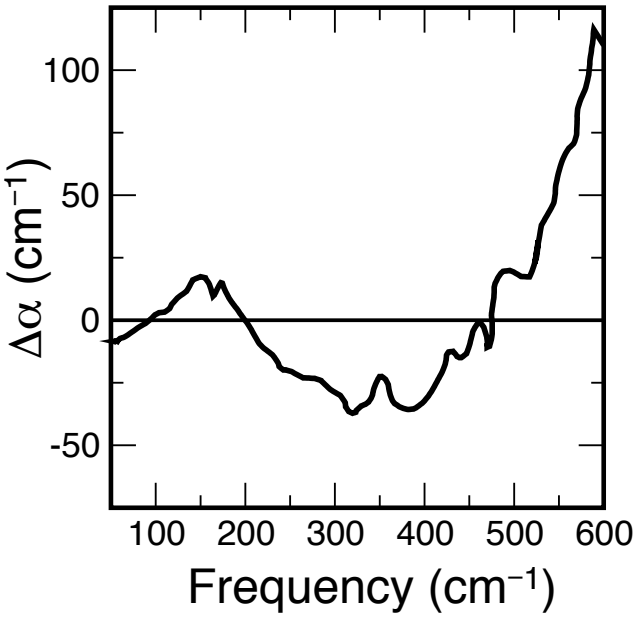

- ✓ Low frequency density of st[illegible]: > 95% of the entropy of water
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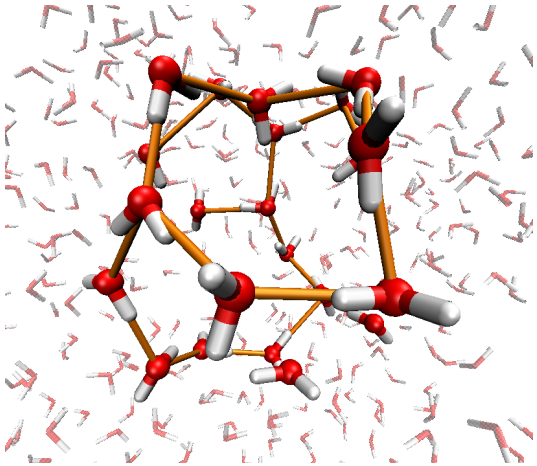
How?

A 2-step thermodynamic cycle for solvation

S. Pezzotti et al. **Angew. Chem., Int. Ed.**, e202203893 (2022)

A 2-step thermodynamic cycle for solvation

1. Create cavity



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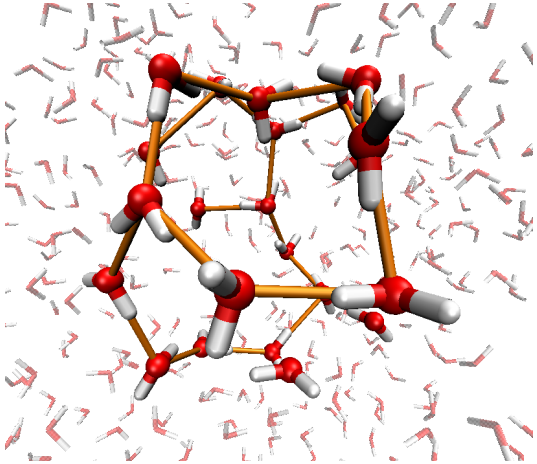
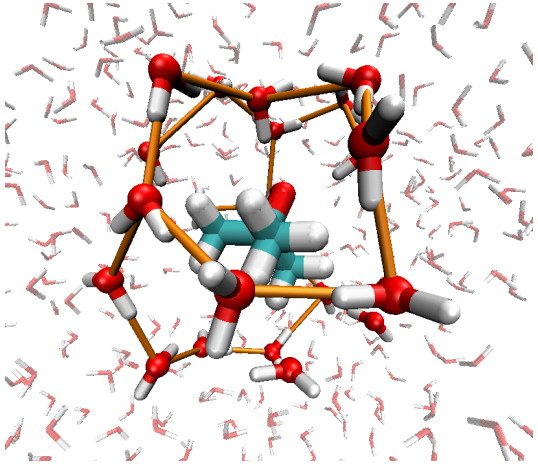


2. Insert solute



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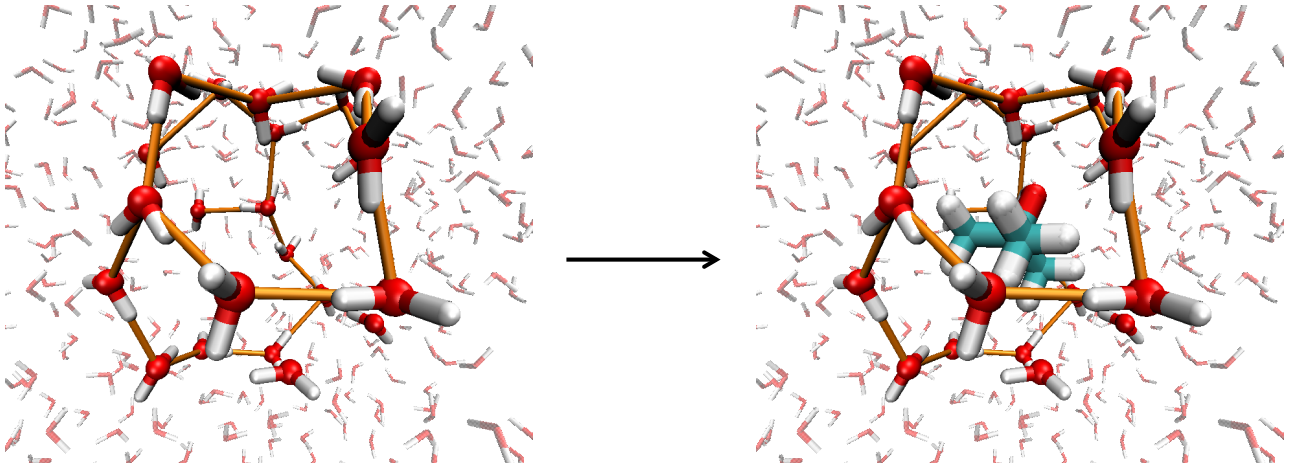

$$\Delta G_{solv} = \underline{\Delta G_{cavity}} + \underline{\Delta G_{bound}}$$

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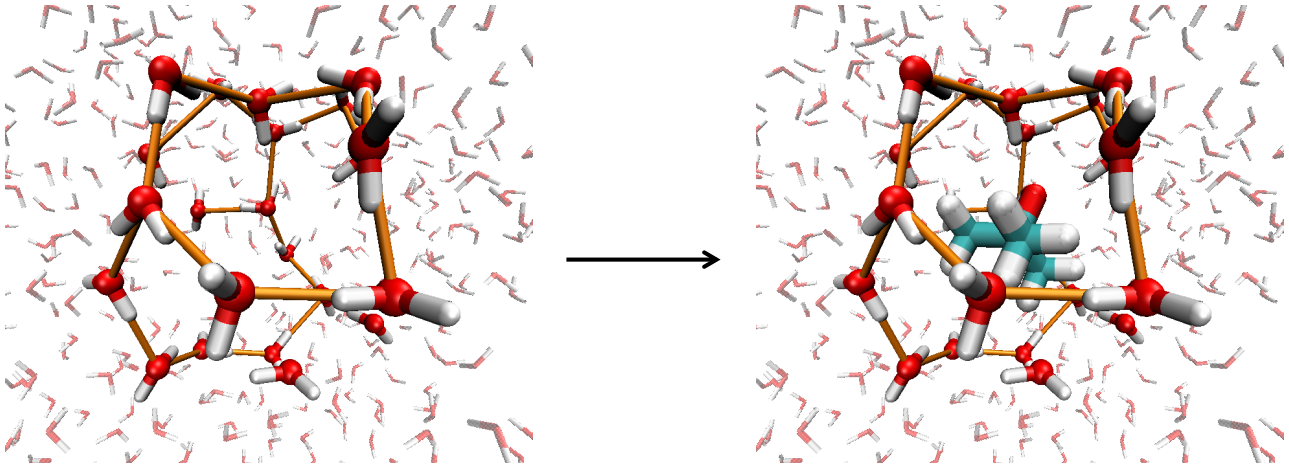


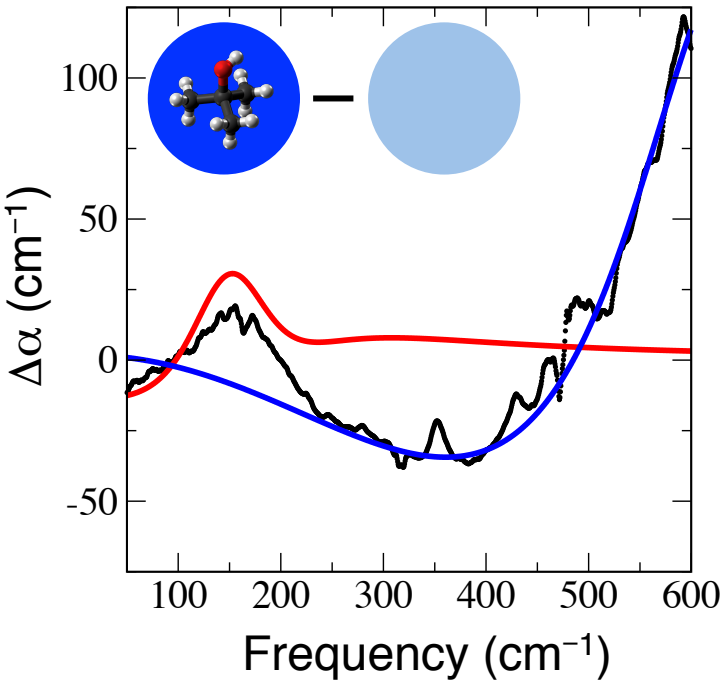
$$\Delta G_{solv} = \Delta G_{cavity} + \Delta G_{bound}$$

Hydrophobic
Cost to perturb water HB-network

Hydrophilic
Gain from solute-water interactions

S. Pezzotti et al. **Angew. Chem., Int. Ed.**, e202203893 (2022)

THz fingerprints of the 2-steps: 1. cavity-wrap



Spectral dissection from
theoretical spectroscopy (DFT-MD)

V. C. Nibali et al., **JPCL**, 11, 4809 (2020).
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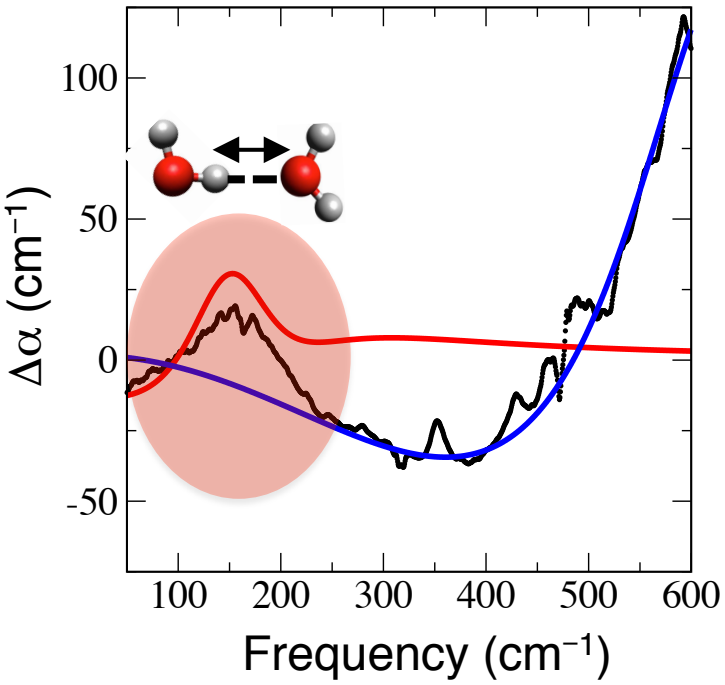
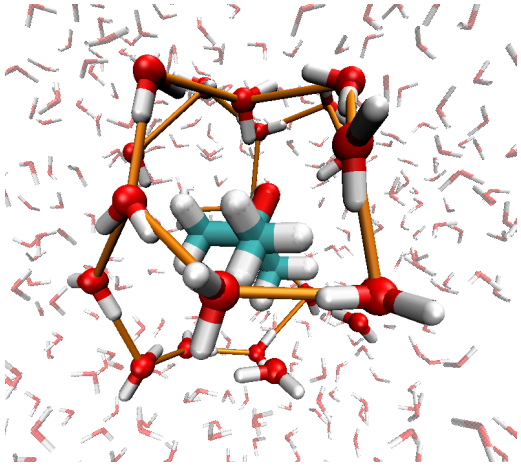


Cavity-wrap = Hydrophobic



Collective HB-stretching

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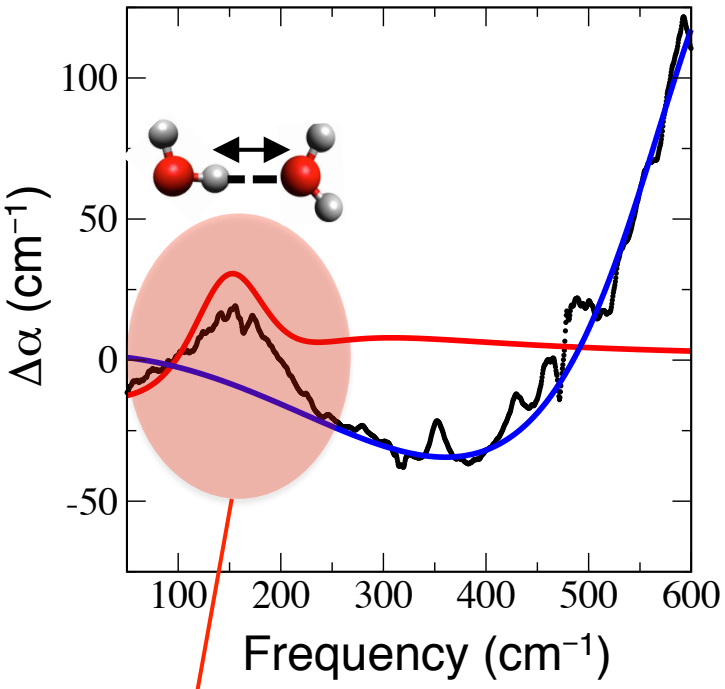

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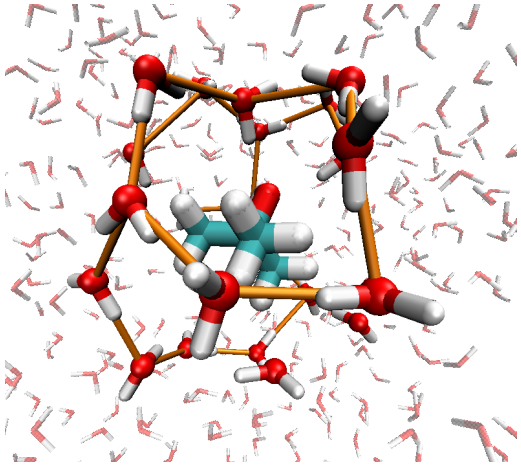

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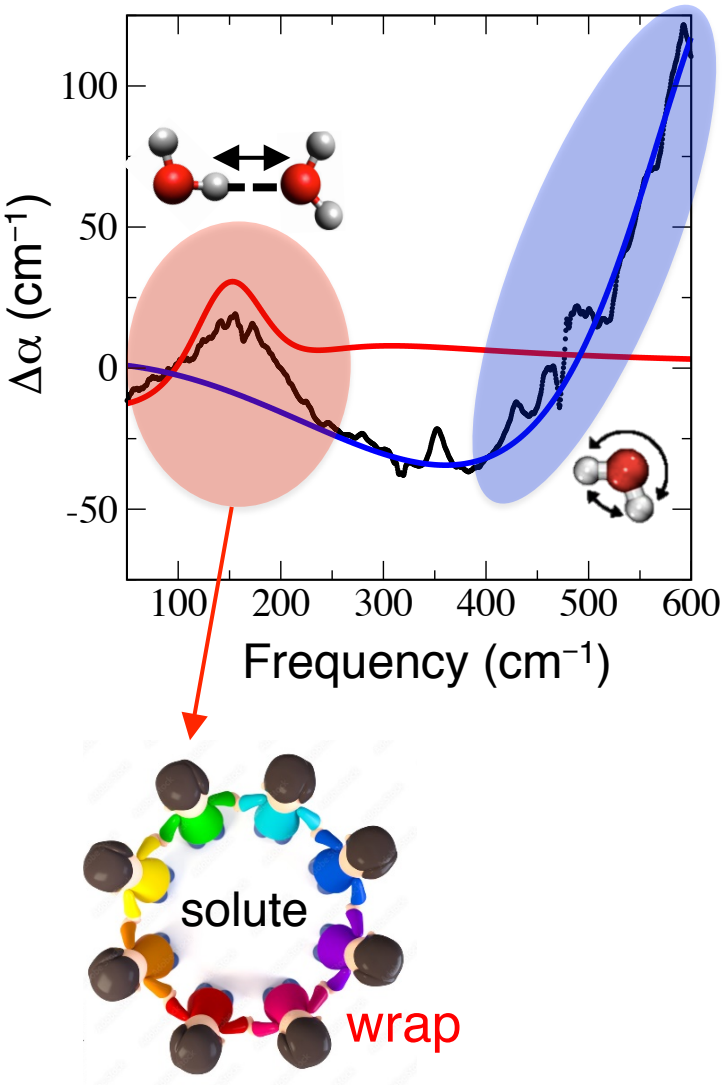
THz fingerprints of the 2-steps: 2. Bound water



Bound = Hydrophilic

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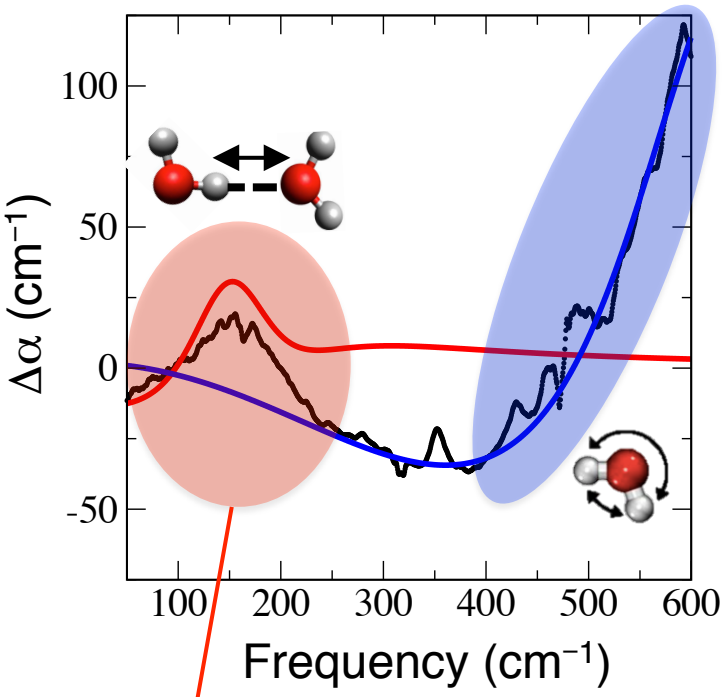

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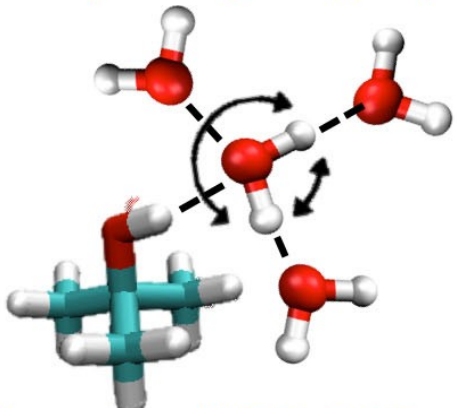

Hindered rotations

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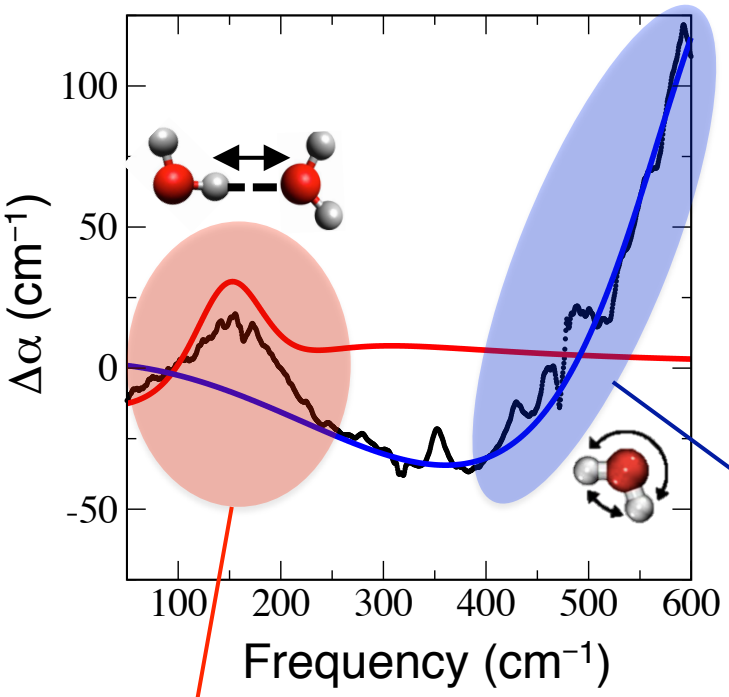

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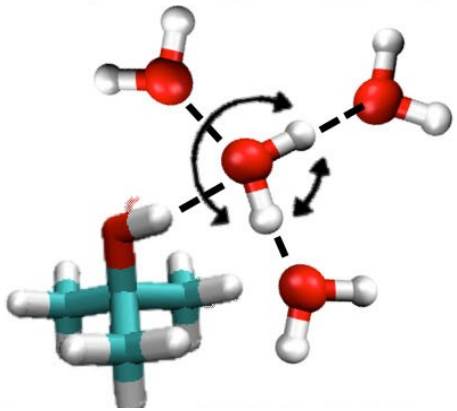

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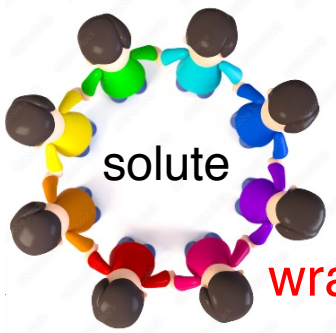

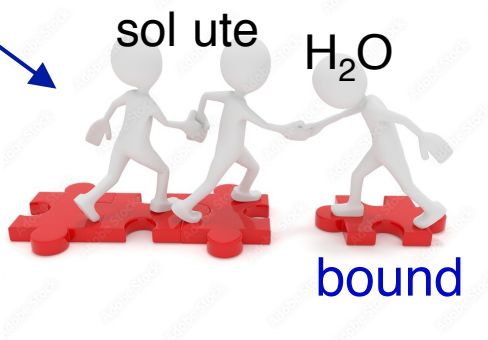

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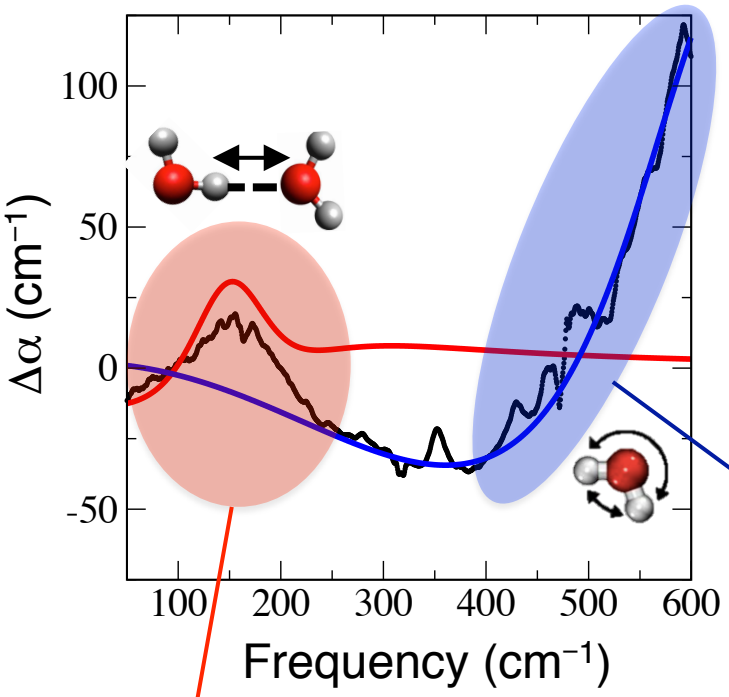

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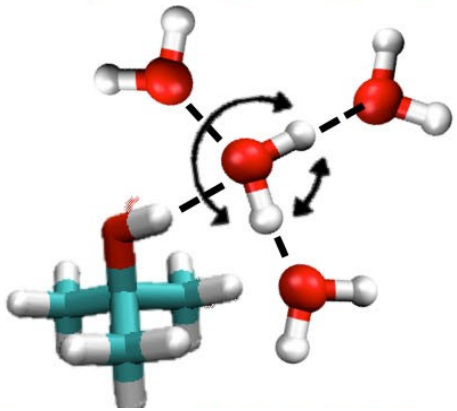

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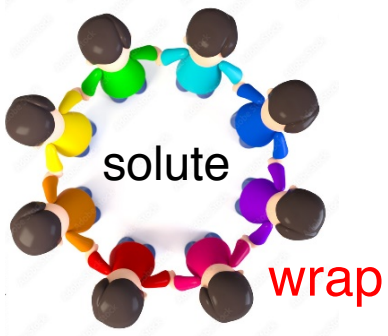
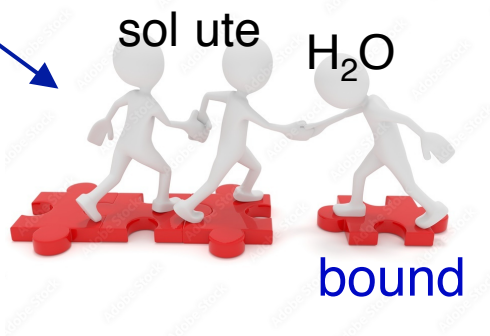


Same fingerprints
for > 20 solutes



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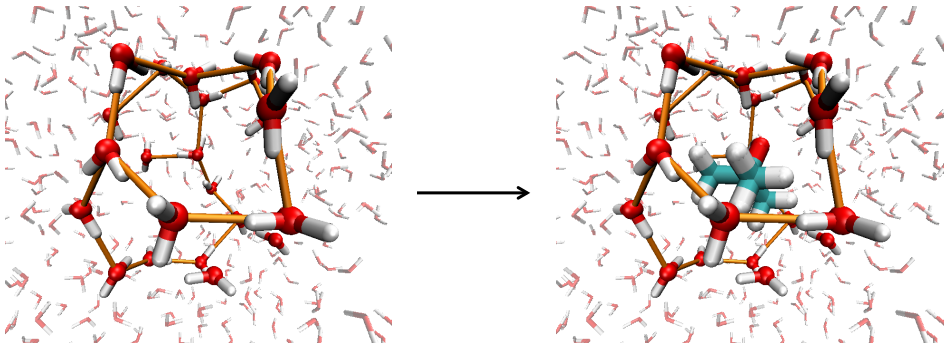
From $\Delta\alpha$ to ΔS and ΔH: the equations of THz-calorimetry

1. Create cavity

2. Insert solute



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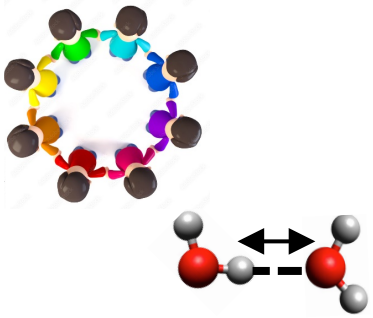

Δα wrap
amplitude

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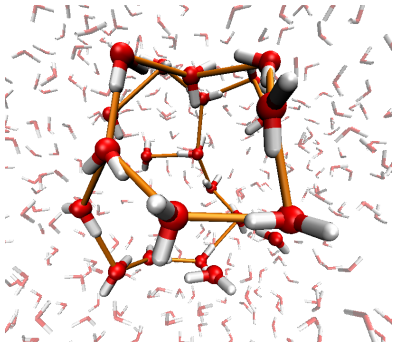

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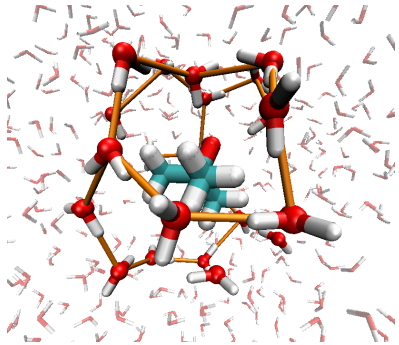
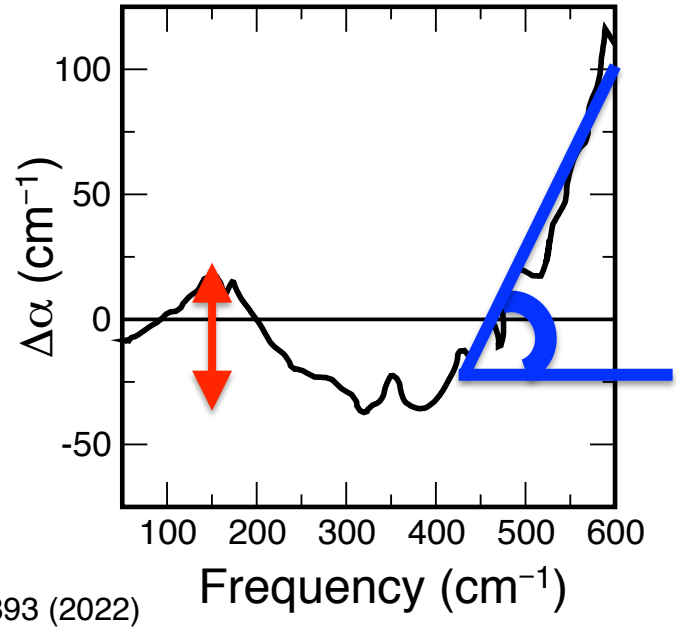


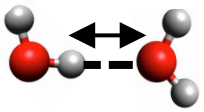
Δα bound
slope

$$\Delta G_{solv} = \Delta G_{cavity} + \Delta G_{bound}$$



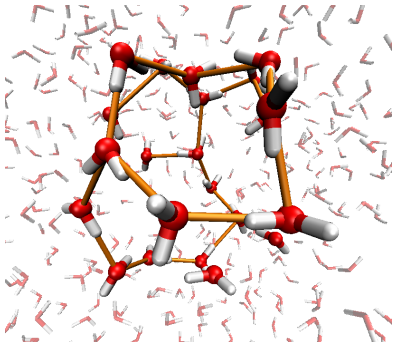
S. Pezzotti et al. **Angewandte**, e202203893 (2022)

From Δα to ΔS and ΔH: the equations of THz-calorimetry

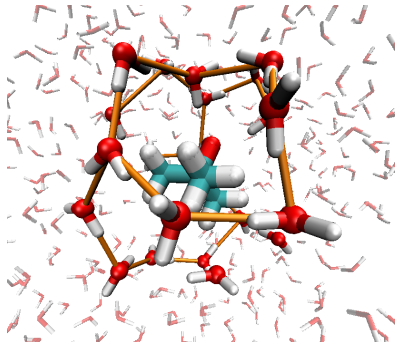


Δα wrap
amplitude

1. Create cavity



2. Insert solute



Δα bound
slope

$$\Delta G_{solv} = \Delta G_{cavity} + \Delta G_{bound}$$

$$\Delta G_{cavity} = \Delta\alpha_{wrap}\Delta\overline{H}_{wrap} - T\Delta\alpha_{wrap}\Delta\overline{S}_{wrap}$$

$$\Delta G_{bound} = \Delta\alpha_{bound}\Delta\overline{H}_{bound} - T\Delta\alpha_{bound}\Delta\overline{S}_{bound}$$

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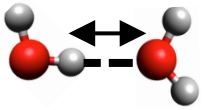

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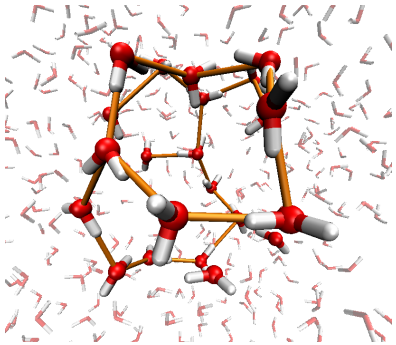

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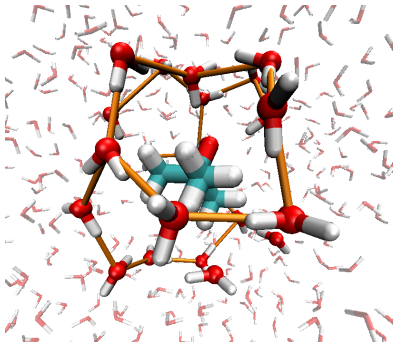

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Measured

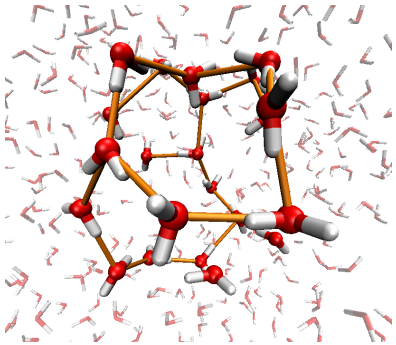
Wrap/bound THz fingerprints

S. Pezzotti et al. **Angewandte**, e202203893 (2022)

From Δα to ΔS and ΔH: the equations of THz-calorimetry

Δα wrap
amplitude

1. Create cavity



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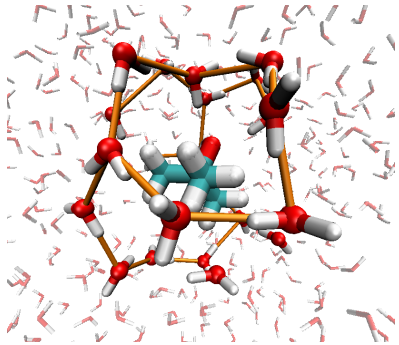

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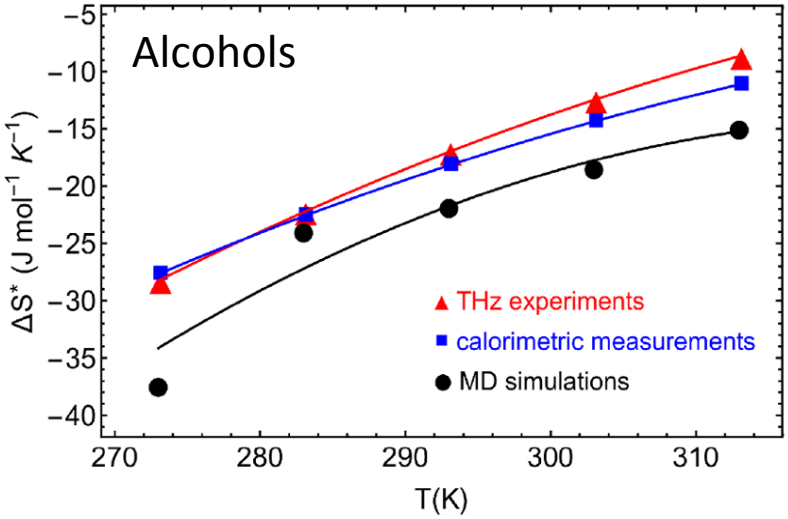
Measured
Wrap/bound THz fingerprints

Parametrized
once and for all

S. Pezzotti et al. **Angewandte**, e202203893 (2022)

What can we do with THz-calorimetry?

✓ Accurate solvation free energies



V. C. Nibali et al., **JPCL**, 11, 4809 (2020).

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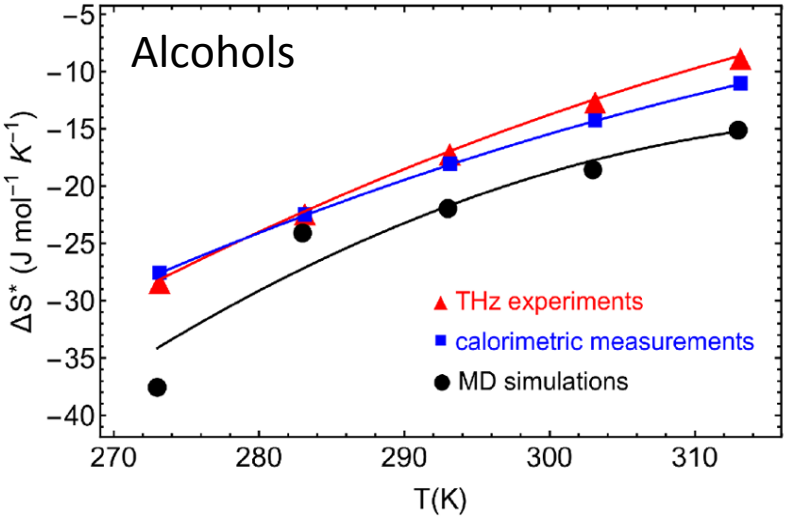

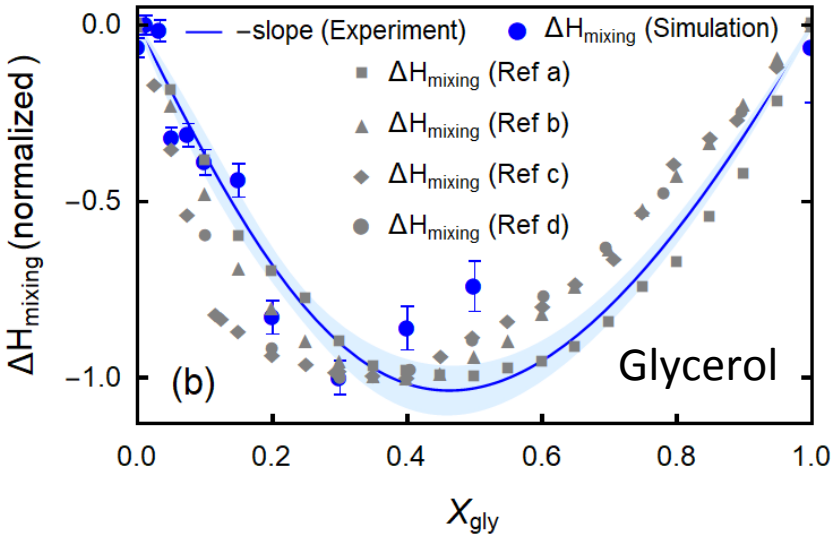

V. C. Nibali et al., **JPCL**, 11, 4809 (2020).
D. Das Mahanta et al., ***Chem. Sci.*** *(2023)*

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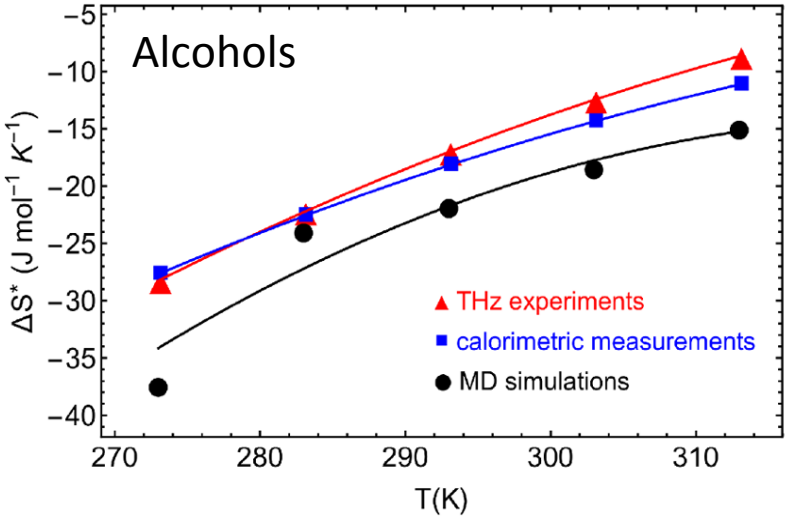
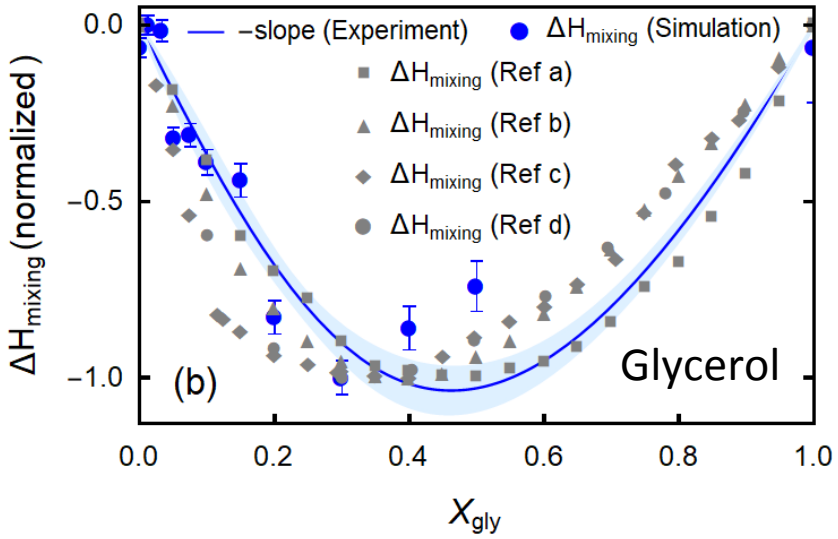
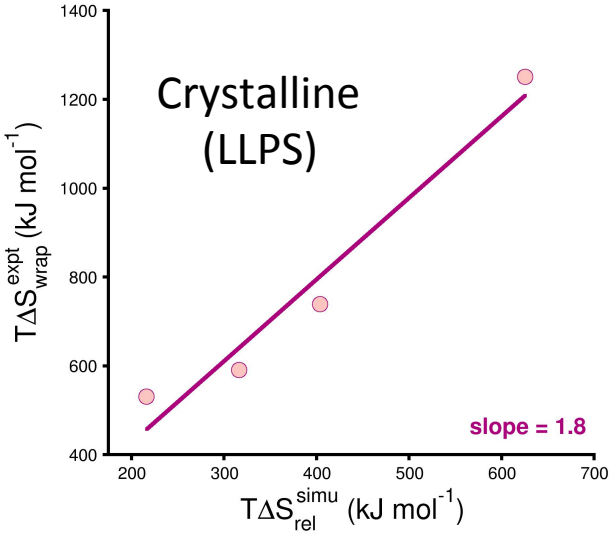


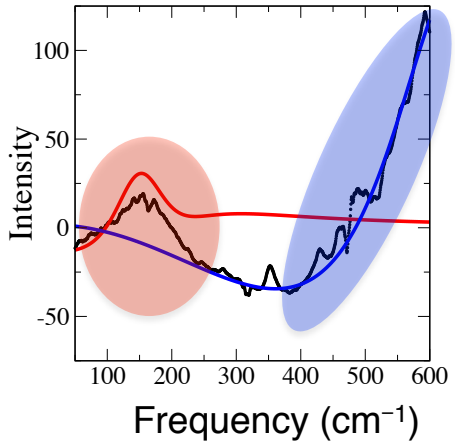


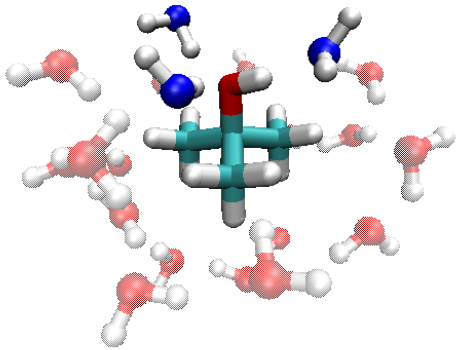


What can we do with THz-calorimetry?

✓ Accurate solvation free energies

✓ Molecular interpretation





S. Pezzotti et al. **Angewandte**, e202203893 (2022)

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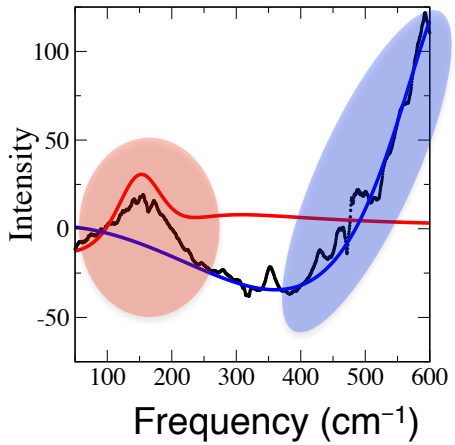

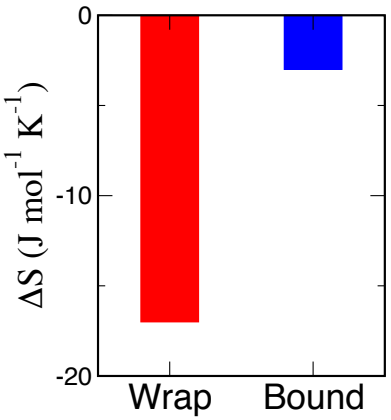

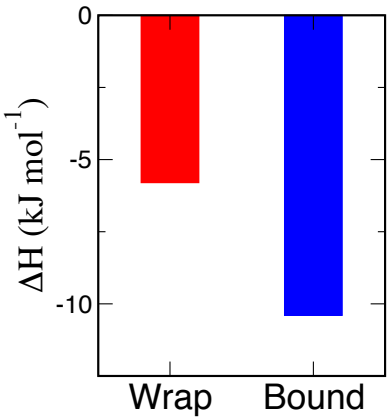

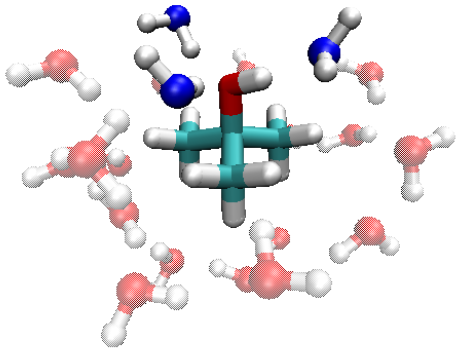

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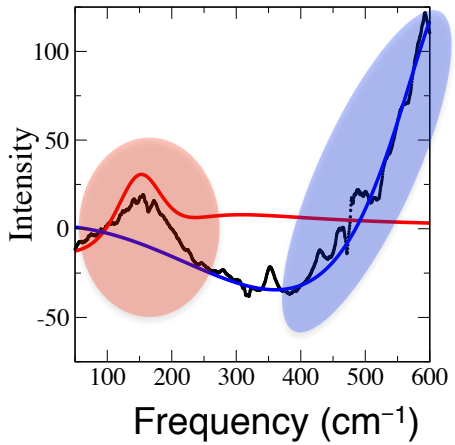
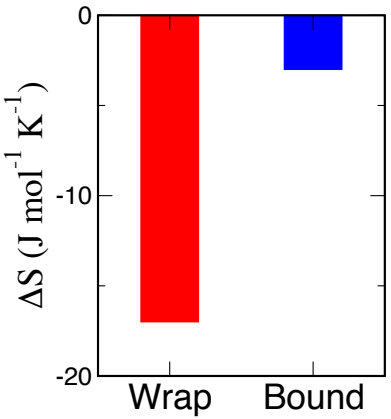
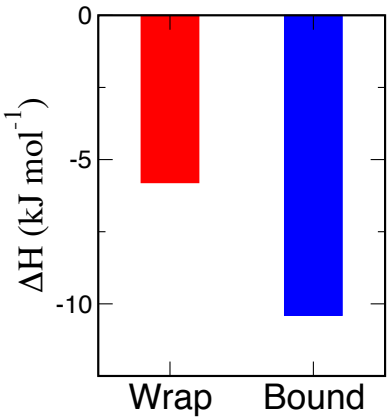
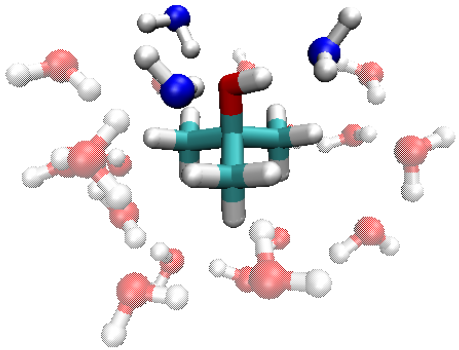








Experimental proof of a key theoretical assumption!

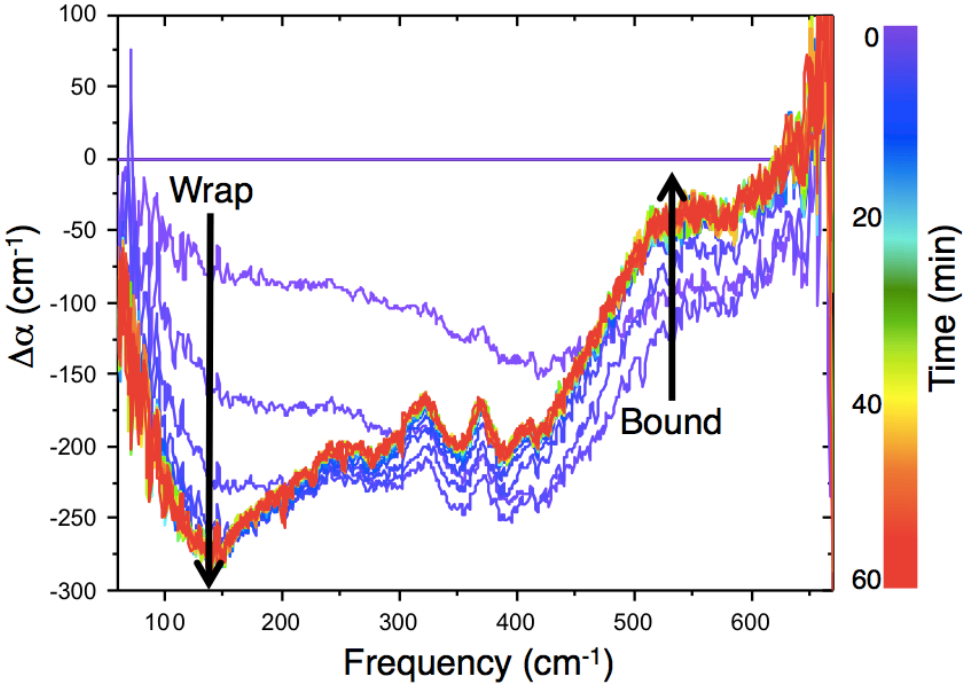
S. Pezzotti et al. **Angewandte**, e202203893 (2022)

What can we do with THz-calorimetry?

✓ Accurate solvation free energies

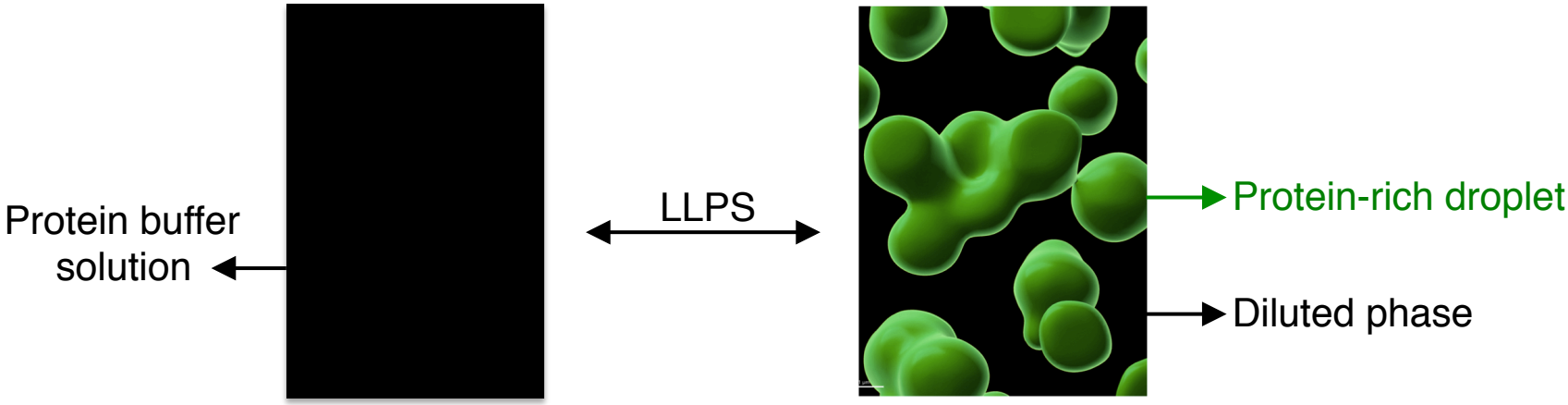
✓ Molecular interpretation

✓ Watch free energy changes in real-time



S. Pezzotti et al. **JPCL**, 14, 1556 (2023)

Liquid-liquid phase separation (LLPS)



fluorescence microscopy of Fused in Sarcoma (FUS) – 10 mM

J. Ahlers et al. **Biophys. J.** 120, 1266 (2021)

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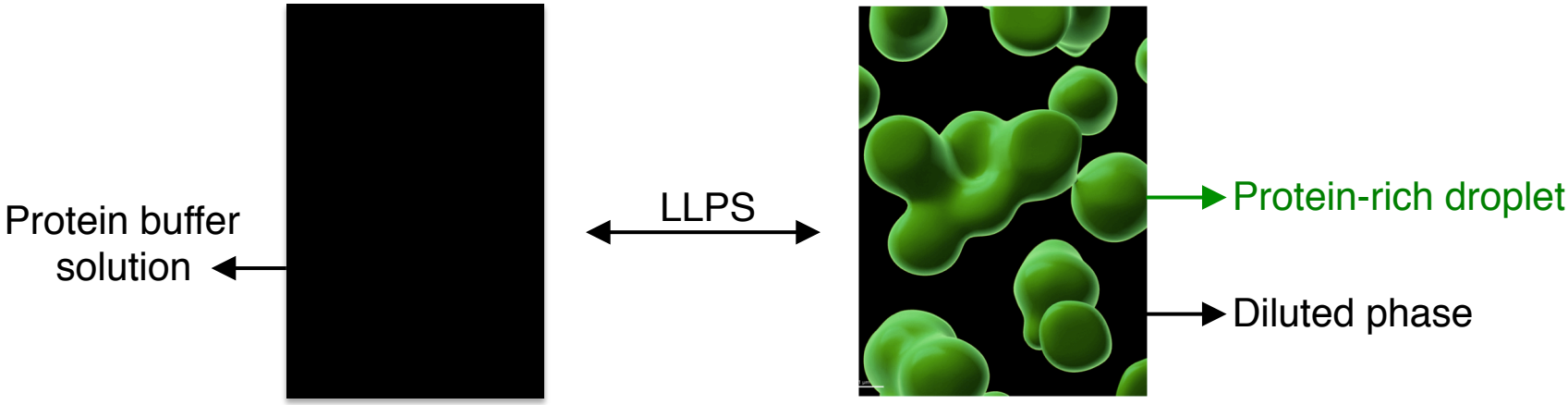

fluorescence microscopy of Fused in Sarcoma (FUS) – 10 mM

- **Biological relevance**: membran-less compartmentalization
- **Medical relevance**: pathological protein aggregates

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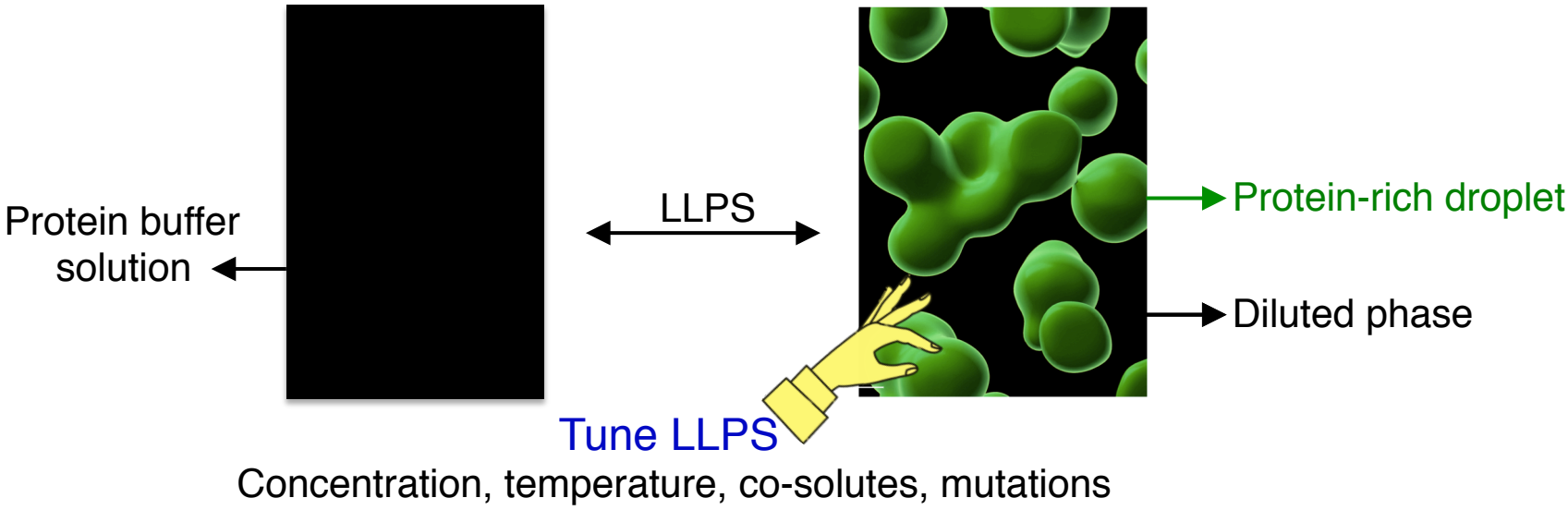

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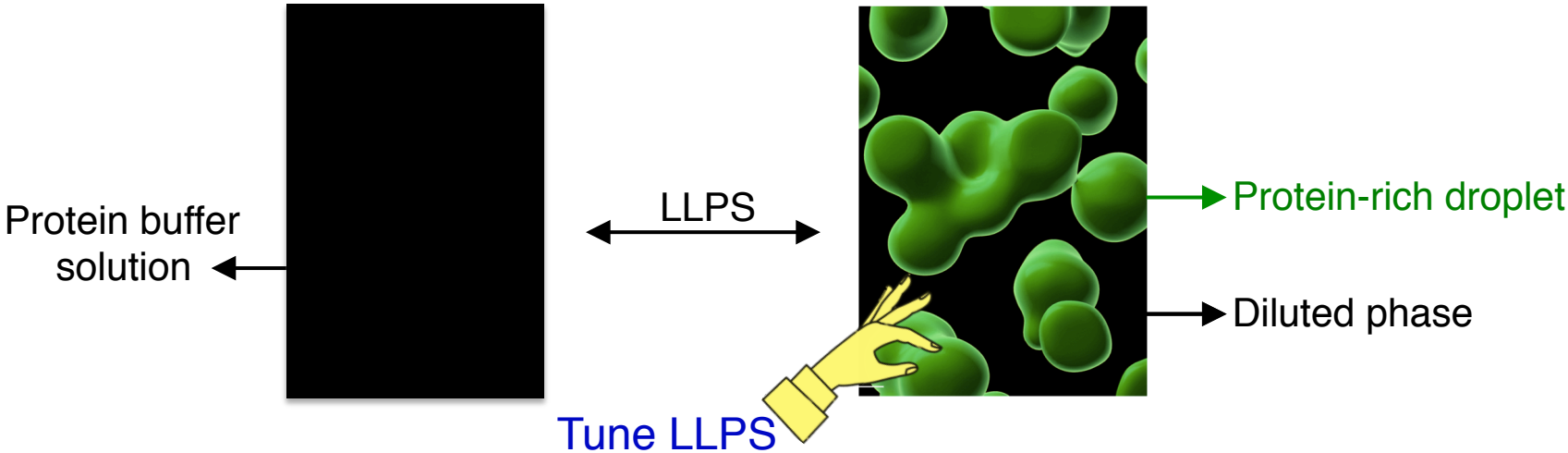
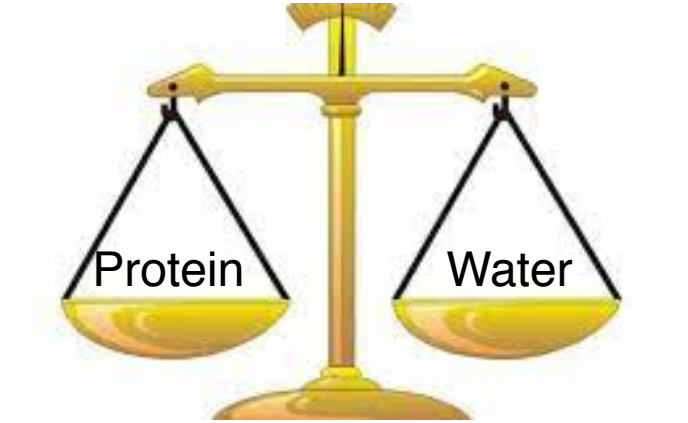


$$\delta G_{LLPS} = \Delta G_{p-p} + \Delta G_{cavity} + \Delta G_{bound}$$



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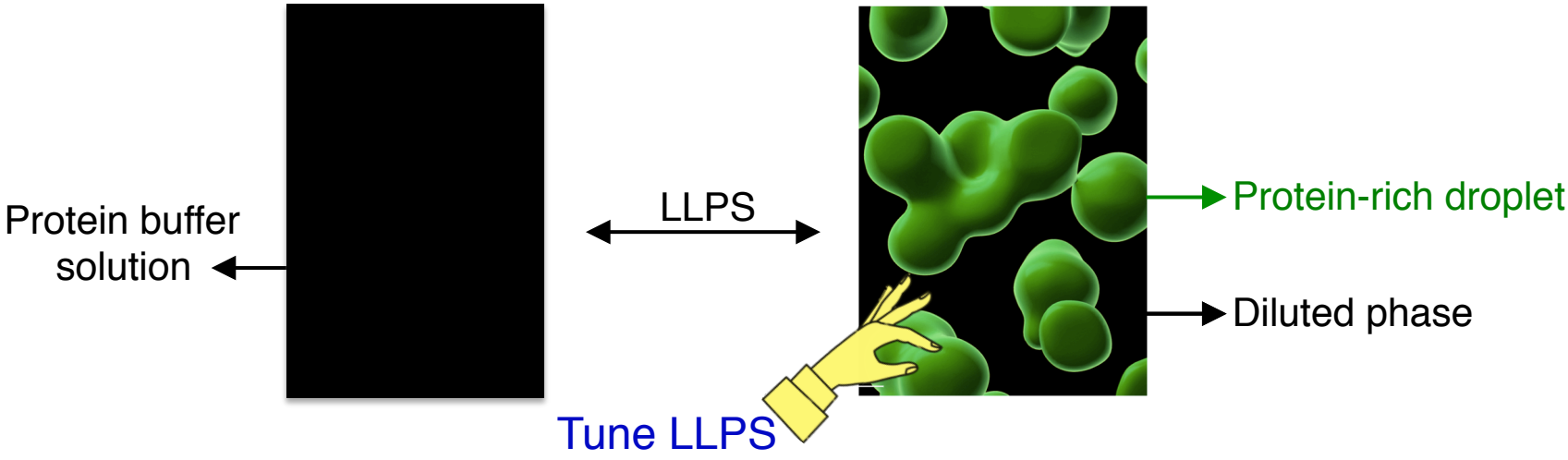

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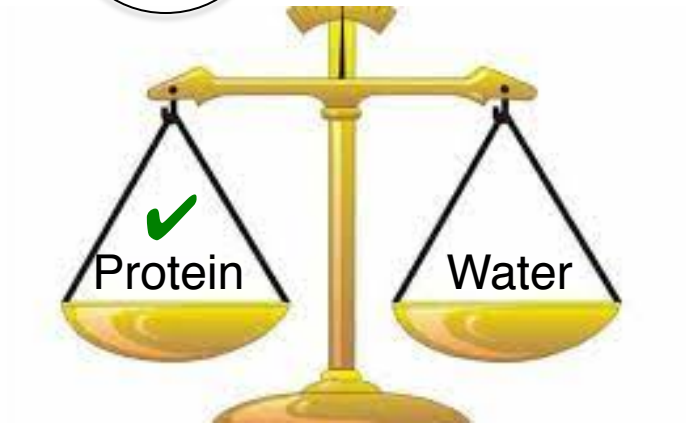

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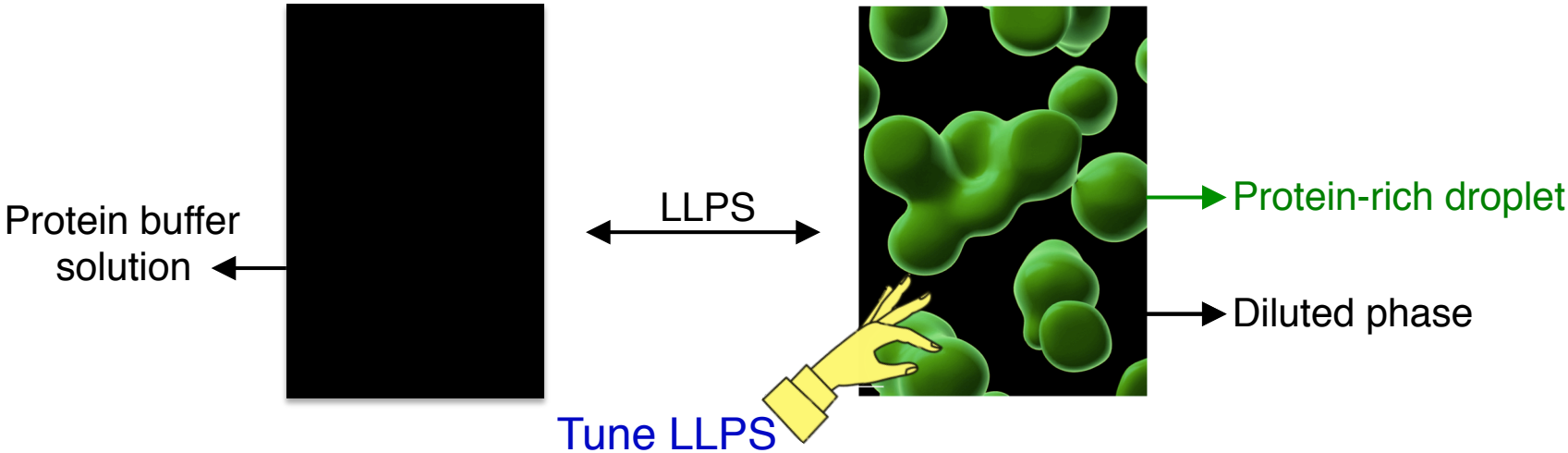

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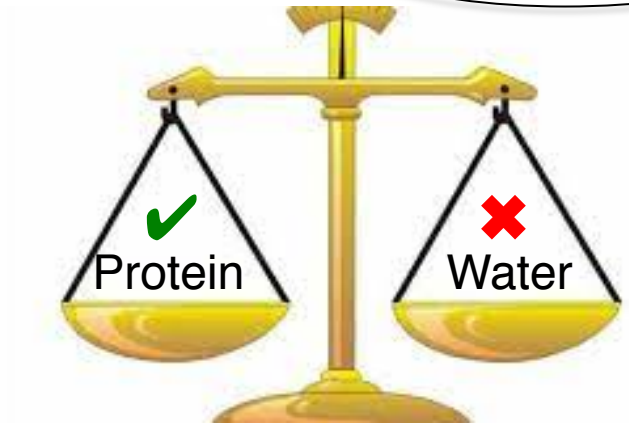
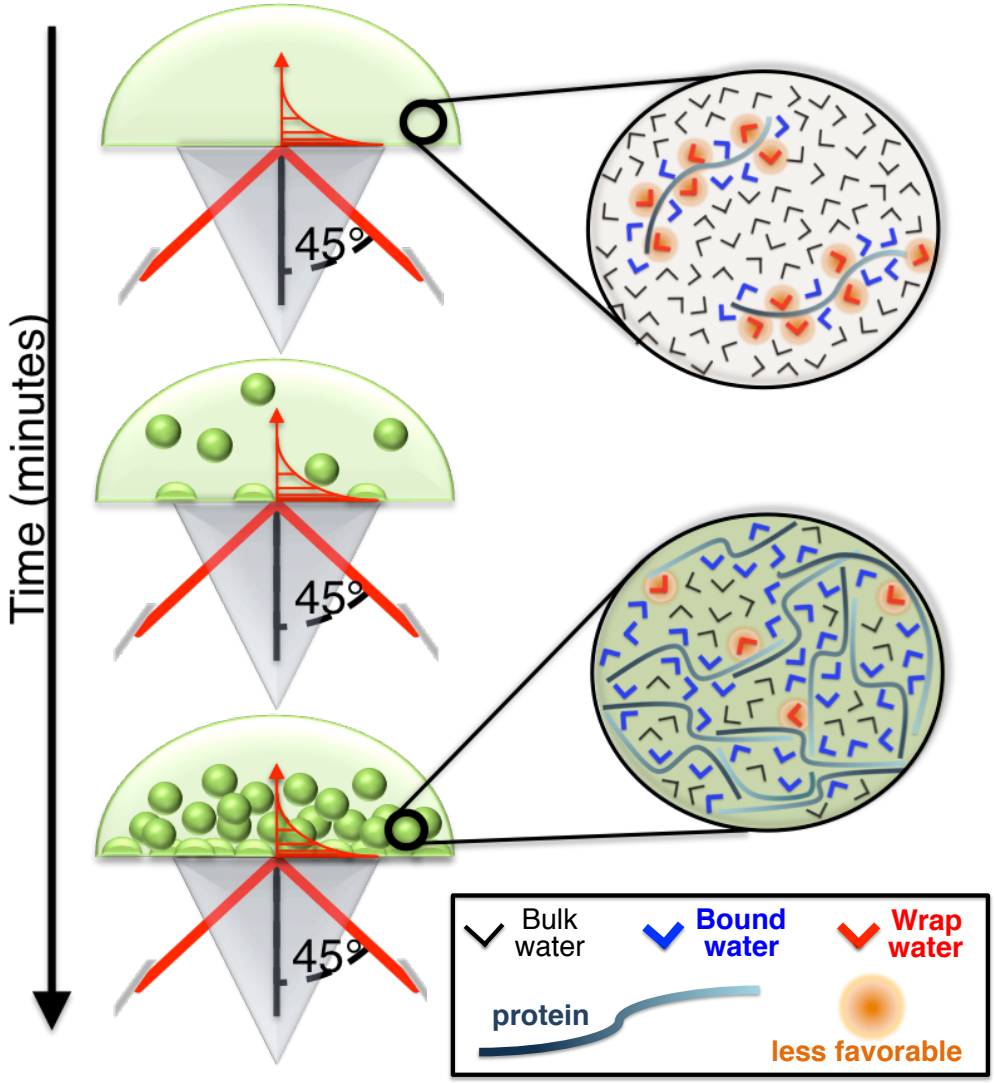


Liquid-liquid phase separation? Ask the water!



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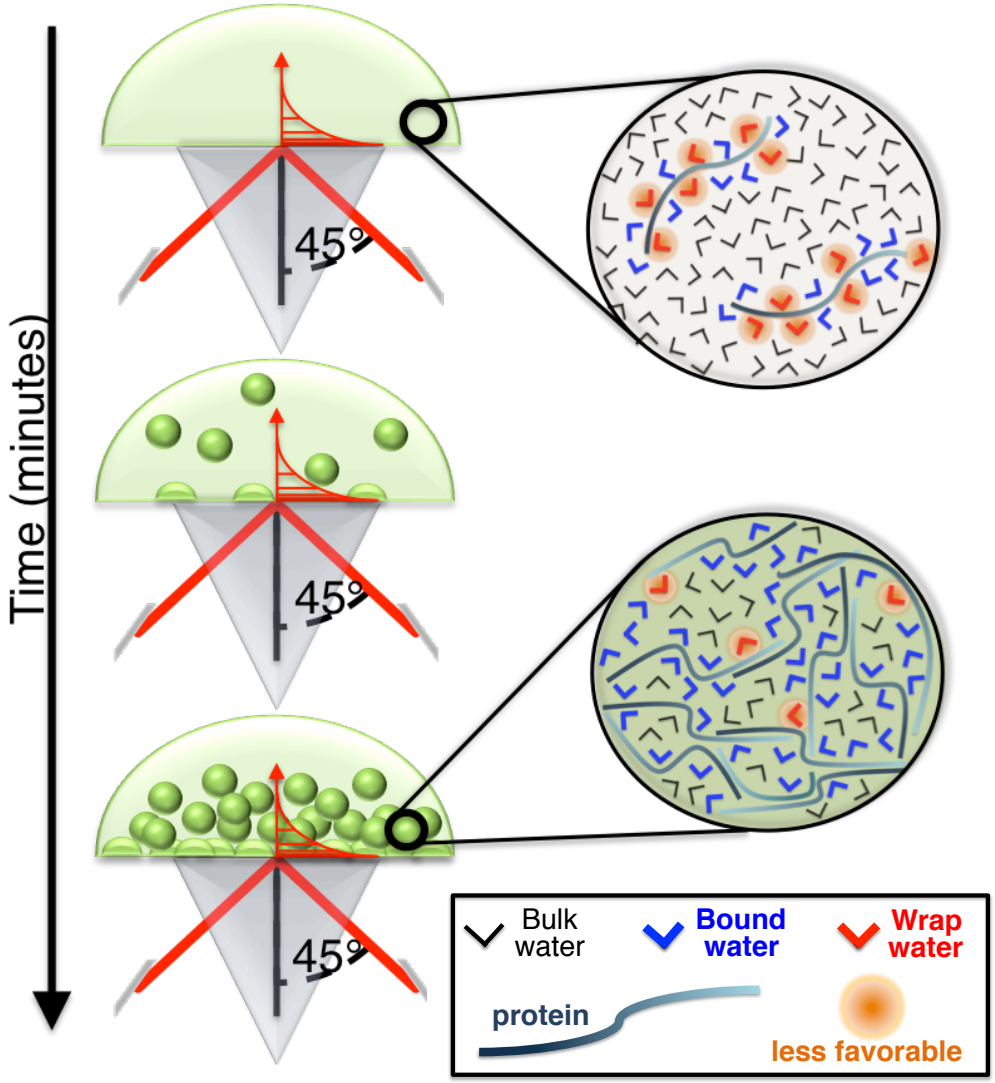

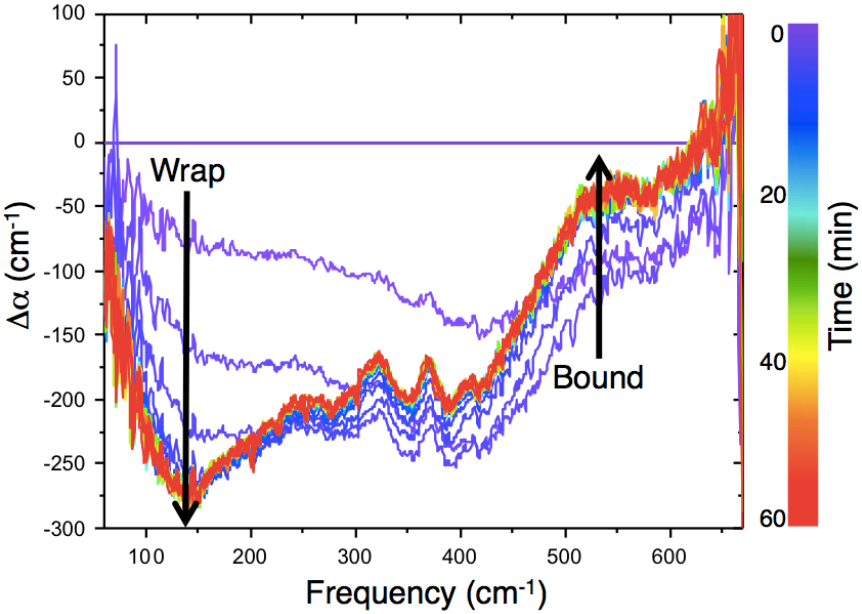

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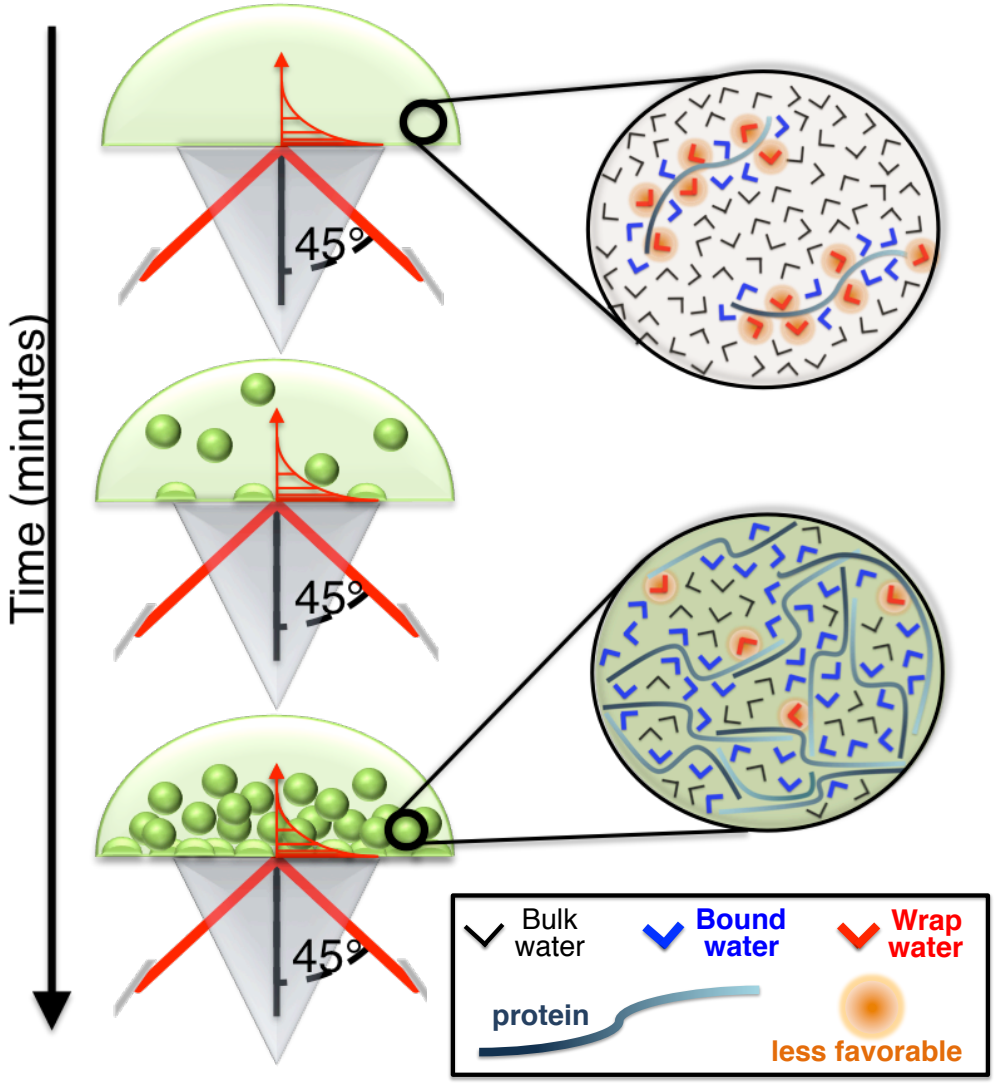

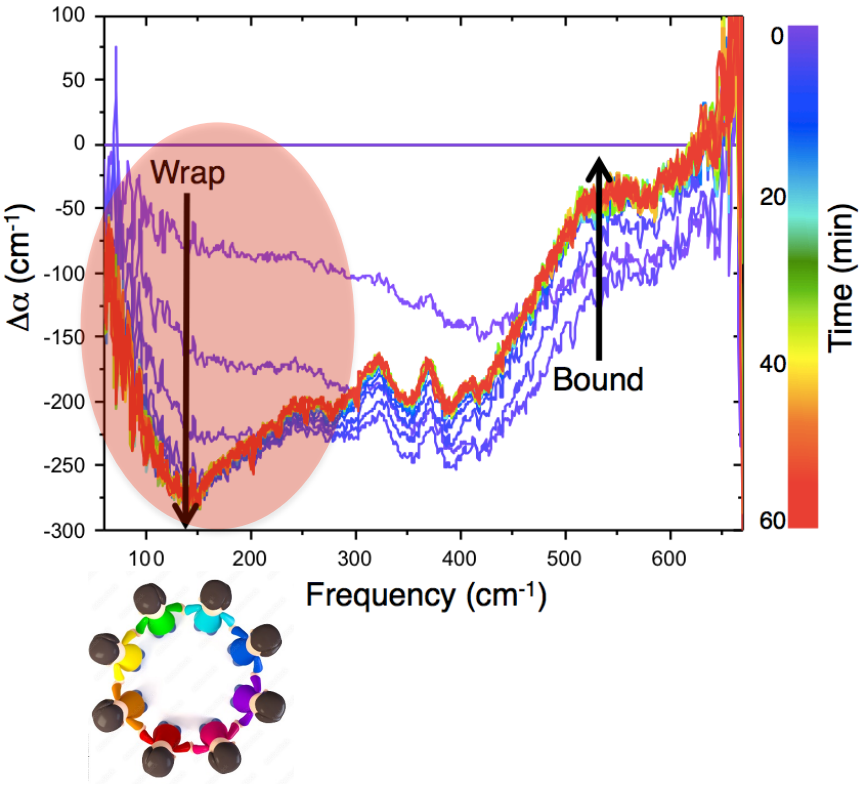

- Wrap (Hydrophobic) water is released

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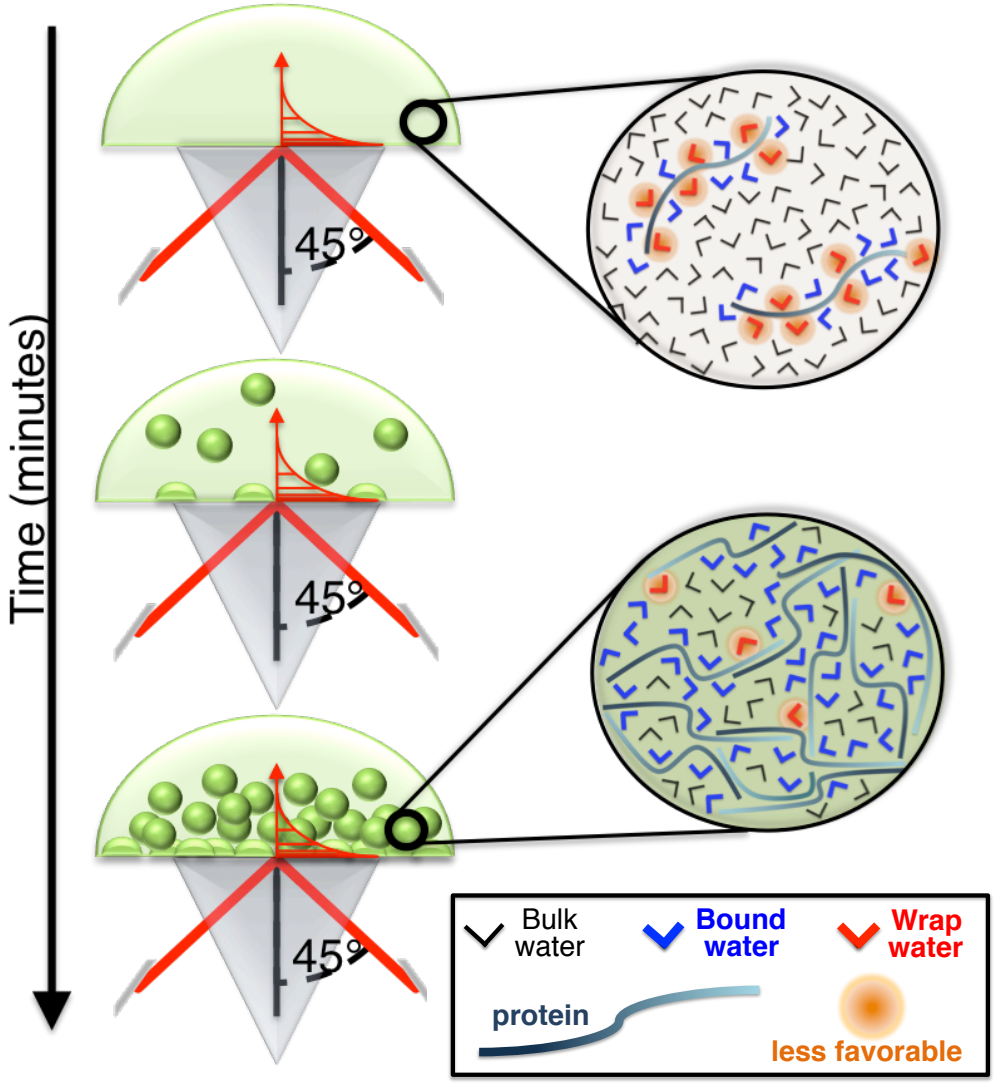

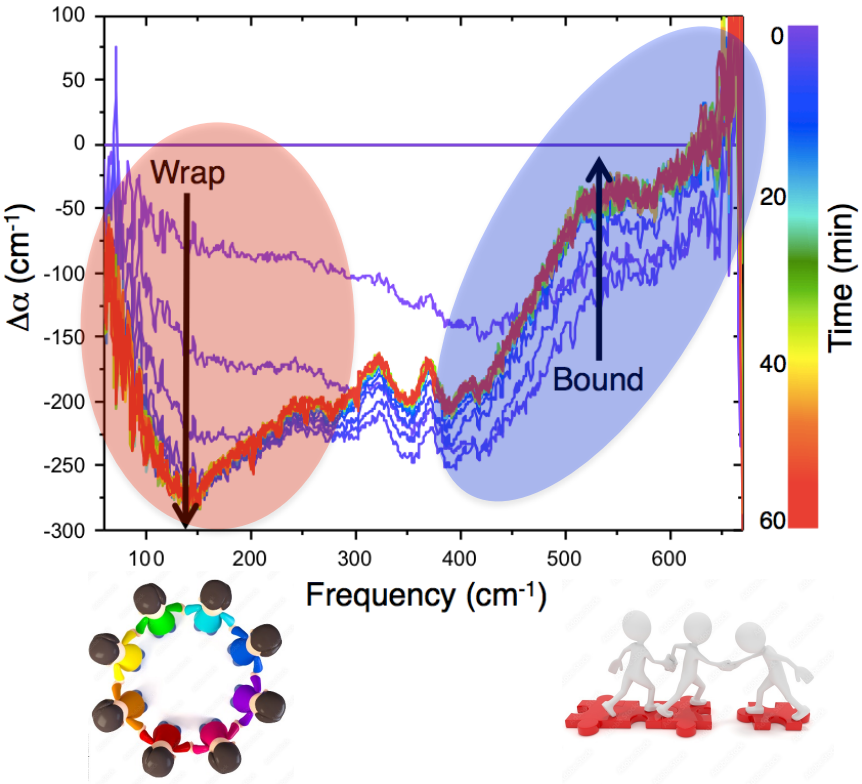

- Wrap (Hydrophobic) water is released
- Bound (Hydrophilic) water is retained

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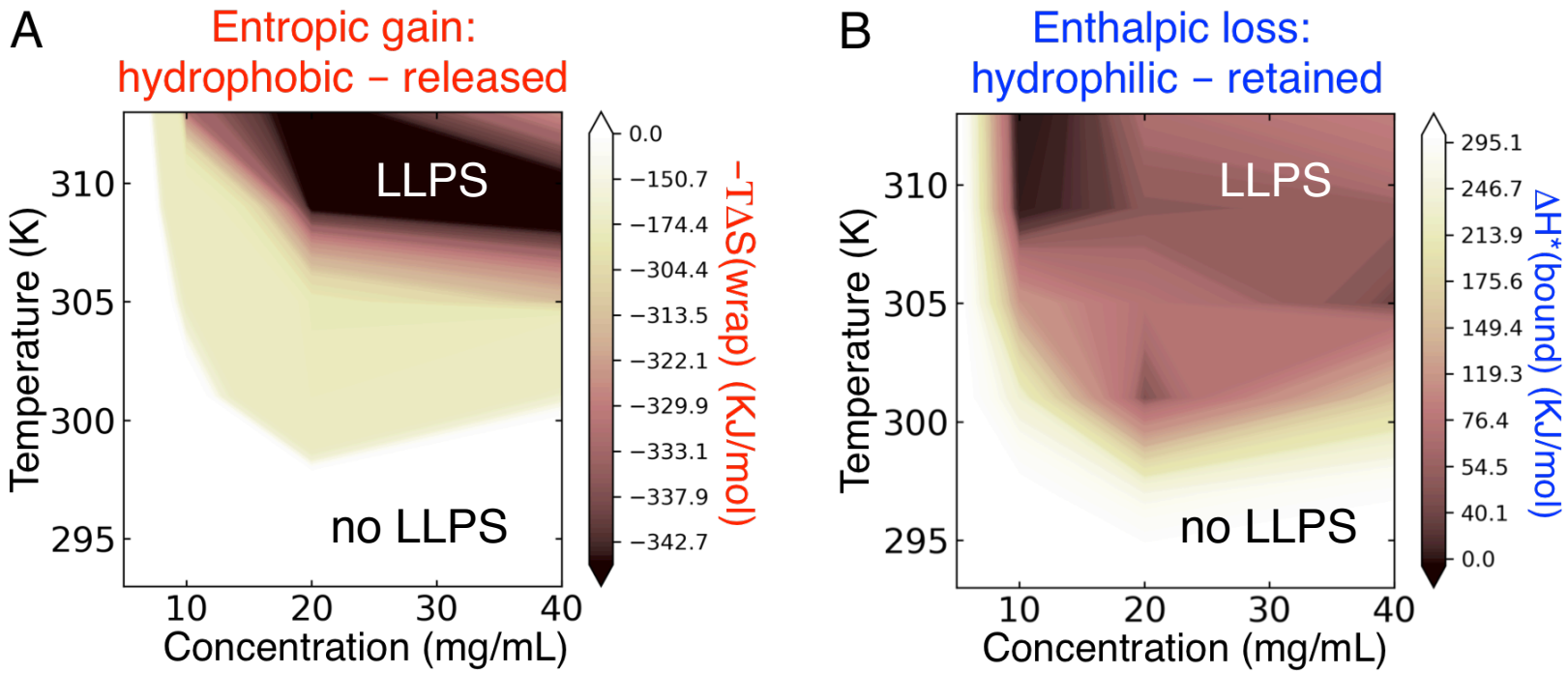
Liquid-liquid phase separation? Ask the water!



König et al. *under review* (2023)
Pezzotti et al. **JPCL**, 14, 1556 (2023)

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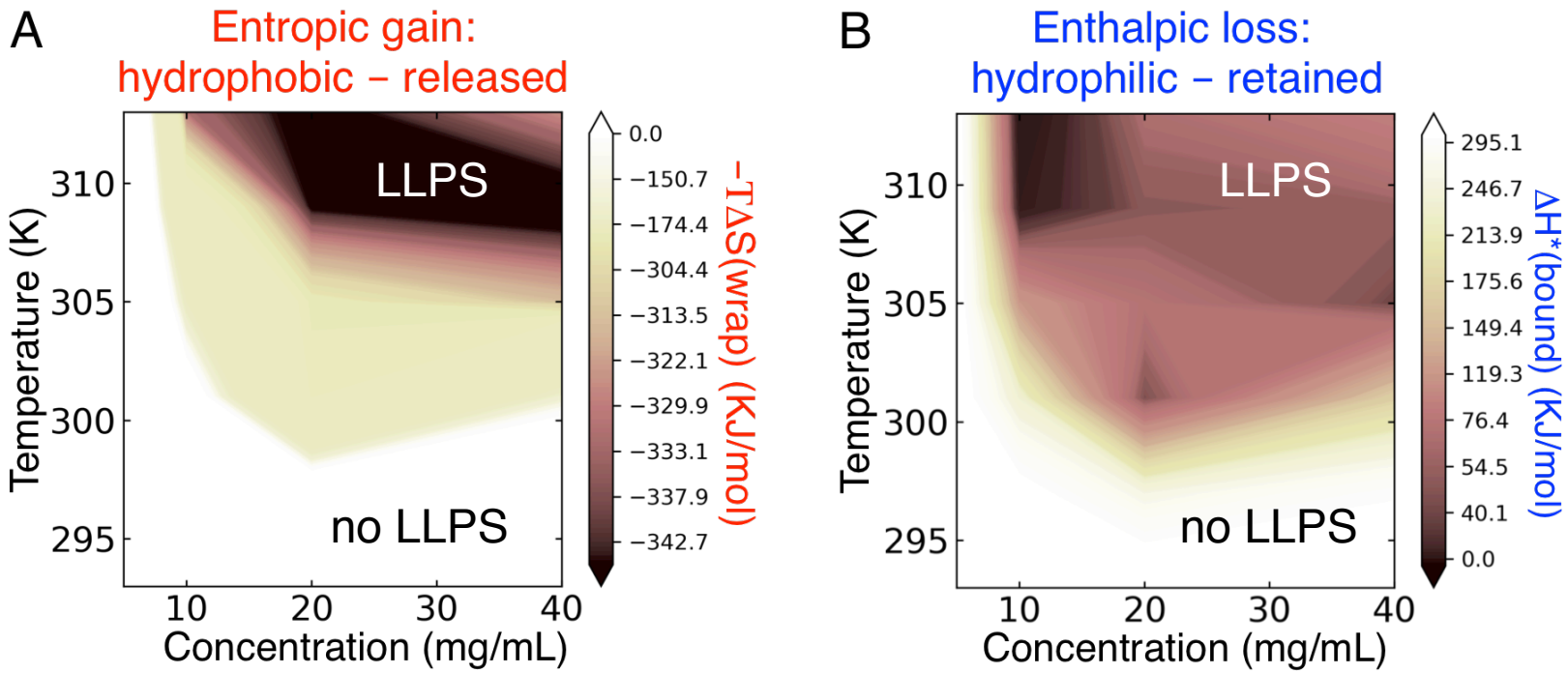

- We can determine if LLPS takes place

König et al. *under review* (2023)
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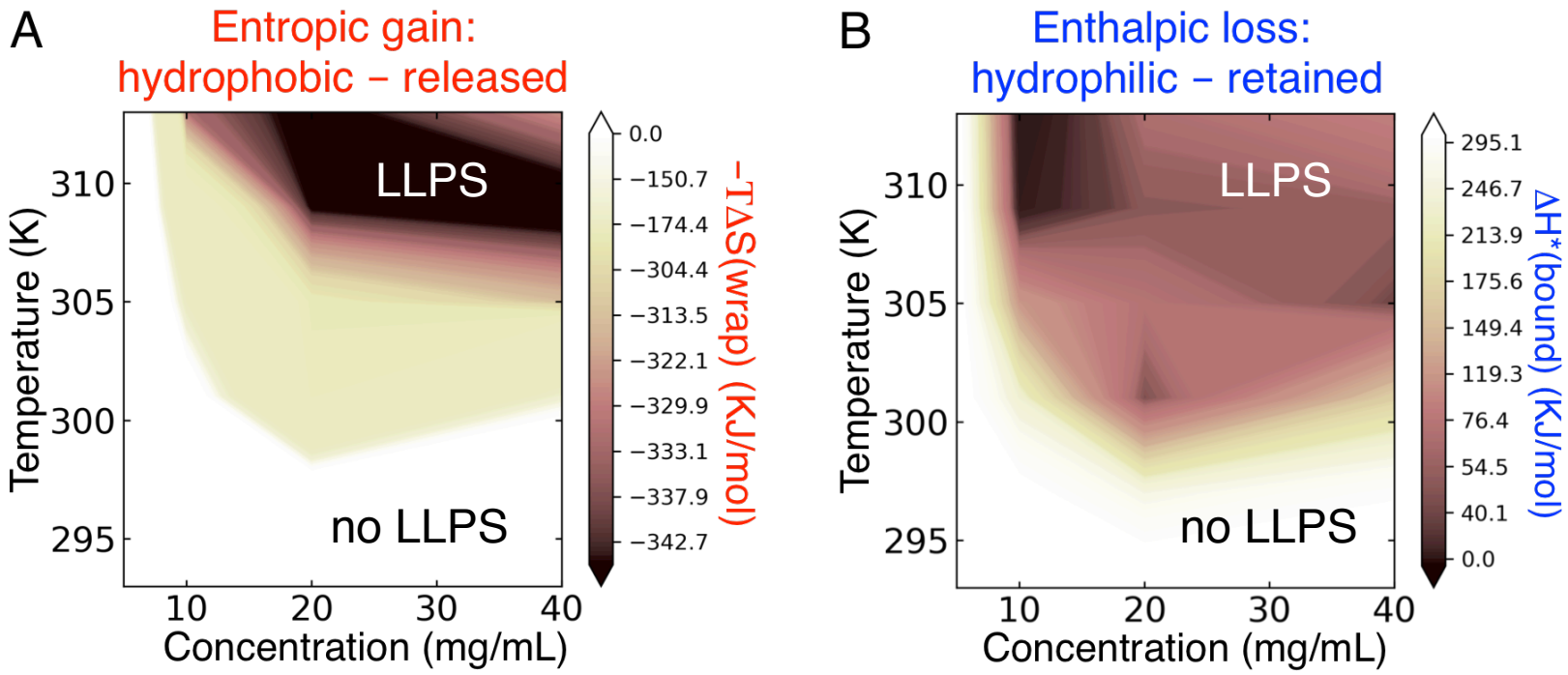

- We can determine if LLPS takes place
- The individual terms are huge, but compensate.

König et al. ***Biophys. J.**, just accepted* (2023)
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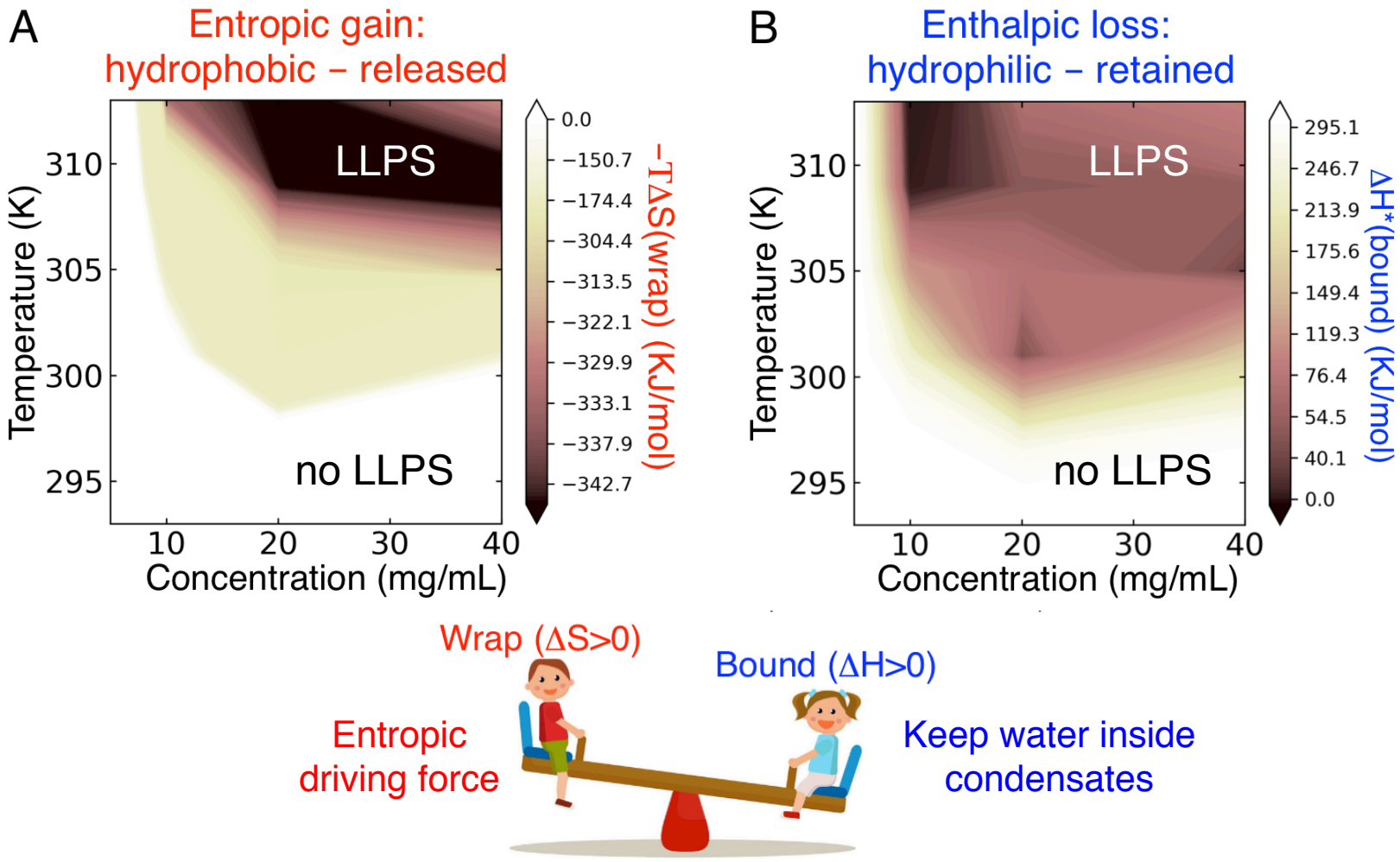
Liquid-liquid phase separation? Ask the water!



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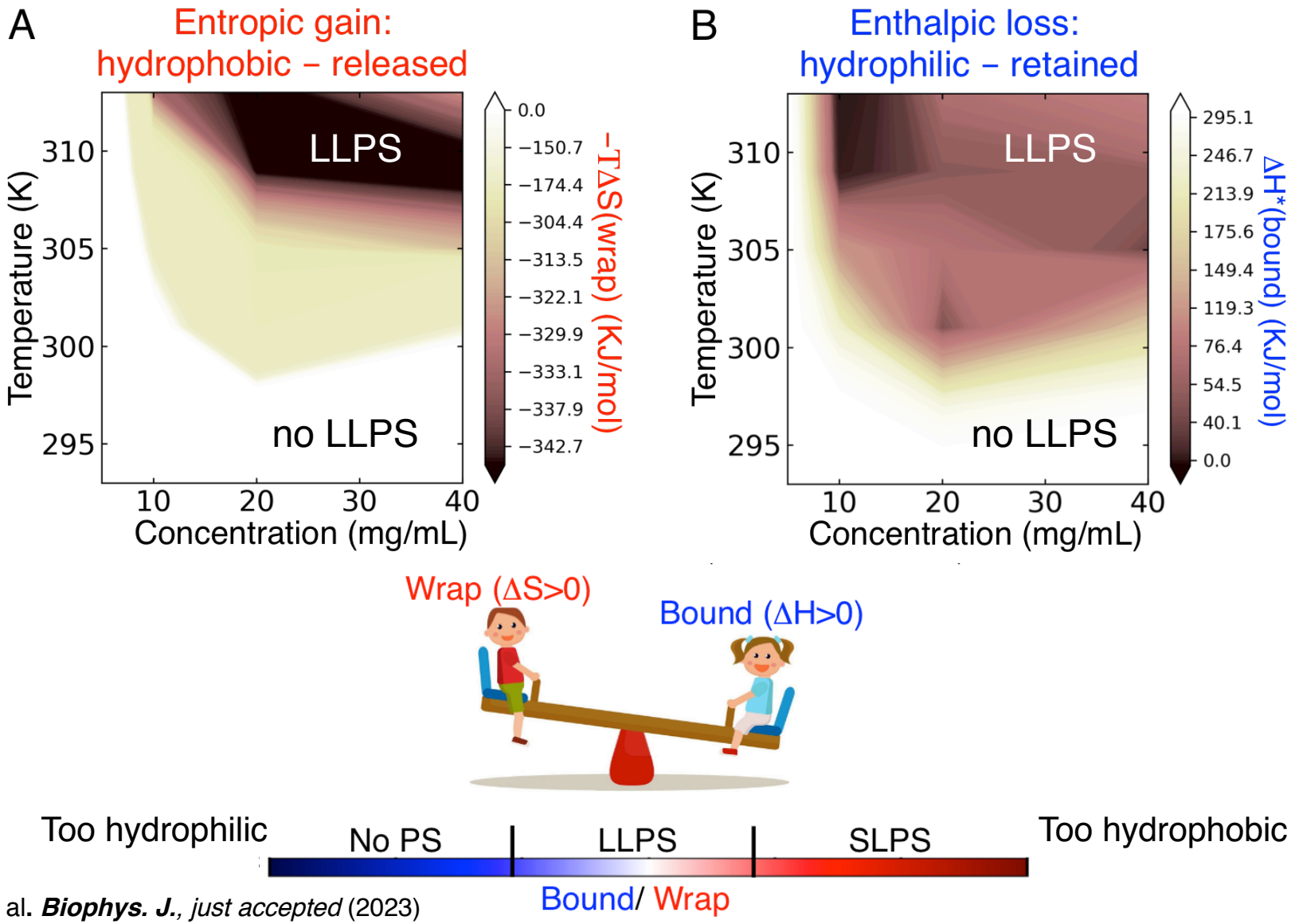

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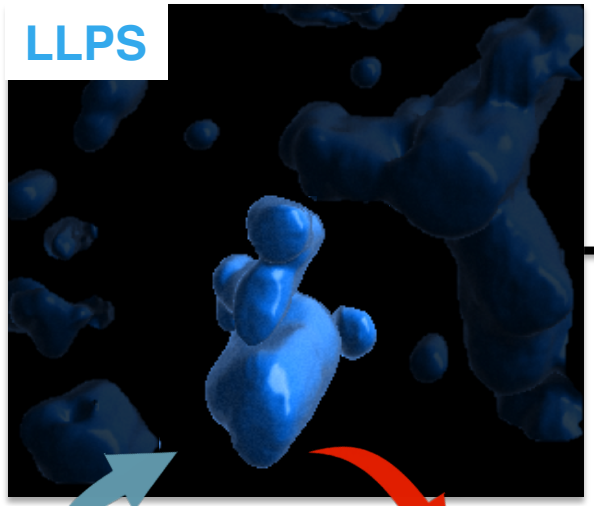
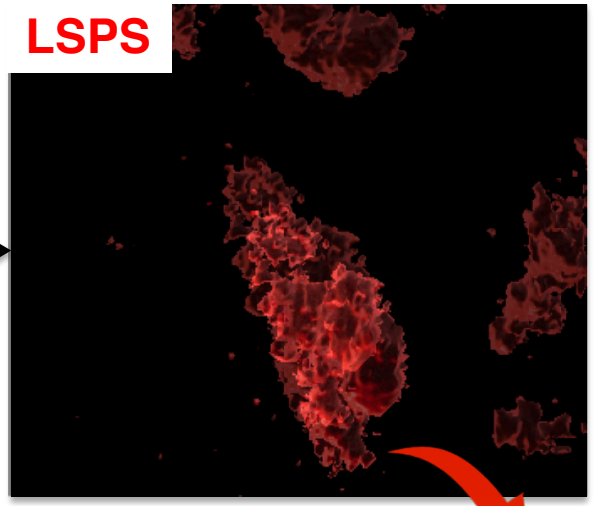
Tuning the hydrophilic/hydrophobic balance



Ad-hoc mutations

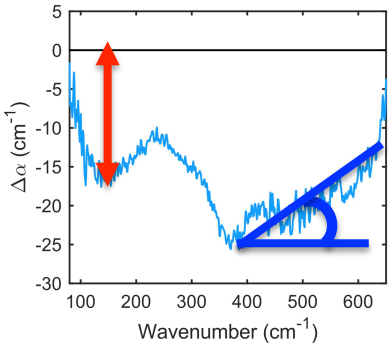


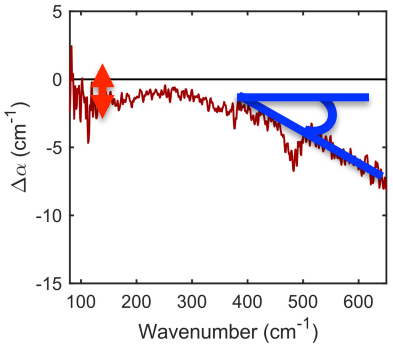
Retained
Bound–Hydrophilic

Released
Wrap–Hydrophobic

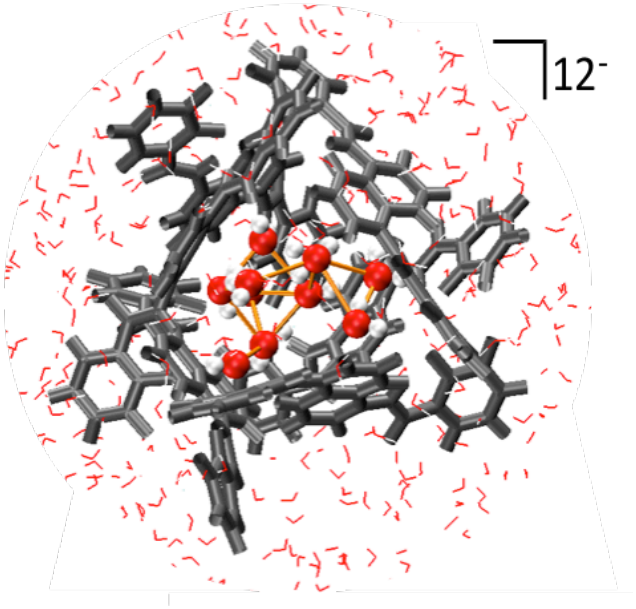
Retained
Bound–Hydrophilic

Released
Wrap–Hydrophobic





Increasing efficiency of a supramolecular catalyst



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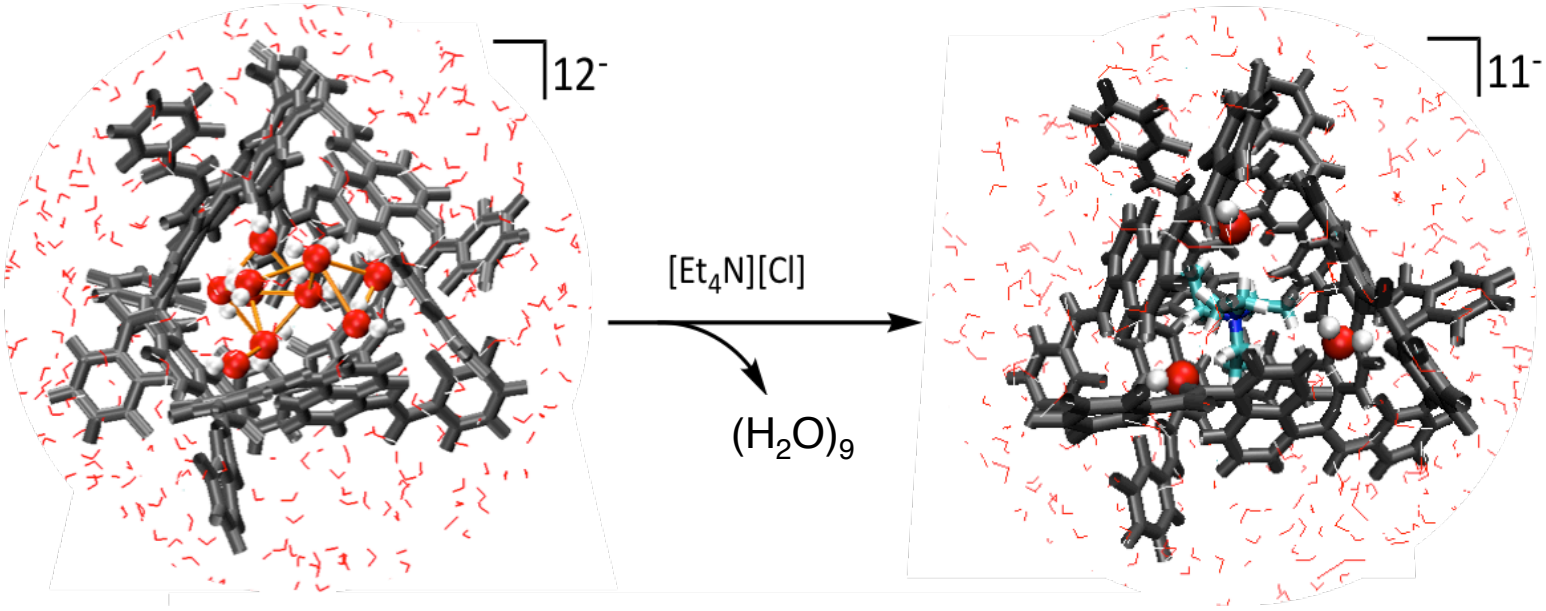

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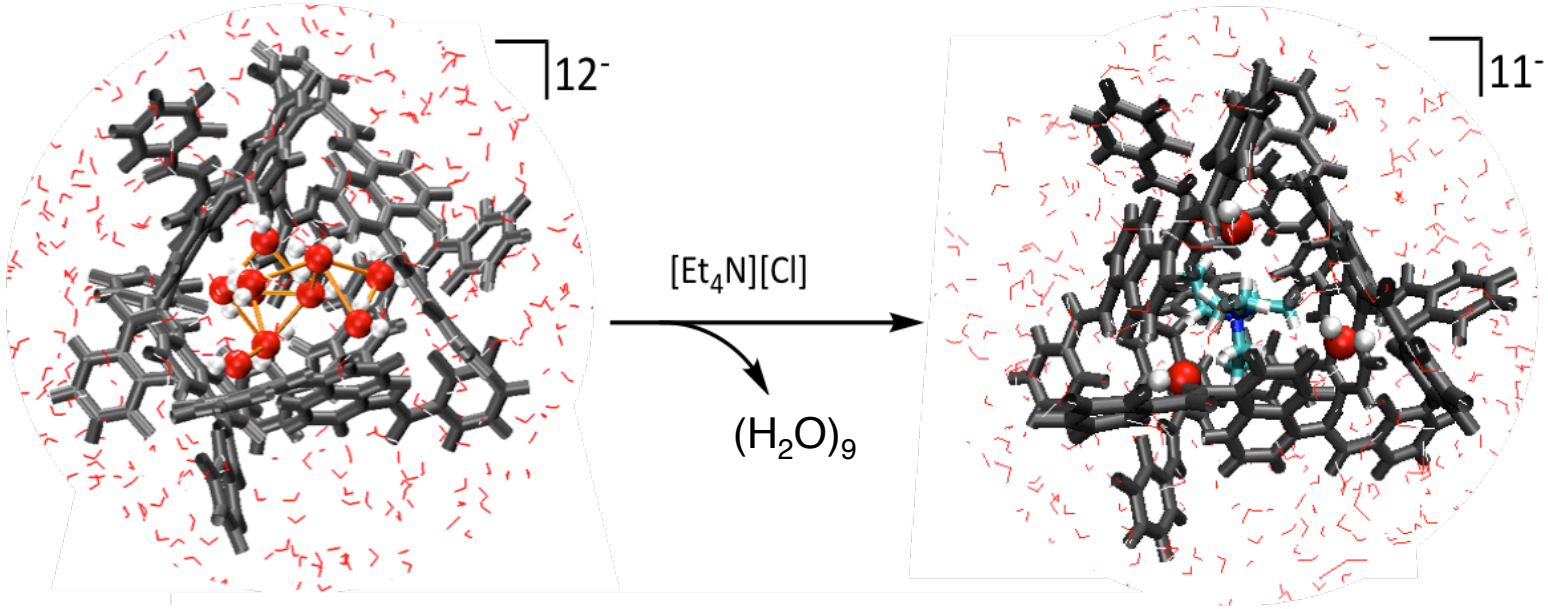
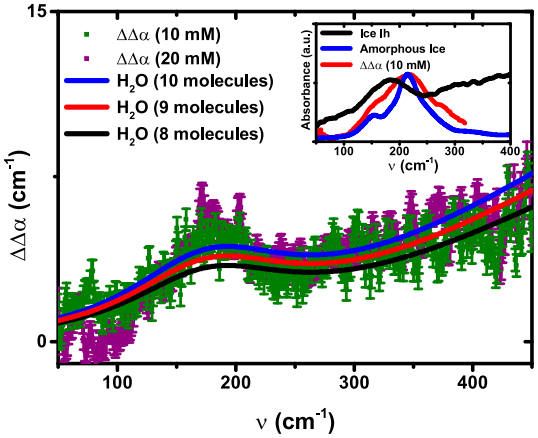
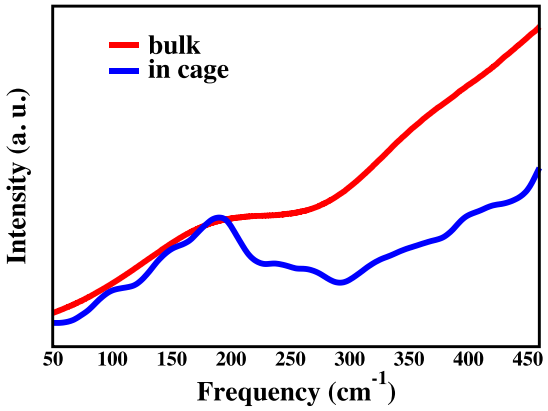


Measured



DFT-MD



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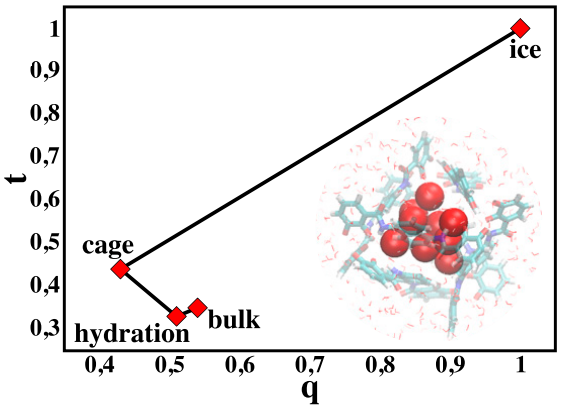

Different from any other water phase

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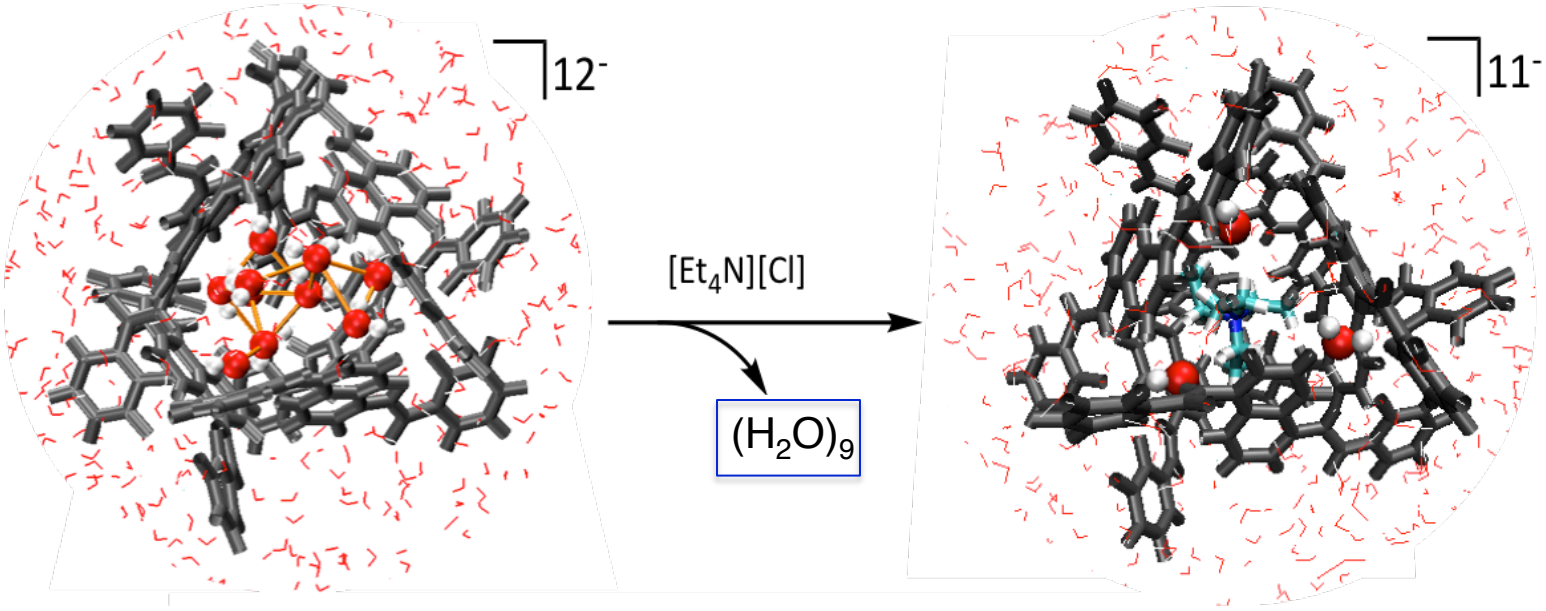
Increasing efficiency of a supramolecular catalyst



Confined water release

=

entropic driving force

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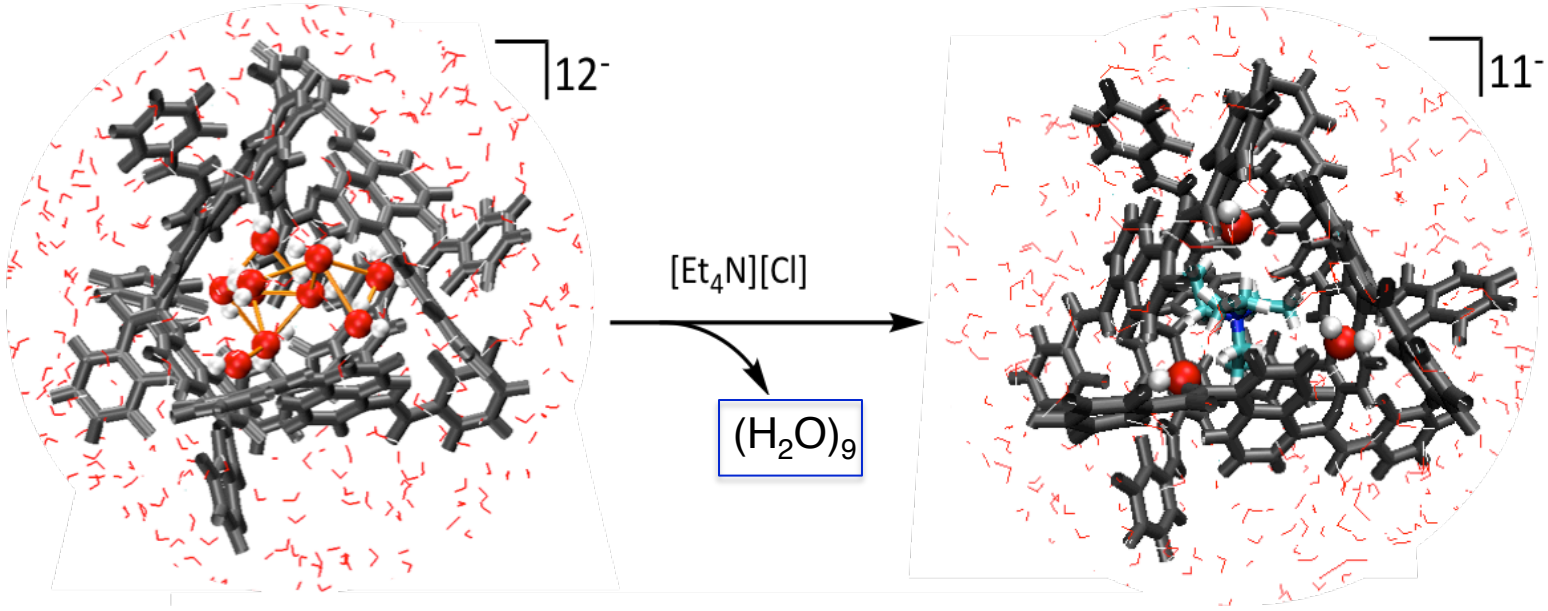
Increasing efficiency of a supramolecular catalyst

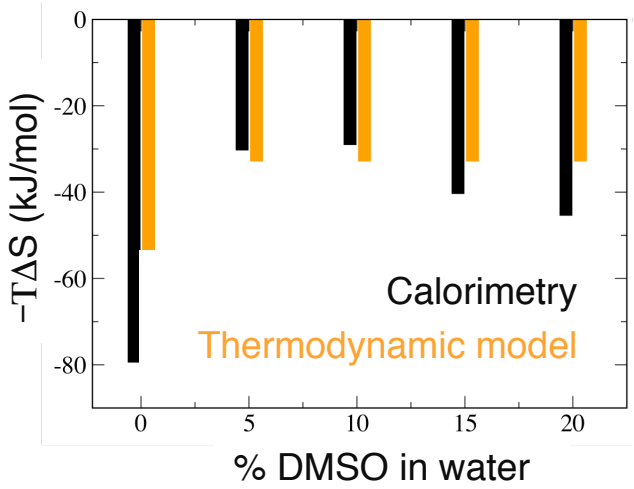


Confined water release

=

entropic driving force

Regulate guest encapsulation



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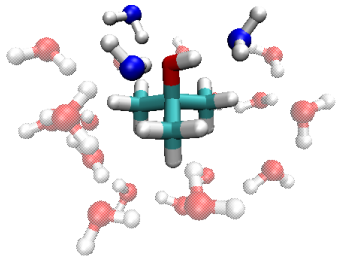
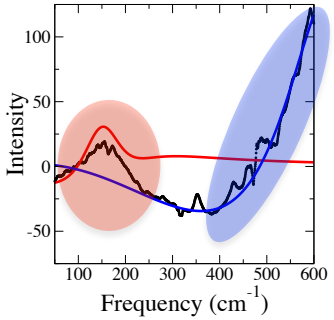
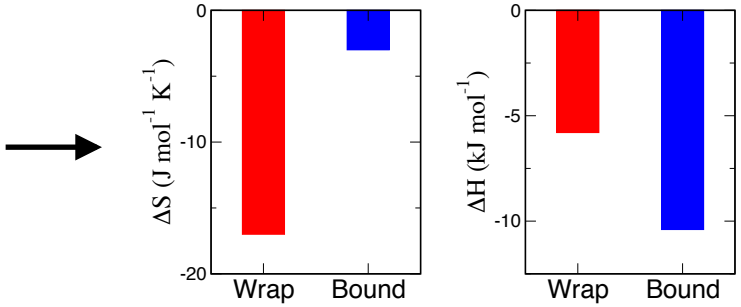
Conclusions on THz calorimetry

- We can directly correlate solvation structure-spectroscopy-thermodynamics
- We can dissect local hydrophobic & hydrophilic hydration contributions







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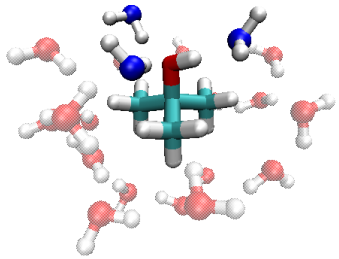
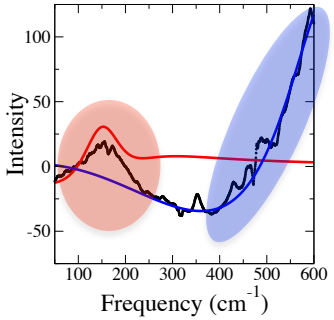
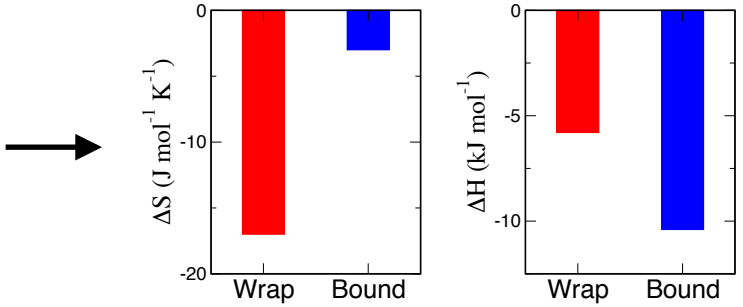
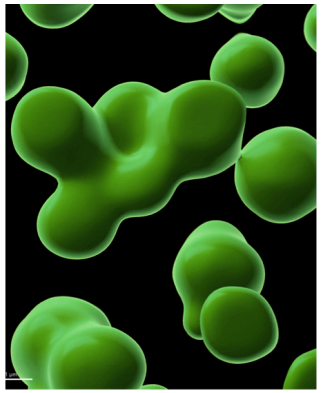


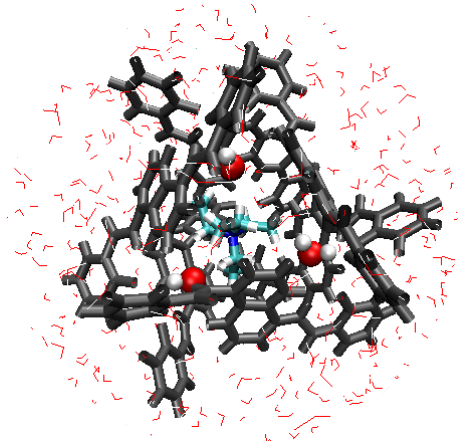




- We can reveal solvation driving forces to catalytic and biological processes in real-time



LLPS



F. Sebastiani, et al., **PNAS**, 2020, 117, 32954

Acknowledgments

Martina Havenith's group

Collaborations

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F. Sebastiani (Italy)
M.-P. Gaigeot (France)
Songi Han (U.S.)
S. Shell (U. S.)

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Thank you for your attention

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