Nonlinear infrared spectroscopy

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Biomolecular Phonotics
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Outline of the course

How do we get microscopic/molecular information from macroscopic measurements?

- Scientific talk (1 hour): application of nonlinear IR spectroscopy
- Fundamentals of nonlinear IR spectroscopy (4 hours):

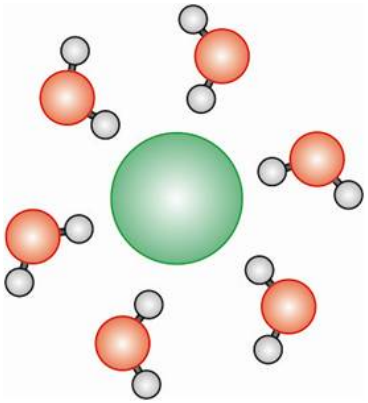
Love-hate relationships with water

Ultrafast spectroscopy
Y. L. A. Rezus & H. J. Bakker

Water and life

Water is essential to life where it plays an important role in many chemical and biological processes:

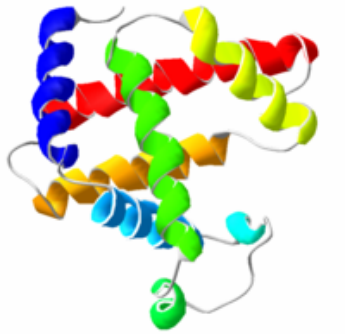
- Ion solvation



- Chemical reactions

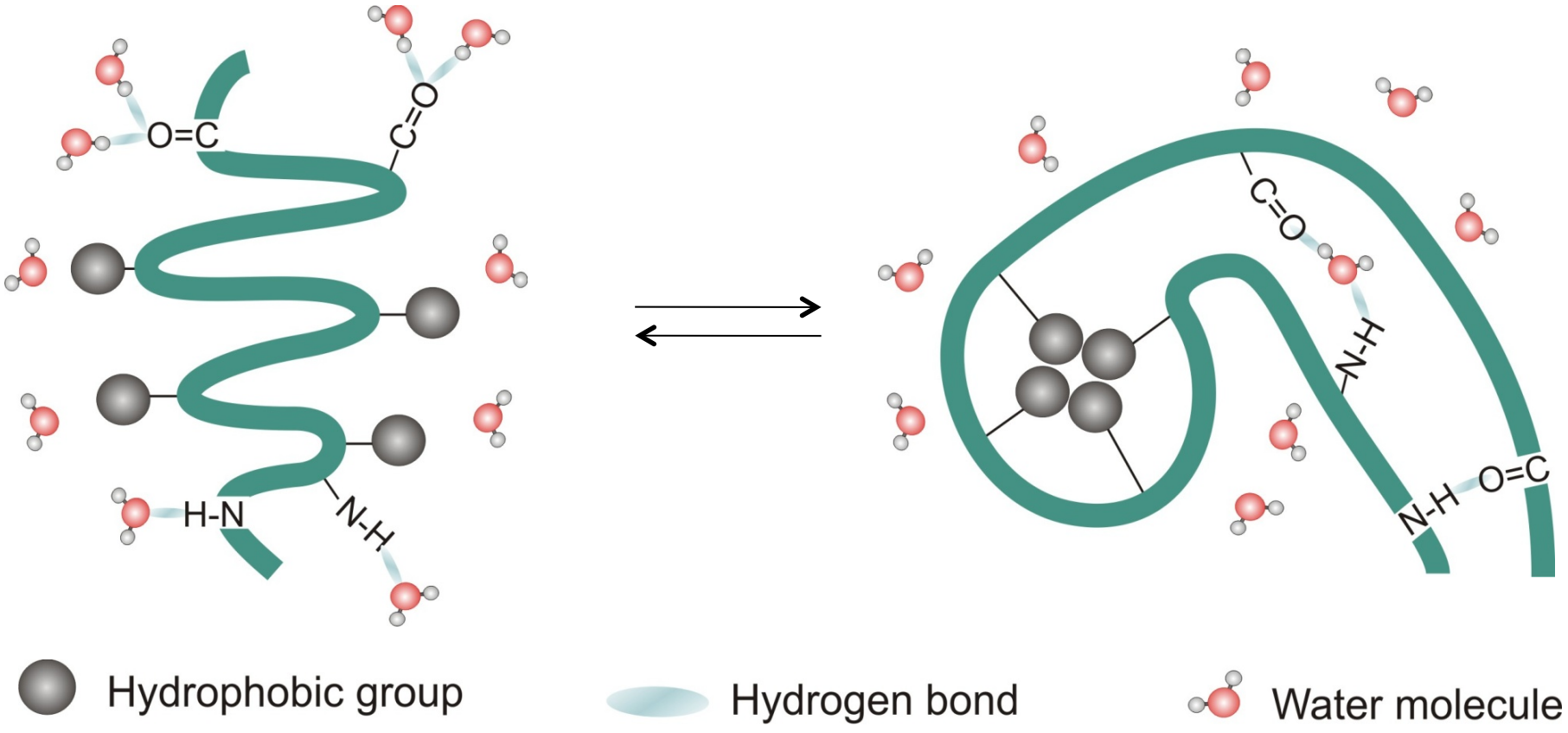
$$A + B \rightarrow C$$

- Protein folding



Water and life

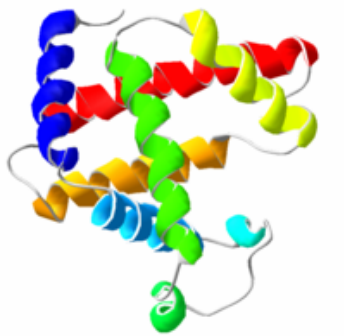
Interactions with water determine protein structure



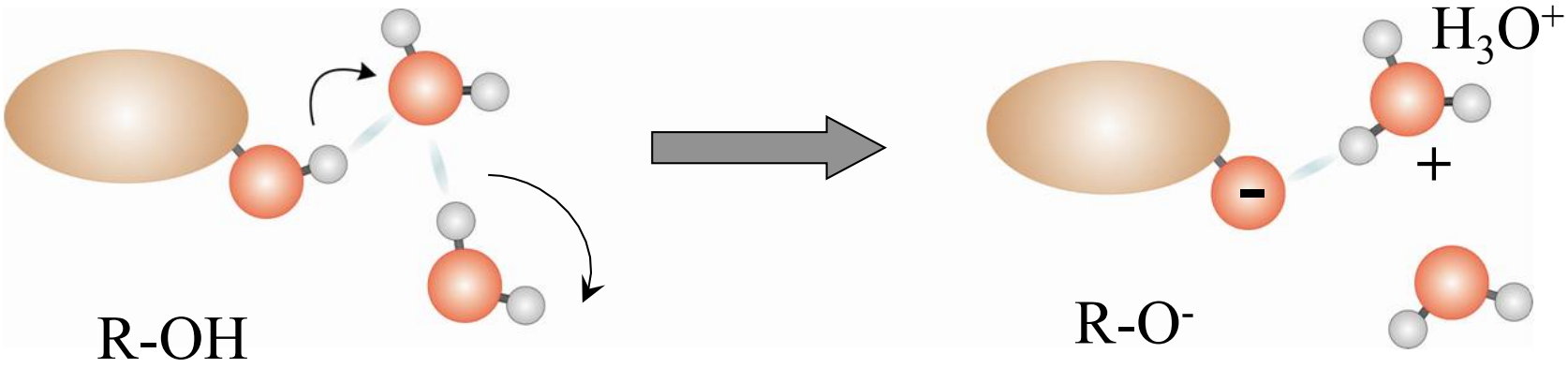
(adapted from: www.exobio.cnrs.fr, Kristin Bartik)

Dynamics of water molecules

•Enzyme function



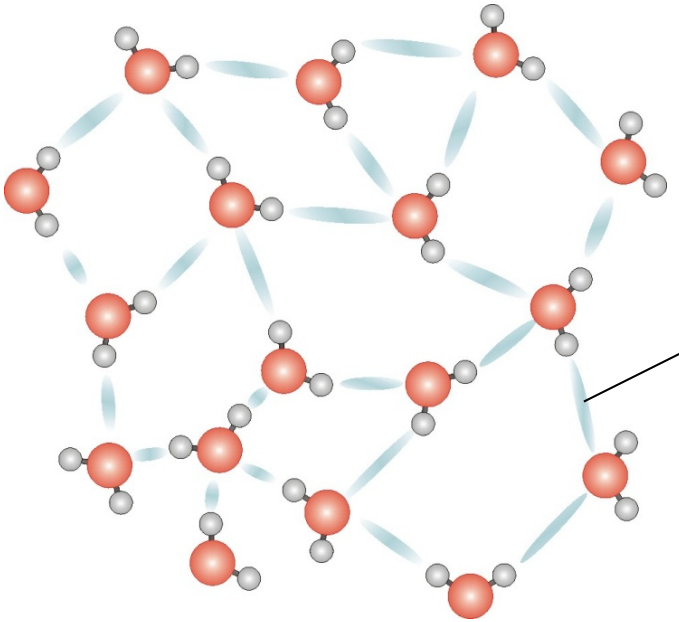
•Acid-base reactions ('Moses mechanism')



Dynamics (i.e. rotations) of water molecules occur on a picosecond timescale!

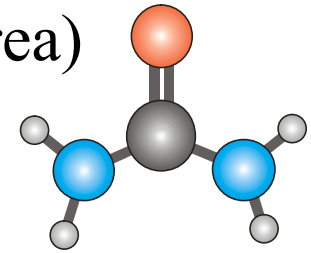
Questions

- Why is water so dynamic?
- How fast can a water molecule reorient?
- How do we measure this?

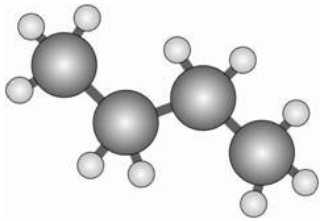


- What is the effect of solutes?

Case 1: polar/hydrophilic solutes (urea)

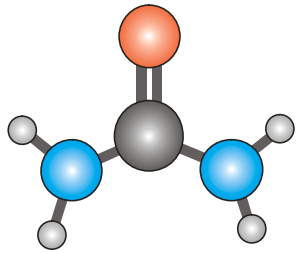


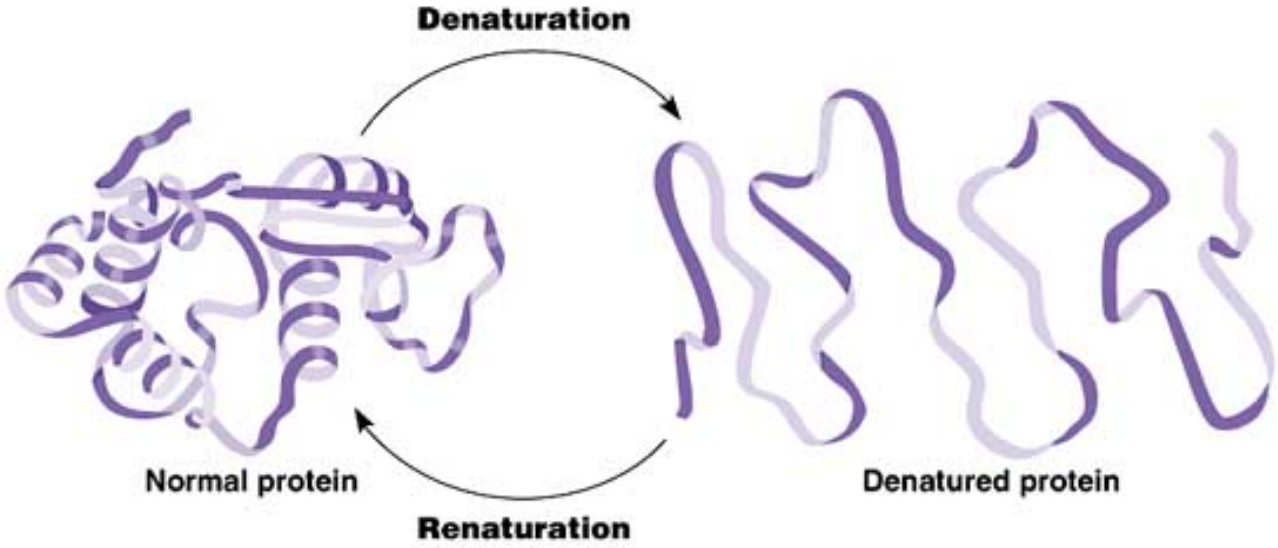
Case 2: apolar/hydrophobic solutes



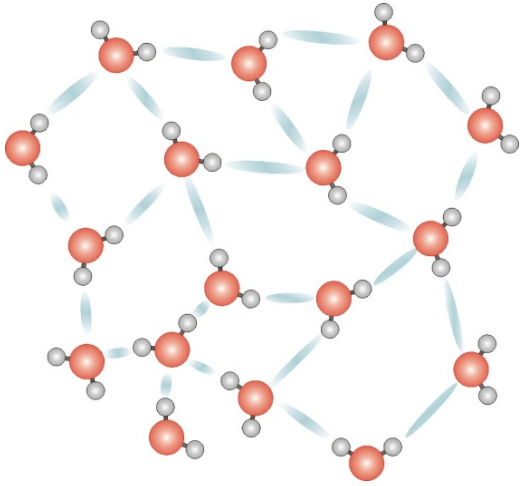
Case 1: aqueous urea

•Reversible denaturation of proteins (routinely used, poorly understood)





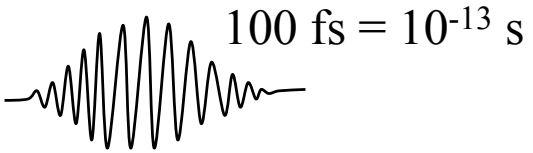
•Restructuring the hydrogen-bond, facilitating the solvation of hydrophobic groups (debated...)



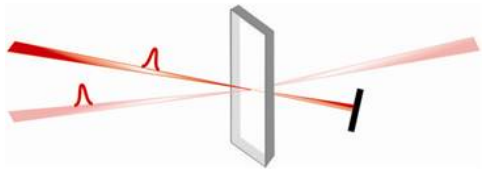
Outline: studying molecular motion

Physics:

- Femtosecond infrared light pulses

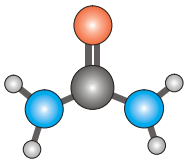


- Pump-probe experiment



Chemistry:

- Hydrophilic solvation



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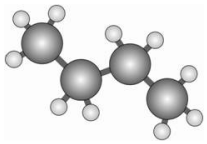
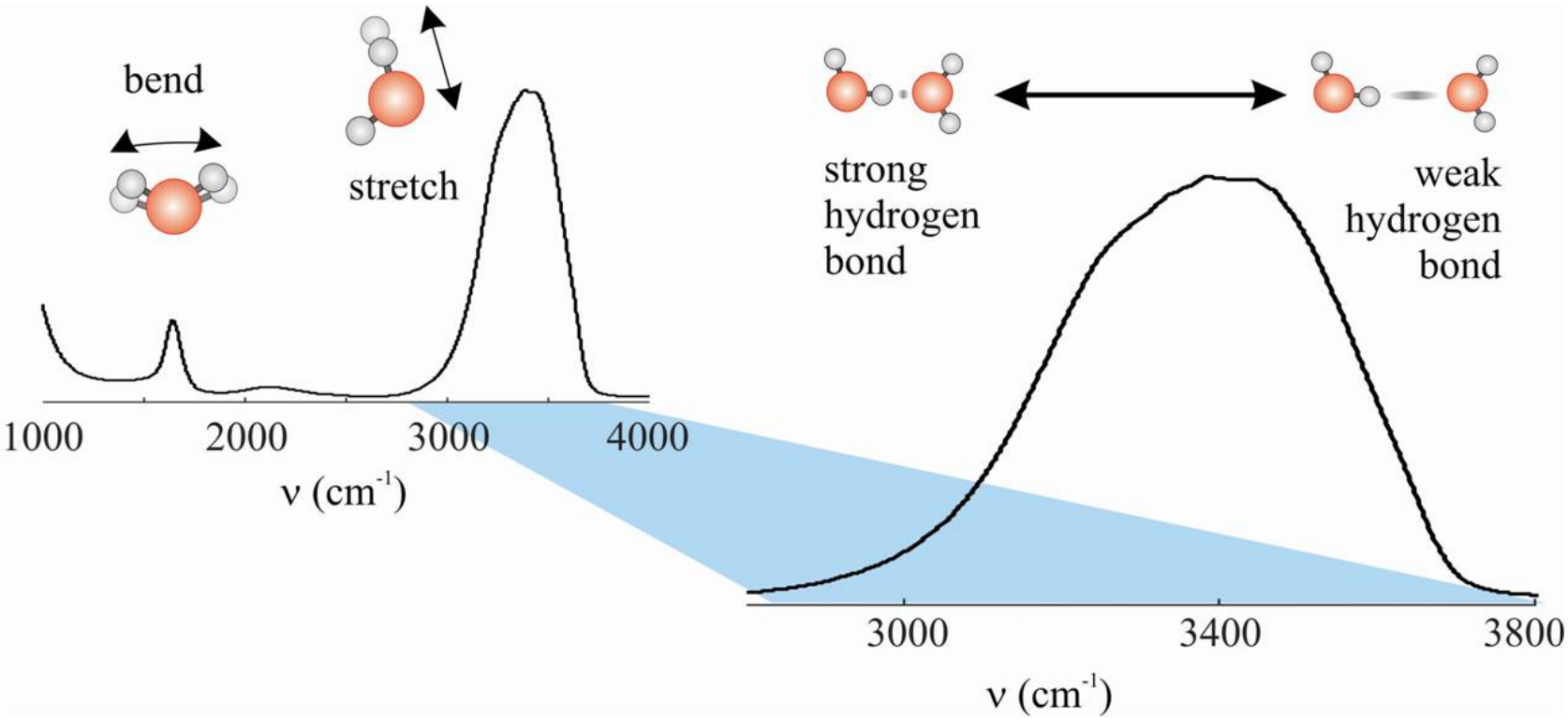


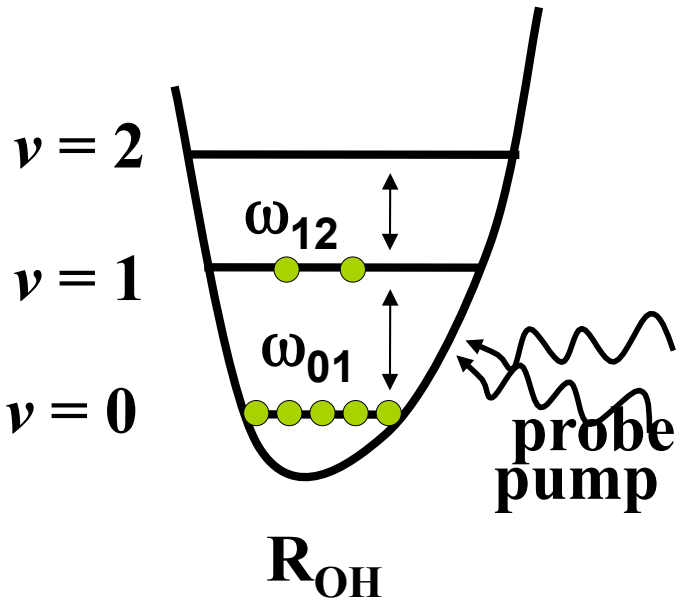
IR spectroscopy



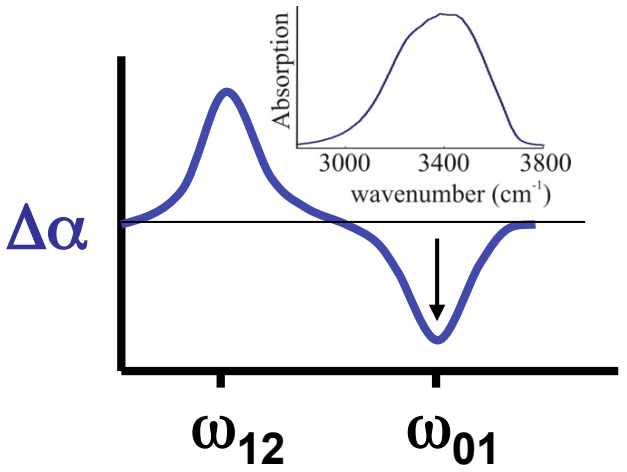
Rotations of water molecules occur on a picosecond timescale: femtosecond infrared (~4 μm) pulses are needed!

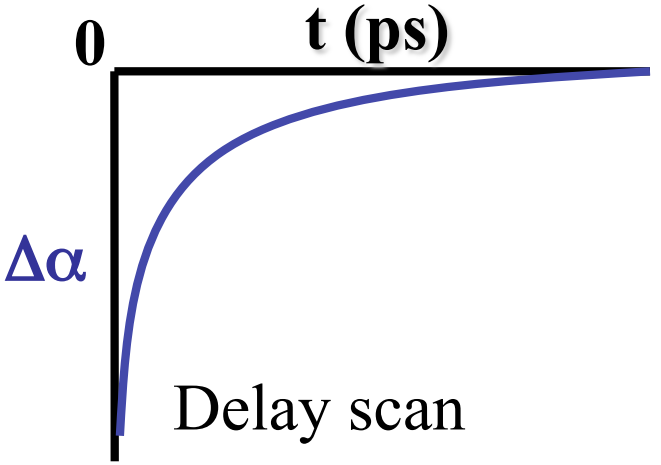
Pump-probe: principle

Energy levels

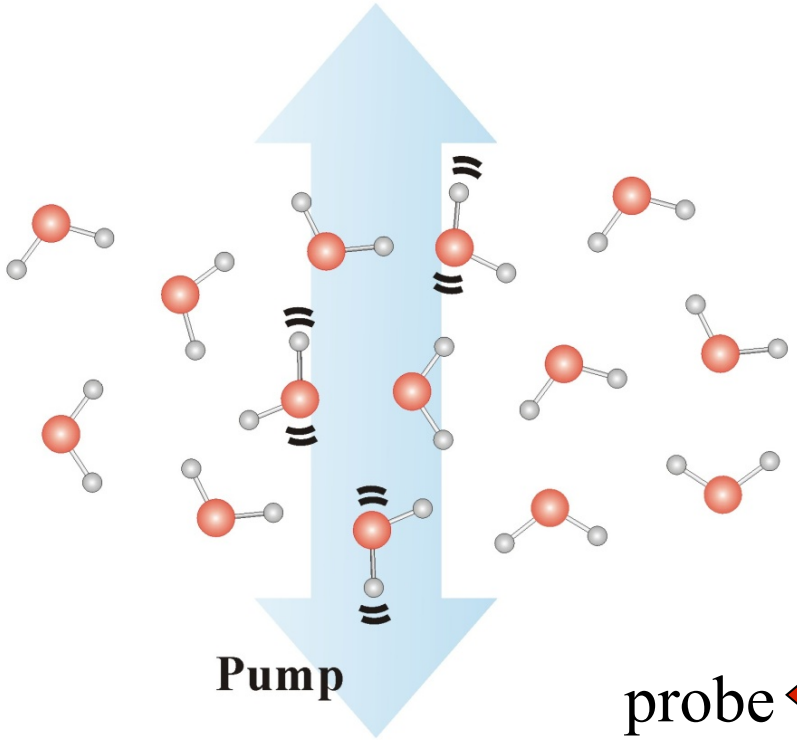


Transient spectrum

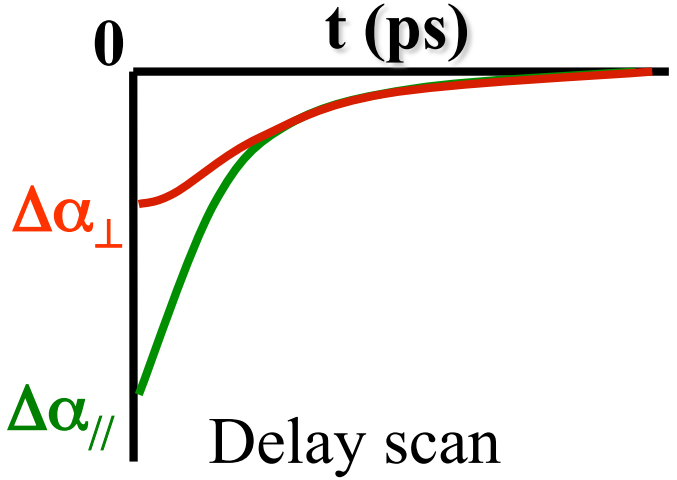




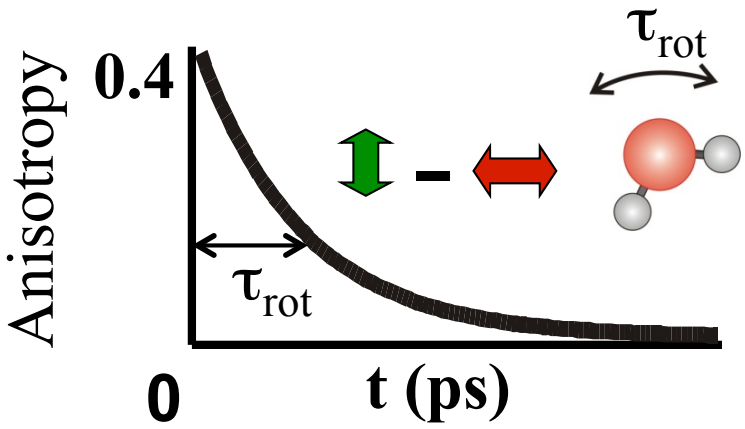
Pump-probe: Anisotropy



probe

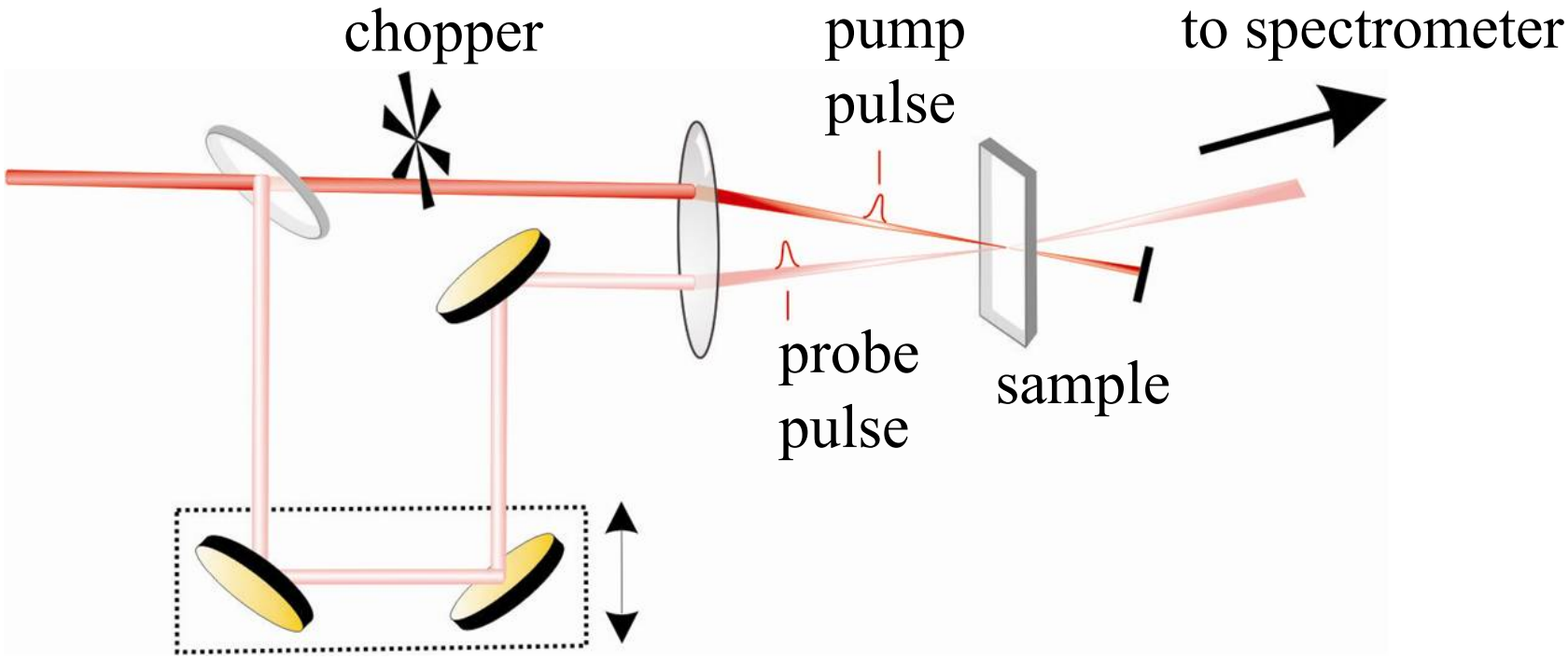


$$\bullet \text{Anisotropy} = \frac{\Delta\alpha_{//} - \Delta\alpha_{\perp}}{\Delta\alpha_{//} + 2\,\Delta\alpha_{\perp}}$$



Pump-probe: experiment

~5 μJ @ 4 μm
(2500 cm^{-1}),
~150 fs, 1kHz



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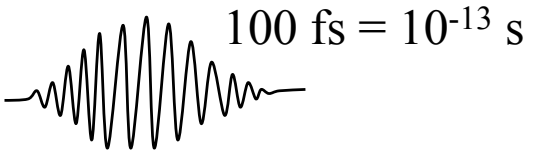

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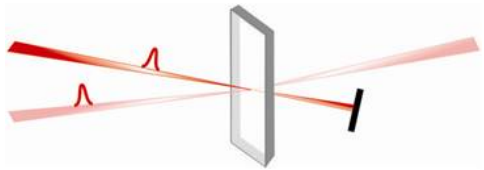

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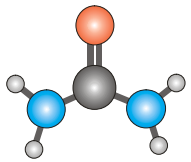
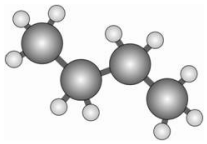
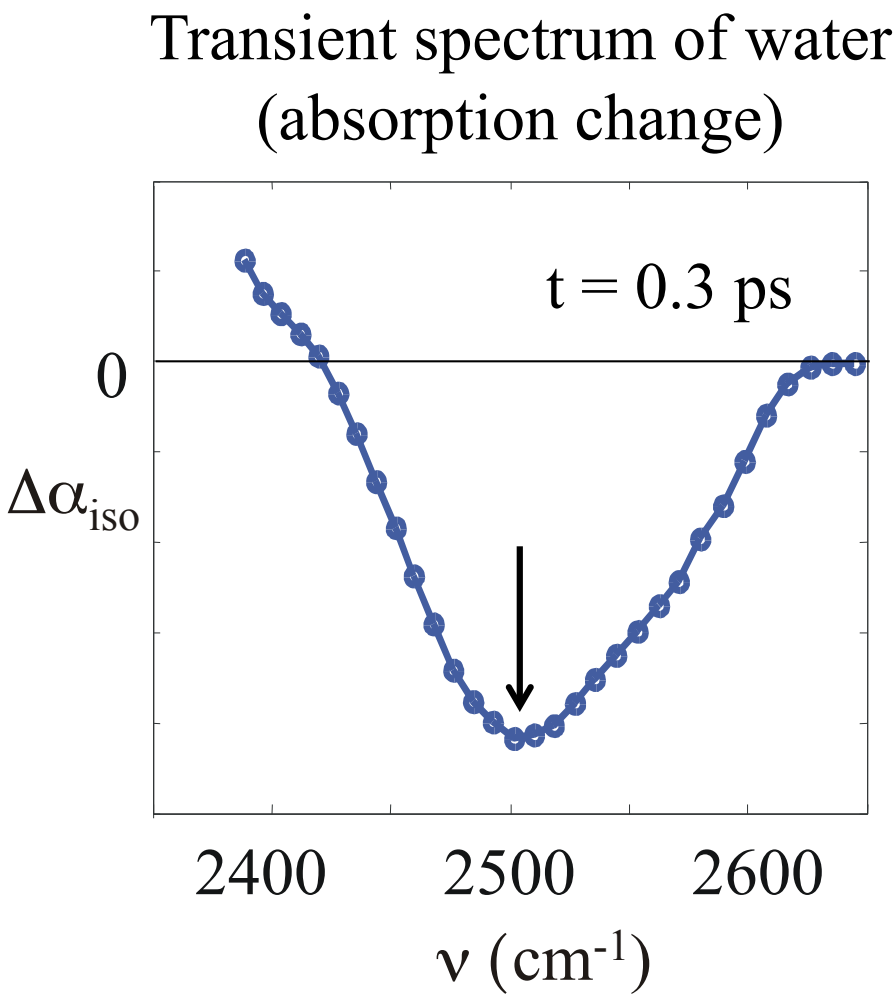
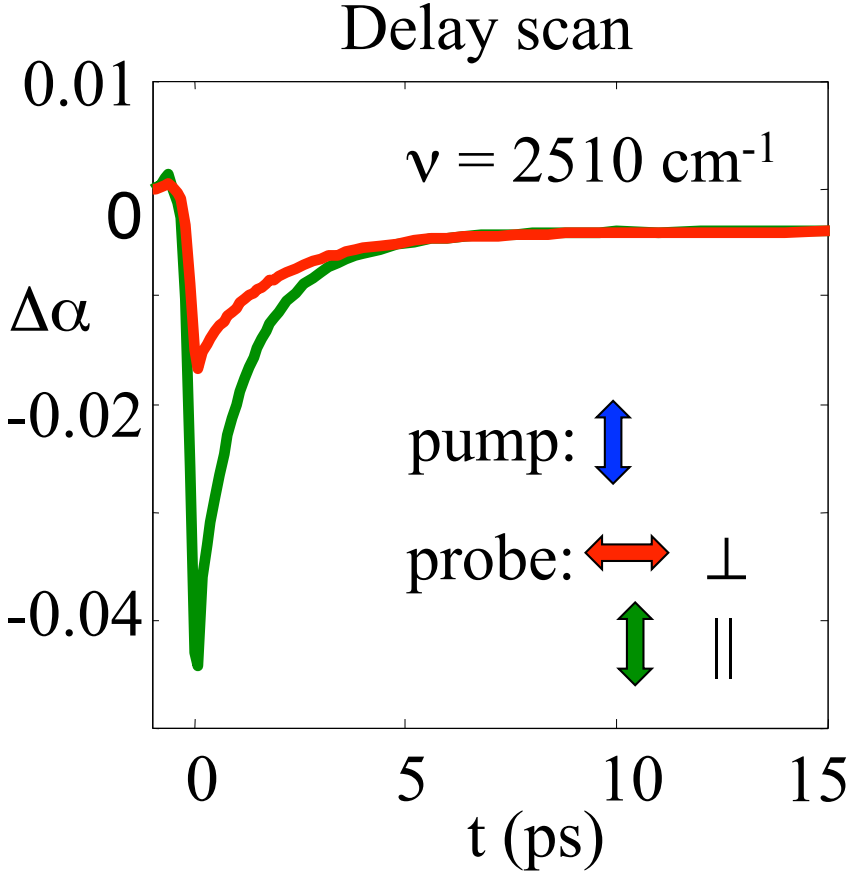


- Hydrophobic solvation

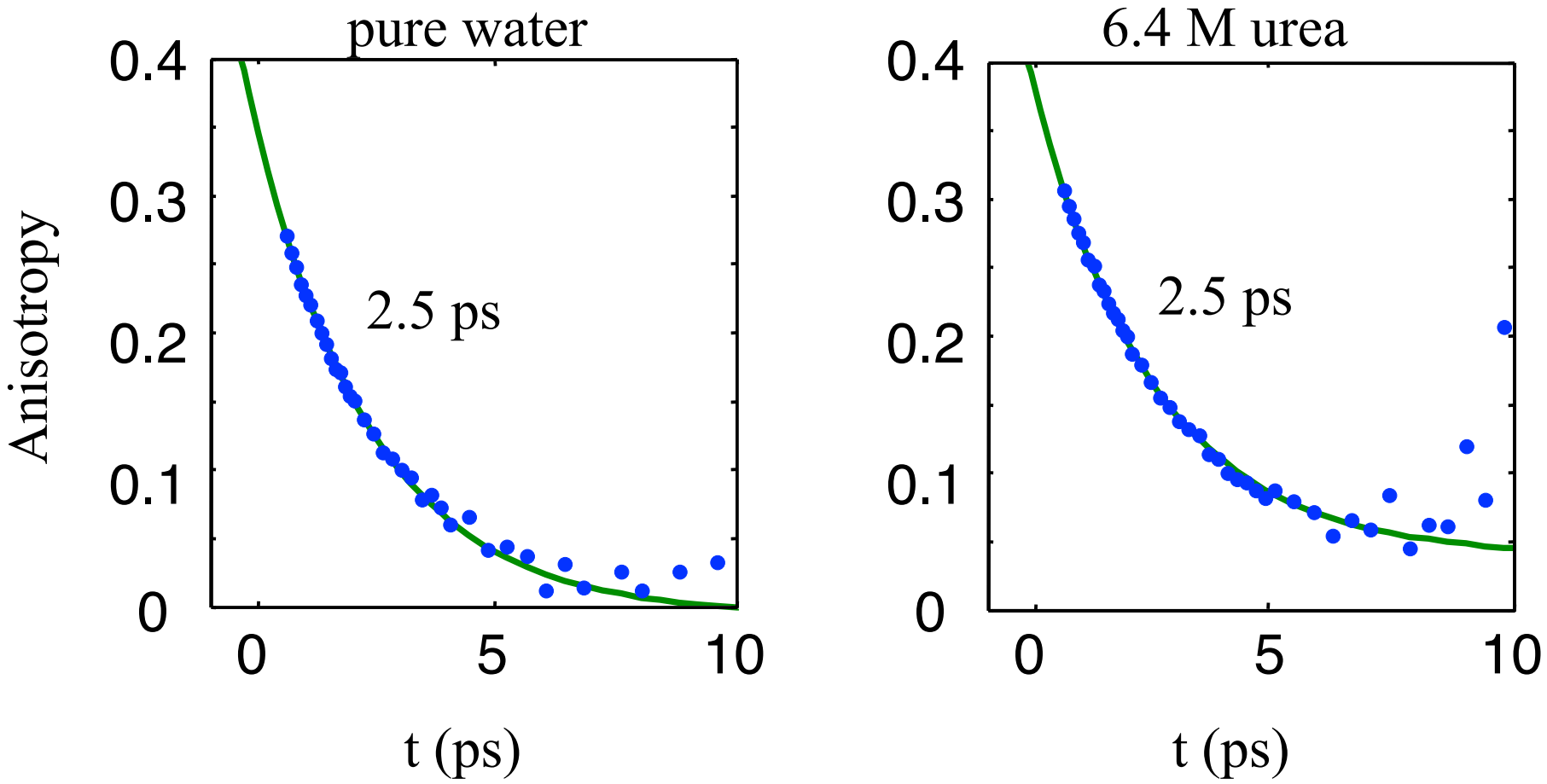


Results

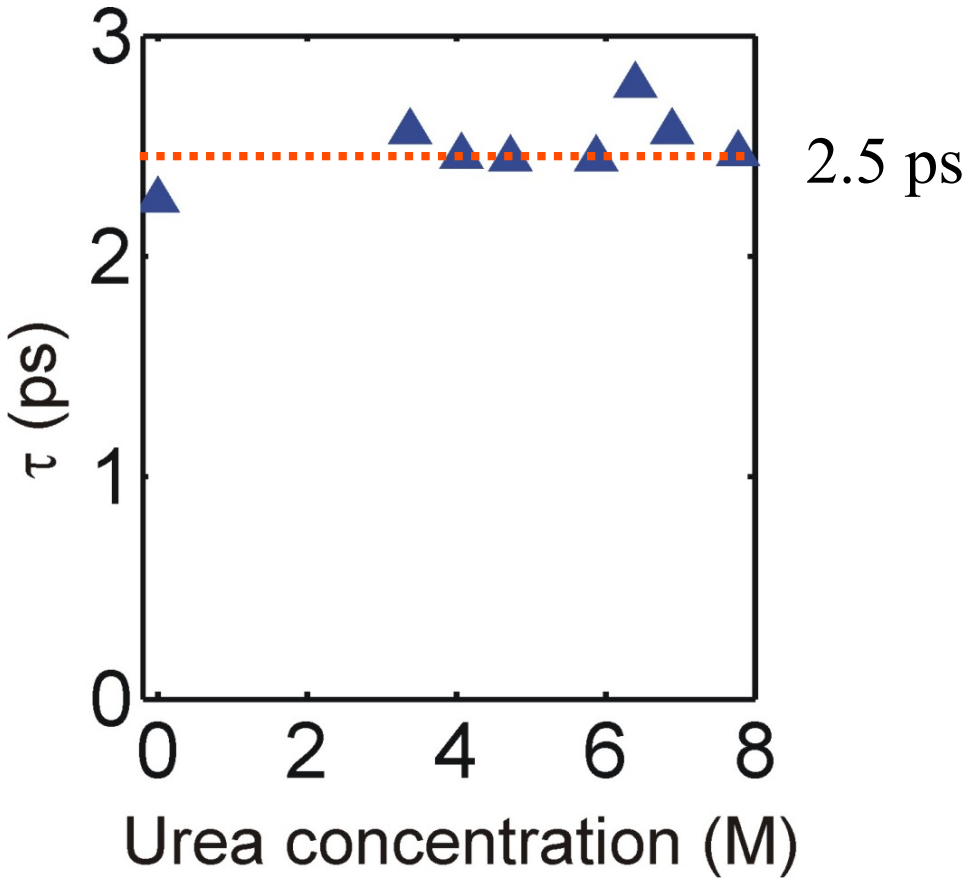

Transient spectrum of water
(absorption change)
t = 0.3 ps
0
Δα_iso
2400
2500
2600
ν (cm-1)


Delay scan
ν = 2510 cm-1
0.01
0
Δα
-0.02
-0.04
pump:
probe:
⊥
||
0
5
10
15
t (ps)

Anisotropy



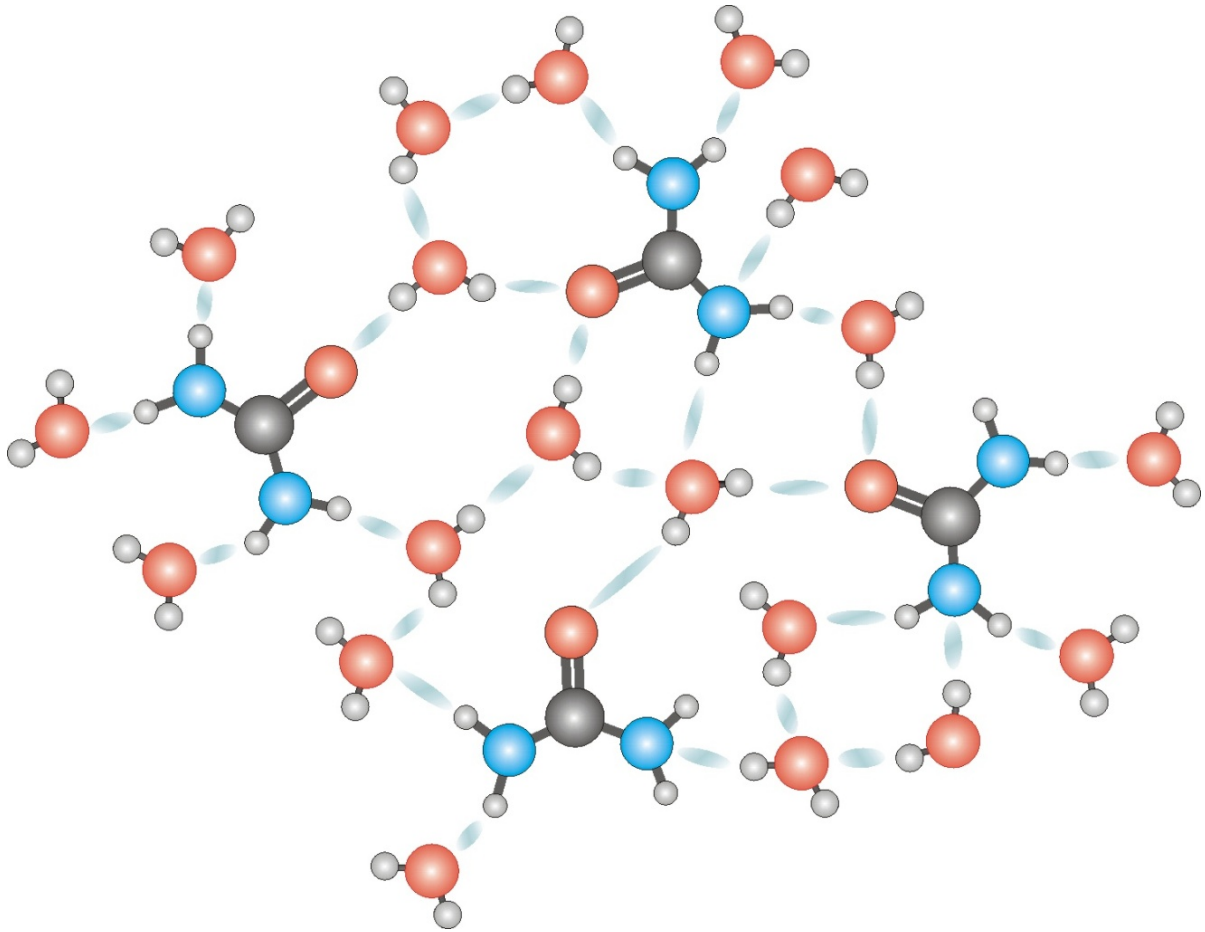
Reorientation time vs. concentration



•Reorientation time of water is unaffected by urea!

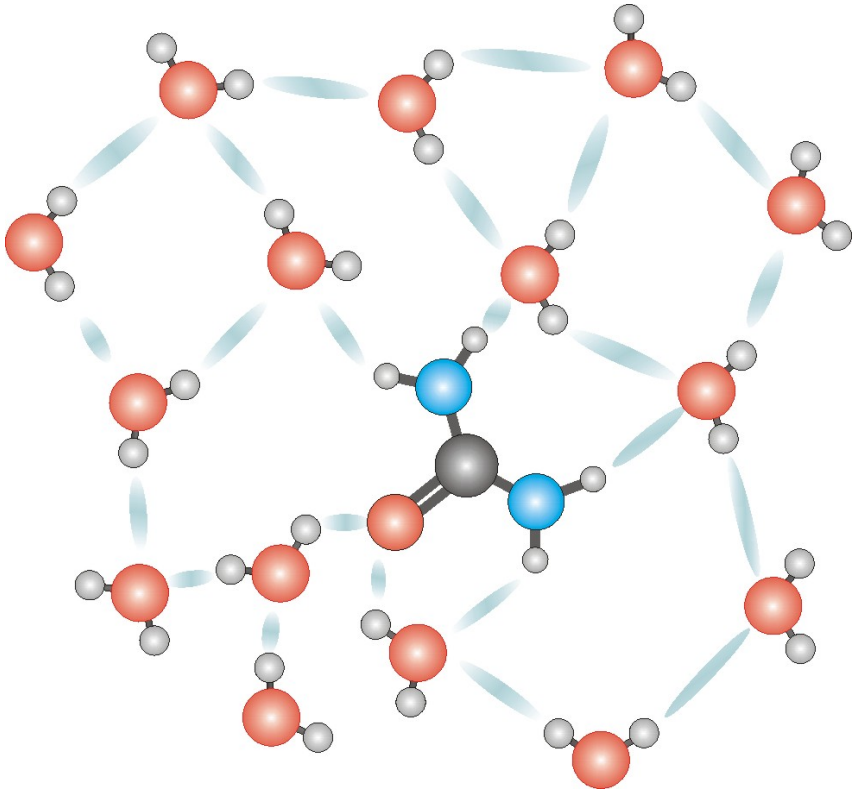
Y.L.A. Rezus and H. J. Bakker, PNAS 103, 18417 (2006)

Concentrated urea solution

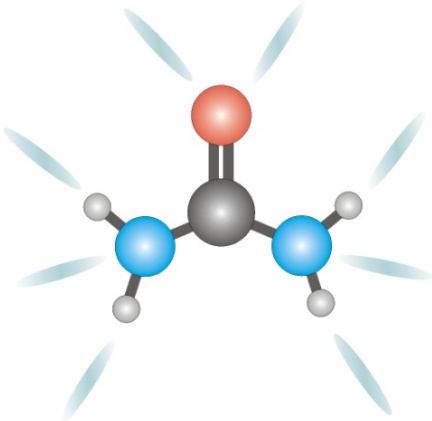


- Urea:water = 1:5, i.e. all water molecules are in contact with urea
- Nevertheless water molecules reorient as in pure water!

Urea substitutes for a water dimer



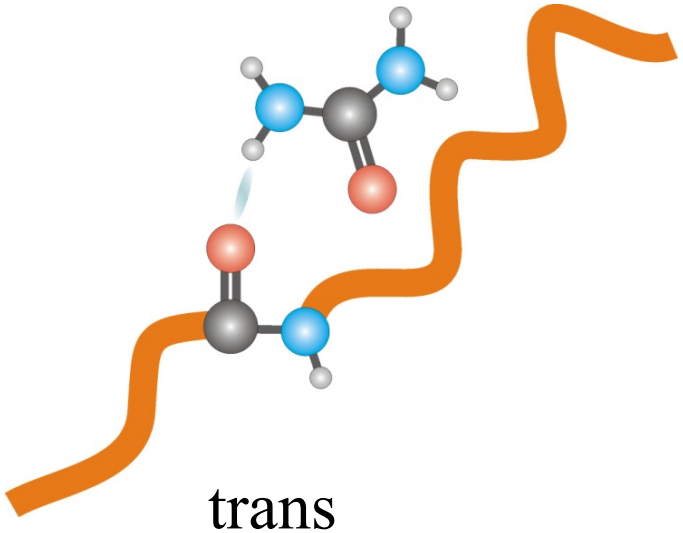
8 H-bonds with water



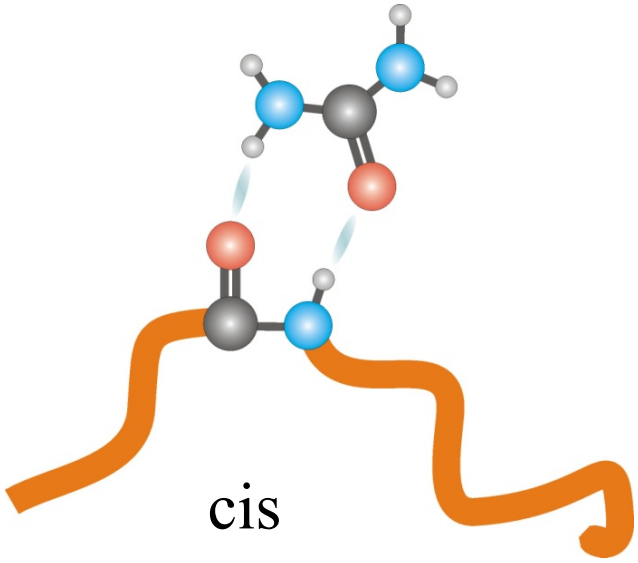
•Urea fits into the H-bond network of water exceptionally well

What about protein denaturation?

- Indirect mechanism seems unlikely
- Urea may cosolvate amino-acid residues
- Double H-bond formation favors cis-conformation of amide groups (unfolded protein)



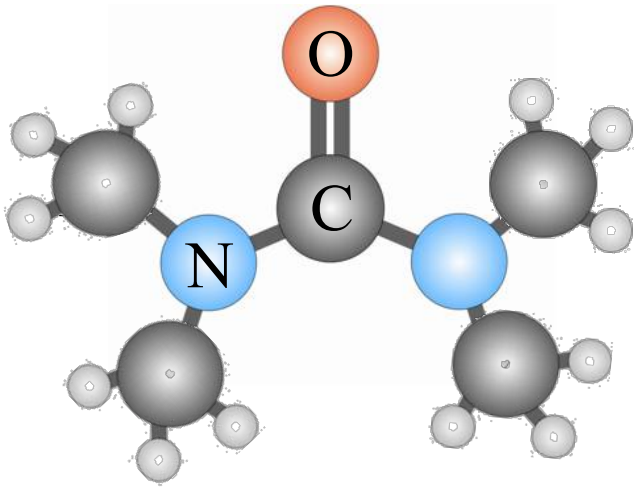
trans



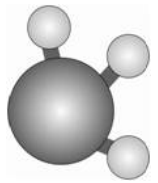
cis

From hydrophilic to hydrophobic…

- Introducing apolar groups



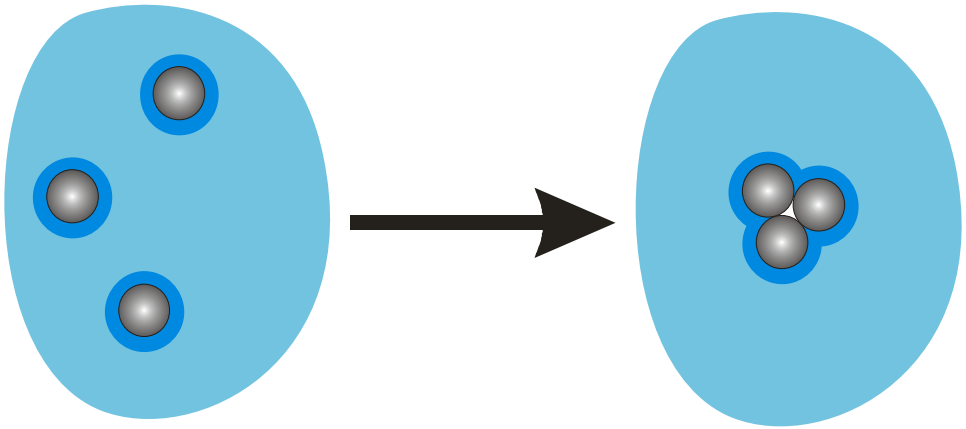
Tetramethylurea



CH_3 (methyl)

Case 2: Hydrophobic solvation

• The tendency of apolar groups to associate in water (hydrophobic interactions)

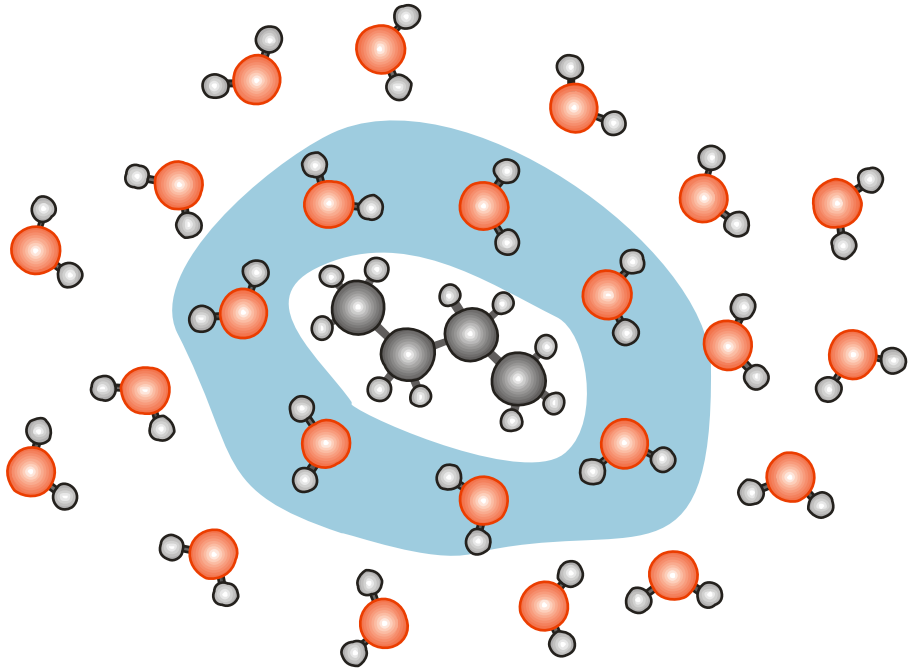


Important driving force in biochemical processes

• folding of proteins
• self-assembly of lipid membranes
• drug-protein assocation

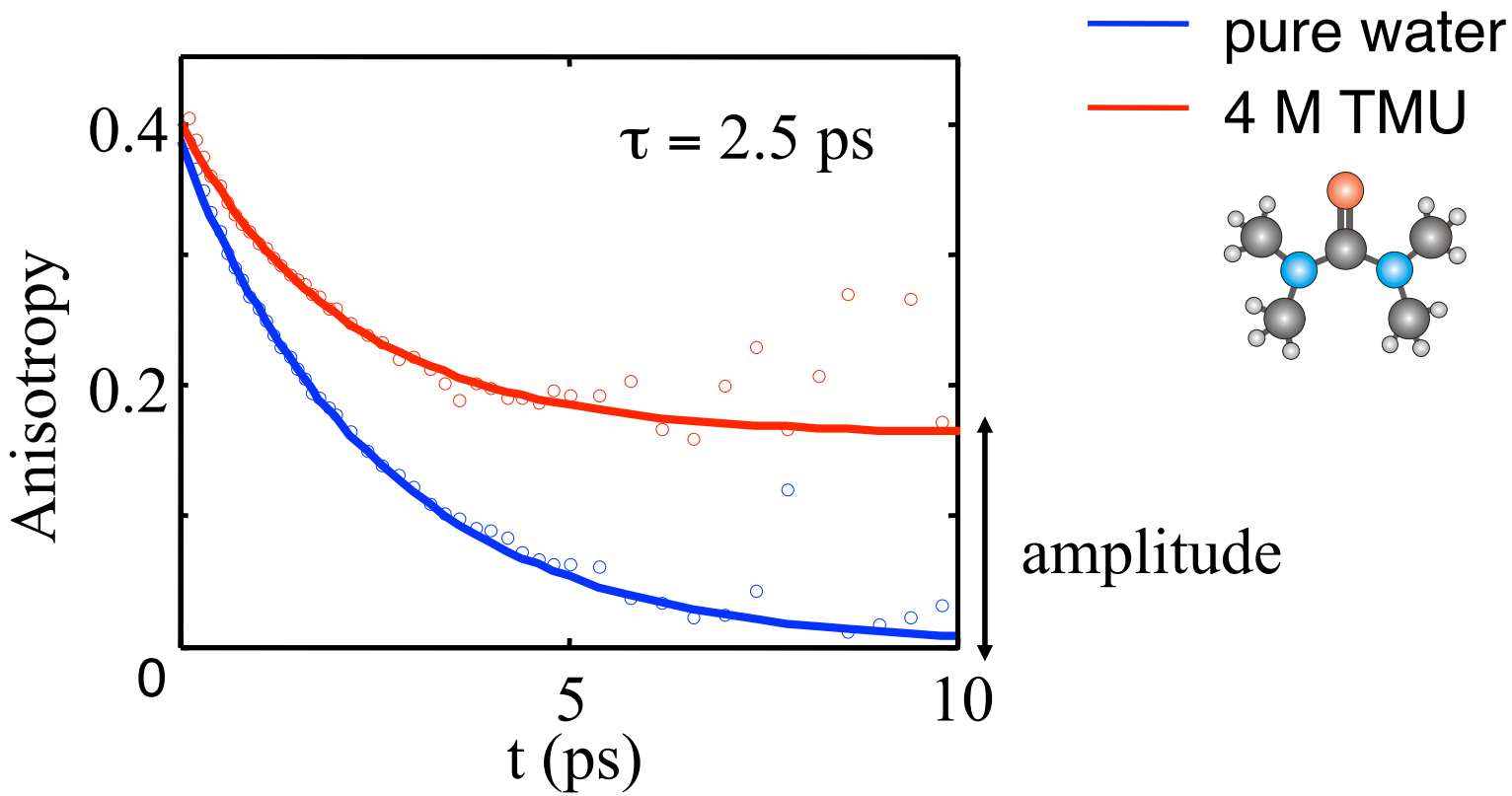
Hydrophobic hydration

- Solvation of apolar groups in water
- Iceberg model of Frank and Evans (1945)



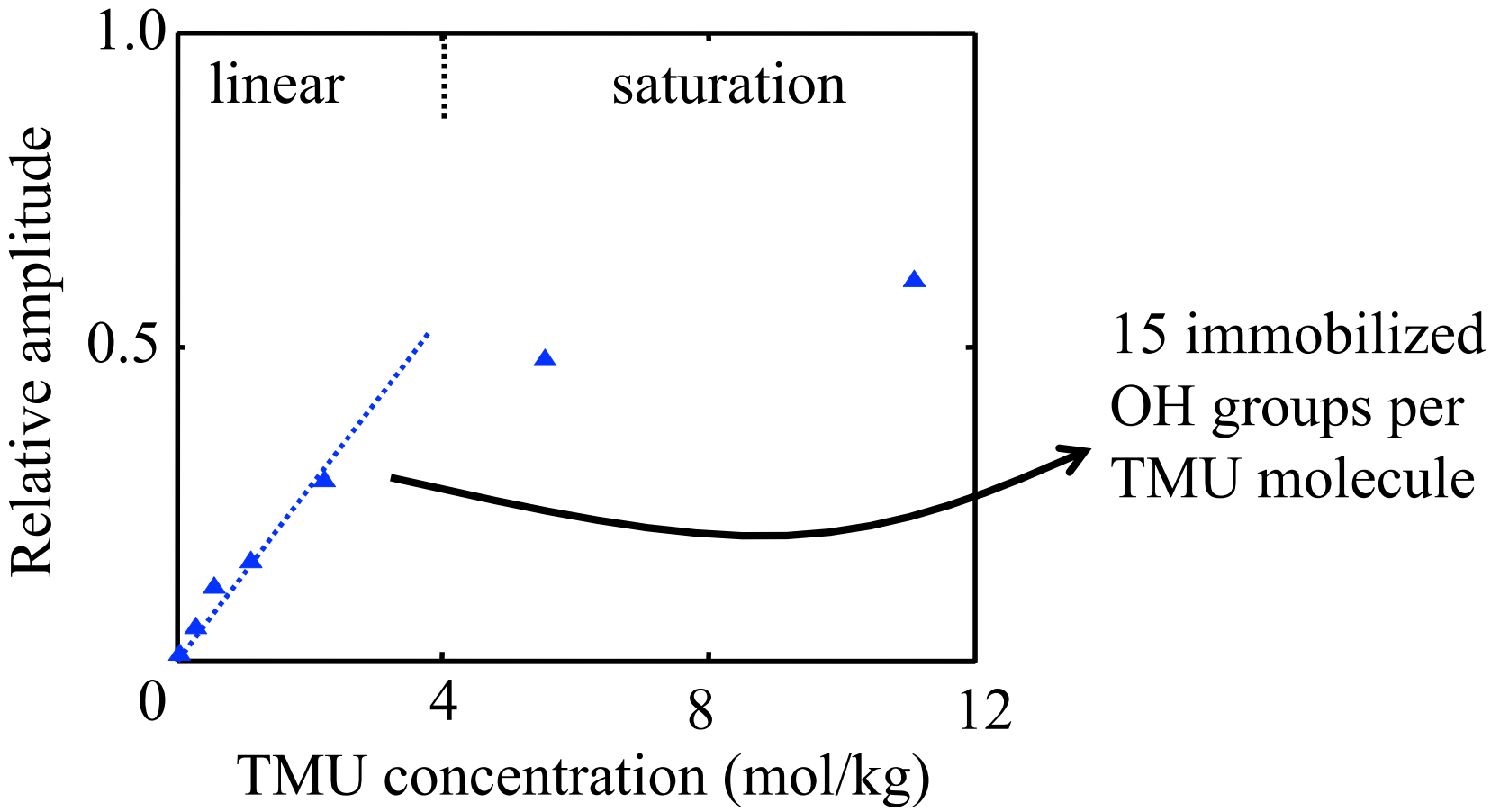
- Controversy: icebergs not observed with structural methods (neutron diffraction, NMR)
- Many techniques determine a time-averaged structure

Anisotropy: measurements



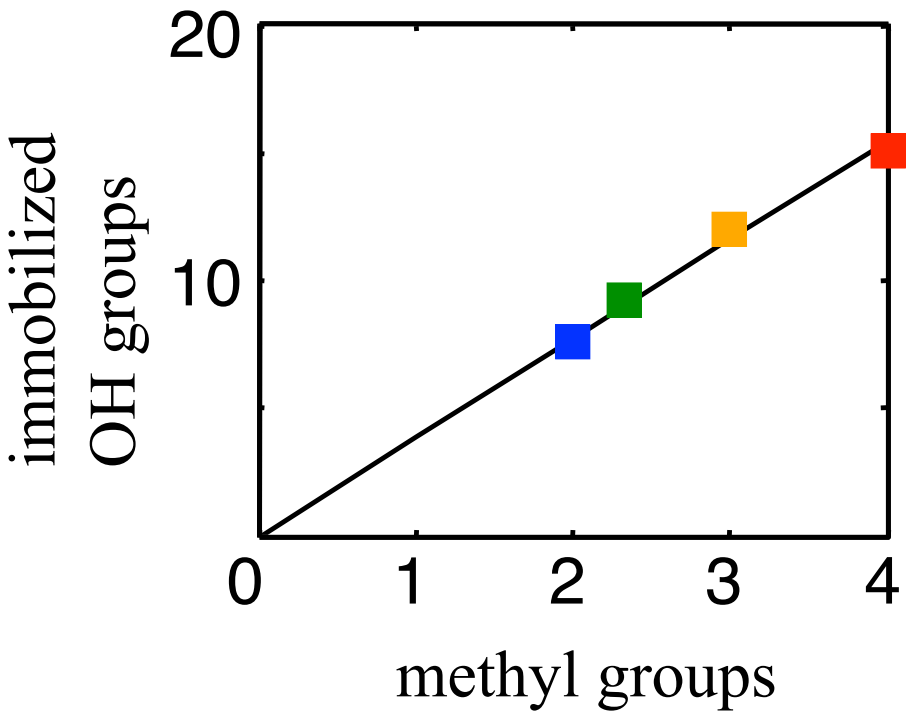
- Appearance of a slow component ($\tau > 10$ ps)!

Slow component: amplitude

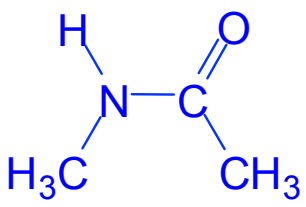


•Slow component is associated with the solvation shell (immobilized water molecules)

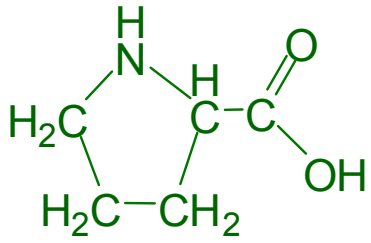
Hydrophobic hydration?



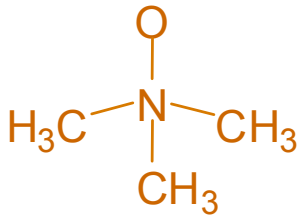
NMA



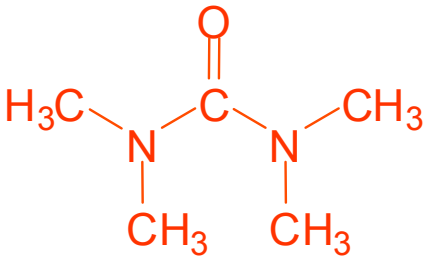
Proline



TMAO



TMU

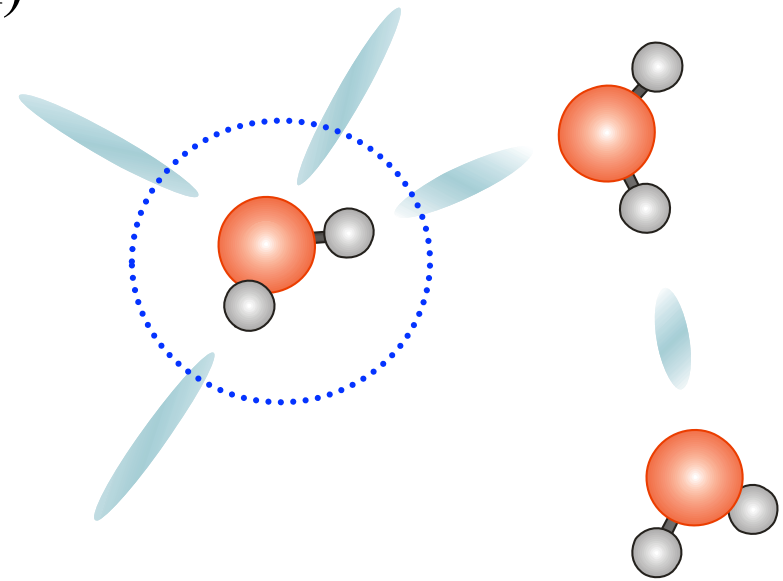


- 4 OH groups are immobilized per methyl group

Y.L.A. Rezus and H.J. Bakker, Phys. Rev. Lett. 99, 148301 (2007)

Physical mechanism

- Immobilized water molecules are not more strongly bound than in the bulk liquid
- Reorientation mechanism of Laage et al.[1] (bifurcated H-bond, 5-fold coordination)



[1]Laage and Hynes, Science 311, 832 (2006)

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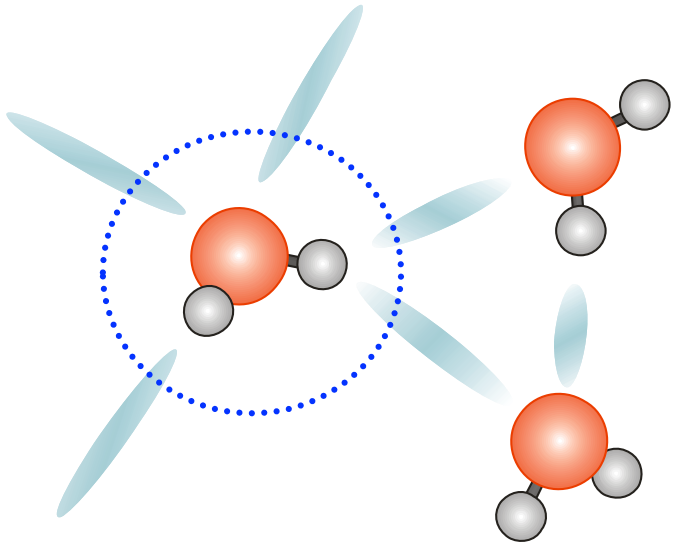

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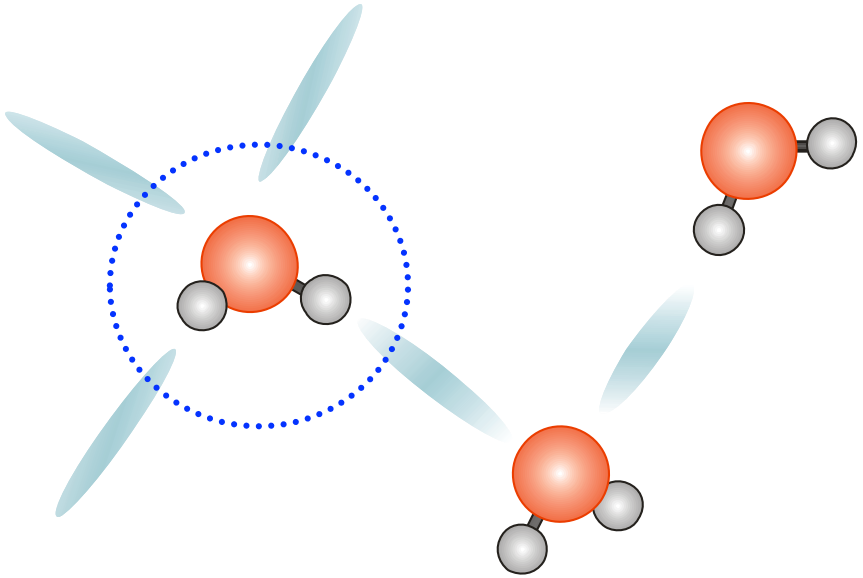

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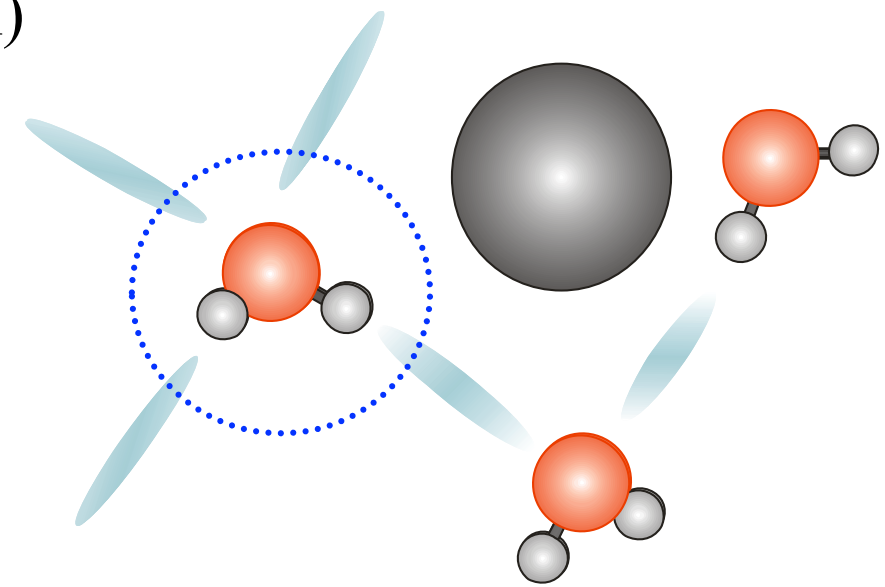

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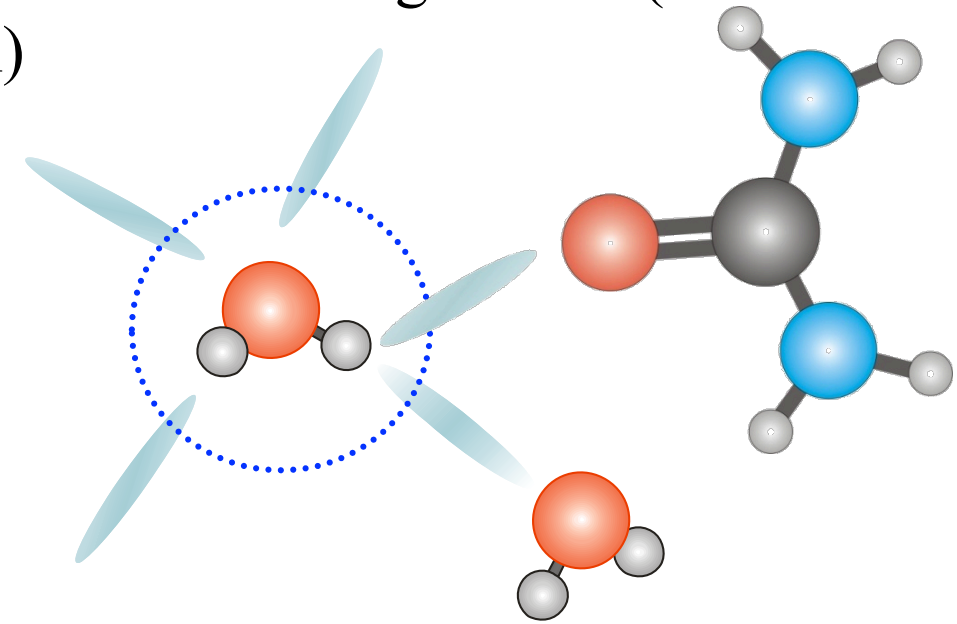
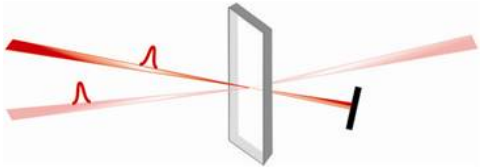
- Immobilized water molecules are not more strongly bound than in the bulk liquid
- Reorientation mechanism of Laage et al.[1] (bifurcated H-bond, 5-fold coordination)



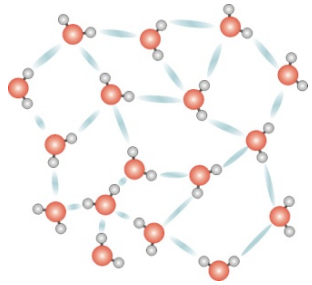
[1]Laage and Hynes, Science 311, 832 (2006)

Summary ‘love-hate relationships’

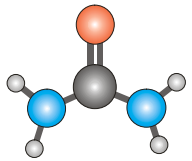
- Femtosecond infrared pump-probe spectroscopy to study molecular dynamics



- Fast dynamics of water depend on high density of H-bonds



- Hydrophilic groups (urea) do not affect water dynamics



- Hydrophobic groups immobilize water (4 OH groups per methyl group).