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Educational, Scientific and
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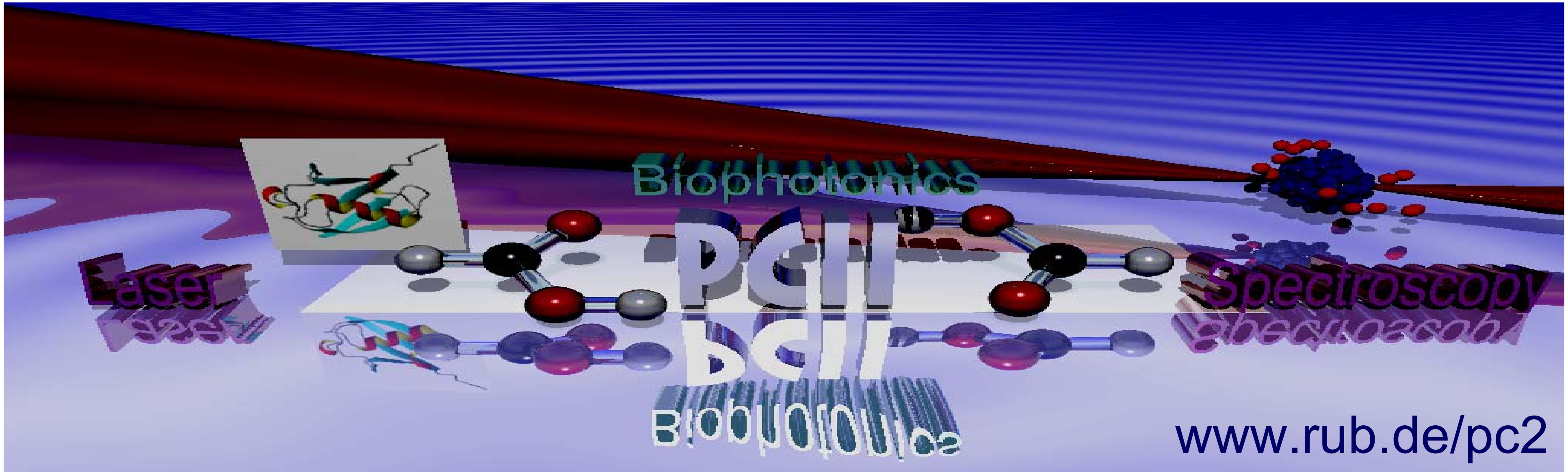
**Winter College on Micro and Nano Photonics for Life Sciences**

***11 - 22 February 2008***

# THz spectroscopy of proteins

Martina Havenith
*Ruhr University Bochum*
*Bochum, Germany*




# The THz dance of water with biomolecules

M. Havenith
Physical Chemistry, Ruhr-Universität Bochum, Germany

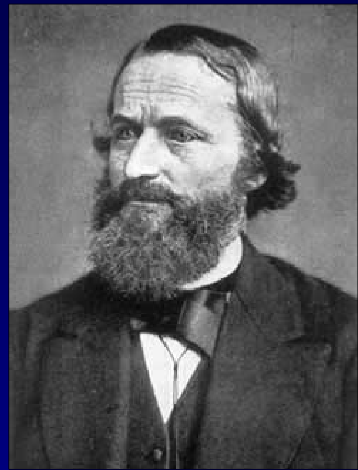
**Funding:**
Human Frontier Science Award 2004

# Physical Chemistry: How it started

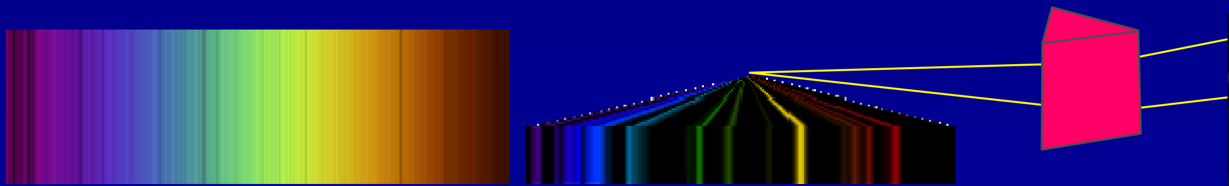
Robert Wilhelm Bunsen
chemist
(1811-1899)

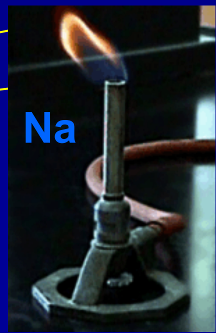
Bunsen: 15. 11. 1859:

„At the moment Kirchhoff and I are busy with a work which keeps us awake all night.
Kirchhoff has made an unexpected wonderful discovery, by finding the reason for the dark lines in the spectrum of the sun."



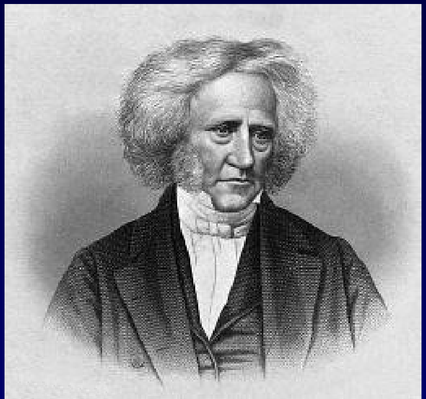
Gustav Kirchhoff
physicist, mathematician
(1824-1887)






Chemische Analyse durch Spectralbeobachtungen (1860)
Abhandlungen über Emission und Absorption (1859-1862)

# Why did it take so long ?

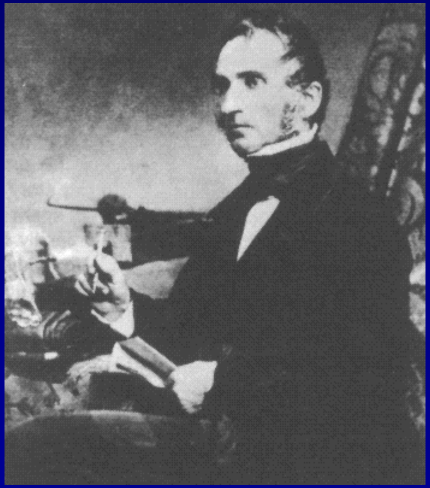


Sir John Frederick Herschel
(1792 - 1871)

**John Frederick Herschel**
**Physiker, Mathematiker (1827)**

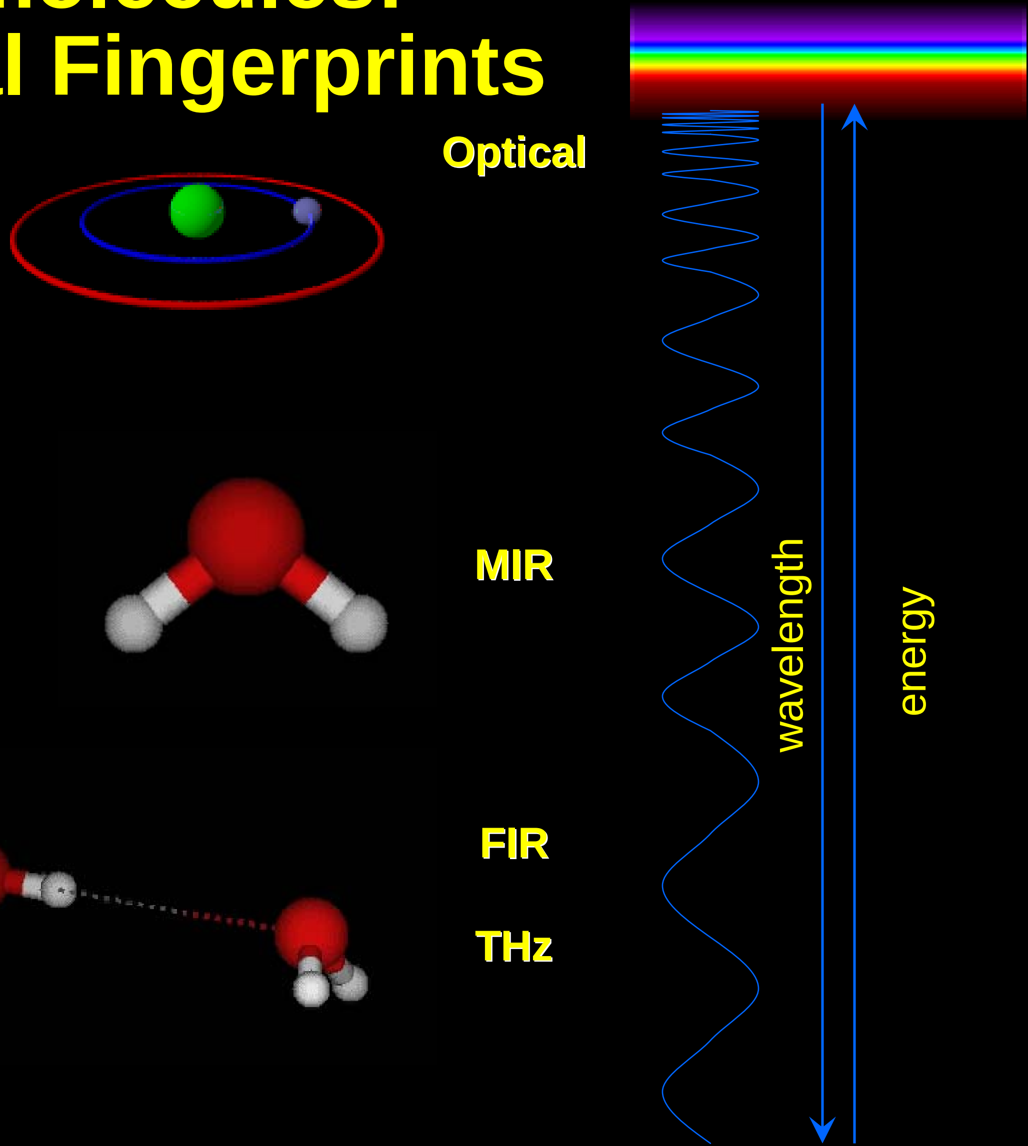
„Their color would be a good means to detect even small quantities of dyed materials. But since this is chemistry one doesn‘t talk about it !“


**Justus von Liebig**
**Chemiker (1840)**
„There is no science which is lacking in soul, with more inability to think, a greater lack of true insight and understanding, more shortsighted and weakness under the cover of knowledge and erudition as mathematics.“

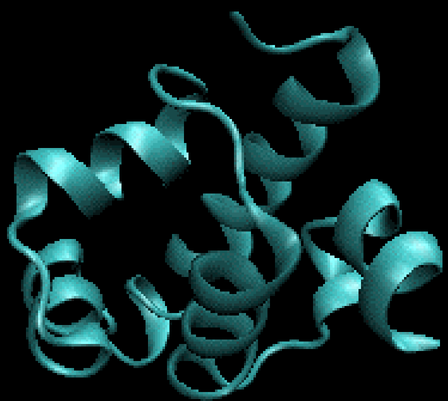


Baron Justus von Liebig
(1803 - 1873)

# Taking a closer look into the world of biomolecules: Spectral Fingerprints




Skeleton motions
Breathing modes
Large amplitude vibrations



**Determination of structure**

# The THz gap
# between electronics and photonics

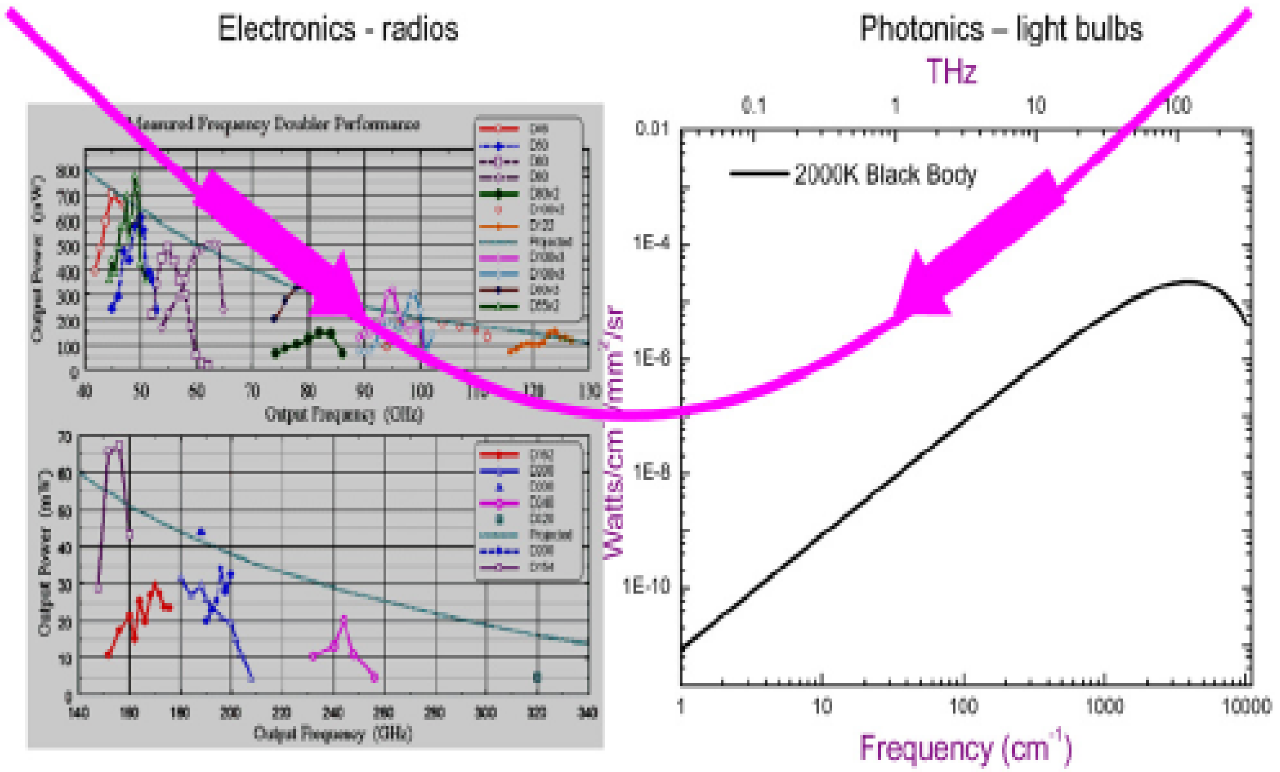
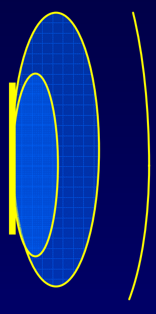


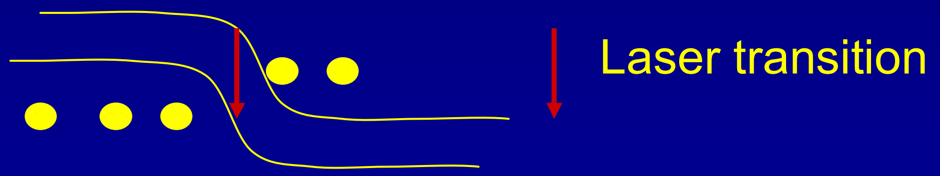

**Figure 2.** Illustration of the real THz gap—the decline in power as electronics goes to higher frequencies, and photonics goes to lower frequencies. Electronic data courtesy of Tom Crowe, Virginia Diodes Inc.

# Entering the THz age

- **Electronic: Acceleration of charges**
  **limited by speed of charge accelaration**
  **THz corresponds to fsec**

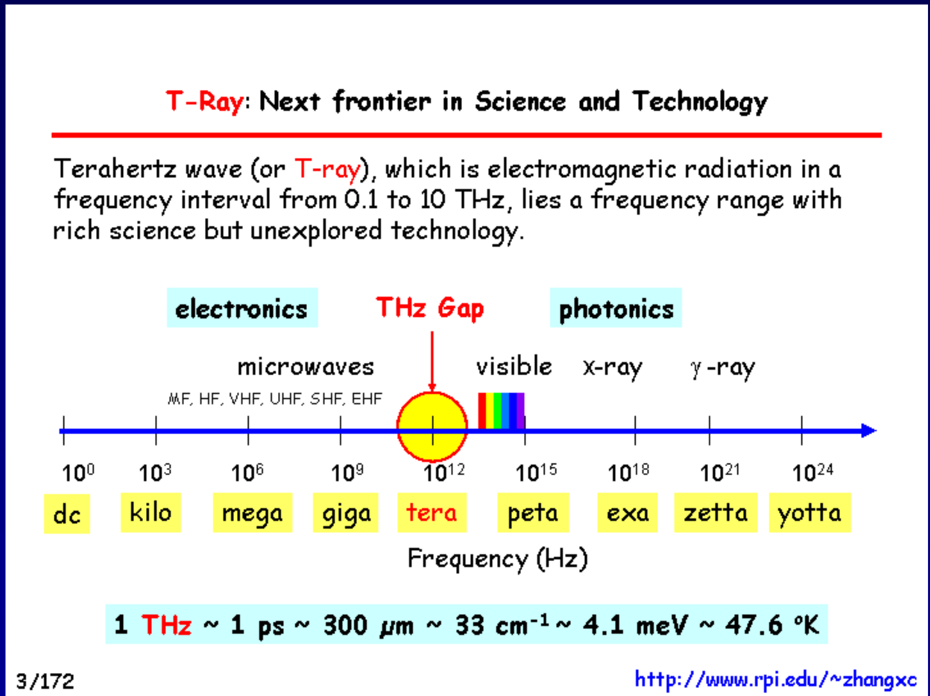


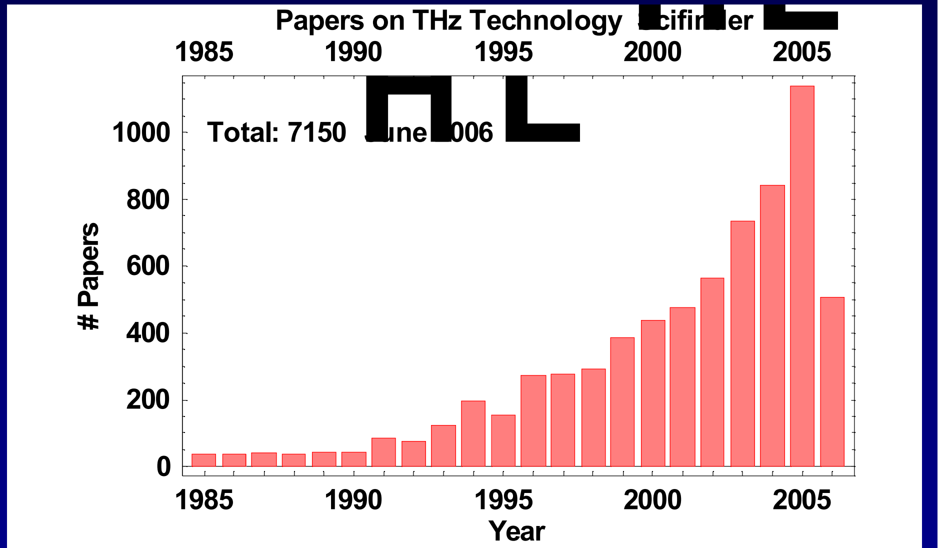
- **Photonic: transition between conduction band and valence band**
  **limited by possible band gap in semiconductors**




- **The new radiation sources:**
  - **p-Ge laser**
  - **THz time domain spectrometer (TDS)**
  - QCL quantum cascade laser (imaging )
  - photomixer
  - Free electron laser

# THz spectroscopy a new spectral window for applications in chemistry, medicine and material science

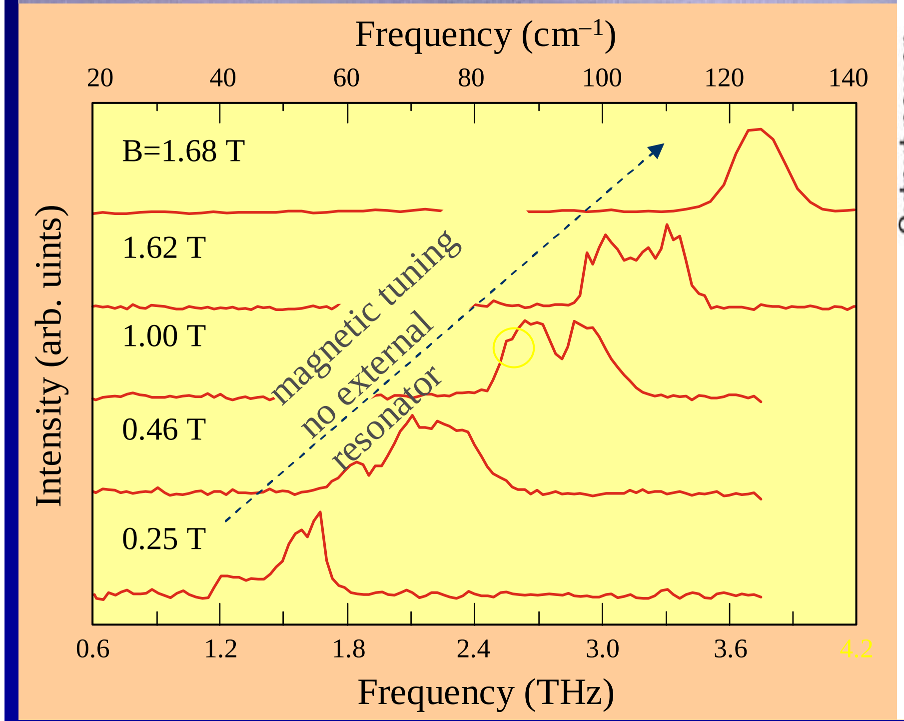


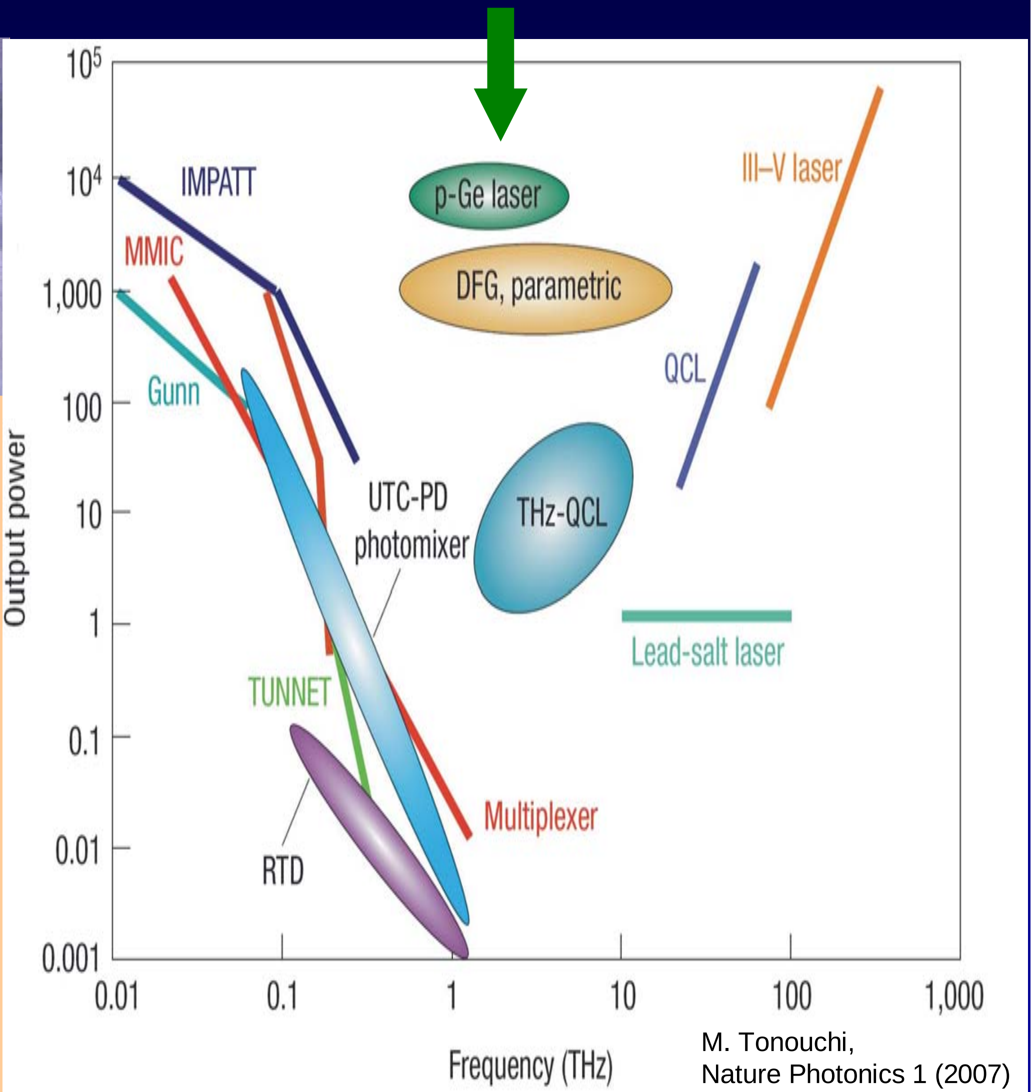




Publications from 1985-2005

*The new sources:*

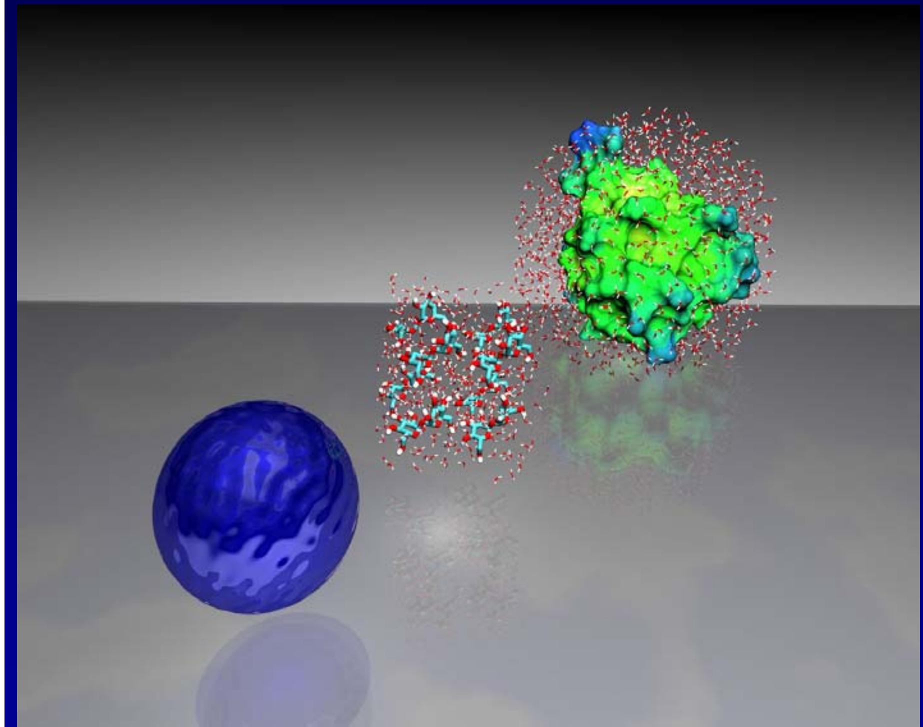
# Tunable THz Germanium Laser - Emission
# - a high power laser in the THz region-


M. Tonouchi,
Nature Photonics 1 (2007)

# A microscopic view on biomolecules



**Do we have a molecular description of macroscopic phenomena ?**
*"How do physical forces and interactions govern biological function, from the molecular to the cellular scale ?"*

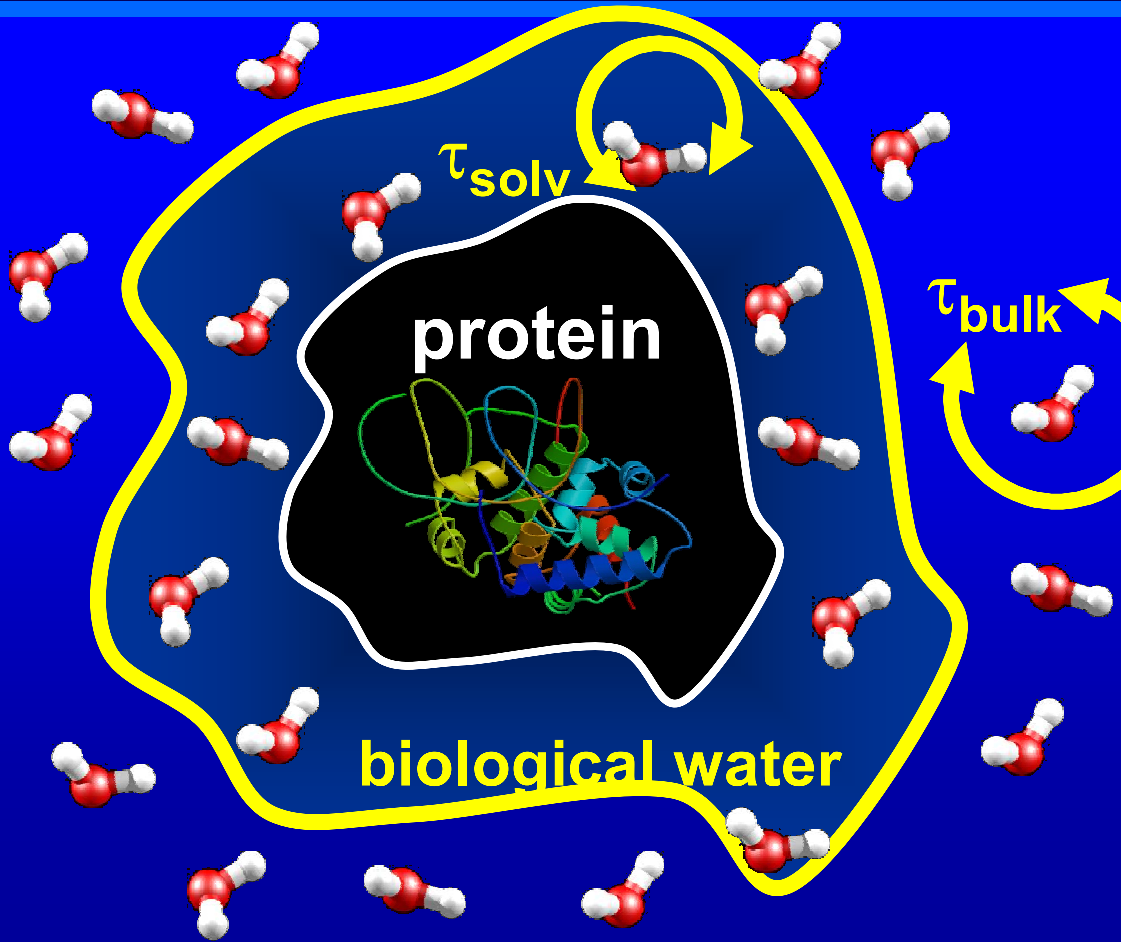
**Can we predict the function of the protein based upon merely the structure ?**

**Additional relevant parameters ?**
**Dynamics? Role of water ?**

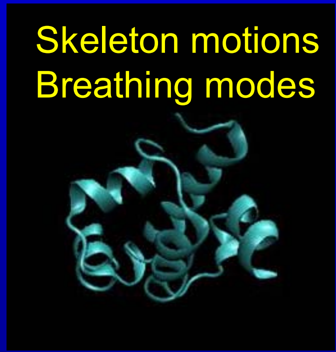
**Can we find adequate models for theoretical description of solvation?**

# Solvation of proteins:

**Expulsion of solvation water the hydrophobic core cause folding barrier**
**Dynamical and structural fluctuations of water molecules contribute to the flexibility of the polypeptide chain : e.g. heme binding site**
**Slaving? Do solvent fluctuations dominate protein function ?**
**How does solvation water differ from bulk water ?**
**What is the size of the hydration layer ?**




**Pal, Peon, Zewail, PNAS 99, 1763 )**



# THz absorption of water yields information on network oscillations – solvation dynamics

Beers law

$I(\omega)=I_0 \cdot e^{-\alpha(\omega)l}$ + const.

Result:

$\alpha \approx 400\ cm^{-1}$ !

f = 2.7 THz
= 90 $cm^{-1}$

## Strong temperature dependence of THz absorption

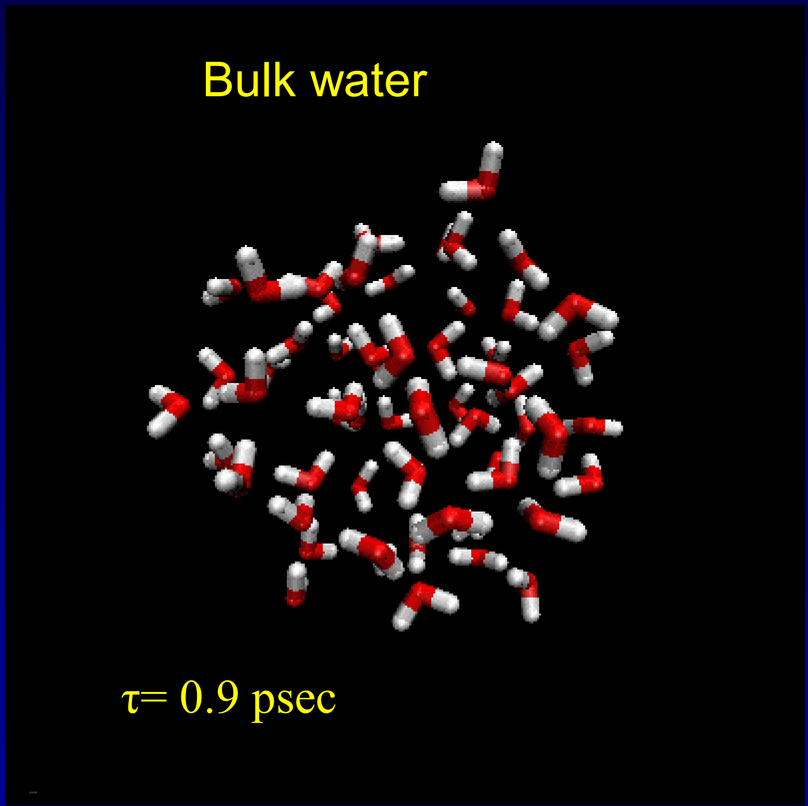
**Ronne, Thrane, Astrand, Wallqvist, Mikkelsen, Keiding,**
**J. Chem. Phys. 107, 5319 (1997)**

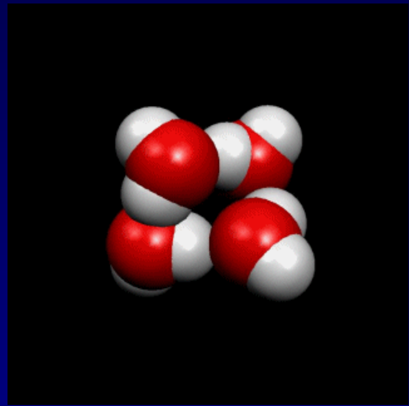
**Water absorption at 1.5 THz for 100 µm water layer:**

**370 K : $I/I_0 = 4 * 10^{-1} = 40\ \%$**

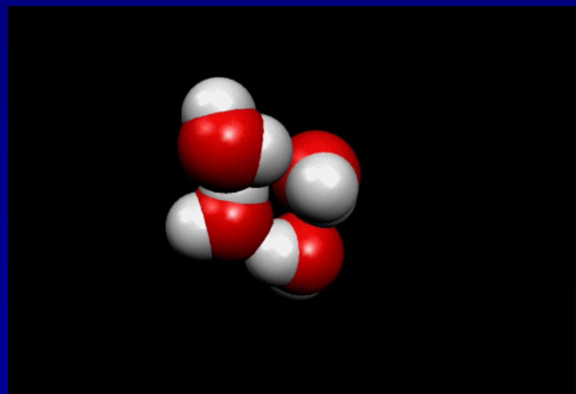
**270 K : $I/I_0 = 7 * 10^{-3} = 0.7\ \%$**

# Molecular dynamic simulationen (MOIL)
# X. Xu, D. Leitner, University of Reno
# water motions at 3 THz (sub-psec dynamic)



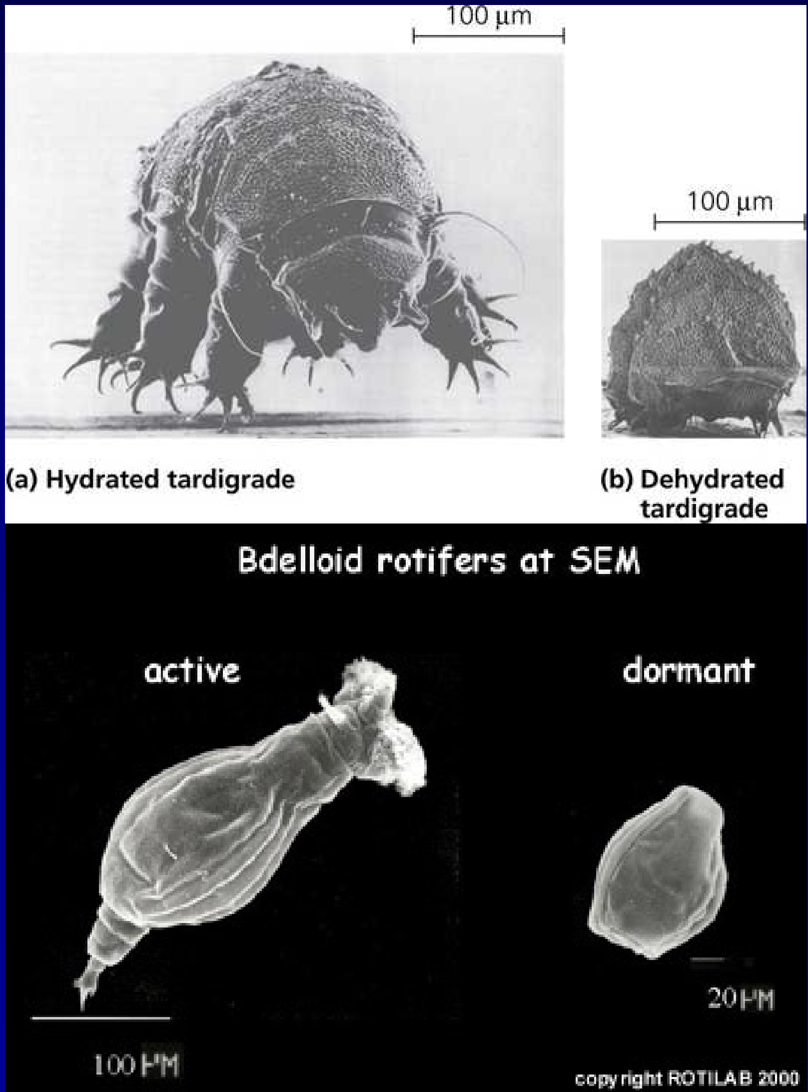



Libration (675 cm-1):
rupture and breaking
of H-bonds



connectivity (200 cm-1):
Longitudial motion of
the H-bond along the axis

# Anhydrobiosis
# "life without water"




- Anhydrobiotes can survive complete dehydration
- For plants we find a correlation with the presence of larger amounts of solubale disaccharides, especially trehalose.
- Although this is used in industrial production processes, the molecular mechanism is remains still unclear, so far.

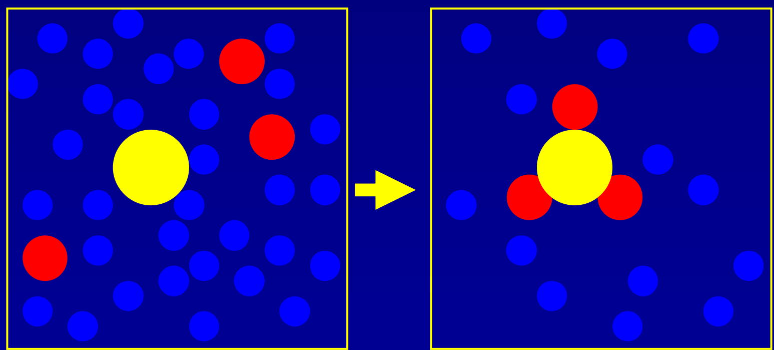
# Well known: “Bioprotection” of Liposomes, proteins and red blood cells by sugar

# Competing molecular description:

- **<u>Water exclusion:</u>**

  H-bridge of the sugar to the protein

  Crowe, Science <u>223</u>, 701 (1984)



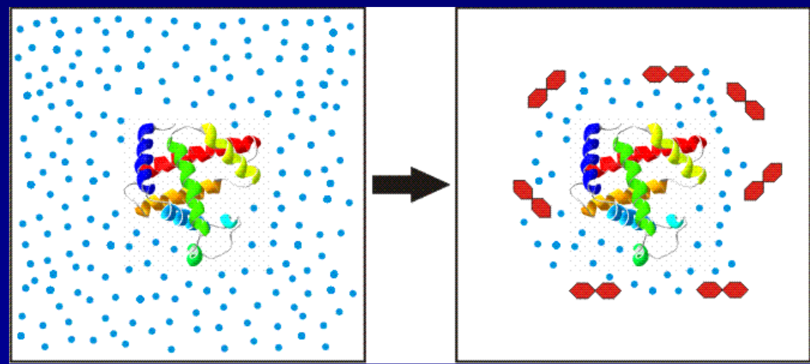
Crowe et al.

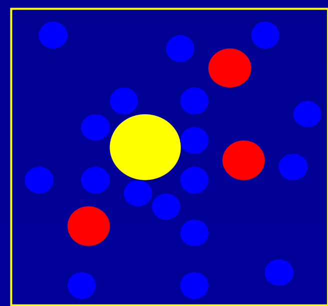
- **<u>crystallisation</u> (Dihydrat Th)**

  **Formation of water channels**

  **Immobilization of water**

  **D. Kilburn et al., Nature mater.** 5, **632 (2006)**





Bio-molecule disaccharide water

**THZ spectroscopy of solvated lactose:**

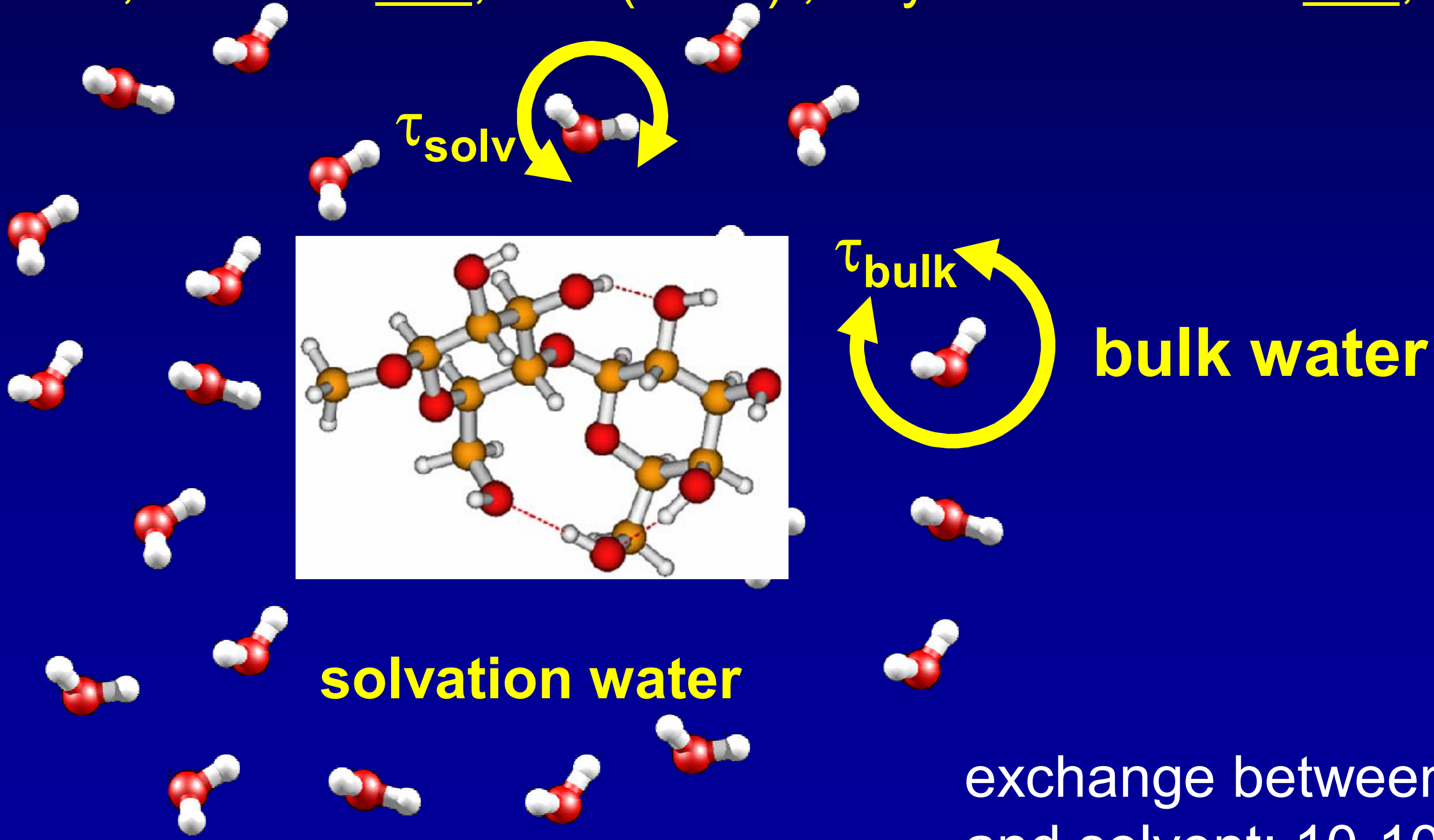
Observed: “Bioprotection” of proteins by sugars

Hydrogen bonding of sugars to proteins or:

Do carbohydrates slow down the dynamics ?

How long ranged is the influence of lactose on a psec scale?

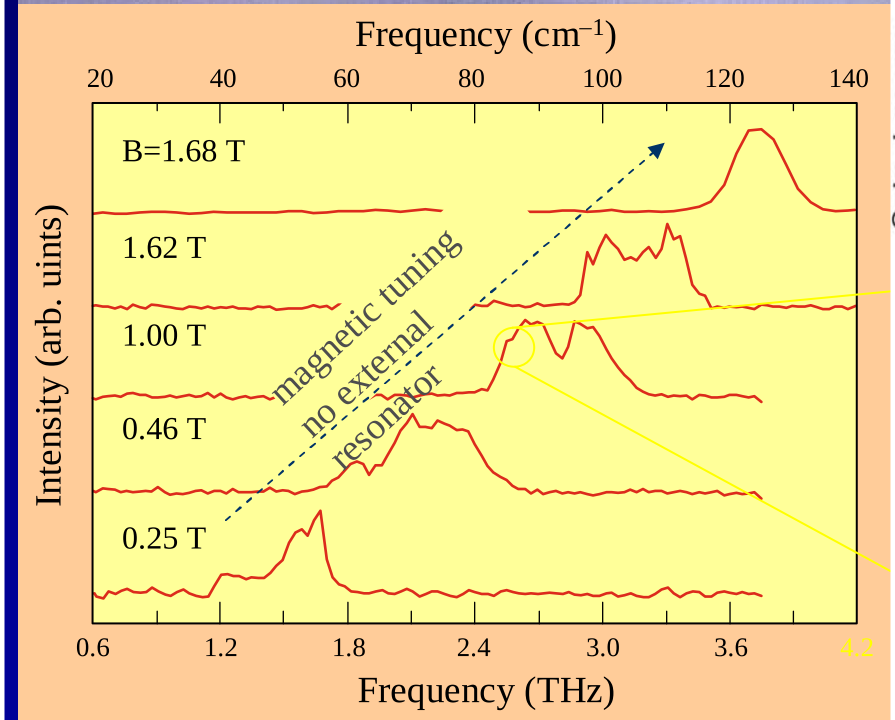
(Crowe, Science 223, 701 (1984) , Fayer *et al.* JACS 127, 14279)

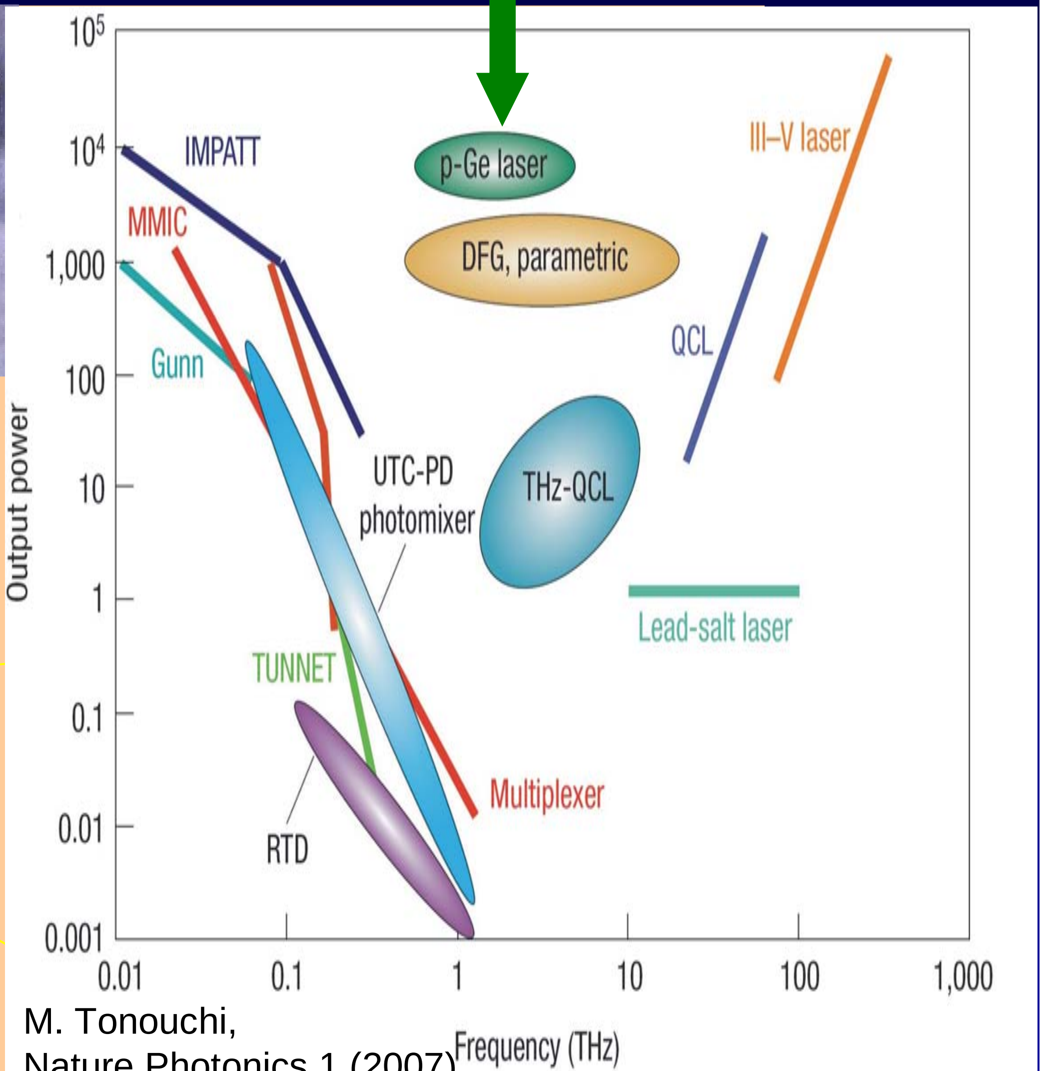



exchange between bulk and solvent: 10-100 psec

*THz spectroscopy*

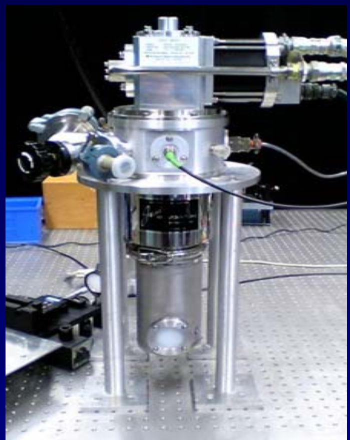
# Tunable THz p-Germanium Laser - Emission
# - a high power (2 W) laser in the THz region-

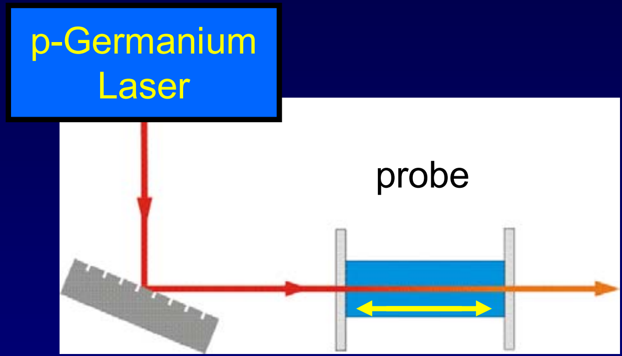

M. Tonouchi,
Nature Photonics 1 (2007)

# THz absorption spectroscopy of solutes
# Set-up of a p-Ge spectrometer

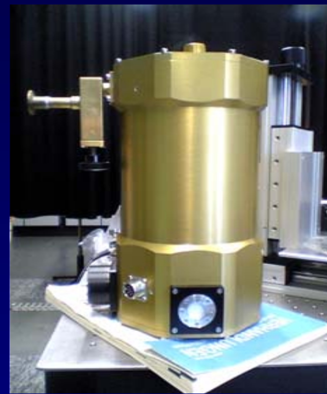


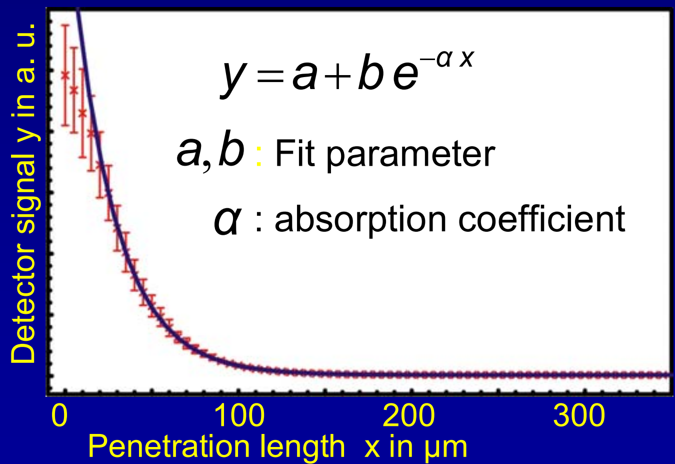
Radiation source
- 2 Watt laser power
- penetrates 150 µm water layer
- tunable from 1 - 4 THz

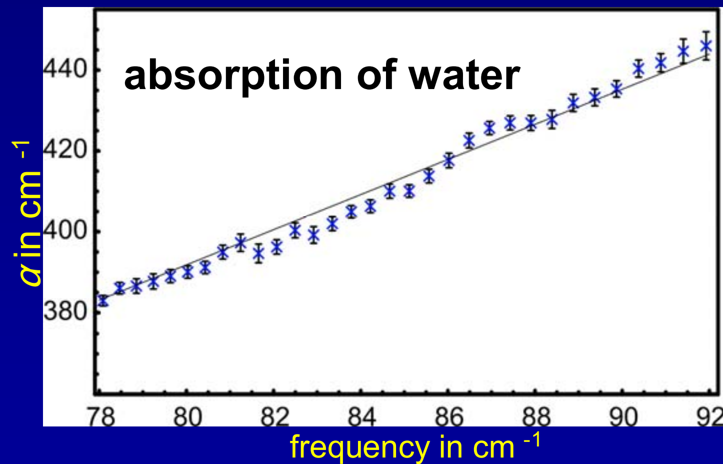
only high power table-top THz radiation source




detector





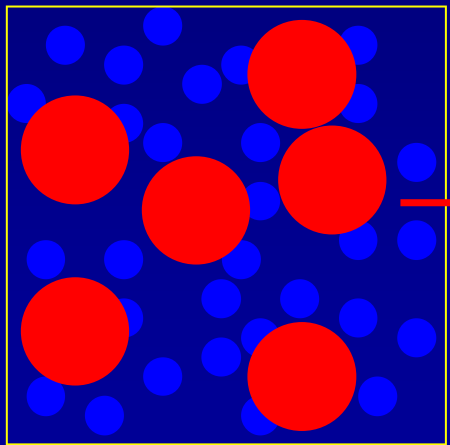

A. Bergner, U. Heugen, E. Bündermann, G. Schwaab, M. Havenith, D. Chamberlain und E. Haller, Rev. Sci. Instrum., 76, 2005

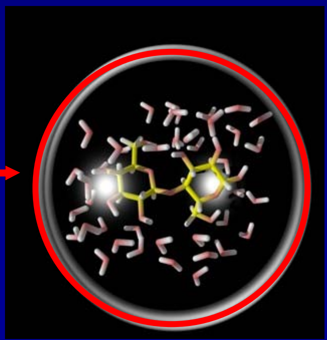
# The THz Spectrum of solvated Lactose

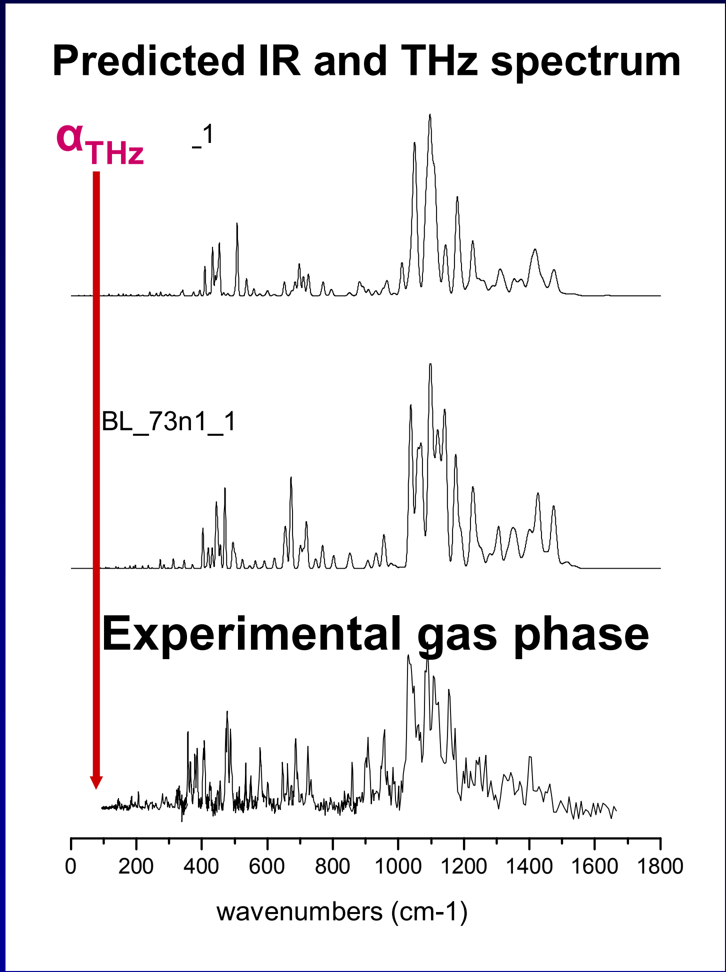
**Lactose (gas phase) is nearly transparent in the THz spectral range**

**simple model:**

**THz absorption is decreased by water displacement**

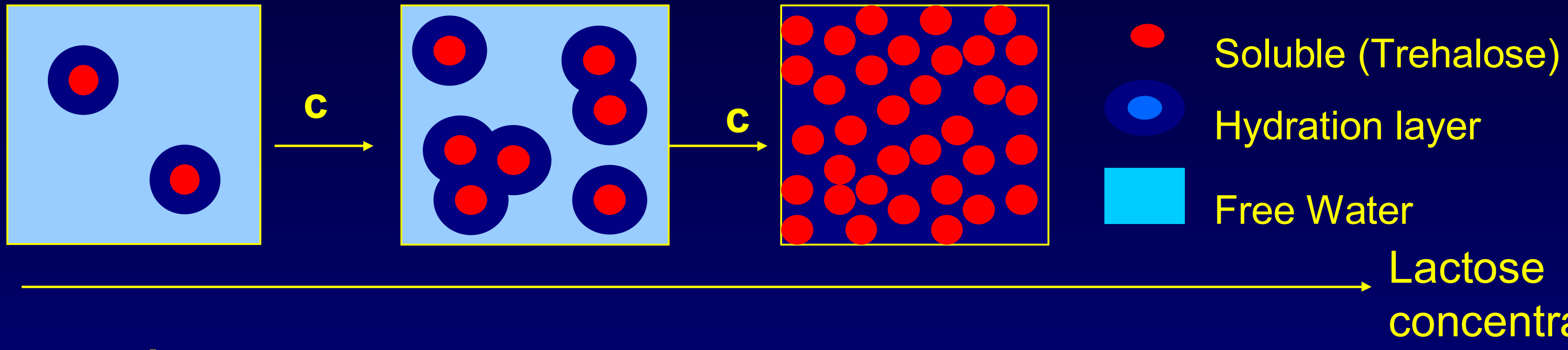






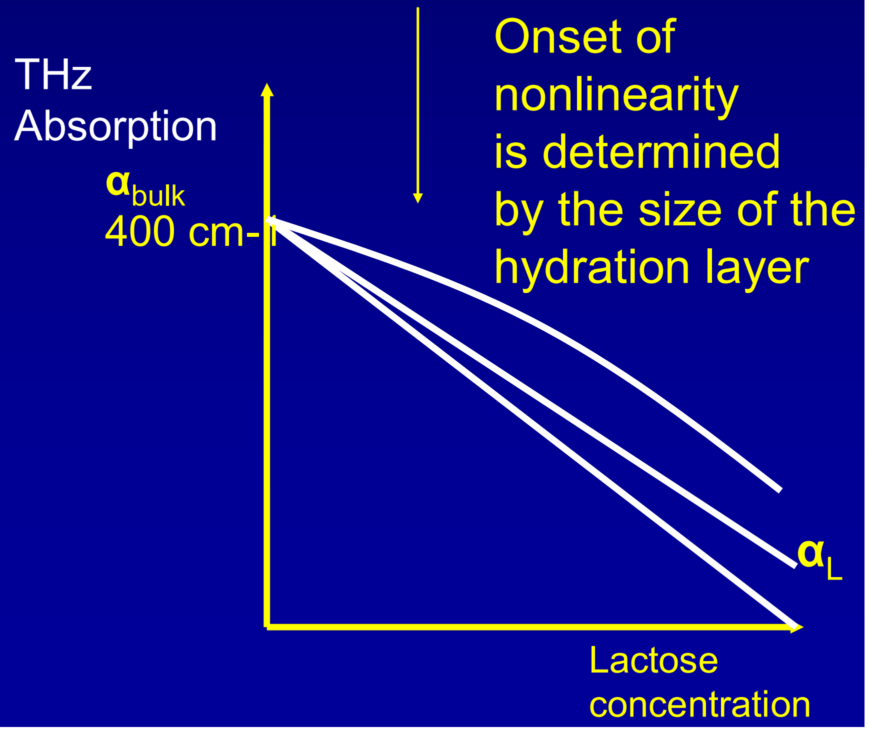

R.A. Jockusch, R.T. Kroemer, F.O. Talbot, L.C. Snoek, P. Çarçabal, J.P. Simons, **M. Havenith,** J.M. Bakker, I. Compagnon, G. Meijer, G. von Helden
**J. Am. Chem. Soc. 126, 5709 (2004)**

# Concentration dependence of the THz spectrum of solvated lactose




**Assumptions:**

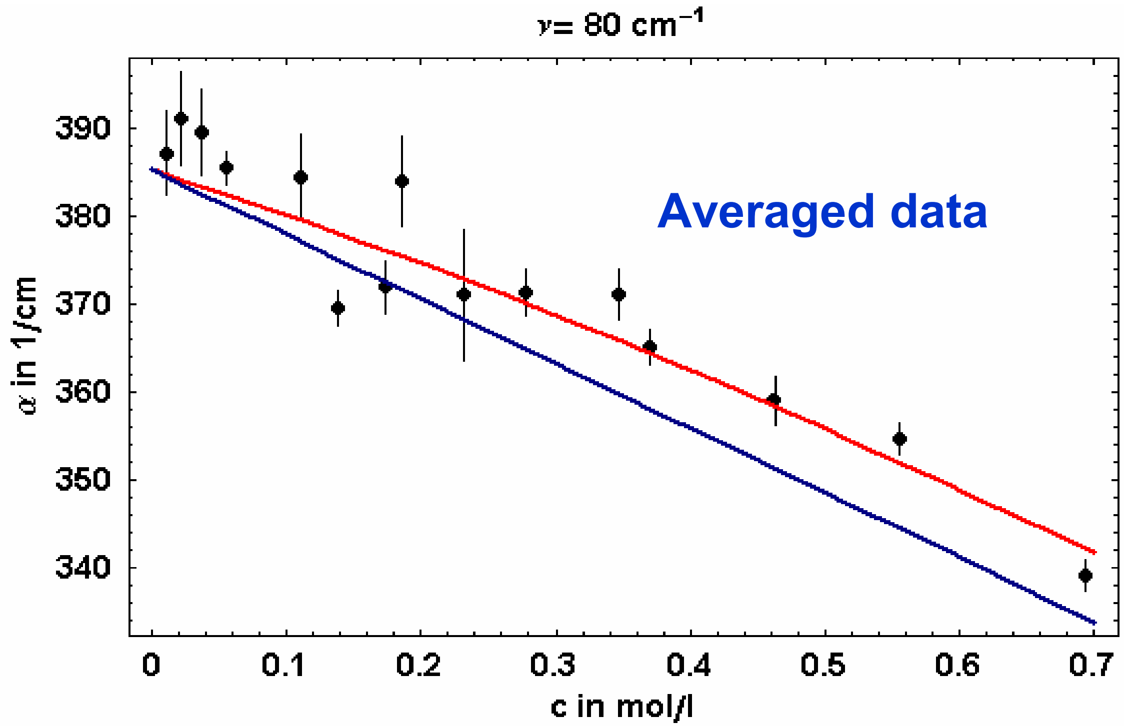
- Excluded volume model:
  ⇨linear decrease of absorption
  $\boldsymbol{\alpha_{total} = (1 - V_{solute}/V)\, \alpha_{bulk}}$
- Each lactose contributes with its THz absorption
  ⇨Linear increase $\boldsymbol{+\, V_{solute}/V\, \alpha_{solute}}$
- Hydration water contributes to total absorption
  ⇨Linear contribution $\boldsymbol{+\, V_{sw}/V\, \alpha_{sw}}$
- overlap of hydration shells
  ⇨deviation from linearity

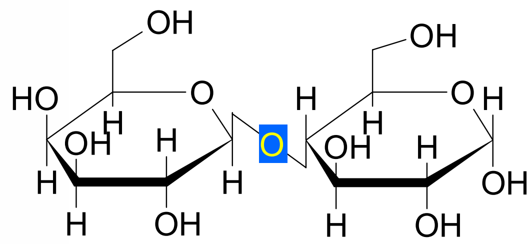


# THz spectroscopy of solvated lactose

## First experimental Results

bulk




**Fitted**
3 component model

Linear:
Water displacemnt

**Fit of:** $V_{sw/V}$ : relative volume of the solvation water
$\alpha_{sw}$ : absorption coefficient of the solvation water

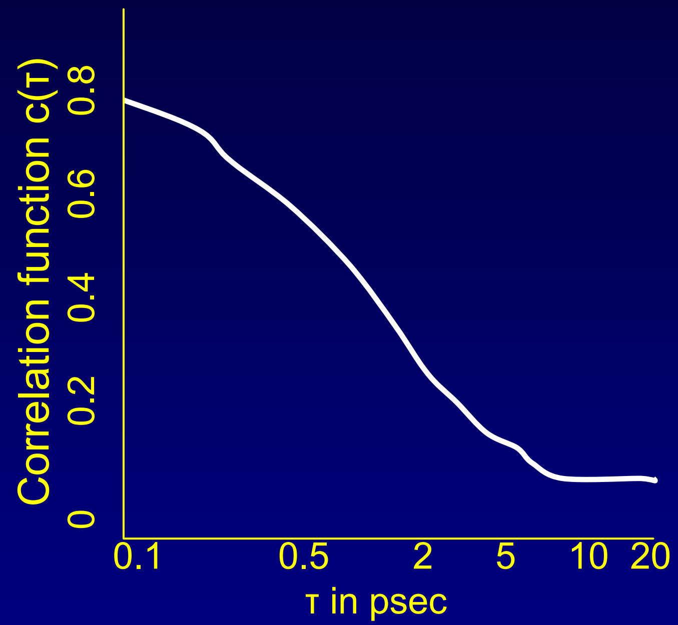
U. Heugen, G. Schwaab, E. Bründermann, M. Heyden, X. Yu, D.M. Leitner, **M. Havenith**
Solute induced retardation of water dynamics:
Hydration water probed directly by THz spectroscopy,
**PNAS 103, 12301 (2006)**

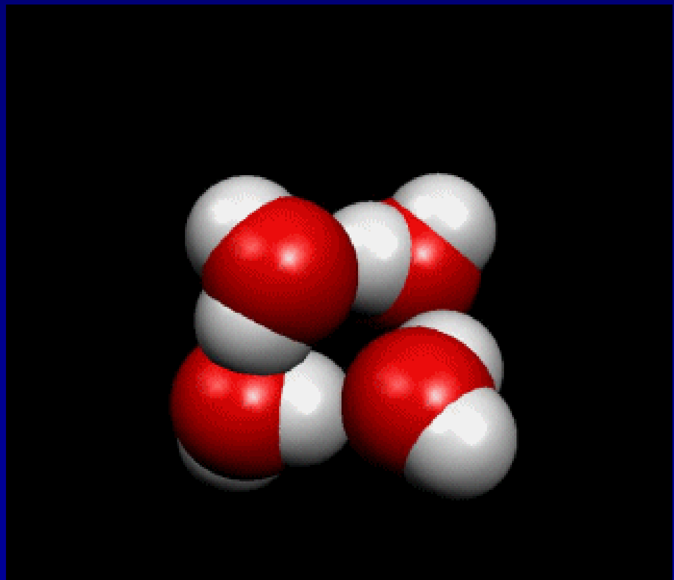
# Quantitative description of solvation dynamicsby correlation function

Probability that an H-bond exists at a time t, if it was intact at t=0

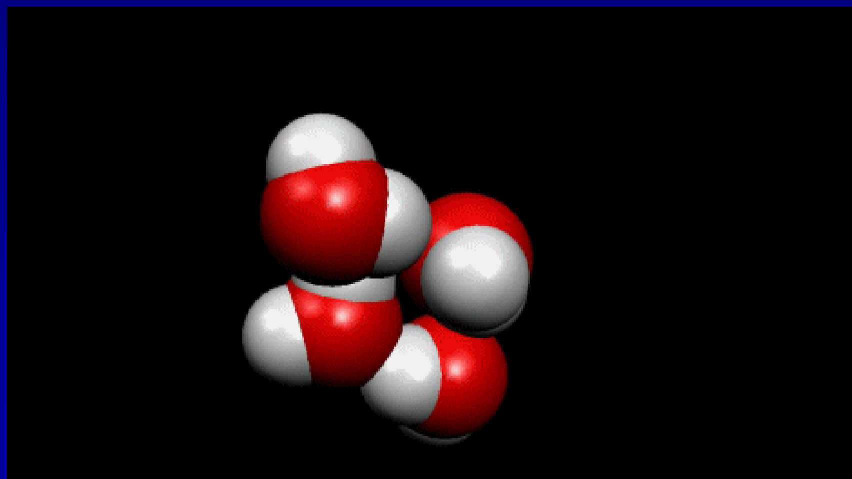
$$C(t)=\langle h(0)h(t)\rangle/\langle h\rangle$$

$$c(t)=\exp(-t/\tau)$$



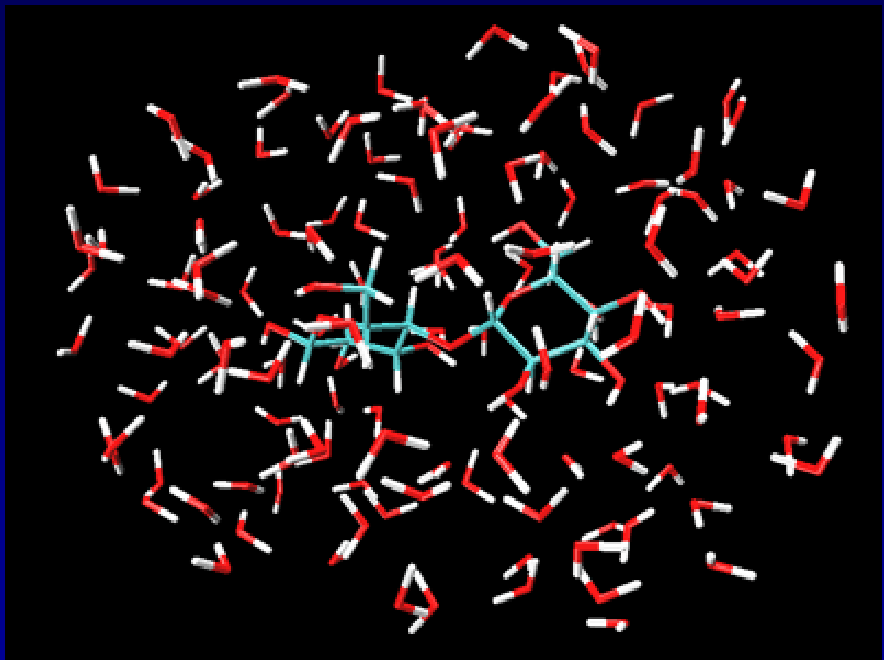
„Rotation“

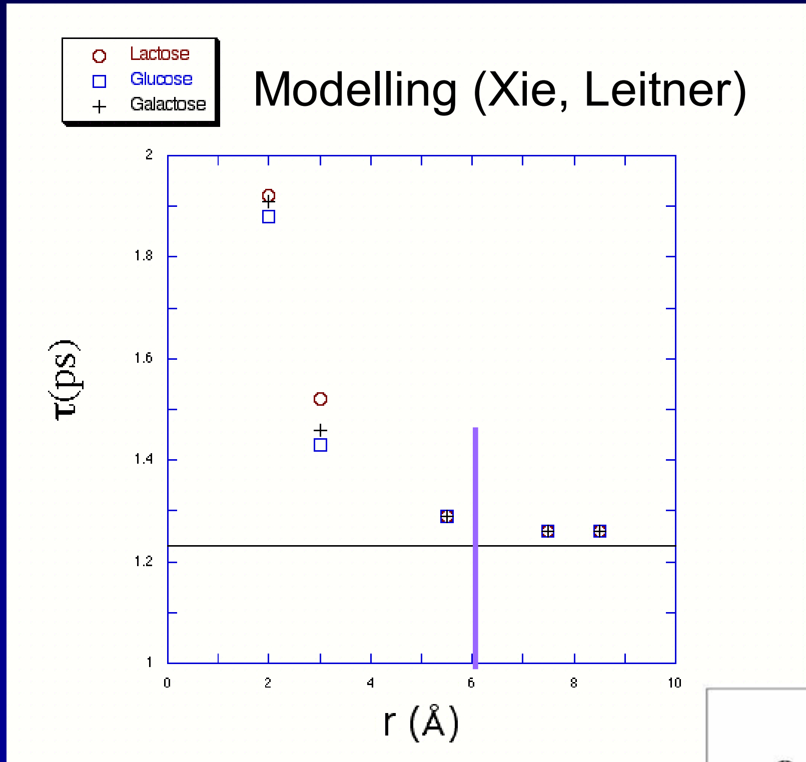


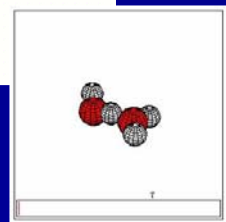
„Diffusion“

# THz spectroscopy as a new tool to probe the dynamical hydration layer (global probe)
# 123 water per one sugar

## Determination of the (sub-psec) solvation radius





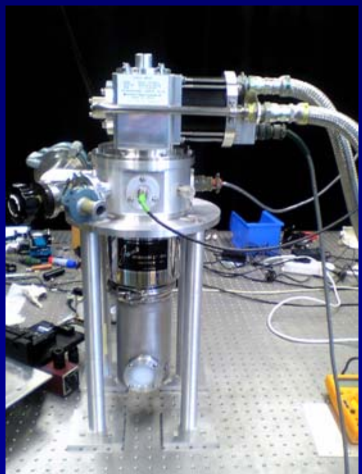



**$R_{solv}$= (5.13±0.24) Å**
**Corresponds to 123 $H_2O$**
(much more than X-ray)

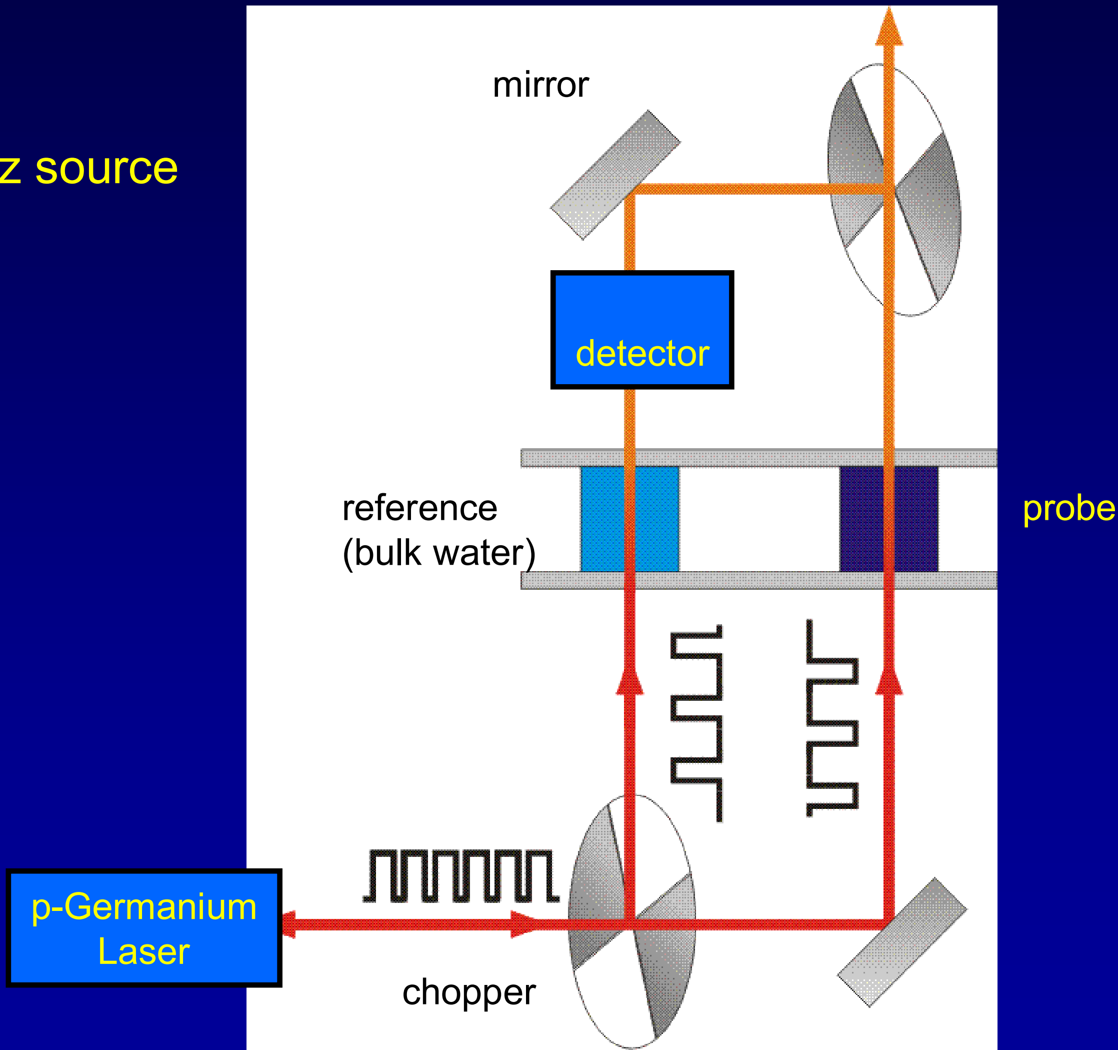
**Significant retardation up to 6 Å (112 $H_2O$)**
**Particullary sluggish for 46 water (4 Å )**

# THz difference absorption spectrometer
## for precise measurements of absorption coefficients

**p-Ge laser:** a unique THz source

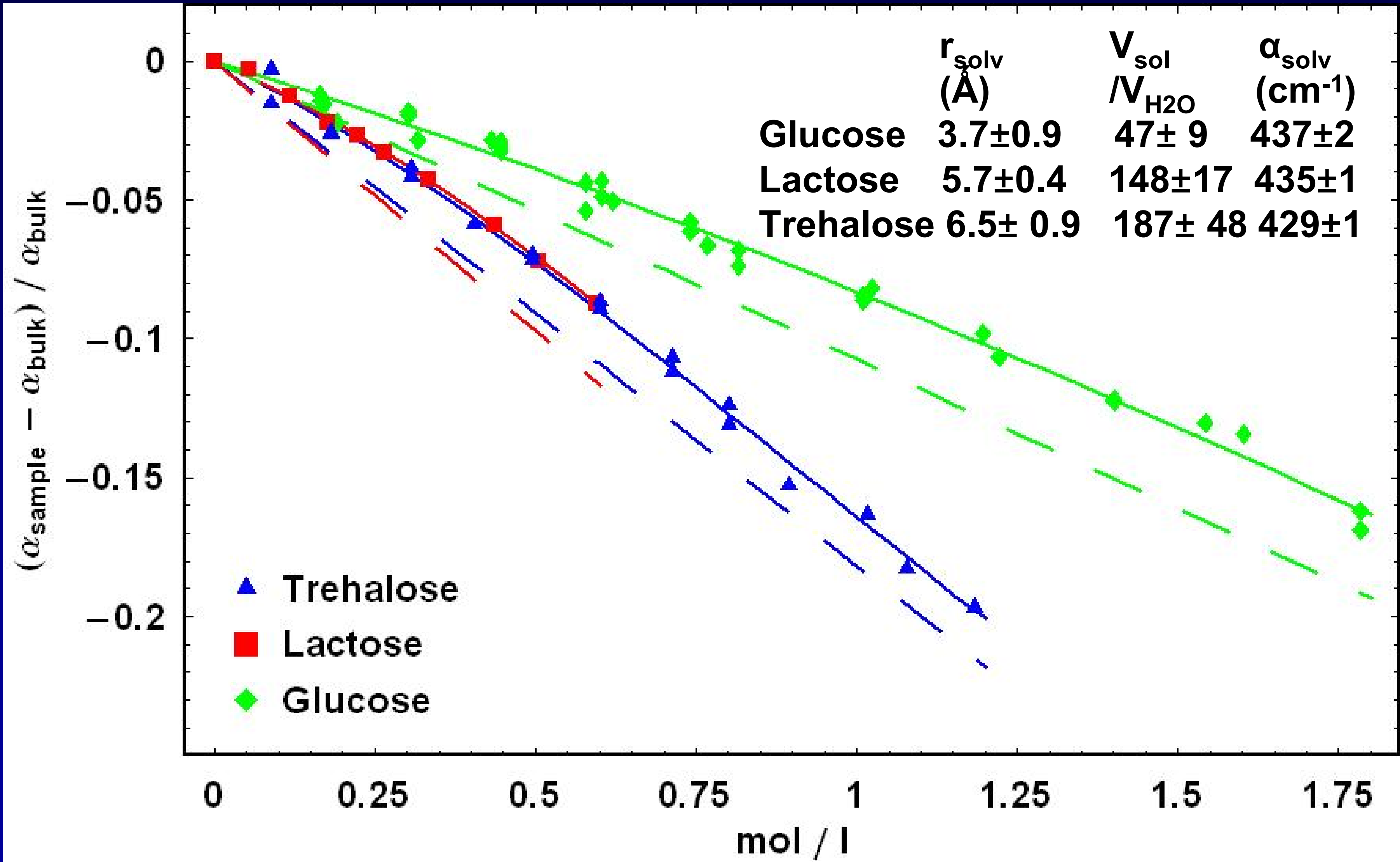


$$\alpha_{probe} = -\frac{\ln(I_{probe}/I_0)}{d}$$

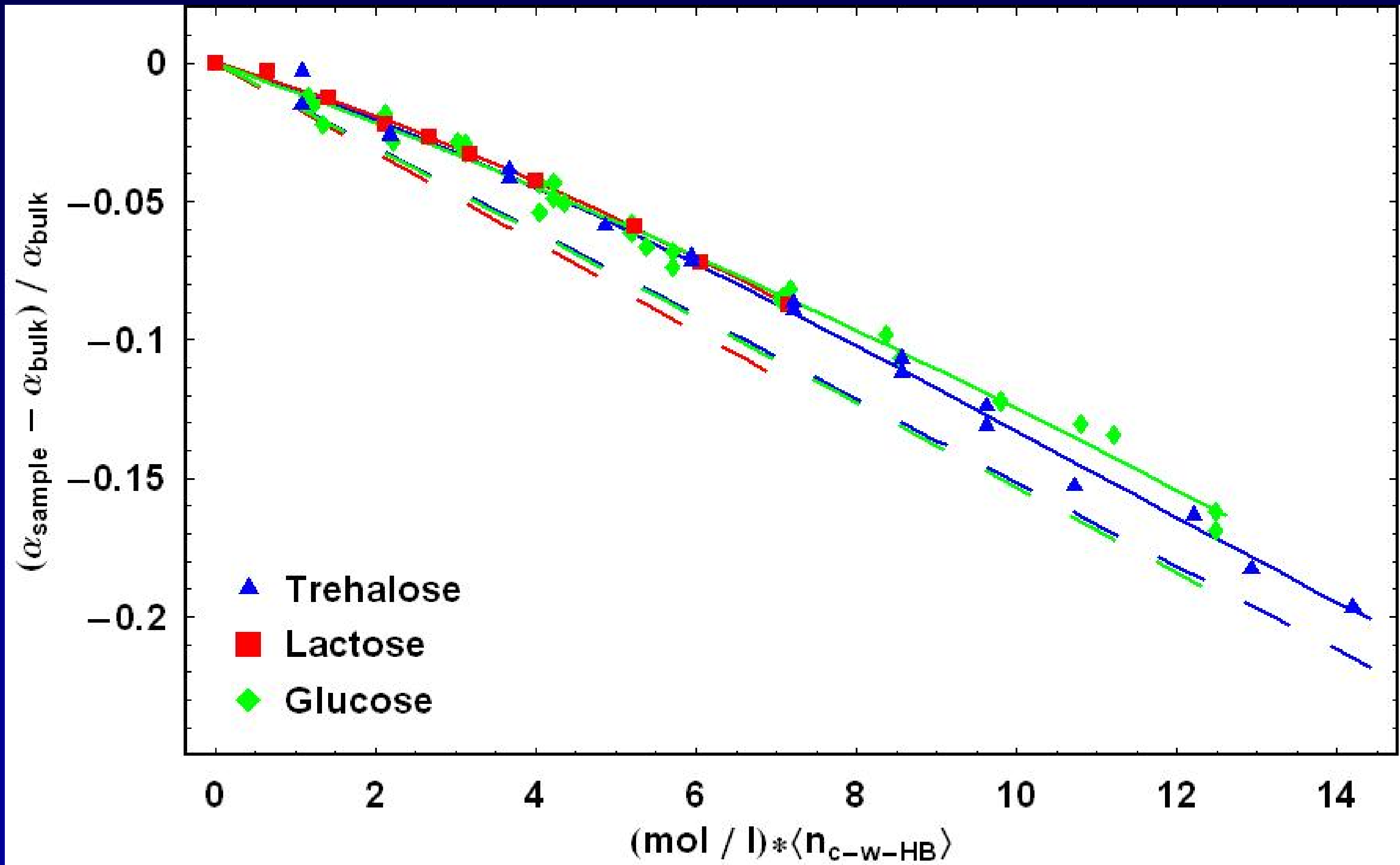


# New Experimental result: Comparison between different carbohydrates:

M. Heyden, U. Heugen, G. Nienhues, E. Bründermann, D. Leitner, M. Havenith, 2008



# Comparison between different sugars: scaling the change of THz absorption with the number of H-bonds ($\langle n_{c\text{-}w}\text{-HB}\rangle$)



# THz spectroscopy as a tool to determine the size and the „nature“ of the hydration shell in the sub-psec regime

**Fit to a three component model:**

$\alpha_{total}=$

$$\mathbf{V_{sw}/V\ \alpha_{sw}} + V_{solute}/V\ \boldsymbol{\alpha}_{\mathbf{solute}} + (1-V_{sw}/V-V_{solute}/V)\ \alpha_{bulk}$$

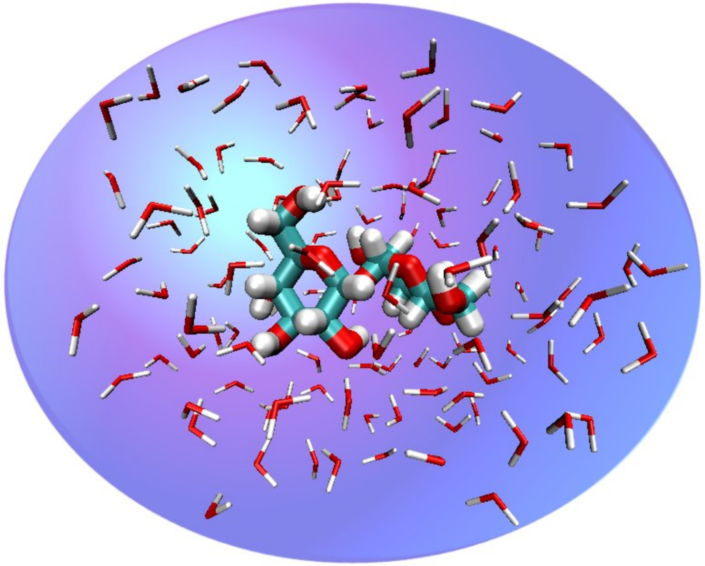
$\mathbf{V_{sw}/V}$ **:** relative volume of the solvation water

$\boldsymbol{\alpha}_{\mathbf{sw}}$: absorption coefficient of the solvation water

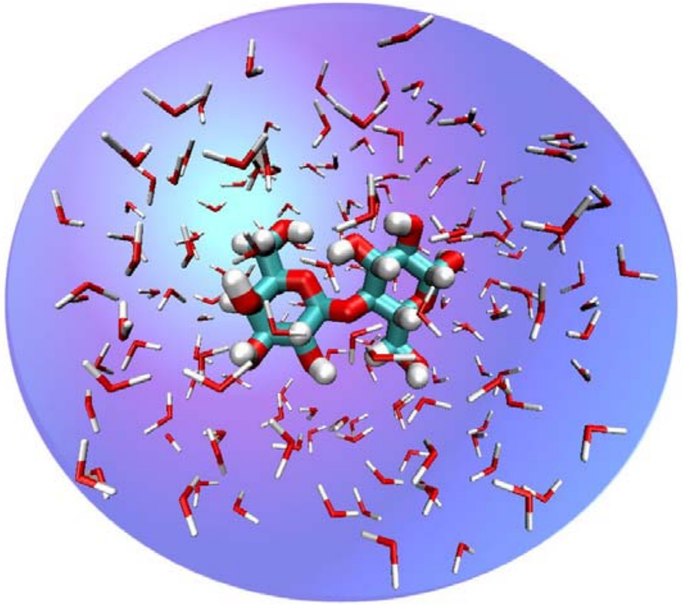
$V_{solute}/V$: relative volume of the solute

$\boldsymbol{\alpha}_{\mathbf{solute}}$: absorption coefficient of the solute

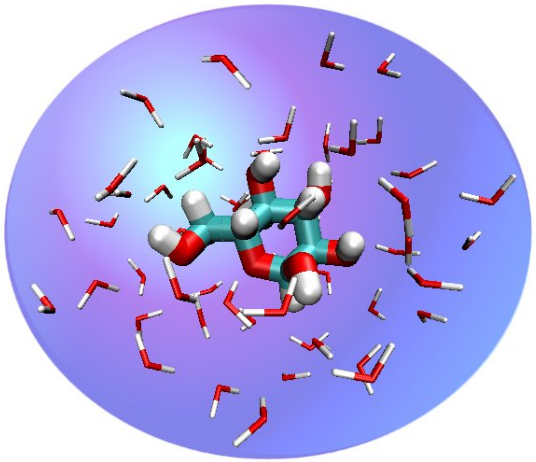
# Comparison of the dynamical hydration shell for different carbohydrates



**Trehalose**
$R_{sol}$= (6.5±0.9) Å
~ 190 $H_2O$



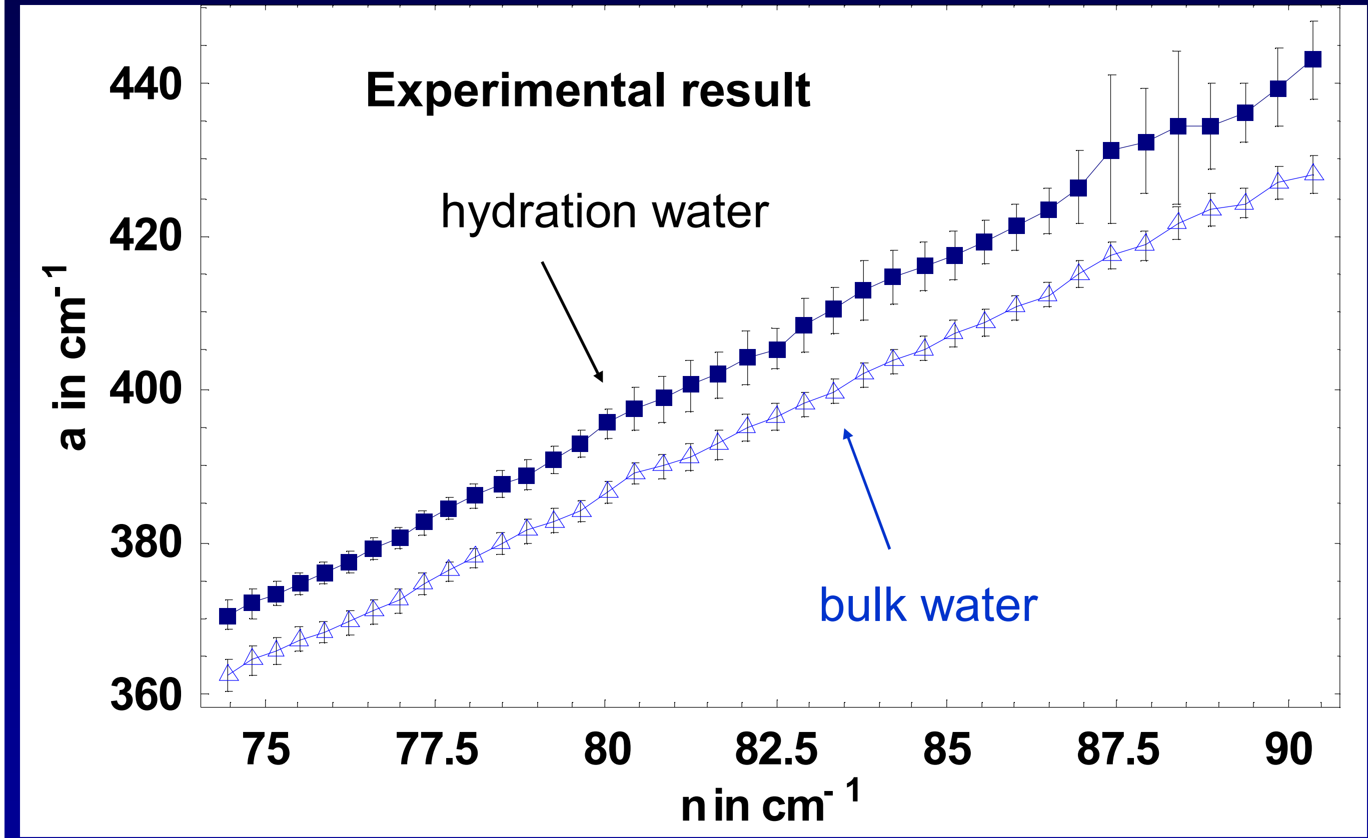
**Lactose**
$R_{sol}$= (5.7±0.4) Å
~ 150 $H_2O$



**Glucose**
$R_{sol}$= (3.7±0.9) Å
~ 50 $H_2O$

# THz absorption of bulk and hydration water

## Hydration water shows increased THz absorption




**Conclusions by comparison to MD:**

**Network vibrations of water molecules near lactose oscillate more in phase than bulk water**

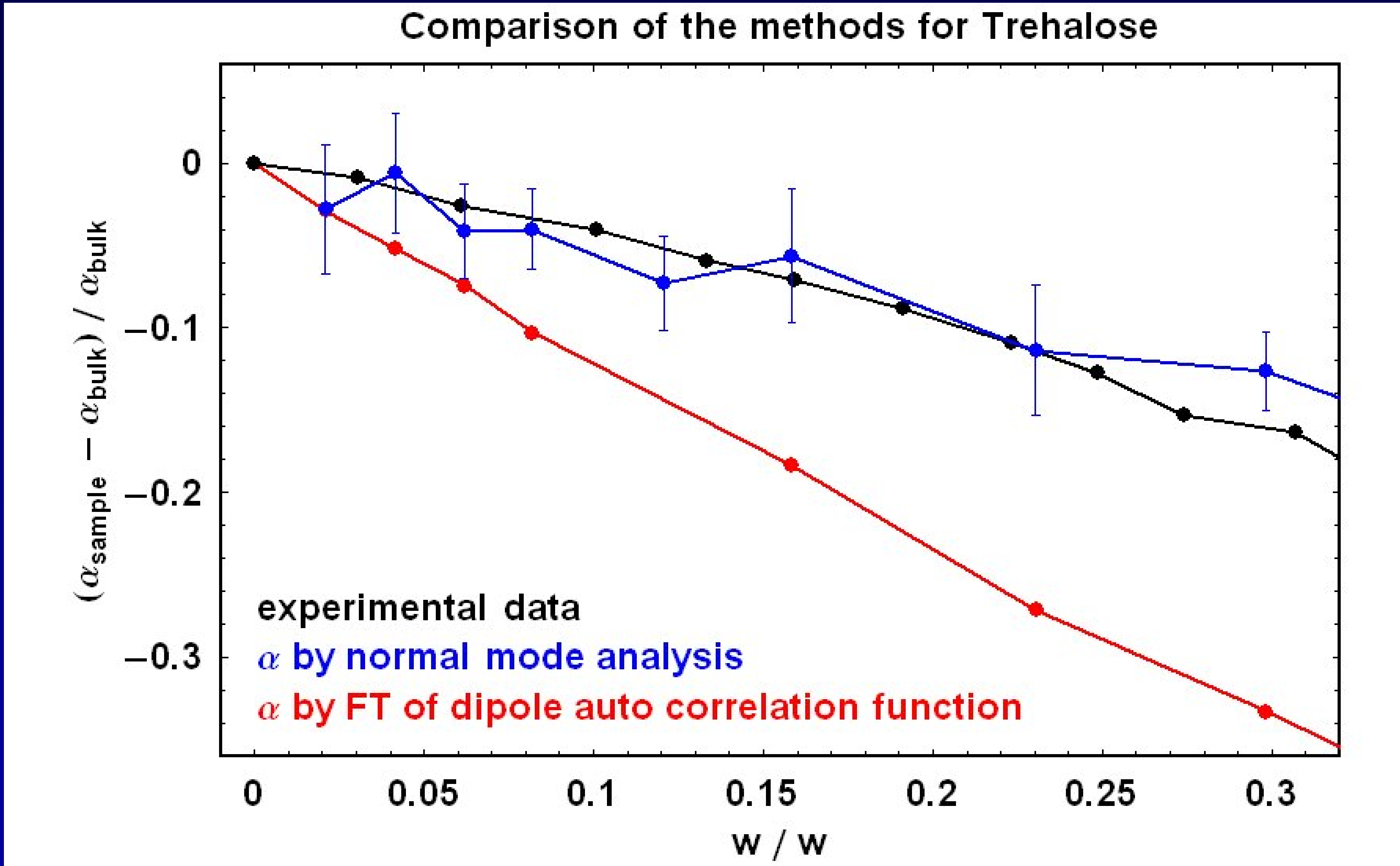
**Lactose and hydration water molecules oscillate coherently**

**Coherent ocsillation yields increased THz absorption**

U. Heugen, G. Schwaab, E. Bründermann, M. Heyden, X. Yu, D.M. Leitner, M. Havenith, PNAS **103**, 12301 (2006)

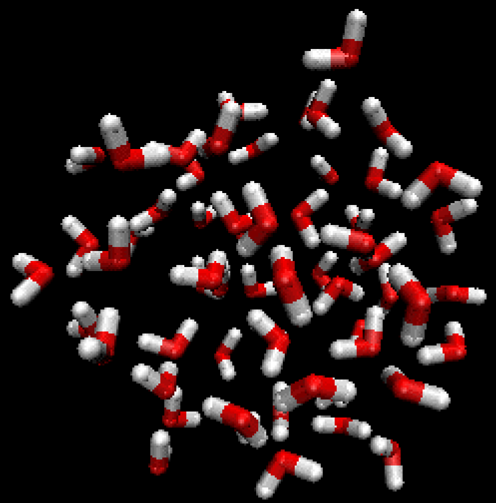
# Mol. Dyn. simulation (3nm x 3nm x 3nm box)

**M. Heyden, U. Heugen, G. Nienhues,E. Bründermann, D. Leitner, M. Havenith, JACS under review**



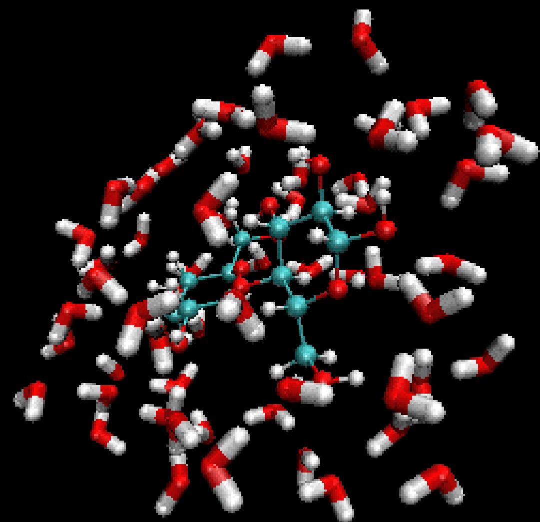
# Molecular dynamic simulation (MOIL)
# X. Xu, D. Leitner, University of Reno
# Motions at 3 THz (sub-psec Dynamik)

Bulk water



$\tau$= 0.9 psec

Lactose and Hydration water



retardation:
$\tau$ = 1.3 psec

**collective motions of water molecules in the network**

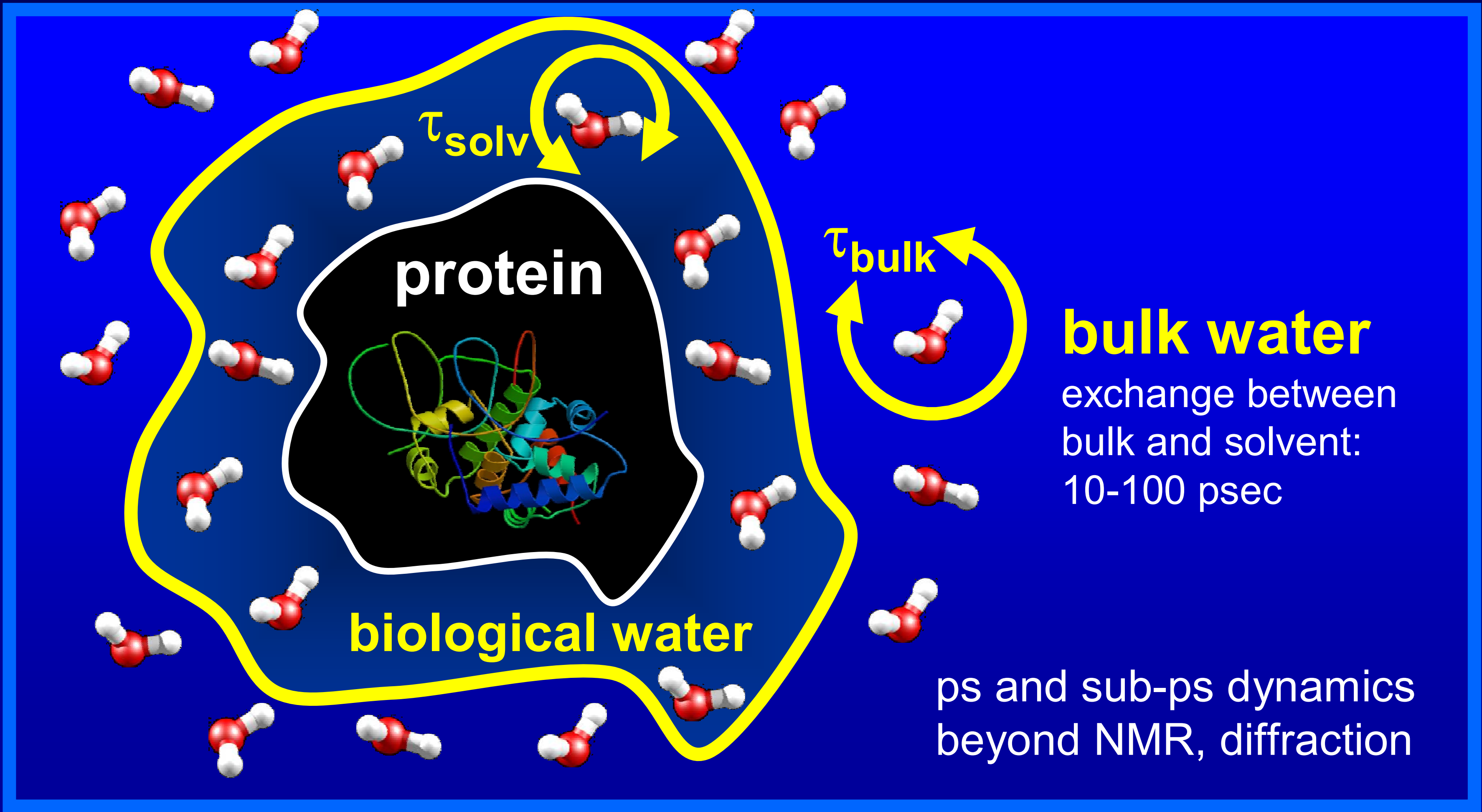
# Individual *versus* collective oscillations

**Bulk water: Uncorrelated, incoherent motion**

# Individual *versus* collective motions

**Hydration water: Coherent, in phase motion**

How about proteins?
What absorbs more proteins of the water ?
Do we see “biological water” ?



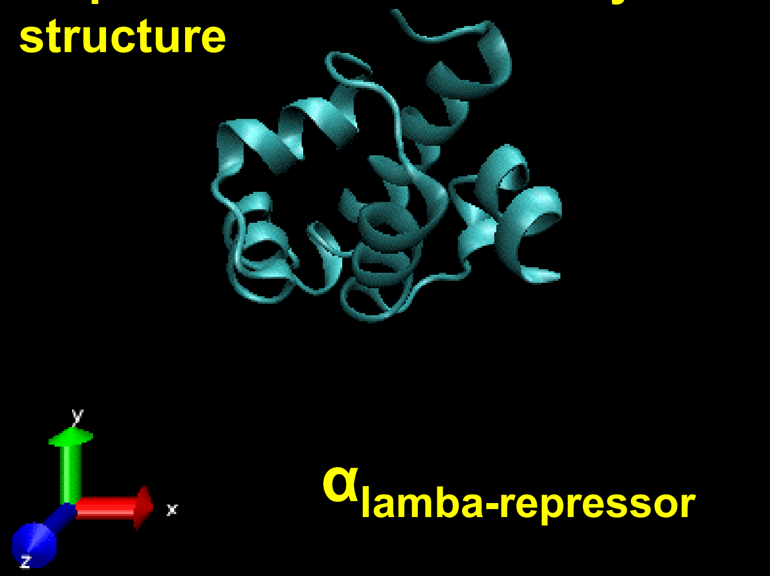
# Taking a closer look on protein and water dynamics:

D.M. Leitner, M. Havenith M. Gruebele

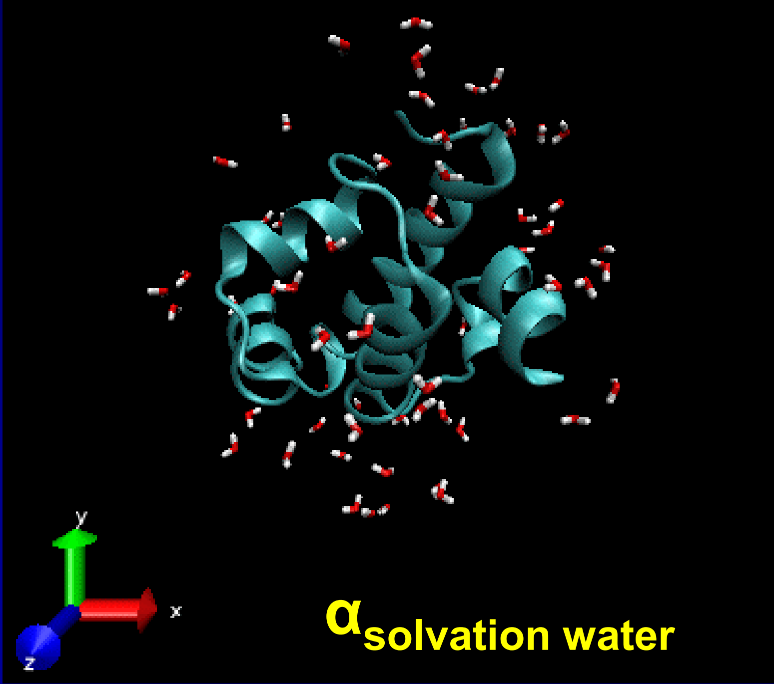
Intl. Reviews in Physical Chemistry 25, 553 (2006)

## THz modes at 80 cm$^{-1}$

breathing modes of helices
displacement in secondary structure

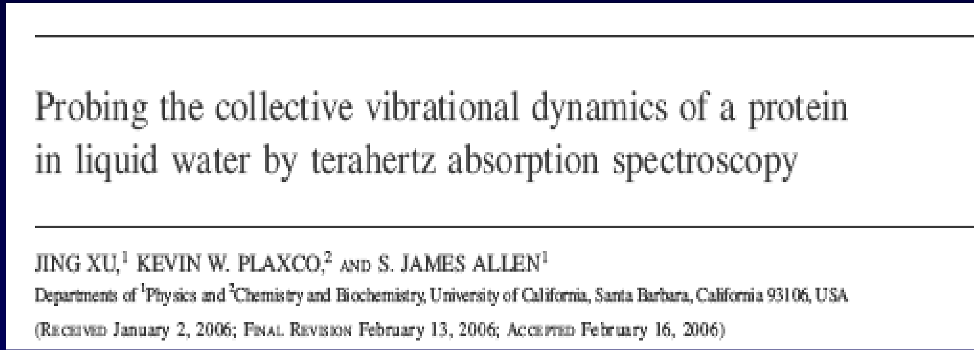



## THz dance of water




molecular dynamics simulation (MOIL)  and figures by: X. Xu, D. Leitner

# Previous study on THz spectroscopy of proteins



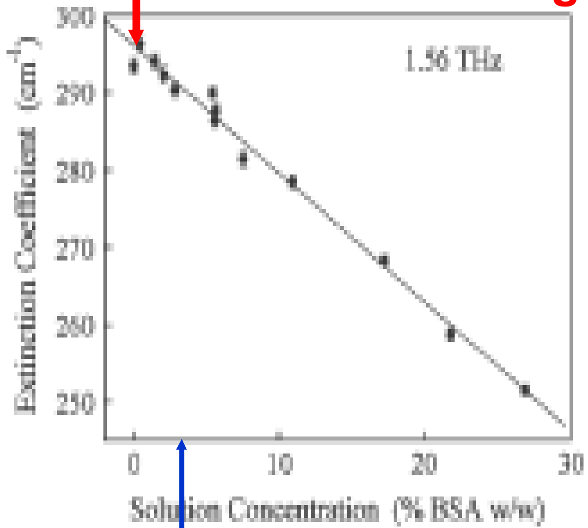
Probing the collective vibrational dynamics of a protein in liquid water by terahertz absorption spectroscopy

JING XU,[1] KEVIN W. PLAXCO,[2] AND S. JAMES ALLEN[1]
Departments of [1]Physics and [2]Chemistry and Biochemistry, University of California, Santa Barbara, California 93106, USA

(RECEIVED January 2, 2006; FINAL REVISION February 13, 2006; ACCEPTED February 16, 2006)

Protein science
15, 1175 (2006)

Is this a bending point ?




THz absorption of
BSA solutions at
1.56 THZ

Highest concentration in our experiment

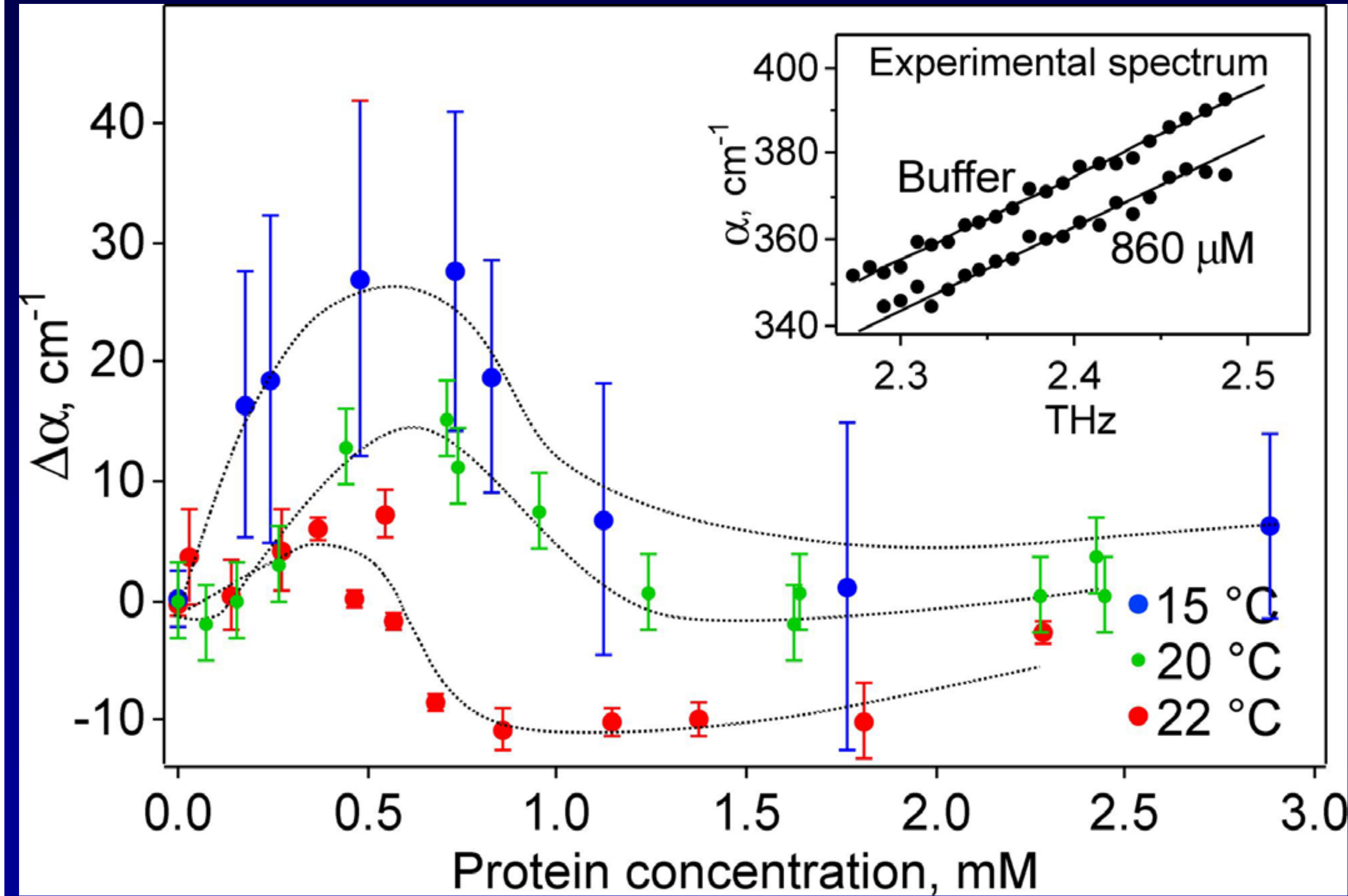
Conclusion:
Linear decrease with protein
Concentrations
Proteins absorb less than water

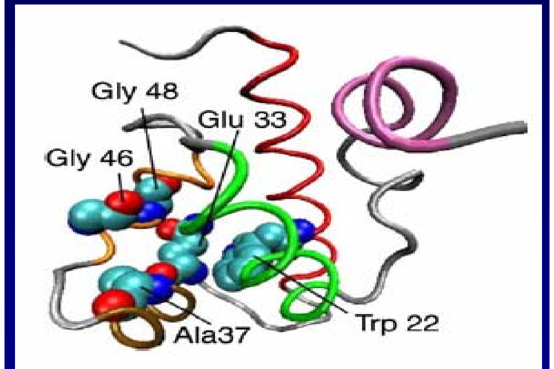
Could deduce the spectrum of the
THz protein :
linear increasing with frequency

Possible coupling of hydration
dynamics to protein dynamics
(Whitmore, Wolpert, Markelz et al.
Biophys. J. 85 , 1269 (2003))

# THz absorption of solvated lamda-repressor

## Experimental results: non monotonic concentration dependence !

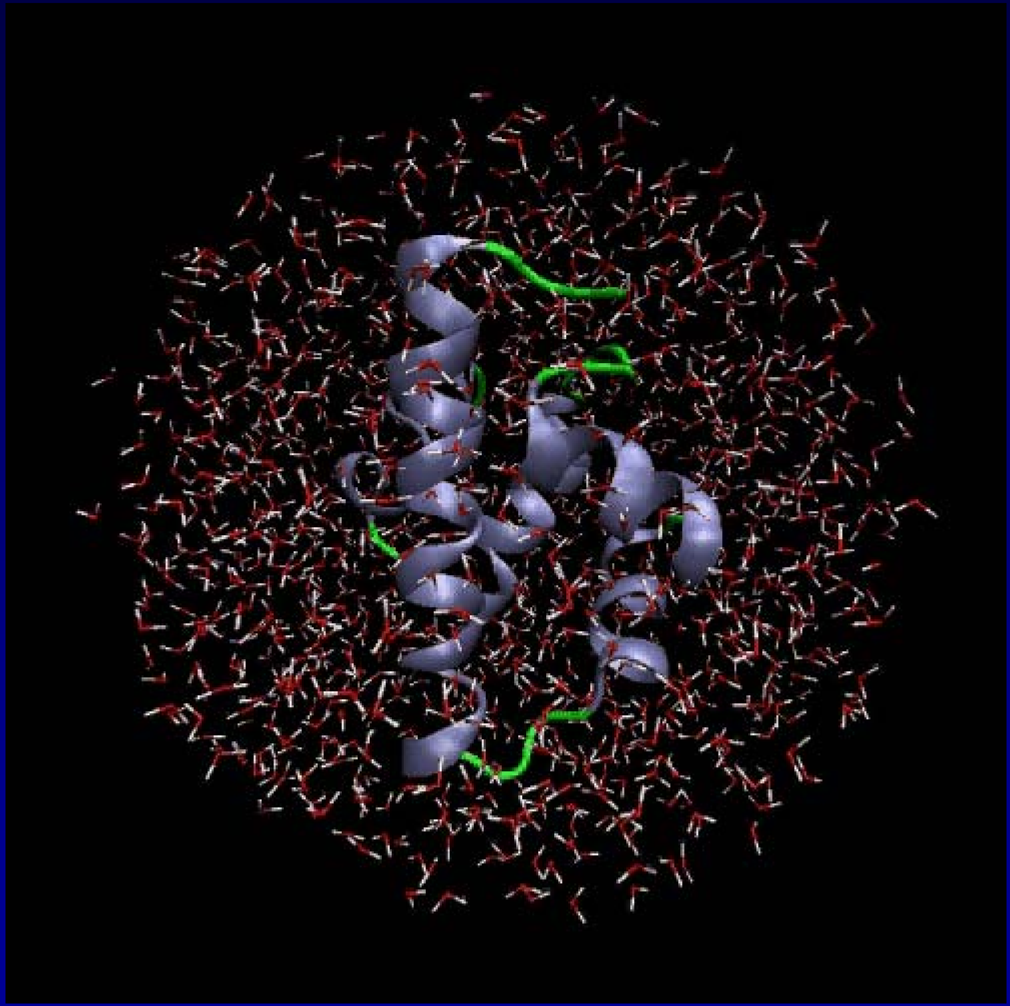



**Structure of the five helix bundle λ-repressor**

*S. Ebbinghaus, S. J. Kim, M. Heyden, X. Yu, U. Heugen, M. Gruebele, D. Leitner, and M. Havenith, PNAS 104, 20749 (2007)*

# The THz dance of the water with the protein



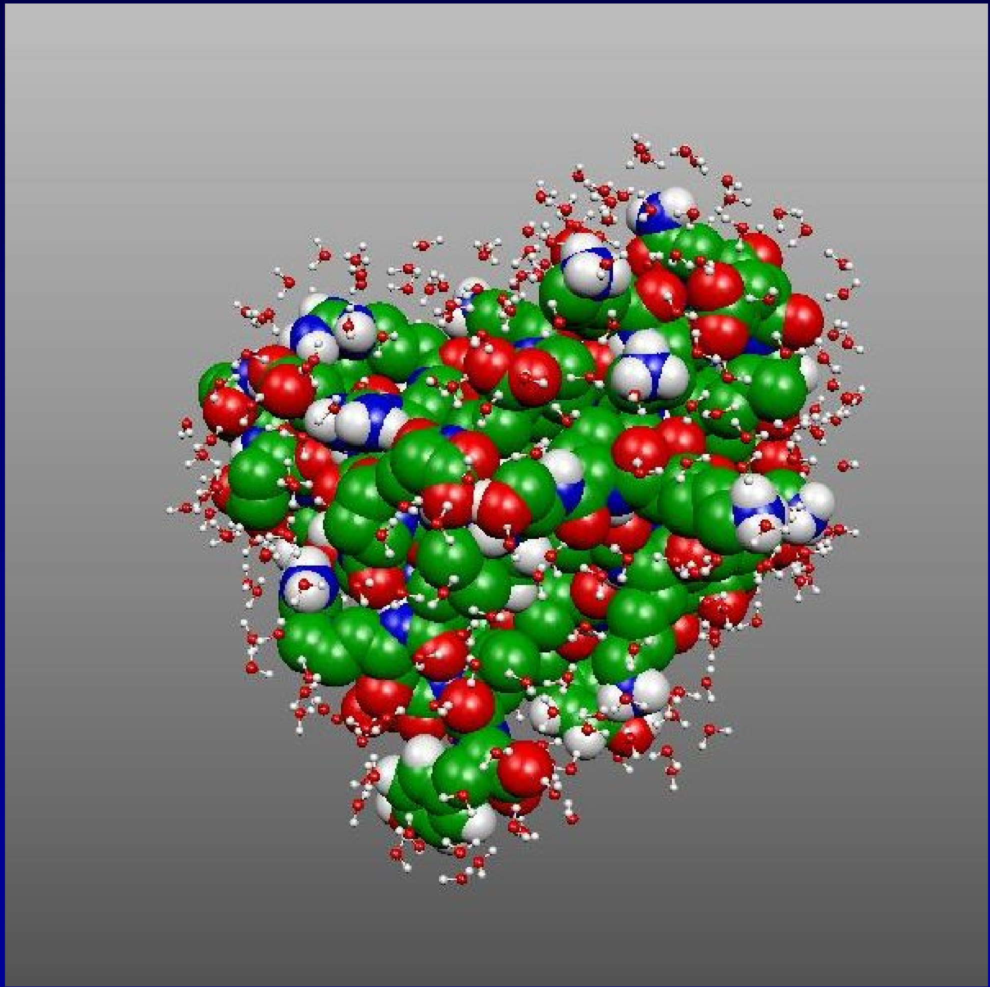
**Result:**

**influence on the correlated water motion of up to 20 Å, greater than the observed structural correlation length**

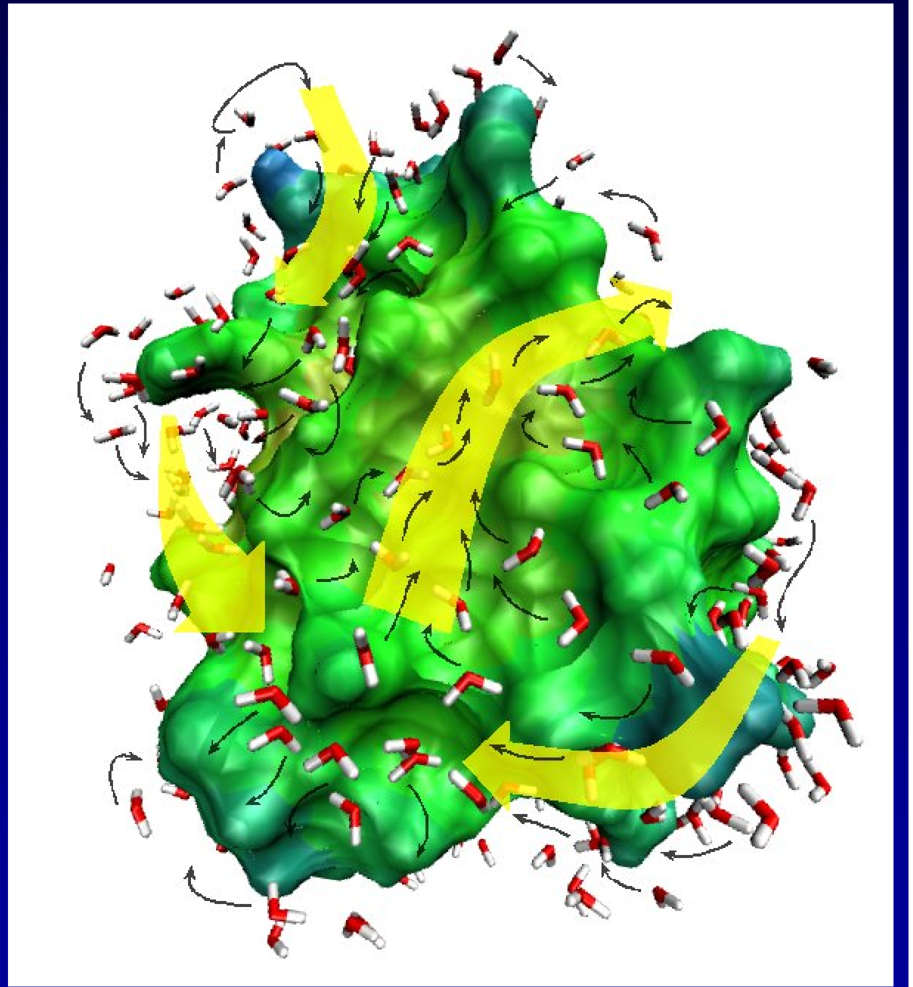
**This corresponds to 1000 $H_2O$**

*S. Ebbinghaus, S. J. Kim, M. Heyden, X. Yu, U. Heugen, M. Gruebele, D. Leitner, and M. Havenith, PNAS 104, 20749 (2007)*

# THz Spectroscopy of a prototype system: λ-repressor

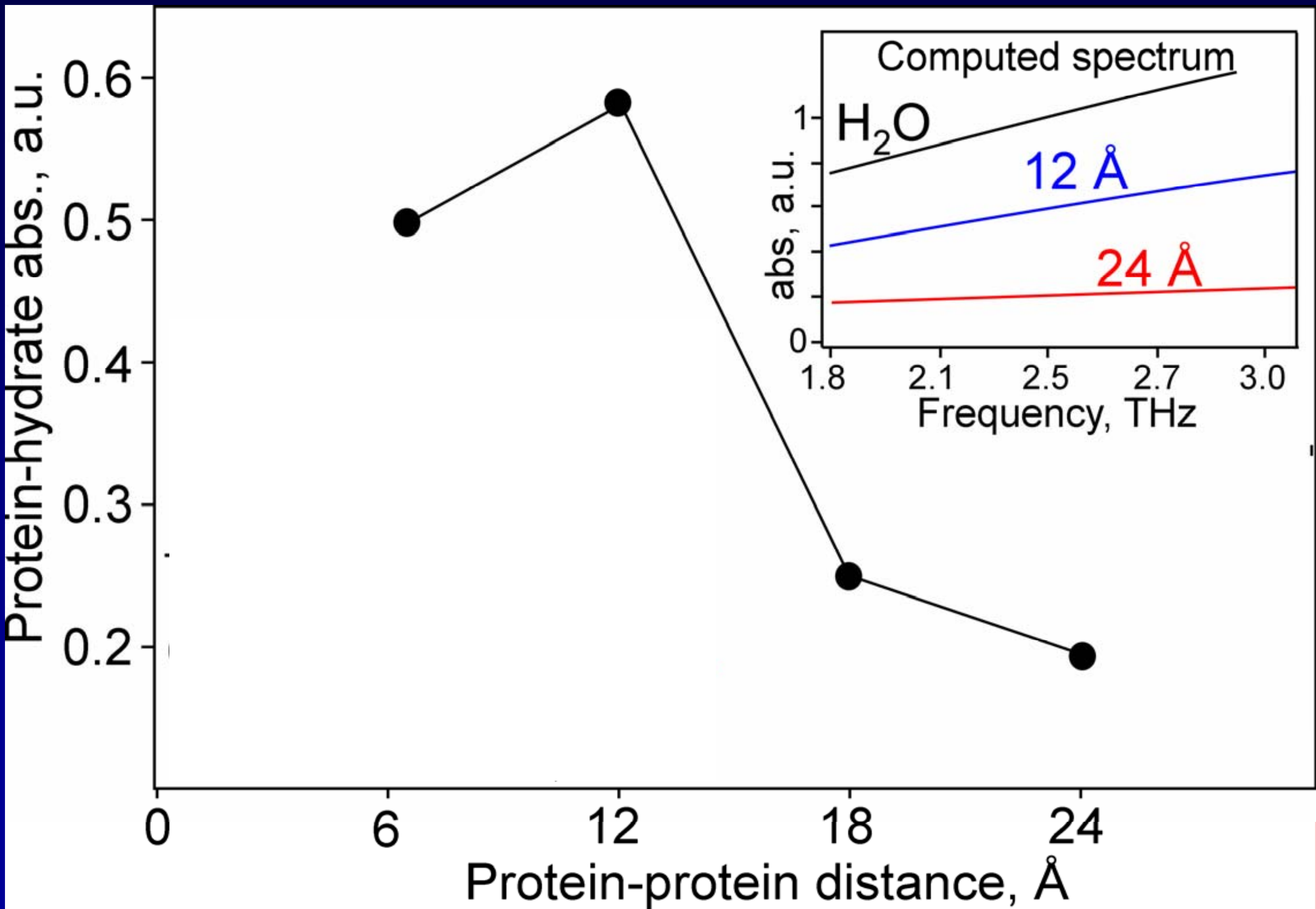


structure (Protein + static hydration shell)



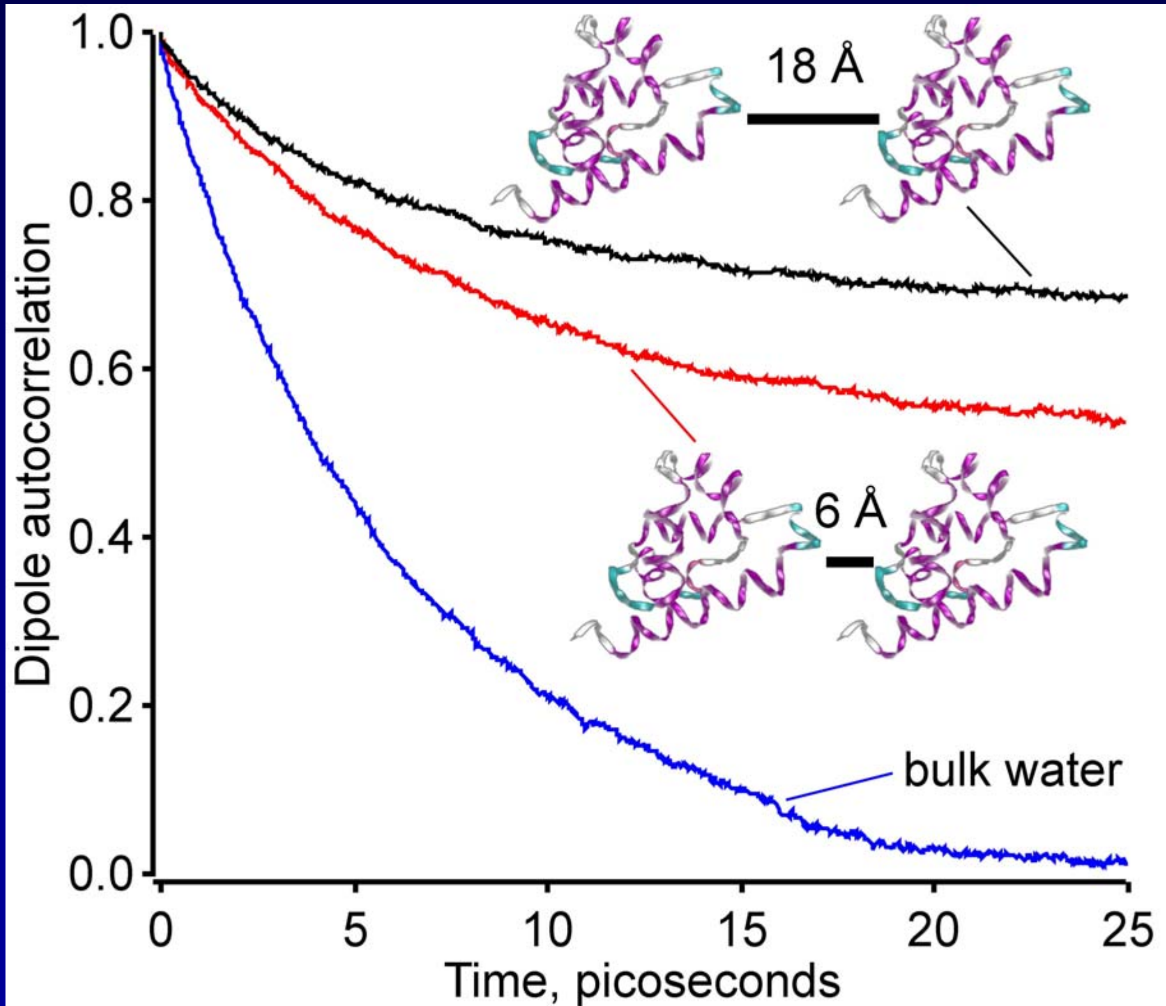
Solvation dynamics

# Molecular modeling confirms:
## Distance dependence of collective THz modes




**Coherent oscillation in the distance,**
**less coherent when hydration shells overlap**

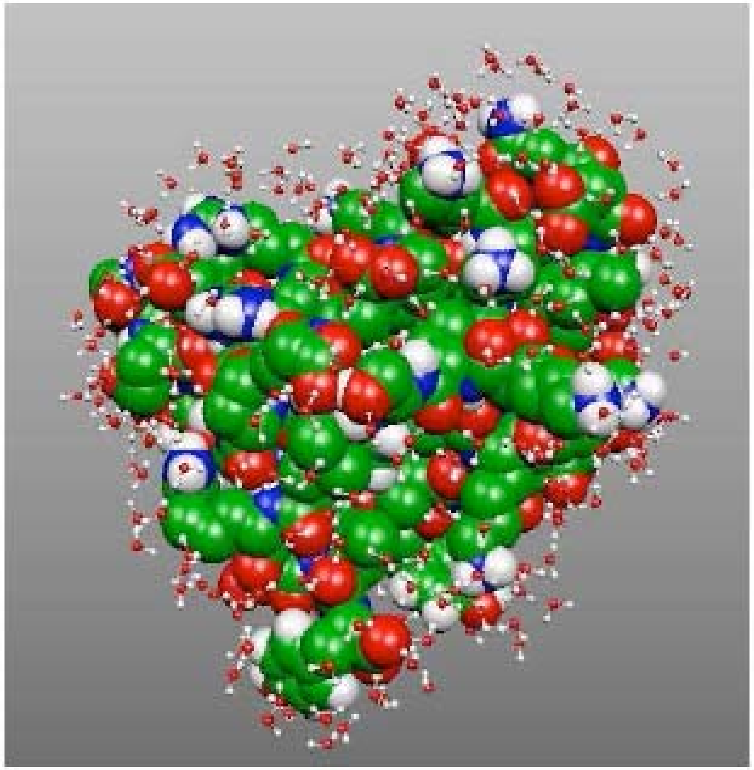
# Dipole auto correlation function for different protein distances

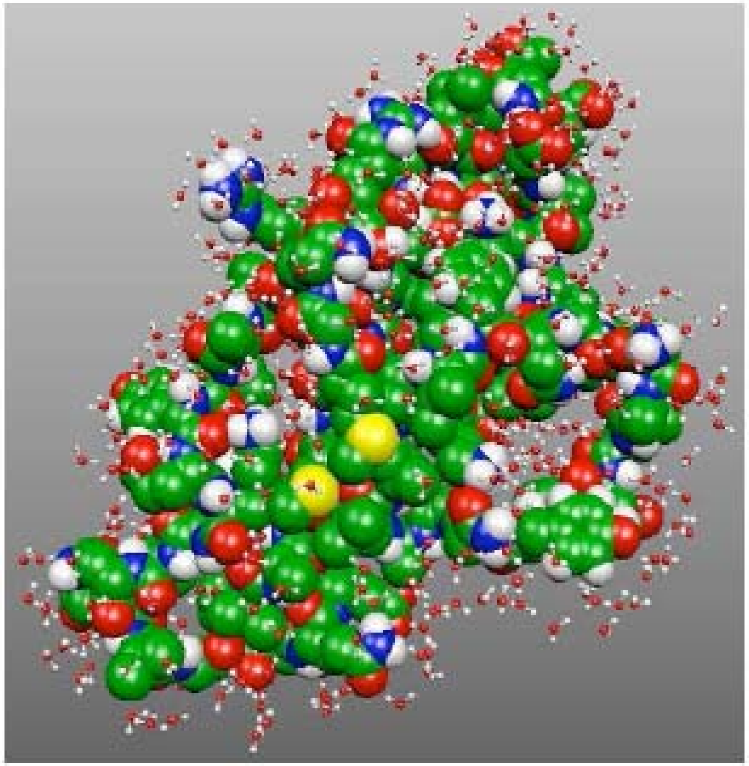



**∫dt exp(-i2Πt)<M(0)M(t)>**

**M is total dipole of system**
***n* is index of refraction**

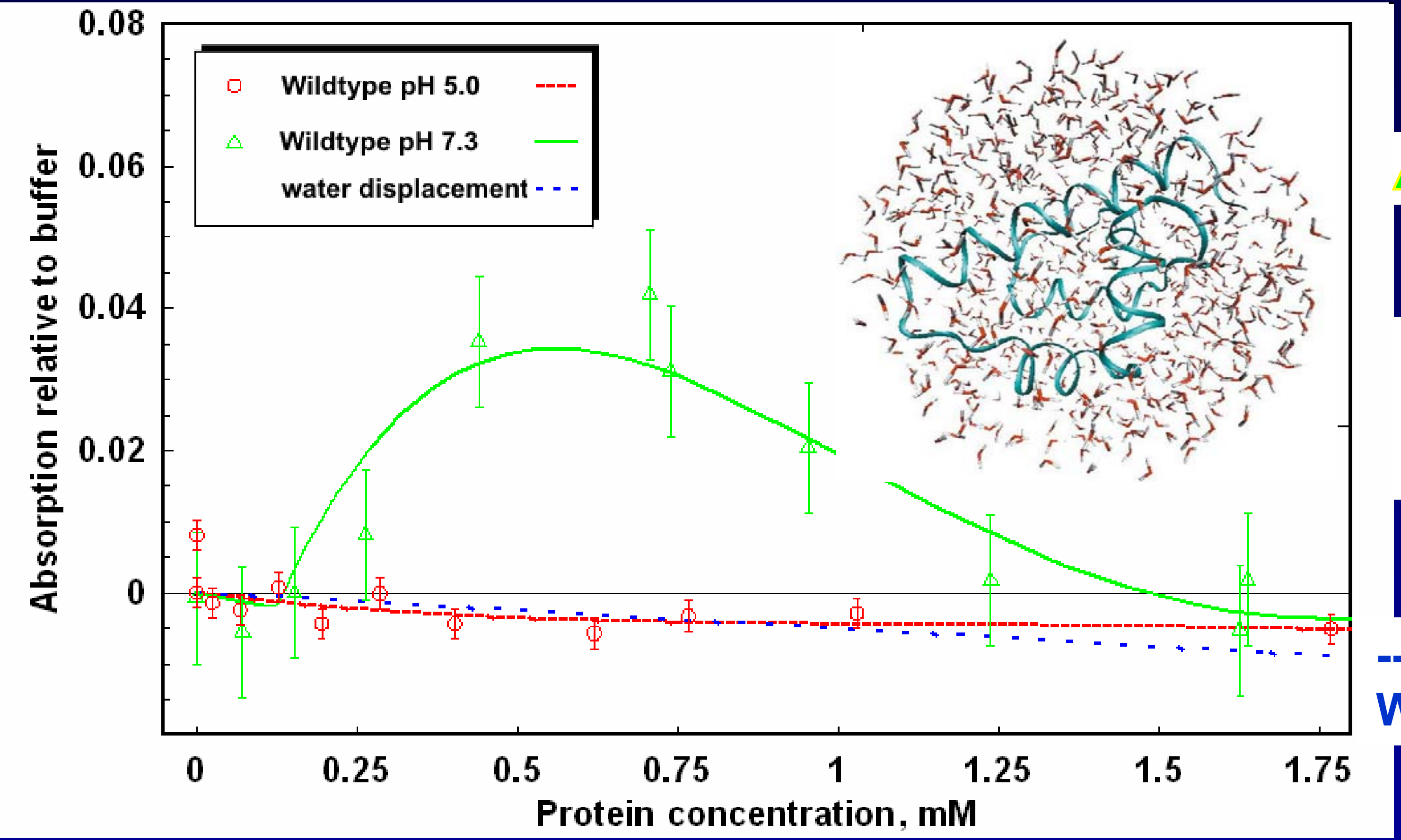
# THz dynamics of hydration water: Dependence on protein sequence and environment





**What about unfolding ? Does it influence the hydration dynamcis ?**

# THz spectroscopy – a new experimental handle for sytematic studies of the long range interaction of proteins



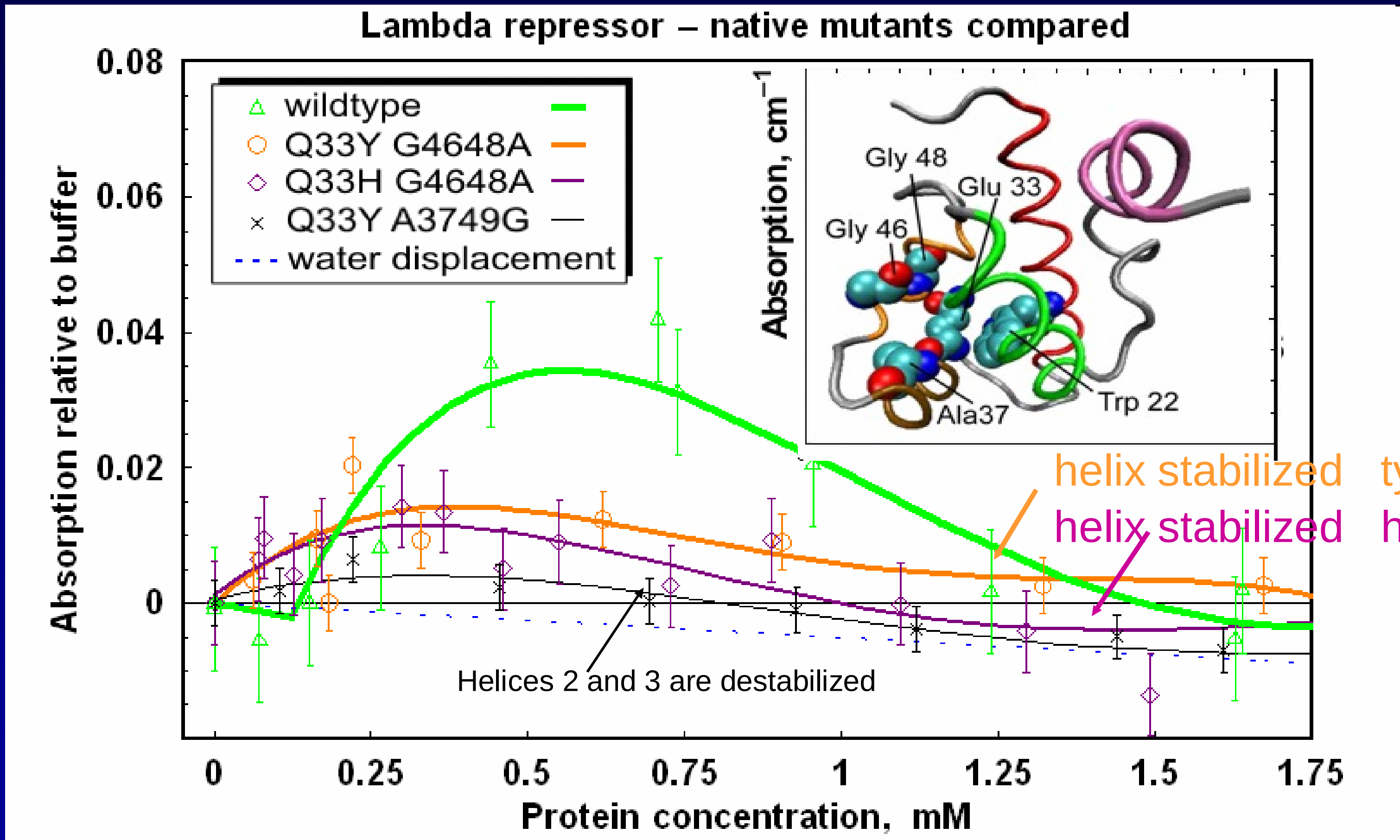

native at pH=7,3

partially denaturated at pH=5

---
Water replacement

**Significant differences observed for the protein induced changes on the solvation water network dynamics when brought closer to unfolding:**
***S. Ebbinghaus, S. J. Kim, M. Heyden, X. Yu, U. Heugen, M. Gruebele, D. Leitner, and M. Havenith, JACS 101021 ja0746520 (2008)***

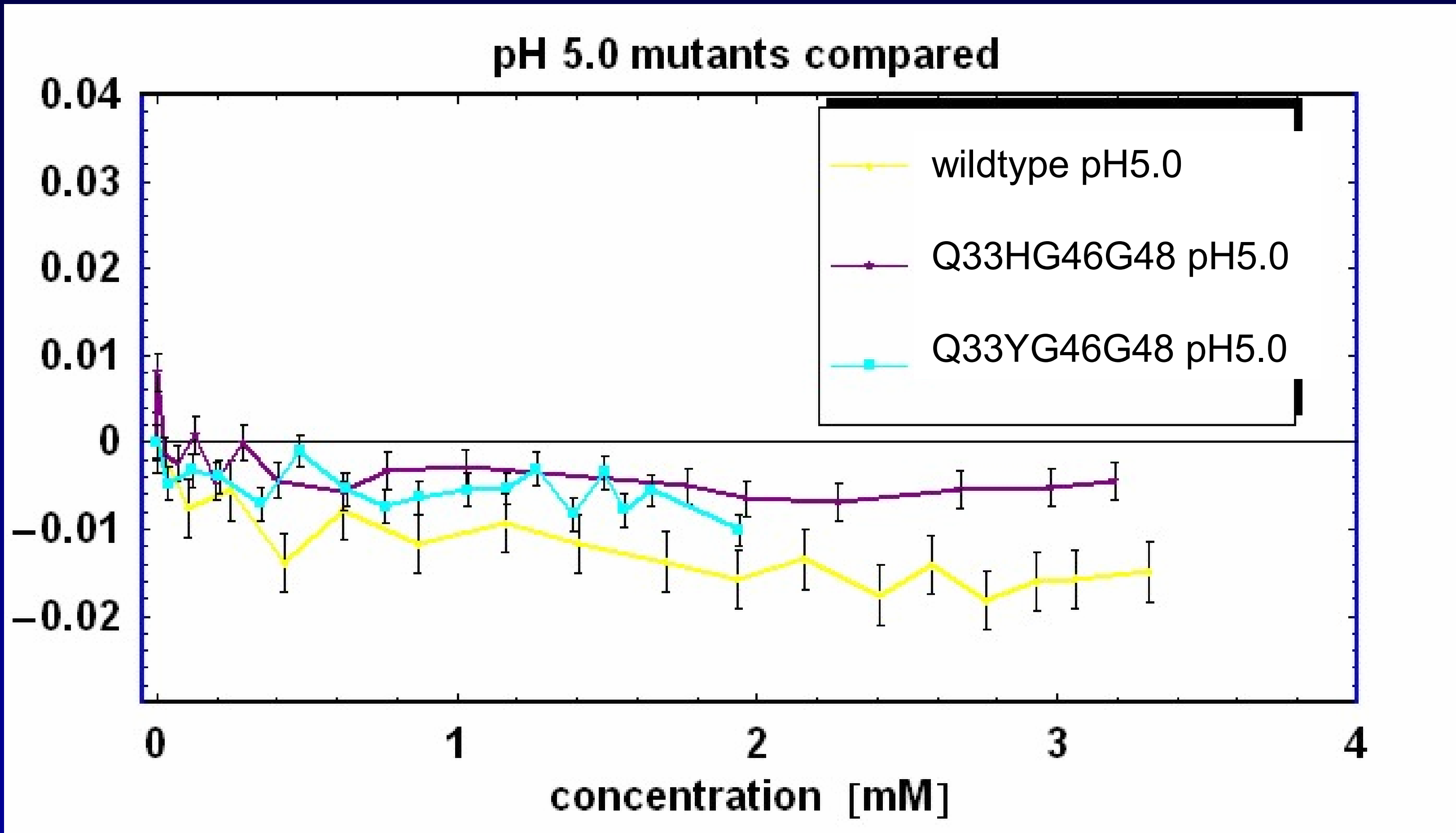
# Comparison of the integrated THz absorbance (between 2.1 and 2.8 THz) of the pseudo-wild type lambda repressor with three mutants (Gln 22 →Tyr) of the protein




pH 7.3

**Site directed mutagenesis:**
**replacement of polar glutamine sidechain by less polar sidechain when stabilizing secondary structures Gly4648Ala or destabilizing Ala3749Gly**

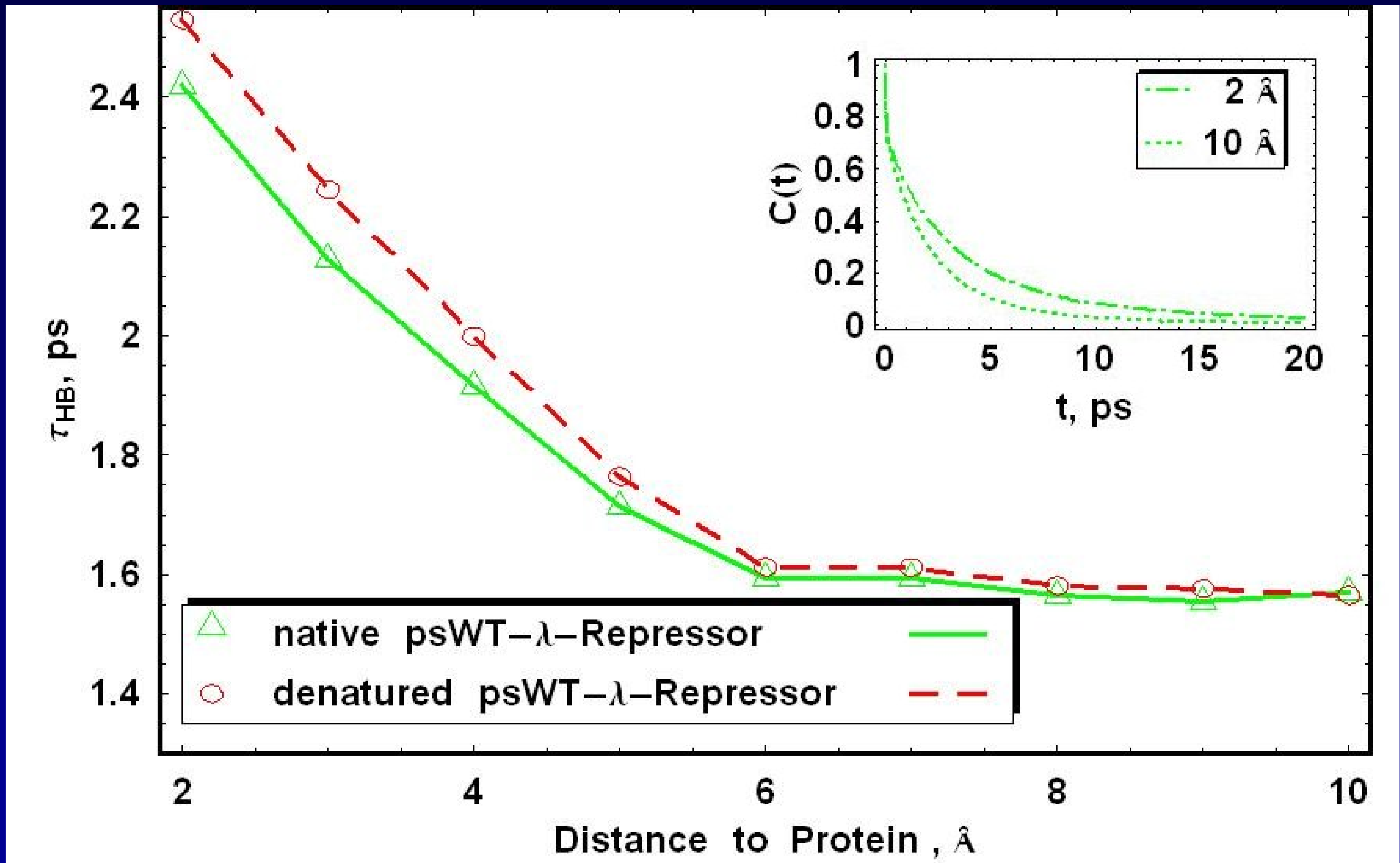
# Long range water structuring if the proteins are closer to the unfolding transition




**Molecular dynamics simulation: retardation of water dynamics caused by exposure of hydrophobic residues to the water**

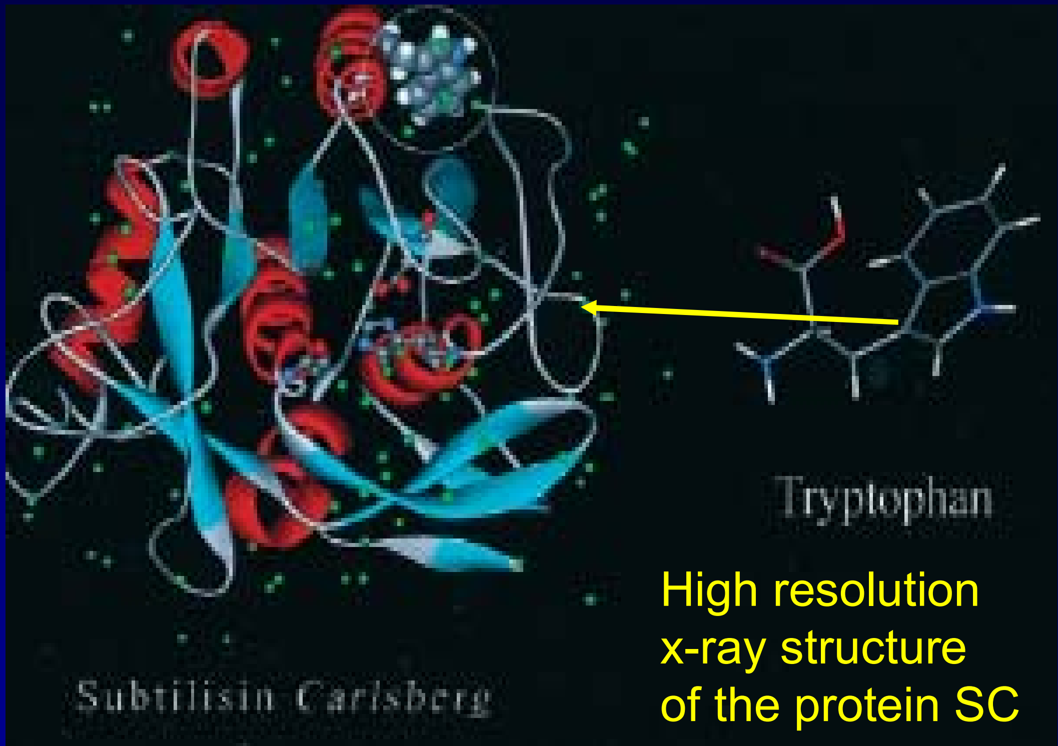
**Is the long range water struturing important for the protein function ????**

# Molecular dynamics simulation



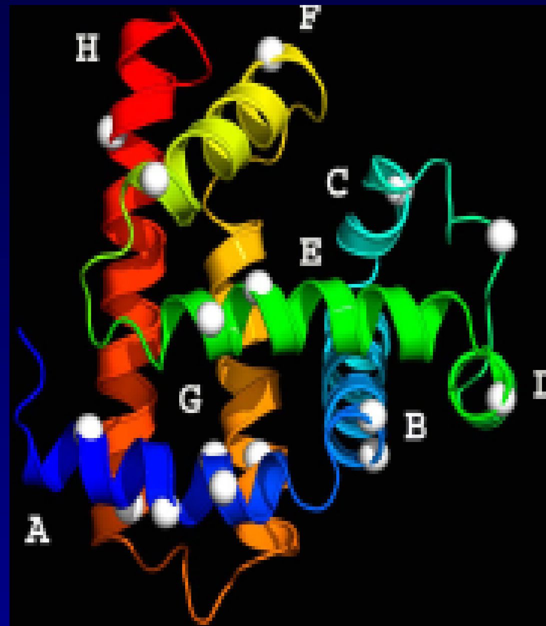

**Water molecules around the denatured state show retardation of dynamics caused by the exposure of hydrophobic residues of the denatured protein**

# X ray structure measures bound water
# THz spectroscopy probes changes in solvation dynamics




High resolution
x-ray structure
of the protein SC

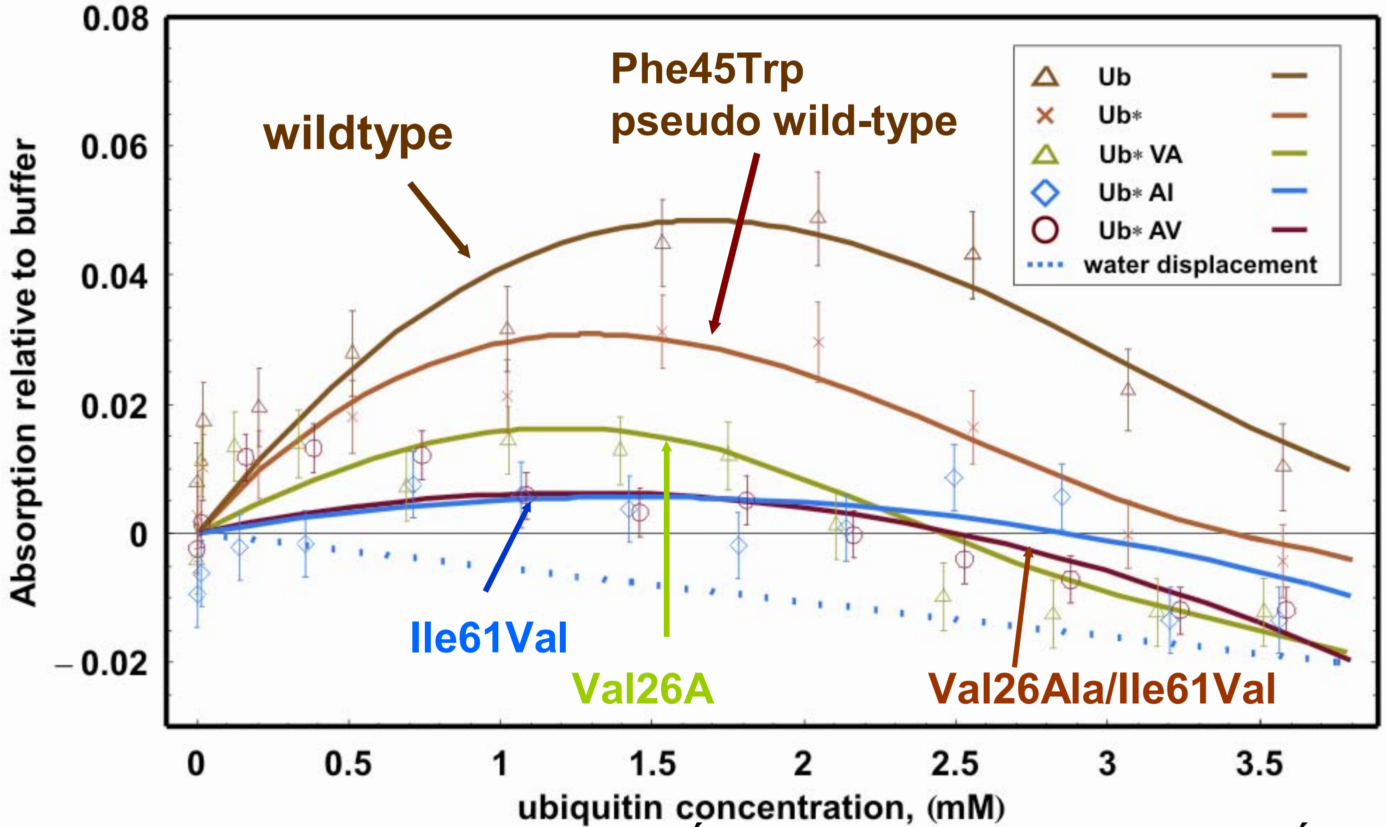
Shown is tryptophan and bound water molecules (green dots)




Mapping of hydration dynamics
Zhang; Wang; Kao; Qui;
Yang; Okobiah; Zhong,
*PNAS* **2007, 104, 18461 - 18466.**

**Distinguish:**
**bound water, H-bond formation , 1. hydration shell**
**Long range effect on water dynamics, extending several layers**

# Heterogeneous solvation dynamics: THz spectroscopy of ubiquitin B. Born, S.J. Kim, M. Gruebele, M. Havenith, 2008




Mean protein-protein distance: 28 Å 17 Å

**Results:**

**Ubiquitin: dynamical hydration layer 10-15 Å**

**Introduction of tryptophan changes hydration dynamics !**

**mutation at surface:**
**$\alpha_{solv}\downarrow$ (at 1.5 mM)**
**flexibility increases**
**$\alpha_{solute}\downarrow$ (at 3.5 mM)**

**Probing heterogeneous solvation dynamics:**
**UB*VA and the Ub*AV mutant show an increased flexibility due to the truncation of the sidechain at position 61; Ile61Ala replacement closer to the protein surface**

# THz spectroscopy of ubiquitin

- Wild type shows the most pronounced effect !
- Dynamical hydration layer exceeds to 10-17 Å

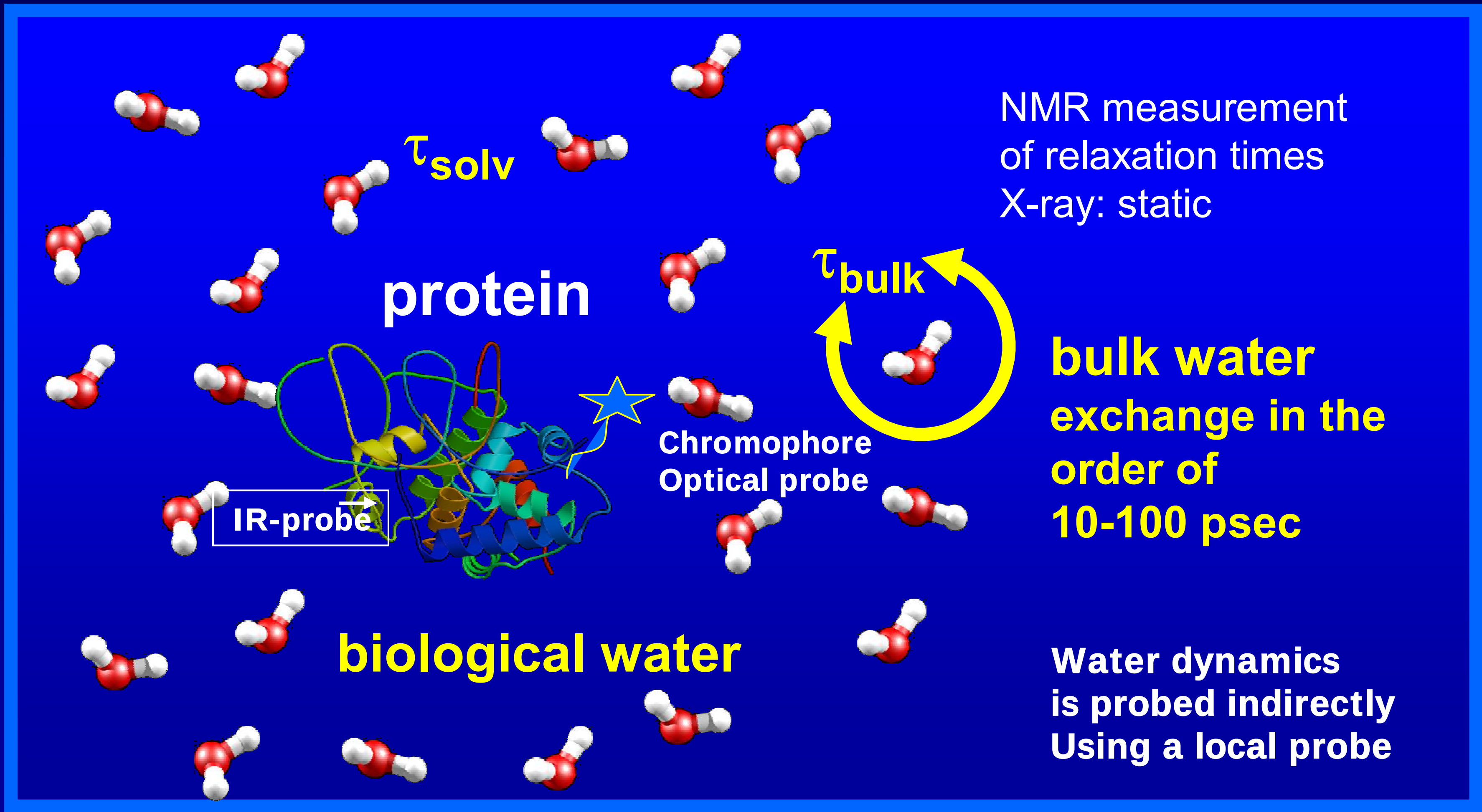
  Smaller than for λ-repressor

- **Replacement of phenylalanine by tryptophan influences hydration dynamics !**

- **Increased surface hydrophobicity of the mutants is a candidate for changes in hydration water yielding bulk water-like THz absorption**

- **largest deviation from Ub* for the more disruptive core mutation Ala26Val, but a smaller effect for the less disruptive Ile61Ala mutation of a near surface**

# Comparison of different experimental techniques to probe solvation dynamics:



## What about THz microscopy in a cell?

<u>Noninvasive</u> three-dimensional characterization of coupled network motions in complex heterogeneous systems in space <u>and</u> time (i.e. living cells and tissue)

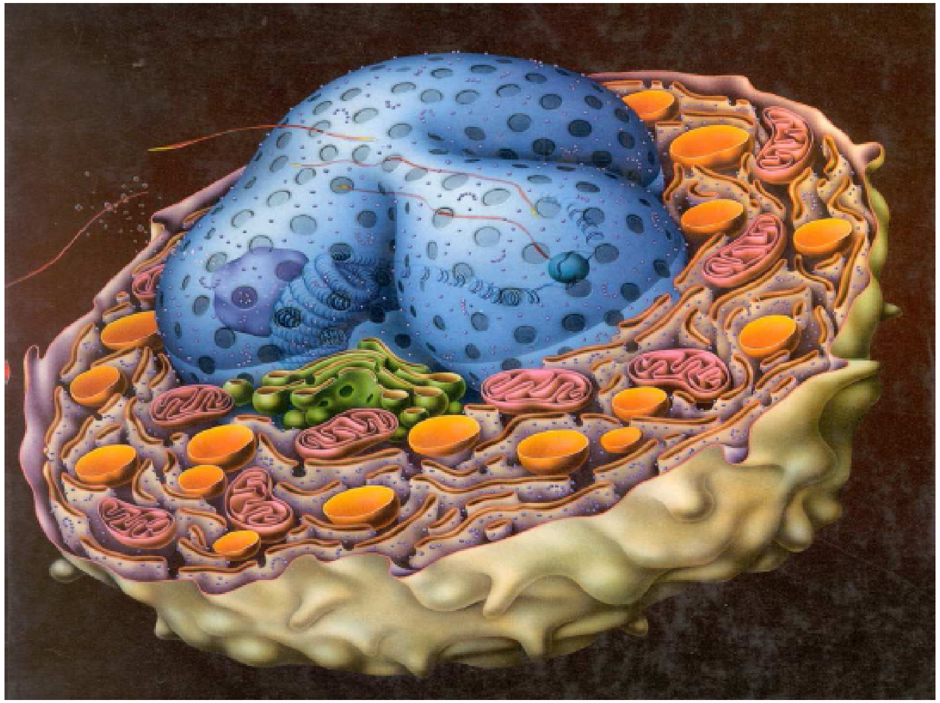
What do we expect ?

Water in cells is not bulk like !

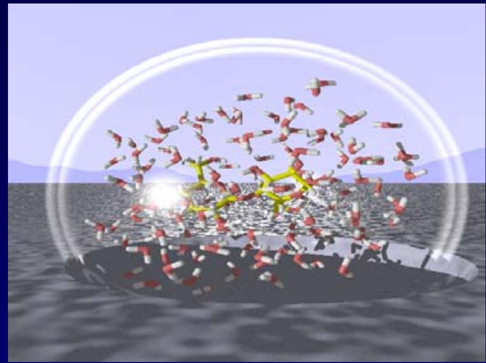
Water dynamics influence protein dynamics

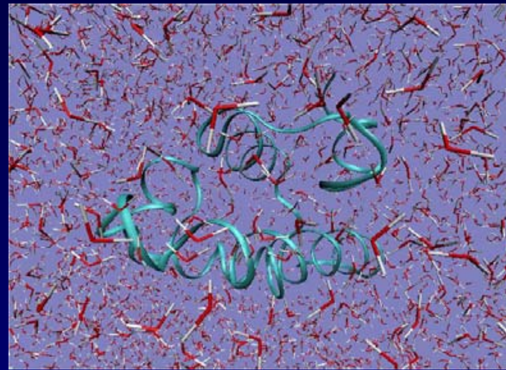
And: Protein influences the water dynamics

Biological relevance ?



# IR and THz spectroscopy: A tool to probe hydration dynamics





- Microsolvation can be probed by IR spectroscopy:
  $(HCl)_x$ $(H_2O)_y$ in He nanodroplets, *J. Phys. Chem. A*, 111, 12192 (2007)
- THz spectroscopy probes the the dynamical size of the hydration layer (retardation of fast dynamics) for carbohydrates, *PNAS* 103, 12301 (2006)
- For proteins a non linear concentration dependence of the THz absorption indicates a long range influence on solvent dynamics of up to 20 Å; *PNAS* **104, 20749 (2007)**
- Systematic study of denaturated proteins and site directed mutants of lamba repressor:
  Probe of inhomogeous hydration dynamics, JACS 2008, in press

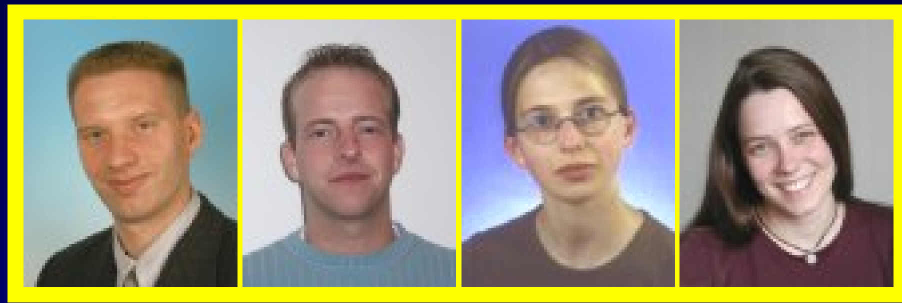
# Laser Spectroscopy & Biophotonics

*(physicists, chemists, biochemists)*

Erik Bründermann

Gerhard Schwaab

**Intermolecular Interaction**



Stephan Rudolph — Markus Ortlieb — Anna Gutberlet — Anja Metzethin

**Funds:**

**State:** HBFG

**German science foundation:**

SP 1116

FOR 618

**EU:**

INTCHEM

**International**

HFSP Award 2004

**THz**

Konstanze Schröck — Matthias Krüger — Simon Ebbinghaus — Udo Heugen — Benjamin Born — Matthias Heyden

**Imaging:**
**Scanning Nearfield**
**Infrared Microscopy**

Jean-Sebastian Samson

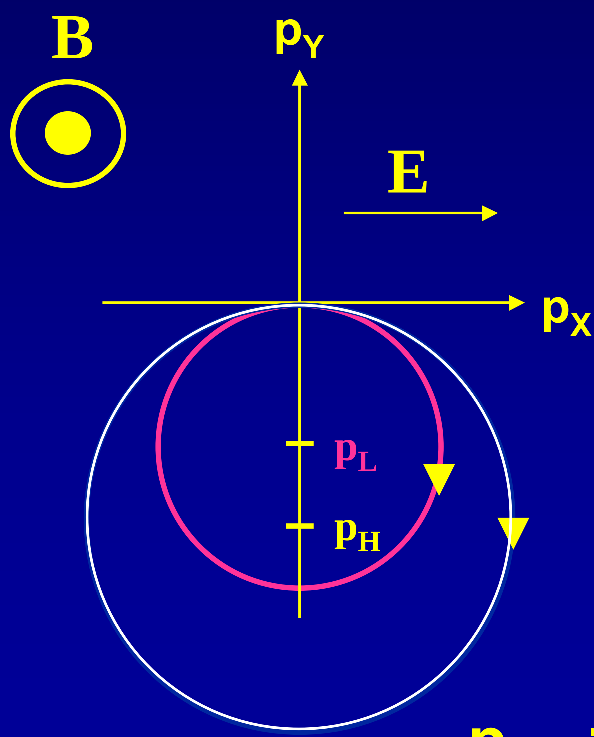
Götz Wollny — Ilona Kopf

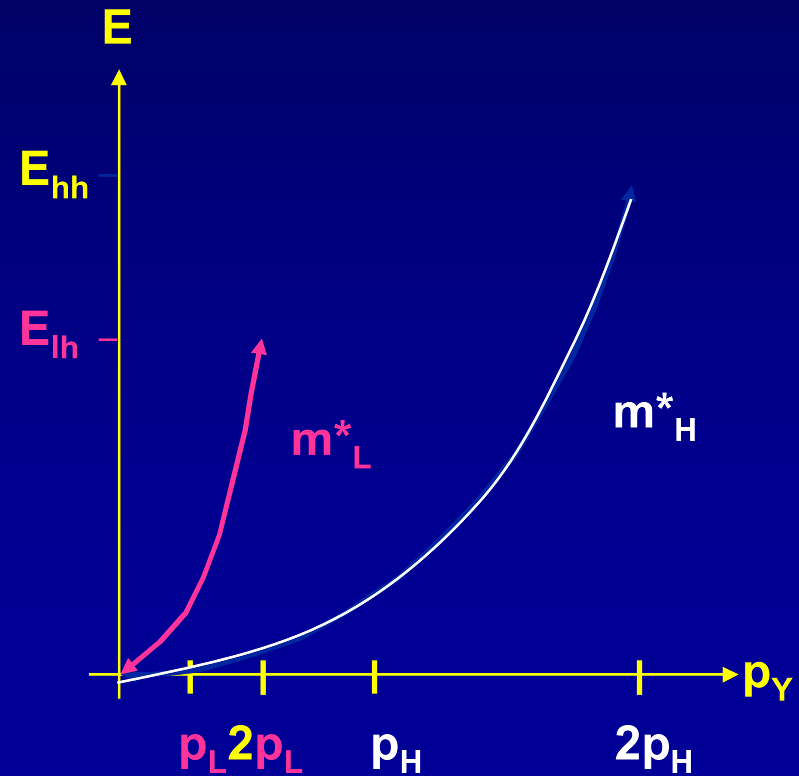

Benjamin Born
Simon Ebbinghaus
Udo Heugen
E. Bründermann
G.
Schwaab
Seung Joong Kim
MG
DL
MH
The human element:
At an HFSP-sponsored meeting of all participants

# Classical Hole Motion in E ⊥ B

**Free hole motion under Lorentz-Force at low temperature (T = 4K)**

$$\vec{F} = m^* \cdot \frac{d\vec{v}}{dt} = e \cdot (\vec{E} + \vec{v} \times \vec{B})$$



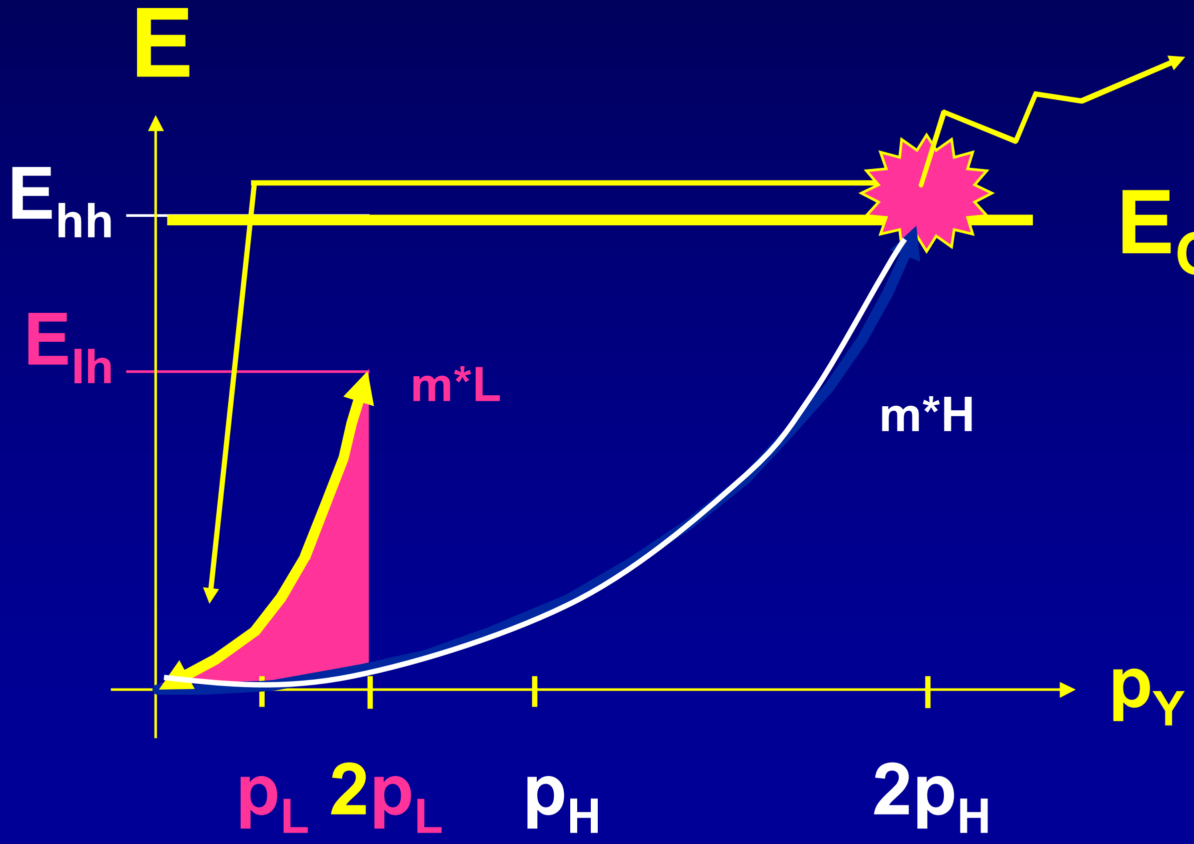




$$p_{L,H} = m^*_{L,H}\, E/B$$

# The p-Ge laser: a tunable high power THz laser

## Classical Hole Motion in E ⊥ B

$$\boxed{\vec{F} = m^* \cdot \frac{d\vec{v}}{dt} = e \cdot (\vec{E} + \vec{v} \times \vec{B})}$$



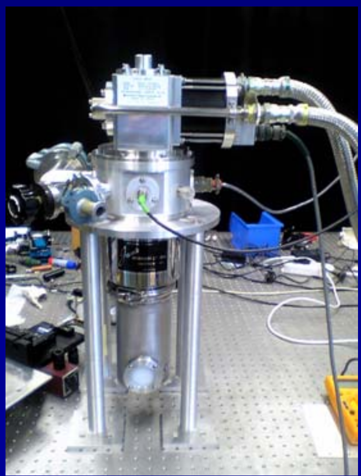

Optical Phonon

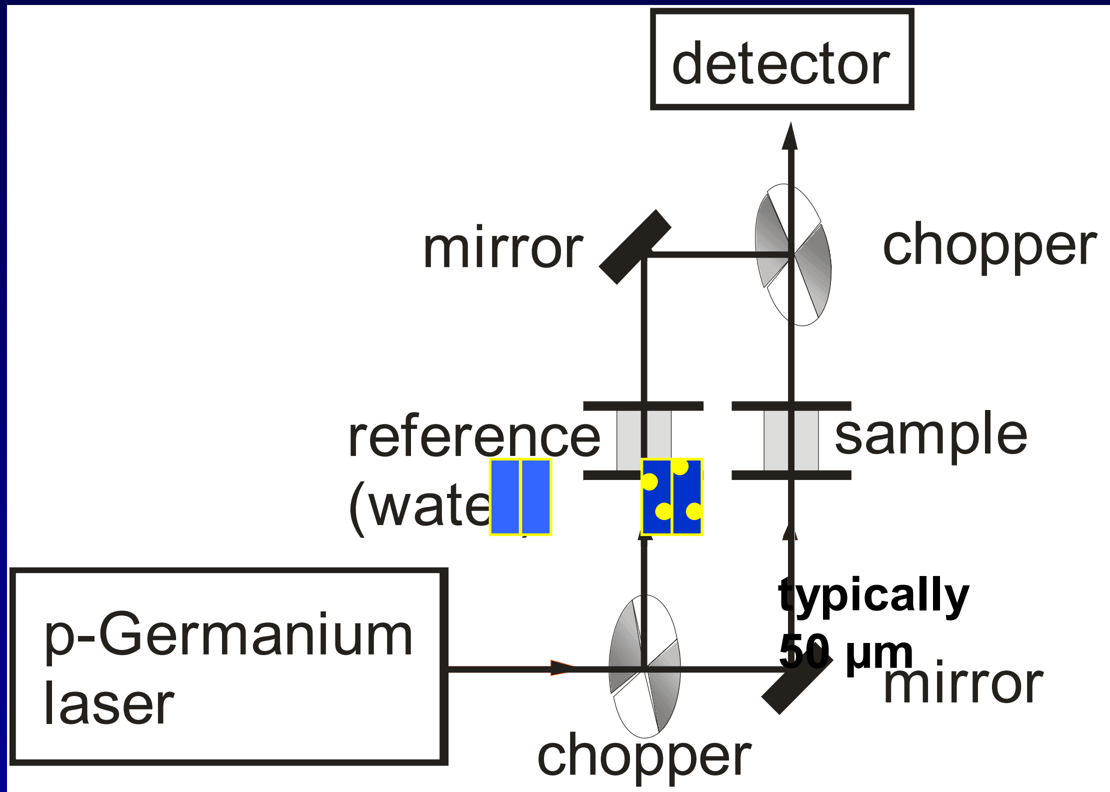
$E_{OP} = 37$ meV

$p_{L,H} = m^*_{L,H}\, E/B$

$p_{L,H} = m^*_{L,H}\, E/B$

# THz difference absorption spectrometer
## for precise measurements of absorption coefficients



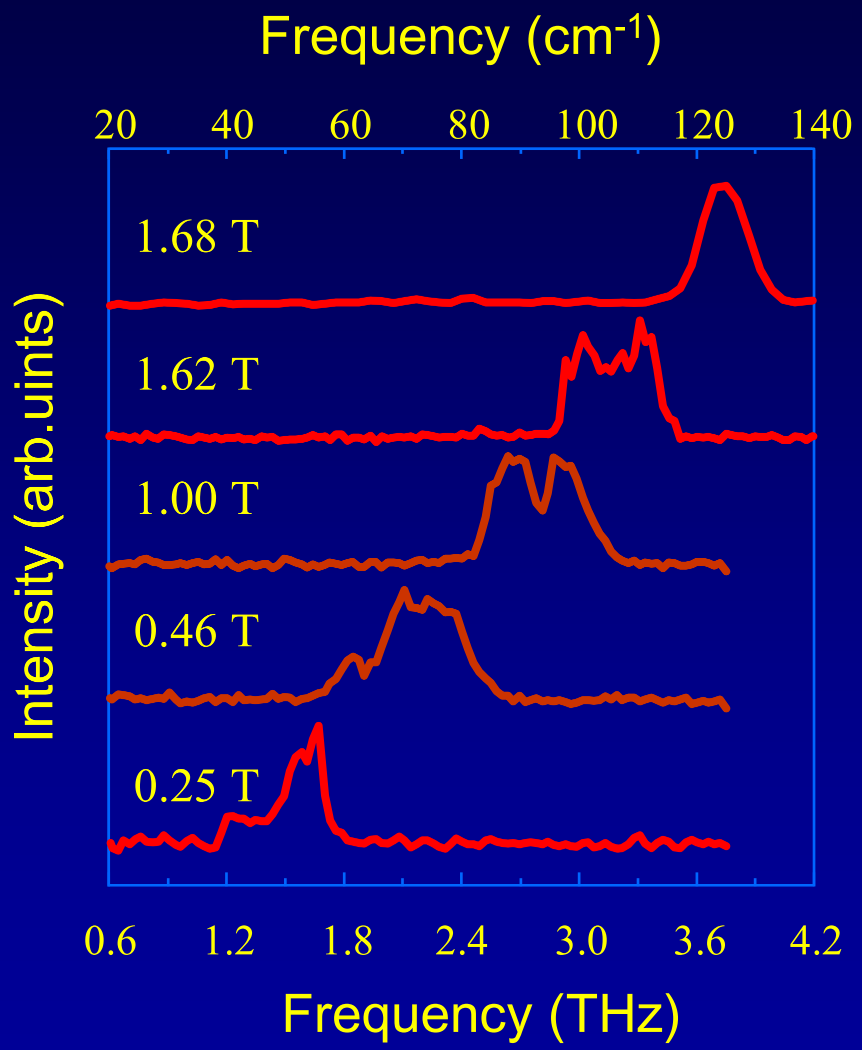



**p-Ge laser:** a unique THz source

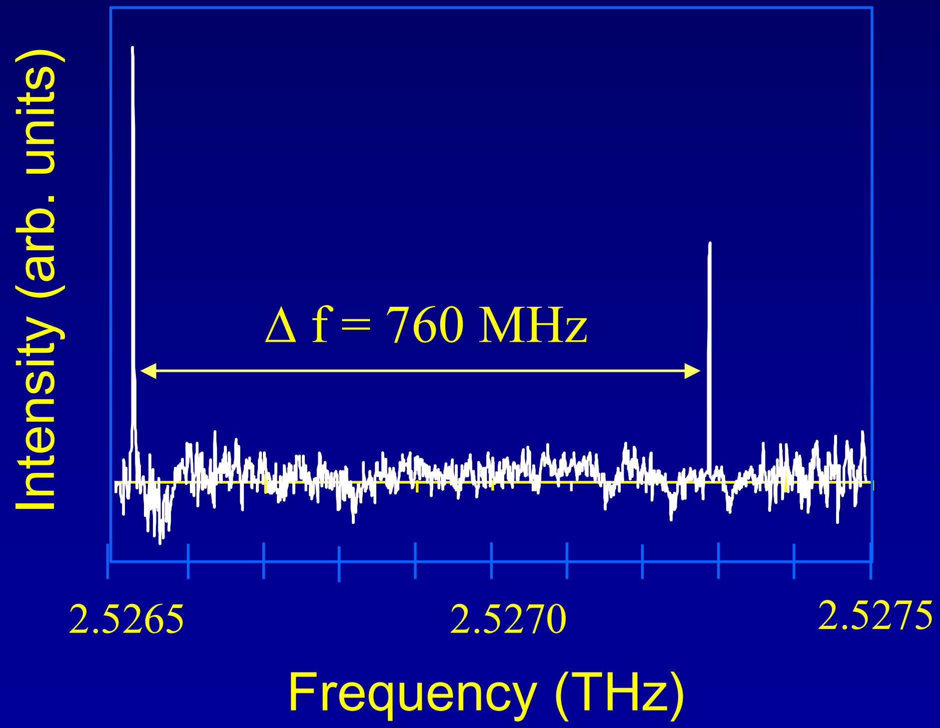
# 1 - 4 THz Ge Laser

*linewidth < 1 MHz*
*puls length from <50 ps to 32 ms*
*duty cycle 5%*
*high power: up to 10 W (Dt = 1 ms)*

$\Delta f/f$: $10^6 - 10^7$




L. A. Reichertz, O. D. Dubon, G. Sirmain, E. Bründermann, W. L. Hansen, D. R. Chamberlin, A. M. Linhart, H.P. Röser, E. E. Haller, Phys. Rev. **B56,** 12069-12072 (1997)




E. Bründermann, H.P. Röser, A.V. Muravjov, S.G. Pavlov, V.N. Shastin, Infrared Phys. Technol. **1**, 59-69 (1995)

# THz spectroscopy as a tool to determine the size and the „nature“ of the hydration shell in the sub-psec regime

**Fit to a three component model:**

$$\alpha_{total} = \mathbf{V_{sw}/V\ \alpha_{sw}} + V_{solute}/V\ \boldsymbol{\alpha}_{\mathbf{solute}} + (1 - V_{sw}/V - V_{solute}/V)\ \alpha_{bulk}$$

**$V_{sw}/V$ :** relative volume of the solvation water

**$\alpha_{sw}$ :** absorption coefficient of the solvation water

$V_{solute}/V$: relative volume of the solute

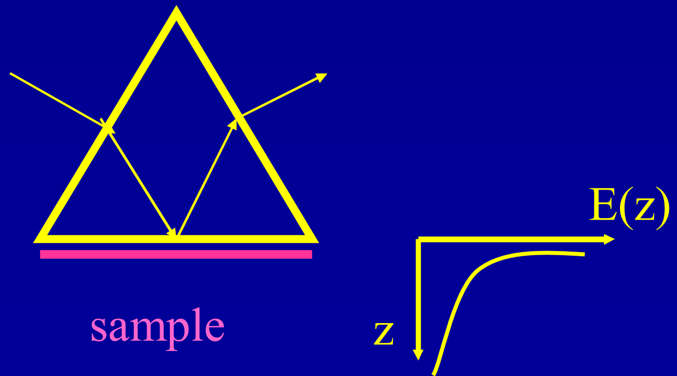
**$\alpha_{solute}$:** absorption coefficient of the solute

# Individual *versus* coherent motions


**Hydration water: The american way**

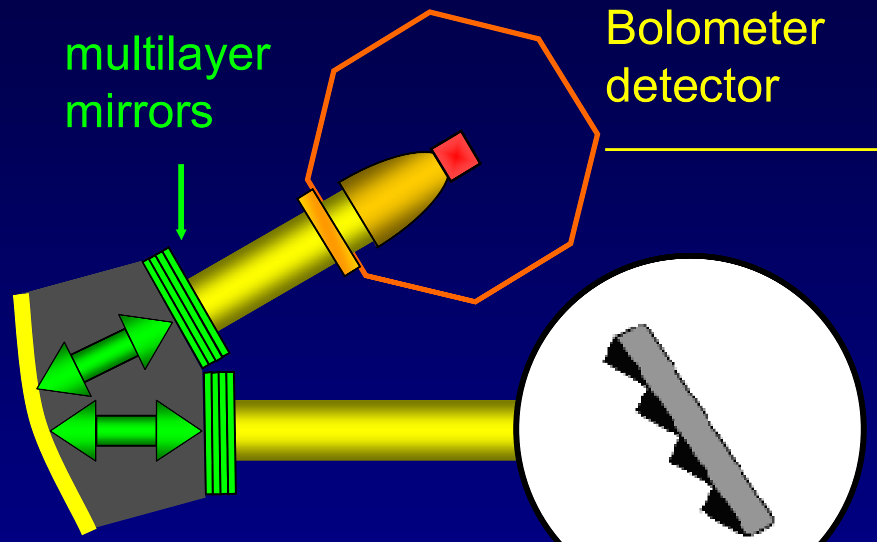
# THz spectroscopy of thin layers and films

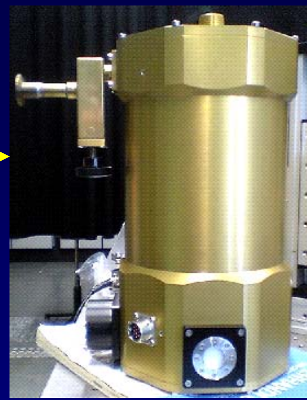
- Measurement of small quantities of a probe
- Measurements on thin films, and liquid drops with high absorption coefficents
- Sensitive detection of thin layers required:
- Solution: attenuated reflection spectroscopy

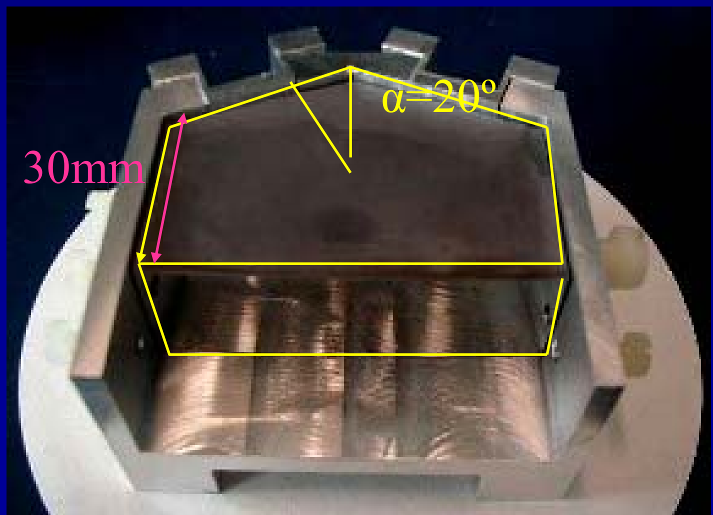



Exponentially decaying electric field
Penetration depth: wavelength depend
d=26 μm @ λ=100 μm

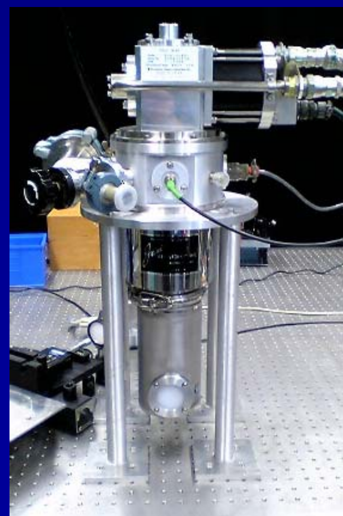
# THz Cavity Enhanced
# Attenuated Total Reflection Spectroscopy






Radius of curvature 500 mm



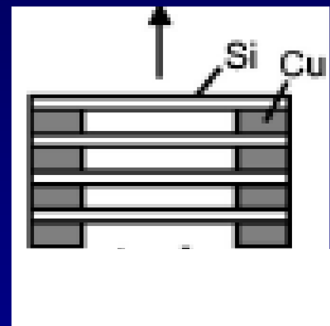
# Increasing sensitivity:

**Design of highly reflecting non absorbing mirrors in the THz**

R. Schiwon, G. Schwaab, E. Bründermann, M. Havenith

**App. Phys. L. 83(20), 4119, ( 2003)**

- Wanted: no absorption, high bandwidth
- Gold: highly reflectant but non transparent
- Dielelectric mirrors for the THz region




**Solution:**

**Reflectivity <99%**

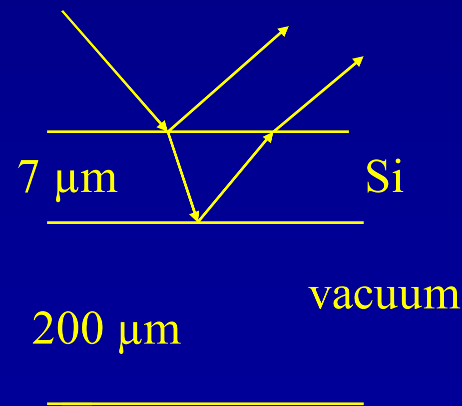
Alternating materials:

spacing: λ/4

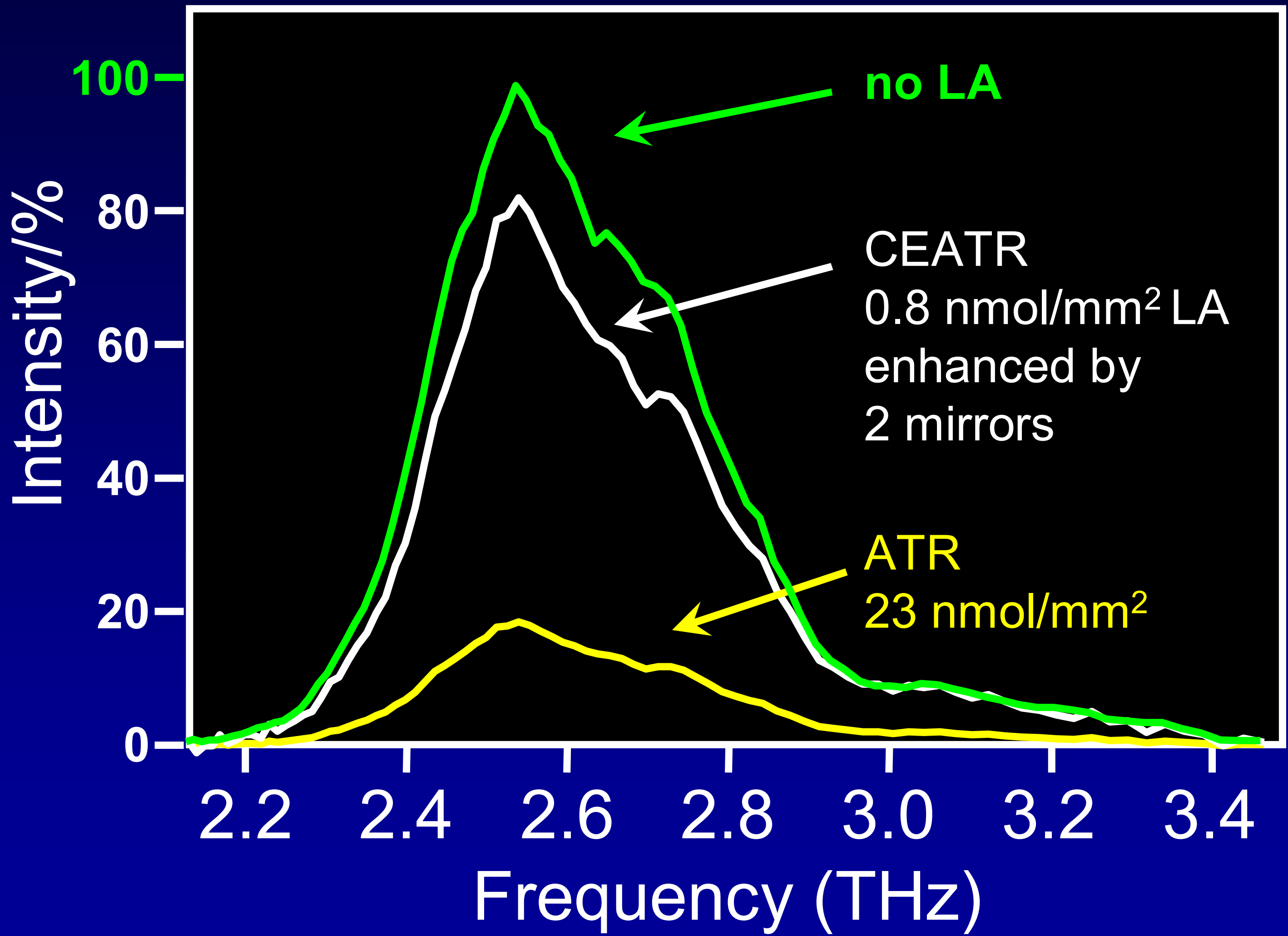
distinct dielectric constant:

silicon (ε=11.7) (n=3.42)

air (ε=1.0) (n=1.0)



# Cavity Enhanced THz ATR Spectroscopy




**23 nmol/mm² film 1.5 μm = 1000 layers of amino acids**

**0.8 nmol/mm2 film of 50 nm = 33 layers of amino acids**

**LA = lactalbumin hydrolysate degraded protein**

# Experimental results:

**R. Schiwon, G. Schwaab, E. Bründermann, M. Havenith**
**Appl. Phys. Lett. 86, 201116 (2005)**

- Lactalbumim hydrolysate: commercially available, degraded protein, found in many biological systems
- Probe dissolved and spread on surface
- Detection limit:

ATR: 23 nmol/mm$^2$: 1500 nm ($d_{penetration}$/17)

corresponding to 1000 layers of free amino acids

CEATR: 0.8 nmol/mm$^2$: 50 nm ($d_{penetration}$/520)

corresponding to 33 layers of free amino acids yielding 15%±3% reduction

- New: THz absorption measurements of thin films and surfaces

# Future potential for medical applications: Monitoring the influence of pharmaceuticals on a single cell level in real time


Chemical Science

Research highlights

by the Royal Society, November 2004

## Tracking drugs in single cells

„A non-invasive method
To quantify water in living
cells is being pioneerd in Germany“

## Future improvements:

Tomography
improved lateral resolution