

# Hydration dynamics investigated by THz spectroscopy

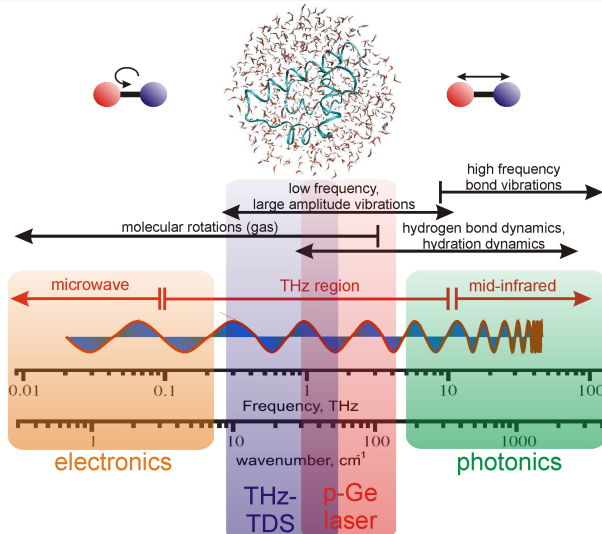
International Symposium on Molecular Spectroscopy 2008

Simon Ebbinghaus, Matthias Heyden, Udo Heugen, Gudrun Niehues, Benjamin Born,  
Erik Bründermann, Gerhard Schwaab, Martina Havenith

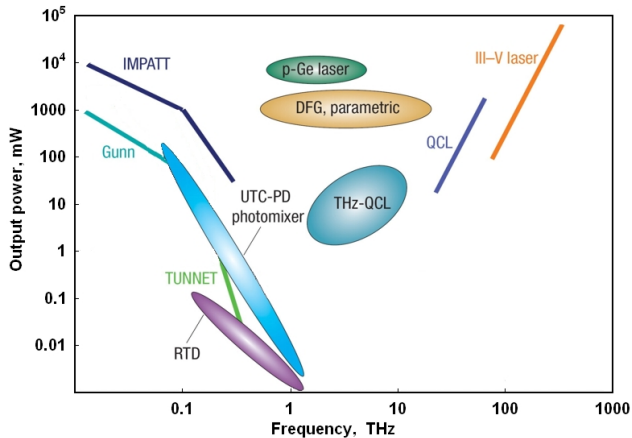
Xin Yu, David Leitner

Seung Joong Kim, Martin Gruebele

# Hydration dynamics investigated by THz spectroscopy

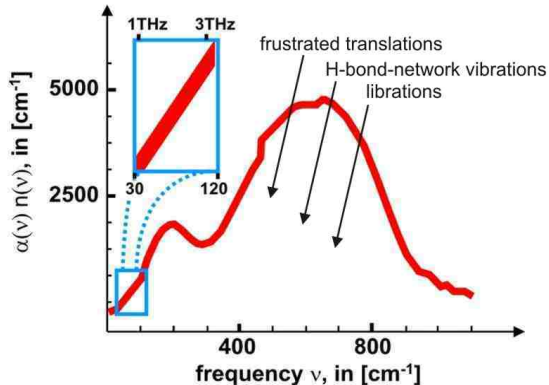


## Using a high power source



M. Tonouchi (2007), *Nature Photonics*, **1**, 97-105

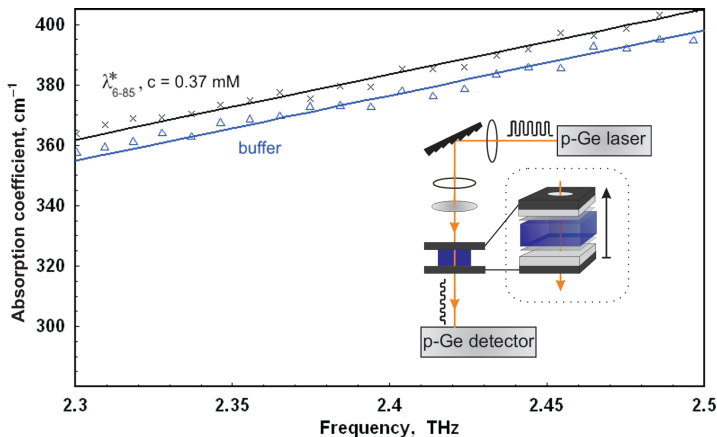
# Absorption spectrum of water



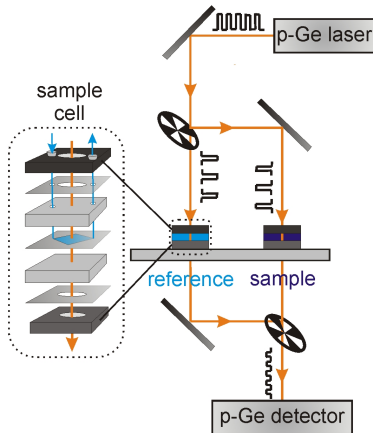
P.L. Silvestrelli, M. Bernasconi and M. Parrinello (1997), *Chem. Phys. Lett.*, **130**,478



# The THz p-Ge laser spectrometer

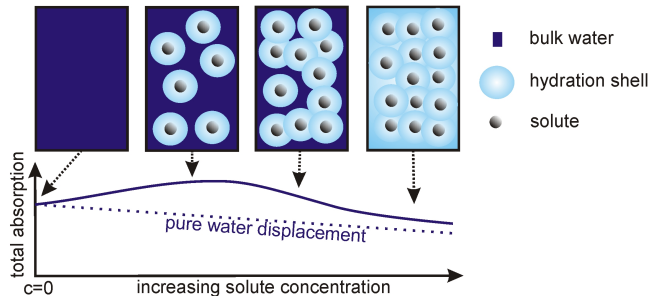


## Difference spectroscopy set-up (p-Ge laser)



- unique high power (up to 10 W)  
p-Ge laser
- transmission spectroscopy from  
2.1 THz to 2.8 THz
- 50  $\mu\text{m}$  sample thickness
- double beam configuration,  
measuring small differences in  
absorption

# Concentration dependent THz response



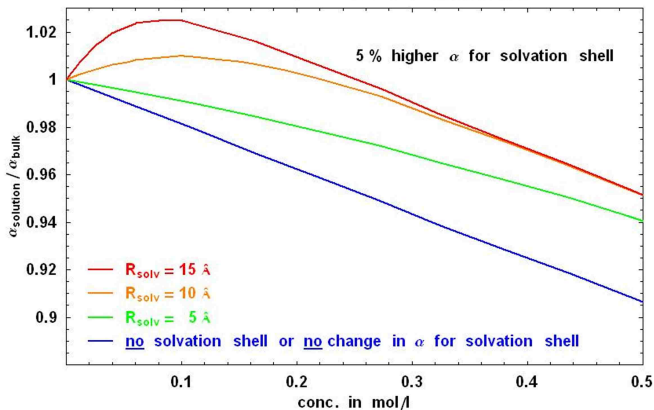
$$\alpha_{total}(2 - comp.) = \frac{V_{solute}}{V_{total}} \alpha_{solute} + \left(1 - \frac{V_{solute}}{V_{total}}\right) \alpha_{bulk}$$

$$\alpha_{total}(3 - comp.) = \frac{V_{solute}}{V_{total}} \alpha_{solute} + \frac{V_{hw}}{V_{total}} \alpha_{hw} + \left(1 - \frac{V_{solute}}{V_{total}} - \frac{V_{hw}}{V_{total}}\right) \alpha_{bulk}$$



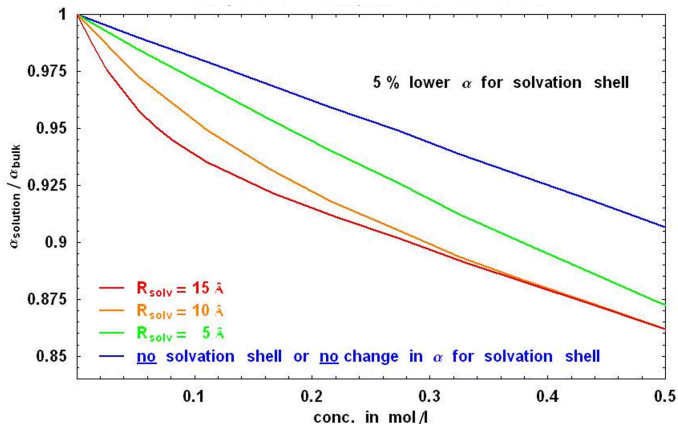
U. Heugen, G. Schwaab, E. Bründermann, M. Heyden, X. Yu, D. M. Leitner, M. Havenith (2006), *Proc. Nat. Acad. Sci. USA*, **103**, 12301-6

## Increased hydration shell absorption



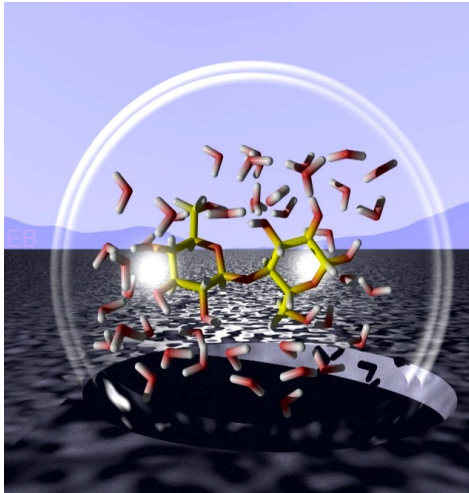
M. Heyden, E. Bründermann, U. Heugen, G. Niehues, D.M. Leitner, M. Havenith (2008), *J. Am. Chem. Soc.*, **130**, 5773-9.

## Decreased hydration shell absorption



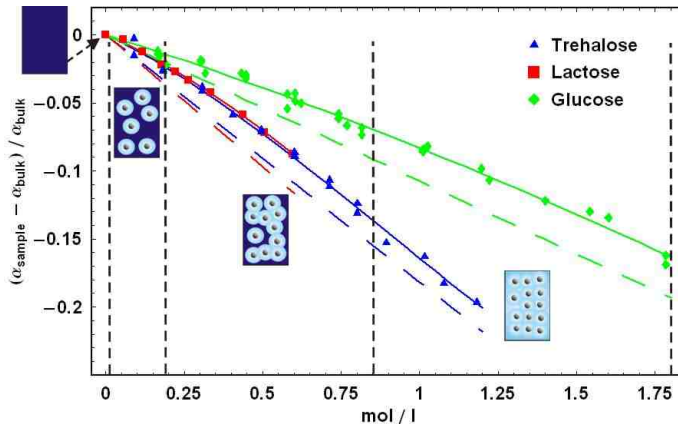
M. Heyden, E. Bründermann, U. Heugen, G. Niehues, D.M. Leitner, M. Havenith (2008), *J. Am. Chem. Soc.*, **130**, 5773-9.

# Motivation



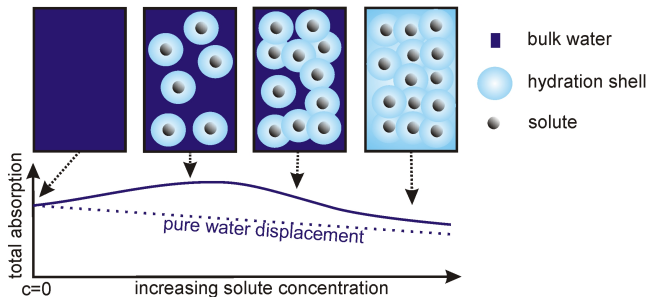
- **anhydrobiosis** (survival of extreme conditions) correlated to presence of disaccharides
- possible mechanism: **retardation of surrounding water** by coupling of hydration water to disaccharide motion
- retardation of water network dynamics by disaccharides can **directly influence protein dynamics** (protein dynamics coupled to water network dynamics (*part II of the talk*))

# THz spectra of saccharide solutions



M. Heyden, E. Bründermann, U. Heugen, G. Niehues, D.M. Leitner, M. Havenith (2008), *J. Am. Chem. Soc.*, **130**, 5773-9.

## Concentration dependent THz response



$$\alpha_{total}(2 - comp.) = \frac{V_{solute}}{V_{total}} \alpha_{solute} + \left(1 - \frac{V_{solute}}{V_{total}}\right) \alpha_{bulk}$$

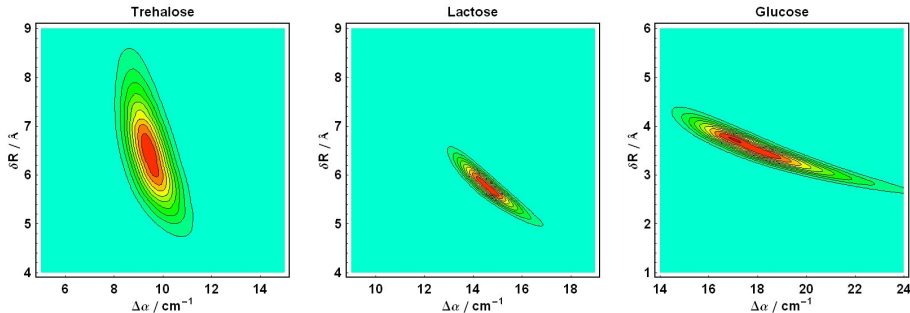
$$\alpha_{total}(3 - comp.) = \frac{V_{solute}}{V_{total}} \alpha_{solute} + \frac{V_{hw}}{V_{total}} \alpha_{hw} + \left(1 - \frac{V_{solute}}{V_{total}} - \frac{V_{hw}}{V_{total}}\right) \alpha_{bulk}$$



U. Heugen, G. Schwaab, E. Bründermann, M. Heyden, X. Yu, D. M. Leitner, M. Havenith (2006), *Proc. Nat. Acad. Sci. USA*, **103**, 12301-6

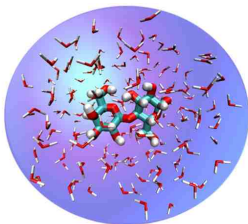


## Normalized statistical likelihood of the 2 fitted parameters



M. Heyden, E. Bründermann, U. Heugen, G. Niehues, D.M. Leitner, M. Havenith (2008), *J. Am. Chem. Soc.*, **130**, 5773-9.

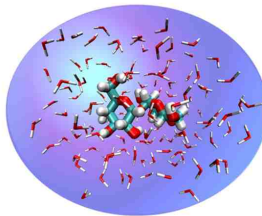
## Comparing the three carbohydrates



Lactose

$$\Delta\alpha = 14.7 \pm 0.49 \text{ cm}^{-1}$$

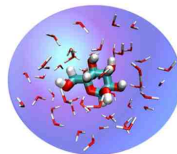
$$\delta R = 5.6 \pm 0.4 \text{ \AA}$$



Trehalose

$$\Delta\alpha = 9.4 \pm 0.7 \text{ cm}^{-1}$$

$$\delta R = 6.4 \pm 0.9 \text{ \AA}$$



Glucose

$$\Delta\alpha = 17.1 \pm 1.8 \text{ cm}^{-1}$$

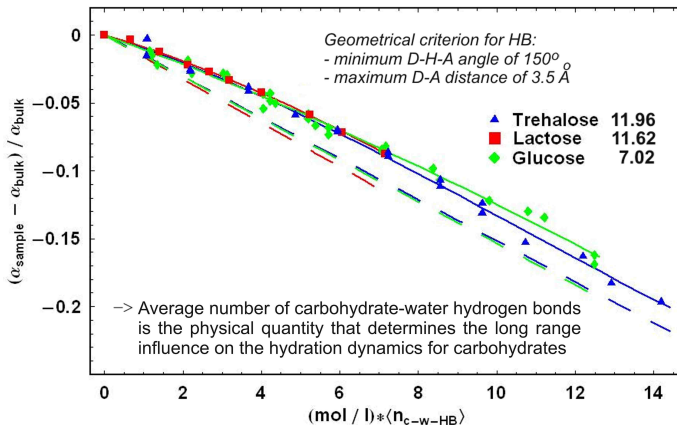
$$\delta R = 3.7 \pm 0.9 \text{ \AA}$$

- > extended dynamic hydration shell for disaccharides
- > enhanced bioprotectors



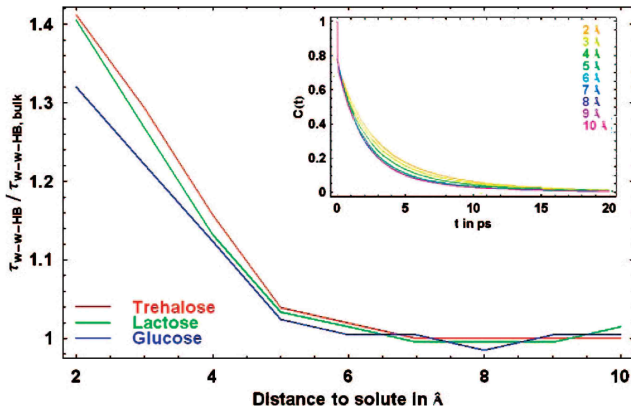
M. Heyden, E. Bründermann, U. Heugen, G. Niehues, D.M. Leitner, M. Havenith (2008), *J. Am. Chem. Soc.*, **130**, 5773-9.

# Correlation to COH-H<sub>2</sub>O hydrogen bonds



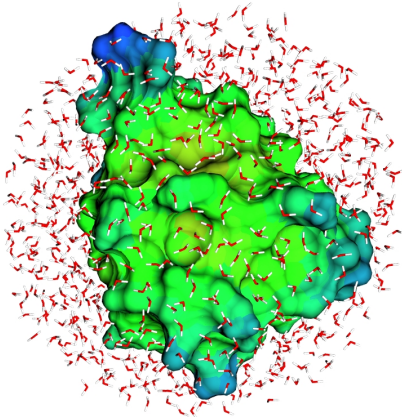
M. Heyden, E. Bründermann, U. Heugen, G. Niehues, D.M. Leitner, M. Havenith (2008), *J. Am. Chem. Soc.*, **130**, 5773-9.

# Interpretation of hydration dynamics by MD simulation



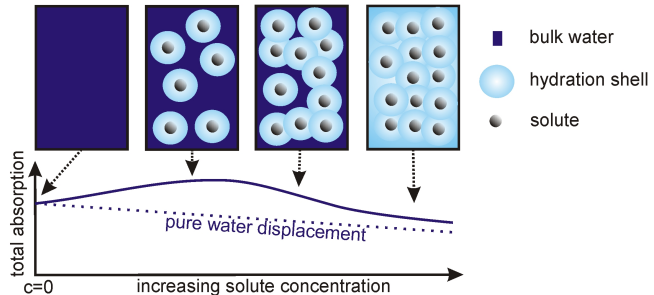
M. Heyden, E. Bründermann, U. Heugen, G. Niehues, D.M. Leitner, M. Havenith (2008), *J. Am. Chem. Soc.*, **130**, 5773-9.

# Motivation



- **dynamic interplay** between biomolecule and the hydration shell involves **large amplitude motions**
  - slaving by  $\alpha$ -fluctuations
  - coupling to  $\beta$ -motions
- dynamic interplay is essential to **protein function and protein folding**
- $\lambda_{6-85}^*$  is the model protein (pseudo wildtype)
- 9.1 kDa protein (completely  $\alpha$ -helical) that controls the  $\lambda$ -switch in E-coli

# Concentration dependent THz response



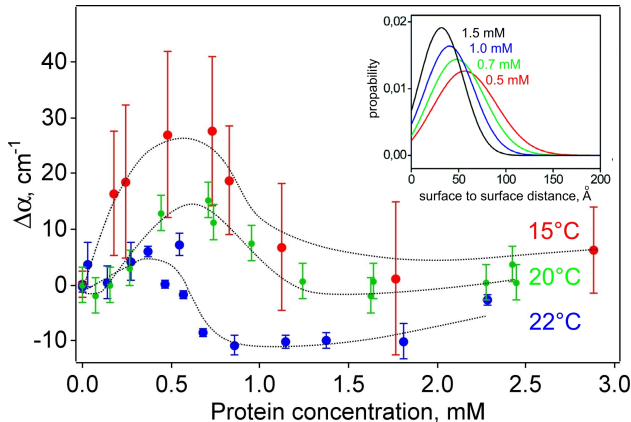
$$\alpha_{total}(2 - comp.) = \frac{V_{solute}}{V_{total}} \alpha_{solute} + \left(1 - \frac{V_{solute}}{V_{total}}\right) \alpha_{bulk}$$

$$\alpha_{total}(3 - comp.) = \frac{V_{solute}}{V_{total}} \alpha_{solute} + \frac{V_{hw}}{V_{total}} \alpha_{hw} + \left(1 - \frac{V_{solute}}{V_{total}} - \frac{V_{hw}}{V_{total}}\right) \alpha_{bulk}$$



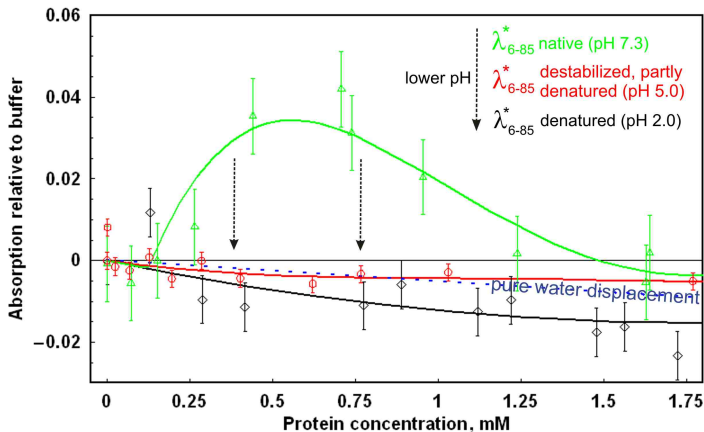
U. Heugen, G. Schwaab, E. Bründermann, M. Heyden, X. Yu, D. M. Leitner, M. Havenith (2006), *Proc. Nat. Acad. Sci. USA*, **103**, 12301-6

# Concentration dependent THz response of $\lambda_{6-85}^*$



S. Ebbinghaus, S. J. Kim, M. Heyden, X. Yu, U. Heugen, M. Gruebele, D. M. Leitner, M. Havenith (2007), *Proc. Nat. Acad. Sci. USA*, **104**, 20749-52

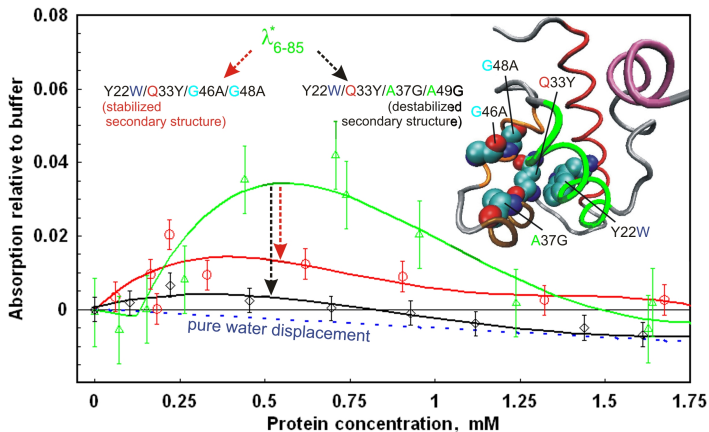
# Global destabilization of $\lambda_{6-85}^*$ by pH



S. Ebbinghaus, S. J. Kim, M. Heyden, X. Yu, M. Gruebele, D. M. Leitner, M. Havenith (2008), *J. Am. Chem. Soc.*, **130**, 2374-5



# Local hydration investigated by site-specific mutation

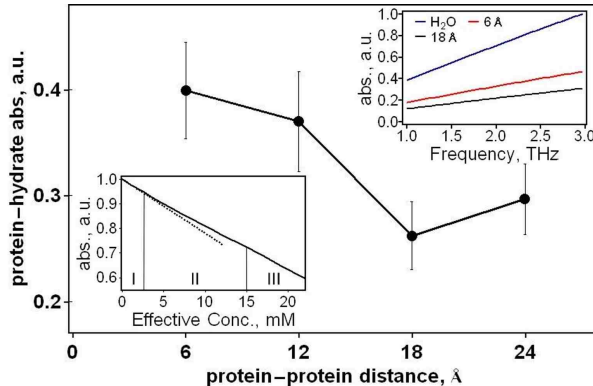


S. Ebbinghaus, S. J. Kim, M. Heyden, X. Yu, M. Gruebele, D. M. Leitner, M. Havenith (2008), *J. Am. Chem. Soc.*, **130**, 2374-5

# Interpretation of hydration dynamics by MD simulation

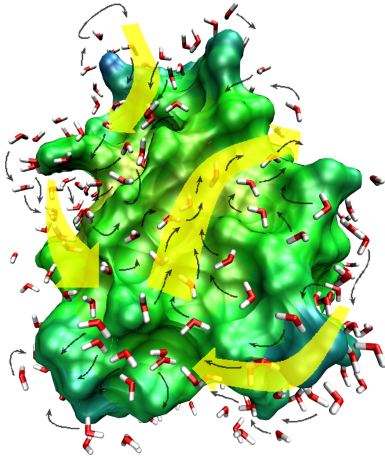
- **MD simulation** using *GROMOS96* force field
- $\lambda_{6-85}^*$  framed into cubic boxes with periodic boundary conditions
- boxes of different sizes, simulating different protein concentrations
- simple point charge (SPC) model water fills up the boxes
- 25 ps trajectory with 30 fs time steps; averaging 2000 trajectories
- fourier transformed dipole autocorrelation function yields THz spectrum

## Dipole autocorrelation



S. Ebbinghaus, S. J. Kim, M. Heyden, X. Yu, U. Heugen, M. Gruebele, D. M. Leitner, M. Havenith (2007), *Proc. Nat. Acad. Sci. USA*, **104**, 20749-52

# Collective Flows around proteins

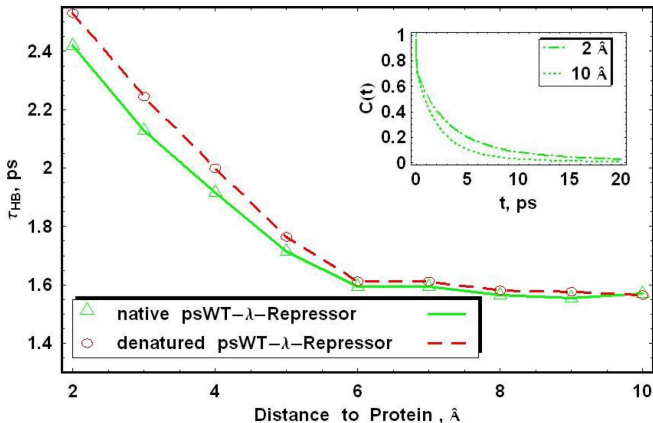


- **collective flows** (currents, vortices, and divergent flows) of water molecules around the protein surface
- observed up to **15 Å** away from protein surface
- **highly fluctuating**: patterns change within a time scale of less than 10 ps



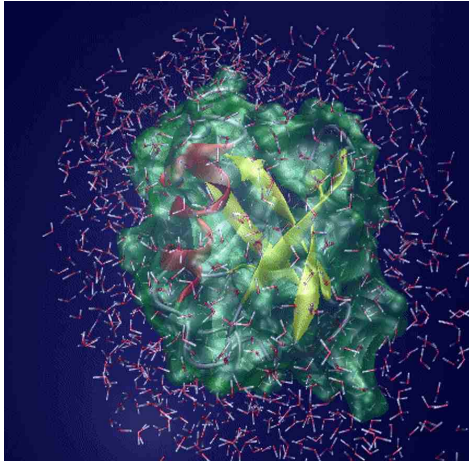
K. Umezawa, H. Junichi,  
S. Shimotakahara, H. Shindo,  
(2007), *J. Chem. Phys.*, **127**, 045101-7

## MD Simulation: Denatured vs. Native $\lambda_{6-85}^*$



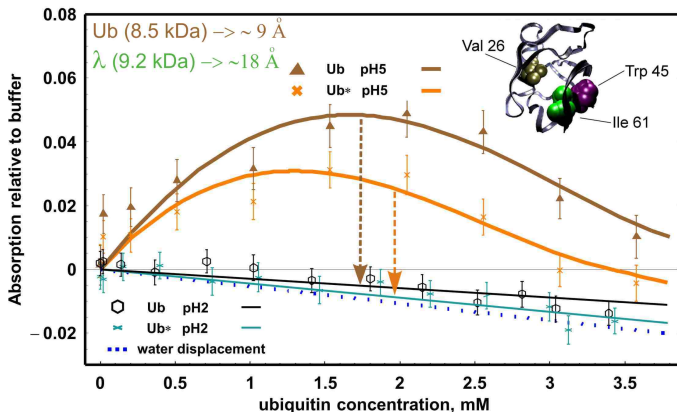
S. Ebbinghaus, S. J. Kim, M. Heyden, X. Yu, M. Gruebele, D. M. Leitner, M. Havenith (2008), *J. Am. Chem. Soc.*, **130**, 2374-5

# Motivation



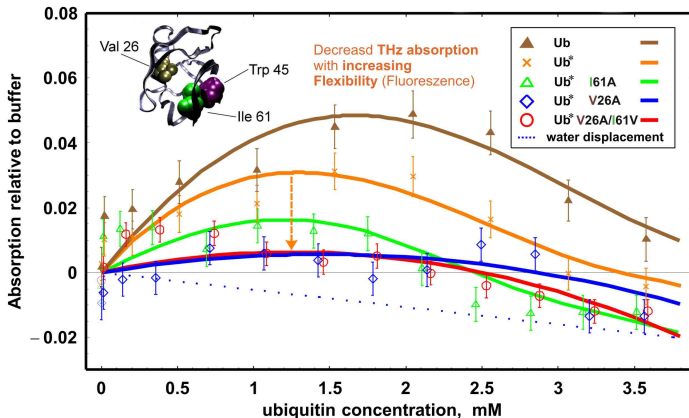
- **Ubiquitin** is a 8.5 kD  $\alpha/\beta$ -protein with one helical segment
- ubiquitously expressed in eukaryotes controls the stability, function, and intracellular localization of a wide variety of proteins
- pseudo-wildtype Ub\* contains Phe45Trp mutation as an independent probe for protein flexibility
- prototype for folding kinetics studies
- probed in the past with emphasis on the backbone and side chains

# Global destabilization of Ubiquitin by pH



B. Born, S. J. Kim, M. Gruebele, M. Havenith (2008), *Faraday Discussion*, accepted

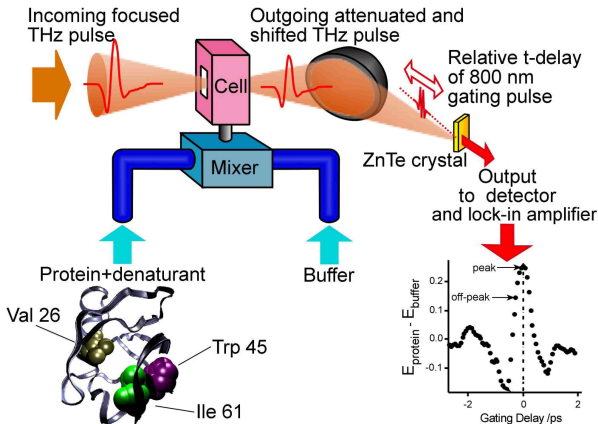
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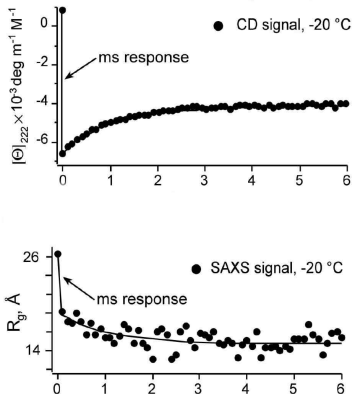
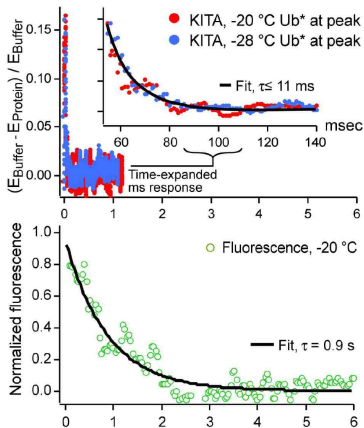


# Kinetic THz Absorption (KITA)



S. J. Kim, B. Born, M. Havenith, M. Gruebele (2008), *Angewandte Chemie*, accepted

# Hydration dynamics during protein folding



S. J. Kim, B. Born, M. Havenith, M. Gruebele (2008), *Angewandte Chemie*, accepted

# Conclusion and Outlook

- 1 long-range influence correlated with hydrogen bonds between the molecule and adjacent water molecules for **carbohydrates** → Disaccharides have larger influence → explaining the enhanced bioprotection
- 2 dynamic hydration layer (up to 20 Å) of **proteins** by THz exceeds by far the values for the static hydration shell ( $\sim 20$  Å)
- 3 Hydration dynamics of proteins sensitive to local and global destabilization ( $\lambda_{6-85}^*$ ) and site chain flexibility (**Ubiquitin**)
- 4 **Real-time detection** of protein-water dynamics upon protein folding by **KITA** → ms relaxation attributed to forming of hydrogen bond during early stage of folding

# Acknowledgement



