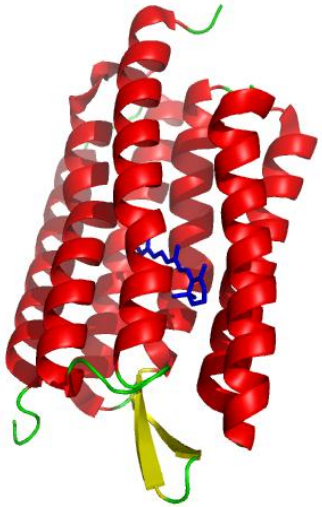
HZDR
HELMHOLTZ ZENTRUM DRESDEN ROSSENDORF

Freie Universität Berlin

WP 3
"Time-resolved IR spectroscopy on proteins"



PIDID Meeting
December 16th 2011

Dr. Christian Bauer

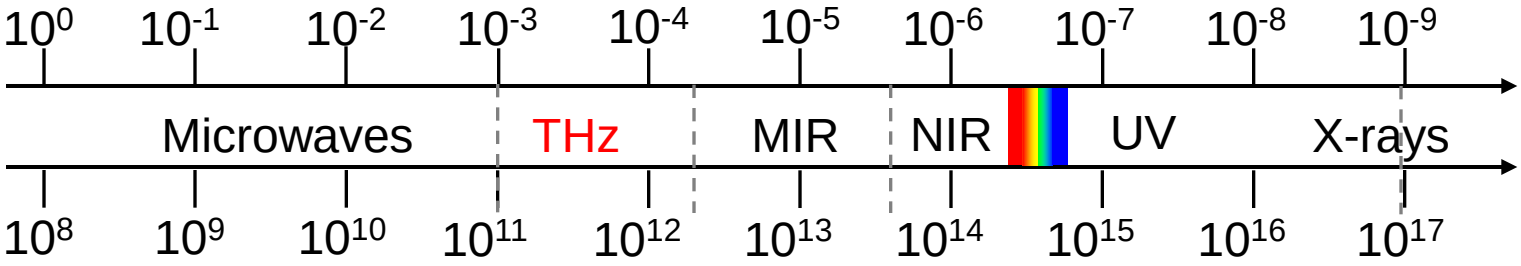
Guideline

1. Motivation for THz spectroscopy of proteins
2. WP3 "Time-resolved IR spectroscopy on proteins"

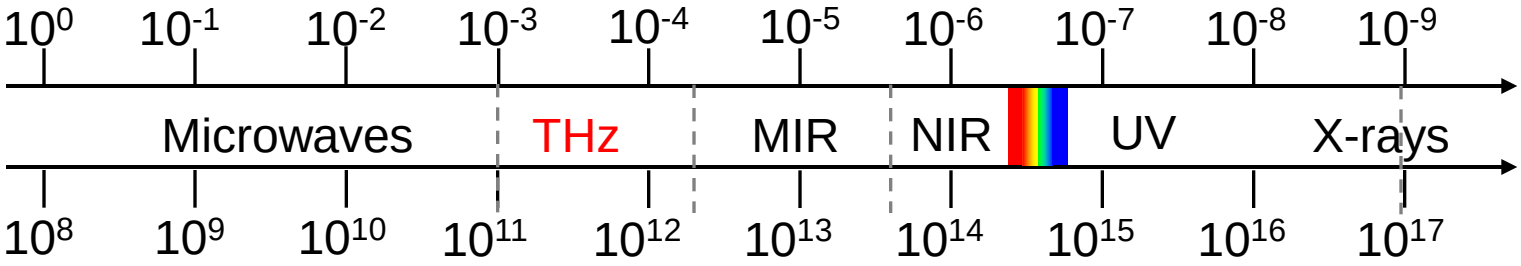
- Advanced pump/probe experiment
- Model protein Bacteriorhodopsin: structure, function and spectroscopic properties
- Experimental setup of advanced pump/probe experiment

3. Results: FIR difference spectroscopy on Bacteriorhodopsin (preliminary work)

Spectroscopy on Proteins in the Infrared



Spectroscopy on Proteins in the Infrared



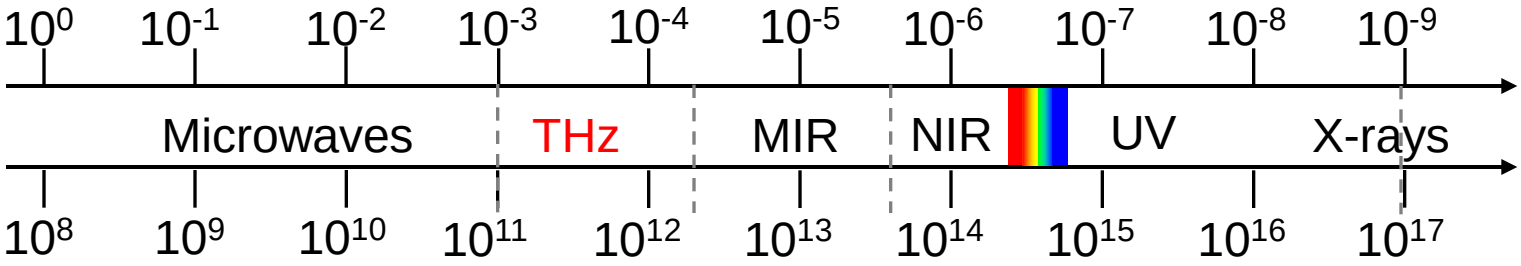
mid infrared: standard method

Detection of change of vibration of

- Chromophores
- Protein backbone
- single amino acid side chains

→ structural analysis on molecular level

Spectroscopy on Proteins in the Infrared



far infrared (THz):
new method of light induced difference absorption spectroscopy

Detection of change of vibrations of

- Chromophores
- Protein backbone
- H-bonding network
- Low energy torsional modes

→ structure analysis on molecular level

mid infrared: standard method

Detection of change of vibration of

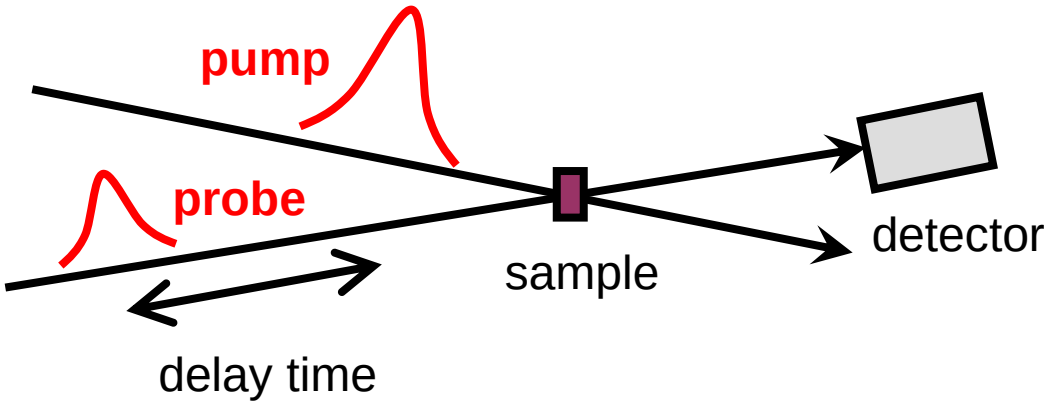
- Chromophores
- Protein backbone
- single amino acid side chains

→ structural analysis on molecular level

Motivation for Spectroscopy on Proteins in the Far Infrared

- Assignment of far IR modes helps to understand the functionality of proteins
- In the far infrared collective vibrations of the chromophore and the H-bonding network are excited
- Far Infrared radiation can be used to perform special multi-wavelengths pump/probe experiments

Classic Pump/Probe Experiment



- pump pulse

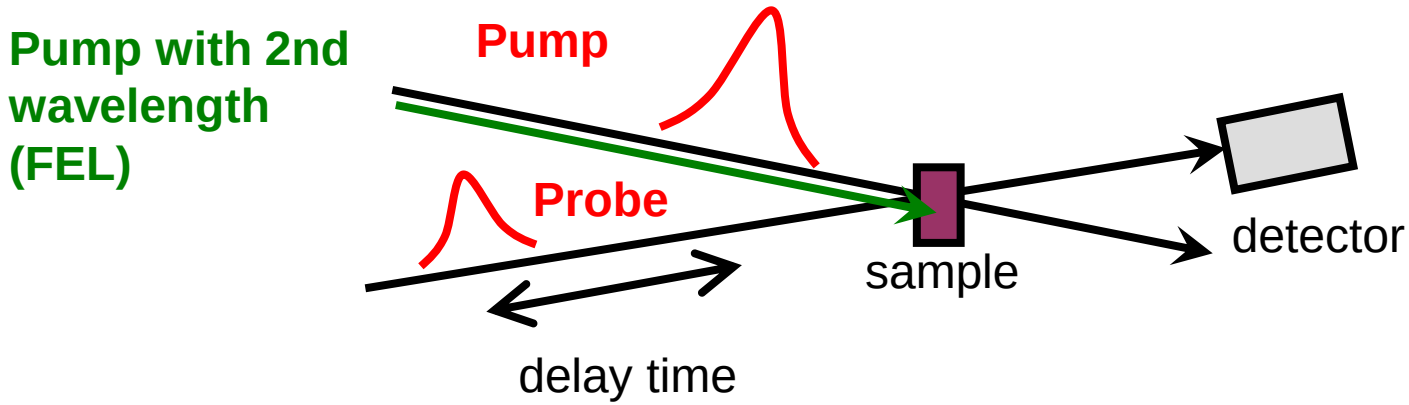
stimulates a process,
reaction, phase transition…

-> preferably by photons!

- probe pulse

measures change of
materials properties at
different times

Modified Pump/Probe Experiment: Control of Photoreaction



- pump pulse

stimulates a process, reaction, phase transition…

-> preferably by photons!

pump pulse with 2nd wavelength

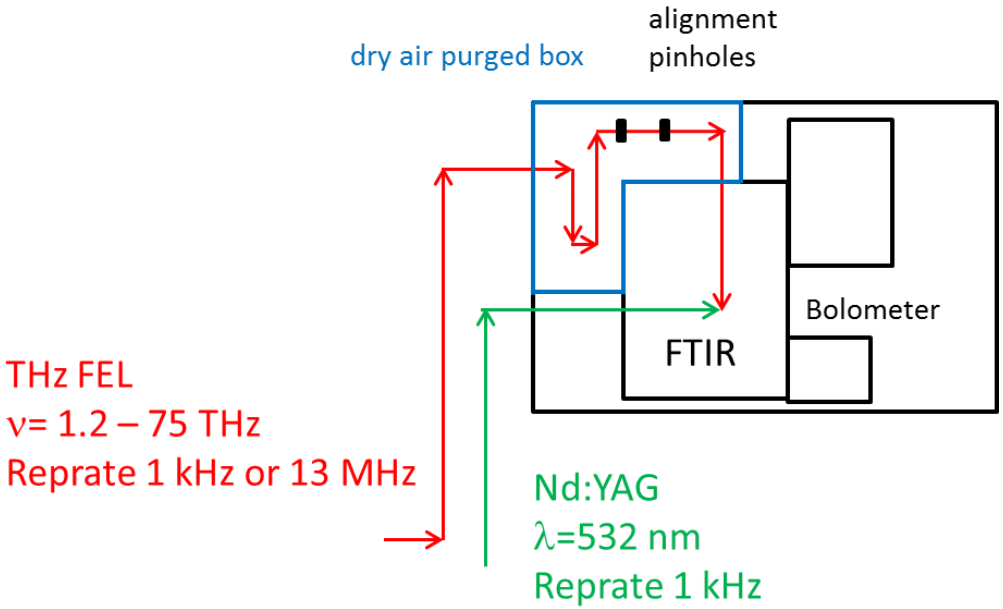
changes the photoreaction

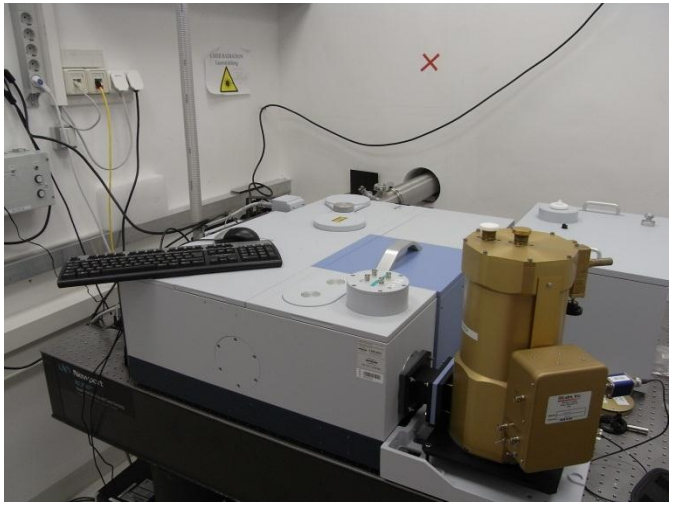
-> control of reaction

- probe pulse

measures change of materials properties at different times

Setup of modified Pump/Probe Experiment



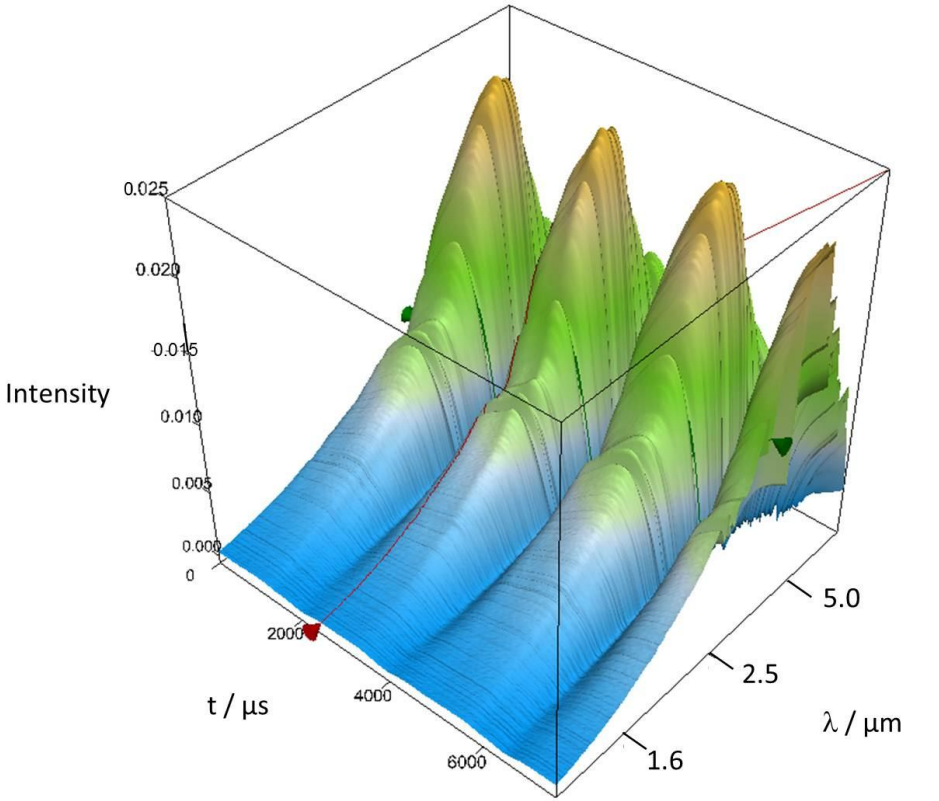


two pump beams: VIS and THz-FEL

one probe beam: IR (FTIR-Globar)

time-rsolved stepscan technique

Time-resolved FTIR measurement: "Stepscan" technique



- Preliminary stepscan experiment without sample
- Proof of principle with "chopper experiment" according to manual

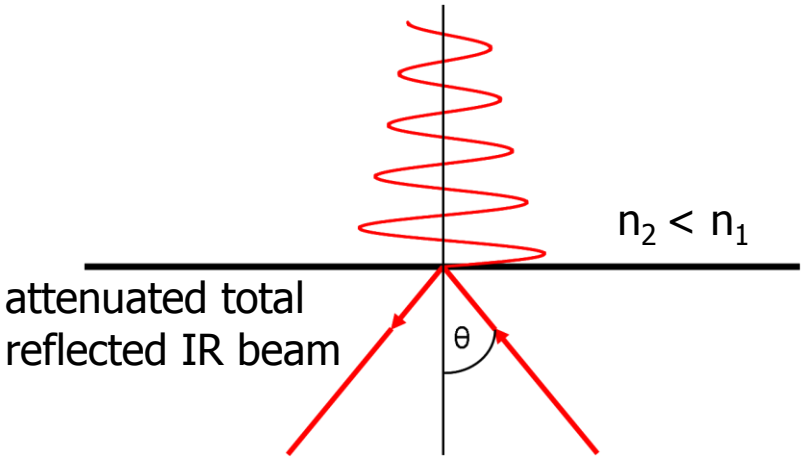
Attenuated Total Reflection (ATR) technique

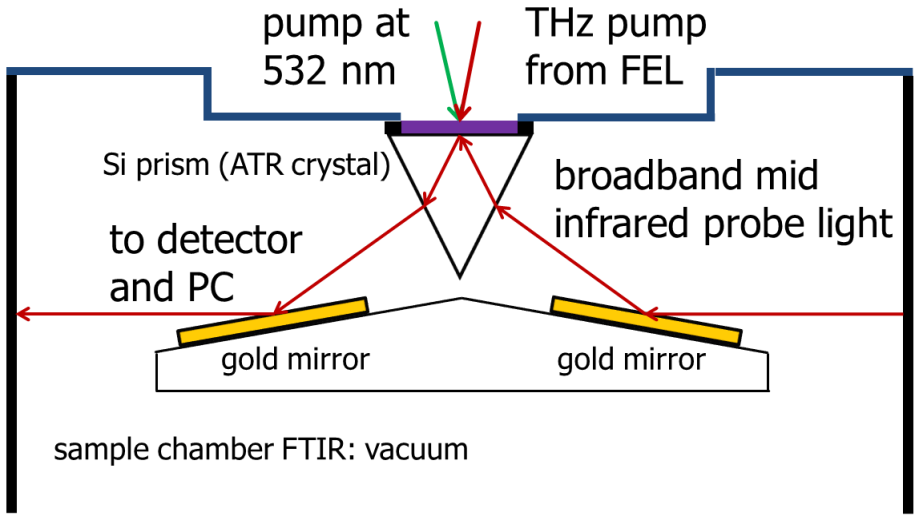
Window material in transmission setup not transparent in the FIR
(KBr below 25 µm, CaF_2 below 12 µm, BaF_2 below 15 µm transparent)

+ Setup vacuum-tight

+ Sample preparation possible as film

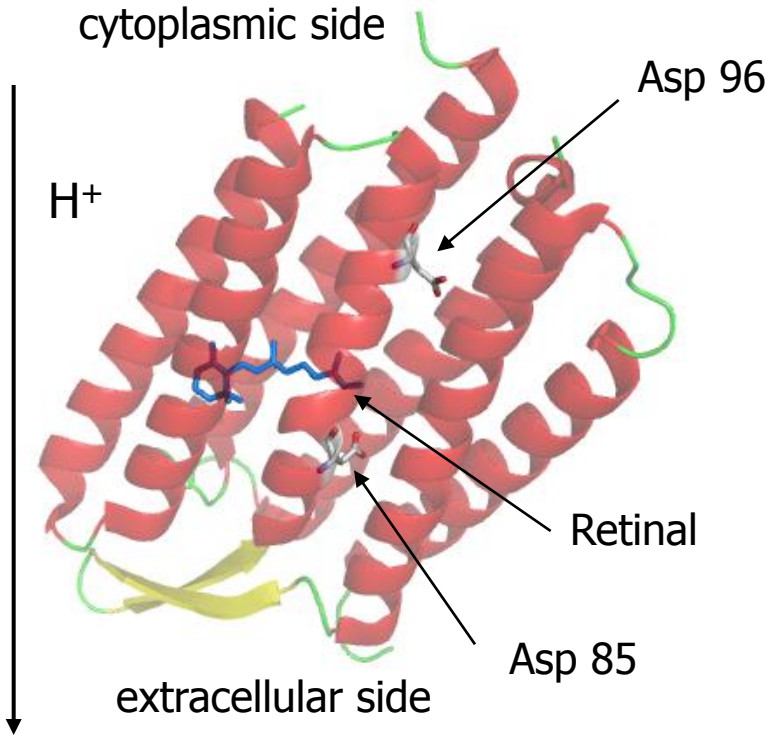
– Lower signal-to-noise ratio as in transmission





Modified Pump/Probe Experiment
Biomolecule: Bacteriorhodopsin (bR)

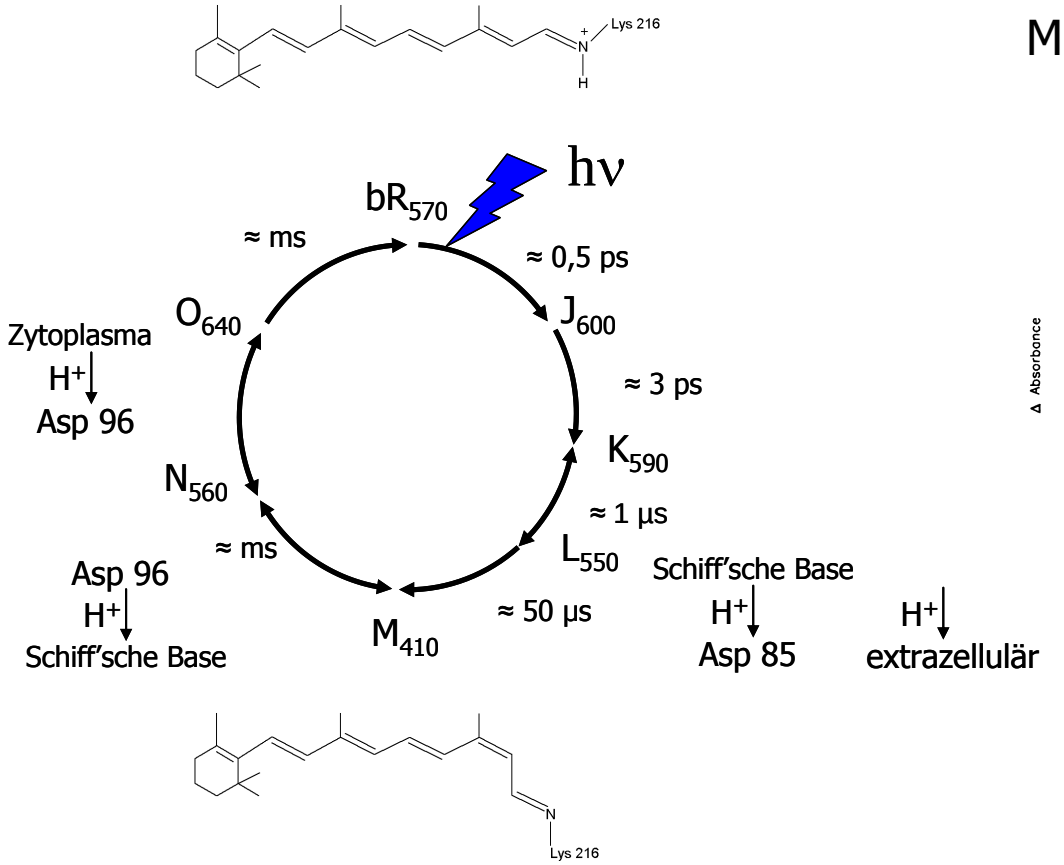
3D structure of Bacteriorhodopsin



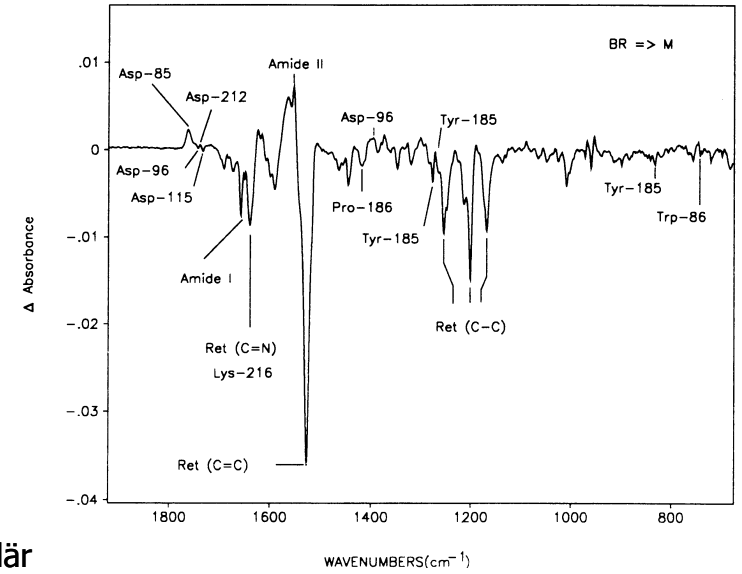
PDB entry: 1C3W

Photocycle of Bacteriorhodopsin

simplified photocycle of Bacteriorhodopsin



M-BR difference absorption spectrum

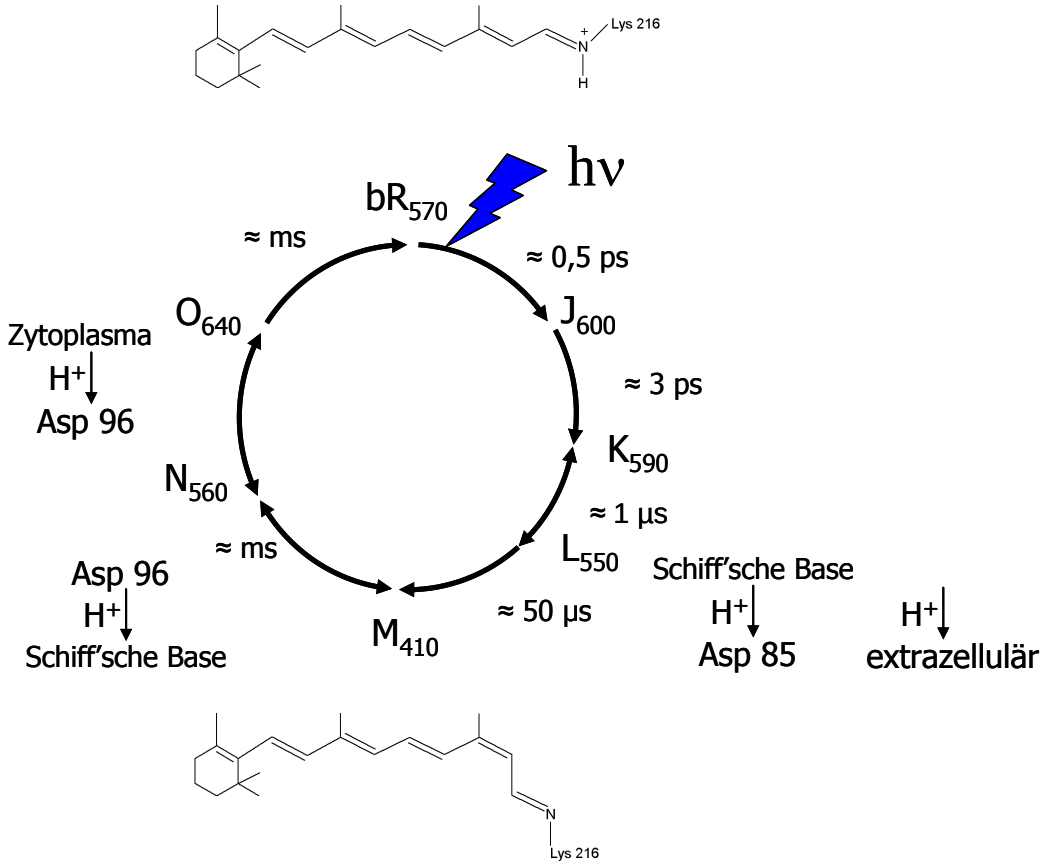


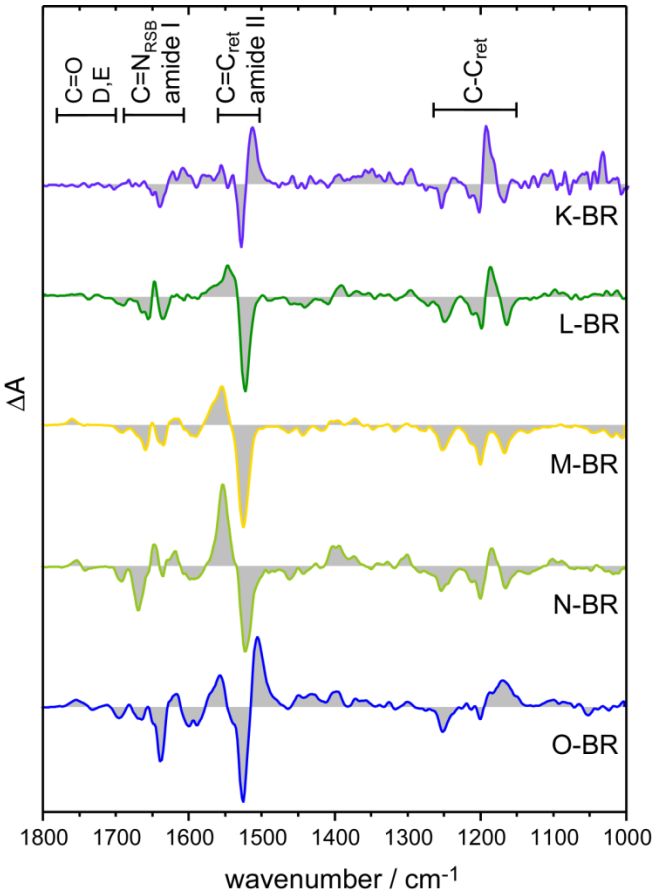
Cycle: Popp *et al.* (1993), Biophys. J. **65**, 1449

Spectrum J. Heberle / FUB

Photocycle of Bacteriorhodopsin

simplified photocycle of Bacteriorhodopsin





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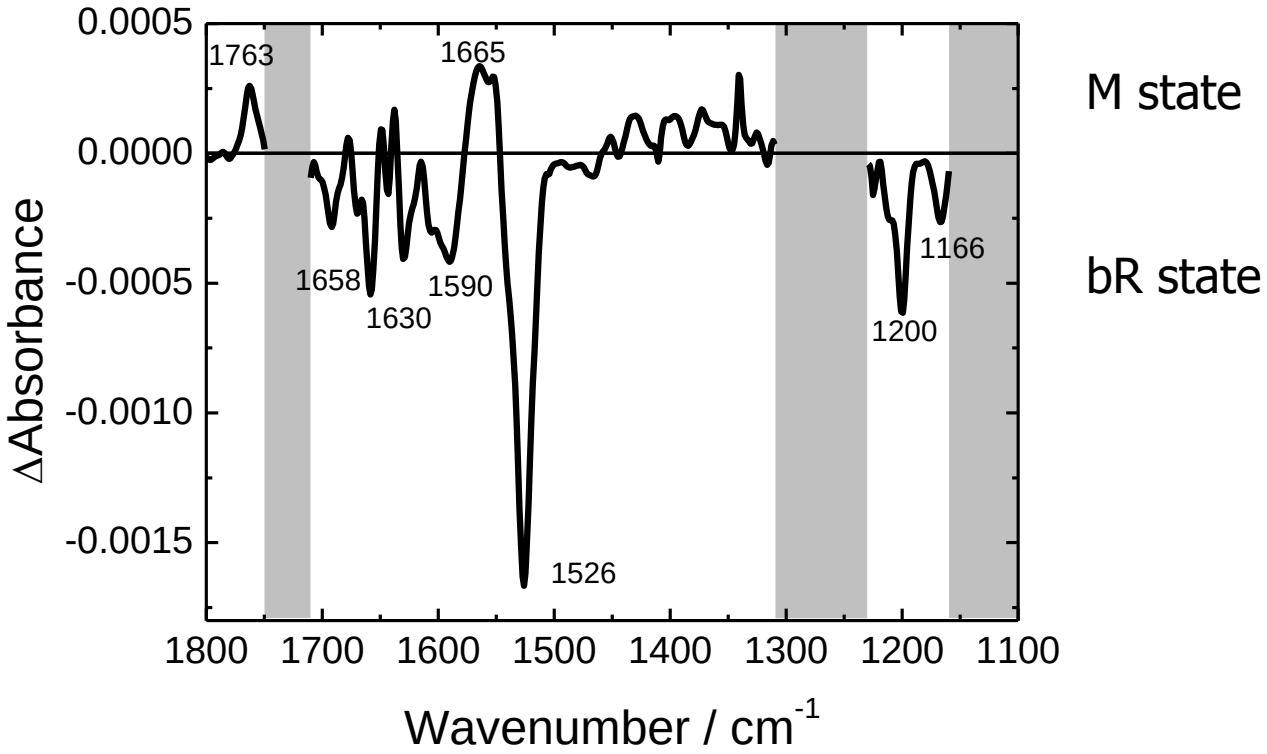
Spectra J. Heberle / FUB

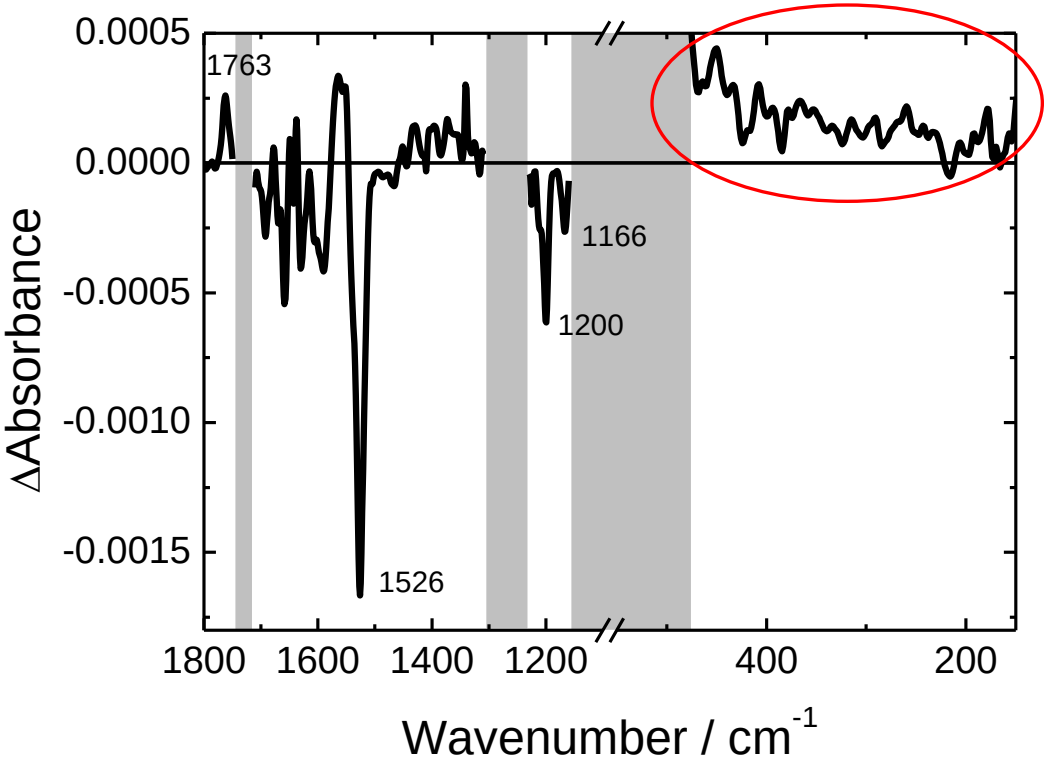
Difference Absorption Spectrum of a Bacteriorhodopsin Film in the Mid Infrared



- Characterization of accumulated intermediate using spectra from literature: M state

- Optimization of sample preparation

Light induced Difference Absorption Spectrum of a Bacteriorhodopsin Film in the Far Infrared

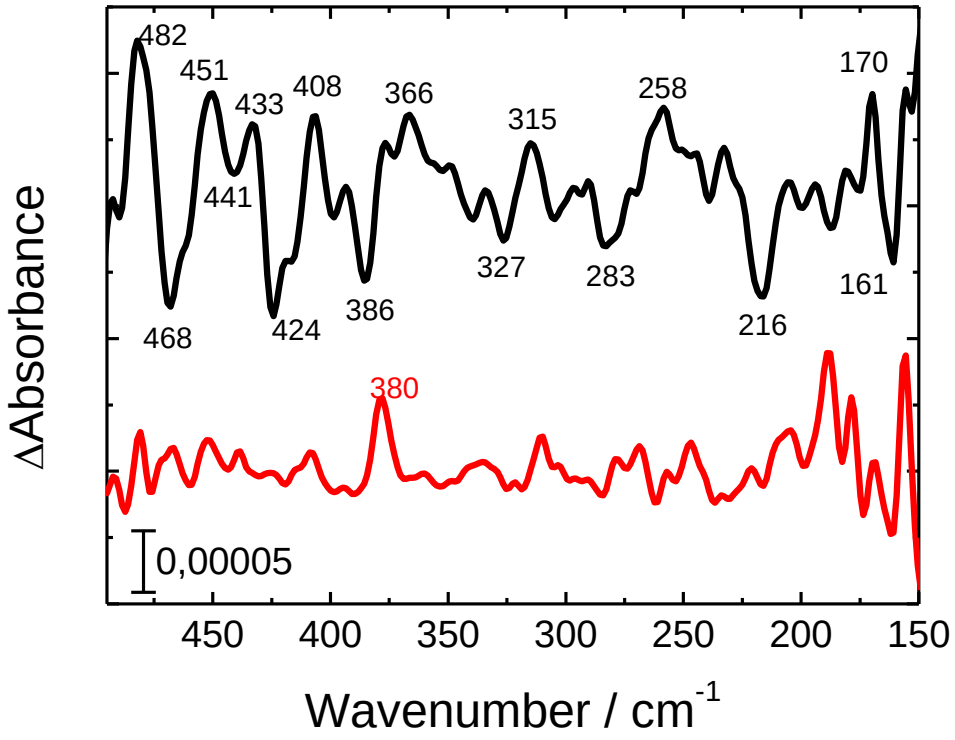


Difference bands in the far IR

Ratio of the amplitude of difference bands in the mid IR and the far IR ≈ 10:1

→ experiment in the FIR takes ≈ 100 times longer than in the mid IR

Light-induced Difference Absorption Spectrum of a Bacteriorhodopsin film in the Far Infrared



M state

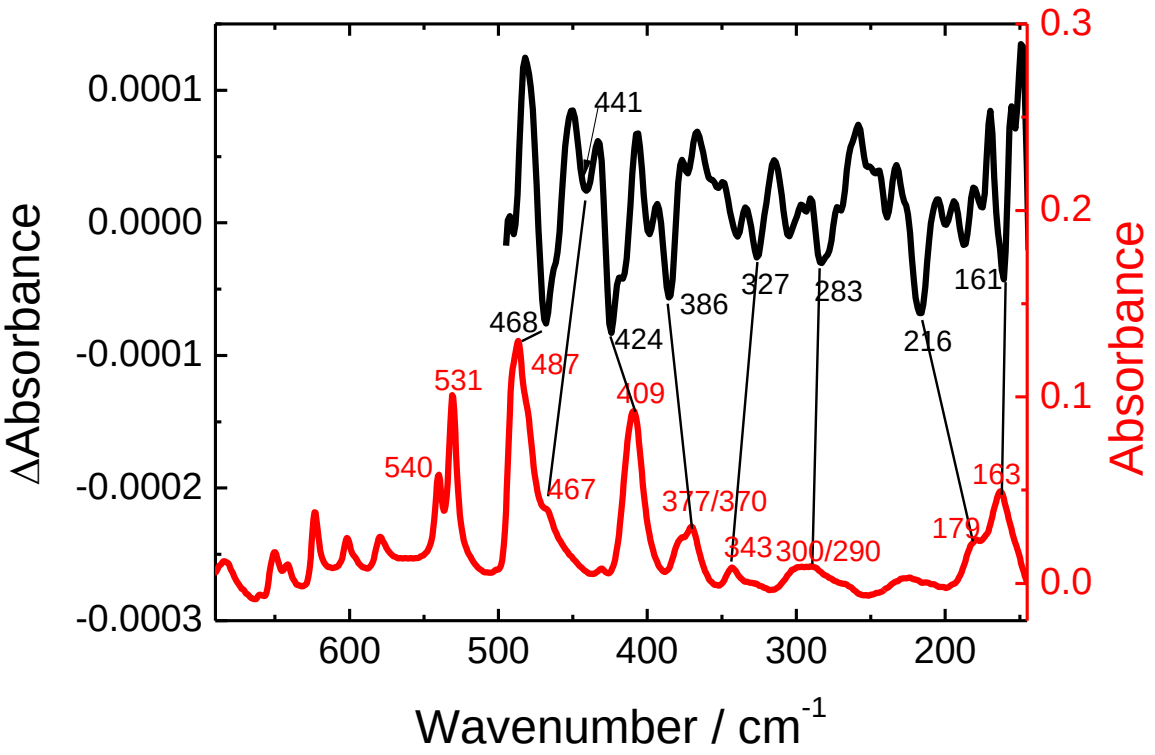
bR state

bR film

without sample

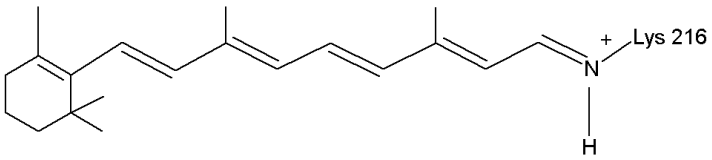
- positive and negative difference bands
- without sample: no difference bands, stronger noise at 380 cm^{-1}

Tentative Assignment of the Negative Difference Bands

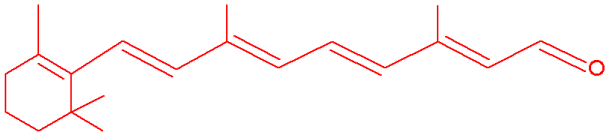


all-*trans* Retinal in bR,

protonated Schiff base



all-*trans* Retinal without apoprotein



→ Similarity in band pattern

Samples comparable despite differences in:

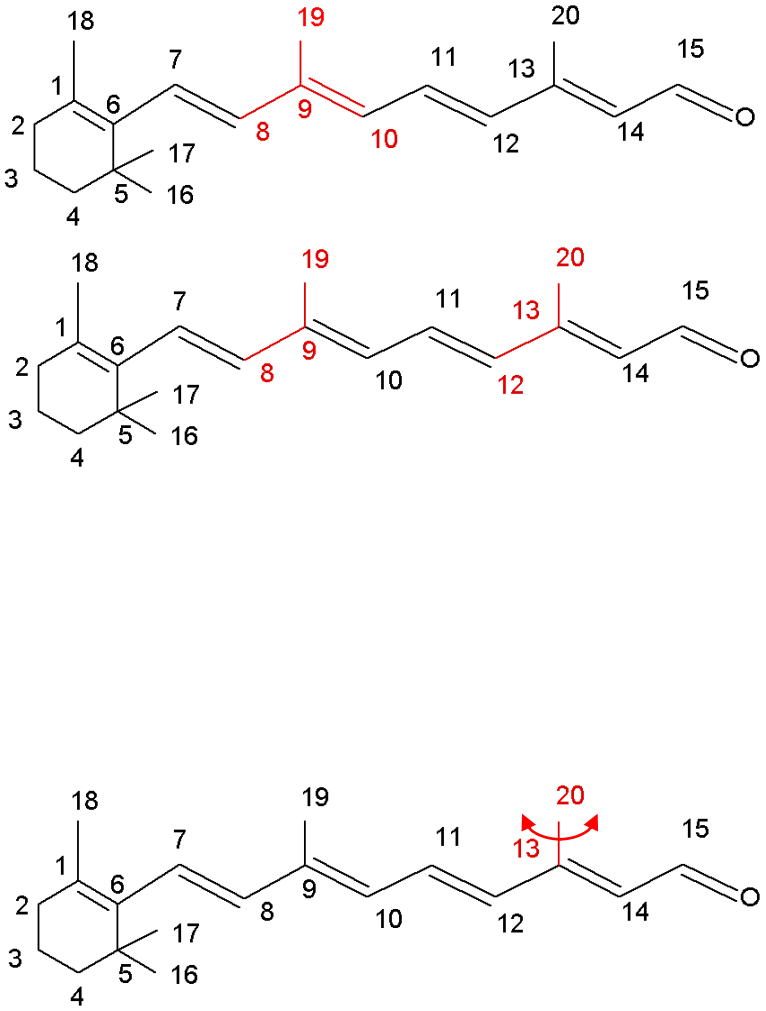
- Structure
- Chromophore environment

Tentative Assignment of the Negative Difference Bands

Difference bands of bR film		all-*trans* Retinal in PE	
positive	**negative**	**this work**	**Gervasio *et al.***
482 cm^{-1}			
	468 cm^{-1}	487 cm^{-1}	487 cm^{-1}
451 cm^{-1}			
	441 cm^{-1}	467 cm^{-1}	466 cm^{-1}
433 cm^{-1}			
	424 cm^{-1}	409 cm^{-1}	409 cm^{-1}
408 cm^{-1}			
	386 cm^{-1}	377/370 cm^{-1}	377/369 cm^{-1}
366 cm^{-1}			
	327 cm^{-1}	343 cm^{-1}	331 cm^{-1}
315 cm^{-1}			
	283 cm^{-1}	300/290 cm^{-1}	301 cm^{-1}
258 cm^{-1}			
	216 cm^{-1}	179 cm^{-1}	180 cm^{-1}
170 cm^{-1}			
	161 cm^{-1}	163 cm^{-1}	162 cm^{-1}

Tentative Assignment of the Negative Difference Bands

Negative difference bands of bR film	all-*trans* Retinal in PE	Assignment of bands by calculations Gervasio *et al.*
468 cm^{-1}	487 cm^{-1}	**C8C9C10 bending + C8C9C10-C19 bending**
441 cm^{-1}	467 cm^{-}	
424 cm^{-1}	409 cm^{-1}	**C8C9C19 bending + C12C13C20 bending**
386 cm^{-1}	377/370 cm^{-1}	
327 cm^{-1}	343 cm^{-1}	
283 cm^{-1}	300/290 cm^{-1}	
216 cm^{-1}	179 cm^{-1}	
161 cm^{-1}	163 cm^{-1}	**C13-C20H_3 torsional**



Conclusion

- Development of an ATR-Setup for spectroscopy in the far IR
- Measurement of light-induced difference spectra of a Bacterio-rhodopsin film in the far IR from 490 to 150 cm^{-1}
- Negative difference bands tentatively assigned to vibrational modes of all-*trans* retinal

Outlook

Band assignment useful knowledge to perform two-colour-pump/probe experiments:

Re-isomerisation of Bacteriorhodopsin´s retinal by resonant excitation in the far IR possible → control of photocycle

Prokhorenko, V. I. *et al.* Science **313**, 1257 (2006)
„coherent control" using fs pulses in the NIR: control of isomerisation yield of Bacteriorhodopsin shown

Acknowledgement

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Thank you for your attention