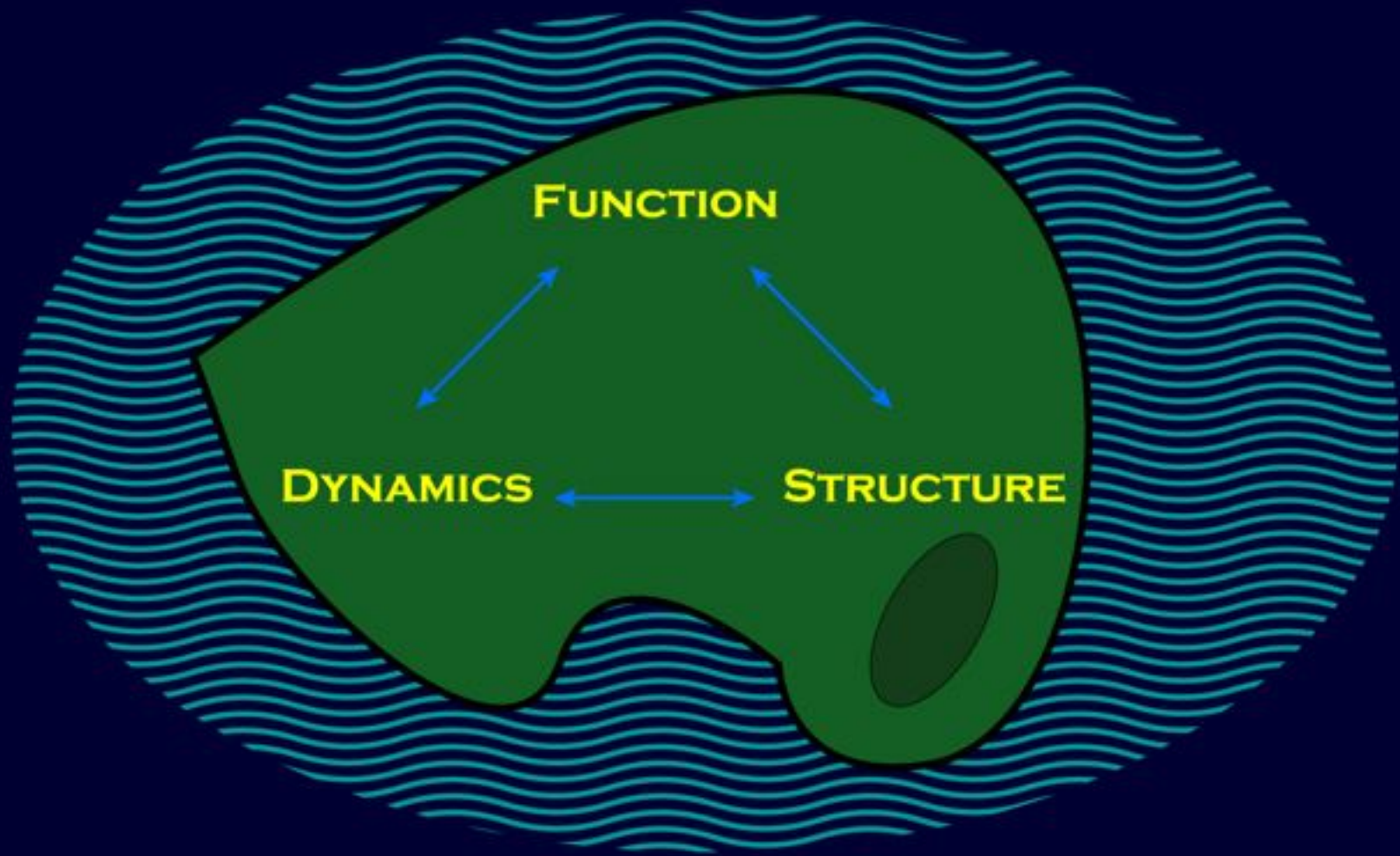


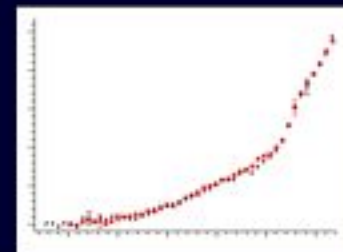
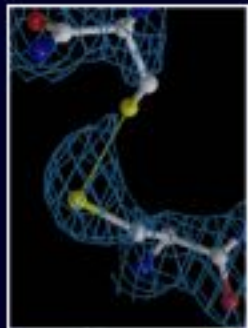
**Protein and solvent dynamics as studied by  
neutron scattering and  
temperature-controlled X-ray crystallography**

**Martin WEIK**  
**Institut de Biologie Structurale**  
**Grenoble, France**



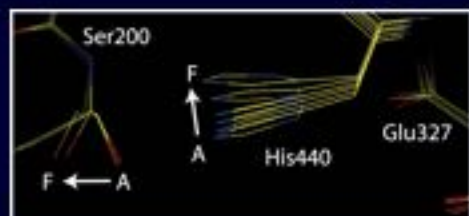
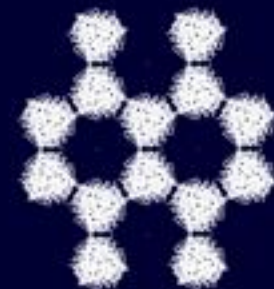


## Neutron scattering probes global protein dynamics



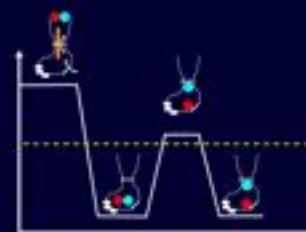
## Specific X-ray radiation damage to proteins

## Solvent behaviour in flash-cooled protein crystals



## Radiation damage probes local protein flexibility

## Strategy to trap enzymatic intermediate states

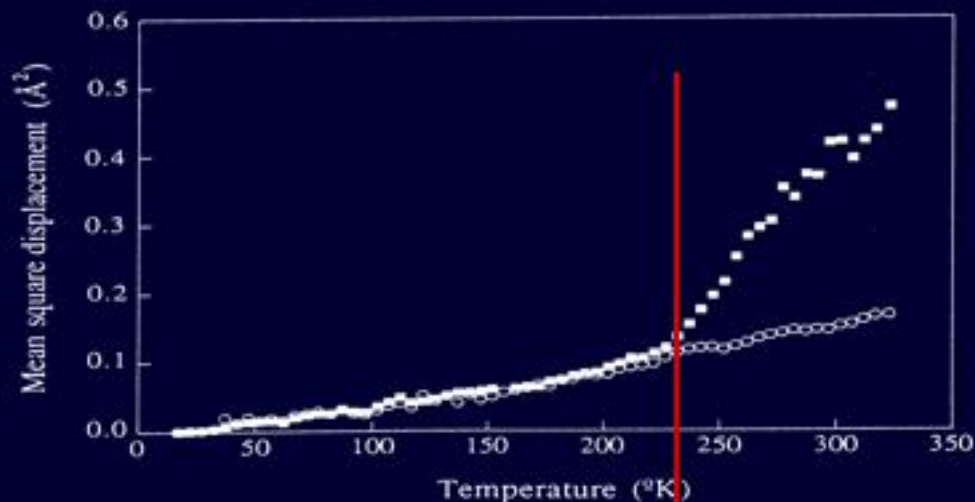


# ① Neutron scattering probes global protein dynamics



# Dynamical transitions in proteins

Neutron scattering - ns - ps motions - purple membranes



**Mössbauer spectroscopy**

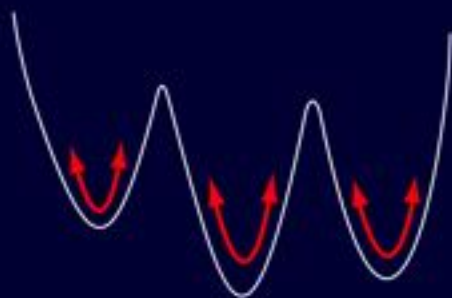
Parak *et al.* (1982) *JMB* 161, 177

**Neutron scattering**

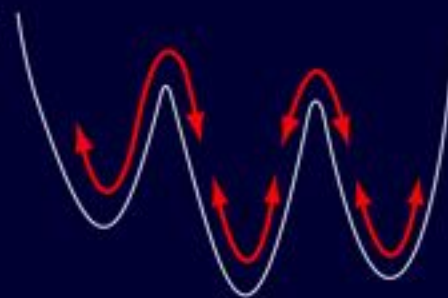
Doster *et al.* (1989) *Nature* 337, 754

Ferrand *et al.* (1993) *PNAS* 90, 9668

**Spectroscopy, MD simulation,  
Crystallography...**



- harmonic motions
- substates frozen out

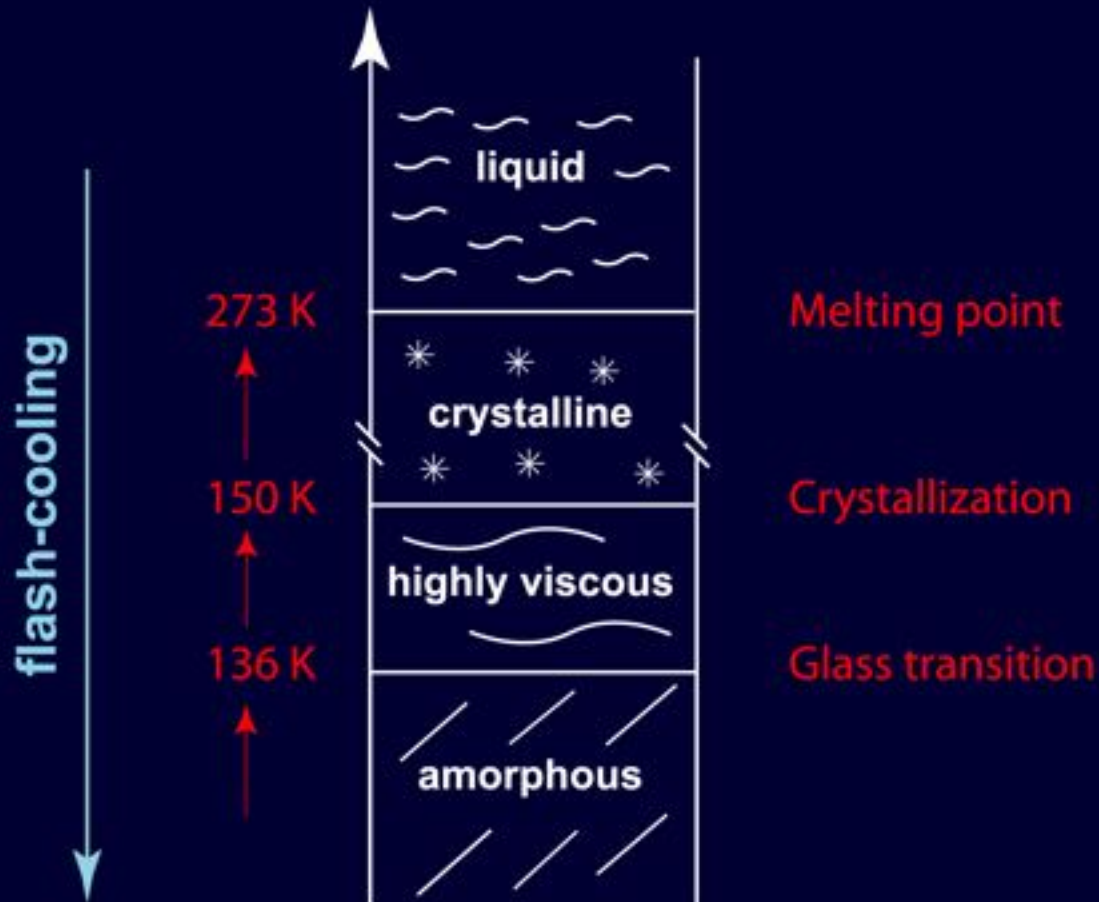


- non-harmonic motions
- transitions between substates

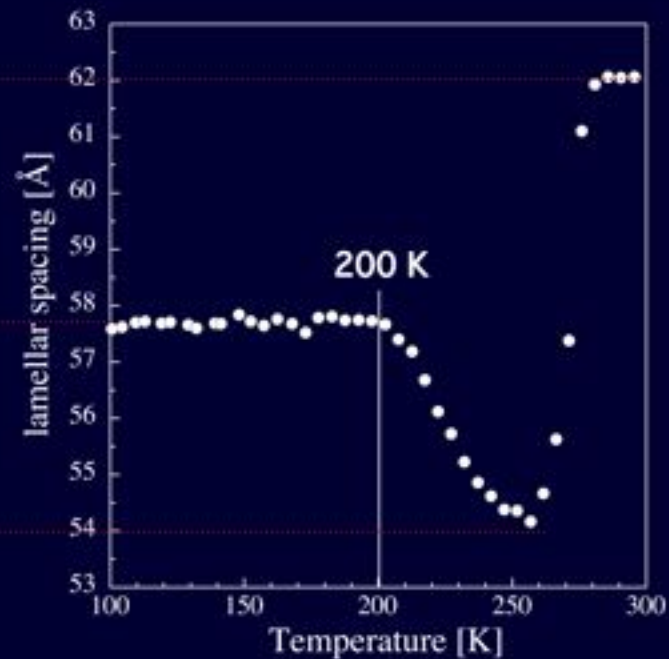
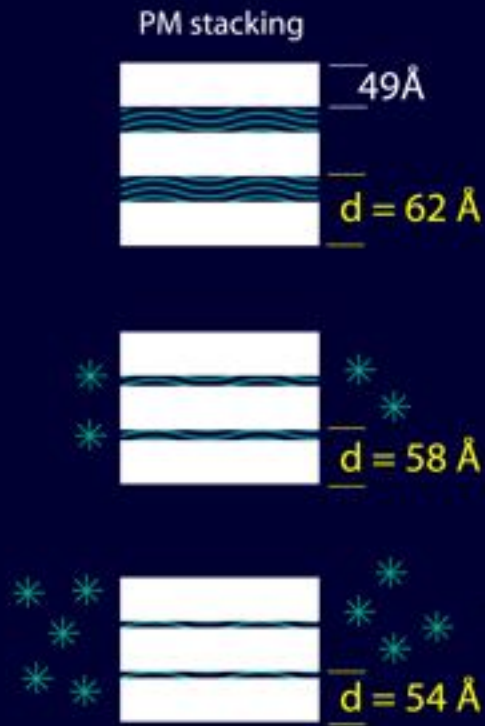
## Purple membrane stacks



# Pure water at cryo-temperatures



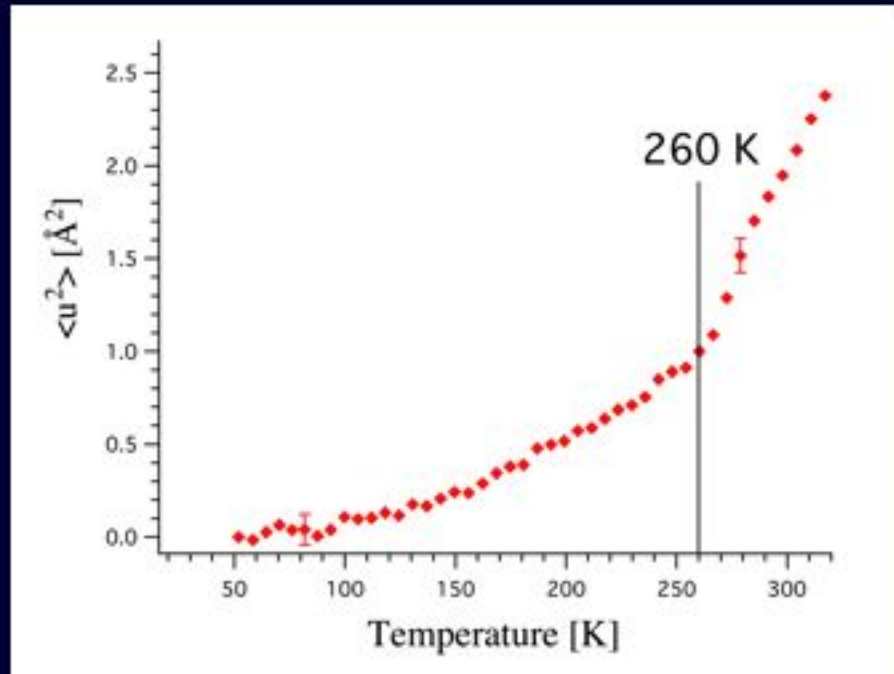
# Ultra-viscous water in stacks of PM at 200 K



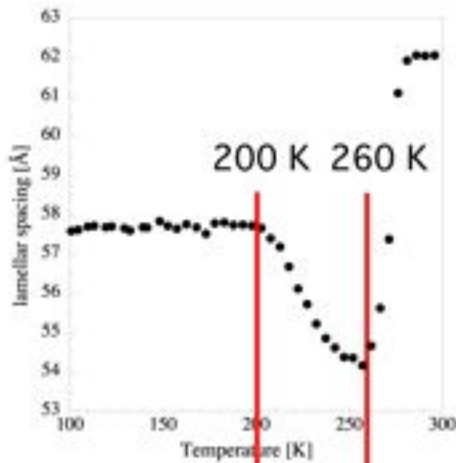
Long-range translational diffusion above GT, concomitant with crystallization

**Does glass transition of inter-membrane water trigger a dynamical transition in PM ?**

# Dynamical transitions of flash-cooled PM measured by IENS (IN16)

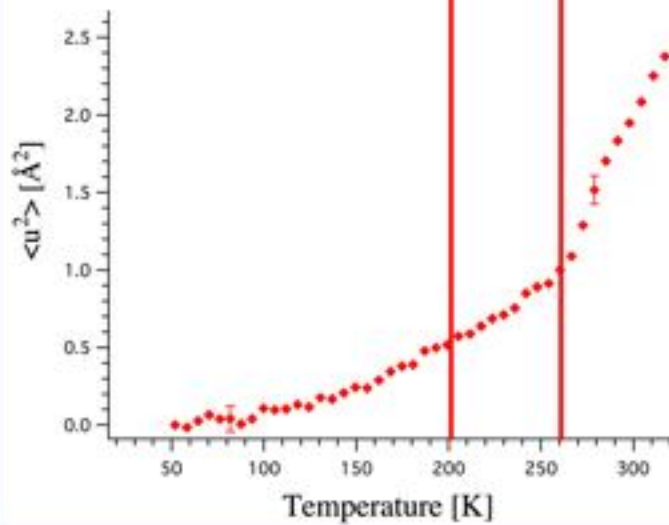


- dynamical transition at 260 K
- no transition at 200 K



Inter-membrane water:

Glass transition at  $\leq 200$  K



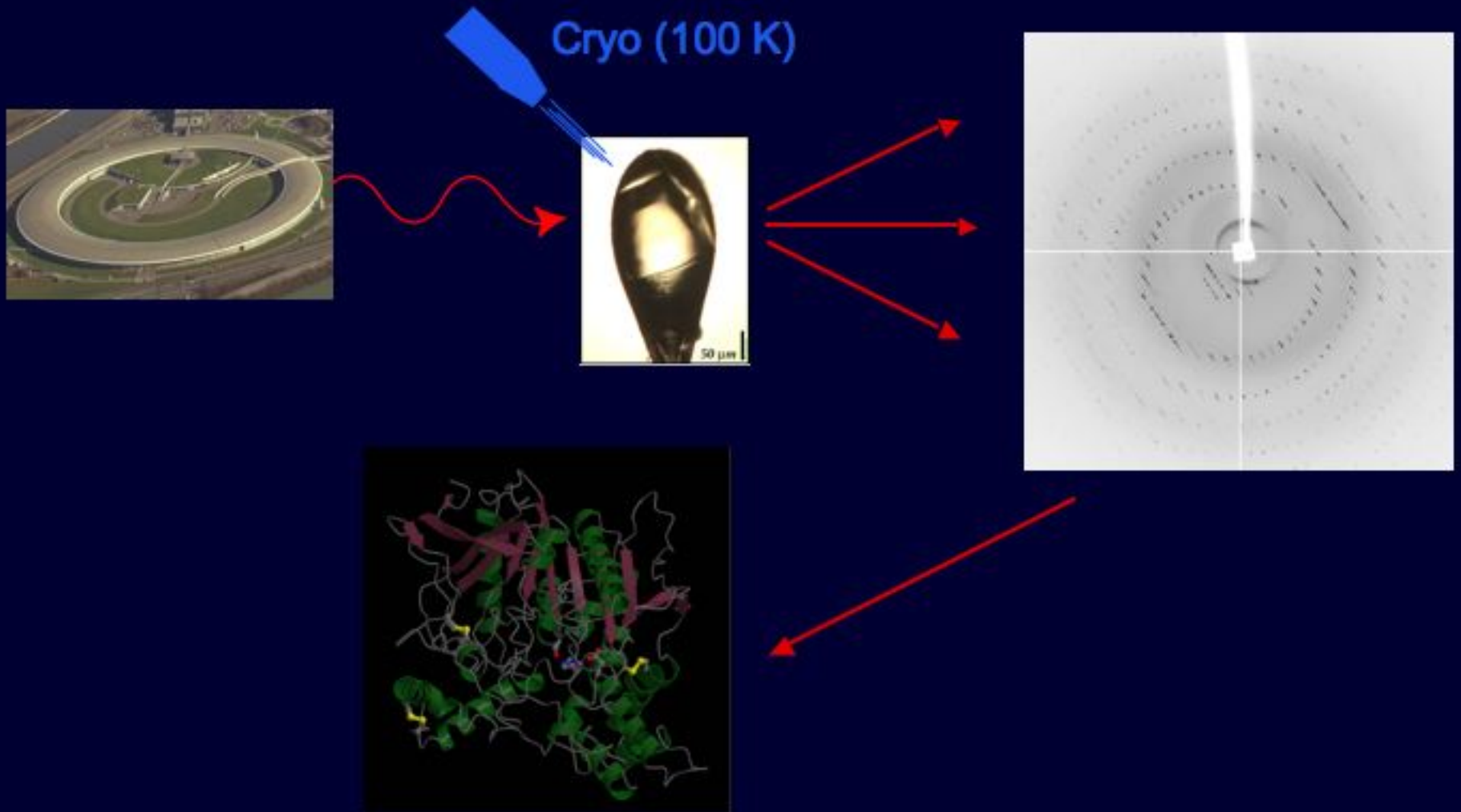
ns-ps motions in PM:

Dynamical transition at and 260 K

**Glass transition of inter-membrane water does not trigger dynamical transition in PM**

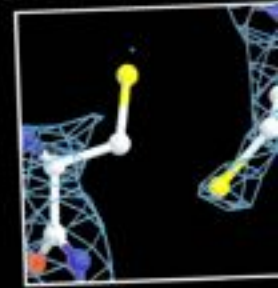
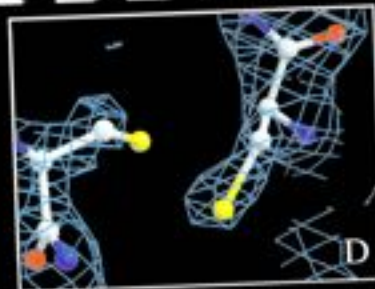
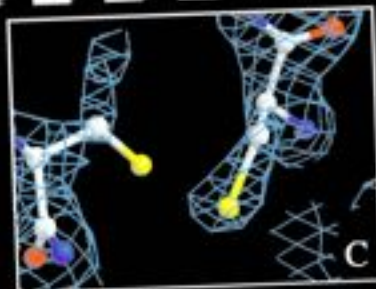
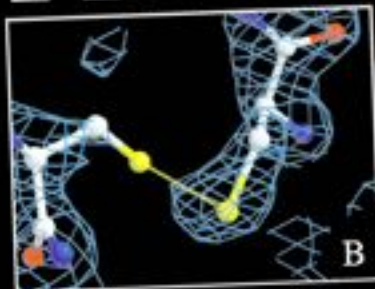
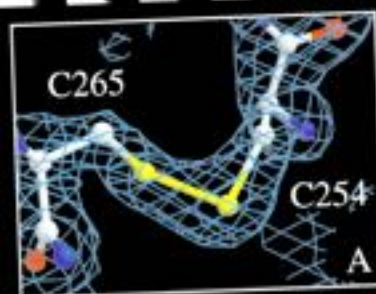
- Behaviour of water confined by stacks of biological membranes at cryo-temperatures?
  - long-range translational diffusion at 200 K
  - glass transition at  $\leq 200$  K
- Does glass transition of inter-membrane water trigger dynamical transition in the membrane?
  - No - but what about local protein flexibility?

# Protein X-ray crystallography



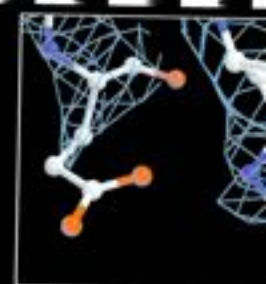
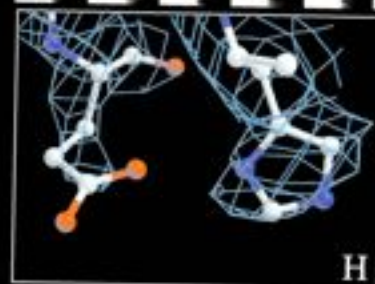
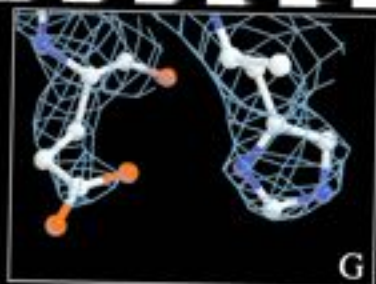
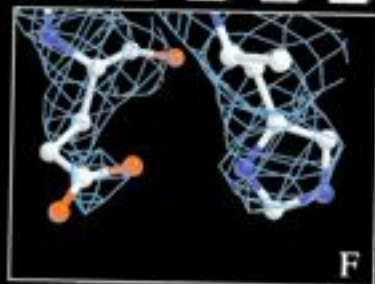
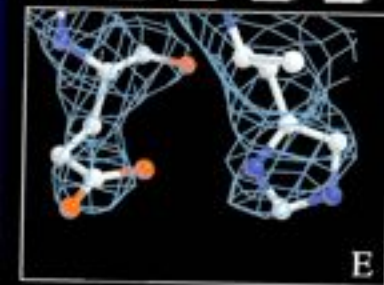
**Synchrotron radiation is a powerful tool, but price must be paid**

## Disulfide breakage



## ② Specific X-ray radiation damage to proteins

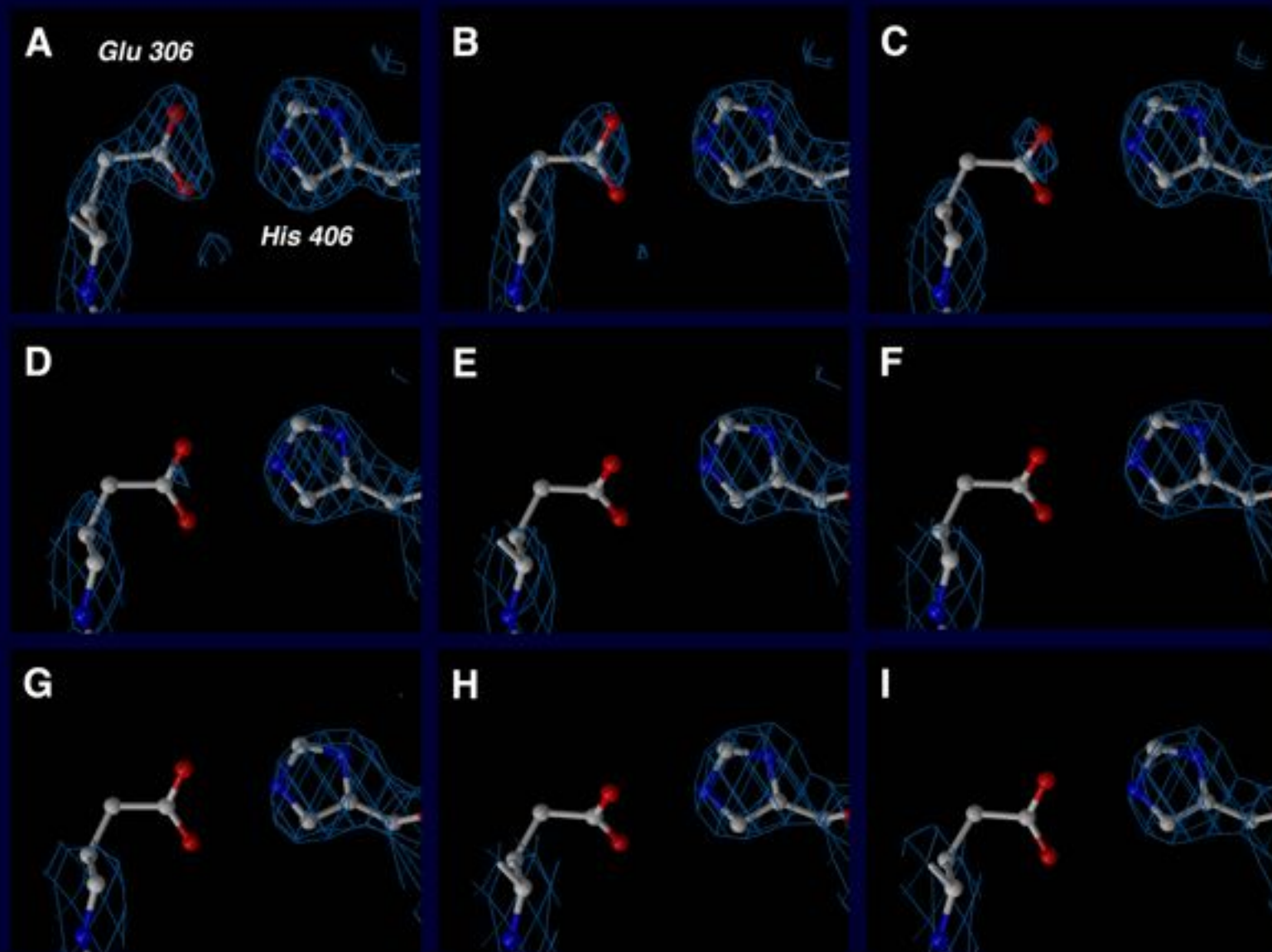
### Decarboxylation



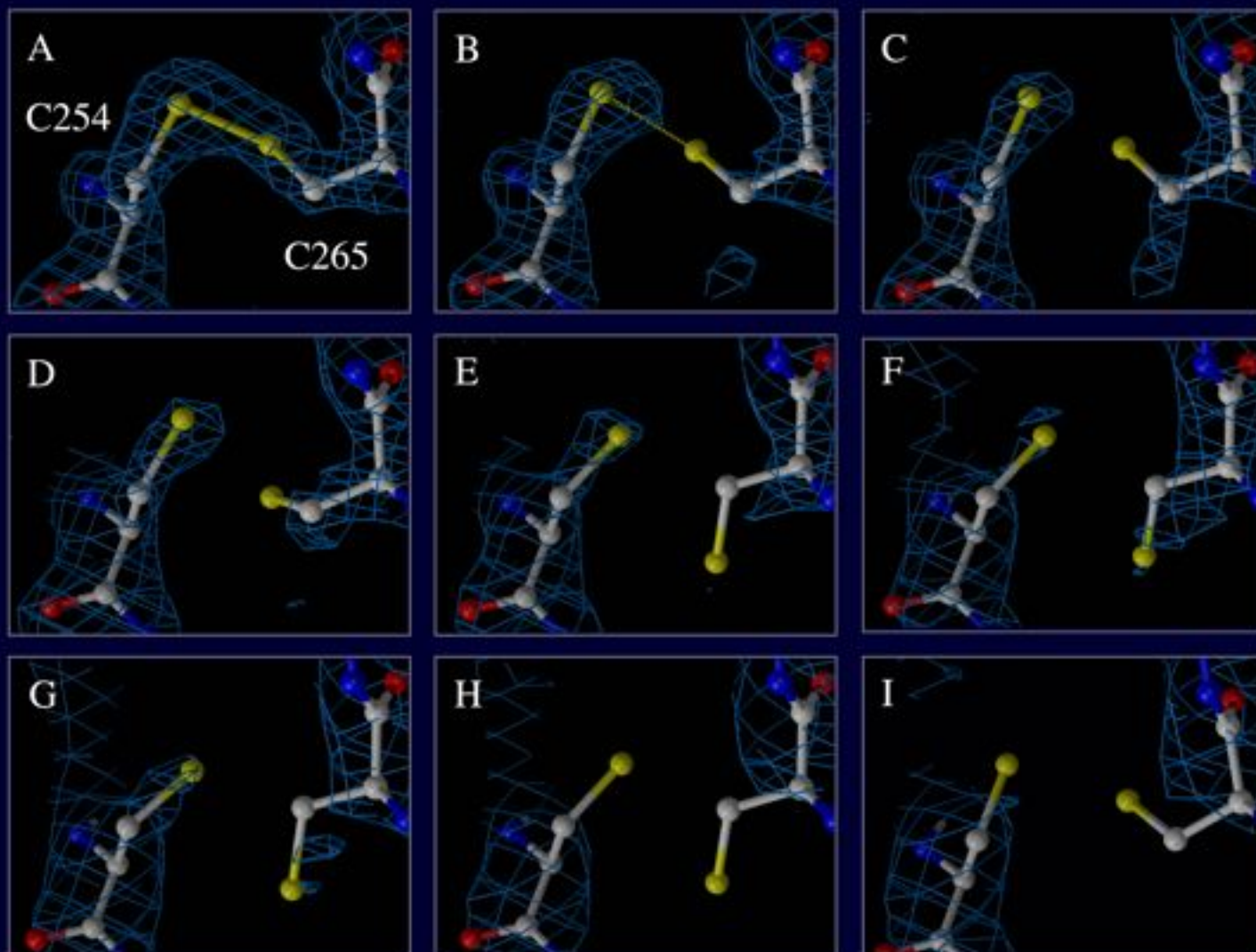
## Data collection series

- crystals of acetylcholinesterase (AChE)
- ESRF undulator beam line ID14 - EH4
- 9 complete data sets (A - I) at 100 K
- dose:  $10^7$  Gy/data set  
(for comparison: natural dose for humans: 0.002 Gy/year )

# Decarboxylation of Glu306, T=100 K

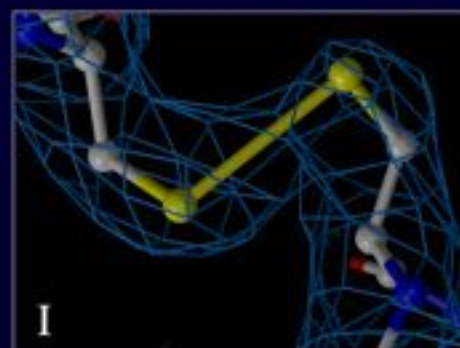
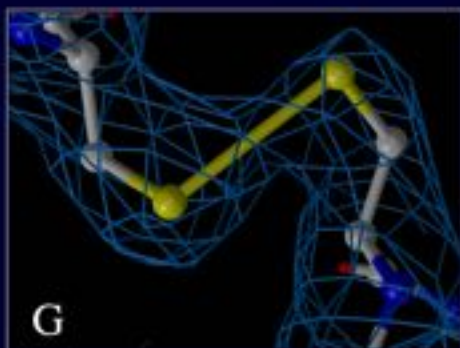
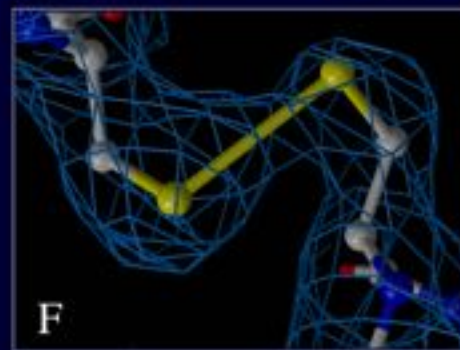
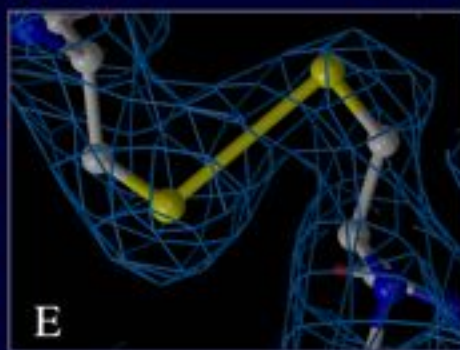
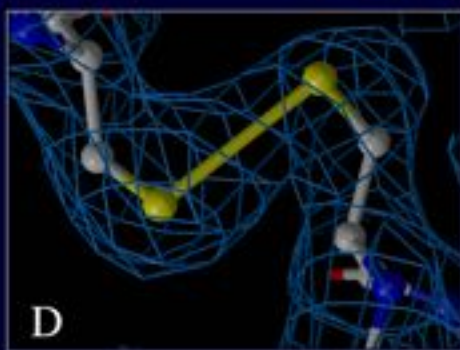
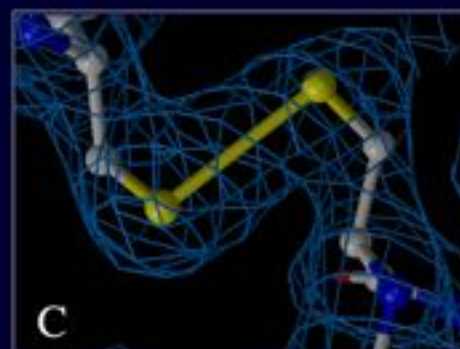
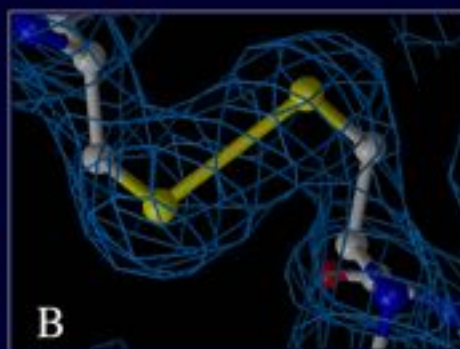
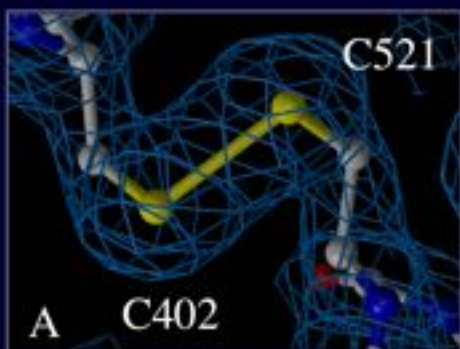


# Disulfide bond C254 - C265 breaks



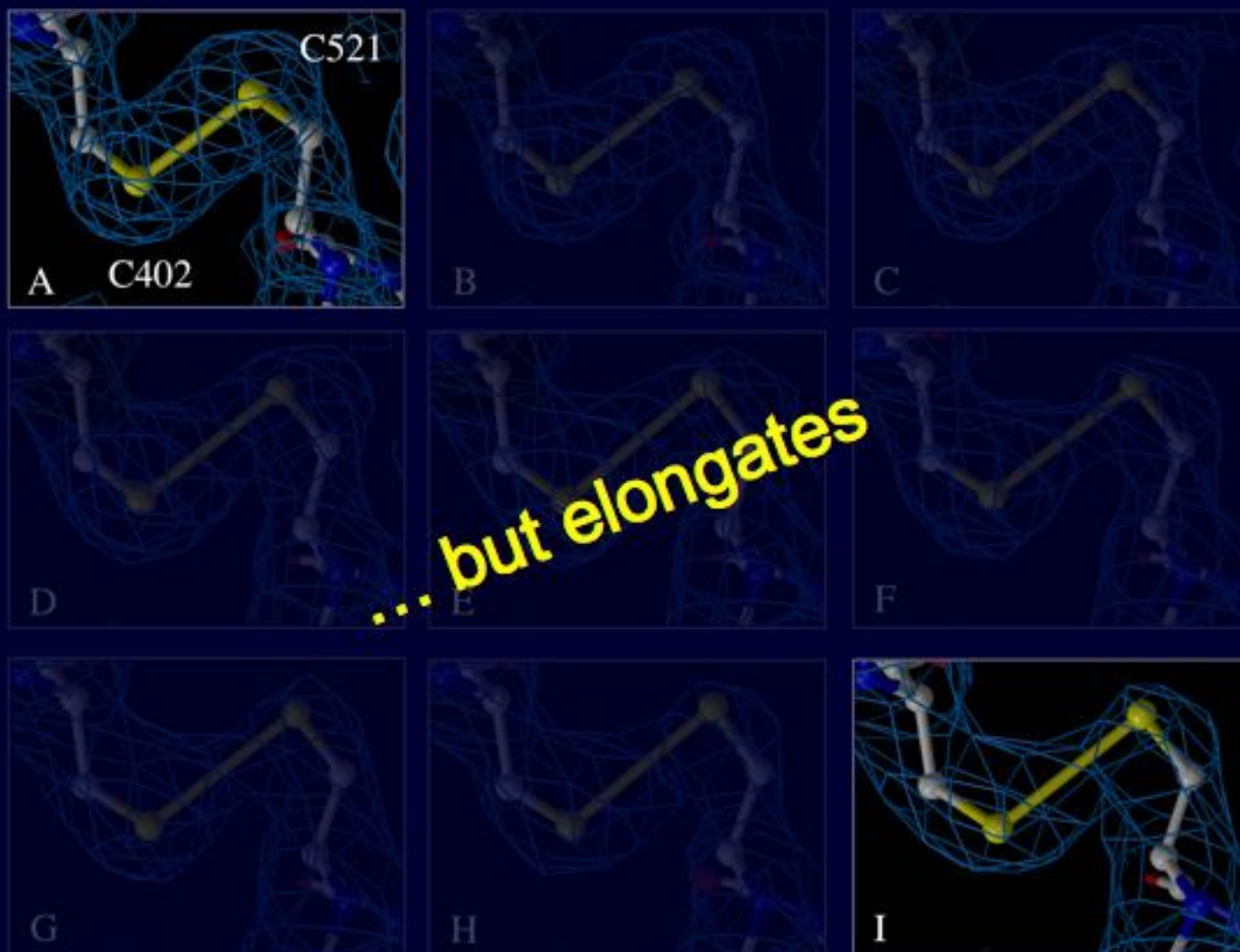
T=100 K

# Disulfide bond C402 - C521 does not break ...



T=100 K

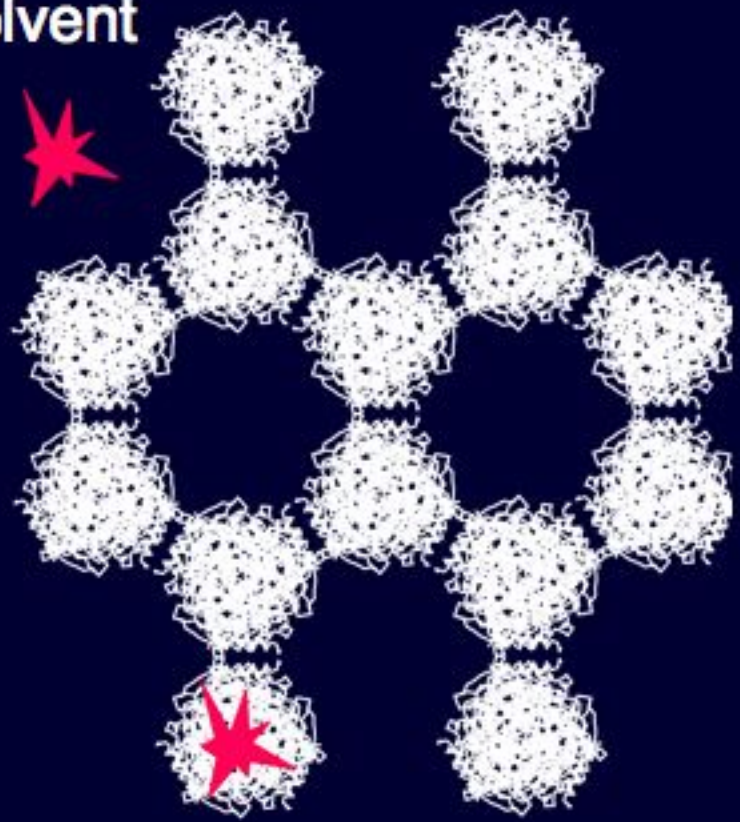
# Disulfide bond C402 - C521 does not break ...



T=100 K

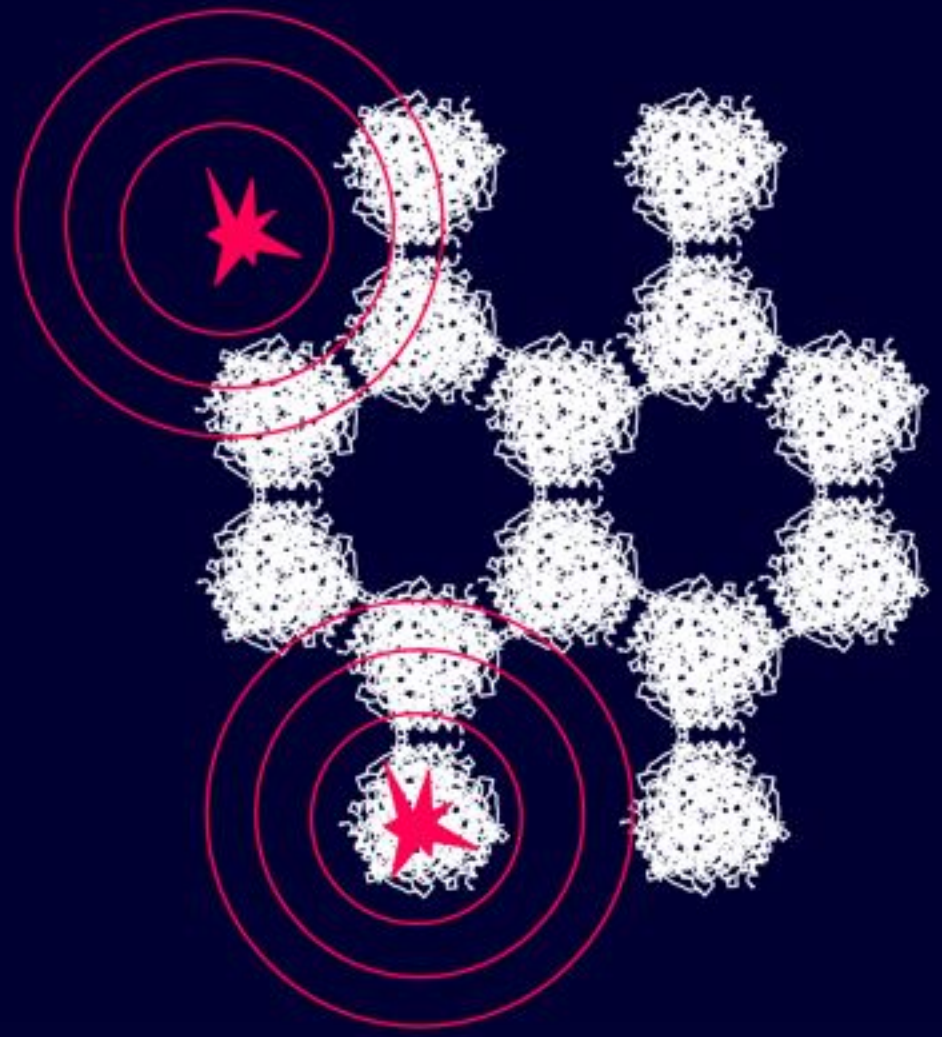
# Primary damage

solvent



protein

# Secondary damage



## AChE: 3 disulfide bonds



## Specific radiation damage

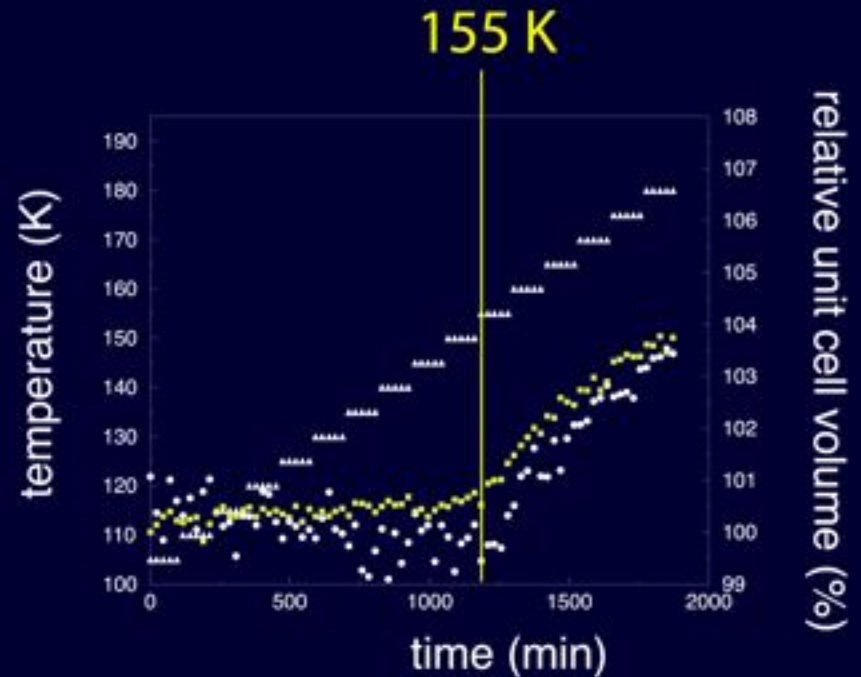
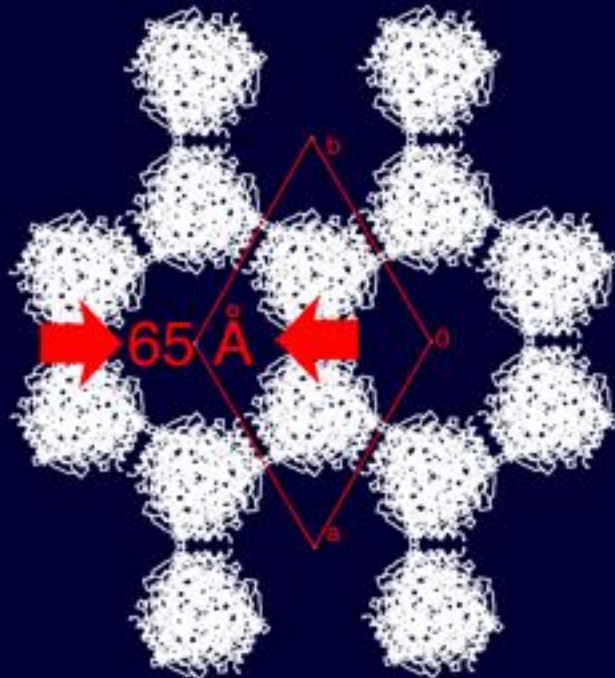
- Synchrotron radiation produces specific damage even at 100 K
  - disulfide bonds, Glu/Asp ...
- Differential sensitivity for chemically identical groups according to their location
- Disulfide radicals can be trapped and identified

## Implications

- Mechanism(s) of radiation damage to biological macromolecules
- Technical aspects of data collection using synchrotron radiation

**③ Radiation damage probes local protein flexibility**

# Solvent behaviour in flash-cooled AChE crystals

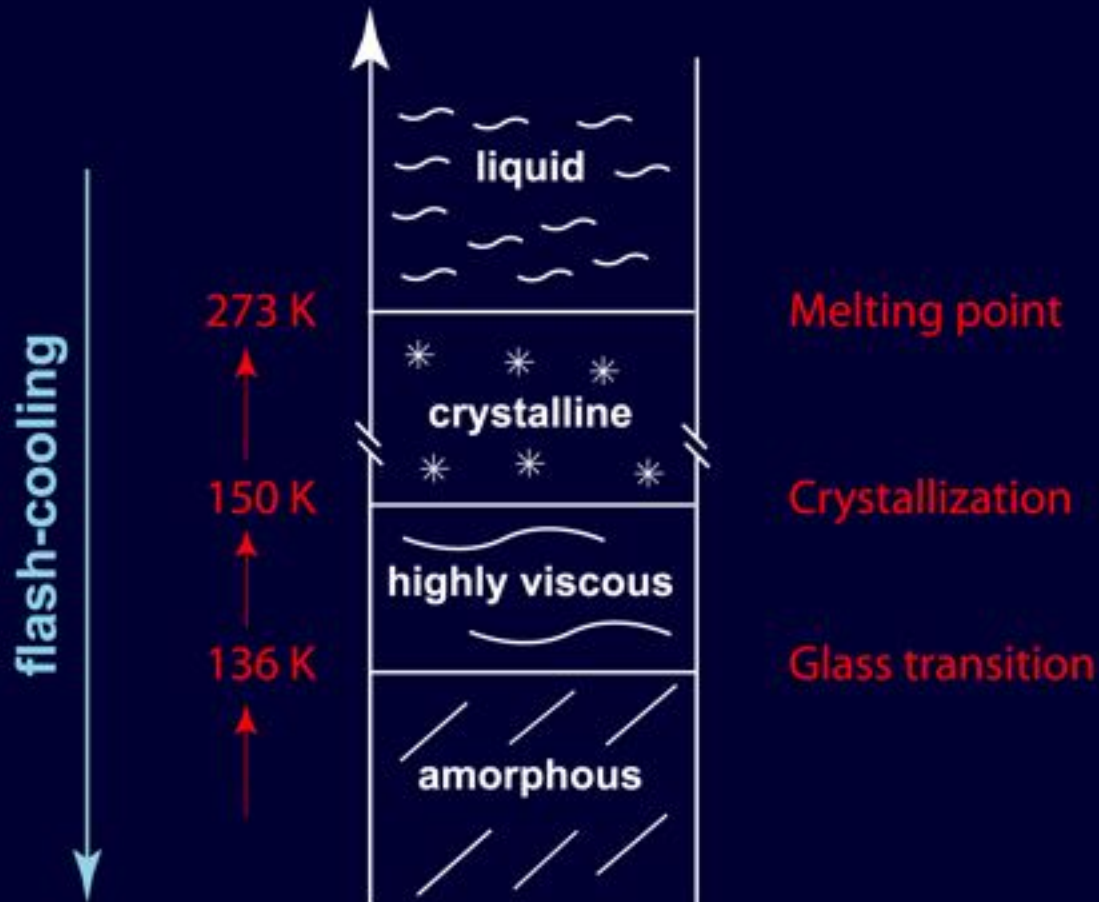


**Abrupt unit-cell volume increase at 155 K**

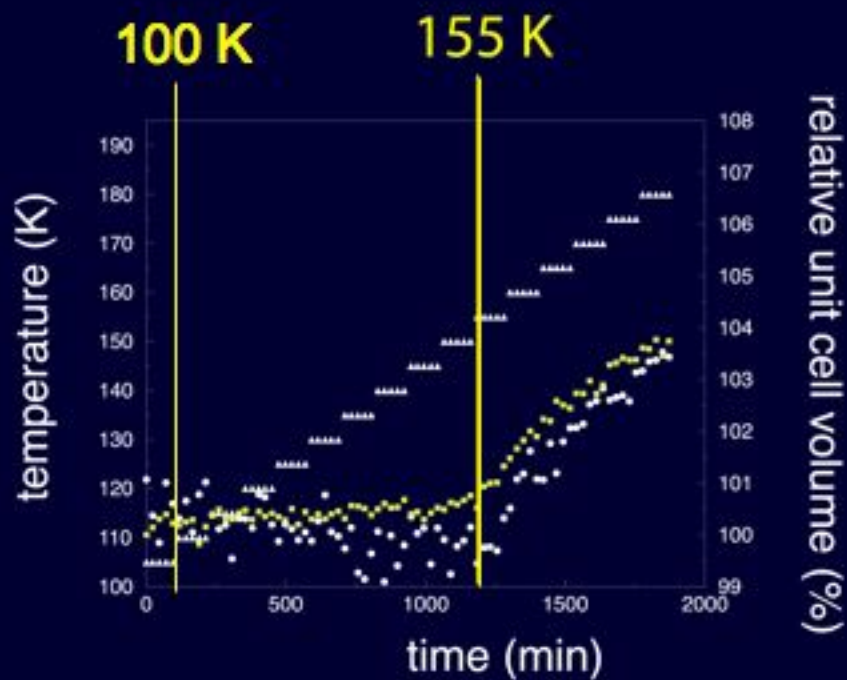
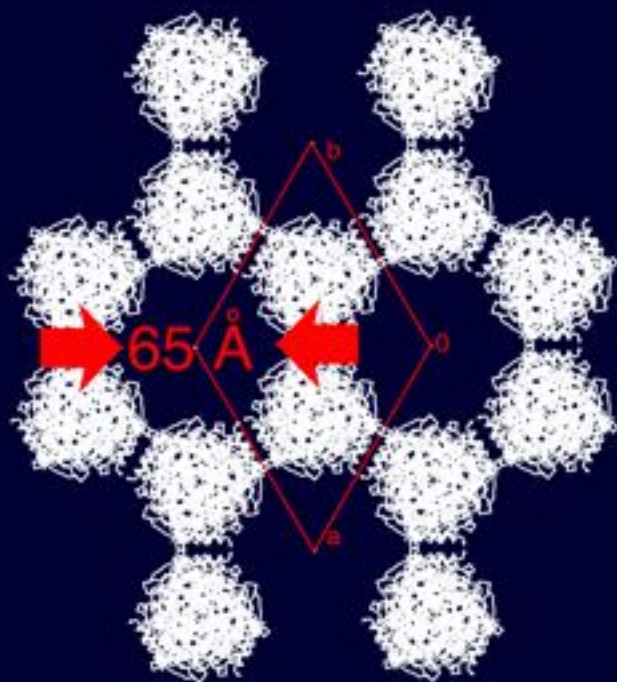
Solvent in large channels :

- ice formation
- glass transition

# Pure water at cryo-temperatures

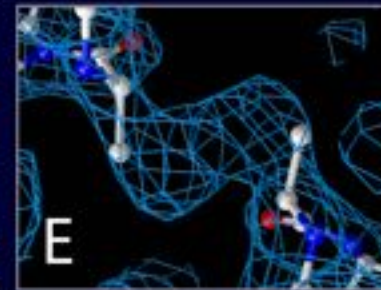
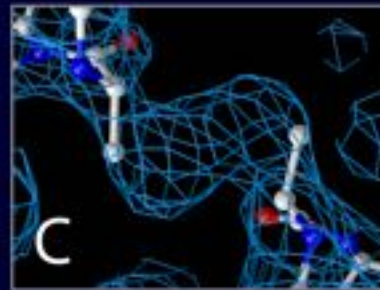
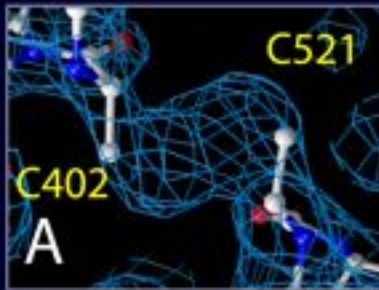


# Solvent behaviour in flash-cooled AChE crystals

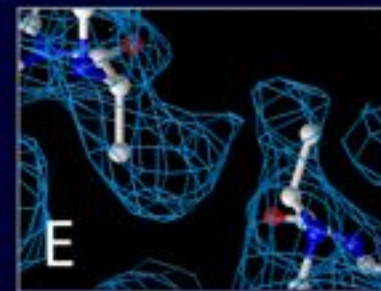
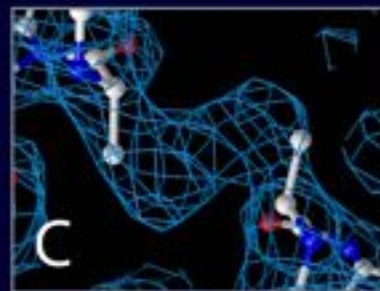
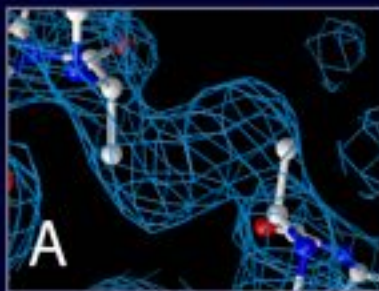


# AChE disulfide bond C402 - C521

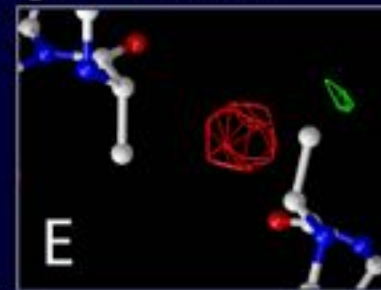
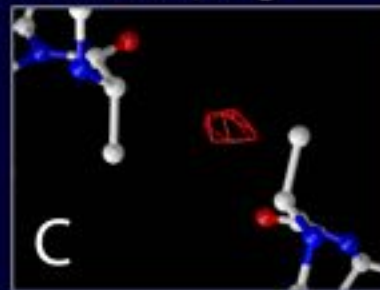
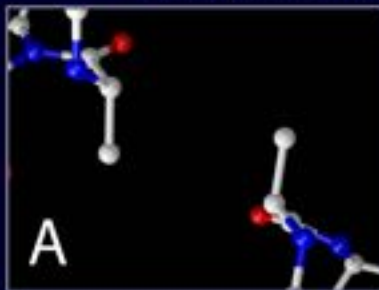
100 K



155 K

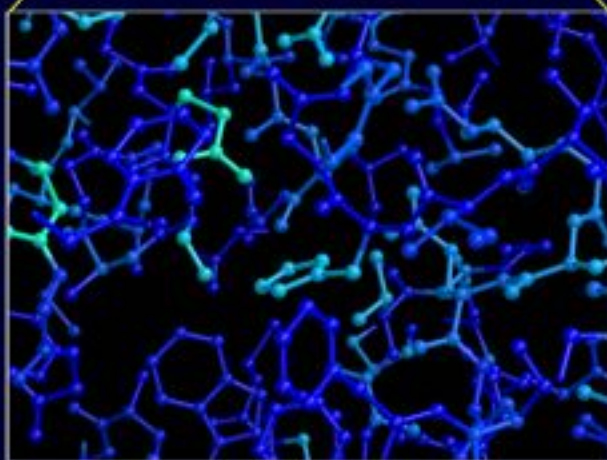
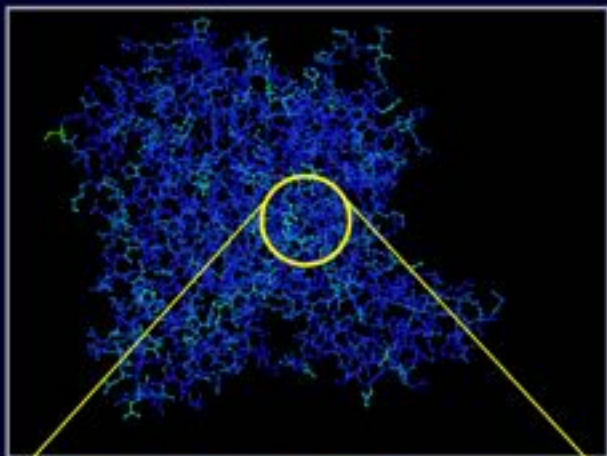


Fourier Difference Map  $F_o^{155K} - F_o^{100K}$  at  $5\sigma$

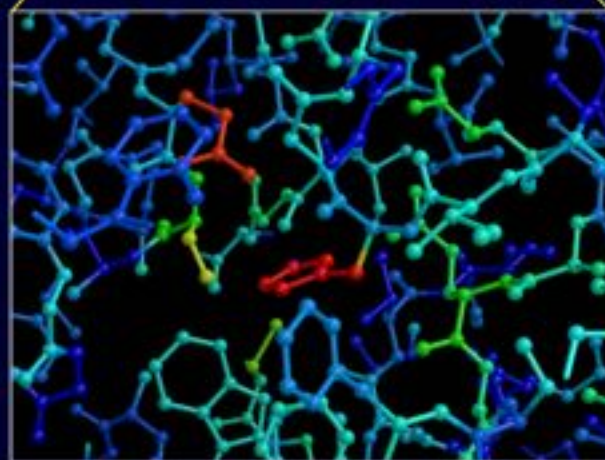
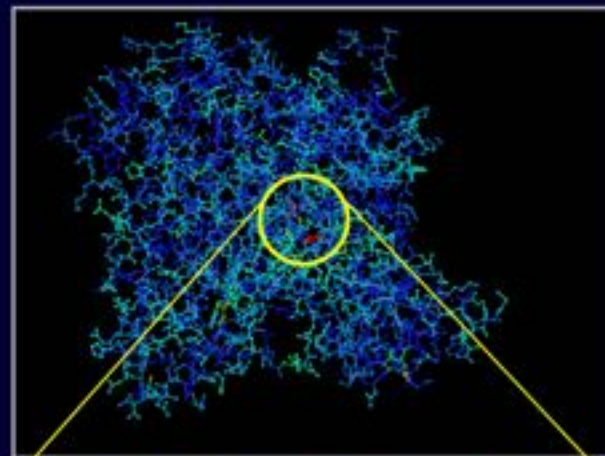


# AChE colored according to B-factor increase $(B_E - B_A)/B_A$

100 K



155 K

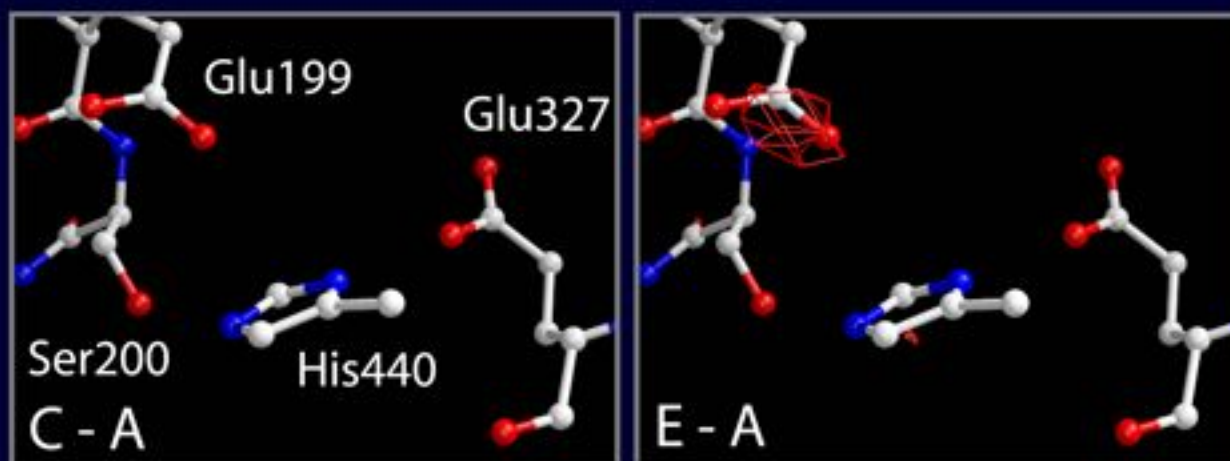


**At 155 K : Active site most affected**

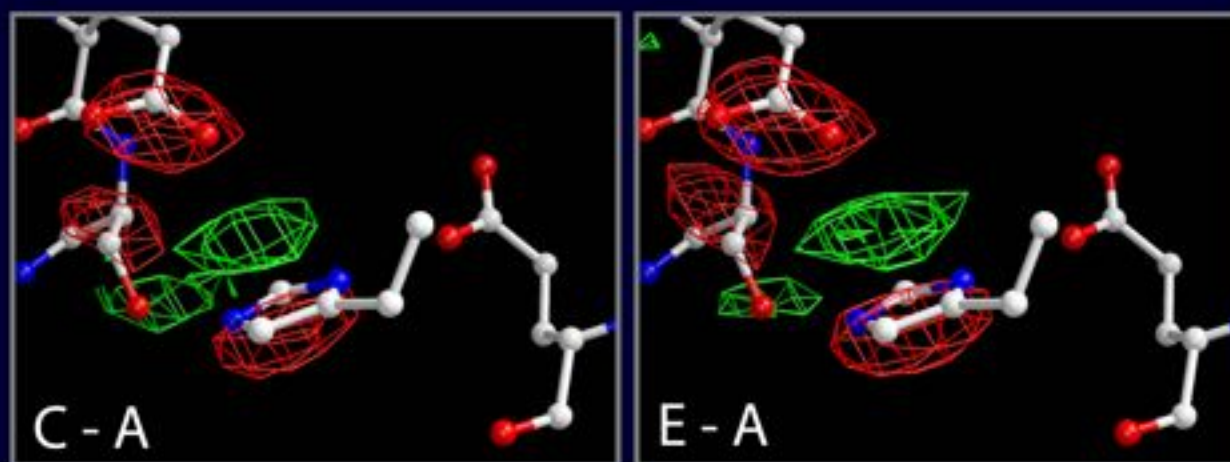
# AChE active site

Sequential Fourier Difference maps at  $5\sigma$

100 K

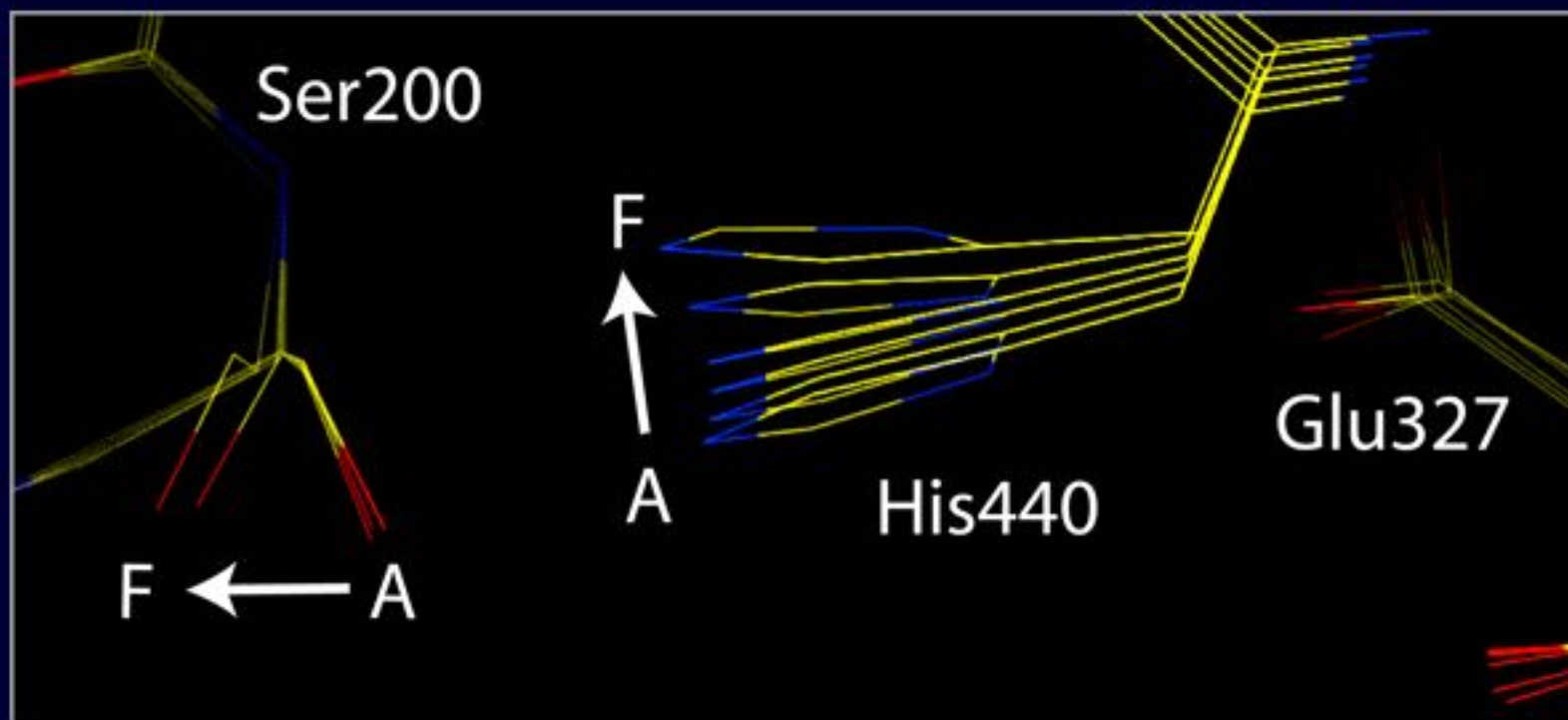


155 K



Conformational changes upon damage at 155 K

# Enzyme radiation-inactivation



155 K

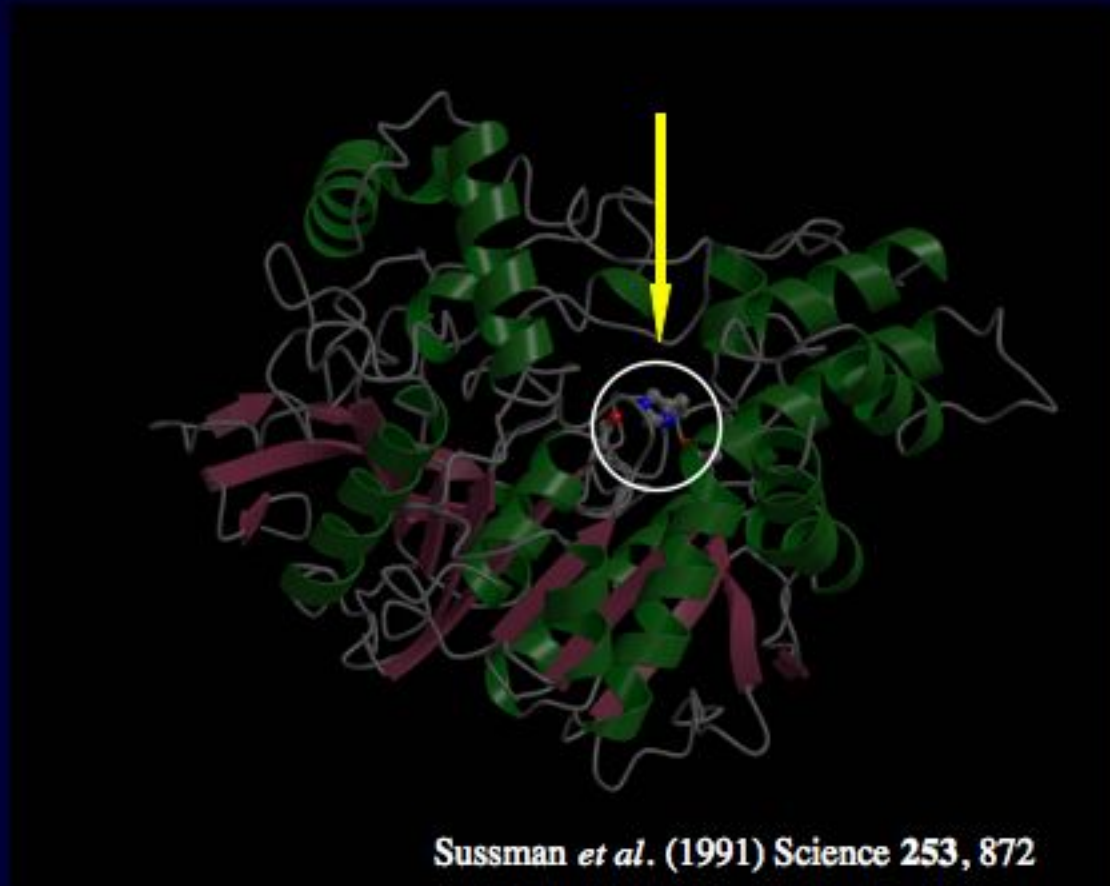
Local protein flexibility is increased above solvent glass transition  
yet still below protein dynamical transition

- X-ray radiation damage is temperature-dependent
- radiation-induced changes --> marker for protein dynamics
- crystal solvent undergoes glass transition
- local protein flexibility is increased above solvent glass transition yet still below global protein dynamical transition

**How to make use of this dynamical information?**

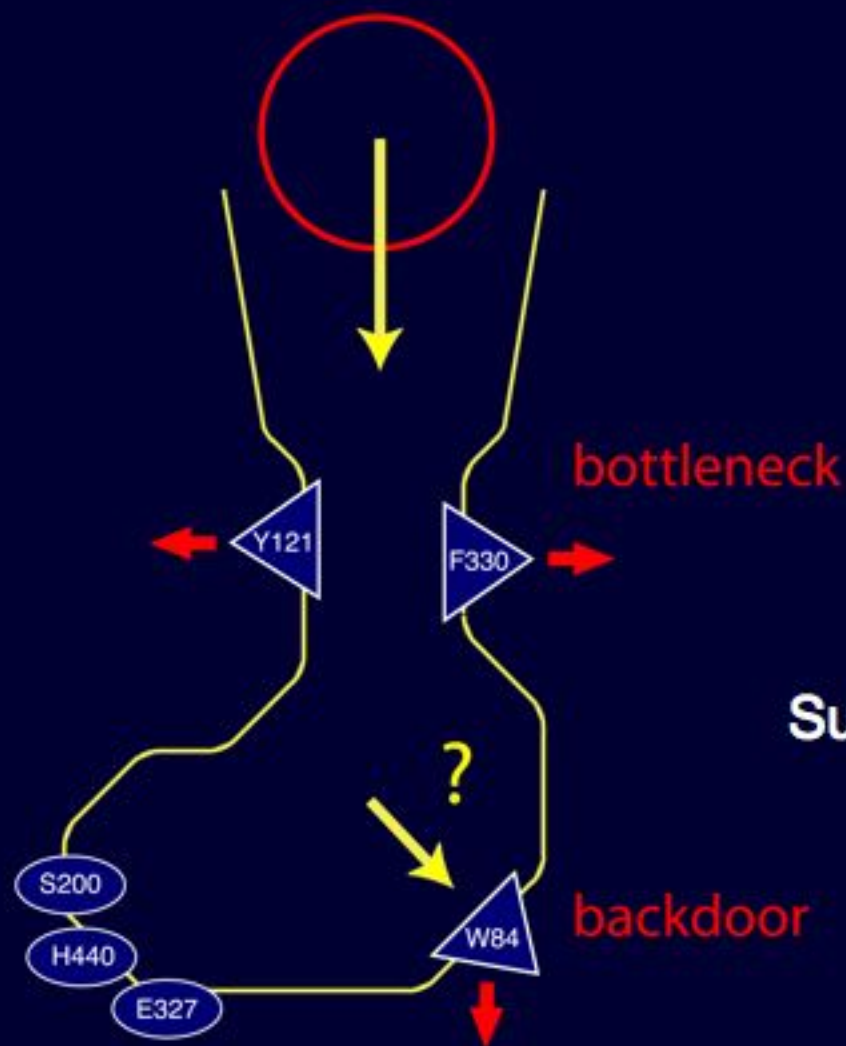
## ④ Strategy to trap enzymatic intermediate states

# Acetylcholinesterase: Structure and Function

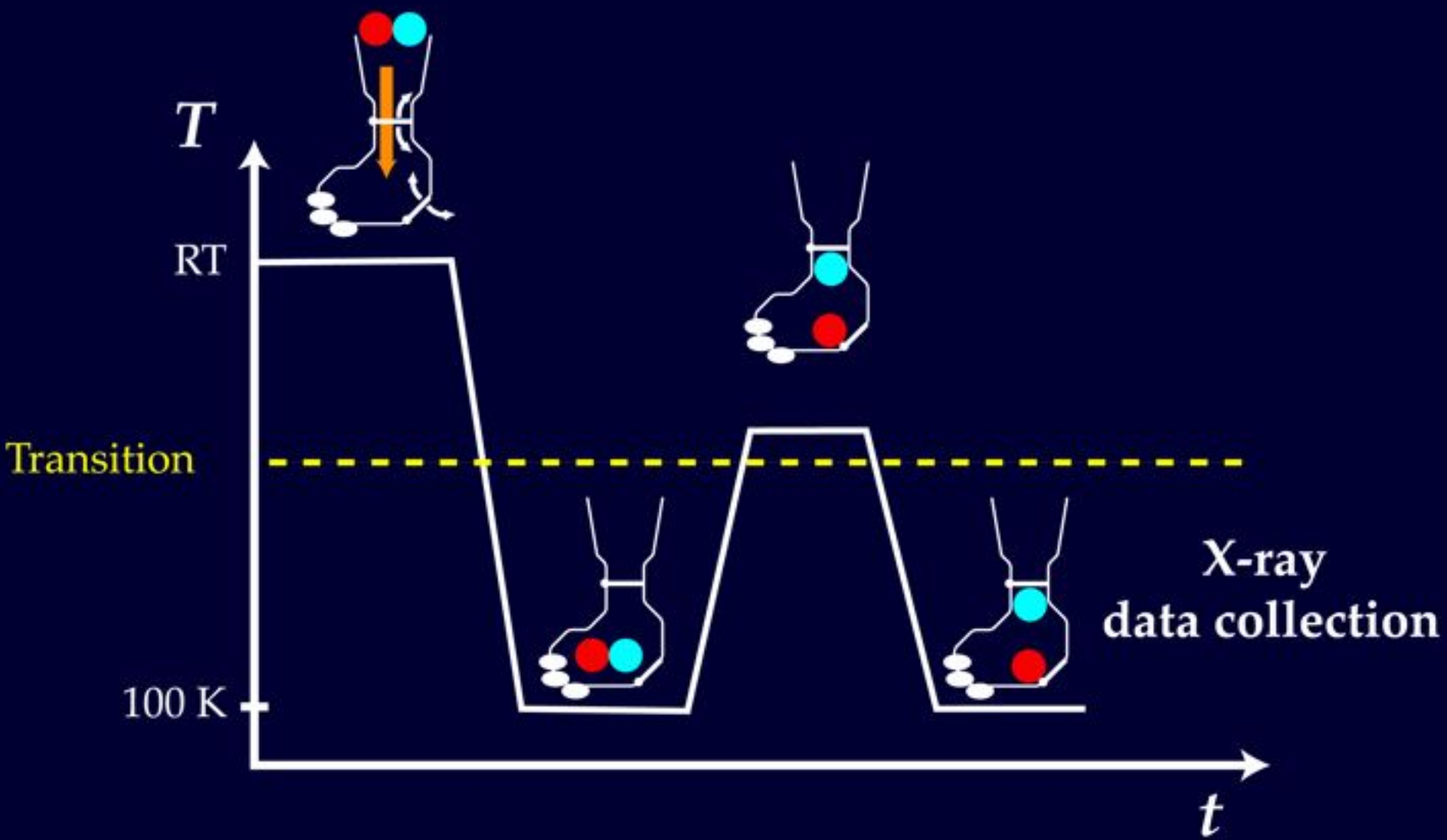


- hydrolyses neurotransmitter acetylcholine
- turnover 20000 s<sup>-1</sup>
- involved in Alzheimer disease
- target of insecticides and nerve gases

# Traffic of substrates and products in AChE

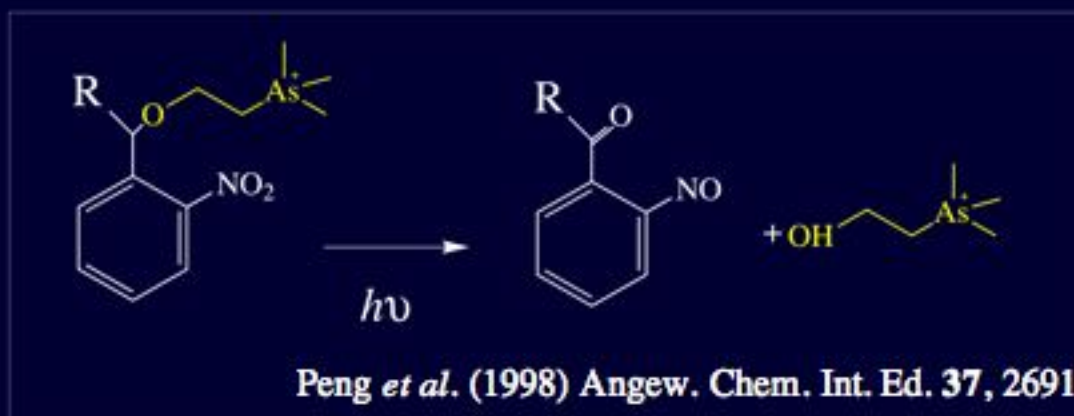


Substantial 'breathing' motions are required

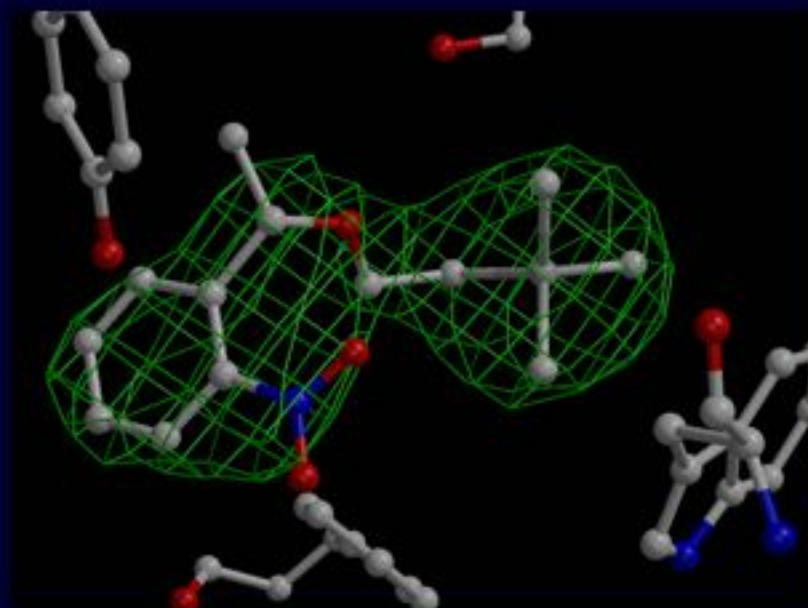


# Photolabile precursor of choline

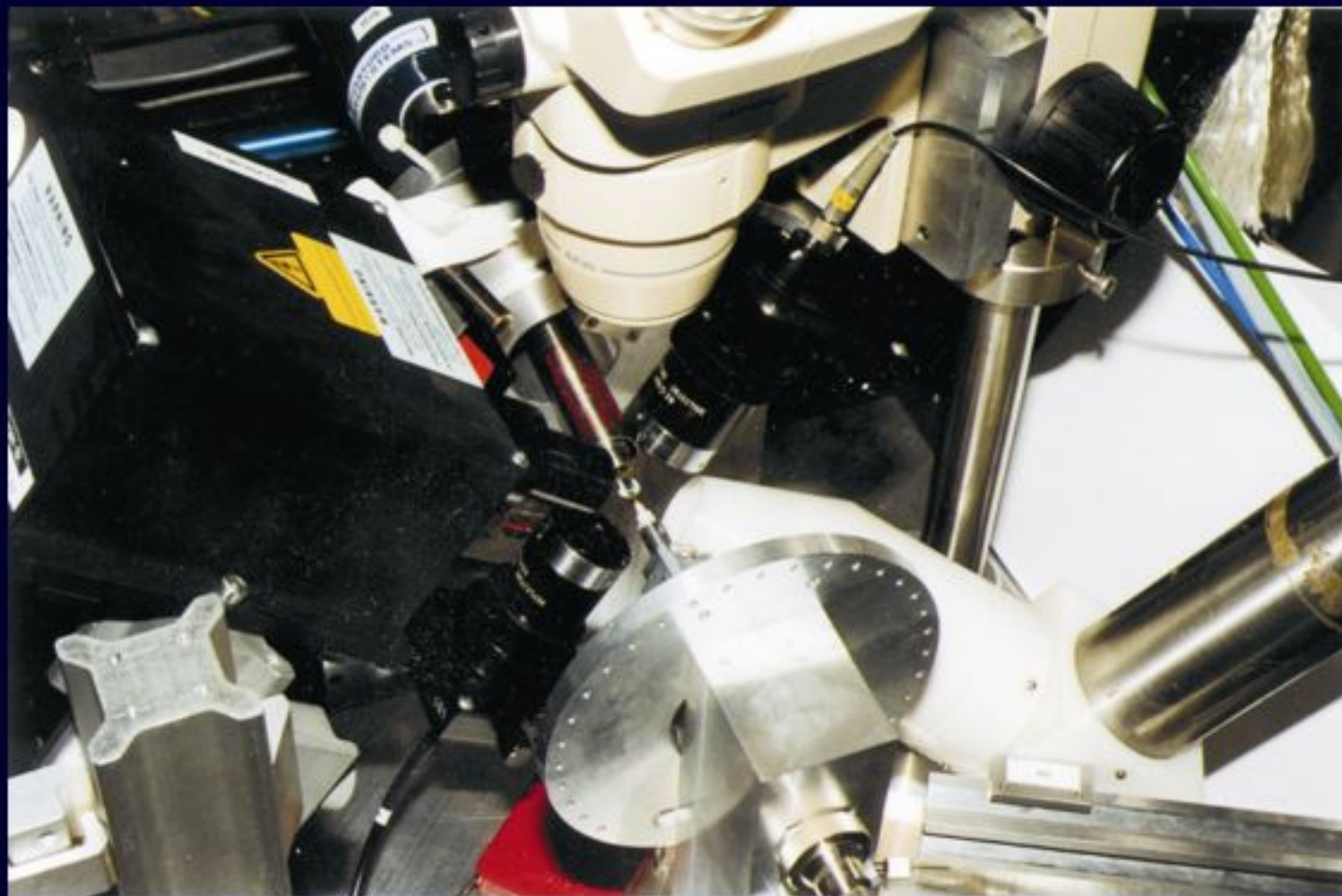
## Photofragmentation



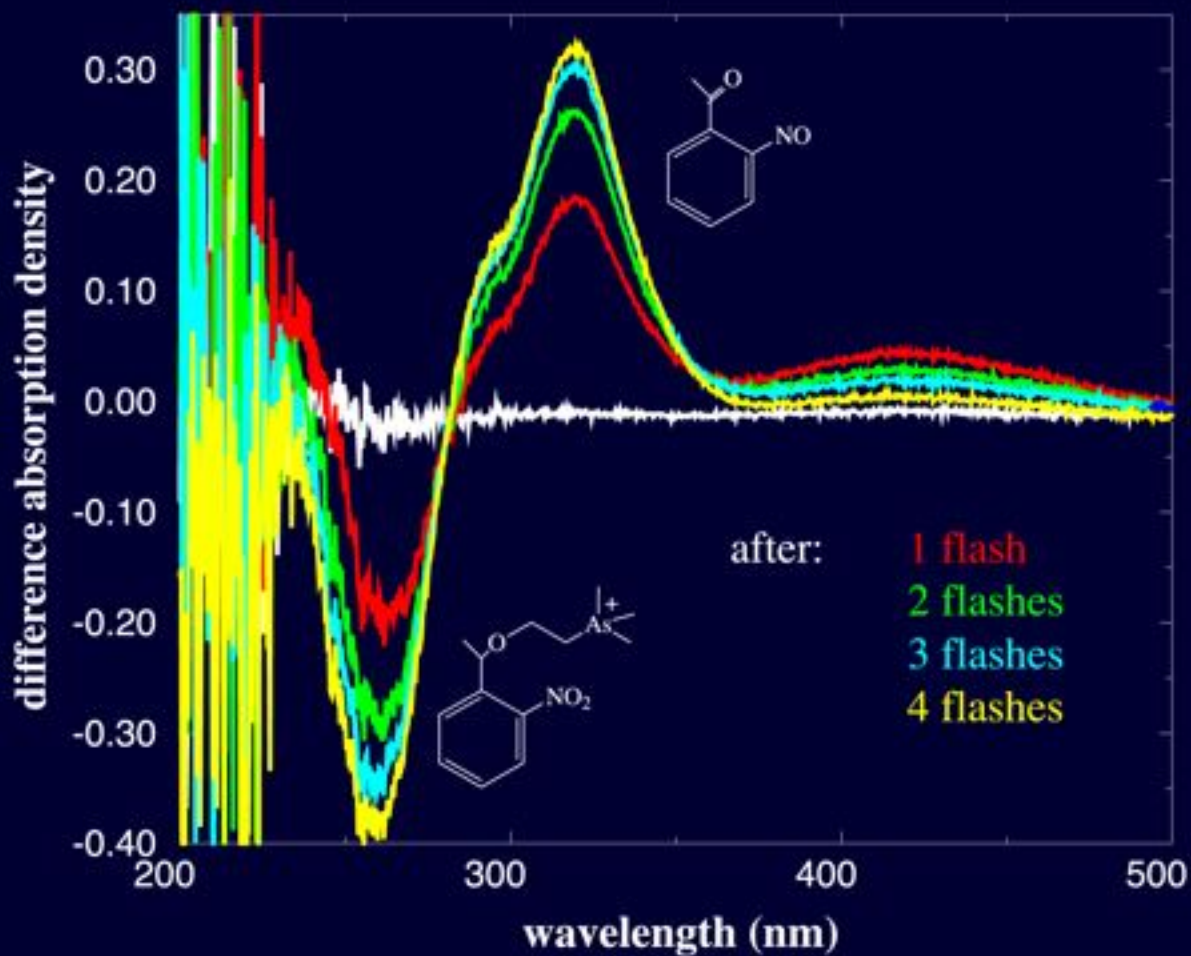
## Bound to AChE



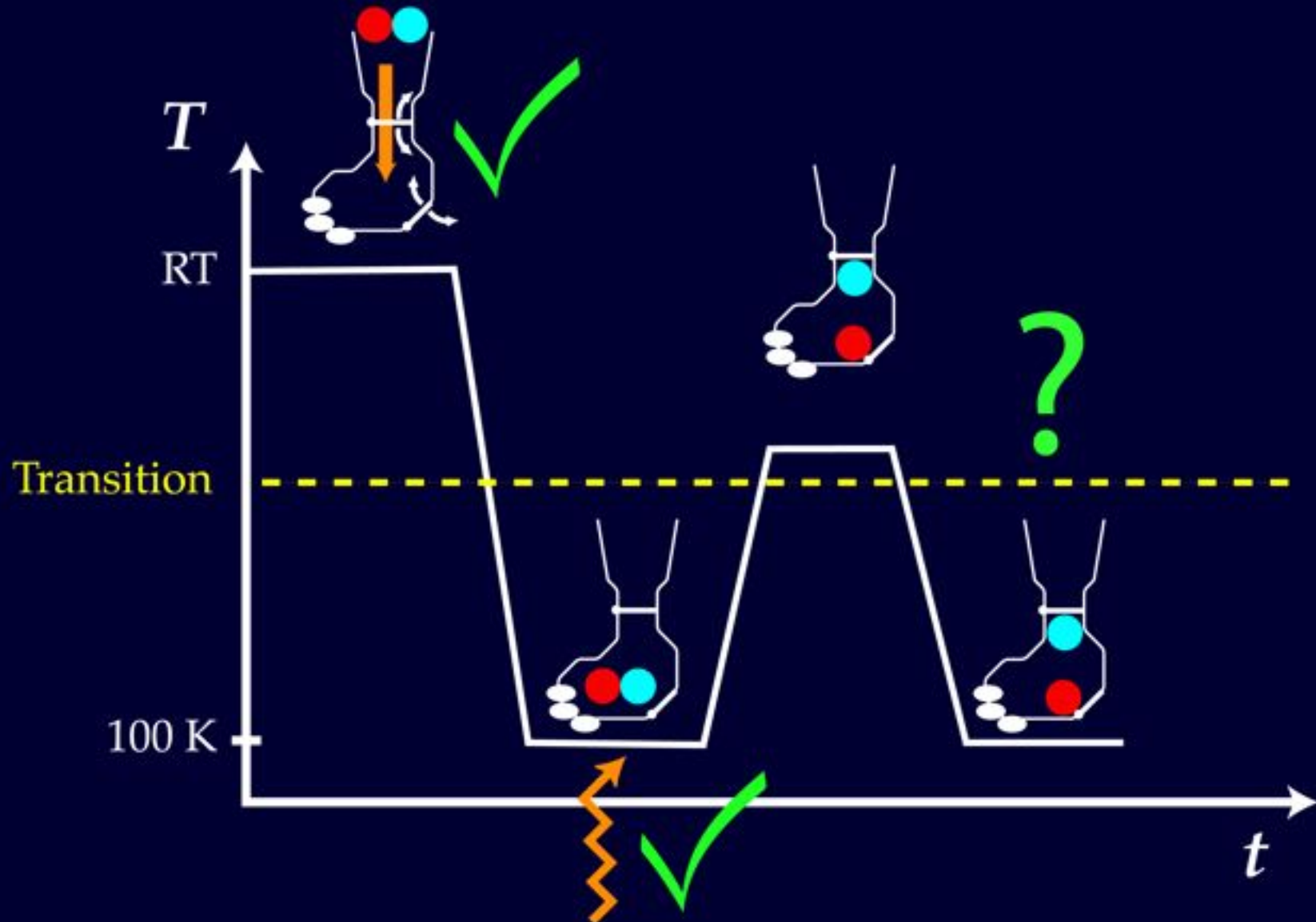
# Microspectrophotometer



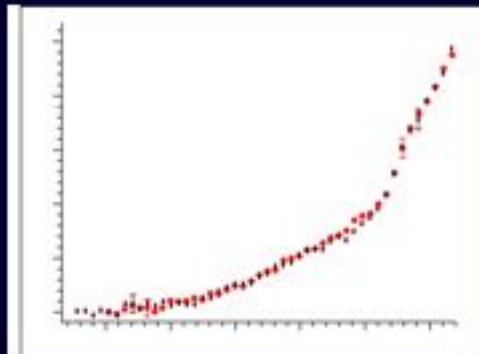
# Photofragmentation of caged arsenocholine at 100 K



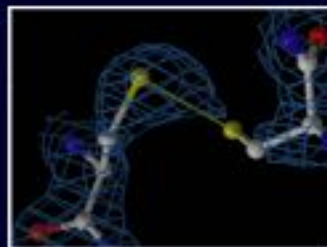
# Strategy to trap structural intermediate states in ChE



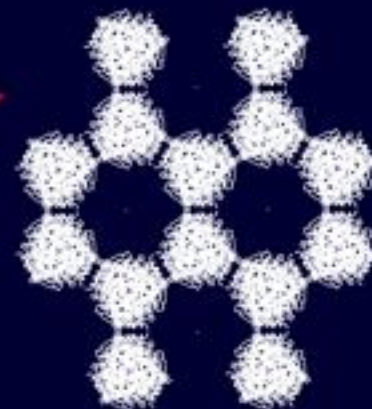
## Global dynamics



## Radiation damage



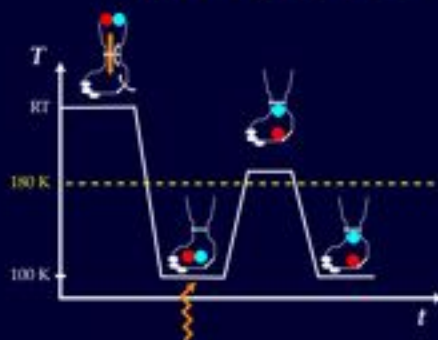
## Solvent behaviour



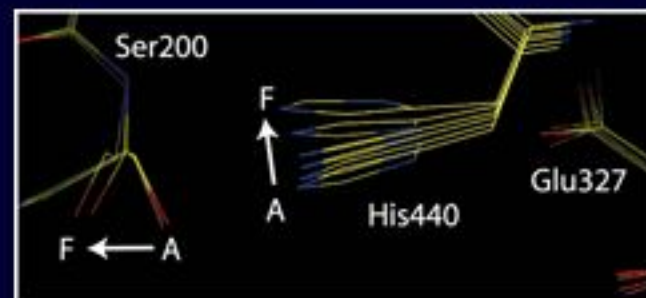
## Water behaviour



## Intermediates



## Local flexibility



IBS, LBM Grenoble

J. Colletier  
U. Lehnert  
G. Zaccai

IBS, LCCP Grenoble

A. Royant  
D. Bourgeois

Université de Strasbourg

A. Specht  
M. Goeldner

Universiteit Utrecht

EMBL, Grenoble

Weizmann Institute, Rehovot

G. Kryger  
M. Harel  
I. Silman  
J. Sussman

T. Schreurs  
B. Bouma  
M. Raves  
P. Gros  
J. Kroon

R. Ravelli

ESRF, Grenoble

S. McSweeney

LMB, Oxford

J. Murray  
E. Garman

MPI, Martinsried

D. Oesterhelt

University of Tromsø

H.-K. Schroder Leiros

Université Paris - Sud

C. Houée-Levin

Université P. et M. Curie, Paris

J. Bergès

