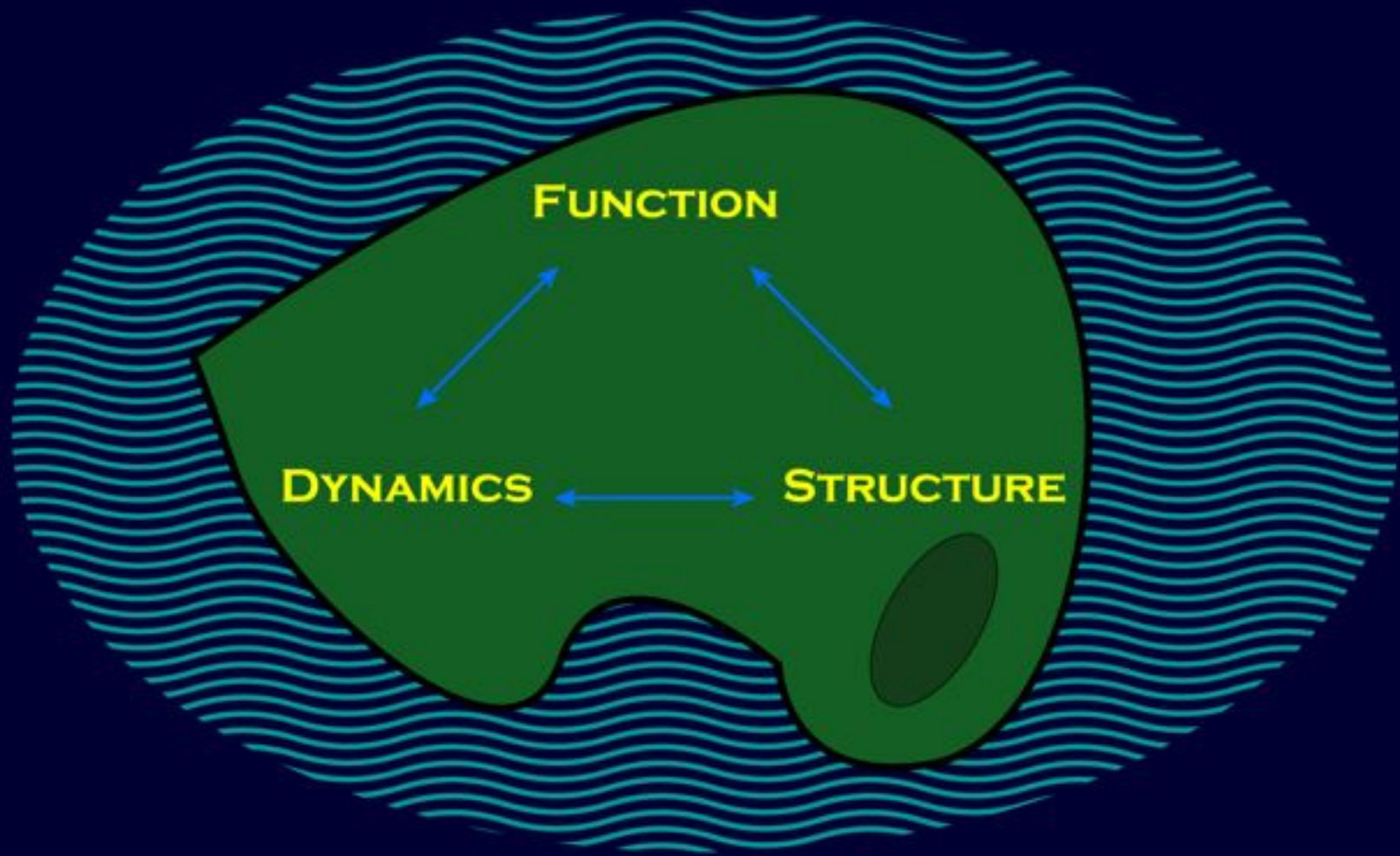


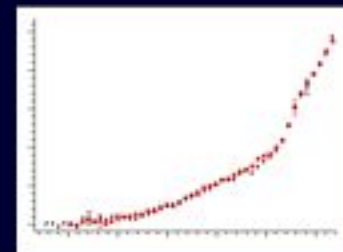
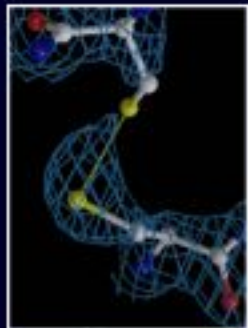
**Protein and solvent dynamics as studied by
neutron scattering and
temperature-controlled X-ray crystallography**

Martin WEIK
Institut de Biologie Structurale
Grenoble, France



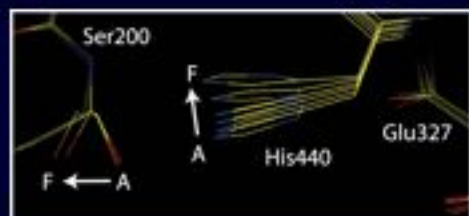
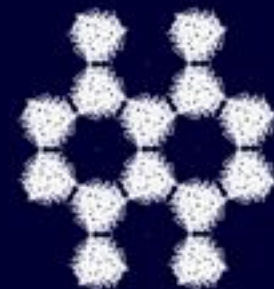


Neutron scattering probes global protein dynamics



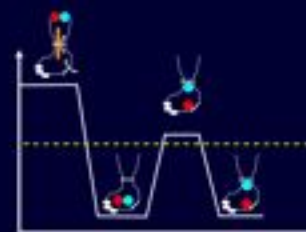
Specific X-ray radiation damage to proteins

Solvent behaviour in flash-cooled protein crystals



Radiation damage probes local protein flexibility

Strategy to trap enzymatic intermediate states

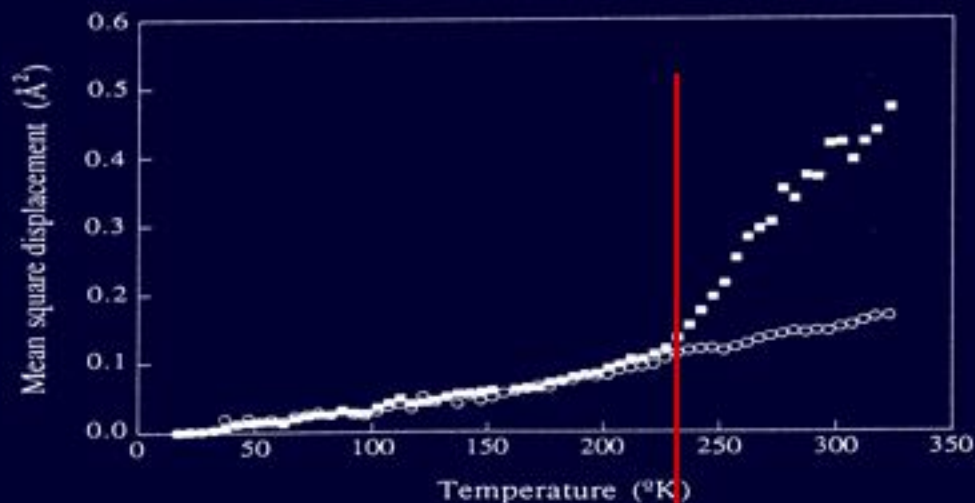


① Neutron scattering probes global protein dynamics



Dynamical transitions in proteins

Neutron scattering - ns - ps motions - purple membranes



Mössbauer spectroscopy

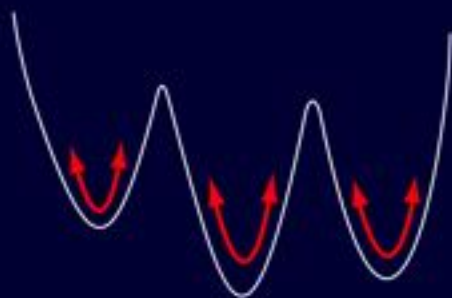
Parak *et al.* (1982) *JMB* 161, 177

Neutron scattering

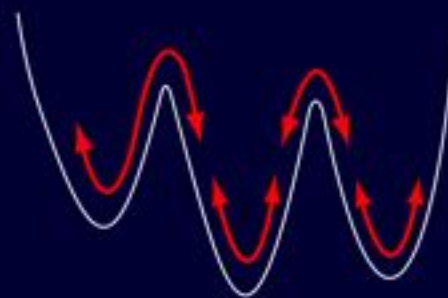
Doster *et al.* (1989) *Nature* 337, 754

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

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



- harmonic motions
- substates frozen out

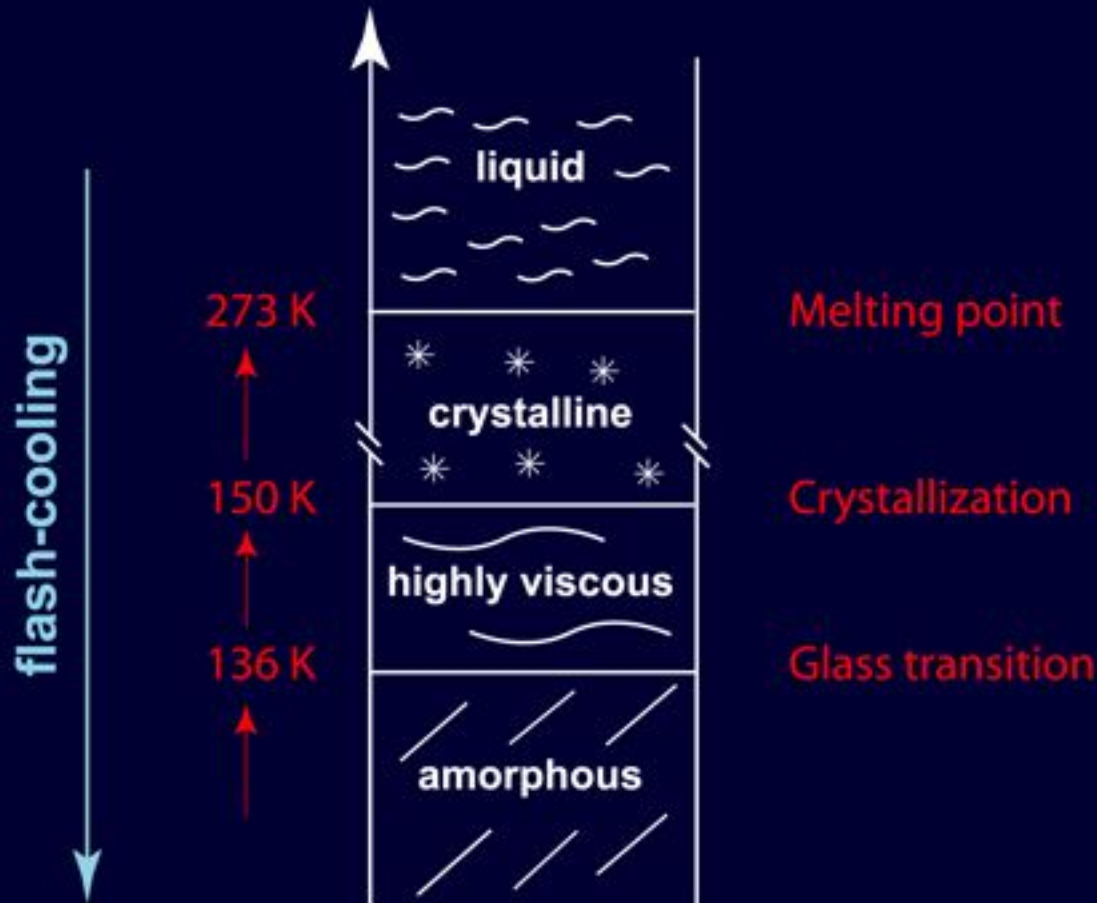


- non-harmonic motions
- transitions between substates

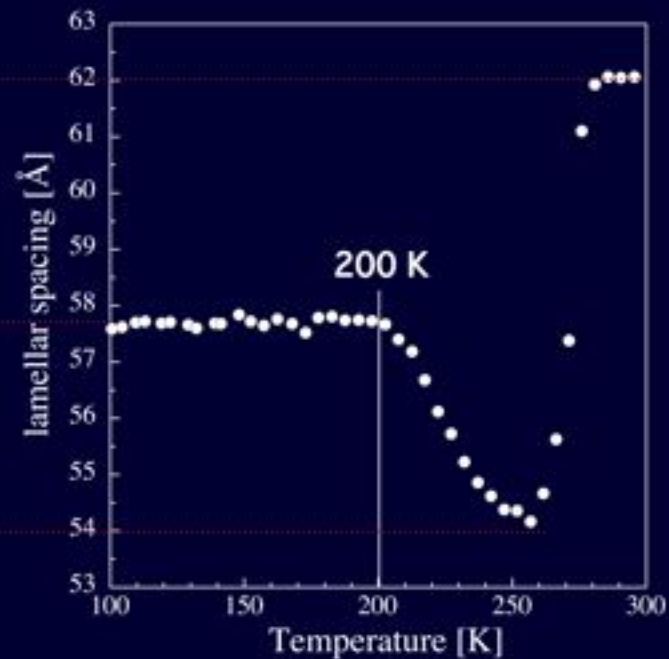
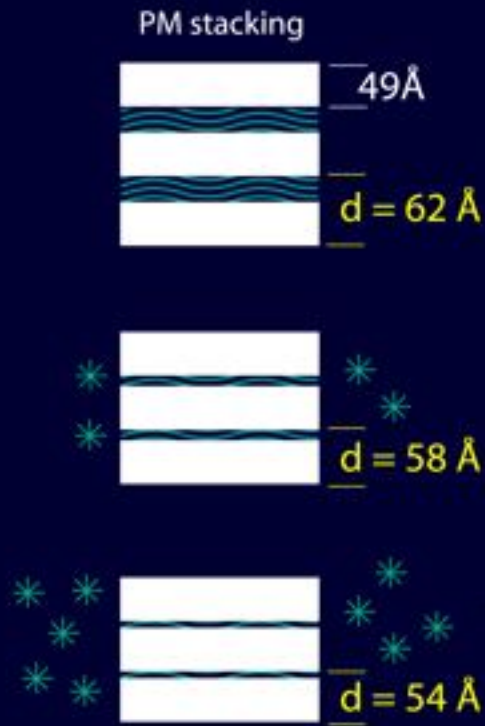
Purple membrane stacks



Pure water at cryo-temperatures



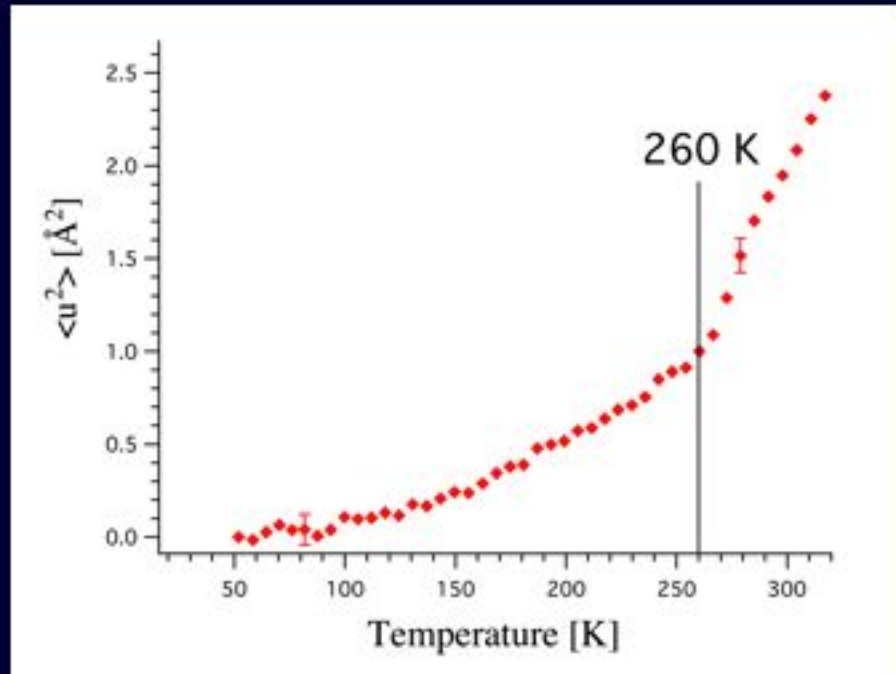
Ultra-viscous water in stacks of PM at 200 K



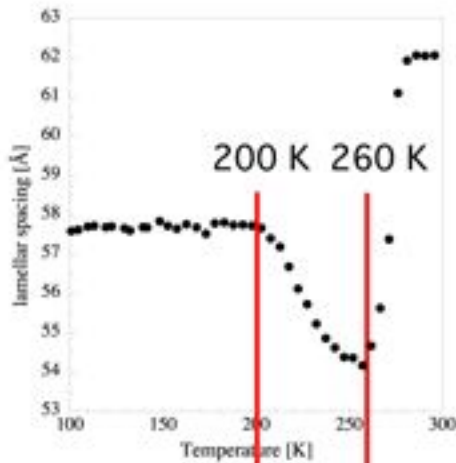
Long-range translational diffusion above GT, concomitant with crystallization

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

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

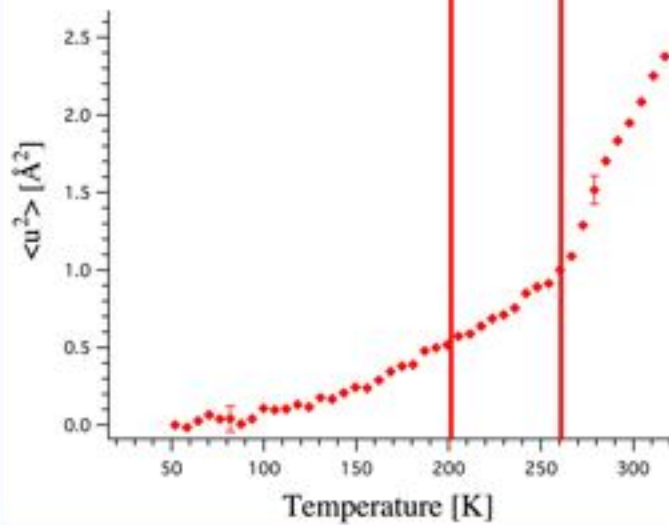


- dynamical transition at 260 K
- no transition at 200 K



Inter-membrane water:

Glass transition at ≤ 200 K



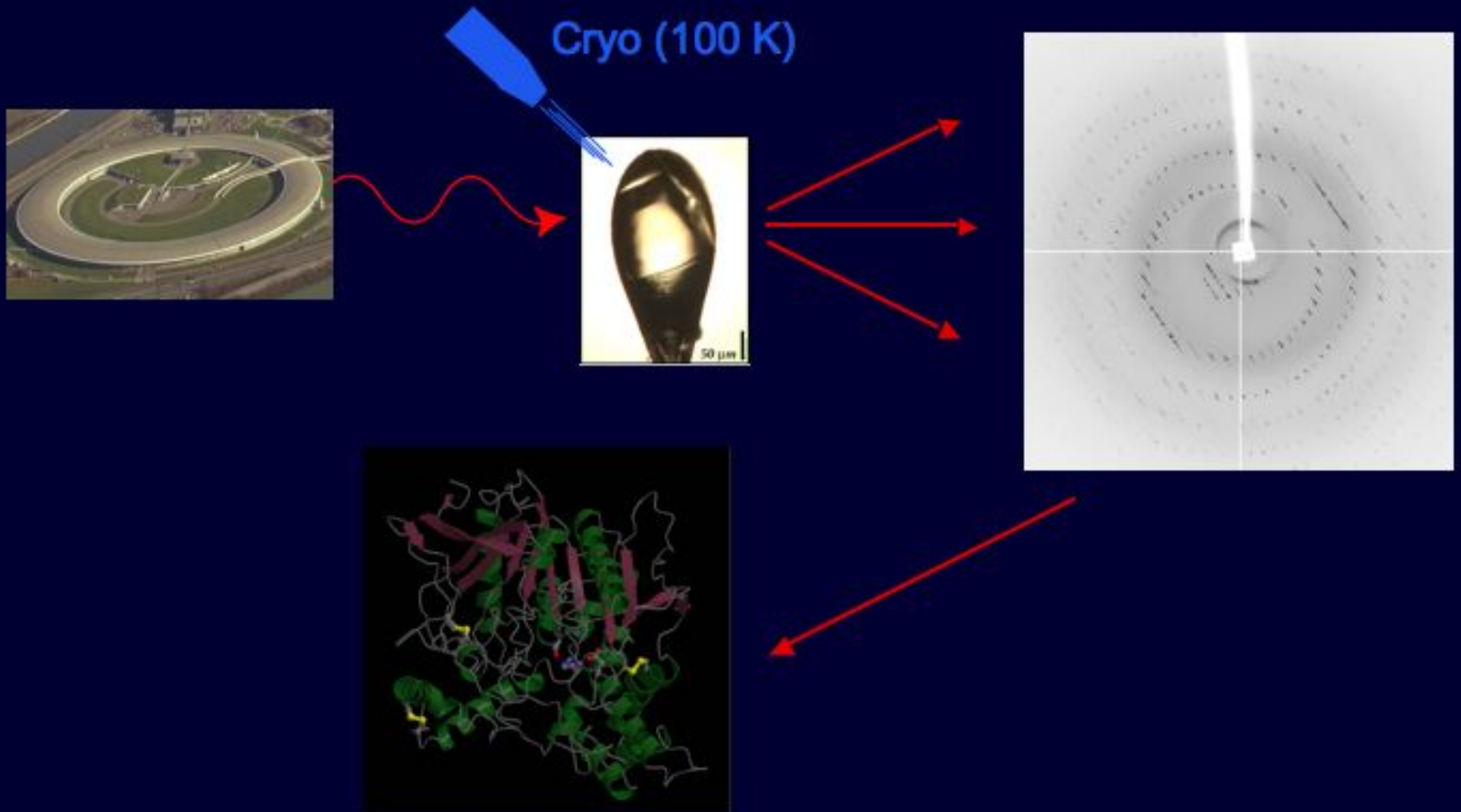
ns-ps motions in PM:

Dynamical transition at and 260 K

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

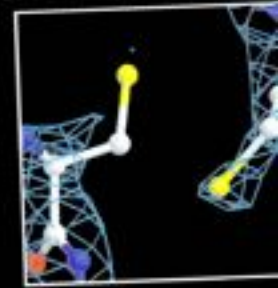
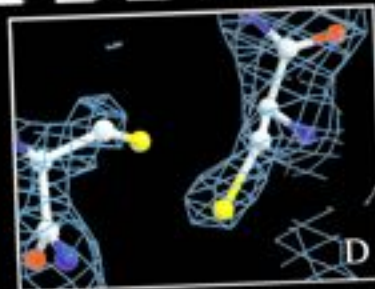
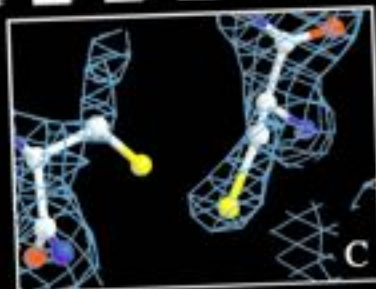
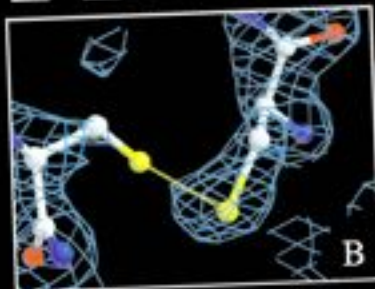
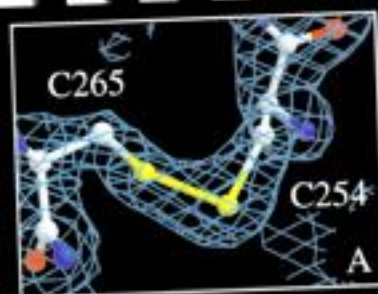
- Behaviour of water confined by stacks of biological membranes at cryo-temperatures?
 - long-range translational diffusion at 200 K
 - glass transition at ≤ 200 K
- Does glass transition of inter-membrane water trigger dynamical transition in the membrane?
 - No - but what about local protein flexibility?

Protein X-ray crystallography



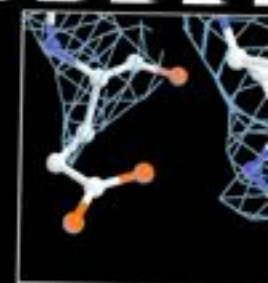
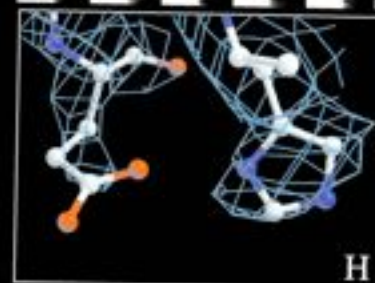
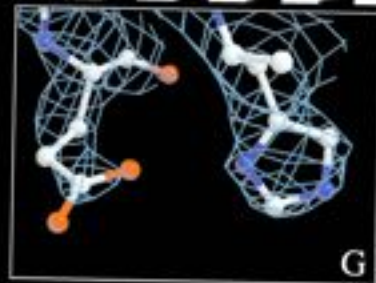
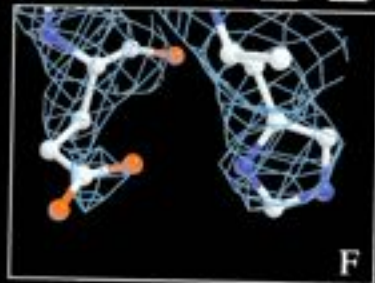
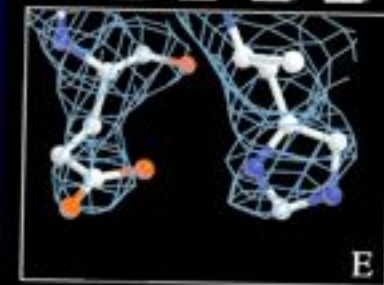
Synchrotron radiation is a powerful tool, but price must be paid

Disulfide breakage



② Specific X-ray radiation damage to proteins

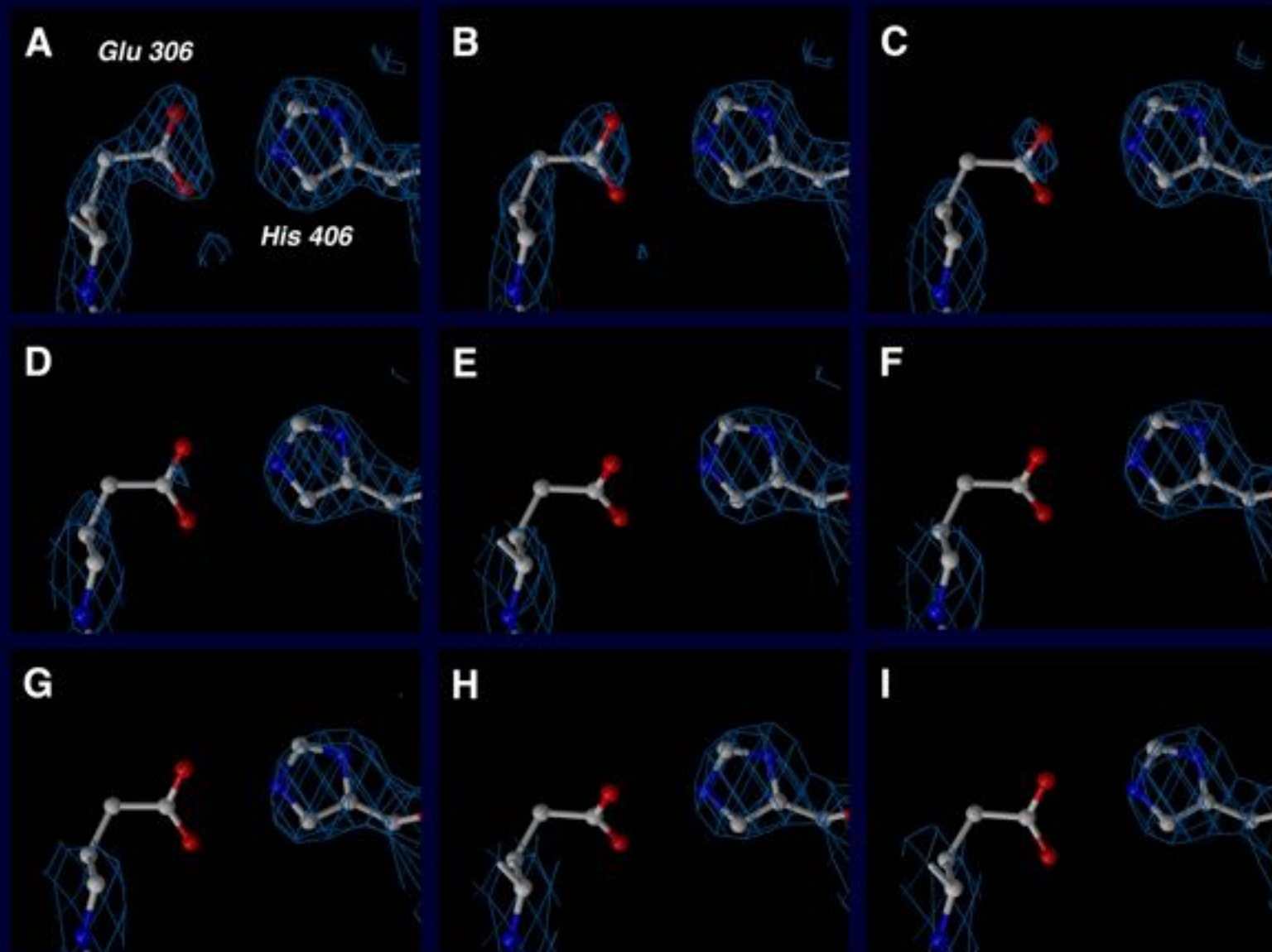
Decarboxylation



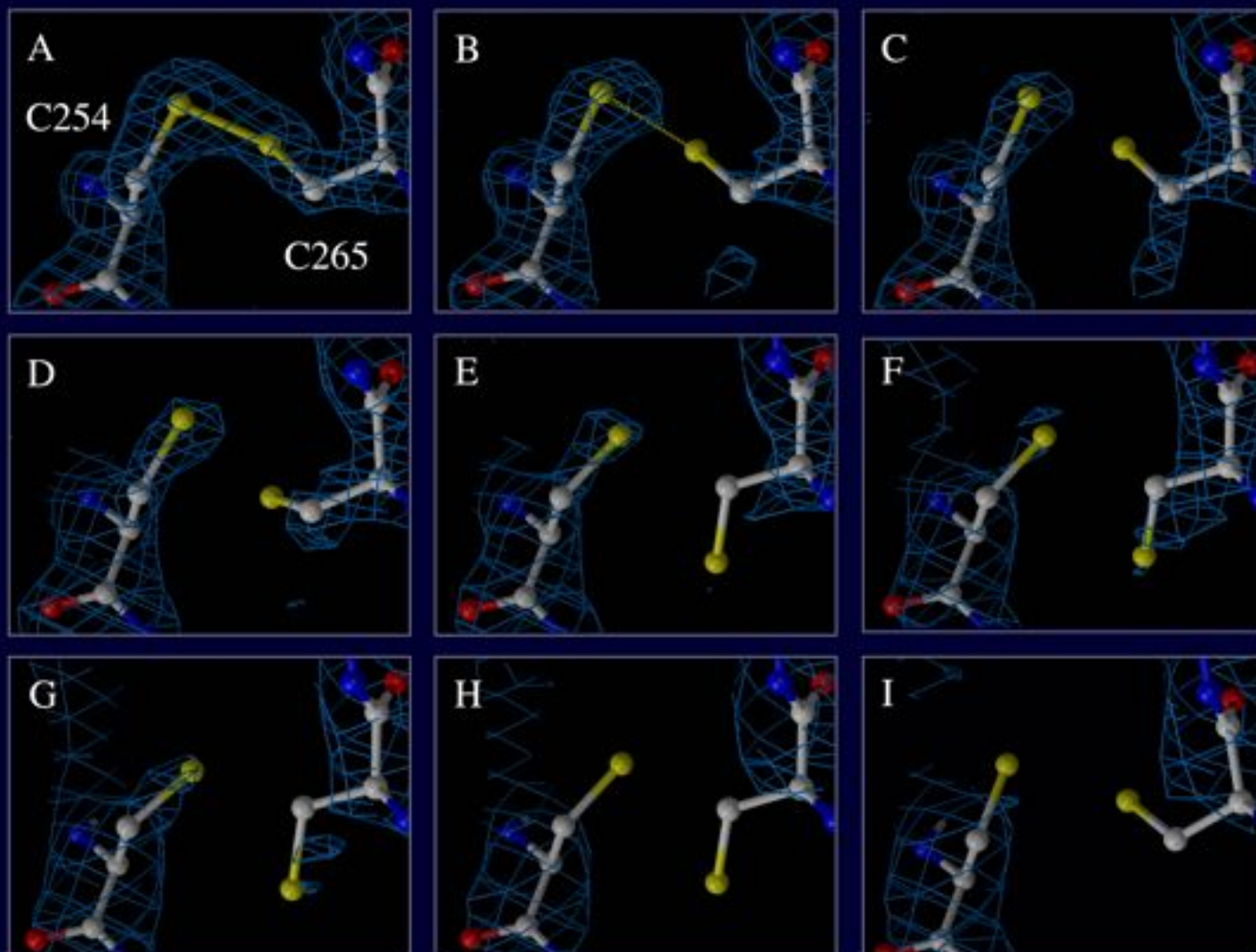
Data collection series

- 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

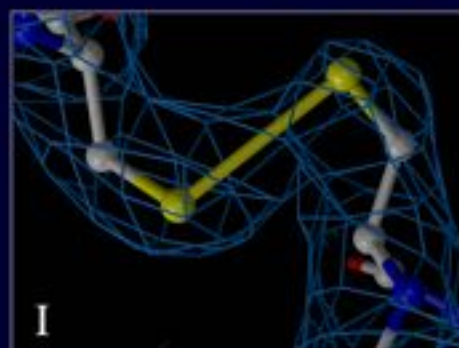
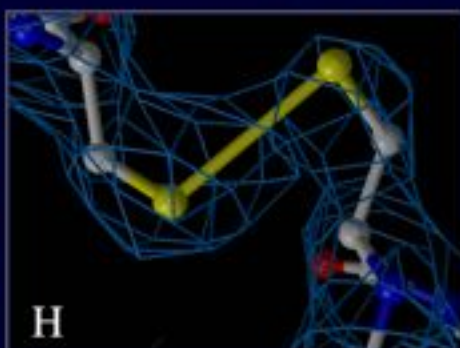
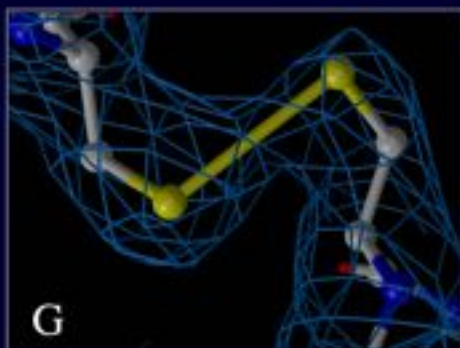
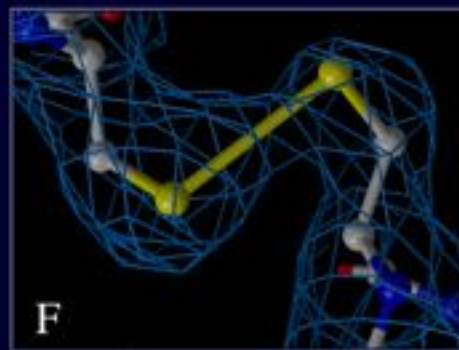
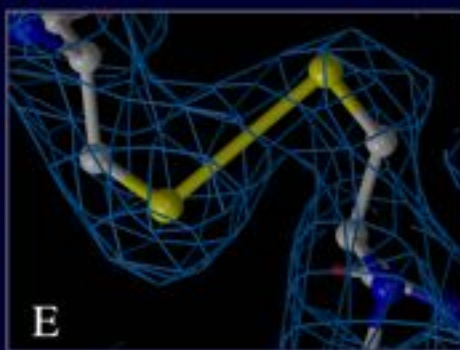
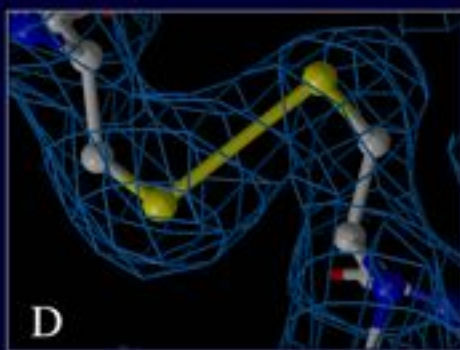
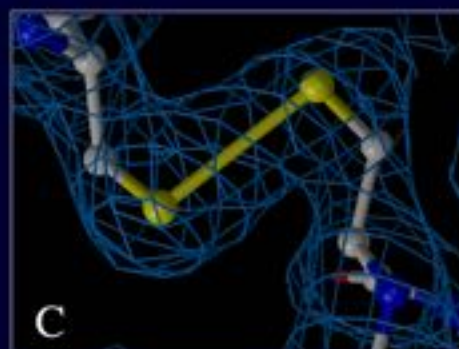
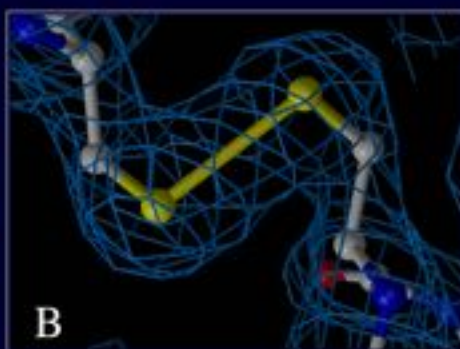
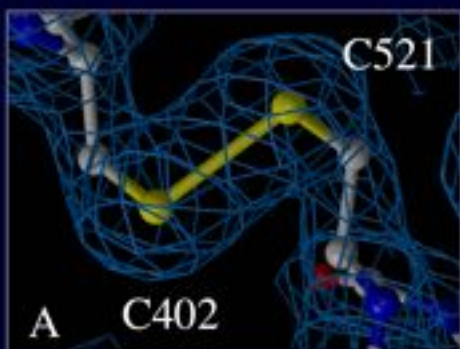


Disulfide bond C254 - C265 breaks



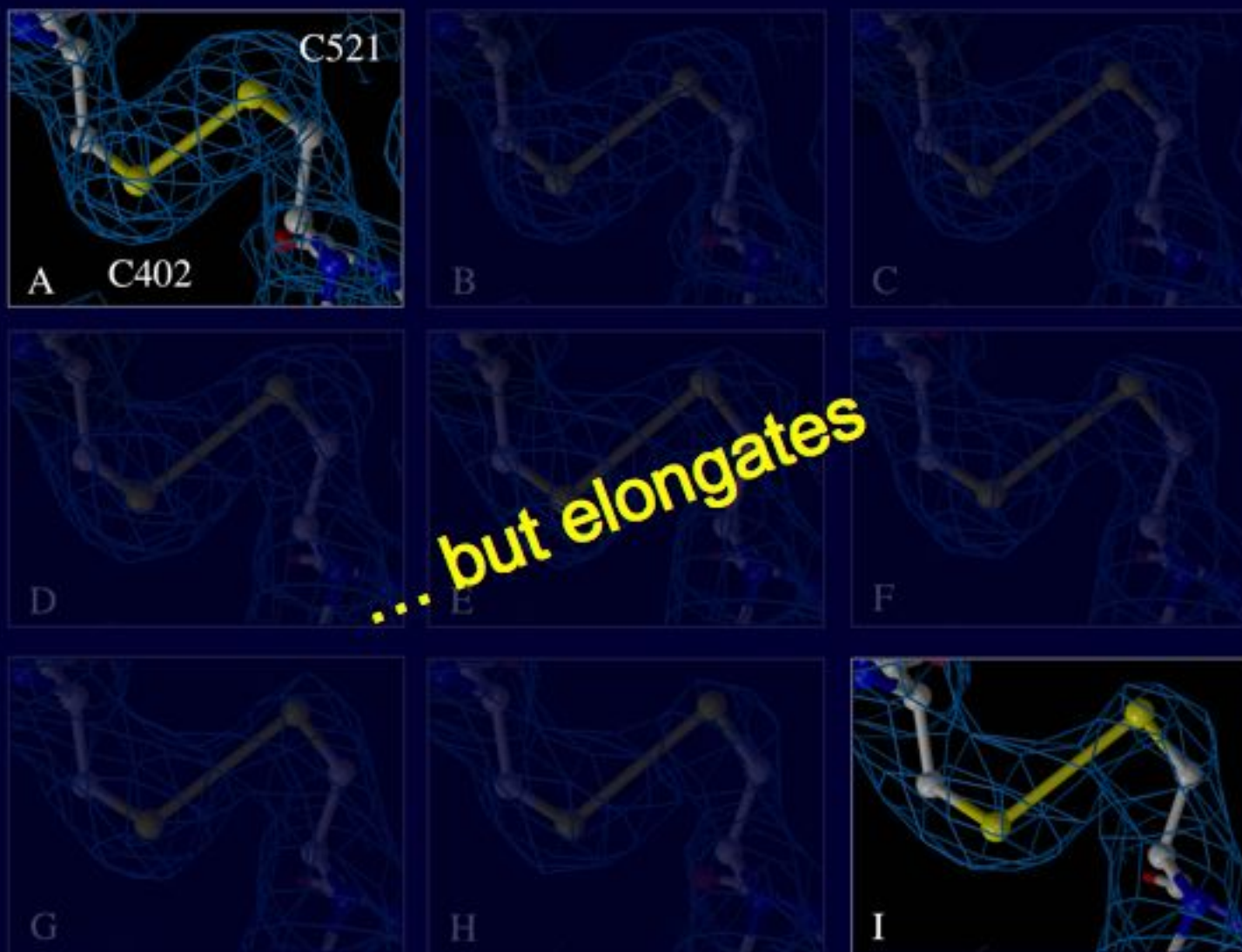
T=100 K

Disulfide bond C402 - C521 does not break ...



T=100 K

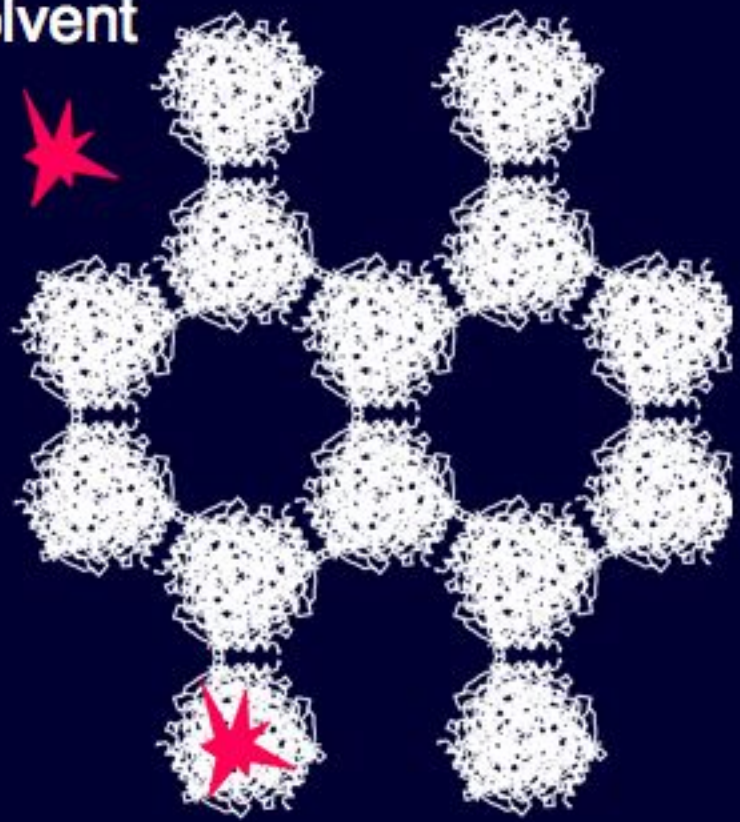
Disulfide bond C402 - C521 does not break ...



T=100 K

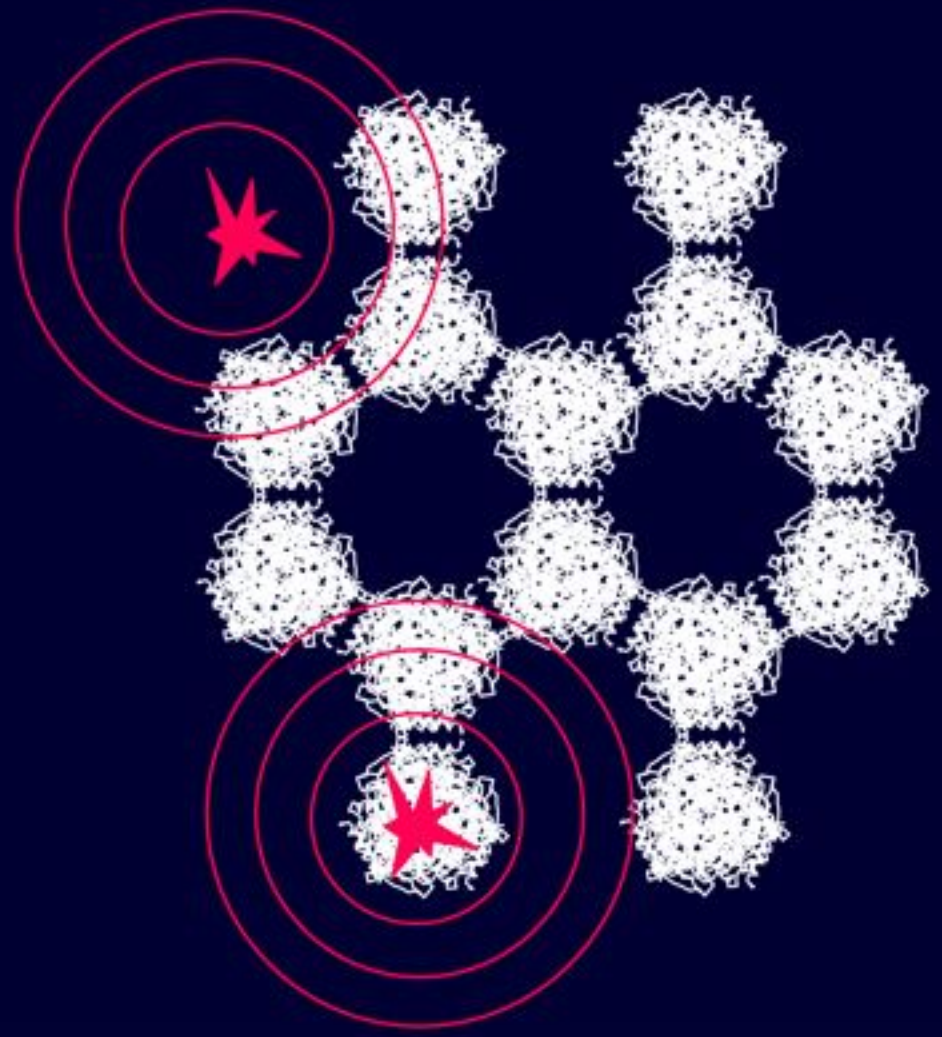
Primary damage

solvent



protein

Secondary damage



AChE: 3 disulfide bonds



Specific radiation damage

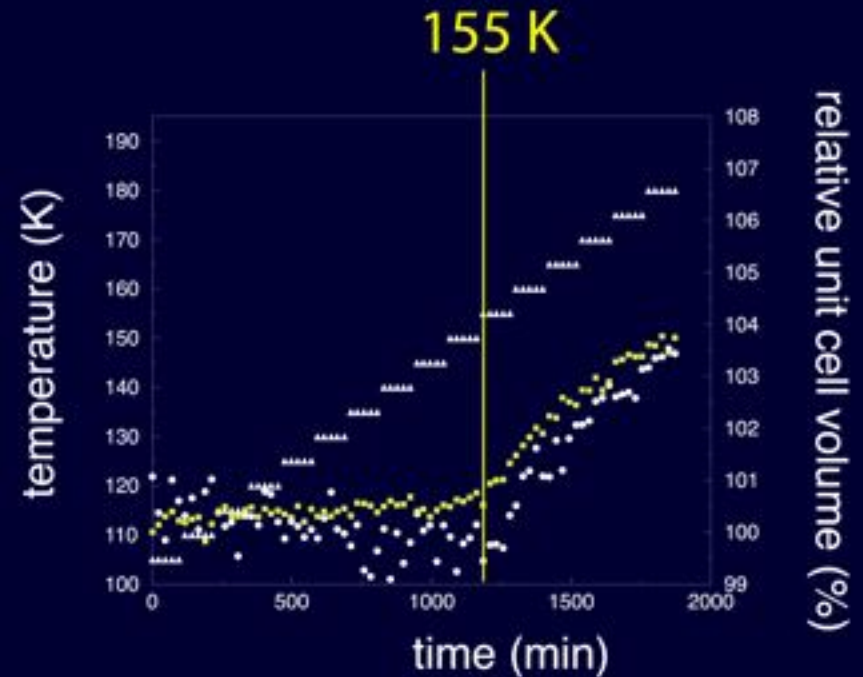
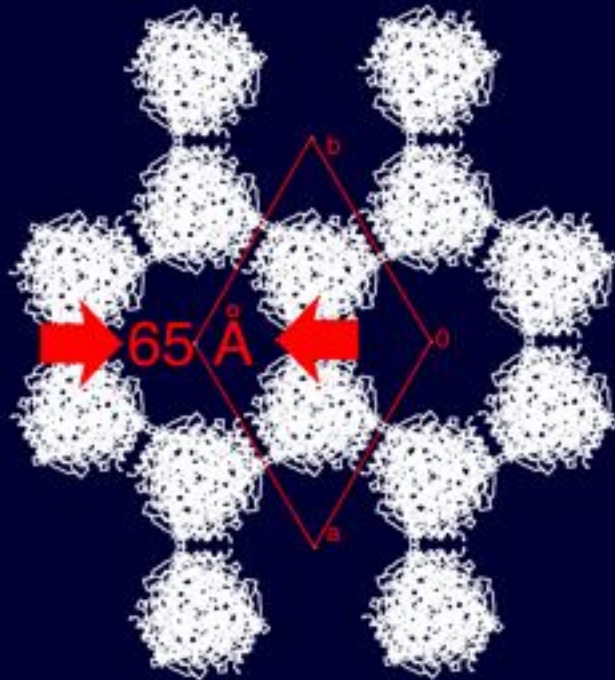
- Synchrotron radiation produces specific damage even at 100 K
 - disulfide bonds, Glu/Asp ...
- Differential sensitivity for chemically identical groups according to their location
- Disulfide radicals can be trapped and identified

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

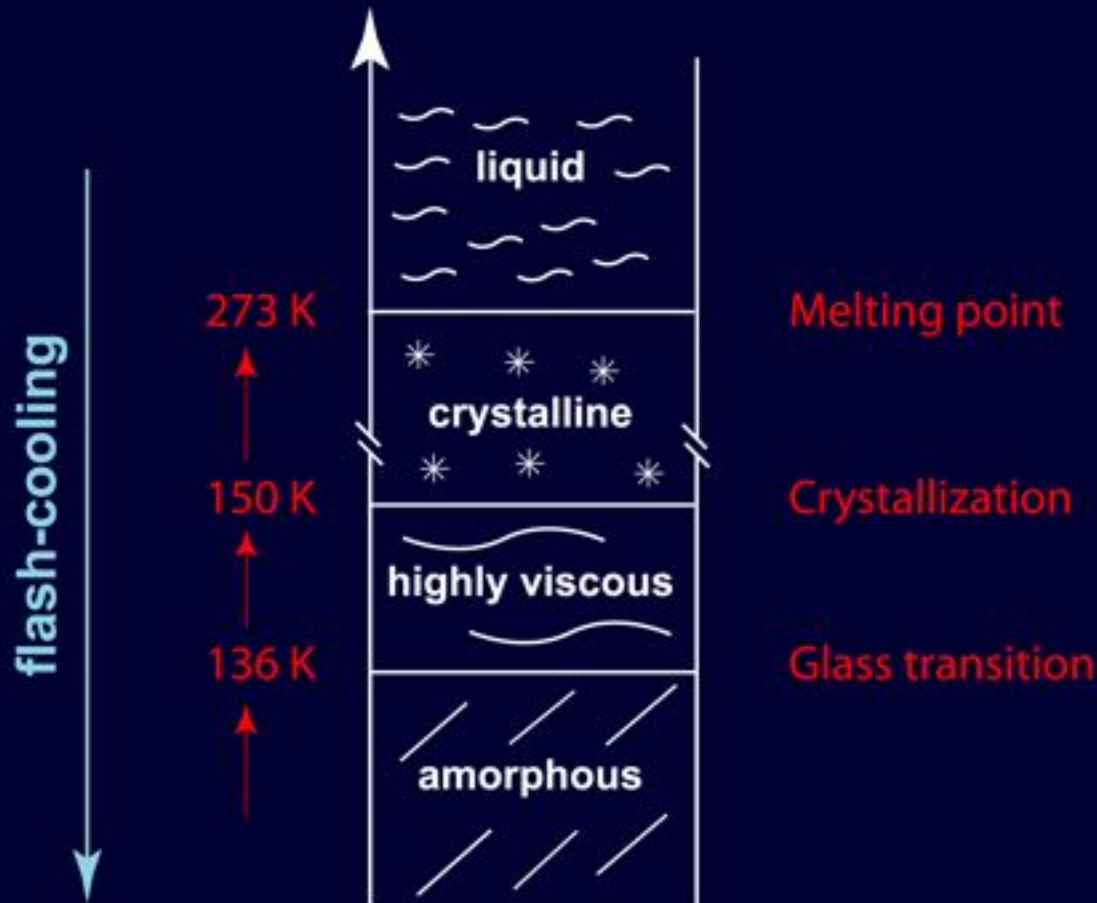


Abrupt unit-cell volume increase at 155 K

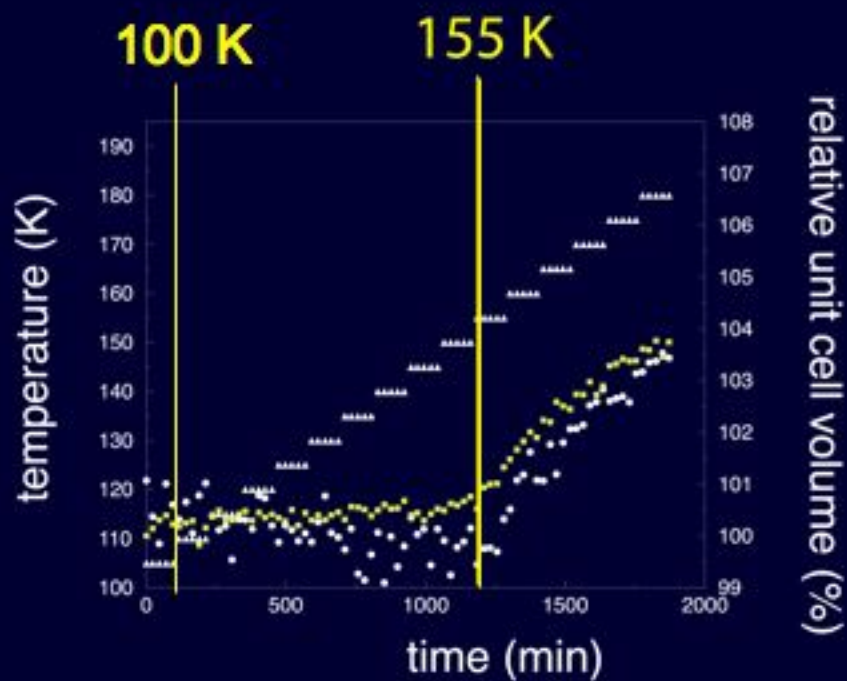
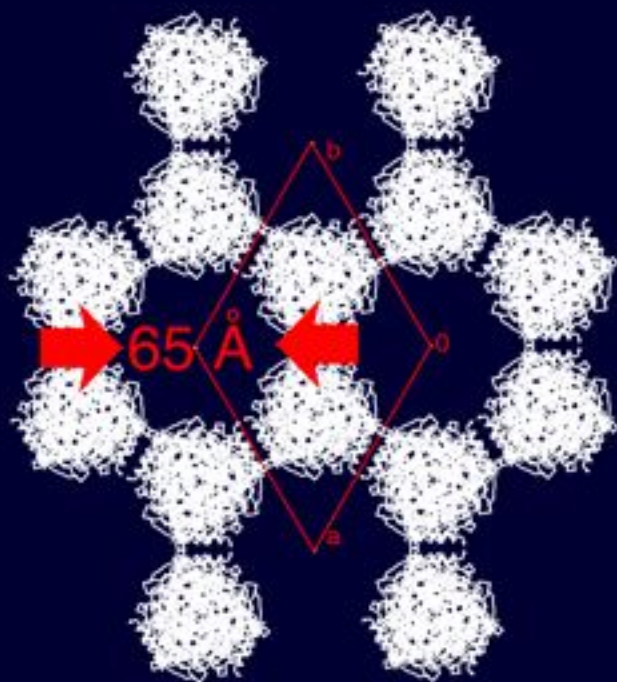
Solvent in large channels :

- ice formation
- glass transition

Pure water at cryo-temperatures

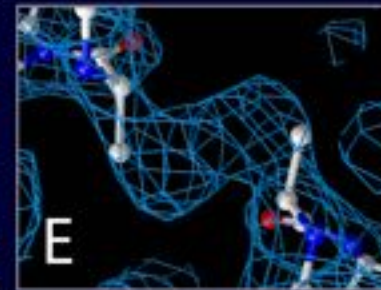
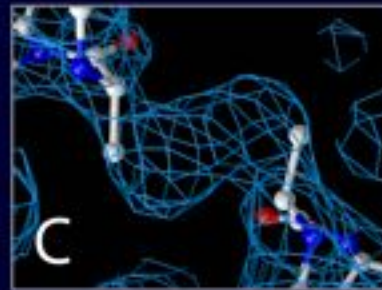
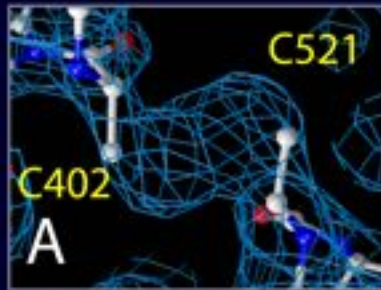


Solvent behaviour in flash-cooled AChE crystals

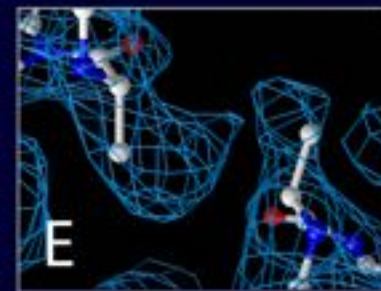
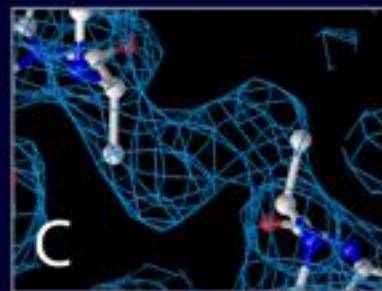


AChE disulfide bond C402 - C521

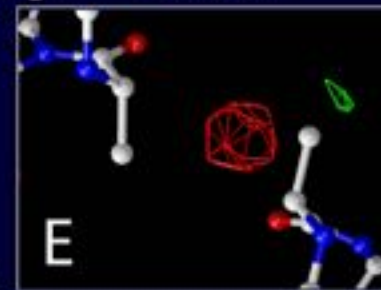
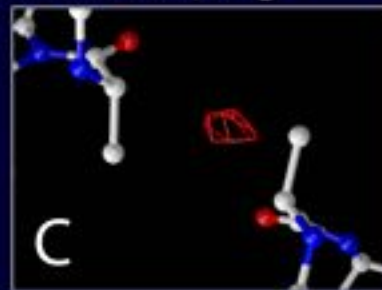
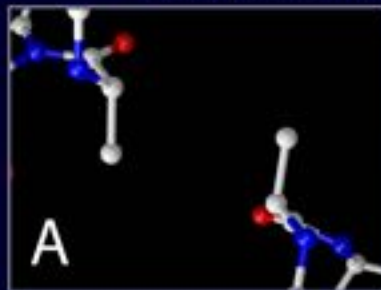
100 K



155 K

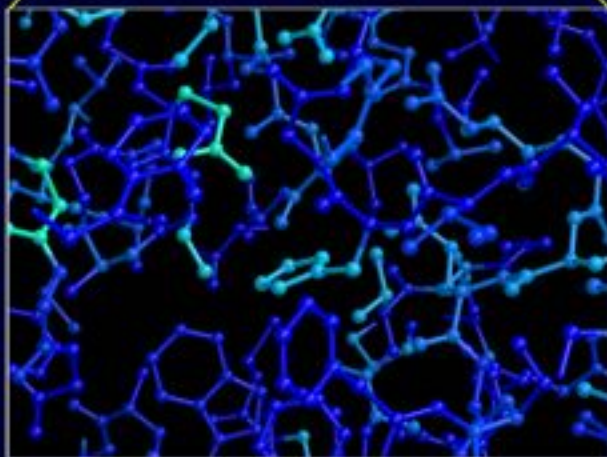
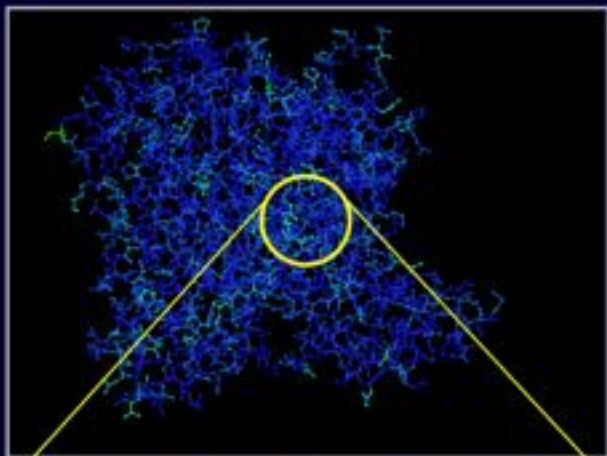


Fourier Difference Map $F_o^{155K} - F_o^{100K}$ at 5σ

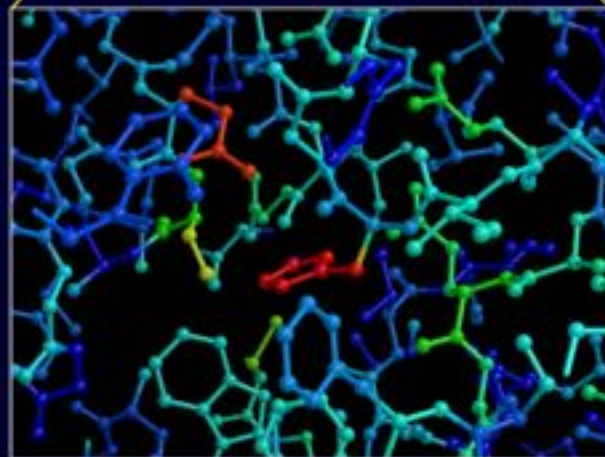
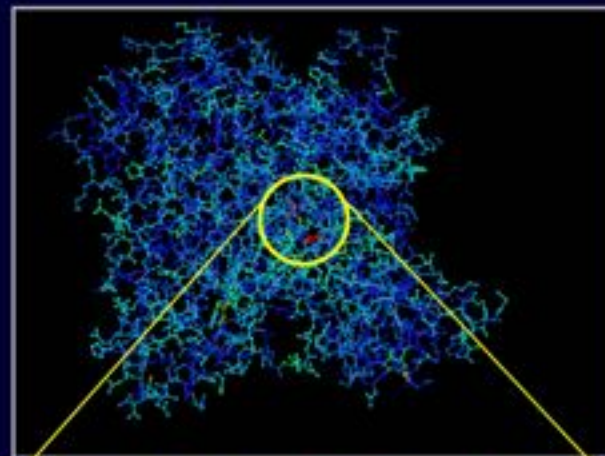


AChE colored according to B-factor increase $(B_E - B_A)/B_A$

100 K



155 K

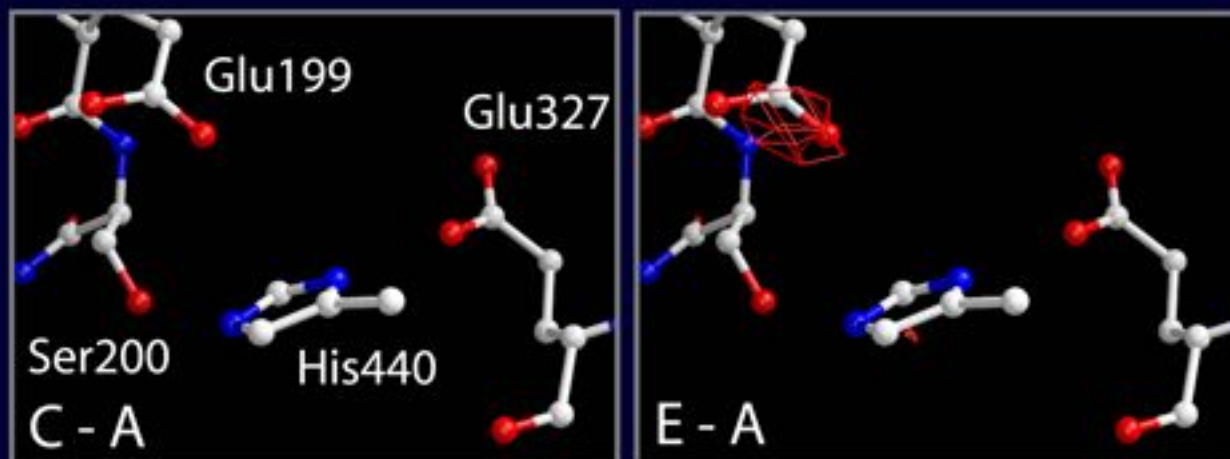


At 155 K : Active site most affected

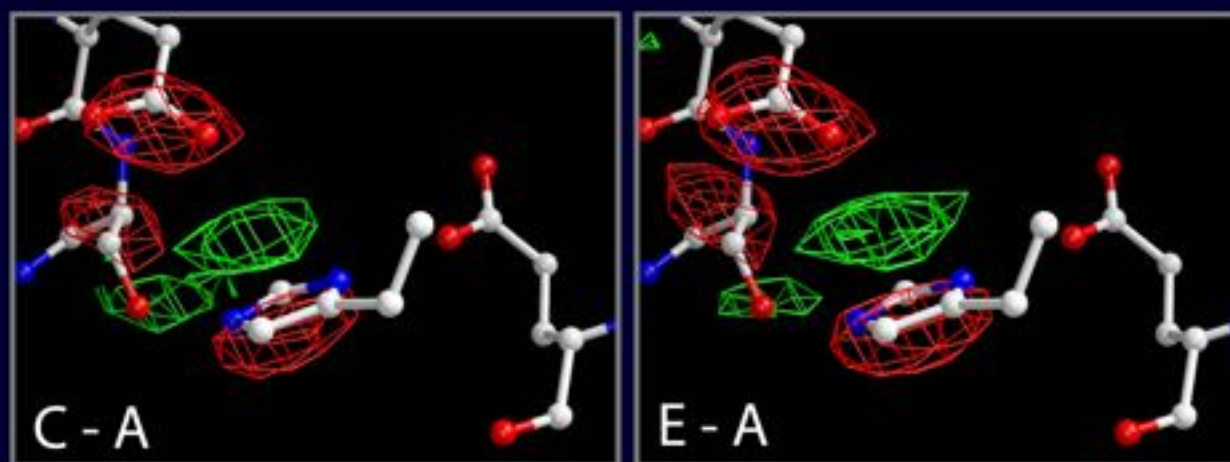
AChE active site

Sequential Fourier Difference maps at 5σ

100 K

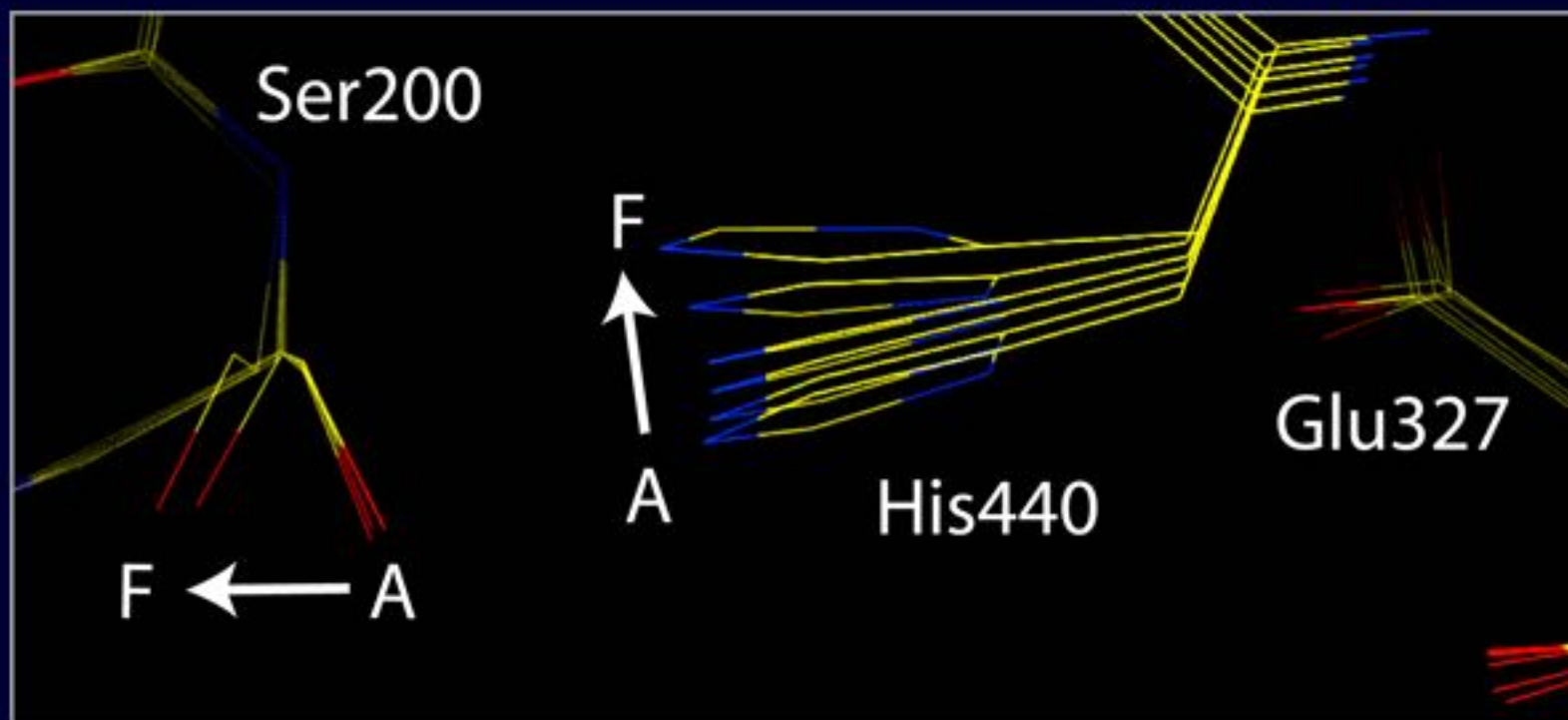


155 K



Conformational changes upon damage at 155 K

Enzyme radiation-inactivation



155 K

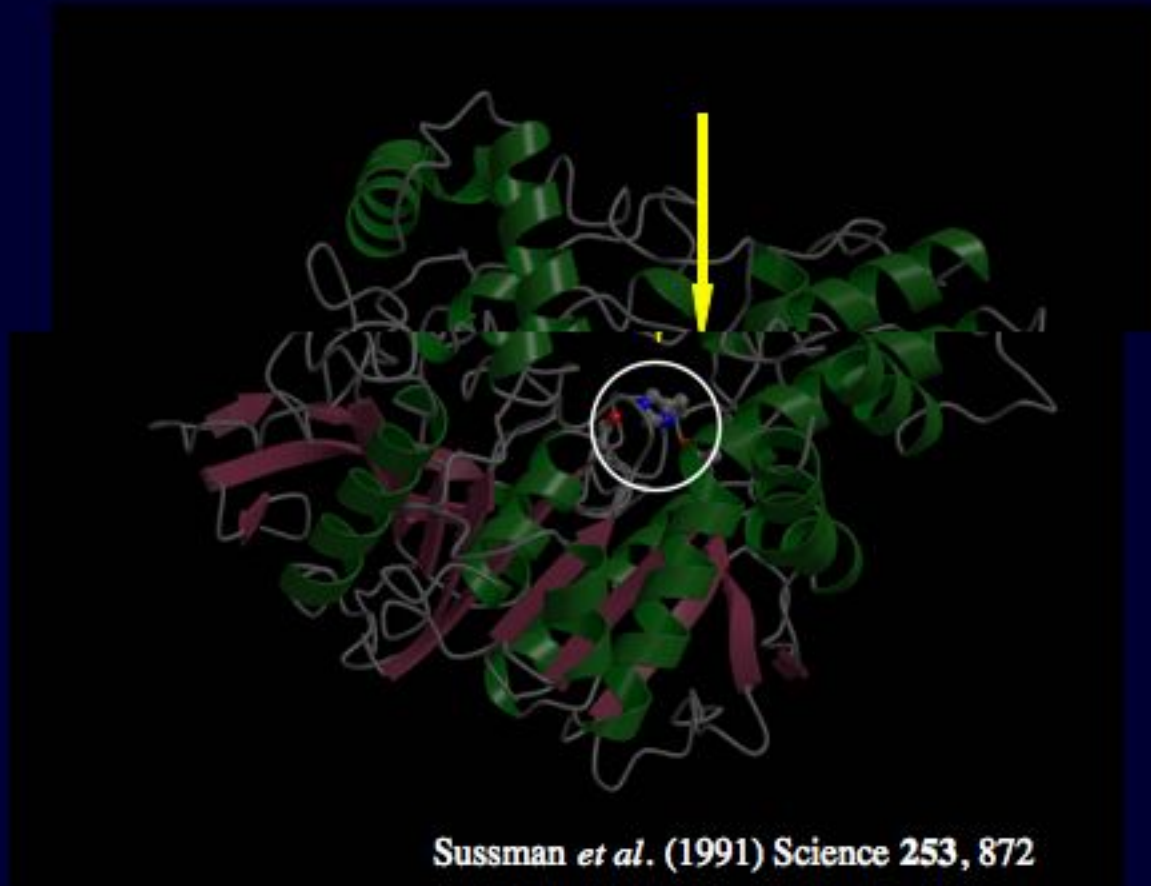
Local protein flexibility is increased above solvent glass transition
yet still below protein dynamical transition

- 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?

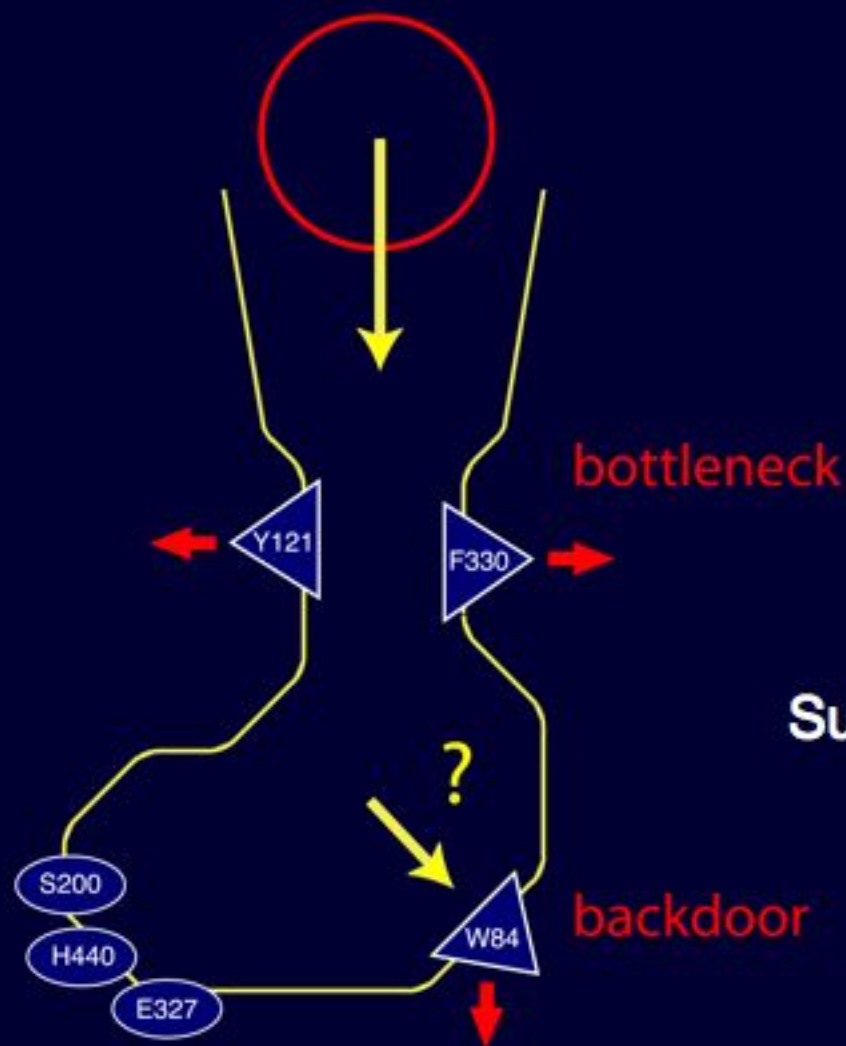
④ Strategy to trap enzymatic intermediate states

Acetylcholinesterase: Structure and Function

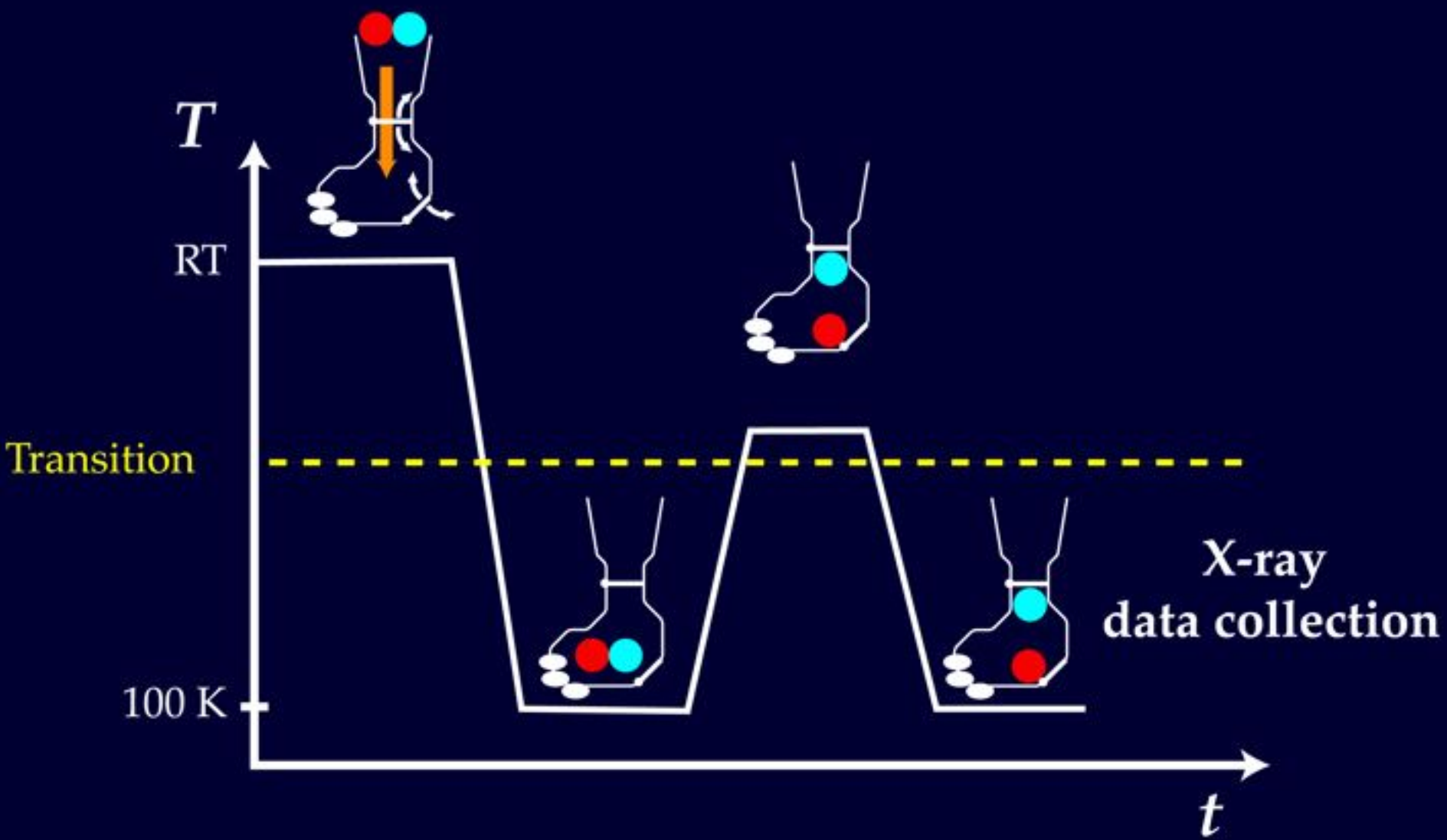


- hydrolyses neurotransmitter acetylcholine
- turnover 20000 s⁻¹
- involved in Alzheimer disease
- target of insecticides and nerve gases

Traffic of substrates and products in AChE

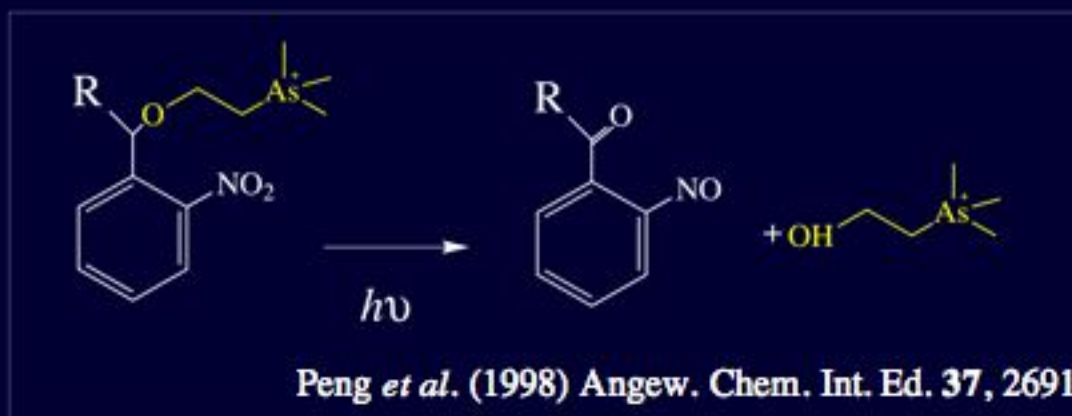


Substantial 'breathing' motions are required

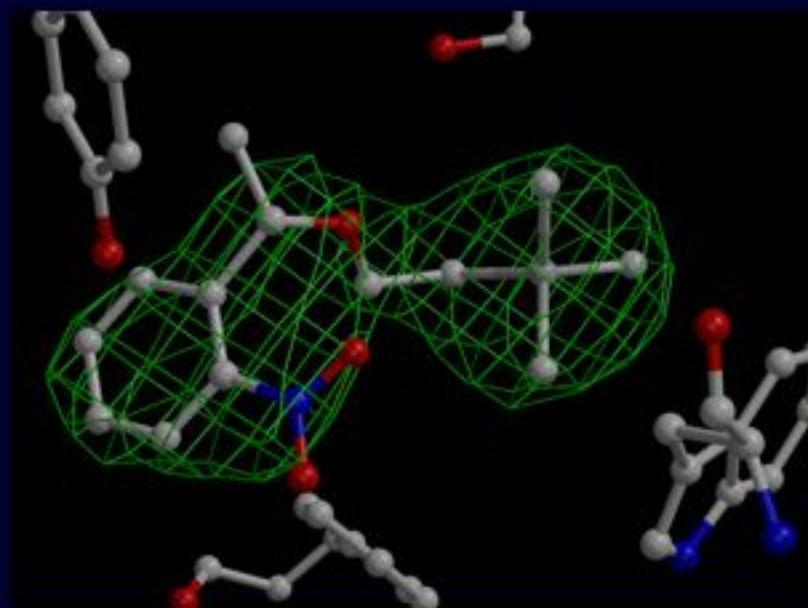


Photolabile precursor of choline

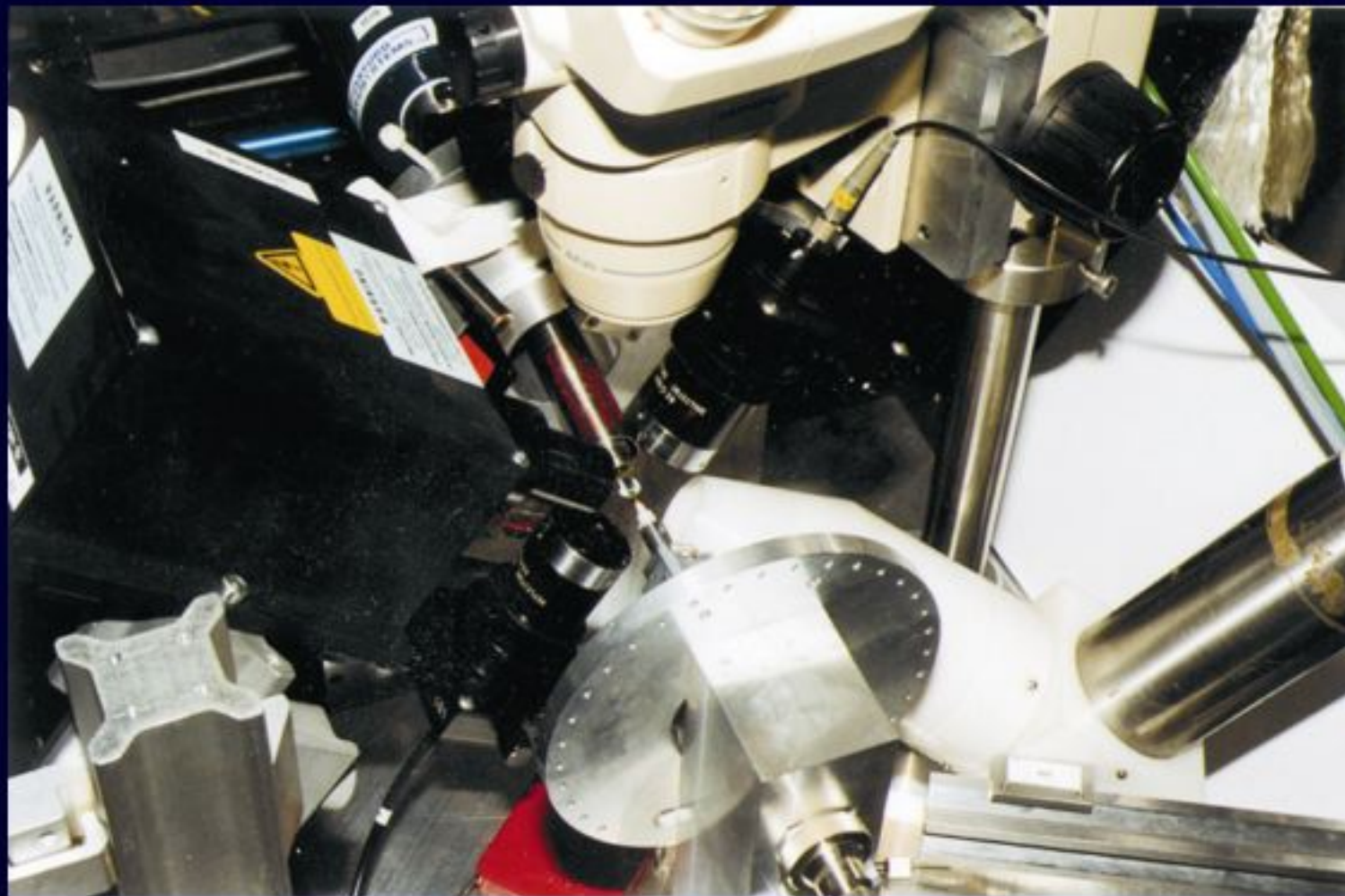
Photofragmentation



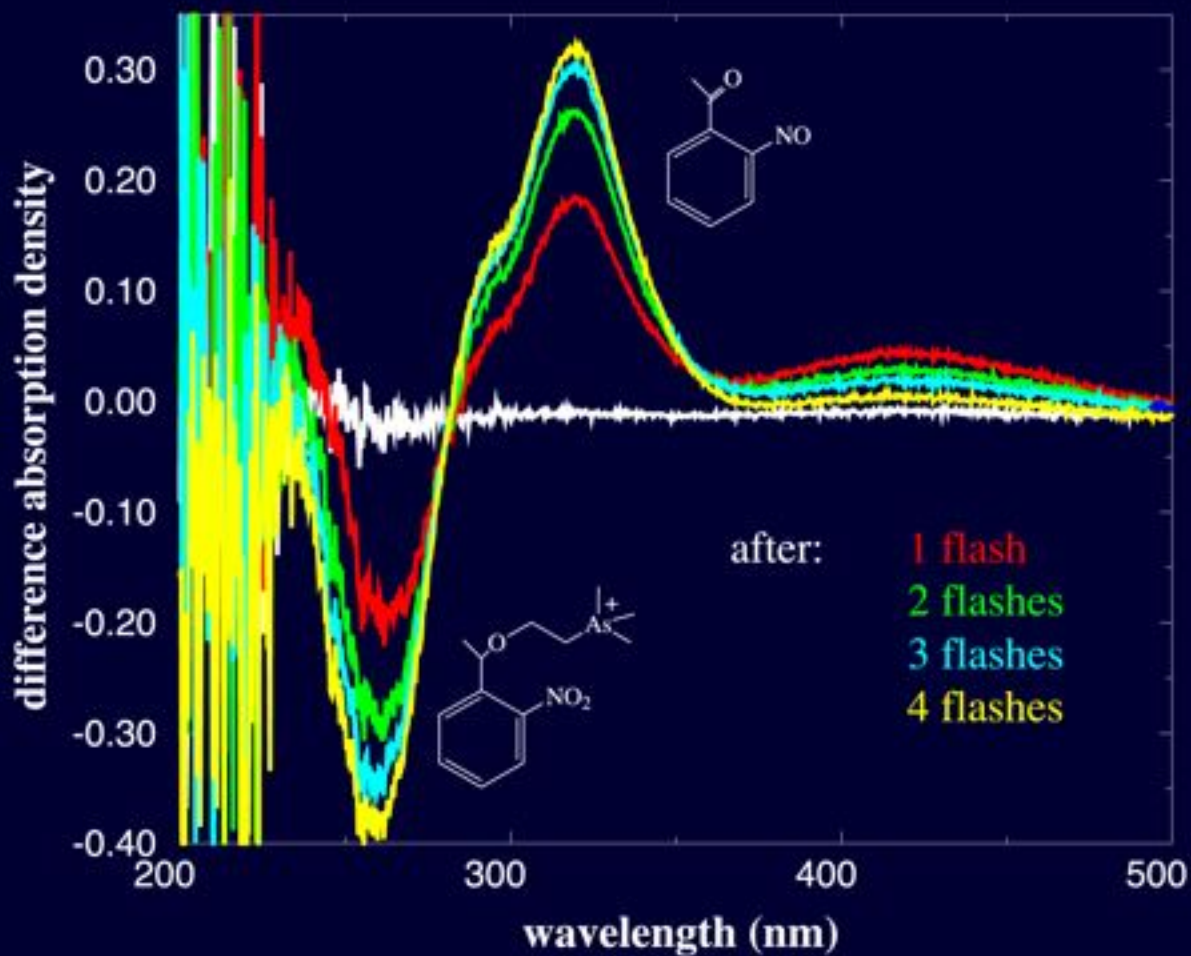
Bound to AChE



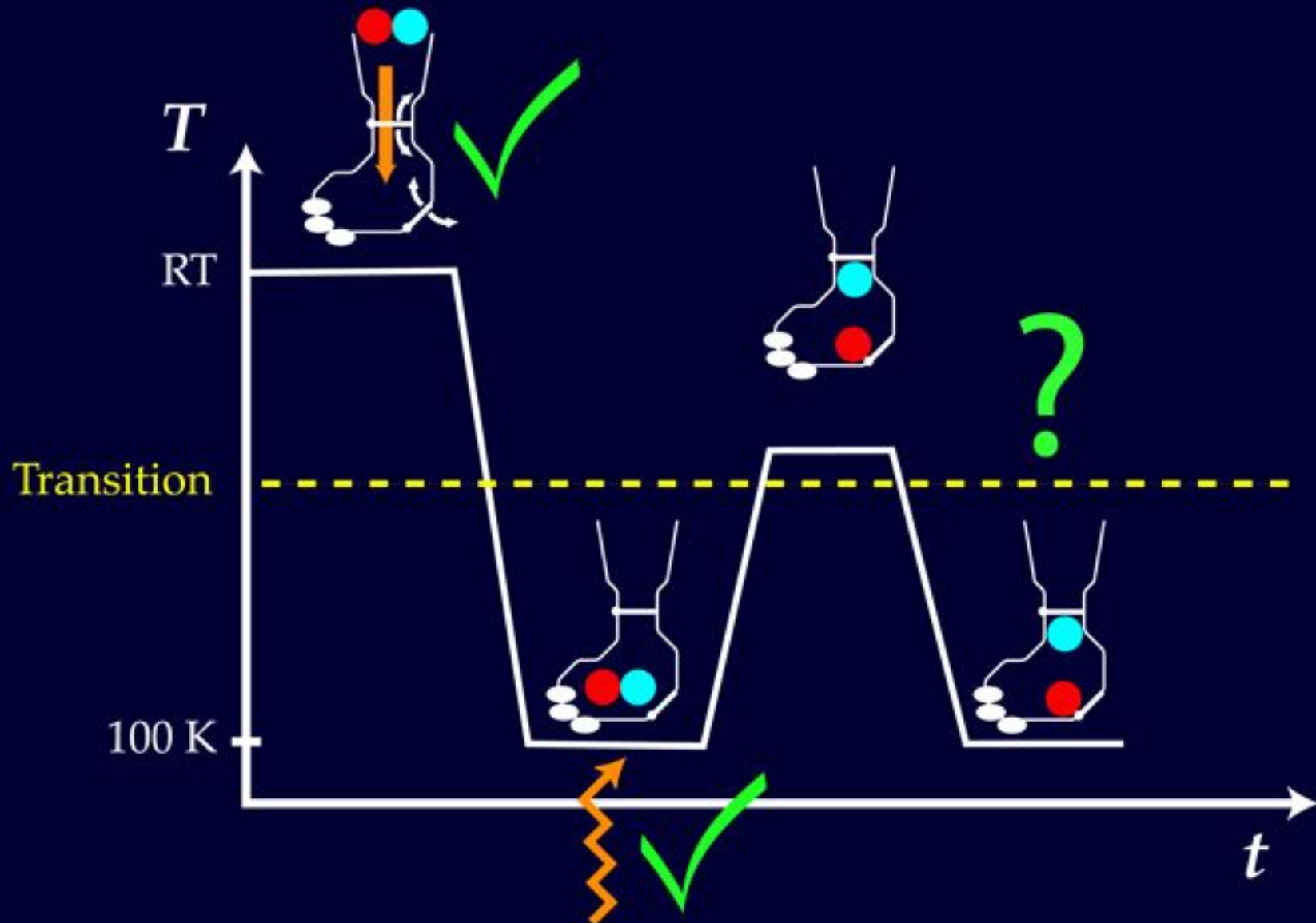
Microspectrophotometer



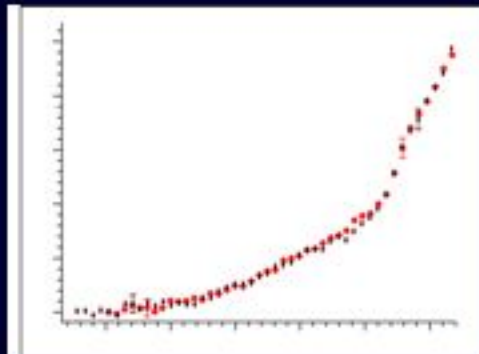
Photofragmentation of caged arsenocholine at 100 K



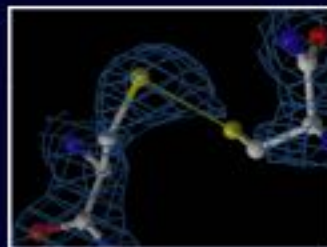
Strategy to trap structural intermediate states in ChE



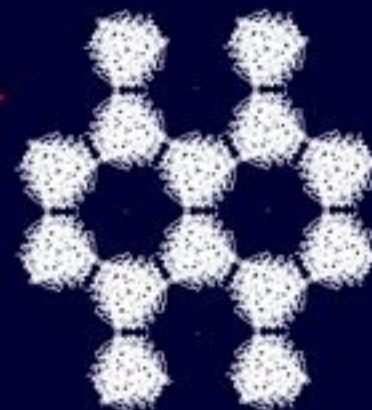
Global dynamics



Radiation damage



Solvent behaviour



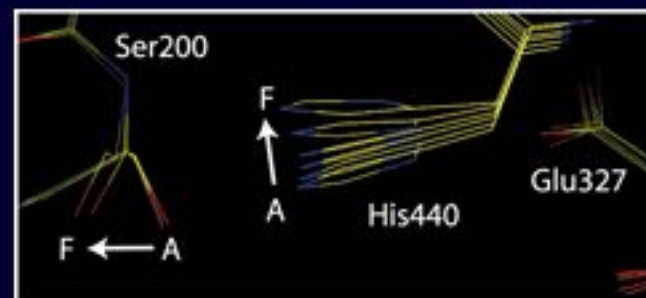
Water behaviour



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

