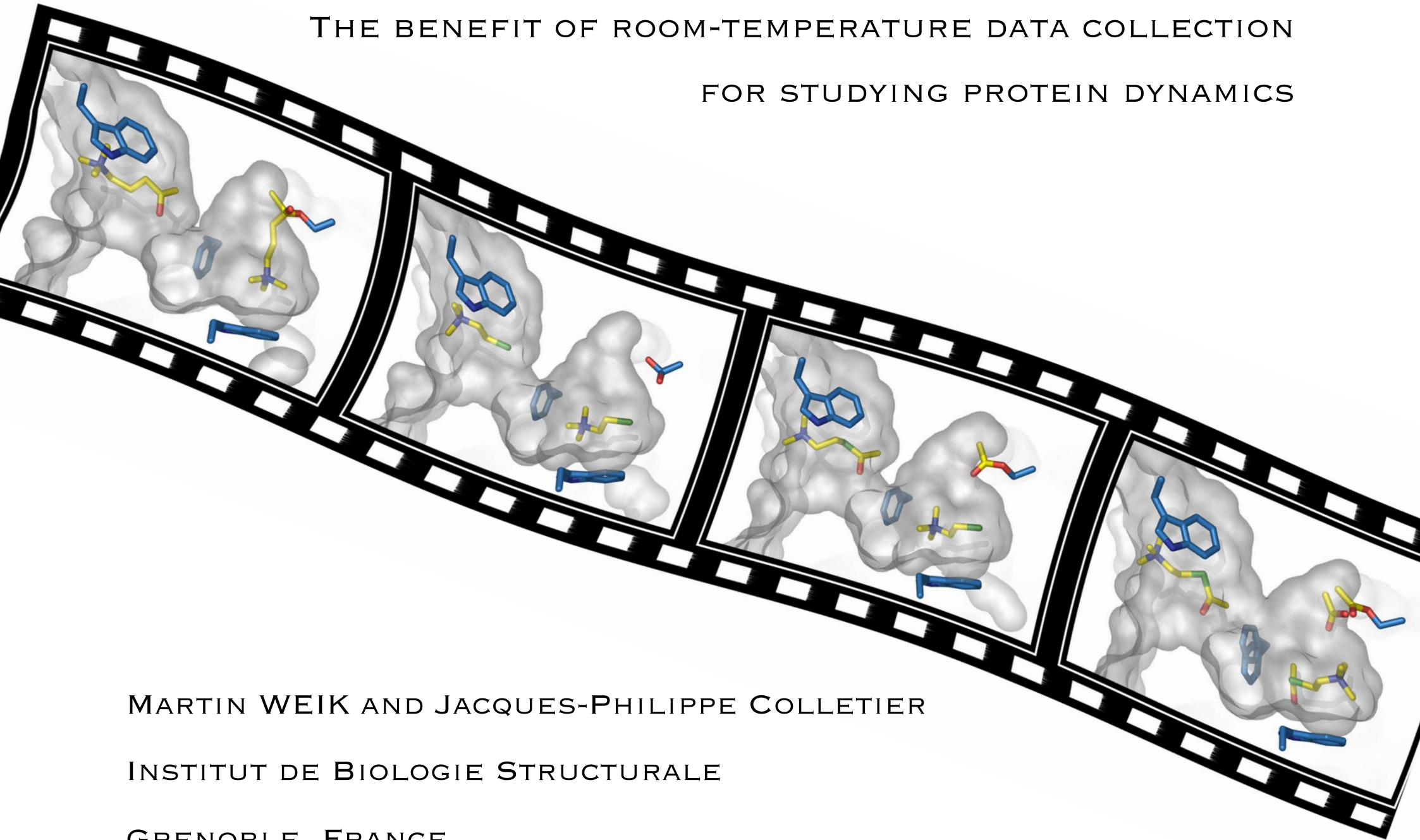


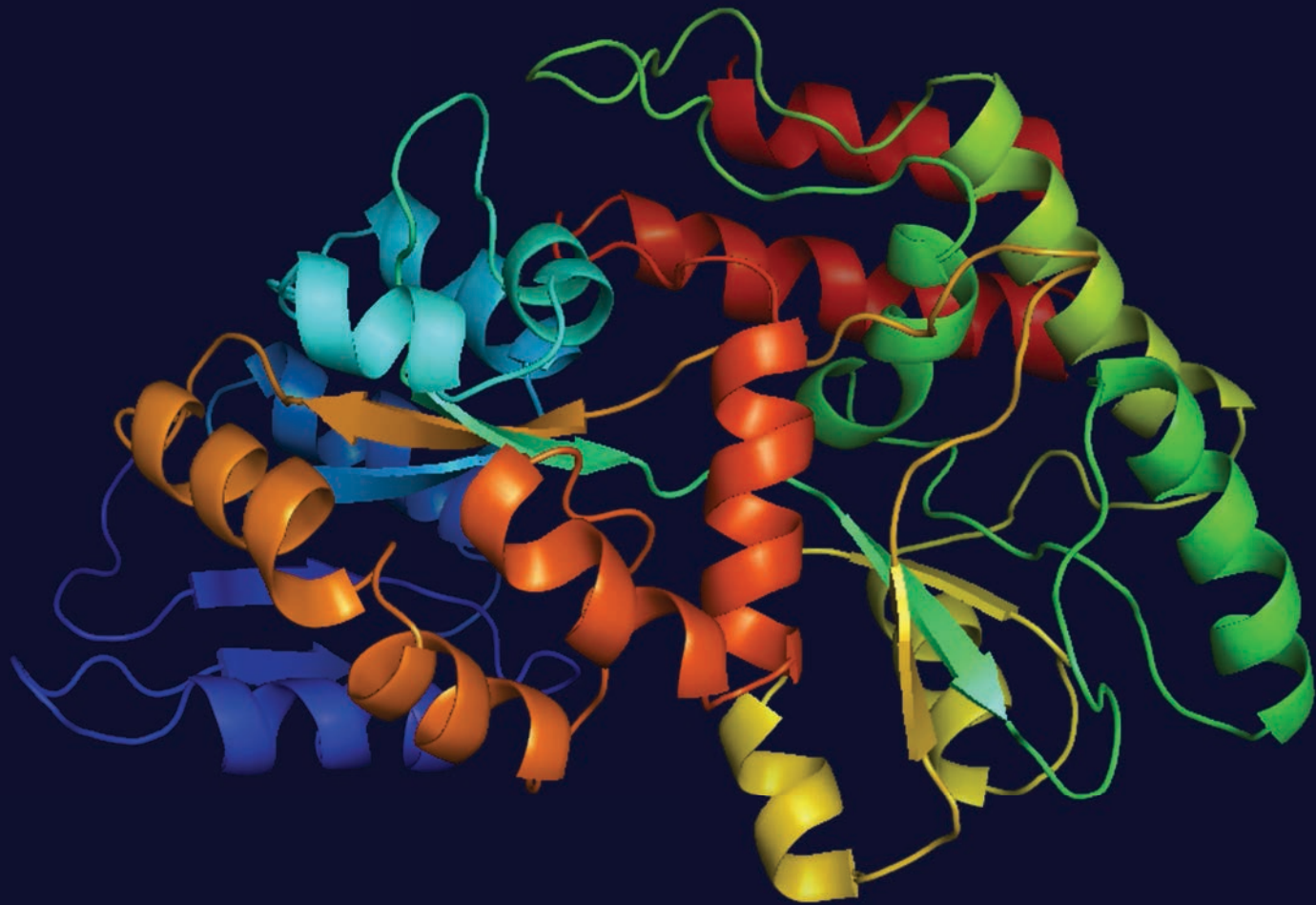
THE BENEFIT OF ROOM-TEMPERATURE DATA COLLECTION  
FOR STUDYING PROTEIN DYNAMICS

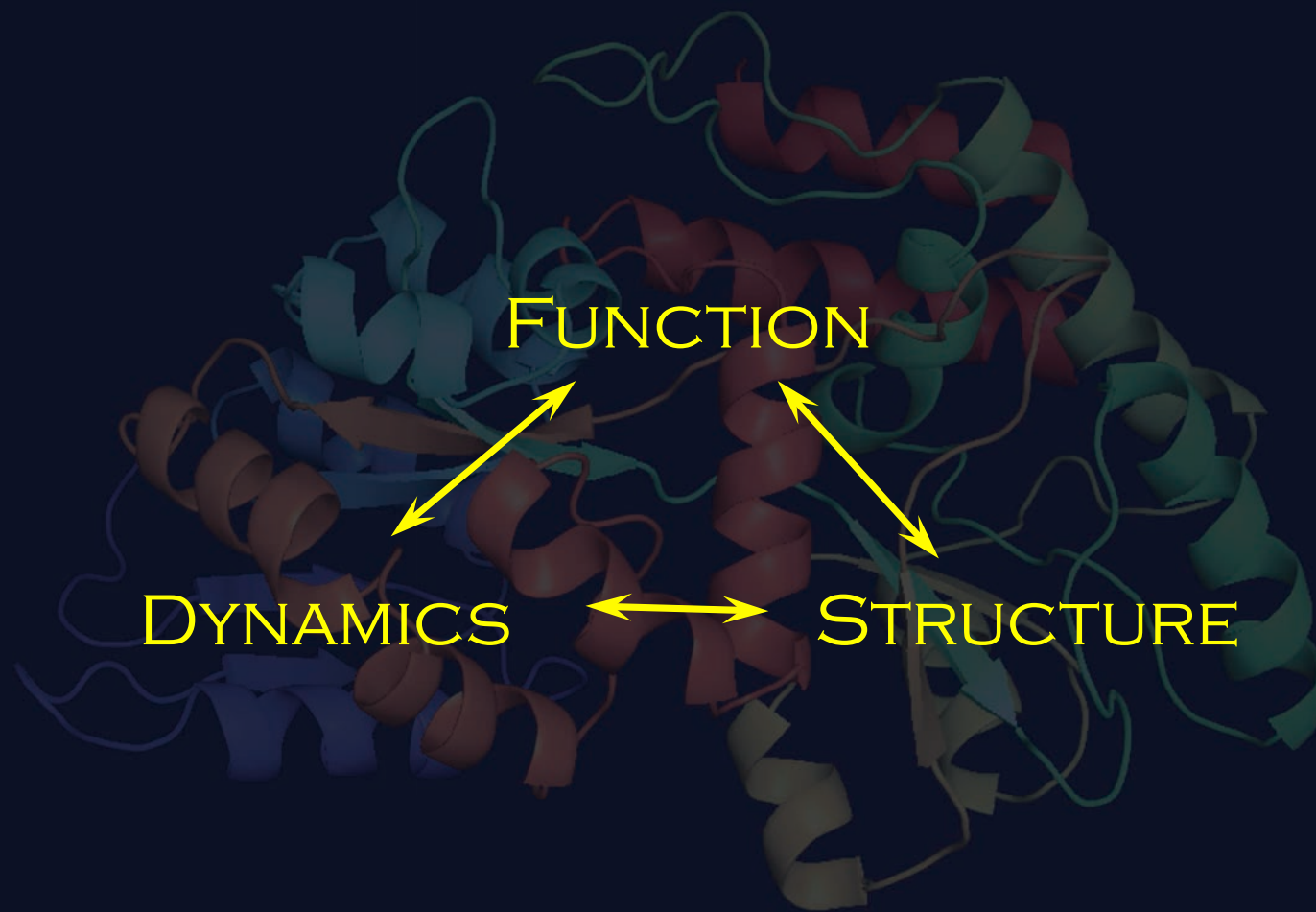


MARTIN WEIK AND JACQUES-PHILIPPE COLLETIER

INSTITUT DE BIOLOGIE STRUCTURALE

GRENOBLE, FRANCE





dynamics

electronic transitions

enzymatic transition states

bond vibrations

side chains motions

domain motions

protein folding

enzyme catalysis

protein synthesis

fs

ps

ns

$\mu$ s

ms

s

technique

neutron spectroscopy

time-res. SAS, EXAFS

Single particle cryo-EM

NMR

THz spectroscopy

EPR spectroscopy

Single molecule experiments

Mössbauer spectroscopy

fluorescence spectroscopy

high-speed AFM

MD simulations / QM/MM

time-res. FTIR

kinetic crystallography (freeze-trapping)

XFEL

time-res. Laue crystallography

# dynamics

electronic transitions

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bond vibrations

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# technique

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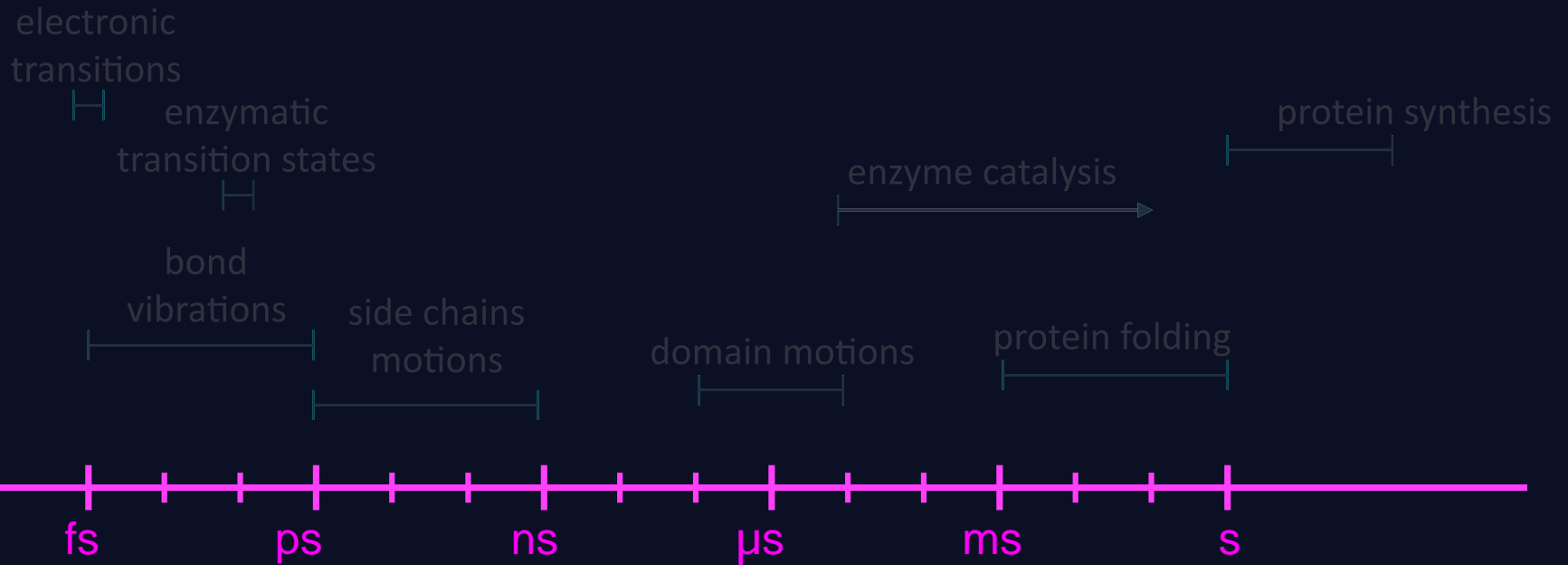
time-res. FTIR

kinetic crystallography (freeze-trapping)

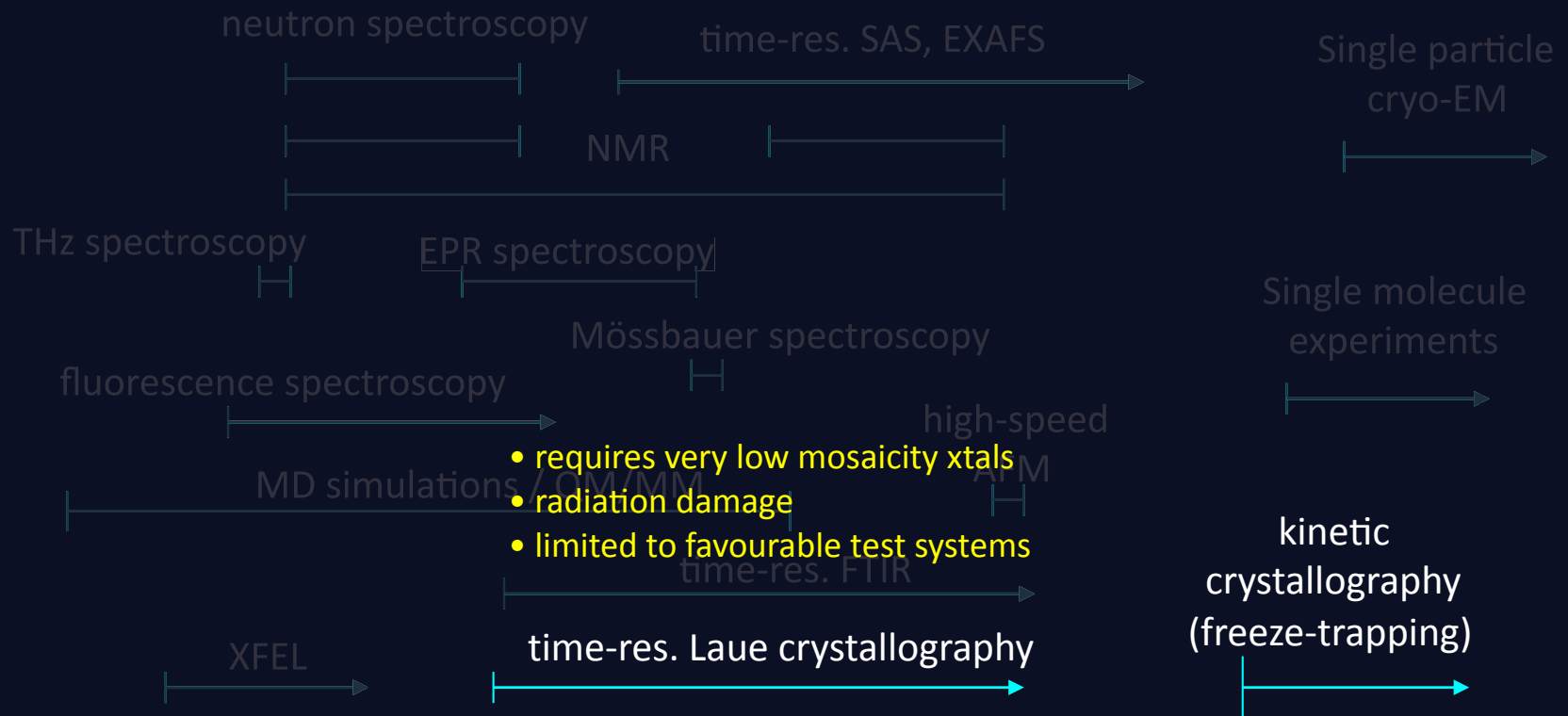
XFEL

time-res. Laue crystallography

# dynamics

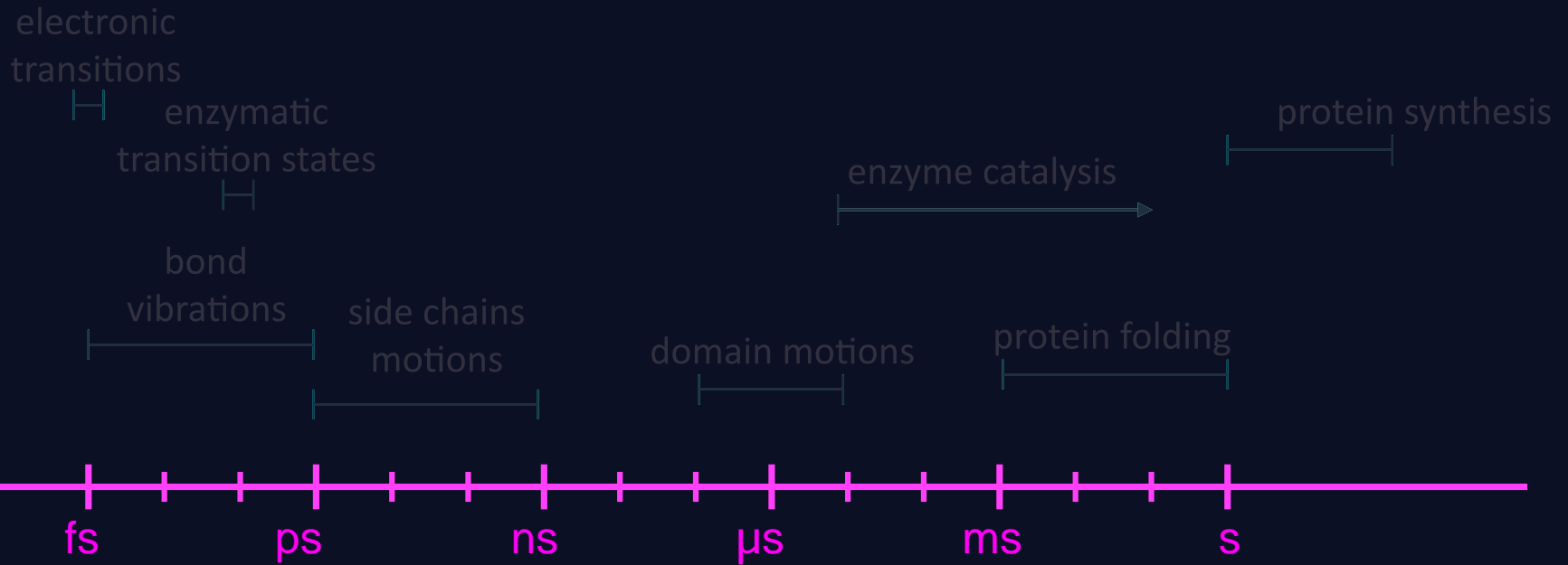


# technique



- requires very low mosaicity xtals
- radiation damage
- limited to favourable test systems

# dynamics



# technique

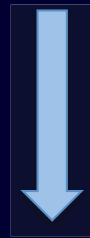


- serial data collection
- damage-free structures
- theoretically: fs time resolution

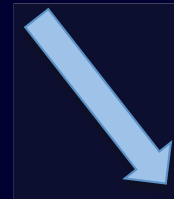
How can high flux-density synchrotron sources  
further contribute to studying protein dynamics ?



Static  
room-temperature  
crystallography



Distribution of side chain dynamics  
(equilibrium fluctuations)



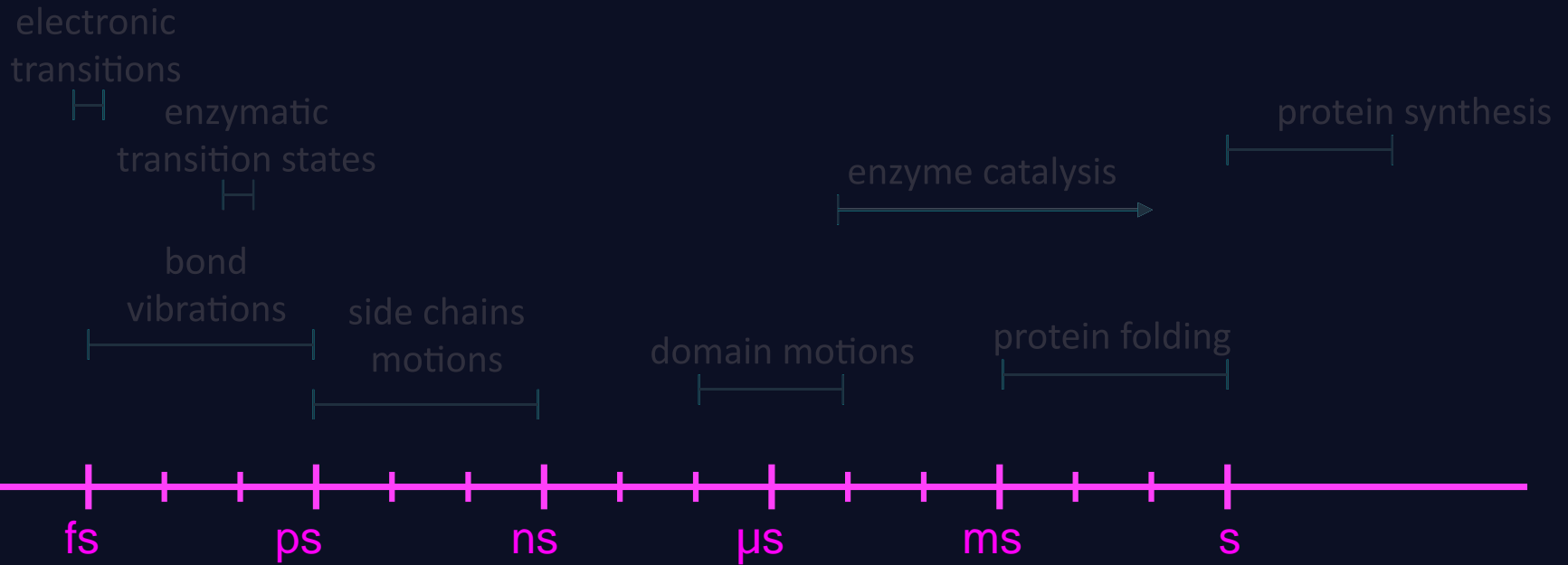
Time-resolved (monochromatic)  
room-temperature  
crystallography



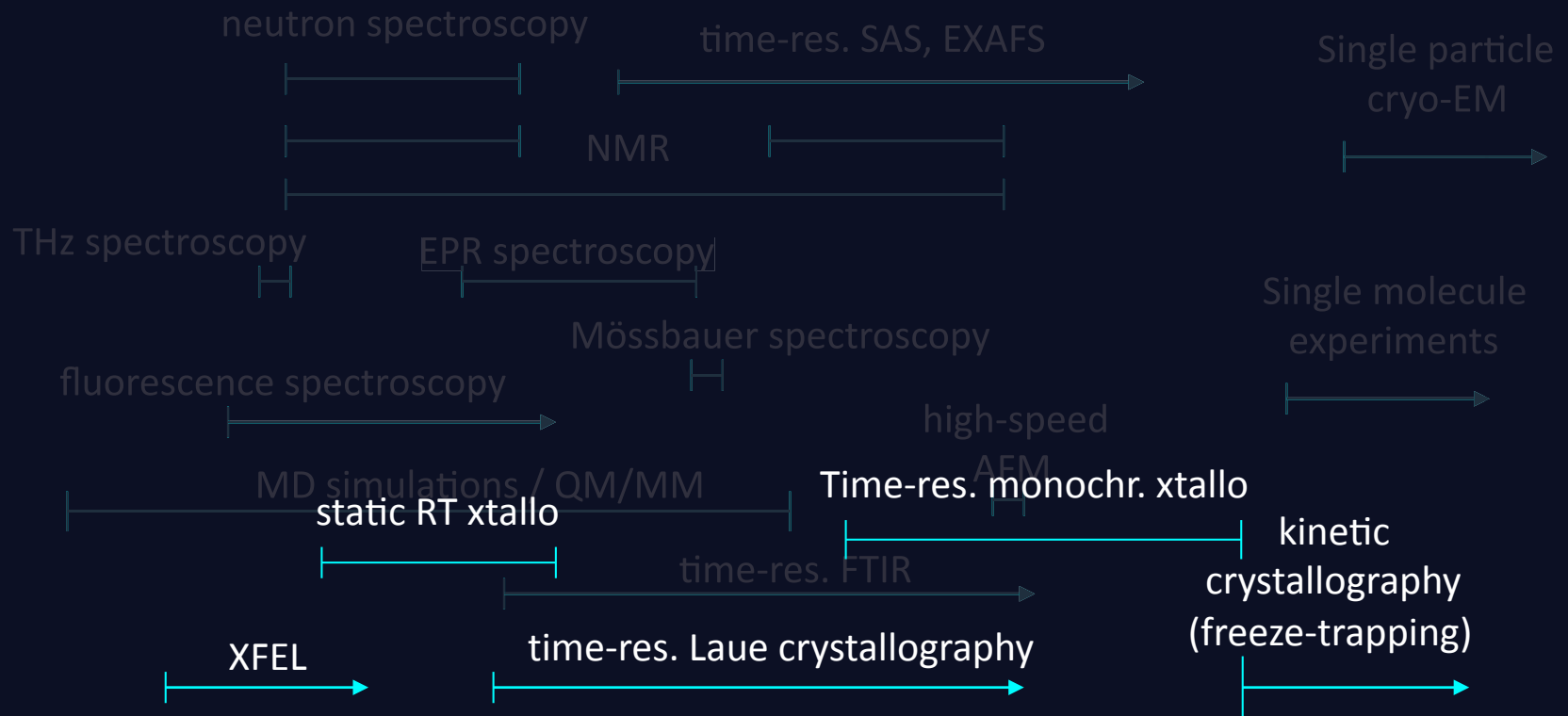
Structural changes  
during protein functioning  
(kinetics)



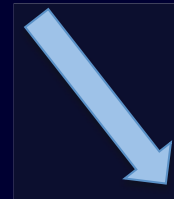
# dynamics



# technique



How can high flux-density synchrotron sources further contribute to studying protein dynamics ?



Static  
room-temperature  
crystallography

Time-resolved (monochromatic)  
room-temperature  
crystallography

X-ray radiation damage  
100x greater at RT than at 100 K



Distribution of side chain dynamics  
(equilibrium fluctuations)

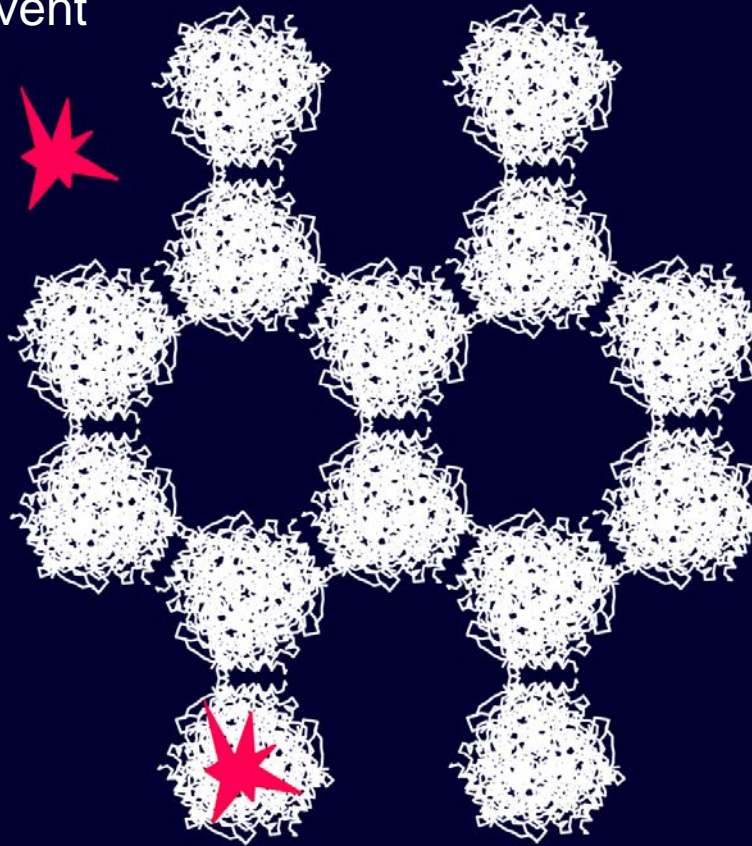
Structural changes  
during protein functioning  
(kinetics)

# Primary and secondary radiation damage

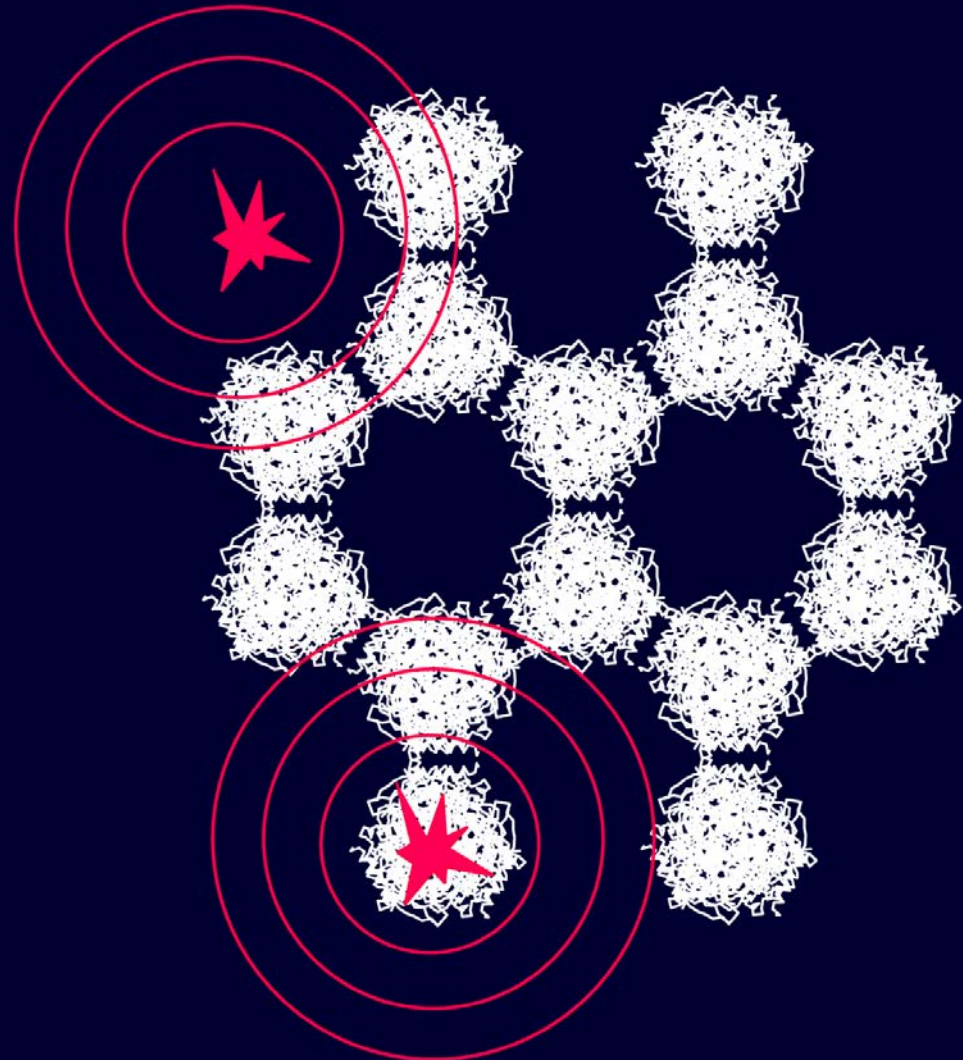
Primary damage

Secondary damage

solvent



protein



# Primary events

at 12.7 keV ( $\lambda=0.98 \text{ \AA}$ )

Murray *et al.* (2005) *J. Synchrotron Rad.* **12**, 268

- 98% of incident photons don't interact at all
- 2% interact:

Elastic (Thomson) scattering (diffraction): 8%

Compton scattering: 8%

Photoelectric effect: 84%

each photoelectron produces **500 ionization events**

Cross sections:

H

C

N

O

S

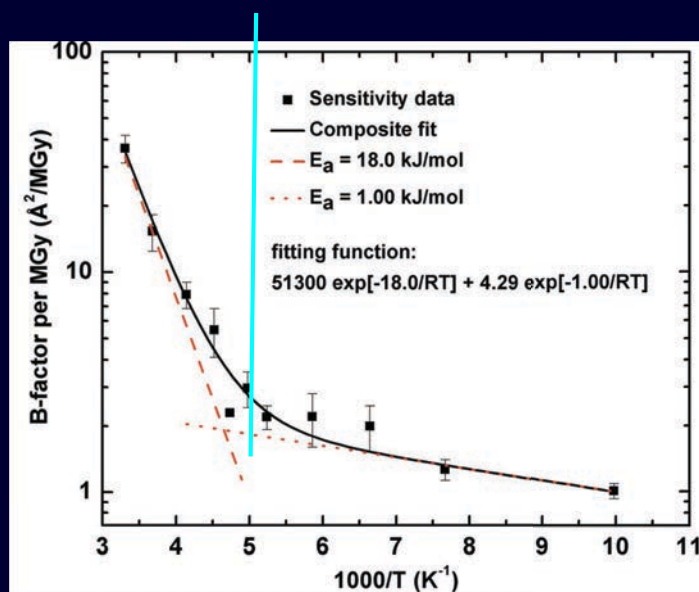
**secondary damage**

Ravelli *et al.* (2005) *J. Synchrotron Rad.* **12**, 276

## Temperature-dependence of radiation sensitivity: transition at 200 K

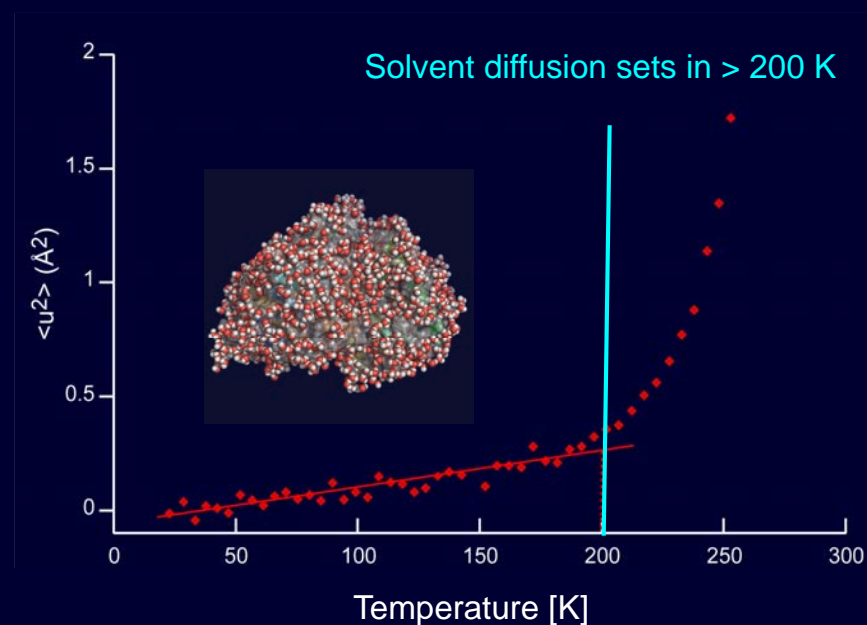
Warkentin, Hopkins, Badeau, Mulichak, Keefe, Thorne (2013) *JSR* 20, 7

Transition in radiation sensitivity at 200 K



Warkentin & Thorne (2010) *Acta Cryst D* 66, 1092

Solvent mean-square displacements from neutron scattering



Wood, Frölich, Gabel, Moulin, Haertlein, Paciaroni, Zaccai, Tobias & Weik (2008) *JACS* 130, 4586

Radical diffusion at RT responsible for increased radiation damage ?

## Temperature-dependence of radical mobility

$T < 115 \text{ K}$  :  $e^-$  are mobile in amorph. ice

$T > 115 \text{ K}$  :  $e^-$  and  $H^\bullet$  are mobile in amorph. ice

Fisher and Devlin (1995) J. Phys. Chem. **99**, 11584

$T > 130 \text{ K}$  :  $e^-$ ,  $H^\bullet$  and  $OH^\bullet$  are mobile in cryst. Ice

Symons (1999) Progr. Reaction Kinetics and Mechanisms **24**, 139

$T > 110 \text{ K}$  :  $e^-$ ,  $H^\bullet$  and  $OH^\bullet$  are mobile in amorph. Ice

Sevilla, private comm.

$T > 160 \text{ K}$  :  $OH^\bullet$  become mobile in protein crystals

Owen et al. (2012) Acta Cryst D68, 810

100 K: only electrons are mobile

> 160 K

(solvent glass transition):  $OH^\bullet$  are mobile

**$OH^\bullet$  are responsible for increased radiation damage at RT**

(Owen et al. (2012) Acta Cryst D68, 810)

## Dose rate effect close to RT

At 260 K: Outrun half of damage by collecting data in 1s (680 kGy / s)

Warkentin et al. (2012) Acta Cryst D68, 124

At RT: Outrun almost half of damage by collecting data at 1 MGy / s with exposure times < 60 ms

Owen et al. (2012) Acta Cryst D68, 810

# Future high flux-density synchrotron sources :

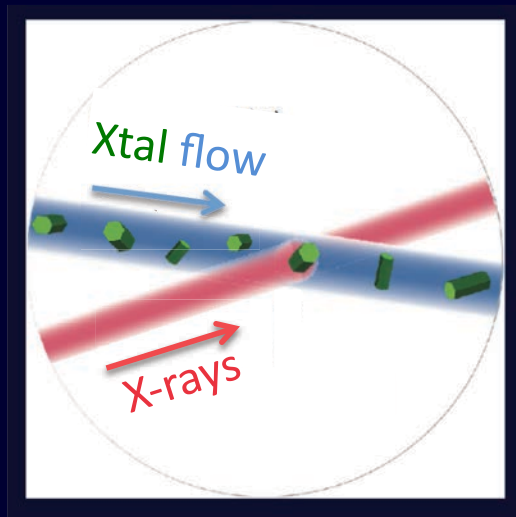
Ultrafast (ms) data collection with ultra-high dose rate  
at RT could reduce radiation sensitivity to the one at 100 K

Warkentin, Hopkins, Badeau, Mulichak, Keefe, Thorne (2013) *JSR* 20, 7  
Owen et al. (2012) *Acta Cryst D* 68, 810

## Serial crystallography à la XFEL SFX

(reviewed by Schlichting & Miao (2012) *COSB* 22, 613):

## Serial Synchrotron Microsecond Crystallography



Extracted from Aquila et al (2012) *Optics Express* 20, 2706

## ID29 after phase II upgrade: (ESRF Upgrade program phase II White Paper)

- 300 000 x increase in brilliance
- Garman limit (30 MGy) reached in less than ms
- possible to collect one frame on  $\mu$ s time scale ?
- needs very fast detector
- sample heating ?

## Sample delivery:

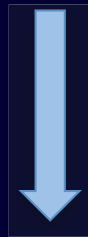
- LCP injector in vacuum (Weierstall et al.):
  - min. speed: 30  $\mu$ m/s. Moves 30 nm in 1 ms
  - successfully used at LCLS (Liu et al (2014) *Science* 342, 1521)
- LCP injector in air (Doak, ..., Schlichting)
- solid support (Zarrine-Afsar et al. (2012) *Acta Cryst D* 68, 321)
- loop mounted – 100 K (Gati et al. (2014) *IUCrJ* 1, 1 )



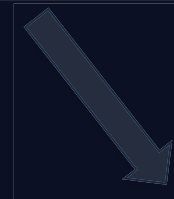
How can high flux-density synchrotron sources contribute to studying protein dynamics ?



Static  
room-temperature  
crystallography



Distribution of side chain dynamics  
(equilibrium fluctuations)



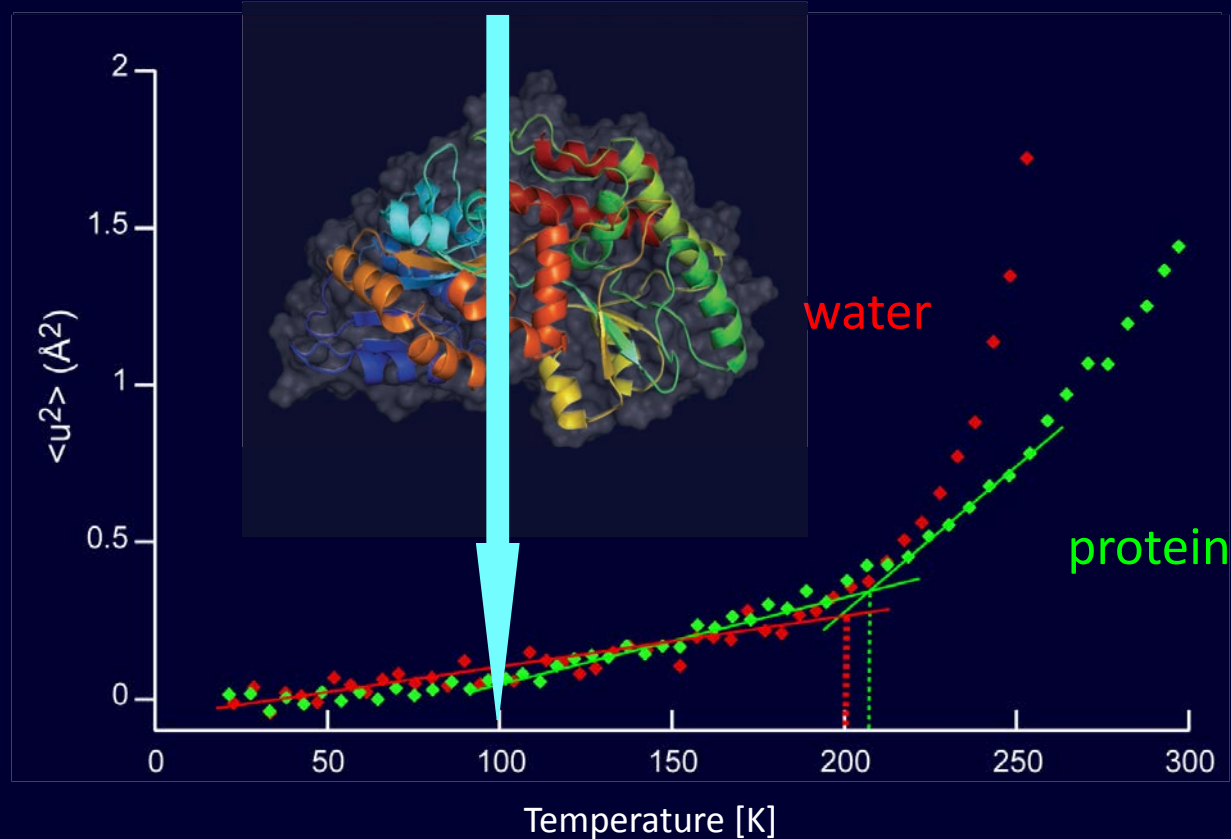
Time-resolved (monochromatic)  
room-temperature  
crystallography



Structural changes  
during protein functioning  
(kinetics)

# Temperature-dependent side-chain flexibility from neutron scattering

Cryo X-ray data collection



Wood, Frölich, Gabel, Moulin, Haertlein, Paciaroni, Zaccai, Tobias & Weik (2008) JACS 130, 4586

Cryo-cooling at 500 K / s : protein conformational changes quenched at 200 K

Halle (2004) PNAS 2004, 4793

# Study of protein dynamics by (temperature-dependent) X-ray crystallography has a long history

Frauenfelder, Petsko, Tsernoglou (1979) *Nature* 280, 558

Temperature-dependent X-ray diffraction as a probe of protein structural dynamics

Singh, Bode, Huber (1980) *Acta Crystallographica Section B* 36, 621

Low-temperature protein crystallography. Effect on flexibility, temperature factor, mosaic spread, ...

Hartmann, Parak, Steigemann, Petsko, Ponzi, Frauenfelder (1982) *PNAS* 79: 4967

Conformational substates in a protein: structure and dynamics of metmyoglobin at 80 K

Tilton, Dewan, Petsko (1992) *Biochemistry* 31: 2469

Effects of temperature on protein structure and dynamics:

X-ray crystallographic studies of the protein ribonuclease-A at nine different temperatures from 98 to 320 K

Ostermann, Waschipky, Parak, Nienhaus (2000) *Nature* 404: 205

Ligand binding and conformational motions in myoglobin

Schlichting, Berendzen, Chu, Stock, Maves, Benson, Sweet, Ringe, Petsko, Sligar (2000) *Science* 287: 1615

The catalytic pathway of cytochrome p450cam at atomic resolution

·  
·  
·

# Protein conformational heterogeneity greater in RT than in 100 K structures

Fraser, van den Bedem, Samelson, Lang, Holton, Echols & Alber (2011) PNAS 108, 16247

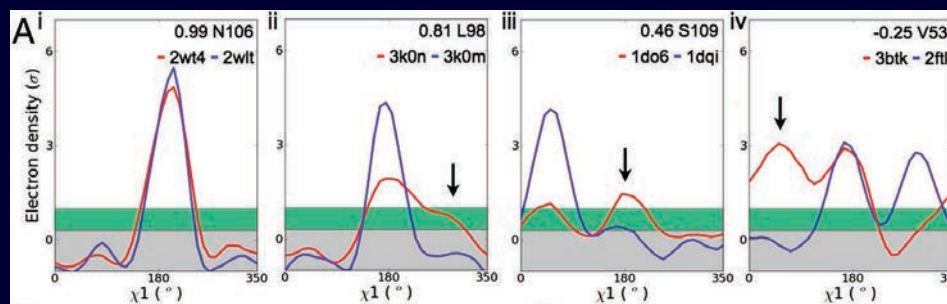


100 K

RT

Alternate conformation of H94  
In H-Ras at RT, but not at 100 K

Cryo-cooling remodels  
conformational distributions in  
35% of all protein side-chains



Tools to analyse conformational heterogeneity in crystal structures:

- **RINGER**: samples e- density around side-chain dihedrals below  $1\sigma$  level (Lang *et al.* (2010) Protein Sci. 19, 1420)
- **qFit**: automates building of alternative polypeptide conformations (van den Bedem *et al.* (2009) Acta Cryst. D65, 1107)
- Time-averaged crystallographically restrained MD **refinement of ensembles** (Burnley *et al.* (2012) eLife 1, e00311)
- **END, RAPID**: place e- density maps on absolute scale and calculate noise at each position in the map (Lang *et al.* (2014) PNAS 111, 237)

electronic  
transitions

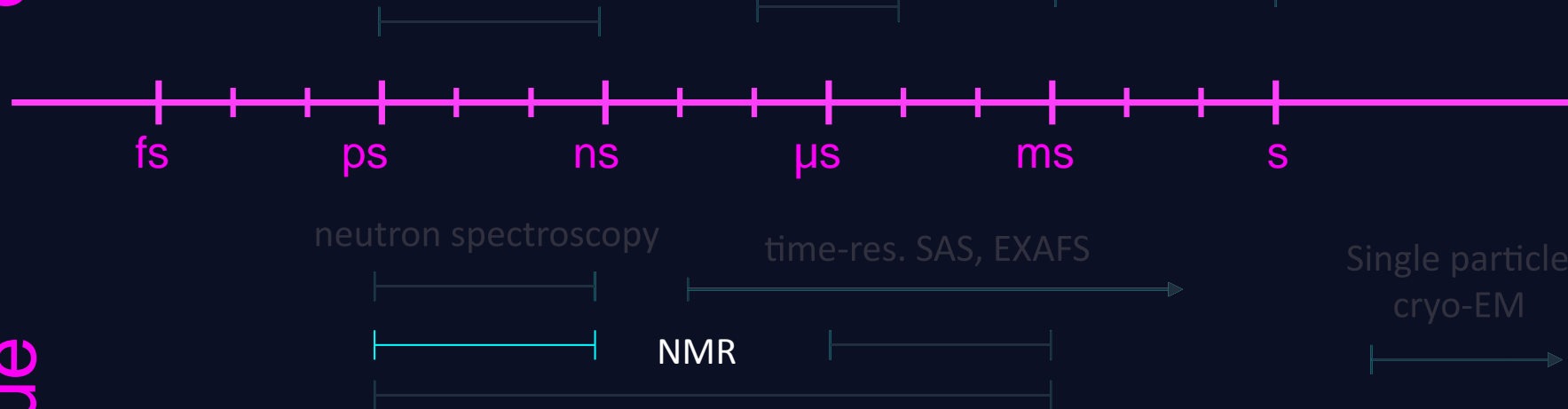
thesis

dynamics

# Hidden alternative structures of proline isomerase essential for catalysis

James S. Fraser<sup>1</sup>, Michael W. Clarkson<sup>2</sup>, Sheena C. Degnan<sup>1</sup>, Renske Erion<sup>1</sup>, Dorothee Kern<sup>2</sup> & Tom Alber<sup>1</sup>

(2009) Nature 462, 669



technique

# Integrated description of protein dynamics from room-temperature X-ray crystallography and NMR

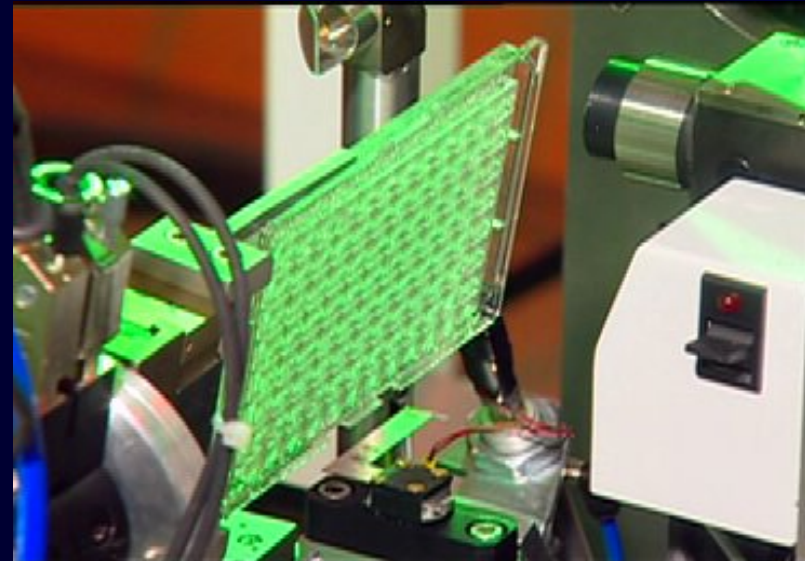
R. Bryn Fenwick<sup>a</sup>, Henry van den Bedem<sup>b</sup>, James S. Fraser<sup>c</sup>, and Peter E. Wright<sup>a,1</sup> (2014) PNAS 111, E445



# (Serial) Room temperature crystallography also important for ...

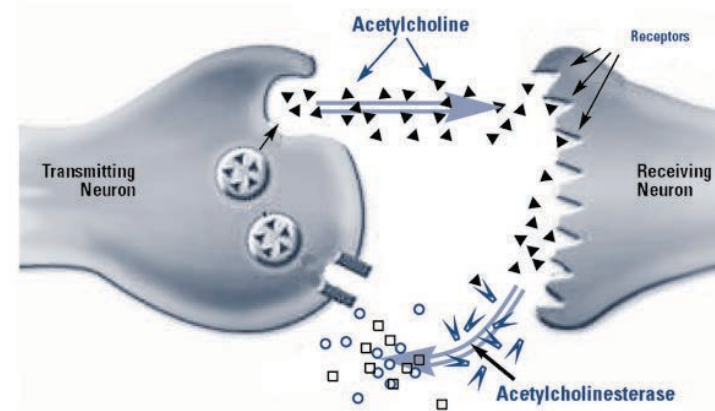
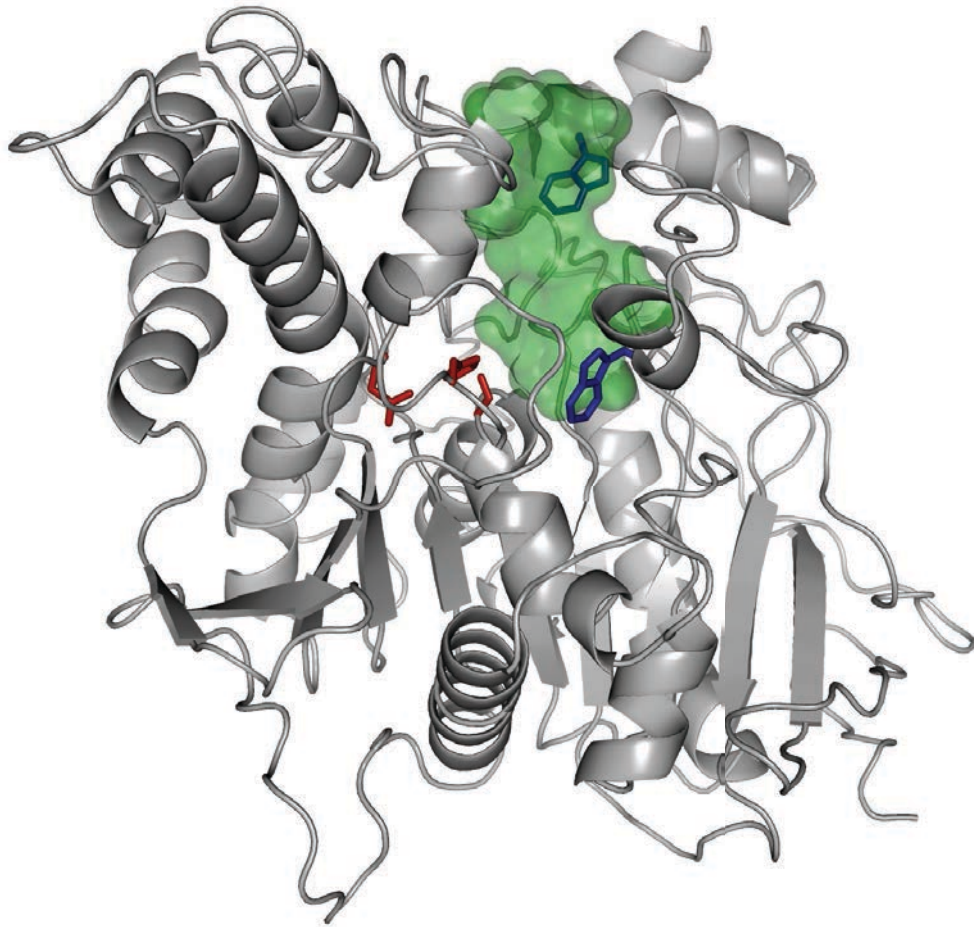
- collecting data from (fully) oxidized structure by spreading dose over many crystals  
(e.g. 400 xtals used to collect fully oxidized cytochrome c oxidase (Aoyama et al. (2009) PNAS 106, 2165))
- *in situ* crystal screening and data collection

FIP (ESRF BM30A)



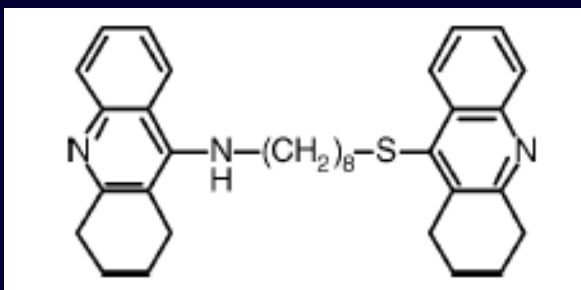


# Drug design and side-chain equilibrium dynamics: Acetylcholinesterase

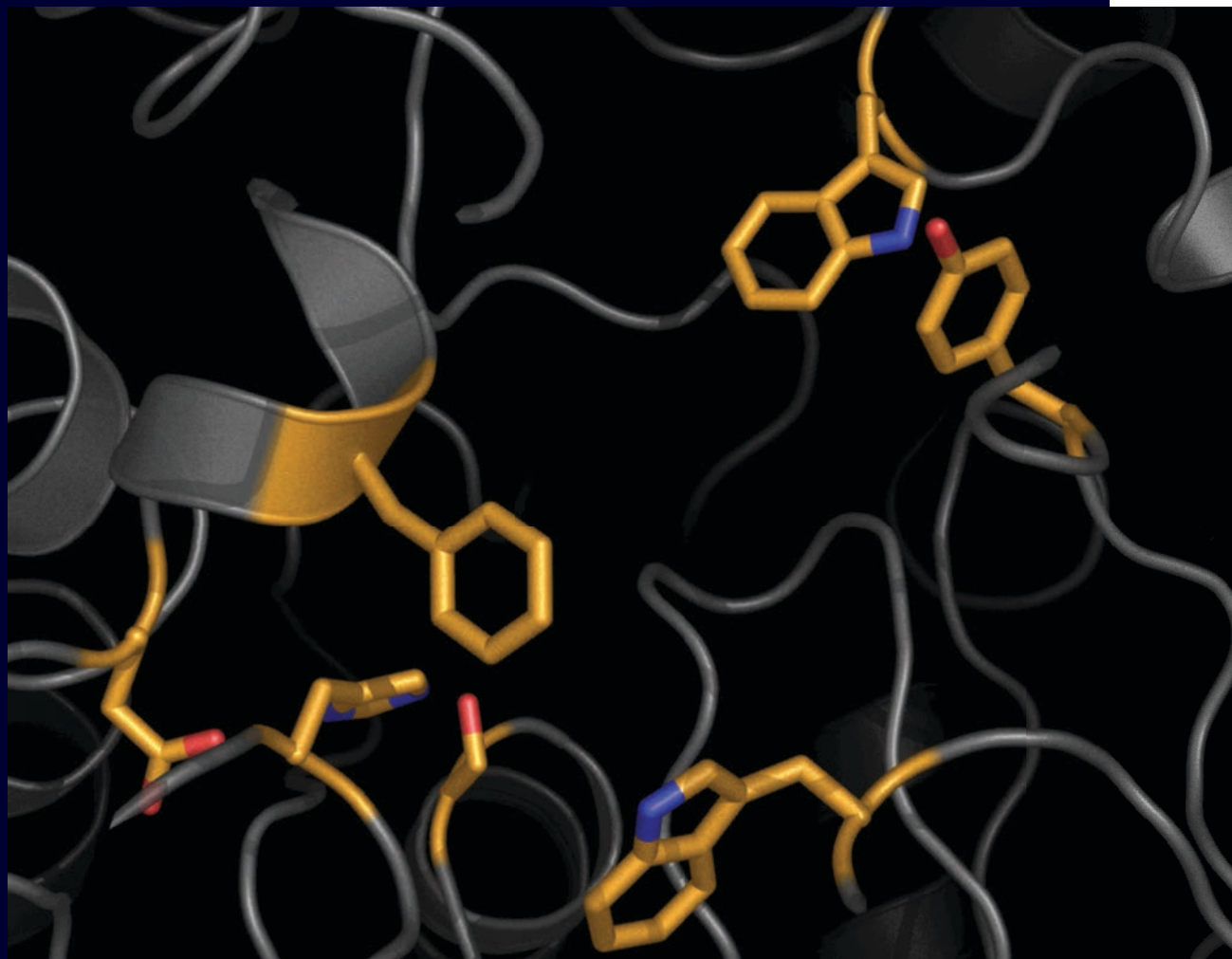
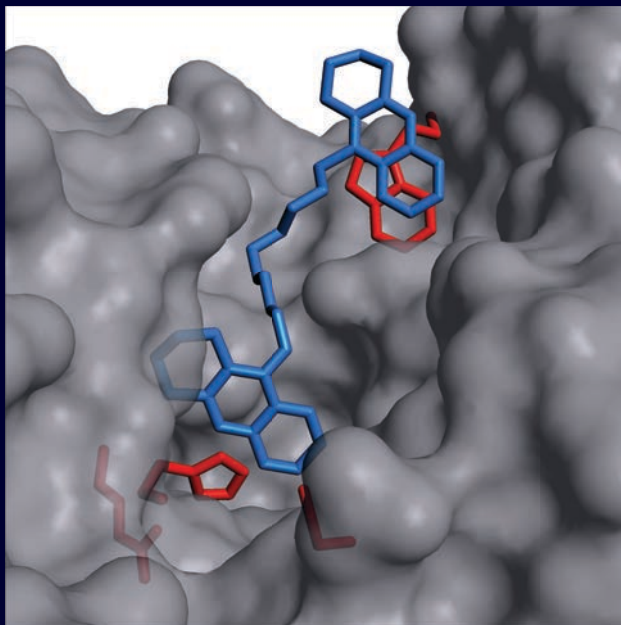


- hydrolyses neurotransmitter acetylcholine
- Nature's most rapid enzyme
- target of palliative Alzheimer drugs
- target of organophosphates

## Tryptophan in binding site undergoes conformational change



Savini et al. (2003) *J Med Chem*, 46, 1



Colletier, Sanson, Nachon, Gebellieri, Fattorusso, Campiani & Weik (2006) *JACS* **128**, 4526



Trp279 movement ...

... induced fit ... ?

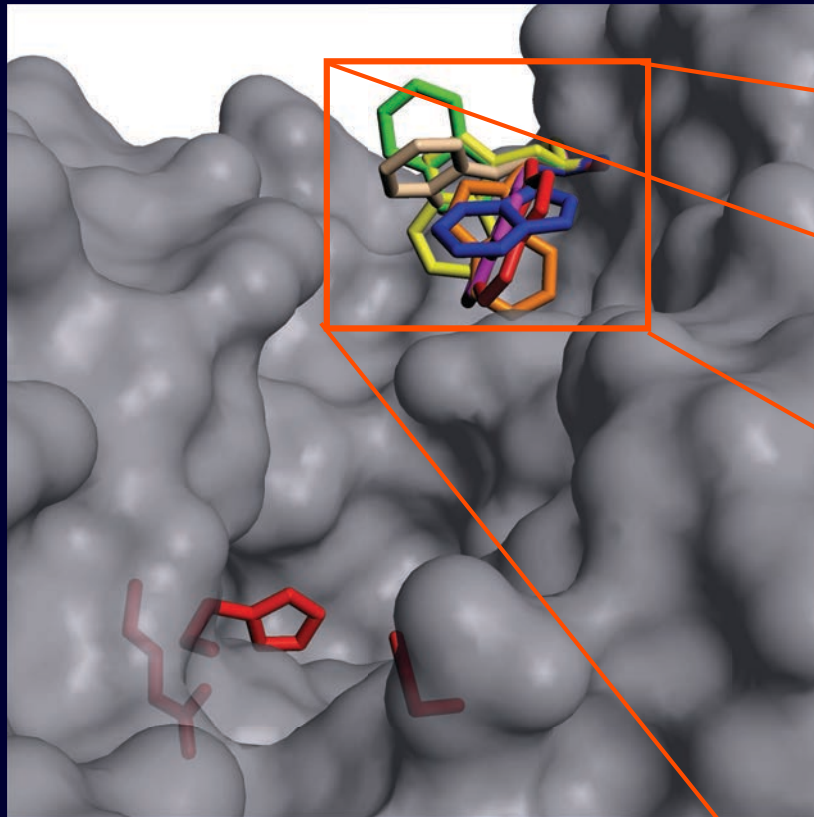
(first binding, then conformational change)



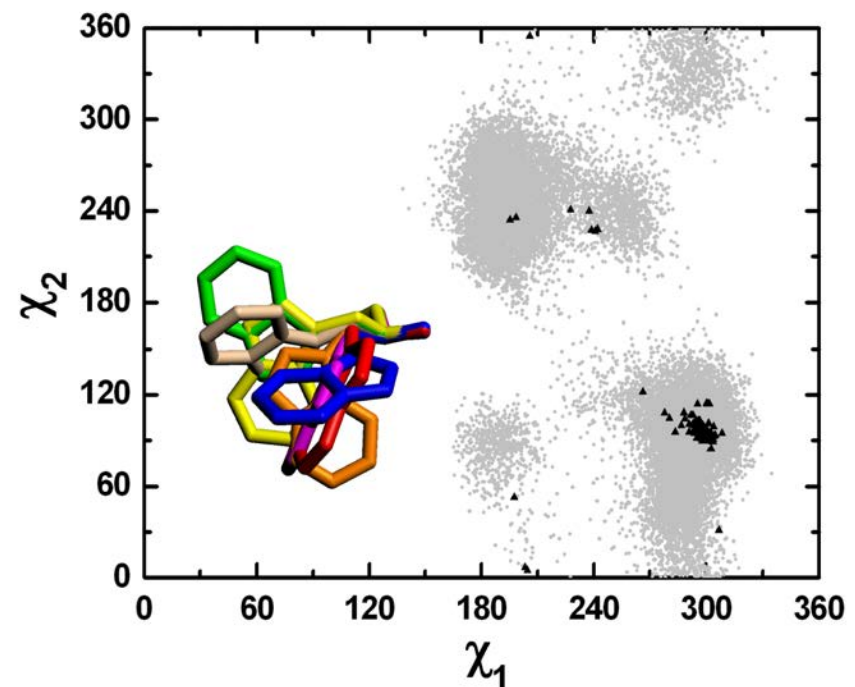
... or pre-existing equilibrium dynamics ?

(conformational fluctuation, then binding)

# Pre-existing equilibrium conformations selected by ligand binding

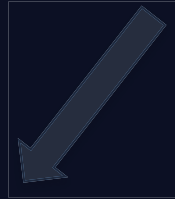


MD simulation of **native** AChE



Xu, Colletier, Jiang, Silman,  
Sussman & Weik (2008) *Protein Sci.* 17, 601

How can high flux-density synchrotron sources contribute to studying protein dynamics ?



Static  
room-temperature  
crystallography



Distribution of side chain dynamics  
(equilibrium fluctuations)



Time-resolved (monochromatic)  
room-temperature  
crystallography

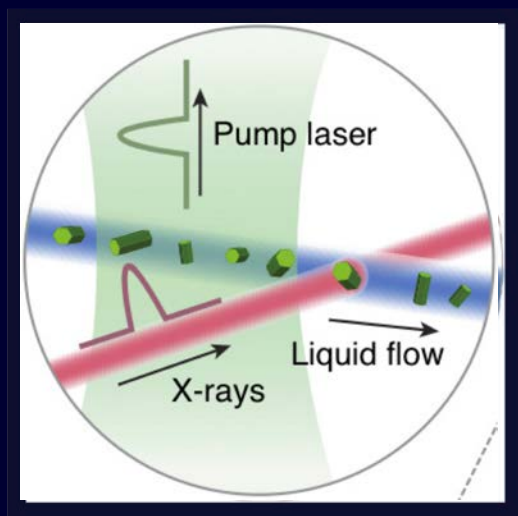


Structural changes  
during protein functioning  
(kinetics)



# Time-resolved Serial Synchrotron Microsecond Crystallography (SSMX)

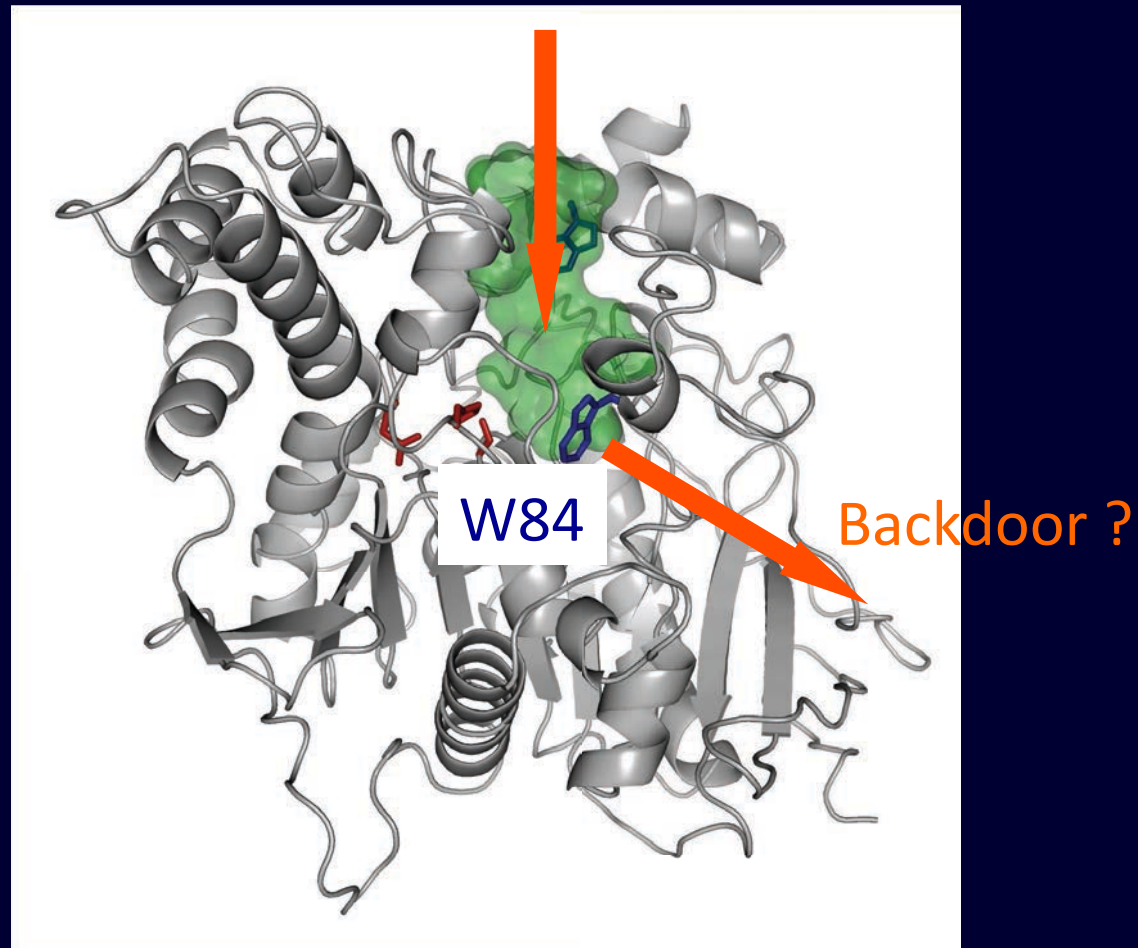
- serial microcrystal delivery (injectors, solid supports, ...)
- reaction trigger: - optical - laser pulse
  - rapid mixing based on microfluidics  
(sub-ms time scale: Graceffa et al. (2013) JSR 20, 820)
- $\mu\text{s}$  – ms X-ray pulses, e.g. with chopper system  
Cammarata et al (2009) Rev. Sci. Instr. 80, 015101



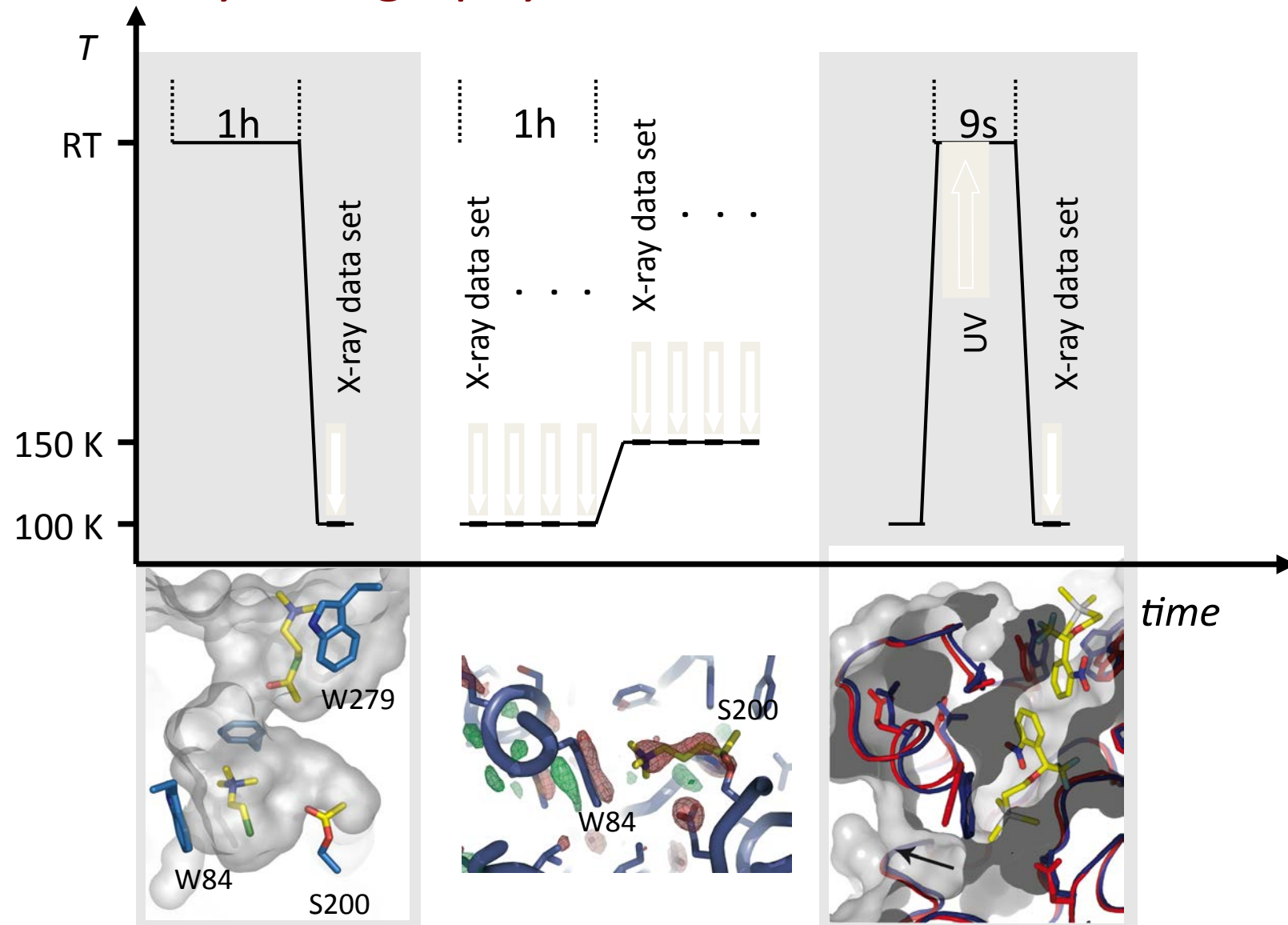
Extracted from Aquila et al (2012) Optics Express 20, 2706

Examples of macromolecular function  
on ms- $\mu\text{s}$  time scale ?

Acetylcholinesterase:  
substrate and product traffic on the  $\mu$ s time scale



# Kinetic crystallography shows small W84 movement



Colletier, Fournier, Greenblatt, Stojan, Sussman, Zaccai, Silman & Weik (2006) *EMBO J.* 25, 2746

Colletier, Bourgeois, Sanson, Fournier, Sussman, Silman & Weik (2008) *PNAS*, 105, 11742

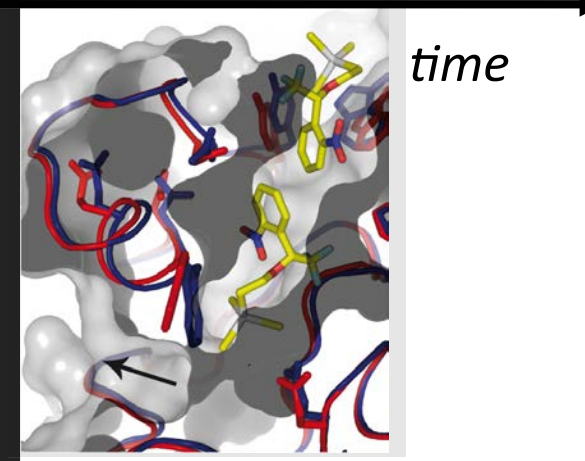
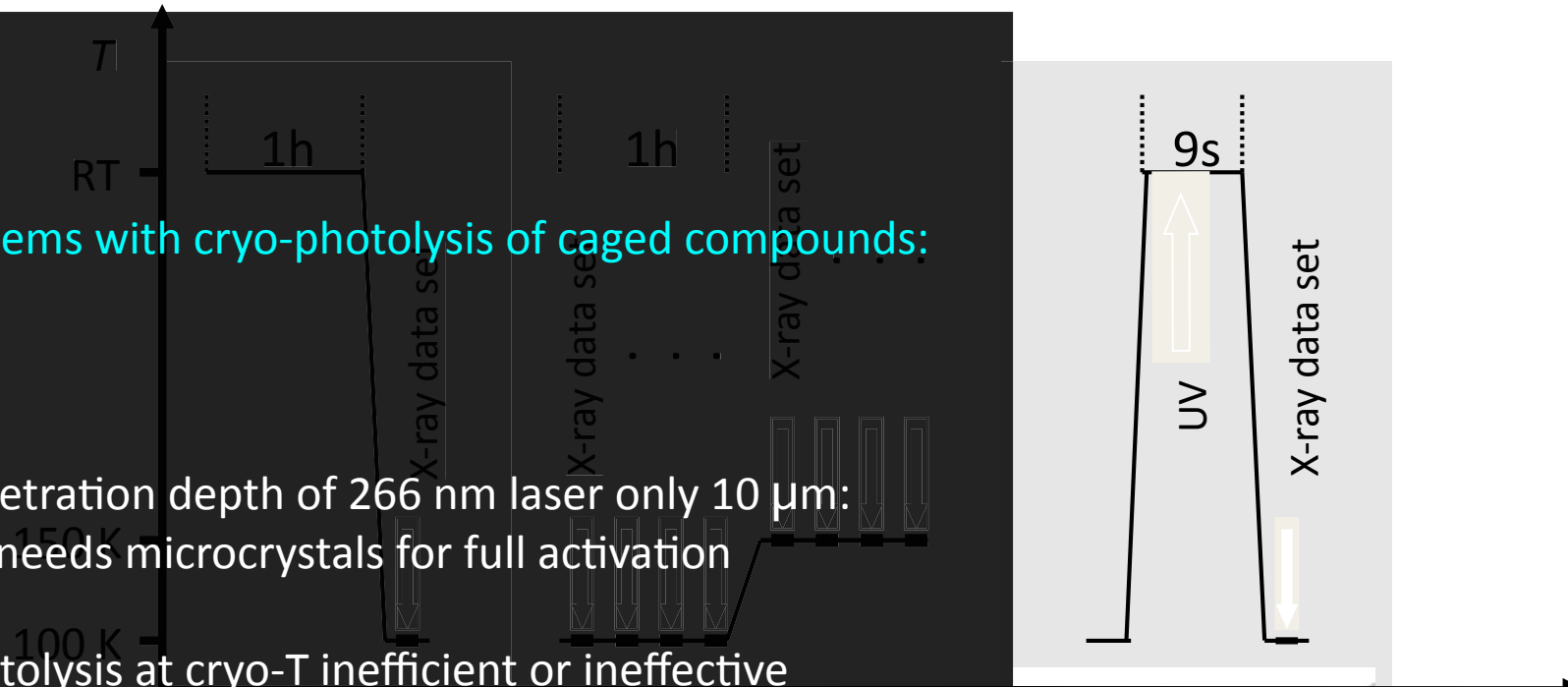
Colletier, Royant, Sanson, Specht, Nachon, Masson, Zaccai, Sussman, Goeldner, Silman, Bourgeois & Weik (2007) *Acta D63*, 1115

# Kinetic crystallography shows small W84 movement

## Problems with cryo-photolysis of caged compounds:

- penetration depth of 266 nm laser only 10  $\mu\text{m}$ : needs microcrystals for full activation
- photolysis at cryo-T inefficient or ineffective
- caged compounds very X-ray sensitive

Time-resolved  
Serial Synchrotron Microsecond Crystallography  
could provide functional snapshots



Colletier, Fournier, Greenblatt, Stojan, Sussman, Zaccai, Silman & Weik (2006) *EMBO J.* 25, 2746

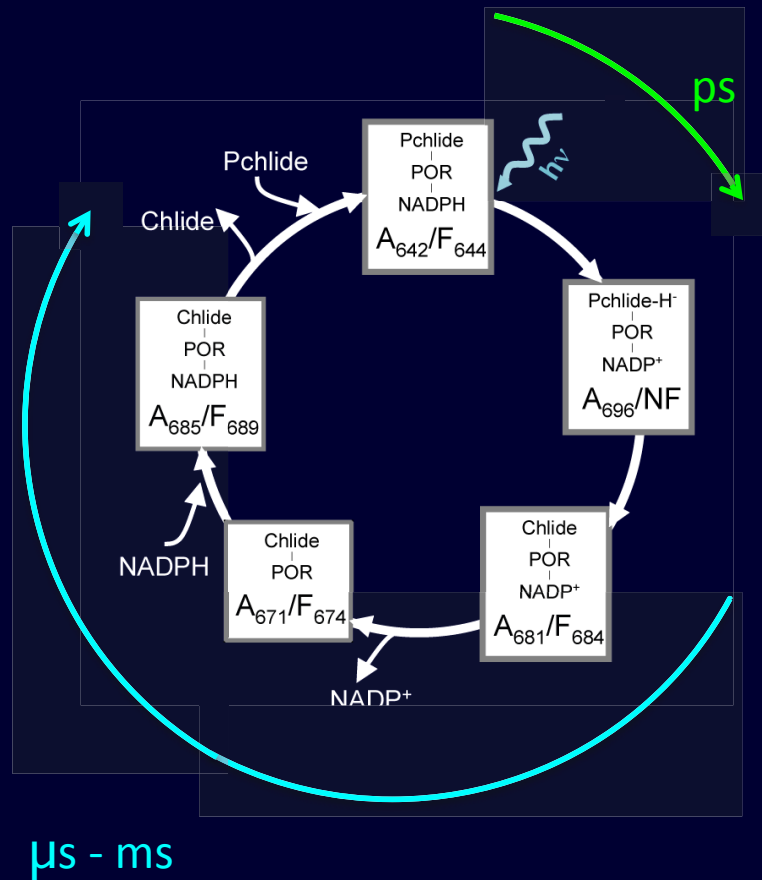
Colletier, Bourgeois, Sanson, Fournier, Sussman, Silman & Weik (2008) *PNAS*, 105, 11742

Colletier, Royant, Sanson, Specht, Nachon, Masson, Zaccai, Sussman, Goeldner, Silman, Bourgeois & Weik (2007) *Acta D63*, 1115

# Pchlide oxidoreductase (POR) : one of two light-activated enzymes in Nature

Heyes & Hunter (2005) *TIBS* 30, 642

POR catalyses generation of chlorophyll precursor chlide



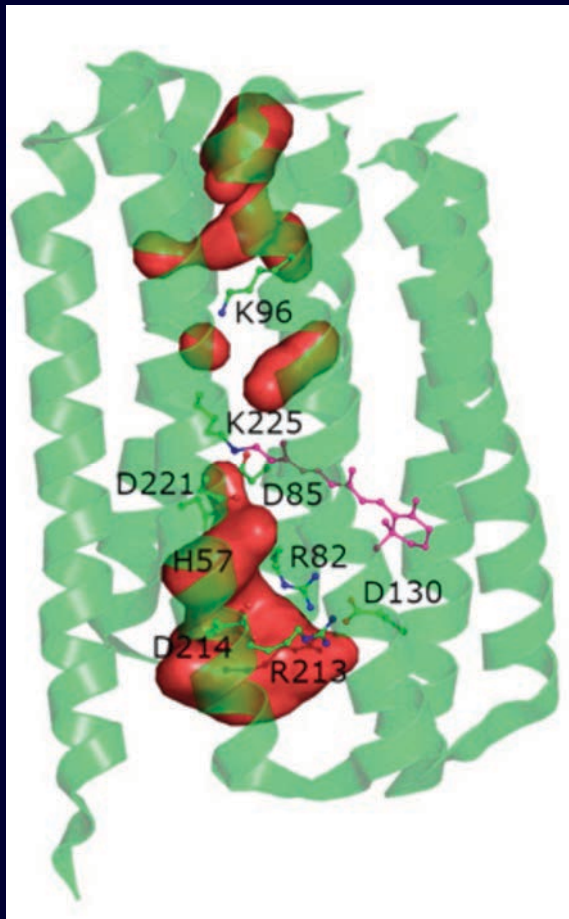
- early excited state events: **ps**
- following proton transfer and product release: **μs – ms**
- problem: no crystals (yet)

Heyes & Hunter (2005) *TIBS* 30, 642

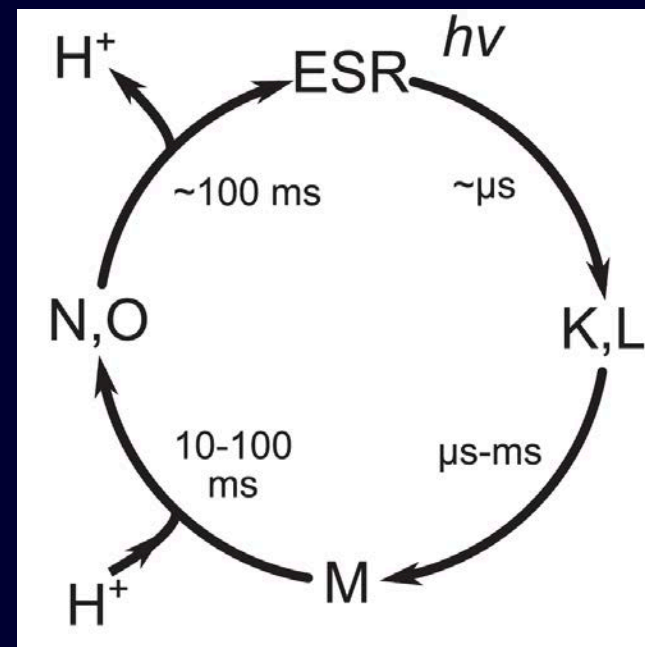


## Proteorhodopsin from nonmarine bacteria

Gushchin, Chervakov, Kuzmichev, Popov, Round, Borshchevskiy, Ishchenko, Petrovskaya, Chupin, Dolgikh, Arseniev, Kirpichnikov, Gordeliy (2013) *PNAS* 110: 12631

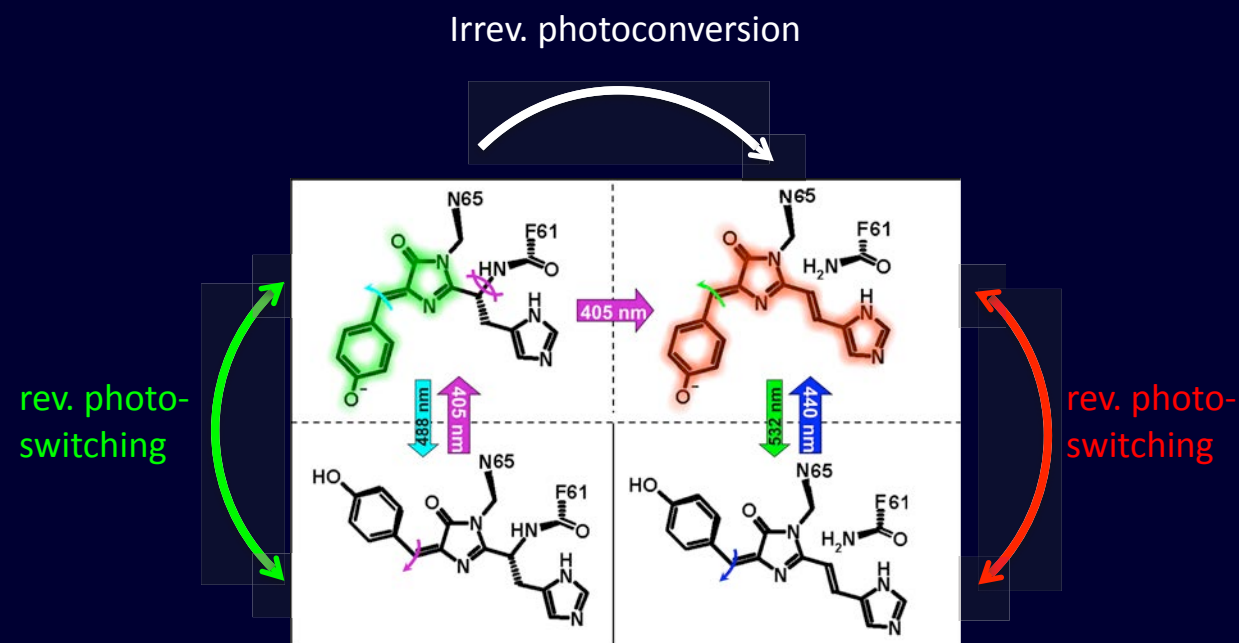


### Photocycle



# Photoswitching of the fluorescent protein IrisFP

Adam, Lelimousin, Boehme, Desfonds, Nienhaus, Field, Wiedenmann, McSweeney, Nienhaus, Bourgeois (2008) *PNAS* **105**, 18343



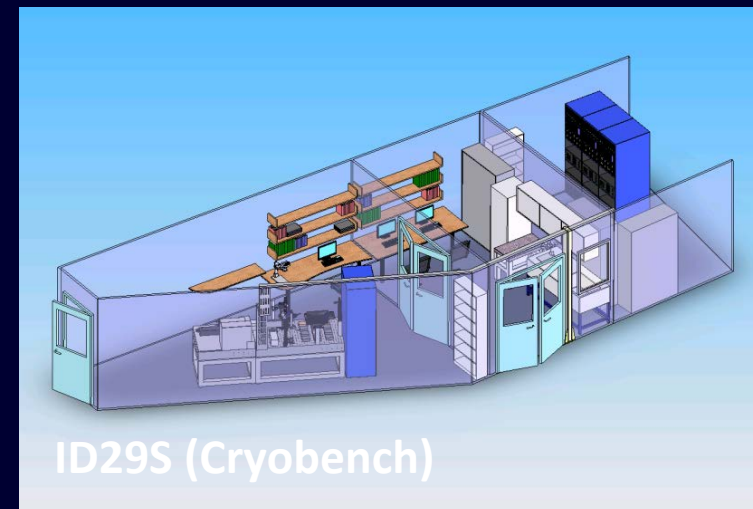
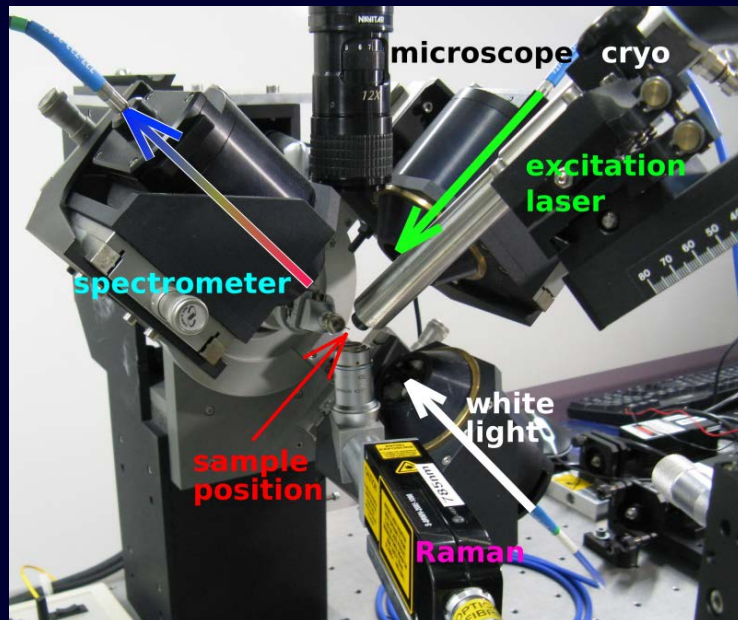
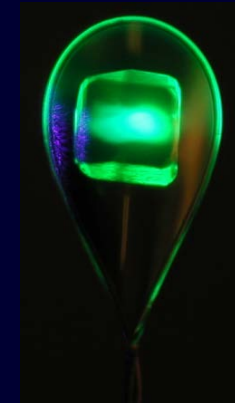
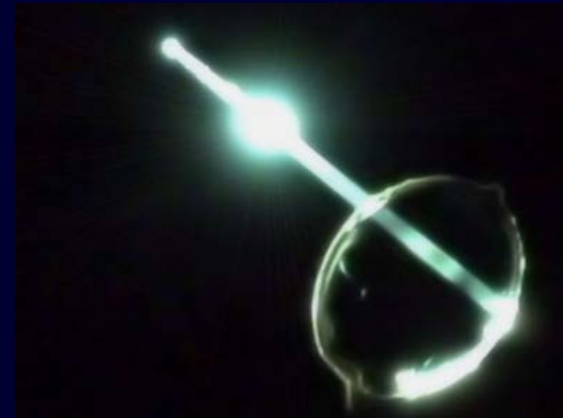
Adam et al. (2008) *PNAS* **105**, 18343

# Complementary methods important for kinetic crystallography: *in crystallo* spectroscopy platform Cryobench (IBS / ESRF) Antoine Royant

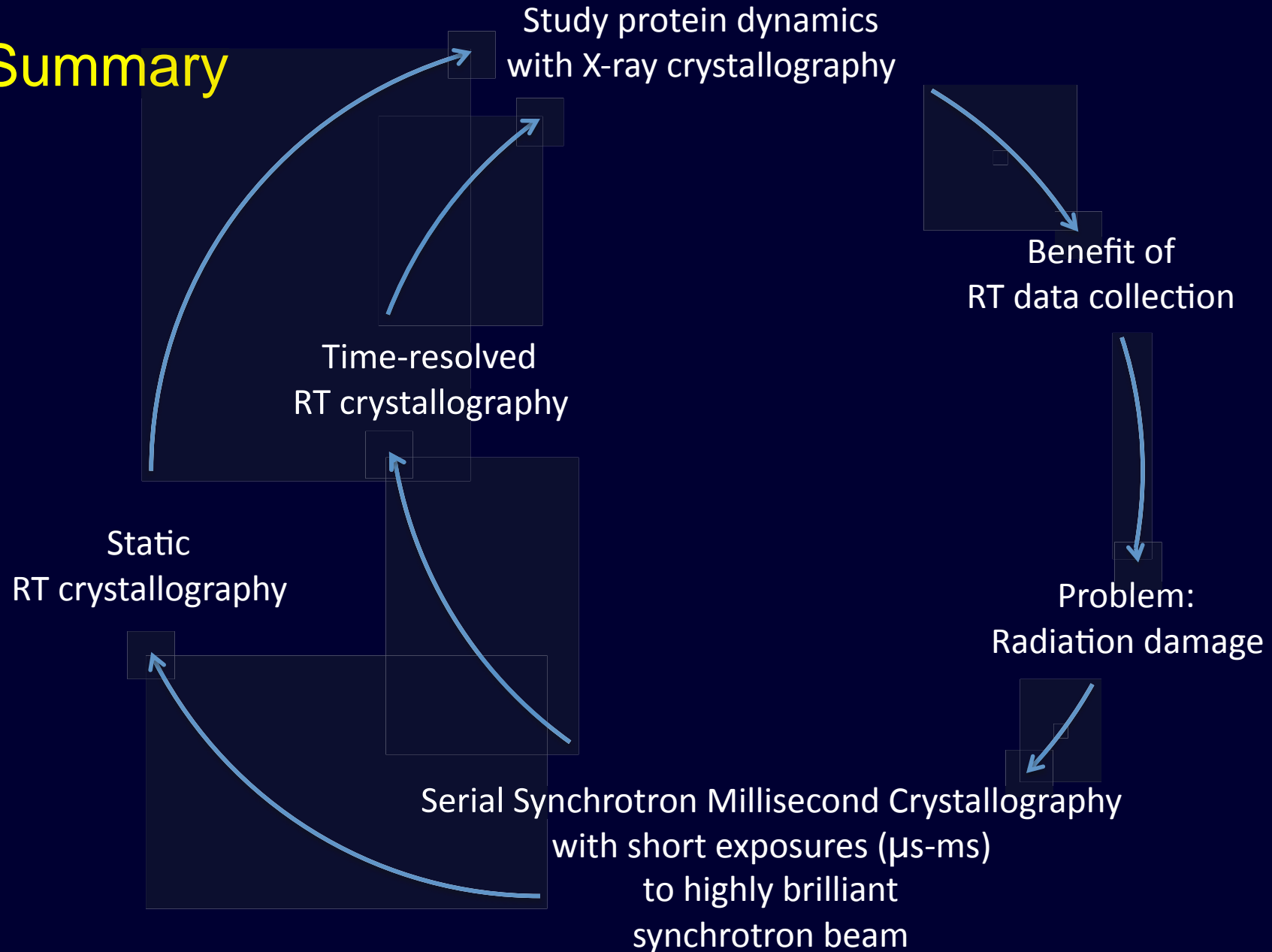
## Spectroscopy:

- UV/vis absorption
- Fluorescence
- Raman

on protein crystals



# Summary





# *Merci à ...*

IBS, SPD

E. Carletti  
J.-P. Colletier  
A. Royant  
B. Sanson  
G. Santoni

IBS, Pixel

D. Bourgeois

Weizmann Institute

I. Silman  
J. Sussman

Univ. Strasbourg

A. Specht  
M. Goeldner

EMBL, Grenoble

R. Ravelli  
(now Maastricht Univ.)

ESRF, Grenoble

S. McSweeney  
(now BNL)

IRBA, La Tronche

F. Nachon  
P. Masson

ESRF MX staff,  
ESRF BAG

Shanghai Institute of  
Materia Medica

Y. Xu



## 8th International Workshop X-ray Radiation Damage to Biological Crystalline Samples

### Organisers

**Elspeth Garman**  
University of Oxford, United Kingdom

**Martin Weik**  
Institut de Biologie Structurale, France

**Gleb Bourenkov**  
EMBL Hamburg, Germany

**Henry Chapman**  
DESY, Germany

**Sean McSweeney**  
BNL, United States of America

**Alke Meents**  
DESY, Germany

**Colin Nave**  
Diamond Light Source, United Kingdom

**Arwen Pearson**  
University of Leeds, United Kingdom

**Raimond Ravelli**  
Leiden University Medical Centre,  
The Netherlands

**Gerd Rosenbaum**  
University of Georgia,  
Argonne National Laboratory,  
United States of America

**Thomas Schneider**  
EMBL Hamburg, Germany

**Soichi Wakatsuki**  
SLAC, Stanford University,  
United States of America

10–12 April 2014

EMBL and DESY, Hamburg  
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# LES HOUCHES-TSRC WORKSHOP ON PROTEIN DYNAMICS

19 - 23 MAY 2014, LES HOUCHES, FRANCE



## CONFIRMED SPEAKERS

T. Barends (DE)	D. Leitner (USA)
M. Blackledge (FR)	F. Mulder (DK)
A.-N. Bondar (DE)	A. Paciaroni (IT)
M. Cammarata (FR)	A. Pearson (GB)
P. Champion (USA)	P. Schanda (FR)
M. Cho (KR)	S. Scheuring (FR)
M. Field (FR)	J.-E. Shea (USA)
J. Fraser (USA)	G. Stock (DE)
P. Hamm (CH)	B. Strodel (DE)
M. Havenith (DE)	M. Tarek (FR)
S. Hayashi (JP)	J. van Thor (GB)
M. Heyden (DE)	J. Wand (USA)
D. Kokh (DE)	J. Zaccai (FR)
T. Komatsuzaki (JP)	

This workshop is a forum for presenting, teaching and discussing results from the application of state-of-the-art experimental (including, but not limited to, optical spectroscopy, NMR spectroscopy, X-ray crystallography, XFELs, electron microscopy and scattering methods), and theoretical and computational approaches to studying protein dynamics.

APPLICATION AT:

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ORGANIZERS

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