The benefit of room-temperature data collection for studying protein dynamics

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The image depicts a flowchart of techniques and dynamics in the study of molecular processes. From top to bottom:

**Dynamics**
- Protein synthesis
- Enzyme catalysis
- Protein folding
- Domain motions
- Side chains motions
- Bond vibrations
- Electronic transitions
- Enzymatic transition states

**Technique**
- Neutron spectroscopy
- Time-res. SAS, EXAFS
- NMR
- THz spectroscopy
- EPR spectroscopy
- Mössbauer spectroscopy
- Fluorescence spectroscopy
- MD simulations / QM/MM
- Single particle cryo-EM
- Single molecule experiments
- High-speed AFM
- Kinetic crystallography (freeze-trapping)
- Time-res. Laue crystallography
- Time-res. FTIR
- XFEL
dynamics

- electronid transitions
- enzymatic transition states
- bond vibrations
- side chain motions
- domain motions
- protein folding

technique

- neutron spectroscopy
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- time-res. FTIR
- XFEU
- time-res. Laue crystallography

- protein synthesis
- enzyme catalysis

- Single particle c|yo-EM|
- Single molecule experiments

- kinetic crystallography (freeze-trapping)
FS: electron transitions, enzymatic transitions, bond vibrations, side chain motions, domain motions, protein folding

PS: enzyme catalysis

NS: protein synthesis

μS: neutron spectroscopy, time-res. SAS, EXAFS

MS: NMR

S: THz spectroscopy, EPR spectroscopy, Mössbauer spectroscopy, fluorescence spectroscopy, Mössbauer, high-speed AFM

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

XFEL: time-res. Laue crystallography (freeze-trapping)
How can high flux-density synchrotron sources further contribute to studying protein dynamics?

Static room-temperature crystallography

Time-resolved (monochromatic) room-temperature crystallography

Distribution of side chain dynamics (equilibrium fluctuations)

Structural changes during protein functioning (kinetics)
How can high flux-density synchrotron sources further contribute to studying protein dynamics?

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 ($\lambda=0.98$ Å)

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

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


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

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

$T > 110 \text{ K}$: $e^-$, $H^\bullet$ 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

$> 160 \text{ K}$
(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)

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 D68, 810

Serial crystallography à la XFEL SFX
(reviewed by Schlichting & Miao (2012) COSB 22, 613):
Serial Synchrotron Microsecond Crystallography

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 μs time scale?
- needs very fast detector
- sample heating?

Sample delivery:
- LCP injector in vacuum (Weierstall et al.):
  - min. speed: 30 μm/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)

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

\[ \langle u^2 \rangle (\text{Å}^2) \]

\[ \text{Temperature [K]} \]

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

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

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

Ligand binding and conformational motions in myoglobin

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

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

Cryo-cooling remodells
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 1σ 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)
Hidden alternative structures of proline isomerase essential for catalysis

James S. Fraser¹, Michael W. Clarkson², Sheena C. Degnan¹, Renske Erion¹, Dorothee Kern² & Tom Alber¹


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

R. Bryn Fenwick⁶, Henry van den Bedem⁷, James S. Fraser⁸, and Peter E. Wright⁹,¹

(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

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

Xu, Colletier, Jiang, Silman,
How can high flux-density synchrotron sources contribute to studying protein dynamics?

- Static room-temperature crystallography
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- Distribution of side chain dynamics (equilibrium fluctuations)
- 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)
- **µs – ms X-ray pulses**, e.g. with chopper system

Examples of macromolecular function
on ms-µs time scale?
Acetylcholinesterase: substrate and product traffic on the μs time scale
Kinetic crystallography shows small W84 movement

Colletier, Bourgeois, Sanson, Fournier, Sussman, Silman & Weik (2008) PNAS, 105, 11742
Kinetic crystallography shows small W84 movement

Problems with cryo-photolysis of caged compounds:

- penetration depth of 266 nm laser only 10 μm: 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, Bourgeois, Sanson, Fournier, Sussman, Silman & Weik (2008) PNAS, 105, 11742
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)
Proteorhodopsin from nonmarine bacteria

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

- Study protein dynamics with X-ray crystallography
- Time-resolved RT crystallography
- Static RT crystallography
- Serial Synchrotron Millisecond Crystallography with short exposures (μs-ms) to highly brilliant synchrotron beam
- Benefit of RT data collection
- Problem: Radiation damage
Merci à ...

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