Can Radiation Damage to Protein Crystals Be Outrun at Synchrotron Source Intensities?

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Protein Crystallography

Pre-1990: Nearly all X-ray data sets collected from crystals at or near room temperature.

Problem: Protein crystals are very radiation sensitive.

- Diffraction spots fade away before a complete data set can be collected.
- Data collected from many crystals must be scaled and merged to determine a structure.
- Room temperature crystals are hard to handle and ship.

Protein Crystallography

1990-2005: Cooling crystals to T=100 K dramatically reduces radiation damage.

- Development of high-throughput cryocrystallographic hardware and methods.
- Data collection from microcrystals

Today:

- Over 98% of all structural data sets are collected from crystals cooled to T=100 K.
- Most under-40 crystallographers have never collected a data set at T=300 K (or any T other than 100 K).
- 2% of MW>200kDa structures at RT, 80% before 2003

But. . .

- Cooling damages protein crystals.
- Cooling may not / does not capture room/biological temperature structural features that may be important in function. (E.g., Fraser et al.)
- Protein motions near 100 K are constrained by frozen solvent to be small and harmonic.
- Proteins at 100 K can't respond to most biologically relevant perturbations.

And. . .

- Protein and crystals are now abundant.
- Scaling and merging data from large numbers of crystals is becoming increasingly routine (e.g., XFELs).
- Tools for harvesting and collecting data at RT are much better.
- Tools for high-throughput RT microcrystallography being developed.
- In most areas of science, temperature is an important "knob".

So...

- Will T=100 K data remain sufficient as the focus shifts from first structures to detailed understanding of mechanism?

- Can radiation damage at and near room/biological temperature be reduced to facilitate structural studies of protein crystals with liquid solvent?

Some Questions

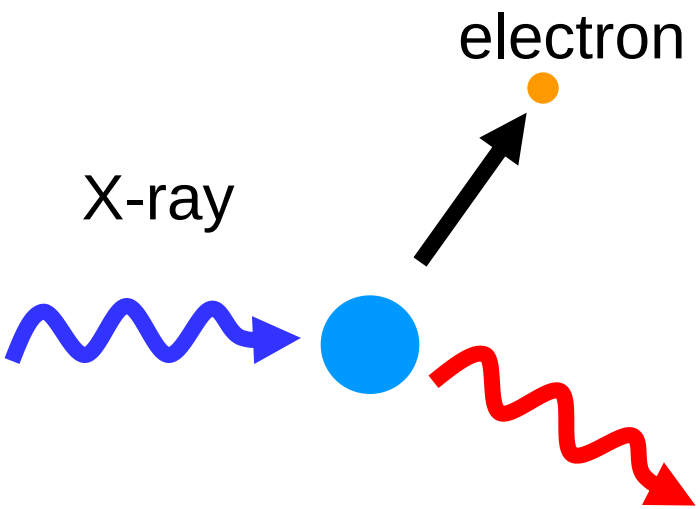
- What are the manifestations of radiation damage in protein crystals?
- What are the mechanisms of radiation damage?
- What are the timescales of these mechanisms?
- How do these mechanisms and their timescales evolve with temperature?
- Which of these mechanisms dominate in determining the manifestations of damage relevant in protein crystallography?

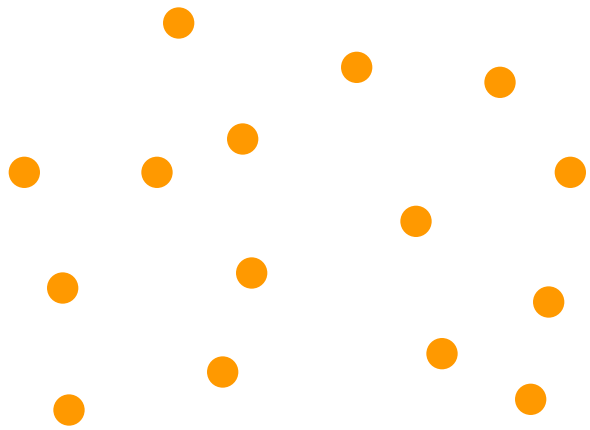
Some Questions

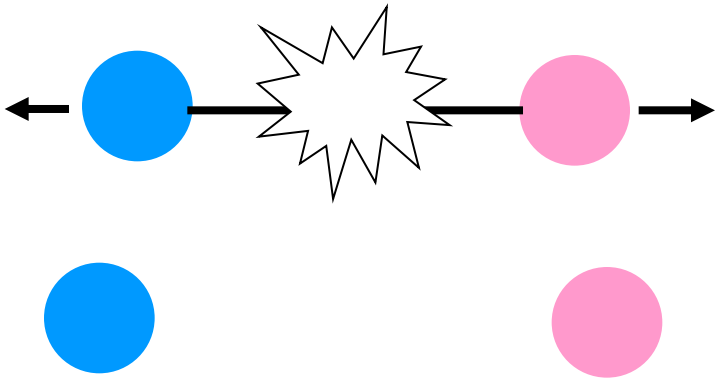
- Can some damage be outrun during data collection at synchrotron source (not FEL) intensities?

- Can radiation damage at and near room/biological temperature be reduced to facilitate structural studies of protein crystals with liquid solvent?

Radiation Damage Mechanisms

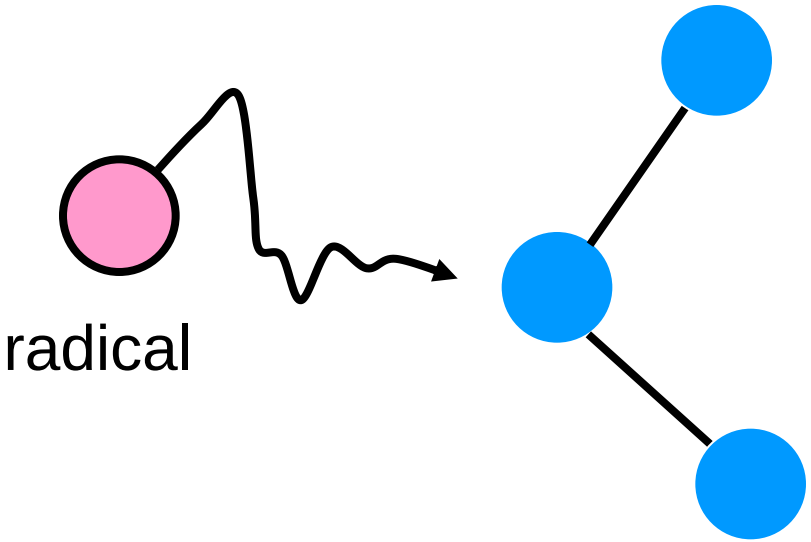


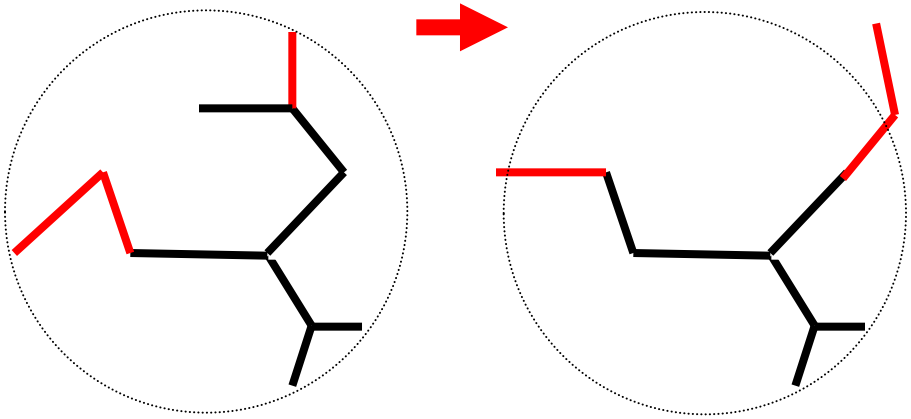


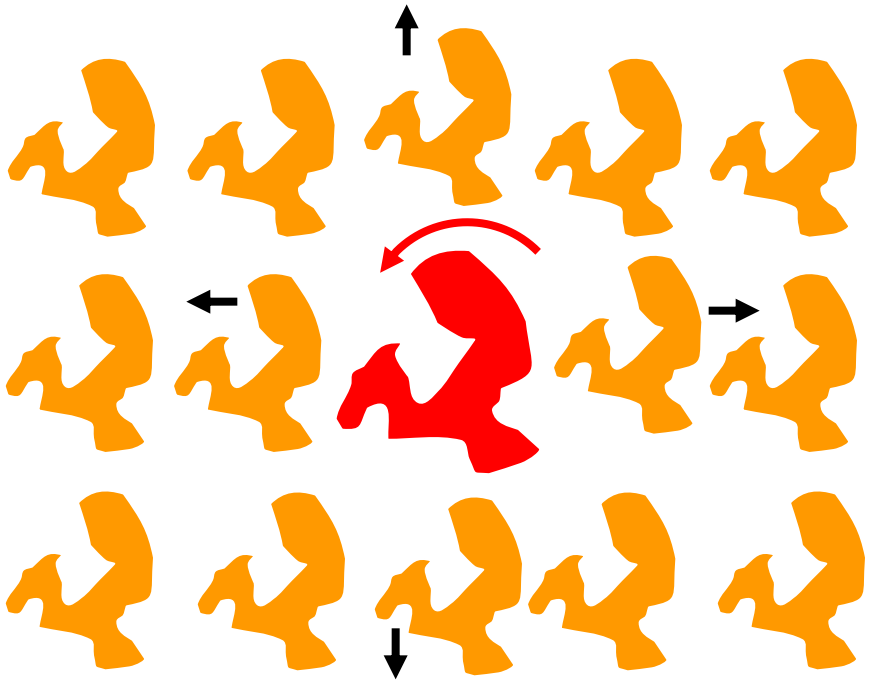


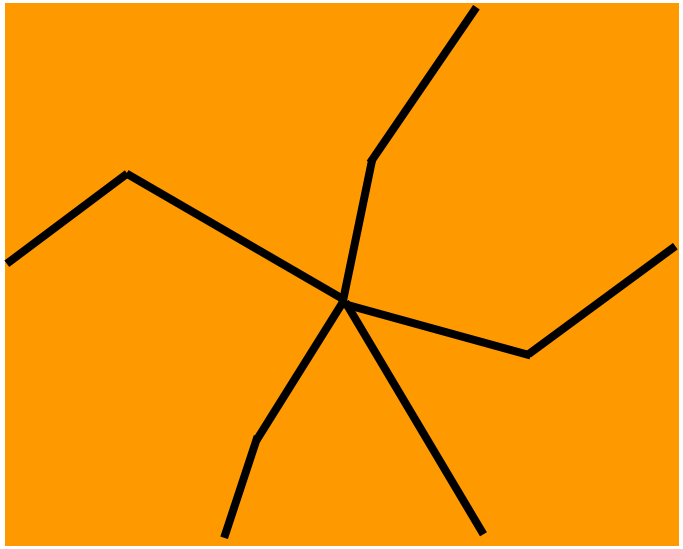
⇒ pressure / stress

⇒ lattice expansion

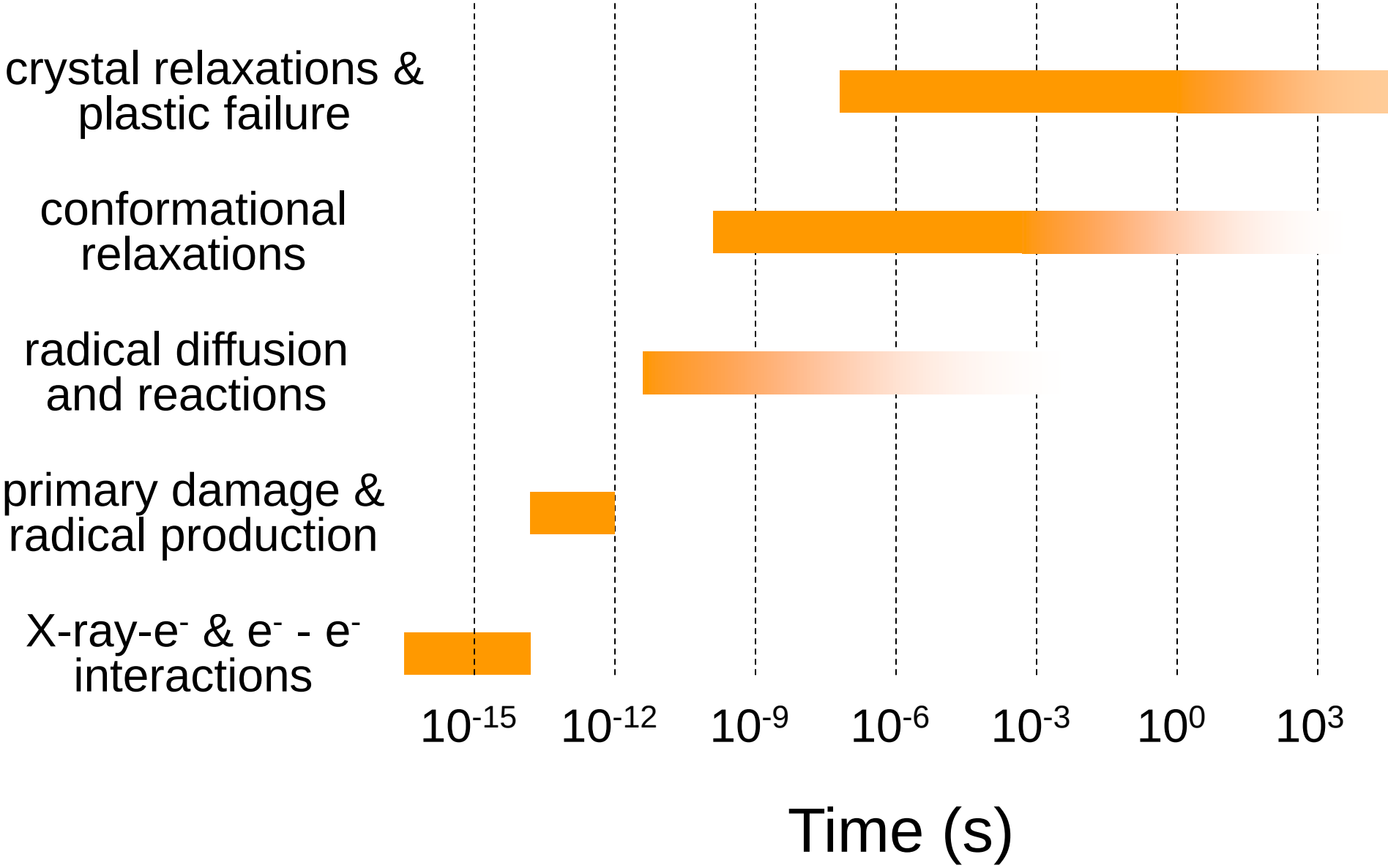








Timescales for Radiation Damage Processes



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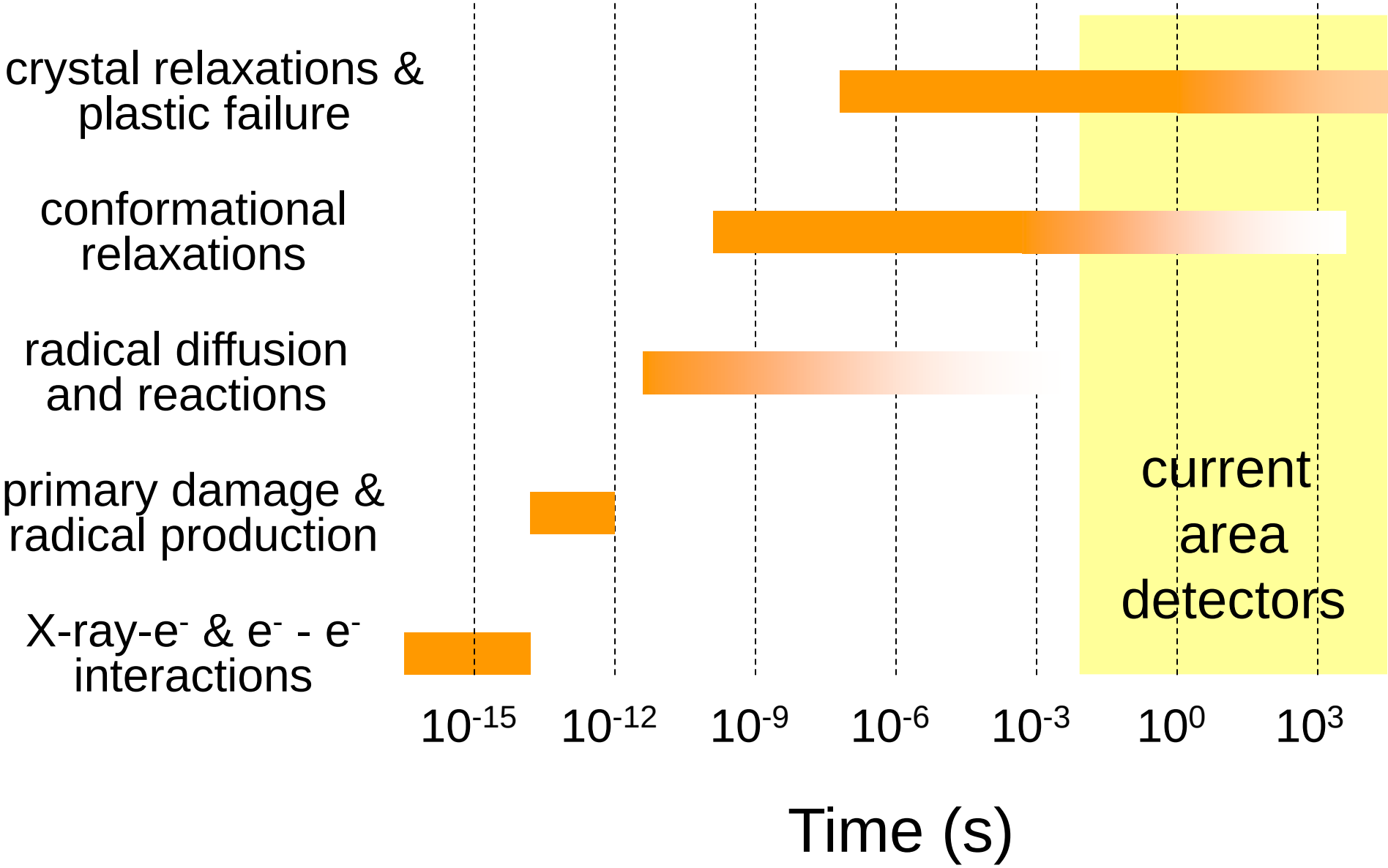
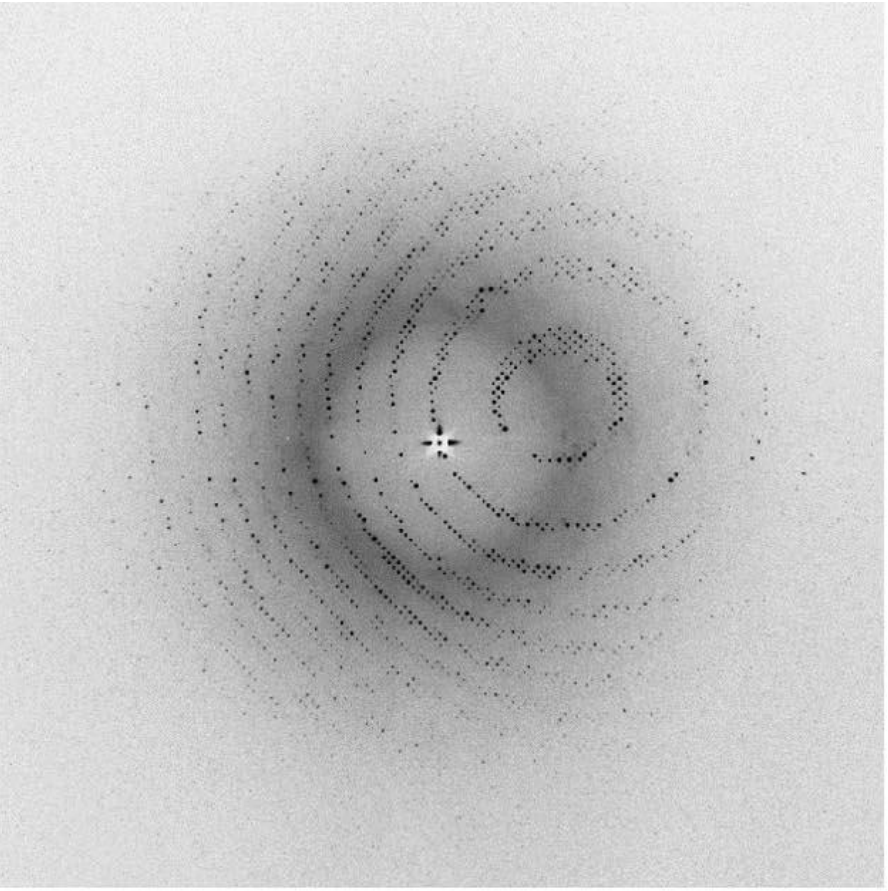
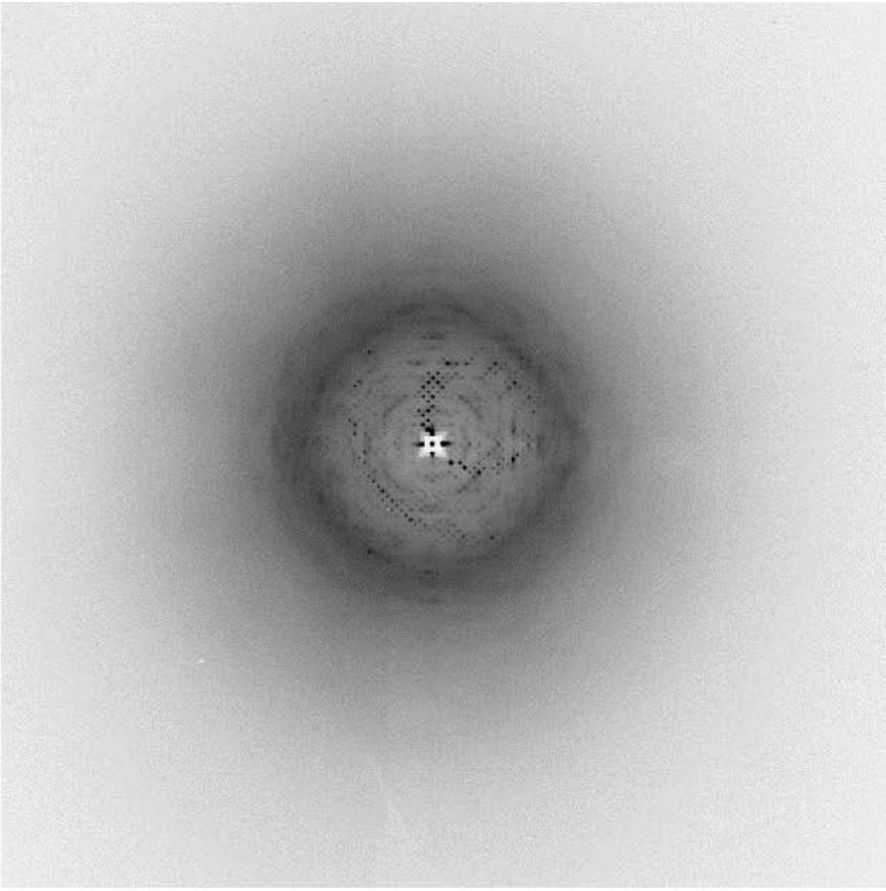


Manifestations of Radiation Damage in Crystallography





Manifestations of Radiation Damage in Crystallography

Global Damage:

- overall variation in diffracted intensities with resolution (k-space metric), versus X-ray dose
- all kinds of damage/disorder contribute, but those that produce the largest atomic displacements dominate

Site-Specific Damage:

- occupancy and conformation changes of specific sites (real-space metric)
- most sensitive to damage that is reproducible from unit cell to unit cell, which is only small part of total damage.

Metrics for Global Damage

Intensity scale factor

Wilson B factor

Scaling B factor

Half-dose $D_{1/2}$ for integrated intensity

R_d, R_R

Unit cell size

Mosaicity

Spectroscopy

Metrics for Global Damage

Intensity scale factor

Wilson B factor

Scaling B factor

Half-dose $D_{1/2}$ for integrated intensity

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Spectroscopy

Data Collection Issues

- Flux density
- Beam profile
- Dose calculation
- Beam drift and intensity fluctuations
- Non-uniform irradiation during sample rotations
- Crystal slippage and vibrations
- Dehydration

+ Inherent crystal-to-crystal variability in damage measured by individual metrics

Data Collection Issues

Consequences:

Accurately evaluating absolute and relative radiation sensitivities is **hard**, and requires measurements on **large numbers** of crystals.

Beware of claims of effects smaller than a factor of two, especially if they are based on measurements on only a few crystals.

Temperature Dependence of Radiation Damage

- **At T=100 K, all protein crystals appear to be comparably radiation sensitive** on a damage per dose (energy absorbed per kg) basis.

Maximum X-ray dose before diffraction becomes unacceptable:

~ 15-20 MGy ("Henderson Limit")

~ 43 MGy ("Garman Limit")

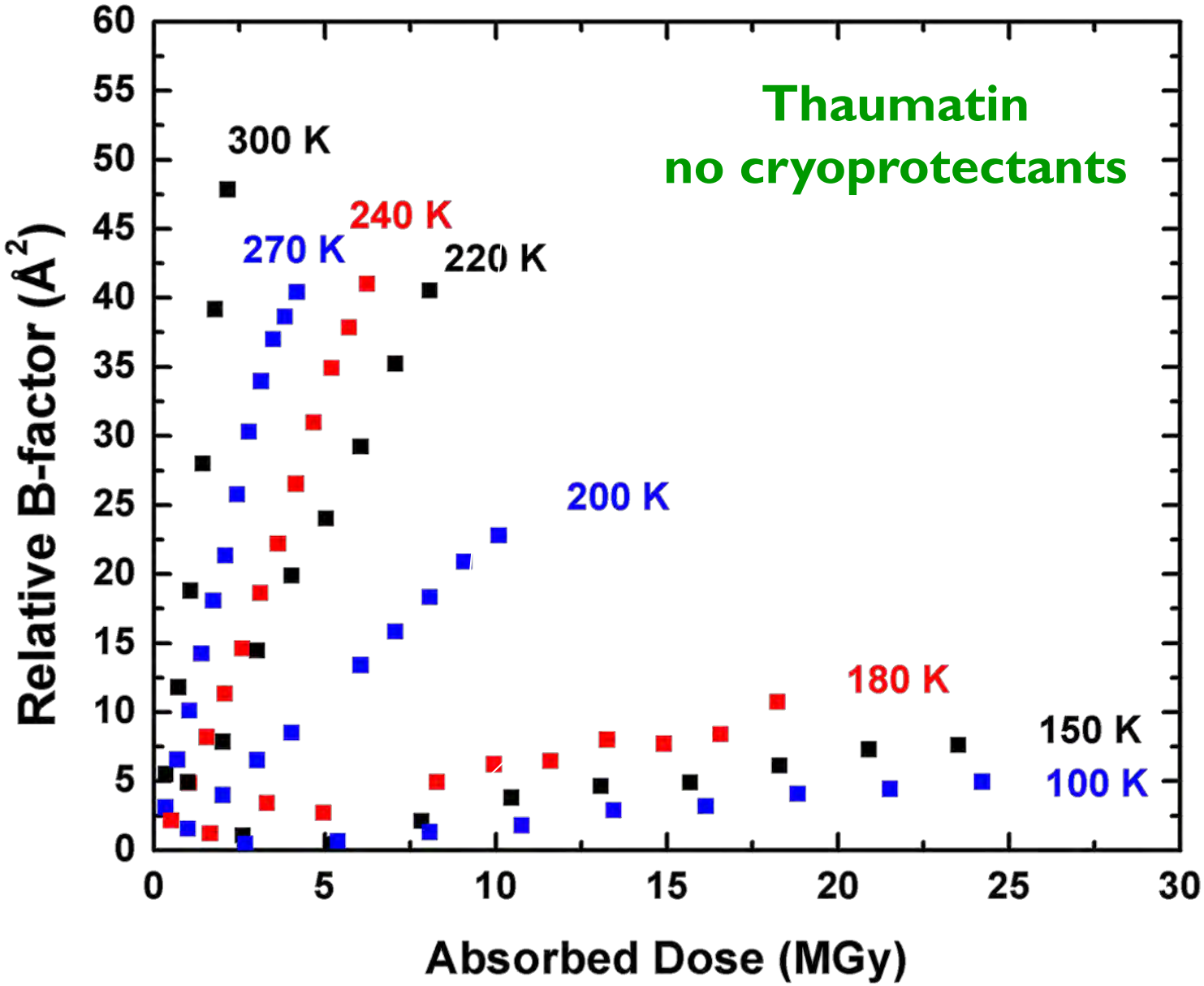
Half-dose $\propto$ initial resolution (Howells et al., 2009)

- At T=300 K, protein crystals are much more radiation sensitive, and the radiation sensitivity varies from protein to protein.

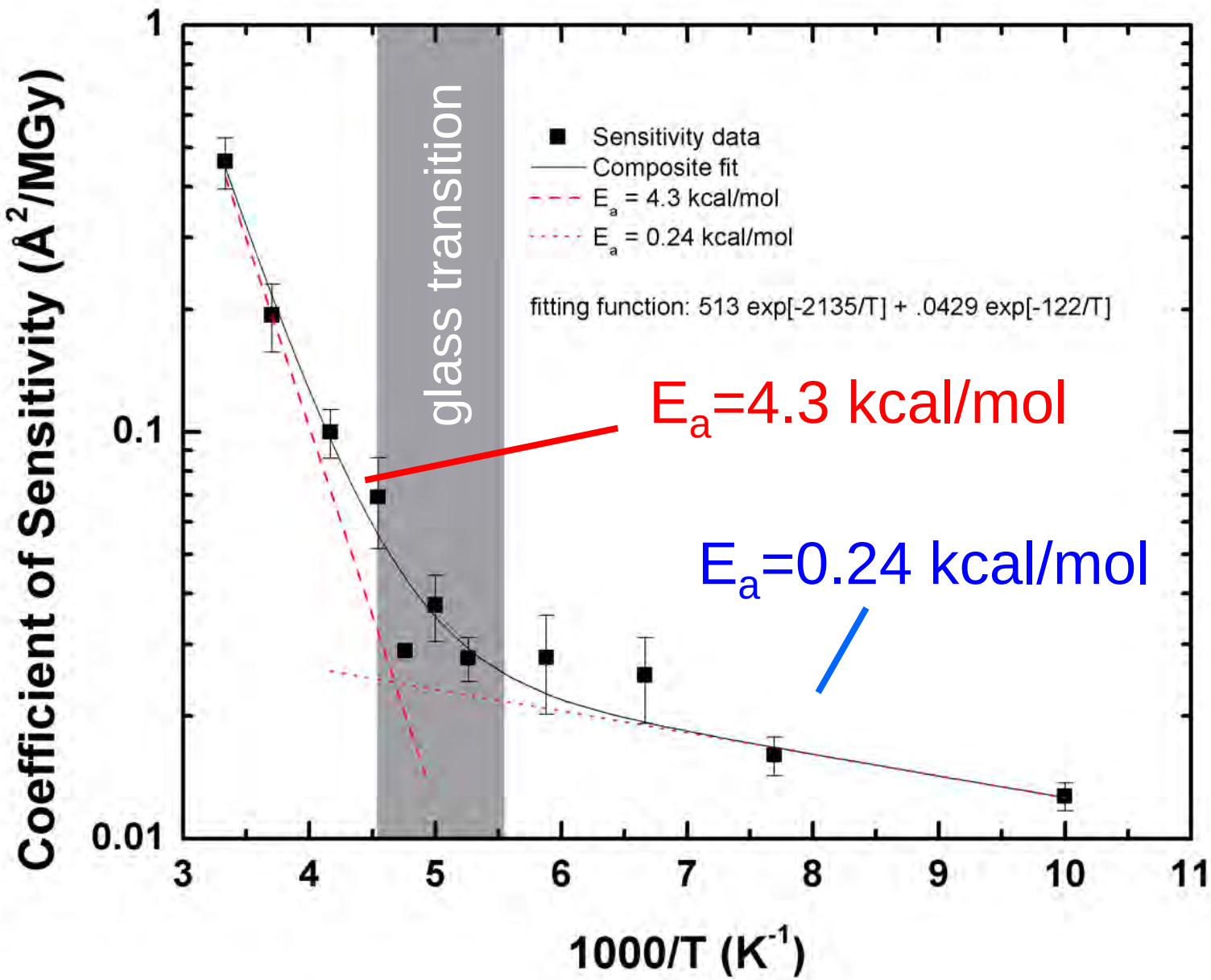
300 K/100 K radiation sensitivity ratios:

lysozyme	(14 kDa, 39% solvent):	48
thaumatin	(22 kDa, 56% solvent):	35
apoferritin	(476 kDa, 61% solvent)	27
urease	(480 kDa, 56% solvent)	35
70s ribosome	(2.2 MDa, 47% solvent)	~1000
TenA	(27 kDa, 80% solvent)	~1000

Relative B versus Dose



Two "activation energies" for radiation damage



Above the **protein-solvent glass transition** (T~200 K):

$$E_a \sim 4.3 \text{ kcal / mol}$$

$\Rightarrow$ radiation damage is dominated by **diffusion controlled radical reactions**, by **conformational relaxations** of damaged protein, and perhaps also by lattice relaxations.

Below the **protein-solvent glass transition**:

E_a ~ 0.24 kcal/mol

~ value for water-free organic compounds

Material	E_a (kcal/mol)
Thaumatin	**0.24**
Paraffin	0.45
Polyethylene	0.28
L-valine	0.41
Ovaline	0.20
Coronene	0.20
Phthalocyanine	0.20
L-asparagine	0.24

$\Rightarrow$ T dependence due to phonon-assisted transitions?

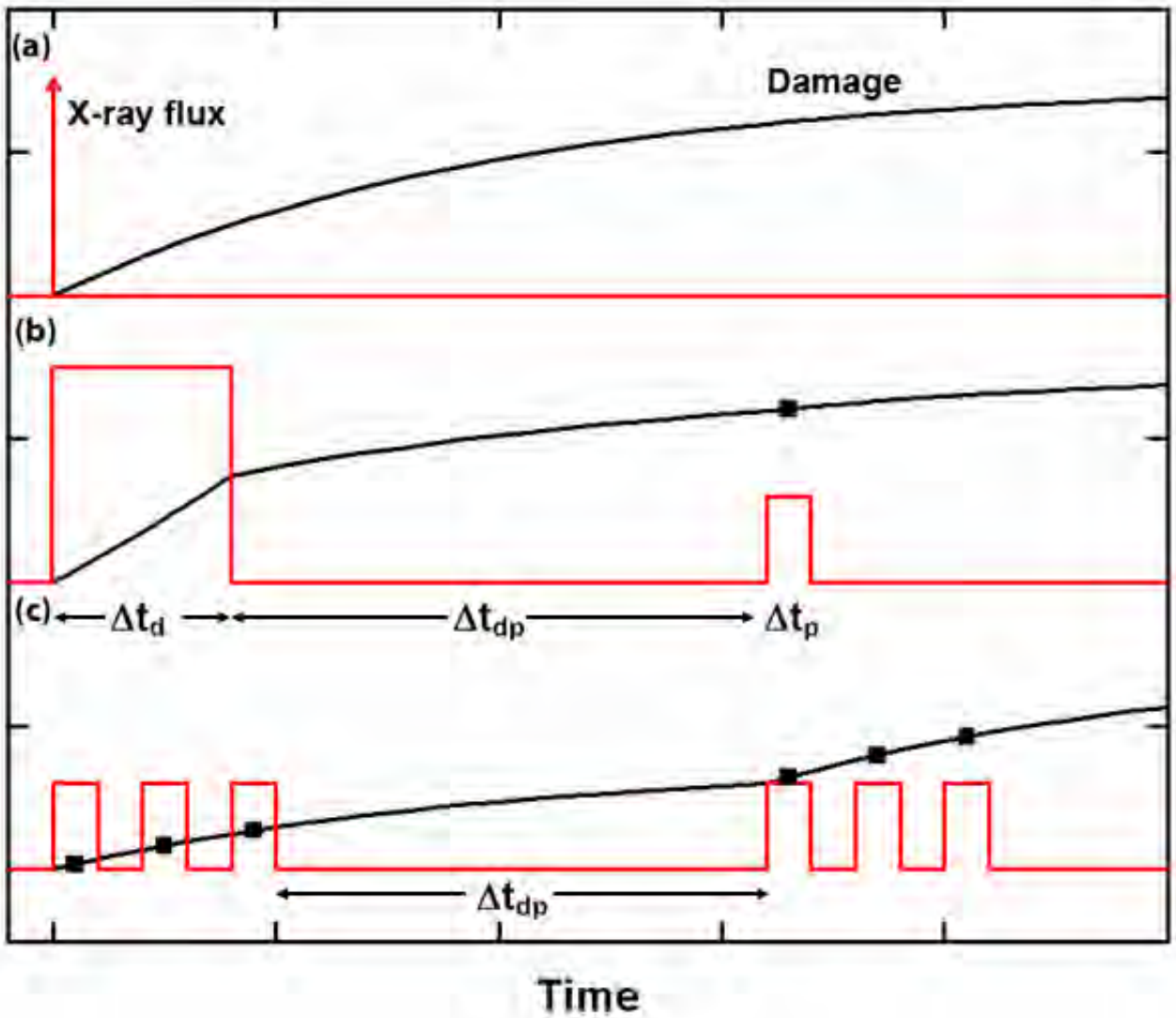
Below T~100 K, global damage is ~T independent.

Time Dependence of Radiation Damage

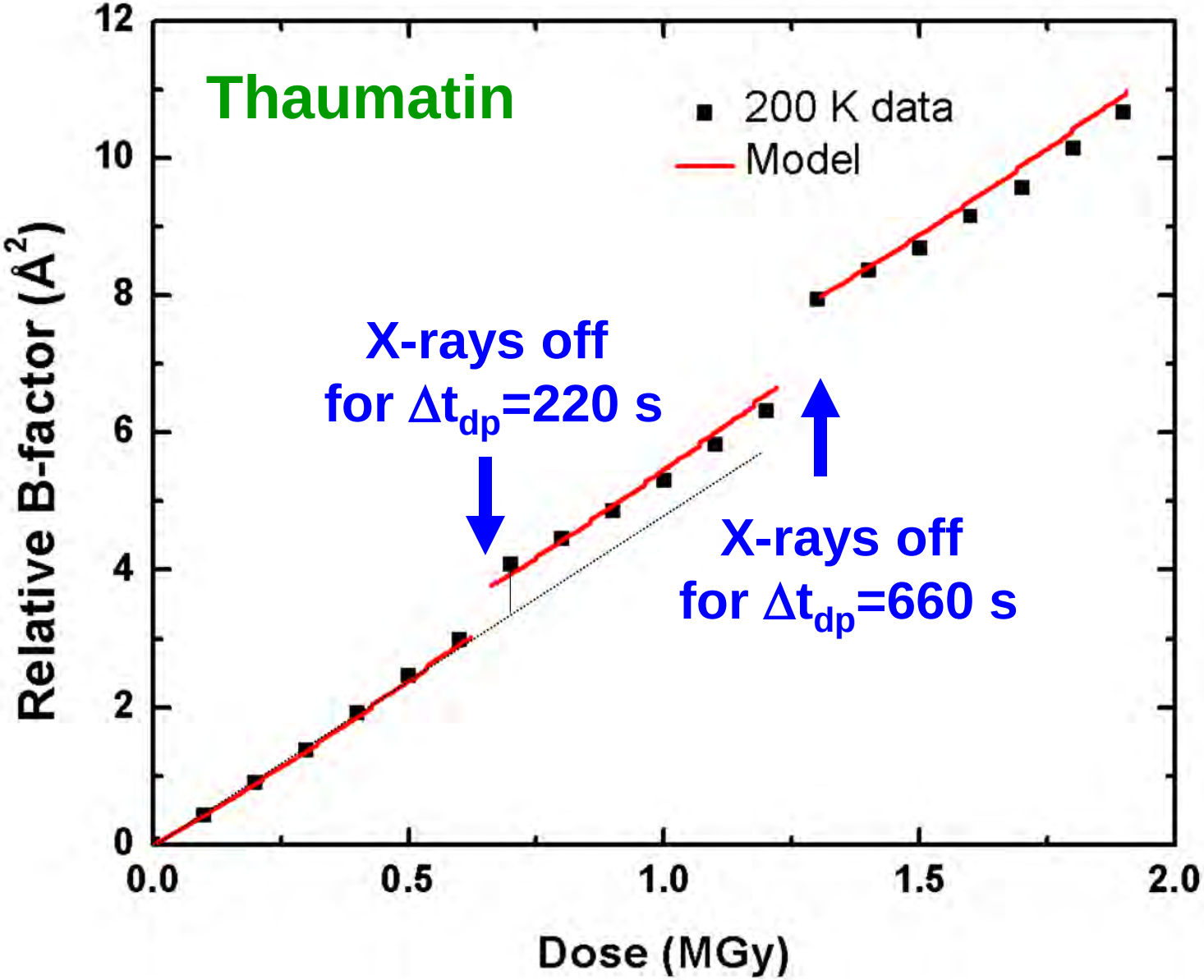
- Timescales of some damage processes should vary strongly with temperature.
- Timescales of solvent-coupled damage processes should diverge near the protein-solvent glass-transition.

Is there a T range where damage evolves on timescales of minutes?

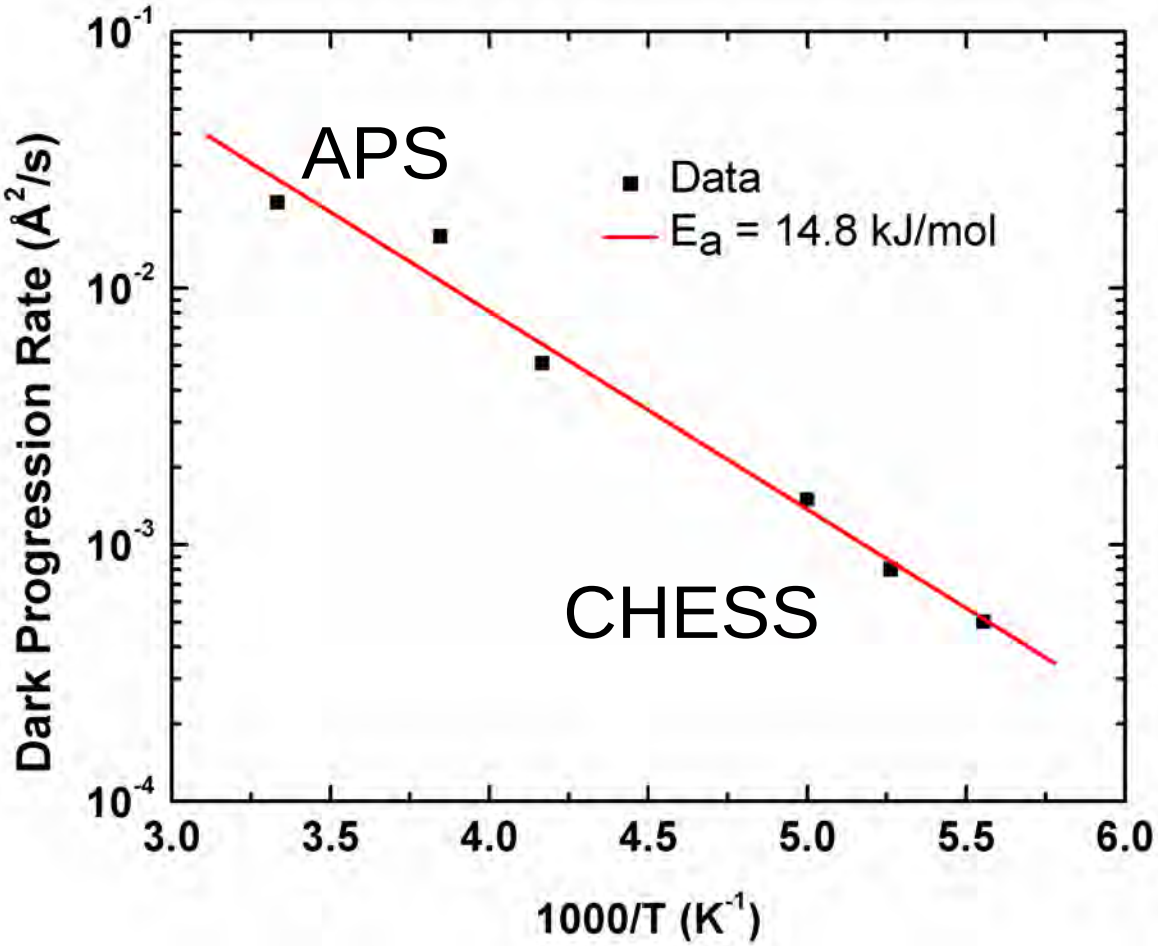
Measuring the Time Evolution of Damage: "Dark Progression"



Dark progression on >200 s timescales is observed between 180 K and 240 K



Dark progression rate has Arrhenius T dependence

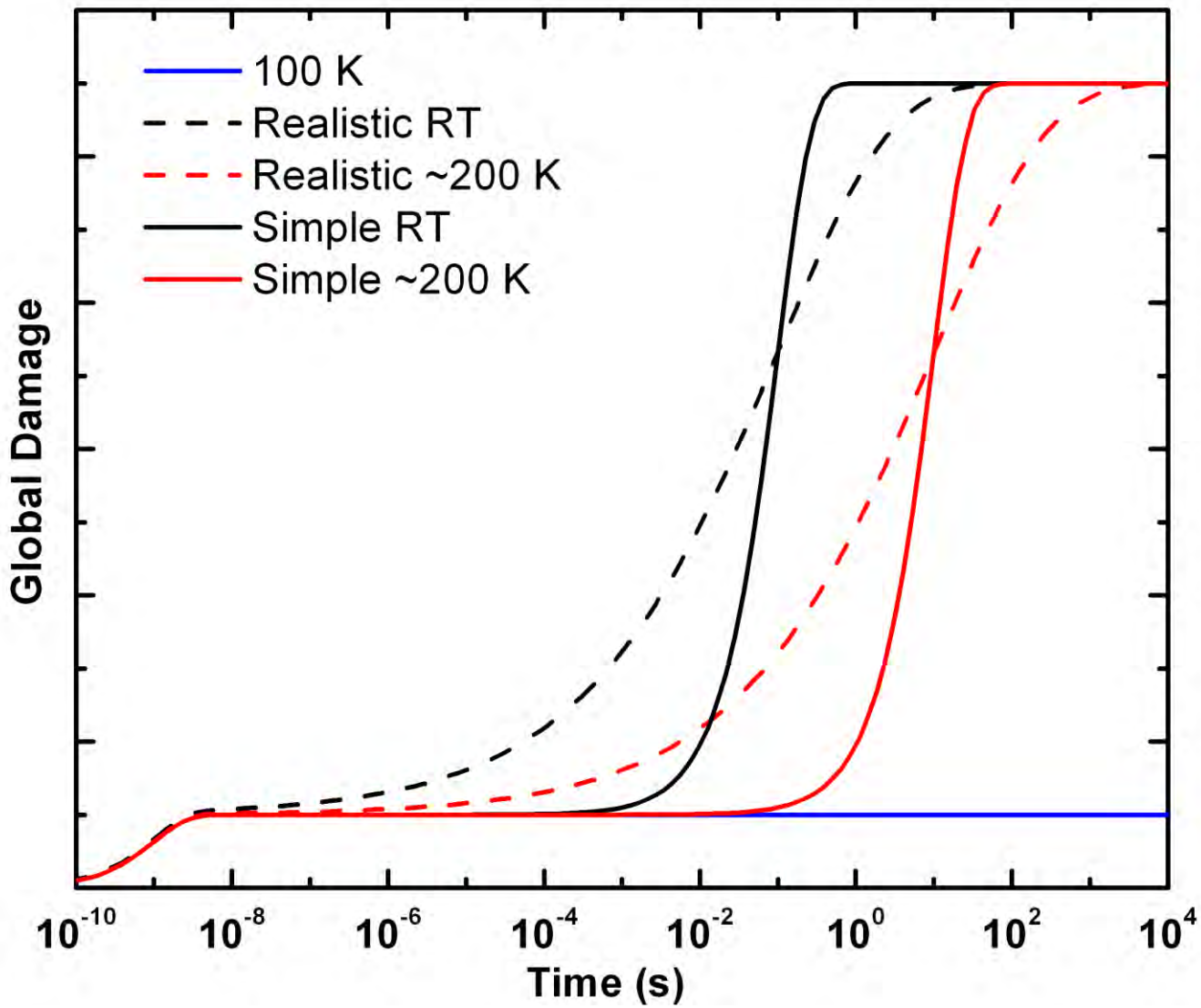


Extrapolated T=300 K damage timescale: **~ 1 s**

>> Timescale for free radical reactions

⇒ Timescale for T=300 K spot fading determined by (slow) structural relaxations (??)

Models for Global Damage vs Time and Temperature



Can Radiation Damage Be Outrun?

Using ultra-high dose rates of FELs? **Yes, in fs**

Using synchrotron sources? **Yes, between 180 K and 240 K in s to minutes**

Can we outrun damage near T=300 K by combining fast data collection and large synchrotron dose rates?

Experiment 1 (2011):

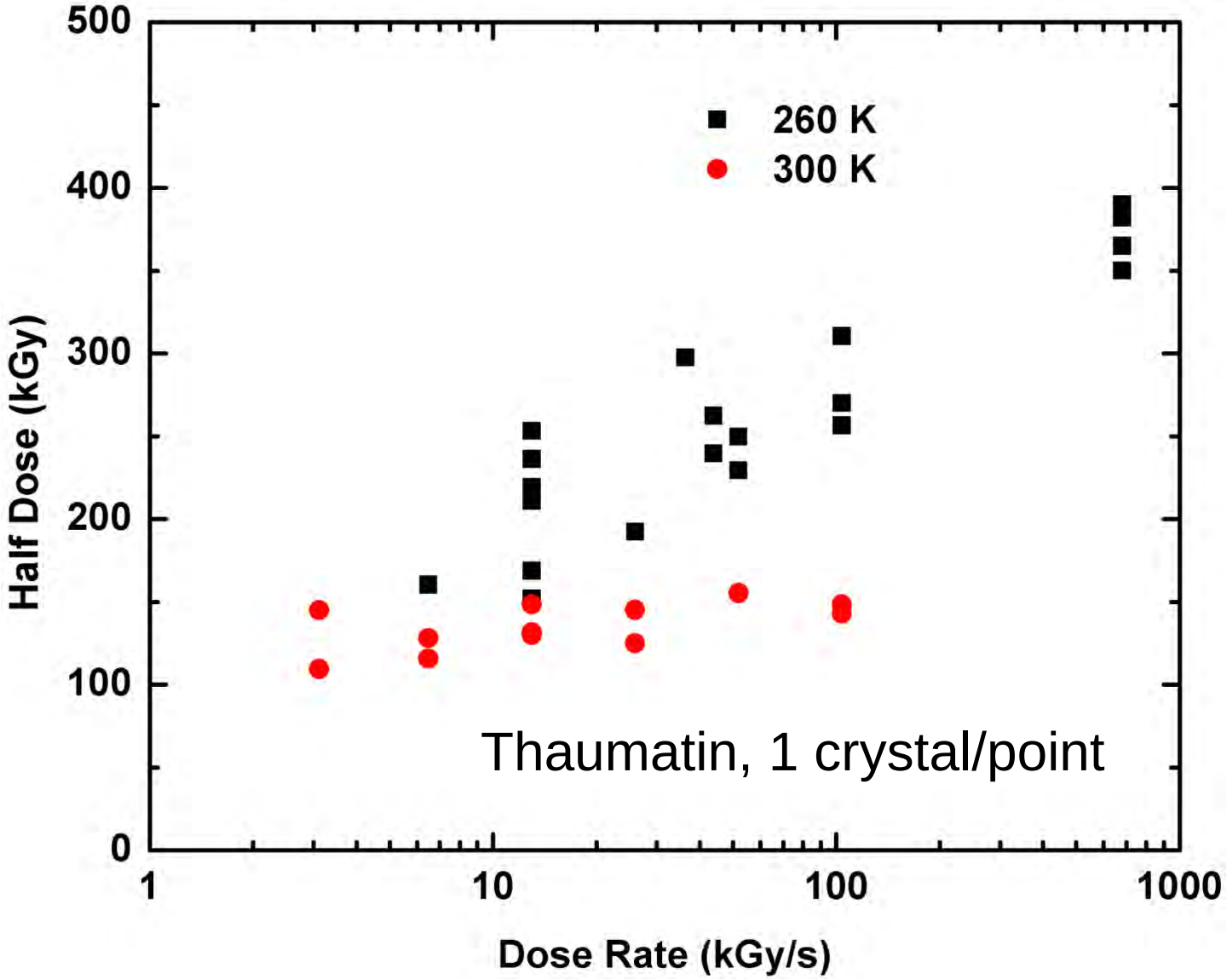
APS 17-ID

1.7×10^{15} ph/s/mm^2

maximum dose rate: **680 kGy/s**

Pilatus 6M detector at 12.5 Hz

Half-Dose versus Dose Rate



How much better can we do?

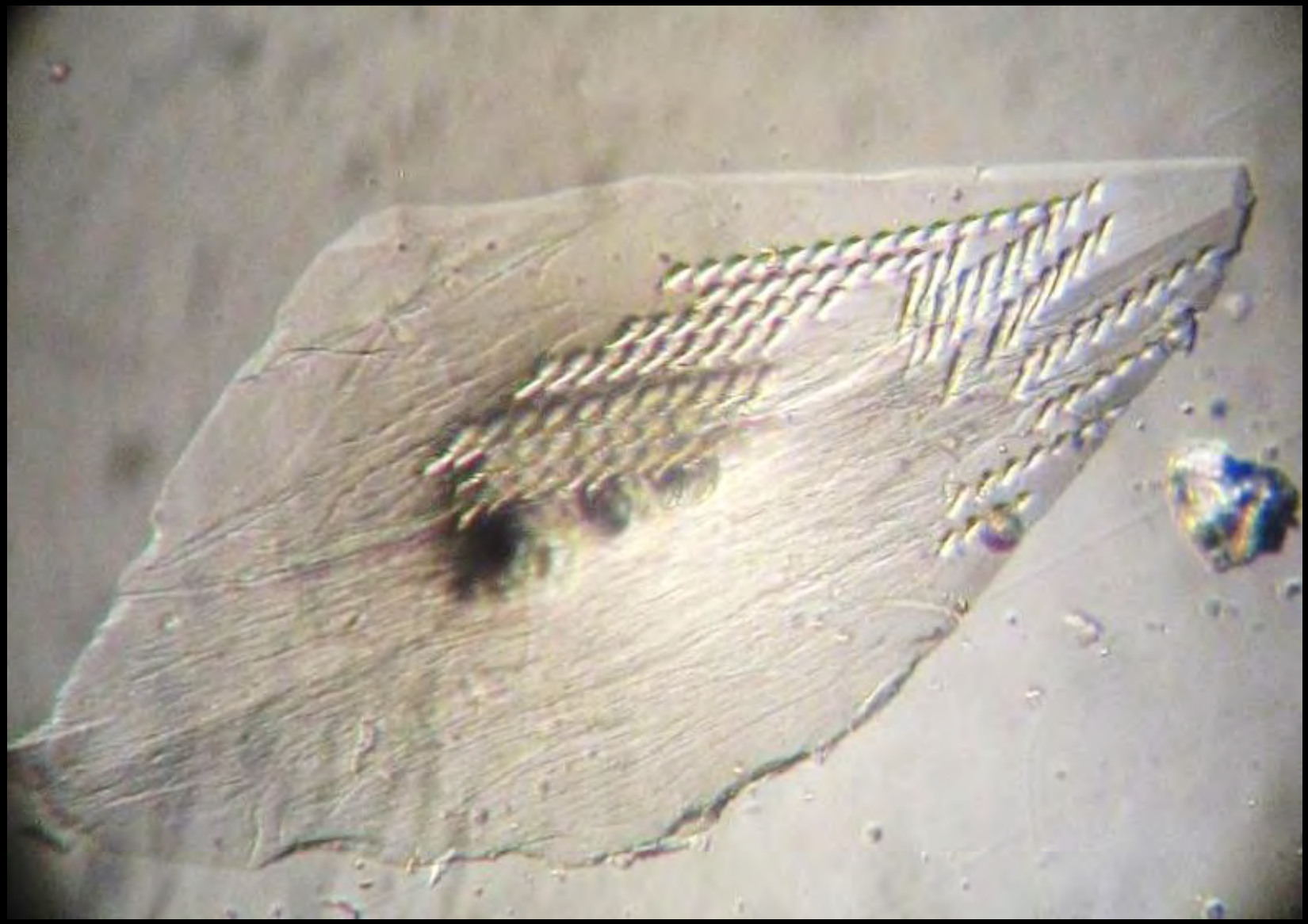
Experiment 11:

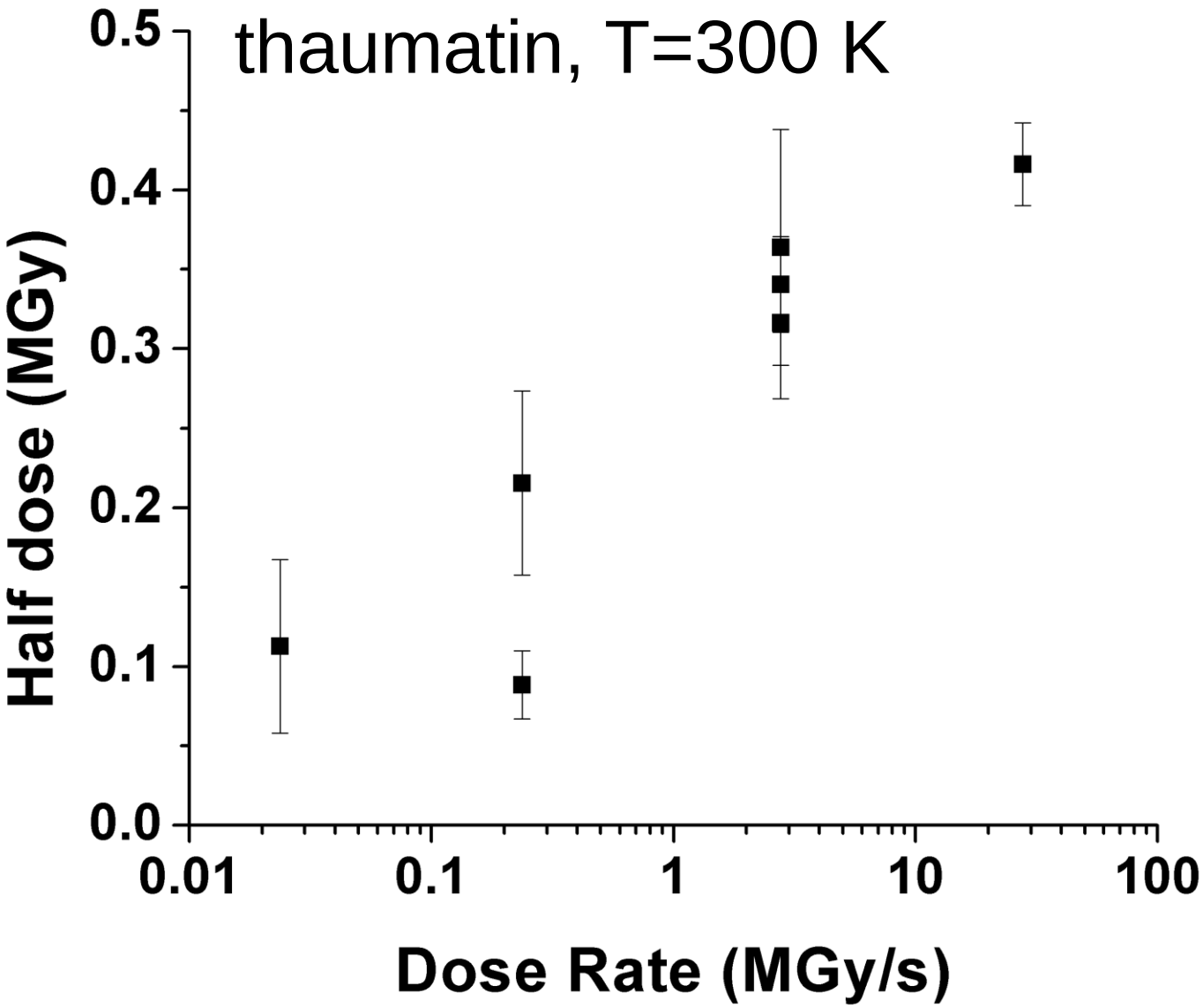
APS 7-ID (August 2013)

~10^{17} ph/s/mm^2 microfocused to 6 μm spot

Maximum dose rate ~40 MGy/s
(~4000 x larger than in standard crystallography)

Pilatus3 300 K detector at 500 Hz



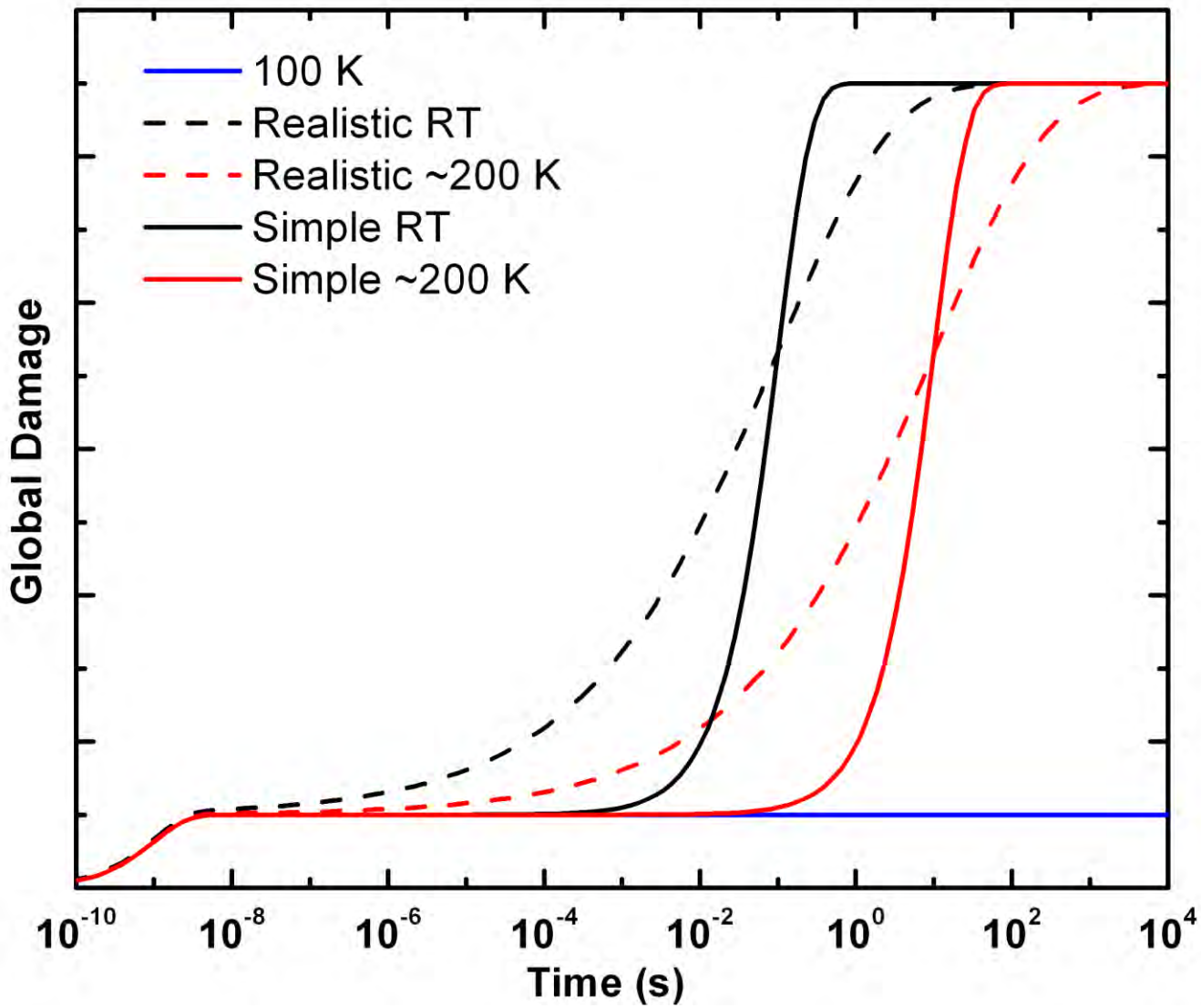

thaumatin, T=300 K
Half dose (MGy)
0.0
0.1
0.2
0.3
0.4
0.5
0.01
0.1
1
10
100
Dose Rate (MGy/s)

- At T=300 K, half-dose of thaumatin and lysozyme crystals is increased by 2-4x by collecting data in ~50 ms

- No increase in half dose with dose rate is observed at T=100 K.

- Half-dose increase with dose rate is consistent with "realistic" model for radiation damage timescale distribution

Models for Global Damage vs Time and Temperature



How much better can we do?

- Larger reductions expected for more radiation sensitive proteins.

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How much better can we do?

- Larger increases in half-dose expected for more radiation sensitive proteins.
- Cooling to ~260 K should give another factor of ~2-3.
- Additional focusing to 2-3 microns should give another factor of ~2-3 (in part due to photoelectron escape)

At ~50 MGy/s, time to collect data to the half-dose at 300 K:

~ 50 ms

Current goniometer rotation speeds:

10,000 rpm or 60° / ms

EIGER detector (Dectris):

10 kHz frame rate

0.1 ms frames

Data collection times determined by sample loading and positioning.

Summary

- The timescale for manifestation of most radiation damage in diffraction patterns is ~1 s near room temperature.

Microfocused synchrotron beams with dose rates of 1-100 MGy/s

+ kHz framing detectors

+ infrastructure for high throughput sample handling and data collection

should facilitate a large expansion in diffraction studies of proteins under more nearly native conditions.

Matt Warkentin

Jesse Hopkins

References:

Glass transition in thaumatin crystals revealed through temperature dependent radiation-sensitivity measurements. M. Warkentin & R. E. Thorne. (2010). Acta Cryst. D66, 1092-1100.

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