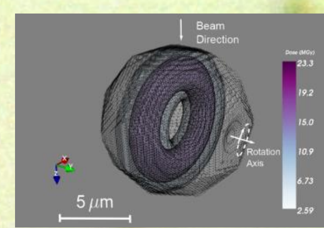
RADIATION DAMAGE:

why do we care?

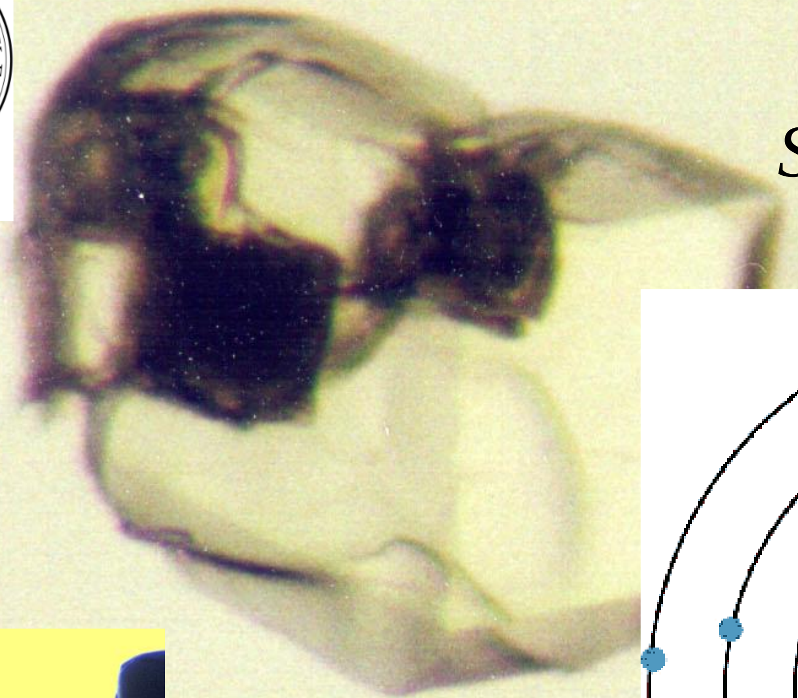


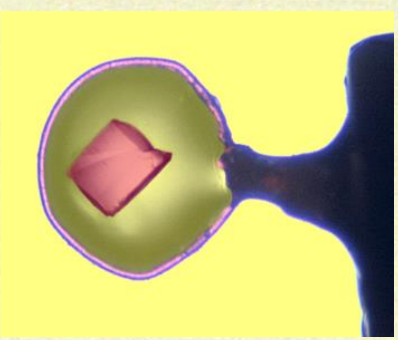
DLS/CCP4 Diamond/CCP4

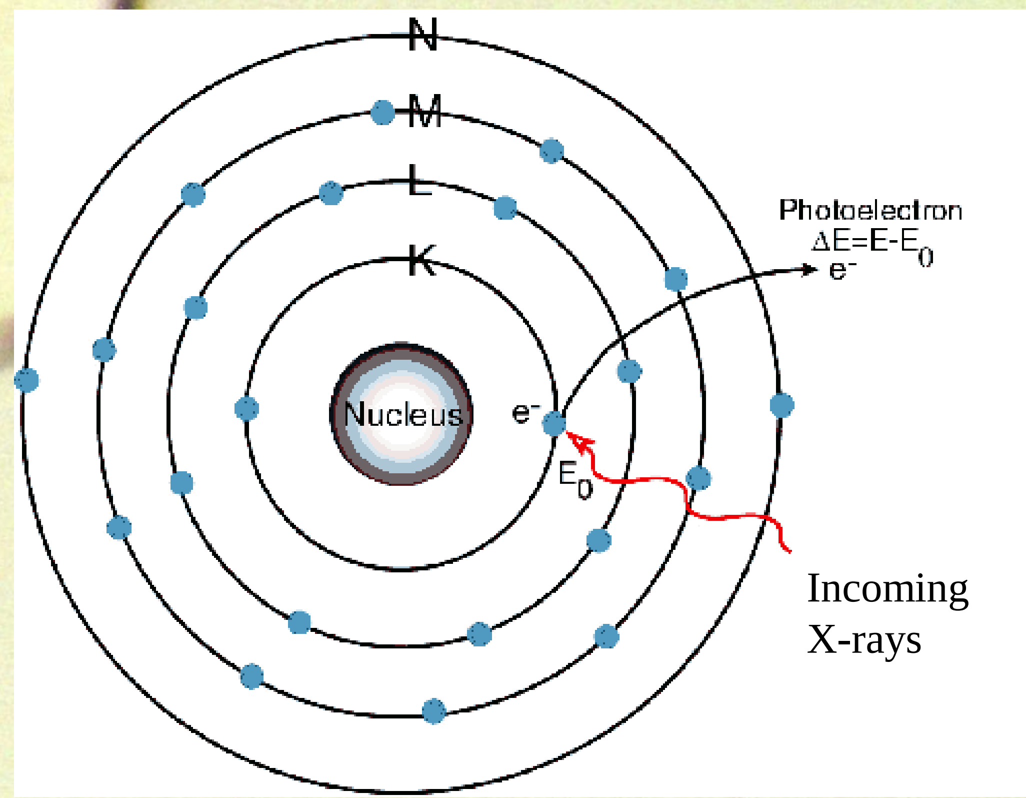
Data Collection and

Structure Solution Workshop

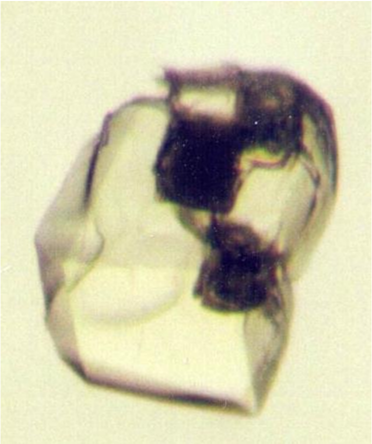
21st November 2023







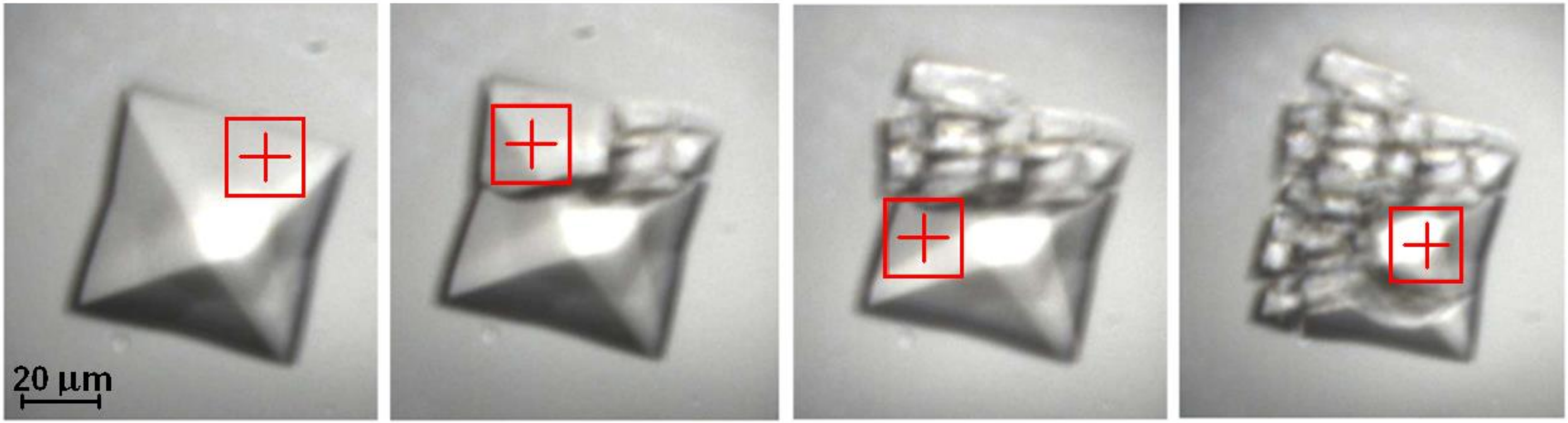
elspeth.garman@bioch.ox.ac.uk

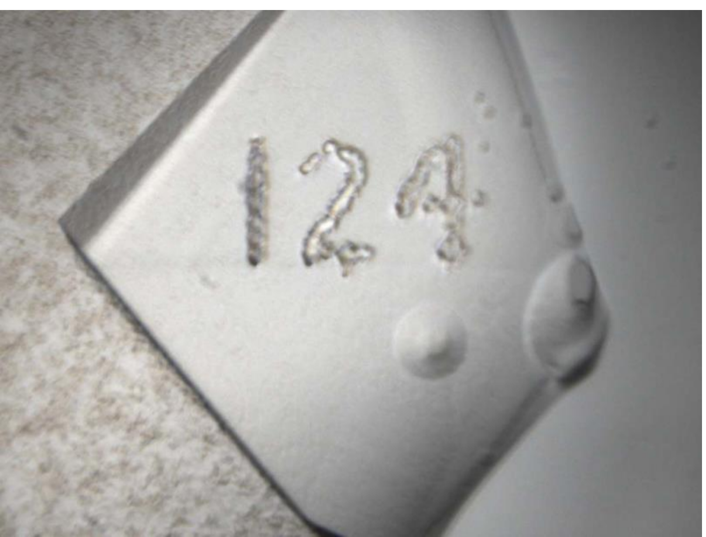


Radiation damage:
The Plan:

- **What are the symptoms?**
- What is it?
- Why do we care? Effect on MAD/SAD.
- How do we estimate the Dose?
- What do we know/would like to know?
- A new RD metric

I24, Diamond, *in situ* data collection from a Bovine Enterovirus 2 crystal, room temperature, 0.5 s 20 μm x 20 μm beam



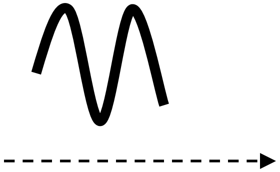


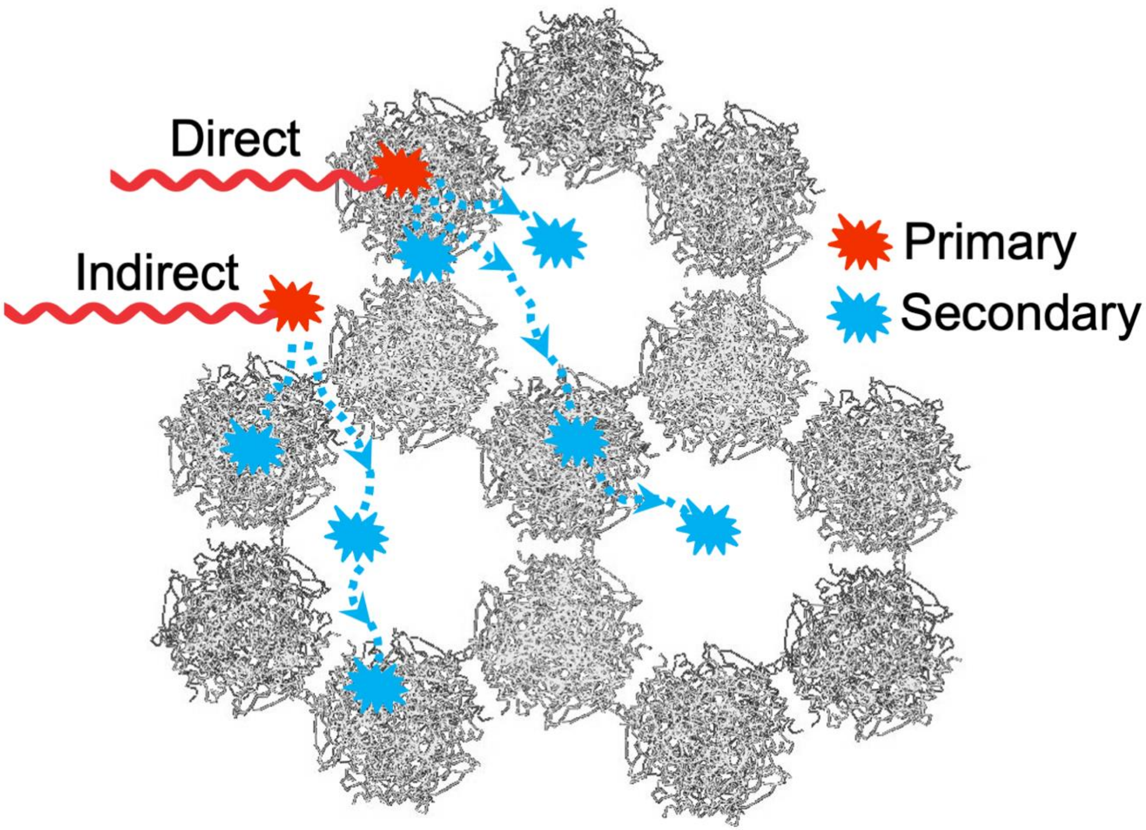
Axford *et al.*,
Acta Cryst D (2012) 592

Beamline logo I24
(Gwyndaf Evans *et al.*)

Radiation damage

Primary
Secondary

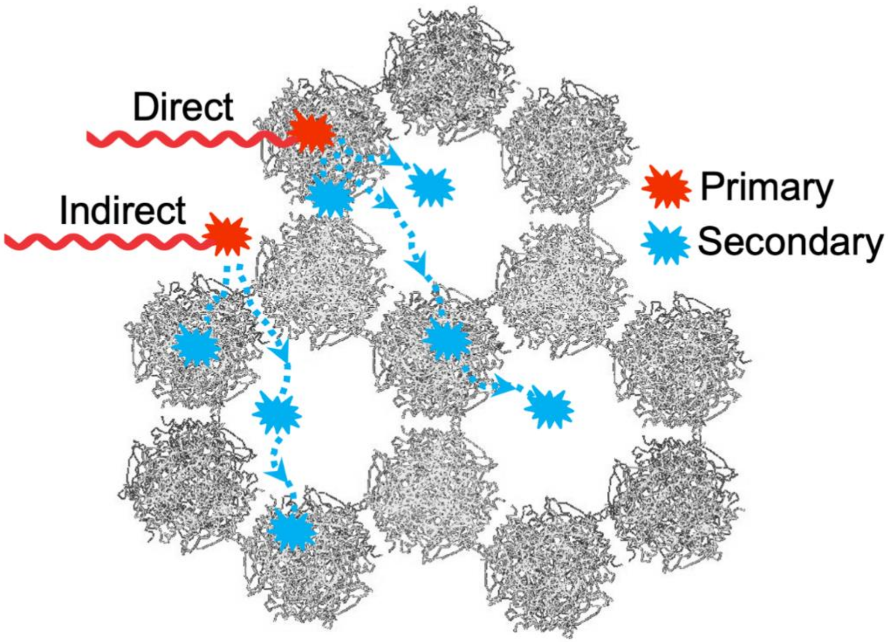


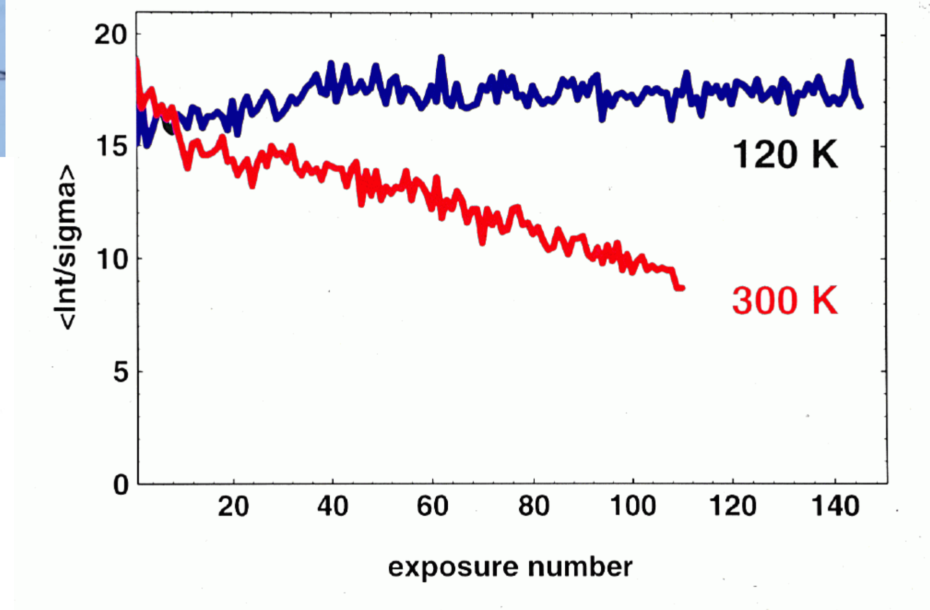


Protein: direct
Solvent: indirect

PRIMARY; inevitable, a fact of physics! Neutralise it?
SECONDARY, can we control it?

Radiation damage

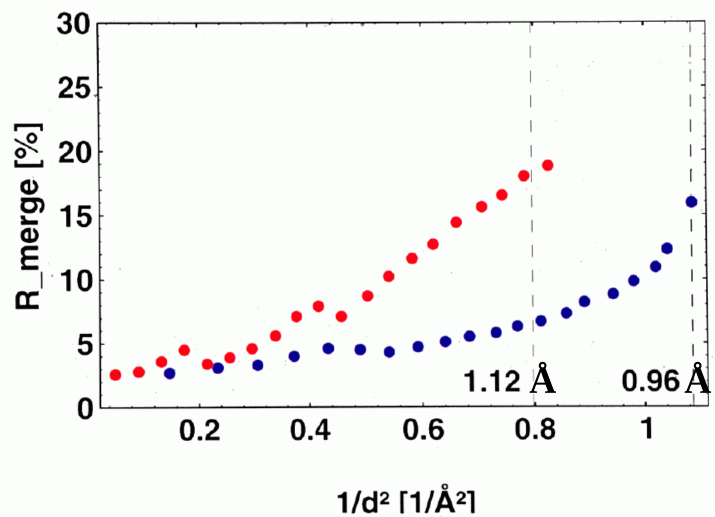




Significantly reduced at 100 K: time factor of ~70

[Nave and Garman *JSR* (2005), **12**, 257-260].

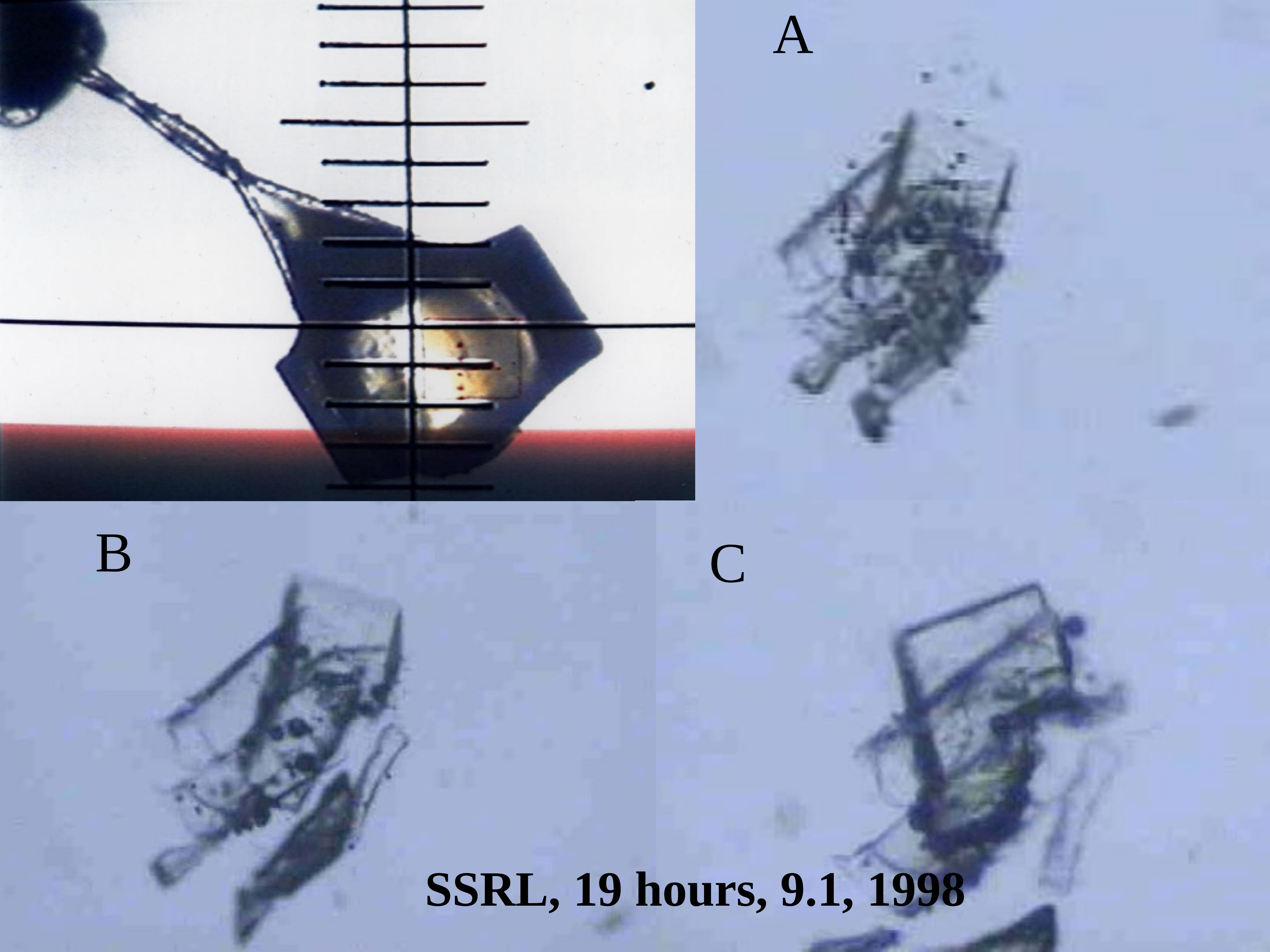
SP445: Data Quality [T.Schneider]



PRIMARY; inevitable, a fact of physics! Proportions?

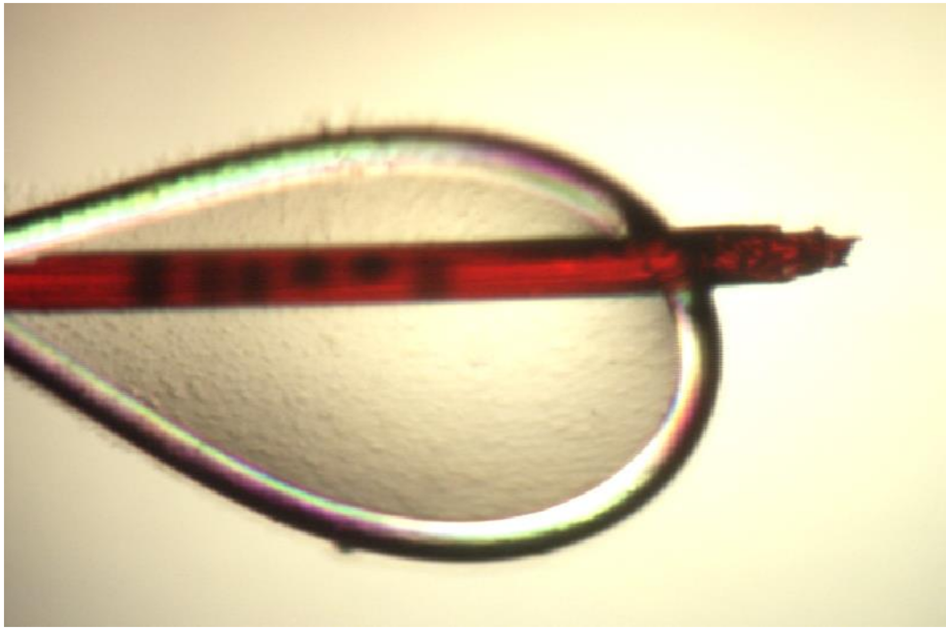
SECONDARY, can we control it?

Garman and Schneider J Appl Cryst 1997

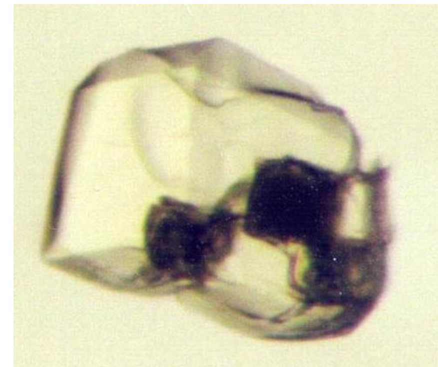


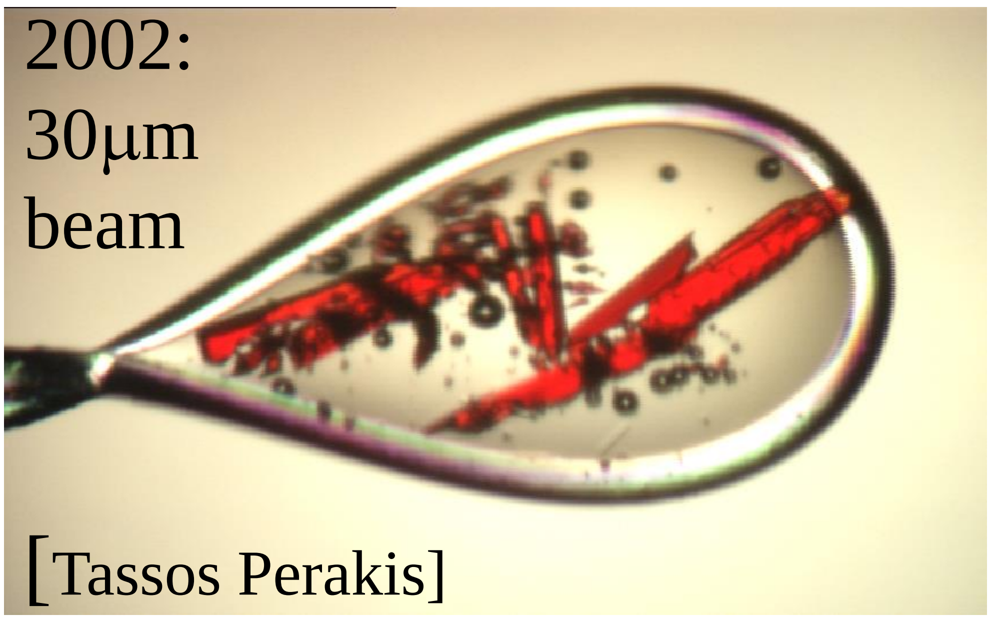
SSRL, 19 hours, 9.1, 1998

1995 onwards: 100 K



BUT THEN, 1999:

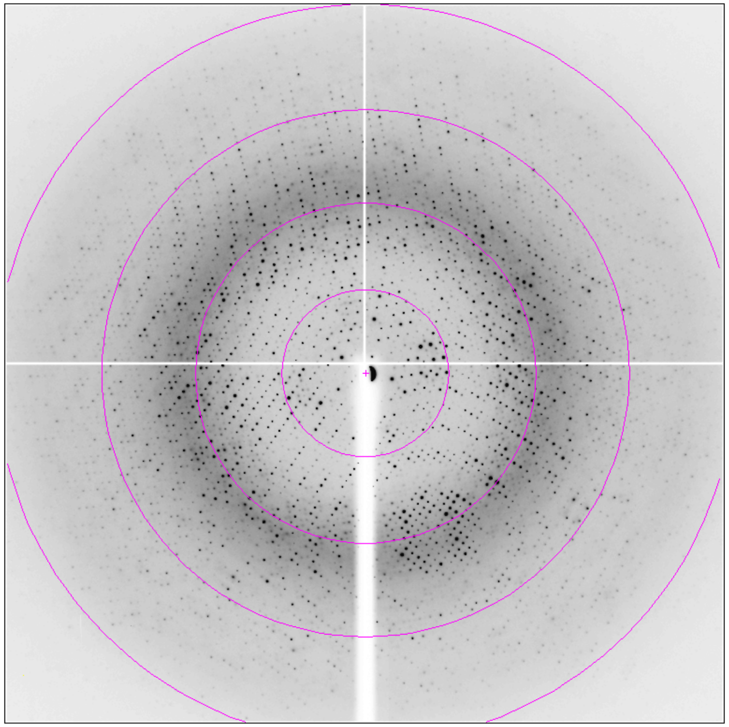




Iron containing protein, ESRF

Also observe spectral changes

Garman & Owen (2006), Acta D62

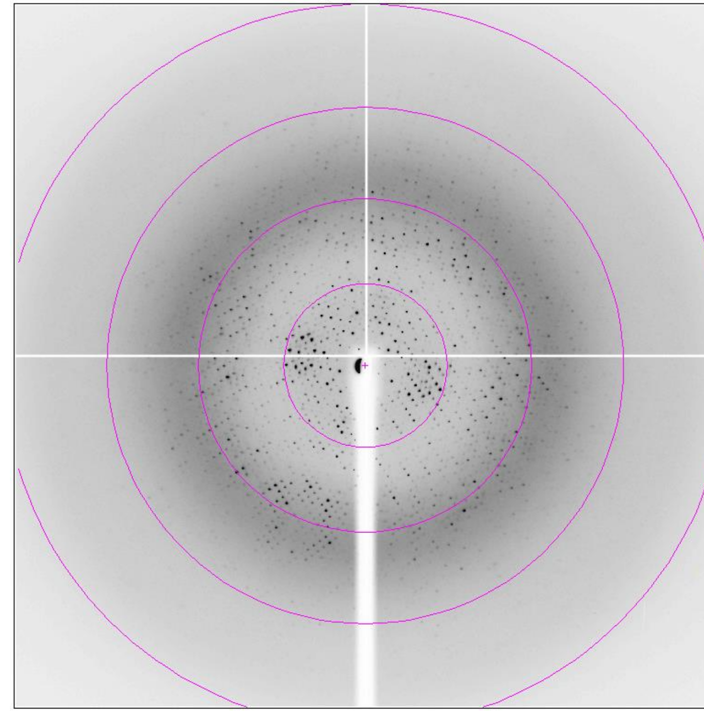


At 100 K

Intensity decrease

Loss of diffraction

Incomplete data from crystals



Happens during 1 dataset at 100K for many crystals

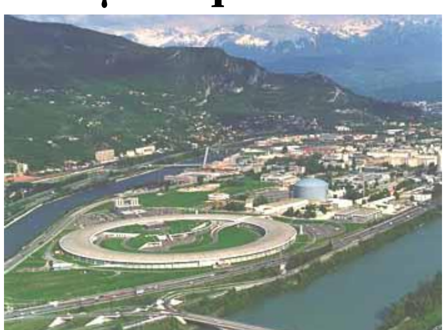
Unit cell volume expansion

Increase in Wilson B factors, Rmerge

Increase in mosaicity

‘GLOBAL’ damage

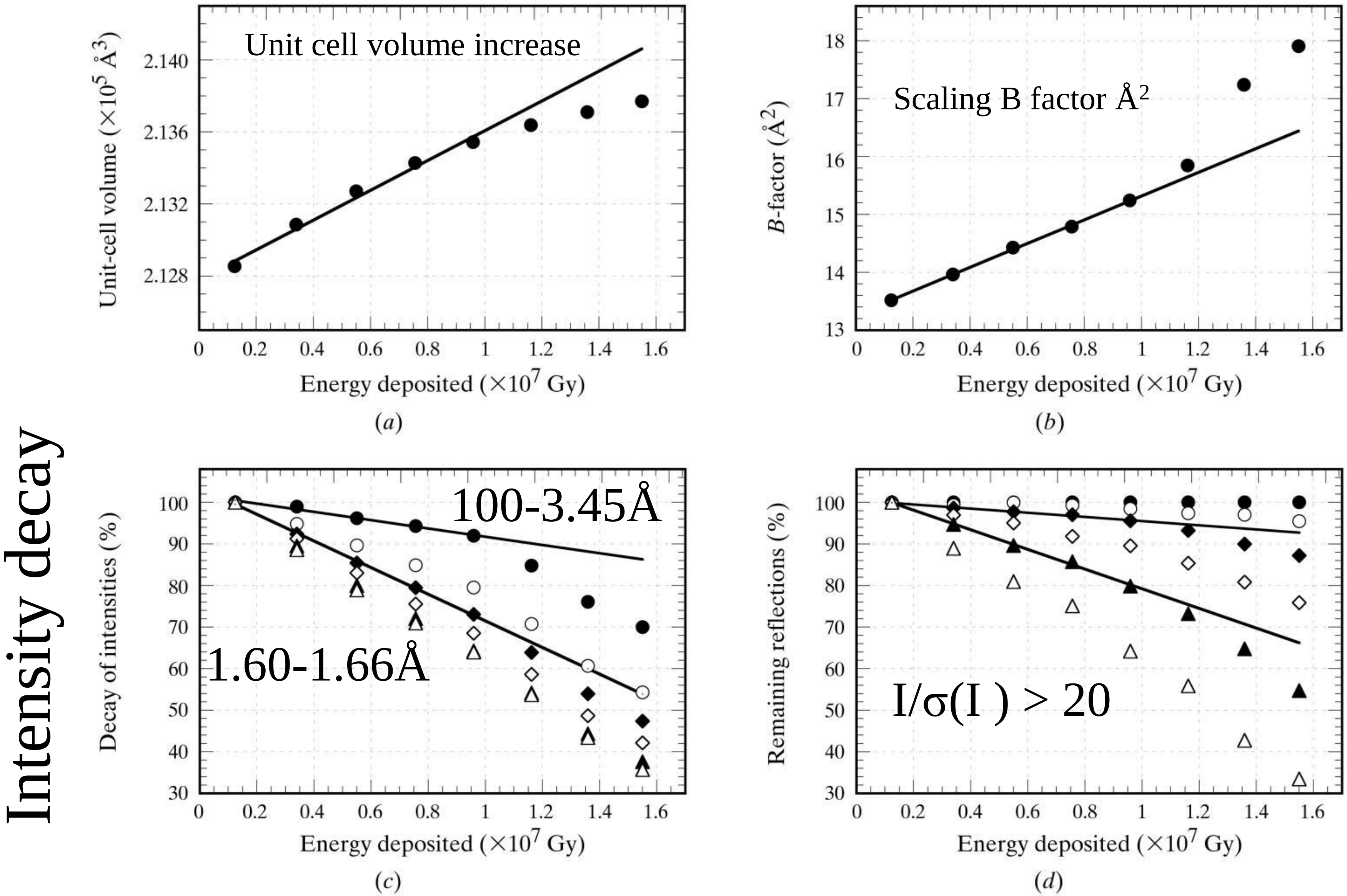
ESRF 2000:
1×10^{12} ph s^{-1} into 100μm square slits



Diamond Light Source:
3×10^{12} ph s^{-1} into 7μm × 6μm
[7×10^{14}]

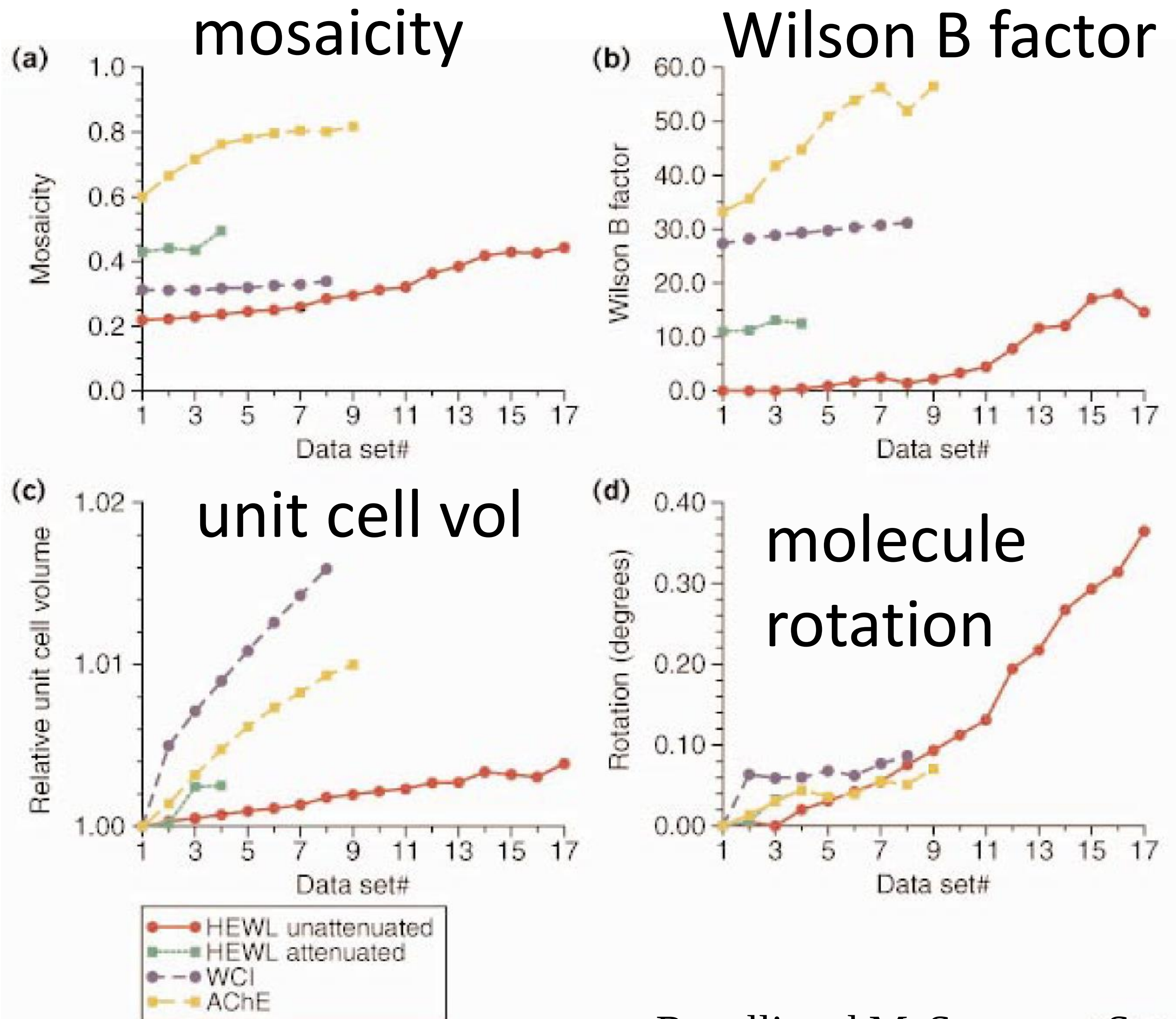
Australian synch.
3×10^{13} ph s^{-1} into 50μm × 70μm [10^{14}]

RadDam signatures in reciprocal space 100 K



Teng and Moffat *JSR* (2000)

Effects at 100 K



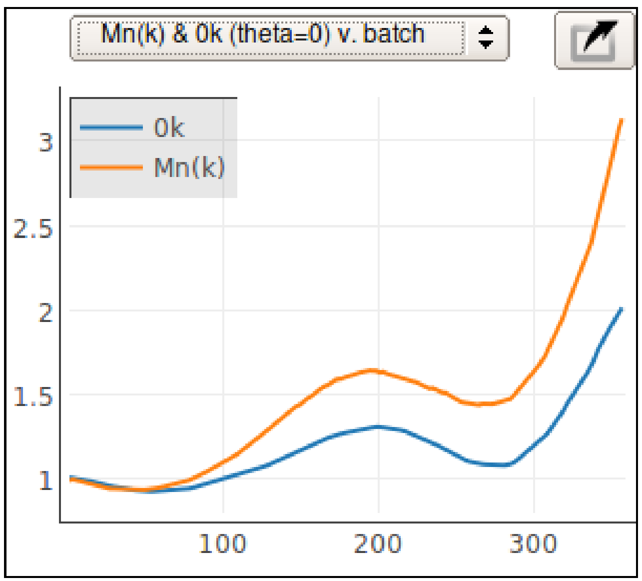
Raimond Ravelli 1968-2023

Ravelli and McSweeney, Structure (2000) 8, 315

RadDam signatures in reciprocal space

Characteristic ‘U’ type shape in scale factors plotted against image number:

ID14-4, ESRF
Ed Lowe



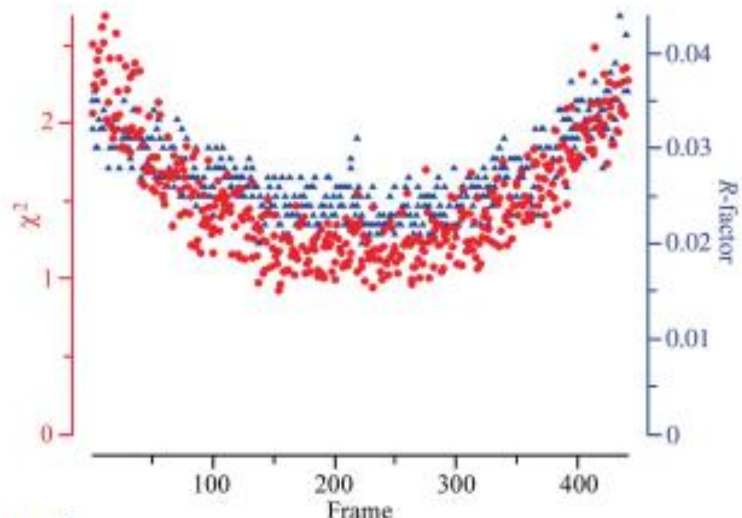


Figure 5
Statistics of scaling the individual images with the average of the data. The presence of radiation change creates a characteristic U-shape in the scaling statistics. Circles indicate $\chi^2 = \langle (I_n - \langle I \rangle)^2/\sigma_n^2 \rangle$ values for individual diffraction images, triangles show the traditional scaling R-factor $R = \Sigma_n |I_n - \langle I \rangle| / \Sigma_n \langle I \rangle$, where in both cases sums are evaluated for all measurements and where the centroid of the diffraction spot is in a particular image frame.

Borek and J.SR (2007). 14, 24–33

Data Parameters affected by Radiation Damage

- $I/\sigma(I)$ or resolution limit ↓
- R_{merge} ↑
- Scaling B factors ↑
- Mosaicity ↑
- Unit Cell expansion a) function of dose ↑

  b) function of cryogen temperature ↑

Could this be an on-line damage metric?

[Ravelli and McSweeney, (2000) Structure]

No!

[Murray & Garman (2002) JSR, Ravelli et al (2002) JSR]

What global damage metric should we use and against what should we plot it?

- I_n/I_1
- Not I/sigma(I)
- Scaling B factors?
- An R_{meas} type measure?

To monitor the damage we need an *x*-axis!

for comparisons across synchrotrons &
even between beamlines at the same synchrotron:
we can't use time or image number.

DOSE estimation

$$\text{Dose} = \frac{\text{energy absorbed}}{\text{unit mass}} = \frac{\text{J}}{\text{kg}} = \text{Gy (gray)}$$

Fundamental metric against which to plot damage.

Cannot be measured, can only be estimated.

Takes care of the physics but NOT the chemistry.

RADDOSE-3D: www.raddo.se

MX experiment

1 MGy/s absorbed by a 100 μm cubed metal free crystal in a

$100 \times 100\ \mu m^2$ beam of

12.4 keV (1 Å) X-rays

flux: 10^{13} photons s^{-1}

3 Gy

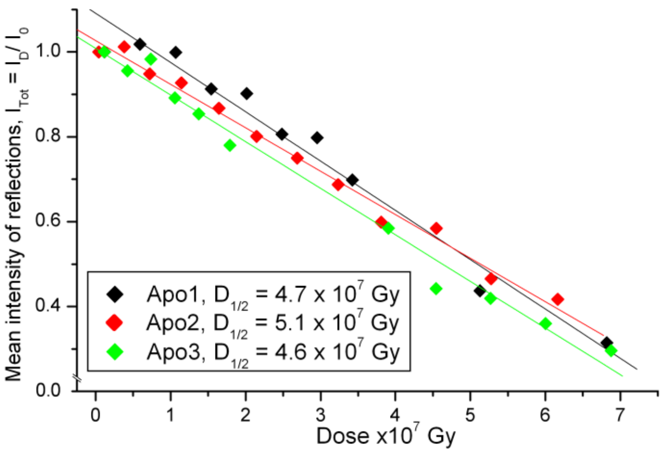
MX at 100 K: 30 MGy experimental dose ‘limit’ reached in ~30 s:
4^{th} generations sources <<1s,
XFELs: <80 fs

Intensity Decay at 100K

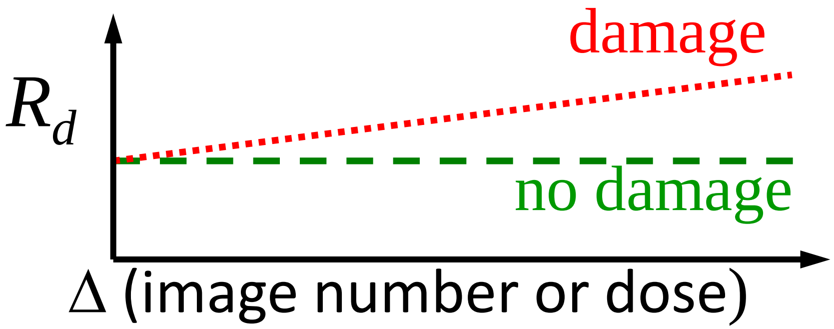
Normalised Intensity vs Dose:

apoferritin

[Owen et al 2006, PNAS]



[Deiderichs 2006 Acta D59, 903]



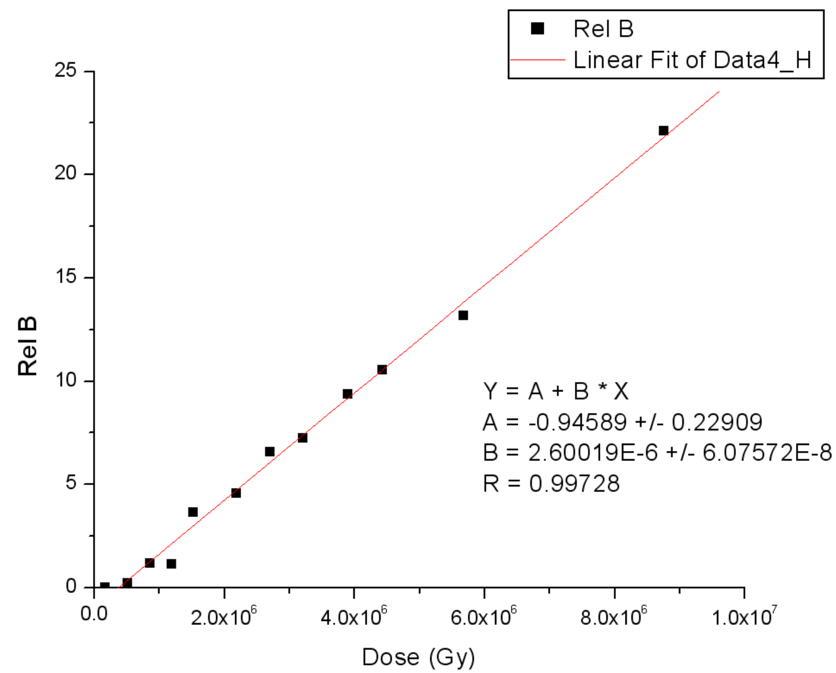
Coefficient of sensitivity α

change in relative isotropic B factor:

$$s_{AD} = \Delta B_{rel} / 8\pi^2 \Delta D$$

(e.g. HEWL@100 K = 0.012 Å^2/Gy)

[Kmetko et al 2006, Acta D62, 1030]

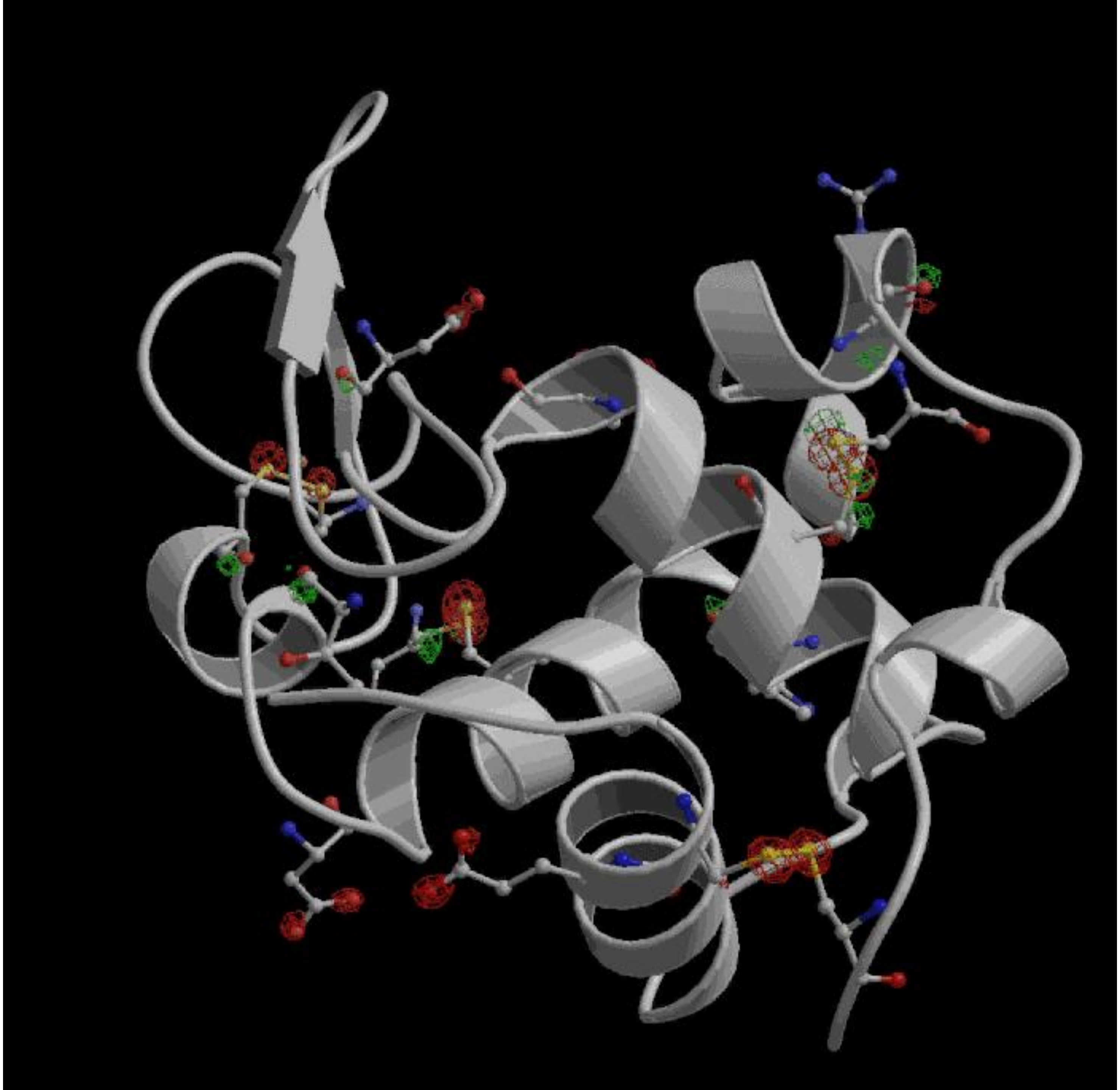


R_d : pair wise R factor between identical and symmetry-related reflections occurring on different diffraction images

Global damage: summary

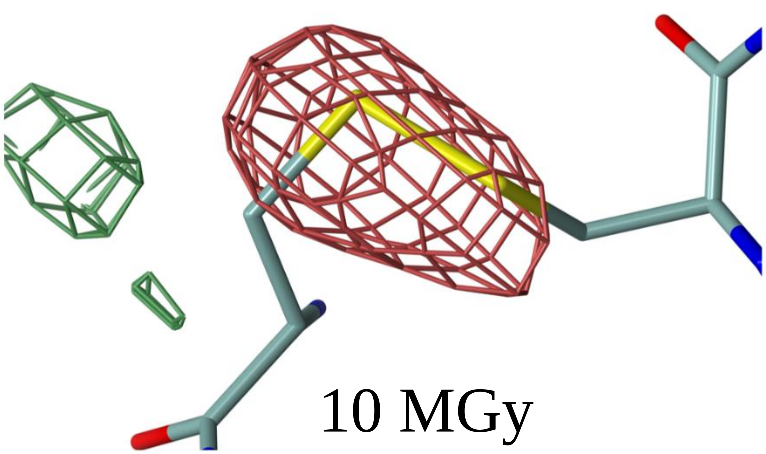
- Incomplete data/lost resolution/scaling 'dip'
- Causes non-isomorphism within a dataset (unit cell grows)
- No significant* ($< \times 2$) dose rate effect at 100 K at current flux densities (10^{15} ph/s/mm^2).
- No significant ($< \times 2$) temperature dependence below 100 K, but weak minimum at around 50 K.
- Damage to lattice due to hydrogen abstraction and then build up?
- Heating not significant at current flux densities.
- For a particular system is predictable/can be modelled (using a sacrificial crystal)

* James Holton criterion for a significant result



Specific Damage

- Specific damage occurs in a predictable hierarchy in proteins
- Reduction of metallocentres
- Breakage of disulphide bonds
- Asp and Glu decarboxylation



Difference map Fo_4-Fo_1

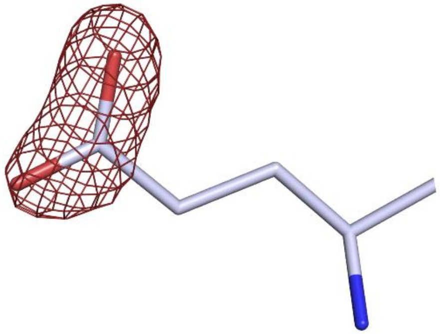
Weik et al. 2000, Burmeister 2000
Ravelli and McSweeney 2000,

Specific Damage

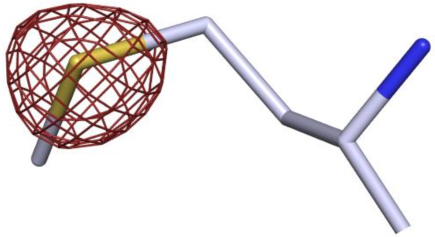
- Specific damage occurs in a predictable hierarchy in proteins
- Reduction of metallocentres
- Breakage of disulphide bonds
- Asp and Glu decarboxylation
- Cleavage of S—C bond in MET
- Rupture of covalent bonds to heavier atoms:

  C-Br, C-I, S-Hg

- **Note** that if this were due to primary damage alone, damage would be in order of absorption cross sections of atoms, which it is not.



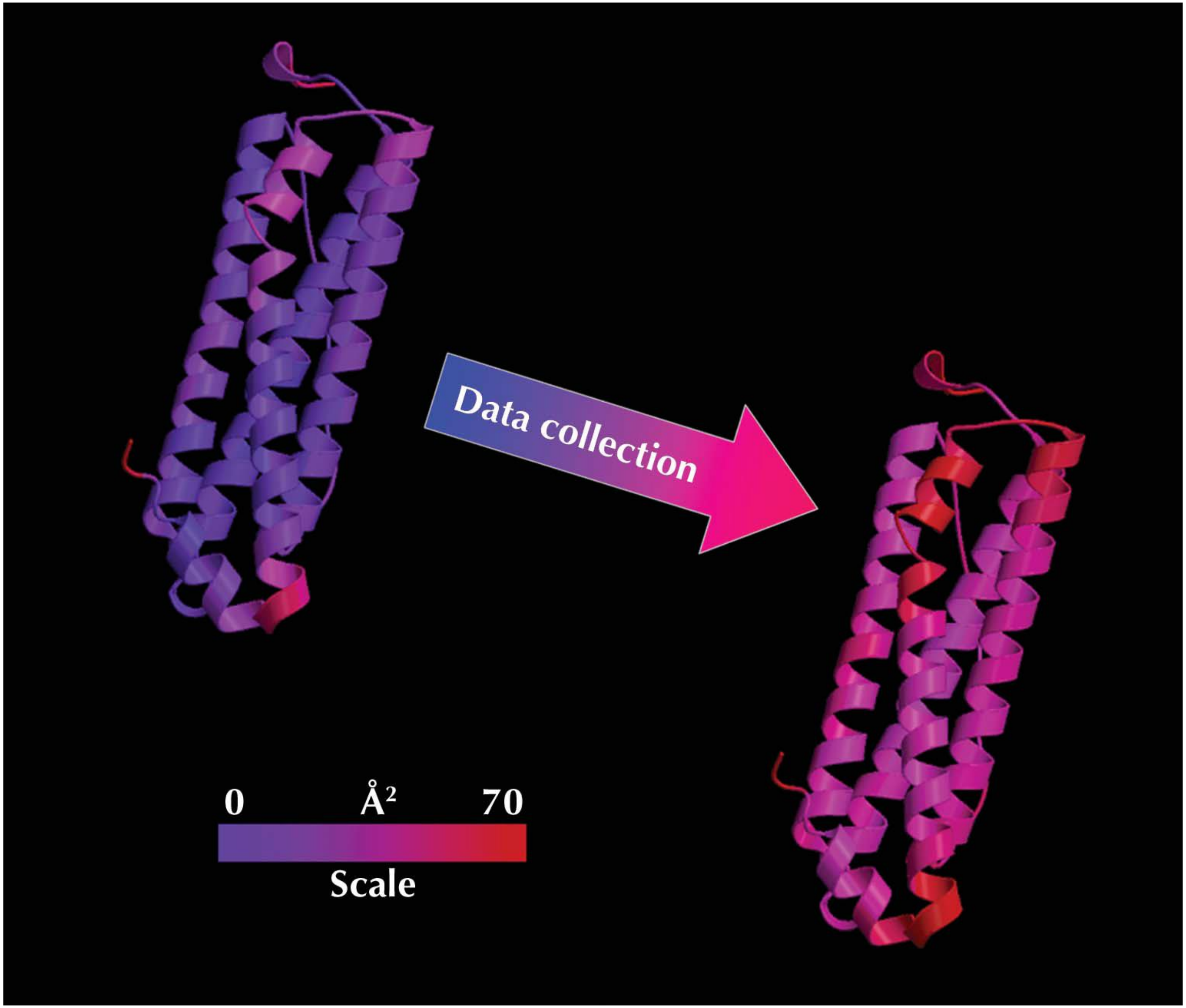
GLU $\pm 4\sigma$–level difference map: Red =negative density



MET $\pm 4\sigma$–level difference map: Red = negative density

Bury et al., JSR 2015

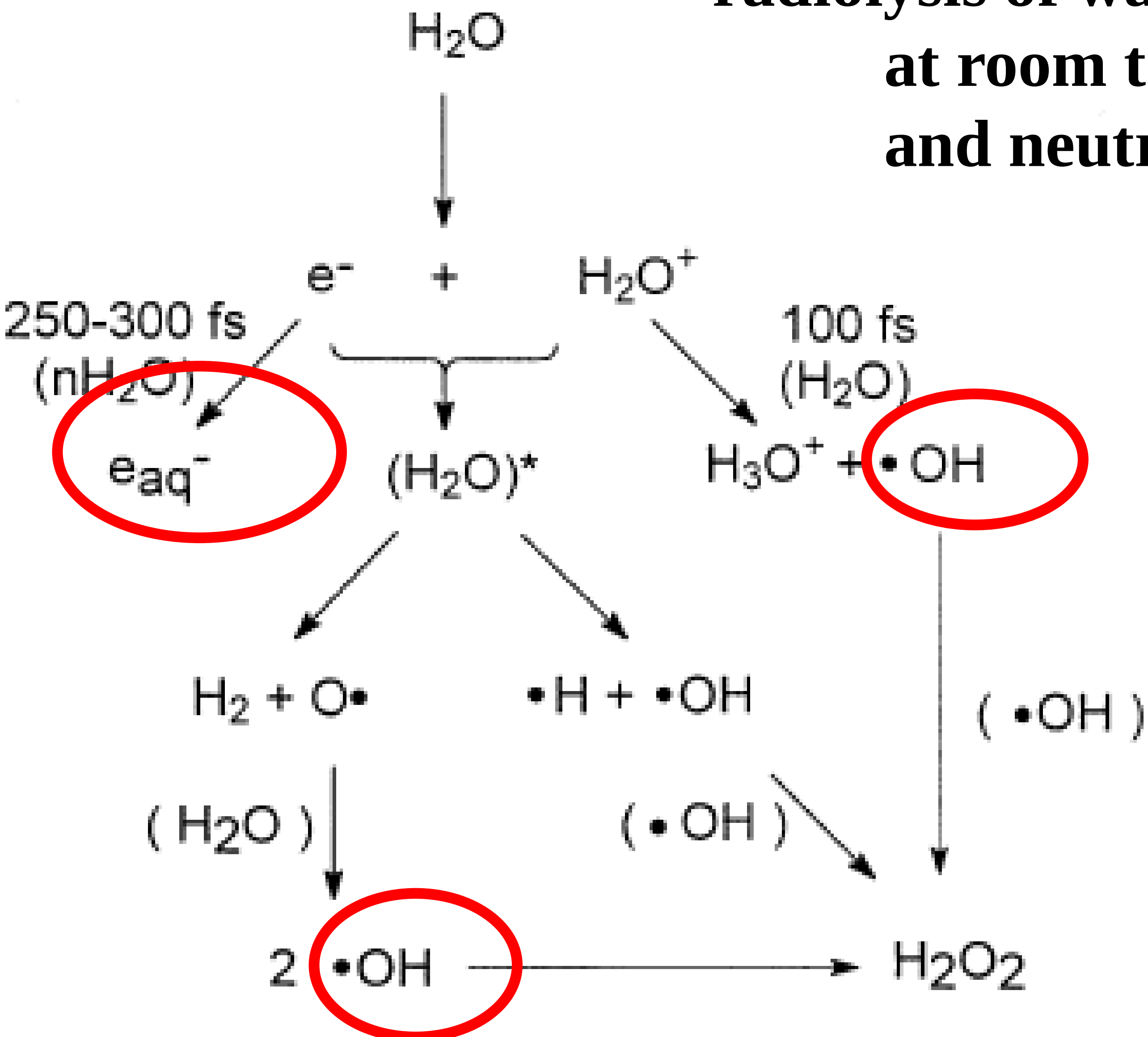
ALSO:
Atomic B-factors increase:



Damage: the Radiation Chemistry

1) INDIRECT RADIATION DAMAGE :

radiolysis of water at room temperature and neutral pH:

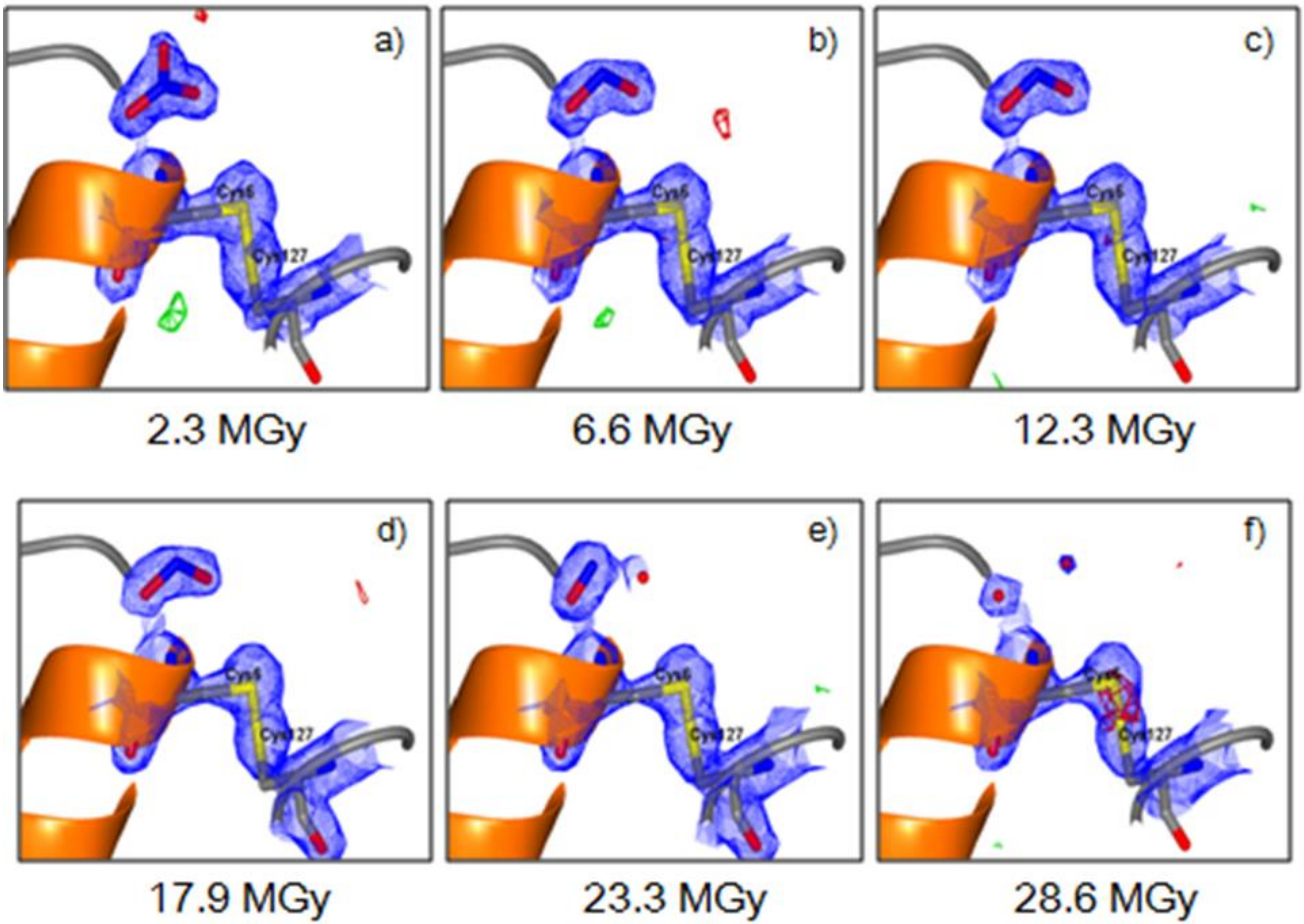


OH thought not to be mobile in glasses below 110K

(Owen et al Acta D 2012)

Hiroki, A. Pimblott, S. M. LaVerne, J. A. (2002) *J Phys Chem A* **106**, 9352-9358

Radiation Chemistry in action: Nitrate scavenger

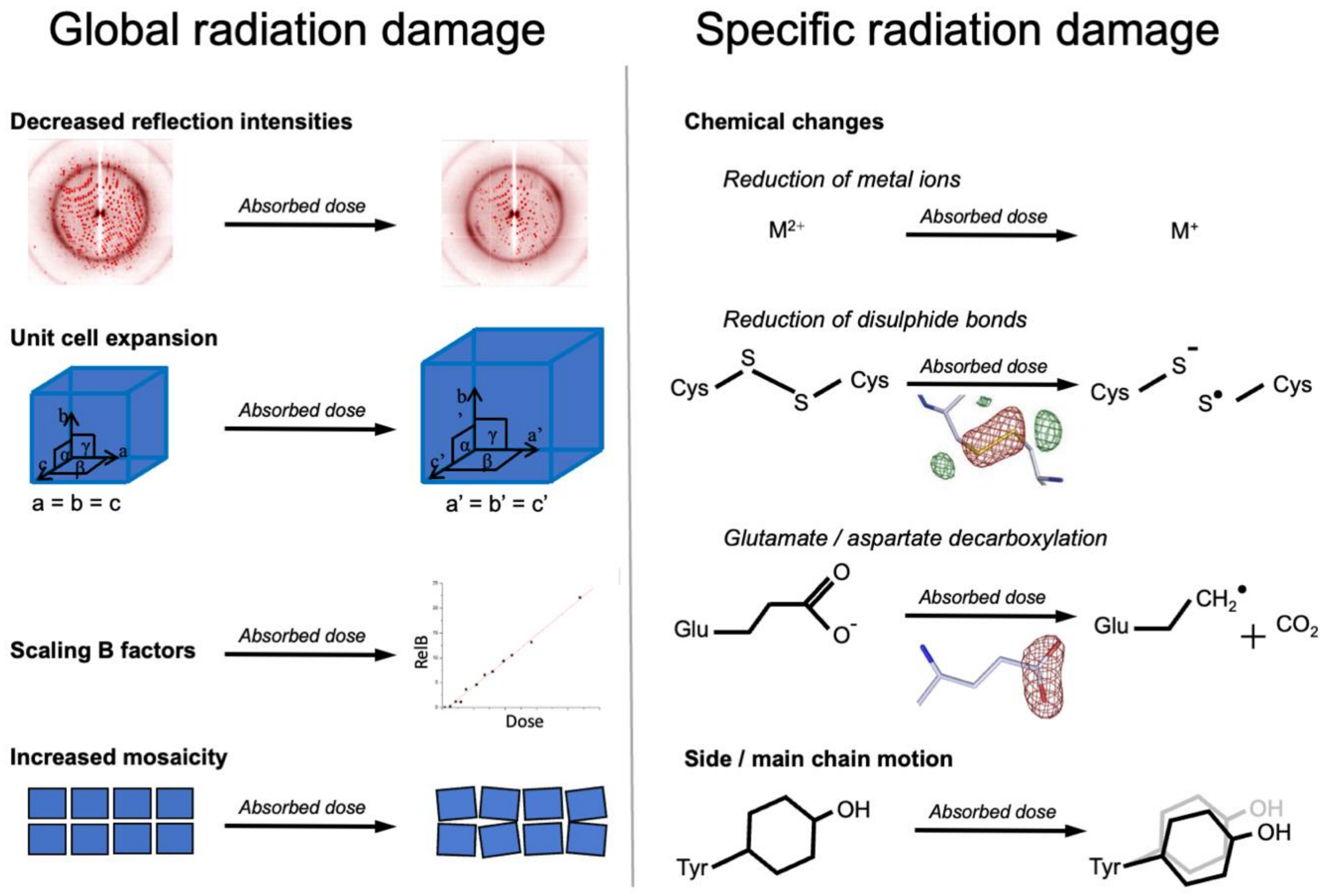


De la Mora, Carmichael, Garman *JSR* (2011) 18, 313

Specific Damage: summary.

- Can compromise biologically relevant observations (e.g. damage enzymatically important glutamates).
- Metallo-enzymes are reduced by X-ray beam.
- Perhaps weakly dose rate dependent ($< \times 2$)
- Perhaps weakly wavelength dependent ($< \times 2$)
- Weakly temperature dependent (varying results) ($< \times 2$)
- Can be reduced with certain scavengers: but very conflicting results (mainly $< \times 2$, benzoquinone RT $\times 9$)
- We DON'T understand pecking order of damage within an amino acid group pH? Solvent accessibility? Neighbouring amino acids?

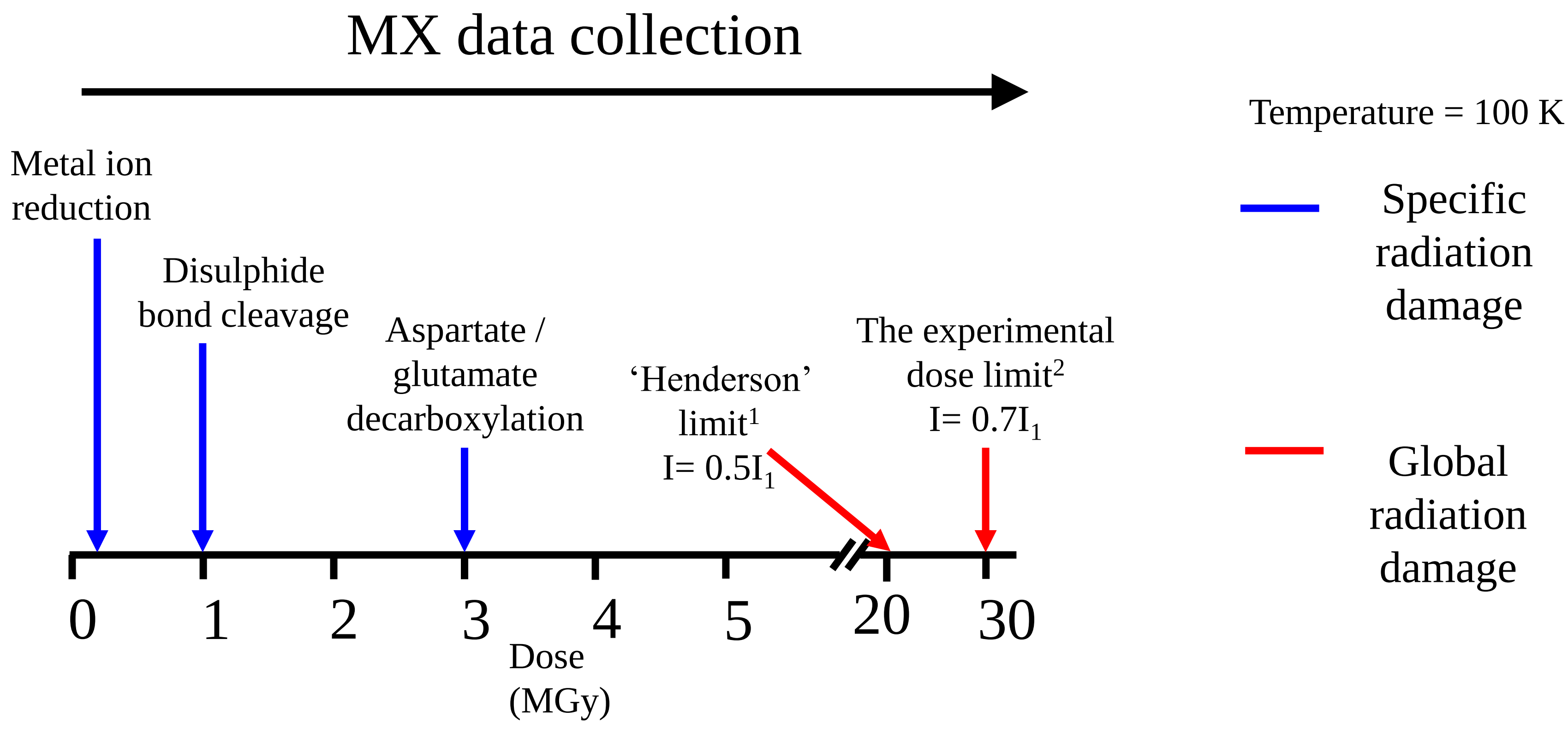
Summary



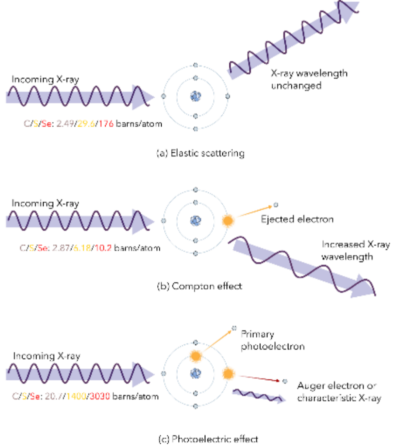
Garman & Weik COSB (2023)

At T=100 K:

- Specific damage effects onset before global damage
- Both classes of damage common in protein crystallography (MX)

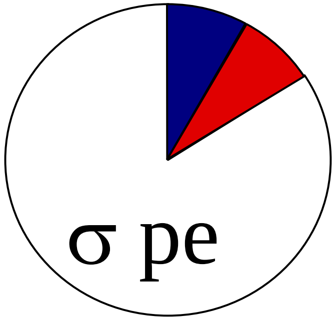


[1. Henderson Phil Trans RS B 1990 2. Owen *et al* PNAS 2006]



Radiation damage:

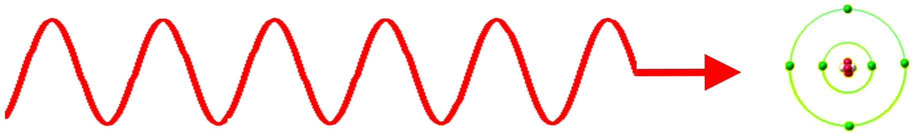
The Plan:



- What are the symptoms?
- **What is it?**
- Why do we care? Effect on MAD/SAD.
- How do we estimate the Dose?
- What do we know/would like to know?
- A new RD metric

A) Primary X-ray interaction processes with crystal and solvent.

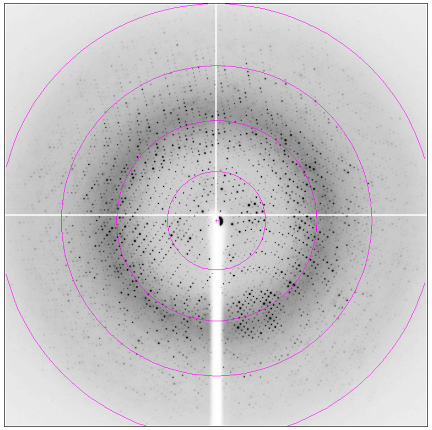
Thomson (Rayleigh, coherent) scattering



[8% at 1Å]

ELASTIC - no energy loss.

PHYSICS of the interaction of X-rays with crystals.



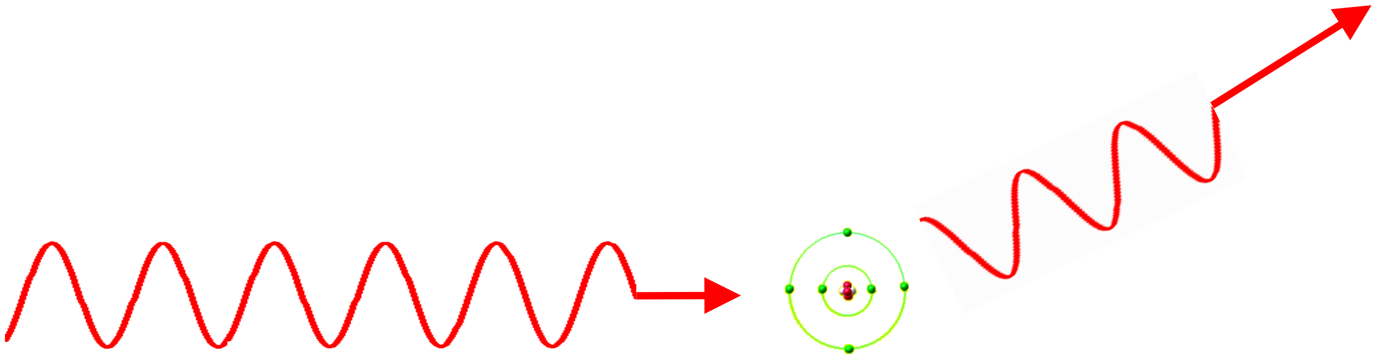
A) Diffraction

B) Absorption = Energy loss

N.B. > 90% of the beam does not interact at all, but goes straight through.

Primary X-ray interaction processes with crystal and solvent.

Thomson (Rayleigh, coherent) scattering

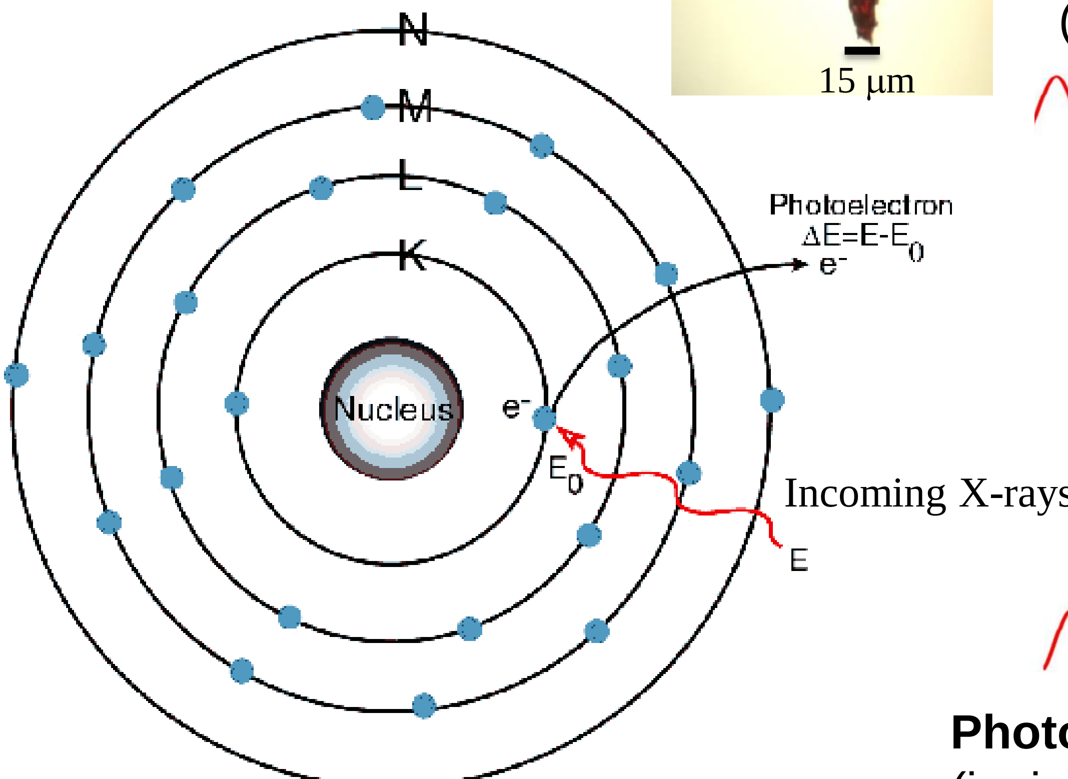


[8% at 1Å]

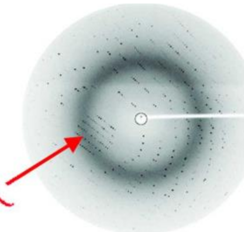
ELASTIC - no energy loss.

Interaction of X-rays with biological samples

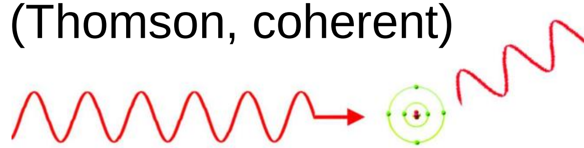
At E_x=12.4 keV, 100 μm protein crystal, only 2% of beam interacts.



8%

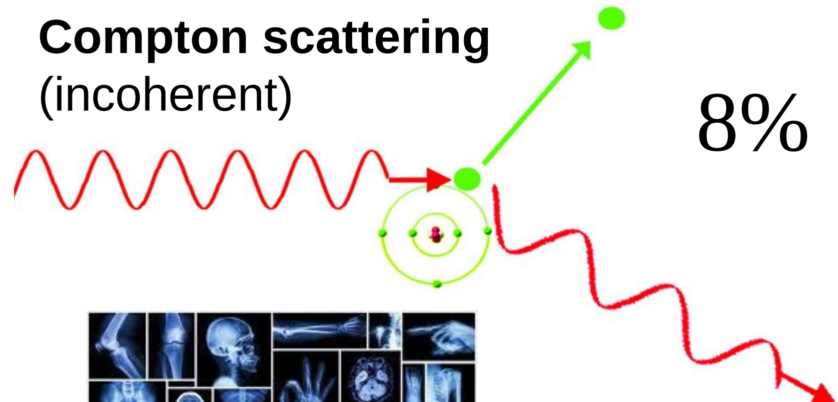


Elastic scattering
(Thomson, coherent)



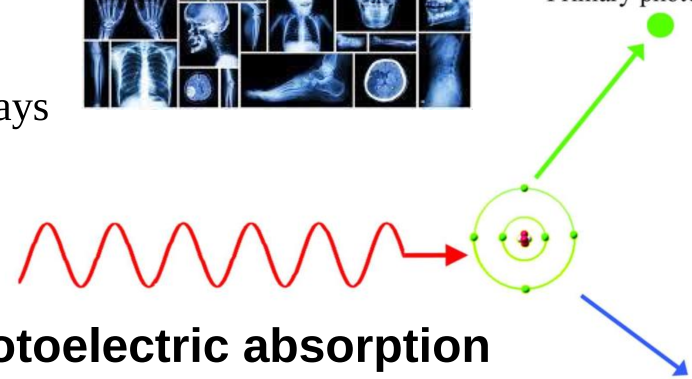
BUT IT IS THE BIT WE WANT!!

Compton scattering
(incoherent)



8%

Primary photoelectron



84%

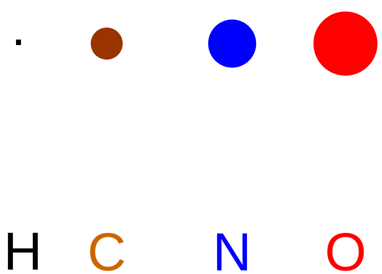
Photoelectric absorption
(ionization of up to 500 other atoms)

[Garman, *Acta Cryst*. (2010). D66, 339–351]

Photoelectric Cross Sections (barns/atom) at 13.1 keV

[1 barn=10^{-28}m^2]

A few heavy atoms can
make a big difference.



[Ravelli *et al*., JSR,(2005) 12]

Photoelectric Cross Sections (barns/atom) at 13.1 keV

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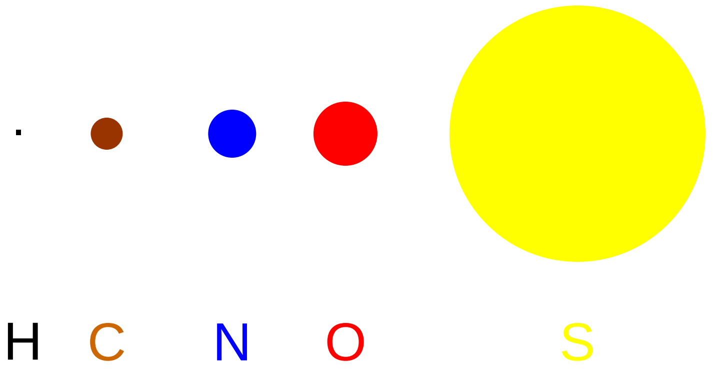

[Ravelli *et al.*, JSR,(2005) 12]

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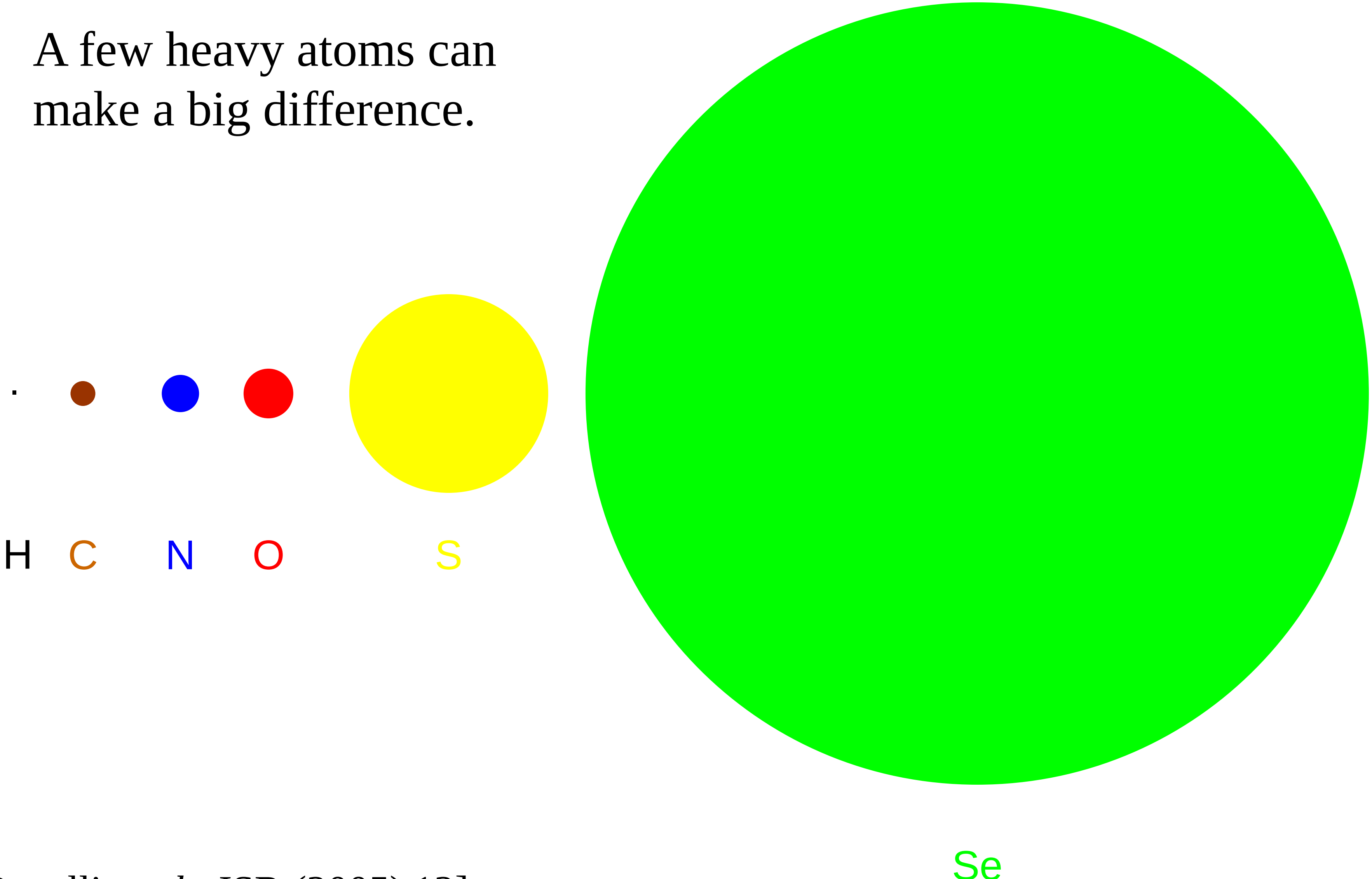
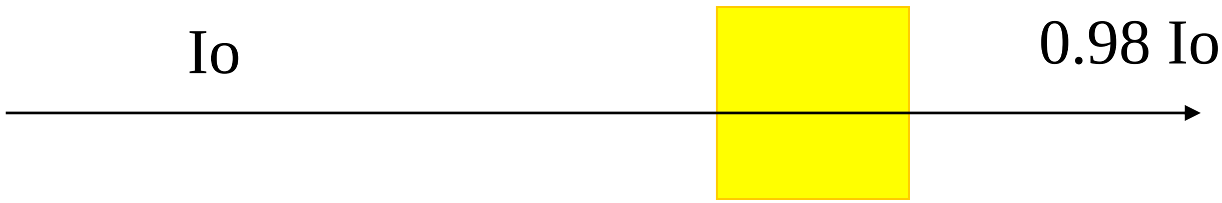


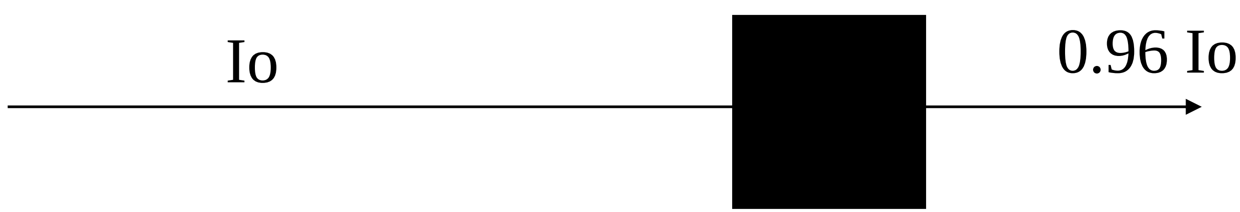
[Ravelli *et al*., JSR,(2005) 12]

Beam absorption (λ=1Å) by a protein crystal

Native HEWL 100 μm thick



Platinum derivatised (1/molecule)
HEWL 100 μm thick



N.B. INCIDENT FLUX is the SAME but the absorbed dose is DOUBLE

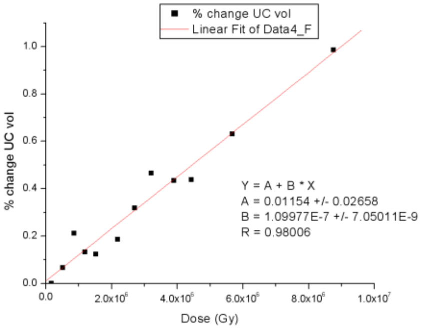
A few heavy atoms in the solvent can make a BIG difference to the absorption cross section and this the dose rate for the SAME flux.

e.g. Cacodylate buffer (arsenic, mass 75 cf selenium = 79)

BACK SOAKING to REMOVE

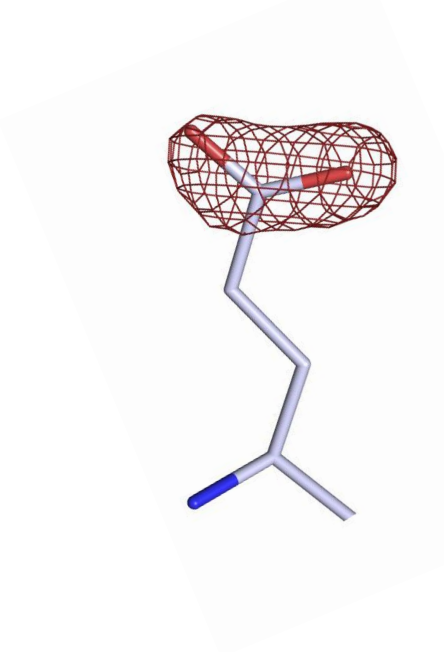
Non-specifically bound heavy atoms

e.g. a brominated DNA-protein complex will radiation damage much faster than a native crystal, and will de-brominate during data collection [Ennfar et al, Acta Cryst D (2002) 1263-1268].



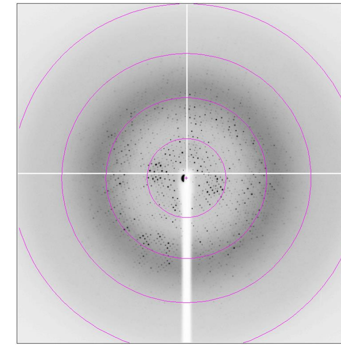
Radiation damage:

The Plan:



- What are the symptoms?
- What is it?
- **Why do we care? Effect on MAD/SAD.**
- How do we estimate the Dose?
- What do we know/would like to know?

Why do we care?



A) We don't get all the data we need!

B) Effect on MAD/SAD phasing methods.

- Failure of structure determination

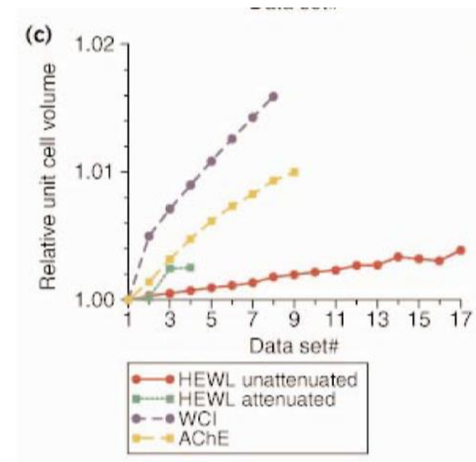
(Multi-wavelength anomalous dispersion MAD, SAD)

due to creeping non-isomorphism –

a) cell expansion and

b) movement of molecule in unit cell

c) structural changes DURING experiment.



i.e. **MAD/SAD phasing** signals (<5%) washed out completely

e.g. for a 0.5% change in all 3 unit cell dimensions of 100Å, reflection intensity changes by **15% at 3Å**

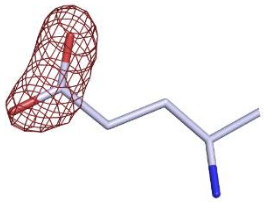
[Crick and Magdoff (1956) Acta Cryst **9**, 901-908]

Why care?

C) Radiation damage can affect our biological results

- Metallo-proteins often photo-reduced during the experiment [e.g. PSII, Yano et al, PNAS (2005)]

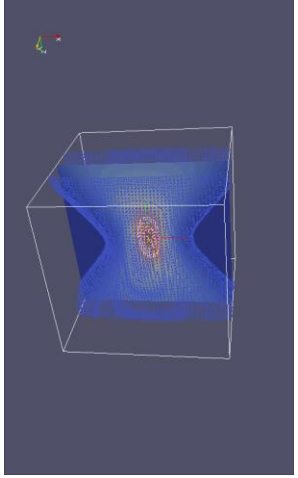
- Decarboxylation of Glu is sometimes part of the protein mechanism, but is indistinguishable from radiation damage at the synchrotron



- X-ray induced structural changes were initially misleading in studies of intermediates
  e.g. Bacteriorhodopsin: orange species

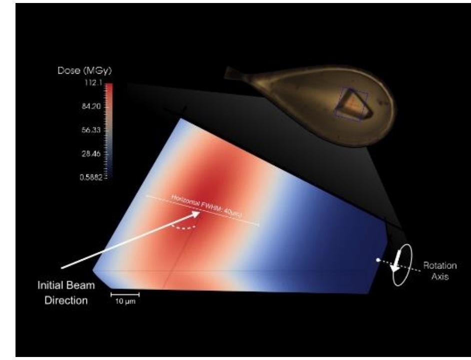
Takeda et al, Crystal structure of the M intermediate of bacteriorhodopsin…JMB (2004),

Wickstrand, et al., Bacteriorhodopsin: Would the real structural intermediates please stand up?, Biochim. Biophys. Acta (2014)



Radiation damage:

The Plan:



- What are the symptoms?
- Why do we care? Effect on MAD/SAD.
- What is it?
- **How do we estimate the Dose?**
- What do we know/would like to know?
- A new RD metric

DOSE Postulate (Henderson 1990):

- There is a MAXIMUM dose for MX postulated as 20 MGy

(Energy absorbed/unit mass: Joules/kg = Gy)

which protein crystals can tolerate: depends only on the PHYSICS of the situation.

Henderson: 20 MGy

Experimental: 43 MGy to $I_{0.5}$ – but don't go lower than $I_{0.7}$, 30 MGy*

- Crystal might not reach that limit due to chemical factors, but it is unlikely to last BEYOND the limit.

- Need to be able to calculate the DOSE conveniently: **RADDOSE-3D**

*Owen, Rudino-Pinera, Garman (2006), PNAS

RADDOSE-3D

V1: Zeldin, Gerstel, Garman (2013) *J.Appl.Cryst*,

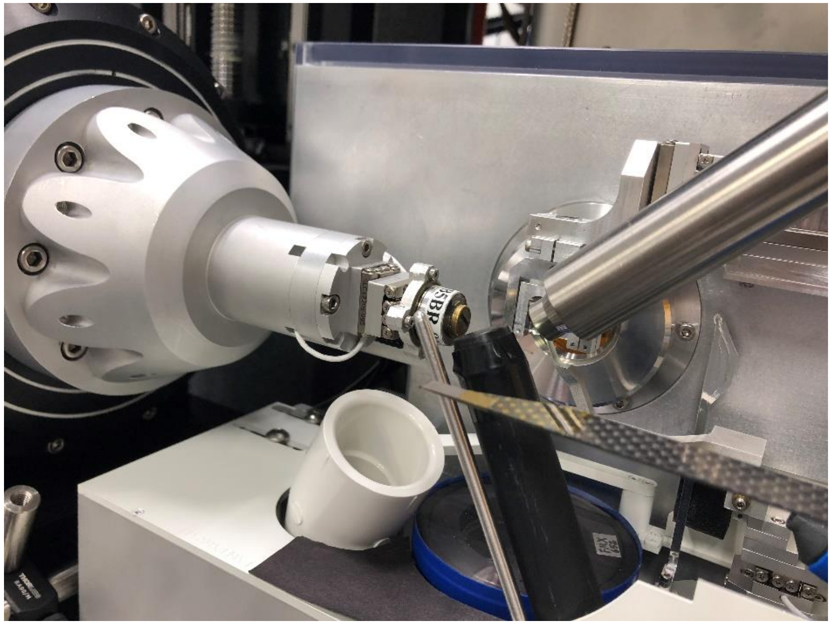
V2: Bury et al (2018*) Protein Science*

V3: Dickerson and Garman (2021) *Protein Science*

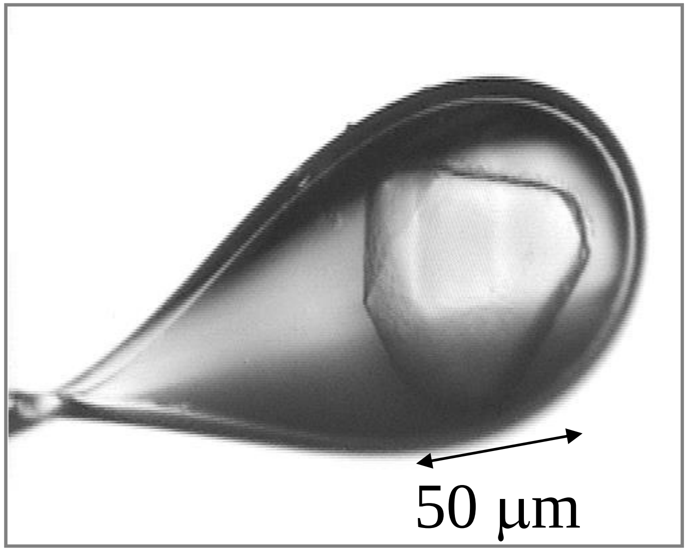
Make the dose calculation convenient for MX (include solvent contribution in mM and heavy atoms explicitly)

To find the energy deposited per unit mass in the crystal, need to characterise two things:

The beam



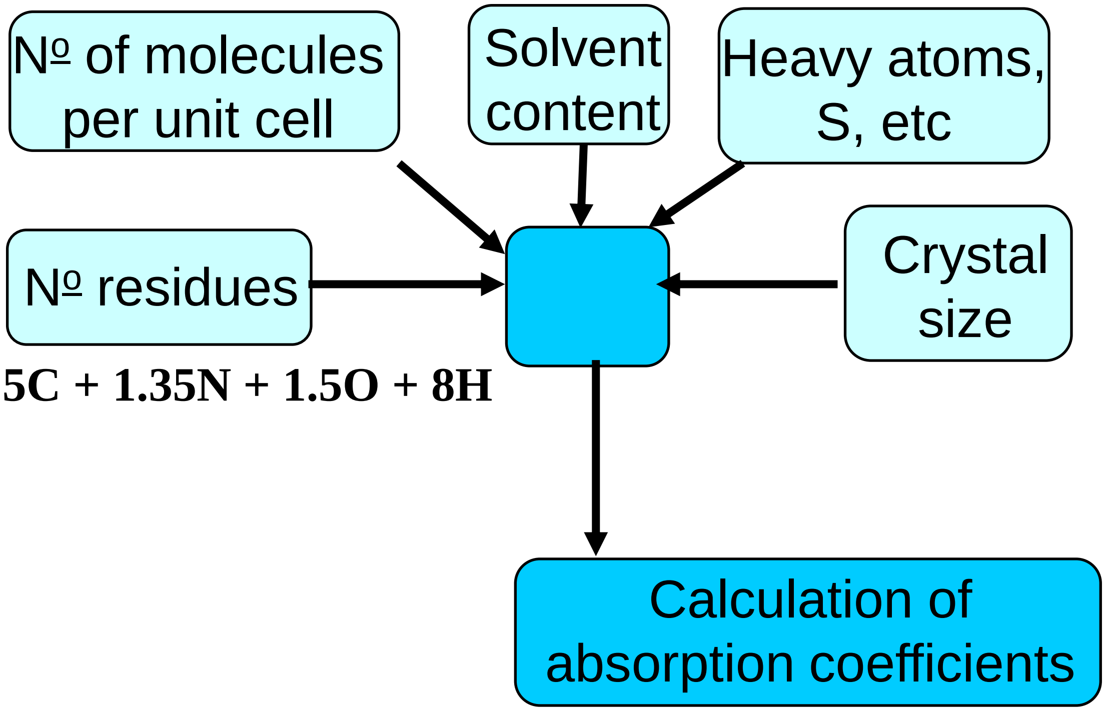
The crystal

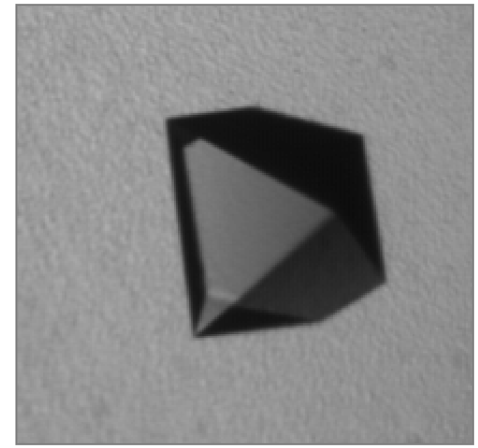


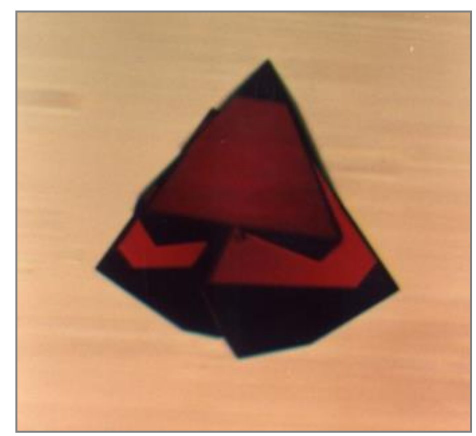
Calculating Dose

(*RADDOSE-3D*)

Crystal Characteristics





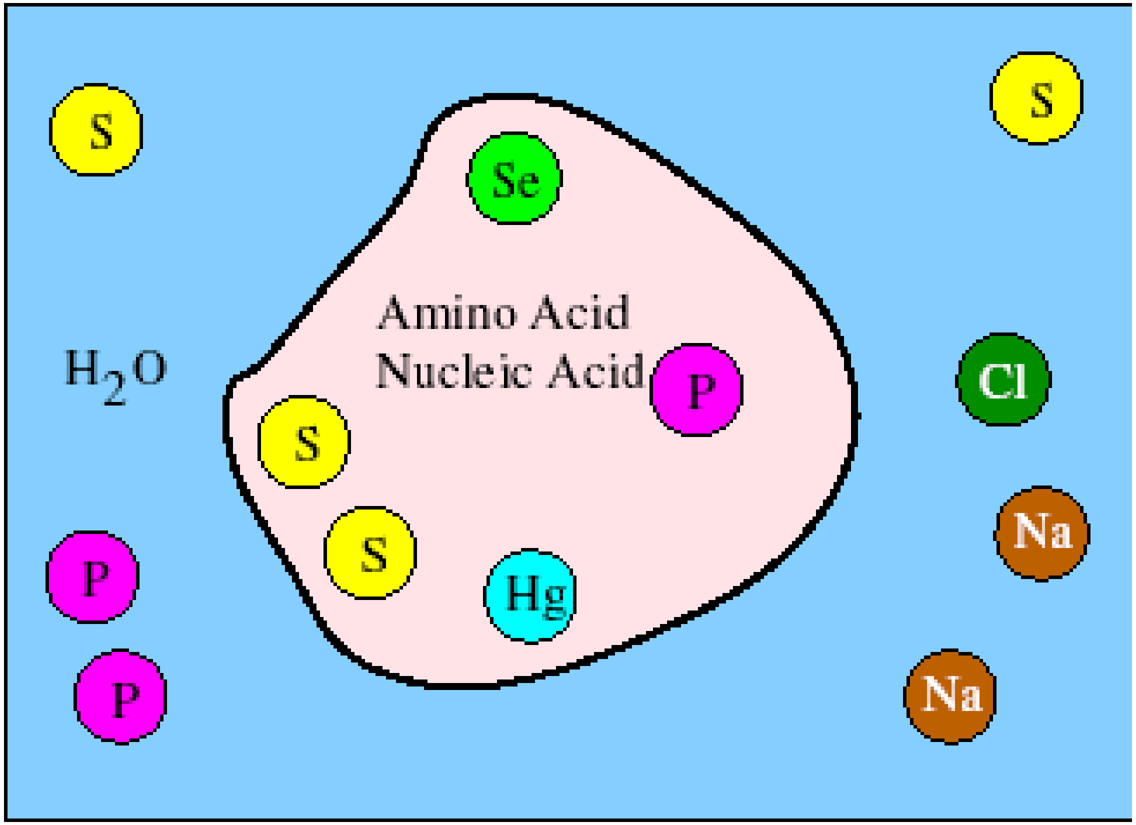


200 µm

absorption coefficients

e.g. apoferritin: 0.406mm^{-1}

holoferritin: 1.133mm^{-1}

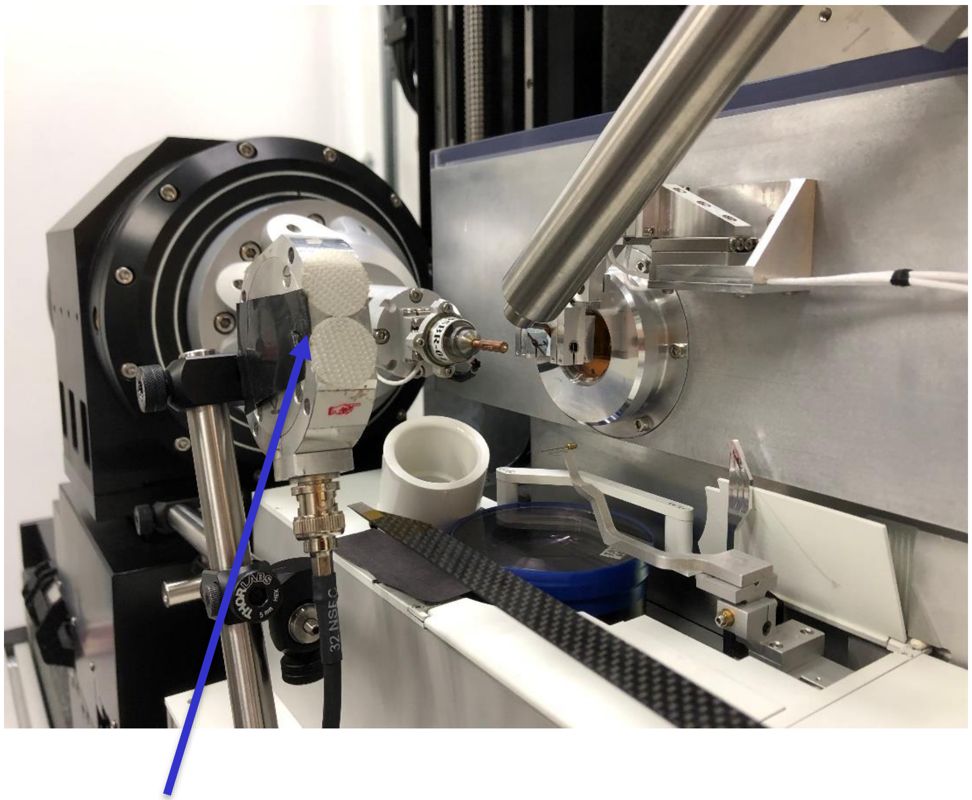


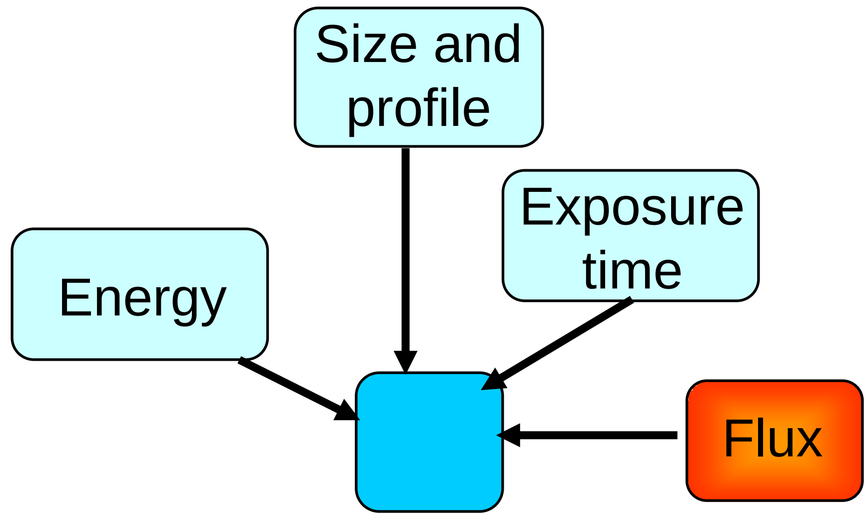
Number of Amino Acids

‘HA’ atoms per monomer, *e.g.* S, Se, Hg

Solvent - concentrations of components, *e.g.* Na^+, Cl^-

Calculating Dose
(*RADDOSE-3D*)
Beam Characteristics





PIN diode to measure flux (ph/s)

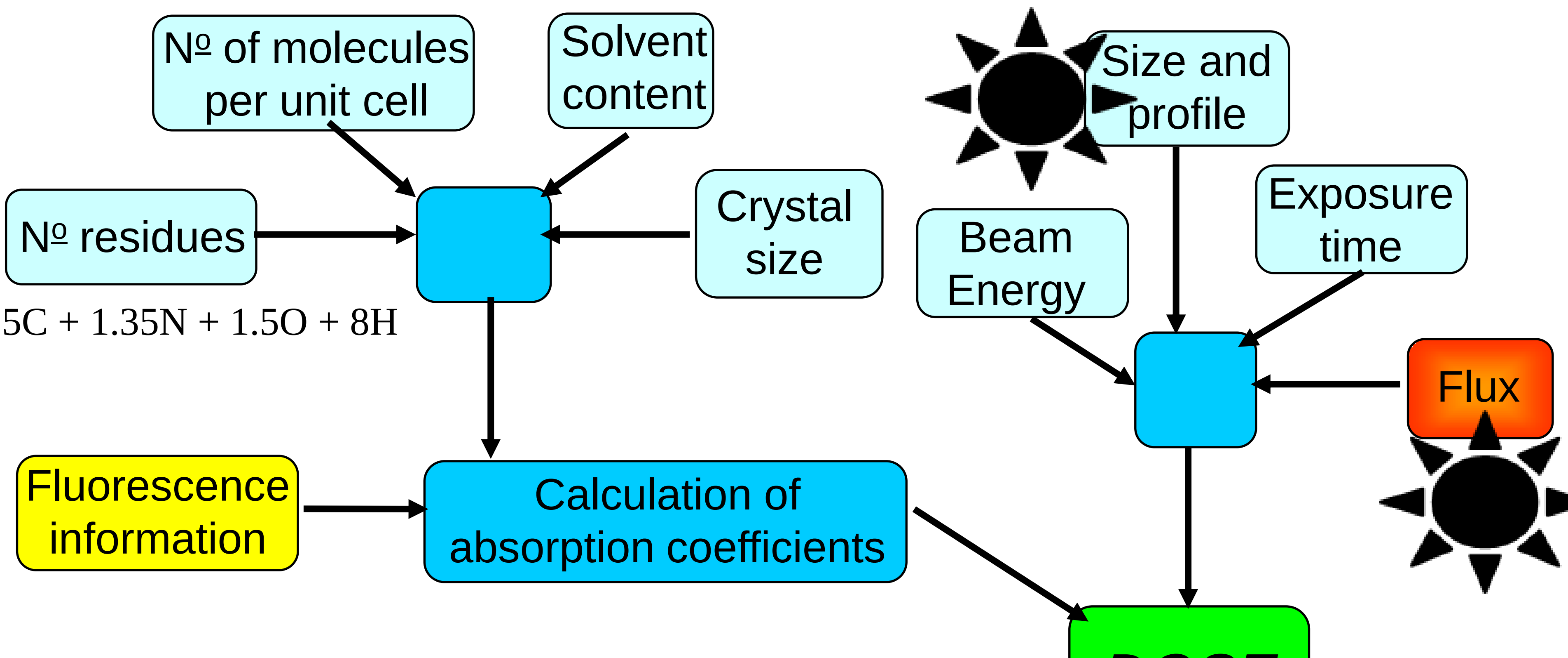
Owen et al JSR 2009

Calculating Dose
(*RADDOSE*)

[R-v1:Murray, Garman & Ravelli, JAC 2004
R-v2: Paithankar, Owen & Garman, JSR 2009,
R-v3: Paithankar & Garman, Acta D 2010]

Crystal Characteristics

Beam Characteristics



e.g. *absorption coefficients*, μ_{abs} , *at 12.7 keV*

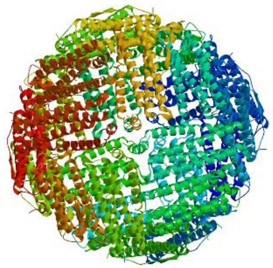
apoferritin: 0.406mm^{-1}

holoferritin: 1.133mm^{-1}

MX experimental dose limit (@ 2Å) measurement: Ferritin

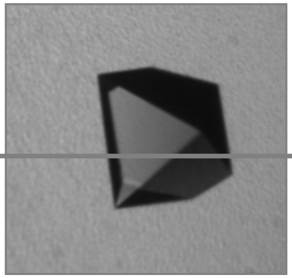
N.B. INCIDENT FLUX is the SAME but the absorbed dose is DOUBLE

The heavy atom (z ≥ 16) content of a crystal is not crystallographically defined, but we need it.



Apoferritin

Io

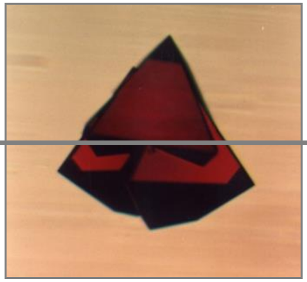


0.98 Io

absorption coefficient
$0.406 mm^{-1}$

Holoferritin

Io



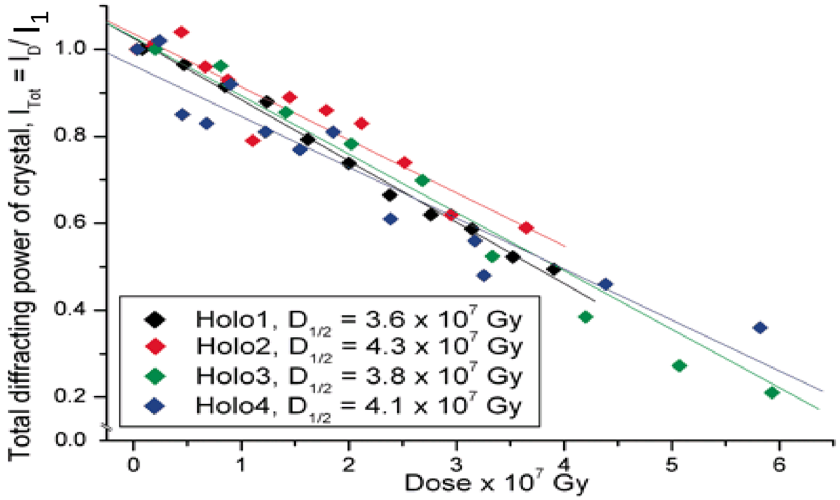
0.96 Io

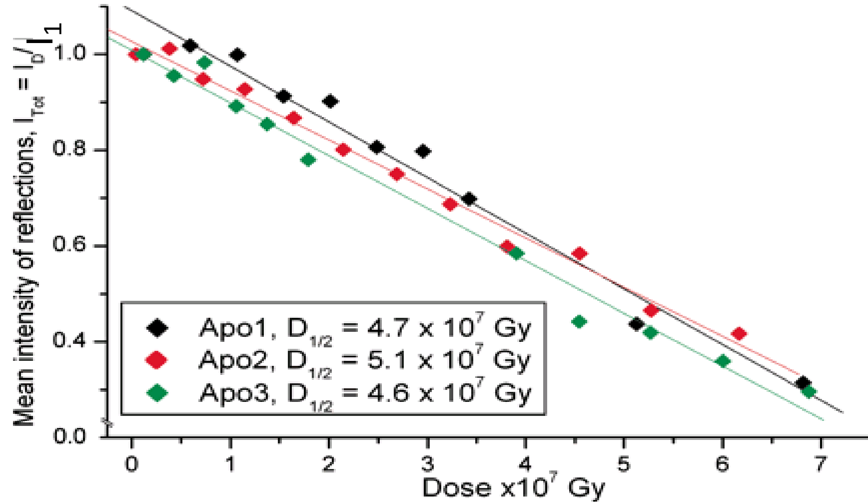
absorption coefficient
$1.133 mm^{-1}$

λ=1Å
100 μm thick xtal

Dose Limit Quantification at 100 K

- Holoferritin & Apoferritin as model: absorption coefficients differ by factor of 2
- Intensity: ~linear dependence on dose
- $D_{1/2}$ is dose to $I_0/2$
- $D_{1/2} = 4.3\ (\pm\ 0.4) \times 10^7$ Gy
  $= 43$ MGy
- Henderson limit, $D_{1/2} = 20$ MGy
- Howells et al (2005): resolution dependent limit of 10 MGy/ Å





Suggested limit to retain biological \`fidelity'

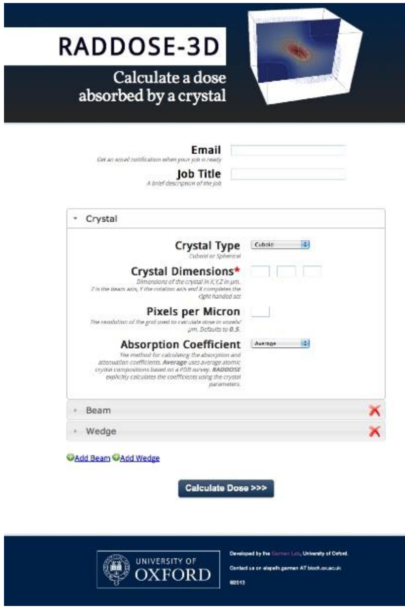
$$D_{0.7} = 3.0 \times 10^7 \text{ Gy} = \mathbf{30\ MGy}$$

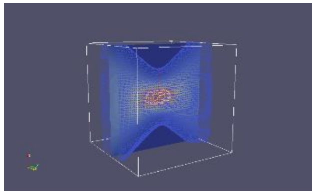
Owen, Rudiño-Piñera, Garman PNAS (2006)

RADDOSE-3D

- TIME- and SPACE-resolved modeling of dose distributions in MX in Java, replaces RADDOSE:
- Full 3-D simulation of dose absorption by the crystal
- Can deal with multiple wedges of data and different energy beams (e.g. MAD)
- Models beam as Top-Hat or Gaussian **or can use measured experimental profiles**
- Engineered for easy extendibility:

  can use any crystal shape
- On server: **www.raddo.se (!!)**
- On github.com/GarmanGroup/RADDOSE-3D



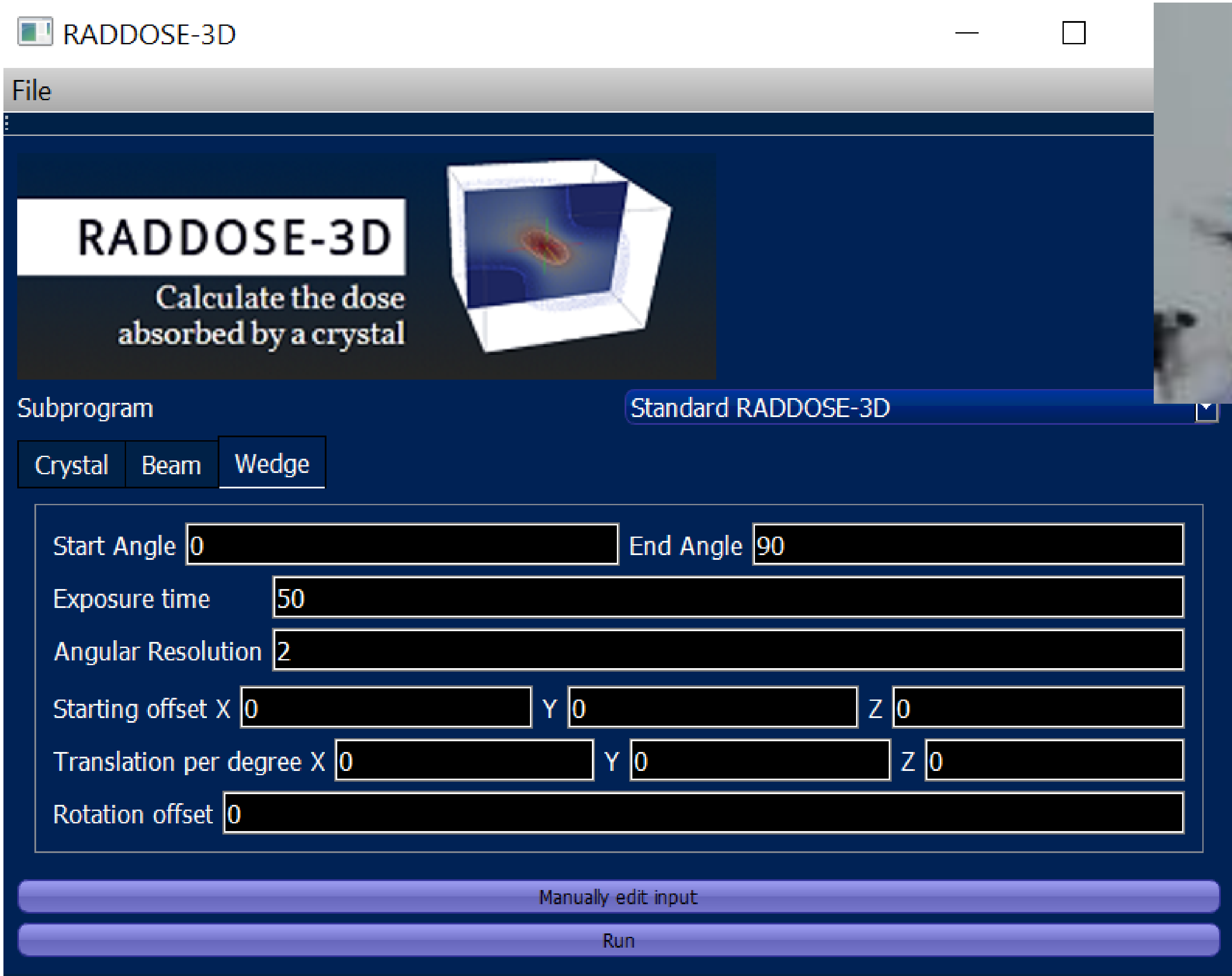


[Zeldin, Gerstel, Garman *JAC* (2013)
Bury, Brooks-Bartlett, Walsh, Garman, *Protein Science* (2018)]

RADDOSE-3D New GUI

Download from: https://github.com/jdickerson95/qt_RADDDOSE-3D/releases

Versions for a PC (Windows_release.zip) and for Linux (Linux_release.zip).



Josh Dickerson

RADDOSE-3D GUI

- Demonstration

RADDOSE-3D (XFEL etc)

TEST our new GUI (Josh Dickerson)!!

To run RADDOSE-3D (or XFEL etc)

Step 1: Download and unzip the RADDOSE-3D GUI from:

https://github.com/jdickerson95/qt_RADDDOSE-3D/releases

There are versions for a PC (Windows_release.zip) and for Linux (Linux_release.zip).

If you have a MAC, there is no new GUI yet, but one will come.

To run the GUI you need to have Java installed which you can get free at

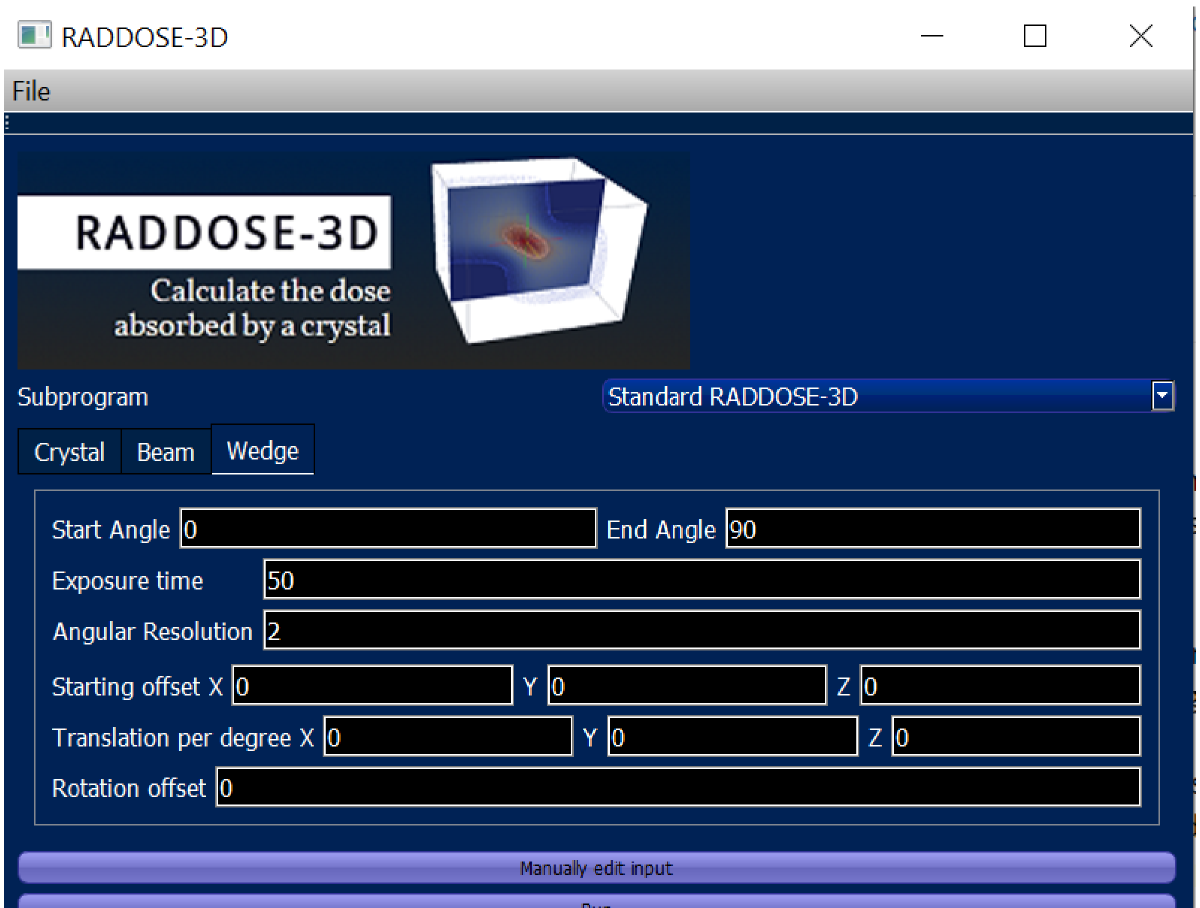
https://www.java.com/download/ie_manual.jsp

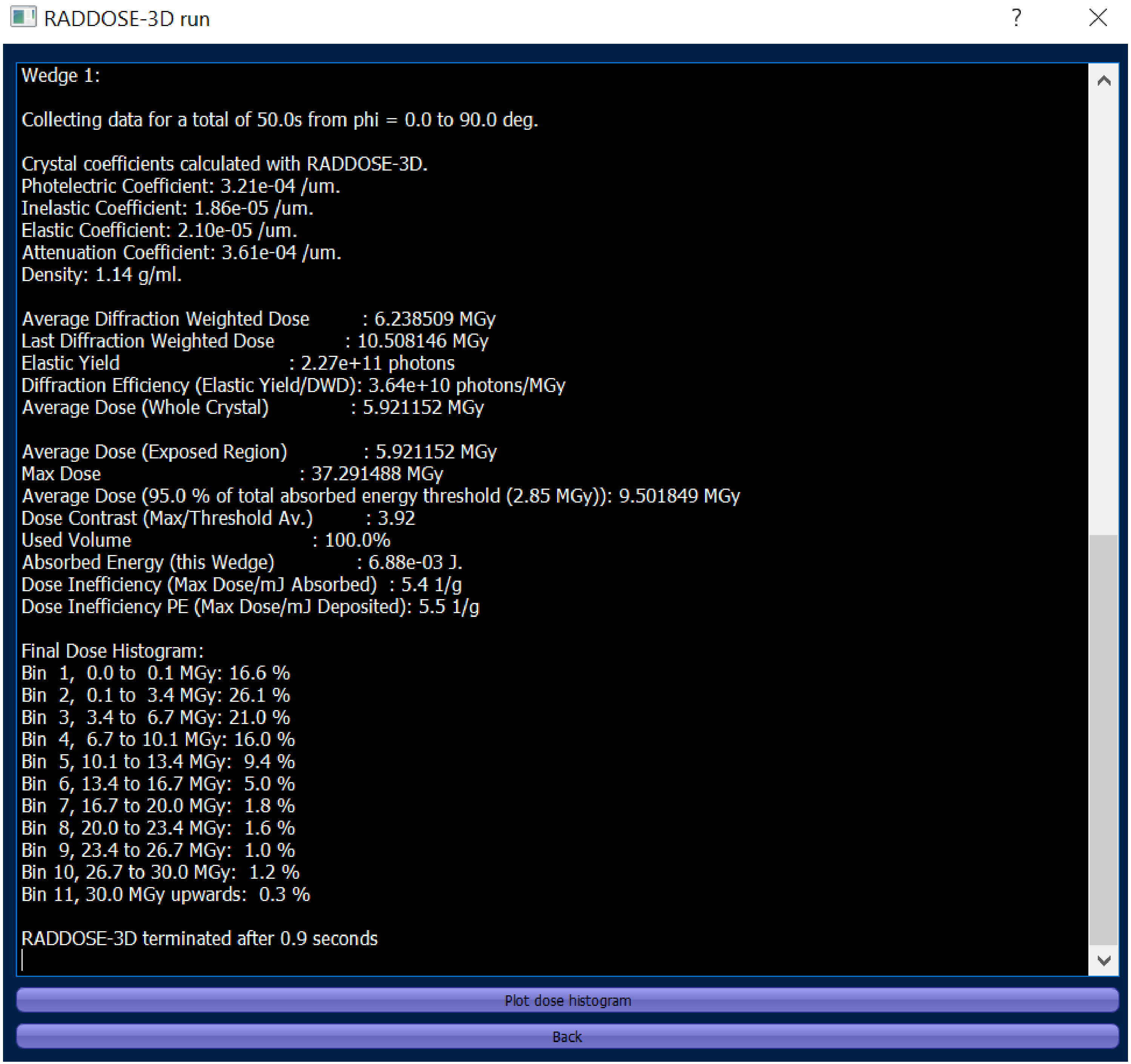
Also, if you have R (https://www.r-project.org/) installed, from the RADDOSE-XFEL output you will be able to produce 3D representations of the dose distribution in your sample.

Step 2: Unzip the file you have downloaded. On Windows machines I recommend the best place to install the executable (e.g. Documents versus Program Files) is in Documents as I tend to avoid putting programs in Program Files unless I am using an installer, to avoid the need for admin privileges and to keep things self-contained.

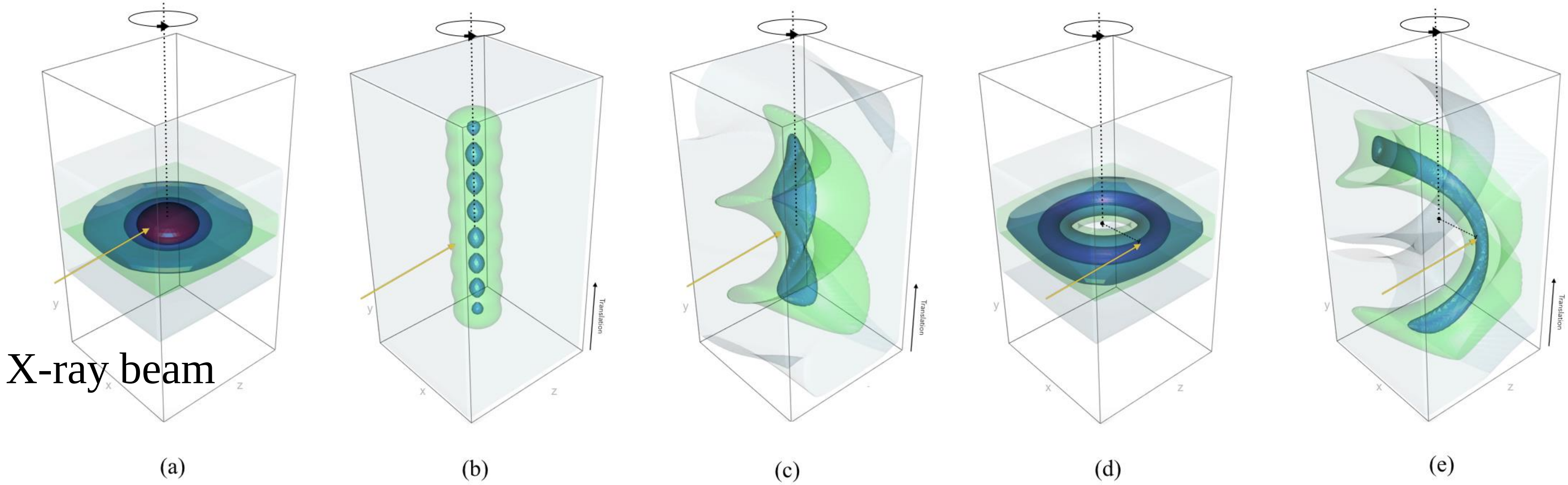
Step 3: Find the file RD3D_GUI.EXE and if on a PC click on it. For Linux run it however you usually run executable files. The GUI should open, and you can enter input on 3 tabs: crystal, beam and wedge.

Under ‘Standard RADDOSE-3D, select ‘XFEL’




RADDOSE-3D run
Wedge 1:
Collecting data for a total of 50.0s from phi = 0.0 to 90.0 deg.
Crystal coefficients calculated with RADDOSE-3D.
Photelectric Coefficient: 3.21e-04 /um.
Inelastic Coefficient: 1.86e-05 /um.
Elastic Coefficient: 2.10e-05 /um.
Attenuation Coefficient: 3.61e-04 /um.
Density: 1.14 g/ml.
Average Diffraction Weighted Dose : 6.238509 MGy
Last Diffraction Weighted Dose : 10.508146 MGy
Elastic Yield : 2.27e+11 photons
Diffraction Efficiency (Elastic Yield/DWD): 3.64e+10 photons/MGy
Average Dose (Whole Crystal) : 5.921152 MGy
Average Dose (Exposed Region) : 5.921152 MGy
Max Dose : 37.291488 MGy
Average Dose (95.0 % of total absorbed energy threshold (2.85 MGy)): 9.501849 MGy
Dose Contrast (Max/Threshold Av.) : 3.92
Used Volume : 100.0%
Absorbed Energy (this Wedge) : 6.88e-03 J.
Dose Inefficiency (Max Dose/mJ Absorbed) : 5.4 1/g
Dose Inefficiency PE (Max Dose/mJ Deposited): 5.5 1/g
Final Dose Histogram:
Bin 1, 0.0 to 0.1 MGy: 16.6 %
Bin 2, 0.1 to 3.4 MGy: 26.1 %
Bin 3, 3.4 to 6.7 MGy: 21.0 %
Bin 4, 6.7 to 10.1 MGy: 16.0 %
Bin 5, 10.1 to 13.4 MGy: 9.4 %
Bin 6, 13.4 to 16.7 MGy: 5.0 %
Bin 7, 16.7 to 20.0 MGy: 1.8 %
Bin 8, 20.0 to 23.4 MGy: 1.6 %
Bin 9, 23.4 to 26.7 MGy: 1.0 %
Bin 10, 26.7 to 30.0 MGy: 1.2 %
Bin 11, 30.0 MGy upwards: 0.3 %
RADDOSE-3D terminated after 0.9 seconds
Plot dose histogram
Back

Dose distribution vs exposure strategy with RADDOSE-3D



20 μm translation

Helical, 0.2 μm/ °

30 μm offset

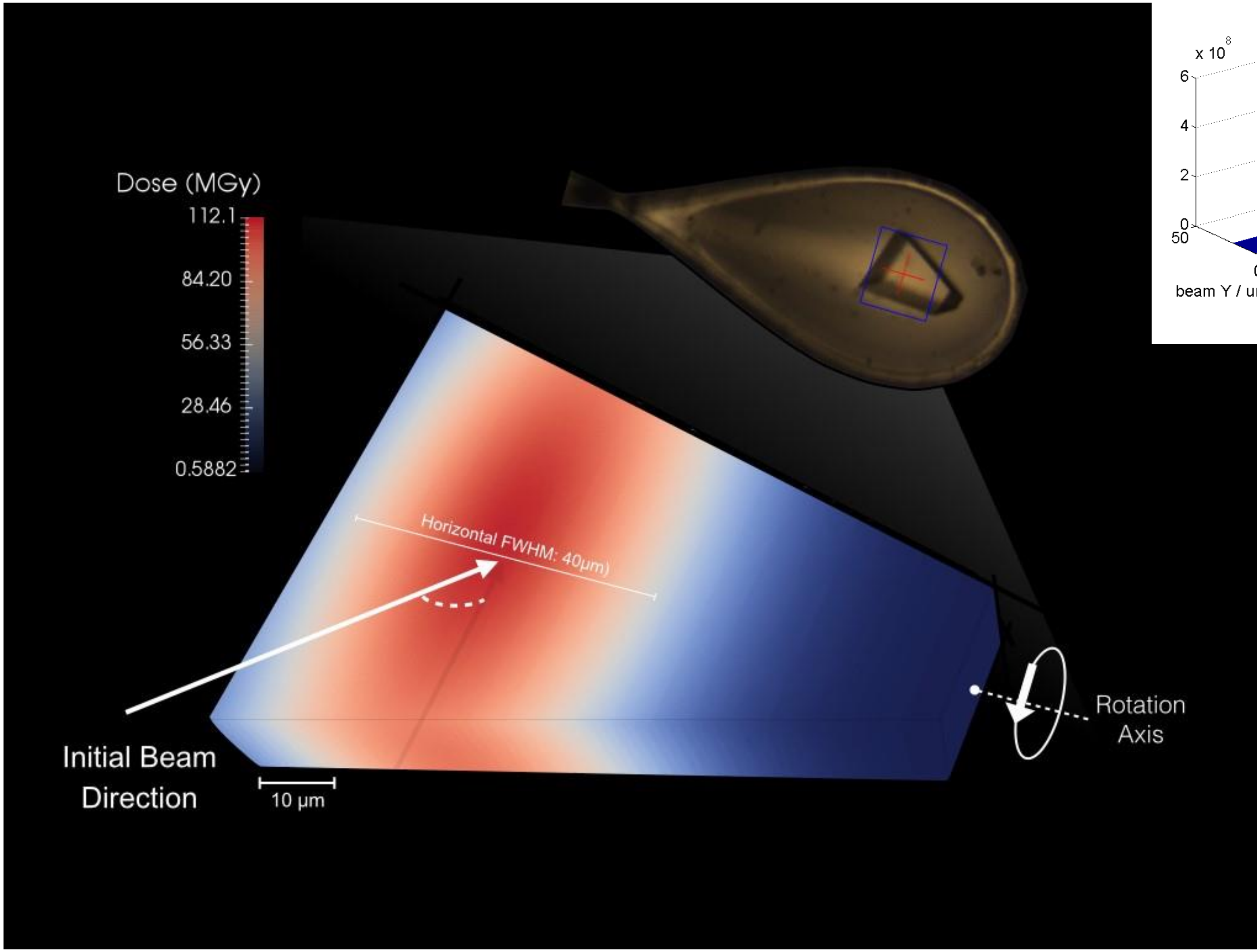
30 μm offset and helical 0.2 μm/ °

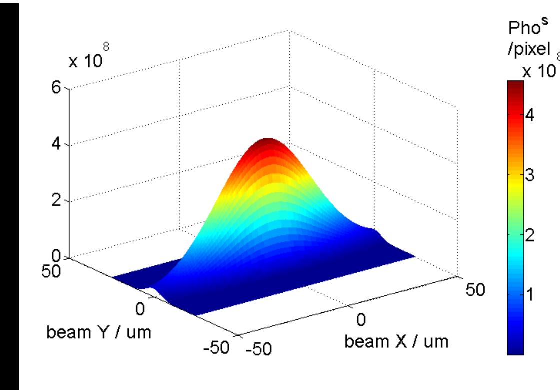
0.0001 MGy (grey),
5 MGy (green),
10 MGy (light blue),
20 MGy (dark blue),
30 MGy (red),
360° rotation
100 μm, 200 μm, 100 μm.
Gaussian beam (FWHM: 20 μm x 20 μm), 12.4 keV, 5 x 10^{11} ph/s,
1 mm x 1 mm rectangular collimation: full crystal bathed in beam

Helical strategy improvement:
Flot *et al* (2010) *JSR* **17**, 107

Zeldin *et al. JSR* (2013), Bury *et al. Protein Science* (2018)

Gaussian Beam Profile



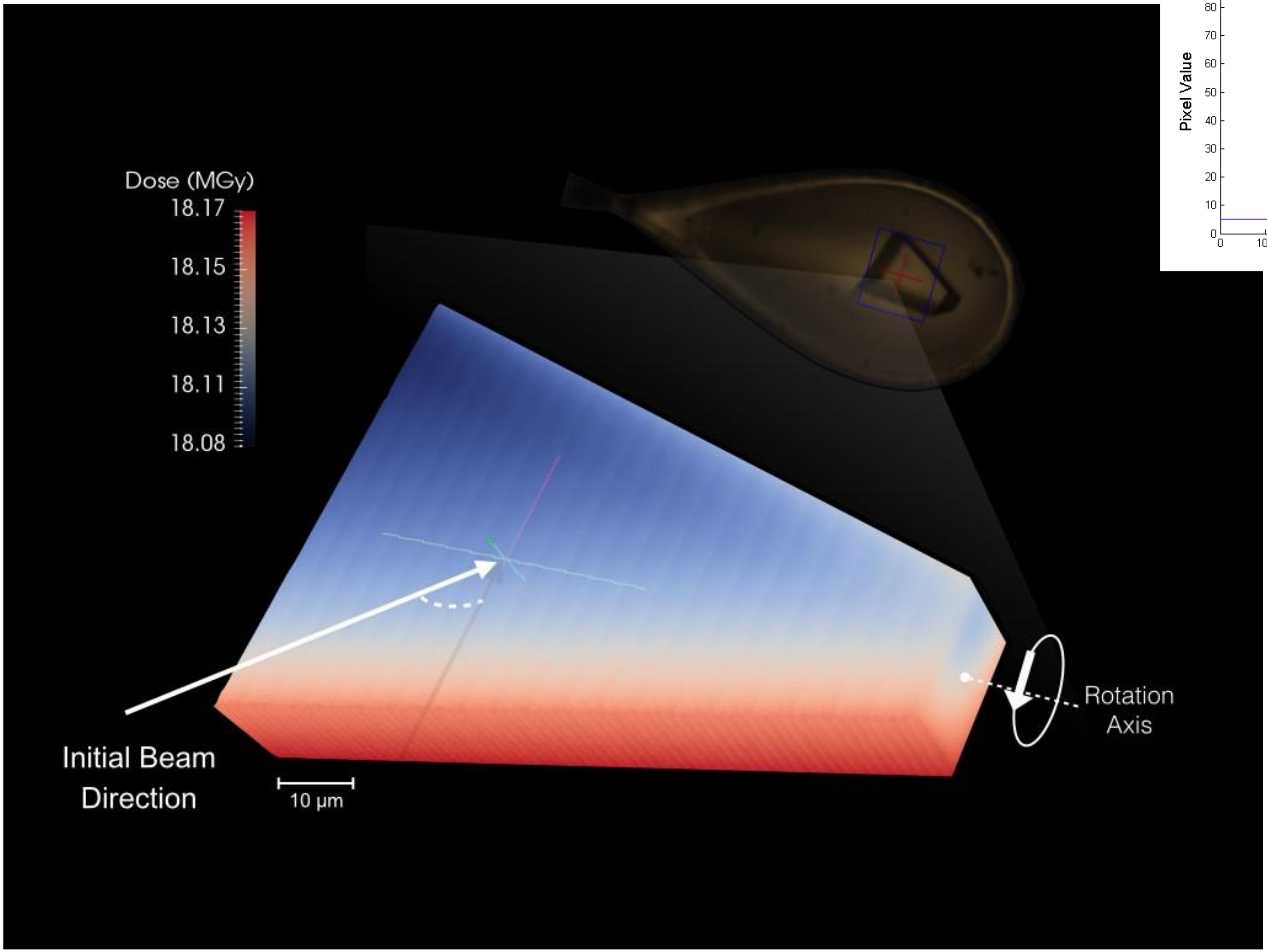


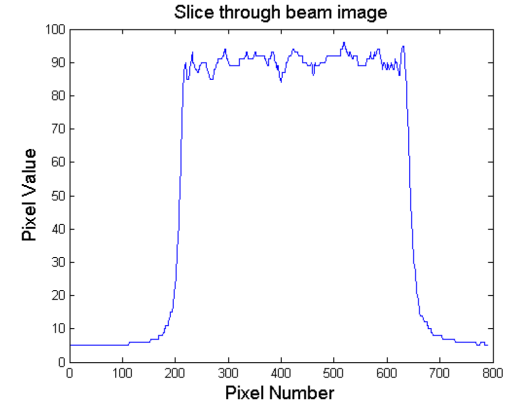
Differential irradiation may lead to differential damage: *get data which merge poorly and are a population of substates*

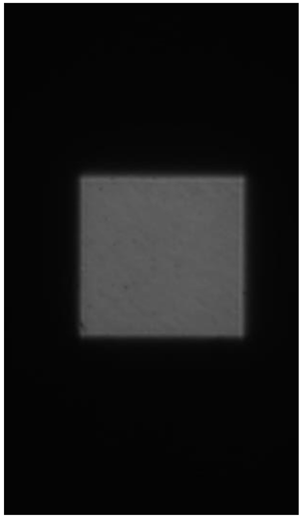
13.2 keV, 60(v)×40μm^2(h) FWHM, 100(h)×160μm^2(v) coll., 5×10^{11} ph/s

Garman & Weik *Meth. Mol. Biol* 1607 (2017)

Top-Hat profile

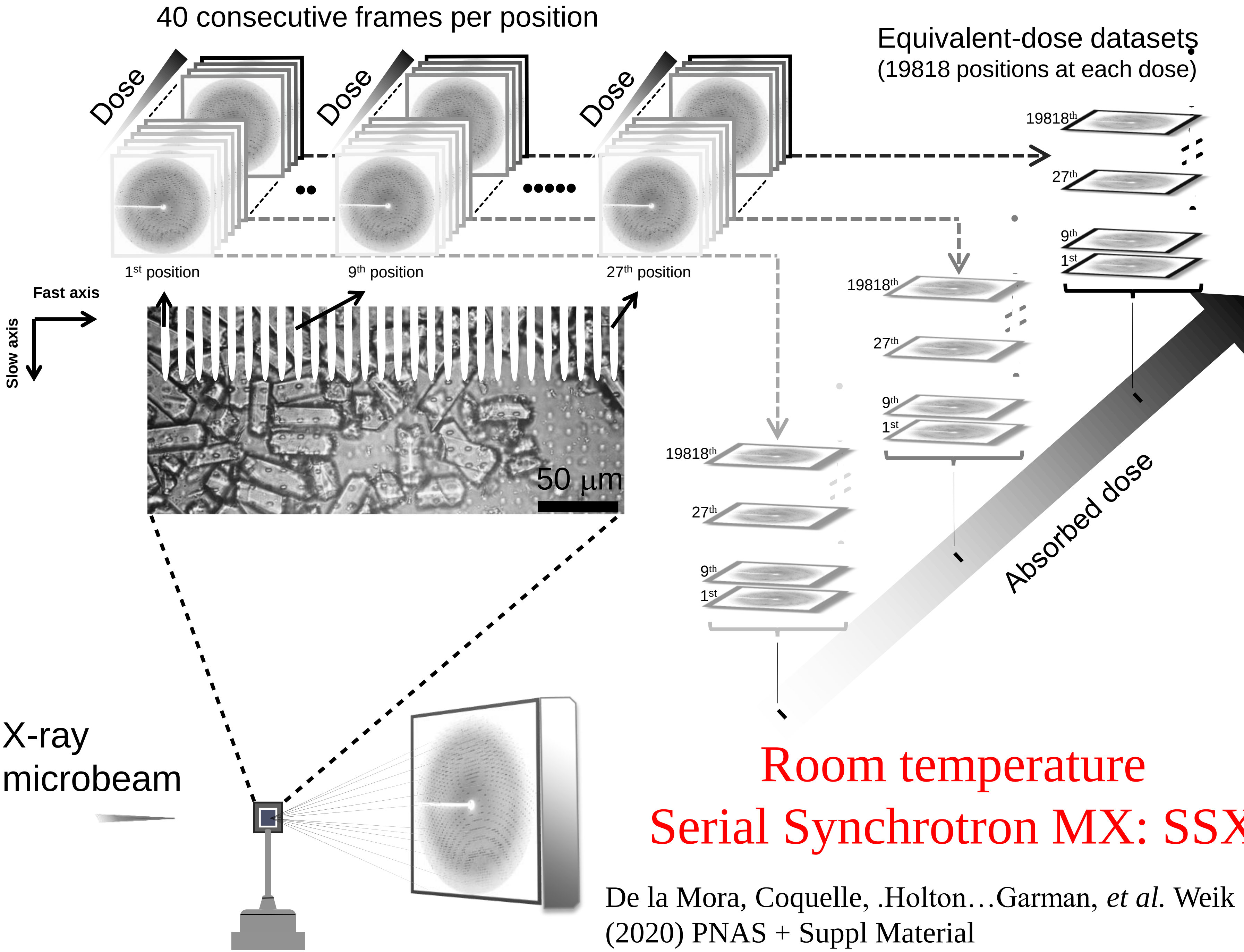






Imaged beam PETRA III, Bourenkov, Schneider

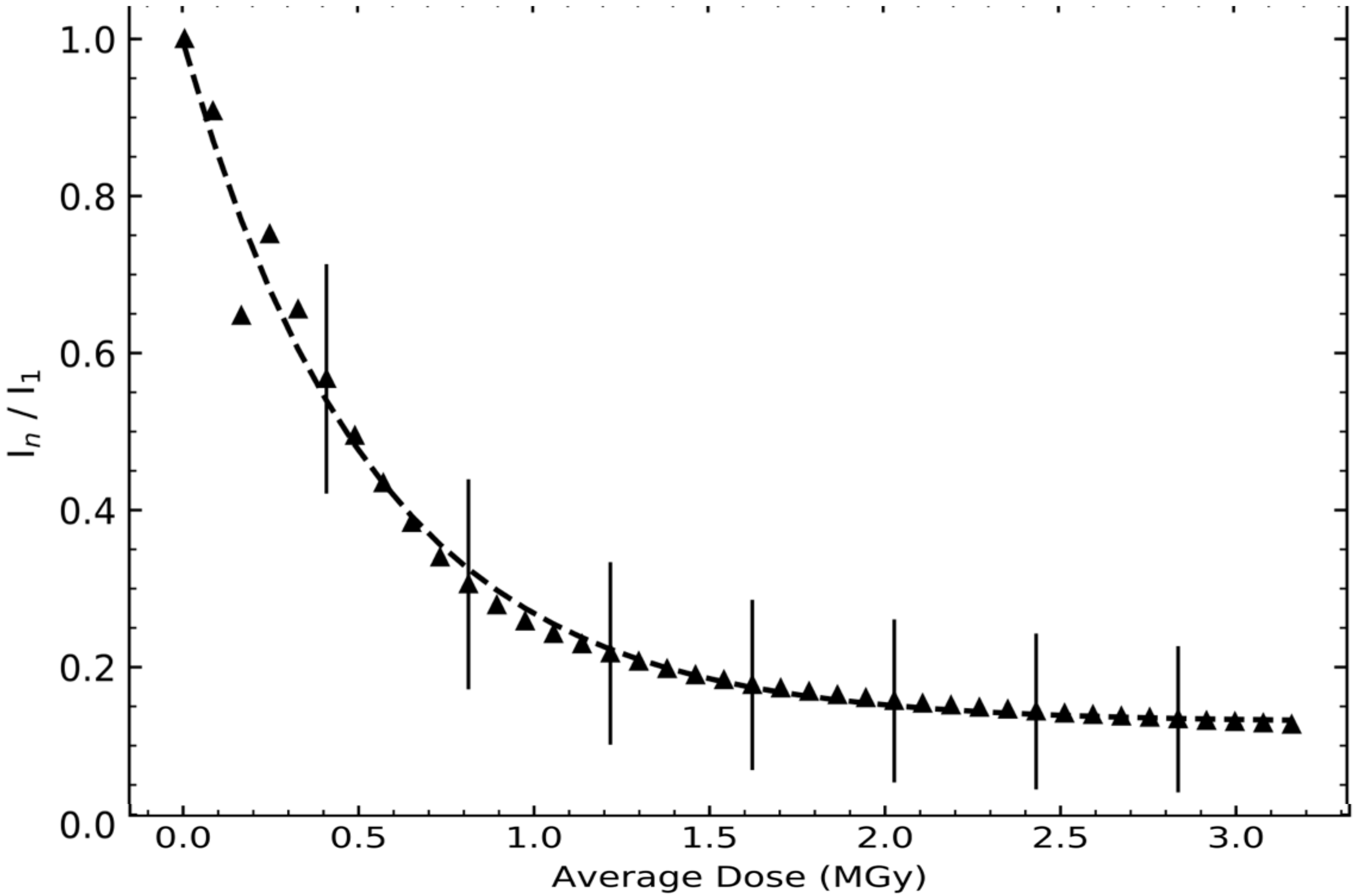
13.2 keV, 100(h) x 160µm^2(v) coll., 5e11 ph/s



Room temperature
Serial Synchrotron MX: SSX

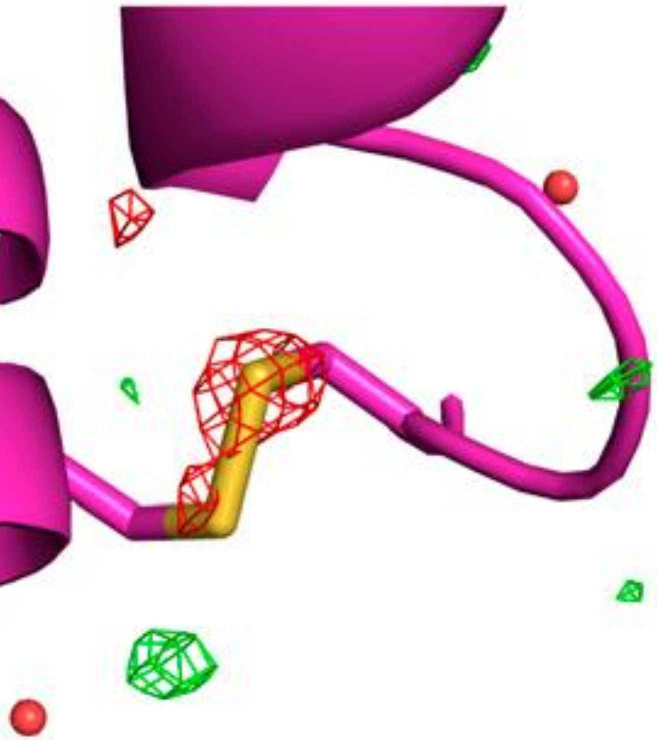
De la Mora, Coquelle, .Holton…Garman, *et al.* Weik (2020) PNAS + Suppl Material

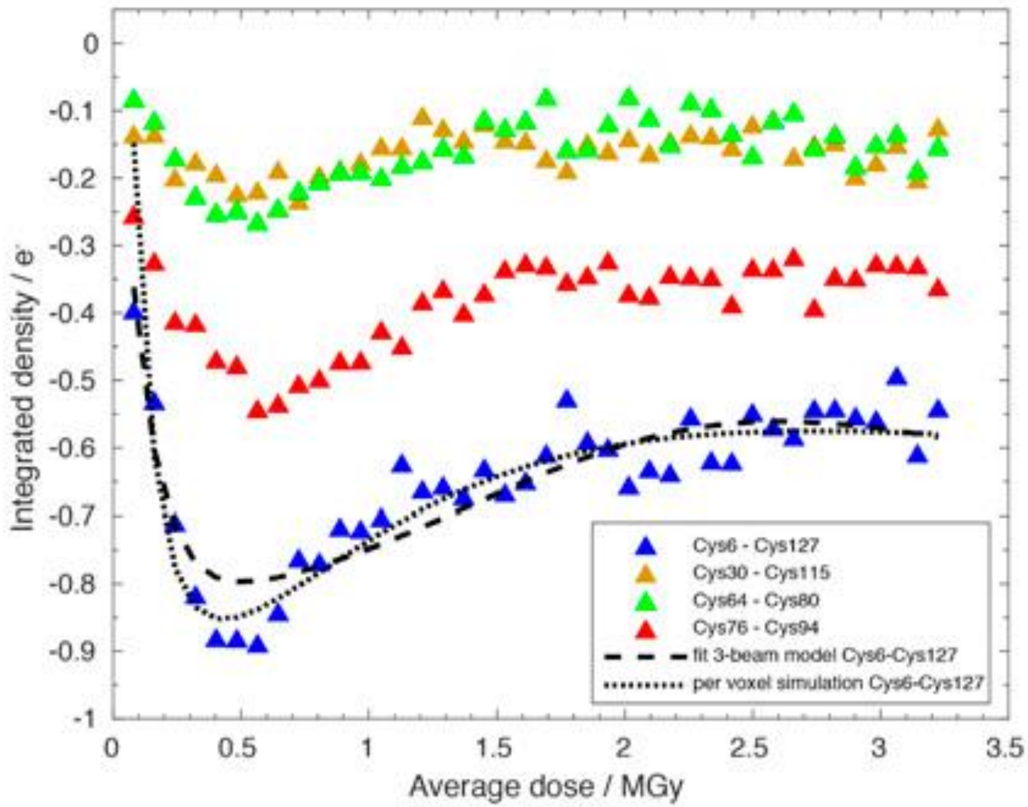
RadDam signatures in reciprocal space (RT MX)



Radiation damage and dose limits in serial synchrotron crystallography at cryo- and room temperatures. De la Mora, Coquelle *et al.* (2020) PNAS

RT high-dose rate ‘recovered’ electron density with dose

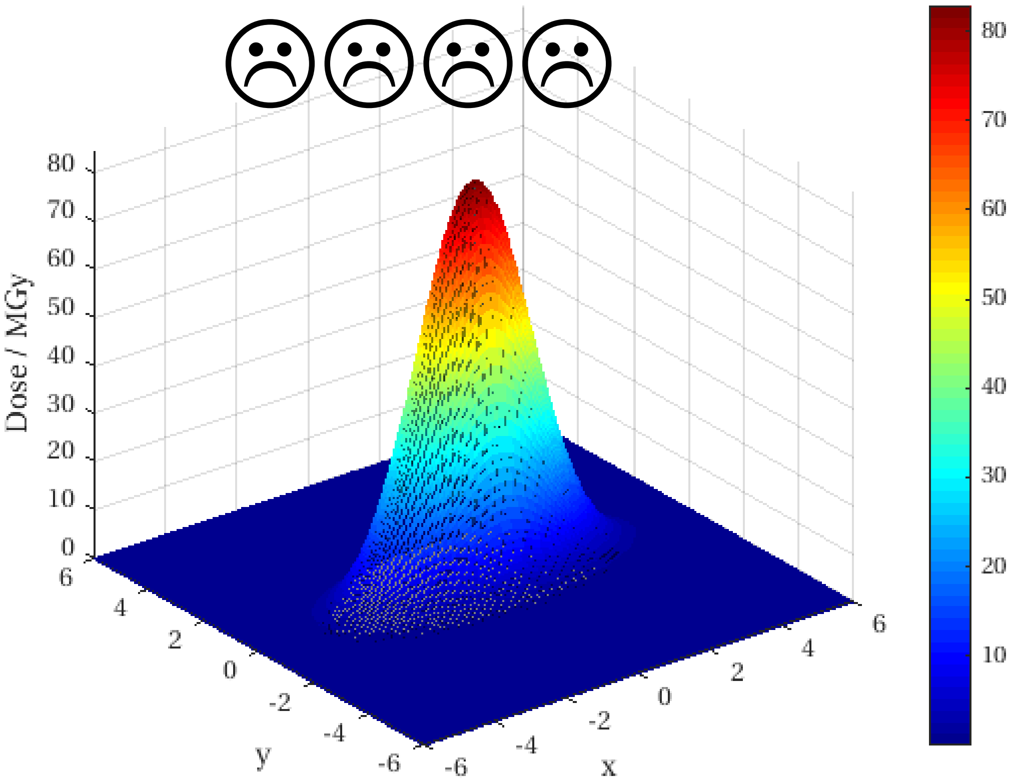


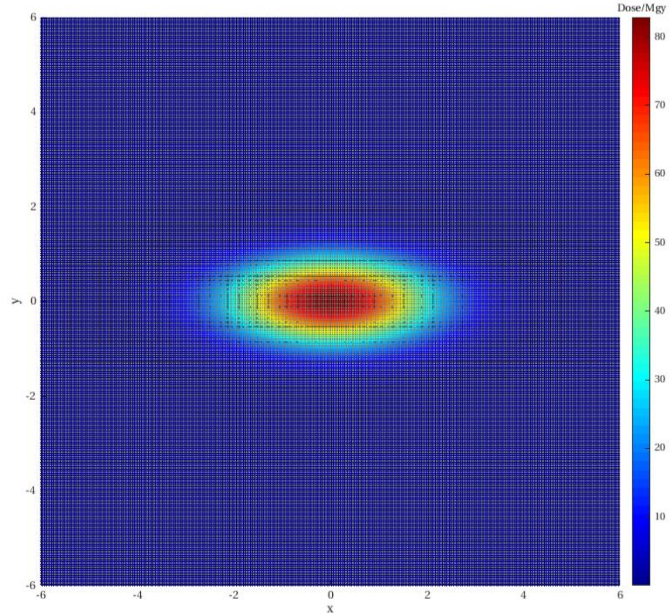


De la Mora, Coquelle *et al.* (2020) PNAS + Suppl Material

Beam shapes

☹☹☹☹

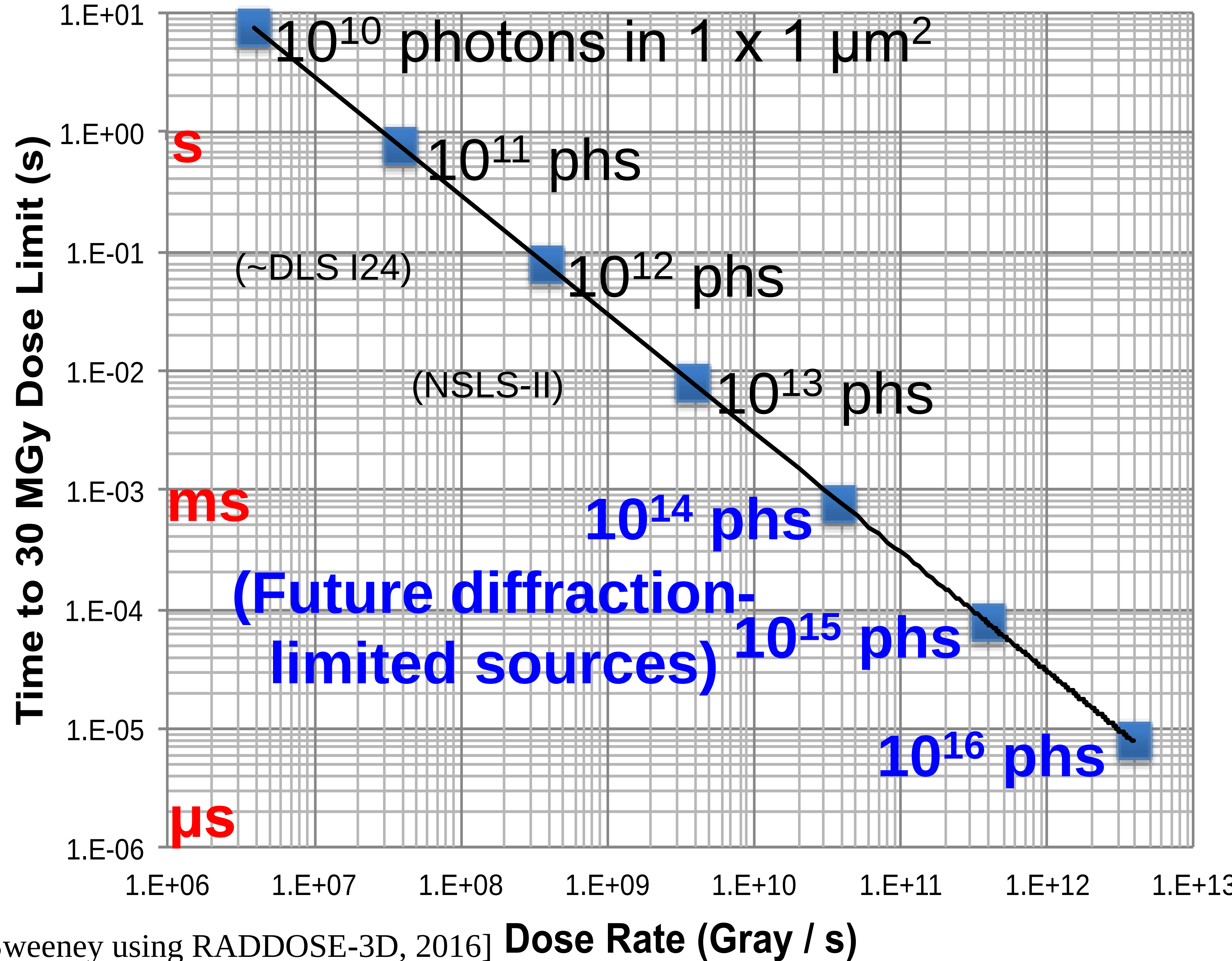




Gaussian/Lorentzian
Pseudo-Voight

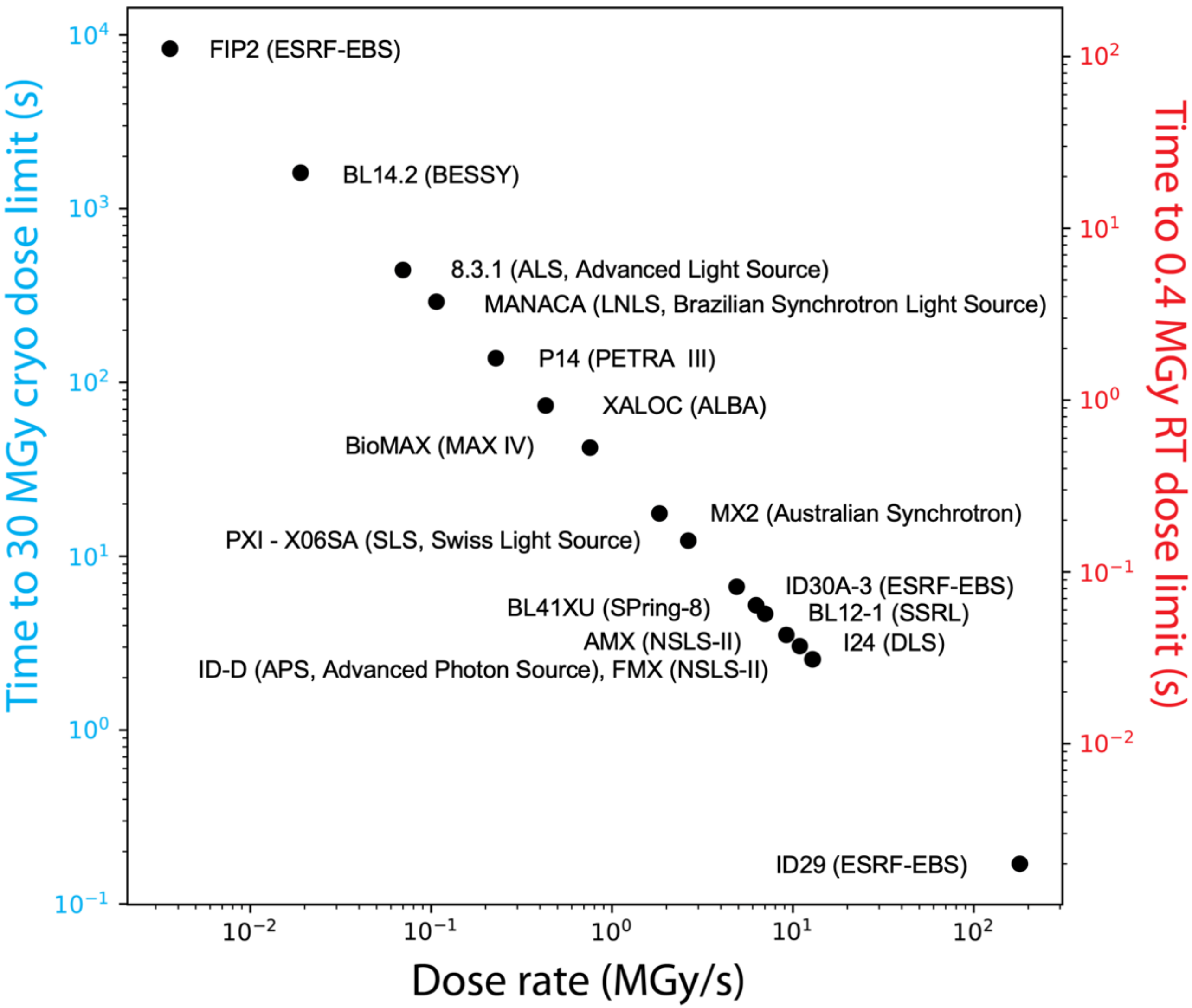
Partition beam
into 3 contributions:
Hot beam
Cold beam
Pedestal beam

De la Mora, Coquelle *et al.* (2020) PNAS + Suppl Material



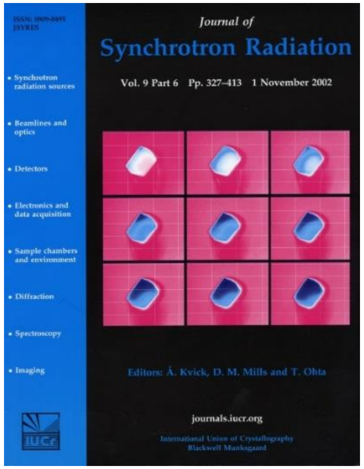
[McSweeney using RADDOSE-3D, 2016]

Time to dose limits at current synchrotrons



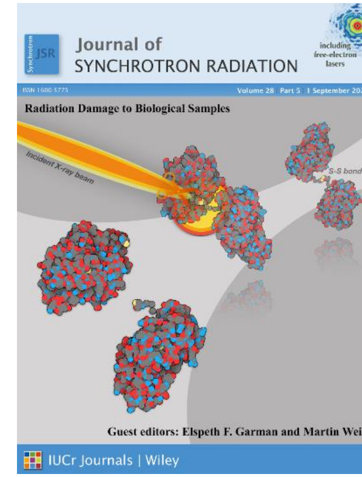
Average diffraction weighted doses (DWD) were calculated for a (50 μm)^3 lysozyme crystal (grown in 100 mM NaAc and 1M NaCl, solvent fraction 38%) rotated 360° in the flux within the FWHMs of the various beams.

Garman & Weik COSB (2023)

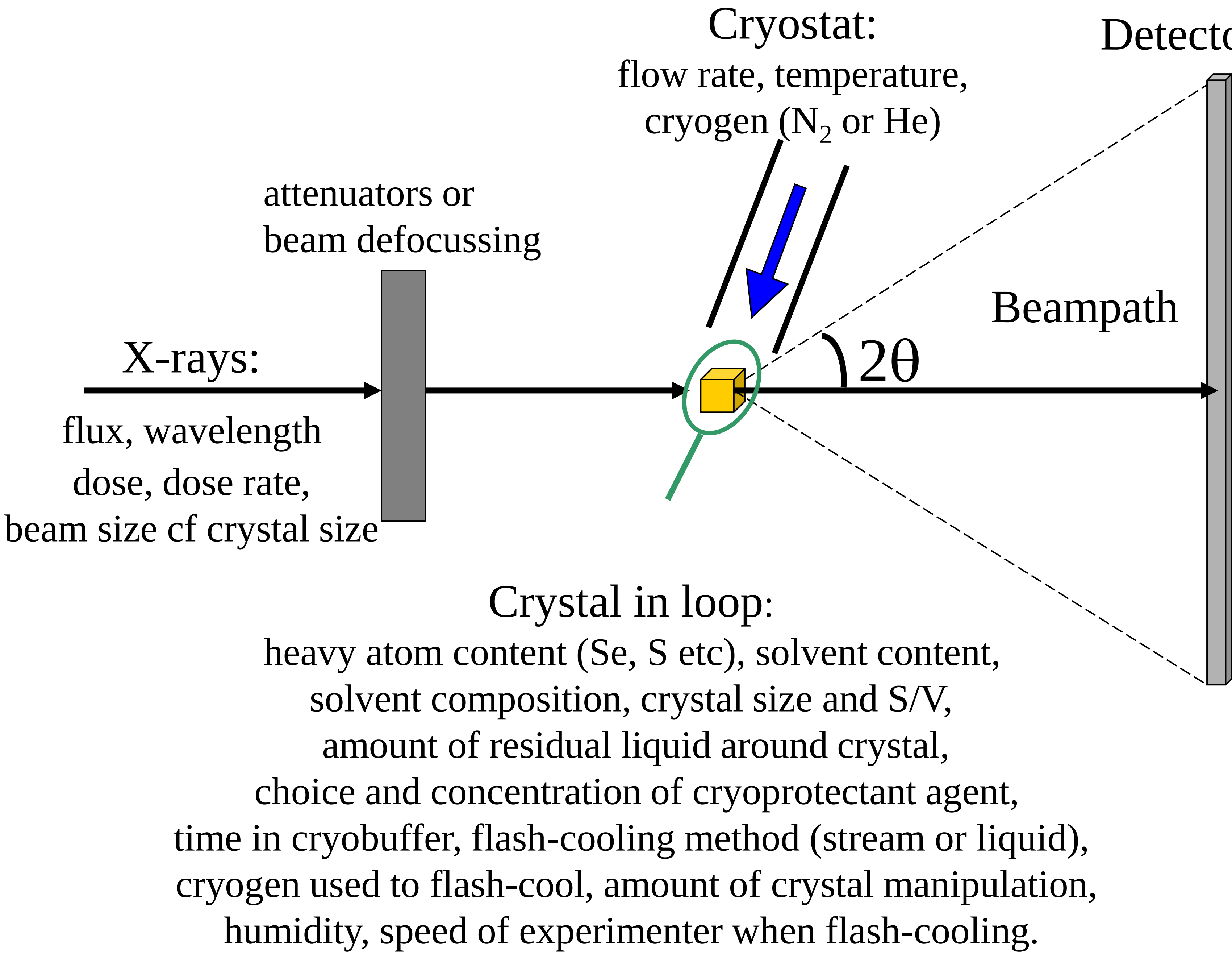


Radiation damage:

The Plan:



- What are the symptoms?
- Why do we care? Effect on MAD/SAD.
- What is it?
- How do we calculate the Dose?
- **What do we know/would like to know?**
- A new RD metric



[Garman, Current Opinion of Structural Biology 2003, **9,** 545-551]

PROBLEM: how do we know that we are making any difference?

- In order to investigate the effects of various parameters on the radiation damage process, we need a robust radiation damage METRIC which is preferably ON-LINE during the diffraction experiment.

No unanimous metric currently/ results from different metrics do not agree.

- Structural changes occur before degradation of diffraction quality is obvious.

Work so far / ongoing:

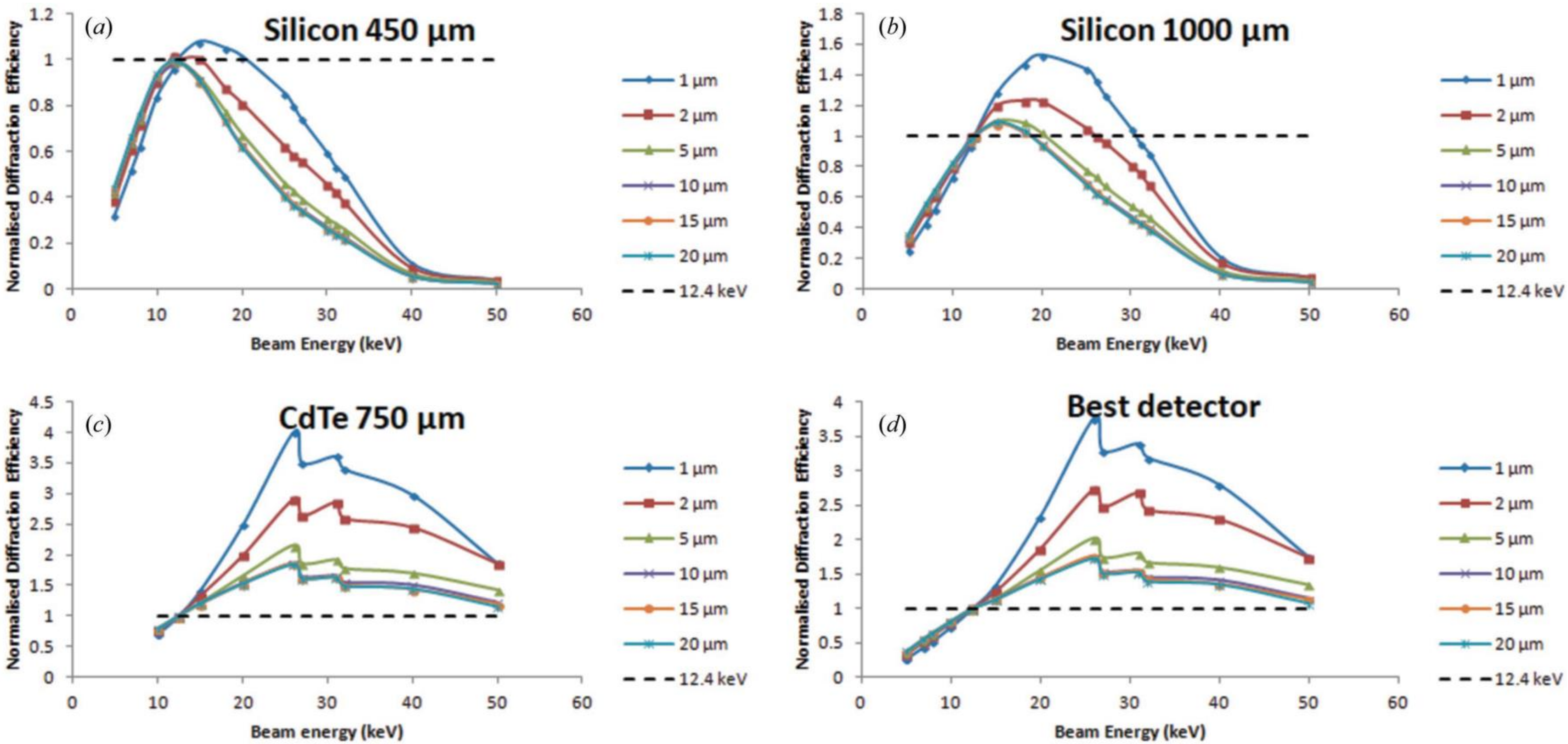
- Lower the cryogen temperature? 40K? 50K? 16K? 140K (no!). Less than a factor of 2 improvement.
- Lower the wavelength/increase X-ray energy)? Lots of anecdote + now some systematic results: no effect on damage rate except for small crystals at higher energies (>20 keV) where photoelectron can escape (needs another talk!

Varying results. Shimizu et al (2007) 14, 4-10, Liebshner et al, Acta Cryst D71(2015), Storm et al (2021) IUCr 8

- Unit cell expansion as a metric? No!
- Change/ regulate the dose/dose rate regime? No, not at current!
- Effect on MAD/SAD? Order of data collection?
- Minimum crystal size? Several papers (see Holton 2009)
- Beam heating. Not a big factor at current flux densities at cryotemperatures (Owen et al JSR 2019).

Optimum incident energy?

Simulations: CdTe detector, 26 keV, diffraction efficiency = diffraction/dose



Dickerson & Garman JSR 2019

Work so far / ongoing:

- X-ray absorption – important parameters defined.
- Remove oxygen? Nothing yet.
- Radiation damage Induced Phasing (RIP)
- Software developments – big progress.
- Add radical scavengers: results disagree.
- Biological implications/applications to mechanistic studies. Now many.
- Room temperature studies: dose rate effects? Results disagree.

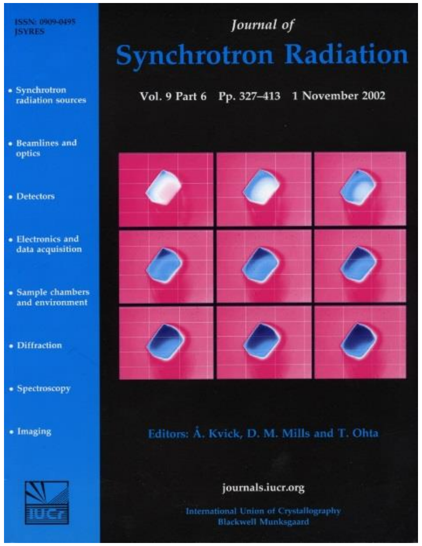
- N.B. Need for **systematic statistically significant** experiments.
- 1999-2021 Series of Radiation Damage Workshops

RD2: Dec 2001

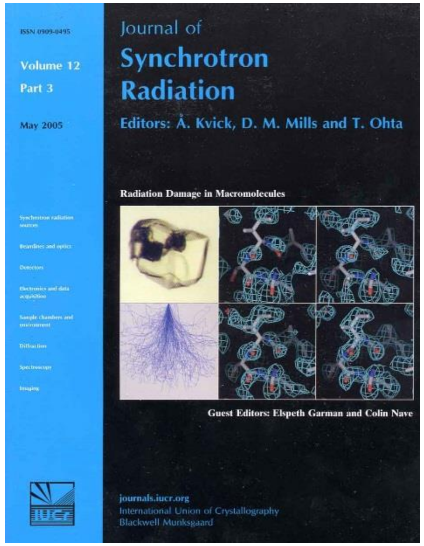
RD3: Nov 2003

RD4: March 2006

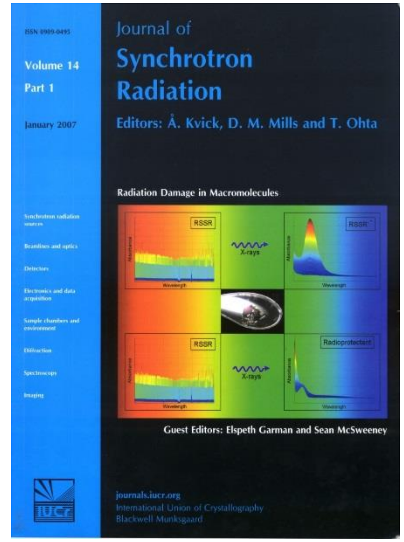
RD5: March 2008



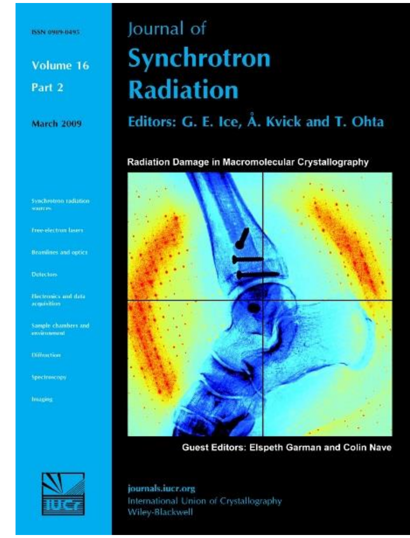
JSR, Nov 2002 (8)



JSR, May 2005 (9)



JSR, Jan 2007 (14)



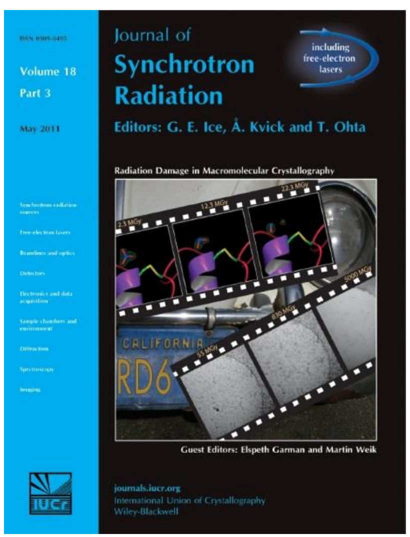
JSR, March 2009 (8)

RD6: Mar 2010

RD7: Mar 2012

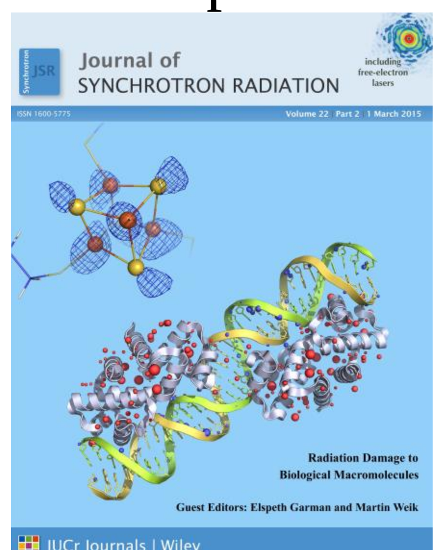
RD8: Apr 2014

RD9: Mar 2016

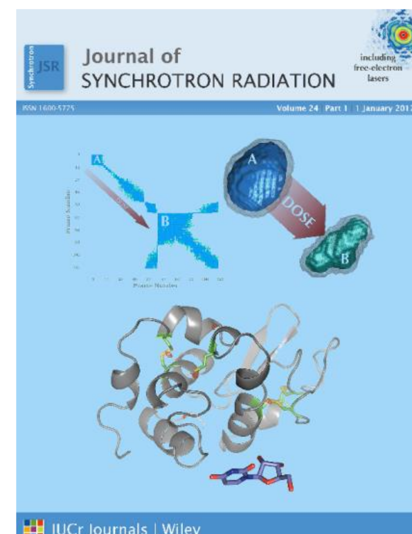


JSR, May 2011 (10)

JSR, Jan 2013 (6)



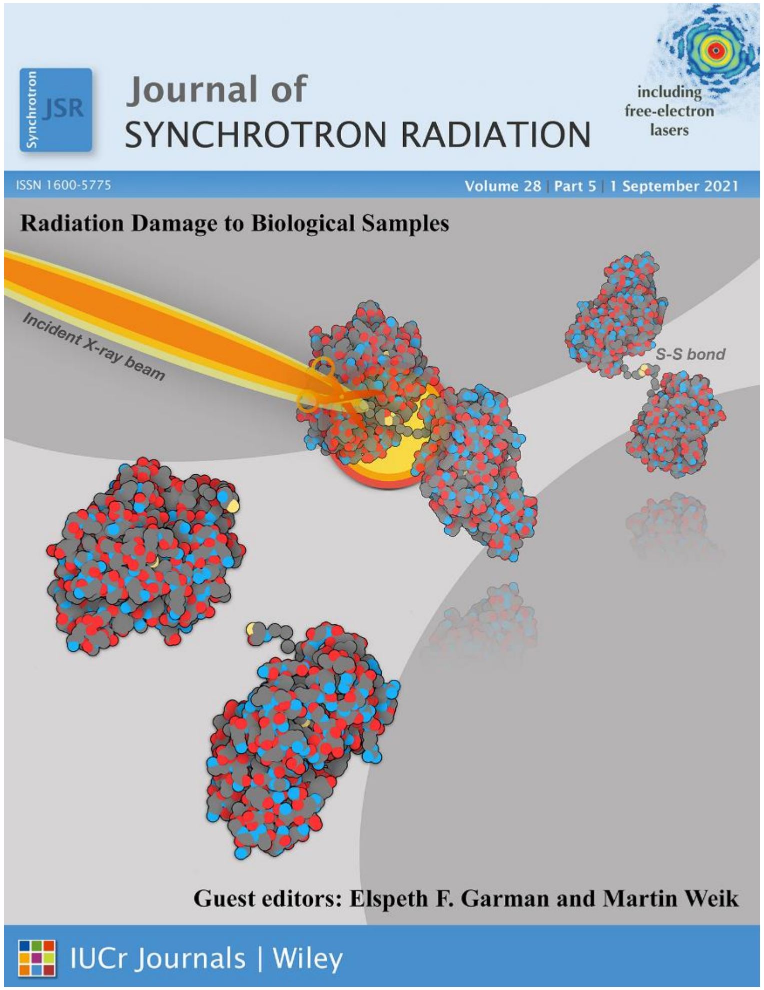
JSR, March 2015 (8)



JSR Jan 2017 (8)

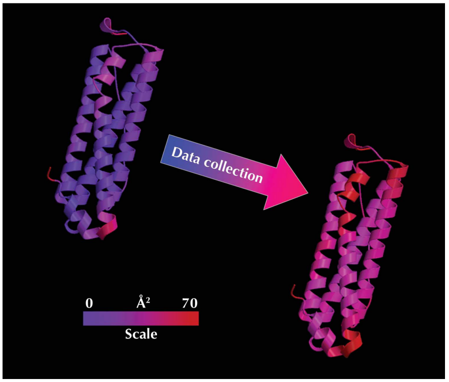
RD10: Sep 2018

RD11: Sep 2021



JSR, July 2019 (9)

RD2 to 11:
Total of 94 papers published in
JSR Special Issues so far



Radiation damage:
The Plan:

- What are the symptoms?
- Why do we care? Effect on MAD/SAD.
- What is it?
- How do we calculate the Dose?
- What do we know/would like to know?
- **A new RD metric**

Question:

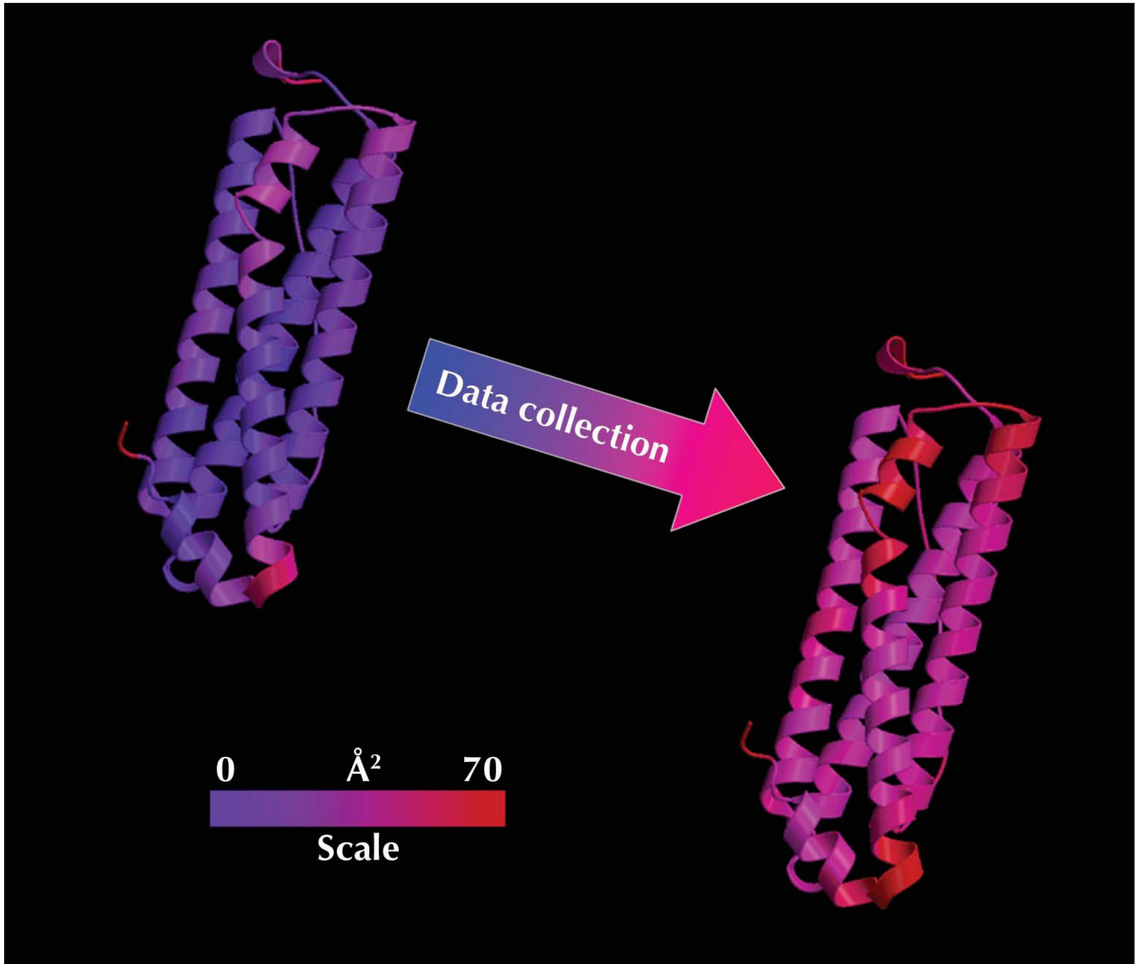
There are <30 ‘radiation damage series’ in the PDB.

Can we give an isolated deposited PDB file a **‘radiation damage index’?**

In the PDB file:

x, y, z, B,

occupancy



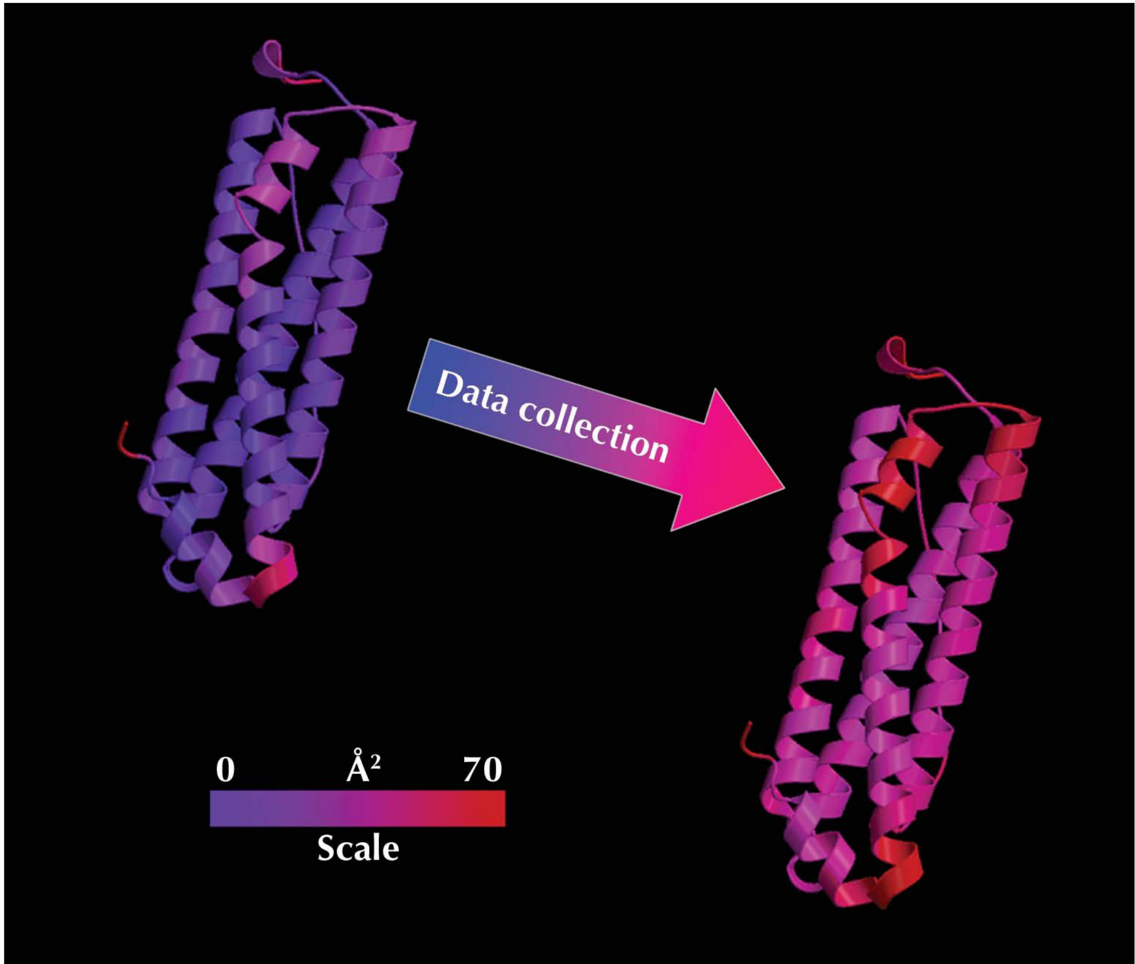
Question:

There are <30 'radiation damage series' in the PDB.

Can we give an isolated deposited PDB file a **'radiation damage index'**?

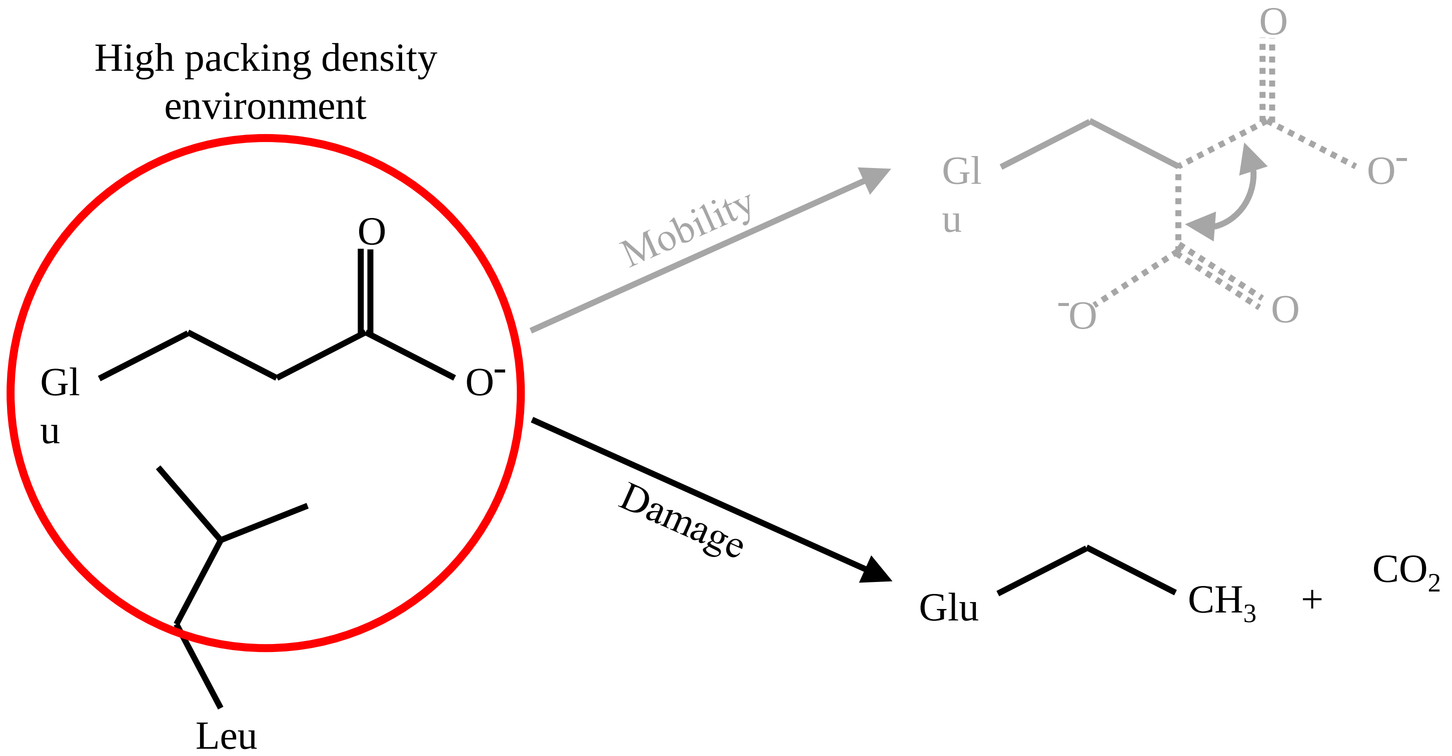
YES!!

Kathryn Shelley



The B_{Damage} metric

- There is a strong correlation between mobility and packing density
- Correcting *B*-factor for packing density enables the distinction of damage from mobility



The B_{Damage} metric

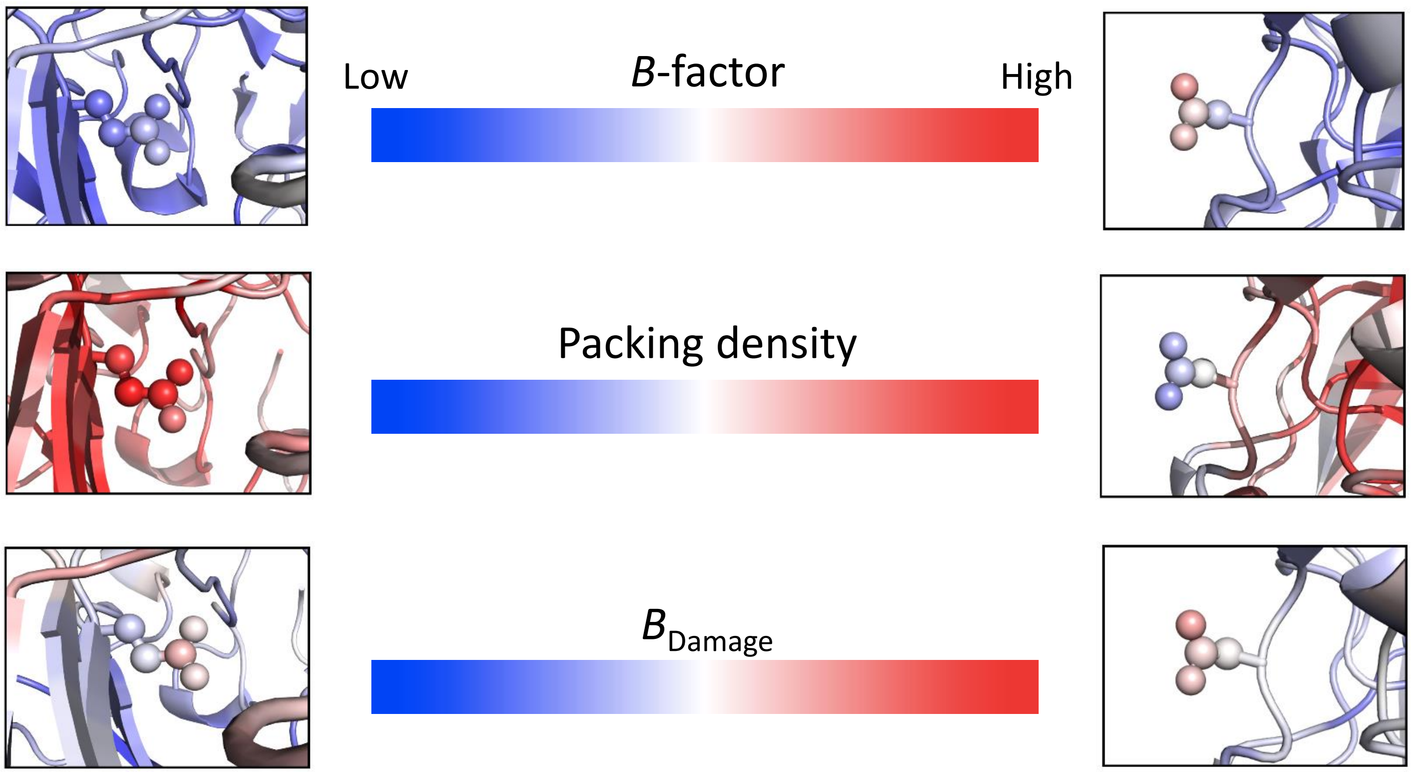
- B_{Damage}[1] is B-factor corrected for packing density

$$B_{Damage\,j} = \frac{B\text{-}factor_{\,j}}{\frac{1}{n}\sum_{i=1}^{n} B\text{-}factor_{\,i}}$$

[1] Gerstel, Deane, Garman (2015) *J Synchrotron Radiat* **22**, 201–212.

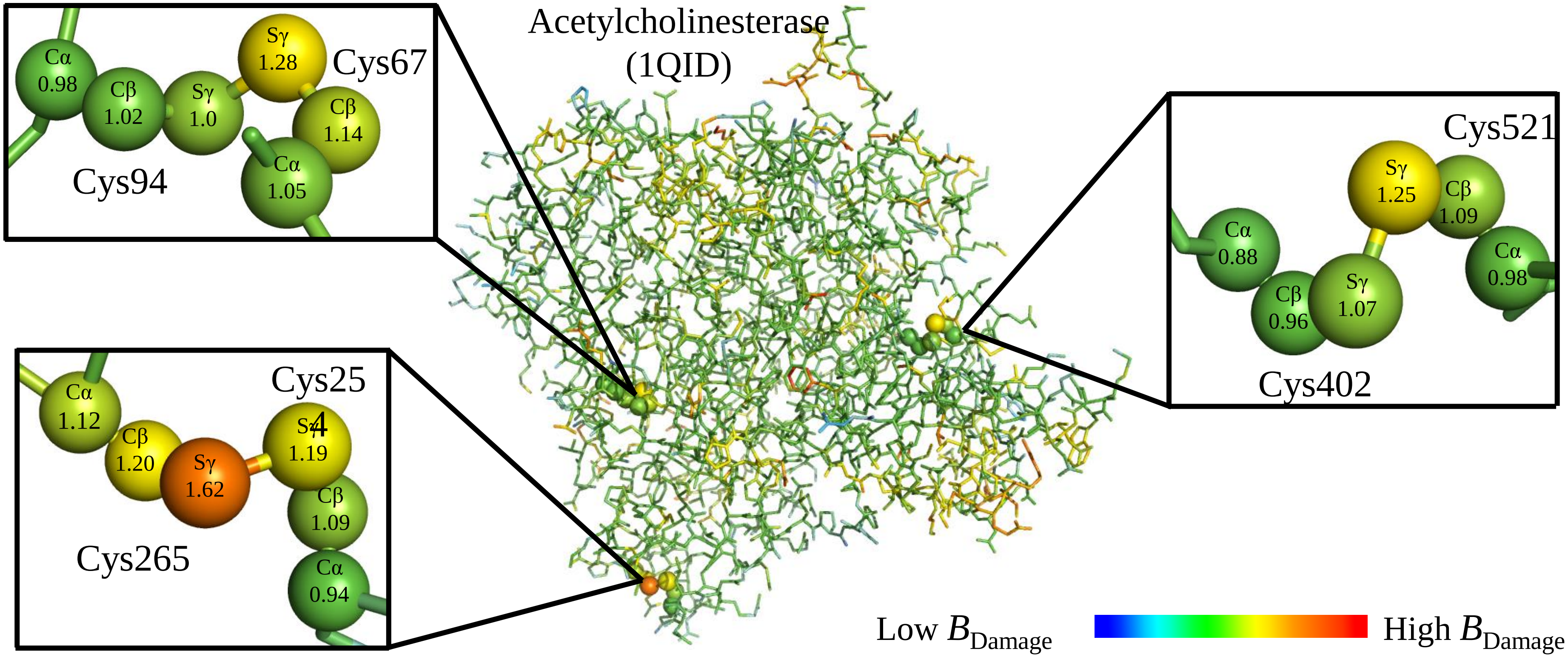
The B_{Damage} metric

- B_{Damage} is B-factor corrected for packing density



RABDAM (now in the CCP4 computing suite)

- RABDAM calculates B_{Damage} for all selected atoms in any standard format PDB file
- B_{Damage} highlights expected sites of specific radiation damage
- RABDAM provides several useful outputs to aid radiation damage evaluation



[Shelley, Brooks-Bartlett, Dixon, Garman, *J. Appl. Cryst* 2018]

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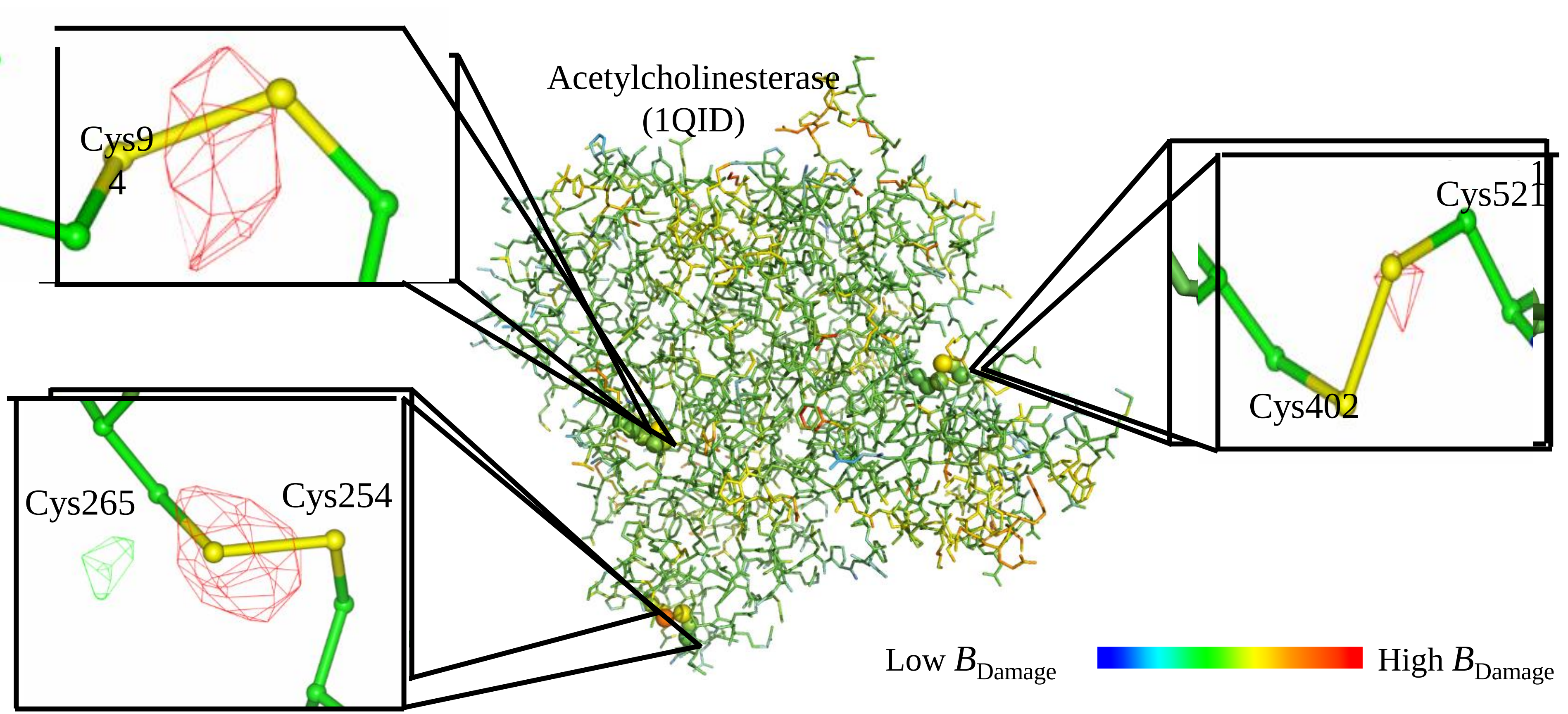
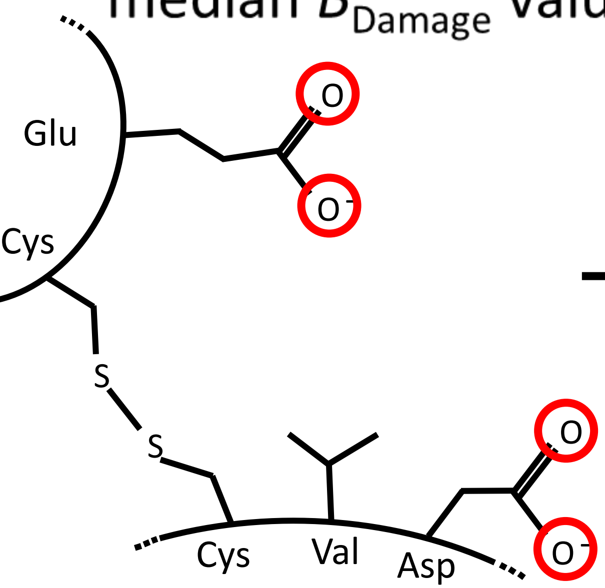


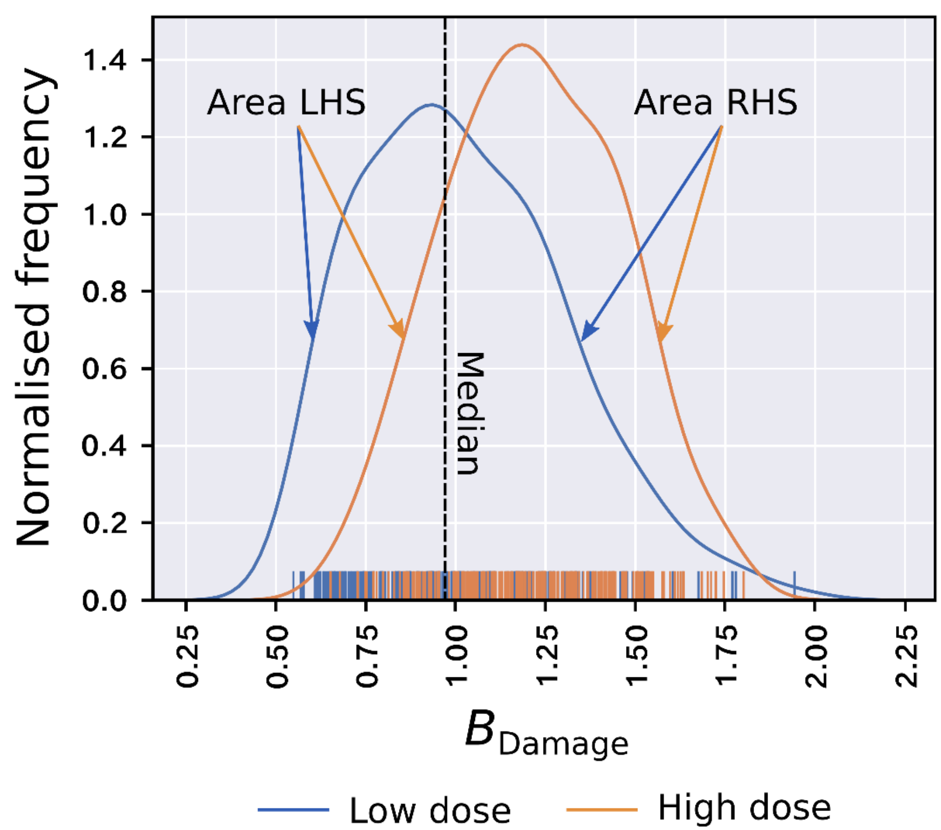
[Shelley, Brooks-Bartlett, Dixon, Garman, *J. Appl. Cryst* 2018]

The B_{net} metric

- B_{net} is calculated from the distribution of the B_{Damage} values of Asp Oδ and Glu Oε atoms
- Equal to the ratio of the area under the curve either side of the median B_{Damage} value of all protein atoms

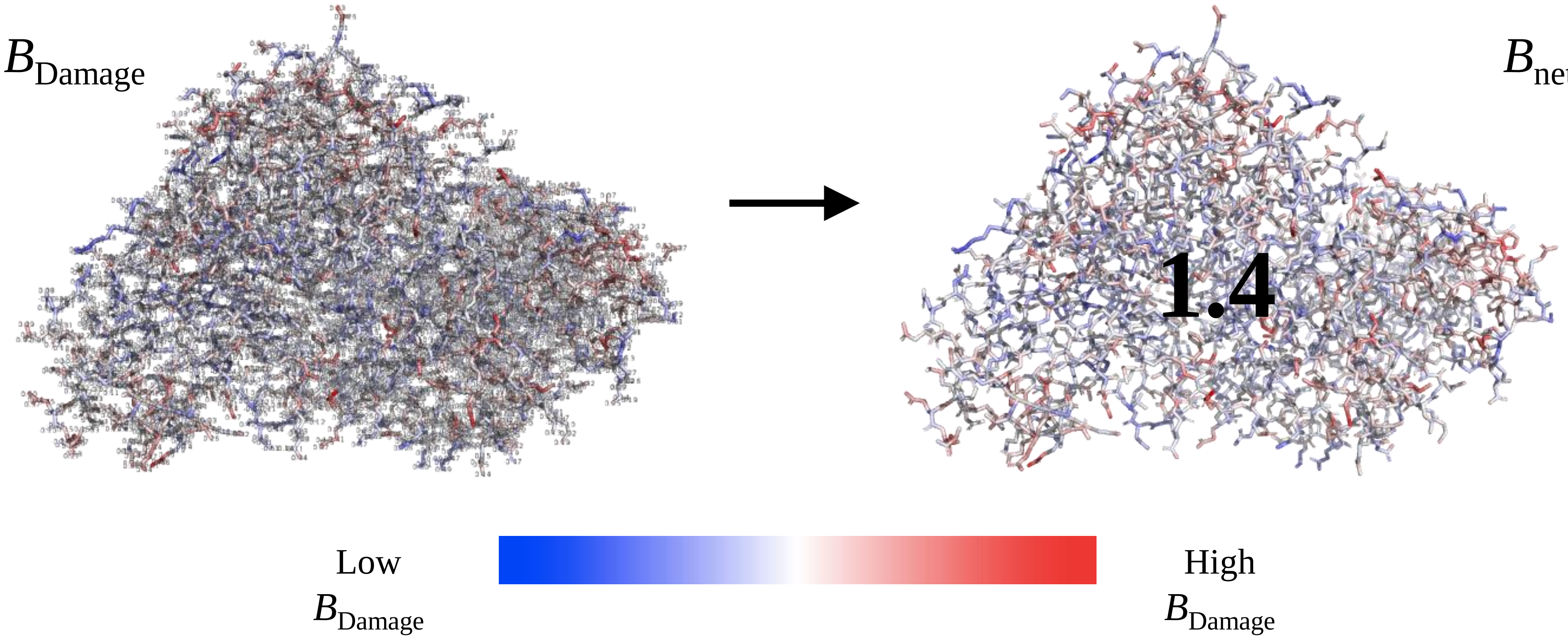


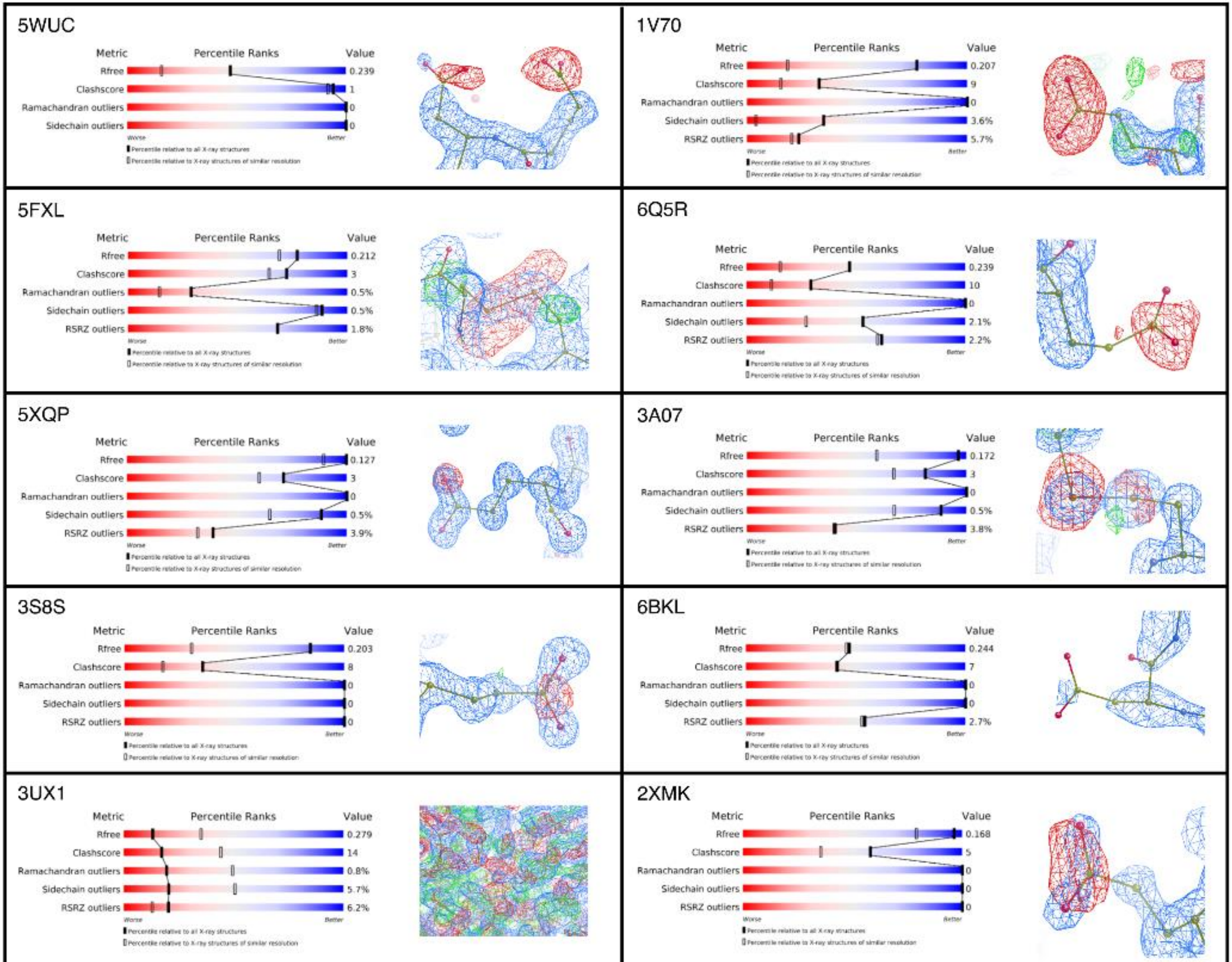
$$B_{net} = \frac{Area\ RHS}{Area\ LHS}$$



The B_{net} metric

- B_{Damage} is a per-atom metric
- The $\boldsymbol{B_{net}}$ metric is a derivative of B_{Damage} that summarises the total extent of specific radiation damage suffered by a PX structure in a single value





5WUC
Metric | Percentile Ranks | Value
Rfree 0.239
Clashscore 1
Ramachandran outliers 0
Sidechain outliers 0
Worse Better
Percentile relative to all X-ray structures
Percentile relative to X-ray structures of similar resolution
1V70
Metric | Percentile Ranks | Value
Rfree 0.207
Clashscore 9
Ramachandran outliers 0
Sidechain outliers 3.6%
RSRZ outliers 5.7%
Worse Better
Percentile relative to all X-ray structures
Percentile relative to X-ray structures of similar resolution
5FXL
Metric | Percentile Ranks | Value
Rfree 0.212
Clashscore 3
Ramachandran outliers 0.5%
Sidechain outliers 0.5%
RSRZ outliers 1.8%
Worse Better
Percentile relative to all X-ray structures
Percentile relative to X-ray structures of similar resolution
6Q5R
Metric | Percentile Ranks | Value
Rfree 0.239
Clashscore 10
Ramachandran outliers 0
Sidechain outliers 2.1%
RSRZ outliers 2.2%
Worse Better
Percentile relative to all X-ray structures
Percentile relative to X-ray structures of similar resolution
5XQP
Metric | Percentile Ranks | Value
Rfree 0.127
Clashscore 3
Ramachandran outliers 0
Sidechain outliers 0.5%
RSRZ outliers 3.9%
Worse Better
Percentile relative to all X-ray structures
Percentile relative to X-ray structures of similar resolution
3A07
Metric | Percentile Ranks | Value
Rfree 0.172
Clashscore 3
Ramachandran outliers 0
Sidechain outliers 0.5%
RSRZ outliers 3.8%
Worse Better
Percentile relative to all X-ray structures
Percentile relative to X-ray structures of similar resolution
3S8S
Metric | Percentile Ranks | Value
Rfree 0.203
Clashscore 8
Ramachandran outliers 0
Sidechain outliers 0
RSRZ outliers 0
Worse Better
Percentile relative to all X-ray structures
Percentile relative to X-ray structures of similar resolution
6BKL
Metric | Percentile Ranks | Value
Rfree 0.244
Clashscore 7
Ramachandran outliers 0
Sidechain outliers 0
RSRZ outliers 2.7%
Worse Better
Percentile relative to all X-ray structures
Percentile relative to X-ray structures of similar resolution
3UX1
Metric | Percentile Ranks | Value
Rfree 0.279
Clashscore 14
Ramachandran outliers 0.8%
Sidechain outliers 5.7%
RSRZ outliers 6.2%
Worse Better
Percentile relative to all X-ray structures
Percentile relative to X-ray structures of similar resolution
2XMK
Metric | Percentile Ranks | Value
Rfree 0.168
Clashscore 5
Ramachandran outliers 0
Sidechain outliers 0
RSRZ outliers 0
Worse Better
Percentile relative to all X-ray structures
Percentile relative to X-ray structures of similar resolution

B_{net} Summary

- B_{Damage} can detect potential sites of specific radiation damage in individual PX structures.
- B_{net} summarises the extent of damage suffered by a PX structure in a single value. Unlike B_{Damage} values, B_{net} values can be fairly compared between different structures.
- Both B_{Damage} and B_{net} values can be calculated with the CCP4 program *RABDAM*.
- *RABDAM* has the capacity to open up the entire PDB for large-scale statistical analysis of specific radiation damage.
- Currently radiation damage is largely overlooked when assessing the quality of structures in the PDB – we hope our metrics will help to change this.

nature COMMUNICATIONS

14th March 2022

ARTICLE

https://doi.org/10.1038/s41467-022-28934-0 OPEN

Quantifying and comparing radiation damage in t
Protein Data Bank

Kathryn L. Shelley [1,2] & Elspeth F. Garman [1]

Summary 1: what can YOU, the experimenter do?

- Do not be afraid to merge data taken from different isomorphous crystals which all had lower doses.
- Back soak non-specifically bound heavier atoms out of your crystals.
- Be ‘absorption aware’ of the contents of your crystal (e.g. Se and buffer) and if possible, avoid cacodylate buffer (arsenic mass=75).
- Match beam size to crystal size

Summary 2: what can YOU, the experimenter do?

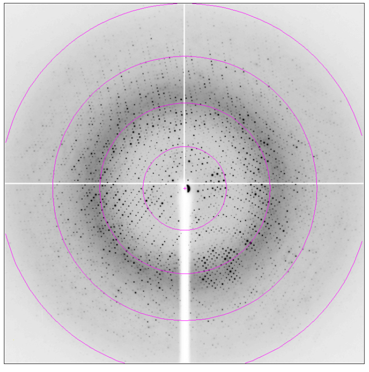
- Scavengers: try electron scavengers at 100 K (nitrate/ascorbate/benzoquinone).

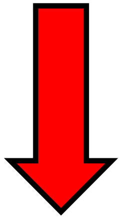
- Dose ‘spreading’: use a tophat profile beam if possible.
  Consider Helical/Translational data collection.

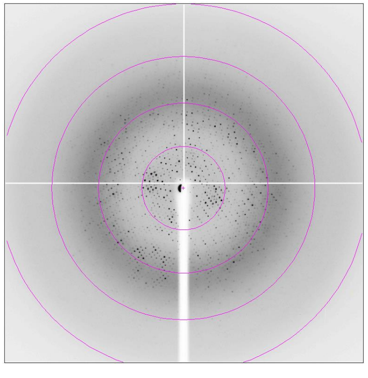
- So you can estimate the dose, ASK at the beamline:
  - What is the flux today at this energy and with this slit size (‘flux density’)?
  - What is the beam profile today at this beam energy? FWHM in *x* and *y*?

Sacrificial crystal to characterise damage rate

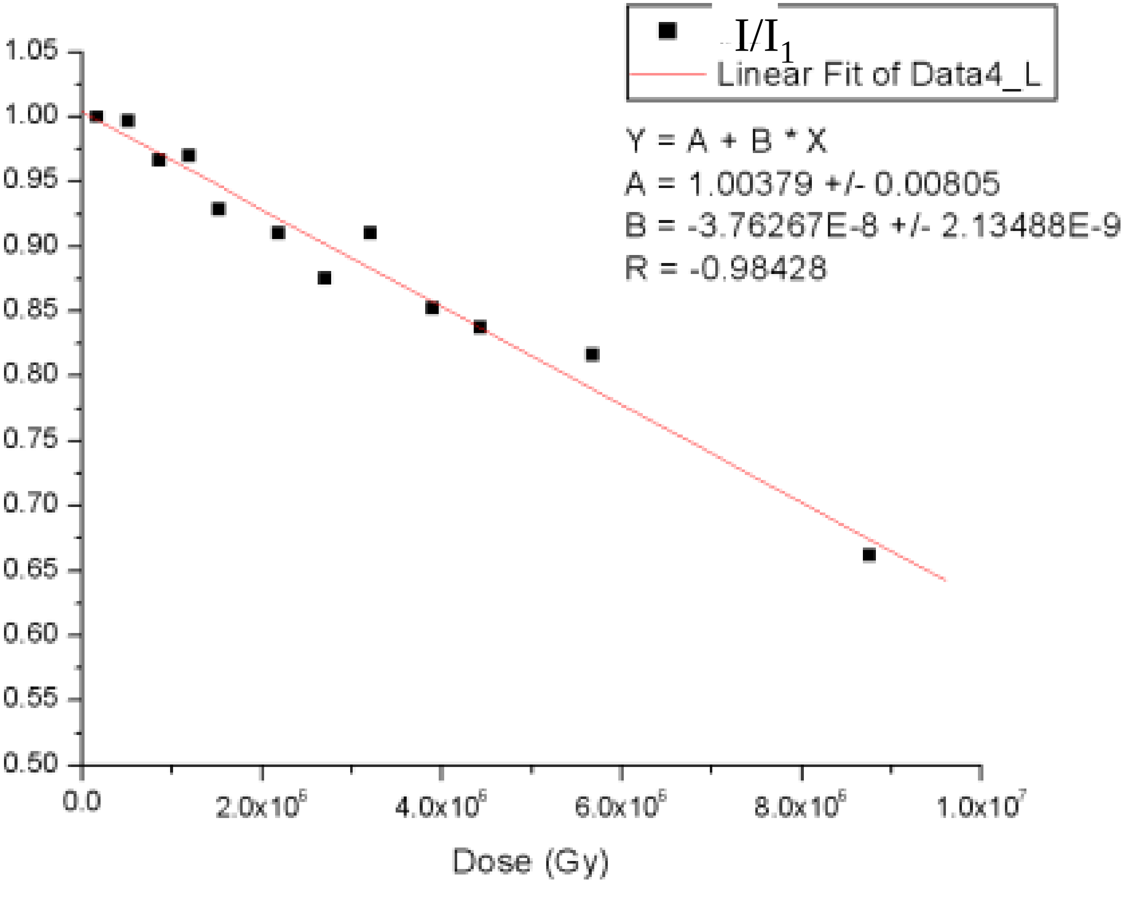
Diffraction fading







I/I_1



Dose = absorbed energy (J) / mass (kg)

Current status: radiation damage in protein crystals

- Understand a lot more than 20 years ago, but still not nearly enough…
- Understand how to do experiments better.
- Research has prompted some very exciting new approaches.
- Many complementary methods now being used on the problem in concert with crystallography
- Experiments must involve more than one sample (!) to get statistically significant results: labour intensive and time consuming. Also MUST know incident FLUX density…
- **Radiation damage has DEFINITELY become a mainstream concern**

FURTHER READING:

- ‘Beginner’s guide to Radiation Damage’ Holton, (2009) *JSR* **16**,133-142
- General summary in: Garman, *Acta D* (2010) **66**, 339-355
- Garman and Weik, Chapter 20 in *‘Protein Crystallography: Methods in Molecular Biology’* (2017) **1607,** 477-489

nature COMMUNICATIONS

14th March 2022

Quantifying and comparing radiation damage in t
Protein Data Bank

Kathryn L. Shelley [1,2] & Elspeth F. Garman [1]

- Garman & Weik Curr. Opin. Struct. Biol. (2023) **82**:102662

I thank my past and present group and our collaborators, and acknowledge their huge contribution to the work

Some Garman Group PhD students
and Postdocs 2000-2018:
next generation Crystallographers…

diamond

ESRF

Graduate students

James Murray (Imperial College)
Robin Owen (DLS)
Eugenio de la Mora Lugo (IBS, Grenoble)
Oliver Zeldin (Facebook)
Markus Gerstel (Cybersecurity)
Helen Ginn (U of Hamburg)
Jonathan Brooks-Bartlett (Spotify)
Charlie Bury (Exscientia)

Postdocs

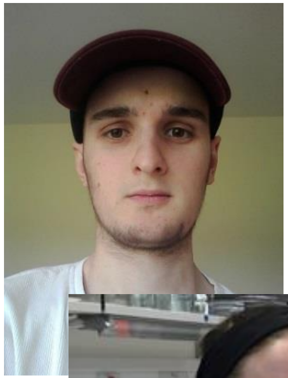
Karthik Paithankar (U. Frankfurt)

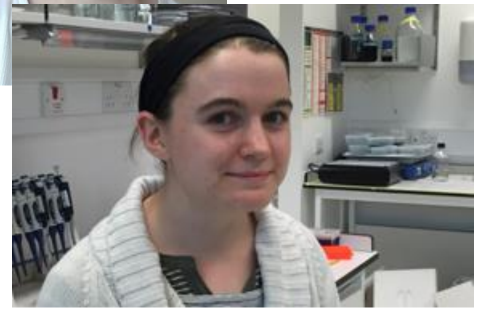
Undergraduate Project students

Kathryn Shelley (RABDAM)
Josh Dickerson (RADDOSE-3D)

Collaborators:

Raimond Ravelli, Maastricht.
Martin Weik, IBS
Ian Carmichael, NDRL, USA
John McGeehan, Portsmouth
James Holton, UCSF





EPSRC
Engineering and Physical Sciences Research Council
SIXTH FRAMEWORK PROGRAMME
European Commission

This was a lot of material delivered very fast so…

questions expected and very welcome

elspeth.garman@bioch.ox.ac.uk

The Crystallographer's DILEMMA:

Rate of damage versus diffraction intensity

RADDOSE-3D

TEST our new GUI!!

To run RADDOSE-3D for MX, SMX or SAXS (which ever you like!)

Step 1: Download and unzip the RADDOSE-3D GUI from:

https://github.com/jdickerson95/qt_RADDDOSE-3D/releases

There are versions for a PC (Windows_release.zip) and for Linux (Linux_release.zip).

If you have a MAC, there is no new GUI yet, but you can run a limited capability RADDOSE-3D from the WWW site:

raddo.se

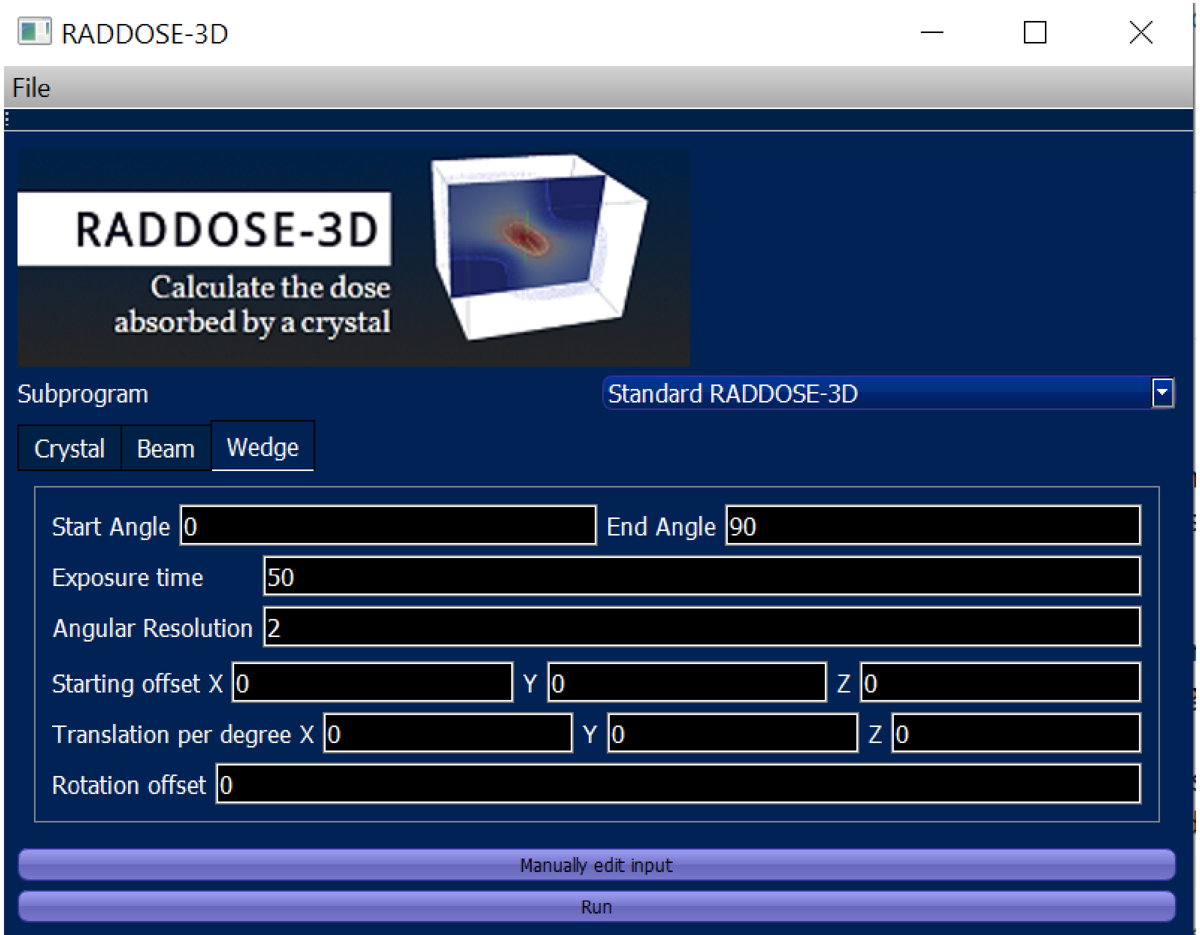
(click on ‘manual interface’ and run the test example first. Then edit the input for a case you would like to try)

To run the GUI you need to have Java installed which you can get free at

https://www.java.com/download/ie_manual.jsp

Also, if you have R (https://www.r-project.org/) installed, from the RADDOSE-3D output you will be able to produce 3D representations of the dose distribution in your sample.

Step 3: Find the file RD3D_GUI.EXE and if on a PC click on it. For Linux run it however you usually run executable files. The GUI should open, and you can enter input on 3 tabs: crystal, beam and wedge.

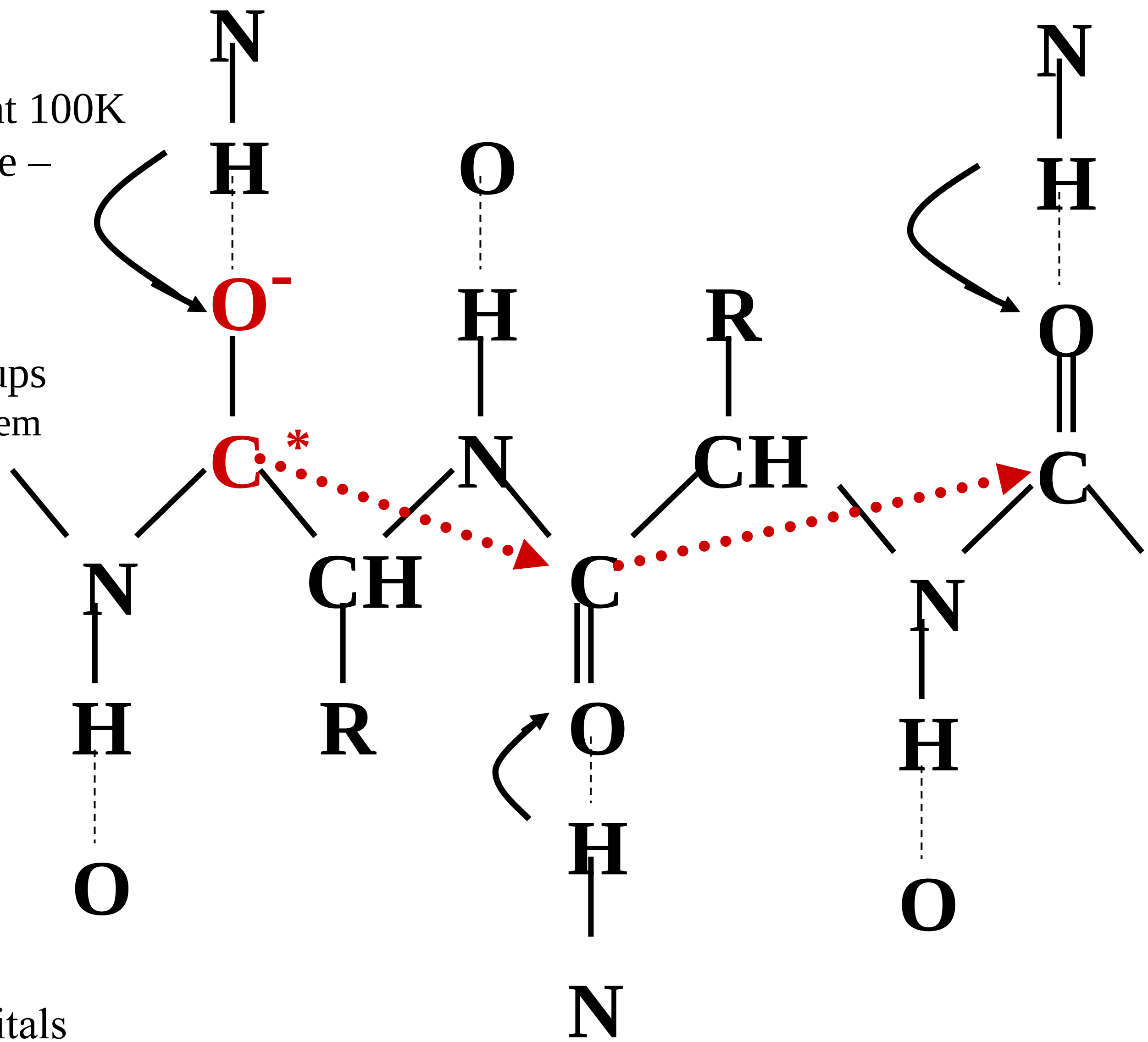


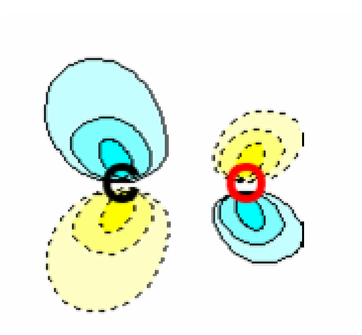
2) DIRECT RADIATION DAMAGE. Protein Redox

a) electron migration and trapping.

Excess electron migrates even at 100K (q.m.tunnelling) along backbone – trapped at a unique C=O

ESR: electrons mobile at 77K
Go to most electron affinic groups
M. Symons 1995, Radiat. Phys. Chem 45, 837-845.





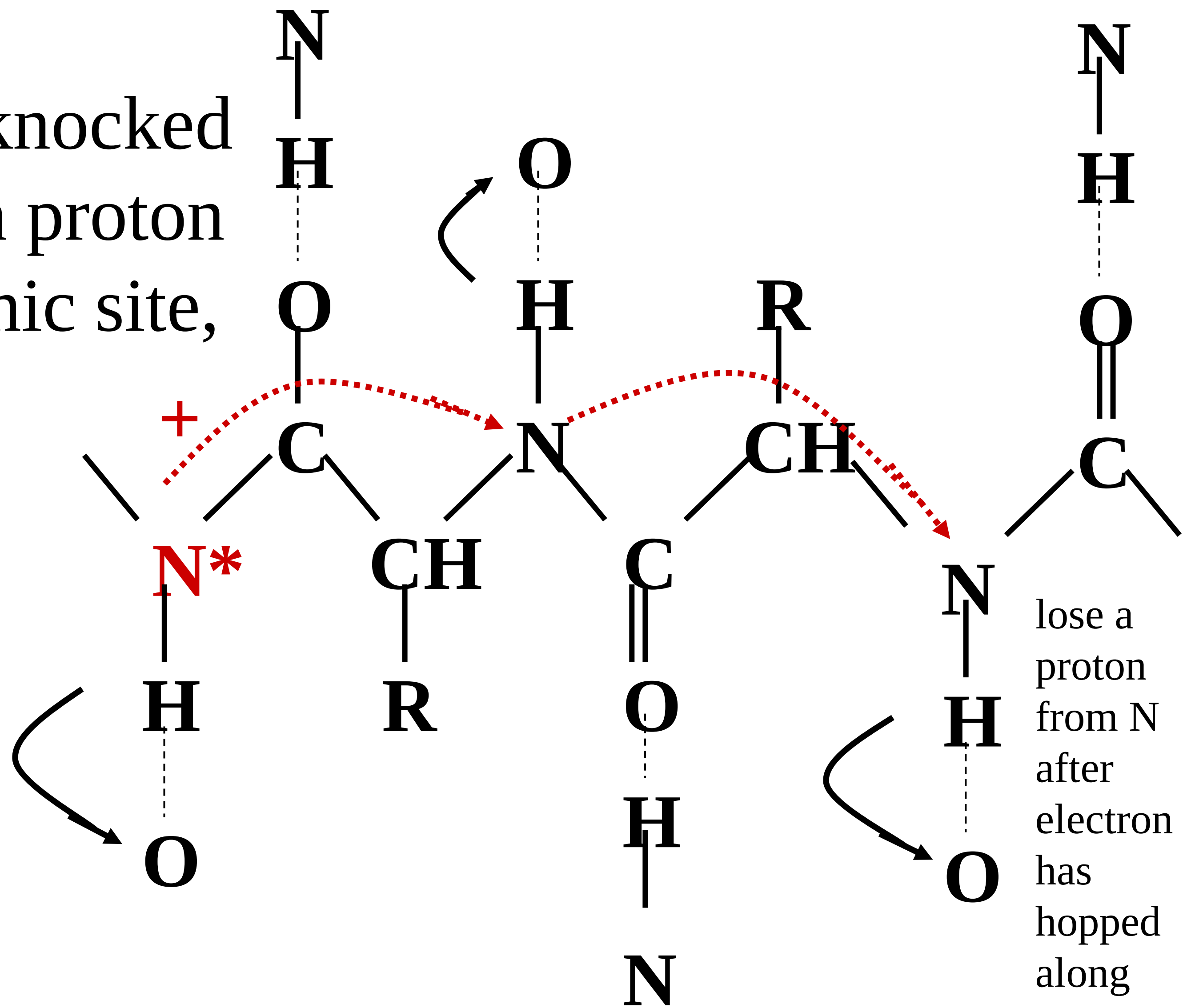
π* orbitals

2) DIRECT RADIATION DAMAGE. Protein Redox-

b) proton hole migration.

Electron gets knocked off, then lose a proton from the cationic site, get a radical.

lose an electron from N



lose a proton from N after electron has hopped along

Interactions of X-rays with atoms in a crystal
Cross sections at E_{inc} 12.4 keV (1 Å)