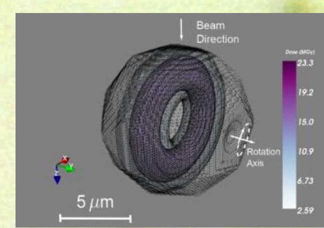
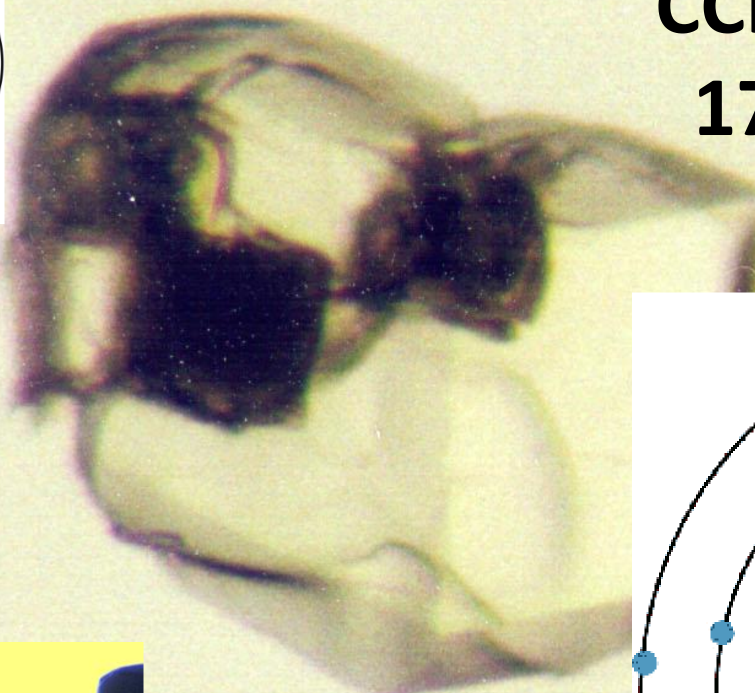
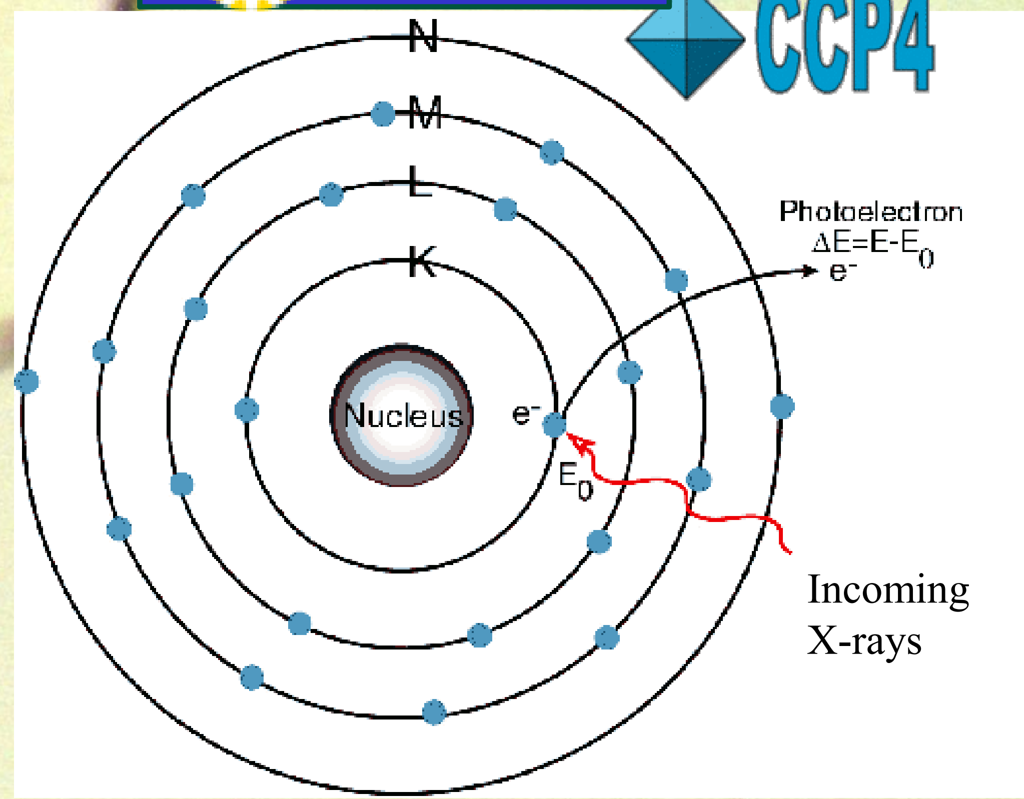
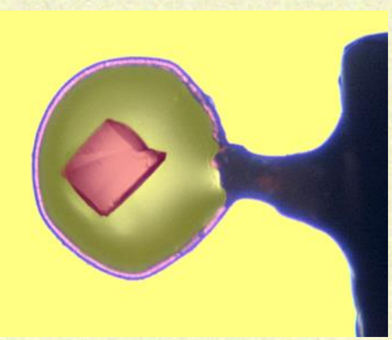
Radiation Damage



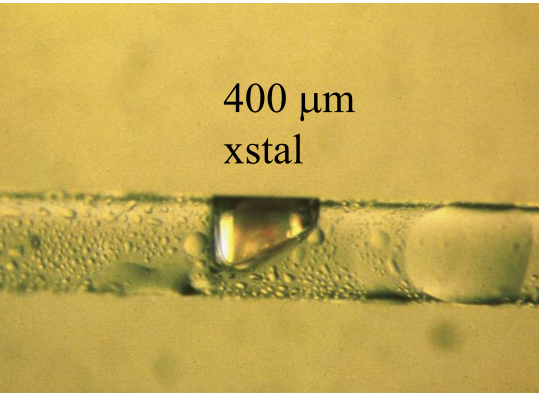
CCP4/DLS Workshop
17 November 2025





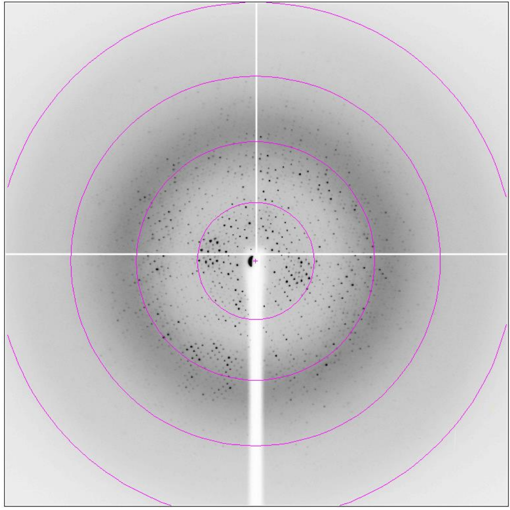


elspeth.garman@bioch.ox.ac.uk

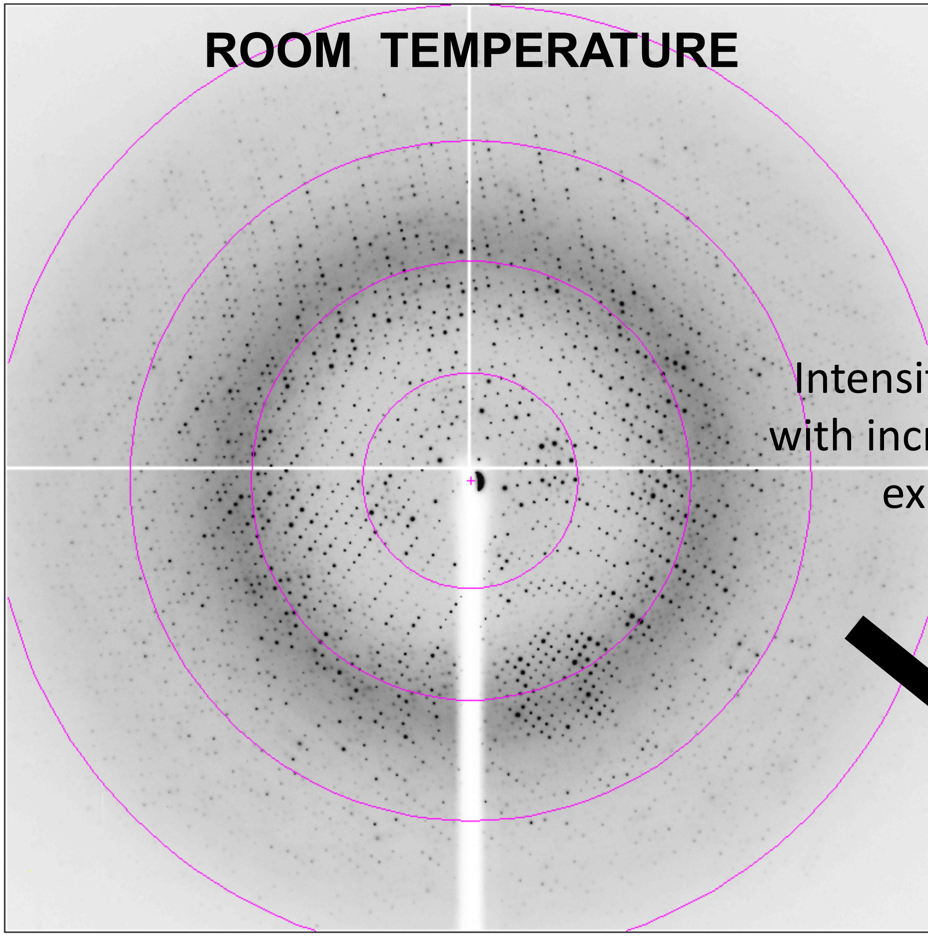


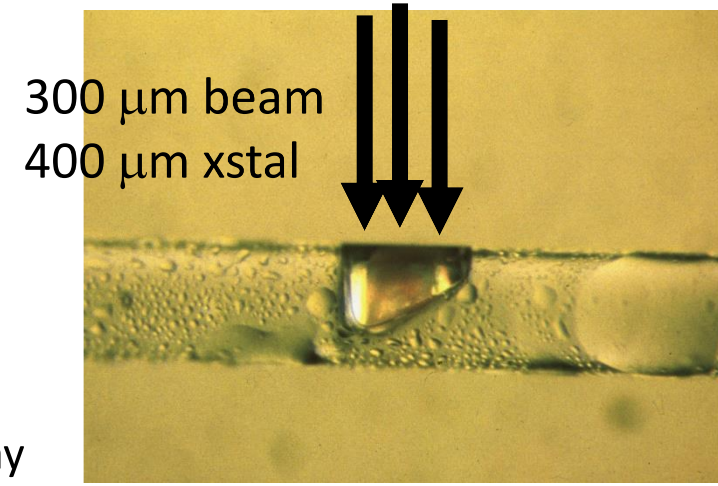
Radiation Damage

The Plan:

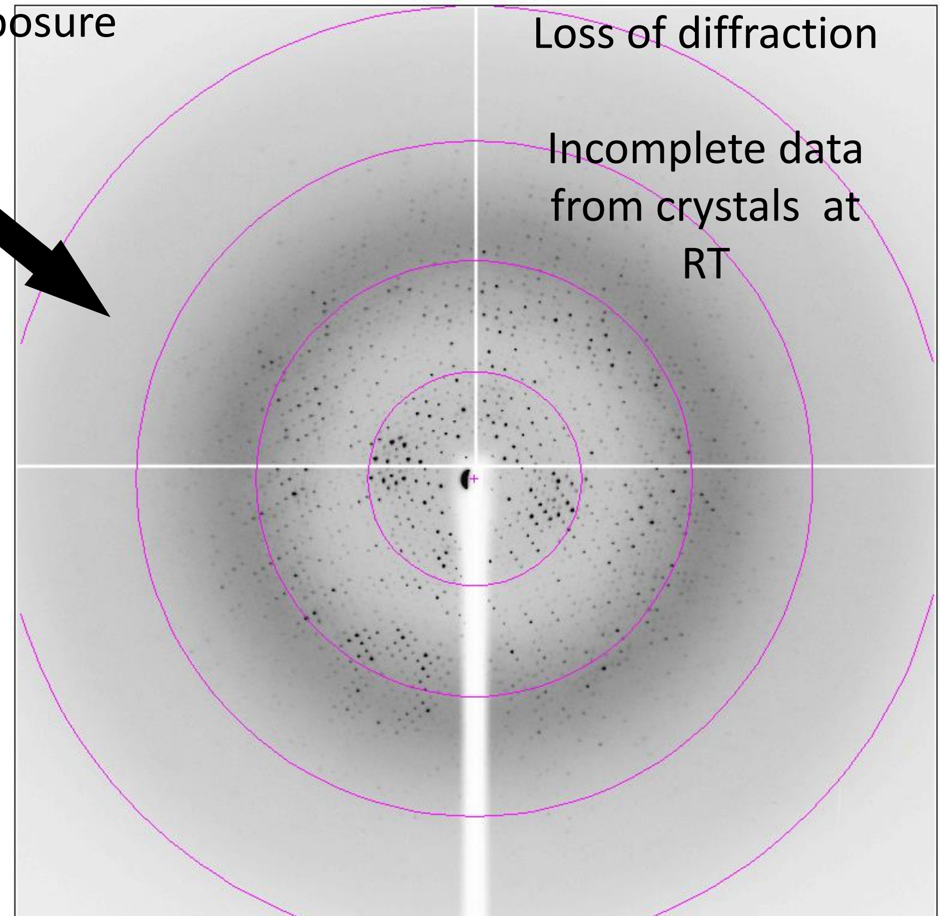


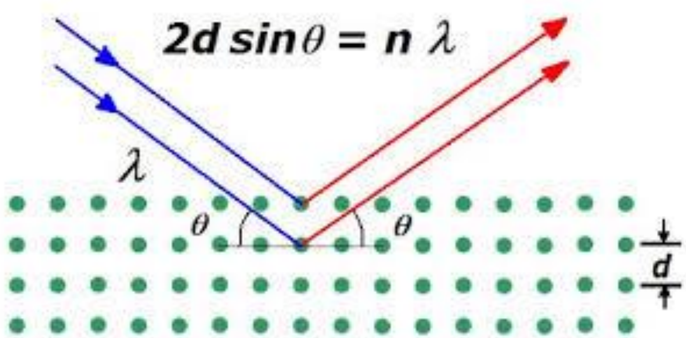
- **Why cool? Radiation damage**
- What are the symptoms?
- What is it?
- Why do we care? Effect on MAD/SAD.
- How do we estimate the Dose?
- The limits
- What can you do to minimise it?



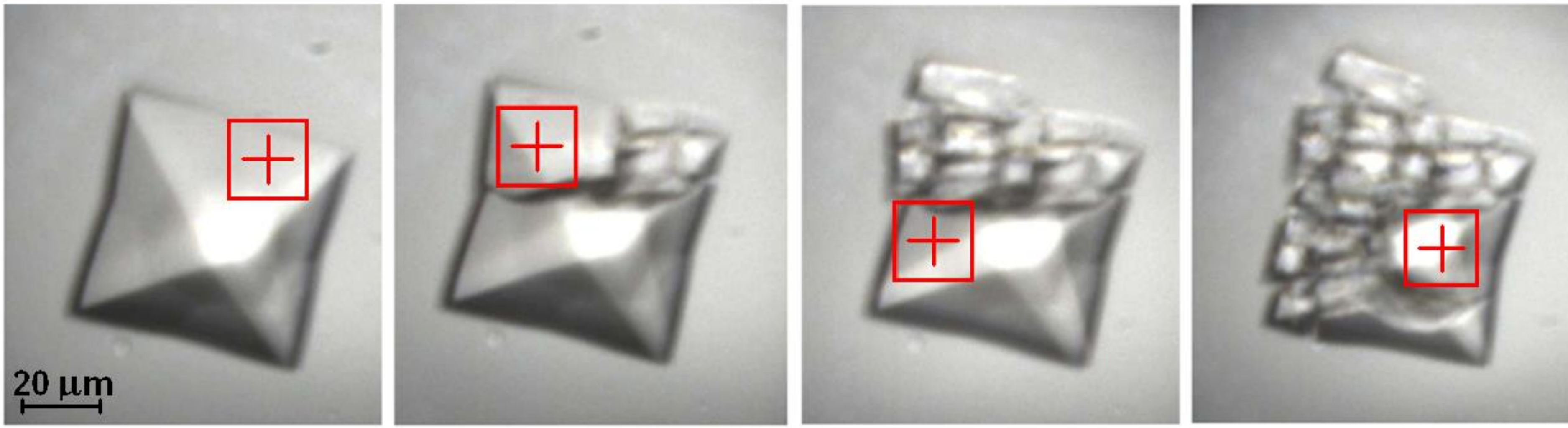


Intensity decrease with increasing X-ray exposure

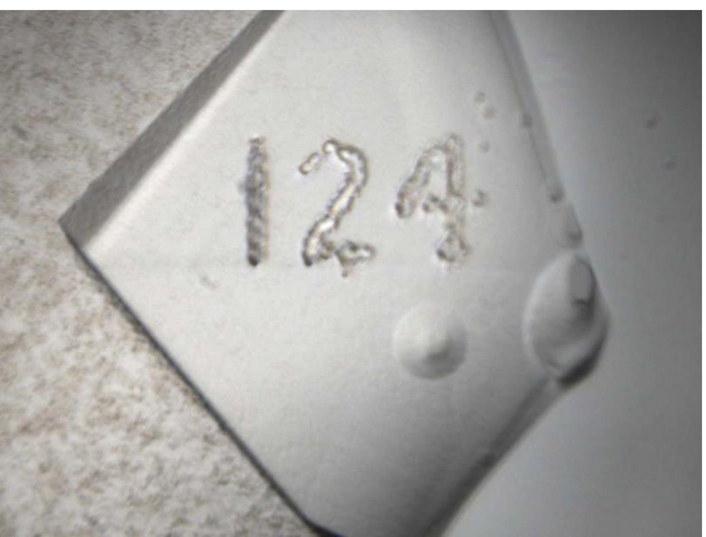




I24, Diamond, *in situ* data collection from a Bovine Enterovirus 2 crystal, room temperature, 0.5 s 20 μm x 20 μm beam



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

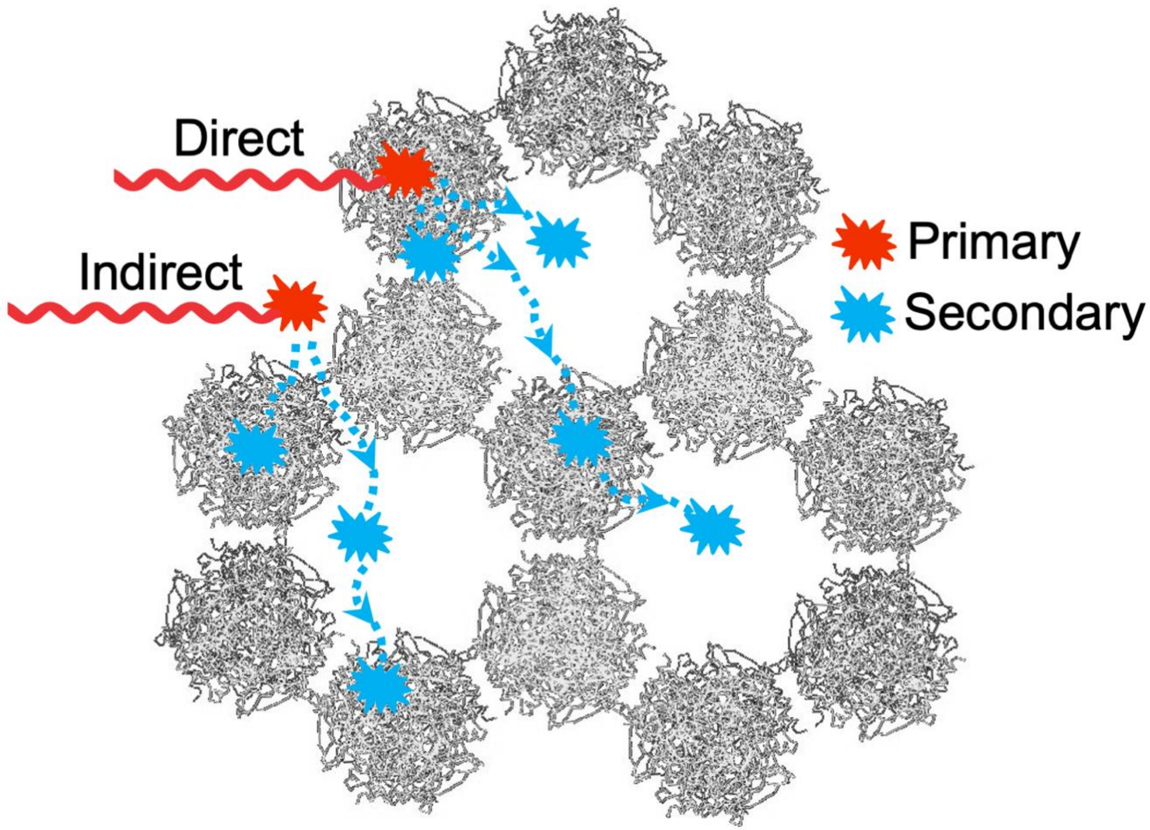


Beamline logo I24
(Gwyndaf Evans *et al.*)

Radiation damage

Primary

Secondary



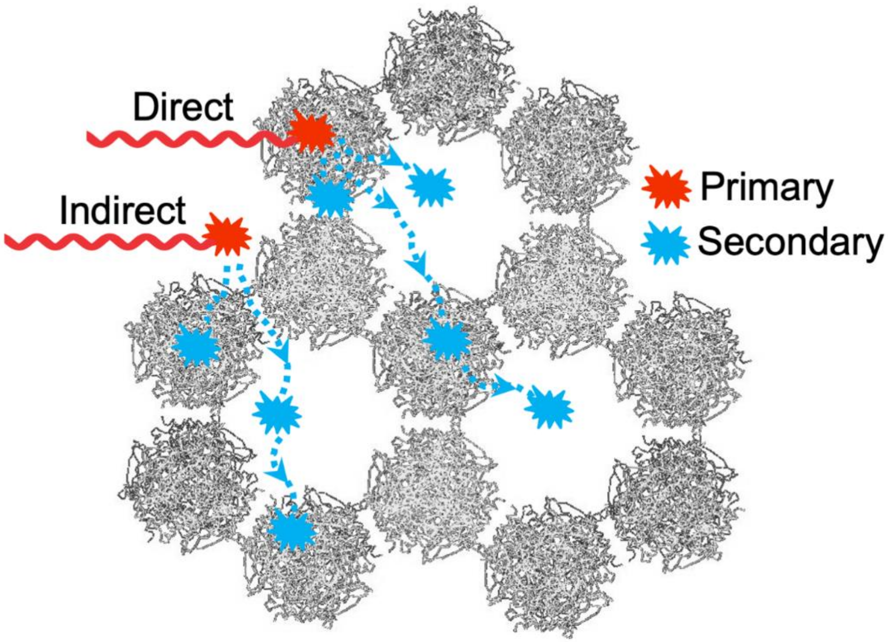
Protein: direct

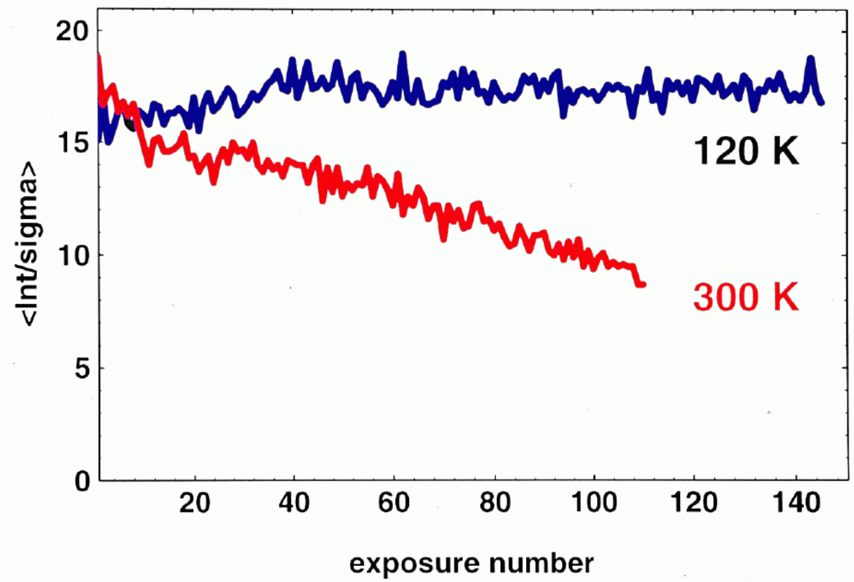
Solvent: indirect

PRIMARY; inevitable, a fact of physics! Neutralise it?

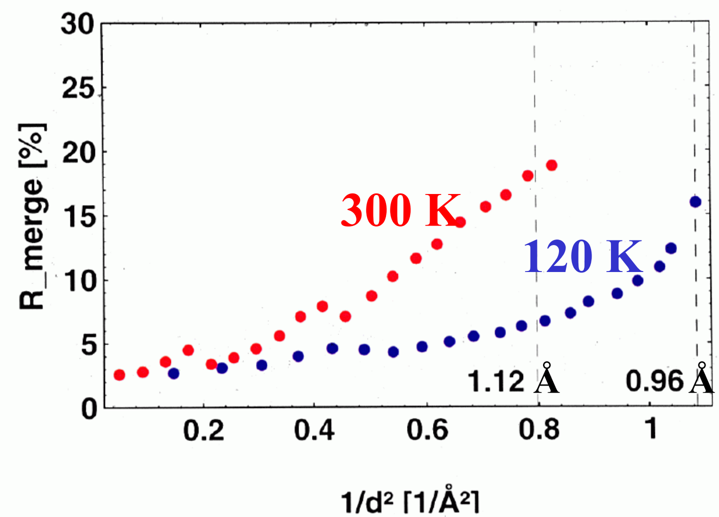
SECONDARY, can we control it?

Radiation damage





SP445: Data Quality [T.Schneider]



Significantly reduced at 100 K: time factor of ~70

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

PRIMARY; inevitable, a fact of physics! Proportions?

SECONDARY, can we control it?

Garman and Schneider J Appl Cryst 1997

BUT we also see degradation at 100 K

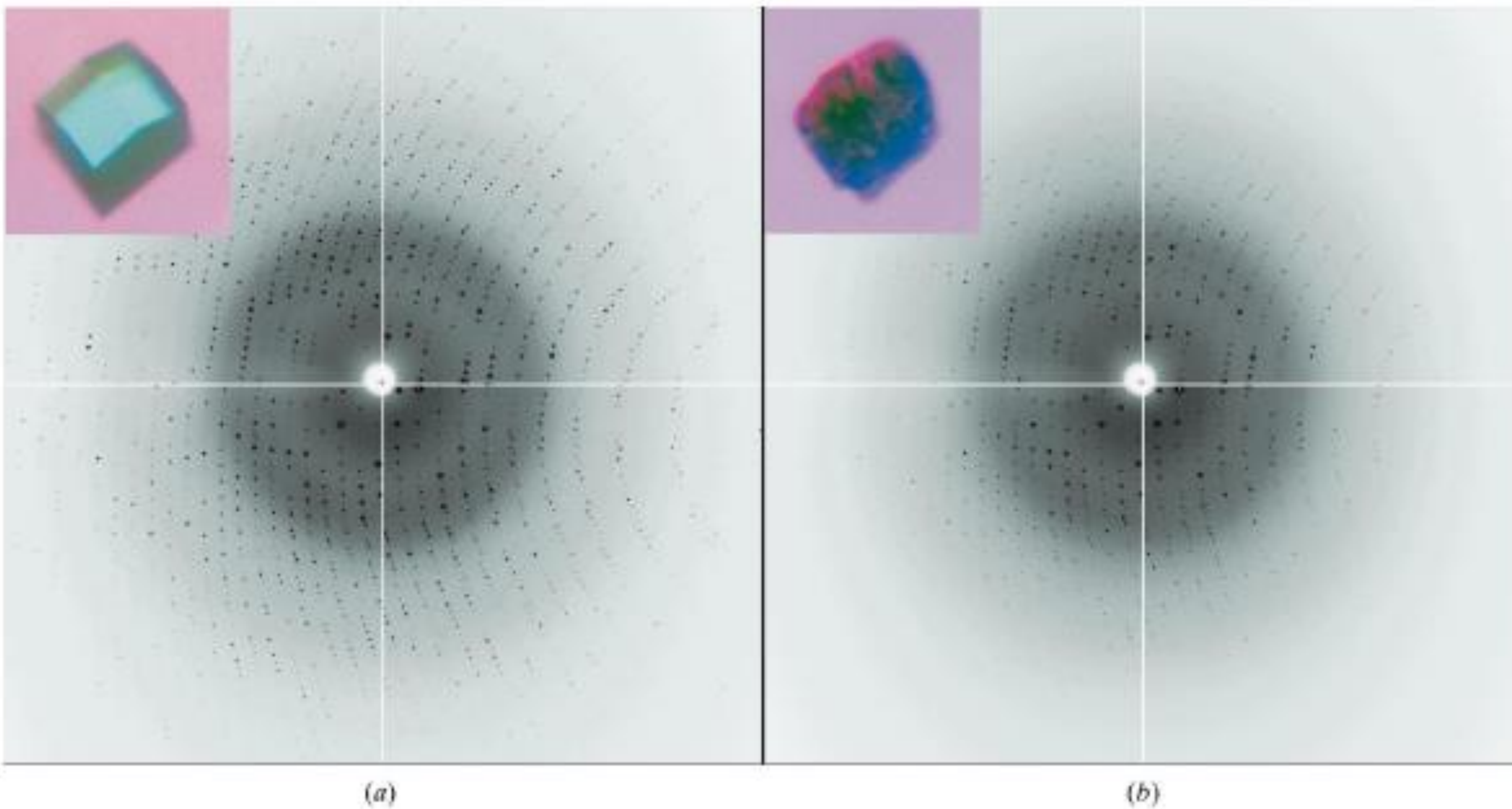
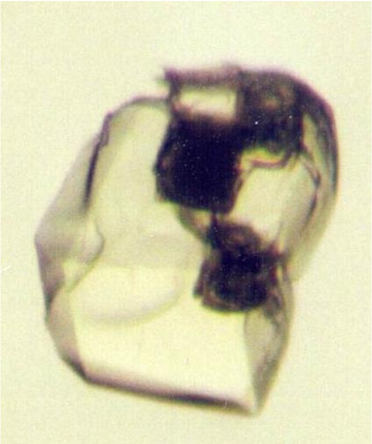


Figure 1
Diffraction images of a lysozyme crystal during a 1.36×10^3 s X-ray exposure at the 14-BM-C beamline. The resolution of diffraction is 1.6 Å at the edge of the image. Two images were taken with identical X-ray dosage. (*a*) The first image; during its exposure 1.2×10^4 Gy were absorbed. (*b*) The last image; after accumulating 1.6×10^7 Gy of absorbed energy. The inserts are photomicrographs of the crystal before and after X-ray exposures. The size of this crystal is ~$110 \times 110 \times 60$ µm. The crystal was maintained at 100 ± 1 K during the experiment.

Teng & Moffatt JSR (2000)



Radiation Damage

The Plan:

- Why cool? Radiation damage
- **What are the symptoms?**
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- The limits
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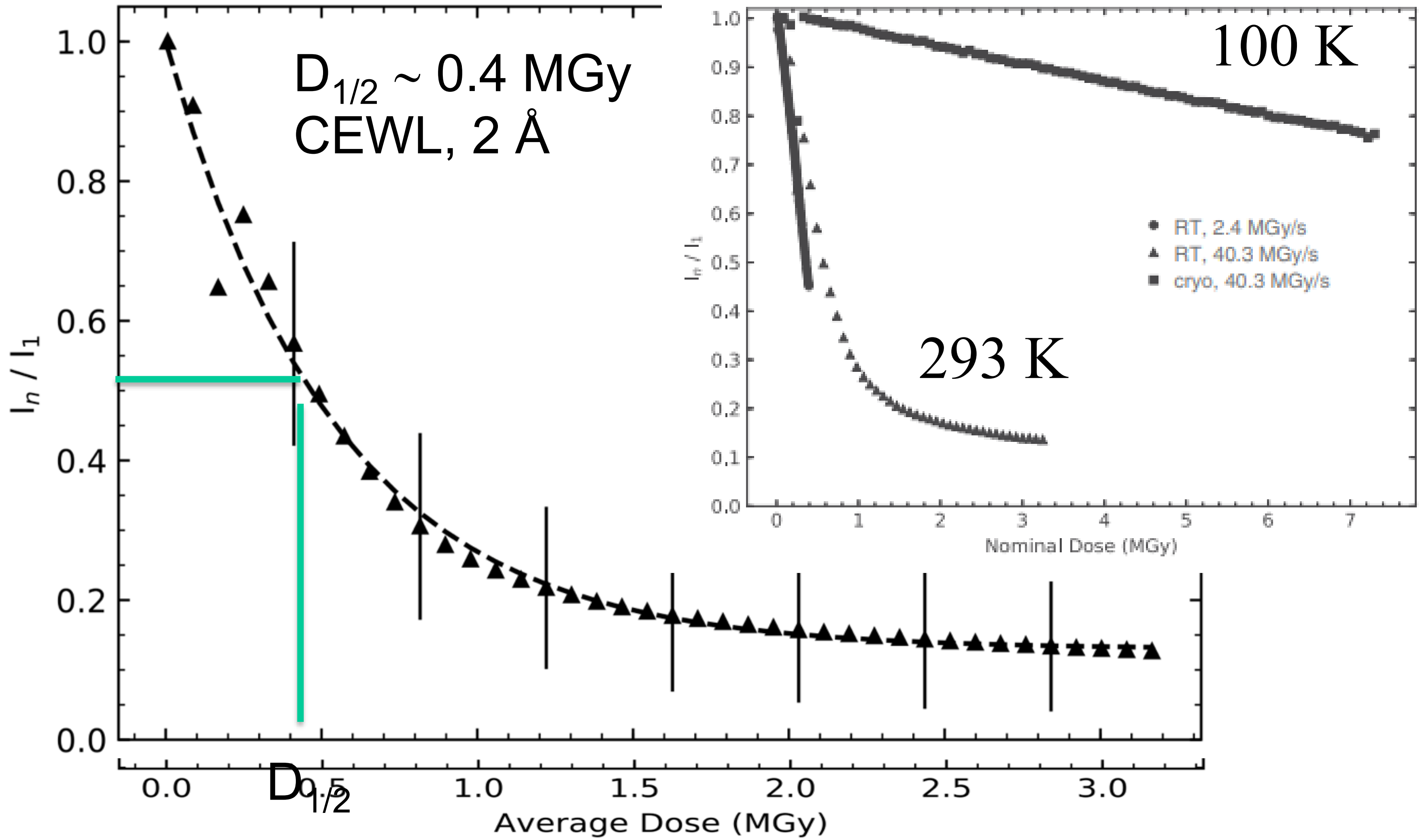
Intensity decay at RT and 100 K



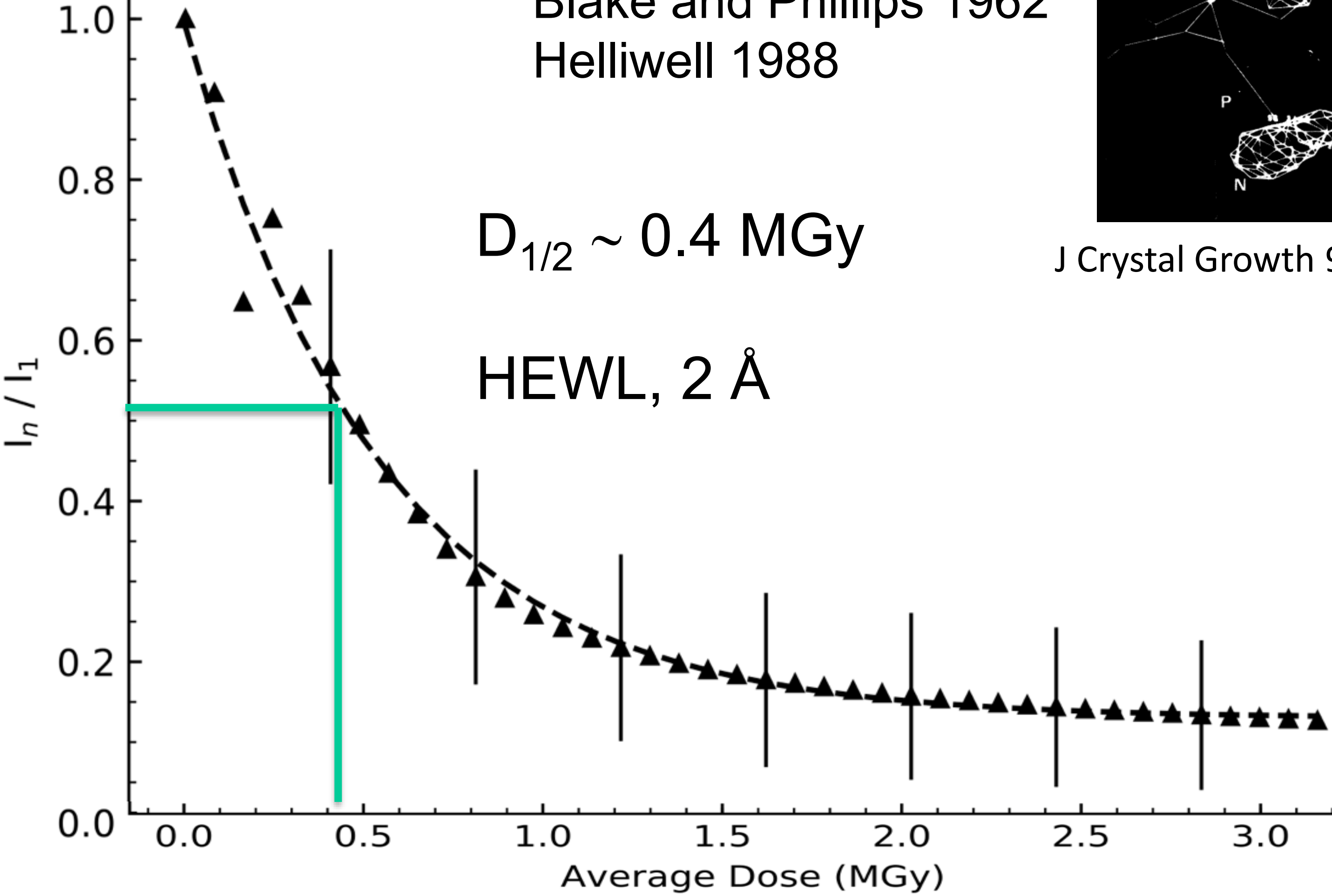
Radiation damage and dose limits in serial synchrotron crystallography at cryo- and room temperatures. De la Mora, Coquelle *et al.* (2020) *PNAS*

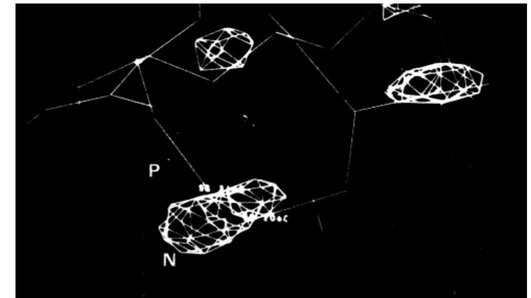
RD signatures in reciprocal space (RT MX)

Blake and Phillips 1962
Helliwell 1988

$D_{1/2} \sim 0.4$ MGy

HEWL, 2 Å

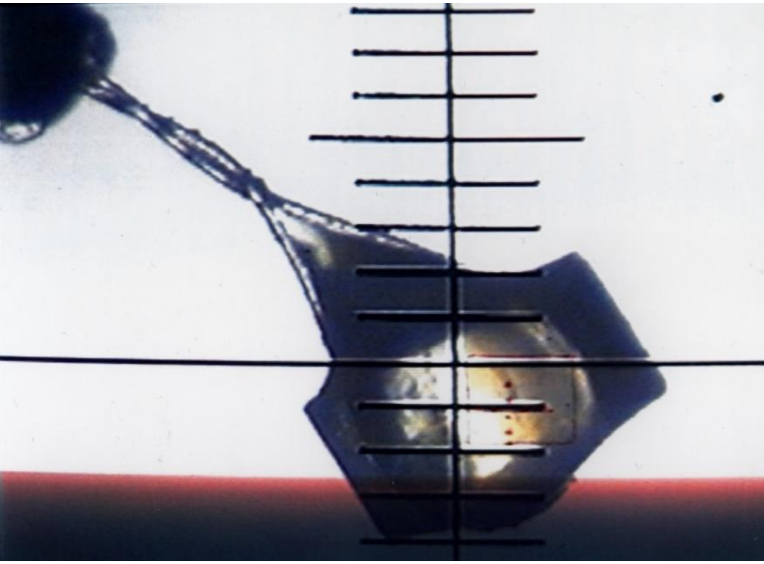


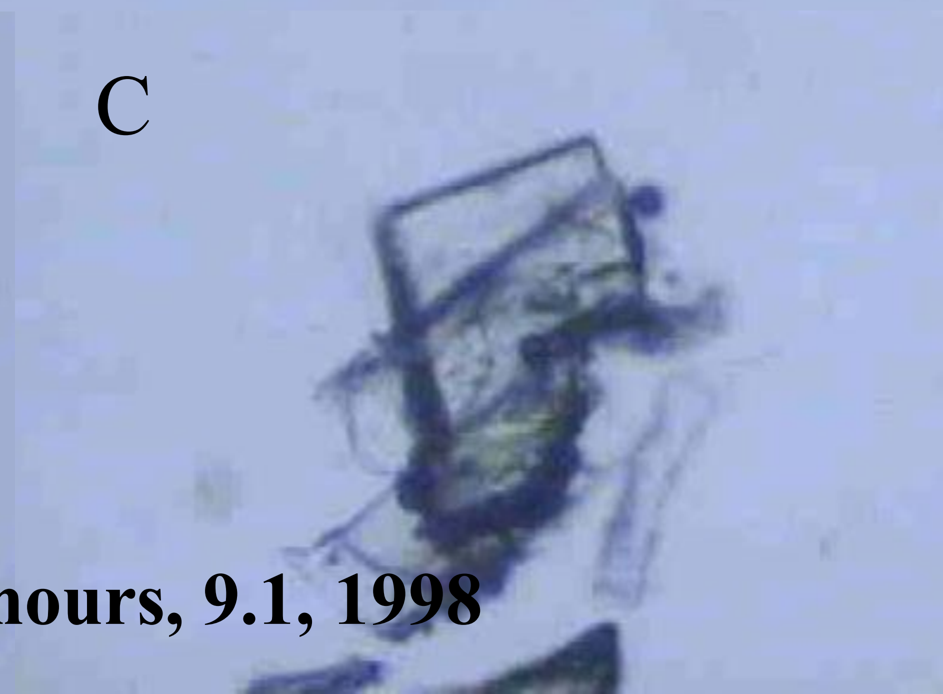


J Crystal Growth 90 (1988) 259-272

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

RadDam signatures (100 K MX)

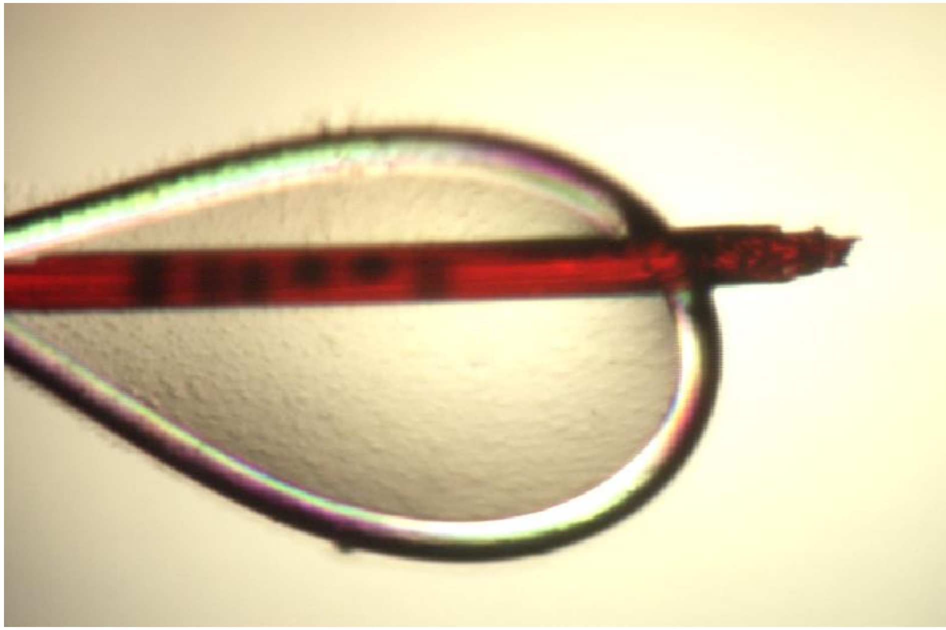


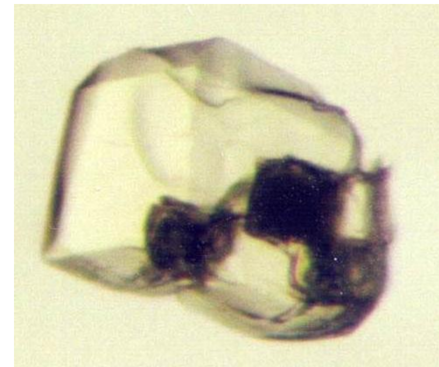


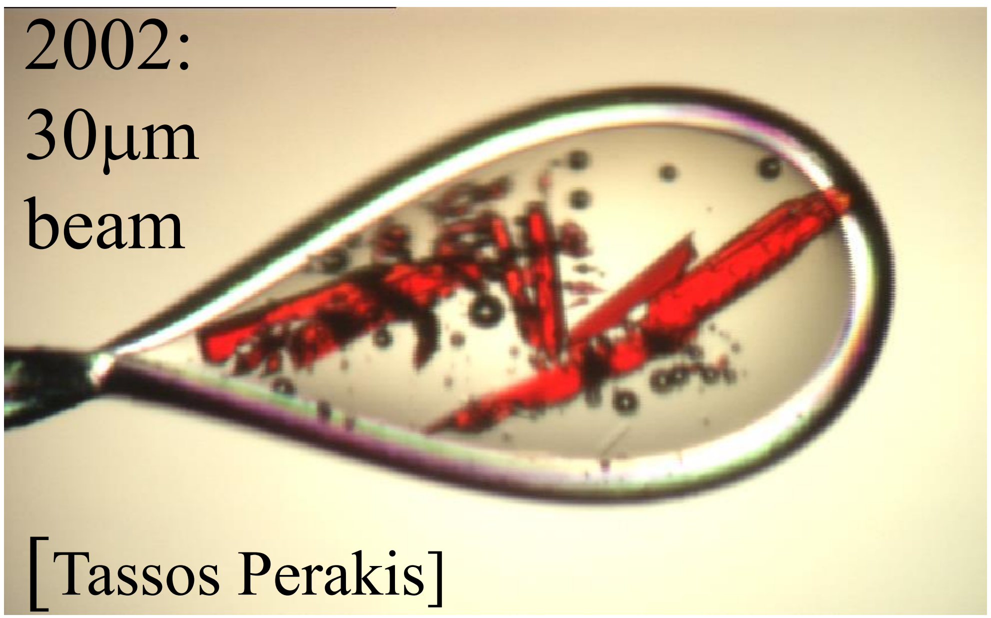
SSRL, 19 hours, 9.1, 1998

1995 onwards: 100 K

BUT THEN, 1999:



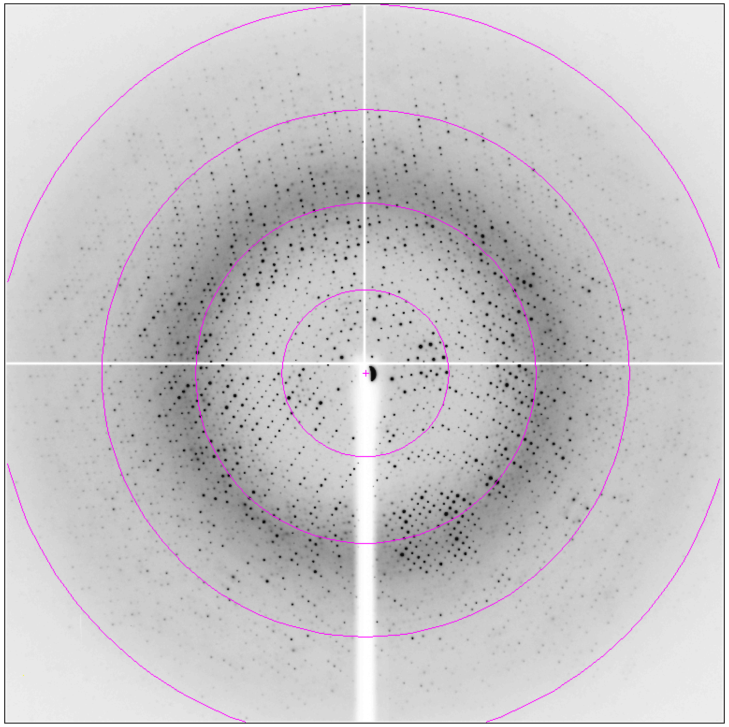




Iron containing protein, ESRF

Also observe spectral changes

Garman & Owen (2006), Acta D62

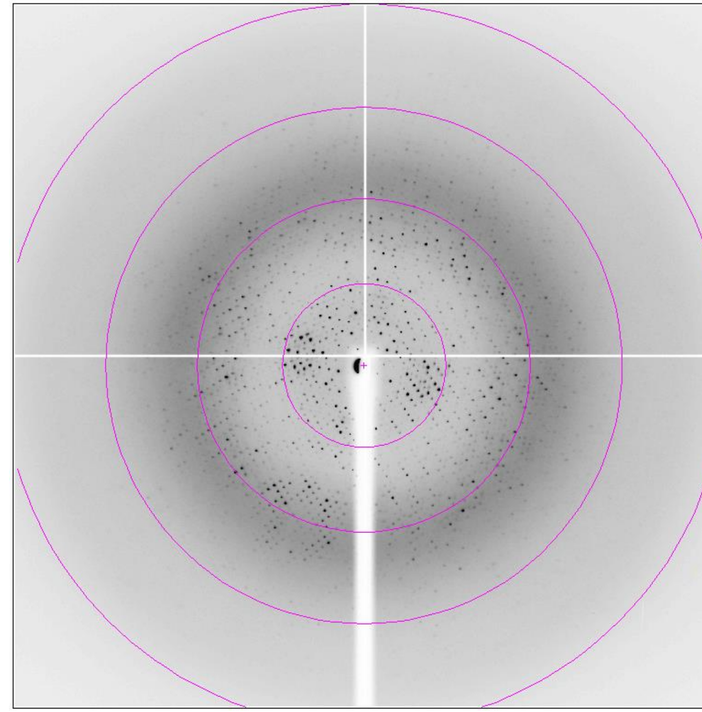


At 100 K

Intensity decrease

Loss of diffraction

Incomplete data from crystals



Happens during 1 dataset at 100K for many crystals

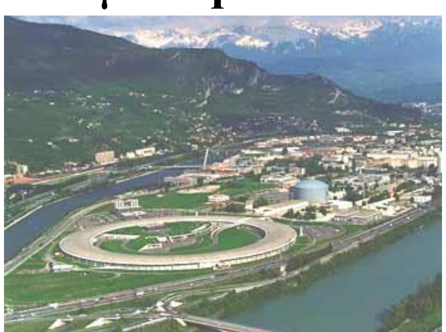
Unit cell volume expansion

Increase in Wilson B factors, Rmerge

Increase in mosaicity

'GLOBAL' damage

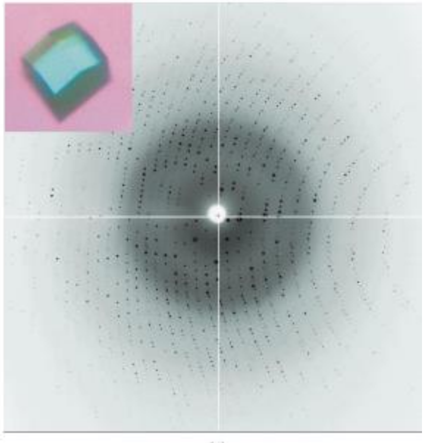
ESRF 2000:
1×10^{12} ph s^{-1} into
100μm square slits

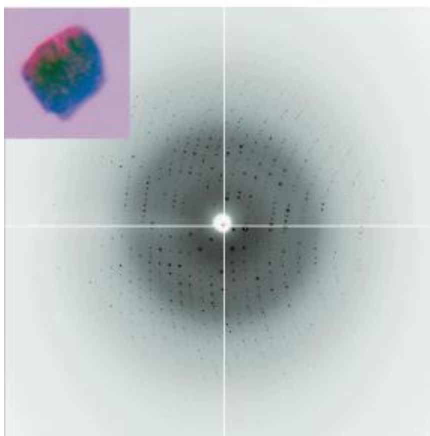


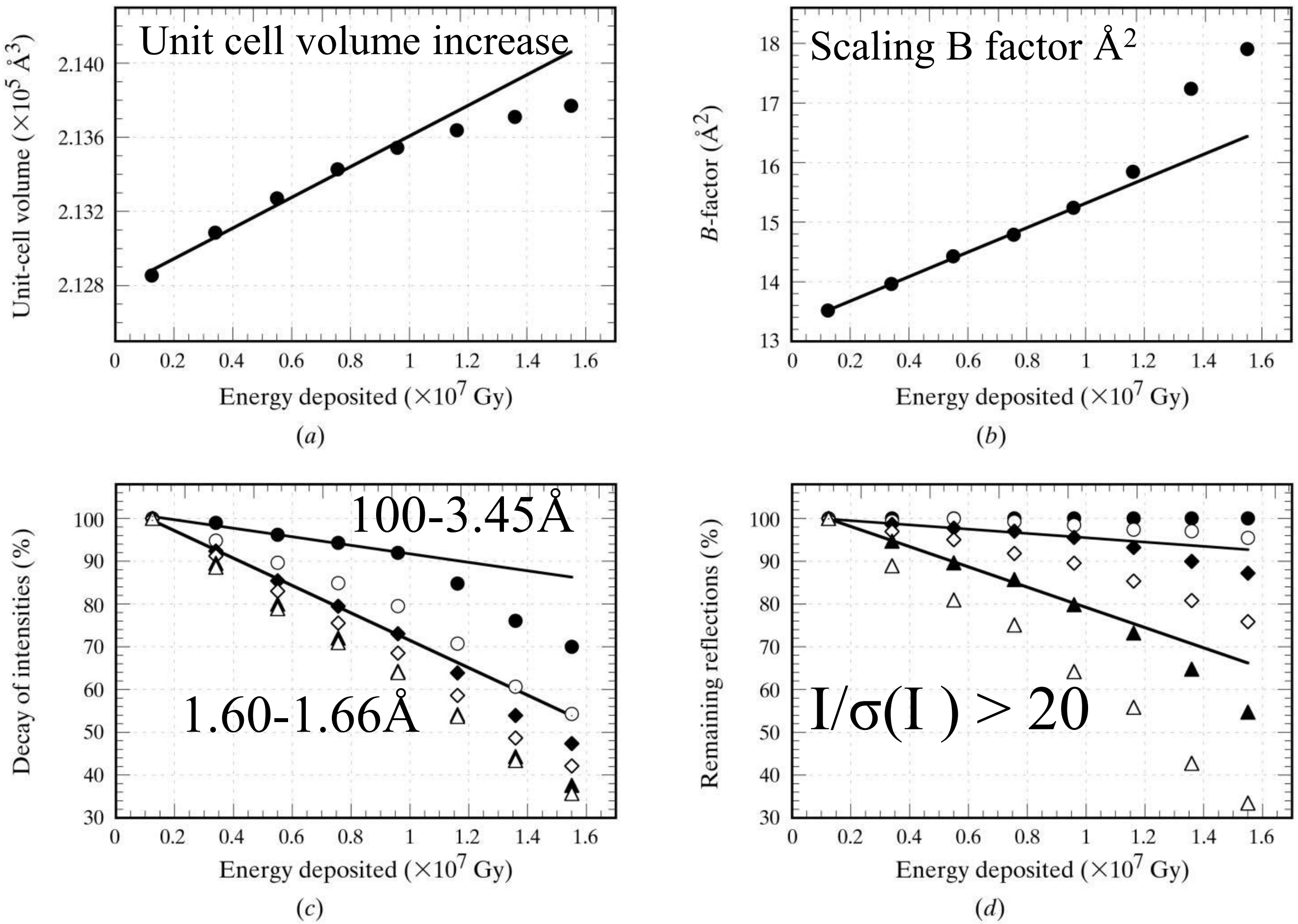
Diamond Light Source
(I24): 3×10^{12} ph s^{-1}
into 7μm × 6μm
[7×10^{14}ph/s/ 100μm^2]

Australian synch.
3×10^{13} ph s^{-1} into
50μm × 70μm
[10^{14} ph/s/ 100μm^2]

RadDam signatures in reciprocal space 100 K

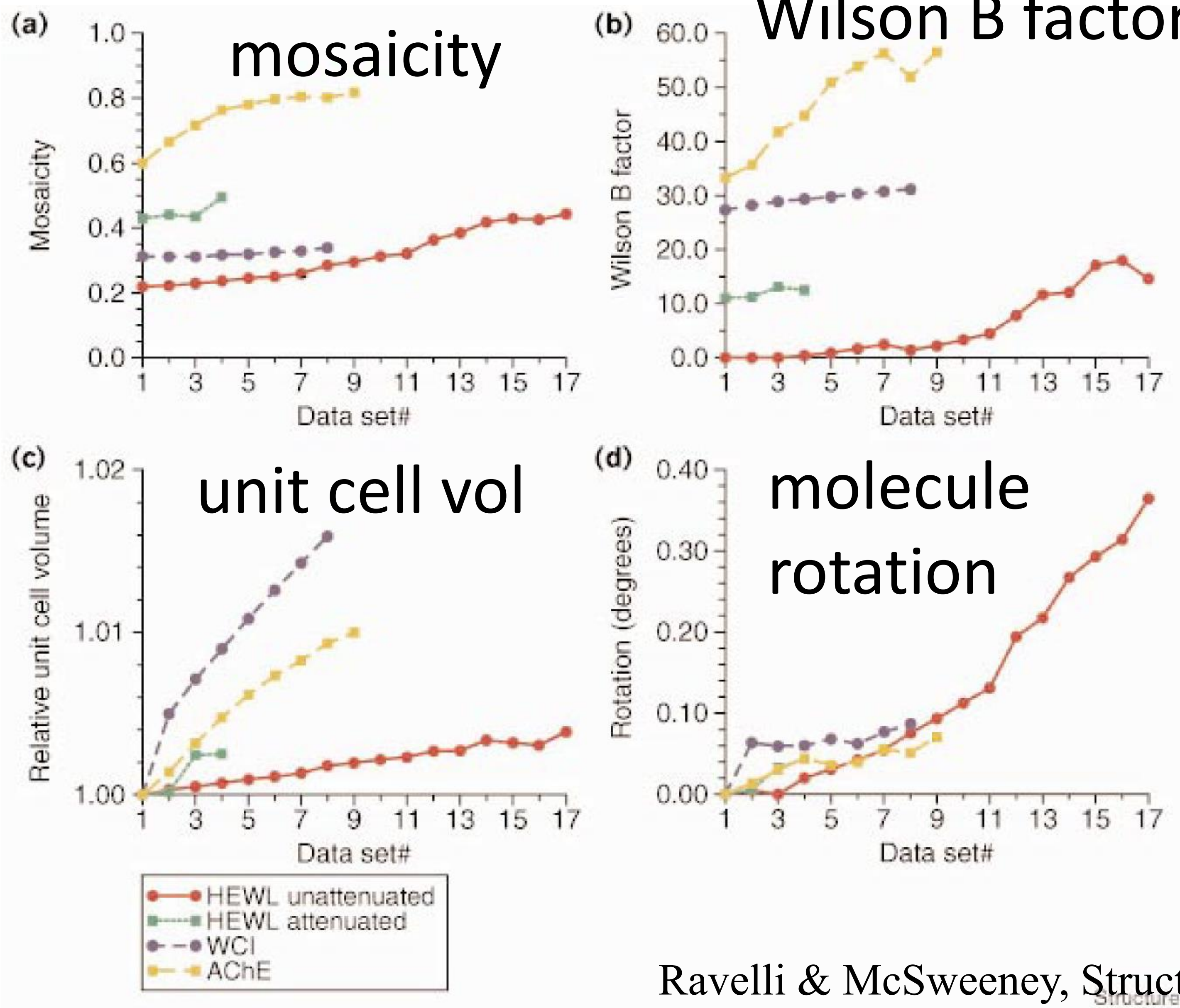






Teng and Moffat *JSR* (2000)

RadDam signatures in reciprocal space 100 K



Raimond
Ravelli
1968-2023

Ravelli & McSweeney, Structure (2000) 8, 315

Data Parameters affected by Radiation Damage

- I /σ(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?

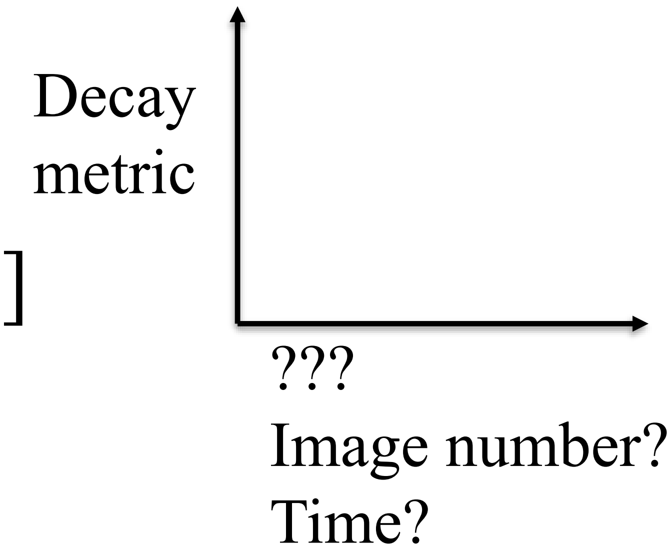
[Ravelli and McSweeney, (2000) Structure]

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 $\downarrow$
- Not I/sigma(I) [I $\downarrow$, sigma(I) $\uparrow$]
- Scaling B factors? $\downarrow$
- An R_{meas} type measure? $\rightarrow$ $\downarrow$



[Diederichs 2006 Acta D59, 903]

To monitor the damage rate, compare results from different experiments and avoid exceeding dose limits, we can't use time or image number.

We need a DOSE estimator

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

Fundamental metric against which to plot damage indicators.

Cannot be measured, can only be estimated.

Takes care of the physics but NOT the chemistry.

RADDOSE-3D: www.raddo.se

and https://bl831.als.lbl.gov/xtallife.html (James Holton)

RADDOSE-3D

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

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

V4: Dickerson and Garman (2021) *Protein Science*

V5: Dickerson et al. (2024) *Protein Science*

Typical MX experiment & ‘limits’

1 MGy/s absorbed by a 100 μm cubed metal free crystal in a 100$\times$100 μm^2 beam of 12.4 keV (1 Å) X-ray flux: 10^{13} photons s^{-1}

MX at synchrotrons RT:
~0.4 MGy experimental dose ‘limit’
(dose to 0.5 I_n/I_1) reached in ~0.4 s

MX at synchrotrons 100 K:
30 MGy experimental dose ‘limit’
(dose to 0.7 I_n/I_1) reached in ~30 s:
4th generations sources <<1s,

MX at XFELs: damage before destruction ?
<80 fs

2-10 Gy: rodent dies in 10-30 days. Radiotherapy for human brain tumour max 60 Gy.

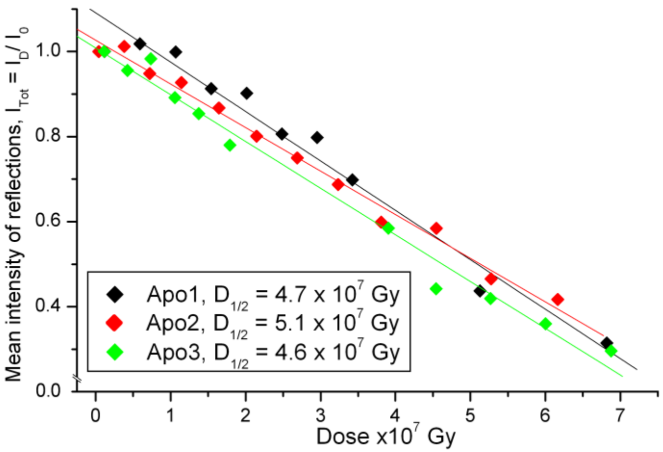
[Shelley & Garman Acta D 2024]

Intensity Decay at 100K

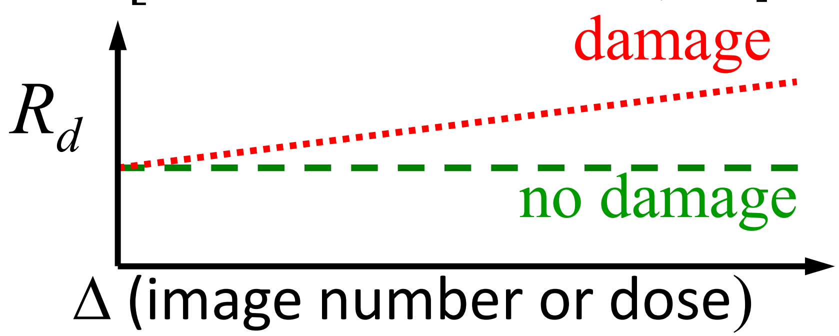
Normalised Intensity vs Dose:

apoferritin

[Owen et al 2006, PNAS]



[Deiderichs 2006 Acta D59, 903]



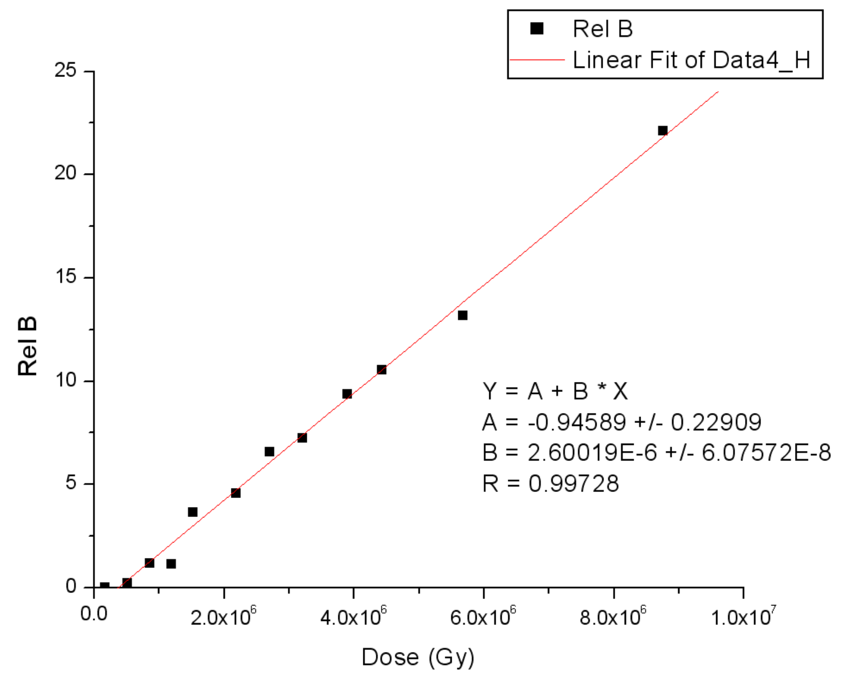
Coefficient of sensitivity α

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)

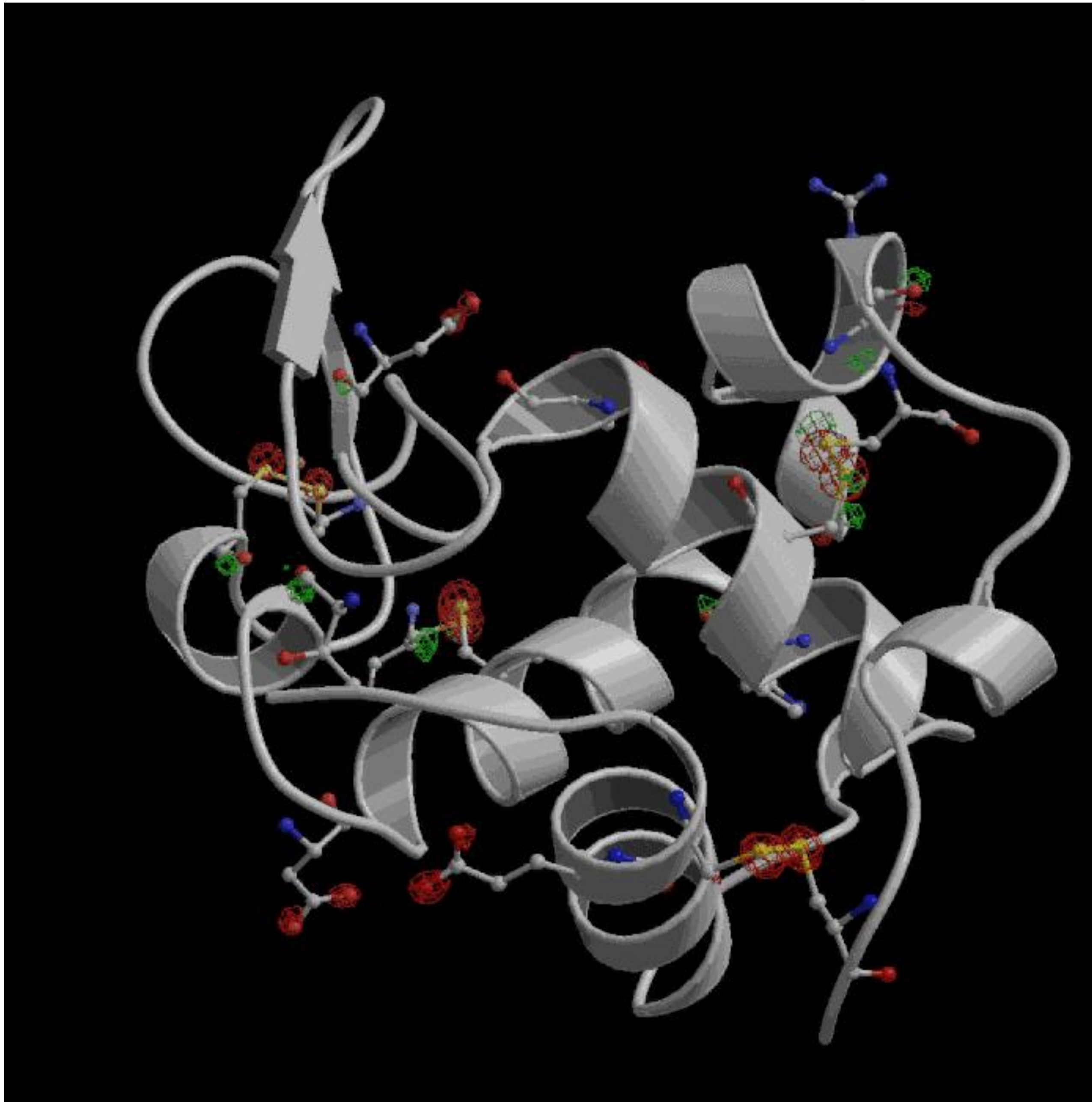
[Kmetko et al 2006, Acta D62, 1030]



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

Real Space: Specific Damage

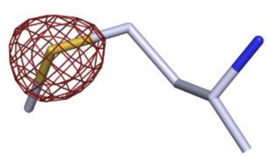
CEWL lysozyme Fo_4-Fo_1

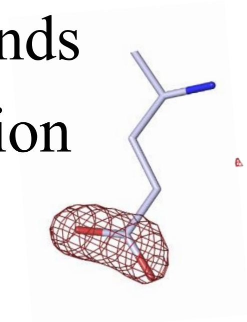


Raimond Ravelli

Real Space: 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

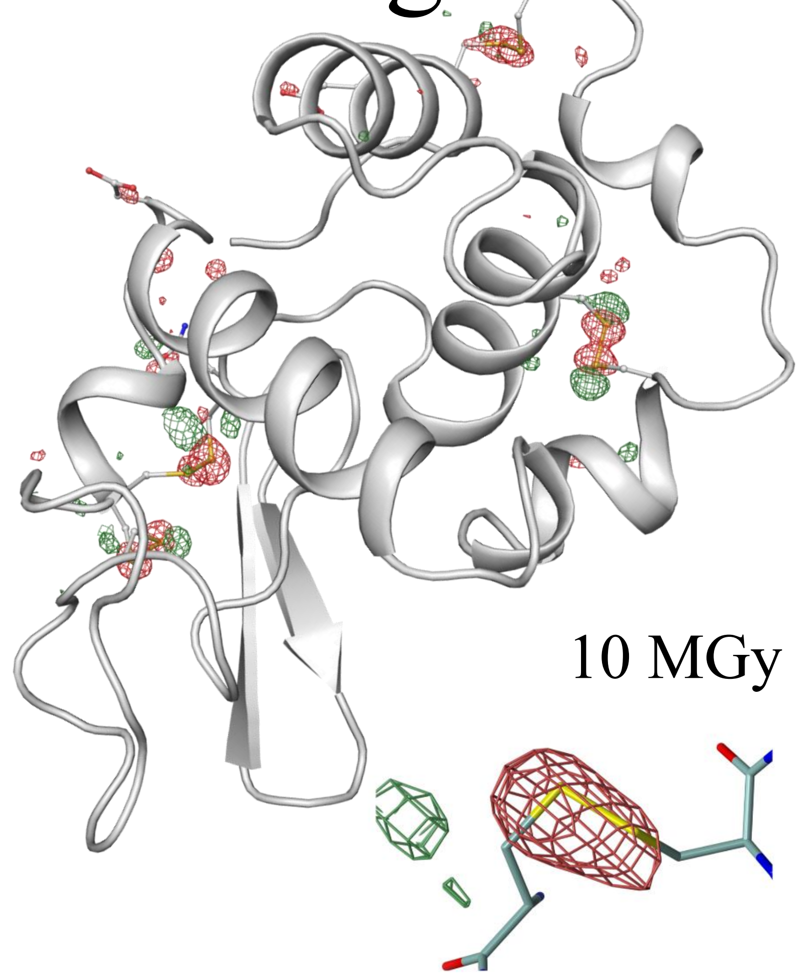




- Rupture of covalent bonds to heavier atoms:

  C-Br, C-I, S-Hg

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



Difference map Fo_4-Fo_1

Weik et al. 2000, Burmeister 2000
Ravelli & McSweeney 2000
Bury et al., JSR 2015

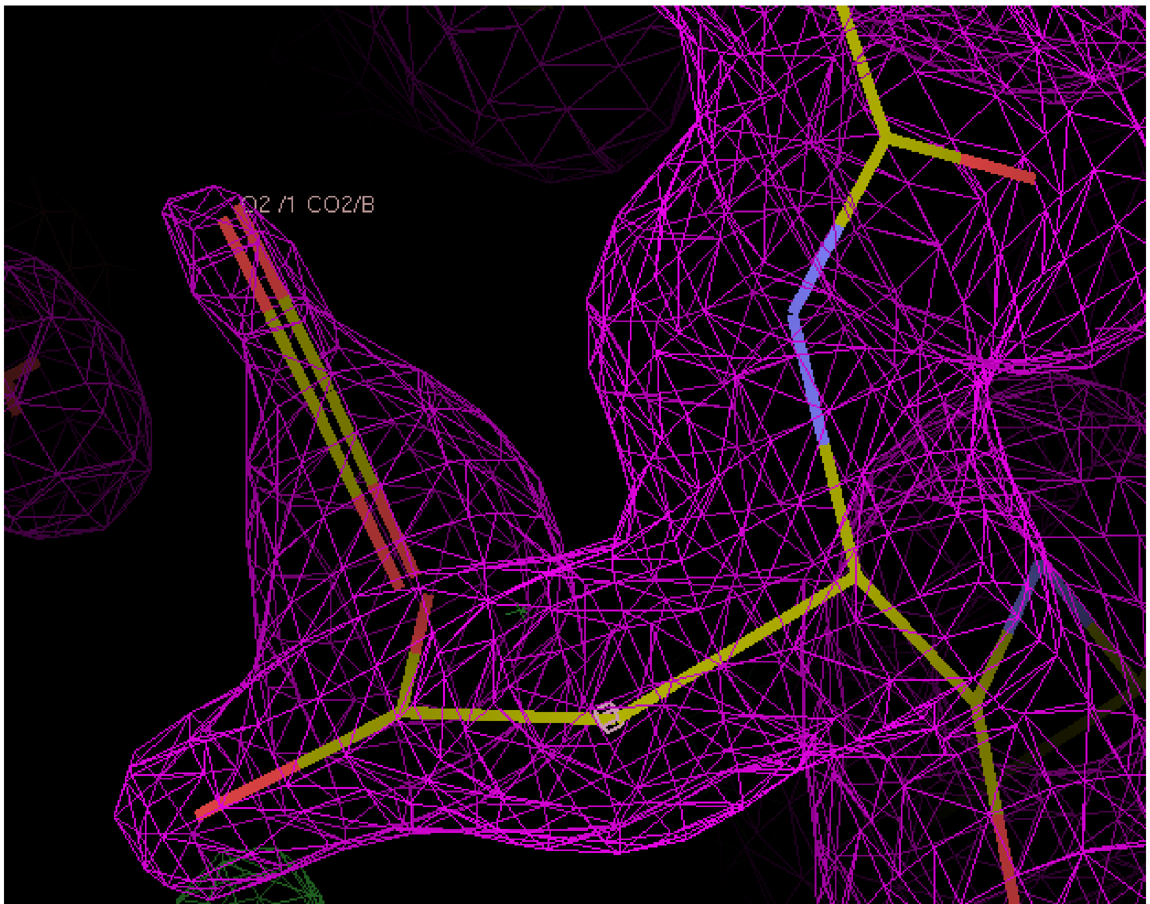
CO_2 from decarboxylation

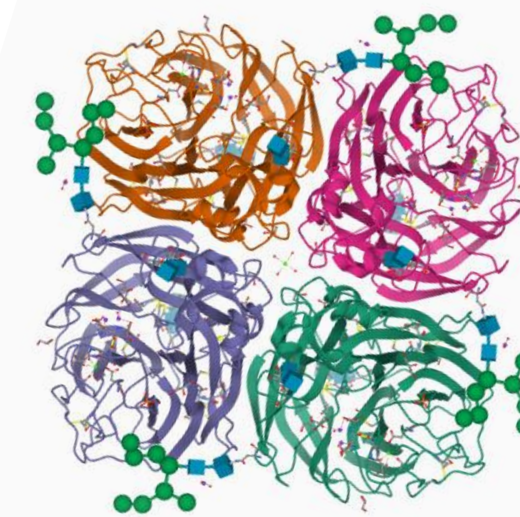
Raddam induced decarboxylation of Asp128.
0.5:0.5 occupancy of C and 2 O atoms involved
N9 influenza neuraminidase, PDB: 6HFC

Resolution: 1.29 Å

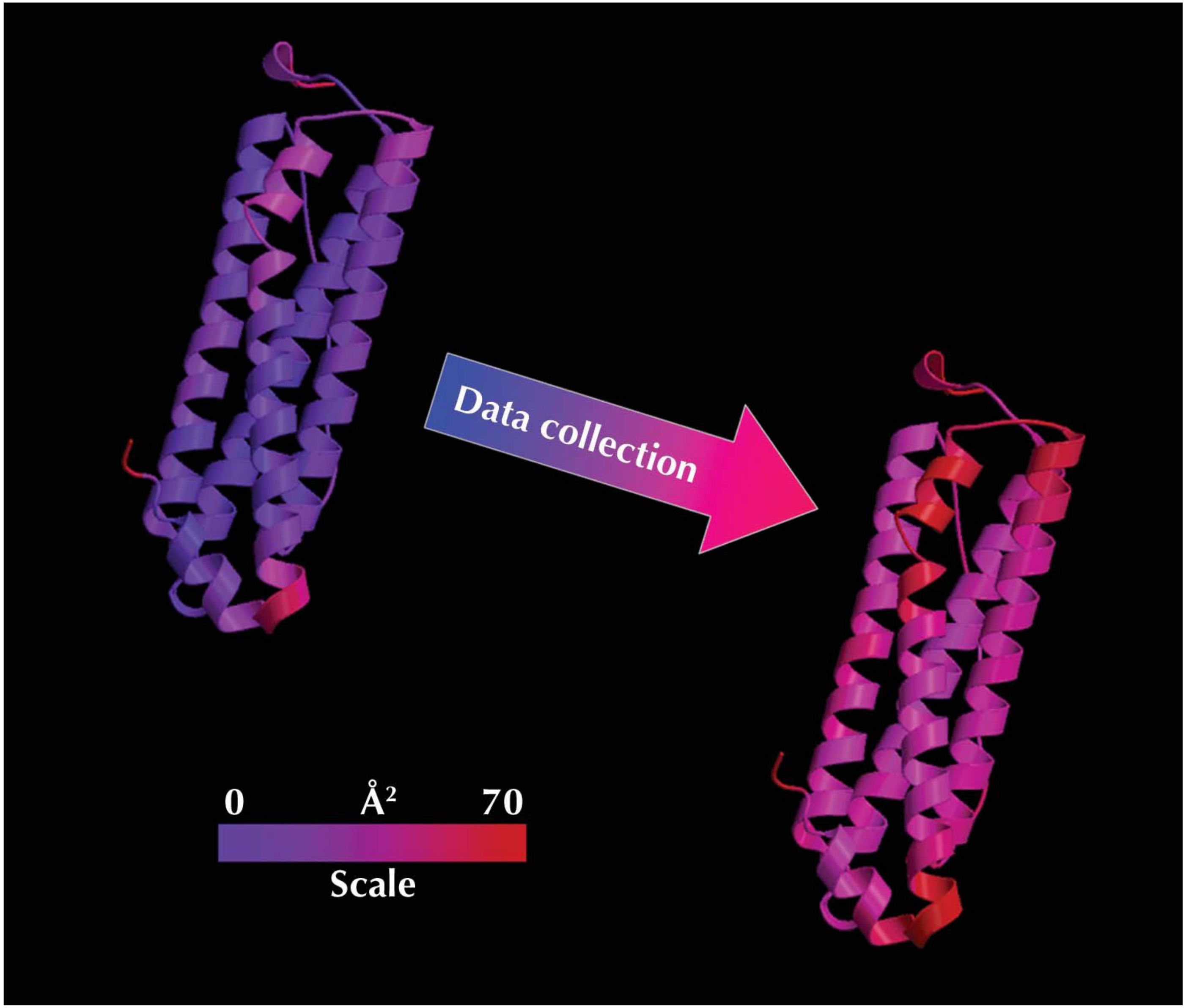
R-Value Free: 0.164

R-Value Work: 0.141

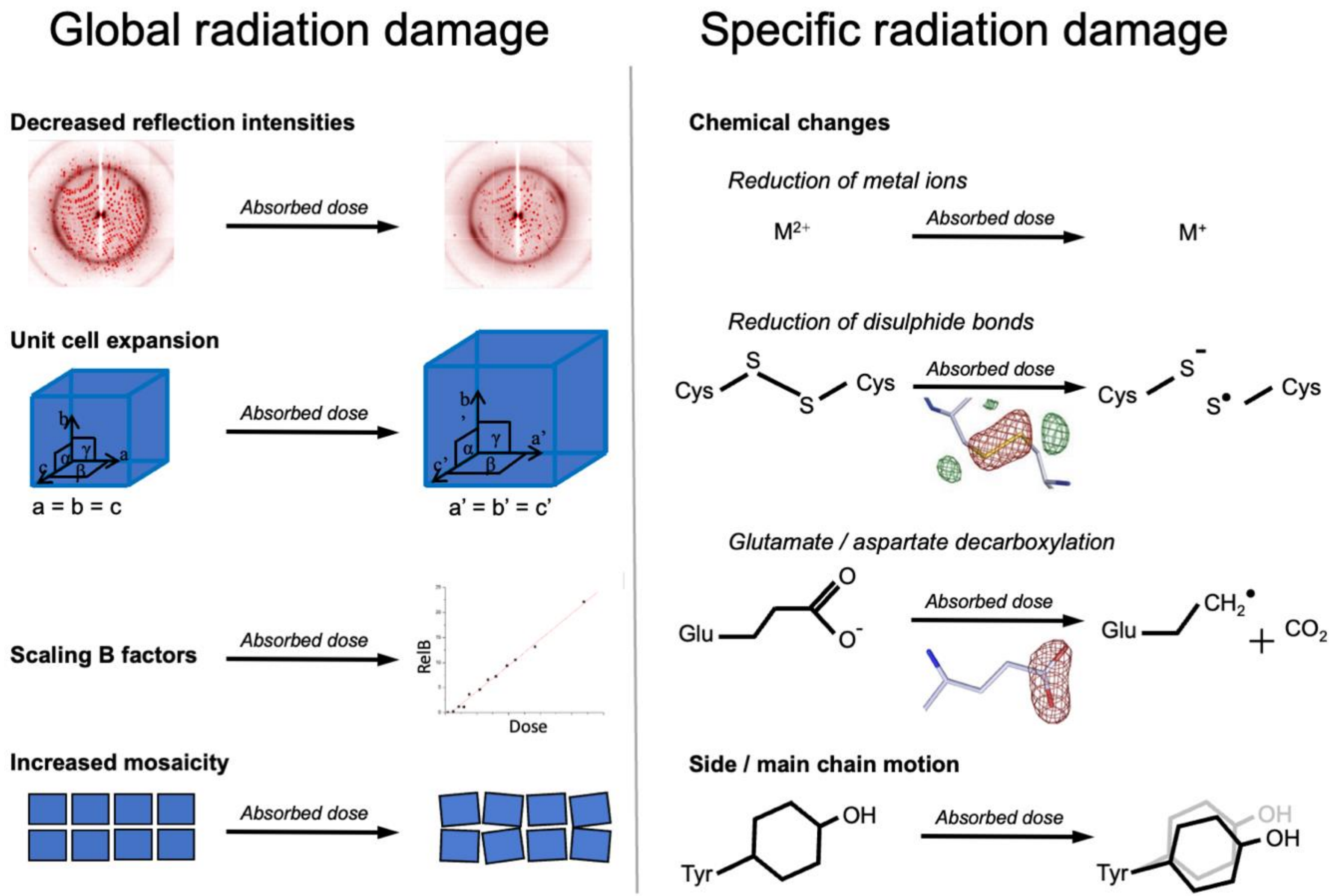




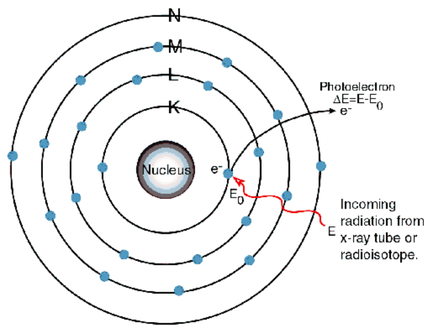
ALSO:
Atomic B-factors increase:



Overall summary of RD effects

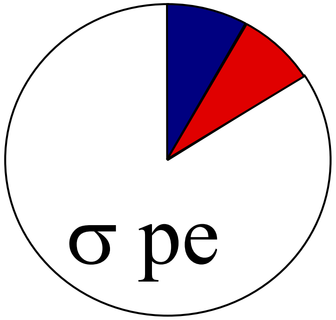


Garman & Weik COSB (2023)



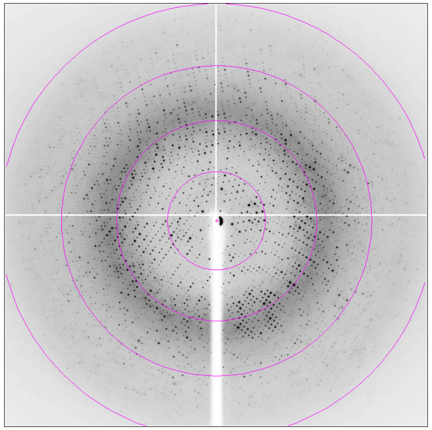
Radiation Damage

The Plan:



- Why cool: radiation damage
- What are the symptoms?
- **What is it?**
- Why do we care? Effect on MAD/SAD.
- How do we estimate the Dose?
- The limits
- What can you do to minimise it?

PHYSICS of the interaction of X-rays with crystals.



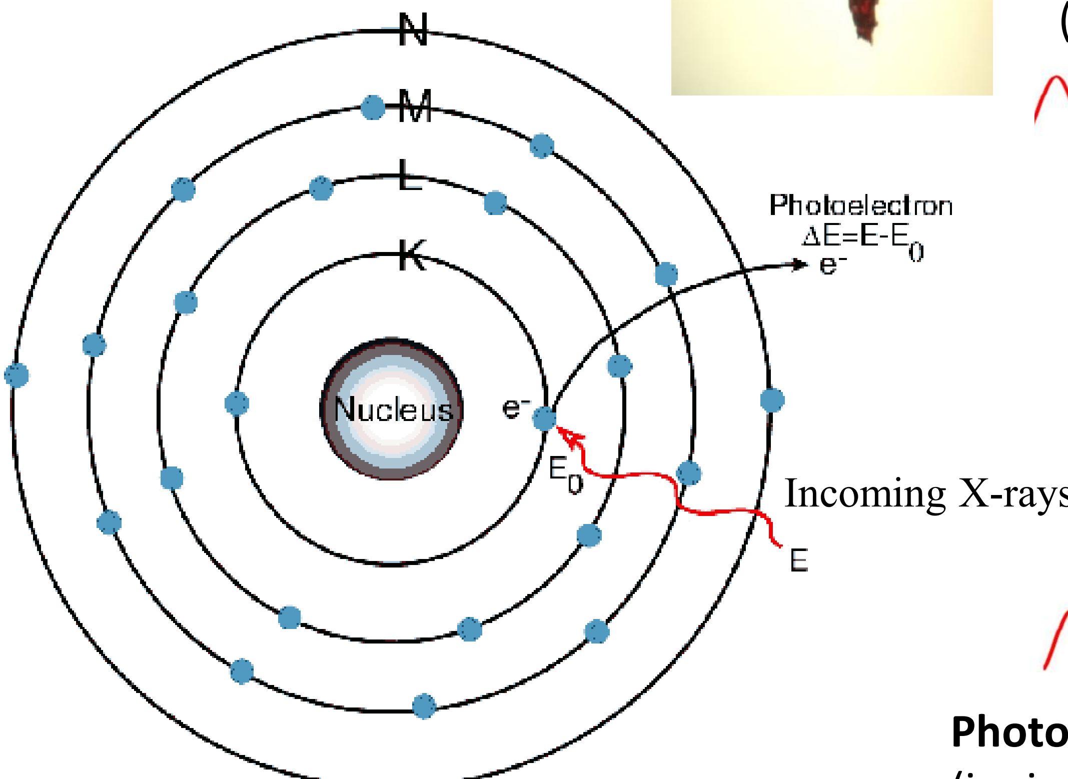
A) Diffraction

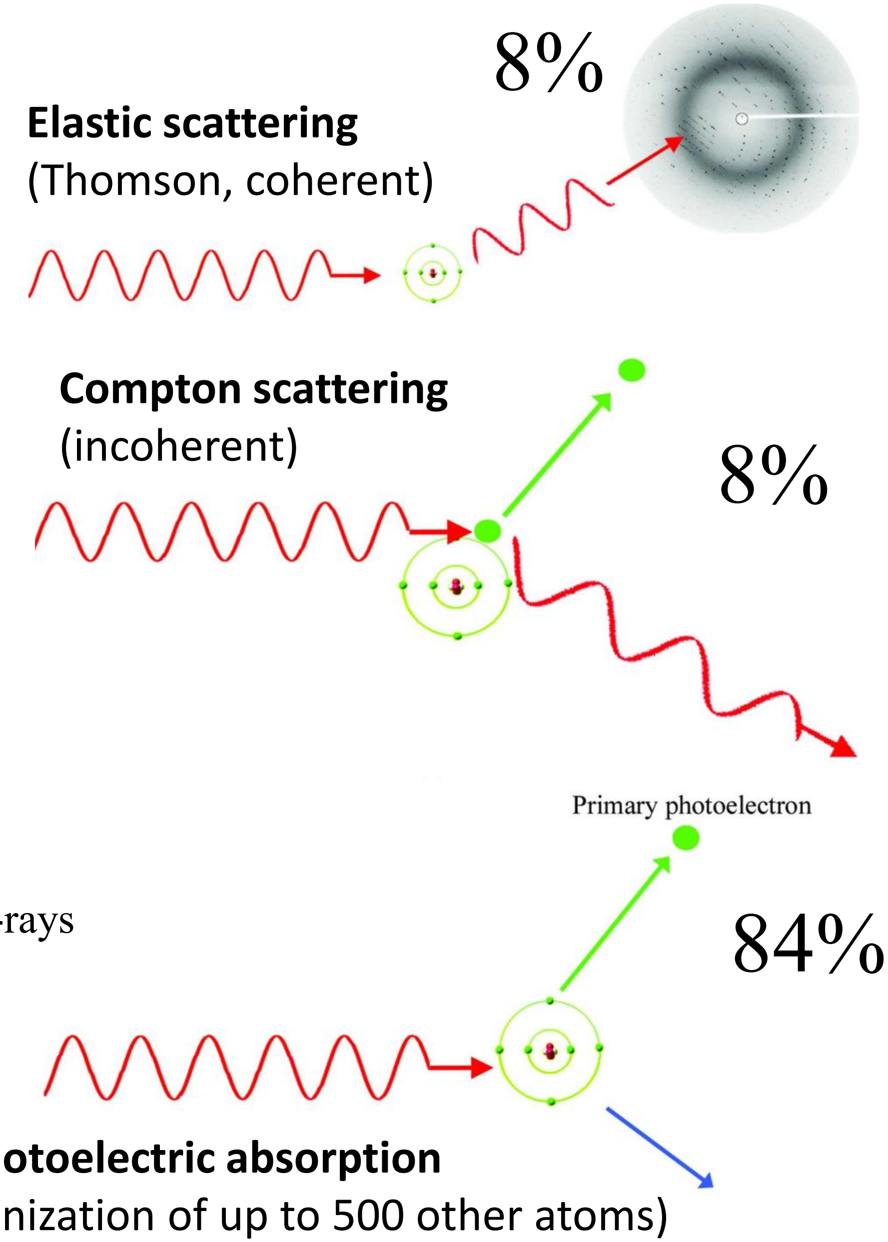
B) Absorption = Energy loss

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

Interaction of X-rays with biological samples

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



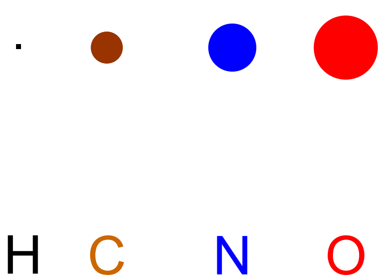


[Garman, *Acta Cryst*. (2010). D66, 339–351]

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]

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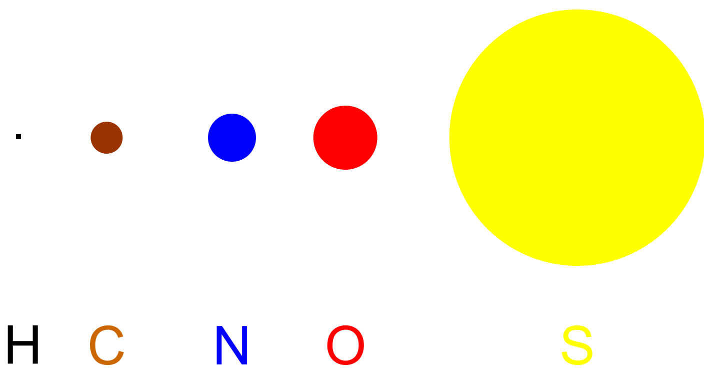

[Ravelli *et al.*, JSR,(2005) 12]

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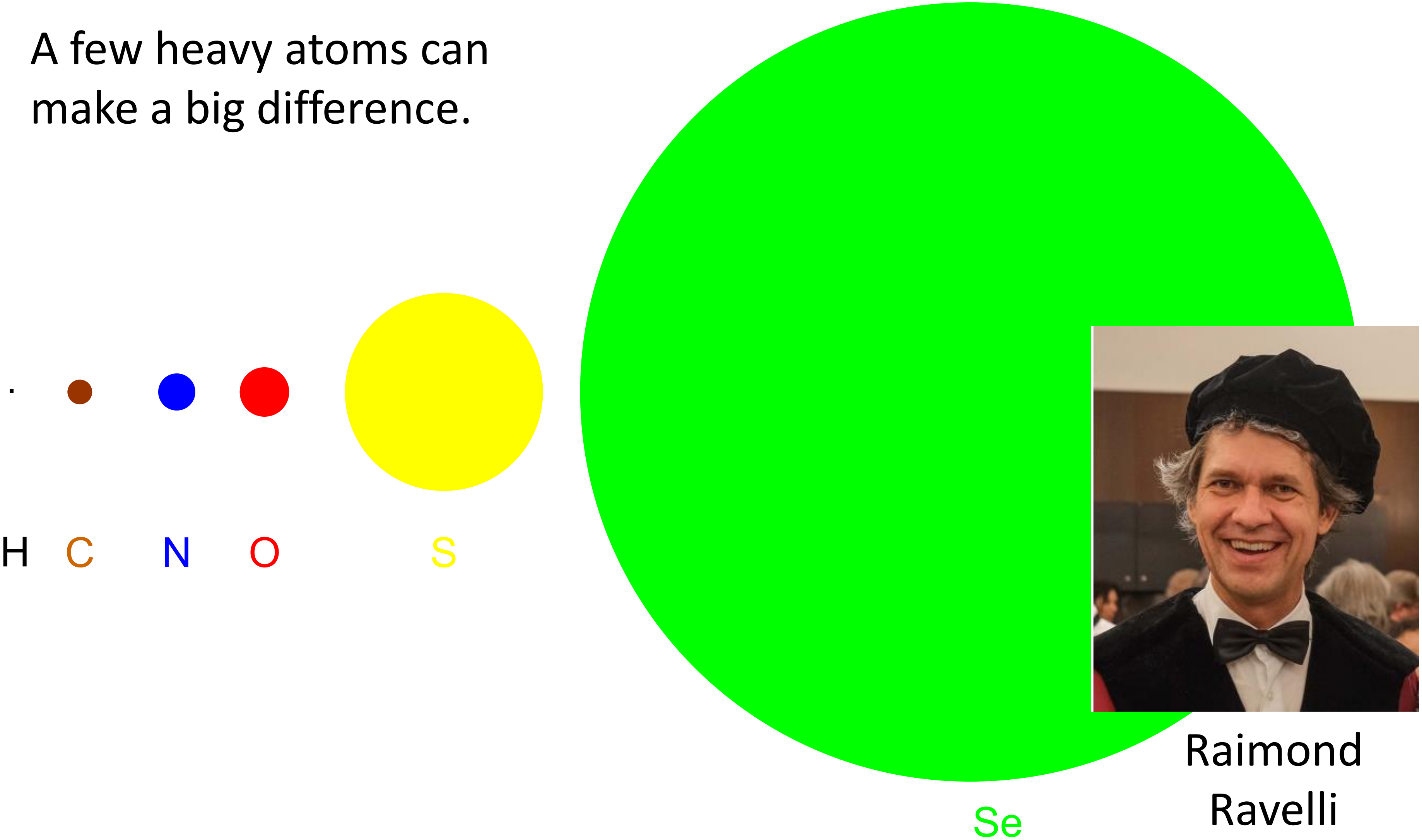
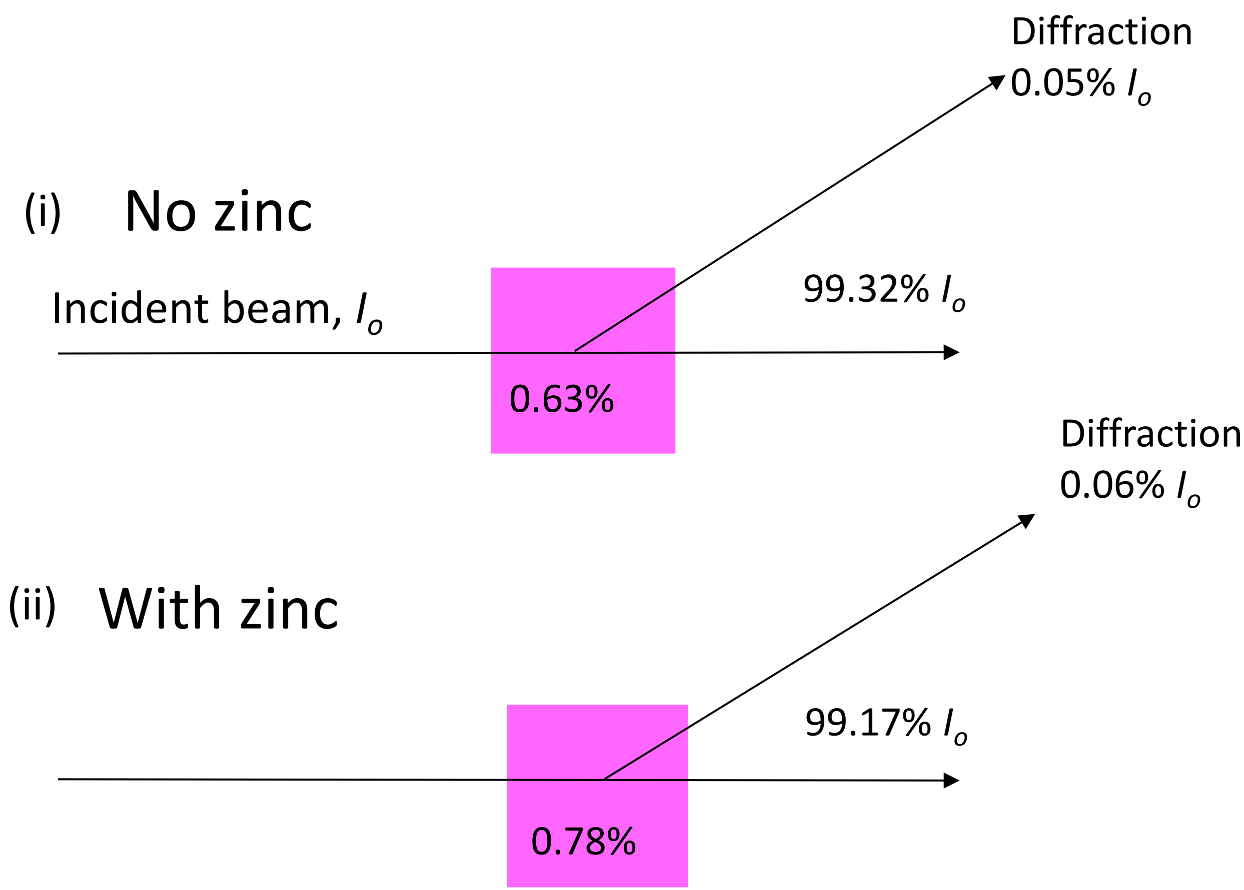


Raimond Ravelli 1968-2023

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

Beam absorption (λ=0.95Å, 13 keV) by a Zn containing protein (metallo-β-lactamase) crystal



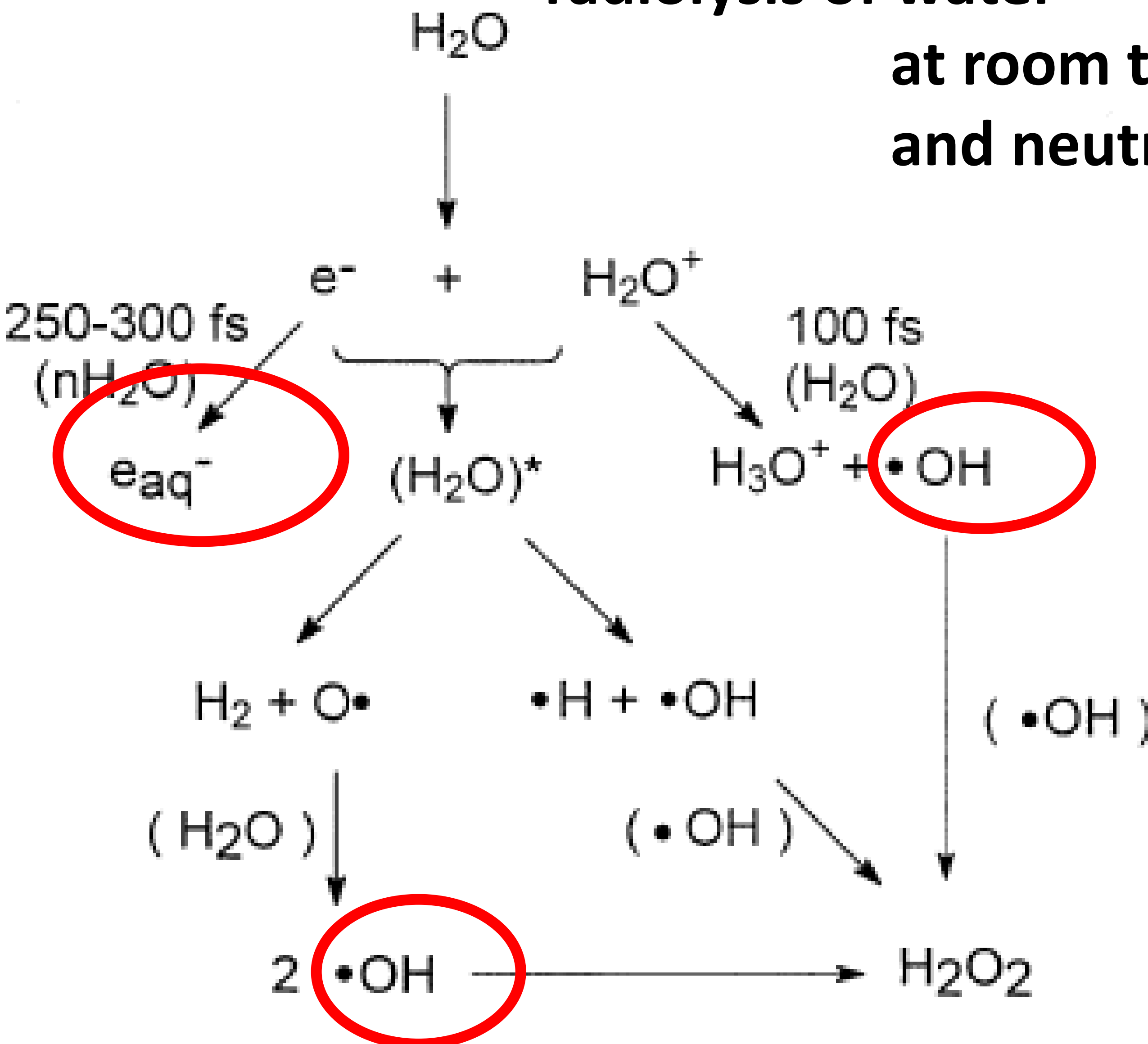
Shelley & Garman, Acta D (2024) 80

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)

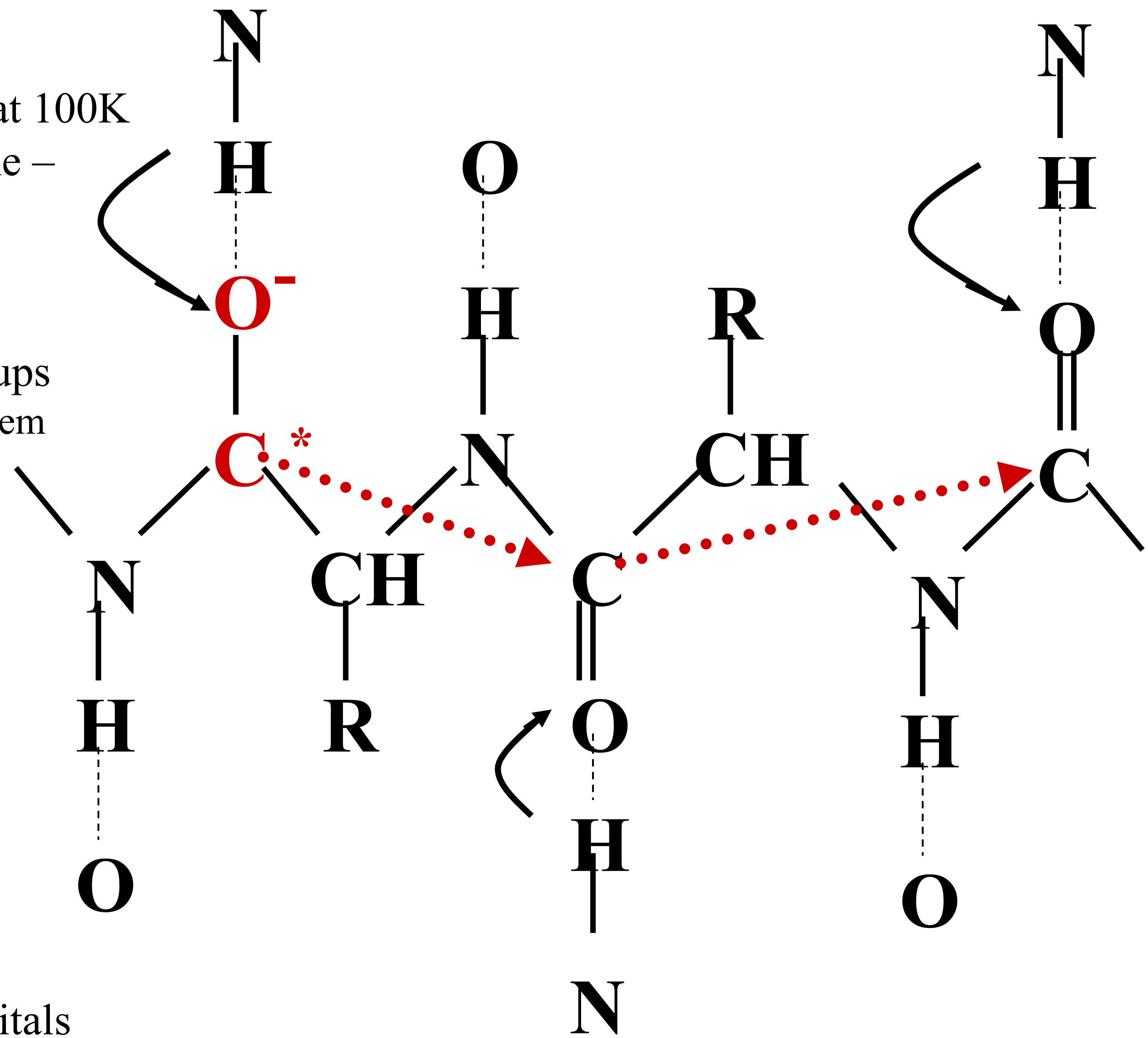
Hiroki, A. Pimblott, S. M. LaVerne, J. A. (2002 *)* *J Phys Chem A* **106**, 9352-9358

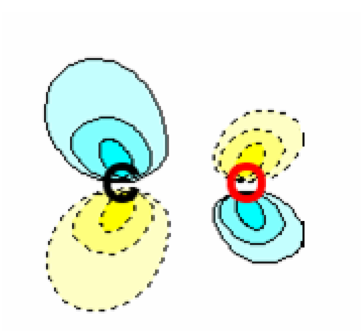
2) DIRECT RADIATION DAMAGE. Protein Redox

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.





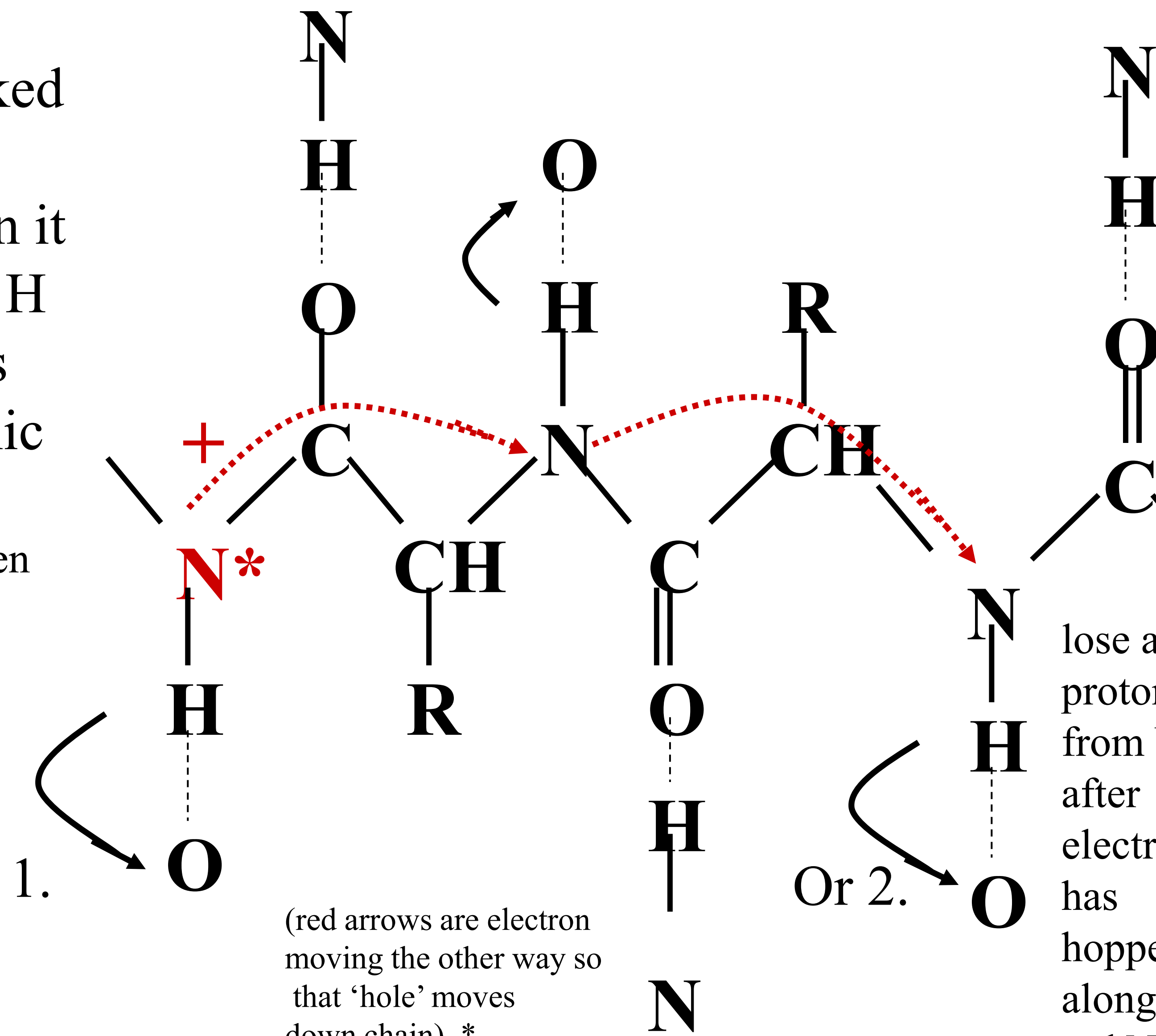
π* orbitals

2) **DIRECT RADIATION DAMAGE.** Protein Redox-

b) apparent hole (lack of electron) migration.

Electron gets knocked off the N, giving a radical, N* and then it pulls electron from H to give H+ which is lost from the cationic site.

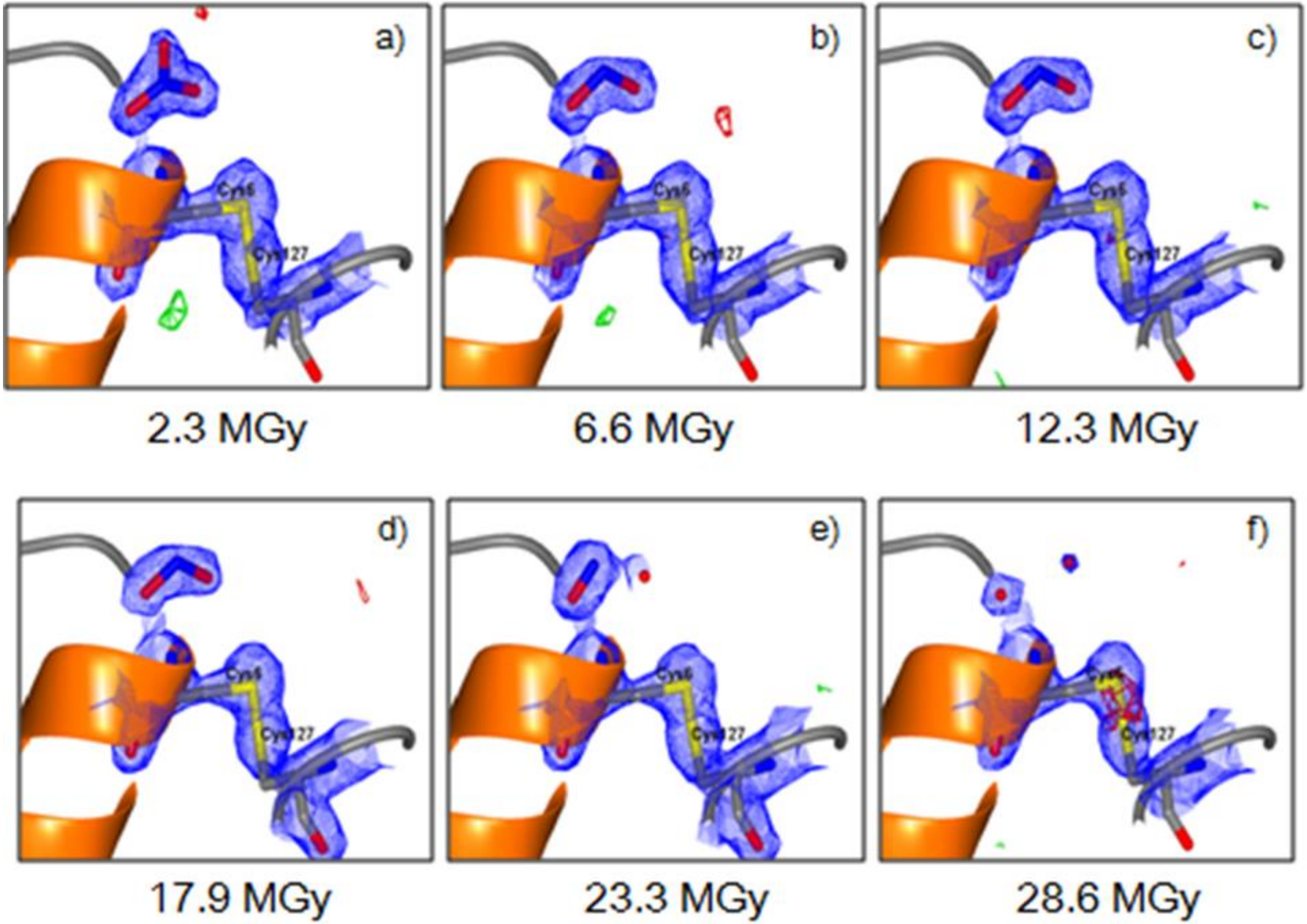
e.g. could add to an oxygen from water.
(Deprotonation = loss of H+ (proton)).



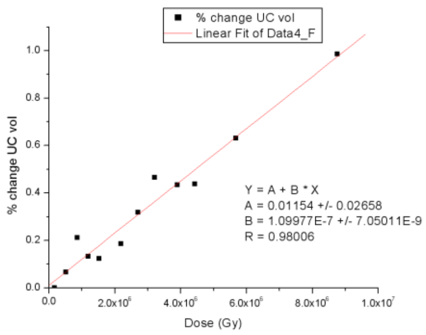
(red arrows are electron moving the other way so that 'hole' moves down chain). *

lose an H+ proton from N after electron has hopped along to red N*

Radiation Chemistry in action: Nitrate scavenger

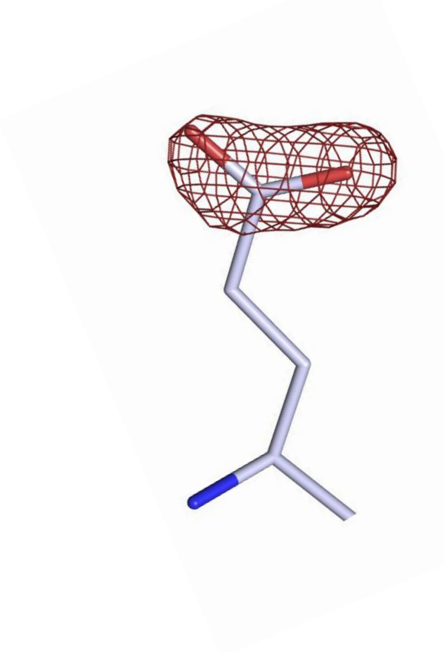


De la Mora, Carmichael, Garman *JSR* (2011) 18, 313



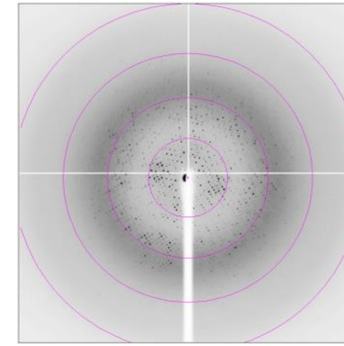
Radiation Damage

The Plan:



- Why cool: radiation damage
- What are the symptoms?
- What is it?
- **Why do we care? Effect on MAD/SAD.**
- How do we estimate the Dose?
- The limits
- What can you do to minimise it?

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

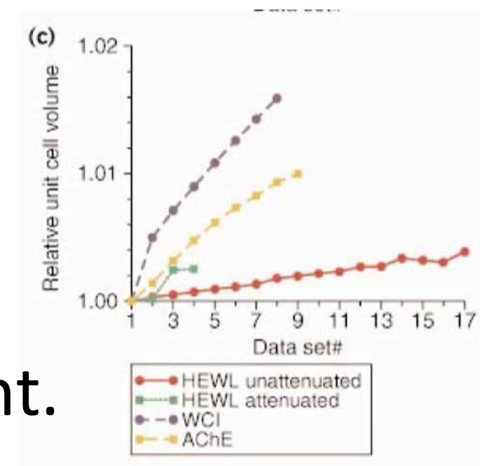
(Multi-wavelength anomalous dispersion MAD, SAD)

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**

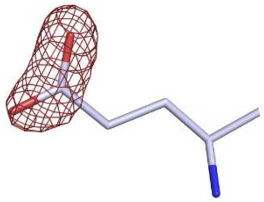
[Crick and Magdoff (1956) Acta Cryst **9**, 901-908]

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)]

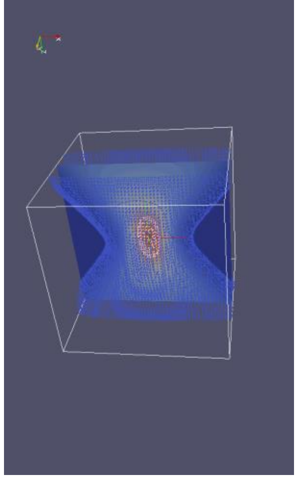
- Decarboxylation of Glu is sometimes part of the protein mechanism, but is indistinguishable from radiation damage at the synchrotron



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

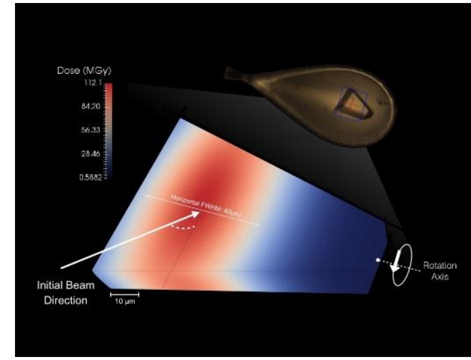
Takeda et al, Crystal structure of the M intermediate of bacteriorhodopsin…JMB (2004),

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



Radiation Damage

The Plan:

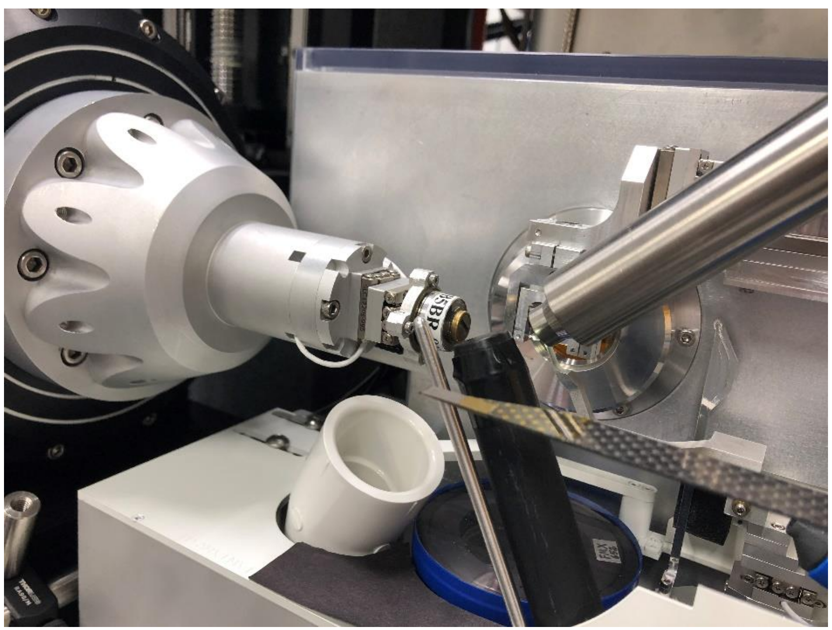


- Why cool: radiation damage
- What are the symptoms?
- What is it?
- Why do we care? Effect on MAD/SAD.
- **How do we estimate the Dose?**
- The limits
- What can you do to minimise it?

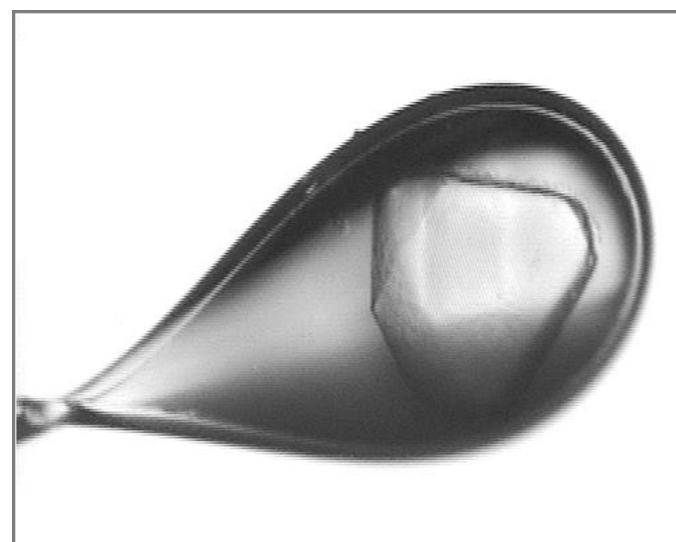
Make the dose calculation convenient for MX (include solvent contribution in mM and heavy atoms explicitly)

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

The beam

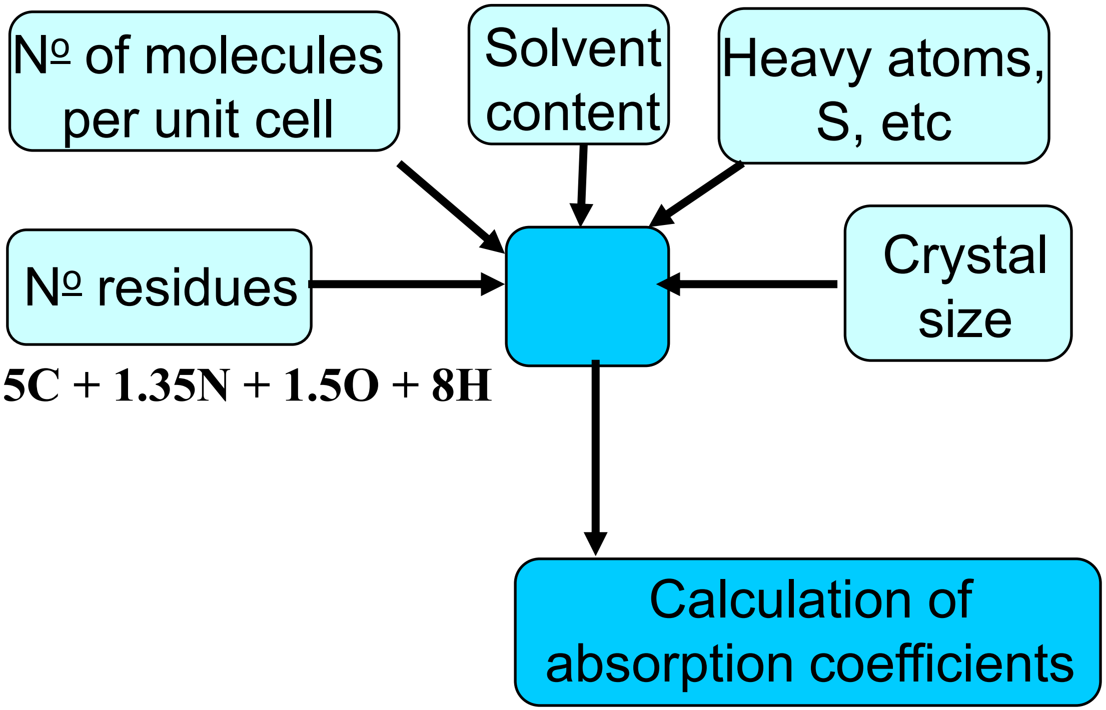


The crystal

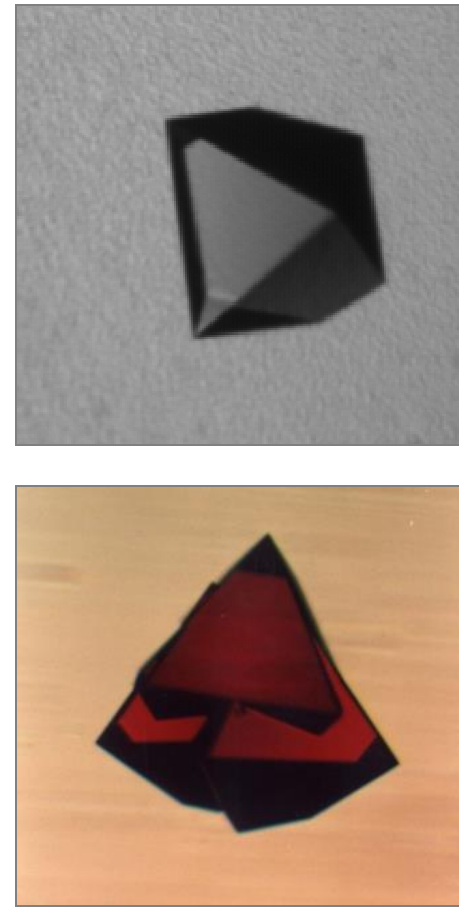


Calculating Dose
(*RADDOSE-3D*)

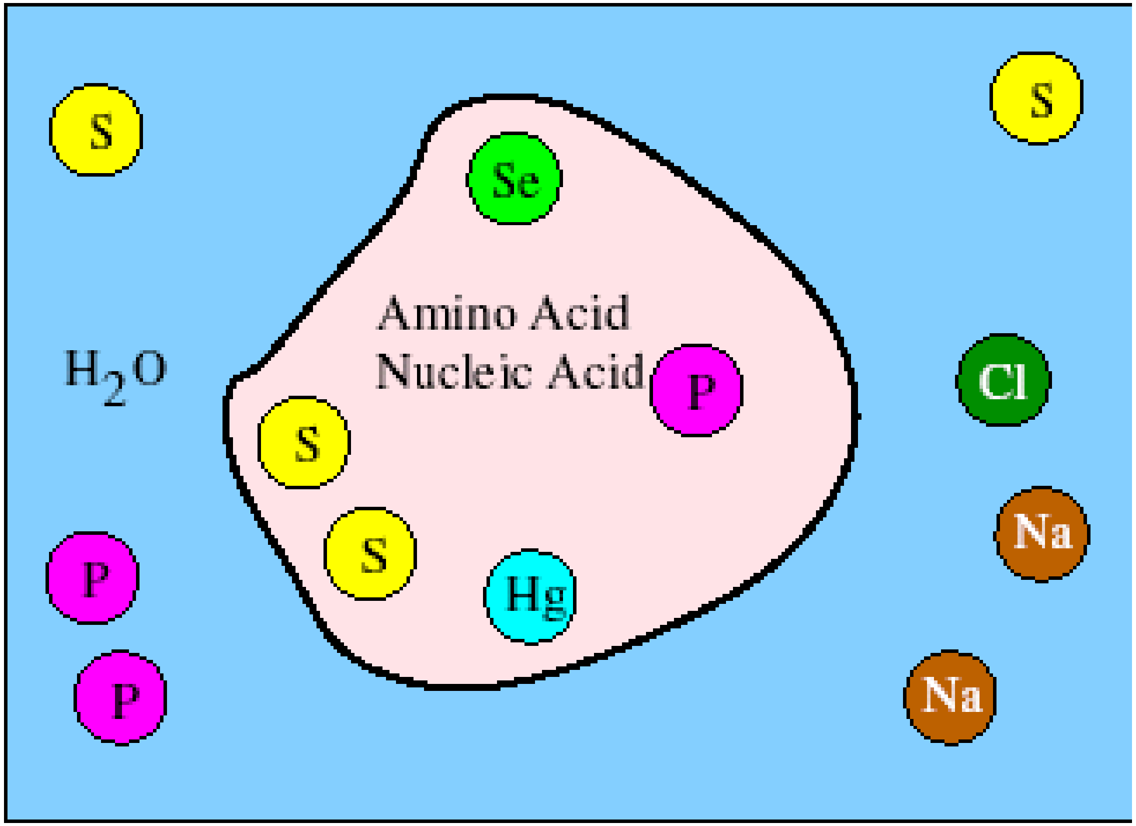
Crystal Characteristics



absorption coefficients
e.g. apoferritin: $0.406 mm^{-1}$
holoferritin: $1.133 mm^{-1}$



200 µm



Number of Amino Acids

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

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

Dose doubling concentrations

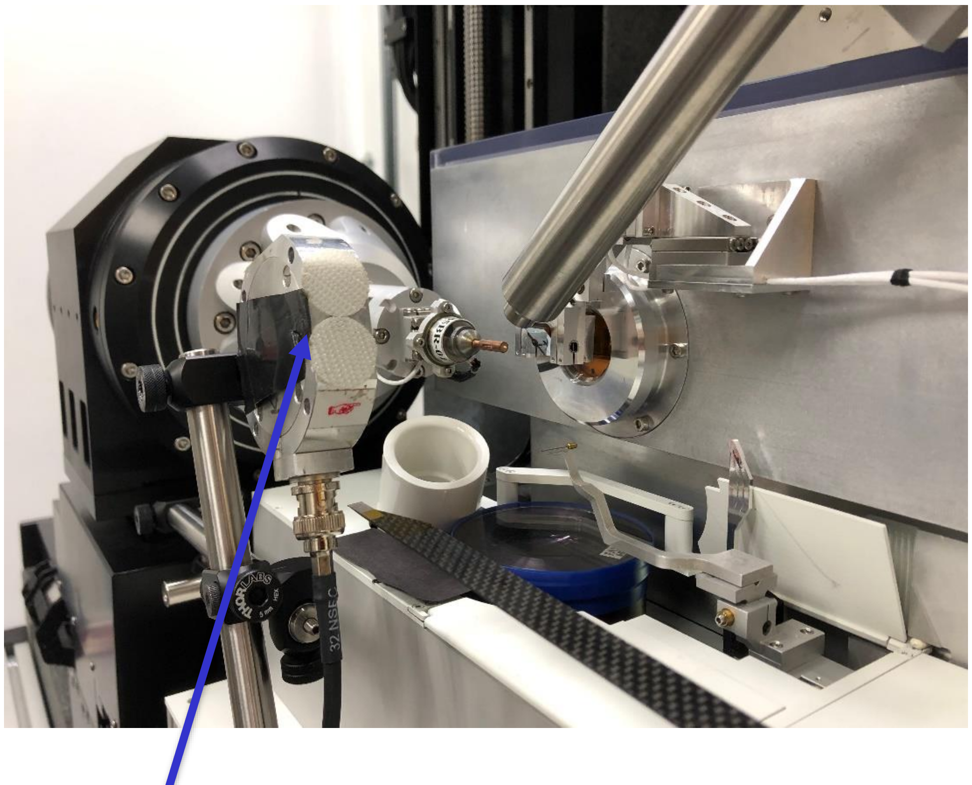
at 12.680 keV/0.9793 Å (Se edge)

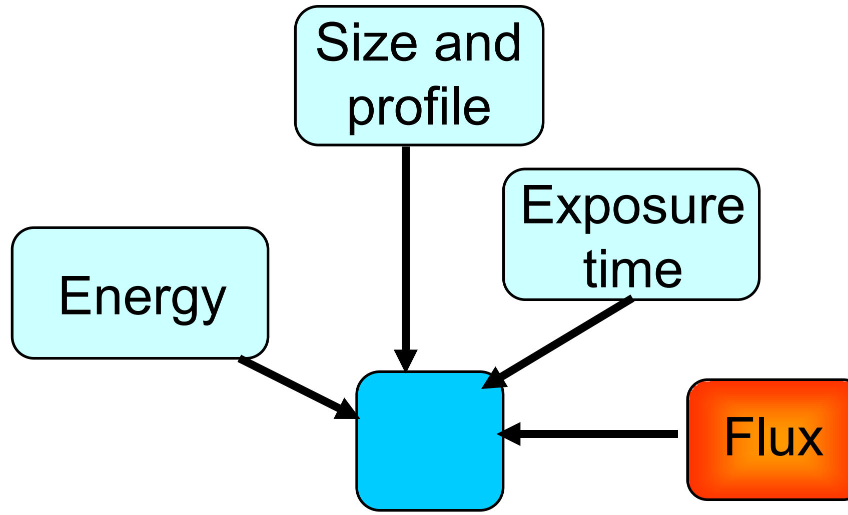
[Holton J. Synchrotron Rad. (2009) 16, 133–142]

using RADDOSE

Na	19 M
Mg	12 M
P	4 M
S	3 M
Cl	2.5 M
K	1.6 M
Ca	1.3 M
Fe	560 mM
Cu	430 mM
Zn	400 mM
As	350 mM
Se	340 mM
Br*	1.2 M (drops to 320 mM at 13.486 keV
I	230 mM
Gd	110 mM
Ta	75 mM
Pt	100 mM
Au	100 mM
Hg	88 mM
U	100 mM

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





PIN diode to measure flux (ph/s)

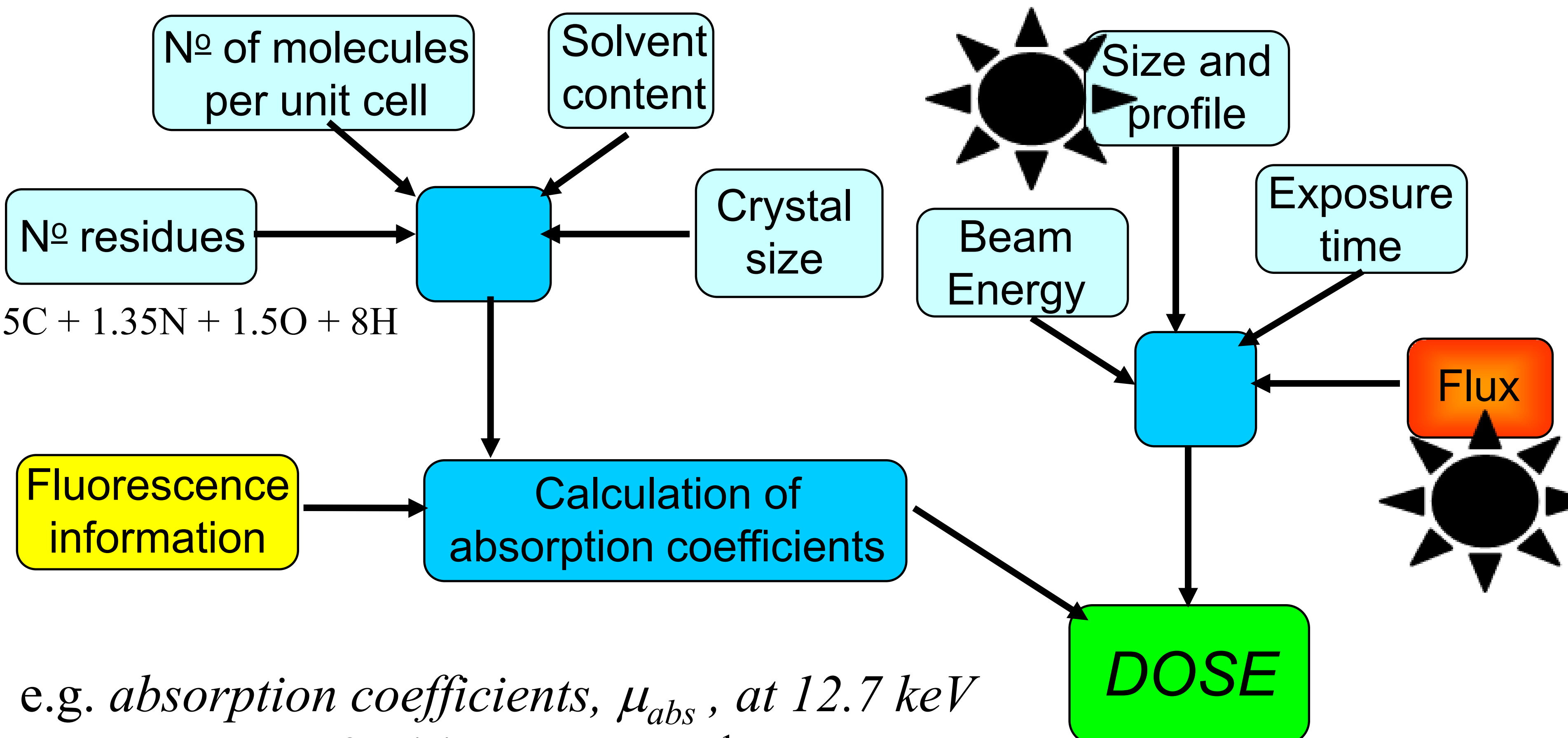
Owen et al JSR 2009

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*

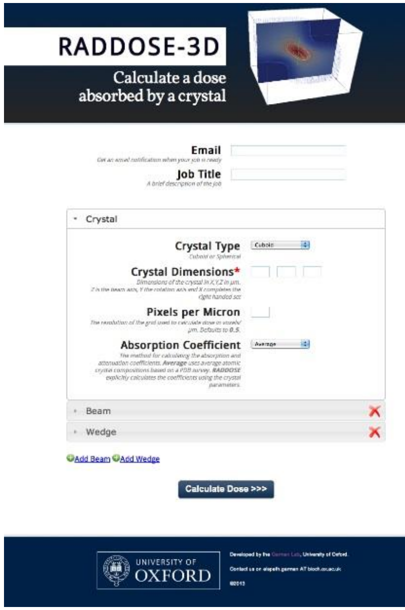
apoferritin: 0.406mm^{-1}

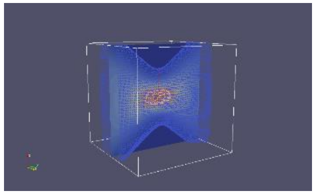
holoferritin: 1.133mm^{-1}

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)]

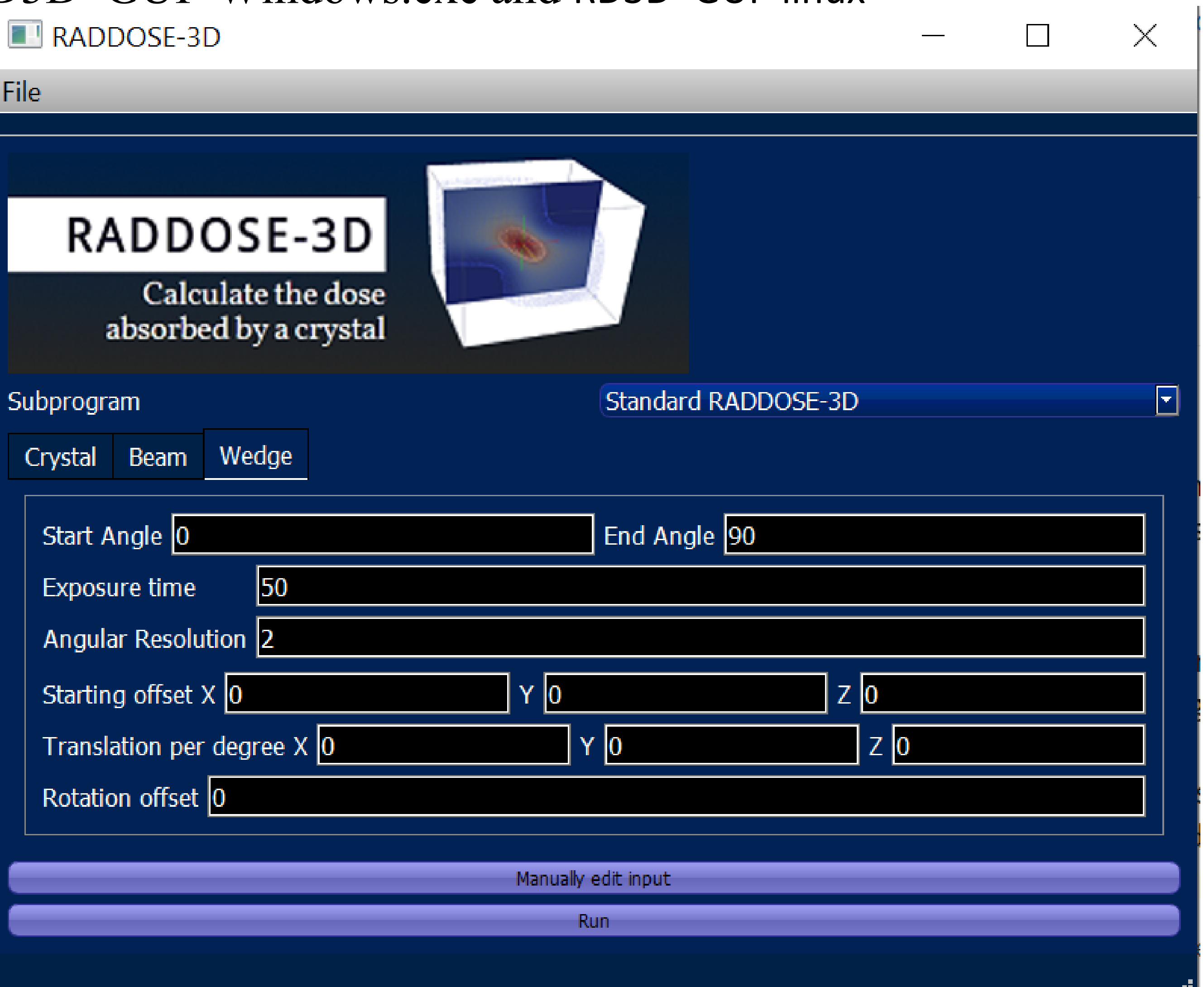
RADDOSE-3D New GUI (tutorial at end of this PDF)

Download from: //https://github.com/GarmanGroup/RADDOSE-3D

Versions for a PC and for Linux, no version for a Mac:

RD3D GUI Windows.exe and RD3D GUI linux

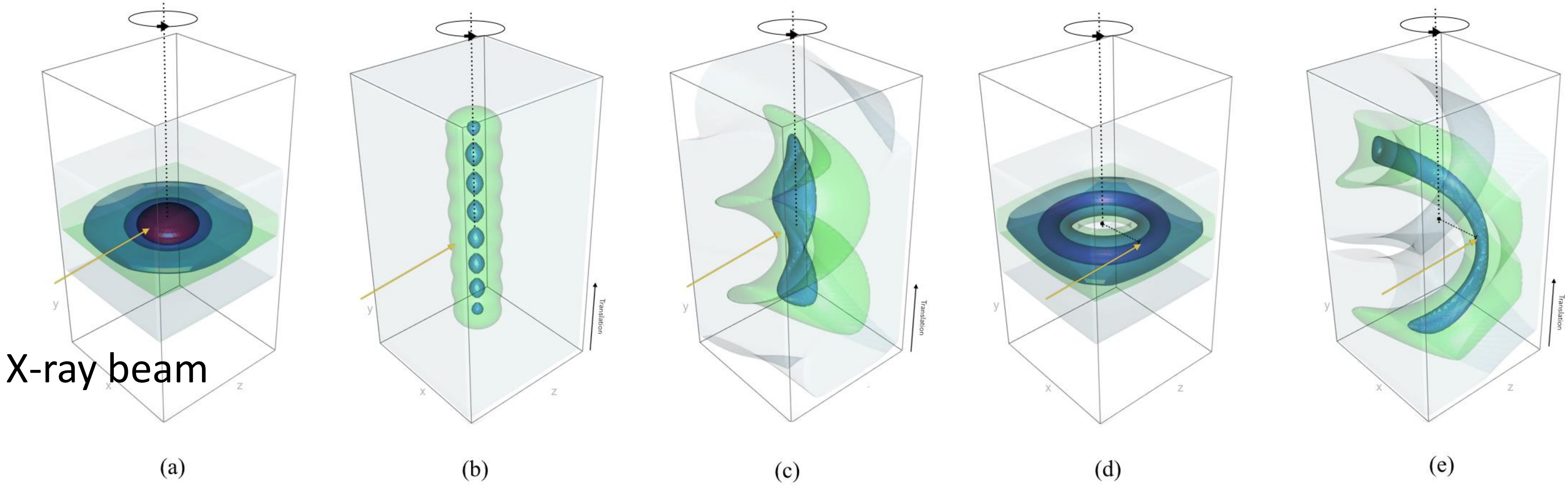
ex



Josh Dickerson

Dickerson *et al* Protein Science (2024) 33.e5005

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)

James Holton's lifetime calculator
https://bl831.als.lbl.gov/xtallife.html

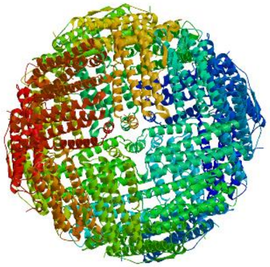
expected crystal lifetime calculator

source =	ALS	8.3.1			
full flux =	1.0e+12 photons/s				
attenuation =	0 %	transmittance =	100 %		
beam $size_{horiz}$ =	61.0 microns	beam $size_{vert}$ =	80.0 microns		
wavelength =	1.1159 Ang	k_{dose} =	1600 photons/micron^2/Gy		
dose rate =	1.3e+5 Gy/s				
experiment goal =	high resolution (cryo)				
resolution =	3 Ang				
dose limit =	30 MGy				
exposure time =	1 seconds/image				
xtal $size_{horiz}$ =	50 microns	xtal $size_{vert}$ =	50 microns	xtal $size_{thick}$ =	50 microns
translation during dataset =	0 microns	rotisserie factor	1	disable warnings	
max images =	234 at damage limit				
inverse beam =	no				
number of wavelengths =	1				
images/wedge =	234				

Instructions:

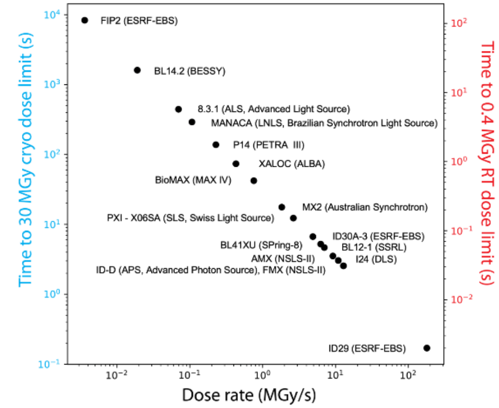
The "horiz" direction is along the spindle axis, "thick" is the direction along the beam path, and "vert" is generally the direction of gravity at a synchrotron.

JM is not using Raddose-3D underneath at ALS 8.3.1. He just uses an equation, as in the web calculator. This is a much more simplistic approach.



Radiation Damage

The Plan:



- Why cool: radiation damage
- What are the symptoms?
- What is it?
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- How do we estimate the Dose?
- **The limits**
- What can you do to minimise it?

DOSE Postulate (Henderson 1990):

- Henderson: postulated a MAXIMUM dose for MX of 20 MGy derived from electron diffraction observations, that protein crystals can tolerate: depends only on the PHYSICS of the situation.

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

Experimental: 43 MGy to $I_{0.5}$ at 2.4 Å

BUT shouldn't go lower than $I_{0.7}$, 30 MGy* (specific damage)

- 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*

V4: Dickerson and Garman (2024) *Protein Science*

Typical MX experiment & ‘limits’

1 MGy/s absorbed by a 100 μm cubed metal free crystal in a 100×100 μm^2 beam of 12.4 keV (1 Å)
X-ray flux: 10^{13} photons s^{-1}

MX at synchrotrons RT:
~0.4 MGy experimental dose ‘limit’
(dose to 0.5 I_n/I_1) reached in ~0.4 s

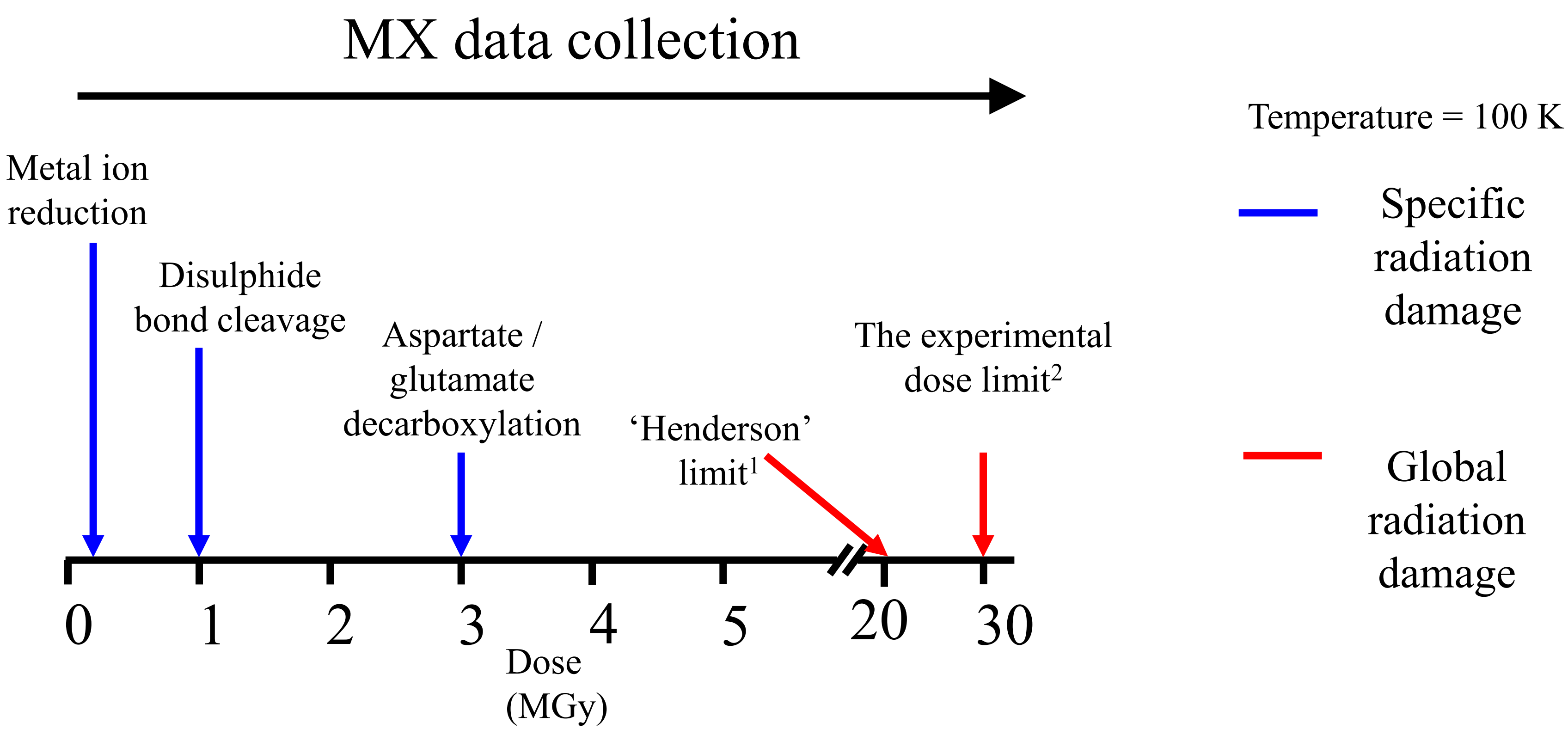
MX at synchrotrons 100 K:
30 MGy experimental dose ‘limit’
(dose to 0.7 I_n/I_1) reached in ~30 s:
4th generations sources <<1s,

MX at XFELs: damage before destruction ?
<80 fs

2-10 Gy: rodent dies in 10-30 days.
Radiotherapy for human brain tumour max 60 Gy.

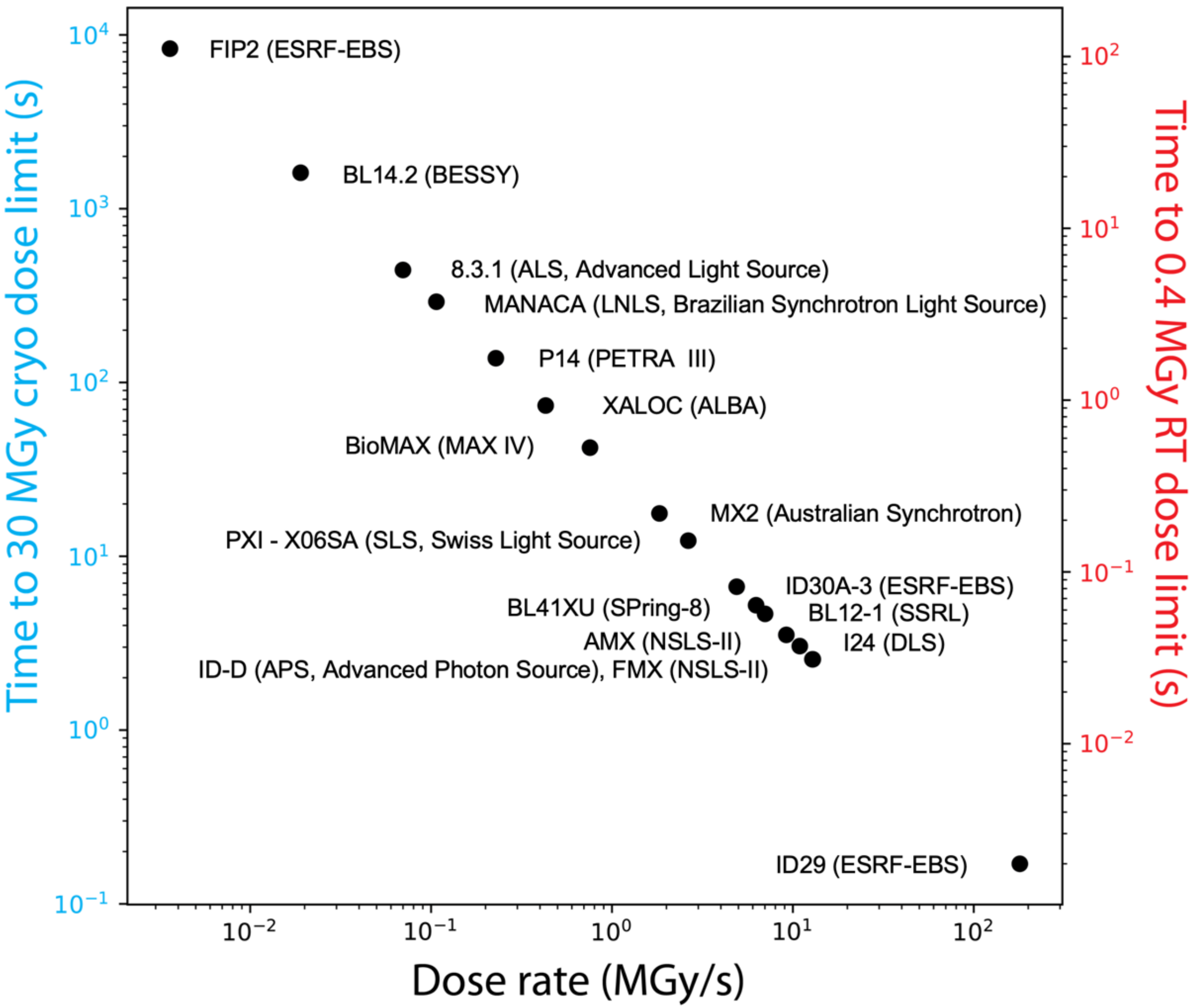
[Shelley & Garman Acta D 2024]

- At 100 K, specific damage effects onset before global damage
- Both classes of damage common in protein crystallography (MX)



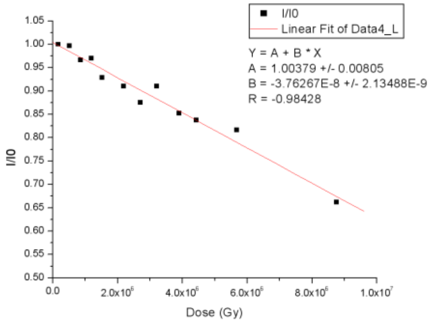
[1. Henderson Phil Trans RS B 1990 2. Owen *et al* PNAS 2006]

Time to dose limits at current synchrotrons

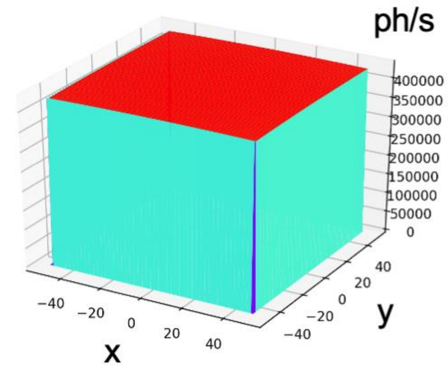


Average diffraction weighted doses (DWD) were calculated for a (50 μm)^3 lysozyme crystal (grown in 100 mM NaAc and 1M NaCl, solvent fraction 38%) rotated 360° in the flux within the FWHMs of the various beams.

Garman & Weik COSB (2023)



Radiation Damage



The Plan:

- Why cool: radiation damage
- What are the symptoms?
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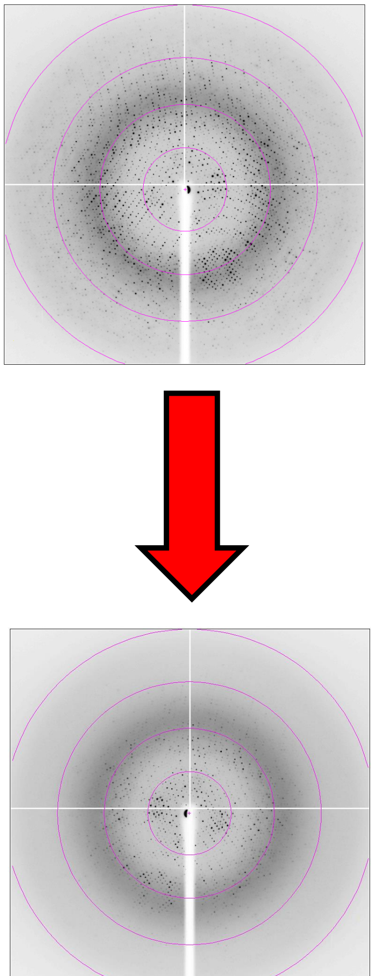
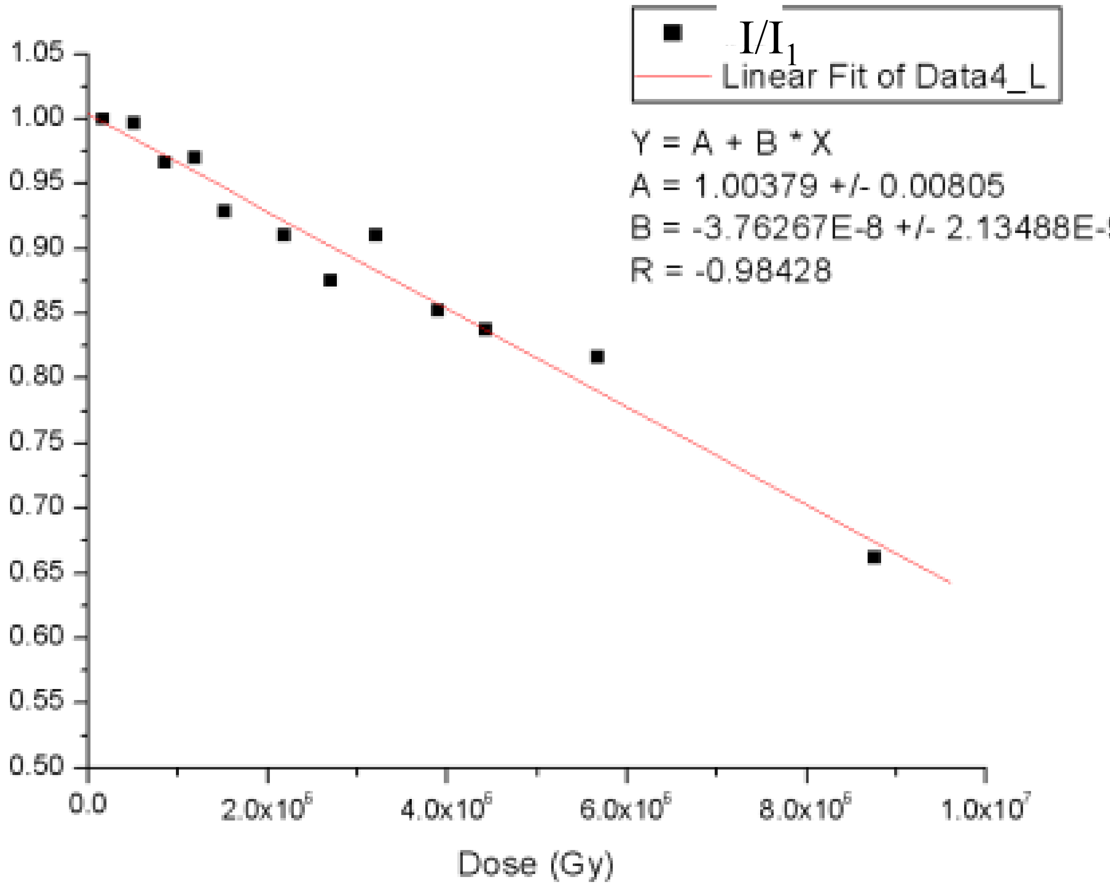
- How do we estimate the Dose?
- The limits
- **What can you do to minimise it?**

1) Sacrificial crystal to characterise damage rate

Diffraction fading



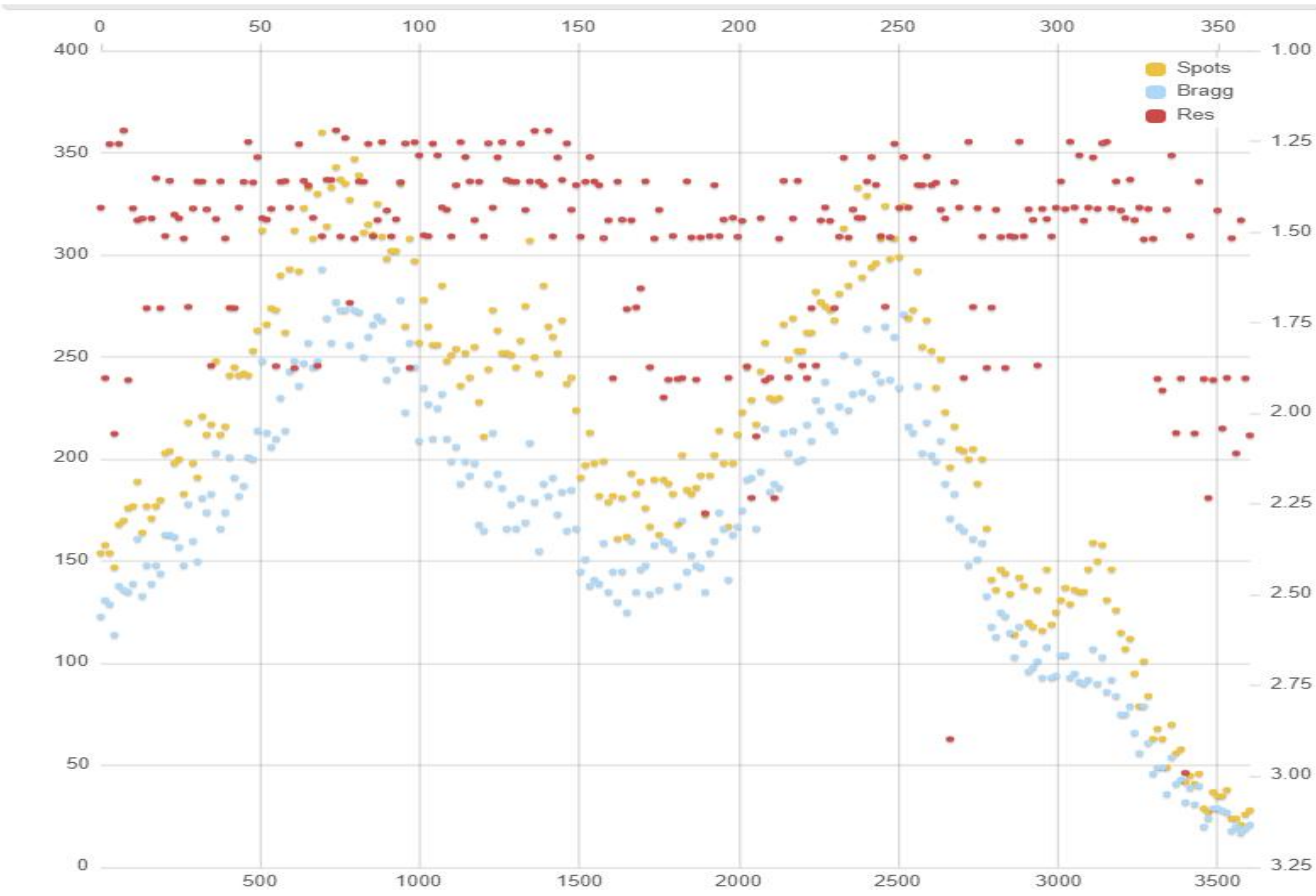
I/I_1

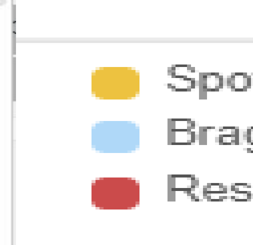


Dose = absorbed energy (J) / mass (kg)

2) During the experiment:

- Very hard to see spot fading by eye on Eiger detector as all reflections are partials. Crystal usually diffracts further out than you can see.
- Eiger has zero dark current background.

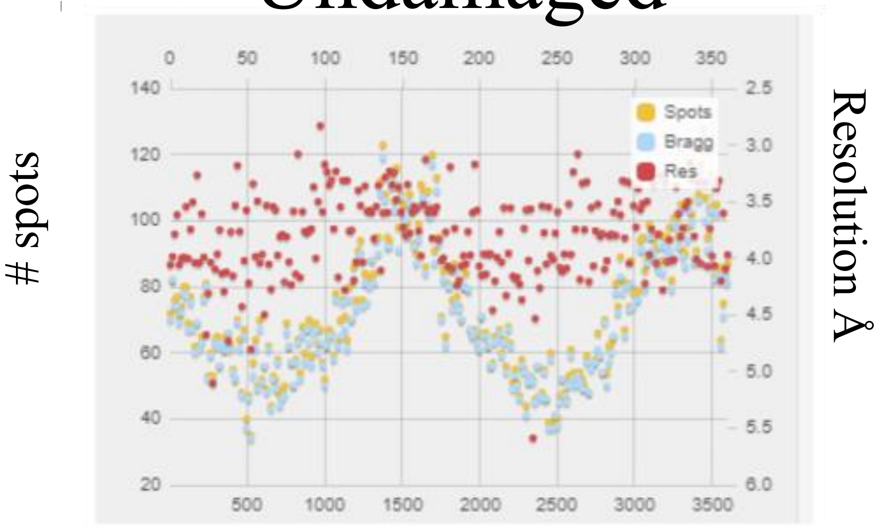




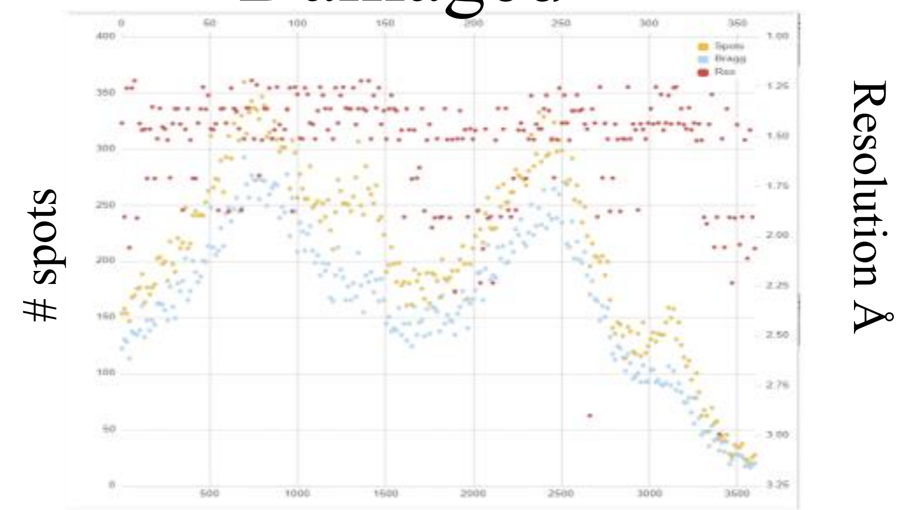
DLS
Ed Lowe

RadDam signatures in reciprocal space

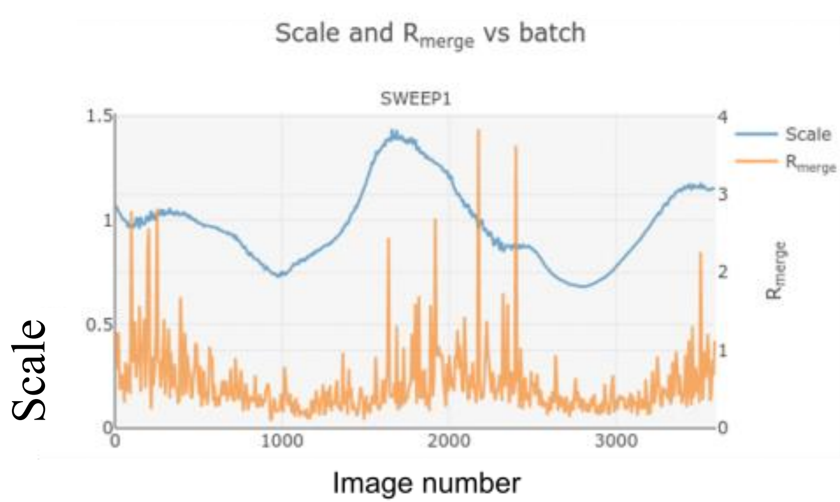
Undamaged

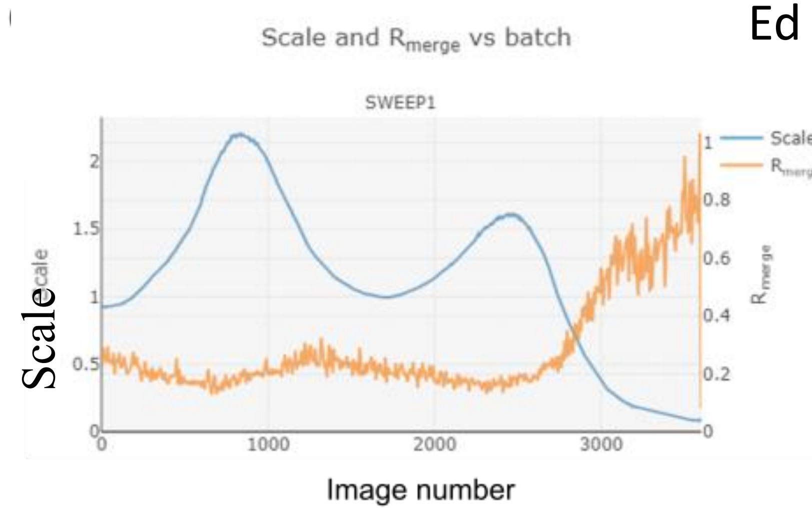


Damaged



DLS
Ed Lowe





Shelley & Garman, Acta Cryst D (2024)

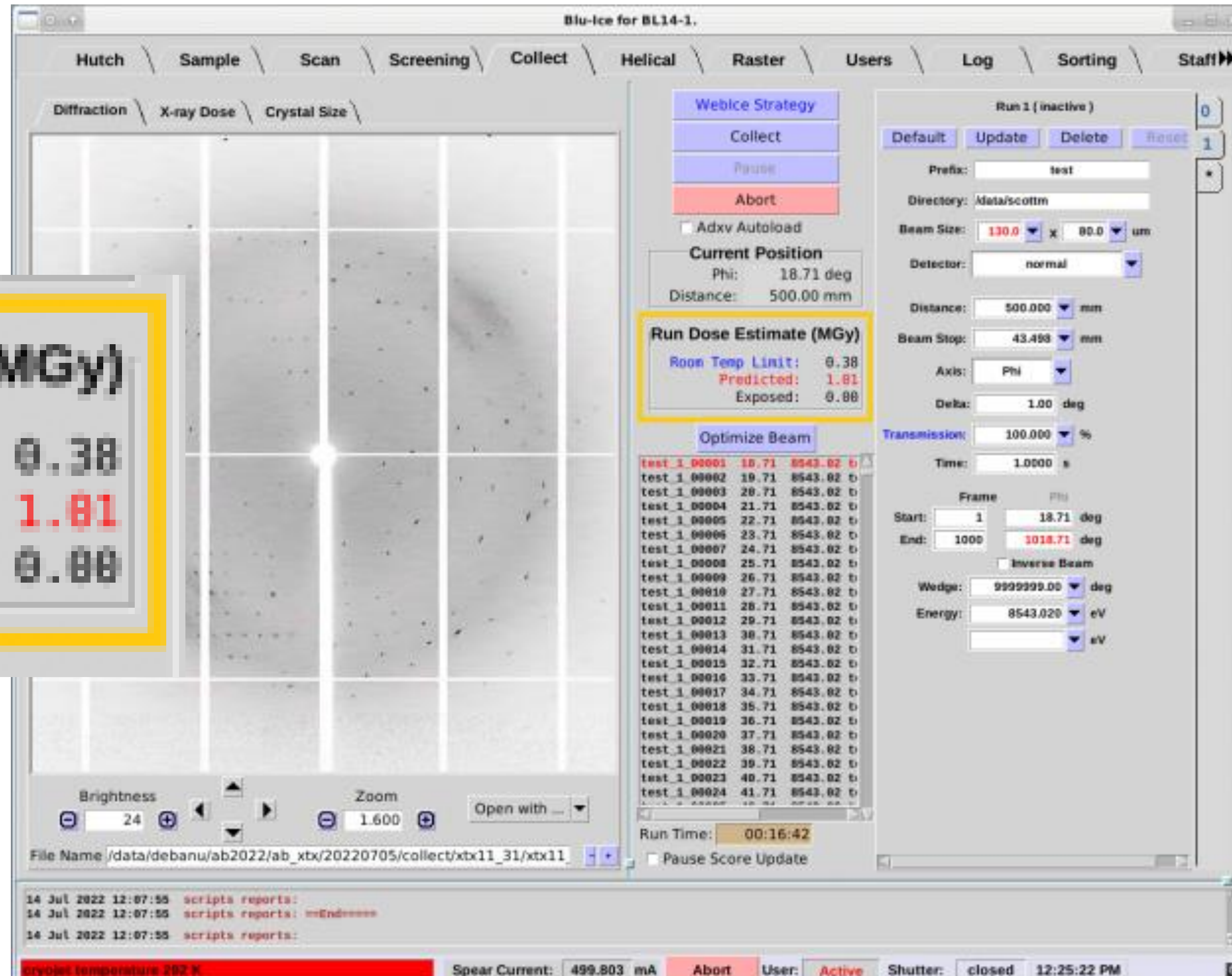
3) DOSE AWARE data collection

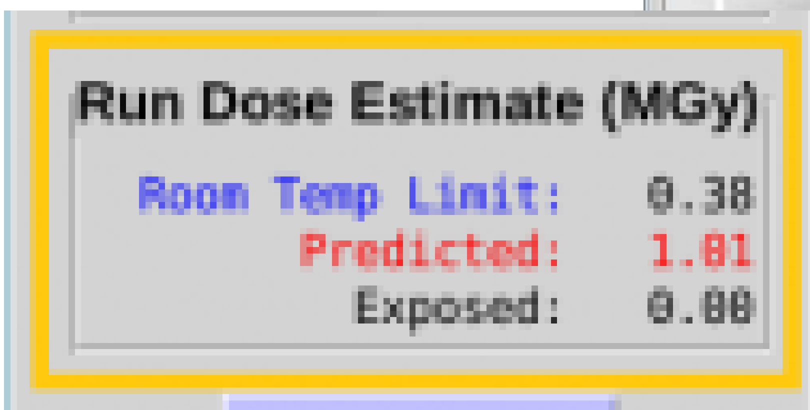
Some beamlines worldwide are running RADDOSE-3D 'underneath' to allow collection by DOSE, not TIME

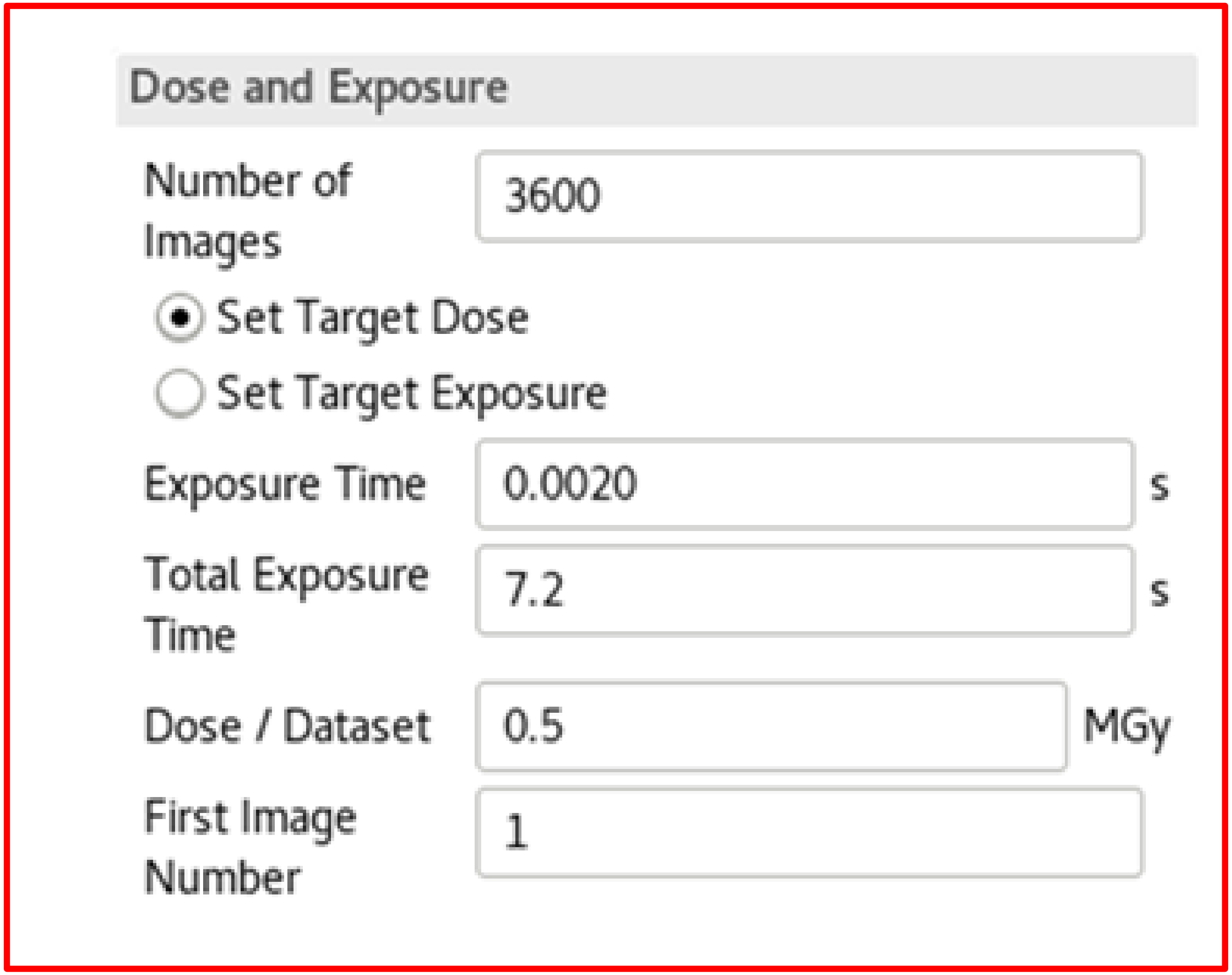
e.g. RADDOSE-3D is now embedded within Blu-Ice at SSRL so runs on all the MX beamlines there.

Also on I04 at DLS

(David Aragao)







Rules of thumb at 100 K (from my colleague Ed Lowe with much experience):
Spread 10 to 15 MGy over 360° to 720°
Max dose: 7 MGy if have 1.4 Å diffraction, can allow more dose for lower resolution
Diminishing returns beyond 15 MGy
AVOID TEMPTATION TO INCREASE THE EXPOSURE TIME or DOSE

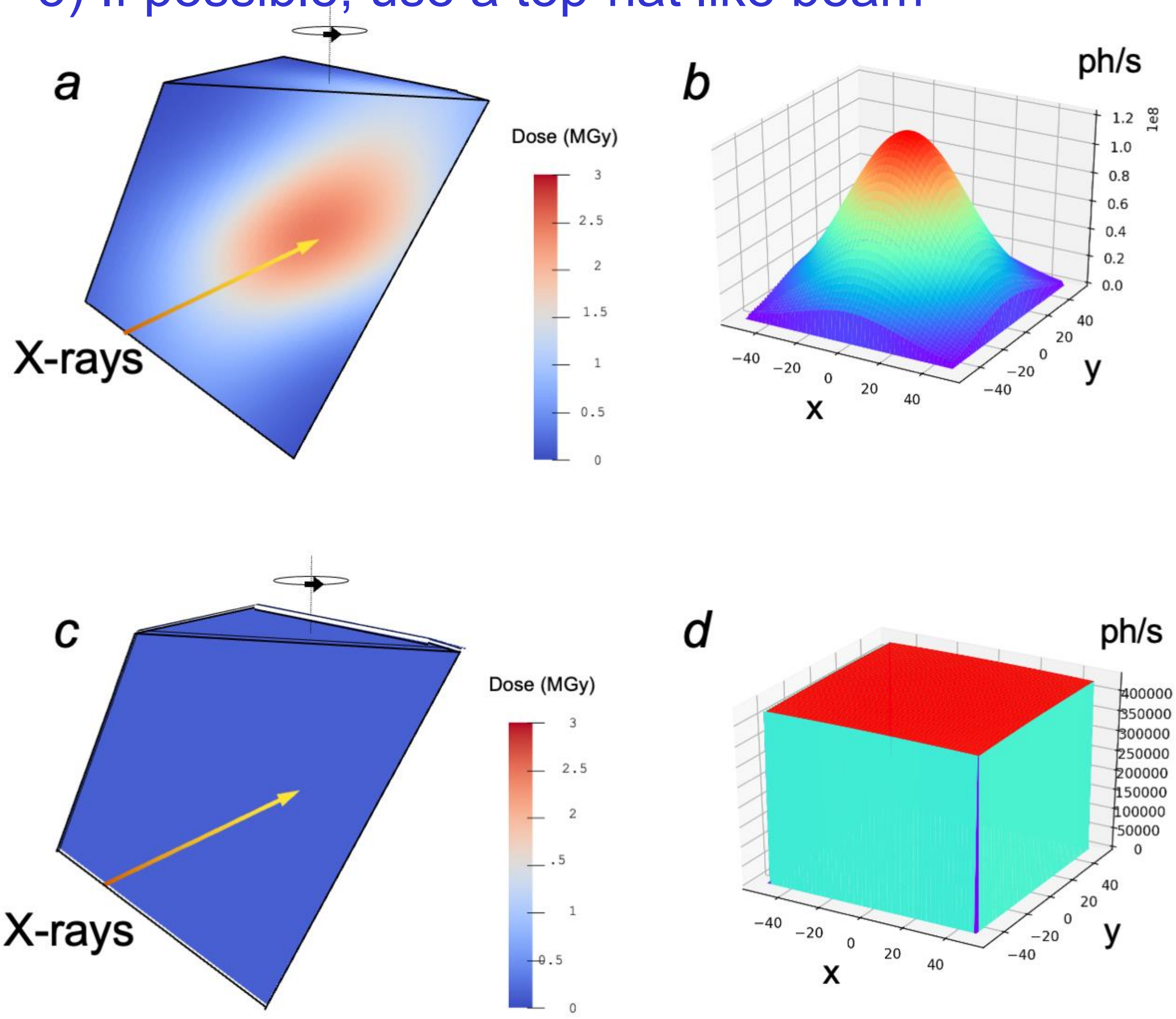
4) BACK SOAK to REMOVE non-specifically bound heavy atoms

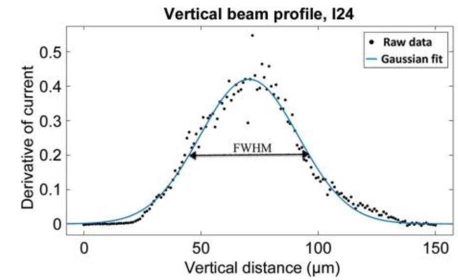
A few heavy atoms in the solvent can make a BIG difference to the absorption cross section and this the dose rate for the SAME flux.

e.g. Cacodylate buffer (arsenic, mass 75 cf selenium = 79) [Tip: avoid cacodylate!]

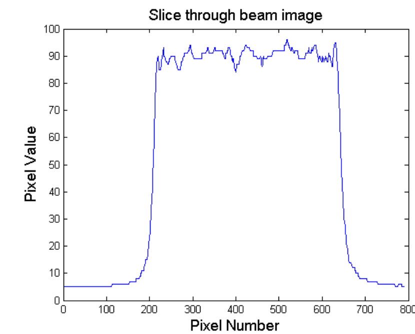
e.g. a brominated DNA-protein complex will radiation damage much faster than a native crystal and will de-brominate during data collection [Ennifar et al, Acta Cryst D (2002) 1263-1268].

5) If possible, use a top-hat like beam





Imaged beam
I24, DLS,
Axford, Owen



Garman & Weik COSB (2023)

Imaged beam
P14, PETRA III,
Bourenkov,
Schneider

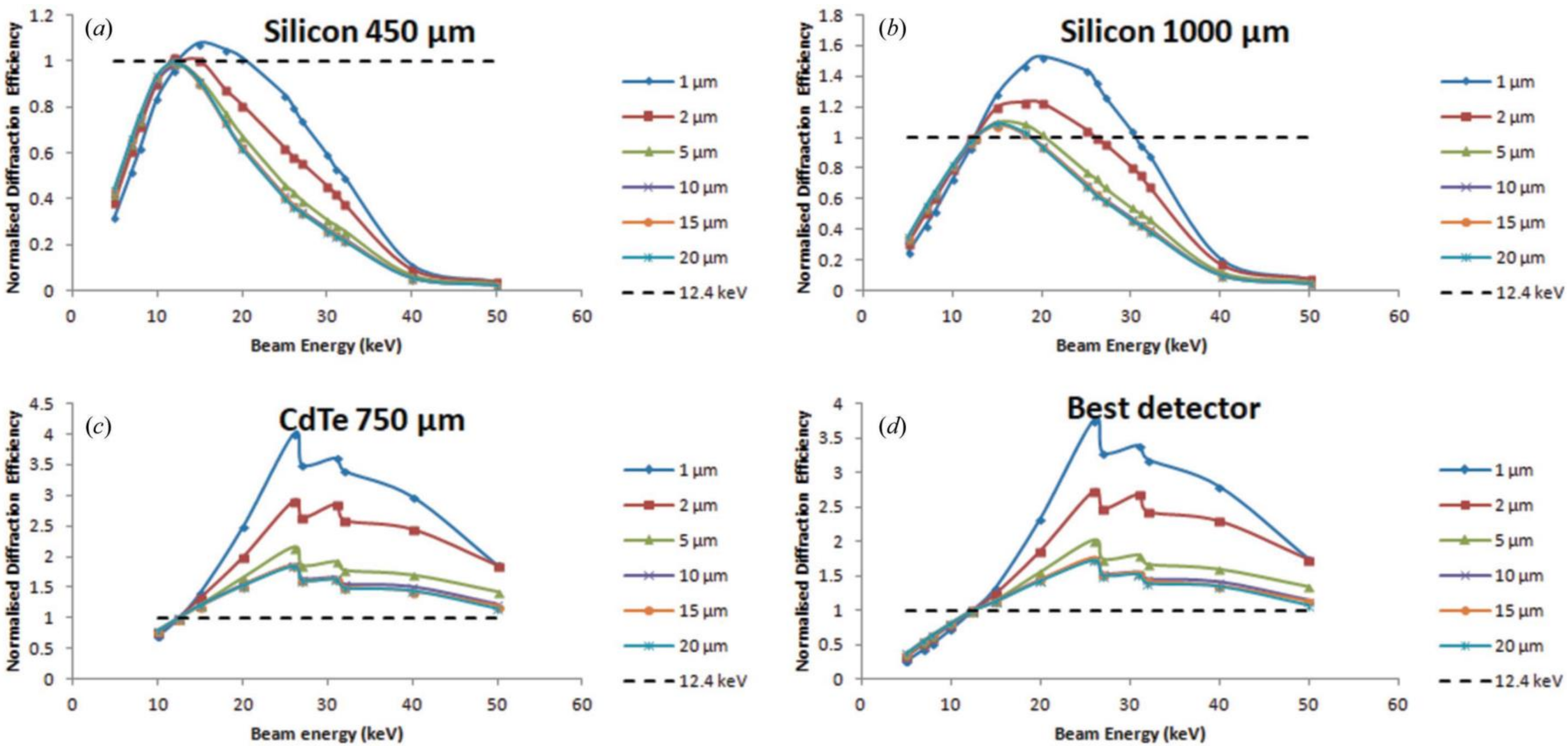
6) For data collection: consider optimising the incident energy

Monte Carlo simulations: CdTe detector, peak in DE@ 26 keV

diffraction efficiency (DE) = diffraction/dose

Photoelectron escape

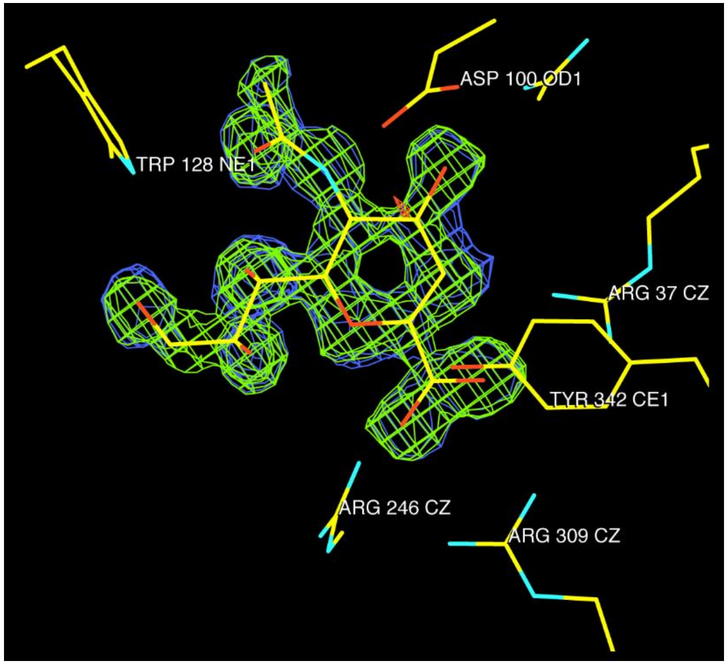
Experimental verification: Storm *et al* IUCrJ 2021



Dickerson & Garman JSR 2019

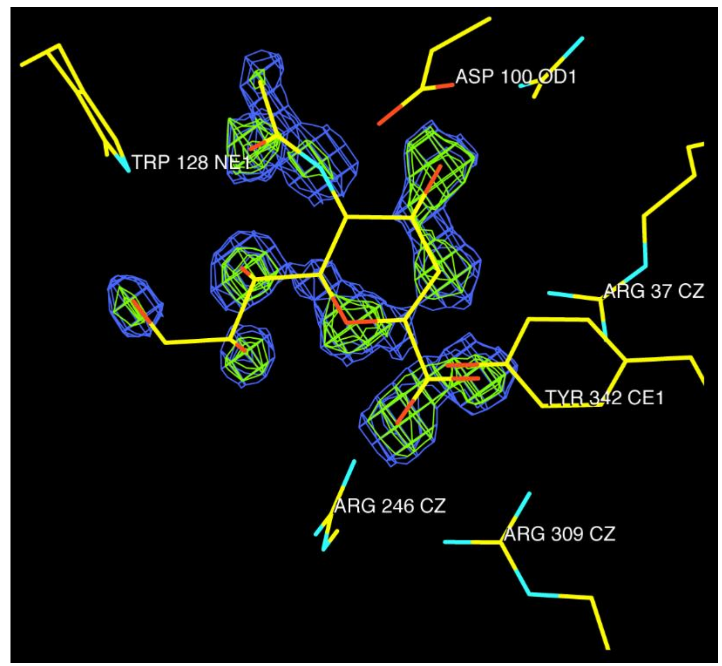
7) Exclude damaged images

High resolution data:



must exclude images showing radiation damage from the dataset

588 images



555 images

(100 K, 1Å resolution)

high, medium, low, high2

Shelley & Garman, Acta Cryst D (2024)

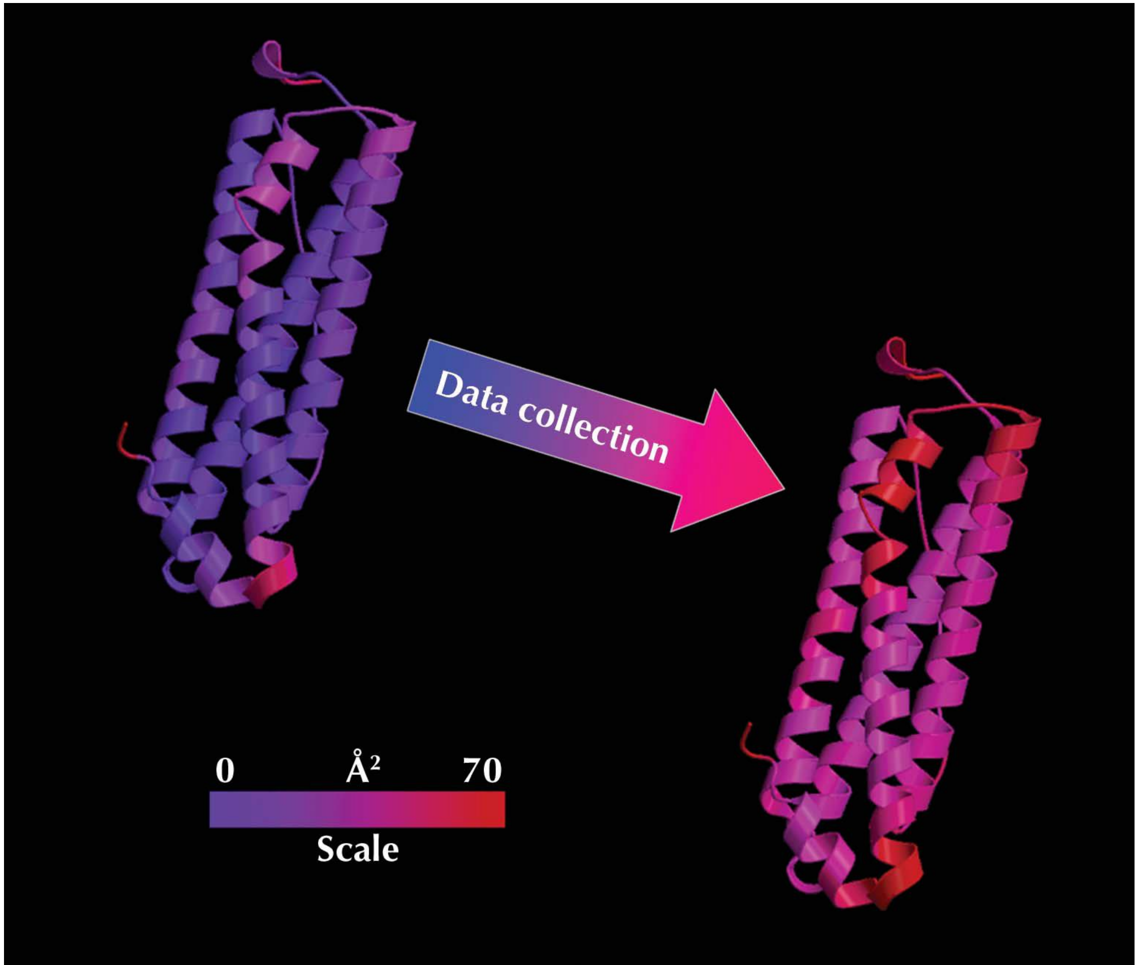
8) During refinement:

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

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

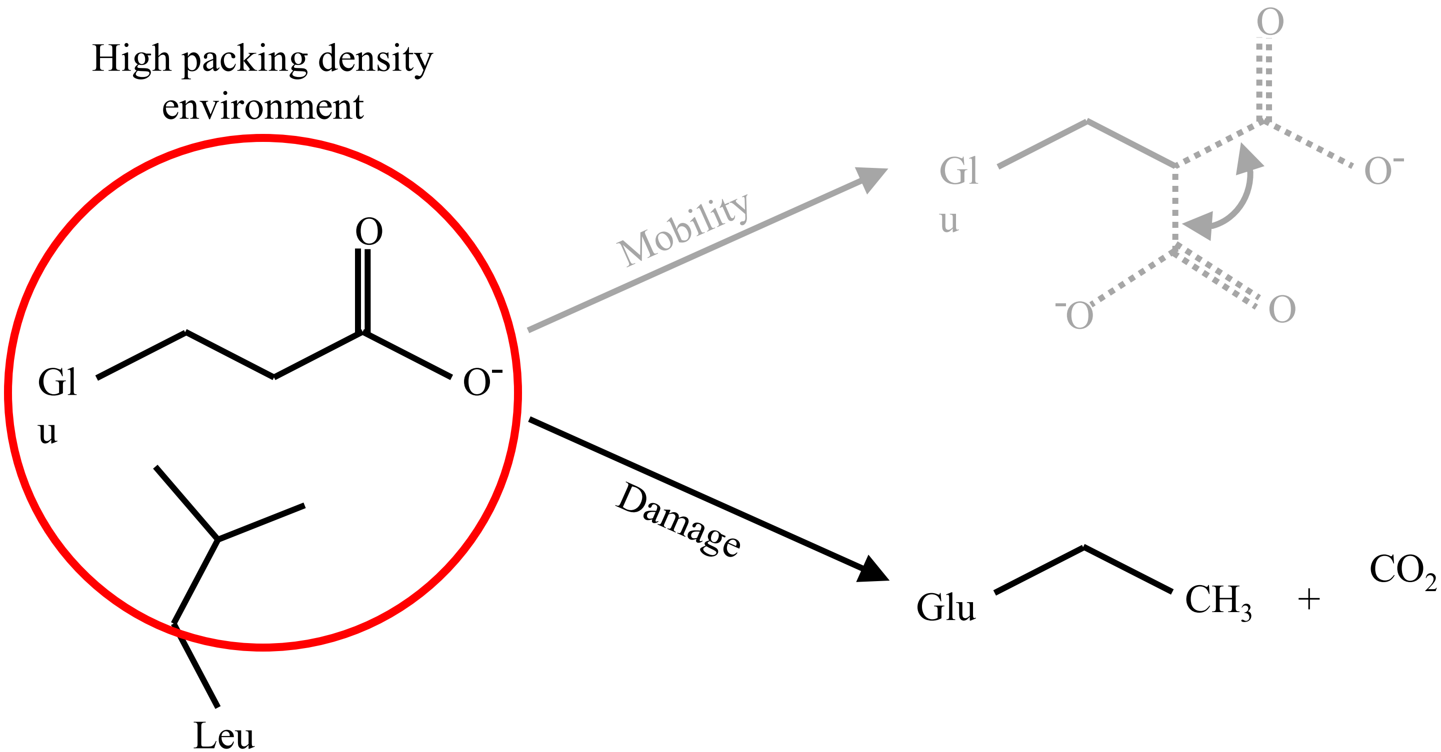
YES!!

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

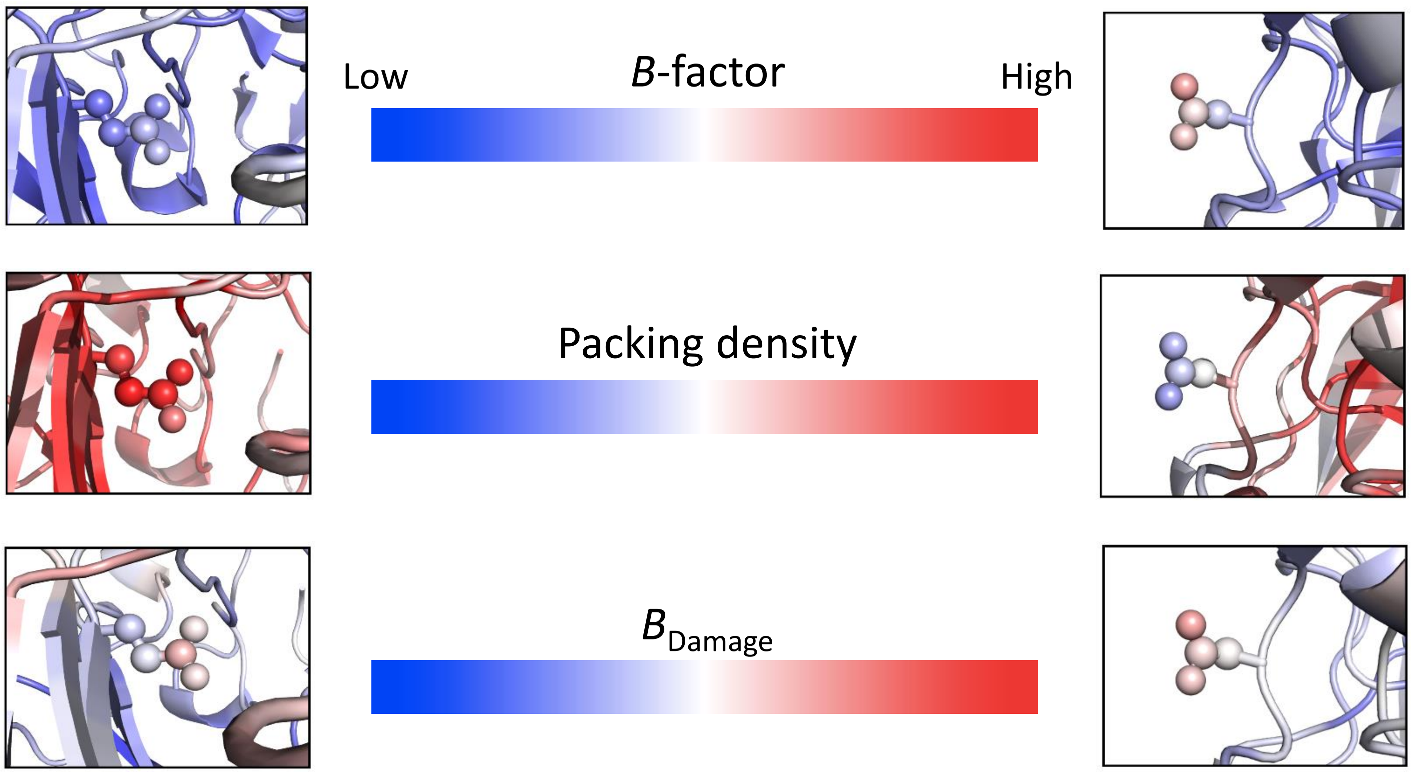
- B_{Damage}[1] is *B*-factor corrected for packing density

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

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

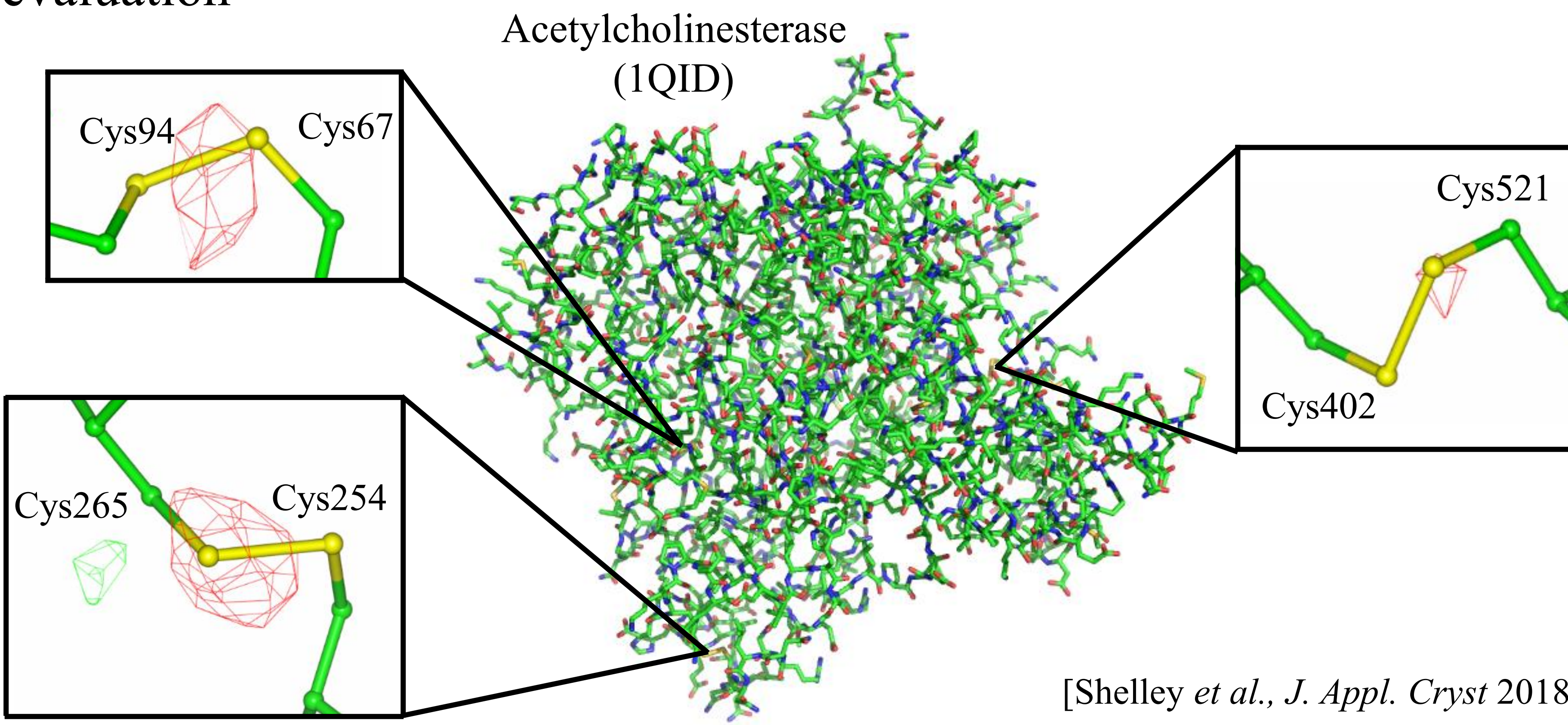
The B_{Damage} metric

- 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



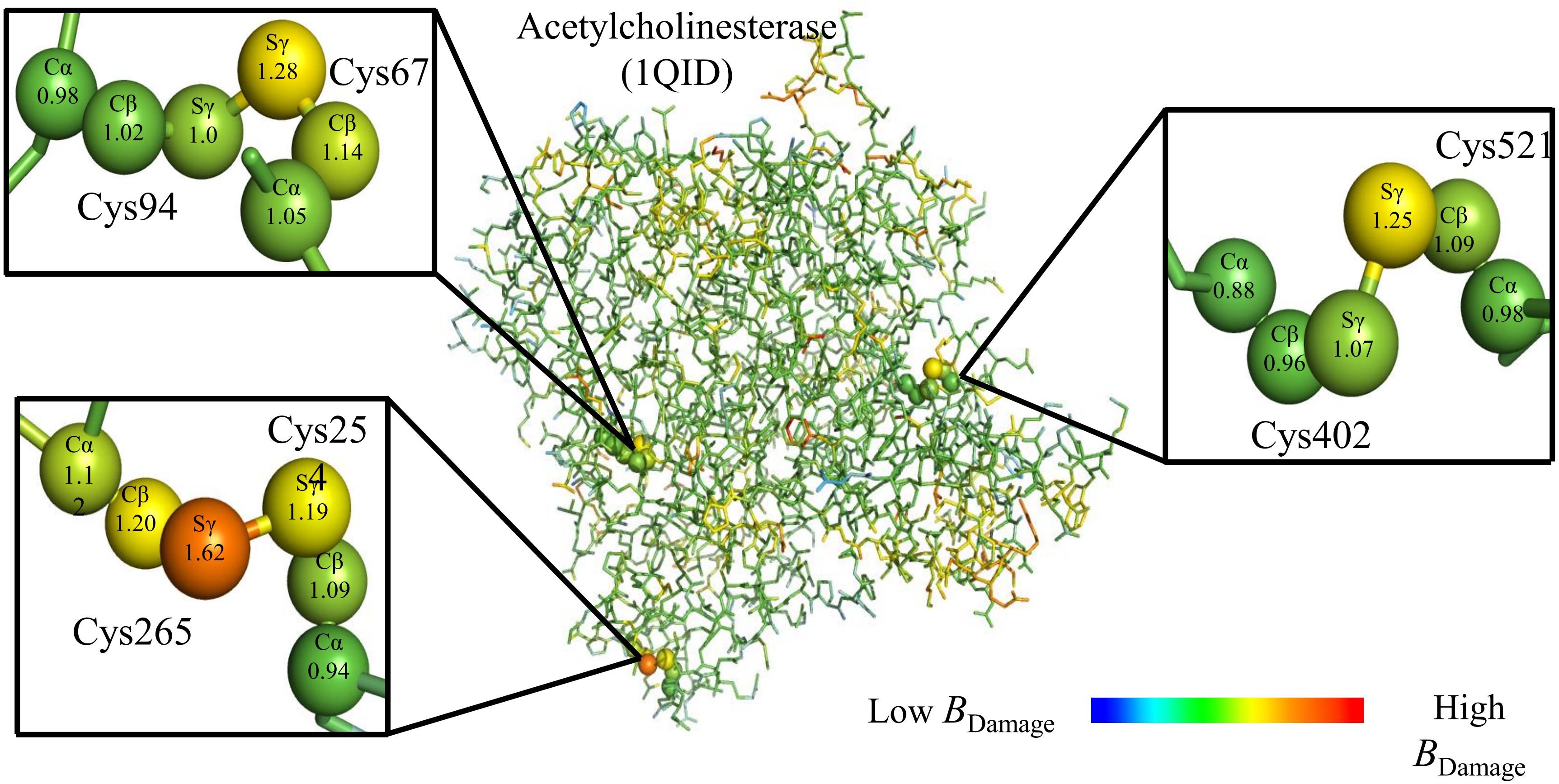
[Shelley *et al.*, *J. Appl. Cryst* 2018]

RABDAM (now in the CCP4 computing suite)

Get a **'radiation damage index,** B_{net}**'** for a 100 K

Use CCP4 program RABDAM. Deconvolutes packing density from *B*-factor to give B_{Damage}

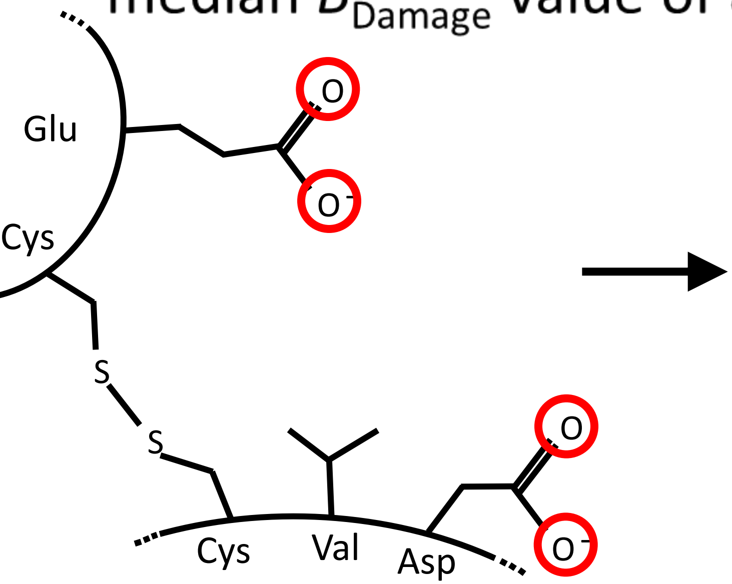
Uses B_{Damage} to compute *Bnet*



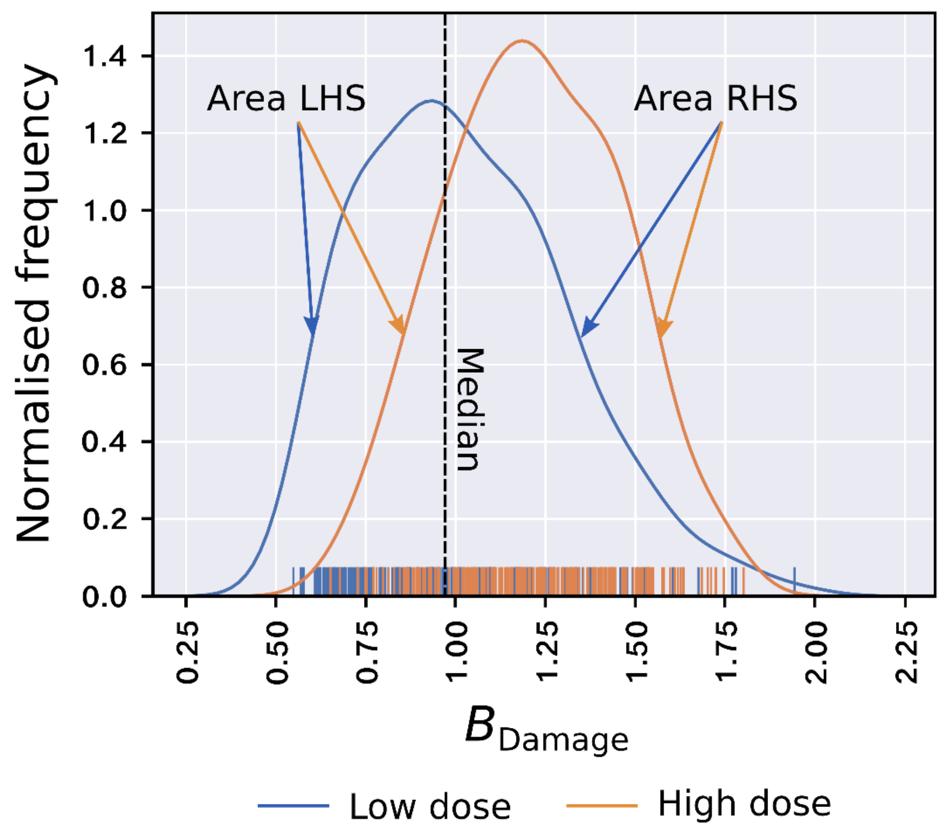
The B_{net} metric

- 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



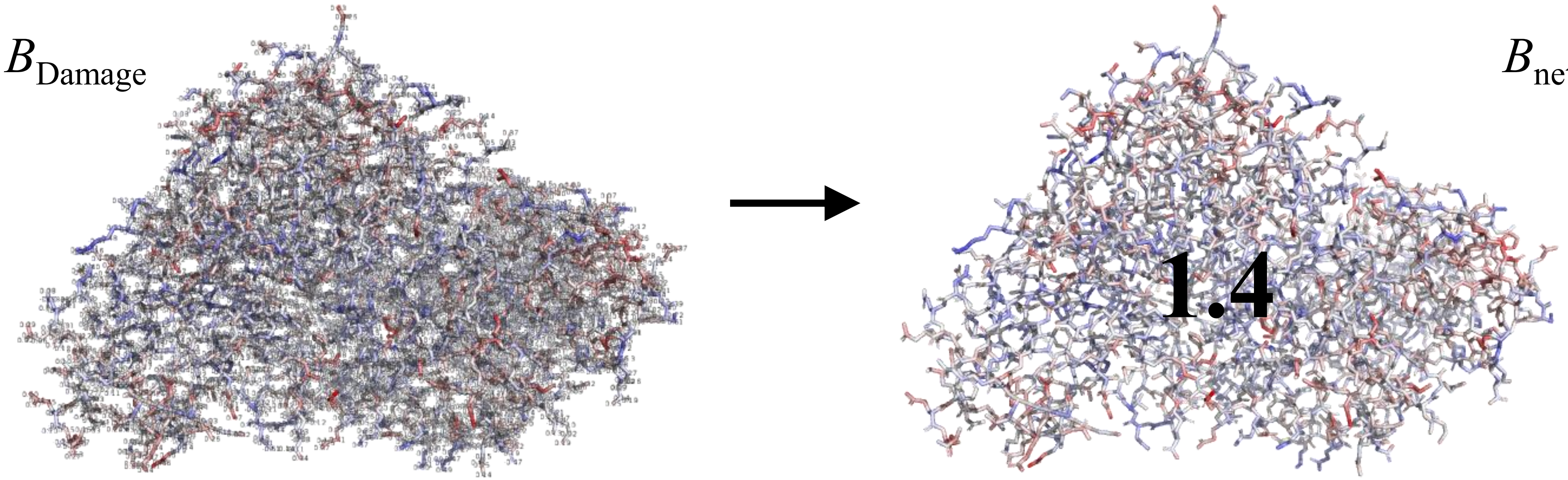
$$B_{net} = \frac{Area\ RHS}{Area\ LHS}$$



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

B_{Damage}



B_{net}

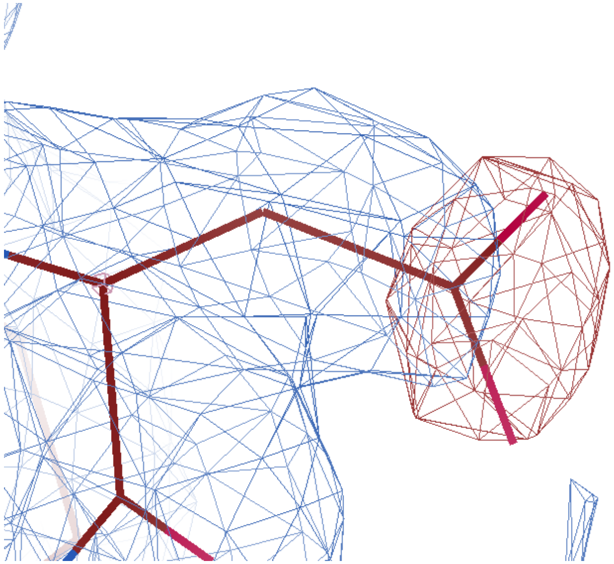
Low B_{Damage}

High B_{Damage}

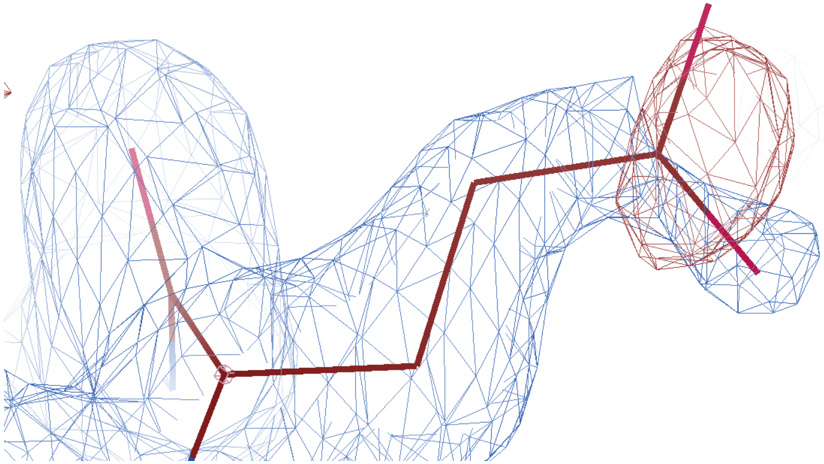
Analysis of the Protein Data Bank: *Bnet*

- Most of the structures with the highest B_{net} values show clear signs of radiation damage in their electron density. Above B_{net} =3 look at structure before you use it!

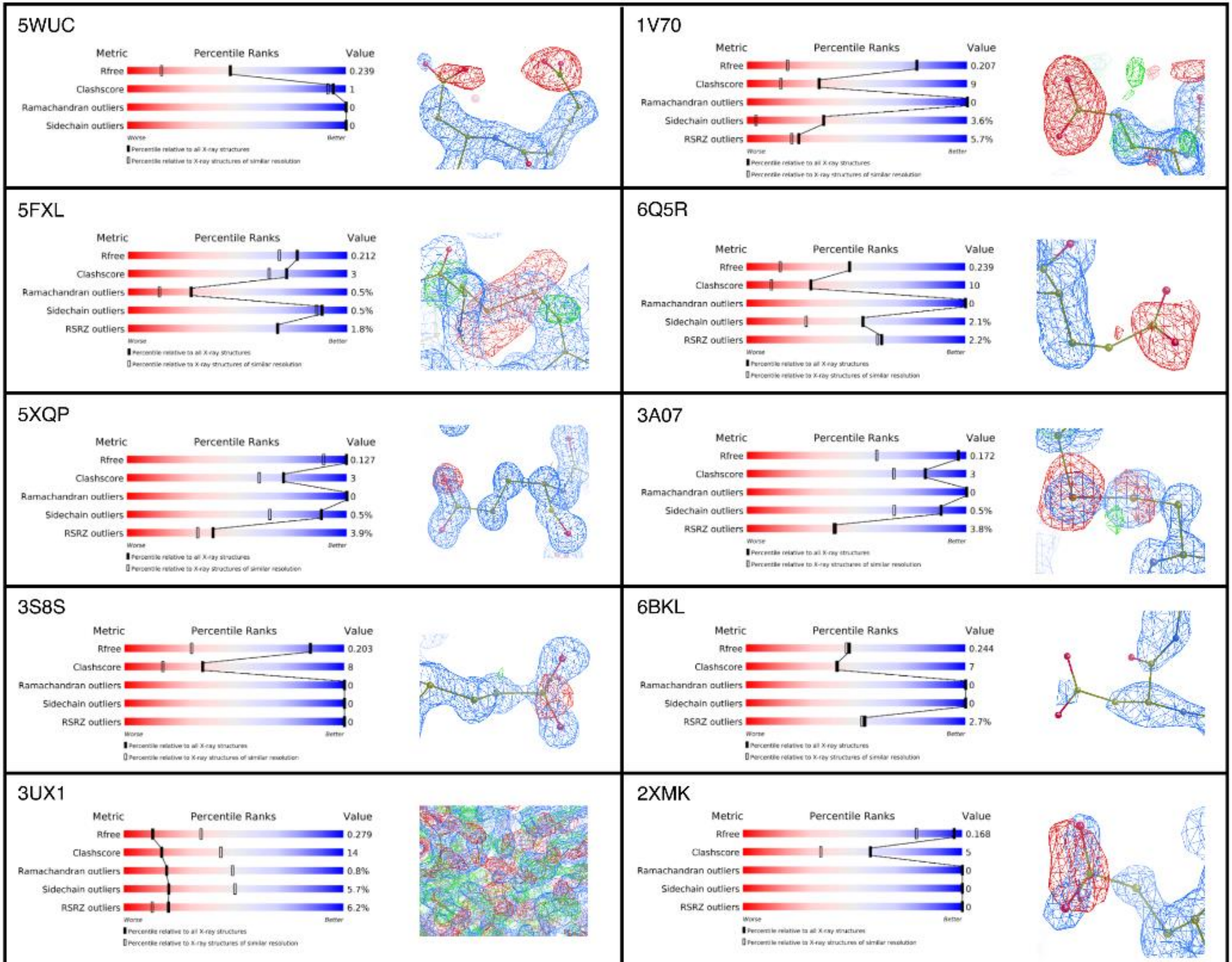
3IWU (B_{net} = 78.2)



5G28 (B_{net} = 66.3)



Shelley & Garman *Nat Comms* (2022) **13**:1314



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

SUMMARY: what YOU, the experimenter, can do.

- Use a low dose, fine slicing, high multiplicity strategy.
- Expose a sacrificial crystal to judge lifetime on that beamline of the day
- Estimate the dose and stay within recommended ranges
- Spread the dose
- Backsoak non-specifically bound heavy atoms out prior to cooling crystal
- Use a top-hat like beam if possible (defocussed top of Gaussian works).
- Do not be afraid to merge data taken from different isomorphous crystals which all had lower doses
- High Energy X-ray data collection (CdTe detector). Sweet spot round 26 keV. (SImulations: Dickerson & Garman JSR 2019, Expt verification: Storm et al IUCrJ 2021)
- Scavengers: try electron scavengers at 100 K (e.g. $NaNO_3$)
- Check your refined structure with RABDAM.

- **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*?**

FURTHER READING:

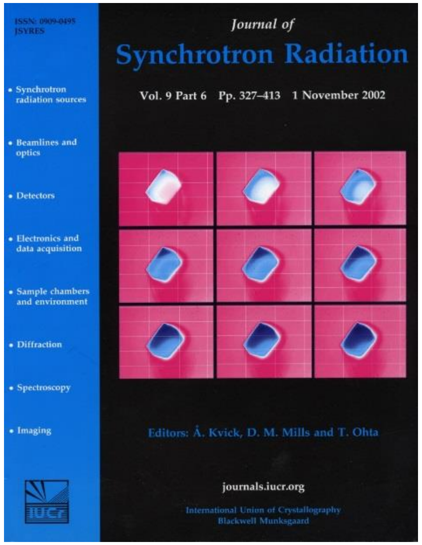
- ‘Beginner’s guide to Radiation Damage’ Holton, (2009) *JSR* **16**,133-142
- General summary in: Garman, *Acta D* (2010) **66**, 339-355
- Warkentin et al *J.Synchrotron Rad*. (2013). **20,** 7-13
- Garman and Weik, Chapter 20 in *‘Protein Crystallography: Methods in Molecular Biology’* (2017) **1607,** 477-489
- nature COMMUNICATIONS

  14th March 2022

  Quantifying and comparing radiation damage in
  Protein Data Bank

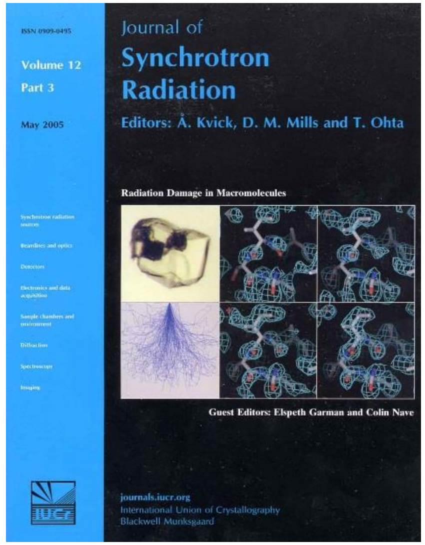
  Kathryn L. Shelley [1,2] & Elspeth F. Garman [1]
- Shelley and Garman, *Acta D* (2024) **80,** 314–332

RD2: Dec 2001



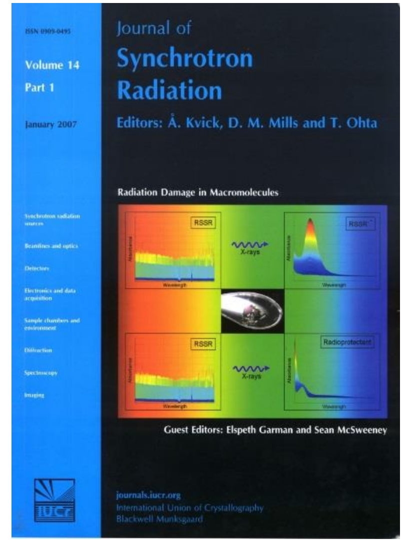
JSR, Nov 2002 (8)

RD3: Nov 2003



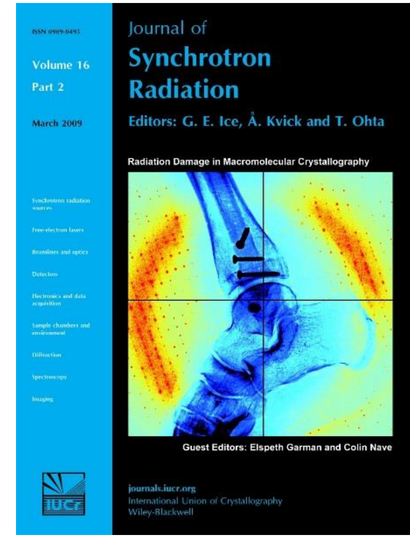
JSR, May 2005 (9)

RD4: March 2006



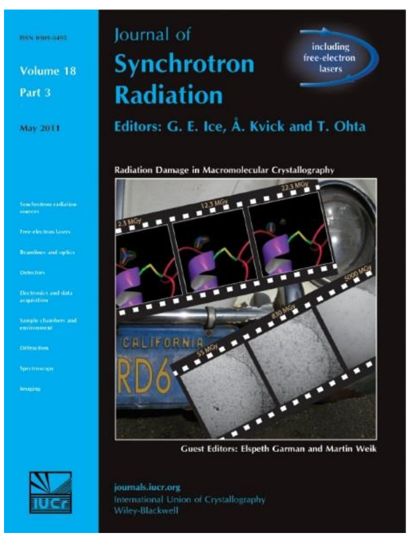
JSR, Jan 2007 (14)

RD5: March 2008



JSR, March 2009 (8)

RD6: Mar 2010

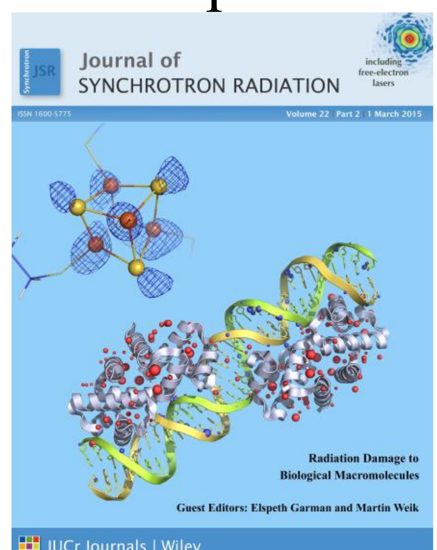


JSR, May 2011 (10)

RD7: Mar 2012

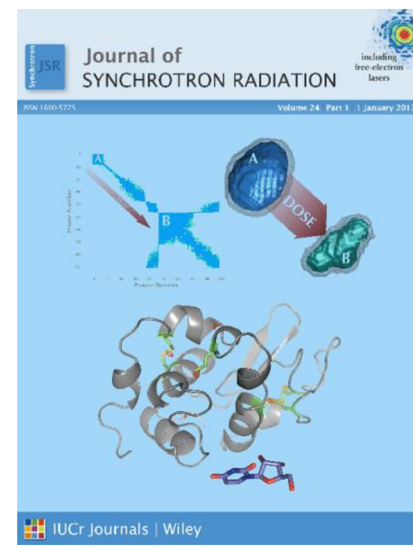
JSR, Jan 2013 (6)

RD8: Apr 2014



JSR, March 2015 (8)

RD9: Mar 2016

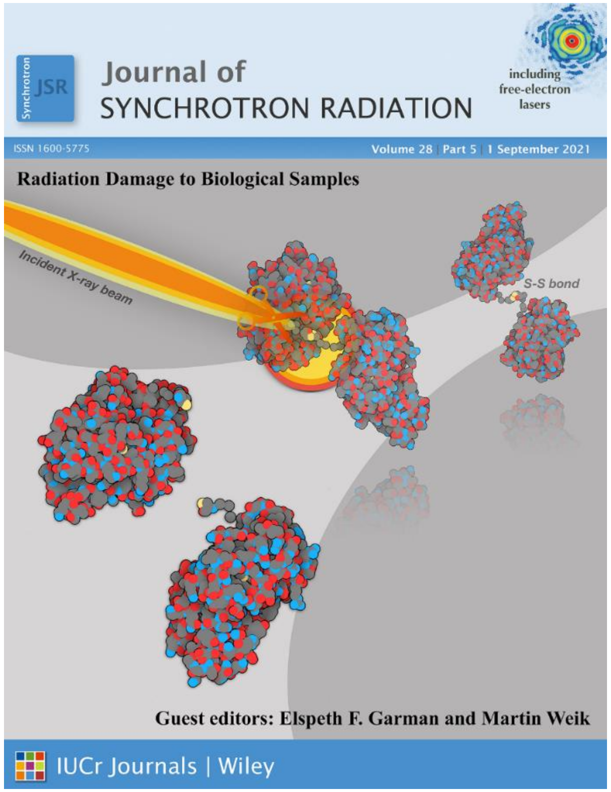


JSR Jan 2017 (8)

RD10: Sep 2018

JSR, July 2019 (9)

RD11: Sep 2021

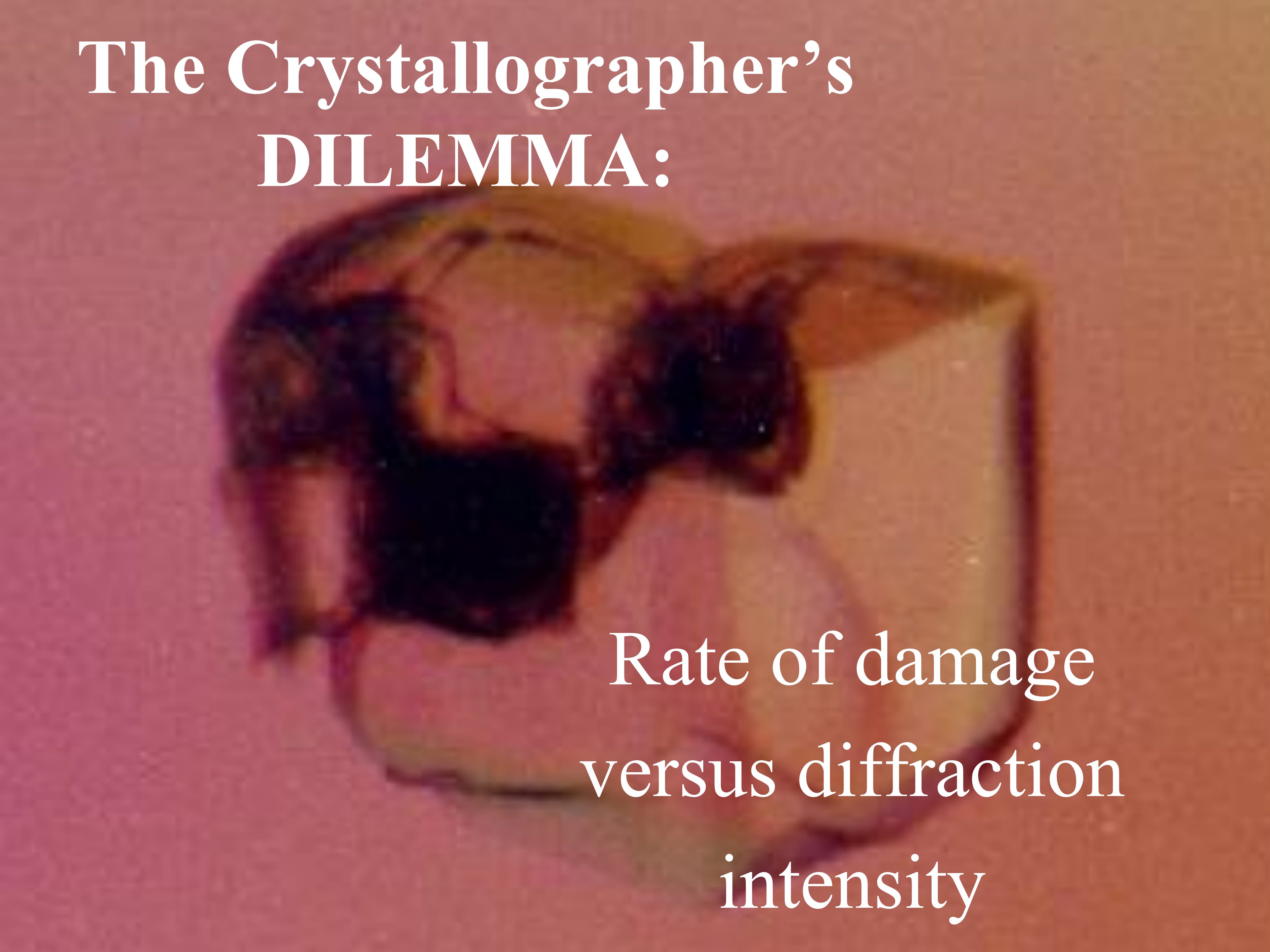


JSR, July 2021 (6)

RD2 to 11: 86 papers published in JSR Special Issues to date

RD12: 3-5th June 2025, PSI, Switzerland

Special Issue of Acta D planned for 2026 (papers being submitted now: -9 already)


The Crystallographer's
DILEMMA:
Rate of damage
versus diffraction
intensity

This was a lot of material delivered quickly so…

Please consider publishing in IUCr Journals Acta D or F: see next slides!

e.g. what is this?

Lorna Dougan, Leeds, LOOminaries

Questions very welcome, now and next 10 week when we meet.

elspeth.garman@bioch.ox.ac.uk

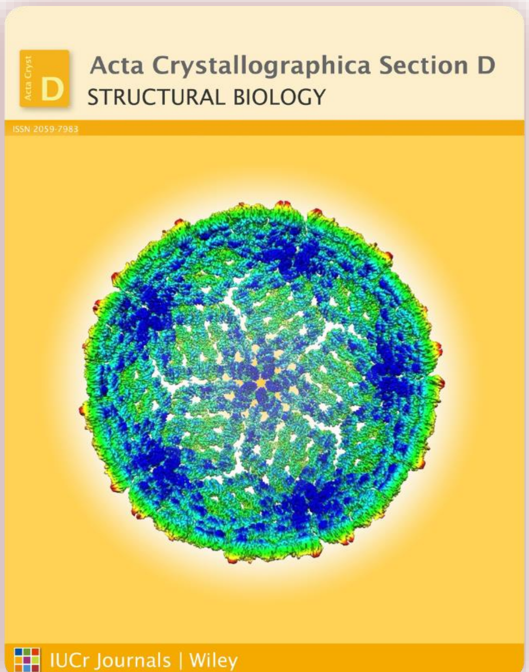
Acta Cryst D

Structural Biology

Acta Crystallographica Section D

journals.iucr.org/d/

@ActaCrystD



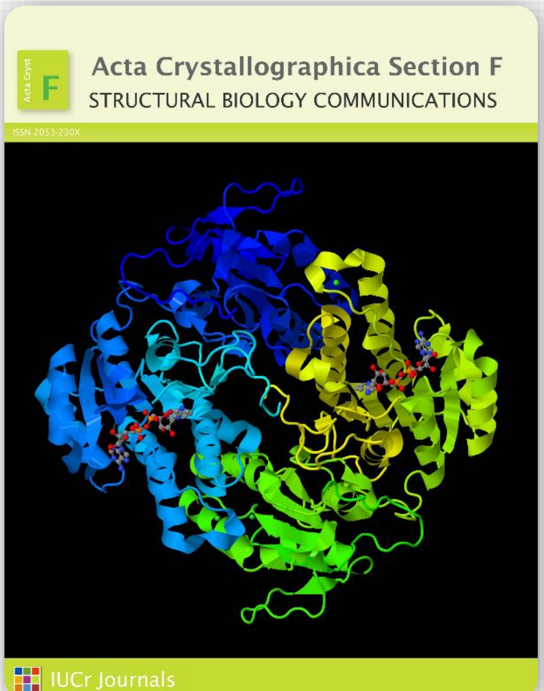
- Society journal established in 1993
- Covers all aspects of structural biology
- New structures of biological importance from any technique
- Reports on the latest software, equipment and methods
- International Editorial Board and trusted expert reviews
- Easy submission with technical editing upon acceptance
- Free to publish – no submission fees or page charges
- Open access option – free if your institution is part of a deal

Structural Biology Communications

Acta Crystallographica Section F

journals.iucr.org/f/

@ActaCrystF



Society journal established in 2005

Covers all aspects of structural biology - concisely and rapidly

Biological structures determined by any technique

Brief reports on the latest software, equipment and methods

International Editorial Board with trusted expert reviewers

Early career researchers are encouraged and highlighted through interviews with our Editors

Free to publish – no submission fees or page charges

Open access option - free if your institution is part of a deal

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/GarmanGroup/RADDOSE-3D

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

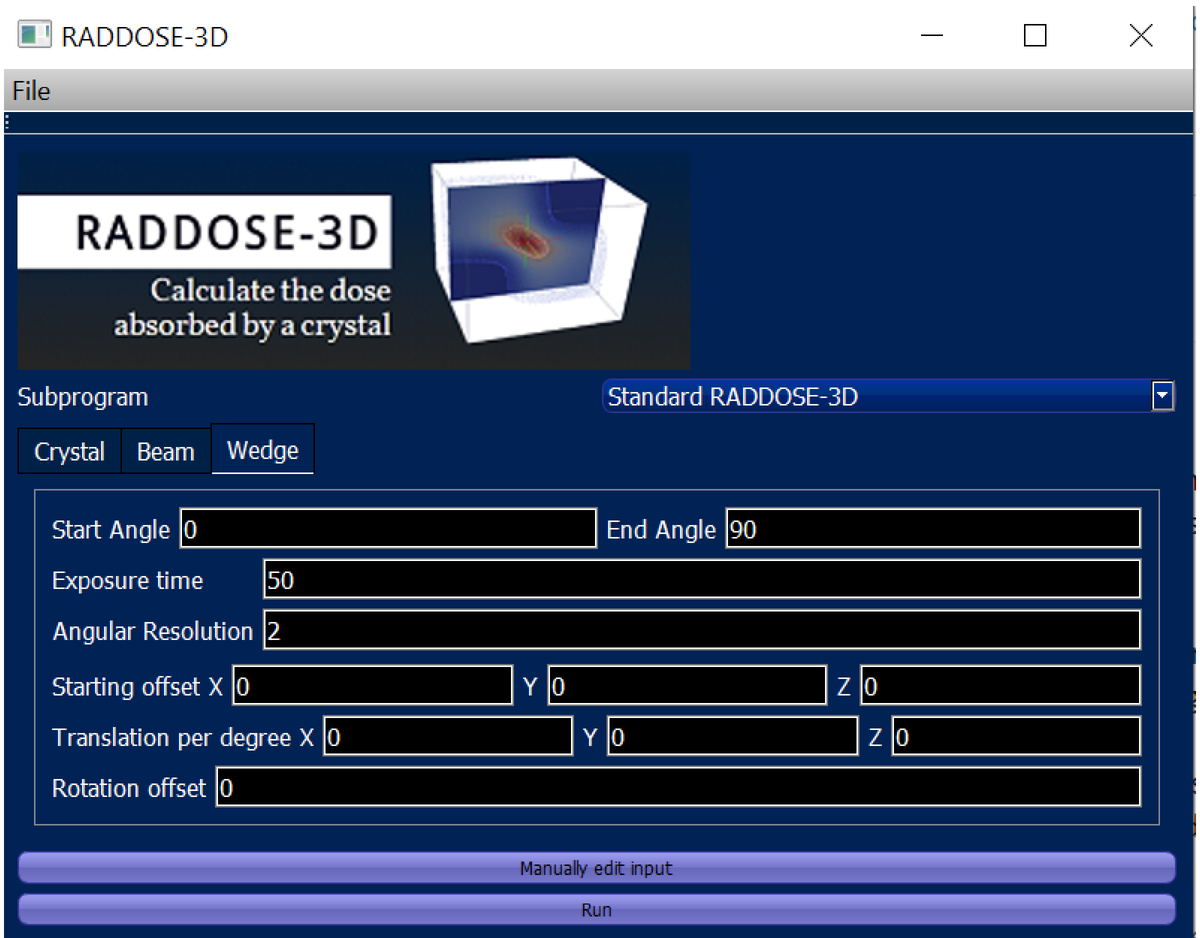
https://www.java.com/download/ie_manual.jsp

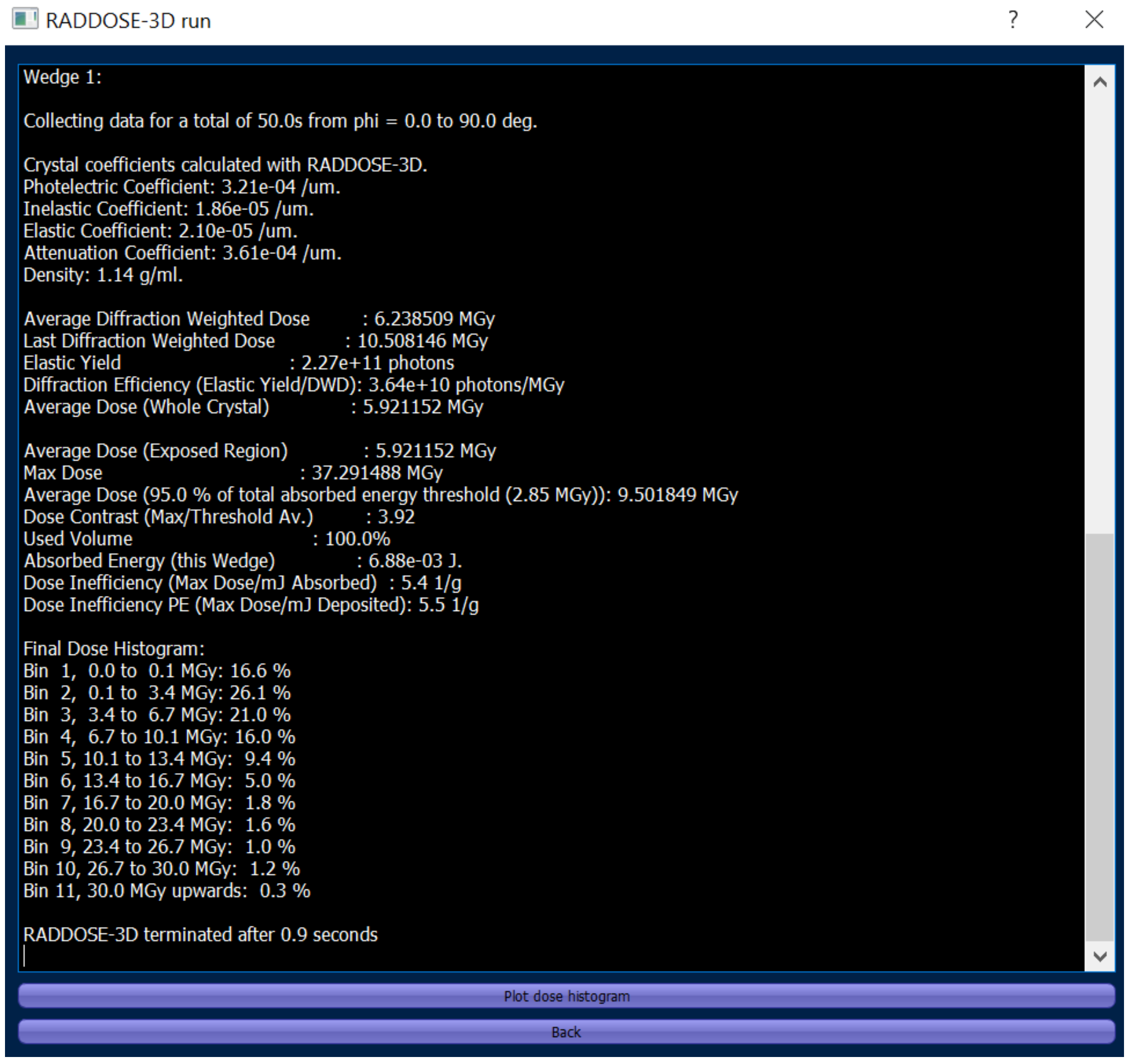
Also, if you have R (https://www.r-project.org/) installed, from the RADDOSE-3D output you will be able to produce 3D representations of the dose distribution in your sample.

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

- Note that installing a third party executable by direct download rather than through an installer might lead your antivirus software to complain.

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.




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

Step 4: Understanding the output! DOSE = ENERGY ABSORBED/MASS

Photoelectric Coefficient: 6.35e-04 /um.
Inelastic Coefficient: 1.87e-05 /um.
Elastic Coefficient: 2.36e-05 /um.
Attenuation Coefficient: 6.77e-04 /um.
Density: 1.16 g/ml.

Average Diffraction Weighted Dose : 11.887135 MGy
Last Diffraction Weighted Dose : 20.040846 MGy
Elastic Yield : 2.51e+11 photons
Diffraction Efficiency (Elastic Yield/DWD): 2.11e+10 photons/MGy
Average Dose (Whole Crystal) : 11.271797 MGy
Average Dose (Exposed Region) : 11.271797 MGy
Max Dose : 71.040785 MGy