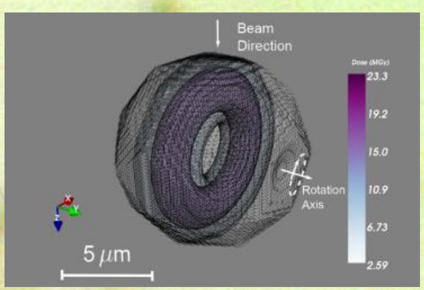
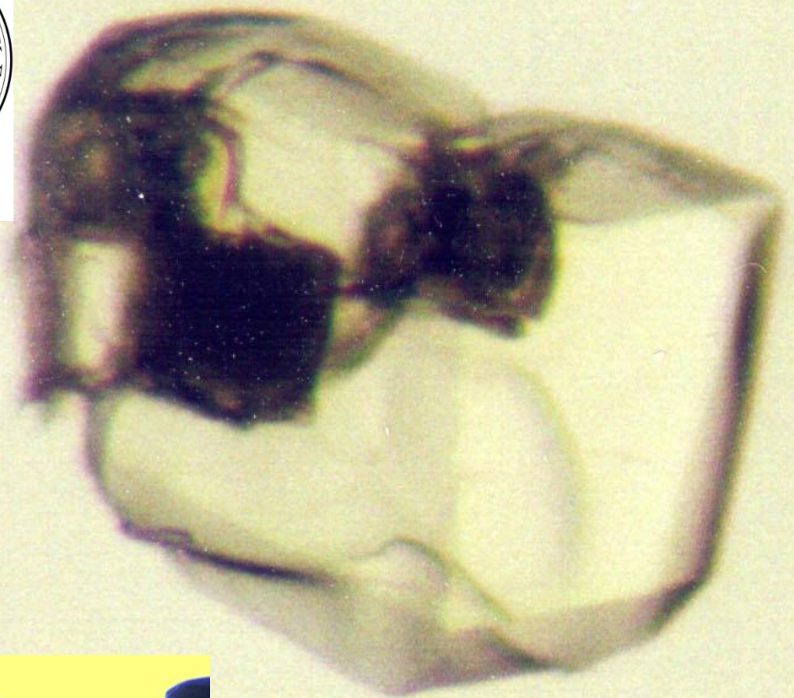
Radiation Damage

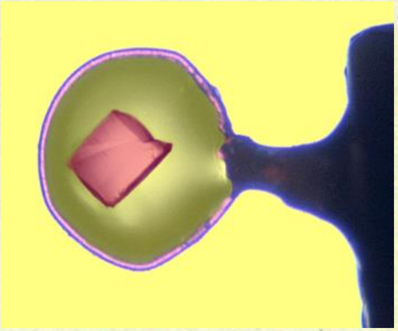
CCP4/DLS MX Workshop:

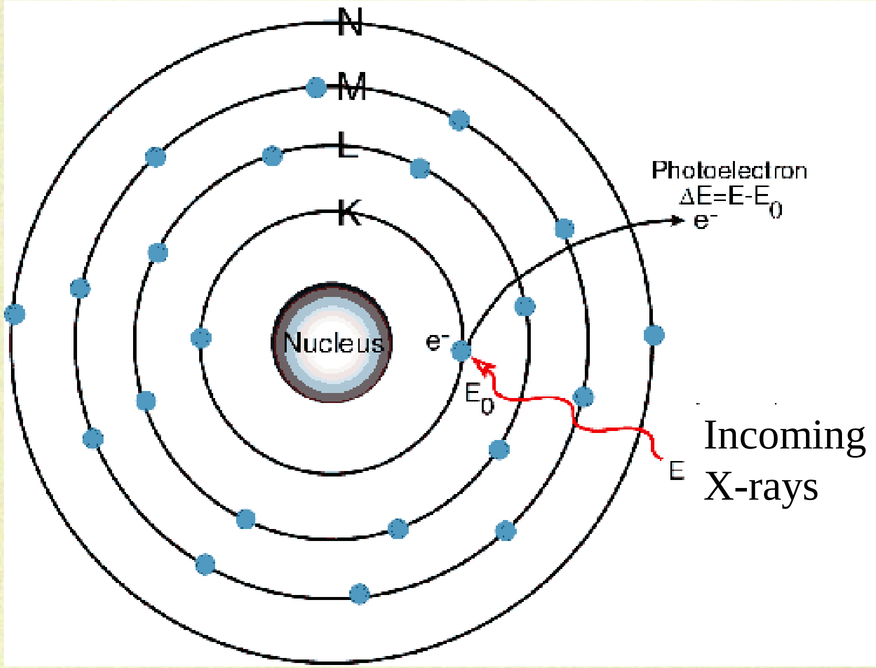




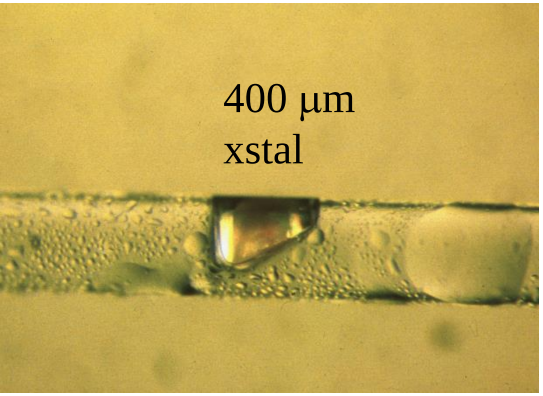
Virtual lecture

18 November 2024



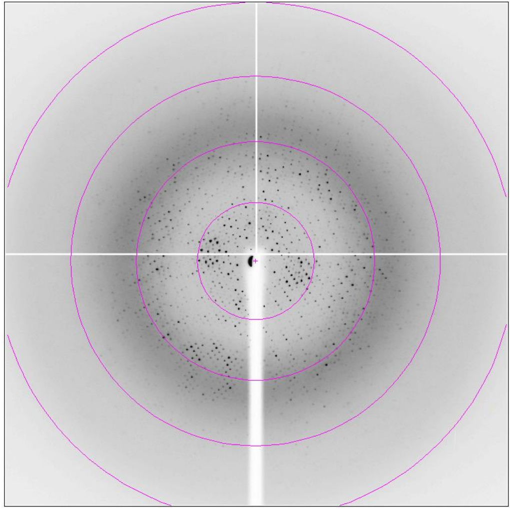


elspeth.garman@bioch.ox.ac.uk



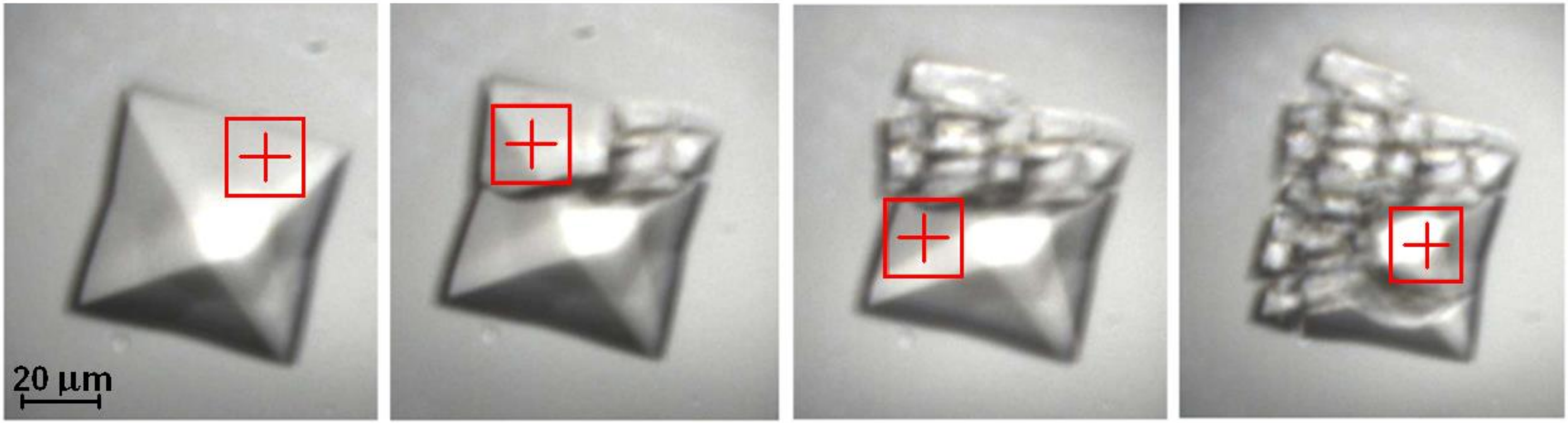
Radiation Damage

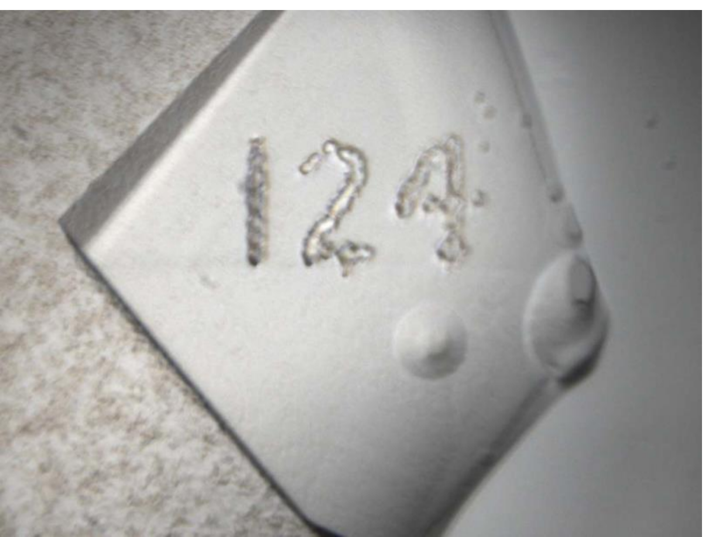
The Plan:



- **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?

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

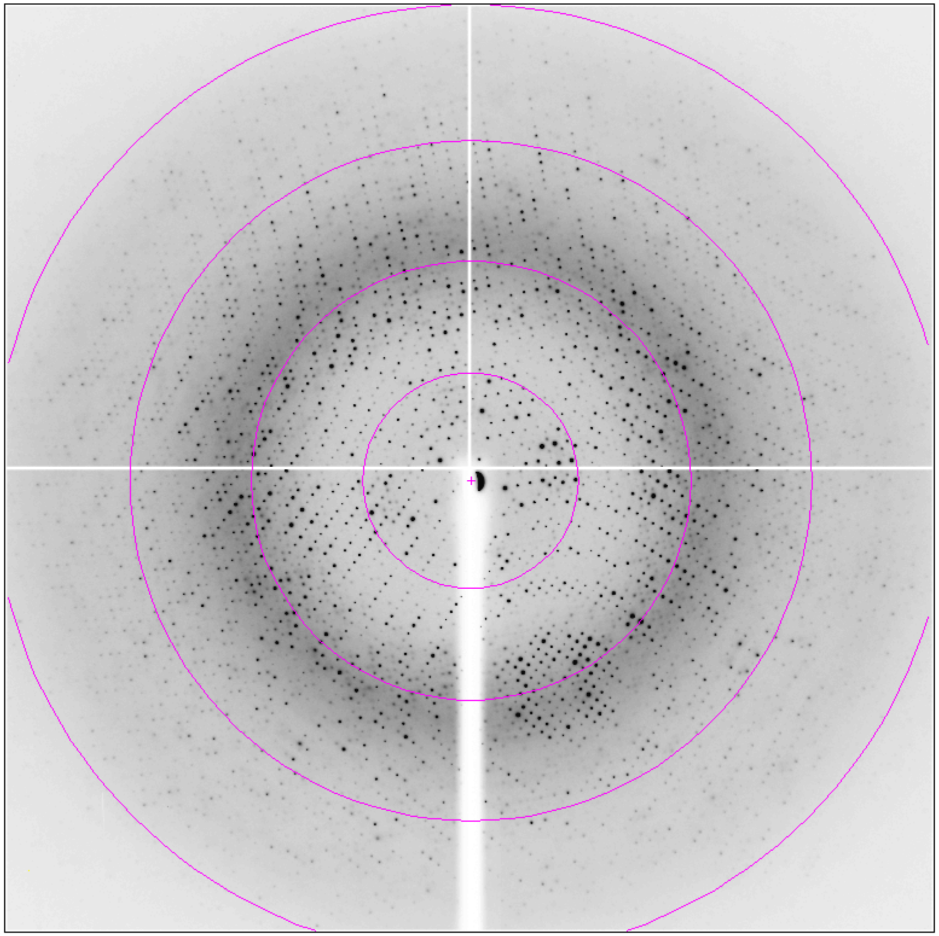




Axford *et al.*,
Acta Cryst D (2012) 592

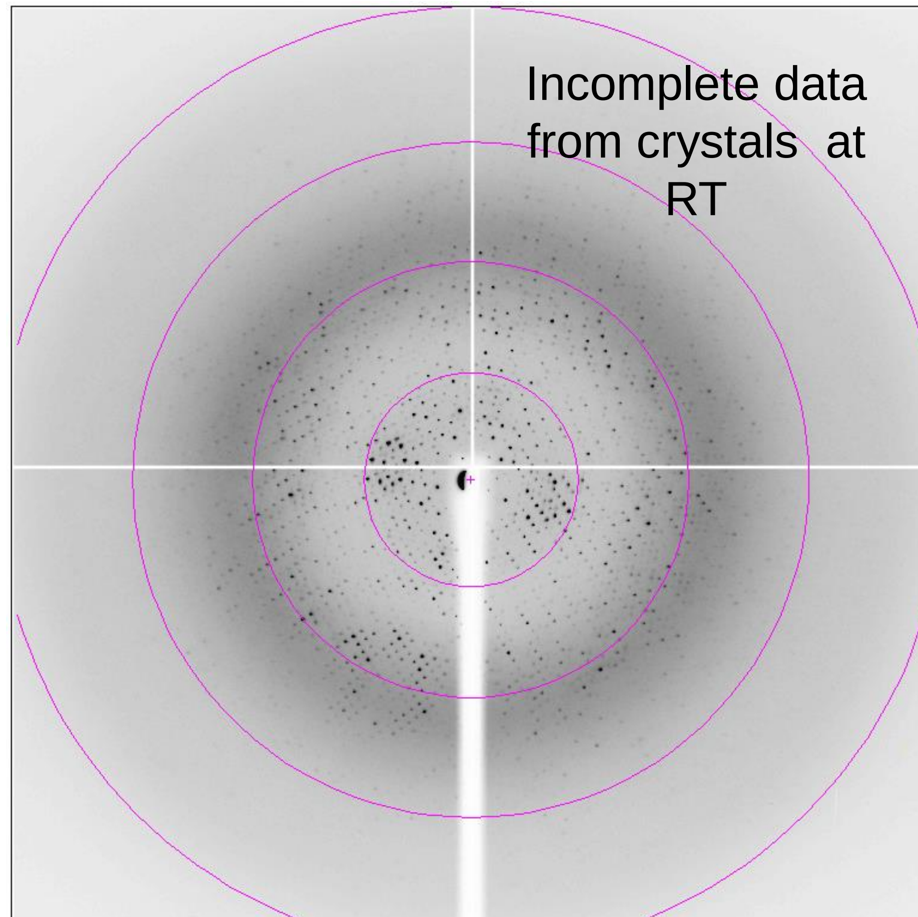
Beamline logo I24, 5 μm beam, large crystal!
(Gwyndaf Evans *et al.*)

ROOM TEMPERATURE

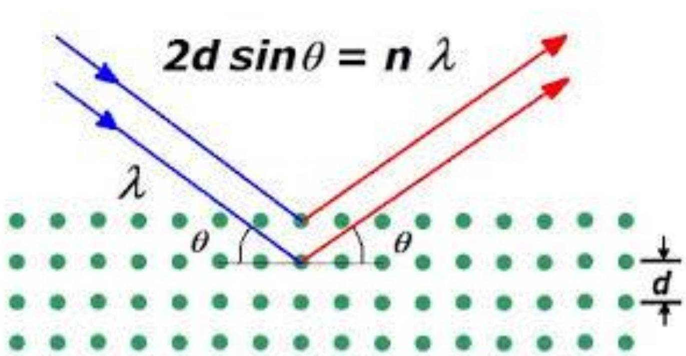


Intensity decrease with dose especially at high Bragg angles

Loss of diffraction

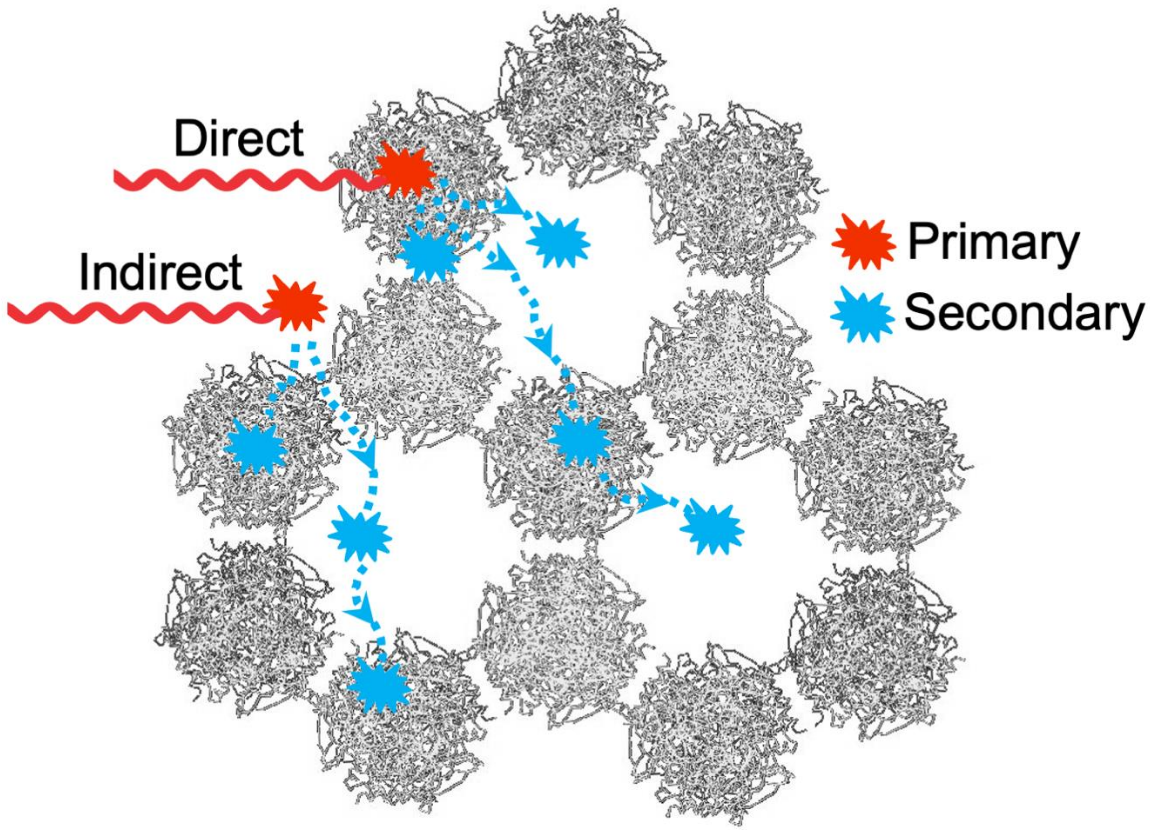


Incomplete data from crystals at RT



Radiation damage

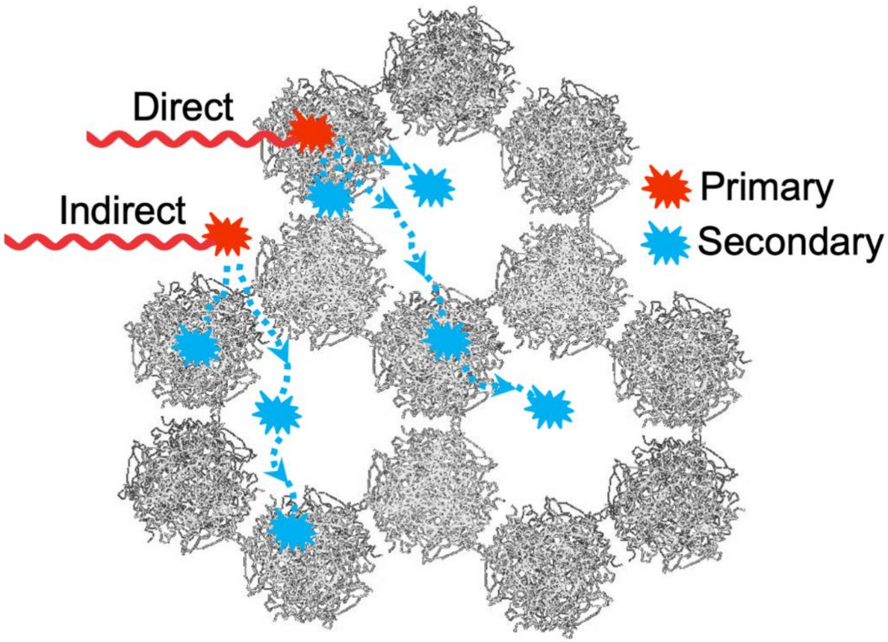
Primary
Secondary

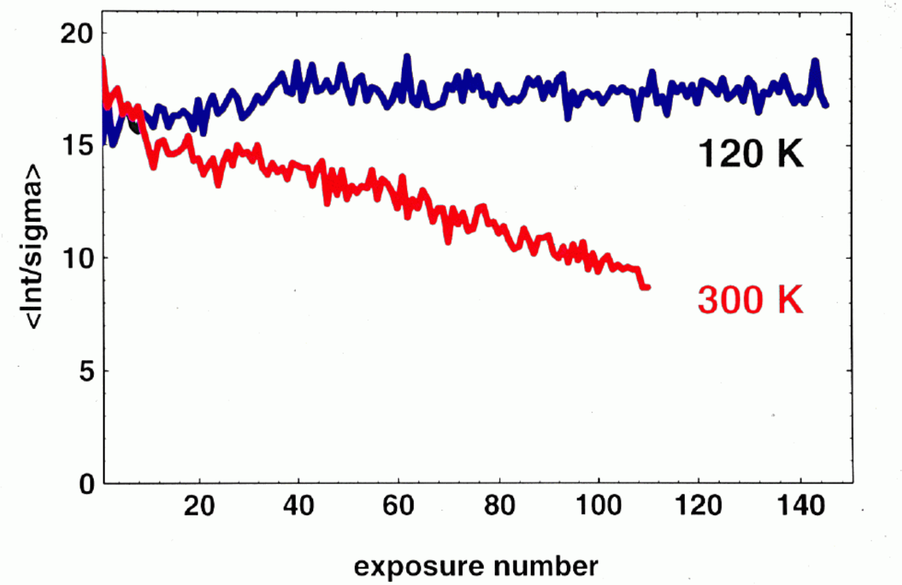


Protein: direct
Solvent: indirect

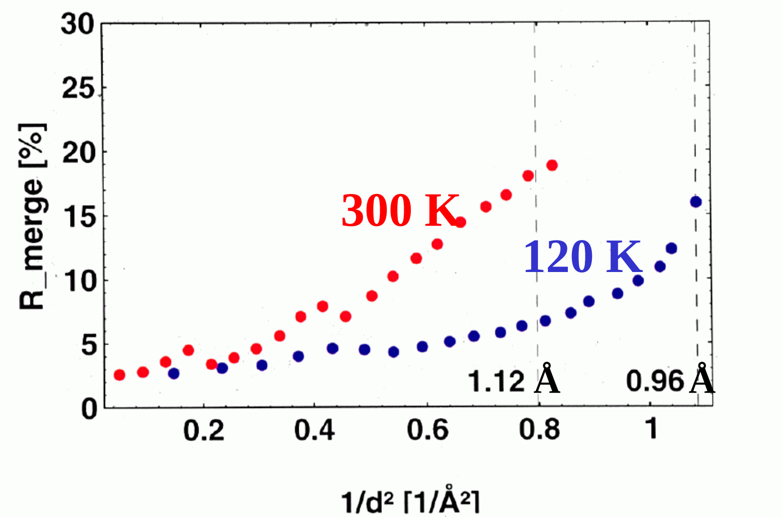
PRIMARY; inevitable, a fact of physics! Neutralise it?
SECONDARY, can we control it?

Radiation damage





SP445: Data Quality [T.Schneider]



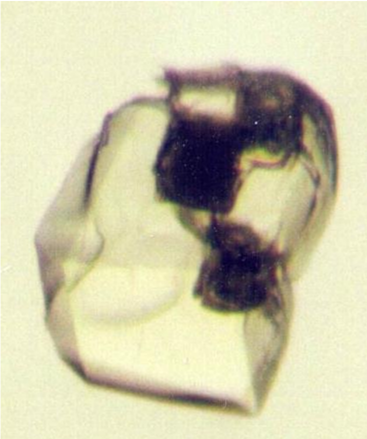
Significantly reduced at 100 K: time factor of ~70

[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



Radiation Damage
The Plan:

- Why cool? Radiation damage
- **What are the symptoms?**
- What is it?
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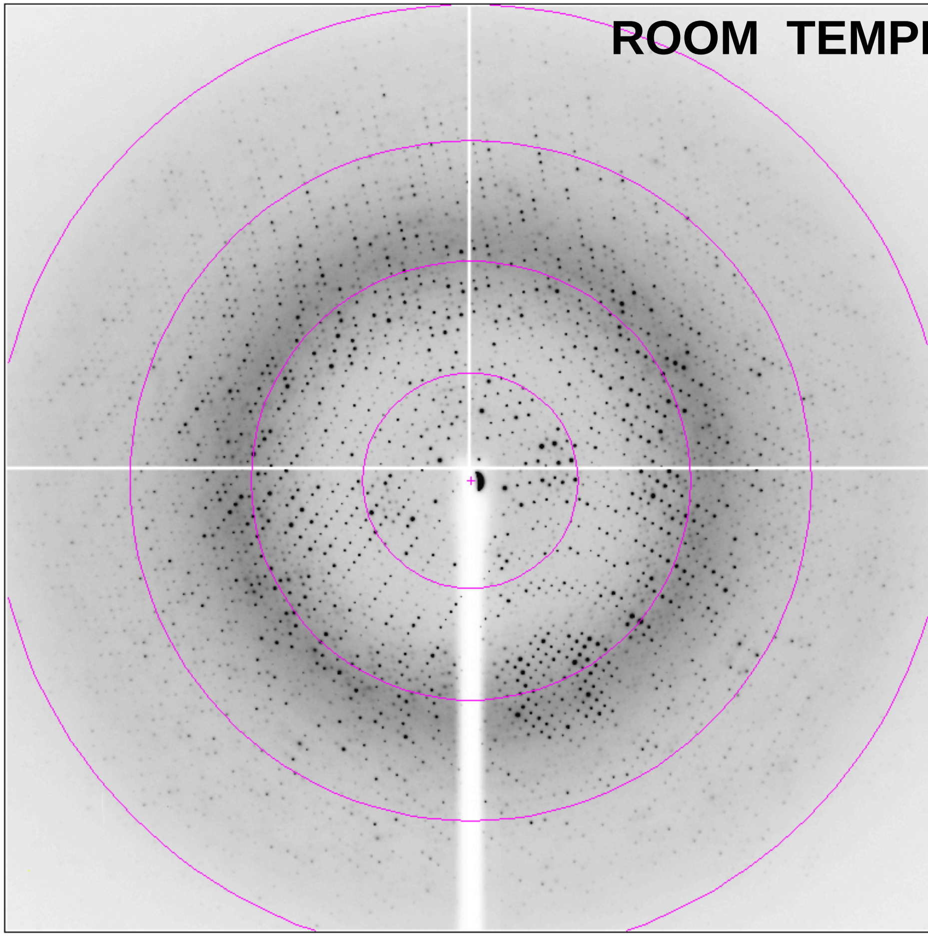
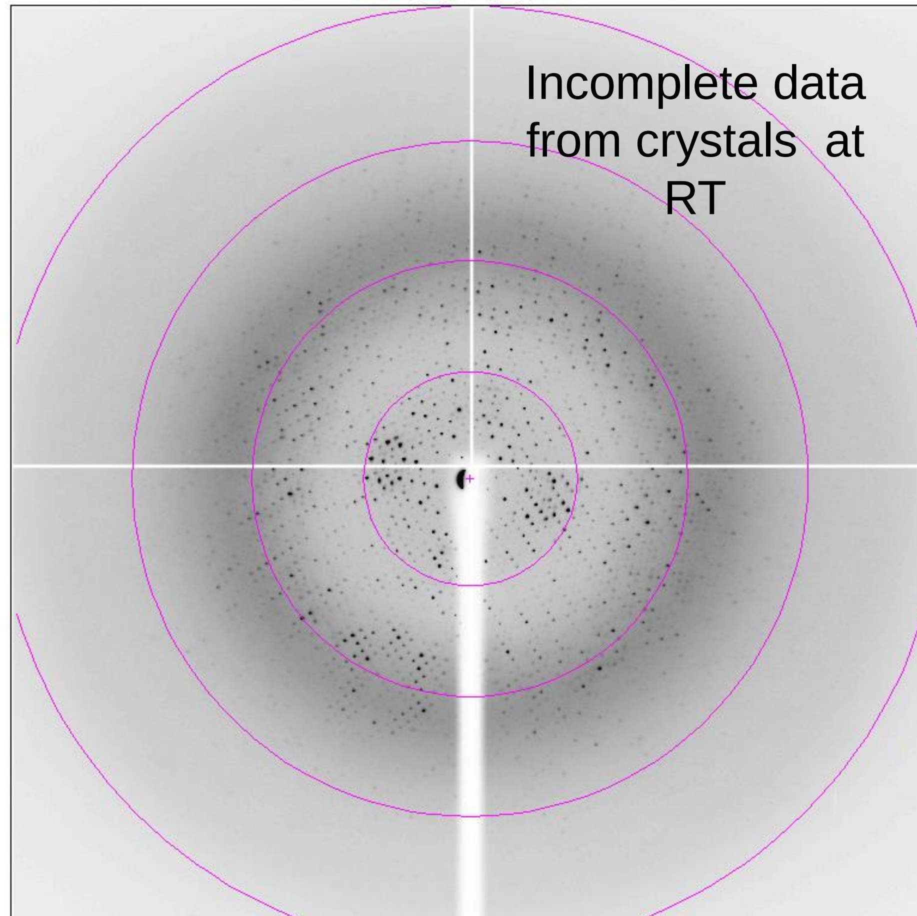
ROOM TEMPERATURE



Intensity
decrease
with dose
especially at high
Bragg angles

Loss of
diffraction



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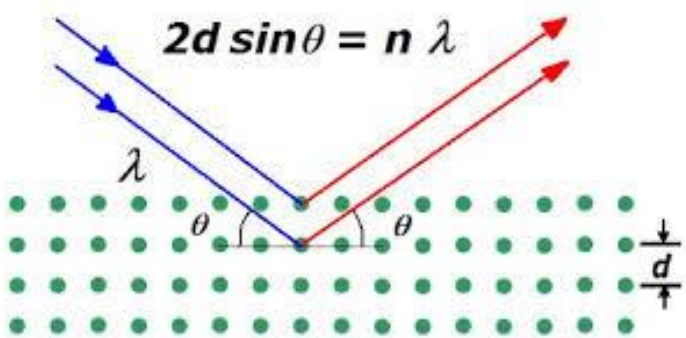
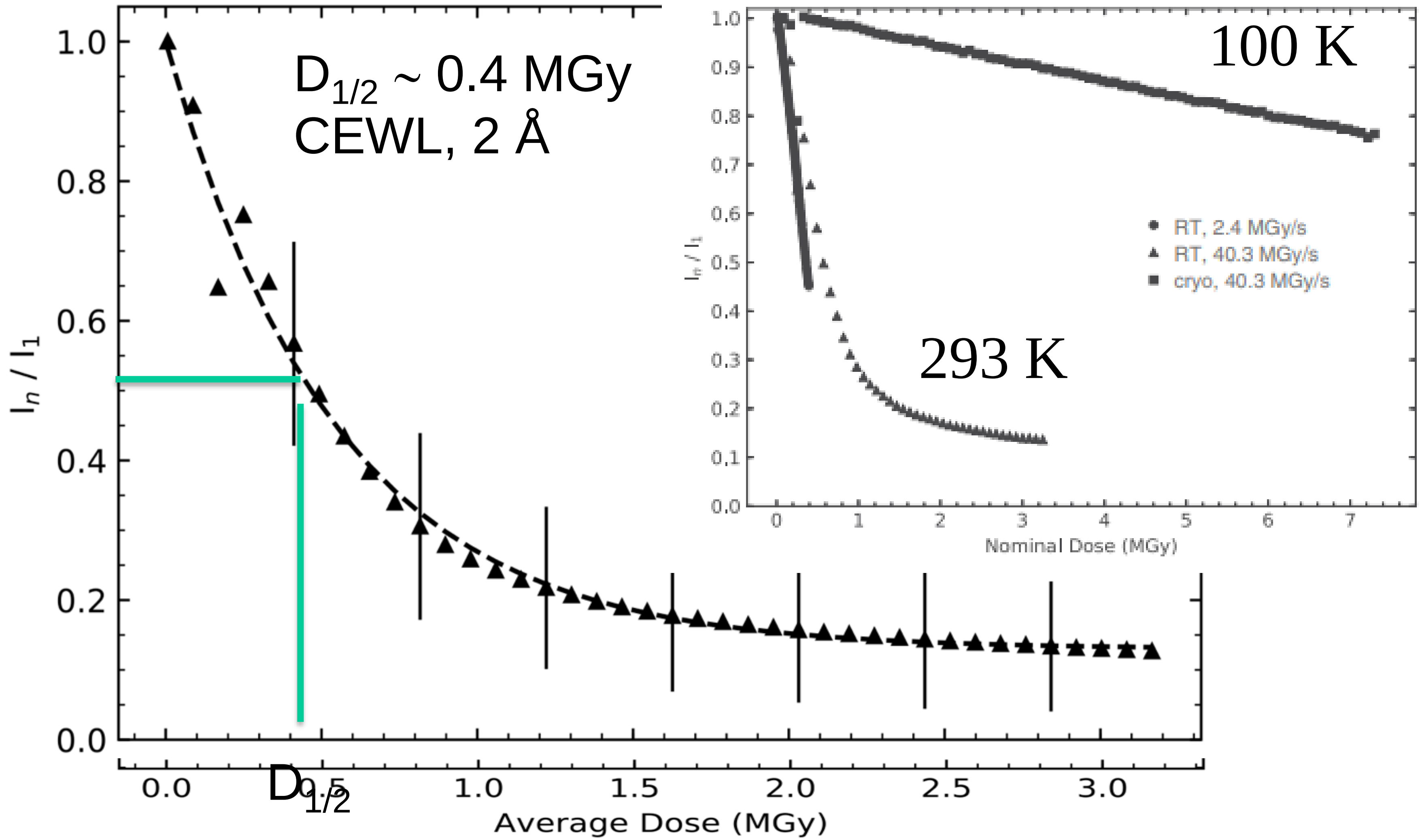
from crystals at
RT

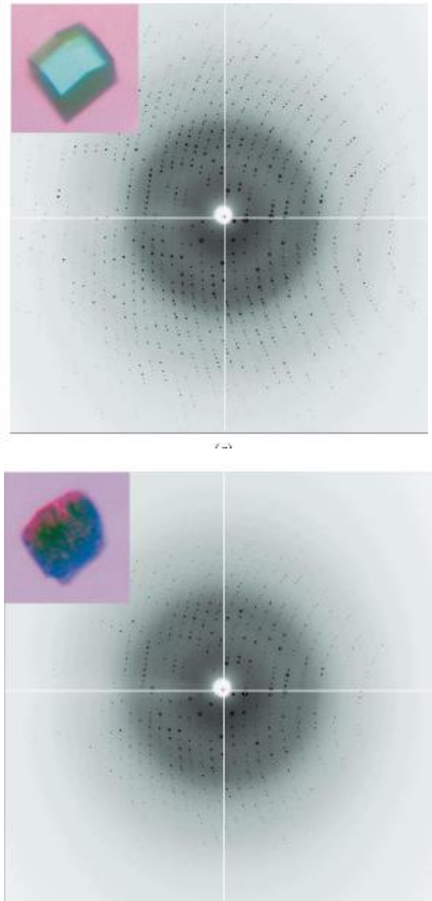


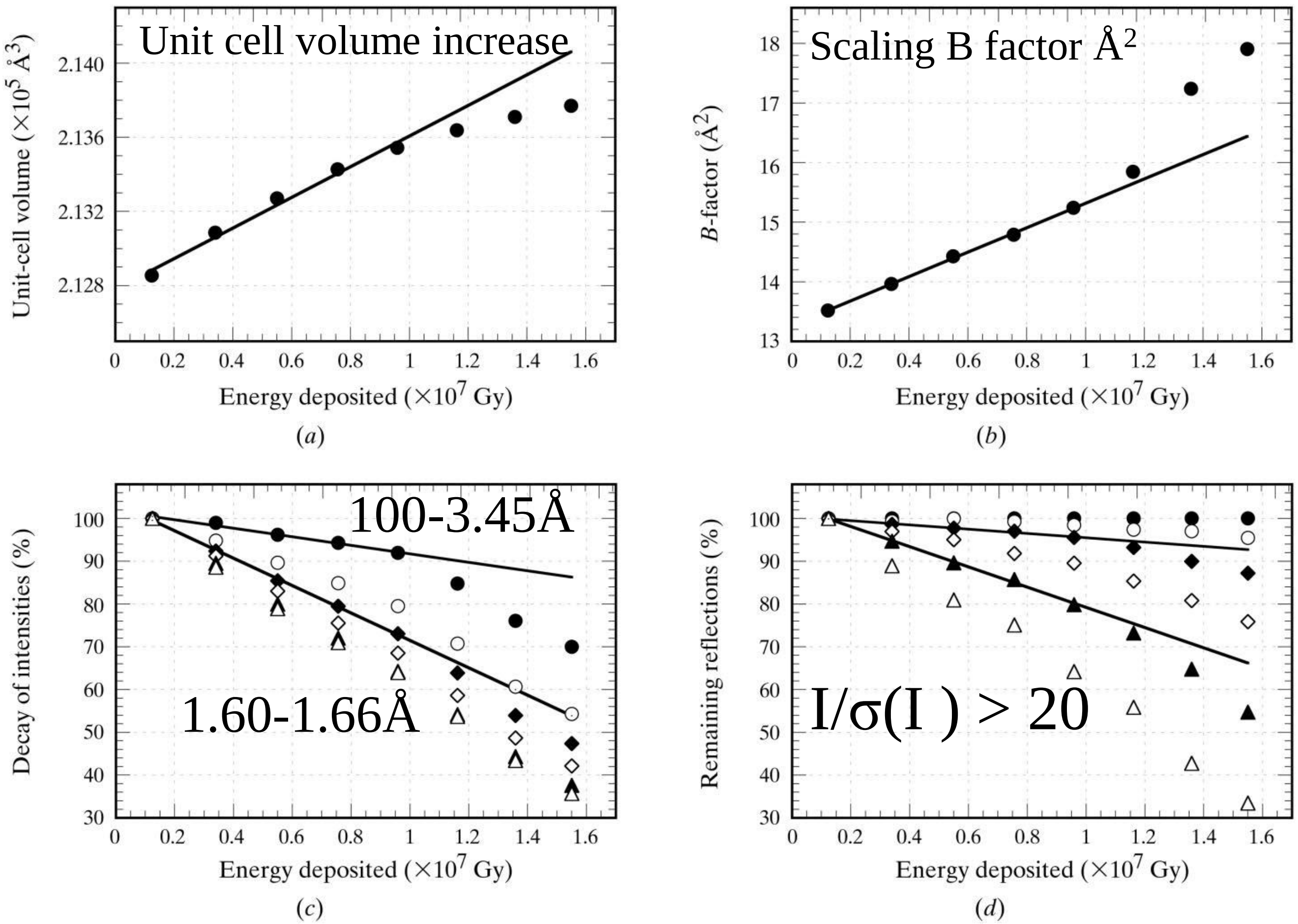
Intensity decay at RT and 100 K



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

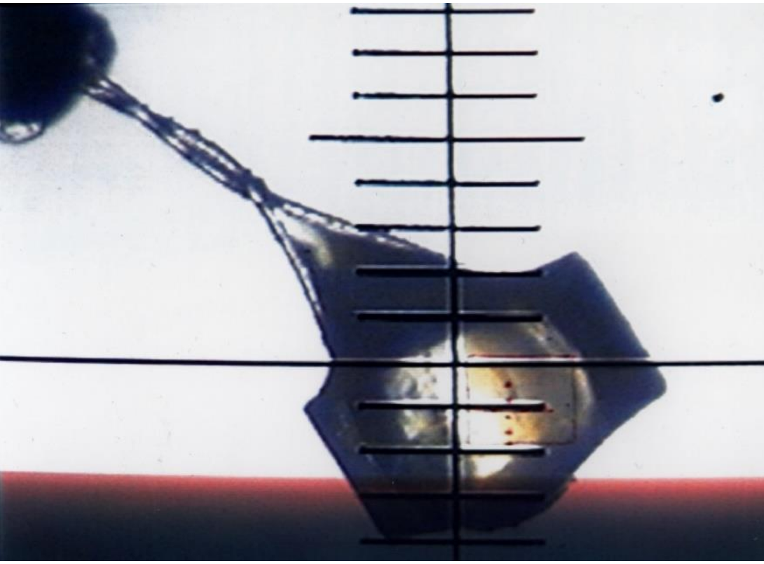
RadDam signatures in reciprocal space 100 K

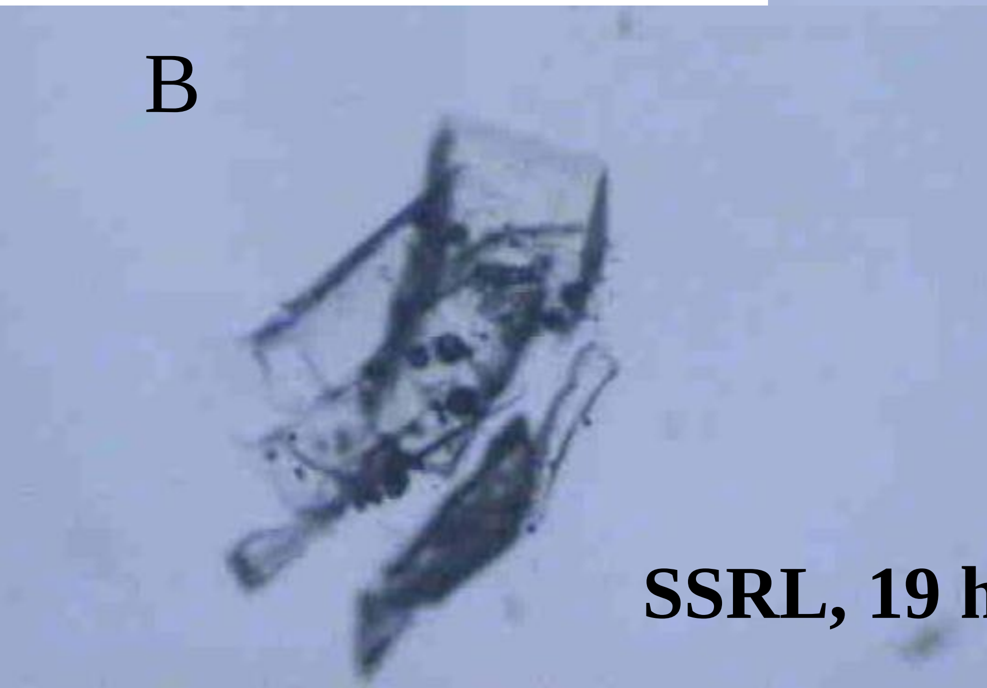


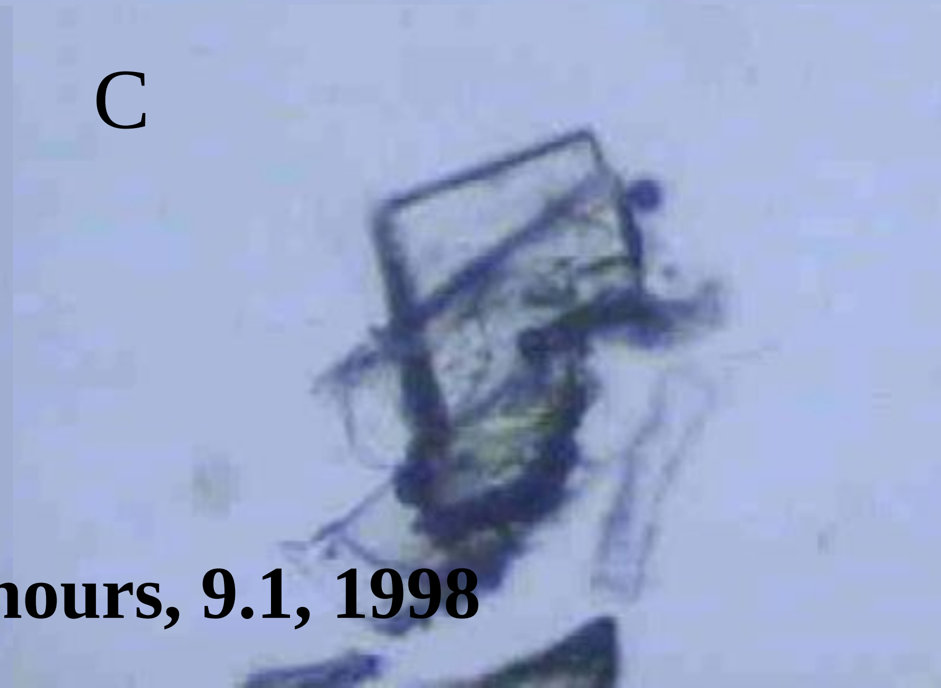


Teng and Moffat *JSR* (2000)

RadDam signatures (100 K MX)

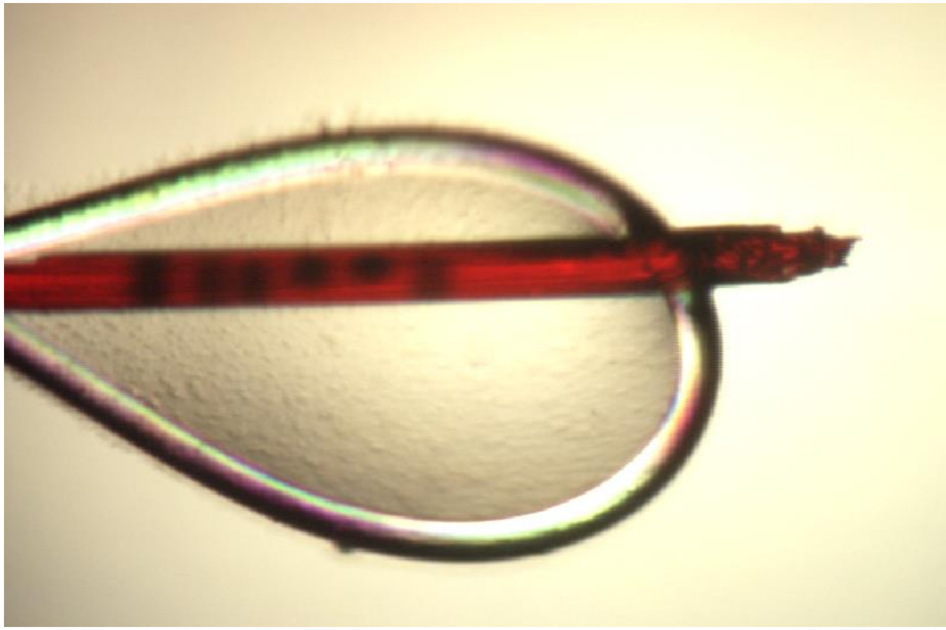




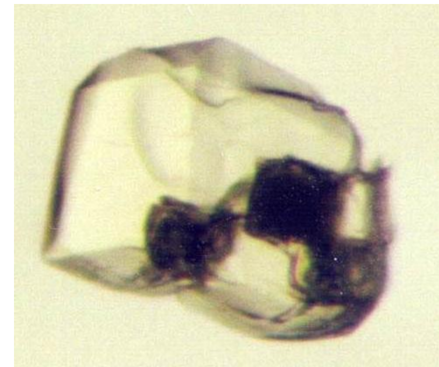


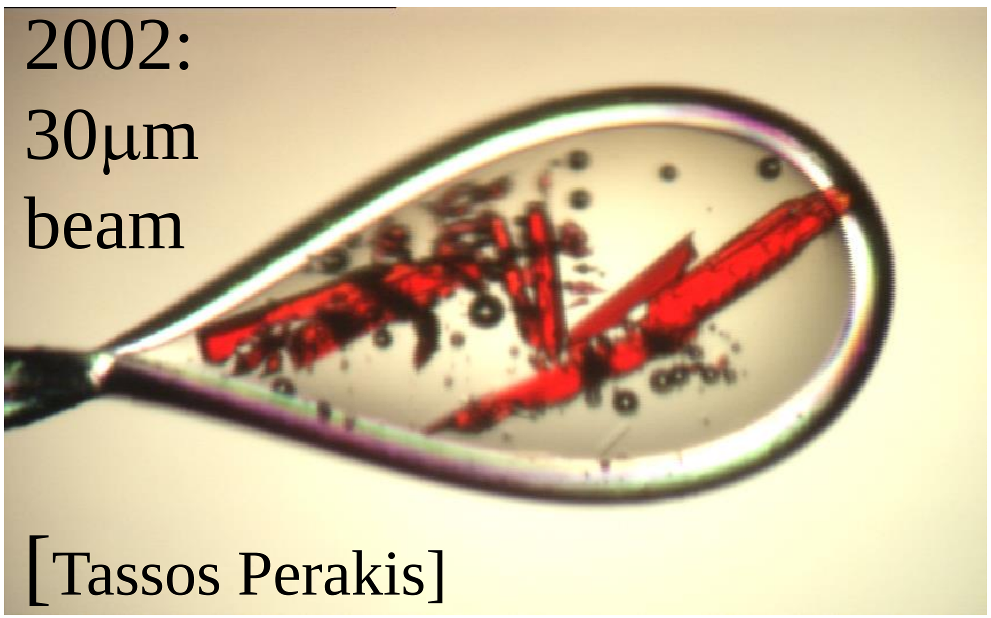
SSRL, 19 hours, 9.1, 1998

1995 onwards: 100 K



BUT THEN, 1999:

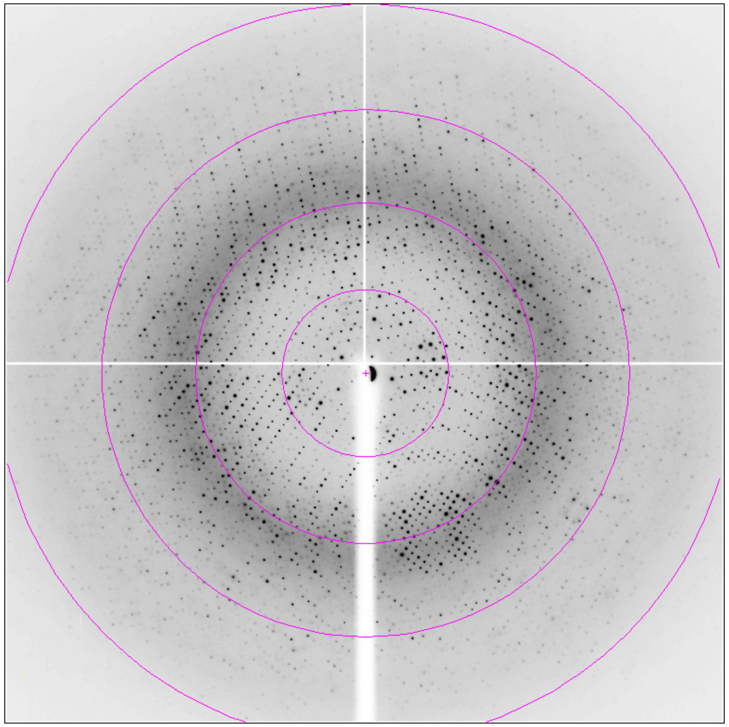




Iron containing protein, ESRF

Also observe spectral changes

Garman & Owen (2006), Acta D62

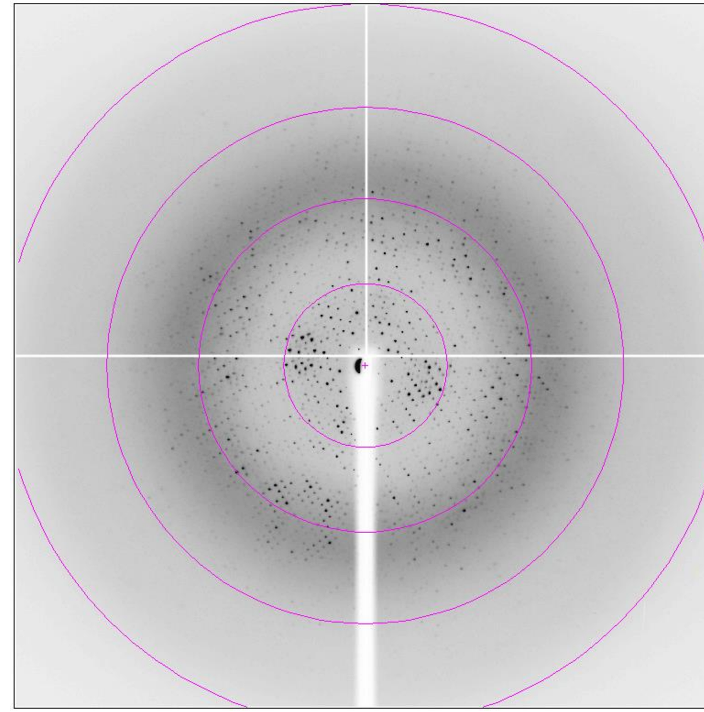


At 100 K

Intensity decrease

Loss of diffraction

Incomplete data from crystals



Happens during 1 dataset at 100K for many crystals

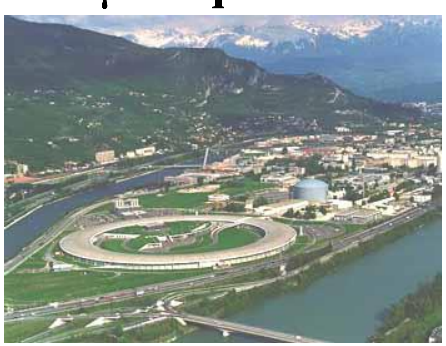
Unit cell volume expansion

Increase in Wilson B factors, Rmerge

Increase in mosaicity

'GLOBAL' damage

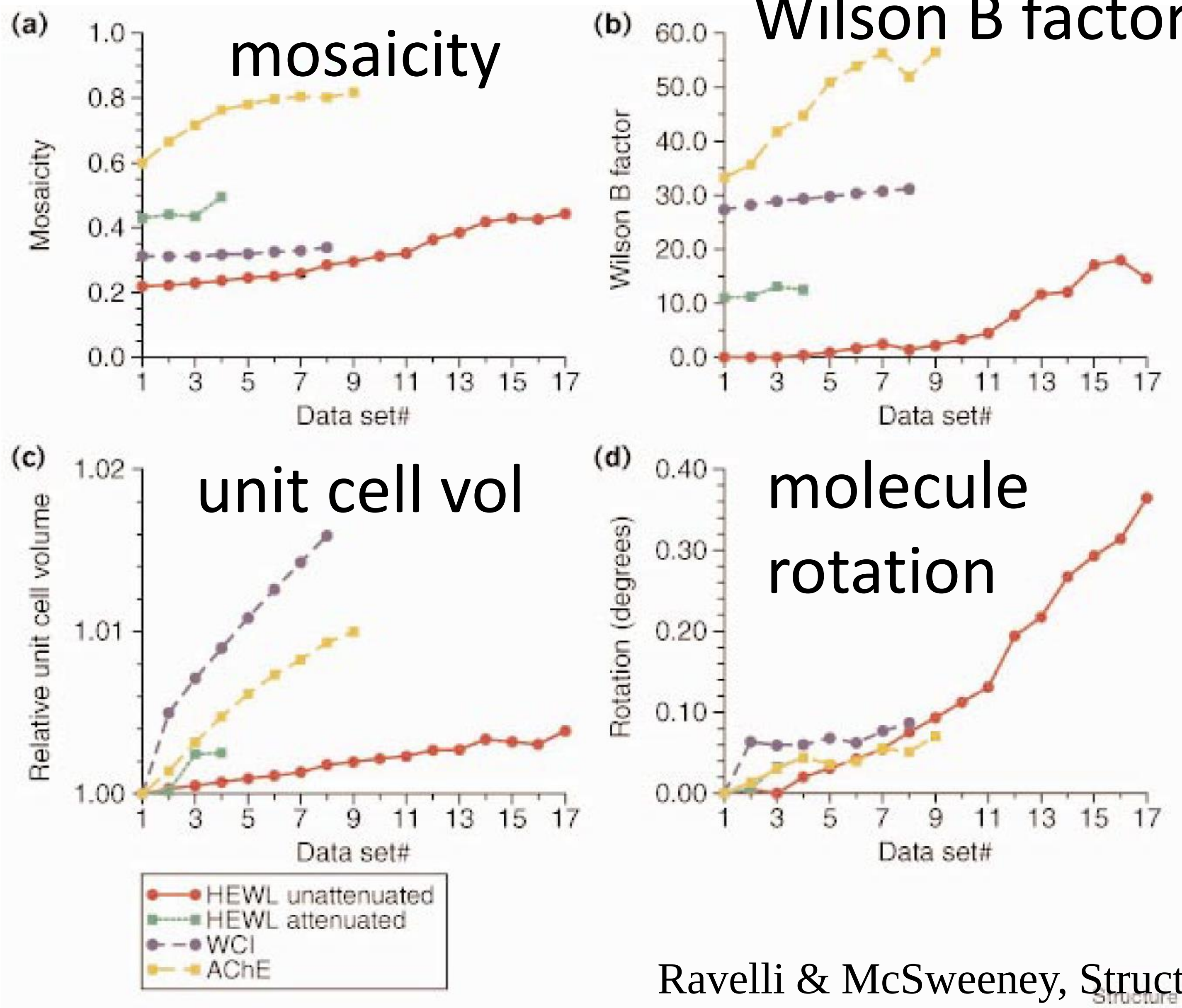
ESRF 2000:
1×10^{12} ph s^{-1} into
100µm square slits



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



Raimond Ravelli 1968-2023

Ravelli & McSweeney, Structure (2000) 8, 315

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?

[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

RADDOSE-3D

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

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

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

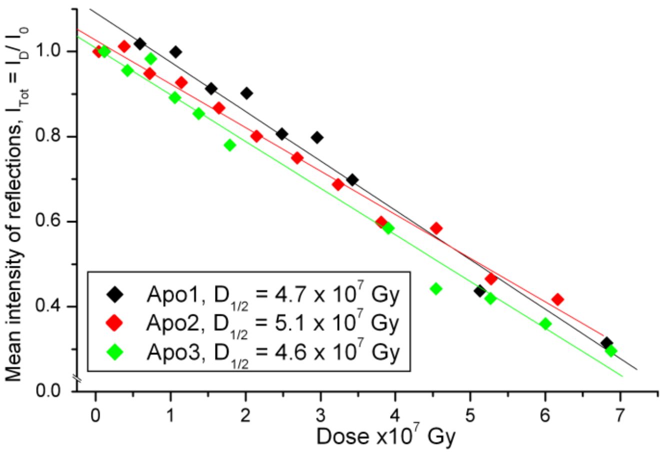
V4: Dickerson et al. (2024) *Protein Science*

Intensity Decay at 100K

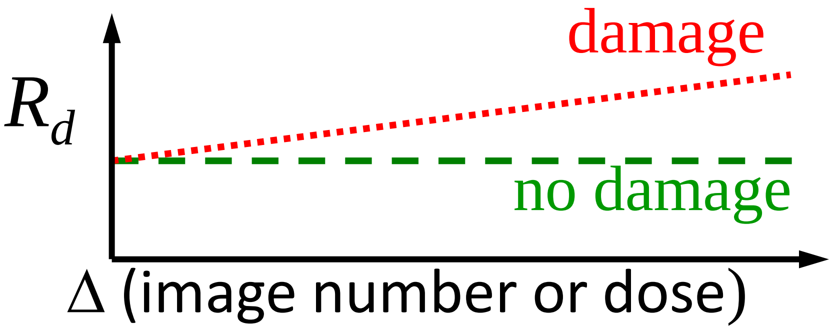
Normalised Intensity vs Dose:

apoferritin

[Owen et al 2006, PNAS]



[Diederichs 2006 Acta D59, 903]



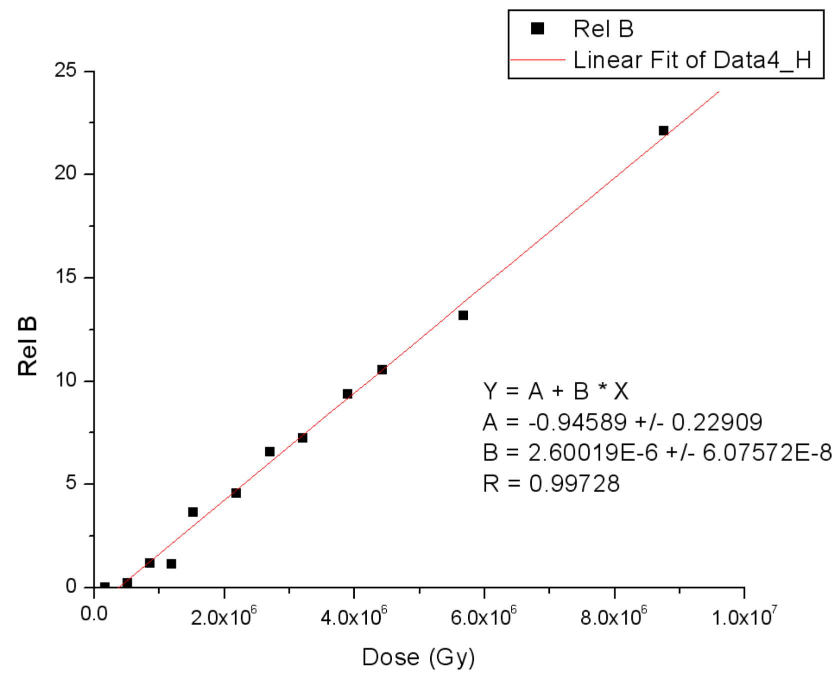
Coefficient of sensitivity α

change in relative isotropic B factor:

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

(e.g. HEWL@100 K = 0.012 Å²/Gy)

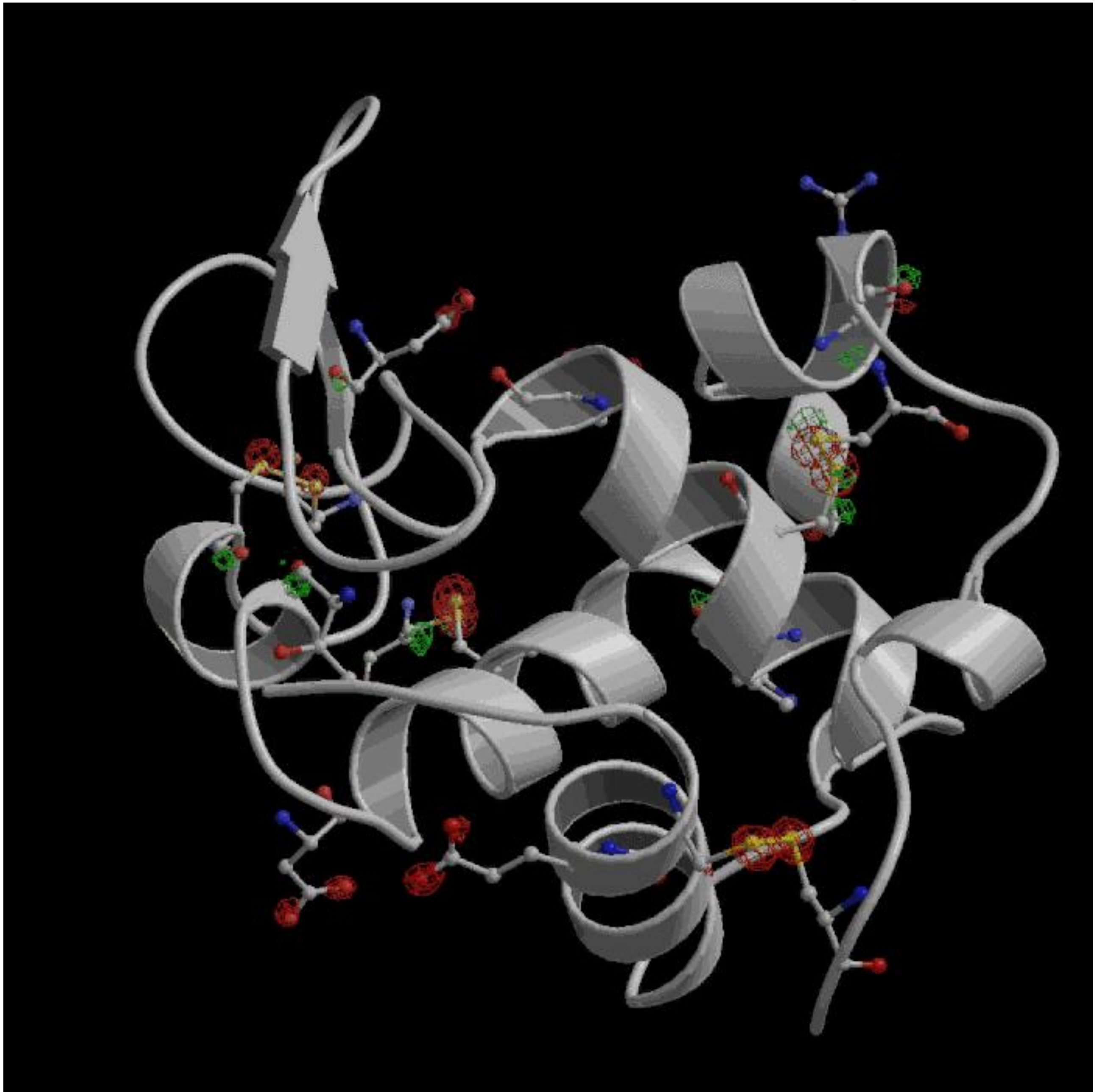
[Kmetko et al 2006, Acta D62, 1030]



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

Real Space: Specific Damage

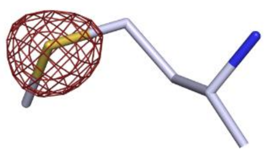
CEWL
lysozyme
Fo_4-Fo_1

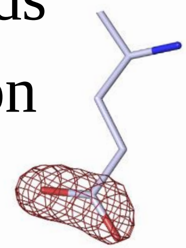


Raimond
Ravelli

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

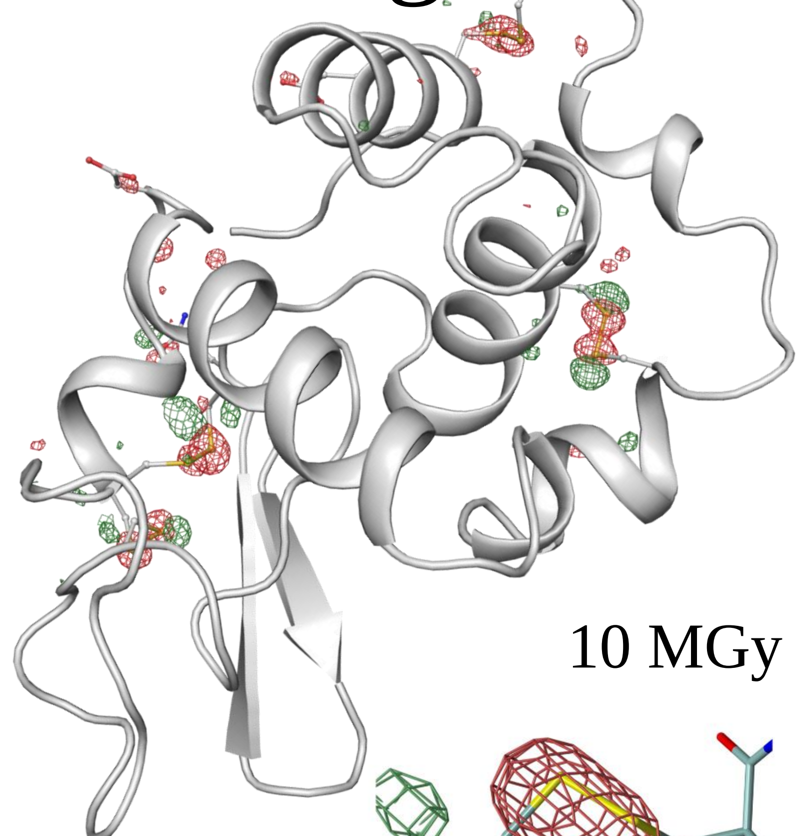




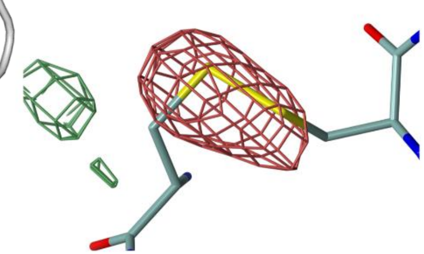
- Rupture of covalent bonds to heavier atoms:

  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.



10 MGy

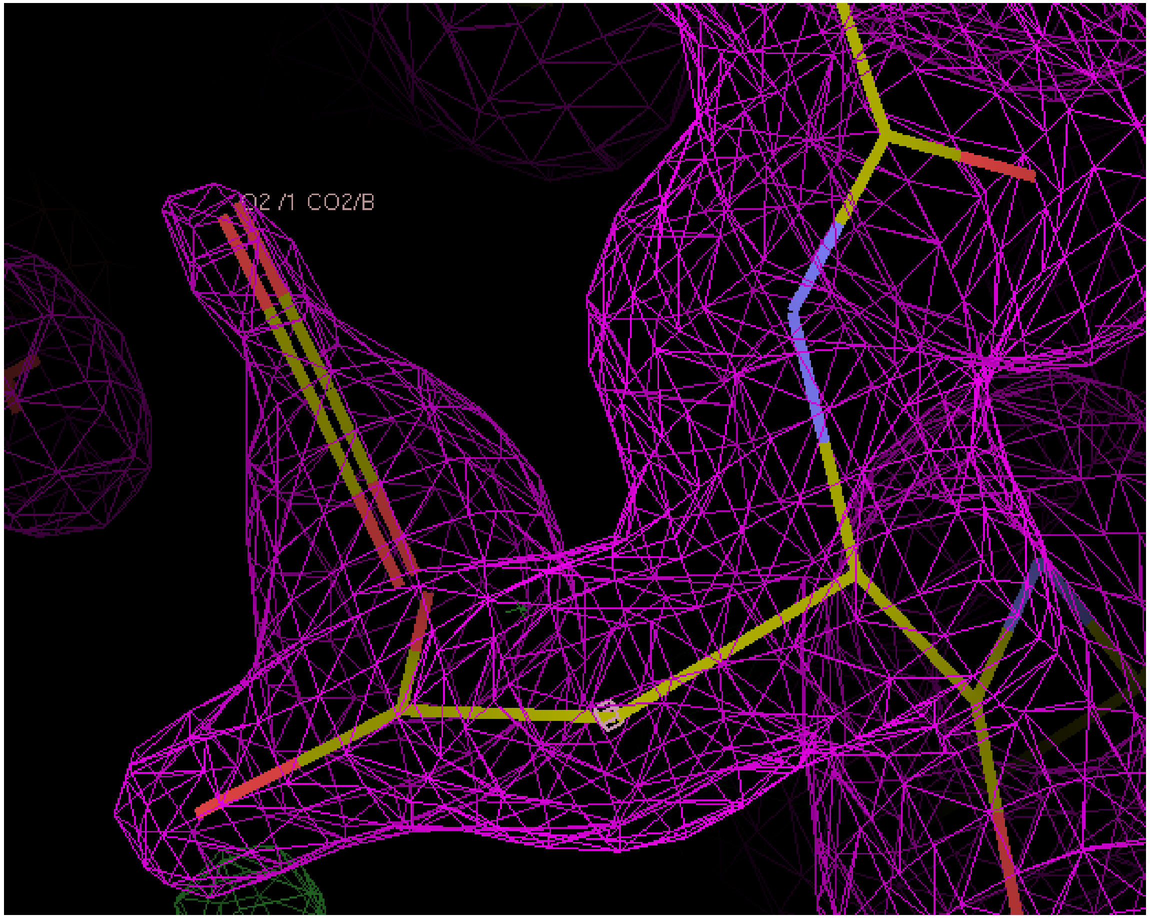


Difference map Fo_4-Fo_1

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

CO_2 from decarboxylation

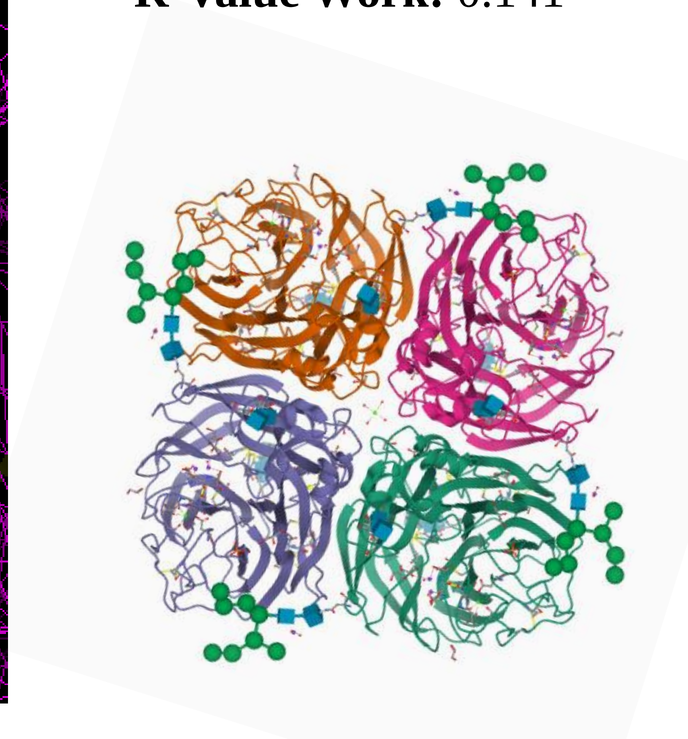
Raddam induced decarboxylation of Asp128.
0.5:0.5 occupancy of C and 2 O atoms involved
N9 influenza neuraminidase, PDB: 6HFC



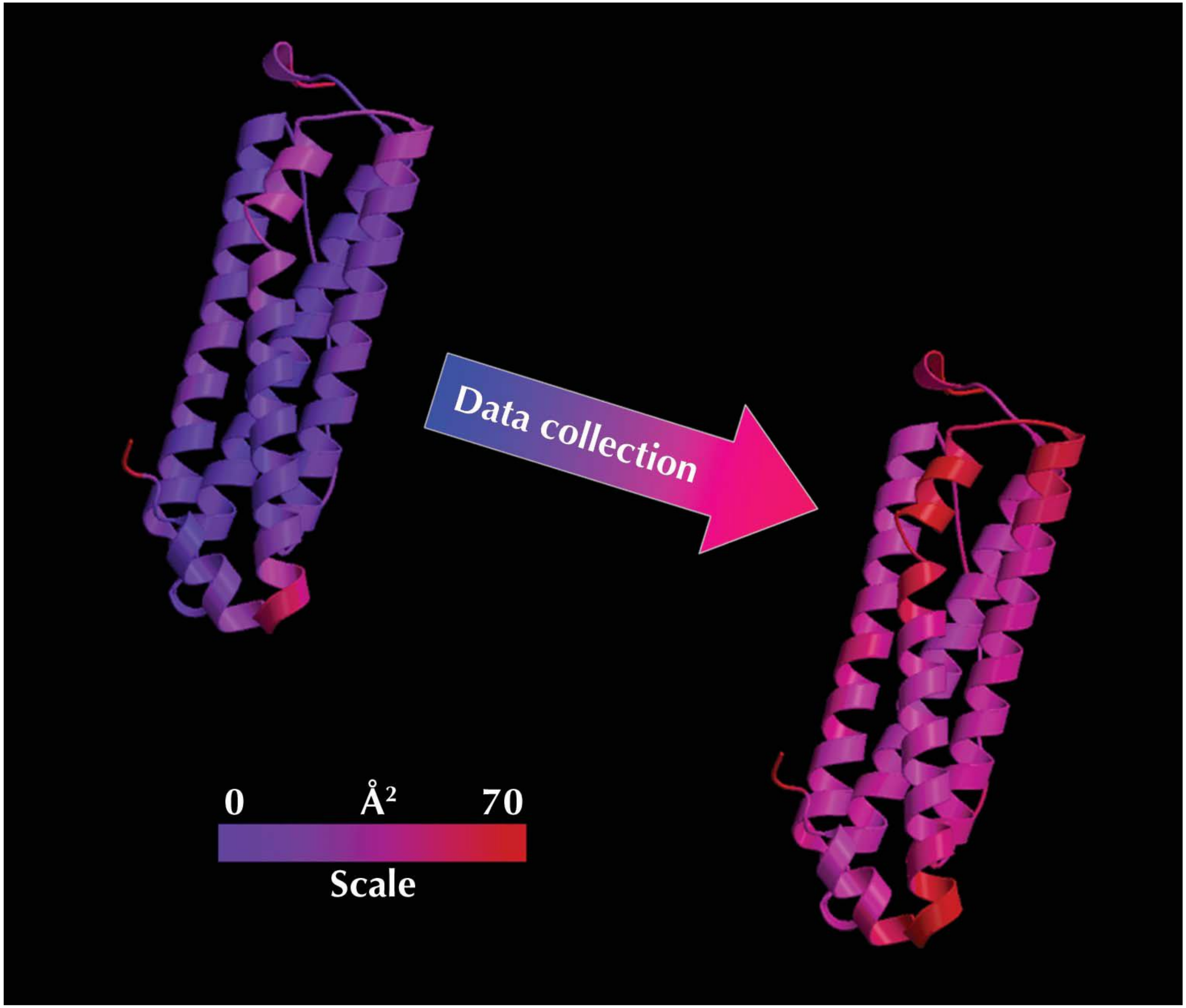
Resolution: 1.29 Å

R-Value Free: 0.164

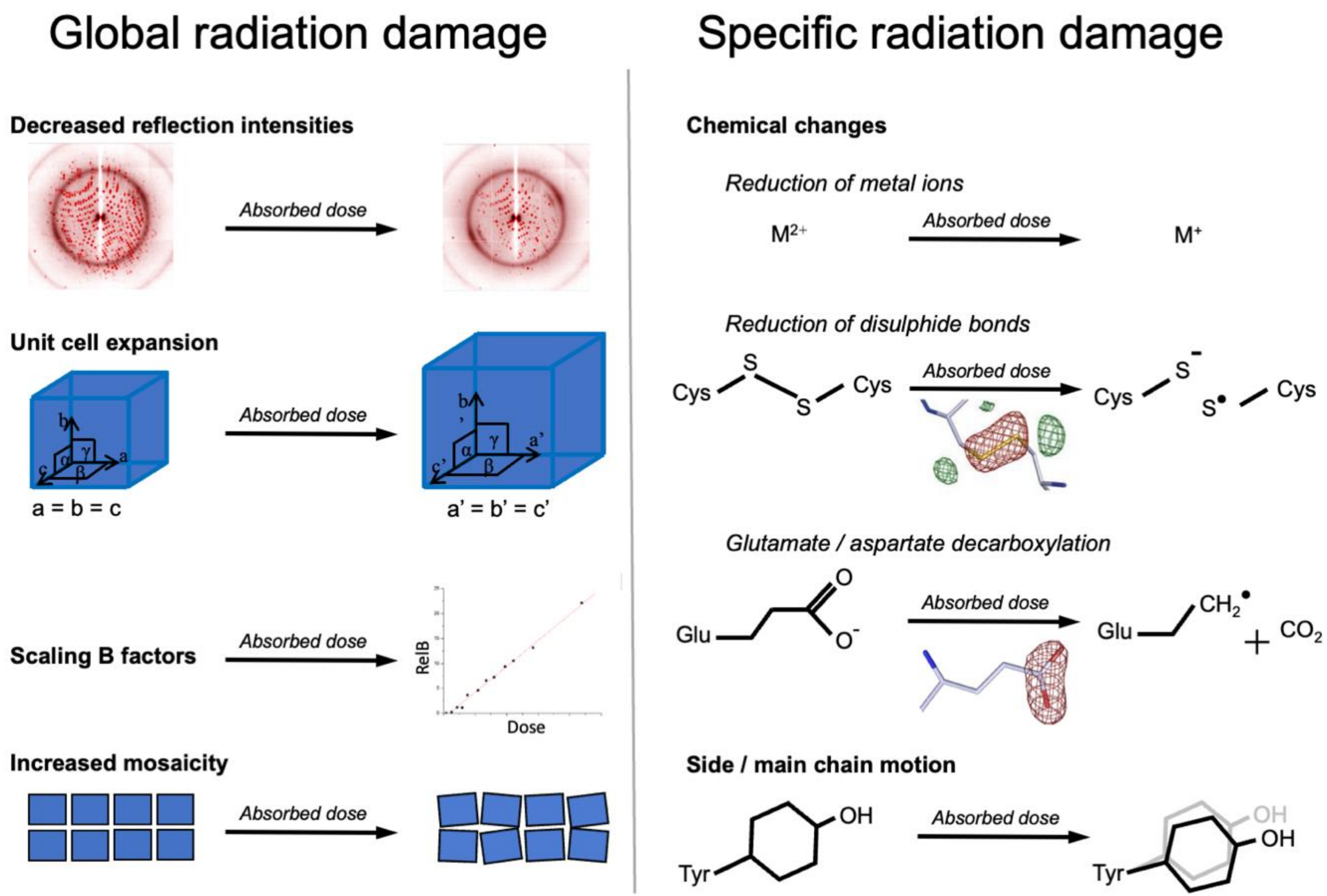
R-Value Work: 0.141



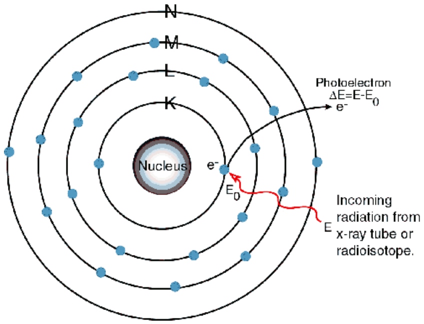
ALSO:
Atomic B-factors increase:



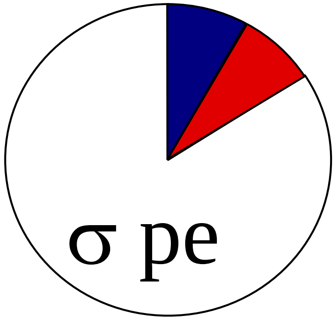
Overall summary of RD effects



Garman & Weik COSB (2023)



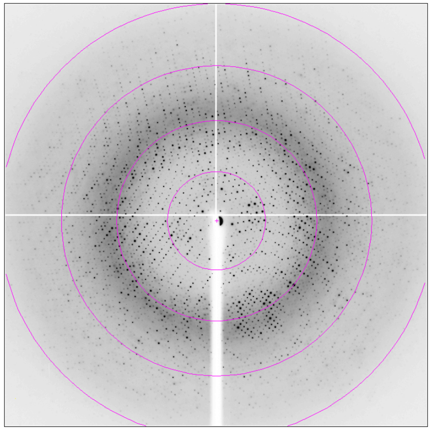
Why we cool…?



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.



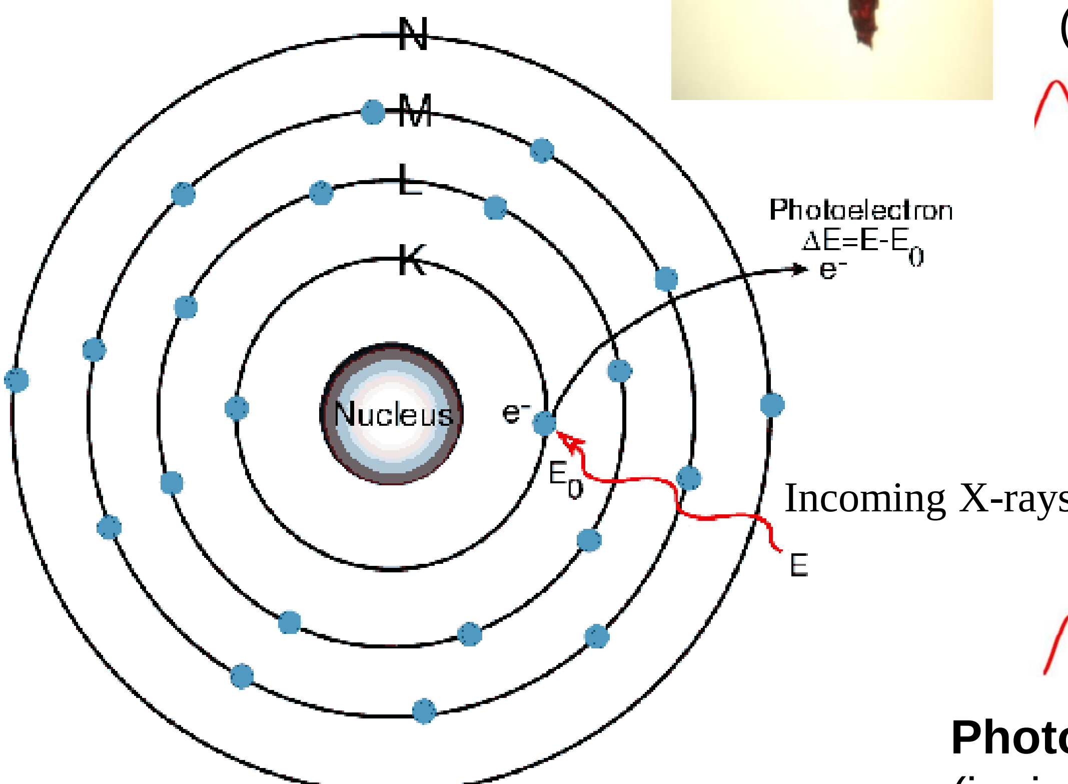
A) Diffraction

B) Absorption = Energy loss

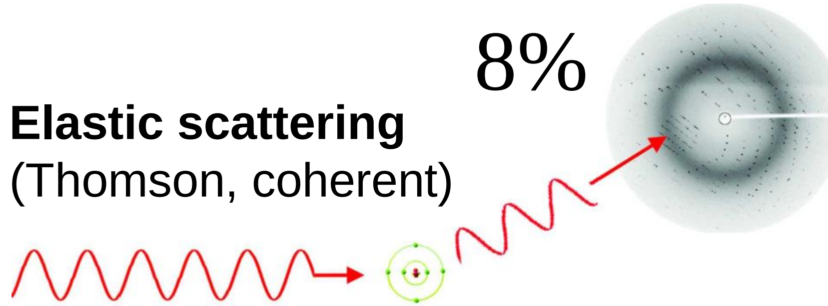
N.B. > 90% of the beam does not interact at all, but goes straight through.

Interaction of X-rays with biological samples

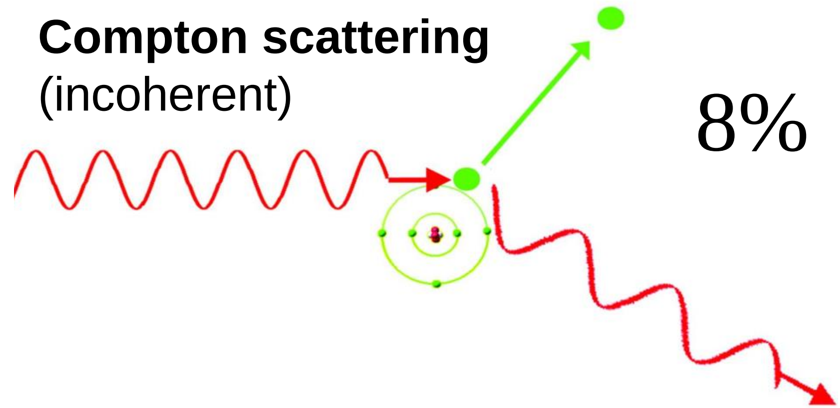
At E_x=12.4 keV, 100 μm protein crystal, only 2% of beam interacts.

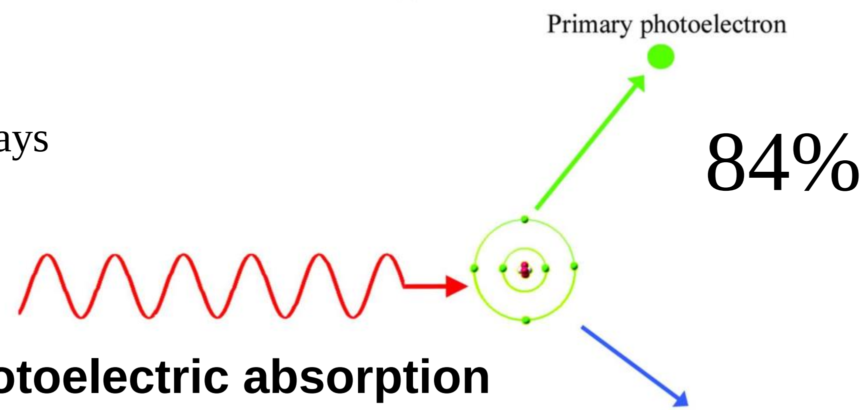


Elastic scattering
(Thomson, coherent)



Compton scattering
(incoherent)





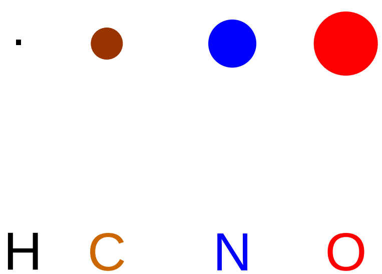
Photoelectric absorption
(ionization of up to 500 other atoms)

[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]

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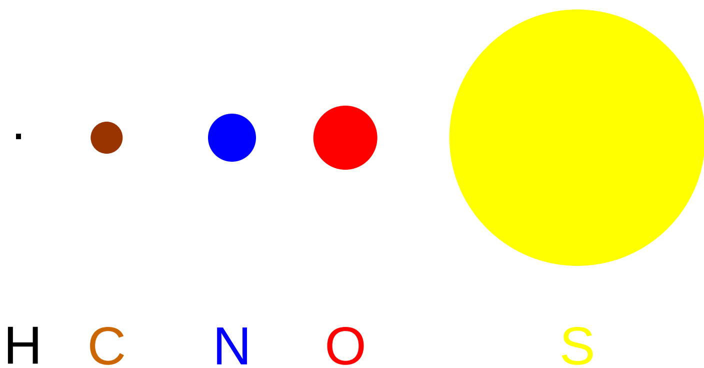

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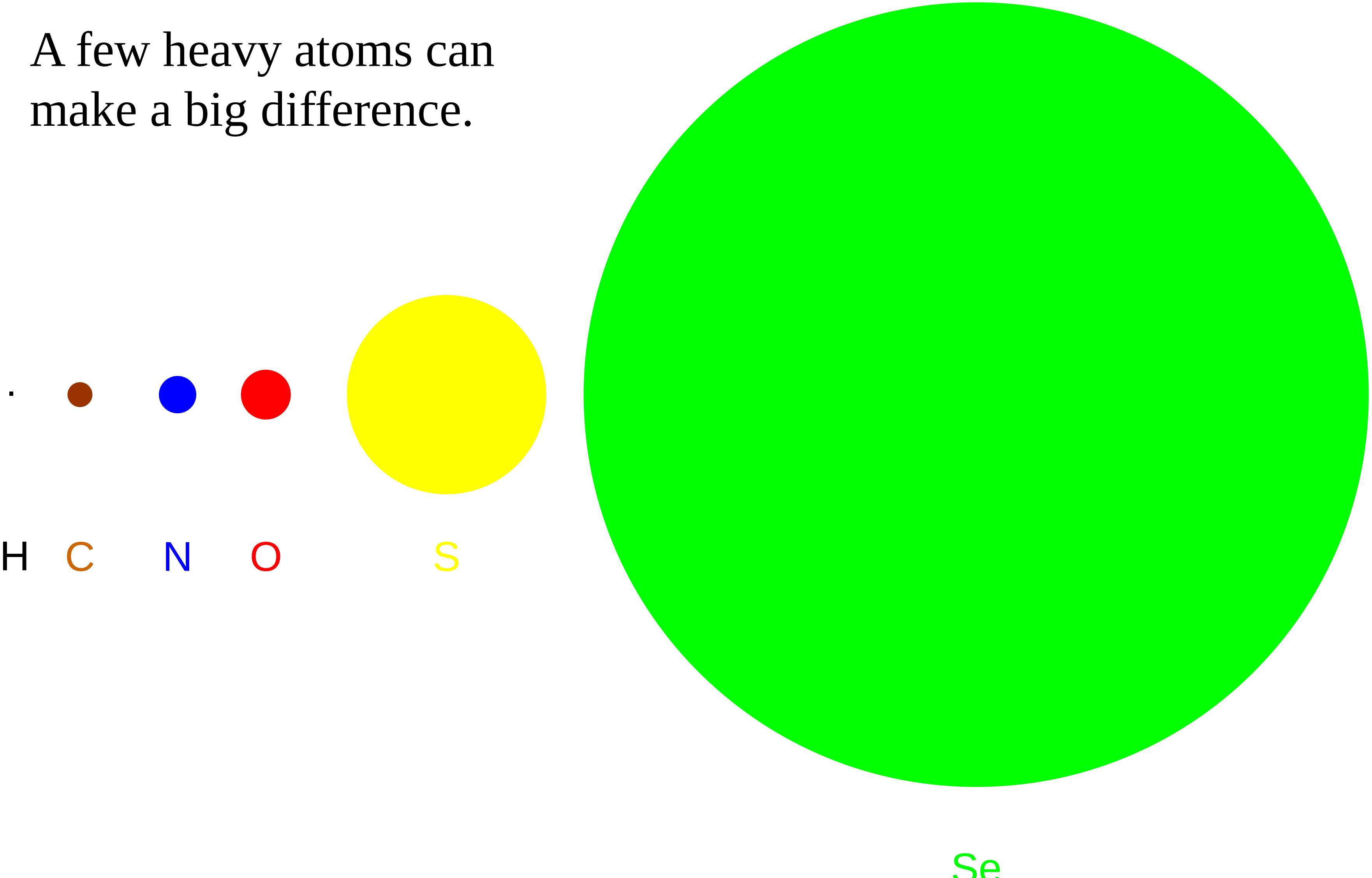
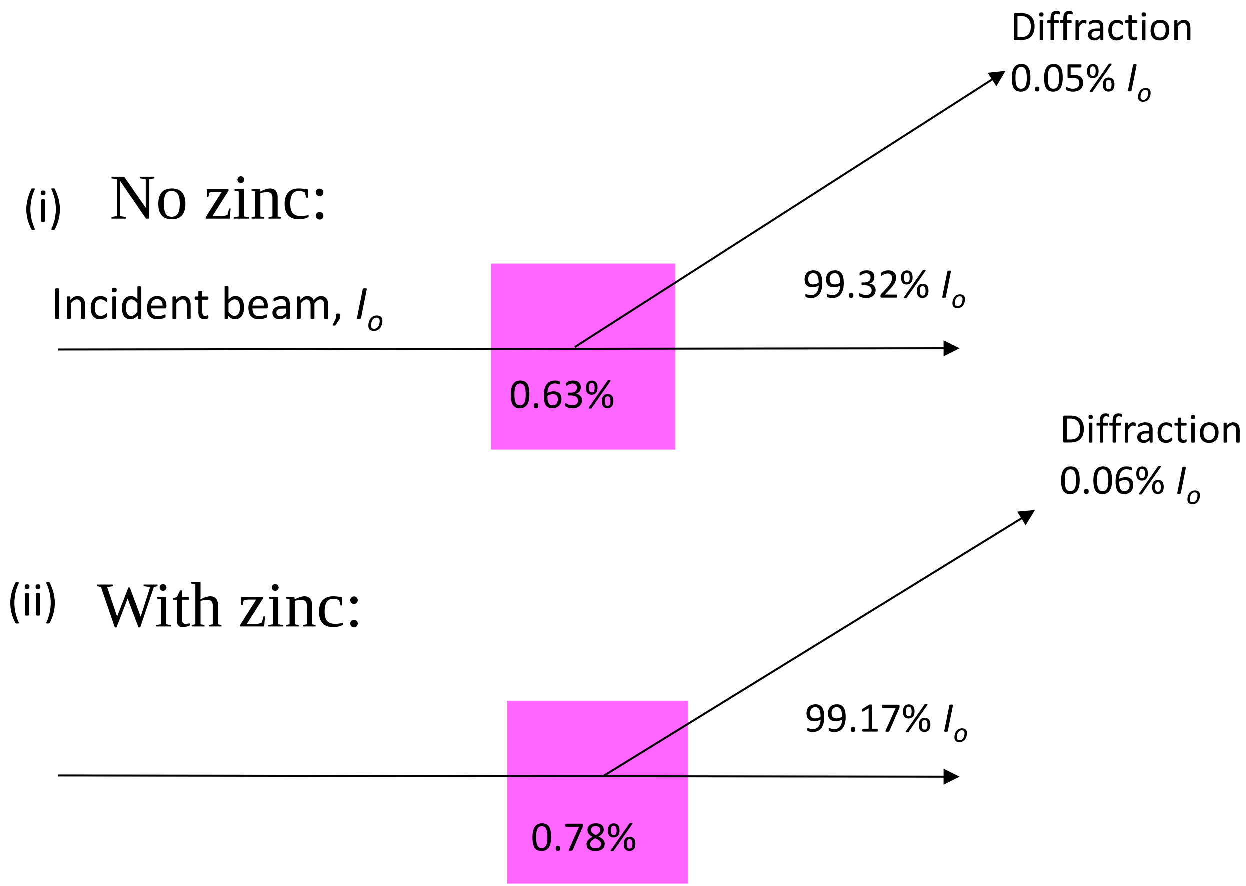


[Ravelli *et al*., JSR,(2005) 12]

Beam absorption (λ=0.95Å, 13 keV) by a Zn containing 30 μm protein (metallo-β- lactamase) crystal



Shelley & Garman, Acta D (2024) 80

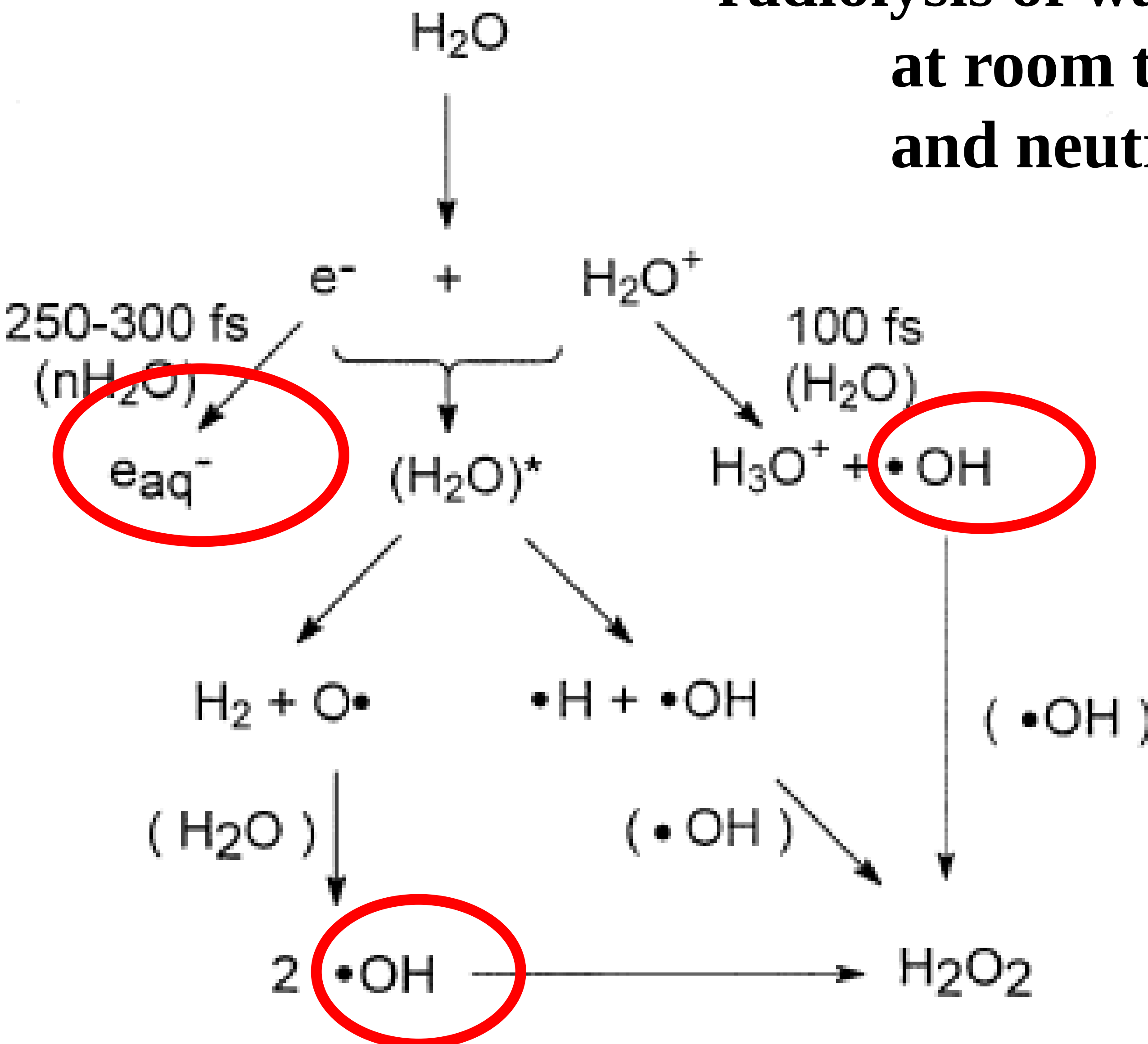
Damage: the Radiation Chemistry

1) INDIRECT RADIATION DAMAGE :

radiolysis of water

at room temperature

and neutral pH:

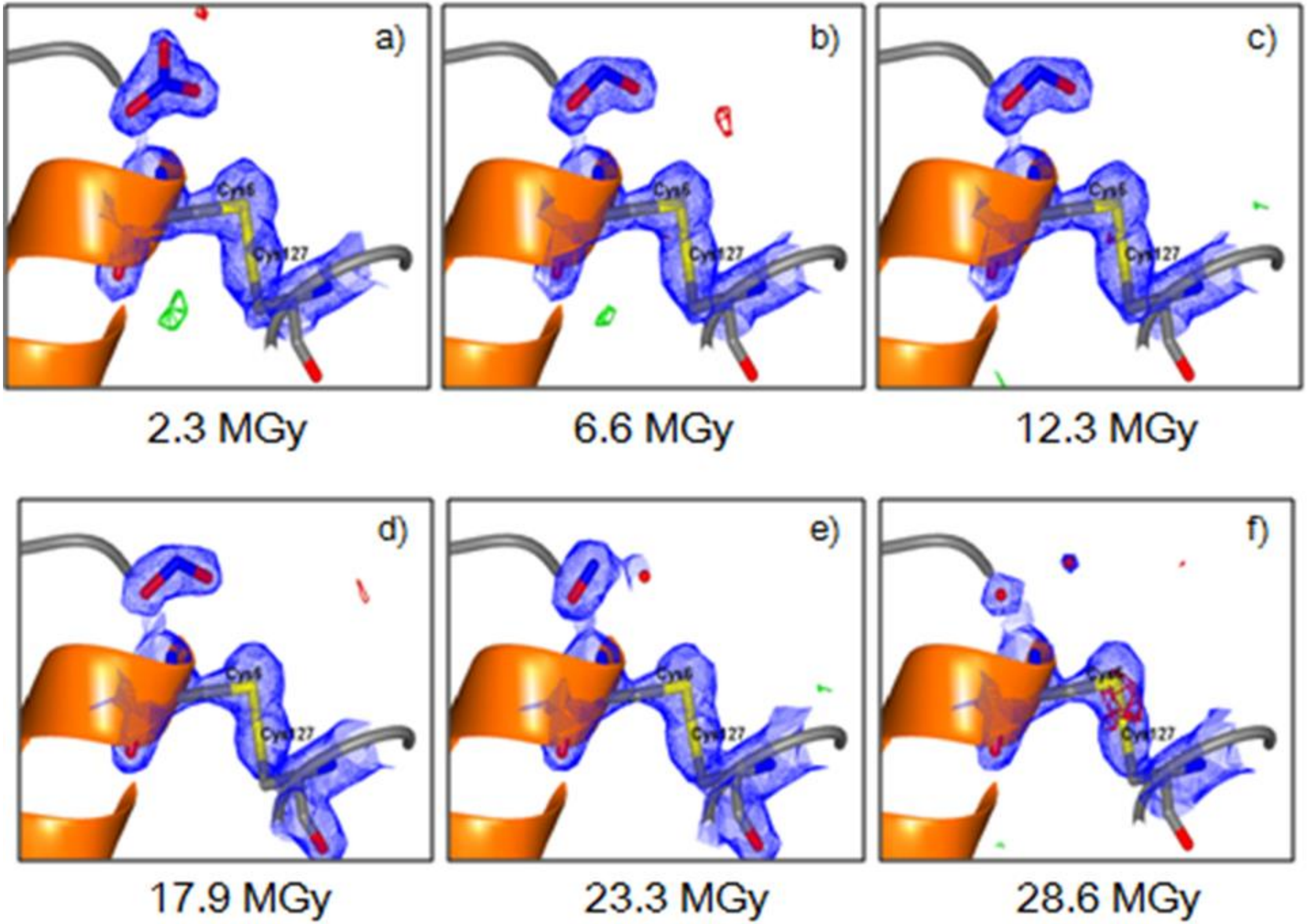


OH thought not to be mobile in glasses below 110K

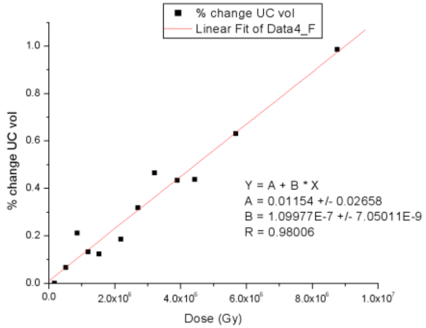
(Owen et al Acta D 2012)

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

Radiation Chemistry in action: Nitrate scavenger

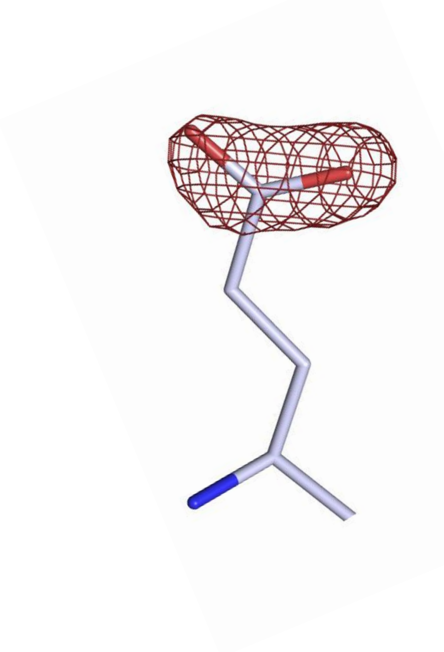


De la Mora, Carmichael, Garman *JSR* (2011) 18, 313



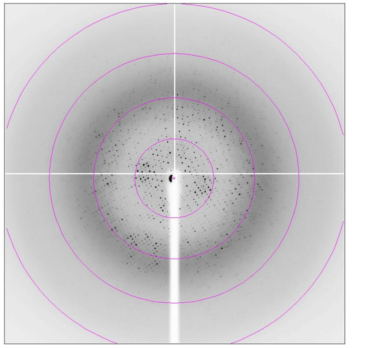
Why we cool…?

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

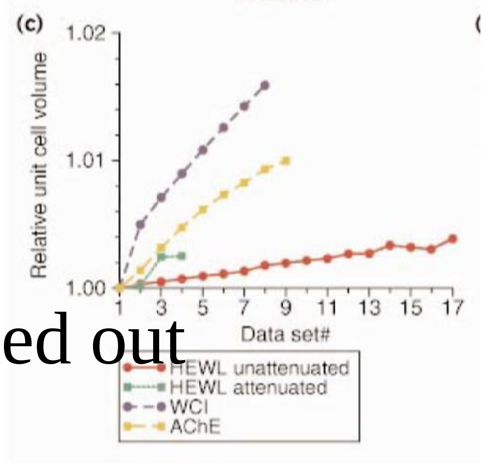
(Multi-wavelength anomalous dispersion MAD, SAD)

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**

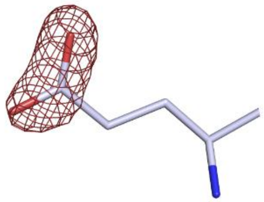
[Crick and Magdoff (1956) Acta Cryst **9**, 901-908]

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)]

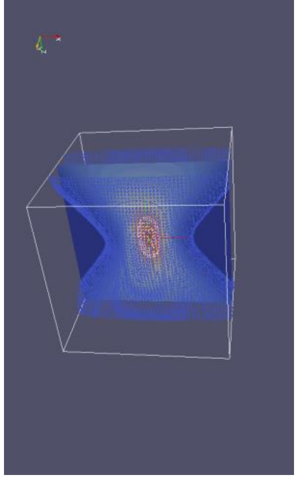
- Decarboxylation of Glu is sometimes part of the protein mechanism, but is indistinguishable from radiation damage at the synchrotron



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

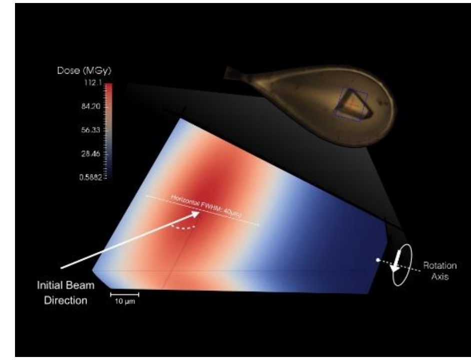
Takeda et al, Crystal structure of the M intermediate of bacteriorhodopsin…JMB (2004),

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



Why we cool…?

The Plan:



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

DOSE Limit Postulate

(Henderson 1990):

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

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

tolerated by protein crystals: depends only on PHYSICS of 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*

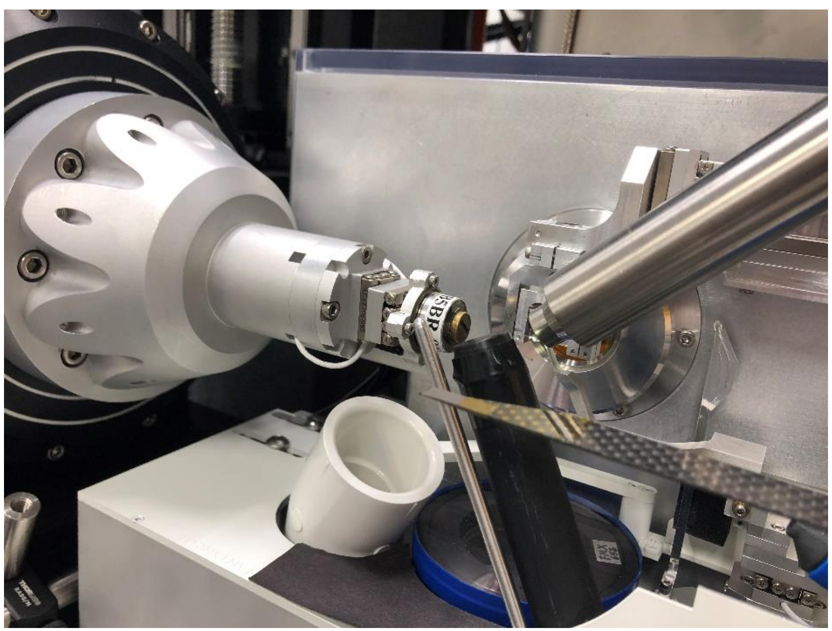
V3: Dickerson and Garman (2021) *Protein Science*

V4: Dickerson et al. (2024) *Protein Science*

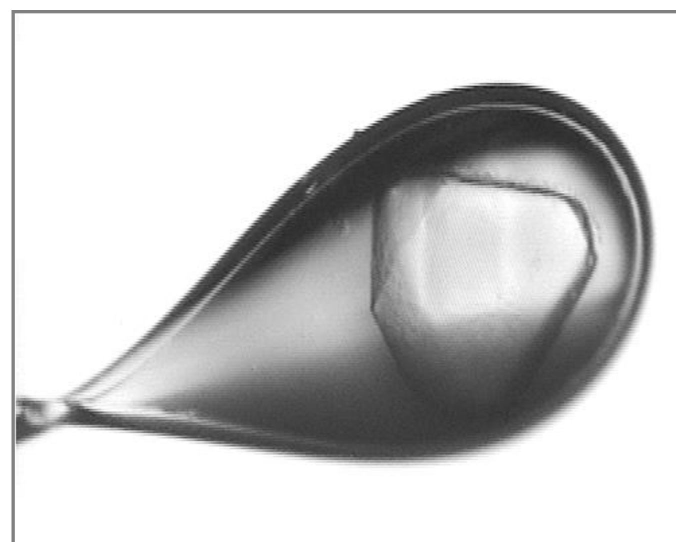
Make the dose calculation convenient for MX (include solvent contribution in mM and heavy atoms explicitly)

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

The beam

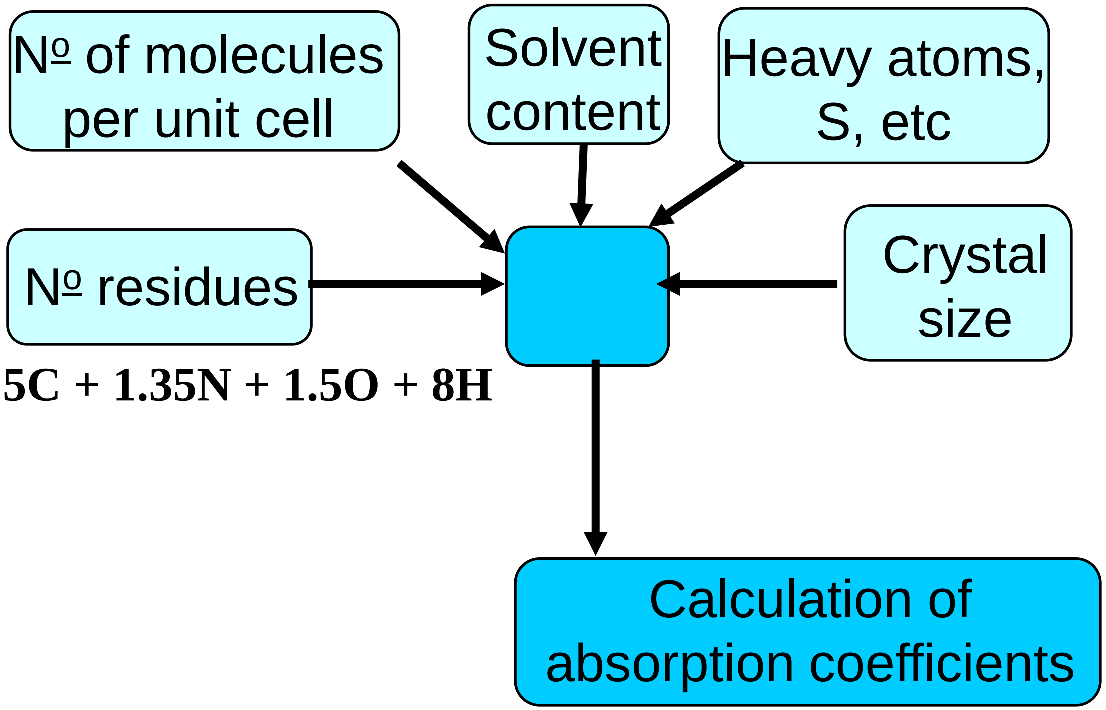


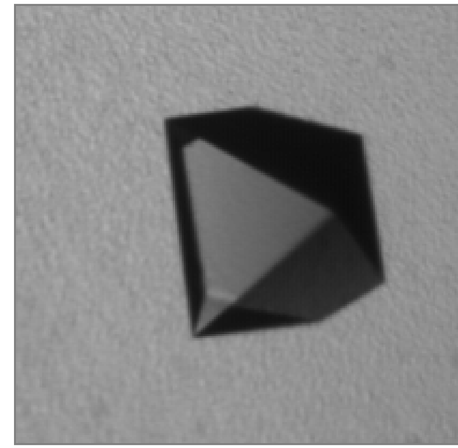
The crystal



Calculating Dose
(*RADDOSE-3D*)

Crystal Characteristics



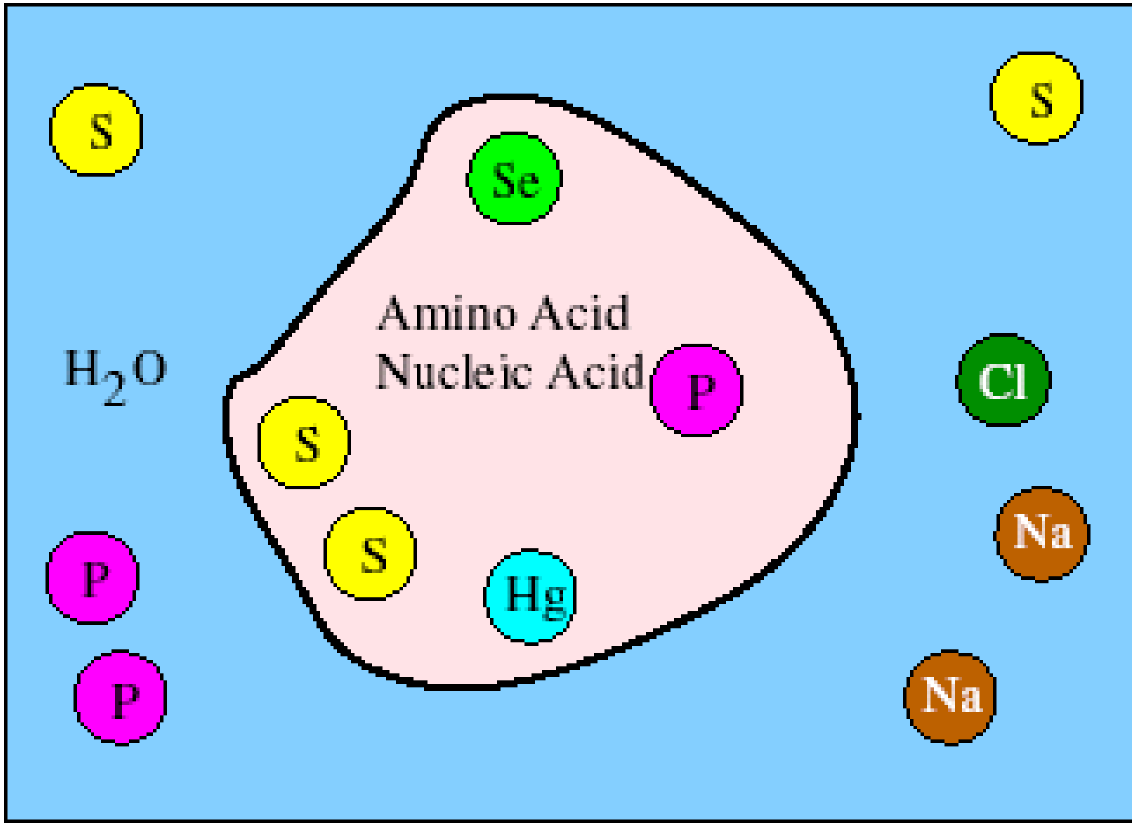


200 µm

absorption coefficients

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

holoferritin: 1.133mm^{-1}

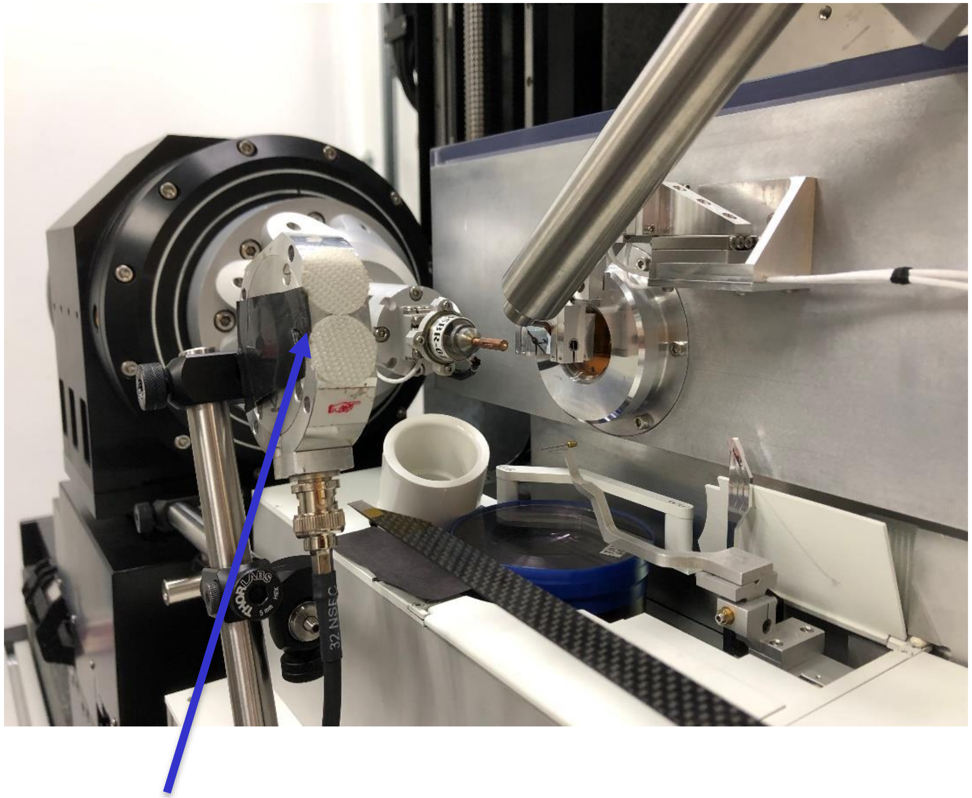


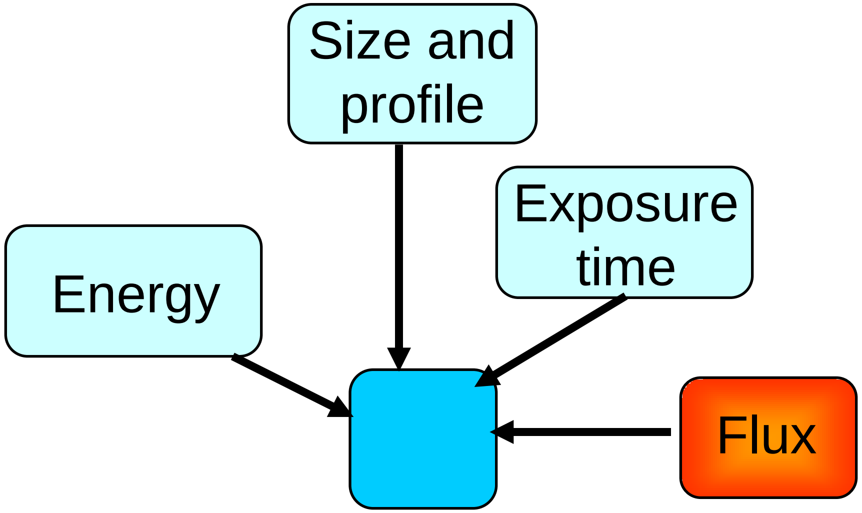
Number of Amino Acids

'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)

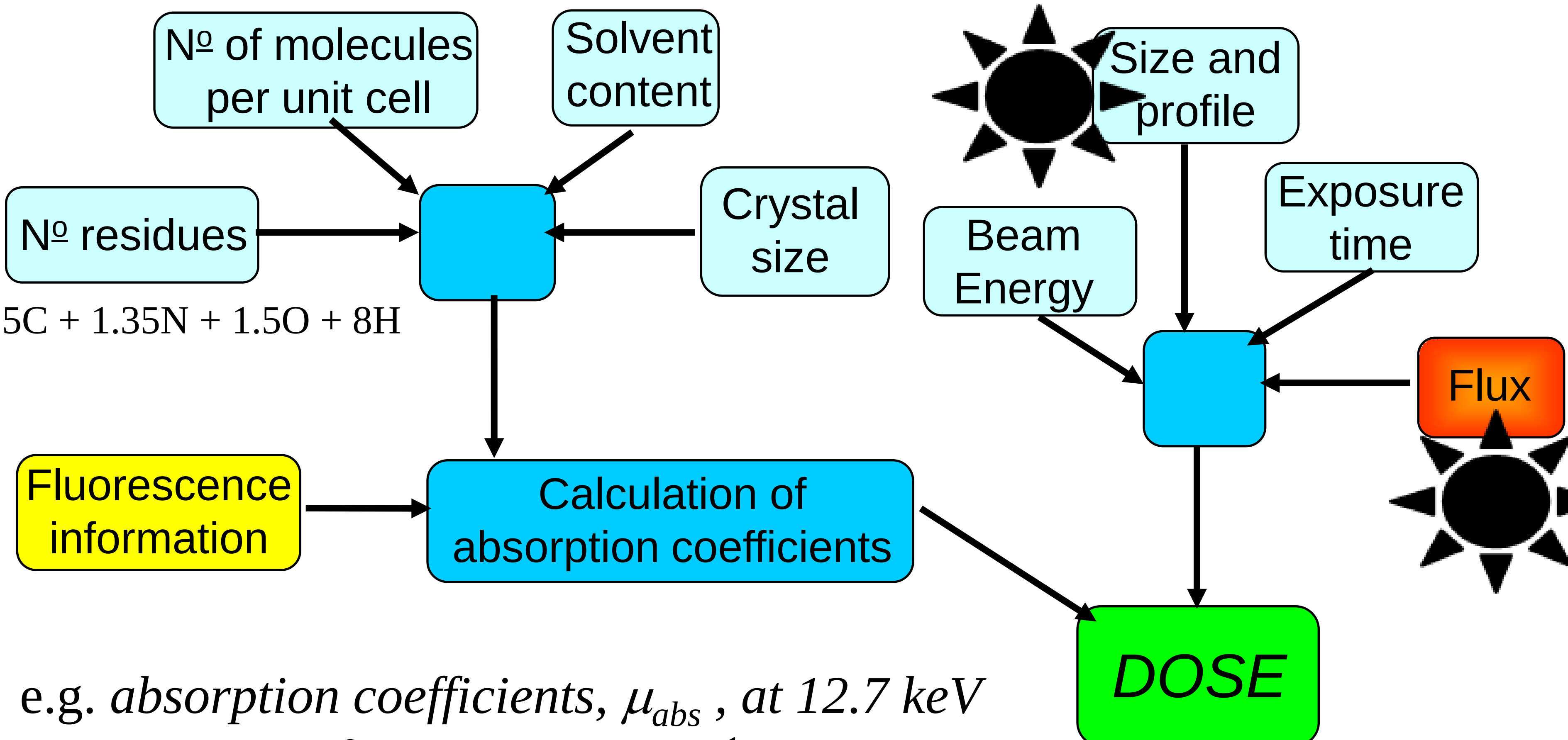
Owen et al JSR 2009

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*

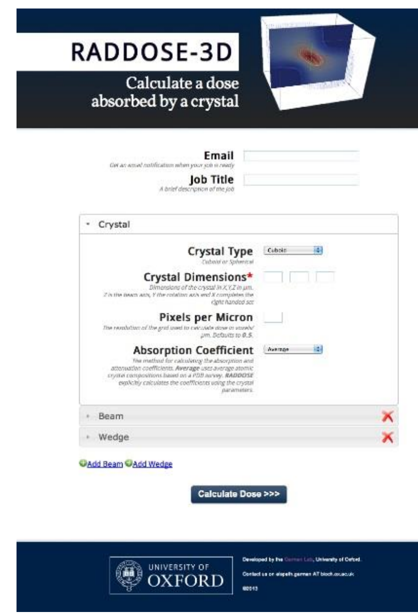
apoferritin: 0.406mm^{-1}

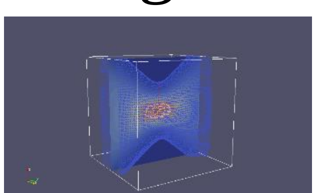
holoferritin: 1.133mm^{-1}

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

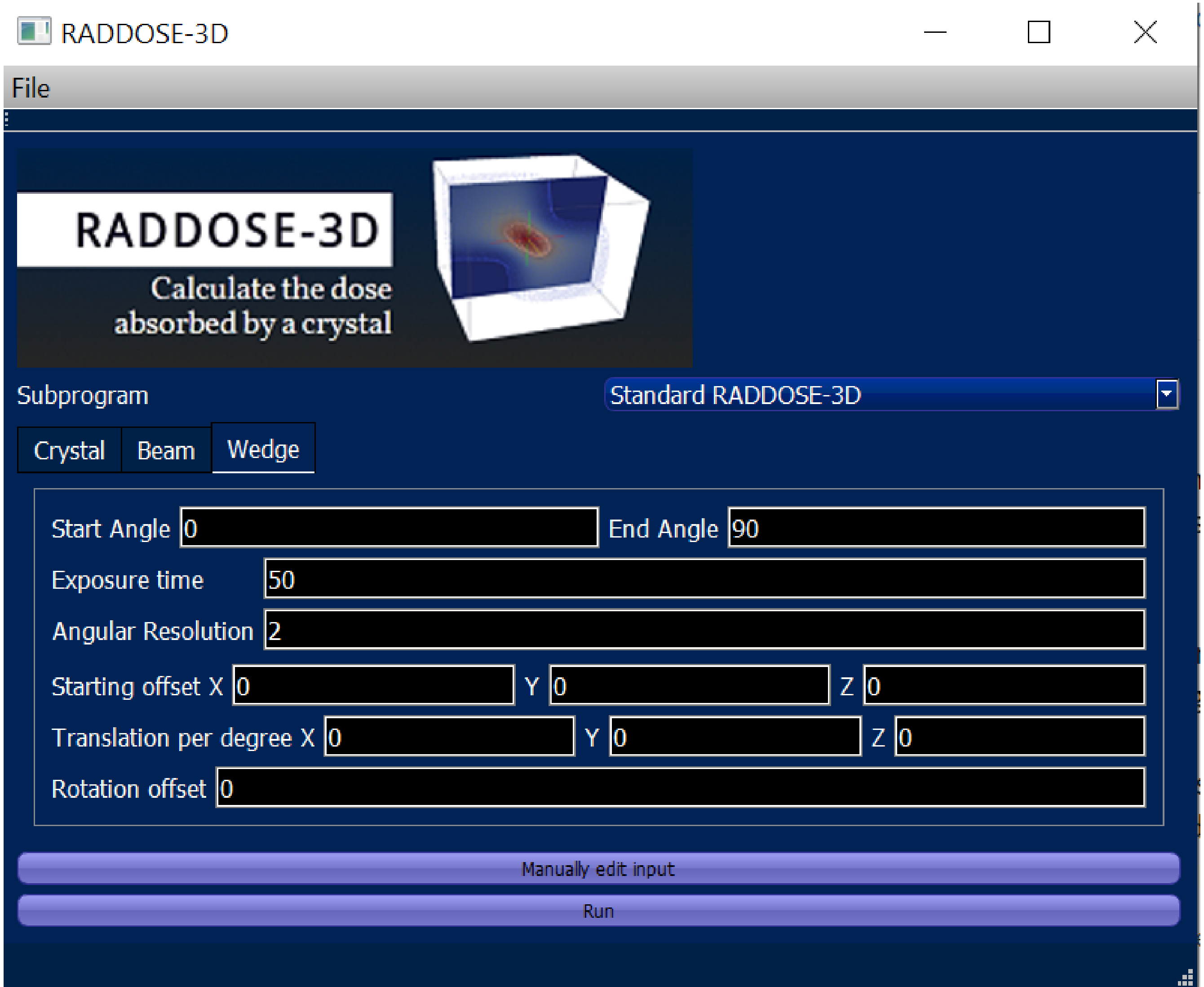




[Zeldin, Gerstel, Garman *JAC* (2013)
Bury, Brooks-Bartlett, Walsh, Garman, *Protein Science* (2018)]

RADDOSE-3D New GUI

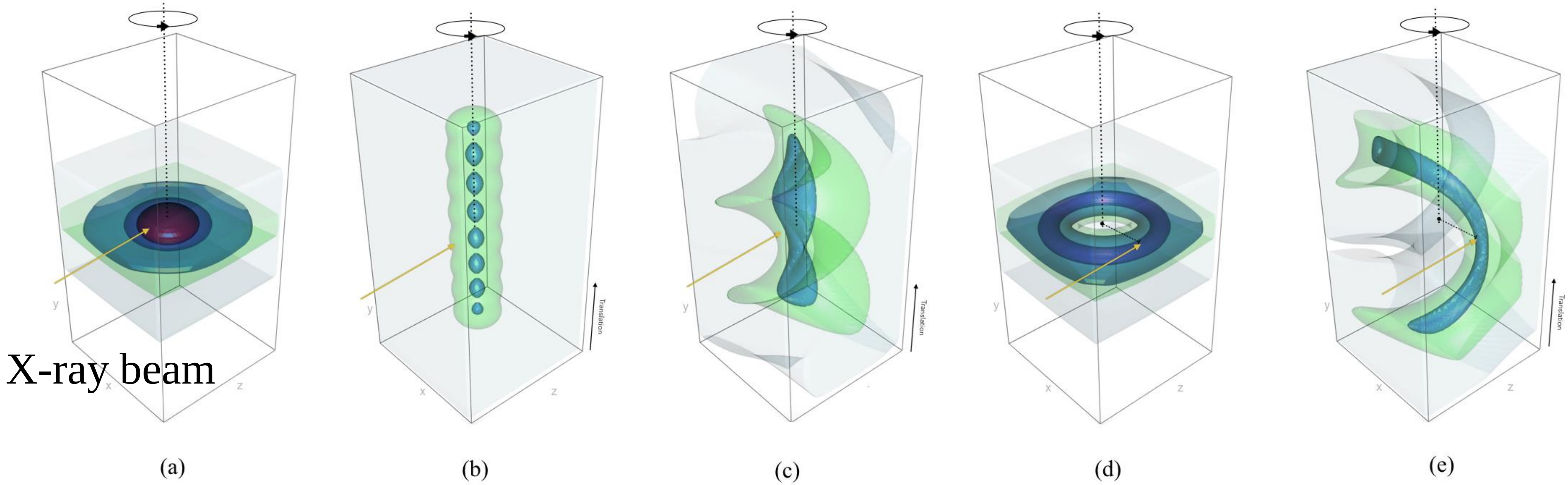
Download from: //https://github.com/GarmanGroup/RADDOSE-3D
Versions for a PC and for Linux, no version for a Mac (anyone interested?!!):
RD3D_GUI_Windows.exe and RD3D_GUI_linux



Josh Dickerson

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

Dose distribution vs exposure strategy with RADDOSE-3D

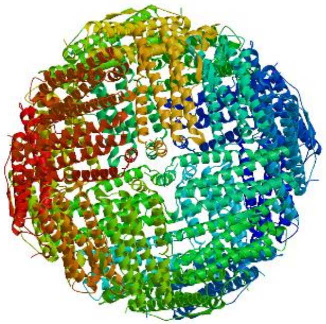


(b)	(c)	(d)	(e)
20 μm translation	Helical, 0.2 μm/ °	30 μm offset	30 μm offset and helical 0.2 μm/ °

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

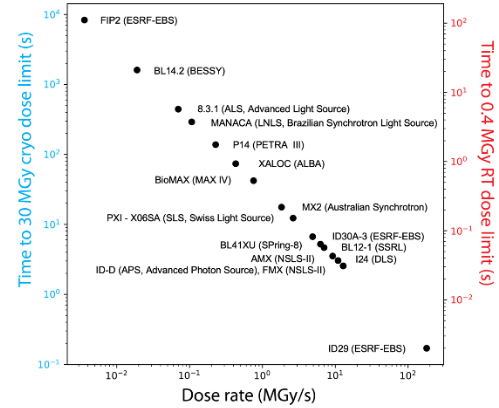
Helical strategy improvement:
Flot *et al* (2010) *JSR* **17**, 107

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



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?

Typical MX experiment

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

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

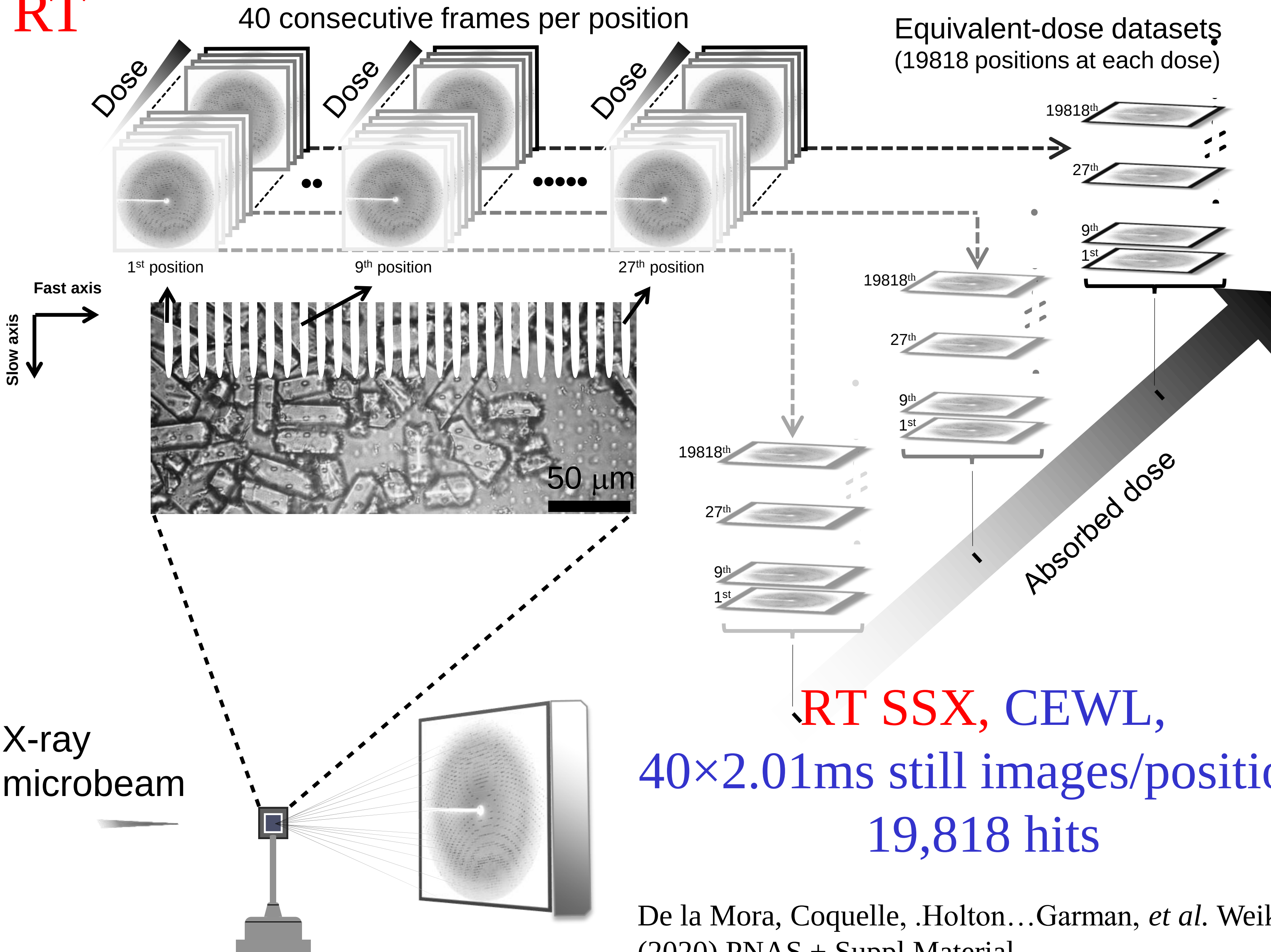
MX at 100 K: 30 MGy experimental dose 'limit' (dose to 0.7 I_n/I_1) reached in ~30 s: 4th generations sources <<1s,

MX at XFELs: damage before destruction ? <80 fs

2-10 Gy, dies in 10-30 days
Radiotherapy on human brain tumour max 60 Gy

[Shelley & Garman Acta D 2024]

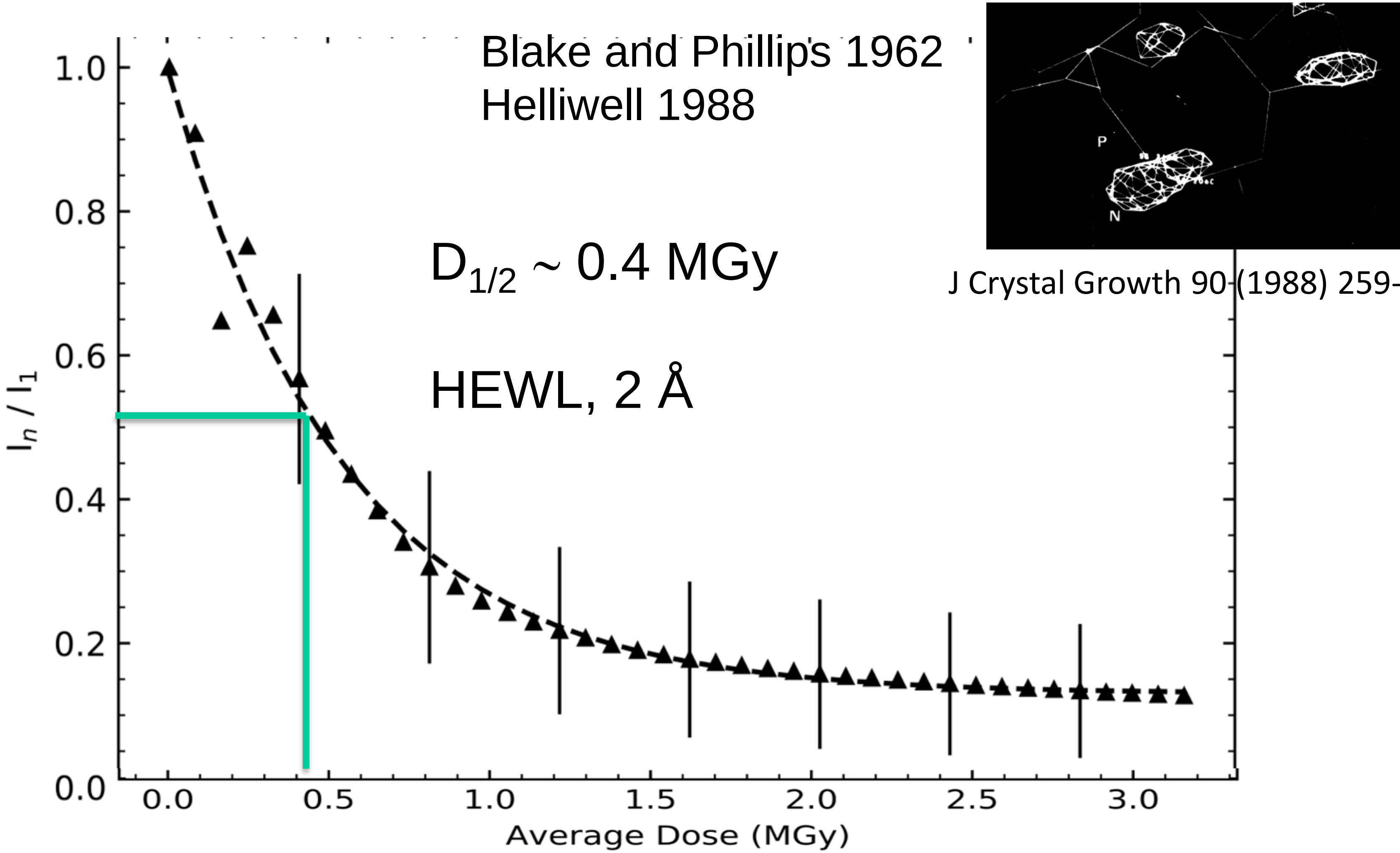
RT



RT SSX, CEWL,
40×2.01ms still images/position,
19,818 hits

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

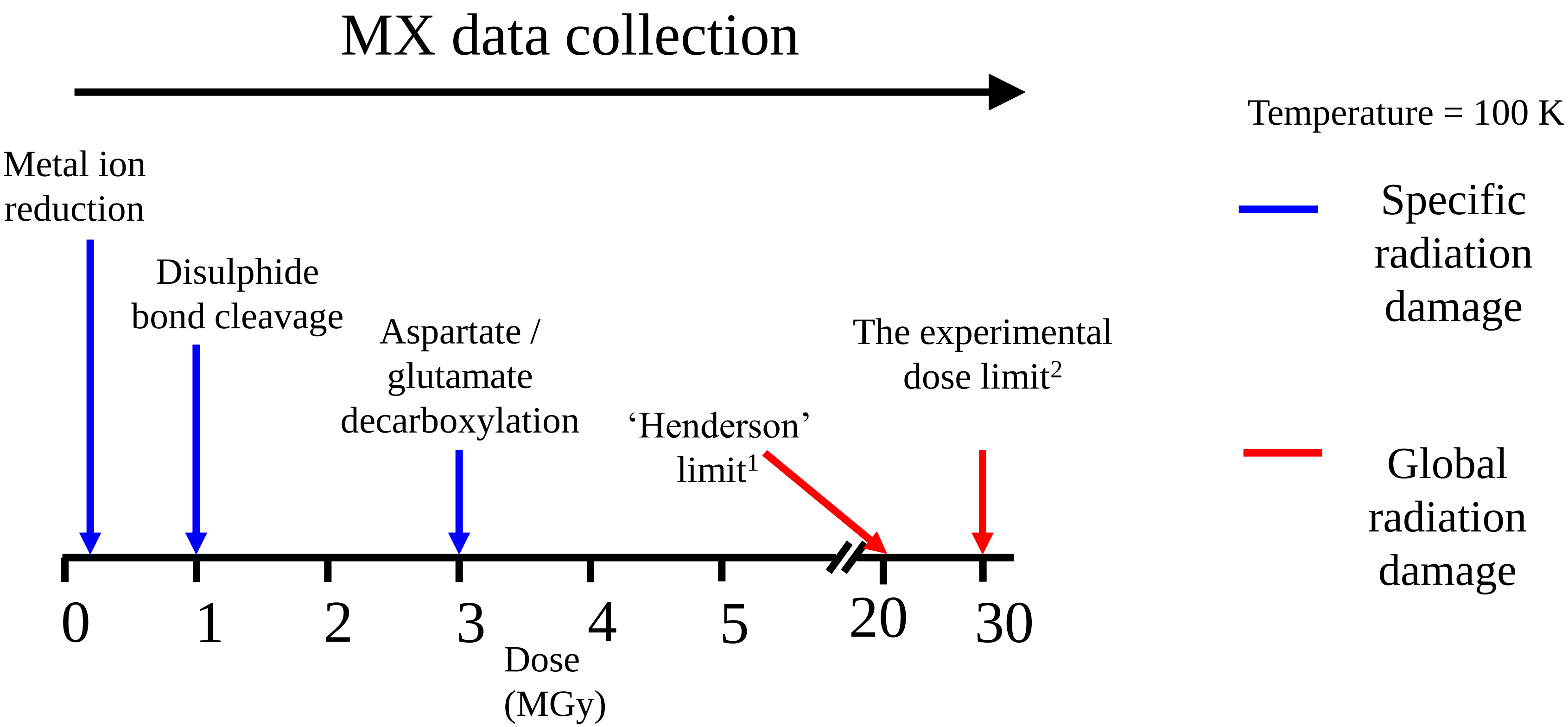
RadDam signatures in reciprocal space (RT MX)



J Crystal Growth 90 (1988) 259-272

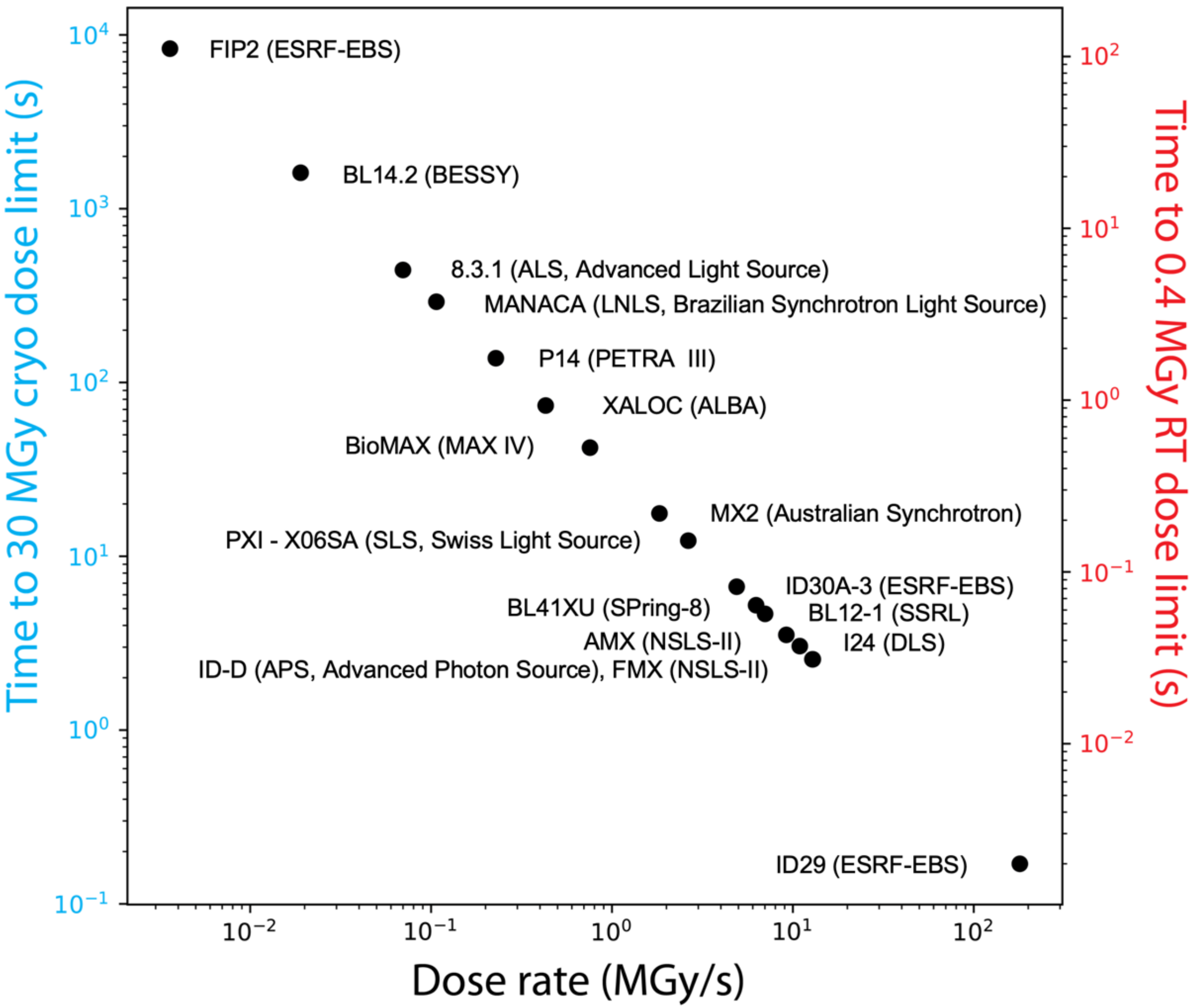
Radiation damage and dose limits in serial synchrotron crystallography at cryo- and room temperatures. De la Mora, Coquelle *et al.* (2020) PNAS

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



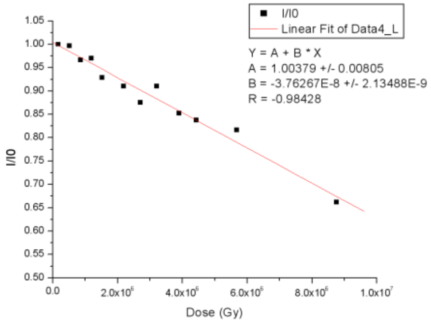
[1. Henderson Phil Trans RS B 1990 2. Owen *et al* PNAS 2006]

Time to dose limits at current synchrotrons



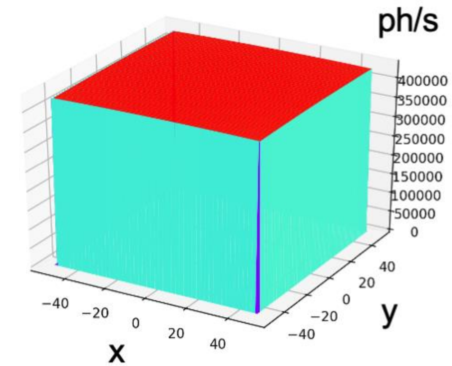
Average diffraction weighted doses (DWD) were calculated for a (50 μm)^3 lysozyme crystal (grown in 100 mM NaAc and 1M NaCl, solvent fraction 38%) rotated 360° in the flux within the FWHMs of the various beams.

Garman & Weik COSB (2023)



Why we cool…?

The Plan:



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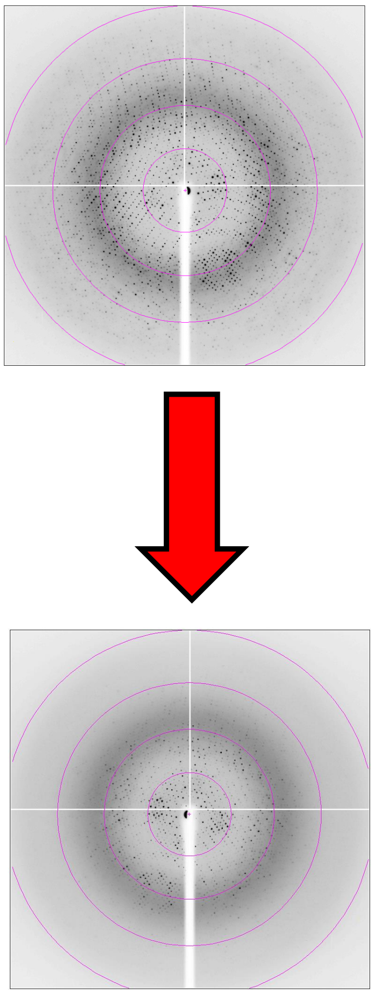
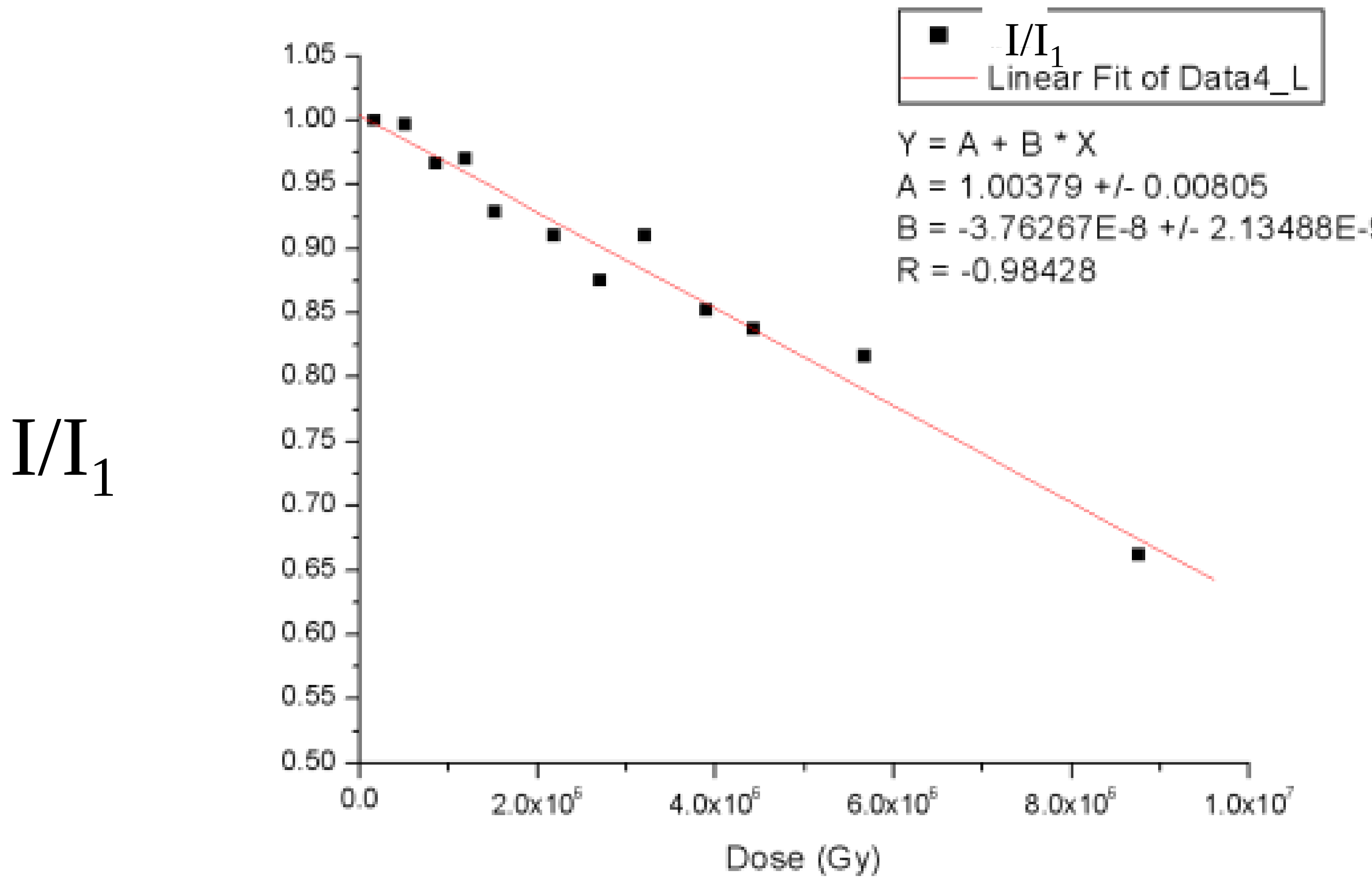
- How do we estimate the Dose?
- The limits
- **What can you do to minimise it?**

1) Sacrificial crystal to characterise damage rate

Diffraction fading

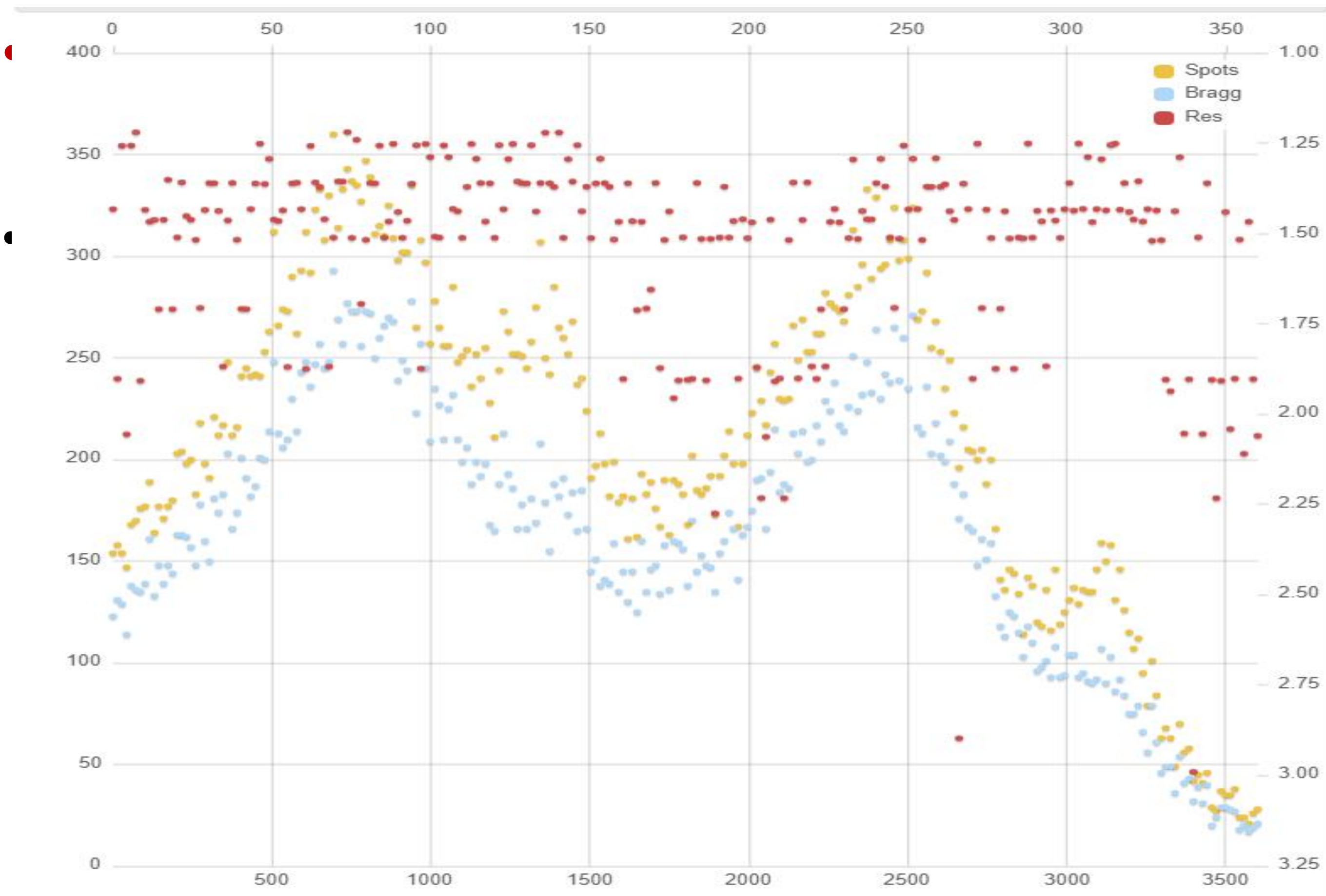


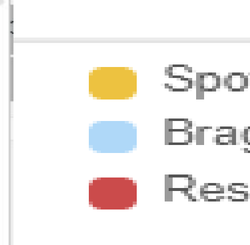


Dose = absorbed energy (J) / mass (kg)

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.





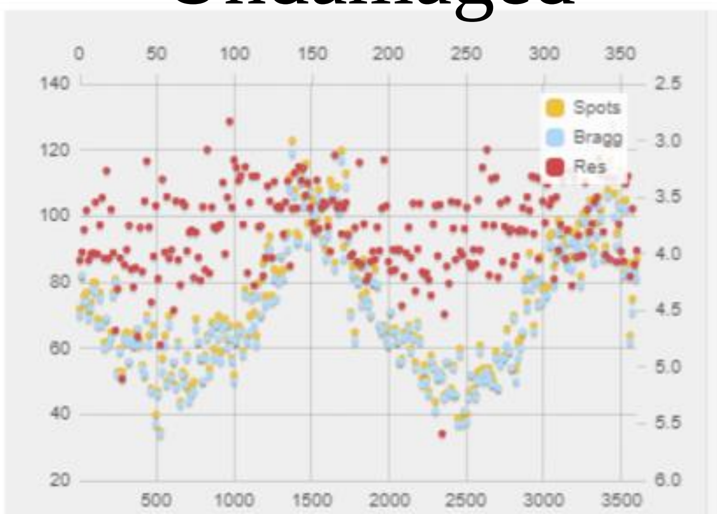
O

DLS
Ed Lowe

RadDam signatures in reciprocal space

Undamaged

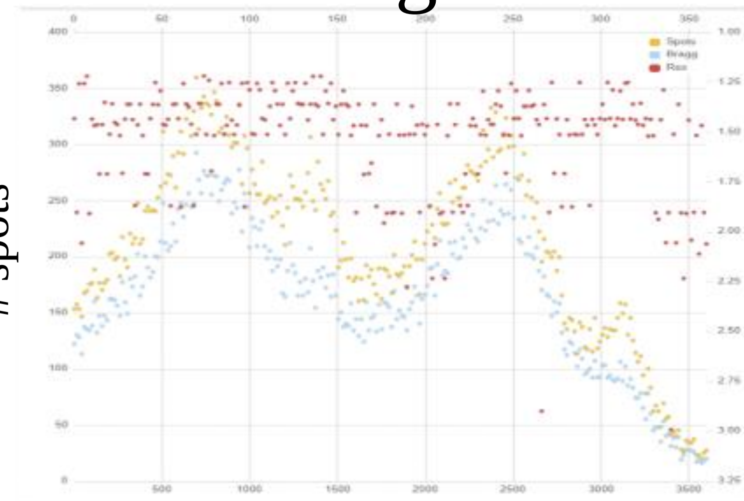
spots



Resolution Å

Damaged

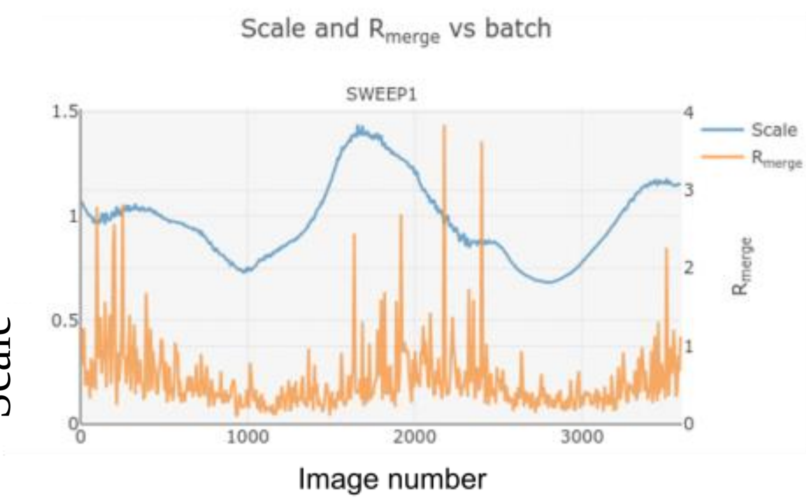
spots



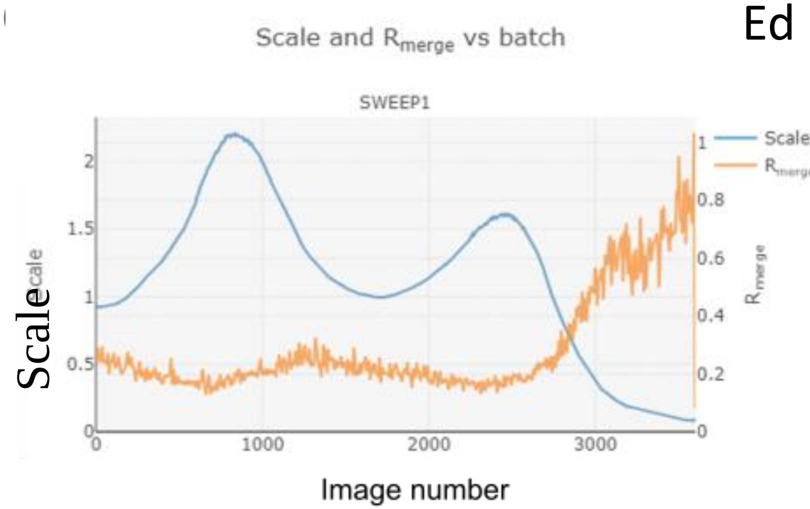
Resolution Å

DLS
Ed Lowe

Scale



Scale



Shelley & Garman, Acta Cryst D (2024)

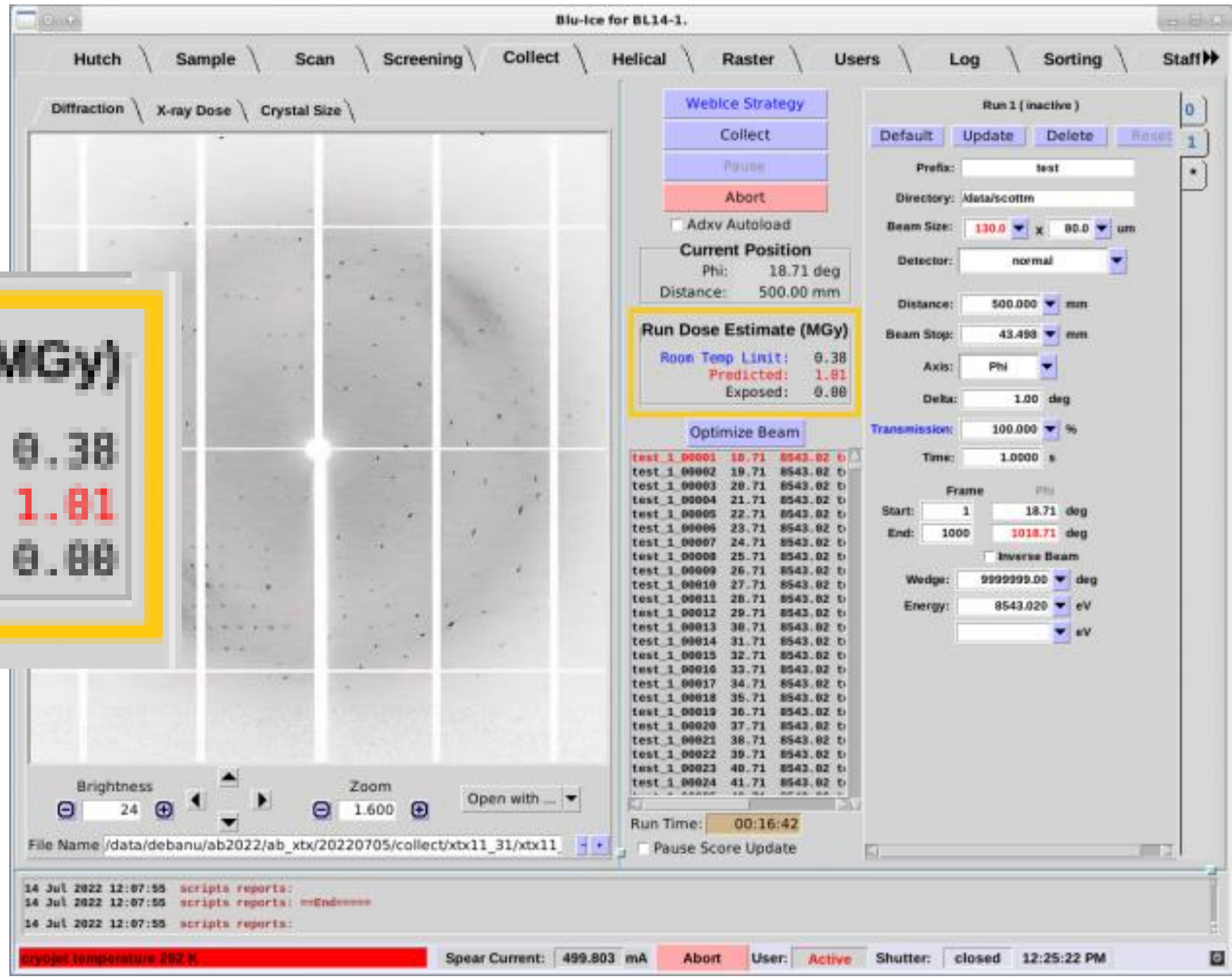
3) DOSE AWARE data collection

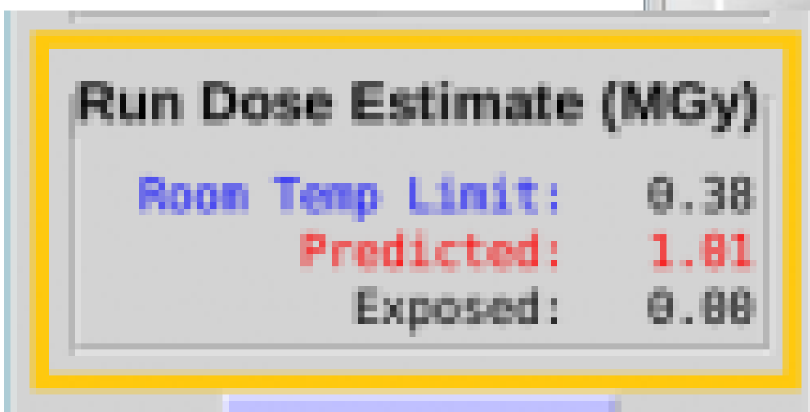
Some beamlines worldwide are running RADDOSE-3D ‘underneath’ to allow collection by DOSE, not TIME

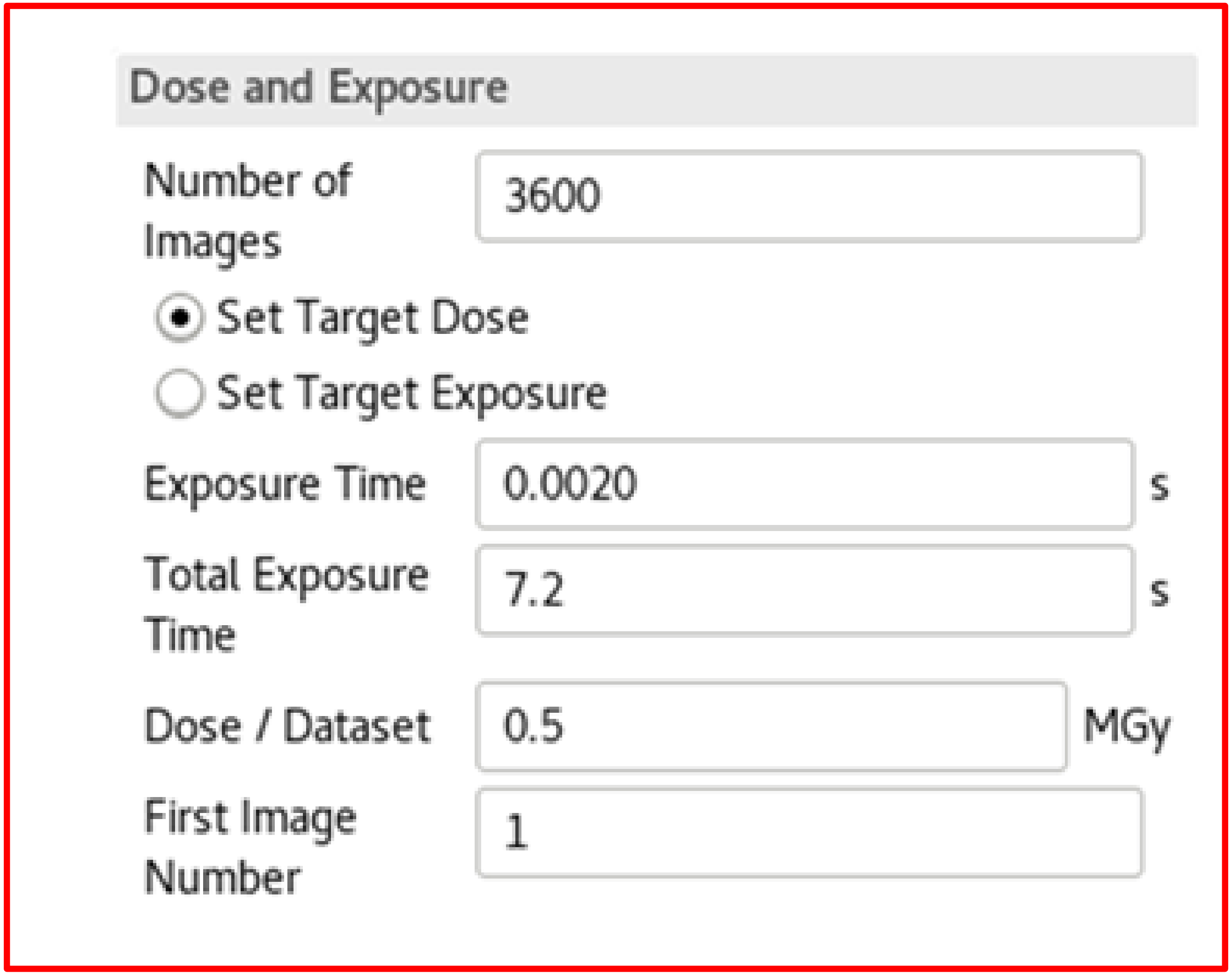
e.g. RADDOSE-3D is now embedded within Blu-Ice at SSRL so runs on all the MX beamlines there.

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

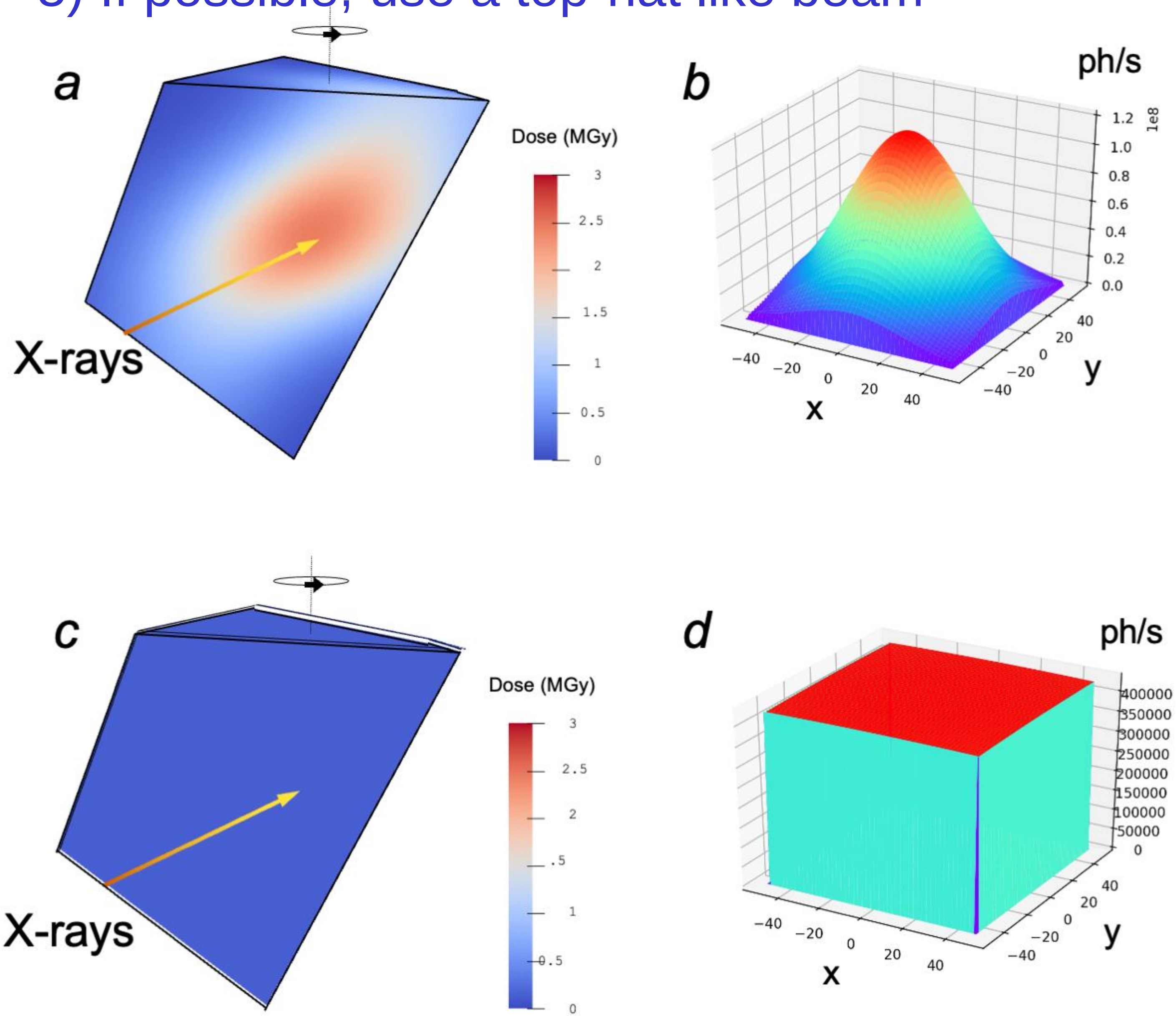
4) BACK SOAK to REMOVE non-specifically bound heavy atoms

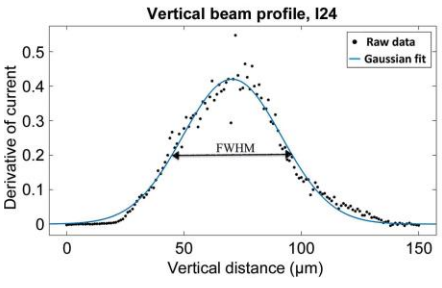
A few heavy atoms in the solvent can make a BIG difference to the absorption cross section and this the dose rate for the SAME flux.

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

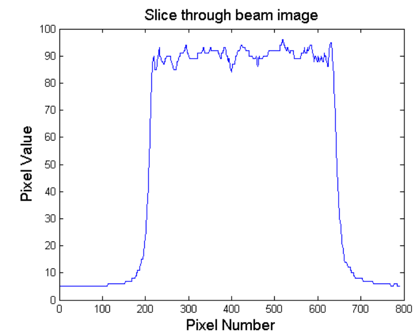
e.g. a brominated DNA-protein complex will radiation damage much faster than a native crystal and will de-brominate during data collection [Ennifar et al, Acta Cryst D (2002) 1263-1268].

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





Imaged beam
I24, DLS,
Axford, Owen



Imaged beam
P14, PETRA III,
Bourenkov,
Schneider

Garman & Weik COSB (2023)

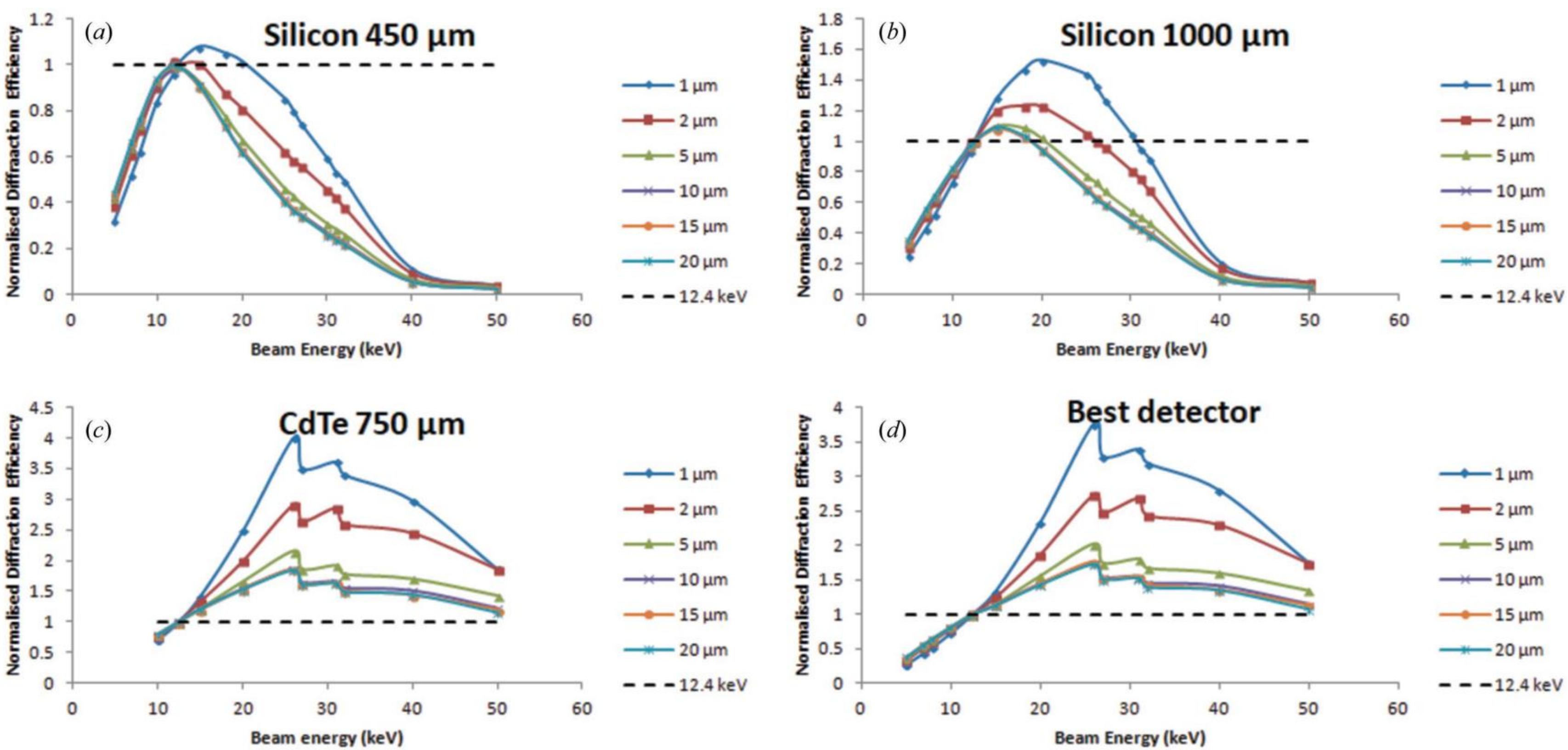
6) For data collection: consider optimising the incident energy

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

diffraction efficiency (DE) = diffraction/dose

Photoelectron escape

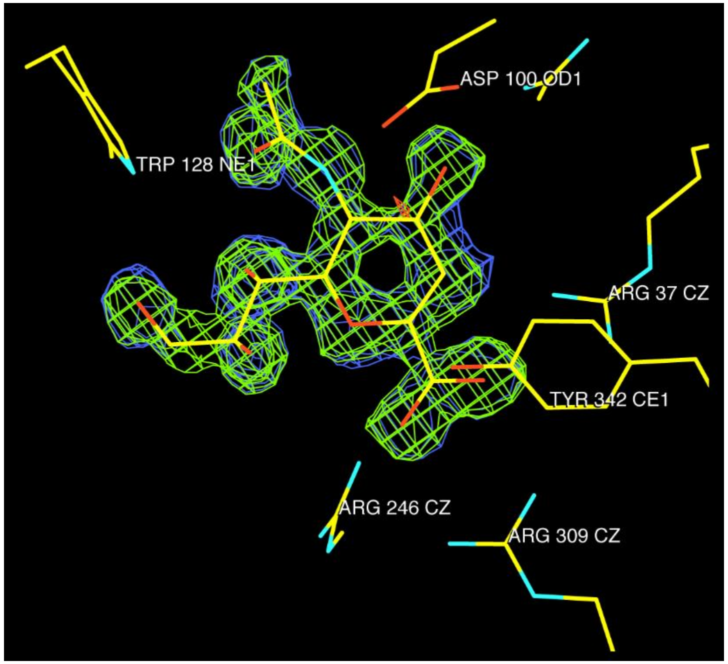
Experimental verification: Storm *et al* IUCrJ 2021



Dickerson & Garman JSR 2019

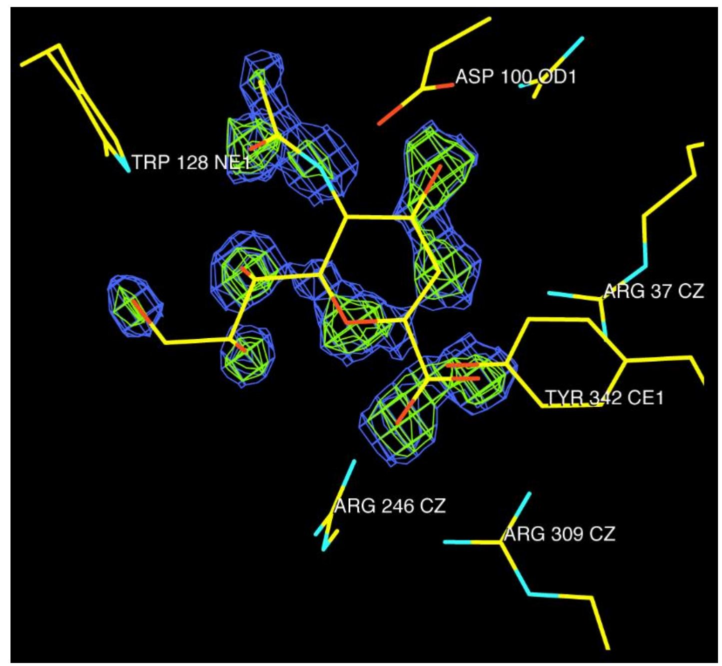
7) Exclude damaged images

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)

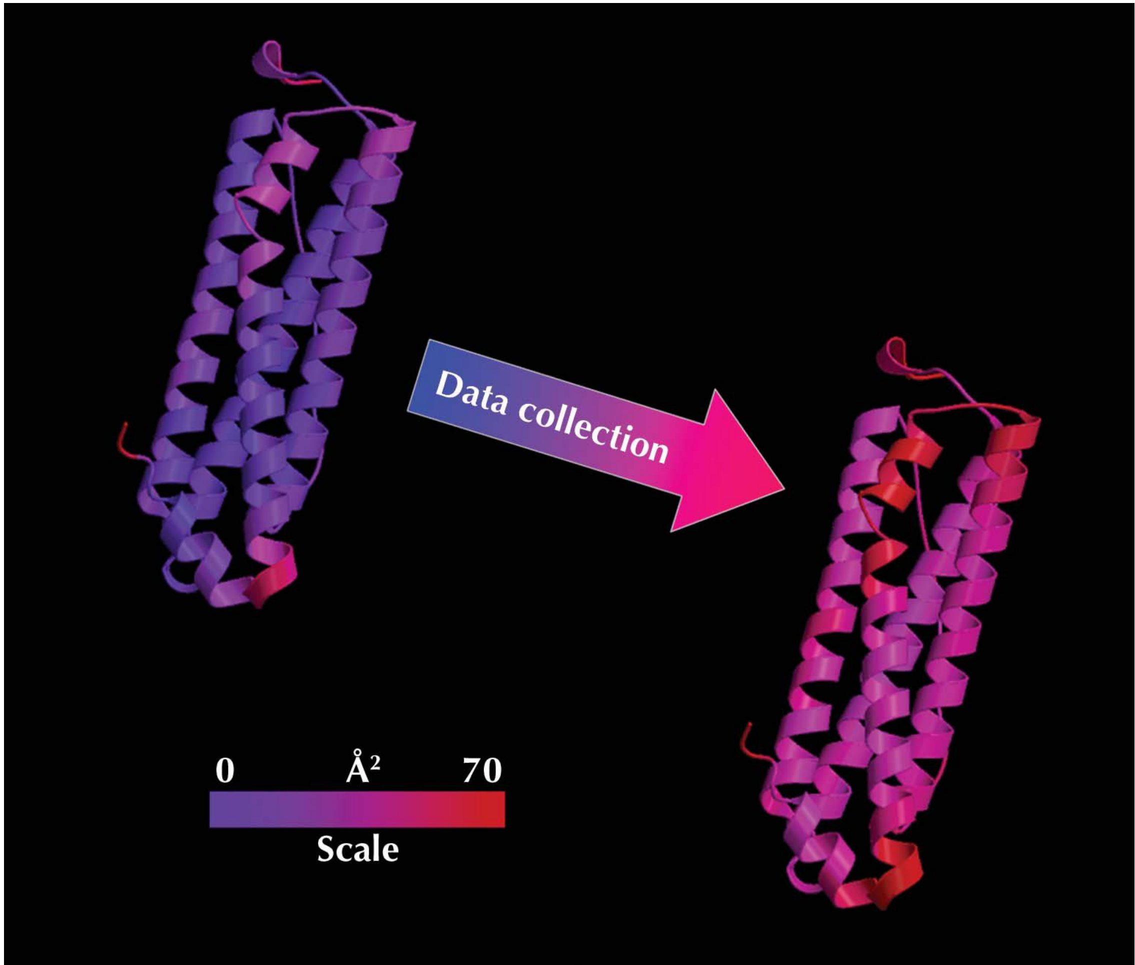
8) During refinement:

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

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

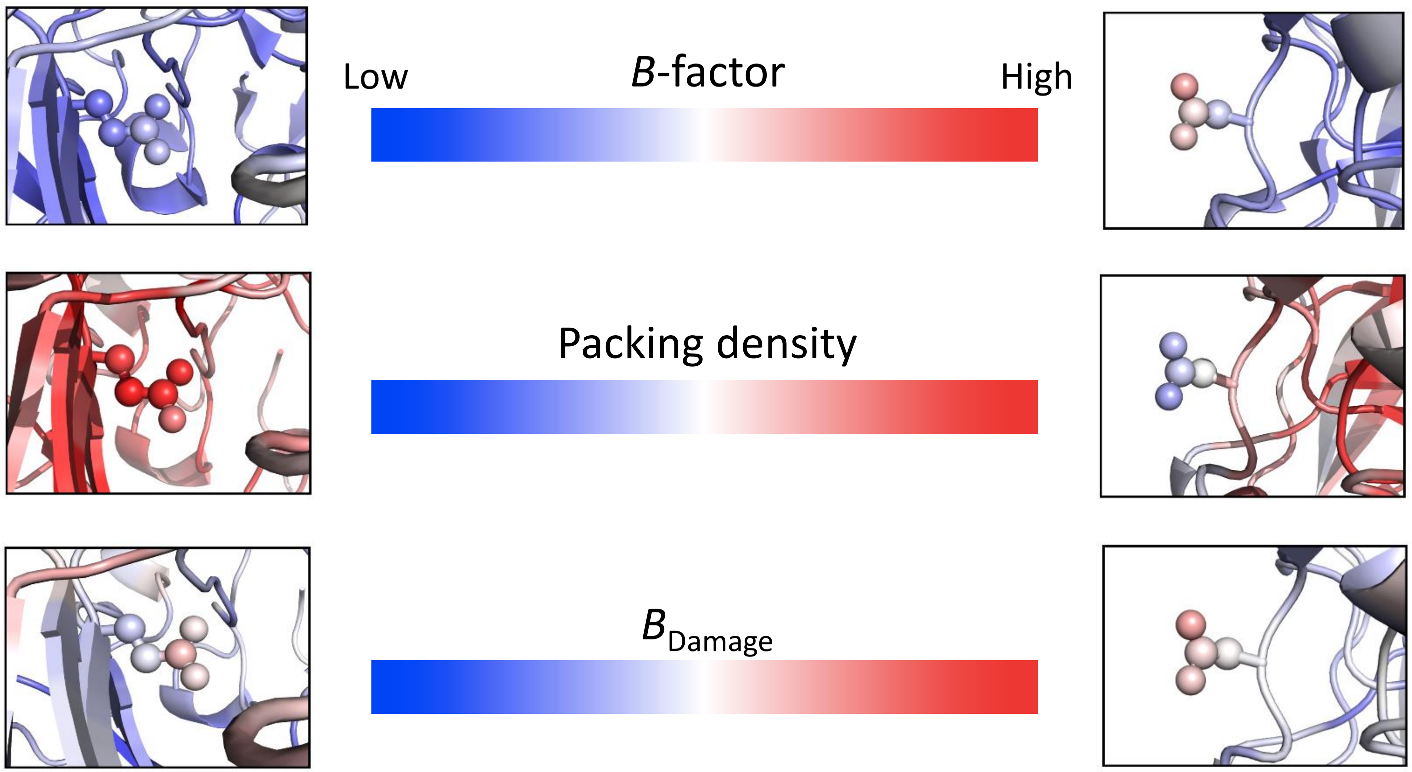
YES!!

Kathryn Shelley



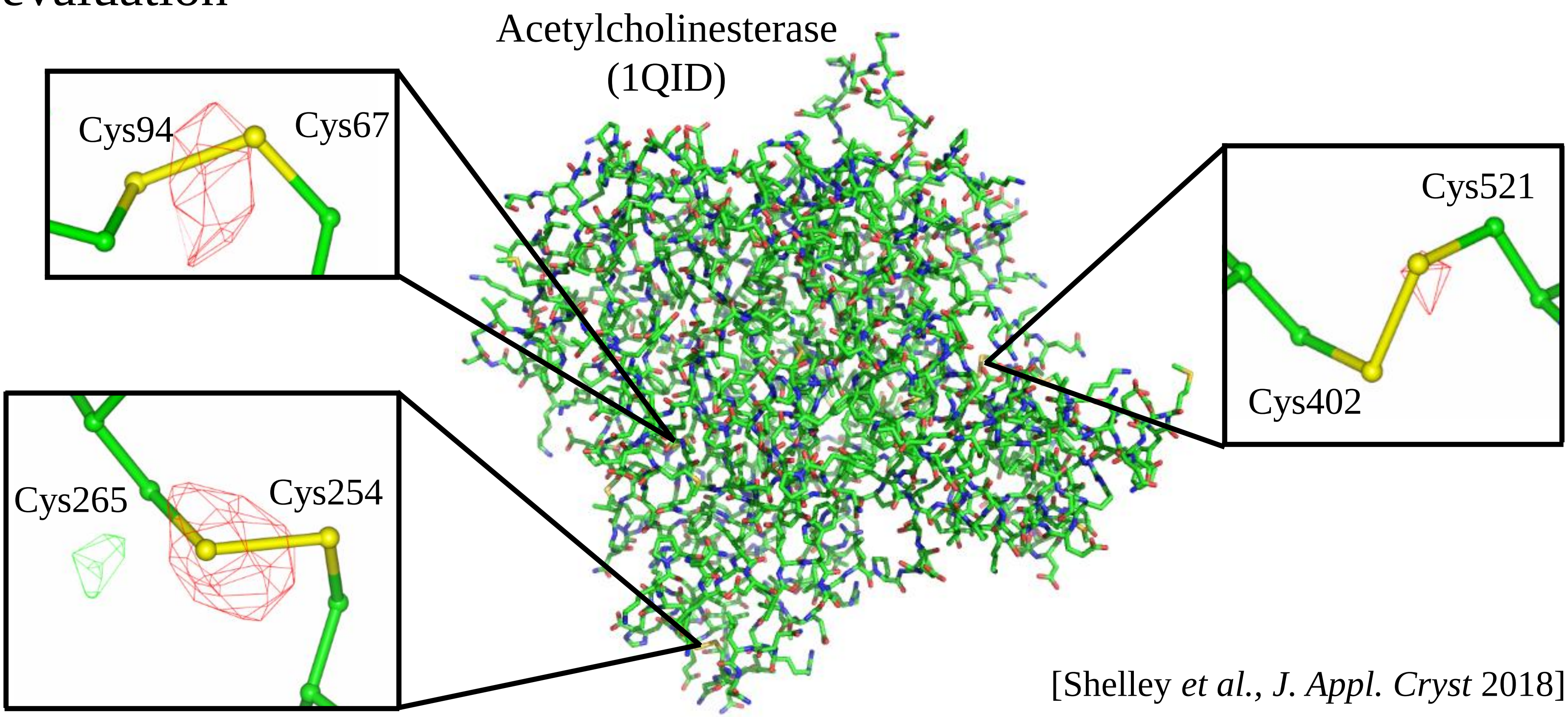
The B_{Damage} metric

- 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



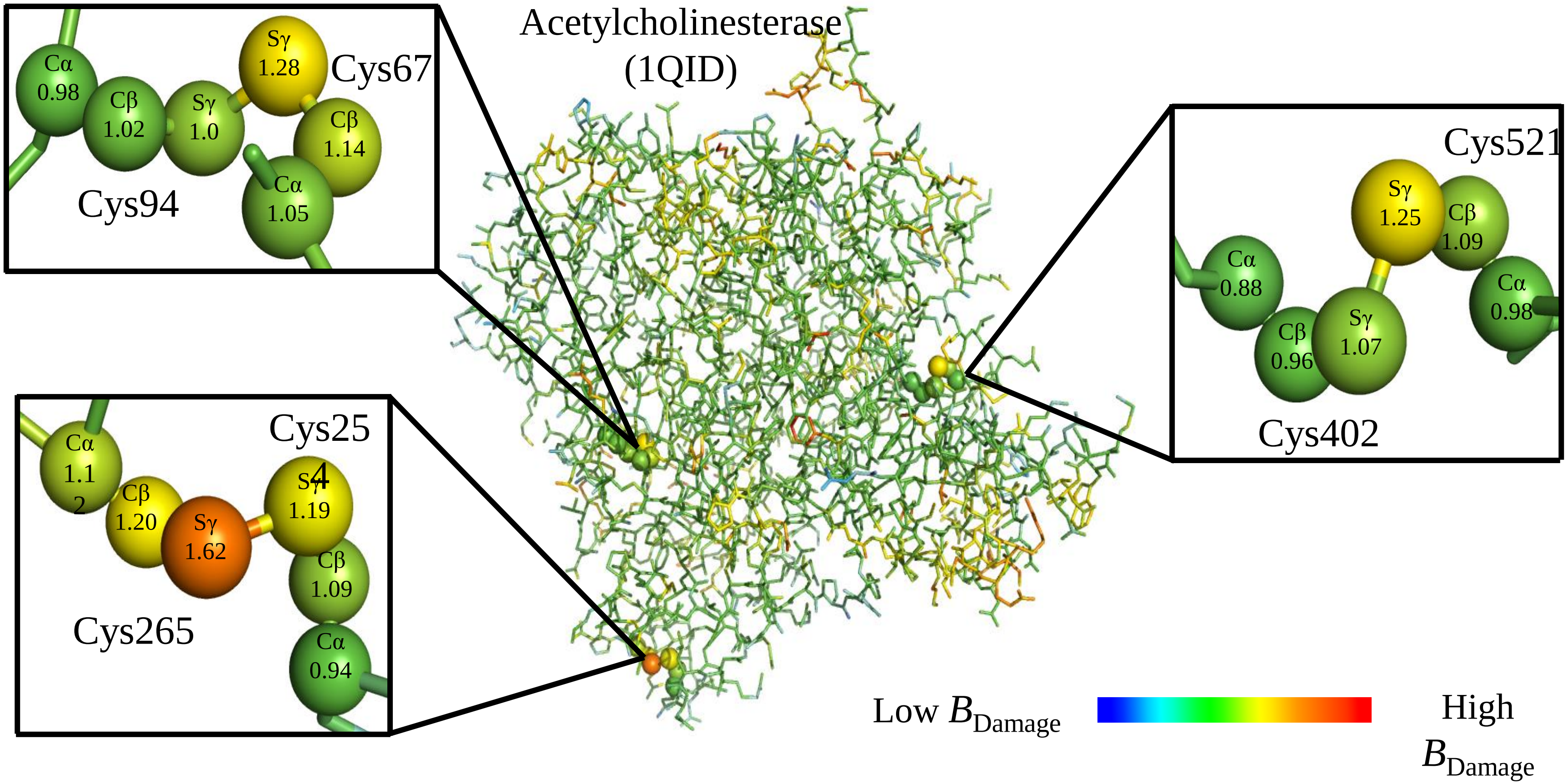
[Shelley *et al.*, *J. Appl. Cryst* 2018]

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}

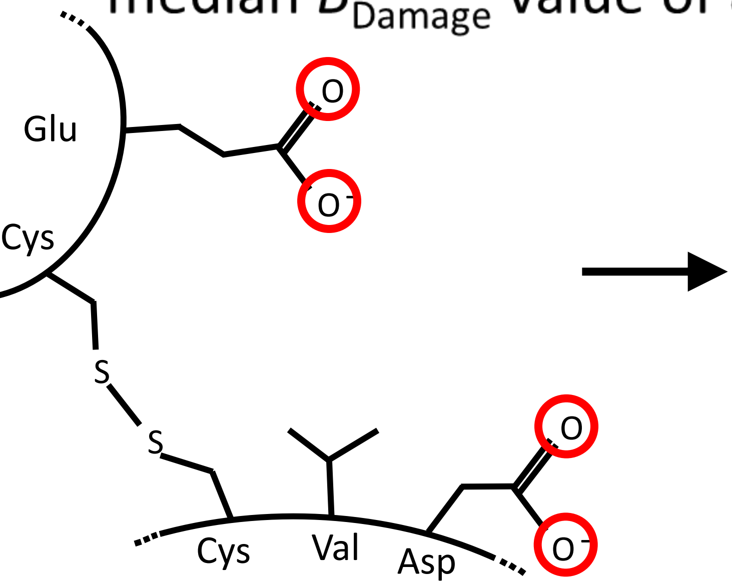
Uses B_{Damage} to compute *Bnet*



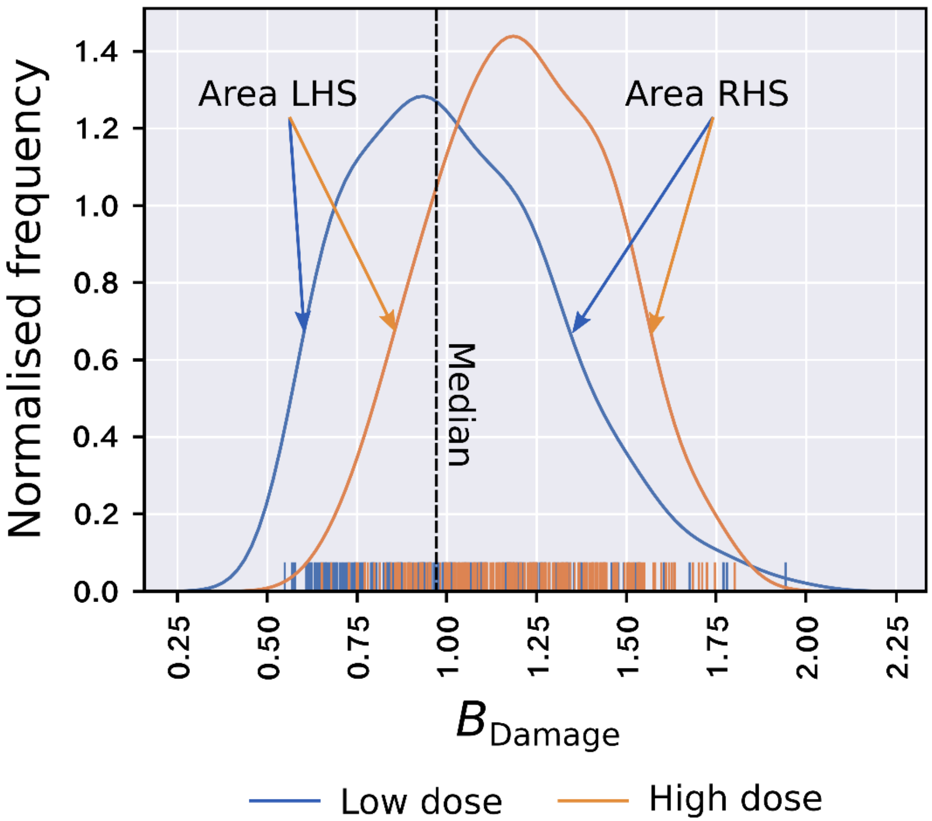
The B_{net} metric

- 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



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

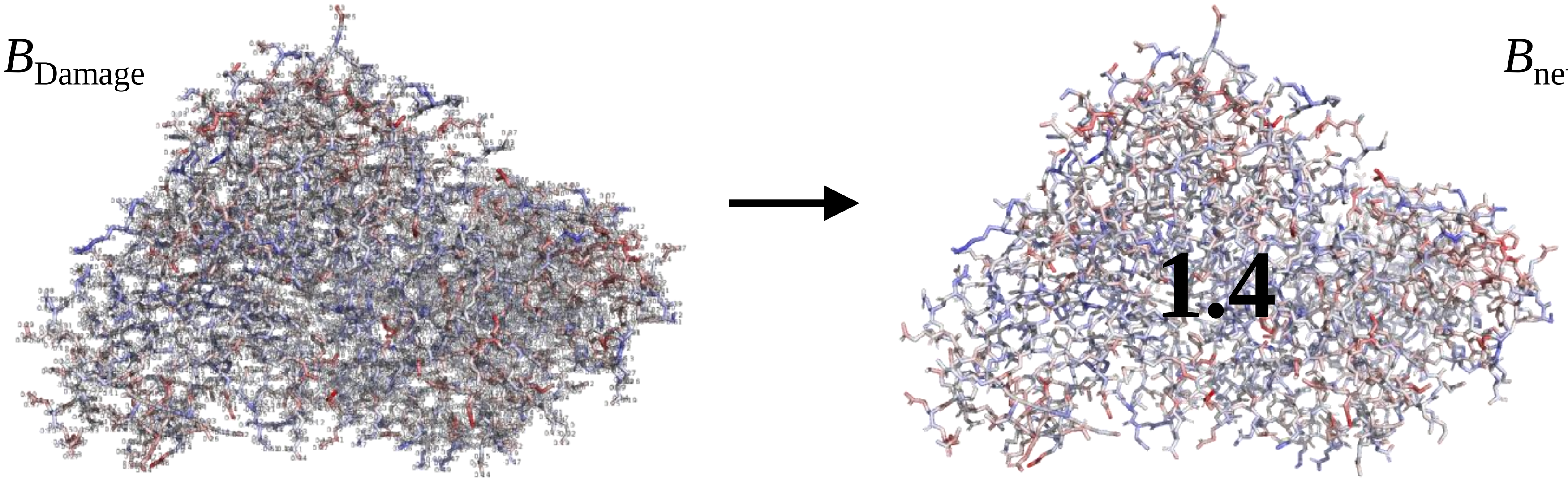


The B_{net} metric

- B_{Damage} is a per-atom metric

- The $\boldsymbol{B}_{\mathbf{net}}$ 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}

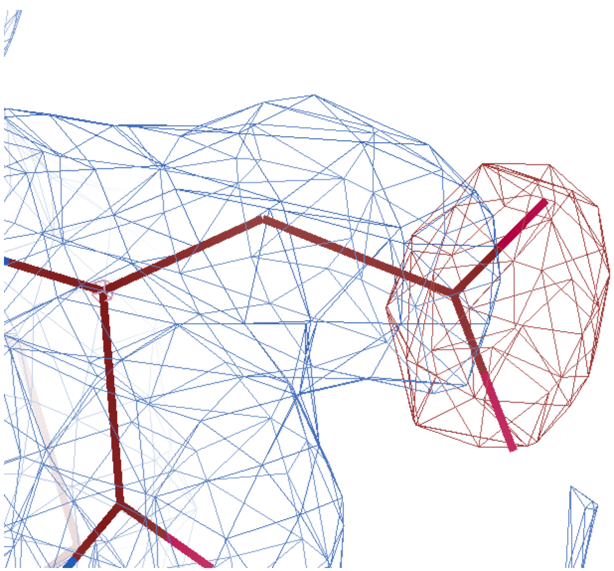
Low B_{Damage}

High B_{Damage}

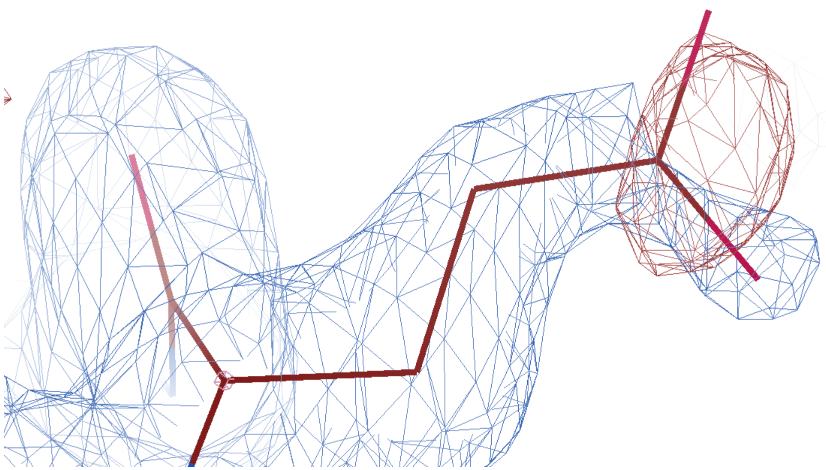
Analysis of the Protein Data Bank: *Bnet*

- 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!

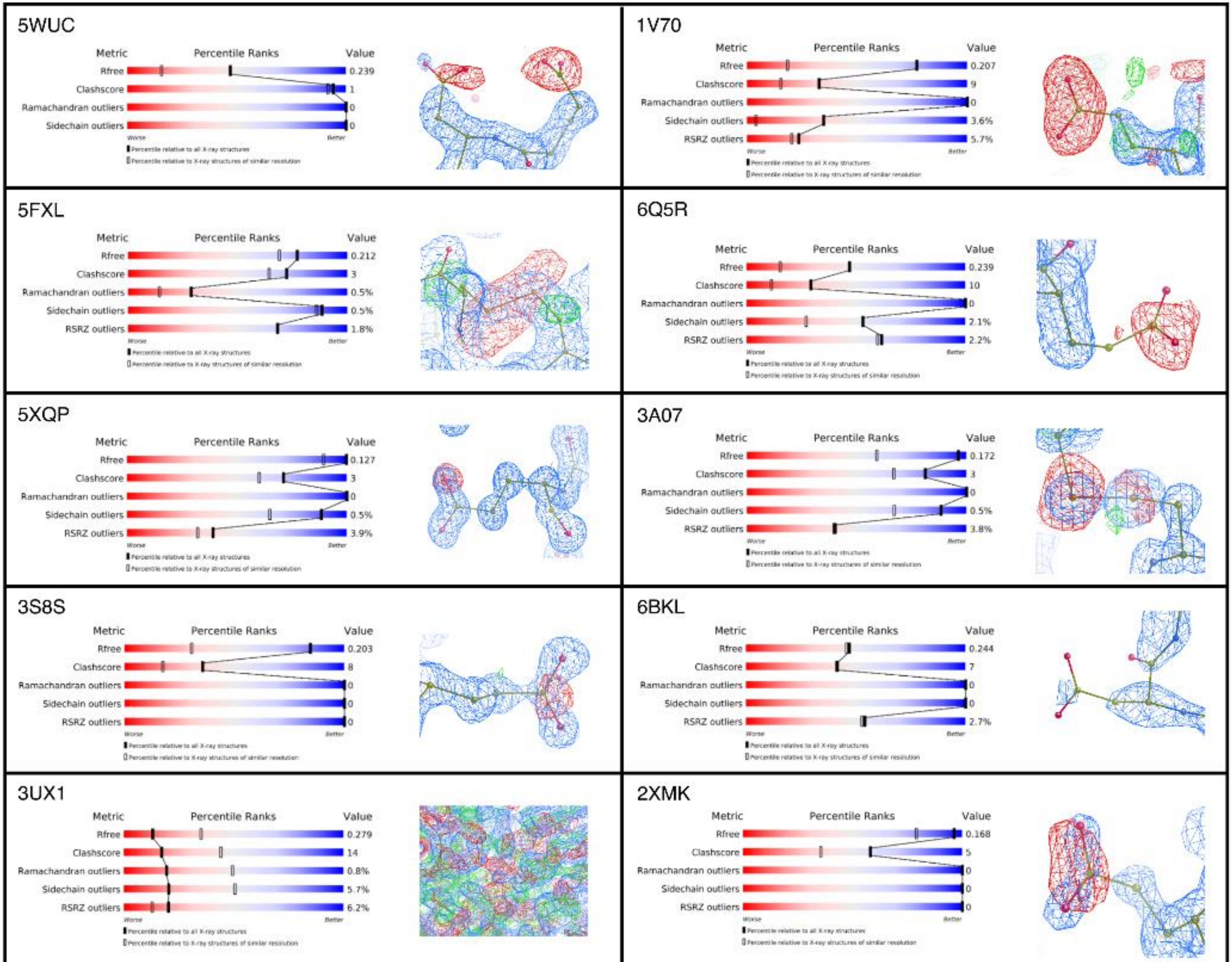
3IWU (B_{net} = 78.2)



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
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

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]
- Shelley and Garman, *Acta D* (2024) **80,** 314–332

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!

EFG by Lorna Dougan, Leeds, LOOminaries

Questions very welcome, now and when we finally meet

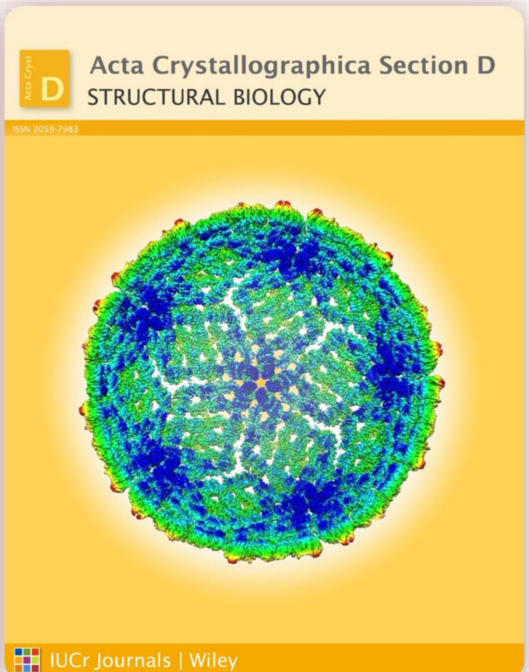
elspeth.garman@bioch.ox.ac.uk

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

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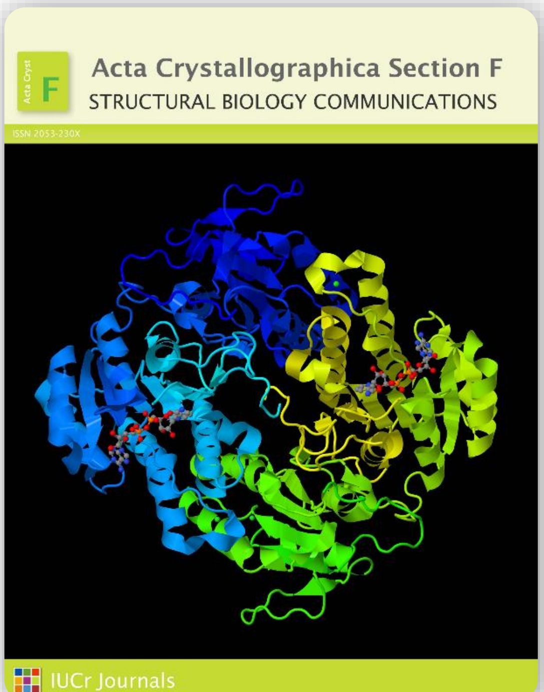
Structural Biology Communications

Acta Crystallographica Section F

journals.iucr.org/f/

@ActaCrystF



Society journal established in 2005

Covers all aspects of structural biology - concisely and rapidly

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Brief reports on the latest software, equipment and methods

International Editorial Board with trusted expert reviewers

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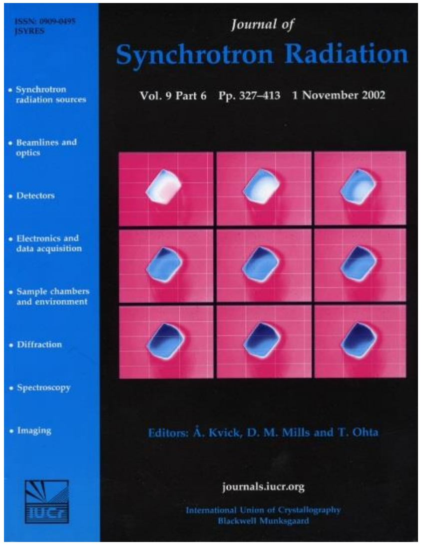
Free to publish – no submission fees or page charges

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

Never too young to start learning crystallography…

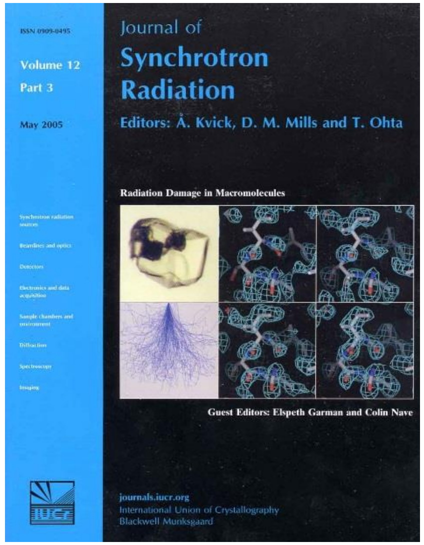
Madeleine, aged 9 months, my granddaughter reading BCA Crystallography News!

RD2: Dec 2001



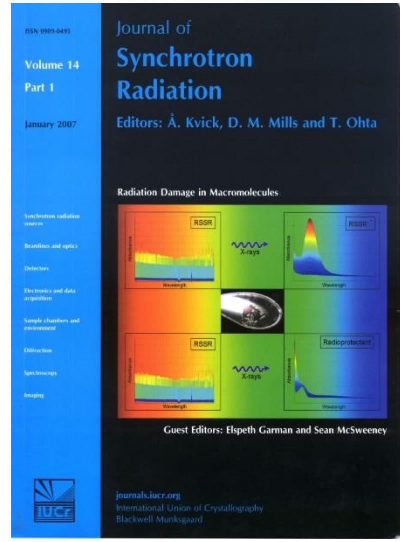
JSR, Nov 2002 (8)

RD3: Nov 2003



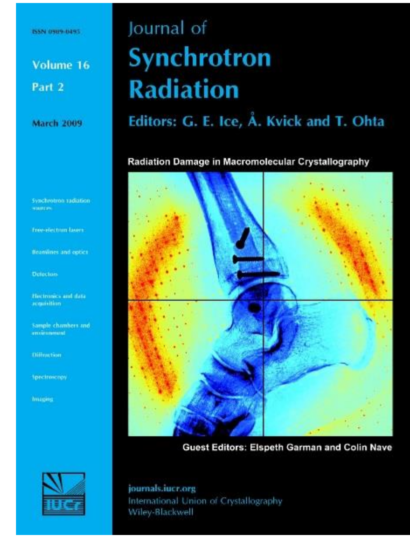
JSR, May 2005 (9)

RD4: March 2006



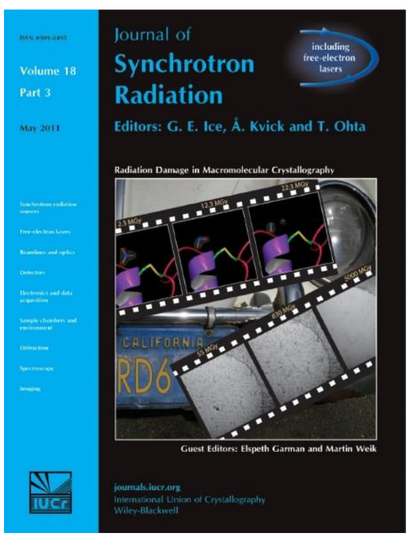
JSR, Jan 2007 (14)

RD5: March 2008



JSR, March 2009 (8)

RD6: Mar 2010

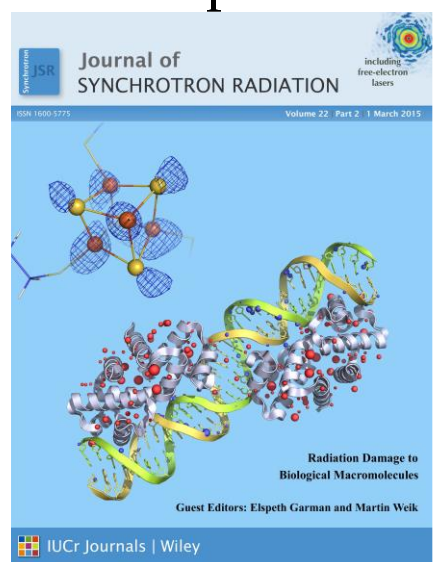


JSR, May 2011 (10)

RD7: Mar 2012

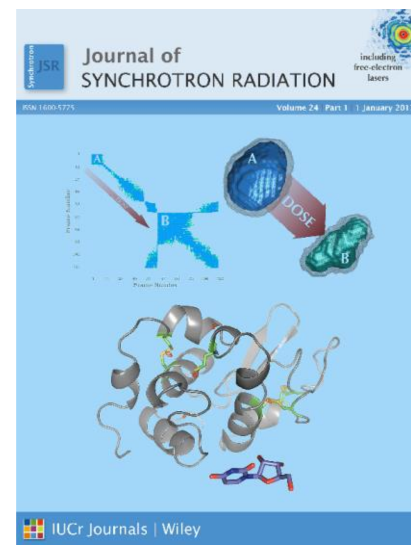
JSR, Jan 2013 (6)

RD8: Apr 2014



JSR, March 2015 (8)

RD9: Mar 2016

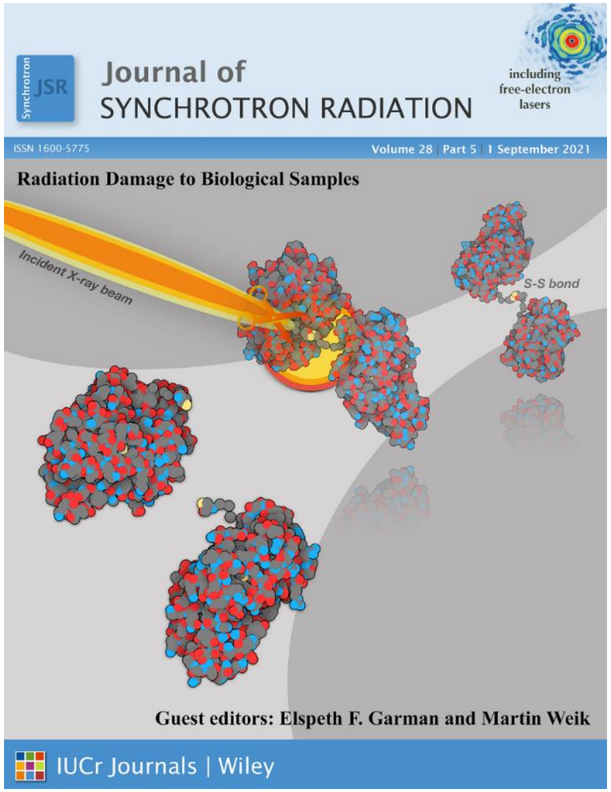


JSR Jan 2017 (8)

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

STOP PRESS!! RD12 3-5th June 2025, PSI, Switzerland

See: **https://indico.psi.ch/e/rd12**

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

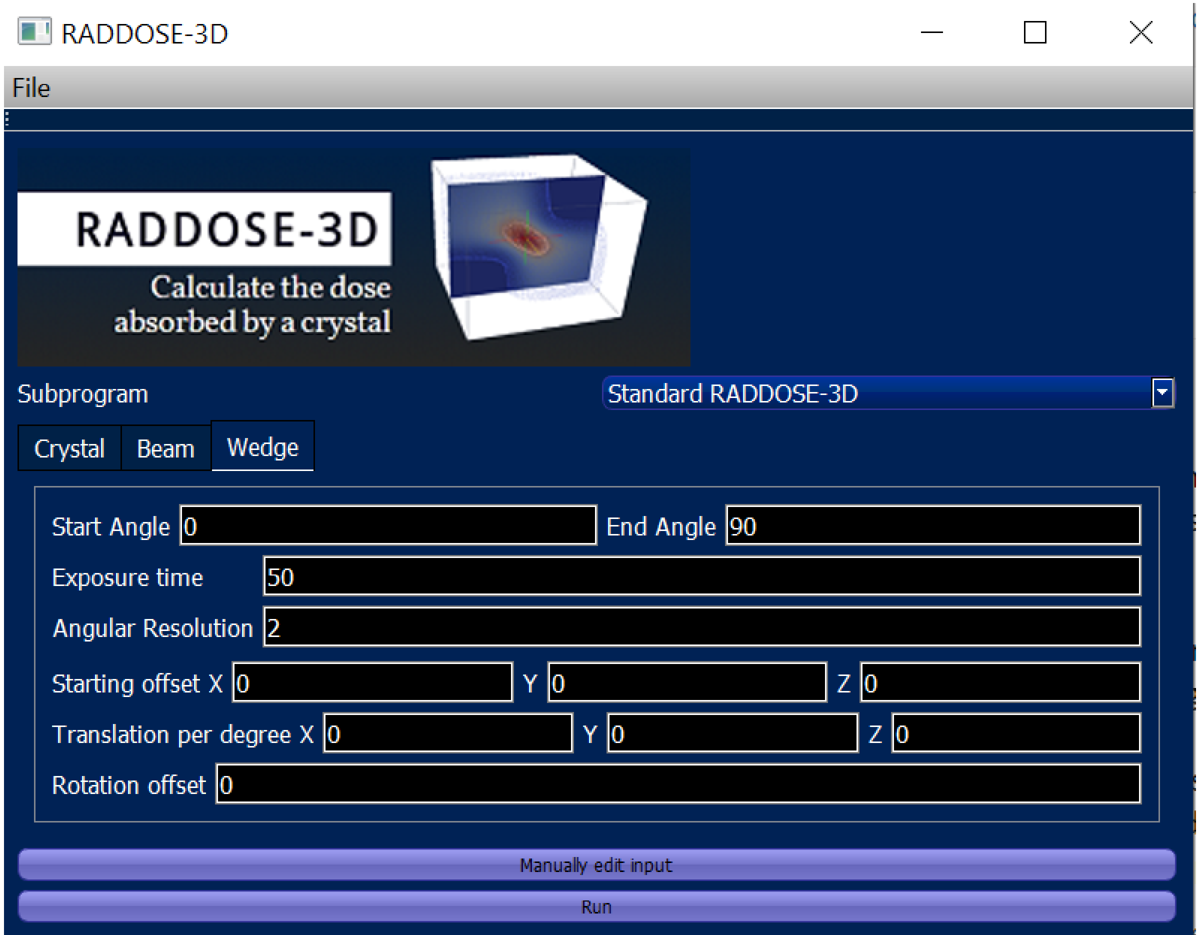
(click on 'manual interface' and run the test example first. Then edit the input for a case you would like to try)

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

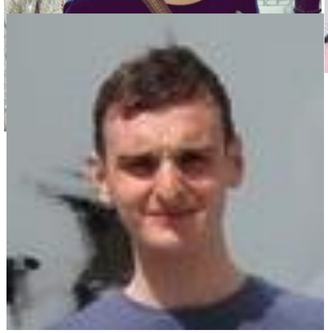
https://www.java.com/download/ie_manual.jsp

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 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.



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

RD Graduate students

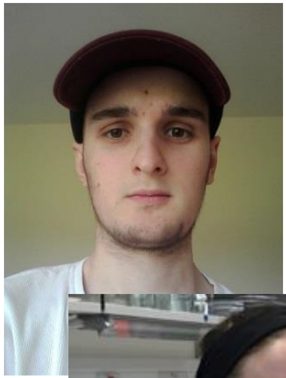
James Murray (Imperial College)
Robin Owen (DLS)
Eugenio de la Mora Lugo (IBS, Grenoble)
Oliver Zeldin (Facebook)
Markus Gerstel (DLS)
Helen Ginn (CFEL, Hamburg)
Jonathan Brooks-Bartlett (Spotify)
Charlie Bury (Medicines Discovery Catapult)

RD Collaborators:

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

RD Postdocs

Karthik Paithankar (U. Frankfurt)



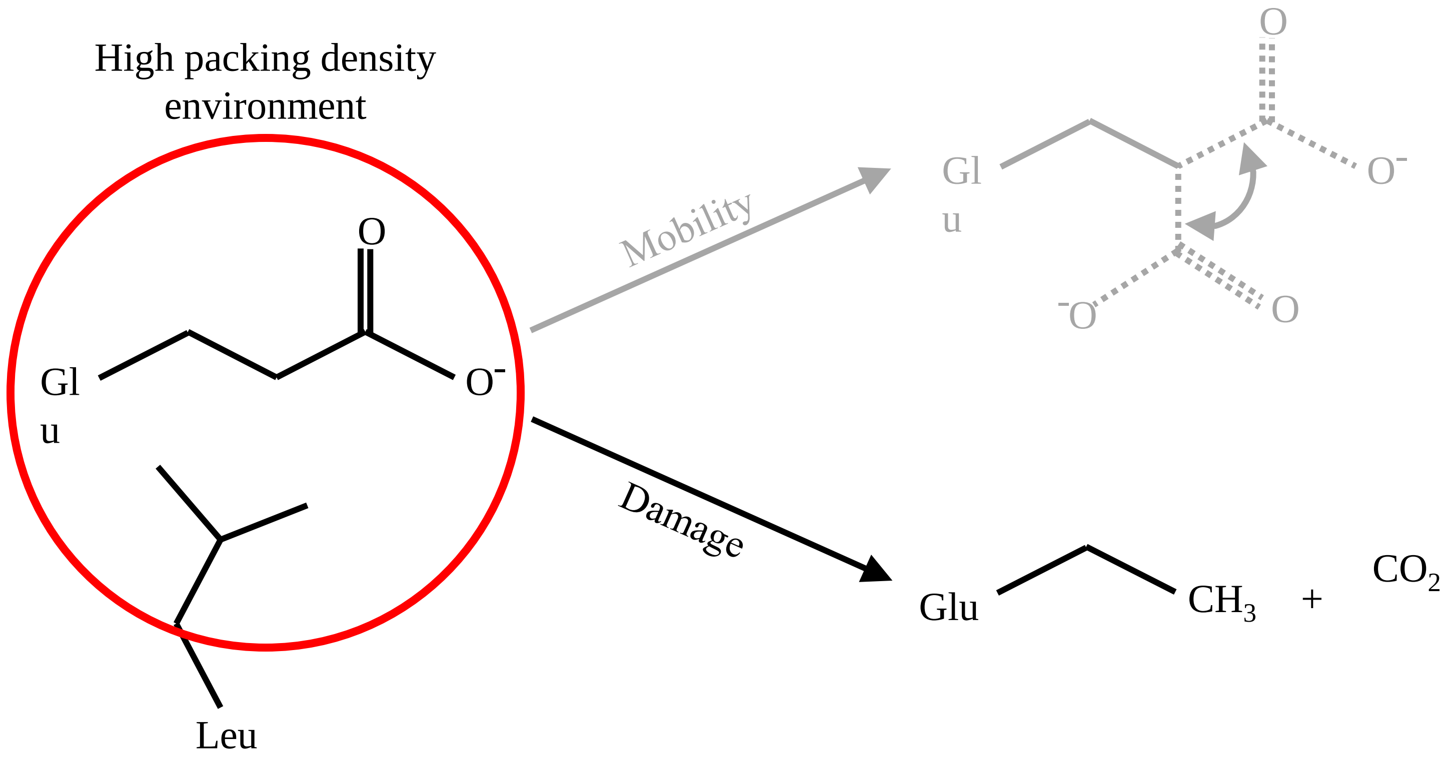
RD Undergraduate Project students

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

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

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



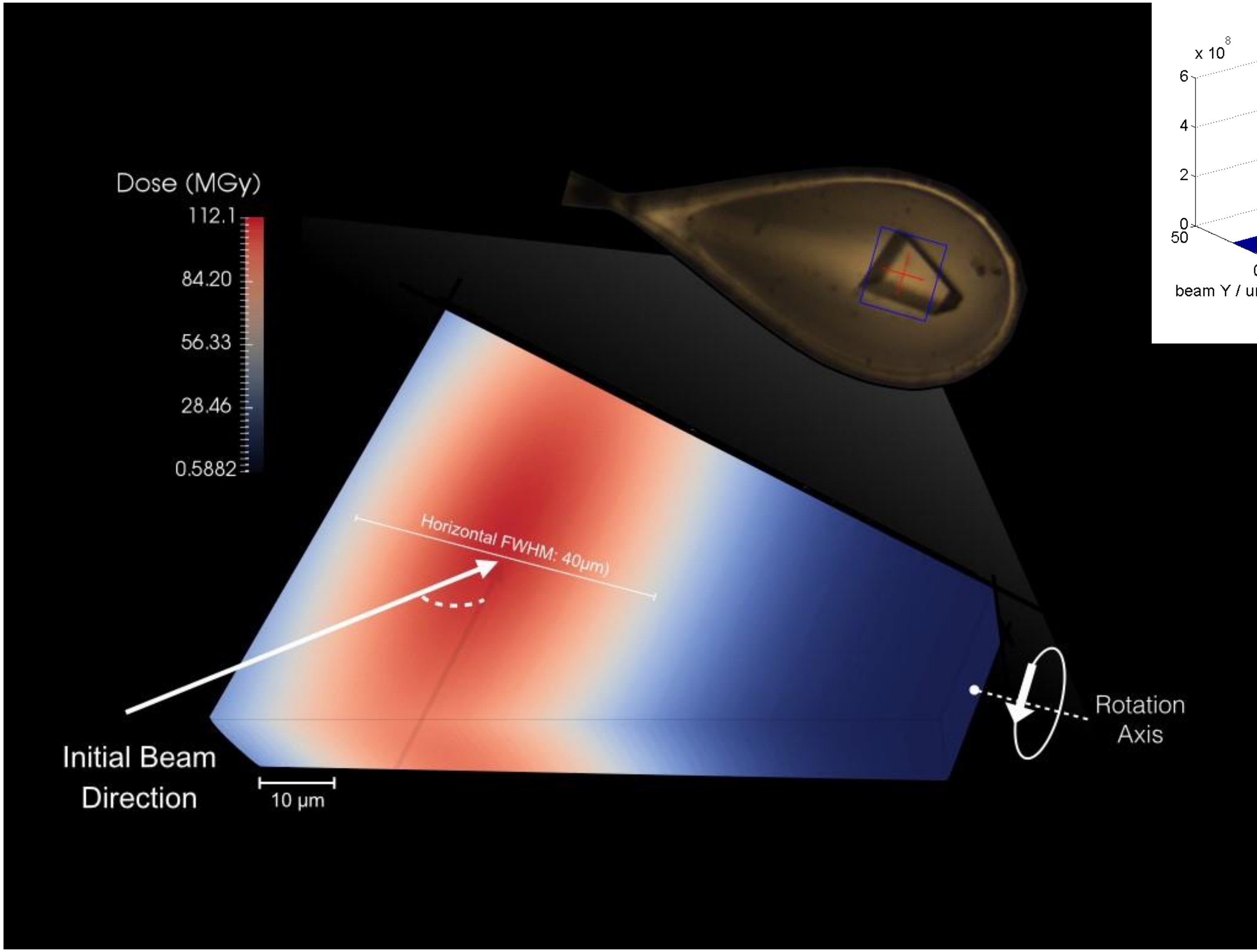
The B_{Damage} metric

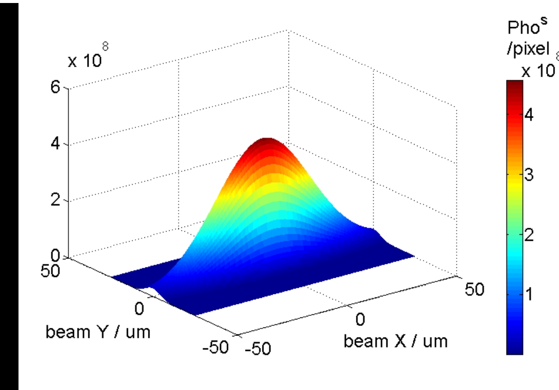
- B_{Damage}[1] is *B*-factor corrected for packing density

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

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

Gaussian Beam Profile



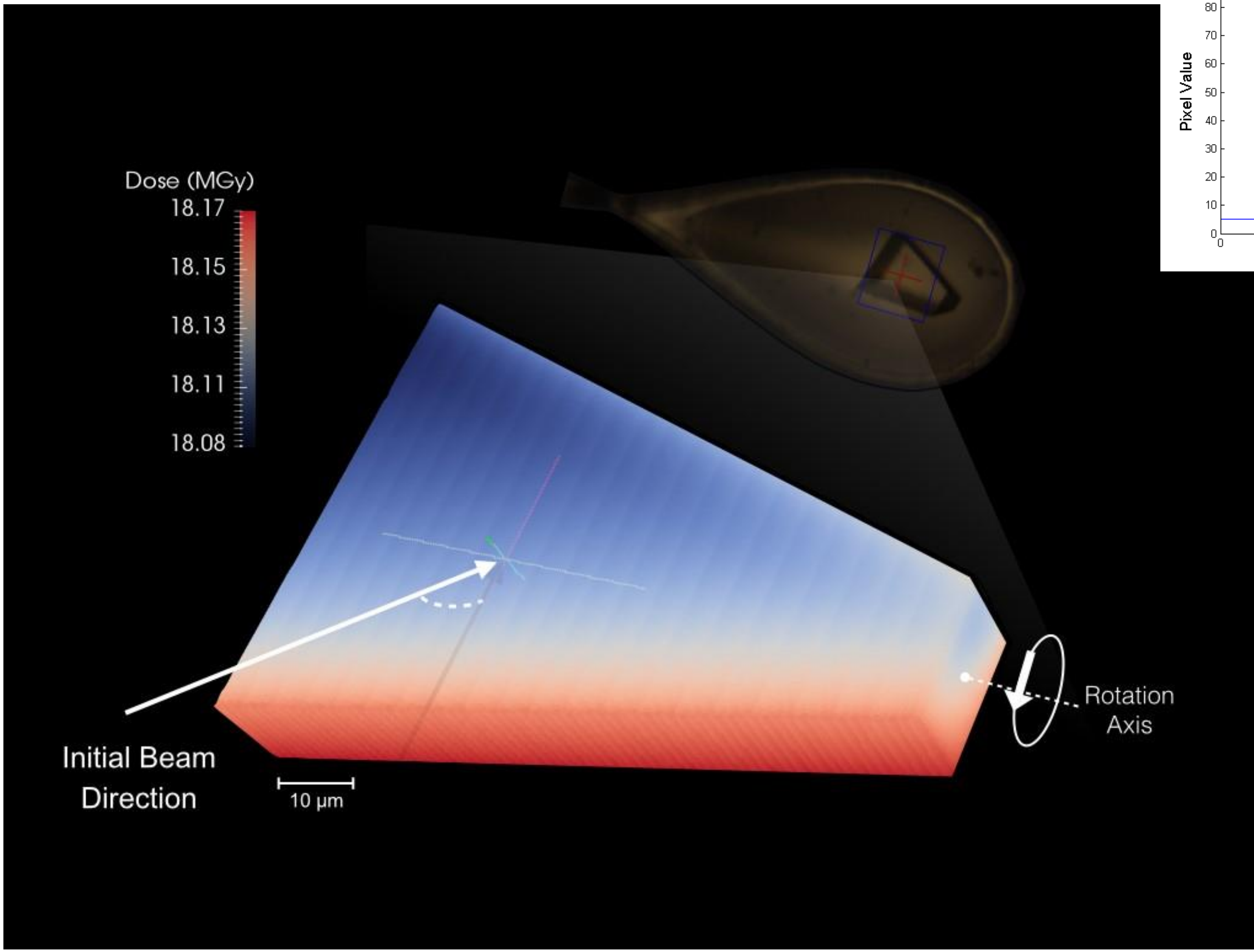


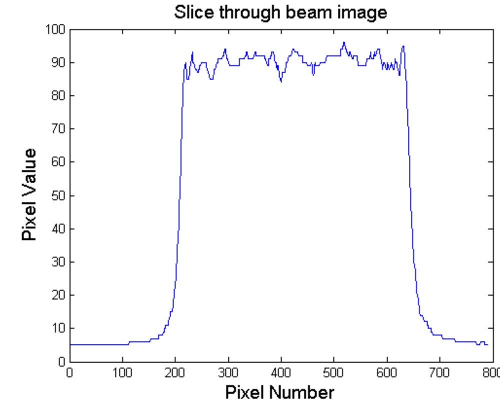
Differential irradiation may lead to differential damage: *get data which merge poorly and are a population of substates*

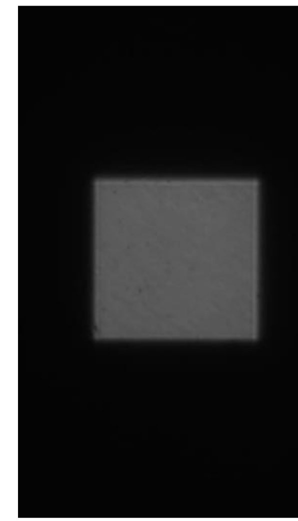
13.2 keV, 60(v)×40µm^2(h) FWHM, 100(h)×160µm^2(v) coll., 5×10^{11} ph/s

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