Radiation damage in MX (and why do we care)

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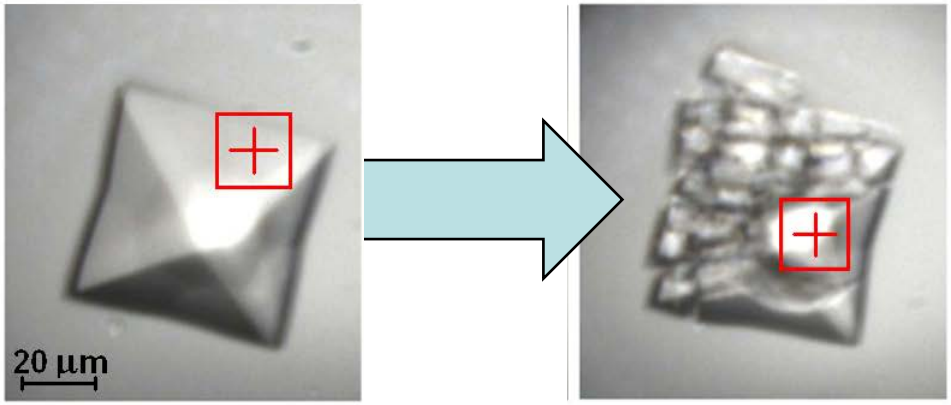
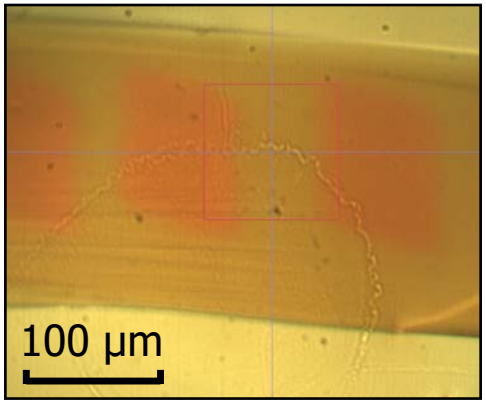
Radiation damage

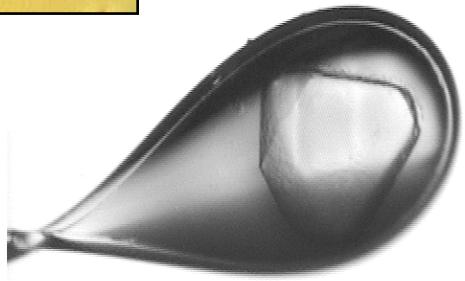
Sometimes this is visible and dramatic…



More commonly, more subtle changes can be seen…



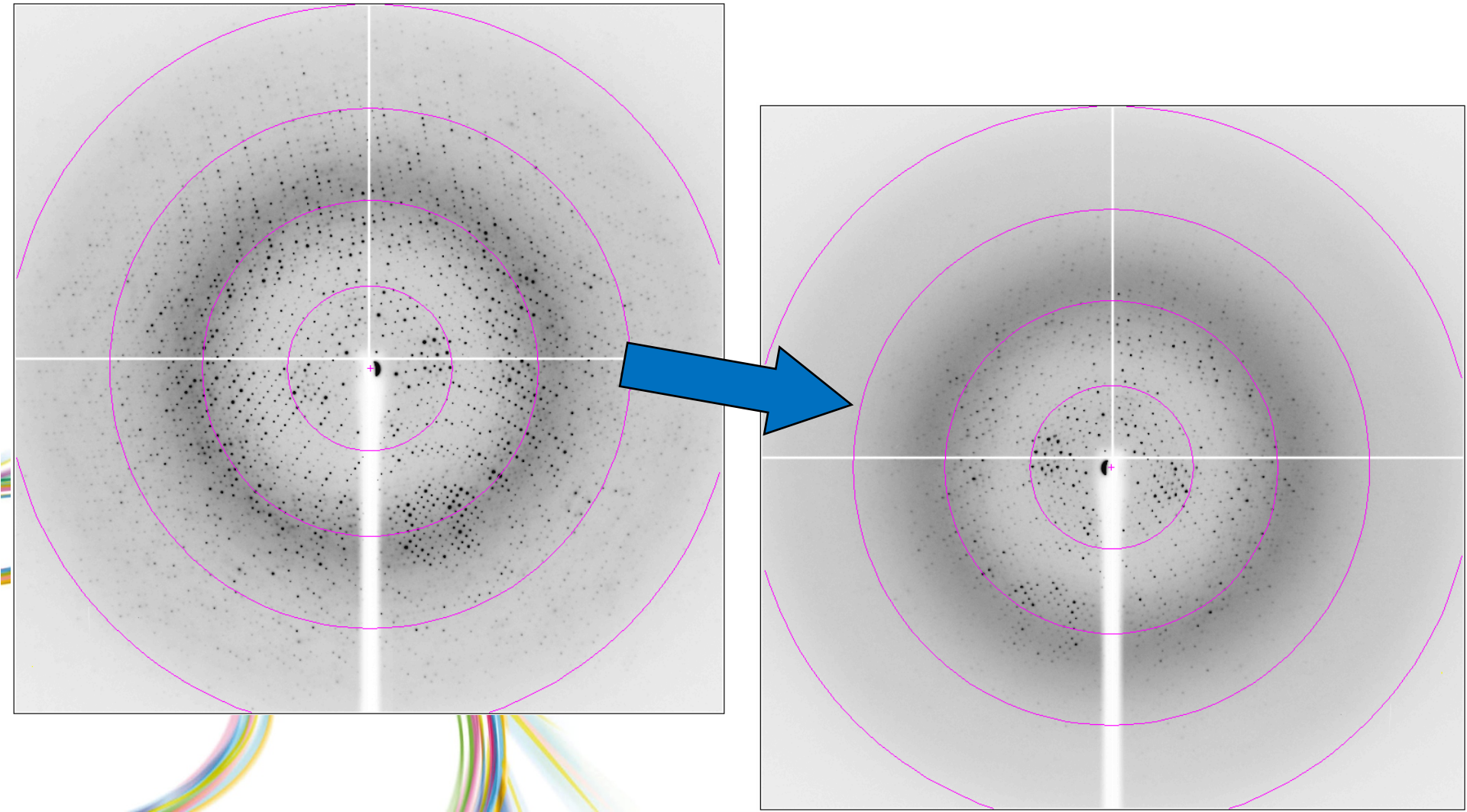
or no change may be visible at all



In all cases, X-ray induced damage occurs

Most obvious symptom:

Intensity decrease with dose / loss of diffraction.
Results in incomplete data



Effects of radiation damage

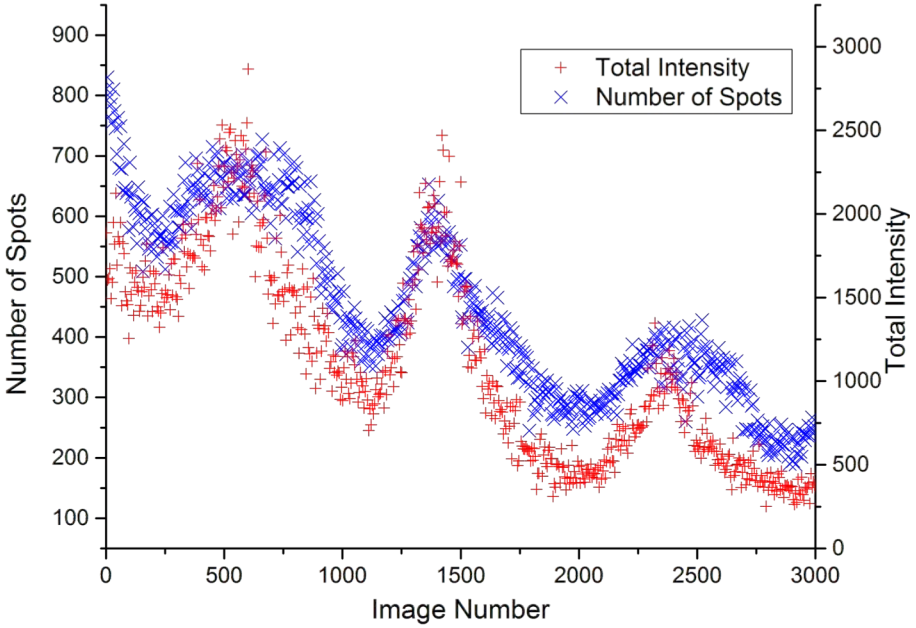
Most obvious signs of radiation damage are

- global intensity decay
- fading of higher resolution data

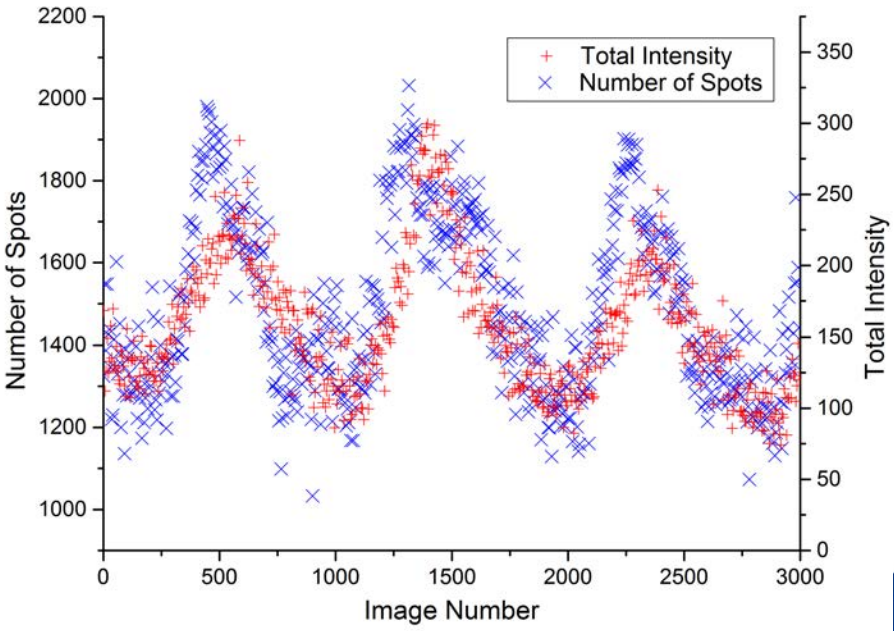
Effects such as site-specific damage harder to spot

- can cause structure solution to fail
- can cause structure to change from biologically relevant form

Dataset collected at 23 % transmission

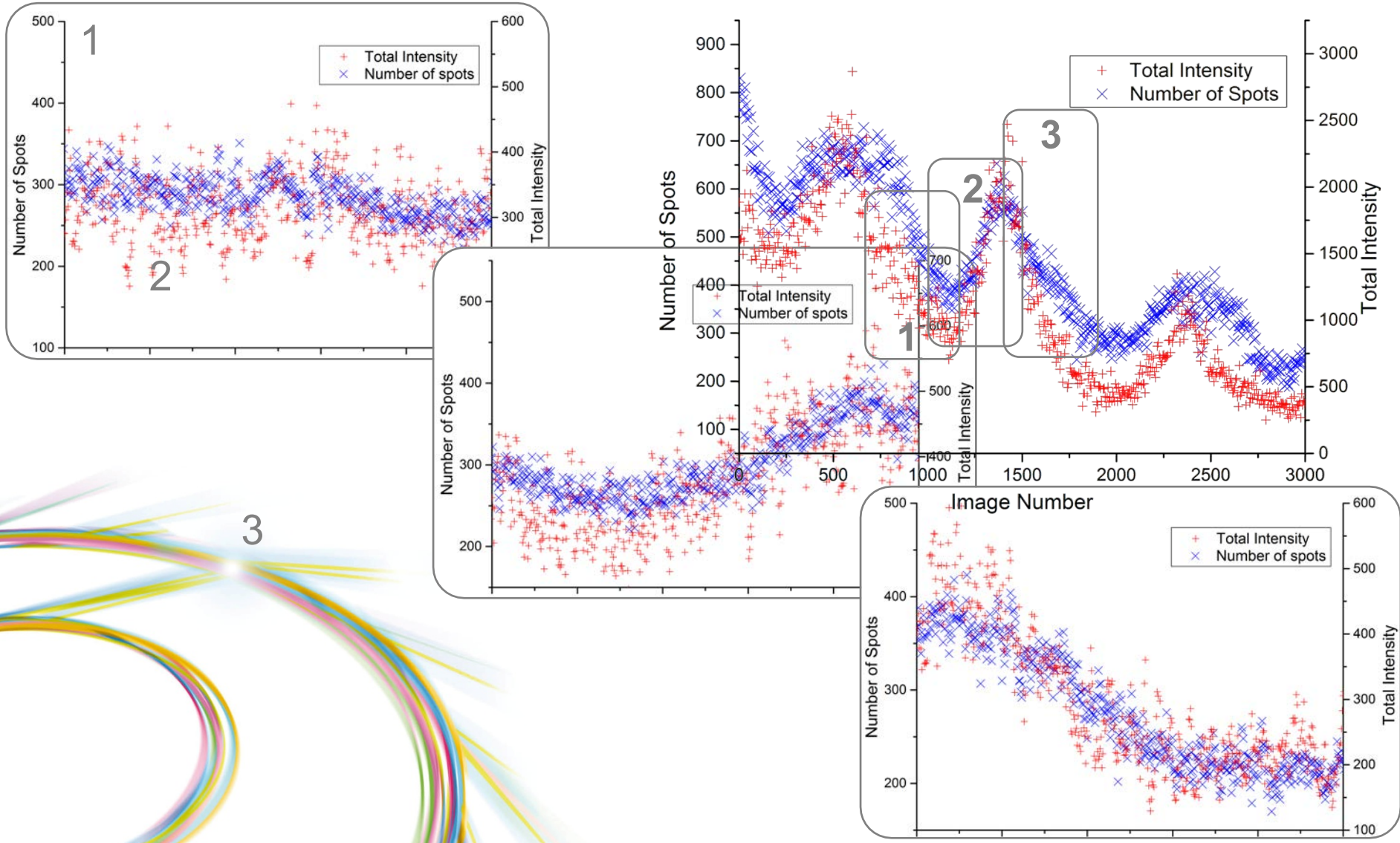


Dataset collected at 2.6 % transmission



Effects of radiation damage

Intensity decay is not a good metric, especially when data collection covers a limited rotation range



Damage is proportional to dose

Absorbed dose, D, is energy absorbed per unit mass
Measured in Gray. 1 Gy = 1 J kg^{-1}

$$D \propto \underbrace{\frac{I_0}{\lambda V}} \left[1 - \exp \underbrace{(-\mu_{abs} t)}\right]$$

Beam properties		Crystal properties
intensity		absorption coefficient
wavelength		crystal thickness
irradiated volume	⟷	irradiated volume

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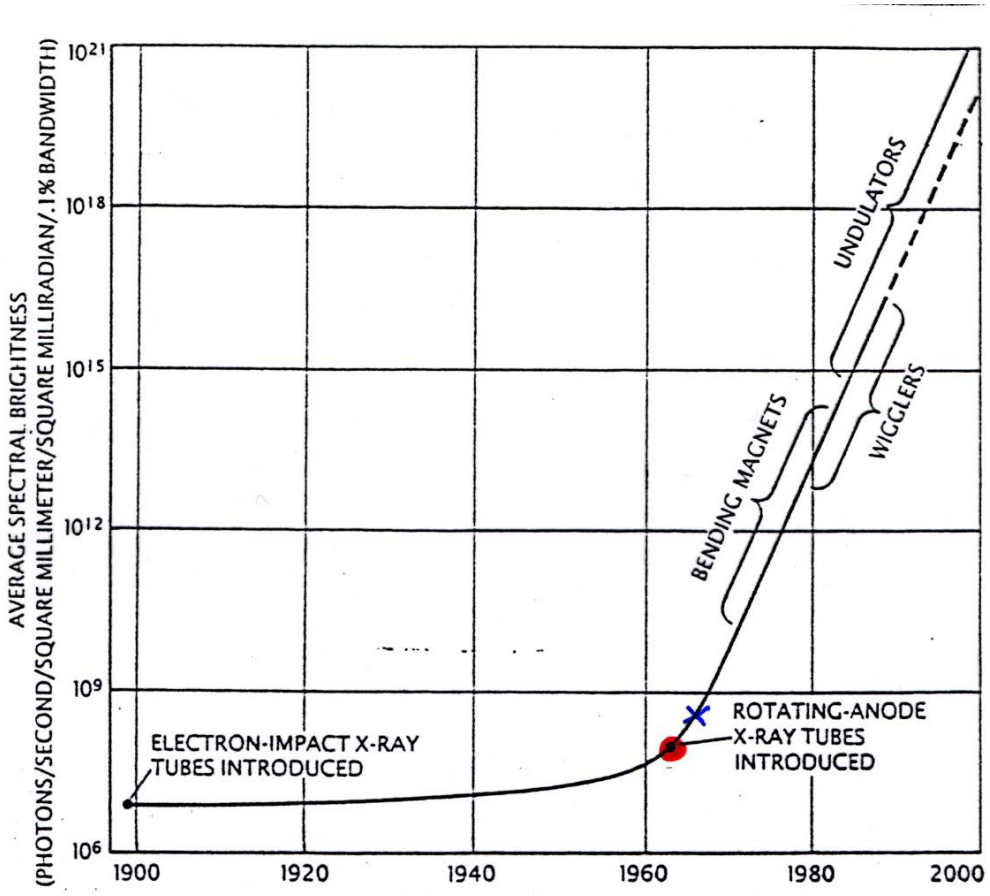
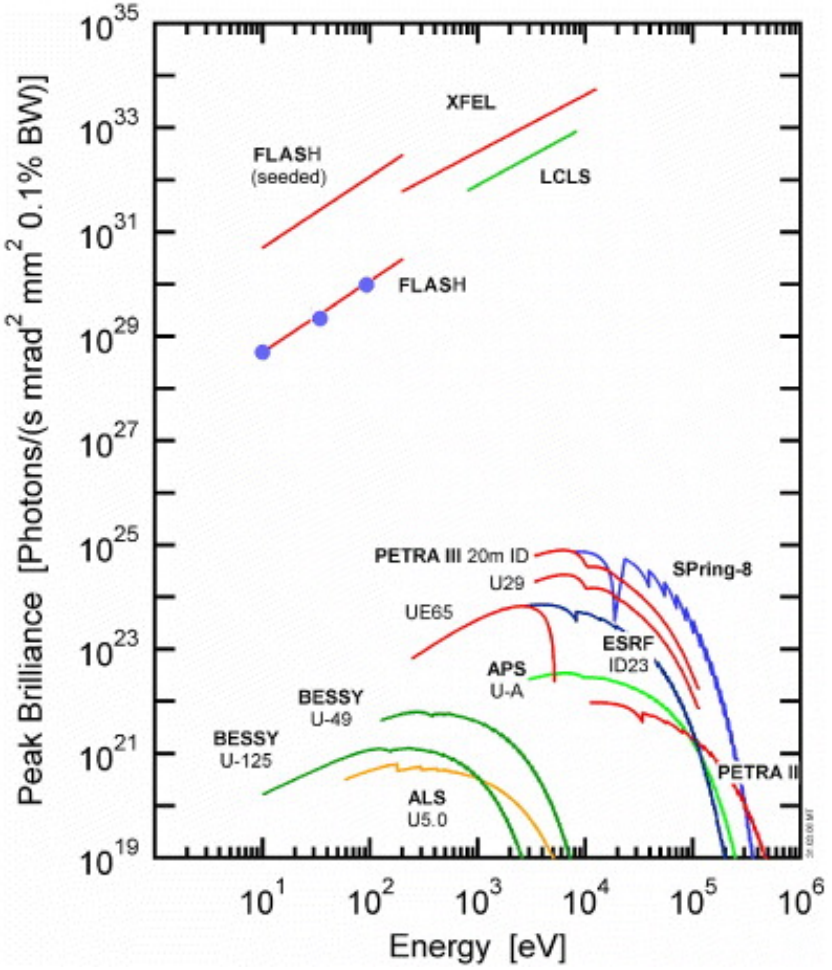
Dose is proportional to beam intensity
(and beam intensity is always increasing)



BRIGHTNESS OF X RAYS has increased by many orders of magnitude since the advent of synchrotron-radiation sources. Undulators in storage rings are the brightest source.



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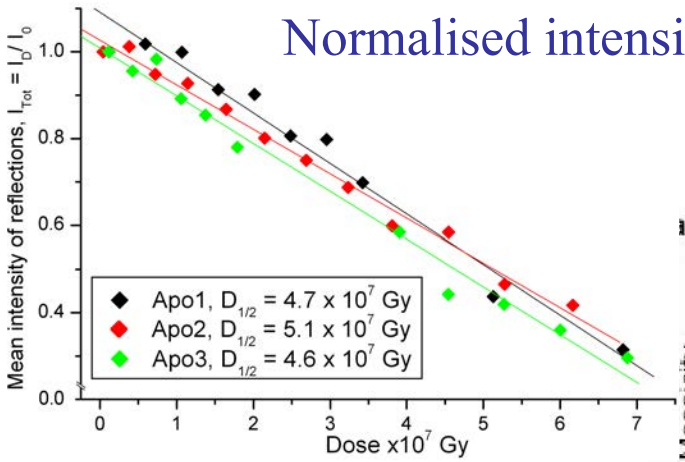
Data Parameters affected by Radiation Damage

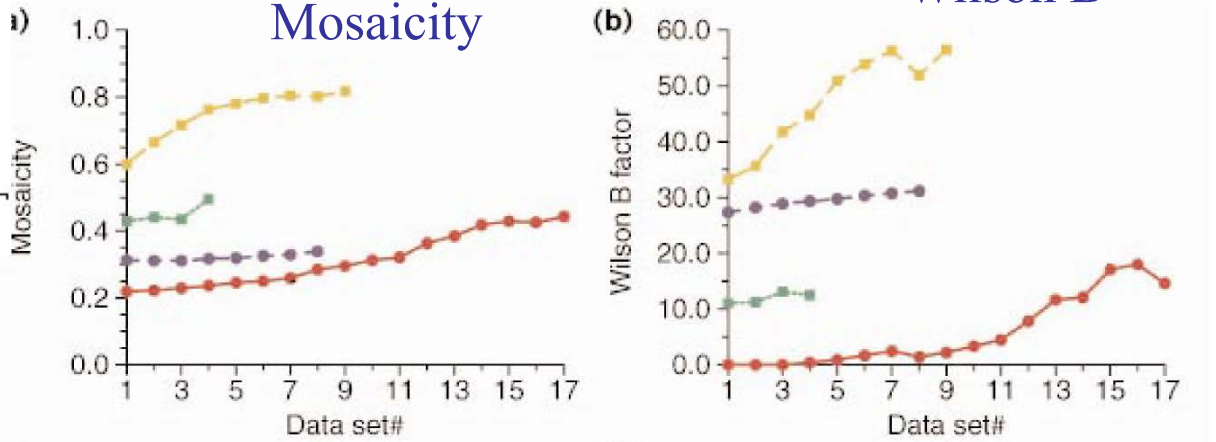
Normalised intensity



Owen, Rudino-Pinera & Garman
PNAS (2006)

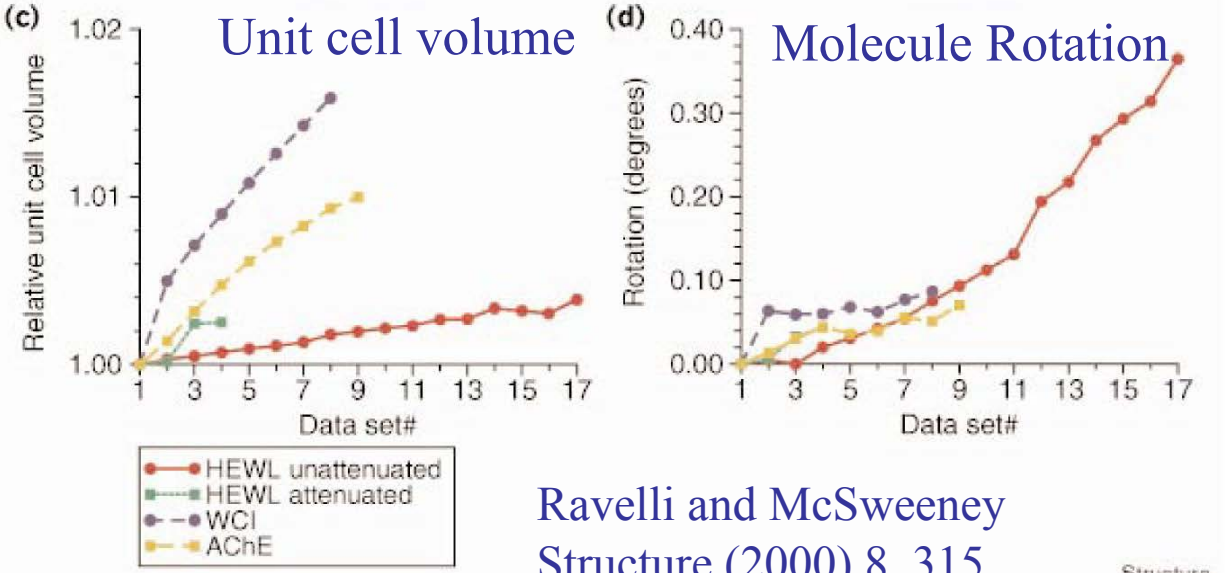
Mosaicity

Wilson B



Unit cell volume

Molecule Rotation



Ravelli and McSweeney
Structure (2000) 8, 315

Data Parameters affected by Radiation Damage

Global damage

- I /σ(I) or resolution limit ⇩
- R_{merge}, R_{meas} ⇧
- 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 and Garman (2002), JSR, Ravelli et al (2002) JSR]

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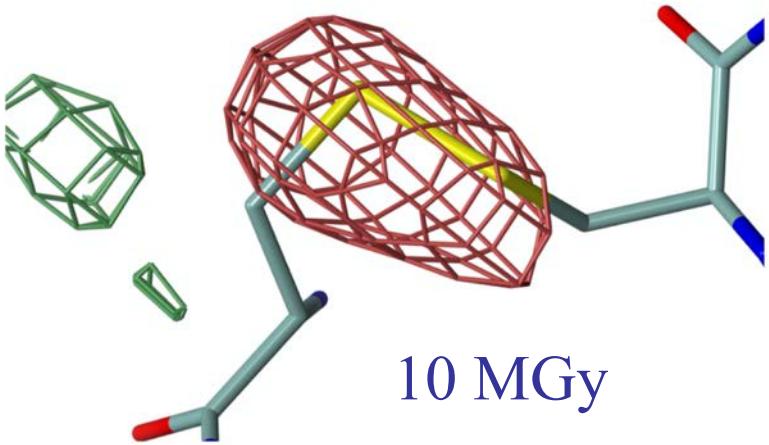
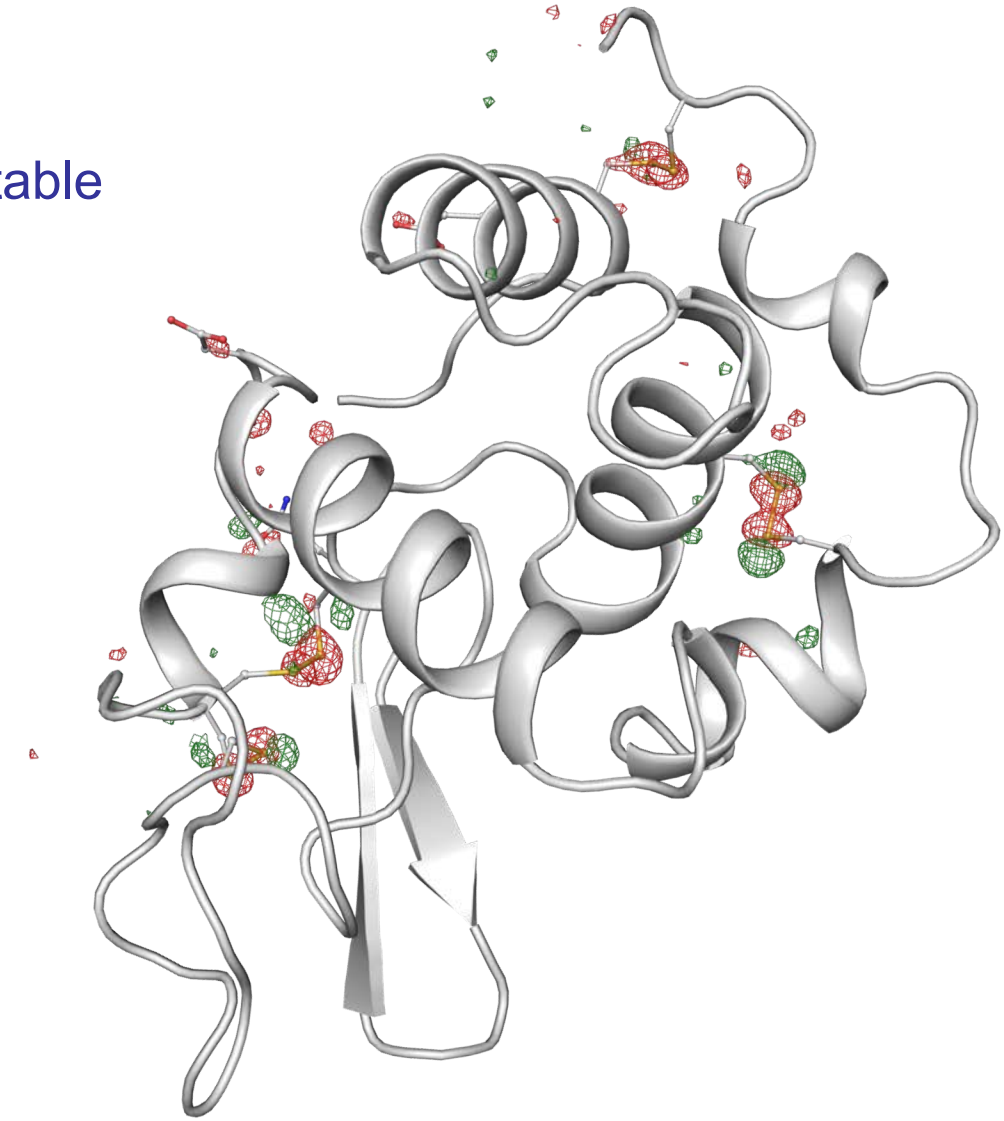
Specific Damage

Specific damage occurs in a predictable hierarchy in proteins

- Reduction of metallocentres
- Breakage of disulphide bonds
- Asp and Glu decarboxylation



Difference map Fo_4-Fo_1



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

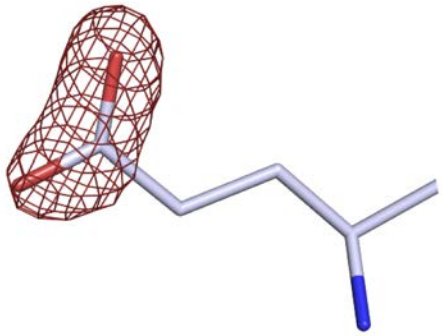
Specific Damage

Specific damage occurs in a predictable hierarchy in proteins

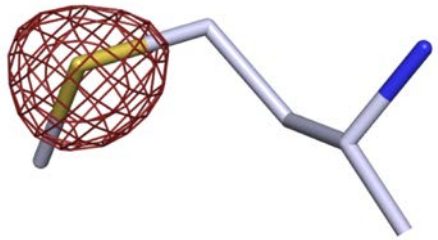
- Reduction of metallocentres
- Breakage of disulphide bonds
- Decarboxylation of ASP and GLU residues
- Cleavage of S—C bond in MET
- Rupture of covalent bonds to heavier atoms:

  C-Br, C-I, S-Hg

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



GLU ±4σ–level difference map: Red =negative density



MET ±4σ–level difference map: Red = negative density

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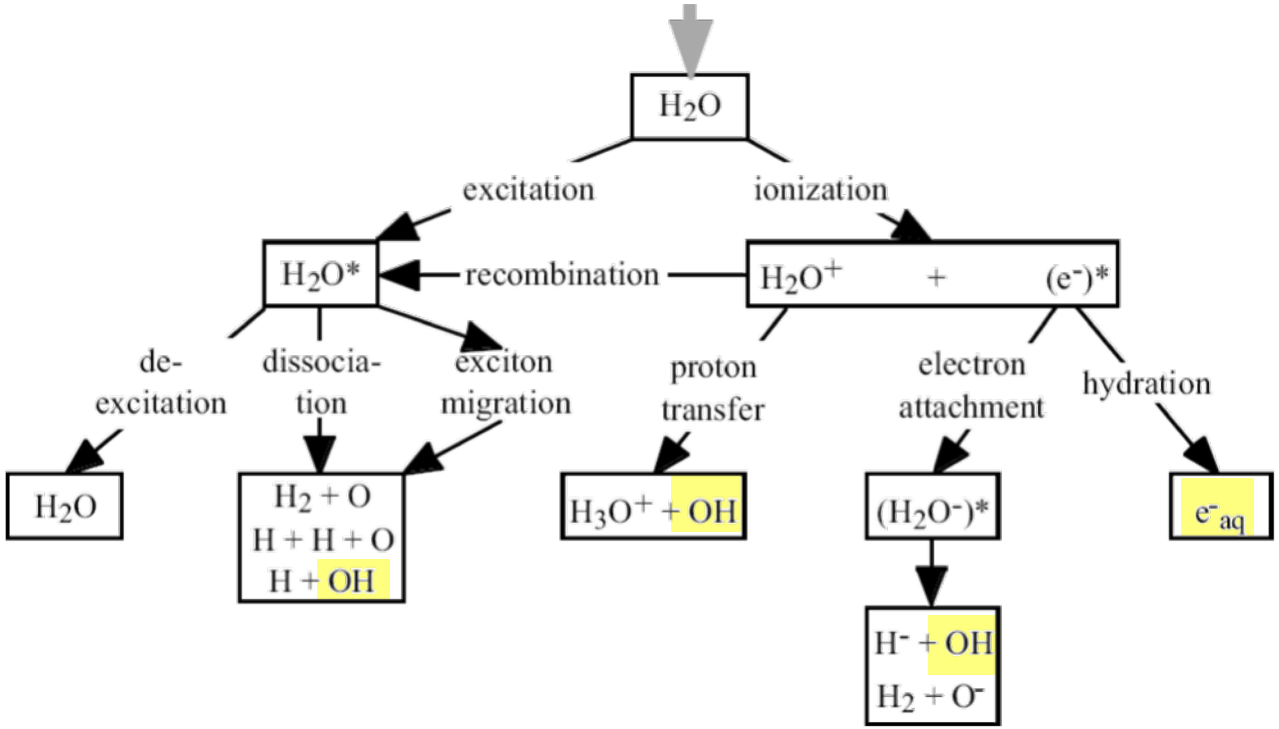
Bury et al., JSR 2015

Site-specific damage

Dose tells us about the physics: as implied earlier lots more (chemistry) going on

Much of energy absorbed by protein crystals results in radiolysis of water: **indirect radiation damage** is occuring

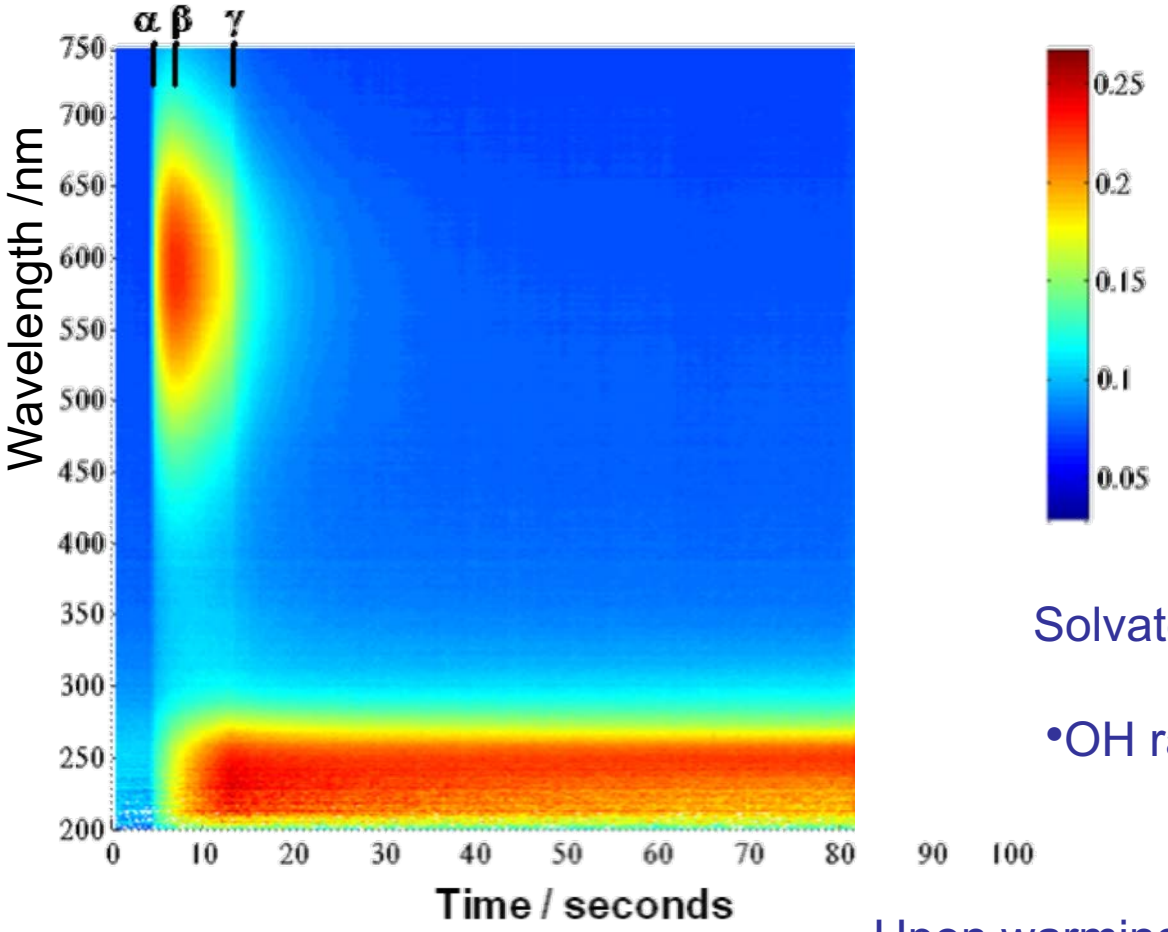


Solvated electrons (e_{aq}) and hydroxyl ($\bullet OH$) of particular interest.

Temperature dependence

in-situ UV-Vis absorption spectroscopy

On-line UV-Vis absorption spectra of FcγRIIIA solution held at 100 K



Solvated e^- mobile at 100K (593 nm)

•OH radicals trapped (240 nm)

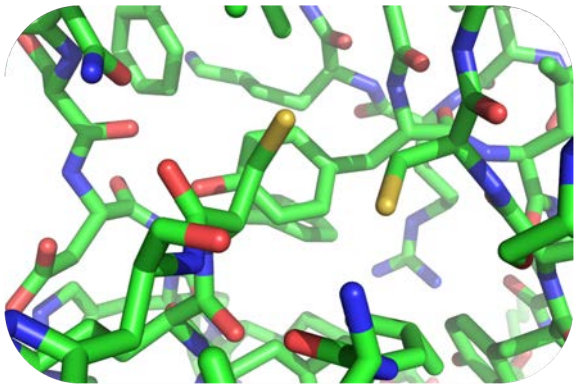
Upon warming •OH become mobile, and diffuse through the sample reacting.

Spectroscopic signature disappears

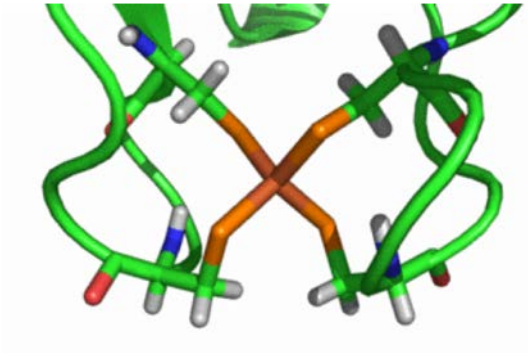
Cryo temperature site-specific damage

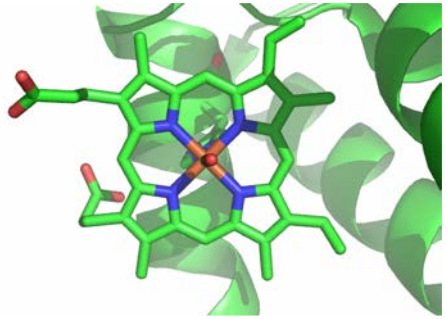
- OH ‘frozen’ and can not diffuse through crystal

solvated electrons still mobile. Can move through solvent, tunnel along backbone and go to most electron affinic groups



disulphide bonds

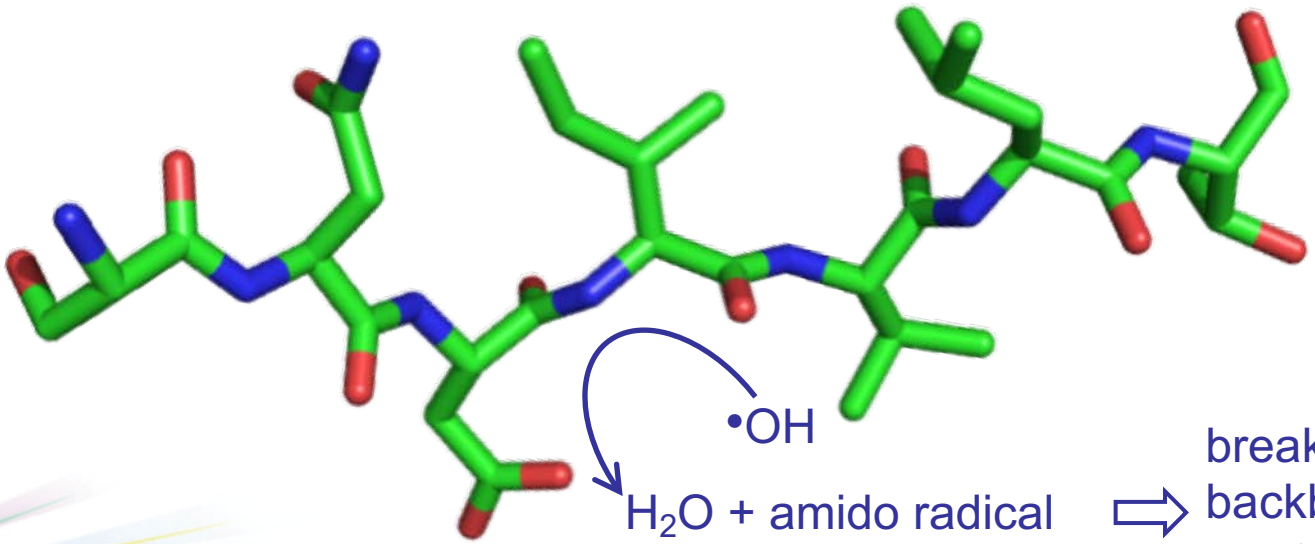




metal centres and active sites

Room temperature site-specific damage

•OH is extremely reactive and reacts at a rate only limited by the rate of diffusion (*Swartz & Swartz (1983)*). Mobility of •OH very low below 130K (*Symons (1999)*) Favoured reaction is abstraction of hydrogen from nitrogen atom of backbone amides (*Rao & Hayon (1974)*).



This is in addition to electron migration and is much more disruptive…

To monitor damage need to know the dose

$$D \propto \underbrace{\frac{I_0}{\lambda V}} \left[1 - \exp\left(\underbrace{-\mu_{abs} t}\right)\right]$$

Beam properties		Crystal properties
intensity		absorption coefficient
wavelength		crystal thickness
irradiated volume	⟷	irradiated volume

…and how X-rays interact with matter

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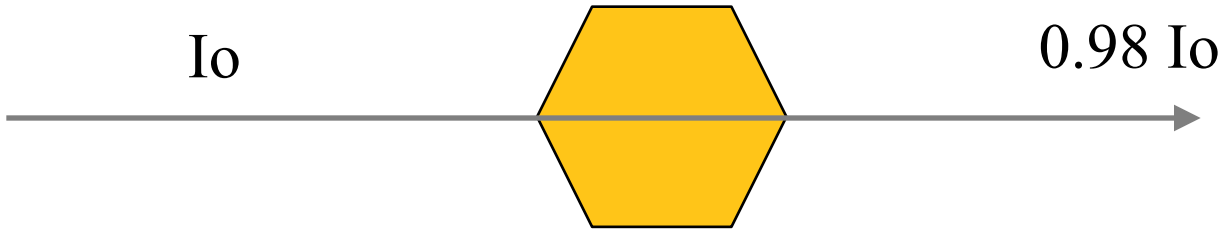
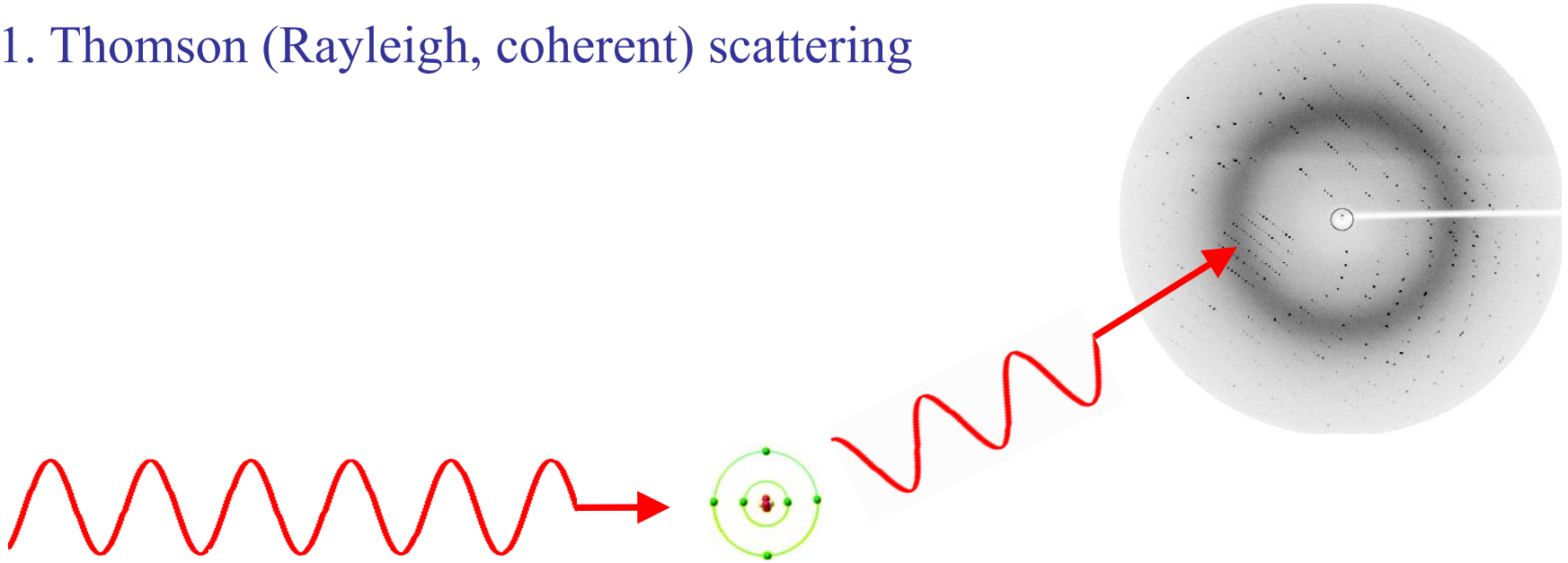
Most X-rays don’t interact

Native HEWL 100 µm thick



Primary X-ray interaction processes

1. Thomson (Rayleigh, coherent) scattering



- ELASTIC - no energy loss.
- Coherent – adds vectorially and gives diffraction pattern.
- Small proportion of total scattering: 8% at 1Å

BUT IT IS THE BIT WE WANT!!

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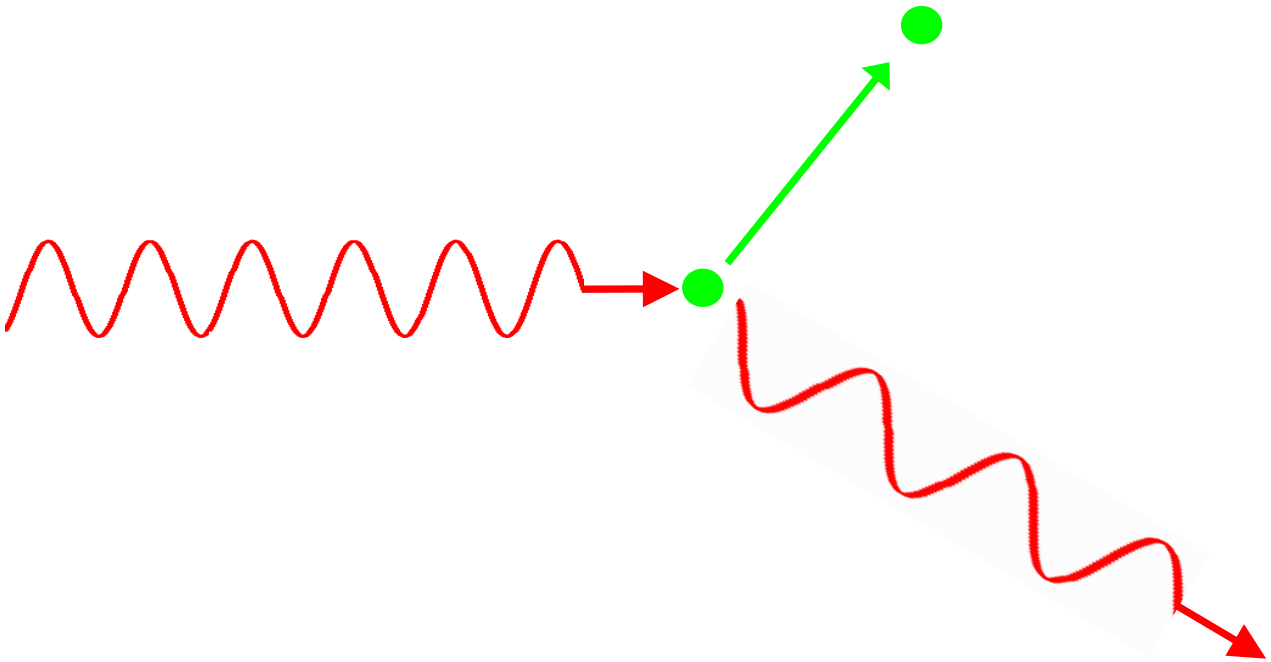
Primary X-ray interaction processes

2. Compton (incoherent) scattering



- X-ray transfers some energy to atomic electron and thus has lower energy (higher wavelength).
- Incoherent – part of X-ray background in images.
- Also a small proportion of total scattering: 8% at 1Å

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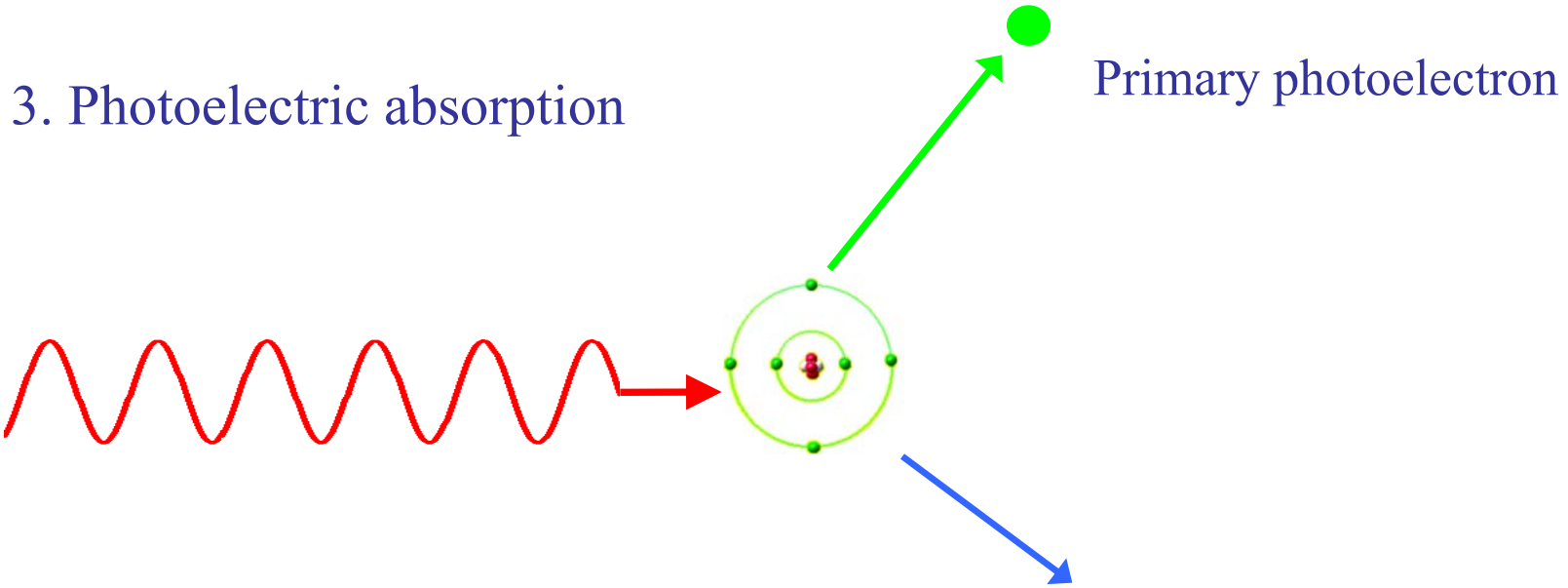
Primary X-ray interaction processes

3. Photoelectric absorption



- The dominant effect: 84% at 12.4 keV
- Inelastic
- X-ray transfers all its energy to an atomic electron, which is then ejected.
- Each 12 keV primary photoelectron can give rise to up to 500 ionisation events.
- Atom can then emit a characteristic X-ray or an Auger electron to return to its ground state.

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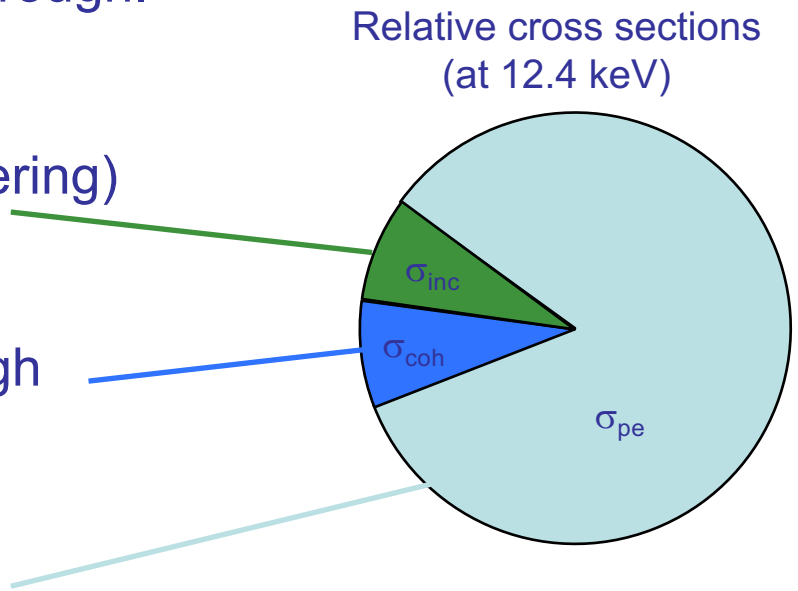
X-ray interactions

More than 90 % of X-rays pass straight through.
Of those that interact:

1. Scattered inelastically (Compton scattering)
   (~8%)

2. Scattered elastically (Thomson/Rayleigh scattering)
   (~8%)

3. Absorbed via the photoelectric effect
   (~84%)

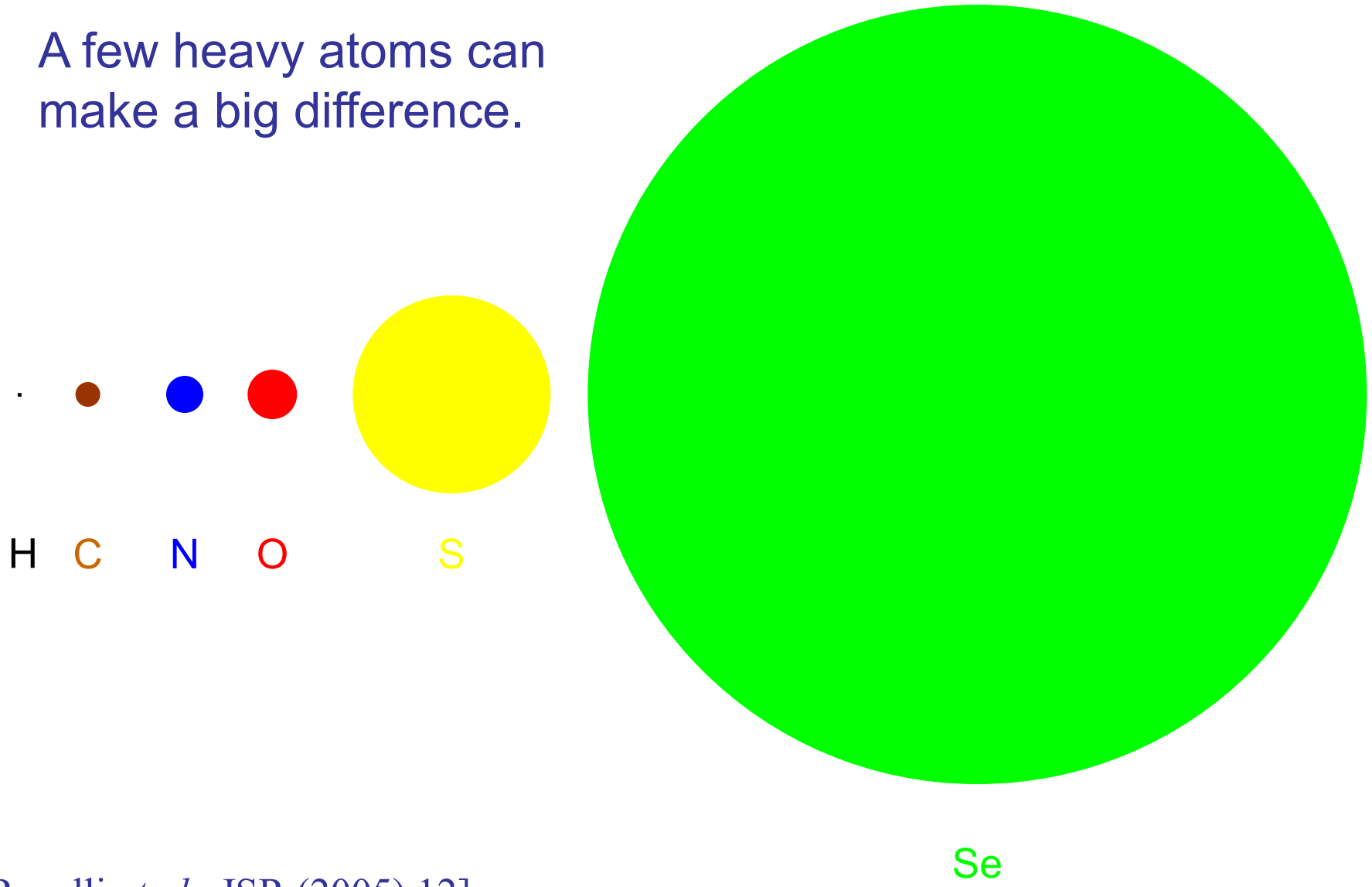


Only (2) contributes to the useful part of the observed diffraction pattern (Bragg spots) but (3) dominates.
Damage is proportional to (3)

Photoelectric cross sections at 13.1 keV

[unit of cross section barn=$10^{-28}m^2$]

A few heavy atoms can make a big difference.

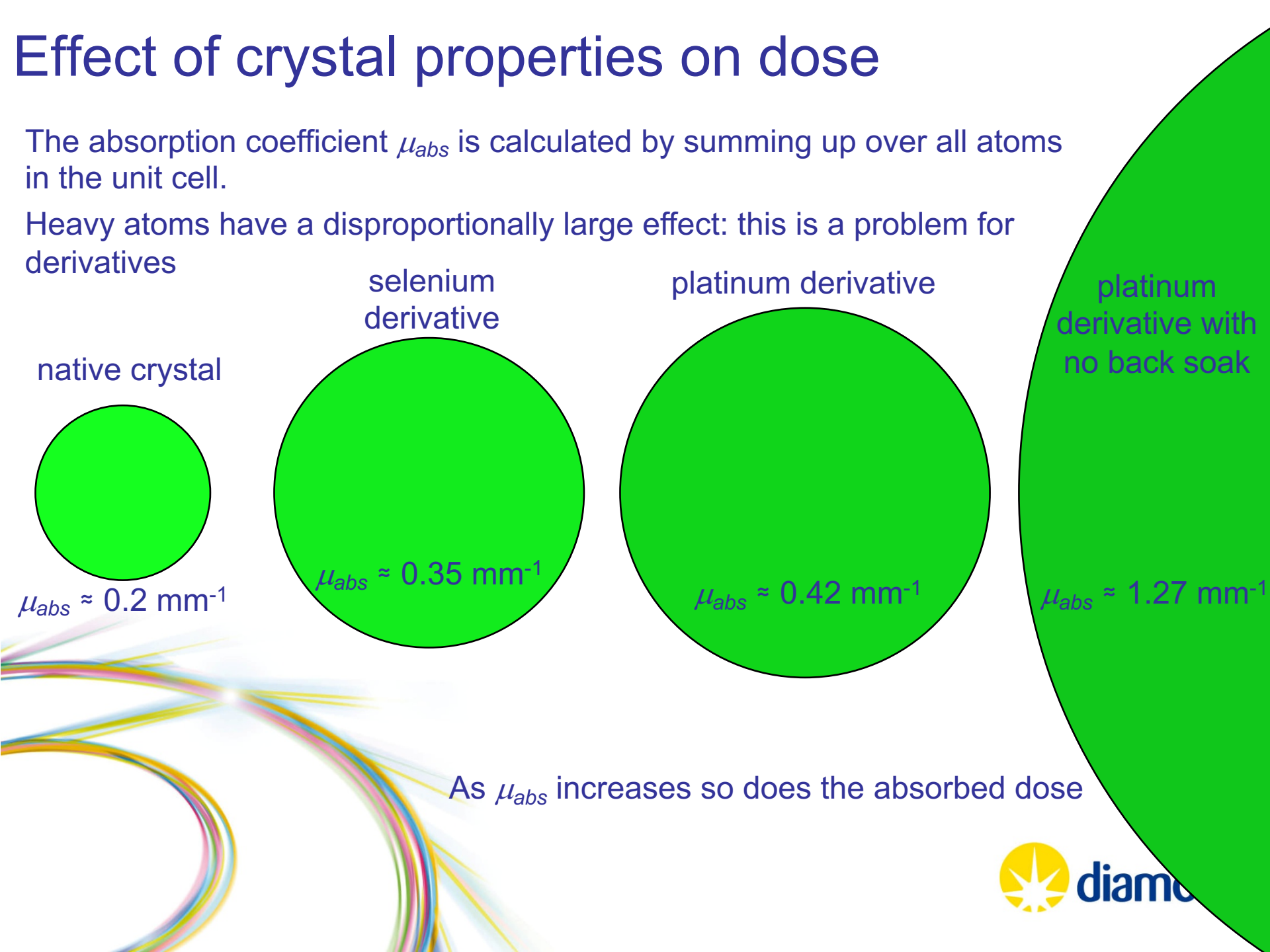


[Ravelli *et al.*, JSR,(2005) 12]

Effect of crystal properties on dose

The absorption coefficient μ_{abs} is calculated by summing up over all atoms in the unit cell.

Heavy atoms have a disproportionally large effect: this is a problem for derivatives



As μ_{abs} increases so does the absorbed dose

Importance of backsoaking

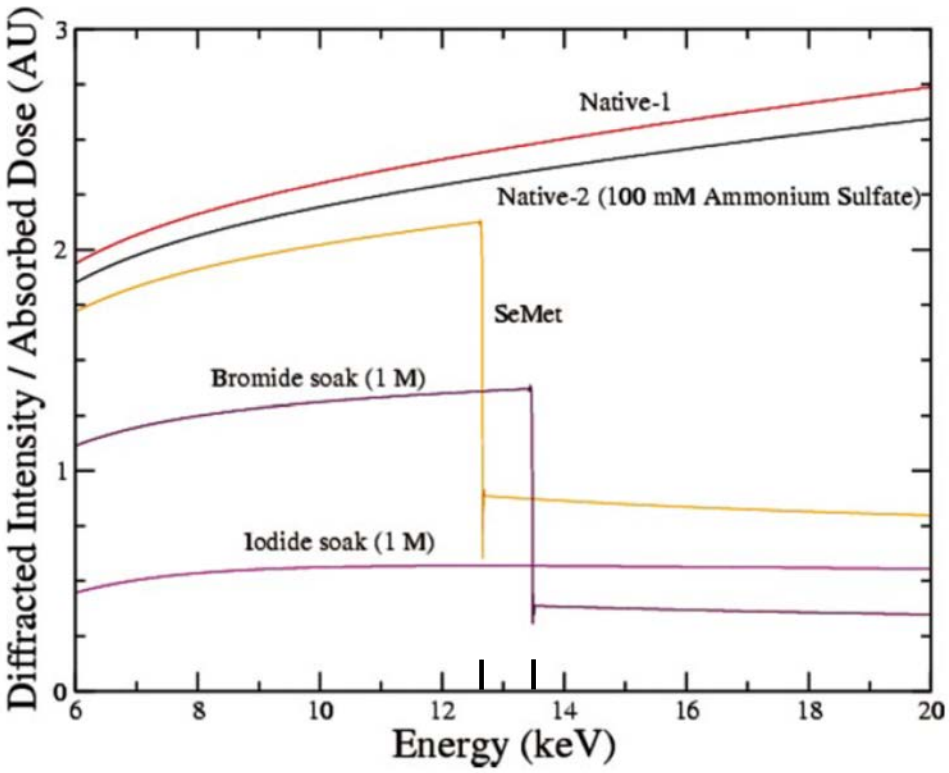
Heavy atoms in surrounding liquid don't contribute to anomalous signal but contribute greatly to absorbed dose

Concentration of heavy atoms in mother liquor to double absorbed dose can be relatively low.

Effect of heavy atoms is most pronounced above absorption edges

cf Bromine dose-doubling concentration

- 1.2 M at 12.68 keV
- 320 mM at 13.486 keV



* Calculated at 12.68 keV

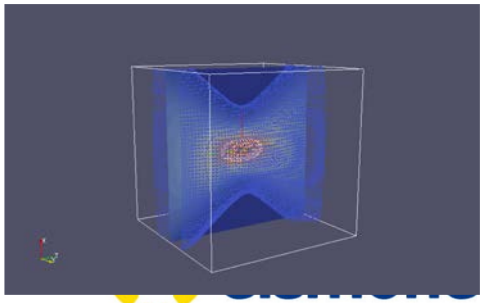
Holton *J. Sync. Rad.* (2009).

Plot from Murray *et al. J. Appl. Cryst.* (2004)

Know your dose: *RADDOSE-3D*

- 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 experimental profiles
- Gives information on dose DISTRIBUTION and TIME COURSE, not just average dose.
- Engineered for easy extendibility: can real beam profile now, and actual crystal shape in the future.
- On server:

www.raddo.se



Zeldin, Gerstel, Garman JAC (2013)

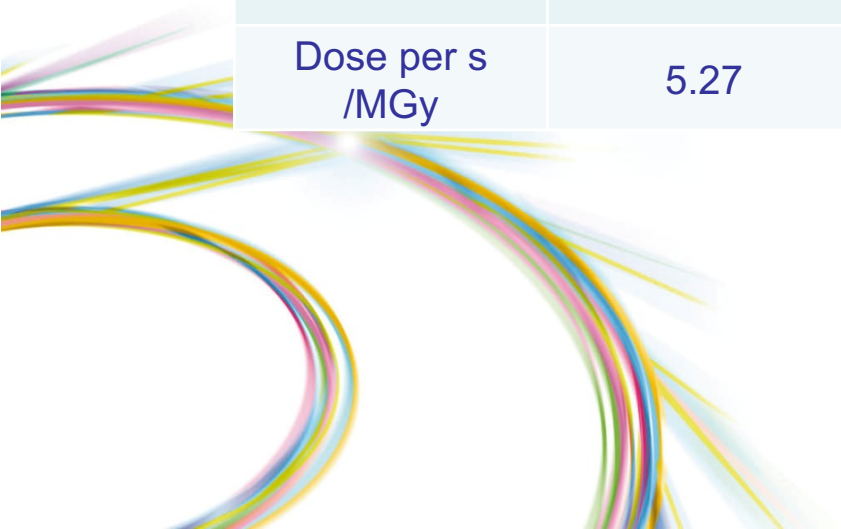
Typical doses for an experiment at DLS

Two proteins:

Small-ish soluble protein FcgRIII (175 aa) 1FNL

ABC transporter (595 aa) 4AYT

	FcgRIII		ABC transporter	
Energy / keV	12.8	12.8	12.8	12.8
Beamsize / mm^2	10×10	80×20	10×10	80×20
Flux / ph s^{-1}	1.5×10^{12}	1.7×10^{12}	1.5×10^{12}	1.7×10^{12}
Dose per s /MGy	5.27	0.37	5.50	0.39



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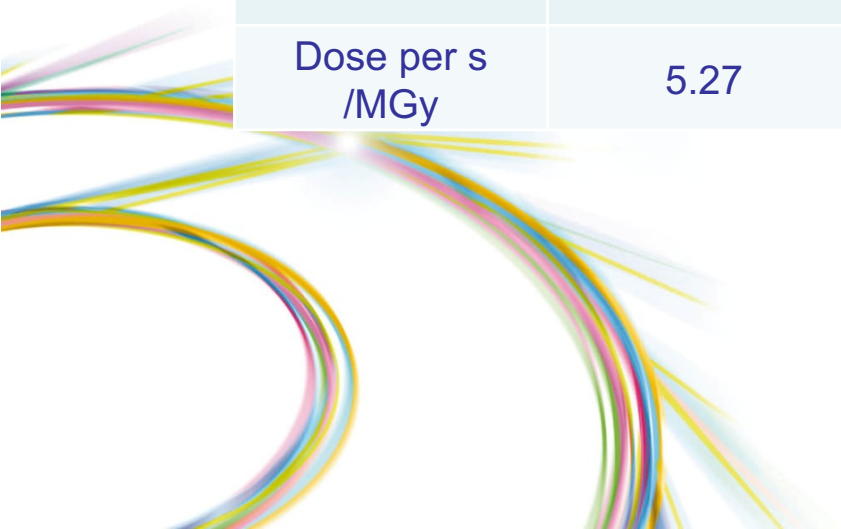
Typical doses for an experiment at DLS

Adding heavy atoms to FcgRIII has a dramatic effect on the absorbed dose.

2 × Pt per monomer

Assumes perfect backsoak

	FcgRIII		FcgRIII Pt derivative	
Energy / keV	12.8	12.8	12.8	12.8
Beamsize / mm^2	10 × 10	80 × 20	10 × 10	80 × 20
Flux / ph s^{-1}	1.5×10^{12}	1.7×10^{12}	1.5×10^{12}	1.7×10^{12}
Dose per s /MGy	5.27	0.37	10.10	0.72



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How much dose is ok?

Several limits suggested in the literature

Global damage

- Experimentally measured dose to half diffracting power 43 MGy (Owen, 2006)
- Resolution dependent limit 10 MGy / Å (Howells, 2005)

Site-specific damage

- Disordering of selenomethionine sidechains 2 MGy (Holton, 2007)
- Reduction of copper nitrate reductase 1.5 MGy (Hough, 2008)
- Cleavage of anomalous scatterers 0.5 MGy (Olieric, 2007)

- Reduction of myoglobin 0.020 MGy (Denisov, 2007)

For reference:

3 Gy kills a hamster

How radiation damage affects intensities

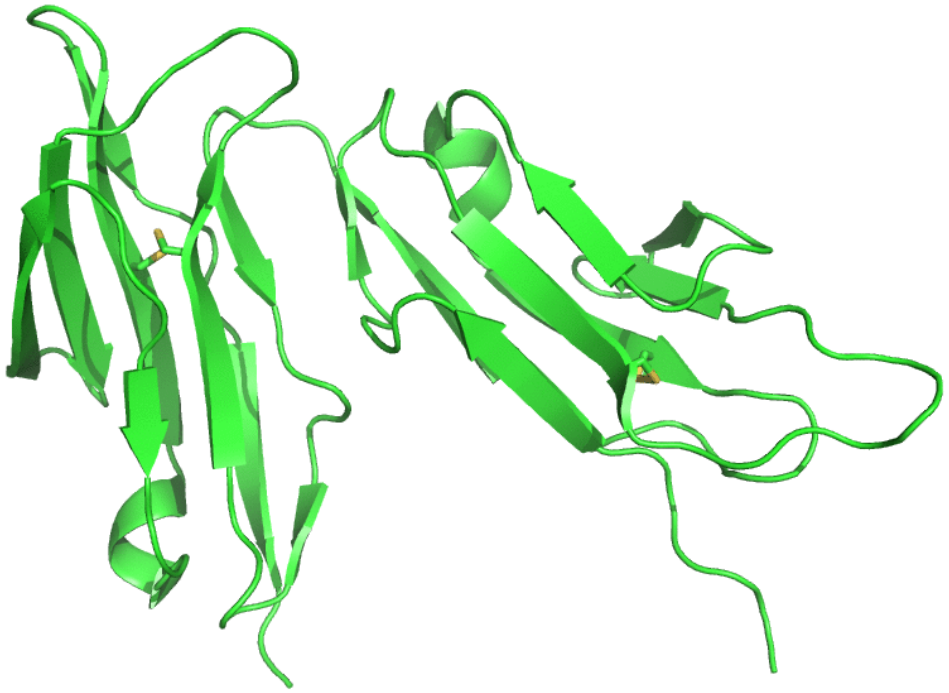
Example: FcγRIIIa
PDB accession code 1FNL

Unit cell 67, 86, 36 Å

$P2_12_12$

175 amino acids; MW 19.95 kDa

Two disulphide bonds



Quantifying changes

The structure factor F_{hkl} can be written as a sum of scattering by all atoms

$$F_{hkl} = \sum_{N} f_i \exp[2\pi i(hx_i + ky_i + lz_i)] \exp[-B_i \sin^2 \theta/\lambda^2]$$

f_i represents scattering by i^{th} atom – the atomic scattering factor

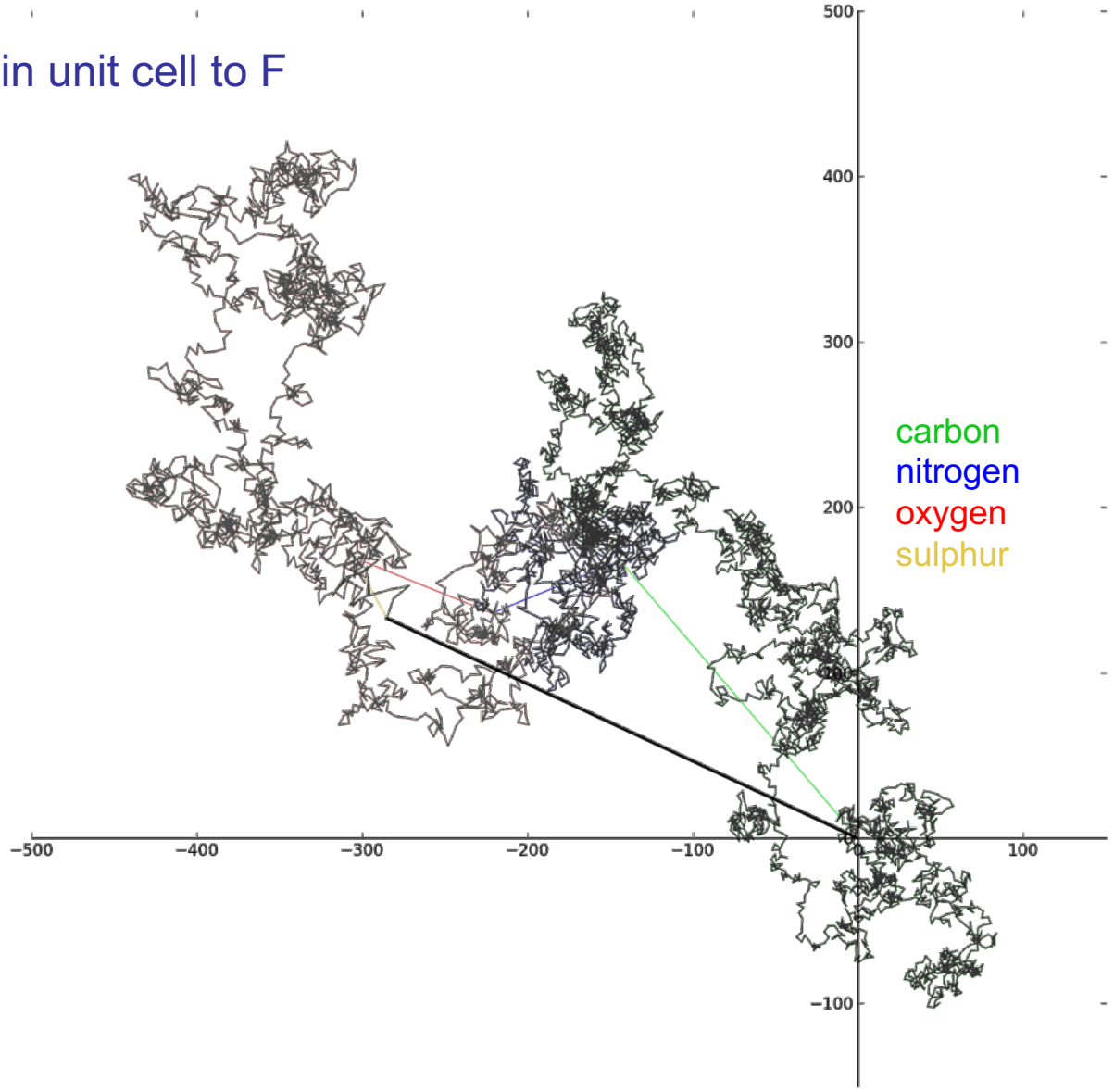
By summing over all N atoms in the unit cell can calculate F.

Argand diagram

Shows contribution of atoms in unit cell to F

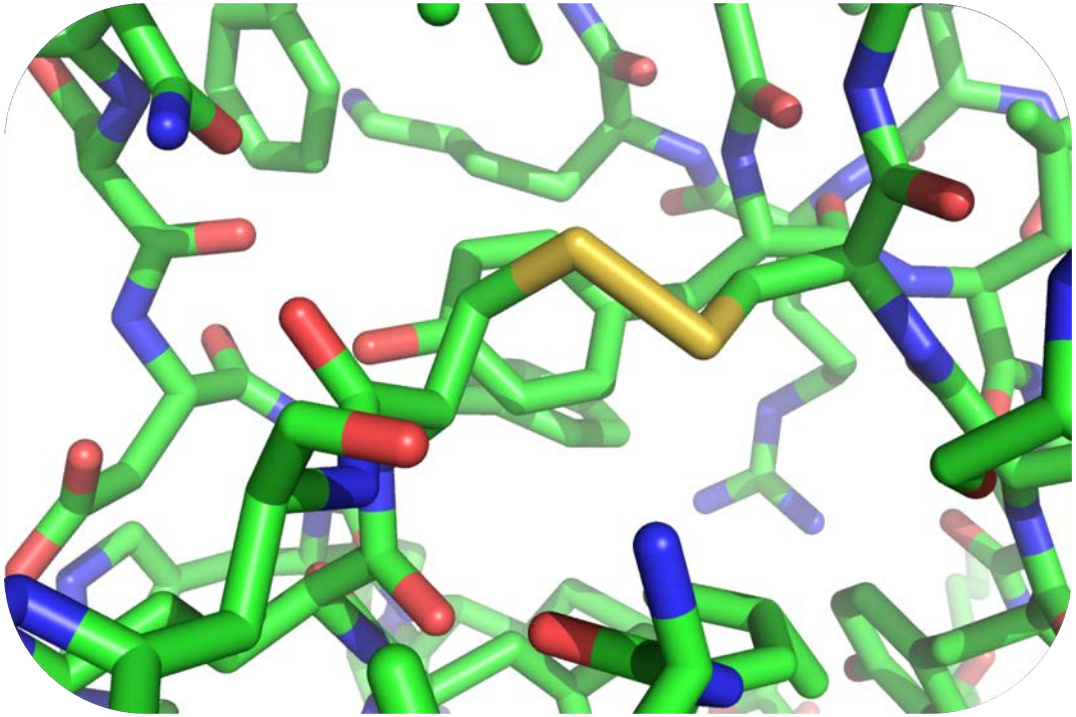
1FNL

reflection (17,7,6)



Changes in structure factor due to:

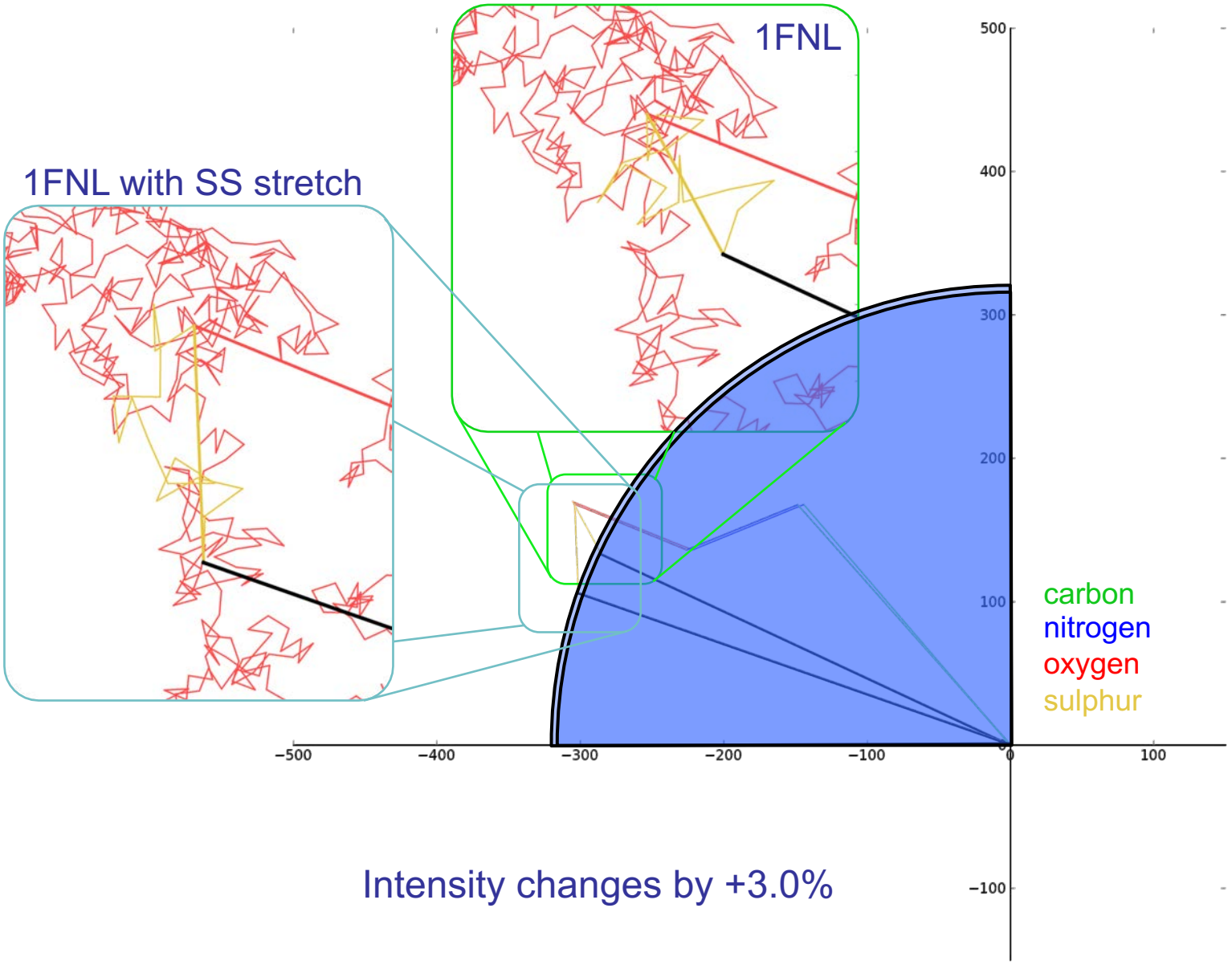
1. Stretched /broken disulphide bonds



Two disulphide bonds per monomer

- C29 – C71 was 2.06 Å, increased to 3.17 Å
- C110 – C154 was 2.04 Å, increased to 3.22 Å (shown above)

Effect of stretching disulphides on reflection (17,7,6)



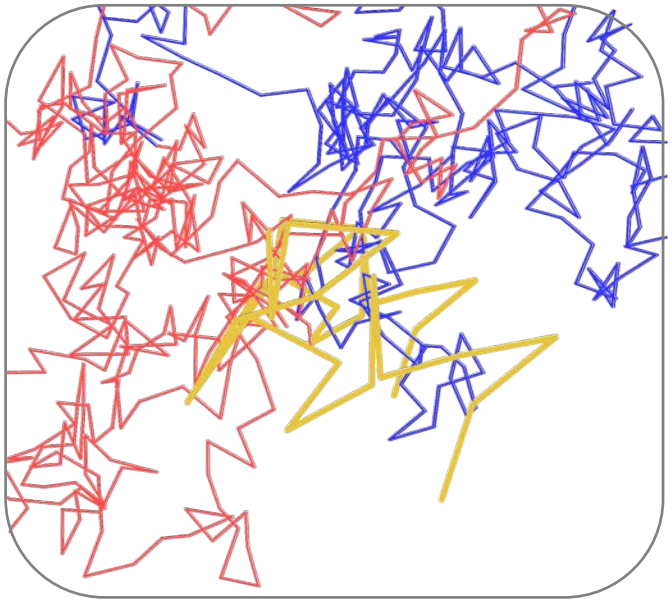
Intensity changes by +3.0%

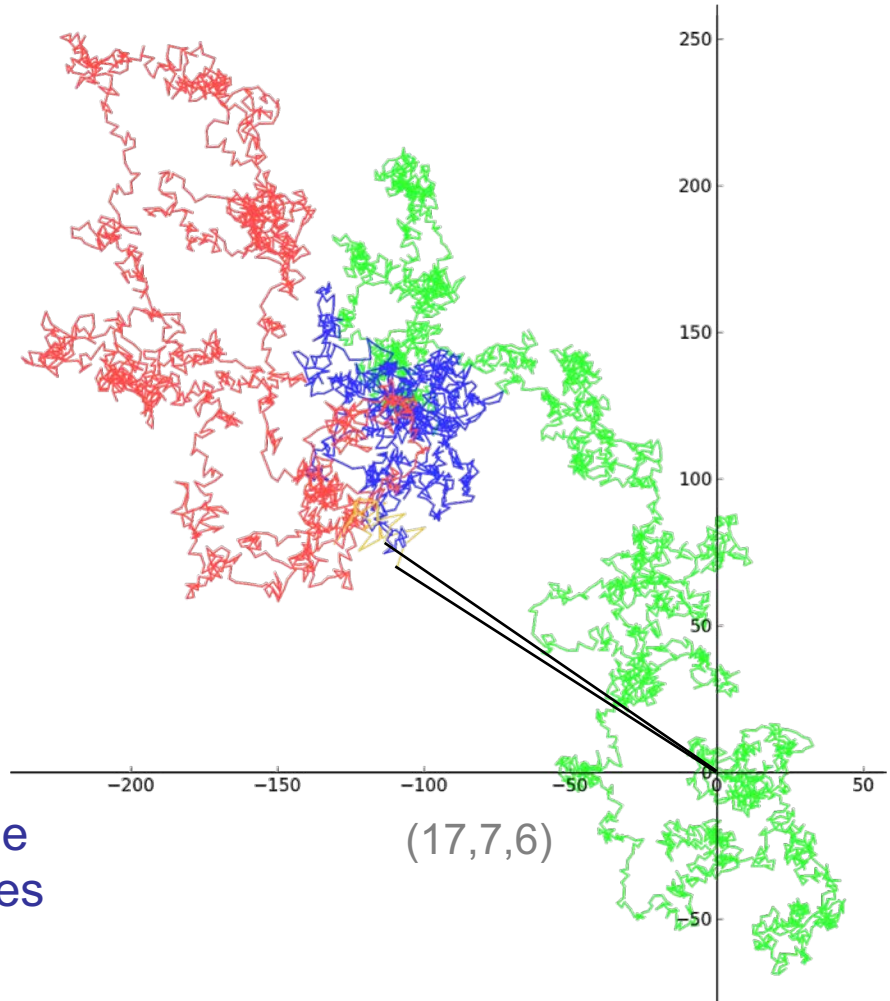
Effect of increasing B-factor

X-ray cross section of sulphur is greater than that of carbon, nitrogen, oxygen

Might expect B-factor to increase more than for other atoms

To illustrate effect, double B-factor of sulphur atoms.





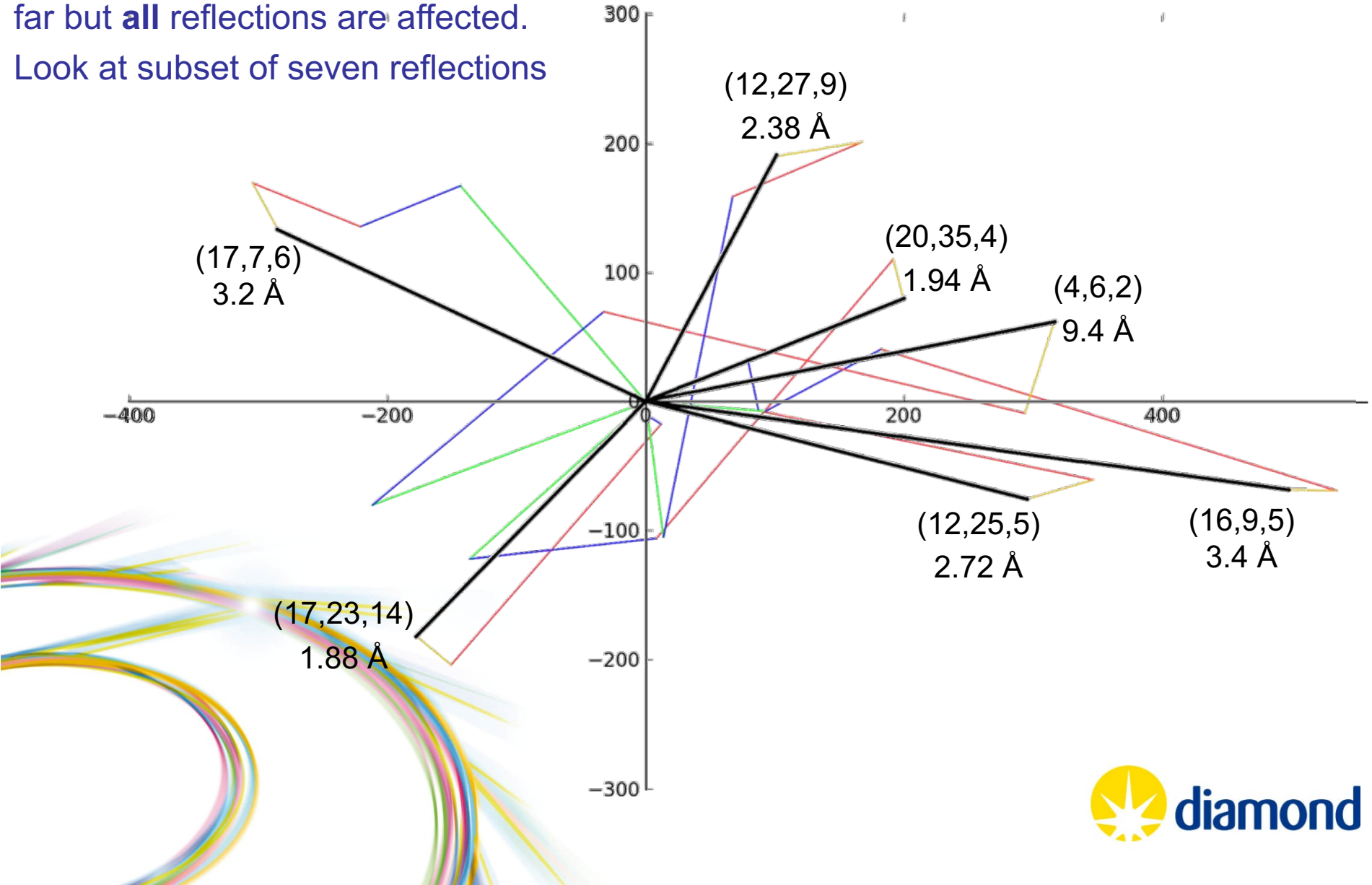
(17,7,6)

For this reflection, result is 6.3 % increase in intensity as sulphur contribution reduces

All reflections are affected

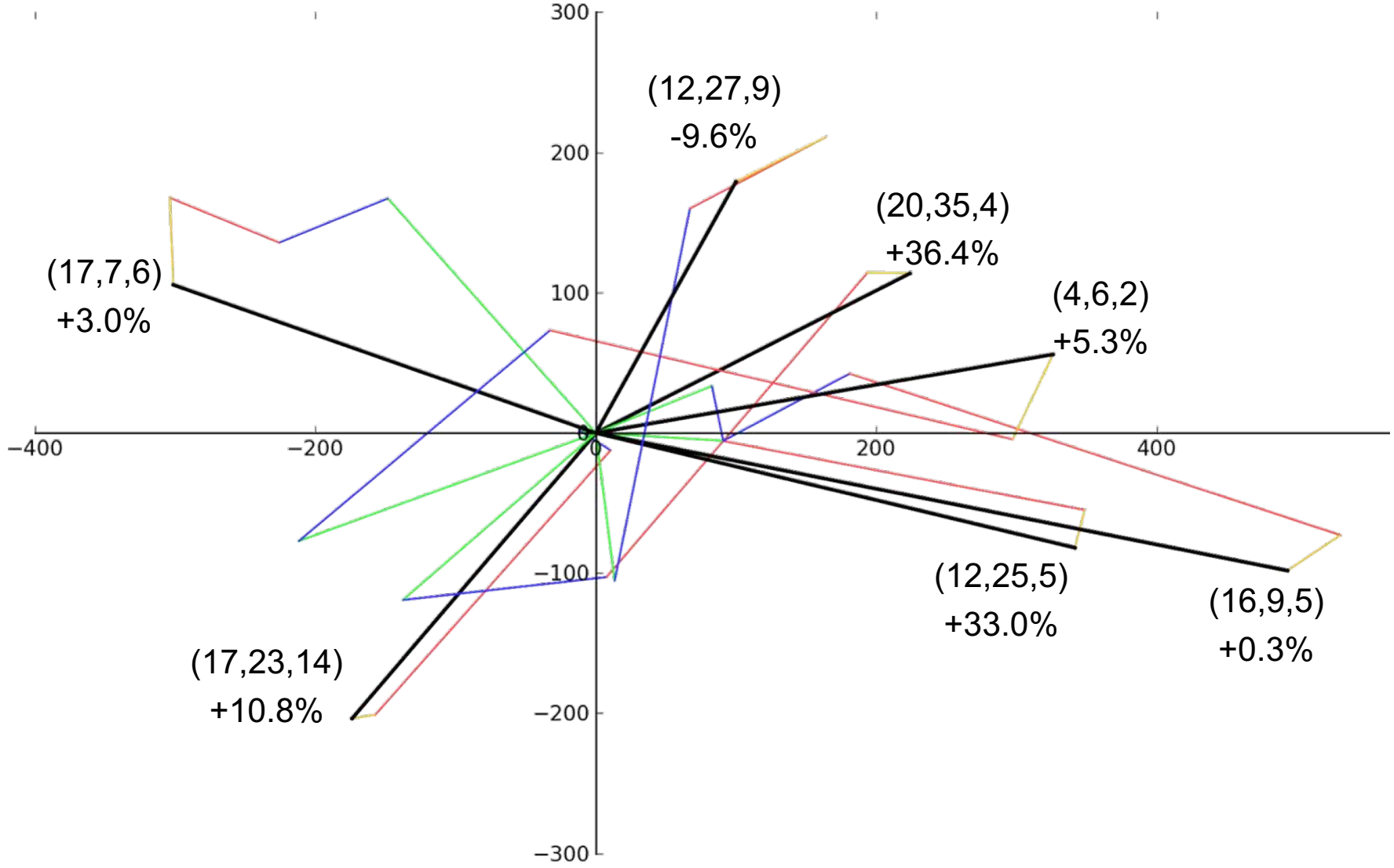
Just looked at a single reflection so far but **all** reflections are affected.

Look at subset of seven reflections



Disulphide stretch

Stretch disulphide as shown for (17,7,6)



Change in unit cell volume

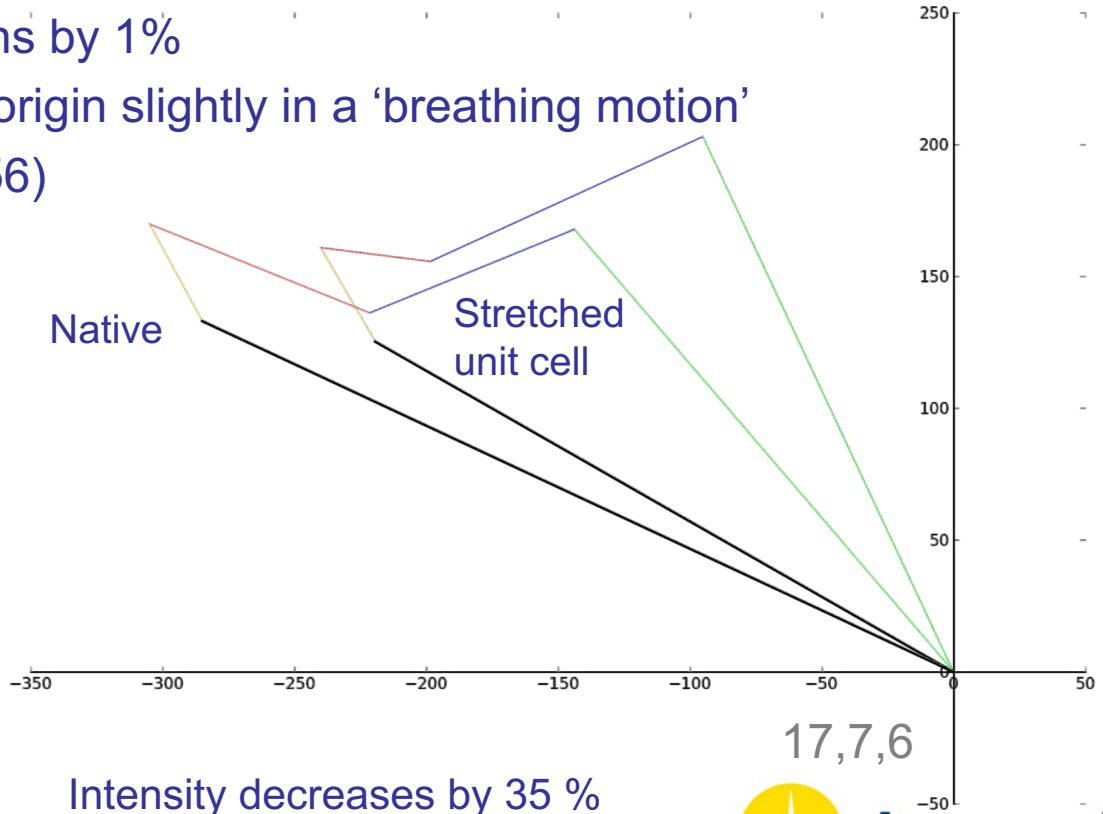
Changes in unit cell dimensions can result from radiation damage

Size and rate of change can be unpredictable

(Murray and Garman 2002, Ravelli *et al*. (2002).)

To illustrate effect on structure factors

- Increase unit cell dimensions by 1%
- Move molecule away from origin slightly in a ‘breathing motion’
  (cf Crick and Magdoff 1956)



17,7,6

Intensity decreases by 35 %

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Changes due to radiation damage

Stretching of disulphides, or preferentially increasing B-factor of groups of atoms, results in different changes in the intensity of reflections

Changes in unit cell volume also causes relative intensity of reflections to change. In this example effect is more dramatic than disulphide breakage

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Comparing radiation damage and derivatisation

Derivatisation also introduces changes to intensities

Can estimate average expected change using

$$\frac{\langle \Delta I \rangle}{I} = \left(\frac{N_E}{N_P}\right)^{1/2} \frac{Z_h}{Z_{eff}}$$

N_E is number of heavy atoms
N_P number of non-H protein atoms
Z_h atomic number of heavy atom
Z_{eff} mean Z of protein (~6.7)

Crick and Magdoff (1956)

<u>Add 2 × Pt to FcγRIIA</u>

- Using the above $\Delta I/I = 0.44$
- Or we can use Argand diagrams to illustrate changes to a subset of reflections.

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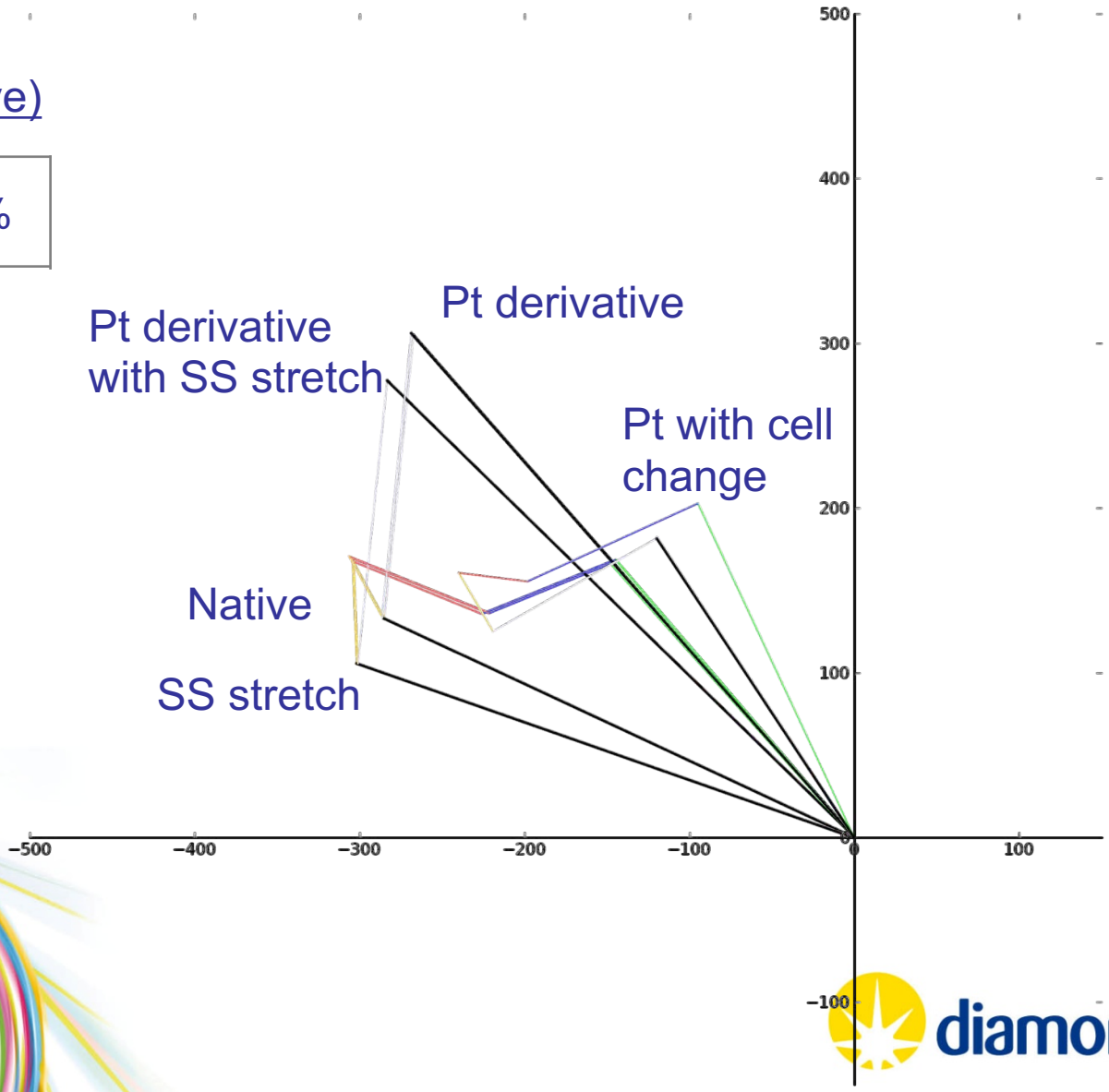
Back to reflection 17,7,6

Change in I (relative to native)

Platinum derivative	+ 66 %



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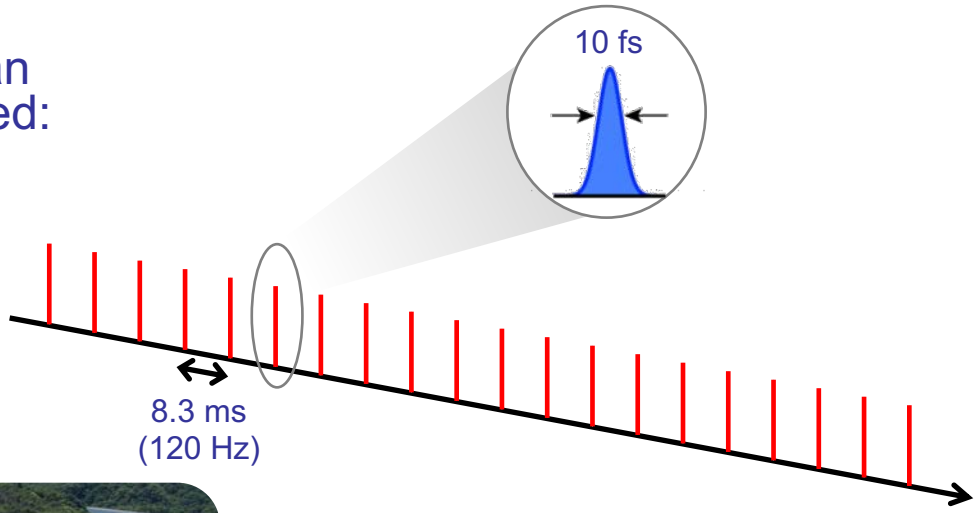
New approaches and new facilities

- Each pulse contains approx the same number of photons as 1s of beam at I24
- Samples are destroyed by a single pulse.
- Short duration of pulse means data can be collected before sample is destroyed:

  “diffraction before destruction”

- A new sample must therefore be presented to each pulse

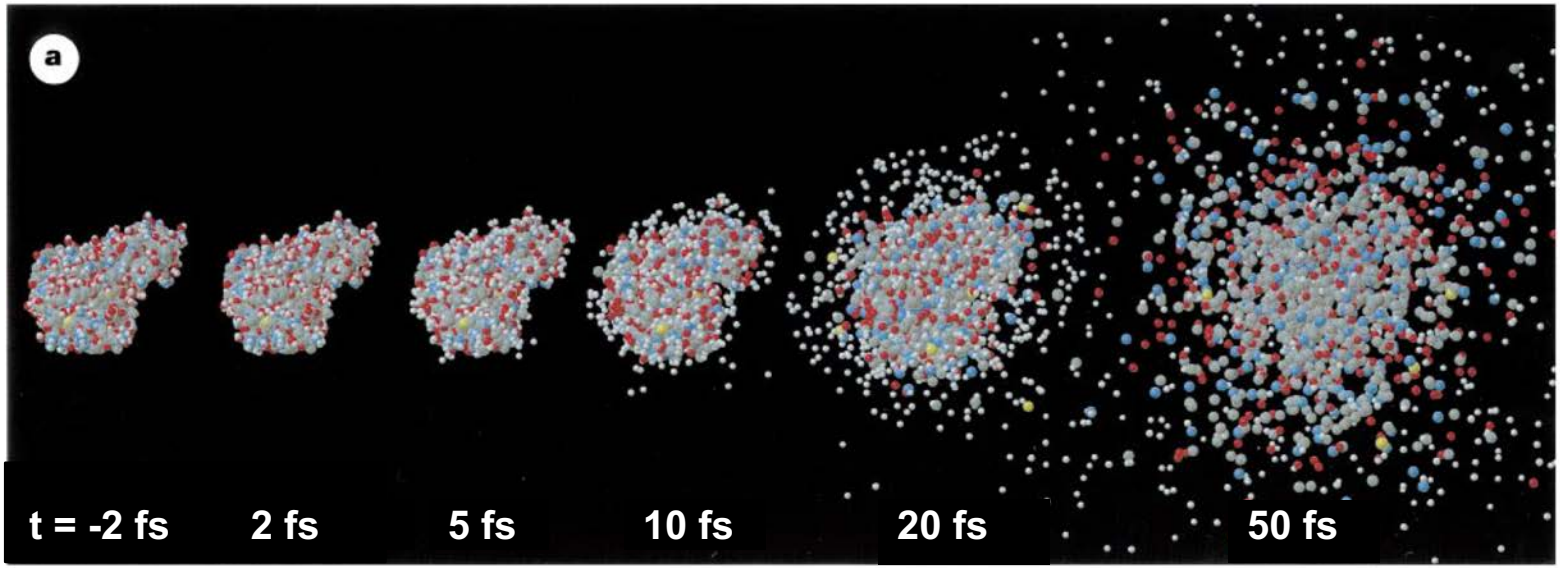
Pulsed structure



Diffraction before destruction

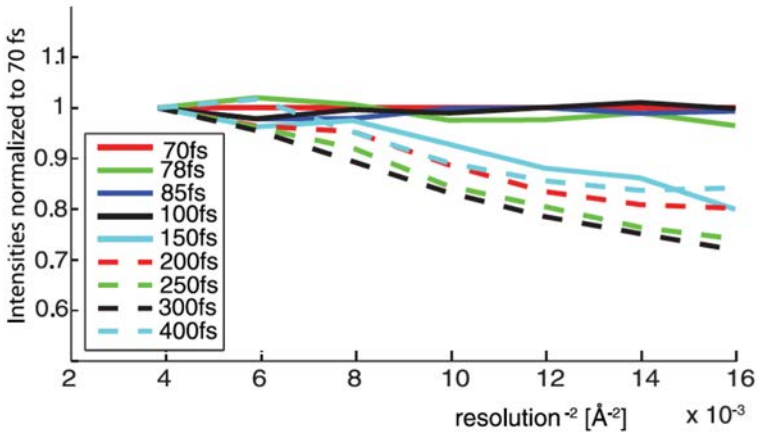
Simulated Coulomb explosion of a protein

Neutze, et al, Nature 406, 752 (2000)



How long is the pulse?

- In principle don't have to worry about damage provided the pulse is short enough
- 100 fs 'threshold' for damage within a pulse

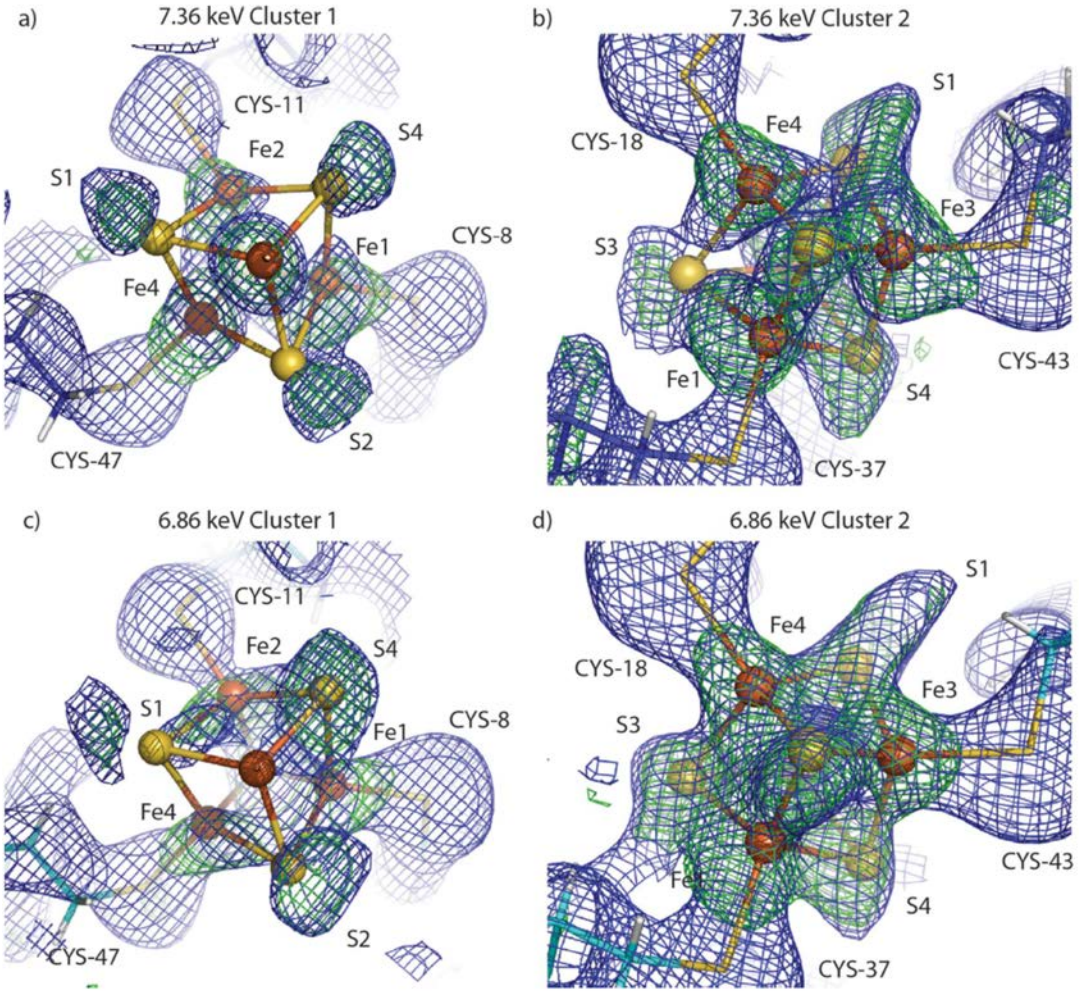


Lomb *et al* Phys Rev B (2011)

At free electron lasers

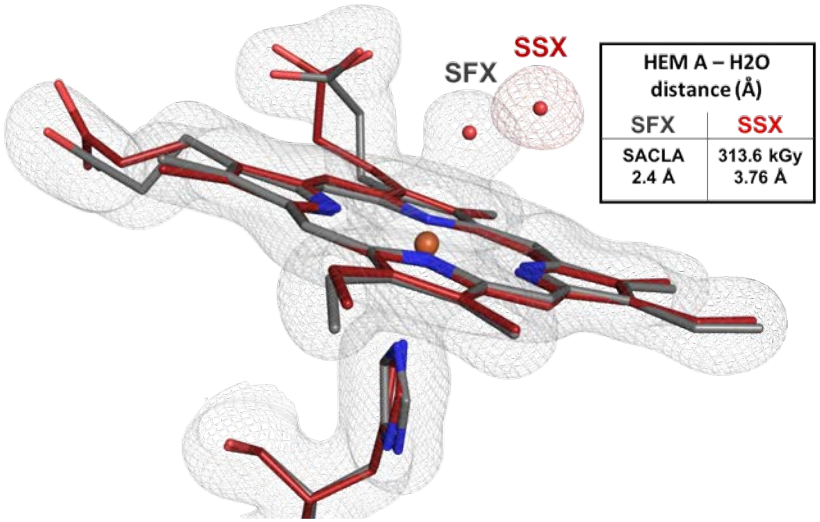
Same principles apply

- damage at heavy atoms sites



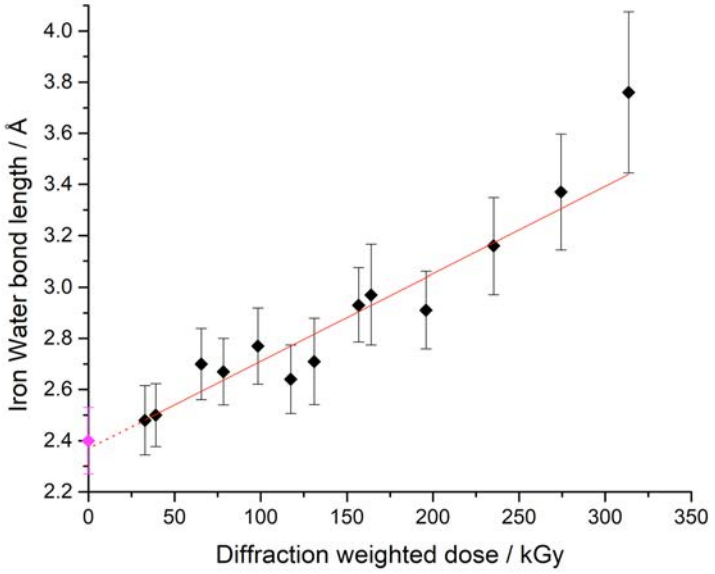
Nass *et al* J. Synch Rad. (2015)

Damage-free structures (?)



If the pulse duration is short, data collection at XFELs (SFX) provides damage free structures which differ significantly from very low dose synchrotron (SSX) structures

Not a simple or win-win experiment however: lots of other things to consider…



Ebrahim *et al*. IUCrJ (2019)

Summary 1: effects of radiation damage

At the synchrotron, radiation damage is unavoidable even at cryo temperatures.

Whether you like it or not you need to be aware of it.

<u>Key points</u>

- Lifetimes approx. $\times$ 150 less at room temperature
- No significant ($< \times 2$) dose rate effect at 100 K at current flux densities (10^{15} ph/s/mm^2).
- No significant ($< \times 2$) temperature dependence below 100 K, but weak minimum at around 50 K.
- Site-specific damage occurs well before changes in global metrics become visible
- Site specific damage causes reflection intensities to change by amounts comparable to the changes induced by bound heavy atoms and socan cause phasing to fail.
- Beam induced heating not significant at current flux densities but likely to be as beamlines get hotter.

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Summary 2: what can YOU, the experimenter do?

For a particular system rates of damage predictable/can be modelled

- Use a sacrificial crystal
- Have a rough idea of the dose (RADDOSE-3D)
    - How do the doses you are using compare to ‘limits’ in the literature?

Reduce the dose

- Every beamline is equiped with attenuators: use them
- Helical data collection
- Merge wedges of data from many crystals. Discard data at the end of each wedge.
- Backsoak non-specifically bound heavier atoms out of your crystals.
- Be ‘absorption aware’ of the contents of your crystal (e.g. Se and buffer)
- Match beam size to crystal size

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Useful literature

Good introductions /overviews

A beginner's guide to radiation damage
James Holton
J. Sync. Rad. (2009), **16**, 133-142

Radiation damage in macromolecular crystallography: what is it and why should we care?

Elspeth Garman

Acta D (2010), **66**, 339-355