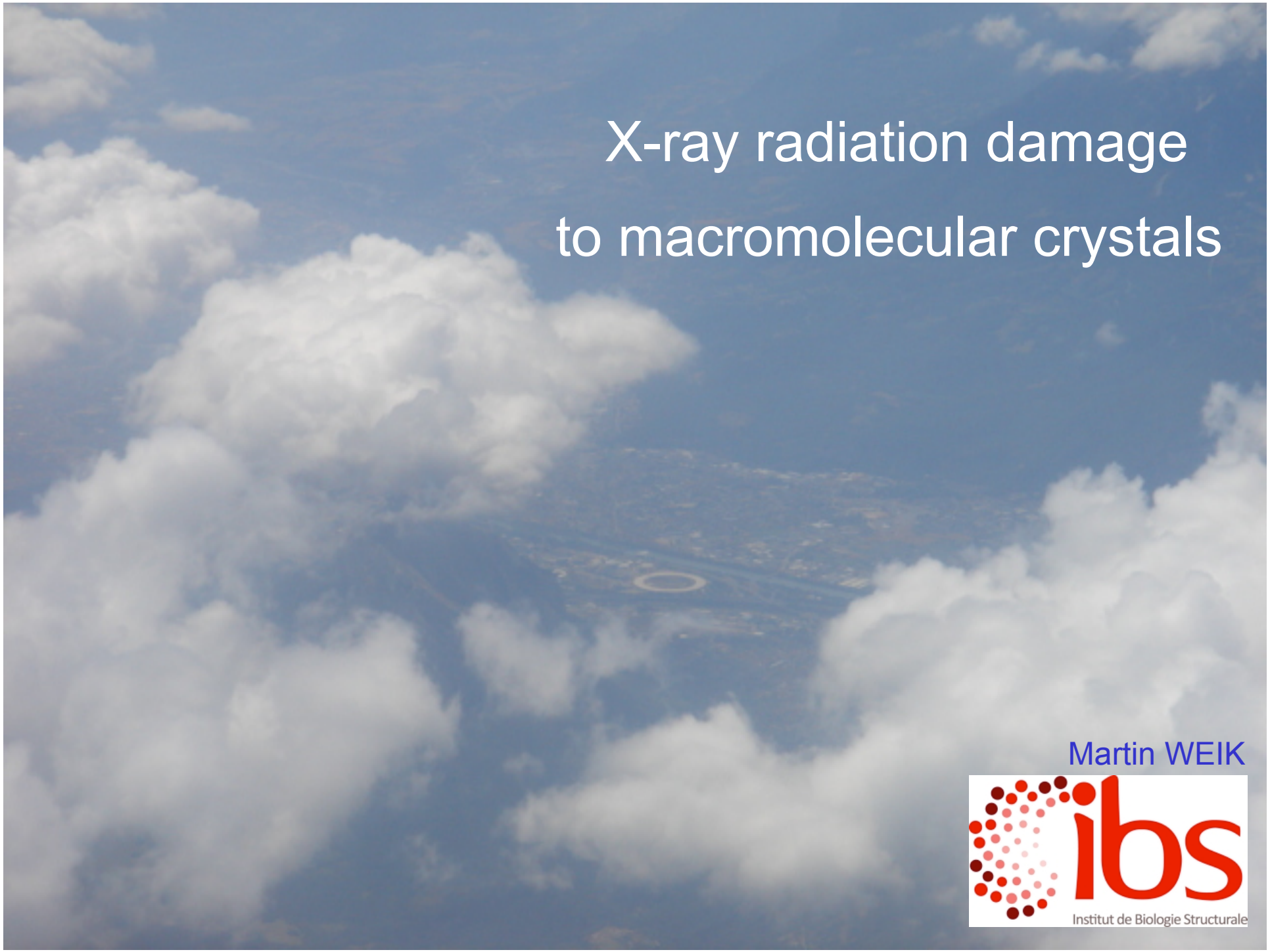
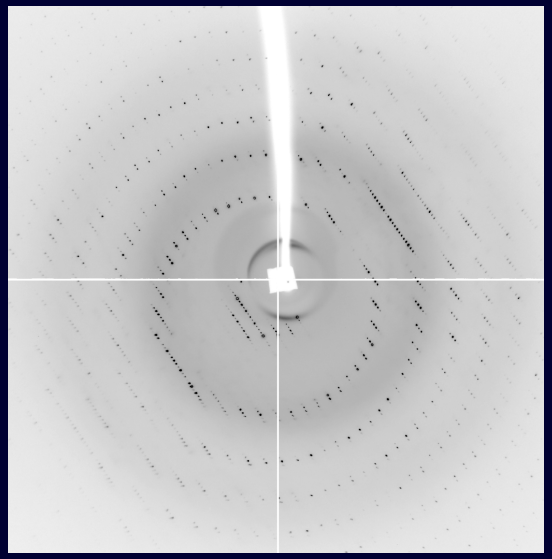

X-ray radiation damage
to macromolecular crystals
Martin WEIK
ibs
Institut de Biologie Structurale

Protein X-ray crystallography

Cryo (100 K)



Synchrotron radiation is a powerful tool, but price must be paid

Outline

- Radiation damage – what is it ?
- Radiation damage – how to limit or avoid it ?

X-ray – matter interaction: primary events

at 12.7 keV (λ=0.98 Å)

Murray *et al.* (2005) *J. Synchrotron Rad.* **12**, 268

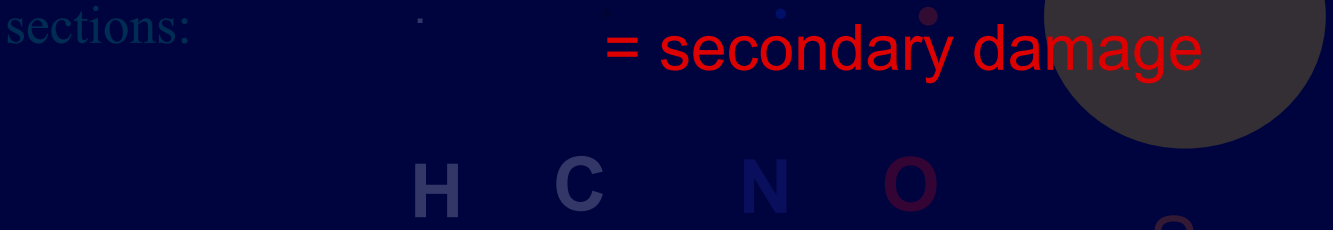
- 98% of incident photons don't interact at all
- 2% interact:

Elastic (Thomson) scattering (diffraction):	8%
Compton scattering:	8%
Photoelectric effect:	84%

each photoelectron produces 500 ionization events

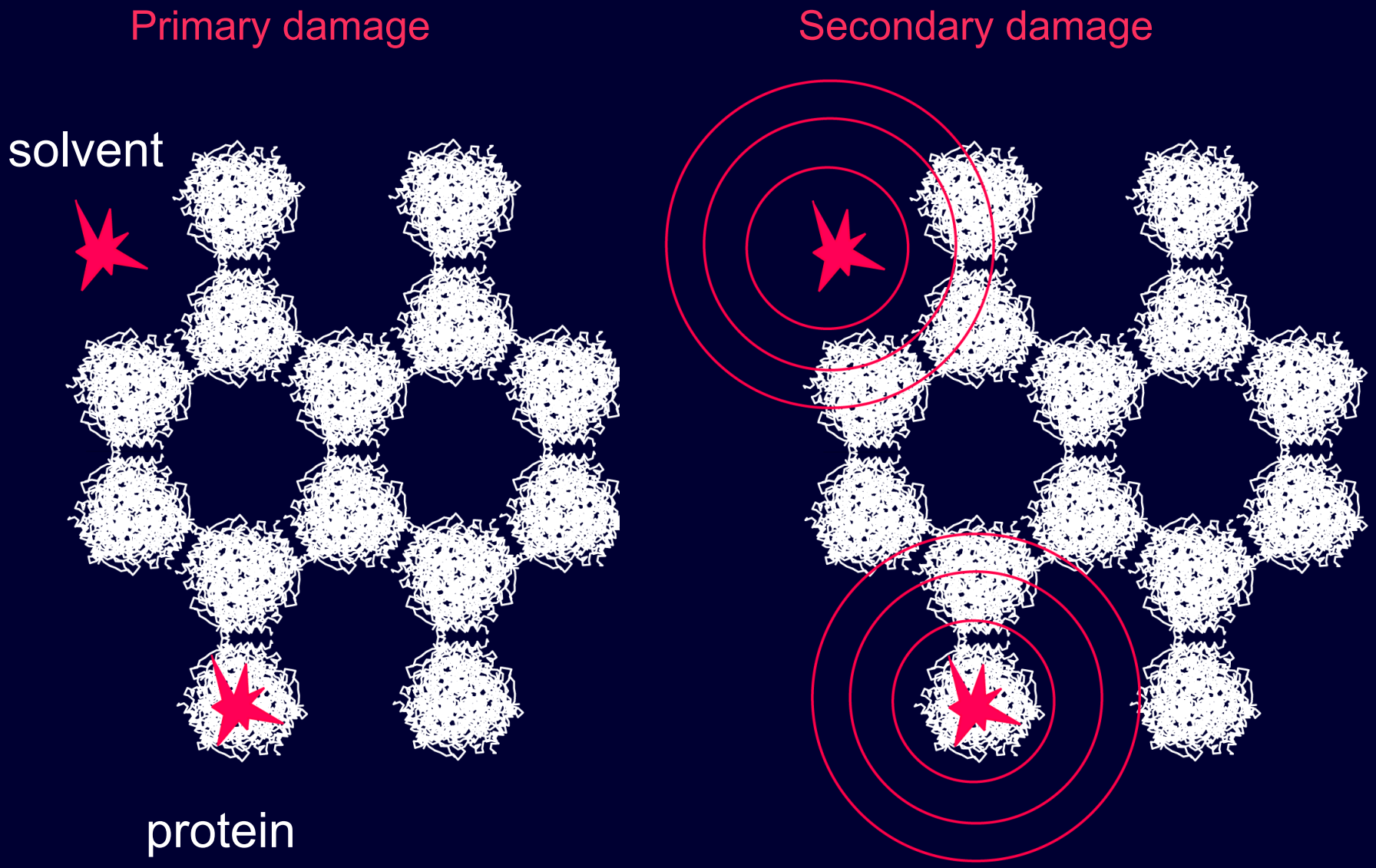
500 ionization events

Cross sections:

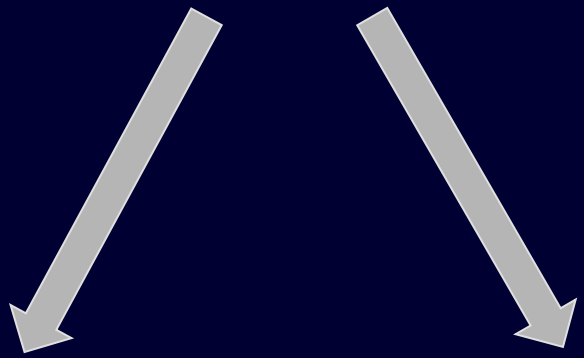


Ravelli *et al.* (2005) *J. Synchrotron Rad.* **12**, 276

primary and secondary radiation damage



secondary radiation damage



global

radiation damage

in reciprocal space

specific

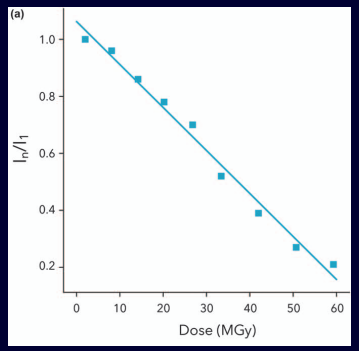
radiation damage

in direct space

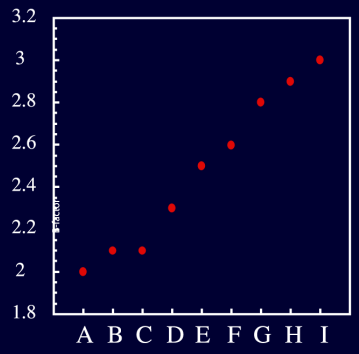
Global radiation damage limits diffraction quality

Gonzalez & Nave (1994) *Acta Crys. D* **50**, 874

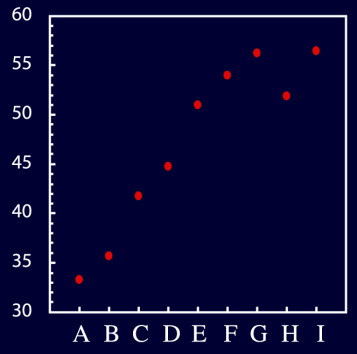
Bragg intensities



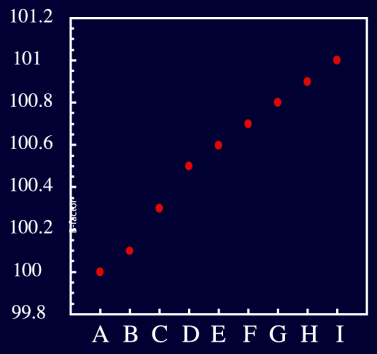
Resolution (Å)



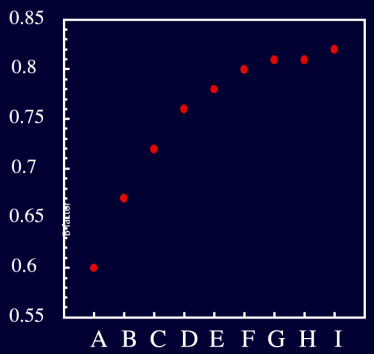
Wilson B-factor (Å^2)



Relative unit cell volume (%)



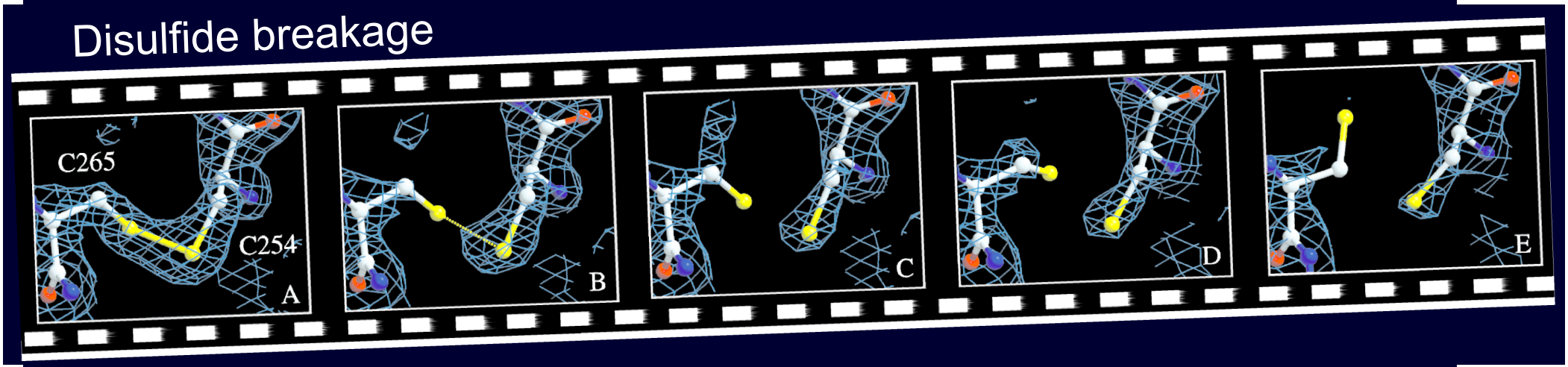
Mosaicity (degrees)



Ravelli & McSweeney (2000) *Structure* **8**, 315; Ravelli *et al*. (2002) *JSR* **9**, 355; Owen et al (2006) PNAS 103, 4912

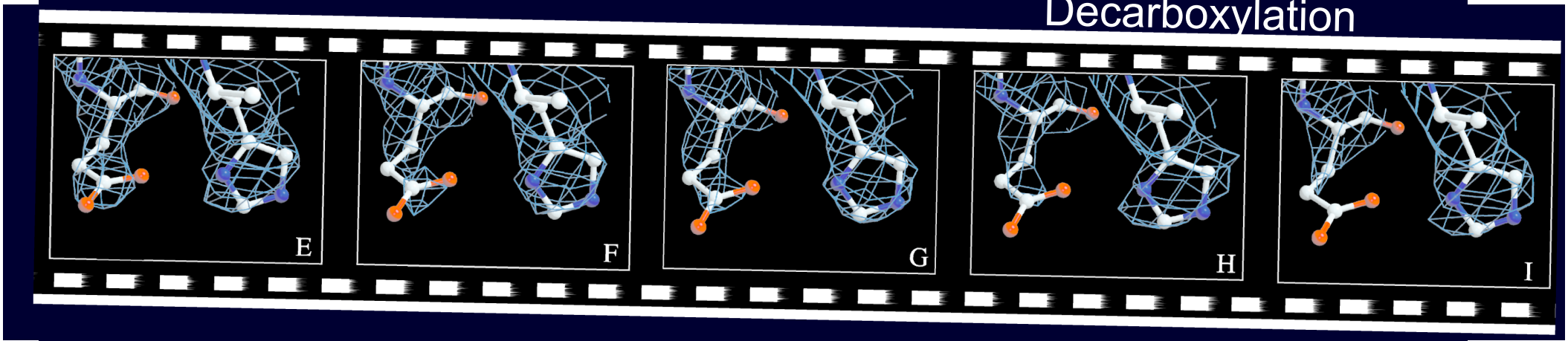
Blake & Philips 1962: ‘ …changes in relative intensities of the X-ray reflections arise from underlying structural changes …’

Disulfide breakage



Specific radiation damage at 100 K

Decarboxylation



Weik, Ravelli et al (2000) PNAS **97**, 623; Ravelli & McSweeney (2000) Structure **8**, 315, Burmeister (2000) Acta Cryst. D**56**, 328

Which radicals cause specific damage at 100 K ?

Radiolysis of water

$$H_2O \xrightarrow{\text{Ionizing radiation}} H_2O^{+\bullet} + e^- \quad \text{(ionization)}$$

$$H_2O \xrightarrow{\text{Ionizing radiation}} H_2O^* \quad \text{(electronic ionization)}$$

$$H_2O^{+\bullet} + H_2O \longrightarrow H_3O^+ + {}^{\bullet}OH$$

$$e^- + nH_2O \longrightarrow e^-_{aq}$$

$$H_2O^* \longrightarrow H^{\bullet} + {}^{\bullet}OH$$

$$e^-_{aq} + H^+ \longrightarrow H^{\bullet}$$

Scheme from Southworth-Davies & Garman (2007) *JSR* **14**, 73

Temperature-dependence of radical mobility

T < 115 K : e^- are mobile in amorph. ice

T > 115 K : e- and $H^\bullet$ are mobile in amorph. ice

Fisher and Devlin (1995) J. Phys. Chem. **99**, 11584

T > 130 K : e^-, $H^\bullet$ and $OH^\bullet$ are mobile in cryst. Ice

Symons (1999) Progr. Reaction Kinetics and Mechanisms **24**, 139

T > 110 K : e^-, $H^\bullet$ and $OH^\bullet$ are mobile in amorph. Ice

Sevilla, private comm.

T > 160 K : $OH^\bullet$ become mobile in protein crystals

Owen et al. (2012) Acta Cryst D68, 810

In cryo-crystallography (100 K): only electrons are mobile

Decarboxylation of Glu/Asp

Oxidation of Glu/Asp by e^- hole

$$R - CH_2 - CO_2^- \longrightarrow R - CH_2 - CO_2^{\bullet} + e^-$$

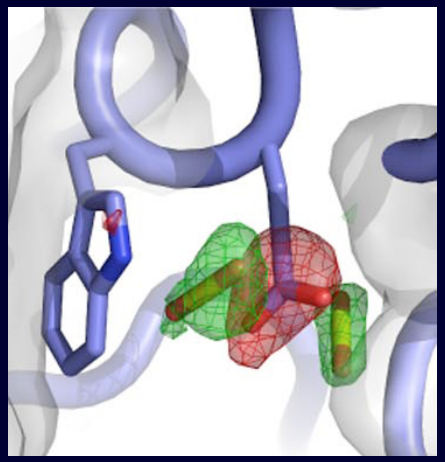
$$R - (CH_2) - CO_2^{\bullet} \longrightarrow R - {}^{\bullet}C\,H_2 + \boxed{CO_2}$$

Sevilla *et al*. (1979) *J. Phys. Chem*. **83**, 2887.
Ravelli & McSweeney (2000) *Structure* **8**, 315

CO_2 formation

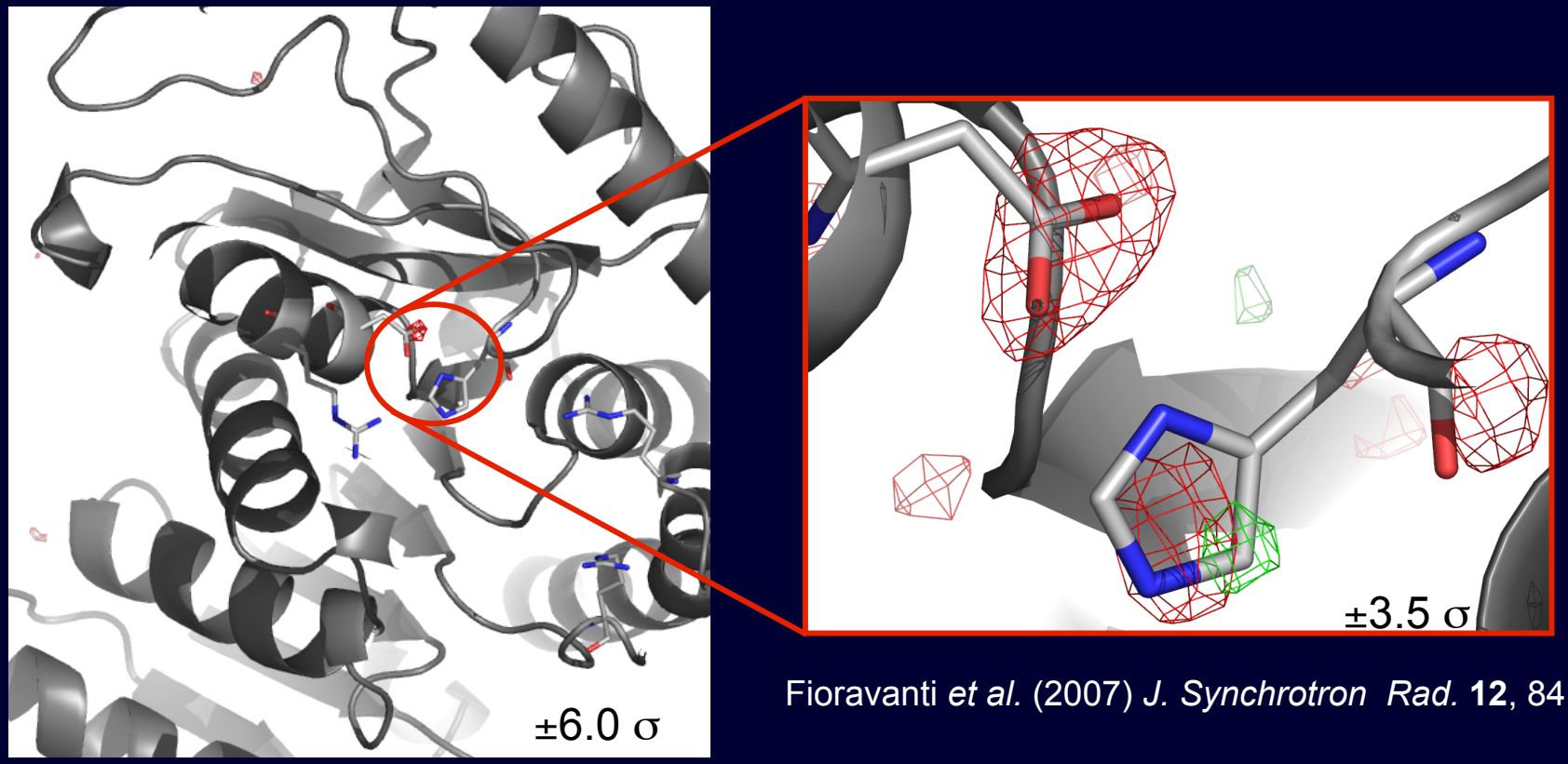
Fourier difference map
between 4th and 1st data set

$F_o^4 - F_o^1$, + 4σ, - 4σ



Colletier *et al.* (2008) *PNAS* 105, 11742

Residues in active sites are very radiation-sensitive



Fioravanti *et al.* (2007) *J. Synchrotron Rad.* **12**, 84

do careful control experiments

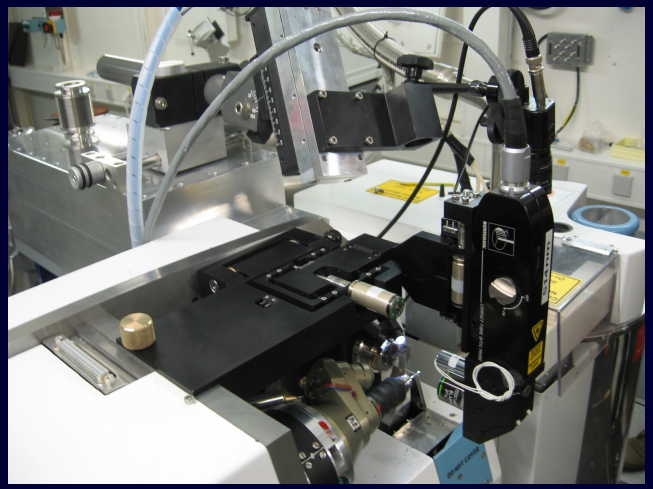
before drawing biological conclusions

Also specific damage: X-ray induced changes in chromophores

- metal complexes (metallo proteins)

- conjugated π systems (flavin- and retinal-containing proteins, fluorescent proteins)

In crystallo spectroscopic methods monitor radiation damage in chromophore-containing proteins



UV-vis

Raman

…. offline or online

on **Cryobench** platform at ESRF

(von Stetten, …., Royant (2015) ActaD 71, 15)

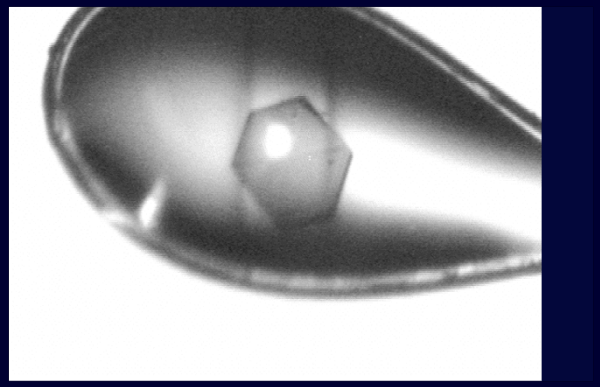
IR (Sage *et al.* (2011) *BBA* 1814, 760)

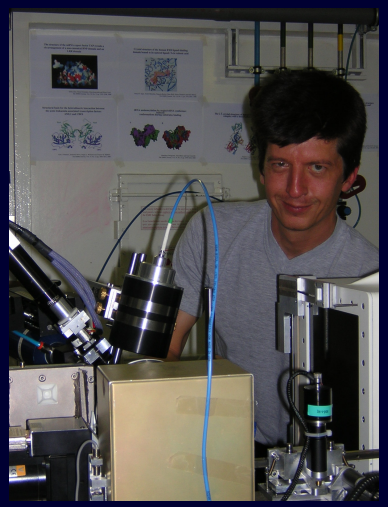
XAS (Hough *et al.* (2008) *J Mol Biol.* **378**, 353)

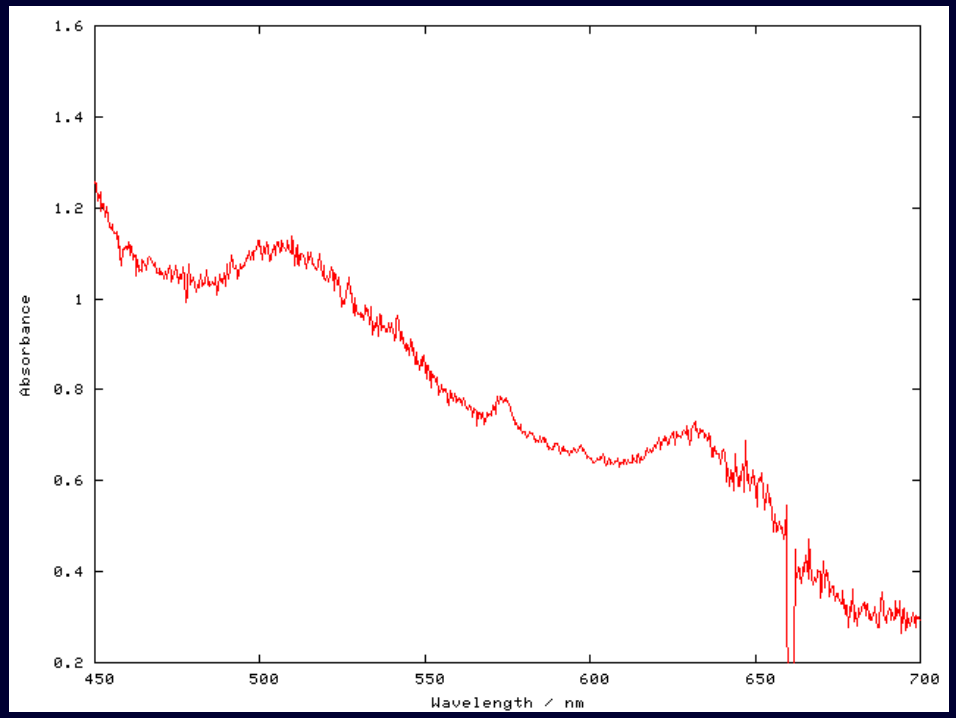
XEOL (Owen *et al.* (2012) *Acta Cryst D.* **68**, 505)

EPR (Utschig et al. (2008) Biochemistry 47, 9251)

X-ray induced reduction of metallo proteins (here Fe $^{3+}$ -> Fe $^{2+}$ in Mb)







Andreas Ostermann (unpublished)

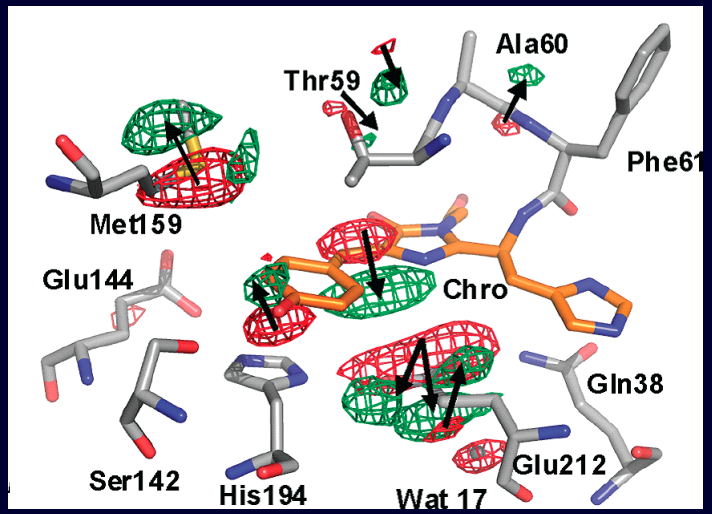
Metal centers are reduced within seconds before full data set is collected

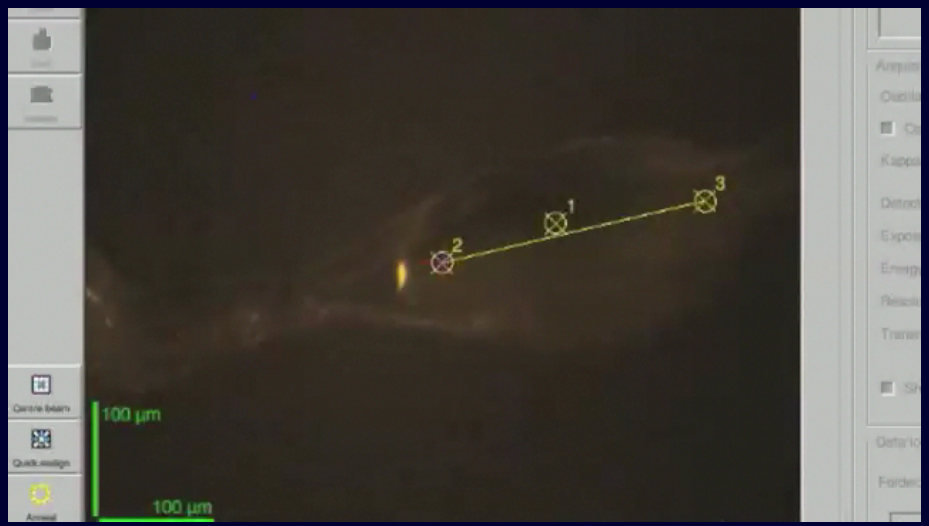
In PDB: numerous protein structures with reduced redox centers

X-ray induced bleaching of fluorescent protein

Adam et al (2009) JACS **131**, 18063

See also
Royant & Noirclerc-Savoye (2011) J Struct Biol 174, 385





How to minimise or avoid X-ray radiation damage ?

Use neutron, not X-ray crystallography

- unlike X-rays, neutrons are not ionizing: no radiation damage
- neutron protein crystallography *sees* hydrogens and water better than X-ray crystallography

But: large crystals are needed

- 1 mm^3 if protein hydrogenated
- 0.1 mm^3 if protein perdeuterated
- data collection: 3 weeks

X-ray crystallography: know and limit absorbed dose

absorbed dose = absorbed energy / mass

1 Gray (Gy) = 1 J / kg

The higher the absorbed dose, the greater the damage

Dose required to reduce diffracted intensity by half at 100 K : $D_{1/2}$= 43 MGy

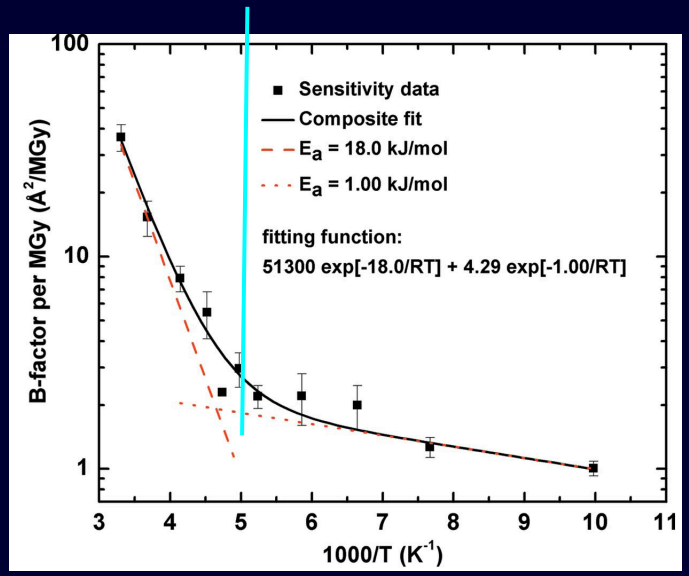
Dose limit

above which biological info is compromised at **100 K**: **D_{ln2}= 30 MGy**

Owen *et al.* (2006) *PNAS 103, 4912*

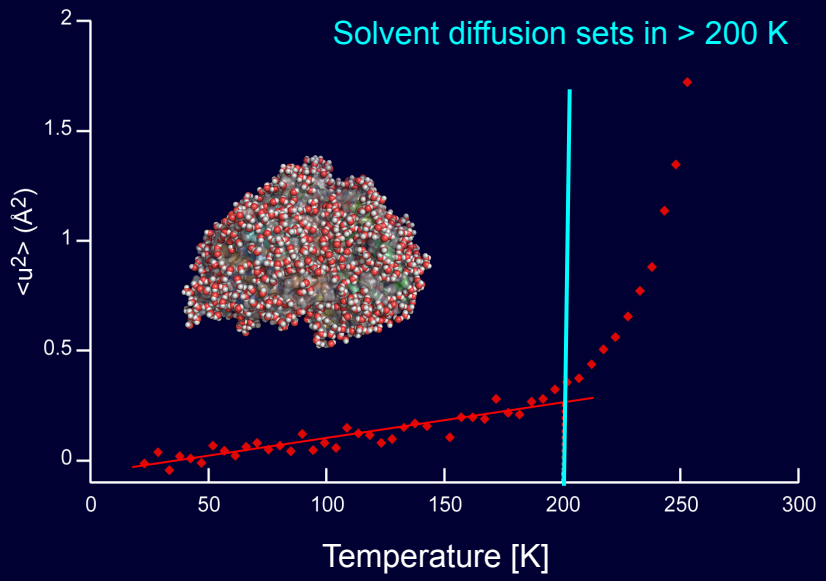
Collect data at cryo-temperature (< 200 K)

Transition in radiation sensitivity at 200 K



Warkentin & Thorne (2010) Acta Cryst D66, 1092

Solvent mean-square displacements from neutron scattering



Wood et al (2008) JACS 130, 4586

Because of radical diffusion: radiation damage 100x higher at RT than at 100 K

Nave & Garman (2005) JSR 12, 257

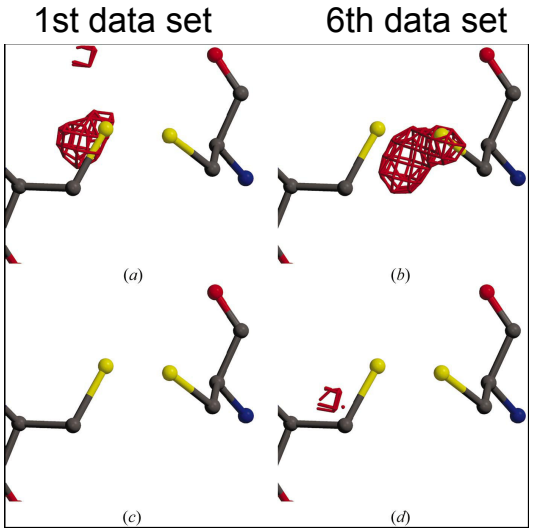
Add radical scavergers

e.g. ascorbate:

Murray & Garman (2002) *JSR* 9, 347

Crystal without ascorbate

Crystal with 1 M ascorbate



Cys76-Cys94
SS bond in HEWL

Further scavengers with some effect:

Quinone, TEMP, reduced DTT (Southworth-Davies & Garman (2007) JSR 14, 73)

Potassium hexacyanoferrate (Macedo *et al.* (2009) JSR. 16, 191)

Nicotinic acid and DTNB (Kauffmann *et al.* (2006) *Structure* **14**, 1099)

Nicotinic acid study not statistically significant (Nowak et al. (2009) Acta Cryst. D65, 1004)

Uridine reduces some global damage (Crosas et al. (2017) JSR 24, 53)

Effectiveness of scavengers **controversial** (Kmetko et al. (2011) Acta Cryst. D67, 881)

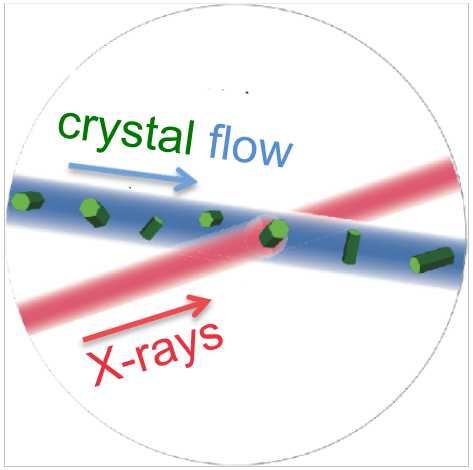
So far, no truly effective scavenger described

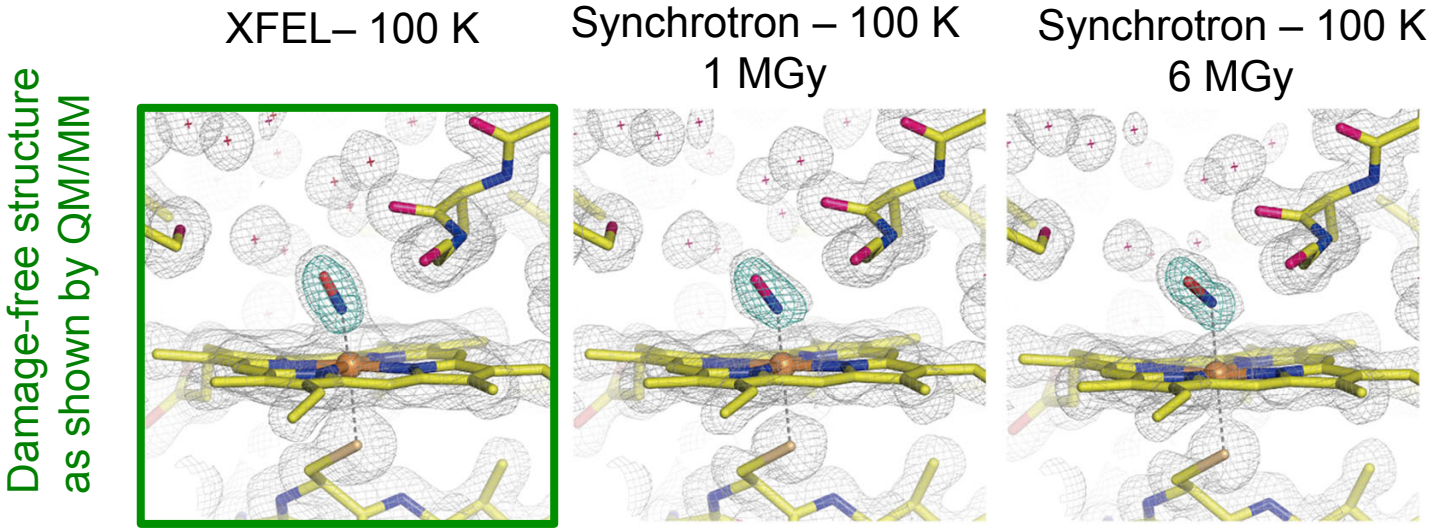
Allan et al. (2013) *JSR* 20, 23

Use serial femtosecond crystallography (SFX) at XFELs

Radiation damage free structures
through
Diffraction-before-destruction

Neutze et al (2000) Nature 406, 752





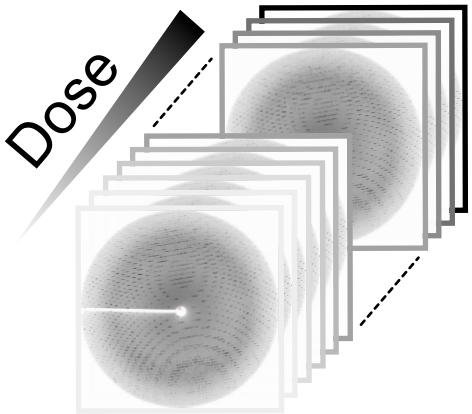
Fe-NO bond length:	1.67 Å	<	1.68 Å	<	2.10 Å
NO bending:	158°	>	147°	>	122°

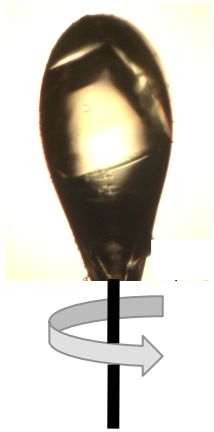
Tosha et al (2017) Nat Commun 8, 1585

Use serial crystallography at synchrotrons

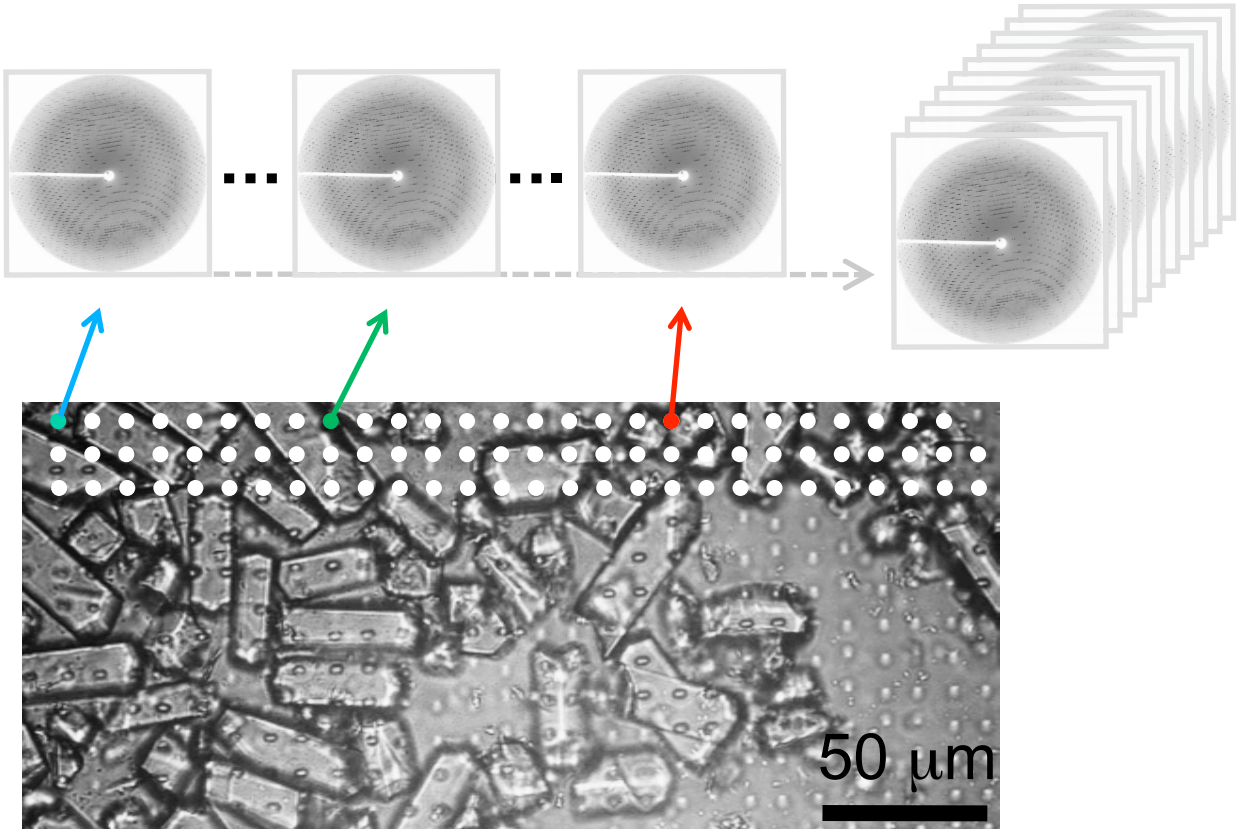
to distribute absorbed dose over thousands of microcrystals

Oscillation crystallography :
large dose per data set





Serial crystallography :
Composite data set of minimal dose



De la Mora, Coquelle *et al*, submitted

Summary

- Radiation damage – what is it ?
  - global damage
  - specific damage: S-S, Glu/Asp
  - active sites very radiation sensitive – biological information altered
  - chromophores highly radiation sensitive
  - online microspectrophotometry complements protein crystallography

- Radiation damage – how to limit or avoid it ?
  - neutron crystallography
  - limit absorbed dose (< 30 MGy at 100 K, < 0.4 MGy at room temperature)
  - collect at cryo-temperatures (< 200 K)
  - use scavengers
  - serial femtosecond crystallography at XFELs: diffraction-before-destruction
  - serial crystallography at synchrotrons

Not covered:

- Practical issues
  - is there a dose-rate effect?
  - wavelength-dependence of radiation damage?
  - radiation damage and MAD
  - how to use it?
  - beamheating
  - raddam in SAXS experiments

- Radiation-induced changes to study macromolecular function