Radiation damage in Serial Femtosecond Crystallography

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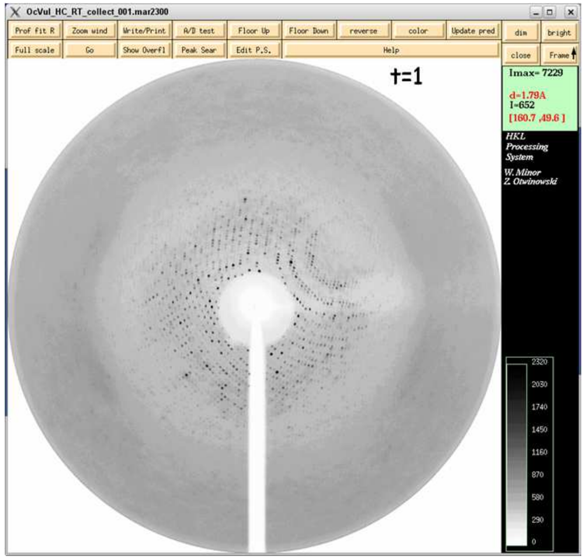
Outline:

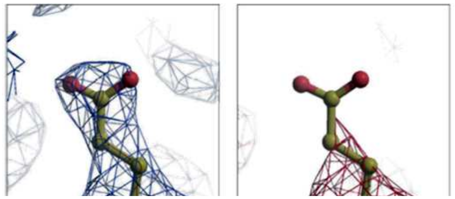
- **Introduction:**
  - Radiation damage in MX at synchrotrons
  - Ultra-fast radiation damage at the FEL
- **SFX experiments:**
  - Overview of previous results
  - Recent results at high intensity
- **Summary & Outlook**

Radiation damage in MX at synchrotrons

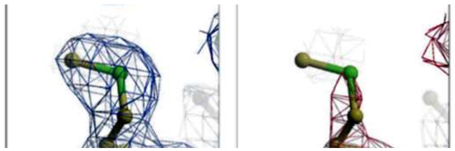
Global damage
- Loss of resolution
- Increase of mosaicity
- Change in unit cell constants

Local damage
- Decarboxylations, S-S bond breakage
- Photo-reduction of redox systems, e.g. metal centers,…





Local damage to structure



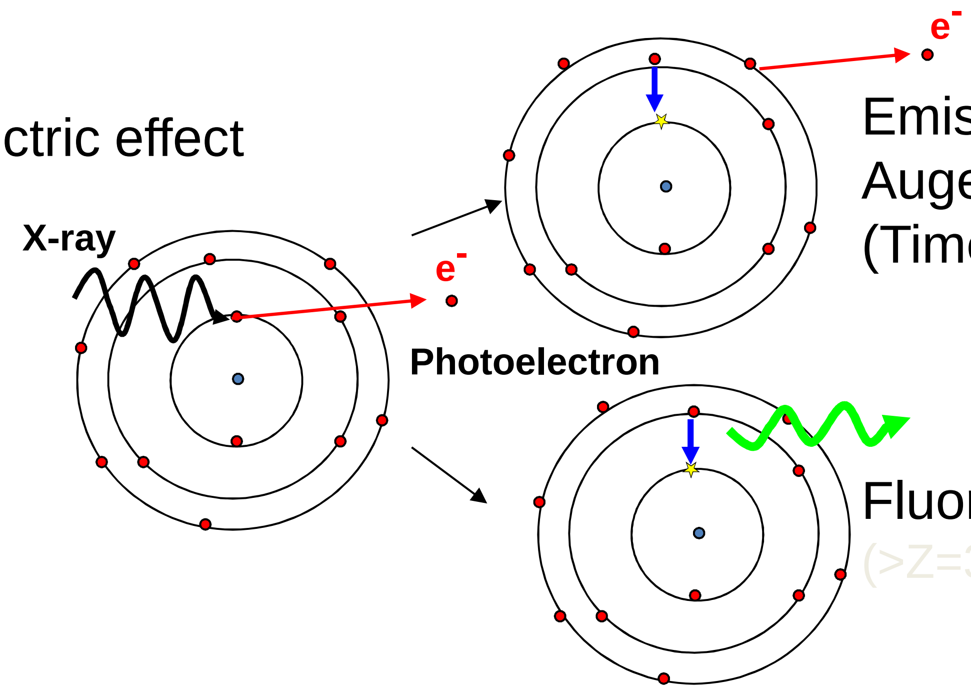
From: Garman and Owen (2005), *Acta Cryst.* D**62**, 32-47

Nine out of ten X-rays cause radiation damage

At 12 keV (λ=1.03 Å)

- 10% elastic scattering
- 10% inelastic scattering
- 80% photoelectric effect

Photoelectric effect



Emission of
Auger electron (low Z atoms)
(Time scale: few fs)

Fluorescence photon
(>Z=30)

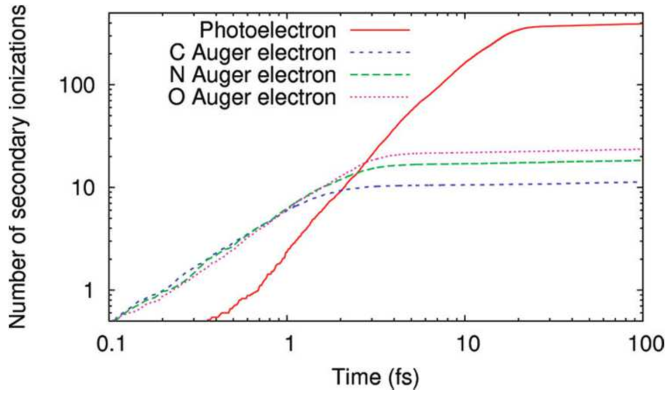
Ultra-fast damage at the X-FEL

Primary ionization processes

- photoionization via photoelectric effect
- Auger decay (typically low Z atoms)

Secondary ionization

- secondary ionization by collisions with Auger and photo-electrons



C. Caleman *et al*. ACS Nano 2011

Degradation of scattered signal

- modification of the electronic structure
- progressing crystal disorder due to Coulomb repulsion

Global and local damage in SFX

Global damage

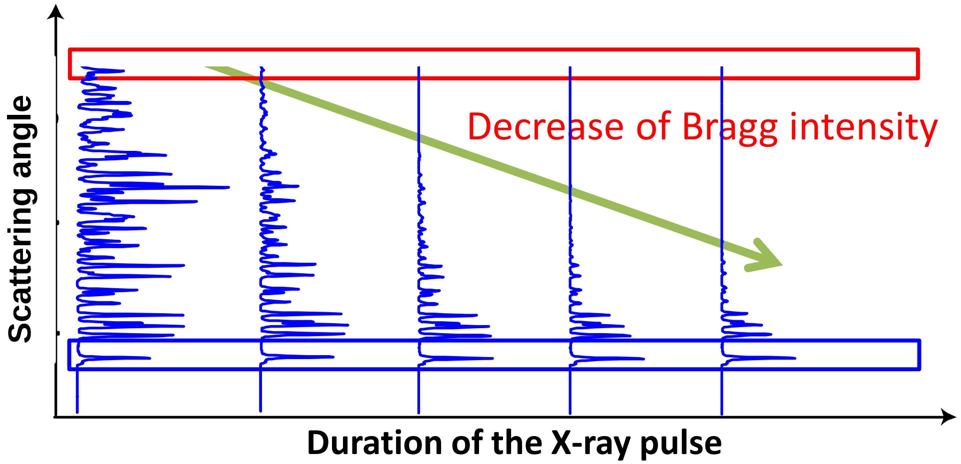
- (multiple) ionization of the whole sample
- decrease of Bragg intensity as the crystal disorders during the X-FEL pulse

Local (specific) damage

- site specific modification of the electronic structure
- “hot spots” of local damage = high Z centers, solvent channels, S-S bridges, due to higher absorption cross sections

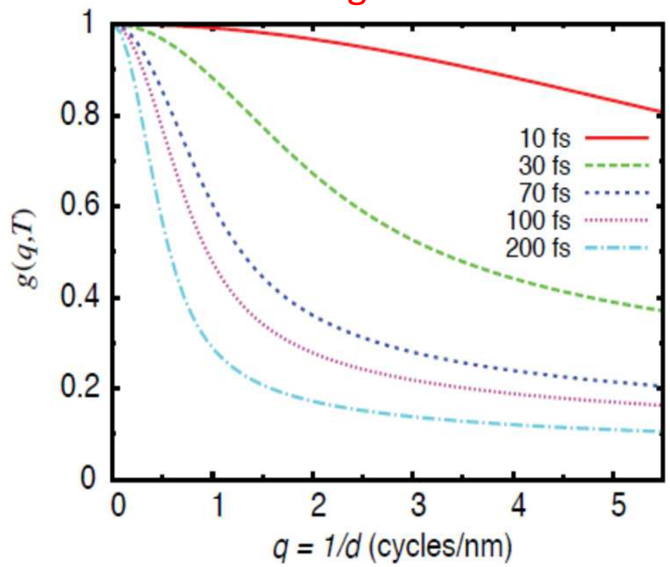
Global radiation damage in SFX

Ionization modifies the scattering form factor and initiates uncorrelated movement of ions, which reduces the order of crystalline lattice in a resolution-dependent fashion during the pulse.



Lomb et al., Phys. Rev. B **84:** 214111 (2011)

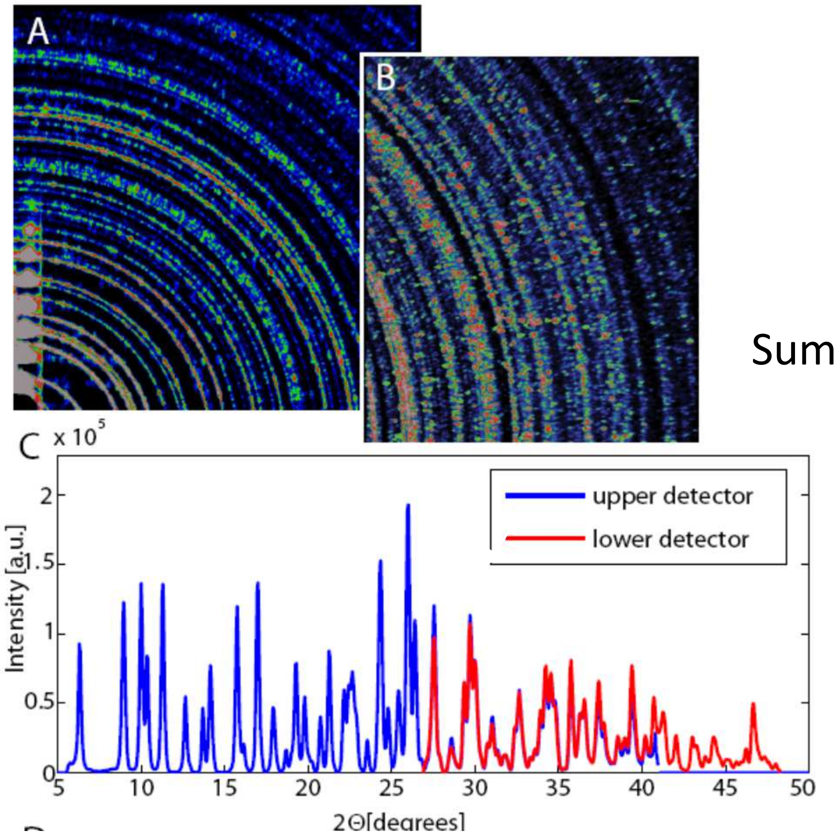
Scaling is possible if the damage distribution
Is homogenous



Barty et al Nature Photonics 6: 35 (2012)

SFX damage experiment with lysozyme nanocrystals

AMO/CAMP, Jun 2010, 2.0 keV, 10 μm^2 focus, 5x10^{16} W/cm^2



Summed single crystal shots

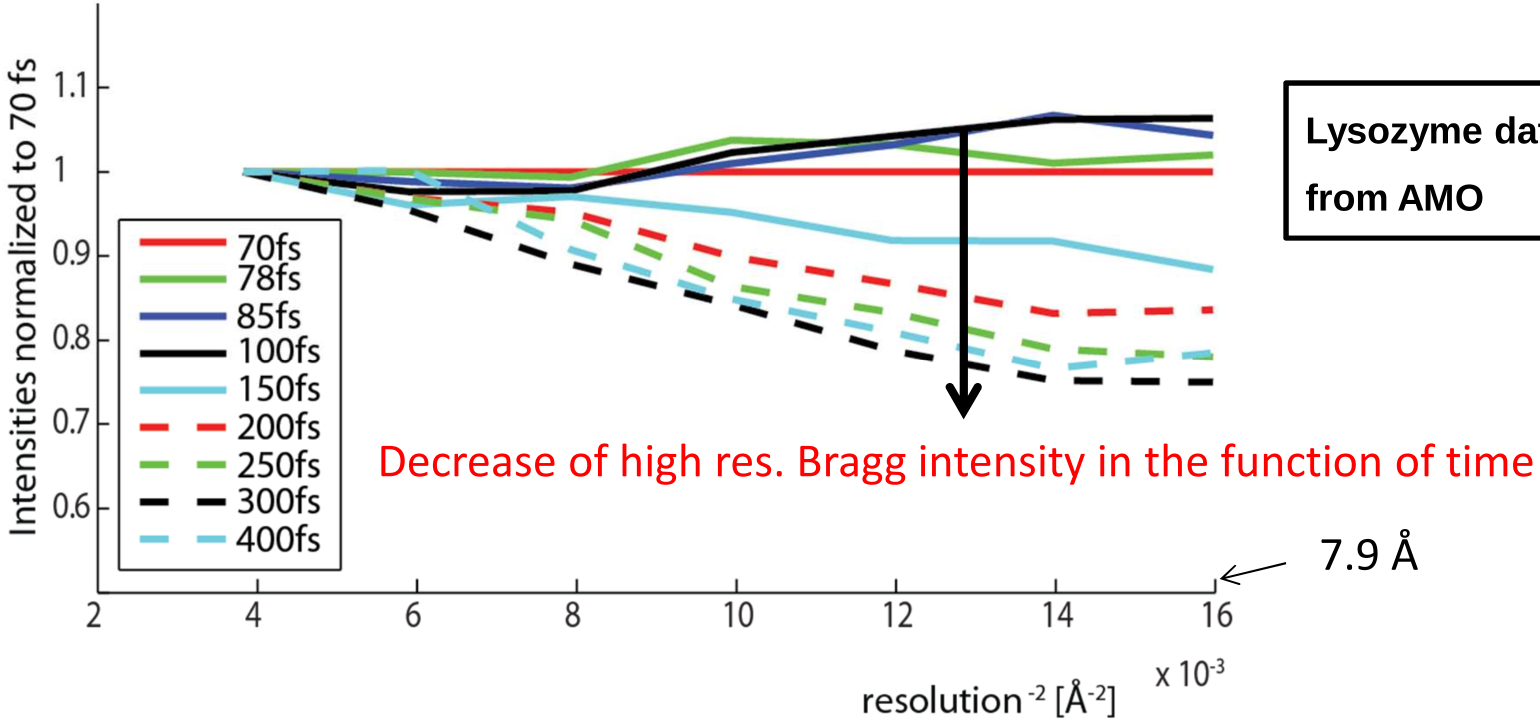
1D powder pattern

- Good correlation with synchrotron powder patterns despite **3 GGy** dose/crystal

Lomb et al PRB, 2011

Outrunning global radiation damage in SFX

Diffraction intensity as a function of pulse length



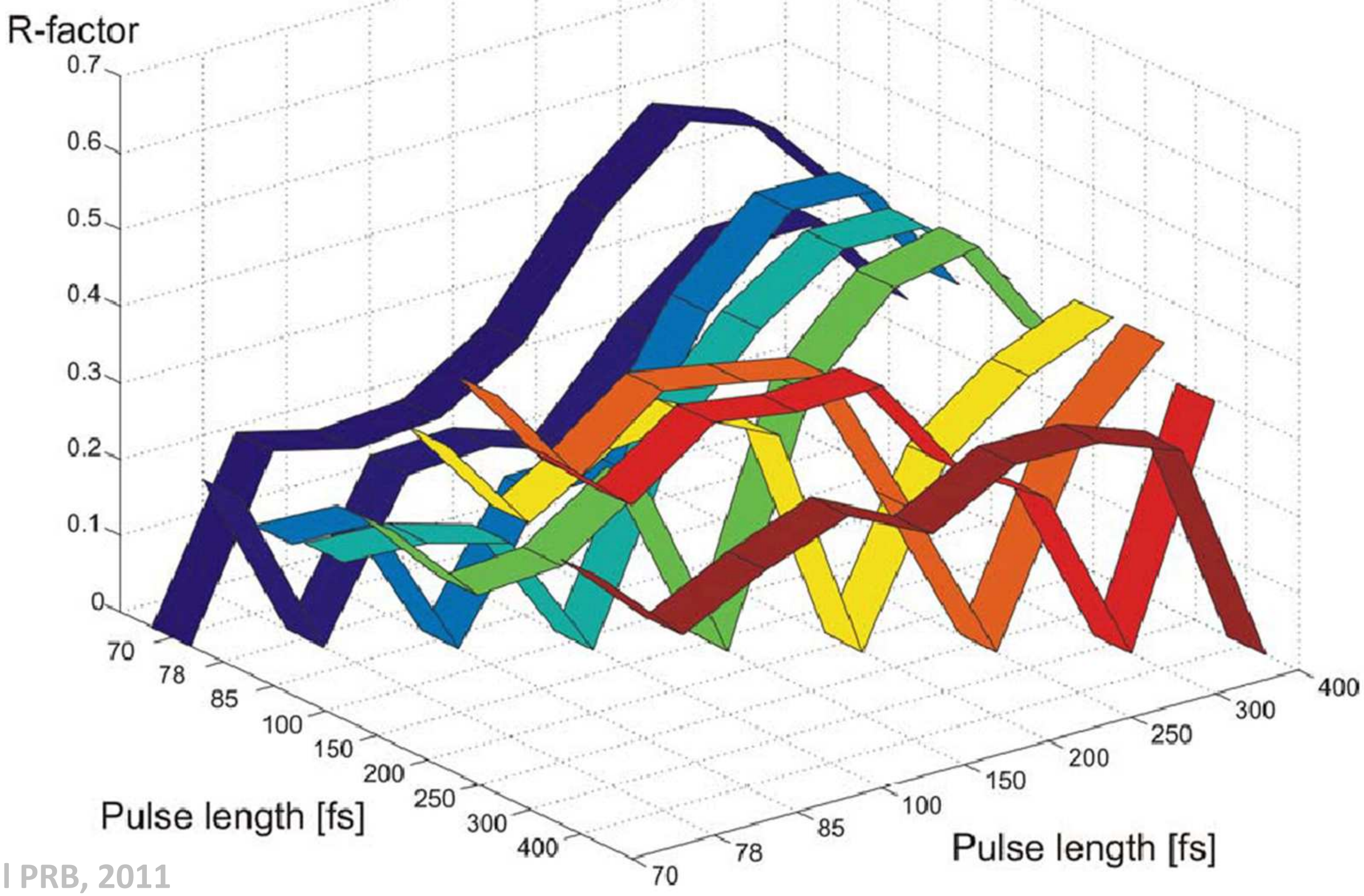
Lysozyme datasets from AMO

Lomb et al PRB, 2011

Predicting local damage "hot spots" in SFX

By scaling lysozyme SFX data sets from different pulse lengths

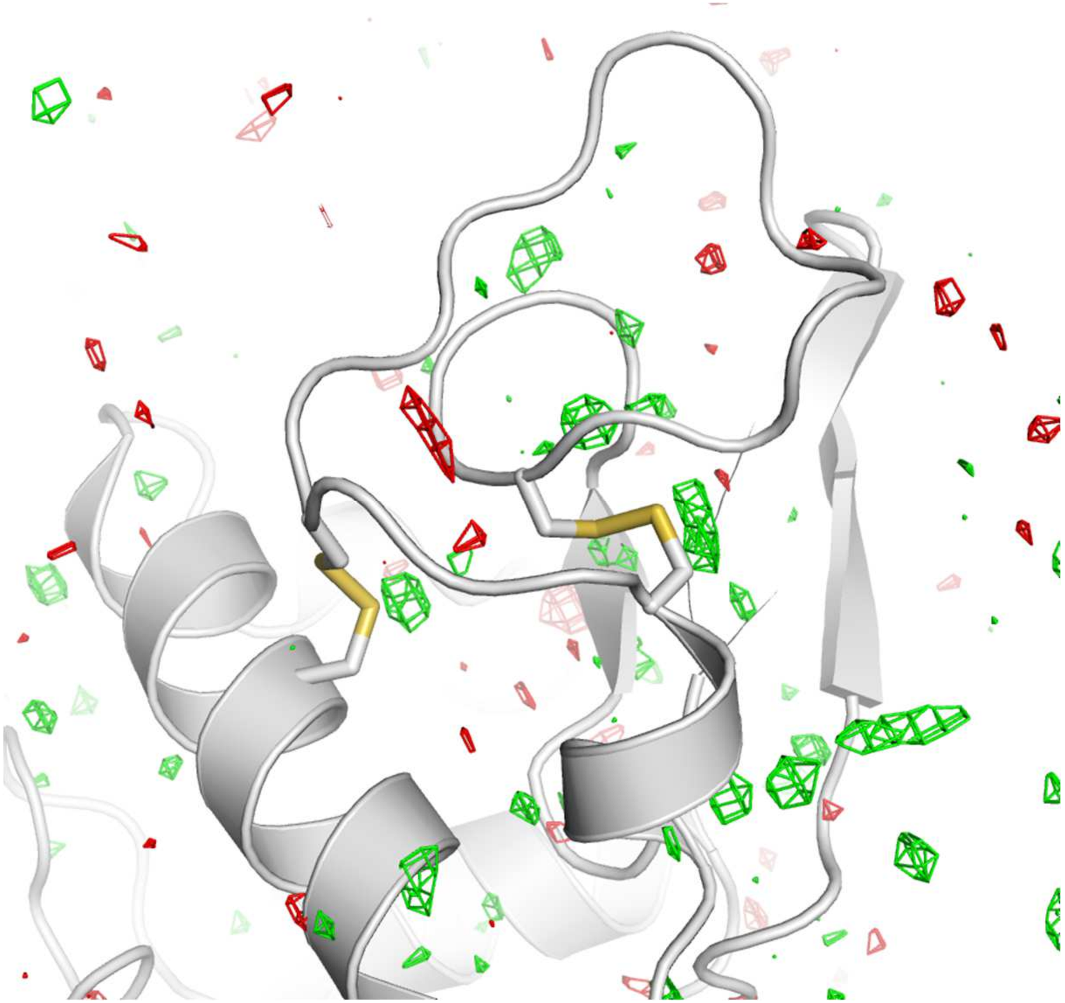
Data sets didn't scale well using Wilson scaling -> damage not uniform



Lomb et al PRB, 2011

First high-resolution structure – no apparent damage

9.3 keV, 40 fs, 10 µm^2 focus, attenuated beam



Lysozyme, 1.9 Å

<- Difference electron density map:

F_{obs}[FEL]-F_{obs}[synchrotron]
+3 σ (green) and -3 σ (red)

Dose = 33 MGy / crystal

Boutet et al. Science (2012)

Radiation damage in SFX. Yes - no - maybe?

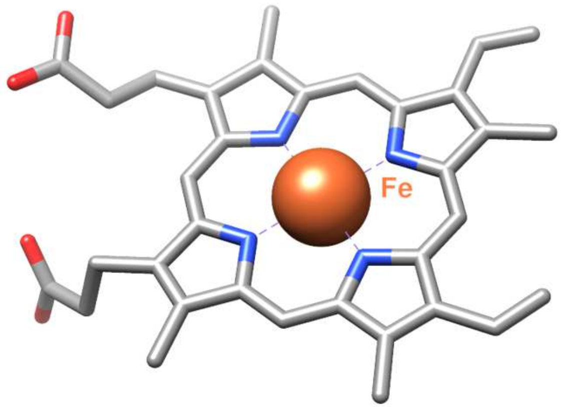
July 2013, 200 nm x 200 nm focus

Motivation:

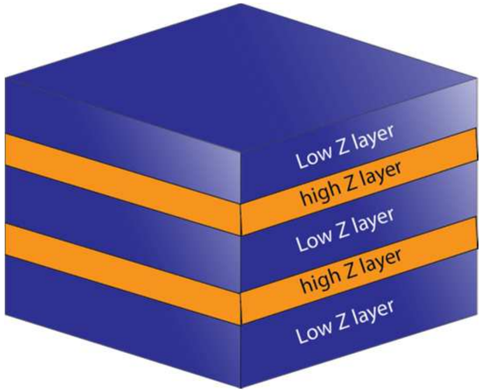
Probe the existence of damage hot spots

- measure above and below Fe-absorption edge (7.1 keV)
- study the dose and dose rate dependency

Metals (especially iron) have huge biological relevance, metal containing centers are prone to radiation damage



“Hot spots” of radiation damage



Plasma code calculations predict preferred, pulse length dependent ionization in the high Z regions of the sample above and below the Fe K-shell edge.

7.4 keV

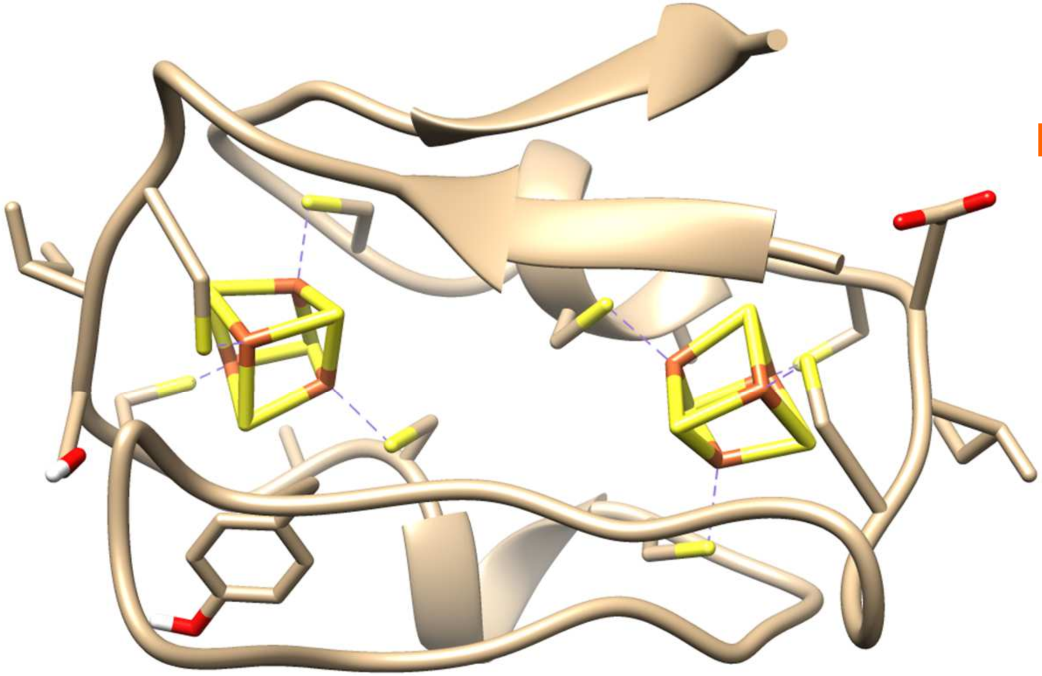
6.4 keV

C. Caleman

Test case for radiation damage in SFX - ferredoxin

- Two Fe_4S_4 clusters are in very similar environment
- 0.94 Å reference structure

Dauter et al *Biochem.* **36**, 16065-16073 (1997).



Fe_4 S_4 clusters as high Z centers

Crystals:
1.6 ± 0.5 × **1.6** ± 0.5 × **17** ± 7.5 **µm³**

***Chlostridium* Ferredoxin**

Ionization dynamics simulated with plasma code

Calculated ionization of Fe, S, C for our experimental conditions:
1.8×10^{19} (W/cm^2), 200 nm × 200 nm focus

Scattering power estimated from the number of bound electrons.

Accumulated signal during the pulse due to the loss of scattering power.

Olof Jönsson,
Nic Timneanu,
Carl Caleman

Localized damage observed in ferredoxin

7.4 keV, 80 fs, 1.5 mJ, 200 nm x 200 nm focus, 31 GGy

10 cycles restrained refinement in Refmac5 without clusters
Superposition of cluster coordinates from reference structure 2FDN

Cluster 1 **Cluster 2**

2*m*Fo-*D*Fc 1 σ
Fo-*D*Fc 2.5 σ

Fe - orange
S - yellow

- Unexpectedly:
- Correlated displacement of S atoms in cluster 1
- Worst than usual intensity distribution (global damage)

Differences between the two clusters

Cluster 1 **Cluster 2**

Correlated displacement of S atoms in cluster 1. At both energies.

LCLS
7.36 keV

Fe - orange
S - yellow

LCLS
6.86 keV

Ferredoxin densities from SLS

Cluster 1 **Cluster 2**

Non-damaged [4Fe – 4S] clusters

The same protein batch as for LCLS

SLS
7.36 keV

Fe - orange
S - yellow

SLS
6.86 keV

Cluster 2 **Cluster 1**

$F_{obs}(SLS) - F_{obs}(LCLS)$

7.36 keV

+ 3 σ
- 3 σ

Cluster 2 **Cluster 1**

$F_{obs}(SLS) - F_{obs}(LCLS)$

6.86 keV

+ 3 σ
- 3 σ

Fe - orange
S - yellow

Anomalous difference Fourier peaks

Cluster 1 **Cluster 2**

3 σ

Cluster 1 **Cluster 2**

Fe - orange
S - yellow

5 σ

Summary & Outlook

- Observation of local “hot spots” of radiation damage in SFX at high intensity, due to inhomogeneity (high Z regions) in the sample
- Unexpected different level of electronic damage between the two clusters
- Unexpected correlated motion of S atoms in one of the clusters
- Possible implications for high intensity phasing method and single particle imaging where intense pulses are necessary

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