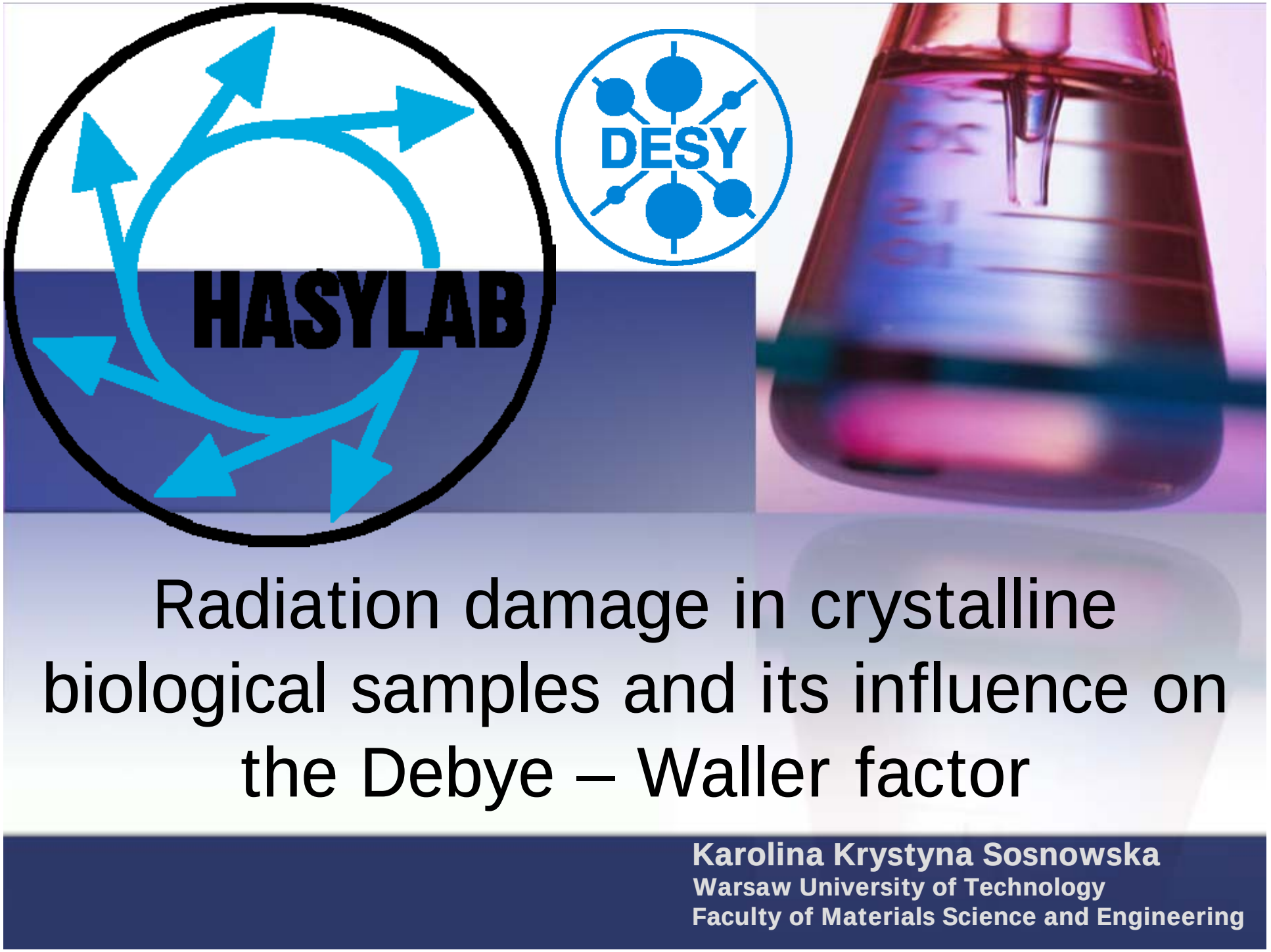

Radiation damage in crystalline biological samples and its influence on the Debye – Waller factor

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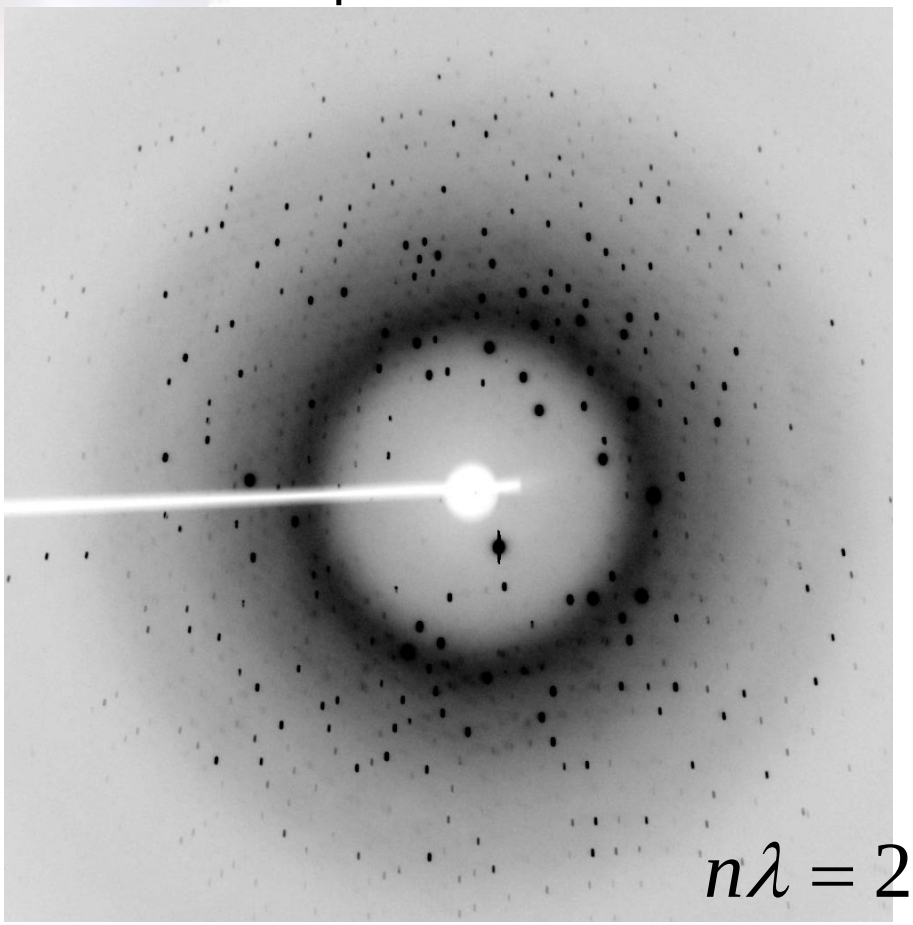
Outlook

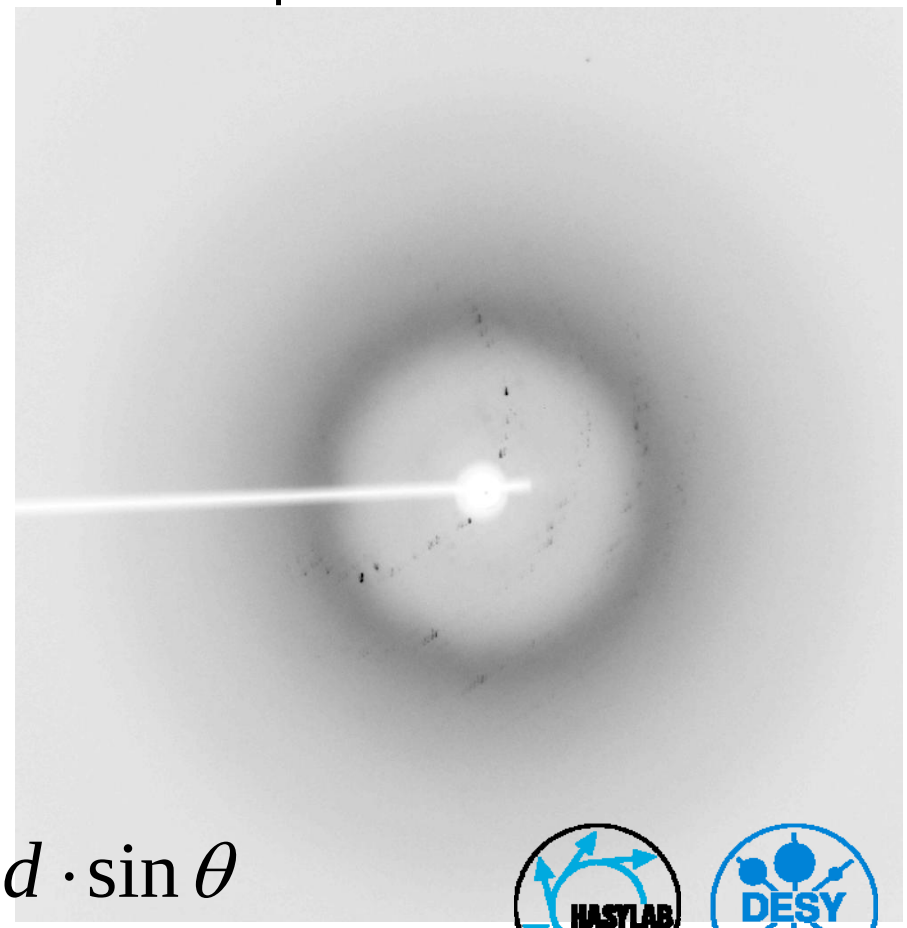
- X – ray diffraction
- Debye – Waller Factor
- Protein structure
- Radiation Damage
- Data Collection
- Results
- Conclusions

Motivation

- before exposure



- after exposure

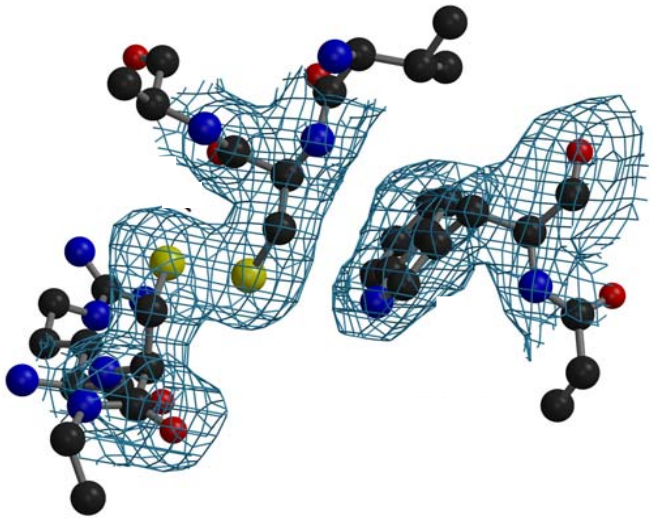


$$n\lambda = 2d \cdot \sin\theta$$

X – ray diffraction

- Electron Density Map

$$\rho(x,y,z) = \frac{1}{V}\sum_{hkl} F_{\underline{h}} \cdot e^{-2\pi i\cdot(hx+ky+lz)}$$



- Structure Factor

$$F_{\underline{h}} = \sum_{m} f_m \cdot e^{2\pi i \cdot \underline{h}\cdot \underline{r}_m}$$

$$I \sim \left|F_{\underline{h}}\right|^2$$

Debye – Waller factor

- Describes the thermal motion of the atoms
- Contains information of static and dynamic disorder

$$F = \sum_n f \cdot \exp\left(-B\frac{\sin^2\theta}{\lambda^2}\right) \cdot \exp\left(i \cdot (2\pi \cdot \underline{h}) \cdot \underline{r}_m\right)$$

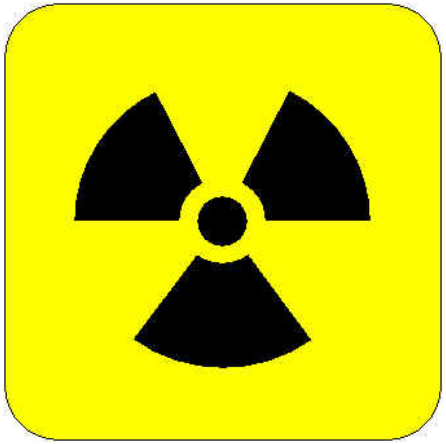
Debye – Waller factor

- B-factor value – measures oscillations and vibrations of an atom

$$B = f\left(\left\langle U_m{}^2 \right\rangle\right)$$

Radiation damage

- Two steps process:

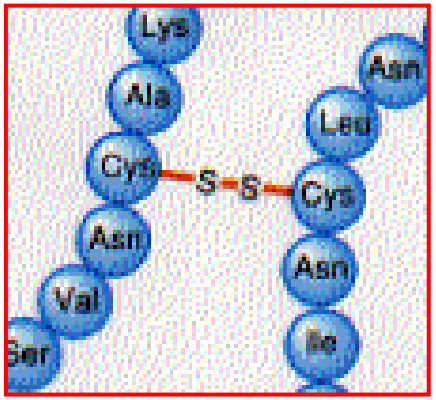


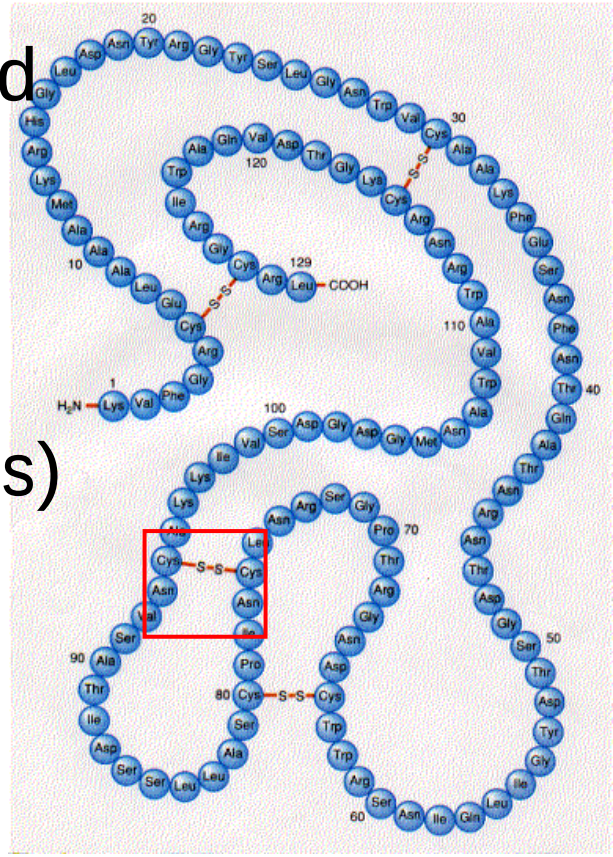
  - Primary damage – exposure to the X–ray beam
  - Secondary damage – chemical reactions in the material

Protein – Lysozyme

- Protein – an amino acid chain fold into unique 3D structure.

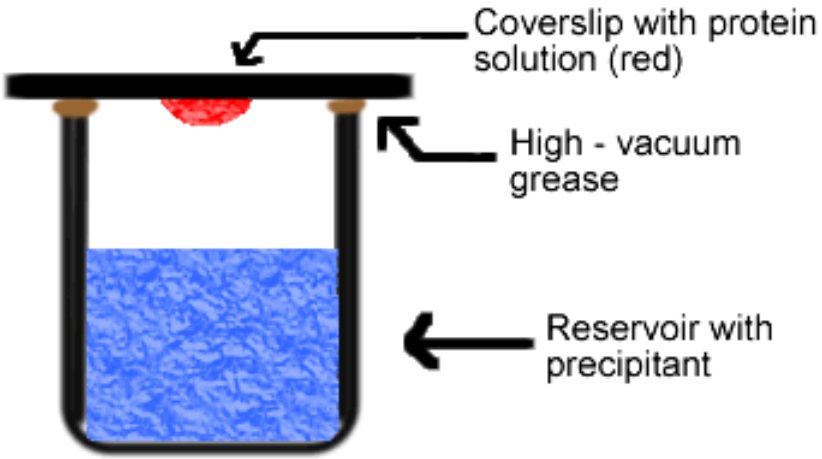
- Lysozyme – a single polypeptide chain of 129 amino acids (residues) with four pairs of di-sulphide bridges.





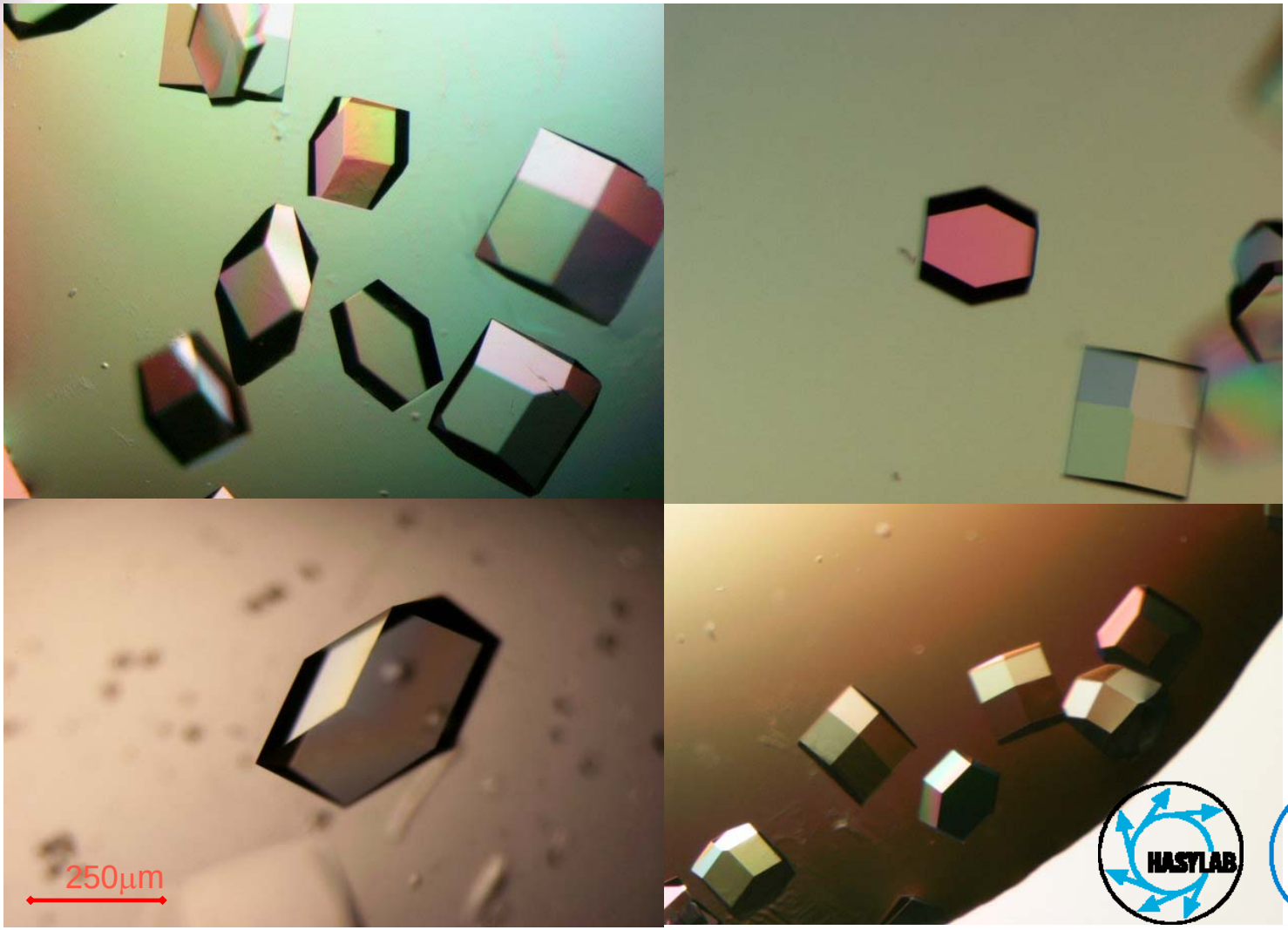
Protein - crystallization

- The hanging drop method



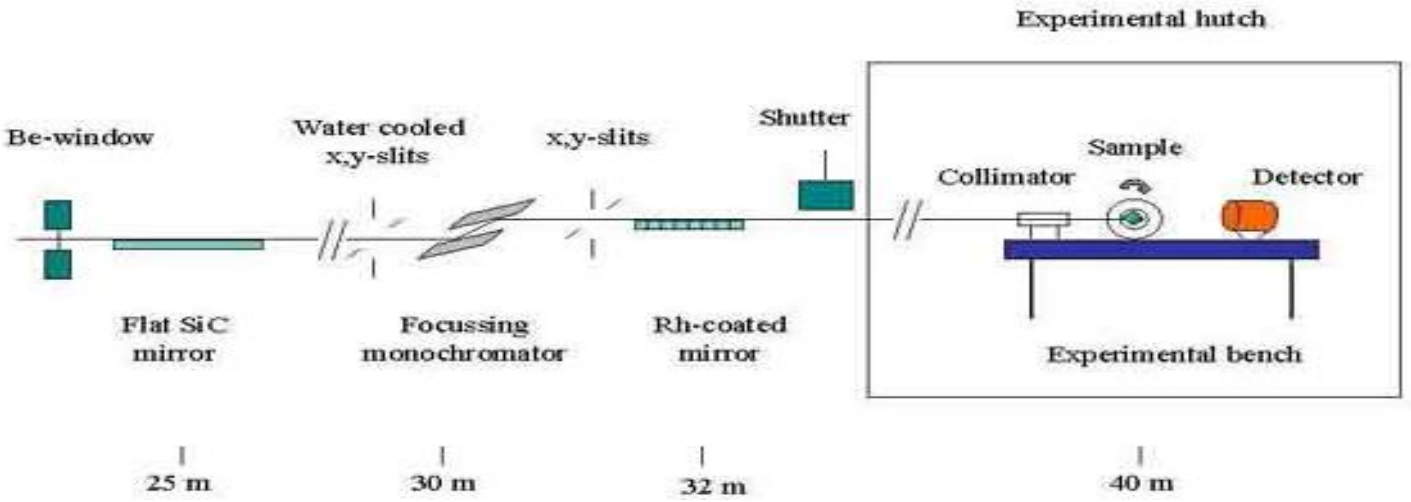
- Parameters:
  - protein purity
  - protein concentration
  - pH
  - temperature
  - precipitants

Protein – Lysozyme crystals



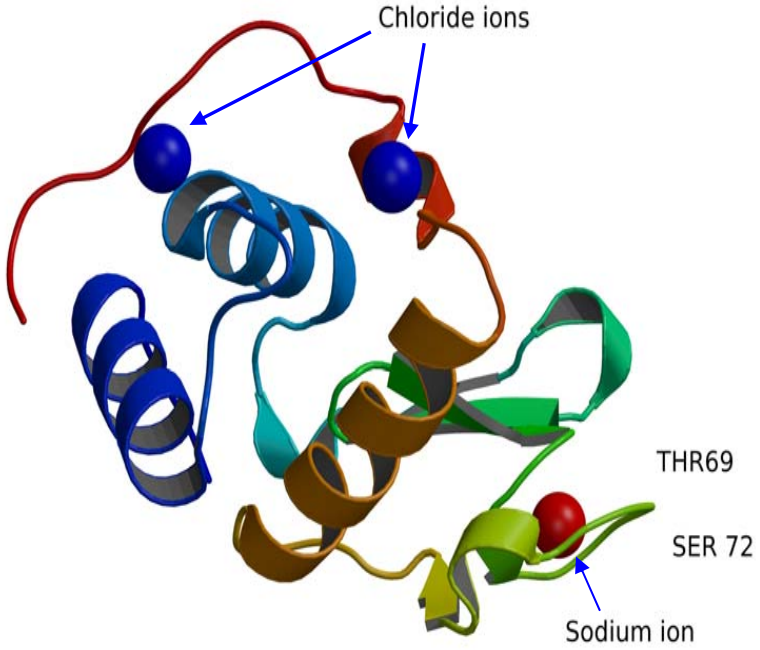
Data collection

- All data were obtained at EMBL beam line X13 at DORIS



- Experiments at 10K and 90K
  - 8 datasets each
  - same dose mode

Results

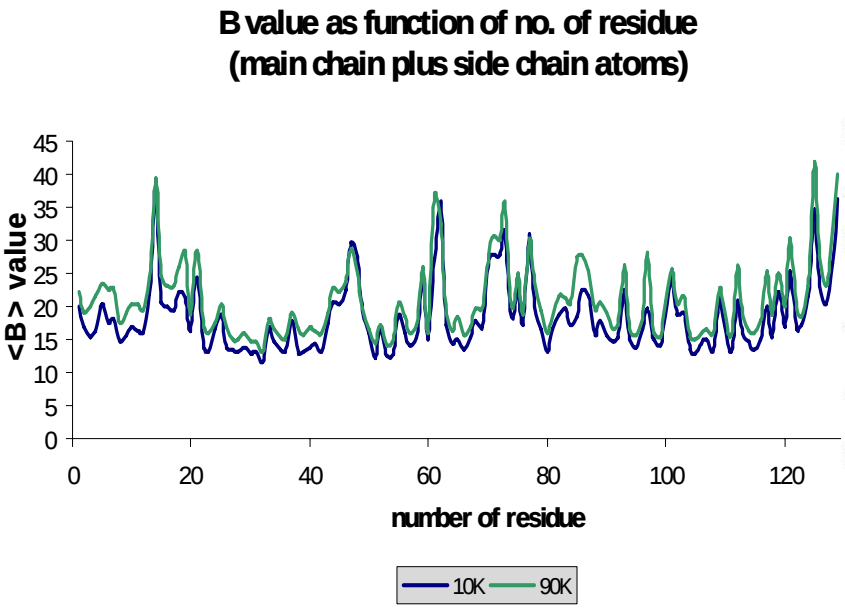


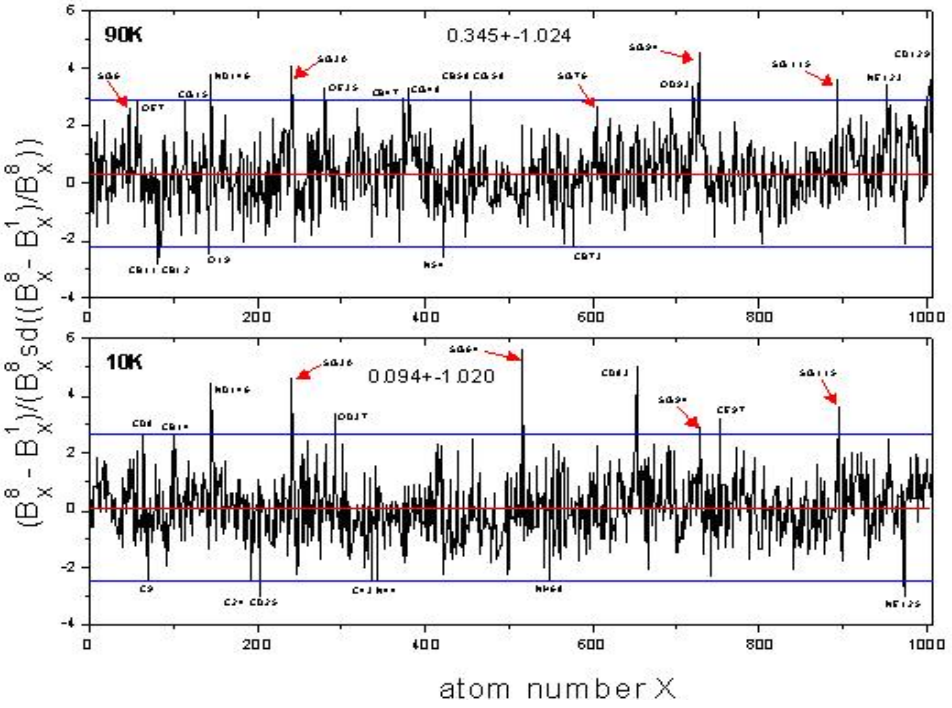
- The result of the refinement is the solved structure:
  - Space group – $P4_32_12$
  - unit cell parameters:

    a = 78,3 Å

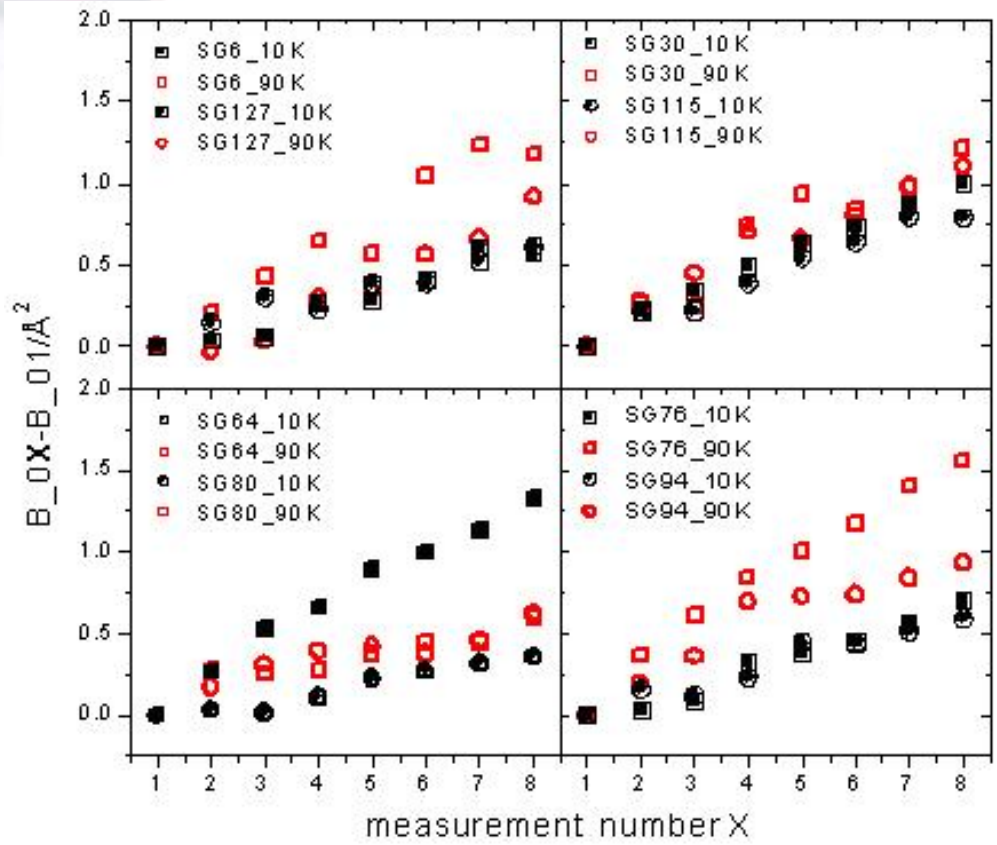
    c = 36,9 Å

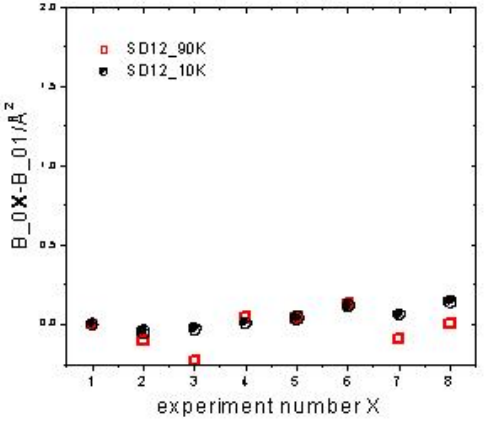
Results

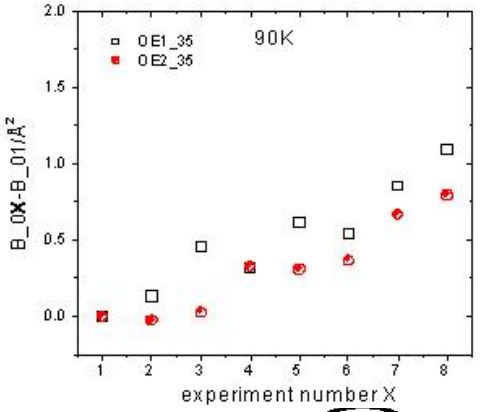

B value as function of no. of residue
(main chain plus side chain atoms)
<B> value
number of residue
10K
90K


90K
0.345+-1.024
10K
0.094+-1.020
atom number X

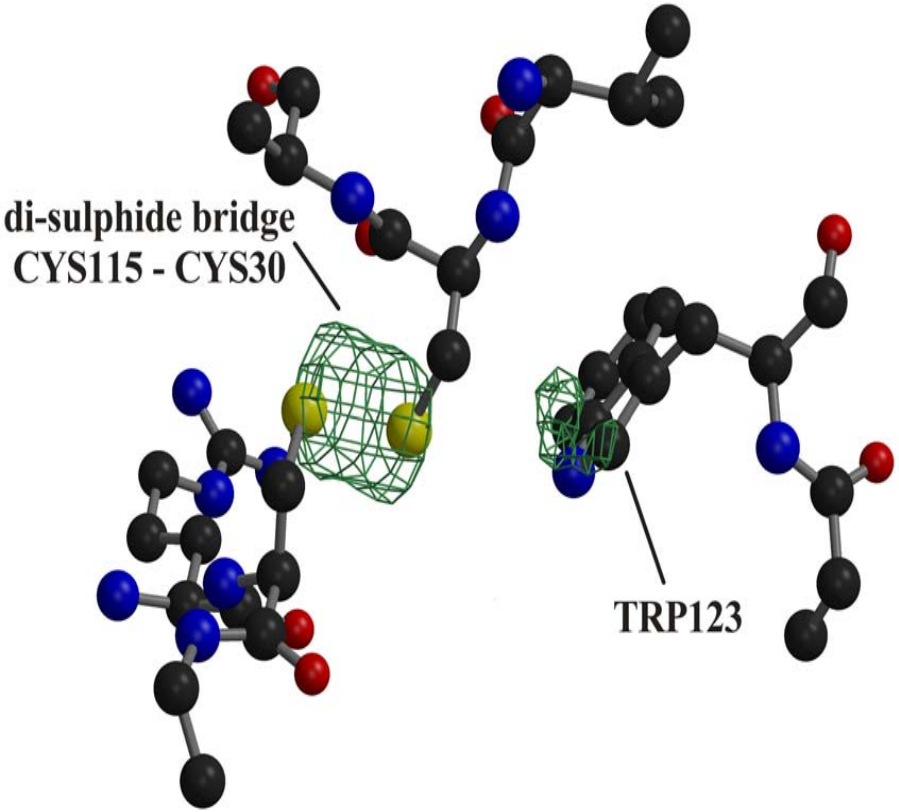
Results


SG6_10K
SG6_90K
SG127_10K
SG127_90K
SG30_10K
SG30_90K
SG115_10K
SG115_90K
SG64_10K
SG64_90K
SG80_10K
SG80_90K
SG76_10K
SG76_90K
SG94_10K
SG94_90K
B_0X-B_01/Å²
measurement number X

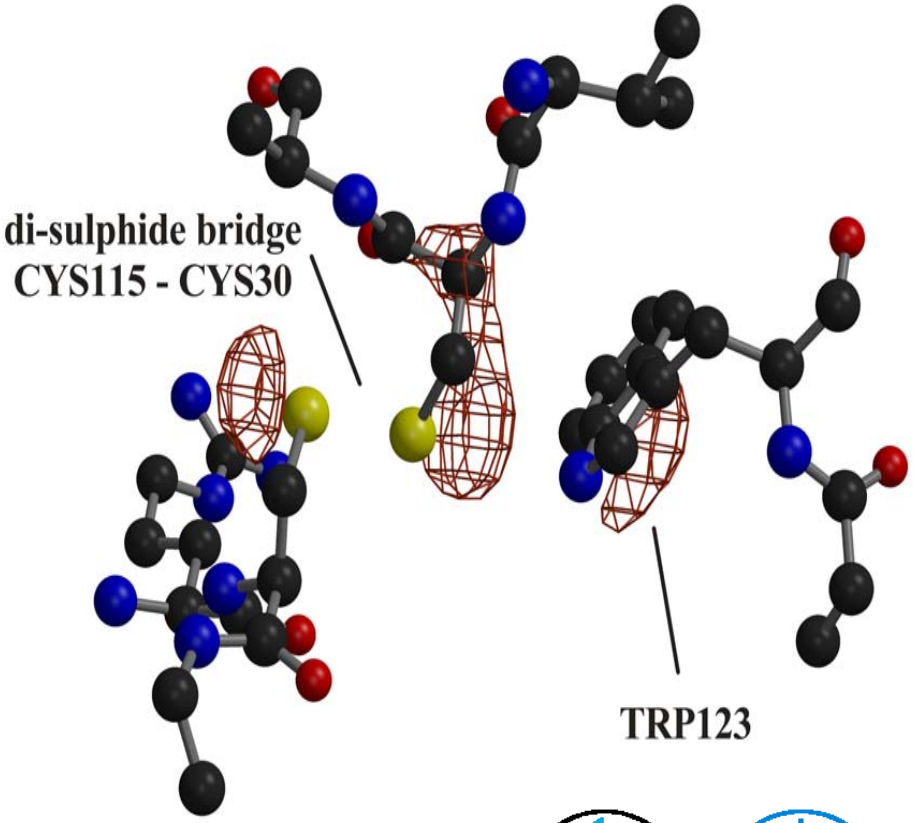

SD12_90K
SD12_10K
B_0X-B_01/Å²
experiment number X


OE1_35
OE2_35
90K
B_0X-B_01/Å²
experiment number X

Results



Negative differential electron density map



Positive differential electron density map

Conclusions

- The influence of radiation damage on the structure is seen in Debye – Waller factor at:
  - di – sulphide bridges
  - carboxyl groups