Study of Biological Macromolecules with a Laboratory XRD system

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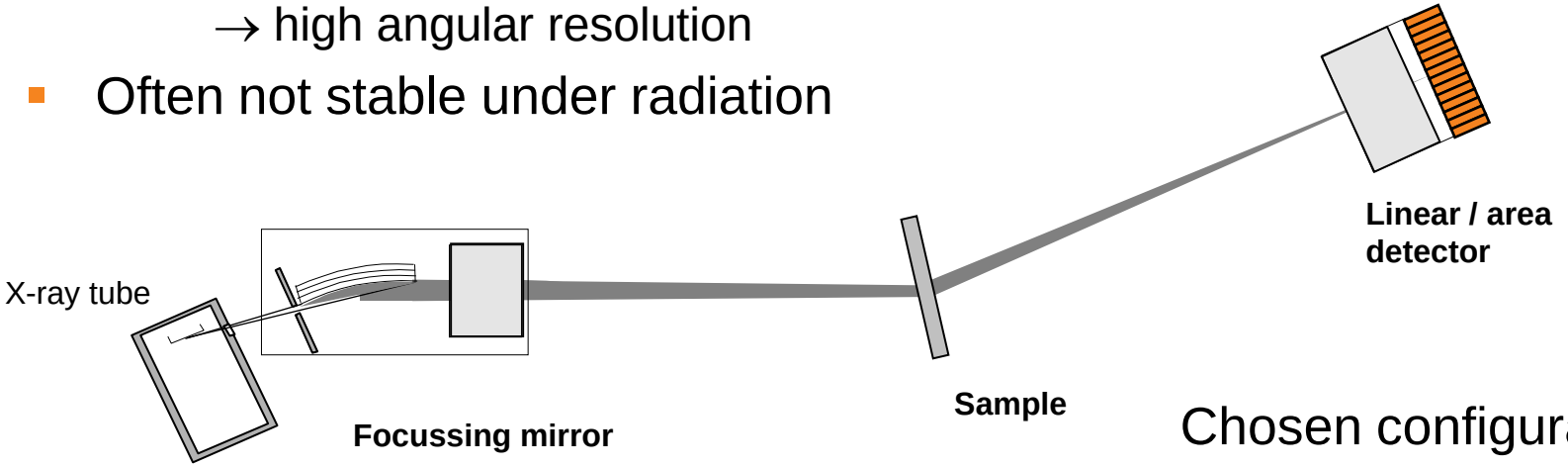
Protein analysis

- Challenges
- Protein poly-crystallography
- *In situ* humidity studies
- SAXS on bio-macromolecules

The challenge

Proteins are challenging samples:

- Weak scatter
  - → high intensity required (and low background)
  - → linear detector / area detector (with high resolution)
- Large molecules / cells
  - → good low angle performance (peak position and asymmetry/ resolution)
  - → high angular resolution
- Often not stable under radiation

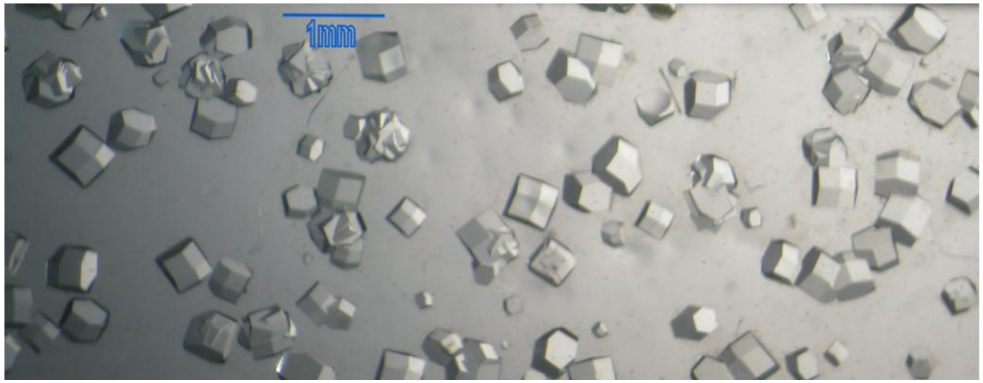


Chosen configuration - high intensity and resolution

HEWL – Sample in capillary

- HEW Lysozyme
  - Can easily be extracted
  - Well-known procedure for crystallization
  - Crystallization after approx. 46 hours
- Pipetting into 0.5 mm capillary (manually compacted)

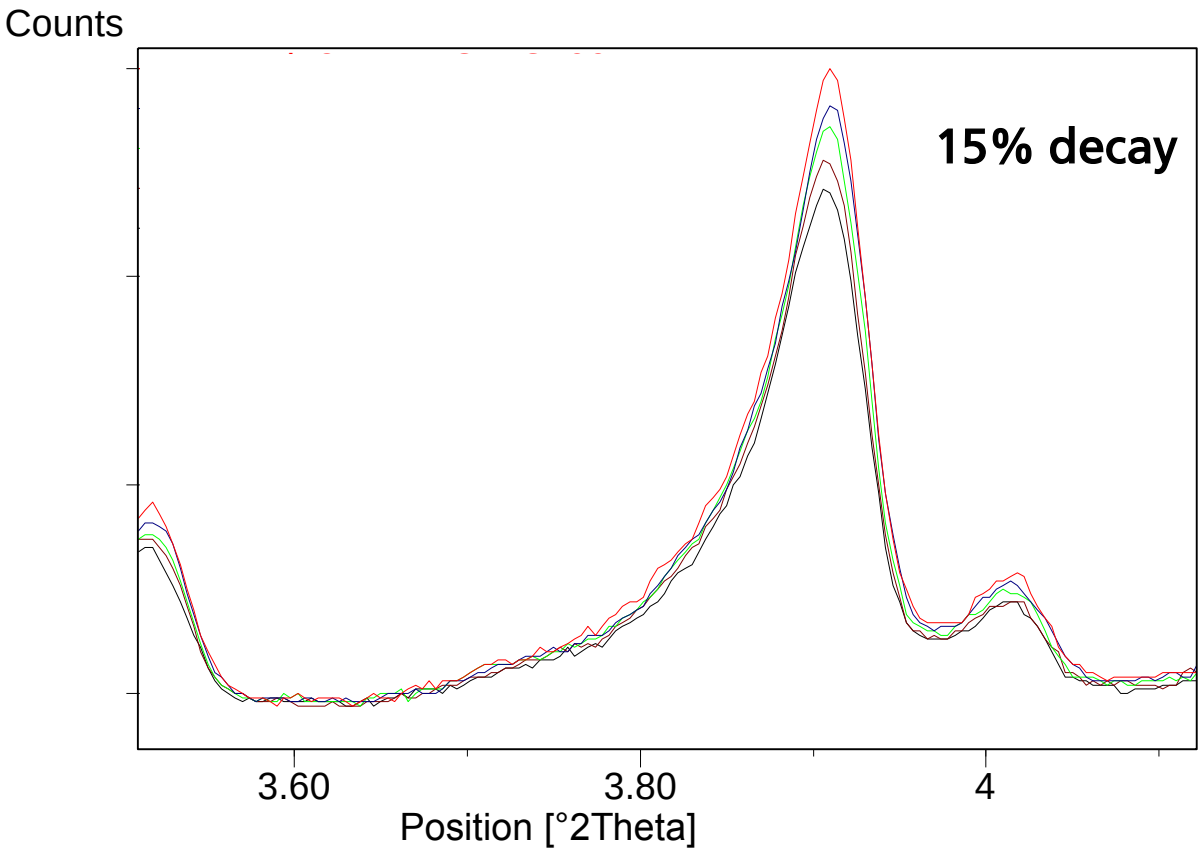
Tetragonal



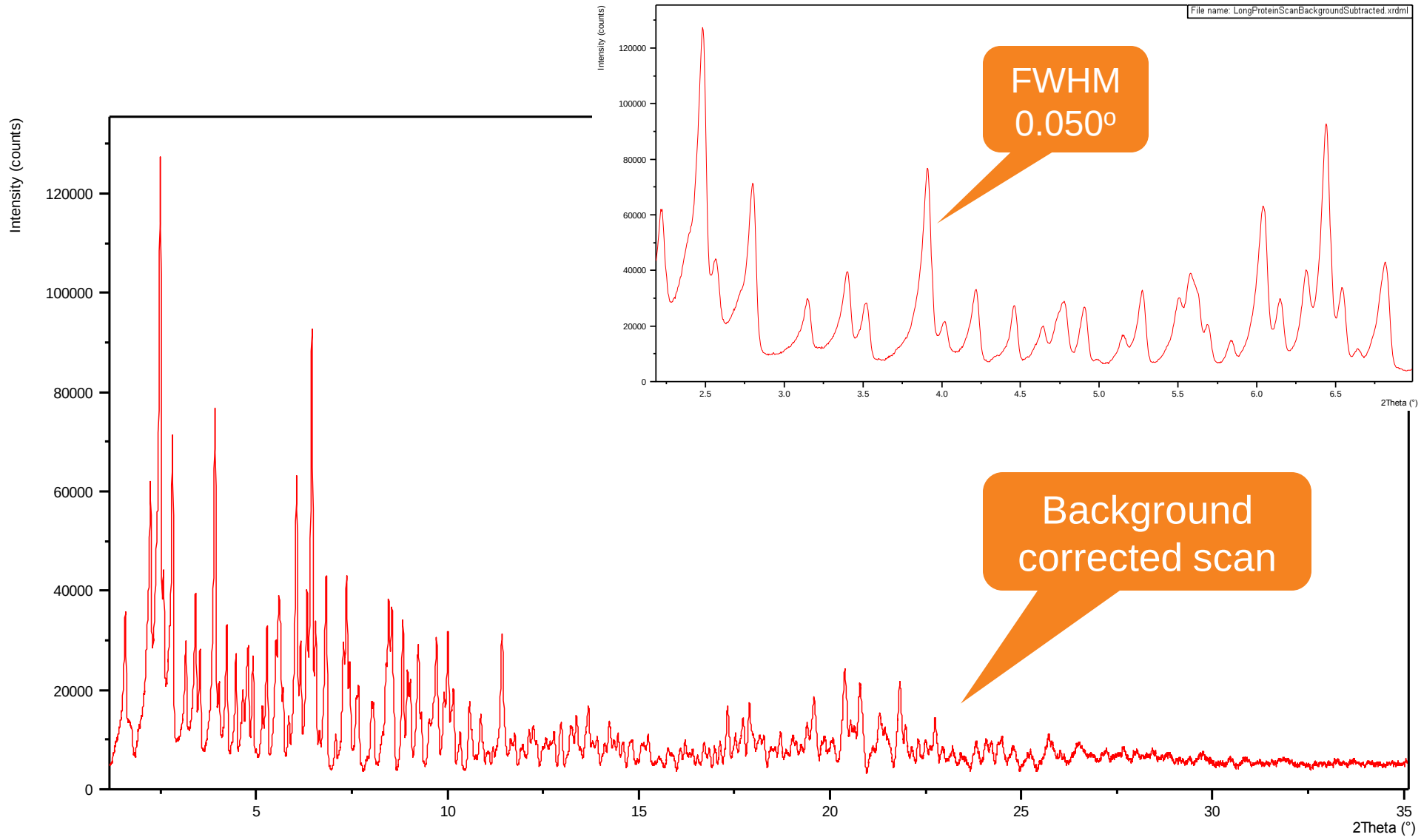
Acknowledgement:
sample supplied by B. Prugovečki , Laboratory of General and Inorganic Chemistry, Faculty of Science, University of Zagreb, Croatia

Sample stability over the time - Lysozyme

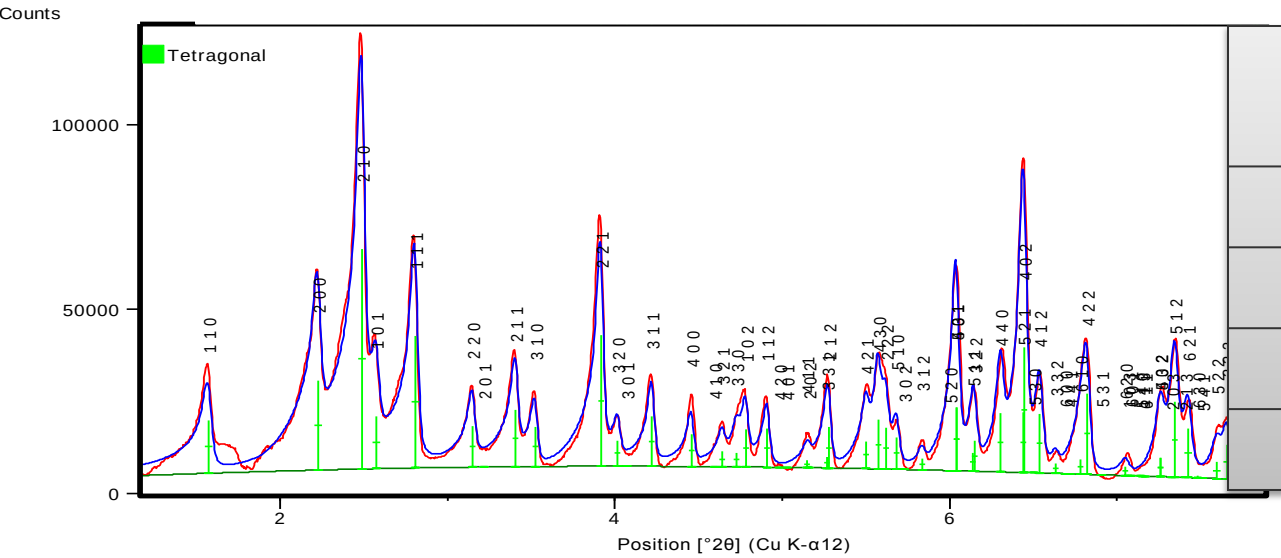
Intensities drop during ~83 hours radiation (5x17 hours)



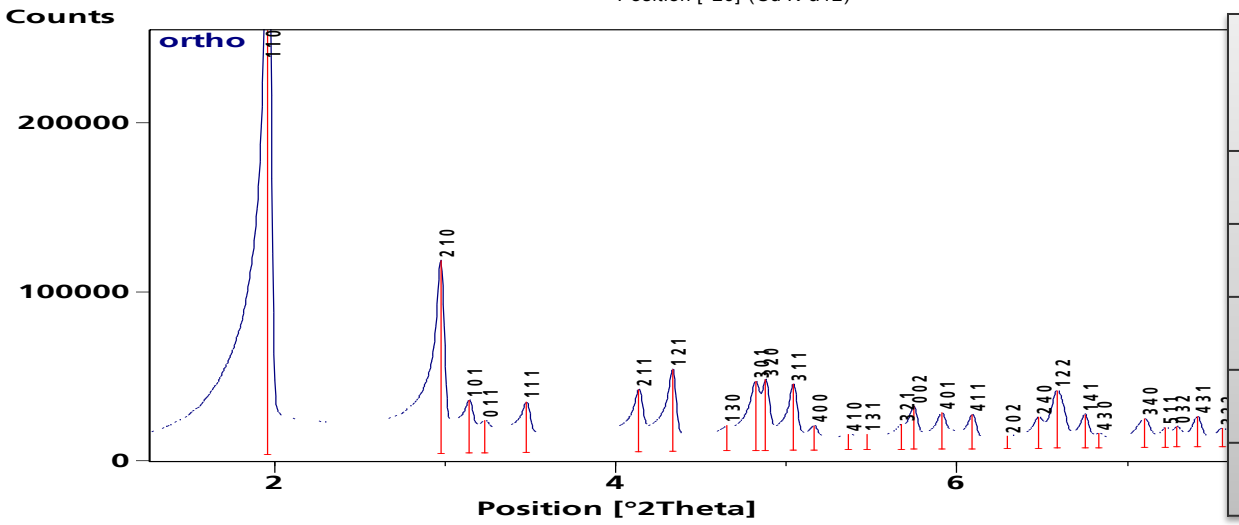
HEWL Lysozyme – capillary data



Tetragonal - Orthorhombic



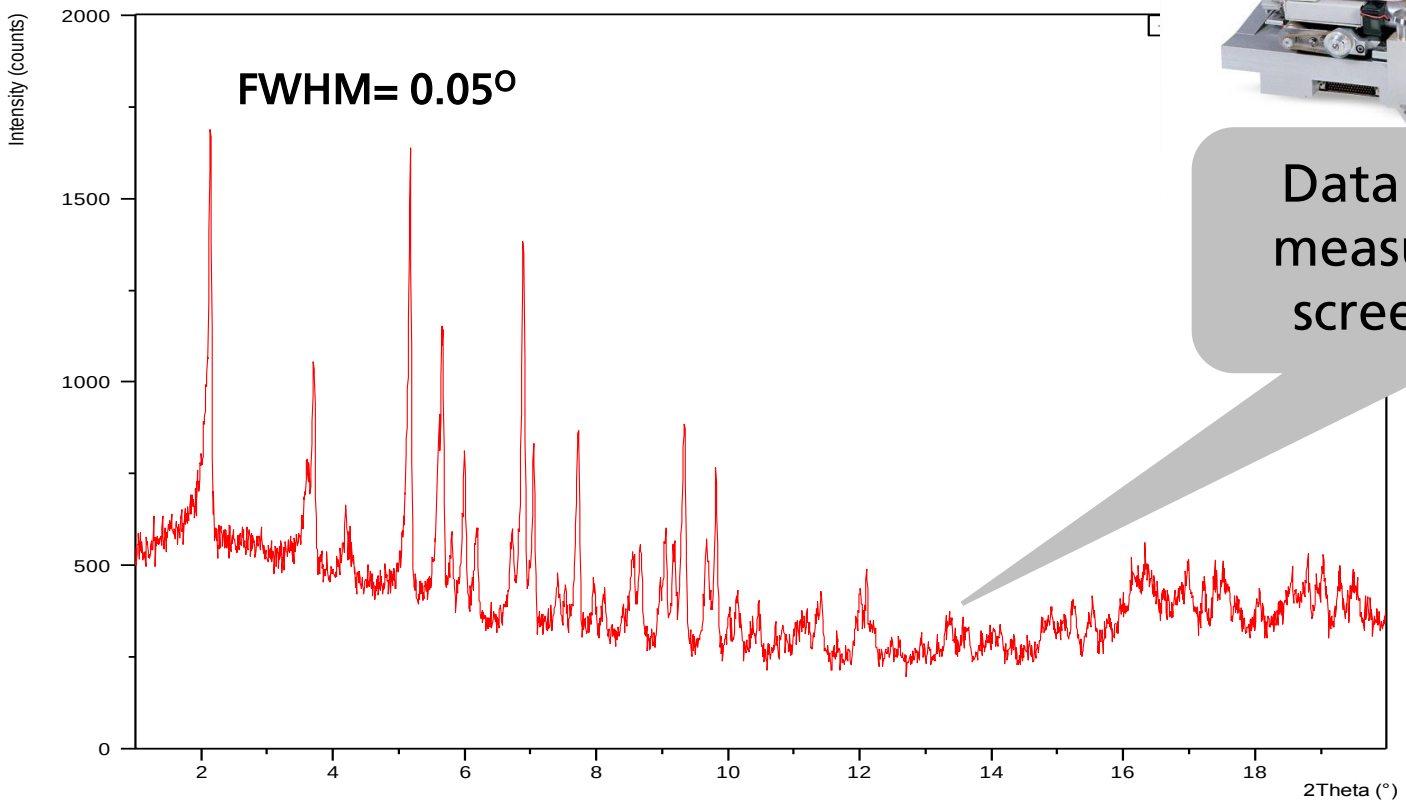
Crystal system:	Tetragonal (Pawley fit)
a (Å)	79.095(6)
c (Å)	37.957(4)
V (Å^3)	237458
FOM	24.8



Crystal system:	Orthorhombic (Pawley fit)
a (Å)	68.418(7)
b(Å)	59.459(7)
c (Å)	30.745(3)
V (Å^3)	125073
FOM	28.7

Bovine Insulin on HTS stage

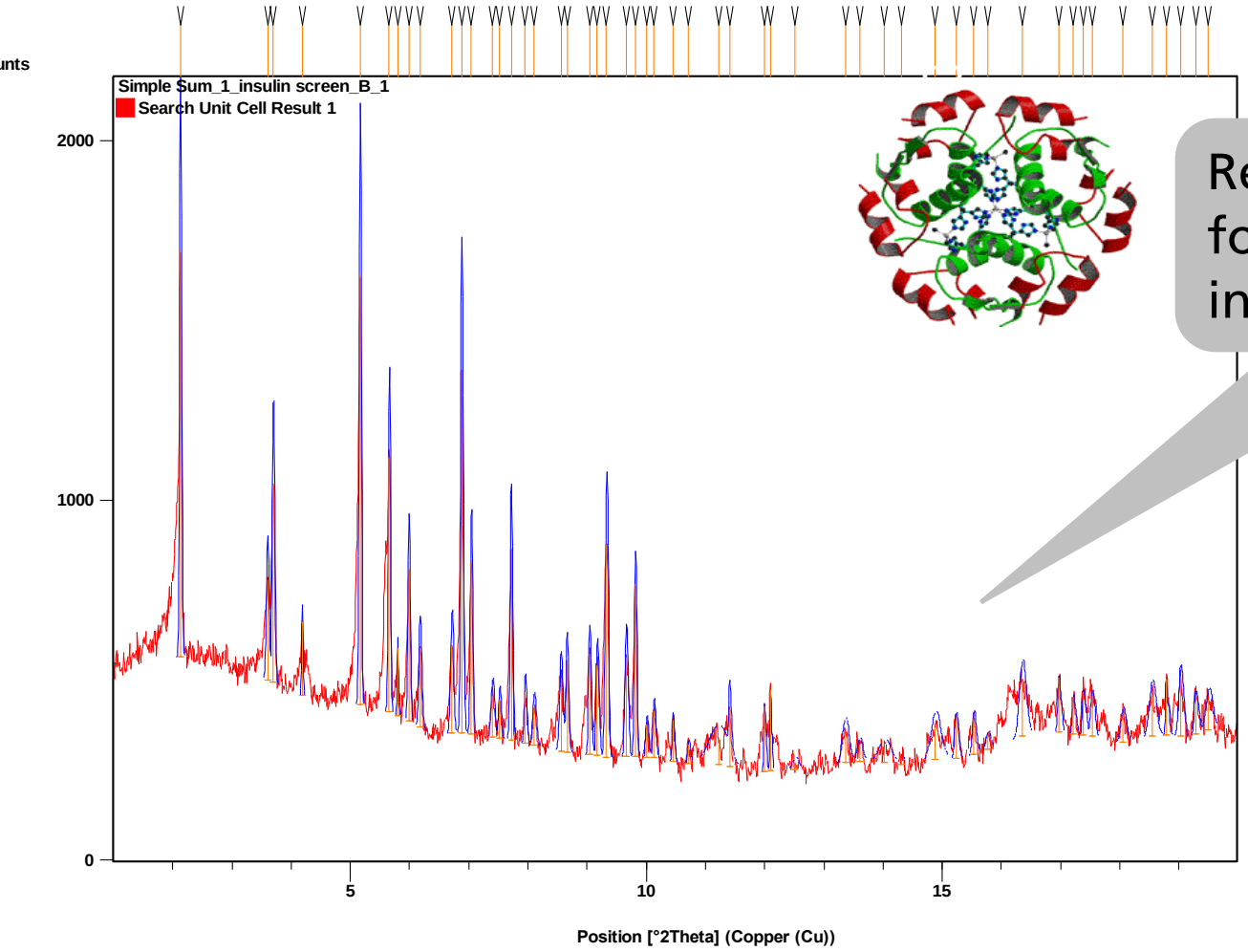
- Crystallized at ph 5.6
- Placed into a HTS-plate



Data of ~20 min measurement on screening plate

Acknowledgement: sample supplied by I. Margiolaki, ESRF, Grenoble, France

Indexing



Resolution sufficient for “automated” indexing of Insulin

Hexagonal crystal system

a = 82.491 Å

c = 33.627 Å

V = 198167 Å^3

Protein polycrystallography

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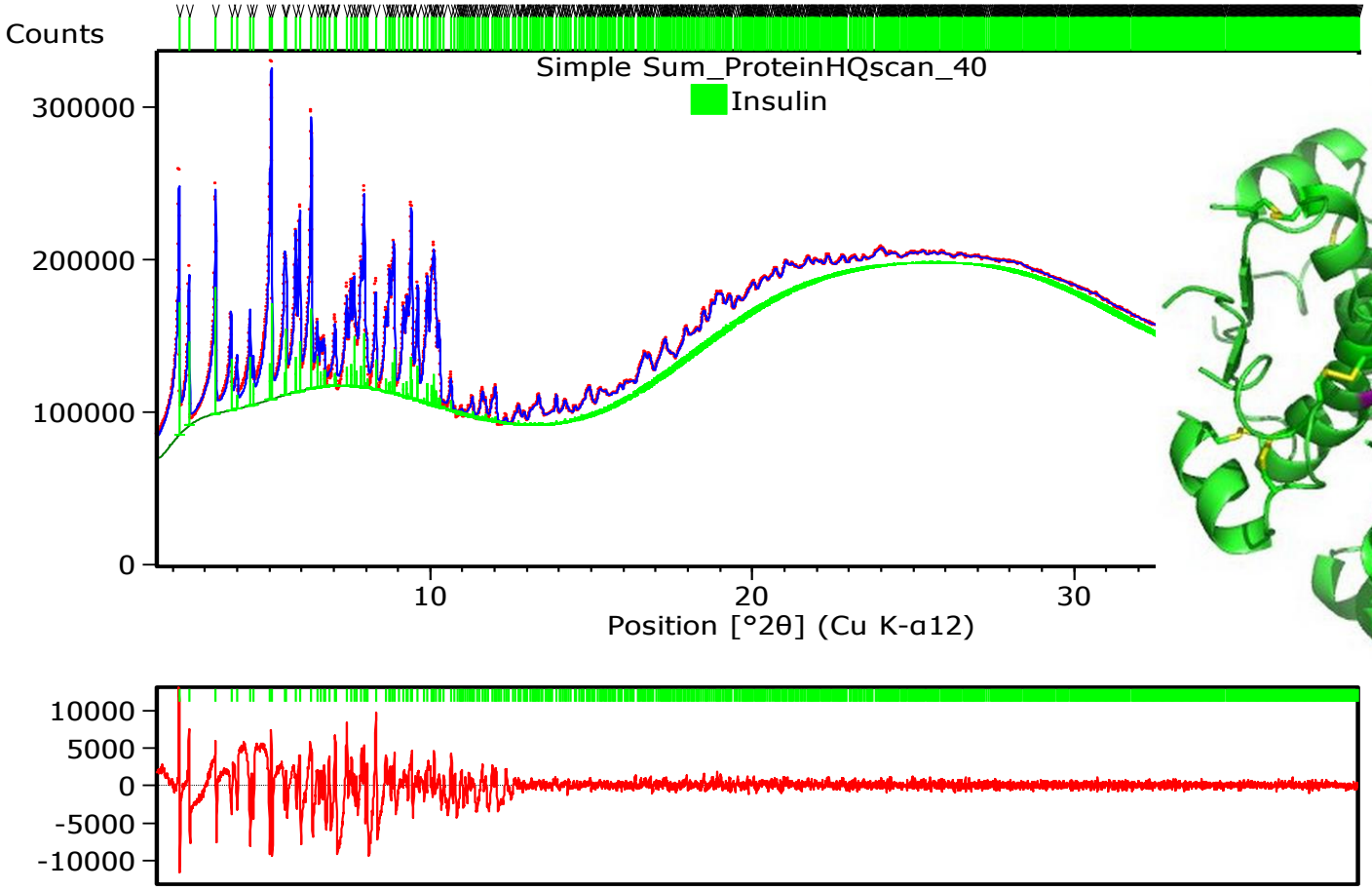
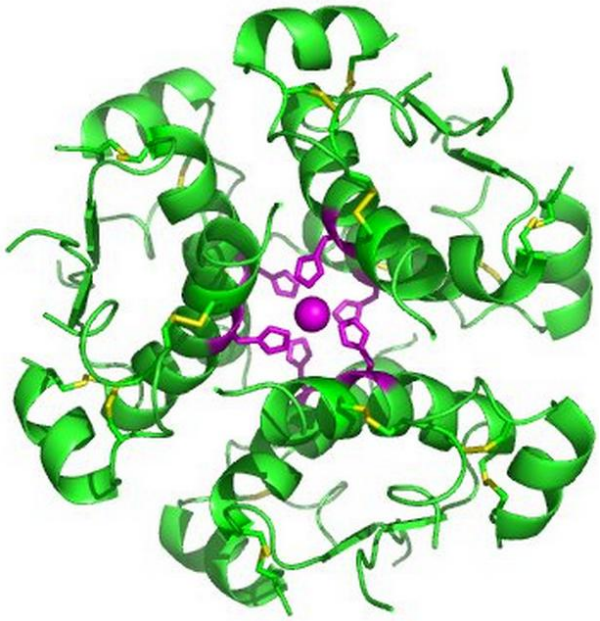
Insulin - Pawley fit, **4563 refined parameters**, **5457 data points**





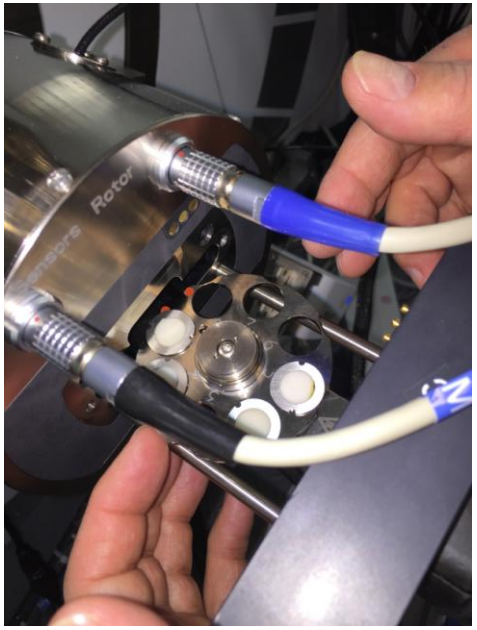
Protein *in situ* humidity study

- Protein molecules in microcrystalline precipitates are surrounded by solvent and their packing arrangement is retained by limited intermolecular contacts.
- A change in the crystal environment first affects the bulk solvent that fills the intermolecular space, with resulting changes in the crystal structure.

Protein *in situ* humidity study

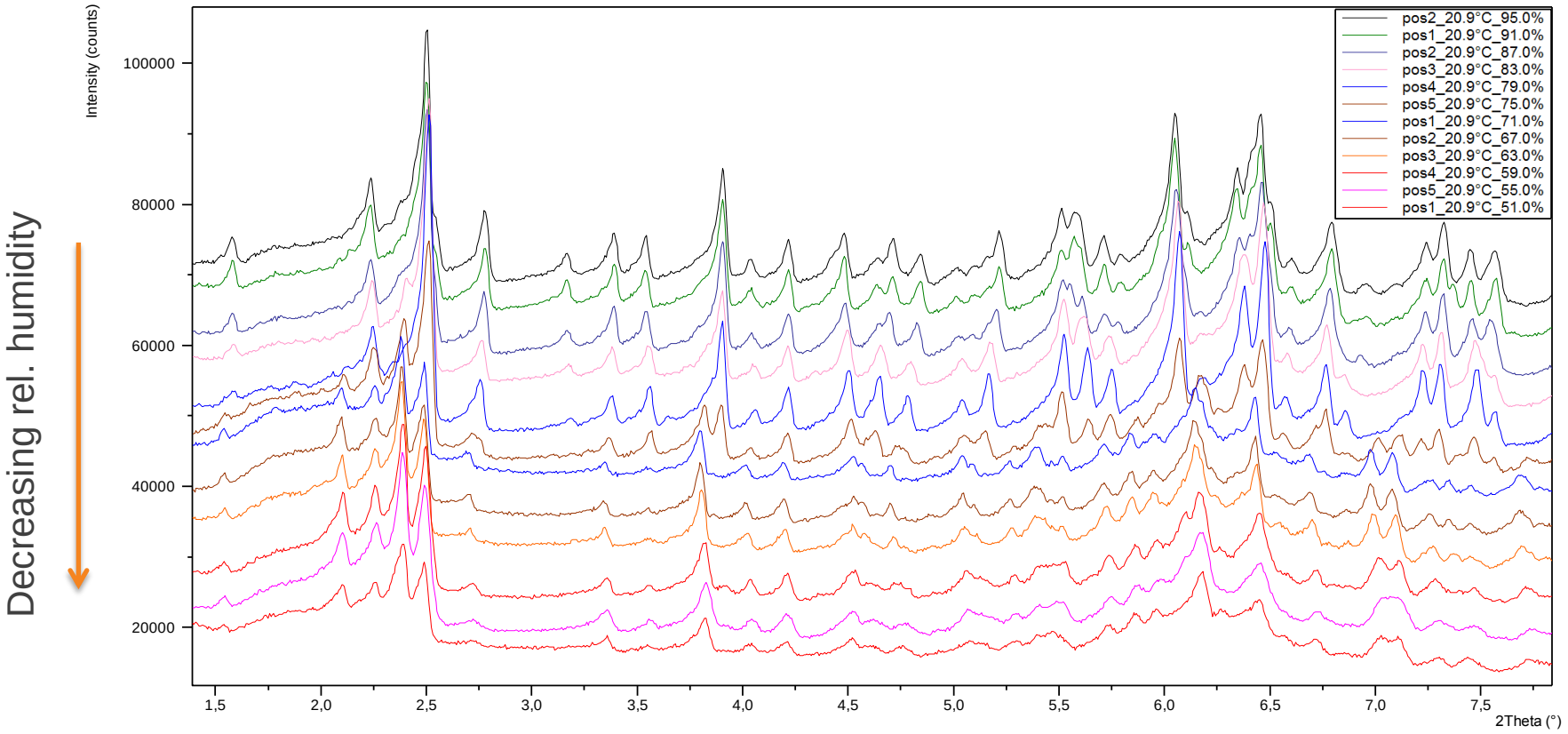
Sample preparation:

- Crystallization (HEWL with 50 mg/ml, pH 4.4)
- Concentration in centrifuge
- Pipetting concentrate onto transmission holders (~100µl precipitate per sample) – sample height varies during humidity cycle
- Multiple samples to reduce radiation damage
- Humidity variation: 95% → 51%



Protein *in situ* humidity study

- 3 hours / humidity step
- 30 min stabilization / 2.5 h data collection (3 scans)
- Here: results of 2nd humidity cycle



In situ humidity study on HEWL

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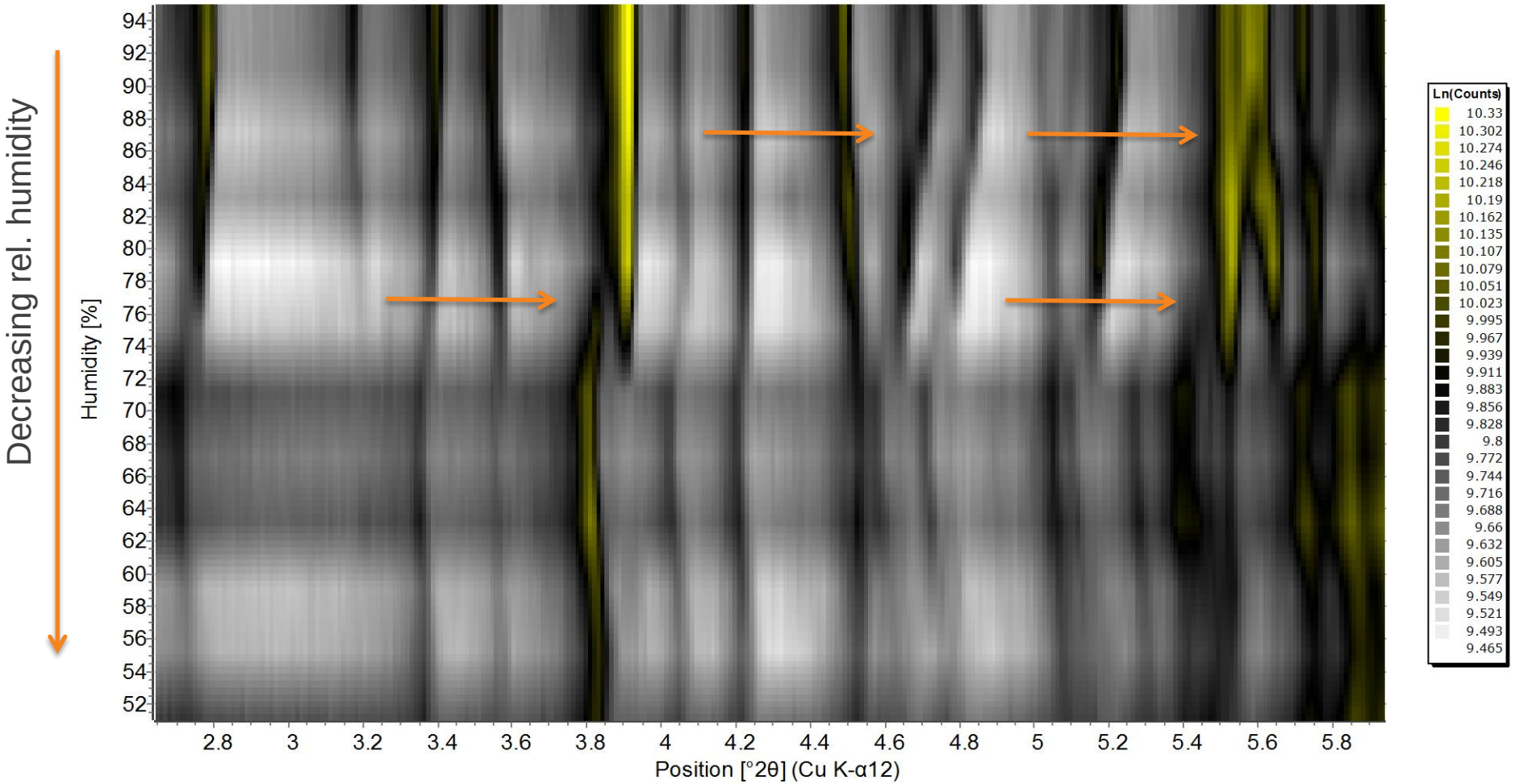
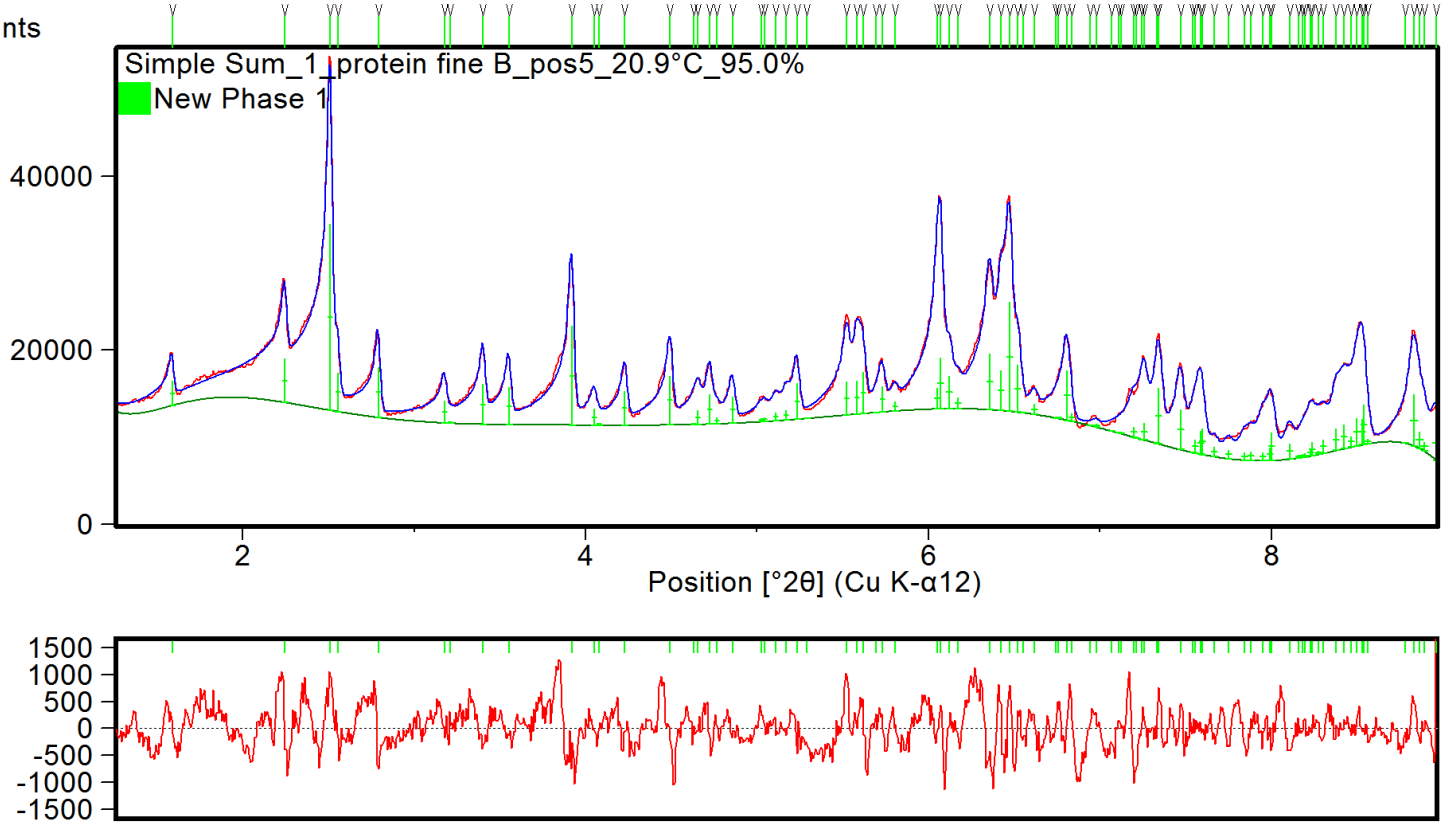
Surface view showing phase transitions



In situ humidity study on HEWL

- Tetragonal HEWL phase at 95% Humidity
- Pawley fit



In situ humidity study on HEWL

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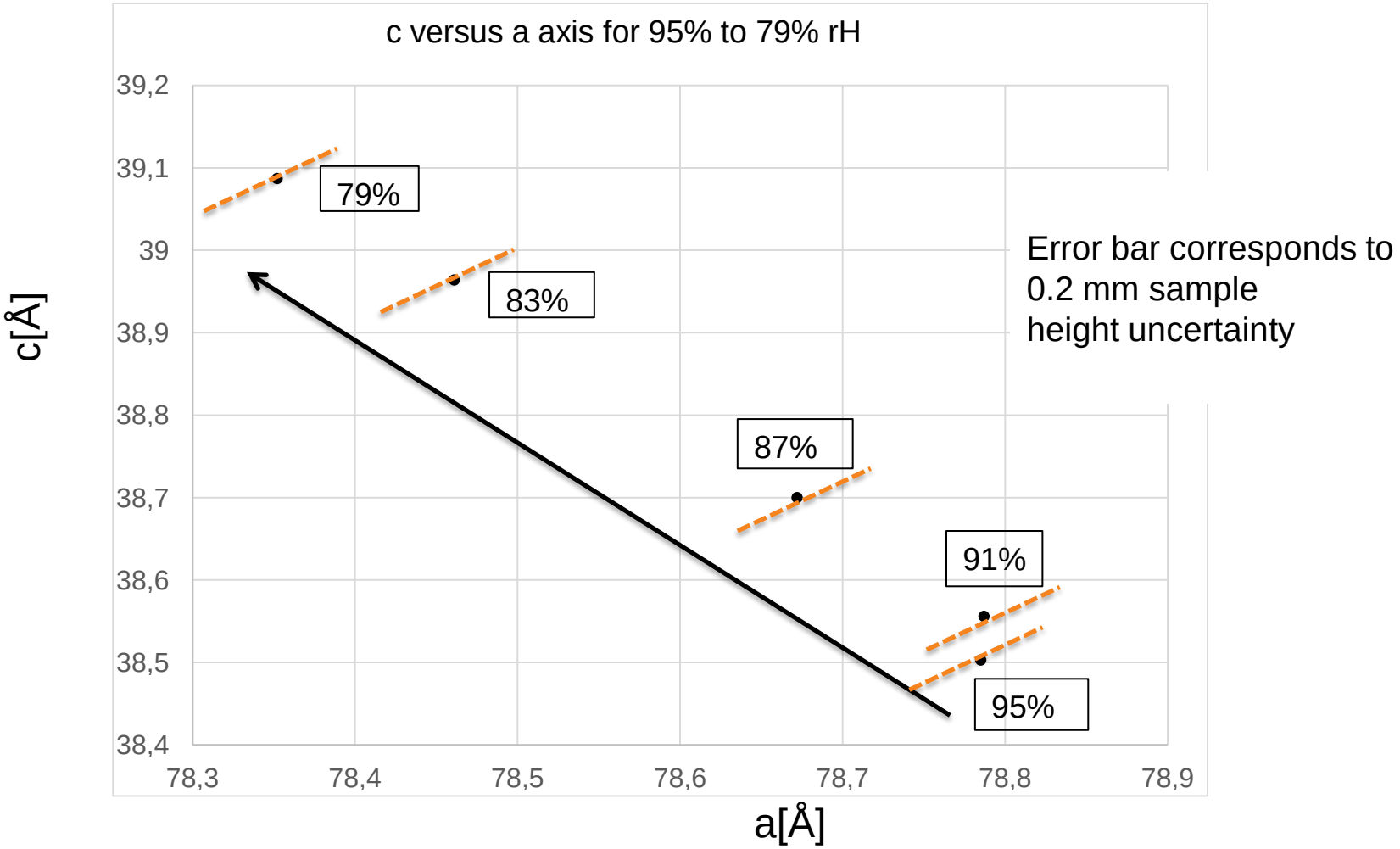
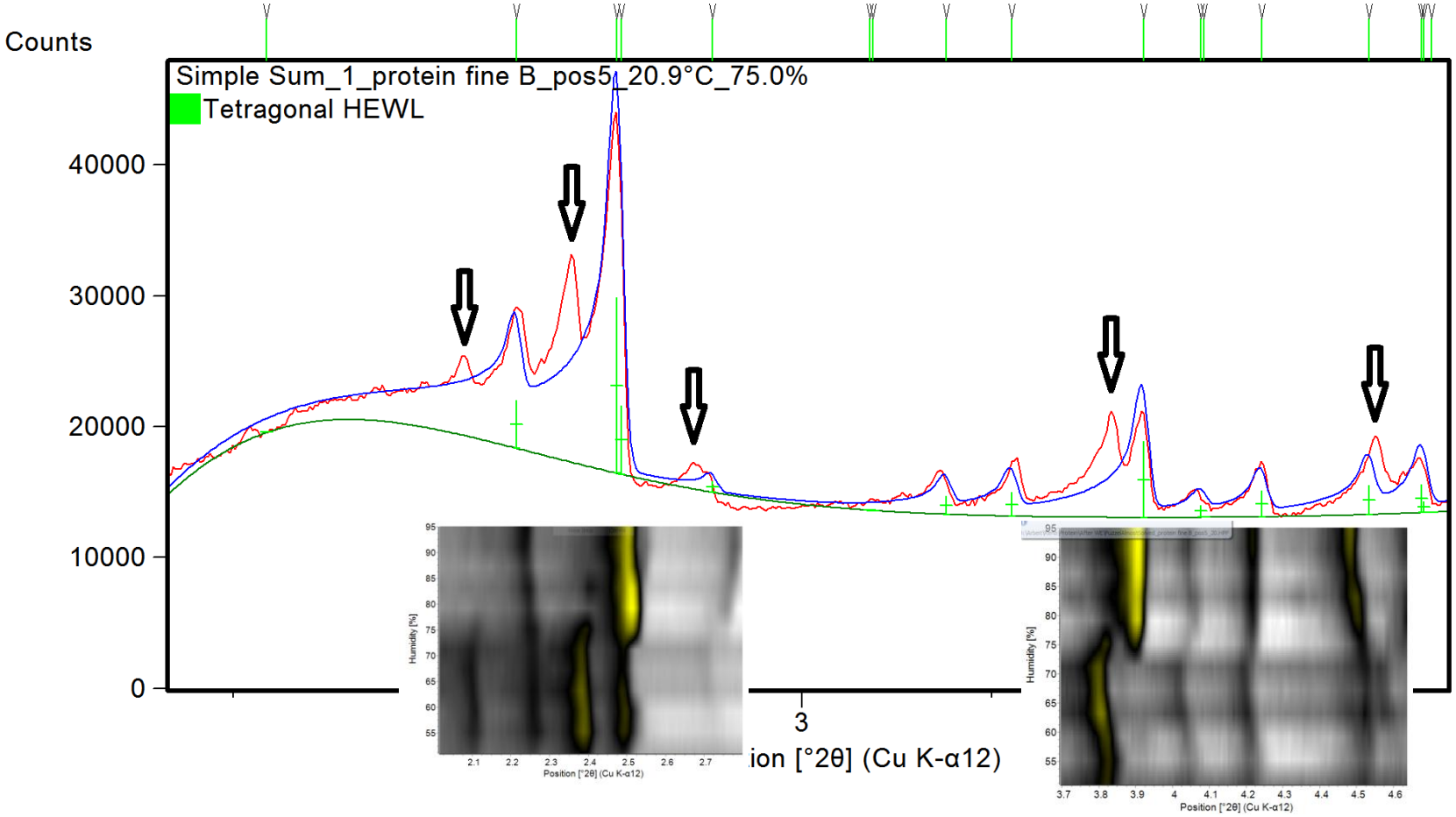
Pawley fit of Tetragonal phase from 95% to 79% rH



In situ humidity study on HEWL

Peaks of an un-indexed HEWL polymorph after Pawley fit of the Tetragonal HEWL cell at 75% RH



In situ humidity study on HEWL

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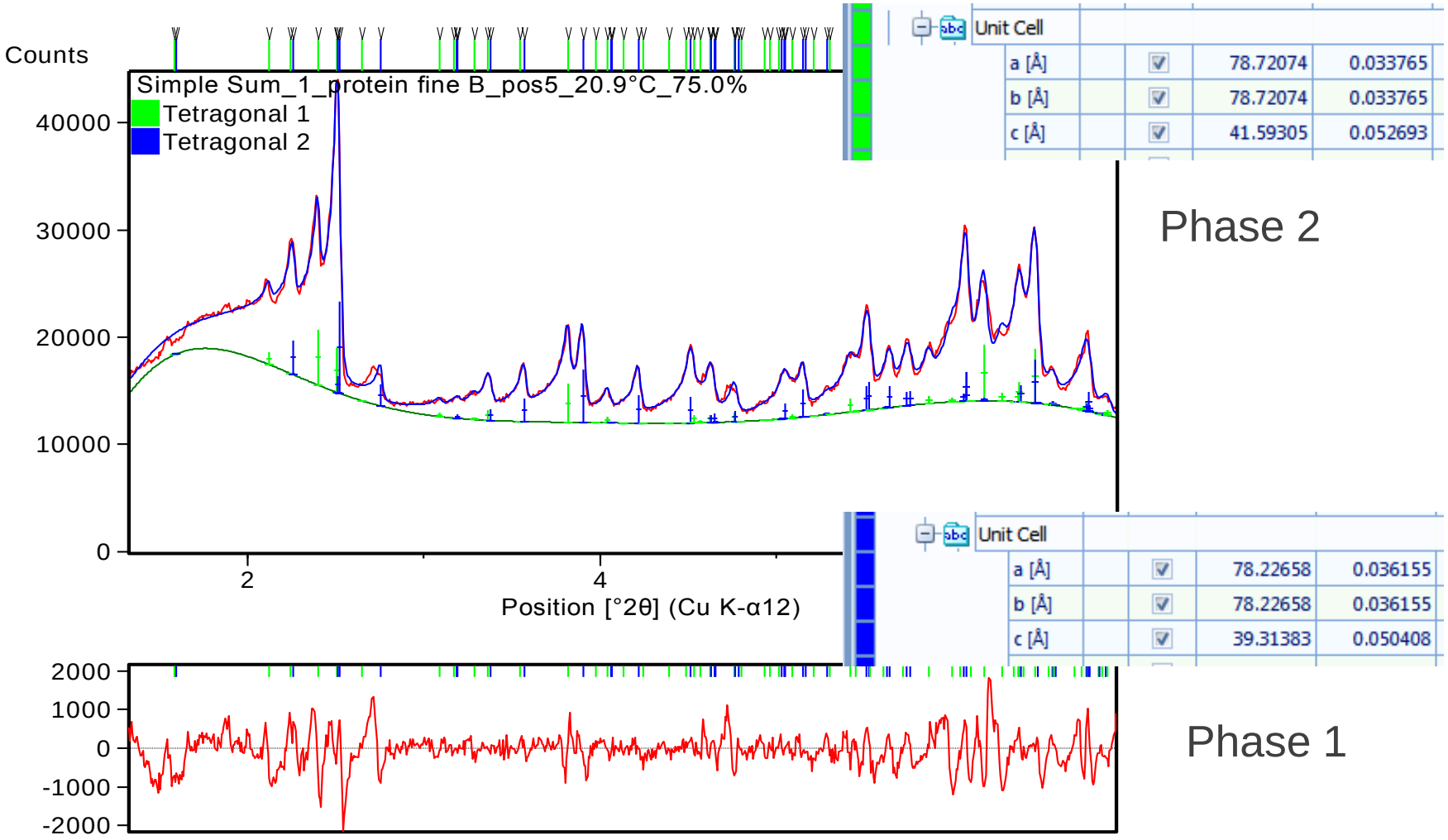
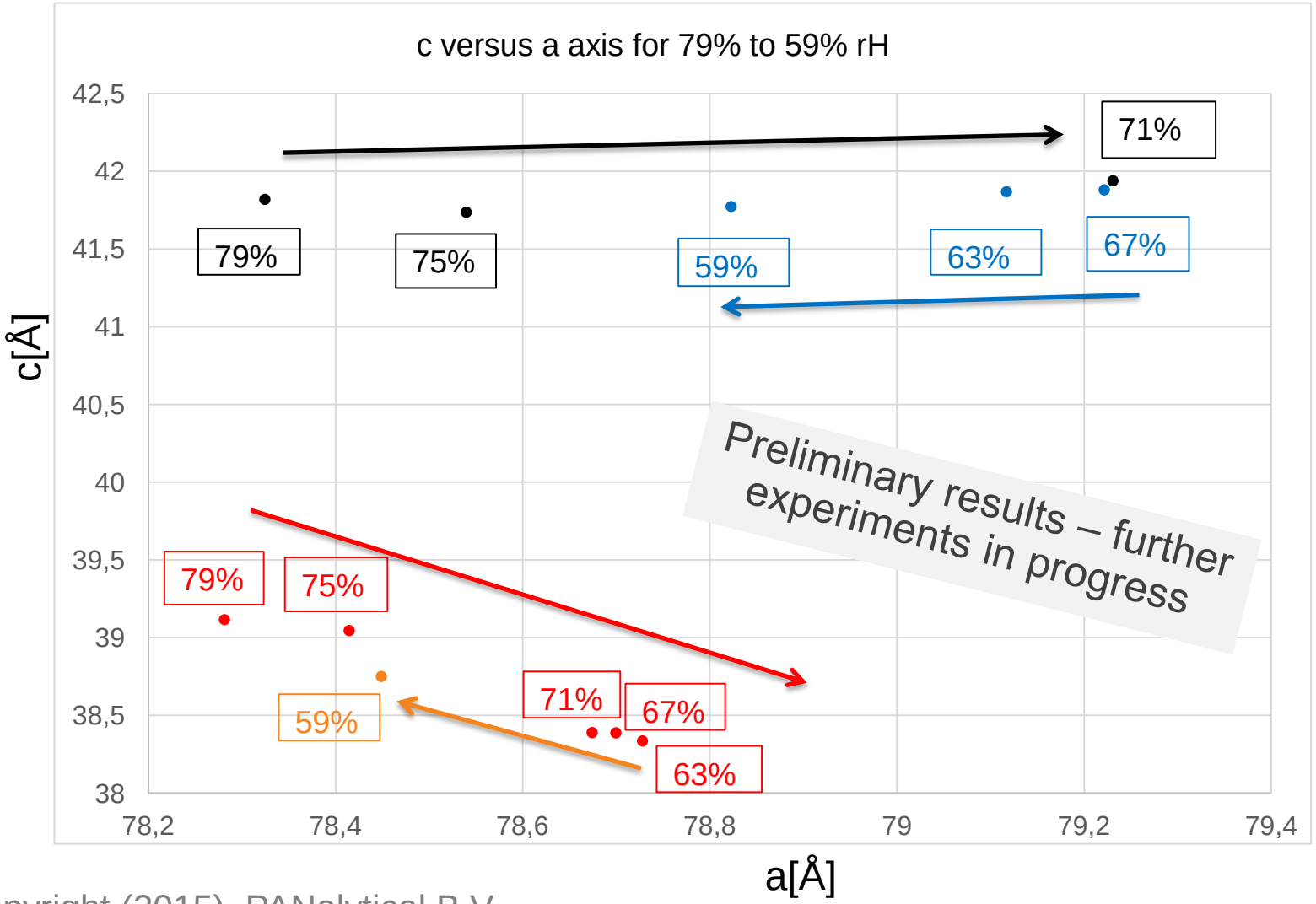
Peaks could be explained by a second tetragonal phases



In situ humidity study on HEWL

Pawley fit with two Tetragonal phases below 79% rH



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BioSAXS

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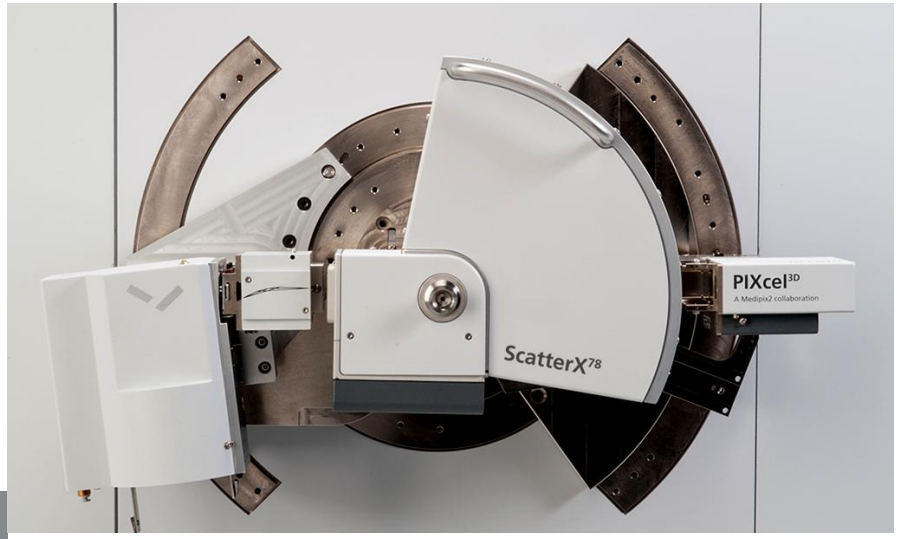
The biomacromolecules are studied in their native environment, i.e. in dilute (approx. 1 wt.%) solution. Crystallization is not required. Available sample quantities are often very small.

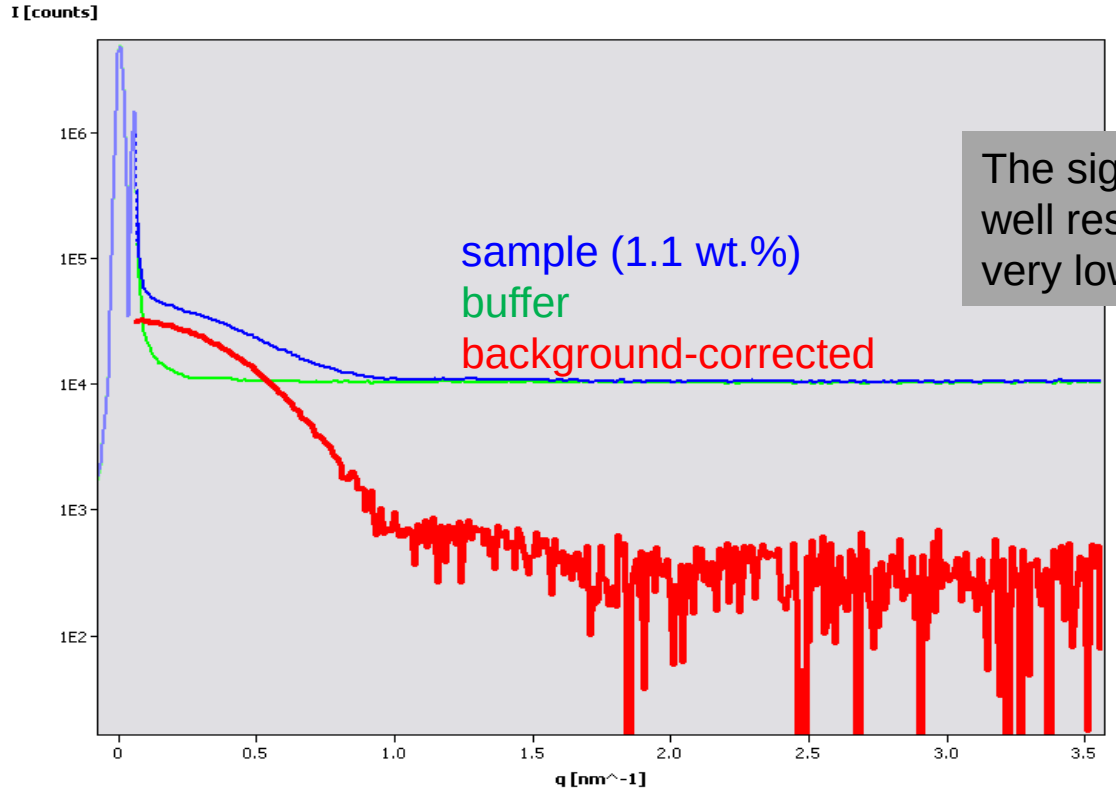
- ✓ Size
- ✓ Shape (3D envelope)
- ✓ Compactness and aggregation behavior
- ✓ Folding / unfolding
- ✓ 2nd virial coefficient (molecule interaction)

These properties can be studied as a function of temperature, pH, buffer composition, etc.



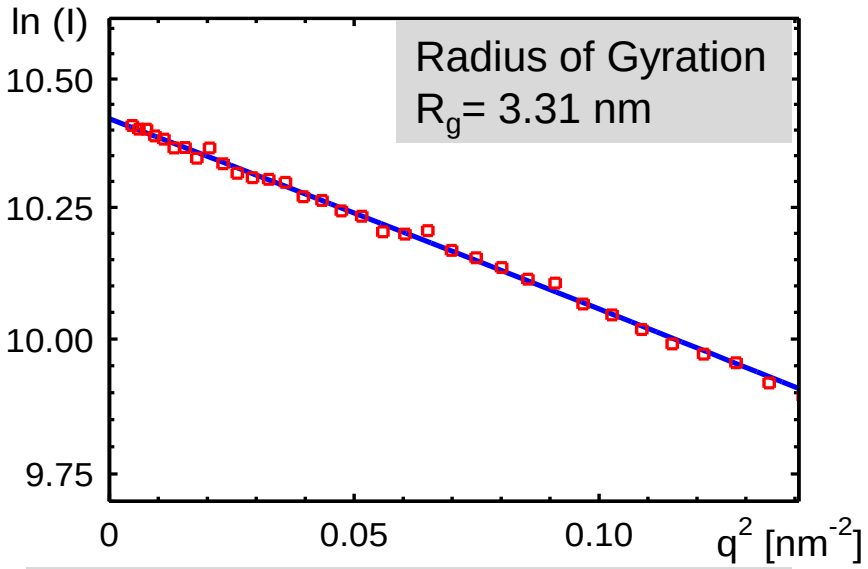
Glucose isomerase

- Enzyme (protein) produced by microorganisms (bacteria)
- Catalyzes the conversion of glucose to fructose
- Massive industrial use for the production of high-fructose syrups
- Popular protein to demonstrate SAXS measurement and analysis capabilities

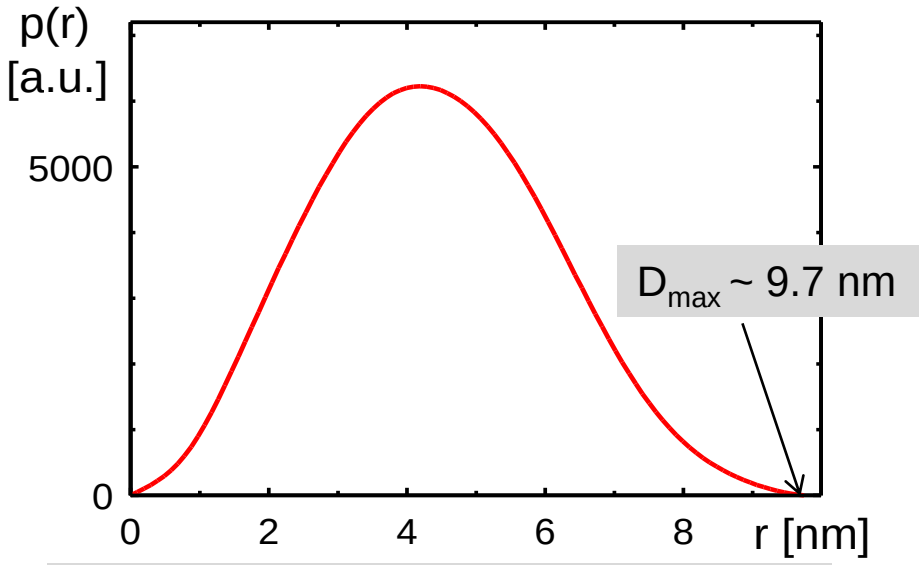


The signal from the protein is well resolved, even in the very low intensity region

Guinier plot / Pair distance distribution function p(r)



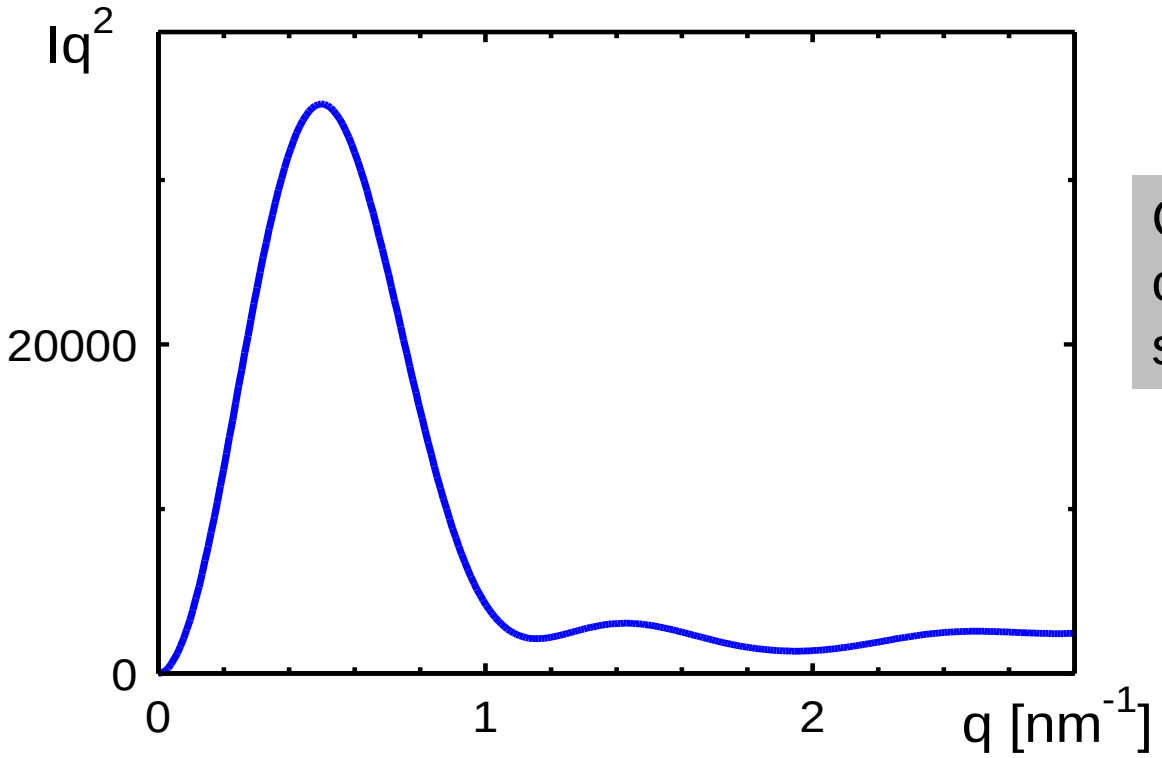
Straight line at smallest angles - no aggregation



p(r) indicates that the protein has an overall symmetric shape; maximum dimension is approx. 9.7 nm

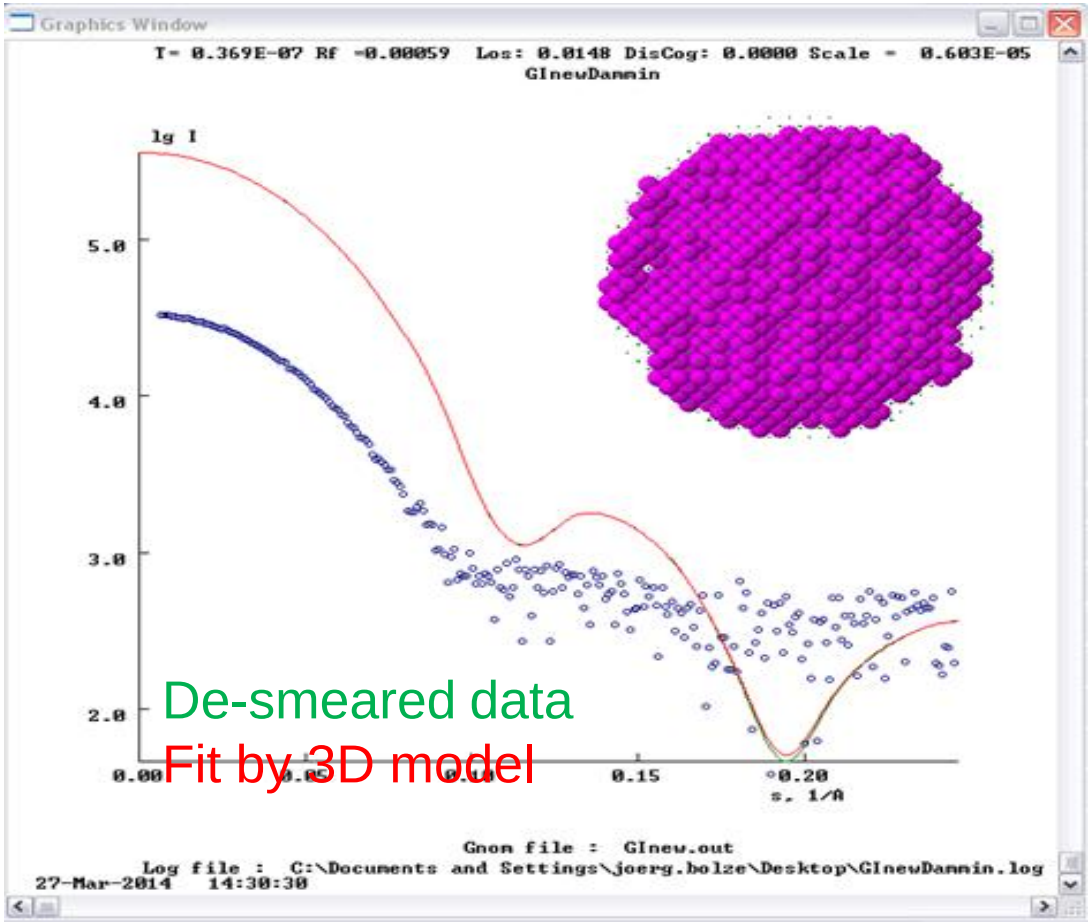
	R_g from Guinier plot	R_g from *p(r)*	D_{max} from *p(r)*
Lab system	3.31 nm	3.32 nm	9.7 nm
Synchrotron (lit)	3.25 nm	n.a.	9.7 nm

Kratky plot



Curve indicates a compact, folded structure

3D-shape reconstruction

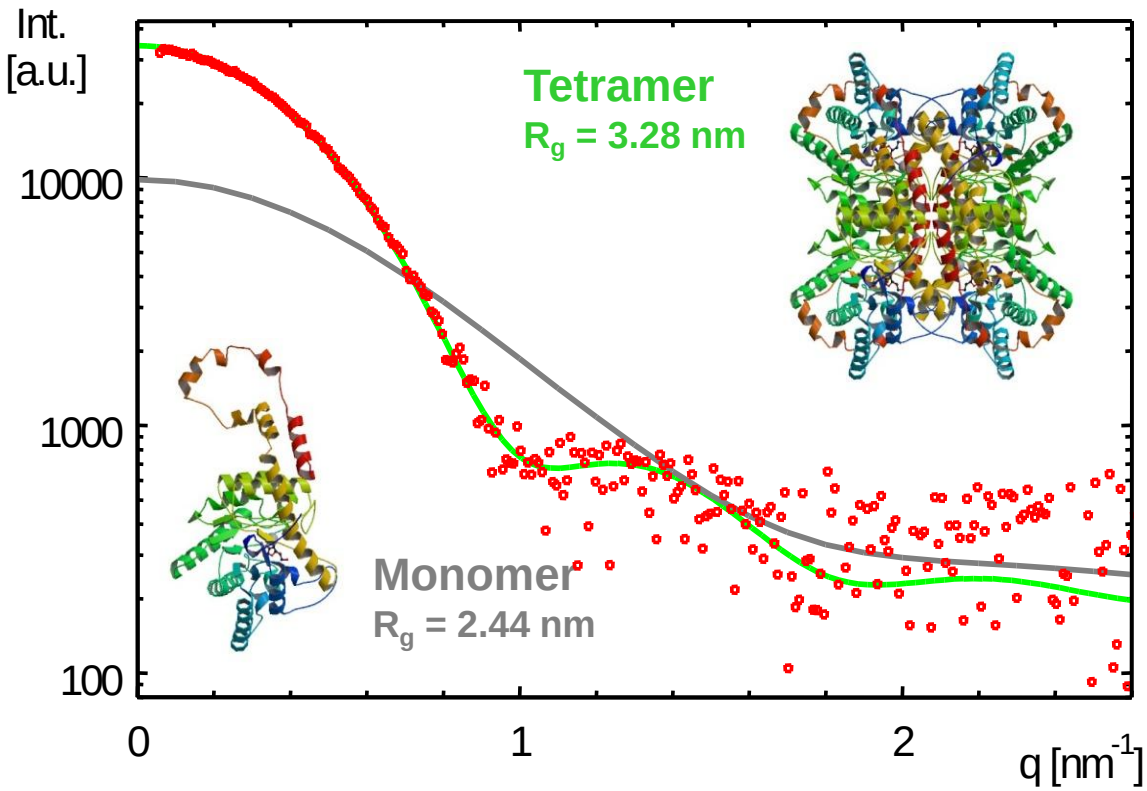


Software:
Dammin (EMBL) [1]

Model fits the experimental data very well

[1] D. I. Svergun (1999). Restoring low resolution structure of biological macromolecules from solution scattering using simulated annealing. *Biophys J. 2879-2886.*

Comparison with results from single crystal diffraction



Simulation of SAXS data from known single crystal structures (using CRYSOL[1] from the EMBL): Experimental SAXS data is in good agreement with the Tetramer structure. (R_g values from simulations)

[1]Svergun D.I., Barberato C. and Koch M.H.J. (1995) CRYSOL - a Program to Evaluate X-ray Solution Scattering of Biological Macromolecules from Atomic Coordinates *J. Appl. Cryst.* , ***28, 768-773.***

Conclusion

Multi-purpose X-ray laboratory diffraction systems offer a range of possibilities to study biological macro molecules:

- Protein polycrystallography (indexing, space group determination)
- Polymorph identification / screening
- Analysis of structural changes of protein precipitates upon dehydration and hydration
- Shape analysis, aggregation and folding of diluted proteins in solution (SAXS).