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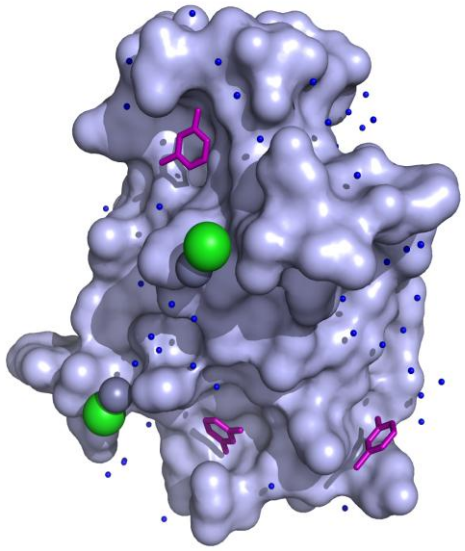
The Power of Powder

Humidity induced phase transitions of HEWLysozyme



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Outline

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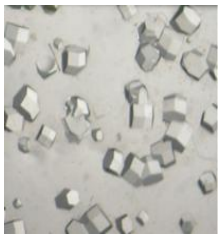
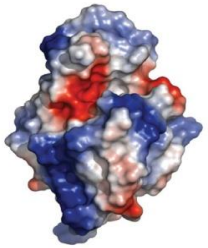
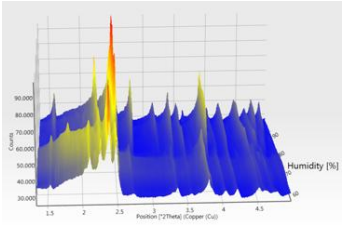


Why powder diffraction?



HEWL – what is known?



Protein *in situ* humidity studies

Why powder diffraction?

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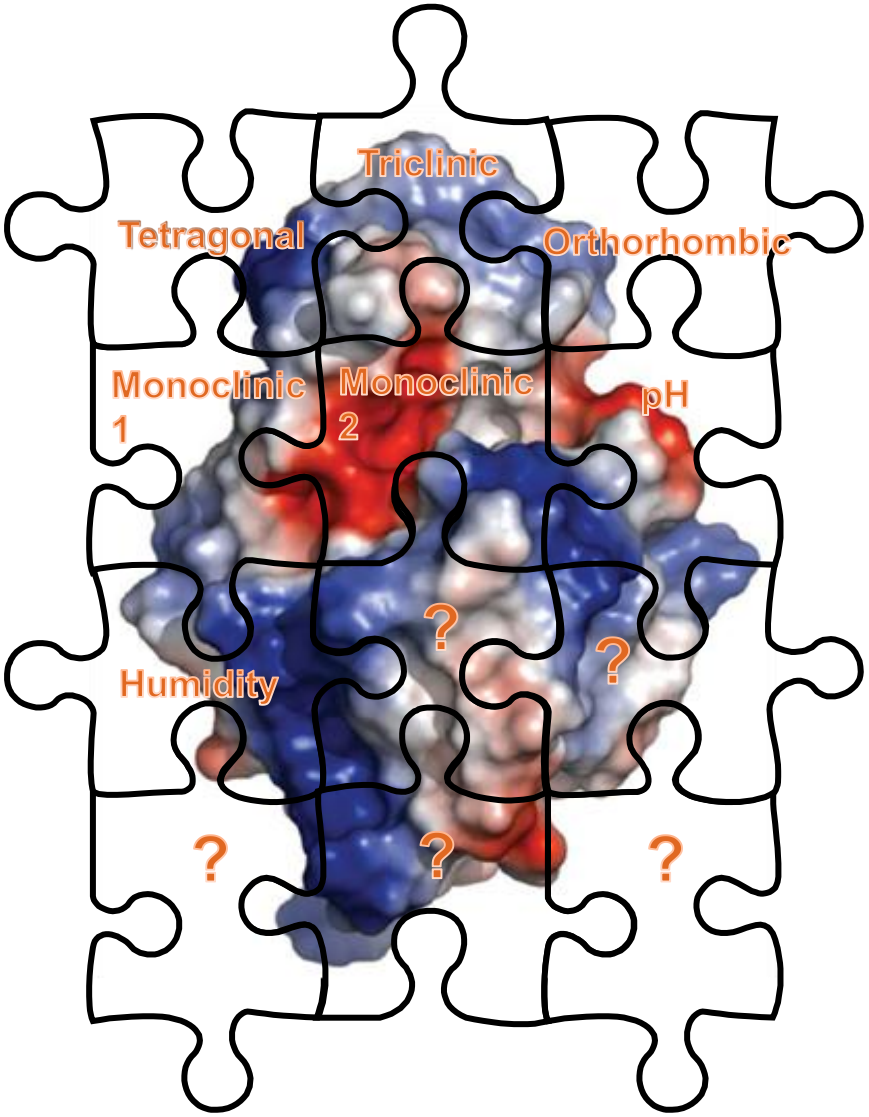
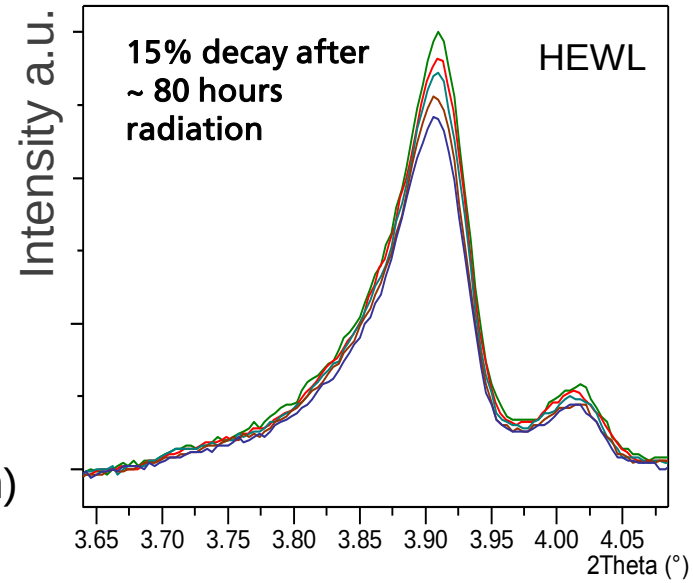


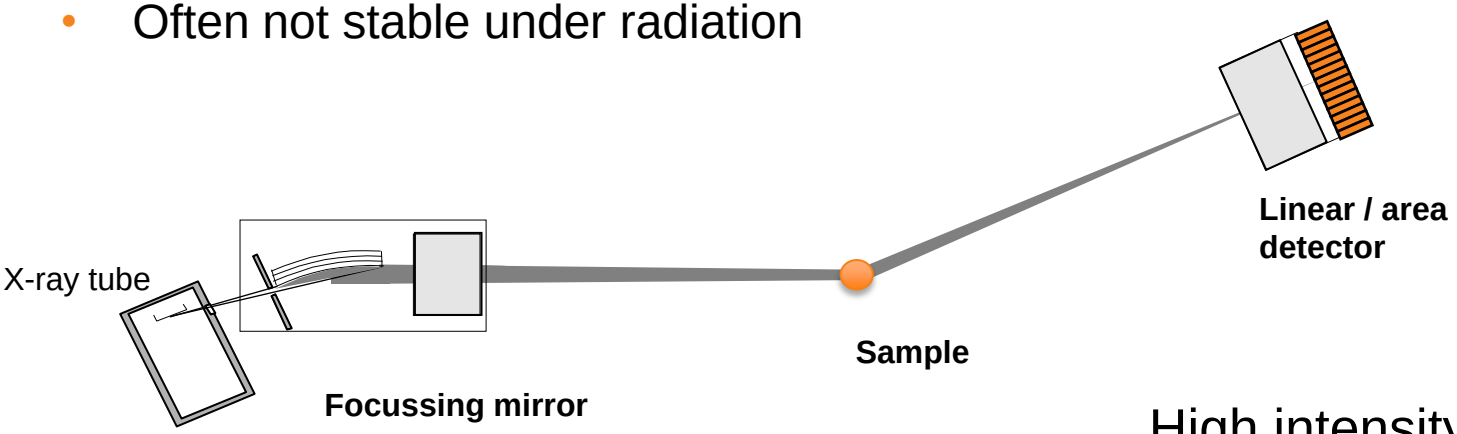
- Known and unknown HEWL Polymorphs
- Polymorph screening is like solving a huge puzzle, without powder diffraction pieces are missing in the big picture.
- Analyzing single crystals is a very selective, kind of “cherry picking" process.
- XRPD is an important tool to screen the landscape and see more from the complex picture.

Laboratory XRD - 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



High intensity and resolution

Data comparison

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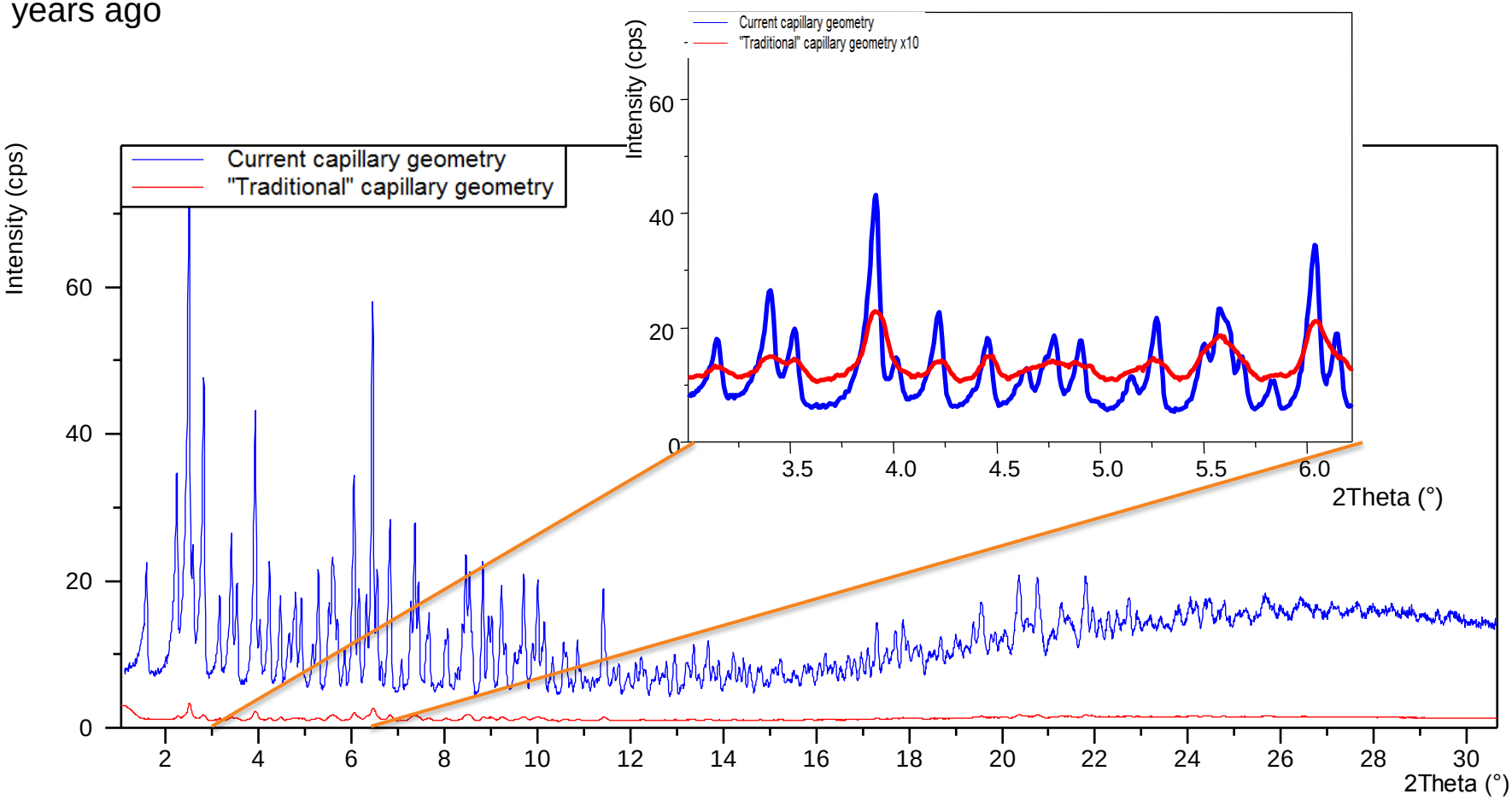
Lab data quality improved significantly with the introduction of focusing mirrors around 10 years ago



Data comparison

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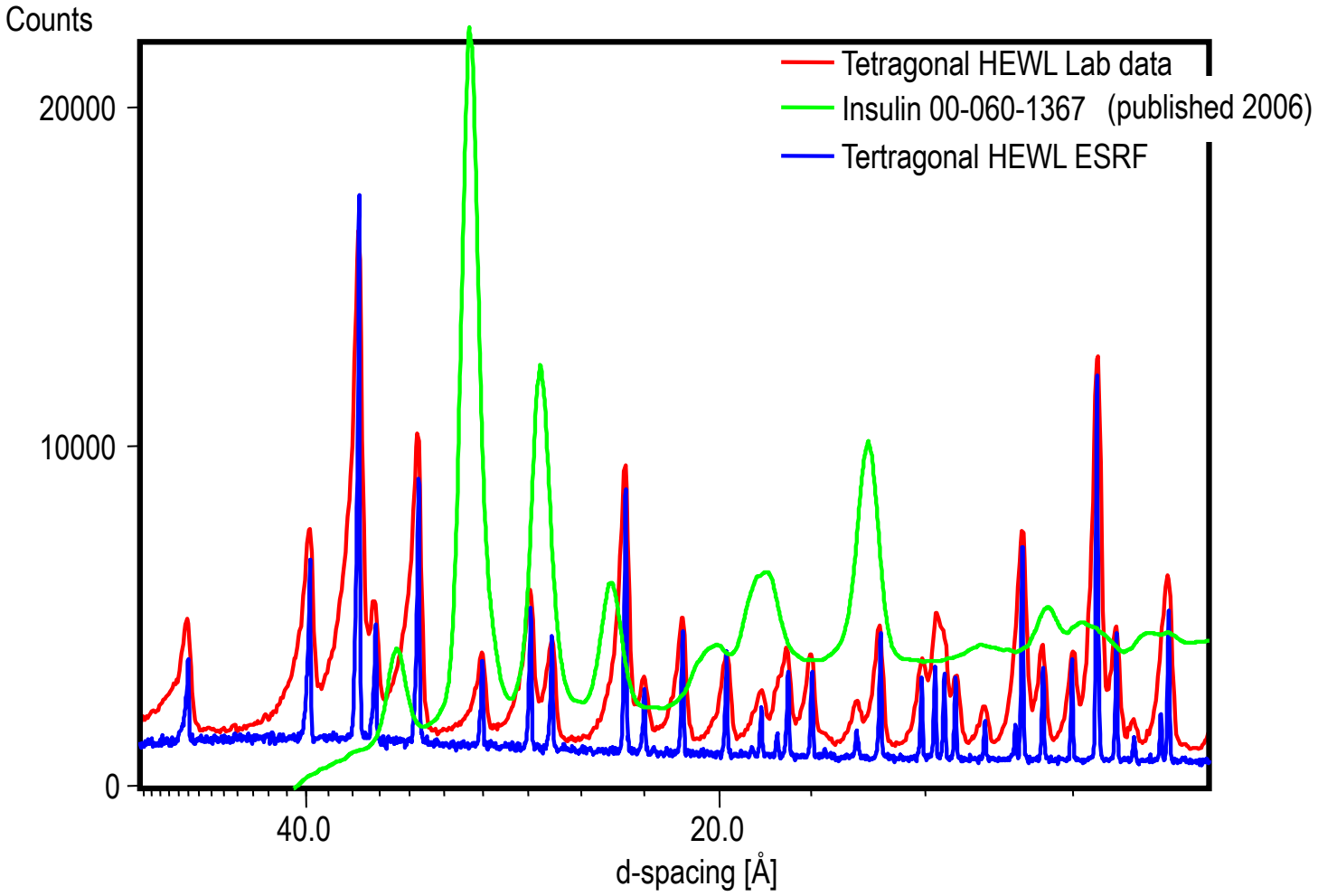

HEWL (Hen-Egg White Lysozme)

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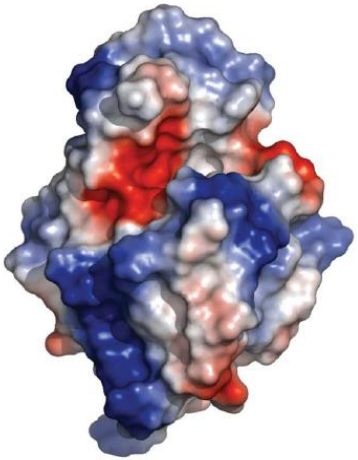
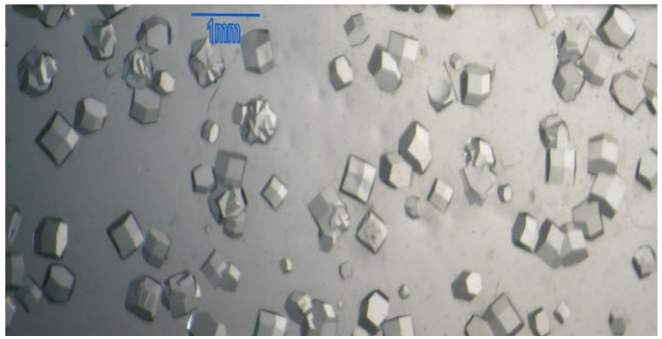
- Lysozyme damages bacterial cell walls and can be found in a number of secretions (tears, saliva, milk,..)
  - Can easily be extracted
  - Well-known procedure for crystallization
  - Crystallization after approx. 24-48 hours
  - Affordable in “normal” quantities





Tetragonal

Published HEWL polymorphs (reported in PDB database)

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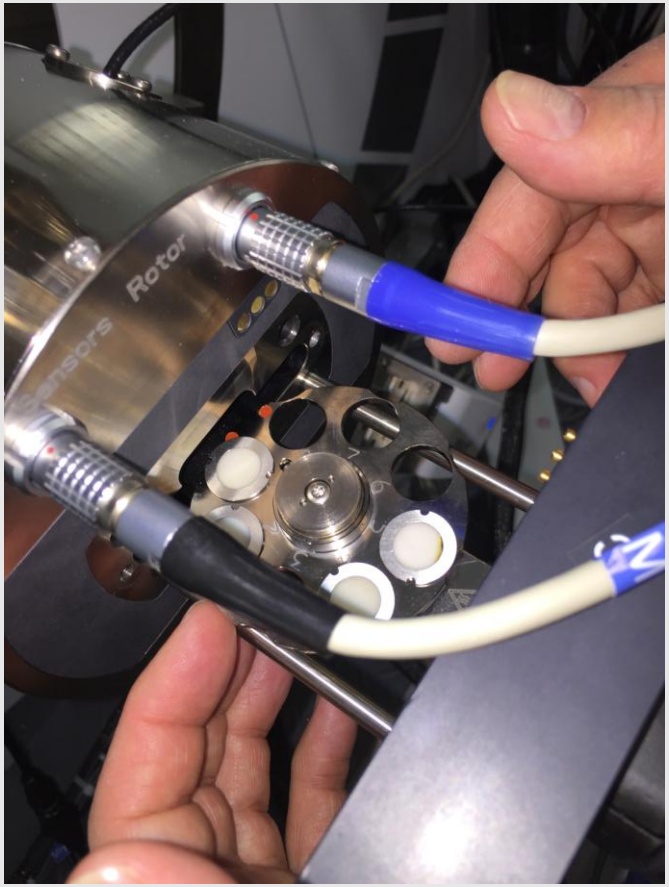
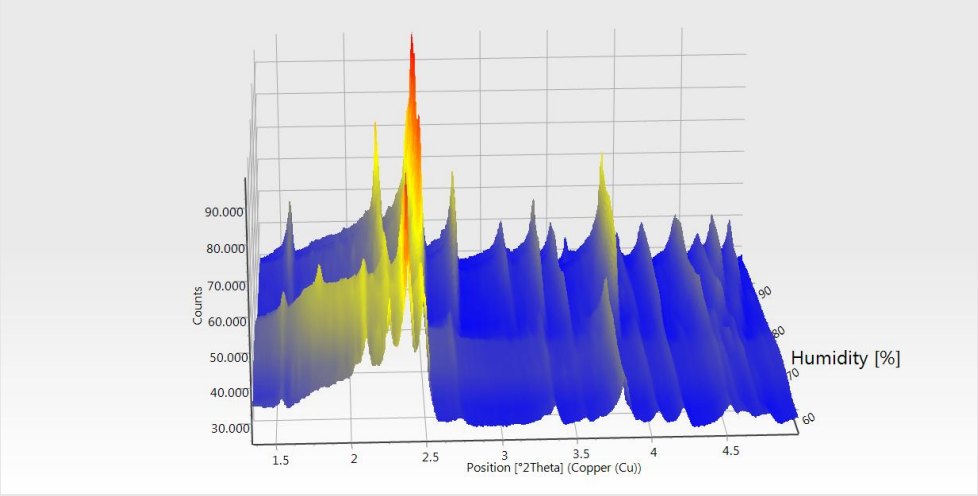
HEWL polymorphs	***a [Å]***	***b[Å]***	***c[Å]***	***α[°]***	***β[°]***	***γ[°]***
Tetragonal	77.21 -79.48	77.21 -79.48	36.7 -38.75	90	90	90
Monoclinic 1	25.32 -26.9	54.73 -58.95	30.68 -31.55	90	109.98 -112.2	90
Monoclinic 2	27.42-28.07	62.71-62.94	60.02-60.94	90	90.42-92.72	90
Orthorhombic	30.47 -30.58	55.39 -59.58	68.26 -68.85	90	90	90
Triclinic	27.07	31.25	33.76	87.98	108	112.11

HEWL *in situ* humidity study





HEWL *in situ* humidity study

Why?

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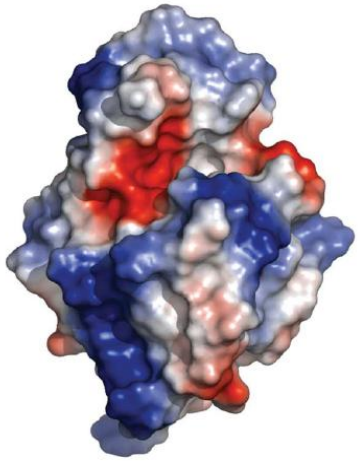
- Protein molecules in microcrystalline precipitates are surrounded by solvent and their packing arrangement is retained by limited intermolecular contacts.

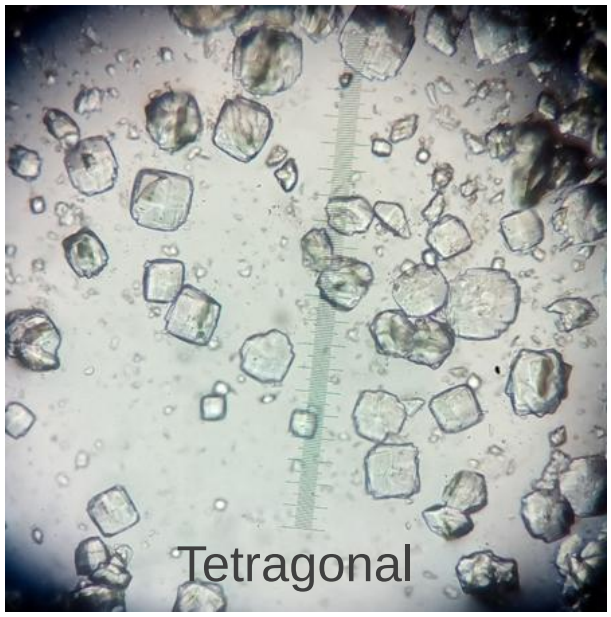
- A change in the crystal environment first affects the bulk solvent that fills the intermolecular space, with resulting changes in the crystal structure.

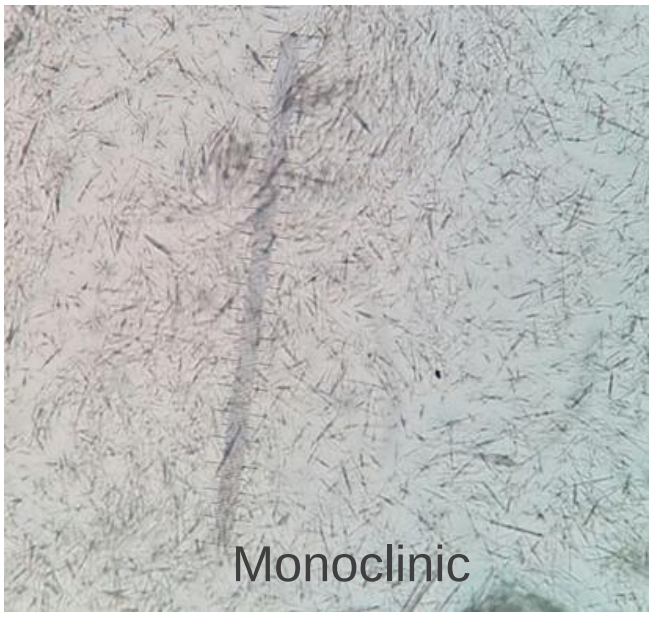
- Careful dehydration may cause large changes in unit-cell parameters and in some crystals, the dehydration induces a molecular arrangement change resulting in a new crystal structure.



HEWL *in situ* humidity study

- Tetragonal HEWL crystallized with PEG 4000
  - 50 mg/ml, pH 4.2
- Tetragonal HEWL crystallized without PEG 4000
  - 50 mg/ml, pH 4.2
- Monoclininc HEWL crystallized without PEG 4000
  - 70 mg/ml, pH 4.5





Sample pre-screening and purity

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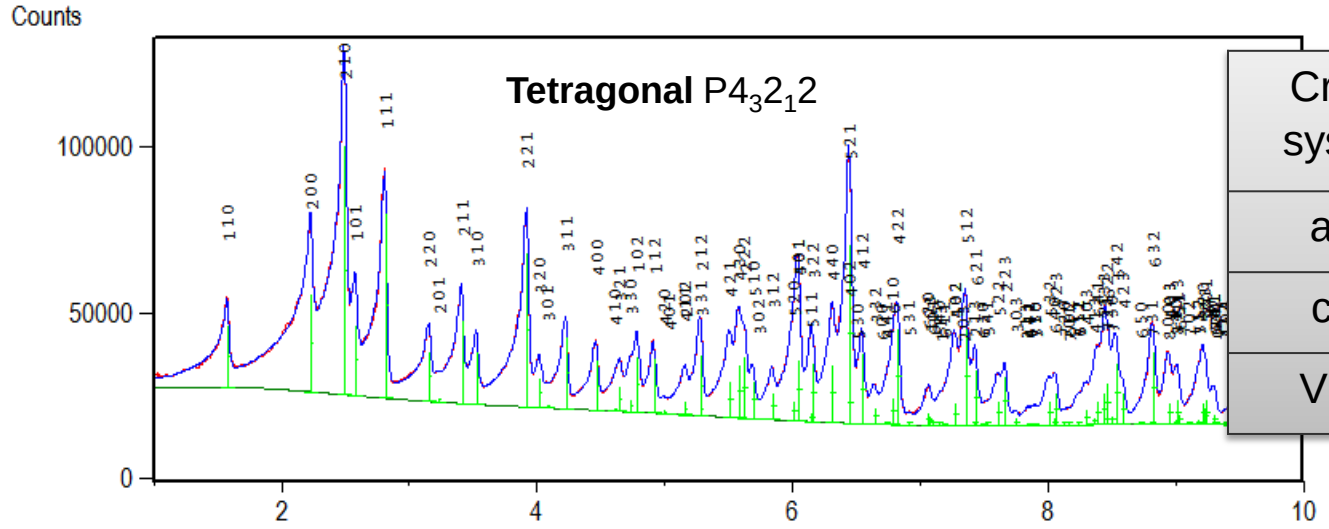
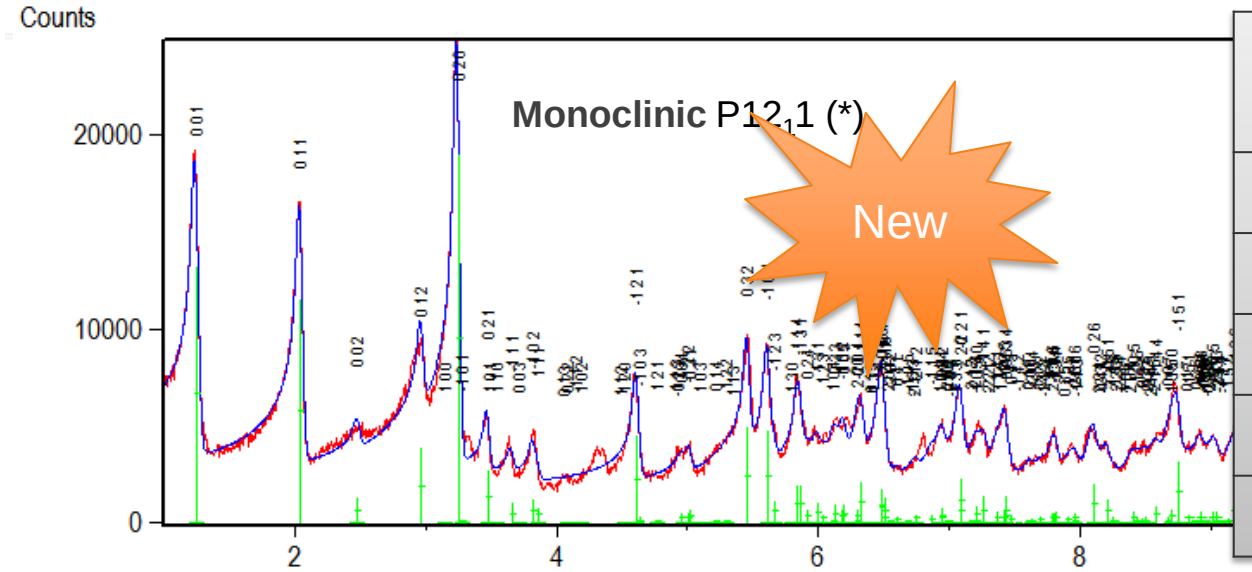


Crystal system:	Tetragonal
a (Å)	79.120(3)
c (Å)	37.935(1)
V (Å^3)	237474(6)



Crystal system:	Monoclinic
a (Å)	28.20(2)
b (Å)	54.50(1)
c (Å)	71.68(4)
β (º)	95.495(3)
V (Å^3)	109660(60)

Pawley fit within HighScore Plus

(*) Highest probability acc. ExtSym

HEWL *in situ* humidity study

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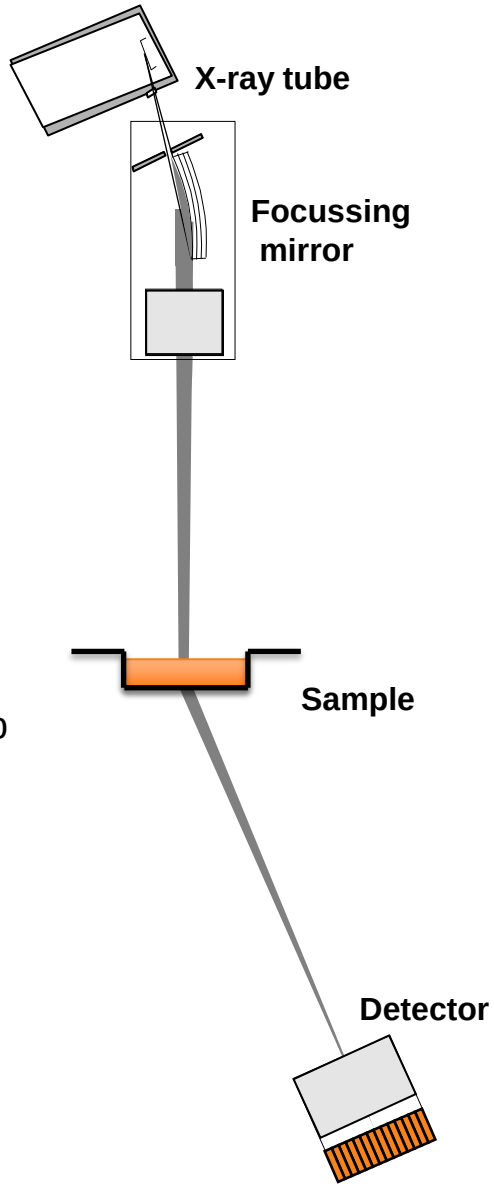
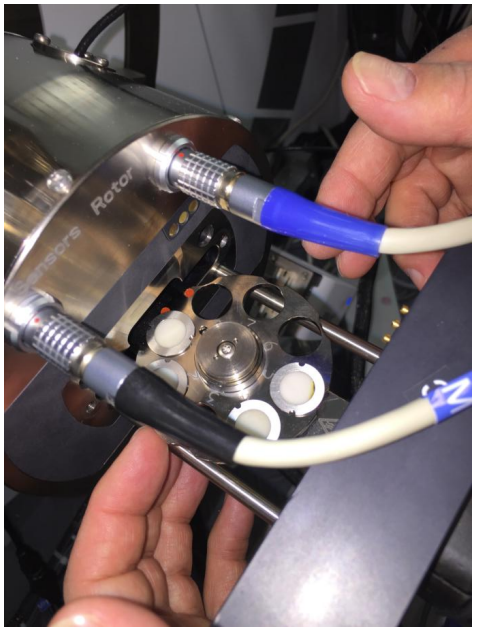
Sample preparation:

- Concentration in centrifuge
- Pipeting concentrate onto transmission holders (~100µl precipitate per sample)
- Multiple samples to reduce radiation damage
- Humidity variation: 95% → 51%





HEWL *in situ* humidity study

Tetragonal HEWL crystallized with PEG4000

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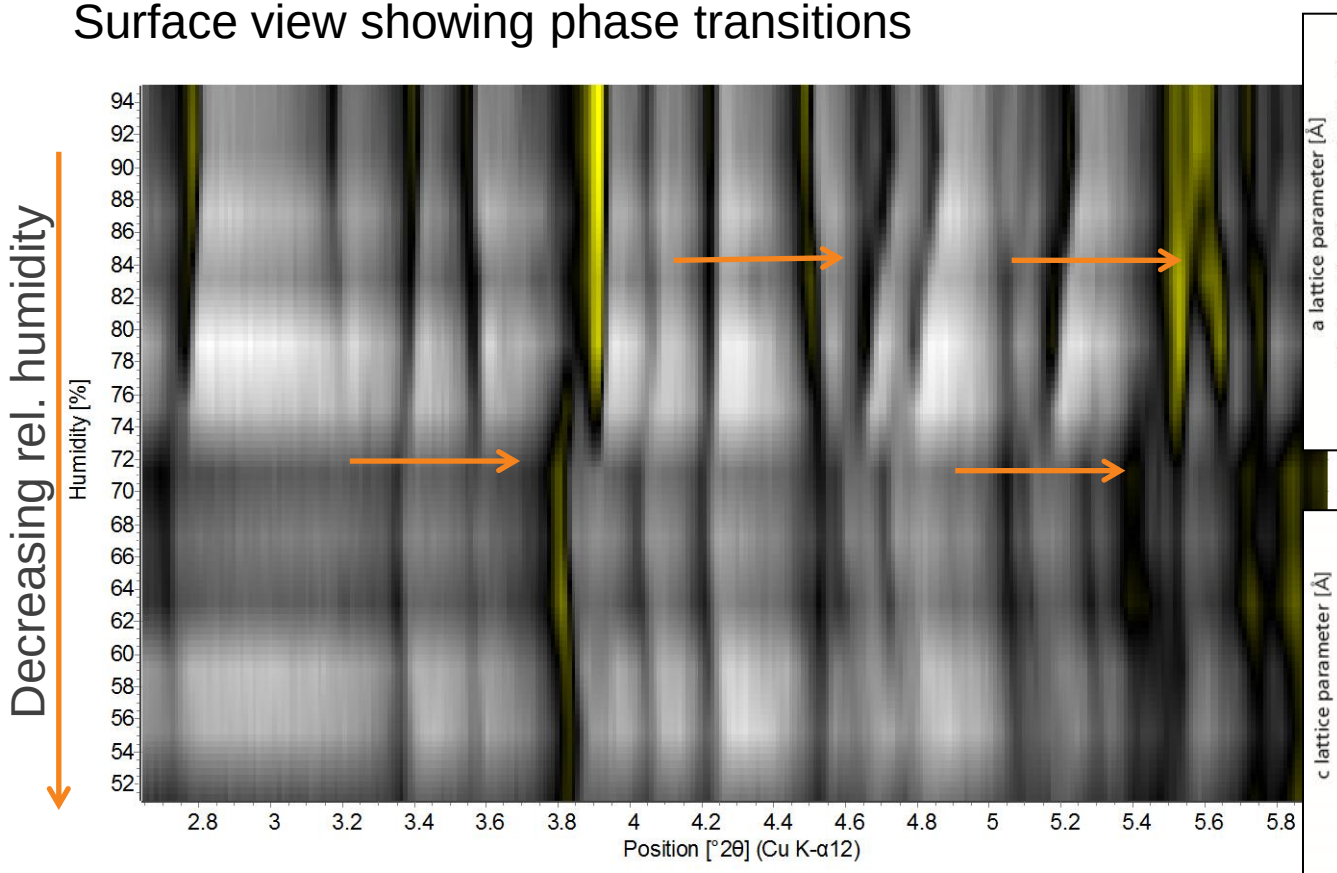
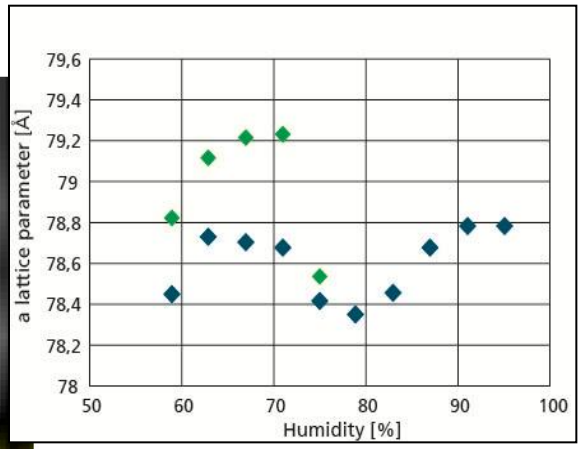
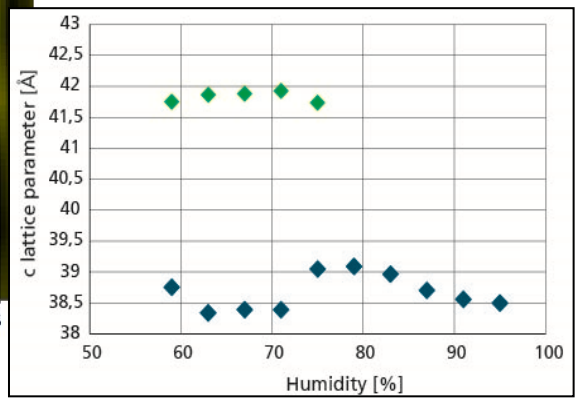
Surface view showing phase transitions







Cell parameter extracted by Pawley fit

HEWL *in situ* humidity study

Pawley fit - second tetragonal phase?

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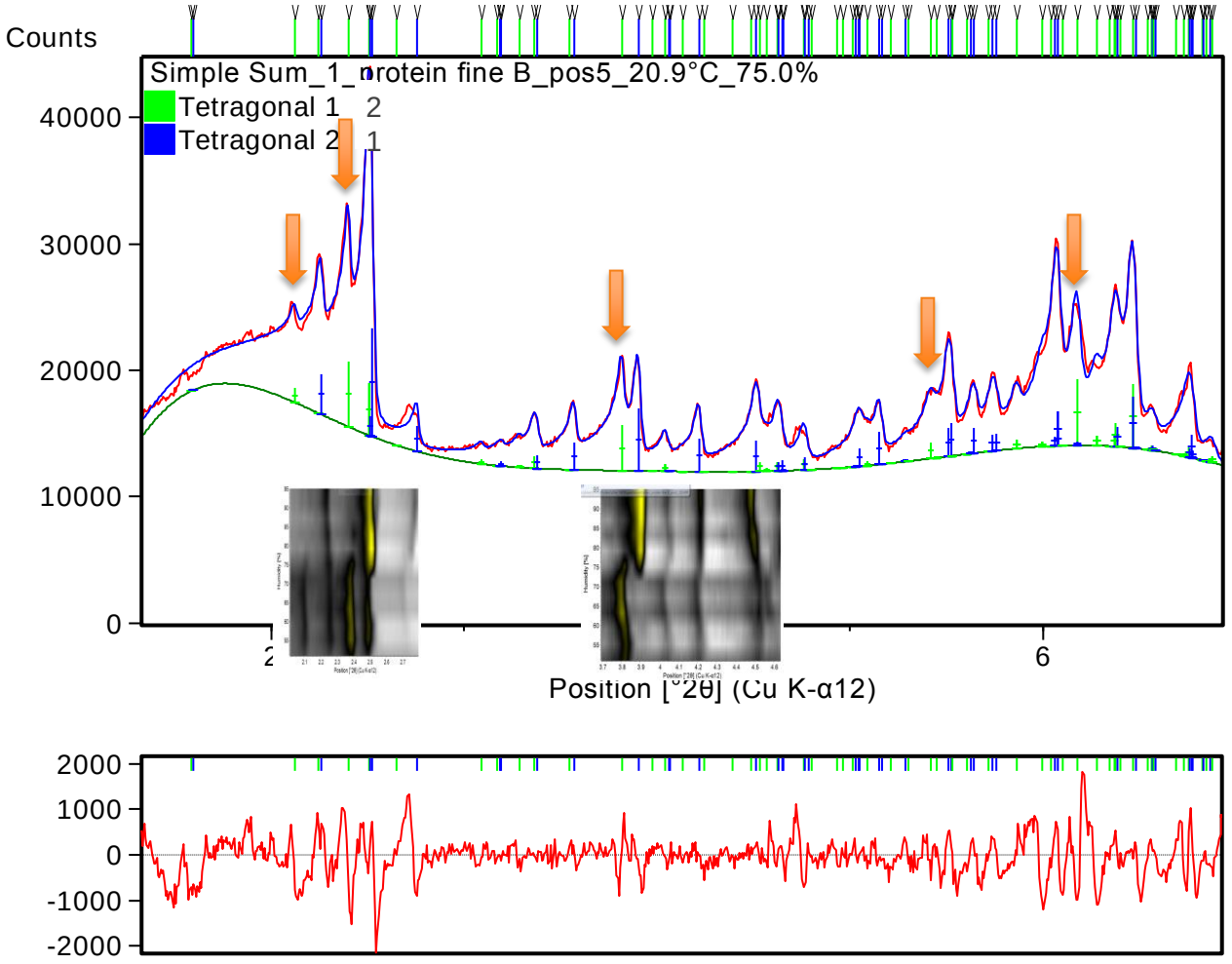

Phase 1

Unit Cell			
a [Å]	☑	78.22658	0.036155
b [Å]	☑	78.22658	0.036155
c [Å]	☑	39.31383	0.050408

Phase 2

Unit Cell			
a [Å]	☑	78.72074	0.033765
b [Å]	☑	78.72074	0.033765
c [Å]	☑	41.59305	0.052693

New

HEWL *in situ* humidity study

Tetragonal HEWL crystallized without PEG4000

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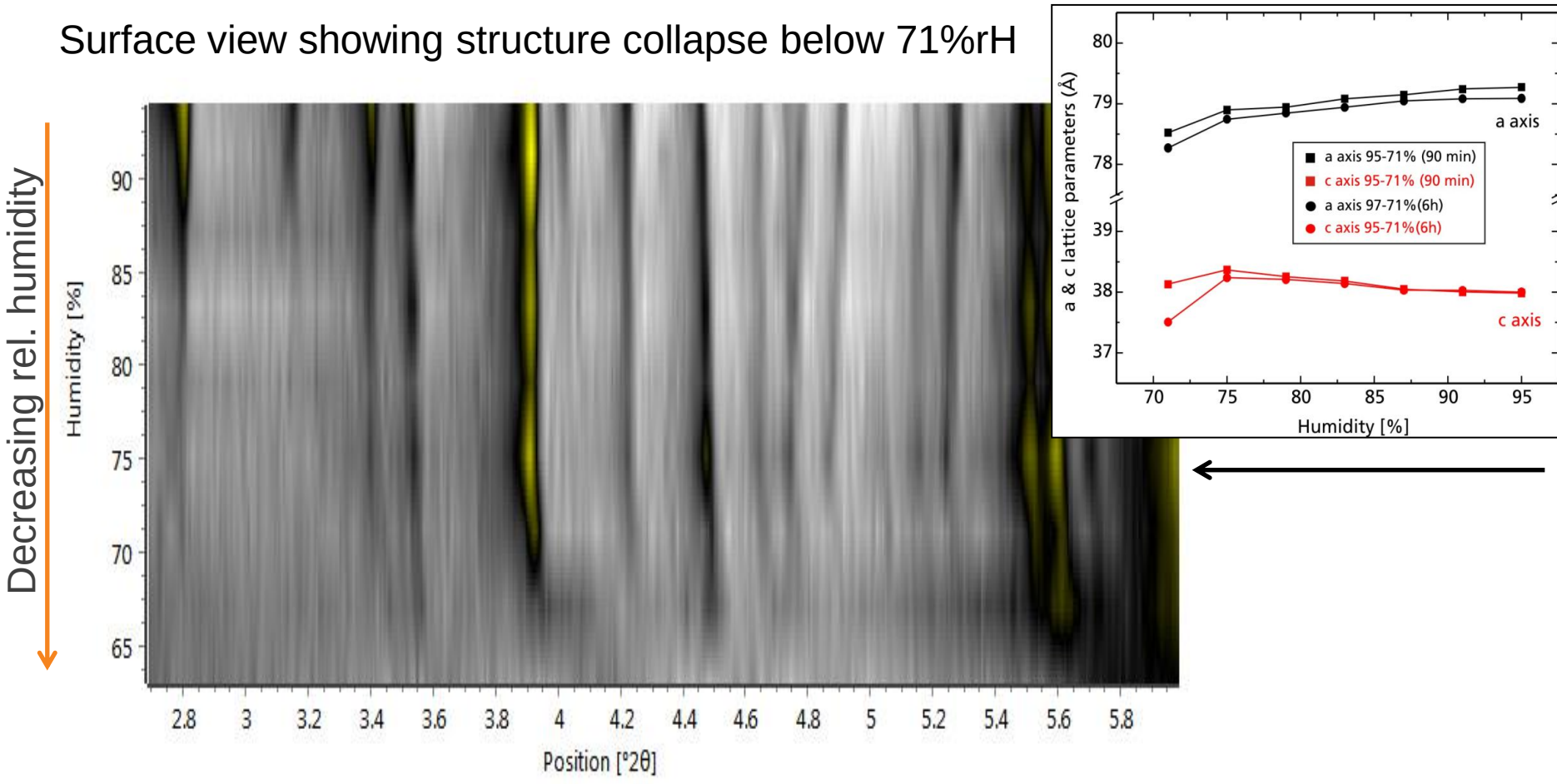
Surface view showing structure collapse below 71%rH



Cell parameter extracted by Pawley fits

HEWL *in situ* humidity study

Tetragonal HEWL crystallized without PEG4000

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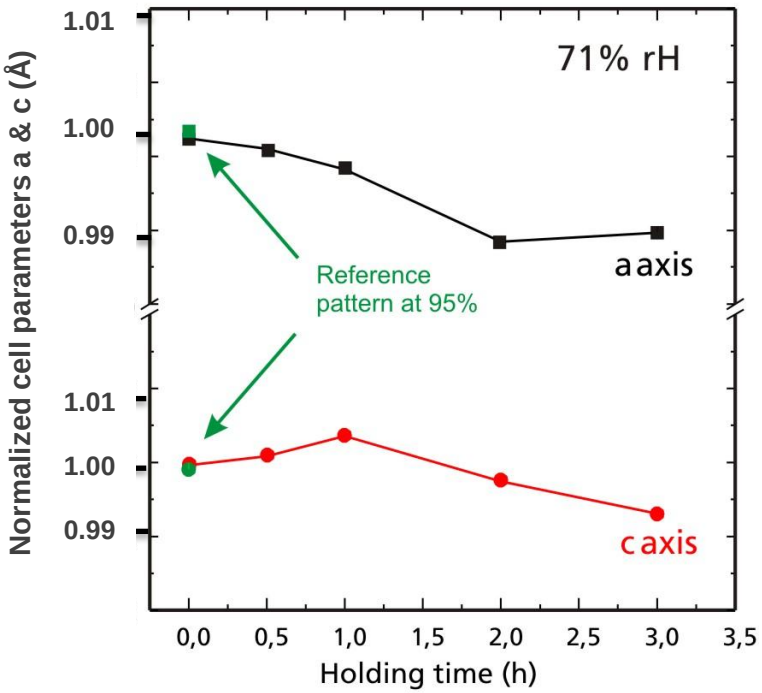
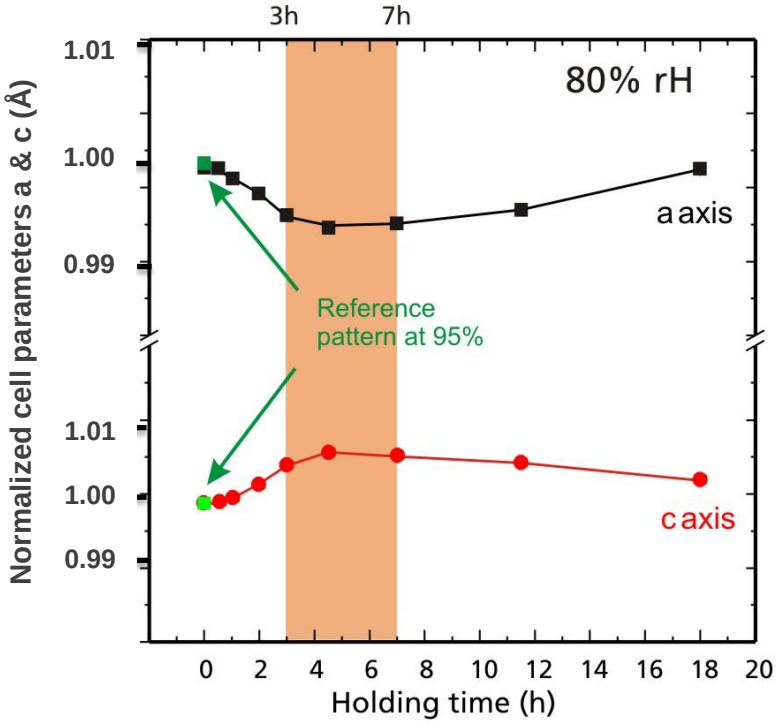
Fast reduction of humidity from 95% rH to 71% rH or 80% rH



Crystalline structure of the HEWL “collapsed” after ~4h (irreversibly)



Sample “survives” more than 24h. After 3h a first equilibrium is achieved, after 7h the sample “adapts” to the new humidity conditions and lattice dimensions move towards original values at high humidity

HEWL *in situ* humidity study

Monoclinic HEWL

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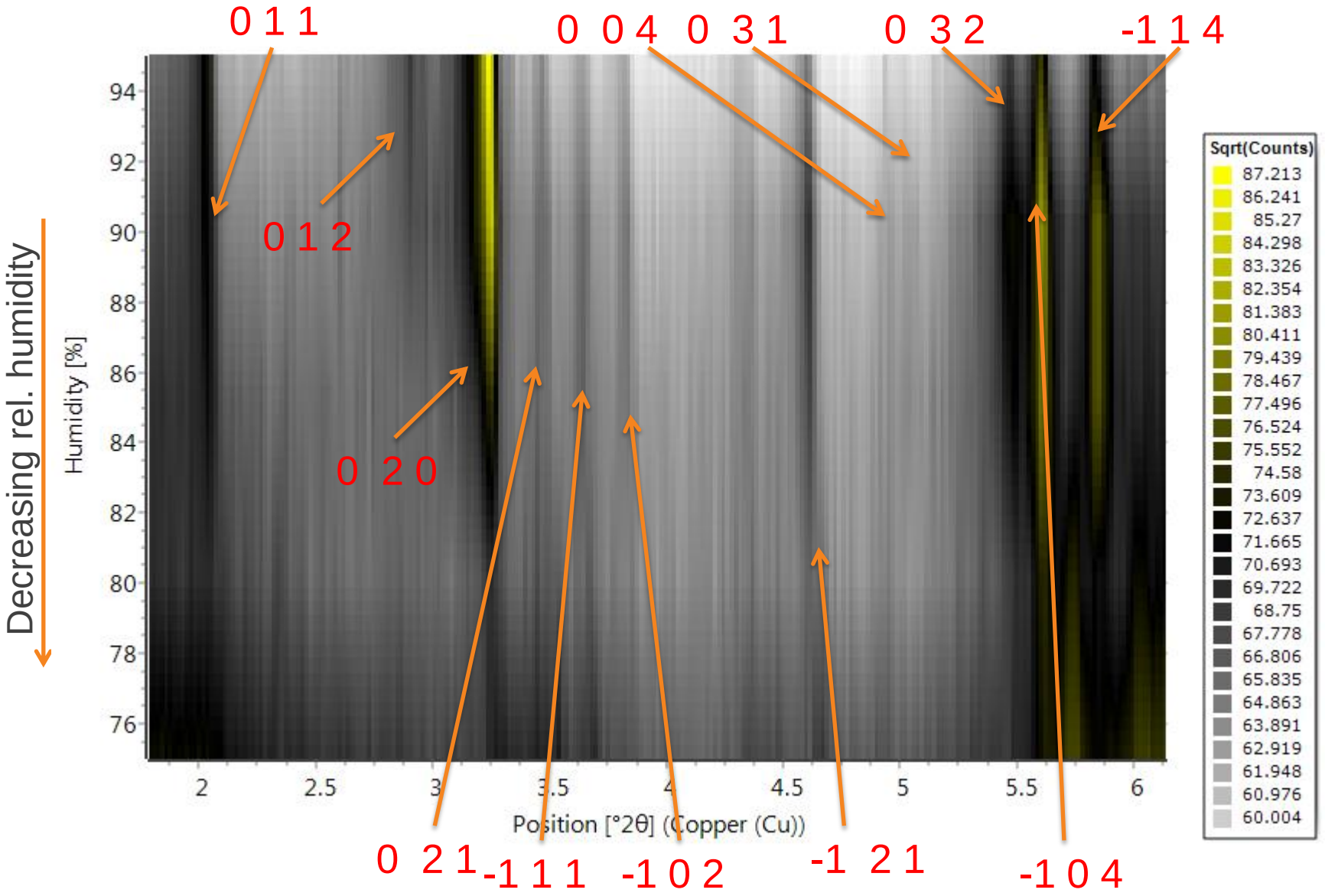
Surface view showing structure collapse below 75% rH



HEWL *in situ* humidity study

Monoclinic HEWL

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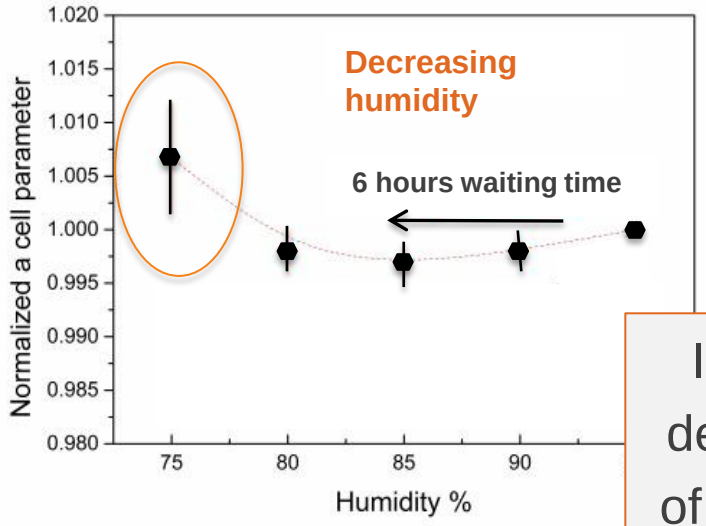
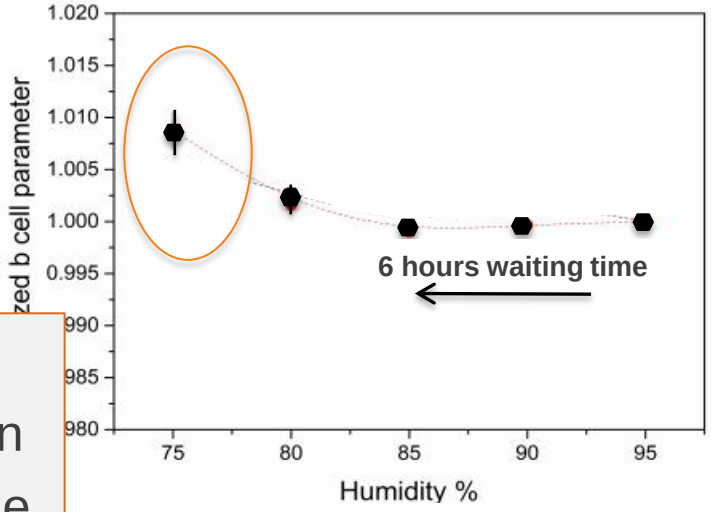
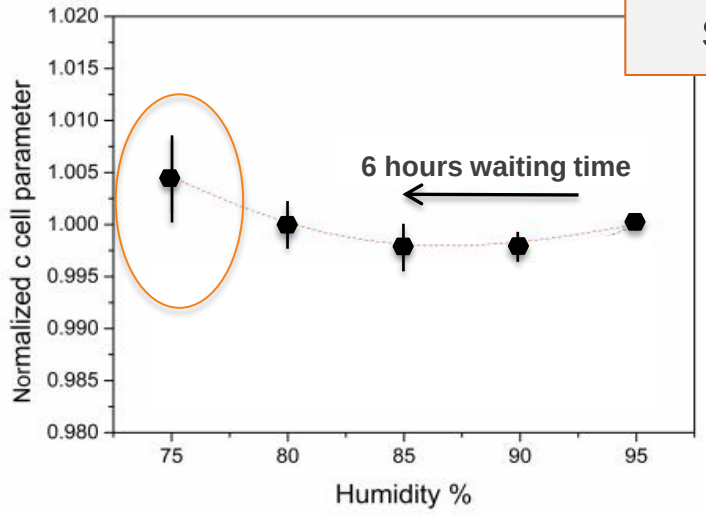
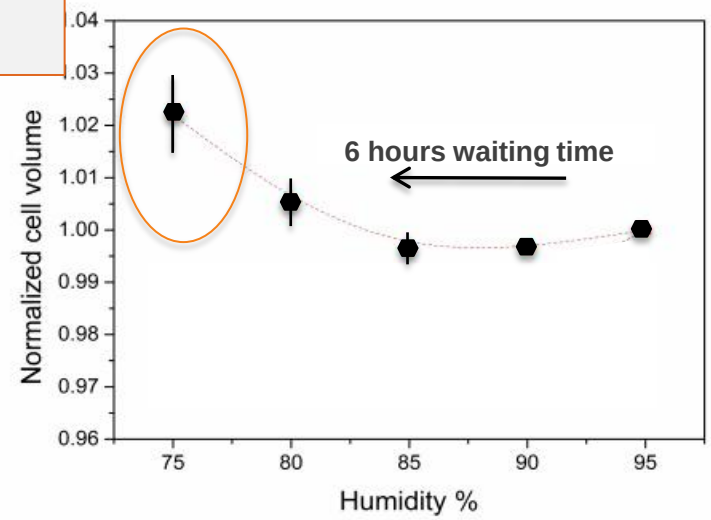
Cell parameter variation in step wise reduction of rH





Indicating degradation of crystalline structure





HEWL *in situ* humidity study

Monoclinic HEWL

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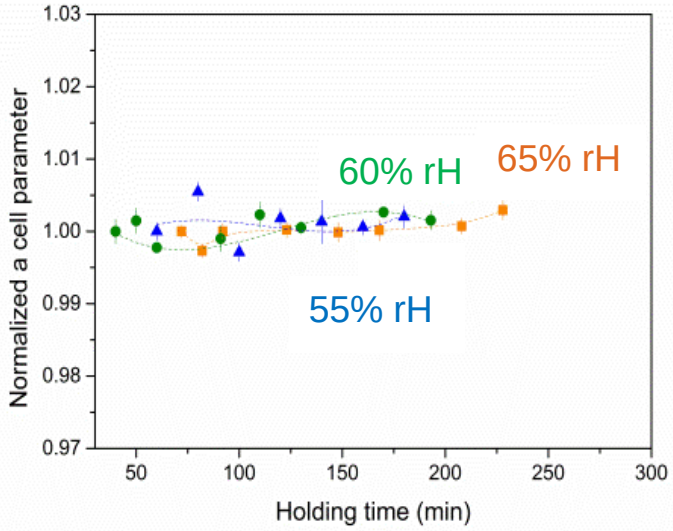
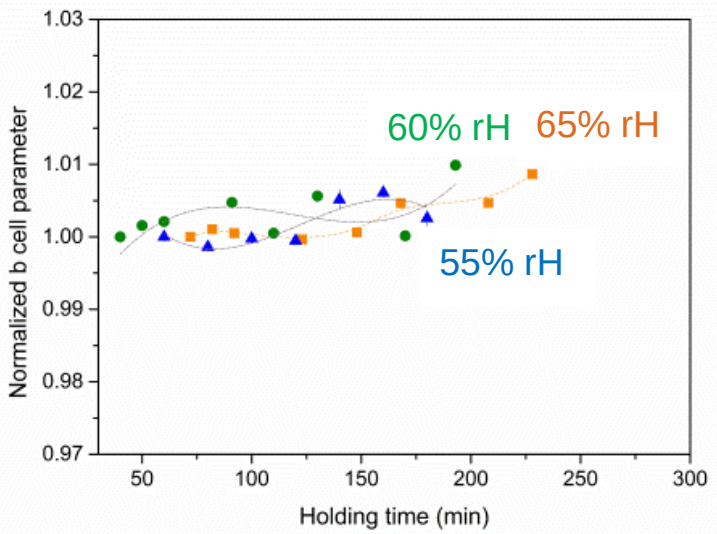
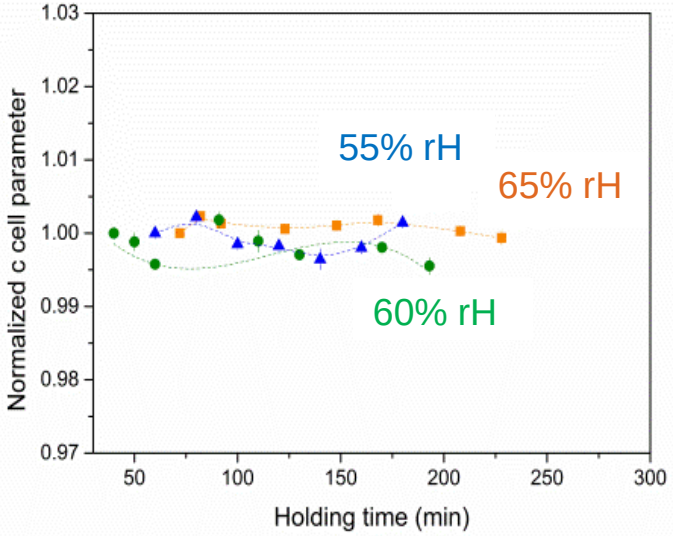
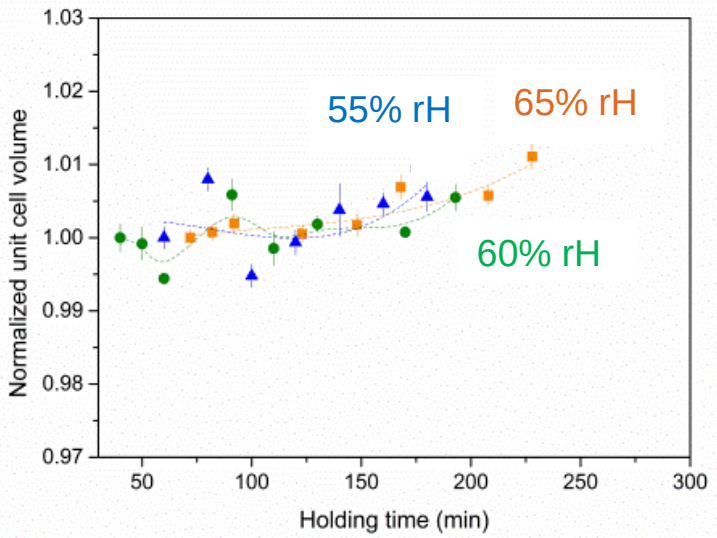
Cell parameter variation after transition to 65%rH, 60%rH, 55%rH









“Survival time” after fast humidity transitions

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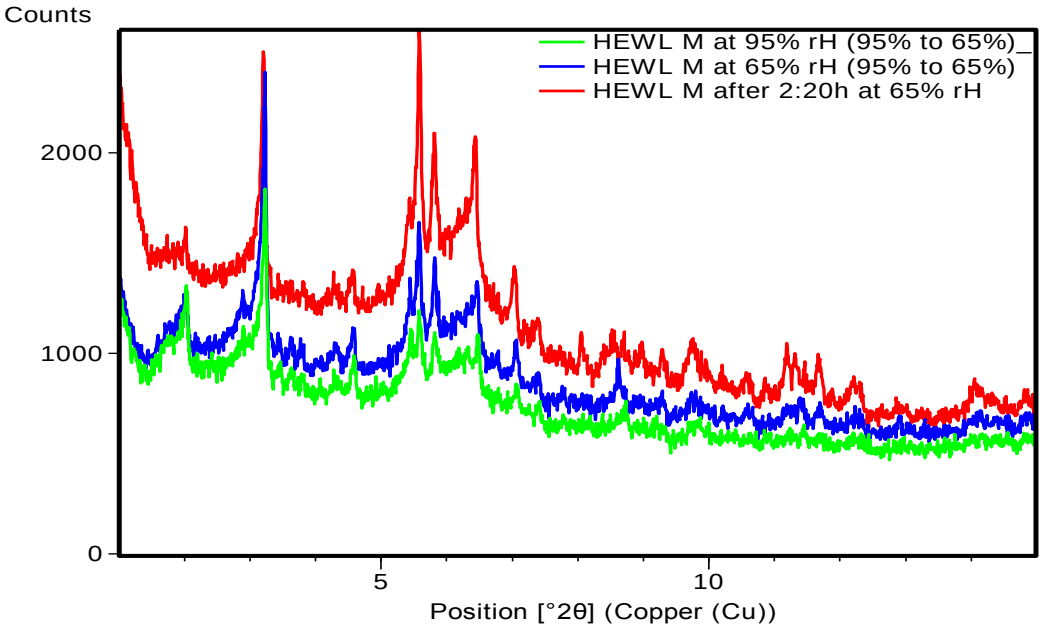
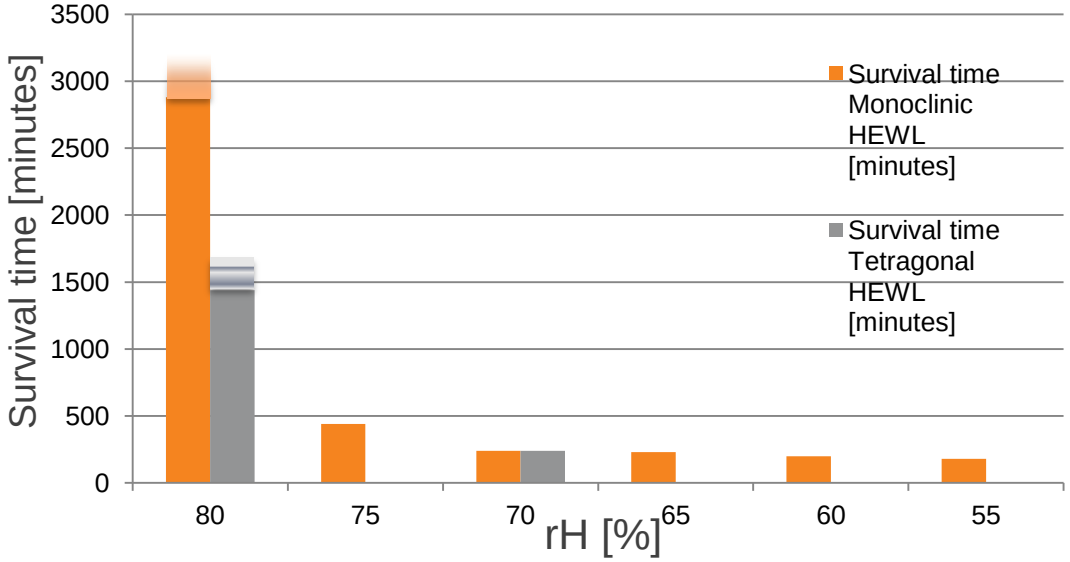
After fast humidity transitions the pattern quality was first improving (crystal growth?) before finally the degradation began.



Only for the Monoclinic HEWL phase we have a complete set of direct transitions from 95% rH to 80, 75, 70, 65, 60 and 55% rH.



Lysozyme polymorphs

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HEWL polymorphs	***a [Å]***	***b[Å]***	***c[Å]***	***α[°]***	***β[°]***	***γ[°]***
Tetragonal 1	77.21 -79.48	77.21 -79.48	36.7 -38.75	90	90	90
Tetragonal 2	**78.7**	**78.7**	**41.6**	90	90	90
Monoclinic 1	25.32 -26.9	54.73 -58.95	30.68 -31.55	90	109.98 -112.2	90
Monoclinic 2	27.42-28.07	62.71-62.94	60.02-60.94	90	90.42-92.72	90
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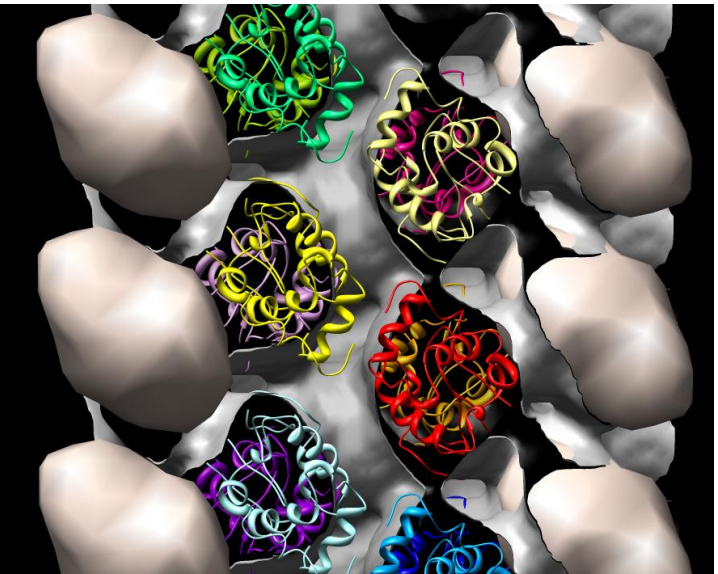
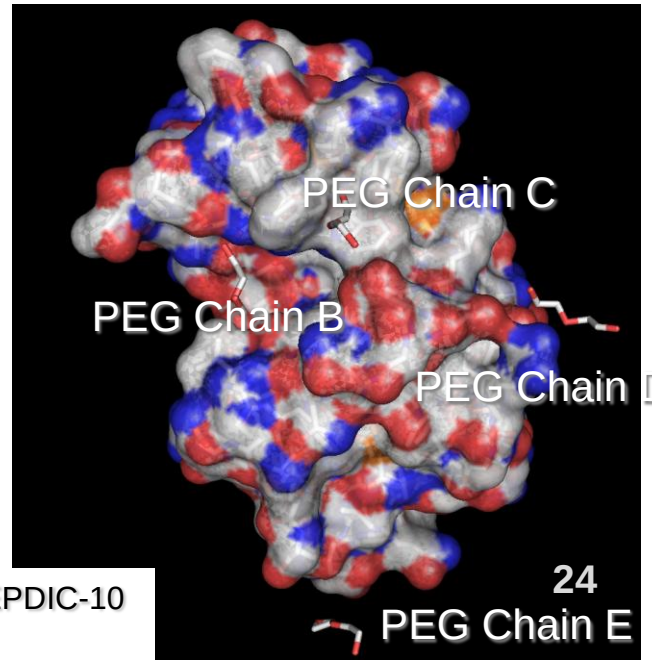
Orthorhombic	30.47 -30.58	55.39 -59.58	68.26 -68.85	90	90	90
Triclinic	27.07	31.25	33.76	87.98	108	112.11

Protein *in situ* humidity study

Conclusions

- Evidence for 2 new phases (tetragonal / monoclinic)
- Results are different from published single crystal data (e.g. Dobrianov et al., Acta Cryst. (2001), D57, 61-68)
  - Isolated single crystal vs. bulk average
  - Effect of the size of the crystal (influencing water diffusion)
- PEG partly replaces water in the solvent channels and can bind to the protein surface (creating intermolecular cross linking) (*) – causing structure stabilization below 75% rH.





(*) I. Margiolaki et al, EPDIC-10

Acknowledgement

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Sofia Trampari, National Kapodistrian University of Athens, Greece

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  - European Union (European Social Fund) in collaboration with the Greek State, under the “ARISTEIA II” Action (MIS Code 4659) of the “Operational Program Education And Lifelong Learning”
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  - EU FP7 REGPOT CT-2011- 285950 ‘SEEDRUG’ project
  - International Atomic Energy Agency (CRP code F12024)
  - COST Action (CM1306)

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