



## Panalytical presentation

### HUMIDITY INDUCED PHASE TRANSITIONS OF HEW LYSOZYME INVESTIGATED BY MICROCRYSTALLINE POWDER DIFFRACTION

D. Beckers<sup>1</sup>, G. Nénert<sup>1</sup>, T. Degen<sup>1</sup>, S. Saslis<sup>2</sup>, S. Logotheti<sup>2</sup>, F. Karavassili<sup>2</sup>, A. Valmas<sup>2</sup>, I. Margiolaki<sup>2</sup>, S. Trampari<sup>3</sup>

<sup>1</sup>PANalytical B.V., Almelo, The Netherlands,

<sup>2</sup>University of Patras, Greece,

<sup>3</sup>Kapodistrian University of Athens, Greece

Proteins often crystallize in microcrystalline precipitates. The protein molecules are then 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. In literature it is reported that protein crystals in controlled humidity environments show a large change in unit-cell parameters when the humidity is decreased [1-2]. When a protein crystal is carefully dehydrated, it is in a metastable state in which the crystal initially still retains the original packing structure [2]. Further dehydration may cause the collapse of the crystal lattice: the crystal no longer maintains its packing structure because of the loss of a large amount of bulk solvent. However in some crystals, the dehydration induces a molecular arrangement change resulting in a new crystal structure. This has been already reported for hen egg-white (HEW) lysozyme [3]. While dehydration can induce structural changes, this is also likely to happen upon hydration of the same crystals.

Here, we present our results of microcrystalline tetragonal and monoclinic HEWL samples on a laboratory X-ray powder diffractometer including in situ measurements under variable relative humidity conditions. The observed gradual structural changes as well as phase transitions upon dehydration and hydration of HEWL are analyzed in the relative humidity range 50% - 95%. (Figure 1.)

Dehydration and hydration processes are reversible in humidity cycles in the range of 95% rH to 75% rH. Without stabilizing PEG the lower limit for dehydration of tetragonal HEWL is around 75% rH. With PEG the tetragonal HEWL samples remain crystalline below 75% rH, but show phase transitions and larger variations of the cell parameters. Below 75% rH a new tetragonal polymorph was discovered.

1. Kiefersauer, R., et al. (2000). *J. Appl. Cryst.* 33, 1223–1230.
2. Dobrianov, I., et al. (1999). *J. Cryst. Growth*, 196, 511–523.
3. K. Harata, T. Akiba, *Acta Cryst.* (2007). D63, 1016–1021.

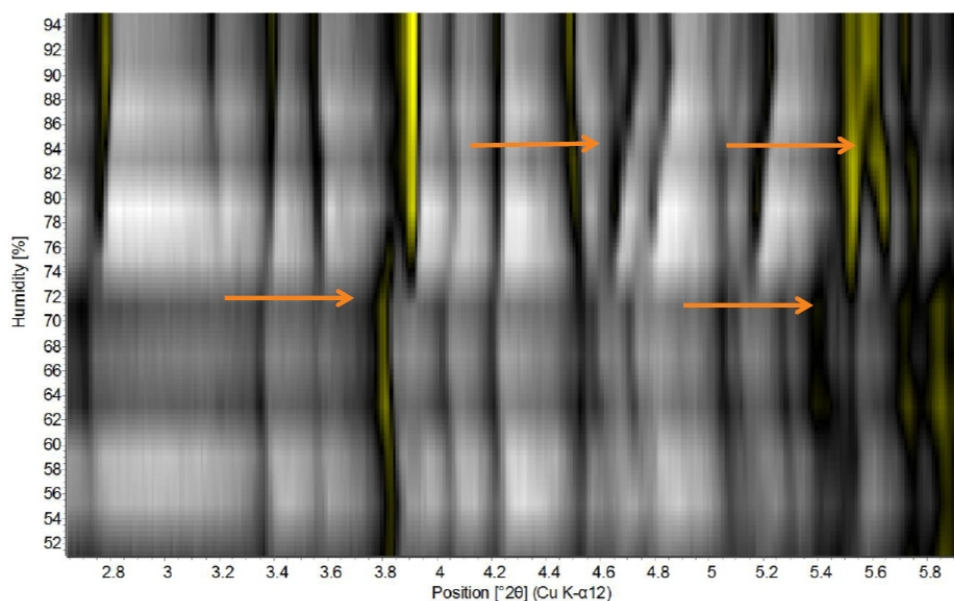


Figure 1. Phase transitions under variable humidity