

In-situ Observation of Elementary Growth Steps on a Protein Crystal, Surface Diffusion of Protein Molecules and Dislocations inside a Protein Crystal

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To elucidate the mechanisms of defect formation in protein crystals, one has to observe in-situ 1) behaviors of individual protein molecules in the vicinity of crystal surface, 2) consequent movements of elementary growth steps, and 3) defects formed in a crystal.

For the process 1), we have applied a single molecule imaging technique, which is popular in the field of biological physics. Using fluorescence labeled lysozyme molecules, we have succeeded in observing individual lysozyme molecules diffusing in the vicinity of tetragonal lysozyme crystals in situ, for the first time. We found that the diffusivity of lysozyme molecules close to the crystal surface is 4 orders of magnitude smaller than that in a bulk solution.

For the process 2), we have combined laser confocal microscopy (LCM) with differential interference contrast microscopy (DIM), and succeeded in observing elementary growth steps (5.6 nm in height) non-destructively [1]. Using this LCM-DIM system, now we are observing bunching processes of elementary growth steps.

For the process 3), we have applied LCM-DIM and phase contrast microscopy, and succeeded in observing dislocations normal to an incident light and inclusions in-situ during growth.

[1] Sasaki G., et al., *J. Crystal Growth*, 2004, **262**, 536.

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