[MS39-04] A mounting method for fragile protein crystals using humid air and glue coating. Takashi Kumasaka, a Seiki Babaa

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Protein crystals are fragile, and it is often laborious to find a condition suitable for handling before conducting X-ray diffraction experiments. In the conventional treatment for cryopreservation of protein crystals, addition of permeable cryoprotectant agents to crystal mother liquor frequently damages the crystals. To address this issue, we developed a new crystal mounting method, the humid air and glue-coating (HAG) mounting method, which involves a combination of controlled humid air and water-soluble polymer glue for crystal coating. By adjusting the sample moisture with exposure to the controlled humid air, most protein crystals coated with the glue were stable at room temperature and were able to be cryocooled under optimized humidity. We successfully applied this method to several protein samples including membrane proteins. In particular, this method was useful when applied to fragile protein crystals known to be sensitive to a subtle change of physicochemical environment. For example, a new crystal form of the bacterial α/β-hydrolase RsbQ was very fragile physically in comparison with the previous form [1], that is, it damaged immediately by soaking with its mother liquor adding any types of cryoprotectants or it degraded gradually by the crystallization droplet exposed to atmosphere. By using the HAG method, we were able to obtain 1.4 Å data from the crystal cryocooled at 69.1 % relative humidity and solve its structure. This effect is attributed to moisture buffering and slow diffusion as characteristics of the polymer glue. Moreover, the glue-coating improved experimental stability and reproducibility in crystal dehydration by humidity change [2, 3]. The changes of the lattice constants in tetragonal lysozyme crystals were reproducible, reversible and gradual in humidity change, but these amounts were smaller than those in the previous experiments without coating [2, 3] and rather similar to the result of the soaking experiment [4]. The results suggest that this method can gently control the lattice constants of protein crystals, thus it will also be useful to prepare a series of isomorphic crystals for multi-crystal data collection at brilliant synchrotron or XFEL experiments.

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