[MS39-P06] Controlled Environment on Data Quality

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Sulphur Single-wavelength Anomalous Diffraction (S-SAD) phasing has become more common place since its first use in 1981 by Hendrickson et al. to solve the structure of crambin [1]. However, phasing using native sulphur is still not routine, with less than 100 novel structures determined since 1981, due to the small anomalous signal produced by sulphur atoms [2, 3]. By using longer wavelength X-rays it is possible to amplify the anomalous signal for sulphur, at the cost of increased X-ray absorption in the sample and mother liquor. This effect can be minimised by removing the excess mother liquor surrounding the sample.

The HC1b setup [4] provides an air stream with a precise relative humidity to allow sample manipulation without reducing diffraction quality. Once the relative humidity of the crystal and mother liquor has been determined the crystal can remain in the air stream indefinitely. This environment has been utilised to test the effects on data quality when transferring protein crystals from a standard nylon loop to kapton sample holders, removing excess mother liquor in the process. This protocol will allow the use of dedicated sample holders optimised for use in long wavelength macromolecular crystallography (MX) that are too fragile for direct fishing. Using micro-manipulators the mother liquor in the nylon loop is brought into contact with the kapton mount from above, excess mother liquor is wicked away from below leaving the crystal to rest on the kapton mount. This provides an alternative to the capillary-top mounted protocol proposed by Kitago et al. (2010) [2].

To test the effects of sample transfer under humidity controlled conditions, three established tests crystal systems were used. The relative humidities of the crystals were determined as defined in Wheeler et al. (2011) [5]. A series of in total 31 lysozyme, 16 ferritin and 26 thaumatin crystals were transferred as described above and flash cooled in liquid nitrogen. 17 controls of each crystal system were prepared by direct fishing and cooling to compare data quality.

Initial data quality was assessed through merging statistics provided by the automated Xia2 pipeline at Diamond Light Source. Average values for overall and outer shell $I/\sigma(I)$, R_{merge}, R_{meas}, $R_{p.i.m}$ and maximum resolution ranges were calculated. Samples subjected to humidity controlled transfer and control samples were found to have similar average values for these parameters within one standard deviation of each other. A two-sample T-test for unpaired data, assuming equal variance, shows that the population means are the same within a 95% confidence interval. This suggests that the manual handling of lysozyme, ferritin and thaumatin crystals under humidity controlled conditions is not detrimental to crystal data quality. Manual processing of these datasets using XDS and more in-depth analysis of data quality indicators is underway. Further experiments are also planned using other proteins and crystals.

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