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**Supporting information for article:**

**A temperature-controlled cold gas humidifier and its application to protein crystals with the HAG method**

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## Supporting information

In the previous experiment (PDB ID 3AG1), CcO crystals were packaged into a glass capillary tube in a 4°C cold room. To confirm the effect of temperature, A CcO crystal was manipulated using the TeC-W workbench at 4°C and maintained in 98%RH at RT using the original humidifier (Baba *et al.*, 2013) during the diffraction experiment. The maximum resolution of the first image was 2.6 Å (Fig. S1a). However, after 10 min, the resolution decreased to 3.5 Å (Fig. S1b). This result suggested that CcO crystal at RT have decreased quality.

**Figure S1** Diffraction images of CcO crystal using the HAG method at RT and 98%RH. The CcO crystal was manipulated at 4°C using the TeC-W workbench. (a) The first diffraction image immediately taken after mounting on a diffractometer. (b) The second diffraction image after 10 min. CcO, cytochrome c oxidase; HAG, humid-air and glue-coating; RT, room temperature; RH, relative humidity.

