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Supplemental information

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An automated platform for structural analysis of membrane proteins through serial crystallography

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Figure S1:

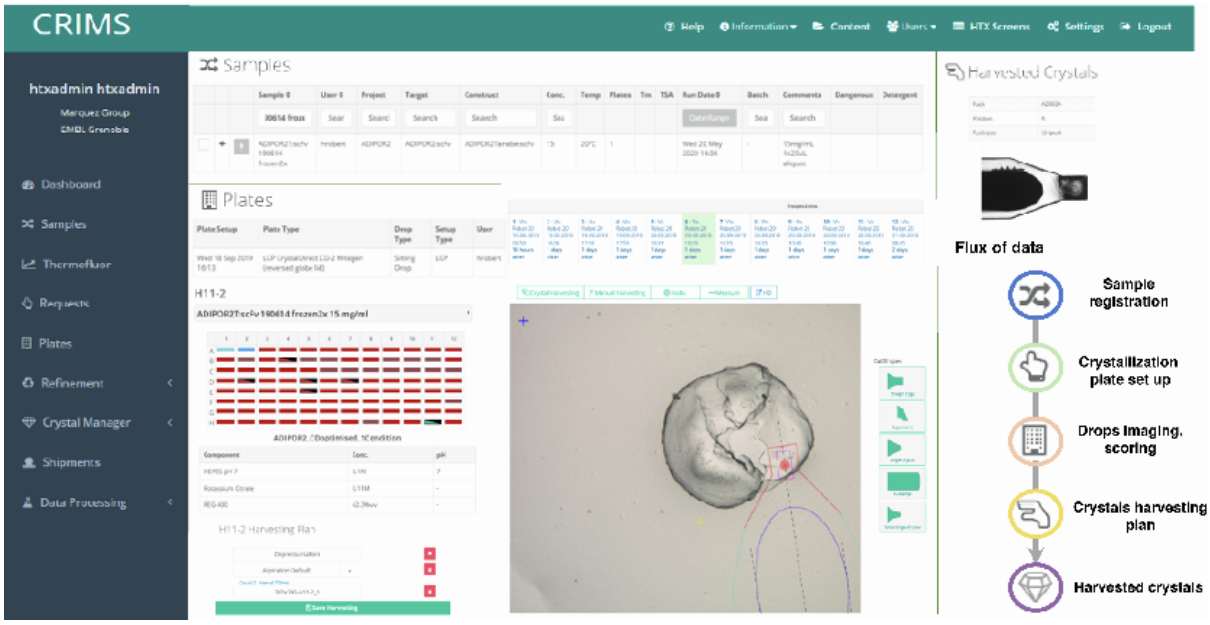

Figure S1. The Crystallographic Information Management System (CRIMS, related to Figure 3) provides automated sample tracking and data management over the whole experimental workflow as illustrated in the right panel. The main panel shows the crystal harvesting interface with a harvesting plan for well H11 of a CrystalDirect plate containing ADIPOR2 crystals.

Figure S2:

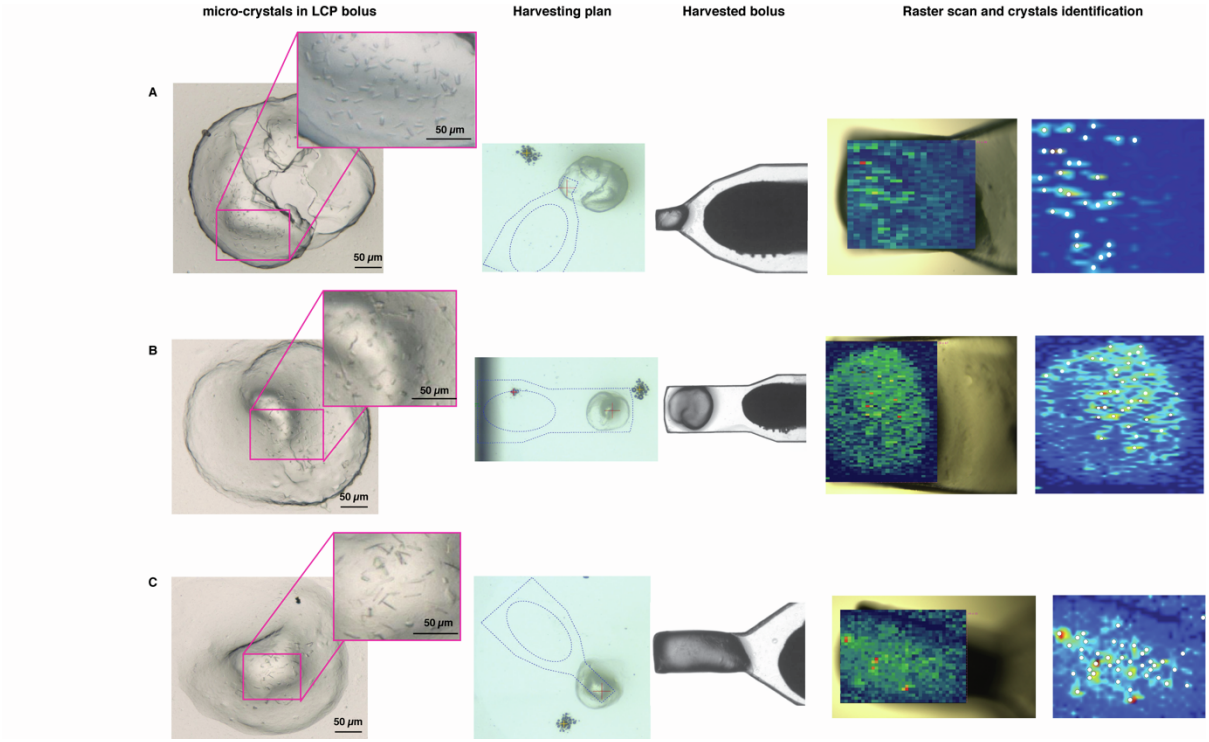

Figure S2: Automated harvesting and rastering of LCP crystals, related to Figures 1 and 4. Crystals grown in mesophase using a CrystalDirect plate. The panels (from left to right) illustrate different steps of the process: First column shows the microcrystals of different membrane proteins in LCP bolus; second column shows automated harvesting plan designed in CRIMS; third column shows samples harvested and cryo-cooled with the CrystalDirect robot; and final column shows the samples at the beam line with the result of the X-ray raster scan superposed at cryogenic temperature. Three representative samples – ADIPOR2, ACER3, and a GPCR target (typical diffraction limit of 4.0 Å) for screening processes involving harvesting of the full bolus or parts of it are shown in **A–C**, respectively.

Figure S3:

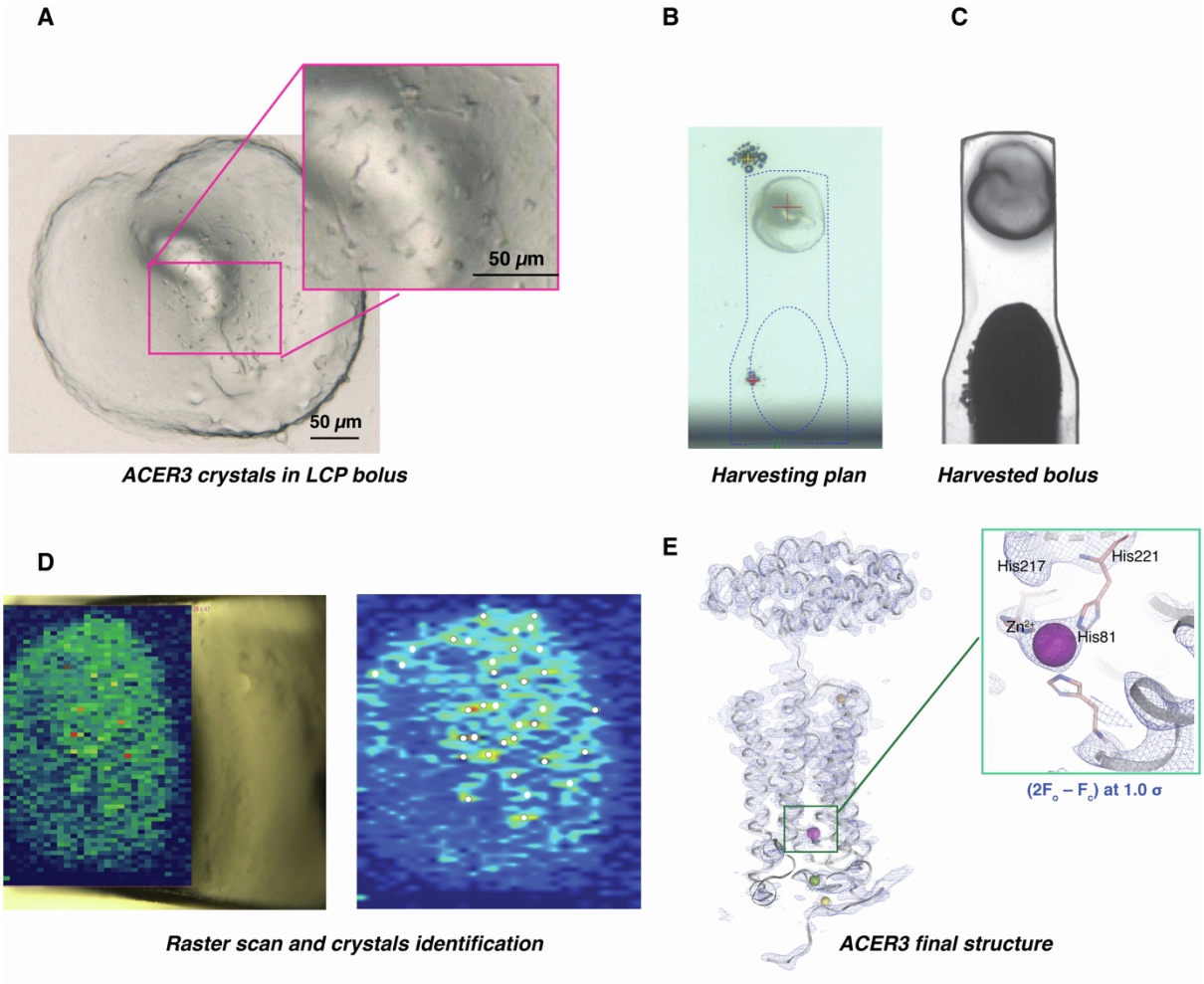

Figure S3. ACER3 structure determination on CrystalDirect harvested micro-crystals, related to Figures 4 and 5. (**A**) ACER3 micro-crystals. (**B**) Image from CRIMS of crystal harvesting plan and (**C**) image from CrystalDirect harvester of harvested crystals in the cryo-steam. (**D**) X-ray diffraction heat map and crystal picking for miniset collection at beamline X06SA (PXI, Swiss Light Source). (**E**) Refined structure and electron density map of ACER3 in this work (PDB: 6YXH).

Figure S4:

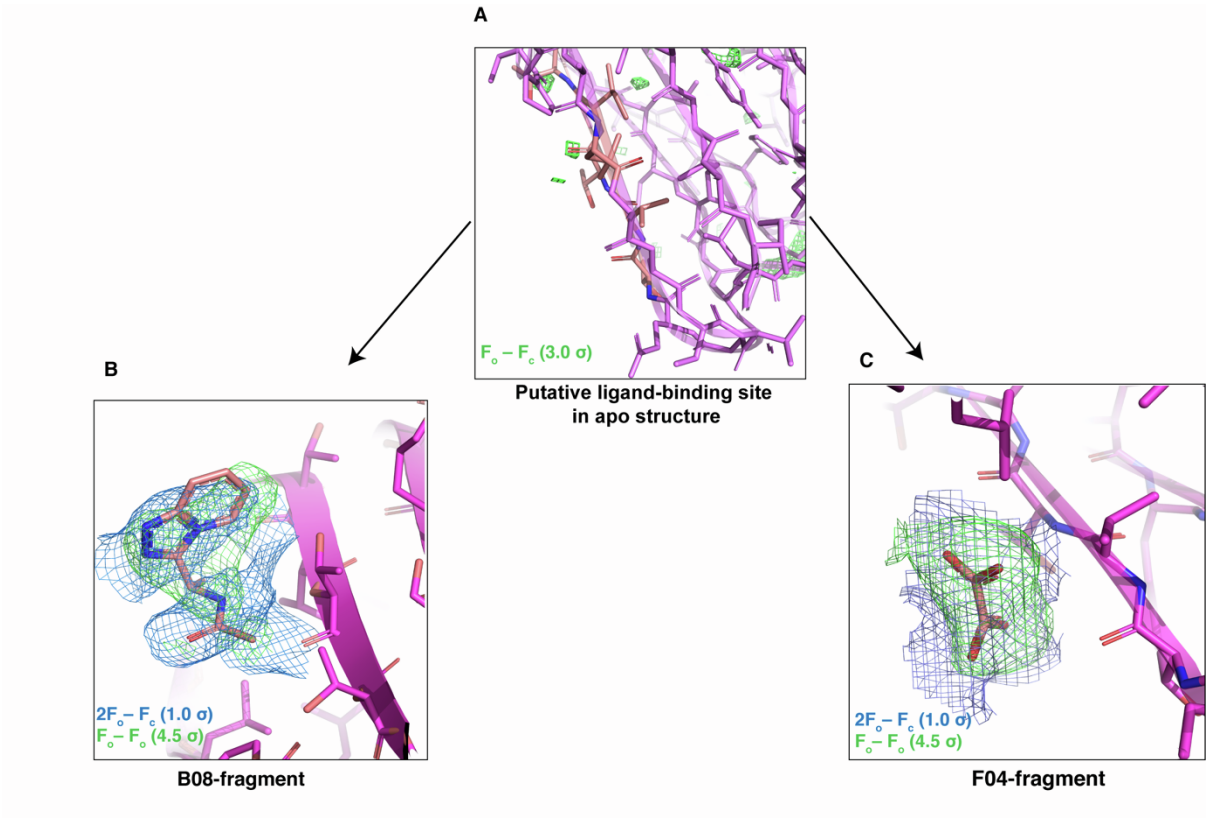

Figure S4. Electron density data for fragment-soaked ADIPOR2 crystals, related to Figure 5. Fo–Fc difference map (green) contoured at 3 sigma (**A**) unsoaked ADIPOR2 crystals. 2Fo–Fc in blue at 1.0 sigma and Fo–Fo in green at 4.5 sigma for soaked fragments B08 and F04 respectively, depicted as stick models (**B**, **C**).

Figure S5:

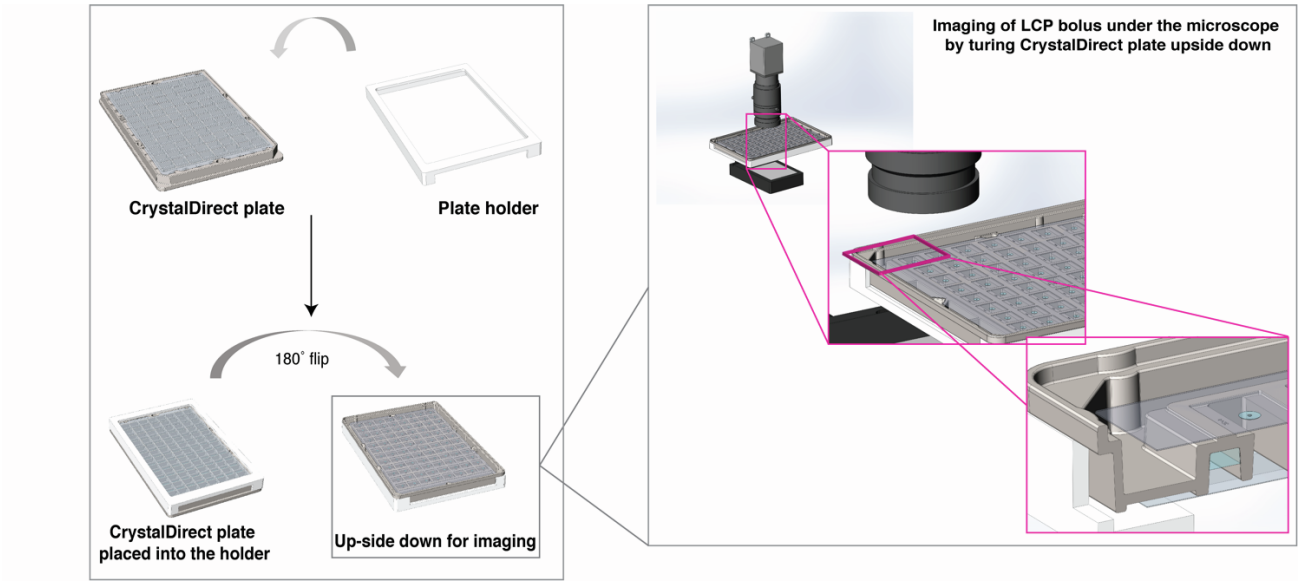

Figure S5. 96-well plate adaptor enabling optimal imaging of LCP experiments with commercial imaging robots, related to the STAR Methods section. Most commercial imaging systems image crystallization plates from the top. However, the top side of the LCP bolus in CrystalDirect plates tends to present a rough structure, which may interfere with crystal imaging. To overcome this and facilitate crystal detection a plate adaptor (plate holder) was developed enabling an inverted configuration during imaging. This adaptor consists of a plastic frame compatible with the SBS-plate format into which the crystallization plate can be inserted (**left panel**) and turned upside-down (crystallization film facing up and towards the camera) whilst guaranteeing safe handling by the imaging robot (**right panel**). Thanks to this adaptor, crystallization experiments are imaged through the film in which the LCP bolus is resting, therefore always presenting a smooth and flat surface to the camera, which notably improves crystal imaging.

Figure S6:

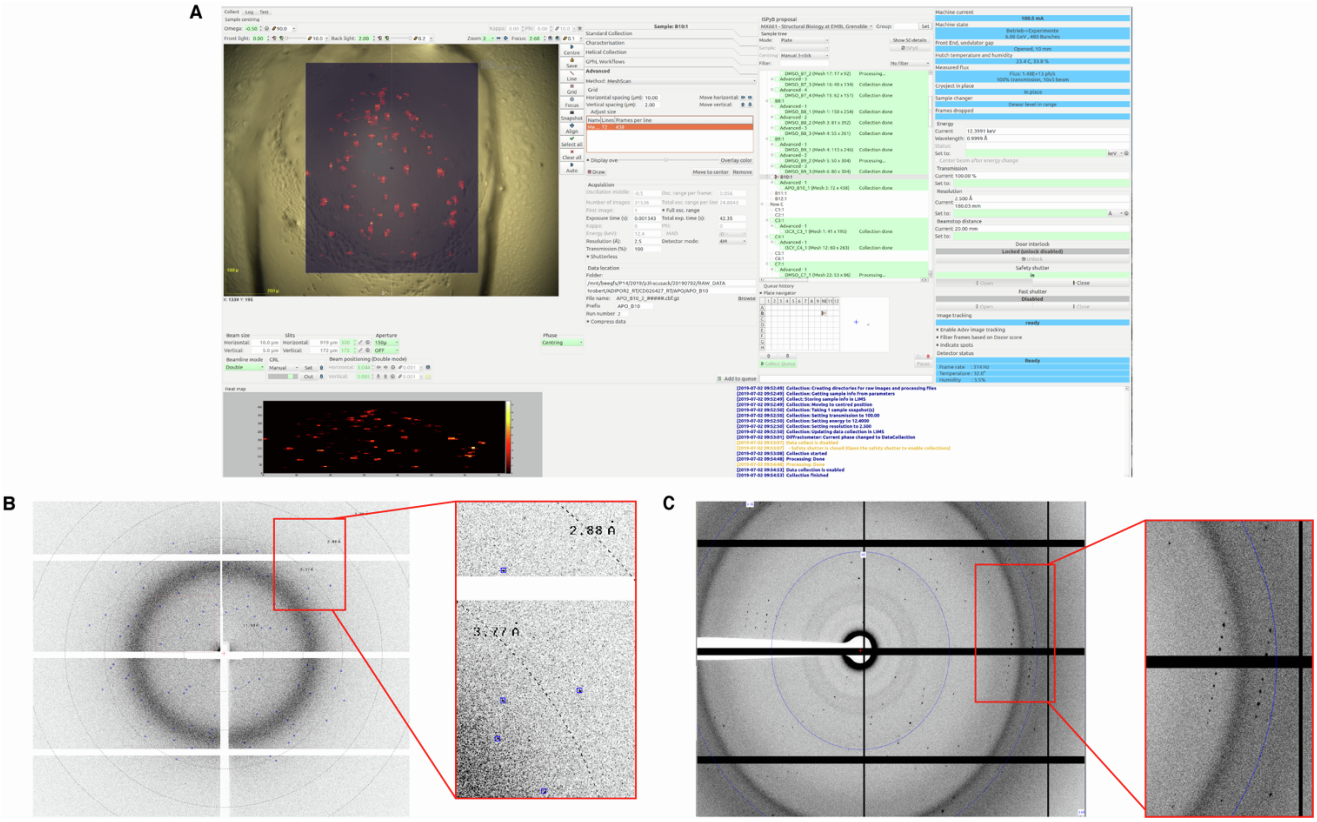

Figure S6. (A) MXCUBE2 data collection GUI at the P14 beamline in PetraIII, Hamburg, Germany, elated to the STAR Methods section. Related to Figures 1 and 5 and Table 1. The GUI shows the room temperature data collection, with raster scan (top hit-map) performed as helical-line scan (See, **STAR Methods**) and the on-the-fly hit finding on the data stream is shown in the bottom 2D-plot. Example of diffraction patterns from the Room Temperature **(B)** and Cryogenic **(C)** data collection on ADIPOR2 microcrystals.