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Why I hate icy conditions···dynamic structural biology of the SARSCoV- 2 Papain-Like protease from room temperature studies

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Biology is complex and has been described as soft, slow, squishy, and/or sticky. Life's reactions are catalyzed by enzymes, which use an active site constellation of atoms from a relatively small fraction of each macromolecule. To achieve the correct outcome, the active site atoms must all be in the right place, at the right time, for long enough, and with sufficient energy to transcend the barriers encountered along each reaction coordinate. Consequently, enzyme function is profoundly supported by the entire macromolecule structure, its surrounding environment, and the dynamics of the whole.
Structural biologists have experimentally determined and released nearly 186,000 atomic models of macromolecules, representing a large cross section of biodiversity. Most derive from traditional crystallographic experimental data collected from samples at cryogenic temperature (e.g. 100 K). Cryocooling effectively "freezes"ground state configuration(s) and restricts dynamics. These ground-state structures and their functional insights are essential to life science R&D efforts globally.
For example, ground-state crystal structures were critical in the recent Pfizer medicinal chemistry campaign that led to the oral antiviral treatment for SARS-CoV-2, Paxlovid - a combination of two antiviral compounds, nirmatrelvir (PF-07321332) and ritonavir (to slow the metabolism of the former). Two SARS-CoV-2 proteases, i.e. the main protease (Mpro) and the papain-like protease (PLpro) hydrolyze the viral polypeptide chains to yield functional non-structural proteins. They are both essential for viral replication. Nirmatrelvir was designed to inhibit Mpro from the original SARS-CoV-2 strain, but it is also a potent inhibitor of the Mpro from Delta and Omicron variants.
Our community-based collaboration ([2695] Time-resolved SFX of Covid-19 proteins including Mpro and PLpro) focused on water soluble substrates or inhibitors ranging in size from 112 –684 Daltons, used mix-inject flow focusing gas dynamic virtual nozzle methods. X-ray photons arrived at the interaction region with a 500 kHz intra-pulse rate. We collected 2020 images per second using the AGIPD 4M, which were bundled into five-minute runs. Calibration of the 606k AGIPD images within each run took ~ 40 minutes to prepare for further analysis. We developed versions of cctbx.xfel and DIALS to exploit parallel processing strategies distributed over many nodes at the Eu.XFEL. The calibrated virtual images were processed with cctbx.xfel and DIALS at 3500 Hz –to our knowledge, setting a world record. We made use of a 96-node computer cluster (80-140 cores per node) within the MAXWELL cluster at DESY, with a dedicated 100 node partition for the live experiment. We indexed and integrated 606k images in 2-3 minutes by sub-filing the run into 12 sequences, each run on 8 nodes. We therefore produced interpretable electron density maps about 4 minutes after calibration was complete. Further optimisation post-beamtime reduced the total nodes to 12-48 while achieving comparable speeds (8-16% of the MAXWELL cluster). This demonstrates that cctbx.xfel and DIALS can utilise the current Eu.XFEL compute resource to produce real-time datasets with minimal impact on other MAXWELL users. Importantly, it allows enough compute resource for other serial processing algorithms to run concurrently. Some of our tr-SFX results of small ligand binding to PLpro will be presented.

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