Supporting Information

Conformational Flexibility of the Acyltransferase from the Disorazole Polyketide Synthase is revealed at an X-Ray Free Electron Laser using a Room-Temperature Sample Delivery Method for Serial Crystallography

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Methods

Preparation of the acyl transferase. Disorazole acyl transferase (AT) protein was prepared as previously described[15]. Small crystals of the AT were grown by mixing equal volumes of 15 mg/mL protein and a crystallization solution consisting of 24% PEG 4K, 0.04 M ammonium acetate, 0.1 M Tris-HCl (pH 7.5). After 4 min the crystals obtained were concentrated by centrifuging at a speed of 2000 rpm for 1.5 min. The filtrate was removed, and the crystals were re-suspended in a storage solution of 12% PEG 4K, 0.04 M ammonium acetate, 0.1 M Tris- HCl (pH 7.5). The solution used for the XFEL experiment contained crystals of approximately 50 µm x 10 µm x 2 µm in size and had a crystal concentration of approximately 75 crystals (various sizes) per 1 µL.

Preparation of lysozyme crystals. 200 µL of a 25-30 mg/mL solution of lysozyme from chicken egg white, purchased from Sigma-Aldrich, was mixed with 400 µL of a precipitant

solution consisting of 2.5 M NaCl, 6% PEG 6K, 0.15 M sodium acetate (pH 4.5). This solution was set aside for around 6 h. The crystals obtained were separated by centrifuging for 2 min at 2000 rpm. The crystals were re-suspended in a solution of 1.5 M NaCl, 5% PEG 6K, 0.1 M sodium acetate (pH 4.5). The crystal solution used for the synchrotron experiment had crystal size variations between 15 and 100 µm with a crystal concentration of about 9 crystals per 1 µL.

Data collection and analysis.

Extractor installation on the beamline

The SE device (Figure 2) is assembled on a kinematic mount (Newport BKL-4), which allows easy attachment to a motorized stage assembly (Figure S1). For our experiments the stage assembly also serves as a connecting base to the cryo-nozzle holder of a goniometer setup used at the XPP and MFX (Macromolecular Femtosecond Crystallography) stations at the LCLS (Figure S2) and the SSRL beam lines (Figure S3). To prepare for SE experiments, the cryo-nozzle may be quickly removed from the kinematic mount and replaced with the SE assembly. The same beam-stop, on-axis sample visualization, and collimation systems as utilized for the goniometer-based experiments can be used for the SE data collection without further modifications.

AT microcrystals at the LCLS XFEL.

The SFX experiments with the AT were carried out at the LCLS XPP instrument at the SLAC National Accelerator Laboratory (Menlo Park, CA). The LCLS X-ray beam, with a pulse duration of 40 fs, was focused to a beam size of 20 x 20 μm^2 full-width at half-maximum with a pulse energy of 2.9 mJ, a photon energy of 9.5 keV and a repetition rate of 10 Hz.

The SE was outfitted with a mesh substrate that carried crystals in a liquid film held within each mesh hole (diameter of 0.9 x 1.1 mm^2). The crystals were observed to rotate freely within the liquid film after extraction. A typical data collection cycle began with the solenoid rapidly submerging, pausing with the substrate submerged for ~0.5 s to replenish crystals, and then rapidly removing the substrate from the crystal solution. This was immediately followed by the use of motorized stages to translate the mesh by 60 µm steps between X-ray exposures,

positioning a new area of the mesh to each X-ray pulse with a total translation of about 2 mm. After a series of X-ray exposures was collected at 10 Hz, the process of loading, extracting, and exposing a fresh batch of crystals within the mesh was repeated.

A total of 22,985 diffraction images (12% of these images were collected while the SE was reloading fresh crystals and therefore could not contain any diffraction) were collected at 10 Hz with a Rayonix HS170 detector, corresponding to 40 min of XFEL beam time. Images containing more than 50 Bragg peaks were considered as diffraction "hits", which yielded a total of 2,075 images corresponding to an approximate hit rate of ~10%. The detector distance was set at 150 mm, with an achievable resolution of 2.50 Å at the edge of the detector (2.0 Å in the corner). Data reduction was carried out using *cctbx.xfel*[1] with an indexing and integration optimization algorithm implemented in *IOTA*[2]. Scaling, post-refinement and merging were performed using *PRIME*[3]. The high symmetry of the $P2_12_12_1$ space group, in which the AT crystallized, ensured high redundancy of the data, which allowed us to reject any images with correlation coefficient (calculated using the internal merged reference dataset) below 50% to maximize the quality of the final merged dataset. As a result, the final merged dataset contained only 771 images, which was nevertheless sufficient to achieve 98.8% completeness and a 14-fold redundancy. While visible diffraction was observed in individual images to resolutions as high as 2.1 Å, both completeness and redundancy abruptly decreased beyond 2.5 Å. Thus, we set the limiting resolution of the merged dataset to 2.5 Å (Table S1).

The structure was determined by molecular replacement using *Phaser*[4] with a search model based on a previously-published *apo*-AT structure (PDB ID: 3RGI) and modified to remove water molecules, heteroatoms and sidechains. The structure was then refined using alternating cycles of automated reciprocal-space refinement in *phenix.refine*[5] and manual rebuilding in real space using *Coot*[6]. The final data processing and refinement statistics are summarized in Table S1.

The structures of the acyltransferase-malonate and acyltransferase-citrate complexes were determined by molecular replacement using MOLREP[7] with the previously determined native AT structure as the search model. The structure was refined using REFMAC[8] and manual

building using *Coot*. The final data processing and refinement statistics are summarized in Table S1. The Ramachandran (phi/psi) plot is shown in Figure S7.

Remote delivery devices may be used to deliver solutions directly into the vial containing the crystal solution and mixing may be carried out in-situ. This would enable the study of enzyme substrate/ cofactor/inhibitor complexes using the same crystal solution (Figure S8). We plan to use the remote delivery device to study PKS reaction dynamics by introducing malonyl- or methylmalonyl-CoA into the native crystal solution.

HEWL crystals at the SSRL synchrotron.

The serial crystallography experiments with lysozyme were carried out at the SSRL beamline 12-2 at the SLAC National Accelerator Laboratory (Menlo Park, CA). During data collection, the substrate is translated vertically by a positioning stage that is part of the SE assembly and horizontally by a stage that is part of the cryo-nozzle holder The wavelength of the 12-2 X-ray beam was 0.980 Å, with a flux of 1.7 x 10^{12} p/s focused to 50 x 20 μm^2 at the sample position. A 23 µm thick Cyclo Olefin Polymer (COP) film (ZeonorFilm from Zeon Chemicals) was used as the carrier substrate. During data collection, the crystal solution was first stirred by two quick solenoid motions, in and out of the solution, in order to distribute the HEWL crystals evenly before removal of the substrate for data collection. The carrier substrate was translated with a velocity of 2.1 mm/sec to bring the crystals to the interaction region. The crystals were exposed for 0.2 s without translating the substrate. The film substrate was then returned into the crystal solution to replenish the sample. The process of solenoid stirring, loading, extracting and exposing fresh crystals was repeated to collect the complete data set.

A total of 2946 diffraction images were collected using a PILATUS 6M detector. Each crystal was exposed to the beam once for 0.2 sec. Individual diffraction-pattern hits were defined as frames containing more than 10 Bragg peaks, which yielded a total of 2208 images corresponding to an average hit rate of 75%. The data were collected to a resolution of 1.62 Å at the edge of the detector (1.28 Å in the corner). Data reduction, structure solution and structure refinement were carried out in the same manner as for the AT. As with the AT, the high

symmetry of the HEWL space group ($P4_32_12$) allowed the application of stringent rejection criteria (CC=50%) while still obtaining 100% completeness and a 29-fold redundancy from only414 images. Using the completeness, redundancy and $CC_{1/2}$ of 91.8% (Table S2), we established 2.0 Å as the limiting resolution of this dataset, and the structure was determined by molecular replacement and refined to yield R_{work} and R_{free} of 20.5% and 23.3%, respectively (Table S2, Figure S6). Although the crystals may not be typically destroyed in the synchrotron beam, the dose received by each crystal was approximately 100 times the room temperature dose limit[9] (i.e. where the overall diffracting power of the crystal is reduced by half). As with the XFEL experiment, crystals that survive the initial exposure and are exposed for a second time, it is expected that the resulting images would be excluded during the normal data analysis process. Furthermore, there was no indication of radiation damage in the resulting electron density map.

While in this case the lysozyme diffraction data was collected in an automated fashion, it should be noted that, when the film substrate is used with a low concentration of crystals, an alternative less automated method for data collection may also be employed, using an on-axis video microscope and motorized translations to position individual crystals on the substrate into the X-ray interaction region before exposure, in a "click and shoot" process.

References

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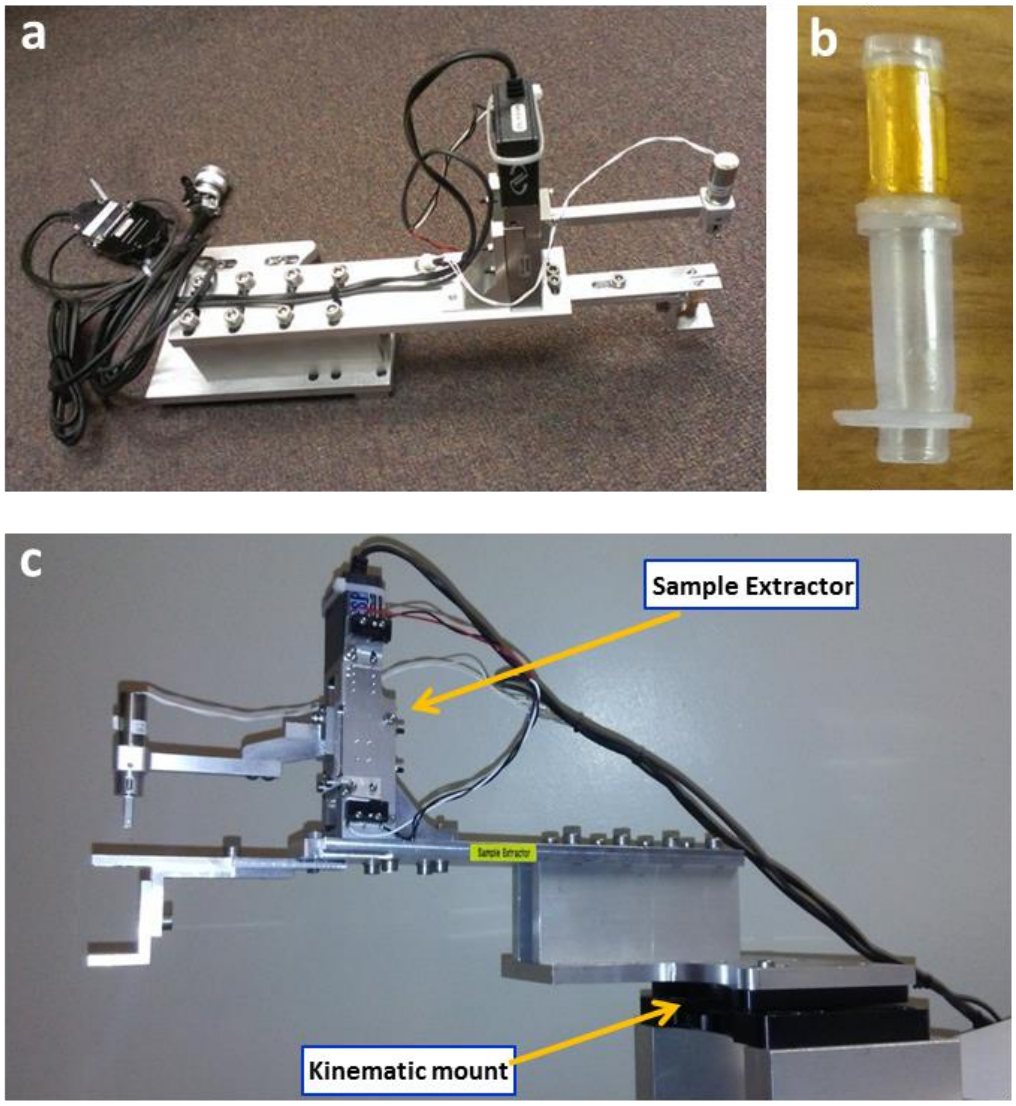
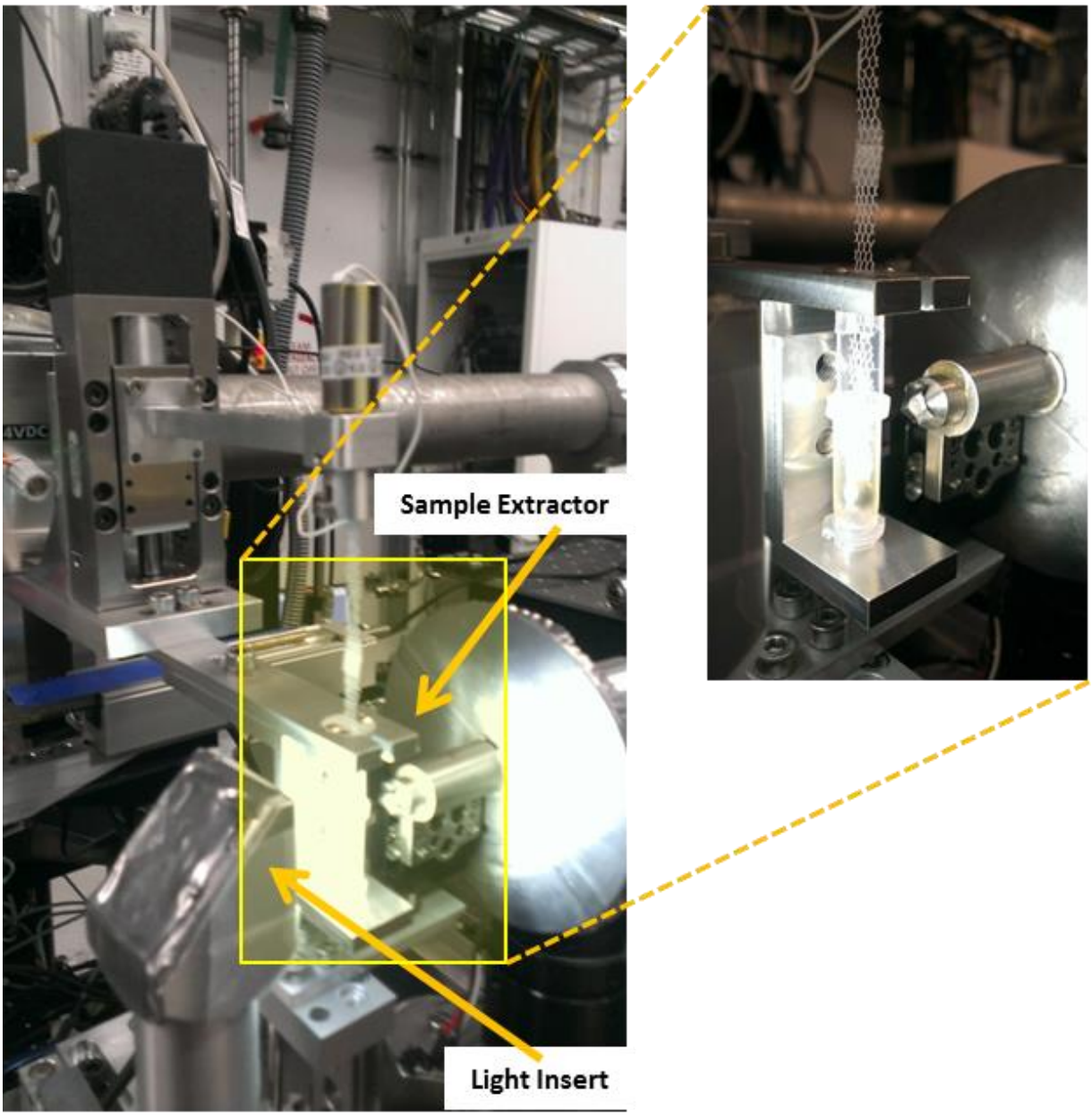
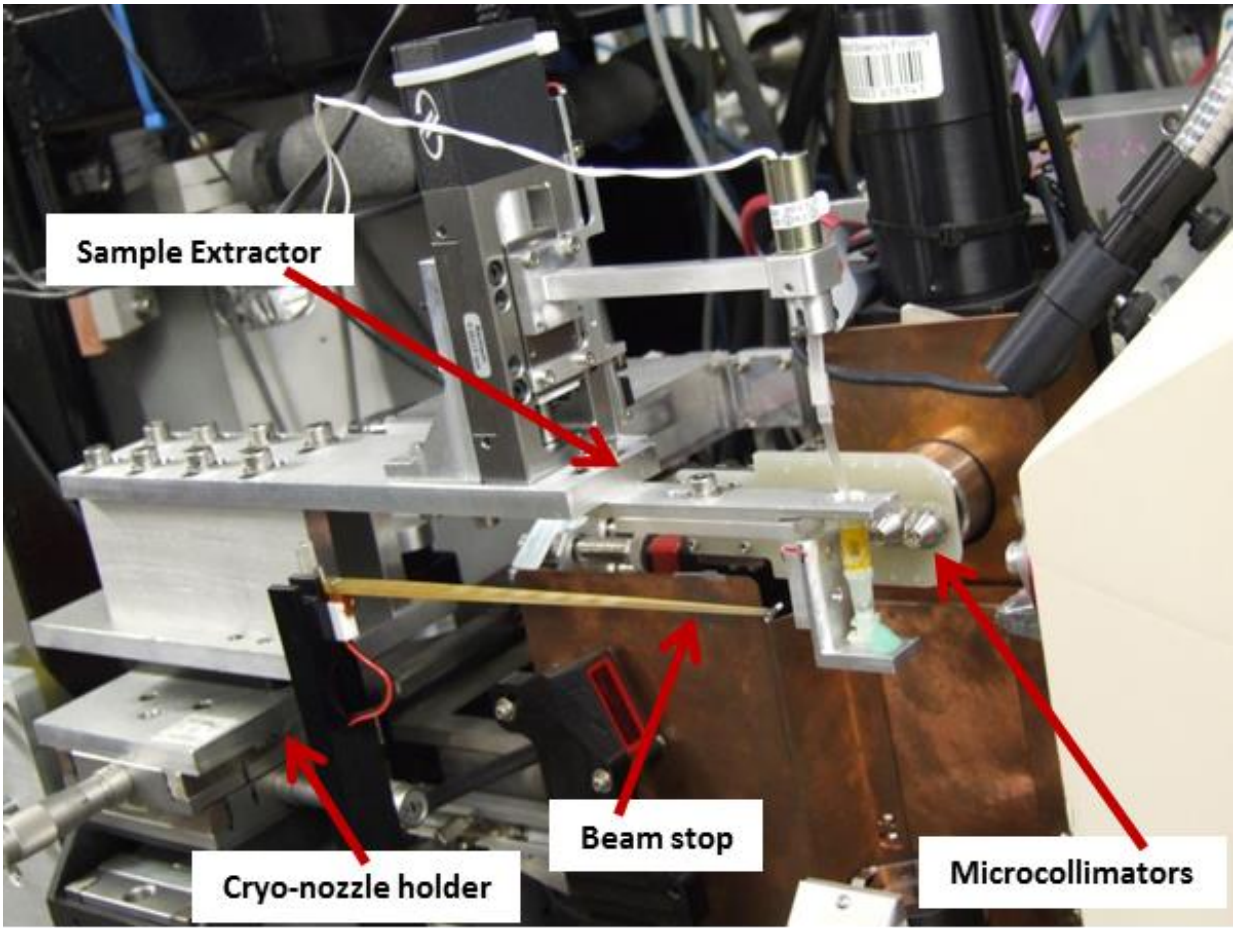
Supplementary Figures



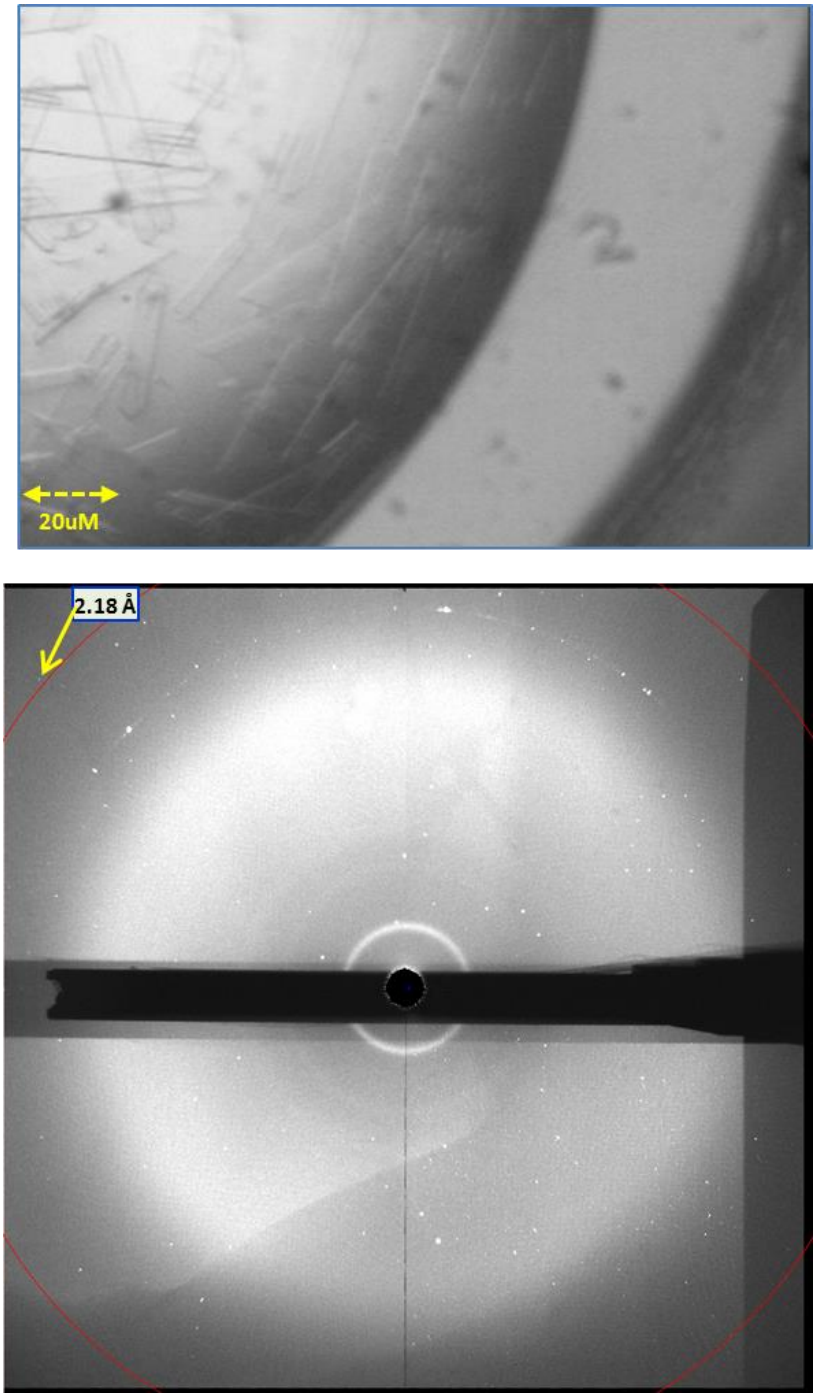
Supporting Figure 1. Sample Extractor. View of the SE (panel A), View through the large opening of the crystal holder (Panel B), and the SE mounted on an adaptor compatible with the beam line kinematic mount (Newport BKL-4) that holds the cryo-nozzle (panel C).



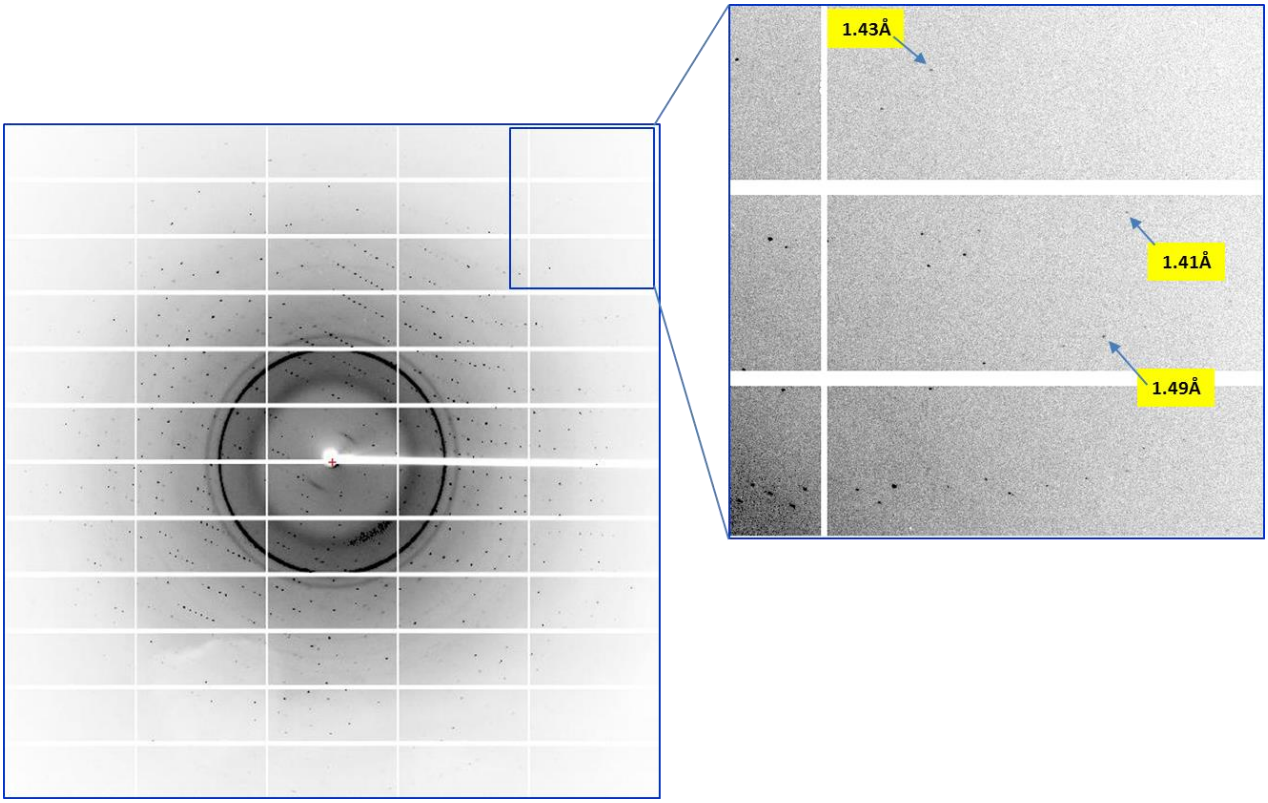
Supporting Figure 2. The SE installed at the XPP instrument of the LCLS. This setup is compatible with the standard LCLS-MFX goniometer setup. A moveable back light, used to illuminate crystals viewed with an on-axis video microscope, is in the inserted position.



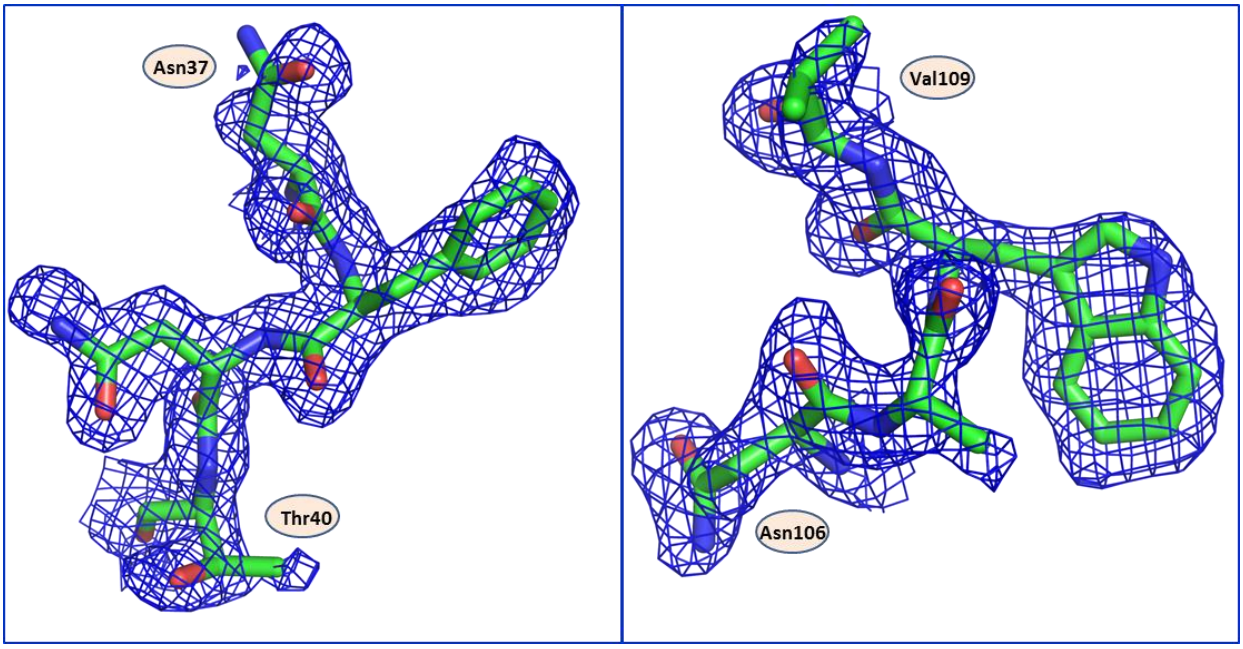
Supporting Figure 3. The SE installed at beamline 12-2 at SSRL. The SE is mounted on a kinematic mount that otherwise holds the beamline cryo-nozzle assembly and the SE generally occupies the same space as the cryo-nozzle. Therefore, the goniometer, video microscope and other equipment do not need to be removed for the SE experiments. The on-axis video microscope, routinely used to view (along the X-ray beam direction), crystals mounted on a goniometer, was also used to view crystals on the SE film substrate.



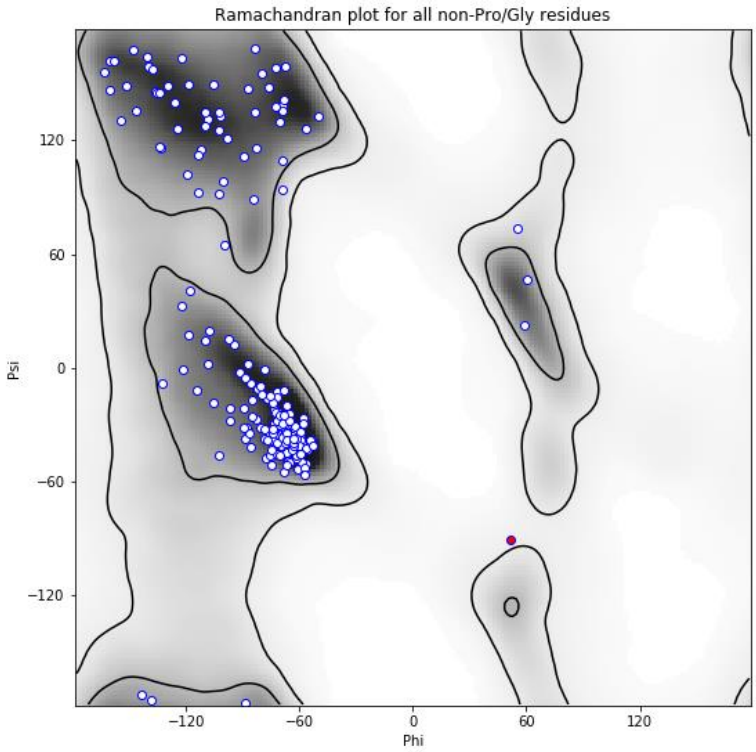
Supporting Figure 4. Crystals of Acyl transferase. AT crystals are shown in the top panel. This sample is taken from the crystal solution used for the experiment. The thin plate morphology of the crystals and their size heterogeneity did not adversely affect the completeness of the data or the performance of the device. The bottom panel shows a diffraction pattern from the AT crystals collected at the XFEL. The arrow indicates spots visible at 2.18 Å resolution.



Supporting Figure 5. Lysozyme diffraction at the synchrotron. The zoomed-in view shows the spots visible at 1.4 Å resolution. The spots were sharp with a good profile. The dark ring near the center of the images, (~4.1 Å) is background scatter from the polymer film substrate.

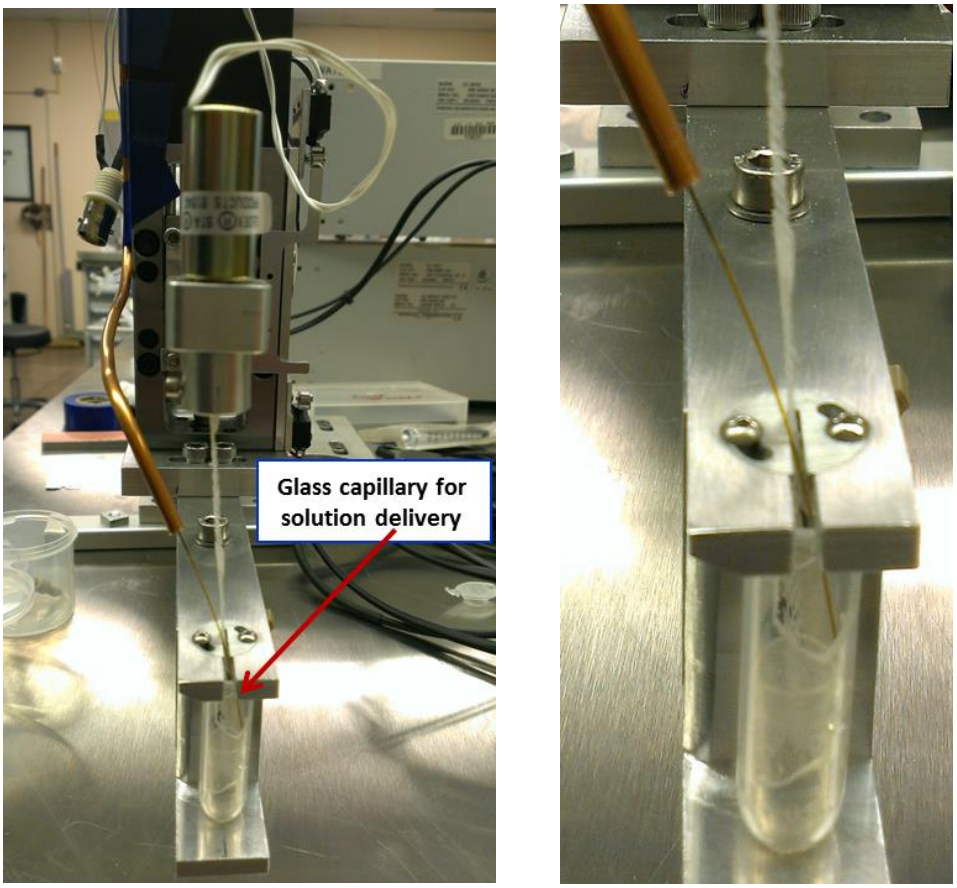


Supporting Figure 6. Lysozyme electron density. The electron density maps for two regions of the lysozyme structure. The simulated annealing composite omit map (contoured at 1.0 sigma) is shown. The simulated annealing composite omit map was calculated using Phenix, with solvent excluded from the omitted region.



Supporting Figure 7. Ramachanran plot for the room temperature XFEL AT structure.

a b



Supporting Figure 8. A setup for remote sample delivery. A. Setup for delivering solutions to the SE remotely. An HPLC pump and a flow control unit enable delivery of microliter amounts of solutions through a glass capillary into the vial holding the crystal solution. After collecting data for the native protein, a substrate, cofactor or inhibitor solution may be delivered into the crystal solution using the remote device. The diffraction data for the substrate complex, substrate-cofactor complex, product complex or inhibitor complex can be collected after incubating the crystals in the respective solutions. This can also be done in a serial fashion to study structures of native enzyme followed by a cofactor soak and/or a substrate soak. The delivering solution could also contain a "small molecule cocktail" for fragment-based screening methods. Since the SE brings the unexposed crystals back into the crystal solution holder, the entire experiment can be completed using the same crystallization solution. B. Zoomed-in view showing the capillary insert.

Supporting Table 1. Crystallographic parameters, data collection and refinement statistics

	Acyltransferase -RT	**Malonate-100°K**	**Citrate – 100°K**
Crystallographic parameters			
Space group	$P2_12_12_1$	$P4_32_12$	$P4_32_12$
Unit-cell dimensions (Å)	44.21, 54.64, 124.15Å, 90,90,90°	104.67, 104.67, 138.63Å, 90,90,90°	104.26, 104.26, 138.69Å, 90,90,90°
Data collection statistics			
Resolution limits (Å)	19.5 – 2.5 (2.54-2.50)	39.2–2.00 (2.05-2.00)	39.2-1.63 (1.67-1.63)
Number of unique reflections	10781	52610	95319
Redundancy	14.2 (4.7)	8.1(8.2)	6.5(6.0)
Completeness			
Overall (outer shell)	98.8 (89.9)	99.9(98.8)	99.8(99.9)
CC1/2	91.8 (79.0)	99.9(83.4)	99.9(69.8)
I/σ			
Overall (outer shell)	4.1 (1.9)	15.4(2.8)	18.9(2.1)
Refinement statistics			
Resolution limits	19.4-2.5 (2.61-2.50)	39.2–2.00 (2.05-2.00)	39.2–1.63 (1.67-1.63)
R_{factor}[a] (%)	23.1 (30.0)	18.4(24.3)	16.8(26.7)
R_{free} (%)	27.1 (35.5)	19.9(25.2)	19.1(29.6)
Model contents/average B(Å^2)			
Protein atoms	2164/32.2	4284/27.9	4319/21.2
Ligand		14/35.6	26/27.7
Ions		11/43.5	10/47.6
Solvent molecules	74/18.6	357/34.3	599/34.3
RMS deviations			
Bond length (Å)	0.003	0.008	0.009
Bond angle (°)	0.536	1.237	1.474
Ramachandran favored (%)	97.5	98.9	99.3
Ramachandran outliers (%)	0.7	0.0	0.0

[a] $R_{factor} = \sum|Fp - Fpcalc|/\sum Fp$, where Fp and Fpcalc are observed and calculated structure factors; R_{free} is calculated with 5% of the data.

Supporting Table 2. Crystallographic parameters, data collection and refinement statistics for HEWL

Crystallographic parameters	
Space group	$P4_32_12$
Unit-cell dimensions (Å)	79.03, 79.03, 38.24Å, 90,90,90°
Data collection statistics	
Resolution limits (Å)	39.5-2.05 (2.09-2.05)
Number of unique reflections	8021
Redundancy	29.1 (19.6)
Completeness	
Overall (outer shell)	100 (100)
CC1/2	90.3 (47.8)
I/σ	
Overall (outer shell)	5.5 (1.2)
Refinement statistics	
Resolution limits	39.4-2.05 (2.18-2.05)
Number of reflections/% (|F|>2σ|F|)	8021 (1165)
Reflections used for R_{free}	803 (130)
R_{factor} [a] (%)	20.5 (25.6)
R_{free} (%)	23.3 (29.3)
Model contents/average B(Å^2)	
Protein atoms	1039/15.2
Ligand	
Ions	1/12.8
Solvent molecules	104/23.1
RMS deviations	
Bond length (Å)	0.003
Bond angle (°)	0.703
Ramachandran favored (%)	97.5
Ramachandran outliers (%)	0.0

[a] $R_{factor} = \sum|Fp - Fpcalc|/\sum Fp$, where Fp and Fpcalc are observed and calculated structure factors; R_{free} is calculated with 5% of the data.