The Conformational Flexibility of the Acyltransferase from the Disorazole Polyketide Synthase Is Revealed by an X-ray Free-Electron Laser Using a Room-Temperature Sample Delivery Method for Serial Crystallography

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Supporting Information

ABSTRACT: The crystal structure of the trans-acyltransferase (AT) from the disorazole polyketide synthase (PKS) was determined at room temperature to a resolution of 2.5 Å using a new method for the direct delivery of the sample into an X-ray free-electron laser. A novel sample extractor efficiently delivered limited quantities of microcrystals directly from the native crystallization solution into the X-ray beam at room temperature. The AT structure revealed important catalytic features of this core PKS enzyme, including the occurrence of conformational changes around the active site. The implications of these conformational changes for polyketide synthase reaction dynamics are discussed.

Polyketide synthases (PKSs) work in a highly modular fashion to synthesize medicinally important natural products with a variety of therapeutic properties.1,2 The basic unit of the module consists of a β-ketosynthase (KS), an acyltransferase (AT), and an acyl carrier protein (ACP). The KS domain receives the growing polyketide chain from the upstream module and subsequently catalyzes chain elongation with an ACP-bound extender unit as the co-substrate (Figure 1). The AT transacylates the extender unit onto the pantetheinyl arm of the ACP (Figure 1). The AT domain is responsible for selection of an appropriate building block (malonyl- or methylmalonyl-CoA) in each chain elongation cycle that is transferred to a dedicated acyl carrier protein (ACP) domain in the same module. Substitution for domains loading other extender units allows introduction of a broadened chemistry into the polyketide backbone.3–5 Considerable research has focused on engineering the PKS system to produce new compounds.6 The observed inefficiency underscores the importance of a deeper understanding of PKS structure–function relationships. The structures of individual PKS enzymes7,8 and multidomain fragments9,10 revealed some mechanistic insights into catalysis; together with recent small-angle X-ray scattering analysis, these structural studies have enabled modeling of the entire assembly.11 However, the lack of high-resolution structures of intact PKS modules limits the ability to improve their synthetic efficiency via engineering approaches. In particular, the difficulty in growing larger crystals and the sensitivity of crystals to X-rays make it difficult to employ conventional crystallographic methods for atomic analysis.

The ultrashort femtosecond X-ray pulses of X-ray free-electron laser (XFEL) sources can produce diffraction data on time scales that mitigate radiation damage processes. This enables structural investigations of extremely small, weakly diffracting, and radiation-sensitive crystals at ambient temperatures.12 Because the exposed crystals are destroyed in the process, serial femtosecond crystallography (SFX) data sets are comprised of still diffraction images collected from many individual crystals delivered serially. The XFEL at the SLAC Linac Coherent Light Source (LCLS) produces hard X-ray pulses at a maximum frequency of 120 Hz, and protein crystals for SFX experiments are most often delivered rapidly into the X-ray beam using a liquid jet injector.13 Significant improvements in liquid jet technology have recently been made, including the use of high-viscosity carrier solutions to minimize sample consumption and to improve reliability.14–17 However, challenges remain, such as the need to alter crystallization conditions and/or transfer crystals into carrier solutions that are compatible with the specific injector technology, a process that often adversely affects diffraction quality. Moreover, to prevent clogging of the injector nozzle, crystal slurries may need to be filtered to limit crystal size. In such circumstances, delicate crystals like the PKS subcomplexes may not survive the shear forces associated with filtration or the injection process.18 Acoustic injectors that deliver drops containing crystals “on demand” show promise as an alternative way to reduce sample demand under-

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consumption. However, compatibility requirements of carrier solutions and limitations in drop size continue to pose technical challenges. Therefore, these techniques are not well suited for AT crystals, which are difficult to produce in large quantities and are sensitive to changes in the crystallization solution.

We developed a new device, the sample extractor, to efficiently deliver microcrystals in native crystallization solutions for serial crystallography (SX) and SFX experiments (Figure 2 and Figure S1). The sample extractor uses a variety of mesh- or thin film-based substrates to transport crystals directly from their mother liquor to the X-ray interaction region. To mitigate dehydration during X-ray diffraction data collection, both the crystalline sample and the substrate are enclosed in a small vial. A small opening in the vial allows the X-ray beam to enter, and a larger opening on the opposite side covered with a thin X-ray transparent film allows the diffracted X-rays to pass through (Figure S1). The substrate is connected through a small slot on top of the vial to a small solenoid that can rapidly move the substrate in and out of the crystal solution. A small stainless steel weight at the end of the substrate provides positional stability and also agitates the solution, thus preventing the crystals from settling to the bottom of the reservoir.

During operation, the substrate is first submerged into a solution containing crystals. Crystals are trapped on the substrate as it is rapidly removed from the solution. The substrate is then translated in a zigzag pattern to expose a “grid” of regularly spaced sites, some of which contain crystals. The substrate can be translated rapidly up to 2.5 mm/s using the current device. After a rastering sweep is completed, the substrate is resubmerged, transferring unexposed crystals back into solution to keep them hydrated and to make them available for subsequent exposures. This crystal recycling approach, currently not available in any other sample delivery method, minimizes the loss of valuable crystalline sample during data collection. Furthermore, the sample extractor does not appear to be sensitive to crystal size or shape, and because it operates at ambient temperature and pressure, there is essentially no mechanical stress on the crystals during delivery. The sample extractor device is compact and easily mounted at the LCLS and SSRL crystallography beamlines. Furthermore, the existing collimation, beam stop, and on-axis sample visualization systems can be used for beam conditioning and sample extractor alignment (see Figures S1–S3).

It has been recently reported that ambient-temperature X-ray crystallography can access protein conformational dynamics that are locked out in low-temperature structures. The serial X-ray diffraction data for AT crystals were collected during protein crystal screening (PCS) time at the LCLS X-ray pump probe (XPP) station using 3.3 mg of protein in 300 μL of native crystallization solution (approximately 75 crystals of ~50 × ~10 × ~2 μM^3 per microliter) (Figure S4). The XFEL operated at a wavelength of 9.5 keV with a pulse length of 40 fs. Still diffraction images were recorded using a Rayonix MX170-HS X-ray detector operating at a frame rate of 10 Hz. A nylon mesh with diamond-shaped holes (1.0 mm × 0.9 mm) was used as the sample extractor carrier substrate. After extracting the substrate from the crystal solution and transferring it into the X-ray beam path, we recorded ~40 exposures while the substrate was translated by 60 μm between each exposure. The detector operated in a continuous mode, yielding five images without diffraction each time the substrate was submerged and the crystals were reloaded. Thus, ~12% of the total 22985 detector images collected in ~40 min lacked diffraction data because the sample was being regenerated. A total of 2075 images contained diffraction patterns with at least 50 peaks [with resolution as high as 2.1 Å (Figure S4)], yielding a hit
rate of ∼10%. Of these, 1482 images could be successfully indexed and integrated. The quality of the merged data set was improved by applying stringent rejection criteria. As a result, the final data set contained 771 of the filtered images and was 98.6% complete to a resolution of 2.5 Å with a redundancy of 14 (space group \( P_{2_1}2_12_1 \)) and a \( CC_{1/2} \) of 91.7% (Table S1). The structure was refined to yield 8\( R_{work} \) and 8\( R_{free} \) values of 23.1 and 27.1%, respectively. Despite the thin plate morphology of the AT crystals (Figure S4), the data set was complete, indicating crystals were not preferentially orienting on the substrate. In comparison, previous experiments using the gas dynamic virtual nozzle liquid injector \(^{13}\) at the LCLS XFEL required 6 mL of AT crystals to produce 66 images with diffraction, the highest observed resolution being only 2.6 Å. The sample extractor described here holds 300 \( \mu L \) of the crystal suspension, and smaller vial designs are in progress. After continuous diffraction data had been collected for ∼40 min using the same sample, there was no observable deterioration in diffraction quality. Because crystals are typically destroyed upon exposure to the X-ray beam, the chance of repeat exposure of

Figure 3. Conformational changes in the AT. (a) The left panel shows the surface representation of the AT active site (apo-LCLS structure), highlighting its deep narrow pocket. The close-up view of the malonate binding site is shown at the right. In the close-up view, the catalytic dyad residues (Ser86 and His191) are labeled with orange backgrounds and the gatekeeper residues (Gln9 and Phe190) are labeled with yellow backgrounds. Thr54 and Thr57, which are involved in the stabilizing Gln9, are also shown. The hydrogen bonds in the room-temperature and 100 K structures are shown as black and yellow dashed lines, respectively. Gln9 in the room-temperature structure is stabilized by a water molecule that bridges to the main chain carbonyl of Ser52. The 2\( F_o - F_c \) maps contoured at 1σ for the room-temperature and 100 K structures are shown as green and blue meshes, respectively (Phenix was used to superpose the electron density maps, ref 5 in the Supporting Information). In the low-temperature structure of the apoenzyme, access to the binding site of the malonate extender unit is blocked off. In contrast, the room-temperature structure shows a higher-energy conformation that appears to be critical for substrate access. The residue equivalent to Gln9 in the AT from the bacillaene PKS (unpublished structure, Protein Data Bank entry 5dz6) shows hydrogen bonding to a glycerol molecule in the malonate binding site. (b) The catalytic dyad and the residues involved in the substrate specificity are shown as sticks. The residues involved in substrate specificity (Phe190 and Gln9) are colored orange and black for the malonate and citrate complexes, respectively. This picture shows the movement of the Phe190 side chain to accommodate larger substrates in the binding pocket.
the same crystal is very low. For the few crystals that survive the beam, the repeat diffraction image will be substantially poorer and will therefore be excluded from the final data set by standard data processing methods.

The resulting AT structure represents the first ambient-temperature structure of a PKS enzyme to date. Comparison of the ambient-temperature structure with the recently published 100 K structure reveals conformational flexibility at ambient temperature that is not observed at low temperatures. A notable difference was found in the active site comprised of Ser86 and His191 (which form the catalytic dyad), Arg111 (which stabilizes the C3 carboxylate of the malonyl extender unit through a salt bridge), and Phe190 and Gln9 (which are believed to control substrate specificity by interacting with C2 and any attendant substituent of the extender unit) (Figure 3). In particular, the Gln9 side chain has undergone a reorientation, resulting in disruption of the hydrogen bond between the Gln9 carboxamide and the side chain of Thr57 (Figure 3). This observed widening of the substrate binding cavity comprised of the Phe190 and Gln9 side chains is intriguing, as it suggests that active site dynamics play an important role in controlling the high substrate specificity of the AT family of enzymes. To test this hypothesis, we compared structures of AT crystals soaked in malonate and citrate solutions, collected using crystals held at 100 K. As shown in Figure 3, the orientation of Gln9 in the room-temperature LCLS structure is analogous to that observed in the malonate- and citrate-bound complexes. The larger substrates are accommodated by the movement of the Phe190 side chain (Figure 3b). Thus, it appears that room-temperature X-ray diffraction analysis of the disorazole apo-AT has allowed the visualization of a higher-energy conformation of this enzyme that may be critical for accommodating its cognate substrate with high specificity.

Additional conformational changes are observed near the active site in the vicinity of Gln156. Gln156 shows alternate conformations in the room-temperature structure and forms a direct hydrogen bond with Asn152 (Figure 4a). While the published structures of the native and acetate complexes had four water molecules connecting these residues (Figure 4b), the structures of the malonate complex and the citrate complex show hydrogen bonds involving only one water molecule (Figure 4c). Flipping of the side chain of Gln156 moves the side chain of nearby Gln276. This reorganization results in a new long-range interaction (16 Å from Cα of Gln276 to His85) in the room-temperature structure. This interaction connects the solvent-exposed surface to the internal active site (Figure 4d). More generally, our findings demonstrate that room-temperature diffraction is capable of uncovering conformational flexibility that may otherwise be locked out at lower temperatures.

In addition, these experiments have established the efficient operation and utility of the sample extractor device to study fragile crystals in limited supply. To broaden the utility of the sample extractor for room-temperature structural analysis, we also tested the device at the Stanford Synchrotron Radiation Lightsource (SSRL) and successfully collected a 2.0 Å data set of hen egg white lysozyme (HEWL) microcrystals (Table S2 and Figures S5 and S6). Use of a film rather than a mesh substrate helped to restrict crystal motion during the relatively long X-ray exposure time of 0.2 s.

In summary, the first room-temperature structure of a polyketide enzyme using the novel sample extractor device was determined to 2.5 Å resolution. Structural comparison with the low-temperature structures revealed conformational flexibility in this complex and a dynamic PKS enzyme. Further experiments are in progress to collect serial crystallography data sets for microcrystals of PKS complexes stabilized by antibodies.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.biochem.7b00711.

Methods, Figures S1–S8, and Tables S1 and S2 (PDF)

**Accession Codes**

Coordinates and structure factors of the acyltransferase (XFEL room temperature), malonate complex, citrate complex, and lysozyme have been deposited in the Protein Data Bank as entries 6APK, 6APG, 6APF, and 6APM, respectively.

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**Author Contributions**

I.I.M. contributed to design, crystallization, data collection, structure refinement, and structure analysis. A.E.C. and M.S. contributed to design, data collection, and structure analysis. K.A. contributed to design, data collection, and SEM data collection. M.H. contributed to design, data collection, and structure analysis. A.E.C. and M.S. contributed to acyltransferase protein and structure analysis.
A.Y.L., M.U., and A.T.B. contributed to data processing, refinement, and structure analysis. The manuscript was prepared by A.E.C., S.E.M., I.I.M., A.Y.L., C.K., and M.U. with input from all authors.

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**Notes**

The authors declare no competing financial interest.

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**REFERENCES**

Supporting Information

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Methods

Preparation of the acyl transferase. Disorazole acyl transferase (AT) protein was prepared as previously described15. 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 \( \mu m \times 10 \mu m \times 2 \mu m \) in size and had a crystal concentration of approximately 75 crystals (various sizes) per 1 \( \mu L \).

Preparation of lysozyme crystals. 200 \( \mu L \) of a 25-30 mg/mL solution of lysozyme from chicken egg white, purchased from Sigma-Aldrich, was mixed with 400 \( \mu L \) 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 µm² 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 mm²). 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\(_{1}2\(_{1}2\(_{1}\)\) 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 $1.7 \times 10^{12}$ p/s focused to 50 x 20 $\mu$m$^2$ at the sample position. A 23 $\mu$m 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_{3}2_{1}2) allowed the application of stringent rejection criteria (CC=50%) while still obtaining 100% completeness and a 29-fold redundancy from only 414 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 (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


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. Ramachandran plot for the room temperature XFEL AT structure.
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

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<tr>
<td></td>
<td>Solvent molecules</td>
<td>74/18.6</td>
<td>357/34.3</td>
</tr>
<tr>
<td>RMS deviations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bond length (Å)</td>
<td>0.003</td>
<td>0.008</td>
<td>0.009</td>
</tr>
<tr>
<td>Bond angle (°)</td>
<td>0.536</td>
<td>1.237</td>
<td>1.474</td>
</tr>
<tr>
<td>Ramachandran favored (%)</td>
<td>97.5</td>
<td>98.9</td>
<td>99.3</td>
</tr>
<tr>
<td>Ramachandran outliers (%)</td>
<td>0.7</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

\( R_{\text{factor}} = \sum |F_p - F_{\text{calc}}|/\sum F_p, \) where \( F_p \) and \( F_{\text{calc}} \) are observed and calculated structure factors; \( R_{\text{free}} \) is calculated with 5% of the data.
Supporting Table 2. Crystallographic parameters, data collection and refinement statistics for HEWL

<table>
<thead>
<tr>
<th>Crystallographic parameters</th>
<th></th>
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<tbody>
<tr>
<td>Space group</td>
<td>P4_2_2</td>
</tr>
<tr>
<td>Unit-cell dimensions (Å)</td>
<td>79.03, 79.03, 38.24Å, 90,90,90°</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Data collection statistics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolution limits (Å)</td>
<td>39.5-2.05 (2.09-2.05)</td>
</tr>
<tr>
<td>Number of unique reflections</td>
<td>8021</td>
</tr>
<tr>
<td>Redundancy</td>
<td>29.1 (19.6)</td>
</tr>
<tr>
<td>Completeness</td>
<td></td>
</tr>
<tr>
<td>Overall (outer shell)</td>
<td>100 (100)</td>
</tr>
<tr>
<td>CC1/2</td>
<td>90.3 (47.8)</td>
</tr>
<tr>
<td>I/σ</td>
<td>5.5 (1.2)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Refinement statistics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolution limits</td>
<td>39.4-2.05 (2.18-2.05)</td>
</tr>
<tr>
<td>Number of reflections/%</td>
<td>8021 (1165)</td>
</tr>
<tr>
<td>(</td>
<td>F</td>
</tr>
<tr>
<td>Reflections used for R_free</td>
<td>803 (130)</td>
</tr>
<tr>
<td>R_factor a (%)</td>
<td>20.5 (25.6)</td>
</tr>
<tr>
<td>R_free (%)</td>
<td>23.3 (29.3)</td>
</tr>
<tr>
<td>Model contents/average B(Å²)</td>
<td></td>
</tr>
<tr>
<td>Protein atoms</td>
<td>1039/15.2</td>
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<tr>
<td>Ligand</td>
<td></td>
</tr>
<tr>
<td>Ions</td>
<td>1/12.8</td>
</tr>
<tr>
<td>Solvent molecules</td>
<td>104/23.1</td>
</tr>
<tr>
<td>RMS deviations</td>
<td></td>
</tr>
<tr>
<td>Bond length (Å)</td>
<td>0.003</td>
</tr>
<tr>
<td>Bond angle (°)</td>
<td>0.703</td>
</tr>
<tr>
<td>Ramachandran favored (%)</td>
<td>97.5</td>
</tr>
<tr>
<td>Ramachandran outliers (%)</td>
<td>0.0</td>
</tr>
</tbody>
</table>

a \(R_{\text{factor}} = \frac{\sum |F_p - F_{\text{calc}}|}{\sum F_p}\), where \(F_p\) and \(F_{\text{calc}}\) are observed and calculated structure factors; \(R_{\text{free}}\) is calculated with 5% of the data.