Accurate chromosome segregation requires timely dissolution of chromosome cohesion after chromosomes are properly attached to the mitotic spindle. Separase is absolutely essential for cohesion dissolution in organisms from yeast to man. It cleaves the kleisin subunit of cohesin and opens the cohesin ring to allow chromosome segregation. Cohesin cleavage is spatiotemporally controlled by separase-associated regulatory proteins, including the inhibitory chaperone securin, and by phosphorylation of both the enzyme and substrates. Dysregulation of this process causes chromosome missegregation and aneuploidy, contributing to cancer and birth defects. Despite its essential functions, atomic structures of separase have not been determined. Here we report crystal structures of the separase protease domain from the thermophilic fungus Chaetomium thermophilum, alone or covalently bound to unphosphorylated and phosphorylated inhibitory peptides derived from a cohesin cleavage site. These structures reveal how separase recognizes cohesin and how cohesin phosphorylation by polo-like kinase 1 (Plk1) enhances cleavage. Consistent with a previous cellular study, mutating two securin residues in a conserved motif that partly matches the separase cleavage consensus converts securin from a separase inhibitor to a substrate. Our study establishes atomic mechanisms of substrate cleavage by separase and suggests competitive inhibition by securin.

Separase belongs to the clan CD family of cysteine proteases which includes caspases. It contains a large amino (N)-terminal armadillo (ARM) repeat domain and a highly conserved carboxy (C)-terminal separase protease domain (SPD) that consists of a pseudo-protease domain (PPD) and an active protease domain (APD) (Fig. 1a and Extended Data Fig. 1). Cohesin forms an asymmetric ring to topologically entrap chromosomes (Fig. 1a). Separase cleaves the kleisin subunit to open the cohesin ring and trigger chromosome segregation. It also cleaves other substrates to regulate anaphase spindle elongation and centriole duplication. High-resolution structures of separase have not been determined more than a decade ago.

Figure 1 | Structure of ctSPD. a, Domains and motifs of separase from Chaetomium thermophilum (top) and schematic drawing of cohesin (bottom). b, Sequence alignment of the cleavage sites of separase substrates; sc, Saccharomyces cerevisiae; sp, Schizosaccharomyces pombe; xl, Xenopus laevis; hs, Homo sapiens. c, Autoradiograph of the ctSPD cleavage assay with 35S-cfScC1 wild type (WT) or non-cleavable mutant (NC) as substrates. For gel source data, see Supplementary Fig. 1. d, Cartoon of the crystal structure of ctSPD. L4 is coloured magenta. Loops with no visible electron densities are indicated by dashed lines. e, Cartoon of caspase 9 (Protein Data Bank accession number 1JXQ), with the bound inhibitor shown as yellow sticks.
since its discovery, hindering our understanding of its mechanism and regulation.

We found that SPD of C. thermophilum (ct) separase could be expressed in large quantities in bacteria without securin (Extended Data Fig. 2a). Recombinant ctSPD, but not the C2110S mutant, cleaved ctScc1 to produce two major fragments (Extended Data Fig. 2b). Separase is known to cleave after the EXXR (X, any residue) consensus motif\(^2\). Charge-reversal mutation of the 212EVGR215 motif in ctScc1 reduced cleavage by separase (Fig. 1b, c). An acyloxymethyl ketone (AMK)-containing peptide inhibitor derived from this cleavage site blocked ctScc1 cleavage in a dose-dependent manner (Extended Data Fig. 4b). Similar to separases from other species\(^6\), longer constructs of ctSPD containing an N-terminal extension underwent autocleavage at the \(^{1643}\)ELAR\(^{1648}\) site (Fig. 1b and Extended Data Fig. 2f). Thus, recombinant ctSPD was active.

We determined the crystal structure of ctSPD (Fig. 1d and Extended Data Table 1). It forms one globular domain with two sub-domains—the PPD and the APD—that pack against each other. APD has an overall fold similar to that of caspases (Fig. 1d, e and Extended Data Figs 3 and 4a). PPD also has a mixed \(\alpha/\beta\) fold, but its central \(\beta\)-sheet has a topology different from that of caspases. One edge of this central sheet of PPD forms an edge-on interaction with that of APD, whereas the other edge is capped by a helical domain in PPD. A prominent helical insert of PPD forms a long coiled-coil and packs against APD.

The catalytic dyad H2083 and C2110 are located in loops L3 and L4 of APD (Fig. 1d and Extended Data Fig. 3a). An important mechanism of pro-caspase activation is the reorganization of L4, which can be achieved through homodimerization, cleavage of an internal linker, or both\(^19,22\). The geometry of the catalytic dyad and the extended conformation of L4 in ctSPD are similar to those in active caspase 9 (Fig. 1d, e), consistent with ctSPD being an active enzyme. Thus, separase activation does not require proteolytic cleavage of L4. Consistent with the importance of the L4 loop, mutations of two residues adjacent to C2110, M2108 and S2112, reduced the activity of ctSPD (Fig. 2a, b and Extended Data Fig. 4b). In contrast, mutations of L4 residues distal to C2110, including E2120 and F2121, enhanced the activity of ctSPD.

A segment of the N-terminal tag of recombinant ctSPD binds to a conserved surface pocket in PPD adjacent to L4 (Figs 1d, 2c and Extended Data Fig. 4c). Although this tag is not required for the activity of ctSPD, mutations targeting residues in the tag-binding pocket altered the activity of ctSPD containing the tag (Fig. 2d and Extended Data Fig. 4d). Similar to mutations of the distal L4 residues, the D1698K and D1960K mutations enhanced the activity of ctSPD. We propose that securin or other regions of separase may bind to this site, altering the conformation of L4, and affect the protease activity of separase. Even without bona fide ligands, binding of an artificial tag to this site can regulate the protease activity of ctSPD in a subtle way.

Unlike active caspase 9, which forms a homodimer\(^19\), separase contains an internal PPD in the same polypeptide chain that packs...
against and stabilizes its APD. In particular, the helical insert of PPD makes extensive contacts with APD and bridges the two sub-domains (Extended Data Fig. 5a, b). Deletion of the helical insert or mutations of key residues at the helical insert–APD interface, including D1805 and W2143, abolished the expression of soluble cSPD in bacteria (Fig. 2e and Extended Data Fig. 5a–c). Several helical-insert residues, including C1782 and H1783, are located close to the active site (Extended Data Fig. 5a). Mutations of these residues did not affect the solubility of cSPD, but reduced the protease activity (Extended Data Fig. 5d). Moreover, residues from the tip of the helical insert, along with residues from APD, form a basic pocket that binds a citrate molecule (Fig. 2f). Mutations of these conserved residues, with the exception of R1794E, diminished separase activity (Fig. 2e and Extended Data Fig. 1). Therefore, the helical insert is critical for both the structure integrity and activity of separase.

Phosphorylation of Scc1 by Plk1 enhances Scc1 cleavage by separase. This cleavage-enhancing phosphorylation is opposed by the hydrophobic/hydrophilic residues.

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Expectedly, the ctsecurin–ctseparase complex was less active in ctsCsc1 cleavage, compared with ctsSPD (Extended Data Fig. 9a, b). A conserved EVE motif in securin matches the separase cleavage consensus at positions P2–P6, but lacks the arginine at P1 and often has a proline at P0 instead of a hydrophilic residue (Fig. 4a). A securin mutant with three residues in this motif mutated was cleaved by separase in fission yeast cells.13 We thus mutated P164 and P165 in ctssecurin to R and D, and the matching ctsCsc1 residues at P1 and P0. The resulting ctssecurinRD mutant was efficiently cleaved by ctsSPD, and this cleavage was inhibited by the AMK inhibitor (Fig. 4b). Mutating the phosphoserine-binding residues in ctsSPD or E159 in ctssecurinRD reduced cleavage (Fig. 4c and Extended Data Fig. 9c), indicating that this artificial substrate bound at the canonical substrate-binding sites of separase. ctssecurin bound tightly to the N-terminal ARM domain of ctsseparase (Extended Data Fig. 9d). A synthetic EVE-containing securin peptide did not inhibit ctsSPD (Extended Data Fig. 9e). We propose that securin acts as a pseudo-substrate to competitively block substrate binding to separase (Fig. 4d). Securin binding to the ARM domain of separase provides the necessary avidity for securin to outcompete authentic substrates for access to the active site. Securin is not cleaved because of incompatible residues at the site of cleavage.

As a crucial protease that triggers chromosome segregation, separase is a potential oncoprotein29. Because of the conserved principles of substrate recognition (Extended Data Fig. 7), our structure of an active fungal separase can guide the rational design of chemical inhibitors of human separase, which may have therapeutic potential.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper. References unique to these sections appear only in the online paper.

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Author Information Atomic coordinates and structure factors have been deposited in the Protein Data Bank under accession numbers 5FBY, 5FC3, and 5FC2. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to H.Y. (hongtao.yu@utsouthwestern.edu).
METHODS

No statistical methods were used to predetermined sample size. The experiments were not randomized. The investigators were not blinded to allocation during experiments and outcome assessment.

Expression and purification of cSPD. The ctsccurin cDNA (GenBank identity 18261092) was synthesized at GenScript USA. For the expression of the cSPD, the cDNA fragment of cSPD was subclone into a modified pET bacterial expression vector. The pET-cSPD vector encoded cSPD with an N-terminal His6 tag following sequence: MGSSHHHHHHSSGLVVLVQPGGSLPR. The pET-cSPD vector was transformed into Escherichia coli strain BL21(DE3). Protein expression was induced with iso- propylthiogalactoside (IPTG) at 18 °C overnight. The bacteria were harvested and resuspended in the lysis buffer (50 mM Tris-HCl, pH 8.0, 200 mM NaCl, 5% glyc erol, 1 mM DTT, and 0.05% Triton X-100). After sonication and centrifugation, the supernatant was applied to Ni2+ -NTA resin (Qiagen). After extensive washing, His6-cSPD was eluted from the Ni2+ -NTA column. His6-cSPD was further purified with a mono Q 5/50 GL anion-exchange column (GE Healthcare) and a Superdex 200 10/300 GL column. The point mutants of cSPD were generated with a QuickChange Lightening Site-Directed Mutagenesis kit (Agilent Technologies). The truncated variants and point mutants of cSPD were expressed and purified similarly. Because cSPD underwent autolysis at the absence or presence of 10% glycerol, 5% DTT, and 0.05% Triton X-100. After sonication and centrifugation, the supernatant was applied to Ni2+ -NTA resin (Qiagen). After extensive washing, His6-cSPD was eluted from the Ni2+ -NTA column. His6-cSPD was further purified with a mono Q 5/50 GL anion-exchange column (GE Healthcare) and a Superdex 200 10/300 GL column. The point mutants of cSPD were generated with a QuickChange Lightening Site-Directed Mutagenesis kit (Agilent Technologies). The truncated variants and point mutants of cSPD were expressed and purified similarly. Because cSPD underwent autolysis at the absence or presence of 10% glycerol, 5% DTT, and 0.05% Triton X-100. After sonication and centrifugation, the supernatant was applied to Ni2+ -NTA resin (Qiagen). After extensive washing, His6-cSPD was eluted from the Ni2+ -NTA column. His6-cSPD was further purified with a mono Q 5/50 GL anion-exchange column (GE Healthcare) and a Superdex 200 10/300 GL column. The point mutants of cSPD were generated with a QuickChange Lightening Site-Directed Mutagenesis kit (Agilent Technologies).

Separase activity assay. The cSCc1 cDNA (GenBank identity 18259702) was synthesized at GenScript USA and was cloned into a modified pcRSV vector with a SP6 promoter. To produce 35S-cSCc1 or its mutants, the pcRSV-cSCc1 plasmids were added to a TNT Quick Coupled Transcription Translation System (Promega) and incubated overnight at room temperature to form covalent complexes as monitored by SDS–PAGE. The complexes were further purified with a Superdex 200 10/300 GL size-exclusion column in the buffer containing 20 mM Tris-HCl (pH 8.0), 200 mM NaCl, and 5 mM DTT. Crystals of cSPD1632–2223–pAMK were grown by mixing equal volumes of the protein solution (1 mg ml−1) with the precipitant solution containing 0.2 M ammonium citrate tribasic (pH 7.0) and 12% PEG3350. For the crystallization of cSPD1693–2223–pAMK complex, the 11 mg ml−1 protein solution was mixed with an equal volume of the precipitant solution containing 0.2 M KCl, 0.3 M HEPES (pH 7.5), 32% pentaerythritol propoxylate (5/4 PO/OH), and 10 mM DTT. Diffraction data were collected for cSPD1632–2223–AMK and cSPD1693–2223–pAMK were collected at beamline 19-ID (SRC-CAT) at the Advanced Photon Source (Argonne National Laboratory) at 100 K and processed with HKL3000 (ref. 32).

FOR CRISTALIZATION AND DATA COLLECTION. All crystallization experiments were performed at 20 °C. Initial screens were performed with a Phoenix crystallization robot (Hauschild) using the commercially available screening kits from Hampton Research, Qiagen, and Molecular Dimensions. Conditions obtained from the initial screens were optimized using hanging-drop vapour diffusion method. Diffraction-quality crystals were obtained by repeated microseeding. All crystals were cryoprotected with a reservoir solution supplemented with 15% glycerol.

Both native and SeMet-labelled cSPD1663–2223 crystals were grown by mixing equal volumes of the protein solution (11 mg ml−1) with the precipitant solution containing 0.2 M ammonium citrate tribasic (pH 7.0), 7.9% PEG3350, and 10 mM DTT. Diffraction data were collected at beamline BL8.2.1 at the Advanced Light Source (Lawrence Berkeley National Laboratory) at the wavelength of 0.9786 Å at 100 K and processed with HKL3000 (ref. 32).

For crystallization of cSPD1632–2223–AMK and cSPD1693–2223–pAMK complexes, the purified cSPD proteins were mixed with the cSCc1–AMK or phospho-cSCc1–AMK peptide inhibitors (KareBay Biochem) at a molar ratio of 1:2.5, and incubated overnight at room temperature to form covariant complexes as monitored by SDS–PAGE. The complexes were further purified with a Superdex 200 10/300 GL size-exclusion column in the buffer containing 20 mM Tris-HCl (pH 8.0), 200 mM NaCl, and 5 mM DTT. Crystals of cSPD1663–2223–pAMK were grown by mixing equal volumes of the protein solution (11 mg ml−1) with the precipitant solution containing 0.1 M ammonium citrate tribasic (pH 7.0) and 12% PEG3350. For the crystallization of cSPD1693–2223–pAMK complex, the 11 mg ml−1 protein solution was mixed with an equal volume of the precipitant solution containing 0.2 M KCl, 0.3 M HEPES (pH 7.5), 32% pentaerythritol propoxylate (5/4 PO/OH), and 10 mM DTT. Diffraction data were collected for cSPD1632–2223–AMK and cSPD1693–2223–pAMK were collected at beamline 19-ID (SRC-CAT) at the Advanced Photon Source (Argonne National Laboratory) at 100 K at wavelengths of 0.9793 Å and 0.9795 Å, respectively, and processed with HKL3000.

Structure determination and refinement. The crystal of SeMet-labelled cSPD1663–2223 disfavored to a minimum Bragg spacing of 2.2 Å and exhibited the symmetry of space group P212121 with cell dimensions of a = 55.67 Å, b = 98.79 Å, c = 107.76 Å. Phases were obtained from the selenium single-wavelength anomalous diffraction method. With data truncated to 2.5 Å, nine of ten possible selenium sites were located and refined with PHENIX AutoSol, resulting in an overall figure of merit of 0.323. The electron density electron map was used to construct an initial model with automated building with PHENIX AutoBuild. As a result, 414 of total 587 residues were built in the initial model, with Rwork and Rfree of 27.74% and 32.79%, respectively. Iterative model building and refinement were performed with Coot (31), Coot (32), and PHENIX. Phases of native cSPD1663–2223 crystals were obtained by molecular replacement with Phaser using the SeMet crystal structure as the search model. Data collection and structure refinement statistics are summarized in Extended Data Table 1. Ramachandran statistics (favoured/allowed/outlier (%)) calculated by MolProbity (35) for cSPD1663–2223, cSPD1632–2223–AMK, and cSPD1693–2223–pAMK were obtained by molecular replacement with Phaser using the SeMet crystal structure as the search model. All structural figures were generated with the program PyMOL (http://www. pymol.org/) using the same colour and labelling schemes.

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Extended Data Figure 1 | Sequence alignment of the SPDs from multiple species. The alignment is generated using the online ESPript 2.0 server. Secondary structural elements of ctSPD are indicated above the sequences, with the same labelling and colour schemes as in Fig. 1d (PPD, blue; APD, green; the helical insert in PPD, cyan). Abbreviations: ct, Chaetomium thermophilum; sc, Saccharomyces cerevisiae; sp, Schizosaccharomyces pombe; xt, Xenopus tropicalis; hs, Homo sapiens.
Extended Data Figure 2 | Purification, activity, inhibition, and autocleavage of active ctSPD. **a**, Coomassie-stained gel of purified recombinant ctSPD wild type (WT) and C2110S. **b**, Autoradiograph of the ctSPD cleavage assay with $^{35}$S-ctSc1 as substrate. **c**, Chemical structure of the acyloxymethyl ketone (AMK) inhibitor derived from the ctSc1 cleavage site. **d**, Autoradiograph of the ctSPD cleavage assay with $^{35}$S-ctSc1 as substrate, in the absence or presence of increasing doses of the AMK inhibitor depicted in **c**. **e**, Coomassie-stained SDS–PAGE gel of purified recombinant ctSPD WT or C2110S treated with the indicated doses of the ctSc1-AMK peptide inhibitor. The positions of unmodified ctSPD and ctSPD–inhibitor conjugates are indicated. **f**, Coomassie-stained gel of recombinant ctSPD$^{1632-2222}$ WT or non-cleavable (NC) mutant. The ctSPD$^{NC}$ mutant contains the E1643R and R1646E mutations. The positions of intact and autocleaved ctSPD proteins are indicated.
Extended Data Figure 3  |  Comparison between the folding topologies of \(ct\)SPD and the caspase 9 dimer (Protein Data Bank accession number 1JXQ). The labelling and colour schemes are the same as in Fig. 1d, e. H, the catalytic histidine; C, the catalytic cysteine.
Extended Data Figure 4 | Contributions of the L4 loop and a surface pocket to the protease activity of ctSPD. a, Cartoon of the crystal structure of ctSPD, with the PPD coloured blue, the APD coloured green, the helical insert in PPD coloured cyan, and an N-terminal tag peptide coloured yellow. The N and C termini are indicated. All secondary structure elements are labelled. Loops with no visible electron densities are indicated by dashed lines. Loop 4 (L4) is coloured magenta. H2083 and C2110 of the catalytic dyad are shown as sticks. The orientation of ctSPD in this figure is related to that in Fig. 1d by a 180° rotation along the vertical axis. c, Cartoon of the crystal structure of ctSPD, in the same orientation as in Fig. 1d. The tag peptide (HSQLEVLFQGP) is shown as sticks, overlaid with its 2Fo−Fc electron density map contoured at 1.0σ. d, Representative autoradiograph of the 35S-ctScc1 cleavage assay by WT ctSPD or the indicated mutants. Bottom: Coomassie-stained gel of ctSPD proteins used in the assay. Quantification of the relative protease activities of ctSPD WT and mutants is shown in Fig. 2b. The protease activity is defined as the ratio between intensities of the two major ctScc1 cleavage products and that of the uncleaved ctScc1.
Extended Data Figure 5 | Interactions between the helical insert and the APD. 

**a, b,** Zoomed-in views of cartoons of ctsPD in two orientations that are related by a 180° rotation along the vertical axis. Residues at the interface between the helical insert of the PPD and the APD are shown in sticks and labelled. 

**c,** Coomassie-stained gel of lysates of bacteria expressing the indicated ctsPD mutants and treated without (−) or with (+) isopropyl β-D-1-thiogalactopyranoside (IPTG) and eluates from Ni²⁺-NTA beads that had been incubated with the IPTG lysates. 

**d,** Autoradiograph of the 35S-ctsC1 cleavage assay by WT ctsPD or the indicated mutants. Bottom: Coomassie-stained gel of ctsPD proteins used in the assay.
Extended Data Figure 6 | Phospho-regulation and specificity determinants of separase-mediated cohesin cleavage. a, Autoradiograph of the ctSPD cleavage reactions of 35S-ctScc1 WT or S210A, treated with or without human (hs) Plk1 or its inhibitor BI2536. b, Autoradiograph of the ctSPD cleavage reactions, with 35S-ctScc1 WT or the phospho-mimicking S210E as substrates. c, Zoomed-in view of the cartoon of ctSPD bound covalently to the ctScc1-AMK inhibitor. The catalytic dyad residues C2110 and H2083 are shown as red sticks. The covalently bound inhibitor is shown as yellow sticks, overlaid with its $2F_o-F_c$ electron density map contoured at 1.0$\sigma$. d, Zoomed-in view of the S4 pocket of ctSPD that recognizes the P4 glutamate. Dashed lines indicate hydrogen bonds or favourable electrostatic interactions. The orange sphere indicates a water molecule. e, Mapping of the aberrant ctScc1 cleavage site by ctSPD D2151A. Top: sequence alignment of the aberrant site of D2151A and the major site of WT. Bottom: autoradiograph of the cleavage reactions of ctSPD WT or ctSPD D2151A with the indicated 35S-ctScc1 proteins as substrates. Asterisk marks the aberrant cleavage product by ctSPD D2151A. f, Charge reversal mutants of ctSPD fail to cleave complementary charge reversal mutants of ctScc1. Autoradiograph of the cleavage assay of WT ctSPD or the indicated mutants, with 35S-ctScc1 WT or mutants as substrates. Bottom: Coomassie-stained gel of ctSPD proteins used in the assay.
Extended Data Figure 7 | Conservation of substrate-binding residues in human separase. a, Two different views of the cartoon of the structure of ctSPD–pAMK (green) and a homology model of human (hs) SPD (magenta). The phospho-AMK peptide is shown in sticks. The homology model of hsSPD was generated with SWISS-MODEL. The coordinates of the model are available upon request. b, c, Zoomed-in views of the S1 and S4 pockets of the hsSPD model.
Extended Data Figure 8 | Structural basis of phosphorylation-stimulated Scc1 cleavage. a, Autoradiograph of the ctSPD cleavage reactions of 35S-ctScc1 WT or I211A, treated with or without hsPlk1. b, Zoomed-in view of the surface drawing of ctSPD–pAMK. The surface is coloured according to the electrostatic potential, with red, blue, and white representing negative, positive, and neutral charges, respectively. The covalently bound peptide is shown as sticks. c, Coomassie-stained gel of the indicated ctSPD proteins used in the assays described in Figs 3f and 4c. d, Quantification of the fold of Plk1 stimulation in ctScc1 cleavage by ctSPD WT and the indicated mutants as described in Fig. 3f. Error bars, s.d. (n = 3 independent experiments).
Extended Data Figure 9 | Interactions between ctscc1 and ctssepase.

a, Coomassie-stained gel of recombinant ctssepase–ctscc1 complexes and ctsPD expressed in insect cells. FL, full length. b, Autoradiograph of the ctscc1 cleavage reactions by the ctssepase–ctscc1 complexes and ctsPD. c, Autoradiograph of the cleavage reactions of 35S-ctscc1 WT or mutants with or without ctsPD. d, Coomassie-stained gel of recombinant Strep-tagged ctssepase1–1500 or the ctssepase1–1500–ctscc1 complex bound to Strep-Tactin beads. e, Autoradiograph of the ctscc1 cleavage reactions by ctsPD, in the absence or presence of varying concentrations of the ctscc1153–177 or ctscc1153–177 3A peptides. The EVE motif is mutated to AAA in the ctscc1153–177 3A peptide.
## Extended Data Table 1 | Data collection and refinement statistics

|                      | ctSPD\(^{1663-2223}\) | ctSPD\(^{1653-2223-AMK}\) | ctSPD\(^{1693-2223-pAMK}\) |
|----------------------|------------------------|-----------------------------|-------------------------------|
| **Data collection**  |                        |                             |                               |
| Space group          | P2\(_1\bar{1}2\_1\)   | P6\(_2\_2\_2\_1\)          | P2\(_1\bar{1}2\_1\)           |
| **Cell dimensions**  |                        |                             |                               |
| \(a, b, c\) (Å)      | 55.56, 98.89, 107.75   | 149.15, 149.15, 115.63      | 56.35, 85.01, 119.27          |
| \(\alpha, \beta, \gamma\) (°) | 90, 90, 90             | 90, 90, 90                  | 90, 90, 90                   |
| Resolution (Å)       | 50.00-1.90 (1.93-1.90) * | 50.00-3.10 (3.15-3.10) | 50.00-1.85 (1.88-1.85)        |
| \(R_{merge}\) (%)    | 7.4 (97.0)             | 15.5 (100)                  | 13.5 (100)                    |
| \(t / \alpha\)       | 27.7 (2.5)             | 14.6 (1.3)                  | 19.8 (1.9)                    |
| Completeness (%)     | 100 (100)              | 99.9 (98.7)                 | 100 (100)                     |
| Redundancy           | 7.2 (7.3)              | 9.6 (8.7)                   | 7.5 (5.8)                     |
| **Refinement**       |                        |                             |                               |
| Resolution (Å)       | 49.38-1.90             | 48.82-3.10                  | 42.51-1.85                    |
| No. reflections      | 46925                  | 14228                       | 50139                         |
| \(R_{work} / R_{free}\) | 17.3 / 20.0            | 19.4 / 25.8                 | 18.0 / 20.7                   |
| No. atoms            |                        |                             |                               |
| Protein              | 3937                   | 3745                        | 3730                          |
| Ligand/ion           | 13                     | 36                          | 134                           |
| Water                | 394                    | 52                          | 454                           |
| **B-factors**        |                        |                             |                               |
| Protein              | 27.4                   | 78.4                        | 26.6                          |
| Ligand/ion           | 32.6                   | 86.0                        | 29.8                          |
| Water                | 37.1                   | 66.3                        | 38.4                          |
| **R.m.s deviations** |                        |                             |                               |
| Bond lengths (Å)     | 0.012                  | 0.010                       | 0.013                         |
| Bond angles (°)      | 1.24                   | 1.13                        | 1.05                          |

Data were collected from one crystal for each structure.

*Highest-resolution shell is shown in parenthesis.