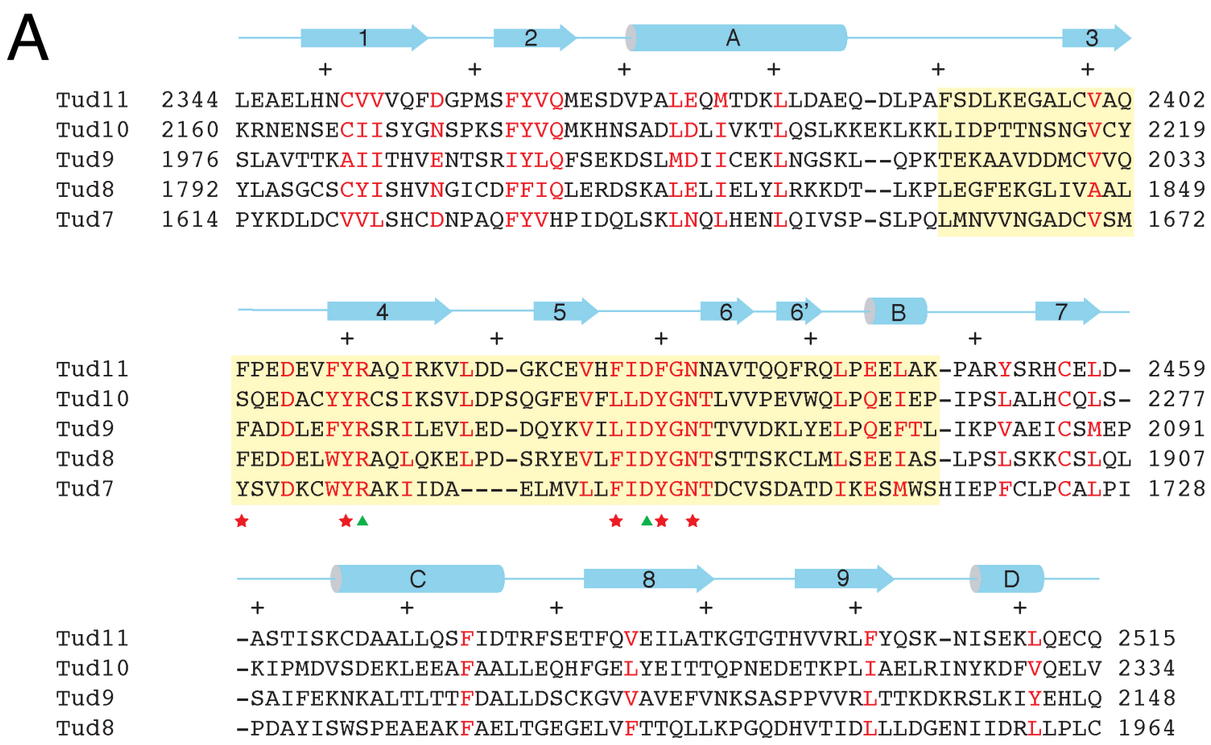
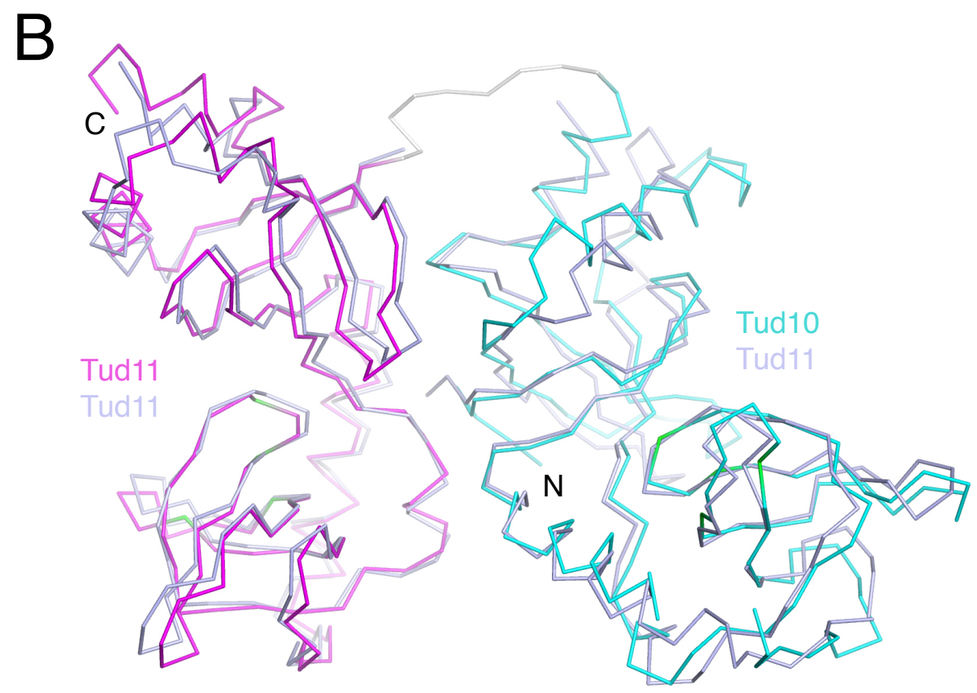
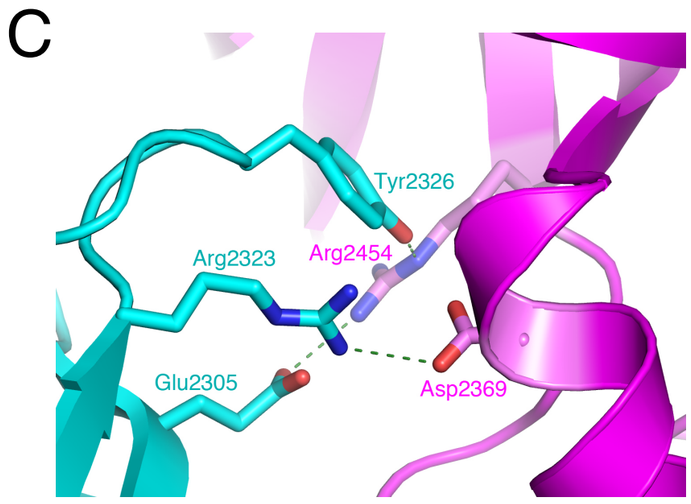
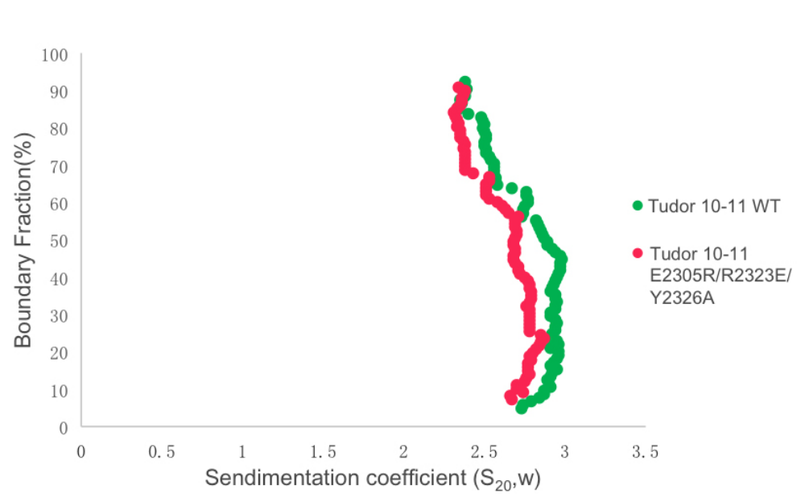
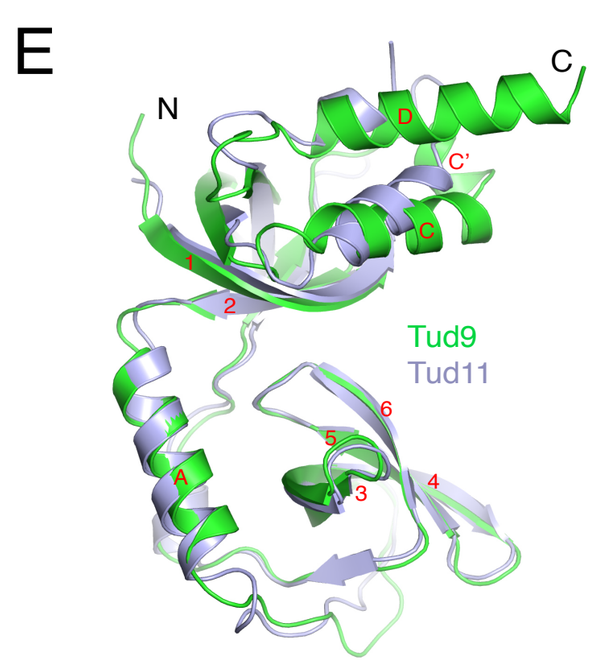
Supplementary Figure S1

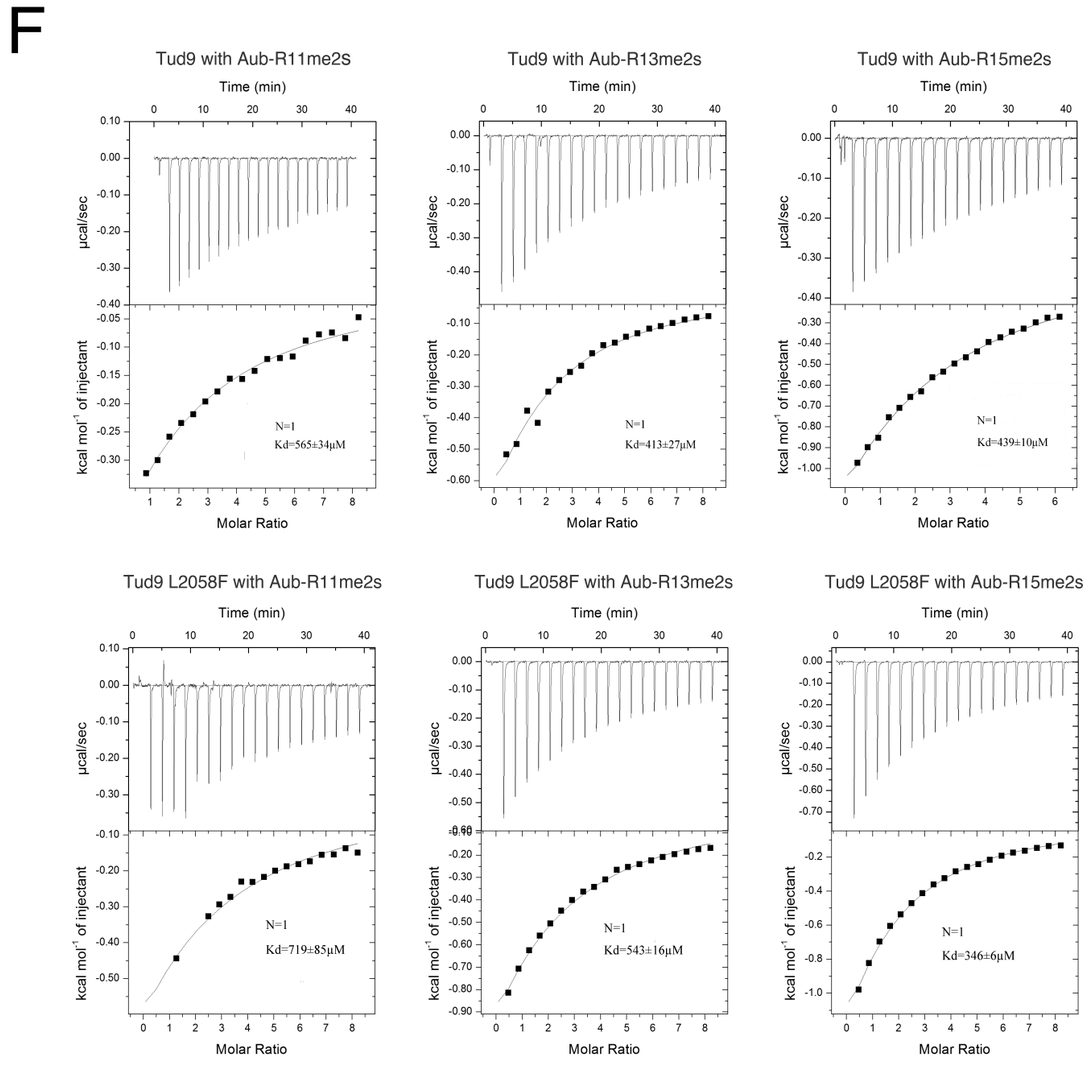












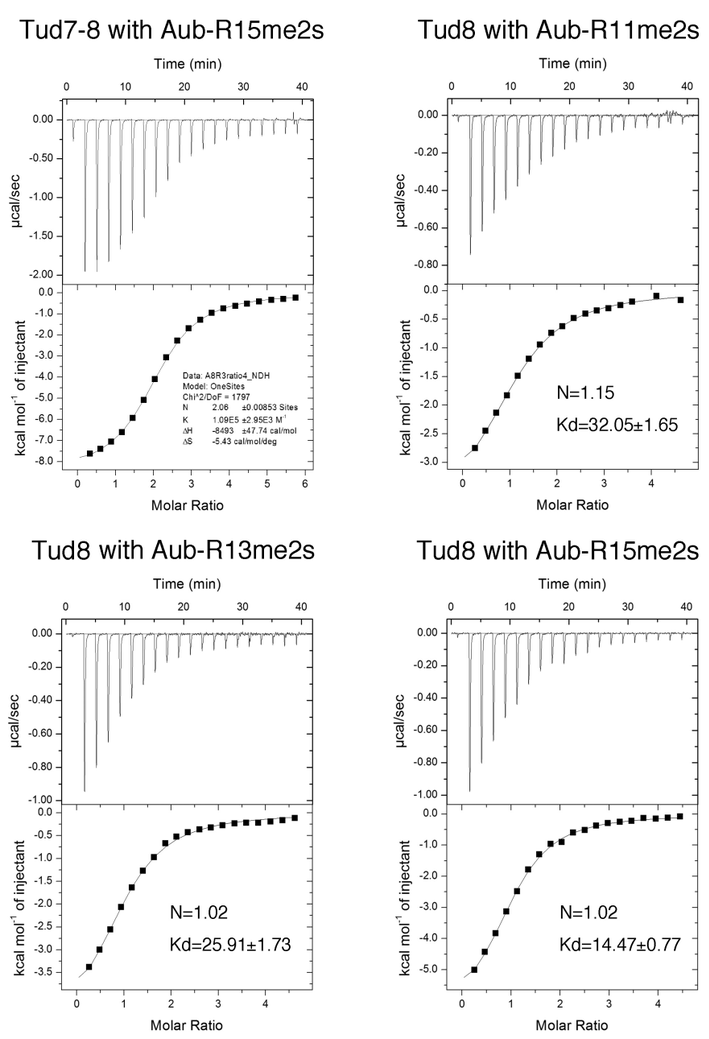


Figure S1. Structural, biochemical and biophysical characterizations of Tud7-11. (A) Sequence alignment of Tud7 to Tud11. Regions correspond to the canonical tudor domain is highlighted in yellow. Amino acids similar in all five domains or identical in four domains are shown in red. An asterisk at the bottom of the sequences marks the position of residues forming the sDMA-binding pocket, and the green triangles mark the conserved pair of charged residues involved in stabilizing the sDMA-binding pocket. Secondary structural elements of Tud11 are indicated above the sequence. (B) Superposition of the Tud11 structure from the Tud11-pep complex (light blue) with that of the eTud10 and eTud11 modules. (C) Interdomain hydrogen bonds and charged interactions between selected Tud10 and Tud11 residues. (D) The E2305R/R2323E/Y2326A mutant (red) and the wild-type (green) Tud10-11 proteins sedimented differently in analytic ultracentrifugation experiments, indicating an altered overall structure of the mutant protein. (E) Superposition of the Tud9 structure (green) with that of Tud11 (light blue) in the Aub-pep complex. (F) ITC measurements of binding affinities of wild-type and L2058F mutant of Tud9 to Aub peptides symmetrically dimethylated at Arg11 (Aub-R11me2s), Arg13 (Aub-R13me2s) and Arg15 (Aub-R15me2s), as indicated. (G) The binding of Tud7-8 and Tud8 to indicated Aub peptides measured by ITC. (H) Tud7-11 model derived from the SAXS envelope. The docked ribbon model of Tud7-11 is superimposed with a semi-transparent surface representation. The sDMA binding pockets are colored blue. The arrow indicates the location of the sDMA-binding pocket of Tud8 in the back.

Supplementary Table S1. Statistics of crystallographic analyses

Data collection	Tud9-Pt	Tud9-Native	Tud10-11-Native
Wavelength (Å)	1.0706	0.9795	0.99985
Space group	$C222_1$	$P2_1$	$I2_12_12_1$
Unit cell			
a, b, c (Å)	58.5, 69.4, 104.4	37.2, 105.7, 52.6	71.2, 125.4, 151.7
α, β, γ (°)	90, 90, 90	90, 90, 100.6	90, 90, 90
Resolution (Å) (outmost shell)	50-2.70 (2.80-2.70)	50-1.80 (1.86-1.80)	50-3.0 (3.05-3.0)
Rmerge	0.108 (0.444)	0.092 (0.531)	0.068 (0.485)
I/σI	32.6 (10.8)	14.8 (2.6)	21.1 (1.9)
Completeness (%)	100 (100)	99.9 (99.9)	97.2 (79.8)
Total/unique reflections	164700/6196	153149/36887	68852/13606
Refinement			
R-work/R-free		18.0/22.6	23.6/27.1
rmsd bonds (Å)		0.009	0.008
rmsd angles (º)		0.976	1.53
B factor (Å2)			
Protein			
Main chain		19.4	89.8
Side chain		23.9	90.5
Ligand/ion		37.2	
Water		30.3	74.7
Ramachandran plot			
favored		352 (98.1%)	262 (81.9%)
allowed		7 (1.9%)	58 (18.1%)
outlier		0	0

Supplementary methods

Protein expression, purification and proteolytic analysis

cDNA segments encoding Drosophila Tud fragments Tud8 (a.a. 1791-1967), Tud9 (1978-2160), Tud10 (a.a. 2163-2340), Tud11 (a.a. 2344-2515), Tud7-8 (a.a. 1617-1961), Tud7-9 (a.a. 1617-2160), Tud9-11 (a.a. 1977-2515), Tud10-11 (a.a. 2163-2515) and Tud7-11 (a.a. 1617-2515) were cloned into the pET28a-Smt3 vector. The L2058F mutant of Tud9 was generated by site-directed mutagenesis. All recombinant polyhistidine-and-sumo-tagged proteins were expressed in the BL21(DE3)-RIL strain of *E. coli* by IPTG (0.5 mM) induction at 16 °C for 20 h. They were purified using the same protocol, namely, chelating the polyhistidine-tagged proteins through nickel resins, followed by cleavage of the sumo tag using sumo protease, and then sequentially through ion-exchange and gel-filtration column chromatography steps. Highly purified fractions were pooled and concentrated in the buffer with 50 mM Tris-HCl (pH 8.0), 100 mM NaCl and 1 mM DTT.

Purified Tud7-11 protein was subjected to analysis by limited proteolysis using V8 (Glu-C). Enzymatic digestion was carried out at room temperature with 10 μl of Tud7-11 stock solution at 1.8 μg/μl in 50 mM Tris pH 8.0, 150 mM NaCl and 1mM DTT and 0.36 μl of V8 protease at 0.1 ug/ul (1:500 w/w enzyme to protein ratio). Time course digestion samples at 1, 2, 5, and 16 hours were collected and analyzed by SDS-PAGE. The proteolytic fragments were transferred onto a PVDF membrane (GE healthcare) and the bands were excised for N-terminal sequencing by Edman degradation.

Crystallization and structure determination

Native Tud9 crystals were grown at 16 °C in 40 mM ammonium sulfate, 0.1 M Hepes (pH 7.5) and 22.5% PEG-3350 by the hanging drop vapor diffusion method. Platinum derivatives were prepared by soaking the crystals in the mother liquor supplemented with 1 mM of K_2PtCl_4 for 24 h. The 1.8 Å native data set was collected at wavelength (λ) of 0.9795 Å at beamline 1W2B of Beijing Synchrotron Radiation Facility (BSRF) using a MAR-165 CCD detector. A Pt-derivative

SAD data set was collected at λ=1.0706 Å at beamline BL17U of Shanghai Synchrotron Radiation Facility (SSRF) using an ADSC Q315r detector. All diffraction data were processed using HKL2000 [16]. The Pt-derivative crystal belongs to the $C222_1$ spacegroup. Two Pt sites were found using SHELXD [17], and the positions were used for phasing with PHENIX [18]. An initial model was built using PHENIX and COOT [19]. The native crystal belongs to the $P2_1$ spacegroup, and there are two molecules per asymmetric unit. The structure was solved by molecular replacement using the SAD-derived Tud9 structure as the search model. Structure refinement and model building were carried out using PHENIX and COOT.

Tud10-11 crystals grew at 20° C in 58%-60% Tacsimate (pH 7.0). A 3.0 Å data set was collected at beamline BL17U of SSRF using a Mar-225 CCD detector. Using a cryoprotectant composed of the mother liquor and 40% ethylene glycol helped improving the diffraction resolution. The crystal belongs to the $I2_12_12_1$ spacegroup, and has one Tud10-11 molecule per asymmetric unit. The structure was solved by molecular replacement using Phaser, with the structure of Tud11 from the Tud11-pep complexes (PDB IDs: 3NTH & 3NTI) as the search model. Model building and structure refinement were carried out using COOT and CNS, respectively [20].

Detailed statistics of crystallographic analyses are shown in Supplementary Table S1. Atomic coordinates of Tud9 and Tud10-11 and associated structure factors have been deposited in the PDB database with accession IDs 4Q5W and 4Q5Y, respectively.

Analytic ultracentrifugation

The E2305R/R2323E/Y2326A triple mutation of Tud10-11 was generated by PCR, and the mutant protein, in a buffer containing 50 mM Tris (pH 7.5) and 200 mM NaCl, was prepared the same way as the wild-type protein. Sedimentation experiments were performed on a Beckman Coulter ProteomeLab XL-I centrifuge with a 4-hole An-60 Ti rotor. The initial absorbance of the samples at the wavelength of 280 nm was ~1.0 and the samples were equilibrated for 1 h at 20℃

under vacuum in the centrifuge prior to analysis by sedimentation. The absorbance at 280 nm was measured by a continuous scan mode at 60,000 rpm in 12 mm double-sector cells. The data were analyzed by enhanced van Holde-Weischet analysis and the Ultrascan II 9.9 revision 1504. The average sedimentation coefficients (S_{ave}) were determined at the boundary midpoint.

Isothermal titration calorimetry (ITC) measurements

Aub peptides used for binding assays were purchased from SciLight Biotechnology. They all span residues 6 to 18, having the amino acid sequence of 6-NPVIARGRGRGRK-18. The three peptides, Aub-R11me2s, Aub-R13me2s and Aub-R15me2s, correspond to substitution of sDMA for unmodified arginine at residue 11, 13 and 15, respectively. ITC measurements were preformed at 25 ºC using an ITC200 calorimeter (MicroCal LLC). Experiments included 25 injections of 2 μl each of peptide solution (1-3 mM) into the sample cell (200 μl) filled with 60-300 μM of protein solution. Both the protein and peptide samples were in the buffer containing 50 mM Tris-HCl (pH 8.0), 150 mM NaCl and 0.1% β-mercaptoethanol. Background heat was measured by injecting the peptide into the buffer without protein added and subtracted from the integrated data. The ITC data were fitted with a one-binding-site model using Origin version 7.0 (OriginLab).

SAXS data collection and analysis

SAXS profile for Tud7-11 was collected at the X33 beamline of the DORIS III storage ring at the Deutsches Elektronen Synchrotron (DESY, Hamburg) [21]. The scattering data were recorded with X-ray beam at $\lambda = 0.15$ nm in the range of momentum transfer between 0.12 and 6 nm^{-1}. The momentum transfer is defined as $s=(4\pi\sin\theta)/\lambda$, where 2θ is the scattering angle. Only data in the range $0.12 < s < 2.2$ nm^{-1} were retained for further calculation due to low signal-to-noise ratio at the high momentum transfer realm. The experiments were preformed in a vacuum cuvette at 10 °C with 8 successive 15-second exposures to lower radiation damage. Data from samples at three different concentrations: 2.0, 4.0 and 6.0 mg/mL were measured, and they

were used for extrapolation of scattering curves to zero concentration for structural analysis. Standard procedures were used to process experimental scattering profiles using the PRIMUS program [22], and the data were corrected for background scattering by the solvent. GNOM [23] was used to compute the distance distribution function p(r), which helps the determination of the maximal size of the protein. Ab initio low-resolution envelope was generated using the program DAMMIN [24], and the crystal and modeled tudor domain structures were initially placed into the SAXS envelope using Chimera [25], followed by manual adjustment in Pymol [26].

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