Supplemental Information

Experimental Procedures

Cloning, expression, purification and mutagenesis

After codon optimization for expression in *E. coli*, the gene fragment of zhd101 was synthesized chemically and cloned into the vector pET-46 Ek/LIC. The *E. coli* strain BL21 (DE3) cells were transformed with pET-46 Ek/LIC-ZHD and grown in LB at 37 °C to an OD_{600nm} of 0.6, normally taking 4 hours. The medium was cooled to 16 °C and then expression of the protein was induced for 48 hours by adding IPTG. The protein rZHD with an N-terminal His-tag (MAHHHHHHVDDDDK) was purified via metal-affinity and ion-exchange chromatography by using Ni-NTA and DEAE-Sepharose Fast Flow columns. Following dialysis in a buffer of 150 mM NaCl and 25 mM Tris, pH 7.5, the protein was concentrated to 100 mg/ml by using Amicon Ultra-10k.

The production of SeMet-containing rZHD used the same *E. coli* strain and plasmid, but the LB medium was supplemented with SeMet. The cells took 24 hours to grow and the protein was expressed for 60 hours. All mutants were produced by using QuickChange Site-Directed Mutagenesis Kit (Strategene, La Jolla, CA) with the mutagenic oligonucleotides listed in Table S1. When verified by sequencing, the correct plasmids were transformed into *E. coli* for expression. Purification of the SeMet and mutant proteins followed that of native rZHD. The final protein concentrations were 77 mg/ml for SeMet and 90 mg/ml for S102A.

Crystallization, data collection and structure determination

Initial screening of crystallization conditions used sitting-drop vapor diffusion method with 22 sets of solutions from Hampton Research. Needle-like crystals of rZHD appeared under many different conditions, among which the PEG-based solutions were most promising. After optimization, the reservoir containing 24% PEG 2000 MME and 0.1 M Bis-Tris pH 6.5 yielded stable rod-like crystals in 1 day at 25 °C. S102A crystals were obtained under the same conditions. For SeMet crystals the PEG concentration was 26%.

Data collection was carried out at beam line BL13B1 of the National Synchrotron Radiation Research Center, Hsinchu, Taiwan. A cryoprotectant solution of 28% PEG 2000 MME, 10% glycerol and 0.1 M Bis-Tris pH 6.5 was used to soak the crystals before flash cooling to 100 K. The S102A/ZEN complex crystal was obtained by soaking the S102A crystals in a cryoprotectant solution that contains 10 mM ZEN for 7 hours. The diffraction intensities were integrated and scaled by using HKL2000 (Otwinowski & Minor 1997).

Using the 3.1 Å-resolution peak data set of SeMet, the heavy atom sites were found by SHELXD (Schneider & Sheldrick 2002) and then refined by SOLVE (Terwilliger 2003). Some statistics can be found in Table S2. All crystals belong to the space group $P6_122$ with three rZHD molecules in an asymmetric unit. The initial phase angles were significantly improved by OASIS (http://cryst.iphy.ac.cn). Subsequent model building by PHENIX (Zwart et al 2008) and BUCCANEER (Cowtan 2006) turned out 582 residues with side chains. The third rZHD molecule was found by

PHASER (McCoy et al 2007). The crystal structures were refined with non-crystallographic symmetry (NCS) restraints by COOT (Emsley & Cowtan 2004), CNS (Brunger et al 1998) and REFMAC (Murshudov et al 2011). The program PyMOL (http://pymol.sourceforge.net/) was used in figure preparation.

Oligomer determination

The rZHD sample was diluted to 0.5 mg/ml using a buffer of 15 mM NaCl and 25 mM Tris pH 7.5. Then 1 ml of the protein solution was loaded onto a HiLoad 16/60 Superdex 200 column (GE Healthcare) and eluted with the same buffer. A marker mixture of conalbumin, ovalbumin, carbonic anhydrase, ribonuclease A and aprotinin was used to estimate the molecular weight of rZHD in solution.

Activity measurement

The ZHD activity was determined by monitoring substrate degradation. Each reaction mixture (210 µl) contained 78.5 µM substrate (25 µg/ml ZEN) and 205 nM enzyme (6 µg/ml ZHD, wild-type or mutant) in a 150 mM NaCl, 25 mM Tris-HCl buffer (pH 7.5). After incubation at 30°C for 10 min, the reaction was terminated by adding 50 µl 1 N HCl and 300 µl methanol. Then 20 µl of the reaction mixture was filtered and analyzed by using a high-performance liquid chromatography system (HPLC; Agilent 1200) equipped with a Welch Ultimate XB-C18 column (4.6 mm X 250 mm, 5 µm; Welch Materials, Inc., Shanghai, China). Samples were eluted with 60% acetonitrile at a flow rate of 0.6 ml/min, and the absorbance was monitored at 254 nm. The amounts of remaining substrate were calculated from the peak areas under the HPLC curves. The assays were carried out in triplicate for the wild-type ZHD and all mutants.

Author Contributions

R.T.G., J.R.L. and Y.Z. designed and directed the research. W.P., Y.Y. and Z.Z purified and crystallized the proteins. T.P.K., C.H.H. and C.C.C. determined the crystals structures. Y.Y., Y.F.Z. and J.W.H. performed the activity assays. R.T.G., T.P.K. and A.H.-J.W. analyzed the structures. T.P.K. wrote the manuscript with input from co-authors. All authors reviewed the manuscript.

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Supp. Table S1. The oligonucleotides for site-directed mutagenesis.

Mutant	Sequence (5' --> 3')
W183H	CATAAAAATTACCCAGTCcacGCAAGAGGTTACCCTAGA
W183F	CATAAAAATTACCCAGTCttcGCAAGAGGTTACCCTAGA
W183A	CATAAAAATTACCCAGTCgcgGCAAGAGGTTACCCTAGA
V153A	TCTAAGATTTTGGCAAATgcgATGTTGAACGATGTATCA
V153D	TCTAAGATTTTGGCAAATgacATGTTGAACGATGTATCA
V153H	TCTAAGATTTTGGCAAATcacATGTTGAACGATGTATCA
V153Q	TCTAAGATTTTGGCAAATcagATGTTGAACGATGTATCA
V158D	TCTAAGATTTTGGCAAATgacATGTTGAACGATGTATCA
V158H	AATGTGATGTTGAACGATcacTCAGGAGGTAGTGAAGCA
P192S	GGTTACCCTAGAACTATTtccCCATCTGCTCCTGTAAAA
L132A	GAATTACCTACAAAGTTAgctGACCACTTATCAAATACA
H134A	CCTACAAAGTTACTGGACgcaTTATCAAATACAGCTGTA
L132A/H134A	TTACCTACAAAGTTAgctGACgcaTTATCAAATACAGCT
I225E/V226E	AGAATCATTTTTTGATAATgaagaaACTGCGACTAAAGCCGGGGTGA
F222R/I225E/V226E	GGCTACACCTACAGAATCATTTcgtGATAATgaagaaACTGCGACTAAAGCCGGGGTGA
S102A	GCCACTGTCTGGGGTTGTgcgAGTGGCGCTTCTACGGTT
S103A	TGTCTGGGGTTGTAGTgctGGCGCTTCTACGGTT
H242A	TTATTGCCAGGGATGgctTTTCCATATGTGTCA
D223A	CCTACAGAATCATTTTTTgctAATATTGTCACTGCGACTA
E126A	TTAGAAACGCTATGTGCCATgctTTACCTACAAAGTTACTGGAC

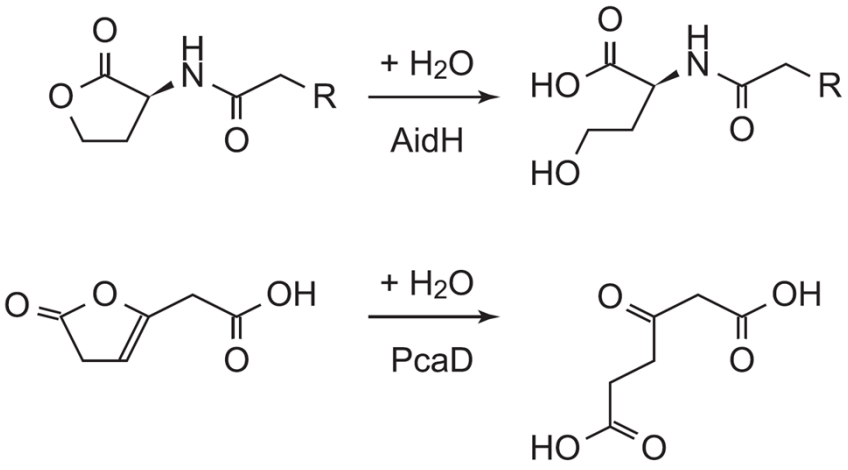
Supp. Table S2. Summary of Se-Met protein data collection and phasing statistics.

Name	Se-Met (peak)
Data collection	
Wavelength (Å)	0.97869
Space group	$P6_122$
Unit-cell a, b, c (Å)	86.0, 86.0, 474.1
Resolution (Å)	25 – 3.1 (3.21 – 3.10)
Unique reflections	19754 (1875)
Average redundancy	17.6 (8.8)
Completeness (%)	98.2 (97.3)
Average <I>/<σ(I)>	18.4 (2.9)
R_{merge} (%)[a]	18.6 (48.0)
Phasing	
No. of sites	14
Figure of merit	0.69

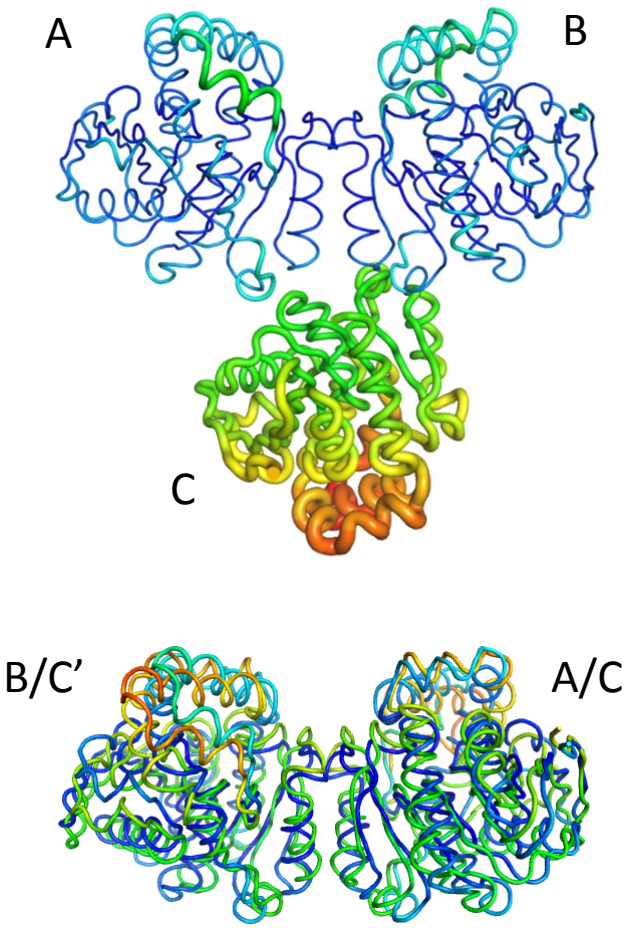
Values in parentheses are for the highest resolution shell.

Supp. Table S3. Data collection and refinement statistics of the rZHD crystals. Numbers in parentheses are for the outmost resolution shells. All positive reflections were used in the refinement.

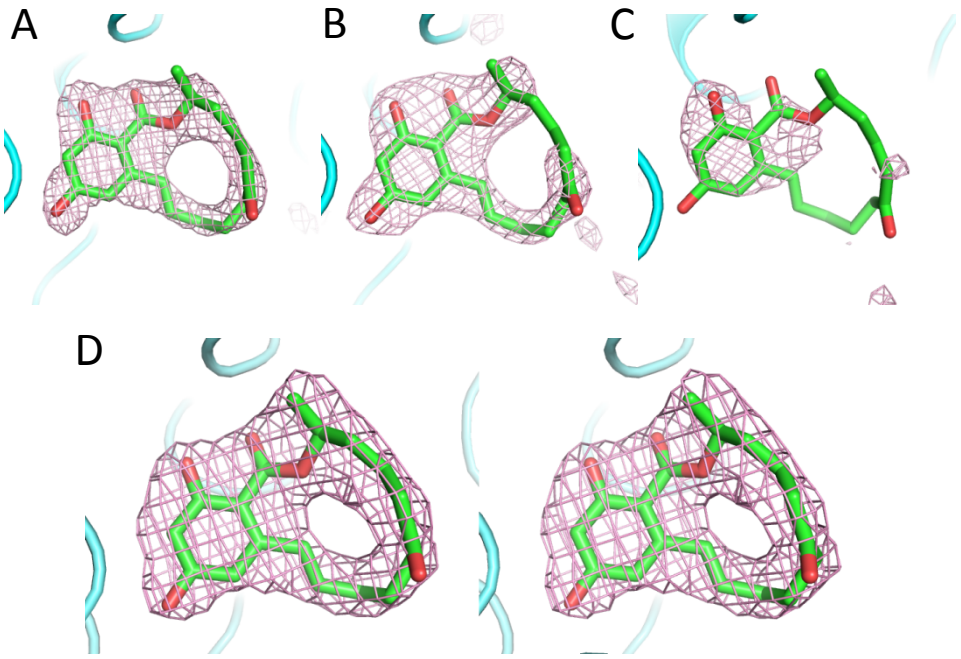
	Native	**S102A/ZEN**
Data collection		
Space group	$P6_122$	$P6_122$
Unit-cell a, b, c (Å)	86.6, 86.6, 474.0	86.8, 86.8, 471.8
Resolution (Å)	25 – 2.6 (2.69 – 2.60)	20 – 2.48 (2.57 – 2.48)
Unique reflections	33747 (3060)	38626 (3680)
Average Redundancy	9.1 (6.9)	6.2 (6.4)
Completeness (%)	99.1 (92.2)	99.4 (97.8)
Average <I>/<σ(I)>	37.6 (4.9)	34.0 (6.2)
R_{merge} (%)	6.5 (24.2)	7.3 (34.5)
Refinement		
No. of reflections	32825 (2767)	36961 (3294)
R_{work} (95% data)	0.211 (0.277)	0.202 (0.244)
R_{free} (5% data)	0.269 (0.327)	0.264 (0.313)
RMSD bonds (Å)	0.019	0.019
RMSD angles (°)	1.7	1.7
Ramachandran plot (%)		
Favored	91.6	94.0
Allowed	7.0	5.4
Outliers	0.4	0.6
Average B ($Å^2$)/non-H atoms		
Protein chains A, B, C	47.9, 47.9, 113.2 / 2022	34.8, 36.8, 91.3 / 2021
Ligands A, B, C		32.7, 35.8, 94.8 / 23
Water molecules	58.5 / 235	55.2 / 426
PDB code	3WZL	3WZM



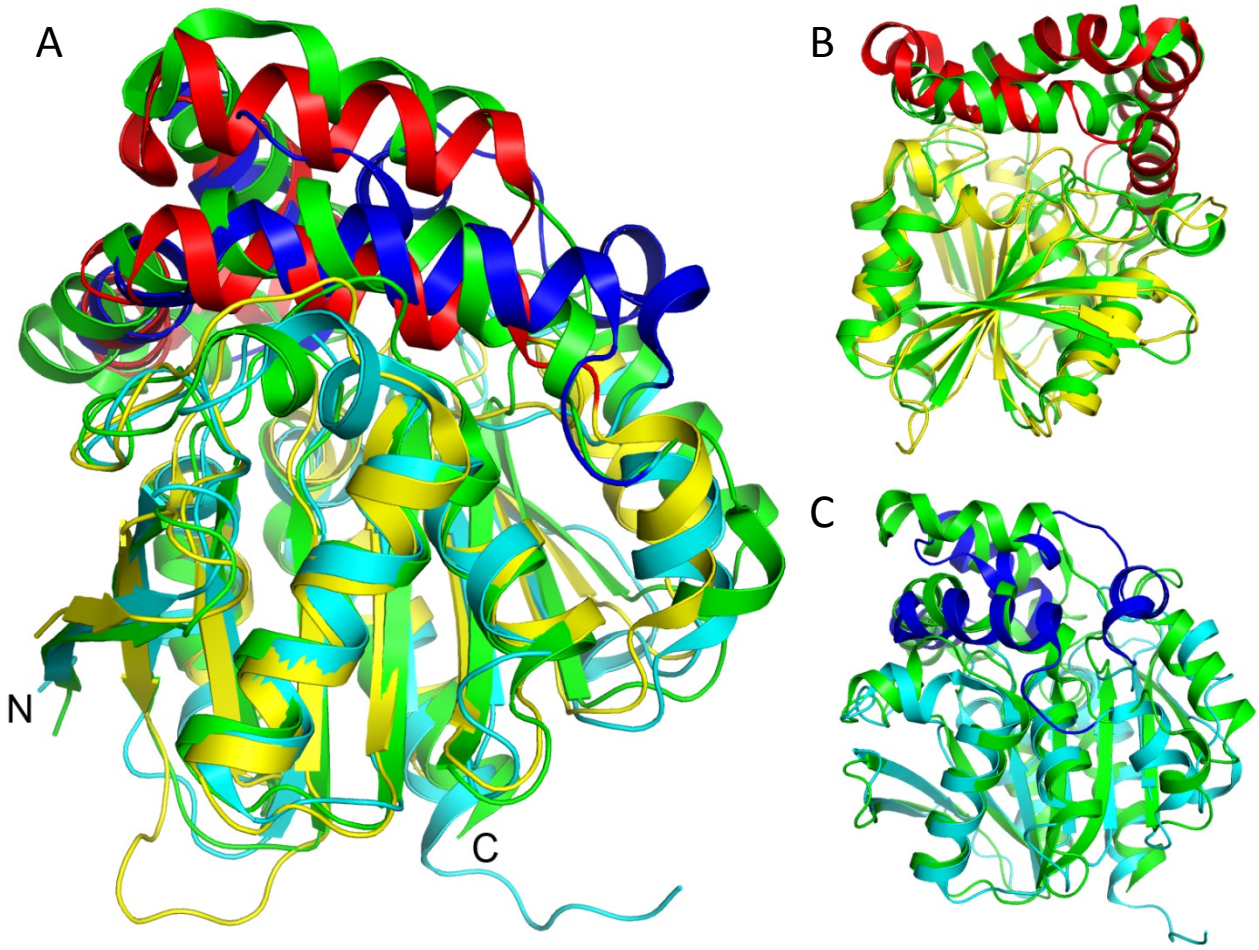
Supp. Figure S1. AHL (AidH) and enol-lactone hydrolase (PcaD) reactions



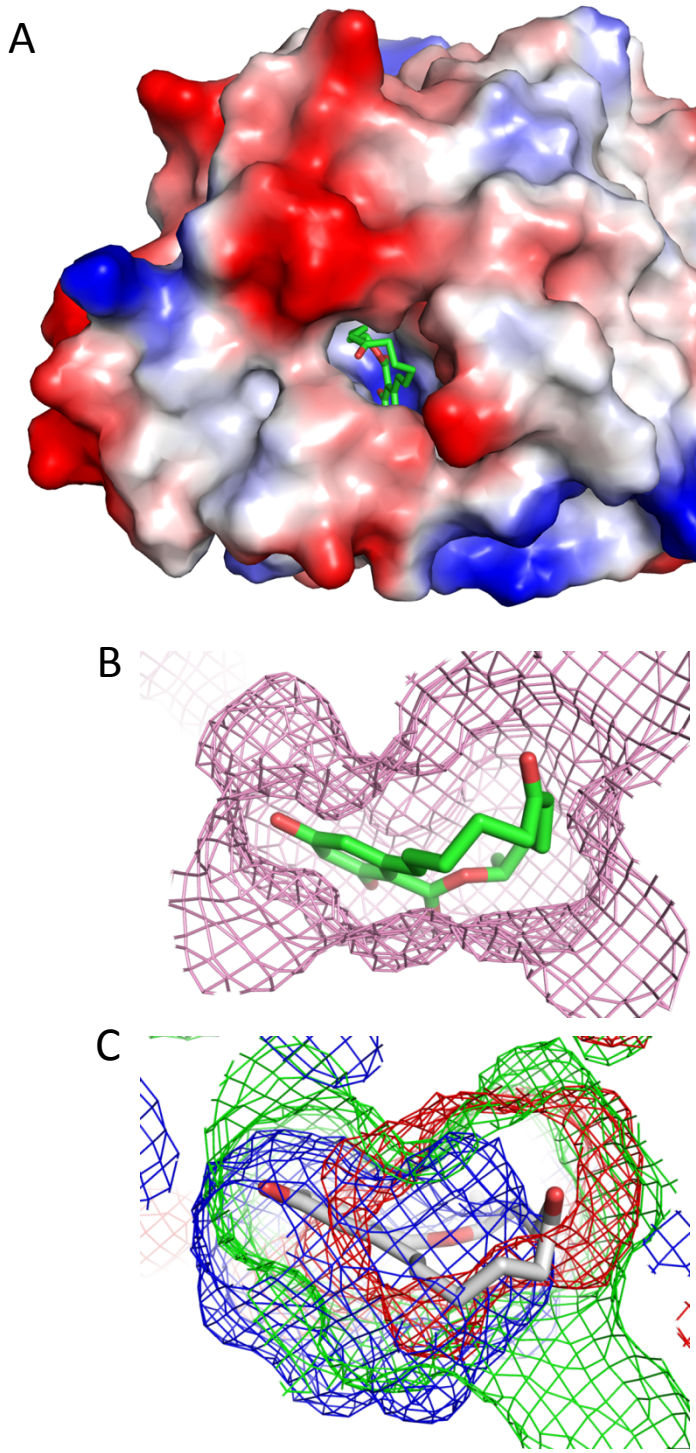
Supp. Figure S2. Top: Temperature factors of the three rZHD monomers in a crystallographic asymmetric unit. The monomers A, B and C are shown as worm models with rainbow colors from blue to red representing the lowest to the highest temperature factors. Above: The dimer formed by monomer C and its symmetry-related monomer C' is superimposed on the dimer of monomer A and B. A rigid-body rotation results in an RMSD of 3.4 Å between the two dimers. Perhaps there was packing imperfectness in the crystal (and thus the higher temperature factor of monomer C).



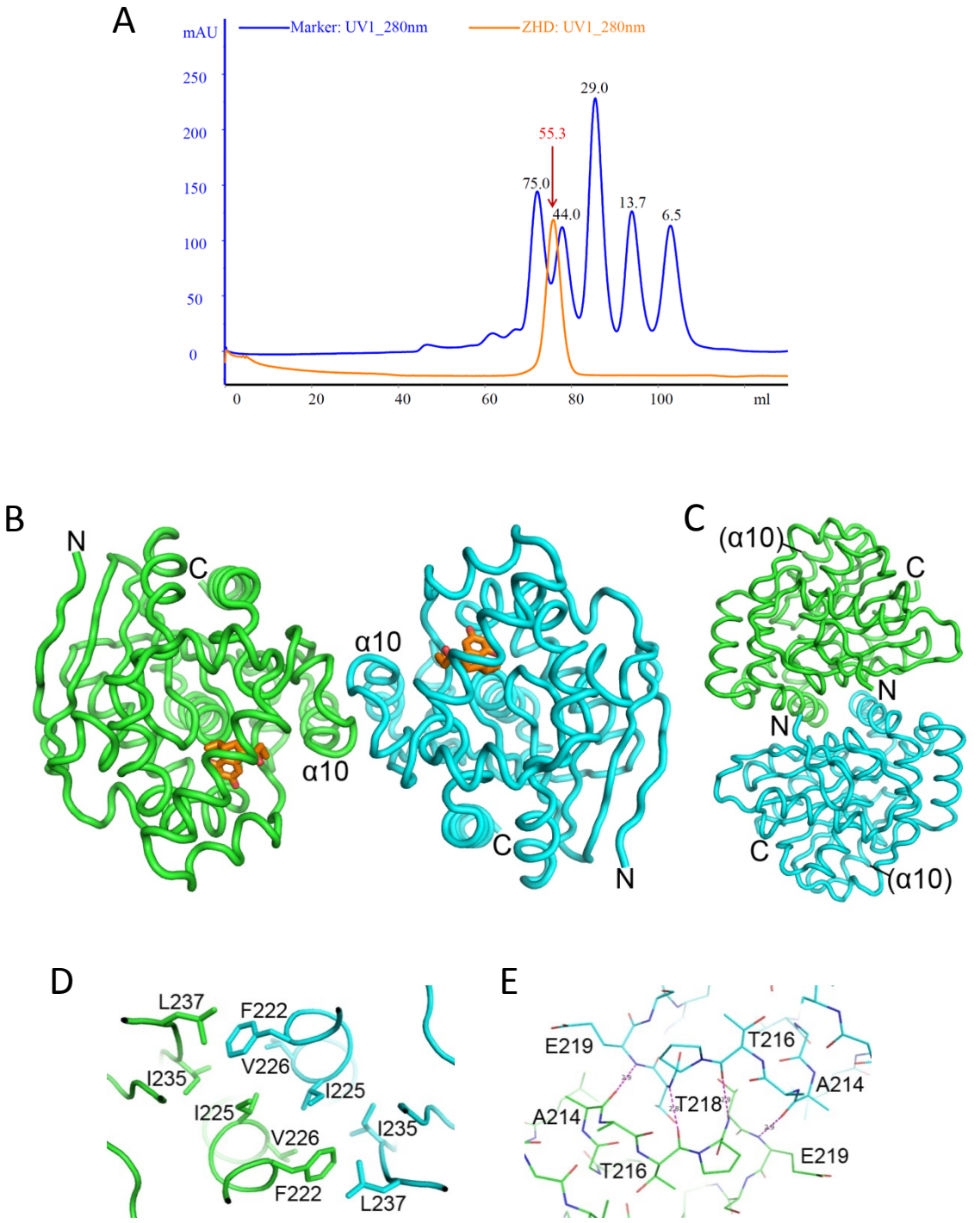
Supp. Figure S3. Electron density of ZEN. (A), (B) and (C) shows the initial Fo-Fc map in the active site of monomer A B and C, contoured at 3-sigma level. (D) shows a stereoview of the final omit map of ZEN bound to monomer A, also contoured at 3 sigma.



Supp. Figure S4. Structural comparison of rZHD with PcaD (PDB 2XUA) and AidH (4G9E). In (A) all three are superimposed. In (B) and (C) the same superpositions are viewed at slightly different angles to show the distinctive features. Color codes: rZHD = green; 2XUA = yellow core, red cap; 4G9E = cyan core, blue cap.



Supp. Figure S5. Substrate binding pockets. In (A) the molecular surface of a ZHD monomer is colored red and blue for electrostatic potential from -60 k_BT to +60 k_BT. The bound ligand is shown as a stick model with green carbon atoms. In (B) the same pocket is shown as a purple mesh in a close-up view. In (C) the ZEN model is in gray and the ZHD pocket green. The overlapped mesh representations are those from PcaD (red) and AidH (blue), after structural superposition.



Supp. Figure S6. Formation of a ZHD dimer. (A) The gel filtration chromatography profile of ZHD sample (orange) is superimposed on that of the mixed marker proteins. (B) Two monomers in a ZHD dimer are shown as worm models in green and cyan, each with a bound ZEN (orange) in the active site. (C) The dimer of PcaD is different from that of ZHD. (D) The ZHD dimer interface is shown in a close-up view centered at the clustered hydrophobic side chains. (E) This view shows the hydrogen bonding interactions, as pink dashes.