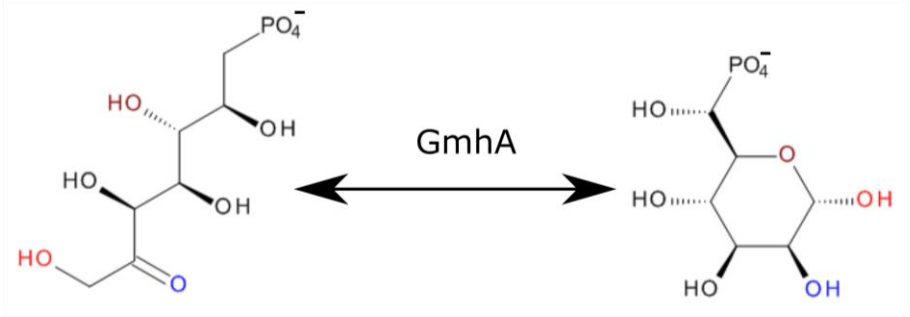
Vivoli, Pang and Harmer: A half-site multimeric enzyme achieves its cooperativity without conformational changes

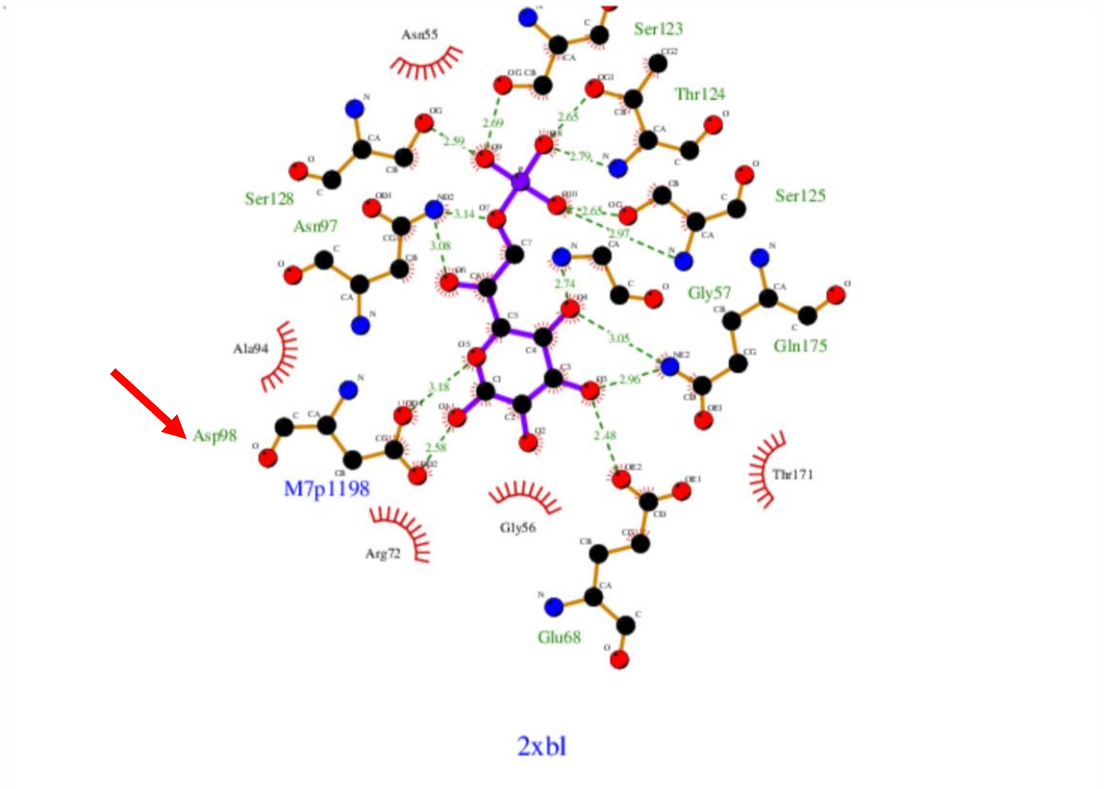
Supplementary information

Supplementary Figure 1: Catalytic action of sedoheptulose-7-phosphate isomerase (GmhA). a: Reaction catalyzed by GmhA. Sedoheptulose-7-phosphate (left, S7P) is isomerized into D-*glycero*-D-*manno*-heptopyranose 7-phosphate (right, M7P). The oxygen atoms that are involved in the reaction are colored red (O1), blue (O2) and brick red (O5) respectively to highlight the alteration in the molecule. **b:** GmhA interactions with product. The product M7P (D-*glycero*-D-*manno*-heptopyranose-7-phosphate) interacts widely with the enzyme active site. In particular, D98 (red arrow) makes hydrogen bonds to both O^1 and O^5. D98 is expected to therefore contribute strongly to the ring cyclization of the M7P product.

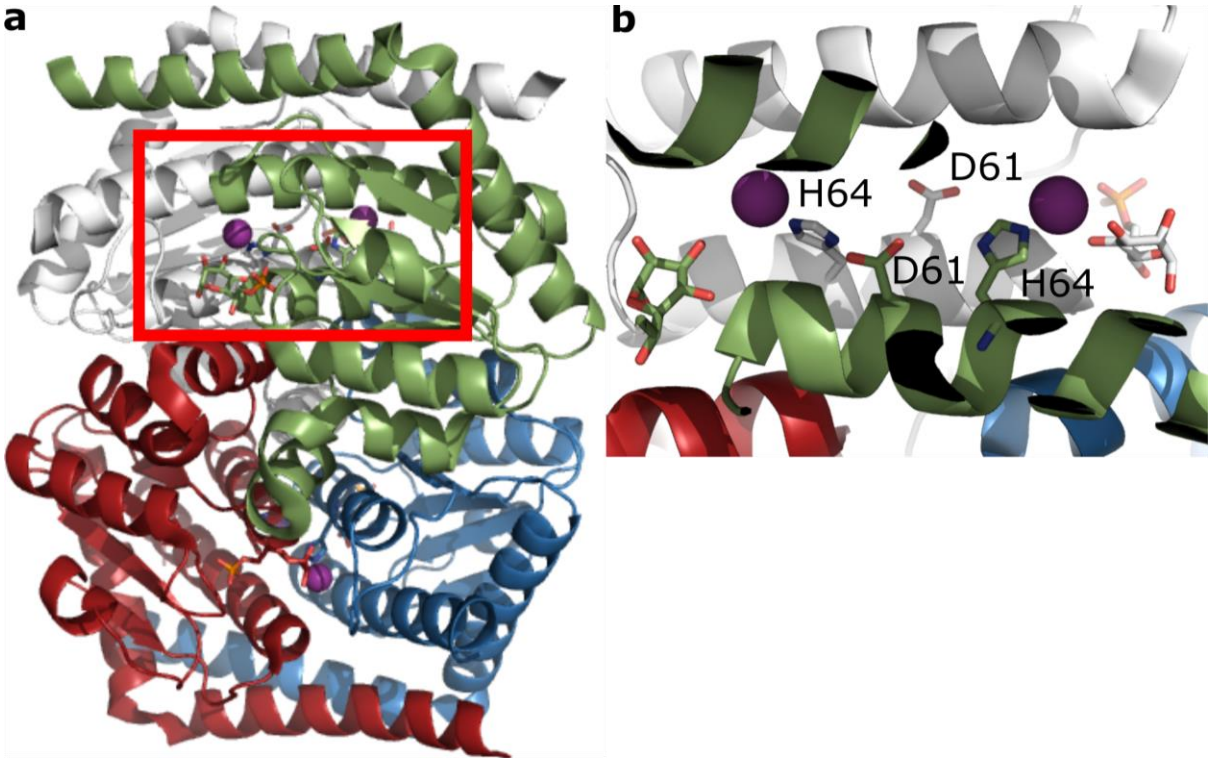
a



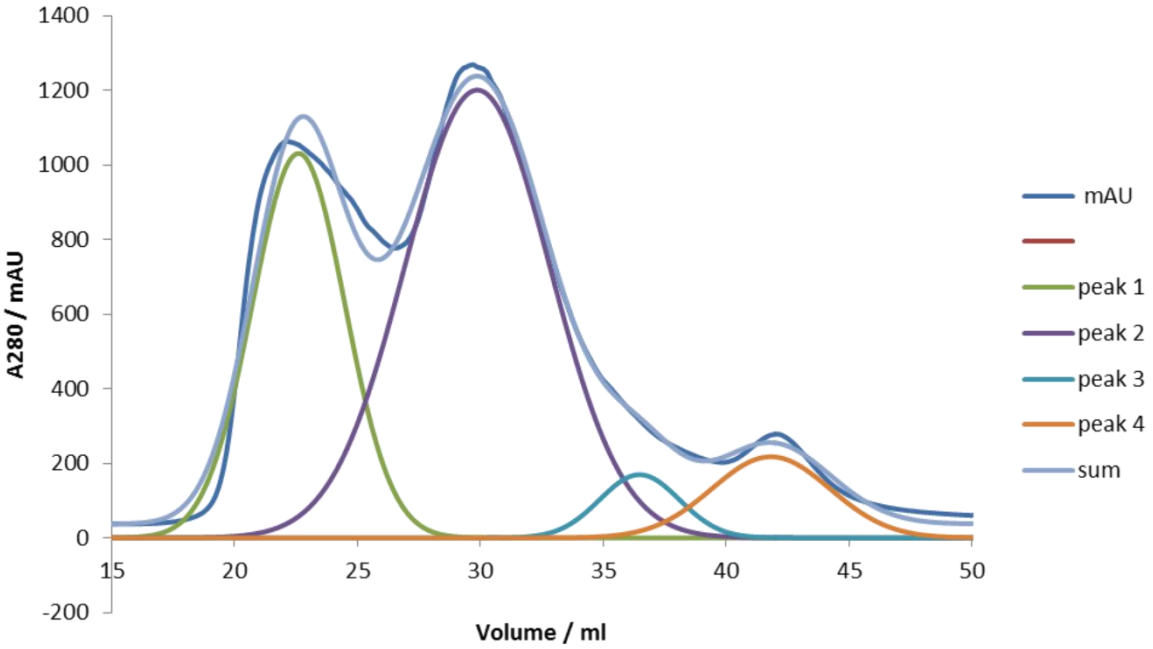
b



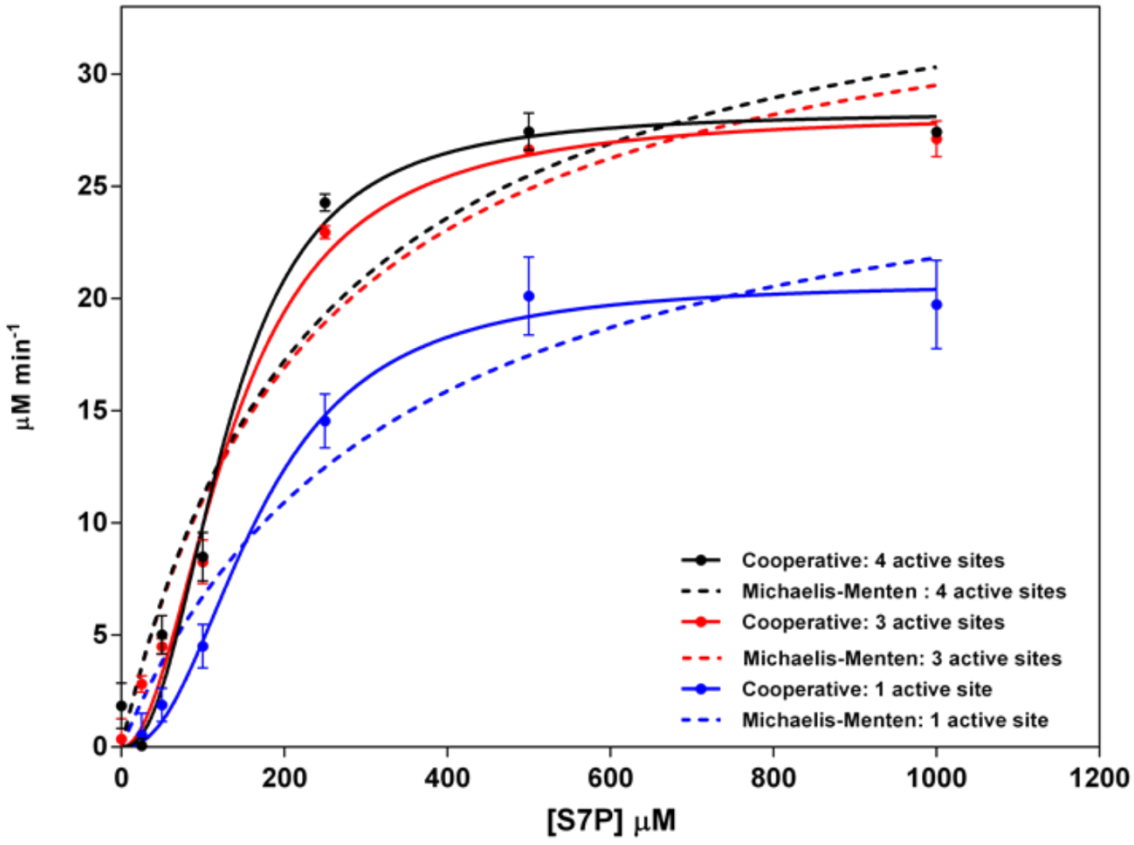
Supplementary Figure 2: A solvent channel links pairs of active sites in GmhA. a: Overview of the structure of GmhA. Pairs of active sites are located in close proximity to one another at the top (red box) and bottom of the molecule. **b:** Close-up, cut away view of the water filled channel linking pairs of active sites. The only hydrophilic side chains in this channel are H64 (coordinating to the catalytic zinc) and D61. Images generated using PyMOL from PDB ID 2XBL [1]. Protein backbone shown as cartoon; product, H64 and D61 as sticks; zinc as spheres. Colors: GmhA chain A, sky blue; chain B, white; chain C, green; chain D, brick red; substrate S7P carbon, cyan; product M7P carbon, yellow; oxygen, red; nitrogen, blue; phosphorus, orange; zinc, purple sphere.



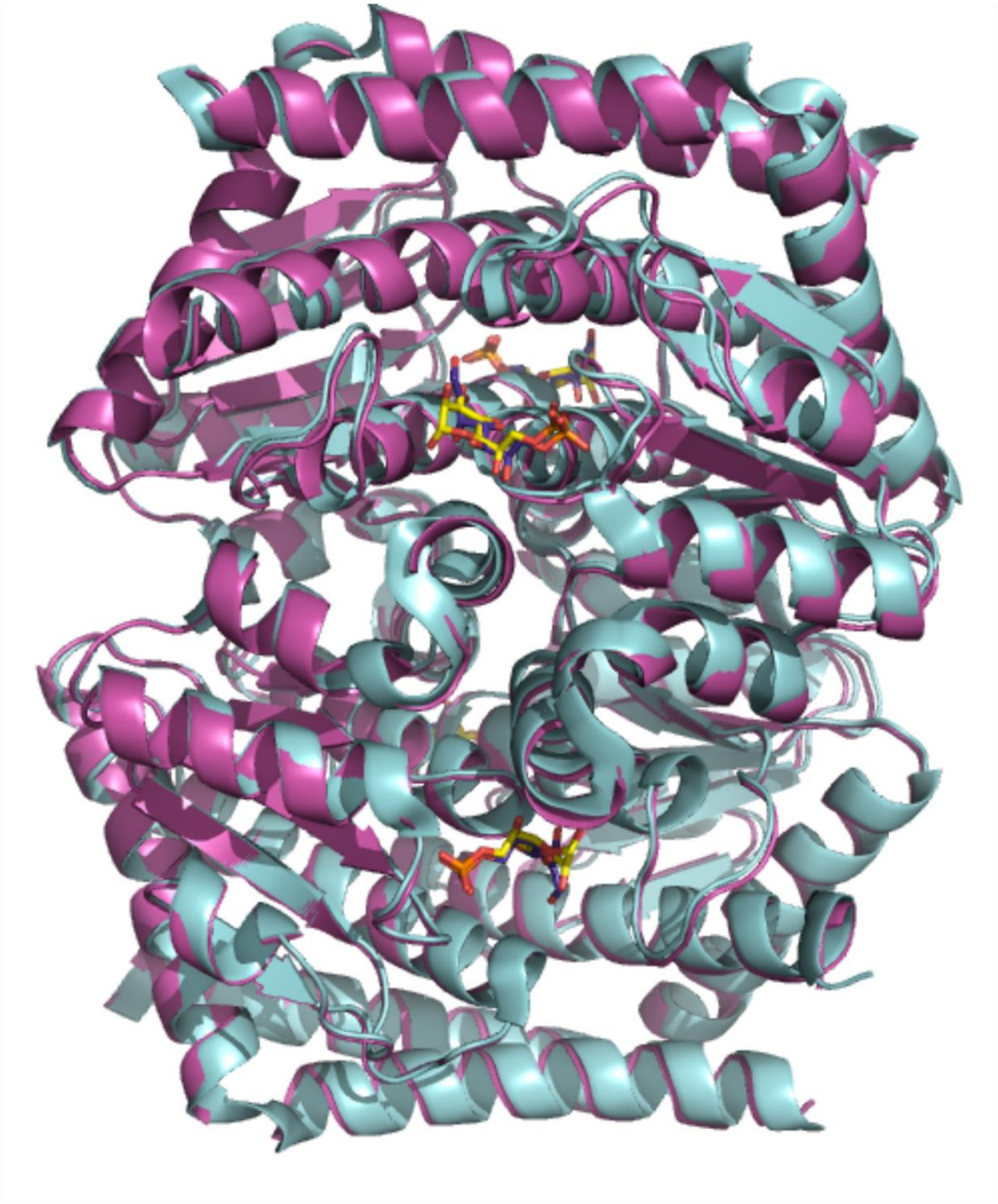
Supplementary Figure 3: Purification of GmhA tetramers with varying numbers of active sites. Two versions of GmhA were co-expressed – 6*His-GmhA E115K (a surface polymorphism seen between the standard K96243 and 1710b strains of *B. pseudomallei*), and GmhA D98N (lacking a critical substrate binding and catalytic residue). The protein was purified by IMAC and size exclusion chromatography; and then further purified over an anion exchange (Q) column, giving the elution profile shown below. The more positively charged E115K mutant is expected to elute earlier than the Four peaks were modelled as Gaussian curves, with the height and width of each peak fitted to the observed data using least-squares fitting using Excel (green, purple, cyan, orange lines respectively). The overall fit of the model (light blue) to the data (blue) is very good. The final fractions for analysis were selected for peaks 1, 2 and 4 to contain less than 1% contaminating material. No fraction contained less than 10% contaminating material for peak 3, so this was not further studied. Mass spectrometry showed that peak 2 contained approximately nine times more of the larger 6*His-GmhA E115K per molecule of GmhA D98N than peak 4. This implies that these represent the 3:1 and 1:3 ratios of 6*His-GmhA E115K and GmhA D98N respectively, as expected from the ion exchange result.



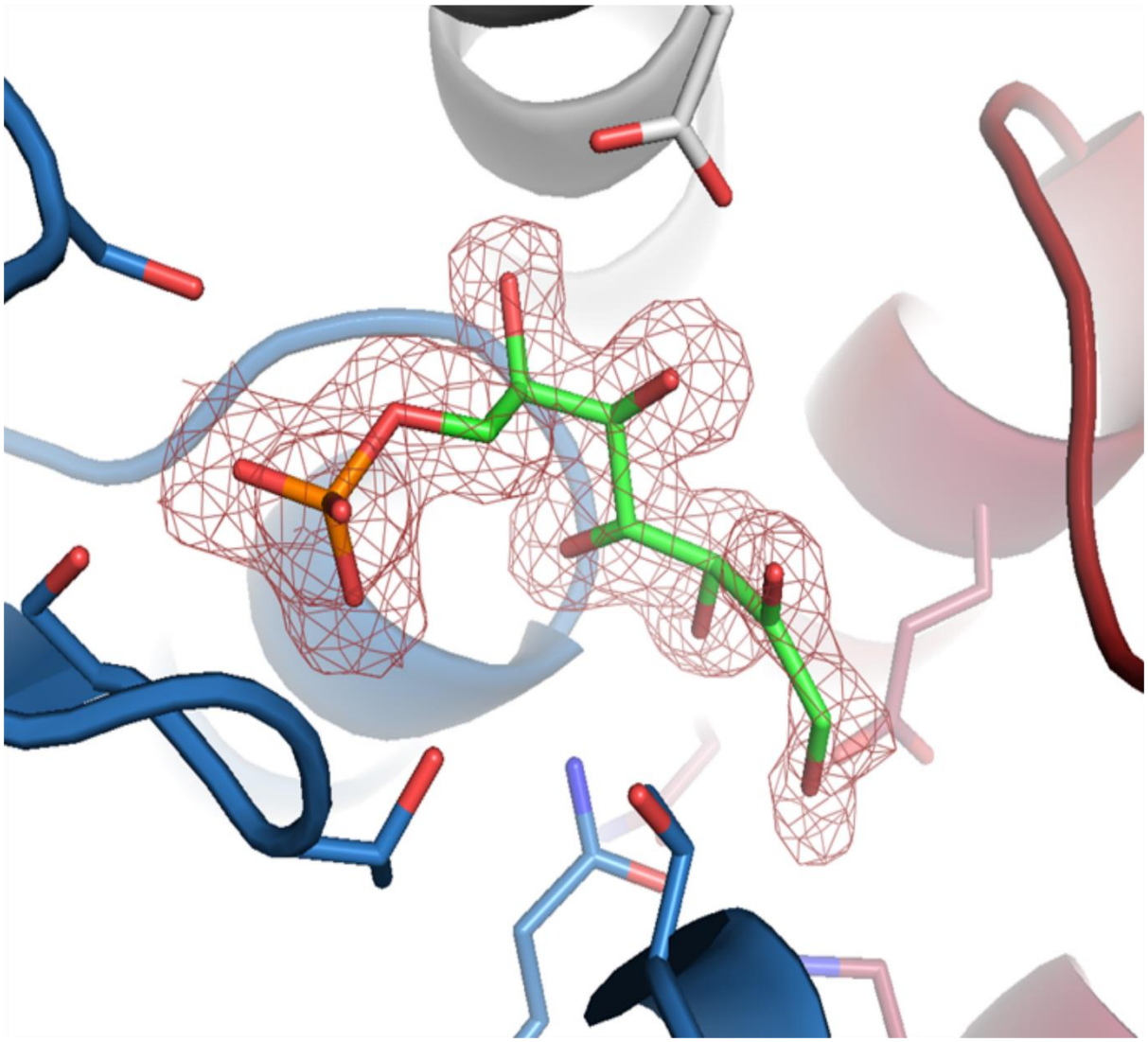
Supplementary Figure 4: GmhA variants with differing numbers of active sites all show cooperativity in their activity. Assay of GmhA variants containing four (black), three (red) or one (blue) active sites all show positive cooperativity in the same manner as wild-type. Best fit cooperative models are shown in full lines; best fit Michaelis-Menten models are shown in dashed lines. Each dataset shows shows positive cooperativity (>99% confidence in cooperative model in all cases, Akaike's information criterion implemented in Graphpad; h = 2.4 ± 0.3, 2.0 ± 0.2, 2.3 ± 0.4 respectively for samples containing four, three and one intact active site). All data shown are biological triplicates, with error bars showing ± SEM.



Supplementary Figure 5: There is no conformational change between substrate and product bound GmhA. The structures of substrate (PDB ID: 5LTZ, magenta, this study) and product (PDB ID: 2XBL, cyan [1]) bound GmhA were superimposed. There is no significant alteration in the structure, with an RMS deviation of 0.189 Å between the 4,967 aligned atoms in the structures. Figure generated using PyMOL. Main chain shown as cartoon, and substrate/product as sticks. Colors: substrate bound cartoon, magenta; product bound cartoon, cyan; substrate carbon, purple; product carbon, yellow; oxygen, red; phosphorus, orange.



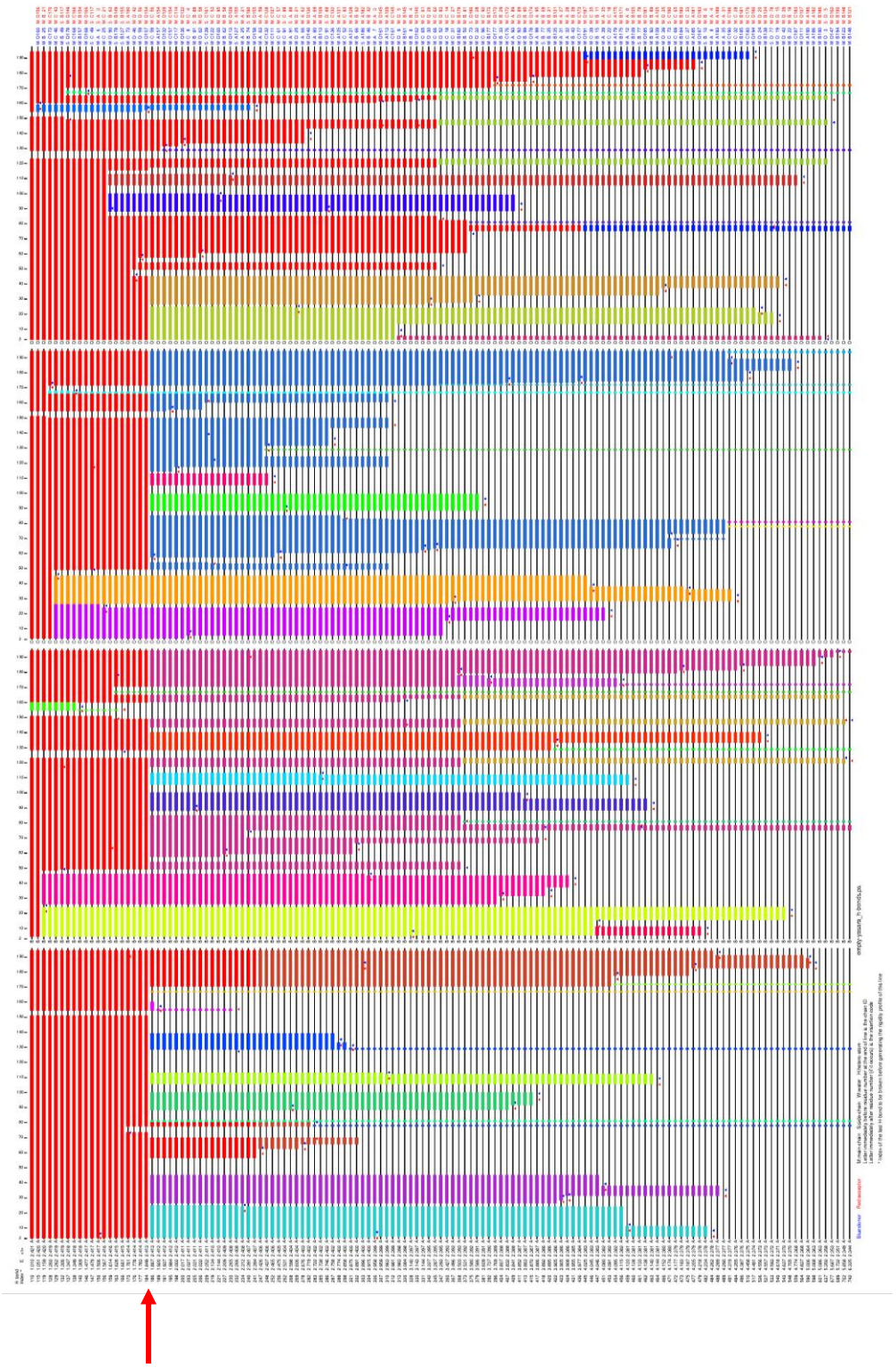
Supplementary Figure 6: The structure of GmhA H64Q shows substrate in the active site. The location of the substrate reflects that of the substrate observed in the E68Q mutant. Similar density is observed in all four active sites. Protein is shown as cartoon, and ligand binding residues as sticks. Density shown as red netting is $2F_o$-F_c, contoured at 1σ. Colors: GmhA chain A, sky blue; chain B, white; chain D, brick red; substrate S7P carbon, green; oxygen, red; nitrogen, blue; phosphorus, orange. Figure generated using PyMOL.



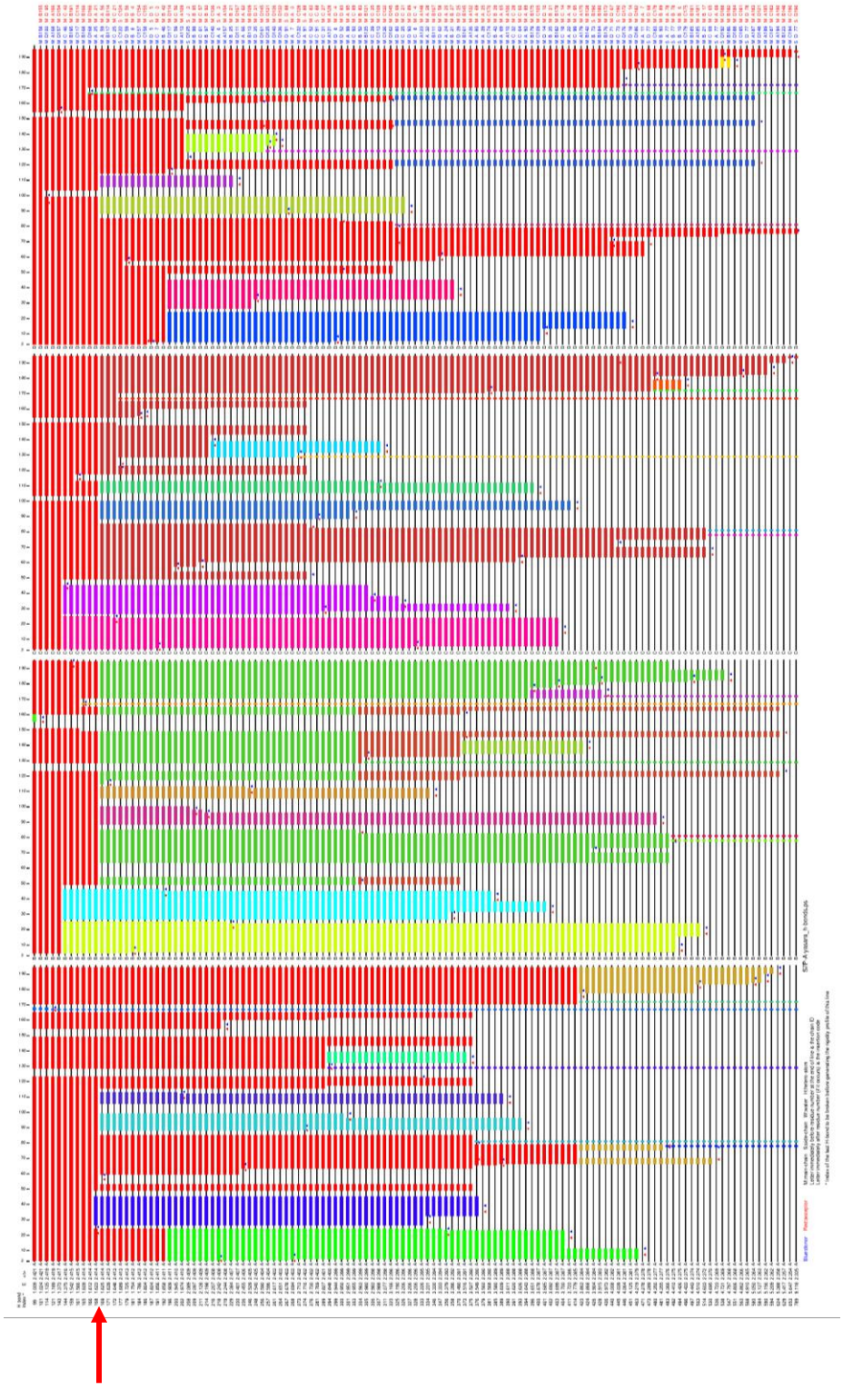
Supplementary Figure 7: Hydrogen bond dilution of GmhA in complex with substrate. The hydrogen-bonding network of GmhA was assessed in the absence of ligands (**a**), or the presence of one S7P molecule (**b**), two S7P molecules on the same face of GmhA (**c**), and two S7P molecules on opposite faces of GmhA (**d**). Hydrogen bonding dilution using PROFLEX [2] was used to highlight strongly and weakly bonded regions of the protein. The output from the hydrogen bonding dilution is shown, with sections of protein bonded together shown in the same color.

In the ligand free state (**a**), the major hydrogen bonding network shows rigidity until hydrogen bonds weaker than 1.9 kcal/mol are broken (red arrow in each panel indicates the point where the major hydrogen bonding network loses integrity). Some external helices break free from this network at lower energies. Binding of one S7P molecule (**b**) causes the network to become weaker, losing rigidity at an energy 0.3 kcal/mol lower. Binding of two molecules of S7P (**c**) to the same face of GmhA (*"cis"* complex) causes the network to lose rigidity at even lower energy (~0.1 kcal/mol earlier). When two S7P molecules bind to opposite faces of GmhA (**d**; "*trans*" complex), in contrast, the network shows much greater stability, maintaining rigidity for another 0.4 kcal/mol beyond the unliganded GmhA.

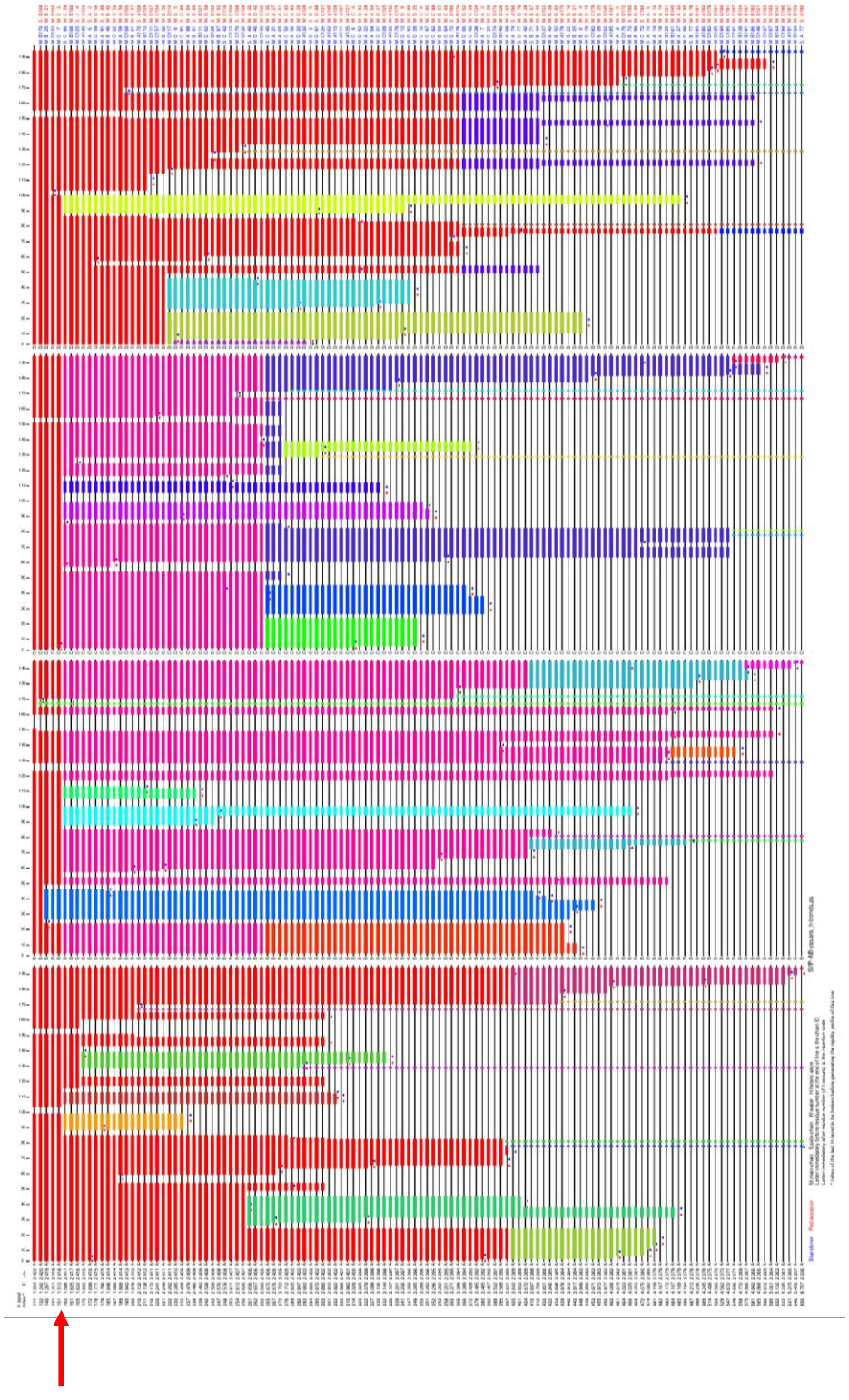
a:



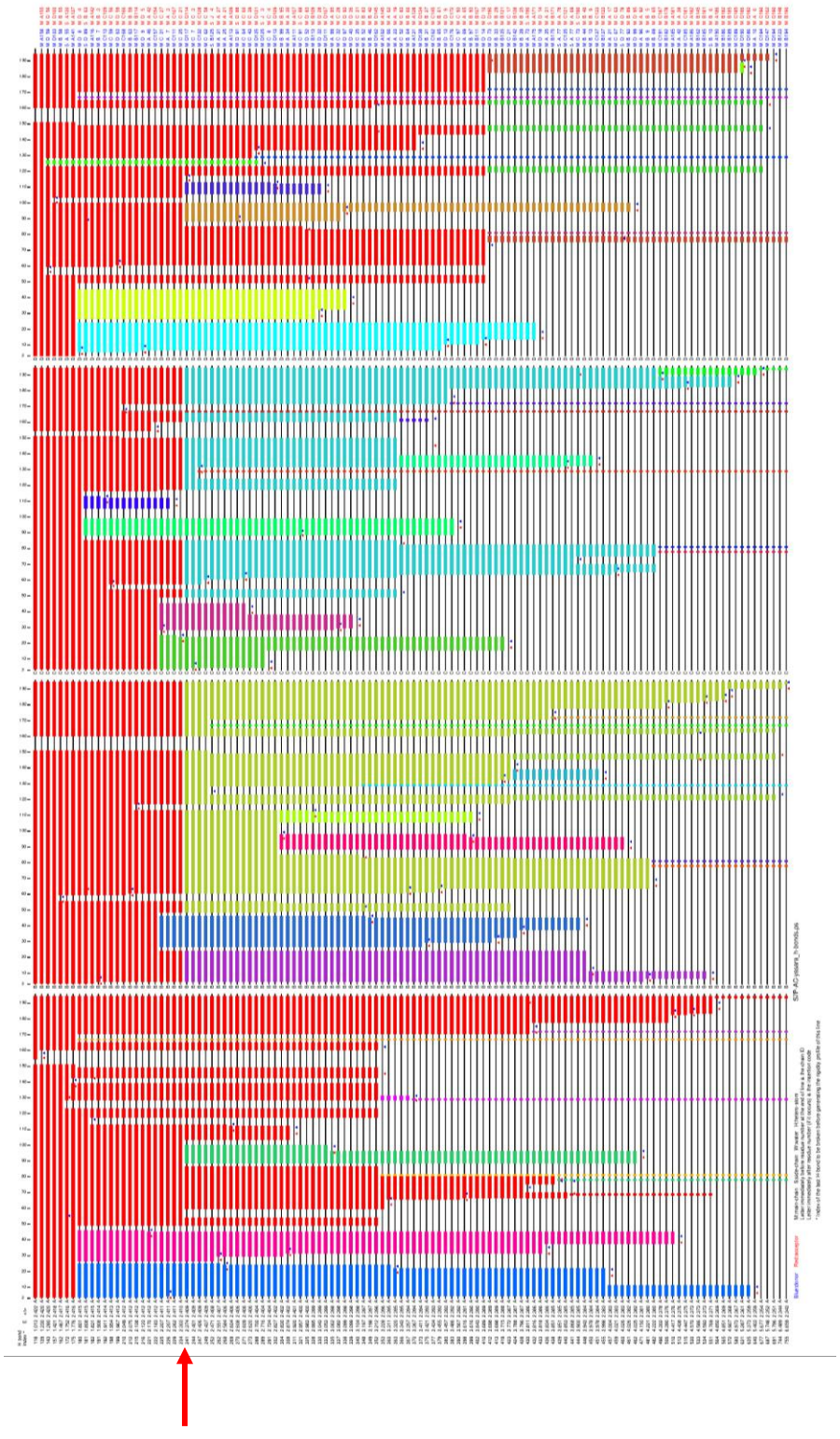
b:



c:



d:



Supplementary Table 1: Crystallographic data collection and refinement statistics.

Mutant	D61A	H64Q	E68Q	Q175E
PDB ID	5LU7	5LU6	5LU5	5LTZ
Crystallisation conditions	50 mM HEPES pH 7.5, 10% (w/w) PEG 3350, 5 mM each $ZnCl_2$, $MgCl_2$, KCl, $CaCl_2$.	50 mM HEPES pH 7.5, 8.5% (w/w) PEG 3350	50 mM HEPES pH 7.0, 7% (w/w) PEG 3350	50 mM MES pH 6.5, 8.5% (w/w) PEG 3350
Cryoprotectant solution and soaking time.	5 mM S7P, 10% (w/w) PEG 3350, 20% (v/v) PEG 400; 30 min	5 mM S7P, 8.5% (w/w) PEG 3350, 21.5% (v/v) PEG 400; 5 min.	25 mM S7P, 35% (v/v) PEG 400; 65 min.	25 mM S7P, 35% (v/v) PEG 400; 55 min.
Data Collection				
Wavelength (Å)	0.97950	0.97950	0.97950	0.97950
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions				
a, *b*, *c* (Å)	73.2, 83.68, 126.10	73.61, 84.61, 127.09	73.20, 83.81, 126.78	72.55, 84.47, 126.68
α, β, γ (°)	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90
Resolution (Å)*	50.33-1.92 (1.97-1.92)	63.70-1.67 (1.76-1.67)	63.40-1.55 (1.59-1.55)	26.25-1.67 (1.71-1.67)
R_{pim} *	0.035 (0.310)	0.057 (0.237)	0.028 (0.302)	0.050 (0.569)
$I / \sigma I$ *	17.3 (2.5)	7.4 (2.2)	17.5 (2.7)	7.6 (1.0)
Completeness (%) *	99.8 (99.7)	88.6 (89.3)	99.5 (99.0)	99.9 (99.9)
Redundancy *	5.5 (5.6)	6.4 (6.2)	6.3 (5.2)	5.6 (5.7)
Refinement				
Resolution (Å)	37.54-1.92	42.31-1.67	63.40-1.55	26.25-1.67
No. reflections	59262	73739	112695	90441
R_{work} / R_{free} (%)	17.03/21.48	19.10/22.60	16.11/18.72	20.75/24.47
No. atoms				
Protein	5733	5763	5795	5750
Ligand/ion	123	120	191	130
Water	557	699	813	380
B-factors				
Protein	25.96	20.02	18.36	33.82
Ligand/ion	34.3	34.4	28.93	40.45
Water	32.31	31.05	30.82	39.57
R.m.s. deviations				

Bond lengths (Å)	0.007	0.019	0.006	0.007
Bond angles (°)	1.047	1.792	1.031	1.059
Ramachandran favoured/ allowed/outlier (%) †	97.8/2.2/0	98.2/1.7/0.1	98.3/1.7/0	97.7/2.0/0.3

*Values in parentheses are for highest-resolution shell. † Calculated using RAMPAGE (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php).

Supplementary Table 2: Summary of the distances and angles for zinc coordination which were obtained at M05 2X/6-31+G*. Points where no value is appropriate for the model are indicated by a dash. The values for the second site of the models with two active sites and two ligands (Model 5 and Model 5^{water}) that are at variance with other liganded sites are shown in bold and red.

		Models									
Distances (Å)	2XBL	1	2	3^A	3^B	4^A	4^B	5^A	5^B	5_wat^A	5_wat^B
Zn-Glu68OE2	1.95	2.06	1.97	2.00	1.97	2.01	1.99	2.02	1.98	1.97	1.97
Zn-His183NE2	2.02	2.03	2.12	2.07	2.05	2.16	2.06	2.13	2.04	2.12	2.07
Zn-His64NE2	2.04	2.04	2.17	2.03	2.05	2.13	2.03	2.11	2.01	2.14	2.01
Zn-Gln175OE2	2.04	2.21	2.05	2.14	2.04	2.06	2.12	2.02	2.00	2.01	2.00
Zn-S7PO2(C=O)	-	-	2.48	-	-	2.66	-	2.69	**3.50**	2.67	2.63
Zn-S7PO1(OH)	-	-	2.16	-	-	2.19	-	2.16	**3.78**	2.24	**3.14**
Angles (°)											
Glu68OE2-Zn-His183NE2	119.44	128.9	91.3	124.0	121.7	88.8	114.5	90.8	109.0	88.3	97.7
His183NE2-Zn-His64NE2	111.79	116.3	107.1	109.1	106.1	110.7	110.4	102.3	110.1	108.2	113.6
His64NE2-Zn-Glu68OE2	110.96	108.7	83.7	113.3	111.6	88.4	109.3	85.3	112.8	91.5	114.9
Gln175OE1-Zn-Glu68OE1	111.91	110.9	177.1	110.6	115.0	169.4	128.5	163.4	121.0	156.7	132.8
Gln175OE1-Zn-His183NE2	98.76	91.7	91.6	96.8	97.2	97.1	92.3	105.0	100.7	108.8	91.4
Gln175OE1-Zn-His64NE2	101.96	91.25	94.9	99.2	102.9	97.6	99.9	96.1	102.4	97.7	103.2
S7PO1-ZN-S7PO2	-	-	70.3	-	-	67.1	-	66.9	**44.5**	67.1	**57.7**
S7PO2-Zn-His64NE2	-	-	88.2	-	-	90.0	-	99.3	**135.1**	92.3	98.7
His183NE2-Zn-S7PO1	-	-	94.4	-	-	91.8	-	90.6	**77.7**	92.3	90.1

Supplementary Table 3: Cartesian coordinates (in xyz format) of all optimized models obtained at M05 2X/6-31+G* level.

Model_1
41

C	-35.189001	68.950999	-4.728000
H	-35.235564	70.009238	-4.476826
H	-35.186251	68.382039	-3.796088
H	-36.092573	68.696296	-5.285476
C	-33.961248	68.688364	-5.528333
N	-33.619808	67.441263	-6.012967
H	-34.156626	66.596209	-5.891478
C	-32.953682	69.512503	-5.939636
H	-32.830845	70.571160	-5.785228
C	-32.452336	67.538661	-6.678905
N	-32.021439	68.783525	-6.650274
H	-31.958412	66.714012	-7.164068
C	-29.742997	72.440985	-4.574013
H	-30.036566	73.424970	-4.930440
H	-30.394740	72.157549	-3.744543
H	-28.714145	72.439092	-4.220527
C	-29.932289	71.422858	-5.688304
O	-30.823441	71.609615	-6.557695
O	-29.228234	70.345789	-5.741554
C	-28.567006	65.393024	-8.102996
H	-28.027979	64.698542	-7.461761
H	-29.550458	64.991730	-8.342375
H	-28.016592	65.522737	-9.036095
C	-28.740629	66.762742	-7.454376
O	-29.527409	67.611639	-7.930508
N	-28.022111	67.036389	-6.364801
H	-28.098545	67.950972	-5.932400
H	-27.371507	66.374922	-5.973542
C	-31.149996	72.623992	-11.993991
H	-31.482297	73.498329	-11.437154
H	-30.308907	72.922224	-12.622691
H	-31.969305	72.300145	-12.638585
C	-30.756124	71.545454	-11.035592
N	-30.288661	70.311874	-11.449202
H	-30.159748	70.024804	-12.407362
C	-30.752790	71.477416	-9.669646
H	-31.038794	72.220276	-8.943866
C	-30.027435	69.555005	-10.366924
N	-30.297719	70.234368	-9.269314
H	-29.658483	68.543958	-10.401789
Zn	-30.287297	69.601058	-7.340796

Model_2
52

C	1.646333	5.125194	-0.478484
H	2.303546	5.687941	0.187093
H	2.190736	4.920317	-1.398750
H	0.784591	5.748218	-0.725236
C	1.214183	3.829108	0.141970
N	0.504006	3.762964	1.326845
H	0.225099	4.550953	1.890464

C 0.224656 2.468653 1.593562
N 0.717610 1.687236 0.655413
H -0.341824 2.144514 2.451792
C 1.336647 2.524321 -0.253785
H 1.797231 2.131560 -1.143967
C 0.061114 0.989020 -3.908752
H 1.043316 1.459990 -3.819479
H -0.688337 1.771772 -3.797529
H -0.017352 0.520242 -4.887303
C -0.083937 -0.009682 -2.808077
O -0.243141 0.509952 -1.657719
O -0.020715 -1.240512 -3.035585
C 1.210662 -1.416023 4.163013
H 2.100193 -1.266422 4.772985
H 0.949216 -2.474246 4.156887
H 0.374915 -0.871322 4.603705
C 1.378165 -0.960930 2.747789
O 0.432597 -1.128886 1.953783
N 2.526838 -0.391553 2.386511
H 2.654957 -0.083807 1.429738
H 3.276870 -0.262159 3.045006
C -5.606304 0.051738 -0.575890
H -6.275969 0.446139 0.190801
H -6.055236 -0.848265 -1.000377
H -5.523426 0.793705 -1.368273
C -4.244609 -0.233581 -0.018949
N -4.025155 -1.133127 1.007645
H -4.734312 -1.676889 1.474246
C -2.700911 -1.185412 1.266053
N -2.048227 -0.365525 0.469626
H -2.252543 -1.823533 2.009279
C -2.997415 0.231541 -0.337943
H -2.710995 0.937201 -1.099213
Zn 0.038515 -0.302934 0.115237
C 3.807875 -2.482586 -1.569633
H 3.993283 -3.510730 -1.253038
H 3.698024 -2.487826 -2.658299
H 4.637871 -1.835386 -1.295192
C 2.514327 -1.968502 -0.983574
O 2.368030 -0.825604 -0.566347
C 1.349015 -2.939039 -0.915477
H 1.522523 -3.598015 -0.057776
H 1.343115 -3.560318 -1.815783
O 0.117667 -2.280566 -0.754232
H -0.112108 -1.939186 -1.702379

Model_3
99

C -39.563653 68.542507 -7.211120
H -38.581129 68.981150 -7.393227
H -39.666336 68.381661 -6.135539
H -40.351606 69.209201 -7.557383
C -39.651419 67.163738 -7.922992
O -38.689092 66.372410 -7.751394
O -40.691133 66.928147 -8.608075
C -35.188897 68.950871 -4.727919
H -34.906866 69.756980 -4.051673
H -35.352393 68.044388 -4.142089
H -36.135888 69.211426 -5.204893

C	-34.116462	68.751864	-5.735894
N	-34.163090	67.815656	-6.741164
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C	-32.927903	69.407075	-5.890295
H	-32.526632	70.226790	-5.319417
C	-33.040212	67.926342	-7.461404
N	-32.252683	68.879398	-6.968408
H	-32.824426	67.302931	-8.313935
C	-29.742971	72.440069	-4.574653
H	-30.167963	73.419237	-4.777899
H	-30.174987	72.030169	-3.659687
H	-28.664378	72.503401	-4.433060
C	-30.059509	71.489900	-5.728468
O	-30.883012	71.803752	-6.609667
O	-29.480014	70.335450	-5.751489
C	-28.567379	65.394261	-8.102668
H	-28.881700	64.356557	-8.009607
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N	-30.675003	65.852984	-6.960446
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H	-31.614494	73.440892	-11.444697
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H	-31.881061	72.231935	-12.703510
C	-30.712661	71.562990	-11.023787
N	-30.077890	70.401123	-11.427705
H	-29.846416	70.156599	-12.377312
C	-30.807602	71.448272	-9.662251
H	-31.224482	72.128303	-8.937933
C	-29.819900	69.643397	-10.341495
N	-30.248483	70.250383	-9.254798
H	-29.332915	68.682653	-10.369490
Zn	-30.355418	69.491193	-7.334519
C	-33.623403	61.484578	-5.654246
H	-32.562865	61.258785	-5.756568
H	-33.847330	61.645174	-4.596571
H	-34.232229	60.652213	-6.008897
C	-33.976298	62.787246	-6.424800
O	-35.190263	62.936574	-6.779225
O	-33.045527	63.610723	-6.609717
C	-38.554910	61.299903	-5.466858
H	-37.657697	60.687497	-5.356582
H	-38.343641	62.272209	-5.017527
H	-39.368858	60.829829	-4.916464
C	-38.938258	61.440105	-6.884146
N	-38.128565	61.975933	-7.857596
H	-37.182731	62.335387	-7.692053
C	-40.097973	61.098034	-7.509683
H	-40.981821	60.641813	-7.095610
C	-38.803593	61.946141	-9.022170
N	-40.008467	61.417158	-8.845367
H	-38.408968	62.320002	-9.951962
C	-43.572723	57.880788	-7.756585
H	-43.130590	56.899329	-7.608791
H	-43.759030	58.365622	-6.797390
H	-44.529404	57.794321	-8.273671

C -42.627914 58.750910 -8.590853
O -41.555335 58.301579 -9.013968
O -43.002581 59.973717 -8.824793
C -42.933860 64.780006 -11.501683
H -42.768484 65.830087 -11.267158
H -42.641535 64.575824 -12.531918
H -43.990170 64.533604 -11.393459
C -42.143177 63.868160 -10.587639
O -42.245814 62.616969 -10.787626
N -41.387271 64.383813 -9.639977
H -40.875694 63.739829 -9.049029
H -41.220844 65.391239 -9.409961
C -38.932353 57.518419 -13.592652
H -38.779144 56.732176 -12.855242
H -37.952248 57.875357 -13.916282
H -39.444423 57.081442 -14.452686
C -39.737348 58.617458 -12.975988
N -40.107421 59.759527 -13.664205
H -39.891365 59.964576 -14.626570
C -40.256486 58.780456 -11.721651
H -40.223706 58.127354 -10.865291
C -40.812708 60.556338 -12.834060
N -40.920042 59.989076 -11.651305
H -41.224527 61.514503 -13.105278
Zn -41.647771 60.861875 -9.942943
O -35.931864 65.801205 -7.175689
H -36.893332 65.905196 -7.342271
H -35.694869 64.854911 -7.055536

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C 5.136753 -1.951802 2.072020
H 5.487952 -2.292242 3.048165
H 4.732391 -2.818065 1.546971
H 4.366083 -1.193169 2.203603
C 6.333666 -1.392450 1.290301
O 6.390661 -0.171762 1.027490
O 7.208374 -2.268315 0.981094
C 1.508863 1.795204 3.078185
H 1.873421 2.438163 3.881880
H 0.580672 1.328645 3.405149
H 2.249082 1.010919 2.907147
C 1.242917 2.577011 1.825994
N 2.184943 3.292937 1.109004
H 3.160401 3.473004 1.322519
C 1.584226 3.848400 0.042961
N 0.293128 3.545385 0.019098
H 2.135711 4.471330 -0.642656
C 0.078979 2.747724 1.130766
H -0.896927 2.348443 1.339998
C -4.085754 1.861616 1.266699
H -3.656004 2.219924 2.205367
H -3.647259 0.886401 1.054359
H -5.165046 1.780231 1.376244
C -3.721404 2.832738 0.177274
O -2.497397 2.811021 -0.167229
O -4.593195 3.589891 -0.309465
C 1.396524 6.958717 -2.952180
H 2.374454 7.220151 -2.546848

H	0.907903	7.859592	-3.331149
H	1.496695	6.251339	-3.773653
C	0.520867	6.361600	-1.891296
O	-0.451177	5.633203	-2.224665
N	0.797361	6.677907	-0.633722
H	0.225609	6.265070	0.092589
H	1.766961	6.942412	-0.401396
C	-2.618775	-0.325059	-4.775590
H	-1.832444	-0.826895	-5.342815
H	-3.475790	-0.161374	-5.431846
H	-2.929765	-0.985685	-3.967943
C	-2.125431	0.975441	-4.191476
N	-1.662516	2.016924	-4.977152
H	-1.605220	2.015574	-5.982735
C	-1.304848	3.045136	-4.173896
N	-1.503502	2.734114	-2.912813
H	-0.921320	3.987750	-4.529067
C	-2.016183	1.449292	-2.908791
H	-2.273661	0.964123	-1.982487
Zn	-1.354745	4.079027	-1.228457
C	-3.734688	7.638804	0.967073
H	-3.131019	7.896219	1.834465
H	-3.945901	8.525083	0.365083
H	-4.690881	7.222357	1.296209
C	-3.031185	6.581758	0.134301
O	-2.108552	5.903107	0.550009
C	-3.527099	6.380053	-1.291398
H	-3.042215	7.138810	-1.914177
H	-4.607552	6.549965	-1.329744
O	-3.205048	5.108504	-1.796540
H	-3.839345	4.457283	-1.329734
C	5.914397	7.247044	-0.138492
H	5.731140	8.101358	0.514955
H	6.040472	7.625911	-1.153850
H	6.811009	6.717298	0.179774
C	4.674654	6.318419	-0.099633
O	4.804737	5.124677	0.286004
O	3.587193	6.850023	-0.474127
C	10.117849	4.046728	1.834473
H	9.449334	3.777453	2.654275
H	9.694152	4.919197	1.332809
H	11.085962	4.324329	2.249882
C	10.286393	2.911839	0.889101
N	9.230996	2.315759	0.239586
H	8.229477	2.542162	0.376394
C	9.710956	1.333703	-0.530542
N	11.032313	1.255101	-0.416925
H	9.084411	0.686009	-1.120368
C	11.401886	2.246371	0.468464
H	12.434081	2.424278	0.715020
C	15.991180	1.693388	0.149293
H	16.708084	0.885898	-0.000959
H	15.814736	1.784558	1.222837
H	16.371384	2.630779	-0.246938
C	14.661459	1.352535	-0.545342
O	14.121149	2.192328	-1.276443
O	14.131830	0.187548	-0.313171
C	9.290532	-3.277168	-1.574132
H	8.345828	-3.408088	-1.049377
H	9.116687	-3.037059	-2.623635

H	9.877298	-4.195982	-1.526498
C	10.104618	-2.156246	-0.927479
O	11.182569	-1.733503	-1.468747
N	9.632870	-1.683894	0.202271
H	10.153900	-0.944106	0.653770
H	8.628750	-1.896554	0.551553
C	13.033844	2.203750	-6.425697
H	12.123219	2.524888	-6.936194
H	13.688966	1.721753	-7.154765
H	13.541166	3.090768	-6.049722
C	12.726490	1.294563	-5.278589
N	12.083261	0.077797	-5.425363
H	11.766145	-0.321974	-6.293751
C	11.948156	-0.490748	-4.208534
N	12.472470	0.290368	-3.287647
H	11.477222	-1.441273	-4.019108
C	12.961154	1.407159	-3.937026
H	13.445103	2.191992	-3.379927
Zn	12.383215	-0.002110	-1.253702
O	6.579380	2.814543	0.803842
H	5.944008	3.521209	0.573038
H	6.152368	1.941145	0.859872

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C	4.750915	-2.222977	1.760012
H	5.009847	-2.422355	2.803894
H	5.028754	-3.095737	1.166428
H	3.678366	-2.046353	1.697307
C	5.525262	-0.981464	1.297225
O	4.922976	0.115002	1.323280
O	6.736356	-1.148210	0.950210
C	1.641008	1.947937	2.709989
H	2.014657	2.644655	3.463022
H	0.814590	1.383168	3.139631
H	2.449282	1.258368	2.453090
C	1.154823	2.676858	1.492434
N	1.967346	3.396724	0.637501
H	2.966897	3.567658	0.721017
C	1.208604	3.900986	-0.350763
N	-0.063192	3.562537	-0.194133
H	1.625453	4.503138	-1.141361
C	-0.100553	2.794386	0.956879
H	-1.025058	2.365377	1.300899
C	-3.871994	2.117009	1.726910
H	-3.313037	2.800484	2.372648
H	-3.329243	1.172969	1.685514
H	-4.863541	1.970148	2.151845
C	-3.936564	2.739798	0.375498
O	-2.856676	2.641347	-0.293692
O	-4.967259	3.340650	-0.000183
C	0.556157	7.043643	-2.318025
H	1.498550	7.449671	-1.952131
H	0.020839	7.798989	-2.896481
H	0.744592	6.201933	-2.986795
C	-0.330460	6.573277	-1.228593
O	-1.412040	6.027633	-1.567369
N	0.043682	6.739219	0.027502

H	-0.521112	6.313843	0.752564
H	1.004850	7.048111	0.232952
C	-3.102002	-0.210964	-4.444894
H	-2.736707	-0.440348	-5.448216
H	-4.184800	-0.351566	-4.426987
H	-2.656694	-0.923095	-3.751980
C	-2.732224	1.179719	-4.026234
N	-3.144225	2.309730	-4.711691
H	-3.696674	2.320425	-5.553667
C	-2.704456	3.401890	-4.048792
N	-2.021643	3.046303	-2.983378
H	-2.919295	4.415144	-4.345364
C	-2.031202	1.664994	-2.958066
H	-1.568738	1.120543	-2.151959
Zn	-1.890026	4.115965	-1.162711
C	-3.693992	7.714960	1.287991
H	-2.965283	8.452955	0.938696
H	-4.693859	8.109409	1.104533
H	-3.536925	7.546329	2.351713
C	-3.450721	6.423239	0.526979
O	-2.687855	5.565678	0.935956
C	-4.170560	6.271924	-0.803449
H	-3.874168	7.114906	-1.434308
H	-5.246118	6.362187	-0.609478
O	-3.888731	5.073569	-1.476771
H	-4.439661	4.338470	-1.033076
C	6.586010	6.982962	-0.323983
H	6.986125	7.372225	0.615315
H	5.909198	7.720778	-0.757373
H	7.424000	6.794968	-0.993699
C	5.865088	5.649777	-0.026296
O	6.596449	4.657256	0.143161
O	4.588825	5.643790	0.049957
C	9.968037	3.533050	1.738997
H	9.594751	3.261733	2.728627
H	9.206015	4.155240	1.263610
H	10.883616	4.113262	1.854065
C	10.242008	2.323962	0.923745
N	9.256949	1.453821	0.530075
H	8.236722	1.579654	0.777481
C	9.817750	0.488599	-0.199185
N	11.135279	0.677741	-0.297818
H	9.253876	-0.326869	-0.619776
C	11.406303	1.835228	0.406081
H	12.394790	2.258207	0.446172
C	15.805009	1.545002	0.422996
H	16.624406	0.860819	0.196719
H	15.383265	1.254321	1.388863
H	16.170429	2.567741	0.485561
C	14.715652	1.424337	-0.613120
O	14.160725	2.411122	-1.086616
O	14.400196	0.191230	-0.884787
C	10.094006	-3.799996	-1.704996
H	9.268788	-4.145854	-1.083753
H	9.718025	-3.450492	-2.665992
H	10.784221	-4.625226	-1.893622
C	10.859825	-2.700361	-1.051396
O	11.669686	-2.046983	-1.758271
N	10.656169	-2.497797	0.237369
H	11.194078	-1.788267	0.715297

H	9.822404	-2.891721	0.694293
C	13.457008	2.389014	-6.213023
H	12.610357	2.719032	-6.819591
H	14.229181	2.000654	-6.881411
H	13.862064	3.259649	-5.699489
C	13.046943	1.376720	-5.200024
N	12.499051	0.147680	-5.520046
H	12.317571	-0.195376	-6.449216
C	12.248998	-0.522244	-4.373578
N	12.604913	0.209327	-3.341208
H	11.829659	-1.512899	-4.317931
C	13.104189	1.394332	-3.836749
H	13.469079	2.157902	-3.167978
C	15.250004	-2.425006	2.018007
H	15.815982	-1.511614	2.224966
H	14.500891	-2.555685	2.796503
H	15.953615	-3.259666	2.017805
C	14.578842	-2.259631	0.677212
O	13.433406	-1.867882	0.579523
C	15.458997	-2.602873	-0.512760
H	16.438349	-2.136818	-0.333512
H	15.616421	-3.686268	-0.483412
O	14.939733	-2.279983	-1.766275
H	14.911465	-1.307592	-1.805758
Zn	12.545823	-0.301831	-1.336397
O	6.810104	2.148961	1.333947
H	6.528540	2.973083	0.884118
H	6.103121	1.465877	1.257805
O	2.768701	7.470945	0.126949
H	3.526653	6.786421	0.069894
H	3.115785	8.187262	0.669699
O	8.114094	-3.335877	1.062694
H	7.867091	-3.785838	1.877594
H	7.526670	-2.500070	1.018991

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C	4.750993	-2.222996	1.760011
H	4.815270	-1.961241	2.819081
H	5.107254	-3.243141	1.623807
H	3.707983	-2.134123	1.453874
C	5.625969	-1.221688	0.958940
O	5.130854	-0.072075	0.775148
O	6.762329	-1.616918	0.579306
C	1.641020	1.947993	2.710013
H	2.603448	2.355711	3.025708
H	0.884247	2.247060	3.434161
H	1.723722	0.859164	2.721853
C	1.255290	2.444523	1.368426
N	1.979327	2.224199	0.217309
H	2.870696	1.741012	0.164680
C	1.316546	2.788867	-0.811849
N	0.202204	3.359715	-0.385708
H	1.678244	2.784179	-1.826534
C	0.159584	3.152594	0.975472
H	-0.665191	3.505506	1.570497
C	-3.871947	2.117082	1.726922
H	-3.328153	2.572230	2.558530

H	-3.437157	1.134788	1.546168
H	-4.923704	2.033822	1.993074
C	-3.683824	2.991493	0.513546
O	-2.537099	2.914026	-0.028459
O	-4.611567	3.740651	0.121847
C	0.726977	7.210948	-3.288936
H	1.745979	7.591138	-3.349546
H	0.025093	8.042422	-3.221252
H	0.490255	6.642814	-4.189123
C	0.516701	6.321319	-2.099984
O	-0.653587	5.864235	-1.953177
N	1.516248	6.069716	-1.278263
H	1.324997	5.463529	-0.491320
H	2.505526	6.387734	-1.360548
C	-3.101998	-0.210987	-4.444985
H	-2.518876	-0.575127	-5.293464
H	-4.156784	-0.195987	-4.727319
H	-2.981349	-0.916512	-3.624450
C	-2.639145	1.144810	-4.002317
N	-2.666859	2.254104	-4.828368
H	-2.980933	2.268819	-5.785254
C	-2.218457	3.323209	-4.132197
N	-1.895586	2.969159	-2.909510
H	-2.159161	4.322724	-4.530823
C	-2.154182	1.616655	-2.812282
H	-1.994284	1.090155	-1.886027
Zn	-1.548531	4.208590	-1.214683
C	-3.693990	7.714975	1.287997
H	-3.045974	7.916597	2.138105
H	-3.869014	8.625904	0.711702
H	-4.662079	7.351879	1.644330
C	-3.086624	6.633606	0.412479
O	-2.211127	5.876410	0.785223
C	-3.639464	6.514537	-1.001833
H	-3.124729	7.255506	-1.620250
H	-4.707921	6.752019	-0.996701
O	-3.419022	5.238860	-1.555155
H	-3.994274	4.591744	-1.003580
C	6.585943	6.982989	-0.324008
H	6.529234	7.954888	-0.811986
H	7.461467	6.428720	-0.664428
H	6.674315	7.126021	0.755662
C	5.301703	6.147672	-0.600704
O	5.357729	4.920789	-0.333082
O	4.292356	6.769238	-1.050426
C	9.967994	3.532988	1.738980
H	9.279697	3.152511	2.496533
H	9.554081	4.469827	1.359922
H	10.924704	3.745010	2.215060
C	10.169309	2.551334	0.650210
N	9.183862	2.069781	-0.177165
H	8.145676	2.247333	-0.130075
C	9.749221	1.210407	-1.032712
N	11.054873	1.103548	-0.800266
H	9.192307	0.672494	-1.782383
C	11.323660	1.947969	0.251844
H	12.319553	2.087258	0.632185
C	15.804987	1.544998	0.422962
H	16.695804	1.181525	-0.095952
H	15.758275	1.061379	1.398571

H	15.880274	2.624583	0.529408
C	14.585677	1.197629	-0.407409
O	14.062021	2.030724	-1.146001
O	14.135717	-0.024001	-0.292412
C	10.094059	-3.799938	-1.705015
H	9.124021	-4.177315	-1.386100
H	10.130533	-3.736191	-2.792346
H	10.884261	-4.476754	-1.378014
C	10.398494	-2.443069	-1.134300
O	11.488127	-1.911290	-1.474708
N	9.539380	-1.875565	-0.303361
H	9.764236	-0.968206	0.080946
H	8.588408	-2.193756	-0.095616
C	13.456984	2.388959	-6.212933
H	12.726324	2.300124	-7.019941
H	14.458241	2.369779	-6.649019
H	13.316044	3.356990	-5.734944
C	13.283218	1.315058	-5.193715
N	13.404311	-0.036228	-5.465963
H	13.630132	-0.441705	-6.360042
C	13.193514	-0.727481	-4.325918
N	12.944657	0.105729	-3.338623
H	13.233809	-1.801660	-4.246441
C	12.997915	1.380355	-3.861931
H	12.858350	2.243146	-3.233122
C	15.249976	-2.425009	2.017991
H	15.051316	-1.379191	2.269616
H	14.556139	-3.059745	2.565744
H	16.281139	-2.650430	2.299925
C	15.033282	-2.578366	0.531397
O	14.000010	-2.994219	0.056502
C	16.208432	-2.149088	-0.336436
H	16.692674	-1.291858	0.147222
H	16.931412	-2.970531	-0.348113
O	15.856202	-1.871711	-1.665273
H	15.136987	-1.223154	-1.607506
Zn	12.483470	-0.184452	-1.371952
O	6.512669	2.260467	-0.188328
H	6.005499	3.097618	-0.160568
H	6.002497	1.495406	0.168024

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