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Insight towards the conserved water mediated recognition of catalytic and structural Zn^{+2} ions in human Matrix Metalloproteinase-8 enzyme: A study by MD-simulation methods

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Abstract:
Human matrix metalloproteinase-8 (hMMP-8) plays a important role in the progression of colorectal cancer, metastasis, multiple sclerosis and rheumetoid arthritis. Extensive MD-simulation of the PDB and solvated structures of hMMP-8 has revealed the presence of few conserved water molecules around the catalytic and structural zinc (Zn_C and Zn_S) ions. The coordination of two conserved water molecules (W and W_S) to Zn_S and the H-bonding interaction of W_S to S_{151} have indicated the plausible involvement of that metal ion in the catalytic process. Beside this the coupling of Zn_C and Zn_S metal ions (Zn_C – W_H (W_1).....W_2 ....H_{162} - Zn_S) through two conserved hydrophilic centers (occupied by water molecules) may also provide some rational on the recognition of two zinc ions which were separated by ~13 Å in their X-ray structures. This unique recognition of both the Zn^{+2} ions in the enzyme through conserved water molecules may be implemented/ exploited for the design of antiproteolytic agent using water mimic drug design protocol.

Key words: Matrix Metalloproteinase, MD simulation, Zn ions, Catalytic mechanism.

Background:
The matrix metalloproteinases (hMMPs) of human are zinc containing endopeptidases and they play some important roles in ovulation, embryogenic growth, tumor growth, metastasis, multiple sclerosis, rheumetoid arthritis etc **[1]**. The human neutrophil collagenase (HNC) or hMMP-8 is mainly produced by neutrophils and involves in colorectal cancer **[2]**. The biological role of hMMP–8 and its actual function / mechanism is incompletely understood. So, hMMP–8 is one of the attractive target for design the inhibitors of extracellular matrix degradation in colorectal cancer and antiproteolytic drug for inflammatory diseases **[1, 3]**. The hMMP-8 is structurally homologous to all other MMPs, consisting of defined functional domains. The crystallographic structures (1BZS, 1JAP, 1ZP5, 1ZVX, 1ZSO) **[4-8]** of hMMP-8 **Table 1 (see supplementary material)** have explored the presence of two (catalytic and structural) zinc ions in catalytic domains of enzyme. Catalytic zinc ion (Zn_C) is bound to three histidine reidues (H_{197}, H_{201}, H_{207}) and different inhibitors (BSI250, HOA4, 2NI128, FIN994, EIN994 respectively), whereas the structural zinc (Zn_S) is coordinated by three histidine residues (H_{147}, H_{162}, H_{175}) and one aspartic acid (D_{149}) which are given in **Table 2 (see supplementary material)**. The catalytic domain also has two calcium ions which are thought to involve in the structural function of enzyme. Furthermore, regarding the mechanism of activity of different hMMPs, the catalytic role of Zn_C is well

known **[9]**; however, the participation of other zinc ion (Zn_S) in the catalytic process has also been indicated in MMP-13 **[10]**.

In this work we perform long time MD simulations in order to study the dynamics of water molecules and their interaction with catalytic and structural zinc (Zn_C and Zn_S) centers. Beside these, the role of Zn_S (structural zinc ion) in the catalytic mechanism / process of hMMP-8 can also be investigated through computational dynamic of solvated enzyme. In addition the simulation could also shade some light on the water mediated recognition between the two zinc centers and their conjugal involvement in the catalytic process. All the results may provide further complementary information on the inhibitor binding chemistry and also enrich the biochemical insight of hMMP-8 enzyme which may be useful for the development of inhibitor topology of hMMP–8.

Methodology:

X-ray structures of hMMP–8 (1BZS, 1JAP, 1ZP5, 1ZVX, 1ZSO) were taken from Protein Data Bank **[11-13]**. All the five structures are monomer and are bonded by several inhibitors (BSI250, HOA4, 2NI128, FIN994, EIN994) given in Table 2. From all the structures, protein, Zn ions and water molecules were isolated using Swiss PDB viewer program **[14, 15]**. The inhibitors were removed from all the structures.

Solvation

All the hMMP-8 structures were solvated using the program CHASA (Conditional Hydrophobic Accessible Surface Area) **[16]** without implying Periodic Boundary Condition (PBC). The explicit hydration have been employed on each structure where approx ~ 800-1000 water molecules have been used to immerse molecules by TIP3 water model.

Energy minimization of the structures

All the five modified PDB and solvated structures were energy minimized by GROMOS 96 force field **[17]** (500 steps of steepest decent followed by 1000 cycles of conjugate gradient) in the Swiss PDB Viewer program with 10Å cut–off distance **[18]** for nonbonded interaction, using a distance dependent dielectric constant. At the initial stage of energy minimization, the protein was kept fixed and the water molecules were only allowed to move freely. Optimization released the local constraints from the structures, the energy minimization was again followed (1000 cycles) by allowing the protein and water molecules to move freely.

Molecular dynamics structures

For investigating the role of water molecules, solvation of native PDB structures was done by creating a water box of dimension 10 × 10 × 10 $Å^3$, using solvate 1.2 plugin within the Visual Molecular Dynamics v. 1.8.6. program **[19]** and MD simulations were followed. Total ~ 2196 water molecules (TIP3P water model) were used to immerse each molecule. The molecular dynamics of each structure was performed by converting the solvated and PDB structures to Protein Structure File (PSF) using the tool Automatic PSF Generation Plugin–1.0 v by applying CHARMM force field **[20, 21]** within the Visual Molecular Dynamics v. 1.8.6. All the ten (five PDB and their solvated) structures were simulated. All the structures were initially energy minimized (100 cycles to eliminate initial contacts which would destabilize the system) using CHARMM force field. After energy minimization all the structures were simulated using Auto Interactive Molecular Dynamics (IMD) v.1.8.6 and Nanoscale Molecular Dynamics v.2.6. **[22]**. During simulation, the whole system was allowed to move freely **[23]**. The molecular simulations were followed upto 5 ns for each structure (where time step was 2fs) at 300K temperature by means of Langevin dynamics **[24]**. The whole system was converged within 500ps and the simulation was adequately converged within 2ns **(Figure 1)**. During the dynamics, several snapshots were recorded (every 2ps) to investigate the detail coordination or interaction of water molecules with the Zinc (Zn_C / Zn_S) ions.

Identification of conserved water molecules

To identify the conserved water molecules in hMMP-8, the five different X-ray crystal structures of the protein (1BZS, 1JAP, 1ZP5, 1ZVX, 1ZSO) were compared using 1BZS as template. All the other structures were superimposed one by one on the template structure by a standard least-square fitting algorithm using Swiss PDB Viewer program. The fit was initially optimized on the backbone atoms and the RMSD values were ~0.5-0.6Å. After superimposing the concerned structure on the template, the conserved water molecular sites were compared and located between the two respective structures. Water molecules that were found to be within 1.5Å **[25, 26]** in between the two structures (template and concerned reference structures) were taken as conserved.

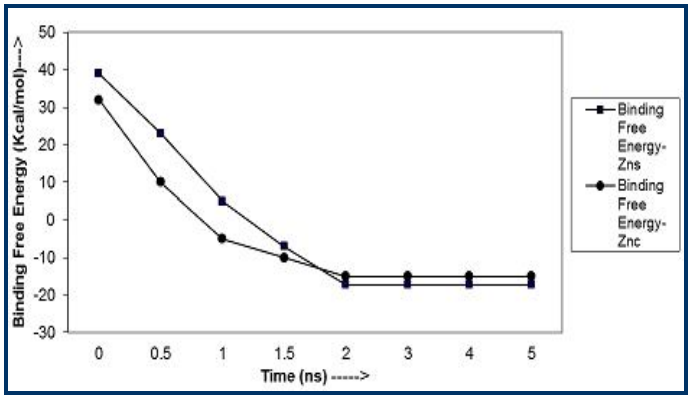

Figure 1: The binding free energy of Znc and Zns in 1 BZS structure during 5ns MD Simulation**.**

Binding free energy and electrostatic free energy of Zn ions

Binding free energy and electrostatic free energy of both the Zn ions in the PDB and MD simulated hMMP-8 structures were calculated by FOLDX program **[27]**. The temperature, ionic strength, pH and VDW (parameters of FOLDX) were assigned as 300K, 0.05 (M), 7.0 and 2.0 Å.

Results:

X-ray Structural information

In this work the catalytic and structural zinc ions are designated by Zn_C and Zn_S. Throughout the work the water molecules which are directly coordinated to Zn_C are abbreviated as W_H and W_I. The W_H (Trans to Zn_C bound H_{207}) is thought to involve in the hydrolysis of substrate **[28]**. The W_I (Trans to Zn_C bound H_{197}) is in close proximity to inhibitor binding site. In the five PDB structures coordination of residues (H_{197}, H_{201}, H_{207}) to Zn_C is same. In these structures Zn_C is also bonded to a competitive inhibitor….(BSI250, HOA4, 2NII8, FIN995, EIN994

respectively). In the structures 1JAP, 1ZVX, 1ZSO the inhibitor molecule has two donor centers, whereas in 1BZS and 1ZP5 the inhibitors have only one donor center. However, the structural zinc ion (Zn_S) is coordinated by three histidines (H_{147}, H_{162}, H_{175}) and one aspartic acid (D_{149}).

Analysis of energy minimized structures

During energy minimization of all the PDB structures, the coordination number of Zn_C changes due to the bonding of incoming water molecules to this metal ion. In the energy minimized native 1BZS, 1JAP, 1ZVX crystal structures, two incoming water molecules (W_H and W_I) form bond with Zn_C, and W_H also forms H–bond to E_{198} (2.5 to 3.5 Å). Whereas in the PDB structures of 1ZP5 and 1ZSO only one incoming water molecule (W_I) is bonded to Zn_C. However, no such water molecular interaction is observed at Zn_S. All the distances are included in **Table 3 (see supplementary material)**. Again, after energy minimization of the two (1BZS, 1JAP) solvated PDB structures two incoming water molecules are observed at the same hydrophilic W_H and W_I sites which are included in **Table 3**. In the rest three (1ZP5, 1ZVX, 1ZSO) solvated structures, only one water molecule appears at W_I site and coordinated to Zn_C. However no water molecular coordination is observed at the structural zinc ion (Zn_S).

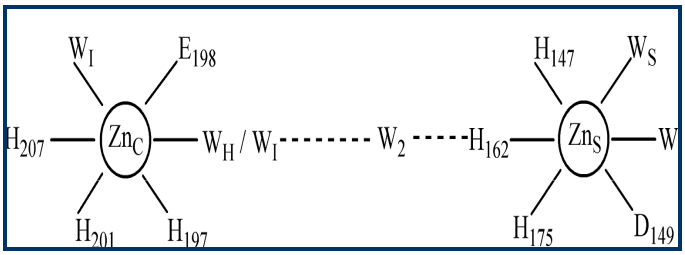

Figure 2: Water mediated recognition of the catalytic and structural zinc ions (Znc and Zns) after MD simulation.

Analysis of MD simulated structures

MD simulation of the different X-ray and solvated structures has also revealed the presence of water molecules at the W_I and W_H hydrophilic centres. Within 500ps, both the water molecules (present at the two hydrophilic centres) and one glutamic acid residue (E_{198}) are appeared to coordinate with Zn_C. In every structure the W_H water molecule forms H–bond to E_{198} (OE1) (~ 2.1 to 2.6 Å). During 5ns simulation, the trigonal geometry around Zn_C has changed to distorted octahedral and the coordination number changed from 3 to 6 **Table 4 (see supplementary material) (Figure 2)**. In the simulated PDB structures, the average Zn_C …..W_1, W_1…..W_2 and W_2…..H_{162} distances are 2.07 – 2.17, 2.14 – 2.80 and 2.23 – 2.87Å respectively. In all the simulated X- ray and solvated structures of hMMP–8 (1BZS, 1JAP, 1ZP5, 1ZVX, 1ZSO) the Zn_S atom is coordinated by two positionally conserved water molecule (W and W_S) and three histidine and one aspartic acid residue. Among the two water molecule one (W_S) also bonded to S_{151}. Within 500ps, these two water molecules appear to coordinate with Zn_S and S_{151} also bonded to W_S. In the simulated X-rays structures the average Zn_S …..W and Zn_S….. W_S distances are 2.08 – 2.15 and 2.02 – 2.21 Å, however in the solvated structures the values are 2.01 – 2.27 and 2.06 – 2.62 Å Moreover, after 2ns, the Zn_C bound W_H / W_1 water molecule bridges to Zn_S bound H_{162} through another W_2 water molecule (Zn_C – W_H (W_1)…..W_2 ….H_{162} - Zn_S). The H–bonding distances are given in **(Figure 2) Table 5 (see supplementary material)**.

The average binding free energy of Zn_C and Zn_S ions in the five PDB structures (1BZS, 1JAP, 1ZP5, 1ZVX, 1ZSO) are 23.88 and 137.1 Kcal/mole, whereas after simulation the values are reduced to -75.7 and -34.5 Kcal/mole. After solvation, the average binding free energy of Zn_C and Zn_S are 87.6 and 94.2 Kcal/mole whereas after simulation (of the solvated structures) the average binding free energy reduced to -51.1 and -48.3 Kcal/mole **(Figure 1) Table 6 (see supplementary material)**. The average electrostatic binding free energy of Zn_C and Zn_S ions of the five PDB structures (1BZS, 1JAP, 1ZP5, 1ZVX, 1ZSO) are -0.848 and -0.682 Kcal/mole respectively, whereas after simulation the values are -6.0 and -10.2 Kcal/mole. After solvation, the average electrostatic binding free energy of Zn_C and Zn_S -8.26 and 13.6 Kcal/mole, whereas after simulation (of solvated structures) the values are reduced to -14.43 and -26.45 Kcal/mole **Table 6**.

Discussion:

The hydrophilic center around both the zinc ions (ZnC and ZnS) in hMMP–8 structures seems to be interesting. The coordination of water molecules present at W_H hydrophilic site which forms H-bond to E_{198} around Zn_C ion in three Emin X-ray structures (1BZS, 1JAP, 1ZVX) and all MD simulated structures are observed to be conserved. Furthermore, the coordination of W_I water molecule in two Emin X-ray structures (1ZP5, 1ZSO) and all MD simulated structures is also conserved. The presence of W_H and W_I hydrophilic centers has been revealed during the simulation of hMMP-8 PDB and solvated structures. These two conserved water molecules may provide some stability to Zn_C ion. Nevertheless, in all the MD simulated X–ray and solvated structures, the Zn_S bound two water molecules occupying the same hydrophilic centers (trans to H_{162}, H_{175}), indicate the possibilities of variable hydration susceptibility of catalytic and structural zinc ions (Zn_C and Zn_S) in the hMMP–8 dynamical structures **Table 4**. The shift or fluctuation of binding and electrostatic free energy of both the zinc ions in the X-ray and simulated structures may imply some possibility of Zn_S centre to act as a catalytic partner.

The variation of coordination geometry from tetrahedral to distorted octahedral through the interaction of two water molecule (W and W_S) directly to Zn_S in all the PDB and solvated MD–simulated structures and the H–bonding of W_S with S_{151}(OG) **Table 7 (see supplementary material)** indicated the possible involvement of metal ion and S_{151} to the catalytic process. Furthermore, the recognition of the Zn_C and Zn_S (which are ~ 13Å apart) through two conserved water molecules (W_H / W_1 and W_2) and the observation of similar type of water mediated conjugation of metal ions in different synthetic Zn–complexes **[29, 30]** may also support the role of two Zn centres in the function and mechanism of enzyme **(Figure 2 & 3)**. Based on the dynamical results of this work the participation of Zn_S in the collagenolysis mechanism of hMMP-8 may be proposed **(Figure 3)**. The Zn_S directly binds to substrate and polarize it and subsequently activate the water molecule which may then act as a nucleophile. The Zn_S bound W_S (water molecule) and S_{151}(OG) may also thought to activate the metal ion in the intermediate transition steeps of the photolytic mechanism.

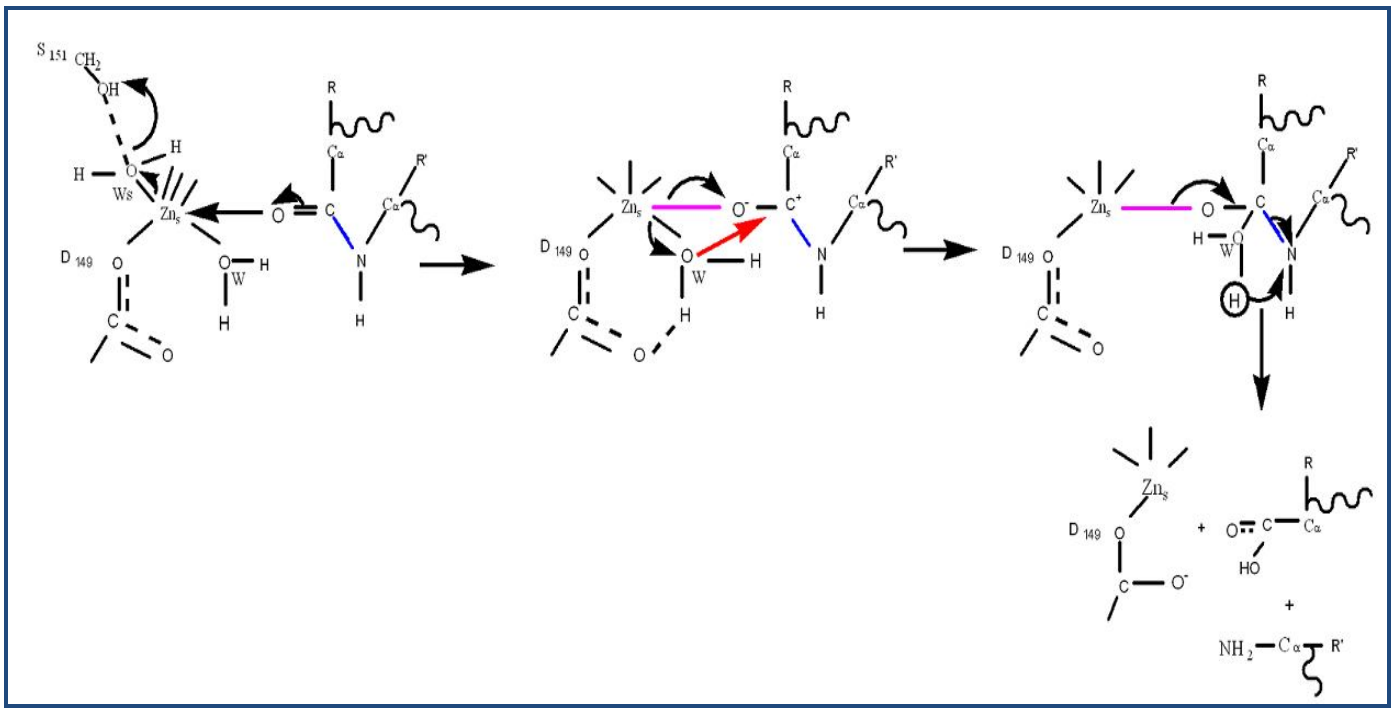

Figure 3: Proposed mechanism on water mediate hydrolysis of substrate in hMMP-8.

Conclusion:

The MD-simulation of the PDB and solvated structures of hMMP-8 have revealed the presence of few conserved water molecules (W and W_S) around the catalytic and structural zinc (Zn_C and Zn_S) ions, which are mostly inaccessible in X-rays structures. The coordination of these two conserved water molecules to Zn_S and the H-bonding interaction of W_S to S_{151} have indicated the plausible involvement of that metal ion in the catalytic process. The differential coordination dynamics of the ligands (water molecules) around both the zinc ions seem to be an interesting feature of hMMP-8. Beside this the coupling of Zn_C and Zn_S metal ions through two conserved hydrophilic centers (occupied by water molecules) may also provide some rational clues on the recognition of two zinc ions which were separated by ~13 Å in their X-ray structures. Possibly, the biochemical signal between the two Zn^{+2} ions may be transmitted through W_1 and W_2 water centers in enzyme.

The unique recognition and stereospecific interaction of both the Zn^{+2} ions through conserved water molecules of both the Zn^{+2} ions in the enzyme (Zn_C – W_H (W_1).....W_2 ....H_{162} - Zn_S) seem to be important for activity and ligand recognition. Possibly, the geometrical and electronic consequences of conserved water molecular interaction W and W_S are very implicative for design the topology of hMMP-8 inhibitor which may be implemented / exploited for the design of antiproteolytic agent using water mimic drug design protocol. Possibly, heterocyclic ligand with flexible structure containing two aliphatic short chains with suitable spacer length may be suggested to design selective inhibitor for hMMP-8.

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Supplementary material:

Table 1: Preliminary X-ray structural data of hMMP- 8

PDBID	No. of protein molecule in asymmetric unit	Number of total amino acid in each chain	N Number of water molecules	Name of inhibitor	R factor	Resolution (Å)	
1BZS	1	165	208	[a]BSI250	0.192	1.70	4
1JAP	1	157	148	[b]HOA4	0.194	1.82	5
1ZP5	1	163	125	[c]2NI128	0.219	1.80	6
1ZVX	1	163	144	[d]FIN994	0.209	1.87	7
1ZS0	1	163	244	[e]EIN994	0.210	1.56	8

a 2-(biphenyl-4-sulfonyl)-1,2,3,4-tetrahydro- isoquinoline-3-carboxylic acid.
bH3 N O.
cN-{2-[(4'-cyano-1,1'-biphenyl-4-yl)oxy]ethyl}- n'-hydroxy-n-methylurea.
d(1R)-1-{[(4'-methoxy-1,1'-biphenyl-4-yl)sulfonyl]amino}- 2-methylpropylphosphonic acid.
e(1S)-1-{[(4'-methoxy-1,1'-biphenyl-4-yl)sulfonyl]amino}- 2-methylpropylphosphonic acid.

Table 2: The residues / Inhibitor (I) forms bond to catalytic and structural zinc atoms (ZnC and ZnS) in X-ray structures of hMMP-8 and their distances in Å.

PDB ID	Residues coordinated to Zn_C		Residues coordinated to Zn_S	
1BZS	H 197	2.12	H 147	2.16
	H 201	2.08	H 162	2.19
	H 207	2.17	H 175	2.23
	I	1.97	D 149	1.93
1JAP	H 197	2.00	H 147	1.94
	H 201	2.12	H 162	2.04
	H 207	2.25	H 175	2.11
	I	1.91	D 149	1.78
	I	2.18		
1ZP5	H 197	2.08	H 147	2.08
	H 201	2.06	H 162	2.05
	H 207	2.11	H 175	2.07
	I	2.26	D 149	1.98
1ZVX	H 197	2.13	H 147	2.05
	H 201	2.09	H 162	2.10
	H 207	2.15	H 175	3.50
	I	1.98	D 149	2.13
	I	3.47		
1ZSO	H 197	2.08	H 147	2.09
	H 201	2.10	H 162	2.05
	H 207	2.16	H 175	2.13
	I	3.27	D 149	2.01
	I	3.22		

Table 3: Water (WI, WH) molecules interact at the ZnC atom and their distances(Å) in the energy minimized (Emin) PDB and solvated hMMP-8 structures.

PDBID	X-raystructures	Energy minimized structures			
		X-ray			
1BZS	-------	[a]W_I	W 207..2.08	W_I	W 426 1.95
		[b]W_H	W 69 2.22	W_H	W 317..1.95
1JAP	--------	[a]W_I	W 23…1.94	W_I	W 408 1.93
		[b]W_H	W 43 2.04	W_H	W 309 1.75
1ZP5	--------	[a]W_I	W 69 1.75	W_I	W 306 2.02

1ZVX	--------	[a]W_I	W 9 1.98	W_I	W 401 1.98
		[b]W_H	W 54 1.99		
1ZSO	--------	[a]W_I	W 41 1.98	W_I	W 412 1.86

aWI indicate the water molecule trans to ZnC boun H197.
bWH indicate the water molecule trans to ZnC boun H207.

Table 4: Recognition of the residues and water molecules to ZnC and ZnS atoms in the 5ns simulated X-ray and solvated structures. All the distances are in Å.

PDBID	Distances of the Zn_C…W / E_{198} and Zn_S…W_S/W			
	X–ray structures		Solvated structures	
	Zn_C…W / E_{198}	Zn_S… W_S / W	Zn_C…W / E_{198}	Zn_S… W_S / W
1BZS	W 162 2.06 W 69 2.17 E_{198} 1.95	W 93(W_S) 2.02 W 33(W) 2.08	W 140 2.04 W 69 2.07 E_{198} 1.99	W 41(W_S) 2.09 W 33(W) 2.12
1JAP	W 25 2.04 W 23 2.09 E_{198} 1.99	W 81(W_S) 2.02	W 25 2.13 W 5409 2.15 E_{198} 1.99	W 4611(W) 2.23
1ZP5	W 69 2.09 W 49 2.14 E_{198} 2.01	W 23 (W_S) 2.21 W 2(W) 2.08	W 76 2.75 W554 2.32 E_{198} 2.11	W 114 (W_S) 2.62 W 736 (W) 2.01
1ZVX`	W 9 2.11 W 144 2.12 E_{198} 2.05	W 60(W_S) 2.08 W 63(W) 2.15	W 205 2.81 W 674 2.04 E_{198} 2.81	W 668(W_S) 2.17 W 1484(W) 2.35
1ZSO	W 49 2.07 W 71 2.18 E_{198} 1.94	W 14(W_S) 2.09 W 48(W) 2.15	W 642 2.12 W 965 2.14 E_{198} 2.45	W 1004(W_S) 2.06 W1513(W) 2.27

* The residential frequency of all the water molecules is 0.75. The water molecule which involves in the interaction with S151 and is coordinate to the ZnS (S151…..WS…..ZnS) is designated by WS.

Table 5: Water mediated (W1, W2) interaction and the distances (Å) between catalytic zinc (ZnC) and H162 (bonded to ZnS) in the MD simulated PDB structures of hMMP-8.

PDB ID	W_1 /Zn_c---W_1(W_H) Distance	W_2 /W_1---- W_2	W_2---- H_{162} (Zn_s)
1BZS	W 69 2.17	W 207 2.31	2.23
1ZP5	W 49 2.14	W 86 2.66	2.87
1ZVX	W 9 2.11	W 115 2.73	2.65
1ZSO	W 149 2.07	W 116 2.80	2.42

Table 6: The binding free energy and electrostatic free energy (in Kcal/mol) of the PDB and simulated (X-ray and solvated) structures.

Binding free energy / Electrostatic free energy of the Zinc ions						
PDBID	**X-ray structures**			**MD Simulated(5 ns) structures**		
				X-ray	**Solvated**	
	Zn_C	Zn_S	Zn_C	Zn_S	Zn_C	Zn_S
1BZS	32 / -0.6	39.2 / -1.83	-15.5 / -1.8	-17.2 / -1.79	-10.2 / -1.94	-8.9 / -4.17
1JAP	28.4 / -1.13	26.8 / -1.97	-9.2 / -0.66	-14.8 / -12.74	-9.2 / -2.75	-12.5 / -4.74
1ZP5	29 / -0.67	35.6 / -0.9	-12.6 / -1.79	6 / -1.00	-6.4 / -3.54	-5.7 / -6.24
1ZVX	22.6 / -0.86	28.7 / -0.79	-4.8 / -2.02	9 / -2.83	-10.5 / -3.04	-14.4 / -6.18
1ZSO	7.4 / -0.98	6.8 / 2.08	-16.8 / -1.05	17.5 / -1.84	-14.8 / -3.16	-6.8 / -5.12

Table 7: Distances (Å) of S151……ZnS, ZnS….WS, and S151(OH)…..WS in the X-ray and MD simulated structures of hMMP-8.

PDB ID	**X-ray**		**MD Simulated (X-ray structures)**		**MD Simulated (Solvated structures)**	
	S151….ZnS	**.ZnS WS**	**S_{151}….Zn_S**	**S151(OH)….WS**	**S151….ZnS**	**S151(OH)….WS**
1BZS	6.05	W 93 7.67	3.92	W 93 3.04	4.08	W 41 2.60
1JAP	6.82	W 81 7.14	3.72	W 81 2.88	3.99	W 4611 2.75
1ZP5	6.81	W 23 12.76	3.89	W 23 2.72	4.21	W114 3.12
1ZVX	6.60	W 60 17.12	4.25	W 60 3.14	3.88	W 668 3.10
1ZSO	6.93	W 14 7.97	4.10	W 14 3.02	4.19	W 1004 2.95

* The ZnS…WS distances in the MD simulated X-ray and solvated structure is given in Table 4.