Document. S1, Main parameters for the bi-zinc active center of NDM-1

1. ZN1-related parameters

1.1 parameters for ZN1

ATOM	RES	RESNAME	NAME	Mass	Charge	GB Radius	GB Screen
3350	229	ZN1	ZN	65.4000	0.6368	1.5000	0.8000

1.2 parameters for the ZN1 coordinate bonds

Atom 1		Atom 2		R	eqFrcCnst
1211	NE2	3350	ZN	2.0285	70.1000
1234	ND1	3350	ZN	1.8140	235.4000
2184	NE2	3350	ZN	2.0396	65.7000

1.3 parameters for ZN1-related bondangle

Atom 1		Atom 2		Atom 3		FrcCnst	Theta eq
1212	CD2	1211	NE2	3350	ZN	50.0000	123.6701
1211	NE2	3350	ZN	1234	ND1	35.0000	99.6900
1211	NE2	3350	ZN	2184	NE2	35.0000	108.4100
1209	CE1	1211	NE2	3350	ZN	50.0000	121.3201
1235	CE1	1234	ND1	3350	ZN	50.0000	126.1701
1234	ND1	3350	ZN	2184	NE2	35.0000	110.6200
1233	CG	1234	ND1	3350	ZN	50.0000	124.9101
2185	CD2	2184	NE2	3350	ZN	50.0000	118.0601
2182	CE1	2184	NE2	3350	ZN	50.0000	132.0401

2. ZN2-related parameters

2.1 parameters for ZN2

ATOM	RES	RESNAME	NAME	Mass	Charge	GB Radius	GB Screen
3351	230	ZN2	ZN	65.4000	0.8113	1.5000	0.8000

2.2 parameters for the ZN2 coordinate bonds

Atom 1		Atom 2		R	eqFrcCnst
1268	OD1	3351	ZN	2.3275	21.0000
2430	SG	3351	ZN	2.3500	52.2000
3040	NE2	3351	ZN	2.1732	29.4000

2.3 parameters for ZN2-related bondangle

Atom 1		Atom 2		Atom 3		FrcCnst	Theta eq
1268	OD1	3351	ZN	2430	SG	35.0000	97.9500
1268	OD1	3351	ZN	3040	NE2	35.0000	88.8400
1267	CG	1268	OD1	3351	ZN	50.0000	141.4301
2430	SG	3351	ZN	3040	NE2	35.0000	107.5700
2427	CB	2430	SG	3351	ZN	70.0000	107.9900
3041	CD2	3040	NE2	3351	ZN	50.0000	122.7301
3038	CE1	3040	NE2	3351	ZN	50.0000	127.7001

Table S1： Zn-water and water-water distance values in the five configurations

PDB/conf*	Res#	dZ	dW1W2	dZN1W1	dZN2W1	dZN1W2	dZN2W2	dW1W3	dW2W3	dZN1W3	dZN2W3	dZN1W4	dZN2W4	dW1W4
3SPU	2.1	3.84	2.21	2.54	2.46	4.19	2.48	-	-	-	-	-	-	-
4TZE	1.57	3.49	3.16	2.05	2.07	3.98	2.12	-	-	-	-	-	-	
4TYF	1.1	3.54	3.07	1.89	2.03	3.97	2.1	-	-	-	-	-	-	-
4TZF	1.22	3.7	3.25	2	2.13	4.57	2.02	-	2.5	4.14	2.07	-	-	-
3Q6X	1.3	4.58	-	2.07	2.95	-	-	-	-	-	-	-	-	-
5N0H	1.9	3.83	2.08	2.15	2.08	3.83	2.71	-	-	-	-	2.94	4.70	3.01
S1[a]-conf	-	4.00±0.17	3.90± 0.50	2.26± 0.11	2.31± 0.11	4.82± 0.33	2.26± 0.09	3.22± 0.13	3.08± 0.34	2.21± 0.08	4.87± 0.51	2.33± 0.07	4.12± 0.61	2.65± 0.84
S2[b]-conf	-	3.80± 0.42	3.62± 0.31	2.12± 0.71	2.39± 0.19	4.91± 0.40	2.24± 0.87	-	-	-	-	-	-	-
I[c]-conf	-	3.60± 0.23	-	-	-	4.53± 0.36	2.27± 0.08	-	-	-	-	-	-	-
sub-free	-	4.10± 0.50	3.88± 0.45	2.27± 0.14	2.43± 0.19	5.05± 0.40	2.30± 0.14	3.02± 0.24	3.28± 0.27	2.46± 0.18	4.74± 0.57	2.37± 0.22	4.08± 0.63	2.77± 0.17

*, configuration; #, resolution (Å); [a], configuration A1; [b], configuration A2; [c], configuration B, C1 and C2

Table S2：Decompostion of binding free energy of MM-PBSA of five enzyme-substrate configurations

Component	A1	A2	B	C1	C2
	avg±std (kcal/mol)	avg±std (kcal/mol)	avg±std (kcal/mol)	avg±std (kcal/mol)	avg±std (kcal/mol)
VDWAALS	-23.55±2.69	-28.02±4.42	-20.40±2.30	-23.89±3.00	-24.53±3.16
EEL	-96.18±10.84	-118.28±12.36	-151.02±8.72	-76.26±8.61	-123.29±11.14
EPB	67.21±9.76	81.25±11.44	114.04±7.66	40.65±7.47	82.77±11.58
ECAVITY	-3.01±0.26	-3.98±0.43	-2.65±0.18	-3.22±0.29	-3.80±0.35
DELTA G gas	-119.73±10.86	-146.30±14.24	-171.41±8.70	-100.15±8.06	-147.82±12.03
DELTA G solv	64.20±9.65	77.27±11.13	111.38±7.60	37.43±7.48	79.00±11.36
TOTAL	-55.53±5.55	-69.04±5.23	-60.03±3.76	-62.73±4.32	-68.84±2.86

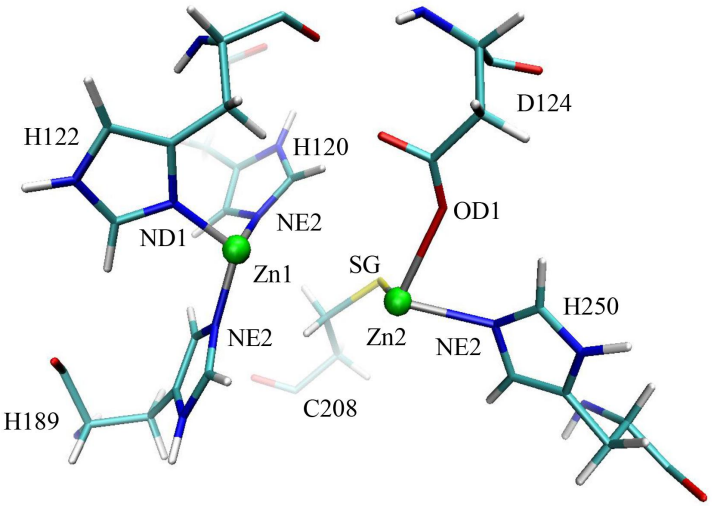

Figure S1. The bonded model of zinc ions active center of NDM-1 built through the method of MCPB.py.

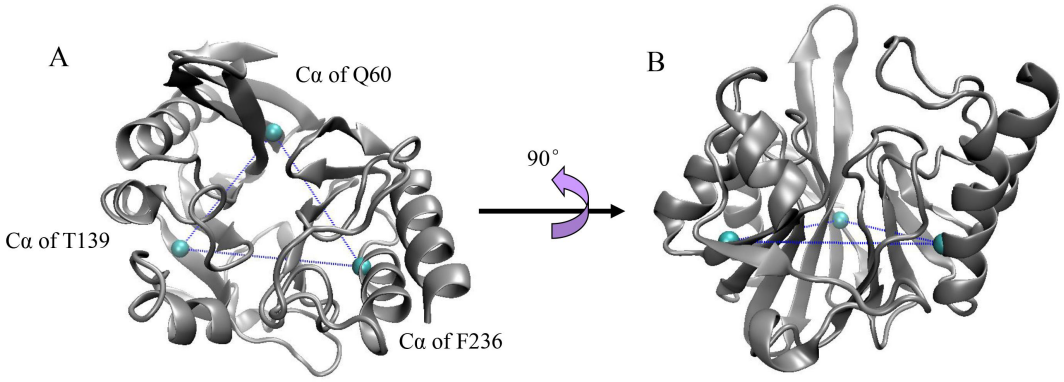

Figure S2. The plane defined by the Cα of Q60, T139 and F236. (A) top view; (B) side view.

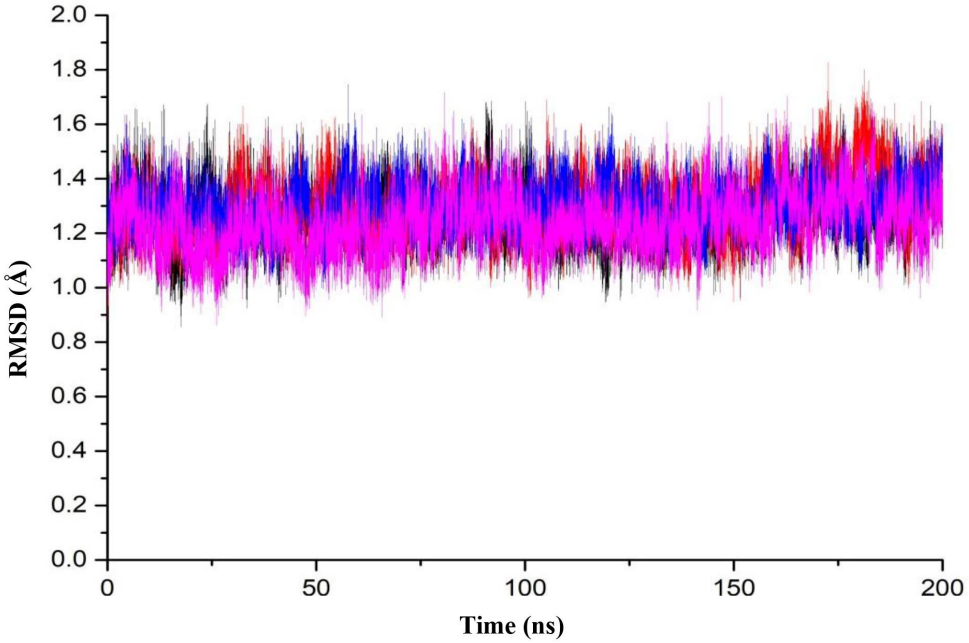

Figure S3. RMSD values of four randomly selected MD trajectories.

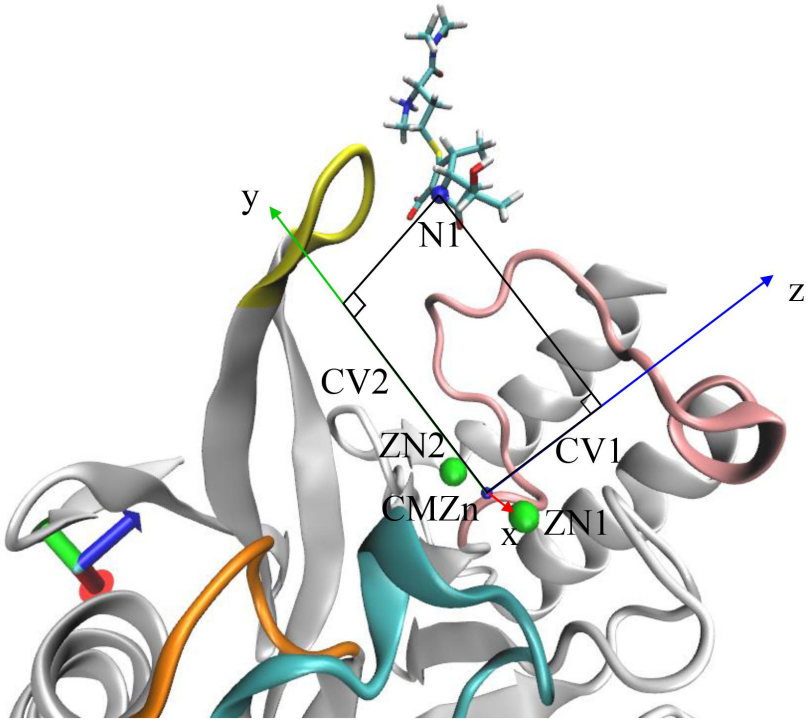

Figure S4. The collective variables in this study. N1 atom of MER molecule is shown as blue ball, ZN1 and ZN2 is shown as green ball, the center of mass of the zinc ions (CMZn) is shown as a small blue ball. The x, y and z axis are shown as red, green and blue arrows. Loop3, 4, 6 and 10 are shown in yellow, orange, cyan and pink. The first CV was the distance between the N1 atom (located in the lactam ring of MER) and the CMZn along the Z axis. The second CV was the distance between N1 atom and the CMZn along the Y axis.

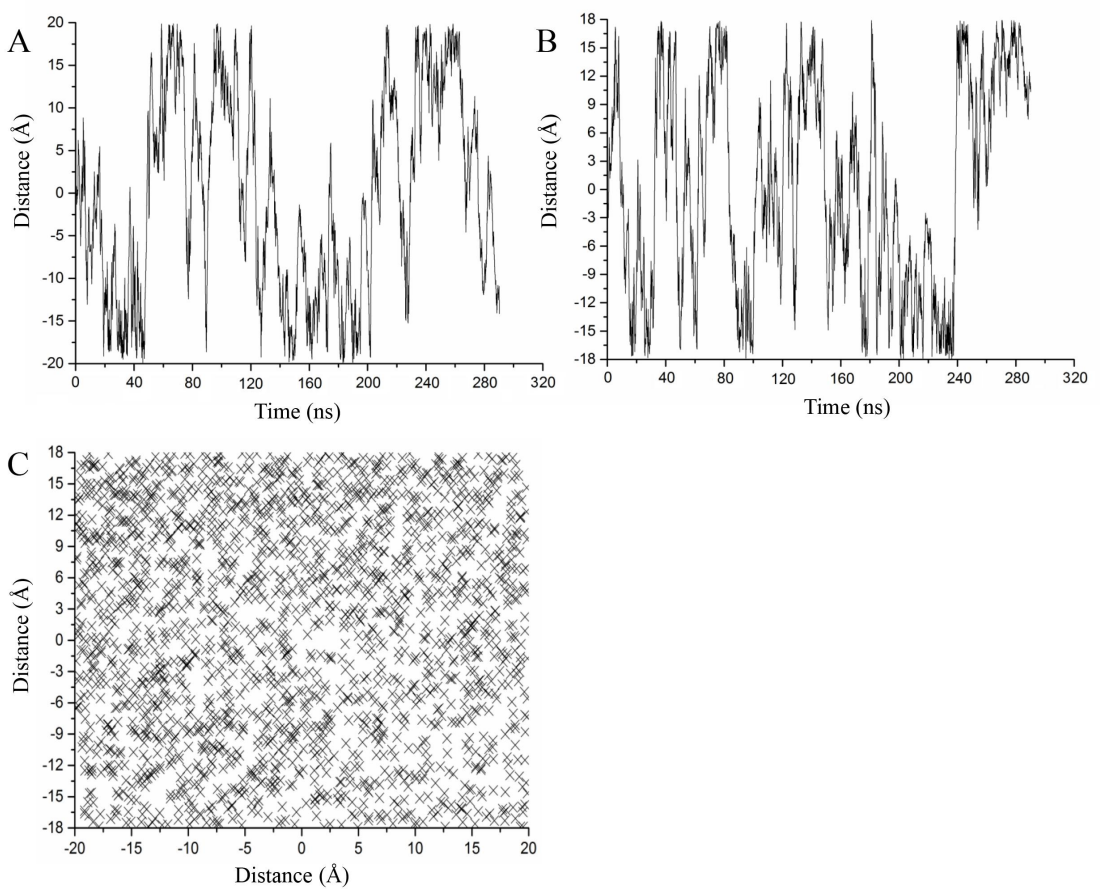

Figure S5. The convergence in metadynamics simulation. The change of CV1 (A) and CV2 (B) along the simulation time, and the distribution of sampling points in the sampling region (C).

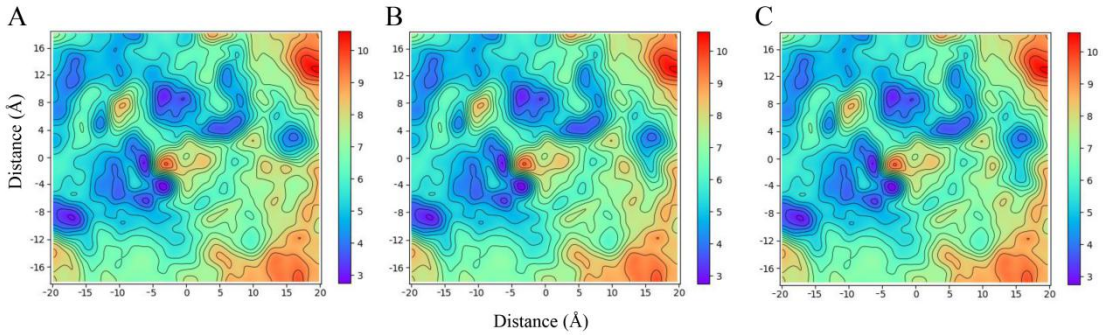

Figure S6. The contour plot of the free energy landscape reconstructed of 270 ns (A), 280 ns(B) and 290 ns (C) of metadynamics simulation respectively. The bar in the right side of each figure indicates the free energies (kcal/mol). As can be seen, the positions of the energy minimas on the surface do not change between frames. The three free energy landscapes are very similar, with the relative energy difference between the minima and only small changes in the regions loop10.

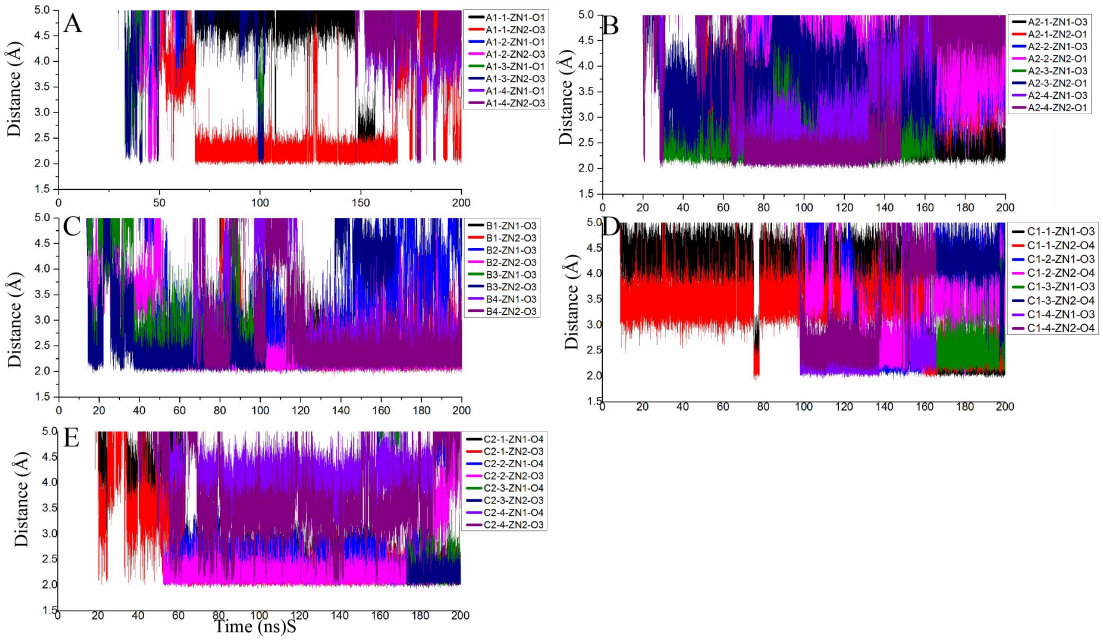

Figure S7. The Zn-O distance change (observed in a range of 1.5-5Å) of five different trajectories produced configuration (A) A1, (B)A2, (C) B, (D) C1 and (E) C2.

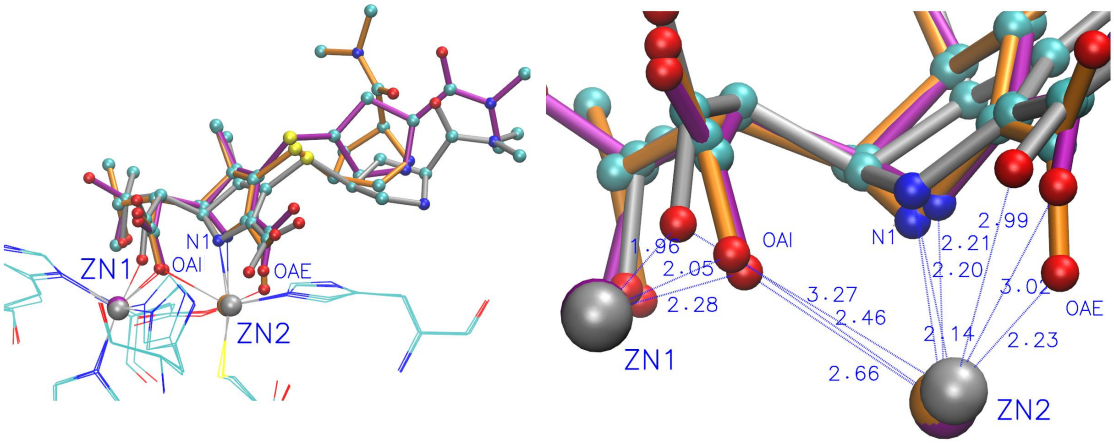

Figure S8. The superimoposition of three published crystal structures of NDM-1 with hydrolyzed meropenem. A, the overviewer of the three crystal structures; B, the detailed distance values (blue numbers) between N1 (of the original β-lactam ring) and carboxylic O atoms in hydrolyzed MER and zinc ions. The bonds of 4EYL, 4RBS and 5N0H are shown as purple, gray and orange.

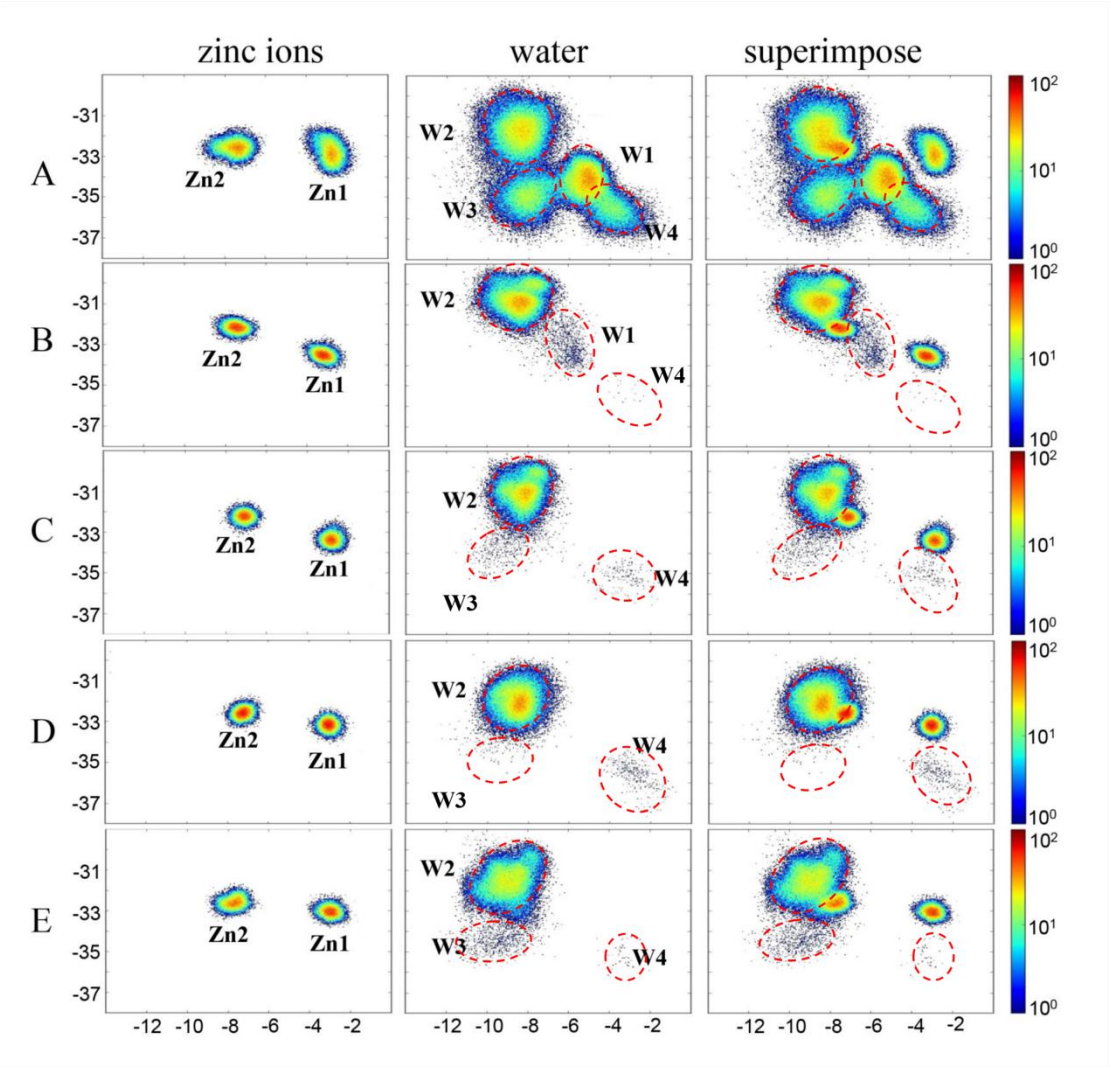

Figure S9. The planar projection of water molecules in the active center of NDM-1 of five different configurations. A to E, the distribution models of water molecules in the AC of NDM-1 of configuration (A) A1, (B)A2, (C) B, (D) C1 and (E) C2. Regions corresponding to W1, W2, W3 and W4 are indicated in red all circled. The bar at the right side of the figures indicated the density of water molecules.

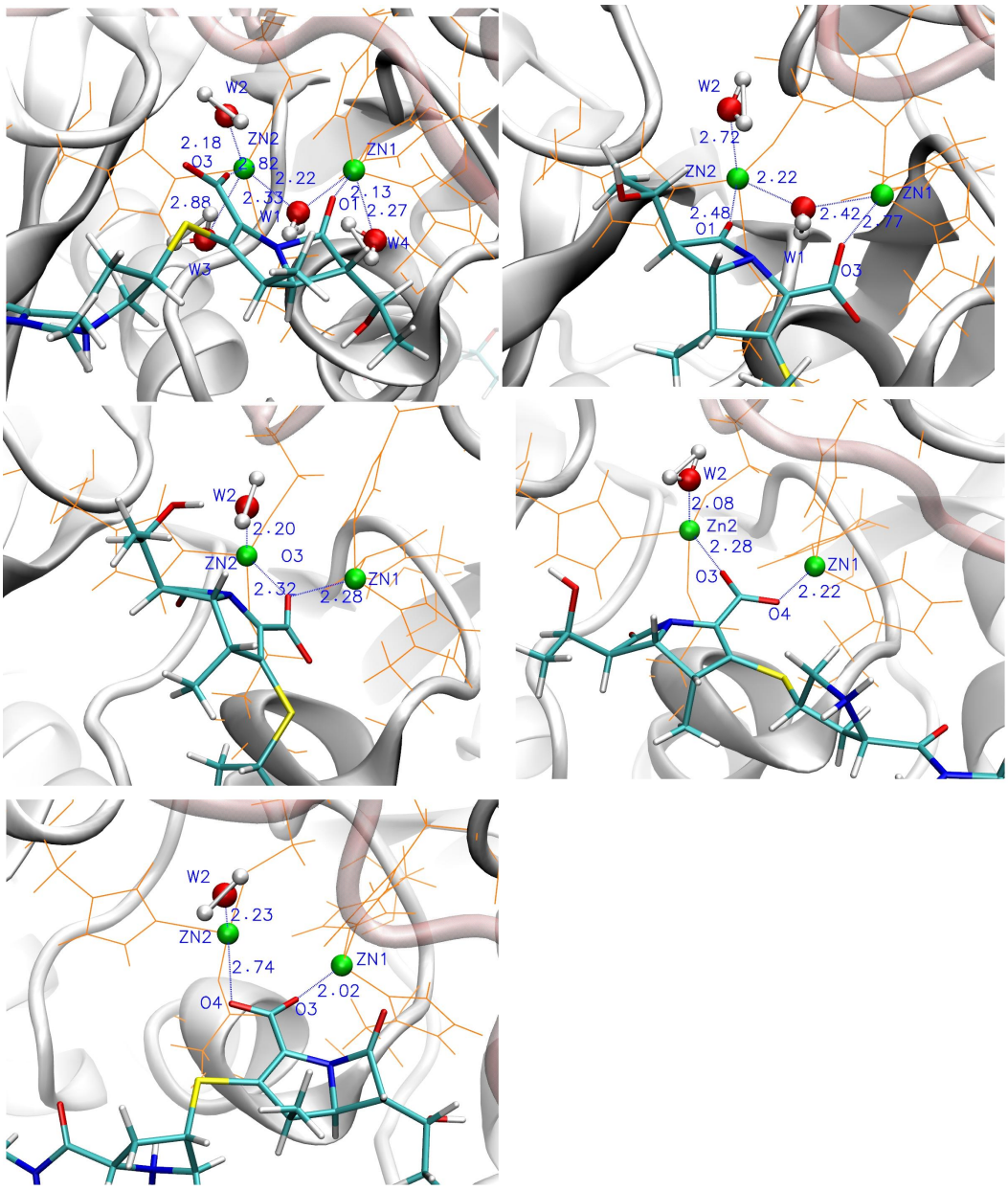

Figure S10. Water and Meropenem molecules in the active center of NDM-1 of five enzyme-substrate binding state configurations. (A)–(E) correspond to the configurations A1, A2, B, C1 and C2 respectively. Meropenem molecule are shown in licorice model; zinc ions are shown in green and CPK model, water molecules are shown in CPC model; residue coordinated to zinc ions are shown in orange lines; key atomic distance values are show in blue line, and distance values are shown beside the blue lines (Å); L10 is shown in pink.

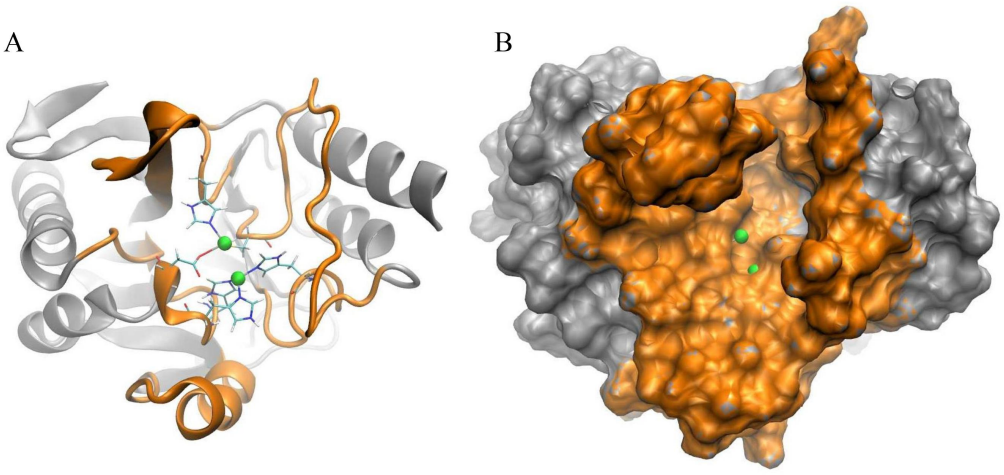

Figure S11. Sampling regions of decomposition of MM-PBSA.. Protein are shown in Newcartoon model and surf model. (A)- (B) Orange areas for decomposition of MM-PBSA.

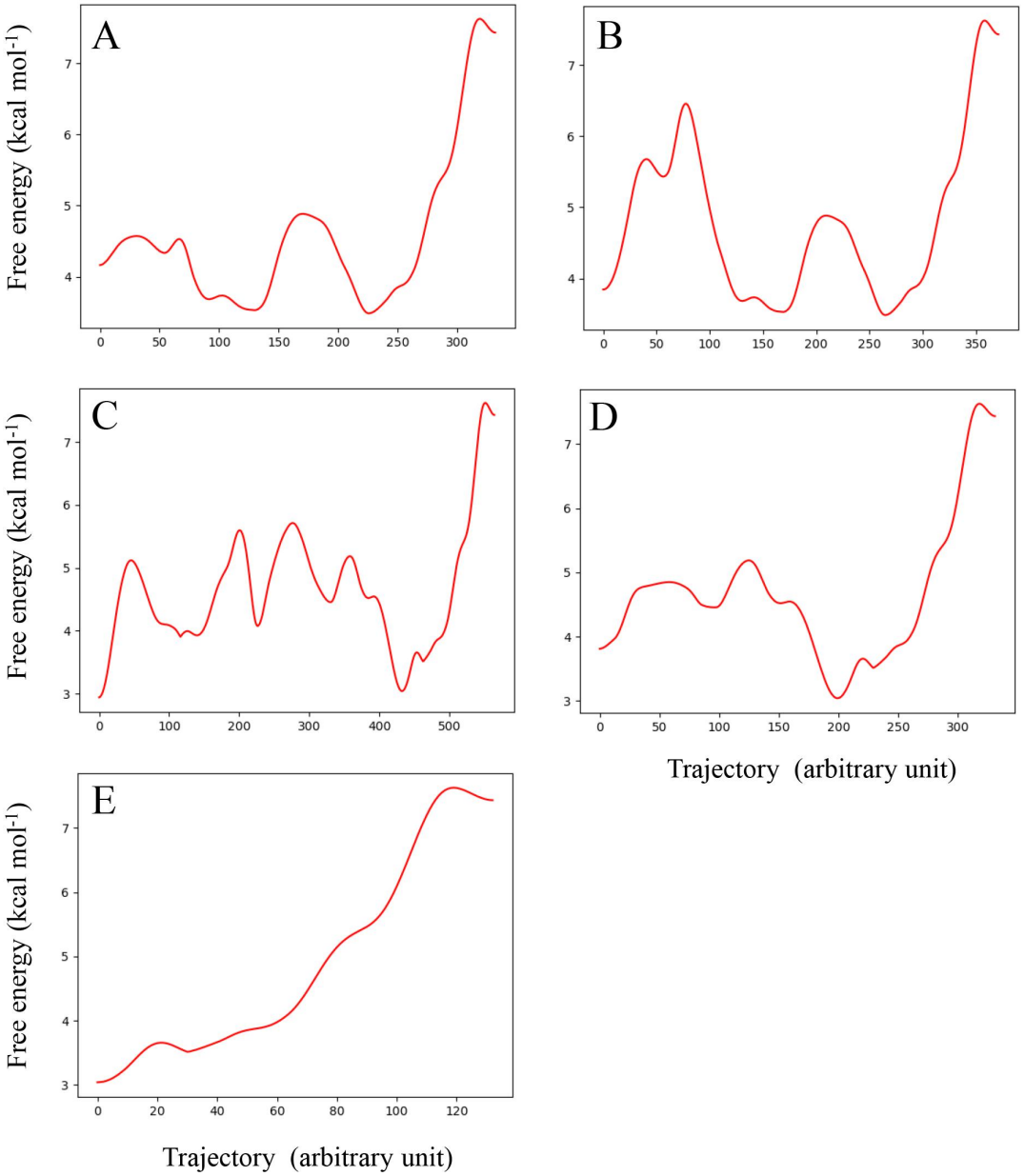

Figure S12. The free energy along the binding pathways. (A)-(E) correspond to the path P1, P2, P3, P4 and P5 respectively.

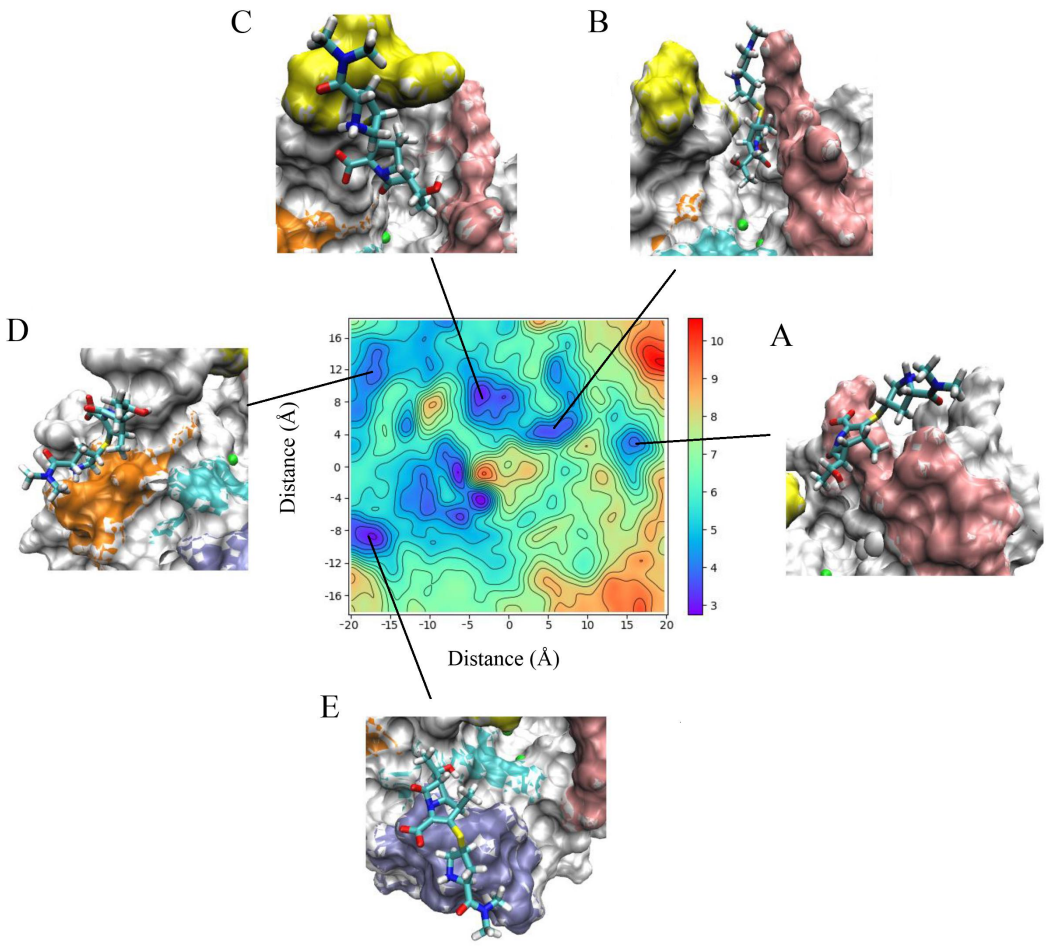

Figure S13. Typical substrate configurations of five regions with relatively lower free energy. A-E are typical substrate configuration located in the five relatively lower free energy regions respectively.