Inhibitory Zinc Ion Binding Site and The Metal Exchange Mechanism in Human DPP III

Sanja Tomić, Antonija Tomić, Hrvoje Brkić and Antonia Matić





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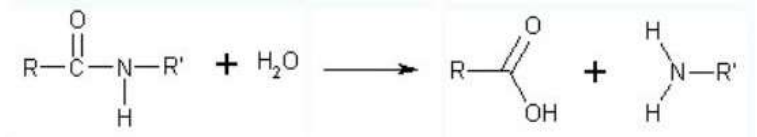
DPP III or M49 enzyme family

Zn dependent metalo enzymes of Molecular mass ~61-97 (103) kDa

widely spread 5 kingdoms: Eubacteria, Protista, Fungi, Plantae, Animalia

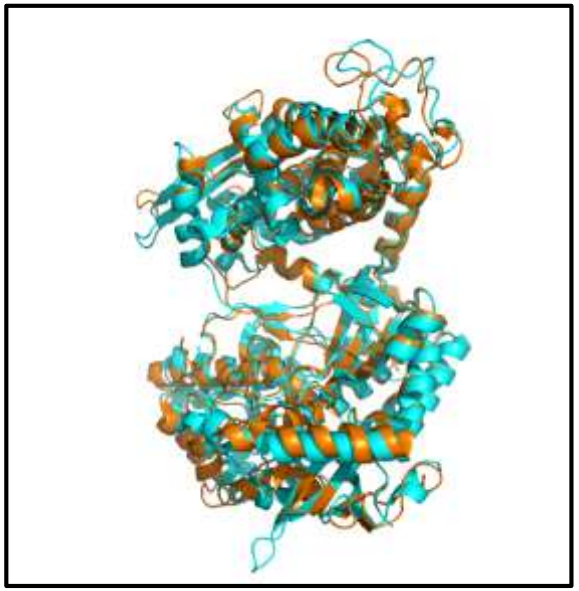


Zinc-exopeptidase hydrolyzes dipeptides from the N-terminal of its substrates, peptides with three AA and more



Human DPP III

2008. → **yeast** DPP III (PBD id 2CSK)
2009. → **human** DPP III (PBD id 3FVY)
2012. → **E451A mutant** of human DPP III with tynorphin (PBD id 3T6B)





Oxidative stress

Pain

Immune Response

Influenza

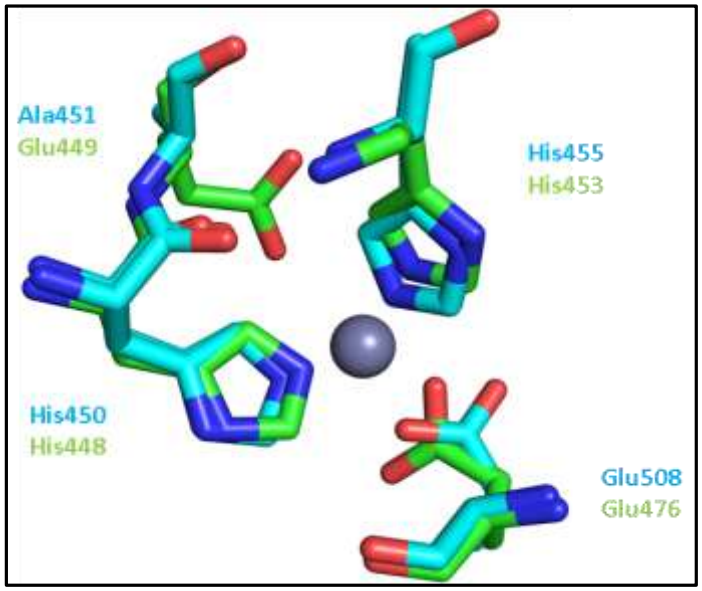
Blood pressure

Cancer

cardiac shock biomarker

Conserved motifs:

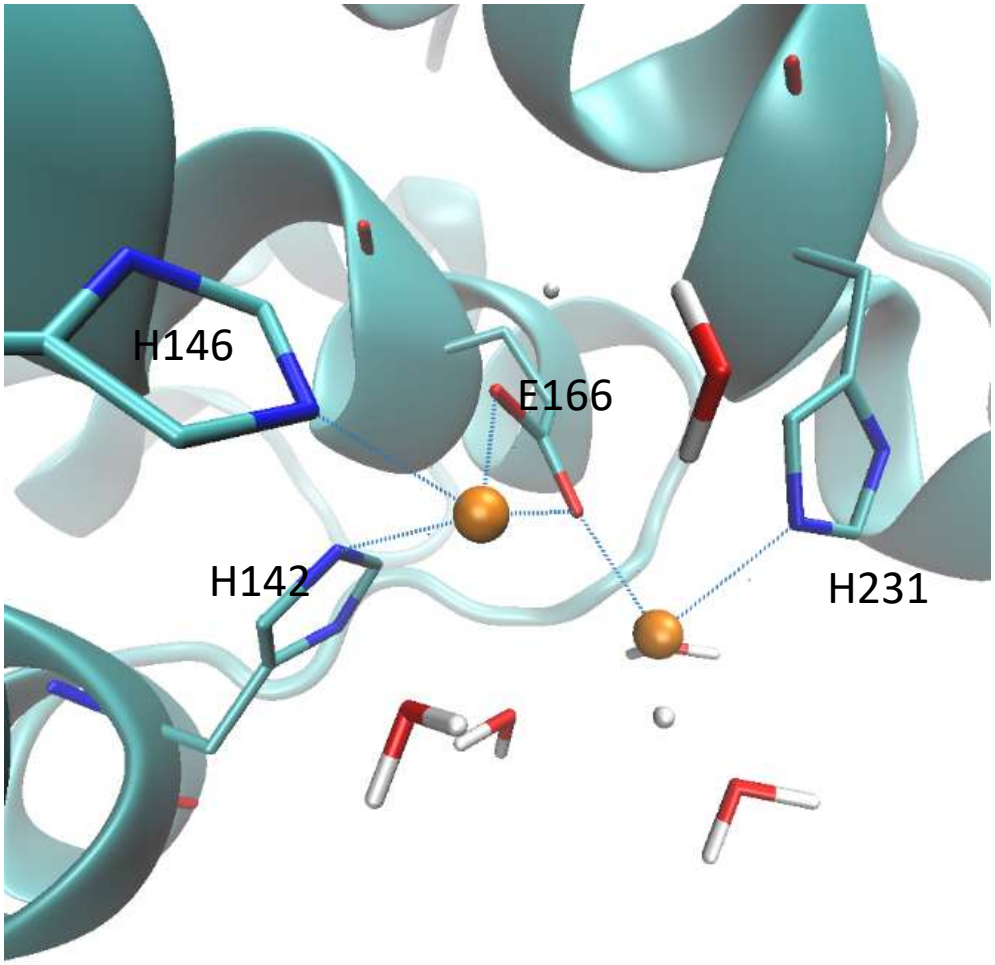
HEXXGH and **EEXR(K)AE(D)** coordinate **Zn ion**



Ehperiments: **excess zinc inhibits hDPP III hydrolytic activity**

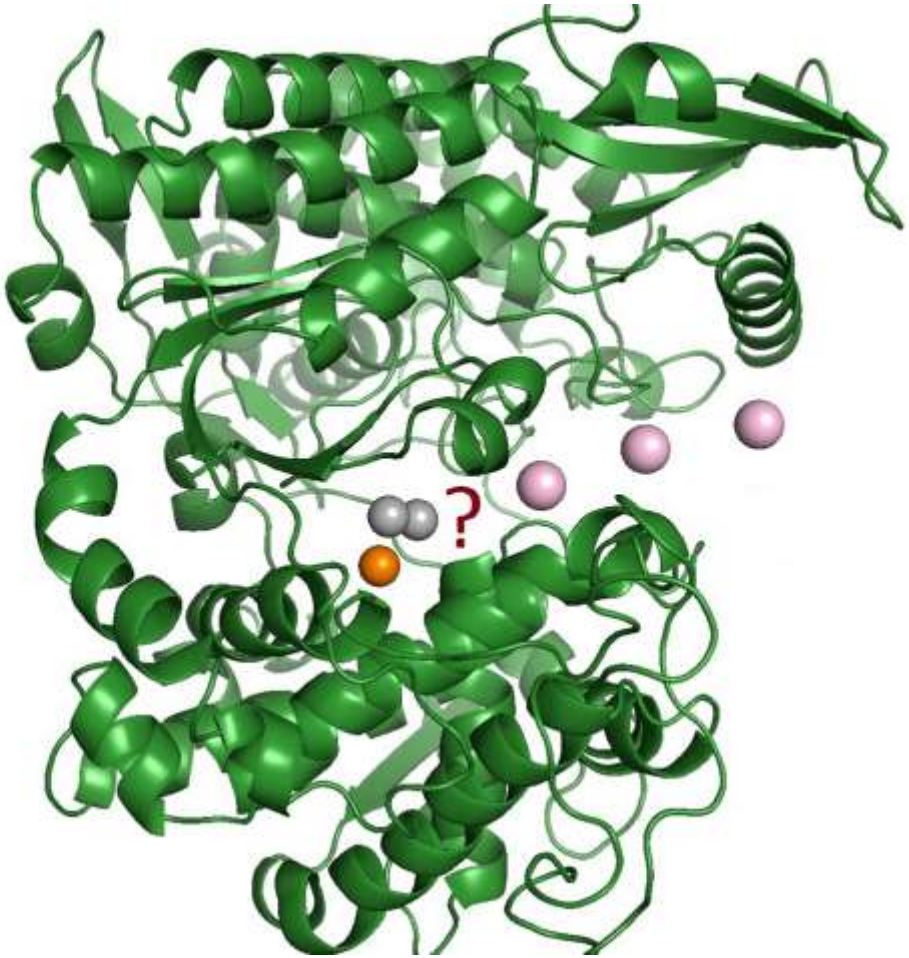
Zn ion added as $Zn(Ac)_2$ to the reaction mixture of pH 8.0, inhibited rat DPP III already at a 1µM level (IC_{50} = 1.8µM), while 10µM $Zn(Ac)_2$ completely abolished its activity.

Thermolysin, PDB_id **1lnd** (TML)

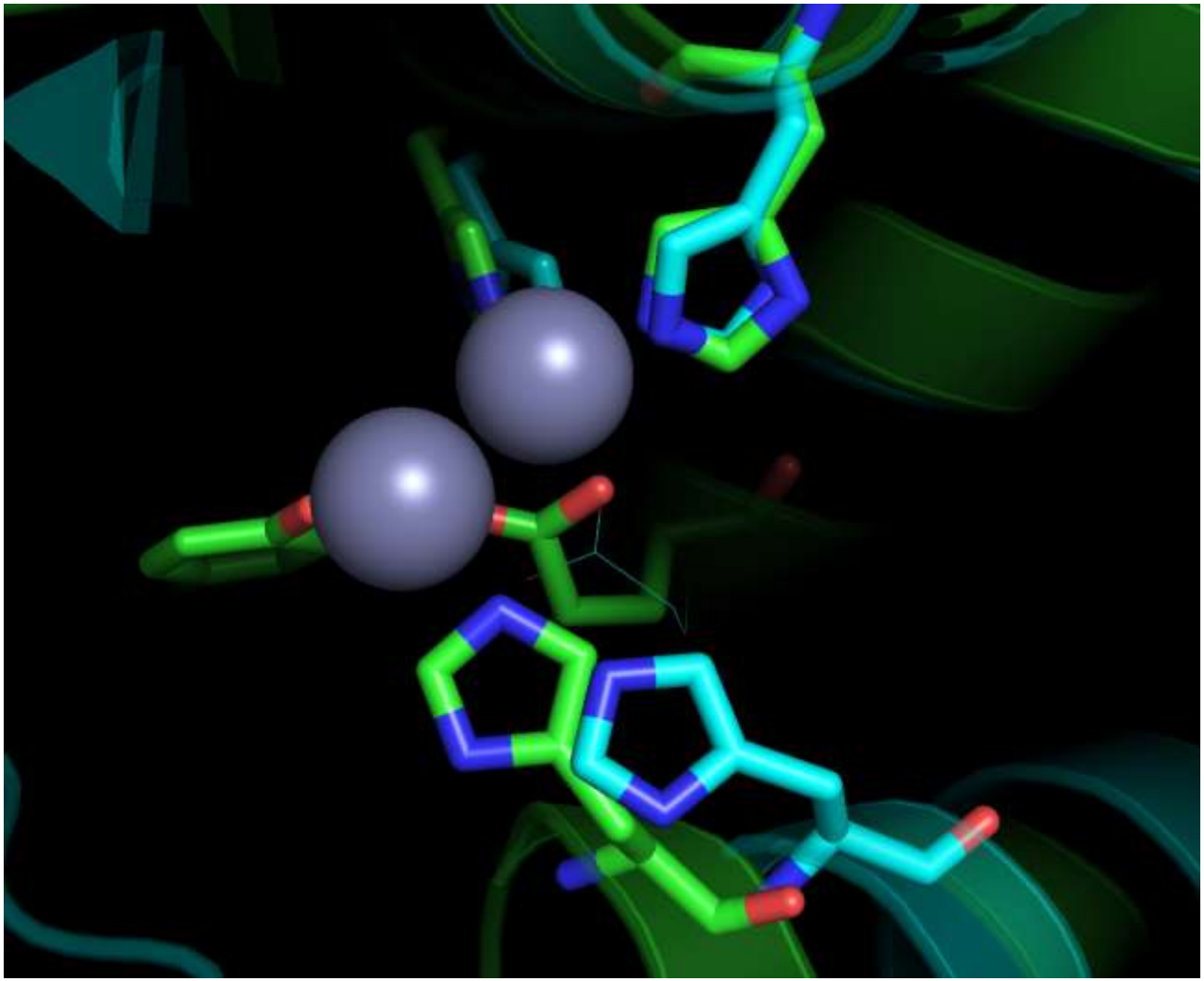


Excess zinc also inhibits other exo- and endometallopeptidases. One example is thermolysin where the inhibitory effect is explained by the binding of a second zinc ion to the catalytically important H231 within 3.2 Å of the zinc bound to native thermolysin.

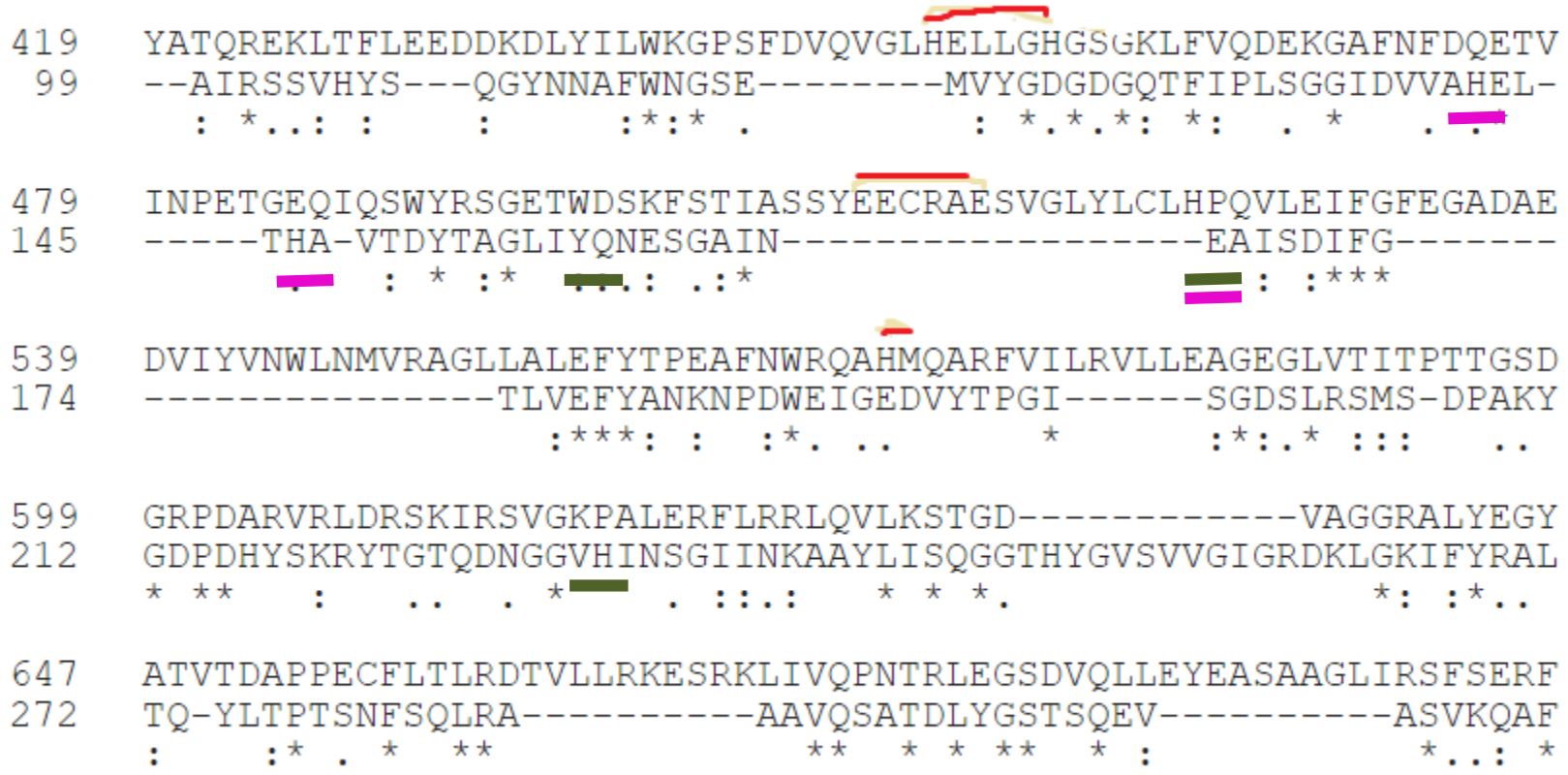
Identifikacation of the inhibitory Zn binding site hDPP III



hDPP III – TML 3D structures - aligment

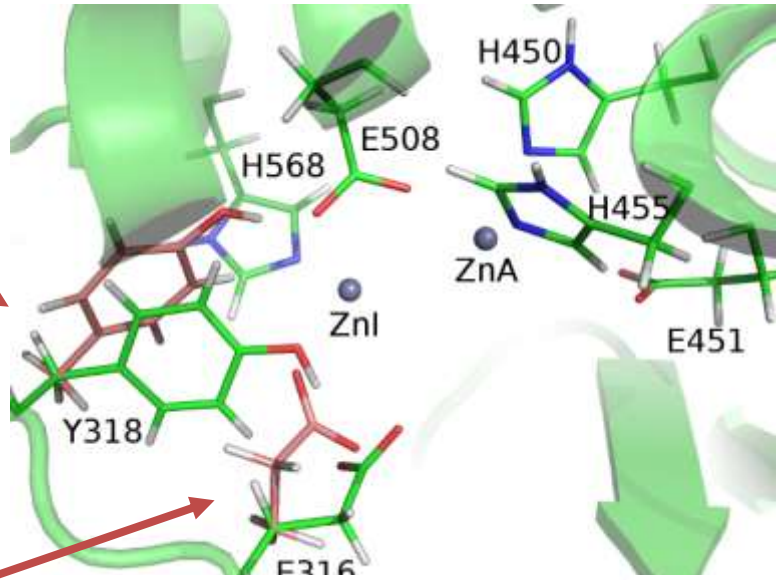


hDPP III – TML hDPP III -sequences - aligment



QMMM calculations (S1, S2, S3)

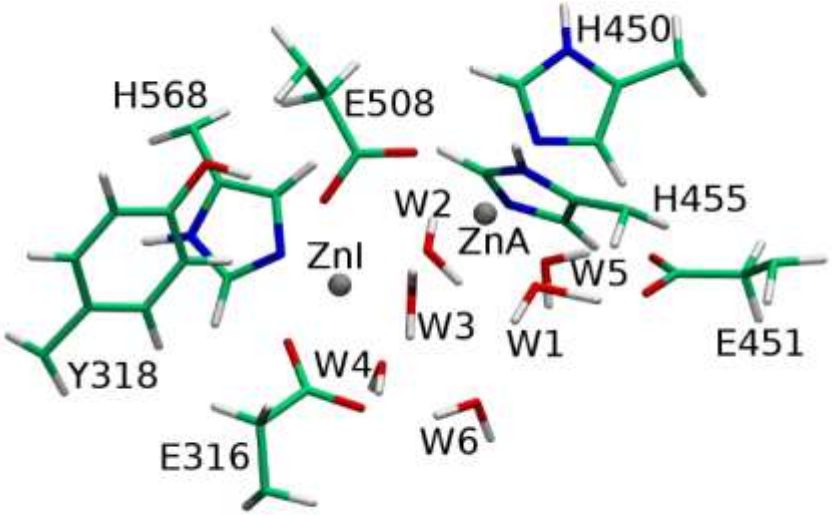
S2

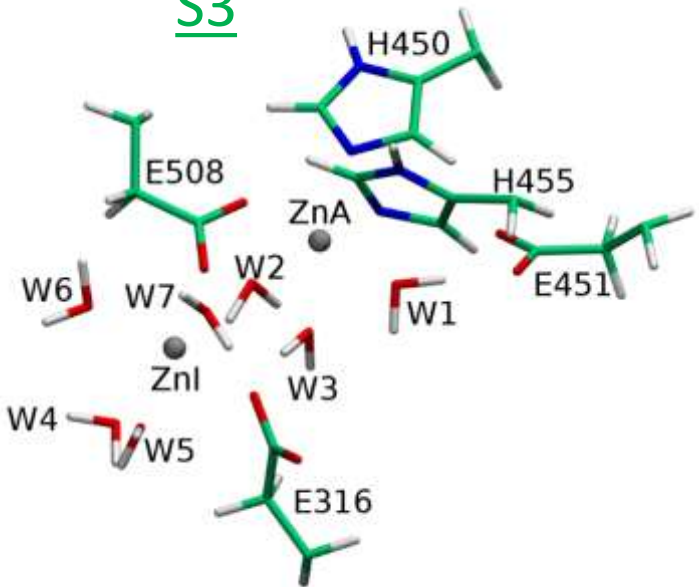


ZNI coordination:

E508, H568 , Y318
E508, H568 , E316
E508, E316,
+ 3W

S3

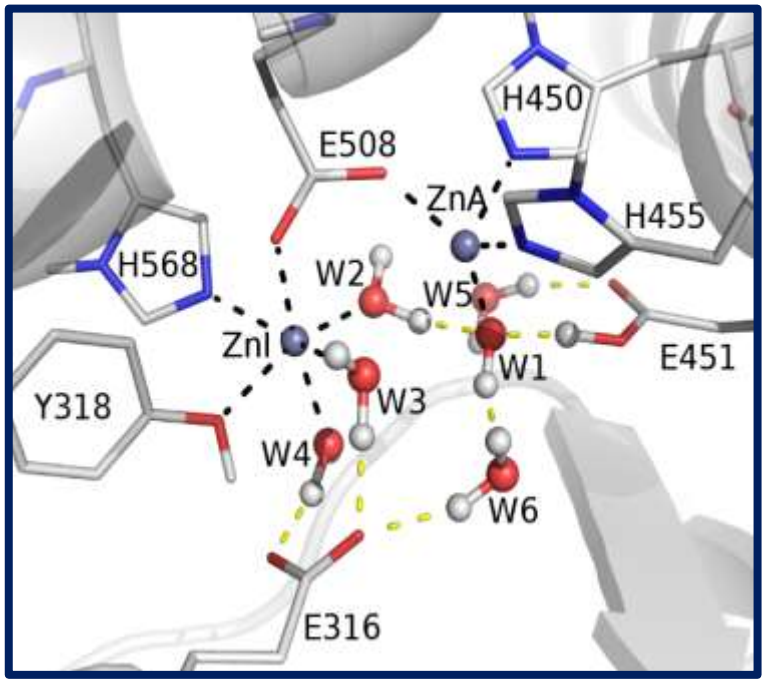


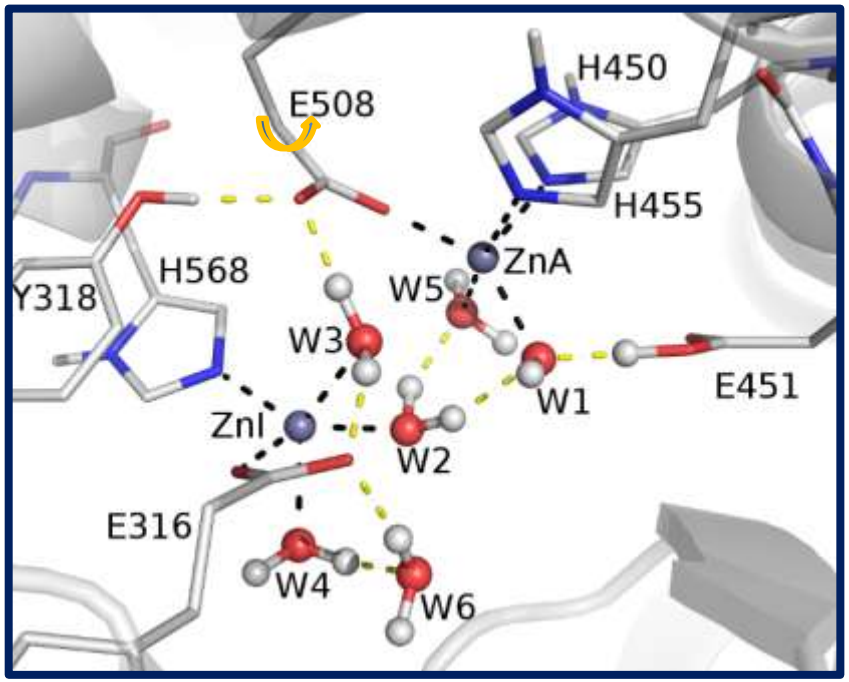


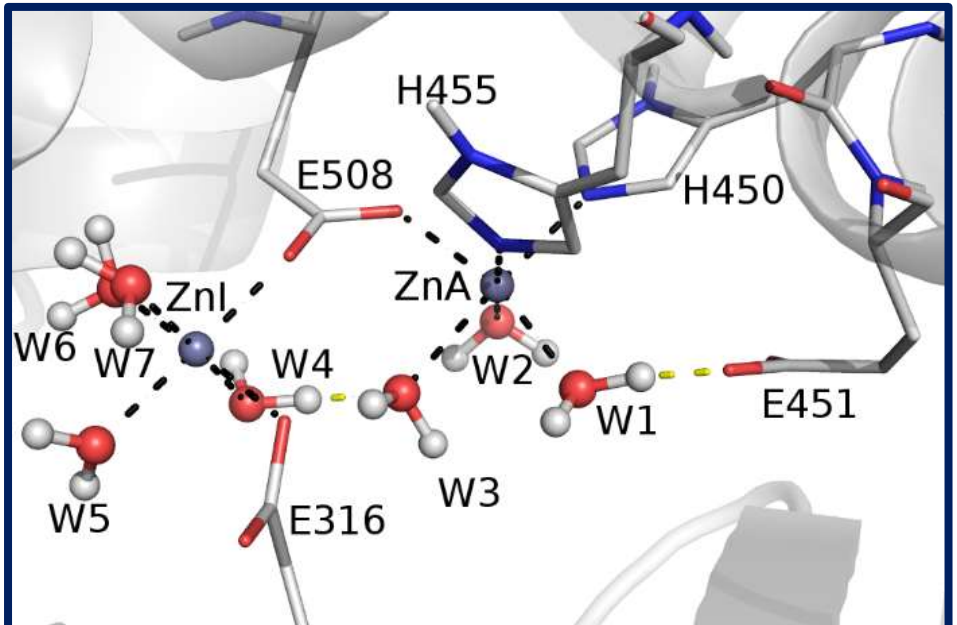
S1 / S2 / S3 initial and QMMM optimized

	d/Å				
	I	**S1**	**S2**	**I**	**S3**
ZnA-ZnI	3.45	3.78	4.31	5.05	4.95
ZnA-H450(ne2)	2.34	2.11	2.14	2.29	2.19
ZnA-H455(ne2)	2.45	2.10	2.21	2.39	2.16
ZnA-E508(oe2)	2.18	2.03	2.06	2.05	2.14
ZnI-E508(oe1)	2.17	2.16	**3.98**	2.06	2.09
ZnI-H568(ne2)	2.35	2.20	2.18	9.59	9.57
ZnI-Y318(oh)	2.53/4.19	2.22	4.79	9.28	9.98
ZnI-E316(oe2)	3.89 /2.75	4.03	**2.10**	2.07	2.11

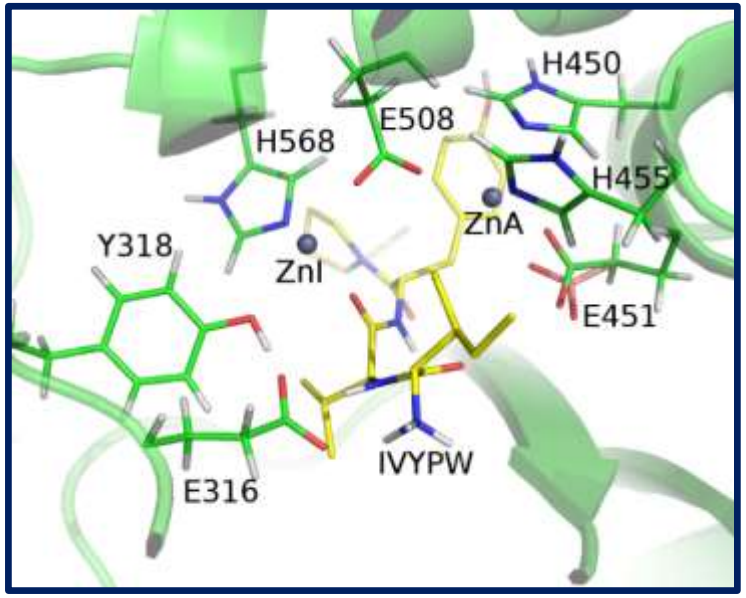
S1 / S2 / S3 QMMM optimized



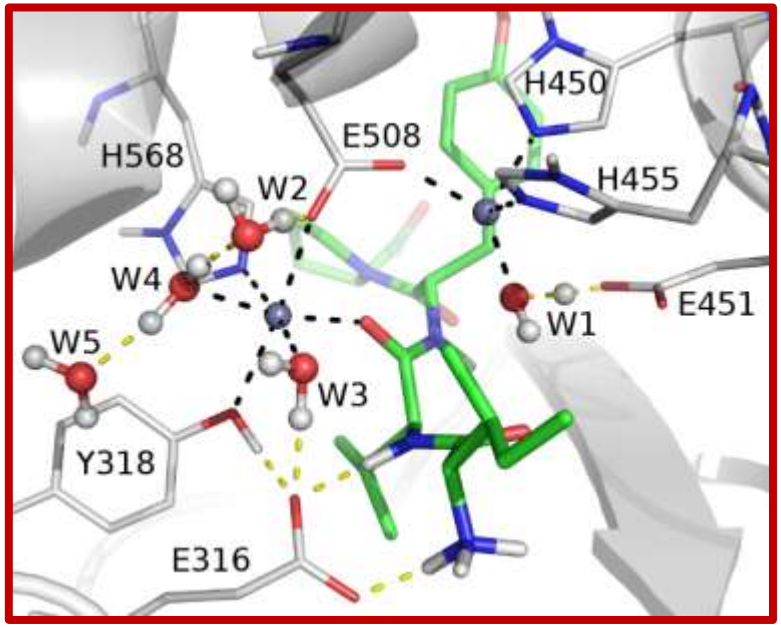


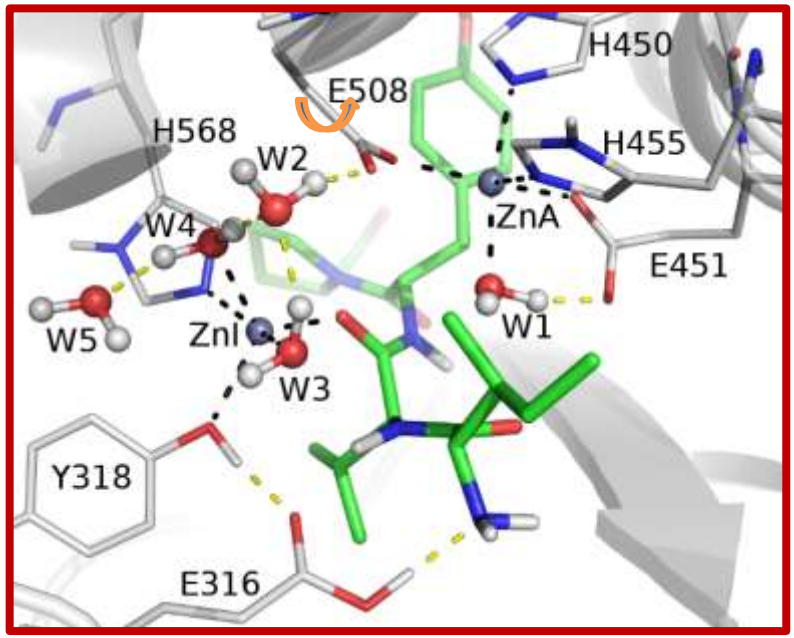


QMMM calculations (CPLX1 / CPLX2)



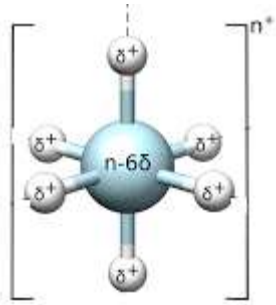
	d/Å		
	I	**CPLX1**	**CPLX2**
ZnA-ZnI	5.30	4.75	5.54
ZnA-H450(ne2)	2.19	2.11	2.15
ZnA-E451(oe2)	2.93 / 2.19	3.67	2.18
ZnA-H455(ne2)	2.29	2.14	2.17
ZnA-E508(oe2)	2.17	2.02	2.10
ZnI-E508(oe1)	2.20	2.29	**4.26**
ZnI-H568(ne2)	2.30	2.23	2.19
ZnI-Y318(oh)	2.60	2.28	2.17

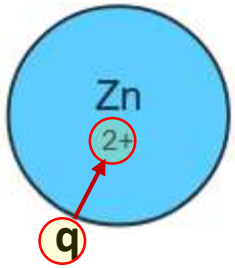


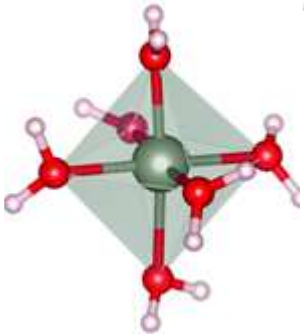


Zn nonbonding parameters models

Model	#	charge/e				
		Zn				E451
Dummy atom		Total	central point (carry vdw parameters)	δ points at z-axis of octahedron	points at x and y axises of octahedron	
	D1[1]	2	-1	0.5	0.5	
	D2	1.325	-0.475	0.1	0.4	-0.65
6-12	1[2]	2.0				
	2	1.375				-0.75
	3	ZnA 1.1, ZnI 0.9				
	3'	1.0				
	3r	ZnA 0.9, ZnI 1.1				







$$V(r^N) = \sum_{veze} \frac{k_l}{2}(l_i - l_{i,0})^2 + \sum_{kutovi} \frac{k_\theta}{2}(\theta_i - \theta_{i,0})^2$$

$$+ \sum_{torzije} \frac{V_n}{2}(1 + \cos(n\omega - \gamma))$$

$$+ \sum_{i}^{N} \sum_{j}^{N} \left(4\varepsilon_{ij} \left[\left(\frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}} \right)^{6} \right] + \frac{q_i q_j}{4\pi\varepsilon r_{ij}} \right)$$

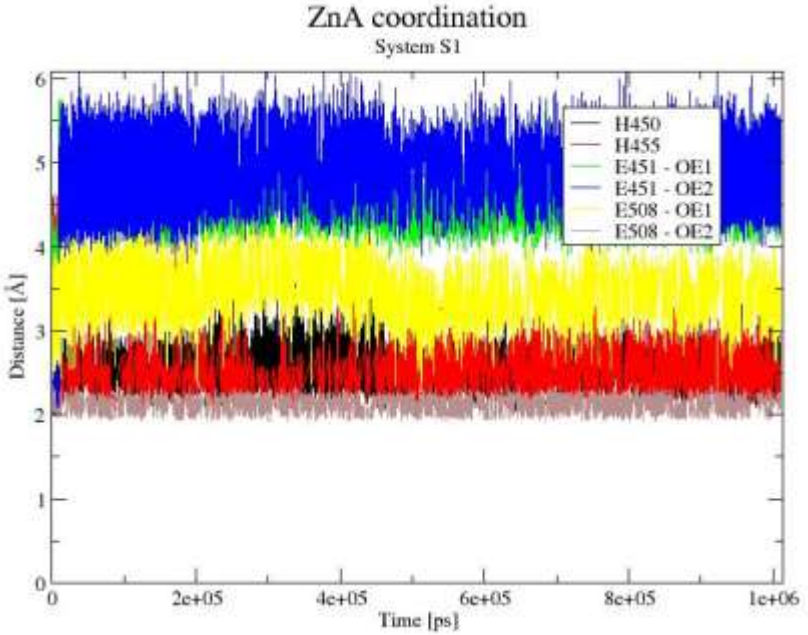
Lorentz-Berthelot rules

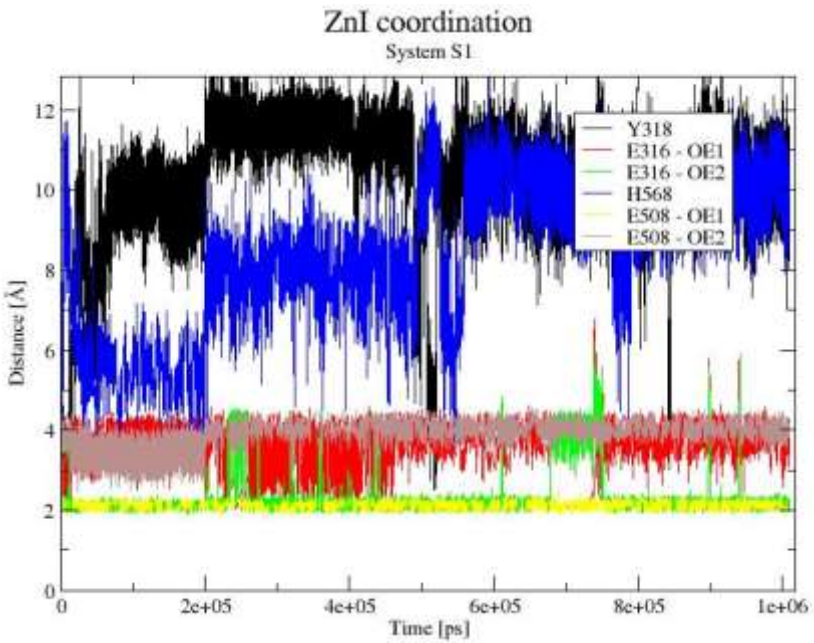
$$\sigma_{ab}^{LB} = \frac{\sigma_{aa} + \sigma_{bb}}{2}, \qquad \varepsilon_{ab}^{LB} = \sqrt{\varepsilon_{aa}\varepsilon_{bb}}.$$

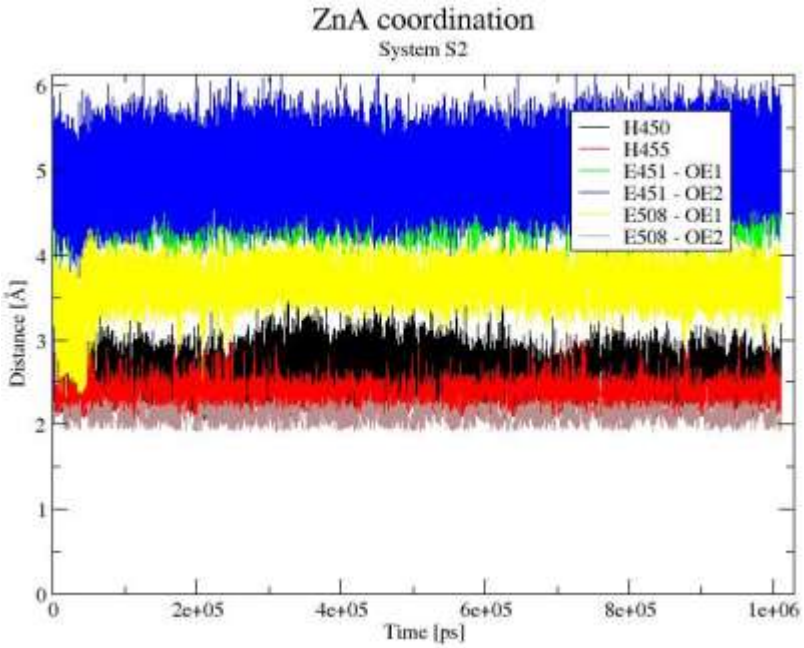
Dummy atom Zn parameter models

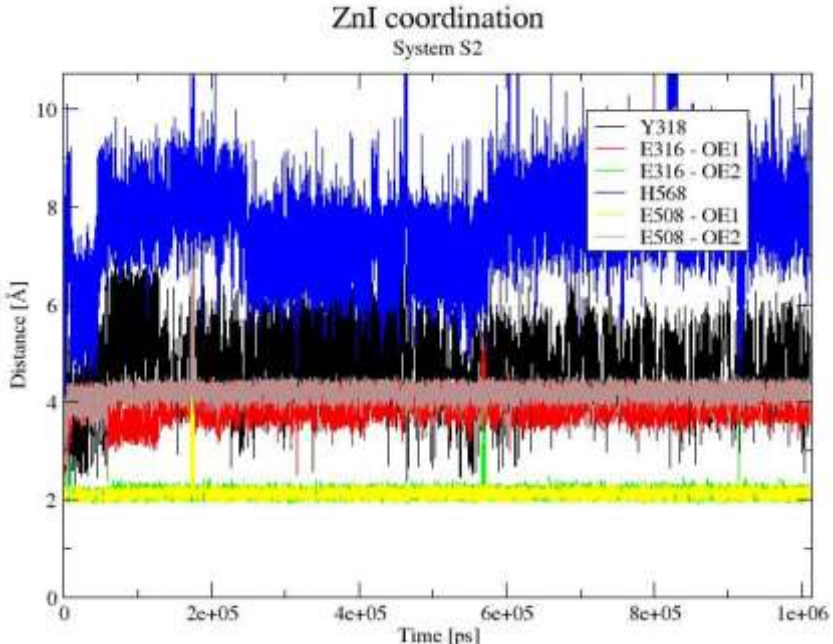
Model #	**SYSTEM** Force field	t_i/t_{total} (μs)	<ZnA–ZnI> (Å)	ZnA ligands	ZnI ligands	MMGBSA(kcal/mol)		
						Receptor: DPP III Ligand: ZnA + ZnI	Receptor: DPP III Ligand: ZnA	Rec: DPP III+ ZnA Ligand: ZnI
D1	**S1** ff14SB	$0.099/0.1^{ZnA}$ $0.095/0.1^{ZnI}$	$<Zn_A–Zn_I>$ (Å)	**H450, H455, E508^{M}**, E451^{M} 2 W	**H568**, E316^{B}, **E508^{M}, Y315** 2W	-292±11	-164±6	-108±7
	S1 ff03	0.1/0.1	5.4±0.1 51% 5.1±0.1 49%	**H450, H455, E508^{M}**, E451^{M} 2W	E316^{M}, **E508^{M},** H568 3W	-320±8	-187±6	-121±6
D2	**S1** ff03	$0.085/0.1^{ZnA}$ $/0.1/0.1^{ZnI}$	3.5±0.2	**E508^{M}, H450, H455** mostly 3W	E316^{M}, **E508^{M}** mostly 3W	-144±6	-80±5	-47±7
	S1 ff03	$0.066/0.1^{ZnA}$ $0.056/0.1^{ZnI}$	4.9±0.5	**E508^{M}, H450, H455** 3W	E316^{M}, **E508^{M}, H568** 3W	-166±8	-81±5	-50±10
	S1 ff14SB	$0.58/1^{ZnA}$ $0.88/1^{ZnI}$	5.4±0.3	**H450, H455, E508^{M}** 3-4W	E316^{M}, **E508^{M}** mostly 4 W	-122±8	-67±5	-48±7
	S2 ff14SB	$0.5/1^{ZnA}$ $0.99/1^{ZnI}$	4.7±0.5	**H450, H455, E508^{M}** 3-4W	**E316^{M}, E508^{M}** 4-5W	-116±7	-68±5	-43±5
	S1 ff14SB	$0.062/0.1^{ZnA}$ $0.1/0.1^{ZnI}$	4.1±0.3	**H450, H455, E508^{M}** 3W	**E508^{M}** 4-5 W	-102±9	-69±4	-23±6
	S1 ff14SB	$0.58/0.25^{ZnA}$ $0.88/0.25^{ZnI}$	4,7±0.1	**H450, H455, E508^{M}**, E451^{M} 1W	**H568, E508^{M}**, V730 2-3W	-160±7	-96±5	-57±5
	CPLX1 ff14SB	$0.5/0.5^{ZnA}$ $0.5/0.5^{ZnI}$	5.2±0.3	**H450, H455, E508^{M,B}**,E451 mostly 1 W	**E508^{M}** mostly 4 W	-114±7	-93±5	-18±4
	CPLX2 ff14SB	$0.5/0.5^{ZnA}$ $0.44/0.5^{ZnI}$	5.4±0.3	**H450, H455, E508^{M,B}** 1W	**H568, E508^{M,B}** **^{c}HM-O2nd** mostly 2 W	-150±8	-85±5	-39±4

D2 model

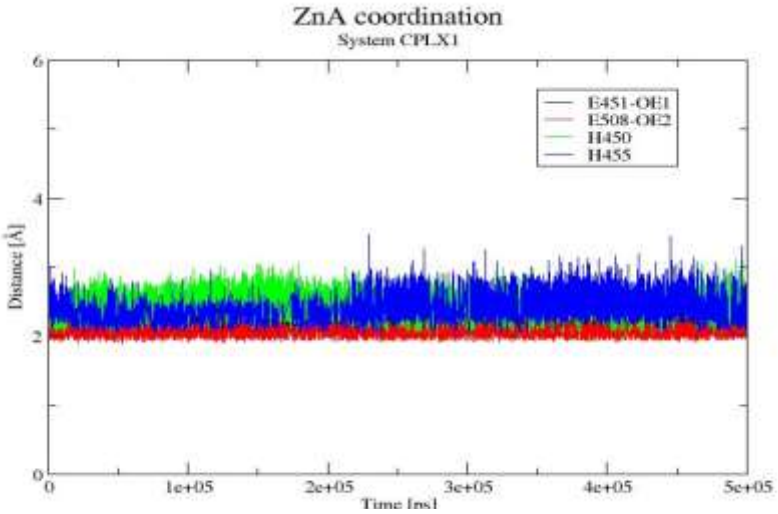

ZnA coordination
System S1
H450
H455
E451 - OE1
E451 - OE2
E508 - OE1
E508 - OE2
Distance [Å]
Time [ps]

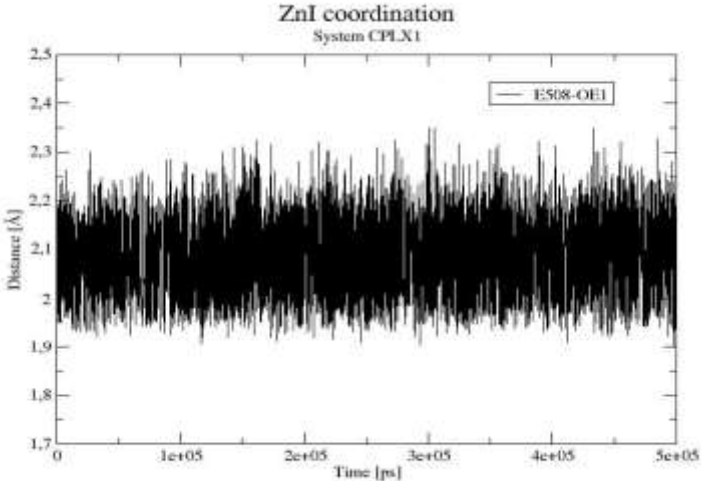

ZnI coordination
System S1
Y318
E316 - OE1
E316 - OE2
H568
E508 - OE1
E508 - OE2
Distance [Å]
Time [ps]

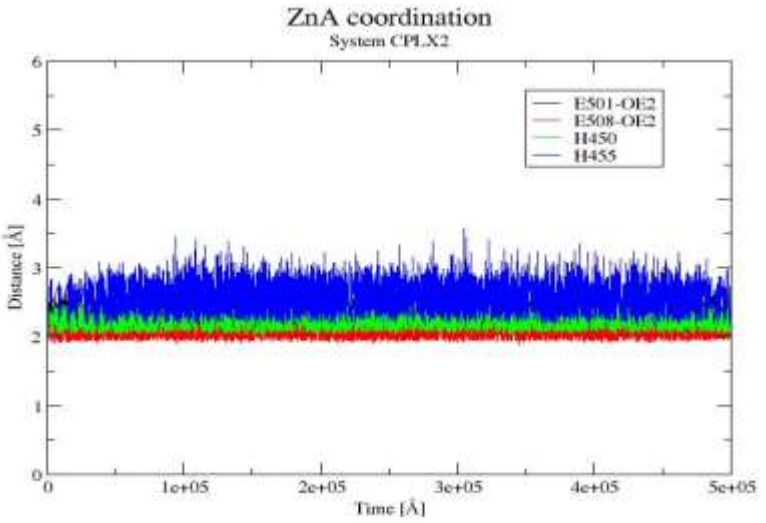

ZnA coordination
System S2
H450
H455
E451 - OE1
E451 - OE2
E508 - OE1
E508 - OE2
Distance [Å]
Time [ps]

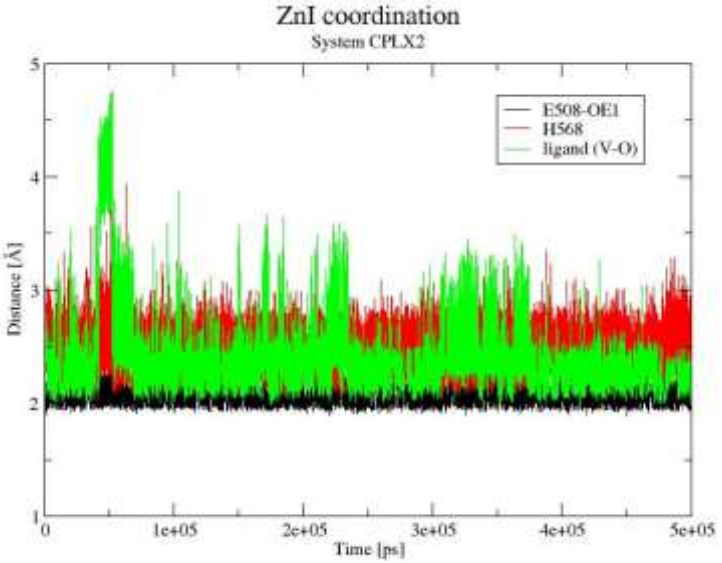

ZnI coordination
System S2
Y318
E316 - OE1
E316 - OE2
H568
E508 - OE1
E508 - OE2
Distance [Å]
Time [ps]

D2 model

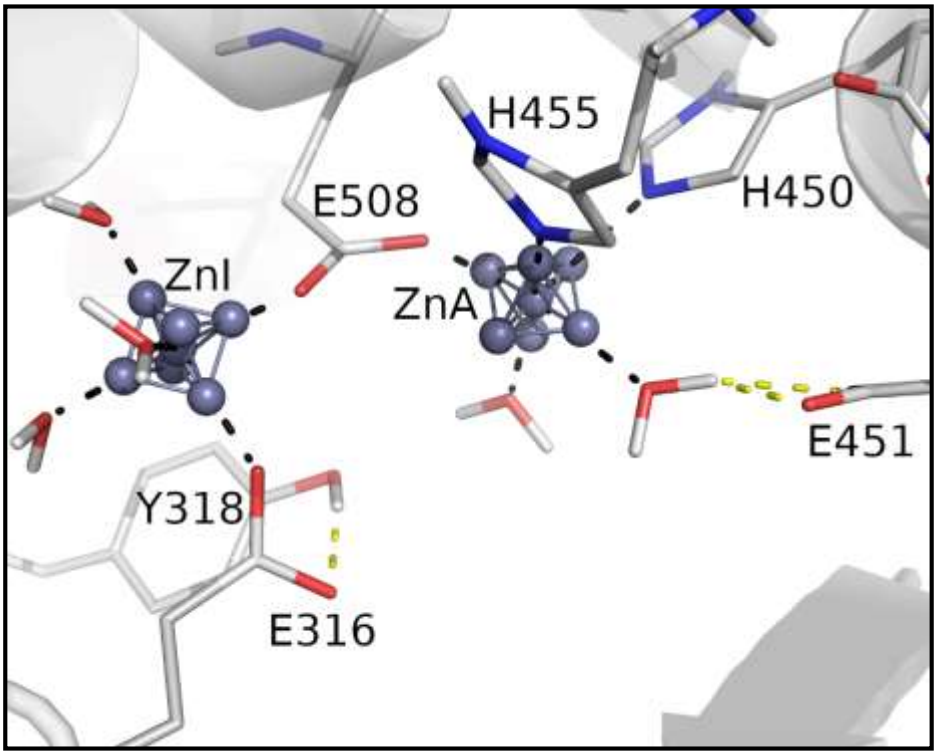

ZnA coordination
System CPLX1
E451-OE1
E508-OE2
H450
H455
Distance [Å]
Time [ps]

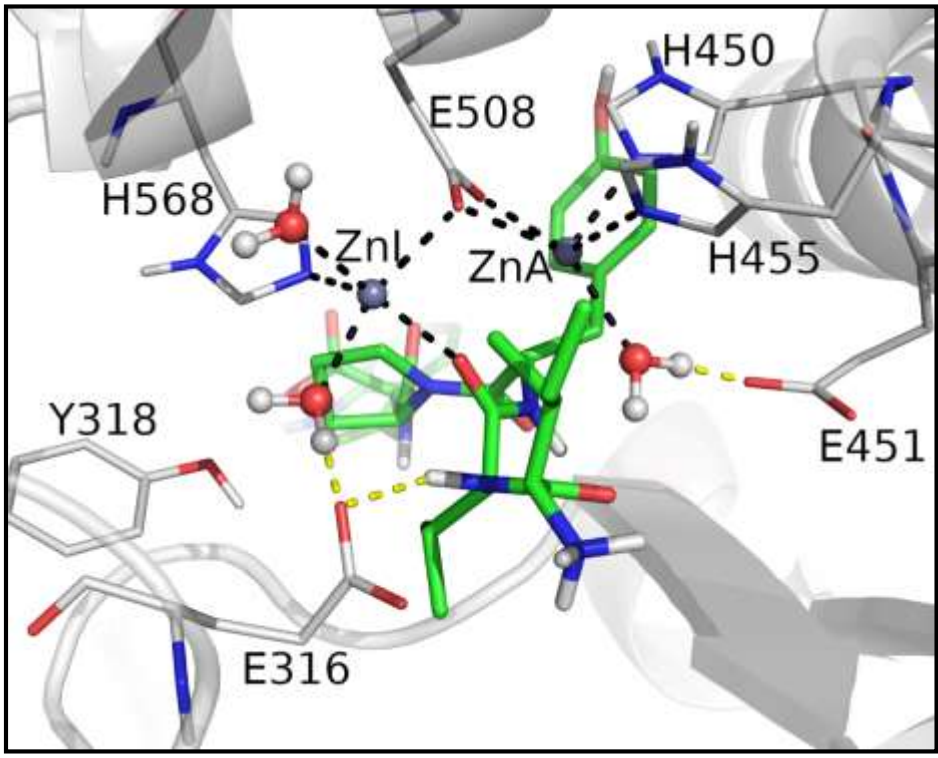

ZnI coordination
System CPLX1
E508-OE1
Distance [Å]
Time [ps]


ZnA coordination
System CPLX2
E501-OE2
E508-OE2
H450
H455
Distance [Å]
Time [Å]


ZnI coordination
System CPLX2
E508-OE1
H568
ligand (V-O)
Distance [Å]
Time [ps]

D2 model, structures of S2 and CPLX2 obtained after 1µs and 500 ns, respectively, of MD simulations



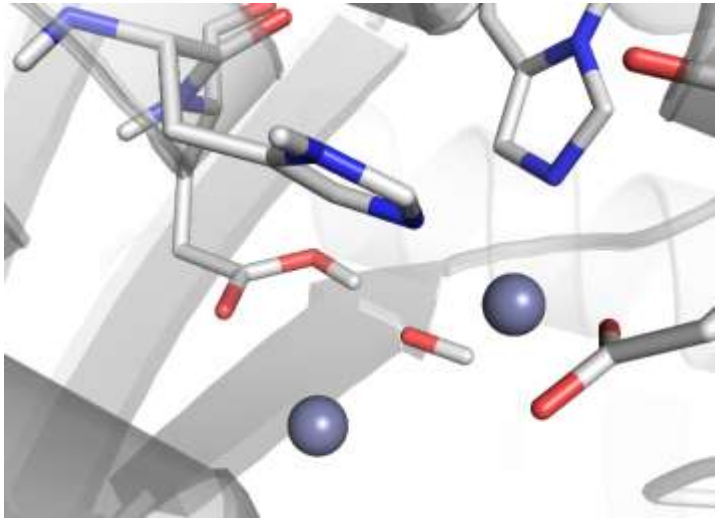


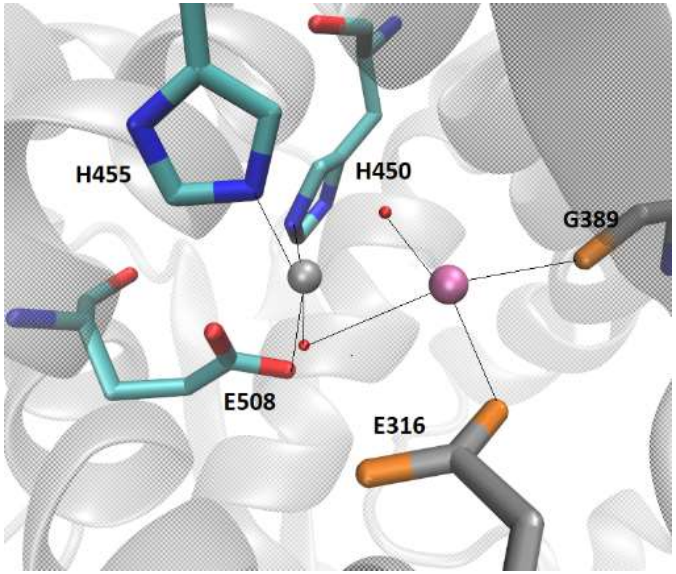
12-6 ZN parameters models

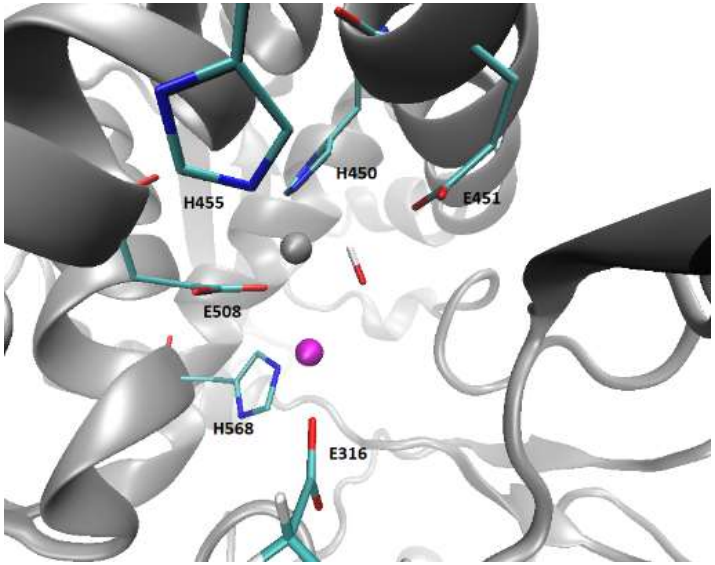
Model #	SIMULATED SYSTEM Force field	t_i/t_{total} [a] (μs/μs)	< ZnA –ZnI> (Å)	ZnA ligands	ZnI ligands (coordinated with)	MMGBSA(kcal/mol) [b]		
						Rec: DPP III Ligand: Zn_A+ Zn_I	Receptor: DPP III + ZnA Ligand: Zn_I	Rec: DPP III Ligand: ZnA
2	**S2** ff03	0.85/1	4.0±0.4	**H450, H455, E508^{M}**, E451^{M} W 1-4	**E508^{B}**, E316^{B} W 1-4	-31±4	-2±2	-27±3
	S1 ff03	0.99/1^{ZnA} 0.81/1^{ZnI}	3.8±0.2	**H450, H455, E508^{M}**, E451^{M} mostly 2 W	**E508^{M}**, E316^{B} mostly 3 W	-32±4	1±3	-26±3
	S1-OH$^-$ ff03	0.25/0.35^{ZnA} 0.21/0.35^{ZnI}	3.5±0.3	**H450, H455, E508^{M}**, E451^{M} , **OH$^-$**	E316^{B} **E508^{B}**, **OH$^-$** mostly 3 W	-24±6	13±5	-27±4
3	**S1**-OH$^-$ ff03	0.40/0.44^{ZnA} 0.17/0.44^{ZnI}	7.0±3.2	**H450**, **H455**, **E508^{B,M}**, **OH$^-$** 0-1 W	E316^{B,M}, 2-3 W	-52±5	-4±3	-43±3
	S1- OH$^-$ ff14SB	0.69/0.7^{ZnA} 0.55/0.7^{ZnI}	3.5±0.2 (last 590 ns)	**H450**, **H455**, **E508^{M}**, **OH$^-$** 1-2 W	E451^{M,B}, **OH$^-$** 2-3 W	-49±4	-11±4	-35±3
	S1- OH$^-$ ^{exc} ff14SB	0.5/1.0^{ZnA} 0.87/1.0^{ZnI}	10.0±0.7 (last 740 ns)	N294, E316^{M} mostly 2-3 W	H450, H455, **E508^{M}** mostly 1-2 W	-27±4	-14±4	-12±2
	CPLX1 ff14SB	1.1/1.1^{ZnA} 1.1/1.1^{ZnI}	4.0±0.2	**H450**, E451^{M,B} **H455**, **E508^{M}**, 1 W	**H568**, **E508^{M,B}** ^{c}**HM-O2nd** mostly 1 W	-34±4	-1±3	-23±3
3'	**CPLX2** ff14SB	0.94/1.0^{ZnA} 1.0/1.0^{ZnI}	3.8±0.2	**H450**, **H455**, **E508^{M,B}** mostly 1 W	**H568**, E508^{M,B} ^{c}**HM-O2nd** mostly 2 W	-11±4	4±3	-8±3
3r	**CPLX2** ^{exc} ff14SB	0.44/0.98^{ZnA} 0.41/0.98^{ZnI}	4.5±0.4	**H450**, **H455**, **E451^{M,B}** mostly 1W	H450, H455, , E508^{M,B} ^{c}**HM-O2nd** mostly 1 W	-23±2	-2±1	-21±2

LJ nonbonding model **3**

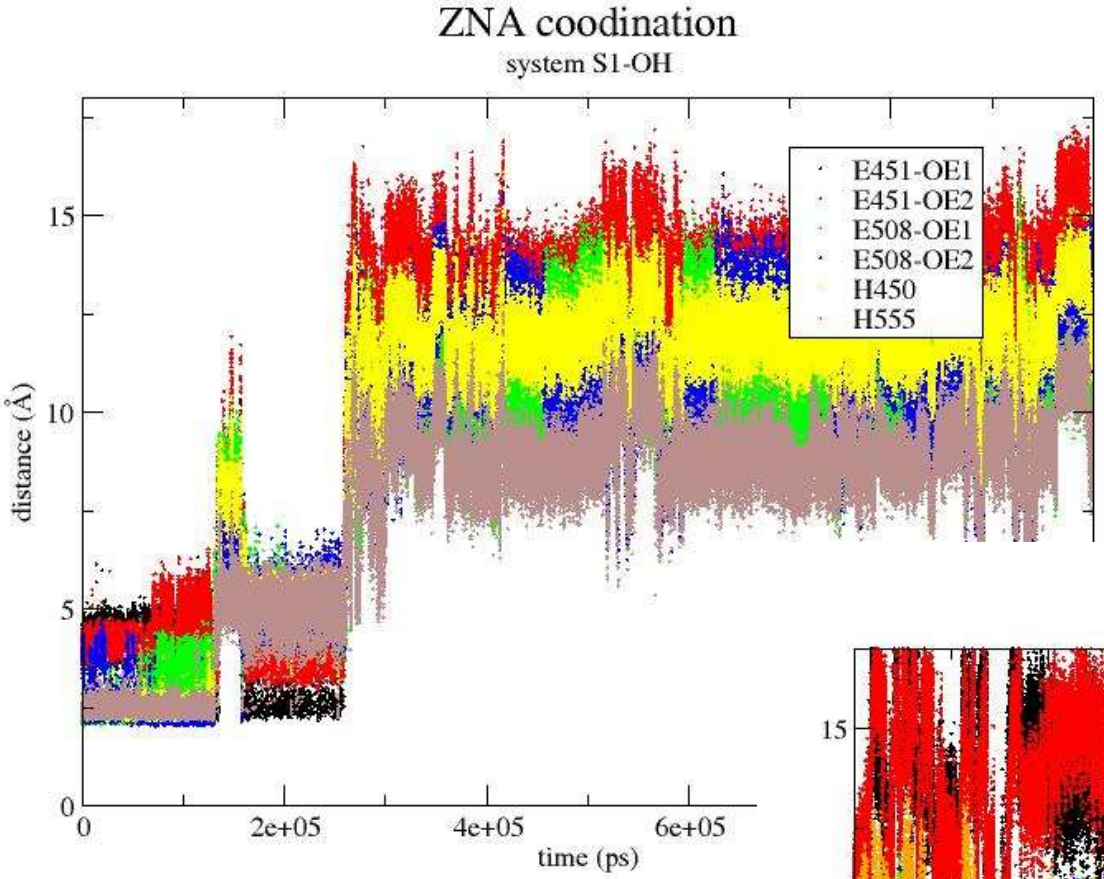
S1-OH$^-$

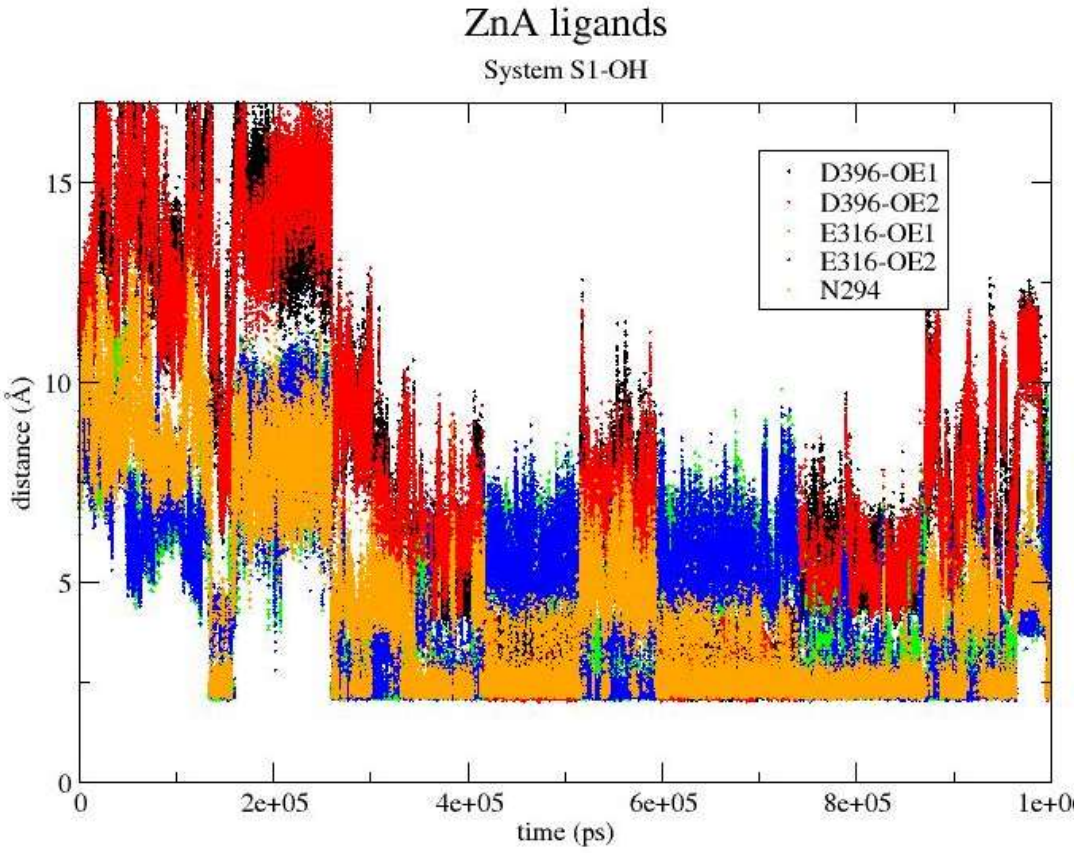




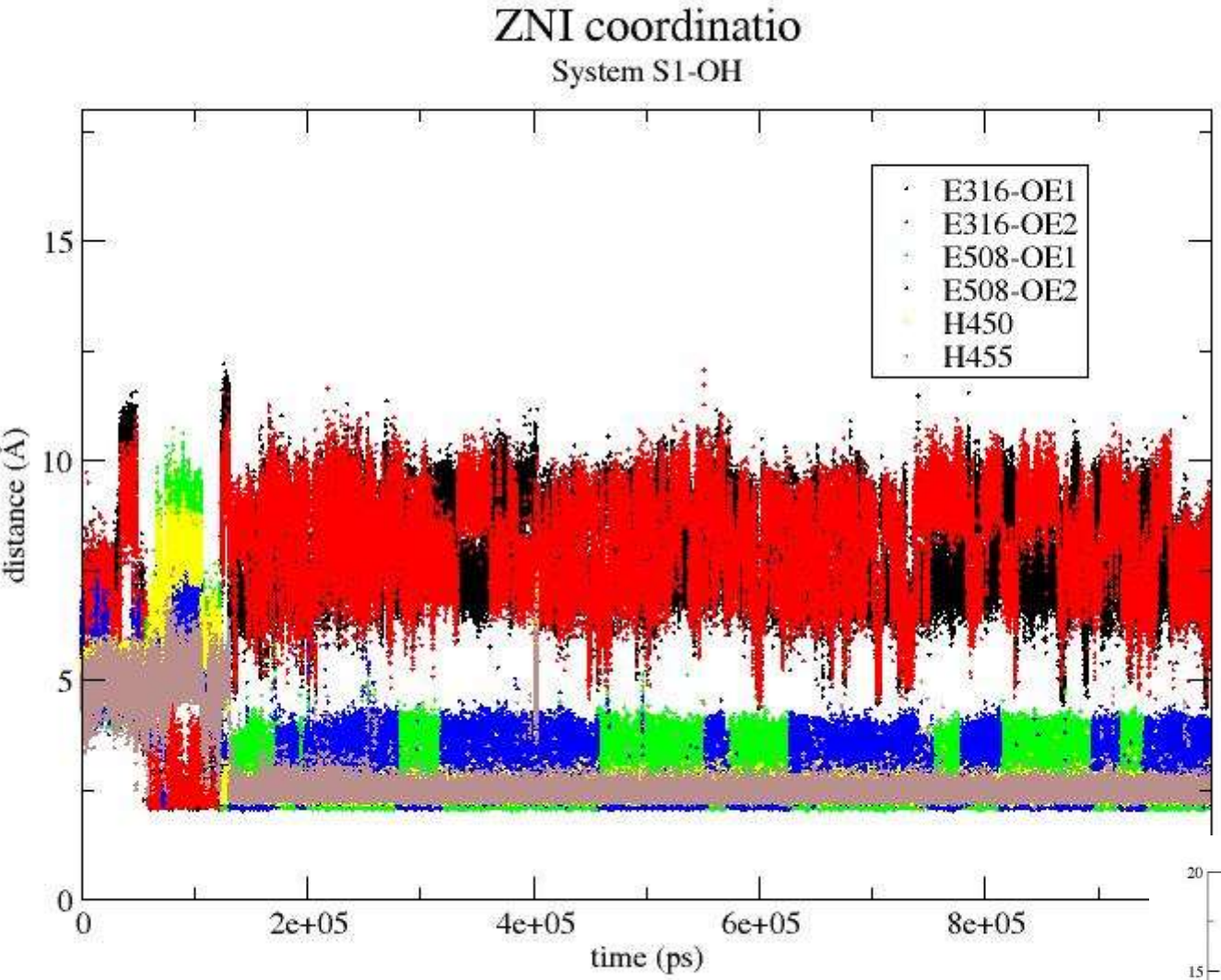


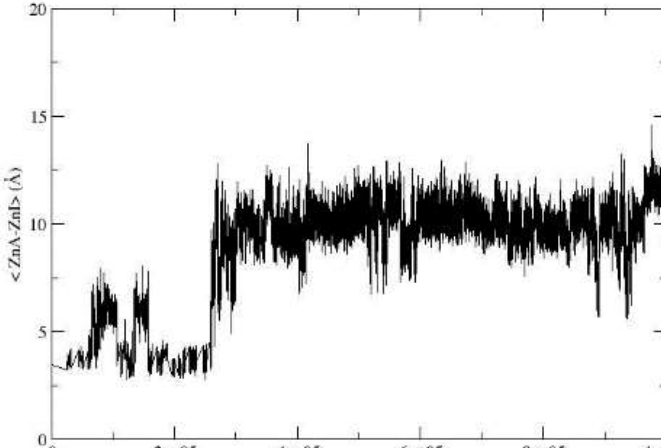
MD-S1


ZNA coodination
system S1-OH
E451-OE1
E451-OE2
E508-OE1
E508-OE2
H450
H555
distance (Å)
time (ps)
0
5
10
15
0
2e+05
4e+05
6e+05

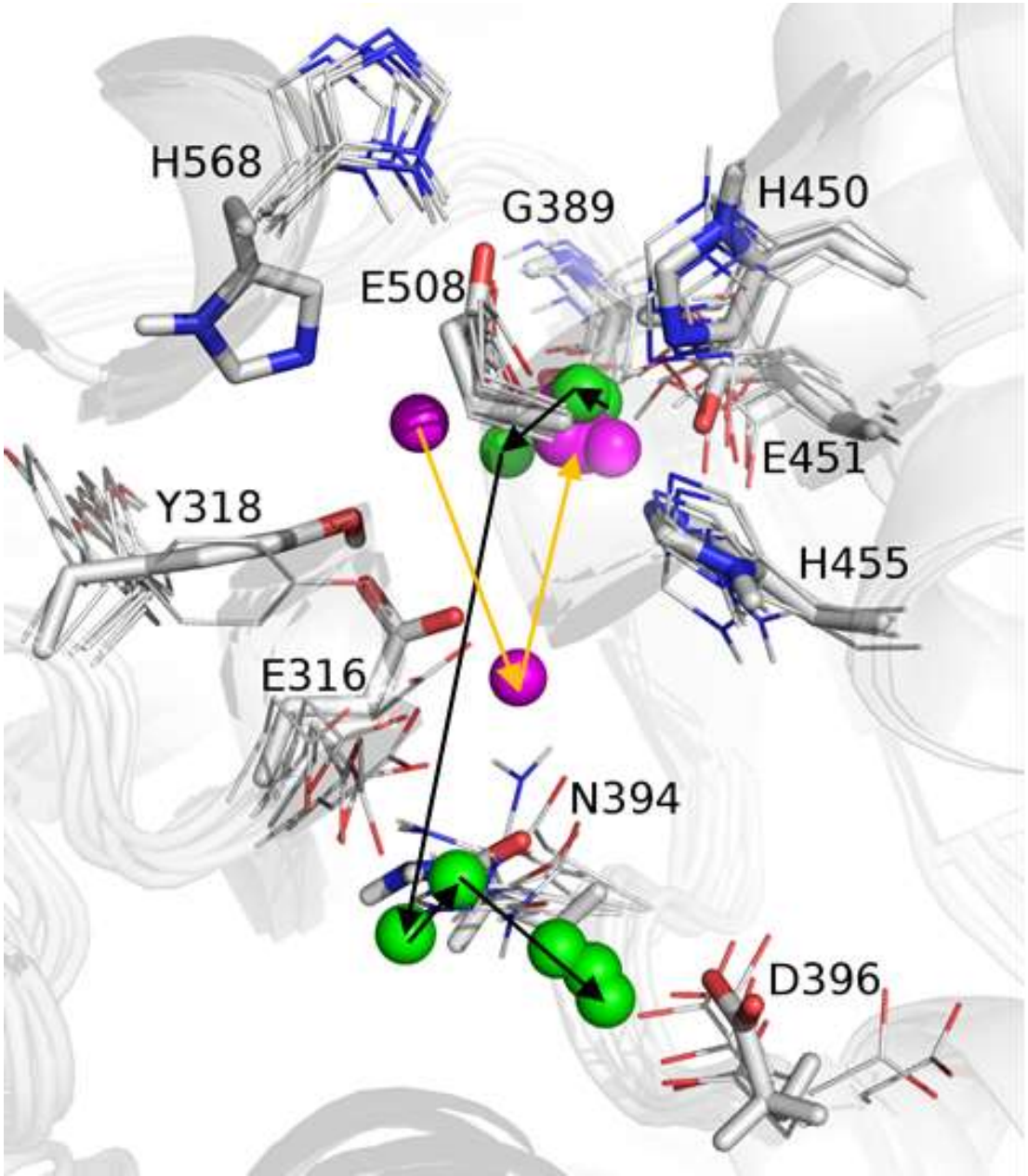

ZnA ligands
System S1-OH
D396-OE1
D396-OE2
E316-OE1
E316-OE2
N294
distance (Å)
time (ps)
0
5
10
15
0
2e+05
4e+05
6e+05
8e+05
1e+06

MD-S1


ZNI coordinatio
System S1-OH
E316-OE1
E316-OE2
E508-OE1
E508-OE2
H450
H455
distance (Å)
time (ps)
0
5
10
15
0
2e+05
4e+05
6e+05
8e+05

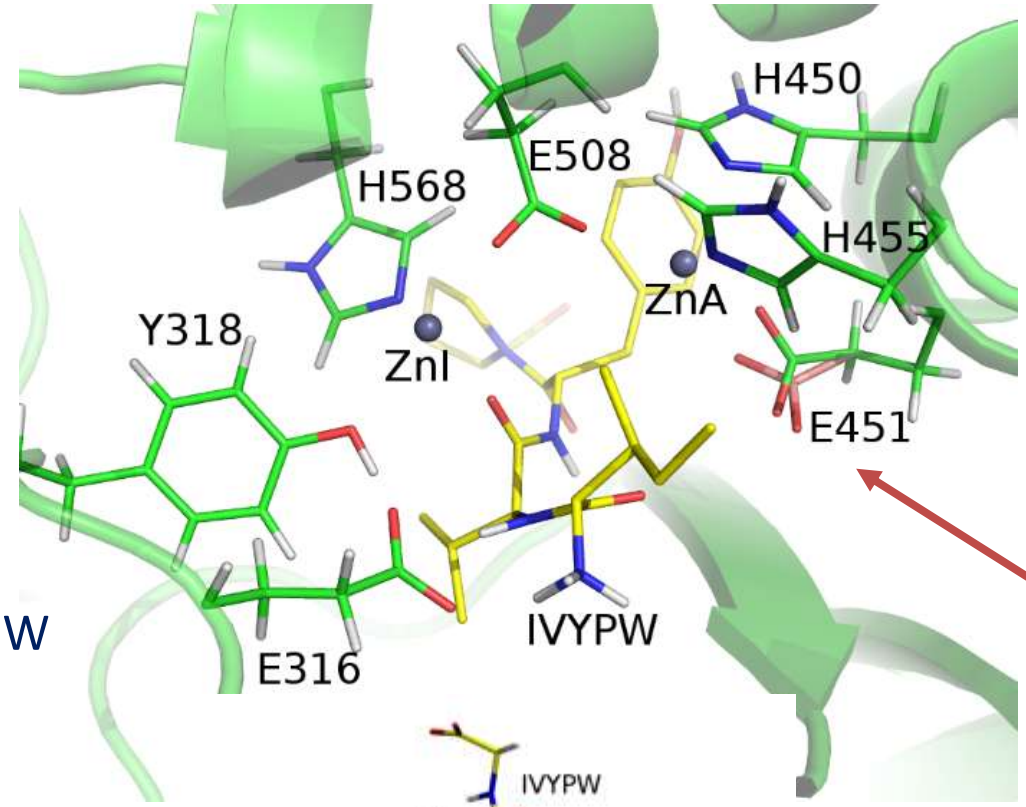

<ZnA-ZnI> (Å)
0
5
10
15
20

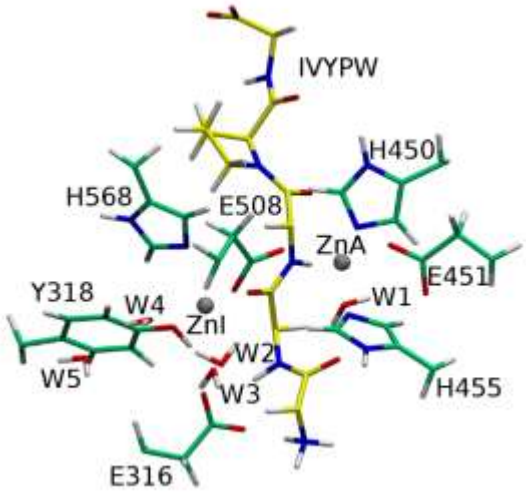
Izmjena ZNA i ZNI tijekom MD



QMMM račun CPLX1 i CPLX2

hDPP III – IVYPW





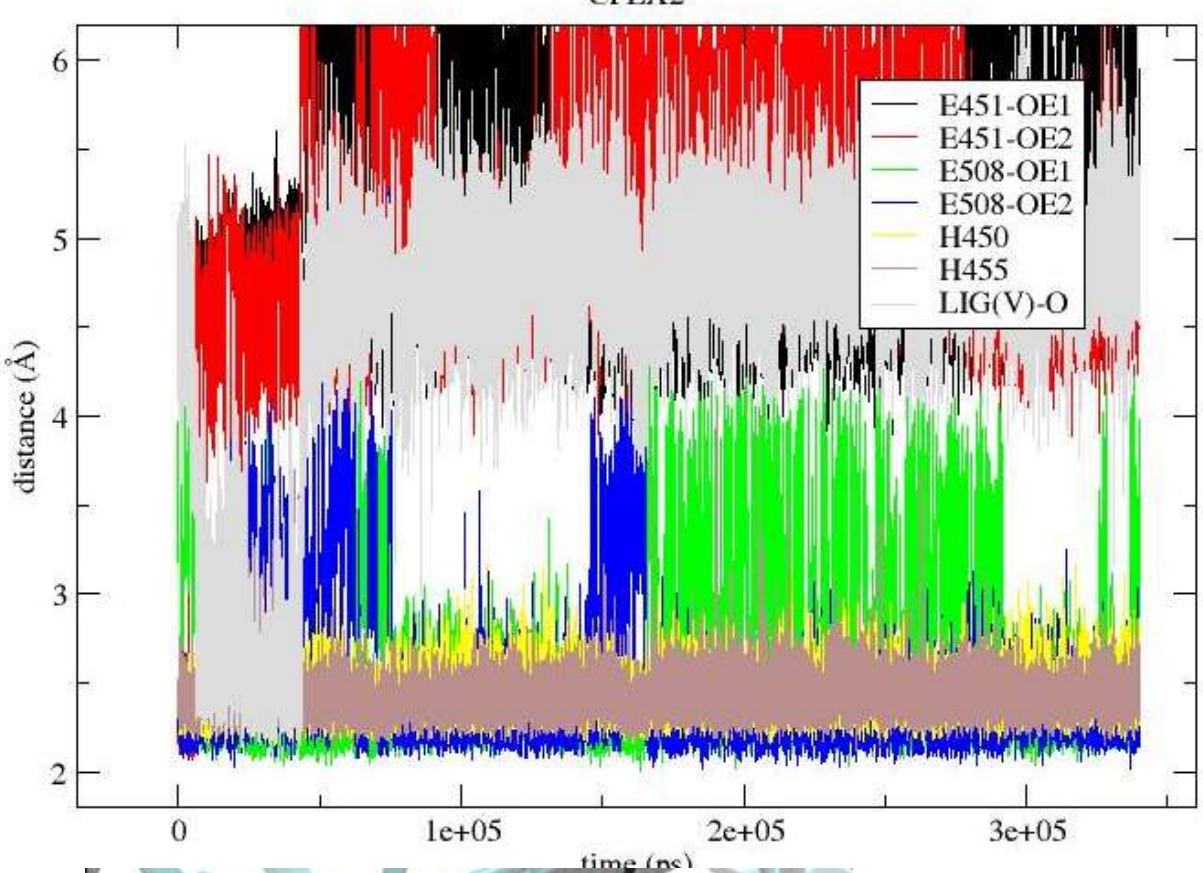
ZNA coordination:

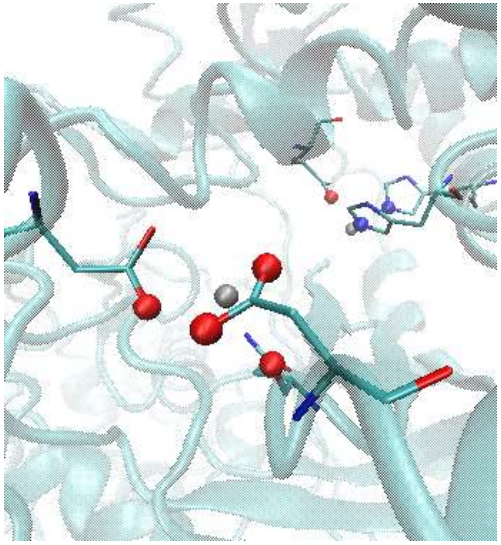
E508, H450, H455
E508, H450, H455, E451

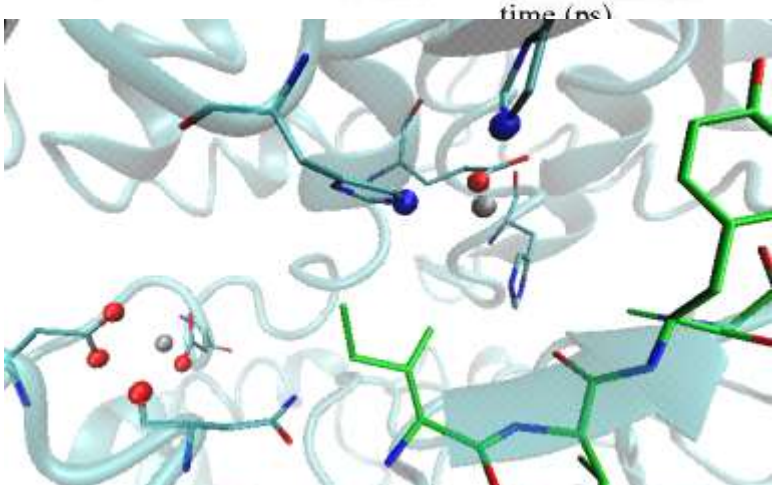
MD- cplx

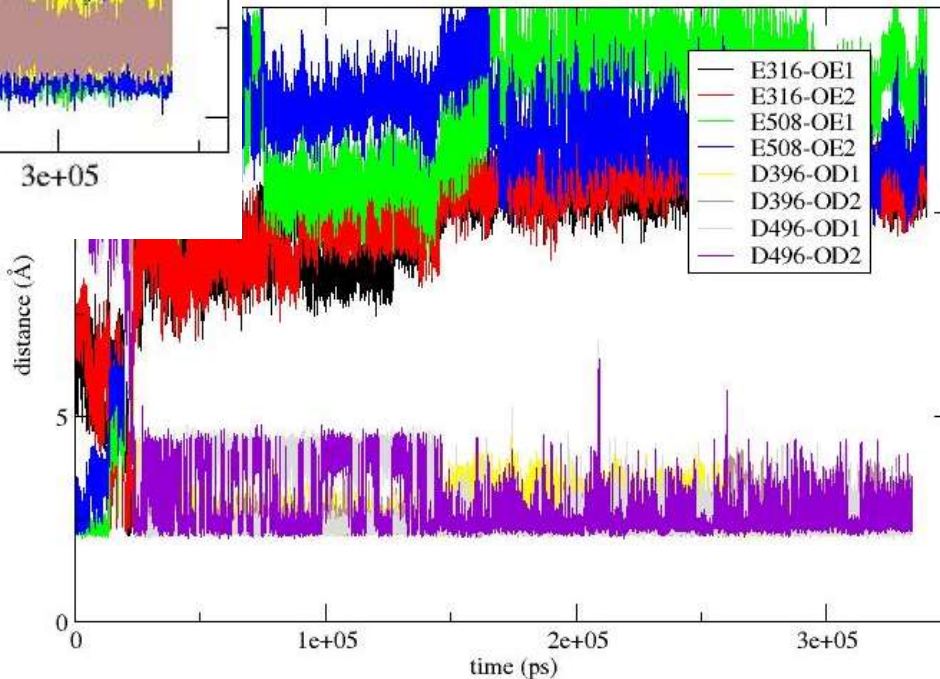
Model #	SYSTEM Force field	t_i/t_{total} (μs)	<ZnA–ZnI> (Å)	ZnA ligands	ZnI ligands	MMGBSA(kcal/mol)		
						Receptor: DPP III Ligand: ZnA + ZnI	Receptor: DPP III Ligand: ZnA	Rec: DPP III+ ZnA Ligand: ZnI
D2	**CPLX1** ff14SB	$0.5/0.5^{ZnA}$ $0.5/0.5^{ZnI}$	5.2±0.3	**H450, H455, E508^{M,B}** mostly 1 W	**E508^{M}** mostly 4 W	-114±7	-93±5	-18±4
	CPLX2 ff14SB	$0.5/0.5^{ZnA}$ $0.44/0.5^{ZnI}$	5.4±0.3	**H450, H455, E508^{M,B}** 1W	**H568, E508^{M,B}** **^{c}HM-O2nd** mostly 2 W	-150±8	-85±5	-39±4
2	**CPLX1** ff03	$0.24/0.25^{ZnA}$ $0.24/0.25^{ZnI}$	5.2±0.3	**H450, H455, E508^{M}**, E451^{M} 1-2 W	**H565, E508^{M}** mostly 2 W	-26±2	0±1	-22±2
3	**CPLX1** ff14SB	$1.1/1.1^{ZnA}$ $1.1/1.1^{ZnI}$	4.0±0.2	**H450, E451^{M}** **H455, E508^{M},** 1 W	**H568, E508^{M,B}** **^{c}HM-O2nd** mostly 1 W	-34±4	-1±3	-23±3
	CPLX1 ff14SB	$1.1/1.2^{ZnA}$ $0.5/1.2^{ZnI}$	4.0±0.2	**H450, E451^{M}** **H455, E508^{M},** 1 W	**H568, E508^{M}** **^{c}HM-O2nd** 1 W	-31±3	-2±2	-22±3
	CPLX2 ff14SB	$0.32/0.34^{ZnA}$ $0.32/0.34^{ZnI}$	11.6±0.9	**H450, H455, E508^{M},** mostly 2 W	D396^{M,B}, D496^{M,B} 1-2 W	-25±3	-12±2	-12±3
	CPLX2 ff14SB	$0.28/0.28^{ZnA}$ $0.08/028^{ZnI}$	18.6±12.7	**H450, H455, E508^{M},** 1 W	**E508^{M}** **^{c}HM-O2nd** 1 W	-21±5	-4±3	-17±3
3'	**CPLX2** ff14SB	$0.94/1.0^{ZnA}$ $1.0/1.0^{ZnI}$	3.8±0.2	**H450, H455, E508^{M,B}** mostly 1 W	**H568, E508^{M,B}** **^{c}HM-O2nd** mostly 2 W	-11±4	4±3	-8±3
3r	**CPLX2** ff14SB	$0.44/0.72^{ZnA}$ $0.21/0.72^{ZnI}$	4.5±0.4	**H450, H455, E451^{M,B}** mostly 1W	**H568, E508^{M,B}** **^{c}HM-O2nd** mostly 1 W	-21±3	-2±2	-14±5

MD-CPLX2-m3

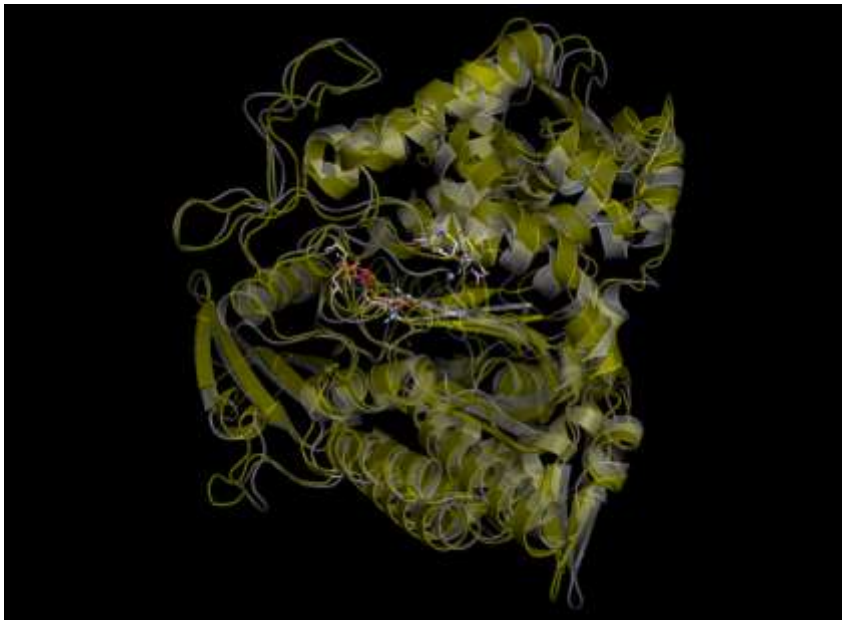

ZNA
CPLX2
E451-OE1
E451-OE2
E508-OE1
E508-OE2
H450
H455
LIG(V)-O
distance (Å)
time (ps)
0
1e+05
2e+05
3e+05
2
3
4
5
6

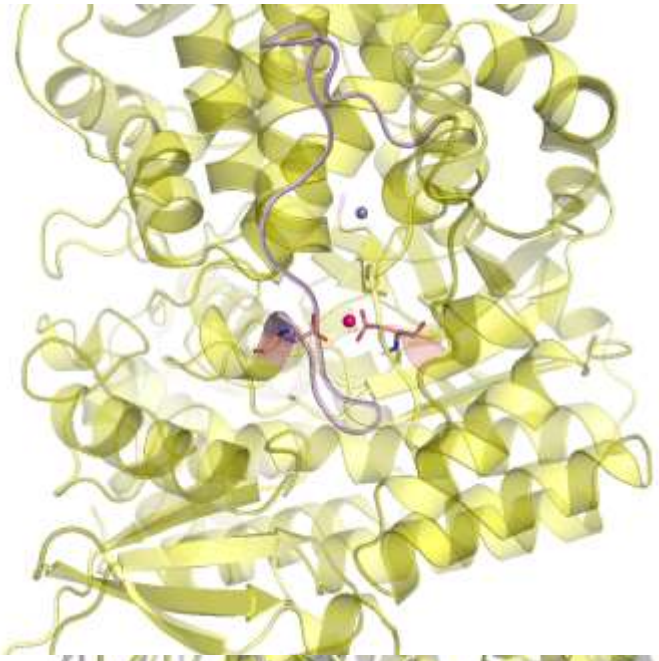


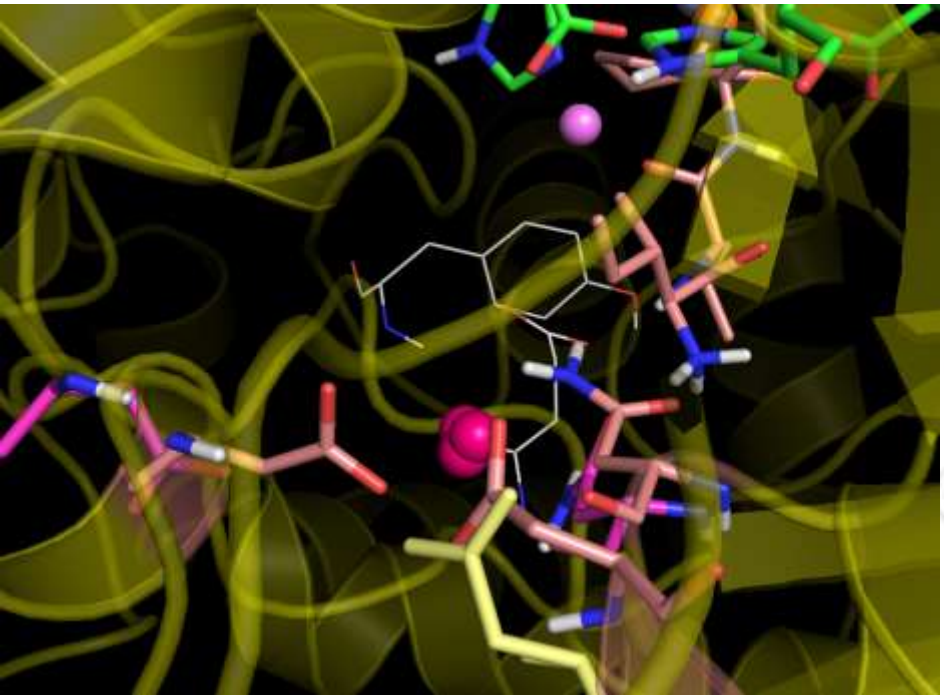


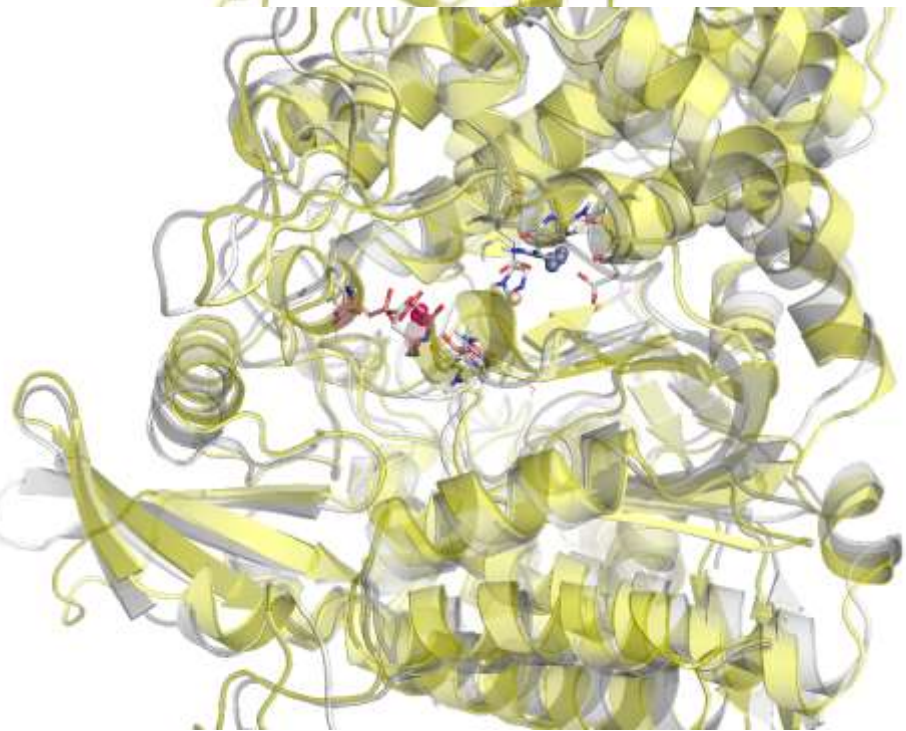

ZNI
CPLX2
E316-OE1
E316-OE2
E508-OE1
E508-OE2
D396-OD1
D396-OD2
D496-OD1
D496-OD2
distance (Å)
time (ps)
0
1e+05
2e+05
3e+05
5

vrata

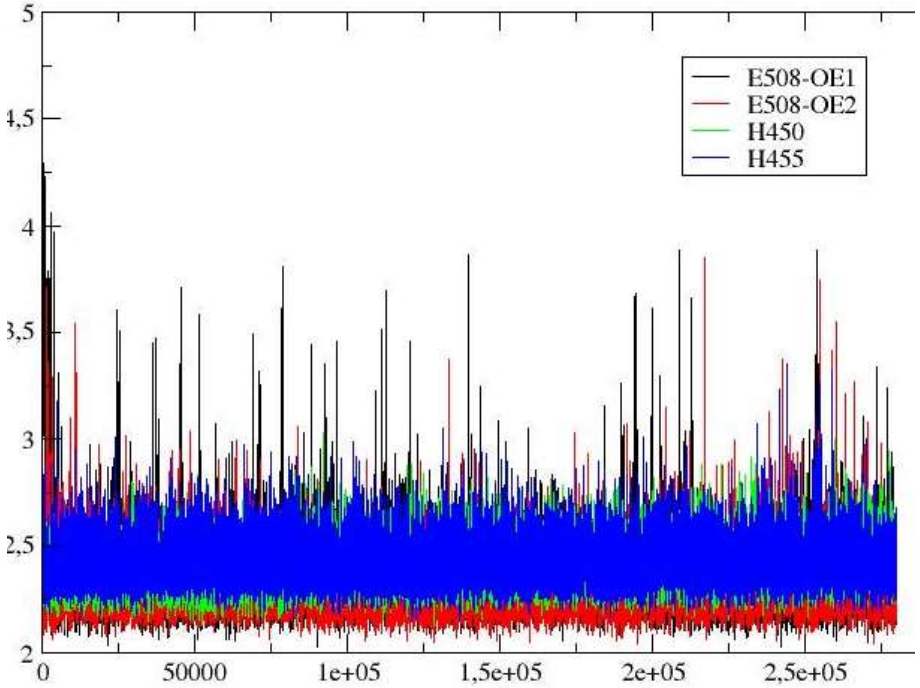


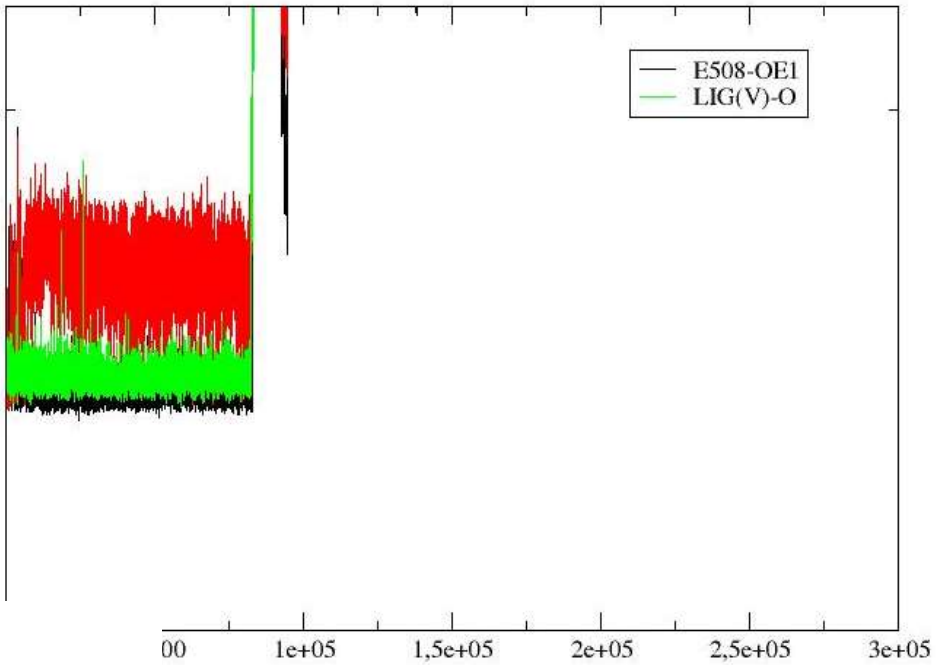


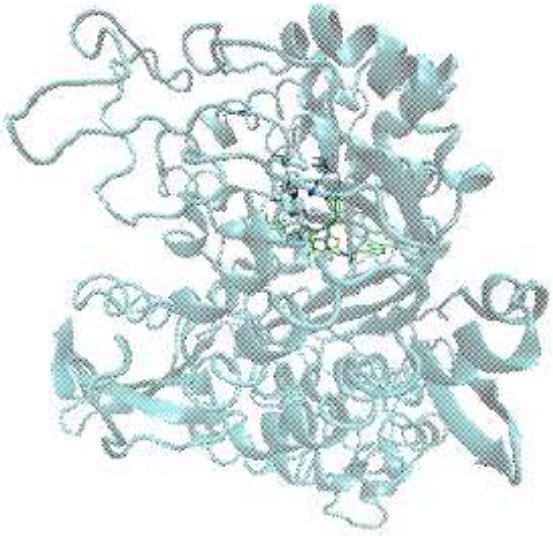


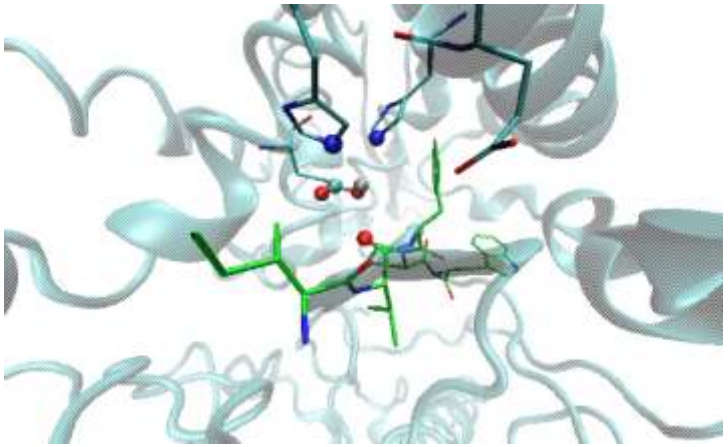


MD-CPLX2-m3

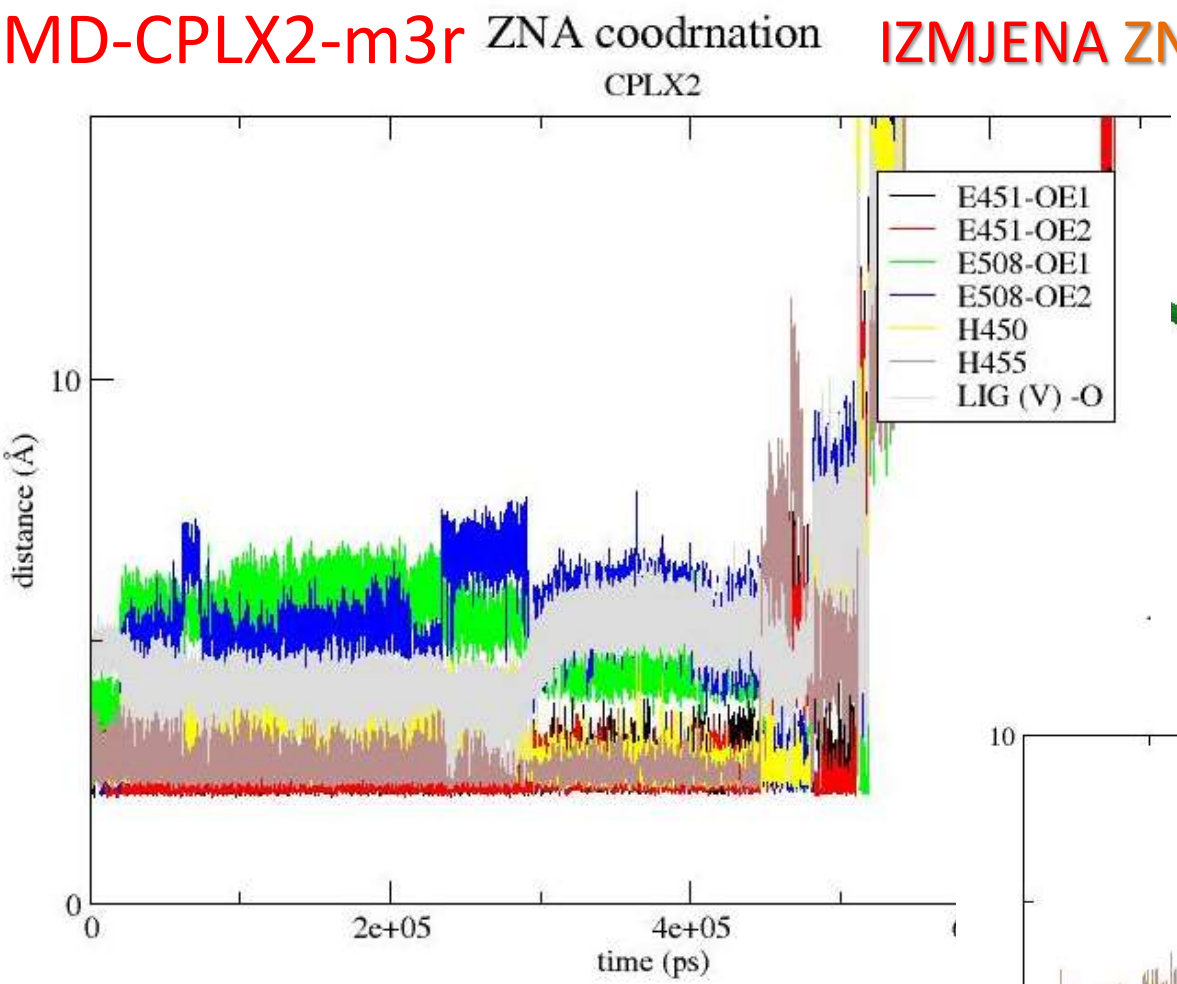

E508-OE1
E508-OE2
H450
H455
5
4,5
4
3,5
3
2,5
2
0
50000
1e+05
1,5e+05
2e+05
2,5e+05


E508-OE1
LIG(V)-O
00
1e+05
1,5e+05
2e+05
2,5e+05
3e+05





MD-CPLX2-m3r



IZMJENA ZNA ZNI

