DIPEPTIDYL PEPTIDASE III INHIBITION TESTS BY METAL DICATIONS

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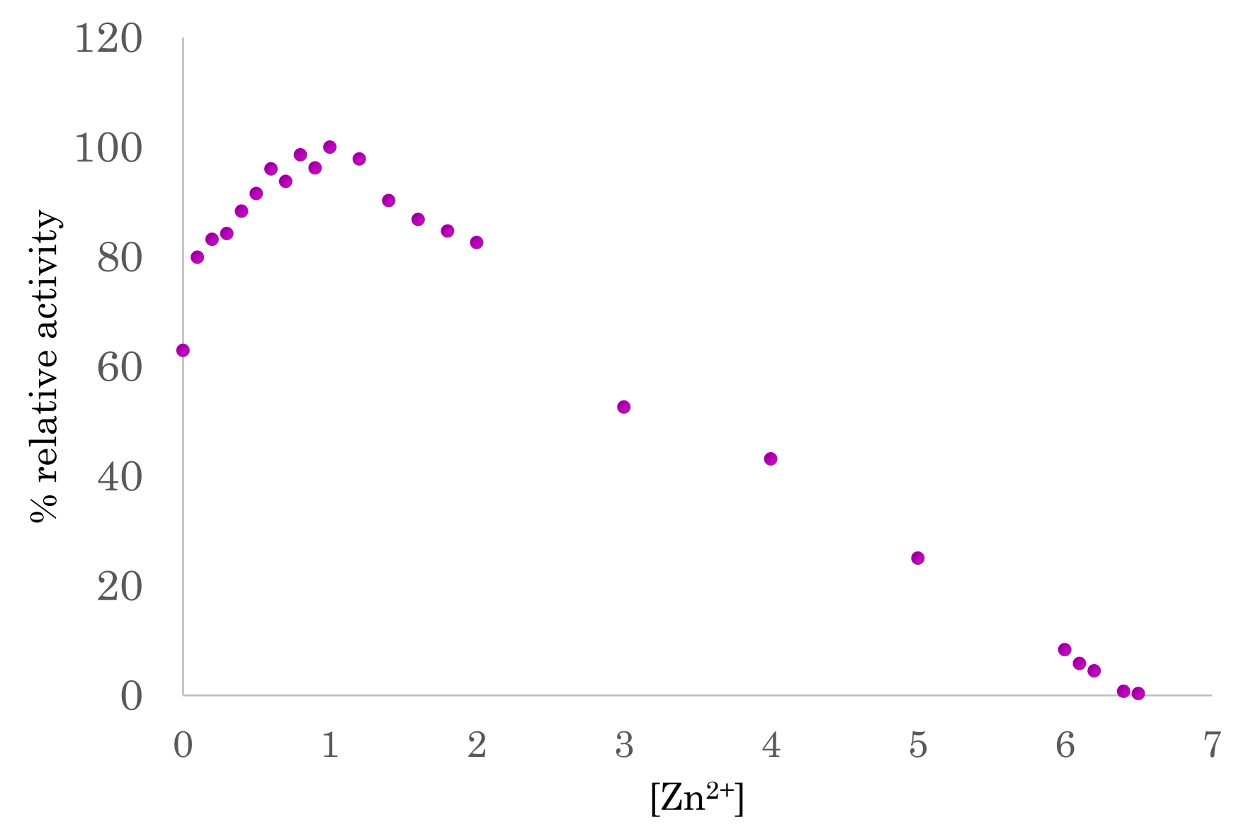
Dipeptidyl peptidase (DPP) III is a zinc-dependent exopeptidase whose motifs, "HEXXGH" and "EEXR(K)AE(D)" are responsible for the binding of metal ions to the active site of the enzyme. The peptidase activity of human dipeptidyl peptidase III (hDPP III) is inhibited by excess zinc ions. The aim of this study is to determine the effects of different concentrations of metal dications: Zn^{2+}, Mn^{2+}, Co^{2+} i Cu^{2+} and Cu^{2+} on the activity of hDPP III, and identify the inhibitory binding site of the metal. The existence of a metal inhibitory binding site was hypothesized based on the structural similarity of the hDPP III active site and thermolysin in which the binding of a second metal ion in the immediate vicinity of a catalytically active ion (PDB: 1LND) has already been demonstrated, and an observed decrease in hDPP III activity at higher zinc concentrations.[1]

The ‘stop flow’ method monitors the decrease in the enzymatic activity of hDPP III caused by an excess of zinc ions.



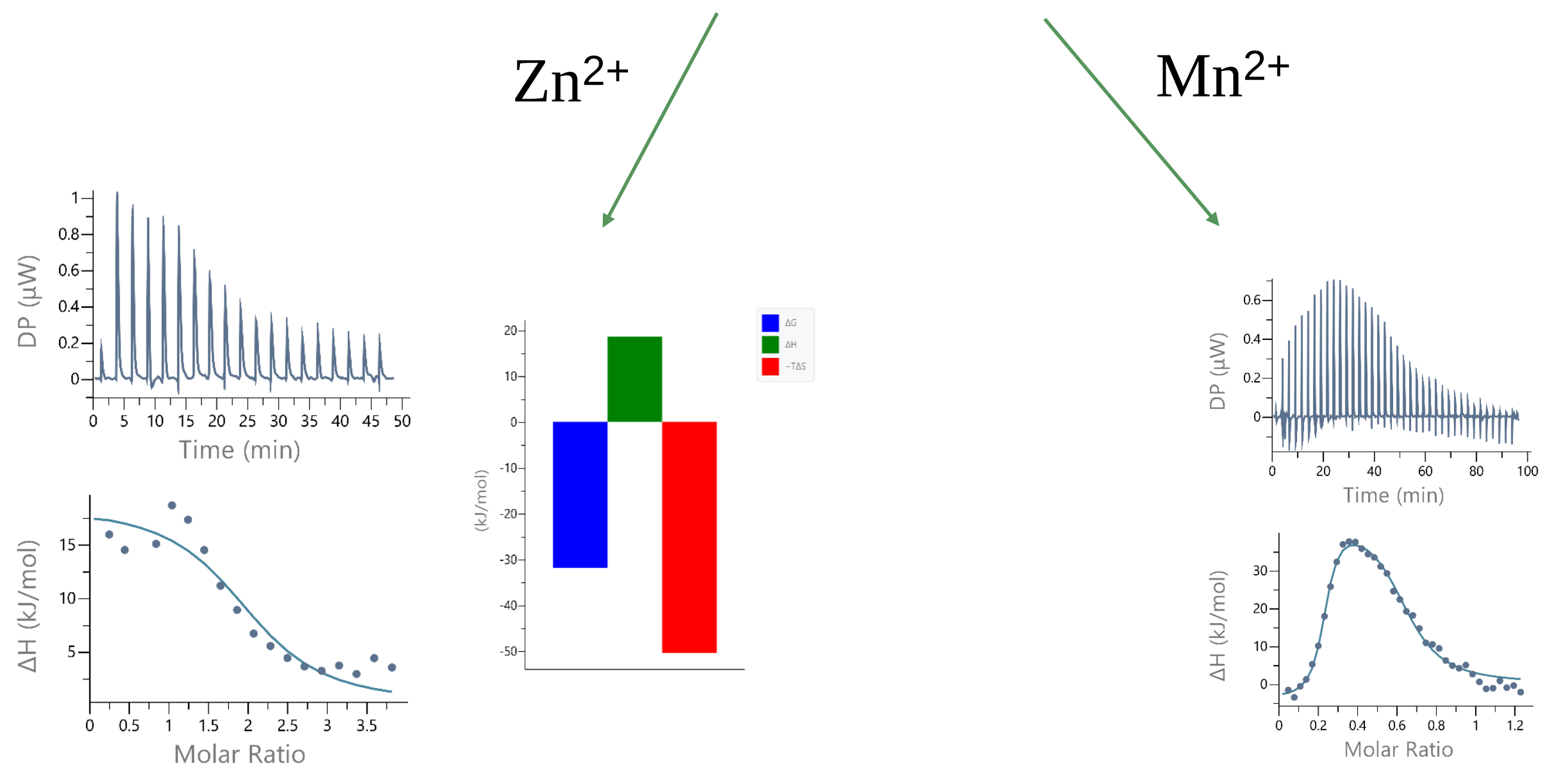
HR-ICP-MS (high resolution mass spectroscopy with inductively coupled plasma) determines ratio of the number of metals and proteins.

apo hDPPIII + M^{2+}	Zn^{2+}	Cu^{2+}	Co^{2+}	Mn^{2+}
apo+Zn^{2+}	1,0	0,0	0,0	0,0
apo+Cu^{2+}	0,0	2,0	0,0	0,0
apo+Co^{2+}	0,2	0,0	0,3	0,0
apo+Mn^{2+}	0,3	0,0	0,0	0,0

Approximately two metal-binding sites are present in human DPPIII.

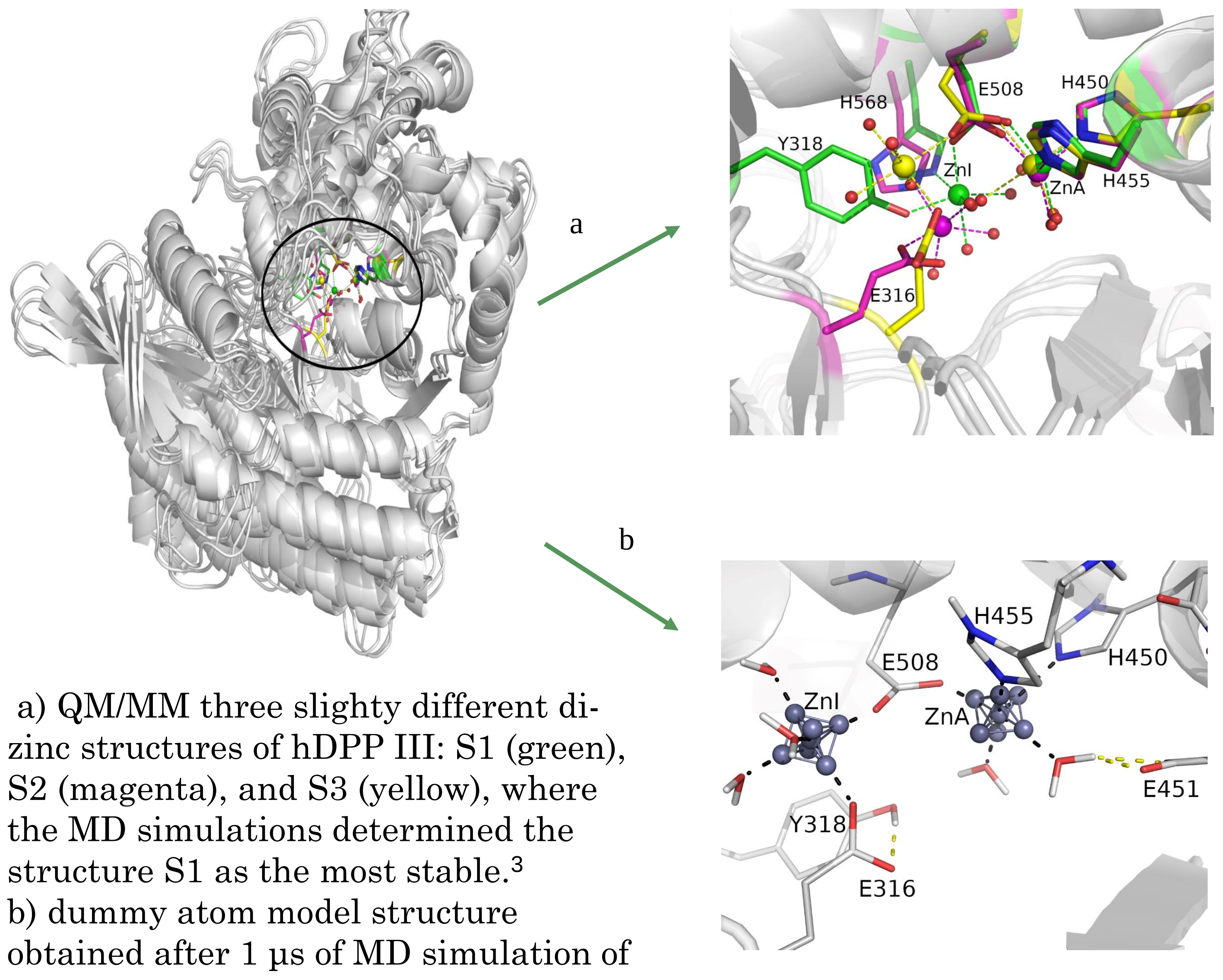
ITC (Isothermal Titration Calorimetry) determination of the number of metal ions per protein molecule

apo hDPPIII + M^{2+}	N
Zn^{2+}	2,00+/-0,08
Cu^{2+}	1,2+/-0,10
Co^{2+}	0,663+/-0,013
Mn^{2+}	0,213+/-0,008
	0,435+/-0,021

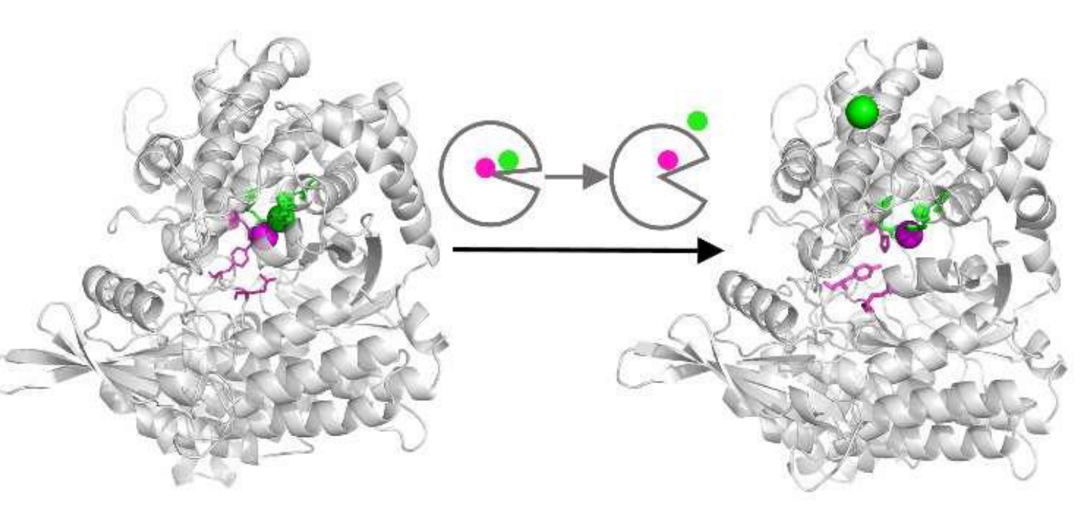


Binding of another metal ion to ‘Metal inhibitory binding site’ located next to the catalytically active site of the enzyme and its influence on the structure and dynamics of hDPP III was identified using molecular dynamics in combination with the calculation of free binding energies and quantum mechanical - molecular mechanical calculations.

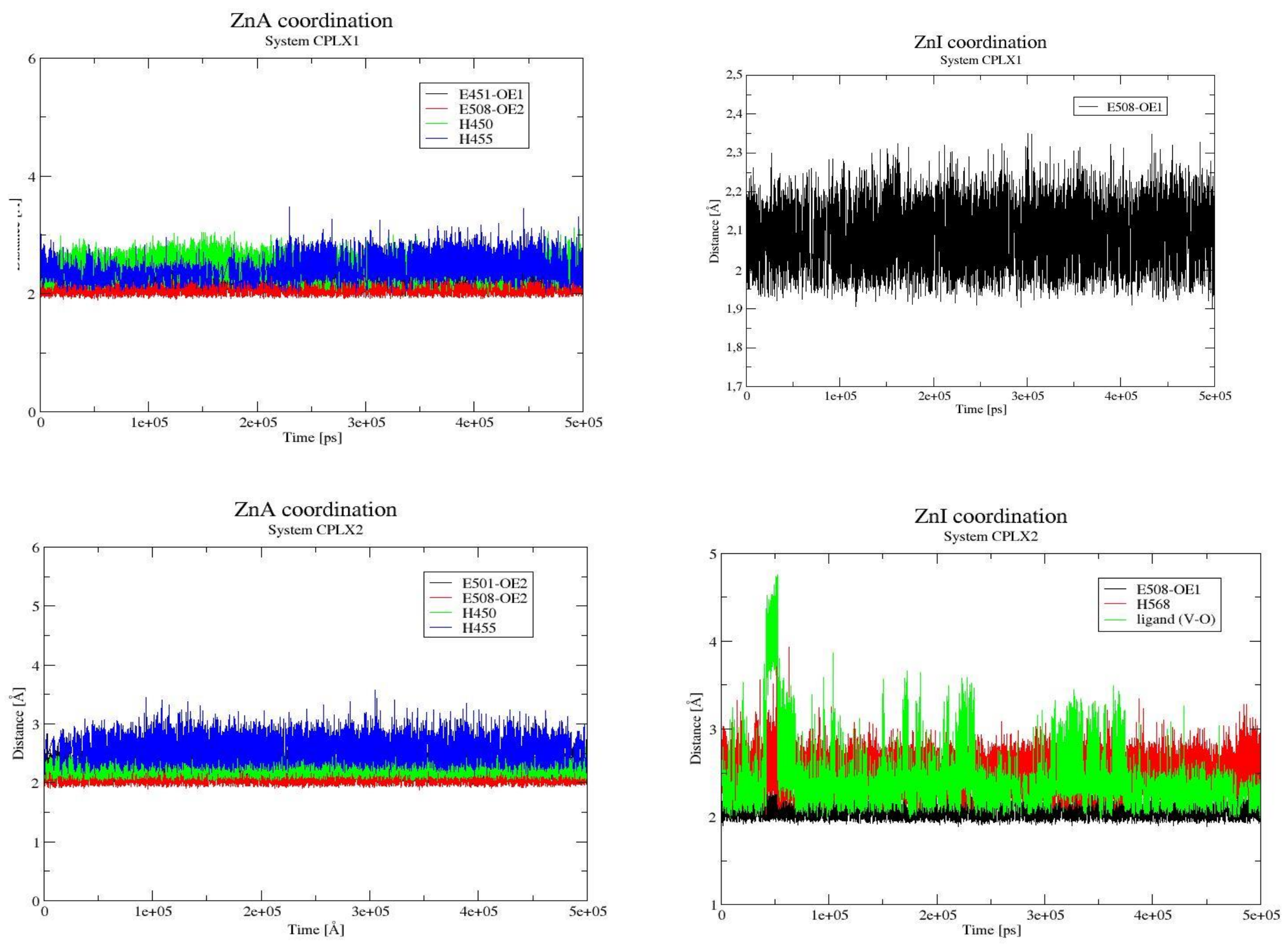
MD simulations of hDPP III (PDB: 5EGY), second binding site of zinc ion (PDB: 1LND and 1CPX).



a) QM/MM three slighty different di-zinc structures of hDPP III: S1 (green), S2 (magenta), and S3 (yellow), where the MD simulations determined the structure S1 as the most stable.[3]
b) dummy atom model structure obtained after 1 μs of MD simulation of the S2 structure using ff14SB force field.[3]



Simulations showed translocation of Zn ion from the inhibitory to the catalytic binding site, accompanied by displacement of the catalytic Zn from the interdomain cleft.[3]



Coordination of zinc ions during 500 ns of MD simulations of the hDPP III – IVYPW complex. The results of two independent runs are given; top – results of run1 and bottom results of run2.[2]

Literature:
1. K.M. Fukasawa, J. Hirose, T. Hata, Bio.Biophys Acta.1804 (2010), 2063-2069.
2. C.J. Young, S. Siemann, Highly dynamic metal exchange in anthrax lethal factor involves the occupation of an inhibitory metal binding site. Chem Commun (Camb). 2016., 52(79):11748-51.
3. A. Tomić, H. Brkić, A. Matić, S. Tomić, Unravelling the inhibitory zinc ion binding site and the metal exchange mechanism in human DPP III, Physical Chemistry Chemical Physics, 2021, DOI: 10.1039/D1CP01302E

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