Zn^{2+} AND Matrix Metalloproteinase

Oscar

Outline

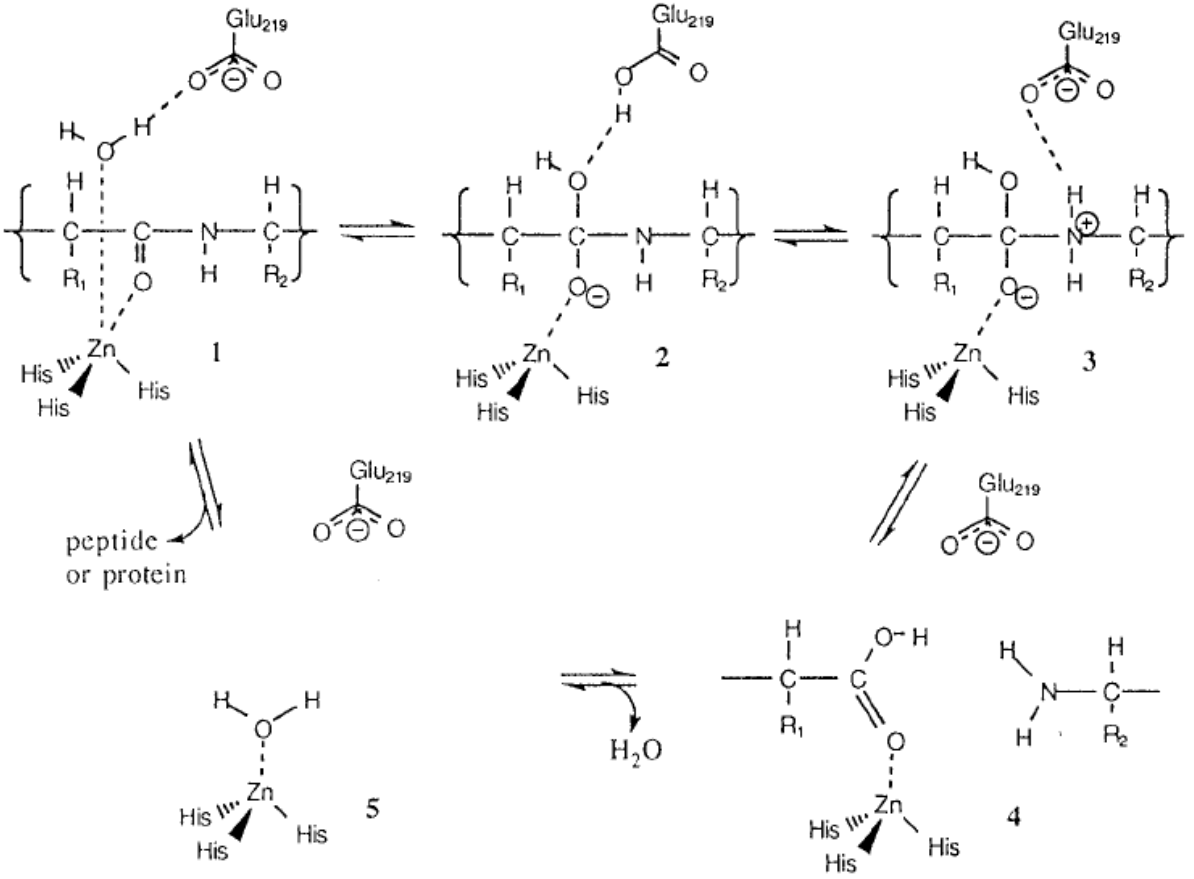
- Background
- Modeling of Zn^{2+}
- Zn^{2+} Parametrization in AMOEBA
- Future work

Zinc and proteins

- Zinc is the most common transition metals found in enzymes.
- Zinc containing proteins play a key role in the biosynthesis and metabolism of a number of bioactive peptides.
- Most commonly investigated zinc-metalloproteinases include carbonic anhydrase (CA), carboxypeptidase A (CPA), alcohol dehydrogenase (AD), matrix metalloproteinase (MMP) and thermolysin (TL).

Matrix Metalloproteinase (MMP)

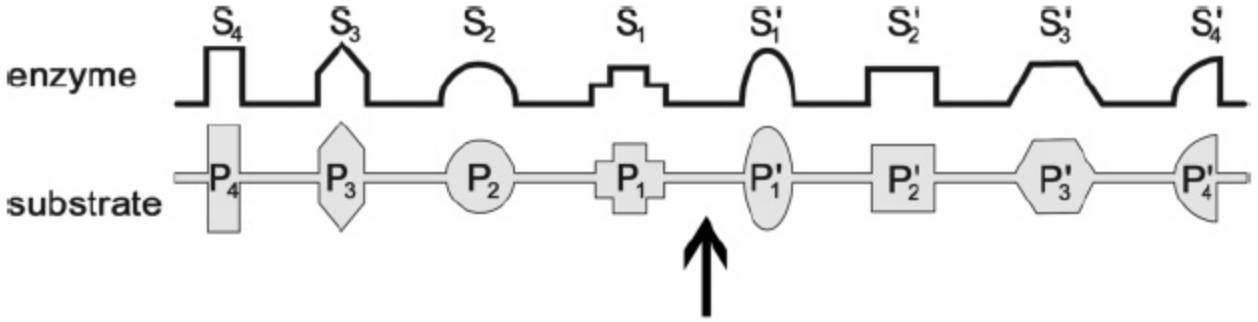
- MMPs are a family of zinc-dependent enzymes that degrade the extracellular matrix (ECM) by effecting amide bond hydrolysis on substrates.



(Toba JMC 1999)

Matrix Metalloproteinase (MMP)

- MMP family is known to include approximately 27 enzymes, divided into four sub-groups based on their substrate specificity:
  - Collagenases (MMP-1 -8 and -13)
  - Gelatinases (MMP-2 and -9)
  - Stromelysins (MMP -3 -10 and -11)
  - Membrane-type



Lauer-Fields JACS 2007

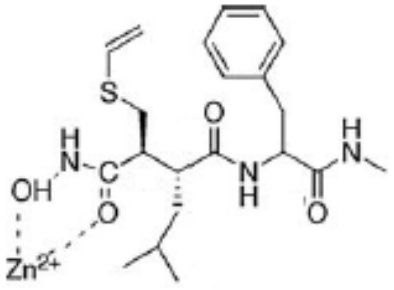
MMP pathologies

- MMP activity was controlled by transcriptional regulation, zymogen activation and endogenous inhibitors under normal physiological conditions.
- Overexpression of MMPs leads to accelarated matrix degradation.
- Excessive activation of MMP is associated with tumor growth, arthritis, cardiovascular diseases, multiple sclerosis, eye and skin diseases and periodontitis.
- MMP family has been a pharmaceutical target for over 20 years.

MMP inhibitors

- Research has mainly focused on the discovery active-site directed inhibitors with zinc-binding groups (ZBG).
  - Hydroxamate ($CONH\text{-}O^-$)
  - Carboxylate (COO^-)
  - Thiolate (S^-)
  - Phosphinyl (PO_2^-)

  Hydroxamate is most commonly used.

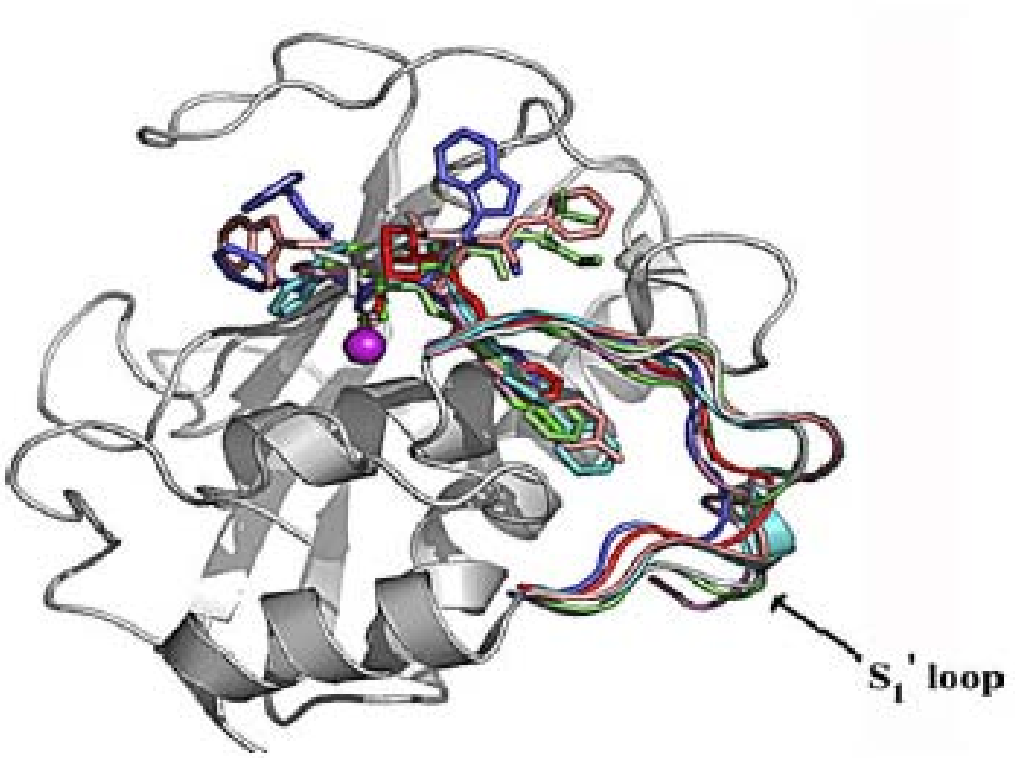


Cuniasse, P et al. Biochimie 2005

Failure of MMP drug design

- Faulty clinical trial design
- Toxicity and side effect
- Lack of specificity

Flexible S1’ Loop



Cuniasse et al. Biochimie (2005)

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Why is it hard to model Zn^{2+}?

- Polarization
- Charge transfer
- Multiple coordination geometries
- Lack of accurate force field

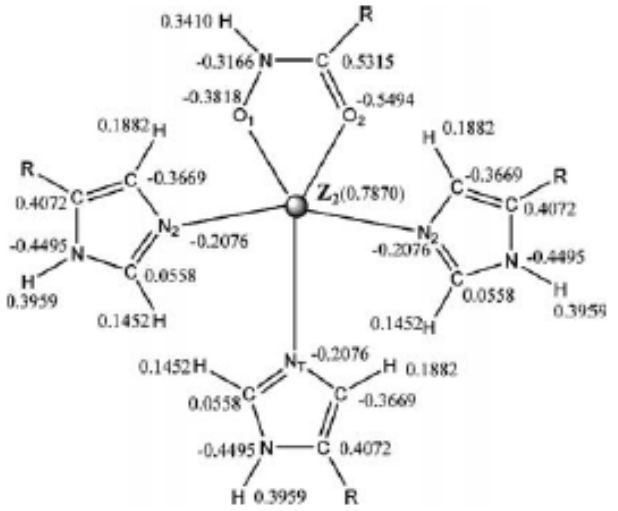
Two models of Zn^{2+}

- Bonded model
- Non-bonded model

Bonded model

- Explicit bond and angle terms are introduced into the potential energy function to account for interactions between the metal and the protein and ligand atoms.
- Bonded model gives better coordination geometry than non-bonded model.
- Inability to allow for coordination changes around the ion during dynamics and limited ion-ligand flexibility.

Bonded model



T. Tuccinardi et al.
Bioorg. Med. Chem.
14 (2006) 4260-4276

Interaction	Bond length	Force constant
Z2–N2	2.1051	80.00
Z2–NT	2.1619	70.00
Z2–O1	2.1070	95.00
Z2–O2	2.1111	103.50
O1–N2	1.4061	520.00

Interaction	Angle	Force constant
N2–Z2–N2	111.8620	8.00
N2–Z2–NT	97.9730	8.00
N2–Z2–O2	123.3505	8.75
N2–Z2–O1	90.2945	9.50
NT–Z2–O2	86.7380	8.25
NT–Z2–O1	165.1020	12.85
O1–Z2–O2	78.3650	9.90
C–N*–Z2	126.0000	26.50
N2–O1-Z2	111.8140	67.00
Z2–O2–C	110.5900	33.00

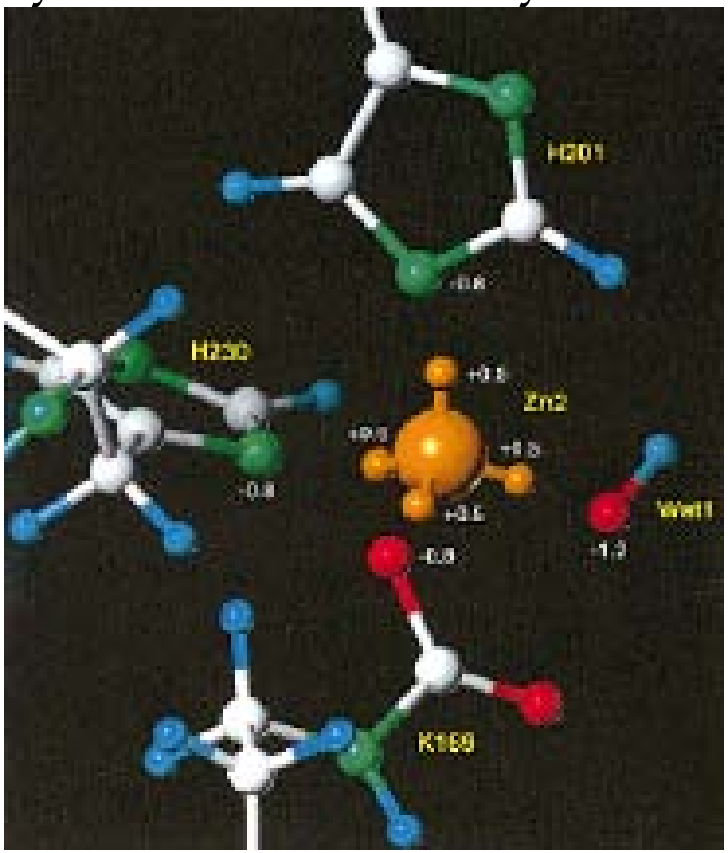
Non-bonded model

- The non-bonded models tend to generate the highest coordination number possible (octahedral).
- They allow the flexibility of ligand conformational sampling.
- Very sensitive to the electrostatic model chosen, and can suffer from the inability to retain low coordination number.

Cationic Dummy Atom Model

- Uses four identical dummy atoms tetrahedrically attached to the zinc ion and transfers all the atomic charge of the zinc divalent cation evenly to the four dummy atoms.

Yuan-Ping Pang.
Proteins: Structure, Function, and Genetics 45:183-189 (2001)



Cationic Dummy Atom Model

Bond	K [kcal/mol Å^2]	R_{eq} (Å)
CT-P	230	1.61
P-O	525	1.48
N2-C	481	1.34
DZ-ZN	540	0.90
DZ-DZ	540	1.470

Angle	K [kcal/mol radian^2)]	T_{eq} (deg.)
CA-CT-P	100	120.5
HC-CT-P	100	120.5
CT-P-OS	100	108.23
CT-N2-C	50	123.2
N2-C-O2	70	120
H-N2-C	35	120
CT-P-O	100	108.23
O-P-OS	100	108.23
DZ-ZN-DZ	55	109.50
DZ-DZ-DZ	55	60.0
DZ-DZ-ZN	55	35.25

Dihedral	IDIVF	V_n/2 (kcal/mol)	γ (deg.)	N
X-CT-P-X	3	0.75	0	3
X-N2-C-X	4	9.6	180	2
ZN-DZ-DZ-DZ	1	0	35.3	2
DZ-ZN-DZ-DZ	1	0	120.0	2
DZ-DZ-DZ-DZ	1	0	70.5	2

Improper dihedral	V_n/2 (kcal/mol)	γ (deg.)	N
X-X-CA-CA	1.1	180	2

VDW	Mass	R^* (Å)	Eps (kcal/mol)	VDW	Mass	R^* (Å)	Eps (kcal/mol)
ZN	53.38	3.1	1E-6	DZ	3.0	0	0

SIBFA model

- Sum of Interactions Between Fragments Ab initio-computed
- Based on a functional representation of distinct electronic effects which control intra- and inter- molecular interactions.
- Reproduce the accurate inhibitor coordination structure.
- Capable of matching quantum chemical binding energies by 2%.

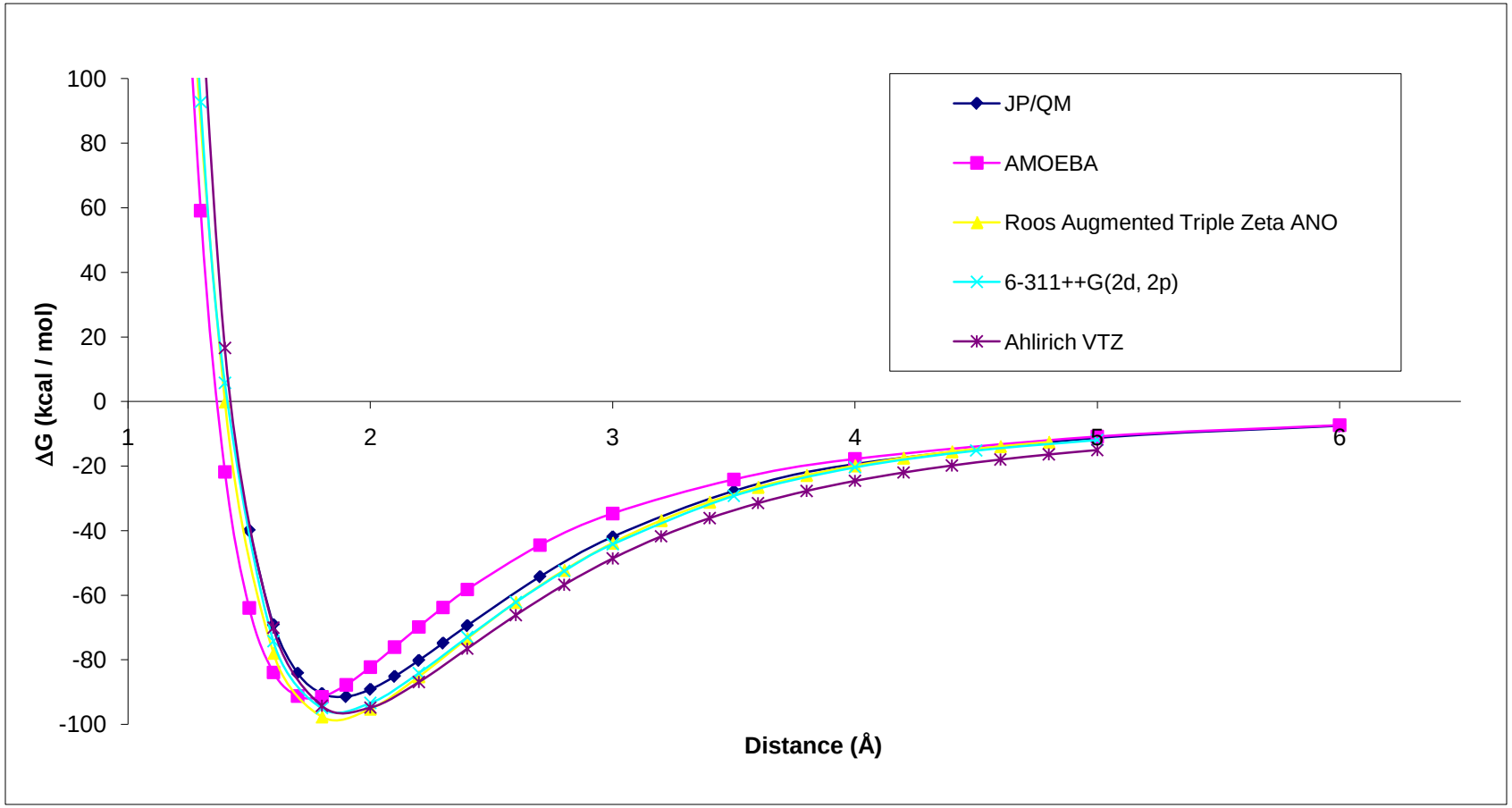
$$\Delta E = E_{MTP} + E_{rep} + E_{pol} + E_{ct} + E_{disp}$$

Garmer, D. R.; Gresh, N and Roques, B. P. *PROTEINS: Structure, Function, and Genetics* 31:42-60 (1998)

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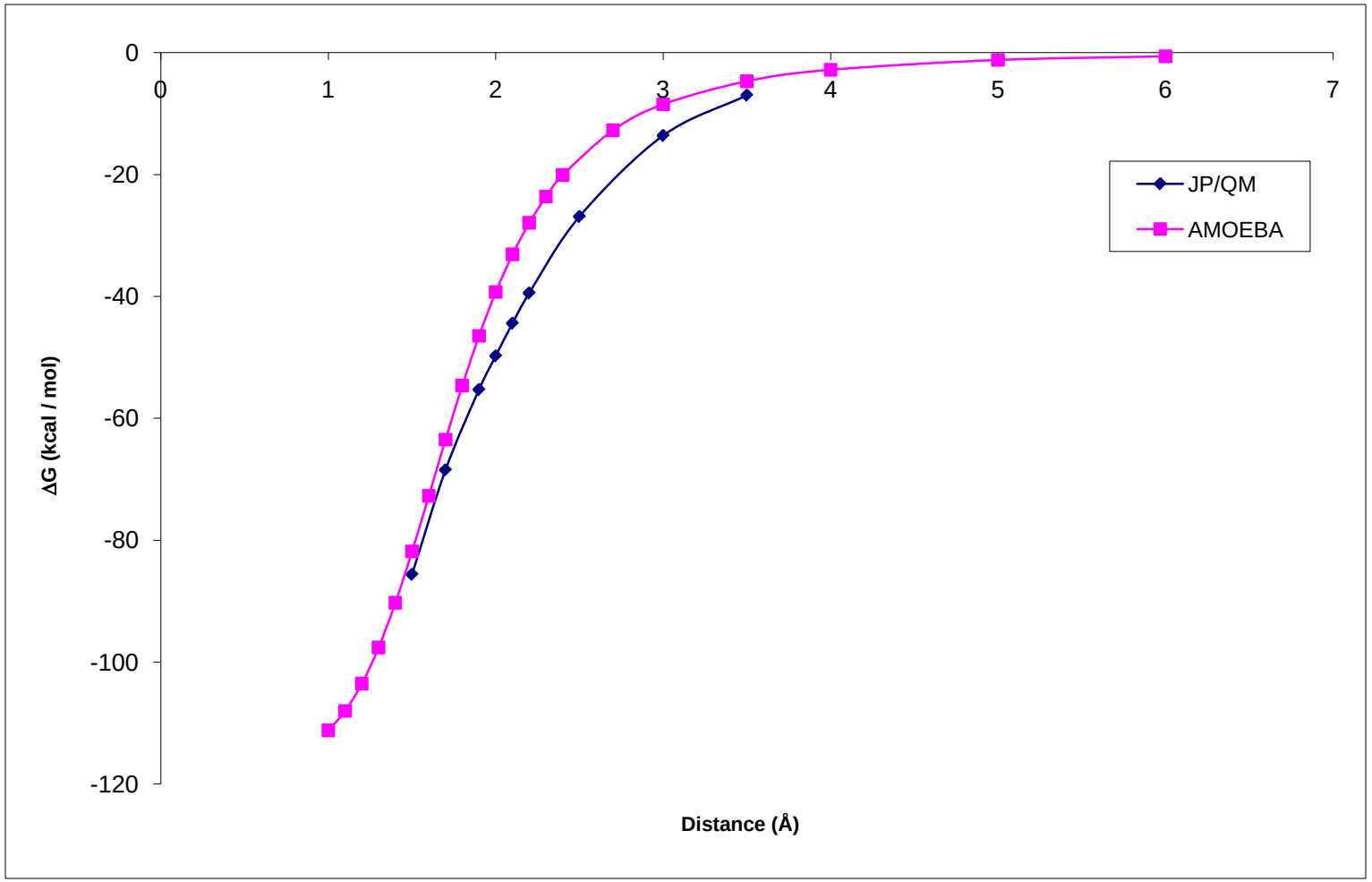
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Parametrization of Zn^{2+}



LANL2DZ basis set. Pang et al

Polarization energy



Solvation free energy of Zn^{2+}

AMOEBA[1]	Experiment[2]	QCA[3]
-457.22	-467.26	-458.1

1. Grossfield, A.; Ren, P. Y.; Ponder, J. W., Ion solvation thermodynamics from simulation with a polarizable force field. *Journal of the American Chemical Society* **2003,** 125, (50), 15671-15682.

2. Marcus, Y., A Simple Empirical-Model Describing the Thermodynamics of Hydration of Ions of Widely Varying Charges, Sizes, and Shapes. *Biophysical Chemistry* **1994,** 51, (2-3), 111-127.

3. Asthagiri, D.; Pratt, L. R.; Paulaitis, M. E.; Rempe, S. B., Hydration structure and free energy of biomolecularly specific aqueous dications, including Zn2+ and first transition row metals. *Journal of the American Chemical Society* **2004,** 126, (4), 1285-1289.

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Future work

- Finalize parameters of Zn in Amoeba.
- Find crystal structures of MMPs with inhibitors.
- Parameterize inhibitors.
- Calculate the absolute and relative free energies of two or more inhibitors binding to the same MMP.
- Investigate the selectivity of one inhibitor towards different MMPs.