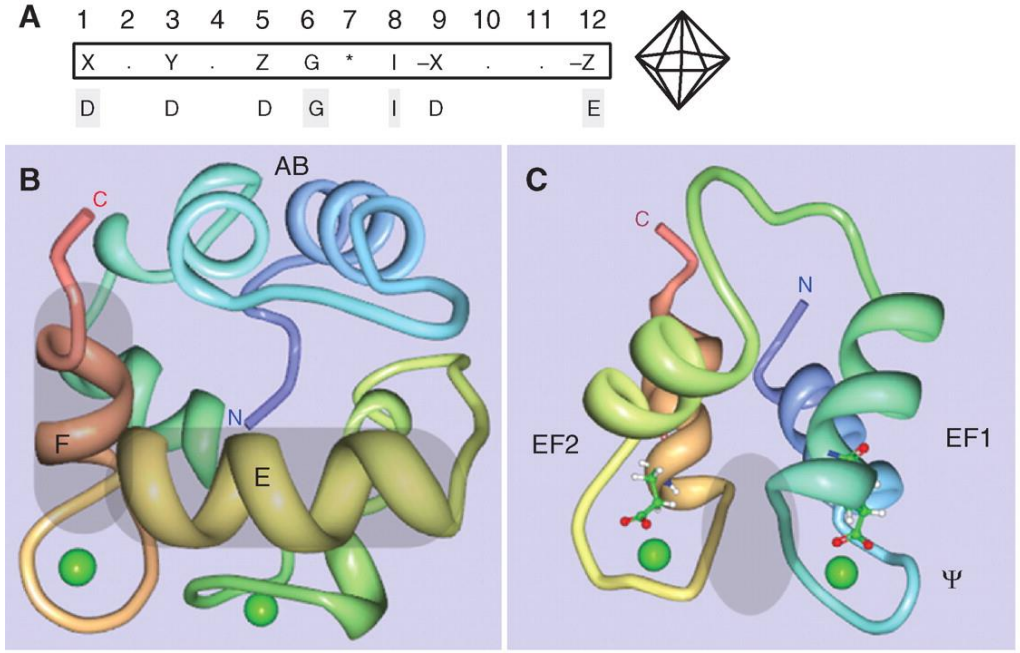
EF hand binding free energy

Zhifeng Jing

2017.3.24

EF hand

- EF-hand is a Ca^{2+}-binding motif which appears in parvalbumin, calmodulin and troponin-C



A. Consensus sequence of EF-hand Ca^{2+}-binding loop
B. Solution structure of Ca^{2+}-bound human α parvalbumin (PV)
C. Solution structure of bovine CB-D9k with a pseudo 14-residue EF-hand (EF1) and a canonical EF-hand (EF2).

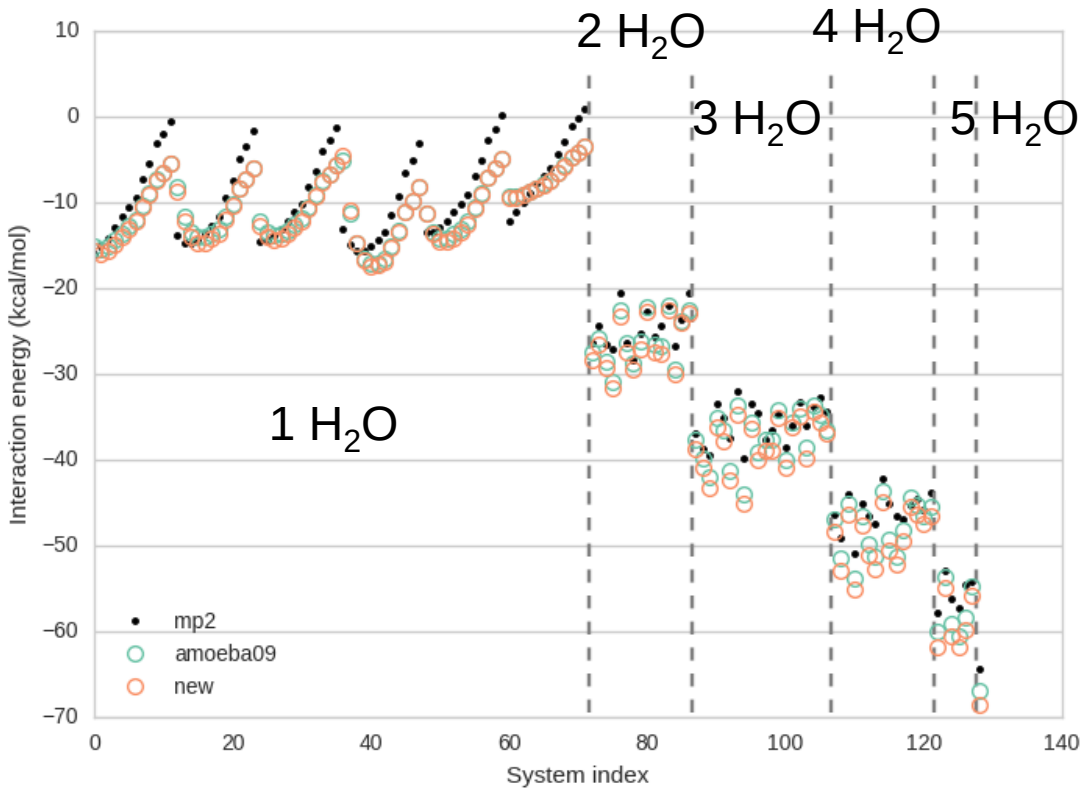
EF hand

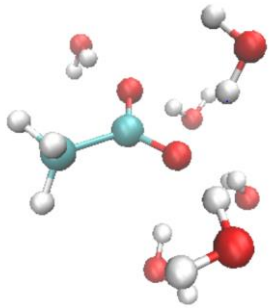
- There are two classes of EF-hands
  - Ca^{2+} specific
  - Ca^{2+}/Mg^{2+} binding

	α PV	β PV
# of functional Ca^{2+} binding sites	2	2
# of Ca^{2+} specific sites	0	1
$K_{D,Ca}$	~4 nM	~40 nM ~600 nM
$K_{D, Mg}$	~30 uM	~200 uM
$K_{D,Ca}$(app) at [Mg^{2+}] of 0.5-1 mM	~200 nM	~300 nM

Acid parameters

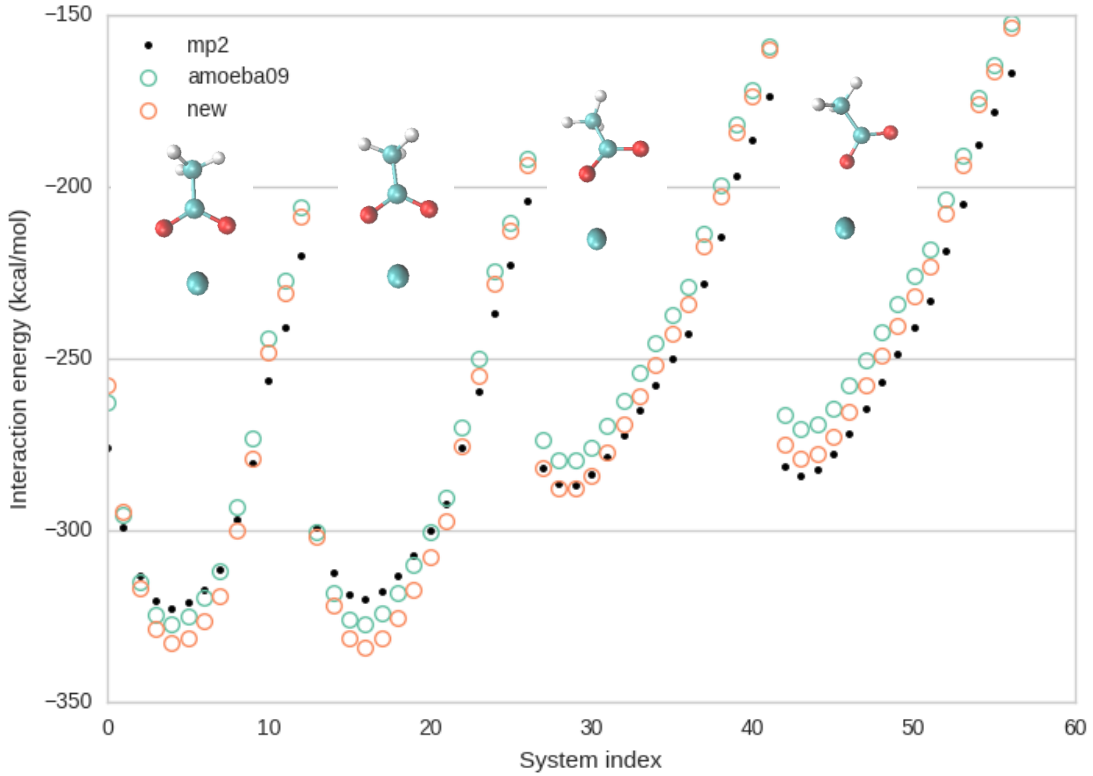
- Acid-water interaction





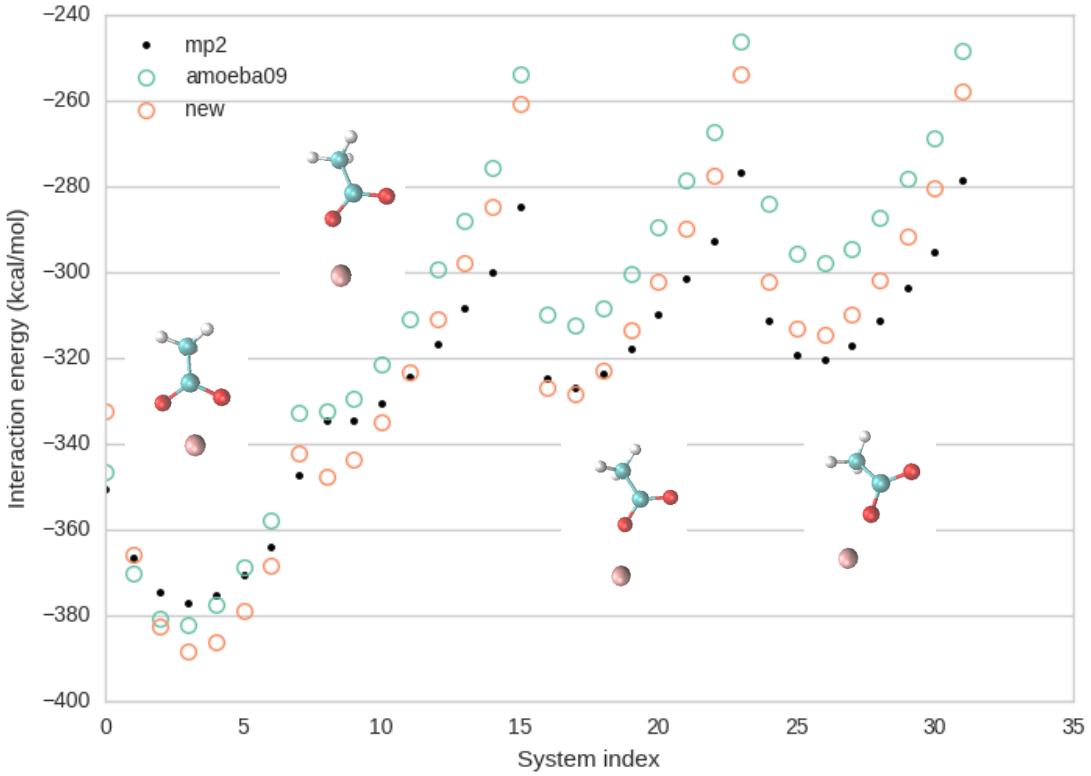
Acid parameters

- Ca^{2+}-acid interaction
  - C vdw



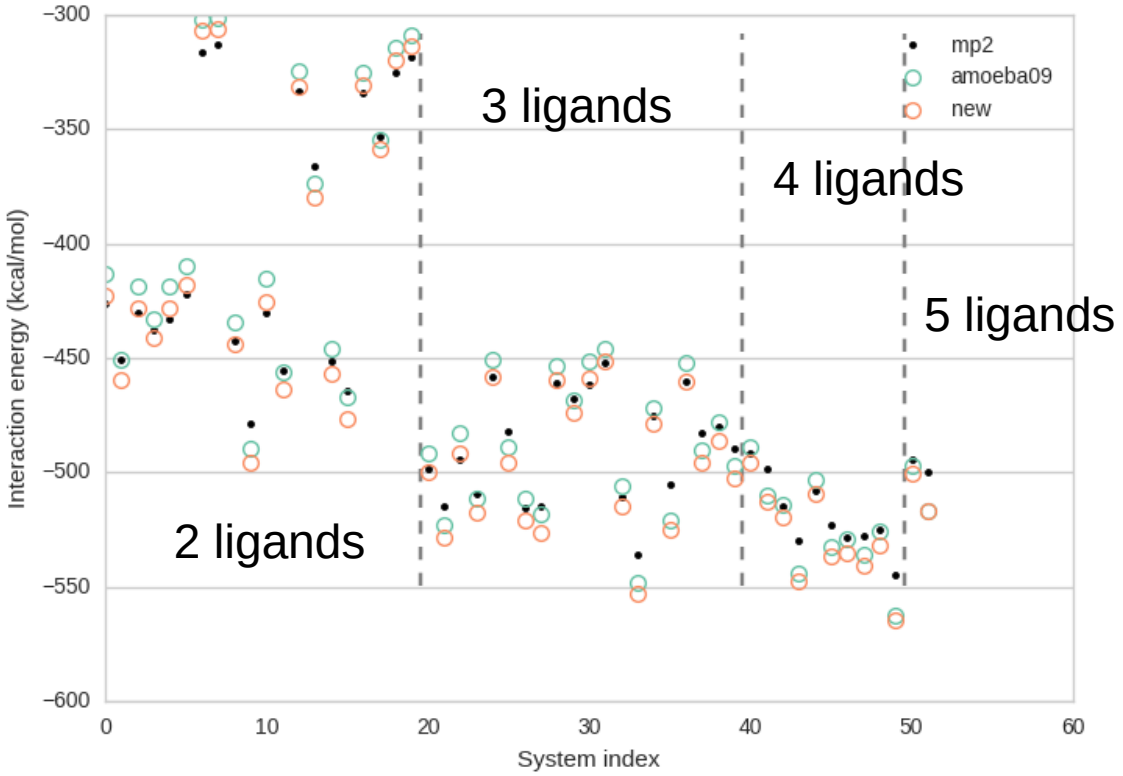
Acid parameters

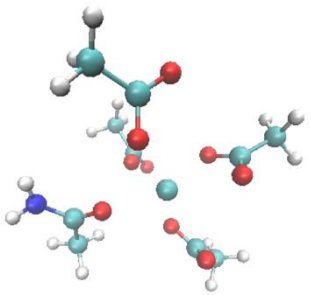
- Mg^{2+}-acid interaction
  - C vdw



Acid parameters

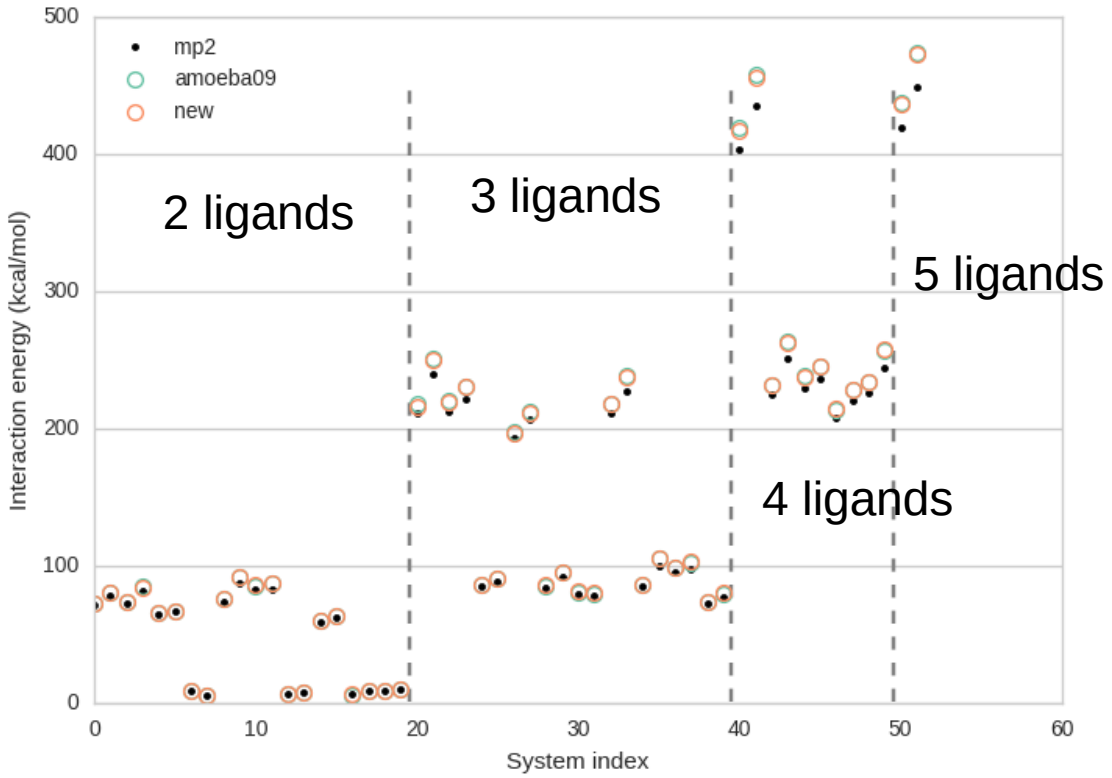
- Ca^{2+}-acid many body interaction
  - polarizability





Acid parameters

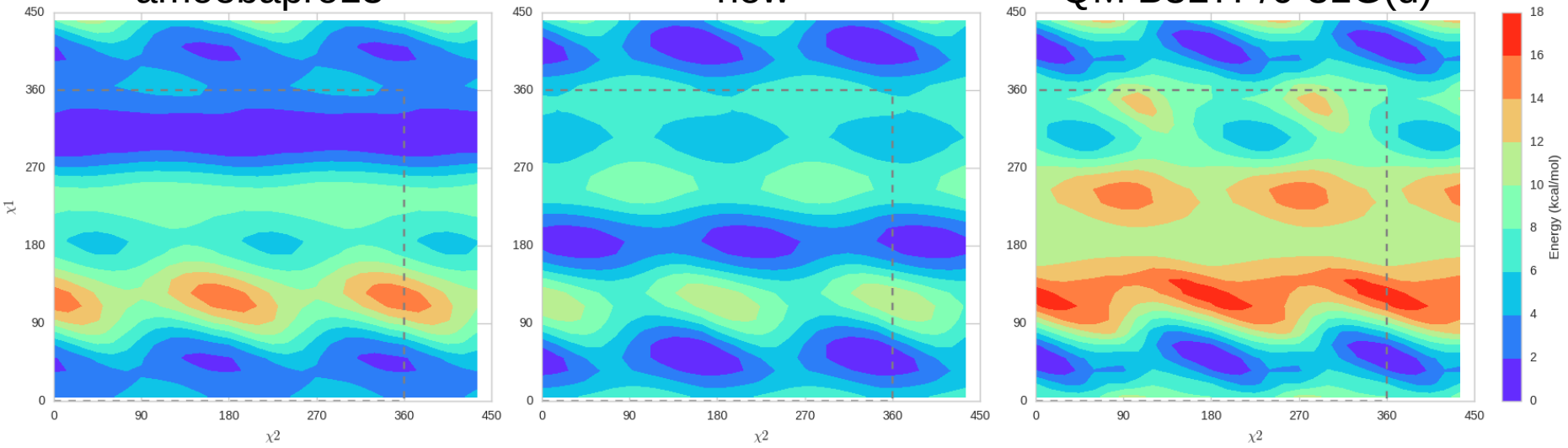
- Acid-acid many body interaction



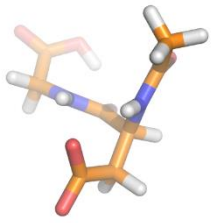
Torsion parameters

- Gly-Asp-Ace alpha-helix conformation

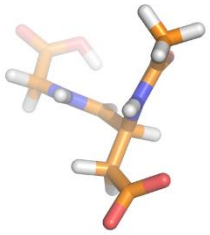
amoebapro13 | new | QM B3LYP/6-31G(d)



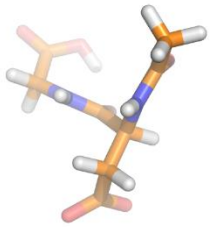
χ1, χ2



50, 165



320, 165

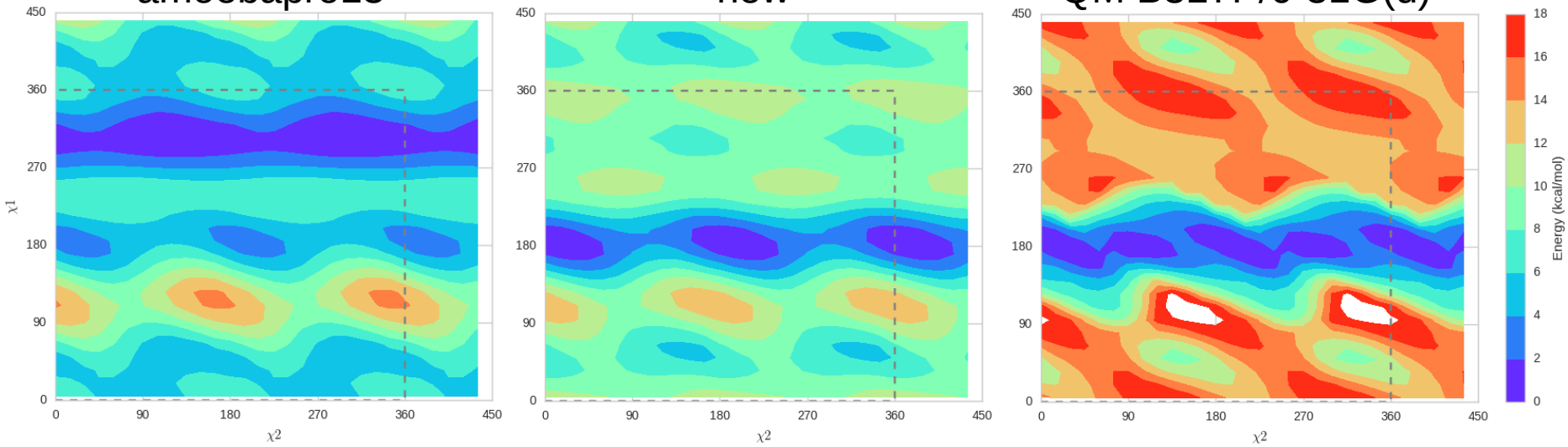


185, 225

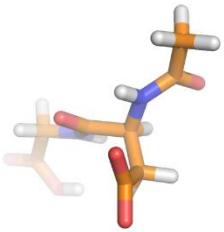
Torsion parameters

- Gly-Asp-Ace beta-sheet conformation

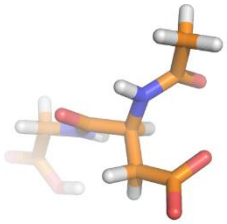
amoebapro13 | new | QM B3LYP/6-31G(d)



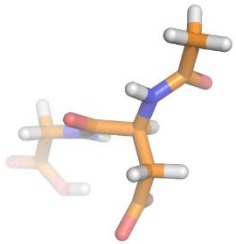
χ1, χ2



50, 165



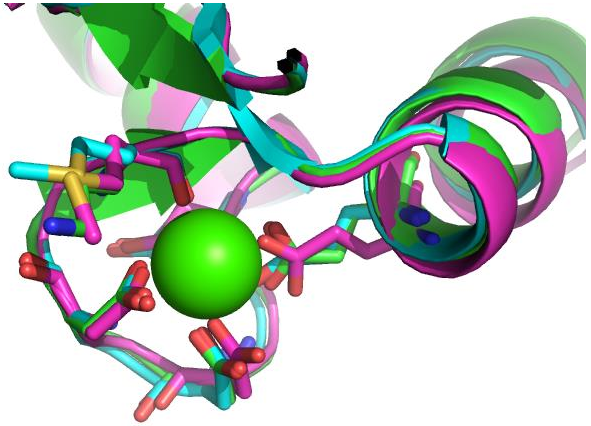
320, 165



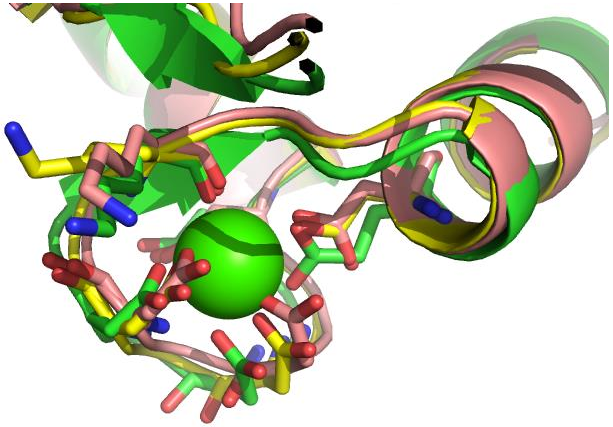
185, 225

MD simulations

- PDB structures



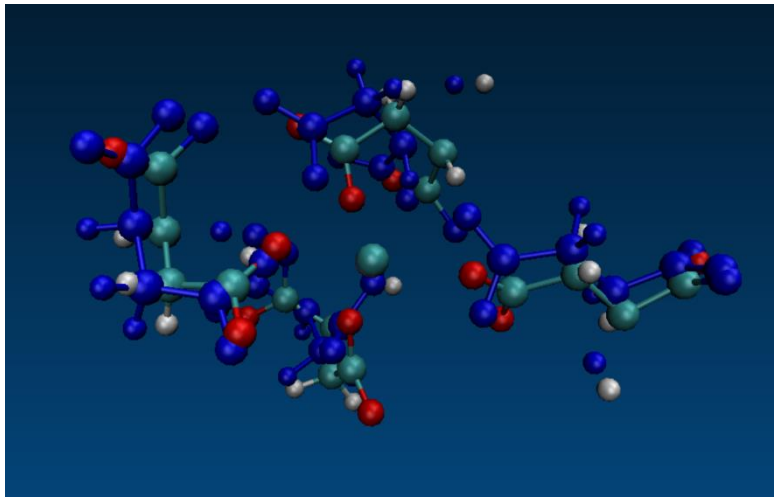
Green: WT PV-Ca^{2+}
Cyan: Pike PV-Ca^{2+}
Magenta: Pike PV-Mg^{2+}



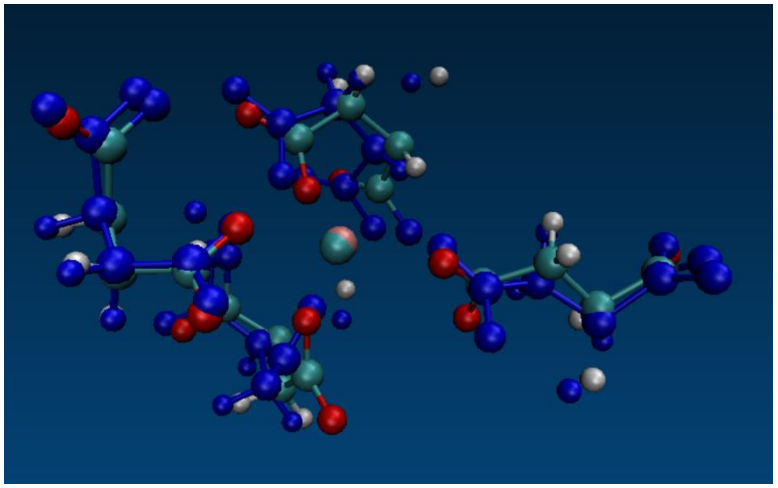
Green: WT PV-Ca^{2+}
Yellow: E101D PV-Ca^{2+}
Pink: E101D PV-Mg^{2+}

MD simulations

- Mutant-Ca^{2+}
  - Coordination number is reproduced with new parameter



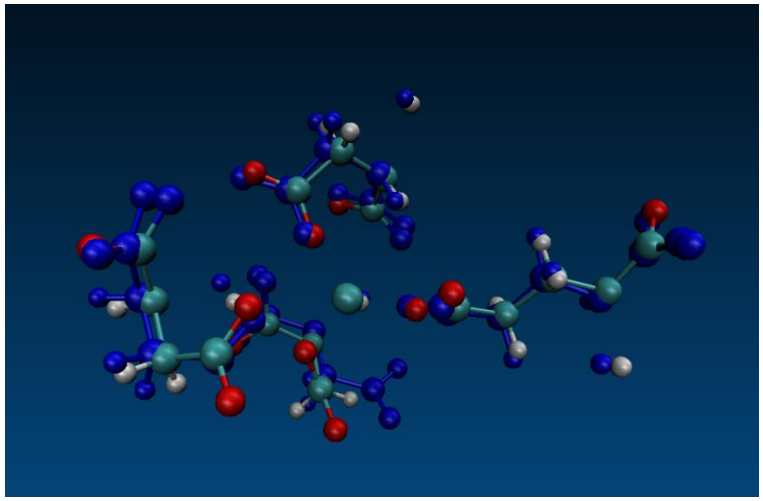
PDB (green) and simulation with amoeba09 (blue)



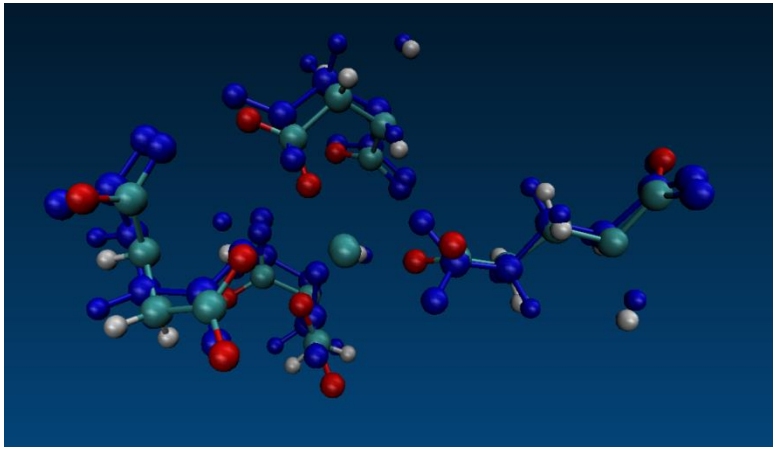
PDB (green) and simulation with new parameters (blue)

MD simulations

- WT-Ca^{2+}
  - Coordination number is reproduced with new parameter



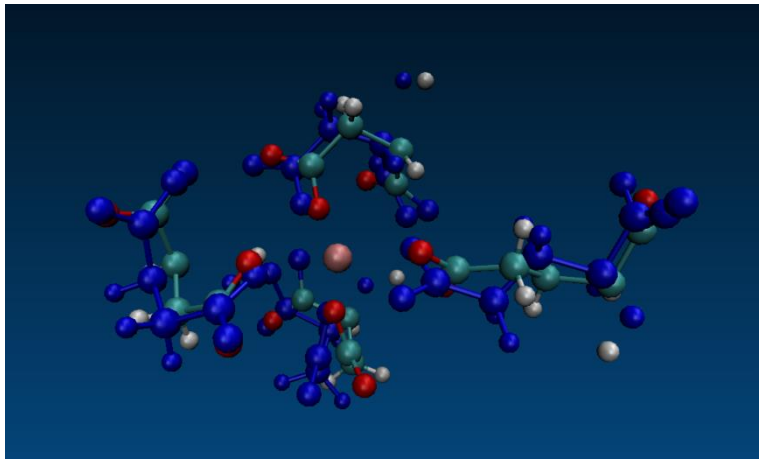
PDB (green) and simulation with amoeba09 (blue)



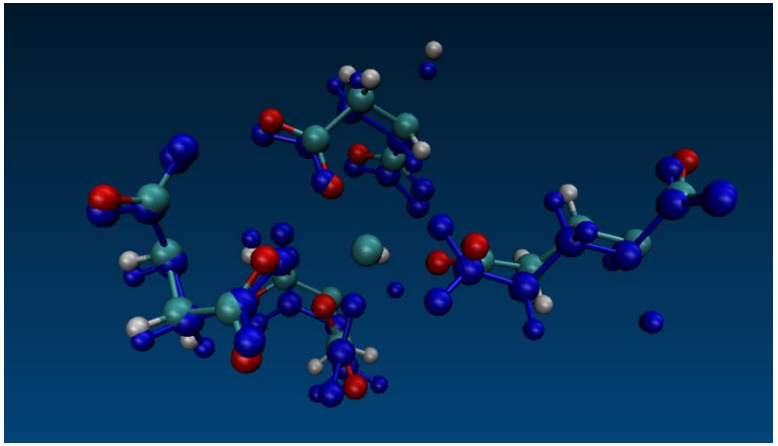
PDB (green) and simulation with new parameters (blue)

MD simulations

- WT and Pike-Mg^{2+}
  - Coordination number changes from 6 to 7



Pike. PDB (green) and simulation with new parameter (blue)



WT. Ca^{2+} PDB (green) and Mg^{2+} simulation with new parameters (blue)

Binding free energy

- Mg/Ca relative binding free energy
- Acid-ion interaction mainly influences enthalpy, while torsion can have influence in entropy (in the case of mutant)

WT				
Mg→Ca (kcal/mol)	Expt	amoeba09	09+acid	09+acid+tors
ΔΔG	-5.5	0.7	3.7	3.1
ΔG		74.5	77.5	76.9
ΔH		89.7	101.5	101.0
-TΔS		-15.2	-24.0	-24.1
Mutant				
Mg→Ca (kcal/mol)	Expt	amoeba09	09+acid	09+acid+tors
ΔΔG	-1.4	-6.5	-1.7	3.7
ΔG		67.2	72.1	77.5
ΔH		77.0	81.3	79.0
-TΔS		-9.7	-9.2	-1.5

Summary and future work

- Summary
  - The coordination between ion and protein is determined by a delicate balance of torsion restraint and ion-ligand interaction
  - Enthalpy contribution dominates the Mg^{2+}/Ca^{2+} relative binding free energy, while entropy also plays a role in determining the specificity

- Future work
  - Optimize Glu side chain torsion parameters based on QM energy and torsional free energy

Torsion

- Parameters

amoebapro13. RMSE 6.5 kcal/mol

a	b	c	d	k1	k2	k3
1	7	8	30	-4.15	2.060	0
3	7	8	30	-2.45	1.688	0
7	8	30	31	0	1.400	0

New param. RMSE 3.2 kcal/mol

a	b	c	d	k1	k2	k3
1	7	8	30	-0.46	2.520	0
3	7	8	30	-8.55	-0.902	0
7	8	30	31	0	1.940	0