



Supplementary Information for

Many-body effect determines the selectivity for Ca^{2+} and Mg^{2+} in proteins

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Supplementary Information Text

Estimation of binding free energy from QM interaction energies

The calculation of $\Delta\Delta G_{\text{bind}}^{\text{calc}} = \Delta\Delta E_{\text{int}} - \Delta\Delta G_{\text{solv}}^{\text{expt}}$ in **Table 1** in the main text used the experimental hydration free energies. The results will be different if QM interaction energy is used. The difference between ion-water cluster (6 water molecules for Mg^{2+} and 7 for Ca^{2+} , see **Figure S1**) interaction energies calculated by RI-MP2/def2-QZVPPD is 72.3 kcal/mol, compared to the difference for experimental hydration free energies of 77.8 kcal/mol.⁵⁰ Since the purpose of **Table 1** is mainly to illustrate the importance of many-body effect, the conclusion will not be affected.

Influence of binding pocket geometry

We also considered the influence of geometry change of the binding pocket, by calculating the energies of Mg^{2+} complexes at Ca^{2+} geometries (**Table S1**). Comparing the energies of Mg^{2+} and Ca^{2+} complexes at the Ca^{2+} geometry, it is evident that the many-body energy strongly favors Ca^{2+} . The relaxation from Ca^{2+} geometry to Mg^{2+} geometry is accompanied by large gain in two-body energy and high cost from many-body energy. Without many-body interaction, the Mg^{2+} complex will be overly stabilized by the geometry relaxation.

Alternative simulations to study the polarization effect

In the results of “AMOEBA no polarization” in the main text, polarization was completely turned off. Additional simulations were performed where only the polarization associated with the metal ion was removed. This was done by setting strong damping parameter $a = 10^{-6}$ and small polarizability $\alpha = 0.01 \text{ \AA}^3$. The results are similar to those of non-polarizable simulations: the smaller ion is always favored and pockets with more negative charges have larger relative binding free energies.

Effect of polarization on binding pocket structure

Polarization is important for maintaining the structure of the binding pockets. In the crystal structure of 1ZOO, there is one Asp side chain that indirectly interacts with the metal ion through a bridging water molecule. The bridging water molecule stayed in the binding pocket in the polarizable simulations, but was quickly released in non-polarizable AMOEBA or AMBER simulations, bringing one more Asp to the first shell. The bridging water molecule is strongly polarized by $\text{Mg}^{2+}/\text{Ca}^{2+}$ and Asp, with a polarization energy of -9.6 kcal/mol (product of induced dipole and permanent electric field at the water) when bound to Ca^{2+} . The polarization energy of the water that only coordinates Ca^{2+} in EF-hand proteins ranges from -4.5 to -6.2 kcal/mol. Therefore, without polarization, the bridging water cannot be stabilized.

Methods

The torsion parameters of Asp χ_1 and χ_2 and Glu χ_1 and χ_3 were refined in this work based on QM calculation with implicit solvation. Special vdW parameters were used to model the strong H-bond between carboxylate and protein backbone. All these parameters have been included in the TINKER release at <https://github.com/jayponder/tinker>.



Figure S1. Structures of $\text{Mg}^{2+}(\text{H}_2\text{O})_6$ and $\text{Ca}^{2+}(\text{H}_2\text{O})_7$. The structures were optimized using B3LYP/6-311++G(2d,2p)/PCM. The ion-water interaction energies calculated by RI-MP2/def2-QZVPPD are -336.67 and -264.38 kcal/mol for Mg^{2+} and Ca^{2+} , respectively.

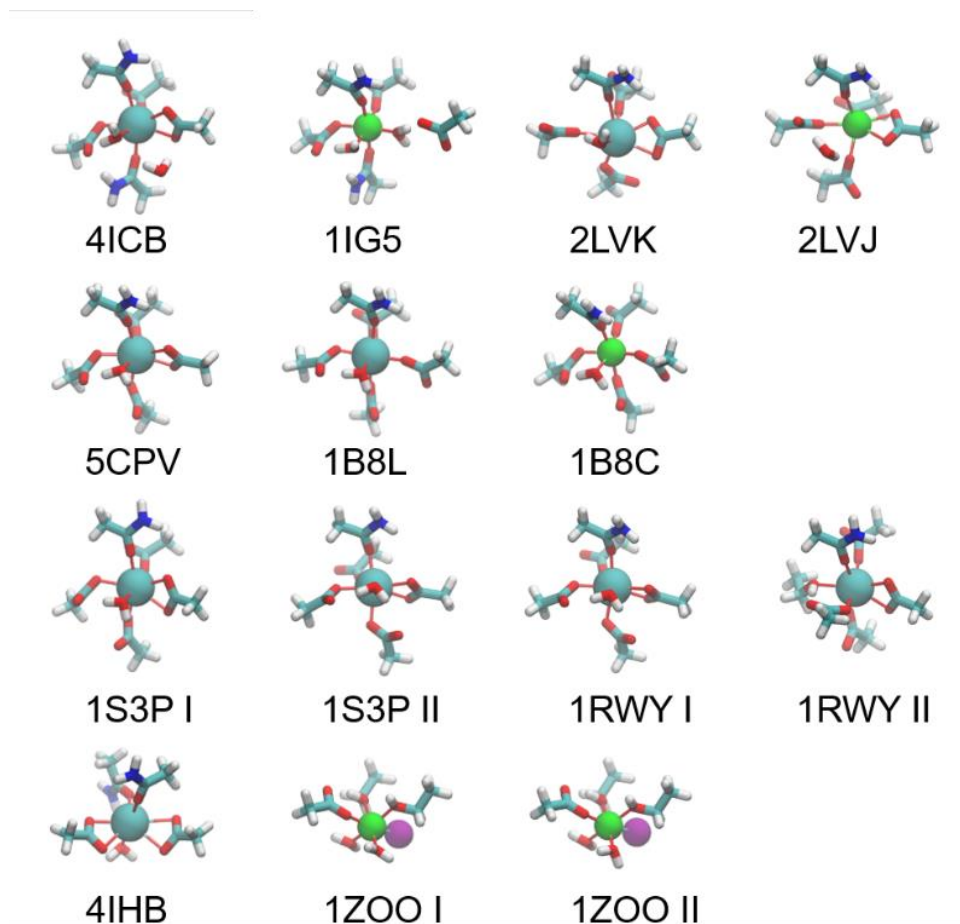


Figure S2. Structures of model compounds of protein-ion complexes. Ligands are represented by sticks, where C, N, O and H are colored by cyan, blue, red and white, respectively. Mg^{2+} , Ca^{2+} and Cl^- ions are represented by green, cyan and purple spheres.

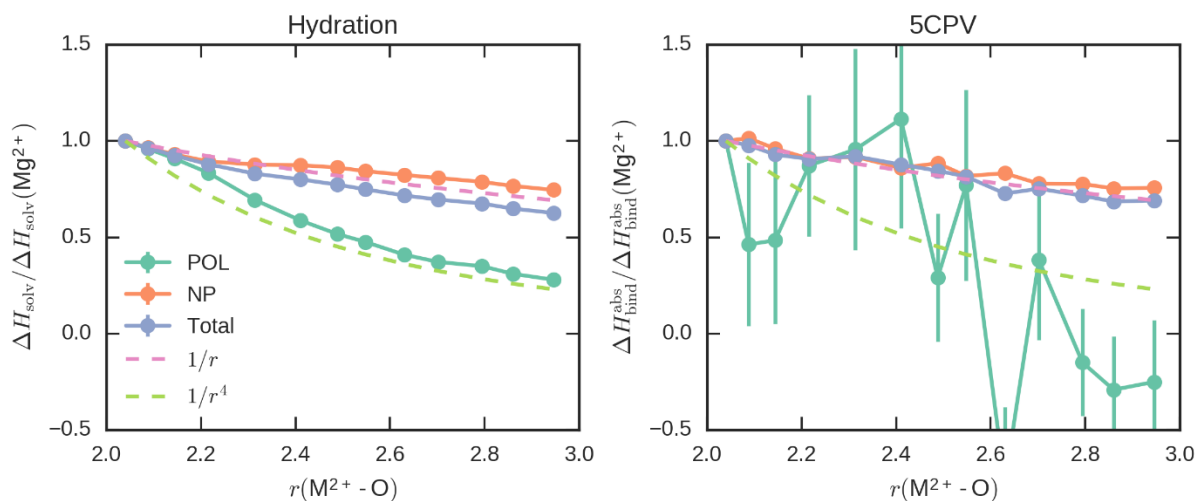


Figure S3. The relative change in hydration and absolute binding enthalpies as a function of ion size. $r(\text{M}^{2+} - \text{O})$ is the distance between the metal ion and first-shell water O in the hydration simulations. All ions have the same polarization parameter as Mg^{2+} but different vdW parameters. The polarization (POL) and permanent (PERM) components of hydration enthalpy decreases roughly as $1/r^4$ and $1/r$, respectively. The total hydration enthalpy decreases slightly faster than $1/r$. The absolute binding enthalpy in 5CPV overlaps with $1/r$, which decreases faster than the total hydration enthalpy. This is because the polarization constitutes a smaller portion in 5CPV binding compared to hydration (**Figure 5B** in main text). Although the polarization enthalpy for 5CPV suffers from large statistical uncertainties, it is clear that the polarization component decreases faster than the permanent component.

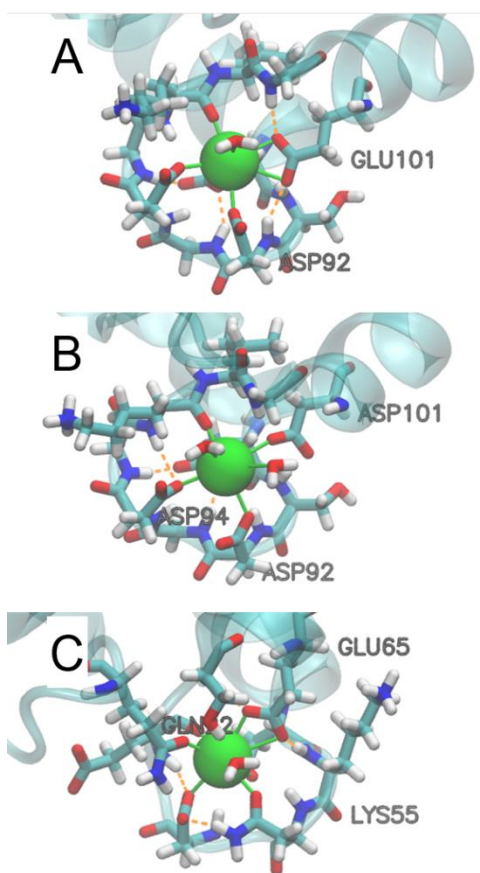


Figure S4. The structures of (A) 5CPV, (B) 1B8L and (C) 4ICB bound to the largest hypothetical ion with vdW diameter $\sigma = 4.5 \text{ \AA}$. The coordination numbers in (A-C) are 7, 7, and 8, respectively.

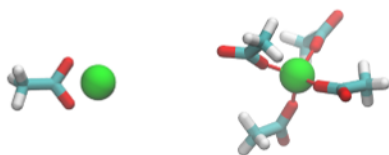


Figure S5. Structures of $\text{Mg}^{2+}\text{Ac}^-$ and $\text{Mg}^{2+}(\text{Ac}^-)_6$ optimized using AMOEBA. In $\text{Mg}^{2+}(\text{Ac}^-)_6$, the four acetates are monodentate ligands and forms a perfect tetrahedral geometry.

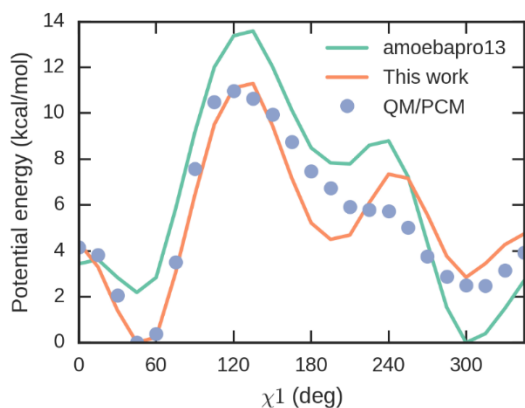


Figure S6. Derivation of torsion parameters for Asp χ_1 . The backbone torsions were fixed at the alpha-helix conformation and χ_2 was fixed at 0 deg. The QM/PCM calculations used wB97x-D/6-311++G(2d,2p)/PCM. AMOEBA calculations employed the GK implicit solvation method. The new parameters better capture the relative energy between the minima around $\chi_1 = 60$ and $\chi_1 = 300$.

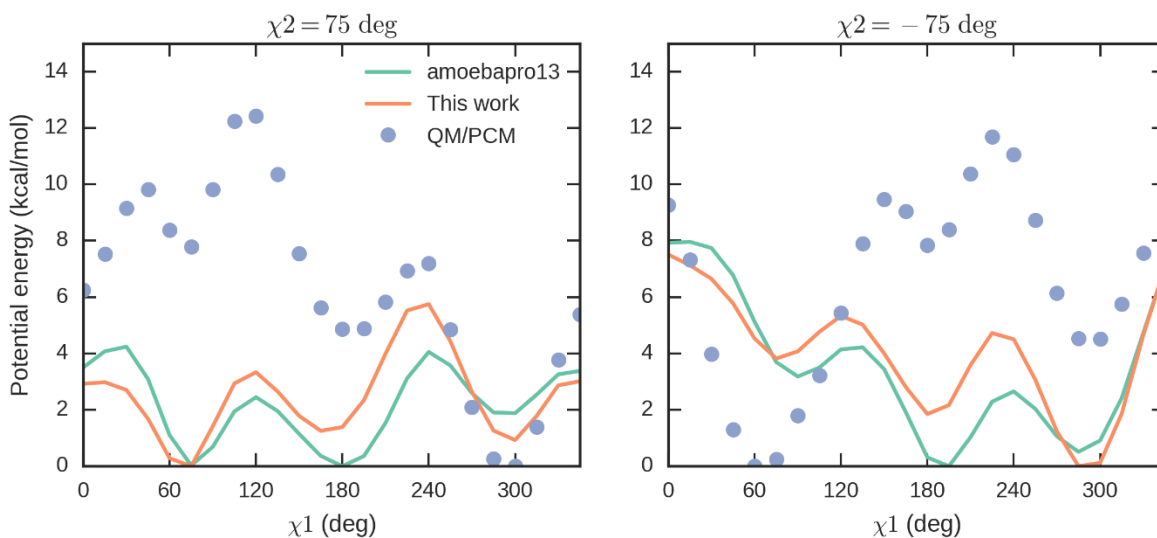


Figure S7. Derivation of torsion parameters for Glu χ_1 . The backbone torsions were fixed at the alpha-helix conformation and χ_3 was fixed at 0 deg. The QM/PCM calculations used wB97x-D/6-311++G(2d,2p)/PCM. AMOEBA calculations employed the GK implicit solvation method. The new parameters better capture the relative energy between the minima around $\chi_1 = 180$ and $\chi_1 = 300$. However, the energies around $\chi_1 = 60$ for different χ_2 angles cannot be captured simultaneously.

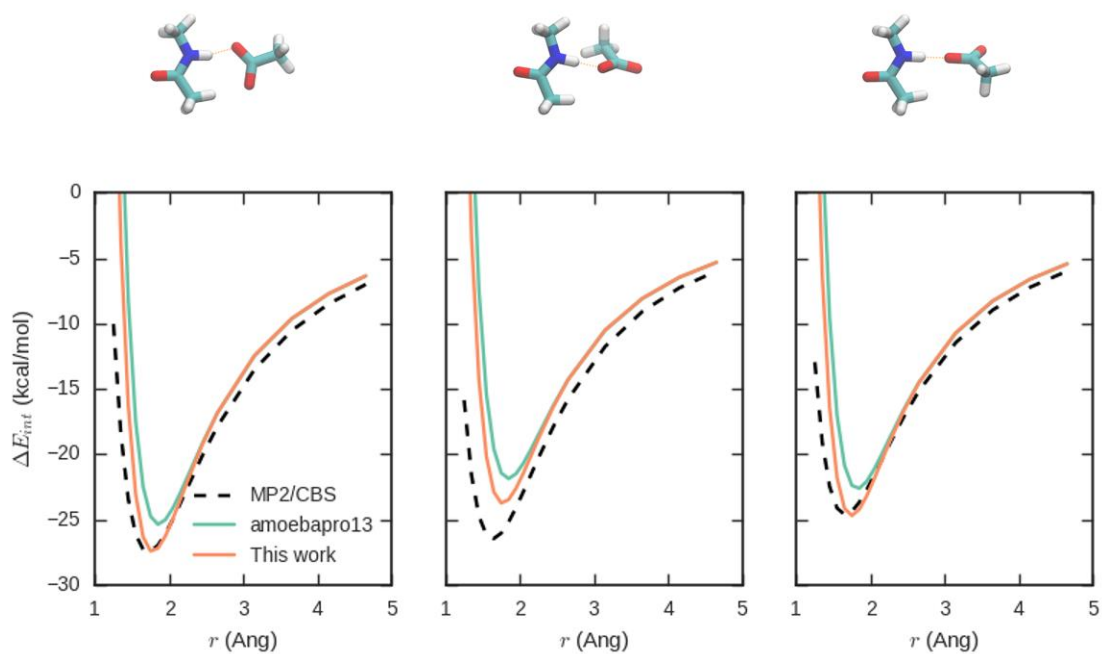


Figure S8. Interaction energy between acetate and N-methylacetamide. The MP2/CBS values were extrapolated from MP2/aug-cc-pVTZ and MP2/aug-cc-pVQZ. In this work, the vdW parameters between carboxylate O and the amide H were $\sigma = 3.10$ Angstrom and $\epsilon = 0.040$ kcal/mol.

Table S1. Energy differences (kcal/mol) between Mg^{2+} and Ca^{2+} complexes at the Ca^{2+} geometry. $\Delta\Delta E = \Delta E (\text{Ca}^{2+}) - \Delta E (\text{Mg}^{2+})$. 2B and MB are two-body and many-body energies, respectively. The definitions of all the terms are same as in **Table 1**.

PDB	Model compounds	$\Delta\Delta E_{2B}$	$\Delta\Delta E_{MB}$	$\Delta\Delta E_{\text{int}}$	$\Delta\Delta E_{2B} - \Delta\Delta G_{\text{hyd}}$	$\Delta\Delta E_{\text{int}} - \Delta\Delta G_{\text{hyd}}$
4ICB	$\text{Ac}_3\text{B}_2\text{W}_2$	111.2	-51.1	60.2	33.4	-17.6
2LVK	$\text{Ac}_4\text{B}_1\text{W}_1$	110.4	-66.9	43.6	32.6	-34.2
1B8L	$\text{Ac}_4\text{B}_1\text{W}_1$	79.3	-52.7	26.6	1.5	-51.2

Table S2. Interaction energy and polarization energy differences (kcal/mol) between Mg^{2+} and Ca^{2+} complexes calculated by AMOEBA. $\Delta\Delta E = \Delta E(\text{Ca}^{2+}) - \Delta E(\text{Mg}^{2+})$. As defined in **Table 1**, 2B and MB are two-body and many-body energies, respectively. NP and POL are nonpolarization and polarization energies, respectively.

PDB	Model compounds	$\Delta\Delta E_{2B, NP}$	$\Delta\Delta E_{2B, POL}$	$\Delta\Delta E_{2B}$	$\Delta\Delta E_{MB}$	$\Delta\Delta E_{int}$	$\Delta\Delta E_{int} - \Delta\Delta G_{hyd}$
4ICB	$\text{Ac}_3\text{B}_2\text{W}_2$	33.4	124.1	157.5	-99.3	58.2	-19.6
2LVK	$\text{Ac}_4\text{B}_1\text{W}_1$	29.3	115.1	144.4	-74.4	70.0	-7.8
1B8L	$\text{Ac}_4\text{B}_1\text{W}_1$	68.3	39.6	108.0	-33.6	74.4	-3.4

Table S3. Comparison of AMOEBA and MP2 interaction energies (kcal/mol).

	Ion	Many-body energy		Total interaction energy	
		MP2	AMOEBA	MP2	AMOEBA
4IHB	Ca	142.67	111.66	-540.29	-532.26
1ZOO I	Mg	248.37	224.82	-638.48	-621.85
1ZOO II	Mg	246.57	220.93	-635.16	-620.62
1S3P	Ca	245.20	210.01	-540.22	-534.62
1S3P	Ca	252.40	217.67	-548.20	-540.43
1RWY I	Ca	268.71	233.53	-554.38	-544.76
1RWY II	Ca	248.33	215.01	-545.28	-540.03
5CPV	Ca	252.09	215.06	-544.45	-536.36
1B8L	Ca	260.87	224.24	-539.82	-537.64
1B8C	Mg	364.22	323.58	-616.78	-595.84
1IG5	Mg	311.59	256.20	-651.00	-650.33
4ICB	Ca	220.73	181.84	-589.29	-580.28
2LVJ	Mg	313.02	233.39	-596.12	-589.31
2LVK	Ca	239.88	199.81	-525.01	-514.94

Table S4. Comparison between experimental and calculated relative free energies $\Delta\Delta G_{\text{bind}}(\text{Mg} \rightarrow \text{Ca})$ and calculated absolute binding free energies of proteins and acetate (in kcal/mol). The standard deviations of all calculated results are 0.2 kcal/mol. The AMBER/MDEC results do not include the electric continuum contribution, which can be chosen to reproduce the experimental hydration free energy.

	Expt.	$\Delta\Delta G_{\text{bind}}(\text{Mg}^{2+} \rightarrow \text{Ca}^{2+})$				AMBER	AMBER/MDEC
		AMOEBA	AMOEBA without polarization	AMOEBA without polarization on/by ion			
5CPV	-5.6	-8.3	9.8	24.5	11.3	-4.4	
1B8L	-1.6	-3.1	10.4	27.9	12.4	-0.6	
4ICB	-6.2	-7.2	7.1	18.6	8.5	-4.4	
2CHE	-0.5	-1.8	6.7	19.2	9.7	-1.0	
4IHB	-1.7	-3.4	4.6	20.0	8.4	-0.4	
1ZOO	1.7	-0.2	4.8	12.6	-2.8	-2.8	
Acetate	0.1	0.2	3.4	6.9			

	Expt.	$\Delta\Delta G_{\text{bind}}^{\text{abs}}(\text{Mg}^{2+} \rightarrow \text{Ca}^{2+})$ or $\Delta\Delta G_{\text{solv}}(\text{Mg}^{2+} \rightarrow \text{Ca}^{2+})$				AMBER	AMBER/MDEC
		AMOEBA	AMOEBA without polarization	AMOEBA without polarization on/by ion			
Hydration	77.8	77.7	39.5	41.1	77.6	45.2	
5CPV		69.5	49.3	65.6	88.8	40.7	
1B8L		74.7	49.8	69.0	89.9	44.6	
4ICB		70.5	46.7	59.7	86.1	40.7	
2CHE		75.9	46.1	60.3	87.3	44.2	
4IHB		74.4	44.1	63.1	86.0	44.8	
1ZOO		78.0	44.3	53.7	74.7	42.3	
Acetate		77.9	42.9	48.0			

Table S5. Enthalpy and entropy contributions to absolute relative binding free energy and relative hydration free energy (kcal/mol). $\Delta\Delta H_{\text{POL}}^{\text{abs}}$ is the relative polarization enthalpy between Mg^{2+} and Ca^{2+} , and $\Delta\Delta H_{\text{NP}}^{\text{abs}}$ is the remaining additive contribution to the relative binding enthalpy.

	$\Delta\Delta H^{\text{abs}}$	$\Delta\Delta H_{\text{POL}}^{\text{abs}}$	$\Delta\Delta H_{\text{PERM}}^{\text{abs}}$	$\sigma(\Delta\Delta H^{\text{abs}})$	$\sigma(\Delta\Delta H_{\text{POL}}^{\text{abs}})$	$\sigma(\Delta\Delta H_{\text{PERM}}^{\text{abs}})$
5CPV	69.23	26.09	43.15	6.49	7.15	3.14
1B8L	92.81	46.84	45.97	5.56	6.56	3.33
4ICB	64.74	38.66	26.08	6.00	6.65	3.50
2CHE	72.70	56.63	16.08	8.34	8.59	5.45
4IHB	67.75	30.75	37.00	13.80	15.26	7.72
1ZOO	82.86	38.92	43.95	9.31	10.41	6.01
Acetate	82.67	38.65	44.02	3.89	3.54	2.37
Hydration	84.50	51.64	32.85	1.81	1.83	1.34

Table S6. Relative binding free energies (kcal/mol) between different combination of vdW and polarization parameters. The damping length is defined by $\left(\frac{\alpha}{a}\right)^{\frac{1}{3}}$, where α is the polarizability and a is the damping parameter in AMOEBA. A longer damping length means weaker polarization. When vdW is fixed, weaker polarization (larger damping length) leads to higher free energy. When polarization parameter is fixed, larger vdW diameter leads to lower free energy.

Initial state		Final state		$\Delta\Delta G_{\text{bind}}$ (kcal/mol)						
vdW (Å)	Damping (Å)	vdW	Damping	5CPV	1B8L	4ICB	2CHE	4IHB	1ZOO	Acetate
2.90	1.39	2.90	1.65	1.3	2.3	2.0	2.5	4.0	0.2	0.9
3.59	1.39	3.59	1.65	2.6	4.0	3.4	4.5	3.7	1.9	2.0
2.90	1.65	3.59	1.65	-9.6	-5.4	-9.2	-4.3	-7.3	0.1	-0.7
2.90	1.39	3.59	1.39	-10.8	-7.0	-10.6	-6.3	-7.1	-1.6	-1.8

Table S7. Parameters and properties artificial ions used in simulations. σ and ϵ are the vdW radius and well depth. α is the polarizability. a is the dimensionless damping parameter. The hydration radius r_{hyd} is calculated by the position of the first M-O peak in RDF minus 1.34 Å. For comparison, the experimental ion radius of Mg^{2+} , Ca^{2+} , Sr^{2+} , Ba^{2+} are 0.72, 1.00, 1.18 and 1.35 Å, respectively. n_{coord} is the coordination number. The errors for the solvation free energies are about 0.2 kcal/mol.

σ (Å)	ϵ (kcal/mol)	α (Å ³)	a	r_{hyd} (Å)	$\langle n_{\text{coord}} \rangle$	ΔG_{solv} (kcal/mol)
2.90	0.28	0.08	0.115	0.704	6.0	-434.8
3.25	0.32	0.08	0.115	0.812	6.0	-399.3
3.59	0.35	0.08	0.115	0.976	6.6	-361.2
3.81	0.37	0.08	0.115	1.134	7.5	-337.2
4.03	0.40	0.08	0.115	1.290	8.1	-313.8
4.26	0.42	0.08	0.115	1.436	8.7	-290.8
4.45	0.44	0.08	0.115	1.582	9.3	-272.9

Table S8. Torsion parameters used in this work. The unit of force constant is kcal/mol.

Torsion	k_1	k_2	k_3
Asp N-CA-CB-CG	-2.900	1.800	0.000
Asp C-CA-CB-CG	-6.950	-1.150	0.000
Asp CA-CB-CG-OD	0.000	1.700	0.000
Glu N-CA-CB-CG	-2.280	0.970	3.700
Glu C-CA-CB-CG	0.160	1.655	-2.520
Glu CB-CG-CD-OE	0.000	1.460	0.000