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Thiol Versus Hydroxamate as Zinc Binding Group In HDAC Inhibition: An Ab Initio QM/MM Molecular Dynamics Study

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Abstract

Zinc-dependent histone deacetylases (HDACs) play a critical role in transcriptional repression and gene silencing, and are among the most attractive targets for the development of new therapeutics against cancer and various other diseases. Two HDAC inhibitors have been approved by FDA as anti-cancer drugs: one is SAHA whose hydroxamate is directly bound to zinc, the other is FK228 whose active form may use thiol as the zinc binding group. In spite of extensive studies, it remains to be ambiguous regarding how thiol and hydroxamate are bound to the zinc active site of HDACs. In this work, our computational approaches center on Born-Oppenheimer ab initio quantum mechanical/molecular mechanical (QM/MM) molecular dynamics with umbrella sampling, which allow for modeling of the zinc active site with reasonable accuracy while properly including dynamics and effects of protein environment. Meanwhile, an improved short-long effective function (SLEF2) to describe non-bonded interactions between zinc and other atoms has been employed in initial MM equilibrations. Our ab initio QM/MM MD simulations have confirmed that hydroxamate is neutral when it is bound to HDAC8, and found that thiol is deprotonated when directly bound to zinc in the HDAC active site. By comparing thiol and hydroxamate, our results elucidated the differences in their binding environment in the HDAC active sites, and emphasized the importance of the linker design to achieve more specific binding towards class IIa HDACs.

1. Introduction

Histone deacetylase enzymes (HDACs), which are responsible for the removal of acetyl groups from acetyl-lysine residues of histone tails, play important roles in regulating many vital cellular functions.^[1–3] Eleven isoforms of zinc-dependent HDACs have been identified in human cells. Their malfunctioning or aberrant over-expression has been mechanistically linked to a variety of diseases, most notably cancer, and accordingly the inhibition of HDAC

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Supporting Information Available: Figures S1–S6. Illustration of our overall computational protocol to simulate enzyme reactions; Molecular Structures of SAHA and FK228; The detailed QM/MM partition scheme for small system and large system of HDAC8-SAHA complex and HDAC4-SAHA complex; Detailed structure for the free energy profile of transition between HIP143 and HIP142 state in HDAC8_S_SAHA complex; Complete free energy profile for the transition between HIP158 and HIP159 state in HDAC4 system; Complete free energy profile for the transition between negative S_SAHA and neutral S_SAHA state; Details of SLEF2 force field parameterization and test.

has emerged as one very promising strategy for the development of new therapeutics.^[4–12] Unlike zinc proteases, such as thermolysin, whose coordination shell only contains one negatively charged amino acid residue, the coordination shell of the catalytic zinc in HDACs includes two negatively Asp residues as well as a neutral histidine residue. Meanwhile, there are two His-Asp dyads nearby that serve important catalytic roles. Based on ab initio QM/MM simulations, we have found that HDAC does not employ the general-acid-base pair mechanism like zinc proteases,^[13,14] instead it uses a proton-shuttle catalytic mechanism similar to serine proteases.^[15] Under the guidance of our characterized HDAC catalytic mechanism, a new class of boronic acid-based HDAC inhibitors have been successfully designed and developed, and very recently we have discovered the first HDAC2-selective inhibitor, β -hydroxymethyl chalcone.^[16]

A well established pharmacophore model for inhibitors of zinc-dependent HDACs consists of a chelator as the zinc binding group, a linker occupying the binding channel, and a cap interacting with the rim region of the pockets. FDA has recently approved two broad spectrum HDAC inhibitors as anticancer drugs: one is SAHA, suberoylanilide hydroxamic acid (vorinostat; Merck Research Laboratories) whose head-group is directly bound to zinc;^[4,17] and the other is FK228, a natural product bicyclic depsipeptide (romidepsin; Cloucester Pharmaceuticals) whose active form is its glutathione reduced disulphdryl metabolite and may use thiol as zinc binding group.^[18] Molecular structures of SAHA and FK228 are shown in Figure S2. In spite of extensive studies, significant questions remain regarding how hydroxamate and thiol are bound to the zinc active site of HDACs in the first place. For SAHA, it is known to be in the neutral state in aqueous environment, and the controversy is whether it would be deprotonated when it is bound to zinc active site of HDACs.^[19,20] For thiol based inhibitors, besides the question about its protonation state, the previous modeling study suggested that sulfur is indirectly bound to zinc through a water molecule^[18], while the more recent structural studies of the largazole-HDAC8 complex indicated sulfur is directly bound to zinc.^[21–23]

In order to elucidate difference as well as similarity between hydroxamate and thiol in their binding to the zinc active site of HDACs, we have carried out Born-Oppenheimer ab initio QM/MM molecular dynamics simulations with umbrella sampling to comparatively characterize binding mechanisms of SAHA and thiol-based SAHA towards HDAC8 and HDAC4, respectively. HDAC8 has been the best characterized structurally among class I HDACs, and is the only isoform whose over-expression in neuroblastoma is correlated with the advanced disease stage.^[9,10] HDAC4 is the best experimentally characterized among class IIa HDACs, and recently there is significant interest in the development of class IIa specific HDAC inhibitors.^[24] Our simulation results demonstrated that hydroxamate is neutral when it is bound to HDAC8, and found that thiol is deprotonated when directly bound to zinc in the HDAC active site. By comparing thiol and hydroxamate, we have elucidated the differences in their binding environment in the HDAC active sites, and emphasized the importance of the link design to achieve more specific binding towards class IIa HDACs.

2. Computational methods

One key obstacle to reliably simulate zinc-dependent HDACs comes from the well known challenge in describing zinc ligand interactions although many models have been built^[25–36]. Our theoretical approaches will center on Born-Oppenheimer ab initio QM/MM molecular dynamics simulations, which allow for modeling of the chemistry at the enzyme active site with reasonable accuracy while properly including dynamics and effects of protein environment. Our overall computational protocol to determine the free energy profile of a specific chemical reaction in a biological system is summarized in Figure S1. First, each reactant system is prepared based on the crystal structure and molecular modeling, and the prepared system is equilibrated by extensive MD simulations with periodic boundary conditions. With an equilibrated MD snapshot, the QM/MM model is prepared, minimized and then employed to map out a reaction path given a chosen reaction coordinate. For each determined structure along the path, long MD simulation with MM force field is performed to equilibrate the MM subsystem with the QM subsystem being frozen. Finally, the resulting snapshot is used as the starting structure for ab initio QM/MM MD simulation with umbrella sampling,^[37,38] in which both QM and MM sub-systems are allowed to move simultaneously. From these biased simulations, the free energy profile for each reaction is obtained with the weighted histogram analysis method (WHAM).^[39–41] This computational protocol has been successfully applied to study a number of biological systems, and some predications have been subsequently confirmed by experimental studies.^[15,42–54]

Meanwhile, recently we introduced a short-long effective function^[49] (SLEF) to describe charge interactions between the zinc ion and all other atoms. In this work, first each initial HDAC-inhibitor model is prepared based on the crystal structure and solvated with explicit water molecules; then each prepared system is equilibrated with classical molecular dynamics simulations with the nonbonded SLEF2 force field for modeling the zinc ion and Amber99SB^[55–57]/GAFF^[58]/TIP3P^[59] to describe protein/ligand/water respectively; and finally each equilibrated HDAC-inhibitor system is investigated by a series of ab initio QM/MM simulations. In the following, first we summarize the SLEF2 force field to describe zinc interactions, and then our detailed simulation protocol is described.

A. Nonbonded SLEF2 force field to model zinc interactions

The SLEF2 function form to describe non-bonded interactions between zinc and other atoms is shown in the following equations:

$$E_{Total}^{i,j} \text{ (i or j=Zn)} = E_{es(SLEF)} + E_{vdw(LJ-12,6)} \quad (1)$$

$$\begin{aligned} E_{es(SLEF)} &= E_S + E_L = (c_S + c_L) \times E_{es(Coulomb)} \\ &= (c_S + c_L) \times \frac{1}{4\pi\epsilon_0} \times \frac{q_i q_j}{r_{ij}} \end{aligned} \quad (2)$$

$$c_s = \frac{1}{1 + \alpha \times \frac{(|q_i| + |q_j|)^2}{(R_i^* + R_j^*)^2} \times \exp(\beta \times r_{ij}^2)} \quad (3)$$

$$c_L = \frac{1}{1 + \frac{(|q_i| + |q_j|)}{q_{Zn}} \times \exp(1 - \lambda \times r_{ij})} \quad (4)$$

The electrostatic interaction term in Eq. 2 is the conventional Coulomb energy multiplied by a factor, which is the sum of the short-range coefficient C_S and the long-range coefficient C_L . C_S is dependent on the atomic charge q , the vdW radius R^* and the distance r of atom i and j . C_L is related to the atomic charge q and the distance r of atom i and j . This SLEF2 short-long effective function has the following attractive features: (1) It is pairwise, additive, and compatible with widely used atomic pairwise force fields for biomolecules modeling. (2) It mostly changes the charge-charge interactions between Zn^{2+} and all other atoms, the vdW interactions between Zn^{2+} and the short-range atoms, but does not affect force field parameters that model other interactions in the system. (3) It is a non-bonded model that is inherently capable to describe different zinc ligands and coordination modes, and it would be easy to be implemented in typical biomolecular modeling suites.

In the SLEF2 force field, a total of four parameters: α (Eq. 3), β (Eq. 3), λ (Eq. 4), and the Zn's vdW radius R_{Zn}^* , have been optimized by employing ab initio QM/MM force matching. The optimized parameters of the SLEF2 force field are presented in Table 1. The detailed fitting procedure is presented in the supporting information.

We have implemented these into Amber suite of program, and carried out extensive MD simulations for a total of 16 zinc-containing proteins (26 zinc coordination shell), including mono-Zn, unattached-bi-Zn, attached-bi-Zn and attached-tri-Zn proteins, as shown in Table S1. It shows that the zinc coordination numbers yielded via long-time MD simulations with the amber99SB-SLEF2 force field are in reasonable agreement with the corresponding crystal structures.

B. System preparation and Equilibration

The initial structures of the enzyme-inhibitor complex were prepared on the basis of a crystal structure of the Y306F HDAC8 mutant-substrate complex (pdb code: 2V5W^[60]) and a crystal structure of HDAC4 (pdb code: 2VQJ^[61]). First, the mutated residue of HDAC8 was changed back into the wild type one with the Swiss-PdbViewer.^[62] Then the modified 2V5W template was superimposed with SAHA-HDAC8 complex (pdb code: 1T69^[63]). The substrate of 2V5W is replaced by SAHA. For the thiol-based SAHA (S_SAHA), the hydroxamate group is replaced by a sulfur atom. Then the protonation states of charged residues were determined via H++ program^[64] by carefully examining their individual local environment. Each prepared system was neutralized by adding Sodium ion, and was solvated into a rectangular box with a 10 Å buffer distance between the solvent box wall and the nearest protein atoms with AMBER tools. Each system consists of ~50 000 atoms. By employing AMBER11 molecular dynamics package,^[65] they are first minimized and

equilibrated, and then simulated for 5 ns with periodic boundary condition, temperature at 300 K, and pressure at 1 atm. The trajectory was stable, and the resulted snapshot was employed for subsequent QM/MM studies. The Amber99SB^[55–57] force field for the protein and TIP3P model^[59] for water molecules were employed, and the force field parameters for ligands was generated from AMBER GAFF force field^[58] by AMBER tools. The zinc ion was modeled with the SLEF2 force field.

C. *ab initio* QM/MM Simulation

With the snapshot taken from the classical MD trajectory, the QM/MM model was prepared by deleting solvent molecules beyond 25 Å of the zinc atom. The resulting system had ~12 000 atoms in total, and it was divided into QM and MM subsystems. The QM subsystem, including the side chains of two HIS interacting with the ligand, 2ASP and 1 HIS coordinating to zinc atom, substrate, and zinc ion and any water between the two HIS and the ligand. (small QM sub-system, see Figure S3 in Supporting Information). The QM region was treated by the B3LYP functional with Stuttgart ECP/basis set57 (SDD) for the zinc atom and 6-31G* basis set for all other QM atoms. This B3LYP(SDD,6-31G*) level of QM treatment has been previously tested and employed successfully to describe zinc coordination shell,^[14,66–70] and it is similar to that used in other contemporary *ab initio* QM/MM studies of Zinc enzymes.^[71,72] The QM/MM boundaries were described by the pseudobond approach.^[73–76] All other atoms were described by the same molecular mechanical force field used in previous classical MD simulations.

The prepared QM/MM system was first minimized, and then relaxed with the unrestraint QM/MM MD for 5ps. After that, the system was employed to map out a minimum energy path with the reaction coordinate driving method and B3LYP(SDD,6-31G*) QM/MM calculations. The reaction coordinate varies from system to system. For each determined structure along the reaction path scan, the MM subsystem was equilibrated with 500 ps molecular mechanical MD simulations with the QM subsystem frozen. Then, the resulting snapshots were used as starting structures for *ab initio* QM/MM MD simulations with umbrella sampling.^[37,38] Each window was simulated for 25 ps. The configurations after 5 ps for each window were collected for data analysis. The probability distributions along the reaction coordinate were determined for each window and put together with the WHAM^[39–41] to determine the free energy profile.

All our *ab initio* QM/MM calculations were performed with modified Q-Chem^[77] and Tinker^[78] programs. For all QM/MM calculations, the spherical boundary condition was applied and the atoms more than 22 Å away from the zinc atom were fixed. The 18 and 12 Å cutoffs were employed for electrostatic and van der Waals interactions, respectively. There was no cutoff for electrostatic interactions between QM and MM regions. The Beeman algorithm^[79] was used to integrate the Newton equations of motion with the time step of 1 fs, and the Berendsen thermostat method^[80] was employed to control the system temperature at 300 K.

3. Results and discussion

HDAC8-SAHA

SAHA (vorinostat; Merck Research Laboratories) is the first HDAC inhibitor that has been approved by FDA as an anti-cancer drug,^[4,17] while HDAC8 is the best characterized structurally among class I HDACs. SAHA is known to be in the neutral state in aqueous environment, but there is controversy regarding whether it would be deprotonated when it is bound to the zinc active site of HDAC8. Our previous ab initio QM/MM molecular dynamics simulations with umbrella sampling, in which the 1T69 crystal structure^[63] is used for building the initial model of HDAC8-SAHA complex and the QM sub-system does not include 2 Asp residues near the active site, have found that the free energy of the neutral SAHA state is 3.8 kcal/mol lower than the deprotonated SAHA state for the HDAC8-SAHA complex.^[19] This indicates that SAHA should be neutral when it is bound to HDAC8. However, recently Chen et al carried out ONIOM QM/MM minimization studies of the same system and discussed whether including two Asp residues in the QM sub-system or not would lead to different results.^[20] They conclude that including two Asp residues in the QM sub-system is necessary in QM/MM studies of HDAC, and SAHA is deprotonated when it is bound to HDAC8. It should be noted that Chen et al's simulation protocol has two main limitations: 1. Only a single starting structure has been employed for the ab initio QM/MM minimization study with atoms outside 2 Å of the active site frozen, and thus results can be very much dependent on the initial structure; 2. No water molecule is included in their QM/MM calculations of the HDAC8-SAHA complex system, and thus water solvation effect has not been accounted for. Thus here we are motivated to revisit the HDAC8-SAHA system.

In order to independently examine how the size of QM sub-system would affect the results of HDAC8-SAHA, we started from the crystal structure of 2V5W^[60], instead of 1T69^[63] that has been used in the previous work. In comparison with 1T69, the structure of 2V5W has a higher resolution, and contains 2 potassium ions and crystal water molecules which are not included in 1T69. We have carried out ab initio QM/MM MD simulations with umbrella sampling to determine free energy profiles for both small QM sub-system (does not including two ASPs) and large QM sub-system (including two ASPs), and have obtained two free energy profiles, as shown in Figure 1a and 1b. We can see that the new result agree with each other very well, and is also consistent with our published result that SAHA is more stable in the neutral state in its binding complex with HDAC8.^[19] Whether using large or small QM subsystems in QM/MM simulations, SAHA is found to be doubly coordinated to the zinc ion at its neutral state, which is consistent with the crystal structure studies. As shown in Figure 1, distance between zinc and O1 of SAHA is $2.37 \pm 0.15 \text{ \AA}$ in small system and $2.36 \pm 0.15 \text{ \AA}$ in large system, distance between zinc and O2 of SAHA is $2.17 \pm 0.11 \text{ \AA}$ in small system and $2.16 \pm 0.10 \text{ \AA}$. In crystal structure (pdb code 1T69), distance between zinc and O1 of SAHA is $1.95 \text{ \AA}$ and distance between zinc and O1 of SAHA is $1.97 \text{ \AA}$. Thus our results demonstrate that including two Asp residues in the QM sub-system is not necessary in QM/MM studies of HDAC, and this conclusion is similar as in the previous study of acetylcholinesterase.^[81] For the rest of QM/MM studies, small QM sub-systems have been employed.

HDAC8-S_SAHA

With the characterization and confirmation of HDAC8-SAHA binding mechanism, our next essential task is to elucidate how the thiol group would be bound to the active site of HDAC8 considering that this zinc binding group has been employed by another FDA approved drug FK228(romidepsin; Cloucester Pharmaceuticals) [18]. Here we have carried out a series of classical MD and ab initio QM/MM MD simulations of the HDAC8-S_SAHA complex.

Considering that the protonation state of S_SAHA in the HDAC8-S_SAHA is not clear and the proton of the thiol group can be transferred to either H142 or H143 if S_SAHA is deprotonated, we have prepared three possible initial structures: 1. the neutral S_SAHA system; 2. the HIP142 system in which H142 is doubly protonated while the S_SAHA is deprotonated; 3. the HIP143 system in which H143 is doubly protonated while the sulfur is deprotonated. For each prepared system, first classical MM minimization and 5 ns MD simulations were performed for equilibration and then ab initio QM/MM MD simulations were carried out. Among three systems, we have found that only the HIP143 system is stable during the 55 ps ab initio QM/MM MD simulations, while for the neutral S_SAHA system, the thiol group of S_SAHA deprotonated very quickly and the proton transferred to H143 during the ab initio QM/MM MD simulation. For the HIP142 system, the proton is found to spontaneously transfer from HIP142 to H143 through a water molecule, which leads to the HIP143 system. These three unrestrained ab initio QM/MM MD simulations suggest that in the HDAC8-S_SAHA binding complex, S_SAHA is deprotonated and H143 is doubly protonated. In order to further confirm that the HIP143 state is more stable than the other two, we have carried out ab initio QM/MM MD simulations with umbrella sampling to determine their free energy differences. From Figure 2, we can see that the HIP143 state is ~7kcal/mol more stable than the HIP142 state, while Figure 3 indicates an uphill free energy profile to transfer proton from HIP143 to S_SAHA. The HIP143 state (negative S_SAHA) is ~13 kcal/mol more stable than the neutral S_SAHA state. Thus, these free energy results are very consistent with unrestrained ab initio QM/MM MD simulations, and both indicate that S_SAHA is deprotonated when binding to zinc of HDAC8, with H143 doubly protonated and H142 singly protonated.

Figure 4a and 4b illustrates binding sites of HDAC8-SAHA and HAC8-S_SAHA respectively. We can see that there are three main differences between them: 1. SAHA is neutral while S_SAHA is negatively charged; 2. SAHA is doubly coordinated to zinc while S_SAHA is singly coordinated; 3. SAHA is directly hydrogen bonded to both H142 and H143, while there is a bridging water molecule between H142 and S_SAHA.

HDAC4-S_SAHA

After characterizing binding mechanism of HDAC8-S_SAHA, we have employed the similar computational protocol to the HDAC4-S_SAHA system, considering that there is significant interest to develop class IIa HDAC inhibitors and HDAC4 is the best experimentally characterized among class IIa enzymes.[24] The calculated free energy profiles from ab initio QM/MM MD simulations with umbrella sampling (See Figure S5 and S6 in supporting information), indicate that S_SAHA is also deprotonated when binding to

the zinc ion of HDAC4 with H158 doubly protonated and H159 singly protonated, which is similar to the binding mechanism of HDAC8-S_SAHA. Regarding their binding environment, the key difference is the number of water molecules near the zinc binding site as well as their hydrogen bonding network. As shown in Figure 5a, only one water molecule is inserted between H142 and sulfur atom in HDAC8, while in HDAC4, there are 3 waters around the sulfur atom, one water is inserted between 2 histidines and the sulfur atom (shown in Figure 5b). The structural origin for more water molecules in the zinc binding site of HDAC4 is that the binding channel of HDAC4 is much wider and shorter than that in HDAC8, as shown in Figure 6a and 6b. The radial distribution of water around the sulfur atom, as shown in Figure 7, comes to the same conclusion: there is one water molecule close to the sulfur of S_SAHA in HDAC8 while there are three in HDAC4. Considering that that Zn^{2+} has a saturated electronic configuration of d^{10} , its coordination with negatively charged sulfur atom would be dominated by electrostatic interactions and thus would be strengthened by the presence of less water molecules. The above results suggest that, for the development of more potent and specific HDAC-IIa inhibitors, a very promising direction would be to design a bulkier nonpolar linker component.

4. Conclusion

By employing Born-Oppenheimer ab initio QM/MM molecular dynamics simulations and umbrella sampling, we have computationally characterized three HDAC-inhibitor complexes: HDAC8-SAHA, HDAC8-S_SAHA and HDAC4-S_SAHA respectively. Our results have further confirmed that SAHA is neutral in complex with HDAC8, while for S_SAHA, it is negatively charged when directly bound to zinc in either HDAC8 or HDAC4. By comparing thiol and hydroxamate, the differences and similarities in their binding environment in the HDAC active sites have been elucidated. In comparison with HDAC8-S_SAHA, the binding channel of HDAC4-S_SAHA is significantly wider and shorter, and contains much more water molecules. This emphasizes the importance of the linker design to achieve more specific binding towards class IIa HDACs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

(a) Complete free energy profile for the deprotonation of SAHA determined by ab initio QM/MM MD simulation and umbrella sampling for small standard QM system (b) Complete free energy profile for the deprotonation of SAHA for enlarged system which include D176 and D183. ($d2 - d1$) was chosen as reaction coordinate. $d1$ is the distance between SAHA: H (on O1) and H142: N^ε. $d2$ is the distance between SAHA: O1 and SAHA: H (on O1). The statistical error is estimated by averaging the free energy difference between 5–15ps and 15–25ps.

Figure 2.

Complete free energy profile for the transition between HIP143 and HIP142 state in HDAC8 system determined by ab initio QM/MM MD simulation and umbrella sampling. The reaction coordinate is chosen as (d4–d3). d4 is the distance between H143: N^ε and H143: H (on N^ε), d3 is the distance between H143: H (on N^ε) and oxygen of the water molecule between H142 and S_SAHA. The statistical error is estimated by averaging the free energy difference between 5–15ps and 15–25ps. The detailed structural characterization is shown in Figure S4 of Supporting Information.

Figure 3. Complete free energy profile for the transition between negative S_SAHA and neutral S_SAHA state in HDAC8 system determined by ab initio QM/MM MD simulation and umbrella sampling. The reaction coordinate is chosen as $-d2$. $d2$ is distance between H143: H (on N^E) and S_SAHA: S1. The statistical error is estimated by averaging the free energy difference between 5–15ps and 15–25ps.

Figure 4. (a) Binding site of HDAC8-SAHA complex. (b) Binding site of HDAC8-S_SAHA complex. All the distance is from 55ps unrestrained QM/MM MD simulation. The Zinc is shown in the light yellow sphere and sulfur is shown in the dark yellow sphere. All hydrogen bonds are shown in magenta dashed line.

Figure 5. (a) Binding site of HDAC8-S_SAHA complex. (b) Binding site of HDAC4-S_SAHA complex. All the distance is from 55ps unrestrained QM/MM MD simulation. The Zinc is shown in the purple sphere and sulfur is shown in the dark yellow sphere. All hydrogen bonds are shown in magenta dashed line.

Figure 6. (a) Pocket view of HDAC8-S_SAHA complex. (b) Pocket view of HDAC4-S_SAHA complex. Zinc is shown in gray sphere.

Figure 7. Integrated water number around sulfur atoms of S_SAHA. The x-axis is the distance between the oxygen of water molecule and sulfur atom of S_SAHA.

Table 1

The parameters of the SLEF2 Force Field.

α ($\text{\AA}^2/e^2$)	β ($\text{\AA}^{-2}$)	λ ($\text{\AA}^{-1}$)	R_{Zn}^* ($\text{\AA}$)	Q_{Zn}
0.11	0.81	0.74	1.36	+2