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Molecular dynamic simulations of alcohol dehydrogenase with a four- or five-coordinate catalytic zinc ion

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MD simulations of alcohol dehydrogenase

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Abstract

A detailed parametrization is presented of a zinc ion with one histidine and two cysteinate ligands, together with one or two water, hydroxide, aldehyde, alcohol and alkoxide ligands. The parametrization is tailored for the active site of alcohol dehydrogenase and is obtained entirely from quantum chemical computations. The force-field reproduces excellently the geometry of quantum chemically optimized zinc complexes as well as the crystallographic geometry of the active site of alcohol dehydrogenase and small organic structures.

The parametrization is used in molecular dynamics simulations and molecular mechanical energy minimisations of alcohol dehydrogenase with a four- or five-coordinate catalytic zinc ion. The active-site zinc ion seems to prefer four-coordination over five-coordination by at least 36 kJ/mole. The only stable binding site of a fifth ligand at the active-site zinc ion is opposite to the normal substrate site, in a narrow cavity behind the zinc ion. Only molecules of the size water or smaller may occupy this site.

There are large fluctuations in the geometry of the zinc coordination sphere. A four-coordinate water molecule alternates frequently (every 7 ps) between the substrate site and the fifth binding site and even two five-coordinate water molecules may interchange ligation sites without prior dissociation. Ligand exchange at the zinc ion probably proceeds by a dissociative mechanism. The results show that it is essential to allow for bond stretching degrees of freedom in molecular dynamics simulations to get a correct description of the dynamics of the metal coordination sphere; bond length constraints may restrict the accessible part of the phase space and therefore lead to qualitatively erroneous results.

Introduction

Alcohol dehydrogenase (EC 1.1.1.1) catalyses the reversible oxidation of primary and secondary alcohols using NAD^+ as the coenzyme[1,2]. The active site of the enzyme contains a zinc ion that is essential for catalysis. Crystallographic studies of the horse liver enzyme and its binary or ternary complexes with coenzyme and different substrates have shown that this zinc ion is bound to the enzyme through two cysteine and one histidine residue and that it, as a rule, is tetrahedrally four-coordinate with one water or substrate molecule (or the corresponding anions, depending on pH) as the fourth first-sphere ligand[2-5].

Several mechanistic proposals have been put forward, however, according to which five-coordinate intermediates play an essential role during catalysis[6-13]. Crystallographic and spectroscopic data showing that binding to zinc of certain bidentate inhibitors is five-coordinate[14,15] have been taken as evidence in favor of these suggestions. Furthermore, spectroscopic studies of metal-substituted alcohol dehydrogenase have indicated that several binary and ternary complexes may be five-coordinate[6-8,15-20], although other spectroscopic investigations contradict these results[21-25]. The kinetic evidence is also scattered and has been taken to favor four-coordination[1,26], as well as five-coordination[6,7,9,11,27], of zinc in the catalytically productive ternary complexes.

Recently, Ryde published an extensive series of quantum chemical geometry optimizations of models of the active site of alcohol dehydrogenase with a varying number of different non-protein ligands[28]. These calculations showed that in vacuum, the zinc ion with ligands similar to those found in the enzyme prefers four-coordination over five-coordination by about 20 kJ/mole. The relevance of these results for the active-site zinc ion in alcohol dehydrogenase is unclear, however, since it is conceivable that five-coordinate zinc complexes may be stabilized by the enzyme. Therefore, some sort of calculations including the enzyme seemed to be necessary.

One obvious choice would be classical Monte Carlo, molecular dynamics, or molecular mechanics simulations. For such methods, a force-field parametrization describing the potential around a zinc ion with a varying number of different ligands would be necessary. Existing parametrizations of the zinc ion[29-32], however, either lack parameters for some of the zinc ligands found in alcohol dehydrogenase or contain unreliable data. Therefore, we decided to construct a new zinc parametrization. The parametrization is obtained entirely on the basis of quantum

chemistry from the Hessian matrix of the optimized structures in ref. 28. The choice of the ligands and the procedure of the parametrization makes the potential an effective force-field for the zinc ion, tailored for the active site of alcohol dehydrogenase. The parametrization is used in molecular dynamics and molecular mechanics simulations of alcohol dehydrogenase with a varying number of non-protein ligands in order to obtain information on the relative stability of four- and five-coordinate complexes, and on the dynamics of the ligands around the zinc ion.

Methods

Quantum chemical computations

$Zn(HS)_2XL(H_2O)_{0-1}$, where X denotes NH_3 or imidazole and L denotes a non-protein ligand, was chosen as a model of the active site of alcohol dehydrogenase. The full geometry of these models was optimized until the change in energy and the coordinates were below 10^{-6} Hartrees and 10^{-3} Bohr or radians, respectively, using analytical gradient methods at the Hartree-Fock level. No symmetry restrictions were imposed. If not otherwise stated, the results refer to computations with basis sets of double-ζ quality for all atoms (H: (31); C, N, O: (5111/31); S, P: (521111/4111); Zn: (62111111/51111/311))[33,34]. In some cases more extensive basis sets were used; For zinc the double-ζ basis (62111111/33111/311) of Ahlrichs et al.[35] was used, enhanced with p, d and f functions with exponents 0.162, 0.132 and 0.390, respectively. For all other atoms, the 6-31+G* series of basis sets were employed[36]. Partial charges were estimated by standard Mulliken analysis. All quantum chemical calculations were performed on an IBM RISC RS/6000 workstation using the semi-direct program package TURBOMOLE[37].

Molecular dynamic and molecular mechanical simulations

Molecular dynamics simulations were performed by the program MUMOD[38]. The energy function in this program is given in Eqn. 1:

$$\sum_{bonds} A_i(r_i - r_{i0})^2 + \sum_{angles} B_i(\alpha_i - \alpha_{i0})^2 + \sum_{dihedrals} \sum_{j=1}^{3} C_{ij}\left(\cos(j\phi_i) + 1\right) + \sum_{non-bonded\_i<j} \left(\frac{D_{ij}}{r_{ij}^{6}} + \frac{E_{ij}}{r_{ij}^{12}} + \frac{q_i q_j}{4\pi\varepsilon_0 \varepsilon r_{ij}}\right) \quad (1)$$

The first three terms represent the energies of bond stretching, angle bending and dihedral torsions, where r_i, α_i and ϕ_i are the actual bond lengths, angles and dihedral angles and r_{i0} and α_{i0} are the corresponding equilibrium values. The fourth term represents the non-bonded interactions, consisting of a Lennard-Jones 6-12 term and a Coulomb term, where r_{ij} is the distance between atom i and j. The force field does not contain any specific terms for hydrogen bonds or improper dihedral angles.

MUMOD integrates the Newton equations of motion using a double time-step Gear predictor-corrector algorithm of order 4. The forces are divided into a slow (the non-bonded and the dihedral terms) and a fast component; The slow forces are updated every five time steps[38]. The time step was 0.05-0.2 fs. If not otherwise stated, no cut-off was used for the non-bonded interaction. The neighbor list was updated every 40 time steps and the temperature was scaled towards 300 K every 250 time steps.

For molecular mechanics simulations, MUMOD was extended with a procedure for energy minimization using a conjugate gradient method using Polak-Ribieres algorithm and line search according to Fletcher[39]. The energy of the structures was minimized until the norm of the gradient was below 0.01 kJ/mole/Å. No cut-off was used for the non-bonded interactions.

Energies reported are total potential energy, excluding interactions between fixed atoms. In the molecular dynamics simulations the energy was sampled every time step after 20 ps equilibration (totally $2\text{-}8\cdot10^6$ configurations). To compare the energy of a four- and five-coordinate zinc ion in alcohol dehydrogenase, we used the fact that the difference in energy of $Zn(HS)_2(imidazole)(H_2O)_2$ with a five- and a four-coordinate zinc ion is accurately known from quantum chemical computations[28]: 25.2 kJ/mole (including thermodynamical corrections). The energy of these two structures was minimized by molecular mechanics (with the same charges and atom types as in the simulations) and the resulting energy difference was subtracted from the quantum chemical energy difference. The result, –105.1 kJ/mole, was added to all energy differences.

Parametrization of the zinc ion

The parametrization of the zinc ion is based on 14 models of the active site of alcohol dehydrogenase that have been geometry optimized by quantum chemistry[28]. The structures are listed and shortly characterized in **Table I**. The cysteine ligands are modeled as HS^- or CH_3S^-. As shown in **Fig. 1** and Table I HS^- gives virtually the same result as CH_3S^-; the root-mean-square deviation of the two structures in Figure 1 is only 0.015 Å and the Zn-S bond lengths and force constants differ by less than 0.6%. The histidine ligand is modeled by either imidazole or ammonia. As can be seen in Table I, these two ligands give slightly different results. Therefore, all parameters involving histidine are derived from the imidazole structures.

Quadratic force-constants were obtained from the diagonal elements of the analytically calculated quantum chemical Hessian, projected into a space of internal coordinates. Several different sets of internal coordinates were tested to ensure that the Cartesian-to-internal transformation does not distort the values. The force-constants were uniformly scaled by a factor 0.81[36].

For bond lengths and angles these quadratic force-constants represent A_i and B_i in Eqn (1) and could be used directly. For dihedral angles, the periodicity of the potential was determined from the values of dihedral angels found in the structures and the C_{ij} constants in Eqn (1) were obtained by multiplying the quadratic force-constants by an appropriate Taylor coefficient (2, 1/2 and 2/9 for 360°, 180° and 120° periodicity, respectively). The equilibrium values of the bond lengths and angles were calculated by averaging the values observed in the any of the structures in ref. no. 28 (about 20 four-coordinate and 30 five-coordinate structures). The van der Waals parameters for the zinc ion were taken from the AMBER force field: σ=1.70 Å and ε=0.0523 kJ/mole[40].

In addition to zinc, five new atom types had to be added to the potential library of MUMOD, in order to describe a negatively charged cysteine residue, NADH, and hydroxide and alkoxide ions; S-: negatively charged sulfur, P: phosphorus, OS: ester oxygen, OH-: oxygen in the hydroxide ion and OC-: alkoxide oxygen. The force-field parameters of P and OS were taken from the AMBER[40] and XPLOR[41] force fields. The parameters of the negatively charged atom types were obtained from quantum chemical computations on HX^-, CH_3X^- and $CH_3CH_2X^-$, X=S or O, using the 6-31+G** basis sets. These latter parameters are listed in **Table II**.

The protein

Throughout, the coordinates of horse liver alcohol dehydrogenase in complex with NADH and dimethylsulfoxide at 1.8 Å resolution (R-factor=0.172)[4] were used. This is at present the most accurate structure of alcohol dehydrogenase. The enzyme is in the closed conformation that is the catalytically interesting conformation and also the one to which most reports of a five-coordinate zinc ion refer. Both subunits of the enzyme were included in the simulations, as well as the coordinates of 509 crystal water molecules; totally 12941 atoms. The dimethylsulfoxide molecule at the active site was substituted by a water molecule. All Asp and Glu residues were treated as anions and all Lys and Arg residues as cations. The protonation state of the His residues was determined by

examination of the hydrogen bonding structure; His34, 67, 138 and 139 were found to have a proton on ND1, His105 on NE2, while His51 and 348 have protons on both nitrogens. The six cysteine zinc ligands, Cys46, 97, 100, 103, 111 and 174, were assumed to be negatively charged, the other eight cysteine residues uncharged. This charge assignment gave a total charge of +4 for the dimer (34 Asp, 42 Glu, 12 Cys, 2 NADH, 2 carboxy terminals, 60 Lys, 24 Arg, 4 His^+, 4 Zn and 2 amino terminals).

Partial charges of the amino-acids were taken from the MUMOD program library. The charges of the adenine, ribose and phosphate moieties of NADH and of histidine protonated on NE2 or on both NE2 and ND1 were taken from the AMBER library[40]. Partial charges of cysteinate (CA: 0.028, HA: 0.017, CB: -0.416, HB: 0.028, SG: -0.603) and of the nicotinamide moiety of NADH (C1'N: 0.264, N1N: -0.707, C2N: 0.347, H2N: 0.187, C3N: -0.341, C7N: 0.634, O7N: -0.529, N7N: 0.733, H7N: 0.280, C4N: -0.177, H4N: 0.146, C5N: -0.217, H5N: 0.116, C6N: 0.221, H6N: 0.143) were obtained from a Mulliken analysis of the quantum chemically optimized structures of CH_3CH_2SH and $NC_5H_4CONH_2$.

The charge on the zinc ion and its ligands were determined by quantum chemical Mulliken analysis on the systems in Table III and in ref. 28. The zinc charge varied by only ±0.1 e for four- and five-coordinate systems with different ligands but the same basis sets. In order to facilitate comparisons, we therefore decided to use the same zinc charge in all simulations, +0.488, which was obtained for the four-coordinate system with the largest basis sets in Table III. Since the charges on the oxygen and imidazole ligands do not change significantly on coordination to a zinc ion, the residual formal zinc charge (1.512) was distributed uniformly on the SG and CB atoms of the two cysteine ligands; the resulting charge was -0.225 and -0.038, respectively.

The positions of the hydrogen atoms were determined with standard algorithms (program MUMIN). Since some positions are not fully determined by these methods, and also in order to determine the solvent water structure around the enzyme, a 1.8 ps molecular dynamics simulation at 0 K was performed. In this simulation, a water bath of 1719 water molecules was included and all heavy atoms was kept fixed except the oxygen atoms of the solvent water molecules. After this simulation all uncharged amino acids and water molecules with a distance larger than 3.0 Å from the large system (see below) were removed, while charged residues were substituted by an integer charge at the position of the NZ, CZ, CG, CD, SG, CE1, ZN and both P atoms for Lys, Arg, Asp,

Glu, Cys^-, His^+, ZN and NADH, respectively. These integer charges were scaled by a dielectric constant ε=4.0, while ε=1.0 in the rest of the system. The remaining 2813 atoms were subjected to two further molecular dynamic simulations, 0.5 ps at 300 K and 2.5 ps at 0 K, followed by an energy minimization to a gradient norm below 0.01 kJ/mole/Å. In these calculations, periodic boundary conditions with a non-bonded cut-off of 10 Å was used and the heavy non-solvent atoms were kept fixed. The resulting coordinates were used as input in all the simulations.

Two different systems were studied. One system, termed the *small* system, comprised all residues of the enzyme within 3.0 Å from the catalytic zinc ion (in the A subunit of the enzyme) and its ligands (including CB of the protein ligands). These are: Cys46, Ser48, Asp49, Gly66, His67, Glu68, Phe93, Phe140, Leu141, Gly173, Cys174, Gly175, Ile318, Arg369, H2O158 and the nicotinamide moiety of NADH. In addition, all charged residues outside this radius were represented by scaled (ε=4.0) integer charges. Totally, the small system contained 400 atoms. The positions of all atoms except the zinc ion and its ligands (including CB) were kept fixed.

The other system, termed *large*, comprised all residues within 3.0 Å from any atom in the small system (except the integer charges), i.e. residues 43-53, 57, 59, 63-64, 66-69, 90, 92-95, 109-110, 115, 116, 139-142, 146, 170-176, 178-179, 202-203, 292, 294, 318-321, 345-348, 359, 368-370, crystal-waters number 5, 8, 21, 35, 55, 58-59, 158-161, 167, 172, the nicotinamide, N-ribose and the pyrophosphate moiety of NADH and residues 309 and 310 from the B subunit of the protein. 176 scaled (ε=4.0) integer charges were also included, leading to a total number of 1224 atoms. The atoms of the small system (not the integer charges) were free to move, while the other atoms were kept fixed.

Results and Discussion

Basis set dependence of the geometry of the zinc coordination sphere

In order to study the impact of the quality of the basis set on the geometry of the zinc coordination sphere, geometry optimizations were performed on $Zn(NH_3)(SH)_2X$, X=H_2O, OH^- and $(H_2O)_2$, with increasing quality of the basis sets. The results, collected in **Table III**, show that the zinc-ligand distances are strongly dependent on the quality of the basis sets. As the basis sets of double-ζ quality are extended by one set of polarizing functions and two sets of diffuse functions, the Zn-N distance increases by 0.03-0.09 Å, the Zn-S distances decrease by 0.03-0.09 Å, the Zn-OH distance increases by 0.05 Å, while the Zn-OH_2 distance increases by as much as 0.20-0.34 Å. The angles and the dihedrals around the zinc ion also change, but to a much smaller extent.

The greatest changes are found for the $Zn(NH_3)(SH)_2(H_2O)_2$ system. As the quality of the basis sets is increased, the lengths of the two Zn-O bonds become increasingly unequal. This is due to weakening of the Zn-O bond and strengthening of an internal hydrogen bond from an ammonia hydrogen to one of the water oxygens. With the largest basis set, this hydrogen bond becomes more favorable than zinc coordination, and the complex reorganizes to a four-coordinate structure with one water molecule in the second coordination sphere of the zinc ion. Apparently, the stability of five-coordinate complexes decreases as the quality of the basis set is increased. Therefore, the relative stability of five-coordinate complexes is overestimated in this work as well as in our earlier studies[28].

In summary, double-ζ + polarization (double-ζ + a p-function on Zn) seems to be an appropriate level to describe the zinc coordination sphere, except perhaps the Zn-O bond. Unfortunately, it was not possible to use such large basis sets in the parametrization. Instead, the zinc-ligand equilibrium bond distances in the zinc parametrization were rescaled to reproduce the values of the most extended basis sets.

Parametrization of the zinc ion

The force-field parameters for a four- and five-coordinate zinc ion are listed in **Table IV**. The bond lengths are scaled to reproduce the values of the extended basis sets; the original values are

given in parenthesis. Several dihedral angles, especially some X-Y-S-H and X-Y-O-H dihedrals, apparently showed a 60° periodicity. Since such a potential is not allowed in the present version of MUMOD, a 120° term with a decreased C_{i3} constant was used instead. Affected angles are marked with [a] in Table IV.

Another problem was encountered for the five-coordinate zinc ion. There exist several different local minima of the quasi trigonal-bipyramidal five-coordinate structures, characterized by different ligands in the axial positions. Each structure has different sets of equilibrium parameters and force-constants and strictly a separate parametrization should be performed at each minimum. The present parametrization is obtained from the global minimum with the two oxygen atoms in the axial positions and is strictly valid only near this structure. As will be seen below and as is also confirmed by combined quantum chemical and molecular mechanic geometry optimisations[42], this is the optimal structure also in the enzyme.

Many force-constant parameters depend on all the ligands around the zinc ion (i.e. also on atoms not explicitly involved in the parameter). The Zn-S distance, for example, changes from 2.32 to 2.43 Å as an aldehyde ligand (which binds weakly) is replaced by a hydroxide ion (which binds strongly). The corresponding force constant, which is almost perfectly linearly dependent on the bond length, changes accordingly, leading to an uncertainty in the equilibrium bond distances and the bonding force constants of about 0.05 Å and 50 kJ/mole/Å^2 in complexes involving the strongest or weakest ligands. Similarly, bond angle parameters of complexes involving alkoxide and hydroxide ligands are very different from those of complexes with uncharged ligands, due to the electrostatic repulsion between the oxygen and the negatively charged sulfide ions. For example, the S-Zn-S angle is 141-162° in uncharged complexes, but only 114-116° in negatively charged complexes.

These problems could, of course, be solved by introducing a special zinc type for every set of zinc ligands. Yet, this would lead to an unfortunately large number of atom types. A more interesting possibility would be to include the electrostatic 1,3-interactions in the potential energy term. Then the bonded parameters of all atoms would need to be recalculated, but the result would probably be much more accurate without any need of new atom types. In the present parametrization no attempt was made to correct these problems (as in all common force fields); The

present parameters are most accurate for an uncharged zinc coordination sphere with water or alcohol ligands.

The van der Waals parameters (D_{ij} and E_{ij} coefficients in Eqn. 1) of the zinc ion provided the largest problems. An attempt was made to estimate these parameters from quantum chemical interaction energies. This turned out to be impossible due to the inappropriateness of the non-bonding potential. The fourth term in Eqn. 1 may be appropriate for interactions of atoms with low charges, but for highly charged atoms such as a zinc ion, significant terms of any negative power of the inter-atomic distance can be expected. At least four r^{-k} terms (including the Coulomb term) turned out to be necessary to achieve an acceptable fit to the calculated interaction potential. Yet, the van der Waals parameters of the zinc ion should be of little significance in the current parametrization since the first sphere ligands are described by bonded parameters. Therefore, van der Waals parameters of the zinc ion were simply taken from literature.

The first simulations of alcohol dehydrogenase gave an eight-coordinate zinc ion with four non-bonded water molecules at unphysically short Zn-O distances (about 1.5 Å). Therefore, the Zn-OH2 van der Waals parameters were recalculated so that a molecular mechanically minimized structure reproduces the quantum chemically optimized Zn-O distance and the change in energy when a water molecule is added to $Zn(NH_3)(SH)_2(H_2O)$ (2.364 Å and -70.52 kJ/mole[28]). This gave D=-1477 kJ $Å^6$ $mole^{-1}$ and B=473500 kJ $Å^{12}$ $mole^{-1}$.

Performance of the parametrization

The quality of the parametrization of the zinc ion was tested by comparing the geometry of 34 small model systems optimized by molecular mechanics using the current zinc parametrization with results obtained quantum mechanically[28]. 14 of these structures were used in the parametrization of the zinc ion (Table I). The charges of the atoms in the molecular mechanical optimizations were obtained by Mulliken analysis from the quantum chemical computations. The differences in bond lengths, bond angles, dihedral angles and inter-atomic distances are presented in **Table V**.

If 1,4-interactions (i.e. interactions involving atoms separated by three bonds) but not 1,3-interactions are included in the non-bonded term in Eqn. 1 (default in MUMOD), the result is moderately impressing. This is due to deficiencies in the treatment of the non-bonded interactions.

In the model systems there are many potential hydrogen bonds between the zinc ligands, i.e. between atoms separated by only three bonds (e.g. H-N-Zn-O). If the attractive 1,4-interaction (hydrogen-acceptor) is included in the potential, but the repulsive 1,3-interaction is excluded, the hydrogen bonds become unphysically short, leading to a highly distorted geometry, as can be seen in **Fig. 2**.

If also the 1,4-interactions are excluded, the performance of the parametrization is greatly improved (row 4-11 in Table V and **Fig. 3**); the errors in the internal coordinates are almost halved. The hydrogen bonds are much better described, even if 1,5-interactions (repulsion between hydrogen atoms) leads to slightly increased zinc ligand distances. Yet, in simulations with more realistic ligands, which have a lower number of hydrogen atoms, this effect is negligible. Therefore, in the following, 1,4-interactions are excluded from the potential. In most other force-fields, 1,4-interactions are either totally excluded, or damped by a factor of about 4.

There is no significant difference between the performance of the zinc parametrization on the structures used in the parametrization and the other structures. In fact, the structures not included perform a little bit better. This indicates that the number of structures included in the parametrization was large enough and that the parametrization is well transferable to other systems. Further, there are no significant difference in the quality of the parametrization of the five-coordinate and the four-coordinate zinc ion. From Table V, it may seem that the four-coordinate systems have slightly better bond distances but worse angles and dihedral angles, but this is an artifact. The difference in bond lengths (and also the rather poor performance of the bond lengths on the whole) is due to the recalibration of the bonds lengths. As discussed above, the change is larger for five-coordinate complexes. The worse angles and dihedrals of the four-coordinate parametrization, on the other hand, are explained by the fact that complexes with negatively charged oxygen-ligands can only be four-coordinate. As discussed above, these systems have distorted bond angles around the zinc ion.

The Zn-N and Zn-S equilibrium bond distances are in excellent conformity with the distances observed in small organic crystals[31] (Zn-N 1.97-2.21 and 2.00-2.39 Å, Zn-S 2.25-2.50 and 2.36-2.51 Å, for four- and five-coordinate structures, respectively). The Zn-O distances, on the other hand, differ significantly (1.91-2.04 and 1.93-2.39 Å, for four- and five-coordinate structures). This can most probably be attributed to the fact that the crystal data do not discriminate between charged

and uncharged ligands and that in alcohol dehydrogenase the two negatively charged sulfide ligands make the bond lengths of the other zinc ligands longer than normal. Compared to the parametrization of Merz et al[32], the equilibrium values (except the Zn-O distances) are similar, but our force constants are usually slightly larger.

Simulations of alcohol dehydrogenase

Molecular dynamics simulations were performed on seven models of alcohol dehydrogenase, differing in the size of the system (large or small), the coordination number of the zinc ion (four or five), and the non-protein zinc ligands (water, methanol, formaldehyde or hydroxide). In addition, at least three molecular mechanical optimizations were performed on each system, differing in the starting structure (the initial structure, the structure at the end of the molecular dynamics simulation, and the structure with the lowest energy in the molecular dynamics simulation). The results of the simulations are collected in **Table VI** and are viewed in **Figs. 4 and 5**.

In the molecular dynamics simulations of a four-coordinate zinc ion, the zinc-bound water molecule alternates frequently between two different coordination sites at the zinc ion (changing sites every 7 ps, c.f. Fig. 6a). One site is the normal substrate site at the bottom of the substrate cleft, where the molecule may make a hydrogen bond to OG of Ser48 (Fig. 4a). The other site (Fig. 4b; termed the *alternative* site below) is on the opposite side of the zinc ion, in a narrow cavity buried behind the zinc ion. In this site the molecule may interact with OD of Asp49, OE of Glu68 or a crystal water molecule (which is hydrogen bonded to Asp49). It is usually very close to the walls of the cavity, especially Cys46 and Glu68.

In the simulations of a five-coordinate zinc ion with two water ligands, the water molecules occupy both water sites (Fig. 5). The two Zn-O distances are very dissimilar. The small size of the cavity at the alternative water site forces the Zn-O distance to be short, 2.12-2.38 Å, while the Zn-O distance of the water molecule in the normal substrate binding site is longer: 2.43-2.56 Å. The two water molecules stays in their sites most of the simulation. Yet, they interchange sites twice during a 100 ps simulation, showing that such interchange in fact is possible without dissociation of any of the water molecules.

Since the cavity around the alternative coordination site is quite narrow, it was of great interest to deduce whether other molecules than water may occupy this site. Therefore simulations were performed on the small system with three other ligands of interest for the reaction mechanism of alcohol dehydrogenase, namely methanol, formaldehyde and hydroxide ion. **Fig. 6** shows the distance between the oxygen atom of these ligands and the CD atom of Glu68. The latter atom is located almost opposite to the substrate site and was kept fixed during the calculation. Therefore, a distance of 3.3-4.0 Å is indicative for coordination at the alternative site, while 6.1-7.1 Å is typical for coordination at the substrate site. With these criteria, it is can be seen from Fig. 1 that water and the hydroxide ion occupy the alternative site fifteen times and once, respectively, during a100 ps simulation, while methanol and formaldehyde never diffuse into the site during the simulations. Apparently, water is the largest molecule that may occupy the alternative site.

The hydroxide ion is smaller than the water molecule, but has nethertheless much lower occupancy at the alternative site than the water molecule. This is probably due the negatively charged thiolate groups of the two cysteine ligands, which impede diffusion of the hydroxide ion to the alternative site of the zinc ion. Furthermore, the carboxyl groups of Asp48 and Glu68 prevent the hydroxide ion to stay in the alternative site; No potential minimum for the hydroxide ion at the alternative site could by found by molecular mechanical energy minimisations.

Zinc-ligand distances

The average zinc-ligand distances in the molecular dynamics simulations of alcohol dehydrogenase are in excellent agreement with the crystal structure of the enzyme[4]. The Zn-N and Zn-S distances are 2.10-2.15 and 2.19-2.31 Å in the simulations, compared to 2.05-2.14 and 2.21-2.34 in the crystal structure (the two subunits). The Zn-O bond length does not show the same agreement. Yet, this is probably due to the fact that the fourth zinc ligand in the crystal structure is dimethylsulfoxide (DMSO) and not water. Quantum chemical geometry optimizations of $Zn(NH_3)(SH)_2$(DMSO) in **Table VII** show that DMSO binds more strongly to the zinc ion than water; the Zn-O distance is 2.12 Å for DMSO compared to 2.26 Å for H_2O with similar basis sets (Table III). If the difference, 0.14 Å, is added to the Zn-O crystallographic distance, we get 2.29-2.33 Å which is in reasonable agreement with 2.24-2.29 Å observed in the molecular dynamics simulations of a four-coordinate zinc water molecule. The Zn-ligand distances in the molecular dynamics

simulations are usually about 0.05 Å longer than in the molecular mechanical optimizations, as a result of the kinetic energy at 300 K.

In all simulations (except the energy minimisations of the large system with a five-coordinate zinc ion) the Zn-S bond of Cys46 is longer than the one of Cys174 while in the crystal structure the state of affairs is the opposite. This discrepancy can probably be attributed to the uncertainty in the crystal coordinates; In the recent 1.9 Å resolution structures of the free form of liver alcohol dehydrogenase and of the 2.1 Å resolution structure of the complex of the copper substituted enzyme with NADH and dimethylsulfoxide[43,44], the trend is the same as in the calculations.

The molecular dynamics simulations show large fluctuations in the Zn-ligand distances. The Zn-O distance varies ±45% of the equilibrium distance with a standard deviation of about 0.4 Å, while the Zn-S and Zn-N distances vary ±35% with a standard deviation of about 0.27 Å. As a comparison the O-H distance of the zinc-bound water molecule varies ±28% with a standard deviation of 0.09 Å. This reflects the lower force constants of the zinc-ligand bonds, which are intermediate between a normal bond and a hydrogen bond. These large fluctuations in the zinc-ligand distances indicate that ligand exchange at the zinc ion probably proceeds by a dissociative mechanism (old ligand dissociates before the new ligand associates); Even in a 100 ps simulation performed with a bonding potential that is unphysically steep at large bond lengths, the maximum Zn-OH_2 distance is 3.4 Å that is the normal distance for a second sphere zinc ligand[28].

The inappropriateness of bond stretching constraints

Molecular dynamics simulations are usually performed with bond lengths constrained to the equilibrium value using the SHAKE algorithm[45]. This approximation is based on the assumption that the frequencies of the bond stretching vibrations and the other vibrations are well separated, and it leads to appreciable savings in computer time by allowing a larger time-step. The present results show, however, that such a constraint may restrict the accessible parts of the phase space. For a zinc-bound water molecule to interchange between the two possible ligand sites, concerted changes in the zinc-ligand bond lengths and angles are necessary. If the bond lengths were constrained, such site-interchange is impossible[46,47], and qualitatively incorrect results would be obtained.

The conclusion must therefore be that bond length constraints cannot be used unless it is explicitly shown that the vibrational frequencies are separated, *and* that the accessible phase space is not changed by such constraints. It seems that a double time-step algorithm such as the one used in MUMOD[38] is preferable over constrained dynamic algorithms for the integration of Newton's equations of macromolecular systems.

The relative stability of four- and five-coordinate zinc complexes

The present calculations may be used to compare the stability of four- and five-coordinate active-site zinc ions in alcohol dehydrogenase. In the large system, the four-coordinate structures are more stable than the five-coordinate structure by 46 kJ/mole in the molecular dynamics simulations and 36 kJ/mole in the molecular mechanics simulations. In the small system, the differences are 67 kJ/mole for the molecular dynamics simulations and 133 kJ/mole for the molecular mechanics simulations, again in favor of the four-coordinate zinc ion. These differences seem mainly to origin from the zinc coordination sphere (the enzyme outside the active site contributes by about 5 kJ/mole). As shown in **Fig. 7**, the five-coordinate structure differs more from the ab initio vacuum structure than the four-coordinate one; the root-mean-square deviation is 0.20 and 0.27 Å, respectively. In particular, the bond between the zinc ion and Cys46 seems to be strained in the five-coordinate structure.

It must thus be concluded that a four-coordinate zinc ion is more stable than a five-coordinate one. This confirms quantum chemical vacuum calculations[28] and shows that four-coordination of the catalytic zinc ion in alcohol dehydrogenase is preferred not only by the chemical properties of the zinc ion and its ligands, but also by the folding of the enzyme at the active site. This conclusion has strong implications on the reaction mechanism of alcohol dehydrogenase. Several mechanisms have been proposed that involve five-coordinate structures as significant intermediates[6-13]. The present calculations do not support such proposals; since a 46 kJ/mole energy difference corresponds to an equilibrium constant of $1 \cdot 10^{-8}$, five-coordinate structures should be very rare. Furthermore, the dynamic data indicate that even the exchange of zinc ligands probably proceeds via a three-coordinate (rather than a five-coordinate) intermediate.

Alcohol dehydrogenase is known to assume two different main conformations, an open and a closed one[1-3]. The present calculations are based on a crystal structure of the closed conformation. It cannot be ruled out that all experimental reports of five-coordination refer to another conformation of the protein, and that the barrier between these two forms is too high or the interchange time is too long, to be bridged in the simulations. Yet, all experimental evidence (crystallographic and spectroscopic) agrees on that the free enzyme in the open conformation is four-coordinate[2-3,10,16,19-22,44]. Further, it is widely assumed that the dehydration of the active site that occurs when the enzyme closes is important for the catalytic mechanism[1,2]. Thus, if five-coordinate structures obtain in other conformations, the kinetic significance of that is unclear.

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Table I. Energy and zinc-ligand distances of the quantum chemically geometry optimized structures[28] used for force-field parametrization of the zinc ion. A and B denote $Zn(HS)_2(NH_3)$ and $Zn(HS)_2$(imidazole), respectively. A "+" in the formula indicates second sphere coordination. The Zn-O distances are ordered after the size. When ambiguity may arise, an [a] marks out the non-water ligand.

Complex	Energy	Distance to Zn (pm)				
	(H)	N	S1	S2	O1	O2
$A(H_2O)$	-2705.984879	212	233	233	211	
$B(H_2O)$	-2874.532927	204	235	237	211	
$A(OH)^-$	-2705.441774	218	243	243	187	
$A(CH_3OH)$	-2744.989145	213	233	233	208	
$A(CH_3O)^-$	-2744.445435	217	243	243	187	
$A(CH_2O)$	-2743.804699	211	232	235	216	
$Zn(CH_3S)_2(NH_3)(H_2O)$	-2783.986830	213	232	232	212	
$A(H_2O)_2$	-2781.977110	212	241	241	216	217
$B(H_2O)_2$	-2950.521465	207	247	247	210	211
$A(H_2O)+(H_2O)$	-2781.984127	212	234	238	204	369
$A(OH)^-+(H_2O)$	-2781.449373	214	243	244	190	358
$A(H_2O)(CH_3OH)$	-2820.980628	212	240	244	212[a]	218
$A(CH_3O)^-+(H_2O)$	-2820.449651	214	242	243	191	355
$A(H_2O)(CH_2O)$	-2819.796592	211	236	240	219	233[a]

Table II. Quantum chemically calculated force-field parameters for the new atom types OH-, OC- and S-. The MUMOD atom types are described in the legend to Table IV.

a. van der Wall parameters, σ_i and ε_i. Compared to D_{ij} and E_{ij} in Eqn. 1:

$$D_{ij} = 4\varepsilon_{ij}\sigma_{ij}^{6}; E_{ij} = 4\varepsilon_{ij}\sigma_{ij}^{12}; \varepsilon_{ij} = \sqrt{\varepsilon_i \varepsilon_j}; \sigma_{ij} = \frac{\sigma_i + \sigma_j}{2}.$$

atom type	σ (Å)	ε (kJ/mole)
OH–	3.17	0.639
OC–	2.94	1.021
S–	4.97	0.998

b. Bond parameters; equilibrium distance A_i and quadratic force constant r_{i0} in Eqn. 1

atom types		A_i (Å)	r_{i0} (kJ/mole/Å^2)
OH–	HO	0.946	2267
OC–	C	1.335	1788
S–	H	1.337	1096
S–	C	1.835	848

c. Angle parameters: equilibrium angle B_i and quadratic force constant α_{i0} in Eqn. 1.

atom types			B_i (°)	α_{i0} (kJ/mole/radian^2)
HC	C	OC–	114.6	528
C	C	OC–	113.9	600
HC	C	S–	109.8	358
C	C	S–	114.0	494

P	OS	P	123.6	543

d. Dihedral parameters: C_{in} in Eqn. 1. C_{ij}=0 for j?n.

atom types				n	C_{in} (kJ/mole/radian^2)
OC–	C	C	HC	3	8.15
S–	C	C	HC	3	7.32

Table III. The dependence of the geometry on the basis sets. Energies, basis sets, coordination numbers and the zinc-ligand distances of structures obtained by quantum chemical geometry optimizations. A denotes $Zn(HS)_2(NH_3)$. The basis sets are described in the Methods.

Complex	Basis	Energy	Distance to Zn				
	(Zn / other)	(H)	N	S1	S2	O1	O2
$A(H_2O)$	dζ / dζ	-2705.984879	212	233	233	211	
$A(H_2O)$	dζ / 6-31G	-2706.140377	212	234	234	210	
$A(H_2O)$	dζ+p / 6-31G**	-2706.279881	218	230	230	226	
$A(H_2O)$	dζ+p / 6-31(+)G**	-2706.284772	218	230	230	225	
$A(H_2O)$	dζ+p / 6-31+G**	-2706.289841	218	230	230	228	
$A(H_2O)$	dζ+pdf / 6-31+G**	-2706.300360	218	229	229	231	
$A(OH)^-$	dζ / dζ	-2705.441774	218	243	243	187	
$A(OH)^-$	dζ+p / 6-31+G**	-2705.743774	228	240	240	193	
$A(OH)^-$	dζ+pdf / 6-31+G**	-2705.753362	227	240	240	192	
$A(H_2O)_2$	dζ / dζ	-2781.977110	212	242	242	217	218
$A(H_2O)_2$	dζ+p / 6-31G*	-2782.271259	214	234	234	238	246
$A(H_2O)_2$	dζ+p / 6-31+G**	-2782.332593	215	233	233	242	252
$A(H_2O)_2$	dζ+pdf / 6-31+G**	-2782.343160	214	231	231	227	327

Table IV. Parametrization of Zn^{2+}. The relevant MUMOD atom types are: HO: polar hydrogen; HC: non-polar hydrogen; H2O: water hydrogen; C sp3 hybridized carbon; Ca: sp2 hybridized carbon; N: amine nitrogen; Na: aromatic nitrogen (in histidine); O=C: carbonyl oxygen; OH: hydroxyl oxygen; OH2: water oxygen, OH-: hydroxide oxygen; OC-: alkoxide oxygen; S-: negatively charged sulfur (in cysteinate); Zn4: four-coordinate zinc ion; Zn5: five-coordinate zinc ion.

a. Bond parameters; equilibrium distance A_i and quadratic force constant r_{i0} in Eqn. 1. The equilibrium bond distances are recalculated to fit the results with the extended basis sets; the original values are given in brackets.

atom types		Zn4		Zn5	
		A_i (Å)	r_{i0} (kJ/mole/Å^2)	A_i (Å)	r_{i0} (kJ/mole/Å^2)
Zn	N	2.181 (2.120)	262	2.150 (2.120)	262
Zn	Na	2.101 (2.040)	358	2.070 (2.069)	327
Zn	O=C	2.359 (2.160)	189	2.583 (2.330)	100
Zn	OH	2.279 (2.080)	243	2.388 (2.135)	201
Zn	OH2	2.309 (2.110)	204	2.418 (2.165)	161
Zn	OH-	1.923 (1.871)	690		
Zn	OC-	1.926 (1.874)	662		
Zn	S-	2.293 (2.343)	342	2.377 (2.427)	270

b. Angle parameters: equilibrium angle α_{i0} and quadratic force constant B_i in Eqn. 1.

atom types			Zn4		Zn5	
			α_{i0} (°)	B_i (kJ/mole/ radian^2)	α_{i0} (°)	B_i (kJ/mole/ radian^2)
HO	N	Zn	109.0	126.5	109.0	126.5
Ca	Na	Zn	123.6	186.3	126.1	186.3
C	O=C	Zn	131.2	83.4	131.2	100.4
C	OH	Zn	133.4	119.9	133.4	119.9
HO	OH	Zn	110.3	120.7	108.5	120.7
H2O	OH2	Zn	119.0	73.4	119.0	81.3
HO	OH-	Zn	135.1	49.5		
C	OC-	Zn	141.4	42.0		
C	S-	Zn	111.6	79.1	111.1	69.4
HO	S-	Zn	109.9	78.6	109.4	68.8
N	Zn	O=C	90.9	95.6	83.6	221.9
N	Zn	OH	98.0	95.6	91.4	221.9
N	Zn	OH2	97.0	95.6	89.2	221.9
N	Zn	OH-	83.5	95.6		
N	Zn	OC-	86.5	95.6		
N	Zn	S-	103.0	127.2	103.0	127.2
Na	Zn	O=C	97.9	67.2	94.6	135.3
Na	Zn	OH	105.0	67.2	102.4	135.3
Na	Zn	OH2	104.0	67.2	100.2	135.3
Na	Zn	OH-	95.0	67.2		
Na	Zn	OC-	98.0	67.2		
Na	Zn	S-	103.0	127.2	103.0	127.2
O=C	Zn	OH2			172.3	37.3
O=C	Zn	S-	104.4	129.6	93.6	129.6
OH	Zn	OH2			173.9	37.3
OH	Zn	S-	99.7	129.6	92.0	129.6
OH2	Zn	OH2			168.5	37.3
OH2	Zn	S-	96.1	129.6	88.8	129.6
OH-	Zn	S-	120.7	129.6		
OC-	Zn	S-	120.3	129.6		
S-	Zn	S-	146.5	207.4	151.8	69.1

c. Dihedral parameters: C_{in} in Eqn. 1. C_{ij}=0 for j?n. [a] indicates that a n=6 term would be more appropriate.

atom types				n	Zn4 C_{in} (kJ/mole /radian^2)	Zn5 C_{in} (kJ/mole /radian^2)
HC	C	OC-	Zn	3	0.27	0.46
C	C	OC-	Zn	3	0.27	0.46
HC	C	S-	Zn	3	0.76	0.76
C	C	S-	Zn	3	0.76	0.76
HC	Ca	Na	Zn	1	-197.8	-195.2
C	Ca	Na	Zn	1	73.8	85.8
Na	Ca	Na	Zn	1	80.0	88.5
HO	N	Zn	O=C	3	-1.03	-0.50
HO	N	Zn	OH	3[a]	-0.75	-0.52
HO	N	Zn	OH2	3[a]	-0.19	-0.13
HO	N	Zn	OH-	3	-5.45	-8.43
HO	N	Zn	OC-	3	-4.02	-8.01
HO	N	Zn	S-	3	-0.79	-0.42
Ca	Na	Zn	O=C	2	11.8	7.82
Ca	Na	Zn	OH	2	11.8	7.82
Ca	Na	Zn	OH2	2	11.8	7.82
Ca	Na	Zn	OH-	2	-11.8	-7.82
Ca	Na	Zn	OC-	2	-11.8	-7.82
Ca	Na	Zn	S-	2	-11.8	-7.82
Zn	O=C	C	HC	2	-7.61	-7.61
Zn	O=C	C	C	2	-7.61	-7.61
C	O=C	Zn	N	3	3.22	-3.52
C	O=C	Zn	Na	3	3.22	-3.52
C	O=C	Zn	OH2	3		-3.52
C	O=C	Zn	S-	3	3.22	-3.52
Zn	OH	C	HC	3	-2.24	-2.68
Zn	OH	C	C	3	-2.24	-2.68
HO	OH	Zn	N	2	15.5	21.6
HO	OH	Zn	Na	2	15.5	21.6
HO	OH	Zn	OH	2		-17.4
HO	OH	Zn	OH2	2		-21.6
HO	OH	Zn	S-	2	-15.6	-21.6
C	OH	Zn	N	2	15.5	21.6
C	OH	Zn	Na	2	15.5	21.6
C	OH	Zn	OH2	2		-21.6

C	OH	Zn	S-	2	-15.6	-21.6
H2O	OH2	Zn	N	2	14.0	18.1
H2O	OH2	Zn	Na	2	11.2	19.1
H2O	OH2	Zn	O=C	2		20.2
H2O	OH2	Zn	OH	2		-20.2
H2O	OH2	Zn	OH2	2		17.4
H2O	OH2	Zn	S-	2	-12.3	-18.3
HO	OH-	Zn	N	2	-1.47	
HO	OH-	Zn	Na	2	-1.47	
HO	OH-	Zn	S-	2	1.47	
C	OC-	Zn	N	2	-1.34	
C	OC-	Zn	Na	2	-1.34	
C	OC-	Zn	S-	2	1.34	
HO	S-	Zn	N	3[a]	-0.00	-0.00
HO	S-	Zn	Na	3[a]	-0.00	-0.00
HO	S-	Zn	O=C	3	-1.37	-1.82
HO	S-	Zn	OH	3[a]	-1.26	-2.07
HO	S-	Zn	OH2	2[a]	2.24	3.04
HO	S-	Zn	OH-	3	1.16	
HO	S-	Zn	OC-	3	1.16	
HO	S-	Zn	S-	2	-2.83	-3.87
C	S-	Zn	N	3[a]	-1.40	-1.82
C	S-	Zn	Na	3[a]	-1.40	-1.82
C	S-	Zn	O=C	3	-1.37	-1.82
C	S-	Zn	OH	3[a]	-1.26	-2.07
C	S-	Zn	OH2	3[a]	-1.12	-1.45
C	S-	Zn	OH-	3	1.16	
C	S-	Zn	OC-	3	1.16	
C	S-	Zn	S-	2	-1.42	-1.83

Table V. Test of the parametrization. All bond lengths, bond angles, dihedral angles and inter atomic distances are compared for 34 zinc complexes, geometry optimized quantum mechanically and molecular mechanically (with the new zinc parametrization and charges obtained from a quantum chemical Mulliken analysis). The complexes are divided into three groups, viz. four-coordinate systems, five-coordinate systems, and systems with ligands in the second coordination sphere. 14 of the complexes were used in the construction of the parametrization of the zinc ion (listed in Table 1). The comparison is done with, as well as without, the 1,4-nonbonded interactions included.

Group	With 1,4-interactions	Used in the parametrization	No.	bonds (Å)	angles (°)	dihedrals (°)	distances (Å)
all	yes	yes	14	0.078	8.9	25.0	0.76
all	yes	no	20	0.057	8.1	29.6	0.79
all	yes	all	**34**	**0.066**	**8.5**	**27.7**	**0.78**
4 coord	no	yes	7	0.040	5.0	12.8	0.51
4 coord	no	no	4	0.041	4.8	12.2	0.49
4 coord	no	all	11	0.040	4.9	12.6	0.50
5 coord	no	yes	4	0.072	4.6	8.3	0.34
5 coord	no	no	2	0.058	4.0	8.3	0.37
5 coord	no	all	6	0.067	4.4	8.3	0.35
second	no	yes	3	0.046	6.2	18.0	0.47
second	no	no	14	0.043	4.3	11.2	0.44
second	no	all	17	0.044	4.6	12.4	0.44
all	no	yes	14	0.050	5.1	12.6	0.45
all	no	no	20	0.044	4.7	11.0	0.44
all	no	all	**34**	**0.047**	**4.7**	**11.7**	**0.45**

Table VI. Results of the molecular dynamics simulations (MD) and the best (lowest energy) molecular mechanical energy minimisations (MM). The system (large or small), the coordination number (CN), the non-protein zinc ligand(s), the energy and the zinc ligand distances are listed. For the molecular dynamics simulations, the standard deviation and the minimum and maximum values (after 20 ps equilibration) are also given. O1is the ligand in the substrate site, O2 the ligand in the alternative site.

System	CN	Ligand	Method	Energy	Distance to Zn				
				kJ/mole	S46	S174	N67	O1	O2
small	4	H_2O	MD 100ps	1561.0	229	225	214	224	
			stdev.	135	26	26	24	37	
			min.	1013.2	155	164	144	116	
			max.	1859.4	293	307	286	343	
			MM	-52.7	223	218	211	226	
small	4	OH^-	MD 100ps	1665.8	224	222	215	192	
			stdev.	79	20	20	20	16	
			min.	1492.9	164	163	153	143	
			max.	1998.5	287	283	280	232	
small	4	CH_3OH	MD 100ps	1462.9	228	219	214	233	
			stdev.	68	19	20	19	25	
			min.	1254.1	171	161	161	154	
			max.	1785.1	277	278	270	308	
small	4	CH_2O	MD 100ps	1445.0	225	219	215	242	
			stdev.	84	22	19	22	31	
			min.	1230.1	165	170	144	160	
			max.	1761.7	286	273	288	334	
small	5	$(H_2O)_2$	MD 100ps	1733.7	238	226	208	256	223
			stdev.	125	23	25	23	35	33
			min.	1435.6	166	150	148	154	125
			max.	2322.6	317	295	276	353	313
			MM	186.8	226	222	207	243	212
large	4	H_2O	MD 40ps	346.8	231	224	210	229	
			stdev.	61	15	14	15	22	
			min.	234.5	192	184	180	179	
			max.	616.1	265	263	241	277	
			MM	-4308.8	225	224	210	226	
large	5	$(H_2O)_2$	MD 40ps	497.5	234	229	207	250	238
			stdev.	63	16	16	13	19	20
			min.	385.4	191	187	175	194	197

			max.	726.4	276	266	254	294	300
			MM	-4167.5	224	228	208	244	238

Table VII. Quantum chemically optimized structures involving dimethylsulfoxide (DMSO). Energies, basis sets, coordination numbers and zinc-ligand distances of structures are listed. A denotes $Zn(HS)_2(NH_3)$. The basis sets are described in the Methods.

Complex	Basis (Zn / other)	Energy (H)	Distance to Zn			
			N	S1	S2	O1
A(DMSO)	dζ / 6-31G(*)	-3181.649259	212	235	238	203
A(DMSO)	dζ+p / 6-31G*	-3181.793933	218	234	235	212

Figure 1. Difference in the quantum chemical geometries of $Zn(CH_3S)_2(NH_3)H_2O$ and $Zn(HS)_2(NH_3)H_2O$ (shaded). The root-mean-square deviation is 0.044 Å.

Figure 2. The influence of 1,4-interactions on the zinc geometry. $Zn(HS)_2(NH_3)OH^-$ was minimized by molecular mechanics using the zinc parametrization, with (shaded atoms) and without 1,4-interactions in the non-bonded potential.

Figure 3. Performance of the parametrization. $Zn(HS)_2$(imidazole)$(H_2O)_2$ was optimized by quantum mechanics and by molecular mechanics (shaded) using the zinc parametrization. The root-mean-square deviation is 0.23 Å.

Figure 4. The energy minimized structure of alcohol dehydrogenase (large system) with a four-coordinate catalytic zinc ion, compared to the crystal structure[4] (without hydrogen atoms). In (a) the water molecule occupies the substrate site, in (b), the alternative site. Only the amino acids in the small system are shown. (c) shows a detail of the zinc coordination sphere in (a). The root-mean-square deviation of the latter structures is 0.16 Å.

Figure 5. The energy minimized structure of alcohol dehydrogenase (large system) with a five-coordinate catalytic zinc ion, compared to the crystal structure[4] (without hydrogen atoms). Only the amino acids in the small system are shown.

Figure 6. The fluctuation of the distance between the CD atom of Glu68 and the oxygen atom of a. water, b. hydroxide ion, c. formaldehyde, and d. methanol during 100 ps molecular dynamics simulations in the small system of alcohol dehydrogenase. Distances shorter than about 4 Å is indicative for coordination in the alternative zinc site.

Figure 7. The geometry of (a) $Zn(HS)_2$(imidazole)(H_2O) and (b) $Zn(HS)_2$(imidazole)$(H_2O)_2$ optimized by quantum mechanics compared to the corresponding energy minimized structures of the active site of alcohol dehydrogenase (large system; shaded). The root-mean-square deviation is 0.20 and 0.27 Å, respectively.