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Inorganic and bioinorganic molecular mechanics modeling—the problem of the force field parameterization

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Contents

Abstract

A short account of the fundamental principles of molecular mechanics modeling of inorganic compounds, based on recent review articles, is followed by a review of methods for the tuning and validation of force fields. As a case study we then concentrate on recent developments in the modeling of blue copper proteins. Advantages and limitations of purely quantum mechanical methods, QM/MM approaches and empirical force field calculations, as well as the problem of the validation of the results are discussed in detail.
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1. Introduction

Rapid advances in computer hardware and theoretical methodology have established the computation of structures and molecular properties of organic compounds as a complementary tool for experimentalists.

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For transition metal complexes and bioinorganic systems the exploitation of modeling as a powerful and reliable routine tool has only just started. This is due to inherent complications, first, to electronic effects, resulting from partially filled d-orbitals, and second, to the size of biological molecules. Depending on the problem to solve high level quantum-mechanical approaches [1–5], empirical force field calculations [6–8] or empirical correlations [3,9] may be appropriate to predict and/or interpret molecular structures and properties. The choice of the method and the emerging accuracy of the results, limits in their interpretation and possible pitfalls are the first and foremost problems in a modeling study. Various general texts are dealing with this basic and fundamental aspect in various ways [3,4,10].

2. Fundamental principles of molecular mechanics

Molecular mechanics is an efficient approach to optimize molecular structures with generally high accuracy and comparably little computational expense. It is based on the classical parameterization of non-classical (quantum) effects. The fundamental principles, the scope and limits have been outlined [11], specific problems and applications in the area of coordination compounds have been described in detail [7] and the subject has been reviewed extensively [11–18]. The assumption of molecular mechanics is that the coordinates of all atoms of a molecule are determined by forces between each atom and all the others. These are defined by a set of potential energy functions. Structures are computed by minimizing the resulting total steric energy (Eq. (1))

$$E_{\mathrm{tot}} = \sum_{\mathrm{molecule}} (E_{\mathrm{bond}} + E_{\mathrm{angles}} + E_{\mathrm{torsion}} + E_{\mathrm{van\ der\ Waals}} + E_{\mathrm{electrostatics}} + \cdots) \quad (1)$$

Generally, simple potentials are used (e.g. harmonic potentials for bonds and angles and the Lennard-Jones potential for van der Waals interactions) but more elaborate methods have also been described. Additional potential functions have been added to Eq. (1), e.g. penalty functions for out-of-plane bending of planar systems, cross-terms for the correction of weak coupling between the various potentials (non-diagonal force fields) and ligand field terms to account for electronic effects due to the open-shell configuration of transition metal ions. All these features are covered in detail in the literature (see Refs., [7,11–18]). The choice of model is, as expected for a fully empirical approach, a determining factor for the accuracy and reliability of the computed structures (note that this is not only dependent on the software but also on options used within a particular program).

Even more important is the question of how the potential energy functions are parameterized. Generally, atom types define the type of bond (single, double, etc.) between a pair of atoms, the angles around each atom (sp, sp^2, sp^3 etc.) and other electronic effects, such as planarity, torsions and charge distributions. That is, information on electronic factors is included in the assignment of the atom types, and bonding potentials for specific pairs of atom types, angular potentials for specific triples of atom types etc. need then to be parameterized for classes of compounds. It follows that the method for the parameterization, the experimental data set on which the parameterization is performed and the data set on which the parameterization is validated, are the basis for successful molecular mechanics modeling.

3. The force field

3.1. Type of force field

The choice of force field, i.e. the potential energy functions and their parameterization, is dictated by the problem to be solved. Possibilities include general force fields which cover wide areas of chemistry or dedicated force fields developed for a specific type of compound. The lack of general applicability in the latter type of parameterization usually is compensated by a higher accuracy. Since molecular mechanics is an interpolative method, a force field tuned by structural, thermodynamic or spectroscopic data may only be used for the computation of the corresponding type of properties. A parameter set developed to produce structural data does not need to include information about the whole potential energy surface (PES) since only the minima need to be found. The computation of isomer distributions, however, depends on the steepness of the potentials (first derivatives), and to compute vibrational spectra, it is essential to know the curvature of the PES (second derivatives) around the energy minima. For most applications, the essential task, and the first step to consider, is, to accurately reproduce structures, and these are also the basis for an accurate computation of the corresponding spectroscopic and thermodynamic properties of inorganic and bioinorganic compounds.

3.2. Choice of functions

The potential energy functions and their parameterization are strongly interdependent and force field parameters alone are meaningless. Of specific importance for inorganic and bioinorganic force fields is the description of bonding, angle bending and torsions about the metal center. Bonding to (transition) metal ions is generally described by the same potentials used in

general purpose force fields. Often, harmonic functions are used, although the weaker coordinative bonds have been found in a number of studies [19–21] to require anharmonic potentials.

Torsions around metal–donor bonds are quite generally neglected. While there is experimental and theoretical evidence that this is justifiable for σ-bonded ligands this must be different for π-bonded system [7,17,22,23].

Angle-bending around metal centers has been studied extensively and is modeled by various approaches [7]. The most simple and rather successful model is to allow only non-bonded interactions between the donor atoms. This leads to the points-on-a-sphere (POS) model, which is based on a fully geometric approach [24] and has been implemented into inorganic molecular mechanics force fields by replacing the harmonic angle bending functions with 1,3-non-bonded interactions [25]. In models which use angle bending functions these need to include multiple minima around the transition metal centers since, generally, there are various structural possibilities (e.g. square pyramidal and trigonal bipyramidal for five-coordinate compounds, with angles of 90, 120, 180°) [12,26]. A number of simple multi minima potential functions have been described [27–29]. More advanced approaches use ligand field terms [21], e.g. the cellular-ligand-field (CLF) model [30].

3.3. Parameter fitting

This involves the careful choice of a reference data set (training set), a set of appropriate potential energy functions and a method to quantitatively compare the computed with the experimental structural parameters (e.g. by a least-squares fit of the deviation between computed and experimental distances, angles etc.). There are automatic optimization procedures for parameters [16,31–33], and methods based on genetic algorithms or neural networks [34,35], but most parameter development is still done manually.

3.4. Parameters based on experimental structures

The common approach to develop force field parameters is to use experimental structural data (generally X-ray). The procedure is to choose a set of structures which represents the entire structural range of the class of compounds for which the model is developed. With an assumed parameter set all relevant structures are optimized and their quality is evaluated. The parameter optimization is generally based on an iterative procedure that involves changes in the parameter field, optimization of the training set and comparison of the computed with the experimental structural data.

3.5. Parameters based on theoretical structures

The fit to a PES, generated by a higher level theoretical method (e.g. approximate density functional theory, DFT) has the advantage that this may be used although no experimental structural data are available or if these data are not of high enough quality. The PES is scanned by a systematic variation of the structural model. There are two possible methods, a rigid PES, which neglects cross-terms between the parameter that is constrained and all the others, and a relaxed PES which includes parameter correlation. An elegant method to extract the PES is to use the first and second derivatives of the energy, available from the Hessian [33,36–39]. A possible disadvantage is that multiple minima (e.g. for valence angles) are not necessarily detected. Alternatively, experimental data from vibrational spectroscopy or a combination of spectroscopic and ab-initio data can be used to extract the PES [40].

3.6. Published force fields and their evaluation

The force fields currently used in inorganic chemistry may be classified into three groups: (i) universal force fields (e.g. the (UFF [41,42], and other rule-based force fields [27]); (ii) general force fields used for broad ranges of compounds (e.g. MOMEC [22]), and force fields which have been set up for a particular class of structures, e.g. porphyrine complexes. These latter parameterization schemes are often based on extensions of force fields used in organic chemistry, e.g. MM2, 3 [43]. A number of recent textbooks include compilations of published parameter sets and programs [4,44], regularily updated lists are given in review series [45,46] and on some relevant homepages. Note here two important points: (i) force field parameters are highly correlated. That is, it is impossible to extract, e.g. the metal-based parameters from a published force field and use them in another force field with a different parameterization of the ligand backbone. (ii) The force field consists of a set of functions and the corresponding parameter set. That is, extraction of the published parameters and their use in a different program with other potential functions may lead to problems. The conclusion is that published parameters may be a useful start for extensive fitting and validation, and this involves experimental (or QM) structures, thermodynamics and/or spectroscopic data (see above).

4. Conclusion

From the short account on the fundamental principles of molecular mechanical modeling it follows that every chemical structure can be optimized, provided that a suitable force field is available. If this is not the case,

there are well established methods for force field development, even for structural problems where no experimental data are available (specific problems with this very general and probably over-optimistic statement are discussed below). Indeed, in the area of inorganic molecular mechanics modeling there are only very few classes of compounds which have not been studied with force field calculations [7]. In many areas molecular mechanics based predictions have led to valuable ideas and results, and computed structures are very often of high quality. Primarily, this is so because empirical force field calculations are extremely efficient. This allows one to carefully screen the conformational space, to take environmental effects into account (solvation, crystal lattice) and to include dynamic effects. Also, the efficiency of the highly parameterized model allows one to carefully tune and validate the force field (potential energy functions and parameter set). One of the most critical points is that molecular mechanics may not be used for extrapolations, i.e. the force field is dedicated to the class of compounds under investigation and the validation of the model is of importance.

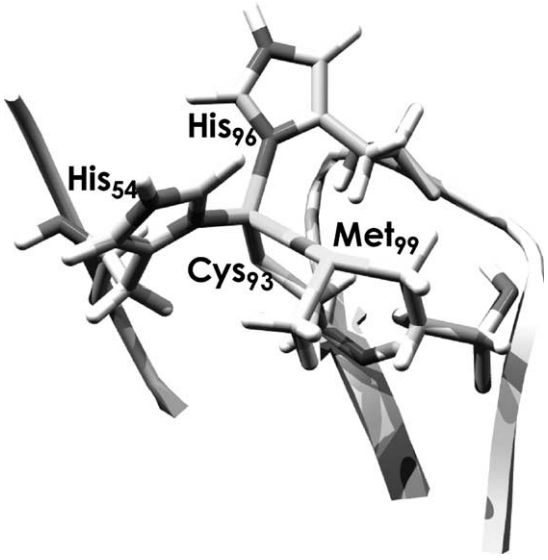

Fig. 1. Active site of Amicyanin (*Paracoccus versutus*, 1AAC), including copper, the donors Cys93, His96, Met99 and His54, and a representation of the C-terminal loop.

In the remaining section we describe the development of a new force field for blue copper proteins [47], for which no general and accurate parmeterization scheme has been available until recently. We will emphasize in particular, where molecular mechanics may be able to help to solve problems in the area of blue copper proteins, which are not efficiently and accurately tackled by other methods and, specifically, where there are problems with respect to the force field development for this class of compounds.

5. Blue copper proteins—a case study

5.1. The problem to solve

Blue copper proteins (cupredoxins, type 1 copper sites) are ca. 10 kDa proteins with a single copper(II/I) site, which is responsible for fast electron transfer, e.g. in plant and bacterial photosynthesis. Their active site structures (see Fig. 1, Table 1), electrochemical and spectroscopic properties (see Table 1) are different from those of classic coordination compounds [48–51]. For these reasons blue copper sites have attracted the attention of coordination chemists for many years. While in the early days of these studies it was believed that the trigonal pyramidal structure of the active site (trigonal bipyramidal in some proteins; various degrees of distortion) is enforced by the protein (entatic state) [52–55] the quite general current believe is that the electronic preference of copper(II) with the given ligand set (a cysteinate, two histidines and a methionine) is for a trigonal pyramidal geometry [47,56–61].

Apart from structure determination the three major objectives of modeling blue copper proteins are: (i) to understand the fundamental reasons for the trigonal pyramidal geometry—a problem which is solved to a large extent [47,59–68]; (ii) to estimate the amount of modulation by the protein of the electronically preferred trigonal pyramidal geometry, and therefore to estimate

Table 1
Properties of wild type blue copper proteins and loop mutants [82–84]

Protein	Cu-plane (Å)	Cu–S_{met} (Å)	A_{460}/A_{600}	EPR	E° (mV)	k_{ese} (M^{-1} s^{-1})
Amicyanin	0.33	2.84	0.10	ax	248	1.2×10^5
Plastocyanin	0.06	2.82	0.12	ax	375	2.1×10^4
AmiPla			0.26	rh	310	3.0×10^3
Azurin	0.11	3.13	0.11	ax	319	1.3×10^6
AmiAzu			0.19	rh	296	1.6×10^4
Pseudoazurin	0.43	2.76	0.41	rh	260	2.8×10^3
AmiPse			0.28	rh	282	1.6×10^4
Rusticyanin	0.30	2.88	0.48	rh	680	1.0×10^4
AmiRus			0.27	rh	360	$< 1 \times 10^4$

the strain induced by the protein backbone (entasis)—there is general agreement that this is relatively small but the quantification still is arguable [59,60,63]; (iii) to correlate the spectroscopic and electron transfer properties with structural features of the copper site (see Table 1 for the variation of some properties)—a task which is largely unfulfilled so far. The computation of molecular properties from structures (experimental or computed) of low molecular weight compounds is routine [20,69–74], and correlations between structures and properties are well established [75]. Structure–property correlations in the area of blue copper proteins have been used extensively [76,77] but these have to be considered with care, see below.

5.2. Scope, limits and general results of various approaches

5.2.1. Experiments

Accurately measured spectroscopic parameters and their thorough interpretation helped to define a structural model of the blue copper site [78,79] before the first crystal structure was solved [80] and proved the model to be correct. More recent sophisticated spectroscopic measurements have, together with quantum mechanical calculations, helped to understand the electronic structure in detail [59,61–68].

A feature which is well established but not generally and not enough appreciated is that, while spectroscopic data of metalloproteins are of similar accuracy to those of low molecular weight compounds, their experimental structures are not [47,60,81]. This clearly emerges from Tables 2 and 3. The variation of bond distances within the chromophore of a specific protein (plastocyanin, see Table 2) is of a similar order of magnitude as the corresponding variation in all relevant blue copper protein structures (see Table 3). Therefore, within the error limit, all experimental structures of the chrompohore of blue copper proteins are close to identical. This is the main reason why it is difficult to correlate molecular properties within the group of blue copper proteins with experimental structural parameters. Note that the accuracy of the individual structures in Table 3 varies considerably; the range of resolution is from 1.3 to 2.8 Å, that of the residuals is from 0.117 to 0.234.

The construction, isolation and characterization of blue copper protein mutants has helped enormously in the understanding of the fundamental properties of cupredoxins [76]. Of specific importance are mutants with a constant donor set, i.e. loop mutants, where the loop which includes three of the donors (Cys93, His96, Met99 in amicyanin, see Fig. 1; the fourth donor, His54 is part of the β-barrel of the protein backbone) is exchanged from one protein by that of another one [82–84]. The induced structural changes lead indeed to a wide variation of the properties (see Table 1) but the experimental structures of these mutants have not been solved so far—and, due to the expected inaccuracies (see above), these would not seem to be very informative.

5.2.2. DFT calculations

The first and most striking result of DFT calculations was that the structure of even a simple model of the blue copper site ($[Cu(imidazole)_2(SCH_3)(S(CH)_3)_2)]^+$) is trigonal pyramidal [64]. The electronic origin of this, for low molecular weight copper(II) compounds uncommon structure, was confirmed by the combination of sophisticated spectroscopic studies with electronic structure calculations and quantum mechanical calculations of other model structures [63,85–87]. Recent DFT studies found that there are two electronic ground states [67,87] with different structures [88], and these have been identified as symmetrical with respect to the two copper-histidine bonds (trig) and asymmetrical (tet), see Table 4 and Fig. 2 [61]. Time-dependent DFT has been used to compute the corresponding UV–vis spectra, and the predicted color change with temperature (temperature-dependent equilibrium between the two structures trig and tet) is in agreement with experimental observations of at least two loop mutants [61].

5.2.3. QM–MM

One of the main differences between computed structures of the copper site of blue copper proteins based on DFT calculations of small models and experi-

Table 2
Selected distances and angles of three different X-ray structures (1PLC [99], 1PNC and 1PND [81]) of plastocyanin (*Pop. nigra*, var. *Italica*) and plastocyanin (*Silene pratensis*; $1BYO_a$, $1BYO_b$) with two different proteins in the unit cell

Cu(II)	Distance to Cu (Å)				L_1–Cu–L_2-angle (°)					
	S_{Cys}	N_b	N_l	S_{Met}	N_b–N_l	S_{Cys}–N_b	S_{Cys}–N_l	S_{Cys}–S_{Met}	S_{Met}–N_b	S_{Met}–N_l
1PLC	2.07	1.91	2.06	2.82	97	132	121	110	89	101
1PNC	2.14	1.97	1.95	2.78	103	132	114	108	88	107
1PND	2.21	2.05	1.96	2.78	102	128	115	107	95	107
$1BYO_a$	2.19	2.02	2.05	2.74	103	134	118	106	84	99
$1BYO_b$	2.11	1.90	2.31	2.79	102	133	117	106	85	108

Table 3
Selected distances and angles of 27 published structures of blue copper proteins (oxidized form) from the PDB data base

Cu(II) model	Distance to Cu (Å)				L_1–Cu–L_2 angle (°)					
	S_{Cys}	N_b	N_l	S_{Met}	N_b–N_l	S_{Cys}–N_b	S_{Cys}–N_l	S_{Cys}–S_{Met}	S_{Met}–N_b	S_{Met}–N_l
1AAC	2.11	1.95	2.03	2.90	105	136	113	111	85	100
1AAN	2.15	1.95	2.00	2.89	109	135	109	109	83	104
1BQK	2.13	1.95	1.92	2.71	100	135	114	107	87	107
1ADW	2.13	2.13	2.11	2.75	101	137	111	113	82	108
1ADW	2.14	2.12	2.15	2.70	100	135	111	114	83	110
1PAZ	2.16	2.16	2.12	2.76	100	136	112	108	87	112
1PMY	2.15	2.07	1.97	2.66	94	144	110	109	86	112
1ZIA	2.14	1.88	1.99	2.71	100	134	114	108	88	110
4PAZ	2.14	1.98	1.96	2.76	103	136	110	108	88	110
6PAZ	2.13	2.01	2.07	2.90	98	135	118	108	85	107
8PAZ	2.13	2.01	2.01	2.71	100	133	114	107	89	111
1RCY	2.26	2.04	1.89	2.88	105	128	119	106	85	106
1AG6	2.15	1.96	2.01	2.88	103	130	120	106	87	102
1BAW	2.10	2.02	2.13	2.73	102	123	120	109	93	107
1BAW	2.09	1.92	2.23	2.77	101	129	116	109	94	103
1BAW	2.16	1.94	2.17	2.70	102	124	114	109	98	107
1BXU	2.14	1.97	2.01	2.94	101	131	121	108	86	125
1BYO	2.11	1.90	2.31	2.79	102	133	117	106	85	108
1BYO	2.19	2.02	2.05	2.74	103	134	118	106	84	99
1BYP	2.15	2.01	2.05	2.84	100	133	119	108	86	104
1IUZ	2.18	2.08	2.06	2.69	96	133	115	113	90	105
1KDJ	2.26	1.93	2.07	2.92	107	126	118	107	81	110
1PCS	2.25	2.03	2.07	2.65	106	130	115	109	85	115
1PLC	2.07	1.91	2.06	2.82	97	132	121	110	89	101
1PNC	2.14	1.97	1.95	2.78	103	132	114	108	88	107
1PND	2.21	2.05	1.96	2.78	102	128	115	107	95	107
7PCY	2.12	1.89	2.17	2.92	104	125	120	108	90	102
Average [a]	2.15	1.99	2.05	2.79	102	132	115	108	87	107
Max [b]	2.26	2.16	2.23	2.94	109	144	121	114	98	125
Min [c]	2.07	1.88	1.89	2.65	94	123	109	106	81	99
σ [d]	0.05	0.07	0.08	0.09	3.3	4.6	3.7	2.2	4.0	5.3

[a] Average of the 27 X-ray structures (not weighted).
[b] Max is the maximum value for each parameter.
[c] Min is the minimum value for each parameter.
[d] σ is the standard deviation for the 27 evaluated X-ray structures.

mental observations is a copper–methionine bond which is significally shorter in the model calculations (see Table 4). This is due to the fact that by these simple DFT model calculations the influence of the protein is not included and the variation of structures and properties (see Table 1) of the various species can therefore not be evaluated. The influence of the protein backbone can be accounted for by its modeling with simple empirical force field calculation, i.e. by a combination of quantum mechanical modeling of the copper site with molecular mechanical modeling of the proteine backbone (QM/MM) [60,89]. The main results of these QM/MM studies

Table 4
Selected distances and angles of the optimized DFT models *trig* and *tet* for the active site of blue copper proteins [61]

Cu(II) model	Distance to Cu (Å)				L_1–Cu–L_2 angle (°)					
	S_{Cys}	N_b	N_l	S_{Met}	N_b–N_l	S_{Cys}–N_b	S_{Cys}–N_l	S_{Cys}–S_{Met}	S_{Met}–N_b	S_{Met}–N_l
trig	2.23	2.07	2.07	2.70	104	120	121	116	95	94
tet	2.24	2.06	2.15	2.52	104	132	104	114	99	100
exp [a]	2.15	1.99	2.05	2.79	102	132	115	108	87	107
σ [b]	0.05	0.07	0.08	0.09	3.3	4.6	3.7	2.2	4.0	5.3

[a] exp is the average of the 27 X-ray structures (see Table 3).
[b] σ is the standard deviation for the 27 evaluated X-ray structures.

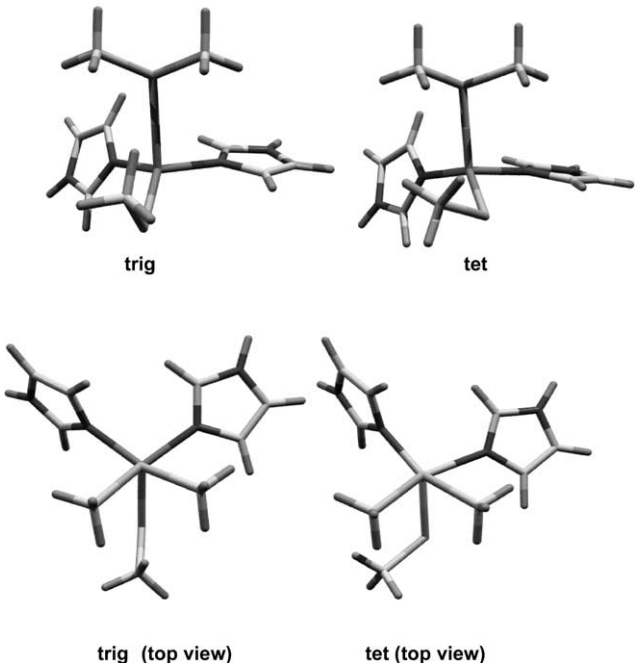

Fig. 2. Two views of the DFT optimized models *trig* and *tet*, parallel to the S_{Cys}–copper bond and parallel to the S_{Met}–copper bond (top view) [61].

are that the computed structures are in agreement with the experimental data (see Table 5) and that the resulting strain energies imposed by the protein (entasis) are in agreement with estimates based on experiments [55,60]. The problem is that it is impossible to judge the accuracy of the computed structures due to the inherent inaccuracy of the experimental data (see above, Tables 2 and 3).

5.2.4. Molecular mechanics

For the efficient computation of structures of a series of blue copper proteins, for conformational analyses and the investigation of dynamic properties there is a need for an accurate and general force field for type 1 copper sites. So far there are only few studies of cupredoxins using force field methods, and most use specific force fields dedicated or tuned to a single species [39,90,91] or have the copper site constrained [92]. An

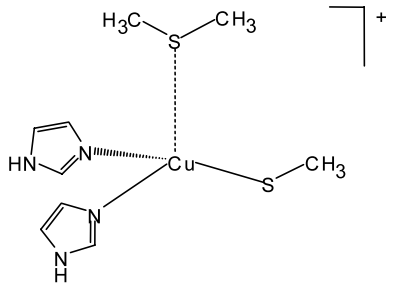

Fig. 3. Structural model $[Cu(Im)_2(SCH_3)(S(CH_3)_2)]^+$, used for the ab-initio and model MM calculations.

attempt to develop a general force field for the copper(II) form of blue copper proteins was recently reported [47]. This uses a DFT-based parameterization scheme for the copper site and the AMBER force field [93] for the protein.

This force field may need further improvement (see below). It uses harmonic potentials for the bonds to and the angles around the copper center. Their parameterization is based on a rigid PES (DFT) of the model structure (see Fig. 3). The resulting potential energy curves (computed energy vs. bond distance (valence angle)) are given in Fig. 4. These were used to fit the corresponding molecular mechanics curves by least-squares fitting and thus developing the corresponding force field parameters. These are given in Table 6, and a comparison of the structures of the model compound (Fig. 3), computed by DFT (structure trig, see Table 4 and Fig. 2) and molecular mechanics is given in Table 7.

Apart from other factors (see below) the parameterization scheme depends heavily on the accuracy of the DFT-based PES. The requirement for the quantum mechanical model is that it produces accurate structures and relative energies. The DFT model used (wave function, basis set, functional) is similar to those used successfully in a broad range of studies in the area of copper coordination chemistry and in computational work on blue copper proteins.

An important question is whether the PES scans should be rigid (as in the published study [47]) or relaxed (i.e. at each point, when varying a specific parameter, the structure is fully optimized). It appears that for the

Table 5
Comparison of the optimized QM/MM structures with the corresponding experimental data [60]

	Distance to Cu (Å)				L_1–Cu–L_2 angle (°)					
	S_{Cys}	N_b	N_l	S_{Met}	N_b–N_l	S_{Cys}–N_b	S_{Cys}–N_l	S_{Cys}–S_{Met}	S_{Met}–N_b	S_{Met}–N_l
Ami [a]	2.23	2.05	2.05	2.92	100	129	118	116	85	102
1AAC	2.11	1.95	2.03	2.90	105	136	113	111	85	100
Rus [b]	2.23	2.05	2.05	2.93	101	133	113	113	81	111
1RCY	2.26	2.04	1.89	2.88	105	128	119	106	85	106

[a] Amicyanin, computed data.
[b] Rusticyanin, computed data.

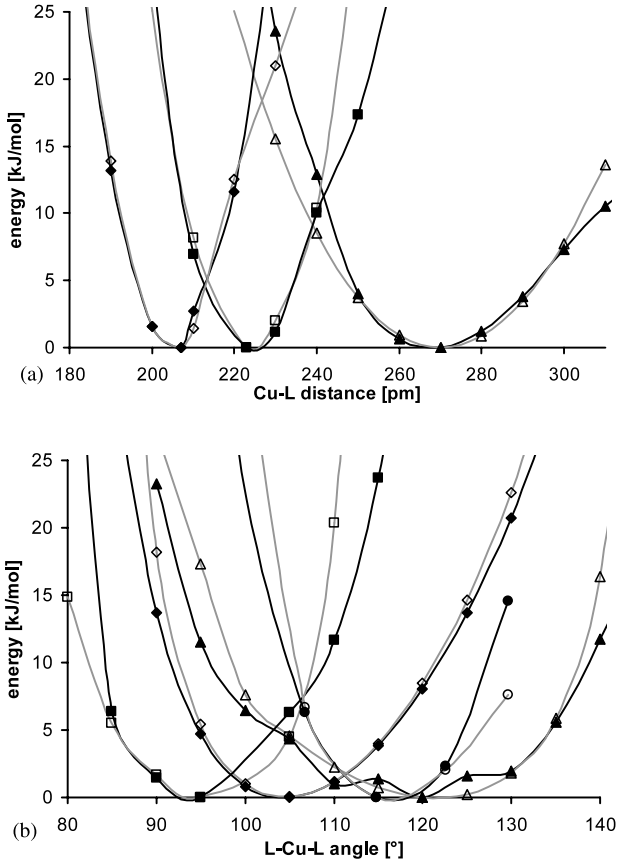

Fig. 4. (a) Cu–L distances (Å) vs. energy (kJ mol^{-1}) of the gas phase model (see Fig. 3); full symbols (black curves): DFT, open symbols (grey curves): MM; diamonds: Cu–N_{His}, squares: Cu–S_{Cys}, triangles: Cu–S_{Met} [47]. (b) L–Cu–L angles (°) vs. energy (kJ mol^{-1}), curves calculated with the model shown in Fig. 3; full symbols (black curves): DFT, open symbols (grey curves): MM; squares: N_{His}–Cu–S_{Met}, diamonds: N_{His}–Cu–N_{His}, circles: S_{Met}–Cu–S_{Cys}, triangles: S_{Cys}–Cu–N_{His} [47].

development of force fields without cross-terms each individual potential is best described by a rigid potential energy scan. This represents a situation where each potential is independent of all the others. Although this is a common concept in molecular mechanics and the basis of the general equation (1), this obviously is not strictly valid [7], and coupling between the various terms has been included in various models as a perturbation by the addition of cross-terms to Eq. (1) [94]. In the molecular mechanics model used [47] no cross terms were included, and this fully represents the situation of a rigid PES computed by DFT.

Another question is whether a harmonic bonding potential can represent the weak copper–methionine interaction. From Fig. 4a it emerges that, in the critical range of $2.5 < Cu–S < 3.0$ Å, there is good agreement between the DFT and MM potential curves. Therefore, the harmonic bonding potential was expected to be suitable to model the copper–methionine interaction [47].

Table 6
(a) Ideal bond distances and stretching force constants, and (b) ideal valence angles and angle bending force constants, introduced to the AMBER force field [47]

Bond	k_b (kcal (mol Å^2)$^{-1}$)	r_0 (Å)
Cu(II)–S_{Met}	15.00	2.700
Cu(II)–S_{Cys}	50.00	2.230
Cu(II)–N_{His}	131.50	2.067
$C_{\gamma,\varepsilon,Met}$–$S_{Met}$	222.00	1.810
$C_{\gamma,His}$–$H_{\gamma His}$	340.00	1.080
Angle	k_θ (kcal (mol rad^2)$^{-1}$)	θ_0 (°)
N_{His}–Cu(II)–N_{His}	22.00	103.00
S_{Cys}–Cu(II)–N_{His}	8.00	115.00
S_{Met}–Cu(II)–N_{His}	5.00	105.00
S_{Cys}–Cu(II)–S_{Met}	5.00	100.00
$C_{\varepsilon 1,His}$–$N_{\delta 1,His}$–Cu(II)	40.00	126.00
$C_{\gamma,His}$–$N_{\delta 1,His}$–Cu(II)	40.00	126.00
$C_{\gamma,\varepsilon,Met}$–$S_{Met}$–Cu(II)	35.00	109.50
$C_{\beta,Cys}$–S_{Cys}–Cu(II)	30.00	109.50
$C_{\gamma,Met}$–S_{Met}–$C_{\varepsilon,Met}$	62.00	98.90
$H_{\beta,Cys}$–$C_{\beta,Cys}$–S_{Cys}	35.00	109.50
$C_{\delta 2,His}$–$C_{\gamma,His}$–$H_{\gamma,His}$	35.00	120.00
$H_{\gamma,His}$–$C_{\gamma,His}$–N_{His}	35.00	120.00
$C_{\beta,Cys}$–S_{Cys}–Cu(II)	30.00	109.50
$C_{\beta,Met}$–$C_{\gamma,Met}$–S_{Met}	50.00	114.70

The most interesting feature emerging from the computed potentials is that, for the N_{His}–Cu–S_{Cys} angle bending, the DFT curve has three local minima at ca. 110, 120 and 130° (see Fig. 4b) [47]. These are rather shallow and in the published first generation force field the MM potential was fitted as a single minimum function with an optimum angle of 120° [47]. From experimental data it emerges that there is an asymmetry in the observed angles with the angle to the loop histidine generally being smaller (ca. 115°) than that to the backbone histidine (ca. 132°, see Tables 3 and 8). From DFT calculations it also emerges that the multi minima potential for the N_{His}–Cu–S_{Cys} angle is not an artifact. Two nearly degenerate ground states have been found [88], and one was refined with trigonal symmetry (two N_{His}–Cu–S_{Cys} angles of 120°), the other with the corresponding angles at 115 and 130°, and these structures and the corresponding computed electronic properties are in agreement with experimental observations [61]. Therefore, a general and accurate second generation force field should include a multiple minima function for this angle potential.

A major problem in developing a general force field for blue copper proteins is its validation. Part of the variation in spectroscopic and redox properties of the blue copper site ($k_{electron\ self\text{-}exchange} \sim 10^3$–$10^6$ M^{-1} s^{-1}; $E^\circ \sim 200$–700 mV; $A_{460}/A_{600} \sim 0.1$–0.5, see Table 1) is believed to be due to structural changes of the copper chromophore. The range of the reported specific bond distances around the copper center is of the order of 0.15 Å and that of valence angles is around 10° (see Tables 3

Table 7
Selected distances and angles of the optimized gas phase $[Cu(Im)_2(SCH_3)(S(CH_3)_2)]^+$ model

	Distance to Cu (Å)				L_1–Cu–L_2 angle (°)					
	S_{Cys}	N_b	N_l	S_{Met}	N_b–N_l	S_{Cys}–N_b	S_{Cys}–N_l	S_{Cys}–S_{Met}	S_{Met}–N_b	S_{Met}–N_l
DFT	2.23	2.07	2.07	2.70	104	120	121	116	95	94
MM	2.23	2.06	2.06	2.69	103	118	115	117	103	99

See Fig. 3; DFT- and MM-optimized structures; the MM-structure was calculated with the S_{Met}–Cu–S_{Cys}–C^a torsion restrained to 0°) [47].

and 8). The accuracy of the reported structures is variable. One of the most accurate structures (1PLC) has standard deviations of the Cu–donor distances of ca. 0.04 Å. If one assumes that all other experimental structures have error limits which are at least as large, the computed structures (Table 8) are of acceptable quality. Unfortunately, no small molecule coordination compounds with a comparable ligand set (two imidazols, a thioether and a thiolate) are available as a more accurate reference for the force field. Therefore, any improvement of the molecular mechanics model (e.g. anharmonic potentials, multiple minima functions etc.) will need other than experimental structural data for the validation [47].

Experimental spectroscopic data and redox reactivities of metalloproteins may be determined very accurately. Therefore, if such properties could be correlated with structural parameters or if such properties could be computed with some accuracy from structures, these correlations or computations might be used to validate the force field. The computation of ligand field spectra (dd transitions) and EPR spectra by angular overlap model calculations, based on computed structures (MM-AOM) is well established [70,95,96], and other similar techniques include the simulation of EPR spectra of weakly coupled oligonuclear systems (MM-EPR) [72,97] and of redox potentials (MM-Redox) [20,71]. The computation of the charge transfer transitions of blue copper proteins by TD-DFT calculations has lead to important new results [61] but the accuracy of the computed transition energies and intensities is, at present, less than satisfactory. There is some hope that a careful analysis of the ligand field transitions [98] by AOM could lead to an MM-AOM model for blue copper proteins and, therefore, to a possibility to further tune force field parameters.

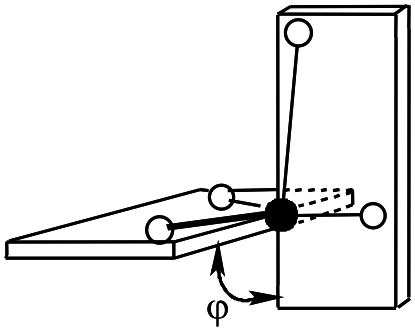

Fig. 5. Twist angle φ between two planes defined by N–Cu–N and S–Cu–S.

Table 8
Selected distances and angles of the optimized (MM) and observed (cr) protein structures

Structure	Distance to Cu (Å)				L_1–Cu–L_2 angle (°)					
	S_{Cys}	N_b	N_l	S_{Met}	N_b–N_l	S_{Cys}–N_b	S_{Cys}–N_l	S_{Cys}–S_{Met}	S_{Met}–N_b	S_{Met}–N_l
$1AAC_{cr}$	2.11	1.95	2.03	2.90	105	136	113	111	85	100
$1AAC_{MM}$	2.23	2.08	2.06	2.80	101	133	113	117	84	102
$1BQK_{cr}$	2.13	1.95	1.92	2.71	100	135	114	107	87	107
$1BQK_{MM}$	2.23	2.07	2.06	2.76	102	133	113	108	84	113
$1RCY_{cr}$	2.26	2.04	1.89	2.88	105	128	119	106	85	106
$1RCY_{MM}$	2.22	2.07	2.06	2.78	102	133	104	114	83	124
$1KDJ_{cr}$	2.26	1.93	2.07	2.92	107	126	118	107	81	110
$1KDJ_{MM}$	2.21	2.07	2.07	2.72	112	119	120	110	80	107
$6PAZ_{cr}$	2.13	2.01	2.07	2.90	98	135	118	108	85	107
$6PAZ_{MM}$	2.22	2.05	2.07	2.75	104	122	122	108	82	111

The coordinates for the X-ray data are from 1AAC for Amicyanin (*Paracoccus denitrificans*), 1RCY for Rusticyanin (*Thiobacillus ferrooxidans*), 1BQK for Pseudoazurin (*Achromobacter cylcloclastes*), 6PAZ for Pseudoazurin (P80I mutant *Alcaligenes faecalis*) [100] and 1KDJ for Plastocyanin (*Dryopteris crasshirizoma*) [47].

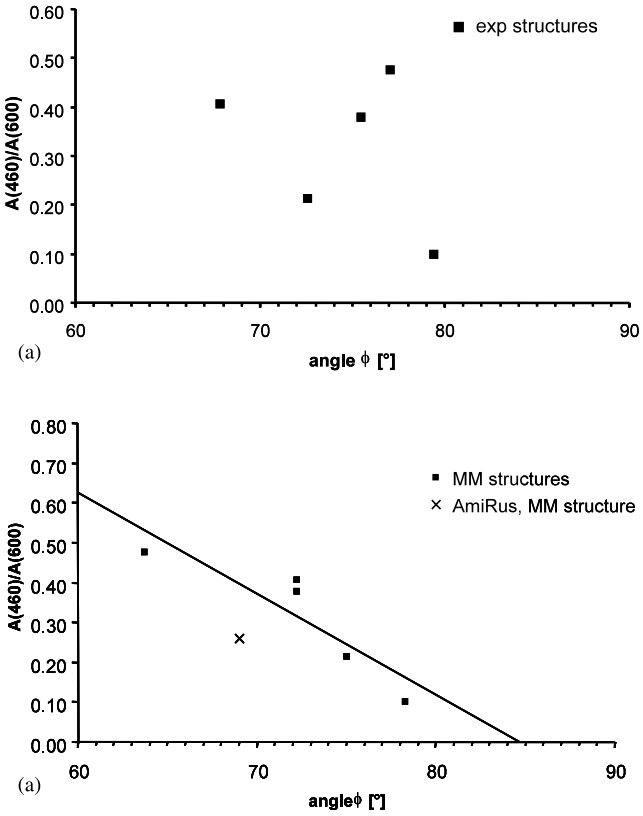

Fig. 6. (a) Plot of the angle φ between the two planes, defined by S_{Met}, S_{cys}, Cu and N_{His}, N_{His}, Cu from X-ray data, with the ratio of the experimentally observed intensities of the two absorption bands at ca. 460 and 600 nm [47]. (b) Correlation of the angle φ between the two planes, defined by S_{Met}, S_{cys}, Cu and N_{His}, N_{His}, Cu from MM data with the ratio of the experimentally observed intensities of the two absorption bands at ca. 460 and 600 nm; structural data from Table 8 [47].

Among the possible correlations of spectroscopic with structural parameters there is the intensity ratio of the two major charge transfer transitions (A_{460}/A_{600}) with the angle φ defined by the metal center and two donor atoms each (see Fig. 5) [67]. An interesting feature is that, while there is no obvious correlation of the experimental data (Fig. 6a), the experimental spectroscopic data are reasonably well correlated with the structural parameters computed by molecular mechanics (Fig. 6b) [47]. This certainly is not strong direct evidence for the quality of the force field but it indicates that the parameterization scheme might be reasonable and, more importantly, it shows where future studies on force fields for metalloproteins might lead to success.

The conclusions of the molecular mechanics study of blue copper proteins [47] are: (i) the results of the MM and of the time consuming QM/MM calculations are very similar (Tables 5 and 8). (ii) There are attractive possibilities to improve the quality of the force field (anharmonic bonding potentials, multiple minima angle bending potentials, electrostatics, solvation). (iii) However, the first and most important problem to solve is to develop a valuable method for the validation of the force field.

6. General conclusions

Molecular mechanics is an empirical technique to compute structures and, in favorable cases, relative stabilities and vibrational spectroscopic parameters. Various approaches are available for the accurate structure optimization of transition metal complexes [7,13,21,22,29]. Therefore, molecular mechanics is an ideal technique for the efficient computation of large systems which include metal centers, such as metalloproteins. It relies on a force field which may be based on experimental observations or ab-initio calculations. As for any model calculations, especially for highly parameterized, fully empirical and interpolative techniques, one of the most important tasks is to validate the model for the class of compounds to be computed, and this usually is done on the basis of experimental structural data. Their accuracy is an important point and needs to be considered thoroughly—too often chemists tend to uncritically believe X-ray data. If no accurate structural data are available, there might be interesting and valuable methods to validate force fields with more easily available and accurate spectroscopic data. The demanding problem in metalloprotein modeling is to develop these tools. In the area of small molecule transition metal coordination compounds, where accurate force fields are available, simple correlations of properties with coordination geometries are well established [75], and more elaborate methods, involving spectra simulations [72,73] and ligand field calculations [70,71,74] have helped to establish empirical modeling methods as a powerful and widely accepted tool. It also has helped to improve and validate force fields, and this is what now is needed in the area of metalloproteins.

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