On The Frontier Bond Location In The QM/MM Description Of Peptides And Proteins.

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Abstract. We propose to consider two different Quantum Mechanics/Molecular Mechanics partitions of peptides. Not only the usual C_α–C_β bond is considered as the frontier but also the less common C–N and N–C peptide bonds are investigated as putative QM/MM boundaries. When the frontier atom is a C atom then the partial double bond character of the peptide bond is naturally taken into account by our scheme. However, when the frontier atom is nitrogen, then special care has to be taken. Instead of the usual mono valence electron description that was previously proposed, the N frontier atom is described with 5 electrons (2 core and 3 valence) to allow him to share part of his valence electrons with the carbon atom, and then to recover the partial double C–N bond of the peptide.

Keywords: Protein, Peptides, Enzyme, hybrid method, QM/MM.
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INTRODUCTION

Since the creation of Quantum Mechanics/Molecular Mechanics (QM/MM) methods in 1976[1], they have been largely used to study peptides, enzymes and proteins[1-6]. They have been developed to handle phenomena that require a quantum chemical treatment, like a chemical reaction, a photon absorption, …, in fact all phenomena sensible to the motion of electrons. The complete description of these systems by means of QM tools only is totally out of reach of current day facilities because of their huge size. However, as chemical reactions or related phenomena are most of the time well localized in space over few atoms, it has been proposed to divide the macromolecules in two parts. The small part (active site) which necessitates the explicit description of the electrons is treated at the QM level of theory. The second part is composed of the remaining atoms of the molecular system, and is called the surroundings. This part cannot be modeled by an uniform dielectric continuum, as we often do to model solvent, since it strongly constrains the geometry of the active part and since the electric fields created by the secondary structures (α-helix, β-sheet, γ-turn, …) are totally different and absolutely non isotropic. Hence MM is used to describe explicitly the surroundings. It is noteworthy that to divide the macromolecule in two fragments, one has to formally cut covalent bonds, leading to the famous "dangling bonds" problem. Several solutions have been proposed to tackle this setback, and they can be classified in two families. The first one uses monovalent atoms to saturate the free valences and will be referred to as the Link Atom (LA) family. The LA is most of the time a hydrogen atom[2-14], or a parametrized atom, like in the pseudo-bond[15], the adjusted connection atom[16] or the capping potentials[17] philosophies. The second family uses Frozen Orbitals (FO) to connect the QM subsystem to the MM one. The first one that we developed since ten years[18-20] at the *ab initio* level uses doubly occupied strictly localized bonding orbital to describe the QM/MM frontier. A similar method was published by Friesner and coworkers[21-23], and a closely related method, named Generalized Hybrid Orbital (GHO) was proposed originally by Gao[24-28] and uses three atomic hybrid orbitals to represent the three dangling bonds. The effective fragment potential (EFP)[29] method defines a buffer region over few bonds and frozen orbitals delocalized over the bonds between the QM and MM parts.

Usually, the QM/MM boundary is set upon a C–C single bond—the C_α–C_β or the C_α–$C_{(=O)}$ bond of the amino acid—since the bond polarity is weak and close to that of a C–H bond (often used in LA schemes). In these cases the

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backbone of the peptide is treated with MM and the side chain with QM. However, one must be aware that these partitions are somehow limited. First, the QM part can be too small (with the C_α–C_β frontier bond) or asymmetric (with the C_α–$C_{(=O)}$ frontier bond) and the chemical process under investigation can be dramatically perturbed by the too close frontier or by the asymmetric description of the surroundings. Second, the remaining classical atomic point charges of the MM force field do not necessary sum up to an integer, and the frontier charges have to be arbitrarily changed or adjusted to keep the charge of the total system consistent[30].

A more natural choice, although more challenging, to divide a peptide would be to slice the macromolecule in amino acids by cutting through the peptide bonds. However, the partial double bond character of the C–N bond cannot be easily tackled by the LA methods (one could hardly think of a C–H double bond!). One attempt even shows some instabilities of the wave function[31]. Nevertheless, this partition would solve the above mentioned limitations. In this communication we will show that the Local Self-Consistent Field scheme[18] can easily be applied to the peptide bond separation without any additional parameter or modification.

The fundamental principles on which our method is based will be recalled briefly in the first section. The solutions we propose for the QM/MM partitions will be gathered in the second section, and the results will be presented in the third one.

LSCF AND LSCF/MM

Let us start with a macromolecule that we cut in two pieces, one to be treated with MM and the other with QM. To do so, one is obliged to formally cut one or several covalent bond. We call these bonds frontier bonds. One atom of the frontier bond will be on the QM side and is denoted X. The atom on the MM side of the frontier bond is labeled Y. If several bonds are cut then the QM frontier atoms are denoted X_i and the MM ones Y_i, with i running from 1 to the number of cut bonds. We proposed[18] that each frontier bond could be represented by a doubly occupied strictly localized bonding orbital (SLBO). The SLBOs are obtained on small model molecules containing the bond of interest, thanks to the transferability principle on which MM methods are based. The SLBOs aim at representing as best as possible the electronic density of the frontier region. They need to remain localized on the frontier bond they are supposed to describe. For that reason we consider them as frozen during the resolution of the self-consistent field (SCF) equations that give the wave function of the QM fragment. Although we recently propose a method that allows the relaxation of the SLBO according to the QM wave function variations[32], here we will consider the SLBO as fixed or frozen. To avoid the tedious care that one is obliged to take when dealing with non orthogonal orbitals, we require that the molecular orbitals (MOs) of the QM fragment are orthogonals to the frozen SLBOs. To solve this problem we have proposed a new way to perform the SCF procedure, called the Local Self-Consistent Field method (LSCF) that is detailed below.

The frozen orbitals (FOs) $|l_i\rangle$, let say L of them, are given as the linear combination of the K basis functions $|\phi_\mu\rangle$ ($\mu = 1, K$):

$$|l_i\rangle = \sum_{\mu}^{K} a_{\mu i} |\phi_\mu\rangle \tag{1}$$

If several FOs are given then they are orthogonalized[33-34]. Note also that the FOs can be of any shape and be occupied or not. The last option is useful to deal with core excited states[33-34]. For the sake of simplicity, let us consider that they are already orthogonal. The first step of the LSCF method is to project out of the subspace span by the FOs each basis function, as shown in equation 2.

$$|\tilde{\phi}_\mu\rangle = N_\mu \left(|\mu\rangle - \sum_{i}^{L} |l_i\rangle\langle l_i|\phi_\mu\rangle \right) \tag{2}$$

where N_μ is a normalization constant. The modified basis set $|\tilde{\phi}_\mu\rangle$ ($\mu = 1, K$) contains K functions that are orthogonal to the L FOs. However, as L independent linear combinations are already defined (the L FOs) the modified set possesses at least L linear dependencies. They are removed during the second step of the LSCF procedure, thanks to the canonical orthogonalization[35] that gives a set of $(K - L)$ orthogonal functions. The two steps are assembled in a unique matrix transformation[18,19] that transforms the initial non orthogonal basis functions in a set of functions that are mutually orthogonal and orthogonal to the FOs. The transformation matrix, B, is rectangular with dimensions $K \times (K - L)$. This matrix is for LSCF what the Löwdin $S^{-1/2}$ matrix is for SCF. One has to note that, so far, the LSCF method is purely a QM method.

The QM/MM adaptation carries the acronym LSCF/MM. In that case, the FOs are doubly occupied SLBOs. The total energy, E_{Total}, can be written as

$$E_{Total} = E_{QM} + E_{MM} + E_{QM/MM} \tag{3}$$

where E_{QM} is the energy of the QM part, E_{MM} the energy of the MM fragment and $E_{QM/MM}$ the interaction between the two subsystems. E_{QM} contains the kinetic energy of the electrons, the electron-nuclei attraction, the electron-electron repulsion and the nuclei-nuclei repulsion of all the QM atoms, Y_i atoms included. Note that the density matrix contains the contribution from the variational MOs but also from the FOs. E_{MM} corresponds to bonded interactions (bond stretchs, valence angle stretchs, dihedral angle torsions, out-of-plan torsion), electrostatic interactions and van der Waals (vdW) interactions between MM atoms[i], Y_i atoms included. The interaction energy, $E_{QM/MM}$, matches the electrostatic electron-classical point charge interaction, the electrostatic nuclei-classical point charge interaction, the vdW interactions between QM and MM atoms[ii], and some bonded MM terms involving MM atoms close to the frontier[36]. The electrostatic interaction between the electrons of the QM subsystem and the classical atomic point charges of the surroundings deserves a special attention. When asking the total energy to be stationary with respect to the variation of the spinorbitals, one has to remember that the electron-point charge interaction depends on the electronic density. Then, naturally, the Fockian incorporates a specific term that will ensure the polarization of the QM fragment due to the remaining part of the system,

$$F_{\mu\nu} = F^0_{\mu\nu} + \sum_c^{MM} \left\langle \phi_\mu \left| \frac{q_c}{r_c} \right| \phi_\nu \right\rangle \tag{4}$$

where $F_{\mu\nu}{}^0$ is the usual Fock matrix element expressed over the basis functions ϕ_μ and ϕ_ν, q_c is the classical point charge of the MM atom, and r_c the electron-point charge distance.

The Y_i atoms require special attention since they have two sides. On one side they are MM atoms since they participate to MM interactions (bonded and non-bonded) with the other MM atoms. On the other side, they can be considered as QM atoms since they possess atomic basis functions and they participate especially to the chemical bonds with the X_i atoms by sharing electrons attracted by the nuclear charges. Since most of the time the frontier bonds can be considered as covalent bonds, each Y_i atom contributes to 1 electron to the bond and then has a corresponding nuclear charge equal to +1 in addition to his classical atomic point charge. However, in dative situations, the Y_i atom can give 2 or 0 electrons, dependent whether it is a donor or an acceptor respectively, and accordingly have a nuclear charge of +2 or 0 respectively. Specific frontier bond potentials, containing five parameters, need to be determined to achieve a correct description of the frontier bond length[19]. Further details can be found somewhere[18-20].

PARTITIONS

In general, the LSCF scheme works perfectly well when the frontier bonds can be correctly represented by localized orbitals[10, 14, 15, 17-19]. For instance, the C_α–C_β bond is a well localized two electrons–two centers bond. *A contrario* the peptide bond can hardly be considered as local. In fact the C–N bond possesses a certain partial double bond character due to the charge transfer from the nitrogen to the carbon (figure 1). The resonance is responsible for the planar geometry of the peptide bond.

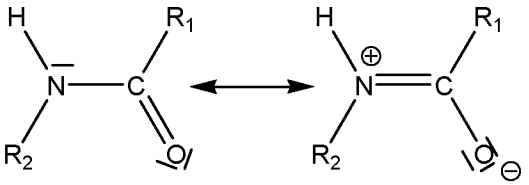


FIGURE 1. Resonance between the two Lewis structures of the peptide bond.

Since the number of electrons between the C and N atoms is not an integer (it is greater than 2) and since it can fluctuate depending on the surroundings (the weight of the zwiterrionic form increases if the O atom is involved in a hydrogen bond for example), one cannot represent it with a frozen electronic density. Two possibilities have to be investigated. Either the C or the N atom is the frontier Y atom. If the C atom is the hybrid Y atom, then the nitrogen

[i] The 1-4 condition is respected.
[ii] The vdW parameters of QM atoms are set to the values defined for the corresponding atom type of the force field.

atom is treated quantum mechanically and the partial delocalization of the nitrogen lone pair is possible. In fact, many basis functions are still available on the carbon atom (few are taken to build the SLBO) to accept the N lone pair. However, if the frontier atom is the nitrogen then the charge transfer is impossible since the N atom possesses only one electron and it is involved in the SLBO.

We recently show that if core electrons of the Y_i atoms are introduced in the QM system, by mean of frozen core orbitals[20] (FCO) or considered as any other electron[36] (self-consistent core orbital, SCCO), then the LSCF/MM method do not require any extra parameters to recover the correct geometry and energetic. Of course, the nuclear charge of the Y_i atoms needs to be augmented by two units for second row elements (Li to Ne) or by ten units for third row elements (Na to Ar), and so on. Here, the N or C hybrid atoms would have a +3 nuclear charge. Pushing the idea one step further, one could think that giving a lone pair to the N frontier atom, and consequently a nuclear charge equal to +5, would allow the partial delocalization.

To illustrate the performance of our method with the usual C_α–C_β frontier bond and with the peptide frontier bond, we decide to investigate the proton affinities of histidine residue engaged in a tetrapeptide. Proton affinities is one of the most delicate properties to correctly obtain since the electrostatic component of the QM/MM interaction is very large[30]. A subtle variation of few tenths of the total electric charge can result in several tens of kcal/mol for the proton affinity.

ILLUSTRATIVE CALCULATIONS

Since our aim is to compare QM/MM results, obtained will different partitions, with pure QM calculations, we decide to compute the proton affinity of a small enough molecule to be able to run QM computations, but also large enough molecule to have a meaningful QM/MM partition. Our choice ends on a tetrapeptide. The three protonation states of the histidine residue are displayed on figure 2, where the histidine amino acid is engaged in a tetrapeptide capped by the acetyl group at the N–terminus, and the N-methylacetamide group at the C–terminus. The three central residues are isoleucine, histidine, and tyrosine (Ace–Ile–His–Tyr–NMe). Since we are looking for the comparison of pure QM and hybrid QM/MM results, the level of theory is not important. It was arbitrarily chosen as RHF/6-311G**.

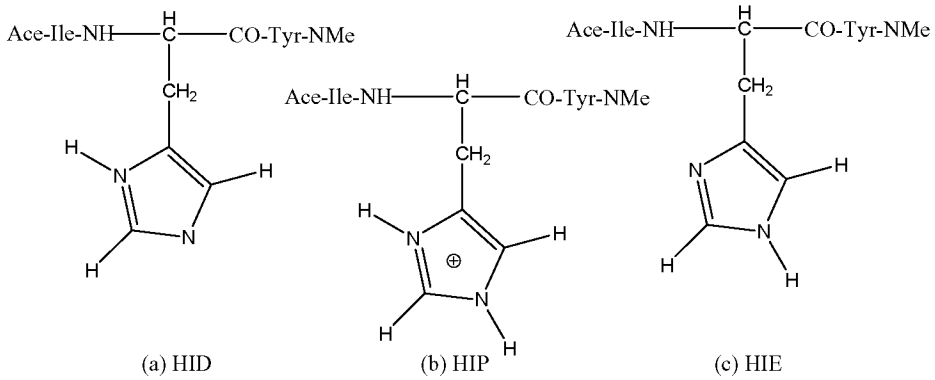
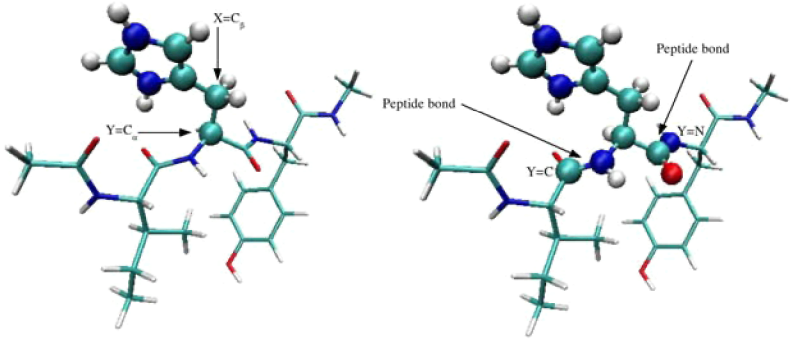


FIGURE 2. Protonation states of the histidine residue. (a) HID; proton at the δ position. (b) HIP: protons at both the δ and ε positions. (c) HIE: proton at the ε position.

When the frontier is located on the C_α–C_β bond of the histidine residue, the Charmm27[37] force field was used since it allows an integer electric charge of the MM and of the QM parts. However, when the frontiers are set on the two peptide bonds surrounding the histidine amino acid the Amber *ff*99[38-39] force field was chosen as it is one of the most frequently employed force field (figure 3). In each case, the Weinstein-Pauncz[40-41] localization criterion was used to determine the SLBO on the ethane model molecule for the C–C bond and on the N-methylacetamide model molecule for the C–N amide bond.

All calculations have been performed with the version of the Gaussian03[42] package that we have modified to carry out LSCF computations, linked to the Tinker[43] software. Several calculations have been envisioned. Standard RHF/6-311G** calculations are used as references. For the C_α–C_β LSCF/MM frontier, two schemes are proposed. The first one uses Y atom with +1 nuclear charge and a specific frontier bond potential[19], and is called LSCF(+1)/MM. The second one sets +3 nuclear charge to the hybrid frontier atom (C_α) and the two core electrons are added to the QM fragment to maintain the electroneutrality. The LSCF(+3)/MM is given to this scheme. For the

two peptide frontier bonds (C_Y–N_X and N_Y–C_X), three schemes are discussed. As previously, LSCF(+1)/MM and LSCF(+3)/MM are carried out. In addition, for the N_Y–C_X frontier, a +5 nuclear charge is attributed to the nitrogen atom with the corresponding two valence electrons added to the QM subsystem. This last scheme is referred as LSCF(+5)/MM, although the C_Y–N_X frontier bond is described with the LSCF(+3)/MM scheme.



(a) C_α–C_β frontier bond. (b) peptide bond frontiers.

FIGURE 3. QM/MM partitions of the Ace-Ile-His-Tyr-NMe tetrapeptide into QM (balls and sticks) and MM (thin sticks).

The geometry of the tetrapeptide was fully optimized at the RHF/6-311G** level of theory and at all LSCF/MM schemes, using the standard convergence criteria of the Gaussian03 package, for all three protonation states.

C_α–C_β Frontier Bond.

Results concerning the C_α–C_β frontier are gathered in Table 1 for the proton affinities and in Table 2 for the geometry. Concerning the proton affinities, one can see that the deviations from the reference RHF calculations are in the range of accuracy one could expect[11]. The error is a little bit larger when the core electrons are included since no specific parameters are used, as is the case for LSCF(+1)/MM calculations. The largest deviation (6.4 kcal/mol) certainly arises because the frontier is too close from the protonation site, and it is then desirable to test the second partition.

TABLE 1. C_α–C_β frontier. Proton affinities (ΔE in kcal/mol) of the histidine residue, calculated at the RHF/6-311G** level of theory, at the LSCF(+1)/MM level, and at the LSCF(+3)/MM level. Deviations from the RHF results are in parenthesis.

	RHF	LSCF(+1)/Charmm27	LSCF(+3)/Charmm27
ΔE(HID – HIP)	257.5	256.0 (1.4)	260.7 (3.2)
ΔE(HIE – HIP)	258.5	260.5 (2.0)	264.8 (6.4)

The comparison of the various geometries is done by means of RMSD expressed in Å. The RMSD are computed following the method of Kabsch[44] implemented in the VMD software[45]. The first remark to be done is that the largest part of the difference between the QM and the QM/MM geometries comes from the geometry of the fragment treated with MM, whatever the LSCF/MM scheme is. This is quite understandable since MM force fields are not devised to reproduce gas phase geometry but condensed phase one. The deformation of the tetrapeptide backbone induced a slight modification of the geometry of the side chain of the histidine residue, described by means of QM. One can also note that the RMSD of the MM fragment are very close whatever the LSCF/MM scheme is. However, the RMSD of the QM subsystem is several times larger for the LSCF(+3)/MM level than with the LSCF(+1)/MM one. The increase of RMSD for the LSCF(+3)/MM method is mainly due to the largest discrepancy of the frontier bond length. One has to recall that this bond needs a specific potential containing five parameters to exhibit the right bond length in the LSCF(+1) approach. One can see that the difference for the LSCF(+1)/MM level is of the order of few hundredths of Å. Substituting this specific frontier bond potential (i.e. LSCF(+1)/MM) by two protons in the nuclei of the Y atom and two electrons in the QM fragment (i.e. LSCF(+3)/MM) leads to a poorer agreement with the reference RHF values (≈2 pm) but are still in reasonable range of accuracy[19]. Anyway, this slight geometric error is certainly responsible for the error in the proton affinities values.

TABLE 2. C_α–C_β frontier. Frontier bond length (in Å) for the three protonation states (HID, HIE, and HIP, see figure 2) of the histidine residue engaged in the Ace-Ile-His-Tyr-NMe tetrapeptide, calculated at the RHF/6-311G** level of theory, at the LSCF(+1)/MM level, and at the LSCF(+3)/MM level. RMSD with respect to the RHF/6-311G** reference geometries are given (in Å) for the QM fragment alone, for the MM part alone, and for the whole system. Deviations from the RHF results are in parenthesis.

	RHF	LSCF(+1)/Charmm27	LSCF(+3)/Charmm27
HIP			
$d(C_\alpha–C_\beta)$	1.546	1.543(-0.003)	1.571(0.025)
$RMSD_{QM}$		0.055	0.186
$RMSD_{MM}$		0.857	0.861
RMSD		0.774	0.788
HID			
$d(C_\alpha–C_\beta)$	1.543	1.541(-0.002)	1.562(0.019)
$RMSD_{QM}$		0.051	0.104
$RMSD_{MM}$		0.422	0.440
RMSD		0.390	0.405
HIE			
$d(C_\alpha–C_\beta)$	1.532	1.536(0.004)	1.560(0.028)
$RMSD_{QM}$		0.037	0.218
$RMSD_{MM}$		0.951	0.960
RMSD		1.009	1.072

C–N and N–C Peptide Frontier Bonds.

Tables 3 and 4 contains the results concerning the peptide bond frontiers. The LSCF(+1) scheme gives proton affinities that are acceptable from the point of view of QM/MM methods[14, 16, 27, 30], although they are in worse agreement with the reference RHF values than those obtain with the previous C_α–C_β QM/MM frontier. Several reasons can be invoqued. First, the peptide bond can not be well represented by a single SLBO, as say before. Second, the nuclear charge of the Y atom (+1), of the C_Y–N_X peptide bond, is not large enough to attract the nitrogen lone pair. Third, the hybrid N_Y atom of the N_Y–C_X peptide bond, don't have any electron to share. Consequently the peptide bonds are not planar and the N_Y–C_X and C_Y–N_X electronic densities are not high enough. The LSCF(+3) method fails completely to recover the correct values! This is certainly due to the weak description of the hybrid nitrogen atom, which has still no lone pair to share. When two additional valence electrons are given to this hybrid N atom, in the LSCF(+5)/MM scheme, the geometry of the peptide bond is planar and the proton affinities are in a striking good agreement with the reference RHF data.

TABLE 3. Peptide bond frontiers. Proton affinities (ΔE in kcal/mol) of the histidine residue, calculated at the RHF/6-311G** level of theory, at the LSCF(+1)/MM level, at the LSCF(+3)/MM level, and at the LSCF(+5)/MM. Deviations from the RHF results are in parenthesis.

	RHF	LSCF(+1)/Amber99	LSCF(+3)/Amber99	LSCF(+5)/Amber99
ΔE(HID – HIP)	257.5	262.5 (5.1)	267.0 (9.5)	255.4 (–2.1)
ΔE(HIE – HIP)	258.5	257.6 (–0.9)	267.5 (9.0)	258.5 (0.0)

The same conclusions that the ones we draw from the C_α–C_β frontier bond can be made concerning the RMSD values. The largest deviation comes from the MM treatment which induces a modification of the QM fragment. However, the frontier peptide bonds are so badly represented by the LSCF(+1)/MM scheme that the specific frontier bond potential is not able to recover the correct geometry. The LSCF(+5)/MM level gives the closest agreement with the reference RHF values, the C–N frontier bond length is 1.38 Å without any extra parameters, to compare with 1.34 Å of the RHF computation. Another source of discrepancy between the full QM and the LSCF/MM calculations is that a hydrogen bond between the hydrogen atom at the δ position and the oxygen atom of the peptide bond linking the histidine to the isoleucine do exist in the full QM computation but disappear in the hybrid treatment whatever the scheme is. This is due to the hybrid description of the peptide bond and not specifically to the LSCF(+5)/MM level. This point will deserve a special car in future work.

TABLE 4. Peptide bond frontiers. Frontier bond lengths (in Å) for the three protonation states (HID, HIE, and HIP, see figure 2) of the histidine residue engaged in the Ace-Ile-His-Tyr-NMe tetrapeptide, calculated at the RHF/6-311G** level of theory, at the LSCF(+1)/MM level, at the LSCF(+3)/MM level, and at the LSCF(+5)/MM level. RMSD with respect to the RHF/6-311G** reference geometries are given (in Å) for the QM fragment alone, for the MM part alone, and for the whole system. Deviations from the RHF results are in parenthesis.

	RHF	LSCF(+1)/Amber99	LSCF(+3)/Amber99	LSCF(+5)/Amber99
HIP				
$d(C_Y–N_X)$	1.344	1.423(0.079)	1.381(0.037)	1.380(0.036)
$d(N_Y–C_X)$	1.339	1.319(-0.020)	1.416(0.077)	1.380(0.041)
$RMSD_{QM}$		0.794	0.650	0.902
$RMSD_{MM}$		1.055	1.117	1.335
RMSD		1.021	1.033	1.335
HID				
$d(C_Y–N_X)$	1.351	1.436(0.085)	1.373(0.022)	1.373(0.022)
$d(N_Y–C_X)$	1.348	1.324(-0.024)	1.419(0.071)	1.380(0.032)
$RMSD_{QM}$		0.197	0.268	0.238
$RMSD_{MM}$		0.838	0.813	1.136
RMSD		0.750	0.778	1.068
HIE				
$d(C_Y–N_X)$	1.345	1.437(0.092)	1.380(0.035)	1.380(0.035)
$d(N_Y–C_X)$	1.343	1.322(-0.021)	1.419(0.076)	1.379(0.036)
$RMSD_{QM}$		0.311	0.583	0.753
$RMSD_{MM}$		0.721	1.098	1.303
RMSD		0.784	1.183	1.474

CONCLUSION

It has been shown that the LSCF/MM method can be applied with success to the study of peptide. The QM/MM frontier has been set on the peptide bonds. This partition is particularly well suited to have a QM fragment of sufficient size to avoid the perturbation of the QM/MM boundary, to have a symmetric description of the amino acid, and to remove the arbitrariness of adjusting the classical point charges to obtain an integer value. We prove that the inclusion of some valence electrons (LSCF(+5) scheme) allows the correct description of the peptide bond (correct bond length, correct electronic density, planar geometry) without any extra parameter. Applications of this new scheme will be investigated in forthcoming papers.

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REFERENCES

1. A. Warshel and D. Lewitt, *J. Mol. Biol.* **103**, 227 (1976).
2. S. Ranganathan and J. Gready. *J. Phys. Chem. B* **101**, 5614 (1997).
3. J. Bentzien, R. Muller, J. Florian, and A. Warshell. *J. Phys. Chem. B* **102**, 2293 (1998).
4. A. Mulholland, P. Lynes, and M. Karplus. *J. Am. Chem. Soc.* **122**, 534 (2000).
5. M. Harrison, N. Burton, I. Hillier, and I. Gould, *Chem. Commun.* **24**, 2769 (1996).
6. P. Cummins, and J. Gready, *J. Phys. Chem. B* **104**, 4503 (2000).
7. F. Maseras and K. Morokuma, *J. Comput. Chem.* **16**, 1170 (1995).
8. M. Svensson, S. Humbel, D.J. Froese, T. Matsubara, S. Sieber and K. Morokuma, *J. Phys. Chem.* **100**, 19357 (1996).
9. S. Dapprich, I. Komárino, K. S. Byun, K. Morokuma, and M. J. Frich, *J. Mol. Struct. (Theochem)* **461**, 1 (1999).
10. U.C. Singh and P.A. Kollman, *J. Comput. Chem.* **7**, 718 (1986).
11. M.J. Field, P.A. Bash and M. Karplus, *J. Comput. Chem.* **11**, 700 (1990).
12. T. Vreven, B. Mennucci, C.O. da Silva, K. Morokuma and J. Tomasi, *J. Chem. Phys.* **115**, 62 (2001).
13. T. Kerdcharoen and K. Morokuma, *Chem. Phys. Lett.* **355**, 257 (2002).
14. D. Das, K.P. Eurenius, E.M. Billings, P. Sherwood, D.C. Chatfield, M. Hodoseek and B.R. Brooks, *J. Chem. Phys.* **117**, 10534 (2002).
15. Y. Zhang, T.-S. Lee and W. Yang, *J. Chem. Phys.* **110**, 46 (1999).

16. I. Antes and W. Thiel, *J. Phys. Chem. A* **103**, 9290 (1999).
17. G.A. DiLabio, M.M. Hurley and P.A. Christiansen, *J. Chem. Phys.* **166**, 9578 (2002).
18. X. Assfeld and J.-L Rivail, *Chem. Phys. Lett.* **263**, 100 (1996).
19. N. Ferré, X. Assfeld and J.-L. Rivail, *J. Comput. Chem.* **23**, 610 (2002).
20. A. Fornili, P.-F. Loos, M. Sironi and X. Assfeld, *Chem. Phys. Lett.* **427**, 236 (2006).
21. D.M. Philipp and R.A. Friesner, *J. Comput. Chem.* **20**, 1468 (1999).
22. R.B. Murphy, D.M. Philipp and R.A. Friesner, *J. Comput. Chem.* **21**, 1442 (2000).
23. R.B. Murphy, D.M. Philipp and R.A. Friesner, *Chem. Phys. Lett.* **321**, 113 (2000)
24. J. Gao, P. Amara, C. Alhambra and M.J. Field, *J. Phys. Chem. A* **102**, 4714 (1998).
25. P. Amara, M.J. Field, C. Alhambra and J. Gao, *Theor. Chem. Acc.* **104**, 336 (2000).
26. D.G. Truhlar, J. Gao, C. Alhambra, M. Garcia-Viloca, J. Corchado, M.L. Sánchez and J. Villá, *Acc. Chem. Res.* **35**, 341 (2002).
27. J. Pu, J. Gao and D.G. Truhlar, *J. Phys. Chem. A* **108**, 632 (2004).
28. J. Pu, J. Gao, and D.G. Truhlar, *J. Phys. Chem. A* **108**, 5454 (2004).
29. V. Kairys, J. H. Jensen, *J. Phys. Chem. A* **104**, 6656 (2000).
30. H. Lin and D.G. Truhlar, *Theor. Chem. Acc.* **117**, 185 (2007).
31. N. Ferré and M. Olivucci, *J. Mol. Struct. (THEOCHEM)* **632**, 71 (2003).
32. P.-F. Loos and X. Assfeld, *J. Chem. Theor. Comp.* **3**, 1047 (2007).
33. N. Ferré et X. Assfeld, *J. Chem. Phys.* **117**, 4119 (2002).
34. P.-F. Loos and X. Assfeld, *Int. J. Quant. Chem.* **107**, 2243 (2007).
35. A. Szabo and N.S. Ostlund, *Modern Quantum Chemistry*, New York: McGraw-Hill, 1989, pp. 144.
36. P.-F. Loos and X. Assfeld, *Comput. Lett.* In press.
37. J. A. D. MacKerell, D. Bashford, M. Bellott, J. R. L. Dunbrack, J. D. Evanseck, M. J. Field, S. Fischer, J. Gao, H. Guo, S. Ha, D. Joseph-McCarthy, L. Kuchnir, K. Kuczera, F. T. K. Lau, C. Mattos, S. Michnick, T. Ngo, D. T. Nguyen, B. Prodhom, I. W. E. Reiher, B. Roux, M. Schlenkrich, J. C. Smith, R. Stote, J. Straub, M. Watanabe, J. Wiórkiewicz-Kuczera, D. Yin, and M. Karplus, *J. Phys. Chem. B* **102**, 3586 (1998).
38. W. D. Cornell, P. Cieplak, C. I. Bayly, I. R. Gould, J. K. M. Merz, D. M. Ferguson, D. C. Spellmeyer, T. Fox, J. W. Caldwell, and P. A. Kollman, *J. Am. Chem. Soc.* **117**, 5179 (1995).
39. J. Wang, P. Cieplak and P.A. Kollman, *J. Comput. Chem.* **21**, 1049 (2000).
40. H. Weinstein and R. Pauncz, *Adv. Atomic. Molec. Phys.* **7**, 97 (1971).
41. H. Weinstein and R. Pauncz, *Symp. Faraday Soc.* **2**, 23 (1968).
42. M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery, T. V. Jr., K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez, and J. A. Pople, Gaussian 03, Revision B.05, Gaussian, Inc., Wallingford, CT, 2004.
43. J. W. Ponder, Tinker, Version 4.2, washington University: St. Louis, MO, 2004.
44. W. Kabsch, *Acta Cryst. A* **34**, 827 (1978).
45. W. Humphrey, A. Dalke, and K. Schulten, *J. Molec. Graphics* **14**, 33 (1996).