Constrained Nuclear-Electronic Orbital QM/MM Approach for Simulating Complex Systems with Quantum Nuclear Delocalization Effects Incorporated

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Abstract

The hybrid quantum mechanics/molecular mechanics (QM/MM) approach, which combines the accuracy of quantum mechanical (QM) methods with the efficiency of molecular mechanics (MM) methods, is widely used in the study of complex systems. However, past QM/MM implementations often neglect or face challenges in addressing nuclear quantum effects, despite their crucial role in many key chemical and biological processes. Recently, our group developed the constrained nuclear-electronic orbital (CNEO) theory, a cost-efficient approach that accurately addresses nuclear quantum effects, especially quantum nuclear delocalization effects. In this work, we integrate CNEO with QM/MM methods through the electrostatic embedding scheme and apply the resulting CNEO QM/MM to two hydrogen-bonded complexes in both gas and aqueous phases. We find that both solvation effects and nuclear quantum effects significantly impact hydrogen bond structures and dynamics. Notably, in the glutamic acid - glutamate complex, which mimics a low barrier hydrogen bond in human transketolase, CNEO QM/MM accurately predicts nearly equal proton sharing between the two residues, with predicted oxygen-hydrogen distance in excellent agreement with experimental results. With an accurate description of both nuclear quantum effects and environmental effects, CNEO QM/MM is a promising new approach for simulating complex chemical and biological systems.

Hybrid quantum mechanics/molecular mechanics (QM/MM) is a powerful tool in computational chemistry.[1–4] It enables the investigation of intricate chemical properties and processes within complex systems and has been widely used in the study of biological problems, including enzymatic processes[4–6] and drug design.[7–9] Addidtionally, it has been used in various other fields such as heterogeneous catalysis[10,11] and nanochemistry.[12,13] The distinctive advantage of QM/MM lies in its multiscale nature, where higher-level accurate QM methods are used for the region of primary interest while lower-level cost-effective MM methods handle environmental regions, thereby minimizing total computational expense.

Despite notable advancements in QM/MM methodologies and its remarkable achievements in practical applications,[12,14–17] the majority of current approaches still treat nuclei in the key QM region classically, resulting in the neglect of nuclear quantum effects. This neglect is particularly problematic in systems where hydrogen, the lightest element, plays a significant role, as seen in many enzymatic reactions involving proton transfer, hydrogen atom transfer, and/or hydride transfer processes[18–22].

To address this challenge, several theories have been developed to conduct QM/MM calculations with nuclear quantum effects included. One such successful approach is path-integral-based methods, which represent quantum systems using ensembles of replicas connected by harmonic springs.[23–29] Path-integral-based QM/MM methods have been successfully applied to study proton transfer,[30] hydride transfer,[31] and RNA cleavage reactions.[32] Although a major limitation of path-integral based methods is the high computational cost, especially if *ab initio* potential energy surfaces for the QM part are to be used, recent developments have been able to accelerate the calculations and reduce their computational costs.[33–37]

Another promising approach is the nuclear-electronic orbital (NEO) method, which employs multicomponent wave functions to simultaneously describe the quantum behavior of both nuclei and electrons.[38–46] When integrated with QM/MM, NEO has provided insights into the impact of nuclear quantum effects on molecular geometries in condensed phases.[47–49] Although static NEO calculations are limited by the assumption of instantaneous quantum nuclear response to the motion of classical nuclei,[47,50,51] the recently-developed real-time NEO QM/MM can address this limitation and incorporate nonadiabaticity between nuclei and electrons, thus offering insights into short-time vibronic dynamics.[48,52,53]

Additionally, semi-classical trajectory methods have been integrated with QM/MM calculations with nuclear quantum effects incorporated.[54] They have been utilized to simulate the vibrational spectra of small biological molecules in condense phases.[54]

Recently, our group developed the constrained nuclear-electronic orbital (CNEO)[55,56] theory to incorporate nuclear quantum effects, particularly quantum nuclear delocalization effects, into classical molecular simulations through a quantum-corrected effective potential energy surface.[57,58] CNEO shows great potential to be a widely-used method for its simple physical picture, high computational efficiency, and accuracy for describing quantum nuclear delocalization effects.[59–65] Due to its similarity to conventional electronic structure methods, with the addition of a more physically accurate quantum delocalized nuclear picture, CNEO is naturally capable of being integrated with the QM/MM framework.

In this work, we develop such an integration using the QM/MM electrostatic embedding scheme. By studying two bimolecular complex systems, one of which is of strong biological relavance, we show that CNEO QM/MM outperforms conventional QM/MM in describing hydrogen bonds and hydrogen-bond dynamics in the condensed phase, aligning well with experimental evidence.

In QM/MM calculations, a key aspect is the effective description of the interactions between the QM and MM regions. Two major embedding schemes are commonly used: mechanical embedding and electrostatic embedding.[1–4,66] In general, the electrostatic embedding scheme offers greater accuracy and is more widely used in computations.[1,2,67,68] In this scheme, the QM system is influenced by the electrostatic potential provided by the MM environment, and the MM portion interacts with the charge obtained from the quantum mechanical calculations of the QM system. Additionally, building upon the electrostatic scheme, polarization effects of the MM system may be considered through polarized embedding using polarizable force fields.[1,69,70]

In the conventional QM/MM electrostatic embedding scheme, the total energy of the whole system can be decomposed into three parts

$$E_{\text{system}} = E_{\text{QM}} + E_{\text{MM}} + E_{\text{QM-MM}}, \tag{1}$$

where E_{QM} and E_{MM} are the energies of the QM and MM regions, respectively, and $E_{\text{QM-MM}}$ represents the QM-MM interaction energy. When QM and MM atoms interact only through non-bonded interactions, $E_{\text{QM-MM}}$ mainly includes two terms: the electrostatic interactions $E_{\text{QM-MM}}^{\text{electrostatic}}$ and the van der Waals interactions $E_{\text{QM-MM}}^{\text{vdW}}$. However, when there are covalent bonds connecting QM and MM atoms, special considerations on the boundary are needed.[4,71–78] In this development, we will focus our discussion on cases where there are no such covalent bonds.

The van der Waals term $E_{\text{QM-MM}}^{\text{vdW}}$ is easier to deal with. It describes both the short-range repulsion and long-range dispersion interactions between QM and MM atoms, and it is often modelled with the Lennard-Jones (L-J) potential

$$E_{\text{QM-MM}}^{\text{vdW}} = \sum_{A}^{N_{\text{QM}}} \sum_{M}^{N_{\text{MM}}} \varepsilon_{NM} \left[\left(\frac{\sigma_{AM}}{|\mathbf{R}_A - \mathbf{R}_M|} \right)^{12} - \left(\frac{\sigma_{AM}}{|\mathbf{R}_A - \mathbf{R}_M|} \right)^{6} \right] \tag{2}$$

Here *ε* and *σ* are pairwise L-J parameters, $\mathbf{R}$ is the position of nuclei, N_{QM} is the number of classical nuclei in the QM region, and N_{MM} is the number of atoms in the MM region.

The electrostatic interaction $E_{\text{QM-MM}}^{\text{electrostatic}}$ describes the Coulombic interactions between the QM system and MM charges. Specifically, it usually includes the Coulombic interactions of electron density and classical nuclear point charges in the QM region with MM charges, denoted by $E_{\mathrm{e-MM}}^{\text{electrostatic}}$ and $E_{\mathrm{nuc-MM}}^{\text{electrostatic}}$, respectively:

$$E_{\text{QM-MM}}^{\text{electrostatic}} = E_{\mathrm{e-MM}}^{\text{electrostatic}} + E_{\mathrm{nuc-MM}}^{\text{electrostatic}} = -\int d\mathbf{r} V_{\mathrm{MM}}^{\mathrm{ext}}(\mathbf{r}) \rho^{\mathrm{e}}(\mathbf{r}) + \sum_{A}^{N_{\mathrm{QM}}} V_{\mathrm{MM}}^{\mathrm{ext}}(\mathbf{R}_A) Z_A \,. \tag{3}$$

Here $\rho^{\mathrm{e}}(\mathbf{r})$ is the electron density, Z_A is the nuclear charge of the *A*-th nucleus, and $V_{\mathrm{MM}}^{\mathrm{ext}}(\mathbf{r})$ is the external potential produced by MM charges. Usually, the MM charges are represented by point charges and the MM potential can be expressed as

$$V_{\mathrm{MM}}^{\mathrm{ext}}(\mathbf{r}) = \sum_{M}^{N_{\mathrm{MM}}} \frac{q_M}{|\mathbf{r} - \mathbf{R}_M|} \,, \tag{4}$$

in which q_{M} is the effective charge of the *M*-th MM atom. Note that instead of point charges, Gaussian charges can also be used, which have been shown to be able to avoid overpolarization of the QM electron density.[71,76,77,79]

Because the QM/MM electrostatic interaction energy depends on the electron density, the solution to the electron density must come from the variational optimization of $E_{\mathrm{QM}} + E_{\text{QM-MM}}^{\text{electrostatic}}$. In practical calculations, if Kohn-Sham density functional theory (DFT) is used, the Kohn-Sham equation will incorporate the MM electrostatic potential $V_{\mathrm{MM}}^{\mathrm{ext}}(\mathbf{r})$ in addition to the external potential generated by classical nuclei in the QM region:

$$\left[-\frac{1}{2}\nabla^2+\int d\mathbf{r}'\frac{\rho^{\mathrm{e}}(\mathbf{r}')}{|\mathbf{r}-\mathbf{r}'|}-V_{\mathrm{MM}}^{\mathrm{ext}}(\mathbf{r})-\sum_{A}^{N_{\mathrm{c}}}\frac{Z_A}{|\mathbf{r}-\mathbf{r}_N|}+V_{\mathrm{xc}}^{\mathrm{e}}(\mathbf{r})\right]\psi_i^{\mathrm{e}}(\mathbf{r})=\varepsilon_i^{\mathrm{e}}\psi_i^{\mathrm{e}}(\mathbf{r}) \tag{5}$$

Upon convergence of self-consistent field (SCF) calculations for $E_{\mathrm{QM}}+E_{\text{QM-MM}}^{\text{electrostatic}}$, the total energy E_{system} can be calculated by adding the MM energy, as well as the van der Waals term $E_{\text{QM-MM}}^{\mathrm{vdW}}$. Afterwards, the forces on QM and MM atoms can be calculated through analytic gradient expressions of the total energy with respect to QM and MM coordinates.

In the past few years, our group developed the CNEO framework to incorporate nuclear quantum effects, especially quantum nuclear delocalization effects, into quantum chemical calculations and molecular dynamics (MD) simulations.[55,56,59–62] This is achieved within the multicomponent quantum chemistry framework by imposing positional constraints on quantum nuclei

$$\left\langle \psi_I^{\mathrm{n}} | \hat{\mathbf{r}}_I | \psi_I^{\mathrm{n}} \right\rangle = \mathbf{R}_I\,, \tag{6}$$

where ψ_I^{n} is the nuclear orbital of the *I*-th quantum nucleus and $\mathbf{R}_I$ is its associated classical position specified by the molecular geometry. In this way, CNEO treats nuclei quantum mechanically but also retains the intuitive classical molecular picture.

With the introduction of constraints on the expectation value of quantum nuclear position operators, the multicomponent electronic Kohn-Sham equation remains the same as conventional NEO theory

$$\left[-\frac{1}{2}\nabla^2+\int d\mathbf{r}'\frac{\rho^{\mathrm{e}}(\mathbf{r}')}{|\mathbf{r}-\mathbf{r}'|}-\sum_{A}^{N_{\mathrm{c}}}\frac{Z_A}{|\mathbf{r}-\mathbf{r}_N|}-\sum_{I}^{N_{\mathrm{q}}}Z_I\int d\mathbf{r}'\frac{\rho_I^{\mathrm{n}}(\mathbf{r}')}{|\mathbf{r}-\mathbf{r}'|}+V_{\mathrm{xc}}^{\mathrm{e}}(\mathbf{r})\right]\psi_i^{\mathrm{e}}(\mathbf{r})=\varepsilon_i^{\mathrm{e}}\psi_i^{\mathrm{e}}(\mathbf{r})\,, \tag{7}$$

where ρ_I^{n} denotes the density of the *I*-th quantum nucleus, and Z_I denotes its charge. N_{c} and N_{q} are the total numbers of classical and quantum nuclei, respectively. The terms in the bracket represent in order the electronic kinetic energy term, Hartree potential, external potential due to classical nuclei, external potential due to quantum nuclei, and exchange-correlation potential for electrons.

In contrast, the nuclear Kohn-Sham equation is modified with an extra term ($\mathbf{f}_I \cdot \mathbf{r}$) associated with the constraint on the expectation position[55]

$$\left[-\frac{1}{2m_I}\nabla^2 - Z_I\int d\mathbf{r}'\frac{\rho^{\mathrm{e}}(\mathbf{r}')}{|\mathbf{r}-\mathbf{r}'|} + Z_I\sum_{A}^{N_{\mathrm{c}}}\frac{Z_A}{|\mathbf{r}-\mathbf{r}_A|} + Z_I\sum_{J\neq I}^{N_{\mathrm{q}}}Z_J\int d\mathbf{r}'\frac{\rho_J^{\mathrm{n}}(\mathbf{r}')}{|\mathbf{r}-\mathbf{r}'|} + V_{\mathrm{c},I}^{\mathrm{n}}(\mathbf{r}) + \mathbf{f}_I\cdot\mathbf{r}\right]\psi_I^{\mathrm{n}}(\mathbf{r}) = \varepsilon_I^{\mathrm{n}}\psi_I^{\mathrm{n}}(\mathbf{r})$$

.(8)

Here the first four terms corresponds to those in Equation (7) but are now for nuclei. $V_{\mathrm{c},I}^{\mathrm{n}}(\mathbf{r})$ is the correlation potential for the ***I***-th quantum nucleus. Note that there is no nuclear exchange within CNEO because of the distinguishible nucleus assumption and nuclear self-Coulomb is explicitly excluded. The Lagrange multiplier $\mathbf{f}_I$ needs to be solved iteratively together with electronic and nuclear orbitals, subject to the geometric constraints on quantum nuclear expectation positions via Equation (6). The converged orbitals can be subsequently used to evaluate the multicoponent energies as a function of both classical and quantum nuclear positions, leading to quantum-corrected effective potential energy surfaces.

Analytic gradients[56] and Hessians[59] with respect to both classical and quantum nuclear positions have also been developed.

For the CNEO-DFT QM/MM development, because some or all nuclei in the QM region are now described quantum mechanically, additional terms in the QM-MM interaction energy $E_{\text{QM-MM}}$ will arise due to the interactions between the quantum nuclei and the MM environment. Specifically, these terms include both the electrostatic interactions and the van der Waals interactions between quantum nuclei and MM atoms.

For the simpler van der Waals interactions, the additional quantum nuclei-MM (q-MM) term can be calculated with

$$E_{\text{q-MM}}^{\text{vdW}} = \sum_{I}^{N_{\text{q}}} \sum_{M}^{N_{\text{MM}}} \varepsilon_{IM} \left[\left(\frac{\sigma_{IM}}{|\mathbf{R}_I - \mathbf{R}_M|} \right)^{12} - \left(\frac{\sigma_{IM}}{|\mathbf{R}_I - \mathbf{R}_M|} \right)^{6} \right]. \tag{9}$$

Then the total QM-MM van der Waals interaction energy becomes

$$E_{\text{QM-MM}}^{\text{vdW}} = E_{\text{c-MM}}^{\text{vdW}} + E_{\text{q-MM}}^{\text{vdW}}. \tag{10}$$

where we now use c-MM to denote the interactions between the classical nuclei in the QM region and MM atoms.

For electrostatic interactions, the additional term can be calculated with

$$E_{\text{q-MM}}^{\text{eletrostatic}} = \sum_{I}^{N_{\text{q}}} Z_I \int d\mathbf{r} V_{\text{MM}}^{\text{ext}}(\mathbf{r}) \rho_I^{\text{n}}(\mathbf{r}), \tag{11}$$

and the total electrostatic QM-MM interaction energy becomes

$$E_{\text{QM-MM}}^{\text{electrostatic}} = E_{\text{e-MM}}^{\text{electrostatic}} + E_{\text{q-MM}}^{\text{electrostatic}} + E_{\text{c-MM}}^{\text{electrostatic}}. \tag{12}$$

Similar to the conventional QM/MM approach, the variational energy minimization of $E_{\text{QM}} + E_{\text{QM-MM}}^{\text{electrostatic}}$ with respect to QM densities leads to Kohn-Sham equations for quantum particles in the QM region. The resulting electronic and nuclear Kohn-Sham equations are highly similar to those in CNEO-DFT, except that the MM potential now enters both the electronic equation (Equation 7) and the nuclear equation (Equation 8) as an additional external potential term.

In CNEO-DFT QM/MM, analytic gradients with respect to the displacement of classical nuclei in the QM region, the displacement of the expectation positions of quantum nuclei in the QM region, and the displacement of MM atom positions can be derived in a similar way to what has been done for conventional DFT QM/MM. These details are provided in the Supporting Information.

We implemented the CNEO-DFT QM/MM in our locally-modified version of PySCF[80–82] with CNEO capability,[83] and the molecular dynamics simulations were carried out in GROMACS.[84–86] In the following calculations, the aug-cc-pVDZ electronic basis set[87] is used for both CNEO-DFT and conventional DFT. For CNEO-DFT calculations, all hydrogen atoms in the QM region are treated as quantum nulclei with the PB4D protonic basis set.[88] The B3LYP[89–91] electronic exchange-correlation functional is used and no electron-proton correlation[92–95] functional or proton-proton correlation functional is used. For molecular mechanics-related calculations, a modified OPLS all-atom force field is used for organic molecules.[96–99] The polar hydrogens (hydrogen in N-H and O-H) of these molecules are assigned Lennard-Jones coefficients from Ref 100. A modified TIP3P model with Lennard-Jones coefficients for hydrogen is used for water.[101] Additional computational details can be found in the Supporting Information.

We first considered a phenol-water complex (Figure 1), which is a hydrogen bonded system that has been studied in the past during the development NEO-DFT QM/MM.[47] In this system, the hydrogen bond to be investigated is the one between the hydrogen atom in the phenol hydroxyl group and the oxygen atom in the water. Therefore, the QM region constitutes the phenol molecule and the hydrogen-bonded water molecule.

Following the procedure in Ref 47, we investigated four key geometric properties for the optimized geometries of the complex in both gas phase and in a water droplet: the OH bond length of the phenol hydroxyl group (O-H), the hydrogen bond distance (O···H), the distance between the two oxygen atoms (O···O), and the ∠OHO bond angle. The results from methods based on pure MM, DFT, and CNEO-DFT are presented in Table 1.

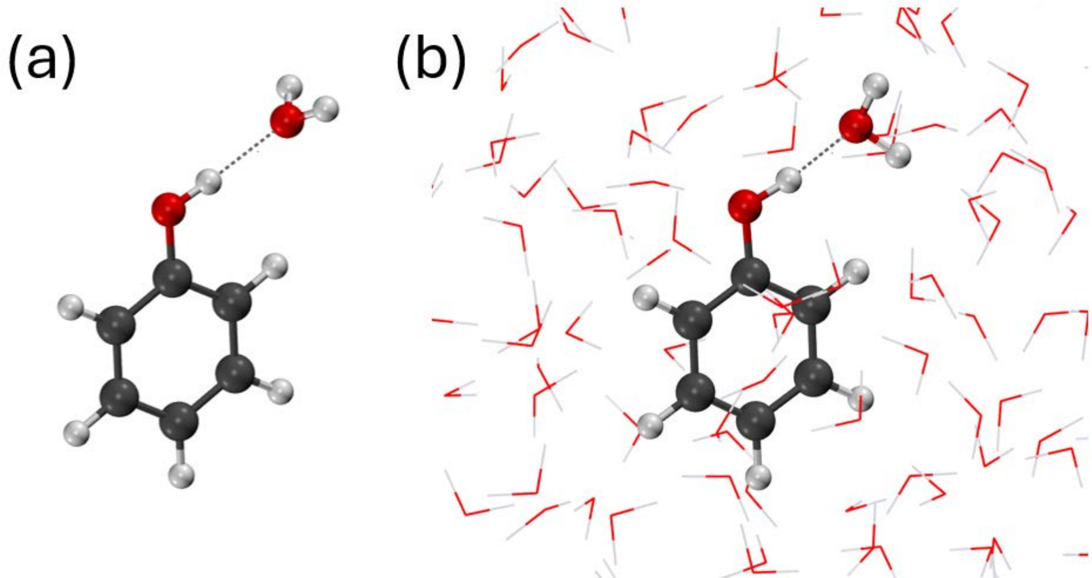

Figure 1. Phenol-water complex in the (a) gas phase and (b) aqueous phase

For all three methods, the hydroxyl O-H bond length is consistently very close to 1 Å. Additionally, switching from gas phase to aqueous phase has little impact on the distance. These results indicate that all three methods can describe the equilibrium bond length well. In contrast, the hydrogen bond O⋯H distance varies dramatically with the underlying method. Specifically, pure MM predicts the largest distance with 1.93 Å in the gas phase and 1.91 Å in the aqueous phase, whereas CNEO predicts the smallest distance with 1.82 Å in the gas phase and 1.59 Å in the aqueous phase. Intrestingly, switching from the gas phase to the aqueous phase barely changes the pure MM results but it leads to a large distance decrease for both DFT QM/MM (0.15 Å) and CNEO-DFT QM/MM (0.23 Å). As to the O⋯O distance, because the ∠OHO bond angle is almost linear and thus the O⋯O distance is roughly the sum of O-H and O⋯H distances, the behavior of the O⋯O distance is similar to that of the O⋯H distance.

Table 1 Geometric Properties of Phenol-Water Complex

Environment	Method	Distance (Å)			∠OHO (degree)
		O–H	O⋯H	O⋯O	

Geometry Optimization (Gas phase)	MM	0.96	1.93	2.88	177
	DFT	0.97	1.88	2.85	173
	CNEO-DFT	1.01	1.82	2.82	172
Geometry Optimization (Aqueous phase)	Full MM	0.96	1.91	2.85	166
	DFT QM/MM	0.99	1.73	2.71	169
	CNEO-DFT QM/MM	1.03	1.59	2.61	171
Molecular Dynamics (Aqueous phase)	Full MM	0.95 ± 0.03	2.89± 0.37	3.44 ± 0.34	120±19
	DFT QM/MM	0.98 ± 0.01	1.88 ± 0.20	2.81 ± 0.19	159±9
	CNEO-DFT QM/MM	1.03 ± 0.03	1.65 ± 0.11	2.67 ± 0.09	168±6

The long hydrogen bond distance by pure MM and its insensitivity to environmental change is a manifestation of its failure in describing hydrogen bonds. This is because using only Coulombic and van der Waals interaction terms in pure MM tends to inadequately capture the intricate nature of hydrogen bonds.[102,103] In constrast, both DFT QM/MM and CNEO-DFT QM/MM can qualitatively describe the significant environmental effect, although CNEO-DFT QM/MM predicts shorter O···H and O···O distances than DFT QM/MM by 0.1-0.14 Å. Additionally, in CNEO-DFT QM/MM, the hydrogen atom is located closer to the center of the two oxygen atoms. Although there is no experimental reference for this phenol-water system, making it difficult to determine which QM/MM method is quantitatively more accurate, these results are qualitatively consistent with previous computational studies that also found neutral hydrogen bond complexes contract when solvated by water.[104]

One notable point is that for this type of static QM/MM geometry optimization, theoretically, CNEO QM/MM and NEO QM/MM should yield the same equilibrium geometry results. This can be confirmed by comparing with the data in Ref 47, which shows that our CNEO QM/MM results match well the optimized geometric parameters obtained from NEO QM/MM, with negligible differences attributed to different basis sets and MM water environment. This consistency in results is a strong indicator that both developments have correctly implemented their respective theories.

Next we performed MD simulations on the phenol-water complex system starting from optimized geometries obtained from the respective methods. All MD simulations are performed for four ps within the *NVT* ensemble at *T*=270K. The first two ps are used for equilibration and the remaining two ps are for data collection. The four geometric properties as well as their standard deviation during the later two ps MD simulations are also shown in Table 1. Note that we only performed the simulation in the aqueous phase because in the gas phase, the phenol-water easily dissociates at the picosecond time scale.

Compared to the geometry optimization results, MD simulations barely change the hydroxyl O-H bond distance on the phenol group and the bond distance standard deviations remain small. This is because the simulation temperature is low compared to the bond strength and thus show negligible thermal fluctuation effects. In contrast, the hydrogen bonded O···H distance becomes larger with MD simulations as a result of the significant thermal fluctuation, which also makes its standard deviation significantly larger than that of the O-H bond distance. Note that with pure MM, the average O···H distance increased by almost 1 Å and the average ∠OHO becomes 120 degrees. This large change is due to the inadequately weak hydrogen bond being broken and re-formed many times during the two ps sampling time. In constrast, the hyrogen bond predicted by both DFT QM/MM and CNEO-DFT QM/MM remains unbroken and is much less affected by thermal fluctuation. Specifically, for the O···H distance, a decrease of 0.15 Å and 0.06 Å is observed for DFT QM/MM and CNEO-DFT QM/MM, respectiviely, and for the bond angle, DFT QM/MM observes a change of about 10 degrees whereas it is as small as three degrees by CNEO-DFT QM/MM. The smaller susceptibility to thermal fluctuation implies a stronger hydrogen bond due to the quantum description on the hydrogen nuclei by CNEO, although as with the geometry optimization part, without experimental references, it remains difficult to conclude if CNEO is quantatitively more accurate.

We note that for MD simulations, CNEO-DFT QM/MM and NEO-DFT QM/MM will be significantly different. CNEO-DFT QM/MM does not assume the instantaneous response of the quantum nuclei to the motion of classical nuclei.[55,59] Therefore, it carries a more physically correct picture and can accurately describe the O-H and O···H vibrational pictures.[60–62] Nonetheless, the recently developed real-time NEO QM/MM dynamics work can mitigate the problem of NEO-DFT QM/MM with the desired capability of describing electron-nuclear nonadiabaticity, although the computational cost will be much higher.[48]

To further demonstrate the power of CNEO-DFT QM/MM and its potential in biological studies, we next investigated a glutamic acid-glutamate complex (Figure 2) in both gas phase and aqueous phase. This complex is a typical low-barrier hydrogen bond system[105–108] in which two glutamate anions share a proton, and it is known to play a vital role in some biological systyems.[107,109–111] For example, in human transketolase, this complex (between E366' and E160) is believed to participate in a proton wire, which is the structural origin of the enzyme's cooperativity,[107] and in bacteriorhodopsin, a proton pump that uses photon energy to establish transcellular proton gradient, this complex (between E194 and E204) is directly involved in the key pump process by releasing the shared proton to the extracellular environment.[109–111]

Due to the high significance in biological systems, the location of the shared proton in this complex and its real-time dynamics is of particular interest. For human transketolase, high-resolution X-ray crystallography concludes that the proton is almost equally shared by the two carboxylic oxygens of E366' and E160 residues, and the O···O distance is 2.55 Å,[107] which is much shorter than that of a normal hydrogen bond (2.7~3.1 Å). For bacteriorhodopsin, although the location of the proton is not exactly know, the determined O···O distance is 2.48 Å,[109] which is even shorter and may imply a more equally shared proton.

To mimic the real biological environment, in principle we should embed the two amino acids in relatively rigid protein backbones. However, as a proof-of-concept study, we simplified the problem by applying distance constraints to two pairs of carbon atoms according to the experimental X-ray structure of human transketolase.[112] Specifically, the distance between the two *α*-carbons is constrained to 7.84 Å and the distance between the two carboxylic carbons is constrained to 9.01 Å. (Figure 2) These constraints approximately serve the same role as the protein backbones, which preserve the intercarboxylic hydrogen bond and prevents strong conformational changes unnatural to protein environment.

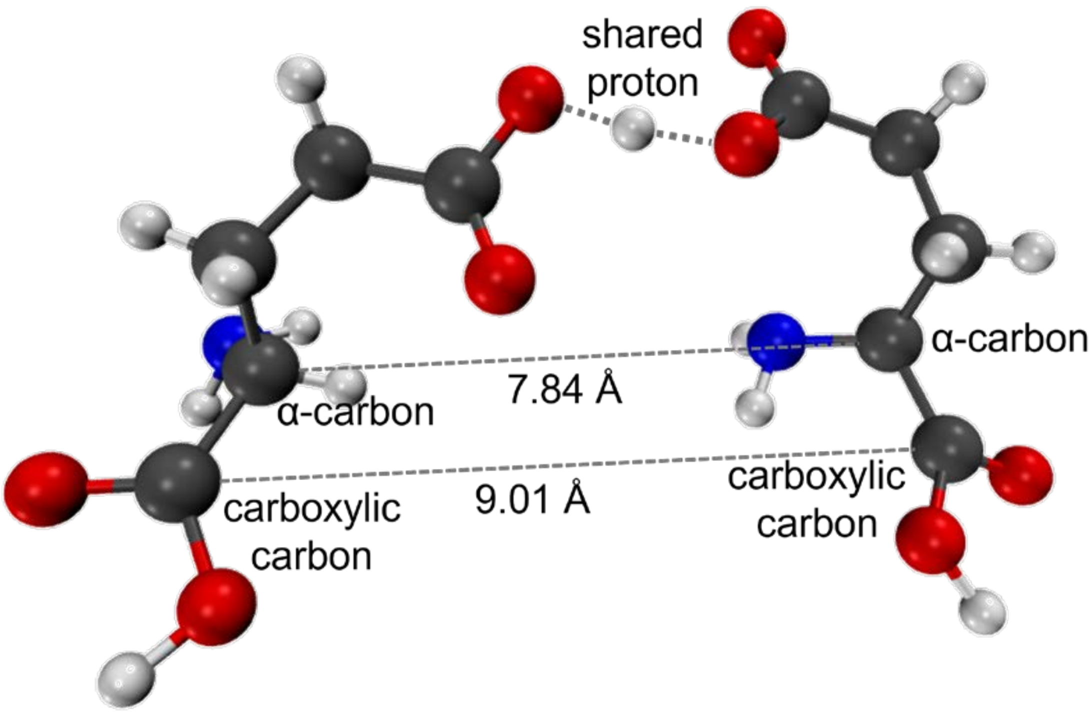

Figure 2 Structure of the glutamic acid-glutamate complex and the applied distance constraints between two α-carbons and two carboxylic carbons to mimic the real structure in the enzyme environment.

As with the phenol-water complex, we first optimized the geometries of the glutamic acid-glutamate complex in both gas phase and aqueous phase. As shown in Table 2, classical MM again yields the longest O···O distances (around

2.65 Å) and the largest difference between O-H and O···H distances (0.7 Å), thus incorrectly predicting the proton to be owned by one residue. With DFT QM/MM, the O···O distance is predicted to be significantly shorter and the length difference between O···H and O-H becomes smaller, which is 0.29 Å in the gas phase and 0.52 Å in solution. For CNEO-DFT QM/MM, the O···O distance is even shorter and the O···H and O-H distance difference further significantly reduces to 0.07 Å and 0.17 Å in the gas phase and aqueous phase, respectively, aligning well with the experimental result that the H is equally shared by the two residues.

There is a major difference between the current glutamic acid-glutamate complex and the previous phenol-water complex: for the phenol-water complex, the hydrogen bond distance and the O···O distance shorten in aqueous phase, whereas for the glutamic acid-glutamate complex, these distances barely change or even becomes larger. This phenomenon has been observed in the past and it was attributed to the differences beween neutral complexes and negatively charged complexes.[104] Heuristically speaking, the charged complex has dipole-ion interactions, which are stronger than dipole-dipole interactions in the neautral complex but are also more susceptible to weakening by the highly polar water solvent. However, more rigorously, the bond length changes reflect an interplay between electronic effects, nuclear quantum effects, and solvation effects. The collective impact of these effects leads CNEO-DFT QM/MM to predict that the hydrogen bond barely changes upon solvation for the glutamic acid-glutamate complex, whereas DFT QM/MM predicts slightly elongated O···H and O···O differences (by ~0.05 Å).

Table 2 Geometric Properties of Glutamic acid-Glutamate Complex

Environment	Method	Distance (Å)			∠OHO (degree)
		O–H	O···H	O···O	
Geometry Optimization (Gas phase)	MM	0.97	1.69	2.65	170
	DFT	1.09	1.38	2.46	171

	CNEO-DFT	1.17	1.30	2.46	170
Geometry Optimization (Aqueous phase)	Full MM	0.97	1.68	2.64	170
	DFT QM/MM	1.05	1.45	2.49	170
	CNEO-DFT QM/MM	1.17	1.29	2.45	170
Molecular Dynamics (Gas phase)	MM	0.97 ± 0.03	1.83 ± 0.21	2.74 ± 0.19	152±27
	DFT	1.10 ± 0.06	1.40 ± 0.11	2.49 ± 0.07	169±5
	CNEO-DFT	1.16 ± 0.05	1.35 ± 0.10	2.49 ± 0.08	169±5
Molecular Dynamics (Aqueous phase)	Full MM	0.96 ± 0.02	1.83 ± 0.17	2.75 ± 0.16	153±26
	DFT QM/MM	1.05 ± 0.05	1.51 ± 0.14	2.55 ± 0.11	170±5
	CNEO-DFT QM/MM	1.13 ± 0.06	1.39 ± 0.13	2.51 ± 0.10	171±5

Next, molecular dynamics simulations were carried out with each method from the corresponding optimized geometries, and the geometric properties are also reported in Table 2. With pure MM, the hydrogen bond becomes considerably longer (~0.15 Å) and inadequately weaker, again indicating the failure of the force field in describing hydrogen bonds. In contrast, the hydrogen bonds treated by both DFT QM/MM and CNEO-DFT QM/MM are stronger with the increase of the hydrogen bond length always within 0.1 Å.

The bond lengths statistics within the *NVT* ensemble do not provide dynamical information in this low-barrier hydrogen bonded system. Therefore, to investigate the important proton transfer dynamics, we performed four ps *NVE* simulations with each method and plotted the O_1-H and O_2-H distances as a function of time. The results are shown in Figure 3.

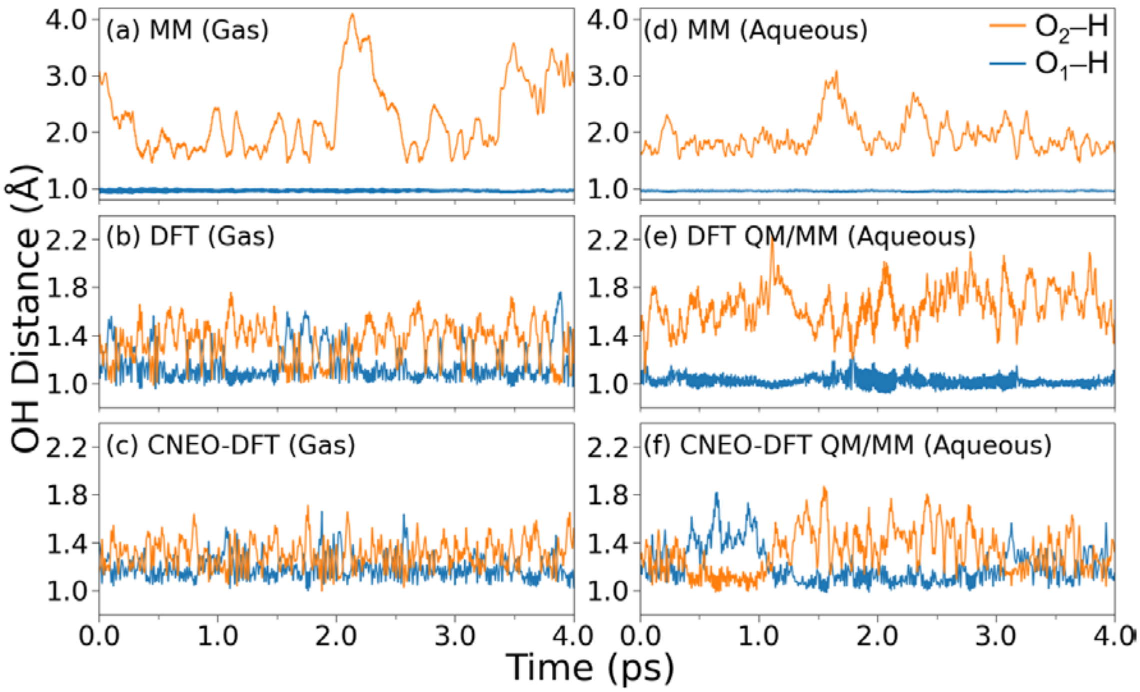

Figure 3 Distances between the shared proton and its two adjacent oxygen atoms during *NVE* simulations of glutamic acid-glutamate complex in the gas and aqueous phases by classical molecular dynamics, DFT-based molecular dynamics, and CNEO-DFT-based molecular dynamics.

Due to the inability to describe bond formation and bond dissociation, proton transfer never occurs in classical MM. The bonded OH always vibrates around its local minimum with a small length variance whereas the hydrogen bonded O···H distance can fluctuate significantly. This fluctuation becomes smaller in the aqueous phase owing to confinement from environmental molecules. In contrast, proton transfer can be observed with both DFT and CNEO-DFT *ab initio* molecular dynamics with the relative O_1-H and O_2-H distances swapped during the gas phase simulation. It is noticeable that proton transfer is more frequent in CNEO-DFT than in DFT. Interestingly, in the aqueous phase, proton transfer is now nearly prohibited in DFT QM/MM simulations but can still frequently take place with CNEO-DFT QM/MM. One obvious reason for this difference between DFT and CNEO-DFT is the smaller O···O distance that facilitates hydrogen sharing and hydrogen transfer by CNEO-DFT, but the deeper reason is the incorporation of quantum nuclear delocalization effects in the CNEO effective surface, which can lower the proton-transfer barrier and accelerate the proton transfer dynamics.

With this dynamic information in mind, we can now reinvestigate the bond length distributions and better interpret the proton location in the glutamic acid-glutamate complex. Because of the occurrence of proton transfer that weakens the identification of hydrogen bond donor and acceptor, we combined the bond length data of O-H and O···H and plotted them in an overall distribution in Figure 4. In DFT simulations, because there is little to no proton transfer, the hydrogen bond tends to be asymmetric with the proton being more possessed by one oxygen, leading to a humped peak in gas phase and distinct double peaks in the aqueous phase. In constrast, with CNEO-DFT simulations, the overall distribution becomes a single peak in both gas phase and aqueous phase due to the much more frequent proton tranfer. The peak positions are both at around 1.2 Å, and the average OH distances are 1.25 Å and 1.26 Å for gas phase and aqueous phase, respectively. This prediction is in excellent agreement with the experiment X-ray results on human transketolase,[112] in which the proton is predicted to be equally shared by the two glutamate residue with the OH distance being 1.28 Å.

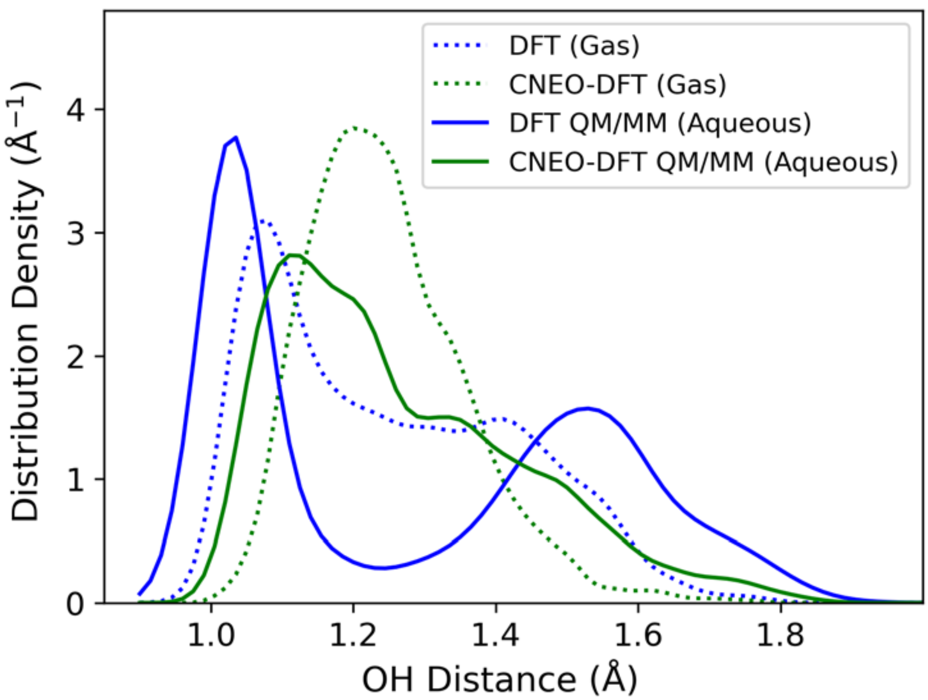

Figure 4 Distance distributions between the shared proton and adjacent oxygen atoms of the glutamic acid-glutamate complex in the gas (dotted line) and aqueous (solid line) phases from DFT-based (blue) and CNEO-DFT-based (green) *NVT* simulations.

We further investigated the correlation between the OH distances and the O···O distance by plotting their joint probability in Figure 5. The lower branch in each panel represents the bonded O-H distance while the higher branch represents the hydrogen bonded O···H distance. It can be observed that the smaller the O···O distance, the more likely that the two branches merge together, facilitating the protron transfer. This observation is conisistent with the conventional understanding of proton transfer processes. However, in general, DFT and DFT QM/MM give larger O···O distances and more distinguishable O-H and O···H distributions, whereas CNEO-DFT and CNEO-DFT QM/MM yield smaller O···O distances and more overlapped O-H and O···H branches that allow more proton transfers.

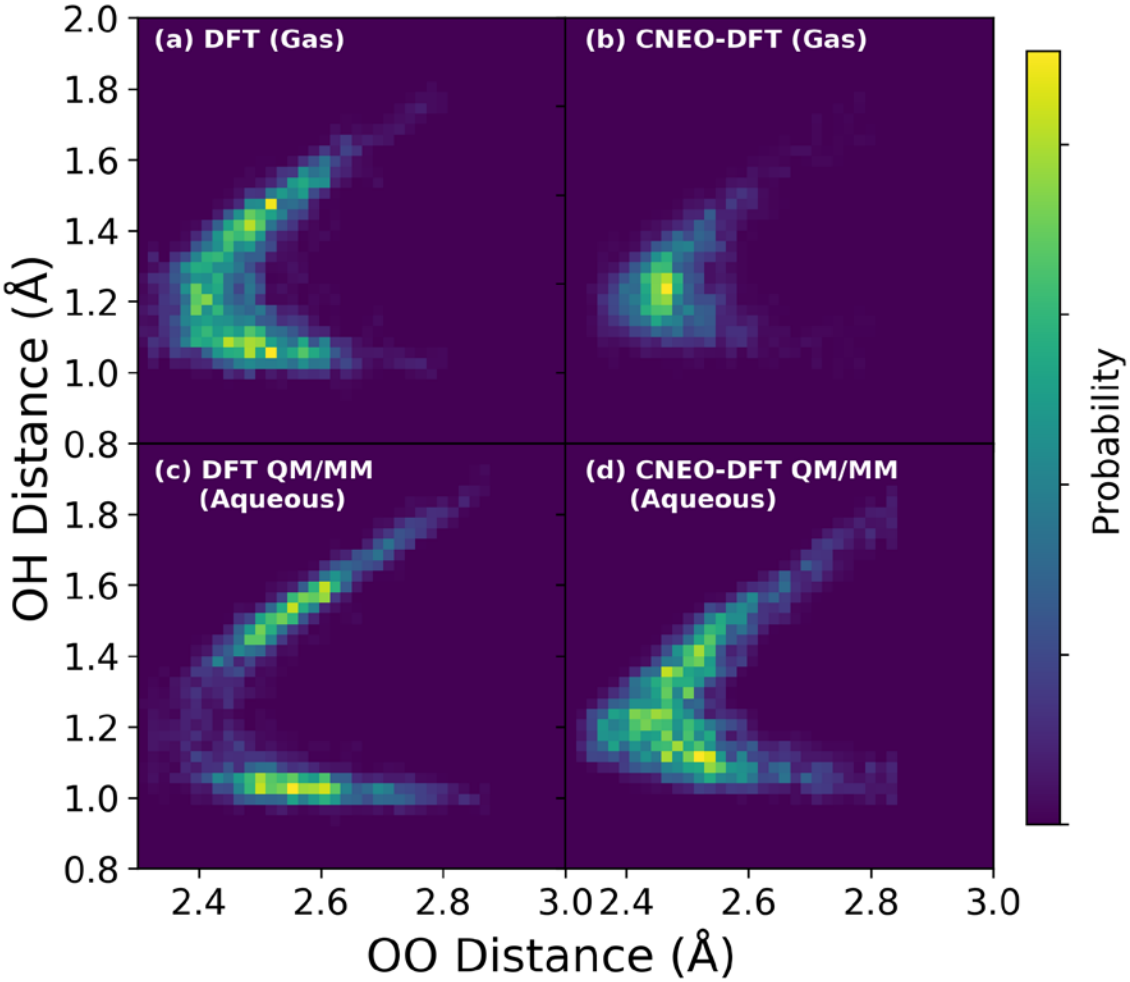

Figure 5 Correlation between oxygen-proton (OH) and oxygen-oxygen (OO) distances in the glutamic acid-glutamate complex in the gas phase and aqueous phases from DFT-based and CNEO-DFT-based *NVT* simulations.

In conclusion, we integrated CNEO with the QM/MM electrostatic embedding scheme and achieved the accurate and efficient incoporation of nuclear quantum effects, particularly quantum delocalizaiton effects, in the QM region of QM/MM simulations. We applied the resulting CNEO QM/MM theory to the calculation of a phenol-water complex and a glutamic acid-glutamate complex in both gas phase and aqueous phase. We investigated the impact of nuclear quantum effects on both optimized geometries and molecular dynamics. For the neutral phenol-water complex, solvation reduces the hydrogen bond distance and the incoporation of nuclear quantum effects through CNEO-DFT leads to a shorter hydrogen bond than that of DFT simulations. In constrast, for the negatively charged glutamic acid-glutamate complex, solvation leads to a large increase for the hydrogen bond distance as predicted by DFT and DFT QM/MM but only a small increase as predicted by CNEO-DFT and CNEO-DFT QM/MM. Through dynamics simulations, we observed much more frequent proton transfer in CNEO-DFT QM/MM simulations than in DFT QM/MM simulations for the glutamic acid-glutamate complex in both gas phase and aqueous phase due to the incorporation of nuclear quantum effects. Additionally, the location of the shared proton predicted by CNEO QM/MM is in great agreement with the experimental observations. All of these results demonstrate the significant impact of the solvation environment and nuclear quantum effects, both of which are key features of the CNEO QM/MM approach. As an accurate and efficient method, CNEO QM/MM holds great promise for future investigation of hydrogen-related processes in complex chemical and biological environments.

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