

Effective yet Reliable Computation of Hyperfine Coupling Constants in Solution by a QM/MM Approach: Interplay Between Electrostatics and Non-electrostatic Effects

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In this paper, we have extended to the calculation of hyperfine coupling constants, the model recently proposed by some of the present authors (*J. Chem. Theory Comput.*, **2017**, *13*, 4854–4870) to include Pauli repulsion and dispersion effects in QM/MM approaches. The peculiarity of the proposed approach stands in the fact that repulsion/dispersion contributions are explicitly introduced in the QM Hamiltonian. Therefore, such terms not only enter the evaluation of energetic properties but propagate to molecular properties and spectra. A novel parametrization of the electrostatic Fluctuating Charges force field has been developed, thus allowing a quantitative reproduction of reference QM interaction energies. Such a parametrization has been then tested against the prediction of EPR parameters of prototypical nitroxide radicals in aqueous solutions.

I. INTRODUCTION

In the last decades multiscale models have been widely used for the study of molecular properties and spectra.^{1–11} In this context, the most successful approaches fall within the class of “focused models”, which aim at accurately modeling both the physico-chemical properties of the target and its interactions with the surrounding environment. The effect of the latter is seen as a perturbation on the target molecule, and is treated at a lower computational level of theory, e.g. by resorting to classical physics, whereas the target molecule is described accurately, generally at the Quantum Mechanical (QM) level. Due to such a partitioning, the computational cost of a QM/classical computation is comparable to that of the corresponding QM isolated system. Such a feature has strongly contributed to the increasing popularity of these models.

QM/Molecular Mechanics (MM) models are among the most renowned classes of QM/classical approaches,^{2,12–18} which have been formalized within different physical frameworks. Beyond the basic mechanical QM/MM embedding, in the last years much effort has been spent to define electrostatic QM/MM embedding approaches, in which a set of fixed charges is placed on the MM moiety (generally on MM atoms) and the interaction between QM and MM portions is modeled by resorting to the Coulomb law. Clearly, in such approaches the QM and MM moieties do not mutually polarize. Mutual polarization, i.e. the polarization of the MM portion arising from the interaction with the QM density and viceversa, can be introduced by employing polarizable force-fields, which can be based on distributed multipoles,^{19–23} induced dipoles,^{24–26} Drude oscillators²⁷ or Fluctuating Charges (FQ).^{8,28,29}

The description of the molecular properties/spectra of embedded systems which is obtained by resorting to polarizable embedding is generally quite accurate.^{22,25,26,30–32} However, such models are deeply based on the assumption that electrostatic energy terms dominate the target/environment interactions. Non-electrostatic (Pauli Repulsion and Dispersion) contributions between the QM and MM portions are roughly modeled by using parametrized functions, e. g. the Lennard-Jones potential,^{33,34} which are however completely independent of the QM density. As a result, they are not taken into account in the QM operators, so that the calculated spectroscopic/response properties are not affected by such interactions. The reasons why such contributions are generally discarded are connected to the presumption of a numerically dominating effect of electrostatic terms. However, non-electrostatic contributions are crucial to get a physically consistent description of any embedded system, also in the case of target/environment interactions dominated by electrostatics.^{35,36}

A way to include non-electrostatic energy terms is to resort to the Effective Fragment Potential (EFP).^{19,20,37–40} The high accuracy of this method is essentially due to the explicit QM calculation of the molecular orbitals of the environment, drifting apart from the concept at the basis of MM Force Field (FF). A similar QM-based approach, namely the Polarizable Density Embedding (PDE), has been recently proposed to only include repulsion effects.^{41,42}

A substantially different way of including non-electrostatic interactions in QM/MM approaches consists of exploiting a model recently developed by some of the present authors,⁴³ which formulates repulsion as a function of an auxiliary density on the MM portion and extends the Tkatchenko-Scheffler (TS) approach to DFT^{44–48} to treat QM/MM dispersion terms. Notice that the formulation of repulsion contributions in terms of gaussian functions placed in the MM region has also been proposed in the so-called Gaussian Electrostatic Model (GEM).^{36,49–51} However, in both the aforementioned PDE and GEM models, repulsion interaction is modeled as a overlap

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one-electron integral. Our approach instead defines repulsion contributions in terms of a two-electron exchange integral, thus physically representing the Pauli repulsion. Moreover, differently from the stand-alone approaches discussed above (EFP, PDE, GEM), our approach can be easily coupled to any kind of QM/MM approach, because repulsion and dispersion are formulated in a way which is totally independent of the choice of the FF to model the electrostatics (i.e. fixed-charges or polarizable embedding). Remarkably, in our model repulsion and dispersion contributions are indeed dependent on the QM density. Thus, an explicit contribution to the QM Fock operator exists and the resulting calculated QM properties/spectra are modified by such interactions.

Our model for non-electrostatics in QM/MM has been so far only challenged on reproducing full QM non-electrostatic interaction energies, for which very good results have been obtained.⁴³ In this paper we start with the extension of the model to spectroscopy. To this end, we report the formulation of non-electrostatic QM/MM terms for EPR, for which environmental effects substantially contribute to the overall observable.⁵²⁻⁵⁴ Environmental (solvent) effects on EPR are usually described by means of continuum models,⁵⁵⁻⁵⁸ and only in few cases by adopting electrostatic QM/MM embedding coupled with a classical Molecular Dynamics (MD) to take into account the fluctuations of both the solute conformations and the solvent molecules.⁵⁹⁻⁶⁵

Nitroxide radicals are among the most thoroughly studied radicals from both experimental and computational points of view due to their remarkable stability coupled to strong sensitivity to the polarity of the surrounding and to the pyramidality of the nitrogen atom. Given their importance, several nitroxide radicals have been synthesized to be either used as spin probes (when dispersed in an environment) or as spin labels (when chemically attached to a biological molecule, e.g. a protein).⁶⁶⁻⁶⁸ High-field EPR spectroscopy provides quite rich information consisting essentially of the nitrogen hyperfine and gyromagnetic tensors.⁶⁶ However, interpretation of these experiments in structural terms strongly benefits from quantum mechanical calculations able to dissect the overall observables in terms of the interplay of several subtle effects.^{59,60,69-75} This situation has prompted us to perform a comprehensive study of prototypical nitroxide radicals in aqueous solution coupling density functional and coupled cluster quantum mechanical computations to molecular dynamics simulations, and average of properties for a sufficient number of snapshots including electrostatic, induction, repulsion and dispersion interactions with the surrounding evaluated by effective quantum mechanical approximations.

To the best of our knowledge, this work presents the first formulation and application of a QM/MM approach accounting at the same time for polarization and non-electrostatic interactions on EPR Hyperfine Coupling Constant (hcc).

The paper is organized as follows: firstly, the theoretical model is presented. Then, the computational approach is applied to the calculation of hcc_N of two nitroxyl radicals (PROXYL and TEMPO) in aqueous solution. Such compounds are characterized by the presence of the N-O group, which has been most widely used as "spin probe" and "spin

label" for the study of structure and dynamics of macro-molecular systems.⁶⁶⁻⁶⁸ Summary and Conclusions end the manuscript.

II. THEORETICAL MODEL

The total energy of a system composed by two interacting moieties, one described at the QM level and the other at the MM level can be expressed as:^{76,77}

$$E_{QM/MM} = E_{QM/MM}^{ele} + E_{QM/MM}^{pol} + E_{QM/MM}^{ex-rep} + E_{QM/MM}^{dis} \quad (1)$$

where, $E_{QM/MM}^{ele}$ accounts for electrostatic interactions and $E_{QM/MM}^{pol}$ is the polarization contribution. Such energy terms are those modeled in the electrostatic embedding approach, and in particular in polarizable QM/MM methods.^{2,12,24-27,78} $E_{QM/MM}^{ex-rep}$ is the exchange-repulsion contribution and $E_{QM/MM}^{dis}$ arises from dispersion interactions.

In this work electrostatic and the polarization terms are modelled by exploiting the Fluctuating Charge (FQ) force field,^{8,30,78-82} whereas non-electrostatic interactions (i.e. the sum of $E_{QM/MM}^{ex-rep}$ and $E_{QM/MM}^{dis}$) are modeled by using the model described in Ref.⁴³. In the next paragraphs, the mathematical formulation of the different energy contributions are discussed.

A. Electrostatic and Polarization Interactions

In order to model electrostatic and polarization terms (see Eq. 1), a polarizable QM/MM embedding needs to be adopted. In such a model, the MM force field adapts to the external field/potential originating from the QM density and electrostatic/polarization terms are included in the QM Hamiltonian, so as to describe the mutual interaction between the QM density and the environment.

In this work we will resort to the FQ force field.⁸ In the resulting QM/FQ model, the electrostatic potential due to the QM density together with the differences in electronegativities between different atoms in the MM region, give rise to a charge fluctuation in the MM region, up to the point that the differences in electrochemical potential between the MM atoms vanish. From a mathematical point of view, this results in the following linear equation:⁸³

$$\mathbf{D}\mathbf{q}_\lambda = -\mathbf{C}_Q - \mathbf{V}(\mathbf{P}_{QM}) \quad (2)$$

where \mathbf{D} is a response matrix, whose diagonal terms are atomic chemical hardnesses, \mathbf{q} is a vector containing the FQs and Lagrangian multipliers. \mathbf{C} contains atomic electronegativities and those constraints which are needed to ensure each MM molecule to have a fixed charge. $\mathbf{V}(\mathbf{P})$ is the potential due to the QM density matrix \mathbf{P} calculated at MM charges positions. We refer the reader to Ref.⁸⁴ for further details.

The interaction between FQ charges and the QM density obeys the Coulomb law:

$$E_{QM/MM}^{ele} + E_{QM/MM}^{pol} = \sum_{j=1}^{N_{FQs}} \int_{\mathbf{R}^3} \frac{\rho_{QM}(\mathbf{r})q_j}{|\mathbf{r} - \mathbf{r}_j|} d\mathbf{r} \quad (3)$$

By differentiating Eq. 3 with respect to the density matrix, $P_{\mu\nu}$, the contribution to the Fock matrix is obtained:⁸³⁾

$$F_{\mu\nu} = \frac{\partial E}{\partial P_{\mu\nu}} = \mathbf{V}_{\mu\nu}^\dagger \mathbf{q} \quad (4)$$

The Fock matrix defined in this way can enter a SCF procedure, so as to finally give a QM density mutually equilibrated with the FQs.

B. Pauli Repulsion Energy

The Exchange-Repulsion energy, $E_{QM/MM}^{ex-rep}$, also known as Pauli Repulsion energy, is formally due the Pauli principle, i.e. wavefunction antisymmetry. From a mathematical point of view, it can be formulated as the opposite of an exchange integral.^{77,85}

$$E_{QM/MM}^{ex-rep} = \frac{1}{2} \int \frac{d\mathbf{r}_1 d\mathbf{r}_2}{r_{12}} \rho_{QM}(\mathbf{r}_1, \mathbf{r}_2) \rho_{MM}(\mathbf{r}_2, \mathbf{r}_1) \quad (5)$$

In order to define the density matrix ρ_{MM} we localize fictitious valence electron pairs for MM molecules in bond and lone pair regions and represent them by s-gaussian-type functions. The expression for ρ_{MM} becomes:

$$\rho_{MM}(\mathbf{r}_1, \mathbf{r}_2) = \sum_{\mathbf{R}} \xi_{\mathbf{R}}^2 e^{-\beta_{\mathbf{R}}(\mathbf{r}_1 - \mathbf{R})^2} \cdot e^{-\beta_{\mathbf{R}}(\mathbf{r}_2 - \mathbf{R})^2} \quad (6)$$

where, \mathbf{R} collects the centers of the gaussian functions used to represent the fictitious MM electrons. The β and ξ parameters are generally different for lone-pairs or bond-pairs, their values being adjusted to the specific kind of environment (MM portion) to be modeled. By substituting Eq. 6 in Eq. 5, the QM/MM repulsion energy reads:

$$E_{QM/MM}^{rep} = \frac{1}{2} \sum_{\mathbf{R}} \int \frac{d\mathbf{r}_1 d\mathbf{r}_2}{r_{12}} \rho_{QM}(\mathbf{r}_1, \mathbf{r}_2) \cdot \left[\xi_{\mathbf{R}}^2 e^{-\beta_{\mathbf{R}}(\mathbf{r}_1 - \mathbf{R})^2} \cdot e^{-\beta_{\mathbf{R}}(\mathbf{r}_2 - \mathbf{R})^2} \right] \quad (7)$$

It is worth noticing that in this formalism, QM/MM Pauli Repulsion energy is calculated as a two-electron integral. Eq. 7 is general enough to hold for any kind of MM environment (solvents, proteins, surfaces ecc.). The nature of the external environments is specified by defining the number of different electron-pair types and the corresponding β and ξ parameters

in Eq. 6. Also, the formalism is general, so that it can be coupled to any kind of QM/MM approach.

By differentiating Eq. 7 with respect to the density matrix, the corresponding contribution to the Fock matrix is obtained:

$$F_{\mu\nu}^{rep} = \frac{\partial E^{rep}}{\partial P_{\mu\nu}} = \frac{1}{2} \int d\mathbf{r}_1 \left[\frac{\chi_{\mu}(\mathbf{r}_1) A_{\nu}(\mathbf{r}_1) + A_{\mu}(\mathbf{r}_1) \chi_{\nu}(\mathbf{r}_1)}{2} \right] \quad (8)$$

where χ_{μ} are atomic basis functions and A_{μ} are calculated as detailed in Ref.⁴³.

C. Quantum Dispersion Energy

To formulate dispersion interactions we start from the Tkatchenko and Scheffler (TS) DFT functional. In this model, the dispersion energy can be written as:

$$E^{dis} = -\frac{1}{2} \sum_{A,B} f_{damp}(R_{AB}, R_A^0, R_B^0) C_{6AB} R_{AB}^{-6} \quad (9)$$

where, R_{AB} is the distance between atoms A and B in a given system, C_{6AB} is the corresponding C_6 coefficient, R_A^0 and R_B^0 are their van der Waals (vdW) radii. The R_{AB}^{-6} singularity at small distances is eliminated by the short-range damping function $f_{damp}(R_{AB}, R_A^0, R_B^0)$.⁴⁴

C_{6AB} coefficients can be expressed in terms of homonuclear parameters C_{6AA} , C_{6BB} , which in turn can be obtained through an Hirshfeld⁸⁶ partition of the density.⁴⁴ Notice that alternative partitioning approaches can in principle be exploited.⁸⁷

Such an approach can be reformulated within a QM/MM formalism,^{43,48} yielding:

$$E_{QM/MM}^{dis} = -\frac{1}{2} \sum_{A \in QM} \sum_{B \in MM} f_{damp}(R_{AB}, R_A^0, R_B^0) \cdot \frac{\eta_A^2 C_{AA}^{free} C_{6BB}^{eff}}{\alpha_A^0 \eta_A^2 C_{AA}^{free} + \alpha_B^0 C_{6BB}^{eff}} R_{AB}^{-6} \quad (10)$$

where C_{6BB}^{eff} are effective homonuclear coefficients of B (MM) atoms and C_{6AA}^{free} are free homonuclear coefficients of A QM atoms. α_A^0 and α_B^0 are static dipole polarizabilities, whereas η_A is a function converting C_{6AA}^{free} into C_{6AA}^{eff} . Further details can be found in Refs.^{43,44,48}

$f_{damp}(R_{AB}, R_A^0, R_B^0)$ in Eq. 10 is a Fermi-type damping function:^{44,88,89}

$$f_{damp}(R_{AB}, R_A^0, R_B^0) = \frac{1}{1 + \exp \left[-d \left(\frac{R_{AB}}{s_R R_{AB}^0} - 1 \right) \right]} \quad (11)$$

where, $R_{AB}^0 = R_A^0 + R_B^0$, and d , s_R are free parameters.

Similarly to what already done for electrostatic and repulsion contributions, by differentiating Eq. 10 with respect to the QM density matrix the dispersion contribution to the Fock matrix is obtained.⁴³

$$F_{\mu\nu}^{dis} = -\frac{1}{2} \sum_{A \in \text{QM}} \sum_{B \in \text{MM}} f_{damp}(R_{AB}) \cdot \frac{2 \frac{\alpha_A^0}{\alpha_B^0} C_{6BB}^2 C_{6AA}^{free} 2\eta_A}{\left(\frac{\alpha_B^0}{\alpha_A^0} C_{6AA}^{eff} + \frac{\alpha_A^0}{\alpha_B^0} C_{6BB} \right)^2} \eta_{A,\mu\nu}^p R_{AB}^{-6} \quad (12)$$

The complete derivation and definition of $\eta_{A,\mu\nu}^p$ can be found in Ref.⁴³.

D. Hyperfine Coupling Constant

The spin Hamiltonian describing the interaction between the electron spin (S) of a free radical containing a magnetic nucleus of spin I and an external magnetic field (B) can be written as:

$$H_S = \mu_B \vec{S} \cdot \mathbf{g} \cdot \vec{B} + \frac{1}{\hbar\gamma_I} \vec{S} \cdot \mathbf{A} \cdot \vec{\mu}_I \quad (13)$$

where the first term is the Zeeman interaction between the electron spin and the external magnetic field through the Bohr magneton μ_B and $\mathbf{g} = g_e \mathbf{1}_3 + \Delta g_{corr}$. Δg_{corr} accounts for the correction to the free electron value ($g_e = 2.0022319$) due to several terms including the relativistic mass (Δg_{RM}), the gauge first-order corrections (Δg_C) and a term arising from the coupling of the orbital Zeeman (OZ) and the spin-orbit coupling (SOC) operator.^{90,91} The second term on the rhs of Eq. 13 describes the hyperfine interaction between S and the nuclear spin I through the hyperfine coupling tensor \mathbf{A} . The latter, which is defined for each nucleus X , can be decomposed into two terms:

$$\mathbf{A}(X) = A_X \mathbf{1}_3 + \mathbf{A}_{dip}(X) \quad (14)$$

The dipolar term $\mathbf{A}_{dip}(X)$ is a zero-trace tensor, whose contribution vanishes in isotropic media (e.g. solutions). The first term A_X (Fermi-contact interaction), which is an isotropic contribution, is also known as hyperfine coupling constant (hcc). It is related to the spin density (ρ_X) at nucleus X by the following relation:

$$A_X = \frac{4\pi}{3} \mu_B \mu_X g_e g_X \langle S_Z \rangle^{-1} \rho_X^{\alpha-\beta} \quad (15)$$

where $\rho_X^{\alpha-\beta}$ can be obtained as:

$$\rho_X^{\alpha-\beta} = \sum_{\mu\nu} P_{\mu\nu}^{\alpha-\beta} \langle \chi_\mu(\mathbf{r}) | \delta(\mathbf{r} - \mathbf{r}_X) | \chi_\nu(\mathbf{r}) \rangle \quad (16)$$

$P^{\alpha-\beta}$ is the difference between α and β density matrices. Because in our approach both electrostatic and non-electrostatic dispersion/repulsion interactions enter the definition of the QM Fock operators (see Eqs. 4, 8 and 12), $P^{\alpha-\beta}$

is modified. Therefore, hyperfine coupling constants with the account of electrostatic, polarization, dispersion and repulsion QM/MM interactions are obtained.

III. COMPUTATIONAL DETAILS

Molecular geometries of PROXYL and TEMPO radicals (Fig. 1) were optimized in vacuo by combining B3LYP and PBE0 hybrid density functionals with both aug-cc-pVDZ and 6-311++G(3df,2pd) basis sets. For all optimized structures the hyperfine coupling constant of Nitrogen atom was calculated by exploiting both B3LYP and PBE0 and the N07D basis set.^{92,93} For the sake of comparison, on the reduced structures depicted in Figure 1, which are obtained by removing ring atoms for both TEMPO and PROXYL but keeping fixed all the geometrical parameters, additional CCSD/EPR-II⁹⁴ calculations were performed.

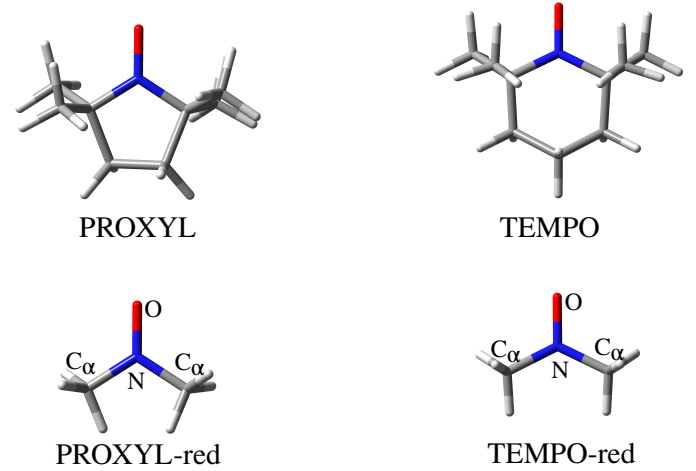


FIG. 1. Top: PROXYL and TEMPO structures. Bottom: reduced structures used for CCSD/EPR-II calculations.

Clusters made of TEMPO and PROXYL radicals with two explicit water molecules (see Fig. 3) were optimized at the PBE0/6-311++G(3df,2pd) level, according to previous studies.⁵⁷ For those structures, the interaction energy between the radicals and the two water molecules was computed by exploiting SAPT0/aug-cc-pVTZ, or jun-ccp-pVDZ or N07D (as implemented in Psi4 1.1⁹⁵) and CCSD(T)/aug-cc-pVTZ, jun-cc-pVDZ and N07D. Counter-Poise corrections were included in CCSD(T) calculations. QM/MM energy calculations were also performed at the PBE0/aug-cc-pVTZ, jun-cc-pVDZ and PBE0/N07D level, by including dispersion and repulsion energies obtained by exploiting our model.⁴³ The QM portion was restricted to the radical, whereas the two water molecules were treated at the MM level. The MM region was described by means of a non-polarizable force field (TIP3P⁹⁶) and the polarizable FQ approach^{8,78} by exploiting two literature parametrizations,^{28,97} and a new parametrization proposed in this work. The parameters used for modeling dispersion and repulsion interactions were taken from Ref.⁴³.

On the same structures, full QM and QM/MM nitrogen hyperfine coupling constants were calculated by exploiting the PBE0/N07D level of theory for treating the QM portion. For the sake of comparison, on the reduced cluster structures depicted in Figure 3, which are obtained by removing ring atoms for both TEMPO and PROXYL but keeping fixed all the geometrical parameters, additional CCSD/EPR-II⁹⁴ hcc_N calculations were performed.

Classical MD simulations were performed with the Amber software (v.12) using the ff99SB force field.^{98,99} Parameters for nitroxides were obtained from a previous study by one of the present authors⁵⁹. The nitroxides were embedded in a cubic box of TIP3P water molecules, which extended to 30 Å from the solute surface. The starting systems were equilibrated following a multistep protocol: i) minimization of the whole system for 10000 steps, ii) heating of the system from 103 to 303 K in 100 ps with a mild restraint of 0.5 kcal/mol Å² on the solute, iii) equilibration in NPT ensemble at a pressure of 1 bar and 303 K for 100 ps. The production phase was then initiated in NVT ensemble and continued for 10 ns. The simulation conditions involved Periodic Boundary Condition (PBC), a 1 fs time step for numerical integration, using SHAKE for constraining bonds involving hydrogens,¹⁰⁰ a 10 Å cut-off for non-bonded interactions, PME for evaluating the long-range electrostatics,¹⁰¹ temperature regulation with Langevin coupling using a collision frequency of 1.0 ps⁻¹, snapshots collection in the trajectory at 1 ps interval.

A total of 200 uncorrelated snapshots were extracted from the MDs (one snapshot every 50 ps). For each snapshot a 13 Å sphere centered at the solute’s geometric center was cut. All hyperfine coupling constants were calculated within the QM/FQ or QM/TIP3P framework at the PBE0/N07D level. The FQ water molecules were modeled both with the SPC FQ parameters,²⁸ the parametrization proposed by some of the present authors⁹⁷ and the parameters proposed in this work. The convergence of the hcc_N values as increasing the number of representative snapshots was checked for both radicals. Dispersion and repulsion contributions to hcc_N were included by exploiting what has been explained in the previous section. All QM/FQ calculations were performed by using a locally modified version of Gaussian 16.¹⁰² Finally, the calculated values were compared with experimental data taken from Refs.^{103,104}.

IV. NUMERICAL RESULTS

In this section we will report the results issuing from the application of the developed methodology to the calculation of the nitrogen hyperfine coupling constant (hcc_N) of PROXYL and TEMPO radicals in aqueous solution. In order to evaluate the role of the different terms (electrostatic/polarization/dispersion/repulsion) concurring to overall solvent effect, we will present the results obtained by exploiting a hierarchy of different approaches, starting from a simple cluster model (isolated radical plus two water molecules) to averaging over a set of representative structures extracted from MD runs, with or without the inclusion of polariza-

tion/dispersion/repulsion solvent contributions. In addition, to allow a direct comparison with experimental hcc_N , reference values for the isolated radicals are discussed.

A. hcc_N of isolated radicals

PROXYL and TEMPO geometries (see Figure 1) were optimized in vacuo at different levels of theory. In particular, B3LYP and PBE0 functionals in combination with aug-cc-pVDZ (BS1) or 6-311++G(3df,2pd) (BS2) basis sets were employed. Selected geometrical parameters are reported in Table I. In particular, the N-O distance, the $C_\alpha NC_\alpha$ angle and the improper dihedral angle $C_\alpha NOC_\alpha$ were taken into consideration (see Figure 1 for atom labeling). Additional data obtained with B3LYP-D3 and PBE0-D3 functionals⁸⁸ can be found in Table S1 given as Supplementary Material (SM). Geometries were also optimized by exploiting the B2PLYP double hybrid functional combined with the maug-cc-pVTZ-d(H) basis set (BS3), which has been reported to reliably describe molecular geometries.¹⁰⁵ The values reported in Table I clearly show that B3LYP/aug-cc-pVDZ and B2PLYP/maug-cc-pVTZ-d(H) perform in a similar way. However, all the considered combinations of functional and basis set do not differ much from the best calculated structure of both radicals. It is worth pointing out that the most relevant difference between PROXYL and TEMPO stands in the value of the improper dihedral angle $C_\alpha NOC_\alpha$, which is related to the Nitrogen atom pyramidalization. In fact, the angle is almost zero for PROXYL and about -21 degrees for TEMPO.

	Parameter	PBE0		B3LYP		B2PLYP
		BS1	BS2	BS1	BS2	BS3
PROXYL	N-O	1.262	1.257	1.274	1.268	1.273
	$\angle C_\alpha NC_\alpha$	115.3	115.2	115.4	115.2	115.2
	$C_\alpha NOC_\alpha$	± 0.0	± 0.0	± 0.0	± 0.0	± 0.0
TEMPO	N-O	1.271	1.266	1.283	1.278	1.282
	$\angle C_\alpha NC_\alpha$	124.1	124.2	124.5	124.6	124.4
	$C_\alpha NOC_\alpha$	± 22.0	± 21.3	± 21.2	± 20.6	± 21.6

TABLE I. Selected geometrical parameters of PROXYL and TEMPO radicals at the different levels of theory. BS1: aug-cc-pVDZ; BS2: 6-311++G(3dp,2pd); BS3: maug-cc-pVTZ-d(H).

For all the optimized structures obtained with PBE0 and B3LYP functionals in conjunction with BS1 and BS2, hcc_N were calculated by exploiting either PBE0 or B3LYP and the N07D basis sets purposely parametrized for both functionals (see Refs.^{92,93} for more details). For the sake of comparison, additional hcc_N calculations were performed at the CCSD/EPR-II⁹⁴ level on the reduced structures depicted at the bottom of Figure 1. All results are reported in Table II.

hcc_N for the two radicals differ by about 3 Gauss at all levels. Such differences are essentially due to the different pyramidalization of the nitroxyl group. The small discrepancies which are reported for the various optimized structures are due to small fluctuations in the improper dihedral angle (see Table I). Notice that all calculated DFT hcc_N are underesti-

mated with respect to CCSD/EPR-II values.

Radical	Optimized structure	PBE0/N07D	B3LYP/N07D	CCSD/EPR-II
PROXYL	B3LYP/BS1	11.8	11.4	12.7
	B3LYP/BS2	12.0	11.3	12.6
	PBE0/BS1	11.8	11.1	12.4
	PBE0/BS2	11.7	11.0	12.3
TEMPO	B3LYP/BS1	15.0	14.4	15.9
	B3LYP/BS2	14.8	14.2	15.7
	PBE0/BS1	14.9	14.3	15.7
	PBE0/BS2	14.7	14.0	15.4

TABLE II. Calculated hcc_N values (Gauss). BS1: aug-cc-pVDZ; BS2: 6-311++G(3dp,2pd).

To further investigate on the role of nitrogen pyramidalization on hcc_N , PBE0/N07D hcc_N values for the reduced TEMPO structure as a function of $C_\alpha NOC_\alpha$ were calculated. The data are graphically reported in Fig. 2.

As it can be noticed, the value computed for PROXYL and TEMPO radicals are almost recovered at zero and ± 20 degrees, respectively. For larger $C_\alpha NOC_\alpha$ values, computed hcc_N values increase up to the maximum value (22 Gauss) at about ± 40 degrees. Such a trend confirms what has already been reported by one of the present authors.⁶⁰ In Figure S1 of the SM, we report for comparison CCSD/EPR-II hcc_N values as a function of $C_\alpha NOC_\alpha$. DFT underestimates spin polarization (which is the only relevant contribution for the planar structure), and at the same time overestimates the SOMO delocalization, which instead increases as the $C_\alpha NOC_\alpha$ increases.

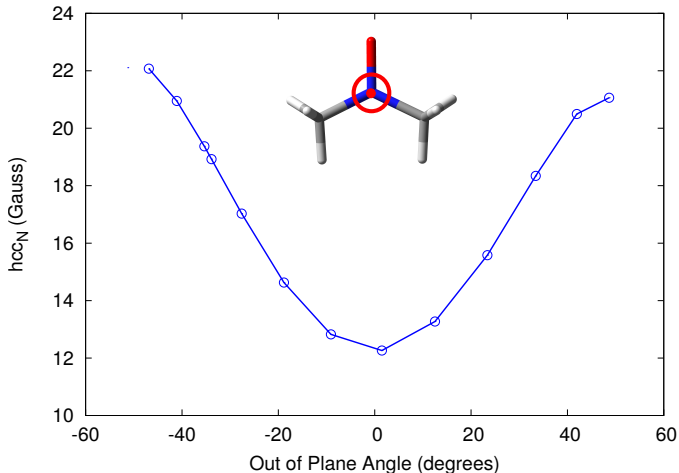


FIG. 2. PBE0/N07D hcc_N values (Gauss) on the reduced TEMPO structure as a function of the out of plane $C_\alpha NOC_\alpha$ angle.

B. hcc_N of PROXYL/TEMPO+water clusters

The most basic method to describe hydrated radicals is to resort to a cluster approach. In particular, due to the presence of the oxygen atom, a natural choice consists of saturating oxygen doublets with two water molecules (see Figure 3).^{57,60} According to what has already been proposed in

previous studies, all structures were optimized at the PBE0/6-311++G(3df,2pd) level.^{57,59}

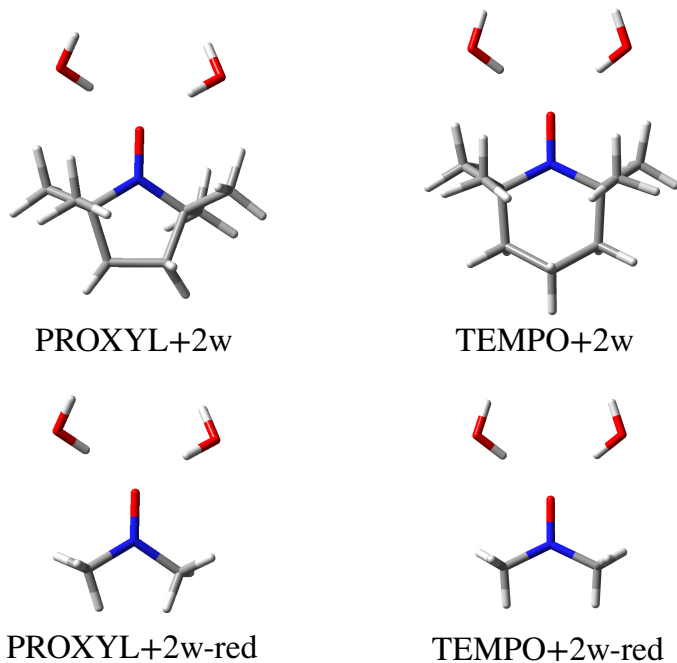


FIG. 3. PBE0/6-311++G(3df,2pd) optimized structures of clusters of PROXYL (top) and TEMPO (bottom) with two water molecules.

To quantify the different contributions to the radical/water interaction energy, Energy Decomposition Analysis (EDA) as formulated in the Symmetry-Adapted Perturbation Theory (SAPT0),^{106,107} was performed by exploiting the aug-cc-pVTZ basis set on the reduced structure of PROXYL cluster (see Figure 3). Additional SAPT0 calculations were performed by exploiting both the jun-cc-pVDZ or N07D basis sets (see Table S2 given as SM). Such additional sets were selected because jun-cc-pVDZ has been reported to provide good results for closed shell systems,¹⁰⁸ whereas N07D is exploited in this study to calculate hcc_N .

SAPT0/aug-cc-pVTZ results are reported in Table III, together with the corresponding values obtained by treating the radical at the QM level (PBE0/aug-cc-pVTZ) and the two water molecules at MM level. QM/MM electrostatic interactions were described by using the FQ approach with three different parametrizations (see Table S3 in SM), whereas QM/MM repulsion and dispersion contributions were modeled as reported above. Additional CCSD(T)/aug-cc-pVTZ calculations including Counter-Poise¹⁰⁹ corrections were also performed to quantify the accuracy of SAPT0 interaction energies.

SAPT0 values show that electrostatic interactions (i.e. the sum of electrostatic and induction terms) give larger contributions with respect to non-electrostatic (repulsion+dispersion). However, non-electrostatic interactions and in particular repulsion cannot be neglected, as it is commonly done in standard QM/MM models.

Moving to QM/FQ, we first notice that the available

parametrizations (FQ^a and FQ^b in Table III) focus on modeling electrostatic interactions, however they can indeed be inadequate whenever non-electrostatic terms are taken into consideration. This is confirmed by our results (Table III): FQ^a and FQ^b electrostatic energies give a qualitatively correct description of SAPT0 or CCSD(T) total interaction energies. On the contrary, FQ^a and FQ^b total interaction energies are unsatisfactory; therefore, a novel FQ parametrization is required (labeled FQ^c in Table III). Differently from FQ^a and FQ^b , which were obtained to reproduce water bulk properties (FQ^a , ref.²⁸) or QM atomic charges (FQ^b , ref.⁹⁷), FQ^c is tuned to the total interaction energy at the CCSD(T) level (with an error of less than 1 kcal/mol). FQ^c yields an accurate description of SAPT0 electrostatic interactions. Notice that similar findings are given by both jun-cc-pVDZ and N07D basis sets (see Table S2 in SM). To end the discussion on interaction energies, it is worth noticing that the analysis reported above is only allowed when non-electrostatic interactions are included in QM/MM calculations, i.e. is not achievable by exploiting common purely electrostatic approaches.

	FQ^a	FQ^b	FQ^c	SAPT0	CCSD(T)
Electrostatic	-20.60	-26.80	-47.06	-31.35	–
Induction	–	–	–	-11.45	–
Repulsion	27.78	28.58	30.99	28.34	–
Dispersion	-3.28	-3.28	-3.28	-9.43	–
Total	3.90	-1.50	-19.35	-23.89	-20.62

TABLE III. PROXYL+2w EDA obtained by exploiting PBE0/FQ with different parametrizations and SAPT0. CCSD(T) calculations include Counter-Poise corrections. All data are reported in mHartree and were obtained by using the aug-cc-pVTZ basis set.

^a FQ parametrization taken from Ref.²⁸

^b FQ parametrization taken from Ref.⁹⁷

^c FQ parametrization proposed in this work

Calculated hcc_N of the PROXYL/TEMPO+2w clusters are reported in Table IV. QM/MM calculations were performed by exploiting both the non-polarizable TIP3P⁹⁶ force field and FQ (with different parametrizations) to describe electrostatic interactions. Two set of QM/MM calculations were performed. The first employs TIP3P or FQ embedding and do not include non-electrostatic interactions. The corresponding results are reported in the first four columns of Table IV. In the second set of calculations, non-electrostatic interactions, as obtained with our model, are included. All results are also compared with full QM calculations, i.e. both the radicals and the two water molecules are described at the QM level (see column 9 in Table IV).

The reported data clearly show that the non-polarizable TIP3P approach gives large errors with respect to full QM calculations; remarkably, the inclusion of non-electrostatic terms does not improve the results. A different picture results from polarizable QM/FQ values. In fact, when only the electrostatic interactions are considered, the FQ^b parametrization gives values which are in fair agreement with the reference full QM data. However, the inclusion of non-electrostatic interactions shifts hcc_N values in the wrong direction, thus increasing the absolute difference with respect to reference val-

ues. This is not surprising, because EDA analysis (see Table III) already showed underestimated electrostatic interactions. The same considerations are also valid for FQ^a , whereas the novel FQ^c parametrization overestimates hcc_N values if only electrostatic interactions are considered. Remarkably, the inclusion of non-electrostatic interactions shifts FQ^c values in the right direction, and the agreement with full QM reference data is almost perfect (0.2 Gauss).

Furthermore, additional PBE0/N07D and CCSD/EPR-II calculations were performed on the reduced structures depicted in Figure 3 (see Table IV). Full QM DFT calculations underestimate CCSD/EPR-II hcc_N value by 0.9 and 0.8 Gauss for PROXYL and TEMPO, respectively. Notice that calculated CCSD/EPR-II hcc_N are still not comparable with experimental values, especially for PROXYL. This confirms that the cluster approach is inadequate to physically describe the solvation phenomenon, which is intrinsically a dynamical process.

C. hcc_N of PROXYL/TEMPO from MD runs

An alternative and more accurate way of modeling solvation is to combine our approach with classical MD. Table V reports selected geometrical parameters (and their standard deviation) obtained by averaging 200 representative snapshots extracted from MD runs performed on PROXYL and TEMPO in aqueous solution. The improper dihedral angle $C_\alpha NOC_\alpha$, which as stated before plays a crucial role in determining EPR parameters, is drastically different with respect to what has been reported for the isolated radicals, especially for TEMPO. Furthermore, due to the dynamical picture given by the MD, the geometrical parameters are accompanied by standard deviations (in brackets), which are large in the case of this angle.

In order to show how the variability in the improper dihedral affects calculated hcc_N values, two different set of calculations were performed. First, all solvent molecules in all snapshots were removed and hcc_N were calculated on the resulting structures. Second, all solvent molecules were indeed included and treated at the FQ level, with the sole inclusion of electrostatic effects (c parametrization). In Figures 4 and 5 the resulting hcc_N values are reported as a function of the out-of-plane $C_\alpha NOC_\alpha$ angle.

As expected, the same picture as already reported for the isolated radicals emerges.

Due to the large variability of hcc_N values as a function of the out of plane angle, the convergence of average values needs to be carefully checked. In Figure 6 QM/FQ hcc_N average values as a function of the number of snapshots are depicted for the two radicals. Clearly, hcc_N is well converged by using 200 snapshots.

Let us now compare our computed data with their experimental counterparts. Table VI collects hcc_N values computed with different approaches. QM indicates calculations performed on the solute-only structures extracted from MD (see above). QM/FQ data were obtained by using the purely-electrostatic polarizable FQ with the c parametrization (the results obtained by exploiting the a, b parametrizations are re-

	PBE0/N07D								Full-QM	Full-QM(red)	CCSD/EPR-II	$\Delta_{CC/PBE0}$	Exp
	Elect.				Elect. + Dis/Rep								
	TIP3P	FQ ^a	FQ ^b	FQ ^c	TIP3P	FQ ^a	FQ ^b	FQ ^c					
PROXYL	13.4	13.1	13.4	14.3	13.1	12.8	13.1	13.9	13.7	13.7	14.6	0.9	16.4
TEMPO	17.9	15.3	15.7	16.7	16.9	14.8	15.1	16.1	15.9	16.3	17.1	0.8	17.3

TABLE IV. hcc_N of PROXYL/TEMPO+2w clusters obtained at different level of theory. All data are reported in Gauss.

^a FQ parametrization taken from Ref.²⁸

^b FQ parametrization taken from Ref.⁹⁷

^c FQ parametrization proposed in this work

	<PROXYL>	<TEMPO>
N-O	1.27 (0.03)	1.27 (0.03)
$\angle C_\alpha NC_\alpha$	115.3 (2.5)	123.6 (2.7)
$C_\alpha NOC_\alpha$	± 0.4 (17.8)	± 5.0 (20.1)

TABLE V. Mean values and standard deviations (in brackets) of selected geometrical parameters of PROXYL and TEMPO structures extracted from MD runs.

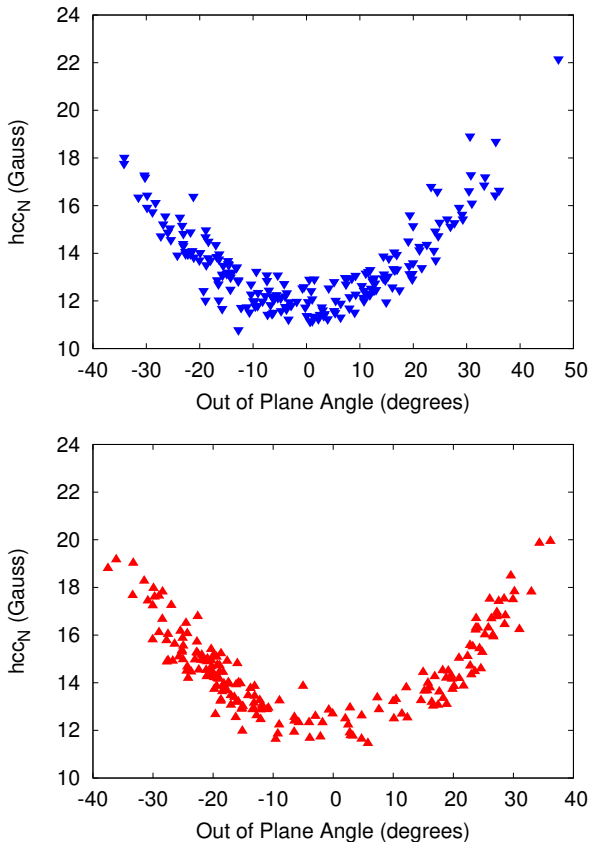


FIG. 4. PBE0/N07D calculated hcc_N (Gauss) on the solute-only structures extracted from MD runs as a function of the out of plane $C_\alpha NOC_\alpha$ angle. (Top: PROXYL; Bottom: TEMPO).

ported in Table S4 in the SM). The contribution to hcc_N due to repulsion interactions is denoted as Δ_{rep} , whereas the contribution to hcc_N of both repulsion and dispersion interactions

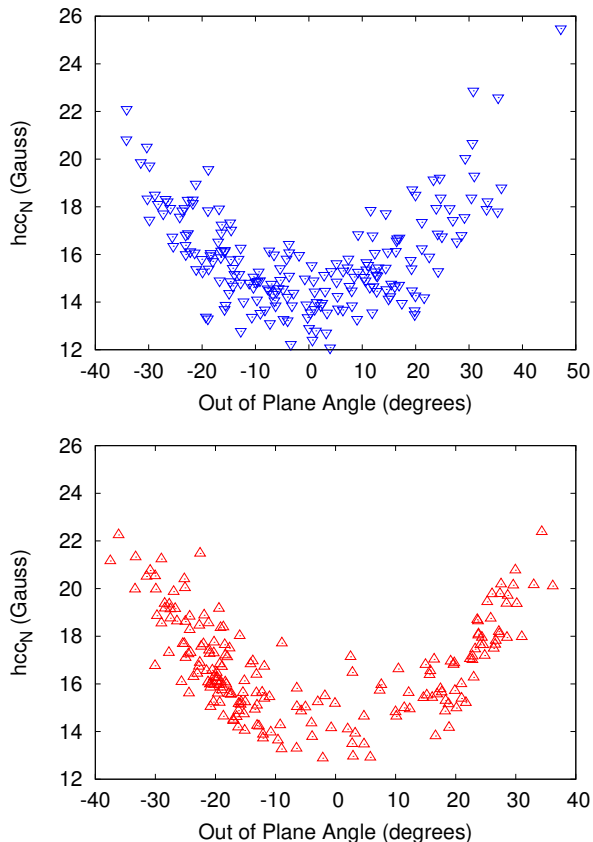


FIG. 5. PBE0/N07D QM/FQ calculated hcc_N (Gauss) on the entire snapshots extracted from MD runs as a function of the out of plane $C_\alpha NOC_\alpha$ angle. (Top: PROXYL; Bottom: TEMPO).

is denoted as $\Delta_{dis-rep}$.

We first notice that, due to the different structural sampling given by the MD, QM data in Table VI differ from what was reported for the isolated radicals (see Table II). The dynamical sampling increases PROXYL and TEMPO hcc_N values by about 2.4 and 2.2 Gauss, respectively. As a result, the difference between hcc_N values of the two radicals (1.1 Gauss) is in good agreement with experimental data (0.9 Gauss).^{103,104} When full solvent effects are included at the purely electrostatic FQ level (2nd column), hcc_N values are increased by about 2.3 Gauss on average for both radicals. This means that attractive interactions increase the computed property. As a

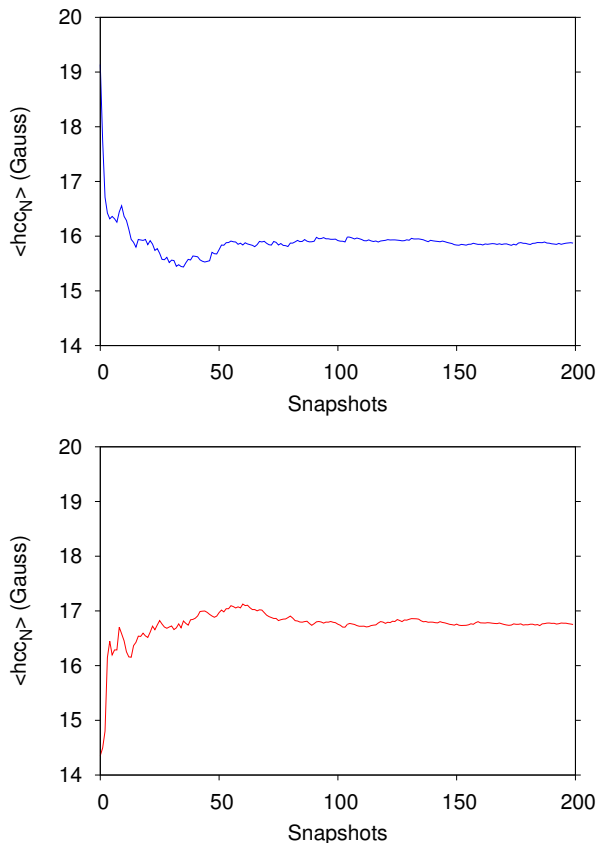


FIG. 6. QM/FQ hcc_N mean value as a function of the number of snapshots extracted from MD runs. (Top: PROXYL; Bottom: TEMPO) All data are reported in Gauss.

result, the inclusion of repulsive interaction terms is expected to decrease computed values, and this is indeed confirmed by the values reported in the third column. In particular, for both radicals hcc_N decreases by 0.4 and 0.5 Gauss, respectively, i.e. of about 17 % and 23 % of the whole solvent effect. The further inclusion of dispersion terms does not affect the difference with FQ average values.

In order to best compare the results of our approach with experimental findings, DFT values were also corrected to account for some intrinsic deficiency. To this end, the difference between full DFT and full CCSD data obtained for clusters ($\Delta_{CC/PBE0}$, see Table IV) was added to the calculated QM/MM value. The resulting values are labeled “Best QM/MM” in Table VI. Remarkably, our best computed values are in excellent agreement with experimental data for both radicals, thus confirming the accuracy and reliability of our approach.

To get further insight into solvent effects on hcc_N values, differences between FQ and QM values are reported as a function of the snapshot in Figure 7. As it can be noticed, for both PROXYL (top) and TEMPO (bottom) the electrostatic solvent contribution to hcc_N is always positive (only in one case a small negative contribution is reported for TEMPO). Notice that this is different from what has been reported for electric properties of higher order.⁸⁴

	QM	FQ	Δ_{rep}	$\Delta_{dis-rep}$	Best QM/MM	Exp.
					$FQ + \Delta_{dis-rep} + \Delta_{CC/PBE0}$	
PROXYL	13.5	15.9	-0.4	-0.4	16.4 ± 0.1	16.4^{103}
TEMPO	14.6	16.8	-0.5	-0.5	17.1 ± 0.1	17.3^{104}

TABLE VI. PBE0/N07D hcc_N mean values calculated on 200 snapshots extracted from MD runs. QM indicates the calculation performed on solute-only structures. FQ refers to the purely electrostatic QM/FQ with c parametrization. Δ_{rep} and $\Delta_{dis-rep}$ are differences between FQ and hcc_N data obtained with our method. Best QM/MM data are obtained by summing FQ, $\Delta_{dis-rep}$ and $\Delta_{CC/PBE0}$ (see Table IV). All values are reported in Gauss.

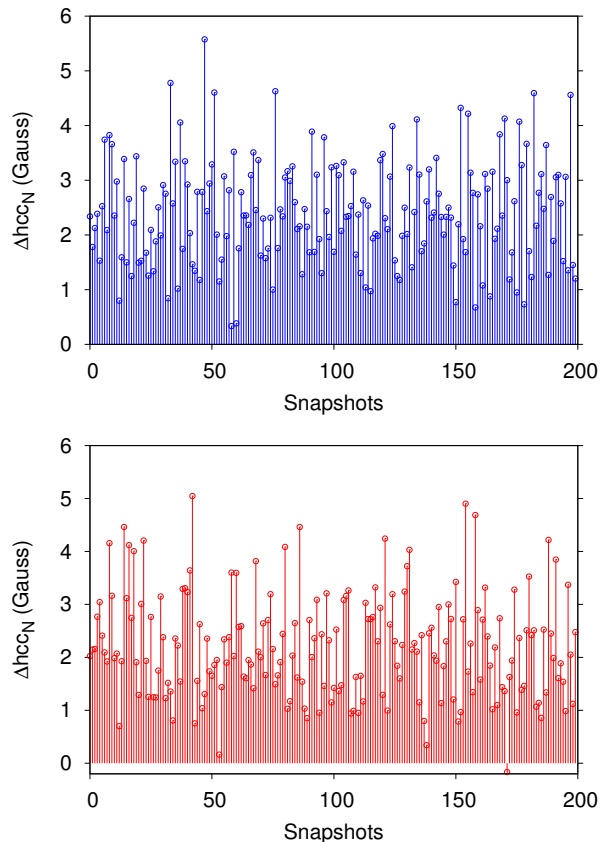


FIG. 7. Calculated solvent effects (see text) on hcc_N as a function of the snapshot extracted from MD runs (Top: PROXYL; Bottom: TEMPO). All data are reported in Gauss.

In Figure 8 the difference between calculated solvent effects on hcc_N as obtained with the purely electrostatic FQ approach or with the further inclusion of the repulsion contribution is reported. Remarkably, repulsion contributions increase or decrease hcc_N value depending on the selected snapshot, thus showing that cluster approaches do not guarantee an adequate modeling of solvent effects. In fact, although repulsion effects give on average a negative contribution to hcc_N , by taking a random snapshot (cluster), a completely different picture could emerge.

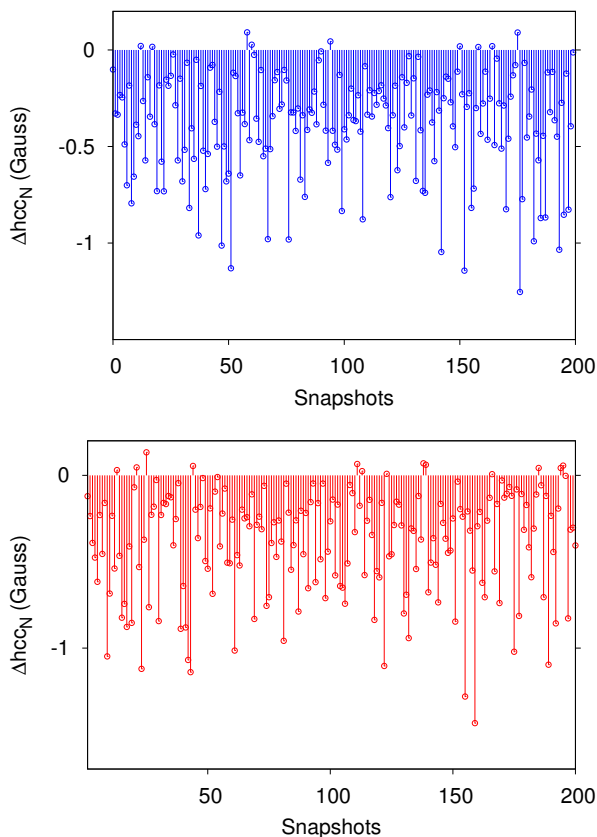


FIG. 8. Difference between FQ and QM/FQ+repulsion solvent effects as a function of the snapshot extracted from the MD (Top: PROXYL; Bottom: TEMPO). All data are reported in Gauss.

V. SUMMARY AND CONCLUSIONS

In this paper, we have extended to the calculation of hyperfine coupling constants, the model proposed in Ref.⁴³ to include Pauli repulsion and dispersion effects in QM/MM approaches. The peculiarity of the proposed approach stands in the fact that repulsion/dispersion contributions are explicitly introduced in the QM Hamiltonian. Therefore, such terms not only enter the evaluation of energetic properties but, remarkably, propagate to molecular properties and spectra. The account of such contributions has permitted a quantitative analysis of QM/MM interaction energies, and this has also required a novel parametrization of the FQ force field, which has been then tested against the prediction of EPR hcc_N of PROXYL and TEMPO in aqueous solutions.

Numerical applications to the two radicals in vacuo, solvated within the so-called cluster approach or as modeled through MD, confirm the well known relevance of solvent effects and a proper account of their dynamical aspects. The further inclusion of dispersion and especially repulsion solute-solvent interactions gives, remarkably, an almost perfect agreement between calculated and experimental values. Therefore, although electrostatic effects have been invoked as dominating the solvation phenomenon in aqueous solution,

we found that non-electrostatic effects are indeed relevant, contributing to 17 % and 23 % of the entire solvent effects on hcc_N for PROXYL and TEMPO, respectively. Remarkably, dispersion interactions seem not to play a crucial role.

To end the discussion, we remark that our model is general enough to be applied to any kind of solvent/environment, pending a reliable parametrization of both electrostatic and non-electrostatic interactions. Also, due to the inclusion of all terms in the molecular Hamiltonian, our approach can be extended to any kind of molecular properties and spectroscopies; this will be the topic of future communications.

VI. SUPPLEMENTARY MATERIAL

PBE0/N07D and CCSD/EPR-II reduced TEMPO hcc_N values as a function of the out of plane $C_\alpha NOC_\alpha$ angle. PROXYL and TEMPO selected geometrical parameters at different levels of theory with the inclusion of Grimme empirical dispersion D3. PROXYL+2w interaction energies calculated at the QM/FQ, SAPT0 and CCSD(T) level. O and H parameters for FQ calculations. PROXYL and TEMPO hcc_N calculated on 200 snapshots extracted from MD runs.

VII. ACKNOWLEDGMENT

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