

Convergence of environment polarization effects in multiscale modeling of excitation energies

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Abstract

We present a systematic investigation of the influence of polarization effects from a surrounding medium on the excitation energies of a chromophore. We use a combined molecular dynamics and polarizable embedding time-dependent density functional theory (PE-TD-DFT) approach for chromophores in proteins and in homogeneous solvents. The mutual polarization between the chromophore and its surroundings is included in the PE-TD-DFT approach through the use of induced dipoles, placed on all atoms in the classical region, and self-consistent optimization of the quantum and classical polarizable regions. By varying the subset of sites in the environment for which atomic polarizabilities are included, we investigate to what distance from the quantum region explicit polarization effects need to be taken into account in order to provide converged excitation energies. Our study gives new insight into the range of polarization interactions for chromophores in different chemical environments. We find that the rate of convergence of excitation energies with respect to polarization cut-off is much slower for chromophores in an ordered environment such as a protein than for chromophores in a homogeneous medium such as a solvent. We show that this in part is related to the (partial) charges in the protein. Our results provide insight into how to define a representation of complex environments of different kinds in an accurate and affordable way.

Keywords: Polarization interactions, Multiscale modeling, Polarizable embedding, QM/MM, Solvent effects on excitation energies

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1. Introduction

The importance of multiscale modeling is firmly established by the Nobel Prize in Chemistry to Karplus, Levitt and Warshel [1]. The basic assumption behind multiscale models is that a molecular system can be divided into smaller subsystems, each of which can be treated using different methods. The most prominent example of a multiscale model is the combined quantum mechanics and molecular mechanics (QM/MM) method [2–4]. Embedding models are an example of the class of focused multiscale models where we pay particular attention to a central part of the system. The simplest embedding models use an (infinite) continuum description of the environment and can be very efficient for solute–solvent systems [5]. However, the extension to more heterogeneous environments, e.g. proteins, is not well defined. Moreover, continuum models suffer from a poor description of more specific solute–solvent interactions such as hydrogen bonds. The QM/MM methods are a generally applicable alternative to the continuum models since the atomistic structure of the environment is retained.

One of the more important aspects of embedding models is the coupling between the central part and the environment. This coupling can be divided into three subclasses [4]: mechanical embedding (ME), electrostatic embedding (EE) and polarizable embedding (PE). The coupling in the ME scheme is performed on a purely classical level and it is therefore only suitable for ground-state energy calculations. The EE scheme, in contrast, includes one-electron operators in the electronic Hamiltonian that describe the interactions between the permanent charge distribution of the environment and the particles, i.e. electrons and nuclei, in the central subsystem. This will directly affect (polarize) the electron density of the central part and thus also the calculated molecular properties. The environment is normally represented by atomic partial charges or multipole moments. The PE scheme is currently the most advanced QM/MM type embedding scheme. Here, the polarization effects in the environment are also taken into account. It is worth noting that the first study by Warshel *et al.* also included polarization effects [2]. Despite this fact, PE schemes have not seen widespread use, most likely because of the complexity of such implementations and also partly due to the added computational costs. Polarization is a many-body effect and thus requires a self-consistent solution. It is therefore necessary to update the electronic Hamiltonian according to the electron density of the central subsystem. The polarization effects, as modeled in QM/MM embedding models, are most frequently based on an induced dipoles model [2, 6–15]. There are, however, also other ways to include classical polarization in embedding models. Some recent examples are the fluctuating charges model [16, 17] and the classical Drude oscillator model [18].

Most QM/MM implementations use standard MM force fields where the electrostatic components have been parametrized to implicitly include polarization effects. The EE schemes can therefore, in principle, also describe the same averaged effects as an explicitly polarizable model on the electronic ground state. The EE schemes cannot, however, describe differential polarization ef-

fects between ground and excited states. This effect becomes important when there is a significant rearrangement of the electronic structure upon electronic excitation [13, 19].

In this study we use the PE model by Olsen *et al.* [13, 20] as implemented in the PE library [21] which has been interfaced with the Gen1Int integral library [22] and the Dalton program [23, 24]. This is a QM/MM-type embedding model that focuses on the calculation of molecular properties, including the use of accurate embedding potentials derived from *ab initio* calculations for each structure explicitly. Currently, the polarization part of this model includes anisotropic polarizabilities that lead to induced dipole moments that can be determined self-consistently with respect to either the ground or the excited states of the central subsystem. The embedding potentials for the proteins are generated using the molecular fractionation with conjugate caps (MFCC) method by Zhang and Zhang [25] as applied to localized properties by Söderhjelm and Ryde [26]. All embedding potential parameters used in this study are derived using DFT for each structure explicitly without using averaged (force field) parameters.

Polarization is generally considered to be a short-range effect, in particular compared to electrostatic interactions. The idea of including polarization only for a subset of atoms in the classical region (those that are closest to the quantum region) has been proposed on several occasions [27–31]. Indeed, Osted *et al.* argue that this can reduce the computational cost while retaining the quality of the results compared to including polarization for the full classical region [27, 28]. Osted *et al.* calculated several properties of liquid water using a combined coupled-cluster / molecular mechanics (CC/MM) approach on a large number of molecular dynamics snapshots [28]. They found that the optimum balance of quality versus computational cost was to include water molecules within a threshold of 10 Å of the quantum region and polarization of the water molecules within a radius of 7 Å from the quantum region.

Söderhjelm *et al.* investigated the distance dependence of several embedding potential parameters on the lowest excitation energy of rhodopsin using CASPT2/CASSCF for the QM region. By removing the polarizabilities or their anisotropy outside a certain threshold, their interaction range was analyzed. The effect of including both the polarizabilities in the first place but in particular also their anisotropy was found to be strong within the first 10 Å from the QM region and levelling out at longer distances [29]. In another study, Söderhjelm *et al.* found that removal of polarizabilities or their anisotropy does not lead to converged protein–ligand interaction energies below a threshold of 20 Å with convergence defined as an absolute error in the interaction energy below 4 kJ/mol [30].

Curutchet *et al.* studied the electronic properties of a light-harvesting protein using the QM/MMpol method. They found the errors to be small with a polarization cut-off of 18 Å compared to using a cut-off of 30 Å. However, this was found to lead to a fourfold decrease in the computational time [31].

These studies [27–31] suggest that the influence of polarization extends over a much longer range in proteins than in simple homogeneous media. The aim

of this study is to investigate this in a systematic manner by exploring to what distance from the quantum region the influence of polarization needs to be taken into account and how far purely electrostatic effects need to be included. We will in particular focus on how this threshold depends on the type of environment by considering both chromophores in proteins and in solution. We will study in more detail the solvent-induced shift of the $n \rightarrow \pi^*$ transition of acetone in different solvents. The goal is to give clear guidelines for how PE calculations should be designed to ensure that the calculations are both accurate and computationally efficient.

In Section 2 we give the computational details for the results presented in Section 3. In Section 4 we discuss the consequences of our computational results on how to design efficient and accurate polarizable embedding calculations for different environments, before we give some concluding remarks in Section 5.

2. Computational details

2.1. Preparation of the structures

We will study two different classes of systems: proteins as models of structured systems with charged side groups, and solute–solvent systems as models for chromophores in a homogeneous environment. The molecular structures for the green fluorescent protein (GFP) and rhodopsin were prepared from the crystal structures 1GFL [32] and 1U19 [33], respectively. 50 snapshots for GFP solvated in water were taken from a 15 ns molecular dynamics (MD) simulation using the CHARMM27 force field [34]. Details of this MD simulation are provided in Ref. 35. For the PE-TD-DFT calculations, the protein was extracted together with a solvation shell consisting of all water molecules with one of the atoms within 8 Å from one of the protein atoms. Starting structures for the solute–solvent systems (uracil and the GFP chromophore in water, acetone in various solvents) were carefully minimized and equilibrated. Structures of the solute in a solvent sphere with radius 20 Å (unless otherwise specified) were subsequently obtained from the MD simulations at intervals of 200 ps. In the solute–solvent calculations, solvent molecules were included in the classical region if one or more of its atoms were within the threshold. For the proteins, only atoms within the threshold were included. In all cases, the chromophore was geometry optimized with QM/MM in the frozen environment of the protein or solvent. A detailed description of the structure preparation is given in Section 1 of the Supporting Information.

2.2. Generation of the embedding potential

The embedding potentials for the polarizable embedding (PE) calculations include coordinates, QM-derived multipole moments up to quadrupoles and QM-derived anisotropic polarizabilities for all atoms outside the QM region. Multipole moments and polarizabilities were calculated for each snapshot separately with DFT using the LoProp [36] approach implemented in Molcas [37, 38]. The generation of the potential was facilitated by the Polarizable Embedding

Assistant Script (PEAS), a python script developed by one of the authors [21]. The details vary slightly between the proteins (GFP and rhodopsin) and solute-solvent structures (uracil and GFP chromophore in water and acetone in different solvents). For the proteins, multipole moments and polarizabilities were obtained from QM calculations on protein fragments from the molecular fragmentation with the conjugated-caps (MFCC) procedure [25] as applied to localized properties in Ref. 26 and described in more detail in Ref. 39. The parameters were calculated with the B3LYP functional [40–43] and the 6-31+G* basis set [44–46]. For the solute-solvent structures, potential parameters were calculated from DFT calculations on each solvent molecule separately using the B3LYP functional [40–43] with the aug-cc-pVDZ basis set [47]. In all cases, the basis set was recontracted to an atomic natural orbital type basis as required for the LoProp approach [36].

2.3. Excitation energy calculations

Vertical excitation energies were calculated with polarizable embedding time-dependent density functional theory (PE-TD-DFT) [13], which has recently become available in the 2013 release of the Dalton program system [23, 24]. The PE-TD-DFT calculations were performed with the CAM-B3LYP exchange-correlation functional [48] in combination with the 6-31+G* basis set [44–46] for the chromophores in the proteins and the aug-cc-pVDZ basis set [47] for the chromophores in the solvents. These basis sets were chosen to be consistent with the basis sets that were used to derive the embedding potentials. Double-zeta basis sets have been shown to perform well for excitation energies of biological chromophores as long as diffuse functions are included in the basis set [49].

In order to investigate the range of polarization interactions, separate PE-TD-DFT calculations were run with different thresholds (1, 2, ... Å) for the inclusion of polarization effects. Polarizabilities (and induced dipoles) were only included for atoms within a certain distance between an atom in the environment and any atom in the chromophore, determined by the polarization threshold R_{pol} . Electrostatic interactions between the environment and the charge density of the quantum region were calculated for all permanent multipoles, i.e. no electrostatic cut-off threshold (R_{el}) was applied, unless otherwise specified.

3. Results

The convergence of the lowest excitation energy with different polarization thresholds is shown in Figure 1 for a solute-solvent system (the $\pi \rightarrow \pi^*$ transition of uracil solvated in water) and for two proteins (GFP and rhodopsin). It is clear from Figure 1 that the influence of polarization in the classical region extends over a longer range in the proteins than in water only. Indeed, the excitation energy is stable above a polarization cut-off of 20 Å for both GFP and rhodopsin, but already above 10 Å for uracil in water. This is clearly demonstrated by the results in Figure 2, which shows the faster convergence for the GFP chromophore in water relative to that of the same chromophore in the

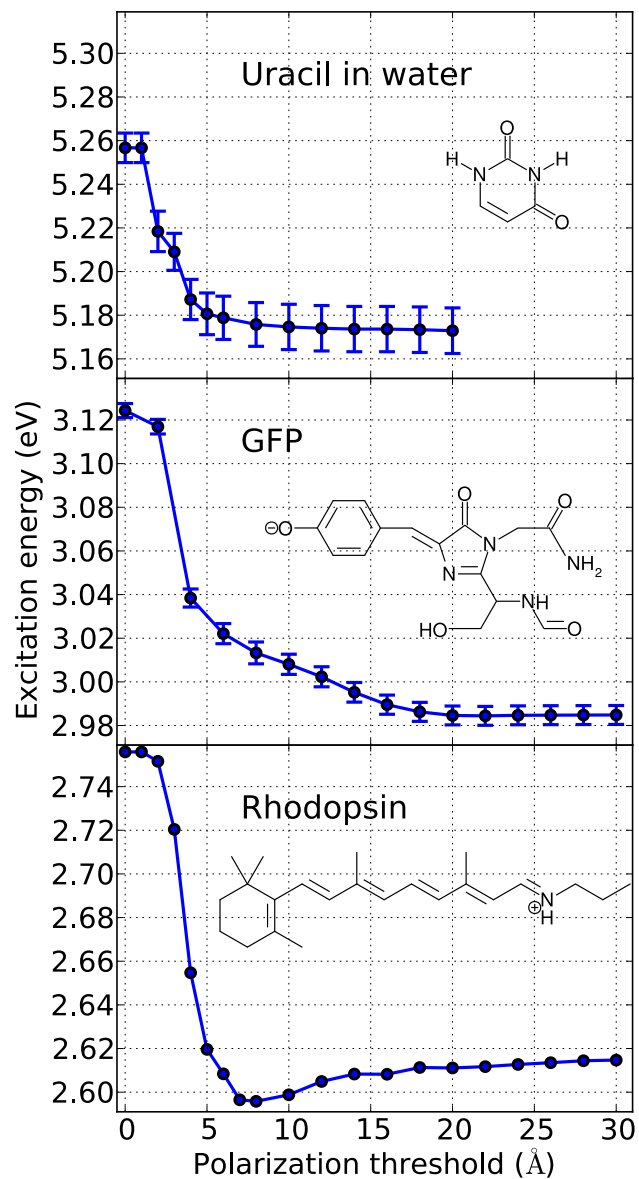


Figure 1: Lowest excitation energies from PE-TD-DFT calculations on different systems using different polarization cut-off thresholds (in Å). Electrostatic interactions were fully included for all atoms in the systems. The results for anionic GFP and uracil in water are averages over 50 snapshots extracted from an MD simulation with standard errors shown as error bars. The results for rhodopsin are based on the crystal structure. Chromophore structures of the quantum region of the PE-TD-DFT calculations are shown in the insets.

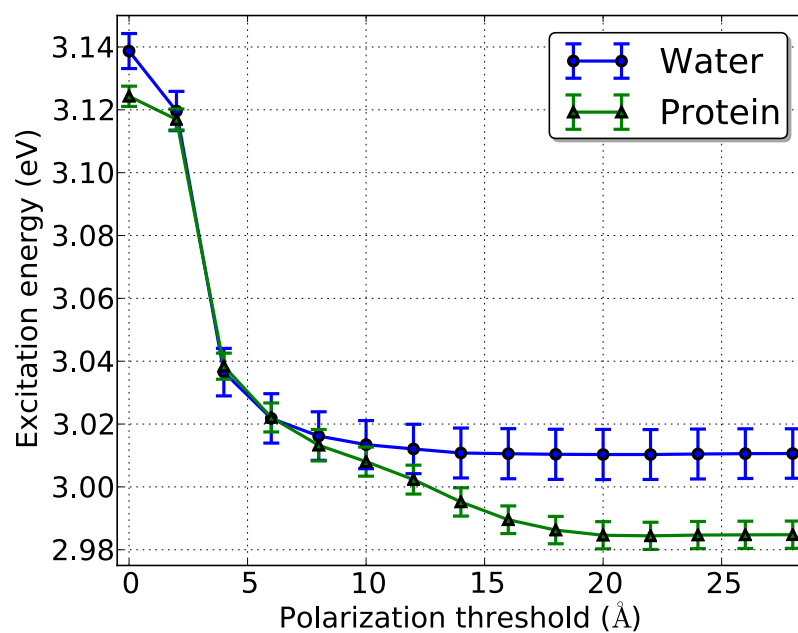


Figure 2: Excitation energies from PE-TD-DFT calculations on the chromophore of GFP in both the native protein environment and solvated in water using different polarization cut-off thresholds (in Å). The results are averages over 50 snapshots extracted from an MD simulation with standard errors shown as error bars. The GFP protein was explicitly solvated in water in both the MD simulation and the PE-TD-DFT calculations. Electrostatic interactions were included for all atoms in the protein and for all water molecules within a sphere with radius 30 Å around the chromophore. Note that the side chains of the chromophore are different in the two models (see Figure SI-1).

native protein environment. For GFP, the same rate of convergence is observed for calculations on the crystal structure and for averages over MD snapshots (Figure 3). Moreover, the type of embedding potential (order of the multipole

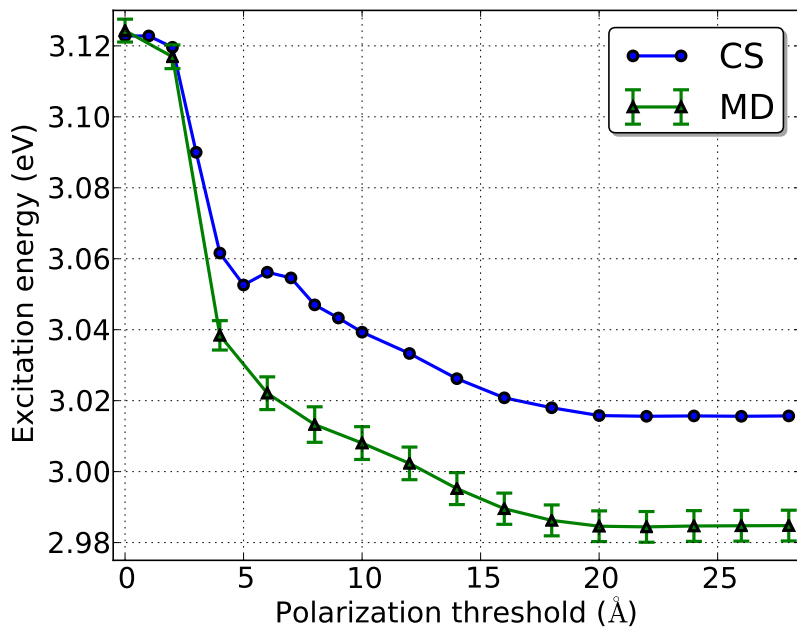


Figure 3: Excitation energies from PE-TD-DFT calculations on anionic GFP using different polarization cut-off thresholds (in Å). The convergence of the excitation energy is compared for calculations based on the crystal structure (CS) and as an average over 50 snapshots extracted from an MD simulation (MD) with standard errors shown as error bars. Electrostatic interactions were fully included for all atoms in the protein.

moment expansion and isotropic *vs.* anisotropic polarizabilities) does not affect the convergence either (see Figures SI-2 and SI-3).

Charged residues are a possible reason for the longer range of polarization interactions in proteins relative to solute-solvent systems [50]. Although the direct effect of polarization is rather short-range, a charged residue at a distance of 20 Å might affect the quantum region *via* other induced dipoles, since these are determined self-consistently and thus include many-body effects. In order to verify this, we made an artificial system where all charged amino acid residues in the classical region of GFP were changed into neutral residues by adding or removing a proton. We then made a new embedding potential for this 'neutralized GFP' and repeated the excitation energy calculations with different polarization cut-off thresholds. The result is a less pronounced effect from the polarization in the classical region (Figure 4). In particular, the effect of adding induced dipoles of the classical sites between 10 and 20 Å is more than

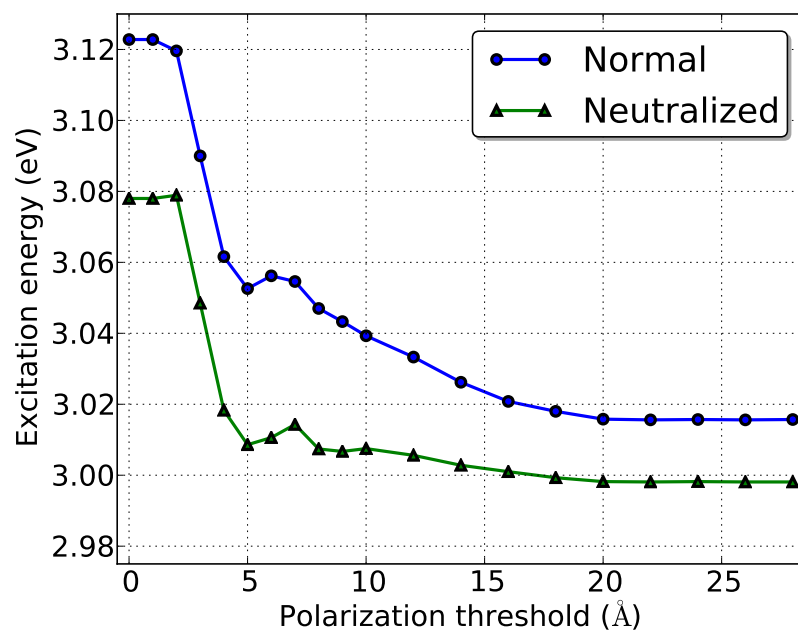


Figure 4: Excitation energies from PE-TD-DFT calculations on the anionic green fluorescent protein (GFP) using different polarization cut-off thresholds (in Å). Calculations were performed both on the crystal structure and on a modified structure in which all the charged amino acid residues were neutralized. Electrostatic interactions were included for all atoms in the protein.

twice as large when the charged residues are kept (0.023 vs. 0.009 eV). The relatively high number of charged side groups in GFP (59 out of 230 residues: 26%) compared to rhodopsin (38 out of 238: 11%)—a membrane protein with therefore many uncharged hydrophobic residues—could explain why the effect of the polarizabilities in the 10 to 20 Å region is almost twice as large for GFP (0.023 eV) as for rhodopsin (0.012 eV). The results for rhodopsin (Figure 1) agree well with a similar study by Söderhjelm *et al.* that showed that the lowest excitation energy is mostly affected by the polarizabilities within a threshold of 10 Å [29].

The solvent shift of the $\pi \rightarrow \pi^*$ transition in uracil solvated in water (-0.21 eV using $\Delta E_{\text{vac}}^{\pi \rightarrow \pi^*} = 5.384$ eV from Ref. 51) is in much better agreement with experiment (-0.31 eV, see Ref. 13) than the results reported in Ref. 13 (-0.12 eV). Since the method (CAM-B3LYP), basis set (aug-cc-pVDZ) and embedding potential (QM-derived multipole moments up to quadrupoles and dipole-dipole polarizabilities) are the same, it is possible that the improvement comes from a better description of the indirect solvent effects, i.e. the effect of the solvent on the geometry of the solute. Indeed, the structures in Ref. 13 were obtained from an MD simulation with a frozen solute whereas the solute was allowed to relax in this work, followed by a QM/MM geometry optimization *in the solvent*.

We also consider the convergence of the lowest excitation energy in uracil solvated in water ($\pi \rightarrow \pi^*$) with the threshold for which electrostatic interactions are calculated (i.e. the size of the system since $R_{\text{pol}} \leq R_{\text{el}}$). Electrostatic interactions are less computationally demanding to include in the PE-TD-DFT calculations compared to polarization effects, but an increased size of the system also leads to increased computational costs through the explicit QM calculation of the embedding potential parameters for each solvent molecule. Errors in the truncation of electrostatic and polarization interactions can cancel each other, or other errors, to *accidentally* give the right answer, thus a systematic approach is needed. The excitation energies and relative errors (compared to including electrostatic and polarization interactions up to 20 Å) are shown in Table 1. We observe that the errors beyond $R_{\text{el}} = 5$ Å and $R_{\text{pol}} = 5$ Å are below 0.01 eV. Moreover, an increase of the thresholds beyond $R_{\text{el}} = 10$ Å and $R_{\text{pol}} = 10$ Å does not lead to significant changes. The differences (on the order of 0.01 eV) are smaller than other sources of error in the calculations. Nevertheless, these small differences are often of interest when considering for instance the effect of different solvents on molecular properties (see below). In these cases, errors in the method (e.g. overestimation of the excitation energy by the CAM-B3LYP density functional) partly cancel and reliable relative solvent shifts can be obtained.

The accuracy of the method should balance the added computational costs, and we therefore also consider the computational times needed for calculations with different polarization cut-off thresholds (Figure 5). The main factor determining the computational time of a QM/MM calculation is of course the size of the quantum region. In fact, without polarization effects (electrostatic embedding) the calculations on the GFP chromophore (39 atoms) in the protein used approximately seven times as much time as the calculations on uracil (10

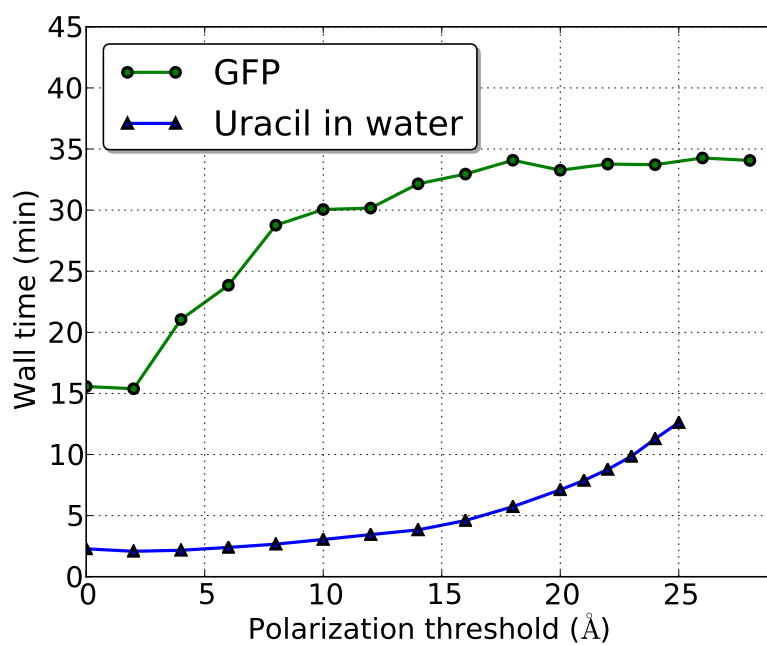


Figure 5: Computational time needed to calculate the lowest four excitation energies in a snapshot of uracil solvated in water and in the anionic GFP chromophore embedded in the protein (crystal structure) using different polarization cut-off thresholds (in Å). Both calculations were performed on 64 cores (4 nodes) on the same machine. Electrostatic interactions were fully included for water molecules up to a distance of 25 Å from uracil and for all atoms in the GFP. The computational time needed to generate the potential parameters is not included here.

Table 1: The lowest excitation energy (ΔE in eV) in uracil solvated in water calculated with different electrostatic (R_{el} in Å) and polarization (R_{pol} in Å) cut-off thresholds. The difference ($\Delta\Delta E$ in eV) to the most accurate calculation ($R_{\text{el}} = 20$ Å, $R_{\text{pol}} = 20$ Å) is shown as a measure of the convergence. The results are averages over 50 snapshots extracted from an MD simulation.

R_{el}	R_{pol}	ΔE	$\Delta\Delta E$
5	0	5.257	+0.084
5	5	5.177	+0.004
10	0	5.258	+0.085
10	5	5.178	+0.005
10	10	5.171	-0.002
15	0	5.258	+0.085
15	5	5.179	+0.006
15	10	5.173	+0.000
15	15	5.174	+0.001
20	0	5.257	+0.084
20	5	5.181	+0.008
20	10	5.175	+0.002
20	15	5.174	+0.001
20	20	5.173	0.000

atoms) in water. Including polarization in the classical region does not lead to an extraordinary increase in the computational time. On the contrary, the calculations take only twice as long when the polarization of water is included up to 15 Å from uracil and only slightly more when polarization is included for the whole GFP (compared to electrostatic embedding only). The computational time increases more rapidly with increasing polarization threshold for uracil in water than for GFP. Indeed, the number of atoms in the classical region increases sharply for uracil in water for thresholds above 15 Å, whereas it levels out for GFP (see Figure SI-4). This is related to the shape of the system: uracil has a *sphere* of water around whereas solvated GFP is shaped like a *barrel*. Thus, the computational time that can be gained by choosing the right polarization threshold depends on the shape of the systems, the largest gain being for spherical systems as they are truly three-dimensional and thus have the largest volume increase with increasing cut-off thresholds. This also includes proteins embedded in a sphere of water molecules. We note that a smaller region including explicit polarization also reduces the computational time needed for explicit calculation of the potential parameters, which is however not the focus in this study.

A remaining question is how the convergence of an excitation energy with varying polarization cut-off threshold depends on the type of solvent. To investigate this, the $n \rightarrow \pi^*$ transition of acetone in five different solvents was calculated as an average over 50 snapshots extracted from MD trajectories. Ex-

PLICIT inclusion of polarization and statistical averaging have been shown to be mandatory to obtain reliable solvent shifts for this system [52]. Our results are shown in Figure 6.

The excitation energy converges slowest for acetone in water. In fact, a polarization threshold of 5 Å is enough for dimethyl sulfoxide (DMSO), diethyl ether (DEE) and hexane, but not for water. We note that comparison with bulky solvents, such as diethyl ether and hexane, should be done with caution. If one of the solvent atoms is within the threshold then the whole molecule is included, extending much farther into the classical region than the threshold. Indeed, only a few extra hexane molecules are included when the threshold is enlarged from 3 to 5 Å. Still, including polarization using $R_{\text{pol}}=5$ captures almost all polarization effects (0.0221 eV / 178 cm^{-1} of the total polarization solvent shift of 0.0237 eV / 191 cm^{-1}) of acetone in a hexane solvent.

The standard errors (and the standard deviations) are largest for the hydrogen-bonding solvents, i.e. water and methanol. In these cases, the number of hydrogen bonds to acetone can change between snapshots, leading to a significant difference in electron density and hence excitation energy. The size of the standard errors (standard deviation of the excitation energy divided by the square root of the number of snapshots) is below the differences between the solvents, indicating that reliable relative solvent shifts can be obtained based on 50 snapshots.

The solvent shifts are separated into indirect solvent effects (comparing a gas-phase calculation with QM calculations on acetone at the 50 solvent geometries), direct electrostatic effects (comparing the QM calculations on the solvent geometries to PE-TD-DFT calculations with $R_{\text{pol}}=0$) and direct polarization effects (comparing PE-TD-DFT calculations with $R_{\text{pol}}=0$ to $R_{\text{pol}}=20$) and compared to experimental solvent shifts [53] in Table 2. The calculated excitation energies (in eV) are tabulated in Section 3 of the Supporting Information.

Table 2: Calculated and experimental solvents shifts (all in cm^{-1}) for the $n \rightarrow \pi^*$ excitation in acetone in five different solvents. The calculated solvent shifts ($\Delta\omega_{\text{tot}}$) are separated into indirect ($\Delta\omega_{\text{ind}}$), direct electrostatic ($\Delta\omega_{\text{elec}}$) and direct polarization ($\Delta\omega_{\text{pol}}$) contributions. Electrostatic and polarization interactions were included for solvent atoms up to a distance of 20 Å from acetone. The experimental values ($\Delta\omega_{\text{exp}}$) are from Ref. 53.

solvent	$\Delta\omega_{\text{ind}}$	$\Delta\omega_{\text{elec}}$	$\Delta\omega_{\text{pol}}$	$\Delta\omega_{\text{tot}}$	$\Delta\omega_{\text{exp}}$ [53]
hexane	-14	-38	191	139	-35 ± 5
diethyl ether (DEE)	-119	188	201	271	180 ± 25
dimethyl sulfoxide (DMSO)	-344	734	265	655	375 ± 10
methanol	-304	1067	349	1111	941 ± 10
water	-393	1673	665	1945	1785 ± 7

The order of the solvent shifts is in agreement with experiment. In fact,

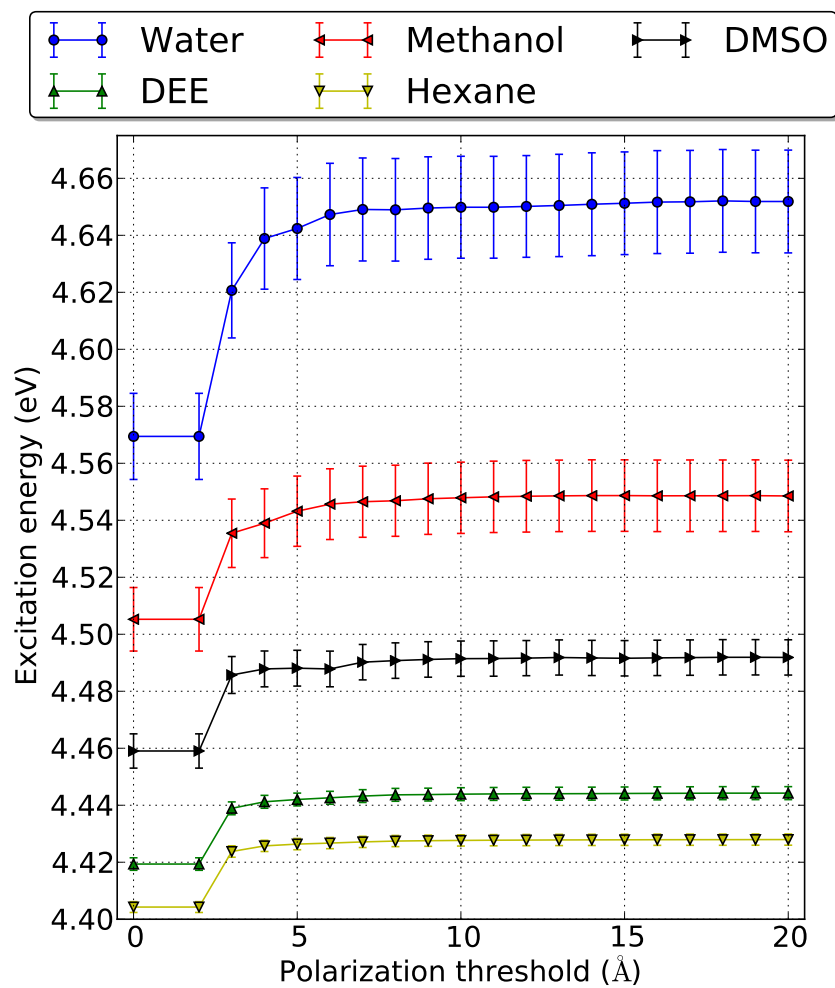


Figure 6: Excitation energies from PE-TD-DFT calculations on acetone ($n \rightarrow \pi^*$) in different solvents using different polarization cut-off thresholds (in Å). The results are averages over 50 snapshots extracted from MD simulations with standard errors shown as error bars. Electrostatic interactions were fully included for solvent atoms up to a distance of 20 Å from acetone in all calculations. The differences between the different solvents in the excitation energies at $R_{\text{pol}}=0$ Å are thus due to electrostatic effects and indirect solvent effects on the geometry of acetone.

the calculated solvent shifts are systematically overestimated by values ranging from 91 (diethyl ether) to 280 (DMSO) cm^{-1} . This can most likely be improved upon by including non-classical interactions, such as dispersion interactions and exchange repulsion, which are especially important for non-polar solvents such as diethyl ether and hexane. This can be done straightforwardly by including the closest solvent molecules in the quantum region. This is, however, computationally expensive. Furthermore, it has been shown that a correct geometry (in particular the C=O bond length) is crucial in order to get an accurate solvent shift for the excitation energy of acetone in water [52]. Improvement of the geometries by more accurate QM/MM geometry optimization is possible in several ways: using a more accurate method (here: B3LYP), using a more flexible basis set (here: 6-31+G*) and including the environment in a more accurate way (here: electrostatic embedding with standard force field charges). Thus, a more rigorous treatment of solvent effects on the $n \rightarrow \pi^*$ excitation in acetone will include at least a treatment of non-classical interactions and use more accurate geometries.

The $n \rightarrow \pi^*$ excitation energy of acetone in different solvents has also been calculated using the same cut-off for electrostatic and polarization interactions (Table SI-II). It is clear that a threshold of 10 Å is enough, not only for the polarization cut-off (as shown in Figure 6), but also for the electrostatic cut-off. Indeed, errors in the calculated solvent-induced shifts are 1 to 2 % (≤ 0.005 eV) compared to including both types of interactions up to 20 Å. Errors of calculated solvent-induced shifts with both cut-offs at 8 Å range from 3 to 5 % (≤ 0.007 eV), still low enough for many purposes.

4. Discussion

A point that emerges from the results presented in this work is that the convergence of excitation energies with polarization cut-off is much slower for chromophores in an ordered medium, such as a protein, than for chromophores in a homogeneous medium, such as a solvent. This is clearly demonstrated in Figure 2 by the faster convergence of the GFP chromophore in water (converged around 10 Å) compared to that of the same chromophore in the protein (converged around 20 Å). We have also shown that this in part is related to the charges in the protein (see Figure 4) and therefore depends on the type of protein, i.e. the abundance of (partial) charges. Indeed, convergence of excitation energies is slower for solvated GFP than for rhodopsin, which is a membrane protein with therefore relatively few (partial) charges. These findings agree well with the work of Söderhjelm *et al.* on rhodopsin, in which explicit polarization in the embedding region was found to be especially relevant within a distance of 10 Å from the chromophore [29]. The results indicate that truncation of an atomistic model of a protein without losing accuracy is advisable only for relatively large proteins with parts that extend further than about 20 Å from the chromophore. Thus, for big proteins—especially with a large solvation shell or embedded in a membrane—truncation can lead to a significant speed-up without noteworthy loss of accuracy. For solute–solvent systems, including solvent

molecules up to 10 Å from the chromophore is enough for accurate embedding calculations.

A smaller cut-off radius for the explicit polarization than for the electrostatics is worthwhile to consider, since the polarization interactions are computationally more demanding than the electrostatic interactions and since the polarization interactions decay faster. In order for such a three-layer ($R_{\text{el}} \neq R_{\text{pol}}$) approach to be worthwhile, it needs to satisfy two criteria: 1) faster convergence of the molecular property with respect to R_{pol} than with respect to R_{el} and 2) significant savings in computational time. As far as the first point is concerned, we have shown for uracil in water (Table 1) and for acetone in various solvents that choosing $R_{\text{el}} > R_{\text{pol}}$ does not lead to an improved convergence of the excitation energies. In fact, induced dipoles relatively far from the chromophore cannot influence the chromophore *directly*, but can do so *via other induced dipoles*. This holds even more for proteins, where the influence of polarization extends to longer distances from the chromophore as shown in this work. Convergence of excitation energies with R_{pol} is thus not faster compared to R_{el} . As far as the second criterion is concerned, for uracil in water there is a possible gain in computational time only for thresholds beyond those that are needed to include the environment in an accurate way. Indeed, including interactions up to 10 Å (which is enough for an accurate description of the environment) leads to a speed-up of a factor of two compared to a 20 Å cut-off. For GFP and rhodopsin, however, including polarization interactions up to 20 Å does not lead to a speed-up of more than 10% compared to inclusion of the whole protein. This is related to the efficient implementation of the PE method that is being used [13], which is different from earlier implementations in which a cut-off of polarization interactions below 10 Å could lead to an increased efficiency [28]. Thus, neither of the two criteria is satisfied and we find that the use of different thresholds for the inclusion of electrostatic and polarization interactions is not necessary for an optimal balance of accuracy and computational efficiency.

We note that the results for excitation energies discussed in this work are not necessarily transferable to other molecular properties. As an example, we computed the one-photon absorption (OPA) oscillator strength and two-photon absorption (TPA) cross section associated with the $\pi \rightarrow \pi^*$ transition in the GFP chromophore (Figures SI-5 and SI-6). The convergence with increasing polarization threshold is faster for the TPA cross-section than for the OPA oscillator strength. Similarly, the convergence of the excitation energy—effectively an energy *difference*—may be faster than the convergence of an *absolute* energy. Indeed, Söderhjelm *et al.* found no clear convergence below 20 Å for the protein–ligand interaction energy when truncating the polarizability in a similar way [30]. It is therefore advisable to do a similar test to decide the size of the classical region needed when another property is being investigated.

We have shown that QM/MM calculations with inclusion of explicit polarization in the classical region are not necessarily very time consuming. A considerable amount of computational time can be won by carefully examining to what extent inclusion of the interactions is needed. Accurate inclusion of all interactions with the surroundings in a computationally efficient way can

be done in other ways as well. First, a three-layer QM/MM/PCM model has been developed in our groups [54]. This combines explicit inclusion of the closest molecules in the environment with the advantages of continuum models and reduces the cost of generating embedding potential parameters and explicit QM-MM interactions in the PE-TD-DFT calculations. Second, explicit calculation of the embedding potential parameters for each unique structure can be avoided by using average parameters for all or for a subset of the classical sites, as previously proposed by Söderhjelm *et al.* [29]. This is relatively straightforward for solute-solvent systems but more involved for e.g. proteins since it requires a general force field tailored towards the calculation of molecular properties. Third, the induced dipoles of the outer region can be frozen at their ground-state values during the response calculation, resulting in a significant speed-up of the PE-TD-DFT calculation. This constitutes an improvement over the approach discussed in this work since the polarization of the outer region is included instead of being truncated. However, the accuracy may be affected if there is a significant difference between the electron densities in the ground and excited states. Fourth, one could define a three-layer approach with inclusion of polarization through induced dipoles up to a certain threshold and *implicit* polarization in the outer region. This would be similar to the approach taken in this work, but with a better description of the polarization of the outer region, with the possible gain of being able to use a smaller threshold. Implicit inclusion of polarization can be done by electrostatic embedding with re-parametrized electric multipoles that capture a part of the polarization effects. All of these approaches rely on the fact that a relatively small region around the quantum region is responsible for most of the perturbation by the environment, which is clearly shown by the results in this work. This observation allows for an efficient scheme for including the rest of the classical region without losing much accuracy.

5. Conclusion

We have systematically investigated to what extent the polarization of the environment has an influence on the excitation energies of a chromophore both in highly ordered and in homogeneous environments. We have found that convergence of excitation energies with polarization cut-off is much slower for an ordered environment, such as a protein, than for chromophores in a homogeneous medium, such as a solvent. Especially for proteins containing many (partial) charges, atoms in the classical region can contribute to the properties of the quantum region through polarization interactions up to a distance of 20 Å. For chromophores in homogeneous solvents, truncation of the environment polarization can be done even below 10 Å as demonstrated by the solvent-induced shift of the $n \rightarrow \pi^*$ transition in acetone in different solvents. Moreover, we have demonstrated that truncating polarization and electrostatic interactions with different cut-offs is not necessary for an optimal balance of accuracy and computational efficiency. Finally, we have discussed other approaches to increase the computational efficiency of polarizable embedding calculations with-

out compromising the accuracy. This work is therefore valuable for the design of computational approaches to include a complex environment in an accurate and affordable way.

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