Good Computational Practice in the Assignment of Absolute Configurations by TDDFT Calculations of ECD Spectra

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Abstract: Quantum-mechanical calculations of chiroptical properties have rapidly become the most popular method for assigning absolute configurations (AC) of organic compounds, including natural products. Black-box TDDFT calculations of ECD spectra are nowadays easily accessible to non-experts. However, an uncritical attitude may easily deliver a wrong answer. We present to the Chirality Forum a discussion on what can be called a good computational practice in running TDDFT ECD calculations, highlighting the most crucial points with several examples from the recent literature.

Keywords: electronic circular dichroism, natural products, density functional theory, chiroptical spectroscopy, structure elucidation

Introduction

The rapid development of computational power[1] has, among many other things, revolutionized the way chemists and other scientists face with the fundamental problem of assigning the absolute configuration (AC) of new molecules.[2] Once based exclusively on the Bijvoet method or chemical correlations, this problem has lately become the main purpose of chiroptical spectroscopies, first of all electronic circular dichroism (ECD).[3] However, for a long time the interpretation of ECD spectra of new compounds has been based entirely on empirical or semi-empirical methods such as spectral comparisons or sector rules.[4,5] Not surprisingly then, non-empirical methods of analysis such as the exciton chirality have become quickly popular and spurred a lot of research.[6,7] The revolution, or renaissance of chiroptical spectroscopies,[8] however arrived when the *ab initio* theoretical simulation of chiroptical properties, including ECD, optical rotation (OR), vibrational CD (VCD), Raman optical activity (ROA), and so on, became a concrete possibility for real-life molecules such as natural products and transition metal complexes.[9-13] Quantum-mechanical (QM) calculations of chiroptical spectra make it possible to assign AC's without the need for any reference system or any chemical derivatization, and often without the necessity of establishing the molecular physico-chemical mechanisms responsible for the observed property (the so-called mechanisms of optical activity). In the specific context of ECD, where a prediction of excited states is necessary for the simulation, a step forward has been taken with the advent of the very cost-effective time-dependent density functional theory (TDDFT) method.[14] The efficiency of TDDFT allows the simulation of the ECD spectrum of a medium-size molecule on a desktop PC in a reasonable time, and the availability of TDDFT ECD calculations in commercial and freely available software has opened the way to a wide usage also by non-experts.[15] The other side of the coin is that a black-box usage of computational tools may easily have unexpected negative consequences on the results.[16] This is especially true when the determination of the AC is concerned: it is obvious that "there are only two possible conclusions in absolute configurational studies: the conclusion is either correct or wrong".[6] The aim of the present contribution to the Chirality Forum is to share our thoughts on what can be called a good computational practice in assigning the AC of organic compounds by means of TDDFT ECD calculations. We will first present a typical flowchart to achieve the AC assignment task, then we will discuss each step with a few examples aimed at highlighting the most critical aspects of the outlined procedure.

Results and Discussion

A TYPICAL COMPUTATIONAL FLOWCHART

The computational study ultimately leading to an ECD calculation of a given molecule starts with the generation of input structures. While the molecular conformation is determined on the way during the computational procedure, all other structural features must be known in advance, including molecular constitution (the so-called "planar" structure) and, very importantly, the relative configuration (RC) of the various chirality elements in the molecule. In principle, this latter piece of information may also be determined by chiroptical spectroscopies, and especially by a combination thereof.[17,18] However, we highly recommend the use of independent tools, including X-ray crystallography (whenever available) and NMR spectroscopy to elucidate as much as possible of the RC. In most cases a careful evaluation of a full set of NMR data sensitive to three-dimensional structure such as scalar J-couplings (especially $^3J_{HH}$ and $^3J_{CH}$) and NOE effects will furnish the relative stereochemistry.[19] It must be stressed that when flexible molecules are concerned, "real" molecular models and not just planar structures should be used to rationalize and

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interpret NOE data. So, we assume that the AC of a molecule with known constitution and RC must be determined, and that a starting molecular model of such a molecule (with the correct RC!) has been generated by a molecular modeling software with an initially arbitrary AC. The typical steps of the ECD computational study are the following (see flowchart in Figure 1).[15,20]
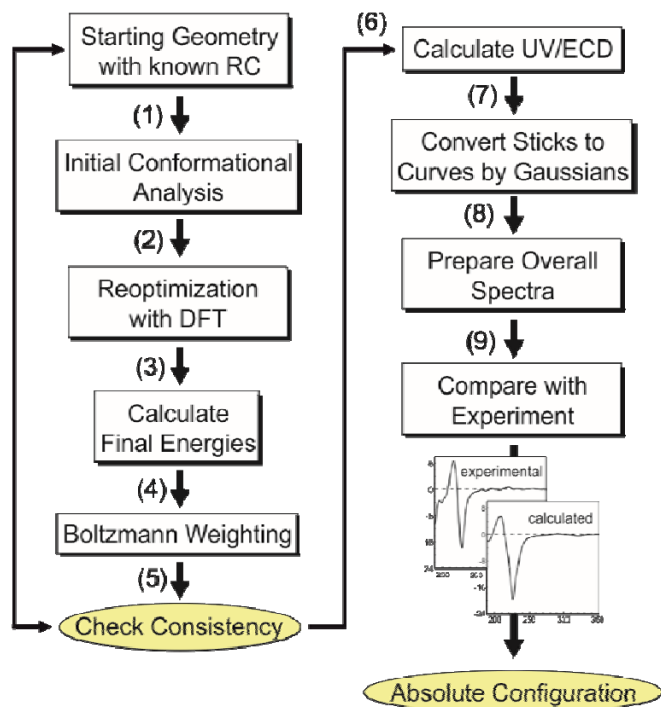

FIGURE 1 Flowchart for the typical steps in ECD calculations.

(1) Perform a thorough conformational search at a low computational level, typically molecular mechanics (MM) with a good force field (FF) such as MMFF (Merck Molecular FF),[21] to investigate the conformational ensemble. This may be done with a conformational analysis protocol such as Monte Carlo or molecular dynamics.[22] It is important that all rotatable bonds are varied and all endocyclic atoms are puckered during the conformational search, in order not to miss any possible conformer.[16]

(2a) Run a preliminary geometry optimization of all conformers found in step (1) at an intermediate level of calculation. This is typically done using the B3LYP/6-31G(d) combination, however the use of a more efficient functional for structure and energetics is recommended,[23] possibly including dispersion correction,[24] in combination with a double-ζ basis set. A different time-efficient approach – especially for large molecules – giving geometries of high quality is the PBEh-3c method proposed by Grimme et al.[25] In this step the MM-structures are screened and a restricted number of low-energy conformations is obtained, falling in an energy window within a certain threshold, i.e. 5 or 10 kcal/mol.

(2b) Run a more refined geometry optimization of the structures selected in (2a) at a higher level of calculation. This may include: (a) a larger basis set such as a triple-ζ or with additional polarization or even diffuse functions. In general, Ahlrichs' basis sets such as def2-SVP or def2-TZVP[26] are better suited than Pople's family (e.g. 6-31G(d) and following); (b) a solvent model such as the Polarizable Continuum Model (PCM),[27] COSMO,[28] or SMD.[29]

(3) Optionally, the set of low-energy structures found in step (2) can be submitted to single-point energy calculations at even higher level (for example using a larger basis sets than in point (2b), or including a solvent model), and/or to frequency calculations to verify their nature of true energy minima and to obtain free energies or zero-point corrected (ZPC) energies. Frequency calculations must be run at the same level of geometry optimizations.

(4) Apply Boltzmann distribution to the set of low-energy minima obtained in steps (2) or (3), by using internal, ZPC or free energies.[20,30,31] A final set of structures is thus obtained together with their respective Boltzmann populations at 298K or 300K. Those falling above a certain population threshold, say 1%, need to be considered in the following steps.

(5) Check the consistency between the calculated conformational ensemble and the whole set of conformation-dependent NMR data (J-couplings, NOE contacts, ring-current shifts). This – in our opinion – very important step is most often overlooked in the current literature. This computational/ experimental consistency check may be hampered or greatly complicated for very flexible molecules, for which experimental data are the average of several low-energy conformations. Such a situation may greatly benefit from low-temperature NMR experiments, which in favorable cases also allow a direct quantification of conformers.[32] Even in the most difficult cases however one should always verify that the most populated conformers do not contradict experimental results, and vice-versa. If this happens, the conformational analysis should be checked for errors and repeated; on the other hand, it may also be a hint of wrong structural assignment, for example of the RC.

(6) Calculate the absorption and ECD spectra for all populated conformers found in step (4). This is often the most computationally demanding step in the whole procedure, and for which the choice of the level of calculation is most crucial.[9,13,14,20,33,34] Restricting only to the TDDFT method, both the functional and the basis set should be chosen carefully. Most importantly, one should never use just a single functional/basis set combination, and different combinations should always be explored. As for the functional, at least two families of functionals, i.e. hybrid and range-separated functionals, should both be tested. Popular hybrid functionals with increasing amounts of "exact" or HF exchange are for example B3LYP (20% HF), PBE0 (25%), M06 (27%), BH&HLYP (50%), and M06-2X (54%).[35] Range-separated functionals such as CAM-B3LYP and ωB97X perform often better than hybrid ones in ECD calculations and should always be considered. Both the HF percentage and the range separation affect, among other things, the absolute and relative transition energies,[36] therefore a correct reproduction of the sequence of bands and their energies is one of the factors dictating the final choice (see "Choice of the TDDFT Functional" section below). As for the basis set, we recommend Ahlrichs' def2-SVP or def2-TZVP,[26] or, more in general, basis sets of double or triple-ζ quality with a sufficiently wide set of polarization functions. Diffuse functions may be necessary whenever Rydberg states contribute substantially to the ECD spectrum.[37] The basis set convergence may be easily verified by using one of the two methods discussed in paragraph "Choice of the TDDFT Basis Set" in the next section. In general, the weaker the computed (and experimental) ECD spectrum, the stronger will be its dependence on the calculation method, including functional, basis set, input geometry, and solvation model. An absorption/ECD calculation is essentially an excited-state

calculation: the number of excited states (roots) to be included must be chosen in a way that the final calculated absorption and ECD spectra, after the wavelength correction (vide infra), extend well below the lowest observed wavelength.

(7) As the ECD calculation run in step (6) actually provides a list of rotational strengths as a function of wavelength or energy (stick plot), generate a true ECD spectrum by applying a band-shape to each rotational strength and sum over the whole spectral range. A Gaussian band-shape is normally used for electronic CD.[38] The choice of the bandwidth, namely the standard deviation of the Gaussian curve, is often done empirically on a best-fit basis; reasonable values for the so-called FWHM (full width at half maximum) are in the range 0.1-0.3 eV. The same applies to the absorption spectrum generated by computed dipolar or oscillator strengths.

(8) Calculate the final Boltzmann-averaged spectrum by weighting each component spectrum obtained in step (7) with the appropriate conformer Boltzmann weight estimated in step (4), and then by adding all weighted spectra to each other.

(9) Compare the experimental absorption/ECD spectrum with the final calculated spectrum obtained in step (8) over the whole available spectral range. In doing so, a wavelength shift may be applied to take into account a systematic over/underestimation of transition energies (sometimes this is called UV correction, as it is simpler to determine the systematic error from a comparison of experimental and calculated UV absorption spectra). If a good match is obtained, the assumed AC is the correct one; if there is a good mirror-image relationship, the opposite AC is correct. Since the ECD spectra of two enantiomers are mirror images by definition, it is not necessary to run the calculation on both enantiomers; when this is done, however, an exact mirror-image relationship must result. Although the comparison between calculation and experiment is often done visually, we recommend the use of a tool allowing for a quantitative comparison.[39,40] This will furnishing a similarity factor which, hopefully, should be sizably larger for the correct AC than the incorrect one (ideally, 1 vs. 0). If the comparison is poor for both possible enantiomers over the available spectral range, the computational procedure should be reexamined, starting first from the choice of functional/basis set combination in step (6) and eventually from the very first step (1) of conformer generation.

EXAMPLES AND IN-DEPTH DISCUSSION

Geometry optimizations and relative energies

One of the major problems connected with QM calculations of molecular properties is the widespread and often uncritical use of the B3LYP hybrid functional.[41] In particular, geometry optimizations and related quantities such as relative internal energies are very commonly estimated at B3LYP/6-31G(d) level. This naïve approach has several drawbacks which cannot be fully treated here. Simply stated, several benchmark studies have demonstrated that B3LYP is not necessarily the most accurate functional for predicting structures and energies of standard organic molecules.[23] Other largely available hybrid and range-separated functionals, including Truhlar M0x and Head-Gordon ωB97 family, perform better in general terms.[42,43] Moreover, when non-covalent interactions come into play, a reliable prediction of conformational energies requires the inclusion of dispersion, either as a correction of normal hybrid or

double-hybrid functionals.[24,44,45] As an example, 2-(3-fluoro-(p-tolyl)propyl)naphthalene (**1**, Scheme 1) may exist in unfolded or folded conformations depending on the presence of an intramolecular π-stacking. Folded conformations are however totally overlooked by B3LYP calculations, while they turn out to be the most stable ones when B2GPPLYP-D3 is used for geometry optimizations in vacuo (Figure 2). As the two conformational families (unfolded and folded) have very different calculated ECD spectra, their relative population has a large impact on the average calculated ECD spectrum.[10]
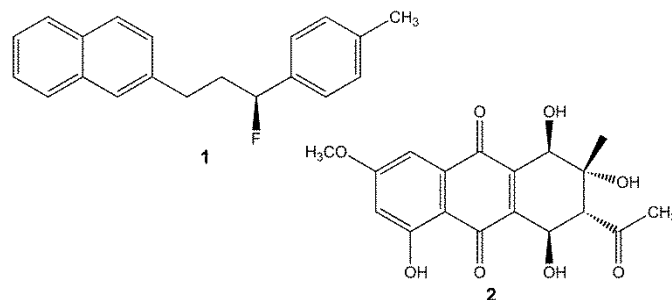
SCHEME 1 Structures of (S)-2-(3-fluoro-(p-tolyl)propyl)naphthalene (**1**) and altersolanol L (**2**) mentioned in the text.

B2GPPLYP-D3:
lowest-energy, **36%** population

B3LYP:
+4.43 kcal/mol, 0% population

B2GPPLYP-D3:
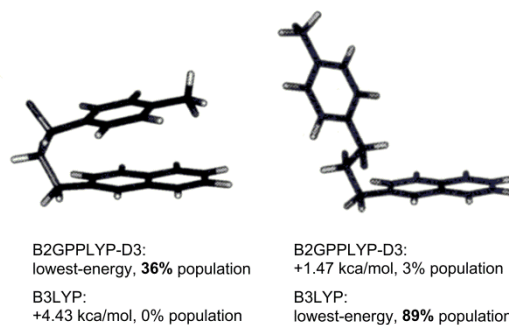+1.47 kcal/mol, 3% population

B3LYP:
lowest-energy, **89%** population

FIGURE 2 The two lowest energy conformations calculated for (S)-2-(3-fluoro-(p-tolyl)propyl)naphthalene (**1**, Scheme 1) at B2GPPLYP-D3/def2-TZVPP and B3LYP/TZVPP' levels (left, folded, and right, unfolded) in vacuo. Adapted from ref.[10], with permission.

TABLE 1 Boltzmann populations (in %) at 300K for the conformers of altersolanol L (**2**) estimated at various levels of calculation (from geometry optimizations).[46]

# [a]	MM	small basis[b] ΔH^c	large basis[b] ΔH^c	large basis[b] ΔH^c +PCM[d]	large basis[b] ΔG^c +PCM[d]
01	78.8	90.5	46.8	3.2	0.3
02	15.9	0.0	0.2	0.6	0.4
03	2.9	2.3	5.7	2.2	1.3
04	1.6	6.2	42.6	62.6	71.3
05	0.6	0.0	0.0	0.0	0.0
06	0.1	0.0	0.2	0.0	0.0
07	0.1	0.3	3.5	28.9	25.6
08	0.1	0.6	0.5	0.5	0.4
09	0.0	0.0	0.0	0.0	0.0
10	0.0	0.1	0.2	2.0	0.7
11	0.0	0.0	0.0	0.0	0.0

[a] Ordered according to MMFF energy. Only conformers with relative B3LYP/6-31G(d) energies within 6 kcal/mol are listed. [b] Small basis set: B3LYP/6-31G(d); large basis set: B3LYP/6-311G+(d,p) [c] ΔH: populations estimated from internal energies; ΔG: populations estimated from ZPC-free energies. [d] PCM model for acetonitrile.

As for the basis set, a double-ζ Pople's type basis set such as 6-31G(d) may be unsuited to calculate geometries and relative energies accurately enough, especially due to the intramolecular

basis set superposition error (BSSE).[47-49] A larger basis set should be considered at least in single-point or frequency calculations to estimate internal or free energies (step 2b in our flowchart). Time-saving approaches such as the resolution of identity method[50] or the chain-of-spheres approximation[51] may be useful in combination with large basis sets. To illustrate how much the calculation method may affect the conformational population even for a relatively rigid organic molecule, we report in Table 1 the Boltzmann population estimated at 300K for altersolanol L (**2**, Scheme 1)[46] with 5 different methods: (a) MMFF energies from step (1) of the flowchart; (b) internal energies calculated at B3LYP/6-31G(d) level, from step (2a); (c) internal energies calculated at B3LYP/6-311+G(d,p) level; (d) internal energies calculated at the same level as in (c), plus including PCM for acetonitrile, from step (2b); (e) ZPC-free energies calculated at the same level as in (d). The dramatic effect of basis set size and solvent model on the conformer population should warn anyone against the naïve approach of obtaining input structures for ECD (or other properties) calculations just by B3LYP/6-31G(d) geometry optimizations.

Another instructive example of the impact of basis set size on relative energies of a set of conformers, and on an average chiroptical property (in this case optical rotation, OR) calculated thereof, is shown in Figure 3.[52] The more general take-home message from this example is that when a reasonable change in conformer population affects the sign of average calculated OR (or some diagnostic CD band), the assignment should be doubted, and an alternative method should be considered.[53]
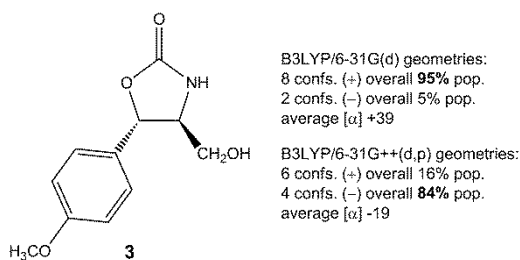

FIGURE 3 Basis-set dependence in the geometry optimization of *trans*-isocytoxazone (**3**) and its consequences on calculated OR.[52]

We'd like to mention here a different approach toward AC assignment based on the comparison between the ECD spectrum measured in the solid state, as microcrystalline KBr or KCl pellet, and that calculated by TDDFT using as input geometry the X-ray structure (possibly after minimal optimization). This method, known as the solid-state ECD/TDDFT approach, skips steps (1)-(5) in the flowchart and, apart from the time-saving, avoids most of the problems related with a correct geometry prediction; on the other hand, it requires crystals suitable for X-ray analysis.[54] In the example of Figure 4, the AC of a natural product (schisanterin A, **4**) was established considering the single solid-state geometry instead of the many low-energy conformers (20 distinct DFT minima with relative internal energies 3 kcal/mol estimated at B3LYP/6-311G+(d,p) level).[55]
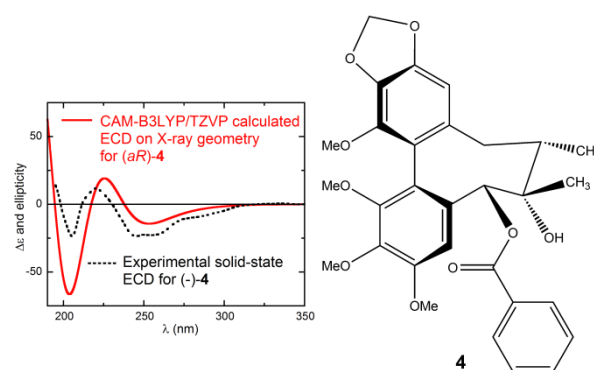

FIGURE 4 AC assignment of schisantherin A (**4**) by means of the solid-state ECD/TDDFT approach.[55]

Boltzmann Distribution

The Boltzmann distribution is universally employed to calculate average molecular properties over a set of conformers each of which has its own calculated property, such as a component ECD spectrum.[20,30,31] One must however be aware that the use of Boltzmann distribution is based on some assumptions: (a) the various conformers are enough separated from each other on the potential energy surface, that is, they lie in sharp energy wells with barriers high enough to keep vibrational states confined to each well; (b) the predicted property (i.e., ECD spectrum) is equivalent for all vibrational states within the energy well. These approximations may easily fail when low-frequency vibrational motions are involved, most notably torsional modes associated with flat potentials, and this may have some consequence on the prediction of ECD and – especially – OR.[56,57]

A second point to be considered is the quantity used in the Boltzmann distribution. As said above, geometry optimizations or single-point calculations afford enthalpies or internal energies; subsequent frequency calculations afford ZPC-energies and free energies. In principle these latter should be more reliable than internal energies because they include vibrational entropic factors. An example of the different distribution obtained by using internal and free energies is shown in Table 1 above. In practice, however, calculated entropies may be very inaccurate, especially for low-energy vibrations, and at least a weighting factor should be used to correct the vibrational energies.[58,59]

Choice of the TDDFT Functional

As we said in the first section, the choice of the functional/basis set combination in TDDFT calculations is the most crucial choice in the whole procedure. On the other hand, this is also one step which can be easily controlled by non-specialists. In fact, the most important thing to do is simply to try different combinations of functionals and basis sets, choosing within a set of popular functionals and reasonable basis sets, and verify the consistency of their results. In fact, the most common and most dangerous error at this step is to use only a single combination, typically again B3LYP with double or triple-ζ basis set, and take its result for granted. It is important to stress that a more systematic search for optimal calculation level does not need to be run on the whole conformational ensemble, but rather the lowest-energy conformer or a limited set of stable conformations usually suffice, with a considerable time-saving.

Provided that the "best" functional for the specific problem should be selected, how is this choice made? Unfortunately there is no general answer to this question. When excited state predictions are concerned as with TDDFT calculations, vertical transition energies and absolute oscillator strengths are normally given a primary importance.[60-62] When full absorption and/or ECD spectra are concerned, however, the relative energy and intensity of the various transition dipole and rotational strengths appear to be more important than absolute values. As a matter of fact, both a wavelength shift (UV correction) and some intensity scaling are normally applied during the computational/experimental comparison in the last step (9) of the flowchart. Therefore, no universal recipe may be given as for the best performing functional/basis set combination for ECD calculations, and the choice must rely on a trial-and-error approach. Generally speaking, both relative transition wavelengths and intensities depend strongly on the HF fraction of hybrid functionals and on the range separation. Consequently, one should pick up different examples from the two families, as suggested in the previous section, and test each of them on one or more selected structures, in association with a good basis set, for example def2-SVP or def2-TZVP. The functional(s) performing better in terms of overall spectrum appearance should be then selected for further basis set testing (if necessary), and finally to run the calculations on the remaining conformers.

In the following we will discuss a few examples which aim at demonstrating the impact of the functional on the quality of the final result, stressing especially the comparison between B3LYP and other functionals.
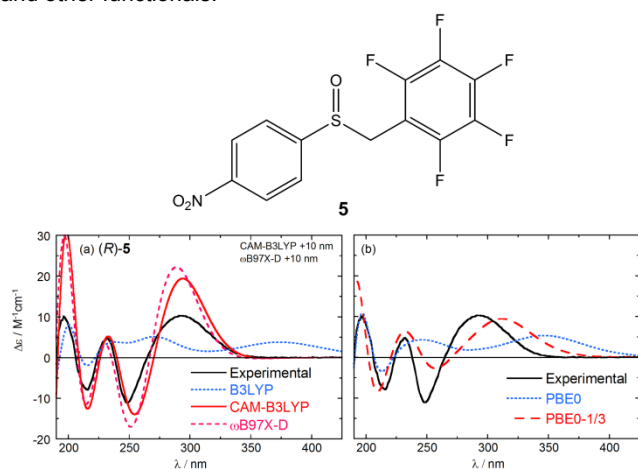

FIGURE 5 Comparison of experimental and TDDFT-calculated ECD spectra for sulfoxide **5** with different functionals. All TDDFT calculations were run with the same basis set (TZVP) using PCM model for acetonitrile. The calculated spectra are the Boltzmann average over 3 structures, geometry optimized at B3LYP/6-311+G(d,p) level in acetonitrile, and UV-corrected.[63]

Several recent reports demonstrate that range-separated functionals like CAM-B3LYP and ωB97X-D are superior to hybrid functionals, first of all B3LYP, in the prediction of chiroptical properties.[63-65] A good example is offered by sulfoxide **5** (Figure 4), for which CAM-B3LYP and ωB97X-D were able to predict the correct sequence of ECD bands, while B3LYP was not, particularly because of a severe underestimation of the energy of the first band.[63] When such a situation occurs, a deeper analysis of transitions and orbitals is desirable to uncover a possible specific functional-related issue. In the case of compound **5** and its analogs, as well as of the bisporphyrin system **6** discussed

below, the major responsible for the failure of B3LYP is the large charge-transfer (CT) character of the "missed" transition(s). B3LYP and many other hybrid functionals are known to perform poorly for CT-like transitions because of an incorrect asymptotic behavior.[62,66,67] This problem may be evidenced by evaluating the CT character of specific transitions by a metric measurement,[36,68] and conveniently tackled e.g. by using a range-separated functional.[36,67]

The same example is also useful to point out the dependence of TDDFT ECD calculations on the exact exchange fraction in hybrid DFT functionals. In fact, a small increase in the amount of HF%, i.e. passing from 25% of PBE0 to 33% of PBE0-1/3,[69] significantly improved the performance of the PBE-based hybrid functional (Figure 5b). This demonstrates how useful it can be to compare similar functionals with different HF%, for example B3LYP (20% HF) vs. BH&HLYP (50%, also known as BHLYP), PBE0 (25%) vs. PBE0-1/3 (33%), M06 (27%) vs. M06-2X (54%) and so on.

Another illustrative case is that of the bis(aminoporphyrin) **6**, for which both the geometry optimization and the TDDFT calculations were sensitive to the used functional.[70] The phenyl rings adjacent to the interporphyrin axis lie above the neighboring porphyrinic macrocycle. The resulting π-stacking is correctly reproduced only by dispersion-corrected functionals such as ωB97X-D. The π-stacking has a strong influence on the dihedral angle of the axis and thus on the ECD spectrum of the found conformers.[70] Moreover, CAM-B3LYP, B3LYP, and BHLYP predict quite different ECD spectra with only the long-range corrected functional CAM-B3LYP reproducing the correct pattern, intensities, and signs of the experimental curve (Figure 5). B3LYP in particular performed poorly due to the already discussed CT-related issue.
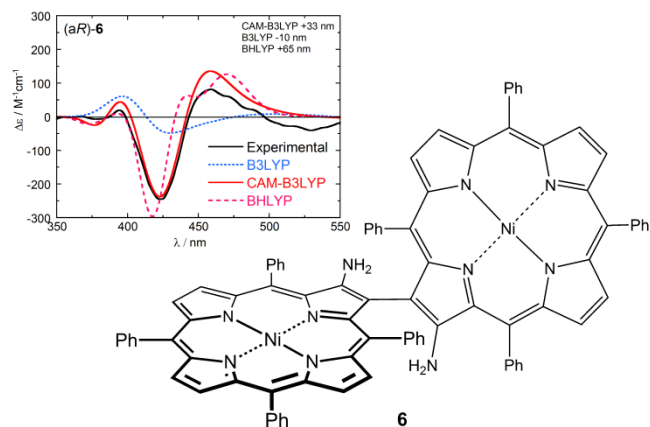

FIGURE 6 Comparison of experimental (intensity scaled to that of the CAM-B3LYP curve) and TDDFT-calculated ECD spectra of the main conformer of the aminoporphyrin dimer **6** calculated with B3LYP, CAM-B3LYP, and BHLYP. All computations were run with the same basis set (6-31G* for C, H, and N and 6-311G* for Ni) and with CPCM for dichloromethane. Optimization was done with ωB97X-D/6-31G* (6-311G* for Ni) and the spectra are UV-corrected.

Choice of the TDDFT Basis Set

The choice of the basis set for the TDDFT calculations is in principle less critical than for the calculation of free energies. The oscillator and rotational strength values are not very sensitive to basis set, moreover two simple pragmatic approaches may help addressing this point. First, rotational strengths calculated from dipole length (DL) and velocity (DV) formalisms converge to each other when approaching basis set completeness,[71] and

most software packages provide results for DL and DV rotational strengths for a direct comparison. Second, one may run several TDDFT calculations on one representative conformer keeping the functional fixed and increasing the basis set size within one family, e.g. using SVP, TZVP, minimally augmented TZVP (ma-TZVP) and augmented TZVP (aug-TZVP),[26,72] and look for the best cost-accuracy compromise. Often double or triple-ζ non-augmented basis sets already give reliable results, but diffuse functions may be required when Rydberg states are involved,[37] or, in general, for anionic species. Apart from the computational cost, diffuse functions often lead to severe problems with linear dependencies in the SCF, thus posing convergence issues. A good compromise are the so-called minimally augmented basis sets (e.g. ma-TZVP, ma-cc-pVTZ) suggested by Truhlar et al.,[72] which significantly lower computational costs at nearly the same accuracy as the large augmented basis sets.[73]

A more difficult and thus more decisive point is the computation of the excitation energies, as they show a higher sensitivity towards the basis set, depending on the nature of the chromophore(s) under investigation. To get the correct absolute values the basis set needs to be very large, including polarization and diffuse functions, keeping in mind that transition energies also depend strongly on the functional.[60-62] However, as said above, for the determination of the absolute configuration it is not necessary to get the correct absolute energy values, instead the relative energies must be reliable. The systematic shift between experimental and weighted-average spectra is usually larger for smaller basis sets, but the UV correction mentioned in step (9) is sufficient to handle it.

Solvent model

The impact of solvent on ECD spectroscopy is usually less dramatic than for other chiroptical spectroscopies such as OR, VCD and ROA. Still, solvent may affect both the relative stability and the component ECD spectrum for each conformer.[74] In the course of both DFT geometry optimizations and TDDFT calculations, non-specific solute-solvent interactions may be accounted for with a continuum solvent model such as PCM[27] or COSMO,[28] whose computational cost is often very reasonable. On the contrary, specific solute-solvent, solute-solute or intramolecular interactions require the use of explicit solvation models,[74] which are more demanding.

It is impossible to discuss solvent effects in any exhaustive way in the present contribution. Even the apparently simple question of whether one should consider or not a solvent model in ECD calculations is far from trivial. Staying on a very pragmatic ground, we recommend the use of a continuum solvent model in single-point calculations of conformational energies (step (3) in the flowchart), and possibly in TDDFT calculations for some selected conformers, at least when moderately polar molecules are concerned in combination with polar solvents. An example of impact of PCM on a conformational population is given in Table 1. As for TDDFT calculations, PCM affects especially relative transition energies and may provide a better spacing between calculated ECD bands.

The inclusion of an explicit solvent model should be considered only when other things fail. For example, in step (5) of the flowchart, the calculated conformational ensemble may contradict independent experimental findings, e.g. NMR data. Very often this is due to missing intramolecular interactions like π-stacking or hydrogen bonds occurring in solution, or strong specific solute-solvent interactions such as hydrogen bonding in water. Their correct simulation needs explicit solvent molecules to be considered in the conformational search, in the geometry optimization and in the TDDFT calculation steps. For examples of such a procedure, which may easily get very complicated and computationally expensive, we refer the interested reader to the literature.[74-77]

Beyond TDDFT

We have only discussed TDDFT as the method of choice for running ECD calculations, in view of its present great popularity. However, several other methods are feasible for excited-state calculations which represent useful alternatives to TDDFT especially in some circumstances.[10,11,13,33,34] Although conventional TDDFT is very time-effective, it is not feasible to calculate the ECD of larger molecules. Here the newly developed simplified TDDFT (sTDDFT) method is a very efficient alternative and allows ECD calculations of molecules with up to 1000 atoms at limited computational cost with good accuracy.[78] Another problem for TDDFT are chromophores with severe long-range CT excitations which can hardly be handled by any functional. Other methods like SCS-CIS(D) (spin-component-scaled configuration interaction singles with perturbative doubles correction), or the coupled-cluster approaches CC2 or EOM-CCSD (equation-of-motion coupled-cluster singles and doubles) may be employed in these cases.[79,80] CC2 in particular has received much attention in ECD calculations.[81-83] Like TDDFT, coupled-cluster methods cannot handle multi-reference wave functions. The use of MRCI (multi-reference configuration interaction) techniques becomes necessary for molecules not fully described by a single electronic configuration.[84] However, these methods are very time demanding and especially MRCI should be applied only by experienced users.

Conclusion

Quantum-mechanical calculations of chiroptical properties are nowadays an easily accessible and powerful tool for assigning absolute configurations of several classes of molecules, in particular small or medium-sized natural products. However, good practice in TDDFT calculations of ECD spectra needs to be more widespread, both to avoid unwanted errors in AC assignments and to favour a more critical use of computational methods. From our discussion it should be clear enough that no computational recipe is universally valid in all cases and for all molecules. In particular, the very popular "B3LYP/6-31G(d)" approach should be discouraged in general, although it may well provide the correct AC assignment. The most important message from the present contribution is that every method of calculation is prone to error, therefore one should never limit to a single level of calculation and should always explore different combinations of e.g. DFT functionals and basis sets. A second crucial point is, in our opinion, that an entirely computational approach is insufficient, especially in the generation of input structures. The conformational ensemble obtained in silico should always be checked against all available experimental data from other techniques such as NMR. We also recommend an independent assessment of relative configuration by means of X-ray diffractometry or again NMR, through a complete set of J-couplings and NOE measurements, whose interpretation is supported by molecular modeling. Finally, in the final comparison between the experimental and the computed ECD spectrum, it is

indispensable not to focus on a single band or a narrow wavelength window, but rather to consider the whole available spectral range. In the same step, a quantitative spectral comparison is also to be preferred over a merely visual one.

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