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DNA Electronic Circular Dichroism on the Inter-Base Pair Scale: An Experimental–Theoretical Case Study of the AT Homo-Oligonucleotide

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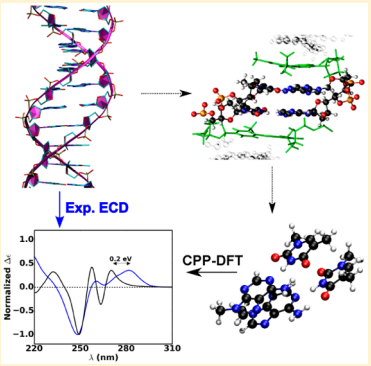
Supporting Information

ABSTRACT: A successful elucidation of the near-ultraviolet electronic circular dichroism spectrum of a short double-stranded DNA is reported. Time-dependent density functional theory methods are shown to accurately predict spectra and assign bands on the microscopic base-pair scale, a finding that opens the field for using circular dichroism spectroscopy as a sensitive nanoscale probe of DNA to reveal its complex interactions with the environment.



DNA is a central molecule of life itself. While it is mainly known for its storage of genetic information, DNA is also involved in a myriad of molecular recognition events and activities of DNA–protein complexes and it has proven capable of maintaining supramolecular structures.[1] Because of the high specificity of the cell machinery, these functions may be significantly affected by slight structural modifications of DNA, and the development of tools to study its secondary, tertiary, and even quaternary structures is essential to monitor DNA-related activity.

A DNA strand is a sequence of nucleosides dA, dT, dC, and dG, where "d" stands for 2′-deoxyribose and A, T, C, and G for bases adenine, thymine, cytosine, and guanine, respectively. The most common types of DNA adopt a double-strand helical structure that can be either right-handed (e.g., B- or A-DNA) or left-handed (e.g., Z-DNA) chiral structures. Because DNA has many π-conjugated moieties, its secondary (and higher) structures can be investigated by electronic circular dichroism (ECD).[2] Over the past decades, ECD signals of DNA have been the subject of particular attention because ECD spectroscopy (i) is sequence-dependent, (ii) is extremely concentration-sensitive, and (iii) elucidates the *in situ* supramolecular arrangement of DNA.[3,4]

DNA conformations are extremely environment-sensitive[5−8] because the secondary structure depends on temperature,[9] nature and concentration of cations,[10−12] and the recognition of systems such as simple molecules[13,14] (e.g., anticancer intercalating drugs), cationic polymers, and biomolecular/supramolecular assemblies[7] (e.g., proteins).[4,6,15,16] For example, linear B-DNA may bind to specific proteins such as histones, leading to a new form of DNA (N-DNA) in nucleosomes.[17] The dichroism signature of N-DNA provides insights into the DNA–protein interactions with respect to the corresponding linear B-DNA structure.

ECD signatures may be used as fingerprints of not only DNA but also the DNA recognition of proteins or synthetic molecules. If the binding structure is modified (e.g., in ligand-protein interactions), the DNA shape is also affected, leading to observed differences in ECD spectra. It must be stressed that in DNA–protein recognition, the use of ECD is particularly relevant because the spectral ranges of DNA and proteins do not overlap strongly, being 220–350 and 150–250 nm for DNA and proteins, respectively.[3]

Even if the literature is rich in experimental studies on ECD signals of DNA (see ref 3 and references therein), it is worth noting that dichroism signatures can only be discussed in terms of comparisons to reference spectra. Therefore, theoretical quantum chemistry appears to be an ideal tool to rationalize macroscopic ECD signals by means of a coupling to the

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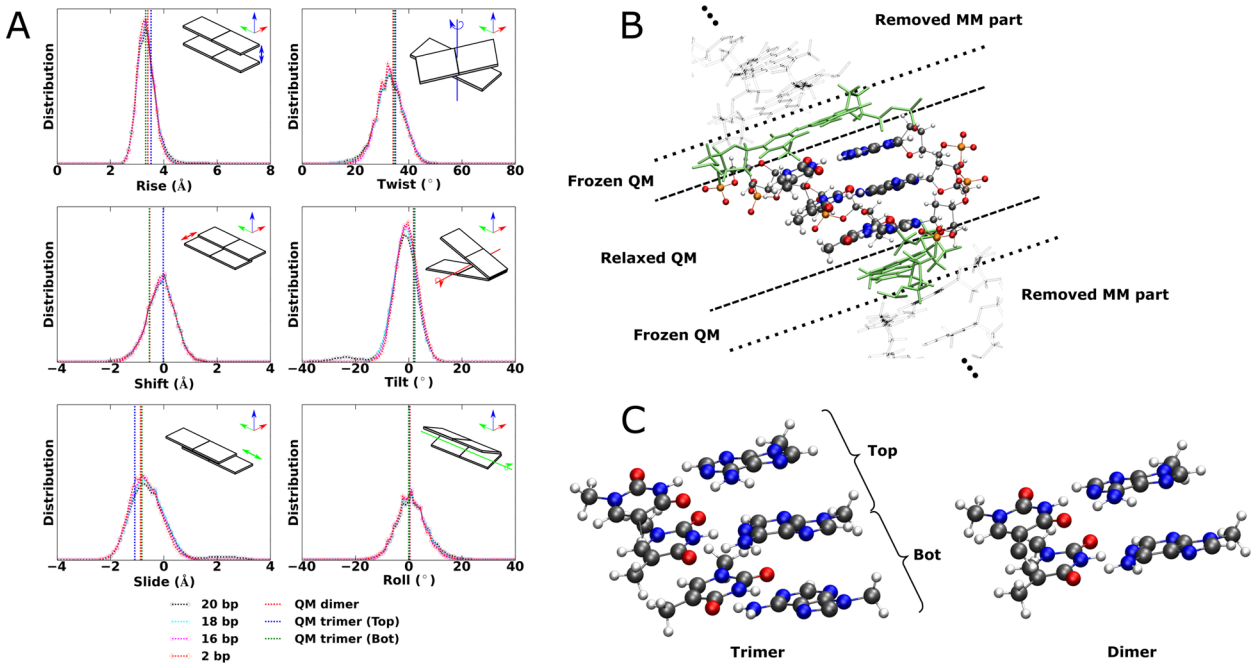

Figure 1. (A) Inter-bp parameter distributions from a 100 ns NVT MD simulation of dsDNA $(dA)_{20}\cdot(dT)_{20}$ including 20, 18, 16, and 2 bps in the statistics. Inter-bp parameters from dimer and trimer QM models are also reported by use of vertical lines. (B) Illustration of the construction of the trimer model. (C) Final QM-optimized dimer and trimer models used for ECD calculations.

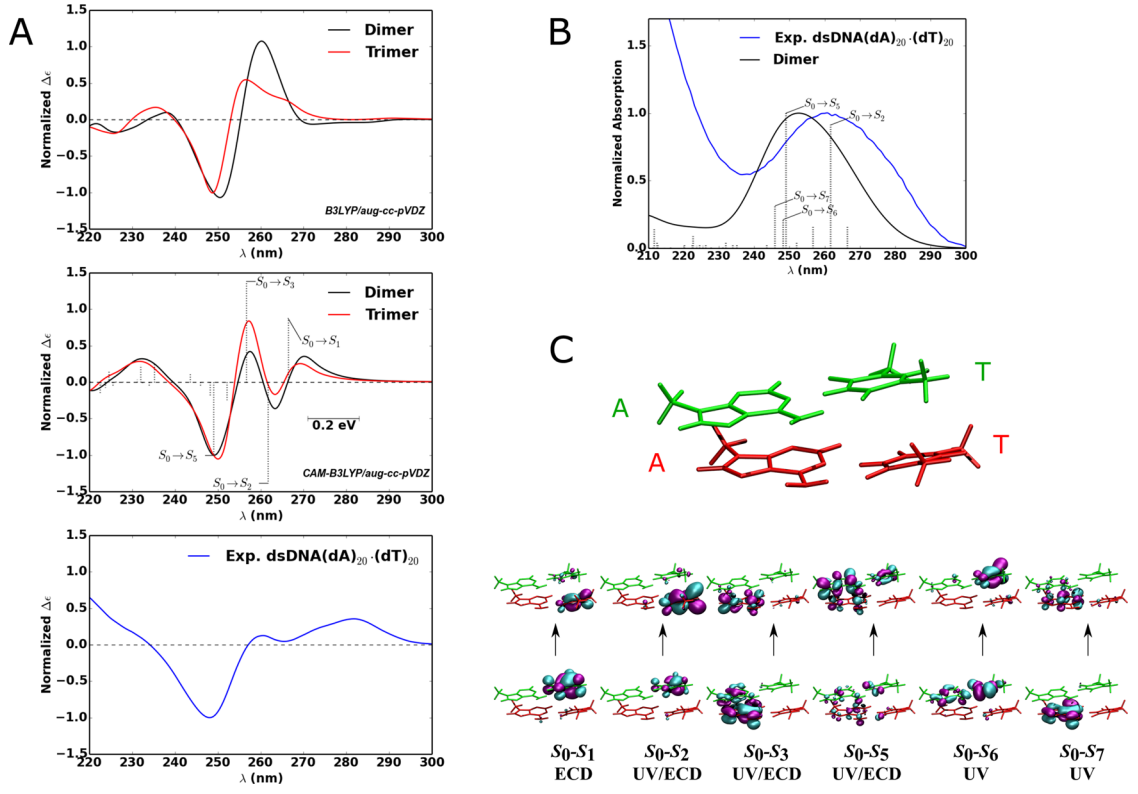

Figure 2. (A) Electronic circular dichroism (ECD) spectra from dimer and trimer model calculations and from experiment. Significant electronic transitions are highlighted and spectra are normalized with respect to the negative band at 249 nm. (B) Comparison of experimental and TD-CAM-B3LYP/aug-cc-pVDZ near-UV absorption spectra. Both spectra are normalized. (C) Weighted sums of Kohn−Sham orbital densities for the dominating electronic transitions in near-UV absorption and ECD spectra. In panels A and B, B3LYP and CAM-B3LYP spectra are shifted by +0.21 and −0.21 eV, respectively.

microscopic base-pair (bp) picture. Even though ECD spectra of DNA components such as uridine derivatives[18] have already been investigated, no elucidation of ECD spectra of DNA has been reported so far at the inter-base pair level. Supported by experiments, we here report an affordable theoretical methodology that accomplishes just that. As an example, we here

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rationalize the ECD spectrum of the B-DNA dsDNA $(dA)_{20}\cdot(dT)_{20}$ sequence. Today, such a large system (1278 atoms) as a whole is out of reach for a treatment with medium/high-level quantum mechanical (QM) methods. Likewise, ECD calculations on single base-pairs are meaningless due their achiral nature. As a first step, we must define the minimal relevant system that captures the chiral features of the double helix. Inter-bp parameters are here of particular importance because they (i) reflect the helix chirality signature and (ii) are expected to strongly influence the ECD responses.[19]

For this purpose, a 100 ns (N,V,T) molecular dynamics (MD) simulation was performed on the B-DNA dsDNA $(dA)_{20}\cdot(dT)_{20}$ sequence using a refined force field for DNA as available in Amber12.[20] Inter-base pair parameters of DNA helical coordinates (Figure 1) were extracted and analyzed for the entire double-stranded DNA and also for fragments of the 18, 16, and 2 central base-pairs (Figure 1A) using Curves+.[19] The helix pitch was extrapolated from the twist and rise parameters resulting in values of 37.3, 36.5, 36.1, and 36.5 Å (i.e., axial rise: 11.0, 11.0, 10.8, and 11.0 bps/turn) with inclusion made of 20, 18, 16, or 2 bps in the statistics, respectively. This is in perfect agreement with the value of 37 ± 1.5 Å recently reported from FM−AFM experiments.[21]

For the six inter-base pair parameters in general, no significant differences could be observed with respect to variations in the number of base-pairs that were analyzed in the statistics (see Figure 1A and Supporting Information), in agreement with recent theoretical studies.[22] This suggests that the $(dA)_2\cdot(dT)_2$ model is sufficient to geometrically represent the secondary structure of an entire short double-stranded DNA in our calculations of ECD spectra.

Our approach is directly related to the simple first-sphere model that has been successfully used in solid-state[23−25] and solvated[26] ECD calculations. This approach is actually based on evaluating and summing two-body contributions between direct neighbors, and, in the simple case of dsDNA $(dA)_{20}\cdot(dT)_{20}$, the first-sphere lattice would thus consist of a base-pair trimer (i.e., for a given bp, one bp above, and the other bp beneath). For this reason, a $(dA)_3\cdot(dT)_3$ trimer model was also constructed as to assess the quality of the dimer model.

Both $(dA)_2\cdot(dT)_2$ and $(dA)_3\cdot(dT)_3$ geometries were optimized at the density functional theory (DFT) B3LYP-D3/6-31G** level of theory based on starting structures taken from an MD snapshot. In the optimization process, additional base-pairs were included to cap the DNA segment (top and bottom), leading to four and five base-pair systems for the dimer and trimer models, respectively. The two supplementary base-pairs were kept frozen (see Figure 1B) to (i) keep the realistic helical shape and (ii) prevent geometrical edge effects (see Supporting Information for further details). Inter-bp parameters of both the dimer and trimer models are in good agreement with MD simulations, validating our new models (see Figure 1A). Because the nucleobases are expected to give the main contribution to the near-ultraviolet (UV) dichroism, the 2′-deoxyribose phosphate moieties were replaced by methyl groups in the ECD calculations (see Figure 1C).

The theoretical ECD spectra shown in Figure 2A have been determined for both dimer and trimer models using a resonance-convergent formulation of response theory known as the complex polarization propagator (CPP) approach[27−29] in conjunction with its DFT implementation in DALTON.[30] The main advantage of the CPP formalism is that excited states are not explicitly addressed, enabling calculations of spectral profiles in regions with high density of states as appearing in ECD calculations on, for example, fullerenes[31] and helical stacks.[32]

The use of a conventional TD-DFT approach can be problematic in the case of DNA because of the need for an explicit resolution of more than 30 excited states even for the small dimer model as to elucidate the complete absorption within the range of 200−350 nm. For example, as the theoretical near-UV absorption spectrum based on the resolution of the 30 lowest excited states is compared with the experimental counterpart (see Figure 2B), it is observed that, even if the lowest energy band is well reproduced, the second band starting from 230 nm is not due to the inclusion of an insufficient number of electronic transitions. For the trimer model the situation is drastically worse and the CPP approach represents the only viable option in this case.

The experimental ECD spectrum of the B-DNA sequence dsDNA $(dA)_{20}\cdot(dT)_{20}$ exhibits two positive bands (283 and 260 nm) followed by a strong negative band at 249 nm (see Figure 2A). The experimental band profile is well reproduced by the CAM-B3LYP calculations. Even if the separation between two positive bands is smaller in the theoretical spectrum than in the experimental one, it should be noted that the discrepancy is as small as 0.2 eV, which is as good as can be expected even with use of state-of-the-art electronic structure theory. The choice of method (here in terms of exchange-correlation functional and basis set) is crucial; a set of functionals was tested and results are reported in the Supporting Information. Particular attention is paid to the highly used functional B3LYP that fails to reproduce the lowest energy band (see Figure 2A). This can be explained by the well-known drawback of conventional hybrid functionals in terms of their inability to describe excited-state charge transfer (ES−CT).[33]

As far as model assessment is concerned, we note a remarkable agreement between spectra obtained with use of the minimal dimer model and the trimer model. This observation represents strong evidence that it is reasonable to attribute the ECD signals of DNA to base-pair dimers, both in a qualitative manner as the origin of the response signal but, more surprisingly, also a *quantitative* manner.

The first experimentally observed positive band is attributed to the S_0-S_1 electronic transition (Figure 2A) that is a pure ES−CT between two neighboring T−T bases. The second positive band is attributed to the S_0-S_3 transition, corresponding to a mixture of local $\pi-\pi^*$ transitions in the A base and ES−CT between neighboring A−A bases (Figure 2C). It should be noted that, in between the positive bands at 260 and 285 nm in the experiment, the ECD signal is strongly reduced. This signal feature is accurately captured in the CAM-B3LYP results and it is assigned to the intense S_0-S_2 transition with a strong negative dichroism. This transition, like the S_0-S_1 transition, is related to ES−CT transitions between neighboring T−T bases. In Figure 2C, it can be seen that accepting orbitals in the formations of states S_1 and S_2 have opposite signs, which is reflected in opposite signs in their respective dichroism signals. The relative intensity of the two positive bands is not correctly described in the CAM-B3LYP spectrum. In the experiment the intensity of the band at 260 nm is lower than the intensity of the band at 285 nm, whereas the opposite trend is observed in the theoretical spectrum. It is clear, however, that the relative intensity depends strongly on the separation of transitions associated with positive (states S_1 and S_3) and

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negative (state S_2) dichroism. As already pointed out, the band separation is too small and the incorrect theoretical description of the relative intensity may well be primarily associated with an underestimation of the separation of states S_2 and S_1.

The main spectral feature in the experiment is the strong negative ECD band at 250 nm. This band is well described with the use of both functionals and it can be attributed to the S_0-S_5 transition that is due to both intra- (n-π^*) and inter-bp transitions, underlining again the ES-CT nature of the low-energy bands. Interestingly, the S_0-S_6 and S_0-S_7 transitions contribute significantly to the near-UV absorption spectrum while they are not involved in the ECD response due to the achiral nature of base pair monomers.

In summary, we report a comprehensive analysis of the near-UV ECD spectrum of B-DNA dsDNA $(dA)_{20}\cdot(dT)_{20}$. Our analysis is based on a building-block principle based on base-pair dimers, a model that is rigorously assessed and verified as accurate against an extended trimer model; a technical prerequisite for achieving high-quality simulations is that the underlying electronic structure method provides accurate treatment of inter-bp charge-transfer transitions. The ramifications of our results are wide as they show that secondary and higher structures of DNA as well as subtle environment interactions can be probed and elucidated by means of a combination of experimental and theoretical near-UV ECD spectroscopy. To mention a few examples: the sequence dependence of ECD signals can be analyzed at the microscopic base-pair level, effects of DNA molecular recognition can be studied (e.g., intercalation of drugs, protein binding, DNA templating), and conformational changes based on specific supramolecular interactions can be monitored.

■ ASSOCIATED CONTENT

Supporting Information

Technical details and benchmarking results. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Widom, J. Role of DNA Sequence in Nucleosome Stability and Dynamics. *Q. Rev. Biophys.* **2001**, *34*, 269−324.

(2) Gray, D. M.; Hung, S.-H.; Johnson, K. H. Absorption and Circular Dichroism Spectroscopy of Nucleic Acid Duplexes and Triplexes. *Methods Enzymol.* **1995**, *246*, 19−34.

(3) Kypr, J.; Kejnovská, I.; Renčiuk, D.; Vorlíčková, M. Circular Dichroism and Conformational Polymorphism of DNA. *Nucleic Acids Res.* **2009**, *37*, 1713−1725.

(4) Berova, N.; Polavarapy, P. L.; Nakanishi, K.; Woody, R. W. *Comprehensive Chiroptical Spectroscopy*; John Wiley & Sons, Inc.: Hoboken, NJ, 2012; p 1840.

(5) Pérez, A.; Luque, F. J.; Orozco, M. Frontiers in Molecular Dynamics Simulations of DNA. *Acc. Chem. Res.* **2011**, *45*, 196−205.

(6) Garrec, J.; Patel, C.; Rothlisberger, U.; Dumont, E. Insights into Intrastrand Cross-Link Lesions of DNA from QM/MM Molecular Dynamics Simulations. *J. Am. Chem. Soc.* **2011**, *134*, 2111−2119.

(7) Lavery, R.; Zakrzewska, K.; Beveridge, D.; Bishop, T. C.; Case, D. A.; Cheatham, T.; Dixit, S.; Jayaram, B.; Lankas, F.; Laughton, C.; et al. A Systematic Molecular Dynamics Study of Nearest-Neighbor Effects on Base Pair and Base Pair Step Conformations and Fluctuations in B-DNA. *Nucleic Acids Res.* **2010**, *38*, 299−313.

(8) Pérez, A.; Lankas, F.; Luque, F. J.; Orozco, M. Towards a Molecular Dynamics Consensus View of B-DNA Flexibility. *Nucleic Acids Res.* **2008**, *36*, 2379−2394.

(9) Meyer, S.; Jost, D.; Theodorakopoulos, N.; Peyrard, M.; Lavery, R.; Everaers, R. Temperature Dependence of the DNA Double Helix at the Nanoscale: Structure, Elasticity, and Fluctuations. *Biophys. J.* **2013**, *105*, 1904−1914.

(10) Williamson, J. R.; Raghuraman, M. K.; Cech, T. R. Monovalent Cation-Induced Structure of Telomeric DNA: The G-Quartet Model. *Cell* **1989**, *59*, 871−880.

(11) Sugimoto, N.; Wu, P.; Hara, H.; Kawamoto, Y. Ph and Cation Effects on the Properties of Parallel Pyrimidine Motif DNA Triplexes. *Biochemistry* **2001**, *40*, 9396−9405.

(12) Duguid, J.; Bloomfield, V. A.; Benevides, J.; Thomas, G. J., Jr. Raman Spectroscopy of DNA-Metal Complexes. I. Interactions and Conformational Effects of the Divalent Cations: Mg, Ca, Sr, Ba, Mn, Co, Ni, Cu, Pd, and Cd. *Biophys. J.* **1993**, *65*, 1916−1928.

(13) Etienne, T.; Very, T.; Perpète, E. A.; Monari, A.; Assfeld, X. A QM/MM Study of the Absorption Spectrum of Harmane in Water Solution and Interacting with DNA: The Crucial Role of Dynamic Effects. *J. Phys. Chem. B* **2013**, *117*, 4973−4980.

(14) Dumont, E.; Monari, A. Benzophenone and DNA: Evidence for a Double Insertion Mode and Its Spectral Signature. *J. Phys. Chem. Lett.* **2013**, *4*, 4119−4124.

(15) Nilsson, K. P. R.; Inganäs, O. Chip and Solution Detection of DNA Hybridization Using a Luminescent Zwitterionic Polythiophene Derivative. *Nat. Mater.* **2003**, *2*, 419−424.

(16) Rubio-Magnieto, J.; Thomas, A.; Richeter, S.; Mehdi, A.; Dubois, P.; Lazzaroni, R.; Clément, S.; Surin, M. Chirality in DNA-π-Conjugated Polymer Supramolecular Structures: Insights into the Self-Assembly. *Chem. Commun.* **2013**, *49*, 5483−5485.

(17) Davey, C. A.; Sargent, D. F.; Luger, K.; Maeder, A. W.; Richmond, T. J. Solvent Mediated Interactions in the Structure of the Nucleosome Core Particle at 1.9Å Resolution. *J. Mol. Biol.* **2002**, *319*, 1097−1113.

(18) Miyahara, T.; Nakatsuji, H.; Wada, T. Circular Dichroism Spectra of Uridine Derivatives: Chirasac Study. *J. Phys. Chem. A* **2014**, *118*, 2931−2941.

(19) Lavery, R.; Moakher, M.; Maddocks, J. H.; Petkeviciute, D.; Zakrzewska, K. Conformational Analysis of Nucleic Acids Revisited: Curves+. *Nucleic Acids Res.* **2009**, *37*, 5917−5929.

(20) Salomon-Ferrer, R.; Case, D. A.; Walker, R. C. An Overview of the Amber Biomolecular Simulation Package. *Wiley Interdiscip. Rev.: Comput. Mol. Sci.* **2013**, *3*, 198−210.

(21) Ido, S.; Kimura, K.; Oyabu, N.; Kobayashi, K.; Tsukada, M.; Matsushige, K.; Yamada, H. Beyond the Helix Pitch: Direct Visualization of Native DNA in Aqueous Solution. *ACS Nano* **2013**, *7*, 1817−1822.

(22) Fresch, B.; Remacle, F. Atomistic Account of Structural and Dynamical Changes Induced by Small Binders in the Double Helix of a Short DNA. *Phys. Chem. Chem. Phys.* **2014**, *16*, 14070−14082.

DOI: 10.1021/jz502696t
J. Phys. Chem. Lett. 2015, 6, 355−359

(23) Pescitelli, G. Solid-State Circular Dichroism and Hydrogen Bonding, Part 2: The Case of Hypothemycin Re-Investigated. *Chirality* **2012**, *24*, 718−724.
(24) Pescitelli, G.; Padula, D.; Santoro, F. Intermolecular Exciton Coupling and Vibronic Effects in Solid-State Circular Dichroism: A Case Study. *Phys. Chem. Chem. Phys.* **2013**, *15*, 795−802.
(25) Padula, D.; Pietro, S. D.; Annunziata, M.; Capozzi, M.; Cardellicchio, C.; Pescitelli, G. Strong Intermolecular Exciton Couplings in Solid-State Circular Dichroism of Aryl Benzyl Sulfoxides. *Chirality* **2014**, *26*, 462−470.
(26) Jurinovich, S.; Pescitelli, G.; Di Bari, L.; Mennucci, B. A TDDFT/MMpol/PCM Model for the Simulation of Exciton-Coupled Circular Dichroism Spectra. *Phys. Chem. Chem. Phys.* **2014**, *16*, 16407−16418.
(27) Norman, P.; Bishop, D. M.; Jensen, H. J. Aa.; Oddershede, J.; Near-Resonant, J. Absorption in the Time-Dependent Self-Consistent Field and Multiconfigurational Self-Consistent Field Approximations. *J. Chem. Phys.* **2001**, *115*, 10323−10334.
(28) Norman, P.; Bishop, D. M.; Jensen, H. J. Aa.; Oddershede, J. Nonlinear Response Theory with Relaxation: The First-Order Hyperpolarizability. *J. Chem. Phys.* **2005**, *123*, 194103−194121.
(29) Norman, P. A Perspective on Nonresonant and Resonant Electronic Response Theory for Time-Dependent Molecular Properties. *Phys. Chem. Chem. Phys.* **2011**, *13*, 20519−20535.
(30) Aidas, K.; Angeli, C.; Bak, K. L.; Bakken, V.; Bast, R.; Boman, L.; Christiansen, O.; Cimiraglia, R.; Coriani, S.; Dahle, P.; et al. The Dalton Quantum Chemistry Program System. *Wiley Interdiscip. Rev.: Comput. Mol. Sci.* **2014**, *4*, 269−284.
(31) Jiemchooroj, A.; Norman, P. Electronic Circular Dichroism Spectra from the Complex Polarization Propagator. *J. Chem. Phys.* **2007**, *126* (134102), 1−7.
(32) Norman, P.; Linares, M. On the Interplay between Chirality and Exciton Coupling: A DFT Calculation of the Circular Dichroism in Π-Stacked Ethylene. *Chirality* **2014**, *26*, 483−489.
(33) Jacquemin, D.; Perpète, E. A.; Scuseria, G. E.; Ciofini, I.; Adamo, C. TD-DFT Performance for the Visible Absorption Spectra of Organic Dyes: Conventional Versus Long-Range Hybrids. *J. Chem. Theory Comput.* **2007**, *4*, 123−135.

DOI: 10.1021/jz502696t
J. Phys. Chem. Lett. 2015, 6, 355−359