Review Article

Applications of the Cartesian Coordinate Tensor Transfer Technique in the Simulations of Vibrational Circular Dichroism Spectra of Oligonucleotides

VALERY ANDRUSHCHENKO* AND PETR BOUŘ*

Institute of Organic Chemistry and Biochemistry, Academy of Sciences, Prague, Czech Republic

Contribution to the CD ISBC 2009 Special Issue

The application of the Cartesian coordinate tensor transfer (CCT) tech-ABSTRACT nique for simulations of the IR absorption and vibrational circular dichroism (VCD) spectra of relatively large nucleic acid fragments is demonstrated on several case studies. The approach is based on direct *ab initio* calculations of atomic tensors, determining molecular properties, for relatively small fragments, and subsequent transfer of these tensors to the larger systems in Cartesian coordinates. This procedure enables precise computations of vibrational spectra for large biomolecular systems, currently with up to several thousands of atoms. The versatile ability of the CCT methods is emphasized on the examples of VCD and IR absorption spectra calculations for B- and Z-forms of DNA, single-, double-, and triple-stranded RNA helices and DNA structures with different base content and sequences. The development and recent improvements of the methodology are followed, including utilization of the constrained normal mode optimization (NMO) strategy and combined quantum mechanics and molecular dynamics simulations. Advantages, drawbacks, and recommendations for future improvements of the CCT method as applied to nucleic acid spectra calculations are discussed. Chiral*ity 22:E96–E114, 2010.* © 2010 Wiley-Liss, Inc.

KEY WORDS: VCD spectroscopy; IR spectroscopy; nucleic acids; DNA; RNA; *ab initio*; molecular dynamics; spectra simulations; normal mode optimization

INTRODUCTION

Optical methods are convenient and versatile tools for molecular structural studies. For example, the electronic circular dichroism (ECD) has been a major chiroptical technique for more than a half of the century.¹ In the last several decades, its analog in the infrared region of spectrum, vibrational circular dichroism (VCD), has emerged and developed into a potent molecular structure determination tool.²⁻¹⁶ VCD is also a chiral spectroscopic technique, which monitors differential absorption of left and right circularly polarized infrared light. Because VCD is derived from infrared absorption (IR), it possesses several advantages over the conventional ECD spectroscopy, originating from ultraviolet absorption (UV). ECD spectrum usually provides relatively limited information on the structure of molecules, contained in a few broad and less resolved bands, which arise from electronic transitions. On the contrary, VCD signal, as the parental IR, arises from normal mode vibrations, often localized on one or several chemical bonds of same type. The resulting spectrum has a large number of relatively well separated bands arising from different molecu-© 2010 Wiley-Liss, Inc.

lar groups (wavenumber and spatial resolution) compared to UV or ECD, which are much easier to analyze and characterize. Thus it provides more specific and rich details of the molecular structure.

Electronic and VCD signal in nucleic acids mostly originates not from an intrinsic chirality of their components (only sugar residues in the backbone are chiral) but from

Contract grant sponsor: Grant Agency of the Czech Republic; Contract grant numbers: 203/01/0031, 203/06/0420, 202/07/0732

Contract grant sponsor: Grant Agency of the Academy of Sciences; Contract grant numbers: A4055104, A400550702, A400550701, M200550902 Contract garnt sponsor: Grant Agency of the Czech Republic; Contract

grant number: P208/10/0559 Contract grant sponsors: Alberta Heritage Foundation for Medical Research (Canada), Institute of Organic Chemistry and Biochemistry

Research (Canada), Institute of Organic Chemistry and Biochemistry (Czech Republic), Natural Sciences and Engineering Research Council of Canada *Correspondence to: Valery Andrushchenko, Institute of Organic Chemis-

try and Biochemistry, Flemingovo nám. 2, 16610, Praha 6, Czech Republic. E-mail: andrushchenko@uochb.cas.cz or Petr Bouř, Institute of Organic Chemistry and Biochemistry, Flemingovo nám. 2, 16610, Praha 6, Czech Republic. E-mail: bour@uochb.cas.cz.

Received for publication 13 December 2009; Accepted 26 March 2010 DOI: 10.1002/chir.20872

Published online 18 May 2010 in Wiley Online Library (wileyonlinelibrary.com).

a through-space dipolar coupling of electronic or vibrational transitions. The coupled dipole-dipole contribution, dominating the spectrum, can be well described by the exciton theory.¹⁷ A typical VCD spectral feature is called "couplet" and contains positive and negative components (bands). Conventionally, a VCD couplet is regarded as positive or negative, depending whether the lower wavenumber component is positive or negative, respectively.⁷ The VCD phenomenon is characterized by locality, i.e., the strongest through-space interaction occurs between the neighboring chromophores and decreases sharply with the distance.¹⁸⁻²⁰ The signal also depends strongly on the mutual orientation of the chromophores.^{18,19} For some systems, including nucleic acids, the VCD intensity increases rapidly with the number of dipoles participating in the coupling.^{18,21} In such cases, the VCD signal is determined at the local level by neighboring dipoles as well as by longrange exciton modes.²¹ Such an exciton propagation is enhanced when the chromophores are arranged in a regular order, which is enabled by an ordered helical structure of nucleic acid molecules.¹⁷ Distortions of the ordered structure lead to decrease of VCD intensity.^{6,14,18,21-23} Such a high sensitivity to minor local and long range variations in a structural arrangement is not observed in conventional IR spectra.

The VCD spectroscopy has thus been extensively employed for studies of nucleic acids starting from 1987.² First applications of the technique were mostly aimed at understanding the nature of nucleic acid VCD and establishing characteristic VCD signatures of different nucleic acid conformations, and were performed mainly by the groups of Keiderling in Chicago^{6,24–29} and Diem in New York.^{14,18,19,30–32} The group of Wieser in Calgary in 1995 expanded the use of VCD in nucleic acid field systematically investigating the effect of the base sequence variation on VCD spectra,³³ studying DNA and RNA interactions with metal ions^{5,23,34–38} and drugs,^{39–43} and exploring atypical nucleic acid structures.⁴⁴ The pioneering work has been performed on home-built VCD instruments.45-47 Nowadays, based on the extensive background acquired by the earlier studies, fast and highly sensitive commercial VCD instruments are also available, such as those from Biotools (USA), Bruker (Germany), and Jasco (Japan). The commercialization stimulated further expansion of VCD spectroscopy applications in the nucleic acid field by many research groups, e.g., Nafie and coworkers in Syracuse,⁴⁸ Polavarapu in Nashville^{49,50} and Urbanova and coworkers in Prague.⁵¹⁻⁵³

Despite the progress in instrumentation, the interpretation of the VCD spectra remains a challenging and often a tedious task, especially for such complex molecules as nucleic acids. Even though an extensive information is gathered up to date about the IR absorption band assignments, some of them are ambiguous or missing due to natural broadening of the spectral lines, band overlap, coupling and mixing of the vibrations of different functional groups.^{54–56} Even less information is available about the assignments of VCD bands due to relatively recent emergence of this technique.^{5,6} This is disappointing, because a complete understanding of the optical response and establishing exact relationship between spectra and molecular structure provides insight not only into the structure, but also into other molecular properties such as conformational behavior, interaction with the solvent and other molecules, or even biological activity.^{57,58} These tasks can be significantly simplified and sometimes completely solved by performing theoretical simulations of the spectra and their comparison with the experimental data.

Initially, empirical and semiempirical models, such as the coupled oscillator (CO) model, were used for the VCD simulations.^{17,19,28,30,32,59} Later, various extension of the CO model were developed and successfully used, such as the DeVoe polarizability theory.^{60–63} While these models could provide a basis for understanding of some VCD features, particularly those observed in the C=O stretching region, they are not applicable generally. Neither the CO models should be used when the chromophores are close in space, which is the case for stacked nucleic acid bases.^{64,65} Today, it is the quantum mechanical approach that appears most viable for the spectral modeling.

Possibility and advantages of the *ab initio* calculations of VCD spectra were first recognized and pioneered by Stephens and coworkers⁶⁶ and Polavarapu⁶⁷ about two decades ago, after the theory of VCD computations has been developed.⁶⁸ In principal, assignments of the vibrational transitions can be done with the help of the calculations, overlapped bands can be resolved, and the extent of the vibrational coupling can be determined. The first principle computations enable prediction of spectra for unknown structures, allow to obtain insight into the nature of interactions with other molecules and solvent, and to select energetically favorable conformations.

Due to the advances in computer technologies and *ab initio* methodology, simulations of VCD spectra within the harmonic approximation for small rigid molecules are relatively straightforward, as implemented in a number of quantum chemistry programs, including the most utilized Gaussian software package.⁶⁹ The Gaussian implementation is based on the magnetic field perturbation theory (MFP) of Stephens⁶⁸ combined with density functional theory (DFT) and field-dependent (gauche invariant) atomic orbitals (GIAO).⁷⁰ Applications of such simulations to small molecules were very successful; in combination with experimental VCD spectra absolute configurations could be determined without the need for much more expensive and time-consuming techniques (e.g., X-ray diffraction).^{10,12,16,71,72}

However, the size and conformational flexibility of the biopolymers complicates the direct *ab initio* modeling of VCD spectra. In some cases, suitably chosen fragments of large biopolymers can provide enough information on a local structure and main spectral features of the molecule due to locality of the VCD phenomenon.^{18,73,74} In general, more universal methods are needed to reliably model spectra for the whole biopolymer without loosing the accuracy of the *ab initio* approach. The Cartesian coordinate tensor transfer (CCT) technique discussed below was developed for this purpose,⁷⁵ as it allows transfer of the most common molecular property tensors (force field, dipole derivatives, atomic axial tensors) from smaller fragments to larger biomolecules. The transfer in Cartesian *Chirality* DOI 10.1002/chir



Fig. 1. Schematics of the Cartesian coordinate tensor transfer (CCT) technique as applied to a DNA octanucleotide. Separate fragments used for *ab initio* calculations are shown as colored blocks. (a) atomic tensors describing some of the inter-base interactions are transferred to the stacked base pairs dimers (orange blocks); (b) missing inter-base pair interactions (white areas between orange blocks in figure "a") are added by transferring tensors for three more base pair dimers (shaded green blocks), covering all inter-base pair interactions; (c) atomic tensors are transferred to the sugar-phosphate dimers (blue blocks); (d) missing sugar-phosphate interactions are added by transferring six more sugar-phosphate dimers (shaded violet blocks). Only interactions between the bases and the sugar-phosphate backbone remain uncovered (white areas in figure "d") since they are not crucial for the particular case, but they may also be included at will by a set of additional *ab initio* computations of whole nucleotide fragments.

coordinates (including translations and rotations) is computationally easier and requires less human input than with nonlinear internal coordinates. This technique allows to expand relatively accurate *ab initio* spectral simulations to large molecules, with several thousands of atoms. It has been earlier successfully applied for simulations of VCD spectra of polypeptides.^{76,77} In the present minireview we will demonstrate how the CCT method can be used to model VCD spectra of nucleic acids, show the main results for different structural forms of DNA and RNA, and discuss the recent advances and developments of the method.

METHODOLOGICAL CONSIDERATIONS

The general procedure in the CCT transfer technique consists of splitting a large molecule into fragments, *ab ini-tio* calculation of atomic tensors for all fragments, and a following transfer of the tensors back to the original large molecule, as an alternative to a direct *ab initio* spectra simulation for the whole large molecule (which may not be feasible at the present state of the computational technologies). It has been shown that if the fragments are properly chosen, the CCT method provides IR absorption and VCD spectra virtually indistinguishable from the fully *ab initio* results.⁷⁸ The method explores the locality of the VCD phenomenon,¹⁸ but it also allows to include some long-range interactions. Additionally, the technique enables parallelization of the computational tasks, which further speeds up the modeling.

The typical steps used in CCT technique are shown in Figure 1 on the example of an octanucleotide. The original structure of the octamer (or any other oligomer) is either an X-ray or NMR structure, usually obtained from Protein Data Bank (PDB).⁷⁹ If such a structure is not available and it represents a canonical A- or B-form nucleic acid confor-*Chirality* DOI 10.1002/chir

mation, the starting geometry can be generated by molecular modeling packages, e.g., Insight II (formerly Biosym; now part of Discovery Studio),⁸⁰ Tinker,⁸¹ AMBER⁸² etc. The octamer structure is split into fragments (colored blocks in Fig. 1), large enough to preserve the important for VCD short-range interactions, but still manageable for direct *ab initio* computations at sufficiently high level of theory. The VCD signal in nucleic acids mainly arises from the dipolar interaction between two stacked base pairs and analogous interactions of the phosphate and sugar residues of the backbone.^{18,19} Therefore, the basic "optical" unit in a nucleic acid, determining the basic VCD pattern, is typically composed of two stacked base pairs and two sugar-phosphate pairs. Considering this, the fragments would contain one or more of such basic units.

Further, the fragments are optimized at *ab initio* level using available quantum chemistry packages, e.g., Gaussian⁶⁹ or Turbomole.⁸³ The optimization is required to relax the initial geometry and bring it close to an energy minimum. Note that frequency calculations within the harmonic approximation are meaningful only for such minimum (equilibrium) geometries.⁶⁹ However, to preserve the initial conformation found in the X-ray, NMR, or canonical structure, larger geometrical changes of the fragments during the optimization must be prevented, e.g., by constraining torsion angles, or by using the constrained normal mode optimization (NMO) method,84 described in more detail later. In most cases freezing of the torsion angles affects low frequency modes outside the experimentally accessible range of interest. Bond lengths and angles most significant for the higher-frequency mid-IR modes are allowed to relax. Typically, DFT electronic methods are used for the quantum chemical computations, combined with a sufficiently large basis set including polarization and, if possible, diffuse functions.



Fig. 2. Canonical B- (a) and Z- (b) forms of $(dG-dC)_4$ octamer used as target templates for atomic tensor transfer; *ab initio* optimized geometries of dimer fragments (GC)*(CG) (c), (CG)*(GC) (d), sugar-phosphate dimer (e), and hydrated monomer fragments G...C with 5 water molecules (f) and sugar-phosphate with 6 water molecules (g).

For the optimized fragments harmonic force field is calculated at the same level of theory as that used for the optimization. For the intensity tensors, computations at different levels are possible, although this usually does not bring a particular advantage. All tensors in Cartesian coordinates from each fragment are then transferred to the original octamer (target structure), atom by atom according to the published procedure⁷⁵ (Fig. 1), using standard translation and rotation transformations. For VCD, the following tensors have to be calculated to obtain the frequencies and intensities:⁶⁸

(i). force constants (Hessian, second derivatives of energy):

$$f_{i\alpha,j\beta} = \frac{\partial^2 E}{\partial r_{i\alpha} \partial r_{j\beta}} \tag{1}$$

(ii). atomic polar tensor (dipole derivatives):

$$P_{\alpha,\,i\beta} = \frac{\partial \mu_{\alpha}}{\partial r_{i\beta}} \tag{2}$$

(iii). atomic axial tensor (magnetic dipole derivatives):

$$A_{\alpha,\,i\beta} = \frac{\partial m_{\alpha}}{\partial p_{i\beta}} \tag{3}$$

The force constants f are related to two atoms (i, j), while the tensors A and P can be thought of as properties of individual atoms i with positions r_i and momenta p_i . The transfer (rotation and translation) is defined by minimizing a root-mean square overlap of chemically similar atoms in the fragments and in the target molecule.⁷⁵ We use the Fortran-based code "cctn" for the tensor manipulations.⁸⁵ Original transfers have been done for periodic repetitive nucleic acid structures. The current version of the program allows for structural irregularities, such as deformations upon drug binding.⁸⁶

Based on the transferred molecular properties, the IR absorption and VCD spectra are computed for the whole target structure. Usually the spectra are simulated by the convolution of calculated intensities with Lorentzian bands ranging from 5 to 10 cm⁻¹ in width. To mimic the experimental conditions (most of the experimental VCD spectra of nucleic acids are measured in D_2O), spectra of deuterated species are simulated, which is computationally a trivial task.

In the following sections we will show how this methodology can be applied to various nucleic acid structures, what results it can provide, underline its strengths and limitations and follow its development over the recent years. Specific computational details for each case will be also provided.

ILLUSTRATIVE EXAMPLES DNA B-Z Transition

The natural conformation of DNA is a right-handed Bform double helix (Fig. 2a).⁸⁷ However, addition of high amounts of metal ions or ethanol to oligomers or polymers consisting of alternating (dG-dC)_n sequences [e.g., (dGdC)₂₀ or poly(dG-dC)] can facilitate a B to Z conformational transition, upon which the handedness of the DNA changes from a right-handed to a left-handed double helix (Fig. 2b).^{88–91} The left-handed sequences have been found in natural DNA and are believed to play a major role in many fundamental life processes, including gene regulation and DNA replication.^{92,93}

One of the first spectroscopic techniques used for investigations of DNA B-Z transition was ECD. Due to opposite handedness of the DNA double helix in B and Z forms, it could be deduced that ECD signal should have an opposite sign for the two forms, which was confirmed experimentally.^{88–90} Similarly, the VCD signal was also shown to change the sign upon the conformational transition.^{27,28,30,34} Thus, the B-form structure produces a major positive VCD couplet (i.e., with a positive component at the lower wavenumber) arising from the C=O stretching vibrations at around 1680 cm⁻¹, while the Z-form structure produces a negative couplet originating from the same vibrations.³⁰ Due to relative simplicity of the systems with alternating d(GpC) sequences (where "p" denotes a phos-*Chirality* DOI 10.1002/chir

E99

CG	
CG	GG
CG) GC
CGCGCGCGCGCGSPSPSPSPSPSPSPSPSPSPSPSPGCGCSPSPCGSPSPGC	P SP SP
CGCGCGCGCGCGSPSPSPSPSPSPSPSPSPSPSPSP	SP SP
CG CG CG CG	P SP SP
00000000000000000000000000000000000000	GC GG

Fig. 3. Schematic representation of a two-step tensor transfer: (a) initial atomic tensors of the target octamer have zero values; (b) step 1: *ab initio* computed atomic tensors for the $(GC)^*(CG)$, $(CG)^*(GC)$ and sugar-phosphate dimers are transferred to the target octamer and replace the zero values in the octamer tensors (orange/yellow areas); (c) step 2: *ab initio* computed atomic tensors for the smaller fragments G...C with 5 water molecules and sugar-phosphate monomer with 6 water molecules (pink areas) are transferred to the target octamer and overwrite the corresponding tensor values transferred in the previous step, thus improving them by accounting for interactions with water molecules. The inter-base pair, sugar-sugar and phosphate interactions (orange/yellow areas) are however preserved from the previous step. Some of the less important interactions (between the bases and the sugar-phosphate backbone) in the target octamer tensors are missing (white areas) and thus neglected.

phodiester linkage between the bases in one strand), the experimental flip of the VCD signal was predicted theoretically fairly satisfactory with the CO model.³⁰ Therefore, in our first attempt of applying the CCT method to nucleic acids we opted for computing VCD spectra of B- and Z-forms of $(dG-dC)_4$ octamer duplex,²⁰ which could be directly compared with the experimentally measured earlier VCD spectra of $(dG-dC)_{20}$.³⁴ We have chosen the octamer structure as it was more convenient for the fragment selection and the CCT procedure than longer sequences and because the experimental VCD spectra of $(dG-dC)_{4}$, $(dG-dC)_{20}$, and poly(dG-dC) were essentially the same.^{30,33,34} The main aim of this work was to verify if the CCT technique could reproduce the experimentally observed sign change of the VCD couplets for the two DNA helices with opposite handedness.

The canonical B- and Z-form geometries of the doublestranded octamer (dG-dC)₄ were generated with the aid of Insight II software,⁸⁰ as shown in Figures 2a and 2b. Because the octamers represent regular d(GpC) sequences with identical geometrical parameters, it was enough to compute the atomic tensors only for a few unique fragments and transfer them to all the other identical fragments in the target octamers. Thus the unique fragments chosen for B-form conformation consisted of stacked $(GC)^{*}(CG)$ and $(CG)^{*}(GC)$ base pairs (Figs. 2c and 2d) and a sugar-phosphate dimer (Fig. 2e). It was necessary to select the two sequences of base pairs because they had different base overlap and, respectively, different base-base interaction in the stack, resulting in different VCD spectra.¹⁸ The same unique fragments were selected for Z-form; however two different configurations of the sugar-phosphate dimer, $Z_{\rm I}$ and $Z_{\rm II}$, had to be used.⁵⁶ In an attempt to account for the influence of solvent, two smaller fragments were created, a single G...C base pair and a sugar-phosphate monomer, both with explicit water molecules, as shown in Figures 2f and 2g. The positions of the water molecules were preoptimized so that oxygen, nitrogen, and acidic hydrogen atoms in the DNA fragments exposed to the solvent could form hydrogen bonds Chirality DOI 10.1002/chir

and were in agreement with other ab initio calculations.94 The 124 water molecules in approximately the same positions with respect to the bases and the sugar-phosphate as in the optimized monomers were also placed around the target octamers with the aid of MCM software.⁹⁵ All the fragments were partially optimized in vacuum with torsion angles constrained to those in the octamer. In the stacked base pair fragments the distances and the mutual orientation of the base pairs were also constrained to maintain their realistic values in the absence of the sugarphosphate linkage. Fragment optimization and calculation of the harmonic force fields, dipole derivatives and atomic axial tensors were done with Gaussian 98 program package 96 at BPW91 97,98 /6–31G** DTF level. The BPW91 GGA functional was chosen over the more common hybrid DFT functional Becke3LYP99 due to better agreement of the computed in vacuum frequencies in the C=Ostretching region with the experiment.⁷⁶ Using this functional does not require any frequency scaling of the computed spectra and they can be directly compared with the experiment.

The transfer of the atomic tensors was performed in two steps. First, the tensors computed for the stacked base pair dimers and the sugar-phosphate dimer were transferred to the target octamers. As a result, the initial zero octamer tensors (Fig. 3a) were replaced with the tensors from the dimers (Fig. 3b, orange/yellow areas). In the second step, the tensors computed for the monomers with explicit water molecules, were transferred to the octamer, overwriting the already transferred from the dimers interactions within a base pair and within a sugar-phosphate monomer, and thus improving them by accounting for the influence of the water molecules on the Watson-Crick hydrogen bonding and the C=O, NH_2 and PO_2 group vibrations (Fig. 3c, pink areas). However, the inter-base pair, sugar-sugar, and phosphate-phosphate interactions transferred from the dimers were preserved (Fig. 3c, orange/yellow areas).

The resulting computed VCD spectra of B- and Z-forms of $(dG-dC)_4$ are compared with the experimental VCD



Fig. 4. Calculated VCD spectra of B- and Z-forms of $(dG-dC)_4$ octamer (bottom) compared with the corresponding experimental spectra of $(dG-dC)_{20}^{34}$ (top). Adapted with permission from Andrushchenko V, Wieser H, Bour P. B-Z conformational transition of DNA monitored by vibrational circular dichroism. *Ab initio* interpretation of the experiment. J Phys Chem B 2002;106:12623–12634. © 2002 American Chemical Society.

spectra of $(dG-dC)_{20}^{34}$ in Figure 4. The main VCD feature of the B-form experimental spectrum is a strong positive couplet at 1691(-)/1678(+) cm⁻¹, arising from the C=O stretching vibrations of guanine bases.³⁴ Upon DNA transition to Z-form this couplet changes its sign to negative with peaks at 1671(+)/1656(-) cm⁻¹. The sign change is accompanied by ~20 cm⁻¹ low-wavenumber shift of the whole couplet, which follows the corresponding shift of the main nitrogen base absorption bands (not shown).

Similarly to the experiment, the calculated spectrum of the B-form octamer is also dominated by a strong positive VCD couplet at 1710(-)/1695(+) cm⁻¹, assigned to the same vibrations based on the analysis of the computed displacements.²⁰ In the calculated Z-form spectrum the main couplet flips the sign to negative at 1707(+)/1693(-) cm⁻¹. Therefore, the main experimental VCD manifestation of the B-Z transition is correctly reproduced by the computations. The right-handed DNA helix is thus intrinsically characterized by a positive carbonyl VCD couplet, while the left-handed helix is characterized by a negative couplet.

Despite this success, some finer spectral features were modeled not correctly or at a full extent. In particular, the large 20 cm⁻¹ low-wavenumber shift of the main VCD couplet and the corresponding absorption band (not shown) observed experimentally was calculated to be only 3 cm⁻¹. Experimental VCD features in the sugar-phosphate vibra-

tional region between 1100 and 900 cm⁻¹, although noisier than the carbonyl features but still well distinguishable, are also not reproduced well computationally. These discrepancies were connected to several points. First, precise modeling of the sugar-phosphate spectroscopic response is intrinsically more challenging than modeling of the stacked nitrogen bases due to larger flexibility of the backbone and its strong influence by the environment (solvent, ions). Second, the explicit hydration scheme was very approximate. Considering significant solvent effect on the DNA spectral response, more refined schemes must be employed. Third, the torsion angle constrains prevented full ab initio optimization of the fragments. Such constrains could have prevented a sufficient relaxation of some sugarphosphate vibrational motions occurring in the relatively low frequency range (below 1300 cm⁻¹). Anharmonic interactions of the sugar-phosphate modes that are not completely relaxed might have affected the harmonic frequency calculations and the resulting spectra. Finally, the employed medium-range basis set might be too small for exact reproduction of the experimental spectra.

Despite the failure of the computations to adequately reproduce the minor experimental spectral features, the most prominent spectral modification accompanying the B-Z transition of DNA was modeled correctly. Thus, this work expanded applicability of the CCT method from peptides onto nucleic acids, and brought up some specific tasks for future improvements of the technique, which were implemented in the follow-up studies.

Single-Stranded Poly(rA) Helix: Introducing the NMO Method

Depending on experimental conditions (temperature, polymer concentration, ion content), poly(rA) and poly(rU) polymers can form a wide range of single, double, and triple helices as well as $coils.^{22,36,100-102}$ The next three sections will be devoted to these RNA forms and their computed VCD spectra. They will be discussed in the order of increasing complexity both from the structural and from the computational points of view.

Due to strong adenine base stacking, single-stranded poly(rA) is capable of forming a relatively stable helical structure in solution in the presence of metal ions, neutralizing the phosphate negative charges.¹⁰³ The presence of a helical structure in poly(rA) was undoubtedly confirmed experimentally by a strong conservative VCD signal.^{22,36,49} Owing to simplicity and relatively small size of a single-stranded chain as well as its ability to form a stable helix, poly(rA) was an ideal model to further test the CCT method for computations of VCD spectra of nucleic acids.¹⁰⁴

Similarly to the B-Z transition spectra modeling, an octamer was used as a target molecule for the CCT technique, in this case (rA)₈. The canonical single-stranded geometry of (rA)₈ was generated with the aid of Insight II software⁸⁰ (Fig. 5a). Because the target octamer represents a regular sequence, the fragments selected for the *ab initio* atomic tensor calculations were: two stacked adenine bases (Fig. 5b) and a sugar-phosphate dimer (Fig. 5c). The fragments were optimized and the tensors calculated with Gaussian 98 program package⁹⁶ at BPW91/6–31G** level of DFT.

E101



Fig. 5. Canonical geometry of the target $(rA)_8$ octamer (a); *ab initio* optimized geometries of the fragments: two stacked adenine bases (b) and a sugar-phosphate dimer (c); calculated IR absorption and VCD spectra of $(rA)_8$ compared to the corresponding experimental spectra of poly(rA) (Tsankov, 2001, unpublished results) (d). Adapted with permission from Andrushchenko V, Wieser H, Bouř P. RNA structural forms studied by vibrational cirular dichroism: *ab initio* interpretation of the spectra. J Phys Chem B 2004;108:3899–3911. © 2004 American Chemical Society.

To preserve the realistic canonical secondary structure geometry of the fragments during the ab initio optimization, a constrained NMO method has been utilized, allowing optimization of molecular geometry in vibrational normal coordinates.⁸⁴ This procedure replaced the constraining of the torsion angles, as such constrains often may not allow for a complete relaxation of the higher-energy smallamplitude movements. In structures partially optimized with internal coordinates some higher-frequency modes may be far from local minima and thus introduce large errors in the modeled spectra. With the NMO method, only the normal modes with lowest frequencies [typically $\omega \in (-300, 300 \text{ cm}^{-1})$ where imaginary frequencies are considered as negative] are frozen. These modes are mostly connected with rotational and translational movements of large groups of atoms and usually do not contribute to measurable spectral range. At the same time, the higher frequency vibrational motions observable in experimental spectra are relaxed completely, unlike for the torsion constraining. In such implementation the NMO procedure typically causes only a minimal change of the fragment geometry. An additional advantage of the NMO method is a much faster and more stable convergence for optimization of noncovalently bound systems, such as, e.g., nitrogen bases in DNA.^{84,105} The range of the fixed normal modes can be chosen arbitrarily or, alternatively, a complete relaxation of the geometry can be performed to exploit the increased speed and stability of the technique for optimization of weakly interacting molecules. Program QGRAD¹⁰⁶ is used for the NMO technique, while the *ab initio* part of the computations can be done with Gaussian,⁶⁹ Dalton,¹⁰⁷ Turbomole⁸³ or similar software. Chirality DOI 10.1002/chir

Because all the atomic tensors of the fragments were calculated at the same level of theory, the transfer of the tensors to the target octamer was performed in one step.

The computed IR absorption and VCD spectra of (rA)₈ are compared with the experimental spectra of poly(rA) (Tsankov 2001, unpublished data) in Figure 5d. The main VCD feature in the experimental spectrum arising from the adenine ring vibrations^{22,36,108,109} is a positive couplet at 1633(-)/1623(+) cm⁻¹, corresponding to the absorption at 1627 cm^{-1} . The calculated positive VCD couplet appears at 1620(-)/1612(+) cm⁻¹ with the corresponding absorption band at 1617 cm^{-1} and is also assigned to the adenine ring stretching vibrations.¹⁰⁴ A weak negative VCD feature measured at 1572(-) cm^{-1} . also originating from the adenine ring vibrations with absorption band at 1572 cm⁻¹, appears in the calculations at a very close wavenumber of 1574(-) cm⁻¹. Almost perfect agreement between the computed and the experimental spectra in the nitrogen base vibrational region in terms of sign, relative intensity and the peak positions was explained by hydrophobicity of the adenine base and, as a result, insignificant effect of the solvent (which was not accounted for in this simulation) on the adenine ring vibrations. Additionally, strong stacking interaction between adenine bases reduces their flexibility. Thus, it can be expected that the real solution structure of a single-stranded poly(rA) helix has geometrical arrangement of the bases very close to the canonical structure reported by X-ray crystallography and used by Insight II software. However, agreement between the experimental and computed spectra is considerably worse in the sugar-phosphate vibrational region. Flexibility of the sin-



Fig. 6. Canonical geometry of the target $(rA)_8^*(rU)_8$ octamer (a); *ab initio* optimized geometries of the fragments: stacked base pair dimer (AA)*(UU) (b) and a sugar-phosphate dimer (c); optimized at PM3 level (ApAp)*(UpUp) dimer (d); calculated IR absorption and VCD spectra of $(rA)_8^*(rU)_8$ compared to the corresponding experimental spectra of poly $(rA)^*$ poly $(rU)^{36}$ (e). Adapted with permission from Andrushchenko V, Wieser H, Bouř P. RNA structural forms studied by vibrational cirular dichroism: *ab initio* interpretation of the spectra. J Phys Chem B 2004;108:3899–3911. © 2004 American Chemical Society.

gle-stranded backbone and its large dynamical fluctuations may result in substantial deviations of the solution structure from the canonical geometry used for the calculations. The inaccuracies arising from such geometrical differences may overshadow the improvements provided by the NMO method in this spectral region. Negligence of the solvent and ion effects can further hamper the modeling of the backbone spectra, which are very sensitive to such interactions.

Double-Stranded Poly(rA)*Poly(rU) Helix

When mixed together in a solution at standard conditions (room temperature, pH7, 0.1 M NaCl), poly(rA) and poly(rU) molecules form a double-stranded helical structure with the bases paired via Watson-Crick hydrogen bonding (Fig. 6a).¹⁰³ The secondary structure of the double-helix is of A-form, as usual for RNA. After the successful modeling of the VCD spectra for a single-stranded (rA)₈ helix, we moved to this computationally more challenging case. Because of the double-stranded structure, the smallest fragments suitable for the calculations of the atomic tensors are much larger and must include two stacked base pairs. The uracil base contains two C=O groups, one of which is involved in Watson-Crick hydrogen bonding and less interacting with a solvent, while the other one is situated in the RNA groove and is fully accessible to a solvent hydrogen bonding, which again makes the system more challenging for modeling.

The canonical double-stranded geometry of $(rA)_8^*(rU)_8$ was generated with the aid of Insight II software⁸⁰ (Fig. 6a). Two fragments were selected for the *ab initio* atomic tensor calculations: a stacked base pairs dimer $(AA)^*(UU)$

(Fig. 6b) and a sugar-phosphate dimer (Fig. 6c). The optimization and the atomic tensor calculation for the fragments were done with Gaussian 98 software⁹⁶ at the BPW91/6–31G^{**} level of DFT. To preserve the fragment geometry corresponding to the canonical structure, the NMO method⁸⁴ was used.

To account for the longer-range coupling, at least in the first approximation, larger fragment was selected, representing a complete dimer (ApAp)*(UpUp) with two stacked A...U base pairs and two sugar-phosphate units (Fig. 6d). Geometry optimization and the atomic tensor calculations for this fragment were performed at semiem-pirical PM3 level, as application of DTF methods to such large fragments was not feasible at that time because of computer hardware restrictions.

The atomic tensors were transferred to the target octamer in two steps. First, the tensors for the larger (ApAp)*(UpUp) dimer calculated at lower level of theory were transferred. Then, the tensors for the smaller fragments calculated at better DTF level were transferred. They overwrote the previously transferred short-range interactions, most important for VCD, while preserving the longerrange interactions and thus improving the resulting target octamer tensors.

The computed IR absorption and VCD spectra of $(rA)_8^*(rU)_8$ are compared to the corresponding experimental spectra of poly $(rA)^*$ poly $(rU)^{36}$ in Figure 6e. As in the case of a single-stranded $(rA)_8$ helix, the best agreement between the computed and experimental spectra is seen for the adenine ring absorption band at 1631 cm⁻¹ with the corresponding positive VCD couplet at 1635(-)/ 1623(+) cm⁻¹, which appear in the calculated spectra at *Chirality* DOI 10.1002/chir ANDRUSHCHENKO AND BOUŘ



Fig. 7. X-ray based geometry of the target $(rU)_8^*(rA)_8^*(rU)_8$ octamer (**a**); *ab initio* optimized geometries of the fragments: base trio U...A...U (**b**), a sugar-phosphate dimer of one of the strands (**c**) and stacked dimer of base trios $(U...A...U)_2$ (**d**); calculated IR absorption and VCD spectra of $(rU)_8^*(rA)_8^*(rU)_8$ compared to the corresponding experimental spectra of poly $(rU)^*$ poly $(rA)^*$ poly(rU) triplex + poly(rA) single helix³⁶ (**e**). Adapted with permission from Andrushchenko V, Wieser H, Bouř P. RNA structural forms studied by vibrational cirular dichroism: *ab initio* interpretation of the spectra. J Phys Chem B 2004;108:3899–3911. © 2004 American Chemical Society.

1633 cm⁻¹ and 1636(-)/1628(+) cm⁻¹, respectively, and are also assigned mostly to the adenine ring vibrations with some admixture of the uracil ring modes.¹⁰⁴ The peak position and sign for these features were reproduced very well, while the relative VCD intensity was somehow overestimated by the computations. Such a good agreement was attributed to the same reasons as for the $(rA)_8$ single-stranded helix, i.e., mainly to the hydrophobicity of the adenine base and minor influence of the solvent on its vibrational modes. Overestimation of the VCD intensity might stem from the admixture of the uracil ring vibrations computed for this mode. Another positive VCD couplet, computed at 1697(-)/1690(+) cm⁻¹ and arising from the absorption band at 1696 cm^{-1} is attributed to the C=O4 vibrations of uracil. It clearly corresponds to the experimental positive VCD couplet at 1677(-)/1665(+) cm⁻¹ with its absorption at 1669 $\rm cm^{-1}$.

While the sign and relative intensity of these features were simulated very closely to the experiment, the peak position was shifted to the higher wavenumbers by about 25 $\rm cm^{-1}$. This relatively large shift was ascribed to the persisting difficulties in modeling of stretching frequencies for highly polar groups, such as C=O, due to substantial influence of solvent, even if the carbonyl is involved in the hydrogen bonding with another base. This is illustrated even better on the C=02 carbonyl, which is fully accessible to the solvent hydrogen bonding. The IR C=O2 vibration band occurs experimentally at 1689 cm⁻¹ with a weak negative VCD band at 1705 cm^{-1} , but it is computed as high as 1740 cm^{-1} with the corresponding VCD band of opposite sign. Indeed, test computations on a single A...U base pair with even simplest accounting for the explicit solvent resulted in downshift of the C=O2 band as much as 25 cm^{-1} .¹⁰⁴ Chirality DOI 10.1002/chir

Surprisingly, the computed absorption and even VCD features in the sugar-phosphate vibrational region reproduced the experimental spectra fairly well, in contrast to the calculations performed for B- and Z-form octamers and single-stranded poly(rA) in this spectral region. Such an improvement was partially connected to the implementation of the NMO method, relaxing the vibrations in this spectral range much better than the torsion angle constraining approach. Compared to a single-stranded poly(rA), the backbone in the double-stranded poly(rA) *poly(rU) is apparently more rigid and its real solution structure could be closer to the canonical geometry, explaining better agreement with the experiment.

Triple-Stranded Poly(rU)*Poly(rA)*Poly(rU) Helix

At the conditions of increased salt concentrations or at the simultaneous action of salt and temperature the double-stranded poly(rA)*poly(rU) helix can disproportionate into a triple-stranded poly(rU)*poly(rA)*poly(rU) helix (Fig. 7a) and a single strand of poly(rA).^{22,36,100,101,103,110} While triple-stranded structures of nucleic acids are somehow intriguing and might be thought of as untypical, they have been shown to play an important biological role as regulators of eukaryotic gene expression.¹¹¹ A site-specific nucleic acid recognition by specially designed oligonucleotides, which can bind to a host DNA molecule forming triple-helical structures (antigene strategy), can be used as a new class of pharmacologically active compounds.^{110,112,113}

There are several possibilities for arranging the individual strands in the triple-helical structure.^{103,110} It is generally accepted that the second poly(rU) strand fits into the major groove of the poly(rA)*poly(rU) double helix with a formation of the Hoogsteen-type base pairs with the poly (rA) strand.^{103,109,110} However, an alternative model, formation of a reverse Hoogsteen-type base pairing, better fits many IR and VCD profiles and their interpretation by the coupled-oscillator model.^{29,36,101}

The triple-helical RNA was the most computationally challenging due to the large size and uncertainty about its solution geometry. Because this structure was not a canonical one, it could not be easily constructed by available molecular modeling software. Therefore we used an experimental X-ray geometry with the Hoogsteen base pairing.¹¹⁴ Unfortunately, no credible model has been found for the reversed Hoogsteen pairing, hence the spectral simulation for this structure could not be tested.

An octamer $(rU)_8^*(rA)_8^*(rU)_8$ (Fig. 7a) was constructed based on the X-ray geometry of a single base trio $U...A...U.^{114}$ The octamer was generated by propagation of the base trio eight times, translating it by 3.05 Å and rotating by 32.7° in each propagation step, according to the X-ray data.¹¹⁴ Five fragments have been chosen for the *ab initio* atomic tensor calculations: a smaller single base trio U...A...U (Fig. 7b), three sugar-phosphate dimers (one for each strand, due to nonequivalent geometries of the sugar-phosphate backbone) (Fig. 7c) and a larger stacked dimer of base trios (U...A...U)₂ (Fig. 7d).

The optimization and the atomic tensor calculation for the fragments were done with Gaussian 98 software.⁹⁶ The BPW91/6–31G** level of DFT was used for the smaller single base trio and sugar-phosphate dimers, while only HF/3–21G level of theory could be used for the larger dimer of base trios. To preserve the fragment geometry corresponding to the X-ray structure, the NMO method⁸⁴ was used. Because of the size restrictions, no larger fragments were feasible to compute even at semiempirical PM3 level, and thus all longer-range interactions had to be neglected.

The atomic tensors were transferred to the target octamer in two steps. First, the tensors for the stacked dimer of base trios $(U...A...U)_2$ calculated at a lower level of theory were transferred. Then, the tensors for the smaller single base trio U...A...U and the sugar-phosphate dimers calculated at better DTF level were transferred. The single base trio tensors overwrote the previously transferred base-base interactions within the trio, while preserving the inter-base trio interactions computed at lower HF level.

The computed IR absorption and VCD spectra of $(rU)_8*(rA)_8*(rU)_8$ are compared to the corresponding experimental spectra of poly(rU)*poly(rA)*poly(rU) triplex + poly(rA) single helix³⁶ in Figure 7e. Although a generally good agreement is observed between the experimental and computed spectra in both nitrogen base and sugarphosphate vibrational regions, it is worse than for the $(rA)_8*(rU)_8$ duplex. The adenine ring band with some uracil contributions at 1627 cm⁻¹ is calculated at 1646 cm⁻¹, thus relatively significantly shifted compared to the single-and double-stranded molecules in Figures 5 and 6. The weak VCD couplet, though also shifted to the higher wave-numbers, is computed with correct sign and relative intensity. The calculated uracil C=O absorption bands are even

more high-frequency shifted, appearing at 1702, 1732, and 1750 cm^{-1} , but can be clearly assigned to the experimental bands at 1658, 1673, and 1696 cm^{-1} . The former band was assigned to the stretching vibrations of the C=O4 carbonyls of both uracil bases, the middle band - to the C=O2 stretch of Watson-Crick-paired uracil coupled with the C=O4 stretch of the Hoogsteen-paired uracil, and the latter band - to the C=O2 stretch of the Hoogsteen-paired uracil.¹⁰⁴ The computed VCD couplets corresponding to all of these carbonyl vibrations are shifted to higher wavenumbers in concert with their parental absorptions, but correctly reproduce the VCD sign (all are positive couplets). The relative intensity of the first two lower-frequency couplets is reproduced faithfully, while the highest frequency couplet has significantly underestimated intensity in the calculations. The absorption profile of the sugar-phosphate backbone is generally reproduced by the computations, but the VCD intensity is considerably underestimated by the simulations.

Somehow worse agreement between the computed and experimental spectra for the triplex structure compared to the duplex was attributed to two major reasons. Firstly, accuracy of the HF/3-21G computations, used of the stacked base trios, is lower than that of the BPW91/6-31G** DFT level used for the calculations of the corresponding duplex fragments. Secondly, only the Hoogsteen-paired model could be tested, although a reversed Hoogsteen model could produce different, possibly more realistic results. Some clues in this direction have been already provided by simpler CO model calculations.²⁹ Test computations of the both types of Hoogsteen base pairing were performed on a single base trio, but strong coupling of the C=O4 and C=O2 vibrations and limited accuracy of the computations prevented reliable discrimination between these models at the utilized level of theory.¹⁰⁴

Accounting for Explicit Solvent and Dynamical Averaging

All the previously described spectra simulations have been performed based either on the canonical nucleic acid geometry (ultimately derived from average crystallographic structures) or directly on a single X-ray geometry (like the triplex structure). However, experimental VCD spectra are usually measured in a solution. The nucleic acid structure in a solution might deviate from a corresponding crystallographic geometry due to dynamical fluctuations, solvent influence, absence of packing forces sometimes influencing the X-ray geometry, and so on. Considering an extremely high VCD sensitivity to even minor structural fluctuations, such deviations might result in different spectra from those predicted for a crystal structure. Consequently, our next attempt in improving the methodology for computing VCD spectra was to account for such dynamical fluctuations. This can be conveniently done by employing molecular dynamics (MD) apparatus to produce more realistic solution structure of a target nucleic acid molecule, used for the transfer of the atomic tensors. MD simulations can also account for the solvent influence on the DNA or RNA structure, and allow to obtain more or less realistic (compared to the ad hoc geometry used for the B-Z transition calculations described above) positions Chirality DOI 10.1002/chir

of water molecules around the nucleic acid in the first hydration shell. These clusters of water molecules can then be included in the *ab initio* computations.

To test this idea, spectra simulations for a series of seven octanucleotides with different base content and base sequence have been performed.¹¹⁵ The most interesting outcome is described in the next two sections. The computed spectra will be compared to the experimental results for the same set of octanucleotides measured earlier.³³ To highlight the effects of dynamical fluctuations and solvent influence, we restrain here to only two octamers with the largest difference in the base content, (CGCGCG CG)*(GCGCGCGC) octamer, abbreviated as CG, and (CGAATTCG)*(GCTTAAGC), abbreviated as CGAT.

Two structures of the octamers have been generated, one representing an ideal canonical geometry (denoted hereafter as the "regular structure") and the other one obtained as an average structure of 5 ns of MD simulations (denoted as the "MD structure").

The regular structure was generated by the Tinker program package⁸¹ with geometrical parameters corresponding to the standard B-DNA double helix. The AMBER force field¹¹⁶ was used as implemented in Tinker. The generated octamer was solvated by a water shell 8 Å thick. The water shell was subjected to a minimization and dynamics with the DNA coordinates fixed to obtain an estimate of the distribution of the water molecules around the octamer helix. The details of the procedure can be found elsewhere.¹¹⁵ For the fragment selection and following *ab initio* computations only water molecules closer than 3.6 Å (the first hydration shell) and hydrogen-bound to DNA were retained.

The MD starting structure was generated within AMBER 4.1 package,¹¹⁷ using Arnott's geometry parameters¹¹⁸ and the AMBER force field.¹¹⁶ The phosphate charges were neutralized by Na⁺ cations and the whole structure was contained in a water box extending by 10 Å from the solute molecules. Standard MD procedure was applied for the system minimization, equilibration and 5 ns production run, details of which can be found elsewhere.¹¹⁵ An average octamer structure was obtained after the MD simulations. The averaging, however, led to a few artifacts in the geometry, which were removed by relaxing the molecule by an additional short minimization. However, before such a minimization, the averaged structure had to be hydrated again and the whole procedure for the hydration shell equilibration had to be repeated.¹¹⁵ As for the regular structures, only hydrogen-bound water molecules of the first hydration shell within a distance of 3.6 Å from the solute were retained for the subsequent *ab initio* computations. The resulting structure for the CG octamer with the first hydration shell is shown in Figure 8a.

Unlike for the octamers with repeating base sequences and regular geometry described until now, for the simulations of irregular DNA geometries, such as MD structures, fragments could not be simply propagated. In this case for each base pair or sugar-phosphate combination a unique fragment must be chosen and subjected to *ab initio* computations. Considering included water molecules from the first hydration shell, such a fragment selection and *Chirality* DOI 10.1002/chir their subsequent matching back to the target octamer after the *ab initio* calculations can be quite troublesome and time consuming. To simplify this task, a possibility for semi-automatic and automatic fragment selection has been added to the MCM software^{95,119} In the latter case the software automatically splits the large molecule into fragments of specified size in such a way that bonds within functional groups and smaller molecules (e.g., solvent molecules) are not broken.

Octamers with both regular and the MD geometries including their first hydration shell were divided into 24 fragments of approximately equal size: 8 fragments for the hydrated base pairs (Fig. 8b) and 16 fragments for the hydrated sugar-phosphate dimers (Fig. 8c). Additionally, seven larger hydrated dinucleotide fragments (Fig. 8d) were chosen to account for longer-range interactions at a lower level of theory.

The fragments were optimized and their atomic tensors were calculated with Gaussian 03 software.⁶⁹ For the smaller fragments (hydrated base pairs and sugar-phosphate dimers) the BPW91/6–31** DFT level was used, while for the larger hydrated dinucleotide fragments only a semiempirical PM3 method could be applied. The NMO method⁸⁴ was used to preserve the original conformational geometry of the fragments.

The transfer of the atomic tensors was performed in two steps. First, the tensors for the dinucleotides, computed at the lower level of theory, were transferred to the target octamers. Then the tensors for the base pairs and the sugar-phosphate dimers calculated at the higher DFT level were transferred, improving the short-range interactions of the target octamers.

The effect of the first hydration shell on the computed IR absorption spectra is shown in Figure 9, where the calculated results for the hydrated and vacuum regular DNA models are compared with the experimental spectra of the CGAT octamer. Inclusion of the realistically distributed explicit solvent molecules, obtained from the MD simulations, significantly improves the overall absorption bandshape of the sugar-phosphate vibrations within 1200-800 cm^{-1} . For the first time the experimental symmetric PO_2^{-1} band at 1085 cm^{-1} and the structurally sensitive sugar mode at 1056 cm^{-1} were clearly reproduced by the calculations at close wavenumbers of 1073 cm⁻¹ and 1051 cm^{-1} , respectively, albeit the relative intensities for these bands were predicted incorrectly. Most of other bands in this region were also computed at close to the experiment wavenumbers. However, there was no real improvement over the vacuum spectrum in the C=O vibrational range between 1700 and 1600 cm^{-1} , except for the slight shift of the most of bands to the lower frequency, closer to the experimental values. Thus, the correct modeling of the carbonyl vibrations still remained problematic. These vibrations are anharmonic, highly susceptible to formation of strong hydrogen bonds with the solvent and other parts of the molecule, and the polar C=O groups strongly polarize the environment.¹²⁰⁻¹²² All these factors apparently complicate the accurate reproduction of the experimental results. Indeed, it was shown that accounting for the anharmonic nature of the carbonyl vibration can downshift it by as



Fig. 8. Examples of the MD averaged octamer and fragment structures used for the atomic tensor transfer: (CGCGCGCG)*(GCGCGCGC) target octamer with the first hydration shell (a); *ab initio* optimized hydrated C...G base pair (b) and hydrated sugar-phosphate dimer (c); PM3 optimized hydrated stacked dimer (CG)*(GC) (d). Adapted with permission from Bouř P, Andrushchenko V, Kabelač M, Maharaj V, Wieser H. Simulations of structure and vibrational spectra of deoxyoctanucleotides. J Phys Chem B 2005;109:20579–20587. © 2005 American Chemical Society.

much as 15–20 cm^{-1} .^{122,123} In contrast to the sugar-phosphate and nitrogen base C=O spectral features, the base ring modes occurring between 1600 and 1500 cm⁻¹ were negatively influenced by the explicit hydration model. The wavenumber and the band shape of the guanine ring absorption computed in vacuum at 1577 cm⁻¹ almost perfectly corresponds to the experimental one at 1575 cm^{-1} . Close agreement is also seen for other guanine and cytosine ring vibrations in this region. These results imply that using explicit solvent models in ab initio calculations of the nitrogen base ring modes might overestimate the effect of water molecules on the ring vibrations. Similar results were obtained earlier for other explicit base pair models, e.g., in the B-Z transition modeling.²⁰ Indeed, the base rings are fairly hydrophobic and on average many of them might not be tightly bound to water molecules, thus better approaching the vacuum results. Such an assumption is confirmed by the perfect agreement of the vacuum computations with the experiment for the poly(rA) with highly hydrophobic adenine bases, described in the above section.¹⁰⁴ Perhaps, more extensive MD averaging of the water distribution around the bases might improve the understanding of this phenomenon.

The effect of the dynamical averaging on the calculated absorption and VCD spectra of the **CG** octamer are shown in Figure 10. The top part of the figure compares the computed IR absorption spectra for the regular and MD structures of the octamer (both including the MD averaged first hydration shell) with the experimental results. There is an obvious improvement of the overall spectral bandshape and the band positions for the MD averaged structure compared to the idealized canonical geometry in both the base and the sugar-phosphate regions. Hence, most of the calculated absorption bands can be clearly assigned to the experimental spectrum. However, the fine interactions between the bases, reflected in the VCD spectra, provide a different picture. As seen from the bottom left part of Figure 10, the main carbonyl VCD couplet between 1700 and 1650 cm^{-1} is predicted completely wrong for the



Fig. 9. Experimental (bottom) and calculated in vacuum (top) and with the first hydration shell (middle) IR absorption spectra of the octamer (CGAATTCG)*(GCTTAAGC) along with the band assignments. The "regular structure" of the octamer (see text) was used in the calculations. Individual transitions are shown for the middle spectrum as red lines. Adapted with permission from Bour P, Andrushchenko V, Kabelač M, Maharaj V, Wieser H. Simulations of structure and vibrational spectra of deoxyoctanucleotides. J Phys Chem B 2005;109:20579–20587. © 2005 American Chemical Society.



Fig. 10. Experimental and calculated IR absorption (top) and VCD (bottom) spectra of the octamer (CGCGCGCG)*(GCGCGCGC). The calculated absorption spectra are shown for the regular canonic geometry (DFT/reg) and for the MD averaged geometry (DFT/MD), both with the MD-averaged first hydration shell. The calculated VCD spectra (bottom) are shown for the MD averaged geometry (a), the regular canonical structure with the MD-averaged first hydration shell (b) and the regular canonical structure in vacuum (c). The VCD spectra for the base (left) and sugar-phosphate (right) vibration regions are shown separately. Adapted with permission from Bout P, Andrushchenko V, Kabelač M, Maharaj V, Wieser H. Simulations of structure and vibrational spectra of deoxyoctanucleotides. J Phys Chem B 2005;109:20579–20587. © 2005 American Chemical Society.

MD structure, having even an opposite sign compared to the experiment. While the regular hydrated structure provides a correct sign, the best correlation with the experiment is obtained for the regular structure in vacuum. Once again, this shows that complete explicit hydration of the bases might overestimate the water influence even on the carbonyl base vibrations, considerably distorting very sensitive to such interactions VCD spectra. Furthermore, it seems that the geometrical distortions of the bases in the MD averaged structure almost completely destroy the correct VCD signal in this spectral range. A possible reason could be that the octamer structure obtained by averaging the MD trajectory might be rather unrealistic and even further away from the real structure than the canonical geometry. Also, a deficiency and inaccuracy of the available empirical force fields might be partially responsible for producing structures with somewhat large (for VCD sensitivity) deviations from the experiment. Alternatively, not the geometrical distortions of the bases, but rather relatively low accuracy of PM3 method used for calculations of highly important for VCD modeling inter-base Chirality DOI 10.1002/chir

pair interactions might result in such unrealistic VCD spectra. In contrast to the nitrogen base region, the calculated sugar-phosphate VCD spectra (bottom right part of Fig. 10) for the MD structure agree well with the experiment. In fact, the band shape of the main VCD couplet at 1062(-)/1042-1022(+) cm⁻¹ matches the experimental couplet at 1089(-)/1072-1089(+) cm⁻¹ the best out of all the compared models. These results demonstrate that while the realistically distributed explicit solvent molecules might be sufficient for the faithful representation of the main IR absorption features in the sugar-phosphate region (cf. Fig. 9), the explicit solvent alone might not be enough for the correct modeling of the more structuresensitive VCD spectra. Flexibility of the backbone and variations in its configurations, provided by the MD simulations, seem to contribute more to the correct description of the coupling, from which the VCD signal arises. Besides, the NMO method was proven again to be indispensable for the spectra modeling of the non-covalently interacting species, especially in the lower-wavenumber region of the sugar-phosphate vibrations.



Fig. 11. Experimental (left) and calculated (right) IR absorption spectra for seven octanucleotides with different base sequence (one strand sequence is given): CGCGCGCG (1); CCTGGTCC (2); CGTATACG (3); CGCATGCG (4); CGCTAGCG (5); CGATATCG (6); CGAATTCG (7). Only stacked base-pairs were used in the calculations. The example model structure is shown at the right-hand side. Adapted with permission from Bour P, Andrushchenko V, Kabelač M, Maharaj V, Wieser H. Simulations of structure and vibrational spectra of deoxyoctanucleotides. J Phys Chem B 2005;109:20579–20587. © 2005 American Chemical Society.

Variations of DNA Sequence: Employing the Continuum Solvent Model

It was interesting to test if the spectra modeling methodology could satisfactory distinguish between nucleic acids with different sequences. For this purpose the computations for a series of seven octanucleotides with different base content and base sequence was performed and the calculated results were compared to the experimental spectra measured earlier.³³ Initially the calculations for all seven octamers have been done similarly to the CG and CGAT octamers, briefly described in the previous section. Unfortunately, it was difficult to model relatively minor spectral differences arising from the variation of the base sequence using the described MD-based approach. These differences were obscured by the errors introduced from the geometry variations, position of the solvent, incomplete MD averaging, low level of theory for description of the inter-base pair interactions and others. To eliminate some of these factors, a simplified system was created. All seven octamers were generated in a canonical B-DNA geometry with the Tinker software.⁸¹ Considering that the backbone absorption and VCD signal virtually does not depend on the base sequence,^{33,43,115} the sugar-phosphate backbone was removed and only eight methylated base pairs preserving the original B-DNA arrangement were retained for each octamer (an example structure is shown in Fig. 11, right-hand side). From these simplified structures the stacked base pair dimers were selected as fragments for the *ab initio* atomic tensor calculations. Removing the backbone and explicit water molecules allowed to

apply the DTF BPW91/6-31G** level directly to the stacked base pair dimers, which supposedly provided an accurate description of the short-range inter-base pair interactions. The fragments were optimized and their atomic tensors were calculated with the Gaussian 03 software.⁶⁹ To preserve the original B-DNA geometry for the fragments, the optimization was done utilizing the NMO method.⁸⁴ Considering significant improvements in development of the continuum solvent models (CPCM), the COSMO¹²⁴⁻¹²⁶ conductor-like continuum model implemented in Gaussian 03 was used instead of the explicit hydration shell. Although the continuum model was not found completely adequate for description of the directional hydrogen bonds,¹²⁷ its usage eliminates the problems with the solvent position averaging and overestimation of the explicit solvent effects, essential for the nitrogen base spectra modeling, as was shown above. The transfer of the atomic tensors was performed in a single step from all fragments to the target octamers.

The employed procedure resulted in much better agreement with the experiment than the MD-based approach as it was implemented above. The calculated and experimental spectra are compared for all the octamers in Figure 11. For most of the sequences, band assignment can be easily done. Relative intensities for the peaks also agree reasonably well with the experiment, especially considering that experimental spectra consist of many overlapping unresolved bands, which are well separated in the computations. Many experimentally observed spectral changes due to variations in

Chirality DOI 10.1002/chir



Fig. 12. VCD (left) and IR absorption (right) spectra of (CGC)*(GCG) base pair trimer computed for different deviations from the canonical Bform helical twist angle τ_0 . The corresponding structure with the definition of the helical twist angle is shown on top. The experimental VCD (left) and IR absorption (right) spectra of (CGCGCGCG)*(GCGCGCGC) octamer are shown beneath the simulated spectra for easy comparison. Adapted with permission from Bour P, Andrushchenko V, Kabelač M, Maharaj V, Wieser H. Simulations of structure and vibrational spectra of deoxyoctanucleotides. J Phys Chem B 2005;109:20579–20587. © 2005 American Chemical Society.

the base sequence can also be seen in the computed spectra. For example, experimental increase in the ring absorption intensity at 1623 cm^{-1} for octamers **3** and **6** is reproduced correctly by the calculated band at 1631 cm⁻¹. Very similar experimental bandshape for the octamers 4 and 5 is reflected in the similar calculated spectral profiles for these octanucleotides. Only slight dependence on the sequence was observed for the lower wavenumber region between 1600 and 1400 cm^{-1} in both the experiment and computations. It should be pointed out that the whole envelope of the calculated C=O absorptions for all octamers was slightly shifted to the lower wavenumbers (by up to 15 cm^{-1}) compared to the experimental spectra. Similar trend was noticed in other calculations where the BPW91 functional was used in combination with the COSMO solvent model.¹²⁸ This shift might be contributed to slight overestimation of the implicit solvent effect on the polar groups by BPW91. We have recently found that the popular for biomolecular calculations hybrid B3LYP⁹⁹ functional when used with COSMO, results in a very similar bandshape as Chirality DOI 10.1002/chir

BPW91, but produces slightly up-shifted frequencies compared to the latter, thus providing closer match with the experimental values.^{86,128} At the same time, BPW91 functional produces frequencies closer to the experiment when used for vacuum or explicit solvent model calculations.^{86,104,115,128}

Refinement of a Molecular Secondary Structure Based on the CCT Method

As was mentioned in Introduction, a combination of the experimental VCD spectroscopy with the ab initio computations was very successful for determination of the absolute configuration of small molecules, and often eliminated the need of more expensive structural methods, such as X-ray crystallography. While such a precision in the structural determination is not possible for large biomolecules at the present state of the computational technologies, the CCT method allows to get insight into many structural features of large molecules. For example, a dependence of the VCD signal pattern on the helical twist angle of simplified (CGC)*(GCG) base pair trimer (Fig. 12, top) was studied.¹¹⁵ The starting geometry of the trimer was gener-ated by Tinker software⁸¹ in a canonical B-form conformation. Only the strongest VCD signal arising from the DNA nitrogen bases (1700...1500 cm⁻¹) was considered and therefore the target system was comprised of three stacked base pairs, while the sugar-phosphate backbone was removed. Such a reduced system also allowed to apply higher level of theory for the *ab initio* calculations. The deviations ranging from -5° to $+20^{\circ}$ from the canonical B-form helical twist angle τ_0 (Fig. 12, top), were introduced to the trimer using the MCM software,95 thus producing a set of trimers with different helical twists. To account for both the CG and GC interactions²⁰ present in the CGC trimer and in the experimentally studied (CGCGCGCG)*(GCGCGCGC) octamer, both (CG)*(GC) and (GC)*(CG) fragments were selected from the target trimers. All the fragments were optimized and their atomic tensors were calculated with the Gaussian 03 package⁶⁹ at the DTF BPW91/6-31G** level with the COSMO implicit solvent model. To preserve the starting geometry, the NMO method⁸⁴ was again utilized. The atomic tensors were transferred from the fragments to the target trimers in a single step.

The resulting IR absorption and VCD spectra computed for different helical twist angles are compared with the experimental spectra of the (CGCGCGCG)*(GCGCGCGC) octamer in Figure 12. It is seen that deviations as large as $+15^0...+20^0$ from the canonical twist angle result in the best agreement of the simulated spectra with the experiment out of all tested angles. This gives a hint that the actual solution geometry of the (CGCGCGCG)*(GCGCGCGC) octamer, for which the experimental VCD spectra were obtained, might deviate from the crystallographycally derived canonical B-DNA structure, with its central part more twisted than in the crystal structure. Although precision of such simulations is limited and the conclusions might be somewhat speculative, this example clearly shows how the CCT method combined with VCD spectroscopy may be applied to refine the geometry of large biopolymers.

GENERAL REMARKS AND CONCLUSIONS

At the present time the direct *ab initio* description of IR absorption and VCD spectra of biomolecules consisting of hundreds and thousands of atoms is not feasible. However, we have shown that it is possible to overcome this limitation by some complementary approaches, exploring physical properties of the systems under study. One of such methods, the CCT transfer technique, was successfully tested multiple times for computing VCD and IR absorption response of large biomolecular systems. Based on the locality of the VCD phenomenon, but also allowing for a long-range propagation of the exciton states, this method can enable spectra modeling for such large systems, almost with the *ab initio* precision. The method opened up possibility to qualitatively and often quantitatively reproduce experimental spectra of the large nucleic acid systems with a wide variety of structures and arbitrary base content and sequence. Furthermore, in some cases it might be possible to refine the starting canonical geometry of macromolecules so that the refined structure results in better agreement with the experimental spectra. It is especially appealing that the technique can be systematically improved in line with the developments in computational technologies and *ab initio* methodology. That is, larger fragments can be subjected for direct ab initio computing, more precise ab initio levels and continuum solvent models can be used, dynamical and explicit solvent effects can be included, solvent-solute interactions and anharmonic contributions can be accounted for, etc.

However, the application of the CCT method is not trivial and requires a certain amount of testing for each particular system. Thus, due to specificity of the nucleic acid structure and building blocks, the direct transfer of the technique as it was implemented for the peptide modeling did not provide optimal results. Furthermore, even within nucleic acids, different approaches might be necessary for proper treating of the interactions within the nitrogen bases and sugar-phosphate backbone. Inclusion of the MD-averaged explicit solvent and dynamical effects generally improved the computed results. Yet, while the complete explicit hydration shell was shown to be important for the sugar-phosphate computations, the nitrogen base calculations might benefit from only partial inclusion of the hydration or, alternatively, from employing implicit continuum solvent models such as COSMO. Dynamical averaging was clearly important for the proper description of the sugar-phosphate backbone flexibility, but it might overestimate the flexibility of the bases and therefore should be used with care, especially with the older and less accurate empirical force fields. A proper selection of the fragments subjected for ab initio computations is crucial. Careful analysis of the short-range interactions important for the VCD signal must be done for this. Another cornerstone is the selection of a proper and sufficiently precise computational method and a basis set. As a rule, at least DTF methods should be used, combined minimally with 6-31G** basis set, for the precise enough calculations of short-range interactions and faithful reproduction of VCD features. It is known, however, that the DFT methods do not correctly reproduce the van der Waals dispersion interactions. Although the direct effect of the dispersion on VCD may be minor, its influence on the optimized geometry might be substantial and therefore this aspect needs to be addressed in future VCD simulations. While the HF method and smaller basis sets might still provide reasonable results, they are generally not advisable for the treating of the short-range interactions due to possible artifacts in the computed spectra. Semiempirical methods, such as PM3, according to our experience, should be avoided at this stage, and might be used only for rough estimation of weak longer-range electrostatic interactions, extending beyond 4-5 Å.²⁰ Proper approach to constraining the original nucleic acid conformation in the fragments during the *ab initio* optimization was also essential. In this respect, the NMO method was indispensable, particularly for the calculations of the sugar-phosphate spectra, and proved to be generally faster and more stable for computations of non-covalently bound and weakly-interacting fragments or molecules.

Despite the unarguable advantages of the CCT transfer technique, such as high precision of the spectra modeling and universality of the method, in some cases the empirical approaches, such as the CO model, can still provide useful answers in a much shorter time; they are also recommended for complementary and conceptual reasons.

ACKNOWLEDGMENTS

The authors thank D. Tsankov for providing experimental absorption and VCD spectra of single-stranded poly(rA).

LITERATURE CITED

- Nakanishi K, Berova N, Woody RW. Circular dichroism: principles and applications, New York: VCH Publishers; 1994. 570 p.
- Deutsche CW, Moscowit A. Optical activity of vibrational origin. I. A model helical polymer. J Chem Phys 1968;49:3257–3272.
- Chabay I, Holzwarth G. Infrared circular-dichroism and linear dichroism spectrophotometer. Appl Opt 1975;14:454–459.
- Nafie LA, Diem M. Optical-activity in vibrational transitions—vibrational circular-dichroism and Raman optical-activity. Acc Chem Res 1979;12:296–302.
- Andrushchenko V, Tsankov D, Wieser H. Vibrational circular dichroism spectroscopy and the effects of metal ions on DNA structure. J Mol Struct 2003;661:541–560.
- Keiderling TA. Vibrational circular dichroism. Applications to conformational analysis of biomolecules. In: Fasman GD, editor. Circular dichroism and the conformational analysis of biomolecules. New York: Plenum Press; 1996. p 555–597.
- Polavarapu PL, Zhao CX. Vibrational circular dichroism: a new spectroscopic tool for biomolecular structural determination. Fresenius J Anal Chem 2000;366:727–734.
- Nafie LA, Freedman TB. Vibrational circular dichroism: an incisive tool for stereochemical applications. Enantiomer 1998;3:283–297.
- Keiderling TA. Protein and peptide secondary structure and conformational determination with vibrational circular dichroism. Curr Opin Chem Biol 2002;6:682–688.
- Freedman TB, Cao XL, Dukor RK, Nafie LA. Absolute configuration determination of chiral molecules in the solution state using vibrational circular dichroism. Chirality 2003;15:743–758.

Chirality DOI 10.1002/chir

- Nafie LA. Vibrational circular dichroism: a new tool for the solutionstate determination of the structure and absolute configuration of chiral natural product molecules. Nat Prod Commun 2008;3:451–466.
- Stephens PJ, Devlin FJ, Pan JJ. The determination of the absolute configurations of chiral molecules using vibrational circular dichroism (VCD) spectroscopy. Chirality 2008;20:643–663.
- Diem M. Solution conformation of biomolecules from infrared vibrational circular dichroism spectroscopy. In: Birge RR, Nafie LA, editors. Biomolecular spectroscopy II, Vol. 1432. SPIE Proceedings; 1991. pp 28–36.
- Diem M. Determination of the solution conformation of biological molecules by infrared CD. Spectroscopy 1995;10:38–43.
- Polavarapu PL. Renaissance in chiroptical spectroscopic methods for molecular structure determination. Chem Rec 2007;7:125–136.
- Uncuta C, Ion S, Gherase D, Bartha E, Teodorescu F, Filip P. Absolute configurational assignment in chiral compounds through vibrational circular dichroism (VCD) spectroscopy. Revista De Chem 2009;60:86–98.
- Tinoco I. Exciton contribution to optical rotation of polymers. Radiat Res 1963;20:133–139.
- Birke SS, Diem M. Conformational studies of the smallest structural motifs of DNA detectable via vibrational circular dichroism: cytidylyl-(3'-5')-guanosine and guanylyl-(3'-5')-cytidine. Biophys J 1995;68: 1045–1049.
- Zhong WX, Gulotta M, Goss DJ, Diem M. DNA solution conformation via infrared circular dichroism: experimental and theoretical results for B-family polymers. Biochemistry 1990;29:7485–7491.
- Andrushchenko V, Wieser H, Bouř P. B-Z conformational transition of DNA monitored by vibrational circular dichroism. Ab initio interpretation of the experiment. J Phys Chem B 2002;106:12623–12634.
- Andrushchenko V, Bour P. Circular dichroism enhancement in large DNA aggregates simulated by a generalized oscillator model. J Comput Chem 2008;29:2693–2703.
- Yang L, Keiderling TA. Vibrational CD study of the thermal denaturation of poly(rA).poly(rU). Biopolymers 1993;33:315–327.
- Andrushchenko V, van de Sande H, Wieser H. DNA interaction with Mn²⁺ ions at elevated temperatures: VCD evidence of DNA aggregation. Biopolymers 2003;69:529–545.
- Annamalai A, Keiderling TA. Vibrational circular-dichroism of poly(ribonucleic acids)—a comparative-study in aqueous-solution. J Am Chem Soc 1987;109:3125–3132.
- 25. Keiderling TA. Vibrational circular dichroism: comparison of techniques and practical considerations. In: Ferraro J. R., Krishnan K., editors. Practical fourier transform infrared spectroscopy industrial and laboratory chemical analysis. San Diego: Academic Press; 1990. p 203–284.
- Wang L, Keiderling TA. Vibrational circular dichroism studies of the A-to-B conformational transition in DNA. Biochemistry 1992;31: 10265–10271.
- Wang L, Keiderling TA. Helical nature of poly(dI-dC).poly(dI-dC). Vibrational circular dichroism results. Nucl Acids Res 1993;21:4127– 4132.
- Wang L, Yang L, Keiderling TA. Vibrational circular dichroism of A-, B-, and Z-form nucleic acids in the PO₂⁻ stretching region. Biophys J 1994;67:2460–2467.
- Wang L, Pančoška P, Keiderling TA. Detection and characterization of triple-helical pyrimidine-purine-pyrimidine nucleic acids with vibrational circular dichroism. Biochemistry 1994;33:8428–8435.
- Gulotta M, Goss DJ, Diem M. IR vibrational CD in model deoxyoligonucleotides: observation of the B–Z phase transition and extended coupled oscillator intensity calculations. Biopolymers 1989;28:2047– 2058.
- 31. Birke SS, Moses M, Kagalovsky B, Jano D, Gulotta M, Diem M. Infrared CD of deoxy oligonucleotides. Conformational studies of 5'd(GCGC)3', 5'd(CGCG)3', 5'd(CCGG)3', and 5'd(GGCC)3' in low and high salt aqueous solution. Biophys J 1993;65:1262–1271.
- Xiang T, Goss DJ, Diem M. Strategies for the computation of infrared CD and absorption spectra of biological molecules: ribonucleic acids. Biophys J 1993;65:1255–1261.

Chirality DOI 10.1002/chir

- Maharaj V, Tsankov D, van de Sande HJ, Wieser H. Ft-Ir-Vcd Spectra of 6-Octadeoxynucleotide. J Mol Struct 1995;349:25–28.
- 34. Andrushchenko VV, van de Sande JH, Wieser H, Kornilova SV, Blagoi YP. Complexes of (dG-dC)₂₀ with Mn²⁺ ions: a study by vibrational circular dichroism and infrared absorption spectroscopy. J Biomol Struct Dyn 1999;17:545–560.
- 35. Andrushchenko V, Leonenko Z, Cramb D, van de Sande H, Wieser H. Vibrational CD (VCD) and atomic force microscopy (AFM) study of DNA interaction with Cr³⁺ ions: VCD and AFM evidence of DNA condensation. Biopolymers 2001;61:243–260.
- Andrushchenko V, Blagoi Y, van de Sande JH, Wieser H. Poly(rA). poly(rU) with Ni(2+) ions at different temperatures: infrared absorption and vibrational circular dichroism spectroscopy. J Biomol Struct Dyn 2002;19:889–906.
- Andrushchenko V, van de Sande JH, Wieser H. Vibrational circular dichroism and IR absorption of DNA complexes with Cu²⁺ ions. Biopolymers 2003;72:374–390.
- Polyanichko AM, Andrushchenko VV, Chikhirzhina EV, Vorob'ev VI, Wieser H. The effect of manganese(II) on DNA structure: electronic and vibrational circular dichroism studies. Nucl Acids Res 2004;32:989–996.
- Maharaj V, Rauk A, van de Sande JH, Wieser H. Infrared absorption and vibrational circular dichroism spectra of selected deoxyoctanucleotides complexed with daunorubicin. J Mol Struct 1997;408:315–318.
- Tsankov D, Kalisch B, van de Sande JH, Wieser H. Cisplatin adducts of d(CCTCTG*G*TCTCC)center dot d(GGAGACCAGAGG) in aqueous solution by vibrational circular dichroism spectroscopy. Biopolymers 2003;72:490–499.
- 41. Tsankov D, Kalisch B, De Sande HV, Wieser H. Cisplatin-DNA adducts by vibrational circular dichroism spectroscopy: Structure and isomerization of d(CCTG*G*TCC)center dot d(GGACCAGG) intrastrand cross-linked by cisplatin. J Phys Chem B 2003;107:6479–6485.
- 42. Pandyra A, Tsankov D, Andrushchenko V, van de Sande JH, Wieser H. Intercalation of daunomycin into d(CG)4 oligomer duplex containing G x T mismatches by vibrational circular dichroism and infrared absorption spectroscopy. Biopolymers 2006;82:189–198.
- Tsankov D, Maharaj V, van de Sande JH, Wieser H. Selected deoxyroctanucleotides and their daunomycin complexes: infrared absorption and vibrational circular dichroism spectra. Vib Spectrosc 2007; 43:152–164.
- 44. Tsankov D, Krasteva M, Andrushchenko V, van de Sande JH, Wieser H. Vibrational circular dichroism signature of hemiprotonated intercalated four-stranded i-DNA. Biophys Chem 2006;119:1–6.
- Keiderling TA. Vibrational circular-dichroism. Appl Spectrosc Rev 1981;17:189–226.
- Diem M, Roberts GM, Lee O, Barlow A. Design and performance of an optimized dispersive infrared dichrograph. Appl Spectrosc 1988; 42:20–27.
- Tsankov D, Eggimann T, Wieser H. Alternative design for improved Ft-Ir/Vcd capabilities. Appl Spectrosc 1995;49:132–138.
- 48. Blagoi Y, Gladchenko G, Nafie LA, Freedman TB, Sorokin V, Valeev V, He Y. Phase equilibrium in poly(rA). Poly(rU) complexes with Cd(2+) and Mg(2+) ions, studied by ultraviolet, infrared, and vibrational circular dichroism spectroscopy. Biopolymers 2005;78:275–286.
- Petrovic AG, Polavarapu PL. Structural transitions in polyriboadenylic acid induced by the changes in pH and temperature: vibrational circular dichroism study in solution and film states. J Phys Chem B 2005;109:23698–23705.
- Petrovic AG, Polavarapu PL. Quadruplex structure of polyriboinosinic acid: dependence on alkali metal ion concentration, pH and temperature. J Phys Chem B 2008;112:2255–2260.
- Novy J, Urbanova M, Volka K. Vibrational and electronic circular dichroism and absorption spectral study of the DNA-5,10,15,20-tetrakis(1-methylpyridinium-4-yl)porphyrin interaction. J Mol Struct 2005; 748:17–25.
- 52. Setnicka V, Urbanova M, Volka K, Nampally S, Lehn JM. Investigation of guanosine-quartet assemblies by vibrational and electronic circular dichroism spectroscopy, a novel approach for studying supramolecular entities. Chemistry 2006;12:8735–8743.

- 53. Novy J, Bohm S, Kralova J, Kral V, Urbanova M. Formation and temperature stability of G-quadruplex structures studied by electronic and vibrational circular dichroism spectroscopy combined with ab initio calculations. Biopolymers 2008;89:144–152.
- Tsuboi M. Infrared and Raman spectroscopy. In: Ts'o POP, editor. Basic principles in nucleic acid chemistry, Vol. 1. New York: Academic Press; 1974. p 399–452.
- Tsuboi M. Application of infrared spectroscopy to structure studies of nucleic acids. Appl Spectrosc Rev 1969;3:45–90.
- Taillandier E, Liquier J, Taboury JA. Infrared spectral studies of DNA conformations. In: Clark RJH, Hester RE, editors. Advances in infrared and Raman spectroscopy, Vol. 12. New York: Wiley-Heyden; 1985. p 65–114.
- Hamm P, Zurek M, Mantele W, Meyer M, Scheer H, Zinth W. Femtosecond infrared-spectroscopy of reaction centers from *Rhodobacter*sphaeroides between 1000 and 1800 Cm(-1). Proc Natl Acad Sci USA 1995;92:1826–1830.
- Hick JM. Chirality: physical chemistry, Vol. 810. New York: Oxford University Press; 2002. 356 p.
- Holzwarth G, Chabay I. Optical activity of vibrational transitions: a coupled oscillator model. J Chem Phys 1972;57:1632–1635.
- DeVoe H. Optical properties of molecular aggregates. 2. Classical theory of refraction absorption and optical activity of solutions and crystals. J Chem Phys 1965;43:3199–3208.
- DeVoe H. Calculations of optical properties of 1-methyluracil crystal by an all-order classical oscillator theory. J Phys Chem 1971;75:1509– 1515.
- Self BD, Moore DS. Nucleic acid vibrational circular dichroism, absorption, and linear dichroism spectra.1. A DeVoe theory approach. Biophys J 1997;73:339–347.
- Self BD, Moore DS. Nucleic acid vibrational circular dichroism, absorption, and linear dichroism spectra. II. A DeVoe theory approach. Biophys J 1998;74:2249–2258.
- Charney E. The molecular basis of optical activity. New York: Wiley; 1979. 376 p.
- Bouř P, Keiderling TA. Computational evaluation of the coupled oscillator model in the vibrational circular dichroism of selected small molecules. J Am Chem Soc 1992;114:9100–9105.
- Kawiecki RW, Devlin FJ, Stephens PJ, Amos RD. Vibrational circulardichroism of propilene-oxide. J Phys Chem 1991;95:9817–9831.
- Polavarapu PL, Pickard ST, Smith HE, Black TM, Rauk A, Yang DY. Vibrational circular dichroism and absolute configuration of substituted thiiranes. J Am Chem Soc 1991;113:9747–9756.
- Stephens PJ. Theory of vibrational circular-dichroism. J Phys Chem 1985;89:748–752.
- 69. Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Montgomery J, J. A., Vreven T, Kudin KN, Burant JC, Millam JM, Iyengar SS, Tomasi J, Barone V, Mennucci B, Cossi M, Scalmani G, Rega N, Petersson GA, Nakatsuji H, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Nakajima T, Honda Y, Kitao O, Nakai H, Klene M, Li X, Knox JE, Hratchian HP, Cross JB, Bakken V, Adamo C, Jaramillo J, Gomperts R, Stratmann RE, Yazyev O, Austin AJ, Cammi R, Pomelli C, Ochterski JW, Ayala PY, Morokuma K, Voth GA, Salvador P, Dannenberg JJ, Zakrzewski VG, Dapprich S, Daniels AD, Strain MC, Farkas O, Malick DK, Rabuck AD, Raghavachari K, Foresman JB, Ortiz JV, Cui Q, Baboul AG, Clifford S, Cioslowski J, Stefanov BB, Liu G, Liashenko A, Piskorz P, Komaromi I, Martin RL, Fox DJ, Keith T, Al-Laham MA, Peng CY, Nanayakkara A, Challacombe M, Gill PMW, Johnson B, Chen W, Wong MW, Gonzalez C, Pople JA. Gaussian 03, Revision C.02, Wallingford CT: Gaussian, Inc.; 2004.
- Cheeseman JR, Frisch MJ, Devlin FJ, Stephens PJ. Ab initio calculation of atomic axial tensors and vibrational rotational strengths using density functional theory. Chem Phys Lett 1996;252:211–220.
- Petrovic AG, Polavarapu PL. Chiroptical spectroscopic determination of molecular structures of chiral sulfinamides: *t*-butanesulrinamide. J Phys Chem A 2007;111:10938–10943.
- Abbate S, Burgi LF, Castiblioni E, Lebon F, Longhi G, Toscano E, Caccamese S. Assessment of configurational and conformational

properties of naringenin by vibrational circular dichroism. Chirality 2009;21:436–441.

- Bouř P, Keiderling TA. Ab-initio simulations of the vibrational circular-dichroism of coupled peptides. J Am Chem Soc 1993;115:9602– 9607.
- 74. Silva R, Kubelka J, Bouř P, Decatur SM, Keiderling TA. Site-specific conformational determination in thermal unfolding studies of helical peptides using vibrational circular dichroism with isotopic substitution. Proc Natl Acad Sci USA 2000;97:8318–8323.
- Bouř P, Sopková J, Bednárová L, Maloň P, Keiderling TA. Transfer of molecular property tensors in Cartesian coordinates: a new algorithm for simulation of vibrational spectra. J Comput Chem 1997;18:646– 659.
- Bouř P, Kubelka J, Keiderling TA. Simulations of oligopeptide vibrational CD: effects of isotopic labeling. Biopolymers 2000;53: 380–395.
- Bouř P, Kubelka J, Keiderling TA. Ab initio quantum mechanical models of peptide helices and their vibrational spectra. Biopolymers 2002;65:45–59.
- Kubelka J. Vibrational spectroscopic studies of peptide and protein structures: theory and experiment, Ph.D. thesis, Chicago: University of Illinois, 2002. 455 p.
- 79. Protein Data Bank. http://www.rcsb.org/pdb/home/home.do.
- Insight II (now part of Discovery Studio), San Diego, CA: Accelrys Software Inc.; 1995; 2001–2009.
- Ponder JW. Tinker, software tools for molecular design. 3.8, Saint Louis: Washington University School of Medicine; 2000.
- 82. Case DA, Darden TA, Cheatham III TE, Simmerling CL, Wang J, Duke RE, Luo R, Merz KM, Pearlman DA, Crowley M, Walker RC, Zhang W, Wang B, Hayik S, Roitberg A, Seabra G, Wong KF, Paesani F, Wu X, Brozell S, Tsui V, Gohlke H, Yang L, Tan C, Mongan J, Hornak V, Cui G, Beroza P, Mathews DH, Schafmeister C, Ross WS, Kollman PA. AMBER 9. San Francisco: University of California; 2006.
- Ahlrichs R, Bar M, Haser M, Horn H, Kolmel C. Electronic-structure calculations on workstation computers—the program system turbomole. Chem Phys Lett 1989;162:165–169.
- Bouř P, Keiderling TA. Partial optimization of molecular geometry in normal coordinates and use as a tool for simulation of vibrational spectra. J Chem Phys 2002;117:4126–4132.
- Bouř P Programs and Utilities. http://hanicka.uochb.cas.cz/~bour/ programs/list.html.
- Andrushchenko V, Wieser H, Bouř P. DNA oligonucleotide-cis-platin binding: ab initio interpretation of the vibrational spectra. J Phys Chem A 2007;111:9714–9723.
- Watson JD, Crick FHC. Molecular structure of nucleic acids—a structure for deoxyribose nucleic acid. Nature 1953;171:737–738.
- Pohl FM, Jovin TM. Salt-Induced cooperative conformational change of a synthetic DNA—equilibrium and kinetic studies with Poly(Dg-Dc). J Mol Biol 1972;67:375–396.
- van de Sande JH, Mcintosh LP, Jovin TM. Z* DNA, the left-handed helical form of poly[d(G-C)] in MgCl2-ethanol, is biologically active. EMBO J 1982;1:115–120.
- Rossetto FE, Nieboer E. The interaction of metal-ions with synthetic DNA—induction of conformational and structural transitions. J Inorg Biochem 1994;54:167–186.
- Taboury JA, Bourtayre P, Liquier J, Taillandier E. Interaction of Z form poly(dG-dC).poly(dG-dC) with divalent metal ions: localization of the binding sites by I.R. spectroscopy. Nucl Acids Res 1984;12: 4247–4258.
- Nordheim A, Rich A. Negatively supercoiled simian virus 40 DNA contains Z-DNA segments within transcriptional enhancer sequences. Nature 1983;303:674–679.
- Wang G, Vasquez KM. Z-DNA, an active element in the genome. Front Biosci 2007;12:4424–4438.
- 94. Zhanpeisov NU, Leszczynski J. Specific solvation effects on the structures and properties of Watson-Crick and reverse Watson-Crick isocytosine-cytosine and guanine-cytosine base pairs: a theoretical ab initio study. J Mol Struct Theochem 1999;487:107–115.

- Bouř P, Maloň P. MCM molecular graphics. Prague: Academy of Sciences; 1995–2009.
- Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Montgomery J, J. A., Vreven T, Kudin KN, Burant JC, Millam JM, Iyengar SS, Tomasi J, Barone V, Mennucci B, Cossi M, Scalmani G, Rega N, Petersson GA, Nakatsuji H, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Nakajima T. Honda Y, Kitao O, Nakai H, Klene M, Li X, Knox JE, Hratchian HP, Cross JB, Bakken V, Adamo C, Jaramillo J, Gomperts R, Stratmann RE, Yazyev O, Austin AJ, Cammi R, Pomelli C, Ochterski JW, Ayala PY, Morokuma K, Voth GA, Salvador P, Dannenberg JJ, Zakrzewski VG, Dapprich S, Daniels AD, Strain MC, Farkas O, Malick DK, Rabuck AD, Raghavachari K, Foresman JB, Ortiz JV, Cui Q, Baboul AG, Clifford S, Cioslowski J, Stefanov BB, Liu G, Liashenko A, Piskorz P, Komaromi I, Martin RL, Fox DJ, Keith T, Al-Laham MA, Peng CY, Nanavakkara A, Challacombe M, Gill PMW, Johnson B, Chen W, Wong MW, Gonzalez C, Pople JA. Gaussian 98, Revisions A.7 and A.9. Pittsburgh, PA: Gaussian, Inc.; 1998.
- Becke AD. Density-functional thermochemistry. 3. The role of exact exchange. J Chem Phys 1993;98:5648–5652.
- Perdew JP, Wang Y. Accurate and simple analytic representation of the electron-gas correlation-energy. Phys Rev B 1992;45:13244– 13249.
- Becke AD. A new mixing of Hartree–Fock and local density-functional theories. J Chem Phys 1993;98:1372–1377.
- Felsenfeld G, Davies DR, Rich A. Formation of a 3-stranded polynucleotide molecule. J Am Chem Soc 1957;79:2023–2024.
- Miles HT. The structure of the three-stranded helix poly(A+2U). Proc Natl Acad Sci USA 1964;51:1104–1109.
- Dagneaux C, Liquier J, Taillandier E. Sugar conformations in DNA and RNA-DNA triple helices determined by FTIR spectroscopy: role of backbone composition. Biochemistry 1995;34:16618–166123.
- Saenger W. Principles of nucleic acid structure. New York: Springer-Verlag; 1984. 556 p.
- 104. Andrushchenko V, Wieser H, Bouř P. RNA structural forms studied by vibrational circular dichroism: ab initio interpretation of the spectra. J Phys Chem B 2004;108:3899–3911.
- 105. Bour P. Convergence properties of the normal mode optimization and its combination with molecular geometry constraints. Collect Czech Chem Commun 2005;70:1315–1340.
- 106. Bouř P. QGRAD. Prague: Academy of Sciences; 2002-2009
- 107. Angeli C, Bak KL, Bakken V, Christiansen O, Cimiraglia R, Coriani S, Dahle P, Dalskov EK, Enevoldsen T, Fernandez B, Hattig C, Hald K, Halkier A, Heiberg H, Helgaker T, Hettema H, Jensen HJA, Jonsson D, J^J/grgensen P, Kirpekar S, Klopper W, Kobayashi R, Koch H, Ligabue A, Lutnìs OB, Mikkelsen KV, Norman P, Olsen J, Packer MJ, Pedersen TB, Rinkevicius Z, Rudberg E, Ruden TA, Ruud K, Salek P, Sanchez de Meras A, Saue T, Sauer SPA, Schimmelpfennig B, Sylvester-Hvid KO, Taylor PR, Vahtras O, Wilson DJ, Agren H. Dalton, a molecular electronic structure program, Release 2.0. 2005. Available at http://www.kjemi.uio.no/software/dalton.html.
- Tsuboi M, Kyogoku Y, Shimanouchi T. Infrared absorption spectra of protonated and deprotonated nucleosides. Biochim Biophys Acta 1962;55:1–12.
- 109. Ohms J, Ackermann T. Thermodynamics of double- and triple-helical aggregates formed by self-complementary oligoribonucleotides of the type rAxUy. Biochemistry 1990;29:5237–5244.

- Duca M, Vekhoff P, Oussedik K, Halby L, Arimondo PB. The triple helix: 50 years later, the outcome. Nucl Acids Res 2008;36:5123– 5138.
- 111. Wells RD, Collier DA, Hanvey JC, Shimizu M, Wohlrab F. The chemistry and biology of unusual DNA structures adopted by oligopurine. Oligopyrimidine sequences. FASEB J 1988;2:2939–2949.
- Maher LJ. Prospects for the therapeutic use of antigene oligonucleotides. Cancer Invest 1996;14:66–82.
- 113. Praseuth D, Guieysse AL, Helene C. Triple helix formation and the antigene strategy for sequence-specific control of gene expression. Biochim Biophys Acta (BBA) Gene Struct Expression 1999;1489:181– 206.
- Chandrasekaran R, Giacometti A, Arnott S. Structure of poly (U)center dot poly (A)center dot poly (U). J Biomol Struct Dyn 2000;17:1023– 1034.
- 115. Bouř P, Andrushchenko V, Kabelač M, Maharaj V, Wieser H. Simulations of structure and vibrational spectra of deoxyoctanucleotides. J Phys Chem B 2005;109:20579–20587.
- 116. Cornell WD, Cieplak P, Bayly CI, Gould IR, Merz KM, Ferguson DM, Spellmeyer DC, Fox T, Caldwell JW, Kollman PA. A 2nd generation force-field for the simulation of proteins, nucleic-acids, and organic-molecules. J Am Chem Soc 1995;117:5179–5197.
- 117. Pearlman DA, Case DA, Caldwell JW, Ross WS, Cheatham III TE, Ferguson DM, Seibel GL, Singh UC, Singh P, Weiner P, Kollman PA. AMBER 4.1. San Francisco, CA: University of California; 1995.
- 118. Arnott S, Campbell-Smith PJ, Chandrasekaran R. Atomic coordinates and molecular conformations for DNA-DNA, RNA-RNA and DNA-RNA helices, In: Fasman GP, editor. CRC Handbook of biochemistry and molecular biology: Section B Nucleic Acids, 3rd ed. Vol. 2. Cleveland: CRC Press; 1976. p 411–422.
- Bouř P. A cluster model of liquid water and its IR spectroscopic response. Chem Phys Lett 2002;365:82–88.
- 120. Kubelka J, Keiderling TA. Ab initio calculation of amide carbonyl stretch vibrational frequencies in solution with modified basis sets. 1. N-methyl acetamide. J Phys Chem A 2001;105:10922–10928.
- 121. Bouř P, Keiderling TA. Empirical modeling of the peptide amide I band IR intensity in water solution. J Chem Phys 2003;119:11253– 11262.
- 122. Andrushchenko V, Matejka P, Anderson DT, Kaminsky J, Hornicek J, Paulson LO, Bour P. Solvent dependence of the *N*-methylacetamide structure and force field. J Phys Chem A 2009;113:9727–9736.
- Zanni MT, Asplund MC, Hochstrasser RM. Two-dimensional heterodyned and stimulated infrared photon echoes of N-methylacetamide-D. J Chem Phys 2001;114:4579–4590.
- Klamt A, Jonas V, Burger T, Lohrentz JCW. Refinement and parametrization of COSMO-RS. J Phys Chem A 1998;102:5074–5085.
- 125. Eckert F, Klamt A. Fast solvent screening via quantum chemistry: COSMO-RS approach. AIChE J 2002;48:369–385.
- 126. Barone V, Cossi M, Tomasi J. Geometry optimization of molecular structures in solution by the polarizable continuum model. J Comput Chem 1998;19:404–417.
- 127. Bouř P. On the influence of the water electrostatic field on the amide group vibrational frequencies. J Chem Phys 2004;121:7545–7548.
- 128. Andrushchenko V, Bour P. Infrared absorption detection of metal ion-deoxyguanosine monophosphate binding: experimental and theoretical study. J Phys Chem B 2009;113:283–291.