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# Bisignate resonance Raman optical activity: a pseudo breakdown of the single electronic state model of RROA?

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Raman optical activity (ROA) spectra were calculated for a set of conformers of astaxanthin, which is a non-rigid molecule exhibiting strong resonance enhancement in the visible range. Single electronic state theory of the Resonance ROA (RROA) predicts the spectrum to be monosigned. For astaxanthin, it appeared that some of the conformers exhibit different sign of the bands than the other conformers. As a result, the conformer population averaged spectrum of astaxanthin can exhibit both signs of the bands, or be monosigned depending on which conformers are dominating, that reflects a departure from the single electronic state approximation. Moreover, use of different basis sets and/or density functional theory (DFT) functionals results in different conformer populations, thus yielding again either monosigned ROA spectrum or with bands of both signs. Consequences of these two findings for the astaxanthin RROA spectrum are discussed. Copyright © 2014 John Wiley & Sons, Ltd.

**Keywords:** astaxanthin; conformation; DFT; RROA

## Introduction

A small difference in the Raman scattering of right-circularly and left-circularly polarized incident (incident circular polarization), Raman scattered (scattered circular polarization), or both incident and scattered (in-phase dual circular polarization DCPI, or out of phase dual circular polarization, DCPII) radiation gives rise to different observations of the Raman optical activity (ROA) spectra of a chiral molecule.<sup>[1,2]</sup> Tremendous potential of Raman scattering is even more enhanced by possibility of registration of resonance effects.<sup>[3]</sup>

In contrast to ROA and other chiroptic spectra, the Resonance ROA (RROA) spectrum derived from single electronic state (SES) is 1/monosignate, 2/ is opposite in sign to the electronic circular dichroism (ECD) of the lone resonant excited electronic state, 3/ has the same relative intensities of the parent RR spectrum, and 4/ is smaller than the RR spectrum by the ratio of the corresponding ECD to its parent absorbance intensity (*g*-value).<sup>[4,5]</sup> Consideration of incident resonance with two electronic states showed, however, that both signs of different RROA bands may occur originating from resonances with different electronic states exhibiting the opposite ECD signs.<sup>[6,7]</sup> An example of a molecule, in which one RROA band exhibited the sign opposite to that of the remaining bands governed by the dominant electronic state, was recently demonstrated.<sup>[8]</sup>

However, there may be yet another reason for a bisignate pattern in the RROA spectrum of a compound: its non-rigidity. Recently, we have been studying carotenoids including chiral astaxanthin (3,3'-dihydroxy- $\beta,\beta$ -carotene-4,4'-dione, AXT) (Fig. 1 top), which is a red food colorant of industrial importance but also a superpotent antioxidant beneficial in context of cardiovascular, immune, inflammatory, and neurodegenerative diseases.<sup>[9,10]</sup> Carotenoids are composed of a large polyene chain often ended with a singly unsaturated ring, which may be chiral as in AXT. The synthetic AXT consists of 3R,3'R : 3R,3'S : 3S,3'S forms in the 1:2:1 ratio, while in *Haematococcus pluvialis* algae, AXT, synthesized

from  $\beta$ -carotene upon stress conditions, exists primarily as a monoestrified 3S,3'S isomer.<sup>[10,11]</sup> The polyene chain enables carotenoids to be observable in minute amounts in living tissues by using RR (or pre-resonance) spectra,<sup>[12]</sup> while the chiral ends make AXT observable by chiroptic spectroscopy methods. Very surprisingly, a computational prediction of the RROA of AXT appeared to be difficult, demanding, and not necessarily leading to unequivocal results. This short communication is signaling problems with prediction of RROA spectra of non-rigid compounds produced by both molecular conformation and unreliability of some density functional theory (DFT) functionals.

## Calculations

The DFT optimization of geometry, calculations of vibrational frequencies, and ROA intensities at 532, 785, and 1064 nm excitation wavelengths were carried out using the Gaussian 09 D.01 package (Gaussian Inc., Wallingford CT).<sup>[13]</sup> The B3LYP<sup>[14,15]</sup> and M05<sup>[16]</sup> functionals combined with the STO-3G and the 6-31+G(d,p) basis sets were used. The 0.93, 0.97, and 0.9542 scaling factors were used

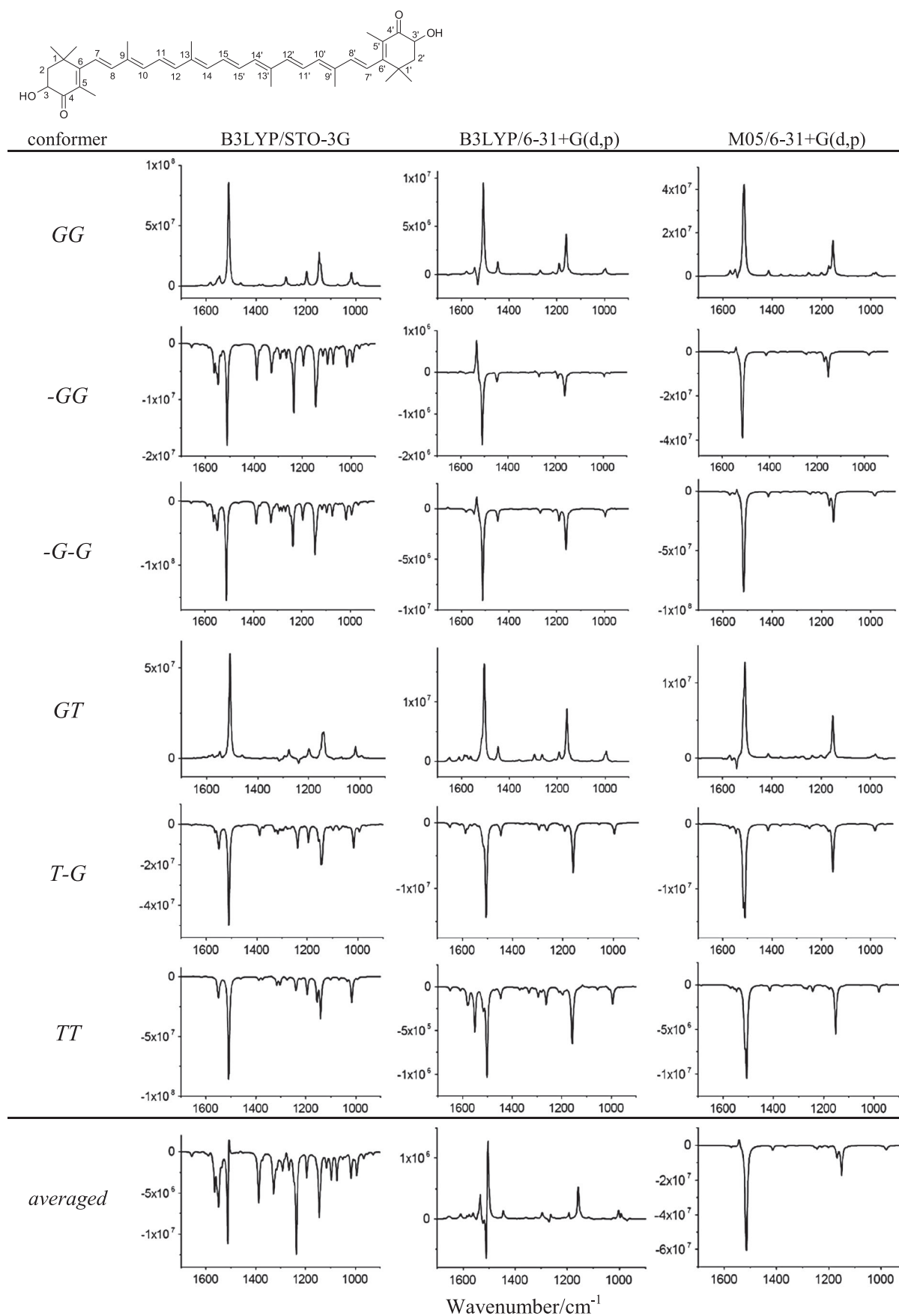
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**Figure 1.** Resonance Raman optical activity spectra calculated for different astaxanthin conformers at different levels of theory and averaged according to Boltzmann contributions of conformers based on Gibbs free energies predicted at 25 °C. The astaxanthin structural formula is presented on the top.

for B3LYP/STO-3G, B3LYP/6-31+G(d,p), and M05/6-31+G(d,p) calculated spectra, respectively, to account for anharmonicity and limitations of basis sets.<sup>[17]</sup> The spectra of 3S,3'S-astaxanthin conformers were averaged using Boltzmann distribution weighted by Gibbs free energies at 25 °C.

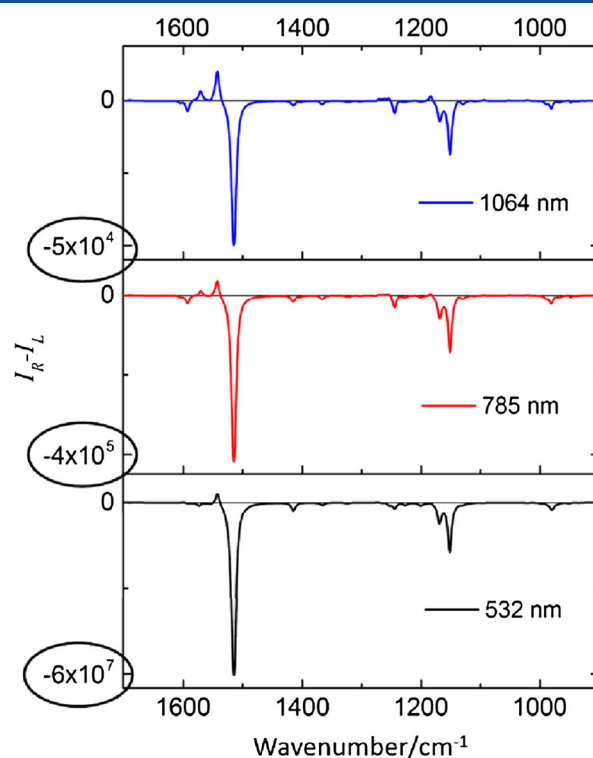
## Results and discussion

The astaxanthin molecule is a good example to illustrate RROA dependence on conformation. It exhibits the resonance Raman spectrum due to polyene chain, the non-rigidity due to single-bonded  $\beta$ -ionone ring ends, and the chirality due to OH substituents (Fig. 1). Two chiral ionone rings give rise to presence of S,S- or R,R- chiral enantiomers and R,S-diastereoisomer. The AXT conformers were already discussed together with the structural changes of AXT in a single algal cell monitored *in situ* by Raman spectroscopy.<sup>[9]</sup> Flexibility of the ionone rings in respect to the chain leads to equilibrium between several symmetrical and asymmetrical conformers, which can be systematized as G (*gauche*), –G (*minus gauche*) and T (*trans*), according to the International Union of Pure and Applied Chemistry convention<sup>[18]</sup> applied to two dihedrals describing ionone rotation with respect to the chain. As reported previously,<sup>[9]</sup> based on B3LYP/6-31+G(d,p) calculations, six independent all-*trans* conformers were found for the 3S,3'S AXT, namely, GG, –GG, –G–G, GT, T–G, and TT, for which the ROA spectra were calculated in this work.

AXT is relatively large molecule for time consuming ROA calculations; therefore, we aimed to verify our first intuition using minimal STO-3G basis set. Then, already at this elementary level, we realized that although all bands in each of the B3LYP/STO-3G predicted RROA spectra have the same sign in agreement with the SES theory of RROA,<sup>[4]</sup> different conformers may exhibit the opposite signs (Fig. 1). Indeed, the GG and GT have positive sign while –GG, –G–G, T–G, and TT exhibit the negative one (Fig. 1). As a result, the Boltzmann factors averaged RROA spectra calculated at the B3LYP/STO-3G level have all but one negative bands. This one, at  $\sim 1500\text{ cm}^{-1}$ , is a mark of a non-negligible presence of the GG or GT conformer in the AXT equilibrium mixture.

It is known that the STO-3G is not of sufficient quality for either vibrational circular dichroism or ROA calculations. It is also established that for ROA, diffuse orbitals are required for the basis set, and in particular, diffuse orbitals on the hydrogens are important. Recalculations of the ROA spectra of conformers using larger 6-31+G(d,p) basis set confirmed variation of the RROA bands sign with conformation and revealed the next problem (Fig. 1). Although in general the sign obtained using the two basis sets remains the same, in three out of the six spectra, namely GG, –GG, and –G–G, one band of the opposite sign has significant intensity (Fig. 1). This may mean that for these conformations, the SES RROA theory is not sufficient, and an additional electronic state, contributing to ECD band of opposite sign to the main electronic resonant band, becomes active. Thus, two different effects may contribute to the bisignate pattern of the RROA spectrum of a flexible molecule. This is clearly observed in the RROA spectrum calculated at the B3LYP/6-31+G(d,p) level averaged and weighted by the conformer population factors (Fig. 1).

Finally, all the conformers were recalculated by using M05 Minnesota global hybrid functional, which is much modern than the reference B3LYP one; for nonpolar, molecules with noncovalent interactions it performs significantly better.<sup>[16,19]</sup> A qualitative picture of RROA spectra obtained with the two functionals combined



**Figure 2.** Resonance Raman optical activity spectra of the astaxanthin conformer equilibrium mixture at 25 °C calculated for three excitation wavelengths at the M05/6-31+G(d,p) level.

with the 6-31+G(d,p) basis set is very similar. However, the opposite bands in the spectra of individual conformers are weaker, and GT form joins the group of systems exhibiting 'the opposite band' (Fig. 1). Yet, the RROA calculations with the M05 functional yield the averaged spectrum definitely different from that obtained using B3LYP one. This is not unexpected: the more modern M05 functional performs better for the conformer abundances than B3LYP (Fig. 1).<sup>[16]</sup> However, one has to be fully conscious of two facts. First, careful population studies require usually post-Hartree-Fock methods to reproduce correctly the experimental conformer abundances.<sup>[20]</sup> Second, reproduction of Raman and ROA intensities depends strongly on the basis set quality and size.<sup>[21]</sup> Still, in this communication, we are qualitatively illustrating problem that can arise when one tries to predict RROA spectrum of a flexible molecule rather than search for quantitative results.

Let us stress at the end that the M05/6-31+G(d,p) predicted spectra averaged for AXT conformer equilibrium mixture (Fig. 2) seem to qualitatively predict both the resonance effect increasing as the wavelength of the incident light is decreased from 1064, through 785, to 532 nm and the sign, which is opposite to that of the ECD spectrum of AXT [data to be published].

## Conclusions

The calculated RROA spectra for different conformers of non-rigid molecule, astaxanthin, demonstrate that the sign of the RROA bands of certain conformers can be opposite to the sign of the bands of the other conformers owing to different signs of the ECD of the resonant electronic state. Such a spectral pattern is not in the contradiction to the SES RROA theory, which predicts the monosigned RROA spectrum for one energetic minimum, i.e. one

conformer. However, as a result, the population averaged RROA spectrum of a flexible molecule can exhibit bands of both signs and therefore suggests the breakdown in SES, although this effect is purely result of Boltzmann averaging of spectra due to existence of various conformers.

The breakdown of the SES approximation is related to the fact that orientation of two  $\pi$ -electron planes of the molecule: the plane of the polyene chain relative to the plane of the ionone ring is different for different conformers that affect *both* ROA and ECD spectra. As a continuation of the research, calculations of ECD spectra will be undertaken for different conformers to demonstrate that the SES model holds for the majority of bands in each conformer even though the sign of the monosignate bands can change between conformers. The overall sign of the RROA spectrum of AXT was preserved for majority of conformers. Yet, an opposite sign band was found in several forms of astaxanthin when a larger basis set was used. This suggests that a multiple electronic state RROA behavior can be possibly revealed when the more adequate basis set is applied. Change of the DFT functional can dramatically change the conformer population averaged RROA spectrum. As a result, depending on the level of theory applied, the predicted RROA spectrum can exhibit bands of both signs or can be almost monosignate.

Additionally it should be stressed that calculations predict the resonance enhancement for AXT at the excitation of 532 nm (that is in the range of the electronic absorption band), manifested as three orders of magnitude increased intensity of ROA bands relatively to the spectrum obtained at the 1064 nm excitation. In the case of approaching SES, the conformationally averaged RROA spectra becomes 'more monosignate' as strong resonance with a SES is approached with laser wavelengths decreasing from 1064 to 785 to 532 nm. In particular, laser excitation at 1064 nm is sufficiently far away from direct strong resonance with the lowest excited electronic state that the ROA spectrum manifests as bisignate, i.e. a significant 'breakdown' of the SES approximation. This effect is nearly gone for 532 nm excitation of AXT, where the spectrum appears nearly monosignate.

To conclude, it is of interest for further studies to identify the source of oppositely signed bands for individual conformers and answer the question if the source of SES breakdown is direct resonance with a second electronic state or is it via Herzberg-Teller coupling of the resonant electronic state with a second non-resonant, vibronically coupled state. Moreover, for a future study, comparison of the relative intensities of the RROA to the parent RR intensities should be undertaken. Even if there is no sign change for the RROA of a given conformer, changes in the relative monosignate RROA bands compared with the relative intensities of the RR bands may be an indication of the breakdown of the SES approximation and the beginning of influence of more than one electronic state.

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