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Gas-Phase Retinal Spectroscopy: Temperature Effects Are But a Mirage

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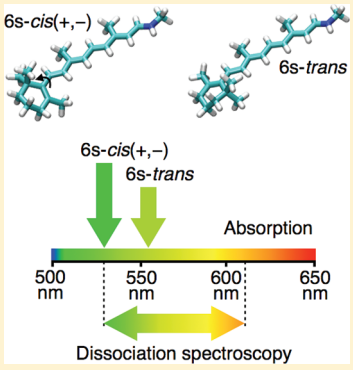
Supporting Information

ABSTRACT: We employ state-of-the-art first-principle approaches to investigate whether temperature effects are responsible for the unusually broad and flat spectrum of protonated Schiff base retinal observed in photodissociation spectroscopy, as has recently been proposed. We first carefully calibrate how to construct a realistic geometrical model of retinal and show that the exchange−correlation M06-2X functional yields an accurate description while the commonly used complete active space self-consistent field method (CASSCF) is not adequate. Using modern multiconfigurational perturbative methods (NEVPT2) to compute the excitations, we then demonstrate that conformations with different orientations of the β-ionone ring are characterized by similar excitations. Moreover, other degrees of freedom identified as active in room-temperature molecular dynamics simulations do not yield the shift required to explain the anomalous spectral shape. Our findings indicate that photodissociation experiments are not representative of the optical spectrum of retinal in the gas phase and call for further experimental characterization of the dissociation spectra.

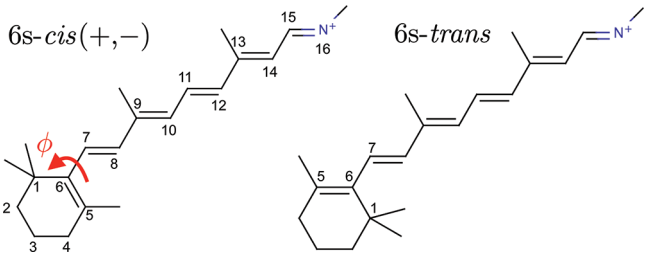


SECTION: Molecular Structure, Quantum Chemistry, General Theory

The retinal protonated Schiff base (RPSB) chromophore represents a fascinating archetype of a photosensitive biological component because it functions as a light detector over a remarkably wide range of absorption energies in visual[1,2] (425−560 nm) and archaeal[3] (480−590 nm) rhodopsins. To understand how the protein tunes absorption over so many wavelengths, it is important to establish the spectral behavior of retinal in the gas phase to discern intrinsic geometric and electronic features from the response of the chromophore to the biological microenvironment. Photoinduced dissociation spectroscopy[4,5] represents, in principle, an ideal experimental technique for this purpose as the measurement can probe charged molecules in the gas phase at low target densities in the absence of external perturbations. For all-trans RPSB, early dissociation spectroscopy experiments[4] produced an absorption spectrum with a strong band at 610 nm and at least two additional shoulders, while a more recent experiment by the same group[5] has obtained a spectrum with a practically flat plateau between 530 and 610 nm. These observations have separately received a high level of attention and have motivated many theoretical attempts to quantitatively reproduce them.[5−12] Unfortunately, the difference between the two spectra was not well-rationalized experimentally nor further characterized, for instance, by intensity dependence studies. This is particularly relevant because retinal is not the only chromophore for which multiple distinct dissociation spectra have been produced depending on experimental factors such as excitation laser power.[13−16] In measurements for the green fluorescent protein chromophore, it has in fact recently been argued that the dissociation spectrum does not reliably represent the optical spectrum of this system.[15,16]

The unusually flat profile of the dissociation spectrum was explained in terms of temperature effects.[5] The RPSB chromophore could easily isomerize between conformers with different orientations of the β-ionone ring with respect to the plane of the conjugate chain (Chart 1). If these configurations

Chart 1. 6s-cis and 6s-trans Conformers of RPSB



are characterized by excitations spanning the appropriate range, temperature fluctuations could be responsible for the observed experimental plateau in the spectrum. The interpretation of the dissociation spectrum of RPSB in terms of temperature effects was however put forward by modeling the conformer structures with the complete active space self-consistent field[17] (CASSCF) method, which largely lacks inclusion of dynamical

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correlation. This theoretical study cannot therefore be considered conclusive. In fact, calculations employing a very different framework based on density functional theory (DFT) indicate that temperature fluctuations might not significantly affect absorption.[18] Consequently, the role of temperature effects on the gas-phase spectroscopy of RPSB remains an open issue due to uncertainties both on the experimental and theoretical sides. Importantly, visual and archaeal rhodopsins differ in their retinal conformers,[3] and it is therefore relevant to assess whether internal variations in the structure or the interactions with the protein pocket are responsible for shifts in the spectrum.

Here, we present a careful investigation of the impact of conformational variations of the chromophore on its absorption properties. Importantly, we do not assume that a given theoretical technique gives us the correct answer but carefully calibrate the approach employed to construct the structural model of the chromophore in the ground state. This is a key point in our work because the role of an appropriate choice of structures has been often neglected or largely overlooked in previous studies. After we identify which structural models are representative of retinal in the gas phase, we then analyze how to accurately compute the excitation energies of the multiple conformers to elucidate the structural dependence of absorption in RPSB.

We first investigate the choice of theoretical method to construct the conformers for the 6s-trans, 6s-cis(+), and 6s-cis(−) orientations of the β-ionone ring (Chart 1) and to estimate the height of the barrier between them. We employ a selected set of modern DFT functionals characterized by a different percentage of exact exchange, namely, BLYP[19,20] (0%), B3LYP[21,22] (20%), M06-2X[23] (54%), and M06-HF[23] (100%). This choice of exchange−correlation approximations is particularly appropriate for retinal because it spans a significant range of percentages of exact exchange, which is one of the most important parameters influencing the structural properties of this chromophore[24] (see also the Supporting Information (SI)).

For each functional, we perform a series of geometrical optimizations at constrained angles of the β-ionone ring, ϕ (Chart 1), while relaxing all other degrees of freedom. As shown in Figure 1a, the resulting potential energy curves are rather different, in particular, as regards the height of the barrier between trans and cis conformers, which decreases when we increase the amount of exact exchange. The relative stability of the conformers also varies, with trans/cis being favored at low/high exchange. Finally, several structural features of the minima are also affected, most notably, the twist angle of the β-ionone ring in the cis orientations and the bond length alternation, both significantly increasing for higher amounts of exchange (see the SI).

To establish which structures most reliably describe retinal, we compute the ground-state energies of all geometries with an accurate highly correlated approach, namely, the complete active space second-order perturbation theory[26,27] (CASPT2). As shown in Figure 1b, the M06-2X functional with its intermediate amount of exact exchange leads to the most accurate description of the geometrical features of retinal. The CASPT2 energies computed on the M06-2X structures are in fact the lowest, while the use of either lower or higher percentages of exact exchange results in higher ground-state energies. The CASSCF structures display instead the worst performance, yielding the highest CASPT2 ground-state

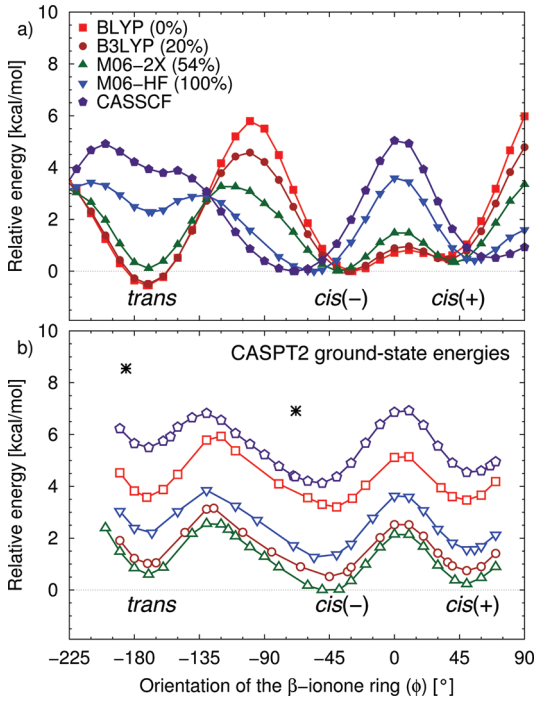


Figure 1. (a) DFT and CASSCF potential energy curves as a function of the orientation of the β-ionone ring, computed with different functionals (full symbols). The percentage of exact exchange is given in parentheses. (b) CASPT2 ground-state energies computed on the DFT and CASSCF ground-state geometries (empty symbols). We also evaluate the CASPT2 energies on the CASSCF ground-state geometries reported in ref 5 (star symbols).[25]

energies. Therefore, CASSCF geometries are not appropriate to describe gas-phase retinal despite having been widely employed for numerous retinal studies in the past decade. It is also important to stress that the interpretation of the broad plateau in the dissociation spectrum in terms of temperature effects was in fact based on the use of CASSCF structural models.[5] For completeness, we compute accurate ground-state energies precisely on the two geometries previously employed to explain the spectrum[5] and obtain the two highest-energy points[25] in Figure 1b.

Having established the reliability of M06-2X to describe the structural features of retinal, we are now in a position to assess whether the presence of different isomers might explain the flat plateau in the photoinduced dissociation spectrum. Because the barriers between the isomers are on the order of 2 kcal/mol, they can be easily overcome at room temperature over the time scales of the action spectroscopy experiments[5] with trapping times of 40 ms. The various conformers can therefore coexist at room temperature, but are their excitations compatible with the spectral features observed in dissociation spectroscopy?

To answer this question, we compute the excitation energies of the RPSB conformers with two highly correlated approaches, namely, the CASPT2 method also employed for the ground state and the n-electron valence-state perturbation theory[28] (NEVPT2). The recently developed NEVPT2 method is more advanced than CASPT2 and generally more accurate. The key results are summarized in Table 1, where we report the vertical excitations computed on our M06-2X structures of the 6s-cis and 6s-trans conformers. Both methods predict that the excitations of the three conformers are comparable, only differing by about 30 nm. Moreover, the vertical excitations are

Table 1. Vertical Excitation Energies (nm) of the Retinal Conformers, Computed with CASPT2 and NEVPT2

geometry	conformer/ϕ	CASPT2	NEVPT2
M06-2X	trans/−170.1	561	551
	cis(−)/−38.3	537	530
	cis(+)/41.8	532	523
CASSCF[a]	trans/−185.7	544	566
	cis(−)/−68.2	473	477

[a]Geometries from ref 5.

clustered on the blue edge of the dissociation spectrum in the range of 520−560 nm. Therefore, these highly correlated approaches strongly support that the excitations of the three conformers are comparable and do not span the experimental plateau extending between 530 and 610 nm.

In Table 1, we also report the excitations computed on the CASSCF geometries from ref 5 because the interpretation of the dissociation spectrum in terms of temperature effects was based on the use of these geometries. The excitations of the 6s-trans and 6s-cis conformers are different, and the value for the cis orientation estimated between 473 and 477 nm falls outside of the experimental plateau. We note that our CASPT2 excitations differ significantly from the values of 547 and 620 nm obtained in ref 5 for the cis and the trans conformers, respectively, with the use of a different zero-order Hamiltonian. The IPEA Hamiltonian[27] employed in this work yields, on average, more accurate excitations, and its superior performance is here corroborated by the good agreement found between our CASPT2 values and the NEVPT2 excitations. Consequently, we stress once again that our calibration of the method to generate the structures as well the excitations clearly indicates that CASSCF is not suitable to describe the structural features of RPSB.

Even though the conformers of retinal are characterized by similar excitations clustering at the blue edge of the spectrum, it is still possible that retinal may visit configurations with red-shifted excitations while fluctuating between the different orientations of the β-ionone. To investigate this possibility, we compute the excitation along the constrained path between the two ground-state minima as reported in Figure 2. We observe that, as retinal rotates between the trans and the cis configurations, the excited-state energy either roughly parallels the ground-state curve or raises even further, yielding higher values for the excitation. Therefore, temperature fluctuations can induce retinal to visit conformations characterized by different angles of the β-ionone ring, but this motion will result in blue-shifted excitations at wavelengths shorter than 520 nm. In summary, temperature fluctuations of the β-ionone ring cannot explain the broad feature in the spectrum.

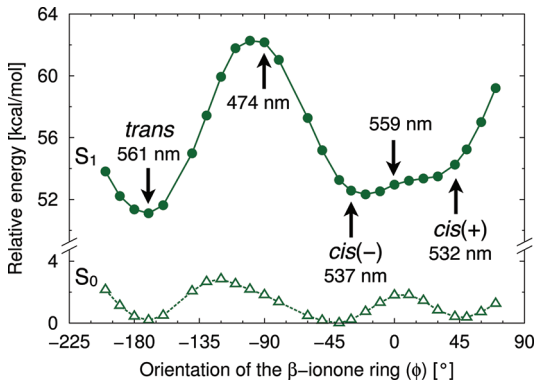
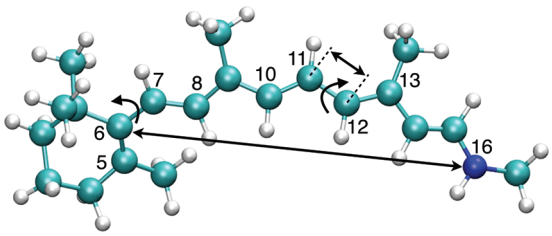


Figure 2. Ground- and excited-state energies computed with CASPT2 on top of M06-2X ground-state geometries.

An obvious question is whether we have correctly identified the β-ionone angle as being the degree of freedom responsible for the anomalous broadening of the absorption spectrum. To investigate the possible influence of other active coordinates, we perform a room-temperature ab initio molecular dynamics simulation of retinal in the cis(−) configuration for about 23 ps. This study is computationally feasible if we employ a generalized gradient approximation (0% exchange) and gives us a good indication of the most relevant degrees of freedom at finite temperatures. In agreement with previous studies,[29] our dynamics reveals the particular activity of three coordinates, (i) the global bend of the chromophore, which can be monitored via the C_6−N_{16} distance, (ii) the dihedral angle C_{10}−C_{11}−C_{12}−C_{13}, and (iii) the length of the C_{11}−C_{12} bond, which is inversely correlated with the bond length alternation in retinal (see Chart 2). We then further analyze these specific coordinates with more accurate approaches. In particular, we employ the M06-2X functional to compute a representative optimal path for constrained values of the coordinate in a range centered on the cis(−) minimum plus/minus twice the fluctuations as estimated along the molecular dynamics run (see the SI). Along these three paths, we then evaluate the CASPT2 excitations. As shown in Figure 3, the variations in these three coordinates do

Chart 2. Active coordinates at Room Temperature[a]



[a]The global bend of the chromophore monitored via the C_6−N_{16} distance, the dihedral angles C_5−C_6−C_7−C_8 and C_{10}−C_{11}−C_{12}−C_{13}, and the C_{11}−C_{12} bond length.

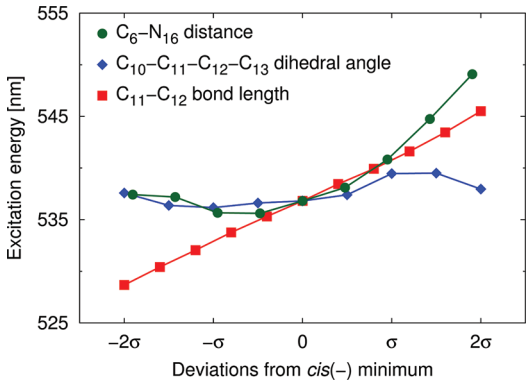


Figure 3. Excitation energies along the coordinates most active at room temperature, computed with CASPT2. The coordinates are centered at the cis(−) minimum, and their room-temperature fluctuations (σ) are 0.2 Å for the C_6−N_{16} distance, 0.025 Å for the C_{11}−C_{12} bond length, and 10° for the C_{10}−C_{11}−C_{12}−C_{13} dihedral angle.

not significantly affect the excitations. A red shift of less than 10 nm is observed when the $C_{11}-C_{12}$ bond is stretched in correspondence to a bond length alternation shorter by about 0.02 Å. A similar red shift is obtained for an increased C_6-N_{16} distance, while varying the $C_{10}-C_{11}-C_{12}-C_{13}$ dihedral angle does not affect the excitation. Consequently, neither the different orientations of the β-ionone ring nor the other degrees of freedom identified as most active at room temperature lead to the required red shift to explain the plateau in the photoinduced destruction absorption spectrum.

In summary, we have employed here state-of-the-art first-principle approaches to investigate the absorption properties of RPSB in the gas phase and the impact of temperature effects on the spectrum. We first demonstrated the fundamental importance to carefully calibrate the method employed to generate the structure of this chromophore and found that M06-2X represents an accurate tool for this purpose, in contrast to the commonly used CASSCF method, which we proved is inadequate. We then investigated the different conformations of the β-ionone ring, which are found to be separated by low barriers and therefore easily accessible at room temperature. These conformers are however characterized by rather similar excitations and clustered at the blue edge of the photodissociation spectrum. Consequently, the different orientations of the ring cannot be responsible for the observed broad and flat plateau in the absorption spectrum. In addition, other degrees of freedom that we identify as active in room-temperature molecular dynamics simulations do not lead to the shift required to explain the anomalous spectral shape in terms of temperature effects.

While the disagreement between our theoretical results and those of photodestruction experiments may appear distressing, we recall that experiments with dissociation spectroscopy appear to be plagued by potential complications such as the possible presence of multiphoton dissociation channels and the consequential nontrivial dependence of the shape of the spectrum on the excitation laser power.[15,16] In recent years, rather distinct spectra have been obtained for the same chromophore,[13−15] retinal included,[4,5] so that the correlation between dissociation and optical spectra is becoming less clear with time. Our calculations for RPSB raise serious concerns on the interpretation of photoinduced dissociation spectroscopy experiments also for retinal. They provide compelling evidence that temperature effects cannot be responsible for the flat plateau between 530 and 610 nm in the spectrum and that these model experiments cannot be considered representative of the optical spectrum of retinal. Our theoretical findings as well as the significant difference between available photo-dissociation spectra of retinal[4,5] call for further experimental investigations and careful characterization of these spectra, in particular, through intensity dependence studies.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Computational details. M06-2X geometries of the RPSB conformers. CASSCF and CASPT2 (with and without the IPEA shift) excitations. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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