Modelling Time-Resolved Two-Dimensional Electronic Spectroscopy of the Primary Photoisomerization Event in Rhodopsin

Ivan Rivalta,*‡§ Artur Nenov,‡ Oliver Weingart,§ Giulio Cerullo,‖ Marco Garavelli,*‡§ and Shaul Mukamel*‡⊥

*Université de Lyon, CNRS, Institut de Chimie de Lyon, École Normale Supérieure de Lyon, 46 Allée d’Italie, F-69364 Lyon Cedex 07, France
‡Dipartimento di Chimica “G. Ciamician”, Università di Bologna, V. F. Selmi 2, 40126 Bologna, Italy
§Institut für Theoretische Chemie und Computerchemie, Heinrich-Heine-Universität Düsseldorf, Universitätsstr. 1, 40225 Düsseldorf, Germany
‖IFN-CNR, Dipartimento di Fisica, Politecnico di Milano, Piazza L. da Vinci 32, 20133 Milano, Italy
⊥Department of Chemistry, University of California, Irvine, California 92697-2025, United States

Supporting Information

ABSTRACT: Time-resolved two-dimensional (2D) electronic spectra (ES) tracking the evolution of the excited state manifolds of the retinal chromophore have been simulated along the photoisomerization pathway in bovine rhodopsin, using a state-of-the-art hybrid QM/MM approach based on multiconfigurational methods. Simulations of broadband 2D spectra provide a useful picture of the overall detectable 2D signals from the near-infrared (NIR) to the near-ultraviolet (UV). Evolution of the stimulated emission (SE) and excited state absorption (ESA) 2D signals indicates that the $S_1 \rightarrow S_N$ (with $N \geq 2$) ESAs feature a substantial blue-shift only after bond inversion and partial rotation along the cis $\rightarrow$ trans isomerization angle, while the SE rapidly red-shifts during the photoinduced skeletal relaxation of the polyene chain. Different combinations of pulse frequencies are proposed in order to follow the evolution of specific ESA signals. These include a two-color 2DVis/NIR setup especially suited for tracking the evolution of the $S_1 \rightarrow S_2$ transitions that can be used to discriminate between different photochemical mechanisms of retinal photoisomerization as a function of the environment. The reported results are consistent with the available time-resolved pump–probe experimental data, and may be used for the design of more elaborate transient 2D electronic spectroscopy techniques.

INTRODUCTION

Rhodopsin (Rh) is a type II opsin found in the photoreceptor rod cells of the retina, where it is used for night and peripheral vision. The chromophore responsible for light reception in Rh is the polyene 11-cis-retinal, which covalently binds the opsin apoprotein through a Schiff base (SB) linkage to the lysine residue Lys296, forming the 11-cis-retinal protonated Schiff base (PSB11) shown in Figure 1. Rh is the only type II opsin for which the X-ray structure has been resolved,1–7 representing a paradigm system for fundamental studies of the primary event in vision and a reference model for the design of biomimetic photochromic devices.8–10 Upon absorption of visible light (with an absorption maximum at $\sim$500 nm, i.e., at a frequency of $\nu_{\text{max}} \approx 20,000 \text{ cm}^{-1}$), PSB11 undergoes ultrafast (200 fs) 11-cis $\rightarrow$ all-trans isomerization (see Figure 1a), triggering protein conformational changes that initiate the signal transduction processes in vision.11 Fundamental understanding of retinal chromophore photochemistry and disentanglement of environmental effects, such as the electrostatic effects of the embedding proteins or the surrounding solvent molecules, are crucial for the design of biomimetic photosensitive devices.

Extensive computational studies aimed to unravel environmental effects which tune the photophysical and photochemical properties of retinals have been recently reviewed,12 indicating possible molecular mechanisms explaining the different behaviors of retinal photoisomerization in various surroundings. Different environmental conditions could, in fact, alter the intrinsic properties of the chromophore by modulating the interplay between the covalent excited states and the ionic “photoactive” $S_1$ state. It has been proposed that, according to the so-called three-state model, the covalent $S_2$ excited state is directly involved in slowing down the photoisomerization reaction in bacteriorhodopsin,13,14 and likely also in solvated...

Special Issue: James L. Skinner Festschrift

Received: March 13, 2014
Revised: May 2, 2014

© XXXX American Chemical Society
retinals, with an avoided crossing between the S₁ and S₀ surfaces giving rise to a transition state (TS) along the S₁ state photoisomerization pathway. Indeed, the presence of an energy barrier on the S₁ energy surface before reaching the conical intersection (CI) with the ground state (GS, or S₀) would slow down the photoreaction rate and make the photoisomerization itself less efficient. In Rh on the other hand, the retinal photoisomerization occurs on a steep and barrierless reaction path involving only the ionic S₁ state and directly leading to a peaked CI, which has the form of a twisted torsional motion of the central double bond characterize the electronic structures of selected critical points along the minimum energy path (MEP) of the S₁ energy surface, see Figure 1b, by means of a state-of-the-art hybrid quantum mechanics (QM)/molecular mechanics (MM) approach based on wave function methods, including complete active space self-consistent field (CASSCF) and second-order multireference perturbation theory (CASPT2). This methodology has shown to provide consistent photophysical and photochemical data for retinals in different environments, including vacuum, protein, and solution, in excellent agreement with chiroptical data, absorption spectroscopy, resonance Raman, and ultrafast optical spectroscopy experiments. A set of data collected from first-principles calculations has been used to simulate time-resolved two-dimensional (2D) electronic spectroscopy experiments of photoactivated rhodopsin. 2D electronic spectroscopy (2DES) is the ultimate third-order nonlinear optical experiment, combining high spectral and temporal resolution and providing a wealth of information which usually remains hidden in conventional pump–probe experiments, such as intra- and intermolecular electronic couplings (that are resolved as off-diagonal cross-peaks in a 2D map), pathway specific signals, homogeneous and inhomogeneous broadening, etc. In 2DES, a sequence of three ultrashort laser pulses interacts with the sample, and the emitted signal field is detected as a function of the three time delays (τ₁, τ₂, and τ₃); see Figure S1 in the Supporting Information. The 2D rephasing (K₁) and non-rephasing (KII) signals can be obtained by double Fourier transformation (FT) with respect to times τ₁ and τ₂, providing 2D signals as a function of two frequencies, the “pump frequency” Ω(τ₁), i.e., Ω₁, and the “probe frequency” Ω(τ₂), i.e., Ω₂, given a fixed value of the “population time” τ₃. For each value of the population time τ₃, the 2DES spectrum can be interpreted, to a first approximation, by associating to the Ω₁ axis the absorption spectrum sensed by the excitation pulses and to the Ω₂ axis the transient absorption spectrum sensed by the probe pulse. A complete description of excited state relaxation is obtained by collecting a sequence of 2D maps as a function of population time τ₃. Thus, with 2DES, the photoisomerization process of retinals can be monitored in proteins and in solution with the best possible combination of spectral and temporal resolution. Such types of experiments should be designed by setting the pump frequency resonant with ω₃, i.e., populating the spectroscopic state S₃, and probing the absorptions and/or the emission of the wave packet evolving on the S₁ surface with different probe frequencies and at various probe delays.

Experimental evidence of two distinct mechanisms for retinal photoisomerization in solution and in Rh is lacking, and the available ultrafast pump–probe experiments, which are intrinsically one-dimensional (1D), do not provide sufficient data to describe the interplay between ionic and covalent states during photoisomerization. Ad hoc designed ultrafast nonlinear optical spectroscopy experiments are therefore needed to shed light on the retinal photoisomerization mechanisms in different environments. In order to guide the design of new time-resolved optical spectroscopy experiments, in this work, we report full characterization of the excited state manifolds of PSB11 in Rh along the retinal photoisomerization pathway. We have characterized the electronic structures of selected critical points along the minimum energy path (MEP) of the S₁ energy surface, see Figure 1b, by means of a state-of-the-art hybrid quantum mechanics (QM)/molecular mechanics (MM) approach based on wave function methods, including complete active space self-consistent field (CASSCF) and second-order multireference perturbation theory (CASPT2). This methodology has shown to provide consistent photophysical and photochemical data for retinals in different environments, including vacuum, protein, and solution, in excellent agreement with chiroptical data, absorption spectroscopy, resonance Raman, and ultrafast optical spectroscopy experiments. A set of data collected from first-principles calculations has been used to simulate time-resolved two-dimensional (2D) electronic spectroscopy experiments of photoactivated rhodopsin. 2D electronic spectroscopy (2DES) is the ultimate third-order nonlinear optical experiment, combining high spectral and temporal resolution and providing a wealth of information which usually remains hidden in conventional pump–probe experiments, such as intra- and intermolecular electronic couplings (that are resolved as off-diagonal cross-peaks in a 2D map), pathway specific signals, homogeneous and inhomogeneous broadening, etc. In 2DES, a sequence of three ultrashort laser pulses interacts with the sample, and the emitted signal field is detected as a function of the three time delays (τ₁, τ₂, and τ₃); see Figure S1 in the Supporting Information. The 2D rephasing (K₁) and non-rephasing (KII) signals can be obtained by double Fourier transformation (FT) with respect to times τ₁ and τ₂, providing 2D signals as a function of two frequencies, the “pump frequency” Ω(τ₁), i.e., Ω₁, and the “probe frequency” Ω(τ₂), i.e., Ω₂, given a fixed value of the “population time” τ₃. For each value of the population time τ₃, the 2DES spectrum can be interpreted, to a first approximation, by associating to the Ω₁ axis the absorption spectrum sensed by the excitation pulses and to the Ω₂ axis the transient absorption spectrum sensed by the probe pulse. A complete description of excited state relaxation is obtained by collecting a sequence of 2D maps as a function of population time τ₃. Thus, with 2DES, the photoisomerization process of retinals can be monitored in proteins and in solution with the best possible combination of spectral and temporal resolution. Such types of experiments should be designed by setting the pump frequency resonant with ω₃, i.e., populating the spectroscopic state S₃, and probing the absorptions and/or the emission of the wave packet evolving on the S₁ surface with different probe frequencies and at various probe delays.

In this scenario, the unique advantage of using 2DES instead of conventional pump–probe spectroscopy is the possibility of resolving inhomogeneous broadening of the different signals along the Ω₁ axis, since there is only one diagonal peak present in the 2D map due to the (GS)S₀ → S₁ transition, and off-diagonal cross-peaks are absent. Unfortunately, computational modeling of the inhomogeneous broadening of the electronic transitions requires a complete sampling of the configurational space of the ground state and the characterization of a very large number of electronic structures along the photoisomerization dynamics, which is challenging, highly computationally demanding, and beyond the scope of the present work. Nevertheless, the simulated 2DES spectra along the “static” MEP of the retinal photoisomerization can provide information on the spectral regions where signals with opposite signs, e.g., excited state absorption (ESA) versus stimulated emission (SE), overlap. In these regions, the inhomogeneous broadening of each electronic transition could significantly affect the shape of the 2D overall signal. Such an effect could be observed

Figure 1. Schematic representation of the cis → trans photoisomerization reaction of PSB11 in rhodopsin (panel a) and the MEP calculated at the CASPT2/CASSCF level (panel b) describing the photoisomerization process from the FC point to the twisted CI funnel, through a point (FCREL) where only skeletal bond stretchings have been relaxed (FCREL) and subsequent structures with increasing (absolute) twisting angles.
experimentally, providing information on the time evolution of the 2D signals, which in conjunction with the simulated 2DES spectra reported here could give “time-resolved” atomistic details of the retinal photoisomerization reaction in Rh. Reliable characterization of temporal evolution of the electronic levels during the photoisomerization reaction requires first-principles simulations. Therefore, accurate simulations of 2DES spectra will be performed using the sum-over-states (SOS) approach,\textsuperscript{31} as implemented in our SOS/QM/MM method,\textsuperscript{32,33} following the evolution of the 2DES map along the photoisomerization path, otherwise not achievable with a more simple parametrized approach based on Frenkel exciton models.\textsuperscript{32,33} Accurate simulations of the 2DES spectra have the advantage of predicting the detectable 2D signals, allowing the design of tailored experiments suited for observation of specific signals. Despite the accuracy of electronic structure calculations of the MEP points, the simulated 2DES spectra still represent a coarse picture of the experimental spectra, since here we assume that during time intervals $t_1$ and $t_2$, the electronic energy levels remain fixed, i.e., a “static” snapshot of the photoisomerizing chromophore can be recorded in an experimental 2D spectrum. To follow more precisely the evolution of the electronic excited state manifold during a real measurement, a nonadiabatic propagation of the eigenstates should be performed, but such a level of complexity is beyond the scope of this work.

Here, we focus on the detection of ESA involving low-lying covalent states of PSB11, because of their relevance in the mechanism of retinal photoisomerization. In particular, with the proposed 2DES experiments, it could be possible to detect the $S_1 \rightarrow S_2$ transitions during the photoisomerization and determine the fluctuations of the $S_1/S_2$ energy gap separation, elucidating the role of the low energy ionic and covalent excited states on retinal photoisomerization in Rh. A comparison between analogous 2DES experiments carried out in other proteins and in solution could finally discriminate between different photochemical mechanisms of retinal photoisomerization as a function of the (bio)chemical environment. Finally, it has been proposed that more elaborate multipulse two-dimensional techniques measuring the fifth-order nonlinear response, i.e., “transient” 2DES, could also be very informative for determining the retinal photoisomerization mechanism in different environments.\textsuperscript{12} The electronic structure calculations and the simulations of time-resolved 2DES spectra reported here represent the groundwork for the design of such elaborate multipulse spectroscopy experiments.

## THEORETICAL METHODS

### Electronic Structure Calculations

Constrained geometry optimizations were performed along the C$_{10}$–C$_{11}$–C$_{12}$–C$_{13}$ torsion coordinate of the PSB11 ranging from $-13^\circ$ to $90^\circ$ at the CI (absolute values of the twisting angle in degrees are used hereafter) were performed using a combined CASSCF(12,12)/6-31G*//Amber (QM/MM) approach and the G09 Benny-algorithm\textsuperscript{44} through COBRAMM.\textsuperscript{35} The QM region was described using Molpro’s CASSCF routines.\textsuperscript{36} The whole $\pi$-system of retinal and the N-terminal methylene group were considered. The $\beta$-ionone ring and its methyl groups were described by Amber99ff parameters.\textsuperscript{22,37} QM and MM regions were connected through hydrogen link-atoms,\textsuperscript{38} the $\beta$-ionone moiety and the two water molecules near the Schiff-base terminus were free to move during optimization, and the rest of the protein pocket was kept fixed at their crystal structure positions.\textsuperscript{39} The ground state equilibrium structure was optimized with single state CASSCF considering only $S_0$ and the torsion path was obtained through state-averaging (SA) involving the $S_0$ and $S_1$ states with equal weight. SA gradients were computed using Molpro’s CPMCSCF routine. As initial structures, we used optimized geometries obtained in previous work.\textsuperscript{40} On top of the newly optimized structures, QM/MM excited state calculations were performed with Molcas 7.7\textsuperscript{41} using the SA-CASSCF(12,12)/6-31G* method followed by energy refinement at the single-state CASPT2 level (hereafter named CASPT2/CASSCF), including all valence $\pi$-electrons and $\pi$-orbitals of the chromophore in the active space, with the MM part of the system treated as external point charges in both CASSCF and CASPT2 calculations. In the CASSCF state-averaging procedure, 70 states were included to ensure that enough roots are comprised in the CASSCF and CASPT2 calculations in order to describe the energy ranges reported for the 2D spectra. An imaginary shift\textsuperscript{32} of 0.2 and a zero-order Hamiltonian IPEA shift of 0.0 were used. Transition dipole moments were calculated at the CASSCF level using the RASSI module of Molcas 7.7.\textsuperscript{43}

### Two-Dimensional Spectroscopy Simulations

The temporal evolution of the nonlinear response reveals electronic and geometrical changes in the system. While the recovery of the ground state bleach (B, blue arrow in Figure 2) depends on the excited state decay rates, the stimulated emission (E, cyan arrow in Figure 2) and excited state absorption (ESA, red arrows in Figure 2) exhibit characteristic shifts along the excited state deactivation pathways. In this work, we employ minimum energy path (MEP) calculations on the first excited state $S_1$ and probe the higher excited manifold and the ground state at selected geometries along the MEP, following the $S_1 \rightarrow S_0$ (with $N \geq 2$) and $S_1 \rightarrow S_N$ transitions at a given MEP point, respectively, while keeping the pump pulse pair in resonance with the GS $\rightarrow S_1$ transition at the Franck–Condon (FC) level.
point. Evolution in the ground state is neglected; thus, the B signal arises by probing the ground state equilibrium geometry. We mainly assume that during time intervals $t_1$ and $t_3$ the electronic energy levels remain fixed, i.e., their propagation is slow with respect to the ultrafast measurement, which allows associating the electronic structure of a static MEP point with its corresponding 2D spectrum. Additional approximations are adopted: the system evolves coherently along $S_1$ following

Table 1. Excited States of the FC, FC<sub>REL</sub>, 30°, 60°, and 90°(CI) Structures<sup>a</sup>  

<table>
<thead>
<tr>
<th>root</th>
<th>type</th>
<th>coeff</th>
<th>$f$</th>
<th>label</th>
<th>root</th>
<th>type</th>
<th>coeff</th>
<th>$f$</th>
<th>label</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>H → L</td>
<td>-0.75</td>
<td>0.73</td>
<td>B</td>
<td>S1</td>
<td>H → L</td>
<td>-0.59</td>
<td>0.81</td>
<td>B</td>
</tr>
<tr>
<td>S2</td>
<td>D(H ⇒ L)</td>
<td>0.47</td>
<td>0.06</td>
<td>D</td>
<td>S2</td>
<td>H → L + 1</td>
<td>-0.40</td>
<td>0.27</td>
<td>D</td>
</tr>
<tr>
<td>S3</td>
<td>D(H, H → 1 ⇒ L)</td>
<td>0.37</td>
<td>0.11</td>
<td>3</td>
<td>S3</td>
<td>D(H, H → 1 ⇒ L)</td>
<td>0.40</td>
<td>0.07</td>
<td>3</td>
</tr>
<tr>
<td>S4</td>
<td>H − 1 ⇒ L</td>
<td>0.37</td>
<td>0.75</td>
<td>4</td>
<td>S4</td>
<td>D(H, H − 2 ⇒ L)</td>
<td>0.39</td>
<td>0.05</td>
<td>i</td>
</tr>
<tr>
<td>S7</td>
<td>H − 5 ⇒ L</td>
<td>0.45</td>
<td>0.13</td>
<td>7</td>
<td>S5</td>
<td>H − 1 ⇒ L</td>
<td>0.54</td>
<td>0.59</td>
<td>4</td>
</tr>
<tr>
<td>S9</td>
<td>H − 2 ⇒ L</td>
<td>-0.52</td>
<td>0.44</td>
<td>9</td>
<td>S6</td>
<td>D(H − 1 ⇒ L)</td>
<td>-0.38</td>
<td>0.08</td>
<td>8</td>
</tr>
<tr>
<td>S10</td>
<td>H − 2 ⇒ L</td>
<td>0.39</td>
<td>0.07</td>
<td>10</td>
<td>S7</td>
<td>H − 5 ⇒ L</td>
<td>-0.38</td>
<td>0.07</td>
<td>7</td>
</tr>
<tr>
<td>S11</td>
<td>H − 2 ⇒ L</td>
<td>0.29</td>
<td>0.07</td>
<td>11</td>
<td>S8</td>
<td>D(H, H ⇒ L + 1)</td>
<td>0.26</td>
<td>0.24</td>
<td>i</td>
</tr>
<tr>
<td>S12</td>
<td>H → L + 1</td>
<td>0.32</td>
<td>0.36</td>
<td>12</td>
<td>S9</td>
<td>H → L + 1</td>
<td>0.46</td>
<td>0.28</td>
<td>12</td>
</tr>
<tr>
<td>S14</td>
<td>H − 3 ⇒ L</td>
<td>0.33</td>
<td>0.14</td>
<td>14</td>
<td>S11</td>
<td>H − 4 ⇒ L</td>
<td>-0.61</td>
<td>0.04</td>
<td>13</td>
</tr>
<tr>
<td>S12</td>
<td>H − 2 ⇒ L</td>
<td>0.35</td>
<td>0.08</td>
<td>ii</td>
<td>S13</td>
<td>H − 5 ⇒ L</td>
<td>0.25</td>
<td>0.08</td>
<td>iii</td>
</tr>
<tr>
<td>S13</td>
<td>H − 4 ⇒ L</td>
<td>0.58</td>
<td>0.39</td>
<td>13</td>
<td>S14</td>
<td>H → L + 2</td>
<td>0.35</td>
<td>0.08</td>
<td>iv</td>
</tr>
<tr>
<td>S15</td>
<td>D(H − 1, H − 3 ⇒ L)</td>
<td>0.25</td>
<td>0.03</td>
<td>13</td>
<td>S15</td>
<td>D(H − 1, H − 3 ⇒ L)</td>
<td>0.25</td>
<td>0.03</td>
<td>13</td>
</tr>
</tbody>
</table>

<sup>a</sup>Only excited states having $S_1 → S_2$ transitions (with $N = 0, >2$) with oscillator strength ($f$) larger than 0.02 are reported. Electron excitations refer to active space orbitals in Figure S2 of the Supporting Information. Labels of $S_1$ and $S_2$ transitions are reported for assignment of 2D peaks in Figures 2–4.
closely the MEP; no population transfer between states occurs before the CI (i.e., signal intensities depend only on the transition dipole moments). It should be noted that MEP calculations do not contain dynamical and temporal information; therefore, we cannot directly extract time delays. For simulation of the 2DES spectra, we have combined the QM/MM methodology, which provides the transition dipole moments at the CASSCF level and CASPT2 corrected transition energies, with the SOS approach, as implemented in our SOS/QM/MM approach documented elsewhere.

SOS calculations were performed with Spectron 2.7, reading the energy levels calculated at each MEP point in order to include the GS bleaching (GSB), i.e., by maintaining the FC $S_0\rightarrow S_1$ energy gap and rigidly shifting all of the $S_n$ energies calculated at the MEP point in order to align the $S_1$ energy with the FC $S_1$ energy. A constant dephasing of 200 cm$^{-1}$ has been employed, with finite transform-limited Gaussian pulse envelopes corresponding to a bandwidth of 5000 cm$^{-1}$. Infinite bandwidth pulses have also been used when specified. We report the combined rephasing and nonrephasing (KI + KII) signals (hereafter named 2D signals), which have absorptive features and can be collected experimentally with either partially collinear pump–probe geometry or heterodyne detected three-pulse photon echo configurations, see Figure S1 in the Supporting Information. Spectra are plotted on a logarithmic scale.

**RESULTS**

*Excited State Manifolds along the Photoisomerization Pathway.* Figure 2 shows the energy levels of PSB11 along the $S_1$ photoisomerization MEP for energies up to 34 000 cm$^{-1}$ (4.2 eV) from the spectroscopic state. The electronic structures calculated at the CASPT2/CASSCF level are reported from the FC region to the CI (at $\sim$90$^\circ$ twisting angle), considering a structure where only skeletal bonds have been relaxed (FC$_{REL}$), and two other structures with intermediate $cis\rightarrow trans$ isomerization angles, i.e., 30 and 60$^\circ$. In the FC region, the first excited state accessible from the spectroscopic $S_1$ state is the double excitation $H\rightarrow L$ (hereafter $D$), i.e., the above-mentioned covalent $S_1$ state (see green bar in Figure 2), located at $\sim$8000 cm$^{-1}$ from $S_0$, in agreement with previous calculations.

The molecular orbital labels, from $H$ to $S$ to $L + S$, refer to the 12 active space orbitals in the CASSCF(12,12) calculations reported in Figure S2 in the Supporting Information. Above 10 000 cm$^{-1}$ from $S_0$, the first two excited states encountered are the covalent $S_1$ state, characterized predominantly by double ($H, H\rightarrow L$) and single ($H\rightarrow L$) excitations, see Table 1, and the ionic $S_1$ state, corresponding to a single $H\rightarrow L$ excitation. The $S_1\rightarrow S_2$ and $S_1\rightarrow S_{13}$ transitions are bright (i.e., with an oscillator strength larger than 0.02, see transitions D, 3, and 4 in Figure 2) and are expected to appear in the near-infrared (NIR) and the visible (red) spectral regions, respectively. At higher energies, in the range 20 000–30 000 cm$^{-1}$ from $S_0$, several excited states are found, generally characterized by single excitations into the lowest virtual orbitals (L and L + 1). In particular, the $S_1\rightarrow S_3$ transition (see transition 7 in Figure 2) has a transition energy close to the fundamental $S_0\rightarrow S_1$ transition frequency in the blue-green region of the visible spectrum, while higher-lying excited states, i.e., $S_{12}$ and $S_{14}$, give rise to a series of ESA bands in the violet and near-UV spectral regions (see transitions 9–12 and 14 in Figure 2).

Upon photoexcitation to the spectroscopic $S_1$ state, the retinal chromophore undergoes skeletal relaxation inducing bond order inversion and formation of a (transient) relaxed state (FC$_{REL}$), which is characterized by an elongated C$_{11}$–C$_{12}$ ("originally double") bond which allows barrier-less rotation driving the $11$-cis $\rightarrow$ all-trans isomerization. As shown in Figure 2, the photoinduced skeletal relaxation induces some significant changes in the electronic structure of the chromophore. As expected, the $S_0/S_1$ energy gap is reduced due to the relaxation along the $S_1$ surface, with consequent red-shift of the $S_1\rightarrow S_0$ SE (hereafter $E$, see cyan line in Figure 2). The relative positions of the covalent $S_1$ and $S_2$ states with respect to $S_1$ are not significantly affected by the bond relaxation, and the corresponding transitions from $S_1$ result in being just slightly red-shifted with respect to FC. It is worth noting, however, that upon bond relaxation the single $H\rightarrow L + 1$ excitation provides a significant contribution to the covalent $S_1$ state and the $H\rightarrow L$ excitation contributes to the $S_1$ state. In contrast, the skeletal relaxation induces a blue-shift of transition 4 by $\sim$2000 cm$^{-1}$. Interestingly, above 20 000 cm$^{-1}$, the double $H\rightarrow L$ excitation, corresponding to the $S_3$ state in the FC manifold (and the $S_4$ state in FC$_{REL}$), gives rise to a bright transition (transition 8) which falls in the same spectral region of transition 7 and close to the fundamental $S_1\rightarrow S_1$ transition frequency. Analogously, the single $H\rightarrow 4\rightarrow L$ excitation (the $S_6$ state in the FC manifold and the $S_{11}$ state in FC$_{REL}$) gives rise to a bright transition just below 30 000 cm$^{-1}$ (see transition 13 in Figure 2), which, in return, is dark (i.e., with an oscillator strength smaller than 0.02) in the FC region. It is worth noting that skeletal relaxation also induces stabilization of some high-lying excited states that were not present in the FC region between 25 000 and 34 000 cm$^{-1}$ from $S_1$ (see magenta lines in Figure 2, i.e., transitions $\rightarrow v$).

Starting from the relaxed FC$_{REL}$ structure, the chromophore undergoes rotation about the C$_{11}$–C$_{12}$ bond until reaching the twisted CI at a torsional angle of $\sim$90$^\circ$. Here, we analyze the variations of the excited state manifold induced by this torsional motion. At 30$^\circ$, we find the electronic structure of the chromophore almost unchanged with respect to the FC$_{REL}$ structure, with a slightly less rich excited state manifold due to the disappearance of the transition 7 ESA band. All the conserved ESA bands, including transitions D, 3–4, 8, 12–13, and 1–iii, are slightly blue-shifted with respect to the FC$_{REL}$ structure, while, as expected, the SE keeps red-shifting. In contrast to the first 30$^\circ$ rotation, a subsequent torsion of 30$^\circ$ induces significant changes in the excited state manifold of the chromophore. In fact, at 60$^\circ$ from the FC$_{REL}$ structure, most of the ESA bands are significantly blue-shifted ($\sim$2700 cm$^{-1}$) with respect to the 30$^\circ$ structure. Interestingly, at 60$^\circ$, we observe a significant localization of the CASSCF active orbitals which significantly affects some of the ESAs. In particular, the first excited state above $S_1$ is now characterized mainly by the single $H\rightarrow L + 1$ and double ($H, H\rightarrow L$) excitations; see Table 1. The $S_1$ state still maintains its covalent character, but the transition from $S_1$ (hereafter named transition $D'$) blue-shifts by $\sim$6000 cm$^{-1}$ with respect to the 30$^\circ$ structure. At 60$^\circ$, the $S_1$ state is mainly characterized by the $H\rightarrow 3\rightarrow L$ excitation (in contrast to $H\rightarrow 2\rightarrow L$ at 30$^\circ$) and the transition from $S_1$ (hereafter named transition $3'$) is blue-shifted by $\sim$2750 cm$^{-1}$ with respect to its energy at 30$^\circ$, now falling nearby the $D'$ transition, in the red region of the visible spectrum (at $\sim$15 000 cm$^{-1}$). At energies above 20 000 cm$^{-1}$ from $S_1$, excited states with mainly double excitation nature are found, see Table 1.
with corresponding bright transitions (named \( l \) and \( l \), see brown lines in Figure 2) lying at \(<23 500 \text{ and } ~27 200 \text{ cm}^{-1} \) and transitions 4 and 8 being blue-shifted to \(~24 300 \text{ and } ~30 000 \text{ cm}^{-1} \), respectively, while transition 13 remains unaffected. It should be noted that also the SE is significantly affected at 60°, red-shifting to the near-IR, well below 10 000 cm\(^{-1} \) (i.e., 1 \( \mu \)m). Finally, at the CI structure with a twisting angle of 90°, only a few bright transitions survive, including transitions \( 3 \), \( 4 \), \( 13 \), and \( D' \). All the surviving ESA bands substantially blue-shift with respect to the 60° structure, in particular transition \( D' \), which now lies in the near-UV at \(~33 800 \text{ cm}^{-1} \). Particularly at the CI, transition \( 3 \) falls nearby the fundamental \( S_0 \rightarrow S_1 \) transition frequency and is expected to overlap with the GSB.

**Two-Dimensional Electronic Spectra.** In the above section, we have shown the changes of the electronic structure of retinal embedded in the Rh protein along the \( S_1 \) isomerization pathway, resulting in several ESA bands from \( S_1 \) to higher states, as well as an SE band from \( S_1 \) to \( S_0 \) which peak at frequencies that change very rapidly in time. Such dramatic spectral evolution of the photoinduced signals can be followed in real time using several advanced ultrafast spectroscopy techniques, such as broadband pump-probe spectroscopy or 2DES. The advantage of 2DES over pump-probe is that the nonlinear response can be resolved along the pump frequency (\( \Omega_p \)), allowing characterization of cross-peaks and inhomogeneous broadenings of each electronic transition. In retinal photoisomerization there is a unique spectroscopic state (\( S_1 \)) on which the photochemical reaction is known to take place. In the present work, thus, we are not interested in cross-peaks that arise from electronic coupling between different excited states (e.g., \( S_1 \rightarrow S_2 \), \( S_1 \rightarrow S_3 \) off-diagonal peaks, etc.), since their characterization requires population of higher excited states (\( S_2 \), \( S_3 \), etc.) that have unknown photophysical or photochemical pathways. We thus focus on the ESA and SE signals of the wave packet evolving only along the \( S_1 \) photoisomerization pathway. In this work, we report 2D spectra that can be experimentally collected with either the partially collinear pump-probe geometry\(^{44,45} \) or the heterodyne detected three-diagonal peaks, etc.\(^{46,47} \) using the simplest nonchiral polarization scheme.

**Broadband 2D Spectra.** In the previous section, we have described the evolution of the excited state manifold along the MEP of the photoisomerization, considering those excited states located below \( 34 000 \text{ cm}^{-1} \) from \( S_1 \). In order to visualize the energy positions of these electronic transitions, the possible overlaps between signals and the energy shifting trends of some important transitions, we report in Figure 3 the broadband 2DES spectra calculated using all three laser pulses centered at the absorption maximum frequency of the chromophore (\( \nu_{\text{max}} \) calculated to be at \( 20 875 \text{ cm}^{-1} \) at the CASPT2/CASSCF level) with infinitely broad bandwidth. Infinitely broad pulses reveal all signals that are “ideally” detectable, helping in the selection of experimental parameters (i.e., central probe frequencies and pulse bandwidths) to be used in tailored experiments for the detection of desired electronic transitions.

Figure 3 shows the broadband 2DES spectra at the FC region, i.e., at \( t_2 = 0 \), with the negative signals of both GSS (peak B) and SE (peak E) overlapping at \( \Omega_2 \sim 20 875 \text{ cm}^{-1} \), i.e., the absorption maximum frequency \( \nu_{\text{max}} \) and several positive signals associated with ESA bands in the \( S_2 \) range from \( 8000 \text{ to } 33 000 \text{ cm}^{-1} \). Following the evolution of the excited state manifolds described in the previous section, all of the 2D signals but the GSB are expected to shift along the MEP during the photoisomerization process. The simulations of 2D spectra at \( t_2 > 0 \) are performed on selected geometries of the “static” MEP that cannot be directly associated with effective values of the excited state evolution time \( t_2 \). However, comparison of the reported 2DES spectra with experimental data could allow association of probe delay times with PSB1 structure, providing unprecedented atomistic details of the retinal photoisomerization.

The transition from \( S_1 \) to the covalent \( S_1 \) state (see green boxes in Figure 3, peak D) appears as a positive signal at \( \sim 8000 \text{ cm}^{-1} \) in the NIR at the FC region. Its transition energy does not change significantly upon skeletal relaxation (small red-shift of \( 1100 \text{ cm}^{-1} \) and \( 30° \) \( C_11-C_11 \) bond rotation (blue-shift of \( 1000 \text{ cm}^{-1} \) with respect to FC). A strong blue-shift of the \( S_1 \rightarrow S_2 \) transition is, instead, observed at a twisted angle of 60°, where the electronic excitation contributing to the covalent \( S_2 \) state changes and the corresponding ESA (peak D') appears in the visible at \(~15 160 \text{ cm}^{-1} \). At the twisted CI, the \( S_2 \) state is highly destabilized and the peak D' lies at very high energies (>30 000 cm\(^{-1} \)), in the near-UV.

The \( S_1 \rightarrow S_3 \) transition (peak 3) behaves similarly to the \( S_1 \rightarrow S_2 \) transition along the photoisomerization path. In fact, up to 30°, its transition energy is almost unchanged (between 13 100 and 13 900 cm\(^{-1} \) with respect to its value in the FC region (i.e., \( 13 820 \text{ cm}^{-1} \)), while a pronounced blue-shift of \( \sim 2500 \text{ cm}^{-1} \) is observed at 60°, with corresponding changes in the electronic excitations contributing to the \( S_3 \) state. Here, peak 3' is found close to peak D' in the red of the visible spectrum. At the CI, the \( S_1 \rightarrow S_3 \) transition is further blue-shifted but it lies at much lower energies than the \( S_1 \rightarrow S_2 \) transition, being located at \(~18 000 \text{ cm}^{-1} \), in proximity to the GSB (B). Indeed, the broadband 2DES spectrum at the CI reported in Figure 3 indicates that the positive peak 3' is canceled by overlap with the strong negative B signal. It is worth noting that peak 3 disappears in the FC\(_{\text{REL}}\) broadband spectrum. This is due to the progressive red-shift of the SE which bumps into low-lying ESA bands that concurrently red-shift during the photoisomerization reaction. Such a signal quenching effect is observed for the \( S_1 \rightarrow S_3 \) transition at the FC\(_{\text{REL}}\) structure, and it is expected for the
$S_1 \rightarrow S_2$ transition at twisting angles slightly larger than 30°, i.e., $\sim 35°$.

The $S_1 \rightarrow S_3$ transition (peak 4) is found as a positive signal at $\Omega_1 \approx 16,000$ cm$^{-1}$ in the FC region, and differently from peaks D and 3, it blue-shifts by $\sim 2100$ cm$^{-1}$ upon bond relaxations. Therefore, the overlap of (positive) peak 4 with the red-shifted (negative) SE is expected to happen at the earliest stages of the photoisomerization, i.e., during the skeletal relaxation and before the bond inversion is completed, corresponding to an energy range of 16,000–18,000 cm$^{-1}$. This result is fully consistent with broadband pump–probe experiments that show the absence of signals in the 16,000–18,000 cm$^{-1}$ range (555–625 nm, in wavelength) at early probe delays, where actually the initial SE was expected to show up. Moreover, at energies $> 18,000$ cm$^{-1}$, in the spectral region of the absorption maximum $\nu_{\text{max}}$ the experimental data at probe delays $< 75$ fs shows the presence of a broad ESA band and its overlap with the GSB signal. Our results also indicate that, in this region of the spectra and at early probe delays (for twisting angles $< 60°$), the negative B signal overlaps with the blue-shifting peak 4 and other two ESAs. Remarkably, at 60°, the calculated 2DES spectrum shows an almost unperturbed negative B signal and the positive peaks D’ and 3 at $\approx 15,200$ and $\sim 16,500$ cm$^{-1}$, respectively, in very nice agreement with ultrafast pump–probe spectroscopy data at around 75 fs probe delay, showing the recovery of the GSB and the appearance of positive signals at energies below 17,400 cm$^{-1}$ (wavelengths $> 575$ nm), with two small spots appearing at $\approx 16,000$ and $\sim 16,900$ cm$^{-1}$. Finally, our 2D spectrum at the twisted CI suggests that the only surviving bright ESA band is the $S_1 \rightarrow S_1$ transition (peak 3′), whose positive intensity is vanishing due to overlap with the negative B signal. No signals are observed below 19,000 cm$^{-1}$, and the first detectable ESA is in the near-UV, above 30,000 cm$^{-1}$, in agreement with pump–probe experiments revealing the presence of just GSB negative signal and the formation of the photoinduced ground state absorption band of the rhodopsin photoprodut in the CI region.

In summary, we showed that the broadband 2DES spectra, obtained using the excited state manifolds calculated at the CASPT2/CASSCF level along the MEP of the retinal photoisomerization, provide useful information on the evolution of ESA signals and their overlap with the SE and GSB signals with opposite sign, in good agreement with the available broadband pump–probe spectroscopy data. In the following section, we consider realistic 2DES combinations of pulse frequencies and bandwidths that can provide useful information on the evolution of specific ESA bands during retinal photoisomerization, including excitations from $S_1$ to low-lying coherent states that have been proposed to interplay with the spectroscopic state in the photoisomerization of retinal in solution.

**One- and Two-Color 2D Spectra.** The simplest 2DES combination of pulses that can be considered involves identical pump and probe pulses (one-color setup), with all frequencies centered at the absorption maximum of the chromophore, $\nu_{\text{max}}$. Figure 4a shows the calculated 2DES spectra simulating such one-color experiment with the pulses’ bandwidth of 5000 cm$^{-1}$. The major contribution to all one-color spectra is, as expected, the negative GSB signal at $\Omega_1 = \nu_{\text{max}} = 20,875$ cm$^{-1}$, peak B. At the FC region, the GSB is accompanied by the SE (at identical frequency) but the simulated 2D spectrum indicates that the expected strong negative peak is partially overlapping with a positive ESA band, peak 7 at $\sim 22,000$ cm$^{-1}$, inducing depletion of the signal and a tiny asymmetry along the $\Omega_1$ frequency axis. At very early probe delays, the wave packet leaves the FC region and the simulated 2DES spectra at the FC$_{\text{REL}}$ and 30° structures indicate that the negative GSB signal will be significantly depleted by a concomitant red-shift of the negative peak E, blue-shift of the positive peak 4 that is approaching $\nu_{\text{max}}$ and lasting positive peaks 7 (at FC$_{\text{REL}}$) and 8 (at FC$_{\text{REL}}$ and 30°). At twisting angles $> 260°$, the GSB signal is almost unperturbed due to the further blue-shift of the ESA band 4, which at 60° lies already at energies $> 24,000$ cm$^{-1}$. At the CI, peak B almost recovers the shape of the 2D isolated signal with a tiny perturbation at energies below $\nu_{\text{max}}$ due to the presence of the blue-shifted $S_1 \rightarrow S_1$ transition. The simulated 2DES spectra along the MEP reported in Figure 4a suggest that with a one-color setup it is possible to observe modulation of the GSB signal at early stages of the photoisomerization, as affected by the evolution of high-lying ESA bands (i.e., the transitions $S_1 \rightarrow S_4$, $S_3$, and $\gamma_{-\gamma}$ in the FC). Probe frequencies lower than $\nu_{\text{max}}$ are necessary in order to track the energy gaps between the $S_1$ state and low-lying coherent excited states (such as $S_2$ and $S_3$), and a two-color setup is required.

Figure 4b shows the calculated 2D spectra from the FC to the CI region, simulating a time-resolved two-color 2DES experiment with pump pulses centered at $\nu_{\text{max}}$ ($\Omega_1 = 20,875$ cm$^{-1}$) and probe pulses centered at $\Omega_1 = 15,000$ cm$^{-1}$, in the red region of the visible spectrum. In the FC region, the $S_1 \rightarrow S_3$ and $S_1 \rightarrow S_4$ transitions, i.e., peaks 3 and 4, are clearly resolved at $\sim 13,800$ and $\sim 16,000$ cm$^{-1}$, respectively, but upon bond relaxation, the red-shifted SE burns the positive peak 3, while peak 4 blue-shifts to the green, at $\sim 18,100$ cm$^{-1}$, and remains detectable. The positive $S_1 \rightarrow S_1$ signal will recover in the two-color spectrum upon torsional motion that further red-shifts the negative E signal. Before reaching the CI region, where the two-color 2D spectrum is essentially signal-free, the D’ transition rapidly shifts to the red and both peaks 3 and D’ are clearly resolved at 60°. The reported two-color 2DES spectrum at 90° suggests that peak 3’ should also be resolved at the CI structure when using a probe pulse centered at 15,000 cm$^{-1}$ with a bandwidth of 5000 cm$^{-1}$. The proposed two-color
CONCLUSIONS

In this work, we analyze the electronic structure changes of the retinal chromophore in bovine rhodopsin along its photoisomerization pathway by characterizing the manifolds of the excited states at critical points of the MEP along the S\textsubscript{1} energy surface, from the FC region to the twisted CI. The electronic structure calculations based on multiconfigurational \textit{ab initio} techniques within a hybrid QM/MM scheme reveal that the S\textsubscript{1}/S\textsubscript{0} energy gap rapidly decreases during the photoinduced skeletal relaxation of the polyene chain, while the evolution of excited state S\textsubscript{1} \rightarrow S\textsubscript{N} (with N \geq 2) absorption energies features a remarkable blue-shift only after bond inversion and partial rotation along the \textit{cis} \rightarrow \textit{trans} isomerization angle, i.e., for twisting angles larger than 30°. We report simulated time-resolved 2DES spectra to track the evolution of the S\textsubscript{1} \rightarrow S\textsubscript{0} SE red-shift and ESA blue-shifts along the MEP. A set of broadband 2DES spectra are reported considering laser pulses with infinite bandwidth, providing a useful picture of the overall detectable 2D signals from the NIR to the near-UV frequency domains. Three different 2D combinations of pulse frequencies have been considered in order to follow the evolution of specific signals: a one-color setup with all pulses centered at the absorption maximum frequency of the chromophore in the blue-green region of the visible (one-color 2DES), a two-color setup with probe pulses centered in the red (two-color 2DVIs), and a two-color setup with probe pulses centered in the NIR (two-color 2DVIs/NIR). We show that a modulation of the GSB signal due to the overlap with high-lying ESA bands, i.e., transitions S\textsubscript{1} \rightarrow S\textsubscript{N} \geq 2, can be observed at early stages of the retinal photoisomerization using a one-color 2DVIs setup. To track, instead, the energy gaps between the S\textsubscript{1} state and the low-lying covalent excited states (such as S\textsubscript{2} and S\textsubscript{3}), a two-color setup is required. The simulated 2D spectra for the two-color 2DVIs setup reveal that the signal quenching of the S\textsubscript{1} \rightarrow S\textsubscript{3} ESA, due to overlap with the red-shifted SE, can be detected in the red spectral window at early probe delays, while the S\textsubscript{3} \rightarrow S\textsubscript{2} transition is unlikely to be observed due to the large and rapid blue-shift of this signal at twisting angles larger than 30°. The S\textsubscript{1} \rightarrow S\textsubscript{2} transition is particularly relevant because it provides direct measurement of the S\textsubscript{1}/S\textsubscript{2} energy gap separation along the photoisomerization path, a parameter that could play a crucial role in slowing down the photoreactivity, determining the photoisomerization mechanism of retinal in solution. Finally, we show that the two-color 2DVIs/NIR setup is best suited for tracking the evolution of the S\textsubscript{1} \rightarrow S\textsubscript{N} and S\textsubscript{1} \rightarrow S\textsubscript{N} transitions along the retinal photoisomerization pathway, and we encourage performing such type of experiments for elucidation of the molecular mechanisms that drive different behaviors of retinal photoisomerization in various environments. The reported results are consistent with the available time-resolved 1D pump–probe experimental data, providing fundamental information for the design of new time-resolved 2D experiments and laying the groundwork for simulation of more elaborate multipulse sequences, such as in transient 2D electronic spectroscopy.

ASSOCIATED CONTENT

Supporting Information

Schematic representations of different 2DES experimental setups, CASSCF(12,12) active space orbitals, and Cartesian coordinates. This material is available free of charge via the Internet at http://pubs.acs.org.
REFERENCES


(34) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci,


