Quantum–classical simulations of rhodopsin reveal excited-state-population splitting and its effects on quantum efficiency

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1. Multiconfigurational quantum chemistry-based QM/MM model construction. A quantum mechanics/molecular mechanics (QM/MM) model of Rh was constructed starting from the 2.2 Å resolution crystallographic structure available in the Protein Data Bank (PDB ID 1U19) and following a reported protocol. The atoms of the retinylidene chromophore, NH group and C₃H₃ substituent form the QM layer, which is treated at CASSCF level of theory with the 6-31G* basis set. The active space involves 12 electrons in 12 orbitals, which comprises the entire π-system of the retinal chromophore moiety and defines the method multiconfigurational wavefunction. The rest of the protein, comprising the atoms of the C₃H₂ moiety belonging to the Lys256 side-chain, form the MM layer, which is described by a modified AMBER94 force field featuring specific Lys296 side-chain parameters. All side-chains and waters with at least
one atom within 4 Å from any atom of the retinal chromophore were kept flexible during the trajectory calculations. All remaining atoms were kept frozen. A representation of the model is given in Fig. 1a. The detailed structure is given in Fig. 1b.

**Fig. 1.** Rh QM/MM Model. (a) Structure of the Rh QM/MM model. (b) Detailed view of the Rh model cavity incorporating the rPSB11 chromophore and comparison with corresponding crystallographic structure. (c) Detailed view of the bathoRh model cavity incorporating the rPSBAT chromophore and comparison with corresponding crystallographic structure.
2. Initial condition generation and population dynamics simulation. The QM/MM model constructed above was employed to simulate a room temperature Boltzmann-like distribution described by 200 initial conditions (geometries and velocities) following a three-step protocol²:

1) A 20 ns room-temperature dynamics simulation was performed at MM level starting from the $S_0$ equilibrium structure of the Rh QM/MM model with GROMACS⁵. The MD relaxations consist of a 50 ps heating followed by 150 ps room-temperature equilibration and 20 ns production with a time interval of 1 fs. The input velocities are generated according to a Maxwell distribution at temperature of 298 K with a random seed and using the Berendsen thermostat. Detailed GROMACS MD parameters are listed in Supplementary section 17.

2) Along the MM dynamics, 200 snapshots (geometries and velocities) were extracted from the 20 ns production MD at every 100 ps time interval.

3) Each snapshot was then employed to start a HF/6-31G*/Amber level 200 fs trajectory followed by a 50 fs a 2-root-state-average CASSCF/6-31G*/Amber level of theory on the $S_0$ PES with time step of 1 fs.

The geometries and velocities of the final (last time step) form the set representing the Boltzmann-like distribution and, therefore, the initial conditions for $S_1$ population dynamics. The generated Boltzmann-like distribution was used to simulate the Rh absorption band. This was done by computing and plotting the $S_0$-$S_1$ vertical excitation energies of each point at the 3-root-state-average CASPT2//CASSCF/6-31G*/Amber level of theory. To test the consistency of the Boltzmann-like distribution we also plotted the 200 corresponding $\alpha$, $\delta_{op}$ and BLA coordinate values. The expected Gaussian-like shape of the distribution was achieved in all cases (see Fig. 2) including the absorption band.
The computed Boltzmann-like distribution provided the initial conditions for propagating 200 semi-classical trajectories. Following the protocol reported in ref. 2, each trajectory was propagated starting from the corresponding initial condition (geometry and velocity) for 200 fs at the 2-root-state-average CASSCF/6-31G*/Amber level of theory with time step of 1 fs. The \( S_1 \) to \( S_0 \) hop event is determined by using the stochastic Tully surface-hop method\(^6\) implemented in the Molcas\(^7\)/Tinker\(^8\) package\(^9\). The Tully parameters we used in this work are: (SUBSTEP=200) to specify 200 substeps of electronic integration to interpolate/extrapolate between two Newton's consecutive steps (i.e., 200 substeps each fs of MD) and (DECOHERENCE=0.1) to deal with the decoherence correction in the population density matrix using a decay factor of 0.1 hartree. The corresponding Molcas/Tinker input is listed in Supplementary section 17. During the initial condition preparation (step 3) and the trajectory propagation, the description of the electrostatic embedding of the QM subsystem into the MM subsystem was treated using the electrostatic potential fitted (ESPF)\(^9\) methodology. The 200 semi-classical trajectories yielded a simulation of the dynamics of the entire population (population dynamics).
Fig. 2. Validation of the room temperature initial conditions. Computed room temperature equilibrium distribution for (a) $S_0$ to $S_1$ absorption wavelengths, (b) $\alpha$ values, (c) $\delta_{op}$ values, (d) BLA values. Vertical red lines mark the corresponding values of the $S_0$ equilibrium structure.
corresponding to the geometrically optimized model. (e) Comparison between experimental (adapted/redrawn from ref. 10) and simulated absorption band. The initial conditions reproduce both \( \lambda_{\text{max}} \) and width of observed absorption band.

3. **Rh model and population dynamics validation.** The validation of the QM/MM model of Rh and of the computed population dynamics, have been carried out by predicting the values of a set of properties relevant to the research objectives and then comparing the predicted and available experimental values. These properties are:

1) \( \lambda_{\text{max}} \) value and corresponding absorption band.

2) Excited state vibrational frequencies for \( \delta_{\text{op}} \) and BLA.

3) Excited state lifetime.

4) Photoproduct appearance time.

5) Photoproduct structure and \( \lambda_{\text{max}} \).

6) Quantum efficiency of Rh wild type and of two of its isotopomers.

7) Conservation of the total energy of the system.

We evaluated the \( \lambda_{\text{max}} \) value (property 1) from the absorption band (see above) computed at the 3-root-state-average CASPT2//CASSCF/6-31G*/Amber level of theory. As reported in Table 1, the computed 500 nm value is in close agreement with the observed value of 498 nm.

The results of the population dynamics allowed to predict property 2-4 and 6. The \( \delta_{\text{op}} \) vibrational frequencies of the 38 fs period, equivalent to 878 cm\(^{-1}\) and BLA (21 fs period, equivalent to 1588 cm\(^{-1}\) are obtained based on their average progressions. These are defined as the numerical average of the corresponding \( \delta_{\text{op}} \) and BLA values at each time point during the excited state.
progression (see Fig. 3a and 3b). The predicted frequency values compare with the observed values 746 cm\(^{-1}\) and 1679 cm\(^{-1}\) respectively.\(^{11}\)

The excited state lifetime is computed by fitting the \(S_1\) population decay with a previously employed Gaussian-plus-exponential equation\(^{12}\) (see Fig. 3c) or as an average value. The predicted 98 fs (from the fitting) and 93 fs as the average decay time values have to be compared with the longer observed 148 fs value.\(^2\)

In order to compute the "photoproduct appearance time" we use both excitation energy as well as geometrical criteria. For the excitation energy we selected the small group of trajectories reaching the \(1S_{1/2}\) within 50 fs (see Fig. 2a) and leading to successful decay events. We define the shortest time employed by those trajectories to match the computed bathoRh absorption \(\lambda_{\text{max}}\) value as shortest time to the photoproduct appearance (see Fig. 3d). We noticed that at this particular time (70 fs) the geometry of the chromophore is still highly distorted with respect to that of an equilibrium bathoRh geometry. In fact, the first trajectory of the group to reach the values of geometrical equilibrated \(\alpha\) and \(\delta_{\text{op}}\) needed 60-85 fs (see Fig. 3e). The 70 fs value may be compared with recent experimental results suggesting photoproduct detection at time scales as low as 30 fs.\(^{11}\)

The bathoRh equilibrium structure (property 5) necessary for the computation of the photoproduct appearance time defined above, was generated by computing a 200 fs Franck-Condon (FC) trajectory (this is a deterministic semi-classical trajectory released from the FC point. i.e. the fully relaxed \(S_0\) equilibrium structure of Rh, without initial velocities), which resulted successful photoproduct. Starting from the trajectory last time step, we performed a geometry optimization at the CASSCF/6-31G*/Amber level obtaining an equilibrium bathoRh
structure whose $\lambda_{\text{max}}$ value was determined via a vertical excitation energy calculation at the 3-root-state-average CASPT2/CASSCF/6-31G*/Amber level. The resulting $\lambda_{\text{max}}$ value of 517 nm is red-shifted with respect to that of the equilibrated Rh model (500 nm) as expected but only qualitatively matching the experimentally determined $\lambda_{\text{max}}$ value of 543 nm. However, the computed bathoRh structure overlapped reasonably well with the corresponding crystallographic structure (see Fig. 1c).

The $\Phi_{\text{cis}-\text{trans}}$ (property 6) was calculated as the ratio of the number of trajectories forming the photoproduct (i.e. this is determined by looking at the $\alpha$ value at the last time step) over the total number of decayed trajectories. The predicted and observed values are 0.68 and 0.67 respectively. The isotope effect on $\Phi_{\text{cis}-\text{trans}}$ is also reproduced with a small set of 50 trajectories of 11-D (deuterium substitution at H11 only) and 11,12-D2 (deuterium substitutions at both H11 and H12) models finding that the $\Phi_{\text{cis}-\text{trans}}$ decreases with mono-deuteration but increases with di-deuteration consistently with the reported experimental and computational results.\(^2\)

To check the conservation of the total energy of the system, the energy progression of the $S_1$ population is plotted in Fig. 3f.

In conclusion, the values of the "observables" 1-6 predicted on the basis of the Rh QM/MM model and population dynamics, indicate a semi-quantitative agreement with the experimental observations (see Supplementary Table 1). These properties have been computed limiting the simulation (mainly due to the high computational cost) to 200 trajectories. Below we will provide evidence that even when using only 100 trajectories one can still obtain, for Rh, acceptable values for properties 1-3 and 6 (the only one tested).
Table 1. Comparison of observed and simulated wavelength of the absorption maximum ($\lambda_{\text{max}}$), photoproduct (bathoRh) appearance time, $S_1$ lifetime, $S_1$ vibrational frequencies and isotopomer isomerization quantum efficiencies.

|                | Rh                               | bathoRh                          |
|----------------|----------------------------------|----------------------------------|
|                | $\lambda_{\text{max}}$ (nm) | $\Phi_{\text{cis-trans}}$ | $\lambda_{\text{max}}$ (nm) |
| product        | $\lambda_{\text{max}}$ (nm) | $S_1$ lifetime (fs) | vibrational freq. (cm$^{-1}$) | $\lambda_{\text{max}}$ (nm) |
| appearance     | lifetime (fs) | vibrational freq. (cm$^{-1}$) | $\delta_{\text{op}}$ | BLA | WT | 11D | 11D,12D |
| Obs.           | 498 | $< 50^{a}$ | $148 \pm 10^{b}$ | 746$^c$ | 1679$^c$ | 0.67$^c$ | 0.45$^b$ | 0.69$^b$ | 543 |
| Comp.          | 500 | $>70^{d,i}$ | $98^{d,ii}$ (93$^{d,iii}$) | 878$^d$ | 1588$^d$ | 0.68$^d$ | 0.62$^e$ | 0.75$e$ | 517$e$ |

a. Observed data from ref. 11. b. Observed data from ref. 2. c. Observed data from ref. 13. d. Based on a 200-trajectory ensemble. d-i. Time range from the first successful decay to the first photoproduct with a bathoRh-like absorption. d-ii. Fitted $S_1$ lifetime of 200 trajectories. d-iii. Average decay time of 200 trajectories. e. Based on a 50-trajectory sample with a Rh model of a full rPSB11 chromophore. f. Based on a 400-trajectory sample with a Rh model of a reduced rPSB11 chromophore from ref. 2. g. Structure computed via geometry optimization on the $S_0$ PES starting from the FC trajectory output.
Fig. 3. Population dynamics analysis. (a) $\delta_{op}$ progression of the $S_1$ population (in black) and corresponding average value (in yellow). The period of the first oscillation is used to compute the $\delta_{op}$ frequency. (b) Same data for BLA. (c) Excited state lifetime fitting. The $S_1$ population decay and the fitting function are given in black and red respectively. (d) Photoproduc (bathoRh) formation from sub-50 fs decay trajectories. The solid curves indicate the excitation energy progression of those sub-50 fs decay trajectories and the dashed red line indicates the $S_0$-$S_1$ excitation energy for the fully relaxed bathoRh structure. (e) Progression along $\alpha$ (in red) and $\delta_{op}$ (in black) for the same trajectory set. The red and black squares indicate the corresponding values for the same fully relaxed bathoRh structure. (f) The $S_0$ potential energy (in red), $S_1$ potential energy (in blue) and total energy (in black) progression at CASSCF level of the $S_1$ population and corresponding average value (in yellow).

4. Population decay statistics: comparison of $\tau$ and $\delta_{op}$. Consistently with previous results\textsuperscript{2,14}, it is possible to demonstrate the existence of a relationship between the sign of the $d\delta_{op}/dt$ velocity and the probability of successful photoisomerization (see Fig. 4a). In fact, the population dynamics analysis shows that 73% of the successful trajectories have, at decay, a positive $d\delta_{op}/dt$. It is also possible to show that by selecting a dihedral angle $\tau$ (approximately) proportional the orbital overlap between the atoms forming the reactive C11=C12 double bond, it is possible to obtain a significantly tighter relationship between sign of the $d\tau/dt$ velocity and the probability of successful photoisomerization. $\tau$ is a linear combination of $\alpha$ and $\delta_{op}$ ($\tau = \alpha - \delta_{op}/2$) and, as demonstrated by the Newman projections in Fig. 1c and 1d in the main text, it is proportional to the overlap between the two $\pi$-orbitals (i.e. $\tau$ mimics the angle between the orbital axis)
involved in the breaking and reconstitution of the C11=C12 double bond. Accordingly, $\tau=-90$ at zero overlap and $\tau=0$ and -180 degrees when the overlap is that of the equilibrium cis and trans bond. As shown in Fig. 4b, the $\tau$ velocity, which depends on both the $\alpha$ and $\delta_{op}$ velocities, is a better reactivity index than the $\delta_{op}$ velocity as 92% of successful trajectories have, at decay, a negative $d\tau/dt$. This indicates that while the majority of the successfully decaying trajectories have a $d\delta_{op}/dt>0$, (i.e., photoproduct is mostly likely forming when $\delta_{op}$ moves in the opposite direction to $\alpha$) ca. 9% of the trajectories reach the photoproduct due to a dominating $d\alpha/dt<0$ value.
Successful trajectories
(73 % with positive $d\delta_{op}/dt$)

Successful trajectories
(92 % with positive $d\tau/dt$)
**Fig. 4.** The photoisomerization outcome is controlled by the $\tau$ and $\delta_{op}$ phase at decay. (a) The distribution of $\delta_{op}$ velocities (degrees/fs) at the decay points for successful and unsuccessful (red and blue, respectively) subpopulations. (b) Same distribution for $\tau$.

**5. The population splitting is independent from the type of model employed and from the initial conditions.** The population splitting event discussed in the main text do not appear to depend on the specific QM/MM model employed, number of excited state trajectories propagated or on the initial conditions. These conclusions are supported by:

1) We observed no significant difference in the splitting behavior when the trajectories are calculated using a QM/MM model constructed starting from the same PDB file, but employing a different construction protocol\textsuperscript{15} defining a larger chromophore cavity (compare Fig. 5a with 5c and 5e with 5g).

2) We observed no significant difference in the splitting behavior when the trajectories are calculated using a model of the human green cone rhodopsin generated from a reported homology model\textsuperscript{15} (compare Fig. 5a with 5d and 5e with 5h).

3) We observed no significant difference in the dynamics when comparing a 100-trajectory subset with the discussed 200 trajectories (compare Fig. 5a with 5b and 5e with 5f).

4) We observed no significant difference in the initial value and velocity of $\alpha$, $\delta_{op}$ and BLA modes of fast and slow trajectory sets, indicating a weak dependence of the splitting event on the initial conditions (see Fig. 6).

In all cases we compare both the $\alpha$ progression and, most importantly the population decay statistics. Again, the results show that there is no significant qualitative difference in terms of
α population splitting. However, the differently constructed models (Fig. 5c and 5g) and the model of the different green cone rhodopsin (Fig. 5d and 5h) differ in the distribution of successful and unsuccessful trajectories. Notice also that the Rh quantum efficiencies $\Phi_{\text{cis-trans}}$ are similar but the one computed for the green cone, featuring a strongly different sequence (41% homology with respect to Rh), is significantly different as expected.
Fig. 5. Splitting along $\alpha$ for (a) 200 trajectories with a Rh model characterized by a 4 Å flexible cavity. (b) 100 trajectories with a Rh model characterized by a 4 Å flexible cavity. (c) 200 trajectories with a Rh model characterized by a flexible cavity defined by computed atlas of surface topography of protein (CASTp). (d) 200 trajectories with human green cone rhodopsin model of CASTp cavity. Population decay oscillatory character for (e) 200 trajectories with Rh model of 4 Å flexible cavity. (f) 100 trajectories with Rh model of 4 Å flexible cavity. (g) 200 trajectories with the Rh model with a CASTp flexible cavity and (h) 200 trajectories with human green cone rhodopsin model of CASTp flexible cavity.
**Fig. 6.** The splitting of $S_1$ population is independent on initial conditions. (a) Distributions of initial room temperature values and velocities of $\alpha$ for the fast (in brown) and slow (in black) trajectory sets. (b) Same data for $\delta_{op}$. and (c) for BLA.

6. The population splits along BLA and then $\alpha$, but not $\delta_{op}$. In the main text we discuss the bifurcating behavior of $\alpha$ showing that only a fraction of the $S_1$ population decays on a short
time scale with a monotonically decreasing $\alpha$. Other fractions invert the $\alpha$ direction from counterclockwise to clockwise and later get back to a counterclockwise direction, resulting in delayed decay events. As shown in Fig. 3a and 3b, such bifurcating behavior is not immediately seen in $\delta_{op}$ and BLA. In order to study the population splitting more quantitatively, we introduce an index describing the dispersion of the three coordinates. Briefly, we calculated the standard deviation of the $\alpha$, $\delta_{op}$ and BLA distributions as a function of time and obtained dispersion index as the ratio between the standard deviation at time $t$ and that at time $0$. A fully vibrationally coherent population would have a dispersion index value equal to 1, as its coherence doesn’t change. As shown in Fig. 7a, unlike $\delta_{op}$ in which the dispersion index remains close to 1, BLA display a relatively larger dispersion index value during the first 15 fs after light absorption. After 20 fs of initial excitation, the rational standard deviation of $\alpha$ increases dramatically. In Fig. 7b we displayed the splitting of the entire $S_1$ population by plotting the average progression of fast, slow and remaining trajectory set along $\alpha$ and stretching motion of N=C15 and C11=C12 component of BLA.
**Fig. 7.** Population splitting. (a) Statistical dispersion of excited state $\alpha$, $\delta_{\text{op}}$ and BLA values. (b) Progression along the average $\alpha$ and C11=C12 and N=C15 bond lengths of the fast trajectory set (black), slow trajectory set (red) and remaining trajectories (purple).

7. **Recalculated trajectories with scaled opsin atomic charges and behavior of the FC trajectory.**

In the main text we show that the electrostatic field imposed by the opsin cavity on rPSB11 modulates the population splitting. In order to provide further support for such a conclusion, we recomputed the entire 200 semi-classical trajectory population dynamics using the same initial conditions but with the opsin atomic charges set at half of their original values. The results in Fig. 8a show that when embedded in such scaled electrostatic field, the $S_1$ population has been completely transferred to the ground state in 110 fs due to a significantly reduced $\alpha$ bifurcation. As expected, such qualitatively different dynamics also leads to a different value of $\Phi_{\text{cis-trans}}$. It is thus demonstrated that when the electrostatic interactions between protein and chromophore are decreased the population evolves and decays more coherently spawning fewer subpopulations, resulting in slightly higher $\Phi_{\text{cis-trans}}$ (see Fig. 8b) while still maintaining the rule that links the $d\delta_{\text{op}}/dt$ sign and the probability of successful chromophore photoisomerization (see Fig. 8c). Such enhanced coherence effect has been documented for both BLA, which respond to the change in electrostatics immediately and $\alpha$ but has a much smaller effect on the $\delta_{\text{op}}$ coherence (see Fig. 8d). As displayed in the Fig. 8e, we also recomputed the entire population dynamics using the same initial conditions of rPSB11 but removing the opsin cavity (so the protein environment effect is only present at the level of initial geometries and velocities). The absence of the cavity and, therefore, the absence of the van der Waals
interactions leads to an even more marked compactness of the trajectories which show no splitting. Such population evolves and decays even more coherently leading to higher $\Phi_{\text{cis-trans}}$ value of 0.78. An additional simulation was also performed after switching off all protein charges while keeping the van der Waals interactions. In this case we still observed a substantial lack of population splitting and a 0.72 quantum efficiency.

In Fig. 3e and 3f in the main text we display the distinct behavior of the fast and slow trajectory sets after 10 fs progression. It is seen that $S_1$ and $S_2$ energies rapidly separate in the fast set but not in the slow set. We also show that the $S_1$ and $S_2$ charge distributions changes monotonically in the fast set while the slow set enters into a region where the trajectories display multiple $S_1$ and $S_2$ charge switching. In Fig. 9 we show that with the scaled electrostatics the behavior of the slow set changes significantly. Indeed, while the $S_1$ and $S_2$ states still lie close to each other, they become more separated and display non-switching charges similar to the situation of the fast trajectory set. This change is consistent with trajectories moving in a region where the $S_1$ and $S_2$ states maintain distinct electronic characters (travel on the same side of the $S_2$/S$_1$ conical intersection in contrast with the situation displayed in Fig. 4c in the main text).

As shown in Fig. 8a, the FC trajectory (see definition above), and therefore a representation of the $S_1$ relaxation dynamics initiated without kinetic energy (i.e. at 0 K), displays similar behavior to the fast set as it decays to $S_0$ in 79 fs with a monotonically decreasing $\alpha$. This implies a weak $S_1$/S$_2$ interaction also found in the fast set. Such similarity explains why the $S_1$/S$_2$ mixing was not detected in previous Rh computational studies$^{16}$ based on single FC trajectories, which thus supported a pure two-state ($S_0$ and $S_1$) photoisomerization mechanism. In other words, the importance of the interaction between $S_2$ and $S_1$ is only evident after a sizable room temperature
sampling. However, the involvement of $S_2$ in the photoisomerization mechanism is limited to the initial relaxation while, after the population splitting, all sub-populations propagate along the $S_1$ PES maintaining a $1B_u$ character. The proposed mechanism is therefore very different from a previously proposed three-state model.\textsuperscript{17}
Fig. 8. Comparison between trajectories calculated with full and halved opsin charges. (a) $\alpha$ progression along a set of 200 trajectories computed (same initial conditions) with a Rh model with original (left) and halved (right) opsin charges. The circles represent decays from $S_1$ to $S_0$. The orange curve indicates the $S_1$ $\alpha$ progression of the FC trajectory. (b) Oscillatory character of the $S_1$ population decay for the same models. (c) Time progression of $\delta_{op}$ along the same trajectories. (d) Statistical dispersion of $\alpha$, $\delta_{op}$ and BLA for the same Rh model with full (solid) and halved (dashed) opsin charges. (e) $\alpha$ progression along a set of 200 trajectories computed (same initial conditions as for the original Rh model) with a Rh model with zeroed opsin charges (left) and of the isolated Rh chromophore (right). The circles represent decays from $S_1$ to $S_0$. The orange curve indicates the $S_1$ $\alpha$ progression of the FC trajectory.
**Fig. 9.** Fast and slow trajectory sets calculated with full and halved opsin charges. (a) $S_1$ and $S_2$ energy profiles for the fast trajectory sets computed with original opsin charges (left) and halved opsin charges (right). The solid and dashed yellow curves indicate the $S_1$ and $S_2$ average energies, respectively. (b) Same data for the slow trajectory set. (c) $S_2$ and $S_1$ charge transfer character (charge present on the CH$_3$-N=C15-C14=C13=C12= moiety) for the fast trajectory sets.
computed with full opsin charges (left) and halved opsin charges (right). (d) Same data for the slow trajectory set.

8. Comparison between MinCI_{S0/S1} and MinCI_{S2/S1} and effect of the scaled opsin atomic charges.

We report the structures of minimum energy S0/S1 and S2/S1 conical intersection (MinCI_{S0/S1} and MinCI_{S2/S1}, respectively). Both conical intersections were obtained at 3-root-state-average CASSCF/6-31G*/Amber level and corresponding geometrical parameters and branching plane vectors are presented in Fig. 10a. Unlike MinCI_{S0/S1}, which features a ca. -90 degree twisted C11=C12 bond and delocalized positive charge along –C10-C9-C8- moiety, MinCI_{S2/S1} displays a planar PSB11 chromophore with positive charge localized in the Schiff base group. The two branching plane vectors of MinCI_{S0/S1} can be described as a BLA stretch and a combination of α and δop twisting while in the case of MinCI_{S2/S1}, the branching plane vectors are comprised by stretching motion of N=C15 and C11=C12 component of BLA.

We also re-optimized the structure of MinCI_{S2/S1} with the opsin atomic charge to be half of the original value. The MinCI_{S2/S1} of scaled charge displays nearly identical branching plane vectors and geometrical features with the one optimized with original charge except a shorter N=C15 bond. Such difference, as shown in Fig. 10b, results in a change of electronic character of the average fast and slow trajectory on the S1 PES. It is clear that originally, the fast and slow trajectory sets travel on opposite sides of MinCI_{S2/S1}. The fast set (Fig. 10b left panel or Fig. 4c in the main text) propagates along the side dominated by a 1B_u character (low charge) while the slow set crosses a region with 2A_g character (large charge) and then re-enters a region with 1B_u character, leading to distinct behavior with respect to the fast set. In contrast, when
embedding in the scaled electrostatic environment (Fig. 10b right panel), both sets propagate along the same side of MinCl$_{S2/S1}$, which is dominated by a 1B$_u$ character, results in closer behaviors.

Fig. 10. Analysis of the S$_1$/S$_0$ and S$_2$/S$_1$ minimum energy conical intersections. (a) Comparison between MinCl$_{S0/S1}$ and MinCl$_{S2/S1}$ optimized structures. The relevant geometrical parameters (in Å and degrees) and the arrow representation of the branching vectors are also given. The values in parenthesis correspond to MinCl$_{S2/S1}$ structure optimized with halved charges. (b) Progression along the average N=C15 and C11=C12 component of BLA (as indicated by the
MinCl_{S2/S1} branching plane vectors) for the fast and slow trajectory sets calculated with original (left) and halved (right) opsin charges. The contour diagram represents the change in charge transfer character (charge present on the CH₃-N=C15-C14=C13-C12= moiety) in the MinCl_{S2/S1} region.

9. **Mapping S₁-S₂ energy gap at CASPT2 and XMS-CASPT2 levels.** In Fig. 4e in the main text we present the change of S₂/S₁ NADC magnitude computed at the 2-root-state-average CASSCF/6-31G*/Amber level of theory level along a 3D cut of S₁ PES. Such a cut is constructed using a series of geometries optimized on S₁ with α, δ₀ and BLA constrained at specific values. In Supplementary Fig. 11, we demonstrate that the highest computed NADC values are located in the regions of low S₁-S₂ energy gap computed at the CASPT2 level of theory. It can be noticed that at the beginning of the S₁ relaxation (low α values) the slow and fast trajectory sets travel the same region of the PES. When α reaches -20 degrees, the slow set moves towards a S₂/S₁ degeneracy region (a cross section of the IS_{S2/S1}) displaying low S₁-S₂ energy gap while such region is abandoned for the fast sets. Such behaviors do not change when the energy profile is evaluated at a high level of theory such as XMS-CASPT2 (see Supplementary Fig. 11c) where the S₁ and S₂ state wavefunctions are now allowed to mix.
Fig. 11. Si-S₄ energy gap mapping. (a) Magnitude of the Si/Si NADC module along a 3D cut of the Si PES. The cut is represented by four 2D cross-sections corresponding to different α values and spanning the δ₀p and BLA coordinates. (b) Si-S₄ energy gap computed at the CASPT2 level

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of theory along the same cut of the $S_1$ PES. (c) Same data computed at the XMS-CASPT2 level of theory

10. **Raman spectra simulations.** Simulations of resonance Raman spectra were based on the assumption that resonance enhancement of vibrational modes is dominated by the FC scattering mechanism. FC factors (B$k$) were obtained within harmonic approximation assuming identical normal modes and vibrational frequencies in the ground and electronic excited states, i.e. neglecting Dushinsky rotation. To derive dimensionless displacements B$k$ of the PES minima along k vibrational modes we used $S_0$ equilibrium geometry, normal modes, and vibrational frequencies, as well excited state gradients at the $S_0$ equilibrium geometry. Rather than using a single-state gradient approach, we used a two-state weighted gradient approximation where both $S_1$ and $S_2$ excited state gradients are involved in the production of an effective weighted gradient that is unique for a particular excitation wavelength. The effective gradient for a given mode can be expressed as:

$$\left( \frac{\partial E}{\partial Q_k} \right)_{\text{eff}} = w_{S1} \left( \frac{\partial E}{\partial Q_k} \right)_{S1} + w_{S2} \left( \frac{\partial E}{\partial Q_k} \right)_{S2}$$  \hspace{1cm} (1)

where $w_{S1}$ and $w_{S2}$ are the weighting factors for the computed gradients of the $S_1$ and $S_2$ excited states, respectively.

To improve the agreement between calculated and experimental frequencies, CASSCF-based vibrational frequencies were scaled by 0.9 to reproduce the position of the most intense band located at $\sim1,550$ cm$^{-1}$. The spectra were obtained as a superposition of Lorentzian curves with a linewidth of 20 cm$^{-1}$. To evaluate resonance Raman intensities, we applied Petrenko and
Neese’s implementation\textsuperscript{19} of Heller’s time-dependent theory.\textsuperscript{23} The spectra were computed for the experimental laser excitation wavelength 488 nm.

The comparison between the theory and the experimental spectrum, shown in Supplementary Fig. 12 and Supplementary Table 2, reveals that pure S\textsubscript{i} gradient is providing the worst comparison with the measured spectrum in terms of intensity ratio with respect to the most intense 1548 cm\textsuperscript{-1} ethylenic stretching mode. The calculated vibrational modes within 1300-1500 cm\textsuperscript{-1} spectral region, the H11-H12 rocking mode at 1272 cm\textsuperscript{-1} (1268 cm\textsuperscript{-1} in experiment), and C11=C12 HOOP mode at 1007 cm\textsuperscript{-1} (970 cm\textsuperscript{-1} in experiment) - all have greatly exaggerated intensities. The admixture of S\textsubscript{2} gradient improves the intensity pattern significantly. On the other hand, a pure S\textsubscript{2} gradient in spectra simulation does a better job than pure S\textsubscript{i} gradient but within 1300-1500 cm\textsuperscript{-1} spectral region there are some bands missing, e.g. 1406 cm\textsuperscript{-1} band. In sum, it seems that using the effective gradient with $w_{S1}=1.00$ and $w_{S2}=0.50$-1.00 reproduces the experimental spectrum definitely better than while using pure gradients in the simulation procedure.
Fig. 12. Effect of the involvement of the S₂ excited state gradient on the simulated resonance Raman spectrum. In each panel, weights used to compute the effective gradient are given for the S₁ and S₂ gradients. Experimental spectrum²¹ is shown in the top panel.
Table 2. Resonance Raman scattering intensity of selected modes relative to the intensity of the most intense mode at 1548 cm⁻¹.

| Experimentala | Calculatedb |
|---------------|-------------|
| **Freq. (cm⁻¹)** | **I/I₁₁₅₄₈** | **Freq. (cm⁻¹)** | **I/I₁₁₅₃₇** |
|               |             | WS₁=1.00 | WS₁=0.00 | WS₂=0.25 | WS₂=0.50 | WS₂=1.00 | WS₂=1.00 |
| 1548          | 1.00        | 1.00     | 1.00     | 1.00     | 1.00     | 1.00     |
| 1451          | 0.042       | 0.35     | 0.23     | 0.18     | 0.15     | 0.09     |
| 1435          | 0.043       | 0.63     | 0.25     | 0.14     | 0.07     | -        |
| 1268          | 0.23        | 1.05     | 0.79     | 0.9      | 0.92     | 0.87     |
| 1214          | 0.21        | 0.53     | 0.45     | 0.41     | 0.42     | 0.39     |
| 970           | 0.26        | 0.5      | 0.38     | 0.32     | 0.33     | 0.29     |

a. Experimental data from ref. 24. Raman scattering was excited by 488 nm laser beam. Experimental error of the I/I₁₁₅₄₈ is 15%. b. Calculated vibrational frequencies are uniformly shifted by a scaling factor of 0.9.

The result above are further supported by a study of the S₂-S₁ energy gap carried out at the CASPT2/6-31G* level of theory for the entire population in the FC region as well as at ultrashort (<10 fs) timescales. As it is apparent in Supplementary Fig. 13, the distribution of energy gaps displays at least 20% of gaps below 20 kcal/mole at the FC point but this percentage is already larger than 50% after just 5 fs relaxation that includes 10% gaps well below 10 kcal/mole.
So geometry optimization starting by random initial conditions generated equilibrium structures displaying $S_2$-$S_1$ energy gaps computed at the CASPT2/6-31G* level as low as 14 kcal/mole (0.643 eV). One of these structures was chosen for an additional So vibrational frequency calculation. The same structure was used to compute the corresponding Resonance Raman spectrum using gradients computed at the 3-root-state-average (3r-SA ($S_0$,$S_1$,$S_2$)) and 2-root-state-average (2r-SA ($S_i$,$S_j$)) CASSCF/6-31G*/Amber levels. The results are displayed in Fig. 14 where we compare the spectra obtained using the weighted gradient approximation (normalized $S_i$ and $S_j$ gradient weights are computed according to Eq. 8 in Jarzecki and coworkers) and the spectra obtained using solely the $S_i$ gradient. In the calculations of the normalized weighting factors we used the following data: $\Gamma = 0.350$ eV for all computed excitations, $\nu_0 = 2.54066$ eV (488 nm), $\mu_{S_1} = 2.6657$, $\mu_{S_2} = 2.6790$, and electronic transition energies: 2.5701 eV ($S_0$-$S_1$) and 3.2133 eV ($S_0$-$S_2$). The calculated absolute values of $w_1=7.056$ and $w_2=1.529$ correspond to normalized values of $w_1=82.2\%$ and $w_2=17.8\%$. It is worth noting that this large difference is related to a 0.643 eV $S_2$-$S_1$ energy difference only because the values
of transition dipole moments for both states are essentially the same (2.6657 for \(S_1\) and 2.6790 for \(S_2\)).

Even though the \(S_2\) gradient contributes only 17.8% it can be seen that the weighted gradient approximation and, therefore, the involvement of the \(S_2\) state improves the spectrum (see the experimental spectrum in Fig. 14 of the main text) very significantly regardless of the level of theory used in the gradient calculation. Therefore, we conclude that these calculations, although not providing a fully accurate simulation of the experimental spectrum, clearly support the involvement of \(S_2\) (i.e. \(S_2/S_1\) mixing) in the initial relaxation of the excited state Rh population that is the target of the present study.

![Graph showing the effect of the involvement of the \(S_2\) excited state gradient on the simulated resonance](image)

**Fig. 14.** Effect of the involvement of the \(S_2\) excited state gradient on the simulated resonance
Raman spectrum vs pure $S_1$ gradient for a geometrically "optimized" snapshot featuring a 0.643 eV $S_2$-$S_1$ energy gap. (A) Experimental spectrum$^{22}$. (B) Simulated spectrum based on excited state gradients calculated using 2r-SA ($S_1$,$S_2$) CASSCF/6-31G*/Amber, (C) Simulated spectrum based on excited state gradients calculated using 3r-SA ($S_0$,$S_1$,$S_2$) CASSCF/6-31G*/Amber. In panels (B) and (C), normalized weights used to compute the effective gradient are given for the $S_1$ and $S_2$ gradients.

11. The $\text{ESP}_{\text{opsin}}$ modulates the FC-MinCIC$_{2/51}$ energy gap. In Fig. 5 we show that the $\text{ESP}_{\text{opsin}}$ is more negative in the Schiff base region than in the region of the $\beta$-ionone, which would preferentially stabilize the $S_2$ state of MinCIC$_{2/51}$ relative to FC as the $S_2$ charges in the Schiff base region are larger in MinCIC$_{2/51}$ with respect to FC. In Supplementary Fig. 15, we demonstrate such $\text{ESP}_{\text{opsin}}$ effect at CASSCF/6-31G*, CASPT2/6-31G*, XMCQDPT2/6-31G*, XMS-CASPT2/6-31G* and XMS-CASPT2/ANO-L-SPDV level of theory (XMCQDPT2 are executed with the FIREFLY$^{25}$ quantum chemical program). The energy profiles of the FC and MinCIC$_{2/51}$ geometries are evaluated with both original and scaling protein charges. The results show that, despite level of theory, the $S_2$ of the MinCIC$_{2/51}$ is better stabilized than the $S_1$, resulting in a more stabilized MinCIC$_{2/51}$ with respect to the FC. The scaled $\text{ESP}_{\text{opsin}}$, on the other hand, result in a destabilized MinCIC$_{2/51}$ with respect to the FC.
**Fig. 15.** Difference in relative energies and energy gaps between the FC and MinClS2/S1. Energy profiles calculated with full protein charges are in blue and with atomic charges multiplied by a 0.5 scaling coefficient are in green. The circles indicate an exchange in state character between $1B_u$ and $2A_g$.

**12. Involvement of $S_2$ in the dynamics of trajectories in the slow set.** In Fig. 3e we show the
various degree of $S_2/S_1$ state mixing during the first 50 fs of $S_1$ relaxation of fast and slow trajectory sets with the $S_2$-$S_1$ energy gap evaluated at CASPT2/6-31G* level of theory. In Supplementary Fig. 16 we show that such variety still exists when the energy profiles are re-evaluated at XMS-CASPT2/ANO-L-VDZP level of theory. It is seen in the plot of the average $S_2$-$S_1$ energy gap in Supplementary Fig. 16b that, despite showing a similar weak $S_2$ involvement the initial 10 fs, the $S_1$ and $S_2$ energies rapidly separate in the fast set but not in the slow set.

In the main text we describe the $S_1$ electronic character along the trajectories with charge distribution. The relatively stronger $S_2/S_1$ state mixing in the slow set can be assigned with a $S_2/S_1$ degeneracy region where the trajectories display multiple $S_1$ and $S_2$ charge switching. To further support our conclusion, we performed Mayer analysis\textsuperscript{26} to monitor the free valence index of C7, C9, C11, C12, C13 and C14 of the retinal chromophore along the trajectories. The average free valences of each atom (calculated as the numerical average value of all trajectories in the fast and slow set) shown in Supplementary Fig. 17 indicates that unlike the fast set, where $S_1$ and $S_2$ show distinct average free valences at the radical center (i.e., C7, C9 and C14), trajectories in the slow set displays similar free valences between the two states.
Fig. 16. Time evolution of $S_2$ and $S_1$ potential energy profiles for the fast and slow sets. (a) The energy profiles of $S_1$ (in blue) and $S_2$ (in black) calculated at XMS-CASPT2/ANO-L-VDZP level of theory. Solid and dashed yellow curves indicated the corresponding average value. (b) The time evolution of average $S_2$-$S_1$ energy gap of fast (in brown) and slow (in black) trajectory sets.
**Fig. 17.** Time evolution of $S_1$ (in blue) and $S_2$ (in black) average free valences for fast (solid curve) and slow (dashed curve) trajectory sets. In the fast set the $2A_g$ diradical character is described by the high coefficients (0.4-0.5) on C9 and, in part, C7. The second radical center is located on C14 so that the system can be depicted with the resonance formula on the top. It is evident that the corresponding centers in the slow set have very similar mixed characters.

13. $S_2$ optimization of an approximate QM/MM model (PSB5) at XMS-CASPT2/ANO-L-VDZP level of theory. In Supplementary section 11 we show that the $S_1$ energy at the CIS$_2$/S$_1$ lies
below that at the FC structure when both geometries are optimized at CASSCF/6-31G* level of theory. Since the investigation of such conclusion with geometries optimized at XMS-CASPT2 level is extremely difficult due to the relatively large (12, 12) active space, we instead perform $S_2$ optimization with the approximate QM/MM model (PSB5) documented in ref. 2. The results in Supplementary Fig. 18b show that such $S_2$ optimization leads to a $S_2$ minima with small $S_2/S_1$ energy gap (10 kcal/mol) and lies below the FC structure in terms of $S_1$ energy.

**Fig. 18.** Energy profiles calculated with PSB5 model. (a) Comparaison between the original Rh and approximated QM/MM PSB5 model. The PSB5 model side-chains originally belonging to the Rh model cavity but located outside the PSB5 cavity are kept frozen during the computations. (b) The energy gaps between the FC and $S_2$ minima.
14. The \(S_1\) initial population dynamics is not significantly sensitive the different level of state averaging (i.e. to the explicit presence of \(S_2\)): I. Two-root \(S_2/S_1\) and three-root \(S_2/S_1/S_0\) state average \(S_1\) progression and \(S_1\) PES mapping.

The involvement of non-adiabatic effects such as transitions from \(S_1\) to higher excited state (e.g. \(S_2\)) and back transitions from \(S_2\) to \(S_1\) are accounted for from the adopted quantum-classical Tully surface hop method. To see if such \(S_1\) to \(S_2\) transitions are frequent and, therefore, have an impact on the proposed mechanistic picture, we have recomputed the entire population dynamics for 50 fs by using two different additional levels of theory: the 3-root-state-average (3r-SA \((S_0,S_1,S_2)\)) and 2-root-state-average (2r-SA \((S_1,S_2)\)) CASSCF/6-31G*/Amber levels which explicitly incorporate the \(S_2\) singlet electronic state in the calculations (see also the Method section in the main text). The results show that \(S_1\) to \(S_2\) hops are detected in both cases but only in limited percentage (14% for the first level and 33% for the second level). Furthermore, \(S_1\) to \(S_2\) surface hop is followed, in almost all cases, by a \(S_2\) to \(S_1\) transition (see Fig. 4e middle and bottom panels in the main text) consistently with a short \(S_2\) lifetime. The \(S_1\) to \(S_2\) hop events occur, as expected, more frequently in slow trajectories (i.e. with respect to \(\alpha\) progression) but are also present in the fast trajectories. Accordingly, we conclude that the slow set described in the main text incorporates a mixed adiabatic and non-adiabatic progression restraining mechanisms. However, the restraining force seems to be characterized by the fact that slower sets transit, effectively, on a more "bounded" (i.e. higher in energy) region of the \(S_1\) potential energy surface (see also next section). This is supported by the data in Fig. 19 that shows only relatively minor differences in the trajectories (top panel) as well as characteristics and topography of the \(S_1\) PES (middle and bottom panels) driving the dynamics. This conclusion is reinforced by the geometry progression along \(\alpha\) and BLA given in the next section).
**Fig. 19.** Dynamics and PES characteristics near MinCl_{S2,S1} computed at different levels of theory

(a) Comparison between the $\alpha$ progression along a set of 200 trajectories of the Rh model computed with the original two-root state average (2r-SA (S1,S2)), three-root state average (3r-SA (S0,S1,S2)) and excited state two-root state average (2r-SA (S1,S2)) level of theory. The left diagram corresponds to the initial part of Fig. 2a. (b) Comparison between the contour diagrams representing the change in charge transfer in the MinCl_{S2,S1} region computed at the same level of theory as a function of the relevant geometrical coordinates. (c) Comparison between the contour diagrams representing the change in $S_1$ potential energy in the MinCl_{S2,S1} region along the same coordinates and for the same levels of theory. The black and red arrows indicate two different relaxation paths created by the presence of the $S_2/S_1$ conical intersection and, roughly,
driving the slow and fast dynamics also discussed in Fig. 4c of the main text.

15. The $S_1$ initial population dynamics is not significantly sensitive the different level of state averaging (i.e. to the explicit presence of $S_2$): II. Comparison of the $S_1$ time evolution of geometries and velocities computed at the two-root $S_1/S_0$ and $S_2/S_1$ and three-root $S_0/S_1/S_0$ state average level.

**Fig. 20.** Initial $\alpha$ and BLA relaxation dynamics computed at different levels of theory after (a)
10 fs, (b) 20 fs and (c) 30 fs dynamics at the original two-root state average 2r-SA ($S_0,S_1$) level and additional 3r-SA ($S_0,S_1,S_2$) and 2r-SA ($S_1,S_2$) levels.

**Fig. 21.** Initial change in $\alpha'/dt$ and dBLA/dt dynamics computed at different levels of theory after (a) 10 fs dynamics, (b) 20 fs dynamics and (c) 30 fs dynamics at the original two-root state average 2r-SA ($S_0,S_1$) level and additional 3r-SA ($S_0,S_1,S_2$) and 2r-SA ($S_1,S_2$) levels.

**16. Comparison between two-root $S_2/S_1$ and three-root $S_2/S_1/S_0$ state average $S_1$ progression.**

Here we compare the surface hop statistics obtained when using two- $S_2/S_1$ and three-root $S_2/S_1/S_0$ state averaging.
Fig. 22. $S_1 \rightarrow S_2$ surface hop statistics. (a) Data for the $2r$-SA ($S_1, S_2$) level of theory. Left. Time distribution of $S_1$ to $S_2$ and $S_2$ to $S_1$ hops and distribution (red line) of $S_2$ lifetime computed as the time separating $S_1$ to $S_2$ and $S_2$ to $S_1$ hops along the same trajectory. Right. Initial $\alpha$ progression along 200 trajectories released along the $S_1$ PES and allowed to hop to the $S_2$ PES. The average progression is marked with a red curve. The average progression of the subset undergoing at least one non-adiabatic transitions or no transitions are in yellow and blue respectively. (b) Same data computed using the $3r$-SA ($S_0, S_1, S_2$) level of theory.

17. MD input parameters.

GROMACS MD parameters:

constraints = none
define = -DFLEXIBLE
integrator = md
dt = 0.001 ps
nsteps = 20200000
nstxout = 1000
nstvout = 1000
nstxtcout = 1000
nstlog = 100
nstenergy = 1000
comm_mode = Angular

; Temperature coupling
gen_vel = yes
gen_temp = 298
gen_seed = 76543
tcoup = Berendsen
tc_grps = System
tau_t = 0.1
ref_t = 0

; Cutoffs and other stuff

cutoff-scheme = group
pbc = no
coulombtype = cut-off
vdwtype = cut-off
rlist = 0
rcoulomb = 0
rvdw = 0
nstlist = 0
ns_type = simple

; Freezing atoms
freezegrps = GroupDyna
freezedim = Y Y Y

; Heating
annealing = single
annealing_npoints = 2
annealing_time = 0 50
annealing_temp = 0 298

**Molcas/Tinker interface input:**
>> EXPORT  MOLCAS_MOLDEN=ON
>> COPY  $InpDir/$Project.xyz  $WorkDir
>> COPY  $InpDir/$Project.JobIph  $WorkDir/$Project.JobOld
>> LINK  FORCE  $Project.JobOld  JOBOLD
>> COPY  $InpDir/$Project.velocity.xyz  .
&GATEWAY
  RICD
  Tinker
  basis = 6-31G*
  group =  C1
>> Foreach  A  in  (1..200)
&SEWARD
  DOANA
&Espf
  External =  Tinker
&RASSCF
    jobi
    C1restart
    nactel=12 0 0
    ras2=12
    inactive=76
    ciroot=2 2 1
    mdrlxroot=2
>> COPY  $Project.JobIph  $Project.JobOld
&surfacehop
  Tully
  DECOHERENCE=0.1
  SUBSTEP  =  200
&MCLR
    iter=100
&ALASKA
&Dynamix
  Velver
  DT=41.3
  velo=1
  Ther=0
>> COPY  $Project.velocity.xyz  $InpDir/$Project.velocity.xyz.new
>> COPY  $Project.xyz  $InpDir/$Project.xyz.new
>> COPY  $Project.JobIph  $InpDir/$Project.JobIph.new
>> COPY  $WorkDir/$Project.RunFile  $InpDir/$Project.RunFileLast
>> End  Do
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