



HAL
open science

Sub-picosecond C=C bond photo-isomerization: Evidence for the role of excited state mixing

Damianos Agathangelou, Partha Pratim Roy, María del Carmen Marín,
Nicolas Ferré, Massimo Olivucci, Tiago Buckup, Jérémie Léonard, Stefan
Haacke

► **To cite this version:**

Damianos Agathangelou, Partha Pratim Roy, María del Carmen Marín, Nicolas Ferré, Massimo Olivucci, et al.. Sub-picosecond C=C bond photo-isomerization: Evidence for the role of excited state mixing. *Comptes Rendus. Physique*, 2021, 22 (2), pp.111-138. 10.5802/crphys.41 . hal-02996783

HAL Id: hal-02996783

<https://hal.science/hal-02996783v1>

Submitted on 9 Nov 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

1 Sub-picosecond C=C bond photo-isomerization:
2 Evidence for the role of excited state mixing
3

4 Photo-isomérisation sub-picoseconde de liaisons C=C:
5 L'importance du mélange des états excités
6

7 Damianos Agathangelou^{1,*}, Partha Pratim Roy^{2, †}, María del Carmen Marín³, Nicolas Ferré⁴,
8 Massimo Olivucci^{3,5}, Tiago Buckup², Jérémie Léonard¹, Stefan Haacke¹
9

10 ¹ Université de Strasbourg – CNRS, Institut de Physique et Chimie des Matériaux de Strasbourg,
11 67034 Strasbourg, France

12 ² Physikalisch-Chemisches Institut, Ruprecht-Karls Universität Heidelberg, 69120 Heidelberg,
13 Germany

14 ³ Biotechnology, Pharmacy and Chemistry Department, University of Siena, Siena 53100, Italy

15 ⁴ Aix-Marseille Univ. - CNRS, ICR, 13397 Marseille, France

16 ⁵ Chemistry Department, Bowling Green State University, Bowling Green, Ohio 43403, United
17 States

18 **Abstract**

19 *Sub-picosecond photo-isomerization is the major primary process of energy conversion in*
20 *retinal proteins and has as such been in the focus of extensive theoretical and experimental*
21 *work over the past decades. In this review article, we revisit the long-standing question as to*
22 *how the protein tunes the isomerization speed and quantum yield. We focus on our recent*
23 *contributions to this field, which underscore the concept of a delicate mixing of reactive and*
24 *non-reactive excited states, as a result of steric properties and electrostatic interactions with*
25 *the protein environment. Further avenues and new approaches are outlined which hold*
26 *promise for advancing our understanding of these intimately coupled chromophore-protein*
27 *systems.*
28

29 **Résumé**

30 *La photo-isomérisation sub-picoseconde est la principale réaction initiant la conversion*
31 *d'énergie dans les protéines de rétinal, si bien qu'elle fait l'objet de travaux théoriques et*
32 *expérimentaux approfondis depuis plus de trente ans. Dans cet article de revue, nous revisitons*
33 *la question toujours ouverte de savoir comment la protéine détermine la vitesse*
34 *d'isomérisation et son rendement quantique. A la lumière de nos contributions récentes en ce*
35 *domaine, nous décrivons le concept d'un mélange d'états excités réactifs et non-réactifs,*
36 *délicatement ajusté par les interactions stériques et électrostatiques avec l'environnement*

* Now at : LUMOS lab, University of Michigan, Department of Physics, USA

† Now at : University of Berkeley, Department of Chemistry, USA

1 *protéique. De nouvelles perspectives et approches prometteuses sont décrites qui pourront*
2 *faire progresser la compréhension de ces systèmes chromophore-protéine intimement couplés.*

3

4 Keywords: Photo-isomerisation, retinal proteins, chromophore-protein coupling, non-
5 adiabatic dynamics, photo-sensory proteins, charge transfer state

6

7

1
2 Many organisms, from microbacteria to vertebrates, use photosensitive retinal proteins for
3 photo-chemical energy conversion or photo-sensory functions [1–3]. Visible light-absorbing
4 retinal proteins rely on the protonated Schiff base of retinal (rPSB) as chromophore, covalently
5 bound to the interior of a barrel-like “opsin” protein. Their biological activity is triggered by a
6 regioselective and stereoselective C=C double bond photoisomerization of rPSB, e.g. 11-*cis* to
7 all-*trans* for the visual pigment rhodopsin (Rho), all-*trans* to 13-*cis* for the proton pump
8 bacteriorhodopsin (bR), or both 13-*cis* to all-*trans* and all-*trans* to 13-*cis* for the bistable,
9 photochromic Anabaena Sensory Rhodopsin (ASR). In all cases, the protein scaffold exerts
10 electrostatic and steric interactions on the chromophore [4–7], which tune the rPSB
11 photoreaction dynamics, e.g. the excited state lifetime (ESL) and its photoisomerization
12 quantum yield (IQY), ultimately determined by the branching ratio between reactive and non-
13 reactive channels. The pronounced influence of the protein interaction on rPSB photo-
14 reactivity is evidenced by comparing with the photoreaction dynamics and IQY of rPSB in other
15 environments, e.g. in solution [8–14] or in vacuum [15].

16 The photoisomerization of rPSB occurs through a conical intersection (CInt), [16,17] which is
17 an intersection seam between the excited (S_1) and ground (S_0) state potential energy surfaces
18 (PES), where the molecule transiently adopts a configuration with a nearly 90° twisted reacting
19 double bond. While it has been shown that there is no correlation between ESL and IQY [18–
20 21], many theoretical predictions agree, that the PESs topography and the nuclear motions at
21 the CInt decide on the branching between reactive and non-reactive pathways, hence on the
22 overall IQY [17,22,23]. There is large scientific interest at investigating the photoreactions of
23 retinal proteins in order to decipher the intra- and inter- molecular parameters which tune
24 the photoreactivity of rPSB, as a model system for non-adiabatic dynamics through a CInt. In
25 addition, such a fundamental understanding would be the key to define chemical design
26 strategies to control the photoreactivity of C=C double bond isomerization in retinal
27 chromophores or similar compounds. In particular, there is broad interest in producing
28 rhodopsin mutants with an enhanced ESL and, as a consequence, fluorescence quantum yield
29 (FQY) for applications in optogenetics [24,25]. Moreover, understanding the dynamic
30 structure-property relationships will most likely indicate new avenues for tailoring molecular
31 devices based on ultrafast C=C double isomerization to perform photoswitching or rotary
32 motion, at the molecular scale, with optimum efficiency [26].

1 Chapter I gives a brief and necessarily incomplete account of recent experimental studies on
 2 the effect of the environment of the photophysics of rPSB's, with an emphasis on the
 3 electrostatic properties, in particular the presence or protonation state of counter ions.
 4 Chapters II to IV concentrate on both electrostatic and steric effects on the ESL and FQY of
 5 rPSB in different retinal proteins and rhodopsin-mimicking photo-switches in solution. Steric
 6 effects, in particular the distortion of the rPSB in the ground state of 11C in Rho as opposed
 7 to the more planar AT in bR is held responsible for the faster photo-isomerization reaction in
 8 Rho [27–32], as will be corroborated in these chapters. In chapter II, we review the concept of
 9 $^1B_u/{}^2A_g$ mixing between the first two excited states (**Error! Reference source not found.**), and
 10 how it translates both steric and electrostatic properties comprehensively. Chapter III then
 11 treats the experimental manifestation and quantitative explanation of these effects for
 12 *Anabaena Sensory Rhodopsin* and some selected mutants. Chapter IV extends this combined
 13 treatment to rhodopsin-mimicking photo-switches, before chapter V summarises the main
 14 concepts and outlines future avenues.

15

16 I. An open question studied on different systems

17 The protonation of the retinal Schiff base in VIS-absorbing retinal proteins makes these
 18 systems distinctively different from their UV-absorbing sisters, which function with a
 19 deprotonated chromophore [33]. The charge-transfer character of the lowest excited state
 20 [34,35], S_1 , is at the heart of the high sensitivity to the presence of counter ions and dynamic

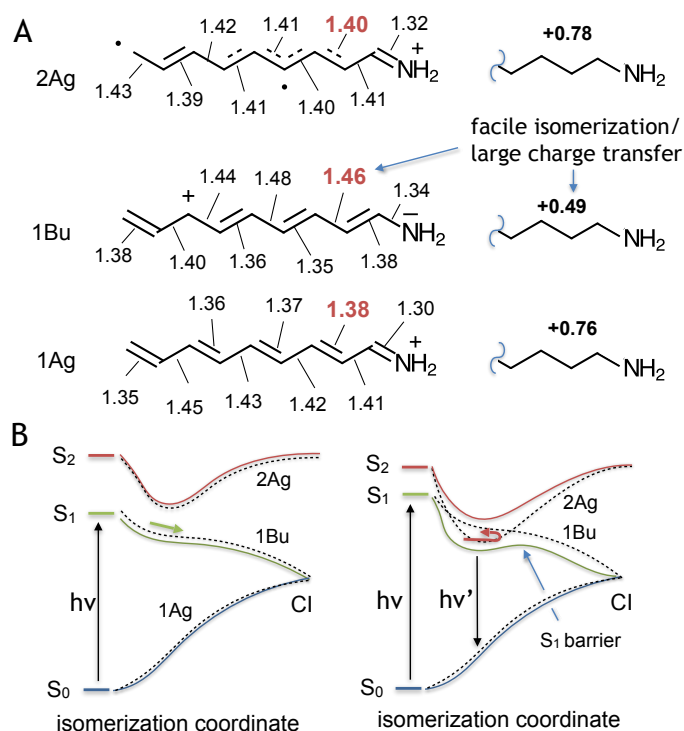


Figure 1: Schematic representation of A: the charge transfer character and bond length alternation (BLA) of the most relevant electronic states S_0 , S_1 and S_2 of rPSB in Franck-Condon geometry, and B : Two extreme cases of weak and strong mixing of the 1B_u and 2A_g character of the first two excited states, presented together with S_0 as a function of an effective isomerization coordinate, limited to the first 90° rotation of the reactive double bond (see also fig. 11). Note the conical intersection (CI) between S_1 and S_0 . Refer to chapter II for a more detailed discussion.

1 electrostatic interactions, thereby controlling the excited state dynamics. This will be
2 highlighted in the following paragraphs.

3

4 ***a. Isolated rPSB's in solution and gas phase***

5 Since 11-*cis* (11C) and all-trans (AT) isomers are the ground state conformations of the visual
6 photo-receptor and of the large majority of microbial retinal proteins, respectively, these were
7 studied quite extensively as isolated molecules in solution and to a lesser extent in the gas
8 phase. It was first noticed that the absorption spectra in solution are significantly blue-shifted
9 with respect to the situation in protein cavities (referred to as the “opsin shift”). It can be
10 quantitatively explained by a stronger polarizability of the protein environment and
11 interaction of the rPSB with its counter ion in solution, reduced though for polar solvents, and,
12 for AT rPSB, by an enhanced ring/chain co-planarization in proteins [36–39]. In solution, the
13 11-*cis* isomer photo-isomerization produces specifically the AT isomer, like in proteins. The
14 IQY is however only about 25% in various solvents versus 67% in Rhodopsin. Conversely, the
15 photo-isomerization of AT in solution produces a variety of product isomers, unlike in proteins
16 where it is 100% bond-selective, and occurs with a total IQY in the range of 15% [40–42].
17 Femtosecond transient absorption and fluorescence spectroscopy consistently showed that
18 the excited state lifetimes and thus the isomerization times are significantly longer than in
19 proteins, with a dominant decay component in the 3-4 ps range, for both the 11C and AT
20 rPSB's [8,10,12,43–45]. For AT, sub-picosecond fluorescence decay components were
21 attributed to internal conversion and the dominant non-reactive back reaction [10,21]. The
22 ESL increase at lower temperatures points to the existence of a small excited state barrier [12].
23 Conversely, in the case of 11C in solution, the sub-ps component was attributed to the
24 minority, barrier-less reactive channel while the 4-ps component would characterize the
25 dominant non-reactive channel [45].

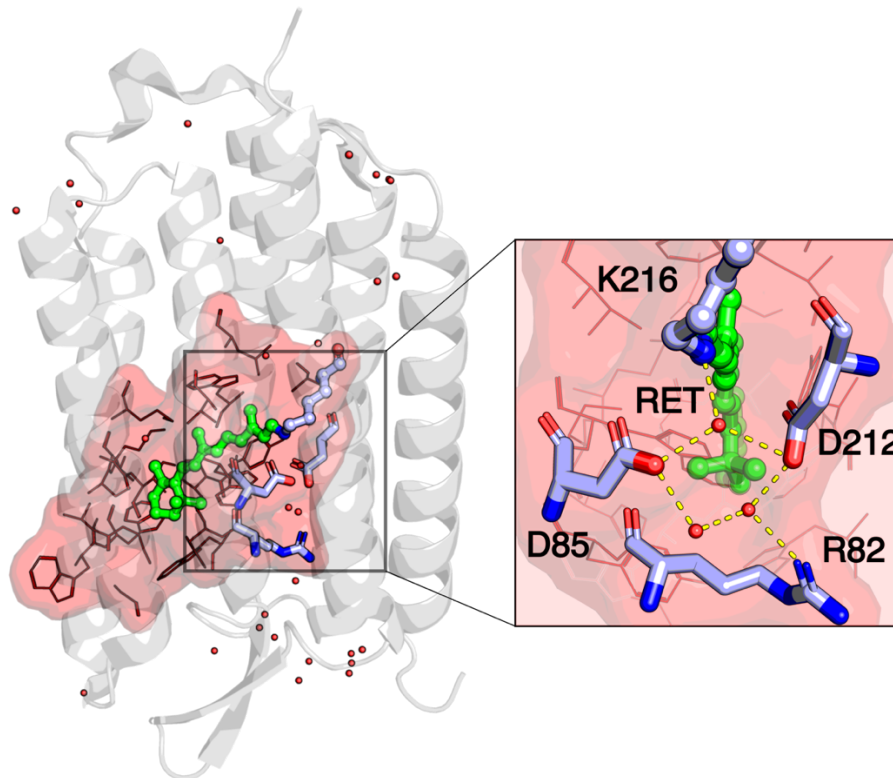
26 In the absence of a counter ion or any polarisable environment, i.e. for rPSB's in gas phase,
27 the absorption spectra of AT and 11C rPSB's are red-shifted with respect to the protein
28 spectra, due to a relative destabilisation of S_0 [46,47]. The energies of the UV/VIS electronic
29 transitions are obtained by recording the photo-fragmentation action spectra of the cationic
30 chromophores held in an ion storage ring. Recently, this approach was extended to two-pulse
31 experiments, which allowed to record the excited states lifetimes *in vacuo* [15]. A first pump
32 pulse resonantly tuned onto the S_0 - S_1 transition excites the molecules, and, after a delay time
33 τ , a second pump pulse at 800 nm promotes the molecules further to S_3 . This additional excess
34 energy stored in the molecule leads to enhanced photo-fragmentation, as compared to the
35 pump pulse-only case. Due to the limited ESL, the fragmentation enhancement is dependent
36 on τ , and the delay-dependent signal measures the time-dependent fraction of excited
37 molecules. A series of comparative experiments, including the effect of temperature
38 concluded that, *in vacuo*, 11C rPSB has an almost barrierless fast decay (≈ 0.4 ps at room
39 temperature), while AT shows a barrier-controlled > 3 ps decay.

40 Hence, both solution and gas phase experiments suggest, that in visual rhodopsin the ESL is
41 inherited from the “intrinsic” properties of the 11C chromophore [15,45], whereas in bacterial
42 rhodopsins, the protein tunes the excited state potential energy surfaces (PES) of AT in terms
43 of barrier height and bond selectivity [15,44]. The origin of these environment-induced
44 modifications of the PES will be discussed in detail in chapter II, on the basis of computational
45 results involving mixing of the first two excited states. While such modelling allows
46 rationalizing the effect of the protein environment on the ESL, the question about how the

1 proteins tune the IQY of 11C and AT chromophores remains largely open. So far, only in the
2 unique case of rhodopsin – showing the fastest photoisomerization reaction - a direct
3 connection was established between the specific vibrational motions driven by the PES
4 topography and the IQY, pointing to the central role of the relative phase between a few
5 essential vibrational modes at the moment of decay at the Clnt [23].

6
7
8

b. Effects of mutations and pH on excited state dynamics of retinal proteins – a selection



9
10
11
12
13

Figure 1: Crystallographic structure of bR (pdb entry 5ZIL), at pH=7, highlighting the retinal chromophore RET (green), covalently bound to Lys216 (blue). The protonated Schiff base linking Lys216 and RET, is connected to the counter ion, the deprotonated aspartic acid D85, via a water molecule. Right : The zoom shows the H-bonding network involving three waters and key residues. Their effects on bR's ESL and IQY was studied by femtosecond spectroscopy (see text).

14 The most studied microbial rhodopsin is bR, a light-activated proton pump, found in
15 *Halobacterium salinarum* [48]. In its light-adapted form, it binds the rPSB primarily in the AT
16 form. In wild-type bR, AT→ 13C photo-isomerization occurs within ≈ 0.5 ps [49–52], and with
17 a quantum yield of $\approx 65\%$ [53–55]. As shown in Figure 1, the crystallographic structure, at pH=7,
18 identifies the deprotonated aspartic acid D85 as the main counter ion of the rPSB connected
19 via a H-bond network with D212 and R82 via three water molecules [28]. First experimental
20 work by El-Sayed and Lanyi [56,57] reported that the replacement of charged residues in the
21 mutants D85N, D212N and R82Q by neutral ones increases the excited state lifetime and thus
22 isomerization time by a factor of 5 to 20. Replacements of uncharged residues in mutants
23 D115N or Y185F did not change the lifetimes significantly. The exact analysis was complicated
24 by the fact that mutations, much like acidification [58], induce a mixture of AT and 13C isomers
25 in the ground state. The observations were explained by a valence bond resonance model
26 describing the excited state as a linear combination of wavefunctions, the electronic densities
27 of which have the positive charge translocated from the Schiff base to different C atoms with
28 odd numbers along the polyene chain. This provokes for every ground state double bond a

1 reactive single bond character in the excited state, but depending on the fraction of local
2 positive charge. Due to the nearby negatively charged D85 and D212, the positive charge is
3 stabilised best on C13 in the excited state of wild-type bR, providing an intuitive explanation
4 of the bond selectivity of the AT \rightarrow 13C isomerization process. The excited state single bond
5 character of C13=C14 allows then for a barrierless and therefore ultrafast isomerization. These
6 effects are absent when D85 and D212 are replaced by neutral residues or if they are
7 protonated (neutral) as in R82Q at pH=4 [56]. The results for D85N were later confirmed and
8 complemented by femtosecond photon echo spectroscopy, which provided evidence for a
9 large sub-100fs dielectric relaxation of the protein environment [59].

10 Another more recent example for large effects on ESL and IQY related to the chromophore
11 environment, was recently reported for KR2, from *Krokinobacter eikastus*. In 2013, it was
12 discovered as the first light-activated Na⁺ pump [60]. Its crystallographic structure was
13 resolved [61,62], highlighting particular motifs responsible for Na⁺ binding at the protein
14 periphery, and most importantly a transient Na⁺ binding site, in the vicinity of the rPSB, made
15 up of D116, N112 and D251, and, with respect to bR, a modified H-bonding network.
16 Comparing the crystal structures at neutral and acidic pH allowed to identify the flipping of
17 the retinal counterion, the aspartic acid D116, as a key element for Na⁺ pumping. At neutral
18 pH, D116 is H-bonded with retinal's Schiff base (rotamer 1), but during the M intermediate of
19 KR2's photocycle, protonation of D116 via the Schiff base (SB) occurs. This would lead to its
20 re-orientation (rotamer 2) and disruption of the otherwise strong H-bonding network, thus
21 enabling Na⁺ translocation [61]. The overall structural changes associated with Na⁺ pumping
22 were very recently analyzed by time-resolved serial X-ray crystallography and QM/MM
23 simulations, covering the pico- to milli-second time scales [63]. However, the changes in the
24 SB-D116 distance were observed to be much smaller than expected, and no clear evidence for
25 the existence of two rotameric states was found in the photocycle at pH=8.0.

26 Regarding ultrafast events, Hontani et al. reported a spectroscopic investigation of the full
27 photo-cycle, and in particular the primary AT \rightarrow 13C isomerization to occur in KR2 within \approx 200
28 fs, at pH=8.0 [64]. This is significantly faster than for other microbial rhodopsins bearing AT
29 rPSB in the ground state and was attributed to steric effects, i.e. distorsion of the AT isomer
30 [65]. Tahara et al. investigated the effect of pH on the primary photo-reaction by femtosecond
31 transient absorption spectroscopy (TAS) [66], with the main objective of obtaining new
32 insights into minority excited state species with > 3 ps lifetimes observed at pH=8.0 [65].
33 Protonation of D116 occurs for pH $<$ 7, and leads to a significant red-shift of the absorption
34 spectrum [60], due to destabilization of the protonated SB ground state (S_0). KR2 at pH $<$ 7 and
35 the D116N mutant show several ps excited state decay components, in addition to the 0.18
36 ps ESL, whose amplitudes increase for lower pH values. The opposite is observed for pH $>$ 7. A
37 good correlation with the pH-dependent IQY showed that only the 0.18 ps decay channel is
38 productive in terms of isomerization. A more detailed analysis of the data suggests that, rather
39 than the protonation state of D116N, the key parameter favoring the fast and efficient excited
40 state pathway is the orientation of that residue and its fraction of population able to form H-
41 bonds with retinal's Schiff base [66]. While a similar co-existence of multiple excited state
42 decay components was previously attributed to reactive and non-reactive excited state
43 pathways [21,44,56,67,68], this study points to the central role of structural inhomogeneities
44 in the ground state, modulated by the protonation of D116N. Similar conclusions were
45 recently drawn by the same authors for the photo-isomerization reactivity in proteorhodopsin
46 [69]. In conclusion, KR2 appears to be a particular retinal protein, affording a flexible and thus
47 disordered Schiff base environment, required for the binding of Na⁺, but at the cost of a

1 relatively low isomerization IQY. Nevertheless, intensive research is under way to use KR2 as
2 a platform for genetic modifications in view of applications in optogenetics [3,24,70,71].
3 Last but not least, it is worth noting that the protein environment should not be considered
4 as a rigid grid of point charges. In addition to ultrafast nuclear motion of the surrounding
5 residues and their related charges [72,73], several experiments performed on bR point to a
6 dynamic electronic response, upon excitation of rPSB, in particular for the polarisable nearby
7 Trp residues, such as Trp86 [74–76]. From a computational point of view, these effects would
8 require a quantum mechanical treatment of the large “rPSB/polarizable residue” system [73]
9 and are out of the scope of the present review.

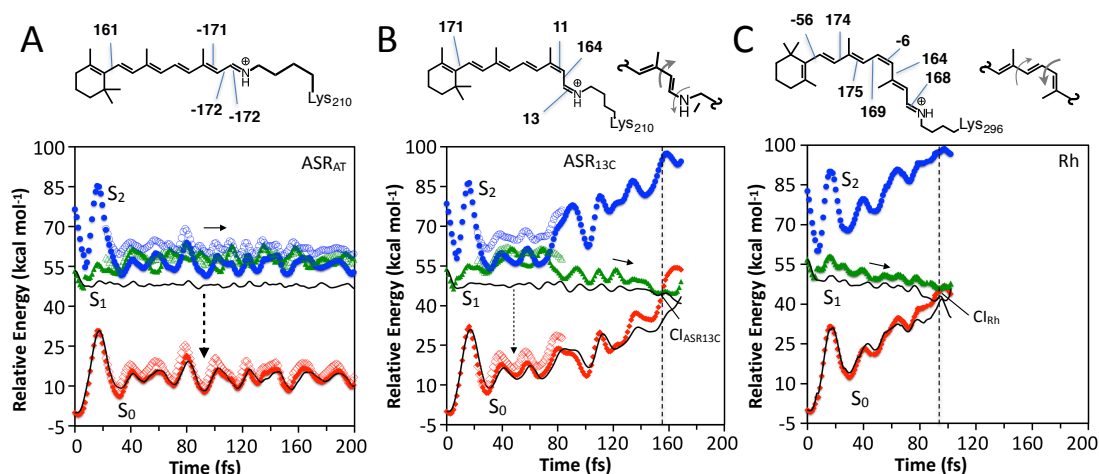
11 II. Theoretical framework: Potential energy surfaces and CInt

12 Since the rPSB is a special case of a linear polyene chain, the role of possible interactions of
13 the first and second excited states (S_1 and S_2) has been frequently evoked and examined in
14 the past [5,77–82]. Adopting the symmetry nomenclature of polyenes, the electronic ground
15 state is referred to 1A_g , and the first excited states to 1B_u and 2A_g symmetry (cf. **Error!**
16 **Reference source not found.**), even though the chromophore is asymmetric due to the
17 positive charge on the Schiff base end. Hence, at the FC point (i.e., at the S_0 equilibrium
18 structure), S_0 has 1A_g covalent character with alternating single/double bond lengths, and a
19 positive charge located on the C=NH₂ framework end. With respect to the ground state, the
20 1B_u state can be described by a bonding π to an anti-bonding π^* electronic transition,
21 delocalized all over the retinal backbone. Such a modification triggers a substantial structural
22 change, characterized by single and double bond inversion (cf. **Error! Reference source not**
23 **found.**), hence the bond length alternation (BLA, defined as the average single bond length -
24 average double bond length) changes sign. Negative BLA is necessary for the isomerization to
25 occur, i.e. 1B_u is the reactive configuration. In addition, the state has a pronounced charge-
26 transfer (CT) character, which implies that the positive charge on the C=NH₂ framework end
27 is reduced and translocated to the polyene backbone. By contrast, the S_2 PES has 2A_g diradical
28 (DIR) character with a strongly reduced BLA. Compared to 1B_u , isomerization is hindered, and
29 the state has non-reactive character. Last, similar to 1A_g , the positive charge remains located
30 on the C=NH₂ Schiff base end [83]. The different charge distributions in these three states lead
31 to a situation where their energy differences, $\Delta E(S_1-S_0)$ and $\Delta E(S_2-S_1)$, are very sensitive to
32 electrostatic interactions and thus to static or dynamic modifications of the environment (see
33 below).

34 From recent quantum chemical computations, it is now well recognized that the energies of
35 the 1B_u and 2A_g states are strongly dependent on the isomerization coordinate, hydrogen-out-
36 of-plane motion and C=C dihedral angles, and that they may even come into degeneracy [84–
37 88]. In such extreme cases, the $^1B_u/{}^2A_g$ mixing is complete, and the system oscillates in S_1
38 between the CT and DIR character, with a frequency determined by BLA. Such a regime was
39 very recently reported to occur in bR, during the first 100 fs after excitation and only if the
40 laser pulse was tuned to shorter wavelengths in order to explore higher vibronic states in S_1
41 [87].

42 In recent years, several papers reported on QM/MM calculations, which addressed the
43 dynamic $^1B_u/{}^2A_g$ coupling along the reaction coordinate, in more general cases. Blending non-
44 reactive 2A_g character into the S_1 state, extends the excited state lifetime. It turns out that the
45 strength of $^1B_u/{}^2A_g$ interaction depends critically on the GS geometry of the rPSB (e.g. 11-*cis*
46 vs. all-*trans*) and on the electrostatic properties of the environment, no matter if it is a solvent
47 or the protein cavity. The then created excited state potential energy landscape may be fully

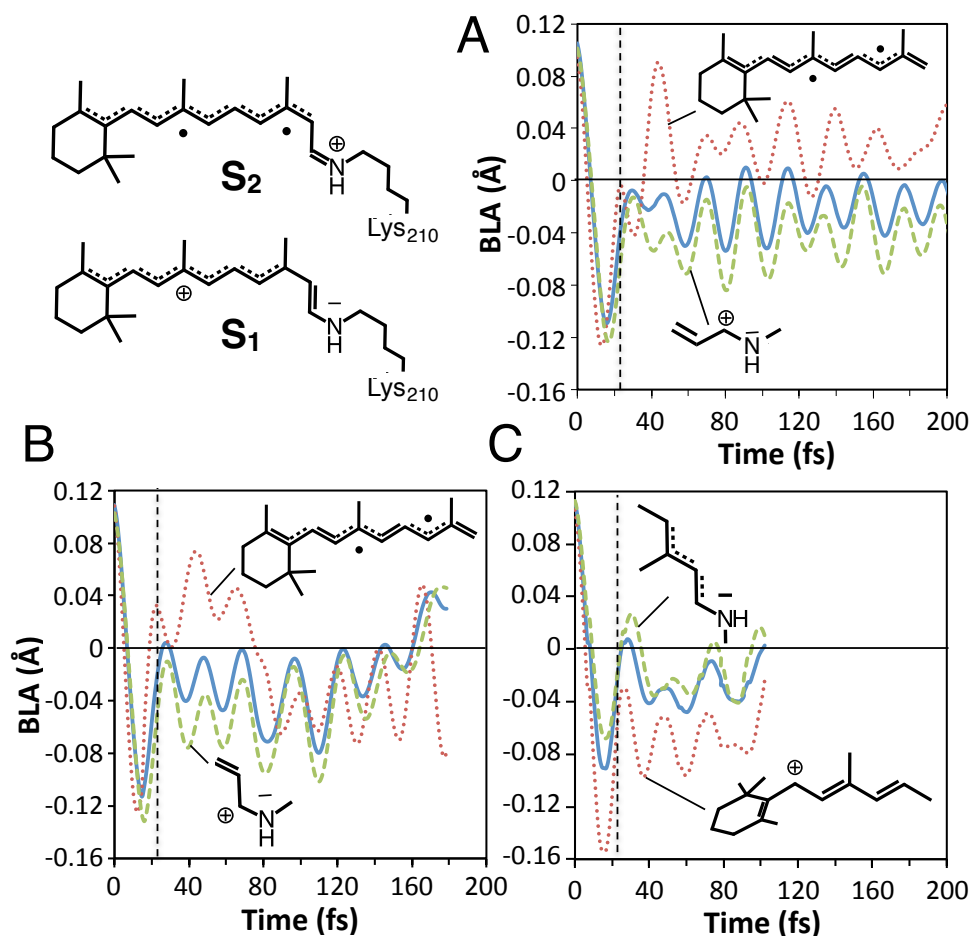
1 reactive or present barriers (cf. **Error! Reference source not found.-B**), and is thereby
 2 determining the ESL and thus the FQY and isomerization reaction speed.
 3 Since 13-*cis* rPSB in microbial protein environments have ESL close to the ones of 11-*cis*
 4 rhodopsin, and both types of chromophores are found to be non-planar, it was concluded that
 5 a pre-twist is synonymous of a displaced excited state potential [30,89–91] (in particular along
 6 hydrogen-out-of-plane motion (HOOP), which display larger Raman activities, cf. section III).
 7 This would lead to a more effective acceleration of the excited state wavepacket along the
 8 reaction coordinate [81,92]. Recent multi-configurational QM/MM simulations go a step
 9 further and rationalise the effect of the rPSB geometry on the excited state energy landscape
 10 computing the S_1 and S_2 energies of the full chromophore within CASSCF or CASPT2
 11 approximations, and embedded in the respective natural protein environments (bovine
 12 rhodopsin for 11-*cis* – 11C/Rho; ASR for 13-*cis* (13C) and all-trans (AT), AMBER force
 13 fields)[86].



14
 15 *Figure 2: QM/MM trajectories of AT/ASR (A), 13C/ASR (B), and 11C/Rho (C). S_0 (red), S_1 (green), and S_2 (blue) The main out-*
 16 *of-plane (deviation larger than $\pm 5^\circ$) dihedral angles of the S_0 equilibrium structure of the rPSBs are given at the top, together*
 17 *with the bicycle pedal motion driving the isomerization on S_1 with reversed bond order. The vertical dashed arrow represents*
 18 *weak fluorescence. Labels ASR_{AT} , ASR_{13C} and Rh correspond, respectively, to AT/ASR, 13C/ASR and 11C/Rho in the text.*
 19 *Reproduced with permission from [86].*

20 Figure 2 shows the QM/MM trajectories for the three cases in the first 200 fs or until the S_1/S_0
 21 Clnt is reached. The computed energies are found to oscillate following the BLA. For 11C/Rho,
 22 S_1 and S_2 are separated at all times and they preserve their 1B_u and 2A_g character, respectively
 23 until the Clnt is reached, i.e. the $^1B_u/{}^2A_g$ coupling is negligible (panel C). S_1 therefore preserves
 24 a strongly reactive CT character, and Figure 3 shows that bond length inversion is complete
 25 (BLA < 0) along the C5=C6–C7=C8–C9=C10–C11=C12 fragment of the chromophore. Both
 26 effects lead in the specific protein environment to a barrierless potential and a rapid down-
 27 hill progression reaching the Clnt within less than 100 fs, in agreement with experiments
 28 [31,93–96].

29



1
2
3
4
5
6
Figure 3: BLA changes in ASR and Rho. (A) BLA changes for the AT/ASR chromophore along the full conjugated chain (solid line), along the C5=C6-C7=C8-C9=C10-C11=C12-C13=C14 fragment (dotted line), and along the -C13=C14-C15=N fragment (dashed line). (B) Same data for the 13C/ASR chromophore. (C) The BLA along the full conjugated chain for the 11C/Rho (solid line), along the C5=C6-C7=C8-C9=C10-C11=C12 fragment (dotted line), and along the -C11=C12-C13=C14-C15=N fragment (dashed line). Reproduced with permission from [86].

7 In 13C/ASR, after an initial BLA displacement, S₁ and S₂ are degenerate in the 40-80 fs time
8 interval, and the dihedral angles, along the C₁₃=C₁₄ & C₁₅=N axes, remain almost planar [86].
9 During the same period, Figure 3 shows that BLA is positive, i.e. S₁ is dominated by a non-
10 reactive DIR character associated with a very limited charge transfer from the -C=N moiety.
11 Only after 80 fs, does S₁ display a negative BLA, a substantial charge transfer and a reactive
12 ¹B_u character, with a down-hill progression towards the CInt by ≈150 fs, in agreement with
13 experiments [19,90,97]. For AT/ASR, S₁ and S₂ are found to remain degenerate during the
14 whole simulation interval of 200 fs (Figure 2). BLA is positive, meaning a non-reactive DIR
15 character prevails (Figure 3-A), explaining the longer ESL observed for AT than for the 13C rPSB
16 in ASR [19,90,97].

17 The above empirical rule stating that the chromophore's isomeric state combined with
18 protein-imposed deformations would control the ESL can now be explained rather intuitively,
19 in terms of the dominant ²A_g or ¹B_u character. In fact, AT/ASR maintains four conjugated
20 double bonds (C5=C6-C7=C8- C9=C10-C11=C12-) with a reduced, but still positive BLA,
21 rather than bond length inversion (Figure 3-A). Such a polyene-like pattern accommodates the
22 non-reactive DIR electronic structure better than the CT structure. On the other hand, due to
23 the pre-twisted β-ionone ring on one side and the rapidly twisting C11=C12 on the other,

1 11C/Rho offers a shorter fragment with two conjugated double bonds (C7=C8–C9=C10–C11)
2 with a negative (inverted) average BLA value (Figure 3-C). As a consequence, $\Delta E(S_2-S_1)$ will
3 rapidly decrease towards a degeneracy along the photoisomerization coordinate in AT/ASR
4 and 13C/ASR but not in Rho.

5 In subsequent publications, the concept of $^1B_u/{}^2A_g$ mixing was further investigated
6 computationally for the environment-induced differences of 11-*cis* rPSB in methanol and in
7 Rho [84], and for the effects of substitutions of the all-trans rPSB in solution with electron-
8 donating or –withdrawing groups [84,88]. Experimental data, regarding the ESL [11,98], where
9 consistently reproduced and explained in terms of the build-up of an excited state barrier or
10 flat, population-stabilising regions due to an increased 2A_g character in the S_1 state, limiting its
11 reactivity. In summary, the concept, which emerges from these theoretical results, is that both
12 steric and electrostatic effects act through the modifications of the S_1/S_2 energy landscape in
13 determining the ESL of rPSB in different protein environments (exemplified in detail in chapter
14 III), and also of rhodopsin-mimicking photo-switches in solution (chapter IV).

15

16 *III. Effects of point mutations: the case of Anabaena Sensory Rhodopsin*

17

18 *Anabaena* Sensory Rhodopsin (ASR) is a membrane protein of 261 residues (26 kDa) found in the
19 eubacterium *Anabaena* (*Nostoc*) sp. PCC7120 [99], and is part of the family of archeal microbial
20 rhodopsin, as much as bR or sensory rhodopsin from *Halobacterium salinarum* [1,100]. The main
21 structural differences with respect to bR are the replacement of Asp212 in bR by Pro206, a non-polar
22 residue, in the vicinity of the rPSB and a hydrogen-bonding network from Lys210 to Glu36 [101].

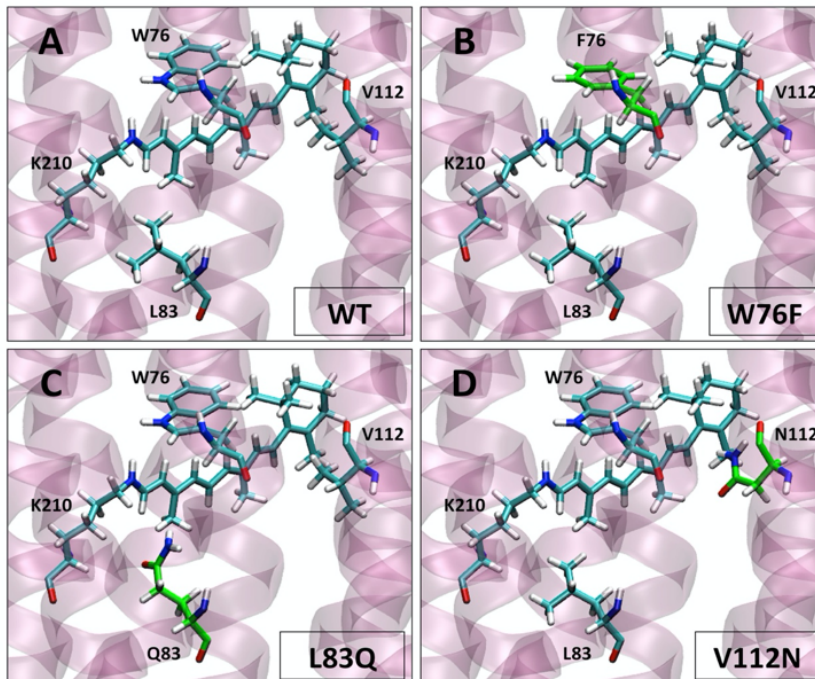
23 Regarding the ultrafast photo-isomerization, it is most notable that ASR bears a mixture of all-
24 trans,15-anti (AT) and 13-cis, 15-syn (13C) rPSB isomers, the relative content of which depends
25 on the illumination, so-called 'light-adaptation' conditions. When left in the dark, the dark-
26 adapted (DA) form adopts 97% AT, which drops to less than 50% under illumination with
27 orange light, at neutral pH [102]. In the context of obtaining insight into the mechanisms by
28 which retinal proteins catalyze the rPSB photo-isomerization reaction, ASR is a model system
29 allowing to study, within the same protein, the protein–rPSB interactions for two different
30 rPSB conformers at the same time. Interestingly, at pH=7, both isomers display markedly
31 different excited state lifetimes (ESL): ~ 0.7 ps for AT, and approx. 150 fs for 13C [19,90,97,103].
32 This was empirically related to the fact that 13C is twisted in the ground state conformation
33 [101], but different electrostatic or dynamic interactions with the protein charge distribution
34 may also play a role. The different excited state lifetimes were rationalized by quantum
35 chemistry computations of the excited state potential energy surface (PES) along the
36 minimum energy paths in ASR [22], and with excited state trajectory calculation [84,86,104].
37 As shown in Figure 2, it was found that the S_1 PES of 13C is barrierless, while that of the AT
38 form displays a plateau or a slight barrier that could account for a short resting period of the
39 excited state population. The precise origin of these different shapes of the S_1 PES for both
40 isomers, and the mechanism of their fine-tuning by the protein interaction has motivated the
41 research efforts described below. While sections *a* and *b* summarize primarily experimental
42 work on the electronic and vibrational dynamics of AT and 13C/ASR, as a function of
43 mutations, section *c* reviews a combined experimental/theoretical study of two cases of
44 mutations which track the molecular origin of a 12-fold enhancement of the ESL.

45

46 **a. Effect of mutations on the ESL: Rationalising spectral shifts and effects on excited state** 47 **lifetimes**

1 Our first joint paper on mutation-induced effect in ASR, published in 2018, reported a
2 combined experimental and computational study of the spectral shifts, the ground state
3 vibrational spectra and isomerization reaction times of the AT and 13C isomers in the mutants
4 L83Q, V112N and W76F [105].

5 **Effects of the modified protein electrostatics on the isomer-specific absorption spectra.** In
6 L83Q and V112N, the nonpolar residues Valine and Leucine are replaced by the polar but
7 neutral Glutamine (Q) and Asparagine (N), respectively, while the W76F mutation reduces the
8 electron density and removes a small dipole close to the rPSB backbone (Figure 4). All
9 mutations lead to blue-shifts of the DA and LA ground state absorption spectra with respect
10 to wt-ASR [105]. Light- adaptation was carried out either with an orange (OA, 590 nm) or a
11 blue (BA, 460nm) LED, and the light-adaptation with the highest 13C isomer ratio was
12 retained. The latter was higher than 50% in all cases, as for wt-ASR. V112N and W76F exhibit
13 a blue-shift under LA as wt-ASR and many other mutants. The absorption spectrum of the 13C
14 isomer of these mutants shifts by 2 nm (V112N) and 8 nm (W76F). L83Q, the blue-most shifted
15 mutant, surprisingly shows the opposite effect, with the 13C isomer being 6 nm red-shifted
16 with respect to AT.
17

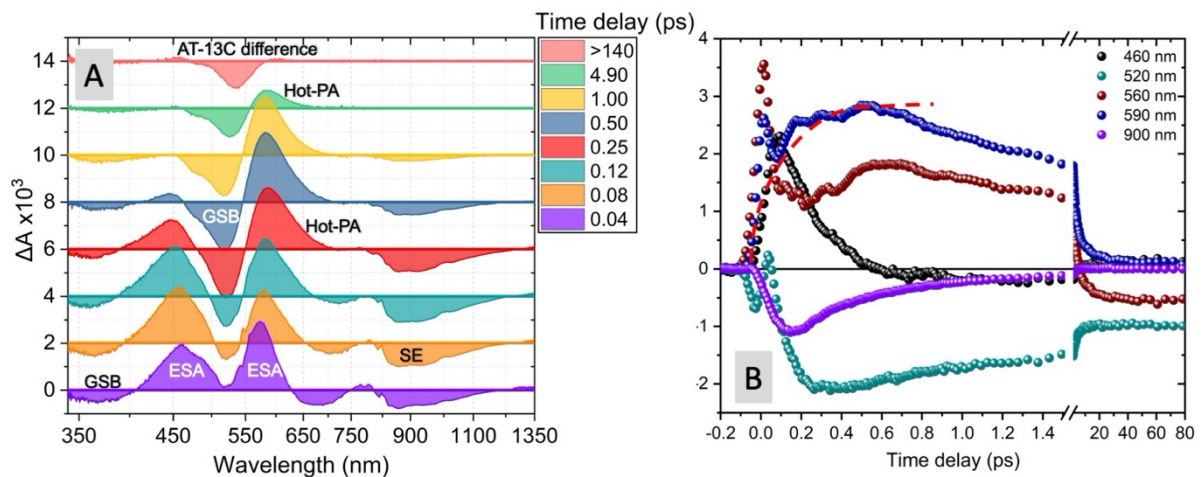


18
19 *Figure 4: Calculated structures of the AT rPSB and the mutated residues L83, W76 and V112N in DA ASR (A). The replacement*
20 *of Trp76 by a Phenylalanine reduces the electron density close to the AT rPSB backbone (B). Introduction of the polar Glu at*
21 *position 83 and Asp at position 112 (C&D) changes the electrostatic interactions with rPSB in both S_0 and S_1 .*

22 From a purely computational structure of the mutated protein-chromophore complexes,
23 obtained by the automatic retinal protein model (ARM) [106], the mutation-induced shifts
24 were correctly reproduced, as well as the amount of LA-induced blue-shifts for W76F and
25 V112N. For L83Q, ARM produced a wrong orientation of the dipoles of Q83, which was
26 corrected “manually”. This produced absorption maxima consistent with the observations,
27 including the inverted LA effect. By comparing both the effects of steric and electrostatic
28 modifications, it was concluded that the latter effects are dominant, in particular for W76F.
29 Indeed, the mutation-induced changes of the rPSB configurations cause, with respect to the
30 wt-ASR, rather a red-shift and not the experimentally observed blue-shifts. For both the
31 V112N and W76F mutants, a change from Valine to Asparagine, near the α -ionone ring of the
12

1 chromophore, and Tryptophan to Phenylalanine produces a destabilization of S_1 and a smaller
2 stabilization of S_0 . Hence, the observed blue-shift of the S_0 - S_1 transition energy is a combined
3 effect of S_1 up-shift and S_0 down-shift.

4 **Effects of the modified protein electrostatics on the reaction kinetics.** The excited state
5 lifetimes and the isomerization reaction times were determined by broadband transient
6 absorption spectroscopy in the 340-750 nm range, covering the characteristic bands of ground
7 state bleach (GSB), excited state absorption (ESA) and stimulated emission (SE), as well the
8 photo-product absorption in the J and K states of both AT and 13C isomers. The latter are
9 obtained by the standard procedure subtracting the experimentally determined contribution
10 of AT excited isomers, from the LA data. Values for ESL were extracted from ESA and SE decay
11 times. For the AT isomers (DA), the ESA decay appeared to be bi-exponential, while it
12 displayed mono-exponential decays for the small signal SE region probed (700-750nm). When
13 the SE is studied at longer wavelengths and maximum signal, all mutants display a multi-
14 exponential excited state decay, as found for wt-ASR [90], and in agreement with the ESA
15 decays (to be published). The fast component was found to be significantly reduced with
16 respect to the 0.75 ps ESL of AT in wt-ASR, with L83Q showing an ESL as short as 120 fs.
17 Whether the second slower component (in the 0.5-0.7 ps range, amplitude $\leq 30\%$) also leads
18 to isomerization is difficult to ascertain since the rise of the photo-product (PPA) or J-state
19 absorption naturally allows to unambiguously determine only the fastest component. A
20 potential slower rise appears to be rather compensated by the signal decay to the J-to-K
21 vibrational relaxation (Figure 5). We therefore quoted the “isomerization reaction time” (IRT)
22 extracted from a combined analysis of GSB, ESA, SE and PPA: L83Q - 120 fs, V112N – 230 fs
23 and W76F 340 fs for the AT isomers. These values are presently reconsidered on the basis of
24 the near-IR SE, which allow to determine ESL with higher accuracy (higher signal and zero
25 background, Figure 5). Nevertheless, the initially reported incidental correlation is confirmed:
26 the larger the spectral blue-shift of the GSA, the smaller the IRT.



27
28 *Figure 5: Transient absorption data for the ASR mutant L83Q in DA conditions ($\approx 100\%$ AT). (A) : Combined UV-VIS and near-IR
29 prob wavelengths, for the delay times indicated in the legend. Ground state bleach (GSB), excited state absorption (ESA), and
30 stimulated emission (SE) highlight signal from S_1 . In the 550-600 nm range ESA turns into “hot” ground state absorption by
31 ≈ 200 fs. (B) Kinetics at selected wavelengths. The red dashed line is a guide to the eye with a 200 fs rise time for the “hot”
32 ground state absorption.*

33 For 13C, the mutations did not alter the IRT as much as for AT, but the values rather indicate
34 the opposite trend: a small but significant increase of ESL and IRT. This an important result,
35 which confirms the initial strategy of choosing ASR as a model system: the protein-
36 chromophore interactions act differently, namely in an opposite fashion, on the two isomer

1 conformations. The molecular basis for this observation is unclear and is the subject on
2 ongoing work both computationally and experimentally. A central question is to figure out in
3 as much LA changes the entire coupled protein-chromophore system in what we call the “13-
4 *cis* isomer”. i.e. both the chromophore and the protein likely undergo LA-induced structural
5 changes.

6 7 **b. Details from femtosecond vibrational spectroscopy**

8 First vibrational spectra obtained by femtosecond non-resonant (pump-)DFWM were also
9 reported in this paper [105] for the isomer ground states in DA and LA, as well as their photo-
10 isomerized K states (pump delay 100 ps). The procedure for extracting AT- and 13C-pure
11 vibrational spectra was explained in detail, and exploited more thoroughly in a subsequent
12 publication [91], summarised and discussed in the following.

13 The vibrational spectra of both AT and 13C isomers in ASR both in the ground and excited
14 states were analysed in detail [91]. The experimental techniques used rely on degenerate four-
15 wave-mixing (DFWM) and impulsive vibrational spectroscopy (IVS), which can be performed
16 also in the excited state, due to the action of an actinic pump pulse as a function of delay time
17 [107–110].

18 The ground state vibrational spectra of both DA and LA forms of ASR were obtained by non-
19 resonant DFWM and IVS, and compared to traditional spontaneous Raman spectra. Non-
20 resonant schemes used a spectrally broad sub-15fs pulse centered at 650-660 nm, and
21 revealed very different resonances in the high-frequency range above 1000 cm^{-1} and, most
22 importantly, a much stronger Raman activity for 13C in the hydrogen-out-of-plane (HOOP)
23 region around $800\text{--}900\text{ cm}^{-1}$. All these observations are in-line with the differences between
24 AT and *cis* rPSB conformations in other retinal proteins, like bR or channel-rhodopsin.
25 Supported by MD simulations on the ground state, it was suggested that the larger distortion
26 hinted on by the HOOP activity is located at the C14-C15 bond, rather than on the reactive
27 C13=C14 bond, in agreement with X-ray data of ASR [101]. As for 11-*cis* rPSB in Rho, this pre-
28 twist of the 13C chromophore, as opposed to the planar AT configuration, was identified as
29 one of the reasons for the shorter ESL of 13C.

30 The structural changes in the excited state were followed by exciting each isomer with an
31 actinic pump. The pure spectra of the excited state was obtained since the GS spectra of each
32 isomer and the photo-products K_{AT} and K_{13C} could be measured separately [91]. In particular,
33 the frequency of the C=C stretch and CH_3 rocking modes showed a transient blue-shift for both
34 isomers, consistent with a reduced conjugation length, induced by the torsional motion.
35 Indeed, in a 90° twisted conformation, the C14–C15–N and C5=C13 moieties are disconnected
36 electronically. Most importantly the dynamics of the transient frequency shifts mirrors the
37 excited state and reaction lifetimes qualitatively. The blue-shift occurs almost immediately
38 upon excitation for 13C and decays with a half-time of 150 fs. The AT frequency shifts reach
39 maximum only after 0.2 ps and adopt the K_{AT} values only by 0.6 ps. These studies also showed
40 that the amplitude of these isomerization-induced changes in the CH_3 rock frequencies were
41 different for AT as for 13C, which is taken as an indication that isomerization causes a larger
42 rotation around the central C13=C14 bond for AT than for 13C, in agreement with previous
43 FTIR results on the cryo-trapped K intermediates [102].

44 In addition, low frequency vibrations ($< 400\text{ cm}^{-1}$), related to excited state modes, identified
45 previously in transient absorption experiments [97], were shown to appear after a short
46 induction time; shorter for 13C than for AT.

1 **Effects of mutations on the ground state vibrational spectra.** The mutants L83Q and V112N
2 were studied by femtosecond DFWM and IVS in the DA and LA states [111], under exactly the
3 same conditions as the ones used in the above TA experiments. It was found that mutations
4 lead to an enhanced HOOP mode activity in the AT ground state of L83Q and V112N indicating
5 a pre-distortion of the chromophore either around the active C13=C14 bond or neighbouring
6 C14-C15 bond of the ground state compared to the AT-isomer in the wt-ASR [111].
7 Additionally, the high frequency vibrations related to C-C single bond or C=C double bond
8 stretch, as well as CH₃ rock modes change consistently with a reduced conjugation length,
9 induced by the pre-distortion. In agreement with previous experimental and theoretical work,
10 the pre-distortion is held responsible for the drastic reduction of the excited state lifetimes
11 for the mutants, as compared to AT in wt-ASR. On the other hand, the mutations do not seem
12 to enhance the pre-distortion already observed for the 13C isomer in wt-ASR, in agreement
13 with a small increase of the ESL for 13C.

14 In summary, time-resolved vibrational spectroscopy, here accessed via degenerate FWM and
15 IVS, provided new insights on structural changes during the isomerization process. For wild-
16 type ASR and its mutants, the observations are perfectly in line with the theoretical
17 predictions outlined in chapter II (Figure 2), underlining here the role of a pre-twist in 13C/ASR
18 for reducing the excited state barrier and leading to the shorter ESL.

19

20

c. Towards a rational prediction of the excited state lifetime of AT rPSB in mutants

21 While a detailed and comprehensive computational analysis of the mutation-induced effects
22 on the excited state lifetimes of AT and 13C for the above mutants is still ongoing, we
23 examined further mutations with the aim of achieving an ASR mutant with an increased
24 excited state lifetime and thus FQY. This is of particular importance in the area of
25 optogenetics, combined, if possible, with an increased absorption of the mutants in the red
26 or near-IR region of the spectrum [24]. We recently reported an almost 10-fold increase of
27 FQY in the ASR double mutant W76S/Y179F, and rationalised the observations within the
28 context of the modified protein electrostatic environment, including the computational
29 analysis of the excited state electronic structure and lifetimes of wt-ASR and the L83Q mutant
30 [112]. The theoretical framework outlined in section II, is central to understanding the
31 mutation-induced modifications of the ¹B_u/²A_g mixing along the reaction coordinate, which, in
32 this paper was traced down to the electrostatic interactions of the mutated residues.

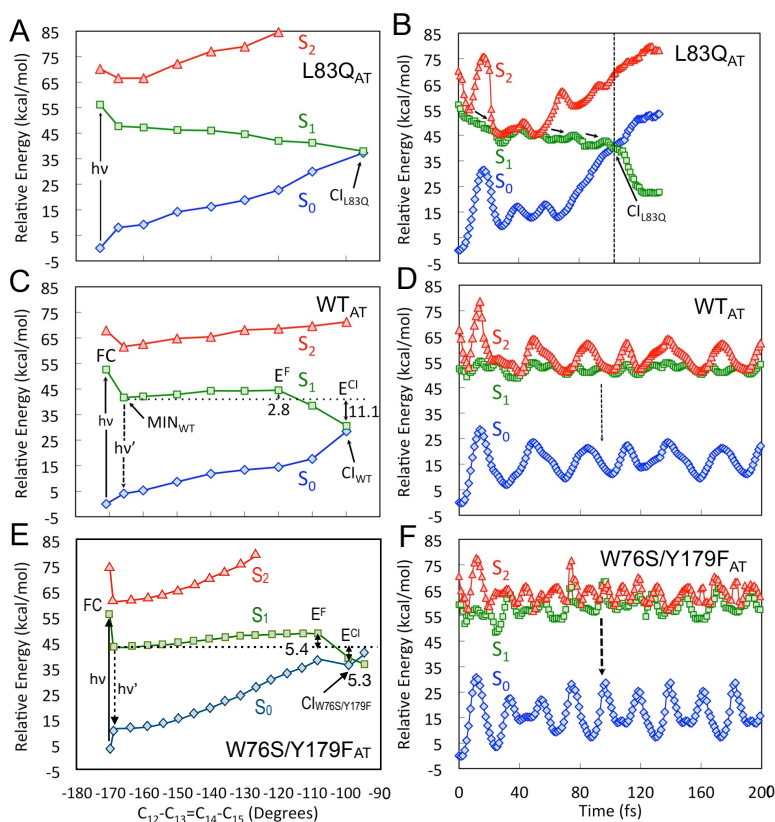
33 The experiments were performed on the DA forms of wt-ASR and on the L83Q and W76S/Y179F
34 mutants, together with two light-adapted forms of the latter. We focused on the mutation effects on
35 the AT isomers, which accumulates to > 95% in DA wt-ASR and L83Q, and is highest in orange-adapted
36 form of W76S/Y179F (39%). While fluorescence of the non-canonical 7- and 9-*cis* isomers was
37 minimised in the double mutant, by excitation with > 520 nm light, contributions from 11- and 13-*cis*
38 cannot be excluded. We found that the FQY was lowest for wt-ASR and increased by a factor of 2.2 for
39 L83Q, and by 7.9 and 6.9 for orange- and green-adapted W76S/Y179F, respectively.

40 The mutation-induced shifts of the ground state absorption and steady-state emission spectra are
41 reported and reproduced with high accuracy by the QM/MM method. The latter is based on the ARM
42 protocol [113], which employs the CASSCF method which is an ab initio (wavefunction-based) multi-
43 configurational quantum chemical (MCQC) method coupled with the Amber molecular mechanics
44 force field to obtain the S₀ QM/MM models of rhodopsins semi-automatically. Vertical excitation
45 energies are then computed using multiconfigurational second-order perturbation theory (CASPT2) to
46 account for the missing dynamic electron correlations. The level of agreement with the observed
47 trends in absorption and fluorescence experiments indicate that the descriptions of the steric and

1 electrostatic effects of the different protein environments on the S_0 and S_1 relaxed structure are
 2 trustful, and can be extended to describing the S_1 dynamics.
 3 These were experimentally probed by measuring the decay of stimulated emission (SE) in the 850-
 4 1250 nm range, with ≈ 60 fs time resolution. The SE decays turned out to be almost wavelength-
 5 independent, and bi-phasic for all studied ASR variants. We thus compared the average values of ESL
 6 with the relative increase of FQY, and found that the FQY increases proportionally to ESL for
 7 W76S/Y179F with respect to wt-ASR, according to $FQY = ESL \times k_r$ with k_r the radiative rate,
 8 generally assumed to be constant. Interestingly, L83Q showed a 2.2-times larger FQY than wt-ASR,
 9 despite an almost 2-fold reduction of the ESL, indicating that L83Q has a higher radiative rate than the
 10 wild-type. This and the effects of the mutations on ESL were explained by calculations of the S_1
 11 relaxation/reaction paths and by computing 200 fs semi-classical trajectories (Figure 6).

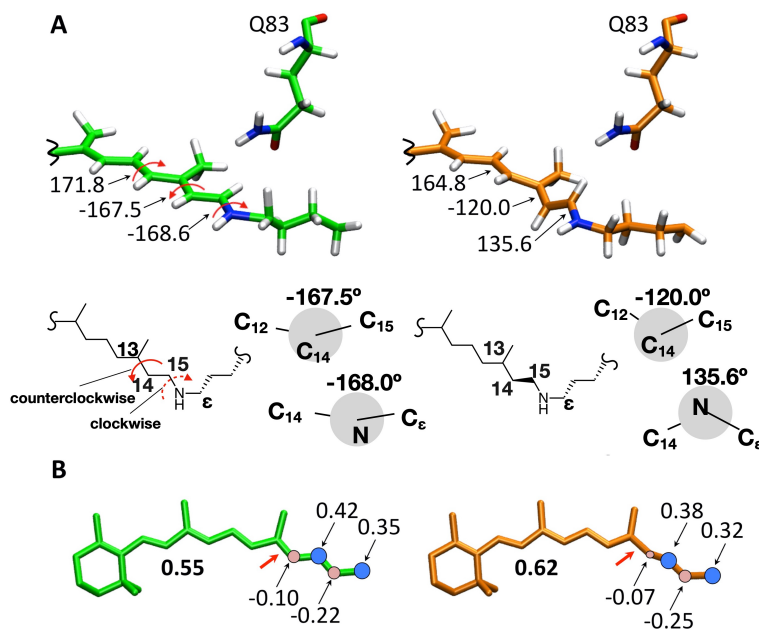
12 Indeed, relaxed scans of the S_0 , S_1 and S_2 energies along the C12–C13=C14–C15 dihedral angle,
 13 computing minimised energies for fixed dihedral angles, indicate that the S_1 profile is
 14 barrierless for L83Q, while it is flatter and displays shallow barriers of ca. 3 and 6 kcal/mol for
 15 wt-ASR and the double mutant, respectively. The barriers have a maximum close to 120°
 16 torsion of the reactive C13=C12 double bond. The excited state lifetimes are best simulated
 17 by trajectories computing the time-dependent energies of the S_1 and S_2 states and their
 18 crossing with S_0 at a conical intersection (Figure 6). Here the kinetic energies in all vibrational
 19 modes of the chromophore are included, leading to different and time-dependent energy
 20 differences, in particular between S_1 and S_2 . Only for L83Q do the computations predict an
 21 ultrafast S_1/S_0 crossing by ≈ 100 fs, consistent with the fastest 0.27 ps decay time observed in
 22 SE. On the other hand, Figure 6 shows that wt-ASR and W76S/Y179F do not reach the CI within
 23 the simulation time, consistent with the observed shortest decay times above 500 fs and with
 24 the computed S_1 barriers in the relaxed energy scans.

25



26

1 Figure 6: (A,C,E): CASPT2//CASSCF/AMBER energy profiles along the S_1 (green squares) isomerization path of L83Q, wt-ASR,
 2 and W76S/Y179F, respectively, computed in terms of a relaxed scan along the C12–C13=C14–C15 dihedral angle. Note the
 3 appearance of an excited state barrier for wt-ASR, and W76S/Y179F. Energy profiles of S_0 and S_2 are indicated by blue
 4 diamonds and red triangles, respectively. (B,D,F): Excited state QM/MM FC trajectories of L83Q, wt-ASR, and W76S/Y179F,
 5 computed at three-root state-averaged-CASSCF/AMBER level of theory and corrected at the CASPT2 level. S_0 (blue diamonds),
 6 S_1 (green squares), and S_2 (red triangles) CASPT2/CASSCF/AMBER energy profiles along the FC trajectories. The reaction path
 7 diagram in panel E is different from the corresponding panel in ref. 112. In fact, the presented path has been computed using
 8 a 3-root CASSCF level - rather than 2-root state average - to be consistent with the results presented in panels A and C.



9
 10 Figure 7: Evolution of the dihedral angles (A) and charges on the Schiff base end (B) along the isomerization path for L83Q.
 11 The C12–C13=C14–C15 dihedral angle is $\approx -170^\circ$ (left) and -120° (right). Adapted from ref. [112].

12 Interestingly, the mutations act through different mechanisms on the ${}^1B_u/{}^2A_g$ mixing. For L83Q, the
 13 reactive character of S_1 is induced through electrostatic interactions imposed by the protein
 14 environment, while in W76S/Y179F the stabilisation of emissive regions on S_1 is due to subtle changes
 15 of the chromophore structure. This was given evidence for by computing the S_1 energy profiles along
 16 the isomerization paths in *vacuo*, i.e. for the isolated rPSB taken with its protein geometry. Indeed, in
 17 the case of the L83Q chromophore structure, the energy profile appears to be very similar to the one
 18 detected for the rPSB in wt-ASR and W76S/Y179F protein environment, which would lead to a
 19 picosecond excited state lifetime and an enhanced emission. Since these are not observed, the short
 20 ESL in L83Q is a consequence of the protein environment. The computations indicate a reorientation
 21 of the π -conjugated chain along the reaction path in a way that enhances the interaction with the polar
 22 side chains of Q83. At the same time, the positive charge on the Schiff base end is further reduced
 23 upon increasing the C12–C13=C14–C15 dihedral angle (Figure 7). Both effects lead to an electrostatic
 24 stabilisation of S_1 along the reaction paths and thus to a barrierless progression on the S_1 PES.

25
 26 On the other hand, the *in vacuo* analysis for wt-ASR and W76S/Y179F shows no qualitative changes in
 27 the energy profiles with respect to the respective protein environments. This indicates that the S_1
 28 barrier is not induced but only marginally modulated by electrostatic interaction. Rather, it is an
 29 intrinsic feature of the rPSB conformation. The increased S_1 barrier in W76S/Y179F is due to subtle
 30 geometrical changes with respect to the rPSB geometry in wt-ASR. Indeed, the computations show
 31 that it is possible to transform the wt-ASR S_2 , S_1 , and S_0 energy profiles into W76S/Y179F-like energy
 32 profiles by simply changing the backbone dihedral angles along the wt-ASR reaction coordinate to the
 33 corresponding W76S/Y179F values. This is remarkable because those are limited changes that indicate

1 the fluorescent tuning in rhodopsins might be achieved also through subtle geometrical (i.e. steric)
2 effects.

3

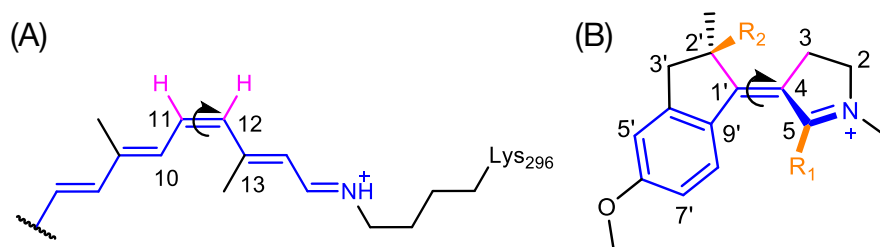
4 In conclusion, this combined experimental and computational study outlines two pathways for a
5 rational design of ASR mutants with enhanced ESL and FQY. It was shown that mutations can induce
6 steric effects that induce a pronounced $^1B_u/{}^2A_g$ mixing, blending non-reactive character into S_1 through
7 geometrical effects. Alternatively, it might be interesting to engineer the mutations such that the
8 dynamic electrostatic interactions with the increasing CT character of the rPSB are of repulsive
9 character so as to destabilise S_1 and maintain a significant $^1B_u/{}^2A_g$ mixing.

10

11 IV. Retinal-inspired molecular switches: towards controlling photoreactivity by 12 synthetic chemical design

13

14 An alternative, complementary strategy to decipher the tuning mechanism of the rPSB
15 photoreactivity in rhodopsins has been to design a model chromophore that mimics – in
16 solution - the electronic structure, PES topologies and eventually photoreactivity of 11-*cis*
17 rPSB in the visual pigment of Rhodospin (Rho) [114,115]. In Rho, the photoisomerization is
18 remarkably fast (<200 fs) and efficient (isomerization quantum yield, IQY = 67%) [93,94].
19 Above all, this photoreaction is vibrationally coherent, a unique mechanism enabling to funnel
20 the energy of the absorbed photon specifically into the isomerization coordinate on a time
21 scale faster than vibrational dephasing, and held responsible for the enhanced IQY in Rho[23].
22 Revealing the chemical design principles underlying such an efficient light to mechanical
23 energy transduction at the molecular scale would be of central interest in the prospect of
24 designing efficient molecular switches and motors [116].



25

26 *Figure 8: Chemical structure of (A) the 11-*cis* isomer of rPSB in Rho, and (B) the Z isomer of the IP compounds. Both IP and*
27 *rPSB have a similar π -electron system (blue molecular backbones) with an S_1 state characterized by a bond length alternation*
28 *and partial reduction of the protonated Schiff base through charge transfer along the carbon backbone. They also have an*
29 *analogous photoreaction coordinate, which involves the torsion around the central C=C double bond (curved arrows) as well*
30 *as out-of-plane motions of the purple bonds. Several IP compounds have been synthesized and studied theoretically and*
31 *experimentally. They differ by the substitutions in orange: R1 = H or Me, R2 = H or Me.*

32 As discussed above in the case of ASR and due to the CT character of the S_1 state, a major
33 parameter influencing the photophysical properties of rPSB is the protein electrostatic
34 environment and the counterion neutralizing its positive charge [56,117]. In the originally
35 proposed N-alkylated IP compounds – which we name MeO-NAIP - the electron-donating
36 MeO group on C6' stabilizes the S_1 state by favoring its CT character, thus mimicking the
37 stabilizing effect of the electrostatic protein environment in Rho [114]. Besides fine-tuning the
38 S_1 CT character, another central ingredient of the biomimetic design is the intramolecular
39 steric hindrance imposed by the two methyl groups on C2' and the methyl group on C5.
40 Altogether they impose the Z configuration of the MeO-NAIP to be the most stable S_0 isomer,
41 in a non-planar - pre-twisted – geometry. Recently, we revealed the effect of the Me
42 substituent on C5 on the IP photoreactivity by investigating the “demethylated” MeO-NAIP

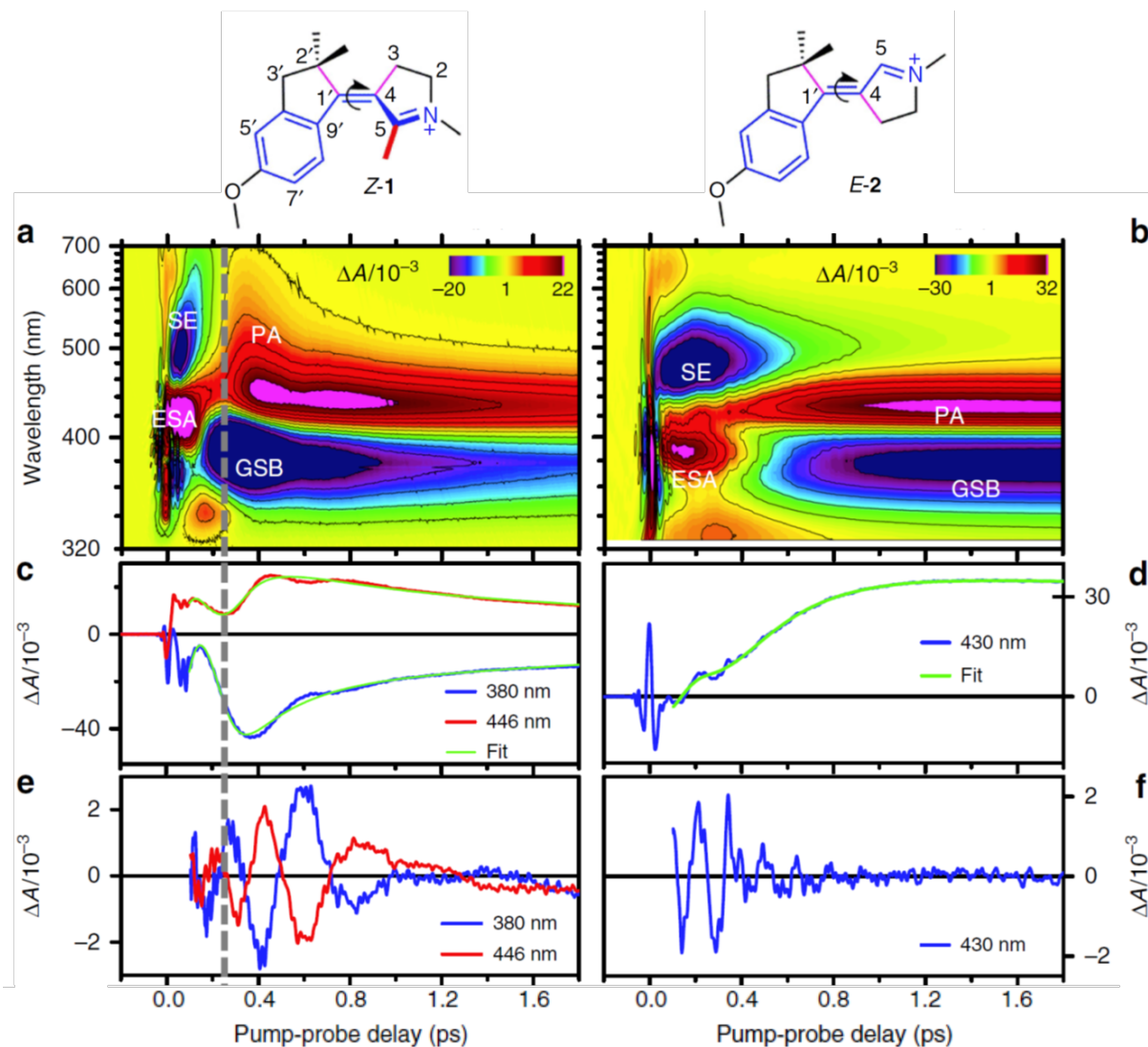
18

1 compound, named dMe-MeO-NAIP, which features no methyl on C5 (R1=H in Figure 8). As we
2 will illustrate below, we demonstrated that while the parent MeO-NAIP undergoes a
3 vibrationally coherent photoreaction mimicking that of rPSB in Rho, this is not the case of
4 dMe-MeO-NAIP. In a way similar to the case of rPSB in retinal proteins and mutants, we could
5 conclude that this difference in photoreactivities observed within the two IP compounds is
6 closely related to the S_1 - S_2 energy gap and mixing, itself controlled by electrostatic and
7 (intramolecular) steric properties.

8 UV-Vis or mid-IR transient absorption (TA) spectroscopy as well as femtosecond fluorescence
9 spectroscopy were used to investigate the ultrafast photoisomerization reaction of a variety
10 of IP compounds in solution [20,115,118–122]. While all compounds undergo a subpicosecond
11 photoisomerization, they can be categorized within two photoisomerization scenarios,
12 corresponding to two qualitatively distinct spectroscopic signatures, as illustrated in Figure 9.
13 For the so-called “parent” NAIP compound [121] and its zwitterionic derivative [119], the
14 excited state absorption (ESA) and stimulated emission (SE) from the S_1 population (Figure 9
15 a) are very short-lived, they spectrally shift to the blue and to the red respectively, and they
16 decay impulsively by 250 fs (vertical dashed line across Figure 9 a,c,e). The ground state
17 photoproduct absorption (PA) band immediately follows, which initially appears after the
18 decay of the SE in the low-energy spectral range (600-700nm), and rapidly blue shifts to
19 ~ 430 nm within ~ 0.5 ps. Such dynamic spectral shifts of the S_1 and early S_0 bands have been
20 interpreted as the signatures of a population which evolves ballistically from the FC region to
21 the photoproduct ground state through the CInt in the form of a vibrational wavepacket [119].
22 A very similar red-shifting SE impulsively followed by a blue shifting PA has been reported in
23 Rhodopsin too and interpreted the same way [94]. Further evolution in the ground state is
24 accompanied by a pronounced low-frequency oscillation (~ 80 cm^{-1} , corresponding to ~ 500 fs
25 period, Figure 9 e) interpreted as the signature of a vibrational wavepacket oscillating around
26 the S_0 PES minimum along the C=C torsion coordinate [119,121]. Importantly this vibrational
27 wavepacket was demonstrated to be triggered by the S_1 reactive motion, meaning that this
28 low-frequency vibrational coherence is preserved through the CInt and exponentially damped
29 (with a ~ 300 fs time constant) after the decay to S_0 [118].

30 Conversely, the “demethylated” NAIP compound, where C₅ carries an H atom in place of a
31 methyl substituent, has a very similar ground state spectrum but qualitatively different TA
32 signatures (see Figure 9 b,d,f): The SE signature does not extend this far to the low-energy
33 range, it is spectrally narrower and longer-lived. The SE and ESA bands decay in concert with
34 the rise of the PA band which appears gradually at 430 nm, rather than impulsively in red part
35 of the probing window. Hence there is no similar indication of a vibrationally coherent reactive
36 motion that would impulsively drive the system through the CInt as discussed above. More
37 precisely, upon light excitation with a very short (8fs) pump pulse, a vibrationally coherent
38 motion is detected in S_1 along (in particular 232 cm^{-1} mode, see Figure 9 f). However, no
39 vibrational coherence is transferred to the ground state, and in particular no vibrational
40 activity is detected at 80 cm^{-1} .

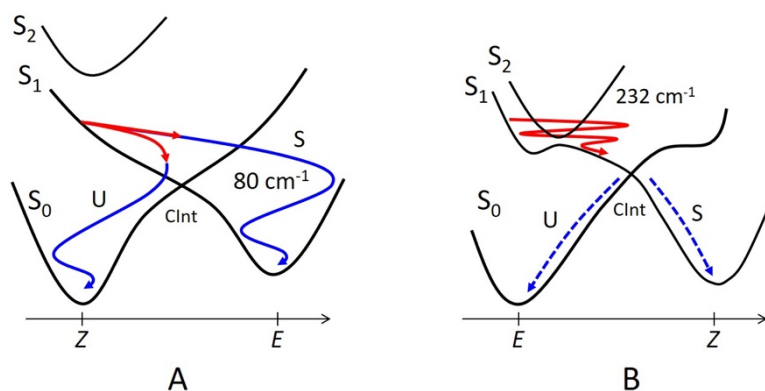
41



1
 2 Figure 9: Transient absorption spectroscopy (TAS) of two representatives of the NAIP compounds family obtained with an 8-
 3 fs, 400-nm pump pulse. Compound Z-1 carries a methyl in C5 and its C=C double bond is pre-twisted in S_0 . Its TAS signatures
 4 (a) are qualitatively different from those of the “demethylated”, planar compound E-2 (b) where the methyl group on C5 is
 5 replaced by a H atom. (c, d) Selection of transient kinetics traces from the above datasets, and their fits. (e, f) The residuals of
 6 the fits are oscillatory signals revealing the vibrational dynamics accompanying the photoreaction. Abbreviations: GSB =
 7 Ground State Bleach; SE = Stimulated Emission; ESA = Excited State Absorption; PA= Photoproduct Absorption. Reproduced
 8 with permission from [118].

9 To rationalize why these two compounds undergo such qualitatively different photoreactions,
 10 we first notice, that the most stable ground state isomer is Z, with a $\sim 15^\circ$ pretwisted C=C bond,
 11 for the C5-methylated compound, but E in a planar conformation for the demethylated
 12 compound. These differences are controlled by the intramolecular steric hindrance
 13 introduced with the methyl substituent on C₅, which also affects the S_1 electronic structure
 14 and photoreactivity of the compounds, as rationalized by QM/MM modeling at the
 15 CASSCF/CASSPT2 level [118]. Indeed, in the planar compound, the S_1 and S_2 states are
 16 computed to be nearly degenerate, resulting in a flat S_1 PES, while in the pre-twisted
 17 compound the degeneracy is lifted and the S_1 PES slope is very steep. Consequently, in the
 18 latter case, the motion along the torsion coordinate is strongly accelerated which drives the
 19 system ballistically towards the Clnt, such that the photoproduct formation occurs faster than
 20 vibrational decoherence of the 80 cm^{-1} mode, resulting in the pronounced oscillatory signal
 21 observed after the decay to S_0 . In the former case, a more diffusive exploration of the S_1 PES
 22 potential would be responsible for an increased excited state lifetime and vibrational

1 decoherence would be effective already by the time the system reaches the CInt and decays
2 to S_0 (Figure 10).



3
4 **Figure 10: Schematic representations of the two distinct photoisomerization scenarios in the NAIP compounds.** (a) The
5 reactive motion of the pretwisted MeO-NAIP is governed by a steep S_1 PES driving the system ballistically towards the CInt.
6 Upon impulsive decay to S_0 , this S_1 motion coherently populates the S_0 vibrational motion along the torsion mode at 80 cm^{-1} .
7 (b) Instead, for the planar demethylated compound, the S_1 and S_2 states are nearly degenerate, resulting in a flat PES providing
8 a much weaker acceleration along the torsion mode, and a more diffusive motion towards the CInt. The crossing of S_1 and S_2
9 leads in fact to an avoided crossing. The corresponding loss of synchronization then explains the observed loss of ensemble
10 coherence. Two decay channels (“U”=“unsuccessful”, and “S”=“successful”) are represented, with the evolutions on the S_1 or
11 S_0 PES represented in red and blue, respectively. The 20 to 25% quantum yield indicates that channel U is dominating. Adapted
12 from [118].

13 By comparing the TA signatures and photoreaction scenarios for each isomer of half a dozen
14 of NAIP derivatives [20], we observe that all compounds under investigation obey either the
15 first or the second photoreaction scenario. Moreover, based on DFT modeling of the
16 compounds S_0 equilibrium structures, we establish a perfect correlation between the C=C
17 double pre-twist and the photoreaction scenario: All compounds featuring a significant ($>10^\circ$)
18 pre-twist obey the first photoreaction scenario, and all compounds featuring a more planar S_0
19 conformation ($<10^\circ$ pre-twist) obey the second one. This allows us to generalize the central
20 role of the intramolecular steric effect and pre-twist at controlling the PES topography via the
21 fine tuning of the S_1 - S_2 state mixing in these retinal-inspired compounds. As reviewed in
22 chapter II&III, the same effect of a pre-twist of the chromophore is operative in 13C/ASR in
23 reducing the excited state barrier [111].

24 A major outcome of our latest study [20], is that while the intramolecular steric hindrance and
25 related S_1 - S_2 state mixing controls the photoreaction dynamics, we observe no correlation
26 between the IQY – in the range 15 % to 35 % for all IP compounds investigated – and
27 observables such as excited state life time, vibrational coherence, absorption wavelengths or
28 degree of pre-twisting. The molecular mechanism controlling the IQY in ultrafast C=C double
29 bound isomerization thus remains an open question so far.

31 V. Summary and conclusions

32
33 In summary, for the sub-picosecond isomerization reactions of rPSB in a protein cavity and for
34 the related bio-mimetic switches in solution, modifications of the ESL can be induced by steric
35 effects (ground state pre-twist) and by changes in the electrostatic environment. The
36 experimental and theoretical studies on the AT and 13C isomers ASR and its mutants have
37 identified both effects, and how they are brought about by point mutations. For the
38 photoswitches, steric [118] and electronic [20,123] effects, brought about by substitutions of
39 the NAIP framework were investigated experimentally. These studies showed, in addition to

1 the change in ESL, a clear influence on the survival of vibrational coherences in the ground
2 state. The computational framework shows that both steric and electrostatic parameters
3 affect the excited state structure due to ${}^1B_u/{}^2A_g$ mixing, and how it determines the CT and
4 reactive character in terms of BLA of the lowest excited state S_1 . A central point is that the
5 simulations allow to predict how this mixing changes dynamically when the chromophores
6 evolve along the reaction coordinate. Shallow excited state barriers appear when S_1 alternates
7 between a reactive CT and non-reactive DIR character, leading to longer ESL and higher FQY.
8 The recent experimental observation of BLA-mediated ${}^1B_u/{}^2A_g$ oscillations demonstrates this
9 effect clearly, within the first 100 fs of the excited state reaction in bR [87].
10 In order to obtain a more direct experimental insight, and a direct comparison with the
11 simulations, measuring the time-dependent S_1 - S_2 splitting would provide new insights for
12 systems with reactive vs. non-reactive excited state dynamics. The S_1 - S_2 transition is expected
13 to lie in the near- to mid-IR region (1000 - 8000 cm^{-1}) [84,118], but the high time resolution
14 required (<50 fs) makes the envisaged VIS-pump/IR-probe spectroscopy challenging.
15 Like mutation effects discussed in chapter III, pH is known to induce small shifts in the ASR
16 absorption maximum wavelength [124]. A new computational protocol has been devised and
17 successfully applied to determine the molecular origin of such a pH effect [125]. The next step,
18 still ongoing, focuses on the pH effect on the ESL. Preliminary results point to a bi-exponential
19 decay from S_1 , modulated by its pH-dependent interaction with S_2 . Further upcoming
20 developments relate to computing the excited state vibrational spectra of the rPSB, in relation
21 with the very informative femtosecond data [91,111], and a possible inclusion of the dynamic
22 polarisation of the nearest neighbour residues [15,74,75]. Unlike the here reported transient
23 spectroscopies, ultrafast X-ray diffraction has the potential of revealing the detailed dynamic
24 structural and electronic changes of the rPSB and its nearest environment, provided these
25 experiments be carried out under single photon excitation conditions [63,72,73,126].
26 Beyond the excited state lifetimes and vibrational coherences, another central property is the
27 reaction quantum yield (IQY), for which we still have to understand and learn the lessons that
28 the outstanding high values found in Rho and bR can teach us, when these are compared with
29 the modest values obtained for the biomimetic switches in solution [20] or the low values
30 ($<2\%$) observed for the crowded alkenes designed as molecular rotary machine [26]. While it is
31 generally accepted that the branching into forward and backward reaction takes place in the
32 vicinity of the conical intersection [23], it remains to be rationalised how chemical
33 substitutions and effects of the environment control the IQY. Femto- and attosecond photo-
34 electron spectroscopy holds the promise to map out the wavepacket evolution across the CI
35 in real time, and one of the main challenges for the next years will be to apply these emerging
36 sophisticated tools for isolated chromophores in gas phase [127,128] and solution [129].
37

38 VI. Acknowledgements

39 The authors acknowledge financial support from the French-German ANR-DFG project
40 “Femto-ASR”, (ANR- 14-CE35-0015-01) and ANR support via grant No. ANR-11-JS04-010-01
41 “IPQCS”, and the “Investissement d’Avenir” program: Labex NIE ANR- 11-LABX-0058_NIE,
42 Labex CSC ANR-10-LABX-0026_CSC, Equipex UNION ANR-10- EQPX-52-01. Other funding came
43 from the Université de Strasbourg for a USIAS Fellowship, from the “Alsace” region, the NSF
44 Grant No. CHE-1710191, and the Italian MIUR for grant PRIN 2015. M.O acknowledges the
45 Ohio Supercomputer Center, the Mésocentre of the Université de Strasbourg and CINECA for
46 granted computer time. The Centre de Calcul Intensif d’Aix-Marseille is acknowledged for granting
47 access to its high performance computing resources.

1 High quality samples for wild-type ASR and its mutants came from H. Kandori and K. Jung and
2 their teams. The contributions of J. Brazard, A. Cheminal and O. Crégut are kindly
3 acknowledged, as well as from S. Fusi, M. Paolino, L. Barneschi, E. Pieri, V. Ledentu and L.
4 Pedraza González.
5

1 REFERENCES

- 2 [1] O.P. Ernst, D.T. Lodowski, M. Elstner, P. Hegemann, L.S. Brown, H. Kandori, *Microbial and Animal*
3 *Rhodopsins: Structures, Functions, and Molecular Mechanisms*, *Chem. Rev.* 114 (2014) 126–163.
4 <https://doi.org/10.1021/cr4003769>.
- 5 [2] M. Kumauchi, T.G. Ebrey, *Visual Pigments as Photoreceptors*, in: *Handb. Photosensory Recept.*,
6 John Wiley & Sons, Ltd, 2005: pp. 43–76. <https://doi.org/10.1002/352760510X.ch3>.
- 7 [3] E.G. Govorunova, O.A. Sineshchekov, H. Li, J.L. Spudich, *Microbial Rhodopsins: Diversity,*
8 *Mechanisms, and Optogenetic Applications*, *Annu. Rev. Biochem.* 86 (2017) 845–872.
9 <https://doi.org/10.1146/annurev-biochem-101910-144233>.
- 10 [4] V. Bonačić-Koutecký, J. Köhler, J. Michl, Prediction of structural and environmental effects on the
11 S1-S0 energy gap and jump probability in double-bond cis–trans photoisomerization, *Chem.*
12 *Phys. Lett.* 104 (1984) 440–443. [https://doi.org/10.1016/0009-2614\(84\)85619-5](https://doi.org/10.1016/0009-2614(84)85619-5).
- 13 [5] A. Cembran, F. Bernardi, M. Olivucci, M. Garavelli, The retinal chromophore/chloride ion pair:
14 Structure of the photo isomerization path and interplay of charge transfer and covalent states,
15 *Proc. Natl. Acad. Sci. U. S. A.* 102 (2005) 6255–6260.
- 16 [6] A. Wand, I. Gdor, J.Y. Zhu, M. Sheves, S. Ruhman, Shedding New Light on Retinal Protein
17 Photochemistry, *Annu. Rev. Phys. Chem.* Vol 64. 64 (2013) 437–458.
- 18 [7] R. Diller, Primary Reactions in Retinal Proteins, in: M. Braun, P. Gilch, W. Zinth (Eds.), *Biol. Med.*
19 *Phys. Biomed. Eng.*, Springer, Berlin Heidelberg, 2008: pp. 243–273.
- 20 [8] H. Kandori, Y. Katsuta, M. Ito, H. Sasabe, Femtosecond fluorescence study of the rhodopsin
21 chromophore in solution, *J. Am. Chem. Soc.* 117 (1995) 2669–2670.
22 <https://doi.org/10.1021/ja00114a040>.
- 23 [9] P. Hamm, M. Zurek, T. Röschinger, H. Patzelt, D. Oesterhelt, W. Zinth, Femtosecond spectroscopy
24 of the photoisomerisation of the protonated Schiff base of all-trans retinal, *Chem. Phys. Lett.* 263
25 (1996) 613–621. [https://doi.org/10.1016/S0009-2614\(96\)01269-9](https://doi.org/10.1016/S0009-2614(96)01269-9).
- 26 [10] G. Zgrablic, K. Voitchovsky, M. Kindermann, S. Haacke, M. Chergui, Ultrafast excited state
27 dynamics of the protonated Schiff base of all-trans retinal in solvents, *Biophys. J.* 88 (2005) 2779–
28 2788. <https://doi.org/10.1529/biophysj.104.046094>.
- 29 [11] T. Sovdat, G. Bassolino, M. Liebel, C. Schnedermann, S.P. Fletcher, P. Kukura, Backbone
30 Modification of Retinal Induces Protein-like Excited State Dynamics in Solution, *J. Am. Chem. Soc.*
31 134 (2012) 8318–8320. <https://doi.org/10.1021/ja3007929>.
- 32 [12] S.L. Logunov, L. Song, M.A. El-Sayed, Excited-State Dynamics of a Protonated Retinal Schiff Base in
33 Solution, *J. Phys. Chem.* 100 (1996) 18586–18591. <https://doi.org/10.1021/jp962046d>.
- 34 [13] J.P. Kraack, T. Backup, M. Motzkus, Vibrational analysis of excited and ground electronic states of
35 all-trans retinal protonated Schiff-base, *Phys. Chem. Chem. Phys.* 13 (2011) 21402–21410.
- 36 [14] J.P. Kraack, T. Backup, M. Motzkus, Coherent High-Frequency Vibrational Dynamics in the Excited
37 Electronic State of All-Trans Retinal Derivatives, *J. Phys. Chem. Lett.* 4 (2013) 383–387.
38 <https://doi.org/10.1021/jz302001m>.
- 39 [15] H.V. Kiefer, E. Gruber, J. Langeland, P.A. Kusochek, A.V. Bochenkova, L.H. Andersen, Intrinsic
40 photoisomerization dynamics of protonated Schiff-base retinal, *Nat. Commun.* 10 (2019) 1210.
41 <https://doi.org/10.1038/s41467-019-09225-7>.
- 42 [16] M. Klessinger, Conical Intersections and the Mechanism of Singlet Photoreactions, *Angew. Chem.*
43 *Int. Ed. Engl.* 34 (1995) 549–551. <https://doi.org/10.1002/anie.199505491>.
- 44 [17] B.G. Levine, T.J. Martínez, Isomerization Through Conical Intersections, *Annu. Rev. Phys. Chem.* 58
45 (2007) 613–634. <https://doi.org/10.1146/annurev.physchem.57.032905.104612>.
- 46 [18] T. Sovdat, G. Bassolino, M. Liebel, C. Schnedermann, S.P. Fletcher, P. Kukura, Backbone
47 Modification of Retinal Induces Protein-like Excited State Dynamics in Solution, *J. Am. Chem. Soc.*
48 134 (2012) 8318–8320. <https://doi.org/10.1021/ja3007929>.
- 49 [19] A. Cheminal, J. Léonard, S.-Y. Kim, K.-H. Jung, H. Kandori, S. Haacke, 100 fs photo-isomerization
50 with vibrational coherences but low quantum yield in Anabaena Sensory Rhodopsin, *Phys. Chem.*
51 *Chem. Phys.* 17 (2015) 25429–25439. <https://doi.org/10.1039/c5cp04353k>.

- 1 [20] M. Gueye, M. Paolino, E. Gindensperger, S. Haacke, M. Olivucci, J. Léonard, Vibrational coherence
2 and quantum yield of retinal-chromophore-inspired molecular switches, *Faraday Discuss.* 221
3 (2020) 299–321. <https://doi.org/10.1039/C9FD00062C>.
- 4 [21] G. Zgrablić, A.M. Novello, F. Parmigiani, Population Branching in the Conical Intersection of the
5 Retinal Chromophore Revealed by Multipulse Ultrafast Optical Spectroscopy, *J. Am. Chem. Soc.*
6 134 (2012) 955–961. <https://doi.org/10.1021/ja205763x>.
- 7 [22] A. Valentini, D. Rivero, F. Zapata, C. García-Iriepa, M. Marazzi, R. Palmeiro, I. Fdez. Galván, D.
8 Sampedro, M. Olivucci, L.M. Frutos, Optomechanical Control of Quantum Yield in Trans–Cis
9 Ultrafast Photoisomerization of a Retinal Chromophore Model, *Angew. Chem. Int. Ed.* 56 (2017)
10 3842–3846. <https://doi.org/10.1002/anie.201611265>.
- 11 [23] C. Schnedermann, X. Yang, M. Liebel, K.M. Spillane, J. Lugtenburg, I. Fernández, A. Valentini, I.
12 Schapiro, M. Olivucci, P. Kukura, R.A. Mathies, Evidence for a vibrational phase-dependent
13 isotope effect on the photochemistry of vision, *Nat. Chem.* (2018).
14 <https://doi.org/10.1038/s41557-018-0014-y>.
- 15 [24] H. Kandori, Retinal Proteins: Photochemistry and Optogenetics, *Bull. Chem. Soc. Jpn.* 93 (2020)
16 76–85. <https://doi.org/10.1246/bcsj.20190292>.
- 17 [25] D. Maclaurin, V. Venkatachalam, H. Lee, A.E. Cohen, Mechanism of voltage-sensitive fluorescence
18 in a microbial rhodopsin, *Proc. Natl. Acad. Sci.* 110 (2013) 5939–5944.
19 <https://doi.org/10.1073/pnas.1215595110>.
- 20 [26] D. Roke, S.J. Wezenberg, B.L. Feringa, Molecular rotary motors: Unidirectional motion around
21 double bonds, *Proc. Natl. Acad. Sci.* 115 (2018) 9423–9431.
22 <https://doi.org/10.1073/pnas.1712784115>.
- 23 [27] T. Okada, M. Sugihara, A.-N. Bondar, M. Elstner, P. Entel, V. Buss, The Retinal Conformation and
24 its Environment in Rhodopsin in Light of a New 2.2 Å Crystal Structure, *J. Mol. Biol.* 342 (2004)
25 571–583. <https://doi.org/10.1016/j.jmb.2004.07.044>.
- 26 [28] H. Luecke, B. Schobert, H.-T. Richter, J.-P. Cartailler, J.K. Lanyi, Structure of bacteriorhodopsin at
27 1.55 Å resolution, *J. Mol. Biol.* 291 (1999) 899–911. <https://doi.org/10.1006/jmbi.1999.3027>.
- 28 [29] R. Mathies, A.R. Oseroff, L. Stryer, Rapid-flow resonance Raman spectroscopy of photolabile
29 molecules: rhodopsin and isorhodopsin, *Proc. Natl. Acad. Sci.* 73 (1976) 1–5.
- 30 [30] G. Eyring, B. Curry, A. Broek, J. Lugtenburg, R. Mathies, Assignment and interpretation of hydrogen
31 out-of-plane vibrations in the resonance Raman spectra of rhodopsin and bathorhodopsin,
32 *Biochemistry.* 21 (1982) 384–393. <https://doi.org/10.1021/bi00531a028>.
- 33 [31] R.W. Schoenlein, L.A. Peteanu, R.A. Mathies, C.V. Shank, The first step in vision: Femtosecond
34 Isomerization of rhodopsin, *Science.* 254 (1991) 412–415.
- 35 [32] J.E. Kim, R.A. Mathies, Anti-stokes Raman study of vibrational cooling dynamics in the primary
36 photochemistry of rhodopsin, *J Phys Chem A.* 106 (2002) 8508–15.
37 <https://doi.org/10.1021/jp021069r>.
- 38 [33] Y. Hontani, M. Broser, M. Luck, J. Weißenborn, M. Kloz, P. Hegemann, J.T.M. Kennis, Dual
39 Photoisomerization on Distinct Potential Energy Surfaces in a UV-Absorbing Rhodopsin, *J. Am.*
40 *Chem. Soc.* 142 (2020) 11464–11473. <https://doi.org/10.1021/jacs.0c03229>.
- 41 [34] G.I. Groma, A. Colonna, J.C. Lambry, J.W. Petrich, G. Váró, M. Joffre, M.H. Vos, J.-L. Martin,
42 Resonant optical rectification in bacteriorhodopsin, *Proc Nat Acad Sci.* 101 (2004) 7971–7975.
- 43 [35] R. Mathies, L. Stryer, Retinal has a highly dipolar vertically excited singlet state: implications for
44 vision, *Proc. Natl. Acad. Sci.* 73 (1976) 2169–2173. <https://doi.org/10.1073/pnas.73.7.2169>.
- 45 [36] H. Houjou, Y. Inoue, M. Sakurai, Physical Origin of the Opsin Shift of Bacteriorhodopsin.
46 Comprehensive Analysis Based on Medium Effect Theory of Absorption Spectra, *J. Am. Chem.*
47 *Soc.* 120 (1998) 4459–4470. <https://doi.org/10.1021/ja973941t>.
- 48 [37] R. Rajamani, J. Gao, Combined QM/MM study of the opsin shift in bacteriorhodopsin, *J. Comput.*
49 *Chem.* 23 (2002) 96–105. <https://doi.org/10.1002/jcc.1159>.
- 50 [38] C. Punwong, J. Owens, T.J. Martínez, Direct QM/MM Excited-State Dynamics of Retinal Protonated
51 Schiff Base in Isolation and Methanol Solution, *J. Phys. Chem. B.* 119 (2015) 704–714.
52 <https://doi.org/10.1021/jp5038798>.

- 1 [39] M. Mališ, J. Novak, G. Zgrablić, F. Parmigiani, N. Došlić, Mechanism of ultrafast non-reactive
2 deactivation of the retinal chromophore in non-polar solvents, *Phys. Chem. Chem. Phys.* 19
3 (2017) 25970–25978. <https://doi.org/10.1039/C7CP03293E>.
- 4 [40] K.A. Freedman, R.S. Becker, Comparative investigation of the photoisomerization of the
5 protonated and unprotonated n-butylamine Schiff bases of 9-cis-, 11-cis-, 13-cis-, and all-trans-
6 retinals, *J. Am. Chem. Soc.* 108 (1986) 1245–1251. <https://doi.org/10.1021/ja00266a020>.
- 7 [41] R.S. Becker, K. Freedman, A comprehensive investigation of the mechanism and photophysics of
8 isomerization of a protonated and unprotonated Schiff base of 11-cis-retinal, *J. Am. Chem. Soc.*
9 107 (1985) 1477–1485. <https://doi.org/10.1021/ja00292a005>.
- 10 [42] Y. Koyama, K. Kubo, M. Komori, H. Yasuda, Y. Mukai, EFFECT OF PROTONATION ON THE
11 ISOMERIZATION PROPERTIES OF n-BUTYLAMINE SCHIFF BASE OF ISOMERIC RETINAL AS
12 REVEALED BY DIRECT HPLC ANALYSES: SELECTION OF ISOMERIZATION PATHWAYS BY RETINAL
13 PROTEINS, *Photochem. Photobiol.* 54 (1991) 433–443. [https://doi.org/10.1111/j.1751-
14 1097.1991.tb02038.x](https://doi.org/10.1111/j.1751-1097.1991.tb02038.x).
- 15 [43] P. Hamm, M. Zurek, T. Röschinger, H. Patzelt, D. Oesterhelt, W. Zinth, Femtosecond spectroscopy
16 of the photoisomerization of the protonated Schiff base of all-trans retinal, *Chem Phys Lett.* 263
17 (1996) 613–621.
- 18 [44] G. Zgrablic, S. Haacke, M. Chergui, Heterogeneity and Relaxation Dynamics of the Photoexcited
19 Retinal Schiff Base Cation in Solution, *J. Phys. Chem. B.* 113 (2009) 4384–4393.
20 <https://doi.org/10.1021/jp8077216>.
- 21 [45] G. Bassolino, T. Sovdat, A. Soares Duarte, J.M. Lim, C. Schnedermann, M. Liebel, B. Odell, T.D.W.
22 Claridge, S.P. Fletcher, P. Kukura, Barrierless Photoisomerization of 11-cis Retinal Protonated
23 Schiff Base in Solution, *J. Am. Chem. Soc.* 137 (2015) 12434–12437.
24 <https://doi.org/10.1021/jacs.5b06492>.
- 25 [46] L. Andersen, I. Nielsen, M. Kristensen, M. El Ghazaly, S. Haacke, M. Nielsen, M. Petersen,
26 Absorption of Schiff-base retinal chromophores in vacuo, *J. Am. Chem. Soc.* 127 (2005) 12347–
27 12350. <https://doi.org/10.1021/ja051638j>.
- 28 [47] J. Langeland Knudsen, A. Kluge, A. V. Bochenkova, H. V. Kiefer, L. H. Andersen, The UV-visible
29 action-absorption spectrum of all- trans and 11- cis protonated Schiff base retinal in the gas
30 phase, *Phys. Chem. Chem. Phys.* 20 (2018) 7190–7194. <https://doi.org/10.1039/C7CP07512J>.
- 31 [48] D. Oesterhelt, W. Stoeckenius, Rhodopsin-like Protein from the Purple Membrane of
32 Halobacterium halobium, *Nature. New Biol.* 233 (1971) 149–152.
33 <https://doi.org/10.1038/newbio233149a0>.
- 34 [49] M.C. Nuss, W. Zinth, W. Kaiser, E. Kölling, D. Oesterhelt, Femtosecond spectroscopy of the first
35 events of the photochemical cycle in bacteriorhodopsin, *Chem Phys Lett.* 117 (1985) 1–7.
- 36 [50] H.J. Polland, M.A. Franz, W. Zinth, W. Kaiser, E. Kölling, D. Oesterhelt, Early picosecond events in
37 the photocycle of bacteriorhodopsin, *Biophys J.* 49 (1986) 651–662.
- 38 [51] M. Du, G.R. Fleming, Femtosecond time-resolved fluorescence spectroscopy of bacteriorhodopsin:
39 Direct observation of excited state dynamics in the primary step of the proton pump cycle,
40 *Biophys. Chem.* 48 (1993) 101–111. [https://doi.org/10.1016/0301-4622\(93\)85002-Y](https://doi.org/10.1016/0301-4622(93)85002-Y).
- 41 [52] J. Herbst, K. Heyne, R. Diller, Femtosecond infrared spectroscopy of bacteriorhodopsin
42 chromophore isomerization, *Science.* 297 (2002) 822–825.
- 43 [53] J. Tittor, D. Oesterhelt, The quantum yield of bacteriorhodopsin, *FEBS Lett.* 263 (1990) 269–273.
44 [https://doi.org/10.1016/0014-5793\(90\)81390-A](https://doi.org/10.1016/0014-5793(90)81390-A).
- 45 [54] R. Govindjee, S.P. Balashov, T.G. Ebrey, Quantum efficiency of the photochemical cycle of
46 bacteriorhodopsin, *Biophys. J.* 58 (1990) 597–608. [https://doi.org/10.1016/S0006-
47 3495\(90\)82403-6](https://doi.org/10.1016/S0006-3495(90)82403-6).
- 48 [55] G. Schneider, R. Diller, M. Stockburger, Photochemical quantum yield of bacteriorhodopsin from
49 resonance Raman scattering as a probe for photolysis, *Chem. Phys.* 131 (1989) 17–29.
50 [https://doi.org/10.1016/0301-0104\(89\)87078-8](https://doi.org/10.1016/0301-0104(89)87078-8).

- 1 [56] L. Song, M.A. El-Sayed, J.K. Lanyi, Protein Catalysis of the Retinal Subpicosecond
2 Photoisomerization in the Primary Process of Bacteriorhodopsin Photosynthesis, *Science*. 261
3 (1993) 891–894. <https://doi.org/10.1126/science.261.5123.891>.
- 4 [57] S.L. Logunov, M.A. ElSayed, L. Song, J.K. Lanyi, Photoisomerization quantum yield and apparent
5 energy content of the K intermediate in the photocycles of bacteriorhodopsin, its mutants D85N,
6 R82Q, and D212N, and deionized blue bacteriorhodopsin, *J. Phys. Chem.* 100 (1996) 2391–2398.
- 7 [58] T. Kobayashi, M. Terauchi, T. Kouyama, M. Yoshizawa, M. Taiji, Femtosecond spectroscopy of
8 acidified and neutral bacteriorhodopsin, 1403 (1991) 407–416.
9 <https://doi.org/10.1117/12.57310>.
- 10 [59] J.T.M. Kennis, D.S. Larsen, K. Ohta, M.T. Facciotti, R.M. Glaeser, G.R. Fleming, Ultrafast Protein
11 Dynamics of Bacteriorhodopsin Probed by Photon Echo and Transient Absorption Spectroscopy,
12 *J. Phys. Chem. B.* 106 (2002) 6067–6080. <https://doi.org/10.1021/jp014681b>.
- 13 [60] K. Inoue, H. Ono, R. Abe-Yoshizumi, S. Yoshizawa, H. Ito, K. Kogure, H. Kandori, A light-driven
14 sodium ion pump in marine bacteria, *Nat. Commun.* 4 (2013) 1678.
15 <https://doi.org/10.1038/ncomms2689>.
- 16 [61] H.E. Kato, K. Inoue, R. Abe-Yoshizumi, Y. Kato, H. Ono, M. Konno, S. Hososhima, T. Ishizuka, M.R.
17 Hoque, H. Kunitomo, J. Ito, S. Yoshizawa, K. Yamashita, M. Takemoto, T. Nishizawa, R. Taniguchi,
18 K. Kogure, A.D. Maturana, Y. Iino, H. Yawo, R. Ishitani, H. Kandori, O. Nureki, Structural basis for
19 Na⁺ transport mechanism by a light-driven Na⁺ pump, *Nature*. 521 (2015) 48–53.
20 <https://doi.org/10.1038/nature14322>.
- 21 [62] I. Gushchin, V. Shevchenko, V. Polovinkin, V. Borshchevskiy, P. Buslaev, E. Bamberg, V. Gordeliy,
22 Structure of the light-driven sodium pump KR2 and its implications for optogenetics, *FEBS J.* 283
23 (2016) 1232–1238. <https://doi.org/10.1111/febs.13585>.
- 24 [63] P. Skopintsev, D. Ehrenberg, T. Weinert, D. James, R.K. Kar, P.J.M. Johnson, D. Ozerov, A. Furrer, I.
25 Martiel, F. Dworkowski, K. Nass, G. Knopp, C. Cirelli, C. Arrell, D. Gashi, S. Mous, M. Wranik, T.
26 Gruhl, D. Kekilli, S. Brünle, X. Deupi, G.F.X. Schertler, R.M. Benoit, V. Panneels, P. Nogly, I.
27 Schapiro, C. Milne, J. Heberle, J. Standfuss, Femtosecond-to-millisecond structural changes in a
28 light-driven sodium pump, *Nature*. (2020) 1–5. <https://doi.org/10.1038/s41586-020-2307-8>.
- 29 [64] Y. Hontani, K. Inoue, M. Klotz, Y. Kato, H. Kandori, J.T.M. Kennis, The photochemistry of sodium ion
30 pump rhodopsin observed by watermarked femto- to submillisecond stimulated Raman
31 spectroscopy, *Phys. Chem. Chem. Phys.* 18 (2016) 24729–24736.
32 <https://doi.org/10.1039/C6CP05240A>.
- 33 [65] S. Tahara, S. Takeuchi, R. Abe-Yoshizumi, K. Inoue, H. Ohtani, H. Kandori, T. Tahara, Ultrafast
34 Photoreaction Dynamics of a Light-Driven Sodium-Ion-Pumping Retinal Protein from
35 *Krokinobacter eikastus* Revealed by Femtosecond Time-Resolved Absorption Spectroscopy, *J.*
36 *Phys. Chem. Lett.* 6 (2015) 4481–4486. <https://doi.org/10.1021/acs.jpcllett.5b01994>.
- 37 [66] S. Tahara, S. Takeuchi, R. Abe-Yoshizumi, K. Inoue, H. Ohtani, H. Kandori, T. Tahara, Origin of the
38 Reactive and Nonreactive Excited States in the Primary Reaction of Rhodopsins: pH Dependence
39 of Femtosecond Absorption of Light-Driven Sodium Ion Pump Rhodopsin KR2, *J. Phys. Chem. B.*
40 122 (2018) 4784–4792. <https://doi.org/10.1021/acs.jpccb.8b01934>.
- 41 [67] M.O. Lenz, R. Huber, B. Schmidt, P. Gilch, R. Kalmbach, M. Engelhard, J. Wachtveitl, First Steps of
42 Retinal Photoisomerization in Proteorhodopsin, *Biophys. J.* 91 (2006) 255–262.
43 <https://doi.org/10.1529/biophysj.105.074690>.
- 44 [68] T. Nakamura, S. Takeuchi, M. Shibata, M. Demura, H. Kandori, T. Tahara, Ultrafast Pump–Probe
45 Study of the Primary Photoreaction Process in pharaonis Halorhodopsin: Halide Ion Dependence
46 and Isomerization Dynamics, *J. Phys. Chem. B.* 112 (2008) 12795–12800.
47 <https://doi.org/10.1021/jp803282s>.
- 48 [69] C.-F. Chang, H. Kuramochi, M. Singh, R. Abe-Yoshizumi, T. Tsukuda, H. Kandori, T. Tahara, Acid–
49 base equilibrium of the chromophore counterion results in distinct photoisomerization reactivity
50 in the primary event of proteorhodopsin, *Phys. Chem. Chem. Phys.* 21 (2019) 25728–25734.
51 <https://doi.org/10.1039/C9CP04991F>.

- 1 [70] M. Karasuyama, K. Inoue, R. Nakamura, H. Kandori, I. Takeuchi, Understanding Colour Tuning Rules
2 and Predicting Absorption Wavelengths of Microbial Rhodopsins by Data-Driven Machine-
3 Learning Approach, *Sci. Rep.* 8 (2018) 15580. <https://doi.org/10.1038/s41598-018-33984-w>.
- 4 [71] B.R. Rost, F. Schneider-Warme, D. Schmitz, P. Hegemann, Optogenetic Tools for Subcellular
5 Applications in Neuroscience, *Neuron*. 96 (2017) 572–603.
6 <https://doi.org/10.1016/j.neuron.2017.09.047>.
- 7 [72] P. Nogly, T. Weinert, D. James, S. Carbajo, D. Ozerov, A. Furrer, D. Gashi, V. Borin, P. Skopintsev, K.
8 Jaeger, K. Nass, P. B ath, R. Bosman, J. Koglin, M. Seaberg, T. Lane, D. Kekilli, S. Br unle, T. Tanaka,
9 W. Wu, C. Milne, T. White, A. Barty, U. Weierstall, V. Panneels, E. Nango, S. Iwata, M. Hunter, I.
10 Schapiro, G. Schertler, R. Neutze, J. Standfuss, Retinal isomerization in bacteriorhodopsin
11 captured by a femtosecond x-ray laser, *Science*. (2018) eaat0094.
12 <https://doi.org/10.1126/science.aat0094>.
- 13 [73] G. Nass Kovacs, J.-P. Colletier, M.L. Gr unbein, Y. Yang, T. Stensitzki, A. Batyuk, S. Carbajo, R.B. Doak,
14 D. Ehrenberg, L. Foucar, R. Gasper, A. Gorel, M. Hilpert, M. Kloos, J.E. Koglin, J. Reinstein, C.M.
15 Roome, R. Schlesinger, M. Seaberg, R.L. Shoeman, M. Stricker, S. Boutet, S. Haacke, J. Heberle, K.
16 Heyne, T. Domratcheva, T.R.M. Barends, I. Schlichting, Three-dimensional view of ultrafast
17 dynamics in photoexcited bacteriorhodopsin, *Nat. Commun.* 10 (2019) 3177.
18 <https://doi.org/10.1038/s41467-019-10758-0>.
- 19 [74] S. Schenkl, F. van Mourik, G. van der Zwan, S. Haacke, M. Chergui, Probing the ultrafast charge
20 translocation of photoexcited retinal in bacteriorhodopsin, *Science*. 309 (2005) 917–920.
21 <https://doi.org/10.1126/science.1111482>.
- 22 [75] J. L eonard, E. Portuondo-Campa, A. Cannizzo, F. van Mourik, G. van der Zwan, J. Tittor, S. Haacke,
23 M. Chergui, Functional electric field changes in photoactivated proteins revealed by ultrafast
24 Stark spectroscopy of the Trp residues, *Proc. Natl. Acad. Sci. U. S. A.* 106 (2009) 7718–7723.
25 <https://doi.org/10.1073/pnas.0812877106>.
- 26 [76] S. Tahara, H. Kuramochi, S. Takeuchi, T. Tahara, Protein Dynamics Preceding Photoisomerization
27 of the Retinal Chromophore in Bacteriorhodopsin Revealed by Deep-UV Femtosecond
28 Stimulated Raman Spectroscopy, *J. Phys. Chem. Lett.* 10 (2019) 5422–5427.
29 <https://doi.org/10.1021/acs.jpcclett.9b02283>.
- 30 [77] P. Tavan, K. Schulten, Electronic excitations in finite and infinite polyenes, *Phys. Rev. B.* 36 (1987)
31 4337–4358. <https://doi.org/10.1103/PhysRevB.36.4337>.
- 32 [78] K.C. Hasson, F. Gai, P.A. Anfinrud, The photoisomerization of retinal in bacteriorhodopsin:
33 Experimental evidence for a three-state model, *Proc. Natl. Acad. Sci.* 93 (1996) 15124–15129.
- 34 [79] A. Mu noz-Losa, M.E. Mart ın, I.Fdez. Galv an, M.L. S anchez, M.A. Aguilar, Solvent Effects on the
35 Radiative and Nonradiative Decay of a Model of the Rhodopsin Chromophore, *J. Chem. Theory*
36 *Comput.* 7 (2011) 4050–4059.
- 37 [80] R. Gonz alez-Luque, M. Garavelli, F. Bernardi, M. Merch an, M.A. Robb, M. Olivucci, Computational
38 evidence in favor of a two-state, two-mode model of the retinal chromophore
39 photoisomerization, *Proc. Natl. Acad. Sci.* 97 (2000) 9379–9384.
40 <https://doi.org/10.1073/pnas.97.17.9379>.
- 41 [81] A. Cembran, R. Gonz alez-Luque, L. Serrano-Andr es, M. Merch an, M. Garavelli, About the intrinsic
42 photochemical properties of the 11-cis retinal chromophore: computational clues for a trap state
43 and a lever effect in Rhodopsin catalysis, *Theor. Chem. Acc.* 118 (2007) 173–183.
44 <https://doi.org/10.1007/s00214-007-0259-9>.
- 45 [82] J.P. Kraack, T. Buckup, M. Motzkus, Evidence for the Two-State-Two-Mode model in retinal
46 protonated Schiff-bases from pump degenerate four-wave-mixing experiments, *Phys. Chem.*
47 *Chem. Phys.* 14 (2012) 13979–13988. <https://doi.org/10.1039/c2cp42248d>.
- 48 [83] S. Gozem, H.L. Luk, I. Schapiro, M. Olivucci, Theory and Simulation of the Ultrafast Double-Bond
49 Isomerization of Biological Chromophores, *Chem. Rev.* 117 (2017) 13502–13565.
50 <https://doi.org/10.1021/acs.chemrev.7b00177>.

- 1 [84] M. Manathunga, X. Yang, Y. Orozco-Gonzalez, M. Olivucci, Impact of Electronic State Mixing on the
2 Photoisomerization Time Scale of the Retinal Chromophore, *J. Phys. Chem. Lett.* 8 (2017) 5222–
3 5227. <https://doi.org/10.1021/acs.jpcclett.7b02344>.
- 4 [85] M. Manathunga, X. Yang, M. Olivucci, Electronic State Mixing Controls the Photoreactivity of a
5 Rhodopsin with all-trans Chromophore Analogues, *J. Phys. Chem. Lett.* 9 (2018) 6350–6355.
6 <https://doi.org/10.1021/acs.jpcclett.8b02550>.
- 7 [86] H.L. Luk, F. Melaccio, S. Rinaldi, S. Gozem, M. Olivucci, Molecular bases for the selection of the
8 chromophore of animal rhodopsins, *Proc Natl Acad Sci U A.* 112 (2015) 15297–302.
9 <https://doi.org/10.1073/pnas.1510262112>.
- 10 [87] S. Gozem, P.J.M. Johnson, A. Halpin, H.L. Luk, T. Morizumi, V.I. Prokhorenko, O.P. Ernst, M.
11 Olivucci, R.J.D. Miller, Excited-State Vibronic Dynamics of Bacteriorhodopsin from Two-
12 Dimensional Electronic Photon Echo Spectroscopy and Multiconfigurational Quantum Chemistry,
13 *J. Phys. Chem. Lett.* (2020) 3889–3896. <https://doi.org/10.1021/acs.jpcclett.0c01063>.
- 14 [88] B. Demoulin, S.F. Altavilla, I. Rivalta, M. Garavelli, Fine Tuning of Retinal Photoinduced Decay in
15 Solution, *J. Phys. Chem. Lett.* 8 (2017) 4407–4412. <https://doi.org/10.1021/acs.jpcclett.7b01780>.
- 16 [89] S. Smith, J.A. Pardo, J. Lugtenburg, R.A. Mathies, Vibrational analysis of the 13-cis-Retinal
17 Chromophore in dark-Adapted Bacteriorhodopsin, *J. Phys. Chemistry.* 91 (1987) 804–819.
- 18 [90] A. Wand, B. Loevsky, N. Friedman, M. Sheves, S. Ruhman, Probing Ultrafast Photochemistry of
19 Retinal Proteins in the Near-IR: Bacteriorhodopsin and Anabaena Sensory Rhodopsin vs Retinal
20 Protonated Schiff Base in Solution, *J. Phys. Chem. B.* 117 (2013) 4670–4679.
- 21 [91] P. Pratim Roy, Y. Kato, R. Abe-Yoshizumi, E. Pieri, N. Ferré, H. Kandori, T. Backup, Mapping the
22 ultrafast vibrational dynamics of all- trans and 13- cis retinal isomerization in Anabaena Sensory
23 Rhodopsin, *Phys. Chem. Chem. Phys.* 20 (2018) 30159–30173.
24 <https://doi.org/10.1039/C8CP05469J>.
- 25 [92] O. Weingart, I. Schapiro, V. Buss, Photochemistry of Visual Pigment Chromophore Models by Ab
26 Initio Molecular Dynamics, *J. Phys. Chem. B.* 111 (2007) 3782–3788.
27 <https://doi.org/10.1021/jp0683216>.
- 28 [93] Q. Wang, R.W. Schoenlein, L.A. Peteanu, R.A. Mathies, C.V. Shank, Vibrationally coherent
29 photochemistry in the femtosecond primary event of vision, *Science.* 266 (1994) 422–4.
- 30 [94] D. Polli, P. Altoe, O. Weingart, K.M. Spillane, C. Manzoni, D. Brida, G. Tomasello, G. Orlandi, P.
31 Kukura, R.A. Mathies, M. Garavelli, G. Cerullo, Conical intersection dynamics of the primary
32 photoisomerization event in vision, *Nature.* 467 (2010) 440–U88.
- 33 [95] P.J. Johnson, A. Halpin, T. Morizumi, V.I. Prokhorenko, O.P. Ernst, R.J. Miller, Local vibrational
34 coherences drive the primary photochemistry of vision, *Nat Chem.* 7 (2015) 980–6.
35 <https://doi.org/10.1038/nchem.2398>.
- 36 [96] P.J.M. Johnson, M.H. Farag, A. Halpin, T. Morizumi, V.I. Prokhorenko, J. Knoester, T.L.C. Jansen,
37 O.P. Ernst, R.J.D. Miller, The Primary Photochemistry of Vision Occurs at the Molecular Speed
38 Limit, *J. Phys. Chem. B.* 121 (2017) 4040–4047. <https://doi.org/10.1021/acs.jpccb.7b02329>.
- 39 [97] A. Wand, R. Rozin, T. Eliash, K.-H. Jung, M. Sheves, S. Ruhman, Asymmetric Toggling of a Natural
40 Photoswitch: Ultrafast Spectroscopy of Anabaena Sensory Rhodopsin, *J. Am. Chem. Soc.* 133
41 (2011) 20922–20932. <https://doi.org/10.1021/ja208371g>.
- 42 [98] G. Bassolino, T. Sovdat, M. Liebel, C. Schnedermann, B. Odell, T.D.W. Claridge, P. Kukura, S.P.
43 Fletcher, Synthetic Control of Retinal Photochemistry and Photophysics in Solution, *J. Am. Chem.*
44 *Soc.* 136 (2014) 2650–2658. <https://doi.org/10.1021/ja4121814>.
- 45 [99] K.-H. Jung, V.D. Trivedi, J.L. Spudich, Demonstration of a sensory rhodopsin in eubacteria, *Mol.*
46 *Microbiol.* 47 (2003) 1513–1522. <https://doi.org/10.1046/j.1365-2958.2003.03395.x>.
- 47 [100] A. Kawanabe, H. Kandori, Photoreactions and structural changes of anabaena sensory rhodopsin,
48 *Sens. Basel.* 9 (2009) 9741–804. <https://doi.org/10.3390/s91209741>.
- 49 [101] L. Vogeley, O.A. Sineshchikov, V.D. Trivedi, J. Sasaki, J.L. Spudich, H. Luecke, Anabaena Sensory
50 Rhodopsin: A Photochromic Color Sensor at 2.0 Å, *Science.* 306 (2004) 1390–1393.
51 <https://doi.org/10.1126/science.1103943>.

- 1 [102] A. Kawanabe, Y. Furutani, K.-H. Jung, H. Kandori, FTIR Study of the Photoisomerization Processes
2 in the 13- *cis* and All- *trans* Forms of *Anabaena* Sensory Rhodopsin at 77 K [†], *Biochemistry*. 45
3 (2006) 4362–4370. <https://doi.org/10.1021/bi052324b>.
- 4 [103] I. Schapiro, S. Ruhman, Ultrafast photochemistry of *Anabaena* Sensory Rhodopsin: Experiment
5 and theory, *Biochim. Biophys. Acta BBA - Bioenerg.* 1837 (2014) 589–597.
6 <https://doi.org/10.1016/j.bbabi.2013.09.014>.
- 7 [104] A. Strambi, B. Durbeej, N. Ferré, M. Olivucci, *Anabaena* sensory rhodopsin is a light-driven
8 unidirectional rotor, *Proc. Natl. Acad. Sci.* 107 (2010) 21322–21326.
9 <https://doi.org/10.1073/pnas.1015085107>.
- 10 [105] D. Agathangelou, Y. Orozco-Gonzalez, M. del Carmen Marin, P.P. Roy, J. Brazard, H. Kandori, K.H.
11 Jung, J. Léonard, T. Buckup, N. Ferre, M. Olivucci, S. Haacke, Effect of point mutations on the
12 ultrafast photo-isomerization of *Anabaena* sensory rhodopsin, *Faraday Discuss.* 207 (2018) 55–
13 75. <https://doi.org/10.1039/c7fd00200a>.
- 14 [106] Y. Orozco-Gonzalez, M. Manathunga, M. del C. Marin, D. Agathangelou, K.-H. Jung, F. Melaccio,
15 N. Ferre, S. Haacke, K. Coutinho, S. Canuto, M. Olivucci, An Average Solvent Electrostatic
16 Configuration Protocol for QM/MM Free Energy Optimization: Implementation and Application
17 to Rhodopsin Systems, *J. Chem. Theory Comput.* 13 (2017) 6391–6404.
18 <https://doi.org/10.1021/acs.jctc.7b00860>.
- 19 [107] J. Hauer, T. Buckup, M. Motzkus, Pump-degenerate four wave mixing as a technique for analyzing
20 structural and electronic evolution: Multidimensional time-resolved dynamics near a conical
21 intersection, *J. Phys. Chem. A.* 111 (2007) 10517–10529.
- 22 [108] T. Buckup, J. Hauer, J. Mohring, M. Motzkus, Multidimensional spectroscopy of beta-carotene:
23 Vibrational cooling in the excited state, *Arch. Biochem. Biophys.* 483 (2009) 219–223.
- 24 [109] T. Buckup, J. Léonard, Multidimensional Vibrational Coherence Spectroscopy, *Top. Curr. Chem.*
25 376 (2018). <https://doi.org/10.1007/s41061-018-0213-4>.
- 26 [110] T. Buckup, M. Motzkus, Multidimensional time-resolved spectroscopy of vibrational coherence
27 in biopolyenes, *Annu. Rev. Phys. Chem.* 65 (2015) 39–57.
- 28 [111] P.P. Roy, R. Abe-Yoshizumi, H. Kandori, T. Buckup, Point Mutation of *Anabaena* Sensory
29 Rhodopsin Enhances Ground-State Hydrogen Out-of-Plane Wag Raman Activity, *J. Phys. Chem.*
30 *Lett.* 10 (2019) 1012–1017. <https://doi.org/10.1021/acs.jpcclett.8b03805>.
- 31 [112] M.D.C. Marin, D. Agathangelou, Y. Orozco-Gonzalez, A. Valentini, Y. Kato, R. Abe-Yoshizumi, H.
32 Kandori, A. Choi, K.-H. Jung, S. Haacke, M. Olivucci, Fluorescence enhancement of a microbial
33 rhodopsin via electronic reprogramming, *J. Am. Chem. Soc.* (2018).
34 <https://doi.org/10.1021/jacs.8b09311>.
- 35 [113] F. Melaccio, M. Del Carmen Marin, A. Valentini, F. Montisci, S. Rinaldi, M. Cherubini, X. Yang, Y.
36 Kato, M. Stenrup, Y. Orozco-Gonzalez, N. Ferre, H.L. Luk, H. Kandori, M. Olivucci, Toward
37 Automatic Rhodopsin Modeling as a Tool for High-Throughput Computational Photobiology, *J*
38 *Chem Theory Comput.* 12 (2016) 6020–6034. <https://doi.org/10.1021/acs.jctc.6b00367>.
- 39 [114] F. Lumento, V. Zanirato, S. Fusi, E. Busi, L. Latterini, F. Elisei, A. Sinicropi, T. Andruniów, N. Ferré,
40 R. Basosi, M. Olivucci, Quantum Chemical Modeling and Preparation of a Biomimetic
41 Photochemical Switch, *Angew. Chem.* 119 (2007) 418–424.
42 <https://doi.org/10.1002/ange.200602915>.
- 43 [115] A. Sinicropi, E. Martin, M. Ryazantsev, J. Helbing, J. Briand, D. Sharma, J. Léonard, S. Haacke, A.
44 Cannizzo, M. Chergui, V. Zanirato, S. Fusi, F. Santoro, R. Basosi, N. Ferre, M. Olivucci, An artificial
45 molecular switch that mimics the visual pigment and completes its photocycle in picoseconds,
46 *Proc. Natl. Acad. Sci. U. S. A.* 105 (2008) 17642–17647.
47 <https://doi.org/10.1073/pnas.0802376105>.
- 48 [116] B.L. Feringa, The Art of Building Small: From Molecular Switches to Motors (Nobel Lecture),
49 *Angew. Chem. Int. Ed.* 56 (2017) 11060–11078. <https://doi.org/10.1002/anie.201702979>.
- 50 [117] T.P. Sakmar, R.R. Franke, H.G. Khorana, Glutamic acid-113 serves as the retinylidene Schiff base
51 counterion in bovine rhodopsin, *Proc. Natl. Acad. Sci.* 86 (1989) 8309–8313.

- 1 [118] M. Gueye, M. Manathunga, D. Agathangelou, Y. Orozco, M. Paolino, S. Fusi, S. Haacke, M.
2 Olivucci, J. Léonard, Engineering the vibrational coherence of vision into a synthetic molecular
3 device, *Nat. Commun.* 9 (2018) 313. <https://doi.org/10.1038/s41467-017-02668-w>.
- 4 [119] J. Briand, O. Braem, J. Rehault, J. Léonard, A. Cannizzo, M. Chergui, V. Zanirato, M. Olivucci, J.
5 Helbing, S. Haacke, Coherent ultrafast torsional motion and isomerization of a biomimetic dipolar
6 photoswitch, *Phys. Chem. Chem. Phys.* 12 (2010) 3178–3187.
7 <https://doi.org/10.1039/b918603d>.
- 8 [120] A.D. Dunkelberger, R.D. Kieda, J.Y. Shin, R. Rossi Paccani, S. Fusi, M. Olivucci, F. Fleming Crim,
9 Photoisomerization and Relaxation Dynamics of a Structurally Modified Biomimetic Photoswitch,
10 *J. Phys. Chem. A.* 116 (2012) 3527–3533. <https://doi.org/10.1021/jp300153a>.
- 11 [121] J. Léonard, I. Schapiro, J. Briand, S. Fusi, R.R. Paccani, M. Olivucci, S. Haacke, Mechanistic Origin
12 of the Vibrational Coherence Accompanying the Photoreaction of Biomimetic Molecular
13 Switches, *Chem.-Eur. J.* 18 (2012) 15296–15304. <https://doi.org/10.1002/chem.201201430>.
- 14 [122] J. Léonard, J. Briand, S. Fusi, V. Zanirato, M. Olivucci, S. Haacke, Isomer-dependent vibrational
15 coherence in ultrafast photoisomerization, *New J. Phys.* 15 (2013).
16 <https://doi.org/10.1088/1367-2630/15/10/105022>.
- 17 [123] I. Schapiro, M. Gueye, M. Paolino, S. Fusi, G. Marchand, S. Haacke, M. Elena Martin, M. Huntress,
18 V. P. Vysotskiy, V. Veryazov, J. Léonard, M. Olivucci, Synthesis, spectroscopy and QM/MM
19 simulations of a biomimetic ultrafast light-driven molecular motor, *Photochem. Photobiol. Sci.*
20 18 (2019) 2259–2269. <https://doi.org/10.1039/C9PP00223E>.
- 21 [124] R. Rozin, A. Wand, K.H. Jung, S. Ruhman, M. Sheves, pH dependence of Anabaena sensory
22 rhodopsin: retinal isomer composition, rate of dark adaptation, and photochemistry, *J Phys Chem*
23 *B.* 118 (2014) 8995–9006. <https://doi.org/10.1021/jp504688y>.
- 24 [125] M. Stenrup, E. Pieri, V. Ledentu, N. Ferré, pH-Dependent absorption spectrum of a protein: a
25 minimal electrostatic model of Anabaena sensory rhodopsin, *Phys. Chem. Chem. Phys.* 19 (2017)
26 14073–14084. <https://doi.org/10.1039/C7CP00991G>.
- 27 [126] M.L. Grünbein, M. Stricker, G. Nass Kovacs, M. Kloos, R.B. Doak, R.L. Shoeman, J. Reinstein, S.
28 Lecler, S. Haacke, I. Schlichting, Illumination guidelines for ultrafast pump–probe experiments by
29 serial femtosecond crystallography, *Nat. Methods.* (2020). [https://doi.org/10.1038/s41592-020-](https://doi.org/10.1038/s41592-020-0847-3)
30 [0847-3](https://doi.org/10.1038/s41592-020-0847-3).
- 31 [127] S. Adachi, T. Schatteburg, A. Humeniuk, R. Mitrić, T. Suzuki, Probing ultrafast dynamics during
32 and after passing through conical intersections, *Phys. Chem. Chem. Phys.* (2019).
33 <https://doi.org/10.1039/C8CP04426K>.
- 34 [128] A.D. Smith, E.M. Warne, D. Bellshaw, D.A. Horke, M. Tudorovskya, E. Springate, A.J.H. Jones, C.
35 Cacho, R.T. Chapman, A. Kirrander, R.S. Minns, Mapping the Complete Reaction Path of a
36 Complex Photochemical Reaction, *Phys. Rev. Lett.* 120 (2018) 183003.
37 <https://doi.org/10.1103/PhysRevLett.120.183003>.
- 38 [129] J. Hummert, G. Reitsma, N. Mayer, E. Ikonnikov, M. Eckstein, O. Kornilov, Femtosecond Extreme
39 Ultraviolet Photoelectron Spectroscopy of Organic Molecules in Aqueous Solution, *J. Phys. Chem.*
40 *Lett.* 9 (2018) 6649–6655. <https://doi.org/10.1021/acs.jpcllett.8b02937>.
- 41
42
43