Coupled HOOP signature correlates with quantum yield of isorhodopsin and analog pigments

Bovee-Geurts, P.H.M.; Lugtenburg, J.; Grip, W.J. de

Coupled HOOP signature correlates with quantum yield of isorhodopsin and analog pigments

Petra H.M. Bovee-Geurts [a], Johan Lugtenburg [b]*, Willem J. DeGrip [a,b]*

a. Department of Biochemistry, 286-Radboud University Medical Center, Radboud Institute for Molecular Life Sciences, P.O. Box 9101, NL-6500 HB, Nijmegen, The Netherlands

b. Department of Biophysical Organic Chemistry, Leiden Institute of Chemistry, Leiden University , P.O. Box 9502, NL-2300 RA Leiden, The Netherlands

* Corresponding authors:

Email addresses:
Petra.HM.Bovee-Geurts@radboudumc.nl, lugtenbu@chemleidenuniv.nl, wim.degrip@radboudumc.nl

ABSTRACT

With a quantum yield of 0.66 ± 0.03 the photoisomerization efficiency of the visual pigment rhodopsin (11-*cis* ⇒ all-*trans* chromophore) is exceptionally high. This is currently explained by coherent coupling of the excited state electronic wavepacket with local vibrational nuclear modes, facilitating efficient cross-over at a conical intersection onto the photoproduct energy surface. The 9-*cis* counterpart of rhodopsin, dubbed isorhodopsin, has a much lower quantum yield (0.26 ± 0.03), which, however, can be markedly enhanced by modification of the retinal chromophore (7,8-dihydro and 9-cyclopropyl derivatives). The coherent coupling in the excited state is promoted by torsional skeletal and coupled HOOP vibrational modes, in combination with a twisted conformation around the isomerization region. Since such torsion will strongly enhance the infrared intensity of coupled HOOP modes, we investigated FTIR difference spectra of rhodopsin, isorhodopsin and several analog pigments in the spectral range of isolated and coupled H-C=C-H wags. As a result we propose that the coupled HOOP signature in these retinal pigments correlates with the distribution of torsion over counteracting segments in the retinylidene polyene chain. As such the HOOP signature can act as an indicator for the photoisomerization efficiency, and can explain the higher quantum yield of the 7,8-dihydro and 9-cyclopropyl-isorhodopsin analogs.

KEYWORDS:

HOOP vibration, photochemistry, infrared spectroscopy, vibrational coherence, chromophore modification, ligand-protein interaction

1. INTRODUCTION

In contrast to the rod visual pigment rhodopsin, a paradigm and name-giver for the opsin family, a major sub-class of the superfamily of G protein coupled receptors, its configurational counterpart isorhodopsin has received much less attention. Rhodopsin binds the 11-*cis* isomer of retinal as its chromophore (Fig. 1), resulting in an absorbance band peaking near 500 nm [1]. Originally, George Wald and coworkers detected that the apoprotein opsin also binds the 9-*cis* isomer of retinal (Fig. 1), be it with five to ten-fold slower kinetics, yielding a pigment with a λmax near 485 nm, which was coined isorhodopsin [2]. The lower binding rate is in line with the about 5 kcal.mol^{-1} higher ground-state enthalpy of isorhodopsin [3,4]. Since for a long time 9-*cis* retinal was not detected in healthy retina's [5,6], isorhodopsin was considered a not too interesting artifact. However, more recently small quantities of isorhodopsin were identified in mice with a mutation in the isomerase (RPE65) that converts the all-*trans* into the 11-*cis* configuration [5]. Furthermore, it was established that oral administration of precursors of the more stable 9-*cis* retinal can rescue the visual defect observed in mice and in human patients, suffering from an inadequate 11-*cis* generating pathway [7,8].

This rescue capacity originates in the very similar photocascade, triggered by illumination of rhodopsin or iso-rhodopsin, culminating within ms in generation of the active state, metarhodopsin II, which mediates signal propagation by binding and activating the visual G protein, transducin (Gt) [1,9,10]. However, next to the spectral sensitivity a major difference between rhodopsin and isorhodopsin lies in their photosensitivity. While the molar absorbance of isorhodopsin is about 5% higher than that of rhodopsin [11], the photoisomerization quantum yield of isorhodopsin is about 60% lower at physiological temperatures (Table 1) [2,12,13].

The remarkably high quantum yield of rhodopsin has eluded a theoretical explanation for several decades. With the development of femtosecond spectroscopic techniques, quantum-chemical calculus and forceful molecular dynamics simulation, a fairly coherent model has slowly emerged [14-28]. Upon photo-excitation of the chromophore into the Franck-Condon state it rapidly relaxes along a barrierless trajectory on the potential energy surface to a minimal energy conical intersection, where effective cross-over to the energy surface of the photoproduct takes place, generating a hot all-transoid state (photorhodopsin) in about 30 fs [14]. This thermalizes within several hundred fs into bathorhodopsin [14-18,20,29], which is stable below 130 K [30], but still contains a highly twisted all-trans configuration for its chromophore, as is evident from strongly enhanced isolated H-wag vibrations in the polyene chain of the chromophore [31-33] (Fig.2).

The cross-over at the conical intersection is facilitated by several structural elements: (1) the opsin binding site, with special crevices for the cyclohexene ring [34,35] and 9-methyl group [35-37], and the Schiff-base linkage to Lys-296, strongly fixates the chromophore [34] and enforces a torsion in its conformation, a.o. resulting in a twist around the C11=C12 bond; (2) upon excitation the electronic distribution in the chromophore is altered, the dipole moment is reversed, and the C11=C12 bond elongates [38,39]; (3) the electronic wave packet on the excited state surface can couple coherently with local nuclear vibrational modes in the hot-spot region (C10-C11=C12-C13), in particular torsional modes of C11=C12 and the coupled hydrogen-out-of-plane (HOOP) vibration of H-C11=C12-H

[14,17,18,21,26,27,40-42]. These combined effects can evolve into rapid crossing of the wave packet at the conical intersection onto the photoproduct surface (also dubbed: quasi-quantum coherent evolution [43]).

With respect to isorhodopsin these elements have been much less deeply investigated [23,44-47]. It was demonstrated that, in contrast to rhodopsin, the photochemical quantum yield of isorhodopsin becomes strongly wavelength dependent at low temperature, at 80 K decreasing from 0.17 to 0.09 at the red wing of the absorbance spectrum [12,23]. This suggests two competing processes. Recent quantum-chemical molecular dynamics simulations indeed propose a split pathway with separate minimal energy conical intersections, consisting of a rapid unreactive pathway, and a slower reactive pathway, possibly coupling with vibrational nuclear modes, that is hindered by sterical interaction with protein residues [45,46]. Even less understood is the intriguing observation that certain modifications of retinal can markedly enhance the quantum yield of the corresponding isorhodopsin analog with little (7,8-dihydro derivative) or strongly adverse (9-cyclopropyl derivative) effects on the corresponding rhodopsin analog [48,49].

As introduced above, there is strong evidence that the H-C11=C12-H HOOP vibration coherently couples with the excited electronic wave packet and thus contributes to the efficacy of crossing at the conical intersection, and thereby the high photochemical quantum yield of rhodopsin. Since the HOOP vibrations originate in the ground state of the pigments, and can be easily identified in FTIR difference spectroscopy [31,33], we searched for a correlation between the coupled HOOP pattern and the photochemical quantum yield in isohodopsin and rhodopsin and some of their analog pigments with deviating quantum yield. This leads us to amend assignments for the HOOP modes in isorhodopsin and rhodopsin, and suggest an explanation for the marked increase in quantum yield in 7,8-dihydro- and 9-cyclopropyl-isorhodopin.

2. MATERIALS AND METHODS

2.1 Retinals.

The chemical structure of the retinals used in this paper is depicted in figure 1. 11-*Cis* retinal was a generous gift from Rosalie Crouch (Medical University of South Carolina, Charleston, U.S.A.). Synthesis and characterization of the other retinals were described in earlier papers [37,48-50].

2.2 Pigments and analogs

Rhodopsin was isolated from bovine eyes. Rod outer segment membranes were prepared from fresh, dark-adapted bovine eyes via sucrose-density gradient centrifugation using dim-red light conditions (> 620 nm; Schott RG620 cut-off filter) [51]. The same procedure was used for apoprotein (opsin) containing membranes after light adaptation of fresh eyes [52]. The regeneration capacity of opsin membranes was estimated from the A280/A500 ratio obtained after incubation in the dark with a three-fold molar excess of 11-*cis* retinal, whereby a ratio of 2.1 ± 0.1 was taken to represent a maximal rhodopsin content. All pigments were prepared with opsin membranes having a

regeneration capacity of 90-100 %. All manipulation with the pigments was performed under dim red light.

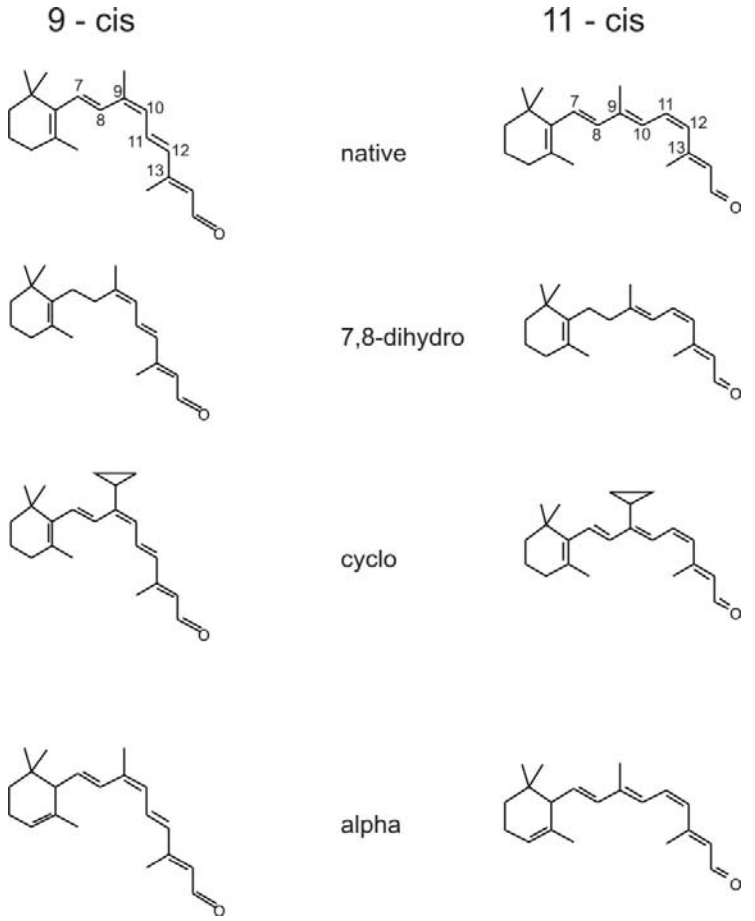

Figure 1: The chemical structures of 9-*cis* and 11-*cis* retinal and their derivatives discussed in this paper. Cyclo stands for 9-cyclopropyl. The numbering of here relevant carbon atoms is indicated in the native retinals. The full numbering is shown in figure S1. The structures are presented in the 12-s-*trans* conformation, which is the conformation induced by the opsin binding site.

Pigments were generated by incubation in the dark with a three-fold molar excess of the retinal during 2 h at room temperature. In case of the poorly incorporating alpha and 9-cyclopropyl retinals another five-fold molar excess was then added and the incubation continued overnight at 4°C. Excess retinal was extracted by incubation with β-cyclodextrin and pigment membranes finally recovered by sucrose step-gradient centrifugation, as described in detail before [48-50,53,54]. Pigments with lower regeneration levels were purified by affinity chromatography and reconstituted into proteoliposomes as described [51,54]. Alpha-rhodopsin exhibited poor stability in detergent solution, however, leading to contaminated preparations with low intact pigment content. The spectral analysis and photochemical characterization of the pigments compiled in table 1 was reported before [48-50].

2.3 FTIR spectroscopy

FT-IR analyses were performed on a Bruker IFS 66/S spectrometer, equipped with a liquid-nitrogen-cooled, narrowband HgCdTe (MCT) detector as described [48,53]. In brief, sample temperature was under computer control using a variable-temperature helium-cooled cryostat (Heliostat, APD Cryogenics Inc.), covered with a set of NaCl windows in the IR light path. Membrane films containing 2-3 nmol of pigment were prepared by isopotential spin drying [55] on AgCl windows (Crystran Limited, UK), except for alpha-rhodopsin where such quantities were not available. The membrane film was rehydrated, sealed using a rubber O-ring spacer and a second AgCl window, and screwed tight in the sample holder of the cryostat. Samples were illuminated in the spectrometer using a modified fiberoptics ring illuminator (Schott) fed by a 150 W halogen light filtered through a 488 ± 10 nm interference filter and a long-pass filter (Schott). Routinely, six consecutive blocks of 1280 scans each were recorded at 4 cm^{-1} resolution, taking about 120 s acquisition time per block to generate a spectrum, both before and after 2 min of illumination. No differences in pattern for the subsequent spectra were detected, and routinely the last dark-state spectrum was subtracted from the first spectrum of the photoproduct to generate a difference spectrum. Difference spectra were taken at 80 K, since vibrational peaks are narrower and pigment and photoproduct HOOP frequencies are well separated at that temperature. Temperature stability was ± 0.2 K. Independent assays of the same pigment produced very similar difference spectra, but averaging did not lead to further noise reduction, since with this large number of scans instrumental noise starts to dominate. All spectra were normalized upon major fingerprint vibrations of the 11-*cis* (rhodopsins; about 1205 and/or 1190 cm^{-1}) and 9-*cis* (isorhodopsins; about 1200 cm^{-1}) configuration, and represent approximately the same number of photoreacting molecules.

Isorhodopsin (9-cis chromophore)					Rhodopsin (11-cis chromophore)				
retinal analog	binding level[1]	λ_{max}[2] (nm)	HOOP cm^{-1} (assignment)	Quantum yield[3]	retinal analog	binding level[1]	λ_{max}[2] (nm)	HOOP cm^{-1}, assignment	Quantum yield[3]
Native	> 90 %	486	969 (HC11=C12H) 959 (HC7=C8H)	0.26 ± 0.03	Native	> 90 %	498	969 (HC11=C12H)	0.66 ± 0.03
7,8-dihydro	> 90 %	428	967 (HC11=C12H)	0.39 ± 0.04	7,8-dihydro	> 90 %	426	971 (HC11=C12H)	0.68 ± 0.06
9-cyclopropyl	30-40 %	504	974 (HC11=C12H)	0.39 ± 0.04	9-cyclopropyl	20-30 %	492	977 (HC11=C12H) 988 (HC7=C8H)	0.08 ± 0.04
alpha-	> 90 %	461	979 (HC11=C12H) 962 (HC7=C8H)	0.28 ± 0.04	alpha-	≤ 50 %	469	960 – 970 (??)	n.d.

[1] Binding levels > 90 % indicate full incorporation of the retinal in the available opsin. Binding rates may still differ up to 10-fold. Lower incorporation usually is the result of much lower binding rates due to a poor fit in the binding site, with gradual loss of opsin activity and/or blockage of the entrance site [56].

[2] Average of ≥ 3 experiments. S.D. ± 2 nm

[3] Average of 3-5 experiments with S.D.

n.d. Not determined because of low stability

Table 1: Selected properties of the pigments discussed in this paper. Except for several FTIR spectra and the HOOP assignments, the native data are averaged from [6,12,23,48,53], the 7,8-dihydro data are taken from [48], the 9-cyclopropyl data from [49], and the alpha-data from [50].

3. RESULTS AND DISCUSSION

3.1 Properties of pigments under discussion

The smooth reaction of opsin, the apoprotein of rhodopsin, with 11-*cis* retinal [2,6] reflects an optimally structured binding site. Opsin offers tight-fitting crevices for the ring segment [34,35,57] and 9-methyl element [23,35-37,58,59], together with docking sites for the 13-methyl moiety [49,60] and the aldehyde conclusion (protonated Schiff base with an ε-amino lysine residue [61,62]). This effectuates a strongly immobilized, stable 12-s-*trans* conformation [34,63] (Fig.1), and induces a torsion in particular in the C9-C13 segment of the polyene chain [23,35,42,64-66]. This state is in line with observations, that in the absence of the 9-methyl or 13-methyl moiety (9-desmethyl- and 13-desmethyl-retinal, respectively), still fairly rapid binding with opsin occurs, but the quantum yield decreases significantly, probably because of reduced torsion in the polyene chain [59,66-69].

In contrast, the markedly slower incorporation rate of 9-*cis* retinal relative to 11-*cis* retinal suggests a much poorer fit in the binding site [2,23]. The 9-*cis* configuration leads to friction especially in the ring and 9-methyl binding pockets [70,71] and to a strong decrease in photochemical quantum yield (Table 1). Surprisingly, for several retinal derivatives this situation is altered (Table 1). A beautiful example is presented by the 7,8-dihydro derivative of 9-*cis* and 11-*cis* retinal (Fig. 1). In this case both isomers react equally smoothly with opsin[48]. Furthermore, both pigments now exhibit very similar spectral properties (Table 1), also supporting a very similar occupation of the opsin binding site. Apparently, release of the constraints of the C7=C8 double bond allows also the 9-*cis* configuration to properly dock into the ring and 9-methyl pockets. Moreover, the 7,8-dihydro derivative enhances the photochemical quantum yield of the corresponding isorhodopsin analog by about 50 % (Table 1). Since the molar absorbance coefficient of 7,8-dihydro isorhodopsin is about 20% higher than that of rhodopsin [48], as a result the photosensitivity of this isorhodopsin pigment has increased quite impressively from about 40% to about 70% of that of rhodopsin. Even more stunning effects are observed upon substituting the 9-methyl moiety for the much bulkier cyclopropyl group [49]. This group hardly fits into the 9-methyl crevice and consequently both the 11-*cis* and the 9-*cis* derivative incorporate into opsin at a much lower rate and to a reduced final regeneration level (Table 1). Now, however, the 9-*cis* configuration binds slightly better and the resulting absorbance band is significantly red-shifted from the rhodopsin analog (504 versus 492 nm, respectively). Apparently, in the 9-cis configuration the 9-cyclopropyl retinal encounters less sterical hindrance from protein residues upon entering and docking into the binding site. In this case, completely reverse effects are observed upon the quantum yield. The photosensitivity of this rhodopsin analog has plummeted, while the quantum yield of this isorhodopsin analog increased with again about 50% (Table 1). In the two isorhodopsin analogs discussed above an equally good or better fit into the binding site as compared to the rhodopsin counterpart is accompanied by an improved quantum yield. Hence, we also looked at the alpha-retinals (Fig. 1), where the 9-*cis* analog binds much better than the 11-*cis* analog, as well [50] (Table 1). However, in this case we do not observe a significant effect on the quantum yield of the isorhodopsin pigment, while the rhodopsin analog could not be analyzed because of problems with purification and stability.

In the introduction the available evidence was summarized for a contribution of coupled hydrogen-out-of-plane (HOOP) vibrations to an efficient crossing of the electronic wave packet at the conical intersection, hence to the level of the quantum yield. Consequently, we looked into our FTIR spectra for a correlation between the coupled HOOP pattern and the quantum yield of the pigments discussed above.

3.2 HOOP modes and vibrational signature

The C-H wag vibration in a H-C=C-H unit can be strongly coupled, basically showing two HOOP symmetry combinations with frequencies in the 950-980 and 650-850 cm^{-1} range [72]. In a planar system the corresponding absorbance bands have infra-red intensity, but in a twisted system their dipolar character will increase and they will be very strongly enhanced [32,58]. In contrast, the C-H wag vibration in a R-C=C-H unit (isolated HOOP) is not strongly enhanced upon skeletal deformation [32], and besides absorbs in the 800-930 cm^{-1} range [32,72], which contains very strong photoproduct vibrations (Fig. 2). Because of the plethora of vibrational modes in a large polymer like a protein, the corresponding IR spectrum is extremely complex. A resonance Raman spectrum will mainly yield chromophore vibrations [73], but this is not a proper alternative since then the frequency and intensity of vibrational peaks depend in a complex way on the conformation in the ground and the excited state, which renders interpretation much more complicated. Fortunately, IR spectra can be considerably simplified if local conformational changes can be induced and difference spectroscopy is applied. The obtained difference spectrum only shows the vibrational bands changed during the transition. With retinal proteins this can be comfortably accomplished by illumination at a low temperature, where mainly the vibrational signature of the chromophore is altered [33]. Such difference spectra, covering the range of coupled and isolated ethylenic HOOPs (800-1000 cm^{-1}), are collected for the above described pigments in figure 2.

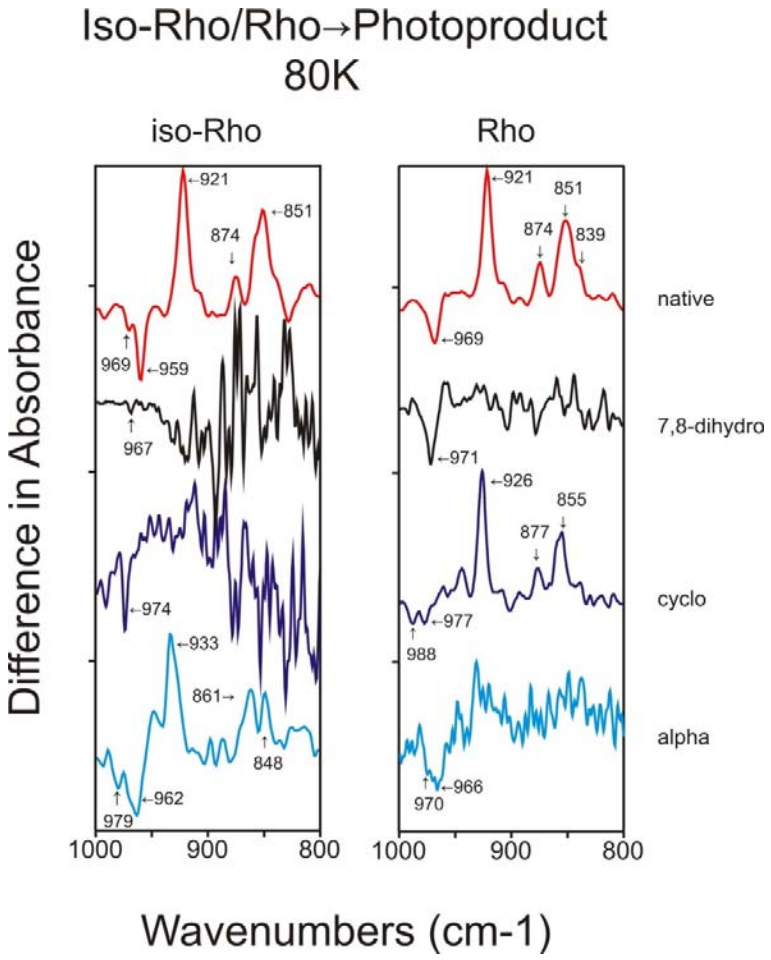

Figure 2: Representative FTIR difference spectra taken at 80 K of the pigments, discussed in this paper, in the spectral range relevant for HOOP vibrations (800-1000 cm^{-1}). Iso-Rho, Rho and cyclo stand for isorhodopsin, rhodopsin and 9-cyclopropyl, respectively. Positive peaks derive from the photoproduct and usually show the presence of individual HOOPs, due to strong skeletal deformation. Negative peaks derive from the ground state pigments. The frequency of selected HOOPs is indicated in wavenumbers (cm^{-1}). The plethora of peaks in the 800-900 cm^{-1} range for the 7,8-dehydro- and 9-cyclopropyl-isorhodopsins probably reflect a dynamical equilibrium at this temperature between two photoproducts differing in skeletal deformation of retinal and in the structure of water molecules in the binding site, since the noise level in the 900-1500 cm^{-1} range of these spectra is much smaller, but again somewhat larger between 1600 and 1700 cm^{-1} (Fig. S2). The spectrum of the alpha-rhodopsin is noisy over the entire 800-1800 cm^{-1} range (Fig. S3), because only small quantities of intact pigment were available (c.f. Materials and Methods). No conclusions were drawn from this spectrum, but it is included for completeness.

As argued above, the isolated HOOP at C10 (Fig. 1) is not relevant for our analysis, but retinals contain two positions where coupled HOOPs can be expected, C7=C8 and C11=C12 (c.f. Fig. 1). In the FTIR difference spectra (Fig. 2) the negative bands derive from the ground state of the pigments. Native isorhodopsin presents two coupled HOOP vibrations at 969 and 959 cm^{-1} [31,50,65]. Using selective ^{2}H and ^{13}C labeling the 959 cm^{-1} peak was assigned to the 7,8 HOOP via elimination [32]. While originally the 969 cm^{-1} peak was not detected in resonance Raman spectra, it was concluded on the basis of the labeling that a weak 11,12 HOOP might be present [32]. Therefore, we assign the strong 959 cm^{-1} vibration to the 7,8 HOOP and the weaker 969 cm^{-1} vibration to the 11,12

HOOP. This assignment is corroborated by the 7,8-dihydro data, where the 7,8 HOOP cannot be present, and the isorhodopsin difference spectrum only shows a peak at 967 cm^{-1} (Fig. 2), which should represent the 11,12 HOOP. Subsequently, 9-cyclopropyl-isorhodopsin also presents a fairly strong single band, now at 974 cm^{-1}, which we assign to the 11,12 HOOP in view of its frequency. Finally, alpha-isorhodopsin again presents two peaks, of which we assign the weaker one at 979 cm^{-1} to the 11,12 HOOP and the stronger one at 969 cm^{-1} to the 7,8 HOOP.

Native rhodopsin only presents a strong single band at 969 cm^{-1} [31,33,73], which, again based upon selective labeling, has been assigned to mainly the *cis*-11,12 HOOP, with possibly a small contribution from the *trans*-7,8 HOOP [32,73]. However, we do not find a rest-peak in derivatives where the 11,12 coupling is abolished (11-methyl, 12-methyl and 12-F rhodopsin [56,74]) or is shifted to lower frequency (10-F rhodopsin[74]). Therefore we assign the 969 cm^{-1} vibration to solely the 11,12 HOOP and conclude that in native rhodopsin the 7,8 HOOP is not enhanced and has not sufficient intensity to be detectable. In 7,8-dihydro-rhodopsin again only a single strong HOOP vibration is observed, now at 971 cm^{-1} , which must be the 11,12 HOOP, since the 7,8-HOOP cannot be present. Surprisingly, 9-cyclopropyl-rhodopsin shows a doublet at 977 cm^{-1} and 988 cm^{-1}, both peaks having about the same intensity (Fig. 2). The solitary wags in the photoproduct of this pigment are all upshifted in frequency, hence we surmise an upshift in the frequency of the coupled HOOPs and assign the 977 cm^{-1} and 988 cm^{-1} peaks to the 7,8 and 11,12 HOOP, respectively. Because of the low quantity of available pigment, the difference spectrum of alpha-rhodopsin is very noisy (Figs. 2 and S3). Hence, it is difficult to assess whether the broad feature near 970 cm^{-1} represents a single HOOP vibration or a doublet. Since we could not reliably determine the quantum yield of this pigment, we refrain from attempting to assign this band.

3.3 Interpretation

The available literature, summarized in the introduction, presents convincing evidence that a twisted conformation of the C10-C13 segment in rhodopsin can prime the pigment for very productive 11-*cis* to all-*trans* photo-isomerization by promoting coherent coupling of torsional and HOOP vibrational modes with the electronic wave packet at the excited state potential surface. On the other hand, we propose that a twist in the C7-C9 segment will be counterproductive, since the 9-methyl moiety remains strongly settled in its binding site pocket [47,70] and cannot really assist in coherent coupling of vibrational modes with a wave packet. The latter argument also holds for the 9-*cis* to all-*trans* photo-isomerization in isorhodopsin. However, the 11,12 HOOP also communicates with the 10-wag, since C10-substituents (10-^{2}H, 10-CH_3, 10-F) downshift the 11,12 HOOP [32,53,74]. Hence, it is conceivable that torsion in the C10-C13 segment can also productively contribute to photo-isomerization of isorhodopsin, be it less effective.

Within this framework we can offer an explanation for the variation in quantum yield observed between isorhodopsin and rhodopsin, respectively, and their analog pigments. In rhodopsin we only detect a strong 11,12 HOOP, indicating that the torsion in the polyene chain is largely located in the C10-C13 segment, which can optimally promote coherent coupling yielding a high quantum yield. The same situation, a strong 11,12 HOOP and a large quantum yield, is also observed in the 7,8-dihydro rhodopsin analog. Since here the C7-C8 bond is flexible,

this supports the concept that in the 11-*cis* configuration the ring and 9-methyl element of retinal snuggle smoothly in their binding site crevice inducing torsion largely in the C10-C13 segment. In 9-cyclopropyl rhodopsin two HOOPs of about equal intensity are observed, indicating that the torsion in the polyene chain now is distributed over the C7-C9 and C10-C13 segment. This must be the consequence of the cyclopropyl group not very well fitting in the 9-methyl crevice when combined with a *cis* C11=C12 bond. The promoting effect of the C10-C13 twist hence will be strongly reduced and the quantum yield severely diminished.

In contrast, native isorhodopsin exhibits a strong 7,8 HOOP with a weaker 11,12 HOOP. This suggest strong torsion in the C7-C9 segment with less torsion in the C10-C13 segment, in concert with a poor fit of the ring and 9-methyl group in combination with a *cis* C9=C10 bond. Together with the less effective coherent coupling through the 10-wag stimulated via C10-C13 vibrational modes, this provides an explanation for the much lower quantum yield of native isorhodopsin relative to rhodopsin (Table 1). Upon release of the C7=C8 constraints in the 7,8-dihydro analog, a better fit of the 9-*cis* isomer in the binding site can be realized, as is obvious from the similar incorporation rates of the 9-*cis* and the 11-*cis* isomer and the very similar spectral properties of the resulting pigments [48]. Only the 11,12 HOOP is then observed, and the corresponding torsion in the C10-C13 segment can strongly enhance the quantum yield of this isorhodopsin analog (Table 1). Surprisingly, the same situation is encountered for the 9-cyclopropyl analog. We have to conclude that in combination with a *cis* C9=C10 bond the cyclopropyl and ring elements apparently can find a docking position, where the torsion is mainly located in the C10-C13 segment of the polyene chain, again strongly enhancing the quantum yield. Finally, in alpha-isorhodopsin two HOOPs are detected, hence the torsion is distributed over the C7-C9 and C10-C13 segment, similar to the parent pigment, and the quantum yield comes at the same level as native isorhodopsin.

3.4 Concluding thoughts

We propose a corrrelation between the degree of torsion in the C7-C9 and C10-C13 segments of the retinylidene chromophore and the photochemical quantum yield of the corresponding pigment. More torsion in the C10-C13 segment results in an increased quantum yield. This can be conveniently deduced from the intensity ratio of the coupled H-C7=C8-H and H-C11=C12-H HOOP vibrations in the 960-990 cm^{-1} range of a FTIR difference spectrum.

It was fortunate that this correlation between HOOP signature and quantum yield became apparent in our selection of analog pigments. The 7,8-dihydro derivative abolishes the H-C7=C8-H HOOP and relaxes the retinal structure to the extent that the 9-*cis* and 11-*cis* isomers incorporate equally well, with very similar spectral properties and a high quantum yield. With respect to the 9-cyclopropyl group, this substituent was selected because of its size being intermediate between the 9-ethyl derivative, which does not really allow discrimination between the 9-*cis* and 11-*cis* isomer [75,76], and the 9-isopropyl derivative, of which both isomers very poorly fit in the binding site [49]. Remarkably, the 9-cyclopropyl derivative completely reverses the isomeric selectivity of the opsin binding site. The 9-*cis* isomer now incorporates slightly better, generating a red-shifted pigment with a much higher quantum yield, as compared to the 11-*cis* isomer (Table 1). This is also reflected in their HOOP signature, the isorhodopsin analog

presenting a single coupled HOOP vibration, and the rhodopsin analog presenting a doublet of about equal intensity (Table 1, Fig. 2). It should be noted that modifications away from the isomerization sites (e.g. positions 13 and 14) may interfere with the mechanism proposed above through destructive interaction with the protein [49]. Further, validation of our assigments will require mass labeling (e.g. ^{2}H or F at position 8 or 11) of all derivatives for both 9-*cis* and 11-*cis* retinal. This is a formidable task and outside the scope of this paper.

An interesting question in this context is whether the value of 0.39, we observe in two cases, is about the maximal quantum yield in reach for isorhodopsin. Such a much lower value than presented by rhodopsin could be the consequence of a less effective coupling of the C9=C10-H wag and other local nuclear modes, requiring stimulation by C10-C13 vibrational modes, with the excited electronic wave packet. This can e.g.be tested on 7,8-dihydro,9-cyclopropyl –isorhodopsin, to evaluate whether the combined modifications can further enhance the quantum yield. Another interesting option in this respect is based upon the report that the ^{2}H-C11=C12-^{2}H double label can enhance the quantum yield of rhodopsin by about 8% [77]. Possibly, a stronger communication of vibrational modes in triple labeled 9-*cis* retinal (^{2}H-C10-(^{2}H)-C11=C12-^{2}H or even F-C10-(F)-C11=C12-F) would enhance the quantum yield of isorhodopsin. In addition, application on isorhodopsin and 7,8-dihydro-isorhodopsin of ultrafast transient-grating spectroscopy [14] and 2D femtosecond stimulated Raman spectroscopy [78] should reveal important intimate details of the photo-isomerisation mechanism in this intriguing photopigment.

In perspective, our concept is an elementary illustration of the subtle and flexible interaction between receptor and ligand, which can modulate either conformational space and thereby the resulting response level.

SUPPORTING INFORMATION

Figure S1: Full carbon numbering of retinal

Figures S2-S3: FTIR difference spectra of the presented pigments over the range 800-1800 cm^{-1}

AUTHOR INFORMATION

Corresponding authors

*Lugtenbu@chem.leidenuniv.nl

*wim.degrip@radboudumc.nl

Notes

The authors declare no competing financial interest.

The study was conceived by JL and WJdG. The data were collected by PHMB-G, and interpreted by all authors. The article was drafted by WJdG with input from the other authors.

ACKNOWLEDGEMENTS

We thank Dr. Rosalie Crouch (Medical University of South Carolina, Charleston, U.S.A.) for a generous gift of 11-*cis* retinal, through financial support from NEI. This research was supported by grants from The Netherlands Foundation for Scientific Research through its Chemical Council (NWO-CW 700.54.008 to W.J.d.G and 700.98.013 to W.J.d.G and J.L.). The funding source had no involvement whatsoever in any element of this study.

REFERENCE LIST

[1] G. Wald, The molecular basis of visual excitation, Nature 219 (1968) 800-807.

[2] R. Hubbard, G. Wald, Cis-trans isomers of vitamin A and retinene in the rhodopsin system, J. Gen. Physiol. 36 (1952) 269-315.

[3] A. Cooper, Energetics of rhodopsin and isorhodopsin, FEBS Lett. 100 (1979) 382-384.

[4] G.A. Schick, T.M. Cooper, R.A. Holloway, L.P. Murray, R.R. Birge, Energy-storage in the primary photochemical events of rhodopsin and isorhodopsin, Biochemistry 26 (1987) 2556-2562.

[5] J. Fan, B. Rohrer, G. Moiseyev, J.-X. Ma, R.K. Crouch, Isorhodopsin rather than rhodopsin mediates rod function in RPE65 knock-out mice, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 13662-13667.

[6] R. Hubbard, P.K. Brown, M.D. Bownds, Methodology of vitamin A and visual pigments, Methods Enzymol. 18C (1971) 615-653.

[7] T. Maeda, A. Maeda, G. Casadesus, K. Palczewski, P. Margaron, Evaluation of 9-cis-retinyl acetate therapy in $RPE65^{-/-}$ mice, Invest. Ophthalmol. Visual Sci. 50 (2009) 4368-4378.

[8] R.K. Koenekoop, R.F. Sui, J. Sallum, L.I. van den Born, R. Ajlan, A. Khan, A.I. den Hollander, F.P.M. Cremers, J.D. Mendola, A.K. Bittner, G. Dagnelie, R.A. Schuchard, D.A. Saperstein, Oral 9-cis retinoid for childhood blindness due to Leber congenital amaurosis caused by *RPE65* or *LRAT* mutations: an open-label phase 1b trial, Lancet 384 (2014) 1513-1520.

[9] H. Kühn, Interactions between photoexcited rhodopsin and light-activated enzymes in rods, Prog. Retinal Res. 3 (1984) 123-156.

[10] K.P. Hofmann, Late Photoproducts and Signaling States of Bovine Rhodopsin, in: D.G. Stavenga, W.J. DeGrip, E.N. Pugh, Jr. (Eds.), Molecular Mechanisms in Visual Transduction, vol. 3, Handbook of Biological Physics, Elsevier Science Pub., Amsterdam, The Netherlands, 2000, pp. 91-142.

[11] G. Wald, P.K. Brown, The molar extinction of rhodopsin, J. Gen. Physiol. 37 (1953) 189-200.

[12] J.B. Hurley, T.G. Ebrey, B. Honig, M. Ottolenghi, Temperature and wavelength effects on photochemistry of rhodopsin, isorhodopsin, bacteriorhodopsin and their photoproducts, Nature 270 (1977) 540-542.

[13] R.S.H. Liu, F. Crescitelli, M. Denny, H. Matsumoto, A.E. Asato, Photosensitivity of 10-substituted visual pigment analogues: Detection of a specific secondary opsin-retinal interaction, Biochemistry 25 (1986) 7026-7030.

[14] P.J.M. Johnson, A. Halpin, T. Morizumi, V.I. Prokhorenko, O.P. Ernst, R.J.D. Miller, Local vibrational coherences drive the primary photochemistry of vision, Nature Chemistry 7 (2015) 980-986.

[15] D.W. McCamant, Re-evaluation of rhodopsin's relaxation kinetics determined from femtosecond stimulated Raman lineshapes, J. Phys. Chem. B 115 (2011) 9299-9305.

[16] I. Rivalta, A. Nenov, O. Weingart, G. Cerullo, M. Garavelli, S. Mukamel, Modelling time-resolved two-dimensional electronic spectroscopy of the primary photoisomerization event in rhodopsin, J. Phys. Chem. B 118 (2014) 8396-8405.

[17] C. Schnedermann, M. Liebel, P. Kukura, Mode-specificity of vibrationally coherent internal conversion in rhodopsin during the primary visual event, J. Am. Chem. Soc. 137 (2015) 2886-2891.

[18] D. Polli, P. Altoè, O. Weingart, K.M. Spillane, C. Manzoni, D. Brida, G. Tomasello, G. Orlandi, P. Kukura, R.A. Mathies, M. Garavelli, G. Cerullo, Conical intersection dynamics of the primary photoisomerization event in vision, Nature 467 (2010) 440-443.

[19] D. Polli, I. Rivalta, A. Nenov, O. Weingart, M. Garavelli, G. Cerullo, Tracking the primary photoconversion events in rhodopsins by ultrafast optical spectroscopy, Photochem. Photobiol. Sci. 14 (2015) 213-228.

[20] Q. Wang, R.W. Schoenlein, L.A. Peteanu, R.A. Mathies, C.V. Shank, Vibrationally coherent photochemistry in the femtosecond primary event of vision, Science 266 (1994) 422-424.

[21] O. Weingart, M. Garavelli, Modelling vibrational coherence in the primary rhodopsin photoproduct, J. Chem. Phys. 137 (2012) 22A523-1-6.

[22] G.E. Busch, M.L. Applebury, A.A. Lamola, P.M. Rentzepis, Formation and decay of prelumirhodopsin at room temperatures, Proc. Natl. Acad. Sci. U. S. A. 69 (1972) 2802-2806.

[23] R.R. Birge, C.M. Einterz, H.M. Knapp, L.P. Murray, The nature of the primary photochemical events in rhodopsin and isorhodopsin, Biophys. J. 53 (1988) 367-385.

[24] L.M. Frutos, T. Andruniów, F. Santoro, N. Ferré, M. Olivucci, Tracking the excited-state time evolution of the visual pigment with multiconfigurational quantum chemistry, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 7764-7769.

[25] P. Kukura, D.W. McCamant, S. Yoon, D.B. Wandschneider, R.A. Mathies, Structural observation of the primary isomerization in vision with femtosecond-stimulated Raman, Science 310 (2005) 1006-1009.

[26] R.A. Mathies, PHOTOCHEMISTRY: A coherent picture of vision, Nature Chemistry 7 (2015) 945-947.

[27] I. Schapiro, M.N. Ryazantsev, L.M. Frutos, N. Ferré, R. Lindh, M. Olivucci, The ultrafast photoisomerizations of rhodopsin and bathorhodopsin are modulated by bond length alternation and HOOP driven electronic effects, J. Am. Chem. Soc. 133 (2011) 3354-3364.

[28] R.W. Schoenlein, L.A. Peteanu, R.A. Mathies, C.V. Shank, The first step in vision: Femtosecond isomerization of rhodopsin, Science 254 (1991) 412-415.

[29] Y.-C. Wu, B. Zhao, S.-Y. Lee, Time-dependent wave packet averaged vibrational frequencies from femtosecond stimulated Raman spectra, J. Chem. Phys. 144 (2016) 054104-1-11.

[30] T. Yoshizawa, G. Wald, Pre-lumirhodopsin and the bleaching of visual pigments, Nature 197 (1963) 1279-1286.

[31] K.A. Bagley, L. Eisenstein, T.G. Ebrey, M. Tsuda, A comparative study of the infrared difference spectra for octopus and bovine rhodopsins and their bathorhodopsin photointermediates, Biochemistry 28 (1989) 3366-3373.

[32] G. Eyring, B. Curry, A. Broek, J. Lugtenburg, R.A. Mathies, Assignment and interpretation of hydrogen out-of-plane vibrations in the resonance Raman spectra of rhodopsin and bathorhodopsin, Biochemistry 21 (1982) 384-393.

[33] K.J. Rothschild, W.A. Cantore, H. Marrero, Fourier transform infrared difference spectra of intermediates in rhodopsin bleaching, Science 219 (1983) 1333-1335.

[34] A.F.L. Creemers, S.R. Kiihne, P.H.M. Bovee-Geurts, W.J. DeGrip, J. Lugtenburg, H.J.M. de Groot, ^{1}H and ^{13}C MAS NMR evidence for pronounced ligand-protein interactions involving the ionone ring of the retinylidene chromophore in rhodopsin, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 9101-9106.

[35] T. Okada, M. Sugihara, A.-N. Bondar, M. Elstner, P. Entel, V. Buss, The retinal conformation and its environment in rhodopsin in light of a new 2.2 Å crystal structure, J. Mol. Biol. 342 (2004) 571-583.

[36] M. Han, M. Groesbeek, S.O. Smith, T.P. Sakmar, Role of the C_9 methyl group in rhodopsin activation: Characterization of mutant opsins with the artificial chromophore 11-*cis*-9-demethylretinal, Biochemistry 37 (1998) 538-545.

[37] Y.-J. Wang, P.H.M. Bovee-Geurts, J. Lugtenburg, W.J. DeGrip, Constraints of the 9-methyl group binding pocket of the rhodopsin chromophore probed by 9-halogeno substitution, Biochemistry 43 (2004) 14802-14810.

[38] O.P. Ernst, D.T. Lodowski, M. Elstner, P. Hegemann, L.S. Brown, H. Kandori, Microbial and animal rhodopsins: Structures, functions, and molecular mechanisms, Chem. Rev. 114 (2014) 126-163.

[39] S.O. Smith, Structure and activation of the visual pigment rhodopsin, Annu. Rev. Biophys. 39 (2010) 309-328.

[40] H.-G. Duan, M. Thorwart, Quantum mechanical wave packet dynamics at a conical intersection with strong vibrational dissipation, J. Phys. Chem. Lett. 7 (2016) 382-386.

[41] T. Kakitani, R. Akiyama, Y. Hatano, Y. Imamoto, Y. Shichida, P.J.E. Verdegem, J. Lugtenburg, Deuterium substitution effect on the excited-state dynamics of rhodopsin, J. Phys. Chem. B 102 (1998) 1334-1339.

[42] S.W. Lin, M. Groesbeek, I. Van der Hoef, P.J.E. Verdegem, J. Lugtenburg, R.A. Mathies, Vibrational assignment of torsional normal modes of rhodopsin: Probing excited-state isomerization dynamics along the reactive C_{11}=C_{12} torsion coordinate, J. Phys. Chem. B 102 (1998) 2787-2806.

[43] R.L. Purchase, H.J.M. de Groot, Biosolar cells: Global artificial photosynthesis needs responsive matrices with quantum coherent kinetic control for high yield, Interface Focus 5 (2015) 20150014-1-16.

[44] A. Strambi, P.B. Coto, L.M. Frutos, N. Ferré, M. Olivucci, Relationship between the excited state relaxation paths of rhodopsin and isorhodopsin, J. Am. Chem. Soc. 130 (2008) 3382-3388.

[45] W.C. Chung, S. Nanbu, T. Ishida, QM/MM trajectory surface hopping approach to photoisomerization of rhodopsin and isorhodopsin: The origin of faster and more efficient isomerization for rhodopsin, J. Phys. Chem. B 116 (2012) 8009-8023.

[46] D. Polli, O. Weingart, D. Brida, E. Poli, M. Maiuri, K.M. Spillane, A. Bottoni, P. Kukura, R.A. Mathies, G. Cerullo, M. Garavelli, Wavepacket splitting and two-pathway deactivation in the photoexcited visual pigment isorhodopsin, Angew. Chem. , Int. Ed. 53 (2014) 2504-2507.

[47] S. Sekharan, M. Sugihara, O. Weingart, T. Okada, V. Buss, Protein assistance in the photoisomerization of rhodopsin and 9-*cis*-rhodopsin-Insights from experiment and theory, J. Am. Chem. Soc. 129 (2007) 1052-1054.

[48] W.J. DeGrip, P.H.M. Bovee-Geurts, I. Van der Hoef, J. Lugtenburg, 7,8-Dihydro-retinals outperform the native retinals in conferring photosensitivity to visual opsin, J. Am. Chem. Soc. 129 (2007) 13265-13269.

[49] W.J. DeGrip, P.H.M. Bovee-Geurts, Y.-J. Wang, M.A. Verhoeven, J. Lugtenburg, Cyclopropyl and isopropyl derivatives of 11-*cis* and 9-*cis* retinals at C-9 and C-13: Subtle steric differences with major effects on ligand efficacy in rhodopsin, J. Nat. Prod. 74 (2011) 383-390.

[50] Y.-J. Wang, P.H.M. Bovee-Geurts, J. Lugtenburg, W.J. DeGrip, Alpha-retinals as rhodopsin chromophores - Preference for the 9-*Z* configuration and partial agonist activity, Photochem. Photobiol. 84 (2008) 889-894.

[51] W.J. DeGrip, F.J.M. Daemen, S.L. Bonting, Isolation and purification of bovine rhodopsin, Methods Enzymol. 67 (1980) 301-320.

[52] W.J. DeGrip, J. VanOostrum, P.H.M. Bovee-Geurts, R. van der Steen, L.J.P. VanAmsterdam, M. Groesbeek, J. Lugtenburg, 10,20-Methanorhodopsins: (7*E*,9*E*,13*E*)-10,20-methanorhodopsin and (7*E*,9*Z*,13*Z*)-10,20-methanorhodopsin - 11-*cis*-Locked rhodopsin analog pigments with unusual thermal and photo-stability, Eur. J. Biochem. 191 (1990) 211-220.

[53] F. DeLange, P.H.M. Bovee-Geurts, J. VanOostrum, M.D. Portier, P.J.E. Verdegem, J. Lugtenburg, W.J. DeGrip, An additional methyl group at the 10-position of retinal dramatically slows down the kinetics of the rhodopsin photocascade, Biochemistry 37 (1998) 1411-1420.

[54] W.J. DeGrip, J. VanOostrum, P.H.M. Bovee-Geurts, Selective detergent-extraction from mixed detergent/lipid/protein micelles, using cyclodextrin inclusion compounds: A novel generic approach for the preparation of proteoliposomes, Biochem. J. 330 (1998) 667-674.

[55] N.A. Clark, K.J. Rothschild, B.A. Simon, D.A. Luippold, Surface induced orientation of multilayer membrane arrays: Theoretical analysis and a new method with application to purple membrane fragments, Biophys. J. 31 (1980) 65-96.

[56] M.A. Verhoeven, P.H.M. Bovee-Geurts, H.J.M. de Groot, J. Lugtenburg, W.J. DeGrip, Methyl substituents at the 11- or 12-position of retinal profoundly and differentially affect photochemistry and signalling activity of rhodopsin, J. Mol. Biol. 363 (2006) 98-113.

[57] P.J.R. Spooner, J.M. Sharples, S.C. Goodall, H. Seedorf, M.A. Verhoeven, J. Lugtenburg, P.H.M. Bovee-Geurts, W.J. DeGrip, A. Watts, Conformational similarities in the β-ionone ring region of the rhodopsin chromophore in its ground state and after photoactivation to the metarhodopsin-I intermediate, Biochemistry 42 (2003) 13371-13378.

[58] G. Eyring, B. Curry, R.A. Mathies, R. Fransen, I. Palings, J. Lugtenburg, Interpretation of the resonance Raman spectrum of bathorhodopsin based on visual pigment analogues, Biochemistry 19 (1980) 2410-2418.

[59] A. Kropf, B.P. Whittenberger, S.P. Goff, A.S. Waggoner, The spectral properties of some visual pigment analogs, Exp. Eye Res. 17 (1973) 591-606.

[60] R. Vogel, S. Lüdeke, F. Siebert, T.P. Sakmar, A. Hirshfeld, M. Sheves, Agonists and partial agonists of rhodopsin: Retinal polyene methylation affects receptor activation, Biochemistry 45 (2006) 1640-1652.

[61] M.D. Bownds, Site of attachment of retinal in rhodopsin, Nature 216 (1967) 1178-1181.

[62] R.A. Mathies, A.R. Oseroff, L. Stryer, Rapid-flow resonance Raman spectroscopy of photolabile molecules: Rhodopsin and isorhodopsin, Proc. Natl. Acad. Sci. U. S. A. 73 (1976) 1-5.

[63] R.H. Callender, A.G. Doukas, R.K. Crouch, K. Nakanishi, Molecular flow resonance Raman effect from retinal and rhodopsin, Biochemistry 15 (1976) 1621-1629.

[64] X. Feng, P.J.E. Verdegem, Y.K. Lee, D. Sandström, M. Edén, P.H.M. Bovee-Geurts, W.J. DeGrip, J. Lugtenburg, H.J.M. de Groot, M.H. Levitt, Direct determination of a molecular torsional angle in the membrane protein rhodopsin by solid-state NMR, J. Am. Chem. Soc. 119 (1997) 6853-6857.

[65] I. Palings, J.A. Pardoen, E.M.M. VanDenBerg, C. Winkel, J. Lugtenburg, R.A. Mathies, Assignment of fingerprint vibrations in the resonance Raman spectra of rhodopsin, isorhodopsin, and bathorhodopsin: Implications for chromophore structure and environment, Biochemistry 26 (1987) 2544-2556.

[66] Q. Wang, G.G. Kochendoerfer, R.W. Schoenlein, P.J.E. Verdegem, J. Lugtenburg, R.A. Mathies, C.V. Shank, Femtosecond spectroscopy of a 13-demethylrhodopsin visual pigment analogue: The role of non-bonded interactions in the isomerization process, J. Phys. Chem. B 100 (1996) 17388-17394.

[67] R. Nelson, J.K. Deriel, A. Kropf, 13-Desmethyl rhodopsin and 13-desmethyl isorhodopsin - Visual pigment analogues, Proc. Natl. Acad. Sci. U. S. A. 66 (1970) 531-538.

[68] U.M. Ganter, E.D. Schmid, D. Perez-Sala, R.R. Rando, F. Siebert, Removal of the 9-methyl group of retinal inhibits signal transduction in the visual process. A Fourier-transform infrared and biochemical investigation, Biochemistry 28 (1989) 5954-5962.

[69] U.M. Ganter, W. Gärtner, F. Siebert, The influence of the 13-methyl group of the retinal on the photoreaction of rhodopsin revealed by FTIR difference spectroscopy, Eur. Biophys. J. 18 (1990) 295-299.

[70] H. Nakamichi, V. Buss, T. Okada, Photoisomerization mechanism of rhodopsin and 9-*cis*-rhodopsin revealed by X-ray crystallography, Biophys. J. 92 (2007) L106-L108.

[71] A.F.L. Creemers, P.H.M. Bovee-Geurts, W.J. DeGrip, J. Lugtenburg, H.J.M. de Groot, Solid-state NMR analysis of ligand-receptor interactions reveals an induced misfit in the binding site of isorhodopsin, Biochemistry 43 (2004) 16011-16018.

[72] N.B. Colthup, L.H. Daly, S.E. Wiberly, Introduction to Infrared and Raman Spectroscopy, Academic Press, New York, U.S.A., 1975.

[73] R.A. Mathies, S.O. Smith, I. Palings, Determination of Retinal Chromophore Structure in Rhodopsins, in: T.G. Spiro (Ed.), Resonance Raman Spectra of Polyenes and Aromatics, vol. Vol. 2, Biological Applications of Raman spectroscopy, John Wiley & Sons, New York, 1987, pp. 59-108.

[74] P.H.M. Bovee-Geurts, I. Fernández Fernández, R.S.H. Liu, R.A. Mathies, J. Lugtenburg, W.J. DeGrip, Fluoro derivatives of retinal illuminate the decisive role of the C_{12}-H element in photoisomerization and rhodopsin activation, J. Am. Chem. Soc. 131 (2009) 17933-17942.

[75] A. Kropf, New visual pigment analogs, Abstr. Annu. Meet. Biophys. Soc. Jap. (1975) 281.

[76] J.W. Lewis, I. Pinkas, M. Sheves, M. Ottolenghi, D.S. Kliger, Structural changes in early photolysis intermediates of rhodopsin from time-resolved spectral measurements of artificial pigments sterically hindered along the chromophore chain, J. Am. Chem. Soc. 117 (1995) 918-923.

[77] R.A. Mathies, C. Schnedermann, M. Liebel, K.M. Spillane, P. Kukura, I. Fernández Fernández, J. Lugtenburg, Hydrogen isotope effect in the primary event in vision: Does vibrational phase play a key role in photoisomerization?, Pacifichem Symp. : Chem. Applic. Ret. Prot. 395 (2015) 71.

[78] D.P. Hoffman, R.A. Mathies, Femtosecond stimulated Raman exposes the role of vibrational coherence in condensed-phase photoreactivity, Acc. Chem. Res. 49 (2016) 616-625.

LEGENDS

Table 1: Selected properties of the pigments discussed in this paper. Except for several FTIR spectra and the HOOP assignments, the native data are averaged from [6,12,23,48,53], the 7,8-dihydro data are taken from [48], the 9-cyclopropyl data from [49], and the alpha-data from [50].

Figure 1: The chemical structures of 9-*cis* and 11-*cis* retinal and their derivatives discussed in this paper. Cyclo stands for 9-cyclopropyl. The numbering of here relevant carbon atoms is indicated in the native retinals. The full numbering is shown in figure S1. The structures are presented in the 12-s-*trans* conformation, which is the conformation induced by the opsin binding site.

Figure 2: Representative FTIR difference spectra taken at 80 K of the pigments, discussed in this paper, in the spectral range relevant for HOOP vibrations (800-1000 cm^{-1}). Iso-Rho, Rho and cyclo stand for isorhodopsin, rhodopsin and 9-cyclopropyl, respectively. Positive peaks derive from the photoproduct and usually show the presence of individual HOOPs, due to strong skeletal deformation. Negative peaks derive from the ground state pigments. The frequency of selected HOOPs is indicated in wavenumbers (cm^{-1}). The plethora of peaks in the 800-900 cm^{-1} range for the 7,8-dehydro- and 9-cyclopropyl-isorhodopsins probably reflect a dynamical equilibrium at this temperature between two photoproducts differing in skeletal deformation of retinal and in the structure of water molecules in the binding site, since the noise level in the 900-1500 cm^{-1} range of these spectra is much smaller, but again somewhat larger between 1600 and 1700 cm^{-1} (Fig. S2). The spectrum of the alpha-rhodopsin is noisy over the entire 800-1800 cm^{-1} range (Fig. S3), because only small quantities of intact pigment were available (c.f. Materials and Methods). No conclusions were drawn from this spectrum, but it is included for completeness.