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Light-driven isomerization of 11-cis retinal and the mediation of color vision

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Abstract

The conversion of electromagnetic energy from photons into chemical energy and then into electrical signals that form vision is a complex process that takes place in the eyes. This event is facilitated by several chemical and biological reactions, such as the photoisomerization of a chromophore and the activation of proteins and enzymes that close nucleotide-gated ion channels. In this review, the chemical processes of the primary events in visual transduction that occur in the retina's photoreceptors are examined to better understand what happens in the human visual system, and the various factors that govern the mediation of color vision. It was found that the photoisomerization of 11-cis retinal is an ultrafast, 100% stereoselective and radiationless decay process in the femtosecond timescale via a singlet excited state and boasts a quantum yield of 67%. The protein environment created from amino acid residues and its electrostatic effects around the Schiff base plays a crucial role in the effectiveness and speed of this reaction, and in color tuning by manipulating the absorbed wavelength of cone photoreceptors by either reducing or increasing the energy gap between HOMO → LUMO in the receptors.

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1 Introduction

Light has shined on Earth since its formation nearly 4.5 billion years ago, constantly bombarding the surface with a plethora of photons every second, bringing about the necessary energy and foundation for life to evolve and flourish. Over such vast timescales, evolution has played its part and adapted to such bright conditions, eventually bringing rise to the visual system. The visual system at first, in its most primitive form, was eyespots found in simple unicellular organisms. Eyespots were only able to discern the difference between light and dark, with no object nor spatial vision. Through millions of years, the eyespot evolved into a highly complex and sensitive sensory system able to detect even a single photon, and in some species like the shrimp mantis - containing 16 different photoreceptor cells that are able to detect UV and polarized light.[1]

Most living creatures on Earth possess sight, albeit with different optical structures with varying efficiency to capture and interpret light. The retina is the highly sensitive tissue of the eye that captures light of differing quantities and frequencies and converts said energy into chemical energy and electrical signals to be further processed. In the human eye, electromagnetic radiation with a wavelength of $\sim$ 380-700 nm, known as visible light, is absorbed by millions of extremely light-sensitive cells known as photoreceptors found in the retina.

Photoreceptors consist of special light-absorbing chromophores, and G-Protein coupled receptors (GPCRs) called opsins which are bound together via a Schiff base[2] thus forming a functional visual pigment able to absorb photons. In humans, two different photoreceptors are used in vision - namely rods and cones, which get their name from their morphological appearance. Both rods and cones contain the same chromophore but are linked to different kinds of opsins. Rods and cones have adapted over millions of years, gaining the ability to capture photons with a difference in light intensity by a factor of 10^8.[3] Cones sacrifice sensitivity for high dynamic resolution and are able to mediate color vision, while rods can only view monochromatic scotopic vision but has tremendous sensitivity.

The basis for the initial steps of vision is based on photochemistry. It is one of the fastest reactions in all of nature, where the photoisomerization of the chromophore 11-cis retinal to its first intermediate state in photorhodopsin happens in approximately 200 femtoseconds.[4] The process of visual transduction is initiated when a visual pigment in the retina photoreceptor absorbs a photon. The energy provided from the photon causes the chromophore to undergo photoisomerization leading to a conformational change in the protein structure of the molecule. This conformational change brings about the closing of cyclic nucleotide–gated ion channels, leading to a subsequent hyperpolarization of the photoreceptor cell membrane. This change in cell potential ushers the formation of electrical signals that eventually are sent to the brain via the optic nerve.

The human eye is able to see millions of colors[5] and perceive and differentiate several thousand various shades of colors[6] due to having three different cone photoreceptors. Each of the three cone receptors responds to and has a unique peak absorption maxima at different wavelengths because of the type of opsin bound to the chromophore. The protein environment around the chromophore plays an integral role in the potency of this photoisomerization and in the color tuning seen in cone photoreceptors. These effects arise from the concepts of structural distortion of the molecule and electrostatic effects resulting from steric interactions and charge delocalization.

This thesis will examine the principles and mechanisms behind the molecular changes that occur from the photochemistry associated with visual perception, and take a look at how the energy from photons is utilized. The main focus will be on the primary vision process, looking at the absorption of light by a chromophore, photoexcitation, photoisomerization, chemical and electrostatic interactions from the protein environment, how it affects speed and efficacy, and the processes which lead to color vision.

2 Theory

2.1 Retinal

The functioning chromophore used in human photoreceptors is the Vitamin A derived aldehyde retinal.[7] In the retina, retinal is linked to an amino acid residue of lysine (lys296) via a Schiff base, covalently bound within the opsin. In the initial stage of vision, retinal begins in the 11-cis retinal conformation and, upon photon absorption, is photoisomerized into the all-trans retinal conformation shown in figure 1:

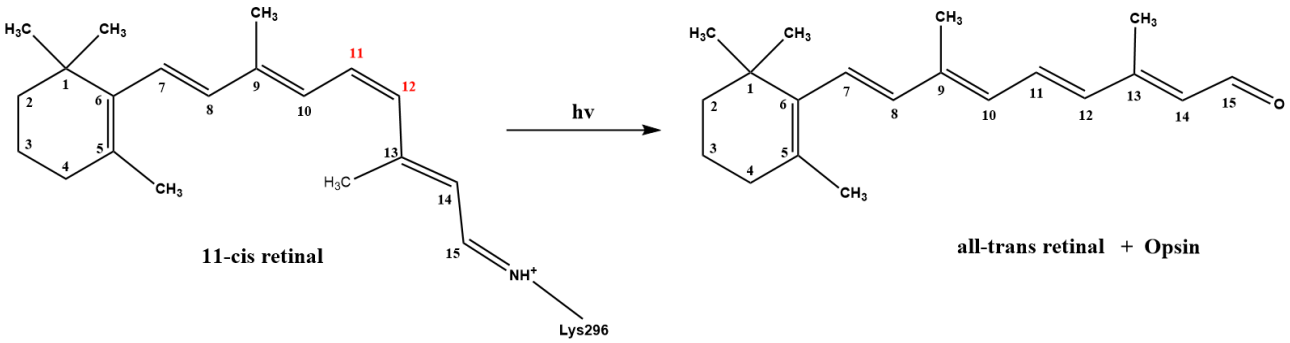

Figure 1: The photoisomerization of 11-cis retinal into all-trans retinal. The Schiff base and lys296 residue have been added for clarity

This change in conformation causes the binding site of the Schiff base to become unstable, and the bound opsin will eventually break off from the retinal molecule, leading to a cascading effect that closes cyclic nucleotide-gate ion channels due to hyperpolarization of the cell membrane.[8] This sends electrical signals to the brain that gets interpreted and turned into what we perceive as vision.

A Schiff base is an organic compound with the general structure of $R_1R_2C{=}NR_3$ which can be see in figure 2. R_1 and R_2 can be hydrogens, but R_3 is either an alkyl or aryl group. Schiff bases are the condensation product of primary amines and carbonyl compounds.[9]

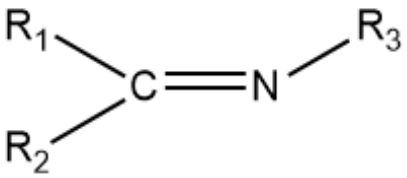

Figure 2: General structure of a Schiff base

2.1.1 Mechanism

Since retinal is a conjugated diene system, the energy gap between its highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) is relatively low, such as the average energy required to excite an electron from the C11-C12 π-bond to $\pi*$ anti-bonding orbital is around 2.3 eV.[10][11] Upon absorption of photons by 11-cis retinal, the energy stored in the light leads to photoexcitation of electrons within the π-bond from its ground state into a higher energy molecular orbital, thus breaking the double-bond and creating a temporary single-bond that is able to rotate freely around its axis.[12] Due to the temporary emergence of a single-bond from the double-bond, the electronic structure of the C11-C12 bond goes from $sp^2 \rightarrow sp^3 \rightarrow sp^2$, leading to bond elongation in the excited state.

2.2 Photoreceptors

2.2.1 Rods

Rod photoreceptors are comprised of the opsin scotopsin and, when bound together with 11-cis retinal, make up the visual pigment called rhodopsin that has a $\lambda_{max} \sim 500$ nm.[13] Rod photoreceptors are extremely sensitive to the degree they are able to react to even a single photon of visible light,[14] and rhodopsin has a quantum efficiency of up to 70%[15] which means that out of three photons absorbed by the retina, on average two will lead to photoisomerization of 11-cis retinal. This profound sensitivity and efficacy make rods the primary receptors for scotopic (nighttime) conditions. Rods are made up of only one type of cell but outnumber cones 20:1, with on average 60 million[16] rod cells in the retina. Rods are easily and quickly saturated in bright conditions, and their regenerative period can be up to 30 minutes.[17] This oversaturation of the rod receptors can be experienced when walking into a dark room after being outside in sunlight, where one is seemingly "blind" for a certain amount of time until sufficient adaptation occurs.

2.2.2 Cones

In the retina, there are roughly 3 million cone photoreceptors.[18] Cones are responsible for vision in photopic (daytime) conditions and exhibit excellent light adaptation, which means they are able to function in various levels of brightness without being oversaturated and rendered ineffective and possess rapid regeneration of 11-cis retinal compared to rod photoreceptors.[19] Cones even increase the speed of processing with increasing brightness.[20] Cone receptors are not sensitive enough to work during low-light conditions, which is why humans do not see color at night or in near darkness. Cone photoreceptors in the human retina are made up of three different visual opsins with different, albeit overlapping absorption spectra. These three opsins are Long-Wavelength sensitive opsin with a λ_{max} 580 nm[21] (Red opsin), Middle-wavelength sensitive opsin λ_{max} 540 nm[22] (Green opsin), and Short-wavelength sensitive opsin λ_{max} 445 nm (Blue opsin)[23] and together makes humans possess trichromacy.[24]

2.3 Color tuning

The process of color tuning is derived from the protein environment around the visual pigments and their interactions with 11-cis retinal and the protonated Schiff base (PSB). There is an intrinsic property of inverse proportionality in photons, meaning that the lower the wavelength that a photon has, the more energy it contains. This means that the absorbed wavelength is inversely proportional to the excitation energy of an electron contained in the ground state of a chromophore. By changing the energy gap between the ground state and excited state, one essentially changes the wavelength at which the photoreceptors absorb most efficiently at.[25]

By reducing the energy gap between the ground state and excited state, one will experience a redshift and an increase in the absorbed wavelength, while increasing the gap will lead to a blueshift and lower wavelength absorption. In the retina, the 11-cis retinal chromophore is protonated and further stabilized by a negatively charged counter-ion derived from amino acid residues that are located around the Schiff base, seen in figure 3.

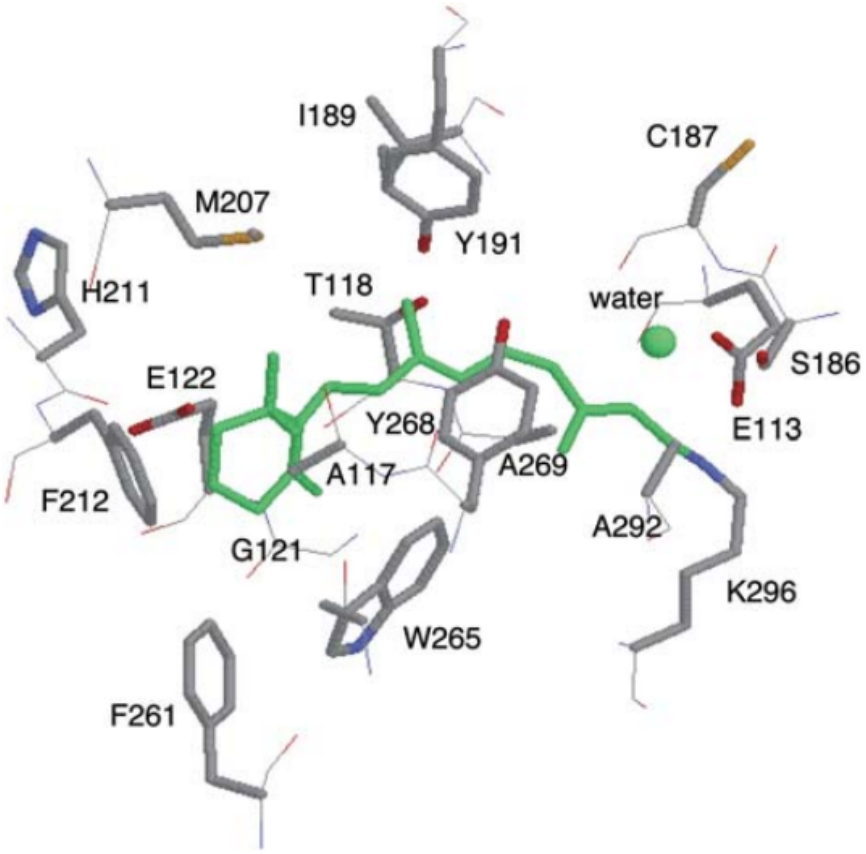

Figure 3: The various amino acid residues found within 4 Å of retinal (Green structure). K296 is the Lysine (Lys296) residue that forms the Schiff base covalent bond with 11-cis retinal, E113 is the Glutamate (Glu113) residue that acts as the counter-ion to the protonated Schiff base and C187 is Cysteine (Cys187)[26]

The distance between the two ion pairs (protonated Schiff base & amino acid residues) decides the energy gap between the ground state and excited state of 11-cis retinal.[27] A protonated 11-cis retinal Schiff base not bound to an opsin absorbs photons in the UV region (λ_{max} 300-370 nm),[28] whereas in the PSB version of 11-cis retinal bound to an opsin forming a protein complex absorbs light in the region of (λ_{max} 380-700 nm)[28] as mentioned in 2.2.2. When a photon is captured by 11-cis retinal, the Schiff base positive charge gets more delocalized

throughout the molecule during the excited state, and this migration leads to either a higher or lower energy gap between the ground state and excited state depending on where the counter-ion is located.[29] As seen by figure 4, the location of the counter-ion pre-excitation decides whether the energy gap of HOMO - LUMO will decrease or increase.

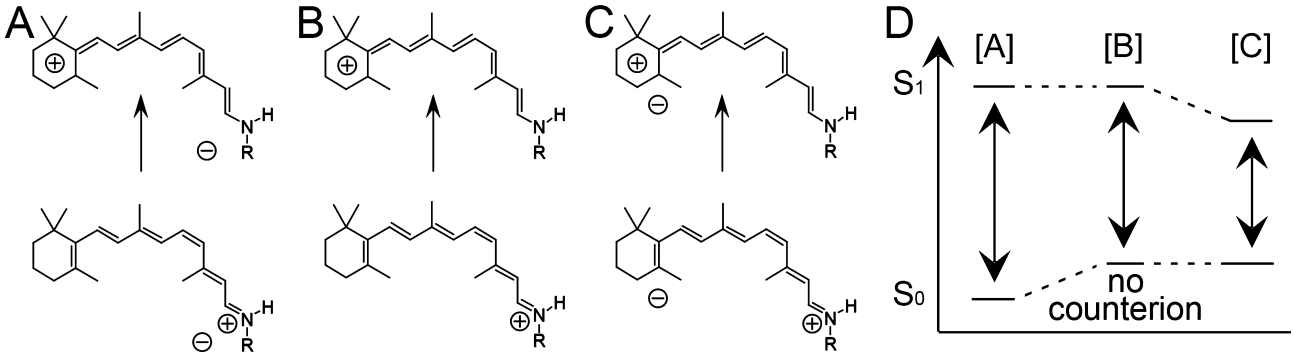

Figure 4: Protonation counter-ion interaction before and after photoexcitation which results in color tuning. Excitation forces the movement of the positive charge located at the Schiff Base towards the β-ionone ring. Figure A) Positive charge migrates away from stabilizing counter-ion, causing an increase in the energy gap between HOMO/LUMO. Figure B) No counter-ion present. Figure C) Counter-ion is located near the β-ionone ring. Excitation forces a positive charge towards a negative counter-ion, stabilizing the molecule and decreasing the energy gap. Figure D) Gaps in energy level pre and post-excitation depending on where counter-ion is located[30]

3 Discussion

3.1 Excited state properties and initial photoprocess

The excited state and its dynamics play a vital role in the efficiency, quantum yield, and the incredible speed of photoisomerization of retinal. A valid question is which excited state, S_1 or T_1 is seen in retinal. Comparative studies[31] on the excited states and isomerization properties of both S_1 and T_1 of unprotonated Schiff base(uPSB) and PSB have been done in alcohol solution. In the T_1 excited state, the quantum yield of intersystem crossing of all-trans retinal not bound to a Schiff base, uPSB all-trans and PSB all-trans retinal was found to be 0.50-0.61, 0.008, and <0.001[32] respectively. Extrapolated from these results, which showed that PSB all-trans retinal had the lowest intersystem crossing quantum yield of the three, it follows that it is highly improbable that the photoisomerization occurs through the T_1 excited state for 11-cis retinal in a natural protein environment.

It is also highly dubious that the T_1 state is preferred taking into account that the first intermediary (photorhodopsin) product during the isomerization is formed after only 200 femtoseconds[33] and that the whole isomerization event takes place in less than eight picoseconds.[34] Just the intersystem crossing between $S_1 \rightarrow T_1$ occurs in the picosecond time frame,[35] meaning that it is slower by a factor of 1000. Another study[36] compared the quantum yields from the isomerization of various retinal PSB isomers in alcohol, including all-trans retinal and 11-cis retinal through both triplet sensitized excitation and by direct singlet excitation. The results of this study produced evidence that $S_0 \rightarrow S_1$ had the highest quantum yield with 0.22 ± 0.05, compared to the T_1 quantum yield of <0.001 as previously mentioned. These studies provide sufficient evidence that the S_1 state is the primary state of excited retinal, albeit there have been some suggestions[37] of triplet-excited "regions" in retinal molecule, but that is beyond

the scope of this review. Experimental studies[38] in solute provided important insight into the dynamics of the de-excitation process. The de-excitation from the S_1 excited state to S_0 ground state happens through radiationless decay that further enhances the quantum yield due to energy conservation. Without a protein environment to essentially "slow down" the retinal molecule on its S_1 potential energy surface, the free-flowing path in the solute paves the way for unrestricted movement. Although this sounds like a more favorable and faster reaction path, the unrestricted motion leads to more frequent collisions with molecules present in the solute. These collisions drain the retinal molecule of energy gained from the absorbed photon, energy that would otherwise be used to drive the isomerization.

The primary reaction in photoisomerization creates the intermediate states shown in figure 5

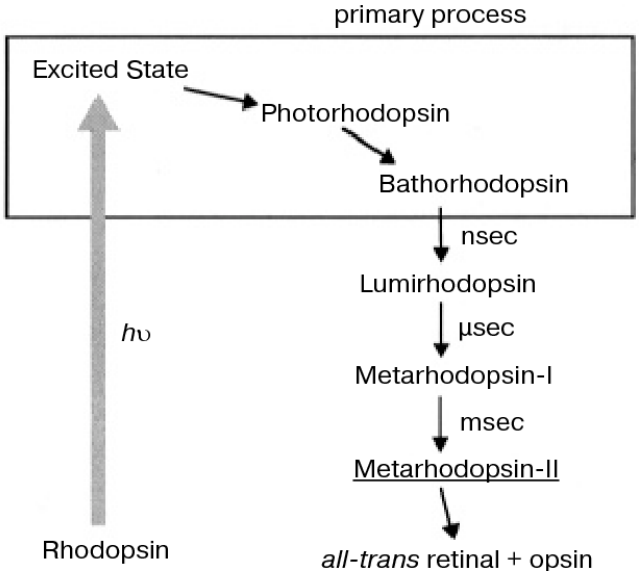

Figure 5: Primary process of photoisomerization of rhodopsin and its intermediate states.[39]

The first photoproducts created in the rhodopsin photocycle, known as the bleaching process, are the intermediates photorhodopsin and bathorhodopsin. Within 200 femtoseconds of photon absorption, photorhodopsin is formed and found to be in a short-lived, highly prominent twisted all-trans conformation. Photorhodopsin is a highly unstable intermediary and can not be stabilized at low temperatures of 120 - 130 K or during its excited state. Absorption spectroscopy shows that photorhodopsin possesses a lower oscillating strength compared to rhodopsin and bathorhodopsin. The lower oscillating strength in photorhodopsin comes from its distorted structure, where the distortion interrupts the conjugation of the double bonds.[40] Photorhodopsin quickly turns into bathorhodopsin via thermal decay and conformational relaxation, and this event happens in less than five picoseconds[41] which makes for difficulties in elucidating photorhodopsins structure.

Even though bathorhodopsin also resides in a highly strained all-trans conformation, there are suggestions that it remains unaltered for as long as 100 ns upon formation from photorhodopsin.[41] On account of the thermal decay and relaxation in photorhodopsin $\rightarrow$ bathorhodopsin, there is evidence pointing towards that bathorhodopsin is the first stable intermediate in the photoprocess, where the actual photon energy (150 kj/mol / $\sim$ 1.5 eV) is stored[42] because of the torsional strain and based on the fact that the conditions around the Schiff base remain unchanged during the rhodopsin $\rightarrow$ bathorhodopsin step.[43] Upon further relaxation of the highly

twisted polyene chain, bathorhodopsin turns into the third intermediary lumirhodopsin. This change accompanies a significant energy transfer from the polyene chain to the opsin moiety as the β-ionone ring experiences relocation due to elongation from the relaxation.

The subsequent decaying of lumirhodopsin $\rightarrow$ metarhodopsin I is linked to the deprotonation of the Schiff base due to a switch of the amino acid residue from Glu113 to Glu181.[44][45] This switch directs the transition from metarhodopsin I $\rightarrow$ metarhodopsin II which finally brings rhodopsin into its active state[46] where phototransduction can begin. Metarhodopsin II interacts and binds to the heterotrimeric G-protein transducin, activating a signal cascade that promotes and catalyses the hydrolysis of 3',5'-cyclic guanosine monophosphate[47] that closes cyclic nucleotide-gated channels and halts the flow of Na^+ and K^+-ions which result in hyperpolarization of the cell membrane of the photoreceptors, leading to the formation of an electrical signal.[48]

It was previously thought that when the measurements of the photoreactions in question went from the nano and pico- time scales to femtoseconds, the full cis-trans isomerization of retinal happened in the 200 fs frame. If that were indeed the case, atoms associated with the movement of the isomerization process in the retinal molecule would have to move a great distance in a very short time. Such cis-trans isomerization dealing with one double bond (C11=C12) seems rather improbable because of atom inertia and from the protein environment physically blocking large movements.[33] Indeed, it was seen in a study[31] that also the C9=C10 and C13=C14 double bonds had a lower barrier for twisting in the S_1 excited state, which would explain how the retinal molecule could stay in the protein pocket of the opsin and also perform a 90° twisting motion of the C11=C12 double bond. The accompanying twisting by C9=C10 and C13=C14 reduces the total geometrical change of the retinal molecule during its isomerization.[49]

3.2 Photoisomerization of 11-cis retinal

In nature, GPCR pigments like rhodopsin and the likes are found to be in an equilibrium state between inactive and active. Usually, in these opsins, thermal vibrations are enough to trigger a shift and transition in the binding of the ligands that cause a change in the equilibrium. Rhodopsin remains a special case as it is covalently bound to 11-cis retinal, which acts as an inverse agonist that shifts the equilibrium almost irreversibly to the inactive state. With this, the only way to turn rhodopsin from its inactive state to its active state is by photoisomerization of 11-cis retinal. As a result of this highly shifted equilibrium, thermal activation of a single rhodopsin cell only happens on average every 420 years.[50]

The incredible sensitivity of rhodopsin, which is found in rod photoreceptors with its ability to capture a single photon, gives rise to its quantum yield of 0.67.[51] The forces behind this efficient system of converting photons into electrical signals come from the extremely fast, 100% stereospecific[51] photoisomerization of 11-cis retinal into all-trans retinal, a process that happens over the time-span of mere femtoseconds,[39] and because of a seemingly barrierless excited state surface[52] due to retinal having a fluorescence quantum yield of $\Phi \sim 10^{-5}$.[53] An important factor that produces the ultrafast reaction and high quantum yield stems from the excited retinal intermediate being bound in the active site at the opsin by covalent bonds between retinal and lys296, hydrogen bonding of nitrogen at the Schiff base, and hydrophobic interactions between the β-ionone ring and hydrophobic regions of the opsin. These three chemical interactions effectively reduce retinals' freedom of movement. This anchoring stores

more than half of the energy from the photon into the first stable intermediate photoproduct bathorhodopsin[54] and is further used to expedite the conformational change in the retinal molecule.

It was found that the transition state of the isomerization of PSB retinal from cis-to-trans with respect to the dihedral angle of the C11=C12-bond happens at 90°, leading to the lowest transition state energy difference of 1.01 eV and the lowest HOMO-LUMO energy gap of 2.04 eV.[55] Upon photon absorption leading to the $S_0 \rightarrow S_1$ excitation, deprotonation occurs at the Schiff base, where the cation migrates away from its initial position near the nitrogen atom and moves towards the conjugated polyene chain and the β-ionone ring.[56] This deprotonation leads to the retinal molecule taking on a charge state of zero which reduces its energy barrier because of charge delocalization, meaning that the full isomerization and active state of metarhodopsin II only occurs after the deprotonation of the PSB retinal molecule.

The quantum yield of modified rhodopsins was shown to be 0.55 for 10-methyl, 0.47 (400 fs formation time) for 13-dimethyl,[57] 0.33 for 10-methyl-13-dimethyl and 0.22 (600 fs) for isorhodopsin[58] From figure 5 there seems to be a linear relationship between the structure of the ground state rhodopsins and the observed quantum yield and speed of isomerization.

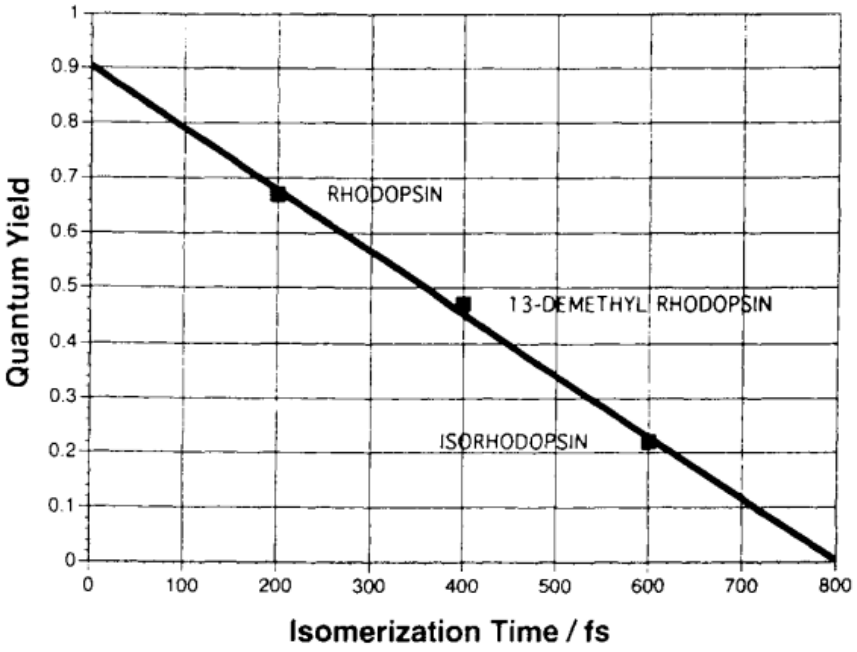

Figure 6: Isomerization time and quantum yield of native rhodopsin and some analogues. A clear linear relationship can be seen regarding quantum yield and the speed of the isomerization[59]

The only thing differentiating these rhodopsin analogues are double bond locations and methyl-group substitutions and their interactions with the protein environment, both through steric and electronic interactions. The unique thing about rhodopsin in a protein environment is that only one chiral conformation is chosen. This conformation selects the C11=C12 double bond for isomerization and which direction the $C_{12}H$ turns during the isomerization,[59] being clockwise or counterclockwise. Seeing as this isomerization happens in a dynamic protein environment, the two rotations are not equivalent, and one direction will be highly favored over the other. This was seen in a study,[60] where it was proposed that there exist two paths for excited

rhodopsin to take. These two paths stem from the location of cys187 (see figure 3) with respect to the hydrogen at C12, where the estimated distance between them is 2.23 Å.[61] When the 11-cis retinal absorbs a photon, it lengthens, and there is atom displacement in the polyene chain, which is specifically directed towards the C11=C12 bend because of the anchoring at each end of the molecule. This targeted displacement sends the H-12 atom on a collision course with cys187. Because cys187 and H-12 cannot be at the same place simultaneously, and cys-187 does not move during the isomerization, the only possible action is the bending of $C_{12}H$. Depending on the direction of the bend, one product will form 70% of the time leading directly to the first intermediary, photorhodopsin. The other product forms 30% of the time and only leads to excited state rhodopsin, which relaxes back to ground state rhodopsin without any transformation occurring.[62]

The observed difference in quantum yield indicates that the protein environment massively enhances the photoisomerization of 11-cis retinal. The amino acid residue of lysine (Lys296) that is bound to retinal, glutamate (Glu113) which acts as the counter-ion that stabilizes the protonation at the Schiff base, and cysteine (Cys187) which acts as a "wall" against the hydrogen atom at C12[63] are the main factors of this.

3.3 Color vision & tuning

A special property of visual pigments is called the principle of univariance. Univariance is why rod photoreceptors, which are only made up of one type of visual opsin, only transmit monochromatic vision in scotopic conditions. Univariance means that the visual pigments in photoreceptors in both rods and cones can not and do not differentiate two photons based on their waveform. This means properties of frequency or polarization; the nature of its wavelength and the subsequent color is not translated during absorption, meaning that a photon with the wavelength of about 400 nm and a photon with the wavelength of 700 nm will affect the visual pigments in the same way.[64] The only thing a photoreceptor will actually "measure" is the number of times a photon photoisomerizes a visual pigment, such as that an increase in photon count for a specific receptor (see 2.2.2) can stem from an increase in intensity, the number of photons that hit, wavelength or both. Because cone photoreceptors consist of three receptors, they mediate color by comparing their response to light stimuli, i.e., how many times the absorbed photons activate Red opsin, Green opsin, or Blue opsin pigments.

Because color vision in humans arises from the unique absorption wavelengths of the Red opsin, Green opsin, and Blue opsin cone visual pigments, there must be a mechanism that controls the absorption maxima of the cone pigments. The three cone photoreceptors in the retina, as previously mentioned, absorb light most effectively with respect to each other at different wavelengths and thus levels of energy (Red opsin 2.20 eV,[65] Green opsin 2.33 eV,[65] and Blue opsin 2.99 eV[66]).

The governing factors that affect the spectral sensitivity of the cone receptors are controlled by primarily three different contributions ordered according to influence:[67] 1) Electrostatic interactions from the protein environment. 2) Structural distortion in the retinal molecule. 3) Quantum effects from the stabilizing counter-ion due to polarization and charge transfer. The electrostatic interaction has the most significant contribution to the absorption energy (0.33 eV redshift in Red opsin & 0.32 eV blueshift in Blue opsin, and negligible in Green opsin)[68] of the three photoreceptors. In contrast, the structural distortion only affects the Blue opsin (0.24

eV blueshift)[68] cone in any meaningful way. The quantum effect barely has any measurable effect at all (< 0.06 eV in any of the three cones).

Regarding the electrostatic effects, the interplay between retinal and charged and or polar amino acid residues modulates the energy levels of the chromophore, as do hydrogen bonds that lead to charge transfer effects. The nature of the first excited state also needs to be considered. The electron transfer from π to $\pi*$ is a single-electron intramolecular transfer[69] where the electron localized at the β-ionone ring (HOMO) migrates to the nitrogen-region of the Schiff base (LUMO). Another factor to consider regarding electrostatic effects is the electrostatic potential caused by the opsin bound to the retinal molecule. Referring to figure 5, Scenario C would lead to a lower energy gap and a redshift towards wavelengths > 600 nm. However, rhodopsin in rod cells absorbs wavelengths at max ≤ 600 nm,[70] meaning that Scenario C is less likely to occur in nature. Scenario A would lead to an increase in energy and an accompanying blueshift, and this state, with a negative counter-ion located at the Schiff base, is observed in crystal structures of rhodopsin.[30]

When the positive charge migrates from the protonated Schiff base to the β-ionone ring, there is a significant change in the dipole moment of the ground state compared to the excited state of retinal. Suppose there is a more negative polarity at the Schiff base. In that case, the positive charge migration is suppressed due to charge delocalization effects because it is more energetically favored to stabilize the Schiff base.[27] The electrostatic potential of retinal is highly negative towards the right (PSB side), which causes the gap between HOMO-LUMO to increase.[71] The big negative region at the Schiff base interacts with the molecular orbitals of retinal in a way that destabilizes the LUMO and its energy level.

Both the HOMO and LUMO are affected and destabilized by the negative electrostatic potential, but the LUMO is more profoundly influenced. The mechanism behind the difference in absorption wavelengths of the three cone receptors is explained by this factor. The extent to which the different pigments' LUMO are destabilized gives rise to the reported LUMO $\rightarrow$ HOMO energy gaps and absorbed wavelength. Figure 7 shows the three different cone pigments and the grade of destabilization of the LUMO:

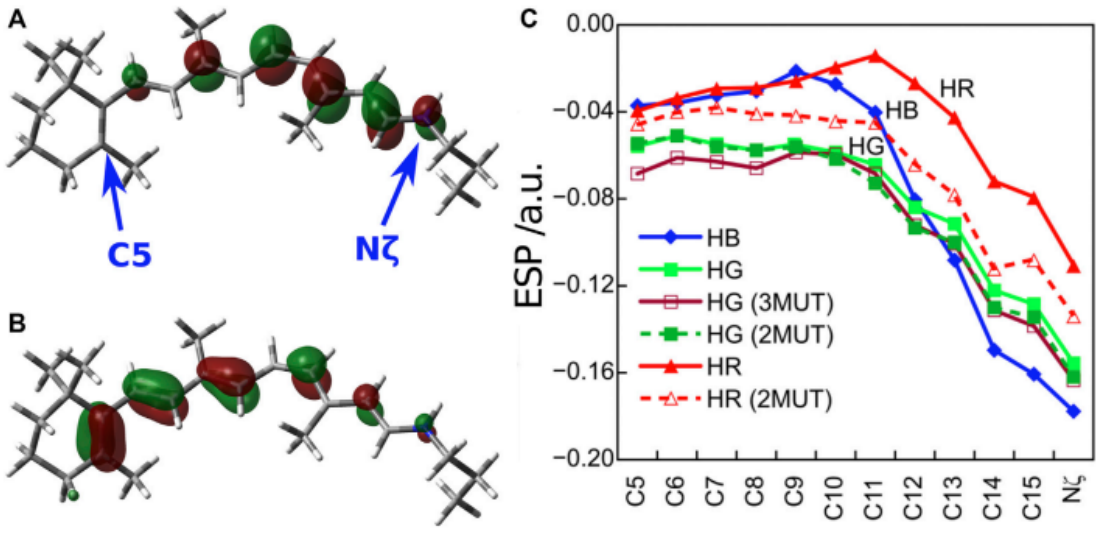

Figure 7: Figure A) Retinal LUMO arrangements. Figure B) Retinal HOMO distribution. Figure C) Electrostatic potential of the three cone pigments' retinal π-chain and their degree of destabilization.[72]

As seen by figure 7, the degree to which the three cone pigments are affected by the electrostatic potential differs. Blue opsin experiences the biggest LUMO destabilization, followed by Human green and Human red.

The structural distortion effects originate from retinal molecule contorting itself to fit in the binding pocket of the relevant opsin.[73] This contorting can lead to either a twist in the polyene chain of retinal such that the conjugation of the π-orbitals is disrupted. This twist leads to a change in the absorption energy in the form of a blueshift of the visual pigment. The structural distortion can also lead to redshift and a lower energy gap. A change in the planarity of 11-cis retinal via steric interactions with the bound opsin leads to a higher degree of conjugation in the molecule and more π-delocalization.[74] For rhodopsin, the C6-C7 σ-bond is found in a 6-s-cis conformation[75] forcing the conjugated polyene chain and the β-ionone ring to become non-planar with respect to each other because of steric hindrance originating from the methyl-group at C5 and the hydrogen at C8, (see figure 1). Because of this interaction, the β-ionone ring is not subjected to the full stabilizing force of the π-electrons.[76] Based on the effects of these stabilizing or destabilizing effects, it can be determined that Blue opsin sees the greatest change in energy gap between its LUMO-HOMO pair compared to the other two pigments and that the electrostatic interactions from the protein environment and structural distortion explain how the three pigments absorb light of different wavelengths and energy.

4 Conclusion

This paper set out to investigate the photochemistry associated with light-absorption in photoreceptors that form the initial process of visual phototransduction and the molecular mechanisms for color vision. This review shows that at the very core of this process is the absorption of photon energy and ensuing photoisomerization of the chromophore 11-cis retinal into all-trans retinal. Energy from the absorbed photons is used to photoexcite an electron from the conjugated π-bond between C11 and C12 into a S_1 singlet excited state. This process is an ultrafast and vibrationally coherent systematic event that occurs in a matter of femtoseconds, making it one of the fastest reactions in nature.

During the isomerization process from 11-cis retinal to all-trans retinal, the molecule forms several intermediaries that initially store almost 60% of the absorbed energy in a twisted polyene chain due to the initial 90° angle between C11 and C12. Via relaxation and thermal decay, the chromophore eventually straightens out into a linear molecule that enables GPCRs to close cyclic nucleotide-gated channels that control the flow of Na^+ and K^+ into the photoreceptor cells. This closure leads to hyperpolarization and subsequent electrical signals that travel to the visual cortex.

High quantum yield and the incredible speed of isomerization are achieved through a barrierless excited state surface, radiationless decay, and a rigid chromophore structure. Even though the C11 - C12 bond is initially at a 90° angle, nearby single and double bonds also contort themselves to alleviate some of the distortions; thus, both movement and steric interactions are reduced.

The protein environment from nearby amino acid residues in and around the retinal molecule plays a crucial part in the speed and quantum yield of the isomerization and the tuning and conveying of color. The protonation and deprotonation of the Schiff base govern the energy

gap between HOMO/LUMO for the three cone photoreceptors due to stabilizing effects from charge delocalization, electrostatic effects like dipole moments, and structural distortion, which affects planarity. Depending on these effects, there will be a shift in the absorbed wavelength to either lower or higher energy, resulting in a redshift or a blueshift, respectively.

Interesting research connected to these findings would be to investigate amino acid and or methyl substitutions to induce stronger electrostatic and steric interactions to 1) Possibly store more energy in the twisted polyene chain. 2) Further enhance the quantum yield.

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