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Grip, W.J. de; Ganapathy, S.

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Rhodopsins: An Excitingly Versatile Protein Species for Research, Development and Creative Engineering

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*Correspondence:

Willem J. de Grip
w.j.de.grip@lic.leidenuniv.nl
Srividya Ganapathy
srganapathy@health.ucsd.edu

†Present address:

Srividya Ganapathy,
Department of Pediatrics and Cellular
and Molecular Medicine, UCSD School
of Medicine, San Diego, CA, United
States

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Willem J. de Grip^{1,2*} and Srividya Ganapathy^{3,†}

¹Leiden Institute of Chemistry, Department of Biophysical Organic Chemistry, Leiden University, Leiden, Netherlands, ²Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, Netherlands, ³Department of Imaging Physics, Delft University of Technology, Netherlands

The first member and eponym of the rhodopsin family was identified in the 1930s as the visual pigment of the rod photoreceptor cell in the animal retina. It was found to be a membrane protein, owing its photosensitivity to the presence of a covalently bound chromophoric group. This group, derived from vitamin A, was appropriately dubbed retinal. In the 1970s a microbial counterpart of this species was discovered in an archaeon, being a membrane protein also harbouring retinal as a chromophore, and named bacteriorhodopsin. Since their discovery a photogenic panorama unfolded, where up to date new members and subspecies with a variety of light-driven functionality have been added to this family. The animal branch, meanwhile categorized as type-2 rhodopsins, turned out to form a large subclass in the superfamily of G protein-coupled receptors and are essential to multiple elements of light-dependent animal sensory physiology. The microbial branch, the type-1 rhodopsins, largely function as light-driven ion pumps or channels, but also contain sensory-active and enzyme-sustaining subspecies. In this review we will follow the development of this exciting membrane protein panorama in a representative number of highlights and will present a prospect of their extraordinary future potential.

Keywords: membrane protein, photoreceptor, retinal protein, visual pigments, optogenetics, ion pumps, microbial, eukaryotic

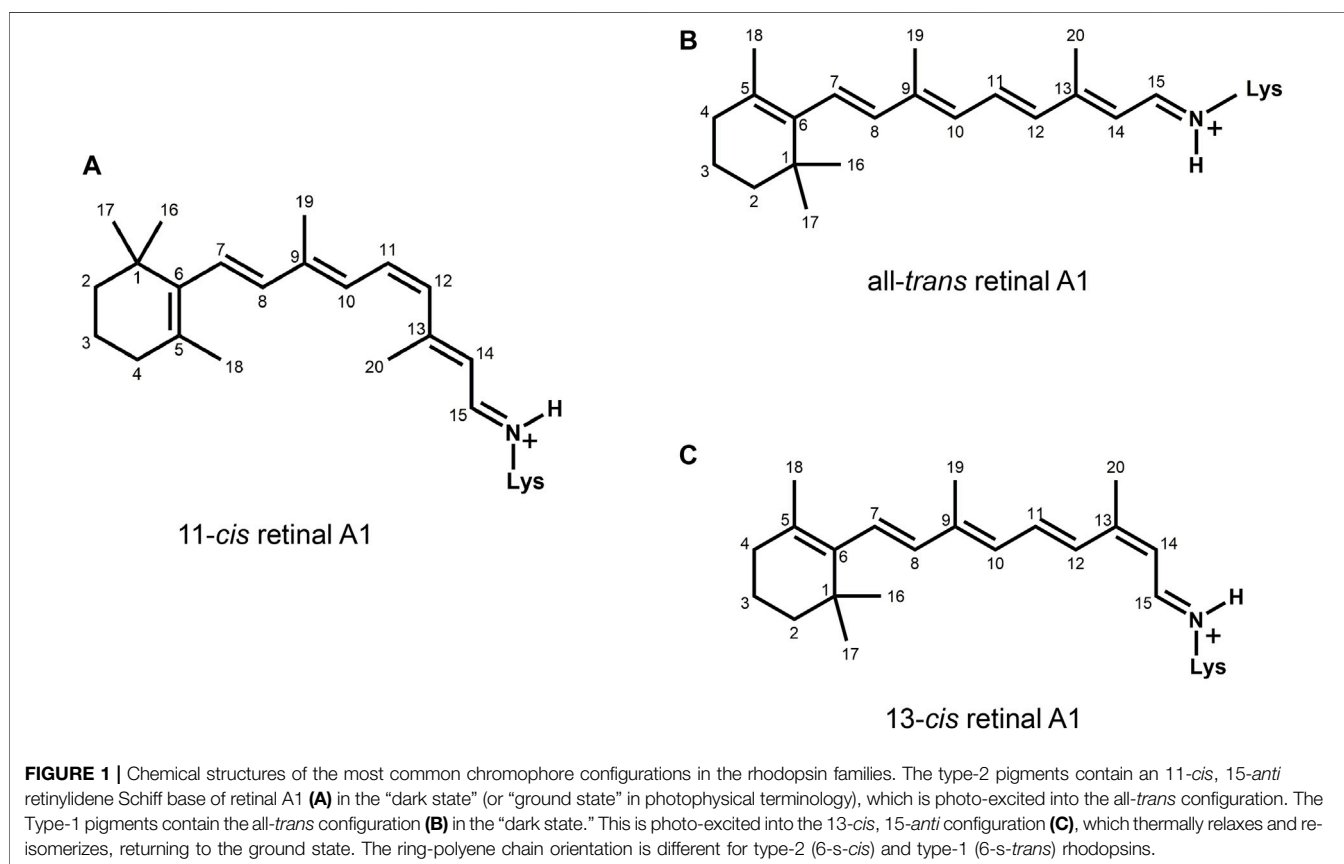
Abbreviations: AFM, Atomic force microscopy; AR3, Archaerhodopsin-3; BR, Bacteriorhodopsin; C1C2, chimera between channelrhodopsin-1 and -2; Cryo-EM, Cryo-electron microscopy; CTAB, Cetyltrimethylammonium bromide; DDM, Dodecylmaltoside; DFT, Density functional theory; DPC, Dodecylphosphocholine; EPR, Electronparamagnetic resonance; FTIR, Fourier-transform infra-red; GR, *Gloeobacter violaceus* rhodopsin; LDAO, Lauryldimethylaminoxide; MSP, Membrane scaffold protein; NG, Nonylglucoside; OG, octylglucoside; PAR, Photosynthetically active region; PM, plasma membrane; RGR, RPE-retinal G protein-coupled receptor; RPE, Retinal Pigment Epithelium; SMA, Styrene-maleic-acid-copolymer; TR, Thermophilic rhodopsin; TR-WAXS, Time-resolved wide-angle X-ray scattering; XFEL, X-ray free electron laser.

INTRODUCTION

The first member and eponym of the rhodopsin family was identified in the 1930s as the visual pigment of the rod photoreceptor cell in the animal retina (Tansley, 1931; Wald, 1935). It turned out to be a membrane protein, owing its photosensitivity to the presence of a covalently bound chromophoric group. This photosensitive group, derived from vitamin A, was appropriately coined retinene, later officially renamed as retinal (Wald, 1935; Morton and Goodwin, 1944; Morton and Pitt, 1957). The visual pigments harboured a special conformer of this polyene compound, in casu the 11-*cis* configuration (Hubbard and Wald, 1952; Hubbard et al., 1971) (**Figure 1**). Upon photo-activation the chromophore was converted into the all-*trans* configuration, which triggered a sequel of conformational changes in the protein, leading to its active state (Wald, 1953; Morton and Pitt, 1957; Dartnall, 1962a). Eventually the chromophore was released as all-*trans* retinal (Dartnall, 1962c; Wald, 1968; Hubbard et al., 1971; Bridges, 1972). Surprisingly, in the 1970s a microbial counterpart of this protein was discovered in the archaeon *Halobacterium salinarum* (at the time referred to as *Halobacterium halobium*), which also harboured retinal as a chromophore, and was named bacteriorhodopsin (Oesterhelt and Stoeckenius, 1971). This membrane protein, however, contained the all-*trans* configuration, which upon photo-activation was converted into the 13-*cis* configuration (Smith

et al., 1985; Oesterhelt, 1998). The resulting active state of the protein in this case thermally decayed in a sequel of steps whereby the chromophore eventually was thermally re-isomerized into the all-*trans* configuration returning to the original starting state (Oesterhelt, 1998; Lanyi, 2004).

Since their discovery a photogenic panorama unfolded, where up to date new members and subspecies with a variety of light-driven functionality have been added to these families. The animal branch, categorized as type-2 rhodopsins, turned out to form part of the major subclass in the superfamily of G protein-coupled receptors (Bennett et al., 1982; Kühn, 1984; Crescitelli, 1991; Hargrave and McDowell, 1992). Currently they have diversified into at least eleven groups (Opn1–Opn9, R-group, Cn-group) most of which are essential to multiple elements of light-dependent animal sensory physiology. Depending on the animal species, they can be located in multiple tissues next to the eye (Terakita, 2005; Davies et al., 2015). Meanwhile, the microbial branch was named as type-1 rhodopsins, which largely function as light-driven ion pumps or channels, but also contain sensory-active and enzyme-sustaining subspecies (Oesterhelt, 1998; Spudich et al., 2000; Ernst et al., 2014; Leung and Montell, 2017; Nagata and Inoue, 2022). The most recent addition to the microbial rhodopsins is the heliorhodopsin family, which is remarkably different from the type-1 family in their inverted orientation in the membrane, with the N-terminal now residing in the intracellular compartment (Pushkarev et al., 2018; Shihoya et al., 2019; Kovalev et al., 2020b; Rozenberg et al., 2021; Chazan



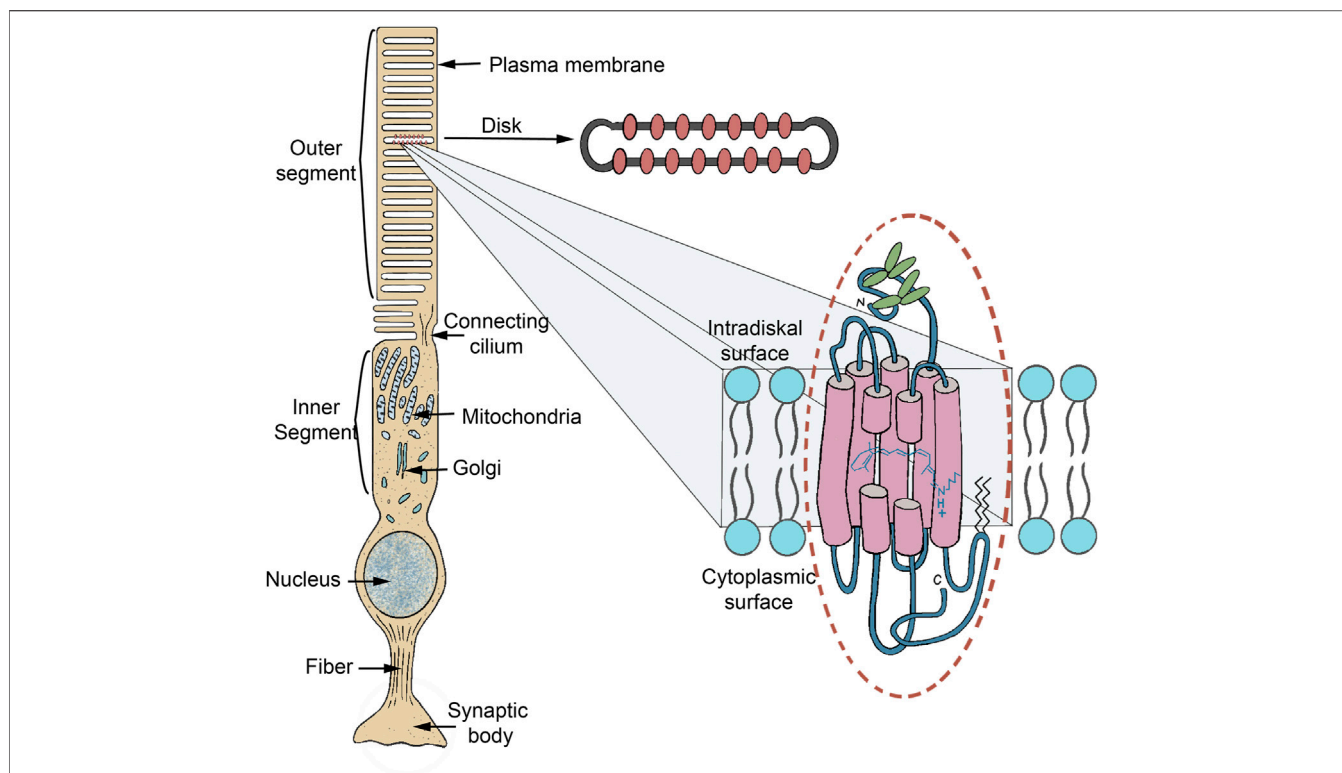


FIGURE 2 | Schematic of a vertebrate rod photoreceptor cell (scotopic vision), zooming in on the location of the rod visual pigment rhodopsin. The rod outer segment (ROS), a ciliary outgrowth, is densely filled with isolated flattened vesicles (discs) which contain rhodopsin as the major (ca 90% w/w) membrane protein. The vertebrate visual pigments are therefore also designated as “ciliary rhodopsins.” Other disc membrane proteins are involved in signal propagation, stabilization of the disc shape and communication with the plasma membrane (PM). The phospholipids in the disc membrane have an exceptionally high content (ca 40%) of highly unsaturated fatty acids (22:6 ω 3) (Daemen, 1973). The discs are continuously generated at the base of the ROS as invaginations of the PM, then are nipped off and move upwards. After 7–10 days they reach the top of the ROS, which is pinched off in a circadian rhythm and degraded in the adjacent retinal pigment epithelium (RPE) (Young, 1976). The vertebrate cone photoreceptor (photopic vision) is organized in a similar fashion, except that the “discs” remain continuous with the PM as invaginations and are not pinched off. The organization of invertebrate visual photoreceptors is roughly similar, but the photoreceptive membranes are organized as numerous microvilli in rhabdomeric structures (Warrant and McIntyre, 1993) and their rhodopsins are also designated as rhabdomeric visual pigments. Only the classical visual pigments (Opn1, Opn2 and R-gene families) are organized in these specialized cellular outgrowths. All other type-2 and all type-1 pigments are targeted to the PM or an eyespot and form only a small part (up to several percent) of that membrane protein population.

et al., 2022). The physiological function of this new family has not become very clear as of yet.

In this review we follow the historical development of this exciting membrane protein panorama in a representative number of highlights and present a prospect of their extraordinary future potential. We broadly outline their functional diversity and physiological relevance, as a comprehensive description is outside the scope of this review. A large number of excellent reviews on the rhodopsin families have been published, many of which we have referred to where appropriate, along with the most relevant early and recent papers. We refrain from presenting many molecular details, and therefore we refer to the following more recent reviews (DeGrip and Rothschild, 2000; Hofmann, 2000; Spudich et al., 2000; Hofmann et al., 2009; Yizhar et al., 2011; Palczewski and Orban, 2013; Ernst et al., 2014; Imamoto and Shichida, 2014; Inoue et al., 2014; Deisseroth, 2015; Hofmann and Palczewski, 2015; Brown and Ernst, 2017; Bando et al., 2019; El Khatib and Atamian, 2019; Dowling, 2020; Kandori, 2020; Kwon et al., 2020; Baillie et al., 2021; Moraes et al., 2021;

Rozenberg et al., 2021; Bondar, 2022; Broser, 2022; Brown, 2022; Khelashvili and Menon, 2022; Nagata and Inoue, 2022).

This review presents a historical perspective and is therefore organized according to the landmark discoveries or progress in the field. In the following sections, we first discuss milestone studies and the common elements of the type-2 and type-1 rhodopsins, followed by individual subsections presenting typical elements for the type-2 and type-1 family, respectively. For the interested reader, we have compiled additional relevant citations in tables accompanying every section.

DISCOVERY

The discovery and identification of rhodopsins was governed by their spectral properties. Since they all absorb photons in the visible spectrum, careful visual observations were the cornerstone for these early studies.

Type-2 Family

Rhodopsin, the founding father of the type-2 family was first identified as the visual pigment of the rod photoreceptor cell. In the 19th century, groundbreaking research on vision by Müller, Boll and Kühne led to the visual perception, that light capture occurred in the distal part of the human retina (**Figure 2**), in particular the outer segments of the photoreceptor cells (Müller, 1855; Boll, 1877; Ewald and Kühne, 1878). The typical red color of this tissue disappeared upon illumination, which was termed “bleaching,” and could to some extent be regenerated upon subsequent dark adaptation of the isolated eyecup. As of the 1930s it became apparent that a membrane-bound protein in the rod photoreceptor cell was responsible for the red color (Tansley, 1931; Bliss, 1948; Wald, 1953). This protein was named rhodopsin, after the ancient Greek words $\rho\omicron\delta\epsilon\omicron\sigma$ (rhodeos, rose-coloured) and $\omicron\psi\omicron\sigma$ (opsis, which appropriately can be translated as sight or eyes). It was found to owe its spectral properties to a covalently bound cofactor, eventually named retinal (Wald, 1935, 1953, 1968; Hubbard et al., 1971). Subsequently, it was discovered that the cone photoreceptors in the vertebrate retina harboured closely related visual pigments (Morton and Pitt, 1957; Dartnall, 1962c; Mustafi et al., 2009). Thereafter, it became known that the invertebrate retina applied structurally very similar, but photochemically slightly differently operating visual pigments (Hara et al., 1967; Suzuki et al., 1993; Gärtner, 2000). Similar “bi-stable” pigments in fact are also active in the vertebrate retina, like the well-known melanopsins (Provencio et al., 1998; Kumbalasisri and Provencio, 2005). Another highlight was the growing insight that the visual pigments form part of the superfamily of G protein-coupled receptors (Kühn, 1984; Hargrave and McDowell, 1992; Palczewski and Orban, 2013). As a matter of fact, rhodopsin is the cornerstone of the major subfamily in this widespread receptor family.

Type-1 Family

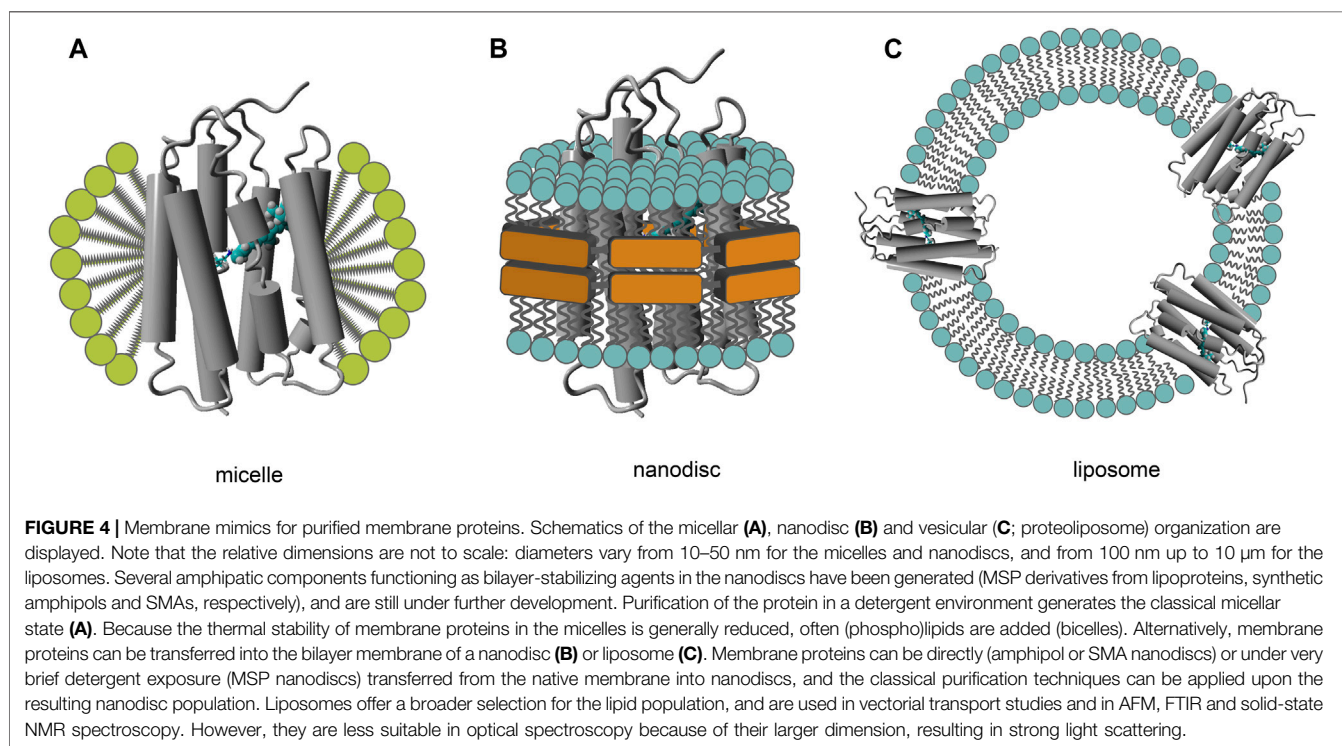
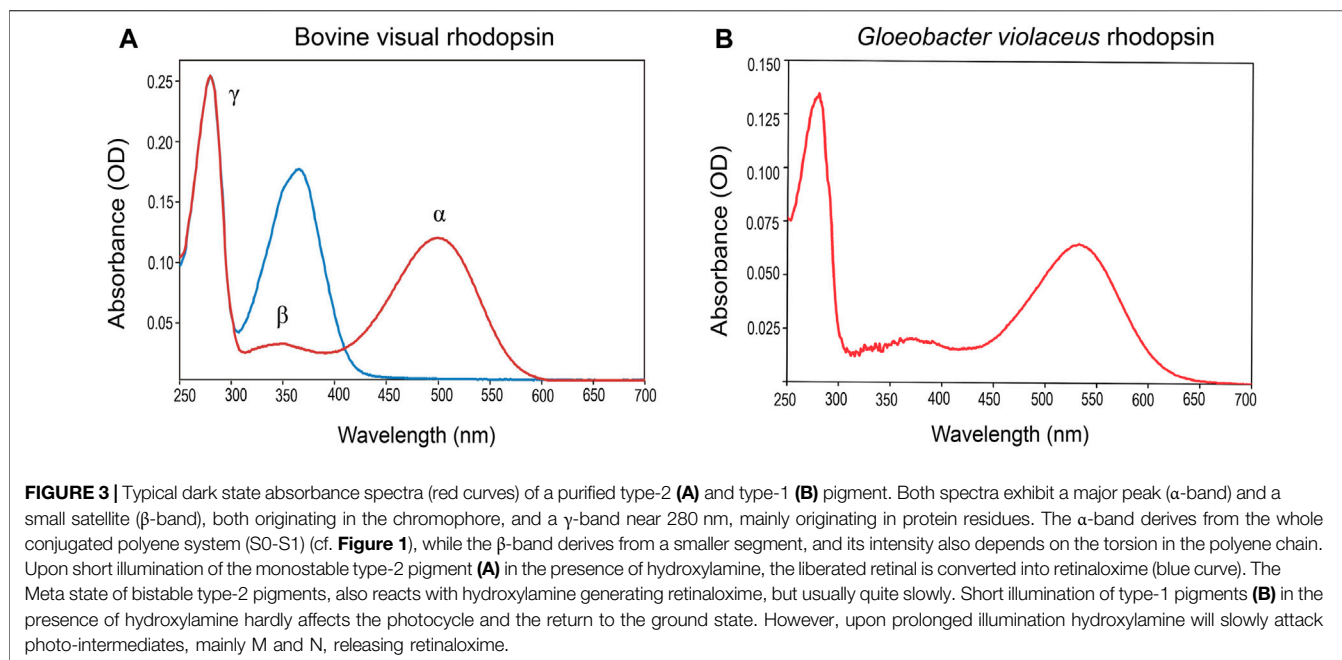
In the early 1970s, fascinated by the dark-purple colonies of the salt lake thriving archaeon *Halobacterium salinarum*, Oesterhelt reported the surprising discovery that an intrinsic membrane protein was dominating purple patches in the cellular membrane of this archaeon and also harboured retinal as the chromophoric cofactor (Oesterhelt and Stoeckenius, 1971; Oesterhelt and Hess, 1973). At that time archaea were considered a subfamily of bacteria, and Oesterhelt coined the name bacteriorhodopsin (BR). Surprisingly, it was discovered that bacteriorhodopsin functions as a light-driven outward-directed proton pump, creating a proton-motive force enabling the cellular ATP-synthase complex to supply the cell with metabolic energy in the form of ATP (Oesterhelt et al., 1991). While bacteriorhodopsin is the dominant photoreceptor in *Halobacterium salinarum*, this archaeon eventually turned out to harbour several related photosensitive proteins, both with ion transport and sensory functions (Oesterhelt, 1998). Since the 1990s this field exploded, with more strains, including eukaryotic organisms like algae and fungi, and other functionalities being revealed every year (Béjà et al., 2000; Spudich et al., 2000; Brown, 2004; Rozenberg et al., 2021; Broser, 2022; Nagata and Inoue,

2022). More recently even viral rhodopsins have been discovered (Philosof and Béjà, 2013; Bratanov et al., 2019; Zabelskii et al., 2020). The overall structure and photochemistry of these pigments are very similar, and they are now considered to be a primary factor in marine phototrophy and solar energy conversion (Kirchman and Hanson, 2013; Gómez-Consarnau et al., 2019).

SPECTRAL AND STRUCTURAL PROPERTIES, AND SOLUBILIZATION

The spectral properties of all rhodopsins were discovered by visual observation, thanks to their absorbance of photons in the visible spectrum (350–750 nm). Accurate recording of their absorbance spectra was complicated in the spectrophotometers available at that time, due to the intense scattering of light by the rhodopsin containing membrane fragments isolated from host cells. Strong chemical reagents or alkaline conditions could dissolve these fragments, but with concomitant denaturation of the proteins and loss of their native spectral properties (bleaching). In the 1950s synthetic surface-active agents, termed detergents, became available, that were able to solubilize these membrane proteins in smaller mixed detergent-lipid-protein micelles, which strongly reduced light scattering (Hallett et al., 1991). Strong detergents like SDS still led to denaturation and release of retinal, but milder detergents were developed to avoid rapid partial unfolding at lab temperature or below. Accurate recording of absorbance spectra could then be established in detergent solutions. If some scattering still remained, or other visible light material interfered, difference spectroscopy was established by recording spectra before and after illumination in the presence of hydroxylamine and taking a difference spectrum. Hydroxylamine captures the released retinal as retinaloxime, which absorbs outside the main absorbance band of most rhodopsins (Wald and Brown, 1953; Hubbard et al., 1971; Kropf, 1975). This usually provides an accurate profile of the main absorbance band or at least the absorbance maximum. (**Figure 3**). A more recent and elegant approach is to insert a membrane protein into small nanodiscs (Civjan et al., 2003; Borch and Hamann, 2009; Ritchie et al., 2009) (**Figure 4**). This also strongly reduces light scattering and has the important advantage of embedding the protein in the more stabilizing lipid bilayer environment (Banerjee et al., 2008; Tsukamoto et al., 2011; Zhou and Cross, 2013; Ganapathy et al., 2020). Nanodiscs can be generated using either lipoproteins and membrane scaffold protein derivatives (MSPs) or small synthetic polymers of the amphipol or styrene-maleic acid copolymer family (SMAs) (Knowles et al., 2009; Popot et al., 2011; Hoi et al., 2021). For MSPs usually a brief detergent solubilization step is still required, while SMAs can extract the protein directly from the membrane, but have a smaller pH-profile (Shirzad-Wasei et al., 2015; Dörr et al., 2016; Kopf et al., 2020; Ueta et al., 2020).

The spectral profile of rhodopsins in the visible and near-UV region is very similar (**Figure 3**). It consists of the most red-



shifted main absorbance band or α -band, a smaller β -band, both originating in the bound retinal, and the γ -band near 280 nm, that largely originates in the aromatic residues of the protein part termed “opsin.” In all rhodopsins retinal is covalently linked to a lysine residue in the seventh transmembrane segment (TM7) via a Schiff base (**Figure 1**) which is mostly protonated. The α -band is strongly red-shifted from the absorbance band of free retinal

(maximum around 380 nm). This unusual polar grouping in the middle of a membrane protein is stabilized by the negatively charged “counterion complex,” containing one, two or occasionally three protein residues (mostly Glu/Asp, sometimes Lys or in anion pumps a Cl^- ion) in a H-bonded network with nearby residues and bound water molecules (Lanyi, 2004; Ernst et al., 2014; Gerwert et al., 2014; Nomura et al., 2018).

Thus, the excitation energy in this retinylidene moiety is strongly reduced, compared to free retinal, which results in a red-shift of the absorbance profile. The magnitude of the red-shift strongly depends on the structure of the H-bonded network and counterion complex involving variable electrostatic interactions with the protonated Schiff base and to a lesser extent on the properties of protein residues in the opsin binding pocket (Lesca et al., 2018; Nikolaev et al., 2020; Shen et al., 2021; Shtyrov et al., 2021; Church et al., 2022a). By modifying these elements Nature created the spectacular broad variance in the spectral profile of rhodopsins, allowing them to cover the entire visible region.

The three-dimensional (3-D) structure of rhodopsins has been extensively investigated by classical electron diffraction on 2-D crystals and X-ray crystallography on large 3-D crystals, by solid-state NMR spectroscopy on membrane fragments and more recently by X-ray free electron lasers (XFEL) on small crystals (Schertler and Hargrave, 1995; Palczewski, 2012; Ladizhansky, 2017; Smith, 2021). Cryo-electron microscopy (Cryo-EM) has been traditionally performed on micellar solutions, but has also evolved to include nanodiscs (Maeda et al., 1991; Bertazolli-Filho et al., 2001; Hasegawa et al., 2018; Zhao et al., 2019; Zhang M. et al., 2021). Only solid-state NMR can be directly applied to membrane suspensions, but overall, there is quite good agreement between the various approaches. The overall structure is quite similar for all rhodopsin families, with the main scaffold consisting of seven closely packed transmembrane α -helices, which creates a tightly fitting binding pocket lined by a lysine residue to covalently bind retinal (Figure 2). The protein N- and C-terminal stretch reside at the extracellular and intracellular side of the membrane, respectively, except for the heliorhodopsin family where this sidedness is reverted (Pushkarev et al., 2018). However, the packing of the α -helices, the size of the loops connecting the α -helices and of the N-terminal and C-terminal stretches outside the membrane differ significantly between the type-1 and type-2 families.

Type-2 Family

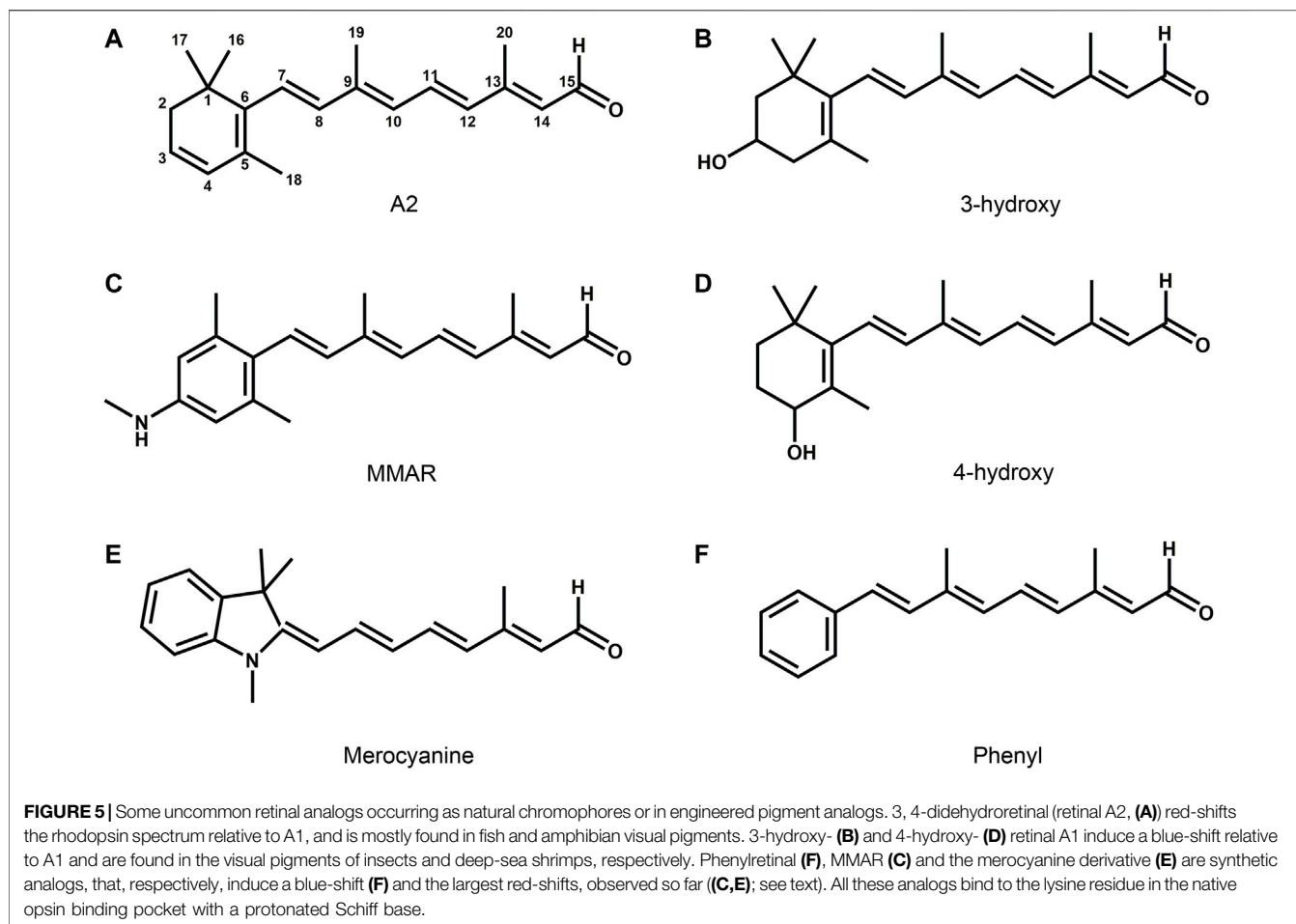
Most type-2 rhodopsins, and in particular cone visual pigments and invertebrate pigments are very sensitive to at least partial denaturation upon solubilization in detergent solution (Bliss, 1948; Kropf, 1982; Okano et al., 1989). While commercial detergents like Triton X-100, CTAB, LDAO and Emulphogene BC-720 could dissolve the vertebrate rod pigment rhodopsin into mixed micelles with none or only very slow loss of spectral properties at room temperature, for most other pigments only the very mild agent digitonin could be applied (Tansley, 1931; Knudsen and Hubbell, 1978; Okano et al., 1989; Hofmann and Palczewski, 2015). This natural compound, a steroidal glycone extracted from *Digitalis purpurea*, however has the disadvantage that its commercial preparations were quite expensive and did vary in composition and aqueous solubility (Bridges, 1977). Major progress was attained in the 1970s upon development of the alkylsaccharide detergents 1-O-n- β -D-octylglucoside (octylglucoside, OG), nonylglucoside (NG) and dodecylmaltoside (DDM) (Stubbs et al., 1976; DeGrip and Bovee-Geurts, 1979). DDM in particular turned out to maintain thermal stability and spectral and photochemical

properties of rhodopsin almost as well as digitonin (DeGrip, 1982; VanAken et al., 1986). Additionally, DDM is well accessible and affordable through organic synthesis, and has therefore become the most popular detergent in the membrane protein field. Also, in case a protein purified in DDM needs to be reconstituted in a lipid bilayer for certain applications (nanodisc or proteoliposome, Figure 4), DDM can be easily extracted via cyclodextrin inclusion (DeGrip et al., 1998). More recently, a large number of novel detergents based upon the structural principle of DDM have been developed, some of which provide better thermal stability or better crystallization conditions for selected membrane proteins than DDM, but all requiring more complex synthesis (Hussain et al., 2016; Nguyen et al., 2018; Ehsan et al., 2020; Urner et al., 2020).

The absorbance band profiles of type-2 rhodopsins are quite similar (Figure 3), but the position of the α -band varies strongly for the visual pigments. The vertebrate rod photoreceptor pigment rhodopsin has quite a broad range in its absorbance maximum (Rh1 subset, 440–520 nm), with fresh-water animals slightly red-shifted and marine animals blue-shifted depending on the depth of their habitat (Lockett, 1977; Luk et al., 2016; Musilova et al., 2019). Vertebrate cone pigments cover the entire visible spectrum, and can be divided into four subsets, the long-wavelength (LWS, absorbance maximum range 520–640 nm), green (Rh2, 460–530 nm), blue (SWS2, 400–470 nm), and UV (SWS1, 350–450 nm) sensitive pigments (Crescitelli, 1991; Yokoyama and Yokoyama, 2000; Imamoto and Shichida, 2014). This classification is not only based upon spectral sensitivity, but also upon sequence similarity (Nathans, 1987; Hunt and Collin, 2014; Jacobs, 2018; El Khatib and Atamian, 2019). Invertebrate visual pigments are more scattered over the visible region and can range from 340 nm up to 600 nm (Gärtner, 2000; Katz and Minke, 2009; Tsukamoto and Terakita, 2010). Non-visual animal rhodopsins are scattered over the 340–550 nm region (Leung and Montell, 2017; Pérez J. H. et al., 2019; Moraes et al., 2021).

The spectral properties of the type-2 rhodopsins depend on the 11-*cis* configuration of the retinylidene chromophore. Next to the standard retinal (retinal A1, Figure 1), several natural modifications occur (analogs). In fresh-water and coastal vertebrates 11-*cis* 3-dehydroretinal (retinal A2) has been observed (Figure 5) (Bridges, 1972; Yoshizawa, 1984; Imai et al., 1999). The longer conjugated chain red-shifts the absorbance maximum by 20–40 nm in rod pigments and up to 70 nm in cone pigments, as compared to retinal A1, to compensate for the lower blue light intensity in their habitat (Dartnall, 1962c; Hubbard et al., 1971). These “A2-rhodopsins” are also referred to as porphyropsins. In insects and some other invertebrates, 11-*cis* 3-hydroxy- and 4-hydroxyretinals have been detected (Figure 5) (Vogt and Kirschfeld, 1984; Matsui et al., 1988; Seki and Vogt, 1998). These modifications blue-shift the absorbance maximum by 20–40 nm, as compared to retinal A1 (Sekharan et al., 2011).

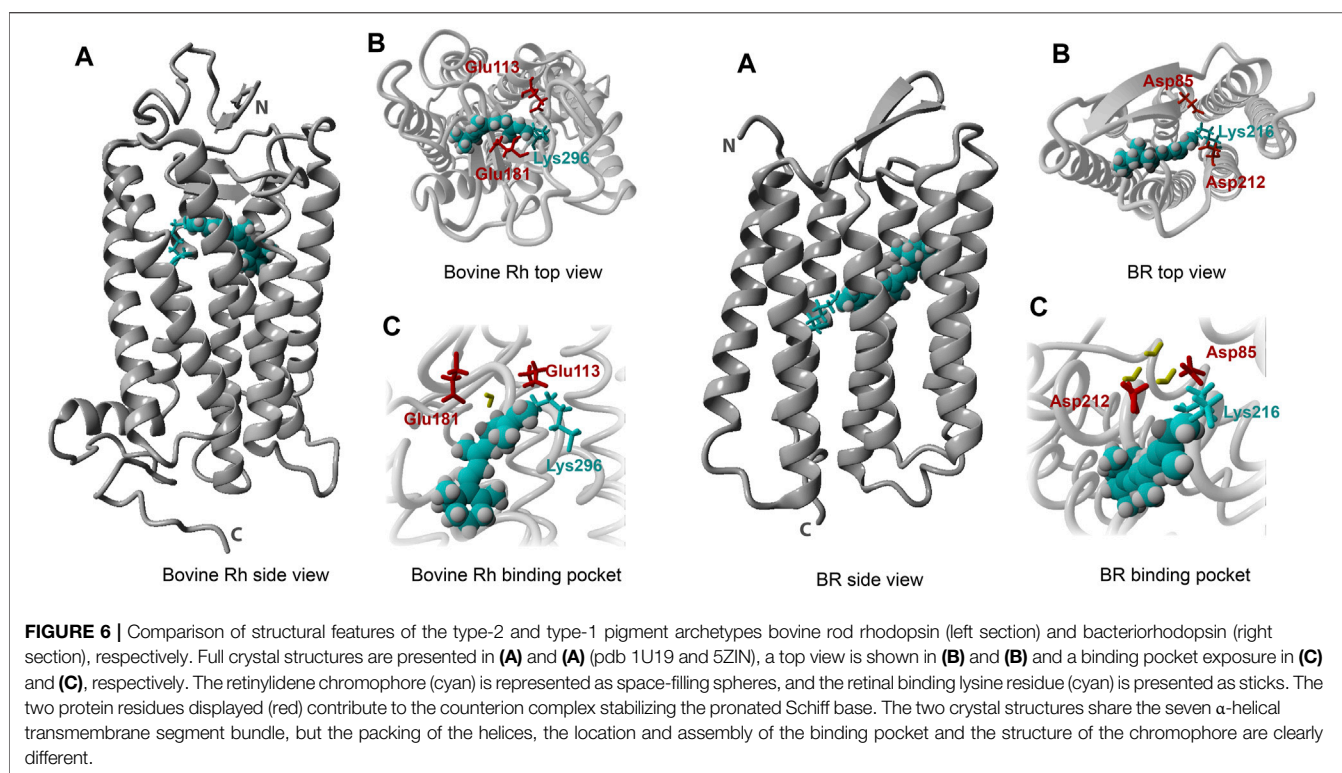
While such natural modifications are exploited to modulate the spectral position of a rhodopsin, the most effective approaches to shift the absorbance spectrum of the rhodopsin chromophore away from that of free retinal (380 nm) are



protonation of the Schiff base and mutation of selected opsin residues lining the retinal binding pocket. For instance, only the Schiff base in UV absorbing rhodopsins, which absorb in the 350–380 nm region, is not protonated, while in all other classes it is protonated (Kusnetzow et al., 2004; Imamoto and Shichida, 2014). The large variation in the spectral properties in the latter classes is mainly due to the combined inductive effect of opsin binding pocket residues, in combination with H-bonding networks involving water molecules. On top of that, some vertebrate LWS visual pigments have developed a unique mutation (Glu197->His) creating a chloride binding site that effectuates a further 20–30 nm red-shift (Wang et al., 1993).

With respect to structural biology, bovine rod rhodopsin was a forerunner among all animal intrinsic membrane proteins, presenting the first detailed 3-D structure via X-ray crystallography in 2000, with many more to follow (Palczewski et al., 2000; Li et al., 2004; Okada et al., 2004). The seven transmembrane α -helical scaffold surrounding an accessible cofactor binding pocket proved to be the general motif for the entire G protein-coupled receptor family (Figure 6) (Sanchez-Reyes et al., 2017). This feat has stimulated advances in many other research fields, including drug design in the pharmaceutical

sciences, study of protein structure-function correlations, and membrane protein-lipid interactions, both from experimental, theoretical and *in-silico* standpoints. Several natural factors concurred to enable this important step forward. First of all, rod rhodopsin is one of the few intrinsic membrane proteins that is available in relatively large quantities in domesticated animals, the most used being cattle (up to 1 mg of rhodopsin per eye), bullfrogs (up to 100 μ g per eye) and chick (up to 100 μ g LWS cone pigment per eye) (DeGrip et al., 1980; Toba and Hanawa, 1985; Yoshizawa and Kuwata, 1991). After enucleation and proper dark adaptation of the eyes, intact rod or cone outer segments (ROS or COS) can be easily isolated in a dark room under dim red light (>650 nm) that will not activate and bleach the pigment (Figure 3). Further, in dark-adapted ROS, rhodopsin makes up about 85% of the total protein content (DeGrip et al., 1980). Eventually, dark-adapted bovine retinae even became commercially available (Hormel Co., Austin, Minnesota, United States). Finally, bovine rod rhodopsin was found to be relatively resistant to destabilization by detergents as compared to most other visual pigments, allowing extensive purification. Likewise, it proved to be sufficiently stable in less mild but more crystal-production-favoring small detergents like OG and



NG to facilitate crystallization trials (Palczewski et al., 2000; Park et al., 2008).

The bovine rhodopsin amino acid sequence was established thanks to heroic protein sequencing efforts (Abdulaev et al., 1982; Hargrave et al., 1983). Over time, sequence information became available more easily via genome mining and c-DNA-sequencing. Thus, it came out that most invertebrate visual pigments are similar in size to the vertebrate pigments (36–42 kD), but mollusc pigments are significantly larger (46–55 kD), because of the presence of a much longer C-terminal (Ovchinnikov et al., 1988b; Gärtner, 2000). This additional stretch is unique in having an insertion of up to eleven copies of a peculiar pentapeptide sequence (Pro-Pro-Gln-Gly-Tyr), which probably helps in immobilization of the protein in the microvillar membrane (Ryba et al., 1993; Gärtner, 2000). Longer C-terminal stretches are also found in non-visual rhodopsins. For instance, the VA-opsin and melanopsin family also show this feature, except that the pentapeptide insertion does not occur. Here the extra sequence probably has a function in complex regulation of signal processing and desensitization (Valdez-Lopez et al., 2020; Contreras et al., 2021). Some VA-opsins and melanopsins are even produced in two or more splicing isoforms, with longer and shorter C-terminals (Davies et al., 2010).

While squid provides fair quantities of visual pigment, the first complete 3-D crystal structures only became available since 2008, both because of the much lower stability of the pigments in detergent solution and since crystallization could only be achieved after proteolytic removal of most of the long C-terminal (Murakami and Kouyama, 2008; Shimamura et al.,

2008). The overall fold of the seven-transmembrane α -helical scaffold is quite similar to bovine rhodopsin, but the structure of the long C-terminal could not be determined, of course. The position of the retinal chromophore is slightly different, since the Glu residue functioning as the direct counterion for the protonated Schiff base is displaced from the site in the vertebrate pigments (Terakita et al., 2004). The first crystal structure of an arthropod rhodopsin (jumping spider) was only recently published in 2019, and again shows the familiar seven α -helical fold with overall high similarity with the squid structure (Varma et al., 2019). So far, crystal structures of non-visual rhodopsins have not been reported.

The crystal unit cell of bovine rod rhodopsin contains a dimer, but its interaction pattern is very different from the natural one (Fotiadis et al., 2006; Palczewski, 2006). In fact, rhodopsin is equally active as a monomer, and the organization in the ROS disc membranes is still debated (monomer, dimer, longer stretches?) (Fotiadis et al., 2004; Chabre and LeMaire, 2005; Mishra et al., 2016; Zhang et al., 2016; Feldman et al., 2019; Zhao et al., 2019). Invertebrate visual rhodopsins are probably rigidly immobilized in their native membrane, which allows to discern the polarization plane of the incoming light (Gärtner, 2000; Stavenga et al., 2000).

The crystal structures are essential to resolve the protein fold of the rhodopsins and have confirmed several conjectures of the binding pocket. Biochemical, vibrational (resonance Raman and FTIR spectroscopy) and solid-state NMR studies already produced very strong evidence that it indeed harboured the 11-*cis* configuration of retinal (Groenendijk et al., 1980; Mathies et al., 1987; Lugtenburg et al., 1988; DeGrip and

TABLE 1 | Selected additional citations for the section “Spectral and structural properties and solubilization”.**Type-1 pigments**

Optical spectroscopy: Roussou et al. (1998); Kanehara et al. (2017); Asido et al. (2021)

Vibrational spectroscopy: Garczarek and Gerwert, (2006); Lórenz-Fonfría and Kandori, (2009); Kraack et al. (2011); Verhoefen et al. (2011); Lórenz-Fonfría et al. (2015a); Ito et al. (2018); Watari et al. (2019); Lórenz-Fonfría et al. (2021)

NMR/EPR spectroscopy: Smith et al. (1989); Shi et al. (2009); Mao et al. (2014); Planchard et al. (2014); Shigeta et al. (2017); Mao et al. (2019); Naito et al. (2019); Friedrich et al. (2020)

Crystallography/EM: Havelka et al. (1995); Kimura et al. (1997); Belhali et al. (1999); Subramaniam et al. (1999); Royant et al. (2001); Vogeley et al. (2004); Luecke et al. (2008); Wada et al. (2011); Kato et al. (2012); Wang et al. (2012); Frank et al. (2014); Kato et al. (2015); Nango et al. (2016); Tsukamoto et al. (2016); Broecker et al. (2017); Hasegawa et al. (2018); Ghanbarpour et al. (2019); Kovalev et al. (2019); Li et al. (2019); Morizumi et al. (2019); Shihoya et al. (2019); Yun et al. (2019); Besaw et al. (2020); Hayashi et al. (2020); Kovalev et al. (2020a); Lu et al. (2020); Bada Juarez et al. (2021); Higuchi et al. (2021); Li et al. (2021); Suzuki et al. (2022); Zhang et al. (2022)

Atomic force microscopy: Müller et al. (2002); Klyszejko et al. (2008); Yu et al. (2017); Heath et al. (2021)

Computational: Hayashi et al. (2001); Fujimoto et al. (2007); Melaccio et al. (2016); Karasuyama et al. (2018); Tsujimura and Ishikita, (2020); Fujimoto, (2021); Shen et al. (2021)

Reviews: Bèjà et al. (2000); Caffrey, (2003); Engel and Gaub, (2008); Bamann et al. (2014); Grote et al. (2014); Neutze et al. (2015); Engelhard et al. (2018); Bibow, (2019); Kwon et al. (2020); Kawasaki et al. (2021)

Solubilization: Yu et al. (2000); Bayburt et al. (2006); Yeh et al. (2018); Ueta et al. (2020)

Other: Tribet et al. (1996)

Type-2 pigments

Optical spectroscopy: Seki et al. (1998); Salcedo et al. (1999); Schafer and Farrens, (2015); Katayama et al. (2019)

Vibrational spectroscopy: Rothschild et al. (1980); Kochendoerfer et al. (1999)

NMR/EPR spectroscopy: Creemers et al. (1999); Carravetta et al. (2004)

Crystallography/EM: Sardet et al. (1976); Davies et al. (1996); Davies et al. (2001); Krebs et al. (2003); Standfuss et al. (2007); Stenkamp, (2008); Hildebrand et al. (2009); Blankenship et al. (2015); García-Nafria and Tate, (2020); Zhang et al. (2021a)

Computational: Nikolaev et al. (2018); Patel et al. (2018)

Reviews: Neitz and Neitz, (1998); Spudich et al. (2000); Sakmar et al. (2002); McDermott, (2009); Smith, (2010); Bickelmann et al. (2015); Guo, (2020)

Solubilization: Kropf, (1982); Sadaf et al. (2015); Frauenfeld et al. (2016); Lee et al. (2020); Grime et al. (2021)

Rothschild, 2000; Mathies and Lugtenburg, 2000). Surely enough, this configuration best fitted the non-protein electronic density in the binding pocket. The same is true for the covalent binding of retinal to a lysine residue, for which the above-mentioned techniques also already provided a wealth of evidence (Bownds, 1967; DeGrip et al., 1973; Creemers et al., 1999; Mathies and Lugtenburg, 2000). However, to firmly establish protonation of the Schiff base the resolution of the crystal structures is not high enough. Instead, the evidence produced by vibrational and NMR spectroscopy is very convincing and in fact was later underpinned by quantum-chemical computation (Palings et al., 1987; Herzfeld and Lansing, 2002; Gascón et al., 2005; Tastan et al., 2014).

Type-1 Family

The sensitivity to detergent action also varies strongly between microbial rhodopsins. For instance, while bacteriorhodopsin (BR) is quite stable in OG, Triton X-100 and dodecylphosphocholine (DPC) even as a monomer, the rhodopsin proton pump from the cyanobacterium *Gloeobacter violaceus* (GR) strongly prefers DDM and is very unstable in DPC (Dencher and Heyn, 1978; Brouillette et al., 1989; Ganapathy et al., 2020). In general, OG and DDM are the preferred agents for solubilization of type-1 rhodopsins.

The spectral range of type-1 rhodopsins (360–690 nm) is comparable to that of type-2. There is less evidence for a clear relation to activity or habitat, an exception being the proton pump proteorhodopsin, which exhibits a blue-shift in deeper marine environments (Bèjà et al., 2001; Bielawski et al., 2004). In a major distinction from type-2, microbial rhodopsins invariably exploit retinal A1 in the all-*trans* configuration as the basis for

their light absorbance. Here, as well, a plethora of experimental evidence has demonstrated retinal binding to a lysine residue via a protonated Schiff base (Haupts et al., 1999).

Advanced angular electron diffraction studies on 2-D BR crystals in membrane patches already afforded a first glimpse into the organization of the helical transmembrane segments of type-1 rhodopsins (Henderson and Unwin, 1975; Mitra et al., 1993; Grigorieff et al., 1996; Heymann et al., 1997; Mitsuoka et al., 1999). The first 3-D crystal structures were reported for BR from 1997 onwards, and at a very high resolution slightly before that of bovine rhodopsin (Luecke et al., 1999; Pebay-Peyroula et al., 2000). This progress was aided by its high stability in detergent solutions and the relatively simple isolation from its native source. Bacteriorhodopsin is organized in large singular patches in the cellular membrane of *Halobacterium salinarum*, which can visually be observed and separated from other membrane fragments quite easily (Oesterhelt and Stoeckenius, 1971). In addition, type-1 rhodopsins complete a full photocycle (see below) and after photo-activation do not release the retinal, but thermally return to the ground state. This obviates the complexity of using dark rooms and shielding all experimental manipulations from room light exposure. Meanwhile, quite a number of crystal structures have been resolved for various classes of type-1 rhodopsins (Table 1). The most recent high resolution 3-D structures actually capitalized on the fantastic progress in cryo-EM (Hirschi et al., 2021; Kishi et al., 2022).

The available type-1 3-D structures show high similarity in protein fold and retinal pocket location. The basic seven α -helical transmembrane organization is comparable to that of type-2 (Figure 6), but for type-1 the helical packing is

somewhat different and more compact. The loop segments connecting the helices are generally shorter and the retinal pocket is positioned differently to accommodate the longer all-*trans* chromophore instead of the curved 11-*cis* one (Figures 1, 6). Aspects of the binding pocket (retinal isomer and binding to a lysine residue via a Schiff base) again were in line with a wealth of evidence generated by biochemical and spectroscopic techniques (Lanyi, 2004). A recent XFEL study of the bacteriorhodopsin photocycle achieved a very high structural (ca 1.5 Å) and temporal (femtosecond) resolution and produced evidence for protonation of the Schiff base (Nogly et al., 2018). Also in the type-1 case the evidence generated by biophysical techniques like vibrational, EPR and NMR spectroscopy and by quantum-chemical computation is most convincing (Ernst et al., 2014; Brown and Ernst, 2017; Ryzantsev et al., 2019; Nagata and Inoue, 2022).

A conspicuous feature of most type-1 rhodopsins is that they organize in homo-oligomers, whether observed in the native membrane or in host cells. The most common arrangement for bacterial and archaeal rhodopsins are trimers or pentamers, though occasionally hexamers do occur as well (Hussain et al., 2015; Shibata et al., 2018; Kao et al., 2019). Circular dichroism spectroscopy provides evidence for exciton coupling between the chromophores (Cassim, 1992; Fujimoto and Inoue, 2020; Fujimoto, 2021). For eukaryotic type-1 rhodopsins, homo-dimeric as well as hetero-dimeric complexes are observed (Mukherjee et al., 2019; Govorunova et al., 2021; Broser, 2022). Isolated type-1 monomers are also functionally active, indicating that the oligomeric assembly probably affords optimal packing and mutual stabilization, and/or the opportunity to modulate monomer activity by inter-subunit interplay (Iizuka et al., 2019).

A novel feature was discovered in the enzyme-rhodopsins i.e. an additional transmembrane segment at the N-terminal (TM8), which functions as a connector with the cognate soluble enzyme domain and seems to be essential for modulating its activity (Ikuta et al., 2020; Tsunoda et al., 2021).

Interestingly, several thermostable microbial rhodopsins have been discovered. The crystal structure of the highly thermophilic rhodopsin (TR) from *Thermus thermophilus* was resolved to be very similar to that of the much less thermally stable xanthorhodopsin (XR) from *Salinibacter ruber*, including the binding crevice for the carotenoid antenna (Tsukamoto et al., 2016). Likewise, the crystal structure of the thermostable rhodopsin proton-pump from *Rubrobacter xylanophilus* (RxR) is very similar to that of bacteriorhodopsin (Hayashi et al., 2020). An unusually widely stable proton pump (pH, detergent, temperature), named Tara76 rhodopsin, was isolated from uncultured bacteria (Shim et al., 2021). Such data shed new light on the design options to increase thermal and environmental stability without a significant sacrifice in dynamics and activity (Hayashi et al., 2020).

Additional selected references relevant for this section have been compiled in Table 1.

FUNCTIONAL DIVERSITY, PHYLOGENY

It was relatively simple in the old days. On one hand, we knew of animal rhodopsins, being G protein-coupled receptors, very nicely developed and evolved into a set of proteins allowing photopic vision (color discrimination) and a single class for extremely sensitive scotopic vision (black-and-white). On the other hand, another class of retinal-proteins had evolved in archaea to exploit solar energy for active transport of protons and chloride ions. However, with the awakening of the genome era, this view became totally obsolete. While the notion that the type-1 and type-2 families probably do not have a common ancestor and have little overlap in physiological function was consistent, over time many new members were discovered and their classification revised (Porter et al., 2012; Yee et al., 2013; Zabelskii et al., 2021). In hindsight, it was to be expected that ahead of the large carotenoid and chlorophyll dependent protein complexes in the photosynthetic reaction centers, Nature would have taken advantage of the abundance of solar energy making maximal use of this fantastic toolbox of retinal-proteins, that are relatively simply to bioproduce and adapt.

It is likely that many products of this toolbox are yet to be discovered, but already the genetic and functional diversity is so vast and complex, that we provide a very general overview below and mostly refer to selected reviews.

Type-2 Family

The animal rhodopsins have meanwhile been classified in at least nine gene families (Opn1–Opn9) and two separate sets with some members still awaiting further assignment (Table 2). Physiological function and tissue distribution show incredible diversity (Janssen et al., 2003; Leung and Montell, 2017; Liebert et al., 2021; Moraes et al., 2021; Calligaro et al., 2022). The classical visual pigments come within Opn1 (cone pigments) and Opn2 (rod pigments). Pigments discovered later in the vertebrate retina, such as melanopsin, VA-opsin or neuropsin, peropsin (RRH) and RGR fall under Opn4, unclassified and Opn5, respectively (Table 2). The common thread still is primary signal transduction via at least one of the available G-protein species (Gt, Go, Gi, Gq, and Gs), with cross-activation, modulation or desensitization via a variety of other mediators. However, RGR and its mollusc counterpart retinochrome are exceptional in this context, since they act as photo-isomerases, binding all-*trans* retinal in the dark state, and releasing 11-*cis* retinal after photo-activation as a supply for regeneration of visual opsins (Hara et al., 1967; Pepe and Cugnoli, 1992; Zhang et al., 2019; Choi et al., 2021; Vöcking et al., 2021). Another remarkable subset are Opn5L, peropsin and Opn7 members, which also bind all-*trans* retinal in the dark state, but that seems to be the active state binding the G protein. Upon illumination they generate the 11-*cis* chromophore, which represents the resting state that in the case of Opn5L members may even thermally revert to the active state (Nagata et al., 2018; Yamashita, 2020; Karapinar et al., 2021; Sakai et al., 2022). An even more surprising observation is that some type-2 pigments may be involved

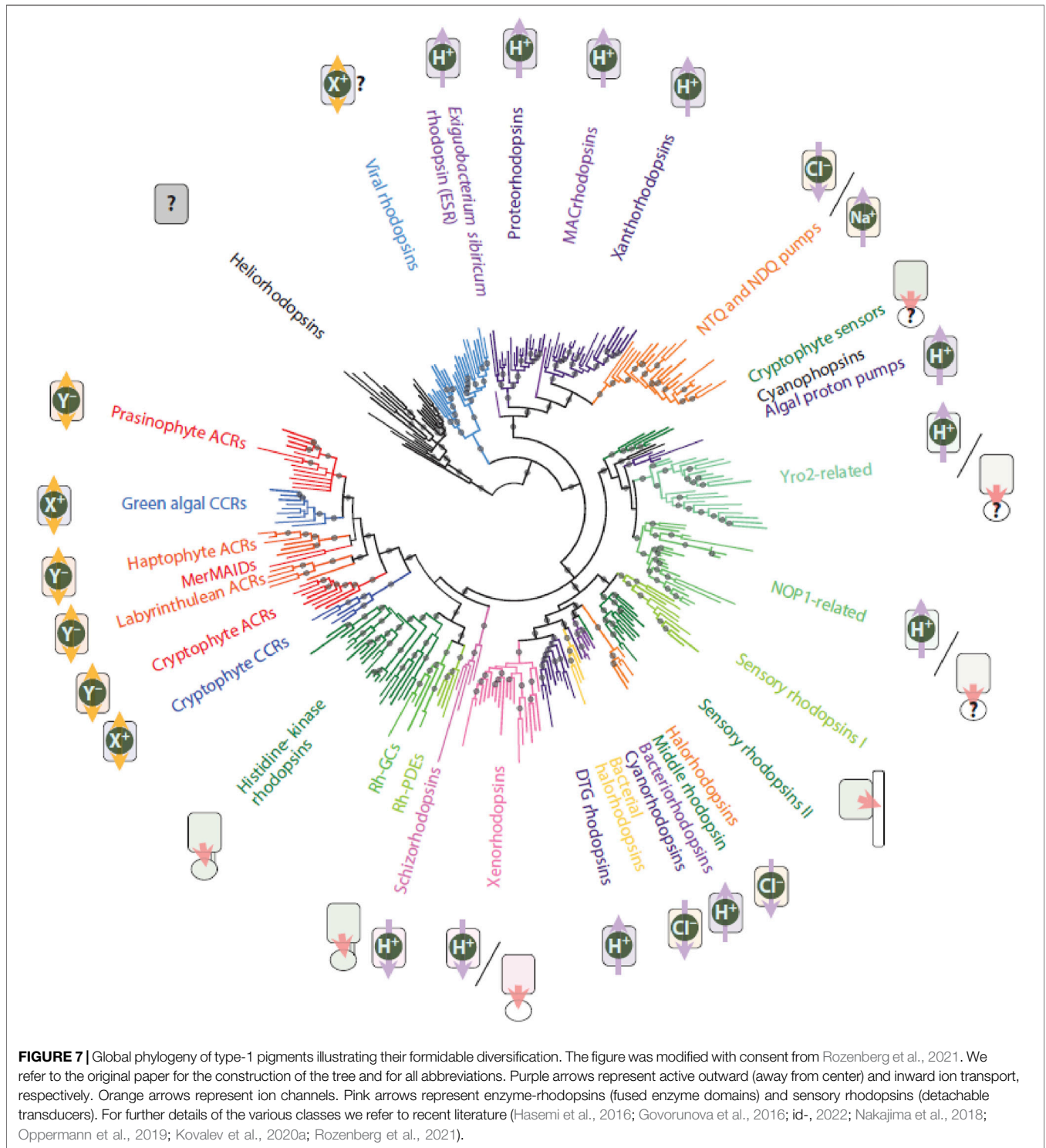
TABLE 2 | Current classification of type-2 rhodopsins.

Gene family or group	Main components ^b	Spectral range ^a	Location	Mono/bi-stable ^a	Special facts	Selected literature
Opn1	Vertebrate cone pigments	350–610 nm	Retina	Mono		Nathans, (1987); Imamoto and Shichida, (2014); Hofmann and Palczewski, (2015); Borgia et al. (2018); Jacobs, (2018); El Khatib and Atamian, (2019); Astakhova et al. (2021)
Opn2	Vertebrate Rod pigments	440–520 nm	Retina, Brain	Mono	Includes exorhodopsin	Ebrey and Koutalos, (2001); Rohrer et al. (2003); Warrant and Lockett, (2004); Tarttelin et al. (2011); Davies et al. (2012); Liu et al. (2019); Ortega and Jastrzebska, (2019)
Opn3	Encephalopsins Panopsins TMT-opsins	Blue-green	Multiple tissues, Extra-ocular	Bi		Blackshaw and Snyder, (1999); Halford et al. (2001); Moutsaki et al. (2003); Leung and Montell, (2017); Lan et al. (2020); Olinski et al. (2020); Xu et al. (2020); Davies et al. (2012); Davies et al. (2021); Liebert et al. (2021)
Opn4	Melanopsins	450–500 nm	Multiple tissues	Bi	Long C-terminals	Provencio et al. (1998); Provencio et al. (2000); Panda et al. (2002); Kumbalasingam and Provencio, (2005); Panda et al. (2005); Giesbers et al. (2008); Davies et al. (2010); Shirzad-Wasei and DeGrip, (2016); Duda et al. (2020); Valdez-Lopez et al. (2020); Contreras et al. (2021)
Opn5 ^c	Neuropsins Peropsins RGR's	UV-blue	Multiple tissues	Bi	11- <i>cis</i> - > all- <i>trans</i> Photoactivation All- <i>trans</i> -> 11- <i>cis</i>	Jiang et al. (1993); Sun et al. (1997); Tarttelin et al. (2003); Yamashita et al. (2010); Yamashita et al. (2014); Nagata et al. (2018); Sato et al. (2018b); Zhang et al. (2019); Yamashita, (2020); Choi et al. (2021); Liu et al. (2021); Calligaro et al. (2022); Fujiyabu et al. (2022)
Opn6		UV-blue	Multiple tissues	Mono and Bi	Zebrafish Monotrenes	Davies et al. (2015)
Opn7		UV-blue	Multiple tissues	Bi and Mono	Zebrafish All- <i>trans</i> -> 11- <i>cis</i>	Davies et al. (2015); Karapinar et al. (2021)
Opn8		UV-blue	Multiple tissues	Bi	Not in mammals	Davies et al. (2015)
Opn9		?	Multiple tissues	?	Zebrafish, long extra-cellular loop	Davies et al. (2015)
R (habdomer) opsins	Molluscs Arthropods	340–600 nm	Mainly ocular	Bi	Molluscs, long C-terminal	Wald, (1953); Hara et al. (1967); Hillman et al. (1983); Vogt and Kirschfeld, (1984); Gärtner, (2000); Stavenga et al. (2000); Furutani et al. (2005); Porter et al. (2012); Nagata et al. (2018); Leung et al. (2020); Nagata and Inoue, (2022)
Cn(iderian) opsins	Jellyfish	?	Multiple tissues	?		Musio et al. (2001); Plachetzki et al. (2012); Porter et al. (2012); Feuda et al. (2014); Davies et al. (2015); Gerrard et al. (2018); Hayashi et al. (2020)
Separate gene groups	VA-opsins Parapinopsins Parietopsins Pinopsins Xenopsins Go-rhodopsins	Blue-green	Multiple tissues	Mostly Bi	Parietopsins mainly in pineal gland Xenopsins and Go-rhodopsins in invertebrates	Okano et al. (1994); Blackshaw and Snyder, (1997); Kojima et al. (1997); Soni and Foster, (1997); Nakamura et al. (1999); Spudich et al. (2000); Foster and Hankins, (2002); Su et al. (2006); Davies et al. (2010); Tsukamoto and Terakita, (2010); Passananeck et al. (2011); Sakai et al. (2012); Koyanagi et al. (2014); Davies et al. (2015); Leung and Montell, (2017); Sato et al. (2018a); Pérez et al. (2019b); Rawlinson et al. (2019); Döring et al. (2020); Eickelbeck et al. (2020); Copits et al. (2021); Rodgers et al. (2021)

^aSpectral range and mono/bistability not always exclusive within a group and very limited known for Opn6-Opn9 and Cn-opsins.

^bCone pigments are mainly involved in color (photopic) vision, rod pigments in (scotopic) dim-light vision. In mammals melanopsins are important for pupillary contraction and circadian regulation. Retinochromes (R-opsins) and peropsins and RGRs (Opn5) have photoisomerase activity (all-*trans* → 11-*cis*).

^cThe Opn5L group (Sato et al., 2018b; Yamashita, 2020) may have been classified wrongly, since they clade within the Opn6-9 framework.



in recognizing temperature differences or mechanical changes, or function as chemosensors or tumorigenic elements, possibly even without requiring their retinal cofactor (Shen et al., 2011; Park et al., 2013; Baker et al., 2015; Pérez-Cerezales et al., 2015; Leung et al., 2020; Xu et al., 2020; Córdova et al., 2021; Moraes et al., 2021).

Type-1 Family

Type-1 rhodopsins have been identified in archaea and eubacteria, including cyanobacteria, as well as in unicellular eukaryotes (algae, fungi, yeast) and more recently also in choanoflagellates and viruses (Lamarche et al., 2017; Bratanov et al., 2019; Zabelskii et al., 2020; Rozenberg et al., 2021;

Govorunova et al., 2022b; Nagata and Inoue, 2022). Most of these pigments function as light-driven ion transporters or ion channels (Figure 7). The newly discovered xenorhodopsins and schizorhodopsins are exceptional as they perform inward-directed proton transport (Inoue et al., 2018; Inoue et al., 2020; Weissbecker et al., 2021; Brown, 2022). However, some type-1 rhodopsins display a photosensory function (sensory rhodopsins) and signal via a cognate transducer protein, which is totally different functionally and structurally from the animal G-proteins (Bogomolni and Spudich, 1991; Krahe et al., 1994; Deininger et al., 1995). Overall, type-1 pigments are the dominant contributors to marine phototrophy (Casey et al., 2017; Larkum et al., 2018; Gómez-Consarnau et al., 2019). In addition, eukaryotic type-1 rhodopsins have been discovered which are intracellularly fused to an enzymatic domain and mediate light-driven enzyme activation (guanylyl cyclase, phosphodiesterase) or inhibition (guanylyl cyclase, based upon histidine kinase activity) (Avelar et al., 2014; Lamarche et al., 2017; Luck et al., 2019; Mukherjee et al., 2019; Tsunoda et al., 2021; Broser, 2022; Tian et al., 2022). These pigments have been termed as enzyme-rhodopsins.

The overall structure and photochemistry of all type-1 rhodopsins present a very similar pattern, though the sequence identity can be as low as 12%, and the kinetics of the photocycle can vary up to at least thousand-fold. The most recent addition, the heliorhodopsins, are not very different in their protein fold from e.g. BR in spite of a very low sequence identity (<10%) (Shihoya et al., 2019). Considering their inverted insertion into the membrane, very long photocycle and so far unknown functionality, they probably are better classified separately as type-3 rhodopsins (Tanaka et al., 2020; Chazan et al., 2022).

HETEROLOGOUS EXPRESSION AND PURIFICATION

The congruent broad heterogeneity in the rhodopsin superfamily offers a fascinating spectrum for mechanistic studies as well as biomimetic adaptation and application. However, mechanistic studies still require large quantities of relatively pure material (at least several mg). With the exception of some visual pigments and archaeal rhodopsins, such quantities are not available from native sources. Besides, purifying minor quantities of rhodopsins out of a large excess of cellular membrane proteins turned out to be a “hell of a job” (Dartnall, 1962b; Hubbard et al., 1971). Furthermore, in-depth mechanistic studies and biomimetic applications need the ability to make modifications biosynthetically at the protein residue level, and synthetically at the chromophore level. And even when *in silico* molecular dynamics and quantum chemical computation would have reached the time-scale of protein conformational changes (femtoseconds to seconds range) and the native accuracy, then still experimental verification is in order. Experimentally modifying rhodopsins in the native organism was completely out of hand at the time, except for some limited success with bacteriorhodopsin mutants in *Halobacterium salinarum* which still did not solve the quantity requirement (Krebs et al., 1993).

Hence, the search for suitable heterologous expression hosts started in the 1980s, and over time it became obvious that the eukaryotic rhodopsins required quite a different perspective.

With the start of the genome era, recombinant DNA technology (genome mining, DNA and c-DNA sequence information and comparison, DNA sequence modification) became accessible and have now become common experimental tools (Khorana, 1979; Khorana et al., 1987). Likewise, total synthesis of retinal isomers and a plethora of derivatives has improved significantly (Dawadi and Lugtenburg, 2010; Liu and Liu, 2011; Álvarez et al., 2014; El-Tahawy et al., 2020).

Type-2 Family

Type-2 rhodopsins can undergo a variety of posttranslational modifications (disulfide-bridge formation, N- and O-glycosylation, methylation, acetylation, myristylation, palmitoylation, phosphorylation), most of which are not properly executed by the bacterial or archaeal biosynthetic machinery (Table 3). Expression of bovine rhodopsin in bacteria and even yeast did not yield promising results (Mollaaghababa et al., 1996; Abdulaev and Ridge, 2000). Hence, for optimal heterologous expression a eukaryotic cell type had to be selected as a host. Attempts have been made to express type-2 pigments and related receptors in the eye of whole organisms (mouse, *Xenopus*) and in *Caenorhabditis elegans* using viral vectors or transgenic animals, but this gave relatively low yields or even led to retinal degeneration (Zhang et al., 2005; Salom et al., 2008; Cao et al., 2012; Salom et al., 2012). Eventually, the best results with sufficient posttranslational modification and targeting to the plasma membrane were obtained in some mammalian cell lines using plasmid transfection (COS, HEK, Neuroblastoma cell lines), in insect cell lines using baculoviral infection (*Spodoptera Sf9* and *Sf12* and *Trichoplusia* “High-Five”) and in *Xenopus* oocytes (Oprian et al., 1987; Janssen et al., 1988; Khorana et al., 1988; Karnik et al., 1993; Kazmi et al., 1996). The highest expression levels of functional pigments, with addition of 11-*cis* retinal during culture or after isolation of the cells, were obtained in suspension culture of insect cells or specially adapted HEK293 cells, with yields up to 130 nmol/L, equivalent to ca 5 mg bovine rhodopsin per liter (Klaassen and DeGrip, 2000; Reeves et al., 2002). Even then the pigment accounts for maximally 5 percent of the total cellular membrane protein, and further purification is inevitable. Eventually, gene manipulation lent a helping hand and it has now become common practice to add a small sequence tag to the pigment c-DNA, encoding a short peptide sequence to easily identify and purify the expressed pigment. Two approaches have become the most popular in the type-2 rhodopsin field. One exploited the availability of a monoclonal antibody against the C-terminal octapeptide of bovine rhodopsin (Molday, 1989). This allows for highly selective immuno-affinity purification using a suitable detergent like DDM for solubilization (Ridge et al., 1995). By adding to or replacing the native C-terminal with this octapeptide, the resulting tagged protein can be comfortably isolated. The second approach involved extending the C-terminal with six to ten histidine residues (His6-tag to

TABLE 3 | Selected additional citations for the section “Heterologous expression and purification”.*Type-1 pigments*

Optical spectroscopy: Chen and Gouaux, (1996); Kwon et al. (2019)
 Posttranslational: Hildebrandt et al. (1991); Müller, (1992); Lang-Hinrichs et al. (1994); Feng et al. (2013)
 Review: LinCereghino and Cregg, (2000); Lichty et al. (2005); Hasegawa et al. (2020)
 Other: Schey et al. (1992)

Type-2 pigments

Optical spectroscopy: Orian et al. (1991); Kojima et al. (1995); Radlwimmer and Yokoyama, (1997); Ma et al. (2001); Melyan et al. (2005); Qiu et al. (2005); Giesbers et al. (2008); Shirzad-Wasei et al. (2013); Kahremany et al. (2019)
 Vibrational spectroscopy: Katayama et al. (2012); Katayama et al. (2017)
 Posttranslational: Hargrave, (1977); Karnik et al. (1988); Ovchinnikov et al. (1988a); Janssen et al. (1991); O'Tousa, (1992); Fujita et al. (1994); Kaushal et al. (1994); Morello and Bouvier, (1996); Nakagawa et al. (1997); Zhang et al. (1997); Katanosaka et al. (1998); Gibson et al. (1999); Hwa et al. (1999); Ridge and Abdulaev, (2000); Maeda et al. (2003); Park et al. (2009); Tam and Moritz, (2009); Salom et al. (2019)
 Expression: Schey et al. (1992); Harada et al. (1994); Townson et al. (1998); Reeves et al. (2002); Peirson et al. (2004); Panda et al. (2005)
 Review: Hargrave, (1982)

His10-tag), which upon solubilization with a suitable detergent allows metal affinity purification over a matrix containing immobilized Ni²⁺ or Co²⁺ complexes (Janknecht et al., 1991; Janssen et al., 1995). Both approaches are very effective with hardly any perturbation of expression level and functionality of the pigment (Reeves et al., 1999; Bosman et al., 2003). Nevertheless, if necessary, a short target peptide sequence for a selective proteolytic enzyme can be introduced in front of the purification tag to remove it after purification (Sarramegna et al., 2006). Most Opn1 and Opn2 pigments can be satisfactorily purified by either procedure (Vissers and DeGrip, 1996; Shirzad-Wasei and DeGrip, 2016; Katayama et al., 2017; Katayama et al., 2019). Some pigments from the other subsets have been difficult to solubilize or are too unstable in detergent solution to survive purification. The alternative option then is to transfer the protein into the stabilizing lipid environment of nanodiscs (Figure 4), which requires hardly any detergent (amphipol or SMA-type) or very brief exposure to a suitable mild detergent (MSP-type). Exploiting the sequence tag on the incorporated protein, the protein-nanodisc unit is then easily purified again by affinity chromatography (Shirzad-Wasei et al., 2015; Cai et al., 2017; Ganapathy et al., 2020).

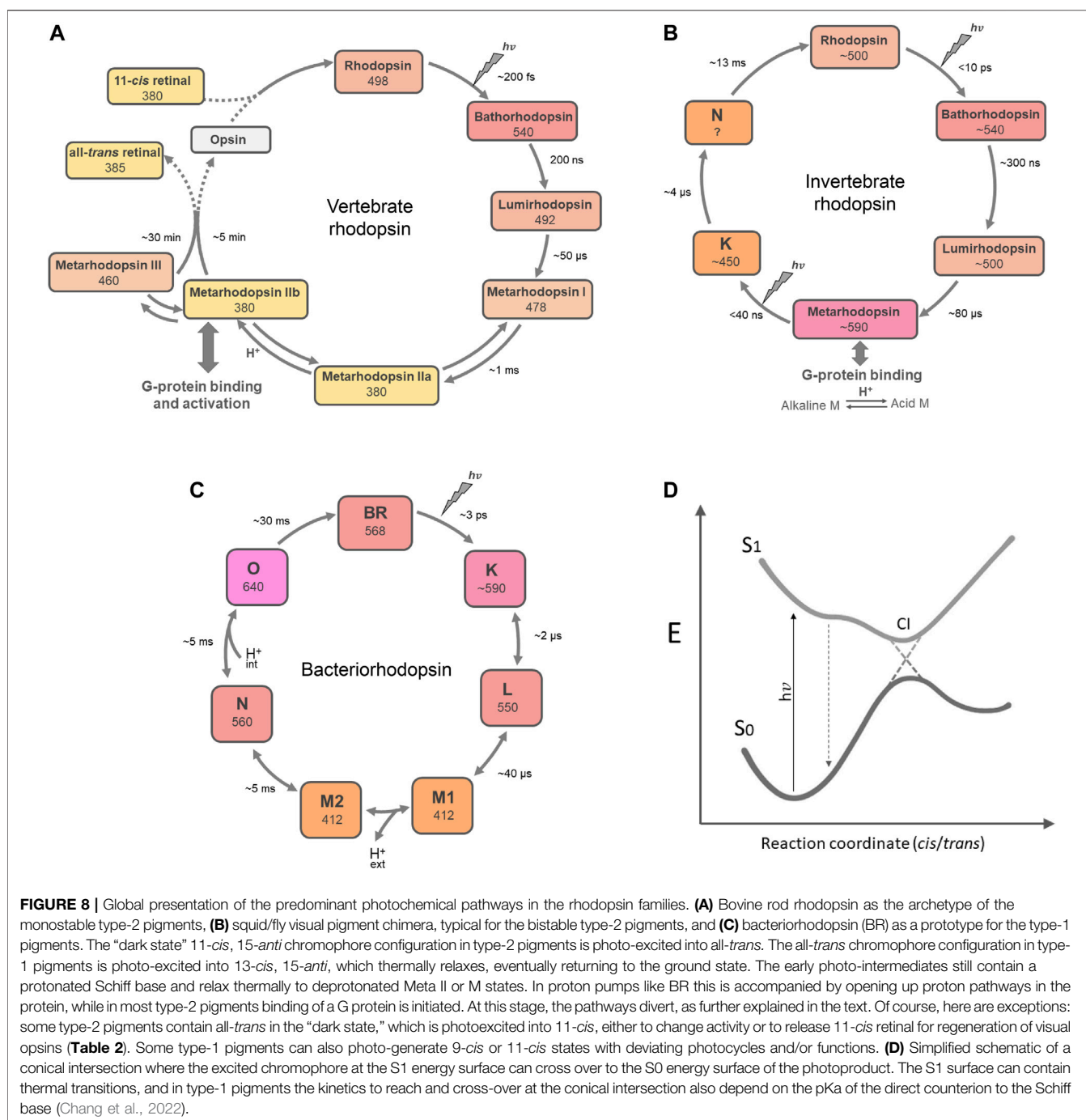
The opportunity to modify, bio-generate and purify type-2 pigments in sufficient quantities has given a tremendous boost to all mechanistic and functional studies. Analysis of the native proteins or binding pocket mutants, often in combination with ²H-, ¹³C- or F-labeling and/or chemical modification of retinal and/or with ¹⁵N- and/or ¹³C-labeling of protein residues or inserting modified amino acids, has provided a wealth of data, underpinning, extending and refining the information obtained from crystal structures (see next sections). Groundbreaking details of dark state structures have been excavated by biochemical (e.g. limited proteolysis, selective chemical modification, selective deuteration, atomic force microscopy, cryo-EM) and biophysical studies (e.g. FTIR and resonance Raman spectroscopy, solid-state NMR spectroscopy, EPR spectroscopy) (Table 3). This also fueled a large body of theoretical and *in-silico* efforts (molecular dynamics, quantum-chemical calculation and modeling) (Ryazantsev et al., 2019; Pedraza-González et al., 2020; Fujimoto, 2021; Mroginski et al., 2021; Church et al., 2022b). As a result of all these

exertions, it has already been possible to construct a highly detailed picture of the dark state of bovine rhodopsin.

Type-1 Family

For the archaeal and bacterial type-1 rhodopsins, a heterologous expression host was more easily identified. *Escherichia coli* strains had already been developed for uncomplicated suspension culture, high productivity, low proteolytic activity and easy transformation. Plasmids with inducible promoters became available, and were further engineered with specific features, like producing the necessary enzymatic machinery to generate all-*trans* retinal from its precursor β -carotene (Kim et al., 2008). Nevertheless, in most cases just supplementing the cell culture with all-*trans* retinal together with inducing opsin expression or even after membrane isolation was sufficient to produce the full equivalent of the corresponding rhodopsin (Spudich et al., 2000; Ganapathy et al., 2015). In this way yields up to 20 mg/L have been reported (Ganapathy et al., 2015; Song et al., 2020). For some archaeal pigments, this straightforward approach only gave low yields and had to be adapted e.g. for bacteriorhodopsin itself (Bratanov et al., 2015; Tu et al., 2018). On the other hand, heterologous expression was more problematic for the eukaryotic type-1 rhodopsins, again because of their more complex posttranslational modification profile. Channelrhodopsins are commonly produced in yeast (*Pichia pastoris*), but successful production of eukaryotic type-1 pigments in insect and mammalian cell lines, *Caenorhabditis elegans* and *Xenopus* oocytes is also reported (Nagel et al., 2003; Bruun et al., 2015; Govorunova et al., 2017). An interesting new approach is using the trypanosome *Leishmania tarentolae* for over-expression (Volkov et al., 2017). For optogenetic applications (see below), functional production and targeting in a mammalian context is imperative, and often requires insertion of trafficking or targeting signals and/or sequence optimization to mammalian genetic code preferences.

The C-terminal His-tag has become the most popular option for purification of archaeal and eubacterial rhodopsins. For eukaryotic type-1 rhodopsins, several tags are used, including the His-tag, although the latter may sometimes interfere with particular electrophysiological or enzymatic analyses



(Govorunova et al., 2021; Rozenberg et al., 2021; Tsunoda et al., 2021; Govorunova et al., 2022b).

Thanks to the powerful combination of the recombinant DNA toolbox with heterologous expression and purification making sufficient protein material available, an astounding repertoire of structural and functional data has also become available for the type-1 rhodopsins (**Table 3**). As a result, bacteriorhodopsin has become the best studied and fathomed membrane protein, with unprecedented insight into its structure and function (Ernst et al., 2014; Larkum et al., 2018; Nogly et al., 2018; Weinert et al., 2019).

Next to that, the type-1 community has delivered prospects for a wealth of biotechnological and biomimical applications, far beyond any prognosis (see below).

PHOTOCHEMICAL PROPERTIES

The initial rapid steps after photoactivation of type-1 and type-2 rhodopsins are quite comparable (**Figure 8**). Ultrafast photoisomerization of the chromophore leads to the first

TABLE 4 | Selected additional citations for the section “Photochemical properties”.**Type-1 pigments**

Optical spectroscopy: Butt, (1990); Ogonah et al. (1991); Chizhov et al. (1996); Inoue et al. (2004); Rupenyan et al. (2008); (2009); Inoue et al. (2011); Bayraktar et al. (2012); Ogren et al. (2015); Tahara et al. (2015); Iyer et al. (2016); Hontani et al. (2017a); Hontani et al. (2017b); Smitienko et al. (2017); Inoue et al. (2018); Chang et al. (2019); Kao et al. (2019); Luck et al. (2019); Tahara et al. (2019b); Hontani et al. (2020); Smitienko et al. (2021); Sugimoto et al. (2021); Chang et al. (2022)

Vibrational spectroscopy: Rothschild et al. (1981); Rothschild and Marrero, (1982); Rothschild et al. (1984); Marrero and Rothschild, (1987); Rödig et al. (1999); McCamant et al. (2005); Amsden et al. (2007); Neumann et al. (2008); Schäfer et al. (2009); Sasaki et al. (2011); Sudo et al. (2011); Saint Clair et al. (2012a); Johnson et al. (2014); Liebel et al. (2014); Kuhne et al. (2015); Lórenz-Fonfría et al. (2015b); Schnedermann et al. (2016); Yi et al. (2017); Roy et al. (2018); Kataoka et al. (2019); Kuhne et al. (2019); Kaufmann et al. (2020); Fischer et al. (2021); Polito et al. (2021)

NMR/EPR spectroscopy: Hu et al. (1998); Ding et al. (2018)

Crystallography/EM: Schobert et al. (2002); Frank et al. (2014); Furuse et al. (2015); Kato et al. (2015a); Wickstrand et al. (2015); Hosaka et al. (2016); Ikuta et al. (2020); Kojima et al. (2020c); Kovalev et al. (2020a); Bada Juarez et al. (2021); Hirschi et al. (2021); Li et al. (2021); Axford et al. (2022); Kishi et al. (2022); Poddar et al. (2022)

Computational: Schapiro and Ruhman, (2014); Feng and Mertz, (2015); Yalouz et al. (2021)

Review: Wand et al. (2013); Kandori et al. (2018); Buhrke and Hildebrandt, (2020)

Type-2 pigments

Optical spectroscopy: Yoshizawa and Wald, (1967); Regan et al. (1978); Shichida, (1986); Imamoto et al. (1989); Lewis et al. (1990); Gärtner et al. (1991); Davidson et al. (1994); Imai et al. (1995); Imamoto et al. (1996); DeLange et al. (1997); Jäger et al. (1997); Lewis et al. (1997); Vought et al. (1999); Kusnetzow et al. (2001); Furutani et al. (2003); Sato et al. (2011); Tarttelin et al. (2011); Gulati et al. (2017); Van Eps et al. (2017); Nagata et al. (2019); Chawla et al. (2021); Sakai et al. (2022)

Vibrational spectroscopy: Rothschild et al. (1976); Rothschild et al. (1983); DeGrip et al. (1985); Pande et al. (1987); DeGrip et al. (1988); Bagley et al. (1989); Masuda et al. (1993); Rath et al. (1993); Hashimoto et al. (1996); Rath et al. (1998); DeLange et al. (1999); Ritter et al. (2004); Yan et al. (2004); Ye et al. (2010); Nonaka et al. (2020); Hanai et al. (2021)

NMR/EPR spectroscopy: Smith et al. (1992); Verhoeven et al. (2001); Struts et al. (2007); Altenbach et al. (2008); Eilers et al. (2012); Brinkmann et al. (2018)

Crystallography/EM: Ruprecht et al. (2004); Schertler, (2005); Nakamichi and Okada, (2006); Scheerer et al. (2008); Choe et al. (2011); Murakami and Kouyama, (2011); (2015); Panneels et al. (2015); Tsai et al. (2019)

Atomic force microscopy: Kawamura et al. (2013)

Computational: Schreiber et al. (2006); Bhattacharya et al. (2008); Tavanti and Tozzini, (2014); Feng et al. (2015); Ren et al. (2016); Tomobe et al. (2017); Demoulin et al. (2021)

Review: Zundel, (1988); Yoshizawa and Kandori, (1991); Farrens, (2010); Smith, (2010); Polli et al. (2015); Vlasov et al. (2020)

Other: Angel et al. (2009); Bayburt et al. (2011)

stable photoproduct within ps. This conversion is extremely efficient with quantum yields between 0.6 and 0.7 for type-2 pigments and varying between 0.3 and 0.7 for type-1 pigments and very low energy loss through fluorescence (Gozem et al., 2017). Often, this red-shifted photoproduct then thermally relaxes via spectrally distinguishable photo-intermediates within ms to a blue-shifted M(eta) intermediate, where the chromophore-binding Schiff base has become deprotonated through transfer of the proton to the direct counterion (Nakagawa et al., 1999; Hofmann, 2000; Tsukamoto and Terakita, 2010; Ernst et al., 2014; Govorunova et al., 2017). This explains the large blue-shift. In some type-1 pigments, a deprotonated M state is not formed, however a protonated L-like equivalent is observed (Spudich et al., 2014; Govorunova et al., 2017; Engelhard et al., 2018). The M or its L-like equivalent intermediate is the active state of the pigment, where the conformational changes in the protein evoke the subsequent cognate activity (grouping with cognate G protein or transducer, opening up an ion channel or vectorial ion pathway, regulating the enzymatic domain, etc.) (Table 4). At the M or L-like stage the type-2 and type-1 pathways take completely different directions.

Type-2 Family

For the type-2 rhodopsins ultrarapid spectroscopy data are limited, and mainly available for Opn1, Opn2, and R-type pigments (Shichida et al., 1978; Shichida, 1990; Schoenlein et al., 1991; Vought et al., 2000; Imamoto and Shichida, 2014; Schnedermann et al., 2018). Generally speaking, two schemes

have been identified: Monostable pigments eventually release all-*trans* retinal (all Opn1 and Opn2 rhodopsins, Figure 8A) following which the opsins require supplementation with retinal re-isomerized elsewhere to regenerate the original “dark” state. Bistable pigments (most other type-2 pigments investigated, Table 2) progress until a stable M-intermediate is reached (all-*trans* chromophore), that requires photo-isomerization to return to the original “dark” state (11-*cis* chromophore) (Figure 8B) (Hillman et al., 1983; Gärtner, 2000; Stavenga et al., 2000).

The photochemical profile of the monostable bovine rod rhodopsin has been explored in great detail. The native pigment and a variety of isotopically labeled and/or mutant pigments have been investigated by femtosecond optical spectroscopy and vibrational and NMR spectroscopy. These studies have revealed intimate details on the kinetics, conformational changes in the chromophore and surrounding H-bonded networks with constrained water molecules, protein-chromophore interplay and Schiff base (de)protonation (Table 4). Overall protein conformational changes have been elucidated by fluorescence, ESR and NMR spectroscopy and TR-WAXS (DeGrip et al., 1999; Kusnetzow et al., 2006; Alexiev and Farrens, 2014; Malmerberg et al., 2015; Van Eps et al., 2017; Smith, 2021). Crystal structures have been resolved for all photo-intermediates and present a broad structural basis (Table 4). The power of theoretical and quantum-chemical calculations has grown immensely, laying a strong foundation for electronic and energetic elements of the process, in

particular (Schapiro et al., 2011; Gozem et al., 2017; Schnedermann et al., 2018; Agathangelou et al., 2021; Nikolaev et al., 2021).

A very effective combination of selectively labeled chromophore with femtosecond spectroscopy and advanced quantum chemical computation resolved many remaining issues in the photoisomerization process of bovine rhodopsin (Schnedermann et al., 2018). The global picture has arisen that after photo-excitation of the chromophore into the Franck-Condon state it rapidly relaxes along a barrierless trajectory on the potential surface to a minimal energy conical intersection (**Figure 8D**). Here, productive resonance of the electronic wave packet at the excited state potential surface with torsional and HOOP vibrational modes in the twisted C10-C13 segment of the 11-*cis* chromophore, can prime very effective cross-over to a ground state energy surface, generating a hot all-*trans*oid state (photorhodopsin) within tens of fs (Johnson et al., 2015). This relaxes thermally in about 200 fs into the photoproduct bathorhodopsin, which contains a still highly twisted all-*trans* chromophore, but is stable below 130 K (Yoshizawa and Wald, 1963). In free retinal, photoexcitation results in formation of several isomers (predominantly all-*trans*, 13-*cis*, 9-*cis*, and 11-*cis*), but in rhodopsins this conversion is remarkably selective from 11-*cis* to all-*trans*. This is clearly facilitated by the constraints of the binding site and the twist in the C10-C13 segment of the chromophore (Bismuth et al., 2007; Weingart, 2007; Schnedermann et al., 2018).

At room temperature, the ca 35 kcal of excitation energy stored in bathorhodopsin (Cooper, 1979) drives further relaxation via several intermediates until the metarhodopsin IIa-IIb equilibrium is reached within ms. This relaxation process subtly rearranges chromophore, protein residues and H-bonded networks up to the metarhodopsin stage, where the Schiff base transfers its proton, the counterion and another Glu at the intracellular side of the protein become protonated and an interhelical activity switch reshuffles helical segments to open up binding residues for the G-protein (Hofmann, 2000; Vogel et al., 2007; Vogel et al., 2008; Pope et al., 2020). The chromophore is subsequently slowly released via hydrolysis of the Schiff base to generate the nearly inactive apoprotein opsin (Wald, 1953; Rothschild et al., 1987; Jastrzebska et al., 2011). *In vivo* the active state is rapidly inactivated through phosphorylation and arrestin binding, however, which blocks activation of the G protein (Ranganathan and Stevens, 1995).

The photochemical profile of other monostable pigments (human rod rhodopsin, several cone pigments) has been investigated to much less depth, but is quite comparable to the bovine rod pigment (Barry and Mathies, 1987; Kusnetzow et al., 2001; Hofmann and Palczewski, 2015; Kazmin et al., 2015). However, the kinetics differ somewhat. For instance, the investigated cone pigments show more rapid kinetics in most steps (Imai et al., 1997; Vissers et al., 1998; Chen et al., 2012; Sato et al., 2012). Ultra-violet absorbing cone pigments may be more complex, as photoisomerization is accompanied by protonation of the Schiff base (Kusnetzow et al., 2004; Mooney et al., 2012).

The photochemical profile of bistable pigments, investigated thus far (squid, octopus and some insect pigments), follow a

scheme similar to the monostable pigments up through formation of the M-intermediate and with comparable kinetics (**Figure 8B**) (Gärtner, 2000; Stavenga et al., 2000; Vought et al., 2000; Murakami and Kouyama, 2015). It is reported that in cephalopods the M-intermediate in fact forms a pH-dependent equilibrium between a protonated (acid M) and a deprotonated state (alkaline M). This involves the Schiff base of the chromophore, and the alkaline M is strongly blue-shifted (Liang et al., 1994; Vought et al., 2000). Photo-reisomerization of the M state to the original “dark” state is again quite efficient with a quantum yield around 0.4 (Stavenga et al., 2000).

Type-1 Family

The “dark” state of type-1 pigments contains a chromophore with the all-*trans*, 15-*syn* configuration (**Figure 1**). Rapid spectroscopy has been performed on quite a number of type-1 pigments, and the global scheme is quite similar to that of bacteriorhodopsin (**Figure 8C**). However, the kinetics of the slower steps (M and subsequent ones) and thereby the overall cycle time can vary considerably from ms up to minutes (Rozenberg et al., 2021; Tsunoda et al., 2021; Broser, 2022; Nagata and Inoue, 2022).

Out of all rhodopsins the photochemistry of BR is understood in most detail (Wickstrand et al., 2015; Nango et al., 2016). Femtosecond XFEL crystallography has even revealed very early responses to photoexcitation of the chromophore (Nogly et al., 2018). The adjacent protein residues and water molecules already react to the charge delocalization in the excited chromophore before the isomerization is initiated (Tahara et al., 2019a). During the isomerization process more of the protein environment becomes involved while the chromophore rapidly relaxes along a 2-state trajectory on the excited state potential surface to a conical intersection, where it effectively crosses in ca 500 fs over to a ground state energy surface into a “hot” transient hybrid state (J) and then relaxes thermally in about 3 ps into the photoproduct K, which contains a still significantly twisted 13-*cis*, 15-*anti* chromophore, but is stable below 150 K (Lanyi, 2004). Here, a major driving force is the elongation of the C13-C14 bond in the excited state in combination with electrostatic re-arrangement and weakening of the hydrated H-bonded network in the Schiff base region. At room temperature, the ca 15 kcal of excitation energy stored in K (this can be higher in sensory rhodopsins) (Birge et al., 1991; Govorunova et al., 2017; Rozenberg et al., 2021) drives further relaxation via the spectrally distinguishable L intermediate until the M states are reached in ca 50 μ s. This relaxation process again subtly re-arranges chromophore, protein helices and H-bonded networks up to the M states, where the Schiff base transfers its proton via a water molecule to the counterion and the hydrated H-bonded network opens up a proton gateway to the extracellular membrane surface. The M-states thermally decay via several intermediates in tens of ms to the BR ground state, during which the Schiff base is reprotonated via proton transfer from residue Asp96, a proton is taken up from the intracellular surface and the chromophore is re-isomerized to the all-*trans*, 15-*syn* configuration. In fact, all-*trans* is the most stable configuration for free retinal (Ganapathy and Liu, 1992). Nevertheless, in some archaeal rhodopsins including BR the chromophore slowly enters an all-*trans*, 15-

anti ↔ 13-*cis*, 15-*syn* equilibrium when stored in the dark (dark adaptation). The latter chromophore is photo-excited in the light and via a separate non-productive photocycle rerouted to the ground state BR (Smith et al., 1989; Oesterhelt et al., 1991). In channelrhodopsins the opposite phenomenon is observed, where prolonged illumination reduces the activity, since an equilibrium between pigments with an all-*trans*, 15-*anti* and a 13-*cis*, 15-*syn* chromophore configuration is generated (light-adaptation with partial desensitization) (Bruun et al., 2015; Kuhne et al., 2019; Rozenberg et al., 2021; Govorunova et al., 2022b).

Using serial synchrotron crystallography, the slower conformational changes from 5 to ca 40 ms were recorded in the BR photocycle and involve small α -helical rearrangements, chromophore re-isomerization and proton uptake, ending in formation of the ground state (Weinert et al., 2019). A very recent study using advanced high-resolution atomic force spectroscopy at the single-molecule level investigated the BR photocycle after M formation (Perrino et al., 2021). It was concluded that a cytoplasmic gate for proton uptake opens up at about 3 ms after photo-excitation lasting for about 14 ms. Surprisingly, this same study observes a “black-out period” of tens of ms before a recycled ground state can be photo-reactivated. This uncovers a very interesting new phenomenon reminiscent of comparable nonresponsive states in animal voltage-regulated channels (Armstrong, 1992). Meanwhile, XFEL studies have also been performed on other ion pumps and channels. A femtosecond XFEL study of the sodium-pumping rhodopsin from *Krokinobacter eikastus* (KR2) again observed photo-isomerization of the chromophore to start in the femtosecond range and completed within 2 ps (Skopintsev et al., 2020). Changes in the local structure of the binding site and early conformational changes in the protein backbone are observed in the early nanosecond range. Further subtle rearrangements result in Schiff base deprotonation in μ s and in the early ms range a gate opens up and transient binding of a Na^+ ion in the vicinity of the Schiff base is observed with release within 20 ms. A femtosecond XFEL study of the chloride pump from the flavobacterium *Nonlabens marinus* follows the conformational adaptations between 1 and 100 ps after photo-excitation (Yun et al., 2021). It shows the final rearrangements of the chromophore to the 13-*cis* configuration within 50 ps, together with the dynamics of the hydrated H-bonded network and deformations in the local α -helical elements. Following chromophore isomerization the chloride ion first dissociates from the protonated Schiff base and then starts to diffuse away. Additional molecular details of the interactions and trajectory of the chloride ion are provided by recent ps up to ms studies using time-resolved serial crystallography in combination with spectral and theoretical analysis (Hosaka et al., 2022; Mous et al., 2022). An XFEL study of the channelrhodopsin chimera C1C2, that photochemically behaves like ChR1, investigated the photo-induced conformational changes from 1 μ s to 4 ms (Oda et al., 2021). Photo-isomerization induces a kink in the chromophore structure, triggering shifts in the retinal binding lysine residue and TM7, starting at around 1 μ s and increasing during formation of the M-state up to 4 ms. This induces small lateral shifts of the chromophore and in TM7 and TM3 at around 50 μ s.

It is postulated that these rearrangements forebode the subsequent opening of the gates in the cation channel pore, although these were not observed in the crystal. The XFEL and serial crystallography studies beautifully illustrate the powerful but subtle design and the broad potential of the photo-driven nanomachinery. Less detailed studies basically show a similar pattern (Table 4). Subtle differences in early kinetics and conformational adaptation in chromophore and adjacent protein elements following photo-excitation are observed in the ultrarapid studies. A cautious interpretation could be that the structure of the hydrated H-bonding network in the complex counterion is an important roadmap for the light-triggered protein activity, which also depends on the pKa of the direct counterion (Hontani et al., 2017b; Oda et al., 2021; Chang et al., 2022).

In this context it should be realized that crystal structures have their limitations (García-Nafria and Tate, 2020; Guo, 2020). Detergent exposure may affect elements of the protein structure, and the crystal will certainly constrain larger conformational alterations in the protein, which may occur in the slower phase of the photocycle (Weinert et al., 2019; Oda et al., 2021; Govorunova et al., 2022b). Hence, it would be preferable to study the slower photocycle phases with experimental approaches that can handle membrane-bound systems as shown in Figure 4, like time-resolved AFM, cryo-EM and vibrational spectroscopy.

The general scheme for the photocycle of BR (Figure 8C) also holds for other type-1 pigments, though the kinetics after M formation can vary significantly (Wand et al., 2013; Tahara et al., 2015; Han et al., 2020; Smitienko et al., 2021). The decay is much slower for sensory rhodopsins, enzyme-rhodopsins and heliorhodopsins, possibly since longer interaction with their cognate partner is required for regulated signal transduction. In fact, some sensory rhodopsins and enzyme-rhodopsins exhibit a bistable photocycle (Kawanabe et al., 2007; Broser et al., 2020) and proton transfer to the counterion may not occur (Bergo et al., 2006).

BIOENGINEERING

This section samples the impressive expansion in the field of rhodopsins bioengineered by creative exploitation of their design principles. Often, similar strategies are utilized for both type-1 and type-2 pigments, and therefore they are clustered together in the following subsections.

Shifts in Spectral And/or Functional Properties Chromophore

Very early on in the 1960s, it was realized that the beautiful design and versatility of rhodopsins could be studied and exploited by modifying the chromophore and changing the spectral properties (Blatz et al., 1969; Kropf et al., 1973). Since protein modeling was not really established at that time, this led to a surge of trial-and-error synthetic efforts to test a large number of retinal analogs on

TABLE 5 | Selected additional citations for the section “Bioengineering”.**Subsection****Chromophore**

Type-1 pigments: Balogh-Nair and Nakanishi, (1982); Muradin-Szweykowska et al. (1984); López et al. (2005); Sineshchekov et al. (2012); Azimi-Hashemi et al. (2014); Ganapathy et al. (2015); Mei et al. (2018); Ganapathy et al. (2019); Hontani et al. (2019); Munro et al. (2019); Chuon et al. (2021)

Type-2 pigments: Arnaboldi et al. (1979); Mollevanger et al. (1987); Friedman et al. (1989); Bhattacharya et al. (1992b); Feng et al. (1997); Huang et al. (1997); DeLange et al. (1998a); Iwasa et al. (1998); Lugtenburg et al. (1999); Verdegem et al. (1999); Wada et al. (2000); Spooner et al. (2004); Wang et al. (2004); Hirano et al. (2006); Verhoeven et al. (2006); DeGrip et al. (2007); Concistrè et al. (2008); Aguilà et al. (2009); Bovee-Geurts et al. (2009); DeGrip et al. (2011); Srinivasan et al. (2014); Alexander et al. (2017); Bovee-Geurts et al. (2017); Buda et al. (2017)

Protein**Type-1 pigments**

Spectral properties: Alexiev et al. (2000); Béjà et al. (2001); Hayashi et al. (2001); Shimono et al. (2001); Bielawski et al. (2004); Mori et al. (2013); Ozaki et al. (2014); Ganapathy et al. (2015); Agathangelou et al. (2018); Oda et al. (2018); Singh et al. (2018); Ganapathy et al. (2019); Inoue et al. (2019); Kuhne et al. (2019); Kojima et al. (2020d); Nakajima et al. (2021); Tsujimura et al. (2021); Shim et al. (2022)

Vibrational spectroscopy: Sonar et al. (1995); Amsden et al. (2007); Ikeda et al. (2007); Yi et al. (2016); Tomida et al. (2020)

NMR/EPR spectroscopy: Steinhoff et al. (1995); Griffiths et al. (2000); Herzfeld and Lansing, (2002); Maly et al. (2008); Shi et al. (2009); Wang et al. (2013); Becker-Baldus et al. (2015); Brown and Ladizhansky, (2015); Shigeta et al. (2017); Azadi-Chegeni et al. (2018); Kaur et al. (2019); Lavington and Watts, (2020); Kawamura et al. (2021); Tomida et al. (2021)

Crystallography/EM: Volkov et al. (2017)

Other: Khorana et al. (1987); Steward and Chamberlin, (1998)

Type-2 pigments

Spectral properties: Nakayama and Khorana, (1991); Chan et al. (1992); Asenjo et al. (1994); Yokoyama, (1995); Hope et al. (1997); Dunham and Farrens, (1999); Kochendoerfer et al. (1999); Hunt et al. (2001); Alexiev et al. (2003); Piechnick et al. (2012); Devine et al. (2013); McKee et al. (2021)

Vibrational spectroscopy: Haris et al. (1992); Lin et al. (1992); DeLange et al. (1998b); Lin et al. (1998); Ye et al. (2009); Rothschild, (2016)

NMR/EPR spectroscopy: Smith et al. (1996); Creemers et al. (1999); Creemers et al. (2002); Eilers et al. (2002); Hubbell et al. (2003); Werner et al. (2007); Altenbach et al. (2008); Hornak et al. (2010)

Computational: Nielsen, (2009); Collette et al. (2018); Peters et al. (2020)

Other: Yokoyama, (2000)

Conversion: Berndt et al. (2014); Vogt et al. (2015); Inoue et al. (2016)

Optogenetics

Type-1 pigments: Tsunoda et al. (2006); Airan et al. (2009); Erbguth et al. (2012); Sudo et al. (2013); Wietek et al. (2015); Alfonsa et al. (2016); Berglund et al. (2016); Berndt et al. (2016); Kulkarni and Miller, (2017); Brown et al. (2018); Pediani et al. (2018); Piatkevitch et al. (2018); Xu et al. (2018); Alabugin, (2019); del Carmen Marín et al. (2019a); Marshel et al. (2019); Jun and Cardin, (2020); Milosevic et al. (2020); Baillie et al. (2021); Hayashi et al. (2021); Kathe et al. (2021); Nakao et al. (2021); Panzer et al. (2021); Zhou et al. (2021); Govorunova et al. (2022a); Guo et al. (2022); Li et al. (2022); Nakao et al. (2022); Shim et al. (2022); Yaguchi et al. (2022)

Type-2 pigments: De Silva et al. (2017); Patriarchi et al. (2018); Berry et al. (2019); Owen et al. (2019); Copits et al. (2021); Hickey et al. (2021); Banskota et al. (2022)

Cell factories: Charvolin et al. (2009); Kim et al. (2012); Schlackmann and Plückthun, (2013); Pinhassi et al. (2016); Lips et al. (2018); Pérez et al. (2019a); Konno et al. (2021); Polito et al. (2021); Zhang et al. (2021); Fujiyabu et al. (2022)

their ability to incorporate into the binding site and to modulate spectral and/or functional properties (Balogh-Nair and Nakanishi, 1982; Derguini and Nakanishi, 1986; Liu and Asato, 1990; Crouch et al., 2002). Initially, this was mainly performed on bovine rod rhodopsin and bacteriorhodopsin, which were easily isolated in sufficient quantities. In this way both bathochromic and hypsochromic spectral shifts up to ca 80 nm could be realized, frequently with retardation of photo-kinetics or total loss of function. For instance, using “locked” retinals (blocking functional photo-transformations) it was confirmed that the photo-isomerization process was essential for the functionality and that the ring-polyene chain connection was 6-*s-cis* in type-2 rhodopsins and 6-*s-trans* in type-1 pigments (**Figure 1**) (Crouch et al., 1984; Fukada et al., 1984; Harbinson et al., 1985; van der Steen et al., 1986; DeGrip et al., 1990; Bhattacharya et al., 1992a; Ganapathy et al., 2015). Also, the remarkable observation was made with bovine opsin, that next to the 11-*cis* and 9-*cis* retinal, also the 7-*cis*, 7, 9-*dicis*, and 7, 9, 13-*tricyclic* retinal isomers could form a functional pigment, inducing a 40–50 nm blue-shift but reducing

thermostability (DeGrip et al., 1976; Liu et al., 1984). In general, it turned out that the bovine opsin binding pocket could better accommodate more voluminous modifications than the bacterio-opsin pocket, suggesting a more constrained character for the latter one. This was later validated in 3-D structures, but other type-1 pigments or photo-intermediates can be less selective (Popp et al., 1993; Inoue et al., 2012; Mori et al., 2013). New analogs are still frequently generated, in particular because recombinant production of mutated opsins modifies the binding pocket constraints. In addition, protein modeling has become more straightforward and for optogenetics larger spectral shifts and other functionalities like higher photosensitivity or higher fluorescence yields are in demand (see below).

Protein Joins In

Once recombinant DNA technology allowed the production of functional opsins in heterologous hosts, one could use this technology to adapt the intrinsic potential of opsins to one’s need and design. Combining synthetic retinal design with

recombinant DNA opsin modification opened up a marvelous toolbox to investigate the structure and functional mechanism of rhodopsins as well as to probe new functionalities and applications. This trend is evolving more and more rapidly. Initially, binding site residues were modified to probe their contribution to the packing, stabilization and spectral tuning of the chromophore (Khorana et al., 1987; Sakmar et al., 1989; Nathans, 1992; Sakmar and Fahmy, 1995; Giesbers et al., 2007). A salient example is the accumulating evidence for type-1 pigments, that three positions around the retinal chromophore, corresponding to L93, P186 and Ala215 in BR, function as natural spectral-tuning modules that systematically shift the absorbance spectrum of the chromophore without affecting molecular function (Table 5). This further inspired detailed analysis with modified and/or labeled retinals (^{13}C , ^2H , F) and protein residues (^{13}C , ^{15}N , F, azido, spin labels) using fluorescence, vibrational, EPR and NMR spectroscopy (Table 5). This profited from as well as steered development of sophisticated theoretical and *in-silico* procedures, like DFT, QM/MM and molecular dynamics (Altun et al., 2008; Collette et al., 2018; Del Carmen Marín et al., 2019b; Dokukina et al., 2019; Pieri et al., 2019; Shao et al., 2020; Nikolaev et al., 2021; Scholz and Neugebauer, 2021; Shen et al., 2021; Pedraza-González et al., 2022). All these elements have already profoundly deepened our insight into the structure and mechanism of bovine rod rhodopsin and bacteriorhodopsin, the frontrunners of type-2 and -1, respectively. However, more members are following up. Some recent conspicuous examples are mentioned in the next section.

Conversion

The manipulations described in the previous subsection frequently revealed surprising conversions in activity profile, exemplifying the versatile design principle of the rhodopsins (Kaneko et al., 2017). An interesting example is presented by the *Nonlabens marinus* inward chloride pump NMR-3 and the *Krokinobacter eikastus* sodium exporter KR2 (Hosaka et al., 2016; Yun et al., 2020). With only 35% sequence identity, the crystal structures are remarkably similar, but the gating residues for Cl^- and Na^+ are located at the opposite site of the membrane (Kato et al., 2015a; Hosaka et al., 2016; Kovalev et al., 2019; Yun et al., 2020). Another example is the huge mutagenesis effort that converted a thermophilic rhodopsin into the best thermally stable rhodopsin available to date, while retaining pump activity (Yasuda et al., 2022). On the other hand, selective mutations in the opsin could convert BR into an inward chloride pump, the sodium pump KR2 into a selective light-driven cation channel, the proton pumps Archaerhodopsin-3 (AR3) and *Coccomyxa subellipsoidea* rhodopsin (CsR) into light-driven proton channels, and the proton pump GR from *Gloeobacter violaceus* into a fluorescent chloride sensor (Sasaki et al., 1995; Brown et al., 1996; Inoue et al., 2015; 2016; Fudim et al., 2019; Vogt et al., 2019; Tutol et al., 2021). Alternatively, a cyanobacterial chloride pump could be converted into a proton pump (Hasemi et al., 2016; Kikukawa, 2021). Novel retinal A1 and A2 analogs with an elongated polyene chain (10 instead of 9 carbons) still could incorporate into the binding pocket of the

ReaChR channelrhodopsin inducing red-shifts up to ca 30 nm (Okitsu et al., 2020). However, when tested upon AR3, one A2 analog induced a 41 nm blue-shift and again converted it into a light-driven proton channel (Takayama et al., 2018). Another novel retinal analog (MMAR, Figure 5.) smoothly incorporated into the binding pocket of the proton pump Green Proteorhodopsin (GPR), inducing a 47 nm red-shift, but when combined with a Phe \rightarrow Ser mutation near the binding pocket, an unprecedented 200 nm red-shift was observed (Ganapathy et al., 2017). This retinal analog not only maintains some pump activity under near-infrared illumination (700–900 nm region; NIR), but also induces strong fluorescence emission in the NIR, probably emitted in the first picoseconds after excitation (Hontani et al., 2018; Mei et al., 2018; 2020). Proton-pumping rhodopsins in several eubacteria (XR, GR and TR) harbor a carotenoid derivative (salinixanthin) close enough to act as an antenna and transfer electronic excitation to the retinal (Balashov et al., 2010; Imasheva et al., 2011; Misra et al., 2019; Jana et al., 2020). This combination significantly broadens the spectral sensitivity of the rhodopsins for blue wavelengths, and the carotenoid binding option can also be introduced into other pigments (Anashkin et al., 2018). Attempts have also been made to generate chimeric pigments with combined functionality. The earliest example was a BR mutant containing loops of rod rhodopsin being able to weakly activate the G protein (Geiser et al., 2006). This concept in BR was further developed (Sasaki et al., 2014; Kaneko et al., 2017; Yoshida et al., 2017) and also found wider application in other rhodopsins (Kaneko et al., 2017). Chimeras could be produced between type-1 and type-2 pigments, often with shared properties and variable potential for G protein activation (Kojima et al., 1996; Geiser et al., 2006; Airan et al., 2009; Nakatsuma et al., 2011; Sasaki et al., 2014; Bedbrook et al., 2017a; Kaneko et al., 2017; Hickey et al., 2021). A remarkable example is that the C1C2 chimera could be crystallized and a high-resolution crystal structure obtained long before its “parent” channelrhodopsins ChR1 and ChR2, (Kato et al., 2012). In a sequel, new chimeric channelrhodopsins with better performance were generated using structure-guided recombination (Bedbrook et al., 2017a). The chimeric concept has also resulted in type-2 recombinants with variable success (Kojima et al., 1996; Giesbers et al., 2007; Hickey et al., 2021).

These selected examples, along with some more references collected in Table 5, already give an impression of the fabulous potential and prospects of the rhodopsin clan. The most impressive flux, however, is noticeable in the optogenetics field.

Optogenetics

Neuronal activity and circuitry are of the essence for multicellular life. Much effort is dedicated to studying activity regulation and circuitry in complex tissues like the brain. This used to be a highly challenging electrophysiological operation, requiring invasive electrodes and precise surgical location. Once it was realized, that rhodopsins could be properly expressed in animal tissues with genetic targeting to specific neurons using selective promoters, it became possible to monitor and regulate neuronal activity by light using endogenously expressed rhodopsins (Boyden et al., 2005). This led to an explosion of

research activity in a new field, coined optogenetics (Deisseroth, 2010, 2015; Rost et al., 2017; Kandori, 2020; Friedman, 2021). Initially, only type-1 rhodopsins were considered, since ion fluxes can directly modulate neuronal activity. Also, all-*trans* retinal is intrinsically available in animal cells and type-1 pigments complete a full photocycle.

In a first breakthrough, a cation-selective channelrhodopsin originally identified in *Chlamydomonas reinhardtii* termed ChR2 (Nagel et al., 2003) was exploited. ChR2 was shown to elicit action potentials in cultured neurons upon illumination (Boyden et al., 2005; Cardin et al., 2010; Klapoetke et al., 2014; Berndt et al., 2016; Deisseroth and Hegemann, 2017). This domain rapidly expanded into ion pumps, which can activate or silence neuronal activity (Chow et al., 2010). Simultaneously, pigments were modified to change spectral range, increase current output, alter photo- and response kinetics, improve membrane targeting, etc. (Lin et al., 2013; Kushibiki et al., 2014; Kato et al., 2015b; Brinks et al., 2016; Govorunova et al., 2017; Cho et al., 2019; Krol et al., 2019; Kojima et al., 2020b; Gong et al., 2020). Eventually, enzyme-rhodopsins as well as bistable type-2 pigments also entered the field, being able to modulate cellular metabolic processes up to gene expression (Mukherjee et al., 2019; Karapinar et al., 2021; Mahn et al., 2021; Rodgers et al., 2021; Tsunoda et al., 2021; Vierock et al., 2021). Bistable type-1 and -2 pigments allow further control, since their activity is triggered by illumination, but ends near the M(eta) stage, which can be photoreversed by illumination in another spectral range (Sheves and Friedman, 1986; Koyanagi and Terakita, 2014; Mederos et al., 2019; Eickelbeck et al., 2020).

A second breakthrough came with the discovery that the intensity of the fluorescence emission of the proton pumps GPR and AR3, be it quite weak, is modulated by the membrane potential (Kralj et al., 2011; Saint Clair et al., 2012b; Kralj et al., 2012). This triggered another burst of research dedicated to improve the voltage sensing of these pumps (minimizing pump activity, shifting spectral range, improving quantum yield, voltage sensing potential, temporal resolution, etc.) by a range of technologies like directed and scanning mutagenesis, multidimensional directed evolution, library screening and machine learning (McIsaac et al., 2014; Engqvist et al., 2015; McIsaac et al., 2015; Abdelfattah et al., 2016; Karasuyama et al., 2018; Kojima et al., 2020a). This was initially mostly performed on AR3, generating a whole family of mutants with different response characteristics (Quasar1 to 3, pa-Quasar3, Novarch, Archon1 and 2, Arch-EEN, Quasar6, Somarchon to name a few) (Piatkevich et al., 2019; Chien et al., 2021). The fluorescence of these voltage sensors most likely originates in late-stage photo-intermediates (Maclaurin et al., 2013). The introduced mutations may even result in a complex bistable photo-equilibrium between a fluorescent and a non-fluorescent state (Mei et al., 2021; Penzkofer et al., 2021). Meanwhile a host of additional voltage sensors have been developed. Next to optimized rhodopsins and chimeric rhodopsin fusions, fusion proteins of light-sensitive opsin cores with other fluorophores, often GFP derivatives or synthetic dyes, and of other voltage sensors with fluorescent rhodopsins have become popular

(Bando et al., 2019; Kannan et al., 2019; Lee et al., 2019; Berglund et al., 2020; Zhang X. M. et al., 2021).

Further control has been sought by combining optogenetics with classical electrophysiology (electro-optogenetics) or combining voltage sensors and neuronal activators and/or silencers both based on rhodopsins (all-optical electrophysiology) (Hochbaum et al., 2014; Afshar Saber et al., 2018; Sridharan et al., 2022). In the latter case, it is important to separate the spectral sensitivities to allow selective control and avoid optical cross-talk. In addition, much effort has been put into shifting the spectral range of the optogenetic tools and sensors as far as possible into the NIR, since NIR radiation penetrates much further into the mammalian brain (up to cm compared to several mm for e.g. blue-green light) (Larkum et al., 2018; Govorunova et al., 2020; Broser, 2022). For this purpose, mutagenesis of far-red absorbing rhodopsins like Crimson and CrimsonSA would be a good starting point (Oda et al., 2018). Another option is the novel channelrhodopsin ChRmine, which has quite unusual properties, including a trimeric structure similar to BR (Marshel et al., 2019; Kishi et al., 2022). A very fascinating example is NeoR, a subunit in the heterodimeric rhodopsin-cyclase from the fungus *Rhizoclostridium globosum*. NeoR is quite exceptional, as it harbors three carboxyl residues near the chromophore and has an absorbance maximum at 690 nm with strong fluorescence emission at 707 nm (Broser, 2022). Other gateways could include special optical technologies or local NIR-converting nanoparticles and two-photon spectroscopy, which are more complicated (Sneskov et al., 2013; Chen, 2019; Matarèse et al., 2019; Yu et al., 2019; Adesnik and Abdeladim, 2021; Lehtinen et al., 2022), or designing special retinal analogs. The latter was quite successful, shifting absorbance maxima up to ca 750 nm with fluorescent emission around 800 nm using merocyanine analogs or MMAR (Figure 5) (Derguini et al., 1983; Hoischen et al., 1997; Liu and Asato, 2003; Herwig et al., 2017; Hontani et al., 2018; Mei et al., 2020). The strong red-shift in these analog chromophores, as well as in the A1 chromophore in NeoR is contributed to extensive delocalization of the positive charge from the protonated Schiff base over the polyene element (Figures 1, 5) (Liu and Asato, 2003; Lutnaes et al., 2004; Ganapathy et al., 2019; Broser, 2022). This will strongly reduce the energy gap between the ground and first excited state. Incorporation of retinal A2 into NeoR-opsin already effectuates a further 69 nm red-shift (Broser et al., 2020). Hence, it would be very interesting to investigate whether the combination of NeoR-opsin or mutants with bathochromic analogs like MMAR would even further red-shift the absorbance band and increase the gap with the emission band. Optogenetic application, however, requires invasive administration of the retinal analog and may need transient depletion of the endogenous A1.

So far, the field of optogenetics has progressed spectacularly, from neuron and brain slice cultures, up to intact animals including insects, *C. elegans*, mice and macaques (Bi et al., 2006; Flytzanis et al., 2014; Inagaki et al., 2014; AzimiHashemi et al., 2019; Babl et al., 2019; Piatkevich et al., 2019; Gong et al., 2020; Wagner et al., 2021; Wright et al., 2021) and is being extended to human disease models (Wright et al., 2017; Williams et al., 2019; Córdova et al., 2021; Fougère et al.,

2021; Lindner et al., 2021). Future prospects will be touched upon in the next section.

Cell Factories

While rhodopsins drive important physiological processes in prokaryotes and eukaryotes, and can contribute significantly to the energy requirement of their hosts, implementing this into biotechnological resources like cell factories has not yet developed very far (Walter et al., 2010). *E. coli* can profit from expression of a rhodopsin proton pump (Martinez et al., 2007; Choi et al., 2014; Na et al., 2015; Wang et al., 2015; Kim et al., 2017; Song et al., 2020). However, the extent to which this can for instance support production of useful consumables or commodity chemicals needs to be established. Cyanobacteria like *Synechocystis* sp. PCC6803 and *Synechococcus* already exploit chlorophyll-based oxidative photosynthesis to gather solar energy and are under intense investigation as cellular factories (Wijffels et al., 2013; Angermayr et al., 2015; Du et al., 2018; Koot et al., 2018; Carpine et al., 2020). They do not have an endogenous opsin, but do produce all-*trans* retinal and can serve as a heterologous host for expression of rhodopsin proton pumps (Chen et al., 2016b; Chen et al., 2017; Chen et al., 2019a). Expression of these pumps was considered as a potential extra energy source, but the contribution of these pumps towards cellular energy production appeared to be limited (Chen et al., 2019b). This may be due to the metabolic constraint of proton fluxes, and/or to the chlorophylls and carotenoids absorbing much of the incoming radiation up to ca 650 nm (the PAR region). Attempts to express the GPR F234S mutant in combination with the retinal analog MMAR were successful in generating a proton pump absorbing in the 700–800 nm range, outside the PAR region. However, this still did not generate sufficient additional energy due to the lower pump activity of this mutant and failed to sustain bacterial growth under NIR illumination (Chen et al., 2018).

PROSPECTS

A major asset of the rhodopsin family is the impressive versatility of the design principle: a relatively simple photosensitive ligand, constrained to allow selective photoisomerization with a high quantum yield, triggering subtle but effective conformational changes in the protein opening up specific binding sites or ion transport pathways.

Genome mining will undoubtedly discover new type-1 and type-2 or related variants, especially considering the still vast reservoir of unexplored microbial and invertebrate life forms. For instance, the apparent non-photoc activity of (rhod)opsins in certain physiological conditions (thermo-, mechano- or chemo-sensing) may add a new chapter to this family saga (Leung and Montell, 2017; Katana et al., 2019; Baden et al., 2020; Fleming et al., 2020; Hasegawa et al., 2020; Mei et al., 2020; Zabelskii et al., 2021; Feuda et al., 2022). Next to that, insight into the effect of pathological mutations will become an ever more important asset in medical diagnostics and potential treatment. This has already been widely explored in the case of rod rhodopsin and retina-degenerative diseases, (Athanasίου et al., 2018). Expression and functional and structural characterization of new

(rhod)opsins or mutants still involves an elaborate effort, but this may be considerably mitigated soon.

The phenomenal progress in *artificial intelligence and machine learning* already culminated in the design of software packages like RoseTTAFold and Alphafold, that are quite successful in predicting the protein fold from the primary sequence (Humphreys et al., 2021; Jumper et al., 2021). Considering the respectable number of crystal structures for type-1 pigments and G protein-coupled receptors already obtained, this *in silico* approach will be of invaluable help to close in on the 3-D structures of rhodopsin sequences identified to date, as well as those yet to be identified. A similar track is conceivable for the assessment of spectral and functional properties. Experimental analyses, in combination with *in-silico* techniques like DFT, machine learning and quantum-chemical computing already made big strides in establishing the contribution of individual opsin residues and water molecules to the spectral tuning of rhodopsins (Kato et al., 2015; Bedbrook et al., 2017b; Karasuyama et al., 2018; Bedbrook et al., 2019; Nikolaev et al., 2020; Inoue et al., 2021; Yang et al., 2022). However, this approach always requires 3-D information. It would be very desirable to build in additional functionalities, e.g. to predict an approximate absorbance maximum, into the sequence-to-structure software packages. This could then be easily expanded towards predicting the effect of mutations and the fit and effects of retinal derivatives or even more distant chromophores. Suggestions for functionality (specific pump or channel, enzymatic domains, thermal stability) probably could also be in reach, though mechanistic details (photoisomerization process, quantum yield of isomerization or fluorescence emission, early conformational changes) may be aiming too high.

Such developments will be a goldmine for *optogenetics*. Rapid prediction of spectral and functional properties and optimal targeting of desired mutants would be very valuable. Likewise, assessment of new constructs like chimeric pigments, fused monomers, oligomeric assemblies, enzyme activating pigments, new signaling partners and the like can be set up *in silico* and will require much less experimental justification (Sasaki et al., 2014; Abdelfattah et al., 2020). This would undoubtedly be accompanied by further physiological expansion of optogenetic tools. A wider spectral range of neuronal activity modulators and voltage sensors together with improved optics will increase the scope for (all)-optical electrophysiological characterization of neural circuitry, also lending insight into neuronal function (and dysfunction) in the brain (Villette et al., 2019; Guimarães Backhaus et al., 2021; Sharma et al., 2021; Zou et al., 2021; Prakash et al., 2022; Sridharan et al., 2022; Tan et al., 2022). Other important medical targets may also arise using optogenetics to correct physiological defects and address pathological conditions, where first steps have already been taken (Braun et al., 1995; Deubner et al., 2019; Shen et al., 2020; Acharya et al., 2021; Cokic et al., 2021; Kathe et al., 2021; Gilhooley et al., 2022; Sun et al., 2022).

Several concepts to utilize rhodopsins in *bioelectronic and biomimic nanotechnology* have already been attempted, but did not yet really come to maturation (Khodonov et al., 2000; Kuang et al., 2014; Hirschi et al., 2019; Arahamian, 2020; Shim et al.,

2021). With the rapidly growing insight in the structural and mechanistic potential of the rhodopsin pigments, this is expected to change at short notice. So far, electro-optical phenomena have been investigated in 2D crystals, lipid films and other matrices (Oesterhelt et al., 1991; Miyasaka et al., 1992; Hong, 1994; Wagner et al., 2013; Zhao et al., 2015; Ji et al., 2017; Gruber et al., 2022). With help of the above mentioned software packages, the design of specific constructs with high performance and stability under the system's conditions will be facilitated.

This would also be the case for application in *cell factories*. The most interesting and rewarding application in this respect is the notion of “synergistic photosynthesis,” the combination of chlorophyll-based oxidative photosynthesis with retinal-based phototrophy, using high-performance rhodopsin proton pumps absorbing in the NIR (Chen et al., 2016a; Chen et al., 2019b). This will also require adaptation of proton regulation in the host cell or introduction of special cellular organelles containing the pump and an ATP-synthase. In eukaryotic cells like algae or fungi, targeting of a proton pump to mitochondria to increase ATP levels for production of commodity chemicals under selected conditions can be further developed (Hoffmann et al., 1994; Hara et al., 2013; Tkatch et al., 2017; Imai et al., 2019; Berry and Wojtovich, 2020). In general, designing highly active ion pumps absorbing in the 700–800 nm region, i.e. outside the PAR region, is essential for productive “synergistic photosynthesis.” Again, artificial intelligence can be a decisive factor here.

EPILOGUE

In roughly 10 years, the rhodopsin field has reached a century's worth of experimental investigation. In this review, we have

REFERENCES

- Abdelfattah, A. S., Farhi, S. L., Zhao, Y., Brinks, D., Zou, P., Ruangkittisakul, A., et al. (2016). A Bright and Fast Red Fluorescent Protein Voltage Indicator that Reports Neuronal Activity in Organotypic Brain Slices. *J. Neurosci.* 36, 2458–2472. doi:10.1523/jneurosci.3484-15.2016
- Abdelfattah, A. S., Valenti, R., Zheng, J., Wong, A., Chuong, A. S., Hasseman, J. P., et al. (2020). A General Approach to Engineer Positive-Going eFRET Voltage Indicators. *Nat. Commun.* 11, 3444–34413448. doi:10.1038/s41467-020-17322-1
- Abdulaev, N. G., Artamonov, I. D., Bogachuk, A. S., Feigina, M. Y., Kostina, M. B., Kudelin, A. B., et al. (1982). Structure of Light-Activated Proteins - Visual Rhodopsin. *Biochem. Int.* 5, 693–703.
- Abdulaev, N. G., and Ridge, K. D. (2000). Heterologous Expression of Bovine Opsin in *Pichia pastoris*. *Meth. Enzymol.* 315, 3–11. doi:10.1016/s0076-6879(00)15831-8
- Acharya, A. R., Vandekerckhove, B., Larsen, L. E., Delbeke, J., Wadman, W. J., Vonck, K., et al. (2021). *In Vivo* blue Light Illumination for Optogenetic Inhibition: Effect on Local Temperature and Excitability of the Rat hippocampus. *J. Neural Eng.* 18, 066038–066031. doi:10.1088/1741-2552/ac3ef4
- Adesnik, H., and Abdeladim, L. (2021). Probing Neural Codes with Two-Photon Holographic Optogenetics. *Nat. Neurosci.* 24, 1356–1366. doi:10.1038/s41593-021-00902-9
- Afshar Saber, W., Gasparoli, F. M., Dirks, M. G., Gunn-Moore, F. J., and Antkowiak, M. (2018). All-Optical Assay to Study Biological Neural Networks. *Front. Neurosci.* 12, 451–451412. doi:10.3389/fnins.2018.00451
- Agathangelou, D., Orozco-Gonzalez, Y., Del Carmen Marín, M., Roy, P. P., Brazard, J., Kandori, H., et al. (2018). Effect of Point Mutations on the Ultrafast Photo-Isomerization of *Anabaena* Sensory Rhodopsin. *Faraday Discuss.* in the press. doi:10.1039/c7fd00200a
- Agathangelou, D., Roy, P. P., Del Carmen Marín, M., Ferré, N., Olivucci, M., Backup, T., et al. (2021). Sub-picosecond C=C Bond Photo-Isomerization: Evidence for the Role of Excited State Mixing. *Comptes Rendus Phys.* 22, 1–28. doi:10.5802/crphys.41
- Aguilá, M., Toledo, D., Morillo, M., Dominguez, M., Vaz, B., Álvarez, R., et al. (2009). Structural Coupling of 11-*Cis*-7-Methyl-Retinal and Amino Acids at the Ligand Binding Pocket of Rhodopsin. *Photochem. Photobiol.* 85, 485–493. doi:10.1111/j.1751-1097.2009.00535.x
- Airán, R. D., Thompson, K. R., Fenno, L. E., Bernstein, H., and Deisseroth, K. (2009). Temporally Precise *In Vivo* Control of Intracellular Signalling. *Nature* 458, 1025–1029. doi:10.1038/nature07926
- Alabugin, A. (2019). Near-IR Photochemistry for Biology: Exploiting the Optical Window of Tissue. *Photochem. Photobiol.* 95, 722–732. doi:10.1111/php.13068
- Alexander, N. S., Katayama, K., Sun, W. Y., Salom, D., Gulati, S., Zhang, J. Y., et al. (2017). Complex Binding Pathways Determine the Regeneration of Mammalian Green Cone Opsin with a Locked Retinal Analogue. *J. Biol. Chem.* 292, 10983–10997. doi:10.1074/jbc.m117.780478
- Alexiev, U., and Farrens, D. L. (2014). Fluorescence Spectroscopy of Rhodopsins: Insights and Approaches. *Biochimica Biophysica Acta-Bioenergetics* 1837, 694–709. doi:10.1016/j.bbabi.2013.10.008
- Alexiev, U., Mollaaghababa, R., Khorana, H. G., and Heyn, M. P. (2000). Evidence for Long Range Allosteric Interactions between the Extracellular and Cytoplasmic Parts of Bacteriorhodopsin from the Mutant R82A and its

mainly touched upon the surface of the phenomenal development in this field, somewhat like molecular force microscopy. In the coming 10 years we expect its expansion to continue and to eventually require an at least ten-volume book series for full documentation. By that time, we will hopefully have a better understanding of how a selection of twenty amino acids can lead a membrane protein domain of 300–400 amino acids surrounding a small chromophoric group to such mechanistic versatility.

AUTHOR CONTRIBUTIONS

WdeG conceptualized and wrote the first draft of the manuscript. SG elaborated on sections of the manuscript and prepared the figures. Both authors contributed to manuscript revision, read, and approved the submitted version.

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- Second Site Revertant R82A/G231C. *J. Biol. Chem.* 275, 13431–13440. doi:10.1074/jbc.275.18.13431
- Alexiev, U., Rimke, I., and Pöhlmann, T. (2003). Elucidation of the Nature of the Conformational Changes of the EF-Interhelical Loop in Bacteriorhodopsin and of the Helix VIII on the Cytoplasmic Surface of Bovine Rhodopsin: A Time-Resolved Fluorescence Depolarization Study. *J. Mol. Biol.* 328, 705–719. doi:10.1016/S0022-2836(03)00326-7
- Alfonsa, H., Lakey, J. H., Lightowers, R. N., and Trevelyan, A. J. (2016). Cl-out Is a Novel Cooperative Optogenetic Tool for Extruding Chloride from Neurons. *Nat. Commun.* 7, 13495–13499. doi:10.1038/ncomms13495
- Altenbach, C. A., Kusnetzow, A. K., Ernst, O. P., Hofmann, K. P., and Hubbell, W. L. (2008). High-resolution Distance Mapping in Rhodopsin Reveals the Pattern of Helix Movement Due to Activation. *Proc. Natl. Acad. Sci. U. S. A.* 105, 7439–7444. doi:10.1073/pnas.0802515105
- Altun, A., Yokoyama, S., and Morokuma, K. (2008). Spectral Tuning in Visual Pigments: An ONIOM(QM : MM) Study on Bovine Rhodopsin and its Mutants. *J. Phys. Chem. B* 112, 6814–6827. doi:10.1021/jp709730b
- Álvarez, R., Vaz, B., Gronemeyer, H., and De Lera, A. R. (2014). Functions, Therapeutic Applications, and Synthesis of Retinoids and Carotenoids. *Chem. Rev.* 114, 1–125. doi:10.1021/cr400126u
- Amsden, J. J., Kralj, J. M., Chieffo, L. R., Wang, X. H., Erramilli, S., Spudich, E. N., et al. (2007). Subpicosecond Protein Backbone Changes Detected during the Green-Absorbing Proteorhodopsin Primary Photoreaction. *J. Phys. Chem. B* 111, 11824–11831. doi:10.1021/jp073490r
- Anashkin, V. A., Bertsova, Y. V., Mamedov, A. M., Mamedov, M. D., Arutyunyan, A. M., Baykov, A. A., et al. (2018). Engineering a Carotenoid-Binding Site in *Dokdonia* Sp PRO95 Na⁺-Translocating Rhodopsin by a Single Amino Acid Substitution. *Photosynth. Res.* 136, 161–169. doi:10.1007/s11120-017-0453-0
- Angel, T. E., Gupta, S., Jastrzebska, B., Palczewski, K., and Chance, M. R. (2009). Structural Waters Define a Functional Channel Mediating Activation of the GPCR, Rhodopsin. *Proc. Natl. Acad. Sci. U. S. A.* 106, 14367–14372. doi:10.1073/pnas.0901074106
- Angermayr, S. A., Rovira, A. G., and Hellingwerf, K. J. (2015). Metabolic Engineering of Cyanobacteria for the Synthesis of Commodity Products. *Trends Biotechnol.* 33, 352–361. doi:10.1016/j.tibtech.2015.03.009
- Arahamian, I. (2020). The Future of Molecular Machines. *Acs Central Sci.* 6, 347–358. doi:10.1021/acscentsci.0c00064
- Armstrong, C. M. (1992). Voltage-dependent Ion Channels and Their Gating. *Physiol. Rev.* 72, S5–S13. doi:10.1152/physrev.1992.72.suppl_4.s5
- Arnaboldi, M., Motto, M. G., Tsujimoto, K., Balogh-Nair, V., and Nakanishi, K. (1979). Hydroretinals and Hydrorhodopsins. *J. Am. Chem. Soc.* 101, 7082–7084. doi:10.1021/ja00517a059
- Asenjo, A. B., Rim, J., and Oprian, D. D. (1994). Molecular Determinants of Human Red/green Color Discrimination. *Neuron* 12, 1131–1138. doi:10.1016/0896-6273(94)90320-4
- Asido, M., Kar, R. K., Kriebel, C. N., Braun, M., Glaubitz, C., Schapiro, I., et al. (2021). Transient Near-UV Absorption of the Light-Driven Sodium Pump *Krokinobacter Eikastus* Rhodopsin 2: A Spectroscopic Marker for Retinal Configuration. *J. Phys. Chem. Lett.* 12, 6284–6291. doi:10.1021/acs.jpcclett.1c01436
- Astakhova, L. A., Novoselov, A. D., Ermolaeva, M. E., Firsov, M. L., and Rotov, A. Y. (2021). Phototransduction in Anuran Green Rods: Origins of Extrasensitivity. *Int. J. Mol. Sci.* 22, 13400. doi:10.3390/ijms222413400
- Athanasiou, D., Aguila, M., Bellingham, J., Li, W. W., Mcculley, C., Reeves, P. J., et al. (2018). The Molecular and Cellular Basis of Rhodopsin Retinitis Pigmentosa Reveals Potential Strategies for Therapy. *Prog. Retin. Eye Res.* 62, 1–23. doi:10.1016/j.preteyeres.2017.10.002
- Avelar, G. M., Schumacher, R. I., Zaini, P. A., Leonard, G., Richards, T. A., and Gomes, S. L. (2014). A Rhodopsin-Guanylyl Cyclase Gene Fusion Functions in Visual Perception in a Fungus. *Curr. Biol.* 24, 1234–1240. doi:10.1016/j.cub.2014.04.009
- Axford, D., Judge, P. J., Bada Juarez, J. F., Kwan, T. O. C., Birch, J., Vinals, J., et al. (2022). Two States of a Light-Sensitive Membrane Protein Captured at Room Temperature Using Thin-Film Sample Mounts. *Acta Crystallogr. Sect. D. Struct. Biol.* 78, 52–58. doi:10.1107/s2059798321011220
- Azadi-Chegeni, F., Schiphorst, C., and Pandit, A. (2018). *In Vivo* NMR as a Tool for Probing Molecular Structure and Dynamics in Intact *Chlamydomonas Reinhardtii* Cells. *Photosynth. Res.* 135, 227–237. doi:10.1007/s11120-017-0412-9
- Azimihashemi, N., Bergs, A. C. F., Schüler, C., Scheiwe, A. R., Costa, W. S., Bach, M., et al. (2019). Rhodopsin-based Voltage Imaging Tools for Use in Muscles and Neurons of *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U. S. A.* 116, 17051–17060. doi:10.1073/pnas.1902443116
- Azimihashemi, N., Erbguth, K., Vogt, A., Riemensperger, T., Rauch, E., Woodmansee, D., et al. (2014). Synthetic Retinal Analogues Modify the Spectral and Kinetic Characteristics of Microbial Rhodopsin Optogenetic Tools. *Nat. Commun.* 5, 5810. doi:10.1038/ncomms6810
- Babl, S. S., Rummell, B. P., and Sigurdsson, T. (2019). The Spatial Extent of Optogenetic Silencing in Transgenic Mice Expressing Channelrhodopsin in Inhibitory Interneurons. *Cell. Rep.* 29, 1381–1395. doi:10.1016/j.celrep.2019.09.049
- Bada Juarez, J. F., Judge, P. J., Adam, S., Axford, D., Vinals, J., Birch, J., et al. (2021). Structures of the Archaeorhodopsin-3 Transporter Reveal that Disordering of Internal Water Networks Underpins Receptor Sensitization. *Nat. Commun.* 12, 629. doi:10.1038/s41467-020-20596-0
- Baden, T., Euler, T., and Berens, P. (2020). Understanding the Retinal Basis of Vision across Species. *Nat. Rev. Neurosci.* 21, 5–20. doi:10.1038/s41583-019-0242-1
- Bagley, K. A., Eisenstein, L., Ebrey, T. G., and Tsuda, M. (1989). A Comparative Study of the Infrared Difference Spectra for octopus and Bovine Rhodopsins and Their Bathorhodopsin Photointermediates. *Biochemistry-USA* 28, 3366–3373. doi:10.1021/bi00434a036
- Baillie, J. S., Stoyek, M. R., and Quinn, T. A. (2021). Seeing the Light: The Use of Zebrafish for Optogenetic Studies of the Heart. *Front. physiology* 12, 748570. doi:10.3389/fphys.2021.748570
- Baker, G. E., De Grip, W. J., Turton, M., Wagner, H.-J., Foster, R. G., and Douglas, R. H. (2015). Light Sensitivity in a Vertebrate Mechanoreceptor? *J. Exp. Biol.* 218, 2826–2829. doi:10.1242/jeb.125203
- Balashov, S. P., Imasheva, E. S., Choi, A. R., Jung, K.-H., Llaeaen-Jensen, S., and Lanyi, J. K. (2010). Reconstitution of *Gloeobacter* Rhodopsin with Echinone: Role of the 4-keto Group. *Biochemistry* 49, 9792–9799. doi:10.1021/bi1014166
- Balogh-Nair, V., and Nakanishi, K. (1982). Synthetic Analogs of Retinal, Bacteriorhodopsin and Bovine Rhodopsin. *Meth. Enzymol.* 88, 496–506. doi:10.1016/0076-6879(82)88067-1
- Bamann, C., Bamberg, E., Wachtveitl, J., and Glaubitz, C. (2014). Proteorhodopsin. *Biochimica Biophysica Acta-Bioenergetics* 1837, 614–625. doi:10.1016/j.bbabi.2013.09.010
- Bando, Y., Grimm, C., Cornejo, V. H., and Yuste, R. (2019). Genetic Voltage Indicators. *BMC Biol.* 17, 71–7112. doi:10.1186/s12915-019-0682-0
- Banerjee, S., Huber, T., and Sakmar, T. P. (2008). Rapid Incorporation of Functional Rhodopsin into Nanoscale Apolipoprotein Bound Bilayer (NABB) Particles. *J. Mol. Biol.* 377, 1067–1081. doi:10.1016/j.jmb.2008.01.066
- Banskota, S., Raguram, A., Suh, S., Du, S. W., Davis, J. R., Choi, E. H., et al. (2022). Engineered Virus-like Particles for Efficient *In Vivo* Delivery of Therapeutic Proteins. *Cell.* 185. doi:10.1016/j.cell.2021.12.021
- Barry, B., and Mathies, R. A. (1987). Raman Microscope Studies on the Primary Photochemistry of Vertebrate Visual Pigments with Absorption Maxima from 430 to 502 Nm. *Biochemistry* 26, 59–64. doi:10.1021/bi00375a009
- Bayburt, T. H., Grinkova, Y. V., and Sligar, S. G. (2006). Assembly of Single Bacteriorhodopsin Trimers in Bilayer Nanodiscs. *Archives Biochem. Biophys.* 450, 215–222. doi:10.1016/j.abb.2006.03.013
- Bayburt, T. H., Vishnivetskiy, S. A., Mclean, M. A., Morizumi, T., Huang, C.-C., Tesmer, J. J. G., et al. (2011). Monomeric Rhodopsin Is Sufficient for Normal Rhodopsin Kinase (GRK1) Phosphorylation and Arrestin-1 Binding. *J. Biol. Chem.* 286, 1420–1428. doi:10.1074/jbc.m110.151043
- Bayraktar, H., Fields, A. P., Kralj, J. M., Spudich, J. L., Rothschild, K. J., and Cohen, A. E. (2012). Ultrasensitive Measurements of Microbial Rhodopsin Photocycles Using Photochromic FRET. *Photochem. Photobiol.* 88, 90–97. doi:10.1111/j.1751-1097.2011.01011.x
- Becker-Baldus, J., Bamann, C., Saxena, K., Gustmann, H., Brown, L. J., Brown, R. C. D., et al. (2015). Enlightening the Photoactive Site of Channelrhodopsin-2 by DNP-Enhanced Solid-State NMR Spectroscopy. *Proc. Natl. Acad. Sci. U. S. A.* 112, 9896–9901. doi:10.1073/pnas.1507713112
- Bedbrook, C. N., Rice, A. J., Yang, K. K., Ding, X. Z., Chen, S. Y., Leproust, E. M., et al. (2017a). Structure-guided SCHEMA Recombination Generates Diverse

- Chimeric Channelrhodopsins. *Proc. Natl. Acad. Sci. U. S. A.* 114, E2624–E2633. doi:10.1073/pnas.1700269114
- Bedbrook, C. N., Yang, K. K., Rice, A. J., Gradinaru, V., and Arnold, F. H. (2017b). Machine Learning to Design Integral Membrane Channelrhodopsins for Efficient Eukaryotic Expression and Plasma Membrane Localization. *Plos Comput. Biol.* 13, 1005786. doi:10.1371/journal.pcbi.1005786
- Bedbrook, C. N., Yang, K. K., Robinson, J. E., Mackey, E. D., Gradinaru, V., and Arnold, F. H. (2019). Machine Learning-Guided Channelrhodopsin Engineering Enables Minimally Invasive Optogenetics. *Nat. Methods* 16, 1176–1184. doi:10.1038/s41592-019-0583-8
- Béjà, O., Aravind, L., Koonin, E. V., Suzuki, M. T., Hadd, A., Nguyen, L. P., et al. (2000). Bacterial Rhodopsin: Evidence for a New Type of Phototrophy in the Sea. *Science* 289, 1902–1906. doi:10.1126/science.289.5486.1902
- Béjà, O., Spudich, E. N., Spudich, J. L., Leclerc, M., and DeLong, E. F. (2001). Proteorhodopsin Phototrophy in the Ocean. *Nature* 411, 786–789. doi:10.1038/35081051
- Belrhali, H., Nollert, P., Royant, A., Menzel, C., Rosenbusch, J. P., Landau, E. M., et al. (1999). Protein, Lipid and Water Organization in Bacteriorhodopsin Crystals: A Molecular View of the Purple Membrane at 1.9 Angstrom Resolution. *Struct. Fold. Des.* 7, 909–917. doi:10.1016/s0969-2126(99)80118-x
- Bennett, N., Michel-Villaz, M., and Kühn, H. (1982). Light-induced Interaction between Rhodopsin and the GTP-Binding Protein: Metarhodopsin-II Is the Major Photoproduct Involved. *Eur. J. Biochem.* 127, 97–103. doi:10.1111/j.1432-1033.1982.tb06842.x
- Berglund, K., Clissold, K., Li, H. F. E., Wen, L., Park, S. Y., Gleixner, J., et al. (2016). Luminopsins Integrate Opto- and Chemogenetics by Using Physical and Biological Light Sources for Opsin Activation. *Proc. Natl. Acad. Sci. U. S. A.* 113, E358–E367. doi:10.1073/pnas.1510899113
- Berglund, K., Fernandez, A. M., Gutekunst, C. a. N., Hochgeschwender, U., and Gross, R. E. (2020). Step-function Luminopsins for Bimodal Prolonged Neuromodulation. *J. Neurosci. Res.* 98, 422–436. doi:10.1002/jnr.24424
- Bergo, V. B., Ntefidou, M., Trivedi, V. D., Amsden, J. J., Kralj, J. M., Rothschild, K. J., et al. (2006). Conformational Changes in the Photocycle of Anabaena Sensory Rhodopsin - Absence Of the Schiff Base Counterion Protonation Signal. *J. Biol. Chem.* 281, 15208–15214. doi:10.1074/jbc.m600033200
- Berndt, A., Lee, S. Y., Ramakrishnan, C., and Deisseroth, K. (2014). Structure-Guided Transformation of Channelrhodopsin into a Light-Activated Chloride Channel. *Science* 344, 420–424. doi:10.1126/science.1252367
- Berndt, A., Lee, S. Y., Wietek, J., Ramakrishnan, C., Steinberg, E. E., Rashid, A. J., et al. (2016). Structural Foundations of Optogenetics: Determinants of Channelrhodopsin Ion Selectivity. *Proc. Natl. Acad. Sci. U. S. A.* 113, 822–829. doi:10.1073/pnas.1523341113
- Berry, B. J., and Wojtovich, A. P. (2020). Mitochondrial Light Switches: Optogenetic Approaches to Control Metabolism. *FEBS J.* 287, 4544–4556. doi:10.1111/febs.15424
- Berry, M. H., Holt, A., Salari, A., Veit, J., Visel, M., Levitz, J., et al. (2019). Restoration of High-Sensitivity and Adapting Vision with a Cone Opsin. *Nat. Commun.* 10, 1221. doi:10.1038/s41467-019-09124-x
- Bertazzolli-Filho, R., Ghosh, S., Huang, W. H., Wollmann, G., and Coca-Prados, M. (2001). Molecular Evidence that Human Ocular Ciliary Epithelium Expresses Components Involved in Phototransduction. *Biochem. Biophysical Res. Commun.* 284, 317–325. doi:10.1006/bbrc.2001.4970
- Besaw, J. E., Ou, W. L., Morizumi, T., Eger, B. T., Sanchez Vasquez, J. D., Chu, J. H. Y., et al. (2020). The Crystal Structures of a Chloride-Pumping Microbial Rhodopsin and its Proton-Pumping Mutant Illuminate Proton Transfer Determinants. *J. Biol. Chem.* 295, 14793–14804. doi:10.1074/jbc.ra120.014118
- Bhattacharya, S., Hall, S. E., and Vaidehi, N. (2008). Agonist-induced Conformational Changes in Bovine Rhodopsin: Insight into Activation of G-Protein-Coupled Receptors. *J. Mol. Biol.* 382, 539–555. doi:10.1016/j.jmb.2008.06.084
- Bhattacharya, S. S., Marti, T., Otto, H., Heyn, M. P., and Khorana, H. G. (1992a). A Bacteriorhodopsin Analog Reconstituted with a Nonisomerizable 13-trans Retinal Derivative Displays Light Insensitivity. *J. Biol. Chem.* 267, 6757–6762. doi:10.1016/s0021-9258(19)50490-2
- Bhattacharya, S. S., Ridge, K. D., Knox, B. E., and Khorana, H. G. (1992b). Light-stable Rhodopsin. I. A Rhodopsin Analog Reconstituted with a Nonisomerizable 11-cis Retinal Derivative. *J. Biol. Chem.* 267, 6763–6769. doi:10.1016/s0021-9258(19)50491-4
- Bi, A. D., Cui, J. J., Ma, Y.-P., Olshevskaya, E. V., Pu, M. L., Dizhoor, A. M., et al. (2006). Ectopic Expression of a Microbial-type Rhodopsin Restores Visual Responses in Mice with Photoreceptor Degeneration. *Neuron* 50, 23–33. doi:10.1016/j.neuron.2006.02.026
- Bibow, S. (2019). Opportunities and Challenges of Backbone, Sidechain, and RDC Experiments to Study Membrane Protein Dynamics in a Detergent-free Lipid Environment Using Solution State NMR. *Front. Mol. Biosci.* 6, 103. doi:10.3389/fmolb.2019.00103
- Bickelmann, C., Morrow, J. M., Du, J., Schott, R. K., Van Hazel, I., Lim, S., et al. (2015). The Molecular Origin and Evolution of Dim-Light Vision in Mammals. *Evolution* 69, 2995–3003. doi:10.1111/evo.12794
- Bielawski, J. P., Dunn, K. A., Sabehi, G., and Béjà, O. (2004). Darwinian Adaptation of Proteorhodopsin to Different Light Intensities in the Marine Environment. *Proc. Natl. Acad. Sci. U. S. A.* 101, 14824–14829. doi:10.1073/pnas.0403999101
- Birge, R. R., Cooper, T. M., Lawrence, A. F., Masthay, M. B., Zhang, C.-F., and Zidovetzki, R. (1991). Revised Assignment of Energy Storage in the Primary Photochemical Event in Bacteriorhodopsin. *J. Am. Chem. Soc.* 113, 4327–4328. doi:10.1021/ja00011a043
- Bismuth, O., Friedman, N., Sheves, M., and Ruhman, S. (2007). Photochemical Dynamics of All-Trans Retinal Protonated Schiff-Base in Solution: Excitation Wavelength Dependence. *Chem. Phys.* 341, 267–275. doi:10.1016/j.chemphys.2007.06.052
- Blackshaw, S., and Snyder, S. H. (1999). Encephalopsin: A Novel Mammalian Extraretinal Opsin Discretely Localized in the Brain. *J. Neurosci.* 19, 3681–3690. doi:10.1523/jneurosci.19-10-03681.1999
- Blackshaw, S., and Snyder, S. H. (1997). Parapinopsin, a Novel Catfish Opsin Localized to the Parapineal Organ, Defines a New Gene Family. *J. Neurosci.* 17, 8083–8092. doi:10.1523/jneurosci.17-21-08083.1997
- Blankenship, E., Vahedi-Faridi, A., and Lodowski, D. T. (2015). The High-Resolution Structure of Activated Opsin Reveals a Conserved Solvent Network in the Transmembrane Region Essential for Activation. *Structure* 23, 2358–2364. doi:10.1016/j.str.2015.09.015
- Blatz, P. E., Lin, M., Balasubramanian, P., Balasubramanian, V., and Dewhurst, P. B. (1969). A New Series of Synthetic Visual Pigments from Cattle Opsin and Homologs of Retinal. *J. Am. Chem. Soc.* 91, 5930–5931. doi:10.1021/ja011049a069
- Bliss, A. F. (1948). The Absorption Spectra of Visual Purple of the Squid and its Bleaching Products. *J. Biol. Chem.* 176, 563–569. doi:10.1016/s0021-9258(19)52673-4
- Bogomolni, R. A., and Spudich, J. L. (1991). Archaeobacterial Rhodopsins: Sensory and Energy Transducing Membrane Proteins. *Mod. Cell. Biol.* 10, 233–255.
- Boll, F. (1877). Zur Anatomie und Physiologie der Retina. *Arch. Anat. Physiol.* 2, 175–286. doi:10.1007/BF02962033
- Bondar, A.-N. (2022). Mechanisms of Long-Distance Allosteric Couplings in Proton-Binding Membrane Transporters. *Adv. Protein Chem. Struct. Biol.* 128, 199–239. doi:10.1016/bs.apcsb.2021.09.002
- Borch, J., and Hamann, T. (2009). The Nanodisc: A Novel Tool for Membrane Protein Studies. *Biol. Chem.* 390, 805–814. doi:10.1515/BC.2009.091
- Borgia, A., Borgia, M. B., Bugge, K., Kissling, V. M., Heidarsson, P. O., Fernandes, C. B., et al. (2018). Extreme Disorder in an Ultrahigh-Affinity Protein Complex. *Nature* 555, 61–66. doi:10.1038/nature25762
- Bosman, G. J. C. G. M., Vanoostrum, J., Breikers, G., Bovee-Geurts, P. H. M., Klaassen, C. H. W., and DeGrip, W. J. (2003). Functional Expression of His-Tagged Rhodopsin in Sf9 Insect Cells. *Meth. Mol. Biol.* 228, 73–86. doi:10.1385/1-59259-400-X:73
- Bovee-Geurts, P. H. M., Fernández Fernández, I., Liu, R. S. H., Mathies, R. A., Lugtenburg, J., and DeGrip, W. J. (2009). Fluoro Derivatives of Retinal Illuminate the Decisive Role of the C₁₂-H Element in Photoisomerization and Rhodopsin Activation. *J. Am. Chem. Soc.* 131, 17933–17942. doi:10.1021/ja907577p
- Bovee-Geurts, P. H. M., Lugtenburg, J., and DeGrip, W. J. (2017). Coupled HOOP Signature Correlates with Quantum Yield of Isorhodopsin and Analog Pigments. *Biochimica Biophysica Acta-Bioenergetics* 1858, 118–125. doi:10.1016/j.bbabi.2016.11.003
- Bownds, M. D. (1967). Site of Attachment of Retinal in Rhodopsin. *Nature* 216, 1178–1181. doi:10.1038/2161178a0

- Boyden, E. S., Zhang, F., Bamberg, E., Nagel, G., and Deisseroth, K. (2005). Millisecond-timescale, Genetically Targeted Optical Control of Neural Activity. *Nat. Neurosci.* 8, 1263–1268. doi:10.1038/nn1525
- Bratanov, D., Balandin, T., Round, E., Shevchenko, V., Gushchin, I., Polovinkin, V., et al. (2015). An Approach to Heterologous Expression of Membrane Proteins. The Case of Bacteriorhodopsin. *PLoS ONE* 10, e0128390. doi:10.1371/journal.pone.0128390
- Bratanov, D., Kovalev, K., Machtens, J.-P., Astashkin, R., Chizhov, I., Soloviov, D., et al. (2019). Unique Structure and Function of Viral Rhodopsins. *Nat. Commun.* 10, 4939. doi:10.1038/s41467-019-12718-0
- Braun, R. D., Linsenmeier, R. A., and Goldstick, T. K. (1995). Oxygen Consumption in the Inner and Outer Retina of the Cat. *Investig. Ophthalmol. Vis. Sci.* 36, 542–554.
- Bridges, C. D. B. (1977). Method for Preparing Stable Digitonin Solutions for Visual Pigment Extraction. *Vis. Res.* 17, 301–302. doi:10.1016/0042-6989(77)90095-5
- Bridges, C. D. B. (1972). "The Rhodopsin-Porphyrin Visual System," in *Photochemistry of Vision*. Editor H. J. A. Dartnall (Berlin: Springer-Verlag), 417–480. doi:10.1007/978-3-642-65066-6_11
- Brinkmann, A., Sternberg, U., Bovee-Geurts, P. H. M., Fernández Fernández, I., Lugtenburg, J., Kentgens, A. P. M., et al. (2018). Insight into the Chromophore of Rhodopsin and its Meta-II Photointermediate by ¹⁹F Solid-State NMR and Chemical Shift Tensor Calculations. *Phys. Chem. Chem. Phys.* 20, 30174–30188. doi:10.1039/c8cp05886e
- Brinks, D., Adam, Y., Kheifets, S., and Cohen, A. E. (2016). Painting with Rainbows: Patterning Light in Space, Time, and Wavelength for Multiphoton Optogenetic Sensing and Control. *Accounts Chem. Res.* 49, 2518–2526. doi:10.1021/acs.accounts.6b00415
- Broecker, J., Eger, B. T., and Ernst, O. P. (2017). Crystallogenesis of Membrane Proteins Mediated by Polymer-Bounded Lipid Nanodiscs. *Structure* 25, 384–392. doi:10.1016/j.str.2016.12.004
- Broser, M. (2022). Far-Red Absorbing Rhodopsins, Insights from Heterodimeric Rhodopsin-Cyclases. *Front. Mol. Biosci.* 8, 806922. doi:10.3389/fmolb.2021.806922
- Broser, M., Spreen, A., Konold, P. E., Peter, E., Adam, S., Borin, V., et al. (2020). NeoR, a Near-Infrared Absorbing Rhodopsin. *Nat. Commun.* 11, 5682. doi:10.1038/s41467-020-19375-8
- Brouillette, C. G., Mcmichens, R. B., Stern, L. J., and Khorana, H. G. (1989). Structure and Thermal-Stability of Monomeric Bacteriorhodopsin in Mixed Phospholipid Detergent Micelles. *Proteins-Structure Funct. Genet.* 5, 38–46. doi:10.1002/prot.340050106
- Brown, J., Behnam, R., Coddington, L., Tervo, D. G. R., Martin, K., Proskurin, M., et al. (2018). Expanding the Optogenetics Toolkit by Topological Inversion of Rhodopsins. *Cell* 175, 1131–1140. doi:10.1016/j.cell.2018.09.026
- Brown, L. S., and Ernst, O. P. (2017). Recent Advances in Biophysical Studies of Rhodopsins - Oligomerization, Folding, and Structure. *Biochimica Biophysica Acta-Proteins Proteomics* 1865, 1512–1521. doi:10.1016/j.bbapap.2017.08.007
- Brown, L. S. (2004). Fungal Rhodopsins and Opsin-Related Proteins: Eukaryotic Homologues of Bacteriorhodopsin with Unknown Functions. *Photochem. Photobiological Sci.* 3, 555–565. doi:10.1039/b315527g
- Brown, L. S., and Ladizhansky, V. (2015). Membrane Proteins in Their Native Habitat as Seen by Solid-State NMR Spectroscopy. *Protein Sci.* 24, 1333–1346. doi:10.1002/pro.2700
- Brown, L. S. (2022). Light-driven Proton Transfers and Proton Transport by Microbial Rhodopsins - A Biophysical Perspective. *Biochimica Biophysica Acta-Biomembranes* 1864, 183867. doi:10.1016/j.bbamem.2022.183867
- Brown, L. S., Needleman, R., and Lanyi, J. K. (1996). Interaction of Proton and Chloride Transfer Pathways in Recombinant Bacteriorhodopsin with Chloride Transport Activity: Implications for the Chloride Translocation Mechanism. *Biochemistry* 35, 16048–16054. doi:10.1021/bi9622938
- Bruun, S., Stoeppler, D., Keidel, A., Kuhlmann, U., Luck, M., Diehl, A., et al. (2015). Light-dark Adaptation of Channelrhodopsin Involves Photoconversion between the All-Trans and 13-cis Retinal Isomers. *Biochemistry* 54, 5389–5400. doi:10.1021/acs.biochem.5b00597
- Buda, F., Keijer, T., Ganapathy, S., and De Grip, W. J. (2017). A Quantum-Mechanical Study of the Binding Pocket of Proteorhodopsin: Absorption and Vibrational Spectra Modulated by Analogue Chromophores. *Photochem. Photobiol.* 93, 1399–1406. doi:10.1111/php.12800
- Buhrke, D., and Hildebrandt, P. (2020). Probing Structure and Reaction Dynamics of Proteins Using Time-Resolved Resonance Raman Spectroscopy. *Chem. Rev.* 120, 3577–3630. doi:10.1021/acs.chemrev.9b00429
- Butt, H.-J. (1990). Quantum Efficiency of Native and Mutant Bacteriorhodopsin Obtained from Blue Light Induced Relaxation Experiments. *Eur. Biophys. J.* 19, 31–39. doi:10.1007/bf00223571
- Caffrey, M. (2003). Membrane Protein Crystallization. *J. Struct. Biol.* 142, 108–132. doi:10.1016/s1047-8477(03)00043-1
- Cai, Y. Y., Liu, Y. T., Culhane, K. J., Devree, B. T., Yang, Y., Sunahara, R. K., et al. (2017). Purification of Family B G Protein-Coupled Receptors Using Nanodiscs: Application to Human Glucagon-like Peptide-1 Receptor. *PLoS ONE* 12, 0179568. doi:10.1371/journal.pone.0179568
- Calligaris, H., Dkhissi-Benyahya, O., and Panda, S. (2022). Ocular and Extraocular Roles of Neoropsin in Vertebrates. *Trends Neurosci.* 1776, 1–12. doi:10.1016/j.tins.2021.11.008
- Cao, P. X., Sun, W., Kramp, K., Zheng, M., Salom, D., Jastrzebska, B., et al. (2012). Light-sensitive Coupling of Rhodopsin and Melanopsin to G_{i/o} and G_q Signal Transduction in *Caenorhabditis elegans*. *FASEB J.* 26, 480–491. doi:10.1096/fj.11-197798
- Cardin, J. A., Carlen, M., Meletis, K., Knoblich, U., Zhang, F., Deisseroth, K., et al. (2010). Targeted Optogenetic Stimulation and Recording of Neurons *In Vivo* Using Cell-type-specific Expression of Channelrhodopsin-2. *Nat. Protoc.* 5, 247–254. doi:10.1038/nprot.2009.228
- Carpine, R., Olivieri, G., Hellingwerf, K. J., Pollio, A., and Marzocchella, A. (2020). Industrial Production of Poly-Beta-Hydroxybutyrate from CO₂: Can Cyanobacteria Meet This Challenge? *Processes* 8, 323–321323.
- Carravetta, M., Zhao, X., Johannessen, O. G., Lai, W. C., Verhoeven, M. A., Bovee-Geurts, P. H. M., et al. (2004). Protein-induced Bonding Perturbation of the Rhodopsin Chromophore Detected by Double-Quantum Solid-State NMR. *J. Am. Chem. Soc.* 126, 3948–3953. doi:10.1021/ja039390q
- Casey, J. R., Ferrón, S., and Karl, D. M. (2017). Light-Enhanced Microbial Organic Carbon Yield. *Front. Microbiol.* 8, 2157. doi:10.3389/fmicb.2017.02157
- Cassim, J. Y. (1992). Unique Biphasic Band Shape of the Visible Circular Dichroism of Bacteriorhodopsin in Purple Membrane. Excitons, Multiple Transitions or Protein Heterogeneity? *Biophys. J.* 63, 1432–1442. doi:10.1016/s0006-3495(92)81701-0
- Chabre, M., and Lemaire, M. (2005). Monomeric G-Protein-Coupled Receptor as a Functional Unit. *Biochemistry* 44, 9395–9403. doi:10.1021/bi050720o
- Chan, T., Lee, M., and Sakmar, T. P. (1992). Introduction of Hydroxyl-Bearing Amino Acids Causes Bathochromic Spectral Shifts in Rhodopsin - Amino Acid Substitutions Responsible for Red-Green Color Pigment Spectral Tuning. *J. Biol. Chem.* 267, 9478–9480. doi:10.1016/s0021-9258(19)50115-6
- Chang, C.-F., Kuramochi, H., Singh, M., Abe-Yoshizumi, R., Tsukuda, T., Kandori, H., et al. (2022). A Unified View on Varied Ultrafast Dynamics of the Primary Process in Microbial Rhodopsins. *Angew. Chem. Int. Ed.* 61, e202111930. doi:10.1002/anie.202111930
- Chang, C. F., Kuramochi, H., Singh, M., Abe-Yoshizumi, R., Tsukuda, T., Kandori, H., et al. (2019). Acid-base Equilibrium of the Chromophore Counterion Results in Distinct Photoisomerization Reactivity in the Primary Event of Proteorhodopsin. *Phys. Chem. Chem. Phys.* 21, 25728–25734. doi:10.1039/c9cp04991f
- Charvolin, D., Perez, J.-B., Rouvière, F., Giusti, F., Bazzacco, P., Abdine, A., et al. (2009). The Use of Amphipols as Universal Molecular Adapters to Immobilize Membrane Proteins onto Solid Supports. *Proc. Natl. Acad. Sci. U. S. A.* 106, 405–410. doi:10.1073/pnas.0807132106
- Chawla, U., Perera, S. M. D. C., Fried, S. D. E., Eitel, A. R., Mertz, B., Weerasinghe, N., et al. (2021). Activation of the G-Protein-Coupled Receptor Rhodopsin by Water. *Angew. Chemie-International Ed.* 60, 2288–2295. doi:10.1002/anie.202003342
- Chazan, A., Rozenberg, A., Mannen, K., Nagata, T., Tahan, R., Yaish, S., et al. (2022). Diverse Heliorhodopsins Detected via Functional Metagenomics in Freshwater *Actinobacteria*, *Chloroflexi* and *Archaea*. *Environ. Microbiol.* 2022, 15890. doi:10.1111/1462-2920.15890
- Chen, G.-Q., and Gouaux, J. E. (1996). Overexpression of Bacterio-opsin in *Escherichia coli* as a Water-Soluble Fusion to Maltose Binding Protein: Efficient Regeneration of the Fusion Protein and Selective Cleavage with Trypsin. *Protein Sci.* 5, 456–467. doi:10.1002/pro.5560050307

- Chen, M.-H., Kuemmel, C., Birge, R. R., and Knox, B. E. (2012). Rapid Release of Retinal from a Cone Visual Pigment Following Photoactivation. *Biochemistry* 51, 4117–4125. doi:10.1021/bi201522h
- Chen, Q., Arents, J. C., Schuurmans, J. M., Ganapathy, S., De Grip, W. J., Cheregi, O., et al. (2019b). Combining Retinal-Based and Chlorophyll-Based (Oxygenic) Photosynthesis: Proteorhodopsin Expression Increases Growth Rate and Fitness of a Δ PSI Strain of *Synechocystis* Sp. PCC6803. *Metab. Eng.* 52, 68–76. doi:10.1016/j.ymben.2018.11.002
- Chen, Q., Arents, J., Ganapathy, S., DeGrip, W. J., and Hellingwerf, K. J. (2017). Functional Expression of *Gloeobacter* Rhodopsin in *Synechocystis* Sp PCC6803. *Photochem. Photobiol.* 93, 772–781. doi:10.1111/php.12745
- Chen, Q., Arents, J., Schuurmans, J. M., Ganapathy, S., De Grip, W. J., Cheregi, O., et al. (2019a). Functional Expression of *Gloeobacter* Rhodopsin in PSI-Less *Synechocystis* Sp. PCC6803. *Front. Bioeng. Biotechnol.* 7, 67. doi:10.3389/fbioe.2019.00067
- Chen, Q., Montesarchio, D., and Hellingwerf, K. J. (2016a). “Direct Conversion: Artificial Photosynthesis with Cyanobacteria,” in *Artificial Photosynthesis*. Editor R. Bruno, 43–61. doi:10.1016/bs.abr.2016.03.001
- Chen, Q., Van Der Steen, J. B., Arents, J. C., Hartog, A. F., Ganapathy, S., De Grip, W. J., et al. (2018). Deletion of *Sll1541* in *Synechocystis* Sp Strain PCC 6803 Allows Formation of a Far-Red-Shifted *Holo*-Proteorhodopsin *In Vivo*. *Appl. Environ. Microbiol.* 84, e024351–0241714. doi:10.1128/AEM.02435-17
- Chen, Q., Van Der Steen, J. B., Dekker, H. L., Ganapathy, S., De Grip, W. J., and Hellingwerf, K. J. (2016b). Expression of *Holo*-Proteorhodopsin in *Synechocystis* Sp PCC 6803. *Metab. Eng.* 35, 83–94. doi:10.1016/j.ymben.2016.02.001
- Chen, S. (2019). Optical Modulation Goes Deep in the Brain. *Science* 365, 456–457. doi:10.1126/science.aay4350
- Chien, M.-P., Brinks, D., Testa-Silva, G., Tian, H., Brooks, F. P. I., Adam, Y., et al. (2021). Photoactivated Voltage Imaging in Tissue with an Archaerhodopsin-Derived Reporter. *Sci. Adv.* 7, eabe3216. doi:10.1126/sciadv.abe3216
- Chizhov, I., Chernavskii, D. S., Engelhard, M., Mueller, K.-H., Zubov, B. V., and Hess, B. (1996). Spectrally Silent Transitions in the Bacteriorhodopsin Photocycle. *Biophysical J.* 71, 2329–2345. doi:10.1016/s0006-3495(96)79475-4
- Cho, Y. K., Park, D., Yang, A. M., Chen, F., Chuong, A. S., Klapoetke, N. C., et al. (2019). Multidimensional Screening Yields Channelrhodopsin Variants Having Improved Photocurrent and Order-Of-Magnitude Reductions in Calcium and Proton Currents. *J. Biol. Chem.* 294, 3806–3821. doi:10.1074/jbc.ra118.006996
- Choe, H.-W., Kim, Y. J., Park, J. H., Morizumi, T., Pai, E. F., Krauß, N., et al. (2011). Crystal Structure of Metarhodopsin II. *Nature* 471, 651–655. doi:10.1038/nature09789
- Choi, A. R., Shi, L. C., Brown, L. S., and Jung, K.-H. (2014). Cyanobacterial Light-Driven Proton Pump, *Gloeobacter* Rhodopsin: Complementarity between Rhodopsin-Based Energy Production and Photosynthesis. *PLoS ONE* 9, e110643. doi:10.1371/journal.pone.0110643
- Choi, E. H., Daruwalla, A., Suh, S., Leinonen, H., and Palczewski, K. (2021). Retinoids in the Visual Cycle: Role of the Retinal G Protein-Coupled Receptor. *J. Lipid Res.* 62, 1000–1040. doi:10.1194/jlr.tr120000850
- Chow, B. Y., Han, X., Dobry, A. S., Qian, X. F., Chuong, A. S., Li, M. J., et al. (2010). High-performance Genetically Targetable Optical Neural Silencing by Light-Driven Proton Pumps. *Nature* 463, 98–102. doi:10.1038/nature08652
- Chun, K., Kim, S. Y., Meas, S., Shim, J.-G., Cho, S.-G., Kang, K.-W., et al. (2021). Assembly of Natively Synthesized Dual Chromophores into Functional Actinorhodopsin. *Front. Microbiol.* 12, 652328. doi:10.3389/fmicb.2021.652328
- Church, J. R., Amoyal, G. S., Borin, V. A., Adam, S., Olsen, J. M. H., and Schapiro, I. (2022). Deciphering the Spectral Tuning Mechanism in Proteorhodopsin: The Dominant Role of Electrostatics Instead of Chromophore Geometry. *Chem. - A Eur. J.* 28, e202200139. doi:10.1002/chem.202200139
- Church, J. R., Haugaard Olsen, J. M., and Schapiro, I. (2022b). The Impact of Retinal Configuration on the Protein-Chromophore Interactions in Bistable Jumping Spider Rhodopsin-1. *Molecules* 27, 71. doi:10.3390/molecules27010071
- Civjan, N. R., Bayburt, T. H., Schuler, M. A., and Sligar, S. G. (2003). Direct Solubilization of Heterologously Expressed Membrane Proteins by Incorporation into Nanoscale Lipid Bilayers. *BioTechniques* 35, 556–563. doi:10.2144/03353rr02
- Cokic, M., Bruegmann, T., Sasse, P., and Malan, D. (2021). Optogenetic Stimulation of G_i Signaling Enables Instantaneous Modulation of Cardiomyocyte Pacemaking. *Front. physiology* 12, 768495. doi:10.3389/fphys.2021.768495
- Collette, F., Renger, T., Müh, F., and Schmidt Am Busch, M. (2018). Red/Green Color Tuning of Visual Rhodopsins: Electrostatic Theory Provides a Quantitative Explanation. *J. Phys. Chem. B* 122, 4828–4837. doi:10.1021/acs.jpcc.8b02702
- Concistrè, M., Gansmüller, A., Mclean, N., Johannessen, O. G., Marin-Montesinos, I., Bovee-Geurts, P. H. M., et al. (2008). Double-quantum ¹³C Nuclear Magnetic Resonance of Bathorhodopsin, the First Photointermediate in Mammalian Vision. *J. Am. Chem. Soc.* 130, 10490–10491. doi:10.1021/ja803801u
- Contreras, E., Nobleman, A. P., Robinson, P. R., and Schmidt, T. M. (2021). Melanopsin Phototransduction: beyond Canonical Cascades. *J. Exp. Biol.* 224, 224–221214. doi:10.1242/jeb.226522
- Cooper, A. (1979). Energetics of Rhodopsin and Isorhodopsin. *FEBS Lett.* 100, 382–384. doi:10.1016/0014-5793(79)80375-0
- Copits, B. A., Gowrishankar, R., O’neill, P. R., Li, J.-N., Girven, K. S., Yoo, J. J., et al. (2021). A Photoswitchable GPCR-Based Opsin for Presynaptic Inhibition. *Neuron* 109, 1791–1809. doi:10.1016/j.neuron.2021.04.026
- Córdova, C., Lozano, C., Rodríguez, B., Marchant, I., Zúñiga, R., Ochova, P., et al. (2021). Optogenetic Control of Cancer Cell Survival in Chr2-Transfected HeLa Cells. *Int. J. Exp. Pathology* 102, 242. doi:10.1111/iep.12426
- Creemers, A. F. L., Kiihne, S. R., Bovee-Geurts, P. H. M., DeGrip, W. J., Lugtenburg, J., and De Groot, H. J. M. (2002). ¹H and ¹³C MAS NMR Evidence for Pronounced Ligand-Protein Interactions Involving the Ionone Ring of the Retinylidene Chromophore in Rhodopsin. *Proc. Nat. Acad. Sci. U. S. A.* 99, 9101–9106. doi:10.1073/pnas.112677599
- Creemers, A. F. L., Klaassen, C. H. W., Bovee-Geurts, P. H. M., Kelle, R., Kragl, U., Raap, J., et al. (1999). ¹⁵N Solid State NMR Evidence for a Complex Schiff Base Counterion in the Visual G Protein-Coupled Receptor Rhodopsin. *Biochemistry-USA* 38, 7195–7199. doi:10.1021/bi9830157
- Crescitelli, F. (1991). The Natural History of Visual Pigments: 1990. *Prog. Retin. Res.* 11, 1–32. doi:10.1111/j.1749-6632.1958.tb39548.x
- Crouch, R. K., Kefalov, V. J., Gärtner, W., and Cornwall, M. C. (2002). Use of Retinal Analogues for the Study of Visual Pigment Function. *Meth. Enzymol.* 343, 29–48. doi:10.1016/s0076-6879(02)43126-6
- Crouch, R. K., Nodes, B. R., Perlman, J. I., Pepperberg, D. R., Akita, H., and Nakanishi, K. (1984). Cycloheptatrienylidene Analog of 11-*cis* Retinal. Formation of Pigment in Photoreceptor Membranes. *Investig. Ophthalmol. Vis. Sci.* 25, 419–428.
- Daemen, F. J. M. (1973). Vertebrate Rod Outer Segment Membranes. *Biochimica Biophysica Acta* 300, 255–288. doi:10.1016/0304-4157(73)90006-3
- Dartnall, H. J. A. (1962a). “The Chemical Structure and Photochemistry of the Visual Pigments,” in *The Visual Process*. Editor H. Davson. 1 ed (New York, U.S.A. Academic Press), 427–471.
- Dartnall, H. J. A. (1962b). “The Identity and Distribution of Visual Pigments in the Animal Kingdom,” in *The Visual Process*. Editor H. Davson (New York, U.S.A. Academic Press), 367–426.
- Dartnall, H. J. A. (1962c). “The Properties of Visual Pigments in Photoreceptors,” in *The Visual Process*. Editor H. Davson. 1 ed (New York, U.S.A. Academic Press), 473–533.
- Davidson, F. F., Loewen, P. C., and Khorana, H. G. (1994). Structure and Function in Rhodopsin: Replacement by Alanine of Cysteine Residues 110 and 187, Components of a Conserved Disulfide Bond in Rhodopsin, Affects the Light-Activated Metarhodopsin II State. *Proc. Nat. Acad. Sci. U. S. A.* 91, 4029–4033. doi:10.1073/pnas.91.9.4029
- Davies, A., Gowen, B. E., Krebs, A. M., Schertler, G. F. X., and Saibil, H. R. (2001). Three-dimensional Structure of an Invertebrate Rhodopsin and Basis for Ordered Alignment in the Photoreceptor Membrane. *J. Mol. Biol.* 314, 455–463. doi:10.1006/jmbi.2001.5167
- Davies, A., Schertler, G. F. X., Gowen, B. E., and Saibil, H. R. (1996). Projection Structure of an Invertebrate Rhodopsin. *J. Struct. Biol.* 117, 36–44. doi:10.1006/jsbi.1996.0067
- Davies, W. I. L., Collin, S. P., and Hunt, D. M. (2012). Molecular Ecology and Adaptation of Visual Photopigments in Craniates. *Mol. Ecol.* 21, 3121–3158. doi:10.1111/j.1365-294x.2012.05617.x
- Davies, W. I. L., Hankins, M. W., and Foster, R. G. (2010). Vertebrate Ancient Opsin and Melanopsin: Divergent Irradiance Detectors. *Photochem. Photobiological Sci.* 9, 1444–1457. doi:10.1039/c0pp00203h

- Davies, W. I. L., Sghari, S., Upton, B. A., Nord, C., Hahn, M., Ahlgren, U., et al. (2021). Distinct Opsin 3 (Opn3) Expression in the Developing Nervous System during Mammalian Embryogenesis. *eNeuro* 8, 0141–0121. doi:10.1523/eneuro.0141-21.2021
- Davies, W. I. L., Tamai, T. K., Zheng, L., Fu, J. K., Rihel, J., Foster, R. G., et al. (2015). An Extended Family of Novel Vertebrate Photopigments Is Widely Expressed and Displays a Diversity of Function. *Genome Res.* 25, 1666–1679. doi:10.1101/gr.189886.115
- Dawadi, P. B. S., and Lugtenburg, J. (2010). Synthesis and Use of Stable Isotope Enriched Retinals in the Field of Vitamin A. *Molecules* 15, 1825–1872. doi:10.3390/molecules15031825
- De Silva, S. R., Barnard, A. R., Hughes, S., Tam, S. K. E., Martin, C., Singh, M. S., et al. (2017). Long-term Restoration of Visual Function in End-Stage Retinal Degeneration Using Subretinal Human Melanopsin Gene Therapy. *Proc. Natl. Acad. Sci. U. S. A.* 114, 11211–11216. doi:10.1073/pnas.1701589114
- DeGrip, W. J., Bonting, S. L., and Daemen, F. J. M. (1973). The Binding Site of Retinaldehyde in Cattle Rhodopsin. *Biochimica Biophysica Acta* 303, 189–193. doi:10.1016/0005-2795(73)90162-1
- DeGrip, W. J., and Bovee-Geurts, P. H. M. (1979). Synthesis and Properties of Alkylglucosides with Mild Detergent Action: Improved Synthesis and Purification of β -1-octyl-, -nonyl- and -Decyl-Glucose. Synthesis of β -1-undecylglucose and β -1-dodecylmaltose. *Chem. Phys. Lipids* 23, 321–335. doi:10.1016/0009-3084(79)90110-0
- DeGrip, W. J., Bovee-Geurts, P. H. M., Van Der Hoef, I., and Lugtenburg, J. (2007). 7, 8-Dihydro-Retinals Outperform the Native Retinals in Conferring Photosensitivity to Visual Opsin. *Jacs* 129, 13265–13269. doi:10.1021/ja074937c
- DeGrip, W. J., Bovee-Geurts, P. H. M., Wang, Y.-J., Verhoeven, M. A., and Lugtenburg, J. (2011). 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, 383–390. doi:10.1021/np100744v
- DeGrip, W. J., Daemen, F. J. M., and Bonting, S. L. (1980). Isolation and Purification of Bovine Rhodopsin. *Meth. Enzymol.* 67, 301–320. doi:10.1016/s0076-6879(80)67038-4
- DeGrip, W. J., DeLange, F., Klaassen, C. H. W., Verdegem, P. J. E., Wallace-Williams, S. E., Creemers, A. F. L., et al. (1999). “Photoactivation of Rhodopsin: Interplay between Protein and Chromophore,” in *Rhodopsins and Phototransduction*. Editor J. A. Goode (Chichester, UK: John Wiley & Sons), 102–118.
- DeGrip, W. J., Gillespie, J., and Rothschild, K. J. (1985). Carboxyl Group Involvement in the Meta I and Meta II Stages in Rhodopsin Bleaching. A Fourier Transform Infra-red Spectroscopic Study. *Biochim. Biophys. Acta* 809, 97–106. doi:10.1016/0005-2728(85)90172-0
- DeGrip, W. J., Gray, D., Gillespie, J., Bovee-Geurts, P. H. M., Vandenberg, E. M. M., Lugtenburg, J., et al. (1988). Photoexcitation of Rhodopsin: Conformation Changes in the Chromophore, Protein and Associated Lipid, as Determined by FTIR Difference Spectroscopy. *Photochem. Photobiol.* 48, 497–504. doi:10.1111/j.1751-1097.1988.tb02852.x
- DeGrip, W. J., Liu, R. S. H., Ramamurthy, V., and Asato, A. E. (1976). Rhodopsin Analogues from Highly Hindered 7-*cis* Isomers of Retinal. *Nature* 262, 416–418. doi:10.1038/262416a0
- DeGrip, W. J., and Rothschild, K. J. (2000). “Structure and Mechanism of Vertebrate Visual Pigments,” in *Molecular Mechanisms in Visual Transduction*. Editors D. G. Stavenga, W. J. DeGrip, and E. N. Pugh Jr. (Amsterdam, Netherlands: Elsevier Science Pub.), 1–54. doi:10.1016/s1383-8121(00)80004-4
- DeGrip, W. J. (1982). Thermal Stability of Rhodopsin and Opsin in Some Novel Detergents. *Meth. Enzymol.* 81, 256–265. doi:10.1016/s0076-6879(82)81040-9
- DeGrip, W. J., VanOostrum, J., and Bovee-Geurts, P. H. M. (1998). 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, 667–674. doi:10.1042/bj3300667
- DeGrip, W. J., VanOostrum, J., Bovee-Geurts, P. H. M., Van Der Steen, R., Van Amsterdam, L. J. P., Groesbeek, M., et al. (1990). 10, 20-Methanorhodopsins: (7E, 9E, 13E)-10, 20-methanorhodopsin and (7E, 9Z, 13Z)-10, 20-methanorhodopsin - 11-*Cis*-Locked Rhodopsin Analog Pigments with Unusual Thermal and Photo-Stability. *Eur. J. Biochem.* 191, 211–220. doi:10.1111/j.1432-1033.1990.tb19112.x
- Deiningner, W., Kröger, P., Hegemann, U., Lottspeich, F., and Hegemann, P. (1995). Chlamyrodopsin Represents a New Type of Sensory Photoreceptor. *EMBO J.* 14, 5849–5858. doi:10.1002/j.1460-2075.1995.tb00273.x
- Deisseroth, K. (2010). Controlling the Brain with Light. *Sci. Am.* 303, 48–55. doi:10.1038/scientificamerican1110-48
- Deisseroth, K., and Hegemann, P. (2017). The Form and Function of Channelrhodopsin. *Science* 357, eaan5544. doi:10.1126/science.aan5544
- Deisseroth, K. (2015). Optogenetics: 10 Years of Microbial Opsins in Neuroscience. *Nat. Neurosci.* 18, 1213–1225. doi:10.1038/nn.4091
- Del Carmen Marín, M., Agathangelou, D., Orozco-Gonzalez, Y., Valentini, A., Kato, Y., Abe-Yoshizumi, R., et al. (2019a). Fluorescence Enhancement of a Microbial Rhodopsin via Electronic Reprogramming. *J. Am. Chem. Soc.* 141, 262–271. doi:10.1021/jacs.8b09311
- Del Carmen Marín, M., De Vico, L., Dong, S. J. S., Gagliardi, L., Truhlar, D. G., and Olivucci, M. (2019b). Assessment of MC-PDFT Excitation Energies for a Set of QM/MM Models of Rhodopsins. *J. Chem. Theory Comput.* 15, 1915–1923. doi:10.1021/acs.jctc.8b01069
- DeLange, F., Bovee-Geurts, P. H. M., Pistorius, A. M. A., Rothschild, K. J., and DeGrip, W. J. (1999). Probing Intramolecular Orientations in Rhodopsin and Metarhodopsin II by Polarized Infrared Difference Spectroscopy. *Biochemistry-USA* 38, 13200–13209. doi:10.1021/bi9909501
- DeLange, F., Bovee-Geurts, P. H. M., Vanoostrum, J., Portier, M. D., Verdegem, P. J. E., Lugtenburg, J., et al. (1998a). An Additional Methyl Group at the 10-position of Retinal Dramatically Slows Down the Kinetics of the Rhodopsin Photocascade. *Biochemistry-USA* 37, 1411–1420. doi:10.1021/bi972397y
- DeLange, F., Klaassen, C. H. W., Wallace-Williams, S. E., Bovee-Geurts, P. H. M., Liu, X.-M., DeGrip, W. J., et al. (1998b). Tyrosine Structural Changes Detected during the Photoactivation of Rhodopsin. *J. Biol. Chem.* 273, 23735–23739. doi:10.1074/jbc.273.37.23735
- DeLange, F., Merckx, M., Bovee-Geurts, P. H. M., Pistorius, A. M. A., and DeGrip, W. J. (1997). Modulation of the Metarhodopsin I/metarhodopsin II Equilibrium of Bovine Rhodopsin by Ionic Strength - Evidence for a Surface Charge Effect. *Eur. J. Biochem.* 243, 174–180. doi:10.1111/j.1432-1033.1997.0174a.x
- Demoulin, B., Maiuri, M., Berbasova, T., Geiger, J. H., Borhan, B., Garavelli, M., et al. (2021). Control of Protonated Schiff Base Excited State Decay within Visual Protein Mimics: A Unified Model for Retinal Chromophores. *Chemistry-A Eur. J.* 27, 16389. doi:10.1002/chem.202102383
- Dencher, N. A., and Heyn, M. P. (1978). Formation and Properties of Bacteriorhodopsin Monomers in the Nonionic Detergents Octyl- β -D-Glucoside and Triton X-100. *FEBS Lett.* 96, 322–326. doi:10.1016/0014-5793(78)80427-x
- Derguini, F., Caldwell, C. G., Motto, M. G., Balogh-Nair, V., and Nakanishi, K. (1983). Bacteriorhodopsins Containing Cyanine Dye Chromophores - Support for the External Point-Charge Model. *J. Am. Chem. Soc.* 105, 646–648. doi:10.1021/ja00341a068
- Derguini, F., and Nakanishi, K. (1986). Synthetic Rhodopsin Analogs. *Photobiochem. Photobiophys.* 13, 259–283.
- Deubner, J., Coulon, P., and Diester, I. (2019). Optogenetic Approaches to Study the Mammalian Brain. *Curr. Opin. Struct. Biol.* 57, 157–163. doi:10.1016/j.sbi.2019.04.003
- Devine, E. L., Oprian, D. D., and Theobald, D. L. (2013). Relocating the Active-Site Lysine in Rhodopsin and Implications for Evolution of Retinylidene Proteins. *Proc. Natl. Acad. Sci. U. S. A.* 110, 13351–13355. doi:10.1073/pnas.1306826110
- Ding, X. Y., Sun, C., Cui, H. L., Chen, S. J., Gao, Y. J., Yang, Y. A., et al. (2018). Functional Roles of Tyrosine 185 during the Bacteriorhodopsin Photocycle as Revealed by *In Situ* Spectroscopic Studies. *Biochimica Biophysica Acta-Bioenergetics* 1859, 1006–1014. doi:10.1016/j.bbabi.2018.05.011
- Dokukina, I., Nenov, A., Garavelli, M., Marian, C. M., and Weingart, O. (2019). QM/MM Photodynamics of Retinal in the Channelrhodopsin Chimera C1C2 with OM3/MRCI. *ChemPhotoChem* 3, 107–116. doi:10.1002/cptc.201800185
- Döring, C. C., Kumar, S., Tumu, S. C., Kourtesis, I., and Hausen, H. (2020). The Visual Pigment Xenopsin Is Widespread in Protostome Eyes and Impacts the View on Eye Evolution. *Elife* 9, e55193. doi:10.7554/eLife.55193
- Dörr, J. M., Scheidelaar, S., Koorengevel, M. C., Dominguez, J. J., Schäfer, M., Van Walree, C. A., et al. (2016). The Styrene-Maleic Acid Copolymer: A Versatile

- Tool in Membrane Research. *Eur. Biophysics J. Biophysics Lett.* 45, 3–21. doi:10.1007/s00249-015-1093-y
- Dowling, J. E. (2020). Vitamin A: its Many Roles - from Vision and Synaptic Plasticity to Infant Mortality. *J. Comp. Physiology a-Neuroethology Sens. Neural Behav. Physiology* 206, 389–399. doi:10.1007/s00359-020-01403-z
- Du, W., Caicedo Burbano, P., Hellingwerf, K. J., and Branco Dos Santos, F. (2018). “Challenges in the Application of Synthetic Biology towards Synthesis of Commodity Products by Cyanobacteria via “Direct Conversion”,” in *Synthetic Biology of Cyanobacteria*. Editors W. Zhang and X. Song (Gateway East, Singapore: Springer Nature Singapore Pte Ltd.), 3–26. doi:10.1007/978-981-13-0854-3_1
- Duda, M., Domagalik, A., Orlowska-Feuer, P., Krzysztynska-Kuleta, O., Beldzik, E., Smyk, M. K., et al. (2020). Melanopsin: From a Small Molecule to Brain Functions. *Neurosci. Biobehav. Rev.* 113, 190–203. doi:10.1016/j.neubiorev.2020.03.012
- Dunham, T. D., and Farrens, D. L. (1999). Conformational Changes in Rhodopsin - Movement of Helix F Detected by Site-specific Chemical Labeling and Fluorescence Spectroscopy. *J. Biol. Chem.* 274, 1683–1690. doi:10.1074/jbc.274.3.1683
- Ebrey, T. G., and Koutalos, Y. (2001). Vertebrate Photoreceptors. *Prog. Retin. Eye Res.* 20, 49–94. doi:10.1016/s1350-9462(00)00014-8
- Ehsan, M., Katsube, S., Cecchetti, C., Du, Y., Mortensen, J. S., Wang, H. Q., et al. (2020). New Malonate-Derived Tetraglucoside Detergents for Membrane Protein Stability. *ACS Chem. Biol.* 15, 1697–1707. doi:10.1021/acscchembio.0c00316
- Eickelbeck, D., Rudack, T., Tennigkeit, S. A., Surdin, T., Karapinar, R., Schwitalla, J. C., et al. (2020). Lamprey Parapinopsin (“UVLamP”): a Bistable UV-Sensitive Optogenetic Switch for Ultrafast Control of GPCR Pathways. *ChemBioChem* 21, 612–617. doi:10.1002/cbic.201900485
- Eilers, M., Goncalves, J. A., Ahuja, S., Kirkup, C., Hirshfeld, A., Simmerling, C., et al. (2012). Structural Transitions of Transmembrane Helix 6 in the Formation of Metarhodopsin I. *J. Phys. Chem. B* 116, 10477–10489. doi:10.1021/jp3019183
- Eilers, M., Ying, W. W., Reeves, P. J., Khorana, H. G., and Smith, S. O. (2002). Magic Angle Spinning Nuclear Magnetic Resonance of Isotopically Labeled Rhodopsin. *Meth. Enzymol.* 343, 212–222. doi:10.1016/s0076-6879(02)43137-0
- El Khatib, S., and Atamian, A. (2019). *Evolution of Color Vision in Vertebrates*. Delhi, India: Akinik Publications, 19–42.
- El-Tahawy, M. M. T., Conti, I., Bonfanti, M., Nenov, A., and Garavelli, M. (2020). Tailoring Spectral and Photochemical Properties of Bioinspired Retinal Mimics by In Silico Engineering. *Angew. Chemie-International Ed.* 59, 20619–20627. doi:10.1002/anie.202008644
- Engel, A., and Gaub, H. E. (2008). Structure and Mechanics of Membrane Proteins. *Annu. Rev. Biochem.* 77, 127–148. doi:10.1146/annurev.biochem.77.062706.154450
- Engelhard, C., Chizhov, I., Sieber, F., and Engelhard, M. (2018). Microbial Halorhodopsins: Light-Driven Chloride Pumps. *Chem. Rev.* 118, 10629–10645. doi:10.1021/acs.chemrev.7b00715
- Engqvist, M. K. M., McIsaac, R. S., Dollinger, P., Flytzanis, N. C., Abrams, M., Schor, S., et al. (2015). Directed Evolution of *Gloeobacter Violaceus* Rhodopsin Spectral Properties. *J. Mol. Biol.* 427, 205–220. doi:10.1016/j.jmb.2014.06.015
- Erbguth, K., Prigge, M., Schneider, F., Hegemann, P., and Gottschalk, A. (2012). Bimodal Activation of Different Neuron Classes with the Spectrally Red-Shifted Channelrhodopsin Chimera C1V1 in *Caenorhabditis elegans*. *PLoS ONE* 7, e46827. doi:10.1371/journal.pone.0046827
- Ernst, O. P., Lodowski, D. T., Elstner, M., Hegemann, P., Brown, L. S., and Kandori, H. (2014). Microbial and Animal Rhodopsins: Structures, Functions, and Molecular Mechanisms. *Chem. Rev.* 114, 126–163. doi:10.1021/cr4003769
- Ewald, A., and Kühne, W. (1878). “Untersuchungen über den Sehpurpur,” in *Untersuchungen aus dem Physiologischen Institute der Universität Heidelberg*. Editor W. Kühne, 248–290.
- Farrens, D. L. (2010). What Site-Directed Labeling Studies Tell Us about the Mechanism of Rhodopsin Activation and G-Protein Binding. *Photochem. Photobiological Sci.* 9, 1466–1474. doi:10.1039/c0pp00283f
- Feldman, T. B., Ivanov, O. I., Kuklin, A. I., Murugova, T. N., Yakovleva, M. A., Smitienko, O. A., et al. (2019). Small-angle Neutron and X-Ray Scattering Analysis of the Supramolecular Organization of Rhodopsin in Photoreceptor Membrane. *Biochimica Biophysica Acta-Biomembranes* 1861, 183000. doi:10.1016/j.bbame.2019.05.022
- Feng, J., Brown, M. F., and Mertz, B. (2015). Retinal Flip in Rhodopsin Activation? *Biophysical J.* 108, 2767–2770. doi:10.1016/j.bpj.2015.04.040
- Feng, J., and Mertz, B. (2015). Proteorhodopsin Activation Is Modulated by Dynamic Changes in Internal Hydration. *Biochemistry* 54, 7132–7141. doi:10.1021/acs.biochem.5b00932
- Feng, S., Powell, S. M., Wilson, R., and Bowman, J. P. (2013). Light-stimulated Growth of Proteorhodopsin-Bearing Sea-Ice Psychrophile *Psychroflexus Torquis* Is Salinity Dependent. *ISME J.* 7, 2206–2213. doi:10.1038/ismej.2013.97
- Feng, X., Verdegem, P. J. E., Lee, Y. K., Sandström, D., Edén, M., Bovee-Geurts, P. H. M., et al. (1997). Direct Determination of a Molecular Torsional Angle in the Membrane Protein Rhodopsin by Solid-State NMR. *J. Am. Chem. Soc.* 119, 6853–6857. doi:10.1021/ja970710d
- Feuda, R., Menon, A. K., and Göpfert, M. C. (2022). Rethinking Opsins. *Mol. Biol. Evol.* 39, msac033. doi:10.1093/molbev/msac033
- Feuda, R., Rota-Stabelli, O., Oakley, T. H., and Pisani, D. (2014). The Comb Jelly Opsins and the Origins of Animal Phototransduction. *Genome Biol. Evol.* 6, 1964–1971. doi:10.1093/gbe/evu154
- Fischer, P., Mukherjee, S., Peter, E., Broser, M., Bartl, F., and Hegemann, P. (2021). The Inner Mechanics of Rhodopsin Guanylyl Cyclase during cGMP-Formation Revealed by Real-Time FTIR Spectroscopy. *eLife* 10, e71384. doi:10.7554/eLife.71384
- Fleming, J. F., Feuda, R., Roberts, N. W., and Pisani, D. (2020). A Novel Approach to Investigate the Effect of Tree Reconstruction Artifacts in Single-Gene Analysis Clarifies Opsin Evolution in Nonbilaterian Metazoans. *Genome Biol. Evol.* 12, 3906–3916. doi:10.1093/gbe/evaa015
- Flytzanis, N. C., Bedbrook, C. N., Chiu, H., Engqvist, M. K. M., Xiao, C., Chan, K. Y., et al. (2014). Archaeorhodopsin Variants with Enhanced Voltage-Sensitive Fluorescence in Mammalian and *Caenorhabditis elegans* Neurons. *Nat. Commun.* 5, 4894. doi:10.1038/ncomms5894
- Foster, R. G., and Hankins, M. W. (2002). Non-rod, Non-cone Photoreception in the Vertebrates. *Prog. Retin. Eye Res.* 21, 507–527. doi:10.1016/s1350-9462(02)00036-8
- Fotiadis, D., Jastrzebska, B., Philippsen, A., Müller, D. J., Palczewski, K., and Engel, A. (2006). Structure of the Rhodopsin Dimer: A Working Model for G-Protein-Coupled Receptors. *Curr. Opin. Struct. Biol.* 16, 252–259. doi:10.1016/j.sbi.2006.03.013
- Fotiadis, D., Liang, Y., Filipek, S., Saperstein, D. A., Engel, A., and Palczewski, K. (2004). The G Protein-Coupled Receptor Rhodopsin in the Native Membrane. *FEBS Lett.* 564, 281–288. doi:10.1016/s0014-5793(04)00194-2
- Fougère, M., Van Der Zouwen, C. I., Boutin, J. A., Neszvecsko, K., Sarret, P., and Ryczko, D. (2021). Optogenetic Stimulation of Glutamatergic Neurons in the Cuneiform Nucleus Controls Locomotion in a Mouse Model of Parkinson’s Disease. *Proc. Natl. Acad. Sci. U. S. A.* 118, e2110934118. doi:10.1073/pnas.2110934118
- Frank, M., Carlson, D. B., Hunter, M. S., Williams, G. J., Messerschmidt, M., Zatspein, N. A., et al. (2014). Femtosecond X-Ray Diffraction from Two-Dimensional Protein Crystals. *IUCr* 1, 95–100. doi:10.1107/s2052252514001444
- Frauenfeld, J., Löving, R., Armache, J.-P., Sonnen, A. F.-P., Guettou, F., Moberg, P., et al. (2016). A Saposin-Lipoprotein Nanoparticle System for Membrane Proteins. *Nat. Methods* 13, 345–351. doi:10.1038/nmeth.3801
- Friedman, J. M. (2021). How the Discovery of Microbial Opsins Led to the Development of Optogenetics. *Cell.* 184, 5266–5270. doi:10.1016/j.cell.2021.08.022
- Friedman, N., Sheves, M., and Ottolenghi, M. (1989). Model Systems for Rhodopsins: The Photolysis of Protonated Retinal Schiff-Bases, Cyanine Dye, and Artificial Cyanine-Bacteriorhodopsin. *J. Am. Chem. Soc.* 111, 3203–3211. doi:10.1021/ja00191a015
- Friedrich, D., Perodeau, J., Nieuwkoop, A. J., and Oschkinat, H. (2020). MAS NMR Detection of Hydrogen Bonds for Protein Secondary Structure Characterization. *J. Biomol. NMR* 74, 247–256. doi:10.1007/s10858-020-00307-z
- Fudim, R., Szczepek, M., Vierock, J., Vogt, A., Schmidt, A., Kleinau, G., et al. (2019). Design of a Light-Gated Proton Channel Based on the Crystal Structure of *Coccomyxa* Rhodopsin. *Sci. Signal.* 12, eaav4203. doi:10.1126/scisignal.aav4203

- Fujimoto, K. J. (2021). Electronic Couplings and Electrostatic Interactions behind the Light Absorption of Retinal Proteins. *Front. Mol. Biosci.* 8, 752700. doi:10.3389/fmolb.2021.752700
- Fujimoto, K. J., Hayashi, S., Hasegawa, J., and Nakatsuji, H. (2007). Theoretical Studies on the Color-Tuning Mechanism in Retinal Proteins. *J. Chem. Theory Comput.* 3, 605–618. doi:10.1021/ct6002687
- Fujimoto, K. J., and Inoue, K. (2020). Excitonic Coupling Effect on the Circular Dichroism Spectrum of Sodium-Pumping Rhodopsin KR2. *J. Chem. Phys.* 153, 04510. doi:10.1063/5.0013642
- Fujita, S., Endo, T., Ju, J.-M., Kean, E. L., and Kobata, A. (1994). Structural Studies of the N-Linked Sugar Chains of Human Rhodopsin. *Glycobiology* 4, 633–640. doi:10.1093/glycob/4.5.633
- Fujiyabu, C., Sato, K., Nishio, Y., Imamoto, Y., Ohuchi, H., and Shichida, Y., T. (2022). Amino Acid Residue at Position 188 Determines the UV-Sensitive Bistable Property of Vertebrate Non-visual Opsin Opn5. *Commun. Biol.* 5, 63. doi:10.1038/s42003-022-03010-x
- Fukada, Y., Shichida, Y., Yoshizawa, T., Ito, M., Kodama, A., and Tsukida, K. (1984). Studies on Structure and Function of Rhodopsin by Use of Cyclopentatrienylidene 11-Cis-Locked-Rhodopsin. *Biochemistry* 23, 5826–5832. doi:10.1021/bi00319a023
- Furuse, M., Tamogami, J., Hosaka, T., Kikukawa, T., Shinya, N., Hato, M., et al. (2015). Structural Basis for the Slow Photocycle and Late Proton Release in *Acetabularia* Rhodopsin I from the Marine Plant *Acetabularia Acetabulum*. *Acta Crystallogr. Sect. F-Structural Biol.* D71, 2203–2216. doi:10.1107/s1399004715015722
- Furutani, Y., Kandori, H., and Shichida, Y. (2003). Structural Changes in Lumirhodopsin and Metarhodopsin I Studied by Their Photoreactions at 77 K. *Biochemistry* 42, 8494–8500. doi:10.1021/bi034438y
- Furutani, Y., Terakita, A., Shichida, Y., and Kandori, H. (2005). FTIR Studies of the Photoactivation Processes in Squid Retinochrome. *Biochemistry* 44, 7988–7997. doi:10.1021/bi050219w
- Ganapathy, S., Bécheau, O., Venselaar, H., Frölich, S., Van Der Steen, J. B., Chen, Q., et al. (2015). Modulation of Spectral Properties and Pump Activity of Proteorhodopsins by Retinal Analogues. *Biochem. J.* 467, 333–343. doi:10.1042/bj20141210
- Ganapathy, S., Kratz, S., Chen, Q., Hellingwerf, K. J., De Groot, H. J. M., Rothschild, K. J., et al. (2019). Redshifted and Near-Infrared Active Analog Pigments Based upon Archaeorhodopsin-3. *Photochem. Photobiol.* 95, 959–968. doi:10.1111/php.13093
- Ganapathy, S., and Liu, R. S. H. (1992). Photoisomerization of Sixteen Isomers of Retinal. Initial Product Distribution in Direct and Sensitized Irradiation. *Photochem. Photobiol.* 56, 959–964. doi:10.1111/j.1751-1097.1992.tb09718.x
- Ganapathy, S., Opdam, L., Hontani, Y., Frehan, S., Chen, Q., Hellingwerf, K. J., et al. (2020). Membrane Matters: The Impact of a Nanodisc-Bilayer or a Detergent Microenvironment on the Properties of Two Eubacterial Rhodopsins. *Biochimica Biophysica Acta-Biomembranes* 1862, 183113. doi:10.1016/j.bbame.2019.183113
- Ganapathy, S., Venselaar, H., Chen, Q., De Groot, H. J. M., Hellingwerf, K. J., and De Grip, W. J. (2017). Retinal-based Proton Pumping in the Near Infrared. *J. Am. Chem. Soc.* 139, 2338–2344. doi:10.1021/jacs.6b11366
- García-Nafria, J., and Tate, C. G. (2020). Cryo-Electron Microscopy: Moving beyond X-Ray Crystal Structures for Drug Receptors and Drug Development. *Annu. Rev. Pharmacol. Toxicol.* 60, 51–71.
- Garczarek, F., and Gerwert, K. (2006). Functional Waters in Intraprotein Proton Transfer Monitored by FTIR Difference Spectroscopy. *Nature* 439, 109–112. doi:10.1038/nature04231
- Gärtner, W. (2000). “Invertebrate Visual Pigments,” in *Molecular Mechanisms in Visual Transduction*. Editors D. G. Stavenga, W. J. DeGrip, and E. N. Pugh Jr. (Amsterdam, Netherlands: Elsevier Science Pub.), 298–388.
- Gärtner, W., Ullrich, D., and Vogt, K. (1991). Quantum Yield of CHAPSO-Solubilized Rhodopsin and 3-Hydroxy-Retinal Containing Bovine Opsin. *Photochem. Photobiol.* 54, 1047–1055.
- Gascón, J. A., Sproviero, E. M., and Batista, V. S. (2005). QM/MM Study of the NMR Spectroscopy of the Retinyl Chromophore in Visual Rhodopsin. *J. Chem. Theory Comput.* 1, 674–685. doi:10.1021/ct0500850
- Geiser, A. H., Sievert, M. K., Guo, L. W., Grant, J. E., Krebs, M. P., Fotiadis, D., et al. (2006). Bacteriorhodopsin Chimeras Containing the Third Cytoplasmic Loop of Bovine Rhodopsin Activate Transducin for GTP/GDP Exchange. *Protein Sci.* 15, 1679–1690. doi:10.1110/ps.062192306
- Gerrard, E., Mutt, E., Nagata, T., Koyanagi, M., Flock, T., Lesca, E., et al. (2018). Convergent Evolution of Tertiary Structure in Rhodopsin Visual Proteins from Vertebrates and Box Jellyfish. *Proc. Natl. Acad. Sci. U. S. A.* 115, 6201–6206. doi:10.1073/pnas.1721333115
- Gerwert, K., Freier, E., and Wolf, S. (2014). The Role of Protein-Bound Water Molecules in Microbial Rhodopsins. *Biochimica Biophysica Acta-Bioenergetics* 1837, 606–613. doi:10.1016/j.bbabi.2013.09.006
- Ghanbarpour, A., Nairat, M., Nosrati, M., Santos, E. M., Vasileiou, C., Dantus, M., et al. (2019). Mimicking Microbial Rhodopsin Isomerization in a Single Crystal. *J. Am. Chem. Soc.* 141, 1735–1741. doi:10.1021/jacs.8b12493
- Gibson, S. K., Parkes, J. H., and Liebman, P. A. (1999). Phosphorylation Alters the pH-dependent Active State Equilibrium of Rhodopsin by Modulating the Membrane Surface Potential. *Biochemistry-USA* 38, 11103–11114. doi:10.1021/bi990411w
- Giesbers, M. E., Bosman, G. J. C. G. M., Bovee-Geurts, P. H. M., and DeGrip, W. J. (2007). Introduction of a Rod Aromatic Cluster Does Not Improve the Structural Stability of the Human Green Cone Pigment. *J. Struct. Biol.* 159, 222–227. doi:10.1016/j.jsb.2007.01.010
- Giesbers, M. E., Shirzad-Wasei, N., Bosman, G. J. C. G. M., and DeGrip, W. J. (2008). Functional Expression, Targeting and Ca²⁺ Signaling of a Mouse Melanopsin-eYFP Fusion Protein in a Retinal Pigment Epithelium Cell Line. *Photochem. Photobiol.* 84, 990–995. doi:10.1111/j.1751-1097.2008.00347.x
- Gilhooley, M. J., Lindner, M., Palumaa, T., Hughes, S., Peirson, S. N., and Hankins, M. W. (2022). A Systematic Comparison of Optogenetic Approaches to Visual Restoration. *Mol. Ther. Methods & Clin. Dev.* 25, 111. doi:10.1016/j.omtm.2022.03.003
- Gómez-Consarnau, L., Raven, J. A., Levine, N. M., Cutter, L. S., Wang, D. L., Seegers, B., et al. (2019). Microbial Rhodopsins Are Major Contributors to the Solar Energy Captured in the Sea. *Sci. Adv.* 5, eaaw8855. doi:10.1126/sciadv.aaw8855
- Gong, X., Mendoza-Halliday, D., Ting, J. T., Kaiser, T., Sun, X. Y., Bastos, A. M., et al. (2020). An Ultra-sensitive Step-Function Opsin for Minimally Invasive Optogenetic Stimulation in Mice and Macaques. *Neuron* 107, 38–51. doi:10.1016/j.neuron.2020.03.032
- Govorunova, E. G., Gou, Y. Y., Sineshchekov, O. A., Li, H., Wang, Y. M., Brown, L. S., et al. (2022a). Kalium Rhodopsins: Natural Light-Gated Potassium Channels. *bioRxiv*. doi:10.1101/2021.09.17.460684
- Govorunova, E. G., Sineshchekov, O. A., Li, H., and Spudich, J. L. (2017). Microbial Rhodopsins: Diversity, Mechanisms, and Optogenetic Applications. *Annu. Rev. Biochem.* 86, 845–872. doi:10.1146/annurev-biochem-101910-144233
- Govorunova, E. G., Sineshchekov, O. A., Li, H., Wang, Y., Brown, L. S., Palmateer, A., et al. (2021). Cation and Anion Channelrhodopsins: Sequence Motifs and Taxonomic Distribution. *mBio* 12, e0165621. doi:10.1128/mbio.01656-21
- Govorunova, E. G., Sineshchekov, O. A., Li, H., Wang, Y. M., Brown, L. S., and Spudich, J. L. (2020). RubyACRs, Nonalgal Anion Channelrhodopsins with Highly Red-Shifted Absorption. *Proc. Natl. Acad. Sci. U. S. A.* 117, 22833–22840. doi:10.1073/pnas.2005981117
- Govorunova, E. G., Sineshchekov, O. A., and Spudich, J. L. (2022b). Emerging Diversity of Channelrhodopsins and Their Structure-Function Relationships. *Front. Cell. Neurosci.* 15, 800313. doi:10.3389/fncel.2021.800313
- Govorunova, E. G., Sineshchekov, O. A., and Spudich, J. L. (2016). Structurally Distinct Cation Channelrhodopsins from Cryptophyte Algae. *Biophysical J.* 110, 2302–2304. doi:10.1016/j.bpj.2016.05.001
- Gozem, S., Luk, H. L., Schapiro, I., and Olivucci, M. (2017). Theory and Simulation of the Ultrafast Double-Bond Isomerization of Biological Chromophores. *Chem. Rev.* 117, 13502–13565. doi:10.1021/acs.chemrev.7b00177
- Griffiths, J. M., Bennett, A. E., Engelhard, M., Siebert, F., Raap, J., Lugtenburg, J., et al. (2000). Structural Investigation of the Active Site in Bacteriorhodopsin: Geometric Constraints on the Roles of Asp-85 and Asp-212 in the Proton-Pumping Mechanism from Solid-State NMR. *Biochemistry* 39, 362–371. doi:10.1021/bi991106d
- Grigorieff, N., Ceska, T. A., Downing, K. H., Baldwin, J. M., and Henderson, R. A. (1996). Electron-crystallographic Refinement of the Structure of Bacteriorhodopsin. *J. Mol. Biol.* 259, 393–421. doi:10.1006/jmbi.1996.0328
- Grime, R. L., Logan, R. T., Nestorow, S. A., Sridhar, P., Edwards, P. C., Tate, C. G., et al. (2021). Differences in SMA-like Polymer Architecture Dictate the

- Conformational Changes Exhibited by the Membrane Protein Rhodopsin Encapsulated in Lipid Nano-Particles. *Nanoscale* 13, 13519–13528. doi:10.1039/d1nr02419a
- Groenendijk, G. W. T., DeGrip, W. J., and Daemen, F. J. M. (1980). Quantitative Determination of Retinals with Complete Retention of Their Geometric Configuration. *Biochim. Biophys. Acta* 617, 430–438. doi:10.1016/0005-2760(80)90009-0
- Grote, M., Engelhard, M., and Hegemann, P. (2014). Of Ion Pumps, Sensors and Channels - Perspectives on Microbial Rhodopsins between Science and History. *Biochimica Biophysica Acta-Bioenergetics* 1837, 533–545. doi:10.1016/j.bbabi.2013.08.006
- Gruber, E., Kabylda, A. M., Brøndsted Nielsen, M., Rasmussen, A. P., Teiwes, R., Kusochek, P. A., et al. (2022). Light Driven Ultrafast Bioinspired Molecular Motors: Steering and Accelerating Photoisomerization Dynamics of Retinal. *J. Am. Chem. Soc.* 144, 69–73. doi:10.1021/jacs.1c10752
- Guimarães Backhaus, R., Fu, T., Backhaus, H., and Stroh, A. (2021). Pipeline for 2-photon All-Optical Physiology in Mouse: From Viral Titration and Optical Window Implantation to Binarization of Calcium Transients. *Star. Protoc.* 2, 101010. doi:10.1016/j.xpro.2021.101010
- Gulati, S., Jastrzebska, B., Banerjee, S., Placeres, A. L., Miszta, P., Gao, S. Q., et al. (2017). Photocyclic Behavior of Rhodopsin Induced by an Atypical Isomerization Mechanism. *Proc. Natl. Acad. Sci. U. S. A.* 114, E2608–E2615. doi:10.1073/pnas.1617446114
- Guo, J., Wu, Y., Gong, Z., Chen, X., Cao, F., Kala, S., et al. (2022). Photonic Nanosheet-Mediated Optogenetics. *Adv. Sci.* 9, e2104140. doi:10.1002/adv.202104140
- Guo, Y. (2020). Be Cautious with Crystal Structures of Membrane Proteins or Complexes Prepared in Detergents. *Crystals* 10, 86. doi:10.3390/cryst10020086
- Halford, S., Freedman, M. S., Bellingham, J., Inglis, S. L., Poopalasundaram, S., Soni, B. G., et al. (2001). Characterization of a Novel Human Opsin Gene with Wide Tissue Expression and Identification of Embedded and Flanking Genes on Chromosome 1q43. *Genomics* 72, 203–208. doi:10.1006/geno.2001.6469
- Hallett, F. R., Watton, J., and Krygman, P. (1991). Vesicle Sizing. Number Distributions by Dynamic Light Scattering. *Biophys. J.* 59, 357–362. doi:10.1016/s0006-3495(91)82229-9
- Han, S., Kim, S.-H., Cho, J. C., Song, J., Bleckner, G., and Jung, K.-H. (2020). Photochemical Characterization of Flavobacterial Rhodopsin: The Importance of the Helix E Region for Heat Stability. *Biochimica Biophysica Acta-Bioenergetics* 1861, 148092. doi:10.1016/j.bbabi.2019.148092
- Hanai, S., Katayama, K., Imai, H., and Kandori, H. (2021). Light-induced Difference FTIR Spectroscopy of Primate Blue-Sensitive Visual Pigment at 163 K. *Biophysics Physicobiology* 18, 40–49. doi:10.2142/biophysico.bppb-v18.005
- Hara, K. Y., Wada, T., Kino, K., Asahi, T., and Sawamura, N. (2013). Construction of Photoenergetic Mitochondria in Cultured Mammalian Cells. *Sci. Rep.* 3, 1635. doi:10.1038/srep01635
- Hara, T., Hara, R., and Takeuchi, J. (1967). Vision in Octopus and Squid. *Nature* 214, 572–575. doi:10.1038/214572a0
- Harada, Y., Senda, T., Sakamoto, T., Takamoto, K., and Ishibashi, T. (1994). Expression of octopus Rhodopsin in *Escherichia coli*. *J. Biochem. Tokyo* 115, 66–75. doi:10.1093/oxfordjournals.jbchem.a124307
- Harbison, G. S., Smith, S. O., Pardeo, J. A., Courtin, J. M. L., Lugtenburg, J., Herzfeld, J., et al. (1985). Solid-state ¹³C NMR Detection of a Perturbed 6-S-Trans Chromophore in Bacteriorhodopsin. *Biochemistry* 24, 6955–6962. doi:10.1021/bi00345a031
- Hargrave, P. A., McDowell, J. H., Curtis, D. R., Wang, J. K., Juszczak, E., Fong, S.-L., et al. (1983). The Structure of Bovine Rhodopsin. *Biophys. Struct. Mech.* 9, 235–244. doi:10.1007/bf00535659
- Hargrave, P. A., and McDowell, J. H. (1992). Rhodopsin and Phototransduction - A Model System for G-Protein-Linked Receptors. *FASEB J.* 6, 2323–2331. doi:10.1096/fasebj.6.6.1544542
- Hargrave, P. A. (1982). Rhodopsin Chemistry, Structure and Topography. *Prog. Retin. Res.* 1, 2–51. doi:10.1016/0278-4327(82)90003-7
- Hargrave, P. A. (1977). The Amino-Terminal Tryptic Peptide of Bovine Rhodopsin. A Glycopeptide Containing Two Sites of Oligosaccharide Attachment. *Biochim. Biophys. Acta* 492, 83–94. doi:10.1016/0005-2795(77)90216-1
- Haris, P. I., Robillard, G. T., Vandijk, A. A., and Chapman, D. (1992). Potential of ¹³C and ¹⁵N Labeling for Studying Protein-Protein Interactions Using Fourier Transform Infrared Spectroscopy. *Biochemistry-USA* 31, 6279–6284. doi:10.1021/bi00142a016
- Hasegawa, M., Hosaka, T., Kojima, K., Nishimura, Y., Nakajima, Y., Kimura-Someya, T., et al. (2020). A Unique Clade of Light-Driven Proton-Pumping Rhodopsins Evolved in the Cyanobacterial Lineage. *Sci. Rep.* 10, 16752. doi:10.1038/s41598-020-73606-y
- Hasegawa, N., Miki, K., and Takeda, K. (2018). X-ray Structure Analysis of Bacteriorhodopsin at 1.3 Å Resolution. *Sci. Rep.* 8, 13123. doi:10.1038/s41598-018-31370-0
- Hasemi, T., Kikukawa, T., Kamo, N., and Demura, M. (2016). Characterization of a Cyanobacterial Chloride-Pumping Rhodopsin and its Conversion into a Proton Pump. *J. Biol. Chem.* 291, 355–362. doi:10.1074/jbc.m115.688614
- Hashimoto, S., Takeuchi, H., Nakagawa, M., and Tsuda, M. (1996). Ultraviolet Resonance Raman Evidence for the Absence of Tyrosinate in octopus Rhodopsin and the Participation of Trp Residues in the Transition to Acid Metarhodopsin. *FEBS Lett.* 398, 239–242. doi:10.1016/s0014-5793(96)01250-1
- Haupts, U., Tittor, J., and Oesterheld, D. (1999). Closing in on Bacteriorhodopsin: Progress in Understanding the Molecule. *Annu. Rev. Biophys. Biomol. Struct.* 28, 367–399. doi:10.1146/annurev.biophys.28.1.367
- Havelka, W. A., Henderson, R. A., and Oesterheld, D. (1995). Three-dimensional Structure of Halorhodopsin at 7 Å Resolution. *J. Mol. Biol.* 247, 726–738. doi:10.1016/s0022-2836(05)80151-2
- Hayashi, M., Kojima, K., Sudo, Y., and Yamashita, A. (2021). An Optogenetic Assay Method for Electrogenic Transporters Using *Escherichia coli* Co-expressing Light-Driven Proton Pump. *Protein Sci.* 30, 2161–2169. doi:10.1002/pro.4154
- Hayashi, S., Tajkhorshid, E., Pebay-Peyroula, E., Royant, A., Landau, E. M., Navarro, J., et al. (2001). Structural Determinants of Spectral Tuning in Retinal Proteins-Bacteriorhodopsin vs Sensory Rhodopsin II. *J. Phys. Chem. B* 105, 10124–10131. doi:10.1021/jp011362b
- Hayashi, T., Yasuda, S., Suzuki, K., Akiyama, T., Kanehara, K., Kojima, K., et al. (2020). How Does a Microbial Rhodopsin RxR Realize its Exceptionally High Thermostability with the Proton-Pumping Function Being Retained? *J. Phys. Chem. B* 124, 990–1000. doi:10.1021/acs.jpcc.9b10700
- Heath, G. R., Kots, E., Robertson, J. L., Lansky, S., Khelashvili, G., Weinstein, H., et al. (2021). Localization Atomic Force Microscopy. *Nature* 594, 385–390. doi:10.1038/s41586-021-03551-x
- Henderson, R., and Unwin, P. N. T. (1975). Three-dimensional Model of Purple Membrane Obtained by Electron-Microscopy. *Nature* 257, 28–32. doi:10.1038/257028a0
- Herwig, L., Rice, A. J., Bedbrook, C. N., Zhang, R. J. K., Lignell, A., Cahn, J. K. B., et al. (2017). Directed Evolution of a Bright Near-Infrared Fluorescent Rhodopsin Using a Synthetic Chromophore. *Cell. Chem. Biol.* 24, 415–425. doi:10.1016/j.chembiol.2017.02.008
- Herzfeld, J., and Lansing, J. C. (2002). Magnetic Resonance Studies of the Bacteriorhodopsin Pump Cycle. *Annu. Rev. Biophysics Biomol. Struct.* 31, 73–95. doi:10.1146/annurev.biophys.31.082901.134233
- Heymann, J. B., Müller, D. J., Mitsuoka, K., and Engel, A. (1997). Electron and Atomic Force Microscopy of Membrane Proteins. *Curr. Opin. Struct. Biol.* 7, 543–549. doi:10.1016/s0959-440x(97)80120-0
- Hickey, D. G., Davies, W. I. L., Hughes, S., Rodgers, J., Thavanesan, N., Maclaren, R. E., et al. (2021). Chimeric Human Opsins as Optogenetic Light Sensitizers. *J. Exp. Biol.* 224, 240580. doi:10.1242/jeb.240580
- Higuchi, A., Shihoya, W., Konno, M., Ikuta, T., Kandori, H., Inoue, K., et al. (2021). Crystal Structure of Schizorhodopsin Reveals Mechanism of Inward Proton Pumping. *Proc. Natl. Acad. Sci. U. S. A.* 118, 2016328118. doi:10.1073/pnas.2016328118
- Hildebrand, P. W., Scheerer, P., Park, J. H., Choe, H.-W., Piechnick, R., Ernst, O. P., et al. (2009). A Ligand Channel through the G Protein Coupled Receptor Opsin. *PLoS ONE* 4, e4382. doi:10.1371/journal.pone.0004382
- Hildebrandt, V., Polakowski, F., and Büldt, G. (1991). Purple Fission Yeast: Overexpression and Processing of the Pigment Bacteriorhodopsin in *Schizosaccharomyces pombe*. *Photochem. Photobiol.* 54, 1009–1016. doi:10.1111/j.1751-1097.1991.tb02123.x

- Hillman, P., Hochstein, S., and Minke, B. (1983). Transduction in Invertebrate Photoreceptors - Role of Pigment Bistability. *Physiol. Rev.* 63, 668–772. doi:10.1152/physrev.1983.63.2.668
- Hirano, T., Fujioka, N., Imai, H., Kandori, H., Wada, A., Ito, M., et al. (2006). Assignment of the Vibrational Modes of the Chromophores of Iodopsin and Bathiodopsin: Low-Temperature Fourier Transform Infrared Spectroscopy of ^{13}C and ^2H -Labeled Iodopsins. *Biochemistry* 45, 1285–1294. doi:10.1021/bi0517077
- Hirschi, S., Fischer, N., Kalbermatter, D., Laskowski, P. R., Ucurum, Z., Müller, D. J., et al. (2019). Design and Assembly of a Chemically Switchable and Fluorescently Traceable Light-Driven Proton Pump System for Bionanotechnological Applications. *Sci. Rep.* 9, 1046. doi:10.1038/s41598-018-37260-9
- Hirschi, S., Kalbermatter, D., Ucurum, Z., Lemmin, T., and Fotiadis, D. (2021). Cryo-EM Structure and Dynamics of the Green-Light Absorbing Proteorhodopsin. *Nat. Commun.* 12, 4107. doi:10.1038/s41467-021-24429-6
- Hochbaum, D. R., Zhao, Y., Farhi, S. L., Klapoetke, N. C., Werley, C. A., Kapoor, V., et al. (2014). All-optical Electrophysiology in Mammalian Neurons Using Engineered Microbial Rhodopsins. *Nat. Methods* 11, 825–833. doi:10.1038/nmeth.3000
- Hoffmann, A., Hildebrandt, V., Heberle, J., and Büldt, G. (1994). Photoactive Mitochondria: *In Vivo* Transfer of a Light-Driven Proton Pump into the Inner Mitochondrial Membrane of *Schizosaccharomyces pombe*. *Proc. Nat. Acad. Sci. U. S. A.* 91, 9367–9371. doi:10.1073/pnas.91.20.9367
- Hofmann, K. P. (2000). “Late Photoproducts and Signaling States of Bovine Rhodopsin,” in *Molecular Mechanisms in Visual Transduction*. Editors D. G. Stavenga, W. J. DeGrip, and E. N. Pugh Jr. (Amsterdam, Netherlands: Elsevier Science Pub.), 91–142. doi:10.1016/s1383-8121(00)80006-8
- Hofmann, K. P., Scheerer, P., Hildebrand, P. W., Choe, H. W., Park, J. H., Heck, M., et al. (2009). A G Protein-Coupled Receptor at Work: the Rhodopsin Model. *Trends Biochem. Sci.* 34, 540–552. doi:10.1016/j.tibs.2009.07.005
- Hofmann, L., and Palczewski, K. (2015). Advances in Understanding the Molecular Basis of the First Steps in Color Vision. *Prog. Retin. Eye Res.* 49, 46–66. doi:10.1016/j.preteyeres.2015.07.004
- Hoi, K. K., Bada Juarez, J. F., Judge, P. J., Yen, H.-Y., Wu, D., Vinals, J., et al. (2021). Detergent-free Lipodisq Nanoparticles Facilitate High-Resolution Mass Spectrometry of Folded Integral Membrane Proteins. *Nano Lett.* 21, 2824–2831. doi:10.1021/acs.nanolett.0c04911
- Hoischen, D., Steinmüller, S., Gärtner, W., Buss, V., and Martin, H.-D. (1997). Merocyanines as Extremely Bathochromically Absorbing Chromophores in the Halobacterial Membrane Protein Bacteriorhodopsin. *Angew. Chem. Int. Ed.* 36, 1630–1633.
- Hong, F. T. (1994). “Retinal Proteins in Photovoltaic Devices,” in *Molecular and Biomolecular Electronics*. Editor R. R. Birge (Washington, DC, USA: American Chemical Society), 1–27.
- Hontani, Y., Broser, M., Luck, M., Weissenborn, J., Kloz, M., Hegemann, P., et al. (2020). Dual Photoisomerization on Distinct Potential Energy Surfaces in a UV-Absorbing Rhodopsin. *J. Am. Chem. Soc.* 142, 11464–11473. doi:10.1021/jacs.0c03229
- Hontani, Y., Broser, M., Silapetere, A., Krause, B. S., Hegemann, P., and Kennis, J. T. M. (2017a). The Femtosecond-To-Second Photochemistry of Red-Shifted Fast-Closing Anion Channelrhodopsin PsACR1. *Phys. Chem. Chem. Phys.* 19, 30402–30409. doi:10.1039/c7cp06414d
- Hontani, Y., Ganapathy, S., Frehan, S., Kloz, M., De Grip, W. J., and Kennis, J. T. M. (2019). Photoreaction Dynamics of Red-Shifting Retinal Analogues Reconstituted in Proteorhodopsin. *J. Phys. Chem. B* 123, 4242–4250. doi:10.1021/acs.jpcc.9b01136
- Hontani, Y., Ganapathy, S., Frehan, S., Kloz, M., De Grip, W. J., and Kennis, J. T. M. (2018). Strong pH-dependent Near-Infrared Fluorescence in a Microbial Rhodopsin Reconstituted with a Red-Shifting Retinal Analogue. *J. Phys. Chem. Lett.* 9, 6469–6474. doi:10.1021/acs.jpclett.8b02780
- Hontani, Y., Marazzi, M., Stehfest, K., Mathes, T., Van Stokkum, I. H. M., Elstner, M., et al. (2017b). Reaction Dynamics of the Chimeric Channelrhodopsin C1C2. *Sci. Rep.* 7, 7217. doi:10.1038/s41598-017-07363-w
- Hope, A. J., Partridge, J. C., Dulai, K. S., and Hunt, D. M. (1997). Mechanisms of Wavelength Tuning in the Rod Opsins of Deep-Sea Fishes. *Proc. R. Soc. B-Biological Sci.* 264, 155–163. doi:10.1098/rspb.1997.0023
- Hornak, V., Ahuja, S., Eilers, M., Goncalves, J. A., Sheves, M., Reeves, P. J., et al. (2010). Light Activation of Rhodopsin: Insights from Molecular Dynamics Simulations Guided by Solid-State NMR Distance Restraints. *J. Mol. Biol.* 396, 510–527. doi:10.1016/j.jmb.2009.12.003
- Hosaka, T., Nomura, T., Kubo, M., Nakane, T., Fangjia, L., Sekine, S.-I., et al. (2022). Conformational Alterations in Unidirectional Ion Transport of a Light-Driven Chloride Pump Revealed Using X-Ray Free Electron Lasers. *Proc. Natl. Acad. Sci. U. S. A.* 119, e2117433119. doi:10.1073/pnas.2117433119
- Hosaka, T., Yoshizawa, S., Nakajima, Y., Ohsawa, N., Hato, M., Delong, E. F., et al. (2016). Structural Mechanism for Light-Driven Transport by a New Type of Chloride Ion Pump, Nonlabens Marinus Rhodopsin-3. *J. Biol. Chem.* 291, 17488–17495. doi:10.1074/jbc.m116.728220
- Hu, J. G. G., Sun, B. Q. Q., Bizounok, M., Hatcher, M. E., Lansing, J. C., Raap, J., et al. (1998). Early and Late M Intermediates in the Bacteriorhodopsin Photocycle: A Solid-State NMR Study. *Biochemistry-USA* 37, 8088–8096. doi:10.1021/bi973168e
- Huang, L., Deng, H., Koutalos, Y., Ebrey, T. G., Groesbeck, M., Lugtenburg, J., et al. (1997). A Resonance Raman Study of the C=C Stretch Modes in Bovine and octopus Visual Pigments with Isotopically Labeled Retinal Chromophores. *Photochem. Photobiol.* 66, 747–754. doi:10.1111/j.1751-1097.1997.tb03219.x
- Hubbard, R., Brown, P. K., and Bownds, M. D. (1971). Methodology of Vitamin A and Visual Pigments. *Meth. Enzymol.* 18C, 615–653. doi:10.1016/s0076-6879(71)18045-7
- Hubbard, R., and Wald, G. (1952). Cis-trans Isomers of Vitamin A and Retinene in the Rhodopsin System. *J. General Physiology* 36, 269–315. doi:10.1085/jgp.36.2.269
- Hubbell, W. L., Altenbach, C., Hubbell, C. M., and Khorana, H. G. (2003). Rhodopsin Structure, Dynamics, and Activation: A Perspective from Crystallography, Site-Directed Spin Labeling, Sulfhydryl Reactivity, and Disulfide Cross-Linking. *Adv. Protein Chem.* 63, 243–290. doi:10.1016/s0065-3233(03)63010-x
- Humphreys, I. R., Pei, J. M., Baek, M., Krishnakumar, A., Anishchenko, I., Ovchinnikov, S., et al. (2021). Computed Structures of Core Eukaryotic Protein Complexes. *Science* 374, 1340.
- Hunt, D. M., and Collin, S. P. (2014). “The Evolution of Photoreceptors and Visual Photopigments in Vertebrates,” in *Evolution of Visual and Non-visual Pigments*. Editor D. M. Hunt (New York: Springer Science+Business Media New York), 163–217. doi:10.1007/978-1-4614-4355-1_6
- Hunt, D. M., Dulai, K. S., Partridge, J. C., Cottrill, P., and Bowmaker, J. K. (2001). The Molecular Basis for Spectral Tuning of Rod Visual Pigments in Deep-Sea Fish. *J. Exp. Biol.* 204, 3333–3344. doi:10.1242/jeb.204.19.3333
- Hussain, H., Du, Y., Scull, N. J., Mortensen, J. S., Tarrasch, J., Bae, H. E., et al. (2016). Accessible Mannitol-Based Amphiphiles (MNAs) for Membrane Protein Solubilisation and Stabilisation. *Chemistry-A Eur. J.* 22, 7068–7073. doi:10.1002/chem.201600533
- Hussain, S., Kinnebrew, M., Schonenbach, N. S., Aye, E., and Han, S. G. (2015). Functional Consequences of the Oligomeric Assembly of Proteorhodopsin. *J. Mol. Biol.* 427, 1278–1290. doi:10.1016/j.jmb.2015.01.004
- Hwa, J., Reeves, P. J., Klein-Seetharaman, J., Davidson, F. F., and Khorana, H. G. (1999). Structure and Function in Rhodopsin: Further Elucidation of the Role of the Intradiscal Cysteines, Cys-110, -185, and -187, in Rhodopsin Folding and Function. *Proc. Nat. Acad. Sci. U. S. A.* 96, 1932–1935. doi:10.1073/pnas.96.5.1932
- Iizuka, A., Kajimoto, K., Fujisawa, T., Tsukamoto, T., Aizawa, T., Kamo, N., et al. (2019). Functional Importance of the Oligomer Formation of the Cyanobacterial H+ Pump Gloeobacter Rhodopsin. *Sci. Rep.* 9, 10711. doi:10.1038/s41598-019-47178-5
- Ikeda, D., Furutani, Y., and Kandori, H. (2007). FTIR Study of the Retinal Schiff Base and Internal Water Molecules of Proteorhodopsin. *Biochemistry* 46, 5365–5373. doi:10.1021/bi700143g
- Ikuta, T., Shihoya, W., Sugiura, M., Yoshida, K., Watari, M., Tokano, T., et al. (2020). Structural Insights into the Mechanism of Rhodopsin Phosphodiesterase. *Nat. Commun.* 11, 5605. doi:10.1038/s41467-020-19376-7
- Imai, H., Hirano, T., Terakita, A., Shichida, Y., Muthyala, R. S., Chen, R.-L., et al. (1999). Probing for the Threshold Energy for Visual Transduction: Red-Shifted Visual Pigment Analogs from 3-Methoxy-3-Dehydroretinal and Related Compounds. *Photochem. Photobiol.* 70, 111–115. doi:10.1111/j.1751-1097.1999.tb01956.x

- Imai, H., Imamoto, Y., Yoshizawa, T., and Shichida, Y. (1995). Difference in Molecular Properties between Chicken Green and Rhodopsin as Related to the Functional Difference between Cone and Rod Photoreceptor Cells. *Biochemistry-USA* 34, 10525–10531. doi:10.1021/bi00033a026
- Imai, H., Terakita, A., Tachibanaki, S., Imamoto, Y., Yoshizawa, T., and Shichida, Y. (1997). Photochemical and Biochemical Properties of Chicken Blue-Sensitive Cone Visual Pigment. *Biochemistry-USA* 36, 12773–12779. doi:10.1021/bi970809x
- Imai, Y., Inoshita, T., Meng, H. R., Shiba-Fukushima, K., Hara, K. Y., Sawamura, N., et al. (2019). Light-driven Activation of Mitochondrial Proton-Motive Force Improves Motor Behaviors in a *Drosophila* Model of Parkinson's Disease. *Commun. Biol.* 2, 424. doi:10.1038/s42003-019-0674-1
- Imamoto, Y., Kandori, H., Okano, T., Fukada, Y., Shichida, Y., and Yoshizawa, T. (1989). Effect of Chloride Ion on the Thermal Decay Process of the Batho Intermediate of Iodopsin at Low Temperature. *Biochemistry* 28, 9412–9416. doi:10.1021/bi00450a025
- Imamoto, Y., and Shichida, Y. (2014). Cone Visual Pigments. *Biochimica Biophysica Acta-Bioenergetics* 1837, 664–673. doi:10.1016/j.bbabi.2013.08.009
- Imamoto, Y., Yoshizawa, T., and Shichida, Y. (1996). Chromophore Configuration of Iodopsin and its Photoproducts Formed at Low Temperatures. *Biochemistry* 35, 14599–14607. doi:10.1021/bi9614850
- Imasheva, E. S., Balashov, S. P., Wang, J. M., and Lanyi, J. K. (2011). Removal and Reconstitution of the Carotenoid Antenna of Xanthorhodopsin. *J. Membr. Biol.* 239, 95–104. doi:10.1007/s00232-010-9322-x
- Inagaki, H. K., Jung, Y., Hoopfer, E. D., Wong, A. M., Mishra, N., Lin, J. Y., et al. (2014). Optogenetic Control of *Drosophila* Using a Red-Shifted Channelrhodopsin Reveals Experience-Dependent Influences on Courtship. *Nat. Methods* 11, 325–U311. doi:10.1038/nmeth.2765
- Inoue, K., Del Carmen Marin, M., Tomida, S., Nakamura, R., Nakajima, Y., Olivucci, M., et al. (2019). Red-shifting Mutation of Light-Driven Sodium-Pump Rhodopsin. *Nat. Commun.* 10, 1993. doi:10.1038/s41467-019-10000-x
- Inoue, K., Karasuyama, M., Nakamura, R., Konno, M., Yamada, D., Mannen, K., et al. (2021). Exploration of Natural Red-Shifted Rhodopsins Using a Machine Learning-Based Bayesian Experimental Design. *Commun. Biol.* 4, 362. doi:10.1038/s42003-021-01878-9
- Inoue, K., Nomura, Y., and Kandori, H. (2016). Asymmetric Functional Conversion of Eubacterial Light-Driven Ion Pumps. *J. Biol. Chem.* 291, 9883–9893. doi:10.1074/jbc.m116.716498
- Inoue, K., Reissig, L., Sakai, M., Kobayashi, S., Homma, M., Fujii, M., et al. (2012). Absorption Spectra and Photochemical Reactions in a Unique Photoactive Protein, Middle Rhodopsin MR. *J. Phys. Chem. B* 116, 5888–5899. doi:10.1021/jp302357m
- Inoue, K., Sasaki, J., Morisaki, M., Tokunaga, F., and Terazima, M. (2004). Time-resolved Detection of Sensory Rhodopsin II-Transducer Interaction. *Biophysical J.* 87, 2587–2597. doi:10.1529/biophysj.104.043521
- Inoue, K., Sudo, Y., Homma, M., and Kandori, H. (2011). Spectrally Silent Intermediates during the Photochemical Reactions of Salinibacter Sensory Rhodopsin I. *J. Phys. Chem. B* 115, 4500–4508. doi:10.1021/jp2000706
- Inoue, K., Tahara, S., Kato, Y., Takeuchi, S., Tahara, T., and Kandori, H. (2018). Spectroscopic Study of Proton-Transfer Mechanism of Inward Proton-Pump Rhodopsin, *Parvularcula Oceani* Xenorhodopsin. *J. Phys. Chem. B* 122, 6453–6461. doi:10.1021/acs.jpcc.8b01279
- Inoue, K., Tsukamoto, T., Shimono, K., Suzuki, Y., Miyauchi, S., Hayashi, S., et al. (2015). Converting a Light-Driven Proton Pump into a Light-Gated Proton Channel. *J. Am. Chem. Soc.* 137, 3291–3299. doi:10.1021/ja511788f
- Inoue, K., Tsukamoto, T., and Sudo, Y. (2014). Molecular and Evolutionary Aspects of Microbial Sensory Rhodopsins. *Biochimica Biophysica Acta-Bioenergetics* 1837, 562–577. doi:10.1016/j.bbabi.2013.05.005
- Inoue, K., Tsunoda, S. P., Singh, M., Tomida, S., Hososhima, S., Konno, M., et al. (2020). Schizorhodopsins: A Family of Rhodopsins from Asgard Archaea that Function as Light-Driven Inward H⁺ Pumps. *Sci. Adv.* 6, 2441. doi:10.1126/sciadv.aaz2441
- Ito, S., Iwaki, M., Sugita, S., Abe-Yoshizumi, R., Iwata, T., Inoue, K., et al. (2018). Unique Hydrogen Bonds in Membrane Protein Monitored by Whole Mid-IR ATR Spectroscopy in Aqueous Solution. *J. Phys. Chem. B* 122, 165–170. doi:10.1021/acs.jpcc.7b11064
- Iwata, T., Colmenares, L. U., Hirata, K., Arime, Y., Nakagawa, M., Kikkawa, S., et al. (1998). ¹⁹F-NMR and UV-Vis Absorption Spectroscopic Studies of Fluorinated octopus Rhodopsin and its Photoproducts. *J. Phys. Chem. A* 102, 5602–5610. doi:10.1021/jp9802477
- Iyer, E. S. S., Misra, R., Maity, A., Liubashevski, O., Sudo, Y., Sheves, M., et al. (2016). Temperature Independence of Ultrafast Photoisomerization in Thermophilic Rhodopsin: Assessment versus Other Microbial Proton Pumps. *J. Am. Chem. Soc.* 138, 12401–12407. doi:10.1021/jacs.6b05002
- Jacobs, G. H. (2018). Photopigments and the Dimensionality of Animal Color Vision. *Neurosci. Biobehav. Rev.* 86, 108–130. doi:10.1016/j.neubiorev.2017.12.006
- Jäger, S., Lewis, J. W., Zvyaga, T. A., Szundi, I., Sakmar, T. P., and Kliger, D. S. (1997). Chromophore Structural Changes in Rhodopsin from Nanoseconds to Microseconds Following Pigment Photolysis. *Proc. Nat. Acad. Sci. U. S. A.* 94, 8557–8562. doi:10.1073/pnas.94.16.8557
- Jana, S., Jung, K. H., and Sheves, M. (2020). The Chirality Origin of Retinal-Carotenoid Complex in Gloeobacter Rhodopsin: a Temperature-dependent Excitonic Coupling. *Sci. Rep.* 10, 13992. doi:10.1038/s41598-020-70697-5
- Janknecht, R., Demartynoff, G., Lou, J., Hipskind, R. A., Nordheim, A., and Stunnenberg, H. G. (1991). Rapid and Efficient Purification of Native Histidine-Tagged Protein Expressed by Recombinant Vaccinia Virus. *Proc. Nat. Acad. Sci. U. S. A.* 88, 8972–8976. doi:10.1073/pnas.88.20.8972
- Janssen, J. J. M., Bovee-Geurts, P. H. M., Merckx, M., and DeGrip, W. J. (1995). Histidine Tagging Both Allows Convenient Single-step Purification of Bovine Rhodopsin and Exerts Ionic Strength-dependent Effects on its Photochemistry. *J. Biol. Chem.* 270, 11222–11229. doi:10.1074/jbc.270.19.11222
- Janssen, J. J. M., Mulder, W. R., DeCaluwé, G. L. J., Vlak, J. M., and DeGrip, W. J. (1991). *In Vitro* expression of Bovine Opsin Using Recombinant Baculovirus: The Role of Glutamic Acid (134) in Opsin Biosynthesis and Glycosylation. *Biochim. Biophys. Acta* 1089, 68–76. doi:10.1016/0167-4781(91)90086-2
- Janssen, J. J. M., VandeVen, W. J. M., VanGroningen-Luyben, W. a. H. M., Roosien, J., Vlak, J. M., and DeGrip, W. J. (1988). Synthesis of Functional Bovine Opsin in Insect Cells under Control of the Baculovirus Polyhedrin Promotor. *Mol. Biol. Rep.* 13, 65–71. doi:10.1007/bf00539052
- Janssen, J. W. H., David-Gray, Z. K., Bovee-Geurts, P. H. M., Nevo, E., Foster, R. G., and DeGrip, W. J. (2003). A Green Cone-like Pigment in the 'blind' Mole-Rat *Spalax Ehrenbergi*: Functional Expression and Photochemical Characterization. *Photochem. Photobiol. Sci.* 2, 1287–1291. doi:10.1039/b300059c
- Jastrzebska, B., Palczewski, K., and Golczak, M. (2011). Role of Bulk Water in Hydrolysis of the Rhodopsin Chromophore. *J. Biol. Chem.* 286, 18930–18937. doi:10.1074/jbc.m111.234583
- Ji, L. L., Ma, B. F., Meng, Q., Li, L. J., Liu, K., and Chen, D. L. (2017). Detergent-resistant Oligomeric *Leptosphaeria* Rhodopsin Is a Promising Bio-Nanomaterial and an Alternative to Bacteriorhodopsin. *Biochem. Biophysical Res. Commun.* 493, 352–357. doi:10.1016/j.bbrc.2017.09.018
- Jiang, M. S., Pandey, S., and Fong, H. K. W. (1993). An Opsin Homologue in the Retina and Pigment Epithelium. *Investig. Ophthalmol. Vis. Sci.* 34, 3669–3678.
- Johnson, P. J. M., Halpin, A., Morizumi, T., Brown, L. S., Prokhorenko, V. I., Ernst, O. P., et al. (2014). The Photocycle and Ultrafast Vibrational Dynamics of Bacteriorhodopsin in Lipid Nanodiscs. *Phys. Chem. Chem. Phys.* 16, 21310–21320. doi:10.1039/c4cp01826e
- Johnson, P. J. M., Halpin, A., Morizumi, T., Prokhorenko, V. I., Ernst, O. P., and Miller, R. J. D. (2015). Local Vibrational Coherences Drive the Primary Photochemistry of Vision. *Nat. Chem.* 7, 980–986. doi:10.1038/nchem.2398
- Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O., et al. (2021). Highly Accurate Protein Structure Prediction with AlphaFold. *Nature* 596, 583–589. doi:10.1038/s41586-021-03819-2
- Jun, N. Y., and Cardin, J. A. (2020). Activation of Distinct Channelrhodopsin Variants Engages Different Patterns of Network Activity. *Eneuro* 7, 0222–0218. doi:10.1523/ENEURO.0222-18.2019
- Kahremany, S., Sander, C. L., Tochtrop, G. P., Kubas, A., and Palczewski, K. (2019). Z-isomerization of Retinoids through Combination of Monochromatic Photoisomerization and Metal Catalysis. *Org. Biomol. Chem.* 17, 8125–8139. doi:10.1039/c9ob01645g
- Kandori, H., Inoue, K., and Tsunoda, S. P. (2018). Light-Driven Sodium-Pumping Rhodopsin: A New Concept of Active Transport. *Chem. Rev.* 118, 10646–10658. doi:10.1021/acs.chemrev.7b00548
- Kandori, H. (2020). Retinal Proteins: Photochemistry and Optogenetics. *Bull. Chem. Soc. Jpn.* 93, 76–85. doi:10.1246/bcsj.20190292

- Kanehara, K., Yoshizawa, S., Tsukamoto, T., and Sudo, Y. (2017). A Phylogenetically Distinctive and Extremely Heat Stable Light-Driven Proton Pump from the Eubacterium *Rubrobacter Xylanophilus* DSM 9941^T. *Sci. Rep.* 7, 44427. doi:10.1038/srep44427
- Kaneko, A., Inoue, K., Kojima, K., Kandori, H., and Sudo, Y. (2017). Conversion of Microbial Rhodopsins: Insights into Functionally Essential Elements and Rational Protein Engineering. *Biophys. Rev.* 9, 861–876. doi:10.1007/s12551-017-0335-x
- Kannan, M., Vasan, G., and Pieribone, V. A. (2019). Optimizing Strategies for Developing Genetically Encoded Voltage Indicators. *Front. Cell. Neurosci.* 13, 53. doi:10.3389/fncel.2019.00053
- Kao, Y.-M., Cheng, C.-H., Syue, M.-L., Huang, H.-Y., Chen, I.-C., Yu, T.-Y., et al. (2019). Photochemistry of Bacteriorhodopsin with Various Oligomeric States in Controlled Membrane Mimicking Environments: A Spectroscopic Study from Femtoseconds to Milliseconds. *J. Phys. Chem. B* 123, 2032–2039. doi:10.1021/acs.jpcc.9b01224
- Karapinar, R., Schwitalla, J. C., Eickelbeck, D., Pakusch, J., Mücher, B., Grömmke, M., et al. (2021). Reverse Optogenetics of G Protein Signaling by Zebrafish Non-visual Opsin Opn7b for Synchronization of Neuronal Networks. *Nat. Commun.* 12, 4488. doi:10.1038/s41467-021-24718-0
- Karasuyama, M., Inoue, K., Nakamura, R., Kandori, H., and Takeuchi, I. (2018). Understanding Colour Tuning Rules and Predicting Absorption Wavelengths of Microbial Rhodopsins by Data-Driven Machine-Learning Approach. *Sci. Rep.* 8, 15580–15511. doi:10.1038/s41598-018-33984-w
- Karnik, S. S., Ridge, K. D., Bhattacharya, S. S., and Khorana, H. G. (1993). Palmitoylation of Bovine Opsin and its Cysteine Mutants in COS Cells. *Proc. Nat. Acad. Sci. U. S. A.* 90, 40–44. doi:10.1073/pnas.90.1.40
- Karnik, S. S., Sakmar, T. P., Chen, H.-B., and Khorana, H. G. (1988). Cysteine Residues 110 and 187 Are Essential for the Formation of Correct Structure in Bovine Rhodopsin. *Proc. Nat. Acad. Sci. U. S. A.* 85, 8459–8463. doi:10.1073/pnas.85.22.8459
- Katana, R., Guan, C. L., Zanini, D., Larsen, M. E., Giraldo, D., Geurten, B. R. H., et al. (2019). Chromophore-Independent Roles of Opsin Apoproteins in *Drosophila* Mechanoreceptors. *Curr. Biol.* 29, 2961–2969. doi:10.1016/j.cub.2019.07.036
- Katanosaka, K., Tokunaga, F., Kawamura, S., and Ozaki, K. (1998). N-linked Glycosylation of *Drosophila* Rhodopsin Occurs Exclusively in the Amino-Terminal Domain and Functions in Rhodopsin Maturation. *FEBS Lett.* 424, 149–154. doi:10.1016/s0014-5793(98)00160-4
- Kataoka, C., Inoue, K., Katayama, K., Bèjà, O., and Kandori, H. (2019). Unique Photochemistry Observed in a New Microbial Rhodopsin. *J. Phys. Chem. Lett.* 10, 5117–5121. doi:10.1021/acs.jpclett.9b01957
- Katayama, K., Furutani, Y., Imai, H., and Kandori, H. (2012). Protein-bound Water Molecules in Primate Red- and Green-Sensitive Visual Pigments. *Biochemistry* 51, 1126–1133. doi:10.1021/bi201676y
- Katayama, K., Gulati, S., Ortega, J. T., Alexander, N. S., Sun, W. Y., Shenouda, M. M., et al. (2019). Specificity of the Chromophore-Binding Site in Human Cone Opsins. *J. Biol. Chem.* 294, 6082–6093. doi:10.1074/jbc.ra119.007587
- Katayama, K., Nonaka, Y., Tsutsui, K., Imai, H., and Kandori, H. (2017). Spectral Tuning Mechanism of Primate Blue-Sensitive Visual Pigment Elucidated by FTIR Spectroscopy. *Sci. Rep.* 7, 4904. doi:10.1038/s41598-017-05177-4
- Kathe, C., Michoud, F., Schönle, P., Rowald, A., Brun, N., Ravier, J., et al. (2021). Wireless Closed-Loop Optogenetics across the Entire Dorsal Spinal Cord in Mice. *Nat. Biotechnol.* 40, 198. doi:10.1038/s41587-021-01019-x
- Kato, H. E., Inoue, K., Abe-Yoshizumi, R., Kato, Y., Ono, H., Konno, M., et al. (2015). Structural Basis for Na⁺ Transport Mechanism by a Light-Driven Na⁺ Pump. *Nature* 521, 48–53. doi:10.1038/nature14322
- Kato, H. E., Kamiya, M., Sugo, S., Ito, J., Taniguchi, R., Orito, A., et al. (2015). Atomistic Design of Microbial Opsin-Based Blue-Shifted Optogenetics Tools. *Nat. Commun.* 6, 7177. doi:10.1038/ncomms8177
- Kato, H. E., Zhang, F., Yizhar, O., Ramakrishnan, C., Nishizawa, T., Hirata, K., et al. (2012). Crystal Structure of the Channelrhodopsin Light-Gated Cation Channel. *Nature* 482, 369–374. doi:10.1038/nature10870
- Katz, B., and Minke, B. (2009). *Drosophila* Photoreceptors and Signaling Mechanisms. *Front. Cell. Neurosci.* 3, 2. doi:10.3389/fncel.2009.03.002.2009
- Kaufmann, J. C. D., Krause, B. S., Adam, S., Ritter, E., Schapiro, I., Hegemann, P., et al. (2020). Modulation of Light Energy Transfer from Chromophore to Protein in the Channelrhodopsin ReaChR. *Biophysical J.* 119, 705–716. doi:10.1016/j.bpj.2020.06.031
- Kaur, J., Kriebel, C. N., Eberhardt, P., Jaktetchai, O., Leeder, A. J., Weber, I., et al. (2019). Solid-state NMR Analysis of the Sodium Pump Krokobacter Rhodopsin 2 and its H30A Mutant. *J. Struct. Biol.* 206, 55–65. doi:10.1016/j.jsb.2018.06.001
- Kaushal, S., Ridge, K. D., and Khorana, H. G. (1994). Structure and Function in Rhodopsin: The Role of Asparagine-Linked Glycosylation. *Proc. Nat. Acad. Sci. U. S. A.* 91, 4024–4028. doi:10.1073/pnas.91.9.4024
- Kawamura, I., Seki, H., Tajima, S., Makino, Y., Shigeta, A., Okitsu, T., et al. (2021). Structure of a Retinal Chromophore of Dark-Adapted Middle Rhodopsin as Studied by Solid-State Nuclear Magnetic Resonance Spectroscopy. *Biophysical J.* 120, 177–185. doi:10.1016/j.bpj.2021.03.019
- Kawamura, S., Gerstung, M., Colozo, A. T., Helenius, J., Maeda, A., Beerenwinkel, N., et al. (2013). Kinetic, Energetic, and Mechanical Differences between Dark-State Rhodopsin and Opsin. *Structure* 21, 426–437. doi:10.1016/j.str.2013.01.011
- Kawanabe, A., Furutani, Y., Jung, K.-H., and Kandori, H. (2007). Photochromism of *Anabaena* Sensory Rhodopsin. *J. Am. Chem. Soc.* 129, 8644–8649. doi:10.1021/ja072085a
- Kawasaki, Y., Konno, M., and Inoue, K. (2021). Thermostable Light-Driven Inward Proton Pump Rhodopsins. *Chem. Phys. Lett.* 779, 138868. doi:10.1016/j.cplett.2021.138868
- Kazmi, M. A., Dubin, R. A., Oddoux, C., and Ostrer, H. (1996). High-level Inducible Expression of Visual Pigments in Transfected Cells. *BioTechniques* 21, 304–311. doi:10.2144/96212rr05
- Kazmin, R., Rose, A., Szczepek, M., Elgeti, M., Ritter, E., Piechnick, R., et al. (2015). The Activation Pathway of Human Rhodopsin in Comparison to Bovine Rhodopsin. *J. Biol. Chem.* 290, 20117–20127. doi:10.1074/jbc.m115.652172
- Khelashvili, G., and Menon, A. K. (2022). Phospholipid Scrambling by G Protein-Coupled Receptors. *Annu. Rev. Biophys.* 51, 39–61. doi:10.1146/annurev-biophys-090821-083030
- Khodonov, A. A., Shevyakov, S. V., Mironova, E. V., Shvets, V. I., Alexeeva, S. G., Demina, O. V., et al. (2000). Bacteriorhodopsin Analogs, Bearing Modified Chromophore as a Basis for the Photochromic Materials. *Mol. Cryst. Liq. Cryst.* 345, 641–646. doi:10.1080/10587250008023938
- Khorana, H. G., Braiman, M. S., Chao, B. H., Doi, T., Flitsch, S. L., Gilles-Gonzalez, M. A., et al. (1987). Site-specific Mutagenesis in Structure - Function Studies of Bacteriorhodopsin. *Chem. Scr.* 27B, 137–147.
- Khorana, H. G., Knox, B. E., Nasi, E., Swanson, R., and Thompson, D. A. (1988). Expression of a Bovine Rhodopsin Gene in *Xenopus* Oocytes: Demonstration of Light-dependent Ionic Currents. *Proc. Nat. Acad. Sci. U. S. A.* 85, 7917–7921. doi:10.1073/pnas.85.21.7917
- Khorana, H. G. (1979). Total Synthesis of a Gene. *Science* 203, 614–625. doi:10.1126/science.366749
- Kikukawa, T. (2021). Unique Cl⁻ Pump Rhodopsin with Close Similarity to H⁺ Pump Rhodopsin. *Biophysical J.* 120, 317–326. doi:10.1016/j.bpj.2021.03.038
- Kim, H.-J., Kwon, Y. D., Lee, S. Y., and Kim, P. (2012). An Engineered *Escherichia coli* Having a High Intracellular Level of ATP and Enhanced Recombinant Protein Production. *Appl. Microbiol. Biotechnol.* 94, 1079–1086. doi:10.1007/s00253-011-3779-0
- Kim, H. A., Kim, H. J., Park, J., Choi, A. R., Heo, K., Jeong, H., et al. (2017). An Evolutionary Optimization of a Rhodopsin-Based Phototrophic Metabolism in *Escherichia coli*. *Microb. Cell. Factories* 16, 111. doi:10.1186/s12934-017-0725-6
- Kim, S.-Y., Waschuk, S. A., Brown, L. S., and Jung, K.-H. (2008). Screening and Characterization of Proteorhodopsin Color-Tuning Mutations in *Escherichia coli* with Endogenous Retinal Synthesis. *Biochimica Biophysica Acta-Bioenergetics* 1777, 504–513. doi:10.1016/j.bbabi.2008.03.010
- Kimura, Y., Vassilyev, D. G., Miyazawa, A., Kidera, A., Matsushima, M., Mitsuoka, K., et al. (1997). High Resolution Structure of Bacteriorhodopsin Determined by Electron Crystallography. *Photochem. Photobiol.* 66, 764–767. doi:10.1111/j.1751-1097.1997.tb03221.x
- Kirchman, D. L., and Hanson, T. E. (2013). Bioenergetics of Photoheterotrophic Bacteria in the Oceans. *Environ. Microbiol. Rep.* 5, 188–199. doi:10.1111/j.1758-2229.2012.00367.x

- Kishi, K. E., Kim, Y. S., Fukuda, M., Inoue, M., Kusakizako, T., Wang, P. Y., et al. (2022). Structural Basis for Channel Conduction in the Pump-like Channelrhodopsin ChRmine. *Cell* 185, 1–18. doi:10.1016/j.cell.2022.01.007
- Klaassen, C. H. W., and DeGrip, W. J. (2000). Baculovirus Expression System for Expression and Characterization of Functional Recombinant Visual Pigments. *Meth. Enzymol.* 315, 12–29. doi:10.1016/s0076-6879(00)15832-x
- Klapoetke, N. C., Murata, Y., Kim, S. S., Pulver, S. R., Birdsey-Benson, A., Cho, Y. K., et al. (2014). Independent Optical Excitation of Distinct Neural Populations. *Nat. Methods* 11, 338–U333. doi:10.1038/nmeth.2836
- Klyszejko, A. L., Shastri, S., Mari, S. A., Grubmüller, H., Müller, D. J., and Glaubitz, C. (2008). Folding and Assembly of Proteorhodopsin. *J. Mol. Biol.* 376, 35–41. doi:10.1016/j.jmb.2007.11.030
- Knoet, C. J., Ungerer, J., Wangikar, P. P., and Pakrasi, H. B. (2018). Cyanobacteria: Promising Biocatalysts for Sustainable Chemical Production. *J. Biol. Chem.* 293, 5044–5052. doi:10.1074/jbc.r117.815886
- Knowles, T. J., Finka, R., Smith, C., Lin, Y.-P., Dafforn, T., and Overduin, M. (2009). Membrane Proteins Solubilized Intact in Lipid Containing Nanoparticles Bounded by Styrene Maleic Acid Copolymer. *J. Am. Chem. Soc.* 131, 7484–7485. doi:10.1021/ja810046q
- Knudsen, P., and Hubbell, W. L. (1978). Stability of Rhodopsin in Detergent Solutions. *Membr. Biochem.* 1, 297–322. doi:10.3109/09687687809063853
- Kochendoerfer, G. G., Lin, S. W., Sakmar, T. P., and Mathies, R. A. (1999). How Color Visual Pigments Are Tuned. *Trends biochem. Sci.* 24, 300–305. doi:10.1016/s0968-0004(99)01432-2
- Kojima, D., Imai, H., Okano, T., Fukada, Y., Crescitelli, F., Yoshizawa, T., et al. (1995). Purification and Low Temperature Spectroscopy of Gecko Visual Pigments Green and Blue. *Biochemistry-USA* 34, 1096–1106. doi:10.1021/bi00003a047
- Kojima, D., Oura, T., Hisatomi, O., Tokunaga, F., Fukada, Y., Yoshizawa, T., et al. (1996). Molecular Properties of Chimerical Mutants of Gecko Blue and Bovine Rhodopsin. *Biochemistry* 35, 2625–2629. doi:10.1021/bi9511548
- Kojima, D., Terakita, A., Ishikawa, T., Tsukahara, Y., Maeda, A., and Shichida, Y. (1997). A Novel Go-Mediated Phototransduction Cascade in Scallop Visual Cells. *J. Biol. Chem.* 272, 22979–22982. doi:10.1074/jbc.272.37.22979
- Kojima, K., Kurihara, R., Sakamoto, M., Takanashi, T., Kuramochi, H., Zhang, X. M., et al. (2020a). Comparative Studies of the Fluorescence Properties of Microbial Rhodopsins: Spontaneous Emission versus Photointermediate Fluorescence. *J. Phys. Chem. B* 124, 7361–7367. doi:10.1021/acs.jpcc.0c06560
- Kojima, K., Miyoshi, N., Shibukawa, A., Chowdhury, S., Tsujimura, M., Noji, T., et al. (2020c). Green-sensitive, Long-Lived, Step-Functional Anion Channelrhodopsin-2 Variant as a High-Potential Neural Silencing Tool. *J. Phys. Chem. Lett.* 11, 6214–6218. doi:10.1021/acs.jpclett.0c01406
- Kojima, K., Shibukawa, A., and Sudo, Y. (2020b). The Unlimited Potential of Microbial Rhodopsins as Optical Tools. *Biochemistry* 59, 218–229. doi:10.1021/acs.biochem.9b00768
- Kojima, K., Ueta, T., Noji, T., Saito, K., Kanehara, K., Yoshizawa, S., et al. (2020d). Vectorial Proton Transport Mechanism of RxR, a Phylogenetically Distinct and Thermally Stable Microbial Rhodopsin. *Sci. Rep.* 10, 282. doi:10.1038/s41598-019-57122-2
- Konno, M., Inoue, K., and Kandori, H. (2021). Ion Transport Activity Assay for Microbial Rhodopsin Expressed in *Escherichia coli* Cells. *Bio-Protocol* 11, 4115. doi:10.21769/bioprotoc.4115
- Kopf, A. H., Dörr, J. M., Koorengel, M. C., Antoniciello, F., Jahn, H., and Killian, J. A. (2020). Factors Influencing the Solubilization of Membrane Proteins from *Escherichia coli* Membranes by Styrene-Maleic Acid Copolymers. *Biochimica Biophysica Acta-Biomembranes* 1862, 183125. doi:10.1016/j.bbamem.2019.183125
- Kovalev, K., Astashkin, R., Gushchin, I., Orekhov, P., Volkov, D., Zinovev, E. V., et al. (2020a). Molecular Mechanism of Light-Driven Sodium Pumping. *Nat. Commun.* 11, 21371. doi:10.1038/s41467-020-16032-y
- Kovalev, K., Polovinkin, V., Gushchin, I., Alekseev, A., Shevchenko, V., Borshchevskiy, V., et al. (2019). Structure and Mechanisms of Sodium-Pumping KR2 Rhodopsin. *Sci. Adv.* 5, eaav2671. doi:10.1126/sciadv.aav2671
- Kovalev, K., Volkov, D., Astashkin, R., Alekseev, A., Gushchin, I., Haro-Moreno, J. M., et al. (2020b). High-resolution Structural Insights into the Heliorhodopsin Family. *Proc. Natl. Acad. Sci. U. S. A.* 117, 4131–4141. doi:10.1073/pnas.1915888117
- Koyanagi, M., and Terakita, A. (2014). Diversity of Animal Opsin-Based Pigments and Their Optogenetic Potential. *Biochimica Biophysica Acta-Bioenergetics* 1837, 710–716. doi:10.1016/j.bbabi.2013.09.003
- Kraack, J. P., Buckup, T., and Motzkus, M. (2011). Vibrational Analysis of Excited and Ground Electronic States of All-Trans Retinal Protonated Schiff-Bases. *Phys. Chem. Chem. Phys.* 13, 21402–21410. doi:10.1039/c1cp22245g
- Krah, M., Marwan, W., Verméglio, A., and Oesterhelt, D. (1994). Phototaxis of *Halobacterium Salinarium* Requires a Signalling Complex of Sensory Rhodopsin I and its Methyl-Accepting Transducer HtrI. *EMBO J.* 13, 2150–2155. doi:10.1002/j.1460-2075.1994.tb06491.x
- Kralj, J. M., Douglass, A. D., Hochbaum, D. R., Maclaurin, D., and Cohen, A. E. (2012). Optical Recording of Action Potentials in Mammalian Neurons Using a Microbial Rhodopsin. *Nat. Methods* 9, 90–95. doi:10.1038/nmeth.1782
- Kralj, J. M., Hochbaum, D. R., Douglass, A. D., and Cohen, A. E. (2011). Electrical Spiking in *Escherichia coli* Probed with a Fluorescent Voltage-Indicating Protein. *Science* 333, 345–348. doi:10.1126/science.1204763
- Krebs, A., Edwards, P. C., Villa, C., Li, J.-D., and Schertler, G. F. X. (2003). The Three-Dimensional Structure of Bovine Rhodopsin Determined by Electron Cryomicroscopy. *J. Biol. Chem.* 278, 50217–50225. doi:10.1074/jbc.M307995200
- Krebs, M. P., Mollaaghababa, R., and Khorana, H. G. (1993). Gene Replacement in *Halobacterium Halobium* and Expression of Bacteriorhodopsin Mutants. *Proc. Nat. Acad. Sci. U. S. A.* 90, 1987–1991. doi:10.1073/pnas.90.5.1987
- Krol, A., Lopez-Huerta, V. G., Corey, T. E. C., Deisseroth, K., Ting, J. T., and Feng, G. P. (2019). Two eARCHT3.0 Lines for Optogenetic Silencing of Dopaminergic and Serotonergic Neurons. *Front. Neural Circuits* 13, 4. doi:10.3389/fncir.2019.00004
- Kropf, A. (1982). A New Detergent for the Study of Visual Pigments. *Vis. Res.* 22, 495–497. doi:10.1016/0042-6989(82)90199-7
- Kropf, A. (1975). The Nature of the Chromophore-Protein Interaction in Visual Pigments as Studied by Visual Pigment Analogues. *Abstr. Annu. Meet. Biophysical Soc. Jpn.* 31, 281–282.
- Kropf, A., Whittenberger, B. P., Goff, S. P., and Waggoner, A. S. (1973). The Spectral Properties of Some Visual Pigment Analogs. *Exp. Eye Res.* 17, 591–606. doi:10.1016/0014-4835(73)90088-2
- Kuang, L. J., Fernandes, D. A., O'halloran, M., Zheng, W., Jiang, Y. J., Ladizhansky, V., et al. (2014). Frozen[®] Block Copolymer Nanomembranes with Light-Driven Proton Pumping Performance. *ACS Nano* 8, 537–545. doi:10.1021/nn4059852
- Kühn, H. (1984). Interactions between Photoexcited Rhodopsin and Light-Activated Enzymes in Rods. *Prog. Retin. Res.* 3, 123–156.
- Kuhne, J., Eisenhauer, K., Ritter, E., Hegemann, P., Gerwert, K., and Bartl, F. J. (2015). Early Formation of the Ion-Conducting Pore in Channelrhodopsin-2. *Angew. Chemie-International Ed.* 54, 4953–4957. doi:10.1002/anie.201410180
- Kuhne, J., Vierock, J., Tennigkeit, S. A., Dreier, M.-A., Wietek, J., Petersen, D., et al. (2019). Unifying Photocycle Model for Light Adaptation and Temporal Evolution of Cation Conductance in Channelrhodopsin-2. *Proc. Natl. Acad. Sci. U. S. A.* 116, 9380–9389. doi:10.1073/pnas.1818707116
- Kulkarni, R. U., and Miller, E. W. (2017). Voltage Imaging: Pitfalls and Potential. *Biochemistry* 56, 5171–5177. doi:10.1021/acs.biochem.7b00490
- Kumbalasisri, T., and Provencio, I. (2005). Melanopsin and Other Novel Mammalian Opsins. *Exp. Eye Res.* 81, 368–375. doi:10.1016/j.exer.2005.05.004
- Kushibiki, T., Okawa, S., Hirasawa, T., and Ishihara, M. (2014). Optogenetics: Novel Tools for Controlling Mammalian Cell Functions with Light. *Int. J. Photoenergy* 2014, 895039. doi:10.1155/2014/895039
- Kusnetzow, A. K., Altenbach, C., and Hubbell, W. L. (2006). Conformational States and Dynamics of Rhodopsin in Micelles and Bilayers. *Biochemistry-USA* 45, 5538–5550. doi:10.1021/bi060101v
- Kusnetzow, A. K., Dukkipati, A., Babu, K. R., Ramos, L., Knox, B. E., and Birge, R. R. (2004). Vertebrate Ultraviolet Visual Pigments: Protonation of the Retinylidene Schiff Base and a Counterion Switch during Photoactivation. *Proc. Natl. Acad. Sci. U. S. A.* 101, 941–946. doi:10.1073/pnas.0305206101
- Kusnetzow, A. K., Dukkipati, A., Babu, K. R., Singh, D., Vought, B. W., Knox, B. E., et al. (2001). The Photobleaching Sequence of a Short-Wavelength Visual Pigment. *Biochemistry* 40, 7832–7844. doi:10.1021/bi010387y
- Kwon, S.-K., Jun, S.-H., and Kim, J. F. (2020). Omega Rhodopsins: A Versatile Class of Microbial Rhodopsins. *J. Microbiol. Biotechnol.* 30, 633–641. doi:10.4014/jmb.1912.12010

- Kwon, Y. M., Patra, A. K., Chiura, H. X., and Kim, S.-J. (2019). Production of Extracellular Vesicles with Light-Induced Proton Pump Activity by Proteorhodopsin-Containing Marine Bacteria. *MicrobiologyOpen* 8, e808. doi:10.1002/mbo3.808
- Ladizhansky, V. (2017). "Advances in Solid-State NMR Studies of Microbial Rhodopsins," in *Modern Magnetic Resonance*. Editor G. A. Webb (New York City: Springer International Publishing AG), 1–19. doi:10.1007/978-3-319-28275-6_65-1
- Lamarche, L. B., Kumar, R. P., Trieu, M. M., Devine, E. L., Cohen-Abeles, L. E., Theobald, D. L., et al. (2017). Purification and Characterization of RhoPDE, a Retinylidene/Phosphodiesterase Fusion Protein and Potential Optogenetic Tool from the Choanoflagellate *Salpingoeca Rosetta*. *Biochemistry* 56, 5812–5822. doi:10.1021/acs.biochem.7b00519
- Lan, Y., Wang, Y., and Lu, H. (2020). Opsin 3 Is a Key Regulator of Ultraviolet A-Induced Photoaging in Human Dermal Fibroblast Cells. *Br. J. Dermatology* 182, 1228–1244. doi:10.1111/bjd.18410
- Lang-Hinrichs, C., Queck, I., Büldt, G., Stahl, U., and Hildebrandt, V. (1994). The Archaeobacterial Membrane Protein Bacterio-Op sin Is Expressed and N-Terminally Processed in the Yeast *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* 244, 183–188. doi:10.1007/bf00283521
- Lanyi, J. K. (2004). Bacteriorhodopsin. *Annu. Rev. Physiology* 66, 665–688. doi:10.1146/annurev.physiol.66.032102.150049
- Larkum, A. W. D., Ritchie, R. J., and Raven, J. A. (2018). Living off the Sun: Chlorophylls, Bacteriochlorophylls and Rhodopsins. *Photosynthetica* 56, 11–43. doi:10.1007/s11099-018-0792-x
- Lavington, S., and Watts, A. (2020). Lipid Nanoparticle Technologies for the Study of G Protein-Coupled Receptors in Lipid Environments. *Biophys. Rev.* 12, 1287–1302. doi:10.1007/s12551-020-00775-5
- Lee, H. J., Huang, K.-C., Mei, G. X., Mamaeva, N., DeGrip, W. J., Rothschild, K. J., et al. (2019). "Pre-resonance Stimulated Raman Scattering Spectroscopy and Imaging of Membrane Potential Using Near-Infrared Rhodopsins," in *Multiphoton Microscopy in the Biomedical Sciences*. Editors M. Periasamy, P. T. C. So, and K. König (Bellingham, U.S.A.: SPIE), 81. doi:10.1117/12.2506833
- Lee, S., Ghosh, S., Jana, S., Robertson, N., Tate, C. G., and Vaidehi, N. (2020). How Do Branched Detergents Stabilize GPCRs in Micelles? *Biochemistry* 59, 2125–2134. doi:10.1021/acs.biochem.0c00183
- Lehtinen, K., Nokia, M. S., and Takala, H. (2022). Red Light Optogenetics in Neuroscience. *Front. Cell. Neurosci.* 15, 778900. doi:10.3389/fncel.2021.778900
- Lesca, E., Panneels, V., and Schertler, G. F. X. (2018). The Role of Water Molecules in Phototransduction of Retinal Proteins and G Protein-Coupled Receptors. *Faraday Discuss.* 207, 27–37. doi:10.1039/c7fd00207f
- Leung, N. Y., and Montell, C. (2017). Unconventional Roles of Opsins. *Annu. Rev. Cell. Dev. Biol.* 33, 241–264. doi:10.1146/annurev-cellbio-100616-060432
- Leung, N. Y., Thakur, D. P., Gurav, A. S., Kim, S. H., Di Pizio, A., Niv, M. Y., et al. (2020). Functions of Opsins in *Drosophila* Taste. *Curr. Biol.* 30, 1367–1379. doi:10.1016/j.cub.2020.01.068
- Lewis, J. W., Hug, S. J., Wallace-Williams, S. E., and Kliger, D. S. (1990). Direct Evidence for an Equilibrium between Early Photolysis Intermediates of Rhodopsin. *J. Am. Chem. Soc.* 112, 6711–6712. doi:10.1021/ja00174a040
- Lewis, J. W., Van Kuijk, F. J. G. M., Carruthers, J. A., and Kliger, D. S. (1997). Metarhodopsin III Formation and Decay Kinetics: Comparison of Bovine and Human Rhodopsin. *Vis. Res.* 37, 1–8. doi:10.1016/s0042-6989(96)00138-1
- Li, H., Huang, C.-Y., Govorunova, E. G., Schafer, C. T., Sineshchekov, O. A., Wang, M. T., et al. (2019). Crystal Structure of a Natural Light-Gated Anion Channelrhodopsin. *eLife* 8, e41741. doi:10.7554/eLife.41741
- Li, H., Huang, C.-Y., Govorunova, E. G., Sineshchekov, O. A., Yi, A., Rothschild, K. J., et al. (2021). The Crystal Structure of Bromide-Bound GtACR1 Reveals a Pre-activated State in the Transmembrane Anion Tunnel. *Elife* 10, 65903. doi:10.7554/eLife.65903
- Li, J., Edwards, P. C., Burghammer, M., Villa, C., and Schertler, G. F. X. (2004). Structure of Bovine Rhodopsin in a Trigonal Crystal Form. *J. Mol. Biol.* 343, 1409–1438. doi:10.1016/j.jmb.2004.08.090
- Li, L. Z., Lu, L. H., Ren, Y., Tang, G., Zhao, Y., Cai, X., et al. (2022). Colocalized, Bidirectional Optogenetic Modulations in Freely Behaving Mice with a Wireless Dual-Color Optoelectronic Probe. *Nat. Commun.* 13, 839. doi:10.1038/s41467-022-28539-7
- Liang, J., Steinberg, G., Livnah, N., Sheves, M., Ebrey, T. G., and Tsuda, M. (1994). The pK_a of the Protonated Schiff Bases of Gecko Cone and octopus Visual Pigments. *Biophys. J.* 67, 848–854. doi:10.1016/s0006-3495(94)80544-2
- Lichty, J. J., Malecki, J. L., Agnew, H. D., Michelson-Horowitz, D. J., and Tan, S. (2005). Comparison of Affinity Tags for Protein Purification. *Protein Expr. Purif.* 41, 98–105. doi:10.1016/j.pep.2005.01.019
- Liebel, M., Schnedermann, C., Bassolino, G., Taylor, G., Watts, A., and Kukura, P. (2014). Direct Observation of the Coherent Nuclear Response after the Absorption of a Photon. *Phys. Rev. Lett.* 112, 238301. doi:10.1103/PhysRevLett.112.238301
- Liebert, A., Pang, V., Bicknell, B., Mclachlan, C., Mitrofanis, J., and Kiat, H. (2021). A Perspective on the Potential of Opsins as an Integral Mechanism of Photobiomodulation: It's Not Just the Eyes. *Photobiomodul. Photomed. Laser Surg.* 40, 123–135. doi:10.1089/photob.2021.0106
- Lin, J. Y., Knutsen, P. M., Muller, A., Kleinfeld, D., and Tsien, R. Y. (2013). ReaChR: a Red-Shifted Variant of Channelrhodopsin Enables Deep Transcranial Optogenetic Excitation. *Nat. Neurosci.* 16, 1499–1508. doi:10.1038/nn.3502
- Lin, S. W., Kochendoerfer, G. G., Carroll, H. S., Wang, D., Mathies, R. A., and Sakmar, T. P. (1998). Mechanisms of Spectral Tuning in Blue Cone Visual Pigments - Visible and Raman Spectroscopy of Blue-Shifted Rhodopsin Mutants. *J. Biol. Chem.* 273, 24583–24591. doi:10.1074/jbc.273.38.24583
- Lin, S. W., Sakmar, T. P., Franke, R. R., Khorana, H. G., and Mathies, R. A. (1992). Resonance Raman Microprobe Spectroscopy of Rhodopsin Mutants: Effect of Substitutions in the Third Transmembrane Helix. *Biochemistry-USA* 31, 5105–5111. doi:10.1021/bi00137a003
- Lincereghino, J., and Cregg, J. M. (2000). Heterologous Protein Expression in the Methylophilic Yeast *Pichia pastoris*. *FEMS Microbiol. Rev.* 24, 45–66. doi:10.1111/j.1574-6976.2000.tb00532.x
- Lindner, M., Gilhooley, M. J., Peirson, S. N., Hughes, S., and Hankins, M. W. (2021). The Functional Characteristics of Optogenetic Gene Therapy for Vision Restoration. *Cell. Mol. Life Sci.* 78, 1597–1613. doi:10.1007/s00018-020-03597-6
- Lips, D., Schuurmans, J. M., Branco Dos Santos, F., and Hellingwerf, K. J. (2018). Many Ways towards 'solar Fuel': Quantitative Analysis of the Most Promising Strategies and the Main Challenges during Scale-Up. *Energy & Environ. Sci.* 11, 10–22. doi:10.1039/c7ee02212c
- Liu, R. S. H., and Asato, A. E. (1990). "The Binding Site of Opsin Based on Analog Studies with Isomeric, Fluorinated, Alkylated, and Other Modified Retinals," in *Chemistry and Biology of Synthetic Retinoids*. Editors M. I. Dawson and W. H. Okamura (Boca Raton, FL, U.S.A.: CRC Press), 52–75.
- Liu, R. S. H., and Asato, A. E. (2003). Tuning the Color and Excited State Properties of the Azulenic Chromophore: NIR Absorbing Pigments and Materials. *J. Photochem. Photobiol. C Photochem. Rev.* 4, 179–194. doi:10.1016/j.jphotochemrev.2003.09.001
- Liu, R. S. H., and Liu, J. (2011). Fluorinated Retinoids and Carotenoids. *J. Nat. Prod.* 74, 512–517. doi:10.1021/np1006394
- Liu, R. S. H., Matsumoto, H., Kini, A., Asato, A. E., Denny, M., Kropf, A., et al. (1984). Seven New Hindered Isomeric Rhodopsins - A Reexamination of the Stereospecificity of the Binding-Site of Bovine Opsin. *Tetrahedron* 40, 473–482. doi:10.1016/s0040-4020(01)88435-0
- Liu, Y., Cui, Y. M., Chi, H., Xia, Y., Liu, H. N., Rossiter, S. J., et al. (2019). Scotopic Rod Vision in Tetrapods Arose from Multiple Early Adaptive Shifts in the Rate of Retinal Release. *Proc. Natl. Acad. Sci. U. S. A.* 116, 12627–12628. doi:10.1073/pnas.1900481116
- Liu, Y. Z., Zhang, W., Du, X. X., Liu, Y. X., Qu, J. B., Liu, X. B., et al. (2021). Genome-wide Identification of Nonvisual Opsin Family Reveals Amplification of RPE-retinal G Protein Receptor Gene (RGR) and Offers Novel Insights into Functions of RGR(s) in *Paralichthys olivaceus* (Paralichthyidae, Teleostei). *J. Exp. Zoology Part B Mol. Dev. Evol.* 334, 25–36. doi:10.1002/jezb.22914
- Locket, N. A. (1977). "Adaptations to the Deep-Sea Environment," in *The Visual System in Vertebrates*. Editor F. Crescitelli (Berlin: Springer-Verlag), 67–192.
- López, S., Rodriguez, V., Montenegro, J., Saá, C., Alvarez, R., López, C. S., et al. (2005). Synthesis of N-Heteroaryl Retinals and Their Artificial Bacteriorhodopsins. *ChemBioChem* 6, 2078–2087. doi:10.1002/cbic.200500148
- Lórenz-Fonfría, V. A., Bamann, C., Resler, T., Schlesinger, R., Bamberg, E., and Heberle, J. (2015a). Temporal Evolution of Helix Hydration in a Light-Gated Ion Channel Correlates with Ion Conductance. *Proc. Natl. Acad. Sci. U. S. A.* 112, E5796–E5804. doi:10.1073/pnas.1511462112

- Lórenz-Fonfría, V. A., and Kandori, H. (2009). Spectroscopic and Kinetic Evidence on How Bacteriorhodopsin Accomplishes Vectorial Proton Transport under Functional Conditions. *J. Am. Chem. Soc.* 131, 5891–5901. doi:10.1021/ja900334c
- Lórenz-Fonfría, V. A., Schultz, B.-J., Resler, T., Schlesinger, R., Bamann, C., Bamberg, E., et al. (2015b). Pre-gating Conformational Changes in the ChETA Variant of Channelrhodopsin-2 Monitored by Nanosecond IR Spectroscopy. *J. Am. Chem. Soc.* 137, 1850–1861. doi:10.1021/ja5108595
- Lórenz-Fonfría, V. A., Yagi, K., Ito, S., and Kandori, H. (2021). Retinal Vibrations in Bacteriorhodopsin Are Mechanically Harmonic but Electrically Anharmonic: Evidence from Overtone and Combination Bands. *Front. Mol. Biosci.* 8, 749261. doi:10.3389/fmolb.2021.749261
- Lu, Y., Zhou, X. E., Gao, X., Wang, N., Xia, R. X., Xu, Z. M., et al. (2020). Crystal Structure of Heliorhodopsin 48C12. *Cell. Res.* 30, 88–90. doi:10.1038/s41422-019-0266-0
- Luck, M., Velázquez Escobar, F., Glass, K., Sabotke, M.-I., Hagedorn, R., Corellou, F., et al. (2019). Photoreactions of the Histidine Kinase Rhodopsin Ot-HKR from the Marine Picoalga *Ostreococcus Tauri*. *Biochemistry* 58, 1878–1891. doi:10.1021/acs.biochem.8b01200
- Luecke, H., Schobert, B., Richter, H.-T., Cartailier, J.-P., and Lanyi, J. K. (1999). Structure of Bacteriorhodopsin at 1.55 Å Resolution. *J. Mol. Biol.* 291, 899–911. doi:10.1006/jmbi.1999.3027
- Luecke, H., Schobert, B., Stagno, J., Imasheva, E. S., Wang, J. M., Balashov, S. P., et al. (2008). Crystallographic Structure of Xanthorhodopsin, the Light-Driven Proton Pump with a Dual Chromophore. *Proc. Natl. Acad. Sci. U. S. A.* 105, 16561–16565. doi:10.1073/pnas.0807162105
- Lugtenburg, J., Creemers, A. F. L., Verhoeven, M. A., Van Wijk, A. a. C., Verdegem, P. J. E., Monnee, M. C. F., et al. (1999). Synthesis of ¹³C-Labeled Carotenoids and Retinoids. *Pure Appl. Chem.* 71, 2245–2251. doi:10.1351/pac199971122245
- Lugtenburg, J., Mathies, R. A., Griffin, R. G., and Herzfeld, J. (1988). Structure and Function of Rhodopsins from Solid State NMR and Resonance Raman Spectroscopy of Isotopic Retinal Derivatives. *Trends biochem. Sci.* 13, 388–393. doi:10.1016/0968-0004(88)90181-8
- Luk, H. L., Bhattacharyya, N., Montisci, F., Morrow, J. M., Melaccio, F., Wada, A., et al. (2016). Modulation of Thermal Noise and Spectral Sensitivity in Lake Baikal Cottoid Fish Rhodopsins. *Sci. Rep.* 6, 38425. doi:10.1038/srep38425
- Lutnaes, B. F., Kildahl-Andersen, G., Krane, J., and Liaaen-Jensen, S. (2004). Delocalized Carotenoid Cations in Relation to the Soliton Model. *J. Am. Chem. Soc.* 126, 8981–8990. doi:10.1021/ja0492541
- Ma, J.-X., Kono, M., Xu, L., Das, J., Ryan, J. C., Hazard, E. S., Iii, et al. (2001). Salamander UV Cone Pigment: Sequence, Expression, and Spectral Properties. *Vis. Neurosci.* 18, 393–399. doi:10.1017/s0952523801183057
- Maclaurin, D., Venkatachalam, V., Lee, H., and Cohen, A. E. (2013). Mechanism of Voltage-Sensitive Fluorescence in a Microbial Rhodopsin. *Proc. Natl. Acad. Sci. U. S. A.* 110, 5939–5944. doi:10.1073/pnas.1215595110
- Maeda, A., Sasaki, J., Pfefferle, J. M., Shichida, Y., and Yoshizawa, T. (1991). Fourier Transform Infrared Spectral Studies on the Schiff Base Mode of All-Trans Bacteriorhodopsin and its Photointermediates-K and Photointermediates-L. *Photochem. Photobiol.* 54, 911–921. doi:10.1111/j.1751-1097.1991.tb02111.x
- Maeda, T., Imanishi, Y., and Palczewski, K. (2003). Rhodopsin Phosphorylation: 30 Years Later. *Prog. Retin. Eye Res.* 22, 417–434. doi:10.1016/s1350-9462(03)00017-x
- Mahn, M., Saraf-Sinik, I., Patil, P., Pulin, M., Bitton, E., Karalis, N., et al. (2021). Efficient Optogenetic Silencing of Neurotransmitter Release with a Mosquito Rhodopsin. *Neuron* 109, 1621–1635. doi:10.1016/j.neuron.2021.03.013
- Malmerberg, E., Bovee-Geurts, P. H. M., Katona, G., Deupi, X., Arnlund, D., Wickstrand, C., et al. (2015). Conformational Activation of Visual Rhodopsin in Native Disc Membranes. *Sci. Signal.* 8, ra26. doi:10.1126/scisignal.2005646
- Maly, T., Debelouchina, G. T., Bajaj, V. S., Hu, K.-N., Joo, C.-G., Mak-Jurkauskas, M. L., et al. (2008). Dynamic Nuclear Polarization at High Magnetic Fields. *J. Chem. Phys.* 128, 052211. doi:10.1063/1.2833582
- Mao, J. F., Aladin, V., Jin, X. S., Leeder, A. J., Brown, L. J., Brown, R. C. D., et al. (2019). Exploring Protein Structures by DNP-Enhanced Methyl Solid-State NMR Spectroscopy. *J. Am. Chem. Soc.* 141, 19888–19901. doi:10.1021/jacs.9b11195
- Mao, J. F., Do, N.-N., Scholz, F., Reggie, L., Mehler, M., Lakatos, A., et al. (2014). Structural Basis of the Green-Blue Color Switching in Proteorhodopsin as Determined by NMR Spectroscopy. *J. Am. Chem. Soc.* 136, 17578–17590. doi:10.1021/ja5097946
- Marrero, H., and Rothschild, K. J. (1987). Conformational Changes in Bacteriorhodopsin Studied by Infrared Attenuated Total Reflection. *Biophys. J.* 52, 629–635. doi:10.1016/s0006-3495(87)83254-x
- Marshel, J. H., Kim, Y. S., Machado, T. A., Quirin, S., Benson, B., Kadmon, J., et al. (2019). Cortical Layer-specific Critical Dynamics Triggering Perception. *Science* 365, eaaw5202. doi:10.1126/science.aaw5202
- Martinez, A., Bradley, A. S., Waldbauer, J. R., Summons, R. E., and DeLong, E. F. (2007). Proteorhodopsin Photosystem Gene Expression Enables Photophosphorylation in a Heterologous Host. *Proc. Natl. Acad. Sci. U. S. A.* 104, 5590–5595. doi:10.1073/pnas.0611470104
- Masuda, S., Morita, E. H., Tasumi, M., Iwasa, T., and Tsuda, M. (1993). Infrared Studies of octopus Rhodopsin - Existence of a Long-Lived Intermediate and the States of the Carboxylic Group of Asp-81 in Rhodopsin and its Photoproducts. *FEBS Lett.* 317, 223–227. doi:10.1016/0014-5793(93)81280-d
- Matarèse, B. F. E., Feyen, P. L. C., De Mello, J. C., and Benfenati, F. (2019). Sub-millisecond Control of Neuronal Firing by Organic Light-Emitting Diodes. *Front. Bioeng. Biotechnol.* 7, 278. doi:10.3389/fbioe.2019.00278
- Mathies, R. A., and Lugtenburg, J. (2000). “The Primary Photoreaction of Rhodopsin,” in *Molecular Mechanisms in Visual Transduction*. Editors D. G. Stavenga, W. J. DeGrip, and E. N. Pugh Jr. (Amsterdam, Netherlands: Elsevier Science Pub.), 55–90. doi:10.1016/s1383-8121(00)80005-6
- Mathies, R. A., Smith, S. O., and Palings, I. (1987). “Determination of Retinal Chromophore Structure in Rhodopsins,” in *Resonance Raman Spectra of Polyenes and Aromatics*. Editor T. G. Spiro (New York: John Wiley & Sons), 59–108.
- Matsui, S., Seidou, M., Uchiyama, I., Sekiya, N., Hiraki, K., Yoshihara, K., et al. (1988). 4-Hydroxyretinal, a New Visual Pigment Chromophore Found in the Bioluminescent Squid, *Watasenia Scintillans*. *Biochimica Biophysica Acta* 966, 370–374. doi:10.1016/0304-4165(88)90087-6
- Mccamant, D. W., Kukura, P., and Mathies, R. A. (2005). Femtosecond Stimulated Raman Study of Excited-State Evolution in Bacteriorhodopsin. *J. Phys. Chem. B* 109, 10449–10457. doi:10.1021/jp050095x
- Mcdermott, A. E. (2009). Structure and Dynamics of Membrane Proteins by Magic Angle Spinning Solid-State NMR. *Annu. Rev. Biophysics* 38, 385–403. doi:10.1146/annurev.biophys.050708.133719
- Mcisaac, R. S., Bedbrook, C. N., and Arnold, F. H. (2015). Recent Advances in Engineering Microbial Rhodopsins for Optogenetics. *Curr. Opin. Struct. Biol.* 33, 8–15. doi:10.1016/j.sbi.2015.05.001
- Mcisaac, R. S., Engqvist, M. K. M., Wannier, T., Rosenthal, A. Z., Herwig, L., Flytzanis, N. C., et al. (2014). Directed Evolution of a Far-Red Fluorescent Rhodopsin. *Proc. Natl. Acad. Sci. U. S. A.* 111, 13034–13039. doi:10.1073/pnas.1413987111
- Mckee, A. G., Kuntz, C. P., Ortega, J. T., Woods, H., Most, V., Roushar, F. J., et al. (2021). Systematic Profiling of Temperature- and Retinal-Sensitive Rhodopsin Variants by Deep Mutational Scanning. *J. Biol. Chem.* 2021, 101359. doi:10.1016/j.jbc.2021.101359
- Mederos, S., Hernández-Vivanco, A., Ramírez-Franco, J., Martín-Fernández, M., Navarrete, M., Yang, A., et al. (2019). Melanopsin for Precise Optogenetic Activation of Astrocyte-Neuron Networks. *Glia* 67, 915–934. doi:10.1002/glia.23580
- Mei, G. X., Cavini, C. M., Mamaeva, N., Wang, P., DeGrip, W. J., and Rothschild, K. J. (2021). Optical Switching between Long-Lived States of Opsin Transmembrane Voltage Sensors. *Photochem. Photobiol.* 97, 1001–1015. doi:10.1111/php.13428
- Mei, G. X., Mamaeva, N., Ganapathy, S., Wang, P., DeGrip, W. J., and Rothschild, K. J. (2020). Analog Retinal Redshifts Visible Absorption of QuasAr Transmembrane Voltage Sensors into Near-Infrared. *Photochem. Photobiol.* 96, 55–66. doi:10.1111/php.13169
- Mei, G. X., Mamaeva, N., Ganapathy, S., Wang, P., DeGrip, W. J., and Rothschild, K. J. (2018). Raman Spectroscopy of a Near Infrared Absorbing Proteorhodopsin: Similarities to the Bacteriorhodopsin O Photointermediate. *Plos One* 13, e0209506. doi:10.1371/journal.pone.0209506
- Melaccio, F., Del Carmen Marin, M., Valentini, A., Montisci, F., Rinaldi, S., Cherubini, M., et al. (2016). Toward Automatic Rhodopsin Modeling as a Tool for High-Throughput Computational Photobiology. *J. Chem. Theory Comput.* 12, 6020–6034. doi:10.1021/acs.jctc.6b00367

- Melyan, Z., Tarttelin, E. E., Bellingham, J., Lucas, R. J., and Hankins, M. W. (2005). Addition of Human Melanopsin Renders Mammalian Cells Photoresponsive. *Nature* 433, 741–745. doi:10.1038/nature03344
- Milosevic, M. M., Jang, J., Mckimm, E. J., Zhu, M. H., and Antic, S. D. (2020). *In Vitro* Testing of Voltage Indicators: Archon1, ArcLightD, ASAP1, ASAP2s, ASAP3b, Bongwoori-Pos6, BeRST1, FlicR1, and Chi-VSFP-Butterfly. *eNeuro* 7, 0060. doi:10.1523/eneuro.0060-20.2020
- Mishra, A. K., Gragg, M., Stoneman, M. R., Biener, G., Oliver, J. A., Miszta, P., et al. (2016). Quaternary Structures of Opsin in Live Cells Revealed by FRET Spectrometry. *Biochem. J.* 473, 3819–3836. doi:10.1042/bcj20160422
- Misra, R., Eliash, T., Sudo, Y., and Sheves, M. (2019). Retinal-Salinixanthin Interactions in a Thermophilic Rhodopsin. *J. Phys. Chem. B* 123, 10–20. doi:10.1021/acs.jpcc.8b06795
- Mitra, A. K., Miercke, L. J. W., Turner, G. J., Shand, R. F., Betlach, M. C., and Stroud, R. M. (1993). Two-dimensional Crystallization of *Escherichia Coli*-Expressed Bacteriorhodopsin and its D96N Variant: High Resolution Structural Studies in Projection. *Biophys. J.* 65, 1295–1306. doi:10.1016/s0006-3495(93)81169-x
- Mitsuoka, K., Hirai, T., Murata, K., Miyazawa, A., Kidera, A., Kimura, Y., et al. (1999). The Structure of Bacteriorhodopsin at 3.0 Å Resolution Based on Electron Crystallography: Implication of the Charge Distribution. *J. Mol. Biol.* 286, 861–882. doi:10.1006/jmbi.1998.2529
- Miyasaka, T., Koyama, K., and Itoh, I. (1992). Quantum Conversion and Image Detection by a Bacteriorhodopsin- Based Artificial Photoreceptor. *Science* 255, 342–344. doi:10.1126/science.255.5042.342
- Molday, R. S. (1989). Monoclonal Antibodies to Rhodopsin and Other Proteins of Rod Outer Segments. *Prog. Retin. Res.* 8, 173–209.
- Mollaaghbabab, R., Davidson, F. F., Kaiser, C., and Khorana, H. G. (1996). Structure and Function in Rhodopsin: Expression of Functional Mammalian Opsin in *Saccharomyces cerevisiae*. *Proc. Nat. Acad. Sci. U. S. A.* 93, 11482–11486. doi:10.1073/pnas.93.21.11482
- Molvenger, L. C. P. J., Kentgens, A. P. M., Pardo, J. A., Courtin, J. M. L., Veeman, W. S., Lugtenburg, J., et al. (1987). High-resolution Solid-State ¹³C-NMR Study of Carbons C-5 and C-12 of the Chromophore of Bovine Rhodopsin: Evidence for a 6-S-Cis Conformation with Negative-Charge Perturbation Near C-12. *Eur. J. Biochem.* 163, 9–14. doi:10.1111/j.1432-1033.1987.tb10729.x
- Mooney, V. L., Szundi, I., Lewis, J. W., Yan, E. C. Y., and Kliger, D. S. (2012). Schiff Base Protonation Changes in Siberian Hamster Ultraviolet Cone Pigment Photointermediates. *Biochemistry* 51, 2630–2637. doi:10.1021/bi300157r
- Moraes, M. N., Monteiro De Assis, L. V., Provencio, I., and De Lauro Castrucci, A. M. (2021). Opsins Outside the Eye and the Skin: a More Complex Scenario Than Originally Thought for a Classical Light Sensor. *Cell. Tissue Res.* 385, 519–538. doi:10.1007/s00441-021-03500-0
- Morello, J.-P., and Bouvier, M. (1996). Palmitoylation: A Post-translational Modification that Regulates Signalling from G Protein-Coupled Receptors. *Biochem. Cell. Biol.* 74, 449–457. doi:10.1139/o96-049
- Mori, A., Yagasaki, J., Homma, M., Reissig, L., and Sudo, Y. (2013). Investigation of the Chromophore Binding Cavity in the 11-*cis* Acceptable Microbial Rhodopsin MR. *Chem. Phys.* 419, 23–29. doi:10.1016/j.chemphys.2012.11.020
- Morizumi, T., Ou, W.-L., Van Eps, N., Inoue, K., Kandori, H., Brown, L. S., et al. (2019). X-ray Crystallographic Structure and Oligomerization of *Gloeobacter* Rhodopsin. *Sci. Rep.* 9, 11283–112811215. doi:10.1038/s41598-019-47445-5
- Morton, R. A., and Goodwin, T. W. (1944). Preparation of Retinene *In Vitro*. *Nature* 153, 405–406. doi:10.1038/153405a0
- Morton, R. A., and Pitt, G. A. J. (1957). Visual Pigments. *Fortschritte Chem. Org. Naturst.* 14, 244–316. doi:10.1007/978-3-7091-7164-6_6
- Mous, S., Gotthard, G., Ehrenberg, D., Sen, S., Weinert, T., Johnson, P. J. M., et al. (2022). Dynamics and Mechanism of a Light-Driven Chloride Pump. *Science* 375, 845. doi:10.1126/science.abj6663
- Moutsaki, P., Whitmore, D. H., Bellingham, J., Sakamoto, K., David-Gray, Z. K., and Foster, R. G. (2003). Teleost Multiple Tissue (Tmt) Opsin: A Candidate Photopigment Regulating the Peripheral Clocks of Zebrafish? *Mol. Brain Res.* 112, 135–145. doi:10.1016/s0169-328x(03)00059-7
- Mroginski, M.-A., Adam, S., Amoyal, G. S., Barnoy, A., Bondar, A.-N., Borin, V. A., et al. (2021). Frontiers in Multiscale Modeling of Photoreceptor Proteins. *Photochem. Photobiol.* 97, 243–269. doi:10.1111/php.13372
- Mukherjee, S., Hegemann, P., and Broser, M. (2019). Enzymerhodopsins: Novel Photorelated Catalysts for Optogenetics. *Curr. Opin. Struct. Biol.* 57, 118–126. doi:10.1016/j.sbi.2019.02.003
- Müller, D. J., Kessler, M., Oesterheld, F., Möller, C., Oesterheld, D., and Gaub, H. (2002). Stability of Bacteriorhodopsin α -helices and Loops Analyzed by Single-Molecule Force Spectroscopy. *Biophysical J.* 83, 3578–3588. doi:10.1016/S0006-3495(02)75358-7
- Müller, H. (1855). Über die entoptische Wahrnehmung der Netzhautgefäße, insbesondere als Beweismittel für die Lichtperception durch die nach hinten gelegene Netzhautelemente. *Verhandlungen. Physikalisch-Medizinische Gesellschaft Würzburg* 5, 411–447.
- Müller, M. (1992). Proteolysis in Protein Import and Export: Signal Peptide Processing in Eu- and Prokaryotes. *Experientia* 48, 118–129.
- Munro, R. A., De Vlugt, J., Ward, M. E., Kim, S. Y., Lee, K. A., Jung, K.-H., et al. (2019). Biosynthetic Production of Fully Carbon-13 Labeled Retinal in *E. coli* for Structural and Functional Studies of Rhodopsins. *J. Biomol. NMR* 73, 49–58. doi:10.1007/s10858-019-00225-9
- Muradin-Szweykowska, M., Peters, A. J. M., and Lugtenburg, J. (1984). The Interaction of Bacterioopsin with 11, 14-bridged Retinals - the Synthesis of 13-demethyl-11, 14-Imino-Retinal, 13-Demethyl-N-Methyl-11, 14-imino, 13-demethyl-11, 14-Thio-Retinal, 13-demethyl-11, 14-Etheno-Retinal, 11, 14-Imino-Retinal and Their Binding with Bacterioopsin. *Recl. Des. Trav. Chim. Des. Pays-Bas* 103, 105–109.
- Murakami, M., and Kouyama, T. (2008). Crystal Structure of Squid Rhodopsin. *Nature* 453, 363–U333. doi:10.1038/nature06925
- Murakami, M., and Kouyama, T. (2011). Crystallographic Analysis of the Primary Photochemical Reaction of Squid Rhodopsin. *J. Mol. Biol.* 413, 615–627. doi:10.1016/j.jmb.2011.08.044
- Murakami, M., and Kouyama, T. (2015). Crystallographic Study of the LUMI Intermediate of Squid Rhodopsin. *PLoS ONE* 10, e0126970. doi:10.1371/journal.pone.0126970
- Musilova, Z., Cortesi, F., Matschiner, M., Davies, W. I. L., Patel, J. S., Stieb, S. M., et al. (2019). Vision Using Multiple Distinct Rod Opsins in Deep-Sea Fishes. *Science* 364, 588–592. doi:10.1126/science.aav4632
- Musio, C., Santillo, S., Taddei-Ferretti, C., Robles, L. J., Vismara, R., Barsanti, L., et al. (2001). First Identification and Localization of a Visual Pigment in *Hydra* (Cnidaria, Hydrozoa). *J. Comp. Physiology A - Sens. Neural Behav. Physiology* 187, 79–81. doi:10.1007/s003590100180
- Mustafi, D., Engel, A. H., and Palczewski, K. (2009). Structure of Cone Photoreceptors. *Prog. Retin. Eye Res.* 28, 289–302. doi:10.1016/j.preteyeres.2009.05.003
- Na, Y.-A., Lee, J.-Y., Bang, W.-J., Lee, H. J., Choi, S.-I., Kwon, S.-K., et al. (2015). Growth Retardation of *Escherichia coli* by Artificial Increase of Intracellular ATP. *J. Industrial Microbiol. Biotechnol.* 42, 915–924. doi:10.1007/s10295-015-1609-6
- Nagata, T., and Inoue, K. (2022). Rhodopsins at a Glance. *J. Cell. Sci.* 134, jcs258989. doi:10.1242/jcs.258989
- Nagata, T., Koyanagi, M., Lucas, R., and Terakita, A. (2018). An All-*Trans*-Retinal-Binding Opsin Peropsin as a Potential Dark-Active and Light-Inactivated G Protein-Coupled Receptor. *Sci. Rep.* 8, 3535. doi:10.1038/s41598-018-21946-1
- Nagata, T., Koyanagi, M., Tsukamoto, H., Mutt, E., Schertler, G. F. X., Deupi, X., et al. (2019). The Counterion-Retinylidene Schiff Base Interaction of an Invertebrate Rhodopsin Rearranges upon Light Activation. *Commun. Biol.* 2, 180–181189. doi:10.1038/s42003-019-0409-3
- Nagel, G., Szellas, T., Huhn, W., Kateriya, S., Adeishvili, N., Berthold, P., et al. (2003). Channelrhodopsin-2, a Directly Light-Gated Cation-Selective Membrane Channel. *Proc. Natl. Acad. Sci. U. S. A.* 100, 13940–13945. doi:10.1073/pnas.1936192100
- Naito, A., Makino, Y., Shigetani, A., and Kawamura, I. (2019). Photoreaction Pathways and Photointermediates of Retinal-Binding Photoreceptor Proteins as Revealed by *In Situ* Photoirradiation Solid-State NMR Spectroscopy. *Biophys. Rev.* 11, 167–181. doi:10.1007/s12551-019-00501-w
- Nakagawa, M., Iwasa, T., Kikkawa, S., Takao, T., Shimonishi, Y., and Tsuda, M. (1997). Identification of Two Palmitoyl Groups in octopus Rhodopsin. *Photochem. Photobiol.* 65, 185–189. doi:10.1111/j.1751-1097.1997.tb01897.x
- Nakagawa, M., Iwasa, T., Kikkawa, S., Tsuda, M., and Ebrey, T. G. (1999). How Vertebrate and Invertebrate Visual Pigments Differ in Their Mechanism of

- Photoactivation. *Proc. Nat. Acad. Sci. U. S. A.* 96, 6189–6192. doi:10.1073/pnas.96.11.6189
- Nakajima, Y., Pedraza-González, L., Barneschi, L., Inoue, K., Olivucci, M., and Kandori, H. (2021). Pro219 Is an Electrostatic Color Determinant in the Light-Driven Sodium Pump KR2. *Commun. Biol.* 4, 1185. doi:10.1038/s42003-021-02684-z
- Nakajima, Y., Tsukamoto, T., Kumagai, Y., Ogura, Y., Hayashi, T., Song, J., et al. (2018). Presence of a Haloarchaeal Halorhodopsin-like Cl⁻ Pump in Marine Bacteria. *Microbes Environ.* 33, 89–97. doi:10.1264/jsme2.me17197
- Nakamichi, H., and Okada, T. (2006). Crystallographic Analysis of Primary Visual Photochemistry. *Angew. Chem. Int. Ed.* 45, 4270–4273. doi:10.1002/anie.200600595
- Nakamura, A., Kojima, D., Imai, H., Terakita, A., Okano, T., Shichida, Y., et al. (1999). Chimeric Nature of Pinopsin between Rod and Cone Visual Pigments. *Biochemistry-USA* 38, 14738–14745. doi:10.1021/bi9913496
- Nakao, S., Kojima, K., and Sudo, Y. (2021). Microbial Rhodopsins as Multi-Functional Photoreactive Membrane Proteins for Optogenetics. *Biol. Pharm. Bull.* 44, 1357–1363. doi:10.1248/bpb.b21-00544
- Nakao, S., Kojima, K., and Sudo, Y. (2022). Phototriggered Apoptotic Cell Death (PTA) Using the Light-Driven Outward Proton Pump Rhodopsin Archaeorhodopsin-3. *J. Am. Chem. Soc.* 144, 3771. doi:10.1021/jacs.1c12608
- Nakatsuma, A., Yamashita, T., Sasaki, K., Kawanabe, A., Inoue, K., Furutani, Y., et al. (2011). Chimeric Microbial Rhodopsins Containing the Third Cytoplasmic Loop of Bovine Rhodopsin. *Biophysical J.* 100, 1874–1882. doi:10.1016/j.bpj.2011.02.054
- Nakayama, T. A., and Khorana, H. G. (1991). Mapping of the Amino Acids in Membrane-Embedded Helices that Interact with the Retinal Chromophore in Bovine Rhodopsin. *J. Biol. Chem.* 266, 4269–4275. doi:10.1016/s0021-9258(20)64317-4
- Nango, E., Royant, A., Kubo, M., Nakane, T., Wickstrand, C., Kimura, T., et al. (2016). A Three-Dimensional Movie of Structural Changes in Bacteriorhodopsin. *Science* 354, 1552–1557. doi:10.1126/science.aah3497
- Nathans, J. (1987). Molecular Biology of Visual Pigments. *Annu. Rev. Neurosci.* 10, 163–194. doi:10.1146/annurev.ne.10.030187.001115
- Nathans, J. (1992). Rhodopsin - Structure, Function, and Genetics. *Biochemistry* 31, 4923–4931. doi:10.1021/bi00136a001
- Neitz, M., and Neitz, J. (1998). “Molecular Genetics and the Biological Basis of Color Vision,” in *Color Vision - Perspectives from Different Disciplines*. Editors W. Backhaus and R. Kliegl (Berlin, Germany: Walter de Gruyter & Co.), 101–119.
- Neumann, K., Verhoeven, M.-K., Weber, I., Glaubitz, C., and Wachtveitl, J. (2008). Initial Reaction Dynamics of Proteorhodopsin Observed by Femtosecond Infrared and Visible Spectroscopy. *Biophysical J.* 94, 4796–4807. doi:10.1529/biophysj.107.125484
- Neutze, R., Brändén, G., and Schertler, G. F. X. (2015). Membrane Protein Structural Biology Using X-Ray Free Electron Lasers. *Curr. Opin. Struct. Biol.* 33, 115–125. doi:10.1016/j.sbi.2015.08.006
- Nguyen, K.-A., Peuchmaur, M., Magnard, S., Haudecoeur, R., Boyère, C., Mounien, S., et al. (2018). Glycosyl-Substituted Dicarboxylates as Detergents for the Extraction, Overstabilization, and Crystallization of Membrane Proteins. *Angew. Chemie-International Ed.* 57, 2948–2952. doi:10.1002/anie.201713395
- Nielsen, M. B. (2009). Model Systems for Understanding Absorption Tuning by Opsin Proteins. *Chem. Soc. Rev.* 38, 913–924. doi:10.1039/b802068j
- Nikolaev, D. M., Manathunga, M., Orozco-Gonzalez, Y., Shtyrov, A. A., Omar Guerrero Martinez, Y., Gozem, S., et al. (2021). Free Energy Computation for an Isomerizing Chromophore in a Molecular Cavity via the Average Solvent Electrostatic Configuration Model: Applications in Rhodopsin and Rhodopsin-Mimicking Systems. *J. Chem. Theory Comput.* 17, 5885–5895. doi:10.1021/acs.jctc.1c00221
- Nikolaev, D. M., Shtyrov, A. A., Mereshchenko, A. S., Panov, M. S., Tveryanovich, Y. S., and Ryazantsev, M. N. (2020). An Assessment of Water Placement Algorithms in Quantum Mechanics/molecular Mechanics Modeling: the Case of Rhodopsins' First Spectral Absorption Band Maxima. *Phys. Chem. Chem. Phys.* 22, 18114–18123. doi:10.1039/d0cp02638g
- Nikolaev, D. M., Shtyrov, A. A., Panov, M. S., Jamal, A., Chakchir, O. B., Kochemirovsky, V. A., et al. (2018). A Comparative Study of Modern Homology Modeling Algorithms for Rhodopsin Structure Prediction. *ACS Omega* 3, 7555–7566. doi:10.1021/acsomega.8b00721
- Nogly, P., Weinert, T., James, D., Carbajo, S., Ozerov, D., Furrer, A., et al. (2018). Retinal Isomerization in Bacteriorhodopsin Captured by a Femtosecond X-Ray Laser. *Science* 361, 145–151. doi:10.1126/science.aat0094
- Nomura, Y., Ito, S., Teranishi, M., Ono, H., Inoue, K., and Kandori, H. (2018). Low-temperature FTIR Spectroscopy Provides Evidence for Protein-Bound Water Molecules in Eubacterial Light-Driven Ion Pumps. *Phys. Chem. Chem. Phys.* 20, 3165–3171. doi:10.1039/c7cp05674e
- Nonaka, Y., Hanai, S., Katayama, K., Imai, H., and Kandori, H. (2020). Unique Retinal Binding Pocket of Primate Blue-Sensitive Visual Pigment. *Biochemistry* 59, 2602–2607. doi:10.1021/acs.biochem.0c00394
- O'tousa, J. E. (1992). Requirement of N-Linked Glycosylation Site in *Drosophila* Rhodopsin. *Vis. Neurosci.* 8, 385–390. doi:10.1017/s0952523800004910
- Oda, K., Nomura, T., Nakane, T., Yamashita, K., Inoue, K., Ito, S., et al. (2021). Time-resolved Serial Femtosecond Crystallography Reveals Early Structural Changes in Channelrhodopsin. *eLife* 10, 62389. doi:10.7554/eLife.62389
- Oda, K., Vierock, J., Oishi, S., Rodriguez-Rozada, S., Taniguchi, R., Yamashita, K., et al. (2018). Crystal Structure of the Red Light-Activated Channelrhodopsin Chrimson. *Nat. Commun.* 9, 3949. doi:10.1038/s41467-018-06421-9
- Oesterhelt, D., Bräuchle, C., and Hampp, N. (1991). Bacteriorhodopsin - A Biological Material for Information Processing. *Quart. Rev. Biophys.* 24, 425–478. doi:10.1017/s0033583500003863
- Oesterhelt, D., and Hess, B. (1973). Reversible Photolysis of Purple Complex in Purple Membrane of *Halobacterium Halobium*. *Eur. J. Biochem.* 37, 316–326. doi:10.1111/j.1432-1033.1973.tb02990.x
- Oesterhelt, D., and Stoeckenius, W. (1971). Rhodopsin-like Protein from the Purple Membrane of *Halobacterium Halobium*. *Nat. New Biol.* 233, 149–152. doi:10.1038/newbio233149a0
- Oesterhelt, D. (1998). The Structure and Mechanism of the Family of Retinal Proteins from Halophilic Archaea. *Curr. Opin. Struct. Biol.* 8, 489–500. doi:10.1016/s0959-440x(98)80128-0
- Ogonah, O., Shuler, M. L., and Granados, R. R. (1991). Protein Production (β -Galactosidase) from a Baculovirus Vector in *Spodoptera Frugiperda* and *Trichopolusia Ni* Cells in Suspension Culture. *Biotechnol. Lett.* 13, 265–270. doi:10.1007/bf01041482
- Ogren, J. I., Yi, A., Mamaev, S., Li, H., Lugtenburg, J., DeGrip, W. J., et al. (2015). Comparison of the Structural Changes Occurring during the Primary Phototransition of Two Different Channelrhodopsins from *Chlamydomonas* Algae. *Biochemistry* 54, 377–388. doi:10.1021/bi501243y
- Okada, T., Sugihara, M., Bondar, A.-N., Elstner, M., Entel, P., and Buss, V. (2004). The Retinal Conformation and its Environment in Rhodopsin in Light of a New 2.2 Å Crystal Structure. *J. Mol. Biol.* 342, 571–583. doi:10.1016/j.jmb.2004.07.044
- Okano, T., Fukada, Y., Artamonov, I. D., and Yoshizawa, T. (1989). Purification of Cone Visual Pigments from Chicken Retina. *Biochemistry-USA* 28, 8848–8856. doi:10.1021/bi00448a025
- Okano, T., Yoshizawa, T., and Fukada, Y. (1994). Pinopsin Is a Chicken Pineal Photoreceptive Molecule. *Nature* 372, 94–97. doi:10.1038/372094a0
- Okitsu, T., Yamano, Y., Shen, Y. C., Sasaki, T., Kobayashi, Y., Morisawa, S., et al. (2020). Synthesis of One Double Bond-Inserted Retinal Analogs and Their Binding Experiments with Opsins: Preparation of Novel Red-Shifted Channelrhodopsin Variants. *Chem. Pharm. Bull.* 68, 265–272. doi:10.1248/cpb.c19-01005
- Olinski, L. E., Lin, E. M., and Oancea, E. (2020). Illuminating Insights into Opsin 3 Function in the Skin. *Adv. Biol. Regul.* 75, 100668. doi:10.1016/j.jbior.2019.100668
- Oppermann, J., Fischer, P., Silapetere, A., Liepe, B., Rodriguez-Rozada, S., Flores-Uribe, J., et al. (2019). MerMAIDs: a Family of Metagenomically Discovered Marine Anion-Conducting and Intensely Desensitizing Channelrhodopsins. *Nat. Commun.* 10, 3315. doi:10.1038/s41467-019-11322-6
- Oprian, D. D., Asenjo, A. B., Lee, N., and Pelletier, S. L. (1991). Design, Chemical Synthesis, and Expression of Genes for the Three Human Color Vision Pigments. *Biochemistry* 30, 11367–11372. doi:10.1021/bi00112a002
- Oprian, D. D., Molday, R. S., Kaufman, R. J., and Khorana, H. G. (1987). Expression of a Synthetic Bovine Rhodopsin Gene in Monkey Kidney Cells. *Proc. Nat. Acad. Sci. U. S. A.* 84, 8874–8878. doi:10.1073/pnas.84.24.8874
- Ortega, J. T., and Jastrzebska, B. (2019). The Retinoid and Non-retinoid Ligands of the Rod Visual G Protein-Coupled Receptor. *Int. J. Mol. Sci.* 20, 6218. doi:10.3390/ijms20246218

- Ovchinnikov, Y. A., Abdulaev, N. G., and Bogachuk, A. S. (1988a). Two Adjacent Cysteine Residues in the C-Terminal Cytoplasmic Fragment of Bovine Rhodopsin Are Palmitylated. *FEBS Lett.* 230, 1–5. doi:10.1016/0014-5793(88)80628-8
- Ovchinnikov, Y. A., Abdulaev, N. G., Zolotarev, A. S., Artamonov, I. D., Bepalov, I. A., Dergachev, A. E., et al. (1988b). Octopus Rhodopsin - Amino Acid Sequence Deduced from C-DNA. *FEBS Lett.* 232, 69–72. doi:10.1016/0014-5793(88)80388-0
- Owen, S. F., Liu, M. H., and Kreitzer, A. C. (2019). Thermal Constraints on *In Vivo* Optogenetic Manipulations. *Nat. Neurosci.* 22, 1061–1065. doi:10.1038/s41593-019-0422-3
- Ozaki, Y., Kawashima, T., Abe-Yoshizumi, R., and Kandori, H. (2014). A Color-Determining Amino Acid Residue of Proteorhodopsin. *Biochemistry* 53, 6032–6040. doi:10.1021/bi500842w
- Palczewski, K. (2012). Chemistry and Biology of Vision. *J. Biol. Chem.* 287, 1612–1619. doi:10.1074/jbc.r111.301150
- Palczewski, K. (2006). G Protein-Coupled Receptor Rhodopsin. *Annu. Rev. Biochem.* 75, 743–767. doi:10.1146/annurev.biochem.75.103004.142743
- Palczewski, K., Kumasaka, T., Hori, T., Behnke, C. A., Motoshima, H., Fox, B. A., et al. (2000). Crystal Structure of Rhodopsin: A G Protein-Coupled Receptor. *Science* 289, 739–745. doi:10.1126/science.289.5480.739
- Palczewski, K., and Orban, T. (2013). From Atomic Structures to Neuronal Functions of G Protein-Coupled Receptors. *Annu. Rev. Neurosci.* 36, 139–164. doi:10.1146/annurev-neuro-062012-170313
- Palings, I., Pardo, J. A., Vandenberg, E. M. M., Winkel, C., Lugtenburg, J., and Mathies, R. A. (1987). Assignment of Fingerprint Vibrations in the Resonance Raman Spectra of Rhodopsin, Isorhodopsin, and Bathorhodopsin: Implications for Chromophore Structure and Environment. *Biochemistry-USA* 26, 2544–2556. doi:10.1021/bi00383a021
- Panda, S., Nayak, S. K., Campo, B., Walker, J. R., Hogenesch, J. B., and Jegla, T. (2005). Illumination of the Melanopsin Signaling Pathway. *Science* 307, 600–604. doi:10.1126/science.1105121
- Panda, S., Sato, T. K., De Lauro Castrucci, A. M., DeGrip, W. J., Rollag, M. D., Hogenesch, J. B., et al. (2002). Melanopsin (*Opn4*) Is Required for Circadian Phase Shifting under Low Light Conditions. *Science* 298, 2213–2216. doi:10.1126/science.1076848
- Pande, C., Pande, A., Yue, K. T., Callender, R. H., Ebrey, T. G., and Tsuda, M. (1987). Resonance Raman Spectroscopy of octopus Rhodopsin and its Photoproducts. *Biochemistry* 26, 4941–4947. doi:10.1021/bi00390a009
- Panneels, V., Wu, W. T., Tsai, C.-J., Nogly, P., Rheinberger, J., Jaeger, K., et al. (2015). Time-resolved Structural Studies with Serial Crystallography: A New Light on Retinal Proteins. *Struct. Dyn.* 2, 041718. doi:10.1063/1.4922774
- Panzer, S., Zhang, C., Konte, T., Bräuer, C., Diemar, A., Yogendran, P., et al. (2021). Modified Rhodopsins from *Aureobasidium Pullulans* Excel with Very High Proton-Transport Rates. *Front. Mol. Biosci.* 8, 750528. doi:10.3389/fmolb.2021.750528
- Park, J. H., Morizumi, T., Li, Y. F., Hong, J. E., Pai, E. F., Hofmann, K. P., et al. (2013). Opsin, a Structural Model for Olfactory Receptors? *Angew. Chemie-International Ed.* 52, 11021–11024. doi:10.1002/anie.201302374
- Park, J. H., Scheerer, P., Hofmann, K. P., Choe, H.-W., and Ernst, O. P. (2008). Crystal Structure of the Ligand-free G-Protein-Coupled Receptor Opsin. *Nature* 454, 183–187. doi:10.1038/nature07063
- Park, P. S.-H., Sapra, K. T., Jastrzebska, B., Maeda, T., Maeda, A., Pulawski, W., et al. (2009). Modulation of Molecular Interactions and Function by Rhodopsin Palmitylation. *Biochemistry* 48, 4294–4304. doi:10.1021/bi900417b
- Passamanek, Y. J., Furchheim, N., Hejnol, A., Martindale, M. Q., and Lüter, C. (2011). Ciliary Photoreceptors in the Cerebral Eyes of a Protostome Larva. *EvoDevo* 2, 6. doi:10.1186/2041-9139-2-6
- Patel, J. S., Brown, C. J., Ytreberg, F. M., and Stenkamp, D. L. (2018). Predicting Peak Spectral Sensitivities of Vertebrate Cone Visual Pigments Using Atomistic Molecular Simulations. *Plos Comput. Biol.* 14, e1005974. doi:10.1371/journal.pcbi.1005974
- Patriarchi, T., Shen, A., He, W., Baikoghli, M., Cheng, R. H., Xiang, Y. K., et al. (2018). Nanodelivery of a Functional Membrane Receptor to Manipulate Cellular Phenotype. *Sci. Rep.* 8, 3556. doi:10.1038/s41598-018-21863-3
- Pebay-Peyroula, E., Neutze, R., and Landau, E. M. (2000). Lipidic Cubic Phase Crystallization of Bacteriorhodopsin and Cryotrapping of Intermediates: Towards Resolving a Revolving Photocycle. *Biochimica Biophysica Acta-Bioenergetics* 1460, 119–132. doi:10.1016/s0005-2728(00)00134-1
- Pediani, J. D., Ward, R. J., Marsango, S., and Milligan, G. (2018). Spatial Intensity Distribution Analysis: Studies of G Protein-Coupled Receptor Oligomerisation. *Trends Pharmacol. Sci.* 39, 175–186. doi:10.1016/j.tips.2017.09.001
- Pedraza-González, L., Barmeschi, L., Padula, D., De Vico, L., and Olivucci, M. (2022). Evolution of the Automatic Rhodopsin Modeling (ARM) Protocol. *Top. Curr. Chem.* 380 (21), 21–48. doi:10.1007/s41061-022-00374-w
- Pedraza-González, L., Del Carmen Marin, M., Jorge, A. N., Ruck, T. D., Yang, X. C., Valentini, A., et al. (2020). Web-ARM: A Web-Based Interface for the Automatic Construction of QM/MM Models of Rhodopsins. *J. Chem. Inf. Model.* 60, 1481–1493.
- Peirson, S. N., Bovee-Geurts, P. H. M., Lupi, D., Jeffery, G., DeGrip, W. J., and Foster, R. G. (2004). Expression of the Candidate Circadian Photopigment Melanopsin (*Opn4*) in the Mouse Retinal Pigment Epithelium. *Mol. Brain Res.* 123, 132–135. doi:10.1016/j.molbrainres.2004.01.007
- Penzkofer, A., Silapetere, A., and Hegemann, P. (2021). Photocycle Dynamics of the Archaeorhodopsin 3 Based Fluorescent Voltage Sensor Archon2. *J. Photochem. Photobiol. B-Biology* 225, 112331. doi:10.1016/j.jphotobiol.2021.112331
- Pepe, I. M., and Cugnoli, C. (1992). Retinal Photoisomerase - Role in Invertebrate Visual Cells. *J. Photochem. Photobiol. B-Biol* 13, 5–17. doi:10.1016/1011-1344(92)80035-t
- Pérez, A. A., Chen, Q., Pineda Hernández, H., Branco Dos Santos, F., and Hellingwerf, K. J. (2019a). On the Use of Oxygenic Photosynthesis for the Sustainable Production of Commodity Chemicals. *Physiol. Plant.* 166, 413–427. doi:10.1111/ppl.12946
- Pérez, J. H., Tolla, E., Dunn, I. C., Meddle, S. L., and Stevenson, T. J. (2019b). A Comparative Perspective on Extra-retinal Photoreception. *Trends Endocrinol. Metabolism* 30, 39–53. doi:10.1016/j.tem.2018.10.005
- Pérez-Cereales, S., Boryshpolets, S., Afanar, O., Brandis, A., Nevo, R., Kiss, V., et al. (2015). Involvement of Opsins in Mammalian Sperm Thermotaxis. *Sci. Rep.* 5, 16146. doi:10.1038/srep16146
- Perrino, A. P., Miyagi, A., and Scheuring, S. (2021). Single Molecule Kinetics of Bacteriorhodopsin by HS-AFM. *Nat. Commun.* 12, 7225. doi:10.1038/s41467-021-27580-2
- Peters, L., D. M., Kussmann, J., and Ochsenfeld, C. (2020). Combining Graphics Processing Units, Simplified Time-dependent Density Functional Theory, and Finite-Difference Couplings to Accelerate Nonadiabatic Molecular Dynamics. *J. Phys. Chem. Lett.* 11, 3955–3961. doi:10.1021/acs.jpclett.0c00320
- Philosof, A., and Bèjà, O. (2013). Bacterial, Archaeal and Viral-like Rhodopsins from the Red Sea. *Environ. Microbiol. Rep.* 5, 475–482. doi:10.1111/1758-2229.12037
- Piatkevich, K. D., Bensussen, S., Tseng, H.-A., Shroff, S. N., Lopez-Huerta, V. G., Park, D., et al. (2019). Population Imaging of Neural Activity in Awake Behaving Mice. *Nature* 574, 413–417. doi:10.1038/s41586-019-1641-1
- Piatkevich, K. D., Jung, E. E., Straub, C., Linghu, C. G., Park, D., Suk, H.-J., et al. (2018). A Robotic Multidimensional Directed Evolution Approach Applied to Fluorescent Voltage Reporters. *Nat. Chem. Biol.* 14, 352–360. doi:10.1038/s41589-018-0004-9
- Piechnick, R., Ritter, E., Hildebrand, P. W., Ernst, O. P., Scheerer, P., Hofmann, K.-P., et al. (2012). Effect of Channel Mutations on the Uptake and Release of the Retinal Ligand in Opsin. *Proc. Natl. Acad. Sci. U. S. A.* 109, 5247–5252. doi:10.1073/pnas.1117268109
- Pieri, E., Ledentu, V., Sahlin, M., Dehez, F., Olivucci, M., and Ferré, N. (2019). CpHMD-Then-QM/MM Identification of the Amino Acids Responsible for the Anabaena Sensory Rhodopsin pH-dependent Electronic Absorption Spectrum. *J. Chem. Theory Comput.* 15, 4535–4546. doi:10.1021/acs.jctc.9b00221
- Pinhassi, J., Delong, E. F., Bèjà, O., González, J. M., and Pedrós-Alió, C. (2016). Marine Bacterial and Archaeal Ion-Pumping Rhodopsins: Genetic Diversity, Physiology, and Ecology. *Microbiol. Mol. Biol. Rev.* 80, 929–953. doi:10.1128/mmr.00003-16
- Plachetzki, D. C., Fong, C. R., and Oakley, T. H. (2012). Cnidocyte Discharge Is Regulated by Light and Opsin-Mediated Phototransduction. *BMC Biol.* 10, 17. doi:10.1186/1741-7007-10-17
- Planchard, N., Point, E., Dahmane, T., Giusti, F., Renault, M., Le Bon, C., et al. (2014). The Use of Amphipols for Solution NMR Studies of Membrane

- Proteins: Advantages and Constraints as Compared to Other Solubilizing Media. *J. Membr. Biol.* 247, 827–842. doi:10.1007/s00232-014-9654-z
- Poddar, H., Heyes, D. J., Schiro, G., Weik, M., Leys, D., and Scrutton, N. S. (2022). A Guide to Time-Resolved Structural Analysis of Light-Activated Proteins. *FEBS J.* 289, 576–595. doi:10.1111/febs.15880
- Polito, R., Temperini, M. E., Ritter, E., Puskar, L., Schade, U., Broser, M., et al. (2021). Conformational Changes of a Membrane Protein Determined by Infrared Difference Spectroscopy beyond the Diffraction Limit. *Phys. Rev. Appl.* 16, 014048. doi:10.1103/physrevapplied.16.014048
- Polli, D., Rivalta, I., Nenov, A., Weingart, O., Garavelli, M., and Cerullo, G. (2015). Tracking the Primary Photoconversion Events in Rhodopsins by Ultrafast Optical Spectroscopy. *Photochem. Photobiological Sci.* 14, 213–228. doi:10.1039/c4pp000370e
- Pope, A. L., Sanchez-Reyes, O. B., South, K., Zaitseva, E., Ziliox, M., Vogel, R., et al. (2020). A Conserved Proline Hinge Mediates Helix Dynamics and Activation of Rhodopsin. *Structure* 28, 1004–1013. doi:10.1016/j.str.2020.05.004
- Popot, J.-L., Althoff, T., Bagnard, D., Banères, J.-L., Bazzacco, P., Billon-Denis, E., et al. (2011). Amphipols from A to Z. *Annu. Rev. Biophysics* 40, 379–408. doi:10.1146/annurev-biophys-042910-155219
- Popp, A., Wolperdinger, M., Hampp, N., Bräuchle, C., and Oesterheld, D. (1993). Photochemical Conversion of the O-Intermediate to 9-Cis-Retinal-Containing Products in Bacteriorhodopsin Films. *Biophys. J.* 65, 1449–1459. doi:10.1016/s0006-3495(93)81214-1
- Porter, M. L., Blasic, J. R., Jr., Bok, M. J., Cameron, E. G., Pringle, T., Cronin, T. W., et al. (2012). Shedding New Light on Opsin Evolution. *Proc. R. Soc. B-Biological Sci.* 279, 3–14. doi:10.1098/rspb.2011.1819
- Prakash, M., Murphy, J., St Laurent, R., Friedman, N., Crespo, E. L., Bjorefeldt, A., et al. (2022). Selective Control of Synaptically-Connected Circuit Elements by All-Optical Synapses. *Commun. Biol.* 5, 33. doi:10.1038/s42003-021-02981-7
- Provencio, I., Jiang, G., DeGrip, W. J., Hayes, W. P., and Rollag, M. D. (1998). Melanopsin: An Opsin in Melanophores, Brain and Eye. *Proc. Nat. Acad. Sci. U. S. A.* 95, 340–345. doi:10.1073/pnas.95.1.340
- Provencio, I., Rodriguez, I. R., Jiang, G., Hayes, W. P., Moreira, E. F., and Rollag, M. D. (2000). A Novel Human Opsin in the Inner Retina. *J. Neurosci.* 20, 600–605. doi:10.1523/jneurosci.20-02-00600.2000
- Pushkarev, A., Inoue, K., Larom, S., Flores-Uribe, J., Singh, M., Konno, M., et al. (2018). A Distinct Abundant Group of Microbial Rhodopsins Discovered Using Functional Metagenomics. *Nature* 558, 595–599. doi:10.1038/s41586-018-0225-9
- Qiu, X. D., Kumbalasingi, T., Carlson, S. M., Wong, K. Y., Krishna, V. R., Provencio, I., et al. (2005). Induction of Photosensitivity by Heterologous Expression of Melanopsin. *Nature* 433, 745–749. doi:10.1038/nature03345
- Radlwimmer, F. B., and Yokoyama, S. (1997). Cloning and Expression of the Red Visual Pigment Gene of Goat (*Capra hircus*). *Gene* 198, 211–215. doi:10.1016/s0378-1119(97)00316-8
- Ranganathan, R., and Stevens, C. F. (1995). Arrestin Binding Determines the Rate of Inactivation of the G Protein-Coupled Receptor Rhodopsin *In Vivo*. *Cell* 81, 841–848. doi:10.1016/0092-8674(95)90004-7
- Rath, P., Decaluwé, G. L. J., Bovee-Geurts, P. H. M., DeGrip, W. J., and Rothschild, K. J. (1993). Fourier Transform Infrared Difference Spectroscopy of Rhodopsin Mutants: Light Activation of Rhodopsin Causes Hydrogen-Bonding Changes in Residue Aspartic Acid-83 during Meta II Formation. *Biochemistry* 32, 10277–10282. doi:10.1021/bi00090a001
- Rath, P., DeLange, F., DeGrip, W. J., and Rothschild, K. J. (1998). Hydrogen Bonding Changes of Internal Water Molecules in Rhodopsin during Metarhodopsin I and Metarhodopsin II Formation. *Biochem. J.* 329, 713–717. doi:10.1042/bj3290713
- Rawlinson, K. A., Lapraz, F., Ballister, E. R., Terasaki, M., Rodgers, J., McDowell, R. J., et al. (2019). Extraocular, Rod-like Photoreceptors in a Flatworm Express Xenopsin Photopigment. *eLife* 8, e45465. doi:10.7554/eLife.45465
- Reeves, P. J., Kim, J.-M., and Khorana, H. G. (2002). Structure and Function in Rhodopsin: A Tetracycline-Inducible System in Stable Mammalian Cell Lines for High-Level Expression of Opsin Mutants. *Proc. Natl. Acad. Sci. U. S. A.* 99, 13413–13418. doi:10.1073/pnas.212519199
- Reeves, P. J., Klein-Seetharaman, J., Getmanova, E. V., Eilers, M., Loewen, M. C., Smith, S. O., et al. (1999). Expression and Purification of Rhodopsin and its Mutants from Stable Mammalian Cell Lines: Application to NMR Studies. *Biochem. Soc. Trans.* 27, 950–955. doi:10.1042/bst0270950
- Regan, C. M., DeGrip, W. J., Daemen, F. J. M., and Bonting, S. L. (1978). Sulfhydryl Group Reactivity as a Probe of Transient Protein Conformational Changes during Rhodopsin Photolysis. *Biochim. Biophys. Acta* 537, 145–152. doi:10.1016/0005-2795(78)90609-8
- Ren, Z., Ren, P. X., Balusu, R., and Yang, X. J. (2016). Transmembrane Helices Tilt, Bend, Slide, Torque, and Unwind between Functional States of Rhodopsin. *Sci. Rep.* 6, 34129. doi:10.1038/srep34129
- Ridge, K. D., and Abdulaev, N. G. (2000). Folding and Assembly of Rhodopsin from Expressed Fragments. *Meth. Enzymol.* 315, 59–70. doi:10.1016/s0076-6879(00)15834-3
- Ridge, K. D., Lu, Z. J., Liu, X.-M., and Khorana, H. G. (1995). Structure and Function in Rhodopsin. Separation and Characterization of the Correctly Folded and Misfolded Opsins Produced on Expression of an Opsin Mutant Gene Containing Only the Native Intradiscal Cysteine Codons. *Biochemistry-USA* 34, 3261–3267. doi:10.1021/bi00010a016
- Ritchie, T. K., Grinkova, Y. V., Bayburt, T. H., Denisov, I. G., Zolnerciks, J. K., Atkins, W. M., et al. (2009). Reconstitution of Membrane Proteins in Phospholipid Bilayer Nanodiscs. *Methods Enzym.* 464, 211–231. doi:10.1016/s0076-6879(09)64011-8
- Ritter, E., Zimmermann, K., Heck, M., Hofmann, K. P., and Bartl, F. J. (2004). Transition of Rhodopsin into the Active Metarhodopsin II State Opens a New Light-Induced Pathway Linked to Schiff Base Isomerization. *J. Biol. Chem.* 279, 48102–48111. doi:10.1074/jbc.m406857200
- Rodgers, J., Bano-Otalora, B., Belle, M. D. C., Paul, S., Hughes, R., Wright, P., et al. (2021). Using a Bistable Animal Opsin for Switchable and Scalable Optogenetic Inhibition of Neurons. *Embo Rep.* 22, 51866. doi:10.15252/embr.202051866
- Rödiger, C., Chizhov, I., Weidlich, O., and Siebert, F. (1999). Time-resolved Step-Scan Fourier Transform Infrared Spectroscopy Reveals Differences between Early and Late M Intermediates of Bacteriorhodopsin. *Biophys. J.* 76, 2687–2701. doi:10.1016/S0006-3495(99)77421-7
- Rohrer, B., Goletz, P. W., Znoiko, S. L., Ablonczy, Z., Ma, J.-X., Redmond, T. M., et al. (2003). Correlation of Regenerable Opsin with Rod ERG Signal in *RPE65(-/-)* Mice during Development and Aging. *Investigative Ophthalmol. Vis. Sci.* 44, 310–315. doi:10.1167/iovs.02-0567
- Rost, B. R., Schneider-Warme, F., Schmitz, D., and Hegemann, P. (2017). Optogenetic Tools for Subcellular Applications in Neuroscience. *Neuron* 96, 572–603. doi:10.1016/j.neuron.2017.09.047
- Rothschild, K. J., Andrew, J. R., DeGrip, W. J., and Stanley, H. E. (1976). Opsin Structure Probed by Raman Spectroscopy of Photoreceptor Membranes. *Science* 191, 1176–1178. doi:10.1126/science.1257742
- Rothschild, K. J., Cantore, W. A., and Marrero, H. (1983). Fourier Transform Infrared Difference Spectra of Intermediates in Rhodopsin Bleaching. *Science* 219, 1333–1335. doi:10.1126/science.6828860
- Rothschild, K. J., DeGrip, W. J., and Sanches, R. (1980). Fourier Transform Infrared Study of Photoreceptor Membrane. I. Group Assignments Based on Rhodopsin Delipidation and Reconstitution. *Biochim. Biophys. Acta* 596, 338–351. doi:10.1016/0005-2736(80)90121-2
- Rothschild, K. J., Gillespie, J., and DeGrip, W. J. (1987). Evidence for Rhodopsin Refolding during the Decay of Meta II. *Biophys. J.* 51, 345–350. doi:10.1016/s0006-3495(87)83341-6
- Rothschild, K. J., Marrero, H., Braiman, M. S., and Mathies, R. A. (1984). Primary Photochemistry of Bacteriorhodopsin - Comparison of Fourier-Transform Infrared Difference Spectra with Resonance Raman-Spectra. *Photochem. Photobiol.* 40, 675–679. doi:10.1111/j.1751-1097.1984.tb05359.x
- Rothschild, K. J., and Marrero, H. (1982). Infrared Evidence that the Schiff Base of Bacteriorhodopsin Is Protonated: bR570 and K Intermediates. *Proc. Nat. Acad. Sci. U. S. A.* 79, 4045–4049. doi:10.1073/pnas.79.13.4045
- Rothschild, K. J. (2016). The Early Development and Application of FTIR Difference Spectroscopy to Membrane Proteins: A Personal Perspective. *Biomed. Spectrosc. Imaging* 5, 231–267. doi:10.3233/bsi-160148
- Rothschild, K. J., Zagaeski, M., and Cantore, W. A. (1981). Conformational Changes of Bacteriorhodopsin Detected by Fourier Transform Infrared Difference Spectroscopy. *Biochem. Biophys. Res. Commun.* 103, 483–489. doi:10.1016/0006-291x(81)90478-2
- Rouso, I., Gat, Y., Lewis, A., Sheves, M., and Ottolenghi, M. (1998). Effective Light-Induced Hydroxylamine Reactions Occur with C-13 = C-14 Nonisomerizable Bacteriorhodopsin Pigments. *Biophysical J.* 75, 413–417. doi:10.1016/s0006-3495(98)77526-5

- Roy, P. P., Kato, Y., Abe-Yoshizumi, R., Pieri, E., Ferré, N., Kandori, H., et al. (2018). Mapping the Ultrafast Vibrational Dynamics of All-*Trans* and 13-*cis* Retinal Isomerization in Anabaena Sensory Rhodopsin. *Phys. Chem. Chem. Phys.* 20, 30159–30173. doi:10.1039/c8cp05469j
- Royant, A., Nollert, P., Edman, K., Neutze, R., Landau, E. M., Pebay-Peyroula, E., et al. (2001). X-ray Structure of Sensory Rhodopsin II at 2.1-Å Resolution. *Proc. Natl. Acad. Sci. U. S. A.* 98, 10131–10136. doi:10.1073/pnas.181203898
- Rozenberg, A., Inoue, K., Kandori, H., and Béjà, O. (2021). Microbial Rhodopsins: The Last Two Decades. *Annu. Rev. Microbiol.* 75, 427–447. doi:10.1146/annurev-micro-031721-020452
- Rupenyau, A., Van Stokkum, I. H. M., Arents, J. C., Van Grondelle, R., Hellingwerf, K. J., and Groot, M. L. (2008). Characterization of the Primary Photochemistry of Proteorhodopsin with Femtosecond Spectroscopy. *Biophysical J.* 94, 4020–4030. doi:10.1529/biophysj.107.121376
- Rupenyau, A., Van Stokkum, I. H. M., Arents, J. C., Van Grondelle, R., Hellingwerf, K. J., and Groot, M. L. (2009). Reaction Pathways of Photoexcited Retinal in Proteorhodopsin Studied by Pump-Dump-Probe Spectroscopy. *J. Phys. Chem. B* 113, 16251–16256. doi:10.1021/jp9065289
- Ruprecht, J. J., Mielke, T., Vogel, R., Villa, C., and Schertler, G. F. X. (2004). Electron Crystallography Reveals the Structure of Metarhodopsin I. *EMBO J.* 23, 3609–3620. doi:10.1038/sj.emboj.7600374
- Ryazantsev, M. N., Nikolaev, D. M., Struts, A. V., and Brown, M. F. (2019). Quantum Mechanical and Molecular Mechanics Modeling of Membrane-Embedded Rhodopsins. *J. Membr. Biol.* 252, 425–449. doi:10.1007/s00232-019-00095-0
- Ryba, N. J. P., Hoon, M. A., Findlay, J. B. C., Saibil, H. R., Wilkinson, J. R., Heimburg, T., et al. (1993). Rhodopsin Mobility, Structure, and Lipid-Protein Interaction in Squid Photoreceptor Membranes. *Biochemistry* 32, 3298–3305. doi:10.1021/bi00064a012
- Sadaf, A., Cho, H. C., Byrne, B., and Chae, P. S. (2015). Amphipathic Agents for Membrane Protein Study. *Meth. Enzymol.* 557, 57–94. doi:10.1016/bs.mie.2014.12.021
- Saint Clair, E. C., Ogren, J. I., Mamaev, S., Kralj, J. M., and Rothschild, K. J. (2012a). Conformational Changes in the Archaeorhodopsin-3 Proton Pump: Detection of Conserved Strongly Hydrogen Bonded Water Networks. *J. Biol. Phys.* 38, 153–168. doi:10.1007/s10867-011-9246-4
- Saint Clair, E. C., Ogren, J. I., Mamaev, S., Russano, D., Kralj, J. M., and Rothschild, K. J. (2012b). Near-IR Resonance Raman Spectroscopy of Archaeorhodopsin 3: Effects of Transmembrane Potential. *J. Phys. Chem. B* 116, 14592–14601. doi:10.1021/jp309996a
- Sakai, K., Imamoto, Y., Su, C.-Y., Tsukamoto, H., Yamashita, T., Terakita, A., et al. (2012). Photochemical Nature of Parietopsin. *Biochemistry* 51, 1933–1941. doi:10.1021/bi2018283
- Sakai, K., Shichida, Y., Imamoto, Y., and Yamashita, T. (2022). Creation of Photocyclic Vertebrate Rhodopsin by Single Amino Acid Substitution. *eLife* 11, 75979. doi:10.7554/eLife.75979
- Sakmar, T. P., and Fahmy, K. (1995). Properties and Photoactivity of Rhodopsin Mutants. *Isr. J. Chem.* 35, 325–337. doi:10.1002/ijch.199500034
- Sakmar, T. P., Franke, R. R., and Khorana, H. G. (1989). Glutamic Acid-113 Serves as the Retinylidene Schiff Base Counterion in Bovine Rhodopsin. *Proc. Natl. Acad. Sci. U. S. A.* 86, 8309–8313. doi:10.1073/pnas.86.21.8309
- Sakmar, T. P., Menon, S. T., Marin, E. P., and Awad, E. S. (2002). Rhodopsin: Insights from Recent Structural Studies. *Annu. Rev. Biophys. Biomol. Struct.* 31, 443–484. doi:10.1146/annurev.biophys.31.082901.134348
- Salcedo, E., Huber, A., Henrich, S., Chadwell, L. V., Chou, W. H., Paulsen, R., et al. (1999). Blue- and Green-Absorbing Visual Pigments of *Drosophila*: Ectopic Expression and Physiological Characterization of the R8 Photoreceptor Cell-specific Rh5 and Rh6 Rhodopsins. *J. Neurosci.* 19, 10716–10726. doi:10.1523/jneurosci.19-24-10716.1999
- Salom, D., Cao, P. X., Sun, W. Y., Kramp, K., Jastrzebska, B., Jin, H., et al. (2012). Heterologous Expression of Functional G-Protein-Coupled Receptors in *Caenorhabditis elegans*. *FASEB J.* 26, 492–502. doi:10.1096/fj.11-197780
- Salom, D., Jin, H., Gerken, T. A., Yu, C., Huang, L., and Palczewski, K. (2019). Human Red and Green Cone Opsins Are O-Glycosylated at an N-Terminal Ser/Thr-Rich Domain Conserved in Vertebrates. *J. Biol. Chem.* 294, 8123–8133. doi:10.1074/jbc.ra118.006835
- Salom, D., Wu, N., Sun, W. Y., Dong, Z., Palczewski, K., Jordan, S., et al. (2008). Heterologous Expression and Purification of the Serotonin Type 4 Receptor from Transgenic Mouse Retina. *Biochemistry* 47, 13296–13307. doi:10.1021/bi8018527
- Sanchez-Reyes, O. B., Cooke, A. L. G., Tranter, D. B., Rashid, D., Eilers, M., Reeves, P. J., et al. (2017). G Protein-Coupled Receptors Contain Two Conserved Packing Clusters. *Biophysical J.* 112, 2315–2326. doi:10.1016/j.bpj.2017.04.051
- Sardet, C., Tardieu, A., and Luzzati, V. (1976). Shape and Size of Bovine Rhodopsin: A Small-Angle X-Ray-Scattering Study of A Rhodopsin-Detergent Complex. *J. Mol. Biol.* 105, 383–407. doi:10.1016/0022-2836(76)90100-5
- Sarramegna, V., Muller, I., Milon, A., and Talmont, F. (2006). Recombinant G Protein-Coupled Receptors from Expression to Renaturation: A Challenge towards Structure. *Cell. Mol. Life Sci.* 63, 1149–1164. doi:10.1007/s00018-005-5557-6
- Sasaki, J., Brown, L. S., Chon, Y.-S., Kandori, H., Maeda, A., Needleman, R., et al. (1995). Conversion of Bacteriorhodopsin into a Chloride Ion Pump. *Science* 269, 73–75. doi:10.1126/science.7604281
- Sasaki, J., Takahashi, H., Furutani, Y., Kandori, H., and Spudich, J. L. (2011). Sensory Rhodopsin-I as a Bidirectional Switch: Opposite Conformational Changes from the Same Photoisomerization. *Biophysical J.* 100, 2178–2183. doi:10.1016/j.bpj.2011.03.026
- Sasaki, K., Yamashita, T., Yoshida, K., Inoue, K., Shichida, Y., and Kandori, H. (2014). Chimeric Proton-Pumping Rhodopsins Containing the Cytoplasmic Loop of Bovine Rhodopsin. *PLoS ONE* 9, e91323. doi:10.1371/journal.pone.0091323
- Sato, K., Yamashita, T., Imamoto, Y., and Shichida, Y. (2012). Comparative Studies on the Late Bleaching Processes of Four Kinds of Cone Visual Pigments and Rod Visual Pigment. *Biochemistry* 51, 4300–4308. doi:10.1021/bi3000885
- Sato, K., Yamashita, T., Kojima, K., Sakai, K., Matsutani, Y., Yanagawa, M., et al. (2018a). Pinopsin Evolved as the Ancestral Dim-Light Visual Opsin in Vertebrates. *Commun. Biol.* 1, 156. doi:10.1038/s42003-018-0164-x
- Sato, K., Yamashita, T., Ohuchi, H., and Shichida, Y. (2011). Vertebrate Ancient-Long Opsin Has Molecular Properties Intermediate between Those of Vertebrate and Invertebrate Visual Pigments. *Biochemistry* 50, 10484–10490. doi:10.1021/bi201212z
- Sato, K., Yamashita, T., Ohuchi, H., Takeuchi, A., Gotoh, H., Ono, K., et al. (2018b). Opn5L1 Is a Retinal Receptor that Behaves as a Reverse and Self-Regenerating Photoreceptor. *Nat. Commun.* 9, 125. doi:10.1038/s41467-018-03603-3
- Schafer, C. T., and Farrens, D. L. (2015). Conformational Selection and Equilibrium Governs the Ability of Retinals to Bind Opsin. *J. Biol. Chem.* 290, 4304–4318. doi:10.1074/jbc.m114.603134
- Schäfer, G., Shastri, S., Verhoeven, M.-K., Vogel, V., Glaubitz, C., Wachtveitl, J., et al. (2009). Characterizing the Structure and Photocycle of PR 2D Crystals with CD and FTIR Spectroscopy. *Photochem. Photobiol.* 85, 529–534. doi:10.1111/j.1751-1097.2008.00491.x
- Schapiro, I., and Ruhman, S. (2014). Ultrafast Photochemistry of Anabaena Sensory Rhodopsin: Experiment and Theory. *Biochimica Biophysica Acta-Bioenergetics* 1837, 589–597. doi:10.1016/j.bbabi.2013.09.014
- Schapiro, I., Ryazantsev, M. N., Frutos, L. M., Ferré, N., Lindh, R., and Olivucci, M. (2011). The Ultrafast Photoisomerizations of Rhodopsin and Bathorhodopsin Are Modulated by Bond Length Alternation and HOOP Driven Electronic Effects. *J. Am. Chem. Soc.* 133, 3354–3364. doi:10.1021/ja1056196
- Scheerer, P., Park, J. H., Hildebrand, P. W., Kim, Y. J., Krauß, N., Choe, H.-W., et al. (2008). Crystal Structure of Opsin in its G-Protein-Interacting Conformation. *Nature* 455, 497–502. doi:10.1038/nature07330
- Schertler, G. F. X., and Hargrave, P. A. (1995). Projection Structure of Frog Rhodopsin in Two Crystal Forms. *Proc. Natl. Acad. Sci. U. S. A.* 92, 11578–11582. doi:10.1073/pnas.92.25.11578
- Schertler, G. F. X. (2005). Structure of Rhodopsin and the Metarhodopsin I Photointermediate. *Curr. Opin. Struct. Biol.* 15, 408–415. doi:10.1016/j.sbi.2005.07.010
- Schey, K. L., Papac, D. I., Knapp, D. R., and Crouch, R. K. (1992). Matrix-assisted Laser Desorption Mass Spectrometry of Rhodopsin and Bacteriorhodopsin. *Biophys. J.* 63, 1240–1243. doi:10.1016/s0006-3495(92)81699-5
- Schlinkmann, K. M., and Plückthun, A. (2013). Directed Evolution of G-Protein-Coupled Receptors for High Functional Expression and Detergent Stability. *Meth. Enzymol.* 520, 67–97. doi:10.1016/b978-0-12-391861-1.00004-6
- Schnedermann, C., Muders, V., Ehrenberg, D., Schlesinger, R., Kukura, P., and Heberle, J. (2016). Vibronic Dynamics of the Ultrafast All-*Trans* to 13-*cis*

- Photoisomerization of Retinal in Channelrhodopsin-1. *J. Am. Chem. Soc.* 138, 4757–4762. doi:10.1021/jacs.5b12251
- Schnedermann, C., Yang, X., Liebel, M., Spillane, K. M., Lugtenburg, J., Fernández, I., et al. (2018). Evidence for a Vibrational Phase-dependent Isotope Effect on the Photochemistry of Vision. *Nat. Chem.* 10, 449–455. doi:10.1038/s41557-018-0014-y
- Schober, B., Cupp-Vickery, J., Hornak, V., Smith, S. O., and Lanyi, J. K. (2002). Crystallographic Structure of the K Intermediate of Bacteriorhodopsin: Conservation of Free Energy after Photoisomerization of the Retinal. *J. Mol. Biol.* 321, 715–726. doi:10.1016/S0022-2836(02)00681-2
- Schoenlein, R. W., Peteanu, L. A., Mathies, R. A., and Shank, C. V. (1991). The First Step in Vision: Femtosecond Isomerization of Rhodopsin. *Science* 254, 412–415. doi:10.1126/science.1925597
- Scholz, L., and Neugebauer, J. (2021). Protein Response Effects on Cofactor Excitation Energies from First Principles: Augmenting Subsystem Time-dependent Density-Functional Theory with Many-Body Expansion Techniques. *J. Chem. Theory Comput.* 17, 6105–6121. doi:10.1021/acs.jctc.1c00551
- Schreiber, M., Sugihara, M., Okada, T., and Buss, V. (2006). Quantum Mechanical Studies on the Crystallographic Model of Bathorhodopsin. *Angew. Chem. Int. Ed.* 45, 4274–4277. doi:10.1002/anie.200600585
- Sekharan, S., Yokoyama, S., and Morokuma, K. (2011). Quantum Mechanical/Molecular Mechanical Structure, Enantioselectivity, and Spectroscopy of Hydroxyretinals and Insights into the Evolution of Color Vision in Small White Butterflies. *J. Phys. Chem. B* 115, 15380–15388. doi:10.1021/jp208107r
- Seki, T., Isono, K., Ozaki, K., Tsukahara, Y., Shibata-Katsuta, Y., Ito, M., et al. (1998). The Metabolic Pathway of Visual Pigment Chromophore Formation in *Drosophila melanogaster* - All-Trans (3S)-3-Hydroxyretinal Is Formed from All-Trans Retinal via (3R)-3-Hydroxyretinal in the Dark. *Eur. J. Biochem.* 257, 522–527. doi:10.1046/j.1432-1327.1998.2570522.x
- Seki, T., and Vogt, K. (1998). Evolutionary Aspects of the Diversity of Visual Pigment Chromophores in the Class Insecta. *Comp. Biochem. Physiol. B* 119, 53–64. doi:10.1016/S0305-0491(97)00322-2
- Shao, Y. H., Mei, Y., Sundholm, D., and Kaila, V. R. I. (2020). Benchmarking the Performance of Time-dependent Density Functional Theory Methods on Biochromophores. *J. Chem. Theory Comput.* 16, 587–600. doi:10.1021/acs.jctc.9b00823
- Sharma, K., Jäckel, Z., Schneider, A., Paul, O., Diester, I., and Ruther, P. (2021). Multifunctional Optrode for Opsin Delivery, Optical Stimulation, and Electrophysiological Recordings in Freely Moving Rats. *J. neural Eng.* 18, 066013. doi:10.1088/1741-2552/ac3206
- Shen, C., Jin, X., Glover, W. J., and He, X. (2021). Accurate Prediction of Absorption Spectral Shifts of Proteorhodopsin Using a Fragment-Based Quantum Mechanical Method. *Molecules* 26, 4486. doi:10.3390/molecules26154486
- Shen, W. L., Kwon, Y., Adegbola, A. A., Luo, J. J., Chess, A., and Montell, C. (2011). Function of Rhodopsin in Temperature Discrimination in *Drosophila*. *Science* 331, 1333–1336. doi:10.1126/science.1198904
- Shen, Y., Campbell, R. E., Cote, D. C., and Paquet, M. E. (2020). Challenges for Therapeutic Applications of Opsin-Based Optogenetic Tools in Humans. *Front. Neural Circuits* 14, 41. doi:10.3389/fncir.2020.00041
- Sheves, M., and Friedman, N. (1986). Influence of External Negative Charges on the Absorption Maxima of Symmetrical Cyanines. A Study with Model Compounds and Artificial Bacteriorhodopsin Pigments. *Angewandte Chemie-International Ed. Engl.* 25, 284–286. doi:10.1002/anie.198602841
- Shi, L. C., Ahmed, M. a. M., Zhang, W. R., Whited, G., Brown, L. S., and Ladizhansky, V. (2009). Three-dimensional Solid-State NMR Study of a Seven-Helical Integral Membrane Proton Pump-Structural Insights. *J. Mol. Biol.* 386, 1078–1093. doi:10.1016/j.jmb.2009.01.011
- Shibata, M., Inoue, K., Ikeda, K., Konno, M., Singh, M., Kataoka, C., et al. (2018). Oligomeric States of Microbial Rhodopsins Determined by High-Speed Atomic Force Microscopy and Circular Dichroic Spectroscopy. *Sci. Rep.* 8, 8262. doi:10.1038/s41598-018-26606-y
- Shichida, Y., Kobayashi, T., Ohtani, H., Yoshizawa, T., and Nagakura, S. (1978). Picosecond Laser Photolysis of Squid Rhodopsin at Room and Low-Temperatures. *Photochem. Photobiol.* 27, 335–341. doi:10.1111/j.1751-1097.1978.tb07609.x
- Shichida, Y. (1986). Primary Intermediates of Photobleaching of Rhodopsin. *Photobiochem. Photobiophys.* 13, 287–307.
- Shichida, Y. (1990). Ultra-fast Laser Spectroscopy of Visual Pigments. *Photochem. Photobiol.* 52, 1179–1185. doi:10.1111/j.1751-1097.1990.tb08456.x
- Shiget, A., Ito, S., Inoue, K., Okitsu, T., Wada, A., Kandori, H., et al. (2017). Solid-State Nuclear Magnetic Resonance Structural Study of the Retinal-Binding Pocket in Sodium Ion Pump Rhodopsin. *Biochemistry* 56, 543–550. doi:10.1021/acs.biochem.6b00999
- Shihoya, W., Inoue, K., Singh, M., Konno, M., Hososhima, S., Yamashita, K., et al. (2019). Crystal Structure of Heliorhodopsin. *Nature* 574, 132–136. doi:10.1038/s41586-019-1604-6
- Shim, J.-G., Kang, N.-R., Chuon, K., Cho, S.-G., Meas, S., and Jung, K.-H. (2022). Mutational Analyses Identify a Single Amino Acid Critical for Color Tuning in Proteorhodopsins. *FEBS Lett.* 596, 784. doi:10.1002/1873-3468.14297
- Shim, J.-G., Soum, V., Kang, K.-W., Chuon, K., Cho, S.-G., Kim, J.-H., et al. (2021). Discovery of a Microbial Rhodopsin that Is the Most Stable in Extreme Environments. *iScience* 24, 102620. doi:10.1016/j.isci.2021.102620
- Shimamura, T., Hiraki, K., Takahashi, N., Hori, T., Ago, H., Masuda, K., et al. (2008). Crystal Structure of Squid Rhodopsin with Intracellularly Extended Cytoplasmic Region. *J. Biol. Chem.* 283, 17753–17756. doi:10.1074/jbc.c800040200
- Shimono, K., Ikeura, Y., Sudo, Y., Iwamoto, M., and Kamo, N. (2001). Environment Around the Chromophore in *Pharaonis* Phoborhodopsin: Mutation Analysis of the Retinal Binding Site. *Biochimica Biophysica Acta-Biomembranes* 1515, 92–100. doi:10.1016/S0005-2736(01)00394-7
- Shirzad-Wasei, N., and DeGrip, W. J. (2016). Heterologous Expression of Melanopsin: Present, Problems and Prospects. *Prog. Retin. Eye Res.* 52, 1–21. doi:10.1016/j.preteyeres.2016.02.001
- Shirzad-Wasei, N., Van Oostrum, J., Bovee-Geurts, P. H. M., Kusters, L. J. A., Bosman, G. J. C. G. M., and DeGrip, W. J. (2015). Rapid Transfer of Overexpressed Integral Membrane Protein from the Host Membrane into Soluble Lipid Nanodiscs without Previous Purification. *Biol. Chem.* 396, 903–915. doi:10.1515/hsz-2015-0100
- Shirzad-Wasei, N., Van Oostrum, J., Bovee-Geurts, P. H. M., Wasserman, M., Bosman, G. J. C. G. M., and DeGrip, W. J. (2013). Large Scale Expression and Purification of Mouse Melanopsin-L in the Baculovirus Expression System. *Protein Expr. Purif.* 91, 134–146. doi:10.1016/j.pep.2013.07.010
- Shtyrov, A. A., Nikolaev, D. M., Mironov, V. N., Vasin, A. V., Panov, M. S., Tveryanovich, Y. S., et al. (2021). Simple Models to Study Spectral Properties of Microbial and Animal Rhodopsins: Evaluation of the Electrostatic Effect of Charged and Polar Residues on the First Absorption Band Maxima. *Int. J. Mol. Sci.* 22, 3029. doi:10.3390/ijms22063029
- Sineshchekov, O. A., Govorunova, E. G., Wang, J. H., and Spudich, J. L. (2012). Enhancement of Long-Wavelength Sensitivity of Optogenetic Microbial Rhodopsins by 3, 4-dehydroretinal. *Biochemistry* 51, 4499–4506. doi:10.1021/bi2018859
- Singh, M., Inoue, K., Pushkarev, A., Bèjà, O., and Kandori, H. (2018). Mutation Study of Heliorhodopsin 48C12. *Biochemistry* 57, 5041–5049. doi:10.1021/acs.biochem.8b00637
- Skopintsev, P., Ehrenberg, D., Weinert, T., James, D., Kar, R. K., Johnson, P. J. M., et al. (2020). Femtosecond-to-millisecond Structural Changes in a Light-Driven Sodium Pump. *Nature* 583, 314–322. doi:10.1038/s41586-020-2307-8
- Smith, S. O., Aschheim, K., and Groesbeck, M. (1996). Magic Angle Spinning NMR Spectroscopy of Membrane Proteins. *Quart. Rev. Biophys.* 29, 395–449. doi:10.1017/S0033583500005898
- Smith, S. O., De Groot, H. J. M., Gebhard, R., Courtin, J. M. L., Lugtenburg, J., Herzfeld, J., et al. (1989). Structure and Protein Environment of the Retinal Chromophore in Light-Adapted and Dark-Adapted Bacteriorhodopsin Studied by Solid-State NMR. *Biochemistry* 28, 8897–8904. doi:10.1021/bi00448a032
- Smith, S. O., De Groot, H. J. M., Gebhard, R., and Lugtenburg, J. (1992). Magic Angle Spinning NMR Studies on the Metarhodopsin II Intermediate of Bovine Rhodopsin: Evidence for an Unprotonated Schiff Base. *Photochem. Photobiol.* 56, 1035–1039. doi:10.1111/j.1751-1097.1992.tb09726.x
- Smith, S. O. (2021). Deconstructing the Transmembrane Core of Class A G Protein-Coupled Receptors. *Trends Biochem. Sci.* 46, 1017. doi:10.1016/j.tibs.2021.08.006

- Smith, S. O., Myers, A. B., Mathies, R. A., Pardo, J. A., Winkel, C., Vandenberg, E. M. M., et al. (1985). Vibrational Analysis of the All-Trans Retinal Protonated Schiff Base. *Biophys. J.* 47, 653–664. doi:10.1016/s0006-3495(85)83961-8
- Smith, S. O. (2010). Structure and Activation of the Visual Pigment Rhodopsin. *Annu. Rev. Biophys.* 39, 309–328. doi:10.1146/annurev-biophys-101209-104901
- Smittenko, O. A., Feldman, T. B., Petrovskaya, L. E., Nekrasova, O. V., Yakovleva, M. A., Shelaev, I. V., et al. (2021). Comparative Femtosecond Spectroscopy of Primary Photoreactions of *Exiguobacterium Sibiricum* Rhodopsin and *Halobacterium Salinarum* Bacteriorhodopsin. *J. Phys. Chem. B* 125, 995–1008. doi:10.1021/acs.jpcc.0c07763
- Smittenko, O. A., Nekrasova, O. V., Kudriavtsev, A. V., Yakovleva, M. A., Shelaev, I. V., Gostev, F. E., et al. (2017). Femtosecond and Picosecond Dynamics of Recombinant Bacteriorhodopsin Primary Reactions Compared to the Native Protein in Trimeric and Monomeric Forms. *Biochemistry-Moscow* 82, 490–500. doi:10.1134/S0006297917040113
- Sneskov, K., Olsen, J. M. H., Schwabe, T., Hättig, C., Christiansen, O., and Kongsted, J. (2013). Computational Screening of One- and Two-Photon Spectrally Tuned Channelrhodopsin Mutants. *Phys. Chem. Chem. Phys.* 15, 7567–7576. doi:10.1039/c3cp44350g
- Sonar, S., Liu, X.-M., Lee, C.-P., Coleman, M., He, Y.-W., Pelletier, S., et al. (1995). Site-directed Isotope Labeling and FT-IR Spectroscopy: The Tyr 185/Pro 186 Peptide Bond of Bacteriorhodopsin Is Perturbed during the Primary Photoreaction. *J. Am. Chem. Soc.* 117, 11614–11615. doi:10.1021/ja00151a041
- Song, Y. Z., Cartron, M. L., Jackson, P. J., Davison, P. A., Dickman, M. J., Zhu, D., et al. (2020). Proteorhodopsin Overproduction Enhances the Long-Term Viability of *Escherichia coli*. *Appl. Environ. Microbiol.* 86, 02087–02019. doi:10.1128/AEM.02087-19
- Soni, B. G., and Foster, R. G. (1997). A Novel and Ancient Vertebrate Opsin. *FEBS Lett.* 406, 279–283. doi:10.1016/s0014-5793(97)00287-1
- Spooner, P. J. R., Sharples, J. M., Goodall, S. C., Bovee-Geurts, P. H. M., Verhoeven, M. A., Lugtenburg, J., et al. (2004). The Ring of the Rhodopsin Chromophore in a Hydrophobic Activation Switch within the Binding Pocket. *J. Mol. Biol.* 343, 719–730. doi:10.1016/j.jmb.2004.08.049
- Spudich, J. L., Sineschekov, O. A., and Govorunova, E. G. (2014). Mechanism Divergence in Microbial Rhodopsins. *Biochimica Biophysica Acta-Bioenergetics* 1837, 546–552. doi:10.1016/j.bbabi.2013.06.006
- Spudich, J. L., Yang, C.-S., Jung, K.-H., and Spudich, E. N. (2000). Retinylidene Proteins: Structures and Functions from Archaea to Humans. *Annu. Rev. Cell. Dev. Biol.* 16, 365–392. doi:10.1146/annurev.cellbio.16.1.365
- Sridharan, S., Gajowa, M. A., Ogando, M. B., Jagadisan, U. K., Abdeladim, L., Sadahiro, M., et al. (2022). High-performance Microbial Opsins for Spatially and Temporally Precise Perturbations of Large Neuronal Networks. *Neuron* 110, 1–17. doi:10.1016/j.neuron.2022.01.008
- Srinivasan, S., Ramon, E., Cordoní, A., and Garriga, P. (2014). Binding Specificity of Retinal Analogs to Photoactivated Visual Pigments Suggest Mechanism for Fine-Tuning GPCR-Ligand Interactions. *Chem. Biol.* 21, 369–378. doi:10.1016/j.chembiol.2014.01.006
- Standfuss, J., Xie, G. F., Edwards, P. C., Burghammer, M., Oprian, D. D., and Schertler, G. F. X. (2007). Crystal Structure of a Thermally Stable Rhodopsin Mutant. *J. Mol. Biol.* 372, 1179–1188. doi:10.1016/j.jmb.2007.03.007
- Stavenga, D. G., Oberwinkler, J., and Postma, M. (2000). “Modeling Primary Visual Processes in Insect Photoreceptors,” in *Molecular Mechanisms in Visual Transduction*. Editors D. G. Stavenga, W. J. DeGrip, and E. N. Pugh Jr. (Amsterdam, Netherlands: Elsevier Science Pub.), 527–574. doi:10.1016/s1383-8121(00)80013-5
- Steinhoff, H.-J., Mollaaghababa, R., Altenbach, C. A., Khorana, H. G., and Hubbell, W. L. (1995). Site Directed Spin Labeling Studies of Structure and Dynamics in Bacteriorhodopsin. *Biophys. Chem.* 56, 89–94. doi:10.1016/0301-4622(95)00019-t
- Stenkamp, R. E. (2008). Alternative Models for Two Crystal Structures of Bovine Rhodopsin. *Acta Crystallogr. D-biol. Cryst.* 64, 902–904. doi:10.1107/s0907444908017162
- Steward, L. E., and Chamberlin, A. R. (1998). Protein Engineering with Nonstandard Amino Acids. *Meth. Mol. Biol.* 77, 325–354. doi:10.1385/0-89603-397-X:325
- Struts, A. V., Salgado, G. F. J., Tanaka, K., Krane, S., Nakanishi, K., and Brown, M. F. (2007). Structural Analysis and Dynamics of Retinal Chromophore in Dark and Metal States of Rhodopsin from ^2H NMR of Aligned Membranes. *J. Mol. Biol.* 372, 50–66. doi:10.1016/j.jmb.2007.03.046
- Stubbs, G. W., Smith, H. G., and Litman, B. J. (1976). Alkyl Glucosides as Effective Solubilizing Agents for Bovine Rhodopsin - A Comparison with Several Commonly Used Detergents. *Biochim. Biophys. Acta* 426, 46–56. doi:10.1016/0005-2736(76)90428-4
- Su, C.-Y., Luo, D.-G., Terakita, A., Shichida, Y., Liao, H.-W., Kazmi, M. A., et al. (2006). Parietal-eye Phototransduction Components and Their Potential Evolutionary Implications. *Science* 311, 1617–1621. doi:10.1126/science.1123802
- Subramaniam, S., Lindahl, I., Bullough, P. A., Faruqi, A. R., Tittor, J., Oesterheld, D., et al. (1999). Protein Conformational Changes in the Bacteriorhodopsin Photocycle. *J. Mol. Biol.* 287, 145–161. doi:10.1006/jmbi.1999.2589
- Sudo, Y., Ihara, K., Kobayashi, S., Suzuki, D., Irieda, H., Kikukawa, T., et al. (2011). A Microbial Rhodopsin with a Unique Retinal Composition Shows Both Sensory Rhodopsin II and Bacteriorhodopsin-like Properties. *J. Biol. Chem.* 286, 5967–5976. doi:10.1074/jbc.m110.190058
- Sudo, Y., Okazaki, A., Ono, H., Yagasaki, J., Sugo, S., Kamiya, M., et al. (2013). A Blue-Shifted Light-Driven Proton Pump for Neural Silencing. *J. Biol. Chem.* 288, 20624–20632. doi:10.1074/jbc.m113.475533
- Sugimoto, T., Katayama, K., and Kandori, H. (2021). Role of Thr82 for the Unique Photochemistry of TAT Rhodopsin. *Biophysics Physicobiology* 18, 108–115. doi:10.2142/biophysico.bppb-v18.012
- Sun, H., Gilbert, D. J., Copeland, N. G., Jenkins, N. A., and Nathans, J. (1997). Peropsin, a Novel Visual Pigment-like Protein Located in the Apical Microvilli of the Retinal Pigment Epithelium. *Proc. Nat. Acad. Sci. U. S. A.* 94, 9893–9898. doi:10.1073/pnas.94.18.9893
- Sun, Y., Li, M. J., Cao, S., Xu, Y., Wu, P., Xu, S., et al. (2022). Optogenetics for Understanding and Treating Brain Injury: Advances in the Field and Future Prospects. *Int. J. Mol. Sci.* 23, 1800. doi:10.3390/ijms23031800
- Suzuki, E., Katayama, E., and Hirosawa, K. (1993). Structure of Photoreceptive Membranes of *Drosophila* Compound Eyes as Studied by Quick-Freezing Electron Microscopy. *J. Electron Microscop.* 42, 178–184.
- Suzuki, K., Del Carmen Marín, M., Konno, M., Bagherzadeh, R., Murata, T., and Inoue, K. (2022). Structural Characterization of Proton-Pumping Rhodopsin Lacking a Cytoplasmic Proton Donor Residue by X-Ray Crystallography. *J. Biol. Chem.* 298, 101722. doi:10.1016/j.jbc.2022.101722
- Tahara, S., Kuramochi, H., Takeuchi, S., and Tahara, T. (2019a). Protein Dynamics Preceding Photoisomerization of the Retinal Chromophore in Bacteriorhodopsin Revealed by Deep-UV Femtosecond Stimulated Raman Spectroscopy. *J. Phys. Chem. Lett.* 10, 5422–5427. doi:10.1021/acs.jpcclett.9b02283
- Tahara, S., Singh, M., Kuramochi, H., Shihoya, W., Inoue, K., Nureki, O., et al. (2019b). Ultrafast Dynamics of Heliorhodopsins. *J. Phys. Chem. B* 123, 2507–2512. doi:10.1021/acs.jpcc.9b00887
- Tahara, S., Takeuchi, S., Abe-Yoshizumi, R., Inoue, K., Ohtani, H., Kandori, H., et al. (2015). Ultrafast Photoreaction Dynamics of a Light-Driven Sodium-Ion-Pumping Retinal Protein from *Krokinobacter Eikastus* Revealed by Femtosecond Time-Resolved Absorption Spectroscopy. *J. Phys. Chem. Lett.* 6, 4481–4486. doi:10.1021/acs.jpcclett.5b01994
- Takayama, R., Kaneko, A., Okitsu, T., Tsunoda, S. P., Shimono, K., Mizuno, M., et al. (2018). Production of a Light-Gated Proton Channel by Replacing the Retinal Chromophore with its Synthetic Vinylene Derivative. *J. Phys. Chem. Lett.* 9, 2857–2862. doi:10.1021/acs.jpcclett.8b00879
- Tam, B. M., and Moritz, O. L. (2009). The Role of Rhodopsin Glycosylation in Protein Folding, Trafficking, and Light-Sensitive Retinal Degeneration. *J. Neurosci.* 29, 15145–15154. doi:10.1523/jneurosci.4259-09.2009
- Tan, P., He, L., Huang, Y., and Zhou, Y. (2022). Optophysiology: Illuminating Cell Physiology with Optogenetics. *Physiol. Rev.* 102, 1263. doi:10.1152/physrev.00021.2021
- Tanaka, T., Singh, M., Shihoya, W., Yamashita, K., Kandori, H., and Nureki, O. (2020). Structural Basis for Unique Color Tuning Mechanism in Heliorhodopsin. *Biochem. Biophysical Res. Commun.* 533, 262–267. doi:10.1016/j.bbrc.2020.06.124
- Tansley, K. (1931). The Regeneration of Visual Purple: its Relation to Dark Adaptation and Night Blindness. *J. Physiology* 71, 442–458. doi:10.1113/jphysiol.1931.sp002749

- Tarttelin, E. E., Bellingham, J., Hankins, M. W., Foster, R. G., and Lucas, R. J. (2003). Neuropsin (Opn5): A Novel Opsin Identified in Mammalian Neural Tissue. *FEBS Lett.* 554, 410–416. doi:10.1016/s0014-5793(03)01212-2
- Tarttelin, E. E., Fransen, M. P., Edwards, P. C., Hankins, M. W., Schertler, G. F. X., Vogel, R., et al. (2011). Adaptation of Pineal Expressed Teleost Exo-Rod Opsin to Non-image Forming Photoreception through Enhanced Meta II Decay. *Cell. Mol. Life Sci.* 68, 3713–3723. doi:10.1007/s00018-011-0665-y
- Tastan, O., Dutta, A., Booth, P. J., and Klein-Seetharaman, J. (2014). Retinal Proteins as Model Systems for Membrane Protein Folding. *Biochimica Biophysica Acta-Bioenergetics* 1837, 656–663. doi:10.1016/j.bbabi.2013.11.021
- Tavanti, F., and Tozzini, V. (2014). A Multi-Scale-Multi-Stable Model for the Rhodopsin Photocycle. *Molecules* 19, 14961–14978. doi:10.3390/molecules190914961
- Terakita, A., Koyanagi, M., Tsukamoto, H., Yamashita, T., Miyata, T., and Shichida, Y. (2004). Counterion Displacement in the Molecular Evolution of the Rhodopsin Family. *Nat. Struct. Mol. Biol.* 11, 284–289. doi:10.1038/nsmb731
- Terakita, A. (2005). The Opsins. *Genome Biol.* 6, 213. doi:10.1186/gb-2005-6-3-213
- Tian, Y., Yang, S., Nagel, G., and Gao, S. Q. (2022). Characterization and Modification of Light-Sensitive Phosphodiesterases from Choanoflagellates. *Biomolecules* 12, 88. doi:10.3390/biom12010088
- Tkatch, T., Greotti, E., Baranuskas, G., Pendin, D., Roy, S., Nita, L. I., et al. (2017). Optogenetic Control of Mitochondrial Metabolism and Ca²⁺ Signaling by Mitochondria-Targeted Opsins. *Proc. Natl. Acad. Sci. U. S. A.* 114, E5167–E5176. doi:10.1073/pnas.1703623114
- Toba, Y., and Hanawa, I. (1985). Photoreceptor Sensitivity as a Function of Rhodopsin Content in the Isolated Bullfrog Retina. *Jpn. J. Physiology* 35, 483–494. doi:10.2170/jjphysiol.35.483
- Tomida, S., Ito, S., Mato, T., Furutani, Y., Inoue, K., and Kandori, H. (2020). Infrared Spectroscopic Analysis on Structural Changes Around the Protonated Schiff Base upon Retinal Isomerization in Light-Driven Sodium Pump KR2. *Biochimica Biophysica Acta-Bioenergetics* 1861, 148190. doi:10.1016/j.bbabi.2020.148190
- Tomida, S., Kitagawa, S., Kandori, H., and Furutani, Y. (2021). Inverse Hydrogen-Bonding Change between the Protonated Retinal Schiff Base and Water Molecules upon Photoisomerization in Heliorhodopsin 48C12. *J. Phys. Chem. B* 125, 8331–8341. doi:10.1021/acs.jpcc.1c01907
- Tomobe, K., Yamamoto, E., Kholmurodov, K., and Yasuoka, K. (2017). Water Permeation through the Internal Water Pathway in Activated GPCR Rhodopsin. *PLoS ONE* 12, e0176876. doi:10.1371/journal.pone.0176876
- Townson, S. M., Chang, B. S. W., Salcedo, E., Chadwell, L. V., Pierce, N. E., and Britt, S. G. (1998). Honeybee Blue-And Ultraviolet-Sensitive Opsins: Cloning, Heterologous Expression in *Drosophila*, and Physiological Characterization. *J. Neurosci.* 18, 2412–2422. doi:10.1523/jneurosci.18-07-02412.1998
- Tribet, C., Audebert, R., and Popot, J.-L. (1996). Amphipols: Polymers that Keep Membrane Proteins Soluble in Aqueous Solutions. *Proc. Natl. Acad. Sci. U. S. A.* 93, 15047–15050. doi:10.1073/pnas.93.26.15047
- Tsai, C.-J., Marino, J., Adaixo, R., Pamulal, F., Muehle, J., Maeda, S., et al. (2019). Cryo-EM Structure of the Rhodopsin-Gai-Bγ Complex Reveals Binding of the Rhodopsin C-Terminal Tail to the Gβ Subunit. *Elife* 8, 46041. doi:10.7554/elifelife.46041
- Tsujimura, M., and Ishikita, H. (2020). Insights into the Protein Functions and Absorption Wavelengths of Microbial Rhodopsins. *J. Phys. Chem. B* 124, 11819–11826. doi:10.1021/acs.jpcc.0c08910
- Tsujimura, M., Noji, T., Saito, K., Kojima, K., Sudo, Y., and Ishikita, H. (2021). Mechanism of Absorption Wavelength Shifts in Anion Channelrhodopsin-1 Mutants. *Biochimica Biophysica Acta-Bioenergetics* 1862, 148349. doi:10.1016/j.bbabi.2020.148349
- Tsukamoto, H., Szundi, I., Lewis, J. W., Farrens, D. L., and Kliger, D. S. (2011). Rhodopsin in Nanodiscs Has Native Membrane-like Photointermediates. *Biochemistry* 50, 5086–5091. doi:10.1021/bi200391a
- Tsukamoto, H., and Terakita, A. (2010). Diversity and Functional Properties of Bistable Pigments. *Photochem. Photobiological Sci.* 9, 1435–1443. doi:10.1039/c0pp00168f
- Tsukamoto, T., Mizutani, K., Hasegawa, T., Takahashi, M., Honda, N., Hashimoto, N., et al. (2016). X-ray Crystallographic Structure of Thermophilic Rhodopsin-1. *Implications For High Thermal Stability and Optogenetic Function.* *J. Biol. Chem.* 291, 12223–12232. doi:10.1074/jbc.m116.719815
- Tsunoda, S. P., Ewers, D., Gazzarrini, S., Moroni, A., Gradmann, D., and Hegemann, P. (2006). H⁺-pumping Rhodopsin from the Marine Alga *Acetabularia*. *Biophysical J.* 91, 1471–1479. doi:10.1529/biophysj.106.086421
- Tsunoda, S. P., Sugiura, M., and Kandori, H. (2021). “Molecular Properties and Optogenetic Applications of Enzymerhodopsins,” in *Optogenetics: Light-Sensing Proteins and Their Applications in Neuroscience and beyond*. Editors H. Yawo, H. Kandori, A. Koizumi, and R. Kageyama. 2nd ed (Singapore: Springer), 153–165. doi:10.1007/978-981-15-8763-4_9
- Tu, C.-H., Yi, H.-P., Hsieh, S.-Y., Lin, H.-S., and Yang, C.-S. (2018). Overexpression of Different Types of Microbial Rhodopsins with a Highly Expressible Bacteriorhodopsin from *Haloarcula Marismortui* as a Single Protein in *E. coli*. *Sci. Rep.* 8, 14026. doi:10.1038/s41598-018-32399-x
- Tutul, J. N., Lee, J., Chi, H. C., Faizuddin, F. N., Abeyrathna, S. S., Zhou, Q., et al. (2021). A Single Point Mutation Converts a Proton-Pumping Rhodopsin into a Red-Shifted, Turn-On Fluorescent Sensor for Chloride. *Chem. Sci.* 12, 5655–5663. doi:10.1039/d0sc06061e
- Ueta, T., Kojima, K., Hino, T., Shibata, M., Nagano, S., and Sudo, Y. (2020). Applicability of Styrene-Maleic Acid Copolymer for Two Microbial Rhodopsins, RxR and HsSRI. *Biophysical J.* 119, 1760–1770. doi:10.1016/j.bpj.2020.09.026
- Urner, L. H., Liko, I., Yen, H.-Y., Hoi, K.-K., Bolla, J. R., Gault, J., et al. (2020). Modular Detergents Tailor the Purification and Structural Analysis of Membrane Proteins Including G Protein-Coupled Receptors. *Nat. Commun.* 11, 564. doi:10.1038/s41467-020-14424-8
- Valdez-Lopez, J. C., Petr, S. T., Donohue, M. P., Bailey, R. J., Gebreeziabher, M., Cameron, E. G., et al. (2020). The C-Terminus and Third Cytoplasmic Loop Cooperatively Activate Mouse Melanopsin Phototransduction. *Biophysical J.* 119, 389–401. doi:10.1016/j.bpj.2020.06.013
- Van Der Steen, R., Biesheuvel, P. L., Mathies, R. A., and Lugtenburg, J. (1986). Retinal Analogs with Locked 6-7 Conformations Show that Bacteriorhodopsin Requires the 6-S-*Trans* Conformation of the Chromophore. *J. Am. Chem. Soc.* 108, 6410–6411. doi:10.1021/ja00280a060
- Van Eps, N., Caro, L. N., Morizumi, T., Kusnetzov, A. K., Szczepke, M., Hofmann, K. P., et al. (2017). Conformational Equilibria of Light-Activated Rhodopsin in Nanodiscs. *Proc. Natl. Acad. Sci. U. S. A.* 114, E3268–E3275. doi:10.1073/pnas.1620405114
- VanAken, T., Foxall-Vanaken, S., Castleman, S., and Ferguson-Miller, S. (1986). Alkyl Glycoside Detergents: Synthesis and Applications to the Study of Membrane Proteins. *Methods Enzym.* 125, 27–35. doi:10.1016/s0076-6879(86)25005-3
- Varma, N., Mutt, E., Mühle, J., Panneels, V., Terakita, A., Deupi, X., et al. (2019). Crystal Structure of Jumping Spider Rhodopsin-1 as a Light Sensitive GPCR. *Proc. Natl. Acad. Sci. U. S. A.* 116, 14547–14556. doi:10.1073/pnas.1902192116
- Verdegem, P. J. E., Bovee-Geurts, P. H. M., DeGrip, W. J., Lugtenburg, J., and De Groot, H. J. M. (1999). Retinylidene Ligand Structure in Bovine Rhodopsin, Metarhodopsin I, and 10-methylrhodopsin from Internuclear Distance Measurements Using ¹³C-Labeling and 1-D Rotational Resonance MAS NMR. *Biochemistry-USA* 38, 11316–11324. doi:10.1021/bi983014e
- Verhoeven, M.-K., Schäfer, G., Shastri, S., Weber, I., Glaubitz, C., Mäntele, W., et al. (2011). Low Temperature FTIR Spectroscopy Provides New Insights in the pH-dependent Proton Pathway of Proteorhodopsin. *Biochimica Biophysica Acta-Bioenergetics* 1807, 1583–1590. doi:10.1016/j.bbabi.2011.09.001
- Verhoeven, M. A., Bovee-Geurts, P. H. M., De Groot, H. J. M., Lugtenburg, J., and DeGrip, W. J. (2006). Methyl Substituents at the 11- or 12-position of Retinal Profoundly and Differentially Affect Photochemistry and Signalling Activity of Rhodopsin. *J. Mol. Biol.* 363, 98–113. doi:10.1016/j.jmb.2006.07.039
- Verhoeven, M. A., Creemers, A. F. L., Bovee-Geurts, P. H. M., DeGrip, W. J., Lugtenburg, J., and De Groot, H. J. M. (2001). Ultra-high-field MAS NMR Assay of a Multispin Labeled Ligand Bound to its G-Protein Receptor Target in the Natural Membrane Environment: Electronic Structure of the Retinylidene Chromophore in Rhodopsin. *Biochemistry* 40, 3282–3288. doi:10.1021/bi0023798
- Vierock, J., Rodriguez-Rozada, S., Dieter, A., Pieper, F., Sims, R., Tenedini, F., et al. (2021). BiPOLES Is an Optogenetic Tool Developed for Bidirectional Dual-Color Control of Neurons. *Nat. Commun.* 12, 4527. doi:10.1038/s41467-021-24759-5

- Villette, V., Chavarha, M., Dimov, I. K., Bradley, J., Pradhan, L., Mathieu, B., et al. (2019). Ultrafast Two-Photon Imaging of a High-Gain Voltage Indicator in Awake Behaving Mice. *Cell* 179, 1590–1609. doi:10.1016/j.cell.2019.11.004
- Visser, P. M. a. M., Bovee-Geurts, P. H. M., Portier, M. D., Klaassen, C. H. W., and DeGrip, W. J. (1998). Large-scale Production and Purification of the Human Green Cone Pigment: Characterization of Late Photo-Intermediates. *Biochem. J.* 330, 1201–1208. doi:10.1042/bj3301201
- Visser, P. M. a. M., and DeGrip, W. J. (1996). Functional Expression of Human Cone Pigments Using Recombinant Baculovirus: Compatibility with Histidine Tagging and Evidence for N-Glycosylation. *FEBS Lett.* 396, 26–30. doi:10.1016/0014-5793(96)01064-2
- Vlasov, A. V., Maliar, N. L., Bazhenov, S. V., Nikelshparg, E. I., Brazhe, N. A., Vlasova, A. D., et al. (2020). Raman Scattering: From Structural Biology to Medical Applications. *Crystals* 10, 38–3149. doi:10.3390/cryst10010038
- Vöcking, O., Leclère, L., and Hausen, H. (2021). The Rhodopsin-Retinochrome System for Retinal Re-isomerization Predates the Origin of Cephalopod Eyes. *BMC Ecol. Evol.* 21, 1–141. doi:10.1186/s12862-021-01939-x
- Vogel, R., Mahalingam, M., Lüdeke, S., Huber, T., Siebert, F., and Sakmar, T. P. (2008). Functional Role of the "Ionic Lock" - an Interhelical Hydrogen-Bond Network in Family A Heptahelical Receptors. *J. Mol. Biol.* 380, 648–655. doi:10.1016/j.jmb.2008.05.022
- Vogel, R., Sakmar, T. P., Sheves, M., and Siebert, F. (2007). Coupling of Protonation Switches during Rhodopsin Activation. *Photochem. Photobiol.* 83, 286–292. doi:10.1562/2006-06-19-ir-937
- Vogele, L., Sineschekov, O. A., Trivedi, V. D., Sasaki, J., Spudich, J. L., and Luecke, H. (2004). *Anabaena* Sensory Rhodopsin: A Photochromic Color Sensor at 2.0 Å. *Science* 306, 1390–1393. doi:10.1126/science.1103943
- Vogt, A., Guo, Y., Tsunoda, S. P., Kateriya, S., Elstner, M., and Hegemann, P. (2015). Conversion of a Light-Driven Proton Pump into a Light-Gated Ion Channel. *Sci. Rep.* 5, 16450. doi:10.1038/srep16450
- Vogt, A., Silapetere, A., Grimm, C., Heiser, F., Möller, M. A., and Hegemann, P. (2019). Engineered Passive Potassium Conductance in the KR2 Sodium Pump. *Biophysical J.* 116, 1941–1951. doi:10.1016/j.bpj.2019.04.001
- Vogt, K., and Kirschfeld, K. (1984). Chemical Identity of the Chromophores of Fly Visual Pigment. *Naturwissenschaften* 71, 211–213. doi:10.1007/bf00490436
- Volkov, O., Kovalev, K., Polovinkin, V., Borshchevskiy, V., Bamann, C., Astashkin, R., et al. (2017). Structural Insights into Ion Conduction by Channelrhodopsin 2. *Science* 358, eaan8862. doi:10.1126/science.aan8862
- Vought, B. W., Dukkupati, A., Max, M., Knox, B. E., and Birge, R. R. (1999). Photochemistry of the Primary Event in Short-Wavelength Visual Opsins at Low Temperature. *Biochemistry-USA* 38, 11287–11297. doi:10.1021/bi990968b
- Vought, B. W., Salcedo, E., Chadwell, L. V., Britt, S. G., Birge, R. R., and Knox, B. E. (2000). Characterization of the Primary Photointermediates of *Drosophila* Rhodopsin. *Biochemistry* 39, 14128–14137. doi:10.1021/bi001135k
- Wada, A., Fujioka, N., Tanaka, Y., and Ito, M. (2000). A Highly Stereoselective Synthesis of 11Z-Retinal Using Tricarbonyliron Complex. *J. Org. Chem.* 65, 2438–2443. doi:10.1021/jo9916030
- Wada, T., Shimono, K., Kikukawa, T., Hato, M., Shinya, N., Kim, S.-Y., et al. (2011). Crystal Structure of the Eukaryotic Light-Driven Proton-Pumping Rhodopsin, *Acetabularia* Rhodopsin II, from Marine Alga. *J. Mol. Biol.* 411, 986–998. doi:10.1016/j.jmb.2011.06.028
- Wagner, M. J., Savall, J., Hernandez, O., Mel, G., Inan, H., Romyantsev, O., et al. (2021). A Neural Circuit State Change Underlying Skilled Movements. *Cell* 184, 3731–3747. doi:10.1016/j.cell.2021.06.001
- Wagner, N. L., Greco, J. A., Ranaghan, M. J., and Birge, R. R. (2013). Directed Evolution of Bacteriorhodopsin for Applications in Bioelectronics. *J. R. Soc. Interface* 10, 201301971. doi:10.1098/rsif.2013.0197
- Wald, G., and Brown, P. K. (1953). The Molar Extinction of Rhodopsin. *J. General Physiology* 37, 189–200. doi:10.1085/jgp.37.2.189
- Wald, G. (1935). Carotenoids and the Visual Cycle. *J. General Physiology* 19, 351–371. doi:10.1085/jgp.19.2.351
- Wald, G. (1953). The Biochemistry of Vision. *Annu. Rev. Biochem.* 22, 497–526. doi:10.1146/annurev.bi.22.070153.002433
- Wald, G. (1968). The Molecular Basis of Visual Excitation. *Nature* 219, 800–807. doi:10.1038/219800a0
- Walter, J. M., Greenfield, D., and Liphardt, J. (2010). Potential of Light-Harvesting Proton Pumps for Bioenergy Applications. *Curr. Opin. Biotechnol.* 21, 265–270. doi:10.1016/j.copbio.2010.03.007
- Wand, A., Gdor, I., Zhu, J. Y., Sheves, M., and Ruhman, S. (2013). Shedding New Light on Retinal Protein Photochemistry. *Annu. Rev. Phys. Chem.* 64, 437–458. doi:10.1146/annurev-physchem-040412-110148
- Wang, N., Wang, M. T., Gao, Y. Y., Ran, T. T., Lan, Y. L., Wang, J., et al. (2012). Crystallization and Preliminary X-Ray Crystallographic Analysis of a Blue-Light-Absorbing Proteorhodopsin. *Acta Crystallogr. Sect. F-Structural Biol. Cryst. Commun.* 68, 281–283. doi:10.1107/s1744309111043612
- Wang, S. L., Munro, R. A., Shi, L. C., Kawamura, I., Okitsu, T., Wada, A., et al. (2013). Solid-state NMR Spectroscopy Structure Determination of a Lipid-Embedded Heptahelical Membrane Protein. *Nat. Methods* 10, 1007–1012. doi:10.1038/nmeth.2635
- Wang, Y.-J., Bovee-Geurts, P. H. M., Lugtenburg, J., and DeGrip, W. J. (2004). Constraints of the 9-methyl Group Binding Pocket of the Rhodopsin Chromophore Probed by 9-halogeno Substitution. *Biochemistry* 43, 14802–14810. doi:10.1021/bi048404h
- Wang, Y., Li, Y., Xu, T., Shi, Z. Y., and Wu, Q. (2015). Experimental Evidence for Growth Advantage and Metabolic Shift Stimulated by Photophosphorylation of Proteorhodopsin Expressed in *Escherichia coli* at Anaerobic Condition. *Biotechnol. Bioeng.* 112, 947–956. doi:10.1002/bit.25504
- Wang, Z. Y., Asenjo, A. B., and Oprian, D. D. (1993). Identification of the Cl⁻ Binding Site in the Human Red and Green Color Vision Pigments. *Biochemistry-USA* 32, 2125–2130. doi:10.1021/bi00060a001
- Warrant, E. J., and Locket, N. A. (2004). Vision in the Deep Sea. *Biol. Rev.* 79, 671–712. doi:10.1017/s1464793103006420
- Warrant, E. J., and Mcintyre, P. D. (1993). Arthropod Eye Design and the Physical Limits to Spatial Resolving Power. *Prog. Neurobiol.* 40, 413–461. doi:10.1016/0301-0082(93)90017-m
- Watari, M., Ikuta, T., Yamada, D., Shihoya, W., Yoshida, K., Tsunoda, S. P., et al. (2019). Spectroscopic Study of the Transmembrane Domain of a Rhodopsin-Phosphodiesterase Fusion Protein from a Unicellular Eukaryote. *J. Biol. Chem.* 294, 3432–3443. doi:10.1074/jbc.ra118.006277
- Weinert, T., Skopintsev, P., James, D., Dworkowski, F., Panepucci, E., Kekilli, D., et al. (2019). Proton Uptake Mechanism in Bacteriorhodopsin Captured by Serial Synchrotron Crystallography. *Science* 365, 61–65. doi:10.1126/science.aaw8634
- Weingart, O. (2007). The Twisted C11=C12 Bond of the Rhodopsin Chromophore - A Photochemical Hot Spot. *J. Am. Chem. Soc.* 129, 10618–10619. doi:10.1021/ja071793t
- Weissbecker, J., Boumrifak, C., Breyer, M., Wiessalla, T., Shevchenko, V., Mager, T., et al. (2021). The Voltage Dependent Sidedness of the Reprotonation of the Retinal Schiff Base Determines the Unique Inward Pumping of Xenorhodopsin. *Angew. Chemie-International Ed.* 60, 23010–23017. doi:10.1002/anie.202103882
- Werner, K., Lehner, I., Dhiman, H. K., Richter, C., Glaubitz, C., Schwalbe, H., et al. (2007). Combined Solid State and Solution NMR Studies of a ϵ -¹⁵N Labeled Bovine Rhodopsin. *J. Biomol. NMR* 37, 303–312. doi:10.1007/s10858-007-9143-0
- Wickstrand, C., Dods, R., Royant, A., and Neutze, R. (2015). Bacteriorhodopsin: Would the Real Structural Intermediates Please Stand up? *Biochimica Biophysica Acta-General Subj.* 1850, 536–553. doi:10.1016/j.bbagen.2014.05.021
- Wietek, J., Beltramo, R., Scanziani, M., Hegemann, P., Oertner, T. G., and Wiegert, J. S. (2015). An Improved Chloride-Conducting Channelrhodopsin for Light-Induced Inhibition of Neuronal Activity *In Vivo*. *Sci. Rep.* 5, 14807. doi:10.1038/srep14807
- Wijffels, R. H., Kruse, O., and Hellingwerf, K. J. (2013). Potential of Industrial Biotechnology with Cyanobacteria and Eukaryotic Microalgae. *Curr. Opin. Biotechnol.* 24, 405–413. doi:10.1016/j.copbio.2013.04.004
- Williams, R. H., Tsunematsu, T., Thomas, A. M., Bogoy, K., Yamanaka, A., and Kilduff, T. S. (2019). Transgenic Archaerhodopsin-3 Expression in Hypocretin/Orexin Neurons Engenders Cellular Dysfunction and Features of Type 2 Narcolepsy. *J. Neurosci.* 39, 9435–9452. doi:10.1523/jneurosci.0311-19.2019
- Wright, P., Rodgers, J., Wynne, J., Bishop, P. N., Lucas, R. J., and Milosavljevic, N. (2021). Viral Transduction of Human Rod Opsin or Channelrhodopsin Variants to Mouse on Bipolar Cells Does Not Impact Retinal Anatomy or Cause Measurable Death in the Targeted Cells. *Int. J. Mol. Sci.* 22, 13111. doi:10.3390/ijms222313111

- Wright, W., Gajjeraman, S., Batabyal, S., Pradhan, S., Bhattacharya, S., Mahapatra, V., et al. (2017). Restoring Vision in Mice with Retinal Degeneration Using Multicharacteristic Opsin. *Neurophotonics* 4, 041505. doi:10.1117/1.nph.4.4.049801
- Xu, C., Wang, R. X., Yang, Y. F., Xu, T. Y., Li, Y., Xu, J., et al. (2020). Expression of OPN3 in Lung Adenocarcinoma Promotes Epithelial-Mesenchymal Transition and Tumor Metastasis. *Thorac. Cancer* 11, 286–294. doi:10.1111/1759-7714.13254
- Xu, Y. X., Peng, L. X., Wang, S. C., Wang, A. Q., Ma, R. R., Zhou, Y., et al. (2018). Hybrid Indicators for Fast and Sensitive Voltage Imaging. *Angew. Chemie-International Ed.* 57, 3949–3953. doi:10.1002/anie.201712614
- Yaguchi, M., Jia, X., Schlesinger, R., Jiang, X., Ataka, K., and Heberle, J. (2022). Near-Infrared Activation of Sensory Rhodopsin II Mediated by NIR-To-Blue Upconversion Nanoparticles. *Front. Mol. Biosci.* 8, 782688. doi:10.3389/fmolb.2021.782688
- Yalouz, S., Senjean, B., Günther, J., Buda, F., O'Brien, T. E., and Visscher, L. (2021). A State-Averaged Orbital-Optimized Hybrid Quantum-Classical Algorithm for a Democritic Description of Ground and Excited States. *Quantum Sci. Technol.* 6, 024004. doi:10.1088/2058-9565/abd334
- Yamashita, T., Ohuchi, H., Tomonari, S., Ikeda, K., Sakai, K., and Shichida, Y. (2010). Opn5 Is a UV-Sensitive Bistable Pigment that Couples with Gi Subtype of G Protein. *Proc. Natl. Acad. Sci. U. S. A.* 107, 22084–22089. doi:10.1073/pnas.1012498107
- Yamashita, T., Ono, K., Ohuchi, H., Yumoto, A., Gotoh, H., Tomonari, S., et al. (2014). Evolution of Mammalian Opn5 as a Specialized UV-Absorbing Pigment by a Single Amino Acid Mutation. *J. Biol. Chem.* 289, 3991–4000. doi:10.1074/jbc.m113.514075
- Yamashita, T. (2020). Unexpected Molecular Diversity of Vertebrate Nonvisual Opsin Opn5. *Biophys. Rev.* 12, 333–338. doi:10.1007/s12551-020-00654-z
- Yan, E. C. Y., Ganim, Z., Kazmi, M. A., Chang, B. S. W., Sakmar, T. P., and Mathies, R. A. (2004). Resonance Raman Analysis of the Mechanism of Energy Storage and Chromophore Distortion in the Primary Visual Photoproduct. *Biochemistry* 43, 10867–10876. doi:10.1021/bi0400148
- Yang, X., Manathunga, M., Gozem, S., Léonard, J., Andruniów, T., and Olivucci, M. (2022). Quantum-classical Simulations of Rhodopsin Reveal Excited-State Population Splitting and its Effects on Quantum Efficiency. *Nat. Chem.* 14, 441. doi:10.1038/s41557-022-00892-6
- Yasuda, S. I., Akiyama, T., Kojima, K., Ueta, T., Hayashi, T., Ogasawara, S., et al. (2022). Development of an Outward Proton Pumping Rhodopsin with a New Record in Thermostability by Means of Amino Acid Mutations. *J. Phys. Chem. B* 126, 1004. doi:10.1021/acs.jpcc.1c08684
- Ye, S. X., Huber, T., Vogel, R., and Sakmar, T. P. (2009). FTIR Analysis of GPCR Activation Using Azido Probes. *Nat. Chem. Biol.* 5, 397–399. doi:10.1038/nchembio.167
- Ye, S. X., Zaitseva, E., Caltabiano, G., Schertler, G. F. X., Sakmar, T. P., Deupi, X., et al. (2010). Tracking G-Protein-Coupled Receptor Activation Using Genetically Encoded Infrared Probes. *Nature* 464, 1386–1U14. doi:10.1038/nature08948
- Yee, D. C., Shlykov, M. A., Västermark, A., Reddy, V. S., Arora, S., Sun, E. I., et al. (2013). The Transporter-Opsin-G Protein-Coupled Receptor (TOG) Superfamily. *FEBS J.* 280, 5780–5800. doi:10.1111/febs.12499
- Yeh, V., Lee, T.-Y., Chen, C.-W., Kuo, P.-C., Shiue, J., Chu, L.-K., et al. (2018). Highly Efficient Transfer of 7TM Membrane Protein from Native Membrane to Covalently Circularized Nanodisc. *Sci. Rep.* 8, 13501. doi:10.1038/s41598-018-31925-1
- Yi, A., Li, H., Mamaeva, N., De Cordoba, R. E. F., Lugtenburg, J., DeGrip, W. J., et al. (2017). Structural Changes in an Anion Channelrhodopsin: Formation of the K and L Intermediates at 80 K. *Biochemistry* 56, 2197–2208. doi:10.1021/acs.biochem.7b00002
- Yi, A., Mamaeva, N., Li, H., Spudich, J. L., and Rothschild, K. J. (2016). Resonance Raman Study of an Anion Channelrhodopsin: Effects of Mutations Near the Retinylidene Schiff Base. *Biochemistry* 55, 2371–2380. doi:10.1021/acs.biochem.6b00104
- Yizhar, O., Fenno, L. E., Davidson, T. J., Mogri, M., and Deisseroth, K. (2011). Optogenetics in Neural Systems. *Neuron* 71, 9–34. doi:10.1016/j.neuron.2011.06.004
- Yokoyama, S. (1995). Amino Acid Replacements and Wavelength Absorption of Visual Pigments in Vertebrates. *Mol. Biol. Evol.* 12, 53–61. doi:10.1093/oxfordjournals.molbev.a040190
- Yokoyama, S. (2000). Molecular Evolution of Vertebrate Visual Pigments. *Prog. Retin. Eye Res.* 19, 385–419. doi:10.1016/s1350-9462(00)00002-1
- Yokoyama, S., and Yokoyama, R. (2000). “Comparative Molecular Biology of Visual Pigments,” in *Molecular Mechanisms in Visual Transduction*. Editors D. G. Stavenga, W. J. DeGrip, and E. N. Pugh Jr. (Amsterdam, Netherlands: Elsevier Science Pub.), 257–296. doi:10.1016/s1383-8121(00)80009-3
- Yoshida, K., Yamashita, T., Sasaki, K., Inoue, K., Shichida, Y., and Kandori, H. (2017). Chimeric Microbial Rhodopsins for Optical Activation of Gs-Proteins. *Biophysics physciobiology* 14, 183–190. doi:10.2142/biophysico.14.0_183
- Yoshizawa, T., and Kandori, H. (1991a). Primary Photochemical Events in the Rhodopsin Molecule. *Prog. Retin. Res.* 11, 33–55. doi:10.1016/0278-4327(91)90023-u
- Yoshizawa, T., and Kuwata, O. (1991b). Iodopsin, a Red-Sensitive Cone Visual Pigment in the Chicken Retina. *Photochem. Photobiol.* 54, 1061–1070. doi:10.1111/j.1751-1097.1991.tb02130.x
- Yoshizawa, T. (1984). Photophysiological Functions of Visual Pigments. *Adv. Biophysics* 17, 5–67. doi:10.1016/0065-227x(84)90024-8
- Yoshizawa, T., and Wald, G. (1967). Photochemistry of Iodopsin. *Nature* 214, 566–571. doi:10.1038/214566a0
- Yoshizawa, T., and Wald, G. (1963). Pre-lumirhodopsin and the Bleaching of Visual Pigments. *Nature* 197, 1279–1286. doi:10.1038/1971279a0
- Young, R. W. (1976). Visual Cells and the Concept of Renewal. *Investig. Ophthalmol. Vis. Sci.* 15, 700–725.
- Yu, H., Siewny, M. G. W., Edwards, D. T., Sanders, A. W., and Perkins, T. T. (2017). Hidden Dynamics in the Unfolding of Individual Bacteriorhodopsin Proteins. *Science* 355, 945–949. doi:10.1126/science.aah7124
- Yu, N., Huang, L., Zhou, Y. B., Xue, T., Chen, Z. G., and Han, G. (2019). Near-Infrared-Light Activatable Nanoparticles for Deep-Tissue-Penetrating Wireless Optogenetics. *Adv. Healthc. Mater.* 8, 1801132. doi:10.1002/adhm.201801132
- Yu, S. M., McQuade, D. T., Quinn, M. A., Hackenberger, C. P. R., Krebs, M. P., Polans, A. S., et al. (2000). An Improved Tripod Amphiphile for Membrane Protein Solubilization. *Protein Sci.* 9, 2518–2527. doi:10.1110/ps.9.12.2518
- Yun, J.-H., Li, X. X., Park, J.-H., Wang, Y., Ohki, M., Jin, Z. Y., et al. (2019). Non-cryogenic Structure of a Chloride Pump Provides Crucial Clues to Temperature-dependent Channel Transport Efficiency. *J. Biol. Chem.* 294, 794–804. doi:10.1074/jbc.ra118.004038
- Yun, J.-H., Li, X. X., Yue, J. N., Park, J.-H., Jin, Z. Y., Li, C. F., et al. (2021). Early-stage Dynamics of Chloride Ion-Pumping Rhodopsin Revealed by a Femtosecond X-Ray Laser. *Proc. Natl. Acad. Sci. U. S. A.* 118, 2020486118. doi:10.1073/pnas.2020486118
- Yun, J.-H., Ohki, M., Park, J.-H., Ishimoto, N., Sato-Tomita, A., Lee, W., et al. (2020). Pumping Mechanism of NM-R3, a Light-Driven Bacterial Chloride Importer in the Rhodopsin Family. *Sci. Adv.* 6, eaay204. doi:10.1126/sciadv.aay2042
- Zabelskii, D., Alekseev, A., Kovalev, K., Rankovic, V., Balandin, T., Soloviov, D., et al. (2020). Viral Rhodopsins 1 Are a Unique Family of Light-Gated Cation Channels. *Nat. Commun.* 11, 5707. doi:10.1038/s41467-020-19457-7
- Zabelskii, D., Dmitrieva, N., Volkov, O., Shevchenko, V., Kovalev, K., Balandin, T., et al. (2021). Structure-based Insights into Evolution of Rhodopsins. *Commun. Biol.* 4, 821. doi:10.1038/s42003-021-02326-4
- Zhang, J. Y., Choi, E. H., Tworak, A., Salom, D., Leinonen, H., Sander, C. L., et al. (2019). Photic Generation of 11-Cis-Retinal in Bovine Retinal Pigment Epithelium. *J. Biol. Chem.* 294, 19137–19154. doi:10.1074/jbc.ra119.011169
- Zhang, L., Salom, D., He, J. H., Okun, A., Ballesteros, J., Palczewski, K., et al. (2005). Expression of Functional G Protein-Coupled Receptors in Photoreceptors of Transgenic *Xenopus laevis*. *Biochemistry* 44, 14509–14518. doi:10.1021/bi051386z
- Zhang, L., Wang, K., Ning, S., Pedersen, P. A., Duelli, A. S., and Gourdon, P. E. (2022). Isolation and Crystallization of the D156C Form of Optogenetic ChR2. *Cells* 11, 895. doi:10.3390/cells11050895
- Zhang, M., Gui, M., Wang, Z.-F., Gorgulla, C., Yu, J. J., Wu, H., et al. (2021a). Cryo-EM Structure of an Activated GPCR-G Protein Complex in Lipid Nanodiscs. *Nat. Struct. Mol. Biol.* 28, 258–267. doi:10.1038/s41594-020-00554-6
- Zhang, S. S., Zheng, S. N., Sun, J. H., Zeng, X. X., Duan, Y. K., Luan, G. D., et al. (2021). Rapidly Improving High Light and High Temperature Tolerances of Cyanobacterial Cell Factories through the Convenient Introduction of an AtpA-C252f Mutation. *Front. Microbiol.* 12, 647164. doi:10.3389/fmicb.2021.647164

- Zhang, T., Cao, L.-H., Kumar, S., Enemchukwu, N. O., Zhang, N., Lambert, A., et al. (2016). Dimerization of Visual Pigments *In Vivo*. *Proc. Natl. Acad. Sci. U. S. A.* 113, 9093–9098. doi:10.1073/pnas.1609018113
- Zhang, X. M., Yokoyama, T., and Sakamoto, M. (2021b). Imaging Voltage with Microbial Rhodopsins. *Front. Mol. Biosci.* 8, 738829. doi:10.3389/fmolb.2021.738829
- Zhang, Y., Iwasa, T., Tsuda, M., Kobata, A., and Takasaki, S. (1997). A Novel Monoantennary Complex-type Sugar Chain Found in octopus Rhodopsin: Occurrence of the Gal β 1-3Fuc Group Linked to the Proxiranal N-Acetylglucosamine Residue of the Trimannosyl Core. *Glycobiology* 7, 1153–1158. doi:10.1093/glycob/7.8.1153
- Zhao, D. Y., Pöge, M., Morizumi, T., Gulati, S., Van Eps, N., Zhang, J. Y., et al. (2019). Cryo-EM Structure of the Native Rhodopsin Dimer in Nanodiscs. *J. Biol. Chem.* 294, 14215–14230. doi:10.1074/jbc.ra119.010089
- Zhao, Z. L., Wang, P., Xu, X. L., Sheves, M., and Jin, Y. D. (2015). Bacteriorhodopsin/Ag Nanoparticle-Based Hybrid Nano-Bio Electrocatalyst for Efficient and Robust H₂ Evolution from Water. *J. Am. Chem. Soc.* 137, 2840–2843. doi:10.1021/jacs.5b00200
- Zhou, H.-X., and Cross, T. A. (2013). Influences of Membrane Mimetic Environments on Membrane Protein Structures. *Annu. Rev. Biophysics* 42, 361–392. doi:10.1146/annurev-biophys-083012-130326
- Zhou, Y., Ding, M. Q., Duan, X. D., Konrad, K. R., Nagel, G., and Gao, S. Q. (2021). Extending the Anion Channelrhodopsin-Based Toolbox for Plant Optogenetics. *Membranes* 11, 287–281212. doi:10.3390/membranes11040287
- Zou, L., Tian, H. H., Guan, S. L., Ding, J. F., Gao, L., Wang, J. F., et al. (2021). Self-assembled Multifunctional Neural Probes for Precise Integration of Optogenetics and Electrophysiology. *Nat. Commun.* 12, 5871–58715879. doi:10.1038/s41467-021-26168-0
- Zundel, G. (1988). “Hydrogen-bond Systems as Proton Wires Formed by Side Chains of Proteins and by Side Chains and Phosphates,” in *Transport through Membranes: Carriers, Channels and Pumps*. Editor A. Pullman (Dordrecht, Netherlands: Kluwer Academic Publishers), 409–420. doi:10.1007/978-94-009-3075-9_27

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