The crowns have eyes: Multiple opsins found in the eyes of the Crown-of-Thorns Starfish *Acanthaster planci*

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Abstract

In the late nineteenth century, the examination of visual pigments led to the discovery of a protein—the opsin—covalently bound to a chromophore. Opsins are G protein-coupled receptors (GPCR) used for both visual and non-visual photoreception, and these proteins evolutionarily date back to the base of the bilaterians. In the current sequencing age, phylogenomic analysis has proven to be a powerful tool, facilitating the increase in knowledge about diversity within the opsin subclasses and so far, nine paralogs have been identified. While phylogeny may help infer function, direct functional studies of opsins in vertebrates, cephalopod mollusks, and fruit flies have shown that there are multiple pathways involving various opsins for visual photoreception along with several other processes. Within echinoderms, opsins have been studied in Echinoidea and Ophiuroidea, but these two groups do not possess proper image forming eyes, but rather widely dispersed dermal photoreceptors. However, most species of Asteroidea, the starfish, possess true eyes and studying them will shed light on the diversity of opsin usage within echinoderms and help resolve the evolutionary history of opsins. Using high-throughput RNA sequencing, we have sequenced and analyzed the transcriptomes of different *Acanthaster planci* tissue samples: eyes, radial nerve, tube feet and a mixture of other organelle tissue. At least 9 opsins belonging to 7 potential opsin paralogs were identified, and seven of them were found significantly differentially expressed in both eyes and radial nerve, providing new important insight into the involvement of opsins in visual and nonvisual photoreception in echinoderms. Of relevance, we found the first evidence of an r-opsin photopigment expressed in a well developed visual eye in a deuterostome animal.

Keywords

Transcriptomics; Asteroidea, vision, photoreceptors, evolution echinoderms

Introduction

Light carries an immense amount of information about the surroundings. Direct light from the sun, the moon, or the stars is used by various animals to set diurnal or annual clocks and to set direction during navigational tasks. Light reflected from the surroundings guides innumerous different behaviours as it provides information about objects with unprecedented details and speed. Light reception is thus widespread in the animal kingdom but interestingly the molecular machinery behind light reception shares many common features across all phyla. In most cases examined so far the first step in the phototransduction, the absorption of the photons in metazoan, is mediated by a specific protein family called opsins [1]. Opsins are seven transmembrane G protein-coupled receptors binding a chromophore, retinal, which undergoes a conformational change upon the absorption of light, thus triggering the rest of the transduction cascade. During the last couple of decades, several molecular studies have examined the diversity of the opsin family and found nine major clades [2]. What the work has also shown is that many animals have a surprisingly high number of opsin gene copies and that they can be expressed in almost any body region or organ [3]. In many of these cases, the functions remain unknown and may well be outside light reception [4,5].

Light reception is known from all major groups of echinoderms and is facilitated by different types of photoreceptors ranging from non-pigmented dermal photoreceptors to proper image forming eyes. A rather special case has been suggested in the brittlestar *Ophiocoma* with dispersed microlenses formed by skeletal elements [6]. Another dermal photoreceptor system is found in sea urchins where it has been proposed to function in a visual context [7,8]. The genome has been sequenced for the sea urchin *Strongylocentrotus purpuratus* and eight opsin genes were found belonging to the opsin clades c-opsins, r-opsins, Go-opsins, peropsin, neuropsin and echinopsins A and B [9,10]. The latter two groups were recently renamed as bathyopsins and chaopsins, respectively, [2]. The r-opsin Sp-opsin4 is expressed in cells at the base of the transparent tube feet and is putatively mediating the directional negative phototaxis described for a couple of species [8,11,12]. The brittle star *Amphiura filiformis* has even higher

opsin diversity with at least 13 gene copies [13], but here little is known about expression patterns and behavioural roles.

Dermal photoreception is also known from several species of starfish (Asteroidea) [14–16] but it is unknown if it is opsin based and if so whether it is the same opsin as found in the photoreceptors of the eyes. Remarkably, these echinoderms also have well defined eyes. They are found in most non-burrowing starfish species at the tip of each arm sitting at the base of the unpaired terminal tube foot as a direct extension of the radial nerve. They are compound eyes and structurally they resemble the eyes of arch clams and fan worms [17,18] with lensless ommatidia typically 20-40 µm in diameter. Depending on species, adult specimens have 50-300 ommatidia in each eye and recent studies have shown that this supports spatial resolution in the range of 8-17 degrees used for navigation [19–21]. These studies have also indicated that the ommatidia have a single population of photoreceptors which utilize an opsin with peak sensitivity in the deep blue part of the spectrum around 470 nm.

Recently, a 384 megabase draft genome of the crown-of-thorns starfish, *Acanthaster planci* was released along with annotations for ~24,500 protein coding genes [22]. Although *A. planci* is not the first Asteroidea with an assembled genome, it is the first species with well defined eyes to have an assembled genome. This presents an opportunity to study the mechanisms behind vision in a species with a well defined eye that is evolutionarily close to species with alternative methods of photoreception. Here we have used tissue specific transcriptomics to investigate the differential expression of opsin genes in *A. planci*. We found at least seven different paralogs and, by comparing expression levels in the eyes, in locomotory tube feet, in the radial nerve, and in a mixture of gonadal, stomach, and epidermal tissue, we are able to infer which opsins are used in vision, in non-visual photoreception, and outside photoreception.

Material and methods

(a) Animals

The specimens of *A. planci* used in this study were hand collected on the Great Barrier Reef off the coast of Cairns, Australia. After collection the animals were kept in holding tanks with running seawater at 26 degrees for 2-3 days and then Flown to Denmark. In Denmark they were kept under similar conditions at the Danish National Aquarium, The Blue Planet, where they were fed three times a week with a past of enriched squid and fish meat. Tissue samples were taken from four specimens with diameters of 15-23 cm. Three terminal tube feet including the eye, 3 locomotory tube feet, approx. 5 cm radial nerve, and pieces of the gonads, the stomas, and the epidermis were sampled from each of the four specimens and stored in RNAlater at 4ºC.

(b) RNA extraction and sequencing

The tissue samples were removed from the RNAlater, immediately frozen with liquid nitrogen and homogenized using a mortar and pestle. Powdered tissues were then dissolved in EuroGOLD RNAPure (EMR 506100) and processed using EUROzol RNA extraction protocol (EMR055100, euroclone), then subjected to LitCl (4M) purification. Library preps and sequencing were done at Università degli Studi di Salerno using SMART-Seq v4 Ultra Low Input RNA Kit.

Sequenced reads were examined using fastqc and then quality filtered and trimmed using trimmomatic (v0.33) [23]. Quality controlled reads were quasi-mapped and quantified to v1 great barrier reef *Acanthaster planci* transcriptome using salmon (v0.7) [24]. Transcripts per million (TPM), the normalized transcript counts [25]. Differentially expressed genes were identified using DESeq2 [26] (FDR $\leq$ 0.05 and -1.5 $\geq$ log2FC $\geq$ 1.5). All scripts can be found at https://github.com/elijahlowe/Acanthaster_opsins.git.

(c) Identification of opsin sequences

Opsin protein sequences were collected from echinoderms [10,13,27–32], hemichordates [33] (Freeman et al. 2008), molluscs [34–36], arthropods [37,38], vertebrates [39–45] and annelids [46] covering 40 species and 159 opsin sequences with an additional 7 melatonin receptor

sequences to be used as an outgroup. These sequences were retrived from various databases including Echinobase [47], and NCBI [48], as well as from publications themselves, as described in (Supp 1). The collected sequences were aligned with MAFFT (v7.215) [49,50] using L-INS-i algorithm which is better designed for divergence sequences and performed well when benchmarked against other multiple sequence aligners [51]. The aligned sequences were then trimmed using trimAl [52], removing gaps that occurred in 10% of the alignments while being sure to retain 60% of the total sequence length. Phylogenetic trees were generated using the aligned sequences with fasttree (v2.1.7) [53] and visualised with figtree (v1.4.3) [54]. Modifications such as additional labels and visual effects were done using inkscape.

Results

Diversity of opsins in *A. planci*

We identified ten putative opsin paralogues in *A. planci*'s proteome using Reciprocal Best Hits (RBH) BLAST, which share high sequence similarity. These sequences were included in a phylogenetic analysis along with 166 sequences spanning 40 different species. Our phylogenetic analysis revealed nine *A. planci* opsins belonging to 7 groups and one sequence identified as a melatonin receptor (figure 1): 1 rhabdomeric opsin (r-opsin), 3 ciliary opsins (c-opsins), 1 peropsin, 1 Go-opsin, 1 RGR opsin, 1 neuropsin, and 1 chaopsin. Chaopsins were first classified as echinoderm specific [9,10] but were later found to group with several cnidarian opsins [2]. *A. planci* possesses representatives of all so far known echinoderm opsin groups with the exception of bathyopsin (former echinopsin A), which thus has yet to be identified in any starfish. *A. planci* opsins grouped closest with those of *Patiria miniata*--an eyeless sea star--followed by *Asteria rubens* opsins.

In *A. planci* eyes, all opsins with the exception of ciliary opsin 1.3 and neuropsin were up-regulated in comparison to the mixed tissues (Figure 2a). This was also the case when comparing opsin expression in the radial nerve to the mixed sample but to a lesser degree (Figure S1). Expression of opsins in the tube feet, however, was not observed to be up-regulated

compared to the mixed tissue, in fact c-opsin 1.3 and neuropsin showed higher expression in the mixed tissue samples (figure 2b).

In order to assess the putative functionality of the *A. planci* identified opsin sequences, an analysis of the key residues necessary for opsin function was performed (Table 1). In most opsins, the retinal binds to the K296 via a Schiff base bond, however the proton in the opsin protein is unstable and a counterion is needed and often supplied by the highly conserved Glutamic acid (E113) residue. There are cases, however, where this residue is replaced by a Tyrosine (Y), Phenylalanine (F), Methionine (M), or Histidine (H) and the other highly conserved Glutamic acid residue, E181, serves as the counterion [55]. This is the case with the majority of the opsins in *A. planci*, where E113 are replaced with a Tyrosine (Y) in the most highly expressed opsins: the r-opsin, chaopsin, Go-opsin, RGR opsin and peropsin. In Ap-c-opsin 1.2, this region appears to be missing and the Go-opsin has a Isoleucine (I) in the position 113.

In addition to nine opsin sequences, we have also observed ten *A. planci* sequences that are potential G protein alpha subunits. Phylogenomic methods classified these sequences as 3 $G\alpha_s$, 1 $G\alpha_o$, 4 $G\alpha_i$, 1 $G\alpha_q$, and 1 $G\alpha_{12}$ (figure S2). All identified G protein alpha subunits with the exception of 1 $G\alpha_s$ (gbr.231.19.t1), 1 $G\alpha_i$ (gbr.143.10.t1) and the $G\alpha_{12}$ are upregulated in the eyes of *A. planci* compare to the mixed tissue samples (figure S3).

(a) Rhabdomeric and ciliary opsins

There are three ciliary opsins identified in the *A. planci* genome, two of which are expressed in the eyes; c-opsin 1.1 and c-opsin 1.2. Both opsins were observed to be significantly differentially expressed in the animal, as neither are expressed in the mixed tissue. Further, observing the expression of c-opsin 1.1 and 1.2 with transcripts per million (TPM) shows that, while differentially expressed, both are expressed at very low levels, less than 20 TPM (figure 2 a & b). C-opsin 1.1 was observed to not have the K296, which is required for the formation of the Schiff base, but instead an Arginine (R) residue is placed in this position. This is the only *A. planci* opsin missing this key residue. While the E113 is present in Ap-c-opsin 1.2 and replaced with tyrosine (Y) in Ap-c-opsin 1.1 and 1.3, the Ap-c-opsin 1.1 and Ap-c-opsin 1.2 are missing other

important motifs, the C187 and C110 disulfide bond motifs [56], respectively, and all three c-opsins are missing the E181 counterion base (table 1).

A. planci's r-opsin, on the other hand, is the most highly differentially expressed of the opsins and of any other genes when comparing eye tissues to mixed tissue (figure 2a). Further, the Ap-r-opsin was observed to be the most up-regulated in the starfish eye tissue and its sequence features the Lysine residue (K296), critical for the Schiff base formation, and a putative counterion (E181) (Table 1).

(b) Chaopsin

Chaopsin is a recently identified group of opsins. Ramirez et al. [2] found the formerly described groups of anthozoa I opsins [57] and the echinoderm echinopsin B [10] to cluster forming the chaopsin group. In agreement with D'Aniello et al. (2015) we found a *A. planci*'s chaopsin to cluster together with other ambulacrarian chaopsins between the r-opsin and c-opsin clades (figure 1). Ap-chaopsin is amongst the highest differentially expressed opsins in our *A. planci* transcriptomes, with ~9.7 $\log_2$ fold changes in the eye compared to the mixed tissue (figure 2a). It is also expressed in the radial nerve tissue, but to a far lesser degree, and appears to not be significantly expressed in the mixed tissues or the tube feet (figure 2b and S1).

(c) Non-visual and photoisomerase opsins

Peropsin and RGR opsin are the highest expressed of the opsin in the tube feet, mixed, and radial nerve, although both still have higher expression in the eyes (figure 2b and S1). The disulfide bond linkage C110/C187, counterion sites and the Lysine for the Schiff base formation are all present. However, both *A. planci* RGR-opsin or peropsin contain variations of the NPxxY motif, NAALQ and NPLMF, respectively (Table 1). Additionaly, peropsin has a variation of the LxxxD motif, ASAGD. Ap-Go-opsin was significantly up-regulated in the eye compared to the mixed tissue sample, and not observed to be expressed in the mixed sample nor the tube feet (figure 2a and b).

Discussion

Light sensing is an important aspect of life and much of it is mediated or initiated by the G-protein-coupled receptor proteins, opsins. The release of the annotated draft genome of *A. planci* has prompted us to investigate its opsin repertoire and expression in a tissue specific manner. This allowed us to classify the specific opsins and to infer possible function and further expand the knowledge of opsin evolution. Nine opsins were identified spanning seven clades: r-opsin, c-opsin, Go-opsin, peropsin, neuropsin, RGR opsin and chaopsin. Through a phylogenomic analysis, it was observed that *A. planci* opsins grouped closest to those in the eyeless species *P. miniata*. This grouping is in accordance with the phylogenetic position of these starfish species [58], with *A. planci* as an Acanthasteridae more closely related to *P. miniata*, an Asterinidae, (both species belonging to Valvatida)- then to *A. rubens*, an Asteridae belonging to Forcipulatacea. However, studies on tissue specific opsin expression will have to investigate in which organs of the eyeless starfish *P. miniata* the respective opsin orthologs are expressed. To this point, it remains unclear if opsins potentially serving a visual function in the eye possessing *A. planci* might have switched functions in the eyeless representative, or if their expression simply persists as a potential evolutionary remain, a finding known e.g. from blind cave salamanders which still possess opsins inside their highly degenerated and pigmentless photoreceptors [59].

Of the several opsins found to be expressed in the eyes of *A. planci*, r-opsin appeared as the highest differentially expressed gene in the eye transcriptome when compared to mixed samples, thus suggesting this opsin is utilized for vision in this starfish. A similar function has been proposed for the sea urchin r-opsin in *S. purpuratus* tube feet [7]. Whereas rhabdomeric opsins have been described in many protostome species as the primary opsin for vision (reviewed in [60]), no deuterostome eye organs have been so far found to bear an r-opsin. As *A. planci* eyes have been previously demonstrated to perform proper spatial vision [19–21], our findings provide first evidence for a deuterostome eye utilizing an r-opsin for spatial vision. Ciliary opsins, on the contrary, which are well known for their role in vertebrate vision and brain

function in some invertebrates [61], appear very poorly expressed in our *A. planci* adult tissue transcriptomes and will not be discussed any further here. Apart from these findings, our differential transcriptomic analysis highlighted specific expression in eye and radial nerve tissues of other less known opsins, the involvement in phototransduction of which has been only little addressed so far. A detailed discussion on these opsins follows.

Chaopsin

In echinoderms, chaopsins (or opsin5) have been identified in Echinoidea, Asteroidea, and Ophiuroidea [10,13,28,32,47]. In the respective species, chaopsins have been found analyzing genomic data or transcriptomes of hypothesized photosensitive tissues, such as the tube feet in *Strongylocentrotus droebachiensis [28]* and *Strongylocentrotus intermedius [32]*. A chaopsin was identified in the genome of the eyeless *P. miniata* but was not found in transcriptome data of the eye possessing starfish *A. rubens*. The lack of chaopsin in *A. rubens* is probably a methodological artifact, since general arm tissue, including radial nerve and tube feet, but not eyes, has been used to generate these transcriptomic data [62]. Chaopsin (Ap-opsin5) is the second most differentially expressed opsin in *A. planci* eyes and it has many of the motifs necessary for phototransduction, including the NPxxY binding motif in the 7 transmembrane domain involved in coupling with the G protein. Little is known up to date about potential chaopsin functions. In the Caribbean elkhorn coral, *Acropora palmata,* opsin3, which along with echinoderm opsin5 belongs to the chaopsin clade [2], has been demonstrated to couple with a Gq-protein in a light-dependent manner [63]. This leads us to hypothesize that the Ap-chaopsin may be important for phototransduction in *A. planci* and potentially in all echinoderms. The exact role of this opsin remains elusive, though.

Peropsin and RGR

Peropsin and RGR were expressed in the tube feet, the mixed tissue, and the radial nerve of *A. planci*, with both having the highest expression in the eyes. Peropsin and RGR opsin are considered as photoisomerase enzymes and not as photopigments, since they bind to all-trans retinaldehyde to regenerate 11-cis-retinoids for pigment regeneration. This has been observed in

the vertebrate retinal pigment epithelium [64], in cephalopod photoreceptors [65] and in the jumping spider [66]. Knock-down mice [67], together with biochemical and spectroscopic studies in amphioxus [68], have demonstrated the same properties for RGR opsin in chordates. This family of opsins is thus important for visual pigment regeneration [69]. While RGR opsins are known to not have the NPxxY binding motif in the 7 transmembrane domain involved in coupling with the G protein, this motif is often found in peropsins [66,68]. This was not the case in *A. planci*, where both RGR opsin and peropsin have varying NPxxY binding motifs. This could alter peropsin's ability to couple with G proteins, further supporting its function as photoisomerase. However, it is worth mentioning that, in chicken, the presence of both peropsin and RGR opsin is thought to serve in the visual cycle of the circadian clock [44].

Go-opsin

In *A. planci* eye and radial nerve transcriptomes, we found significant expression of a Go-opsin along with putative Go alpha subunit proteins. Evidence for Go-opsins in animals is rare. These opsins interact with a specific G-protein that differs from those in the c-opsin as well as the r-opsin transduction cascade [36,70]. In the retina of *Patinopecten yessonensis*, Go-opsin is expressed in a layer of morphologically ciliary receptor cells, which do not express a ciliary opsin. However, in the marine annelid *Platynereis dumerilii*, Go-opsin is coexpressed with two r-opsins in the photoreceptor cells of the larval eye [71]. Knocking down Go-opsin did not lead to absence of phototaxis in *P. dumerilii* but did reduce the sensitivity to the blue-cyan part of the color spectrum (λ_{max}=488 nm). A similar absorbance spectrum (λ_{max}=483 nm) was observed in amphioxus, *Branchinostoma belcheri*, Go-opsin [68,72]. While in *P. yessonensis* the morphological and expression data on Go-opsin point towards a functioning in a visual context, no other studies report of a comparable function. Our data does not reveal if *A. planci* Go-opsin is co-expressed with any other opsins inside the same cells, nonetheless, the fact that this opsin is expressed in the starfish eye along with the results from annelids and amphioxus, opens up the possibility that it is involved in spectral tuning of vision in *A. planci*. Such a tuning would indeed be consisted with the spectral sensitivity curves obtained by electrophysiology in this starfish species [20].

In conclusion, our findings demonstrate that the eye of the starfish *A. planci* expresses an entire set of different opsin proteins. This starfish has recently been demonstrated to perform spatial vision in order to prey on its preferred coral food and r-opsin, as the by far most highly expressed photopigment in its eyes, is likely to facilitate these sophisticated photo behavioural animal responses. *A. planci* is thus not only one more echinoderm possessing a much more complex photoreceptor system than previously assumed, but rather the first deuterostome animal that has been shown to navigate by the use of r-opsin expressing photoreceptors. The variety of opsins found differentially expressed in various starfish light sensitive tissue by our transcriptomic analyses also set the groundwork for comparative studies on evolutionary changes in photoreceptor function that occurred towards the vertebrate eye.

Ethics

Data accessibility

Raw sequencing data will be submitted to NCBI SRA under the accession number ########. Additionally, fasta files containing all sequences can be found in the data section of the github repository https://github.com/elijahlowe/Acanthaster_opsins

Authors' contributions

Collection of tissue samples were done by Anders Garm (AG). RNA extraction was performed by M. Ina Arnone (IA) and Elijah K Lowe (EL). Computational analysis was performed by EL. Writing of the paper was performed by EL, AG, IA and Esther Ullrich-Lüter. All authors contributed to the project design.

Competing interests

The authors declare that we have no competing interest.

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Footnotes

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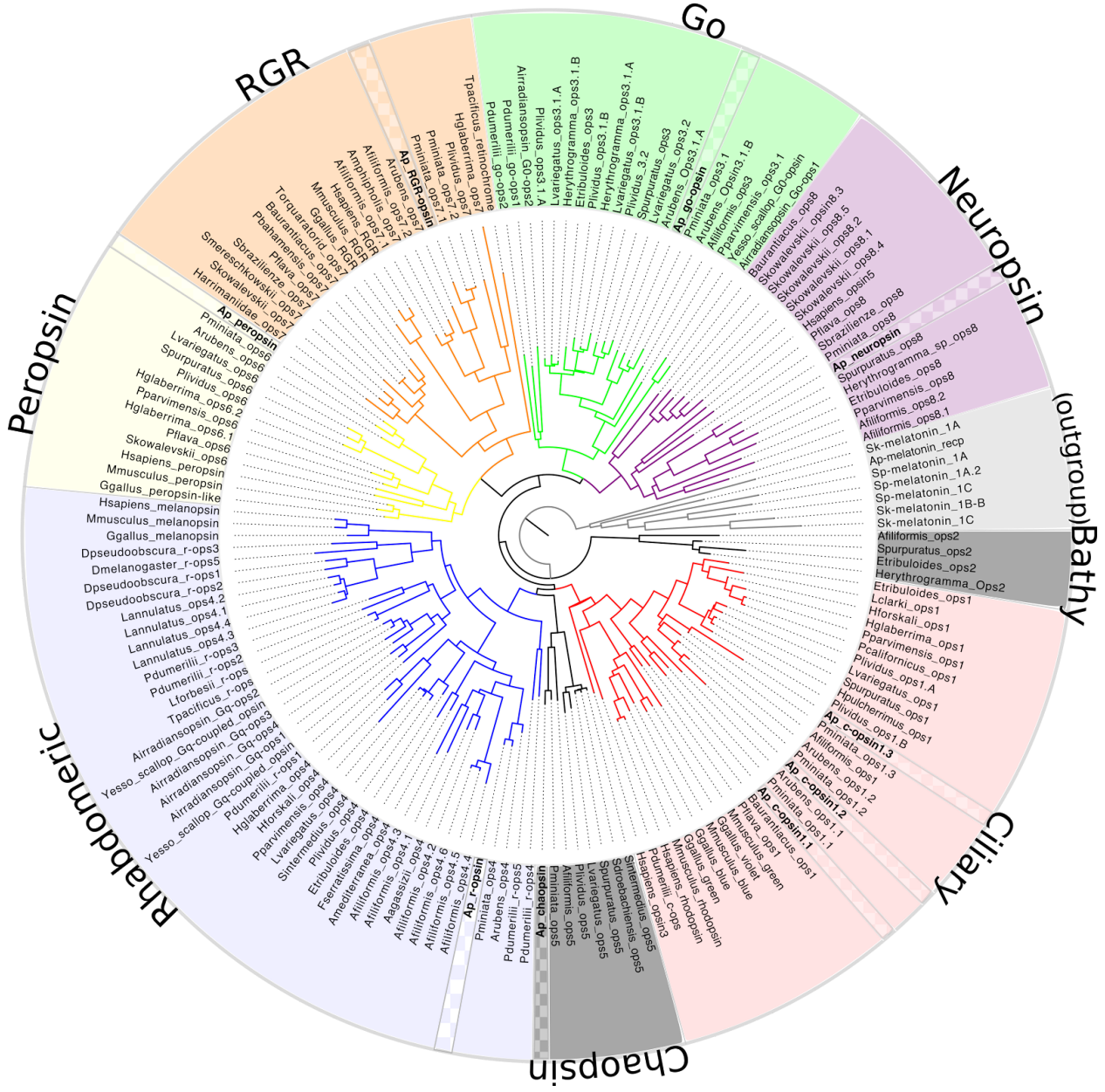
RGR
Go
Neuropsin
(outgroup)Bathy
Ciliary
Chaopsin
Rhabdomeric
Peropsin
Tpacificus_retinochrome
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Pdumerilii_go-ops2
Airradiansopsin_G0-ops2
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Lvariegatus_ops3.1.A
Herythrogramma_ops3.1.B
Etribuloides_ops3
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Ap_go-opsin
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Arubens_Opsin3.1
Afiliformis_ops3
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Yesso_scallop_G0-opsin
Airradiansopsin_Go-ops1
Baurantiacus_ops8
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Skowalevskii_opsin5
Hsapiens_ops8
Pflava_ops8
Sbrazilienze_ops8
Pminiata_ops8
Ap_neuropsin
Spurpuratus_ops8
Herythrogramma_sp_ops8
Etribuloides_ops8
Pparvimensis_ops8
Afiliformis_ops8.1
Afiliformis_ops8.2
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Sk-melatonin_recp
Ap-melatonin_1A
Sp-melatonin_1A.2
Sp-melatonin_1C
Sk-melatonin_1B-B
Sk-melatonin_1C
Afiliformis_ops2
Spurpuratus_ops2
Etribuloides_ops2
Herythrogramma_Ops2
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Lclarki_ops1
Hforskali_ops1
Hglaberrima_ops1
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Lvariegatus_ops1
Spurpuratus_ops1
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Mmusculus_blue
Ggallus_blue
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Mmusculus_rhodopsin
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Pdumerilii_c-ops
Hsapiens_opsin3
Sintermedius_ops5
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Spurpuratus_ops5
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Dpseudoobscura_r-ops3
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Hsapiens_melanopsin
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Mmusculus_peropsin
Hsapiens_peropsin
Skowalevskii_ops6
Pflava_ops6
Hglaberrima_ops6.1
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Hglaberrima_ops6.2
Plividus_ops6
Spurpuratus_ops6
Lvariegatus_ops6
Arubens_ops6
Pminiata_ops6
Ap_peropsin
Harrimaniidae_ops7
Skowalevskii_ops7
Smereschkowskii_ops7
Sbrazilienze_ops7
Pflava_ops7
Pbahamensis_ops7
Baurantiacus_ops7
Torquaratoid_ops7
Mmusculus_RGR
Ggallus_RGR
Hsapiens_RGR
Afiliformis_ops7.1
Amphiphotis_ops7
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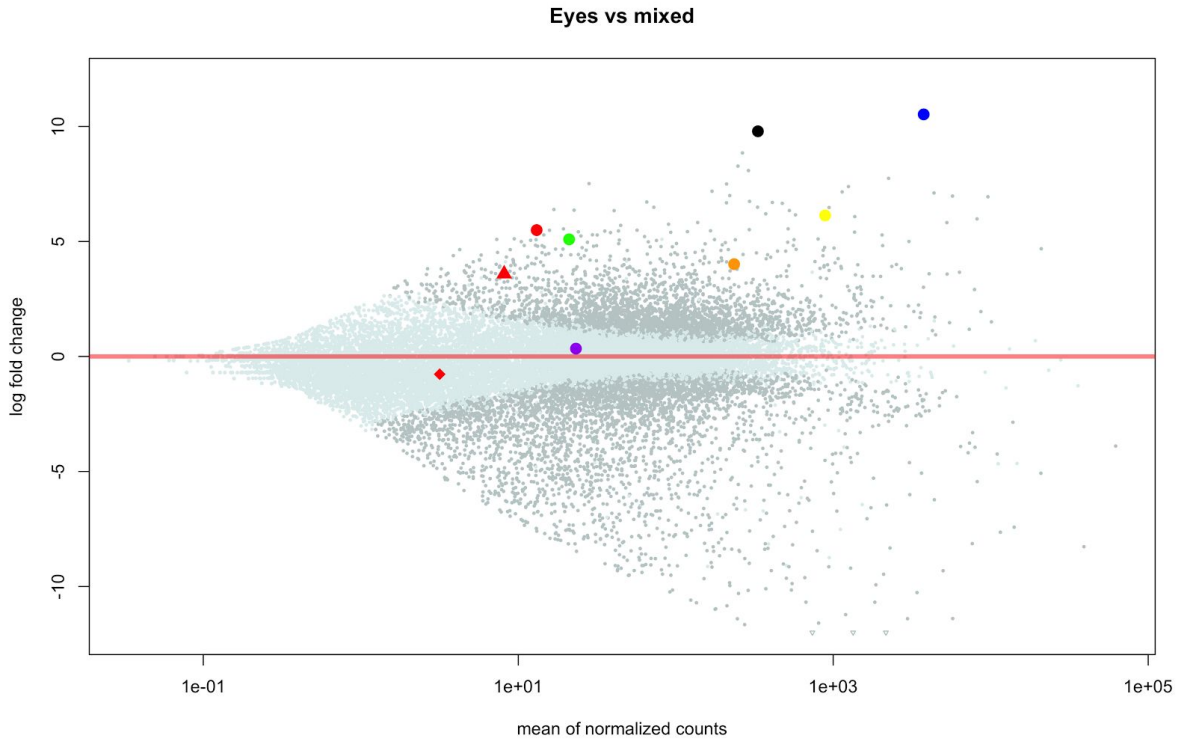
Pminiata_ops7.1
Pminiata_ops7.2

Figure 1.

A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-10
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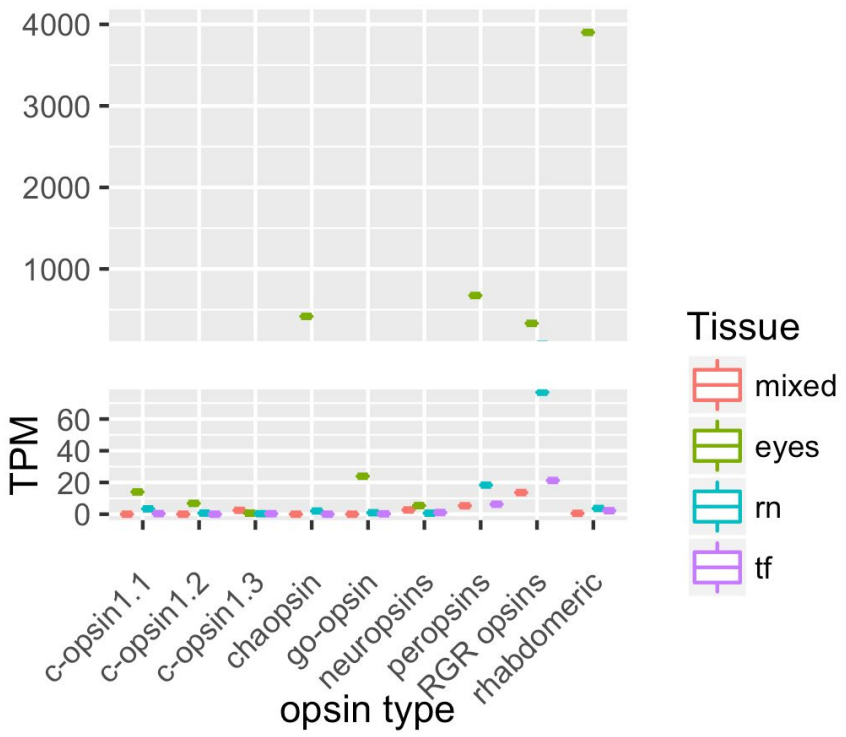
1e+01
1e+03
1e+05
mean of normalized counts

B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1000
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c-opsin1.2
c-opsin1.3
chaopsin
go-opsin
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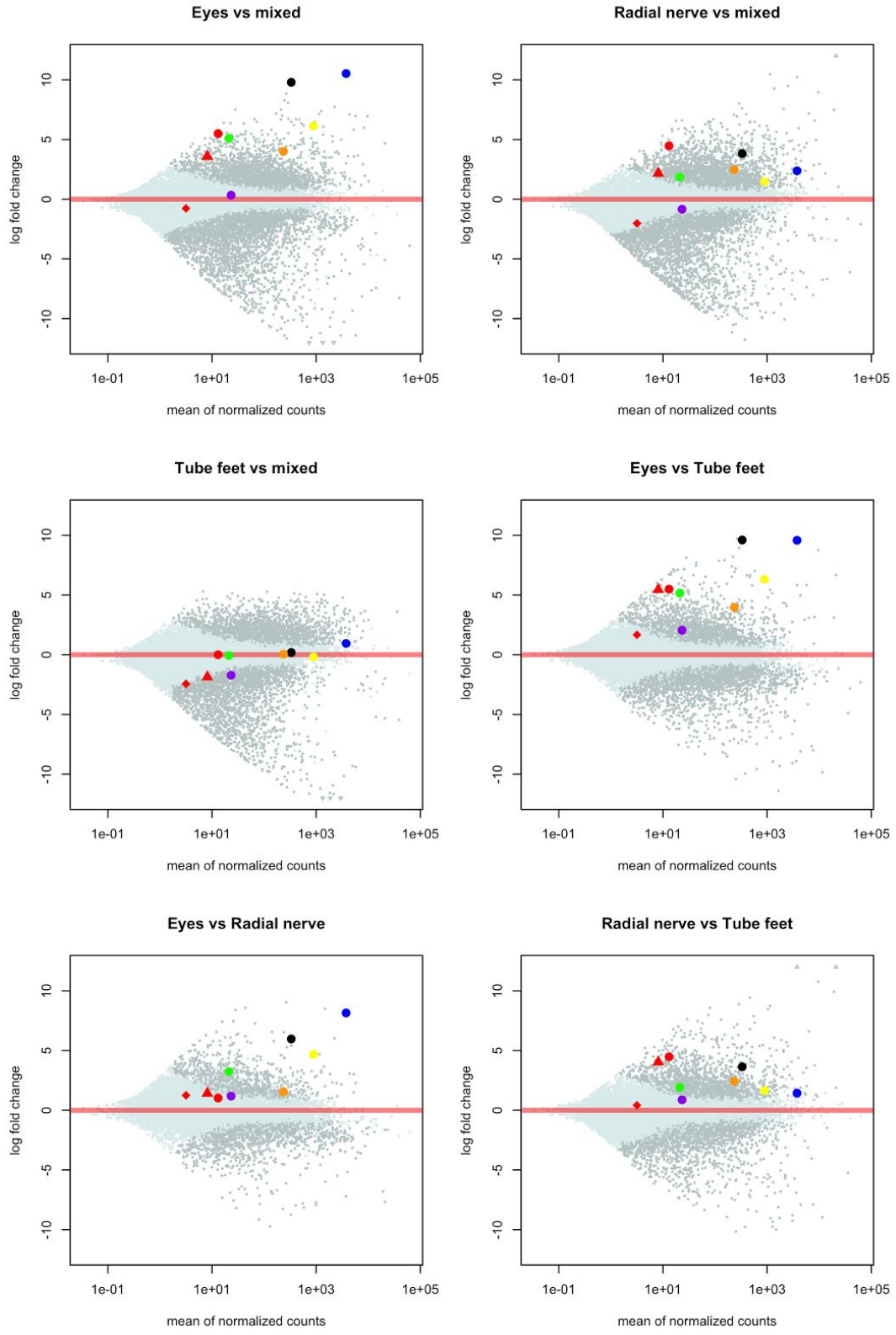
peropsins
RGR opsins
rhabdomeric
opsin type
Tissue
mixed
eyes
rn
tf

Figure 2.


Eyes vs mixed
Radial nerve vs mixed
Tube feet vs mixed
Eyes vs Tube feet
Eyes vs Radial nerve
Radial nerve vs Tube feet
log fold change
mean of normalized counts
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5
0
-5
-10
1e-01
1e+01
1e+03
1e+05

Figure S1

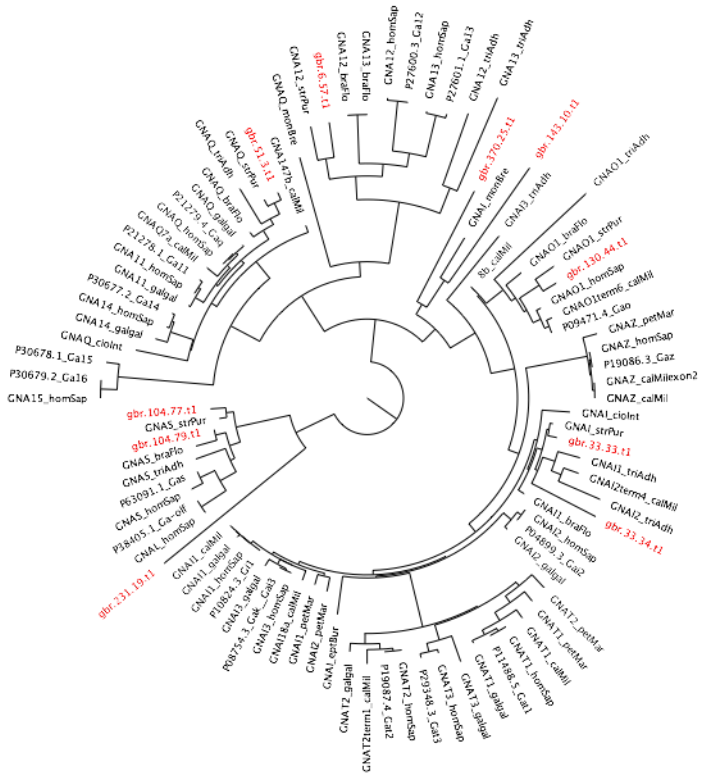

Figure S2

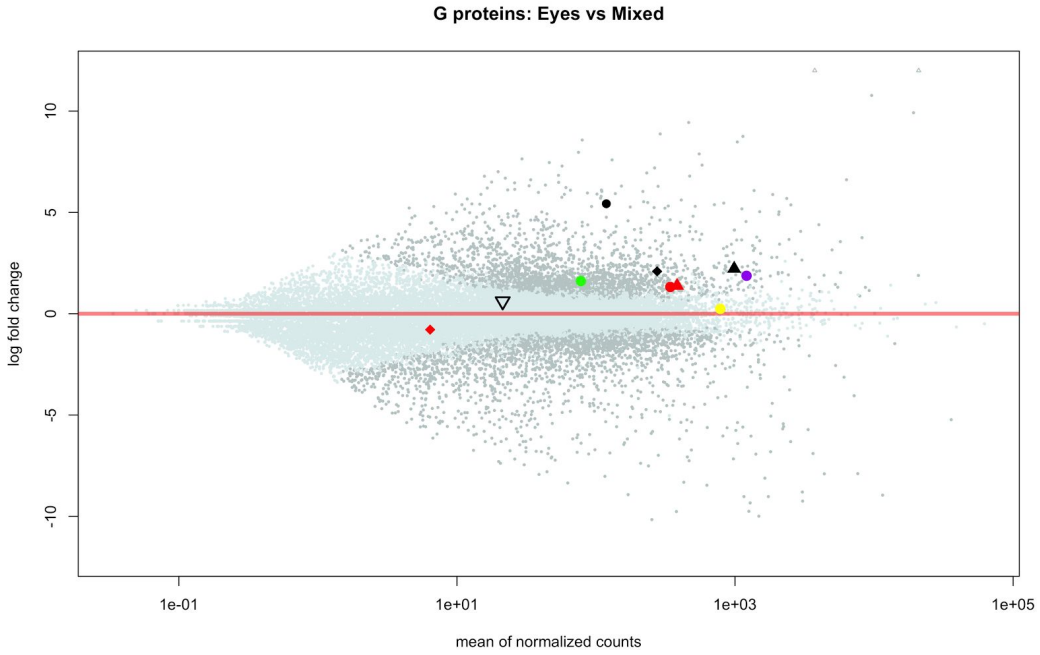

Figure S3

Table 1. Analysis of known typically highly conserved key residues and amino acid motifs

*A. planc*i Opsin	Disulfide bond[a] (C110/C187)	LxxxD[b] (79)	Counterion[c] (E113/E181)	Schiff base[d] (K296)	NPxxY[e] (302)
C-opsin 1.1	C/A	ISVGD	Y/-	R	RPFTA
C-opsin 1.2	H/C	KGNT-	E/-	K	NPIIY
C-opsin 1.3	C/C	VCVAD	Y/-	K	NPVIY
Go-opsin	C/C	MAVSD	I/E	K	NPLIY
R-opsin	C/C	LAFSD	Y/E	K	NPLVY
Chaopsin	C/C	LSGSD	Y/E	K	NPIIY
Peropsin	C/C	ASAGD	Y/E	K	NPLMF
RGR opsin	C/C	LCAGD	Y/E	K	NAALQ
Neuropsin	C/C	LAVSD	Y/E	K	NPIIY

a. Motif required for recognition of rhodopsin by G-protein [73]
b. Motif interacting with NPxxY motif upon receptor activation for structural constrains [74]
c. Glutamic acid residues stabilizing the Schiff base bond
d. Lysine residue forming Shiff base bond with retinal Motif providing structural constraints in response to photoisomerization during formation of the G protein-activating Meta II [75]

Figure Legends

Figure 1. Phylogenomic tree of 166 opsin sequences with melatonin receptors as the outgroup. There are 9 *A. planci* opsins (bold and checkered background) which classify into 7 different groups: 3 c-opsins, 1 chaopsin, 1 r-opsin, 1 peropsin, 1 RGR opsin, 1 go-opsin, and 1 neuropsin. No bathyopsin was found in *A. planci*, which has yet to be identified in any sequenced starfish species. *A. planci* opsin formed a group with *P. miniata* in all cases, with the exception of Go-opsin.

Figure 2. Summary of differential Expression data in various *A. planci* tissue samples focusing on opsin expression. (A) Transcripts per million (TPM) counts of each opsin for each tissue type. For eyes (green) the highest expressed opsins are r-opsin, peropsin, chaopsin, RGR opsin, and go-opsin, with c-opsin 1.1, c-opsin 1.2, and neuropsin being expressed at low amounts, while no expression is observed in c-opsin 1.3. RGR opsin and peropsin were the highest expressed amongst the other tissues. The mixed tissue and tube feet (tf) have little to no expression (TPM < 0.5) of c-opsin 1.1, c-opsin 1.2, go-opsin, and chaopsin. (B) Differential expression of the eyes tissue samples versus the mixed tissue samples. R-opsin is the most differentially expressed, followed by chaopsin. With the exception of c-opsin 1.3 and neuropsin, all opsins are differentially expressed in the eyes of *A. planci* compared to the mixed tissue samples.

Figure S1. Differential gene expression in all *A. planci* tissue samples with the opsins highlighted: c-opsins in red, go-opsins in green, chaopin in black, neuropsin in purple, peropsin in yellow, r-opsin in blue and RGR opsin in orange. The y-axis in the $\log_2$ fold-change, as the distance from the y-axis increases the more differentially expressed a gene is in one tissue versus the other. The x-axis represents counts per million (CPM), an increase on the this axis shows genes with more reads counts.

Figure S2. Phylogenomic tree of 92 G-protein alpha subunit sequences. *A. planci* sequences ID's are highlighted in red. Of the 10 *A. planci* sequences 3 classified as $G\alpha_s$, 1 as $G\alpha_o$, 4 as $G\alpha_i$, 1 as $G\alpha_q$, and 1 as $G\alpha_{12}$.

Figure S3. Differential gene expression in the *A. planci* eye samples compared to mixed with tissues, with the G protein alpha subunits highlighted: 3 $G\alpha_s$ (red), 1 $G\alpha_o$ (green), 4 $G\alpha_i$ (black), 1 $G\alpha_q$ (purple), and 1 $G\alpha_{12}$ (yellow). All identified g protein alpha subunits with the exception of 1 $G\alpha_s$ (gbr.231.19.t1), 1 $G\alpha_i$ (gbr.143.10.t1) and the $G\alpha_{12}$ are upregulated in the eyes of *A. planci* compared to the mixed tissue samples.