OPEN ACCESS

University of BRISTOL

Porter, M. L., Roberts, N. W., & Partridge, J. C. (2016). Evolution under pressure and the adaptation of visual pigment compressibility in deep-sea environments. *Molecular Phylogenetics and Evolution*, *105*, 160-165. https://doi.org/10.1016/j.ympev.2016.08.007

Peer reviewed version

License (if available):
CC BY-NC-ND

Link to published version (if available):
10.1016/j.ympev.2016.08.007

Link to publication record in Explore Bristol Research
PDF-document

University of Bristol - Explore Bristol Research
General rights

Evolution under pressure and the adaptation of visual pigment compressibility in deep-sea environments

Megan L Porter[a,1], Nicholas W Roberts[b,1,2] and Julian C Partridge[c]

[a] Department of Biology, University of Hawai'i at Mānoa, Honolulu, Hawai'i, 96822, USA

[b] School of Biological Sciences, University of Bristol, Tyndall Avenue, Bristol, BS8 1TQ, UK.

[c] School of Animal Biology and the Oceans Institute, University of Western Australia, 35 Stirling Highway, (M317), Crawley, WA 6009, Australia.

[1] both authors contributed equally to this work

[2] Corresponding Author: Nicholas W. Roberts nicholas.roberts@bristol.ac.uk

Abstract

Understanding the link between how proteins function in animals that live in extreme environments and selection on specific properties of amino acids has proved extremely challenging. Here we present the discovery of how the compressibility of opsin proteins in two evolutionarily distinct animal groups, teleosts and cephalopods, appears to be adapted to the high-pressure environment of the deep-sea. We report how in both groups, opsins in deeper living species are calculated to be less compressible. This is largely due to a common set of amino acid sites (bovRH#159, 196, 213, 275) undergoing positive destabilizing selection in six of the twelve amino acid physiochemical properties that determine protein compressibility. This suggests a common evolutionary mechanism to reduce the adiabatic compressibility of opsin proteins. Intriguingly, the sites under selection are on the proteins' outer faces at locations known to be involved in opsin-opsin dimer interactions.

2

1. Introduction

The world's oceans cover approximately 72% of the Earth's surface and, at an average depth of 3800m, the deep-sea represents the major portion of the planet's biosphere (Somero, 1992). As depth increases, so does the hydrostatic pressure, from 0.1 MPa at the surface to over 100 MPa in the deepest oceanic trenches: a thousand-fold range. Life, including macrofauna, is found at all depths, an observation that belies the extent to which pressure can affect biological function. Increasing pressure affects fundamental cellular processes such as enzyme activity, action potential propagation, synaptic transmission, and overall protein function and regulation (Campenot, 1975; Gross and Jaenicke, 1994; Somero, 1992).

In marine animals two types of changes are known to occur that maintain protein function at elevated hydrostatic pressures: extrinsic (changes in cellular and / or membrane composition) and intrinsic (amino acid substitutions leading to modifications of protein structure). An extrinsic change common to many deep-sea animals is that of homeoviscous adaptation to maintain membrane fluidity (Behan et al., 1992; Eguchi et al., 1994; Hazel and Williams, 1990; Somero, 1992). Another is the use of trimethylamine oxide (TMAO) to stabilize proteins (Yancey et al., 2014; Yancey and Siebenaller, 1999, 2015). Intrinsic amino acid changes that increase protein stability are also documented in several marine fish. A number of cytosolic proteins have been investigated in both shallow and deep living species (Stefanni et al., 2014), including lactate dehydrogenase (Brindley et al., 2008; Campenot, 1975; Gross and Jaenicke, 1994; Hennessey and Siebenaller, 1987; Somero, 1992), α-actin (Morita, 2010; Swezey and Somero, 1982; Wakai et al., 2014), and myosin heavy chain proteins (Morita, 2010). These studies have demonstrated that proteins from deep-sea species are functionally less susceptible to increased pressure than those from shallow living species. However, although such studies have suggested that specific amino acid

changes may contribute to protein functional adaptation due to increased hydrostatic pressure, very few have made the link between protein molecular evolution (i.e. changes in amino acid residues), specific amino acid physicochemical properties, and the bulk protein property (i.e. compressibility) that is under natural selection (Wakai et al., 2014).

Transmembrane proteins, and in particular G-protein coupled receptors (GPCRs), are a diverse and extremely important class of proteins involved in cell signaling (Bockaert and Pin, 1999). Several studies have demonstrated that GPCRs are extremely susceptible to perturbation by high pressures (Siebenaller and Garrett, 2002; Siebenaller and Murray, 1999; Somero, 1992). The GPCR (i.e. opsin protein) based visual systems in many deep-sea organisms implies that these proteins have evolved to function within the increased hydrostatic pressures of the deep sea. Studying GPCR function relative to increased pressure is notoriously difficult, as removing the GPCR proteins from the membrane for manipulation alters the overall physiological function of the protein. Therefore, to investigate potential intrinsic changes that have evolved in GPCRs in high-pressure, deep-sea environments, we obtained gene sequences of the transmembrane GPCR opsin from two independent taxonomic groups of marine animals: 122 species of teleost fish and 65 species of cephalopods, representing animals living at a wide range of depths. In fish we investigated the C-type rod opsin, Rh1, and in cephalopods the R-type opsin, representing the two main evolutionarily distinct groups of opsin proteins used in animal vision (Porter et al., 2012). Furthermore, each opsin amino acid sequence provides a convenient analytical way to calculate the adiabatic compressibility of the protein by using well-tested empirical relationships based on the individual physicochemical properties of each constituent amino acid (Gromiha and Ponnuswamy, 1993, and table 1 therein) (Table S1). Using these taxonomically diverse and independently evolved sets of sequences, we reconstructed the phylogenetic history of each set of opsins, calculate the compressibility for each protein, and

use comparative evolutionary methods to look at the evolution of opsin protein compressibility in relation to living depth in order to test the hypothesis that species living at deeper depths have less compressible opsin proteins.

2. Materials and Methods

2.1 Taxon Sampling and Phylogeny Reconstruction.

Opsin sequence data were collected from GenBank for exclusively marine species from two major taxonomic groups: Rh1 sequences from 122 species of fish within the taxon Teleostei and rhodopsin sequences from 65 species of cephalopods (see Supplementary Data for full lists of species and accession numbers). Within both the fish and cephalopod datasets, multiple independent opsin lineages were represented that included shallow living, mid-water, and deep-sea species (Figs. S1, S2, Table S1). Each DNA dataset was translated to amino acid sequence and aligned using the MAFFT v6 online server (Katoh and Toh, 2008). For the fish dataset, this resulted in an alignment of 281 amino acids, spanning from the beginning of transmembrane helix I to the end of transmembrane helix VII, and a cephalopod opsin alignment of 213 amino acids from the beginning of transmembrane helix IV to past the cytoplasmic helix VIII in the C-terminal tail.

The aligned datasets were used to reconstruct maximum likelihood phylogenies (Figures S1 (fish opsin) and S2 (cephalopod opsin) above) using Randomized Axelerated Maximum Likelihood (RAxML) v7.2.8 with rapid bootstrapping (100 iterations) as implemented on the Cyberinfrastructure for Phylogenetic Research (CIPRES) Portal v3.1 (Miller et al., 2010; Stamatakis, 2006). Based on preliminary phylogenetic analyses including non-teleost sarcopterygian and agnathan marine fish (e.g. *Latimeria* and *Petromyzon, respectively*), the opsin from *Clupea harengus* (EU492243) was determined to be the basal marine teleost sequence, and therefore was used to root the phylogeny.

5

Based on preliminary phylogenetic analyses including non-cephalopod molluscs (e.g the bivalve scallop, *Mizuhopecten yessoensis* AB006454), the opsin from *Vampyroteuthis infernalis* (AY545563) was determined to be the basal cephalopod sequence, and therefore was used to root the phylogeny.

2.2 Depth and Compressibility Calculations.

For each species in the opsin datasets, median depth was calculated from catch depth records obtained from the Ocean Biogeographic Information System (OBIS) website (UNESCO) (Table S1). Species with less than five catch records were removed from the dataset. For the remaining species, all records were used to calculate a median catch depth. For the analyses of positive selection, species with median catch depths of 500 m or deeper were considered to be deep-sea species. This was chosen as a conservative measure of depth, given the biased nature of catch depth data, which are skewed towards shallow depths.

Adiabatic compressibility (β_s) is the relative change in the volume of the system per unit adiabatic change in its pressure, and can be estimated for proteins based solely on amino acid sequences (Gromiha and Ponnuswamy, 1993). Briefly, the twelve amino acid properties with the highest correlations to experimentally determined protein compressibility were used to compute a regression equation (Gromiha and Ponnuswamy, 1993, eqn. 5):

$$\beta_s = 56.5064 + 0.3057K^0 + 33.3672P_t - 47.4189P_c - 102.1064F + 14.0347H_t + 1.7694M_w - 0.7816B_t - 0.5990\mu - 11.3579P_\alpha + 5.1392R_a + 29.1553\alpha_n - 2.1465V^0 \,, \tag{1}$$

where the amino acid physicochemical properties are

6

K^0 = compressibility

P_t = turn tendency

P_c = coil tendency

F = mean r.m.s. fluctuational displacement

H_t = thermodynamic transfer hydrophobicity

M_w = molecular weight

B_t = bulkiness

μ = refractive index

P_α = α-helix tendency

R_a = solvent accessible reduction ratio

α_n = power to be at the N-terminus of an α helix

V^0 = partial specific volume.

Using this empirical relationship developed from experimental data, protein adiabatic compressibility can be calculated from its amino acid sequence alone; for each specific property, the total number of each amino acid type, multiplied by that specific property value, is summed across all 20 amino acids. To automate these calculations, a program was written in LabView 2010 (National Instruments) to calculate β_s for a given amino acid sequence (available upon request). To ensure that calculations were comparable across species with different portions of the opsin protein sequence available, we conducted tests comparing the calculated compressibility of an entire opsin protein to compressibility of datasets with the N- and C-terminal tails not included or including just the transmembrane or just the loop regions. These tests showed that the trend of less compressible opsin proteins in deeper living species was detected in all dataset partitions, although strongest in the transmembrane portions of the protein. Therefore, we proceeded to use our program to

calculate the compressibility of every opsin amino acid sequence in both aligned datasets generated for phylogeny reconstruction (see below), representing ~48% of the total cephalopod and ~80% of the complete fish rhodopsin proteins.

2.3 Phylogenetic Comparative Analyses

For both squid and cephalopod datasets, we investigated the relationship between calculated opsin protein compressibility and mean catch depth for all species, using phylogenetic generalized least squares (PGLS), which accounts for the shared evolutionary history of genes. PGLS regressions were run in the 'caper' package of the software program 'R' with ultrametric phylogenies estimated using the 'chronopl' function in the APE package (Orme, 2012; Paradis, 2006; R Core Team, 2014; Sanderson, 2002). The appropriate degree of phylogenetic signal in the residuals was accounted for by simultaneously estimating Pagel's lambda. For analyses, the median depth data were normalized by a $\log_{10}$ transformation.

2.4 Identifying selective influences due to hydrostatic pressure

We used the program TreeSAAP v3.2 (McClellan and Ellison, 2010; Woolley et al., 2003) to test for selection within the aligned opsin datasets used for phylogeny reconstruction on the 12 specific amino acid properties controlling protein compressibility (see above for list). This method is based on the magnitudes of property change due to amino acid residue replacement as inferred across a phylogeny (McClellan et al., 2005), and has been demonstrated to be a more sensitive method for detecting positive selection than more common *dN/dS* based methods (Garb and Hayashi, 2013; McClellan et al., 2005; Porter et al., 2007; Wang et al., 2015). Because we are interested in the evolution of the opsin protein in response to the increased hydrostatic pressures of the deep-sea, we targeted sites identified

8

to be under positive destabilizing selection, defined as selection for radical amino acid property changes (McClellan et al., 2005). In terms of the TreeSAAP analyses, positive destabilizing selection is defined *a priori* as properties with significantly greater amino acid replacements than neutral expectations for the most radical property change categories (e.g., magnitude categories 7 and 8).

We first identified properties within our set of twelve with significantly more observed than expected numbers of changes in radical categories at the $P \leq 0.05$ level. For these properties, we then looked for where the radical changes occurred along branches within the phylogeny leading to deep-sea species and therefore may be contributing to the observed decrease in adiabatic compressibility of the opsin protein with depth. Finally, we identified the specific amino acid residues where the radical changes occurred in the deep-sea lineages. For this study, a deep-sea lineage was defined as a monophyletic clade of opsin sequences from species determined to be deep-sea based on our criterion of a median catch depth of 500m. To investigate the structural and functional consequences of the residues under selection, the identified sites were mapped onto a high-resolution rhodopsin template (e.g. for fish Rh1 the bovine rhodopsin crystal structure, 1U19 (Okada et al., 2004); for cephalopod opsins the squid rhodopsin crystal structure, 2Z73 (Murakami and Kouyama, 2008) from the Protein Data Bank (Berman et al., 2000) using the program Swiss-PdbViewer v3.80b0 (Guex and Peitsch, 1997).

3. Results

3.1 Opsins in Deeper Living Species are Calculated to be Less Compressible

For both the fish and cephalopod groups, we calculated the opsin protein compressibility and compared this with the animals' median recorded living depth within a phylogenetic framework. The reconstructed opsin phylogenies for both fish and cephalopods contained

9

multiple independent lineages inhabiting deep-sea habitats (see Supplementary Figures 1-2). Separate phylogenetic statistical analyses on each group revealed similar relationships between the compressibility of the opsin and the species' living depths. Specifically, a phylogenetic generalized least squares (PGLS) analysis of both fish and cephalopod opsins showed that the calculated protein adiabatic bulk compressibility is significantly lower (p=0.001 and 0.003, respectively) in deeper living species (Fig. 1).

3.2 Positive Selection on the Physiochemical Properties that Define Protein Compressibility

To investigate if this relationship is due to an intrinsic adaptation in protein structure, we used further comparative evolutionary statistical methods (Woolley et al., 2003) to look for evidence of positive destabilizing selection acting on the twelve amino acid properties that define overall protein compressibility (Gromiha and Ponnuswamy, 1993). Positive destabilizing selection is defined as selection for radical change in specific amino acid properties (e.g. large changes in property values), leading to structural or functional shifts in local regions of the protein (McClellan et al., 2005).

As a conservative measure that these molecular adaptations were a common intrinsic mechanism that reduced opsin compressibility, we looked for radical changes occurring across three or more independent deep-sea lineages, either within or across our two datasets. We then identified the opsin residues at which radical changes had occurred across the phylogeny to examine the effect of this positive destabilizing selection on protein structure. Using the criterion of radical change in three independent lineages, we identified seven sites in teleost fish occurring in transmembrane helices I (bovRH# 50), III (bovRH# 114, 124), IV (bovRH# 165), V (bovRH# 213, 217), and VI (bovRH# 271) (Table 1). Some of these sites that contribute to the observed decrease in compressibility in deep-sea species occur on the outer face of the protein, particularly in helices IV and V (Figure 2A). Similarly

in cephalopods, we identified two sites (squidRH# 355 and 356, corresponding to bovRH#341 and 342) in the C-tail that had radical property changes in three deep-sea lineages (Figure 2B). When combining the two datasets, four sites at similar structural positions in extracellular loop II and helices VI, V, and VI were found in three or more deep-sea lineages (squidRH#159, 196, 209, and 284; corresponding to bovRH#160, 198, 212, and 275; Figure 3, Table 1).

Looking across both teleost and cephalopod data, the amino acid sites under selection had a significantly greater than expected number of radical amino acid property changes in 6 of the 12 examined properties that define protein compressibility (Table 1): bulkiness (B_t, cephalopod $p < 0.01$), coil tendency (P_c, fish $p < 0.001$), mean r.m.s. fluctuation displacement (F, fish $p < 0.01$, cephalopod $p < 0.05$), solvent accessible reduction ratio (R_a, fish $p < 0.001$), thermodynamic transfer hydrophobicity (H_t, fish $p < 0.01$, cephalopod $p < 0.001$), and turn tendency (P_t, fish $p < 0.001$, cephalopod $p < 0.05$). Three of these properties (F, H_t, and P_t) were under selection in both fish and cephalopods sites, illustrating that the same amino acid properties have undergone positive selection in both of these phylogenetically distant taxa, resulting in similar effects on opsin compressibility. Interestingly, a large percentage (31.4%) of the significant radical changes observed in deep-sea fish and cephalopod opsins considered together were in the amino acid property, P_t, turn tendency (Table 1), a property of amino acids that indicates their likelihood of being associated with abrupt changes in orientation (Chou and Fasman, 1978).

4. Discussion

4.1 Evolution of Protein Compressibility

By using comparative evolutionary methods, we have calculated that the opsin proteins of deeper living teleost fish and cephalopods are less compressible. This is due to positive

destabilizing selection of the amino acid properties controlling the protein's compressibility, and in particular the property P_t, turn tendency, and through a common motif of opsin sites. Although previous studies have demonstrated that individual amino acid compressibility may play a role in the evolution of visual pigment function in relation to spectral tuning (Porter et al., 2007), this is the first study to link selection on specific amino acid properties to the evolution of overall protein compressibility in high pressure (e.g. deep-sea) environments. Further studies would improve our understanding of whether selection of these particular properties is universal across other GPCRs as an evolutionary adaptation to high-pressure environments. The dominant feature of why hydrostatic pressure affects biological systems, and in particular membrane-based ones, is that physiological or biochemical processes lead to changes in system volume (Benz and Conti, 1986; Somero, 1992). Decreases in protein adiabatic compressibility help maintain normal structural function in response to increases or variation in hydrostatic pressure (Gekko and Hasegawa, 1986). In fish opsins, many of the sites under selection for properties controlling protein compressibility occur in the middle of transmembrane helices and may provide a more stable helix structure under increased pressures (Figure 2A). In particular, two of the identified residues are found in helix III and one in helix VI; both of these helices move to open the G-protein binding site upon visual pigment activation (Farrens et al., 1996). By stabilizing the helix structure these amino acid substitutions may maintain the space required for helical movement upon protein activation. When looking across both fish and cephalopods, the structural placement of the four residues identified as undergoing selection as an adaptive response to increased pressure suggests a second mechanism of maintaining protein function. Many GPCRs are known to form dimers, and this structural conformation is critical to proper function (Bulenger et al., 2005; Comar et al., 2014; Morita, 2010; Swezey and Somero, 1982). Additionally, proteins that form oligomeric assemblies

are highly susceptible to functional changes at even modest pressures (Hazel and Williams, 1990; Morita, 2010). The dimer interface for most GPCRs, including cephalopod and bovine rhodopsins, has been reported to lie on the outer face of helices IV and V (Bockaert and Pin, 1999; Bulenger et al., 2005; Fotiadis et al., 2003; Liang et al., 2003) where the residues that we have identified as being under selection in both fish and cephalopod opsins are located.

These results raise the intriguing possibility that the observed changes at sites on the outer faces of helices IV and V are adaptations to preserve stable dimer interactions by maintaining protein shape and configuration, perhaps to avoid steric clashes (Liang et al., 2003) between amino acids in close adjacency, allowing opsin dimers to function correctly under high hydrostatic pressures. Dimer interactions may be particularly important in opsin activation, as recent evidence has suggested that the activation of the phototransduction cascade relies on the rhodopsin homodimer being part of a heteropentameric rhodopsin / transducin complex (Jastrzebska et al., 2011). Adaptations to maintain dimer interactions under pressure may be a common theme in GPCR evolution in the deep sea. Future studies should use similar methods to look at a broader set of GPCRs to look for evidence of this common theme, as well as use mutational studies to elucidate the role of these sites in dimer interactions and opsin activation. Moreover, molecular dynamics simulations and experimental high-pressure X-ray scattering studies represent two further avenues that should be pursued. Data collected using these techniques would help create a detailed understanding of the effect these site specific amino acid changes have on the system volumes at a range of physiologically relevant pressures.

Acknowledgements

References

Behan, M.K., Macdonald, A.G., Jones, G.R., Cossins, A.R., 1992. Homeoviscous adaptation under pressure: the pressure dependence of membrane order in brain myelin membranes of deep-sea fish. Biochim Biophys Acta 1103, 317-323.

Benz, R., Conti, F., 1986. Effects of hydrostatic pressure on lipid bilayer membranes. II. Activation and reaction volumes of carrier mediated ion transport. Biophys J 50, 99-107.

Berman, H.M., Bhat, T.N., Bourne, P.E., Feng, Z., Gilliland, G., Weissig, H., Westbrook, J., 2000. The Protein Data Bank and the challenge of structural genomics. Nat Struct Biol 7 Suppl, 957-959.

Bockaert, J., Pin, J.P., 1999. Molecular tinkering of G protein-coupled receptors: an evolutionary success. The EMBO journal 18, 1723-1729.

Brindley, A.A., Pickersgill, R.W., Partridge, J.C., Dunstan, D.J., Hunt, D.M., Warren, M.J., 2008. Enzyme sequence and its relationship to hyperbaric stability of artificial and natural fish lactate dehydrogenases. PLoS One 3, e2042.

Bulenger, S., Marullo, S., Bouvier, M., 2005. Emerging role of homo- and heterodimerization in G-protein-coupled receptor biosynthesis and maturation. Trends Pharmacol Sci 26, 131-137.

Campenot, R.B., 1975. The effects of high hydrostatic pressure on transmission at the crustacean neuromuscular junction. Comp Biochem Physiol B 52, 133-140.

Chou, P.Y., Fasman, G.D., 1978. Empirical predictions of protein conformation. Annu Rev Biochem 47, 251-276.

Comar, W.D., Schubert, S.M., Jastrzebska, B., Palczewski, K., Smith, A.W., 2014. Time-resolved fluorescence spectroscopy measures clustering and mobility of a G protein-coupled receptor opsin in live cell membranes. J Am Chem Soc 136, 8342-8349.

Eguchi, E., Ogawa, Y., Okamoto, K., Mochizuki, K., 1994. Fatty acid compositions of arthropod and cephalopod photoreceptors: interspecific, seasonal and developmental studies. Journal of Comparative Physiology B 164, 94-102.

Farrens, D.L., Altenbach, C., Yang, K., Hubbell, W.L., Khorana, H.G., 1996. Requirement of rigid-body motion of transmembrane helices for light activation of rhodopsin. Science 274, 768-770.

Fotiadis, D., Liang, Y., Filipek, S., Saperstein, D.A., Engel, A., Palczewski, K., 2003. Atomic-force microscopy: Rhodopsin dimers in native disc membranes. Nature 421, 127-128.

Garb, J.E., Hayashi, C.Y., 2013. Molecular evolution of alpha-latrotoxin, the exceptionally potent vertebrate neurotoxin in black widow spider venom. Mol Biol Evol 30, 999-1014.

Gekko, K., Hasegawa, Y., 1986. Compressibility-structure relationship of globular proteins. Biochemistry 25, 6563-6571.

Gromiha, M., Ponnuswamy, P., 1993. Relationship between amino acid properties and protein compressibility. Journal of Theoretical Biology 165, 87-100.

Gross, M., Jaenicke, R., 1994. Proteins under pressure. The influence of high hydrostatic pressure on structure, function and assembly of proteins and protein complexes. Eur J Biochem 221, 617-630.

Guex, N., Peitsch, M.C., 1997. SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. Electrophoresis 18, 2714-2723.

Hazel, J.R., Williams, E.E., 1990. The role of alterations in membrane lipid composition in enabling physiological adaptation of organisms to their physical environment. Prog Lipid Res 29, 167-227.

Hennessey, J.P., Jr., Siebenaller, J.F., 1987. Pressure-adaptive differences in proteolytic inactivation of M4-lactate dehydrogenase homologues from marine fishes. J Exp Zool 241, 9-15.

Jastrzebska, B., Ringler, P., Lodowski, D.T., Moiseenkova-Bell, V., Golczak, M., Muller, S.A., Palczewski, K., Engel, A., 2011. Rhodopsin-transducin heteropentamer: three-dimensional structure and biochemical characterization. J Struct Biol 176, 387-394.

Katoh, K., Toh, H., 2008. Recent developments in the MAFFT multiple sequence alignment program. Brief Bioinform 9, 286-298.

Liang, Y., Fotiadis, D., Filipek, S., Saperstein, D.A., Palczewski, K., Engel, A., 2003. Organization of the G protein-coupled receptors rhodopsin and opsin in native membranes. J Biol Chem 278, 21655-21662.

McClellan, D.A., Ellison, D.D., 2010. Assessing and improving the accuracy of detecting protein adaptation with the TreeSAAP analytical software. Int J Bioinform Res Appl 6, 120-133.

McClellan, D.A., Palfreyman, E.J., Smith, M.J., Moss, J.L., Christensen, R.G., Sailsbery, J.K., 2005. Physicochemical evolution and molecular adaptation of the cetacean and artiodactyl cytochrome b proteins. Mol Biol Evol 22, 437-455.

Miller, M., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Gateway Computing Environments Workshop (GCE), 2010, IEEE.

Morita, T., 2010. High-pressure adaptation of muscle proteins from deep-sea fishes, Coryphaenoides yaquinae and C. armatus. Ann N Y Acad Sci 1189, 91-94.

Murakami, M., Kouyama, T., 2008. Crystal structure of squid rhodopsin. Nature 453, 363-367.

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

Orme, D., 2012. CAPER: Comparative Analyses of Phylogenetics and Evolution in R. webpage at http://CRAN.R-project.org/package=caper.

Paradis, E., 2006. Analysis of Phylogenetics with R. Springer-Verlag, New York.

Porter, M.L., Blasic, J.R., Bok, M.J., Cameron, E.G., Pringle, T., Cronin, T.W., Robinson, P.R., 2012. Shedding new light on opsin evolution. Proceedings of the Royal Society B: Biological Sciences 279, 3-14.

Porter, M.L., Cronin, T.W., McClellan, D.A., Crandall, K.A., 2007. Molecular characterization of crustacean visual pigments and the evolution of pancrustacean opsins. Molecular Biology and Evolution 24, 253-268.

R Core Team, 2014. R: A language and environment for statistical computing., . R Foundation for Statistical Computing, Vienna, Austria.

Sanderson, M.J., 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. Mol Biol Evol 19, 101-109.

Siebenaller, J.F., Garrett, D.J., 2002. The effects of the deep-sea environment on transmembrane signaling. Comp Biochem Physiol B Biochem Mol Biol 131, 675-694.

Siebenaller, J.F., Murray, T.F., 1999. Hydrostatic pressure alters the time course of GTP. Biol Bull 197, 388-394.

Somero, G.N., 1992. Adaptations to high hydrostatic pressure. Annu Rev Physiol 54, 557-577.

Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22, 2688-2690.

Stefanni, S., Bettencourt, R., Pinheiro, M., Moro, G.D., Bongiorni, L., Pallavicini, A., 2014. Transcriptome of the Deep-Sea Black Scabbardfish, Aphanopus carbo (Perciformes: Trichiuridae): Tissue-Specific Expression Patterns and Candidate Genes Associated to Depth Adaptation. Int J Genomics 2014, 267482.

Swezey, R.R., Somero, G.N., 1982. Polymerization thermodynamics and structural stabilities of skeletal muscle actins from vertebrates adapted to different temperatures and hydrostatic pressures. Biochemistry 21, 4496-4503.

UNESCO, I.O.C.I.o., The Ocean Biogeographic Information System.

Wakai, N., Takemura, K., Morita, T., Kitao, A., 2014. Mechanism of deep-sea fish alpha-actin pressure tolerance investigated by molecular dynamics simulations. PLoS One 9, e85852.

Wang, J., Yu, X., Hu, B., Zheng, J., Xiao, W., Hao, Y., Liu, W., Wang, D., 2015. Physicochemical evolution and molecular adaptation of the cetacean osmoregulation-related gene UT-A2 and implications for functional studies. Sci Rep 5, 8795.

Woolley, S., Johnson, J., Smith, M.J., Crandall, K.A., McClellan, D.A., 2003. TreeSAAP: selection on amino acid properties using phylogenetic trees. Bioinformatics 19, 671-672.

Yancey, P.H., Gerringer, M.E., Drazen, J.C., Rowden, A.A., Jamieson, A., 2014. Marine fish may be biochemically constrained from inhabiting the deepest ocean depths. Proc Natl Acad Sci U S A 111, 4461-4465.

Yancey, P.H., Siebenaller, J.F., 1999. Trimethylamine oxide stabilizes teleost and mammalian lactate dehydrogenases against inactivation by hydrostatic pressure and trypsinolysis. J Exp Biol 202, 3597-3603.

Yancey, P.H., Siebenaller, J.F., 2015. Co-evolution of proteins and solutions: protein adaptation versus cytoprotective micromolecules and their roles in marine organisms. J Exp Biol 218, 1880-1896.

Figure 1. A univariate phylogenetic generalized least-squares analyses of the relationship between opsin protein compressibility and median depth.

A) In 122 species of teleost fish the protein compressibility is negatively dependent on depth (PGLS, F = 7.248, d.f = 2,120, p = 0.001). B) In 65 species of cephalopods the protein compressibility is negatively dependent on depth (PGLS, F = 6.332, d.f = 2,63, p = 0.003). Solid black lines of A) Compressibility = 13.00-0.20log10(Depth) and B) 12.17-0.24log10(Depth) represent the best fits of the models; a log10 transformation was used to normalize the distribution of the depth data.

Figure 2. Group specific functionally important amino acid residues identified to be under destabilizing selection for properties controlling protein compressibility mapped to the three dimensional structures for (A) bovine rhodopsin (21) and (B) squid rhodopsin (22). In each structure, the portion of the protein analyzed in this study is shown in white, while regions missing from our alignments are in dark grey. All of the amino acid sites shown exhibited radical amino acid property changes in three or more independent deep-sea lineages within either fish (A) or cephalopods (B). The represented amino acid sites have been colored based on domain as follows: transmembrane helix I – red; transmembrane helix III – yellow; transmembrane helix IV – green; transmembrane helix V – light blue; transmembrane helix VI – dark blue; C-tail - pink. The visual pigment chromophore is shown in purple within the trans-membrane palisade of α-helices.

Figure 3. Functionally important amino acid residues common to both fish and cephalopod opsins identified to be under destabilizing selection for properties controlling protein compressibility mapped to the three dimensional structures for (A) bovine rhodopsin (21)

and (B) squid rhodopsin (22). Amino acid residues particularly important in controlling

protein compressibility identified in both taxa (Bovine rhodopsin amino acid #s 159, 196,

213 and 275) are shown as red colored spheres on the helix structure. All four of these sites

exhibited radical amino acid property changes in three or more independent lineages across

both fish and cephalopods. In each structure, regions or the protein missing from our

alignments are in dark grey. The helices containing the identified amino acids have been

colored based on domain as follows: transmembrane helix IV – green; transmembrane helix

V – light blue; transmembrane helix VI – dark blue. The visual pigment chromophore is

shown in purple within the trans-membrane palisade of α-helices.

Table 1. Amino acid sites identified to be under positive destabilizing selection for properties controlling protein compressibility using TreeSAAP analyses (*19*). For each site, the amino acid number in bovine rhodopsin (bovRH#), squid opsin (squidRH#), the opsin protein domain, the number of lineages in which the site was identified (# Fish or # Ceph) out of a total of 11 deep sea teleost fish lineages or 14 deep sea cephalopod lineages, and the properties with radical change are tabulated. The properties are denoted as follows: P_a – alpha helical tendency, B_t – bulkiness, P_c – coil tendency, K^0 – amino acid compressibility, F – mean r.m.s. fluctuation displacement, μ - refractive index, R_a – solvent accessible reduction ratio, H_t – thermodynamic transfer hydrophobicity, P_t – turn tendency. The same structural sites (although not position number) under selection in three or more lineages across both the fish and cephalopod datasets are highlighted in grey.

Bovine RH#	Squid RH#[a]	RH domain[b]	# Fish[c]	# Ceph[c]	Properties								
					Pa	Bt	Pc	K0	F	μ	Ra	Ht	Pt
124		TM3	4	-	X		X						X
159		TM4	3	0				X	X		X		X
160	159	TM4	0	1		X			X				X
165		TM4	4	0	X		X		X	X	X		X
196		EL2	3	0	X		X	X					
198	196	EL2	0	1								X	
209		TM5	3	0					X		X		X
217		TM5	5	0							X		
275	284	TM6	2	1					X		X		X
-	355	C-tail	-	3									X
-	356	C-tail	-	3									X

a – squid rhodopsin site numbers have been provided only for those residues identified in our analyses of cephalopod opsins; b – TM = transmembrane helix, EL = extracellular loop; c – dashes indicate portions of the protein not represented in our opsin sequence dataset.
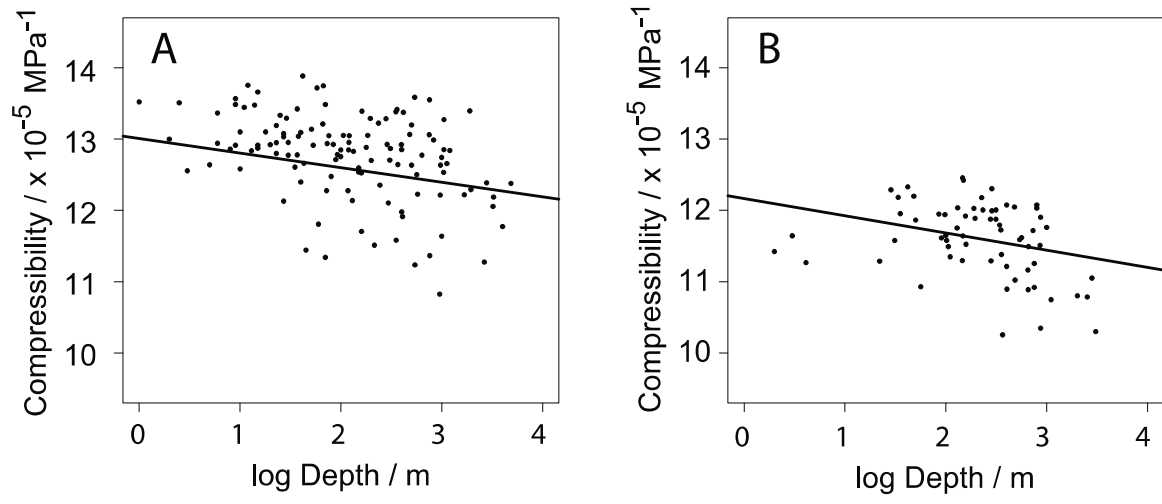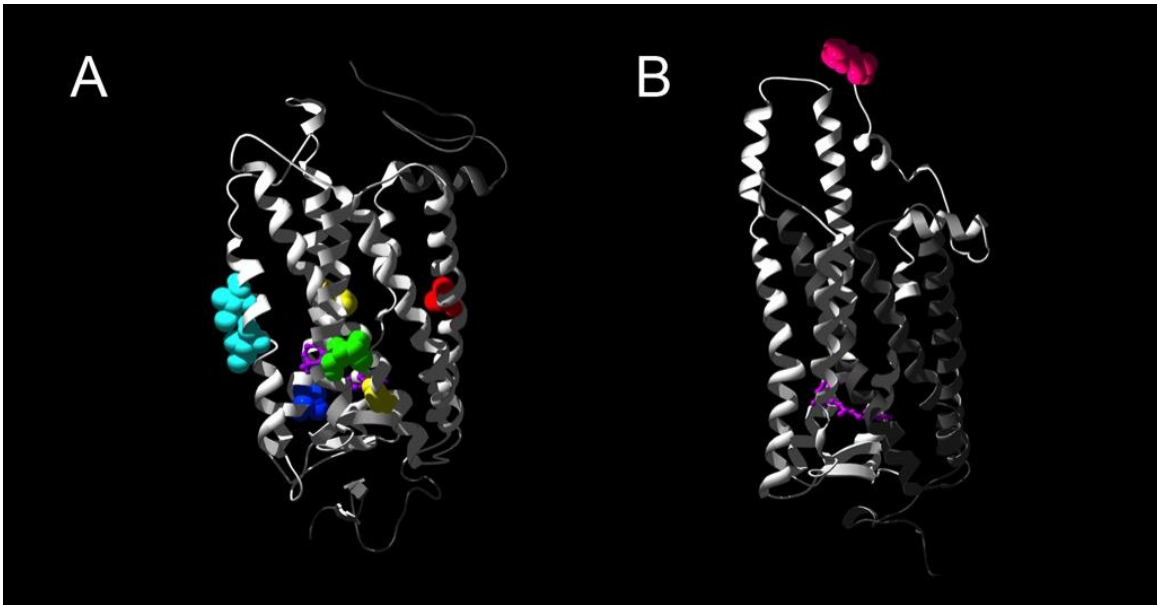
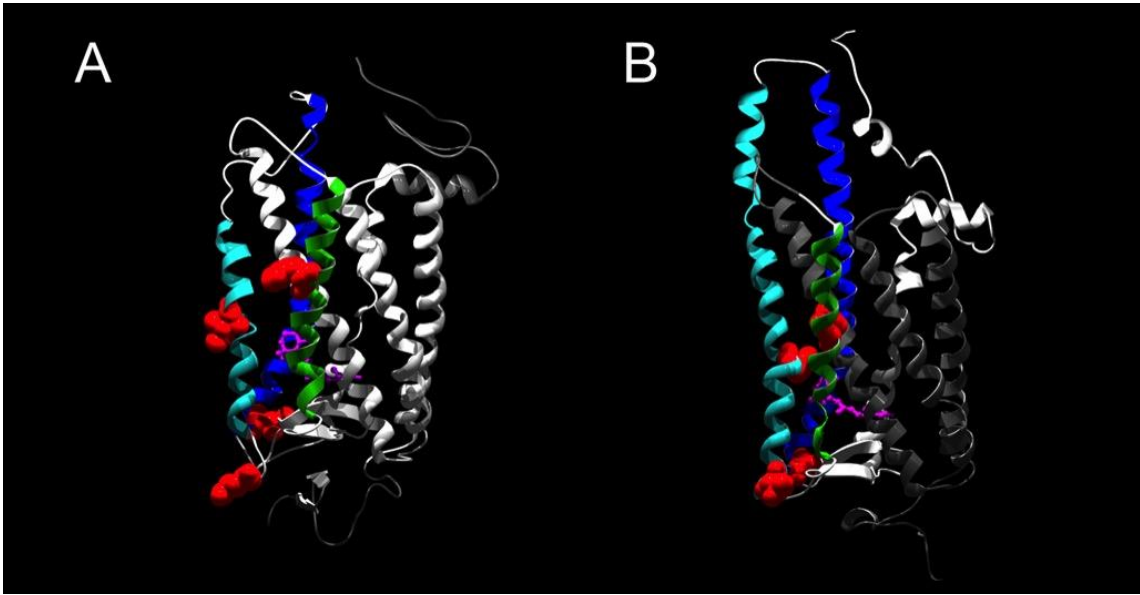**Figures**

Figure 1

Figure 2

Figure 3