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The Organic Crystalline Materials of Vision: Structure–Function Considerations from the Nanometer to the Millimeter Scale

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Vision mechanisms in animals, especially those living in water, are diverse. Many eyes have reflective elements that consist of multilayers of nanometer-sized crystalline plates, composed of organic molecules. The crystal multilayer assemblies owe their enhanced reflectivity to the high refractive indices of the crystals in preferred crystallographic directions. The high refractive indices are due to the molecular arrangements in their crystal structures. Herein, data regarding these difficult-to-characterize crystals are reviewed. This is followed by a discussion on the function of these crystalline assemblies, especially in visual systems whose anatomy has been well characterized under close to in vivo conditions. Three test cases are presented, and then the relations between the reflecting crystalline components and their functions, including the relations between molecular structure, crystal structure, and reflecting properties are discussed. Some of the underlying mechanisms are also discussed, and finally open questions in the field are identified.

1. Introduction

The capability of vision has evolved in different animal phyla, ranging from mollusks to vertebrates. Much of what we know about the structures involved in animal vision is at the larger millimeter length scales, and the foundation for this knowledge was already established in the late 19th Century.[1–3] In the second half of the 20th Century the accumulated information was integrated into a comprehensive body of knowledge, mainly as a result of the work of Land and Lythgoe from the 1960s onward (summarized in refs. [4,5]), but they did not have the ability to image the nano- and microstructure in its native state. The use of electron microscopy to image tissue samples required dehydration, causing dissolution and deformation artifacts. In particular, these preservation artifacts compromised their ability to reliably reconstruct light pathways which is dependent on a detailed and accurate characterization of the eye structure in a close-to in vivo state. Another technical problem that is difficult to resolve to this day is the characterization of the organic crystals that often fulfill key functions in light reflection or scattering. In some cases these crystals are exceedingly small and in all cases radiation sensitive. Furthermore, during microtoming in order to produce TEM thin sections, the crystals are usually lost from the sample. The difficulties are both in the identification of the chemical nature of the material and in the characterization of the crystal morphology, size, and structure. Both these technical problems can now in part be resolved either by using techniques that can provide the appropriate structural information with fresh hydrated samples in loco, such as Raman microspectroscopy, and by using cryofixation techniques,[6] which maintain the tissue in a close-to physiological vitrified state.

This perspective focuses on these two aspects of vision: the organic crystals that are present in many, but not all animal eyes, and the overall 3D anatomy of eyes that were studied in their close-to in vivo state. We then integrate some of this information in terms of the relation between the crystals, the structure and the function, and discuss insights obtained on the underlying mechanisms of image formation.

2. Optical Considerations in Visual Systems

Most man-made imaging systems, from cameras to telescopes, are based on macroscopic lenses and on parabolic mirrors (usually metallic). Lenses form images by refraction of light at an interface where the refractive index is discontinuous, whereas mirrors rely on reflection. Similar lens-based imaging is common in terrestrial animal eyes (as is the case for human vision), but is much less effective under water, where the index contrast between the outside medium (water) and the cornea is much smaller.[4] This calls for alternative solutions which include gradient index lenses, waveguides and mirror-based

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systems. In biology, some of the mirror-based solutions in vision involve crystalline materials, mostly in the form of multiple layers of plate-like crystals separated by cytoplasm or of spherical or quasi-spherical particles. The most common motifs where crystals are used in vision are equivalent to the dielectric mirror, where the high reflectivity derives from multiple thin layers of a dielectric material. In the absence of metallic mirrors, an alternative reflection mechanism is needed. The solution for this is the construction of a multilayer stack composed of alternating low-refractive index (n_l) and high refractive index (n_h) layers. When light impinges upon the stack, a portion of the light (which increases with the index contrast n_h/n_l) is back-reflected at every interface between the high-index and the low-index material. Constructive interference of the backscattered waves from many interfaces leads to enhanced reflectivity. The "ideal" stack, leading to maximal reflection with a minimal number of layers, is the quarter-wave stack, where the optical thickness (the refractive index n times the thickness d) of each layer is exactly a quarter of the wavelength of light ($4nd = \lambda$). In this case, the reflectivity at normal incidence equals

$$|R|^2 = \left| \frac{1 - \left(\frac{n_h}{n_l}\right)^{2N}}{1 + \left(\frac{n_h}{n_l}\right)^{2N}} \right|^2$$

where N is the number of bilayers. This expression also shows that when using an enhanced index contrast, fewer layers are needed to achieve a particular value of the reflectivity. When the layer spacing is less uniform, the stack reflectivity is reduced and spectrally broadened. A completely disordered stack, in turn, reflects a broad spectrum of light.[7]

Another common motif is light scattering from single (usually spherical) particles whose size is comparable to the wavelength of light. This regime was first studied by Mie, who obtained a closed-form expression for the light scattering as a function of the dimensionless parameter $f = 2\pi a/\lambda$, where a is the radius of the sphere. Generally, scattering is stronger for a large index contrast between the scatterer and its surrounding. For $a \ll \lambda$, backscattering and forward scattering are of equal magnitude, while for $a > \lambda$, forward scattering dominates.

3. The Organic Crystals in Vision

Organisms use crystals of organic molecules to produce an extraordinary array of optical phenomena. Crystalline guanine is the most widespread of these materials, being found in at least three different animal phyla. A recent review focused on how organisms use guanine crystals to reflect and scatter light to produce structural colors used in camouflage and display.[8] Here we extend this review to consider other organic materials and particularly to explore the application of such materials in vision. Vision represents a highly complex and "sophisticated" optical function of crystalline materials as this function often imposes additional constraints over the hierarchical organization of the crystals, since, in the case of image-formation, light must not only be reflected, but also focused.

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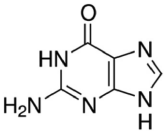
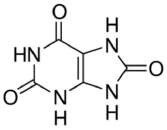
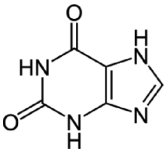
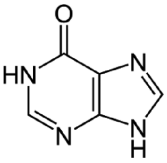
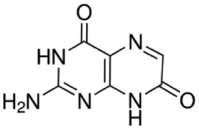
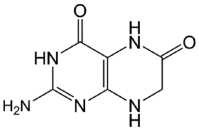
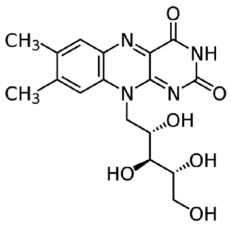
In the 19th century it was already established that many fish eyes contain crystals of guanine.[1,2] Guanine has since been found in the eyes of many other animals, including vertebrates and invertebrates.[4,9] **Table 1** lists just some of the vision systems that use guanine crystals, and there are many more that are known.[10] This in turn raises the question of why guanine is so widely used in vision and for other functions involving light manipulation.[8] Most of the literature in this field has concentrated on tapetum reflectors in eyes and this bias is reflected in the table. The tapetum lucidum (literally the "shiny carpet," sometimes referred to as a "light doubling tapetum") is a retroreflecting layer, lying behind the retina which reflects photons that have not been absorbed by the photoreceptors on the first pass, back to the retina. This backreflection enhances the sensitivity of the eye.

Guanine, however, is not the only organic crystal used in vision. Table 1 shows a number of other crystalline organic compounds that have been reported to be present in eyes. As it is really challenging to identify and characterize these crystals,

Table 1. The currently identified organic crystal components of reflectors used in animal vision and their molecular structures.

Reflecting material	Anatomical location	Taxon	References
Guanine 	Tapetum	Many teleost fish	[1,10]
		Latimeria chalmunae (coelacanth)	[11]
		Squalus acanthias (dogfish)	[12,13]
		Cartilaginous fish (elasmobranchs), sharks	[14]
		Hetrodontus philipi (shark)	[10]
		Some crocodilians	[1,15]
	Various	*Limulus polyphemus* (Horseshoe crab)	[16,17]
	Tapetum	Spiders	[18]
	Mirrors (image-forming)	*Pecten maximus* (scallop)	[19]
Uric acid? 	Tapetum	*Homarus americanus* (lobster)	[16]
		Acheta domesticus House cricket (insect)	[20]
		Hiodon tergisus and *H. alosoides* (fish)	[21]
		Opsanus (Toad fish)	[22]
Xanthine 	Tapetum	*Machilis hrabei* and *Lepismachilis* spp (Jumping bristletails, Insects)	[20]
		Homarus americanus (lobster)	[16]
Hypoxanthine? 	Tapetum	*Homarus americanus* (lobster)	[16]
Isoxanthopterin 	Various reflecting layers	*Penaeus setiferus* (shrimp)	[23]
	Tapetum	*Machrobrachium rosenbergii* (prawn) *Cherax quadricarinatus* (crayfish)	[24]
7,8-Dihydro-xanthopterin? 	Tapetum	*Stizostedion vitreum* (fish)	[25]
Riboflavin 	Tapetum	*Galago crassicaudatus agisymbanus* (lemurs, primate),	[26]
		Ferret	[27]
Zinc cysteinate?	Tapetum	Dogs	[27–29]

the first question is whether these reports are reliable. Most of the characterizations are based on first identifying the presence of reflecting solid bodies. The tissues in which these reflecting bodies are located are then often solubilized and the major organic compounds are identified, usually by histochemical reactions. In some cases the reflecting bodies are isolated and

then identified by histochemical reactions and/or by chromatography, and in very few cases (e.g., the presence of riboflavin crystals[26]) by diffraction. Spectroscopy and/or electron or X-ray diffraction of the reflecting crystals can be regarded as a definitive identification. We have placed question marks in column 1 of Table 1 where we think the identifications need to be confirmed.

Table 1 thus raises two basic questions. Is this list correct, and if so is this list indicative of the range and distribution of reflecting crystals used by animals for vision? We suspect that more crystalline organic compounds will be discovered. Furthermore, do these compounds have a common structural basis that would be consistent with them being used for reflection or scattering?

Although not all the reports of the presence of guanine in eyes may be correct, there is no doubt that guanine is a widespread component of visual systems. Guanine is practically insoluble in water, raising the question of how the large amounts of material needed for crystal deposition can be efficiently transported and concentrated in appropriate compartments where the crystals are deposited. On the other hand, the low solubility of guanine crystals also guarantees their stability in varying environments. In cases where the guanine crystals have been thoroughly examined, it has been shown that the crystals are usually thin plates composed of anhydrous guanine with one specific polymorph type (the β form, **Figure 1**).[30] Why are thin anhydrous guanine crystal plates so well suited to build reflectors? One obvious reason is in their optical properties, specifically the extraordinarily high refractive index $n = 1.83$, measured for light impinging on the crystals perpendicular to the large plate face (note that the value cited here is the mean of the two refractive index values for light polarized within the plate plane, 1.81 and 1.85).[31,32] As discussed above, this high refractive index makes them excellent reflectors. The high refractive index for light polarization within the plane is due to the crystal structure, characterized by hydrogen-bonded layers of the polycyclic conjugated purine rings in the *bc* plane, with the aromatic rings interacting in the a^* direction through π–π stacking (Figure 1). Note that α-anhydrous guanine has the same structure in the hydrogen bonded plane as the β-polymorph, and thus the same high refractive index.[33] The choice of the β form in biology is thus not due to its superior optical properties.

Uric acid belongs to the purine family of molecules, as does guanine. Uric acid crystals appear in more than one phase. The dihydrate form, which crystallizes in biological environments such as bird urine,[34] bladder and kidney stones,[35] is also characterized by planes of hydrogen bonded molecules[36] that may well produce a high refractive index for light perpendicular to the plane. This would be similar to guanine. We note, however, that the crystal phase of uric acid in the eye tapeta (Table 1) has not been determined. Furthermore, even the chemical nature of the compound forming the crystals, whether uric acid or some uric acid salt, is not clear. We can only presume that the phase is uric acid dihydrate. The same uncertainty recurs in most of the cases in which biogenic crystals were identified in animal eyes, and often the biogenic crystal structures, once determined, turn out to be different from those determined in synthetic crystals.[30] The crystal structure of xanthine is not available yet, and the structure of isoxanthopterin crystals extracted from crustacean eyes has only recently been determined.[24] Biogenic isoxanthopterin crystals have the same structural motif of hydrogen bonded planar networks between conjugated heterocyclic pterin molecules, as do guanine and uric acid.

Riboflavin is also a heterocyclic aromatic molecule, with a relatively long hydroxylated sidechain. In the available crystal structures, the aromatic molecules form flat hydrogen-bonded ribbons separated by hydrogen-bonded motifs of the sidechains. Despite the relatively high refractive index along certain crystal axes ($n = 1.74$)[37] the crystals' suitability for light reflection is thus less easy to predict, especially because there are several reported phases and the biogenic crystal structure is not known. Interestingly, riboflavin absorbs light at short wavelengths, but fluoresces at wavelengths similar to rhodopsin absorption (around 500 nm).[10] Thus, tapeta with riboflavin crystals not only reflect light, but the fluorescence can also convert impinging light at short wavelengths into a more biologically useful spectral range.[10,26]

The glaring exception to the planar aromatic hydrogen-bonded heterocyclic character of the crystals used for vision is zinc cysteinate. This molecule has a totally different molecular structure and consequently a different crystal packing arrangement.[38] Because of the molecular structure, under no circumstances can a Zn and cysteine salt have the delocalized electron density and the flat geometry found in the other molecules. The evidence for the presence of zinc cysteinate is based mainly on the presence of high concentrations of both zinc and cysteine

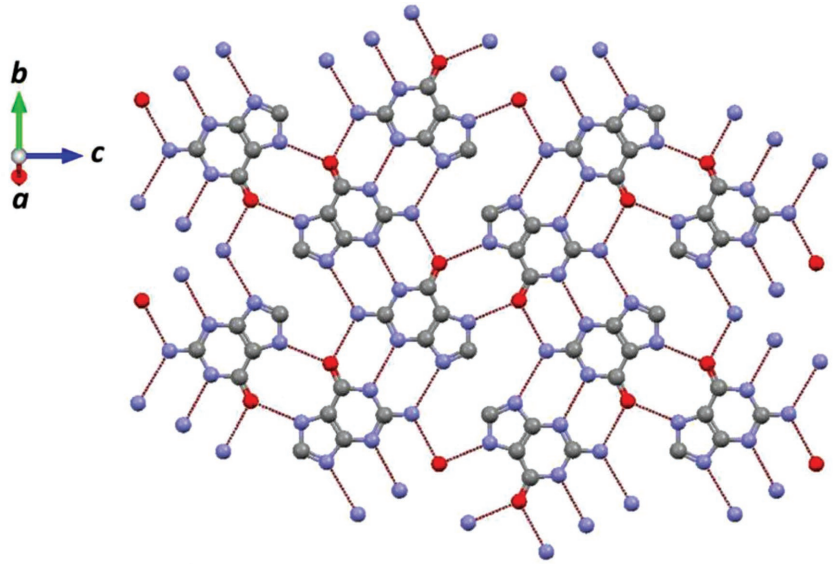


Figure 1. The crystal structure of β-anhydrous guanine in the *bc* plane, showing the rich H-bonding network. C = gray, N = blue, O = red, H-bonds = red lines. The layer structure is perfectly planar. Adapted with permission.[8] Copyright 2017, Wiley-VCH.

in the eye tissues, and in some cases in stoichiometric proportions.[29] No crystals were apparently isolated and characterized. We note that the occurrence of high levels of zinc in the eyes might also be due to the presence of melanophores.[39]

7,8-Dihydroxanthopterin, reported to be present in the tapetum of a fish,[25] is also an exception because the molecule is not planar, and we think that its characterization as a crystalline reflecting material needs to be confirmed.

The shared morphology of biogenic reflector crystals, when known, is plates a few tens of nanometers thick. We note that the plate shape for guanine, and probably some of the other organic crystals listed in Table 1 that have a layered structure similar to guanine, is not the thermodynamically most stable shape.[8] In order to produce thin plates, the crystal formation process has to be well controlled. The little we know about this process is that the crystals are formed by specialized cells (iridophores) and each crystal forms in a membrane-bound vesicle.[40] We also know that at least in one case, the guanine crystals form via a highly disordered precursor phase.[41] One possible scenario is that the so-called amorphous guanine fills the vesicle that is already plate-shaped, and then the amorphous guanine is induced to crystallize. This strategy is used for the production of many inorganic crystals produced in biology.[42] Another possible scenario is that the crystal morphology is controlled by the interaction of specific additives within the growing crystal, which inhibit growth of the guanine crystals along the *a* axis, inducing the formation of plate-shaped crystals.[43]

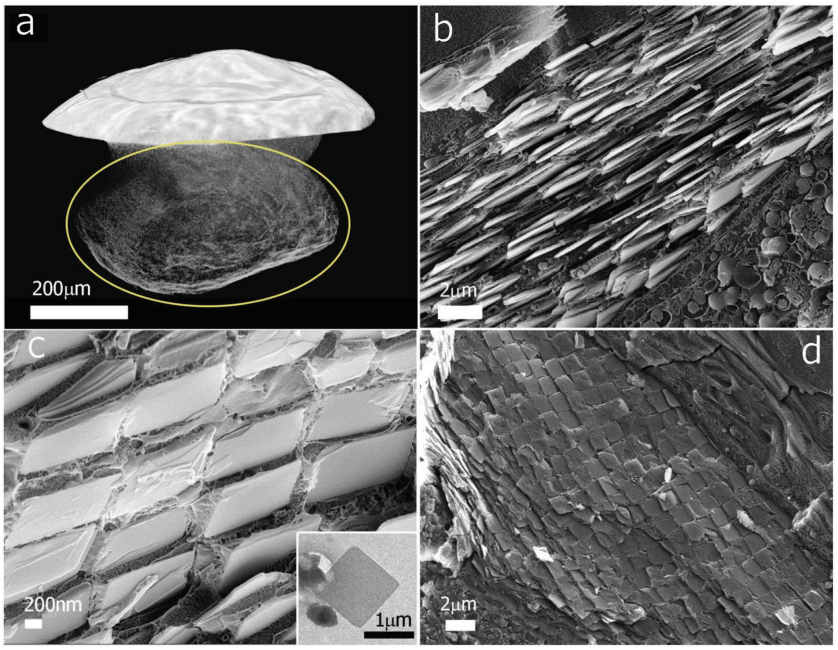


Figure 2. Scallop eyes. a) Oblique 3D microCT image of a hydrated and fixed eye from the scallop *Pecten maximus*. In the upper part of the image is the cornea, under which is located the lens. The curved mirror marked by the yellow ellipse has a flattened bottom. b) Cross section of the mirror, showing the guanine crystals and cytoplasm intercrystalline layers in a high-pressure-frozen and freeze-fractured sample observed by SEM under cryogenic conditions. c) The square guanine crystals seen by cryo-SEM in an oblique fracture through the mirror. Inset: a TEM image of a single crystal extracted from the mirror. d) The perfect tiling of the square crystals seen by cryo-SEM in a fracture almost parallel to the mirror surface. Several layers are exposed by the fracture. Note that in (c) and (d) the crystals appear as parallelepipeds rather than squares, because of the oblique view. Only in the inset in (c) the crystal is viewed from a direction perpendicular to the plate, and appears as a perfect square.

In the next section we present three test cases that we have studied under conditions which preserve the hydrated eye anatomy and where the organic reflecting/scattering crystals have been identified and characterized. In the Discussion section, we mainly use these test cases to highlight what we regard as interesting insights that hopefully have more general relevance. We also identify open questions for further research.

4. Test Case: The Scallop Eye

Figure 2A shows a 3D microCT image of a hydrated, chemically fixed scallop eye. This is one of the tens of eyes lining the scallop's mantle. A cornea that is transparent to light covers the eye. The light passing through the cornea penetrates a lens. This lens, however, has no light focusing power, because its refractive index is practically identical to that of water.[44] It would be extremely difficult to make a lens of this size with a high enough refractive power to focus light onto the retina, especially in sea water where the surrounding medium has a refractive index of $n = 1.33$ (rather than $n = 1$ in air). Presumably, for these reasons the scallop evolved a mirror to focus light, rather than a lens (Figure 2a). The mirrors in the eyes of scallops are extremely efficient light-collectors compared to lenses of the same size.[4] Concave mirrored eyes have the advantage of being able to collect light from a very large field of view and as a result such eyes are often very light-sensitive and well-suited for use in dim light habitats.[45] Similar concave-mirrored eyes have also been found in deep-sea fish[46] and deep sea crustaceans,[47] although the crystalline materials used in these mirrors are not known and in the latter case, the primary function of the mirror is light-collection rather than image-formation.

At the nanometer to tens of nanometers level, the highly reflecting anhydrous guanine crystal plates in the scallop eye are good representatives of the most frequently encountered components of biogenic crystal reflectors, namely multilayers of guanine crystals.[19] The thickness of the crystals in the scallop eye is ≈70 nm, and the thickness of the cytoplasm intercrystal spacings is ≈85 nm, yielding an almost ideal quarter wavelength plate reflecting in the blue-green range (Figure 2b), following the equation

$$2(n_A d_A + n_B d_B) = m\lambda,\ \lambda = 2(74 \times 1.83 + 86 \times 1.33), \approx 500\ \text{nm}$$

The meaning of this equation is that light of wavelengths ≈500 nm is reflected when hitting the mirror perpendicular

to its surface.[48] Interestingly, this corresponds to the spectral range of the light that penetrates the water in southern Wales and in France where the scallops we studied live.[48] It will be interesting to find out whether scallops living in shallower or less attenuating/clearer water have crystals spaced at a larger distance. Larger crystal spacings would reflect light of longer wavelengths corresponding to the light that penetrates shallower or clearer seawater.

At the micrometer to millimeter level, the determining features in the construction of the mirror in the scallop eye are the crystal morphology and the crystal stacking architecture. The crystal morphology is regular squares with a lateral size of close to 1 μm (Figure 2c,d).[19,48] The crystals achieve this regular square morphology through twice-repeated twinning.[49] The complexity of the twinning process required to achieve structural control over the square morphology indicates that producing a crystal of guanine with this morphology is functionally advantageous for the eye. Indeed, it is this morphology, together with the size of each tile, much smaller than the mirror curvature, which allows almost perfect tiling of the curved surface (Figure 2d). This is not the only solution for tiling a curved surface with regular polygons. An alternative solution is tiling with regular hexagons; a solution adopted by copepods for building the reflective layer in their dorsal cuticle surface.[50] The guanine crystals in this latter case also achieve regular hexagonal morphology through twice-repeated twinning.[49]

The overall morphology of the mirror corresponds to a slightly flattened bowl, such that light reflected from the flattened part of the mirror preferentially reflects onto the distal retina, and light reflected from the more curved peripheral parts of the mirror reflects onto the proximal retina. The distal retina is consequently adapted to form images under stronger direct light, whereas the proximal retina is adapted to operate under dimmer peripheral light.[48]

The final product of this complex visual system, is a structure controlled at all hierarchical levels from the Ångstrom to the millimeter scale. We note that the segmented reflecting mirror is structurally similar to mirrors in reflecting telescopes, but of course the tiles are orders of magnitude smaller.[51]

5. Test Case: The Compound Eyes of Decapod Crustaceans

Shrimp, lobsters, crayfish, and prawns are all crustaceans belonging to the order of Decapoda in the arthropod phylum. These crustaceans evolved compound eyes defined as "reflecting superposition eyes."[52,53] The compound eyes are composed of thousands of square-faceted units (**Figure 3**a,b). Each elongated and tapered unit is >500 μm long and only 50 μm wide. Light is reflected from the top of each square-faceted eye unit across a so-called "clear zone" (equivalent to the vitreous humor in mammalian eyes) onto the retina. At grazing incidence light reflection occurs off the walls of the eye units by total internal reflection since the inside of the eye units (n = 1.41) has a slightly higher refractive index than the outside (n = 1.34). At higher angles of incidence, up to ≈40° (with respect to the optical axis of a single eye unit), mirrors lining the top 100 μm of each facet reflect the light. The superposition optics originate from the clear zone that allows light reflected from several hundreds of units to superimpose on the retina.[52,53] The superposition of light from many facets significantly increases the light sensitivity, making the eyes suitable for vision in dim light conditions. Furthermore, a second reflector, the tapetum, which lies immediately behind the retina and extends between the retinal units (rhabdoms), backreflects light that was not absorbed by the retina[53] and is responsible for the observed eye-shine of decapod crustaceans.[54]

Both reflecting apparatuses, the distal mirror and the tapetum, are composed of crystals of isoxanthopterin, but the sizes, morphologies, and arrangements of the crystals are

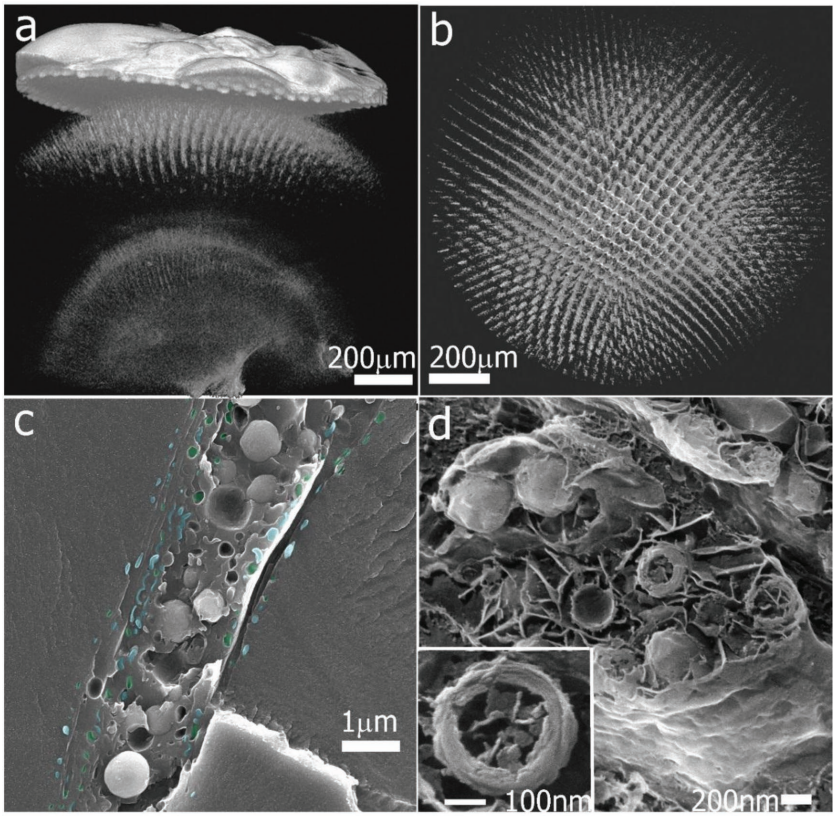


Figure 3. The eyes of decapod crustaceans. a,b): 3D microCT images of a hydrated and fixed eye from the crayfish *Procambarus clarkii*. a) Longitudinal view: from top to bottom are the cornea, followed by the distal mirror, the clear zone and the tapetum. b) View top down onto the square-faceted units of the compound eye, lined by the crystals forming the distal mirror. c) Cryo-SEM micrograph of the sparsely distributed crystals (edge on, colored blue, green) forming the reflecting multilayer lining the square units in the distal mirror of *Cherax quadricarinatus*. The space between the mirrors is occupied by absorbing pigment granules. d) Reflecting granules in the tapetum reflector. The hollow granules are composed of concentric layers of nanocrystals (inset).

completely different.[23,24] In the distal mirror, the crystals are ≈200 nm wide and ≈40 nm thick plates with no well-defined or conserved morphology (Figure 3c).[24] It is however possible that the morphology seen in the image is slightly different in vivo, because the electron sensitive crystals are deformed by exposure to the electron beam. The crystals are distributed in three or four loosely packed rows, where the crystal coverage of the area in each row does not exceed 50%. Such a mirror produces excellent reflectivity for light hitting the mirror at very low angles, and very poor reflectivity for light hitting the mirror at close to normal incidence. At normal incidence the mirror performs poorly because of the sparse distribution of the crystals.[24] Interestingly, the result of this low performance at high angles is that light from high incidence angles, which is affected more by spherical aberration, is preferentially attenuated and contributes less to the image. This phenomenon therefore reduces image-blurring—a process known as "apodization" in optics. The attenuation is assisted by a 1–3 μm thick layer of densely packed, 1 μm large pigment granules located behind the mirror (Figure 3b). These pigment granules efficiently absorb the light entering a square-faceted unit at an angle substantially deviating from its axis. The poor reflectivity of the mirror at high angles also reduces the possibility that light entering at acute angles will be reflected multiple times from the same facet. This would result in light being scattered/reflected stochastically across the clear zone—reducing the image contrast.

Earlier ultrastructural studies reported that the crustacean tapetum is composed of 400 nm reflecting granules.[55] We identified these reflecting granules as densely packed, partly hollow nanoparticles, ≈400 nm in diameter (Figure 3d).[24] Each spherical particle contains several layers of crystals arranged in an onion-like structure. Each crystal is a plate ≈100 nm wide and ≈20 nm thick. These reflecting granules underlie the retina and also penetrate into the spaces between retinal units. The reflecting granules are adapted both in their size and in their structure to provide efficient back-scattering of the dispersed light. The light that is not backscattered onto the rhabdoms is absorbed by pigment granules lying beneath the tapetum reflector as in the distal mirror (Figure 3d). Interestingly, the unique construction and optics of the reflecting superposition compound eye inspired the development of the 'Lobster Eye Telescope' used for imaging X-ray radiation in astronomy.[56]

6. Test Case: The Reflective Fish Iris

The iris is a unique component of the visual system, as it has a dual function. The main function of the iris is visual—blocking light from entering and reaching the retina from unwanted positions or directions. The iris can also have a second communication function, related to the way it is viewed from the outside. This communication function could include attracting potential mates[57] or as part of the external camouflage of an animal.[58]

Iridescence in the irises of fish was reported over a century ago by von Brücke[2] (cited in ref. [58]). Yet only after the invention of the transmission electron microscope was fish iris iridescence shown in the Neon Tetra fish to be associated with a multilayer stack of guanine crystals.[59] Using interference microscopy the crystal thicknesses in the blue and red iridescent layers in the Neon Tetra iris were found to be ≈65 and ≈95 nm respectively, from which it was concluded that the respective iridescent layers are nearly perfect quarter-wave reflectors.[59] The crystals are elongated, with long axes ≈20 μm and short axes a few microns. Direct observation of the details of the iridescent structures of the iris has been hampered, however, by the difficulty in maintaining the structures intact when conducting electron microscopy. A partially ordered structure is present in some regions of the Neon Tetra iris, whereas a seemingly disordered structure was observed in the iris of the pipefish.[60]

The zebrafish eye often serves as a model for disease and vertebrate eye development, and is therefore relatively well studied compared to other fish eyes.[61] An image of the zebrafish eye as observed from outside is presented in **Figure 4**a. The silvery reflectance from the iris is clearly visible and is similar to the reflectance from the neighboring skin. This suggests the presence of a reflector layer where crystals are parallel to one another, as is characteristic of other silvery reflectors, including the zebrafish eye tapetum.[62] A combination of optical and TEM images of eye slices revealed that the zebrafish iris is composed of at least two layers—an outer layer of iridophores and an inner thin layer of pigment cells.[63,64] In the adult zebrafish the iridophore layer is over 10 μm thick, whereas the pigment layer is only several micrometers thick.[63,64] The crystals contained within the iridophores are elongated hexagons having dimensions of about 10 μm by 1 μm and an average thickness of 25 nm (Figure 4b), in good correspondence with previous observations of guanine crystal plates in fish irises and scales.[59,65] The crystals are composed of the β-phase of guanine, as seen by X-ray powder diffraction.[66] Interestingly, both the iridophore layer and the pigment layer are visible at 3 days post fertilization, which is consistent with the fact that the zebrafish larvae already have high visual acuity at 5 days post fertilization (dpf).[67] Taken together, these observations suggest a hybrid reflective-absorptive system for inhibiting light from entering the eye outside the area of the lens.[66] An immediate question that arises relates to the effectiveness of using such a hybrid system. It is well known that the reflectivity of multilayer reflective guanine stacks is still far from unity, even if the layer is composed of a dense arrangement of crystals.[68] Thus further blocking of incident light beyond the ordered reflector is necessary. This function must then be performed by additional functional layers that are present other than the guanine

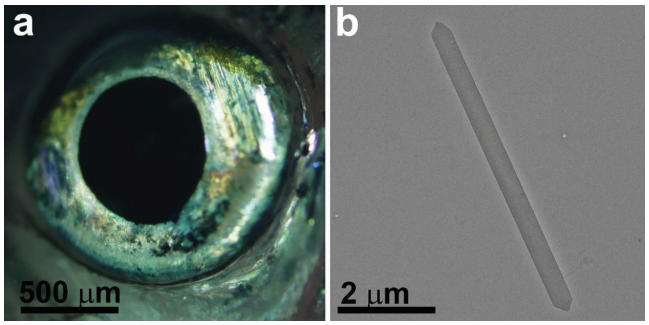


Figure 4. The zebrafish iris. a) A top view of the zebrafish eye showing the silvery iris. b) A TEM image showing a single crystal of guanine extracted from the zebrafish iris argentum.

based reflector. The thin pigmented layer likely serves to absorb remaining light which was not backscattered by the iridophore layer. Notably, this layer is only several microns thick,[63] such that its optical density is relatively low.[69] Yet, combined with the reflector layer and possibly with other scattering elements within the iris (such as xanthophores, clearly visible on top of the iridescent mirror, or less ordered regions of crystals within the iridophore layer), the thin pigmented layer appears to serve as a highly efficient light blocking layer.

7. Discussion

The large variety of crystal morphologies, arrangements, and functions of the reflector and scatterer systems exemplified by the three test cases above, is impressive. Immediate questions that arise are how the choice of structure is related to the optical function (if at all), and what are the guidelines for constructing an efficient functional unit.

Perhaps the first question relates to the widespread use of guanine plates in the reflectors of the visual system. Are the optical properties of guanine unique in any sense?[8] Is this a result of cellular availability or does it have more to do with the ability to control the morphology of guanine? Here we discuss these still open questions further.

The most obvious advantage of guanine is the high refractive index in the plane along which plates are grown. In most configurations, as in the scallop eye,[48] in the zebrafish iris,[66] and in some, but not all fish scales,[68] incident light impinges upon guanine platelets from a perpendicular direction. Thus only the high in-plane refractive index of guanine (n = ≈1.83) plays a role. The high refractive index and crystal anisotropy are not unique properties of guanine. Riboflavin, for example, also has a high refractive index n = 1.74.[37] Another example is isoxanthopterin, from which the reflective structures in the crayfish and shrimp eye are composed. This material has an even higher calculated average refractive index (for light polarized within the plane of the plate) of n = 1.96 and crustaceans produce plate-like crystals of isoxanthopterin.[24] For illustration purposes, **Figure 5** shows the calculated peak reflectivity at normal incidence for these 3 values of the refractive index as a function of the number of double layers (assuming a refractive index of 1.36 for the cytoplasm). As can be seen, for reflectors with more than ten double layers, such as the scallop mirror, the reflectivity already saturates at near-unity even for lower values of the refractive index. Nevertheless, tolerance to small deviations from a perfect quarter-wave stack increases with a higher refractive index and with an increasing number of layers.

The widespread use of guanine is most likely also related to other advantages that guanine has over alternative crystalline reflectors. One possible advantage is cellular availability. Guanine is part of the purine metabolism cycle and is thus readily available in every cell. In certain organisms, including humans, this cycle often results in excretion of uric acid in the urine.[70] In other organisms, such as spiders, the cycle stops at guanine, which is expelled as a solid.[71] Furthermore, as inferred from the large diversity of guanine crystal shapes and sizes, biology has evolved "tools" to control the crystallization of guanine. The "tools" have yet to be understood. Surprisingly, controlled growth

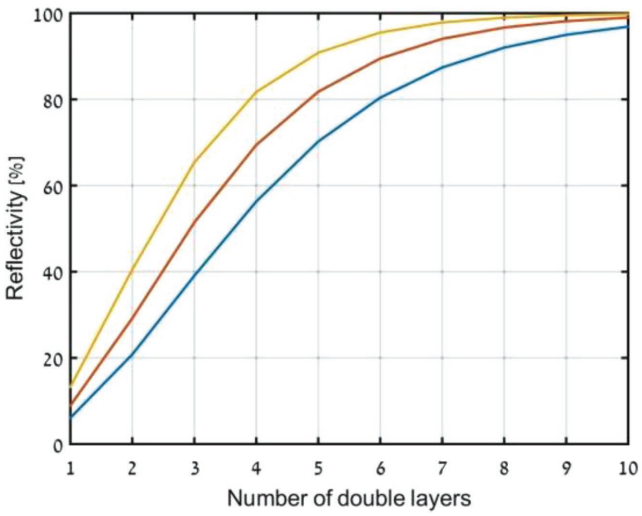


Figure 5. Calculated peak reflectivity at normal incidence as a function of the number of double layers for riboflavin (blue, n = 1.74), guanine (red, n = 1.83), and isoxanthopterin (yellow, n = 1.96). Note that the difference in the refractive index between the two in-plane axes for these crystals is about 0.05, which is much smaller than that observed between different crystals.

of artificial guanine crystals resembling the biogenic ones is extremely difficult.[33] In fact, in vitro growth of the naturally occurring metastable β polymorph of guanine has only recently been reported. Furthermore, when formed in vitro β-guanine is not stable under physiological pH conditions.[33] Further study of the still elusive, yet clearly highly regulated biogenic crystallization and growth mechanism, has promise with respect to the industrial use of guanine and analogous materials.

Guanine exhibits large variability in crystal shapes and sizes in various reflective and scattering systems. Guanine plates can have an area as small as 1 μm^2 (as in the case of the scallop reflectors[48]) and as large as several hundreds of square micrometers (as in the case of tapetum lucidum layer of the coelacanth (*Latimeria chalumnae*)[11]). Plate thickness can vary from ≈20 nm (as in the Koi fish scales[65] to about 100 nm[7] which, considering the high refractive index, can serve as a component in a quarter-wave reflector across the entire UV-vis range.[59] Importantly, within a single species, plate thicknesses are highly regulated and exhibit extremely low variance, especially where narrow band reflection is achieved.[48,72] Interestingly, this variability is often well below the level of variation that would affect its function.

The fact that in a narrow band reflector the low-index layer is comprised of cytoplasm affords tunability of the reflected color while keeping the crystal thickness fixed, and without significant penalty to the reflector efficiency (as has been observed in the tunable reflectors of the sapphirinid copepods[73]). Broadband reflectors are usually based on a disordered (or partially disordered) crystal stack. In this case, the particular choice of crystal thickness is less important, and, in principle, crystal thickness need not be highly regulated. Surprisingly, crystal thickness is highly regulated even in these systems (such as fish scales[65,68]), and disorder is introduced by altering the low-index cytoplasm spacings alone. This hints at the possibility that the process by which guanine plates are formed, at least in fish, may intrinsically regulate crystal thickness.

The shape and the lateral dimensions of guanine plates have a less direct relation to the optical function. A planar reflector can be constructed either from small plates or from large plates without seemingly affecting the reflective properties, as long as the integrity of the crystal stack is not compromised. For example, the scallop mirror[48] and the zebrafish iris[66] seem to have rather similar reflectivities, but very different crystal sizes and shapes. For more demanding applications, such as in the context of vision, which require gap-free coverage of a large area (to avoid loss and optical diffraction artifacts), the shape of the plates must allow for tiling of the plane. For such cases, the lateral dimensions must be strongly regulated too, so that plates are sufficiently uniform to enable tiling. Tiling has been shown for square plates (as in the scallop eye[48]), hexagonal plates (as in the sapphirinid copepods[50]) or elongated hexagons (as in the zebrafish iris[66]). Tiling likely also aids in maintaining the multiple layers in the stack parallel to one another, especially for stacks comprised of multiple layers. For cases where the reflector layer is not tiled, as in fish scales, it is likely that the use of larger plates facilitates maintaining parallelism between the layers, as even rather small angular distortions become sterically forbidden. In contrast, when the reflector is nonplanar, smaller tiles can make it possible to smoothly follow the curved geometry, although the presence of some local defects or gaps between the tiles is unavoidable. Clearly, tiles must be significantly smaller than the radius of curvature of the surface to minimize defects, a condition which is well fulfilled in both the scallop eye and the zebrafish iris (for the latter, at least at late developmental stages). The scallop eye, where the curved mirror surface has a focal length which is only several hundreds of micrometers, is particularly interesting.[48] In this case, large tiles can induce optical aberrations which scale as $d^2/\lambda f$, where d is the lateral dimension of the plate, λ the wavelength of light and f the focal length of the mirror. For visible light, aberrations induced by the planarity of the tiles will become very severe for plates with a lateral dimension of ≈10 µm, and are completely negligible for the 1 µm square plates such as those in the scallop eye.

Another intriguing topic relates to the choice and structure of scattering layers, whose function is especially important in increasing light harvesting efficiencies by photoreceptors.[74] This light harvesting is achieved by backscattering unabsorbed incoming light onto the photoreceptor. In vision, two very different motifs are found in this context. The first are tapeta that rely on highly directional backscattering.[31,58] These ordered layered reflectors are similar to the ones found in the scallop reflective mirror or in fish scales. Their orientation is such that reflected light retraces its path. Upon reflection, light which was not absorbed by the retina gets a second chance to be absorbed and eventually exits the eye (so as not to enhance the background of scattered light within the eye). Such a mechanism can only be operational if the retina geometry is relatively flat such that scattering from it or from the scattering layer is minimal. In other cases, such as the tapetum of the crayfish eye, an alternative motif, employing nondirectional backscattering by a completely disordered reflector is used.[24] In this case, backscattered light covers a broad angular range and is thus significantly less controlled. In the crayfish eye, it is based on Mie backscattering from sub-wavelength sized spheres. A judicious choice of the material and size of the scatterers (with typical sizes of a fraction of a wavelength) can exhibit strong backscattering for certain wavelengths of light. In order to avoid excessive scattering of light into nearby photoreceptors in the light-adapted eye state, pigment granules are combined into the reflective layer, blocking long-range propagation of light within it. Diffusely scattering tapeta have also been observed in fish,[75] crocodiles,[10] and in the opossum.[76] In bird feathers[77] such structures can be especially efficient in backreflecting shorter wavelength blue light, due to the stronger backscattering this light experiences. Size and composition tuning of the scattering layer can, however, induce stronger scattering for other colors as well. It is indeed often useful to combine reflective and absorptive components to improve the overall optical performance of such a system. Such hybrid systems can also be seen in the zebrafish iris and in the reflecting superposition eyes of decapod crustaceans, where remaining light passing through the reflecting layer is readily absorbed by the pigment layer.

8. Concluding Comment

Reflectors are used in eyes to form images, to increase photon capture and for regulating the amount of light reaching the eye. The structure and organization of reflective and scattering organic crystalline components in the eyes of animals, especially those living in water, is multifaceted and depends on a highly organized hierarchical structure. Interestingly, despite the variability in the ways in which reflecting components function, the organic molecules that are used for building reflectors and their characteristic packing modes in the crystal structures, are unexpectedly limited. We are only now beginning to understand the relation between the molecular structure, crystal structure, and reflecting properties. Such an understanding could lead to new applications in the field of material science.

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Conflict of Interest

The authors declare no conflict of interest.

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crystals, eyes, guanine, reflection, vision

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[1] G. Abelsdorf, *Arch. Physiol.* **1898**, 155.
[2] E. W. von Brücke, *Arch. Anat. Physiol. Wiss. Med.* **1845**, 387.
[3] S. Exner, *Die Physiologie der Facettiertern Augen von Krebsen und Insecten*, Franz Deuticke, Leipzig **1891**.
[4] M. F. Land, D.-E. Nilsson, *Animal Eyes*, 2nd ed., Oxford University Press, Oxford **2012**.
[5] J. N. Lythgoe, *The Ecology of Vision*, Clarendon Press, Oxford, **1979**.
[6] J. Dubochet, M. Adrian, J.-J. Chang, J.-C. Homo, J. Lepault, A. W. McDowall, P. Schultz, *Q. Rev. Biophys.* **1988**, *21*, 129.
[7] T. M. Jordan, J. C. Partridge, N. W. Roberts, *J. R. Soc. Interface* **2014**, *11*, 20140948.
[8] D. Gur, B. A. Palmer, S. Weiner, L. Addadi, *Adv. Funct. Mater.* **2017**, *27*, 201603514.
[9] M. F. Land, *Prog. Biophys. Mol. Biol.* **1972**, *24*, 75.
[10] F. J. Ollivier, D. A. Samuelson, D. E. Brooks, P. A. Lewis, M. E. Kallberg, A. M. Komáromy, *Vet. Opthalmol.* **2004**, *7*, 11.
[11] N. A. Locket, *Proc. R. Soc. London, Ser. B* **1974**, *186*, 281.
[12] E. J. Denton, *Philos. Trans. R. Soc., B* **1970**, *258*, 285.
[13] A. Pirie, D. M. Simpson, *Biochem. J.* **1946**, *40*, 14.
[14] W. Kühne, H. Sewall, *Unters. Physiol. Inst. Heidal.* **1880**, *3*, 221.
[15] a) A. Pirie, in *Comparative Ophthalmology* (Ed: O. Graham-Jones), Pergamon Press, Oxford **1966**, p. 57; b) H. Laurens, S. R. Detwiler, *J. Exp. Zool.* **1921**, *32*, 207.
[16] L. H. Kleinholz, *Biol. Bull.* **1959**, *116*, 125.
[17] W. H. Fahrenbach, *Int. Rev. Cytol.* **1975**, *41*, 285.
[18] K. P. Mueller, T. Labhart, *J. Comp. Physiol. A* **2010**, *196*, 335.
[19] M. F. Land, *J. Exp. Biol.* **1966**, *45*, 433.
[20] A. Böhm, G. Pass, *J. Exp. Biol.* **2016**, *219*, 3039.
[21] E. S. Zyznar, F. B. Cross, J. A. C. Nicol, *Proc. R. Soc. London, Ser. B* **1978**, *201*, 1.
[22] J. A. Nicol, H. J. Arnott, *Can. J. Zool.* **1980**, *54*, 114.
[23] E. S. Zyznar, J. A. C. Nicol, *J. Exp. Mar. Biol. Ecol.* **1971**, *6*, 235.
[24] B. A. Palmer, A. Hirsch, V. Brumfeld, E. Aflalo, I. Pinkas, A. Sagi, S. Rozenne, D. Oron, L. Leiserowitz, L. Kronik, S. Weiner, L. Addadi, *Proc. Natl. Acad. Sci. USA* **2018**, *115*, 2299.
[25] E. S. Zyznar, M. A. Ali, *Can. J. Zool.* **1975**, *53*, 180.
[26] A. Pirie, *Nature* **1959**, *183*, 985.
[27] G. Y. Wen, J. A. Sturman, J. W. Shek, *Lab. Anim. Sci.* **1985**, *35*, 200.
[28] G. Weitzel, F.-J. Strecker, U. Roester, E. Buddecke, A.-M. Fretzdorf, *Z. Physiol. Chem.* **1954**, *296*, 19.
[29] G. Y. Wen, J. A. Sturman, H. M. Wisniewski, A. MacDonald, W. H. Niemann, *Invest. Ophthalmol. Vis. Sci.* **1982**, *23*, 733.
[30] A. Hirsch, D. Gur, I. Polishchuk, D. Levy, B. Pokroy, A. J. Cruz-Cabeza, L. Addadi, L. Kronik, L. Leiserowitz, *Chem. Mater.* **2015**, *27*, 8289.
[31] E. J. Denton, *Philos. Trans. R. Soc., B* **1970**, *258*, 285.
[32] W. J. Schmidt, *Giessener Naturwiss. Vorträge* **1949**, *6*, 71.
[33] D. Gur, M. Pierantoni, N. Elool Dov, A. Hirsch, Y. Feldman, S. Weiner, L. Addadi, *Cryst. Growth Des.* **2016**, *16*, 4975.
[34] K. Lonsdale, D. J. Sutor, *Science* **1971**, *172*, 958.
[35] K. Lonsdale, P. Mason, *Science* **1966**, *152*, 1511.
[36] H. Ringertz, *Acta Cryst.* **1966**, *20*, 397.
[37] J. K. Dale, *US 2603633 A*, **1952**.
[38] P. Bell, W. S. Sheldrick, *Z. Naturforsch.* **1984**, *39b*, 1732.
[39] J. M. Bowness, R. A. Morton, *Biochem. J.* **1953**, *53*, 620.
[40] D. L. Fox, *Animal Biochromes and Structural Colours*, Cambridge University Press, Cambridge **1953**.
[41] D. Gur, Y. Politi, B. Sivan, P. Fratzl, S. Weiner, L. Addadi, *Angew. Chem., Int. Ed.* **2013**, *52*, 388.
[42] L. Addadi, S. Raz, S. Weiner, *Adv. Mater.* **2003**, *15*, 959.
[43] I. Weissbuch, L. Addadi, M. Lahav, L. Leiserowitz, *Science* **1991**, *253*, 637.
[44] M. F. Land, *J. Physiol.* **1965**, *179*, 138.
[45] M. F. Land, *J. Opt. A: Pure Appl. Opt.* **2000**, *2*, 44.
[46] a) J. C. Partidge, R. H. Douglas, N. J. Marshall, W. S. Chung, T. M. Jordan, H.-J. Wagner, *Proc. R. Soc. London, Ser. B* **2014**, *281*, 1; b) H. J. Wagner, R. H. Douglas, T. M. Frank, N. W. Roberts, J. C. Partidge, *J. Curr. Biol.* **2009**, *19*, 108.
[47] A. Andersson, D.-E. Nilsson, *Protoplasma* **1981**, *107*, 361.
[48] B. A. Palmer, G. J. Taylor, V. Brumfeld, D. Gur, M. Shemesh, N. Elad, A. Osherov, D. Oron, S. Weiner, L. Addadi, *Science* **2017**, *358*, 1172.
[49] A. Hirsch, B. A. Palmer, N. Elad, D. Gur, S. Weiner, L. Addadi, L. Kronik, L. Leiserowitz, *Angew. Chem., Int. Ed.* **2017**, *56*, 9420.
[50] D. Gur, B. Leshem, M. Pierantoni, V. Farstey, D. Oron, S. Weiner, L. Addadi, *J. Am. Chem. Soc.* **2015**, *137*, 8408.
[51] W. P. McCray, *Giant Telescopes*, Harvard University Press, Cambridge, MA, **2006**.
[52] K. Vogt, *Z. Naturforsch.* **1975**, *30c*, 691.
[53] M. F. Land, *Nature* **1976**, *263*, 764.
[54] P. Kuzne, in *Comparative Physiology and Evolution of Vision in Invertebrates*, Vol. *7* (Ed: H. Autrum), Springer, Berlin **1979**, p. 441.
[55] a) G. Struwe, E. Hallberg, R. Elofsson, *J. Comp. Physiol.* **1975**, *97*, 257; b) P. M. J. Shelton, E. Gaten, C. J. Chapman, *J. Exp. Mar. Biol. Ecol.* **1986**, *98*, 185.
[56] J. R. P. Angel, *Proc. SPIE* **1979**, *0184*, 84.
[57] I. N. Flamarique, C. Bergstrom, C. L. Cheng, T. E. Reimchen, *J. Exp. Biol.* **2013**, *216*, 2806.
[58] G. L. Walls, *The Vertebrate Eye and its Adaptive Radiation*, Cranbrook Institute of Science, Oxford, **1942**.
[59] E. J. Denton, M. F. Land, *Proc. R. Soc. London, Ser. B* **1971**, *178*, 43.
[60] J. N. Lythgoe, J. Shand, *J. Physiol.* **1982**, *325*, 23.
[61] S. C. F. Neuhauss, *Fish. Physiol.* **2010**, *29*, 81.
[62] a) M. Hirata, K. Nakamura, T. Kanemaru, Y. Shibata, S. Kondo, *Dev. Dyn.* **2003**, *227*, 497; b) M. Hirata, K. Nakamura, S. Kondo, *Dev. Dyn.* **2005**, *234*, 293.
[63] K. A. Soules, B. A. Link, *BMC Dev. Biol.* **2005**, *5*, 12.
[64] M. P. Gray, R. S. Smith, K. A. Soules, S. W. M. John, B. A. Link, *Invest. Ophthalmol. Vis. Sci.* **2009**, *50*, 1515.
[65] A. Levy-Lior, B. Pokroy, B. Levavi-Sivan, L. Leiserowitz, S. Weiner, L. Addadi, *Cryst. Growth Des.* **2008**, *8*, 507.
[66] D. Gur, J.-D. Nicolas, V. Brumfeld, O. Bar-Elli, D. Oron, G. Levkowitz, *Adv. Sci.* **2018**, *5*, 1800338.
[67] a) S. S. Easter, G. N. Nicola, *Dev. Biol.* **1996**, *180*, 646; b) V. C. Fleisch, S. C. F. Neuhauss, *Zebrafish* **2006**, *3*, 191.
[68] D. Gur, B. Leshem, D. Oron, S. Weiner, L. Addadi, *J. Am. Chem. Soc.* **2014**, *136*, 17236.
[69] E. V. Koblova, A. N. Bashkatov, E. A. Genina, V. V. Tuchin, V. V. Bakutkin, *Proc. SPIE* **2005**, *5688*, 302.
[70] J. Maiuolo, F. Oppedisano, S. Gratteri, C. Muscoli, V. Mollace, *Int. J. Cardiol.* **2016**, *213*, 8.
[71] J. F. Anderson, *Comp. Biochem. Physiol.* **1966**, *17*, 973.
[72] D. Gur, B. A. Palmer, B. Leshem, D. Oron, P. Fratzl, S. Weiner, L. Addadi, *Angew. Chem., Int. Ed.* **2015**, *54*, 12426.
[73] D. Gur, B. Leshem, V. Farstey, D. Oron, L. Addadi, S. Weiner, *Adv. Funct. Mater.* **2016**, *26*, 1393.
[74] a) A. Gal, V. Brumfeld, S. Weiner, L. Addadi, D. Oron, *Adv. Opt. Mater.* **2012**, *24*, OP77; b) A. Jantschke, I. Pinkas, A. Hirsch, N. Elad, A. Schertel, L. Addadi, S. Weiner, unpublished.
[75] J. A. C. Nicol, in *Vertebrate Photoreceptor Optics* (Eds: J. M. Enoch, F. L. Tobey), Springer-Verlag, Berlin, Germany **1981**, p. 401.
[76] C. R. Braekevelt, *J. Morph.* **1976**, *150*, 213.
[77] H. Noh, S. F. Liew, V. Saranathan, R. O. Prum, S. G. J. Mochrie, E. R. Dufresne, H. Cao, *Adv. Mater.* **2010**, *22*, 2871.