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Patchy proteins form a perfect lens: Squid lenses are formed by a protein gel of varying density

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Most lenses are imperfect: They fail to focus all rays to the same convergence point. In 1854, James Clerk Maxwell proposed a solution for this optical aberration (1). He argued that if the refractive index would decrease parabolically from the center of a spherical lens to its edge, rays would follow curved paths and focus each point on the spherical surface to the opposite point on the same surface. The eyes of fishes and decapod squid are such parabolic lenses, but it has remained unclear how the required molecular architecture is achieved. On page 564 of this issue, Cai *et al.* (2) report that in squid eyes, globular proteins of different sizes form a gel of varying density, thereby building up a refractive index gradient. The findings suggest a role of such protein polydispersity not only in squid and fish lenses, but also in those of vertebrates other than fish, and provide a novel route to smart materials.

The lenses in the eyes of fishes and squids accomplish the task of producing a good retinal image through an internal refractive index gradient, which guides incident rays along curved paths via the lens center to the opposite side of the lens. Eye lenses, including those of fishes and squids, mostly consist of crystallin proteins (3, 4). Squid eyes mainly contain members of the S-crystallin family. A radial protein density gradient leads to a low density (and hence low refractive index) at the edges and a high density (high refractive index) in the center of the lens (5). S-crystallins contain a glutathione *S*-transferase (GST) domain and a flexible, disordered protein loop region. The variable length of the latter suggests a role in determining the refractive index of the lens.

Cai *et al.* sequenced transcriptomes of mature lens tissue from inshore squid (*Doryteuthis pealeii*) and identified more than 40 unique loop sequences that vary in length from 3 to 110 amino acids. It remains to be shown whether this diversity, seen in the messenger RNA composition, is also present at the protein level. The number of unique loops in squid is much higher than in vertebrates, where a handful of α -, β -, and γ -crystallin variants are the main protein components of the eye lens (6). Protein polydispersity has also been reported for α -crystallins in a vertebrate lens, but this polydispersity is encoded in a single protein, involving formation of a range of differently sized protein complexes (7).

Cai *et al.* show that the distribution of protein variants varies between different radial positions. Short sequences are more abundant in the center of the lens, and medium-sized

linkers are more abundant at its edge. S-crystallin variants with long internal loops are found in all radial positions, although they may perform different functions, given their diverse sequence and charge composition.

The study offers a solution for the apparent paradox that the refractive index gradient is highly stable throughout the lens and does not fluctuate locally. If interactions between proteins are isotropic, as commonly assumed, this would allow local fluctuations of protein density and hence refractive index and would distort the transparency of the lens. Cai *et al.* instead propose that the proteins behave as anisotropic colloidal molecules. They consider S-crystallins as “patchy” colloids, which are characterized by localized, short-range attractive surface patches as well as hard-sphere repulsion, with the latter controlling the number of interacting molecules (8, 9). In S-crystallins, the loop regions constitute the attractive surface patches, which mediate interactions between S-crystallin molecules, like nodes in a mesh. The GST domains mediate hardsphere repulsion and ensure that the protein mesh is regularly spaced.

The authors show that small-angle x-ray scattering (SAXS) analysis of individual tissue samples from all regions in the lens confirms the formation of pairwise linked chains of S-crystallin proteins and the presence of multiparticle nodes in the protein network. SAXS analysis of tissue homogenates (a slurry of broken tissue) shows that once formed, the gel does not change its structure upon dilution, which would be expected for isotropic particles.

Hence, the proteins interact as patchy colloids and form an ordered fluid. SAXS and dilution experiments show that the coordination number—that is, the number of connections per node in the protein mesh—increases from around 2 at the edge toward 6 at the center of the lens (see the figure). Such variations in the coordination number might enable the cell to prevent gelation and cessation of protein synthesis below the optimal protein density. According to the authors, gelation only occurs once the optimal coordination number needed to define a certain refractive index is reached throughout the lens.

It remains to be shown how protein sequence, coordination number, and density are related to each other. Future studies should also determine whether the S-crystallin diversity is encoded in the squid genome or achieved by RNA editing; the latter is a common mechanism for recoding proteins in the transcribed genes of squid, octopuses, and cuttlefish (10). Deposited sequences of S-crystallins from the similar squid species *D. opalescens* show a loop diversity resembling that of *D. pealeii*, though lacking long linkers, and show sequence variation in the GST domain. Thus, it seems more likely that the diversity is encoded in the squid genome.

In vitro work using purified proteins is needed to confirm that the disordered loops mediate different coordination numbers and protein densities. Moreover, it is unclear whether the high polydispersity in loop sequence facilitates gel formation and whether polydispersity in vertebrate lenses encoded in single proteins (7) follows a similar concept. Posttranslational modifications have been reported for human crystallins (11). Researchers will need to clarify whether posttranslational modification of the disordered loop constitutes another way through which gel formation is regulated. This knowledge may enable the design of

artificial, aberration-free lenses based on simple materials with properties similar to those of S-crystallins, with potential applications in optical instruments and clinics.

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Protein density,
refractive index,
coordination number

Squid lens

S-crystallin dimer

Crystallin dimers assemble into larger structures, with higher coordination numbers and hence higher protein density in the center of the lens than near the edges.

Architecture of a perfect lens

In squid eye lenses, the refractive index decreases parabolically from the center to the edge.

Cai *et al.* show that this gradient is achieved through assemblies of crystallin S-protein dimers that vary in size from the center to the edge of the lens.

Single refractive index

Graded refractive index

Graded refractive index

The image quality is much lower for an image formed by a spherical glass bead with a single refractive index (top) than through a fish lens with a graded refractive index (bottom).