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STRUCTURAL BASIS FOR EXTREME COLD TOLERANCE IN THE EYE LENSES OF TELEOST FISHES

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EXECUTIVE SUMMARY

Eye lenses of endothermic mammals such as the cow develop cold cataracts at a mild 17°C. In contrast, ectothermic teleost fish lenses remain transparent down to -12°C. Cold-induced cataracts arise from a liquid–liquid phase-separation of lens proteins (crystallins) resulting in a protein-rich and a protein-poor phase. Crystallins are tightly packed at high concentrations to enable refraction of incident light, and teleost lenses are especially protein-dense to achieve a refractive index change in aquatic environments. Attractive forces would enable crystallins to tightly pack in the lens but risk increasing propensity for phase separation. We propose that teleost crystallins are structurally more flexible than mammalian paralogs to minimize the propensity of phase separation at the high concentrations necessary to function in aquatic environments, conferring the observed tolerance to very low temperatures as a side benefit.

RESEARCH CHALLENGE

Attractive forces that are responsible for maintaining proper density of the lens are subject to alterations by physical factors such as low temperature, resulting in the cold cataract phenomenon in endotherms [1]. Reduction in attractive forces can increase cold resilience but would negatively impact the packing density of lens crystallins necessary for the refraction of light in ectothermic teleost fishes. Teleost lens crystallins, therefore, must have evolved adaptive mechanisms to pack at high concentrations, remain soluble, and avoid phase separation. Protein–protein interactions can be attenuated by modulation of flexibility at sites of interaction [2–4], and we propose that the abundant γ-crystallins in fish lenses evolved enhanced flexibility at interaction sites relative to mammalian paralogs.

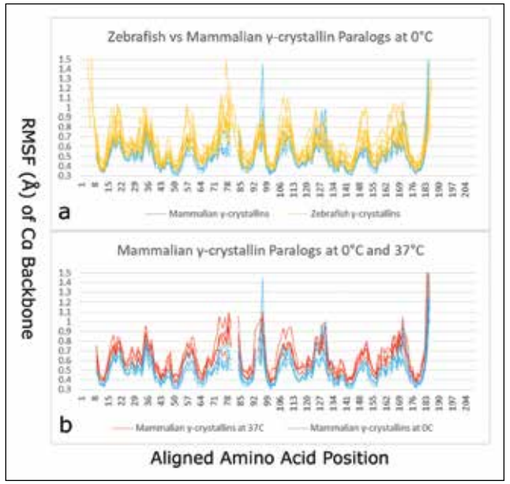

Figure 1: a. Flexibility of five mammalian γ-crystallin isoforms (blue) and 11 zebrafish isoforms (orange) at 0°C. b. Flexibility of five mammalian γ-crystallin isoforms at 0°C (blue) and 37°C (red).

γ-Crystallins have been identified as the mediator for phase separation [5]. Teleost fishes possess a unique γ class of crystallins, the γM, which may confer the ability to maintain homogeneity at very high concentrations and extremely cold temperatures [6]. While mammals typically express between 6 to 7 γ-crystallin isoforms, teleost fishes express between ~20–40 unique isoforms depending on species, all except five belong to the γM class. The large number of γ-crystallin isoforms in teleosts relative to mammals suggests inherent functional importance. Uniform flexibility across all γ-crystallin isoforms may negate the effects of attractive forces necessary for the tight packing of lens crystallins that maintains a high refractive index for teleost fish lenses. Therefore, we additionally propose that flexibility profiles across the lens crystallin landscape will be diverse.

We are currently utilizing the computational power of Blue Waters to run extensive molecular dynamics simulations to address our hypotheses regarding flexibility and extreme cold tolerance. With this resource, we are able to ascertain the potential contribution of flexibility to resist cold cataracts at cold temperatures by assessing the flexibility of a large suite of γ-crystallin isoforms among teleost fishes and mammals.

METHODS & CODES

We ran molecular dynamics (MD) simulations on eleven zebrafish and five mammalian isoforms at a cold temperature (0°C), and 3 zebrafish and 5 mammalian γ-crystallin isoforms at the normal body temperature (25°C and 37°C respectively). Three replicates of each γ-crystallin isoform were simulated for 50 nanoseconds in NAMD 2.12 using CHARMM27 force field parameters. Each of the five mammalian γ-crystallin isoforms was simulated using solved structures obtained from the Protein Data Bank. Simulation of zebrafish γ-crystallin isoforms used one known structure, the γM7-crystallin, and the remaining 10 γ-crystallin isoforms simulated in this study were modeled onto the γM7-crystallin using iTasser. VMD 1.9.3 was used to quantify flexibility via root mean square fluctuations (RMSFs), which measure the average distance (Angstroms) of aligned backbone Cα atoms per residue of a protein over the duration of the simulation. Average RMSF values were taken for each isoform, then formatted based on amino acid sequence alignment generated by MUSCLE 3.8.31 for comparison.

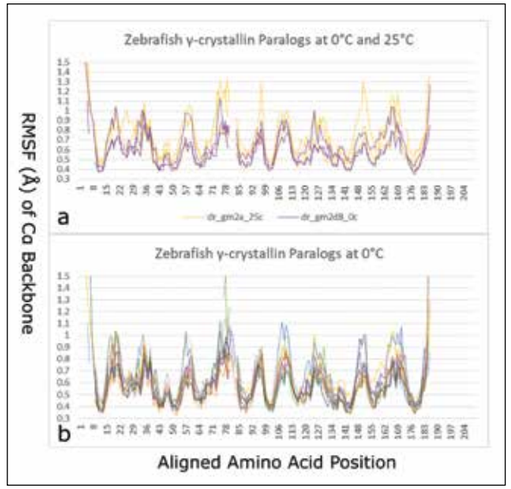

Figure 2: a. Flexibility of three zebrafish γ-crystallin isoforms at 25°C (orange) and 0°C (purple). b. Flexibility of 11 zebrafish γ-crystallin isoforms at 0°C.

RESULTS & IMPACT

At 0°C, it is evident that zebrafish γ-crystallins are largely more flexible than the mammalian isoforms, most notably on surface loops (Fig. 1a). Fig. 1b shows simulations of mammalian γ-crystallins at 37°C and presents flexibility profiles similar in amplitude to zebrafish isoforms at 0°C. The zebrafish γ-crystallin isoforms tested at 25°C exhibit greater flexibility profiles compared to 0°C (Fig. 2a). In accordance with our hypothesis concerning variation of flexibility profiles among the γM-crystallins, the 11 zebrafish γM-crystallins are not identical in amplitude across all isoforms (Fig. 2b). This is unlikely to be due to functionally neutral changes along the γM-crystallin evolutionary trajectory, but, rather, is likely to be due to functional diversity. Identical flexibility at regions with significant attractive forces may mitigate the effects the attractive forces have in maintaining high concentrations necessary for light refraction in teleost fish lenses. Diversity with regard to flexibility, spatial distribution of attractive forces, size, and shape may all be essential parameters in maintaining homogeneity in the lens at high concentrations and over a range of temperatures. Flexibility, in part, appears to explain the large standing question regarding the incredible ability of teleost fish lenses to maintain transparency at extremely cold temperatures.

WHY BLUE WATERS

Our work requires simulating three (3) trials of 49 proteins at two temperatures and over a long timecourse of 50 nanoseconds to detect meaningful molecular behavior. This work is at the core of a Ph.D. project in determining the extreme cold tolerance observed in teleost fish lenses. Only the petascale computational power and resources of Blue Waters could allow us to achieve this core portion of the project in a reasonable amount of time for downstream analyses to test our hypotheses. Without Blue Waters, we would not be able to finish this project in a reasonable time for a P.D. project.