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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 are responsible for maintaining proper density of the lens and 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 that is 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 at temperatures much colder than that faced by mammalian lenses. 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 isoforms.

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 six to seven 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, likely to maintain a refractive index gradient to correct for spherical aberration, with increasing concentration from the cortex to the nucleus. If the propensity of phase separation is due to enhanced flexibility driven by density, we expect elevated flexibility among lens crystallins that predominate in the dense lens nucleus compared to crystallins found in the less concentrated lens cortex.

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.

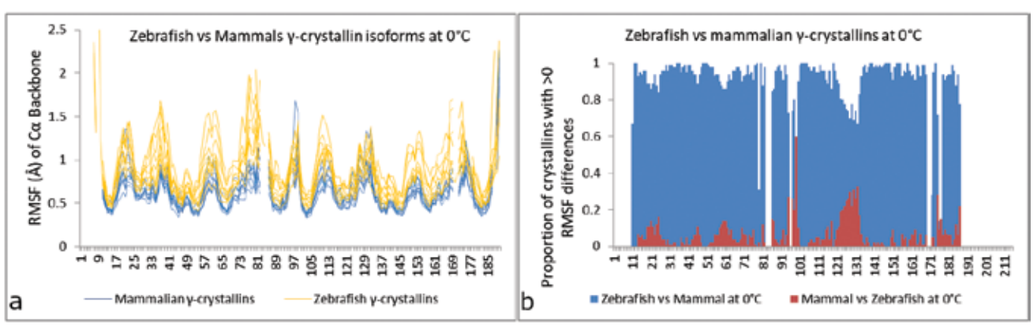

Figure 1: (a) RMSF of eight mammalian γ-crystallin isoforms (blue) and 12 zebrafish isoforms (orange) at 0°C. (b) Proportion of γ-crystallins with RMSF differences among zebrafish γ-crystallins (blue) and mammalian γ-crystallins (orange) that are greater than zero at 0°C.

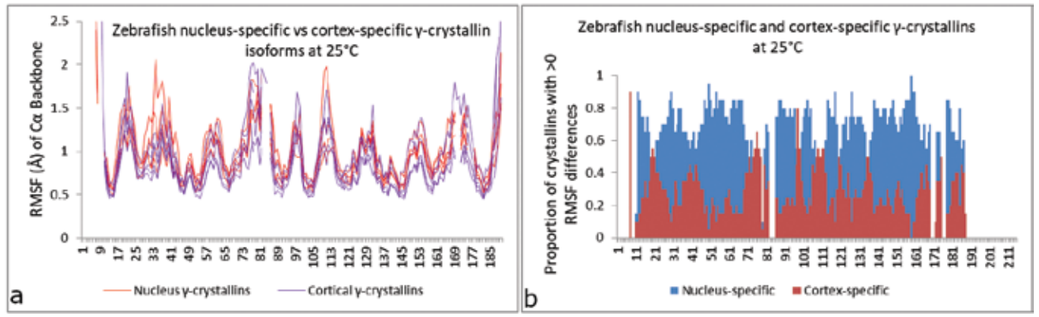

Figure 2: (a) RMSF of zebrafish γ-crystallin isoforms in the dense nucleus (red) and zebrafish γ-crystallin isoforms in the less concentrated cortex (purple) at 25°C. (b) Proportion of zebrafish nucleus γ-crystallins (red) with RMSF differences among zebrafish cortical γ-crystallins (purple) and mammalian γ-crystallins that are greater than zero at 0°C.

METHODS & CODES

We ran molecular dynamics (MD) simulations on twelve zebrafish and eight mammalian isoforms at a cold temperature (0°C), and at the normal body temperature (25°C and 37°C respectively). Three replicates of each γ-crystallin isoform were simulated for 50 nanoseconds (ns) in NAMD [7] 2.12 using CHARMM27 force field parameters. Each of the five mammalian γ-crystallin isoforms were simulated using solved structures obtained from the Protein Data Bank (PDB). Simulation of zebrafish γ-crystallin isoforms used one known structure, the γM7-crystallin, and remaining 11 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 of the last 30 ns were taken for each isoform, then formatted based on amino acid sequence alignment generated by MUSCLE 3.8.31 for comparison. To gauge the proportion of zebrafish γ-crystallins that are more flexible than mammalian γ-crystallins at 0°C, RMSF differences were calculated by each individual crystallin per site, and the sum of each instance was divided by the total number of comparisons. This method was also used to determine the proportion of zebrafish nucleus and cortical-specific γ-crystallins at 25°C.

RESULTS & IMPACT

At 0°C, it is evident that zebrafish γ-crystallins are more flexible than the mammalian isoforms across all sites (Fig. 1a). Zebrafish γ-crystallins demonstrate that nearly all isoforms are more flexible than mammalian crystallins (Fig. 1b). In Fig. 2a, zebrafish γ-crystallins predominately in the nucleus are slightly more flexible than those in the cortex at a majority of sites at 25°C (Fig. 2b). A distinct disparity in flexibility is present among zebrafish and mammalian γ-crystallins, and the elevated flexibility of teleost γ-crystallins may account for their remarkably cold-tolerant lenses. Density may have played a large role toward selection for more flexible crystallins in teleost fishes by preventing interactions among neighboring crystallins that would result in loss of transparency in the lens. In turn, the elevated flexibility may increase the thermodynamic barrier to phase separation at cold temperatures well below thermal habitats occupied by teleost fishes.

WHY BLUE WATERS

Our work requires simulating three trials of 49 proteins at two temperatures, and over a long timecourse of 50 ns to detect meaningful molecular behavior. This work is at the core of a PhD 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.