Blue Waters Final Report

Project Title: 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 bovine develop cold cataract at ~17°C. In contrast, ectothermic teleost fish lenses remain transparent down to -12°C. Cold induced cataract arises 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. We propose that teleost crystallins are structurally more flexible than mammalian isoforms to minimize the propensity of phase separation at their very high concentrations, conferring the observed stability at very low temperatures as a side benefit. Molecular dynamics simulations on a subset of γ-crystallin isoforms from teleost fishes and mammals at normal and cold temperatures lend support to our hypothesis.

Key Challenges

Our project goal is to address the structural basis for extreme cold resistance of the teleost fish lens proteins. Their lenses are approximately twice as dense as mammalian lenses [1], and normal body temperatures are lower than mammalian species, temperatures often colder than the temperatures that induce cold cataracts in a number of mammalian lenses. Remarkably, lenses of teleost fishes resist cold cataracts far below temperatures any species of fish experience in native conditions [2,3]. Adaptations that confer resilience to the extreme cold may have occurred to cope with macromolecular crowding within the highly dense teleost fish lenses, which enabling clear for teleost fishes in a broad range of temperature conditions, but the mechanism remains unknown.

Strong evidence suggests γ-crystallin isoforms, a subclass of lens crystallins, are implicated in the cold opacification of mammalian lenses. Intermolecular interactions can be modulated by modifications in protein flexibility [4-7]. If extreme cold resistance is a product of adaptations to macromolecular crowding, we expect **1)** teleost fish γ-crystallins to be broadly more flexible than mammalian isoforms, and **2)** teleost fish γ-crystallins located in the very dense lens nucleus to be more flexible than γ-crystallins in the less denselens cortex. Assessing flexibility can be accomplished via molecular dynamics (MD) simulations, requiring substantial computational resources to acquire meaningful results.

Why it Matters

A functional lens requires the lens proteins (crystallins) to be packed at high concentrations while maintaining solubility to provide the transparency needed to refract light effectively onto the retina. The eye lenses of endothermic mammals, such as cow, turn opaque when chilled below the animal's normal body temperature, at approximately 17^{o}C [8]. This cold-induced cataract is due to cold-sensitive lens crystallins undergoing a liquid-liquid phase transition (LLPS) resulting in protein-rich phase and protein-poor phase, and is reversible upon warming. In contrast, the teleost fishes inhabiting freezing Antarctic to temperate waters have the remarkable ability to maintain lens transparency well below their thermal ranges, remaining clear as low as -12^{o}C [2,3]. We have additionally discovered that tropical fish lenses also maintain transparency down to -5^{o}C. Of the three major classes of vertebrate crystallin proteins, α, β, and γ, the γ-crystallins have been implicated in the formation of cold-cataracts [9]. Teleost fish lenses are far denser than mammalian lenses, and abundant in an exclusive subclass of methionine-rich γ-crystallins, γM-crystallins [1]. How teleost fishes lenses persist at in transparency at such cold temperatures, far below their mammalian counterpart, is unknown.

Attractive forces are thought to keep the lens crystallins tightly packed within the lens [10]. Reduction in attractive forces may increase cold resilience by reducing overall intermolecular interactions, but would diminish 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. The abundant and diverse teleost fish γM-crystallins exhibit high methionine content on the surface of the lens proteins resulting in lower water density around them, which may account for the higher concentrations of these crystallins in the lens [1]. This poses another problem, as sulfur containing residues increase the propensity for LLPS [11].

Flexibility can play an essential role in modulating intermolecular interactions at sites of interaction [4-7], and phase separation at higher concentrations could be averted by the teleost fish γM-crystallins via elevated flexibility relative to their mammalian isoforms. MD simulations enable us to assess the flexibility of several γ-crystallin isoforms across teleost fishes and mammalian species to test our hypotheses regarding flexibility and transparency in relationship to lens density.

Why Blue Waters

To test our hypothesis on the role of crystallin molecular flexibility in lens transparency requires simulating 3 trials of 47 proteins at two temperatures, and over a long time course 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 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 PhD project.

Accomplishments

We proposed that teleost fish γ-crystallin isoforms have evolved to be more flexible than mammalian γ-crystallins to prevent unfavorable interactions that would result in the LLPS phenomenon leading to cold cataracts, and that teleost fish γ-crystallins in the dense lens nucleus than γ-crystallins in the softer cortical region of the lens. We used the well characterized zebrafish and bovine γ-crystallins as the main representatives of teleost fishes and mammals respectively, while other species are used for additional support. With the node-hours we requested from our 2016-2017 Blue Waters allocation we report here results we obtained from 50 ns simulations for 12 zebrafish and 8 mammalian γ-crystallin isoforms at 0ºC, and 6 zebrafish and 8 mammalian γ-crystallin isoforms at 25ºC and 37ºC respectively. The mammalian γ-crystallins consist of 5 bovine, 1 human, 1 mouse, and 1 rat isoforms.

Results and Impact:

To account if the simulations reflect biologically meaningful differences, and to determine the magnitude of temperature effects on flexibility, we compared the last 30 ns of are simulations via Root Mean Squared Fluctuations (RMSFs) of cold and normal body temperatures for both zebrafish (Fig 1) and mammalian (Fig 2) γ-crystallin isoforms. Between normal and body temperatures, there are very subtle differences in flexibility. This reflects the extreme structural stability of the γ-crystallins, having to persist throughout the duration of the organism's life. Isolated mammalian γ-crystallins have variable phase separation temperatures. While the differences in flexibility are subtle, 0ºC is far below the temperature that mammalian lenses develop cold cataract.

At 0°C, zebrafish γ-crystallin isoforms are more flexible nearly across all sites relative to those in bovine, human, mouse, and rat (Fig 3). The sites with greatest differences in RMSF magnitudes are 28-38, 44-49, 55-61, 74-85, 105-111, 121-125, 136-143, 148-153, 158-173 in zebrafish γ-crystallins. These regions are rich in charged residues, methionine, and aromatic residues. Given the extremely high concentration of γ-crystallins in teleost lenses, the limited mobility may have necessitated the selection for

greater flexibility in order to prevent non-covalent interactions with neighboring γ-crystallins. This is further illustrated when comparing zebrafish γ-crystallin isoforms simulated at 0°C to mammalian isoforms at 37°C, The zebrafish γ-crystallins at 0°C flexibility profiles nearly superimpose onto mammalian γ-crystallins at 37°C (Fig 4).

Our preliminary evidence demonstrate that there are distinct differences in flexibility between zebrafish and mammalian γ-crystallins at 0°C, which may account for the resistance to extreme cold temperatures of teleost fish lenses. Given the large data we have generated, and will continue to generate, we are currently developing methods to more quantitatively summarize the differences between teleost and mammalian γ-crystallins, especially given the difficulty interpreting RMSF graphs with a large number of proteins. We will have a clearer picture when we incorporate the remainder of γ-crystallins in our analyses, which is well on its way with our 2018 Blue Waters allocation.

Figures

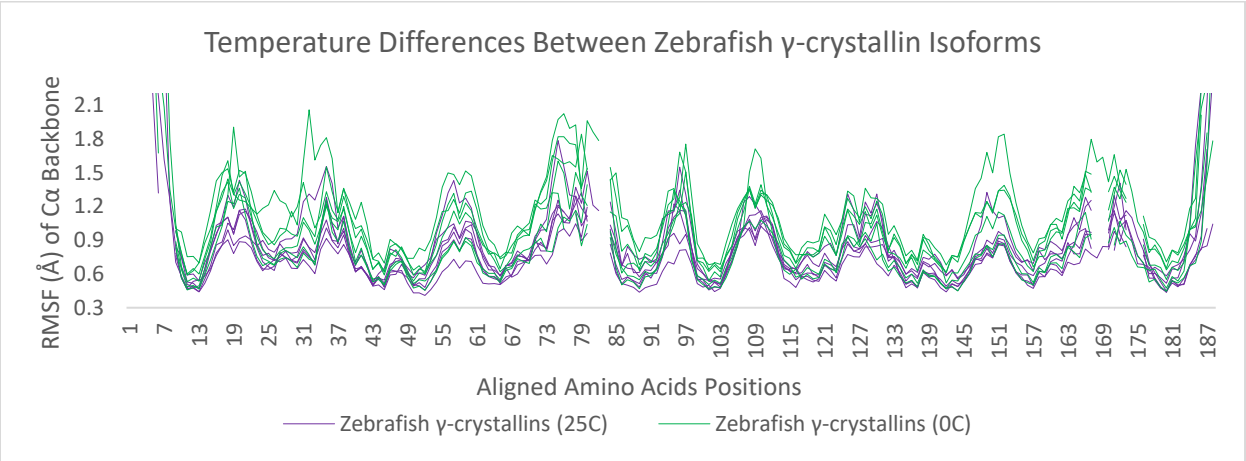

Figure 1. Determination of temperature-dependent differences in root mean squared fluctuations between zebrafish γ-crystallins at 0°C (green) and zebrafish γ-crystallins 25°C (purple) across the last 30 ns of a 50 ns simulation. On the whole, there flexibility profiles are greater among 25°C γ-crystallins.

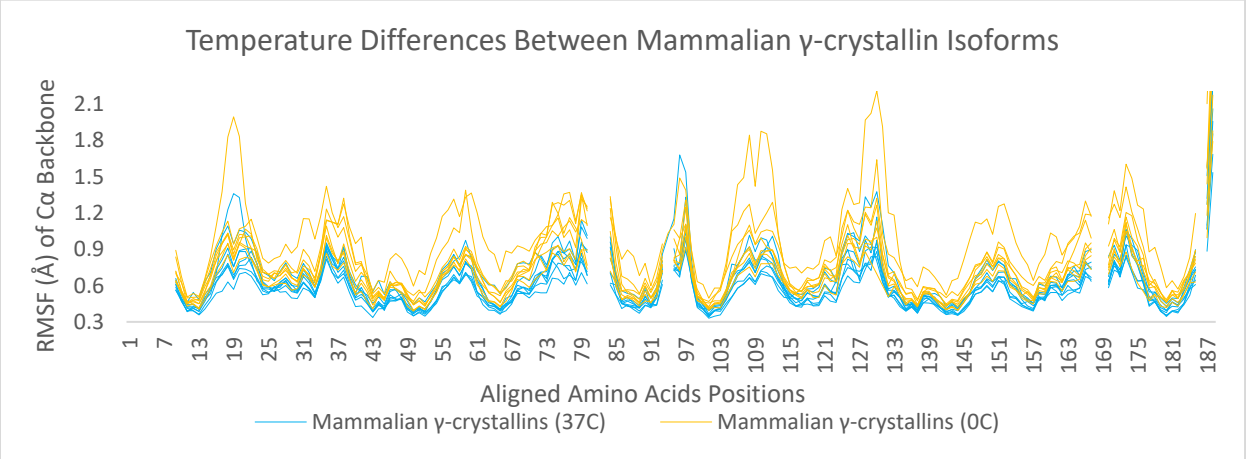

Figure 2. Determination of temperature-dependent differences in root mean squared fluctuations between mammalian γ-crystallins at 0°C (blue) and mammalian γ-crystallins 37°C (orange) across the last 30 ns of a 50 ns simulation. Flexibility at the cold temperature (0°C) across sites are dampened in amplitude relative to normal body temperature (37°C).

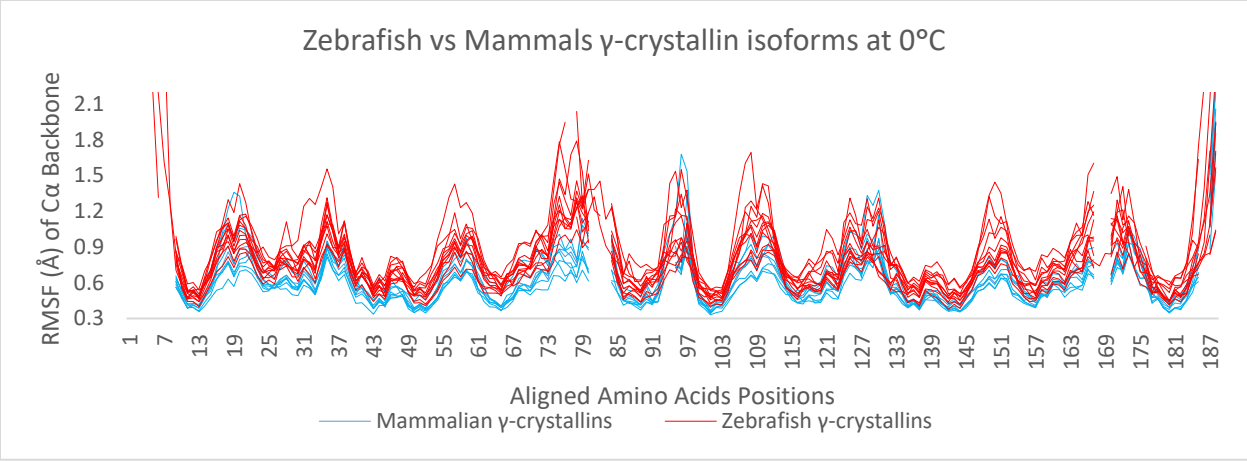

Figure 3. Root mean squared fluctuations between zebrafish γ-crystallins (red) and mammalian γ-crystallins (blue) at 0°C across the last 30 ns of a 50 ns simulation. Collectively, zebrafish γ-crystallins exhibit RMSFs greater than mammalian γ-crystallins at almost every site.

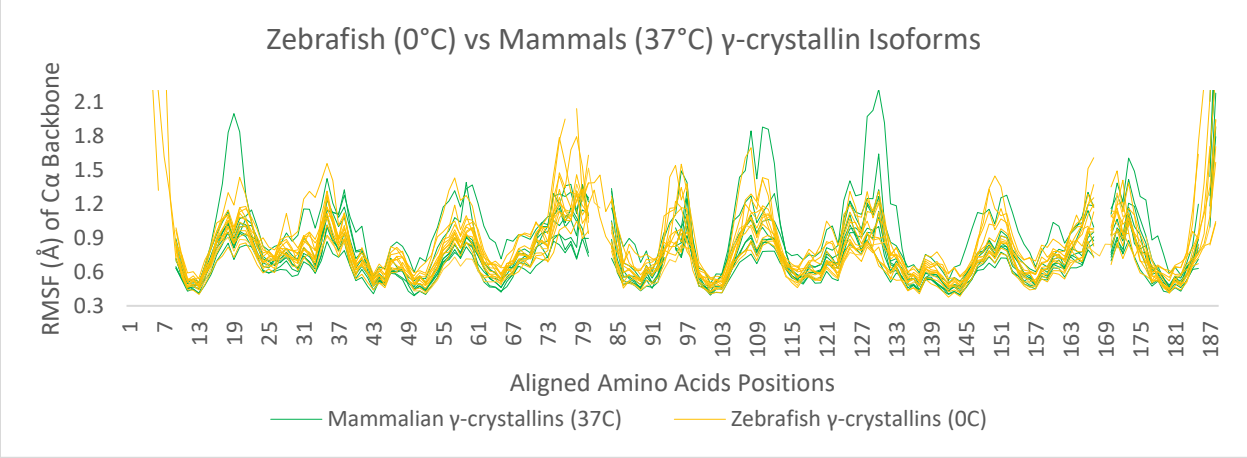

Figure 4. Root mean squared fluctuations between zebrafish γ-crystallins at 0°C (orange) and mammalian γ-crystallins 37°C (green) across the last 30 ns of a 50 ns simulation. The majority of Zebrafish γ-crystallins at 0°C exhibit near identical magnitude of flexibility across sites compared to the mammalian γ-crystallins 37°C.

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