# Investigating the role of cellular membranes in the freeze tolerance of Cope's gray treefrog *Dryophytes chrysoscelis*

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## Abstract


Fig. 1: Cope's gray treefrog *Dryophytes chrysoscelis*. Photo by Clara do Amaral.

The freeze tolerant Cope's gray treefrog Dryophytes chrysoscelis can survive multiple winter freeze-thaw cycles in which up to 70% of extracellular fluids may be frozen solid without apparent detriment to the animal. Previous studies in our lab have shown that post-freeze cell viability in D. chrysoscelis is likely enhanced by biophysical and biochemical properties of cellular membranes, in addition to accumulation of cryoprotectants and upregulation of membrane aquaglyceroporin proteins. Largely composed of lipids, cellular membranes may vary significantly in phospholipid composition and cholesterol content during thermal fluctuation to best preserve membrane integrity and cellular function. The objective of this study is to assess the biochemical and biophysical differences in cellular membranes of treefrogs in discrete stages of the freeze-thaw process to better discern the adaptation of membranes to freezing temperatures. It is hypothesized that lipid biochemistry significantly affects membrane physical conditions, and in combination, the biochemical and biophysical membrane properties actively adapt to and compensate for changes in environmental temperature.


## Background: Freeze tolerance in Cope's gray treefrog

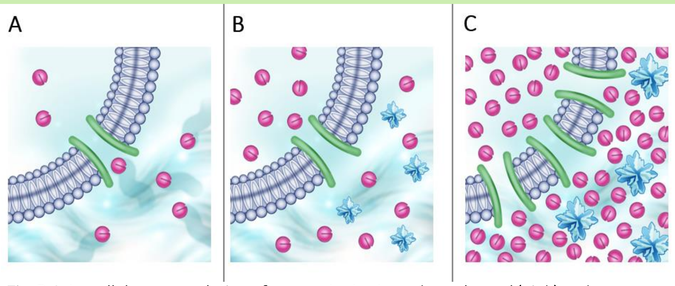



Fig. 2: Intracellular accumulation of cryoprotectants such as glycerol (pink) and evacuation of water molecules by aquaglyceroporin proteins (green) (a) enhances freeze tolerance in *D. chrysoscelis* at very cold and freezing temperatures (b, c).

Previous studies have shown:

- *D. chrysoscelis* is freeze-tolerant only after a period of cold-acclimation.
- Intracellular freezing is avoided by accumulation of cryoprotectants like glycerol (Fig. 2) [1].
- Aquaglyceroporin proteins embedded in the plasma membrane during cold-acclimation and freezing (Fig. 2) allow the passage of glycerol into the cell and water out of the cell [2].
- Aquaglyceroporin proteins increase in number *and* permeability at low temperatures just prior to freezing conditions (Fig. 2C) [2].
- Post-freeze cellular viability is enhanced *without cryoprotectants* under cold-acclimation conditions [3].

## Background: Membrane biochemistry and biophysics

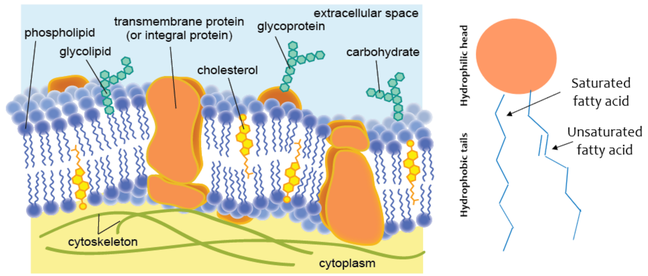
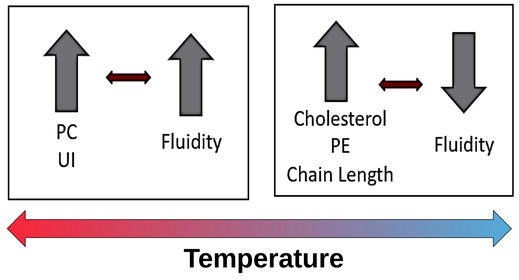



Fig. 3: The basic structure of biological membranes is the phospholipid bilayer. This assembly becomes increasingly complex when all associated lipids, carbohydrates, and proteins are encompassed in the biological membrane organelle (left). Phospholipids are an example of a polar lipid and each has a unique combination of phospholipid head group, fatty acid chain length, fatty acid chain degree of unsaturation, and fatty acid chain symmetry (right).

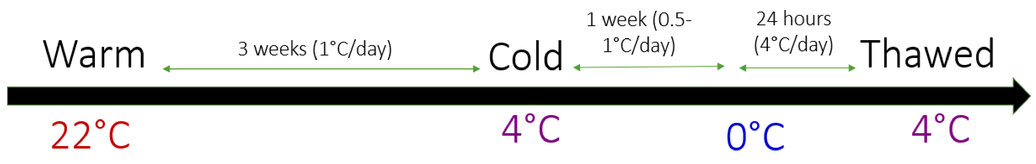
- Membranes vary in neutral (e.g. cholesterol) and polar lipid (e.g. phospholipids) composition (Fig. 3, left).
- Phospholipids are diverse based on head group and fatty acid tails/chains (Fig. 3, right). Head groups vary in size, charge, and shape while fatty acid tails vary in length, unsaturation index (i.e. double bonds), and symmetry.
- The biochemical (lipid) complexity and variety results in an equally diverse range in biophysical microenvironments within the membrane (Fig. 4) [4].
- Temperature also impacts biophysical membrane characteristics (e.g. fluidity) and often results in biochemical lipid remodeling by the cell to preserve membrane integrity (Fig. 4) [5].

Fig. 4: Membrane fluidity is directly influenced by lipid biochemical composition. Temperature also significantly impacts membrane biophysical characteristics, which also coincides with cellular lipid remodeling in the membrane to preserve membrane function.




## Experimental Design & Methods

- Temperature & Experimental Groups: Warm (22°C), Cold (4°C), Thawed (4°C), see below
- Tissue: Biological membranes isolated from liver and skeletal muscle tissues.
- Biophysical Characterization: Membrane fluidity (spectrofluorescence of DPH) [6]
- Biochemical composition:
  - Phospholipid head group ($^{31}$P NMR spectroscopy) [7]
  - Phospholipid fatty acid characterization ($^{1}$H NMR spectroscopy) [8]
  - Cholesterol (fluorescent assay kit)




## Objectives & Aims

- To profile biochemical and biophysical characteristics of membranes in *D. chrysoscelis* at different thermal intervals (see methods) associated with freeze tolerance.
- To determine the extent of membrane lipid remodeling in the freeze tolerance of *D. chrysoscelis* (Fig. 4).
- To assess the potential role of biochemical and biophysical membrane characteristics on membrane permeability to glycerol and water by membrane-associated aquaglyceroporins proteins (Fig. 2).

## Anticipated Results

| Temperature | Warm (22°C) | Cold (4°C) | Thawed (4°C) |
|---|---|---|---|
| Biophysical | ↓Fluidity | ↑Fluidity | ↑↑Fluidity |
| Phospholipid (Fluidizing) | ↓PC | ↑PC | ↑PC |
| Phospholipid (Rigidifying) | ↑PE | ↓PE | ↓PE |
| Fatty Acid UI | ↓UI | ↑UI | ↑↑UI |
| Fatty Acid Chain Length | ↑ACL | ↓ACL | ↓ACL |
| Cholesterol | ↑↑Chol:PL | ↓Chol:PL | ↑Chol:PL |

**Abbreviations:** phosphatidylcholine (PC), phosphatidylethanolamine (PE), unsaturation index (UI), acyl chain length (chain length), cholesterol (Chol), phospholipid (PL)

## References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