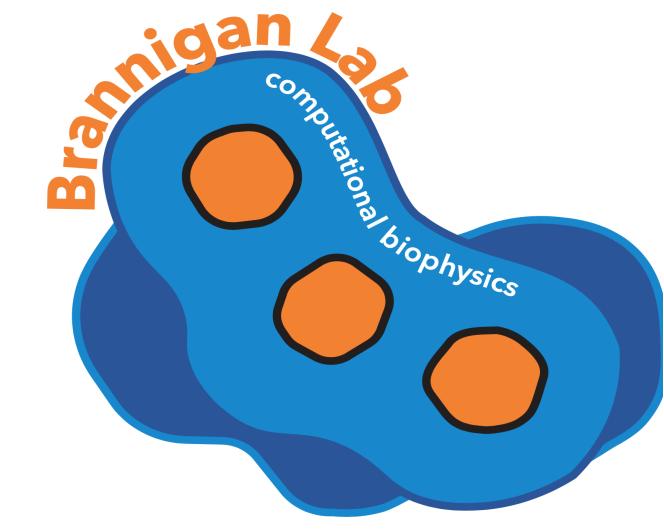


# Molecular Mechanism of Reversed Temperature Dependence of ATP Synthesis in Glacier Ice Worms



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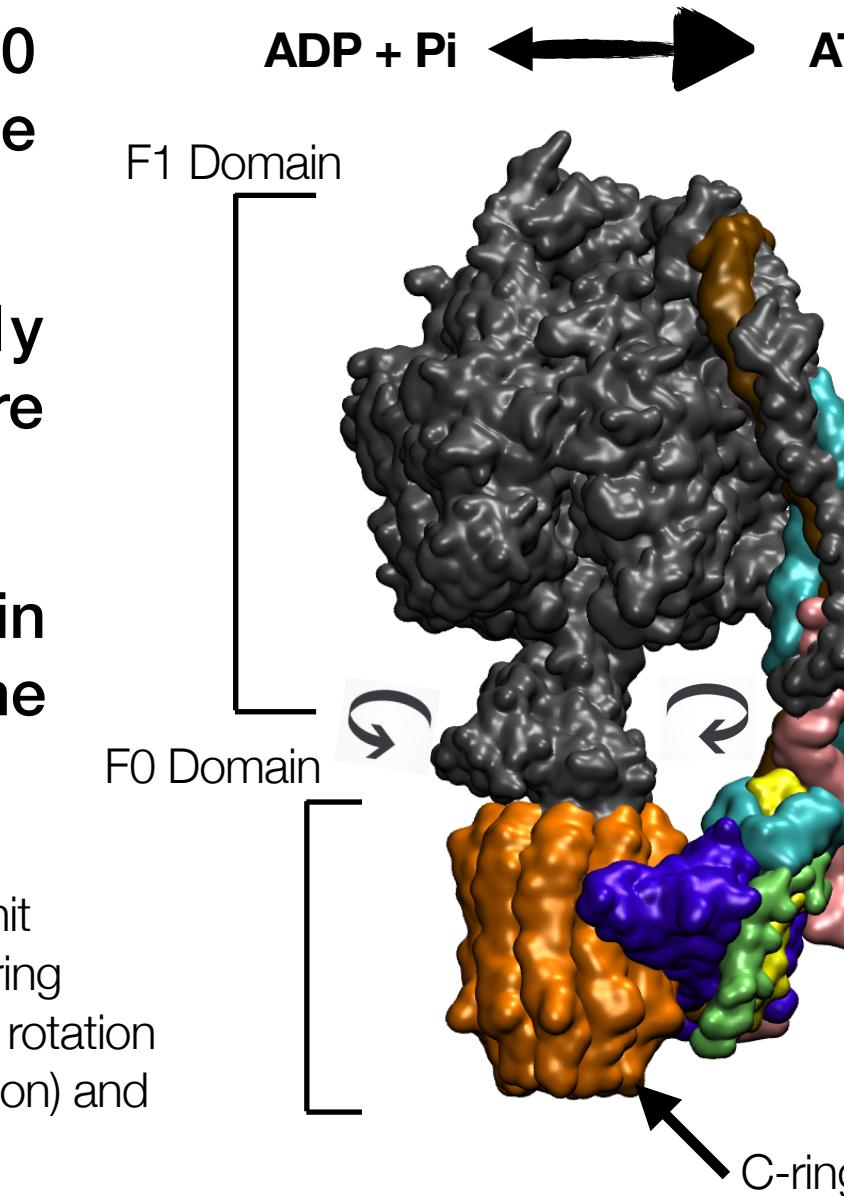
## Abstract

The F0F1 ATP synthase enzyme is highly conserved across species. The F0F1 is a reversible motor, where the clockwise rotation of the rotor portion of F0 (known as the c-ring and present in the membrane-embedded F0 domain) produces ATP, and the counter-clockwise rotation induces ATP hydrolysis. In a surprising contrast with temperate organisms, glacier ice worms display elevated ATP levels as temperatures decline. The increased energy expenditure is used as a strategy for survival at cold temperatures, but the mechanism is unknown. More specifically, an ice worm-specific, 18 amino acid extension with regularly spaced histidine residues was previously found to be fused to the carboxy-terminal of the ATP6 subunit generating a proton shuttling domain projecting away from the F0 exit pore. The role of this C-terminal extension in the temperature dependence ATP synthesis in ice worms is undetermined. To investigate the underlying mechanism of elevated ATP levels in glacier ice worms, we started by investigating the effects of temperature on the dynamics of the F0F1 ATP synthase of yeast as a control. We conducted all-atomistic MD simulations of the F0 domain subunits at different temperatures to evaluate the effect of sequence on temperature dependence. We also measured the rotational diffusion of the c-ring as a function of temperature to evaluate the change in the rotation angles of the c-ring around the z-axis in clockwise and counter-clockwise directions. The ATP6 subunit is attached to the c-ring at the proton exit site. This suggests that the change in the c-ring rotational diffusion, as a function of temperature, can affect the ATP6 conformation, which likely affects the rate of ATP production.

## Primary Research Questions

- 1- How does Temperature affect the rotational diffusion of the C-Ring of the F0 ATP Synthase domain from an extremophile vs. a non-extremophile?
- 2- Are the dynamics of the poorly conserved regions of the ATP6 subunit more sensitive to Temperature?
- 3- How does the ATP6 extension found in the ice worm sequence interact with the rest of the protein?

Fig. 1. Mitochondrial F1F0 ATP Synthase. Rotor-stator subunit distribution in the mitochondrial F1F0-ATP synthase. Rotating c-ring subunits are shown in orange. The arrows indicate the reversible rotation that takes place during ATP synthesis ("clockwise" or right direction) and hydrolysis ("counterclockwise" or left direction).



## Ice Worm F0 Domain Structure Prediction

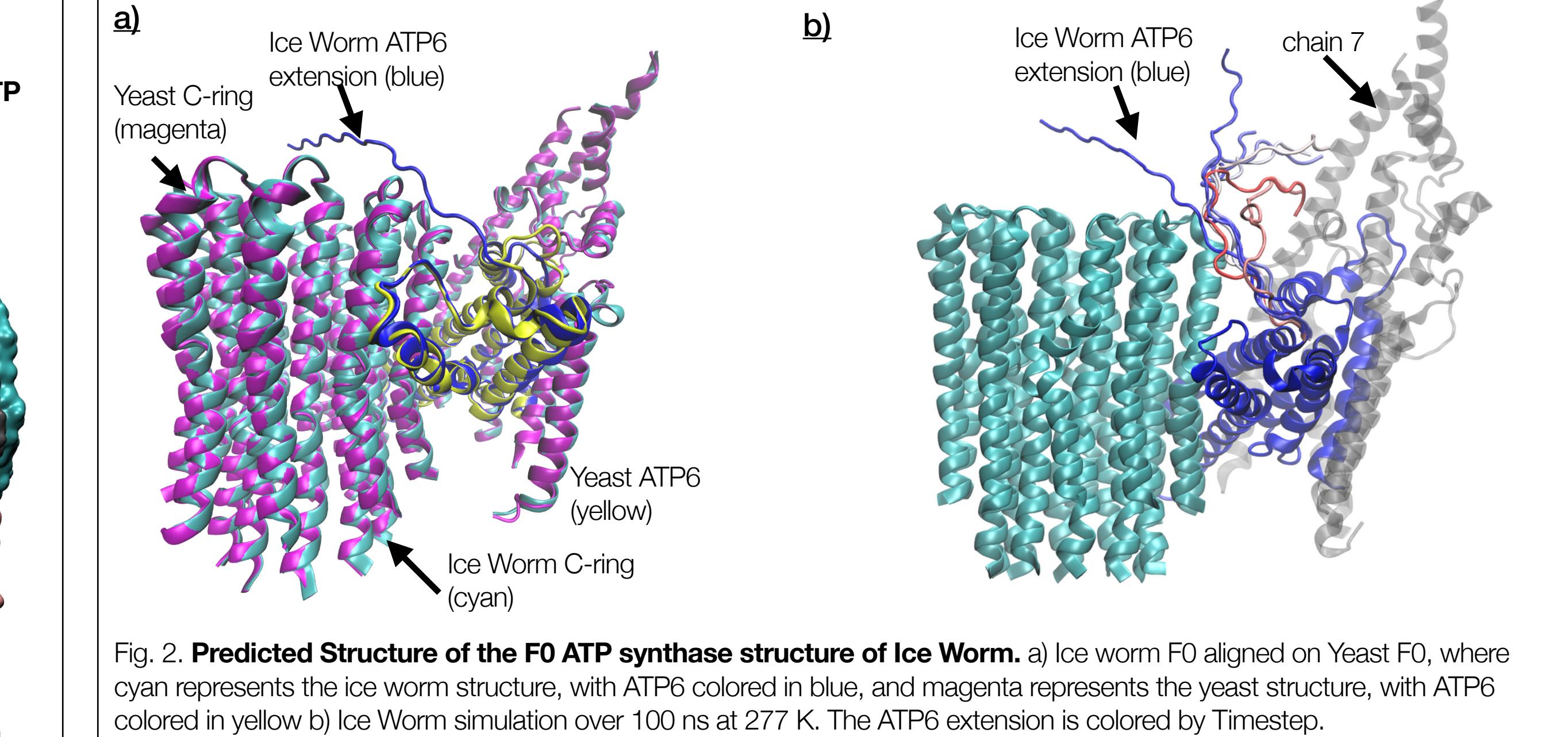


Fig. 2. Predicted Structure of the F0 ATP synthase structure of Ice Worm. a) Ice worm F0 aligned on Yeast F0, where cyan represents the ice worm structure, with ATP6 colored in blue, and magenta represents the yeast structure, with ATP6 colored in yellow b) Ice Worm simulation over 100 ns at 277 K. The ATP6 extension is colored by Timestep.

## Methods

- Using MEGA software, the Multiple Sequence Alignment (MSA) of the ATP6 subunit was calculated using the yeast sequence and worms with homologous sequences.
- Used UGENE Unipro to calculate the conservation scores of ATP6.
- Used CharmmGui to build a POPC lipid bilayer membrane with the F0 ATP synthase embedded.
- Used the Charmm36 model for the proteins, phospholipids, TIP3P waters and ions parameters.
- Ran atomistic molecular dynamics simulations using NAMD v2.14 for 400 ns at 277 K and 310 K.
- Analyzed production simulations using the Visual Molecular Dynamics (VMD) software, specifically the Colvars Dashboard.
- Built an ice worm F0 ATP synthase predicted structure using Modeller. Used the yeast F0 ATP synthase sequence as a template. Target sequences are from ice worms collected in Norway.

## Summary

- We calculated the Multiple Sequence Alignment (MSA) of the ATP6 subunit.
- We ran Atomistic Simulations on the F0 ATP synthase domain of the yeast at 277 K and 310 K.
- We built a homology model for the ice worm F0 ATP Synthase structure.
- In the F0 ATP synthase domain of the yeast, the rotational diffusion coefficient of the c-ring at 310 K is almost two times the rotational diffusion coefficient at 277 K. The results for the ice worm F0 ATP synthase are yet to be determined.
- Poorly conserved residues in yeast have higher RMSF than the highly conserved residues.

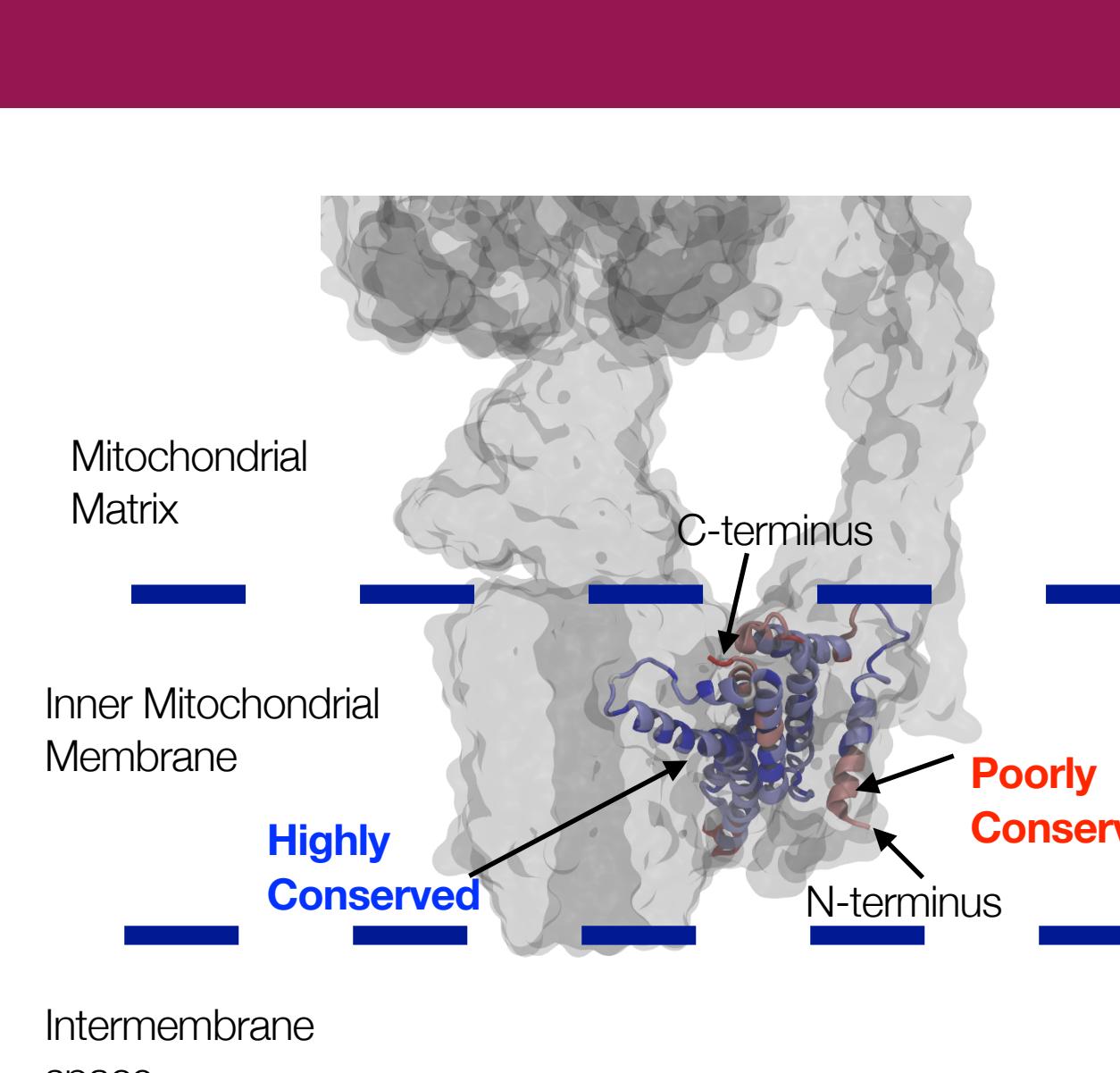


Fig. 3. Poorly Conserved Regions lie at the terminus. The ATP6 Subunit (cartoon structure) lies in the membrane-embedded F0 domain of the F1FOATP Synthase complex (grey). ATP6 is colored by conservation. The conservation scores range from 0.2 (dark red) as the most poorly conserved to 1 (dark blue) as the most highly conserved. Conservation scores were determined via multiple sequence alignment of ATP6 sequences from ice organisms.

## Results and Discussion

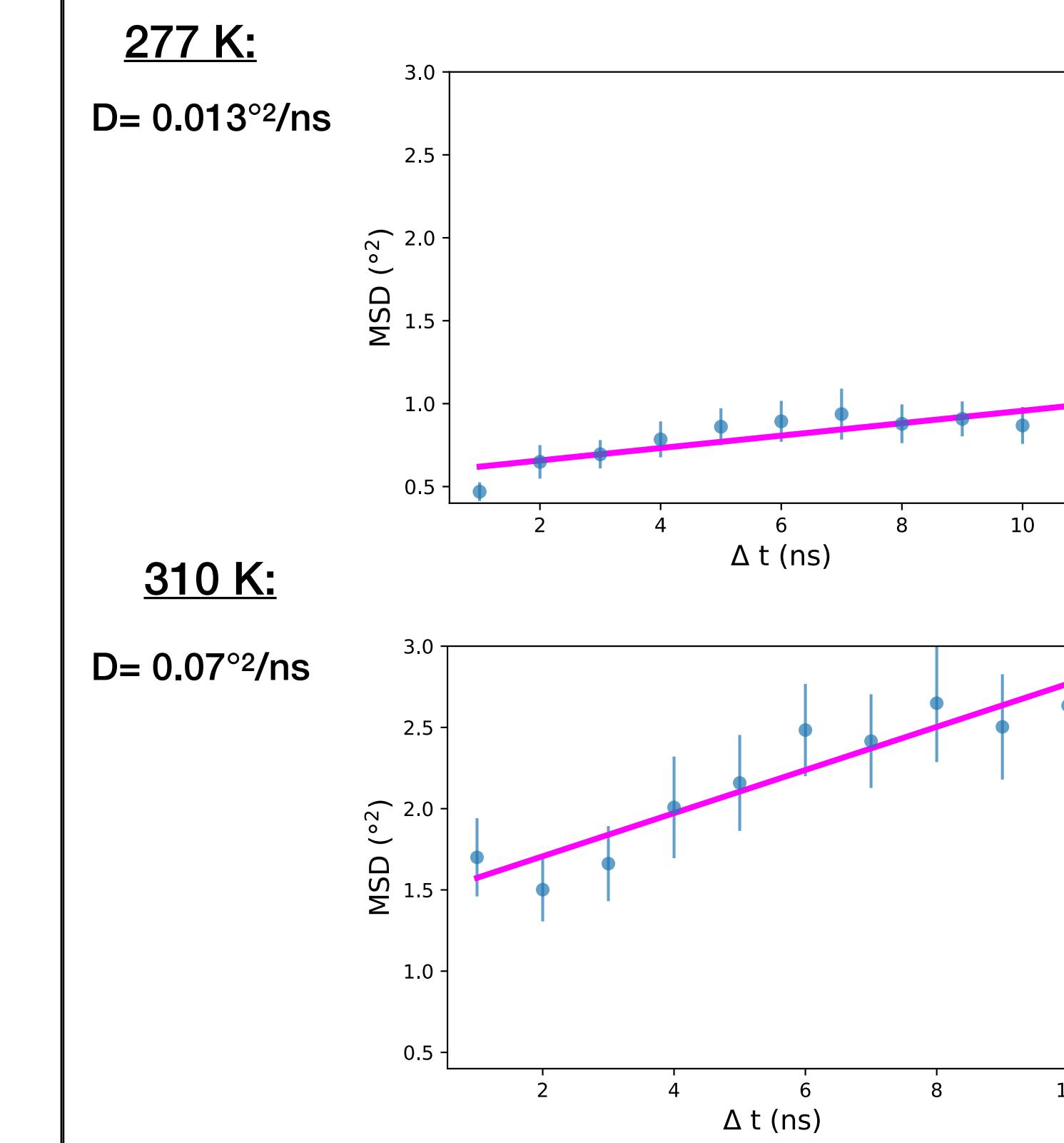


Fig. 4. Faster rotational diffusion at 310 K. MSD of the yeast ATP synthase c-ring rotation around the z-axis as a function of  $\Delta t$  from a NAMD simulation over 125 ns at 277 K and 310 K. The linear  $MSD \propto \Delta t$  dependence (solid magenta line) corresponds to diffusional displacement.

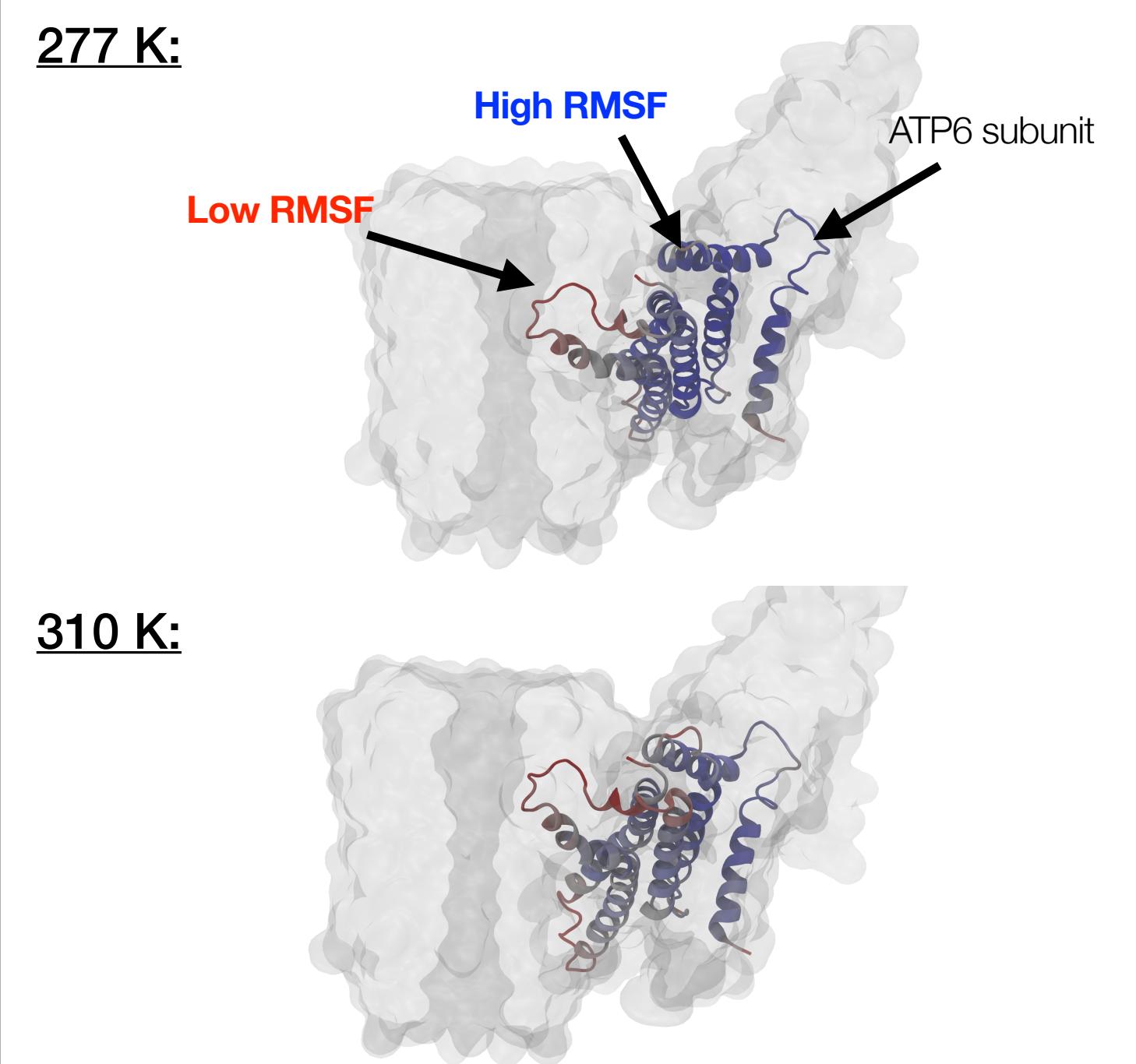


Fig. 5. Highly fluctuating regions lie at the terminus and the loops. The ATP6 Subunit (cartoon structure) lies in the membrane-embedded F0 domain of the F1FOATP Synthase complex (grey). ATP6 is colored by RMSF values. At 277 K, the RMSF values range from 0.81 (dark blue) to 3.07 (dark red) as the least to most fluctuations, respectively. At 310 K, RMSF values range from 0.9 (dark blue) to 3.12 (dark red) as the least to most fluctuations, respectively.

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## References

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