



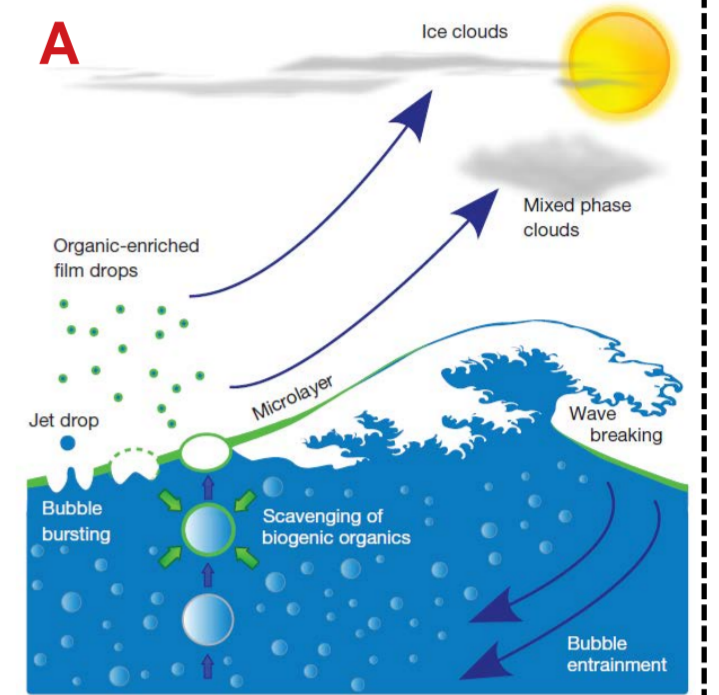
Freezing properties of cyanobacteria mat samples from the Antarctica



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1. MOTIVATION

Aerosols are considered the largest uncertainty in the radiative forcing estimates by the International Panel on Climate Change in their 2014 report¹, in part because their concentration, composition, and size, affect the properties of clouds². Water droplets can remain in super-cooled liquid state down to temperatures of approximately -38°C ³, which means freezing catalysts are required to initiate ice formation at warmer temperatures *via* heterogeneous freezing. Some aerosols act as freezing catalysts and are therefore defined as Ice Nucleating Particles - INPs. INPs can cluster a few molecules of water, orientate them into an ice-like structure, and decrease the energy barrier to nucleation⁴. Sea surface micro-layer, that is, the organic enriched interface between the seawater and the atmosphere, produces aerosols by bubble bursting mechanism and spume droplets created by high winds. Understanding the chemistry and physics of INPs will enable the creation of better meteorological and climate models. The aim of my internship was to investigate the freezing properties of three samples from the Antarctica containing cyanobacteria. Do they contain INPs? If yes, what is their nature?



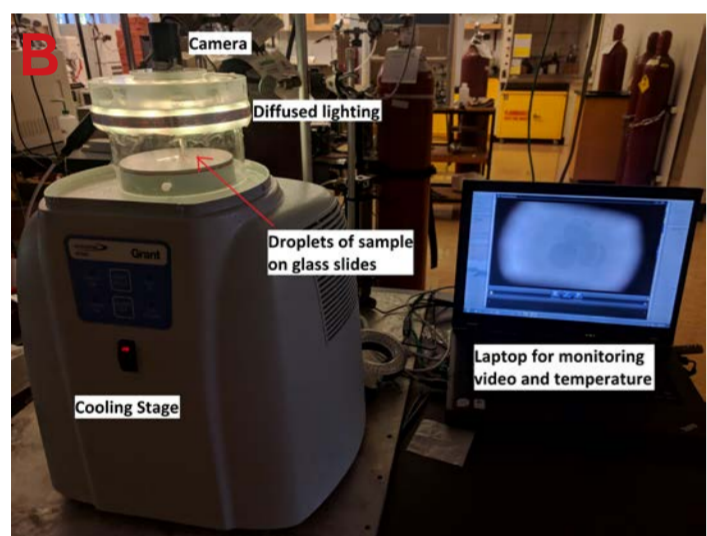
A) Generation of aerosols by bubble bursting mechanism⁵

2. EXPERIMENTAL

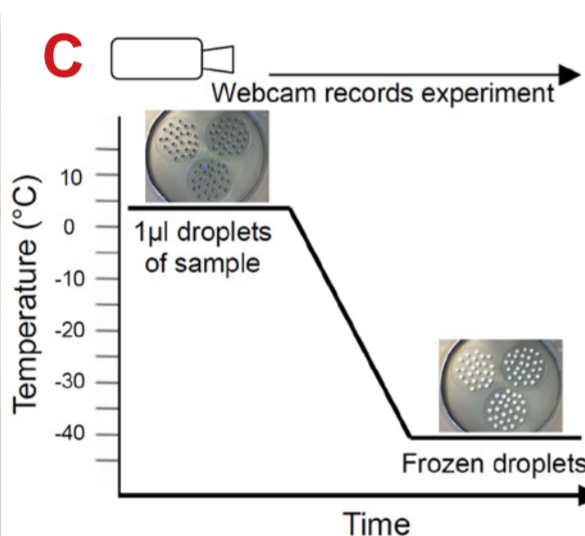
To investigate the freezing properties we compare the T_{50} of our samples with the T_{50} of ultra pure water. The T_{50} is the median freezing temperature, that is the temperature at which 50% of the droplets are frozen. To obtain the T_{50} we set up a freezing experiment.

Freezing experiment

Droplets ($1\mu\text{l}$) of each sample were pipetted onto siliconized glass slides. The droplets were cooled down until they froze. The experiment was recorded to determine the freezing temperature for each droplet.



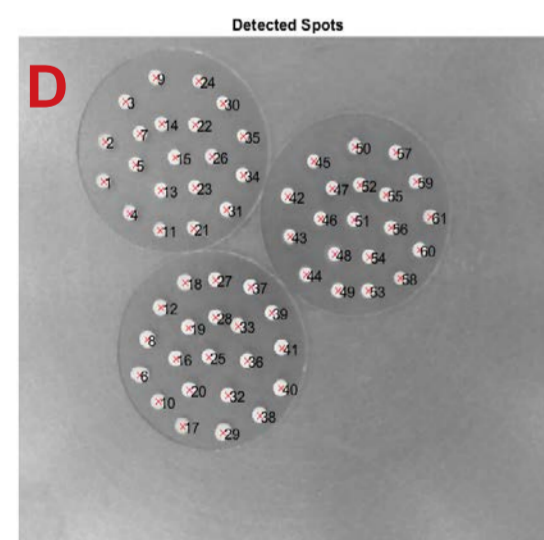
B) Droplet Freezing Setup



C) Experimental Procedure⁶

Matlab automated analysis

To automate the video analysis, a Matlab script was written with the help of Ashton Christy and Luke Melo from the Grant Group. The Matlab script proved to be more accurate, quicker in the execution and less prone to errors than the manual analysis. The freezing recognition strategy relied on the change of brightness of a droplet when it turns to ice.

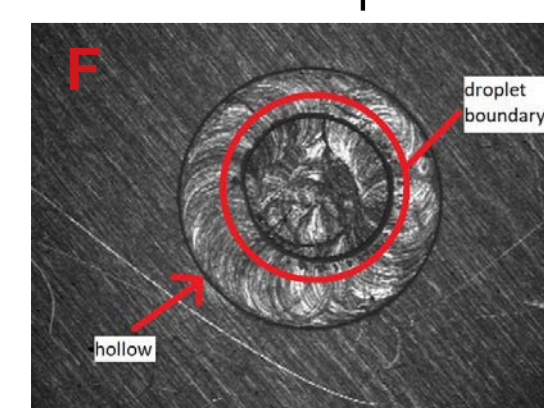


D) Labeled picture of the droplets produced by the script, where even an extra droplet has been correctly detected
E) Output table given by the script. The labels, coordinates, freezing times, and freezing temperatures can be seen.

Spot Number	Spot Position	Freezing Point	Freezing Point Time	Freezing Point Temp	Bioluminescence
41	(18,27)	-15.000	516.1836	677.2456 /f. a.u.	
42	(42,21)	-21.9100	486.8480	1997.2456 /f. a.u.	
43	(42,21)	-23.1000	678.8317	2082.2456 /f. a.u.	
44	(15,19)	-20.0000	495.1586	2062.2456 /f. a.u.	
45	(42,21)	-22.0000	603.1586	2048.2456 /f. a.u.	
46	(42,21)	-22.0000	654.6553	2048.2456 /f. a.u.	
47	(17,20)	-21.7000	643.4320	1983.2456 /f. a.u.	
48	(42,21)	-21.0000	628.1317	1983.2456 /f. a.u.	
49	(30,21)	-20.8000	624.4384	1753.2456 /f. a.u.	
50	(42,21)	-21.2000	642.6460	1983.2456 /f. a.u.	
51	(15,19)	-20.0000	624.6314	1983.2456 /f. a.u.	
52	(15,19)	-20.0000	624.4190	1758.2456 /f. a.u.	
53	(15,19)	-19.0000	606.6961	1974.2456 /f. a.u.	
54	(15,19)	-21.0000	646.3467	1972.2456 /f. a.u.	
55	(15,19)	-14.0000	495.7362	468.2456 /f. a.u.	
56	(15,19)	-20.0000	679.8814	2262.2456 /f. a.u.	
57	(17,20)	-21.5000	639.8372	1983.2456 /f. a.u.	
58	(15,19)	-19.0000	606.6147	2050.2456 /f. a.u.	
59	(15,19)	-16.7000	543.1112	961.2456 /f. a.u.	
60	(17,20)	-23.8000	678.2854	2297.2456 /f. a.u.	

Melting Point Experiment

The T_{50} s have to be corrected for the influence of salts and other electrolytes in the samples. For each sample, one droplet ($0.5\mu\text{l}$) was frozen then slowly reheated, and the melting was recorded. The liquidus temperature was extracted. This temperature enabled us to determine the freezing point depression, the water activity, and to link the latter back to calibrated freezing temperature available in Koop⁷.



F) Recording of a frozen droplet being melted. The experimenter identifies the liquidus temperature by the disappearance of the last opaque inclusion in the droplet.

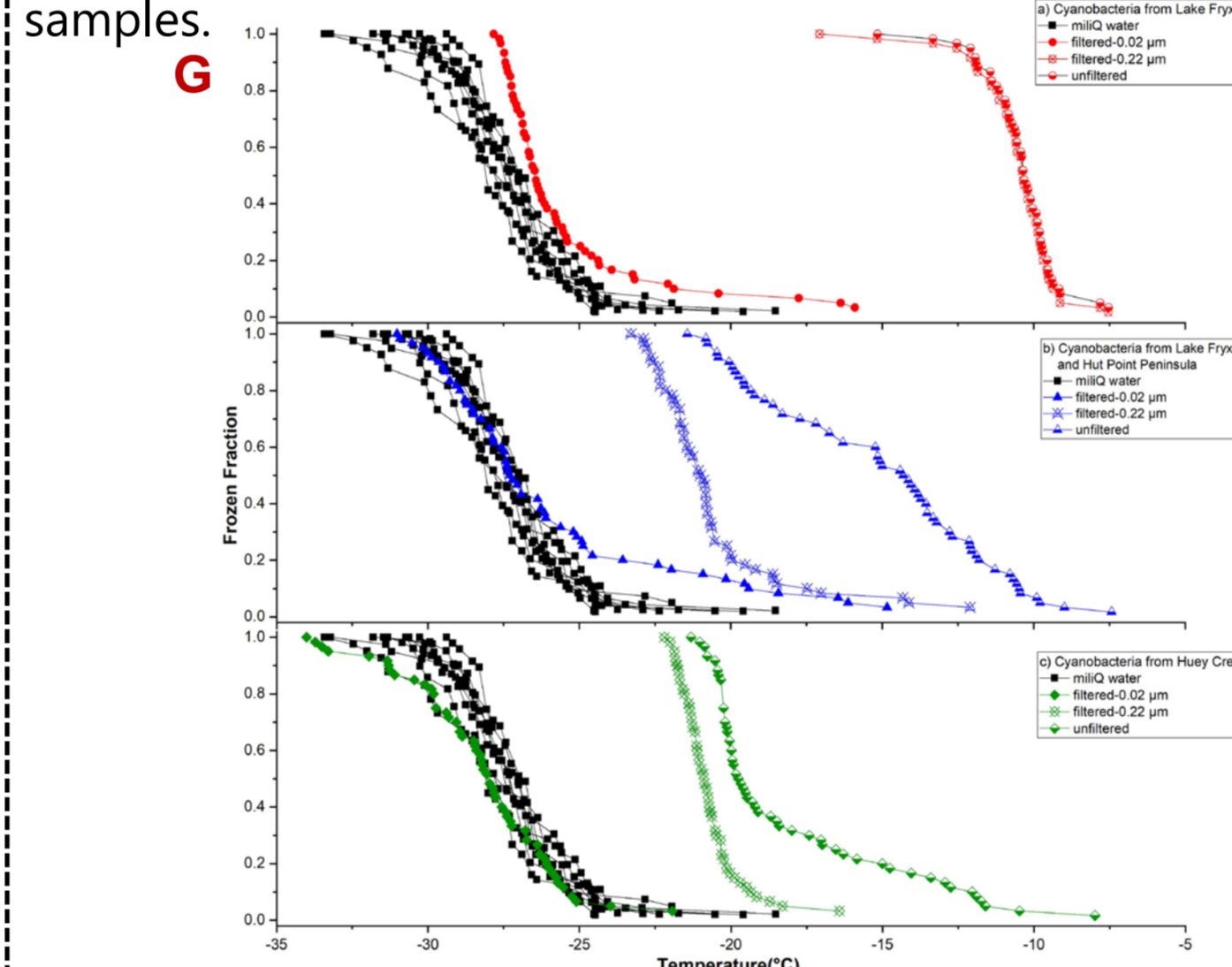
3. RESULTS

Three different treatments were applied to the samples before doing the freezing experiments:

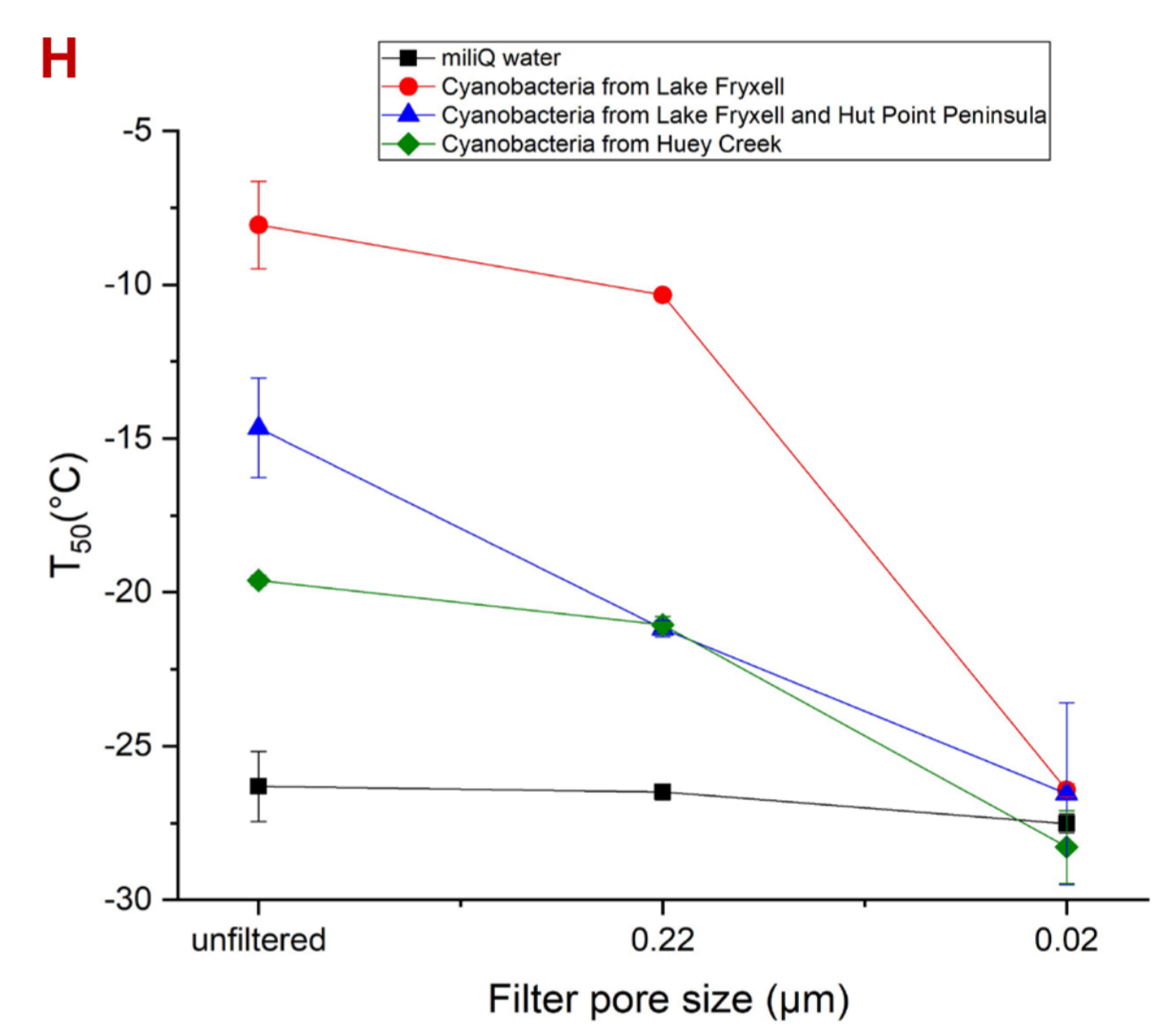
Filtering: helps determining the size range of the INPs

Heating: alters proteinaceous compounds, which should lower the T_{50} of the samples.

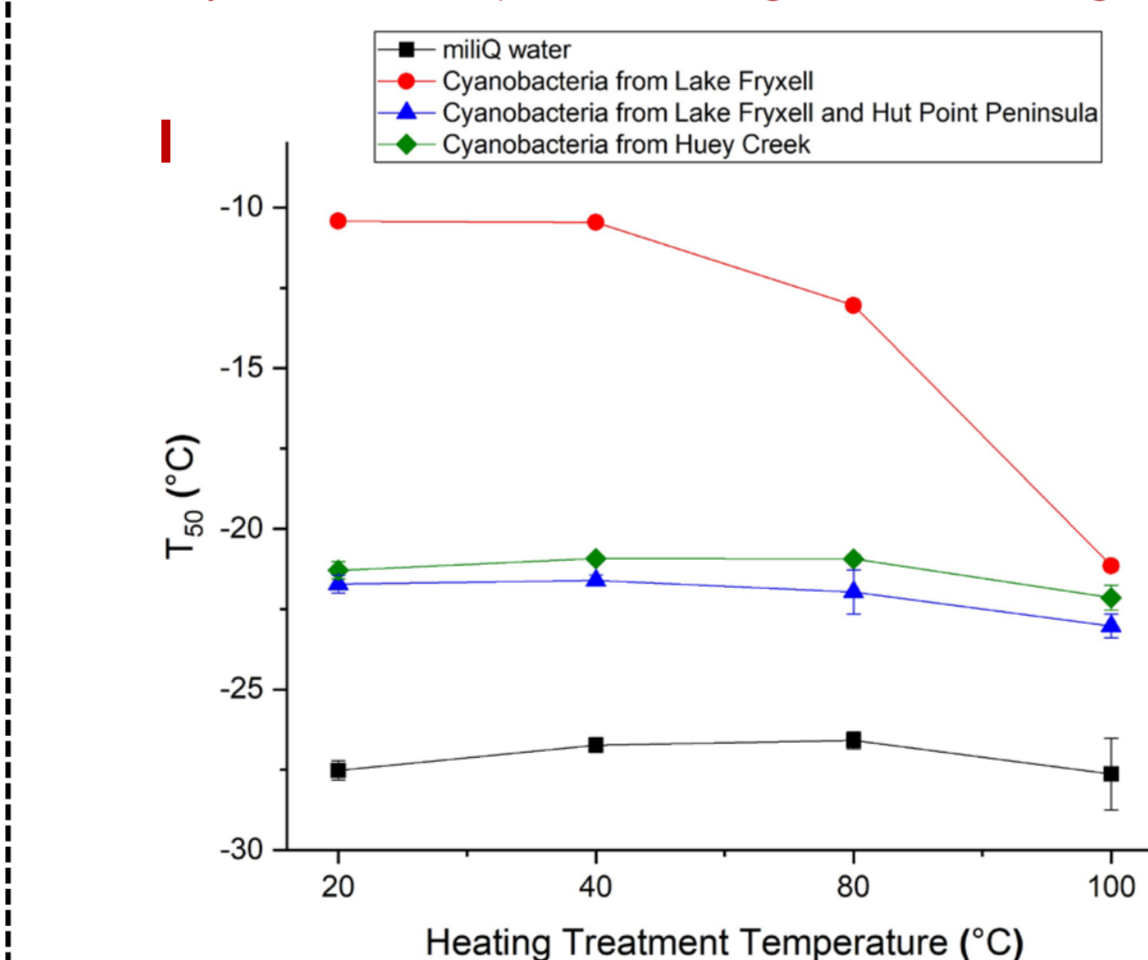
Mixing with Guanidinium Chloride (GCI): alters proteinaceous compounds, which should lower the T_{50} of the samples.



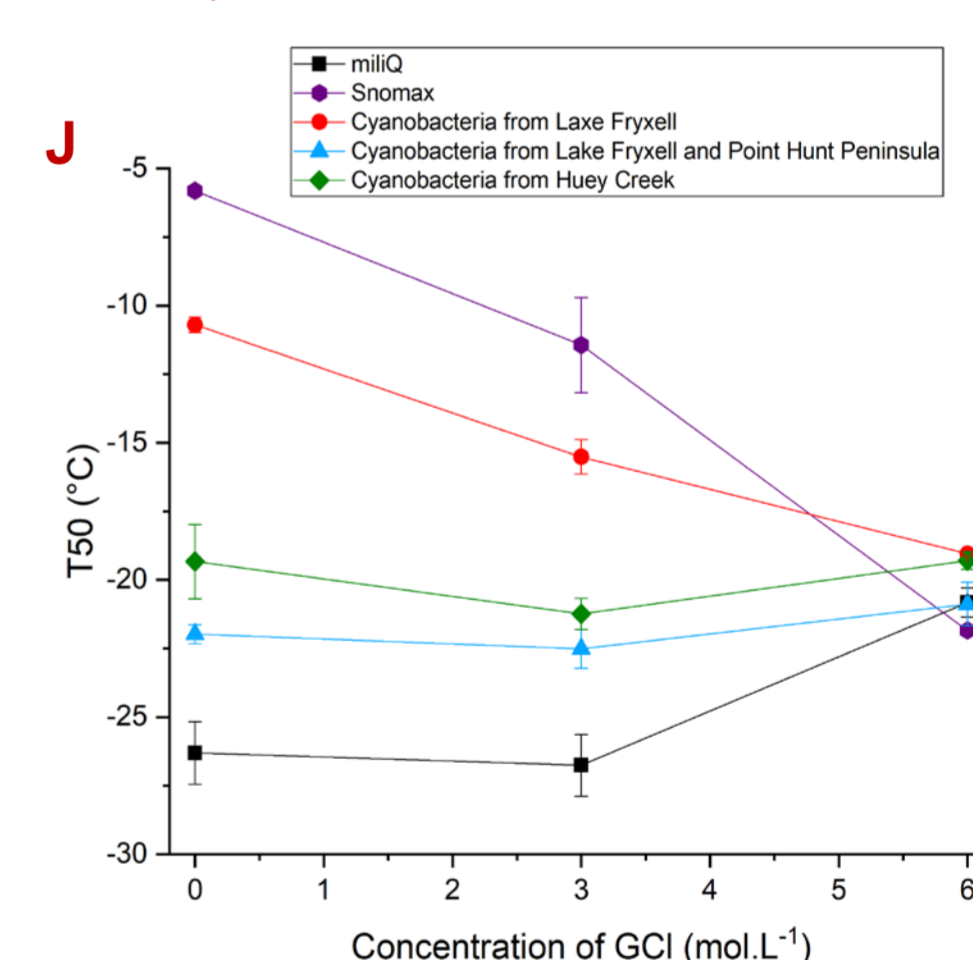
G) Frozen fractions of cyanobacteria samples after filtering, each point showing a freezing event.



H) T_{50} for cyanobacteria samples after filtering, error bars showing 95% confidence intervals based on three duplicate measurements.



I) T_{50} for cyanobacteria samples non-heated and heated to different temperatures for 1 hour, error bars showing 95% confidence intervals based on three duplicate measurements.



J) T_{50} for cyanobacteria samples treated with different concentrations of Guanidinium Chloride, error bars showing 95% confidence intervals based on three duplicate measurements.

4. INTERPRETATION

The three cyanobacteria samples contain INPs. The first sample from Lake Fryxell seems to contain proteinaceous INPs because its T_{50} is lowered both by heating treatment and by GCI treatment, while the two others seem relatively unaffected. As of now, the nature of the INPs in the Hut Point and the Huey Creek samples can't be determined. The INPs in the three samples are however of the same size range: $0.02\mu\text{m}$ - $0.22\mu\text{m}$. The action of GCI is more complex than expected as it seem to create INPs in pure water.

Another filtration could be done, and the retentate could be looked at by scanning electron microscopy, and by Energy-dispersive X-ray spectroscopy, which would help determine if the INPs are organic or inorganic.

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