

## Referee #2

1. Perhaps birch pollen washing water, freezing within a particularly narrow temperature window from  $-17\text{ }^{\circ}\text{C}$  to  $-18\text{ }^{\circ}\text{C}$  (Häusler et al., 2018, <https://www.mdpi.com/2073-4433/9/4/140>), would have been a good third substance to test.

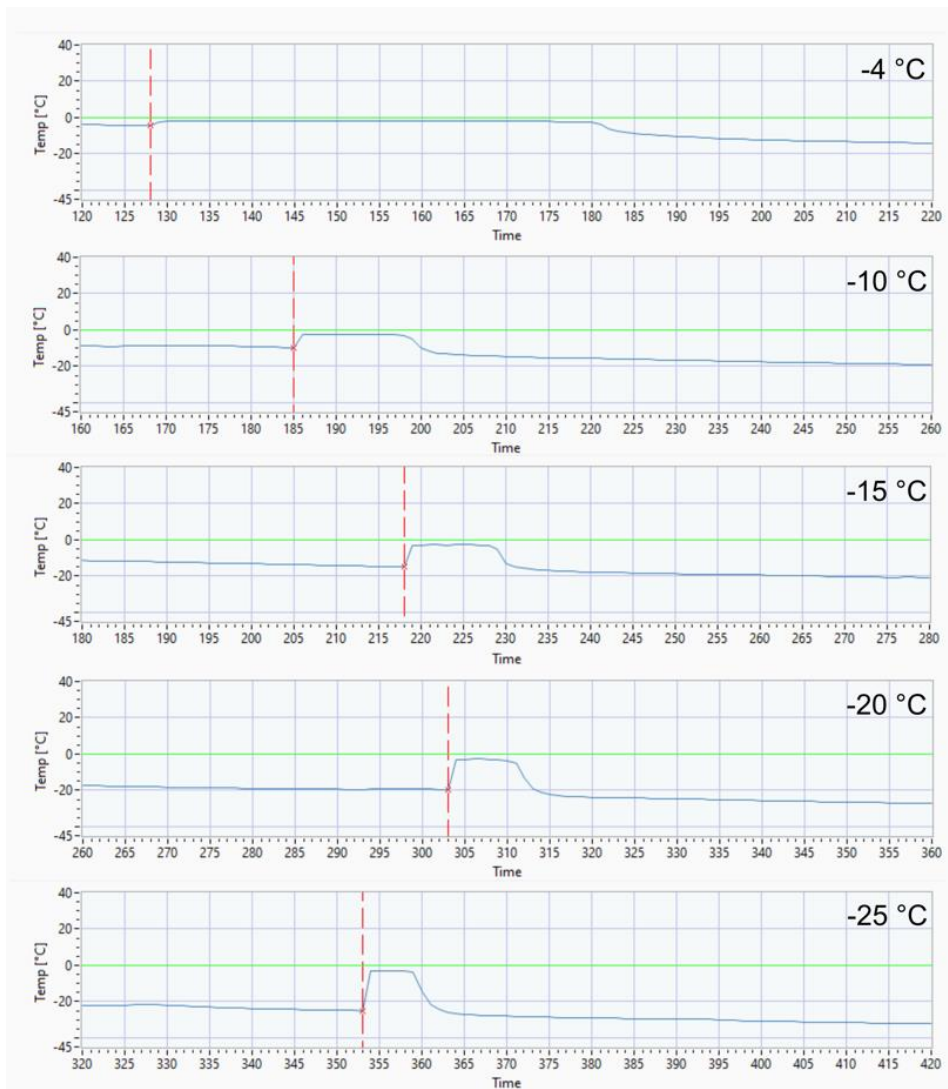
Thank you for this suggestion. It would be interesting to compare the freezing of birch pollen washing water, however this is beyond of the scope of this study. We have decided to use Snomax and Illite as test substance due to the already existing intercomparison studies.

2. A total of 384 droplets provides the opportunity to derive differential freezing spectra (Vali, 2019, <https://doi.org/10.5194/amt-12-1219-2019>) that eventually show the different types of INP discussed in Section 3.3. With little effort a re-analysis of the available data may thereby yield additional insights into ice nucleation active components of snomax and illite that can hardly be gleaned from the cumulative spectra, e.g., in Figures 5, 6, 7, S4, and S5..

Thank you for this suggestion. In our experiments, the 384-well plates were divided to measure several dilutions in the same run. In this way, we measured between 48 to 80 droplets per sample. As the main aim of the article was to validate the PINGUIN instrument, we chose to present cumulative spectra which are also used in intercomparison studies that we refer to.

3. The infrared camera is said to detect the moment of an 'ice nucleation event' (line 68), whereas an optical camera observes a prolonged period, that is the 'change in optical properties such as brightness of the sample during the process of the whole droplet freezing' (lines 68 and 69). Right, but the 'ice nucleation event' can nevertheless be located in time at the beginning of changes in optical properties, no matter how long it took until the droplet was completely frozen. Perhaps I am wrong here, but I would expect to see in the infrared camera record at a warm freezing temperature, say at  $-4\text{ }^{\circ}\text{C}$ , a rise in droplet temperature that is not a sudden step change and that also leaves some room for interpretation regarding the exact onset of freezing. The temperature record shown in Figure 4 is hard to analyse in this respect. Could you please show instead the record of a droplet frozen near  $-4\text{ }^{\circ}\text{C}$ , and narrow the range of the time axis to the minute or so in which the peak occurred?

During the nucleation event the droplet temperature rises to  $0\text{ }^{\circ}\text{C}$  within around 10 seconds. This temperature increase is fast also at high freezing temperatures. However, at high freezing, it takes longer for the droplet to freeze completely and cool down to ambient temperature. Although, it takes around 10 seconds to reach the plateau at  $0\text{ }^{\circ}\text{C}$ , we can detect the nucleation event very precise at the starting point of this temperature increase. We have added Figure S3 to the Supplementary showing the temperature profile for various freezing temperatures. As the temperature increase is higher for droplets freezing at lower temperature, we decided to show a droplet freezing at  $-25\text{ }^{\circ}\text{C}$  in Figure 4. However, we have changed the x-axis to show the freezing event in more detail.



4. Lines 36 and 55: Replace 'high fraction' with 'large fraction' and 'high number' with 'large number'.

We have changed the sentences as suggested.

5. Line 56: Replace 'low number' with 'small number'.

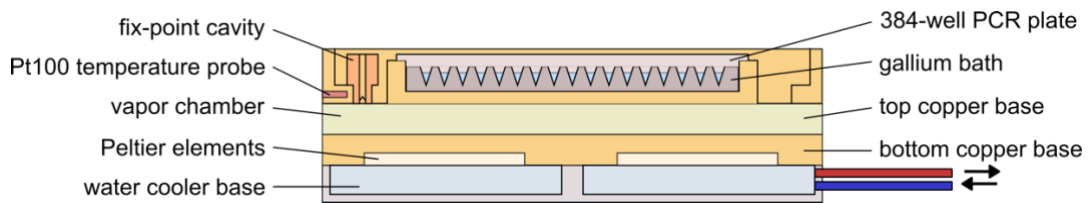
We have changed the sentence as suggested.

6. Lines 90 to 92: Consider rearranging the sentence in this way: 'Furthermore, we address the challenges due to inhomogeneities of the product and due to aging effects and propose a possible solution for using Snomax as a suspension for intercomparison studies and reproducibility measurements.'

We have changed the sentence as suggested.

7. Figure 2: Better use a colour for the vapour chamber (G) that is different from that of the copper components above and below it.

We have updated the figure and changed the color of the vapor chamber.



8. Line 150 onwards: I appreciate the idea to heat the samples for repeated analysis, but how can evaporative loss be prevented, especially in heat treatments near boiling point?

Thank you for this thought. We would need to evaluate this factor once the system modifications allow that heat treatments. If evaporation is a problem, we would cover the PCR plates with an adhesive plastic foil during the heating process.

9. The same question about evaporation arises in the next section, where the flow of dry air is discussed. During the development of the procedure, were sample trays weighed before and after a 40 min run to assess the loss due to evaporation?

Thank you for this suggestion. We have now performed the suggested experiment and found that the loss due to evaporation is 0.36% of the volume and thus negligible. We have added the following sentence after line 180:

"We have evaluated the sample loss due to evaporation and found that this factor is negligible as only 0.36% of liquid was lost during an experiment."

10. Line 175: A 'was' is missing before 'usually'.

Thank you, we have added the missing word.

11. I am not sure whether Section 2.4 is needed because it mostly describes common practice. Consider reducing it to the bare minimum and merging it with the preceding section.

Thank you for this suggestion. We agree that it is common practice, however, we have decided to include a short description to the manuscript to motivate and introduce the equations.

12. Figure 5a: At  $T > -11\text{ }^{\circ}\text{C}$  error bars extend to 1 INP/mg snomax, suggesting that one of the three experiments no or very little freezing events were observed  $> -11\text{ }^{\circ}\text{C}$ . After looking at Figure S4, I understand this is an artefact caused by the assumption of a normal distribution. In principle, you could estimate the multiplicative standard deviation (Limpert et al., 2001, <https://academic.oup.com/bioscience/article/51/5/341/243981>). However, three replicates cannot provide an estimate for that. Therefore, better show in Figure 5 all three replicates and not their mean and normal standard deviation.

We have updated Figure 5 and Figure 6 accordingly and show the 3 individual experiments instead of the mean and standard deviation.

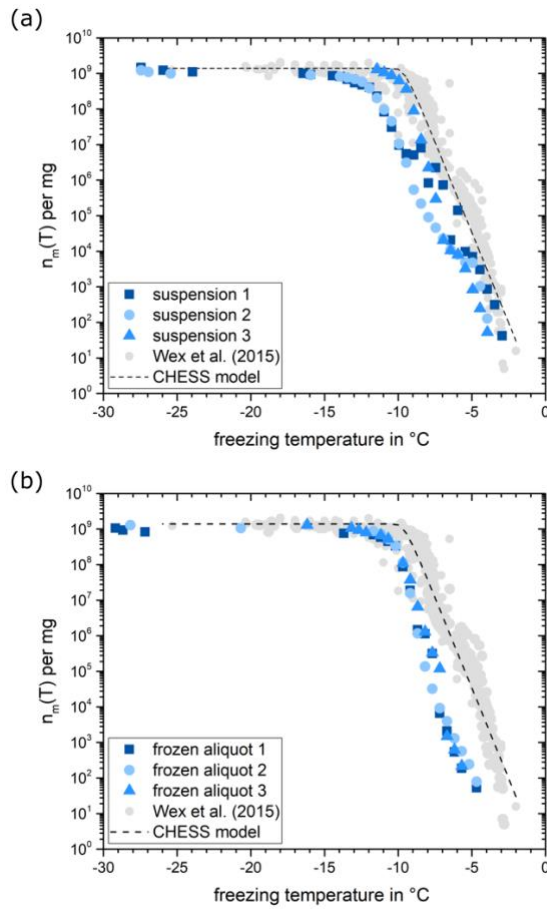
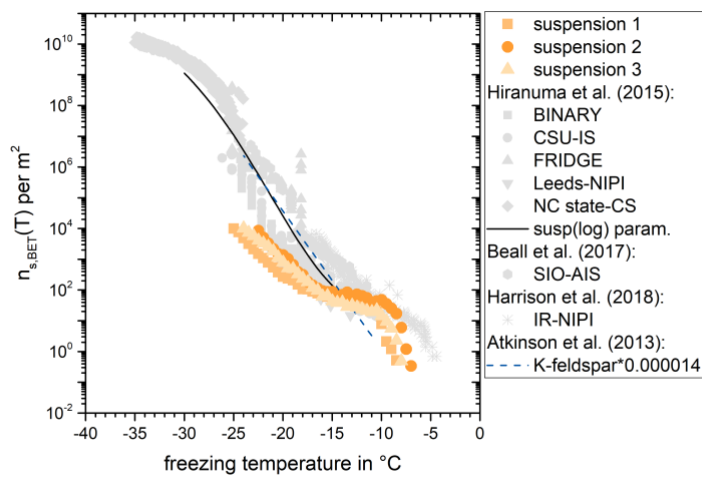


Figure 6:



13. Line 321: Consider to replace 'using' with 'analysing'.

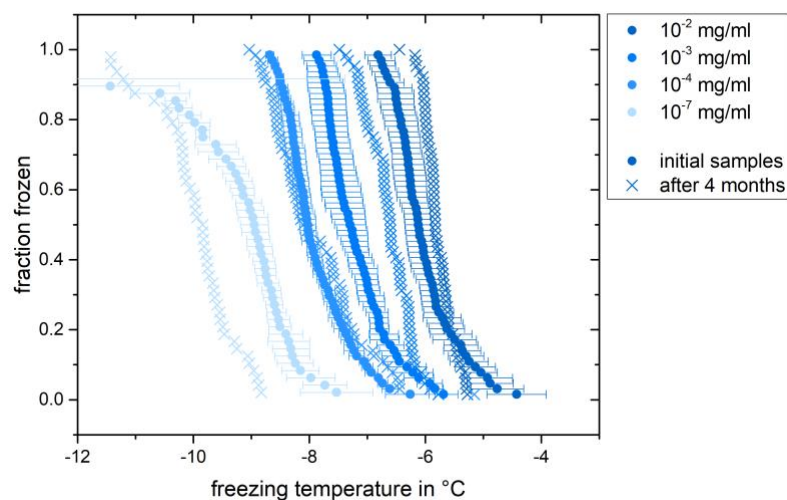
We have changed the sentence accordingly.

14. Line 330: The statement 'are within the range of the concentrations reported therein' has to be narrowed to the temperature range in which it actually applies (-8 °C to -23 °C).

We have modified the sentence: “The measurements obtained in this investigation fall in the lower end of the spectra recorded by other devices using a polydisperse Illite NX suspension (Hiranuma et al., 2015; Beall et al., 2017; Harrison et al., 2018), but are within the range of the concentrations reported therein for temperatures from -8 °C to -23 °C”

15. Figure S5: For fresh and old samples use better distinguishable symbols, e.g. , open circles and crosses, respectively.

We have updated the figure.



16. Lines 376 and 377: Maybe reconsider the statement 'recognition of nucleation events instead of freezing events' (please see my earlier comment above on this issue).

Due to the above explanation, we argue that this statement is true.

17. Line 380: Not sure what is meant by 'intercomparable' here, perhaps 'comparable'?

With “intercomparable” we want to express that it is comparable between the studies.