

Interactive comment on “Activation of Intact Bacteria and Bacterial Fragments Mixed with Agar as Cloud Droplets and Ice Crystals in Cloud Chamber Experiments” by Kaitlyn J. Suski et al.

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Response to Anonymous Referee #1

Suski et al present single-particle mass spectrometry (SPMS) measurements of bacteria and fragments that served as CCN and INPs in the AIDA cloud chamber during immersion cloud freezing experiments. This work tackles the important question of the role of primary biological particles in cloud formation and properties through laboratory experiments. I have questions below regarding interpretation of the data that may impact the results. Otherwise, revisions are recommended below primarily to increase clarity of the manuscript. The main finding of this work is that bacteria fragments

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(mixed with agar) serve as cloud droplet nuclei, whereas intact bacteria rarely nucleate cloud droplets. The miniSPLAT size distributions of particles prior to cloud formation (expansion) within the chamber compared to the cloud droplet residuals support this conclusion. However, I have several questions that may impact the interpretation of the results. Thank you for your detailed comments, suggestions, and questions. Below are our responses.

1) Could intact bacteria burst upon droplet activation or freezing? (Is there any support for this from previous studies?)

There is no evidence that bursting is occurring in the cloud chamber. If the intact cells were bursting during the expansion, they would not be observed post-expansion in roughly equal fractions as they were pre-expansion, as shown in the new Figure 7.

Or, could intact bacteria burst during drying within the CVI? The aerosolized bacteria were dried prior to injection into the AIDA chamber; therefore, if bursting of these specific intact bacterial cells were to occur during drying, it should happen during this initial drying process as well. Consequently, it is unlikely that bursting occurs upon drying in the PCVI or IS-PCVI. Moreover, if intact bacteria activate and then burst in the PCVI, we would expect to see an increase in the number of particles measured by the CPC that is downstream of the PCVI, similar to what is seen during aircraft flights when shattering of large droplets and ice crystals in CVIs occurs. These types of concentration spikes were not observed in the present study.

2) What are the size cuts of the PCVI and IS-PCVI and transmission efficiencies? This information needs to be provided in the experimental section. For each expansion, what fraction of the droplets and ice crystals were and were not transmitted through the CVIs? (Provide in results & discussion.)

The cut-sizes for the PCVI and IS-PCVI used in the 3 expansions have now been included on the expansion plots (Figures 4, 9, and 13), as well as in Table S1 in the supplement. We have also calculated the fraction of droplets and ice crystals

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that were transmitted using the equation below and have plotted the fractions in Figure S1. Fraction of the droplets and ice crystals reached at the $CVI(t) = (PCVI \text{ CPC}(t) \times F_{\text{output}} / F_{\text{input}}) / (W_{\text{elas2}}(t))$ where F_{output} is the CVI output flow (lpm), and F_{input} is the CVI input flow (lpm).

Table S1 also lists the transmission efficiencies of 94.4% for Expansion 1 and 61.9% for Expansion 2 determined according to Figure 3 of Gallavardin et al. (2008) and 91.5% for Expansion 3 determined according to Fig. 13 of Hiranuma et al. (2016).

Gallavardin, S. J., Froyd, K. D., Lohmann, U., Möhler, O., Murphy, D. M., and Cziczo, D. J.: Single particle laser mass spectrometry applied to differential ice nucleation experiments at the AIDA chamber, *Aerosol Sci. Tech.*, 42, 773–791, doi:10.1080/02786820802339538, 2008.

Hiranuma, N., Möhler, O., Kulkarni, G., Schnaiter, M., Vogt, S., Vochezer, P., Jarvinen, E., Wagner, R., Bell, D. M., Wilson, J., Zelenyuk, A., and Cziczo, D. J.: Development and characterization of an ice-selecting pumped counterflow virtual impactor (IS-PCVI) to study ice crystal residuals, *Atmos Meas Tech*, 9, 3817–3836, 10.5194/amt-9-3817-2016, 2016.

3) It is stated that the pressure changes during expansions impacted the miniSPLAT aerosol velocities. What was the dependence of the size dependent miniSPLAT inlet transmission efficiency on these pressure changes during expansions? If particles $>0.5 \mu\text{m}$ were not as efficiently transmitted through the aerodynamic lens at these lower pressures, this would explain why intact bacteria were not observed in the cloud particle residues.

The size-dependent transmission efficiency of the aerodynamic lens inlet, used by the AMS and miniSPLAT, is indeed expected to change with pressure. Liu et al. 2007 presents the results of CFD modeling and experimental data for sampling pressures of 760 torr and 585 torr. It shows that while the model predicts a decreased transmission efficiency for larger particles, measurements on 3 different particle types show

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virtually no change for the transmission efficiency of larger particles. The reference to this paper has also been added to the revised manuscript. Most importantly, our measurements conducted during other expansions in the AIDA chamber show that when larger particles activate more efficiently, it can be clearly observed in the miniSPLAT-measured dva distributions. For example, Figure 1 in the responses shows the normalized miniSPLAT-measured dva size distributions measured before and during an expansion with feldspar present in the chamber. The size distribution of cloud residuals sampled during the expansion clearly shows an enhancement in the relative fraction of larger particles. This suggests that larger particles are effectively transmitted through the PCVI and the miniSPLAT inlet during expansions when pressures are lower than normal atmospheric pressure, consistent with the data reported in Liu et al. 2007.

Peter S. K. Liu , Rensheng Deng , Kenneth A. Smith , Leah R. Williams , John T. Jayne , Manjula R. Canagaratna , Kori Moore , Timothy B. Onasch , Douglas R. Worsnop & Terry Deshler (2007) Transmission Efficiency of an Aerodynamic Focusing Lens System: Comparison of Model Calculations and Laboratory Measurements for the Aerodyne Aerosol Mass Spectrometer, *Aerosol Science and Technology*, 41:8, 721-733, DOI: 10.1080/02786820701422278

Additional Major Comments: - Experimental (Page 5): Please provide additional information about the miniSPLAT operation. What was the LDI power, and did it vary during the study (since ion fragmentation is dependent on this)?

The text has been modified to include this information. “The dual particle detection is also used to generate a trigger for the excimer laser (GAM Lasers Inc., Model EX-5), operated at 193 nm with a constant laser energy of 1.0 ± 0.1 mJ/pulse, which ablates the particles and generates positive and negative ions.”

What was the size dependent inlet transmission efficiency as a function of inlet pressure?

A full size-dependent transmission efficiency curve was not generated for each inlet

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pressure, but as discussed above, larger particles >0.5 μm were transmitted during expansions. Figure 1 shows that the ratio of the two particle types measured by miniSPLAT before the expansion is consistent with the size distributions generated from the SMPS and APS. The important issue here is the difference in the ratio of the concentrations of the two particle types before the expansion and of cloud residuals during the expansion. Size-dependent transmission efficiency curves for two pressures and 3 particle types are given in Liu et al. 2007 (Figures 9 – 11, Table 3). Using the data presented by Liu et al. in Table 3 we plotted their measured averaged transmission efficiencies for two pressures shown in Figure 2 in the response. The miniSPLAT size distributions measured before and after the expansions for the 3 experiments show two clear peaks for two particle types, while the cloud residual size distributions show that the large particle mode is missing. It is not that no large particles were detected in cloud residuals, they are clearly detected for other samples as explained above, but the distinct peak of the intact bacteria mode is not present.

Peter S. K. Liu , Rensheng Deng , Kenneth A. Smith , Leah R. Williams , John T. Jayne , Manjula R. Canagaratna , Kori Moore , Timothy B. Onasch , Douglas R. Worsnop & Terry Deshler (2007) Transmission Efficiency of an Aerodynamic Focusing Lens System: Comparison of Model Calculations and Laboratory Measurements for the Aerodyne Aerosol Mass Spectrometer, *Aerosol Science and Technology*, 41:8, 721-733, DOI: 10.1080/02786820701422278

How were size calibrations conducted (PSLs?) and over what particle size range?

This has been added to the text. “Given that the inlet pressure is changing during the expansions, polystyrene latex spheres (PSLs) of known sizes ranging from 73 nm to 993 μm were used to generate a pressure dependent size calibration curve over a range of pressures from 0.9 to 4 Torr.”

What is the size range of efficient inlet transmission, and does it depend on pressure? (Figure 1 only gives a lower detection limit – no upper limit is provided, although it is

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clear in this figure that transmission appears to drop off above 1 μm .)

The size-dependent transmission efficiency of the inlet used in the SPLAT II and miniSPLAT instruments at normal atmospheric pressure is provided in Zelenyuk et al. (2009). Liu et al. (2007) reports measured size-dependent transmission efficiencies at 2 sampling pressures (Response Figure 2). Our measurements conducted during other expansions in the AIDA chamber show that \sim micron-sized particles like feldspar that activated as CCN and/or IN are transmitted and detected by miniSPLAT.

Zelenyuk, A., Yang, J., Choi, E., Imre, D. (2009). SPLAT II: An Aircraft Compatible, Ultra-Sensitive, High Precision Instrument for In-Situ Characterization of the Size and Composition of Fine and Ultrafine Particles. *Aerosol Science and Technology* 43:411-424.

Peter S. K. Liu , Rensheng Deng , Kenneth A. Smith , Leah R. Williams , John T. Jayne , Manjula R. Canagaratna , Kori Moore , Timothy B. Onasch , Douglas R. Worsnop & Terry Deshler (2007) Transmission Efficiency of an Aerodynamic Focusing Lens System: Comparison of Model Calculations and Laboratory Measurements for the Aerodyne Aerosol Mass Spectrometer, *Aerosol Science and Technology*, 41:8, 721-733, DOI: 10.1080/02786820701422278

How were number concentrations obtained from the miniSPLAT data (what calibration/data processing was done)?

This has been added to the text for clarity. “miniSPLAT-measured particle number concentrations are calculated by dividing the particle detection rate at the first optical detection stage by the pressure-dependent sampling flow rate as described in detail in Vaden et al. (2011a).” This approach was previously applied to aircraft-based measurements and resulted in an average difference of 0.5% between 1-sec concentrations measured by miniSPLAT and a dedicated particle counter (Vaden et al. (2011a)).

- Page 5, Line 31: In addition to different relative peak intensities, there appear to be

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differences in the individual ions present at $> m/z$ 50. This should be discussed.

It is easier to compare the mass spectra of the two particle modes using the new Figure 3 (original Figure S1), in which the lower intensity peaks are magnified. It shows that the mass spectra of the two particle types in the chamber have the same mass spectral peaks, just with different relative intensities.

- To aid interpretation of the mass spectra, please label all ions in Figures 2, 5, 7, 10, and 14. If the chemical identity is not known, please list possibilities and/or at least the numerical m/z .

In the new version of the paper these figures include labeled mass spectral peaks.

Also, please label in the captions whether these mass spectra correspond to averages or representative examples.

They are averages. A note was added to the figure captions.

- Figure S1 is a very informative figure, and the authors may consider moving this to the main text. In particular, the labeling of the ions is useful, and the magnification of the small peaks is helpful. Regarding the comparison here, what differences are observed between the 'agar + bacterial fragments' and 'agar'? This should be discussed in the main text to support the inclusion of agar in the aerosols (Page 6, Line 8). It appears that the main difference may be the presence of m/z 131 in the bacterial fragments. Is this observed in the aerosols and cloud residues? The mass spectra shown in the main text are only shown up to m/z 120, so the reader cannot evaluate this. If m/z 131 is indeed a primary difference between the mass spectra, then I recommend that the authors show the mass spectra in the main text up to at least m/z 135.

We have moved this figure to the main text. Both aerosolized intact bacteria and bacteria fragments mixed with agar are composed of the same compounds, albeit present in different ratios. Therefore, it is not surprising that the mass spectra of all particles in the chamber have the same mass spectral peaks just with different relative inten-

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sities, as shown in the new Figure 3. All of these particles, however, contain distinct phosphorus-containing peaks that separate them from pure agar. The peak at m/z 131 (possibly, $C_7H_{17}NO$) also appears to be characteristic of bacteria and of bacterial fragments. Additionally, one of the findings presented in this manuscript is that the fraction of agar and bacterial fragments is changing with particle size. Figure 12 of the revised manuscript and Figure 3 in the response clearly illustrate this point. The simultaneous measurements of single particle composition and size provides the means to distinguish between the two particle types. However, distinguishing between intact bacteria and large particles composed of bacterial fragments mixed with agar based on their mass spectra is impossible.

- Page 6, Line 3: It is stated here that “intact cells show relatively lower intensities for the metal ions ($23Na^+$, $39/41K^+$, $40Ca^+$)”, but I do not see this in Figure 2.

The mass spectra shown in Figure 2 are normalized to the most intense peak (K^+). The intensity of this peak for intact bacteria represents a much lower fraction of the total mass spectral intensity (sum of all red peaks) as compared to the bacteria fragments mixed with agar. Figure 3 in the response shows the size-dependent mass spectra of bacterial fragments mixed with agar, which were normalized by the total MS intensity. It clearly shows that the relative intensity of K^+ decreases with particle size. Similarly, Figure 12, which used to be Figure 11 in the original version, provides another display of the decreasing relative intensity of the K peak with particle size.

- Page 6, Line 4: Please discuss the organic ions present, as they are not stated here in terms of m/z or ion formula, making interpretation of this statement by the reader not possible. There are many previous SPMS papers on primary biological particles that could aid in this mass spectral interpretation. A greater interpretation of the ions above m/z 41 may aid in the interpretation of the results. Example manuscripts to consult for SPMS biological particle mass spectra include (but are not limited to): Fergenson et al 2004 (Analytical Chem), Czerwiec et al 2005 (Analytical Chem), Srivastava et al 2005 (Analytical Chem). Peaks unique to the bacteria (fragments and intact) and not

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agar should be highlighted in this discussion.

We have expanded this discussion to include more ion identification.

- Page 7, Lines 23-25: It is stated here that “The small differences between the MS of residuals and the small particle mode suggest a slightly higher content of organics in the residuals”, but I do not see this in Figure 5. Please provide additional support/description (e.g. label and discuss specific peaks).

We have added more information about the organic ions in the mass spectra and expanded the discussion.

- Page 9, Line 3: This sentence implies that peaks $> m/z$ 44 are all organics, which contradicts Page 6, Lines 1-2 and previous SPMS biological particle studies that show higher mass inorganic peaks as well. This discussion should consider specific peaks and fix this statement.

We have revised this discussion and modified the original Figure 11 (new Figure 12). There are a number of ways to illustrate that the K^+ peak intensity relative to organic peaks decreases with particle size. Figure 12 and Figure 3 in the response, all illustrate this important point. One of the important findings presented in this paper is that the relative concentrations of agar and bacterial fragments in the small particle mode change with particle size, such that the fraction of agar decreases with particle size.

- Page 9, Lines 28-30: Typically CVIs concentrate cloud residuals during sampling and this transmission is size-dependent. This information needs to be stated in the experimental section, and this needs to be considered in the interpretation of the number concentrations measured after the CVI, as discussed on this page. The reader also does not have knowledge of the transmission differences between the IS-PCVI and PCVI to evaluate the transmission of cloud droplets vs ice crystals as stated here.

The transmission efficiencies and cut-sizes have been added to the text. The size dependent concentration factor would be based on droplet or ice crystal size and not

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residual size. Therefore, calculating the size dependent residual enhancement is not relevant. Average particle concentration enhancement factor is calculated according to Eqn. 6 of Hiranuma et al. (2016) and was 2 for the PCVI and 12 for the IS-PCVI. This information has also been added to the text and the table in the supplement.

- Page 11, Line 31 – Page 12, Lines 1-2: This result was not shown or discussed in the results & discussion, and if included here in the conclusions, it should be shown and discussed.

This result is from unpublished data, so we have added a reference to a personal communication for this result. The n_s value for agar is $7.82 \times 10^8 \text{ m}^{-2}$ at $-22.95 \text{ }^\circ\text{C}$ (250.20 K).

- To aid in comparison of the results between the different expansions, the authors are encouraged to combine (into a multi-panel plot or by stacking the mass spectra) the following mass spectral figures: 5, 7, 10, and 14. Similarly, interpretation would be improved with combining the following size distribution figures: 4, 9, and 13. Figures 1 and 6 could be similarly combined.

We find that data in combined multi-panel plots are difficult to comprehend and interpret. Most importantly, we chose to present the results as three separate experiments to emphasize that the data from three separate experiments and two different bacteria are perfectly reproducible.

- Figure 11 and associated discussion: Since ion intensities in laser desorption ionization are dependent on ionization energies (e.g. Gross et al 2000, Analytical Chem.; Reinard & Johnston 2008, J. ASMS), the ion ratios used here need to be discussed in greater detail as they do not represent quantitative mole or mass ratios. In particular, inorganic ions typically have much lower ionization efficiencies compared to organic ions, which also undergo significant fragmentation at 193 nm. In addition, the organic ions included here in the ratio need to be stated here, in the experimental, or in the results & discussion. While it is clear that the organic contribution increases with size

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(and this is a useful result), clarification needs to be provided for the reader not familiar with LDI. Additionally, the text on page 9 refers to the dva distribution here as a 'schematic representation'. Please clarify in the caption and show actual data instead.

The revised figure (new Figure 12) shows the actual dva distribution. As we discussed above, there are a number of ways to illustrate that the intensity of the K⁺ peak relative to organic peaks decreases with particle size. Figure 12 and Figure 3 in the response, all illustrate this important point. The text also clearly states that the ratios presented in Figure 12 serve as a simple qualitative measure of the relative fraction of bacterial fragments and agar in these particles. We have modified the text to clarify how the ratio shown in the figure was calculated. We agree that this is one of the important findings in this study.

Minor Comments: - Introduction: Many sentences do not include references, which should be added to support the statements. In particular, references are needed on page 2, lines 1, 12, 14, 20, and 21.

References have been added to these sentences.

- Page 3, Line 23: Move this sentence to the end of the previous paragraph, as one sentence does not constitute a full paragraph.

It has been moved.

- Page 4: RH is defined twice here as well as on page 2.

This has been fixed. Thank you.

- Page 4, line 15: Why are the SMPS and APS data converted to volume-equivalent diameters? It would seem more appropriate, for comparison to the miniSPLAT data (in vacuum aerodynamic diameter), to simply convert the SMPS mobility diameters to aerodynamic diameter and leave the APS data in aerodynamic diameter. Also, please provide a reference for the chosen particle density.

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A similar application of volume equivalent diameter in SMPS and APS data for other studies in the AIDA chamber can be found in Hiranuma et al 2015 (Nature Geosci.; DOI: 10.1038/NGEO2374, See Supplemental Information Section 1.3) and many other publications. In addition, the volume-equivalent diameter was used for the SMPS/APS data to be comparable to Welas2-measured metrics (e.g., Hiranuma et al., 2015; Atmos. Chem. Phys., 15, 2489–2518, 2015). Sect. 3.4 of Hiranuma et al. (2016) and references therein describe why volume-equivalent diameter is the best choice for displaying welas2 data, but briefly it is because we do not know the geometry of the ice crystals. The density of 1.4 g/cm³ was used here to arrive at a good overlap in the SMPS and APS size distributions, assuming particle sphericity (Peters et al. (2006)). We have added more clarification on this in the text. The effective density of this bacteria of 1.4 g cm⁻³ has been previously measured in Wex et al. 2015 and is in a good agreement with the effective density of 1.38 g cm⁻³ measured by miniSPLAT for intact pseudomonas syringae during the FIN-1 campaign. Moreover, the vacuum aerodynamic diameter measured by miniSPLAT is not the same as aerodynamic diameter measured by APS. What is important for this study is that 2 distinct particle modes are clearly visible in the miniSPLAT and SMPS/APS-measured size distributions.

Hiranuma, N., Möhler, O., Kulkarni, G., Schnaiter, M., Vogt, S., Vochezer, P., Jarvinen, E., Wagner, R., Bell, D. M., Wilson, J., Zelenyuk, A., and Cziczo, D. J.: Development and characterization of an ice-selecting pumped counterflow virtual impactor (IS-PCVI) to study ice crystal residuals, Atmos Meas Tech, 9, 3817-3836, 10.5194/amt-9-3817-2016, 2016.

Wex, H., Augustin-Bauditz, S., Boose, Y., Budke, C., Curtius, J., Diehl, K., Dreyer, A., Frank, F., Hartmann, S., Hiranuma, N., Jantsch, E., Kanji, Z. A., Kiselev, A., Koop, T., Möhler, O., Niedermeier, D., Nillius, B., Rosch, M., Rose, D., Schmidt, C., Steinke, I., and Stratmann, F.: Intercomparing different devices for the investigation of ice nucleating particles using Snomax (R) as test substance, Atmos Chem Phys, 15, 1463-1485, 10.5194/acp-15-1463-2015, 2015.

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Peters, T. M., Ott, D. & O'Shaughnessy, P. T. Comparison of the Grimm 1.108 and 1.109 388 portable aerosol spectrometer to the TSI 3321 aerodynamic particle sizer for dry particles. 389 Annals of Occupational Hygiene, 50, 843–850, doi:10.1093/annhyg/mel067 (2006).

- Results & Discussion: It would be useful for the reader for sub-headers to be added, for example, 1) Bacteria mass spectral signatures, 2) Expansion 1, 3) Expansion 2. . .

We have added sub-headers to the text.

- Page 5, Line 18: Space needed after (dve).

This has been fixed.

- Page 6, Lines 11-13: This sentence is not clear as written. Laser power should be provided in the experimental section.

This has been clarified as “The negative ion MS presented here and in previous studies are virtually the same, while the positive ion MS presented in this study exhibit significantly higher intensity in organic peaks, most likely due to the differences in the ablation laser power or wavelength (266 nm in Pratt et al. (2009) and 193 nm in this study and Zawadowicz et al. (2017)). Moreover, these studies do not mention the presence of two particle size modes nor distinguish between their mass spectra.” Also, laser power is now listed in the experimental section.

- Page 7, Lines 26-27: Move this sentence to later in the results & discussion where Expansion 2 is discussed.

We feel that this sentence is important. We have it here to inform the reader that we will explain this result in more detail later on in the text.

- Page 7, Last paragraph: This paragraph is all repetition (summary) and could be removed as it doesn't seem necessary.

Thank you for the suggestion. We did remove many repetitive sentences in the revised

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manuscript.

- Page 9, Lines 7-10: Please clarify this sentence.

This has been rewritten to read, “To examine this further, we compare in Figure S2a the MS of cloud residuals smaller than 300 nm to the reference MS of the small particle mode measured before the expansion and find them to be nearly identical. This indicates that they are bacterial fragments mixed with agar. The MS of cloud residuals that are larger than 300 nm have greater ion intensities for the organic peaks (28CO/HNCH⁺, 44CO₂/CH₃COH⁺, 70HNCH(CH₂)₃⁺, 85C₅NH₁₀⁺) than the bacterial fragments mixed with agar, but slightly smaller ion intensities than those of the reference MS of intact bacteria, as shown in Figure S2b. These differences in ion intensities are not sufficient to eliminate the possibility that some of the larger cloud residuals could be smaller intact bacteria.”

- Page 9, Line 19: Should Figure 13 be referred to here instead of Figure 6a (typo)?

No, the correct figure is referenced.

- Page 9, Line 24: Add mention of the number concentration prior to expansion for context.

The text has been revised to read, “Figure 13c displays the welas2-measured total number of activated particles as a function of time (black), which reaches a maximum of ~700 particles cm⁻³. Before the expansion, 1456 cm⁻³ particles were present in the chamber indicating that 47% of the particles activated as CCN.”

- Page 10, Lines 3-4: Move “(light blue)” to after “expansion” in this sentence.

It has been moved.

- Page 10, Lines 10-12: Move sentence to the end of the previous paragraph.

These sentences have been combined with the next paragraph, not with the previous, because the previous paragraph is about size distributions and these sentences are

about the mass spectra.

- Page 11, Line 23: Please provide a reference for the hygroscopicity of intact bacteria.

A reference has been added.

- Figure 9 caption: Mention the type of bacteria used. Make sure this is included in each figure caption.

This information has been added to all figure captions.

- Figure 12: Please clarify the legend of the bottom plot.

The blue trace has been added to the legend.

- Figure 13: Please provide the sample timing for the 'cloud residuals' vs 'cloud residuals late', so that the reader can refer back to Figure 12 for context.

This has been added to the text and the figure caption.

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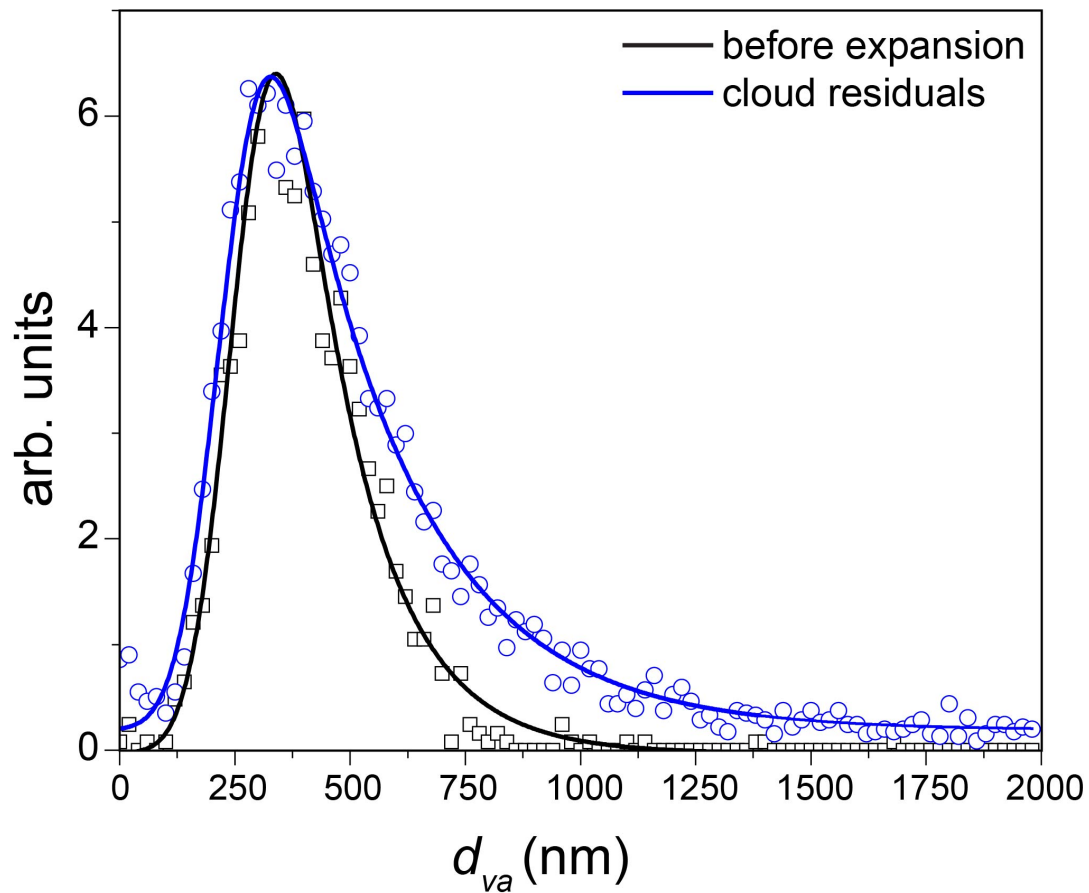


Fig. 1. Response Figure 1

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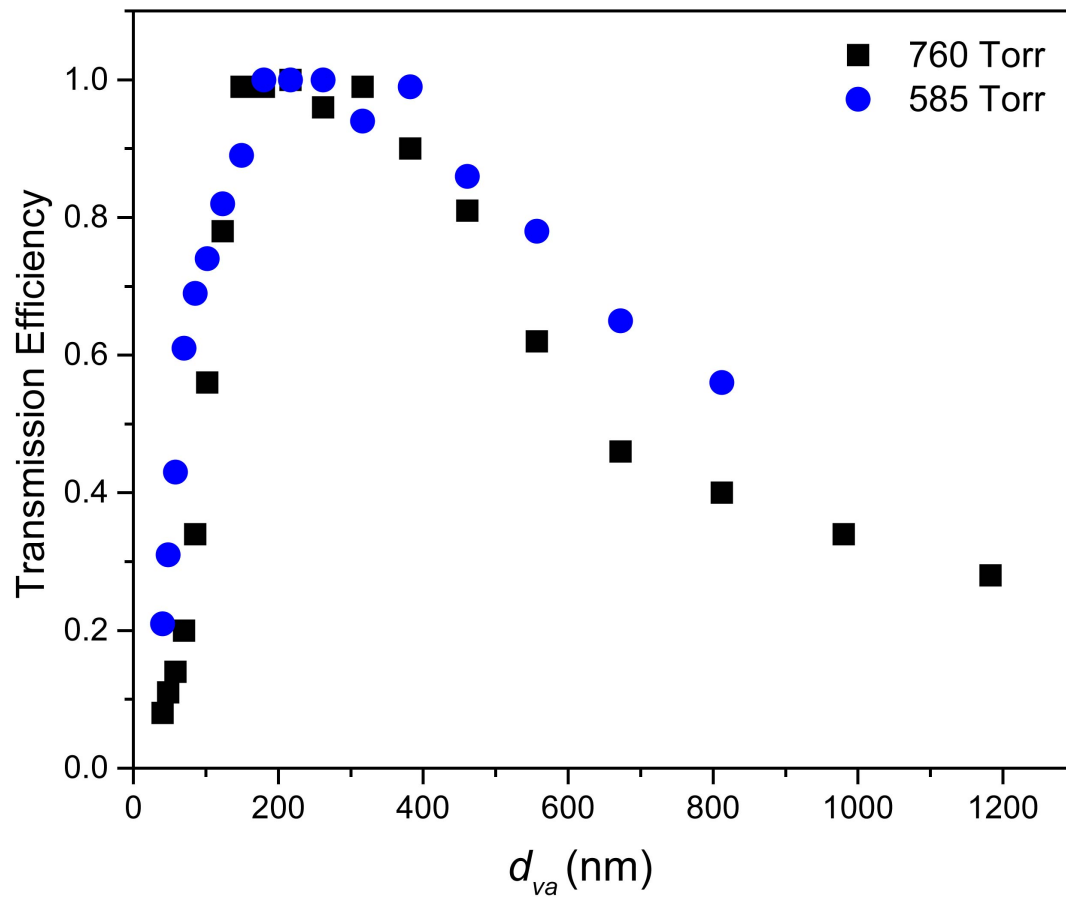
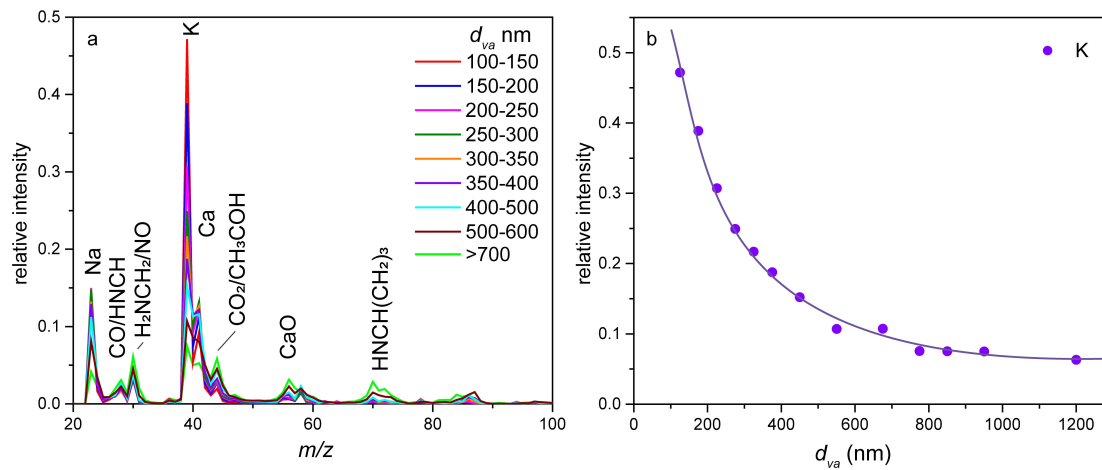


Fig. 2. Response Figure 2

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**Fig. 3.** Response Figure 3[Printer-friendly version](#)[Discussion paper](#)