The authors would like to thank the reviewers for the additional feedback of the manuscript and believe the revised manuscript is much improved and addresses the reviewers' concerns. We have conducted additionally experiments as requested by Reviewer 2. We have made revisions to the manuscript according to the reviewers' comments and the extra experimental findings. The colorings of text in the reviewer response are:

- Light blue: Original reviewer comments
- Dark blue: Text added in the revision while ~~strikethrough words~~ are the text deleted in the revised manuscript.
- Black: Original text in the submitted version of the manuscript and authors' response to the comments and others.

Note that the line number in the response is based on the revised clean-version manuscript.

Since the response includes figures from the original manuscript, support information, first response letter, and the second response letter, we use the following notations to number the figures:
Figure 1, 2,…: Figures used in the original manuscript or added to the revised manuscript.
Figure S1, S2…: Figures used in the original SI (supporting information) or added to the revised SI.
Figure R1, R2…:Figures originally used in the first response letter and reproduced here.
Figure SR1, SR2…: Figures used in the second response letter (this response).

Reviewer 1

Comments: This is a second review of the revised manuscript. The authors have addressed most of my main concerns from the previous version. My remaining concerns are as follows:
Comment 1: The use of the FID measurement to calibrate the instrument should be shown, at least as proof-of-concept. It does not need to be explored fully, but a figure or calculation for at least one peak needs to be included. You have almost done this in lines 230-233; can you simply add some quantitative information for Analyte 1 and Analyte 2?

Response: We thank the reviewer for providing additional feedback for the manuscript. Although the detailed quantification analysis is out of the scope of this manuscript. We agree with the reviewer that a proof-of-concept of quantification for the two analytes should be shown. We have revised the manuscript on Line 226:

"The two peaks highlighted provide an example in the variability of CIMS response: Analyte 1 has a larger FID peak area, indicating a higher mass concentration in the sample mixture than Analyte 2. However, since the CIMS peak area of Analyte 1 is lower, it must be less sensitive than Analyte 2 in an iodide CIMS. With the use of FID in addition to the CIMS detector, calibration of compounds in CIMS without using authentic standards can therefore theoretically be achieved. While implementation of this calibration approach is complex, here, we provide a proof-of-concept quantitative analysis. Hurley et al. demonstrated that the number of moles of an analyte can be calculated from its FID peak area based on a calibration response factor to hydrocarbons, with a correction for oxygenation based on the chemical formula identified by CIMS (specifically, the FID response per carbon atom relative to maximum = -0.54 O/C + 0.99, where O/C is the oxygen to carbon ratio in the target analyte, Hurley et al., 2020). By applying the calibration approach for the two analytes in the example, Analyte 1 (i.e., $C_{10}H_{14}O_3$) is found to be roughly four times more abundant than Analyte 2 (i.e., $C_9H_{14}O_3$) on a per mole basis, but appear substantially lower in its CIMS signal due to a ten times lower sensitivity. ~~Implementation of this calibration approach including detailed methods of quantification and determination of isomer sensitivity is complex and will be addressed in future work.~~ The detailed methods of quantification and

determination of isomer sensitivity will be discussed in future work. This manuscript focuses instead on the descriptions of technical hurdles overcome by TAG-CIMS/FID and its potential value in understanding existing and new ionization chemistries, as well as atmospheric systems."

Comment 2: The multi-reagent-ion chemistry is an important part of this manuscript. Can you add a mass defect plot(s), such as Figure R2, comparing the ions detected with I- to ions detected with the mixed reagent ion chemistry? This could be added to the supplement. It would be helpful to show the abundance of non-adduct ions using the standard iodide ionization scheme, and their enhancement with the mixed ion mode. I think this would also help to address some of the questions from the other reviewer.

Response: We sincerely appreciate the reviewer for suggesting a better way to examine our data and demonstrate the merit of the multi-reagent ionization method. We agree with Reviewer 1 that the manuscript should emphasize the broad signal enhancement in all non-iodide-adduct ions through the use of multi-reagent ionization mode. The comparison of mass defect plots between multi-reagent and iodide ionization mode is a great way to demonstrate such signal enhancement. Therefore, we have removed the original Figure 5 (examination of vanillin) and add instead a comparison of mass defect plots (new Figure 5) to more broadly demonstrate the enhancement in non-iodide-adduct signals after switching from iodide to multi-reagent ionization mode. We have also revised the manuscript on Line 342-376 as excerpted below. The full context of this excerpt is Section 3.3 and excerpted fully in our response to Reviewer 2 on Page 13-16 of the response.

"To examine increases in abundance of non-adduct ions in multi-reagent ionization, all identified ions are plotted as a function of their exact mass and mass defect for iodide ionization (Figure 5a) and multi-reagent ionization (Figure 5b) with the marker area representing the background-subtracted ion abundance. Analysis is limited to only ions that exhibit a chromatographic peak about the level of detection (taken as ten times signal-to-noise in the chromatographic baseline) and with ion abundance higher than 1% of the maximum signal across both systems. The results show that despite slight decreases in their abundance, nearly all of the iodide-adduct ions (green markers within the dashed circle in Figure 5a) are still present after switching to multi-reagent ionization mode. However, signals of non-iodide-adduct ions observed in iodide ionization are enhanced significantly, even for lower-polarity compounds that exhibited non-iodide-adduct ionization pathways in iodide ionization mode. Multi-reagent ionization also generates many new non-adduct ions. While shown summarily as mass defect plots, it is important to remember that all ions are not observed simultaneously, but rather elute as chromatographic peaks comprised of some subset of ions. Figure 5 consequently demonstrates that by using multi-reagent ionization, identification of compounds with iodide adduct signals can be maintained, while additional analytes are accessed through these new ionization pathways, as demonstrated by the increase in peaks observed in Figure 4c. Enhancement of these side reactions expands formula identifications to compounds that do not strongly form iodide adducts in this instrument, due either to inherent chemical limitations (e.g., low polarity) or instrument operating conditions (e.g., adduct declustering). For example, six peaks in labeled in Figure 4c are not detected as iodide adducts, but for which formulas can be assigned using [M-H]- and [M+O_2]- as identifiers, 1: $C_{15}H_{24}O$, 2: $C_9H_{10}O_3$, 3: $C_{12}H_{24}O_2$, 4: $C_{16}H_{32}O_2$, 5: $C_{18}H_{34}O_2$, and 6: $C_{18}H_{36}O_2$. ~~In multi-reagent ionization, the three most abundant ions in the vanillin mass spectrum are deprotonation (i.e., $[M\text{-}H]^-$), the cluster with O_2^- (i.e., $[M+O_2]^-$), and the deprotonated dimer (i.e., $[M_2\text{-}H]^-$). Because of the presence of oxygen in the reagent ion flow, the abundance of $[M\text{-}H]^-$ and $[M+O_2]^-$ is enhanced significantly. Though the $[M+I]^-$ is no longer observed in the spectrum, this is only due to the significant increase in other signals; the actual impact on the iodide adduct formation pathway is minor. To demonstrate, we plot the comparisons of the $[M\text{-}H]^-$ and $[M+I]^-$ of vanillin between the two ionization modes in Figure 5. The peak height of the $[M\text{-}H]^-$ ion of vanillin increases by a factor of 10, from 9.0×10^4 to 90×10^4 ions/s while the $[M+I]^-$ of vanillin reduces by a factor of only 2, from 0.58×10^4 to 0.32×10^4~~

~~ions/s after switching from iodide ionization mode to multi-reagent ionization mode, consistent with the factor of 2 decrease in the reagent I^- ion.~~ The results suggest that the instrument selectivity to other classes of compounds can be enlarged by bringing in O_2^- as an additional reagent ion, without significantly suppressing the iodide ionization pathway. In other words, the sensitivity of compounds that tend to be ionized by O_2^- or other side reactions are significantly enhanced in multi-ionization CIMS with only minor decreases in the sensitivity of compounds typically observed by an iodide-CIMS. As long as individual analytes enter the CIMS at separate times, as in the case of chromatography, combining multiple ionization chemistries can provide additional information or selectivity."

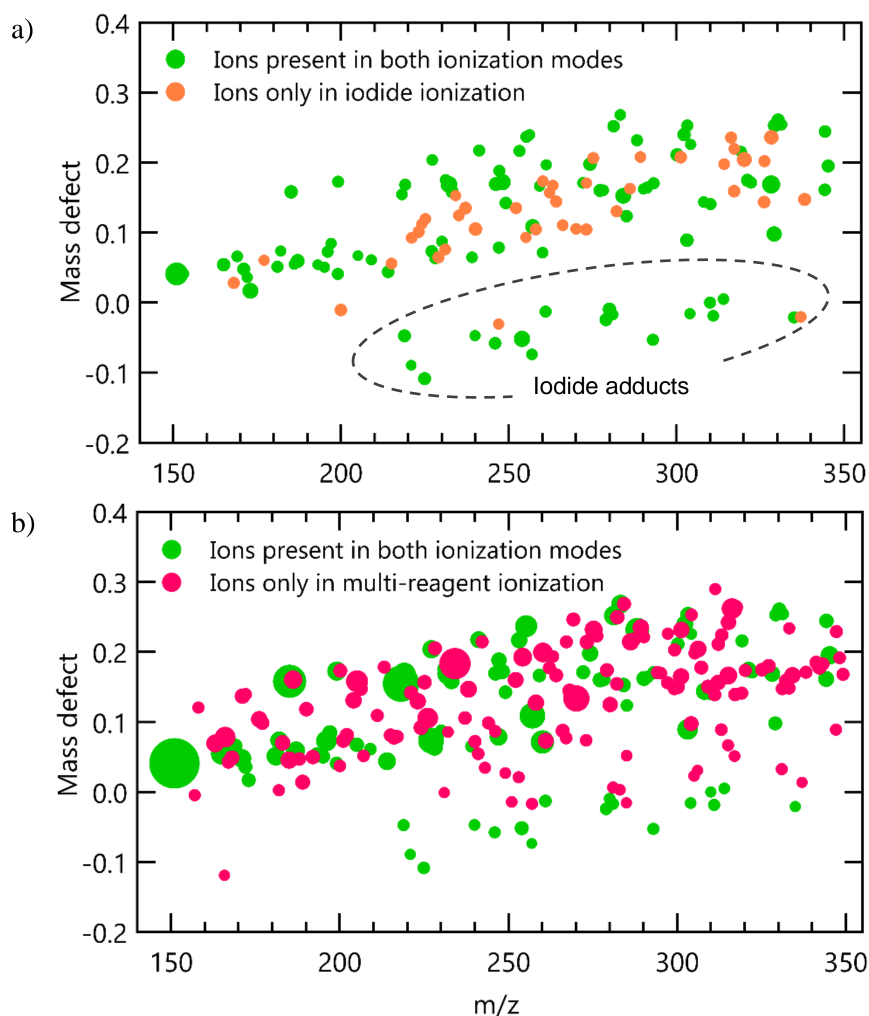

Figure 5. High-resolution mass defect spectrum obtained for liquid mixture samples in a) iodide ionization mode and b) multi-reagent ionization mode. The area of markers is proportional to the ion abundance.

Comment 3: It is not entirely clear which figures were replaced or changed in the revised manuscript. For example, will Figure R1 replace the Figure 2 shown in the corrected manuscript? Will Figure R3 be included somewhere in the main text or in the supplement? Please indicate.

Response: We apologize for not clarifying whether those additional figures are included in the revised manuscript. Since the response includes figures from multiple sources, we use the following notations to number figures:
Figure 1, 2,…: Figures used in the original manuscript or added to the revised manuscript.
Figure S1, S2…: Figures used in the original SI (supporting information) or added to the revised SI.
Figure R1, R2…:Figures originally used in the first response letter and reproduced here.
Figure SR1, SR2…: Figures used in the second response letter (this response).

The reviewer asked "will Figure R3 be included somewhere in the main text or in the supplement?". We will include Figure S3 (previously named as Figure R3 in the first response letter) in the SI. The reviewer asked "will Figure R1 replace the Figure 2 shown in the corrected manuscript?". We do not intend to include Figure R1 in the final manuscript. The comparison of chromatograms between CIMS and FID is already shown and discussed in Figure 2 of the manuscript, and the focus of Figure 4 is the comparison of ionization modes. Our intention in Figure R1 was to answer Reviewer 2's concern, but we do not feel that including the FID chromatogram of Figure 4 in the main text provides additional information or insight than what is already evident from Figure 2, so we have chosen not to add this chromatogram to Figure 4 in the revised manuscript.

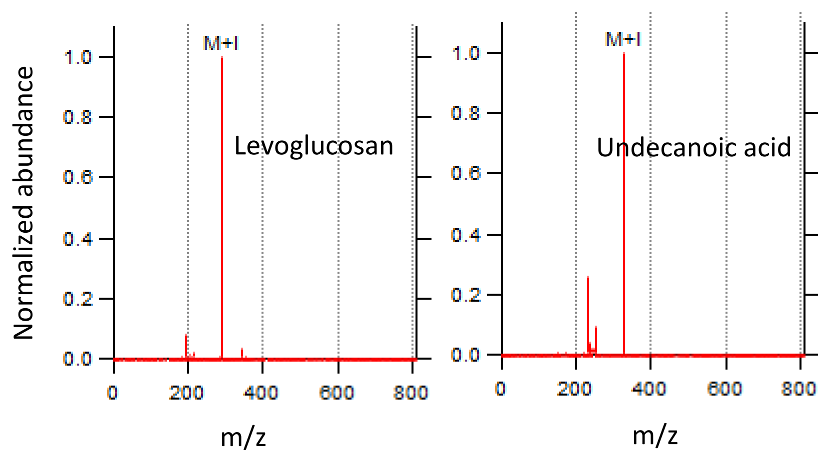

Figure S3. Background-subtracted mass spectra for levoglucosan and undecanoic acid in liquid standard mixture.

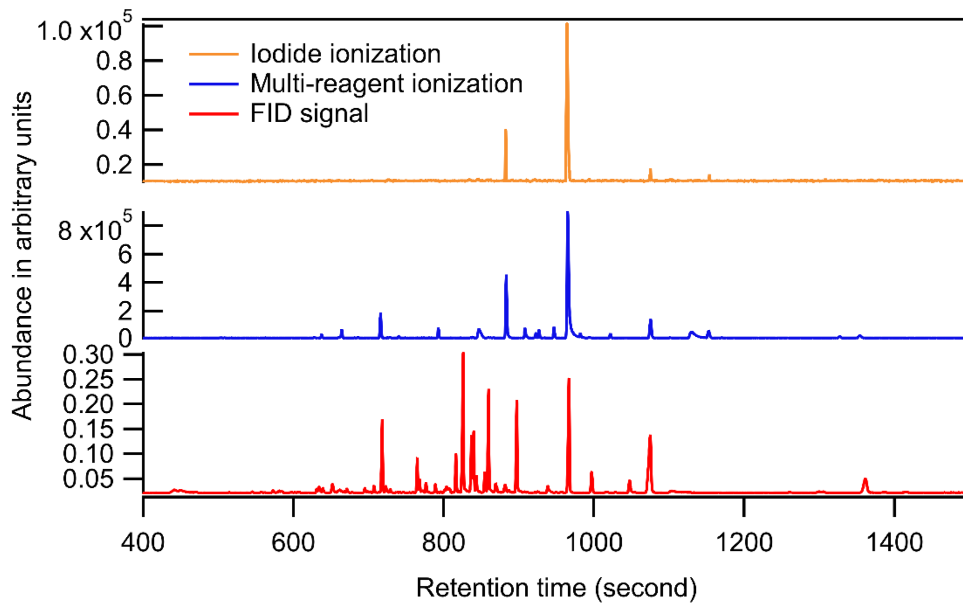

Figure R1: Comparison of chromatograms of analyte total ion counts between CIMS using iodide ionization, CIMS using multi-reagent ionization, and FID. Top two panels are recreated from Figure 4 of the manuscript.

Reviewer 2

I thank the authors for their responses to my comments as "reviewer 2". However, I still find that the there is a clear issue with the Fig. 4a inset, which prevents me from having full confidence that this new instrument is functioning in the way the authors are describing it should. I understand why the authors prefer to keep their main data analysis for a subsequent manuscript, but I think that this manuscript should still be able to show consistency with typical iodide CIMS instruments, and thus give confidence that the subsequent data analysis will be as interpretable and useful as suggested.

Response: The authors would like to thank the reviewer for the additional feedback on the manuscript. As requested, we have conducted additional experiments to examine whether it is the CIMS-related issue or the TAG coupling to make the mass spectrum of vanillin in this study different from what observed in the reviewer's experiments. The new experimental results suggest that the significantly high $[M\text{-}H]^-$ in the mass spectrum of vanillin is related in part to the tuning voltages and could also be influenced by the presence of a small air leak (though the latter is not known for certain). In this case, we have removed the Figure 4 inserts and acknowledged that the CIMS tuning and/or air leaks may result in imperfect mass spectra for less polar compounds like vanillin in the manuscript. Instead of focusing on a single compound, vanillin, we have revised the manuscript to more broadly demonstrate that multi-reagent ionization can boost signals to existing non-iodide-adduct ions and maintain the presence of iodide adducts using the mass defect plots, which was the original intention of showing the vanillin spectrum. We have summarized our experimental findings together with our responses and revisions in the following section.

Comments:

<u>Comment 1:</u> The authors' reply to my comments about vanillin ionization are not entirely satisfying. There are numerous parts of the reply that are inaccurate or irrelevant. For instance, the authors wrote: "A critical difference between this instrument and direct-air sampling instrumentation is the ability to collect "clean" mass spectra of individual analytes,…"

It is indeed straightforward to get 'clean' spectra with a direct-air sampling iodide CIMS, by just wafting a bottle of vanillin or any other single analyte in front of a sampling inlet (impurities in the commercially available vanillin are irrelevant to this discussion). This new instrument is novel because it can produce 'clean' spectra from complex mixtures which is a nice advance (though it should still produce the same spectra as when sampling a single analyte), but that's not what my comment was about. Then the authors say:

"…we believe that a lot of the apparent discrepancy comes from the fact that this instrument specifically provides an ability to see and explore the non-adduct ions, while a typical CIMS does not straightforwardly relate adduct ions to potential non-adduct counterparts."

When sampling just vanillin from a bottle directly into the iodide CIMS like I described, you do get all of the adduct and non-adduct ions, where you can directly explore which non-adduct ions form from ionization of vanillin. Therefore the authors' statement is incorrect (while the new instrument could be useful for identifying the parents of non-adduct ions in a complex mixture, again a nice advance, that's not the issue I am addressing).

Response: We would like to apologize for not clearly describing our arguments on the TAG-CIMS's ability to obtain "clean" mass spectra. We intended to mean that a TAG-CIMS can still provide "clean" mass spectra of individual analytes from a complex sample mixture. We certainly agree with the reviewer that a direct-air-sampling iodide CIMS can see "clean" mass spectra as well if only a single compound is sampled by the CIMS. This can be done by wafting a bottle of liquid standards as suggested by the

reviewer or using permeation tubes with liquid standards filled in. To further avoid such misunderstanding, we have revised the manuscript on Line 305:

"This provides a clean mass spectrum for each chromatographically well-resolved analyte and is particularly useful when analytes are in a complex mixture (Figure S3). Consequently, this technique shows a significant advantage for understanding ionization chemistry."

Comment 2: The main issue that I still have is that when you directly sample vanillin like this with typical iodide ionization, the [M+I]- signal is 100x higher than the [M-H]-- signal (I've measured this myself before), contrary to what is shown in Fig. 4a. The authors describe various things that can affect sensitivity and declustering in a TOF mass spectrometer, e.g. voltages and IMR pressure, but mostly that will only affect whether an adduct stays an adduct or declusters back to a neutral analyte and iodide anion (or further fragmentation e.g. loss of –CO2 or –H2O or –NO2 for some compounds), it won't change the ionization pathway to enhance [M-H]--.
In other words, any properly operating iodide CIMS should always sample vanillin with [M+H]- at much higher signal than [M-H]-, and never with those reversed. Because of this, I still have to conclude that something is amiss with the ionization in your vanillin example in Fig 4. The levoglucosan and undecanoic acid examples given in Fig. R3 do look fine and are as expected, but why is vanillin different? If the answer is some sort of artifact with your instrument design, then I would say there is a potentially major issue with the utility of this instrument, because your iodide CIMS data will not be comparable to other instruments for at least a subset of compounds.
If it really is that you're operating your CIMS in some atypical configuration that gives this spectrum, then I think you need to figure out why and reconfigure to something more standard, otherwise you're negating the benefits of using an iodide CIMS by unnecessarily complicating the ionization chemistry and making it incomparable to other iodide CIMS data. I understand you're arguing that your goal in this manuscript is not to fully understand all of the ionization chemistry (that's the next paper) or to have all the answers, but I would much prefer you don't publish your paper with a Fig 4a inset that is labeled as iodide ionization but is definitely strongly influenced by something else. The purpose of this paper is to show that your new instrument works and briefly show its benefits, but Fig 4a inset tells me something is not working as intended.

That said, it could still be a simple answer. I suggested that the ionization could have changed due to the IMR temperature, but the authors have pointed out that the IMR itself is not heated. It sounds like there could possibly be a surface where the transmission line mates with the IMR that could be at least somewhat heated, but barring this, there are other options. The CIMS could be operating in some strange configuration of voltages or pressures, etc, but I find this unlikely. Is it possible that you just have a lot more O2- impurity in your iodide-only mode than is typical? Is O2 diffusing in or leaking in from your zero air source or even from room air through a leaky fitting? If 225C temps are ruled out, then an O2 leak seems most likely. I'm not sure why this wouldn't show up in your Fig R3 of levoglucosan and undecanoic acid; either O2- is very insensitive to those compounds while being very sensitive to vanillin, or the potential O2 leak was occurring only in your vanillin experiment but not your Fig R3 experiments. The last thing I can think of is that in your Fig4a, that main elution peak for which you're showing the mass spectrum is not actually only vanillin, but may be dominated by some other compound that predominantly forms that non-adduct, which seems unlikely.

In summary, the Fig. 4a inset is definitely not normal iodide ionization of vanillin, and that doesn't convince me as a reader that you have shown this instrument to be sufficiently described for this

publication and subsequent use. I don't know the answer, but I suggest the authors start by sampling vanillin directly with the iodide CIMS to verify they measure mostly [M+I]-.

Response: we again thank the reviewer for their suggestions to improve our publication. To the best of our understandings, the reviewer's main concern can be summarized into two questions: 1) did the iodide CIMS configuration itself or the coupling of a TAG cause the high [M-H]-/[M+I]- ratio for vanillin? 2) if the iodide ionization chemistry is atypical, how can the iodide CIMS data obtained in this study be comparable to other CIMS instruments?

1) Did the iodide CIMS configuration itself or the coupling of a TAG cause the high [M-H]-/[M+I]- ratio for vanillin?

For the first question, the reviewer has pointed out the mass spectrum of vanillin shown in Figure 4 inserts does not agree with previous data collected by themselves and provided methods in the comments to explore the reasons for such discrepancy. The reviewer said "the CIMS could be operating in some strange configuration of voltages or pressures, etc, but I find this unlikely. Is it possible that you just have a lot more O2- impurity in your iodide-only mode than is typical? Is O2 diffusing in or leaking in from your zero air source or even from room air through a leaky fitting?" As suggested, we have completed additional laboratory experiments to specifically examine whether it is the effect of CIMS-related parameters or the coupling of a CIMS to a TAG causes the different ionization regime of vanillin**.** Below, we present data showing two CIMS operating conditions that can strongly affect the $[M-H]^-/[M+I]^-$ ratio of vanillin: 1) tuning, particularly the Short Segmented Quadruple (SSQ) voltages; and 2) as suggested by the reviewer, a small leak of oxygen into the Polonium ionizer.

We find that either, or both, of these instrumental changes can account for the high $[M-H]^-/[M+I]^-$ ratio of vanillin, although not observed for some other analytes. Therefore, we agree with the reviewer that high $[M-H]^-/[M+I]^-$ ratio of vanillin could be due to the CIMS-related settings and issues, but these issues are not related to the coupling to the GC, which is a major focus of this manuscript. We believe that the coupling of a GC did not change the ionization chemistry of vanillin yet the settings of CIMS, the leak of O_2 in CIMS ionizer, or both did.

To examine the effects of CIMS operation on vanillin ionization, we made a non-quantitative solution of HPLC water and vanillin (Sigma-Aldrich; > 99% and used without further purification). This solution was then sublimated into a clean gas flow and injected into the inlet of the CIMS using a liquid calibration system. This provided a constant gas-phase source of vanillin on which instrument parameters could be tested. The CIMS was set up using the standard flow-tube IMR and gas-phase orifice inlet (Bertram et al., 2011). No GC coupling was used. This was a "standard" Iodide CI-TOFMS as described and used frequently in the literature.

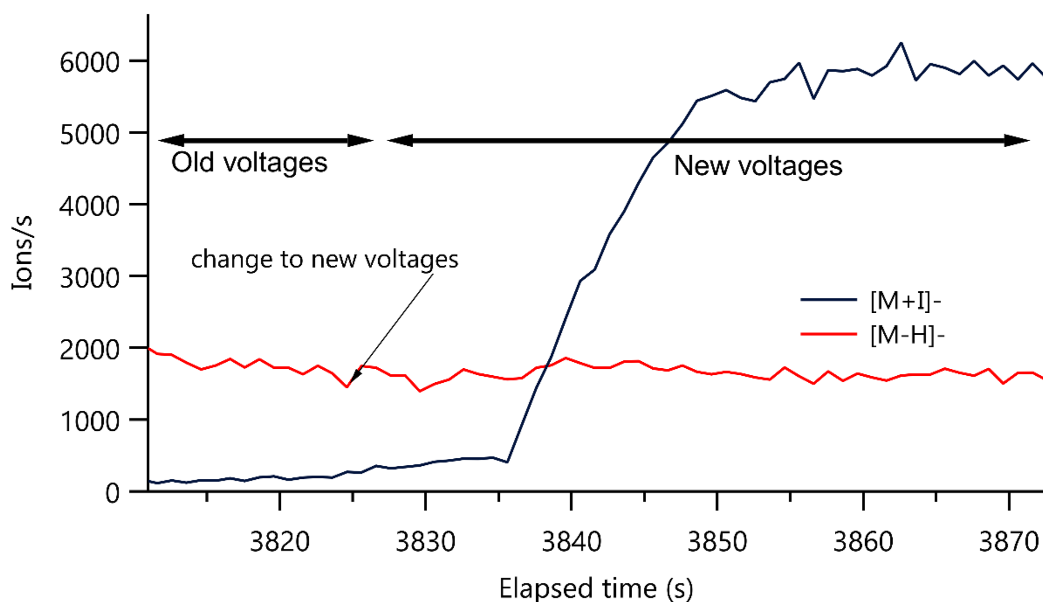

Figure SR1. Abundance of [M+I]- and [M-H]- of vanillin under different CIMS voltage settings.

Figure SR1 shows the raw signals for the [M+I]- and [M-H]- ions of vanillin under the "old" tuning voltages (those used for the manuscript experiments) and a "new" tuning used for the experiments described here. By switching between the old and new voltage settings, we induce an enormous change in the amount of signal observed at m/z 279 $[M+I]^-$, while the amount of $[M-H]^-$ stays the same.

A major region for the difference in the spectra is due to electronically induced declustering of the $[M+I]^-$ adduct ion (which does not affect the non-adduct ions in the same way), which has been previously demonstrated to commonly occur for less-strongly-bound adducts at larger voltage differentials (Lopez-Hilfiker et al., 2016a). To examine this specific tuning effect, we changed the differential voltage between the SSQ and the skimmer (Figure SR2; scatter around central tendencies is simply due to transients between tunings that have not been entirely discarded). The results show that the SSQ voltages, which are determined in the tuning of a CIMS, can strongly impact the $[M-H]^-/[M+I]^-$ ratio of vanillin (largely due to decreases in the $[M+I]^-$ ion, as shown in Figure SR1).

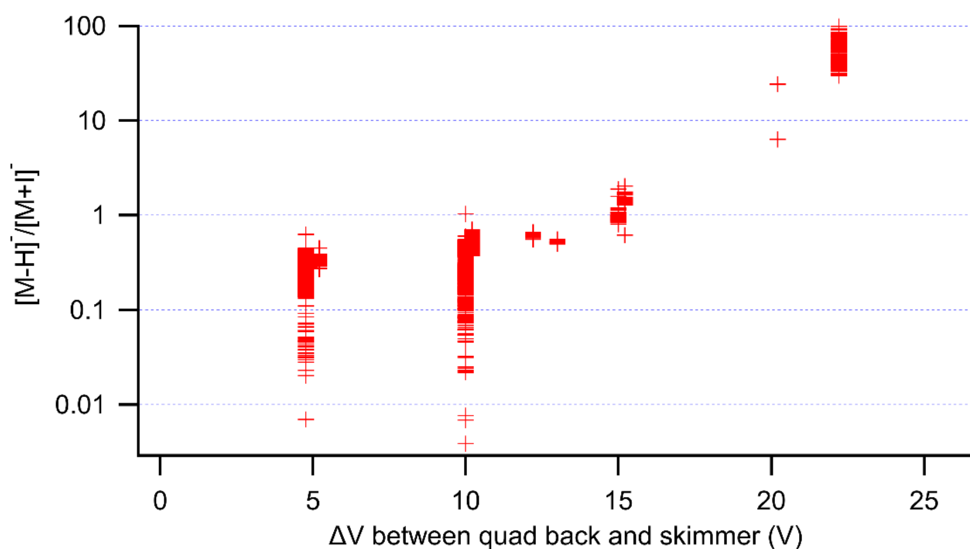

Figure SR2. The effect of differential voltage between the SSQ and the skimmer on the $[M-H]^-/[M+I]^-$ ratio of vanillin.

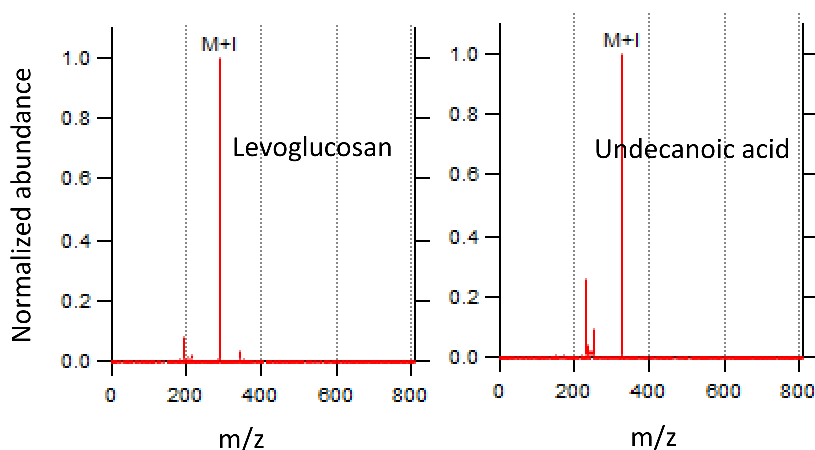

Figure S3. Background-subtracted mass spectra for levoglucosan and undecanoic acid in liquid standard mixture.

Although we use vanillin as an example to demonstrate the possibility of multi-reagent ionization using a TAG-CIMS/FID, the instrument was not intended to specifically measure vanillin. As shown in Figure S3, there are other compounds that have reasonable mass spectra in iodide ionization mode. The use of an instrument tuning that was not optimized to preserve adduct ions of weakly-bound adducts is unfortunate, and not something we would have used if we were trying to extract useful information about the atmosphere or other system, but does not impact our investigation of the GC coupling and our demonstration that GC helps clarify a complex mixture when analyzing with a CIMS. The experimental results suggest the discrepancy of vanillin ionization chemistry between our data and the reviewer's data is not due to the coupling of a GC, but the CIMS-specific parameters, which may vary depending on the application of the users.

Finally, the reviewer suggested the possibility of a leak into the ionizer. We acknowledge this could have happened during our experiments and we tested the effects of a theoretical leak by loosening the connection to the Polonium ionizer and letting a few seconds of room air into the reagent ion system. As shown in Figure SR3, we observe an enormous enhancement in the $[M-H]^-$ signal at m/z 151, relative to the system without the O_2 leak. As the reviewer offers, it is possible that vanillin is unusually susceptible to ionization via O_2- or CO_3-. Though we note that we do not observe a large peak for the carbonate ion in the mass spectra during these experiments, which would have been an indicator of a large leak, so it is not possible to say with certainty whether such a leak existed in the manuscript experiments.

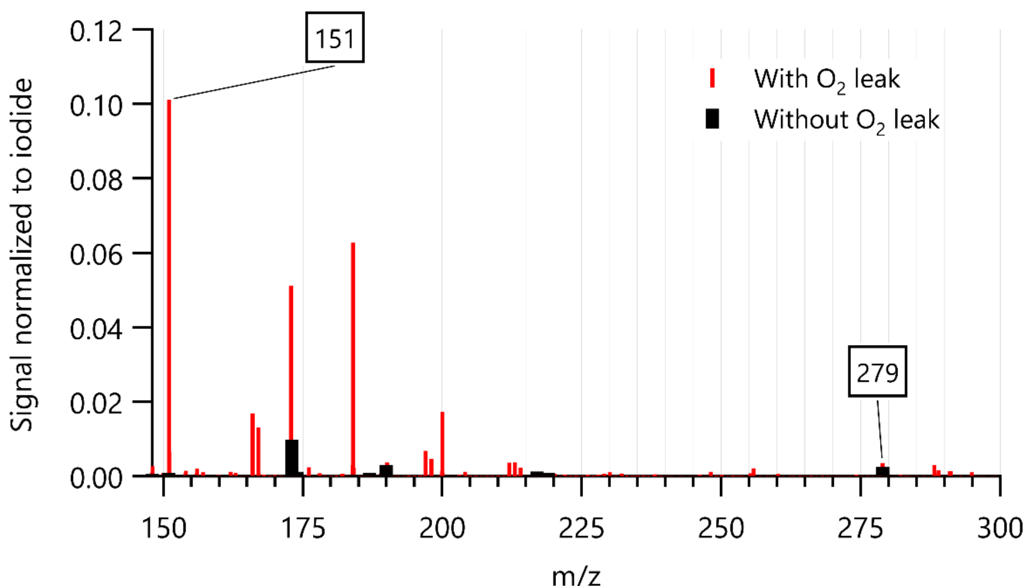

Figure SR3. Normalized mass spectrum of vanillin with and without O_2 leak at the ionizier.

From these experiments, we conclude that the vanillin spectrum of concern to the reviewer is (a) definitely due in part to the tuning of the CIMS, and (b) could be due in part to a small leak at the ionizer (though the support for the latter is a bit mixed and is not known for certain). We hope that this satisfies the reviewer's concerns that the vanillin spectrum shown is in some way a result for the GC coupling and experiments that are the focus of this work. Further work needs to be done to probe the ionization chemistry for compounds with different functional groups, but we hypothesize that less polar compounds are more susceptible to the change of SSQ voltages and O_2 leak due to their weaker iodide-adduct binding enthalpies. Because tuning and physical setup does not change between iodide ionization and multi-reagent ionization, the increase in non-adduct ions observed in the latter is also not due in any way to these issues. We acknowledge the reviewer's concern regarding the vanillin spectrum and have removed Figure 4 inserts from the manuscript. We have also discussed on Line 318 and Line 400 that low signal of the iodide adduct for low-polarity compounds may be due to tuning-related declustering and/or other operating conditions.

On Line 318: "The CIMS voltage settings used in this study were not optimized to minimize declustering of lower-polarity compounds like vanillin, leading to spectra of these compounds in which the iodide adduct significantly is less dominant than the deprotonated form in, even in iodide ionization mode."

On Line 400: "While the iodide-adduct ions do exist in the mass spectrum of individual analytes, we also observe high abundance of non-adduct ions such as $[M-H]^-$ and $[M+O_2]^-$. Although such high abundance of $[M-H]^-$ may be partially resulted by the tuning-driven declustering of low-polarity adduct ions, the observed non-adduct ions likely account for many ions in the non-adduct region of the iodide valley. ~~which likely account for many ions in the non-adduct region of the iodide valley.~~"

2) If the iodide ionization chemistry is atypical, how can the iodide CIMS data obtained in this study be comparable to other CIMS instruments?

Firstly, we would like to note that CIMS-related parameters such as voltage tuning and ion-molecule reaction region (IMR) pressure can vary depending on the application of each study and there is no consensus on a constant and standard sets of voltages used in an iodide CIMS. Ideally, the signal of [M+I]-, which is typically used for quantification in an iodide CIMS, should be tuned to be as high as possible to ensure maximum sensitivity yet maintain good mass resolution. It is clearly not the case for vanillin in this study. However, although we used vanillin in the original manuscript as an example to demonstrate the possibility of multi-reagent ionization using a TAG-CIMS/FID, the purpose of the study is not to solely investigate vanillin ionization chemistry or quantify vanillin. Other compounds should be considered as well. In the iodide ionization mode, we do observe liquid standards (shown in Figure S3) that have [M+I]- as the dominant ion in their mass spectra. A further screening for all 512 compounds identified in the limonene-O_3, limonene-OH, TMB-OH, and eucalyptol-OH experiments shows that the clean mass spectra of those oxidation products have [M+I]- as the dominant ion. Therefore, we agree with the reviewer that the iodide CIMS used in this study might be less sensitive to vanillin and maybe other structurally similar compounds compared to other iodide CIMS that are tuned differently, but do not believe that unoptimized tuning towards a certain category of compounds will lead to incorrect interpretation of all data.

The original purpose of including a "close-up" of vanillin was to explore the effects and potential value of multi-reagent ionization. To address the reviewer's concerns regarding vanillin, and more broadly make this point, we have removed the original Figure 5 (examination of vanillin) and add instead a comparison of mass defect plots (new Figure 5) to more broadly demonstrate the enhancement in non-iodide-adduct signals after switching from iodide to multi-reagent ionization mode. To help the reviewer better examine the new approach, we include below the entire revised Section 3.3 in the response with marked revisions.

“3.3 Exploring new chemistries: multi-reagent ionization

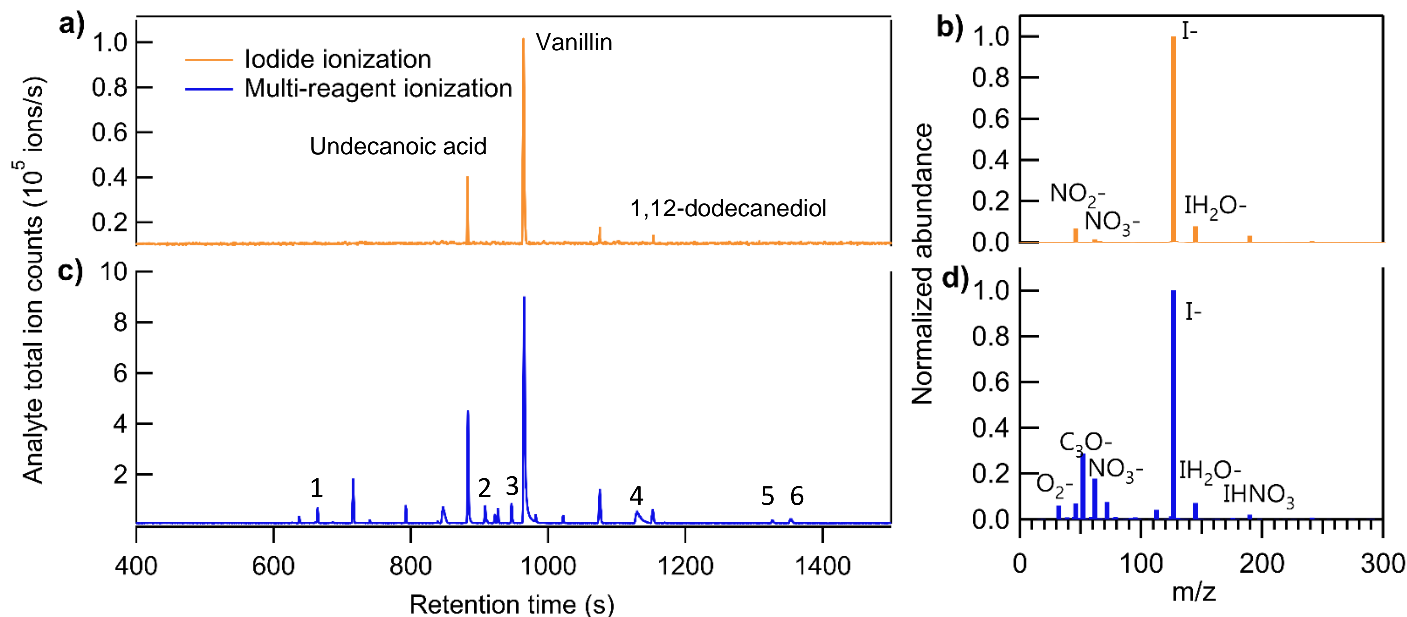

Figure 4. Comparison of chromatograms of analyte total ion counts between a) iodide ionization and c) multi-reagent ionization. Comparison of background mass spectra between b) iodide ionization and d) multi-reagent ionization. The sample introduced in this run is the mixture of liquid chemical standards and six commercially available fragrances.

Unlike direct air sampling by CIMS, in which the mass spectrum at a given time point is the summation of all analytes, the mass spectra of TAG-CIMS/FID analytes are separated in time by chromatography. Consequently, if a chromatographic peak of a compound is well-resolved in CIMS, all signals detected from the ionization of a single analyte are observed at the same chromatographic retention time and unambiguously assignable to that specific compound, including iodide adducts, products of adduct declustering, fragments (generally not from iodide clustering), and any ions produced by simultaneous alternate chemistry with other ions present in the atmospheric pressure interface (e.g., air). This provides a clean mass spectrum for each chromatographically well-resolved analyte and is particularly useful when analytes are in a complex mixture (Figure S3). Consequently, this technique shows a significant advantage for understanding ionization chemistry. Previous work has demonstrated that coupling a GC-interface to a NO^+ CIMS can determine the products ion distributions for VOCs (Koss et al., 2016). This instrument complements this previous work by examining less volatile and more oxidized compounds, as well as other CIMS chemistries. This technique is particularly interesting in the context of iodide CIMS chemistry, as it allows us to explore ions with positive mass defects (i.e., non-adduct ions), which are not particularly well understood (Lee et al., 2014). We demonstrate the capability of the technique by showing the chromatograms of a complex sample containing a mixture of liquid chemical standards and six commercially available fragrances in Figure 4. Three known liquid standards introduced in the mixture – undecanoic acid, vanillin, and 1,12-dodecanediol – are observed in iodide ionization mode using their iodide-adduct ion, i.e., $[M+I]^-$ (Figure 4a). However, while this mode is nominally dominated by ionization by the iodide ion (Figure 4b), other ions are observed in the mass spectrum of these standards, including the deprotonated form of vanillin (i.e., $[M-H]^-$) and its nitrite adduct (i.e., $[M+NO_2]^-$), indicating that the iodide ionization mode used in these experiments includes declustering of iodide

adducts for low-polarity analytes and/or side reactions. The CIMS voltage settings used in this study were not optimized to minimize declustering of lower-polarity compounds like vanillin, leading to spectra of these compounds in which the iodide adduct significantly is less dominant than the deprotonated form in, even in iodide ionization mode. ~~For example, while the analyte mass spectrum of vanillin, shown in Figure 4a, in iodide ionization mode does contain an iodide-adduct ion (i.e., $[M+I]^-$), there are other ions with higher abundance including the predominant deprotonated form of vanillin (i.e., $[M-H]^-$), followed by its nitrite-adduct (i.e., $[M+NO_2]^-$), then the deprotonated form of dimer (i.e., $[M_2-H]^-$). In other words, this compound, which is generally measurable by iodide-CIMS (Gaston et al., 2016), produces a large number of detectable ions. Although the abundance ranking of the produced ions may differ on a compound-by-compound basis, we constantly observe ions other than $[M+I]^-$ in the clean mass spectrum of injected liquid standards such as undecanoic acid, hexadecanoic acid, and 1,12-dodecanediol, as well as more polar and low volatility aerosol constituents produced in the oxidation experiments.~~ However, it is a benefit of the GC-CIMS approach that a clean mass spectrum is obtained for each analyte within this complex mixture and is therefore ~~Application of this instrument to ambient samples and/or selected test systems can therefore be~~ a pathway toward better understanding iodide adduct chemistry as and co-existing side reactions. Other than the introduced standards, which were selected in part due to their tendency to form iodide adducts, few other major analytes are observed, despite the co-introduction of multiple commercially available fragrances known to contain many organic constituents.

Using GC-CIMS not only enables the elucidation of different ionization pathways in the CI source and enables separation of interferences in the quantification, but might also be useful for exploiting these co-existing chemistries to yield additional information. While chemical ionization intrinsically offers selectivity for ease of analysis, selectivity is also negatively limiting (Munson and Field, 1966). Thus, under certain circumstances it may be useful to use multiple reagent ions to detect different classes of compounds using separate, but still soft, ionization methods. Other ions like the deprotonated form of the analyte, $[M-H]^-$ spectra might be better suited for the identification and quantification of some analytes. The deprotonated ions are believed to be produced through the reaction with O_2^- present in the IMR (Dzidic et al., 1975; Hunt et al., 1975). It is reported that the presence of O_2^-, which is commonly found in atmospheric pressure ion sources such as electrospray ionization (ESI) (Hassan et al., 2017), atmospheric pressure chemical ionization (APCI) (McEwen and Larsen, 2009), atmospheric pressure photoionization (APPI) (Song et al., 2007), and direct analysis in real-time (DART) (Cody et al., 2005), may result in the deprotonated molecules through oxidative ionization. Therefore, using multiple reagent ions including I^- and O_2^-, it is possible that low polarity compounds tend to be ionized through proton abstraction by O_2^- while compounds with high polarity can still form iodide adducts. Although we did not find carbonate (as CO_2^- and CO_3^-) in the mass spectrum in this study, we caution that those ions, like O_2^-, can also deprotonate molecules and may interfere with the quantification of the deprotonated ions.

To explore the feasibility of purposefully introducing ~~using~~ multiple simultaneous chemistries (e.g., deprotonation reactions in addition to ~~and~~ iodide adduct formation) to extend the utility of a CIMS with isomer resolution, the CIMS was operated in a multi-reagent ionization mode by adding 100 sccm flow (i.e., 5%) of ultra-zero air to the 2 slpm flow of N_2 for the gas supply of the methyl iodide permeation tube. Figure 4b and 4d show the background ions under the two modes. With no sample introduced into the system (i.e., pure helium as GC effluent), the total ion counts are 2.4×10^6 and 1.4×10^6 ions/s and the I^- ion counts are 1.8×10^6 and 0.7×10^6 ions/s for iodide and multi-reagent ionization, respectively. In other words, by mixing the reagent ion flow with 5% air, the I^- ion reduced by half, while the abundance of additional reagent ions such as O_2^- and NO_3^- increased by approximately an order of magnitude.

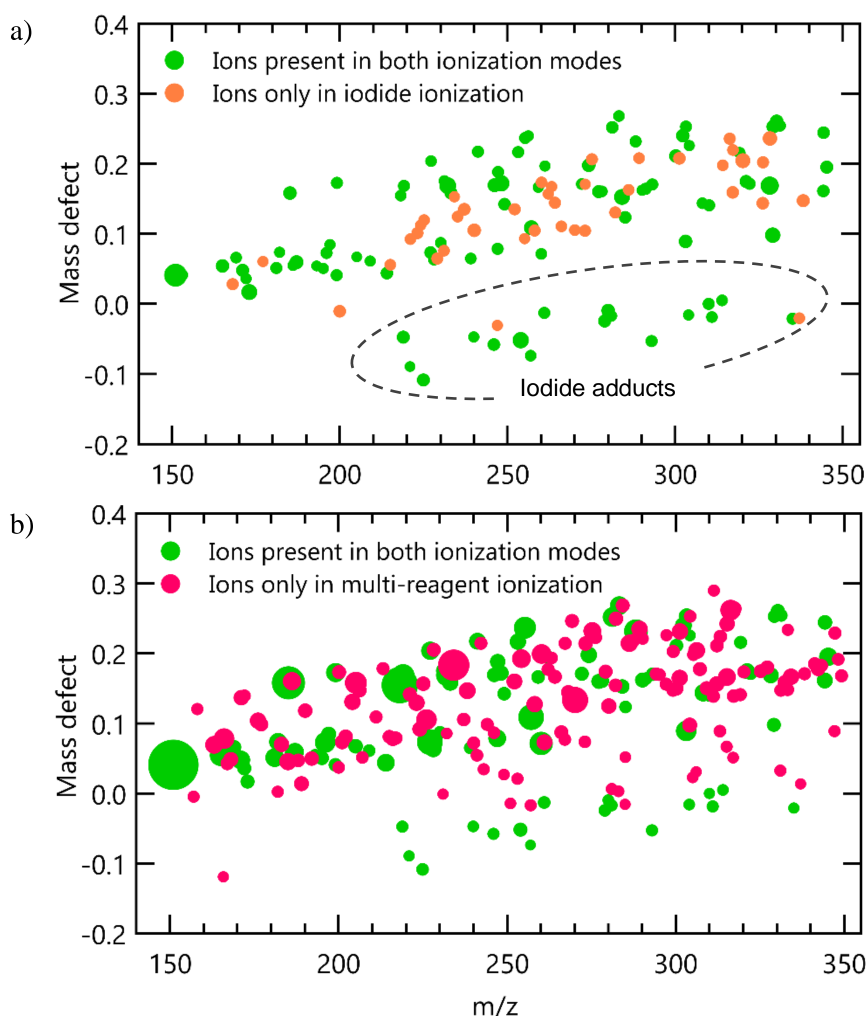

Figure 5. High-resolution mass defect spectrum obtained for liquid mixture samples in a) iodide ionization mode and b) multi-reagent ionization mode. The area of markers is proportional to the ion abundance.

As shown in Figure 4c, for compounds that can be detected by iodide ionization, the total number of ions produced by an analyte increased by a factor of five to ten after switching to multi-reagent ionization mode (note that the scale of y-axis in Figure 4c is a factor of ten higher than that in Figure 4a). ~~For example, the analyte total ion counts of vanillin (retention time = 965 secs), has a peak height of 1.0×10^5 ions/s in iodide ionization mode while the peak height of vanillin in multi-reagent ionization mode is 8.6×10^5 ions/s.~~ This increase in ions is observed to occur almost entirely through the addition of new chemical pathways. To examine increases in abundance of non-adduct ions in multi-reagent ionization, all identified ions are plotted as a function of their exact mass and mass defect for iodide ionization (Figure 5a) and multi-reagent ionization (Figure 5b) with the marker area representing the background-subtracted ion abundance. Analysis is limited to only ions that exhibit a chromatographic peak about the level of detection (taken as ten times signal-to-noise in the chromatographic baseline) and with ion abundance higher than 1% of the maximum signal across both systems. The results show that despite slight decreases in their abundance, nearly all of the iodide-adduct ions (green markers within the dashed circle in Figure 5a) are still present after switching to multi-reagent ionization mode. However, signals of non-iodide-adduct ions observed in iodide ionization are enhanced significantly, even for lower-polarity compounds

that exhibited non-iodide-adduct ionization pathways in iodide ionization mode. Multi-reagent ionization also generates many new non-adduct ions. While shown summarily as mass defect plots, it is important to remember that all ions are not observed simultaneously, but rather elute as chromatographic peaks comprised of some subset of ions. Figure 5 consequently demonstrates that by using multi-reagent ionization, identification of compounds with iodide adduct signals can be maintained, while additional analytes are accessed through these new ionization pathways, as demonstrated by the increase in peaks observed in Figure 4c. Enhancement of these side reactions expands formula identifications to compounds that do not strongly form iodide adducts in this instrument, due either to inherent chemical limitations (e.g., low polarity) or instrument operating conditions (e.g., adduct declustering). For example, six peaks in labeled in Figure 4c are not detected as iodide adducts, but for which formulas can be assigned using [M-H]- and [M+O_2]- as identifiers, 1: $C_{15}H_{24}O$, 2: $C_9H_{10}O_3$, 3: $C_{12}H_{24}O_2$, 4: $C_{16}H_{32}O_2$, 5: $C_{18}H_{34}O_2$, and 6: $C_{18}H_{36}O_2$. ~~In multi-reagent ionization, the three most abundant ions in the vanillin mass spectrum are deprotonation (i.e., $[M-H]^-$), the cluster with O_2^- (i.e., $[M+O_2]^-$), and the deprotonated dimer (i.e., $[M_2-H]^-$). Because of the presence of oxygen in the reagent ion flow, the abundance of $[M-H]^-$ and $[M+O_2]^-$ is enhanced significantly. Though the $[M+I]^-$ is no longer observed in the spectrum, this is only due to the significant increase in other signals; the actual impact on the iodide adduct formation pathway is minor. To demonstrate, we plot the comparisons of the $[M-H]^-$ and $[M+I]^-$ of vanillin between the two ionization modes in Figure 5. The peak height of the $[M-H]^-$ ion of vanillin increases by a factor of 10, from 9.0×10^4 to 90×10^4 ions/s while the $[M+I]^-$ of vanillin reduces by a factor of only 2, from 0.58×10^4 to 0.32×10^4 ions/s after switching from iodide ionization mode to multi-reagent ionization mode, consistent with the factor of 2 decrease in the reagent I^- ion.~~ The results suggest that the instrument selectivity to other classes of compounds can be enlarged by bringing in O_2^- as an additional reagent ion, without significantly suppressing the iodide ionization pathway. In other words, the sensitivity of compounds that tend to be ionized by O_2^- or other side reactions are significantly enhanced in multi-ionization CIMS with only minor decreases in the sensitivity of compounds typically observed by an iodide-CIMS. As long as individual analytes enter the CIMS at separate times, as in the case of chromatography, combining multiple ionization chemistries can provide additional information or selectivity.

~~An example of the benefit of this approach is demonstrated by the detection of compounds not accessible through iodide adduct formation; 4 times as many compounds are observed in multi-reagent ionization mode (with formulas assigned to at least half of them). For example, a known component in the sample of complex fragrance mixtures, eugenol (Peak 2 in Figure 4c), is identified in the multi-reagent ionization mode yet not detected in iodide mode. In Figure 4c, 6 other peaks are labeled that are not detected as iodide adducts, but for which formulas can be assigned using [M-H]- and [M+O2]- as identifiers, 1: C15H24O, 3: C9H10O3, 4: C12H24O2, 5: C16H32O2, 6: C18H34O2, and 7: C18H36O2.~~ A reasonable objection to multi-reagent ionization is that the complexity of adding up signals in multiple ionization chemistry with variable sensitivities may prohibit reasonable CIMS quantification. However, using CIMS for identification of unknowns by formula or other chemical information is valuable on its own, and quantification of many components is achievable using the FID channel of this instrument. This technique is likely only useful when analytes are individually resolved (i.e., isomer resolution), as the resulting mass spectrum of the complete complex mixture would be otherwise too difficult to interpret. We demonstrate here an example of exploring new reagent chemistries: simultaneously using multiple reagent ions is only made possible by the GC separation of analytes, but expands the information provided by this instrument. An in-depth understanding of the competition between reagent chemistries in a multi-reagent system is beyond the scope of this manuscript."

Comment 3: Additionally, about my comment 2 on decomposition products, I thank the authors for the thorough description. I intended to refer to 'upstream decomposition', where heating for desorption (and in the column) could fragment to form smaller product molecules that get transmitted to both the CIMS

and the FID. Then what I'm suggesting is that the iodide CIMS is simply insensitive to these smaller fragmentation products generally, while they do get sampled in the FID. It would be interesting (and valuable for interpretation) to know how much of this signal that gets measured in the FID but not CIMS is due to fragmentation (versus compounds that don't fragment but are not sensitive to iodide). Thank you for showing the FID signal in Fig. R1, but in hindsight that's not actually very useful because that is a mix of several/many known and unknown compounds, so there's still no way of separating out the fragmentation pathway. To answer that question, you'd have to reproduce Fig. R1, but with injections of only a single known compound/isomer at a time. Then, you should have only a single eluting peak that is the known compound/isomer, and any other peaks are fragmentation products that may well show up in just the FID and not the iodide CIMS. I think that including this type of analysis would greatly help the quality of this manuscript, but perhaps it could be included in a future manuscript instead.

Response: We thank the reviewer for clarifying that the comment is based on 'upstream decomposition'. It is true that upstream decomposition may occur when analytes were thermally desorbed from the sampling cell to the GC column. However, we note all 'upstream decomposition' is associated with TAG itself and potentially associated with all instruments using thermal desorption as the sample injection technique. Therefore, the coupling of the CIMS and FID to a TAG is not the cause of such thermal decompostion. We note that the 'upstream decomposition' issue has been reported in earlier work on TAG (Isaacman et al., 2014) and on other instruments like a FIGAERO-CIMS (Stark et al., 2017). Therefore, we agree with the reviewer that thermal decomposition related bias is an important topic and should be carefully examined.

The reviewer's suggestion would be an interesting case study to examine whether the decomposition products of one analyte are observable by iodide ionization or not, and thus might provide one possible explanation for some of the peaks. We note, though, that it is difficult to extend any such examination of one analyte to a general conclusion. For example, one could introduce a single compound that does not decompose in a GC but is ionized by I- (e.g., hexadecanoic acid) and conclude that there is no fragementation, or one could introduce something likely to decompose (e.g., a dimer) and conclude decomposition occurs and, depending on the decomposition products, may or may not be yield the FID/CIMS discrepancy observed here. A large fraction of commercially available standards unfortunately fall into the first category (do not decompose); for example, Hurley et al. introduced ~90 compounds, many of them individually, across a range of O/C up to 1.0 and showed that the peak that came out of the GC corresponded well to the analyte that went into the GC (e.g., did not appear to decompose) (Hurley et al., 2020). Conversely, Isaacman-VanWertz et al. (same PI, and PI of the present work) observed likely effects of decomposition impacting interpretation of results for ambient particles (Isaacman-VanWertz et al., 2016). Consequently, injection of individual analytes provides an exploration of decomposition for that analyte and might hint at some general possibilities, but cannot actually provide us with definitive information on the subject. We agree it would be interesting to find examples of these cases, and will consider doing so in future experiments.

As suggested by the reviewer, we have revised the manuscript on Line 215:

"It is also possible that some of those peaks are thermally decomposed analytes which exhibit low sensitivity in CIMS since all thermal desorption instruments, including the TAG, are known to potentially cause thermal decomposition of samples (Isaacman-VanWertz et al., 2016; Stark et al., 2017)"

We have also revised the manuscript based on a similar comment from Reviewer 2 in the first-round response. Here, we highlight those revisions (not shown in the track-change version of the manuscript since they were already included in the first-round revision) on Line 283:

"Conversely, thermal desorption within TAG may fragment larger accretion products to form analytes not present in the original sample (Buchholz et al., 2019; Isaacman-VanWertz et al., 2016; Lopez-Hilfiker et al., 2016b; Stark et al., 2017)~~(Isaacman-VanWertz et al., 2016; Lopez-Hilfiker et al., 2016b)~~, or may reverse particle-phase oligomerization reactions (Claflin and Ziemann, 2019). These fragments may not represent the actual molecular composition of SOA, though they nevertheless may provide insight into the formation mechanisms of SOA (Isaacman-VanWertz et al. 2016). Consequently, the potential multiple fragments from one parent compound may result in an overestimation of the number of isomers.~~These fragments may be identified as oxidation products in this analysis and consequently overestimate the number of isomers.~~ We note, however, that similar numbers of isomers are observed when using liquid chromatography (Figure 3b), which does not involve thermal desorption. Given these uncertainties, we believe that the results presented are not a floor or a ceiling on the number of isomers in the atmosphere, but a step toward understanding a poorly constrained problem."

And on Line 246:

"Overestimation may occur when large parent molecules decompose to isomers of a smaller formula during thermal desorption. ~~Overestimation may occur because peaks observed might be formed in part by thermal decomposition of analytes during thermal desorption.~~"

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