Enhancement of Lipid Signals in Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry with Ammonium Fluoride as a Matrix Additive

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ABSTRACT: Lipids are essential macromolecules that play a crucial role in numerous biological events. Lipids are structurally diverse which allows them to fulfill multiple functional roles. Matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) is a powerful tool to understand the spatial localization of lipids within biological systems. Herein, we report the use of ammonium fluoride (NH_4F) as a comatrix additive to enhance lipid detection in biological samples, with signal increase up to 200%. Emphasis was placed on anionic lipid enhancement with negative polarity measurements, with some preliminary work on cationic lipids detailed. We observed lipid signal enhancement of $[M-H]^-$ ions with the addition of 500 μM NH_4F additive attributed to a proton transfer reaction in several different lipid classes. Overall, our study demonstrates that the use of NH_4F comatrix additive substantially improves sensitivity for lipid detection in a MALDI system.

INTRODUCTION

Within cells, lipids are one of the most diverse macromolecules. Lipids play a key role in the structural integrity, energy storage, transport, signaling, and a myriad of other functions.[1] With so many essential functions, interest in lipid analysis has risen drastically.[1,2] The gold standard in lipid analysis is liquid chromatography coupled to mass spectrometry (LC-MS).[3] Using LC-MS, lipidomics, the large-scale study of obtaining the lipid profile, can be conducted in both a targeted and an un-targeted manner. Targeted lipidomics provides the absolute quantification of lipid targets, whereas untargeted analyses consider all lipids present within a given sample.[4] Targeted and untargeted approaches collectively aid in elucidating the identification, composition, and quantification of lipids within a given sample. However, LC-MS does not give insight into the localization of lipids *in situ*.

Mass spectrometry imaging (MSI) is a technique used for visualizing the spatial distribution of molecules within a sample. Some of the most common techniques used in MSI include matrix-assisted laser desorption/ionization (MALDI) and desorption electrospray ionization (DESI).[5] Currently, there are about 10-times the number of publications involving MALDI imaging compared to DESI imaging to date, per PubMed and Web of Science searches conducted in January of 2023. MALDI is a popular imaging technique due to its inherently fast analysis, sensitivity, and tolerance to high salts and buffers.[6] In general, MALDI-MSI creates a "chemical map" of specific mass-to-charge (*m/z*) values for a given imaging run. Each pixel in the image is defined by both laser diameter and raster width, which corresponds to a unique mass spectrum collected at that location. MALDI-MSI has been used extensively as a spatial characterization technique in a variety of sample types and disease states.[7,8,9]

One of the challenges in mass spectrometry is the lack of uniformity in the ionization efficiency of macromolecules.[10] Ionization efficiency is dependent on a variety of factors and differs drastically not only between different macromolecules, but also between molecules of the same class, such as lipids.[11,12] Many advances have been made to increase the specificity and sensitivity of lipid species detected in MSI, such as improvements in instrumentation,[13,14] adding washing steps during sample processing,[15,16] matrix composition,[16,17] and introducing additives in matrix application.[9,16,18] Of particular interest to this work are matrix additives, as they represent a simple means to increase lipid sensitivity without the added complexity of extensive protocol modifications and can be applied to existing in-house instrumentation.

Multiple matrix additives have been reported for enhanced detection of macromolecules including trifluoracetic acid (TFA), heptafluorobutyric acid (HFBA), EDTA, and hydrochloric acid (HCl), to name a few.[16,19,20] These studies are often conducted using a targeted approach in positive polarity. Fewer studies have focused on the effect of comatrix additives on lipid signal using both positive and negative polarities. Weigand and co-workers recently reported the use of ammonium fluoride (NH_4F) in nano-DESI MSI experiments.[21] They were able to demonstrate for the first-time using nano-DESI MSI the enhancement of $[M-H]^-$ lipid signal with the addition of NH_4F. In summary, they found a 10- to 110- fold enhancement in lipid signal intensity when using NH_4F as an additive in their solvent spray. The use of NH_4F as an additive has been characterized for both lipidomics and metabolomics applications using LC-MS.[22,23,24] Yanes and colleagues were one of the first groups to demonstrate how the basicity of the fluoride anion results in increased deprotonation of small molecules, such as lipids, using ESI.[24] The addition of NH_4F to the mobile phase resulted in a several fold increase in metabolite detection as compared to not using NH_4F or using other additives.

Within the context of a MALDI system, Cheng & Chan reported the use of ammonium halides as MALDI co-matrix additives to distinguish any effect on signal enhancement for studying oligonucleotides.[25] In their study, they found NH_4F had almost twice the signal enhancement for oligonucleotides relative to the other halides using 2-amino-5-nitrophyridine (ANP) matrix. Cheng & Chan also commented on how the electronegativity of the halide anions appears to play a role in assisting the desorption/ionization process for oligonucleotides. In this study, we demonstrate for the first time to our knowledge, the novel use of NH_4F as a comatrix additive for enhancement of lipid signal using both polarities within a MALDI system.

Herein, we evaluate multiple sample types including cell lysates, lipid standards, liver tissue and single cells as candidates for the application of NH_4F as a broad-spectrum additive in MALDI lipid analysis. Application of the NH_4F additive with NEDC matrix was found to enhance lipid signal intensity for all samples. Interestingly, lipid signals were increased up to 214% in MALDI dry drop analysis of a lipid standard mix and up to 150% in MALDI dry drop analysis of HCT 116 cell lysate. Additionally, enhancements were observed for MALDI-MSI analysis of mouse liver tissue and single-cell imaging of HCT 116 cells. With the use of NH_4F as a comatrix additive in MALDI-MSI applications, researchers will gain increased sensitivity in assessing the spatial localization of lipids within a sample, leading to potential improvements in quantitation for a variety of applications.

MATERIALS AND METHODS

Cell Culture. The HCT 116 human colon carcinoma cell line was purchased from ATCC (Manassas, VA). The cell line was maintained in McCoy's 5A cell culture media (Life Technologies, Grand Island, NY) and supplemented with 10% fetal bovine serum (Thermo Scientific, Gaithersburg, MD), 1% Penicillin-Streptomycin (Fisher Scientific, Hampton, NH), and 1% L-glutamine (Invitrogen, San Diego, CA). Incubation conditions were held at 5% CO_2 at 37°C. The passage of cells was conducted around 75% confluency to ensure passage in the exponential growth phase. The cells were verified by short tandem repeat (STR) sequencing in 2022.

MALDI Dry Drop Sample Preparation. The Avanti EquiSPLASH mix (Avanti Polar Lipids, Alabaster, AL) was used to determine the effect of NH_4F (Sigma-Aldrich, St. Louis, MO) on lipid signal using MALDI. A 10 mg/mL solution of NEDC (Sigma-Aldrich, St. Louis, MO) in 9:1 Methanol (MeOH): H_2O (LC-MS grade) obtained from Honeywell, was prepared and 475 μL was aliquoted into six separate 1.5 mL Eppendorf tubes. Next, NH_4F was added to the matrix solutions to a final volume of 500 μL and final concentrations of 0, 100, 200, 300, 400, and 500 μM NH_4F (Figure S1). Lastly, 1 μL of lipid standard mix was vortexed with 1 μL of matrix solution containing the additive and spotted on a MALDI target plate. Dry drop analysis was conducted on the Bruker timsTOF fleX in using both polarities. Additionally, both MALDI and MALDI-2 were evaluated. It is important to note, when using MALDI-2, we did so with a custom laser setting and not with the M2 preset laser to avoid any power boost associated with the M2 setting.

Dry drop analysis was conducted to determine changes in relative signal intensity for HCT 116 cell lysate in NEDC containing the NH_4F comatrix additive. As mentioned prior, a 10 mg/mL NEDC solution was made with 9:1 MeOH: H_2O. Next, an NH_4F stock solution was prepared in 9:1 MeOH: H_2O and added to the NEDC solution to a final concentration of 500 μM.. An aliquoted amount of the NEDC matrix solution without the additive was used for the 0 μM additive condition. A 1:1 mixture of HCT 116 cell lysate: matrix solution was then spotted on a MALDI stainless steel target plate for analysis on the Bruker timsTOF fleX mass spectrometer using negative ion mode. Instrument-specific parameters can be found in Table S1.

For dry drop experiments using an HTX TM sprayer (HTX Technologies, Carrboro, NC), the NEDC matrix was prepared as previously stated using both a 0 μM and 500 μM condition. These solutions were then sprayed onto an indium-tin-oxide (ITO) slide (Fisher Scientific, Hampton, NH) with dried lipid standards using the TM sprayer nebulizer (Table S2). Briefly, half of an ITO slide was covered with aluminum foil while the other side was sprayed with NEDC containing no additive onto 10 μL of dried EquiSPLASH lipid standard mix. Next, the aluminum foil was shifted to cover the second half of the slide and the second half of the ITO slide was sprayed with NEDC containing 500 μM of NH_4F onto 10 μL of dried lipid standard mix. Again, analysis was conducted on the timsTOF fleX with the same method parameters as previously conducted with the dry drop analysis.

MALDI-MSI Sample Preparation. Mouse liver samples were generously provided by Dr. Maria Mihaylova. The strain used was ad libitum fed wildtype B6 mouse strain. Mice used in this study were under veterinary and husbandry care of the University Laboratory Animal Resources (ULAR) division and approved for use by the IACUC committee at the Ohio State University. Serial sectioning of mouse tissues were collected using a Leica cryostat with an internal chamber temperature of -16°C and a chuck head temperature of -20°C. The stage was set for sectioning at 8 μm thickness. Mouse liver sections were thaw-mounted onto MALDI IntelliSlides (Bruker, Billerica, MA) and then sprayed using a TM sprayer nebulizer.

HCT 116 single-cell analysis was conducted on clean ITO slides. The slides were washed with hexanes (Macron Fine Chemicals, Radnor, PA), followed by MeOH for 10 minutes each in a sonicator. The slides were then coated with 20 μL of a stock poly-D-lysine solution (ThermoFisher, Waltham, MA). The stock solution was prepared by combining 750 μL poly-D-lysine, 750 μL LC-MS grade H_2O, and 1 μL of IGEPAL (Sigma-Aldrich, St. Louis, MO). Slides were placed onto a hot plate for 5 minutes and then washed in LC-MS grade H_2O. After letting the slides completely dry, an 8-well Press-to-Seal Silicone Isolator (Fisher Scientific, Hampton, NH) was applied to the slide. HCT 116 cells were then seeded onto the slides containing the isolators at 10,000 cells/well. The slides were then placed in a 150 x 15 mm petri dish and incubated overnight at 5% CO_2 and 37°C. After incubation, slides were washed twice in phosphate-buffered saline (PBS) (ThermoFisher, Waltham, MA), followed by a single wash in 10% formalin for 10 minutes and then twice in 50 mM ammonium formate (Sigma-Aldrich, St. Louis, MO), and twice in LC-MS grade H_2O, as reported by Cuypers et al.[26] Slides were placed in a -80°C freezer for storage until analysis.

For all spraying procedures, a TM sprayer nebulizer was used to apply the matrix for MALDI-MSI analysis. Briefly, 10 mg/mL NEDC in 9:1 MeOH: H_2O was used for application to tissue sections or single-cell slides. After weighing out the NEDC, a fraction of the matrix was retained for spraying control samples, while NH_4F was added to the remaining matrix at a final concentration of 500 μM. The same spray parameters were used for samples sprayed with and without the additive and can be found in Table S2.

Analysis of Lipid Standards and HCT 116 Cell Lysate with NH_4F Additive. To determine the effect of NH_4F on lipid signal with MALDI, we used an Avanti EquiSPLASH lipid standard mix, which contains 14 lipids covering a range of lipid classes at the same concentration. The major lipid classes observed in the MALDI dry drop analysis with both polarities include phosphatidylinositol (PI), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylglycerol (PG), phosphatidylcholine (PC), and sphingomyelin (SM). In brief, the dry drop analysis was conducted with a 1:1 ratio of lipid standard mix and NEDC matrix solution containing various concentrations of NH_4F additive. Concentrations of 0, 100, 200, 300, 400, and 500 μM NH_4F were examined in both polarities on the Bruker timsTOF fleX mass spectrometer. Additionally, both MALDI and MALDI-2 ionization were examined (Figure S2). For this dry drop analysis, we constructed plots corresponding to the signal intensity of lipid standards as a function of NH_4F concentration to assess how the concentration of the additive affect lipid signal intensity. Figure 1 depicts the plot corresponding to the dry drop analysis using MALDI in negative ion mode and shows the log scale $[M-H]^-$ signal intensity of the lipid standards as a function of NH_4F concentration. All lipid standards detected by dry drop analysis were enhanced by the addition of NH_4F, with a

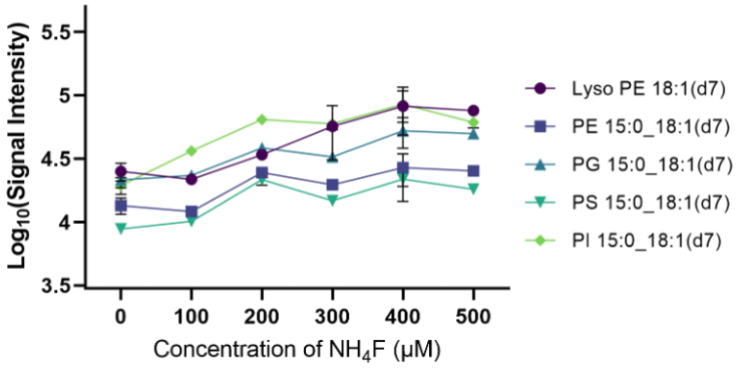

Figure 1. The effect of NH_4F additive concentration on a lipid standard mix by MALDI dry drop analysis. Each standard was observed as an $[M-H]^-$ ion and plotted as the log of the corresponding signal intensity against NH_4F concentration. Each spot represents n = 3.

plateau in signal intensity at concentrations between 400 to 500 μM NH_4F. For example, PI 15:0_18:1(d7), had a 214% increase in signal intensity with the addition of 500 μM NH_4F additive. Lipid standards detected by MALDI-MS had a statistically significant increase ($p \leq 0.05$) in peak signal with the addition of 500 μM NH_4F (Figure 2). Therefore, 500 μM NH_4F was the concentration used in the remaining studies. Interestingly, we noticed enhancement of $[M+H]^+$ ion signal for lipid standards using positive polarity (Figure 2 & Figure S1). Gonzalez-Riano and co-workers described how the use of NH_4F in the mobile phase for LC-MS applications results in the suppression of sodium adducts, $[M+Na]^+$, by the presence of fluoride anions.[23] By suppressing sodium adducts, other

ion species detected, such as $[M+H]^+$, will be influenced by the change in adduct concentration.[27] Our findings show some support for this proposed mechanism, with lipid class dependency. A summary of the dry drop lipid standard analysis and adduct formation can be found in Figure S2 & Figure S3.

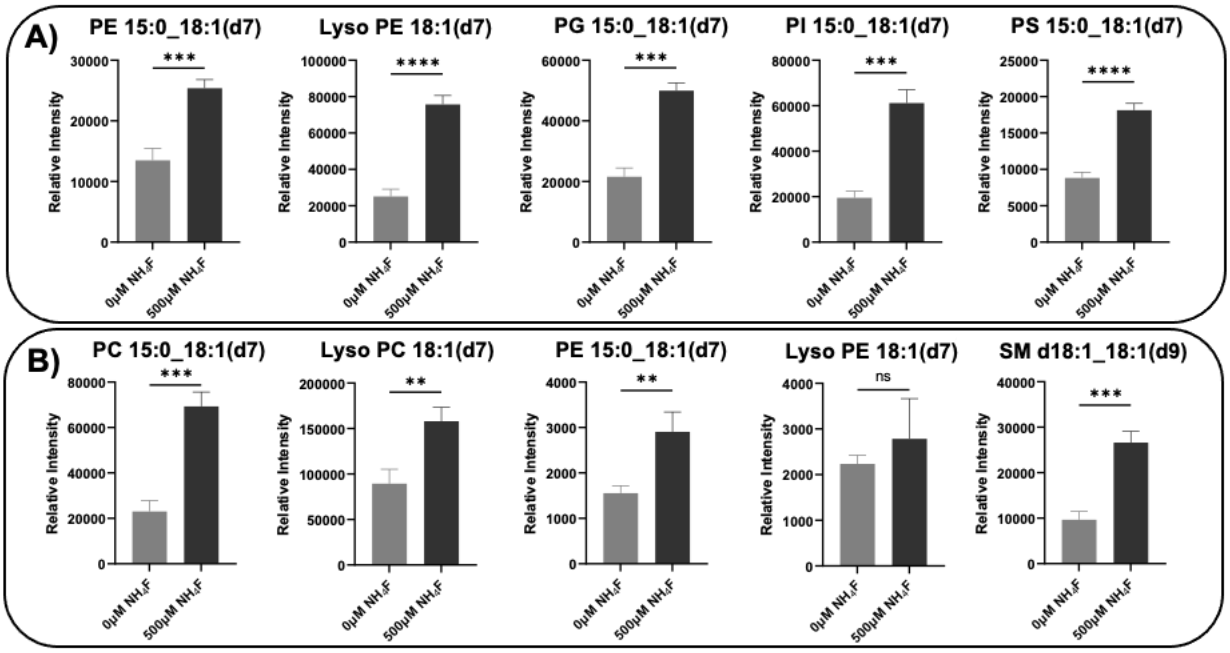

Figure 2. Dry drop analysis of lipid standard mix with NEDC ± 500 μM NH_4F. A) $[M-H]^-$ ions detected using MALDI in negative mode. B) $[M+H]^+$ ions detected using MALDI in positive mode. n = 3 for each condition. (ns = $p > 0.05$, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$)

After evaluating a standard mixture, we next examined whether the same lipid signal enhancement would be observed in a complex biological sample for endogenous lipid species. The HCT 116 colon carcinoma cell line was used for cell lysate analysis of lipid enhancement with the NH_4F comatrix additive. Analysis was conducted on a Bruker timsTOF fleX in negative ionization mode. In total, 25 lipid peaks were manually selected for analysis. 24 of 25 lipid peaks were significantly enhanced ($p \leq 0.05$) and showed as high as a 150% increase in peak signal intensity with the addition of 500 μM NH_4F additive. Lipid peaks selected for analysis were manually validated using an in-house spectral library generated from liquid chromatography-mass spectrometry (LC-MS/MS) data from a HCT 116 cell line. All *m/z* values analyzed, relative intensities, and assignments can be found in the Supporting Information (Figure S4/S5). Similar results were obtained by Weigand et al.[21] They reported anionic lipid signal enhancement with desorption electrospray ionization (DESI) imaging of mouse kidney sections with solvent solutions containing NH_4F. Mechanistically, Weigand and colleagues explain the enhancement is likely due to the basicity of the fluoride anion, which corroborates with previous studies.[22,23] They also hypothesize that the interaction between the anion and lipids occurs at the interface of the ESI droplet. We propose a similar mechanism, where within the MALDI process, the basicity of the fluoride anion interacts with lipid species, causing a proton

transfer reaction.[21,25] The precise mechanism is beyond the scope of this paper and will be explored in future studies; for more information on proton transfer reaction mechanisms within MALDI, see Mirabelli and Zenobi.[28]

MALDI dry drop analysis can show signal variations due to crystal irregularities, often referred to as "hot spots".[29] To determine if the improvement in signal intensity was due to deviations in crystal size between co-crystallization of NEDC with and without NH_4F in dry drop analyses, we also evaluated sprayed matrices, which are commonly used in MALDI imaging applications. With the matrix and comatrix additive nebulized onto samples, we again noticed statistically significant improvement in peak signal intensity of the standards with the addition of 500 μM NH_4F additive relative to 0 μM NH_4F control in both polarities (Figure S6). This enhancement confirmed the increase in signal detected in both the lipid standards and the cell lysate dry drop studies was not due to matrix "hot spot" formation, but due to the presence of the additive. Intriguingly, measurements conducted in positive polarity for the lipid standards were again significantly higher for peak signals containing 500 μM NH_4F in NEDC matrix. The use of MALDI and MALDI-2 were evaluated, where we found a statistically significant increase in lipid signal with the use of MALDI-2 in positive ion mode relative to MALDI, and no change associated with measurements collected in negative ion mode (Figure S6). We postulated the increase in lipid signal with the use of MALDI for both polarities could be due to finer crystal formation occurring with the additive as compared to samples without it. To test this theory, we imaged the matrix crystals using an Echo Rebel Microscope at a 40x objective and noticed the crystals had the same morphology and size both with and without the additive (Figure S7). Based on the observations made by the brightfield images, we determined the data supports crystal formation with NEDC is similar with and without NH_4F additive. This finding confirmed the improvement in lipid ionization likely occurs through interaction with the additive and contributions involving crystal formation are minimal. Since spraying NEDC with additive resulted in a similar trend of increased ionization of lipids, we then examined whether the same trend would be observed with MALDI-MSI experiments.

MALDI-MSI of Mouse Liver. Wildtype mouse liver samples were sectioned and imaged via MALDI-MSI to distinguish the effect of NH_4F use on a heterogeneous sample. Serial sections of the tissue were sprayed with NEDC, with or without 500 μM NH_4F. Analysis was conducted on a Bruker timsTOF fleX in negative ion mode at a spatial resolution of 20 μm. Considerations in mass spectrometer parameters and statistical comparisons can be found in the Supporting Information. A total of 14 putative lipids were selected for analysis after completion of the imaging run. Endogenous lipids analyzed in the tissue sprayed with NEDC containing 500 μM NH_4F displayed a statistically significant increase in peak area as compared to the tissue sprayed with NEDC alone (Figure 3 & Figure S8/S9). The statistically significant increase in lipid signal corresponded to a 10 – 16% signal intensity enhancement for most lipids analyzed between the two conditions. In addition to the improvement in ion signal intensity, the additive appears to provide increased resolution of molecular features associated with the lipids. A wide range of anionic lipids had improved signal, as was the case for both cell lysate and lipid standard analysis with the additive. Lipid classes that were enhanced by the addition of NH_4F include PI, PE, and PS. Figure 3 shows *m/z* 885.5482 (PI 38:4), *m/z* 762.5072 (PE 38:6), and *m/z* 738.5043 (PE 36:4) with a signal enhancement of 12.8%, 16.3%, and 15.6% respectively. Additionally, *m/z* 810.5248 has a putative identification

of PS 38:4 and had a 15.8% increase in signal intensity with the additive (Figure S8). Putative identifications were made for each lipid using high mass accuracy MS[1] spectra collected on the timsTOF fleX and analyzed with LIPID MAPS.

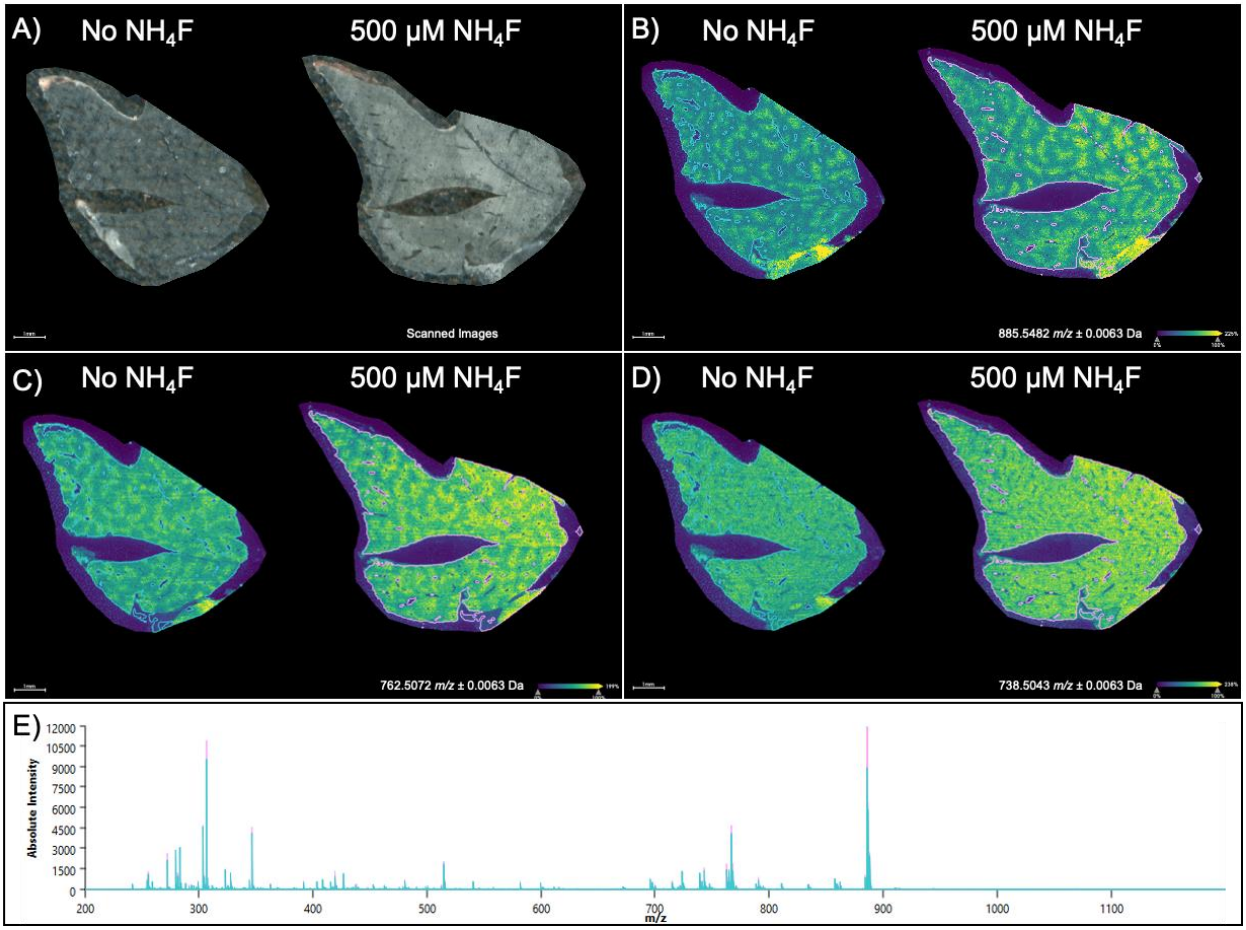

Figure 3. MALDI-MSI of mouse liver sprayed with NEDC ± 500 µM NH_4F, acquired in negative ion mode. A) Scanned images of liver sections used for MALDI-MSI analysis. B) Spatial localization of *m/z* 885.5482 (PI 38:4, Delta = 0.0017) in mouse serial sections. C) Spatial localization of *m/z* 762.5072 (PE 38:6, Delta = 0.0007) in mouse serial sections. D) Spatial localization of *m/z* 738.5043 (PE 36:4, Delta = 0.0036) in mouse serial sections. E) Overlayed mass spectra collected from 200 – 1200 *m/z* for both tissue sections imaged. All ion images were segmented based on the top 2500 intervals and normalized by TIC. Scale bar = 1 mm. LIPID MAPS putative assignments are shown in parenthesis with corresponding delta values. (Blue = No NH_4F Segmentation, Pink = 500 µM NH_4F Segmentation)

Tissues sections are one of the most common samples used for MALDI-MSI analysis.[30] Often tissue samples are imaged to distinguish differences in molecular features, such as lipids, between a diseased state and a non-diseased region or control sample.[31] Therefore, the contribution of an additive to enhance the spatial localization and detection of lipids in a heterogenous tis-sue sample is of great importance. Our findings show that the simple inclusion of NH_4F as a comatrix additive to NEDC results in increased signal intensity for lipids in negative mode for a complex tissue sample.

HCT 116 Single-Cell MALDI Imaging. Recent advances in mass spectrometry instrumentation, such as high spatial resolution and increased sensitivity, have improved the capability for single cell MALDI-MSI.[32] Single cell MALDI-MSI is becoming

significantly important in label-free molecular mapping, drug susceptibility, and biomarker discovery.[29,32] MALDI-MSI of single cells allows a deeper insight into holistic changes in molecular species, such as lipids in different cell types or even determining the effect of a drug treatment option.[33,26] Therefore, it is imperative to continue to advance our ability to detect molecular changes at the single cell level, which can provide insight into a variety of different mechanisms. MALDI-MSI of single cells is often considered challenging due to the need for high spatial resolution, small matrix crystal size, increased sensitivity, and a variety of other factors. Most mammalian cells range from 10 – 100 µm in diameter.[34] Therefore, we performed all single cell imaging at 5 µm spatial resolution using a Bruker timsTOF fleX. Similarly, to the mouse liver imaging, poly-D-lysine coated indium-tin-oxide (ITO) slides were seeded with HCT 116 colorectal carcinoma cells using a silicone isolator and sprayed with NEDC both with and without 500 µM NH_4F. MALDI-MSI revealed several lipid peaks in the single cell imaging analyzed with negative polarity. Interestingly, HCT 116 cells imaged with NEDC and 500 µM NH_4F again displayed enhanced lipid signal relative to NEDC with no additive (Figure 4 & Figure S10/S11). In total 11 lipids were selected for single cell MALDI-MSI analysis. Mainly, PI and PE lipids were detected; for example, *m/z* 885.5473 (PI 38:4) shown in Figure 4, which exhibited a signal enhancement of 37.5% with the additive compared to samples without. Most lipids analyzed in the single cell imaging displayed an enhancement around 20% with the additive. Intriguingly, all PI lipid species analyzed displayed statistically significant increase in lipid signal when imaged with 500 µM NH_4F. Lipid peaks were again manually validated using a spectral library created by LC-MS/MS data.

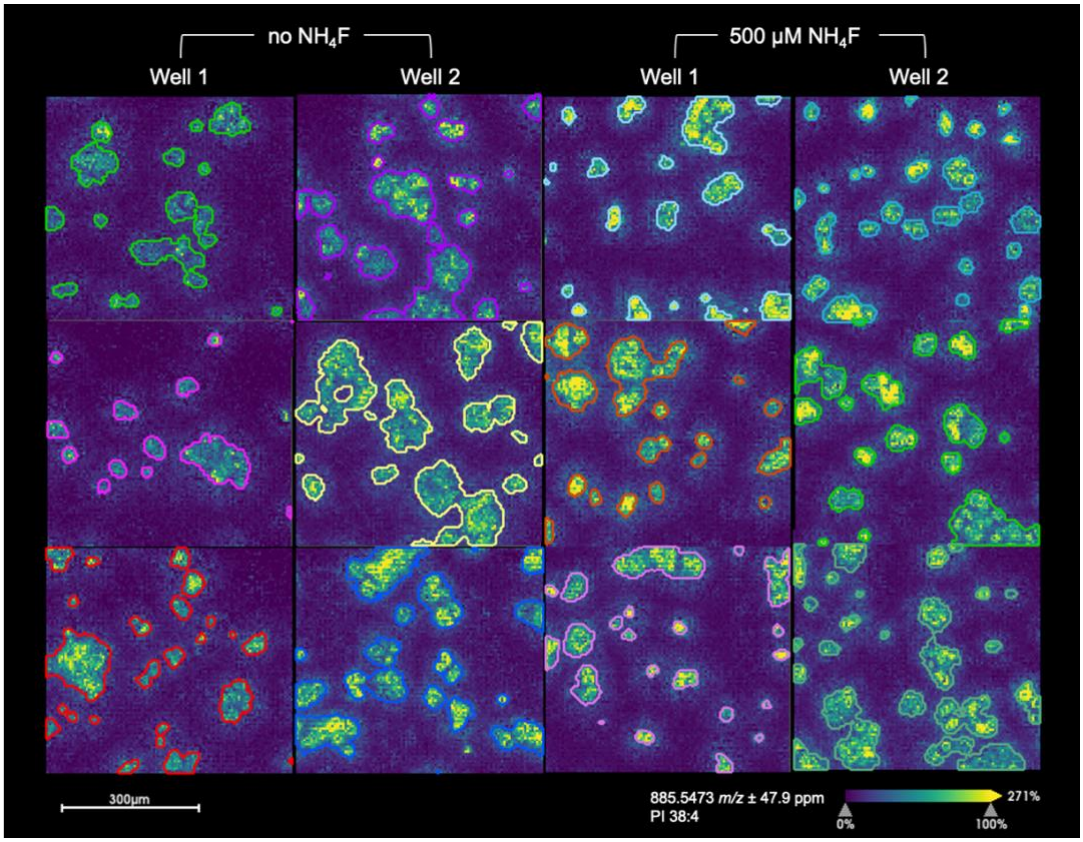

Figure 4. MALDI-MSI of HCT 116 colorectal carcinoma cells sprayed with NEDC ± 500 µM NH_4F, acquired in negative ion mode. Two wells of a Press-to-Seal Silicone Isolator were used for analysis for both conditions with three images collected in each well. The spatial

localization of *m/z* 885.5473 (PI 38:4) can be seen in all 12 images taken. Images were spatially segmented based on the top 100 intervals and normalized by TIC. Scale bar = 300 μm. Lipid assignment was based on LIPID MAPS and a spectral library created with LC-MS/MS data. Statistical analyses were performed with n = 6 for each condition.

Our experimental setup allowed for *in situ* single cell imaging of HCT 116 colon carcinoma cells grown in two-dimensions (2D). Within the same imaging run for each condition, we captured both isolated single cells and clusters of cells typically seen within a 2D cell culture environment. Statistically significant increase in lipid signal was noticed for 8 out of 11 lipids analyzed. We have again showcased how the simple addition of NH_4F as a comatrix additive with NEDC can provide enhanced anionic lipid signal using MALDI-MSI.

CONCLUSION

In this study, we examined the effect of NH_4F as a comatrix additive with NEDC on lipid analysis within different sample types using MALDI. We found that the addition of NH_4F enhances lipid signal intensity in both negative and positive ion modes as compared to NEDC matrix alone. Enhancement of $[M-H]^-$ and $[M+H]^+$ ions was observed in negative and positive polarity respectively with the addition of 500 μM NH_4F additive. In cell lysate dry drop analysis, lipid signal was enhanced 60 – 150% with the addition of 500 μM NH_4F. Lipid standards showed an increase in signal enhancement of 88 – 214% with 500 μM NH_4F additive, depending on lipid class in dry drop analysis in negative mode with the use of MALDI. Spraying NEDC matrix containing 500 μM NH_4F onto lipid standards resulted in a 30 – 83% increase in lipid signal in negative mode with MALDI. Similarly, to dry drop analyses, MALDI-MSI also resulted in heightened signal intensity of anionic lipids from 10 – 16% in mouse liver and 11 – 38% in HCT 116 single cell imaging in negative mode. We postulate the reduction in signal enhancement for MALDI-MSI of the liver tissue is due to the added complexity of imaging a heterogenous tissue, where tissue samples contain many diverse molecules which could interact with NH_4F. Additionally, limited sample ablation with single cell imaging could result in the reduction of signal enhancement with additives for MALDI-MSI experiments. We attribute the overall increase in lipid signal seen in negative polarity to a proton transfer reaction taking place during the MALDI process for all sample types. Moving forward, we aim to investigate the mechanism of the NH_4F additive, along with the effect of NH_4F in combination with other matrices commonly used in lipid analysis with MALDI. In summary, this study introduces a straightforward approach to enhance the sensitivity in lipid detection for MALDI analysis and emphasizes NH_4F usage in MALDI-MSI.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Chemicals, timsTOF fleX parameters, Echo Rebel Microscope parameters, MALDI data analysis, liquid chromatography-mass spectrometry, tables & figures (PDF)

MALDI dry drop results for lipid standards and HCT 116 cell lysate, Sprayed matrix results for lipid standards, sodium adduct formation, MALDI vs. MALDI-2 comparison, statistics for MSI of mouse liver tissue and HCT 116 single cell imaging, liquid chromatography-mass spectrometry (Excel)

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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