DISCOVERY LIPIDOMICS AND MAPPING OF EXOGENOUS FATTY ACID INCORPORATION INTO THE HELA LIPIDOME USING LC-IMS/MS AND LC-IMS/MS/MS

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INTRODUCTION

Odd-chain fatty acids are not produced endogenously and therefore often used as standards for lipid quantification in lipidomics experiments. For structural and dynamics studies of lipid bilayers, several fluorescent probes, including BODIPY, a boron containing fluprophore, have been incorporated into the lipid headgroup or acyl chain. When added to cells, these fatty acids readily incorporate into the cellular lipid fraction. These fatty acid analogs may be metabolized into a range of phospholipids and sphingolipids. A combination of discovery and targeted lipidomics techniques are used to profile the incorporation of these exogenous lipid probes into the HeLa cellular lipidome.

When coupled with liquid chromatography, ion mobility spectrometry (IMS) represents an orthogonal technique that separates ions based on charge, size, and shape. IMS strategies were used to probe the complex mixture of endogenous lipids along with targeted detection of modified lipids present in HeLa cells.

METHODS

Cell Culture

200,000 HeLa cells were plated at 70% confluence using standard protocols.[1] Heptadecanoic acid and C16-BODIPY (16-dipyrrometheneboron difluoride-hexadecenoic acid) were obtained from Avanti Polar Lipids and incubated at 50 μM with the HeLa cells for 24 hours, which were subsequently isolated and frozen with liquid Nitrogen.

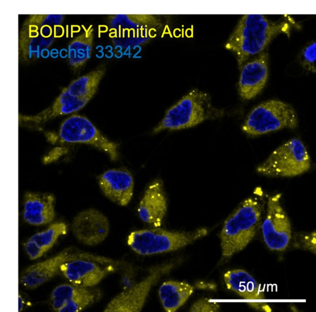
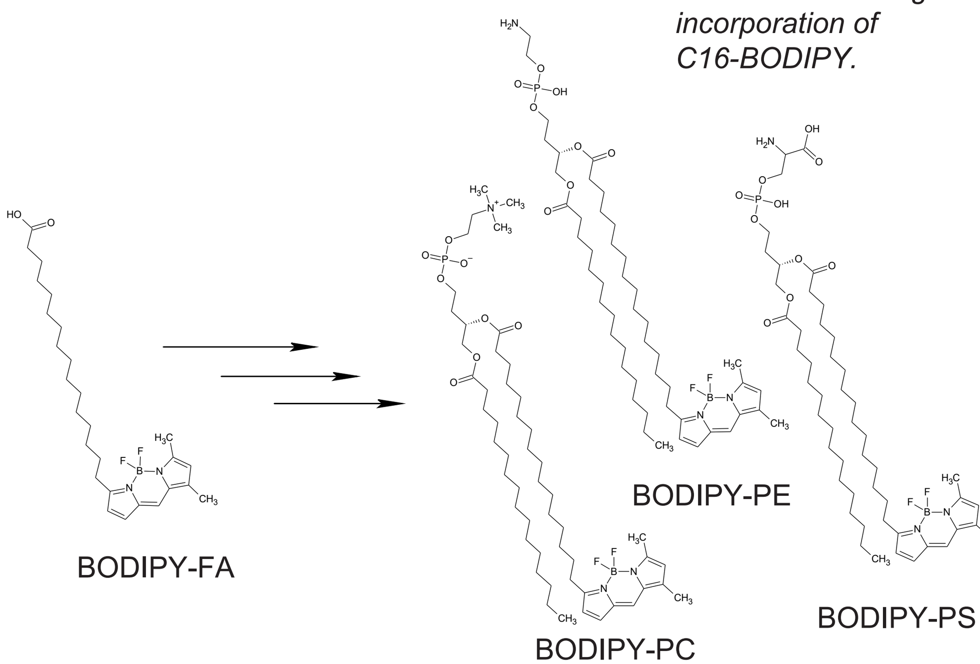


Figure 1. Fluorescence microscopy images of HeLa cells showing incorporation of C16-BODIPY.



Sample Extraction

Resuspend Cells in 70 μL water and vortex

Add 225 μL Methanol and vortex

Add 840 μL Methyl-*tert*-buty ether (MTBE)

Shake on orbital mixer for 60 minutes

Add 140 μL water

Centrifuge at 1000xg for 10 minutes

Remove MTBE layer, dry down under Nitrogen flow and reconstitute in 100 μL 60% Water 40 % Acetonitrile. 2 μL of Avanti SPLASH LipidoMix was added as an internal standard.

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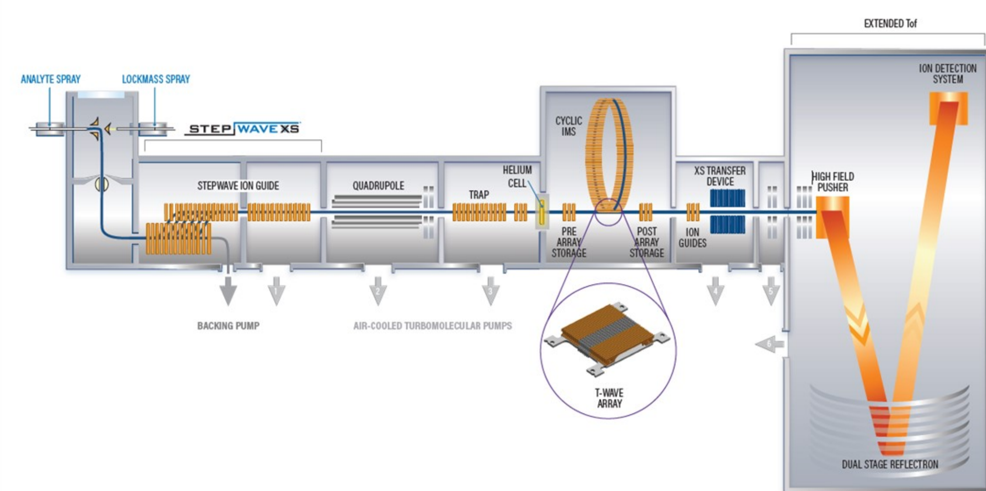


Figure 2. Waters™ SELECT SERIES™ Cyclic™ Notable components are the orthogonal circular (1m pathlength) mobility separator and the array region where ion motion is controlled to move ions into and out of the mobility separator. Analytical figures of merit for this system are described in Reference 2

Liquid Chromatography

Waters ACQUITY™ Premier System

MP A: 60% Aqueous Acetonitrile, 10 mM Ammonium Formate, 0.1% Formic Acid

MP B: 10% Acetonitrile in Isopropanol, 10 mm Ammonium Formate, 0.1% Formic Acid

Column: ACQUITY Premier CSH™ C18, 2.1x100 1.7 μm d_p, operated at 65 C° and flow rate of 400 μL/min

Gradient Elution

Time	% A	% B	Curve
Initial	50	50	
3	47	53	6
16	25	75	6
20	1	99	6
22	1	99	6
22.1	50	50	1
24	50	50	1

Mass Spectrometry

Waters SELECT SERIES Cyclic IMS

Tuned to 60,000 resolution and operated in either ES+ or ES– mode with Leucine enkephalin lock mass

Mobility separations performed in Nitrogen and the CCS scale was calibrated with Major Mix

Single and Multipass HDMS^{E} Experiments using collision energy ramps applied to the XS Transfer Device[2]

Data processing in Waters Connect™

RESULTS FROM HELA CELLS INCUBATED WITH FLUORESCENT LIPIDS

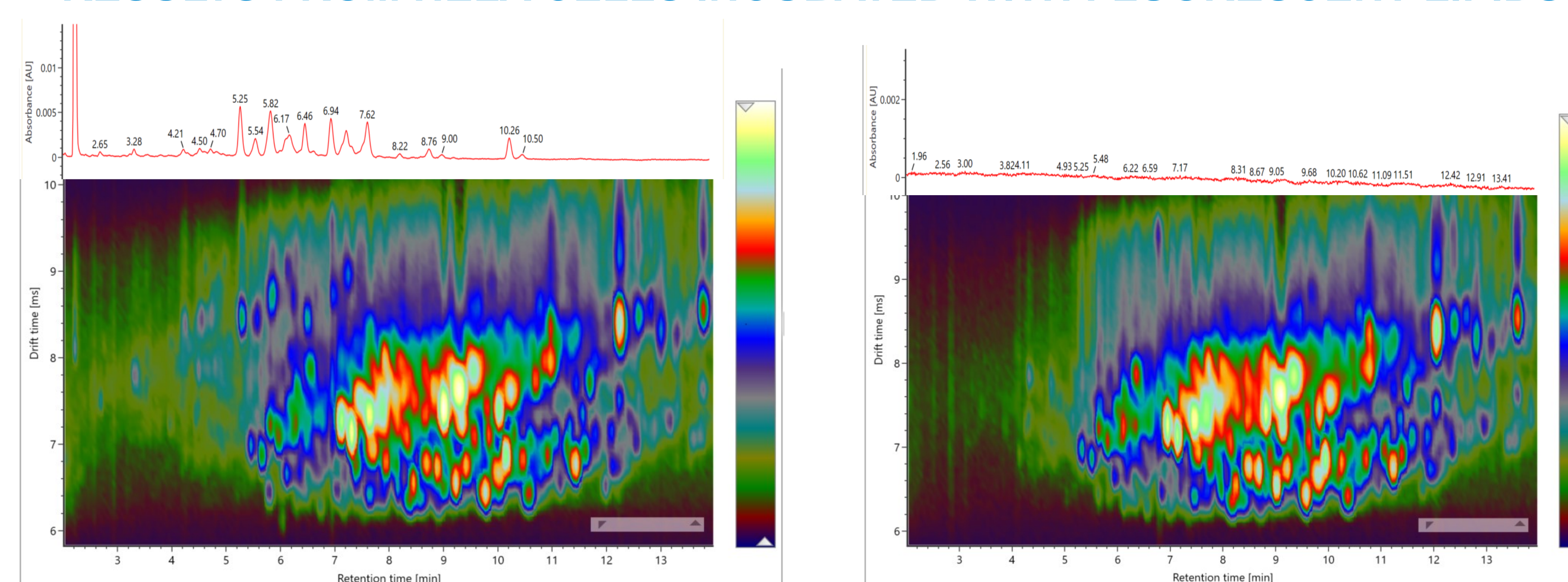


Figure 3. Left Panel: ESI Negative ion 2 dimensional LC/IMS plot of arrival time vs retention time for phospholipids from HeLa cells incubated with C16-BODIPY. Top trace is UV 506 at the main absorption band for the BODIPY ligand. Lipids with C16-BODIPY incorporation range in retention time 5.25 to 7.6 minutes and drift times 8.4 to 9 msec. These lipids are substantially less polar and have larger collisional cross sections compared with the corresponding fatty acyl lipids. Right Panel: Corresponding 2 dimensional plot of arrivaltime vs retention time for phospholipids from control incubations.

	Observed RT (...	Component name	Observed m/z	Mass error (mDa)	Mass error (ppm)	Observed drift (ms)	Observed CCS (Å²)	Detector counts	Response
1	4.30	PS_BODIPY_C226	1024.5799	-0.5	-0.5	9.09	296.08	6031	3926
2	4.52	PS_BODIPY_C204	1000.5797	-0.7	-0.7	8.90	293.17	6028	3892
3	4.59	PS_BODIPY_C182	976.5797	-0.8	-0.8	8.69	290.00	4839	3152
4	4.65	PS_BODIPY_C225	1026.5958	-0.3	-0.3	9.14	296.88	5676	3634
5	5.24	PC_BODIPY_C140	922.6045	-1.8	-2.0	8.41	285.70	59921	34231
6	5.25	PS_BODIPY_C160	952.5790	-1.4	-1.5	8.52	287.34	25468	11996
7	5.51	PC_BODIPY_C226	1022.6358	-1.8	-1.7	9.21	297.90	11492	6335
8	5.52	PS_BODIPY_C181	978.5942	-1.9	-1.9	8.76	291.08	131534	75330
9	5.52	PS_BODIPY_C204	1000.5761	-4.3	-4.3	8.53	287.29	5194	3413
10	5.56	PE_BODIPY_C140	880.5576	-1.7	-1.9	7.79	275.72	3322	3322
11	5.77	PC_BODIPY_C204	998.6361	-1.5	-1.5	9.05	295.52	41375	22415
12	5.86	PE_BODIPY_C226	980.5891	-1.5	-1.6	8.65	289.32	28827	16689
13	5.86	PC_BODIPY_C182	974.6362	-1.4	-1.4	8.83	292.21	17691	10155
14	5.91	PC_BODIPY_C225	1024.6515	-1.7	-1.7	9.24	298.46	6976	4299
15	6.12	PE_BODIPY_C204	956.5890	-1.6	-1.7	8.44	286.07	108657	62425
16	6.23	PE_BODIPY_C182	932.5894	-1.2	-1.3	8.25	282.99	10460	6883
17	6.27	PE_BODIPY_C225	982.6054	-0.8	-0.8	8.69	289.91	11337	6548
18	6.62	PC_BODIPY_C160	950.6363	-1.3	-1.3	8.73	290.63	79515	44242
19	6.62	PC_BODIPY_C183	1018.6237	-3.7	-3.6	9.23	298.33	5525	3520
20	6.66	PS_BODIPY_C180	980.6092	-2.6	-2.6	8.90	293.21	1469	1469
21	6.88	PC_BODIPY_C204	1044.6386	-4.4	-4.2	9.54	303.02	1547	1547
22	6.89	PC_BODIPY_C181	976.6515	-1.7	-1.8	8.96	294.26	105106	57809
23	7.01	PE_BODIPY_C160	908.5902	-0.4	-0.4	8.14	281.36	21193	13020
24	7.27	PE_BODIPY_C181	934.6055	-0.8	-0.8	8.34	284.51	112011	66000

Table 1. Phospholipids containing C16-BODIPY group identified in HeLa cells incubated with C16-BODIPY. ESI Negative Mode. All mass measurements were within 4.5 ppm.

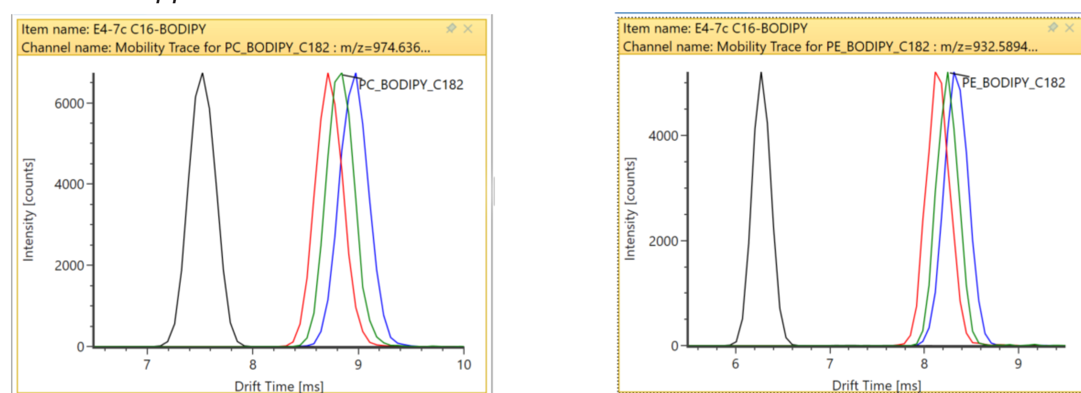


Figure 4. Left Side Overlaid plots of PC C16:0 C18:2 (Black), PC C16-BODIPY:C16:0 (Red) ,PC C16-BODIPY: C18:1 (Green) and PC C16-BODIPY C18:2. (Blue) Right Side Overlaid plots of PE C16:0 C18:2 (Black), PE C16-BODIPY,C16:0 (Red) PE C16-BODIPY C18:1 (Green) and PE C16-BODIPY C18:2.(Blue)

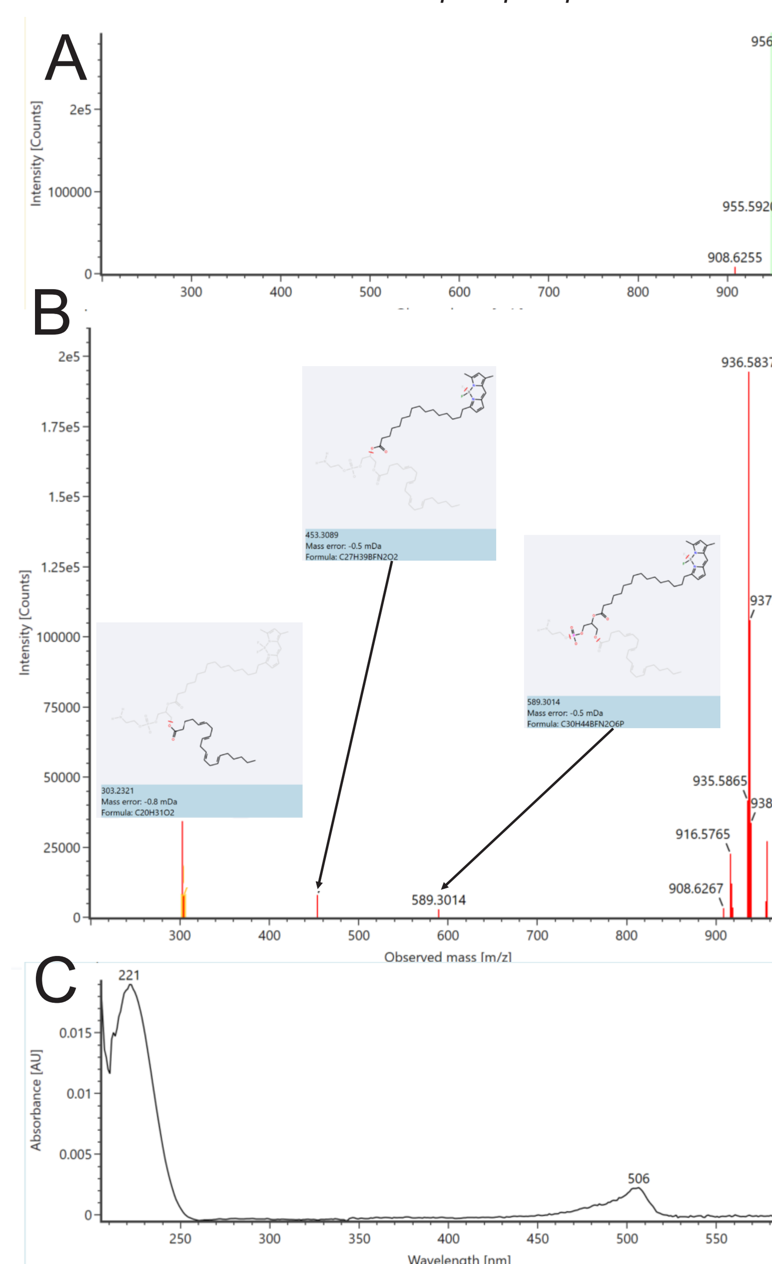
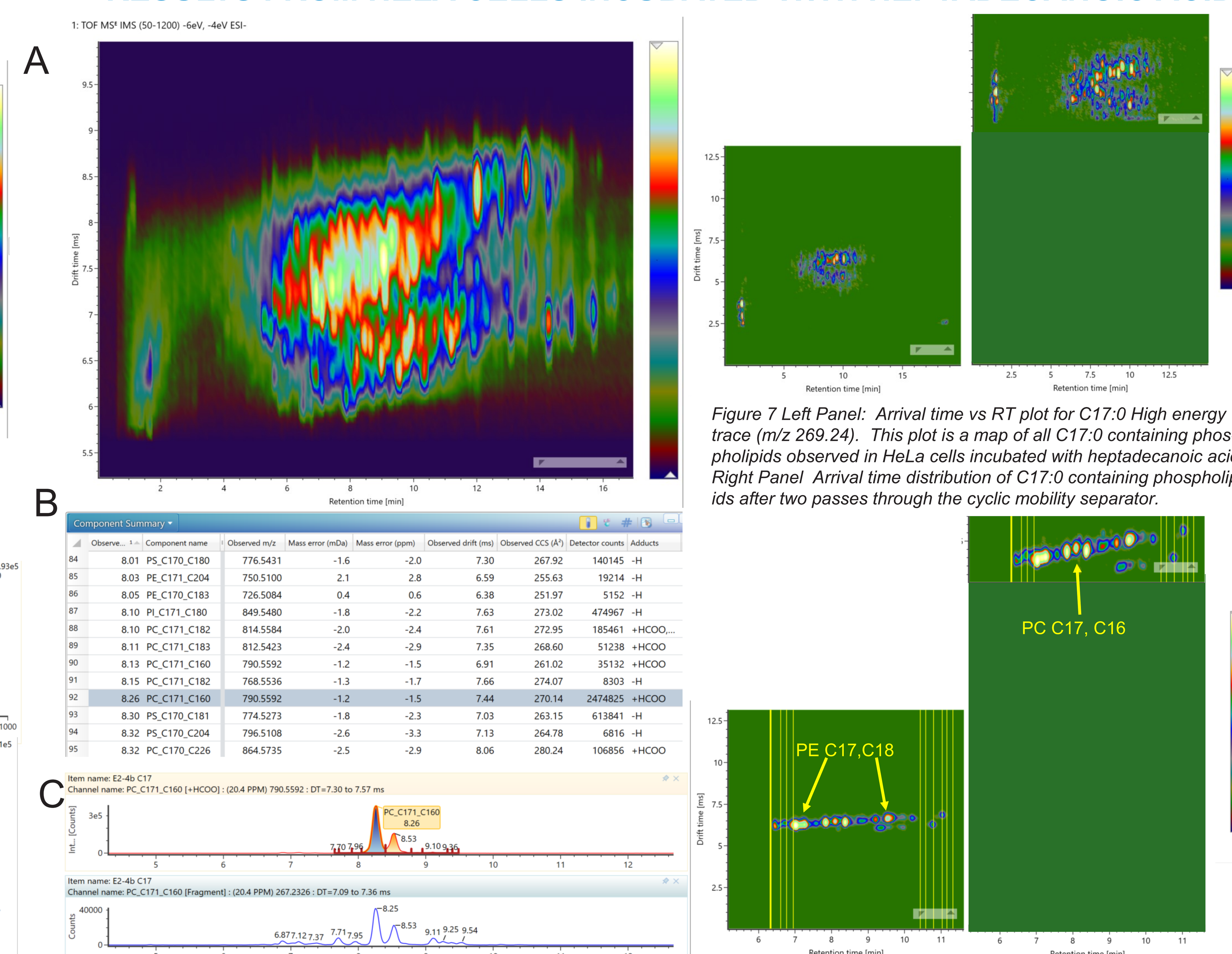


Figure 5A. Mobility filtered low energy spectrum for PE C16-BODIPY:C20:4. B. Annotated mobility filtered high energy spectra. Notable for the BODIPY moiety are losses of HF. C. UV Spectra showing broad absorption band at 506 nm for the BODIPY heterocycle.

RESULTS FROM HELA CELLS INCUBATED WITH HEPTADECANOIC ACID



	Observe...	Component name	Observed m/z	Mass error (mDa)	Mass error (ppm)	Observed drift (ms)	Observed CCS (Å²)	Detector counts	Adducts
84	8.01	PS_C170_C180	776.5431	-1.6	-2.0	7.30	267.92	140145	-H
85	8.03	PE_C171_C204	750.5100	2.1	2.8	6.59	255.63	19214	-H
86	8.05	PE_C170_C183	726.5084	0.4	0.6	6.38	251.97	5152	-H
87	8.10	PI_C171_C180	849.5480	-1.8	-2.2	7.63	273.02	474967	-H
88	8.10	PC_C171_C182	814.5584	-2.0	-2.4	7.61	272.95	185461	+HCOO,...
89	8.11	PC_C171_C183	812.5423	-2.4	-2.9	7.35	268.60	51238	+HCOO
90	8.13	PC_C171_C160	790.5592	-1.2	-1.5	6.91	261.02	35132	+HCOO
91	8.15	PC_C171_C182	768.5536	-1.3	-1.7	7.66	274.07	8303	-H
92	8.26	PC_C171_C160	790.5592	-1.2	-1.5	7.44	270.14	2474825	+HCOO
93	8.30	PS_C170_C181	774.5273	-1.8	-2.3	7.03	263.15	613841	-H
94	8.32	PS_C170_C204	796.5108	-2.6	-3.3	7.13	264.78	6816	-H
95	8.32	PC_C170_C226	864.5735	-2.5	-2.9	8.06	280.24	106856	+HCOO

Figure 6A. Arrival time vs RT plot for HeLa Cells incubated with heptadecanoic acid. B. Table of selected C17 containing phospholipids. C. Extracted ion current chromatograms for PC C17:1, C16:0 and High Energy XIC for C17:1 fragment Ion. D. Low energy and annotated high energy spectra for PC C17:1, C16:0.

Figure 7 Left Panel: Arrival time vs RT plot for C17:0 High energy trace (m/z 269.24). This plot is a map of all C17:0 containing phospholipids observed in HeLa cells incubated with heptadecanoic acid. Right Panel Arrival time distribution of C17:0 containing phospholipids after two passes through the cyclic mobility separator.

Figure 8 Left Panel: Arrival time vs RT plot for PE lipids containing C17 and C18 lipids and PC lipids containing C16 and C17. Right Panel Arrival time distribution PE lipids containing C17 and C18 lipids and PC lipids containing C16 and C17 after three passes through the cyclic mobility separator.

CONCLUSIONS

- **Incorporation of exogenous fatty acids into cellular phospholipid pool.**
- **Observation and characterization of C16-BODIPY containing PC, PE, and PS. Fragmentation patterns driven by loss of HF.**
- **Rapid incorporation of C17 acyl groups into lipid pool followed by extensive desaturation**
- **Use of multipass cIMS to for high resolution separation of C17 containing phospholipids**

References

1. https://www.biorxiv.org/content/10.1101/2022.02.14.480333v1
2. Giles et al. *Anal Chem.* **2019**, 8564-8573.

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