Cell-envelope remodelling as a determinant of phenotypic antibacterial tolerance in *Mycobacterium tuberculosis*

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I. SI Appendix - Material and methods

1. Materials

Unless otherwise stated, all other chemicals and reagents were purchased from Sigma-Aldrich.

2. Bacterial strains and conditions

M. tuberculosis H37Rv (Mtb) was used for most experiments. *M. smegmatis* mc^2155 and *M. bovis* BCG Pasteur were employed in a few metabolomics and NaCl sensitivity experiments. Mtb H37Rv-ATCC (DIM+, SL+, DAT/PAT+); Mtb H37Rv-Pasteur (DIM+, SL+, DAT/PAT-); H37Rv-Pasteur ppsE+pks2 KO PMM127 (DIM-, SL-, DAT/PAT-); H37Rv-Pasteur ppsE KO PMM127:CiE526 (DIM-, SL+, DAT/PAT-); H37Rv-Pasteur pks2 KO PMM127: pMVE (DIM+, SL-, DAT/PAT-); H37Rv-Pasteur pks3/4 PMM127: pWM228 (DIM-, SL-, DAT/PAT+) were grown in 7H9 liquid medium with 10 % OADC enrichment (Difco) supplemented or not with NaCl, to yield final concentrations of sodium chloride of 14.5 mM, 125 mM and 250 mM. Mtb was cultured up to mid-exponential phase in 7H9 liquid medium supplemented with 0.5 g/l Fraction V bovine serum albumin, 0.05% tyloxapol, 0.2% dextrose and 2% glycerol, 10 mM NaCl. For metabolomic profiling studies, Mtb was cultured on 7H10 agar supplemented with 0.5 g/l Fraction V bovine serum albumin, 0.05% tyloxapol, 0.2% dextrose and 0.2% glycerol and 10 mM NaCl. For lipidomics, Mtb was cultured in 7H9 liquid medium without any detergent or exogenous lipid source.

Clinical isolates had the following phenotypic and genotypic profiles:

Clinical Isolate	Phenotypic profile for RIF and INH	Genotypic profile (Mutations in genes related to RIF and INH resistance)
CF46	RIF^R / INH^R	His526Cys (*rpoB*) / Ser315Thr (*katG*)
CF81	RIF^R / INH^R	Asp516Val (*rpoB*) / Ser315Thr (*katG*)
CF85	RIF^R / INH^R	Ser315Thr (*katG*)
CF102	RIF^S / INH^S	*
CF158	RIF^R / INH^R	Ser531Leu (*rpoB*)

R: resistant
S: susceptible
* not found

3. Acid-fast stain

A loop of bacteria from each culture was transferred to a glass microscope slide and allowed to dry and then fixed in formaldehyde overnight. The slides were stained with TB Carbol-Fuchin KF for four minutes and destined with TB decolourizer and counter stained with TB

Brilliant Green K for thirty seconds (Becton Dickinson).The slides were photographed on a Olympus IX70 with QIClick colour camera and the objective was x100 NA1.35.

4. Membrane potential

Mtb was grown to an OD 0.6, spun down and suspended in 7H9 media containing either 10mM, 125mM, 250mM or 1,000mM of NaCl. 1ml aliquots were taken at various time points. The samples were washed in 7H9 media containing no albumin, suspended in 1ml of 7H9 media without albumin and exposed to 15μM DiOC2 for 20 min, at room temperature. Samples were then washed in 7H9 without albumin and suspended in 7H9 media without albumin. Fluorescence was monitored on a SpectroMax Gemini XPS plate reader (Molecular Devices), green 488/530nm and red 488/610nm. The membrane potential was calculated as a ratio of red to green fluorescence.

5. Metabolomics

5.1. Metabolite extraction

For metabolomic profiling studies, Mtb was cultured on 7H10 agar supplemented with 0.5 g/l Fraction V bovine serum albumin, 0.05% tyloxapol, 0.2% dextrose and 0.2% glycerol and 10 mM NaCl. Thus, Mtb was grown initially in 7H9 liquid media containing the carbon source of interest until the late logarithmic phase and then inoculated onto 22 mm 0.22 μm nitrocellulose filters under vacuum filtration. Mtb-laden filters were then placed atop chemically equivalent agar media (described above) and allowed to grow at 37°C for 5 days. Filters were then transfer into a 7H10 plates supplemented with 0.5 g/l Fraction V bovine serum albumin, 0.05% tyloxapol, 0.2% dextrose and 0.2% glycerol containing 10, 250 and 1000 mM NaCl. Bacteria were metabolically quenched by plunging Mtb-laden filters into acetonitrile/methanol/H_2O (2:2:1) pre-cooled to -40 °C and metabolites were extracted by mechanical lysing of the entire Mtb-containing solution with 0.1 mm acid-washed Zirconia beads. Lysates were clarified by centrifugation and then filtered with 0.22 μm Spin-X column filters (Costar®). Bacterial biomass of individual samples was determined by measuring the residual protein content of metabolite extracts using the BCA assays kit (Thermo®).

5.2. Nuclear magnetic resonance - metabolomics

After one Bligh and Dyer extraction[1], aqueous phases were dried under vacuum and suspend with 160μL D_2O containing the internal standard, DSS (4,4-dimethyl-4-silapentane-1-

sulfonic acid), at a final concentration of 100 µM. Samples were loaded into 3mm NMR tubes. NMR spectra were acquired with a Bruker Avance II instrument with a nominal ^{1}H frequency of 800 MHz using a CPTCI cryoprobe. The Bruker pulse program *noesygppr1d* was used with a 1 s pre-saturation pulse (50 Hz bandwidth) centered on the water resonance, 0.1 ms mixing time, and 4 s acquisition time at 25 °C. The datasets were zero-filled, Fourier-transformed, phase corrected and analyzed in Chenomx NMR Suite 7.0. Absolute metabolite concentrations were estimated by comparison with the Chenomx metabolite library, supplemented where necessary with additional spectra of pure standards obtained using identical acquisition parameters.

5.3. Liquid chromatography-mass spectrometry - metabolomics

Aqueous normal phase liquid chromatography was performed using an Agilent 1200 LC system equipped with a solvent degasser, binary pump, temperature-controlled auto-sampler (set at 4°C) and temperature-controlled column compartment (set at 20°C), containing a Cogent Diamond Hydride Type C silica column (150 mm × 2.1 mm; dead volume 315 µl), from Microsolv Technology Corporation. Flow-rate of 0.4 ml/min was used. Elution of polar metabolites was carried out using solvent A consists in deionized water (Resistivity ~ 18 MΩ cm), 0.2% acetic acid and solvent B consists in acetonitrile and 0.2% acetic acid, and the gradient as follows: 0 min 85% B; 0-2 min 85% B; 2-3 min to 80% B; 3-5 min 80% B; 5-6 min to 75% B; 6-7 min 75% B; 7-8 min to 70% B; 8-9 min 70% B; 9-10 min to 50% B; 10-11 min 50% B; 11-11.1 min to 20% B; 11.1-14 min hold 20% B. Accurate mass spectrometry was carried out using an Agilent Accurate Mass 6230 TOF apparatus. Dynamic mass axis calibration was achieved by continuous infusion, post-chromatography, of a reference mass solution using an isocratic pump connected to a Multimode ionization source, operated in the positive-ion mode. ESI capillary and fragmentor voltages were set at 3,500 V and 100 V, respectively. The nebulizer pressure was set at 40 psi and the nitrogen drying gas flow rate was set at 10 L/min. The drying gas temperature was maintained at 250°C. The MS acquisition rate was 1.5 spectra/sec and *m*/*z* data ranging from 80-1,200 were stored. This instrument routinely enabled accurate mass spectral measurements with an error of less than 5 parts-per-million (ppm), mass resolution ranging from 10,000-25,000 over the *m*/*z* range of 121-955 atomic mass units, and a 100,000-fold dynamic range with picomolar sensitivity. Data were collected in the centroid mode in the 4 GHz (extended dynamic range) mode. Detected *m*/*z* were deemed to be identified metabolites on the basis of unique accurate mass-

retention time identifiers for masses exhibiting the expected distribution of accompanying isotopomers. Typical variations in abundance for most of the metabolites stayed between 5 and 10% under these experimental conditions.

5.4. ^{13}C-Labeling Analysis

Under the experimental conditions described above using U-$^{13}C_3$ glycerol and U-$^{13}C_6$ dextrose, the extent of ^{13}C labeling for each metabolite was determined by dividing the summed peak height ion intensities of all ^{13}C labeled species by the ion intensity of both labeled and unlabeled species.

6. Lipidomics

6.1. Lipid extraction

Heat-killed mycobacteria from 50 mL 7H9 liquid medium (0.5 g/l Fraction V bovine serum albumin, 0.2% dextrose and 2% glycerol, 10 mM NaCl), with or without NaCl concentration shift, were first washed 3 times with ddH_2O. The pellets were then submitted to $CHCl_3$/MeOH 1:2 (v/v) extraction for 12h at room temperature followed by one $CHCl_3$/MeOH 1:1 (v/v) extraction and one $CHCl_3$/MeOH 2:1 (v/v) extraction for 3h at room temperature. Pools extracts were concentrated and evaporated to dryness. Total lipids extracted were normalized to the dried weight of lipid extract.

6.2. Lipid analysis by MALDI-ToF

Total lipids were suspended in chloroform-methanol 8:2 v/v, analysed by 1D thin-layer chromatography (TLC) or directly deposited onto a steel target for analysis by MALDI-TOF MS (microflex LRF, Bruker Daltonics, MA, or Ultraflex, Bruker, Bremen, Germany). Spectra were acquired in reflectron mode and mass assigned through external calibration. The 2-(4-hydroxyphenylazo)benzoic acid (HABA) or 2,5-dihydroxybenzoic acid (DHB) matrix (Sigma®) was used at a concentration of ~10 mg/ml in ethanol-water (1:1, v/v). In a typical experiment, 1 µl of total lipid extract in chloroform-methanol (8:2, v/v) and 1 µl of the matrix solution were mixed with a micropipette directly on the target.

6.3. Lipidomic analysis by Q-ToF

The mass spectrometry method and materials used here are according to the analytical platform developed by Layre et al[2]. Collision-induced dissociation mass spectrometry analysis was performed with an Agilent 6520 Accurate Mass Qtof mass spectrometer with an energy of 35 - 100 V and HPLC system with a Varian diol column (3 µm x 150 mm x 2 mm). The column was eluted at 0.15 ml/min with a binary gradient solvent A (hexane:isopropanol, 70:30 [v/v], formic acid 0.02% [m/v], ammonium hydroxide 0.01% [m/v]) and solvent B (isopropanol:methanol, 70:30 [v/v], formic acid 0.02% [m/v], ammonium hydroxide 0.01% [m/v]).

7. Antibacterials

All antibacterial were purchased from Sigma-Aldrich, except kanamycin Sulfate (Gibco 11815-024). Isoniazid (Cat. # I3377), rifampicin (Cat. # R3501), D-cycloserine (Cat. # C6880), streptomycin sulfate salt (Cat. # S6501), gentamicin (Cat. # G1272), amikacin disulfate salt (Cat. # A1774), ciprofloxacin (Cat. # 17850-5G-F), ethambutol dihydrochloride (Cat. # E4630), ethionamide (Cat. # E6005), gatifloxacin (Cat. # G7298), moxifloxacin hydrochloride (Cat. # Y0000703)).

7.1. Growth conditions

The bacteria growth occurred at 37°C until to reach the mid-log phase (OD_{600nm}=0.4-0.6) as an adaptation period. After this period, the bacterial suspensions were prepared as described below and REMA assays were performed.

7.2. REMA assay

The anti-*M. tuberculosis* activity of the compounds in different concentrations of NaCl (14.5 mM, 125 mM and 250 mM) was determined by the REMA (Resazurin Microtiter Assay) method [3]. Stock solutions of the tested compounds (10.000 µg/mL) were prepared in water (for D-cycloserine, kanamycin, streptomycin, gentamicin, amikacin, gatifloxacin, moxifloxacin and isoniazid) and dimethyl sulfoxide (for ciprofloxacin, ethionamide, rifampicin and ethambutol) and diluted in Middlebrook 7H9 broth (Difco) (containing different concentrations of NaCl, in accordance with the assay) supplemented with 10% of OADC (OADC enrichment - BBL/Becton-Dickinson, Sparks, MD, USA), to yield the concentrations of sodium chloride aforementioned. The microdilution of the drugs was performed in 96-well plates by BioTek™ Precision™ XS Microplate Sample Processor

(BioTek®) to obtain final drug concentration ranges of 0.09-25.00 µg/mL. Bacterial suspensions were prepared and their turbidities adjusted to match the optical density of Mcfarland n°1 standard. After further dilution of 1:25 in the respective Middlebrook 7H9 broth supplemented with OADC, 100µL the inoculum was added to each well of a 96-well microtiter plate (Nunc) together with the compounds. Cultures were incubated for 7 days at 37°C, and 30µL of 0.02% resazurin was added. Wells were read after 24 h for color change and measured as the fluorescence (excitation/emission of 530/590 nm filters respectively) in a SPECTRAfluor Plus (Tecan) microfluorimeter. The MIC was defined as the lowest concentration resulting in 90% inhibition of *M. tuberculosis* growth. The presented results are from two independent experiments.

8. Corona Green live staining of Mtb infected macrophages

The mouse macrophage cell line RAW 264.7 (ATCC#TIB-71) was maintained in high glucose Dulbecco's Modified Eagles Medium (DMEM) supplemented with 10% heat inactivated Fetal Calf Serum (FCS) and 2mM L-Glutamine (Gibco) without addition of antibiotics (referred to as complete DMEM) and incubated at 37°C in a 5% CO2 atmosphere. RAW264.7 macrophages were washed with Dulbecco's PBS 1X (Gibco), sub-cultured by scraping three times a week at a 1:10 T-75 vented plug seal capped tissue culture flasks (Sarstedt, Germany).

Cells were seeded at $2x10^5$ on 35-mm glass-bottom dishes (WillCo Wells, Netherlands) one day before infection and incubated or not with 5ng/ml IFN-γ for 12h. Cells were infected with a single cell suspension (OD_{600}: 0.01) of *M. tuberculosis* H37Rv-mCherry for 1h and then washed twice with PBS at 37°C and incubated in DMEM for 2h. Following this protocol, the multiplicity of infection (MOI, defined by the number of bacteria per macrophage) was between 5-10. Next cells were wshed twice and incubated with PBS containing 1uM CoronaGreen AM (Invitrogen) for 45 min, washed twice with PBS and immediately analyzed by live cell imaging.

Imaging was performed using a Leica SP5 Laser Scanning Confocal Microscope (Leica Microsystems, Germany) equipped with AOBS, AOTF and an environmental control chamber (EMBLEM, Germany) providing 37°C temperature, 5 % CO_2 and up to 50 % humidity in a closed chamber during imaging. Temperature corrected immersion oil (Cargille Labs, UK. Type 37) was used for imaging and a single focal plane of the sample was monitored in time (xyt scanning mode) using 63x/1.4 HCX-PLAPO oil objective (Leica Microsystems, Germany). The time-lapse imaging was performed at 200 or 400 Hz scanning speed, 2-3X scanning zoom, 3X line averaging with frames taken at 5 to 30 seconds intervals for up to 2 hours. Time-lapse movies contained in the Leica native (.LIF) format were exported as TIFF image sequences) for analysis.

Analysis was performed by measuring the signal intensity of the green associated to Mtb phagosomes at the single cell level ImageJ (U.S. National Institute of Health, Bethesda, Maryland, USA) Iterative versions of ImageJ used for this work are 1.41m through 1.46m. Fiji is a distribution of ImageJ available at http://fiji.sc. At least 48 phagosomes in 20 different cells from one representative experiment (out of three) were analyzed.

8. References

[1] Bligh, E. G., and Dyer, W. J. (1959) A rapid method of total lipid extraction and purification, *Canadian journal of biochemistry and physiology 37*, 911-917.

[2] Layre, E., Sweet, L., Hong, S., Madigan, C. A., Desjardins, D., Young, D. C., Cheng, T. Y., Annand, J. W., Kim, K., Shamputa, I. C., McConnell, M. J., Debono, C. A., Behar, S. M., Minnaard, A. J., Murray, M., Barry, C. E., 3rd, Matsunaga, I., and Moody, D. B. (2011) A comparative lipidomics platform for chemotaxonomic analysis of Mycobacterium tuberculosis, *Chem Biol 18*, 1537-1549.

[3] Palomino, J. C., Martin, A., Camacho, M., Guerra, H., Swings, J., and Portaels, F. (2002) Resazurin microtiter assay plate: Simple and inexpensive method for detection of drug resistance in Mycobacterium tuberculosis, *Antimicrobial Agents and Chemotherapy 46*, 2720-2722.

II. SI Appendix - Figure Legends

SI Appendix Figure S1: MIC_{90} values for anti-tubercular drugs obtained in the presence of 14.5, 125 and 250 mM NaCl with Mtb (H37Rv) and Clinical Isolates. Asterisks indicate that the values obtained are the lower limit (maximum concentration of drug present in the assay) and not true values. Data are the average of three independent experiments.

SI Appendix Figure S2: Effect of various NaCl concentrations on Mtb viability (CFU/mL) (A), M. *bovis* BCG (B) and *M. smegmatis* mc^{2}155 (C). Bacteria were cultivated to mid-log phase OD_{600} 0.5-0.6 and exposed to 10, 250 and 1,000 mM NaCl. Viable bacteria were determine by determination of the CFU/mL.

SI Appendix Figure S3: Acid-fast staining of Mtb H37Rv exposed to 10, 125, 250 and 1,000 mM NaCl, for 5 days.

SI Appendix Figure S4: ^{1}H-NMR stack plots illustrating time course metabolic responses accompanying halotolerance at 10, 250 and 1,000 mM NaCl (*, glucose; Δ, trehalose; ◊, glycerol; □□, citrulline).

SI Appendix Figure S5: Pool size of citrulline biosynthetic intermediates. a, Pool size of *N*-acetylglutamate and ornithine upon shift to 1,000 mM NaCl for Mtb H37Rv grown on acetate (light blue), dextrose (purple), glycerol (green), dextrose and glycerol (red) and dextrose and glycerol at 10 mM NaCl (dark blue). **b,** Pool size of ornithine and citrulline in *M. smegmatis* mc^{2}155 and *M. bovis* BCG Pasteur upon NaCl shift at 10mM (blue) and 1,000 mM NaCl (red). Y-axis represents the ion abundance normalized to the amount of residual protein in the extracts.

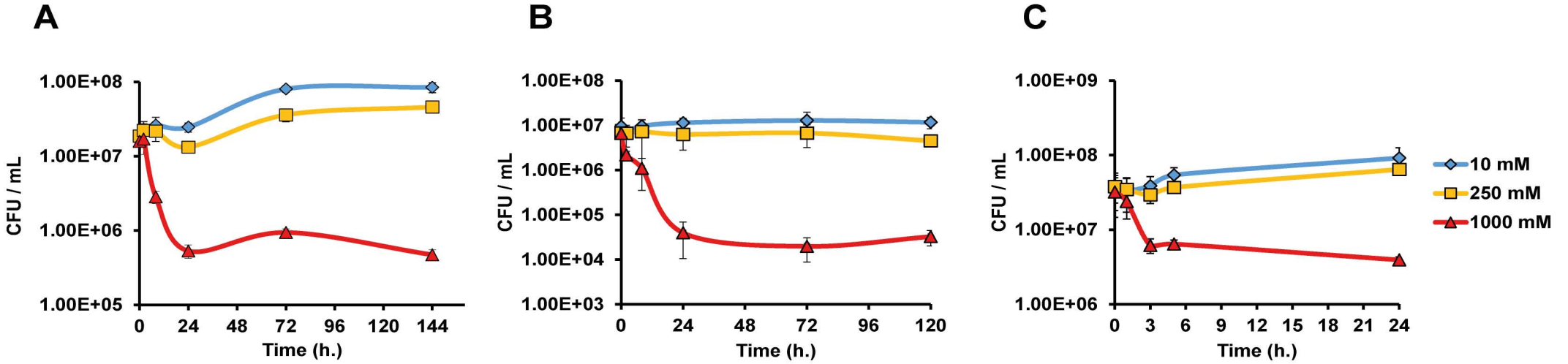
SI Appendix Figure S6: Lipid changes upon NaCl shift. a,. Extracted ion chromatogram (EIC) and MS/MS spectrum of Ac_2PIM_2 $[M-H]^-$ 1694.1774 **b,** Extracted ion chromatogram (EIC) and MS/MS spectrum of Ac_2PIM_6 $[M-H]^-$ 2342.3899. **c,** EIC and MS/MS spectrum of phosphatidylethanolamine $[M-H]^-$ 732.5548. **d,** EIC and MS/MS spectrum of phosphatidylglycerol $[M-H]^-$ 717.4654. **e,** EIC and MS/MS spectrum of tretraacylated sulfoglycolipid $[M-H]^-$ 2478.1683. **f,** EIC and MS spectrum of free fatty acids $[M-H]^-$ 379.3581. **g,** Structure of Ac_xPIM_y, where x represents the number of fatty acids present on the mannose units and y the number of mannose units. **h**, Thin layer chromatography of total lipids from Mtb H37Rv from 0 to 96h NaCl shift to 250 mM NaCl, solvents system 60:25:4 $CHCl_3/CH_3OH/H_2O$ v/v/v and developed by phosphomolybdic acid 5% in ethanol followed by heat 10 min at 100°C. **i**, Thin layer chromatography of total lipids from Mtb H37Rv from 0 to 96h NaCl shift to 1,000 mM NaCl, solvents system 60:25:4 $CHCl_3/CH_3OH/H_2O$ v/v/v and developed by phosphomolybdic acid 5% in ethanol followed by heat 10 min at 100°C. **j**, Bar graph showing the percentage of Ac_xPIM_2 and Ac_xPIM_6 upon NaCl shift at 250 mM NaCl. **k**, Bar graph showing the percentage of Ac_xPIM_2 and Ac_xPIM_6 upon NaCl shift at 1,000 mM NaCl. **l**, MALDI-ToF spectra of Ac_xPIM_y in negative ion mode at 10mM NaCl from 0h to 96h. **m**, MALDI-ToF spectra of AcxPIMy in negative ion mode at 1,000mM NaCl from 0h to 96h. **n**, EIC and MS/MS spectrum of ornithine lipid $[M+H]^+$ 695.6296. **o,** EIC and MS spectrum of menaquinone $[M+H]^+$ 785.6231. **p,** EIC and MS/MS spectrum of mannosylphosphomycoketide $[M-H]^-$ 707.5248. **q,** EIC and MS/MS spectrum of mycobactin $[M-2H+Fe]^{3+}$ 923.4700.

SI Appendix Figure S7: Metabolomic data showing time-dependent pool size and labelling profiles of phosphatidylethanolamine (PE) and phosphatidylinositol (PI) polar heads at 10, 250 and 1,000 mM NaCl, over time.

SI Appendix Table 1: Labelling analysis of metabolites upon NaCl shift

III. SI Appendix - Figures

Strain	[NaCl] (mM)	Average MIC_{90} (μg/mL)							
		ETH	KAN	STR	GEN	AMI	CIP	GAT	MOX
H37Rv	14.5	1.6	3.0	1.0	3.1	1.5	0.8	0.1	0.1
	125	4.7	11.9	6.2	9.5	6.2	1.5	0.2	0.2
	250	18.7	24.7	>25.0	>25.0	12.5	2.7	0.2	0.2
CF46*	14.5	6.2	1.6	3.1	1.6	0.8	0.4	0.1	0.1
	125	6.2	4.7	25	6.25	3.1	1.2	0.2	0.2
	250	12.5	12.5	>25	12.5	12.5	1.2	0.4	0.4
CF81*	14.5	1.6	3.1	12.5	3.1	1.6	0.4	0.1	0.1
	125	6.2	4.7	>25	4.7	1.6	1.9	0.3	0.3
	250	25	18.7	>25	>25	18.7	6.2	1.2	1.6
CF85*	14.5	2.3	9.4	1.6	3.1	3.1	1.2	0.1	0.1
	125	3.1	6.2	3.1	6.2	3.1	1.2	0.2	0.2
	250	18.7	12.5	12.5	12.5	12.5	2.3	1.0	0.4
CF102*	14.5	6.2	3.1	0.8	3.1	1.6	0.8	0.1	0.1
	125	6.2	6.2	3.1	6.2	4.7	0.8	0.1	0.1
	250	12.5	12.5	6.2	12.5	6.2	0.8	0.2	0.1
CF158*	14.5	N.D.	3.0	0.3	1.5	0.8	1.5	N.D.	0.2
	125	N.D.	5.0	2.2	5.2	2.5	1.6	N.D.	0.3
	250	N.D.	21.0	22.8	22.9	21.0	8.1	N.D.	1.0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72
96
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Time (h.)
B
1.00E+08
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
CFU / mL
0
24
48
72
96
120
Time (h.)
C
1.00E+09
1.00E+08
1.00E+07
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9
12
15
18
21
24
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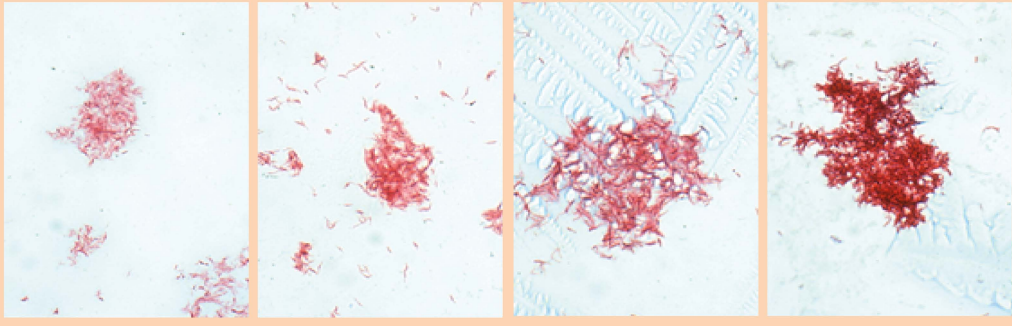
1000 mM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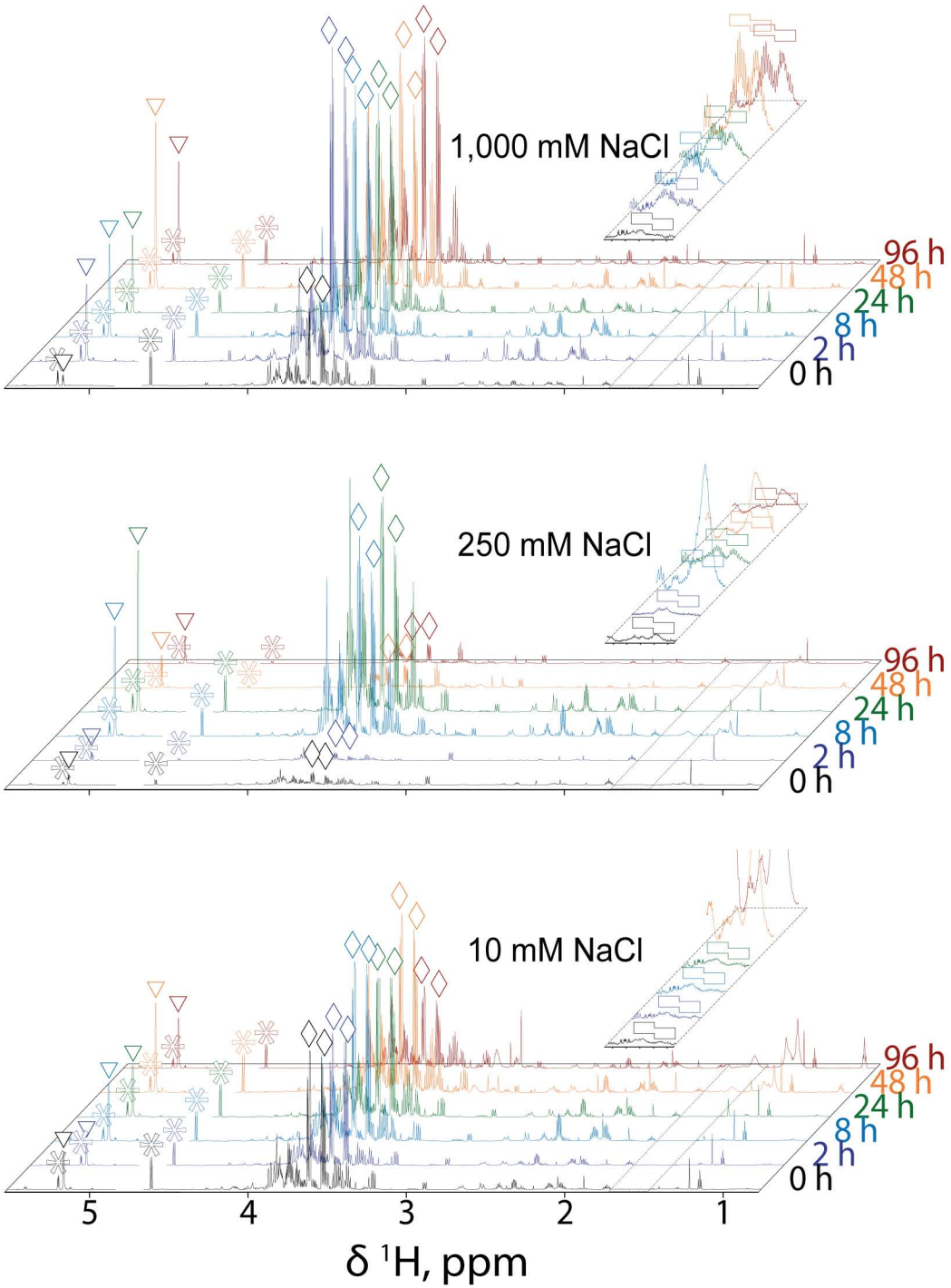
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48 h
24 h
8 h
2 h
0 h
250 mM NaCl
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24 h
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48 h
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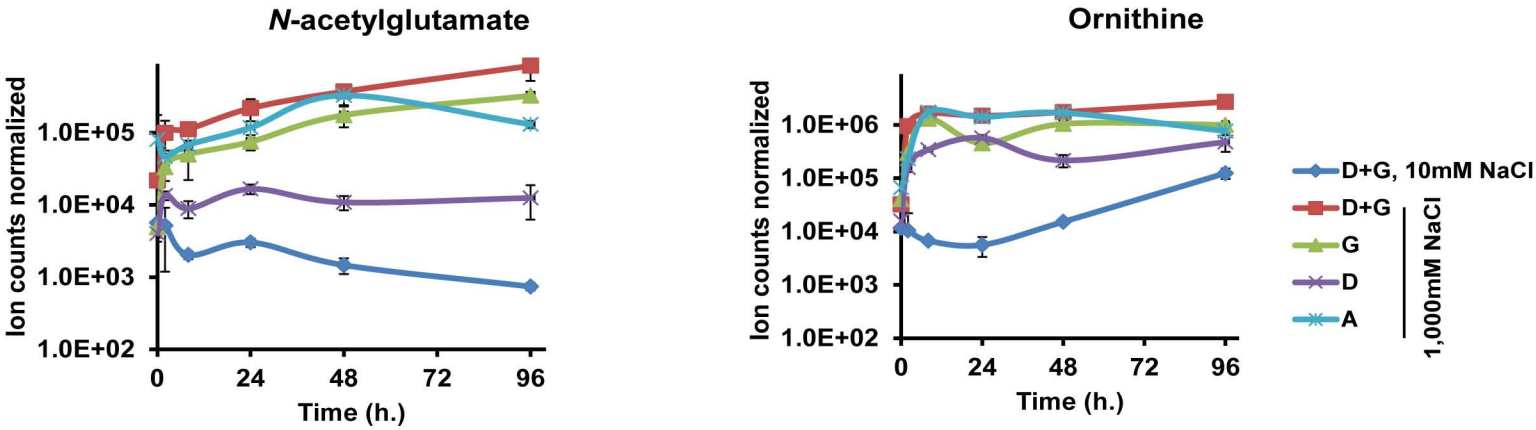
2 h
0 h
5
4
3
2
1
δ ¹H, ppm

A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24
48
72
96
Time (h.)
Ornithine
Ion counts normalized
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1.0E+05
1.0E+04
1.0E+03
1.0E+02
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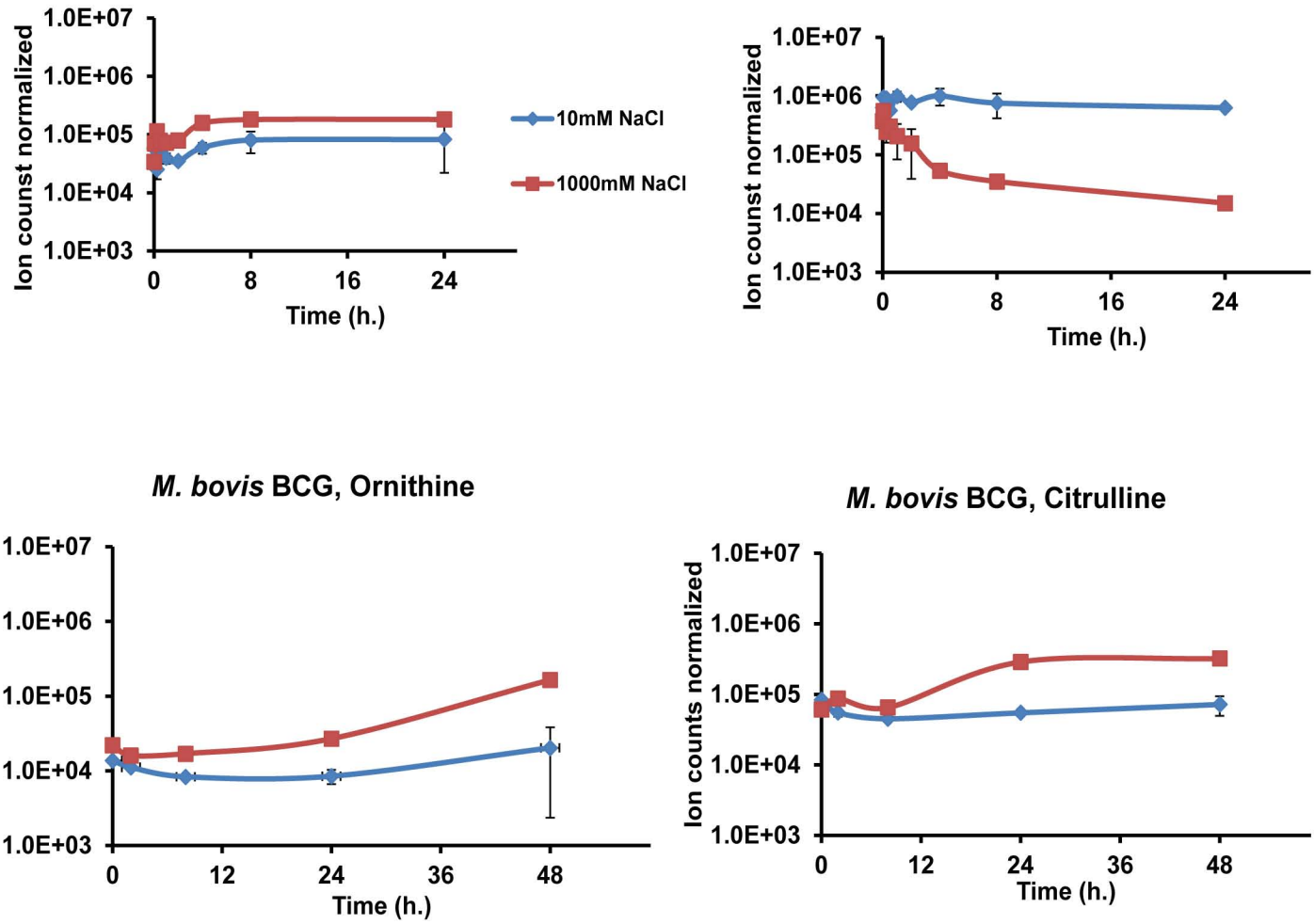
24
48
72
96
Time (h.)
D+G, 10mM NaCl
D+G
G
D
A
1,000mM NaCl

B


M. smegmatis, Ornithine
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8
16
24
Time (h.)
10mM NaCl
1000mM NaCl
M. smegmatis, Citrulline
Ion counst normalized
1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
0
8
16
24
Time (h.)
M. bovis BCG, Ornithine
Ion counts normalized
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1.0E+06
1.0E+05
1.0E+04
1.0E+03
0
12
24
36
48
Time (h.)
M. bovis BCG, Citrulline
Ion counts normalized
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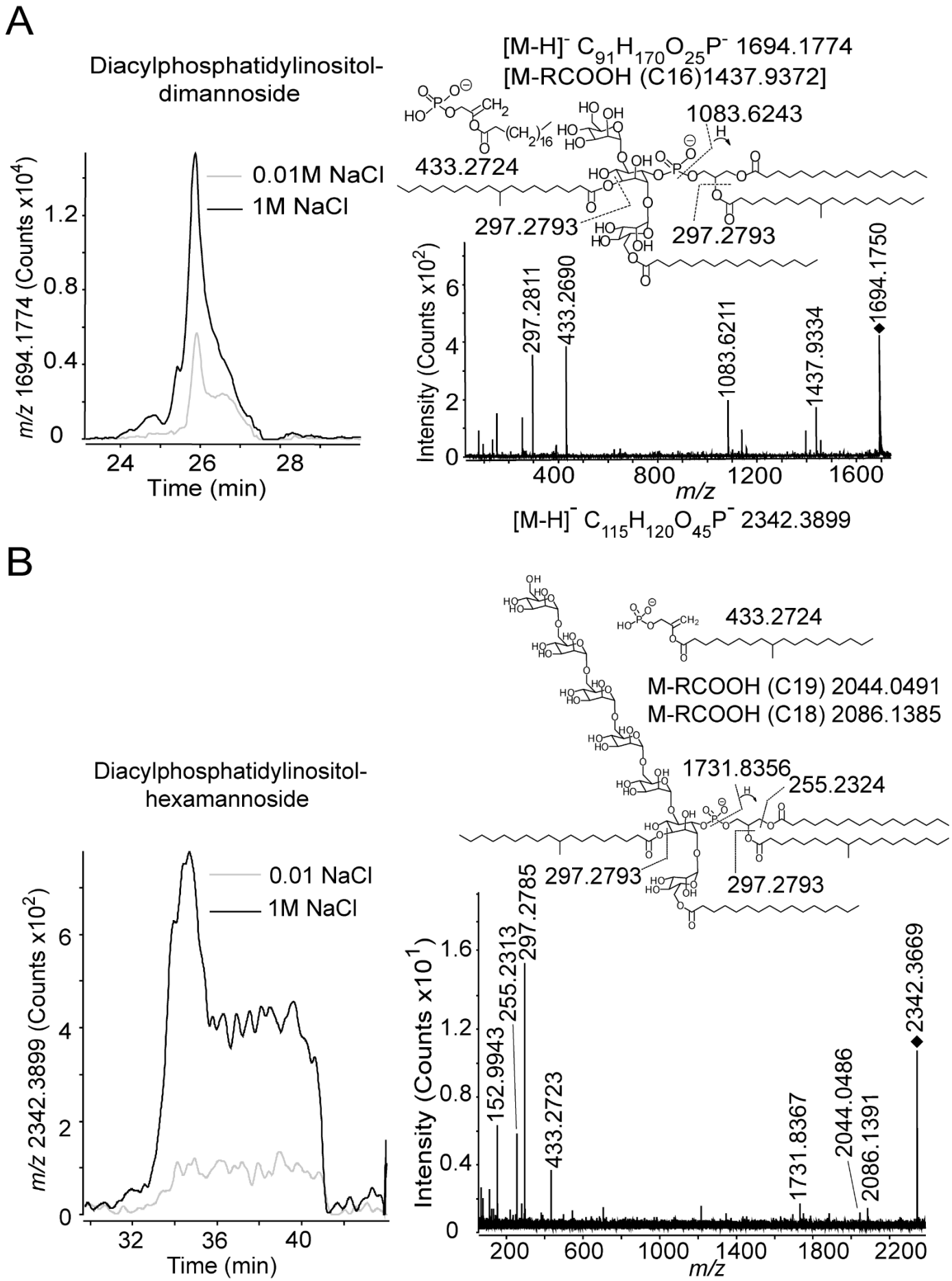
0
12
24
36
48
Time (h.)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Diacylphosphatidylinositol-dimannoside
0.01M NaCl
1M NaCl
m/z 1694.1774 (Counts x10^4)
Time (min)
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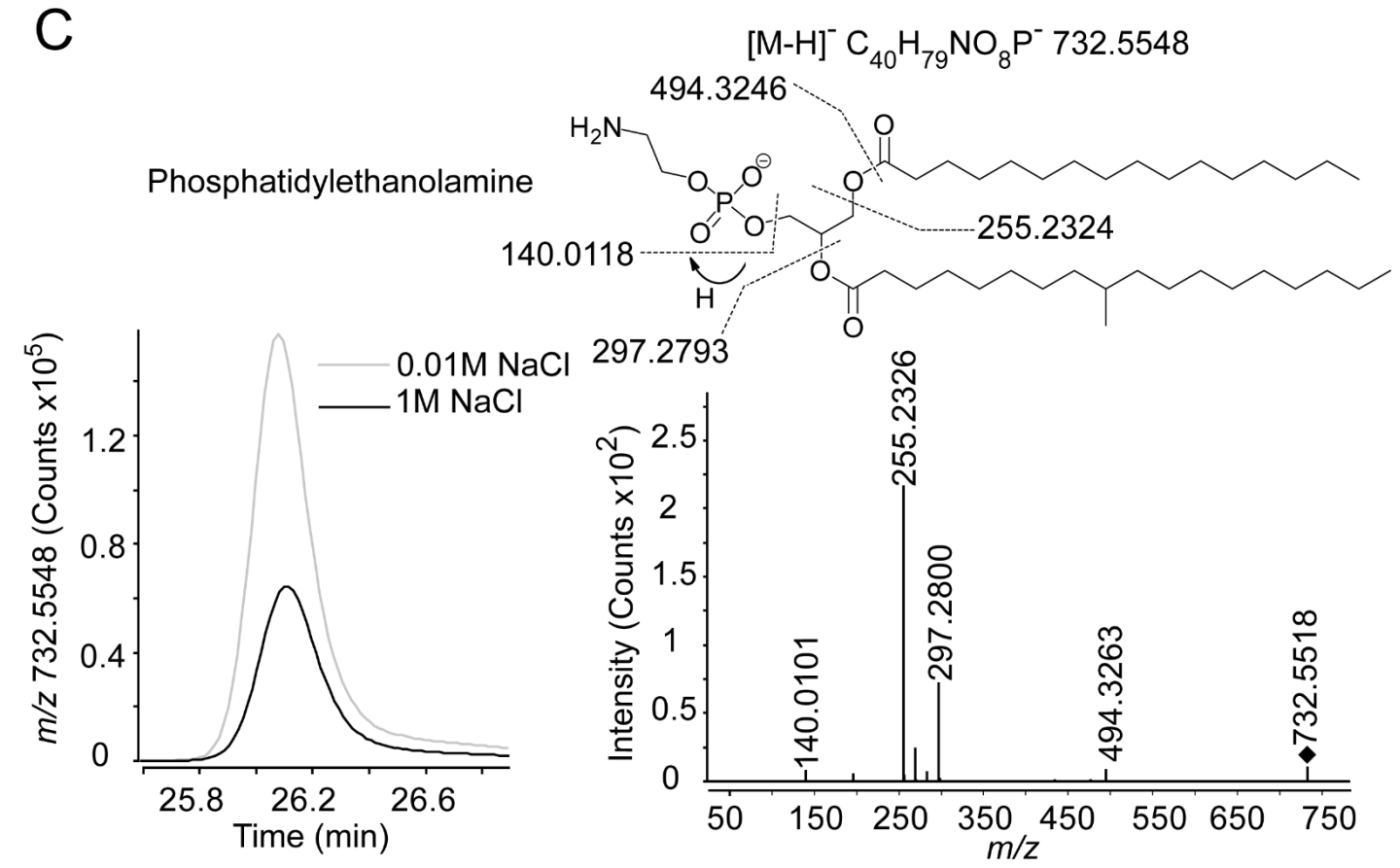
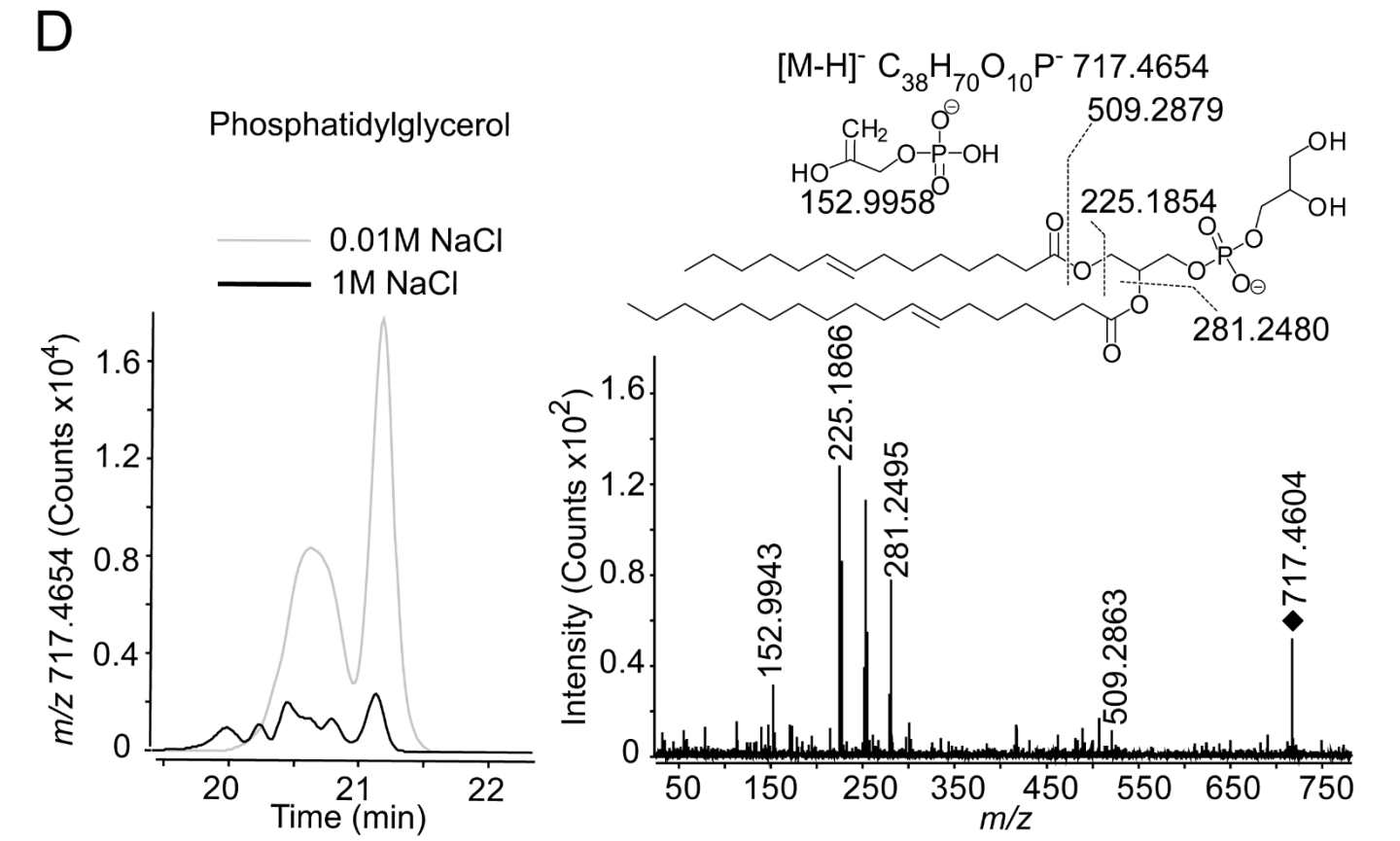
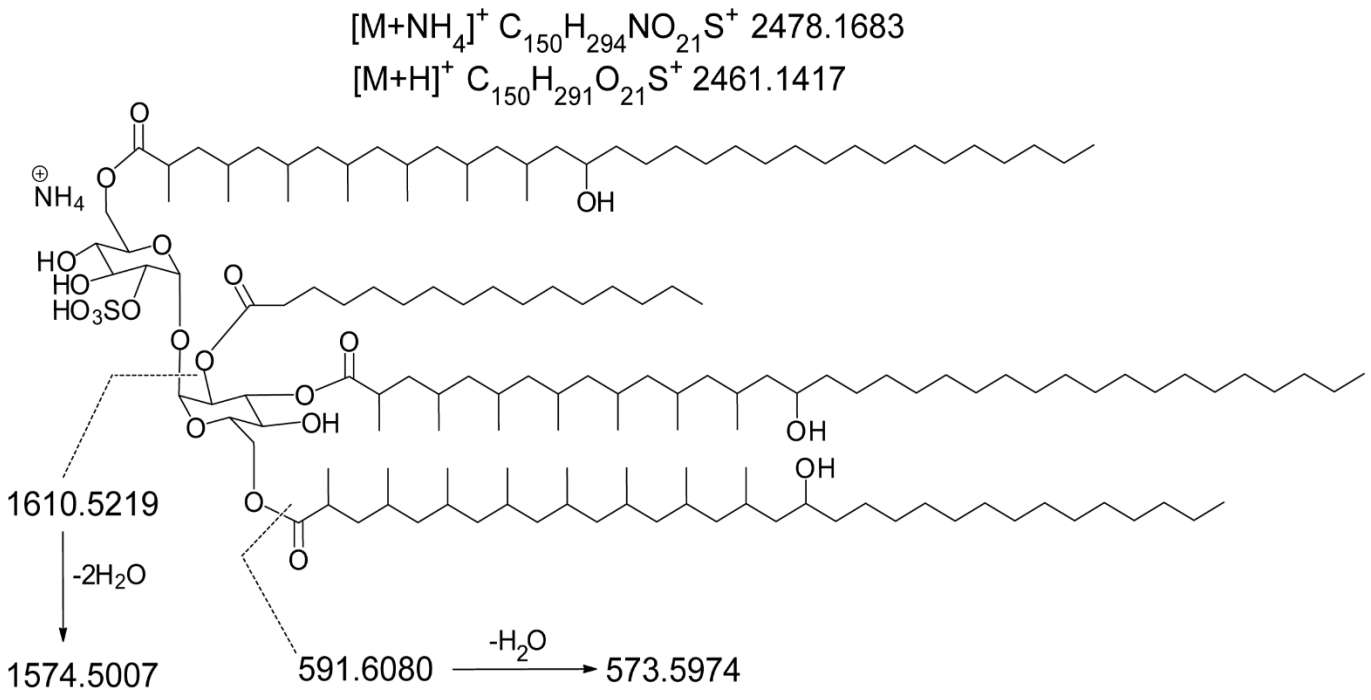
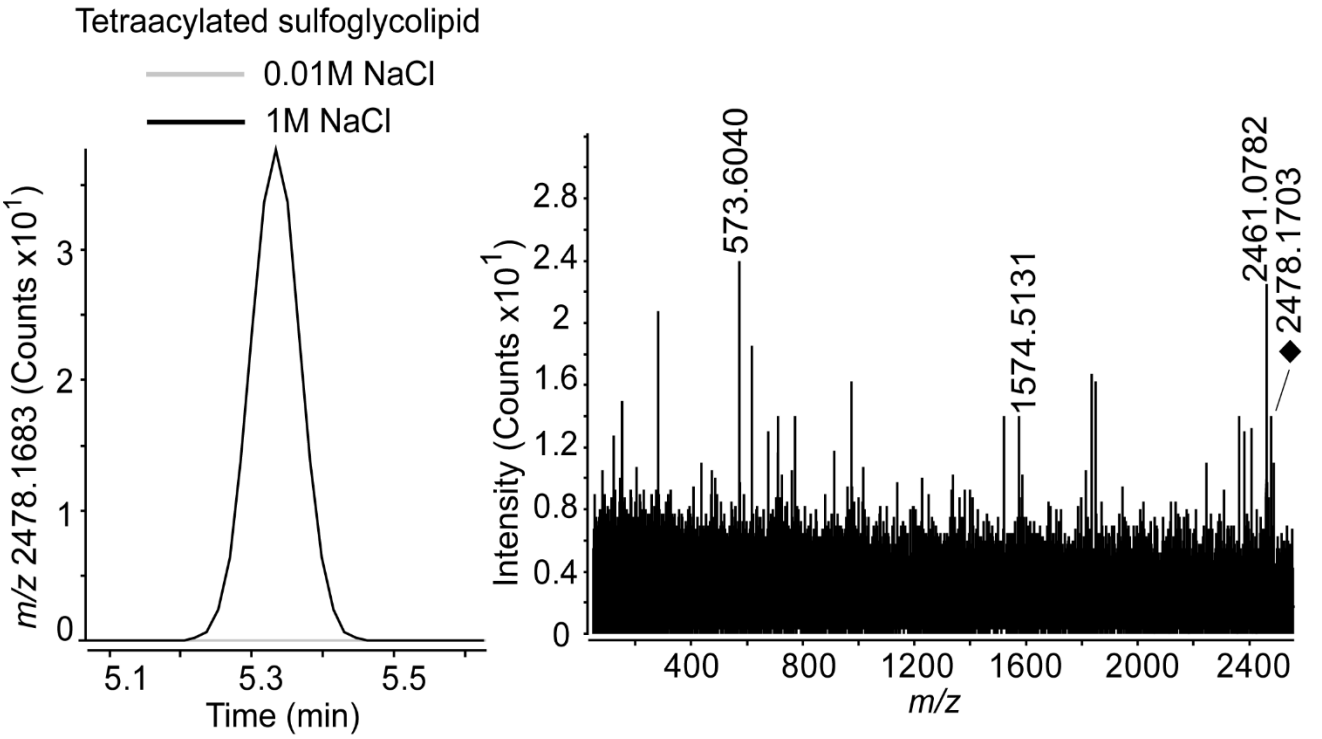
433.2724
297.2793
297.2793
Intensity (Counts x10^2)
297.2811
433.2690
1083.6211
1437.9334
1694.1750
m/z
[M-H]- C115H120O45P- 2342.3899
B
Diacylphosphatidylinositol-hexamannoside
0.01 NaCl
1M NaCl
m/z 2342.3899 (Counts x10^2)
Time (min)
433.2724
M-RCOOH (C19) 2044.0491
M-RCOOH (C18) 2086.1385
1731.8356
255.2324
297.2793
297.2793
Intensity (Counts x10^1)
152.9943
255.2313
297.2785
433.2723
1731.8367
2044.0486
2086.1391
2342.3669
m/z

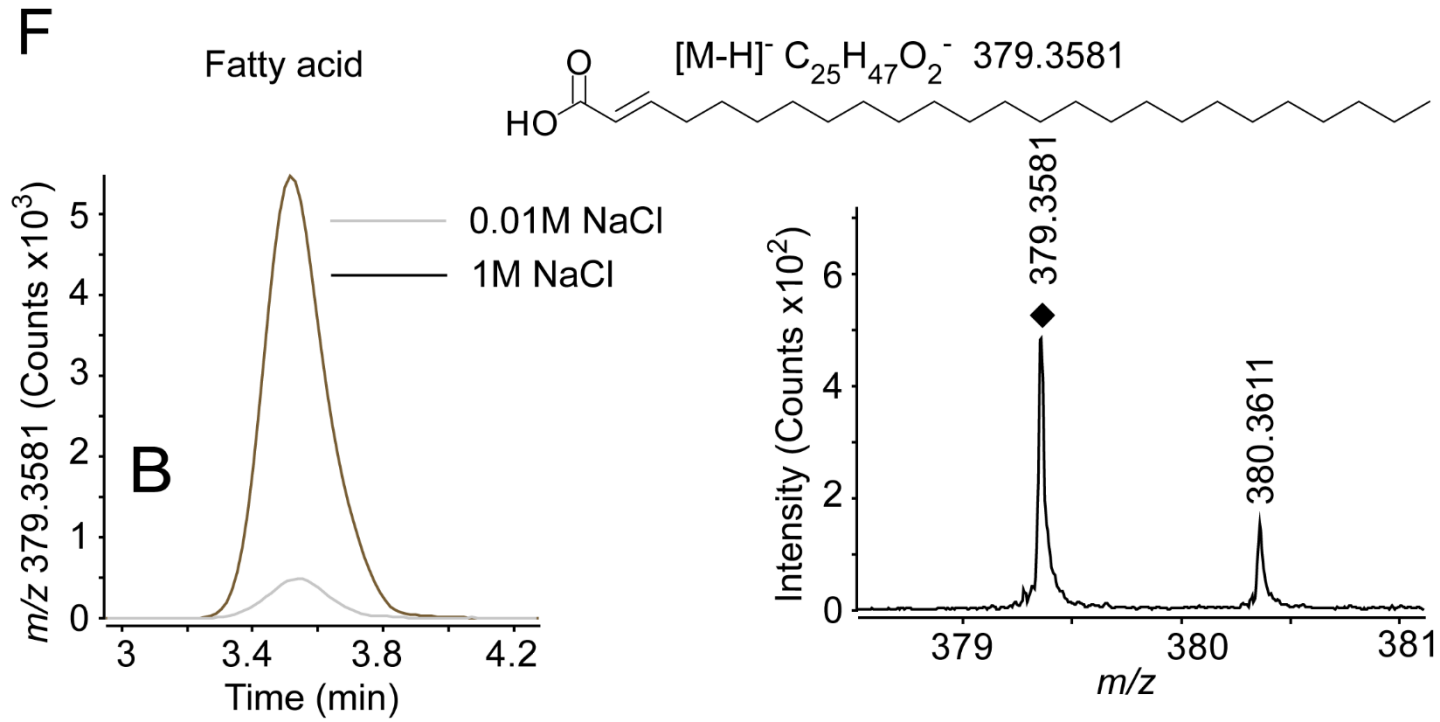

C
[M-H]⁻ C40H79NO8P⁻ 732.5548
494.3246
Phosphatidylethanolamine
255.2324
140.0118
297.2793
0.01M NaCl
1M NaCl
m/z 732.5548 (Counts x10⁵)
Time (min)
Intensity (Counts x10²)
255.2326
297.2800
140.0101
494.3263
732.5518
m/z

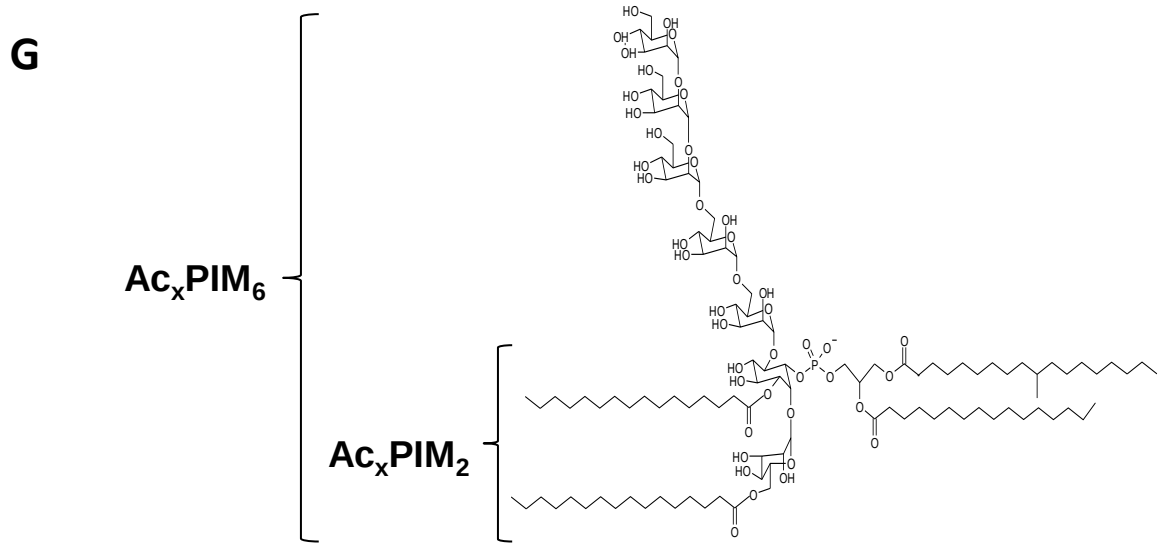

D
[M-H]⁻ C38H70O10P⁻ 717.4654
Phosphatidylglycerol
509.2879
152.9958
225.1854
281.2480
0.01M NaCl
1M NaCl
m/z 717.4654 (Counts x10⁴)
Time (min)
Intensity (Counts x10²)
225.1866
281.2495
152.9943
509.2863
717.4604
m/z

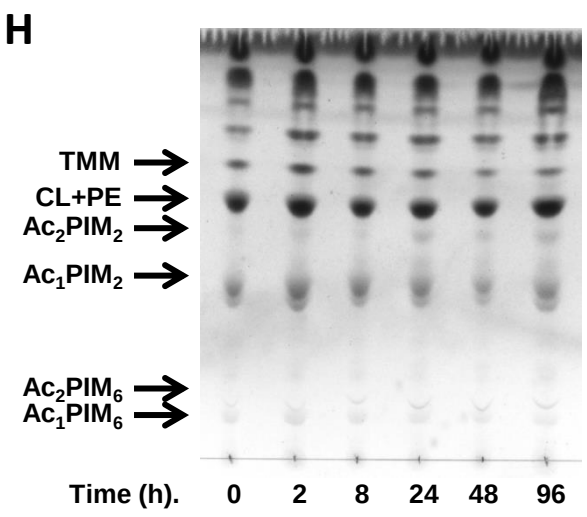
E

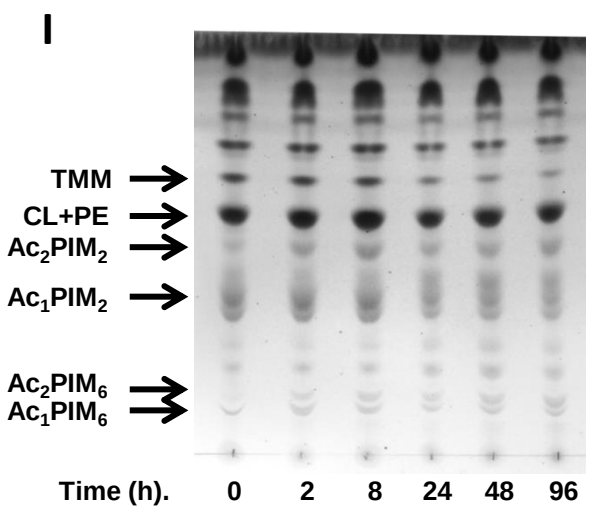

[M+NH4]+ C150H294NO21S+ 2478.1683
[M+H]+ C150H291O21S+ 2461.1417
1610.5219
-2H2O
1574.5007
591.6080
-H2O
573.5974

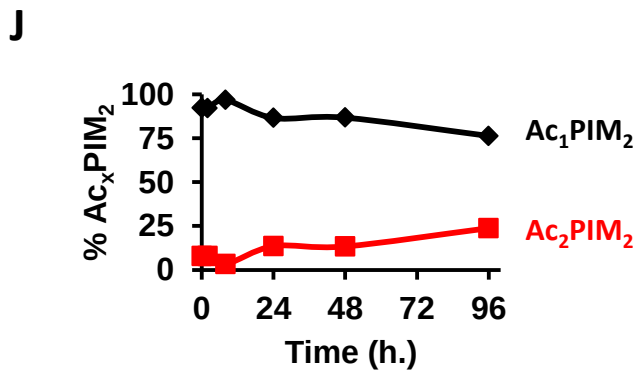

Tetraacylated sulfoglycolipid
0.01M NaCl
1M NaCl
m/z 2478.1683 (Counts x10-1)
Time (min)
Intensity (Counts x10-1)
573.6040
1574.5131
2461.0782
2478.1703
m/z

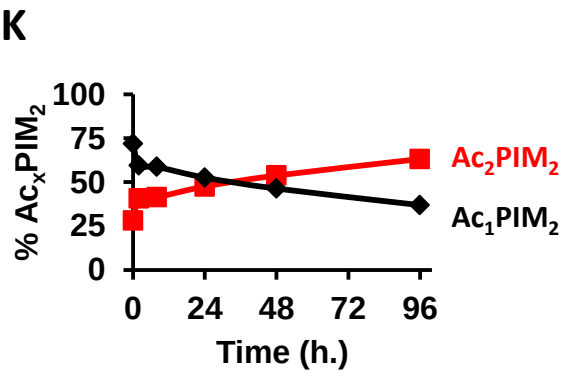

F
Fatty acid
O
HO
[M-H]- C25H47O2- 379.3581
B
0.01M NaCl
1M NaCl
m/z 379.3581 (Counts x10^3)
Time (min)
Intensity (Counts x10^2)
379.3581
380.3611
m/z

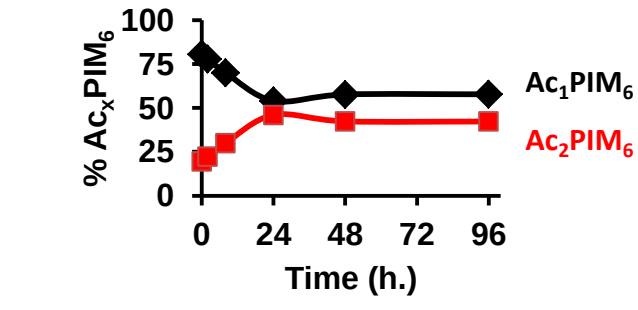

G
Ac$_x$PIM$_6$
Ac$_x$PIM$_2$

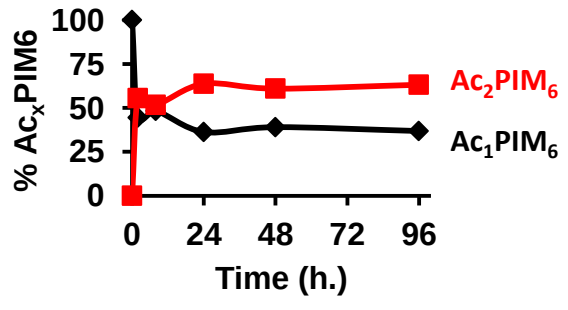

H
TMM
CL+PE
Ac$_2$PIM$_2$
Ac$_1$PIM$_2$
Ac$_2$PIM$_6$
Ac$_1$PIM$_6$
Time (h). 0 2 8 24 48 96

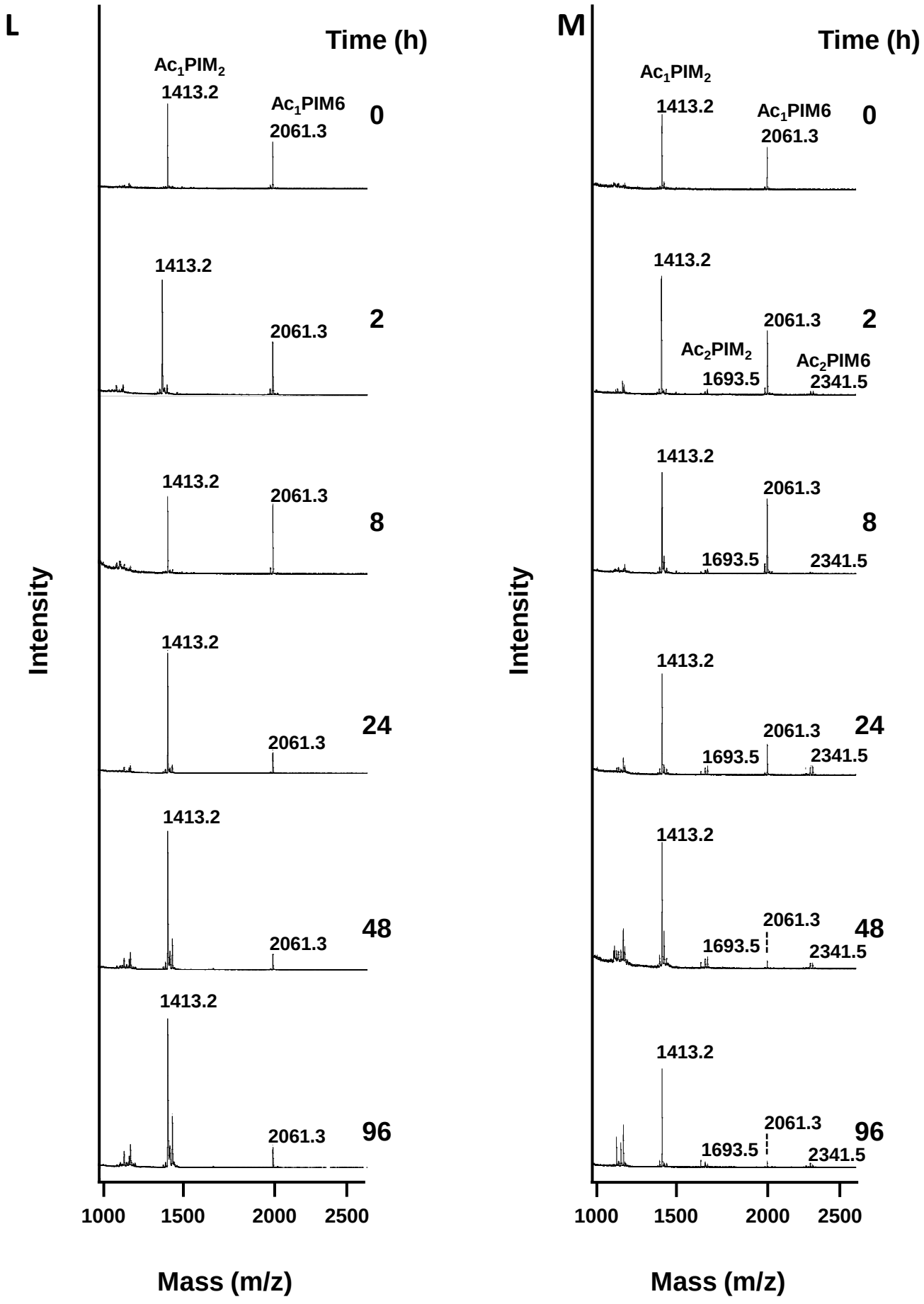

I
TMM
CL+PE
Ac$_2$PIM$_2$
Ac$_1$PIM$_2$
Ac$_2$PIM$_6$
Ac$_1$PIM$_6$
Time (h). 0 2 8 24 48 96


J
% Ac$_x$PIM$_2$
100
75
50
25
0
0 24 48 72 96
Time (h.)
Ac$_1$PIM$_2$
Ac$_2$PIM$_2$

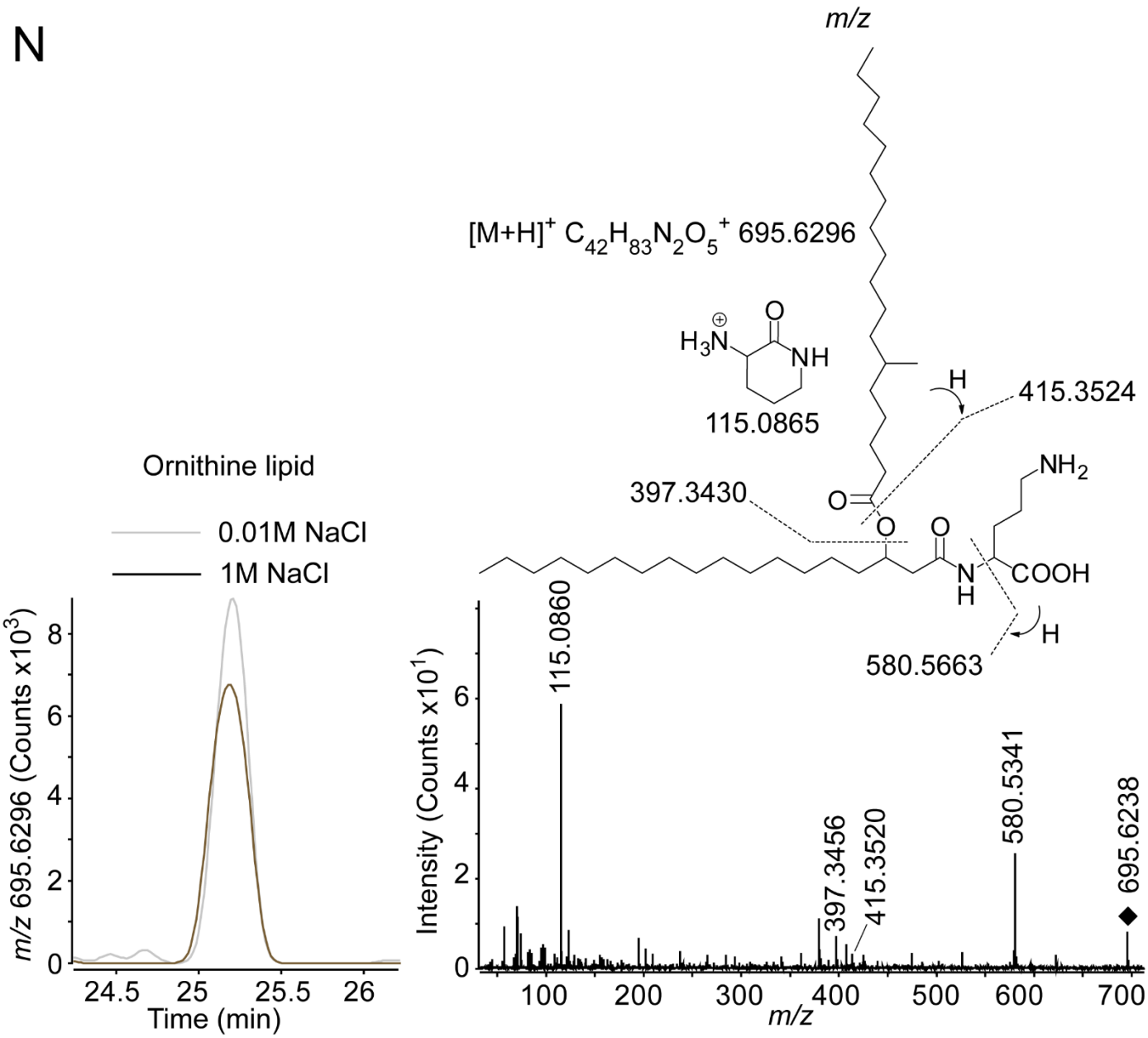

K
% Ac$_x$PIM$_2$
100
75
50
25
0
0 24 48 72 96
Time (h.)
Ac$_2$PIM$_2$
Ac$_1$PIM$_2$

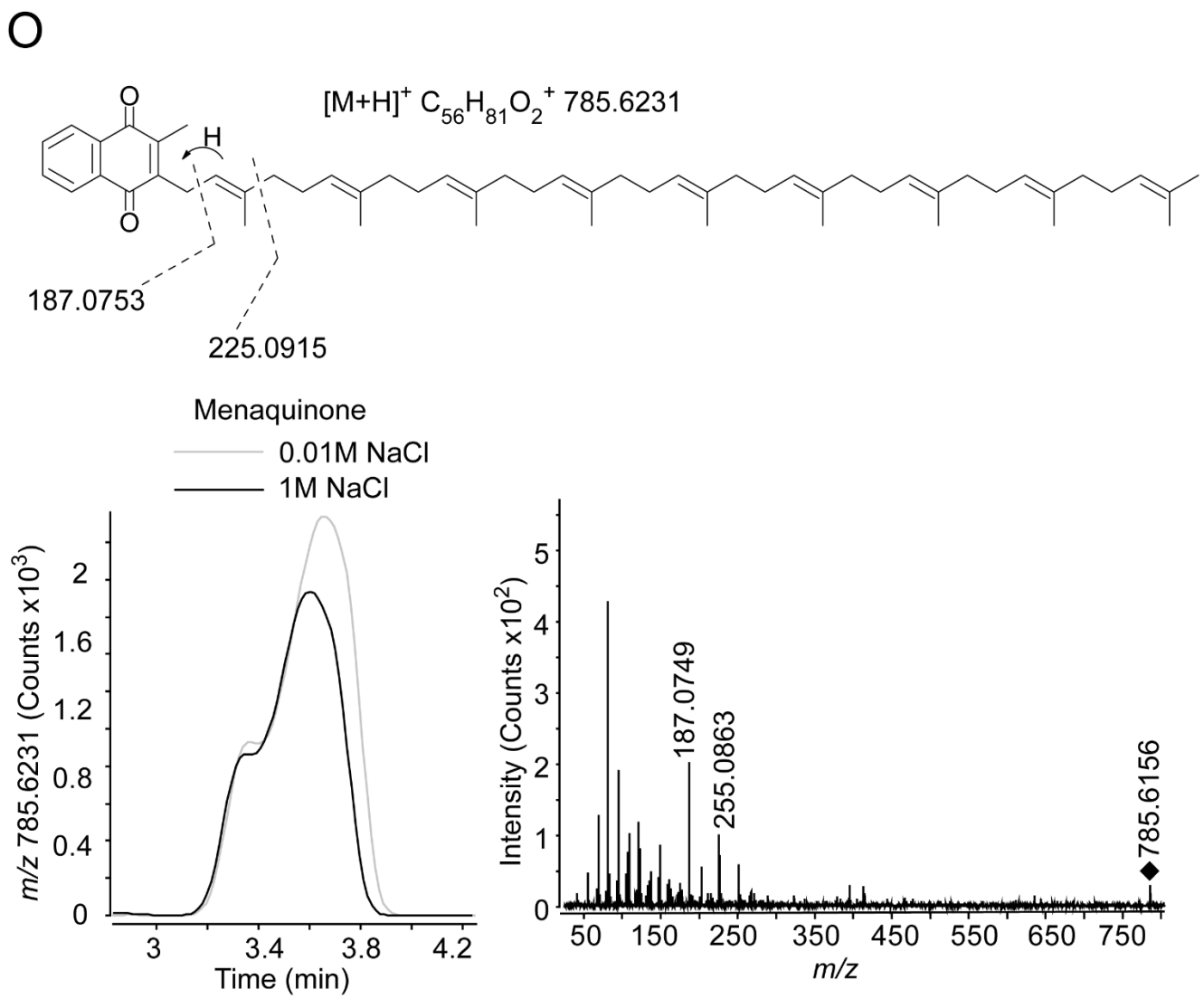

% Ac$_x$PIM$_6$
100
75
50
25
0
0 24 48 72 96
Time (h.)
Ac$_1$PIM$_6$
Ac$_2$PIM$_6$


% Ac$_x$PIM6
100
75
50
25
0
0 24 48 72 96
Time (h.)
Ac$_2$PIM$_6$
Ac$_1$PIM$_6$

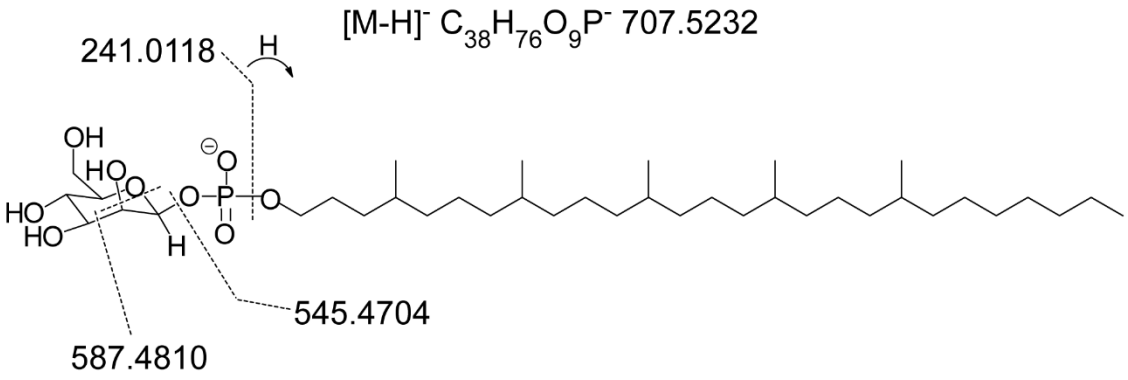

L
Time (h)
Ac1PIM2
1413.2
Ac1PIM6
2061.3
0
1413.2
2061.3
2
1413.2
2061.3
8
1413.2
2061.3
24
1413.2
2061.3
48
1413.2
2061.3
96
Intensity
1000
1500
2000
2500
Mass (m/z)
M
Time (h)
Ac1PIM2
1413.2
Ac1PIM6
2061.3
0
1413.2
2061.3
Ac2PIM2
1693.5
Ac2PIM6
2341.5
2
1413.2
2061.3
1693.5
2341.5
8
1413.2
2061.3
1693.5
2341.5
24
1413.2
2061.3
1693.5
2341.5
48
1413.2
2061.3
1693.5
2341.5
96
Intensity
1000
1500
2000
2500
Mass (m/z)

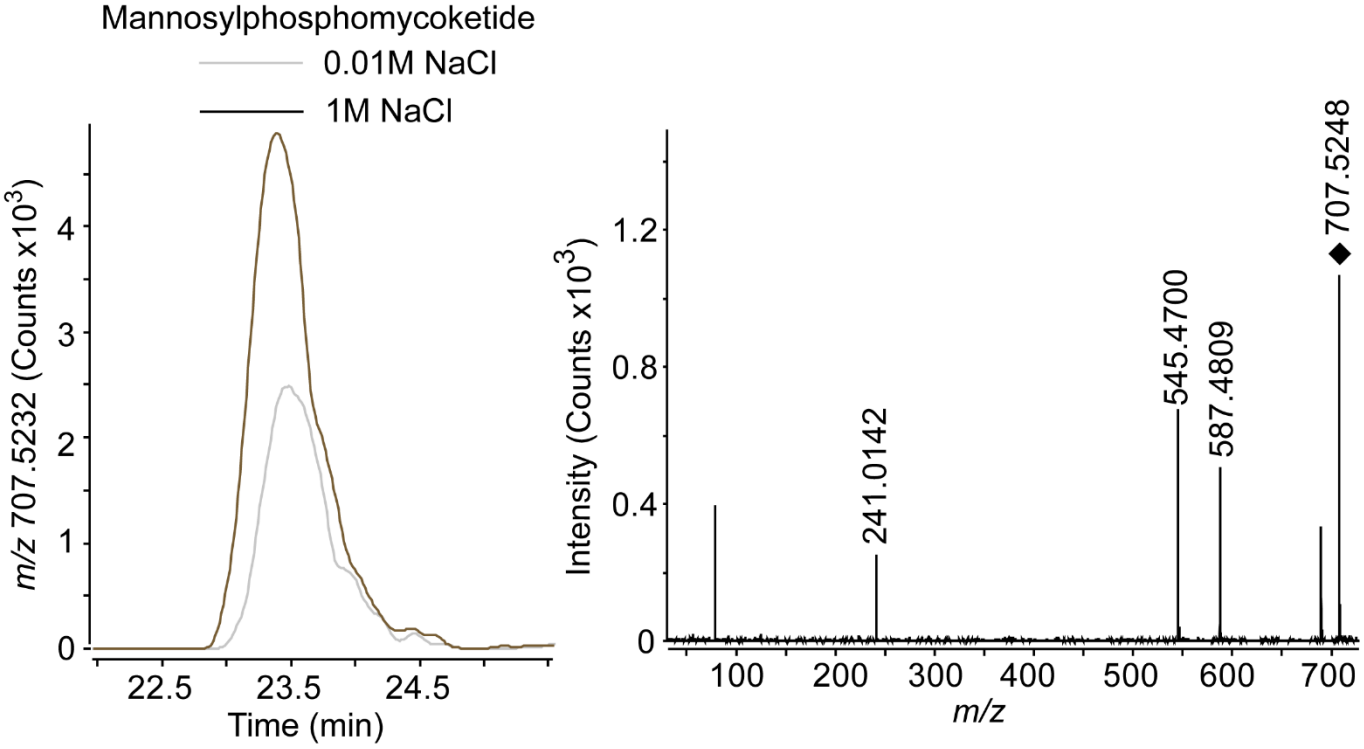
N


m/z
[M+H]+ C42H83N2O5+ 695.6296
H3N
O
NH
115.0865
H
415.3524
397.3430
NH2
O
O
O
N
H
COOH
580.5663
H
Ornithine lipid
0.01M NaCl
1M NaCl
m/z 695.6296 (Counts x10^3)
Time (min)
Intensity (Counts x10^1)
115.0860
397.3456
415.3520
580.5341
695.6238
m/z

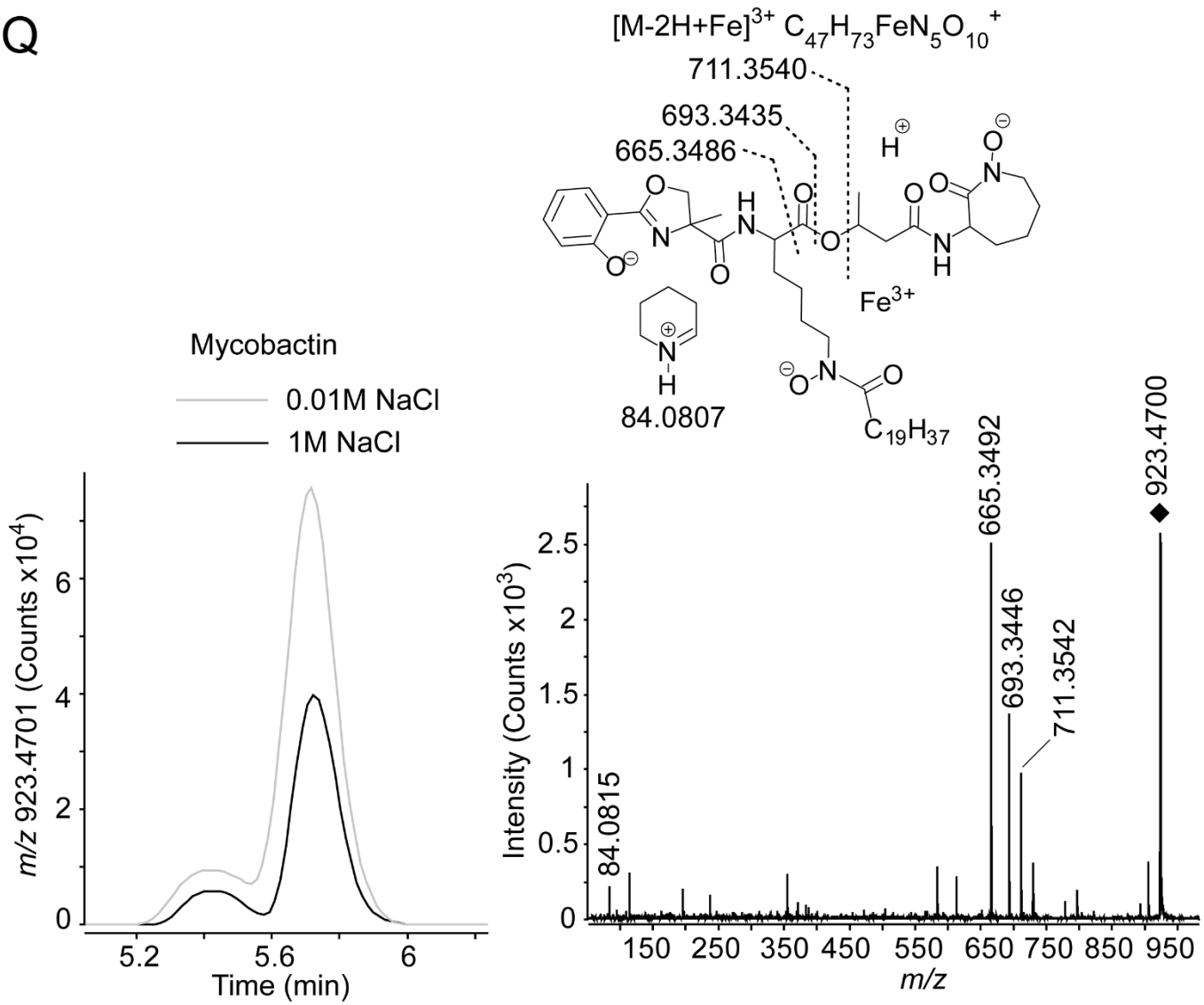

O
O
H
O
[M+H]+ C56H81O2+ 785.6231
187.0753
225.0915
Menaquinone
0.01M NaCl
1M NaCl
m/z 785.6231 (Counts x10^3)
2
1.6
1.2
0.8
0.4
0
3
3.4
3.8
4.2
Time (min)
Intensity (Counts x10^2)
5
4
3
2
1
0
187.0749
255.0863
785.6156
50
150
250
350
450
550
650
750
m/z

P


[M-H]- C38H76O9P- 707.5232
241.0118
H
OH
HO
HO
HO
O
O
O
P
O
O
O
H
545.4704
587.4810


Mannosylphosphomycoketide
0.01M NaCl
1M NaCl
m/z 707.5232 (Counts x10^3)
4
3
2
1
0
22.5
23.5
24.5
Time (min)
Intensity (Counts x10^3)
1.2
0.8
0.4
0
241.0142
545.4700
587.4809
707.5248
100
200
300
400
500
600
700
m/z

Q


[M-2H+Fe]3+ C47H73FeN5O10+
711.3540
693.3435
665.3486
Fe3+
84.0807
C19H37
Mycobactin
0.01M NaCl
1M NaCl
m/z 923.4701 (Counts x10^4)
Time (min)
Intensity (Counts x10^3)
665.3492
693.3446
711.3542
923.4700
84.0815
m/z

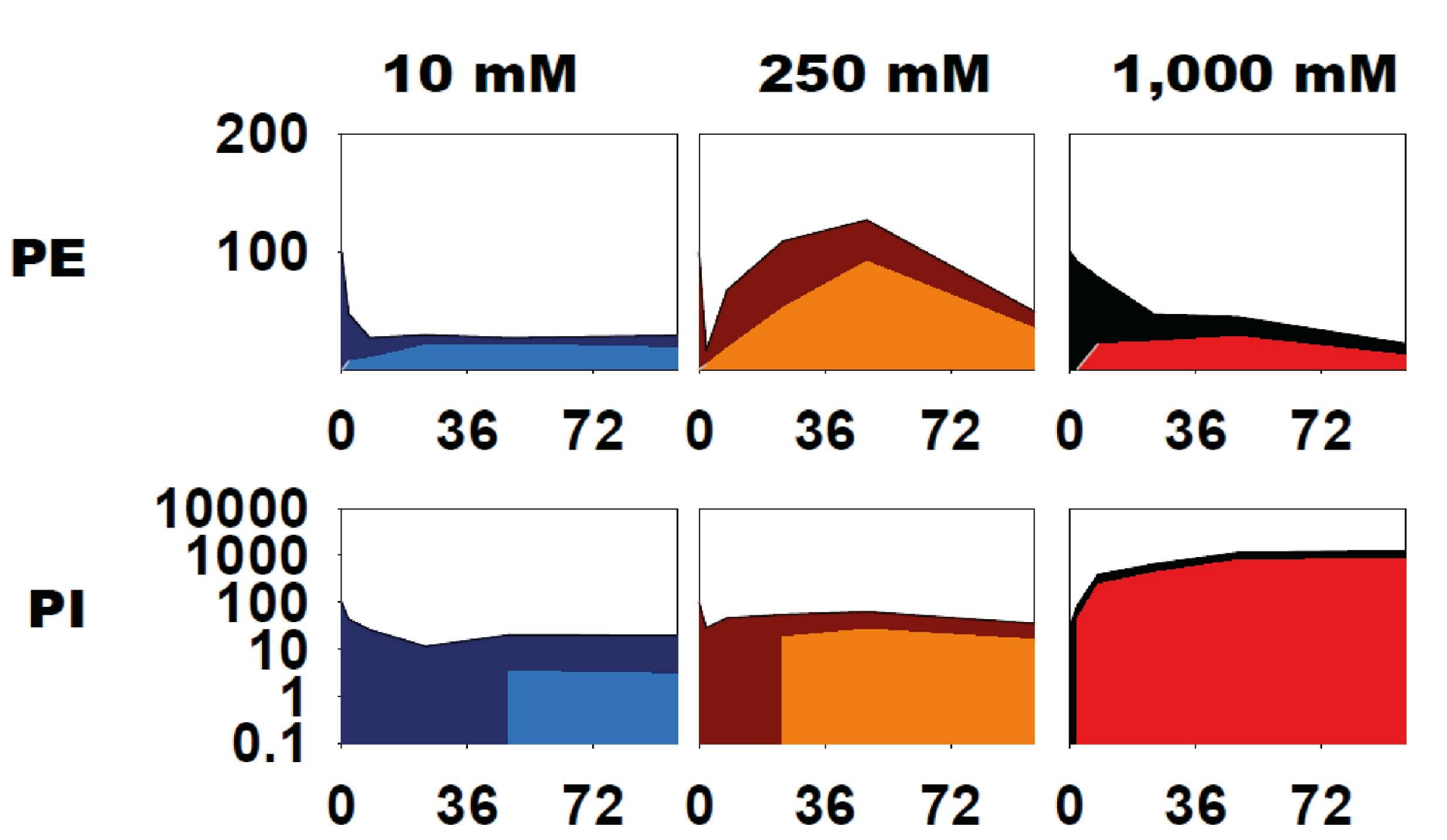

10 mM
250 mM
1,000 mM
PE
200
100
0
36
72
PI
10000
1000
100
10
1
0.1

Table S1: Metabolites analyzed by targeted metabolomics.

Metabolites	Empirical formula	Retention time (min.)	Theoritical m/z positive ion mode	Theoritical m/z negative ion mode	Experimental m/z positive ion mode	Experimental m/z negative ion mode	Error (ppm)
Hexose-phosphate	$C_6H_{13}O_9P$	6.0-14.1	261.0370	259.0224	261.0384	-	5.363
Glycerol 3-phosphate	$C_3H_9O_6P$	6.0-14.0	173.0210	171.0064	173.0216	-	3.468
Alanine	$C_3H_7NO_2$	10.2-11.4	90.0550	88.0404	90.0555	-	5.552
Aspartic acid	$C_4H_7NO_4$	8.2-10.6	134.0448	132.0302	134.0452	-	2.984
(Iso)citrate	$C_6H_8O_7$	0.5-1.1	193.0343	191.0197	-	191.0187	5.180
α-Ketoglutarate	$C_5H_6O_5$	1.1-1.8	147.0288	145.0142	-	145.0139	2.040
Succinate	$C_4H_6O_4$	1.6-2.0	119.0339	117.0193	-	117.0192	0.840
Malate	$C_4H_6O_5$	1.7-3.0	135.0288	133.0142	-	133.0146	2.962
Glutamate	$C_5H_9NO_4$	8.6-10.6	148.0604	146.0459	148.0606	-	1.351
***N*-acetyl glutamate**	$C_7H_{11}NO_5$	1.1-1.6	190.0710	188.0564	190.0724	-	7.366
***N*-acetyl ornithine**	$C_7H_{14}N_2O_3$	11.6-12.8	175.1077	173.0932	175.1084	-	3.998
Ornithine	$C_5H_{12}N_2O_2$	14.9-16.1	133.0972	131.0826	133.098	-	6.011
Citrulline	$C_6H_{13}N_3O_3$	11.7-13.1	176.1030	174.0884	176.1039	-	5.111
Arginine	$C_6H_{14}N_4O_2$	14.0-16.1	175.1190	173.1044	175.1202	-	6.852
Proline	$C_5H_9NO_2$	10.1-11.6	116.0706	114.0561	116.0708	-	1.723
Serine	$C_3H_7NO_3$	9.9-11.4	106.0499	104.0353	106.0501	-	1.886
Glycine	$C_2H_5NO_2$	10.6-12.4	76.0393	74.0248	76.0398	-	6.576
Lysine	$C_6H_{14}N_2O_2$	15.1-16.2	147.1128	145.0983	147.1133	-	3.399