A multimodal system for lipid A structural analysis from a single colony

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Introduction

Lipid A, the hydrophobic anchor of lipopolysaccharide (LPS) is a potent sepsis-inducing molecule that stimulates human immune responses. The structural diversity of lipid A is correlated both with pathogenic capability and adaptation to the unique bacterial growth environments. Thus, understanding the structure of lipid A is important in fundamental microbiology and host-pathogen interactions. Previously, our group developed Fast Lipid Analysis Technique (FLAT) which employs an on-surface extraction combined with conventional Matrix-Assisted Laser Desorption/Ionization Mass Spectrometer (MALDI-MS), allowing for rapid structural characterization of lipid A directly from raw biomass of bacterial cells. Here, we employ tandem MS in both polarities, negative-ion (FLAT^n) and positive-ion (FLAT^{n+}) modes, to elucidate the structure of lipid A of various Gram-negative bacteria form single colonies.

Experimental Section

Bacteria samples: *Escherichia coli* (*E. coli*) ATCC 25922 and *Pseudomonas aeruginosa (P. aeruginosa)* ATCC 31482 isolates were cultivated at 37°C in Lysogeny broth (LB, Becton-Dickenson, Hunt Valley, MD, USA) supplemented with 1.5% Bacto-agar.

Sample preparation: A single colony of each bacterial strain was obtained using an inoculation loop, followed by smearing on a 96-well MALDI plate. Next, FLAT was conducted as shown below.[1]

Mass Spectrometry: A Bruker MALDI (tims TOF) MS used for FLAT^n experiments. This instrument is equipped with a dual ESI/MALDI source with a SmartBeam 3D 10 KHz frequency tripled Nd:YAG laser (355 nm). The system was operated in "qTOF" mode (TIMS deactivated) with ion transfer tuning. MALDI (tims TOF) MS was used for FLAT (direct biomass MS for visualization of lipid A primary molecular ions), FLAT^n (direct biomass MS/MS for fragmentation and structural analysis of lipid A in negative ion mode)[2,] and FLAT^{n+} (direct MS/MS for fragmentation and structural analysis of lipid A in positive ion mode).

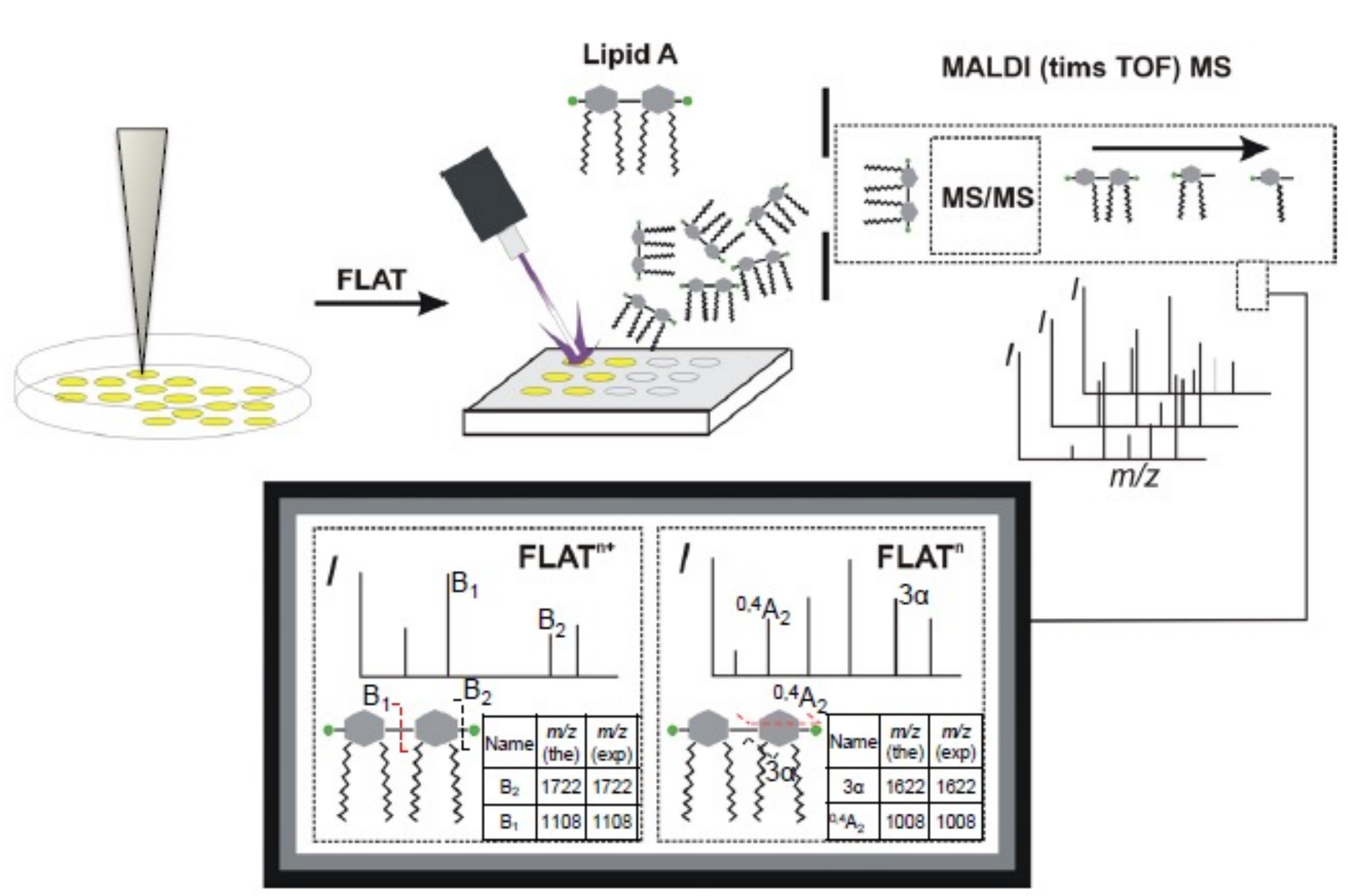


Figure 1. A single colony from each bacterium is spotted on the MALDI target, treated with a mild acid to hydrolyze lipid A, and subjected to MALDI qTOF MS/MS mass spectrometry in both polarities with the matrix norharmane.

Determination of MS and MS/MS efficiency in positive ion mode

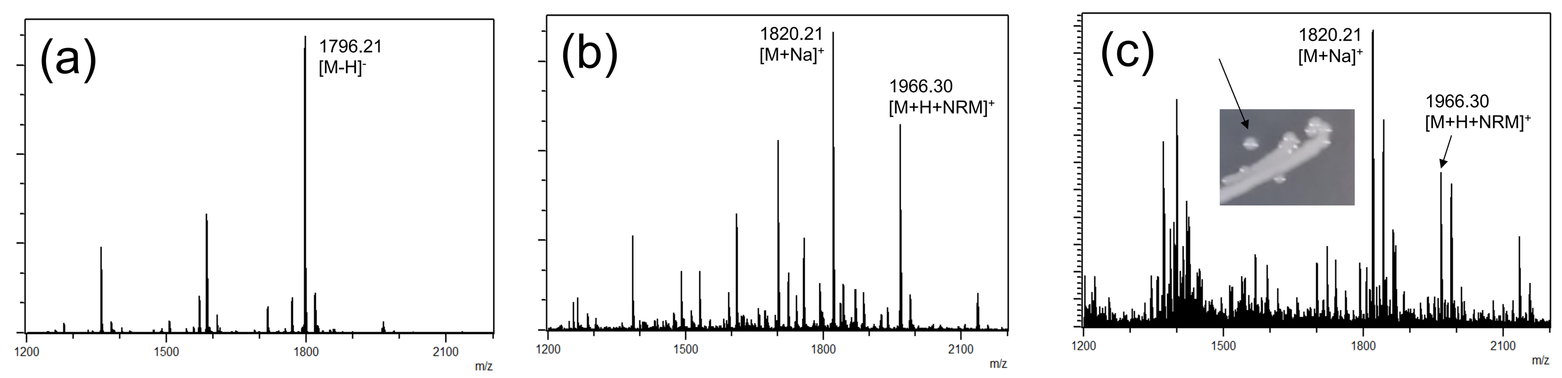
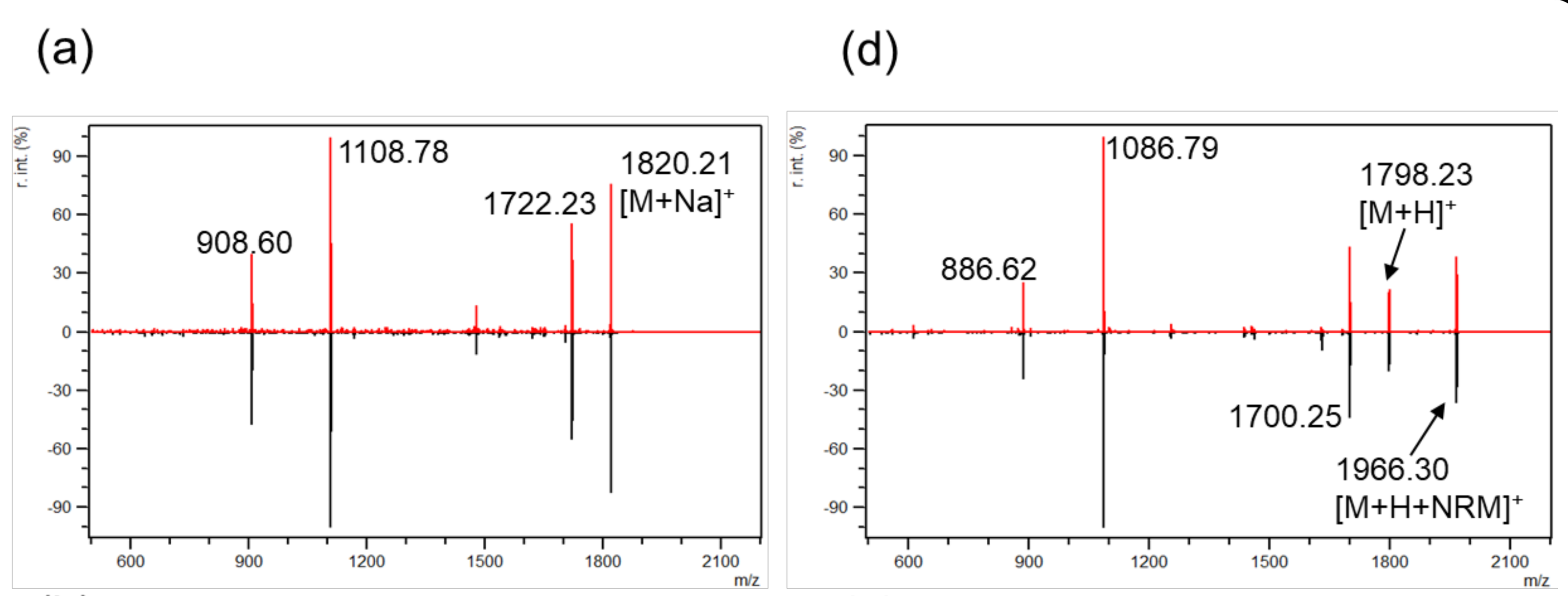


Figure 2. MS spectra of lipid A from commercial standard and a single colony of *E. coli*. Spectra were collected and analyzed as follows. (a) MALDI mass spectrum from the commercially available *E. coli* lipid A as a deprotonated anion [M-H]⁻ at *m/z* 1796.21 in negative ion mode. (b) MALDI spectrum from commercially available *E. coli* lipid A shows sodium [M+Na]⁺ and protonated NRM adduct [M+H+NRM]⁺ cations at *m/z* 1820.21 and 1966.29, respectively, in positive ion mode. (c) FLAT spectrum from a single colony of *E. coli* shows sodium [M + Na]⁺ and protonated NRM adduct [M+H+NRM]⁺ form of cations at *m/z* 1820.21 and 1966.29, respectively, in positive ion mode. The inset photo indicates the single colony used.



(b)

Naming	*m/z* (theory)	*m/z* (expt)	Error (Da)
B_2	1722.23	1722.23	0.00
B_1	1108.78	1108.78	0.00
$B_1 + 2'\varepsilon$	908.60	908.60	0.00

(e)

Naming	*m/z* (theory)	*m/z* (expt)	Error (Da)
$[M+H]^+$	1798.23	1798.22	0.01
B_2	1700.25	1700.25	0.00
B_1	1086.79	1086.79	0.00
$B_1 + 2'\varepsilon$	886.62	886.62	0.00

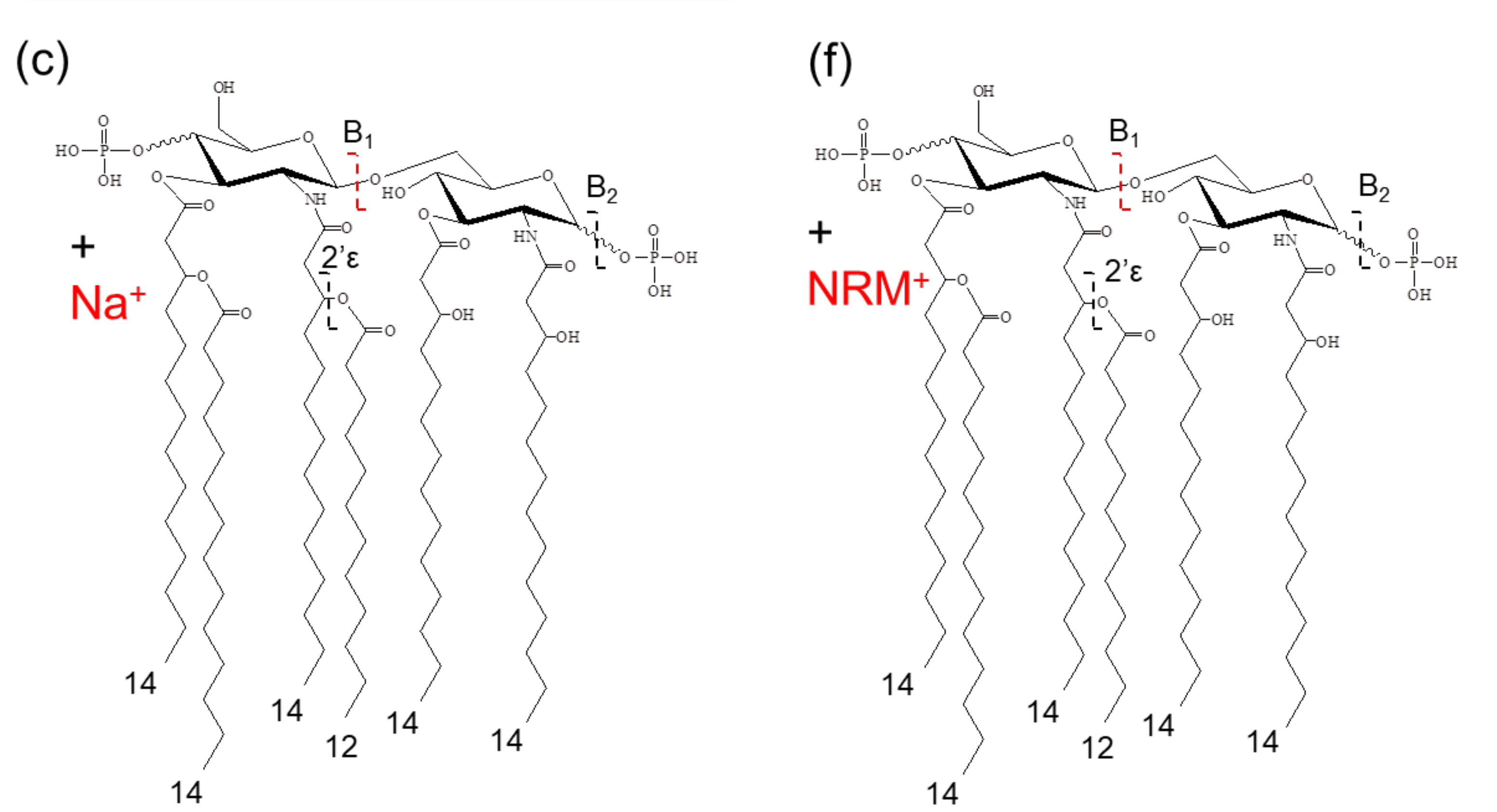
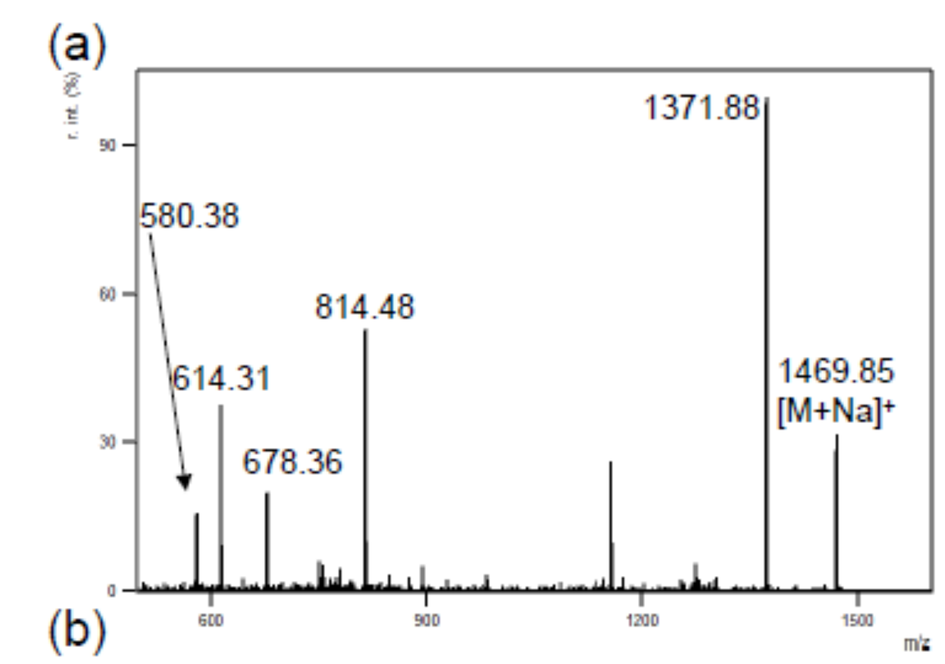


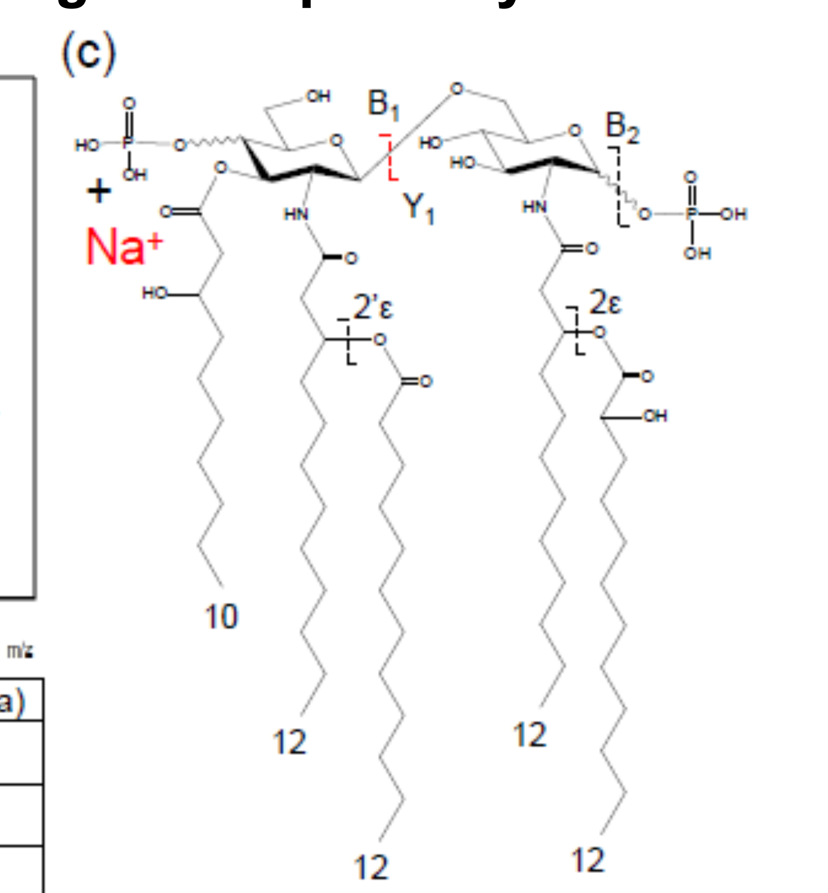
Figure 3. FLAT^{n+} tandem mass spectra of lipid A from a single colony of *E. coli*. Spectra were collected and analyzed as described in the text. This figure shows (a) FLAT^{n+} tandem mass spectrum from the commercial standard (top) and a single colony (bottom) of *E. coli* lipid A as sodium adduct lipid A cations [M+Na]⁺ at *m/z* 1820.21 with almost identical fragments, (b) assignments of fragments, (c) chemical structure of *E. coli* lipid A in the negative mode. (d) FLAT^{n+} tandem mass spectrum from the commercial standard (top) and a single colony (bottom) of *E. coli* lipid A as protonated NRM adduct lipid A cations [M+H+NRM]⁺ at *m/z* 1966.30 with almost identical fragments, (e) assignments of fragments, and (f) chemical structure of *E. coli* lipid A in the positive mode.

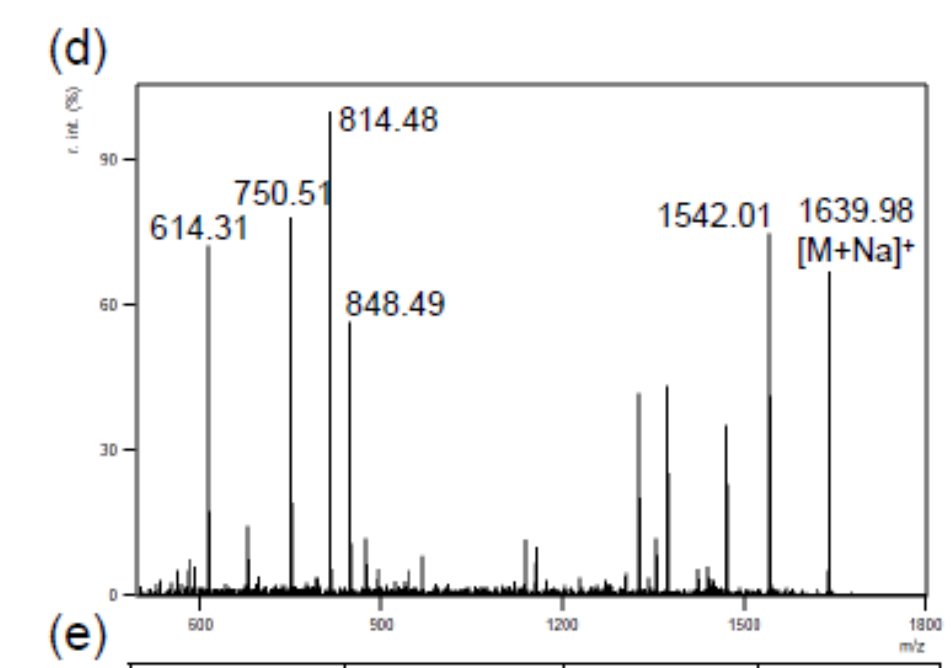
Structure verification of *P. aeruginosa* lipid A by FLAT^{n+}



(b)

Naming	*m/z* (theory)	*m/z* (expt)	Error (Da)
B_2	1371.88	1371.88	0.00
B_1	814.48	814.48	0.00
Y_1	678.36	678.36	0.00
$B_1 + 2'\varepsilon$	614.31	614.31	0.00
$Y_1 + B_2$	580.38	580.38	0.00





(e)

Naming	*m/z* (theory)	*m/z* (expt)	Error (Da)
B_2	1542.01	1542.01	0.00
Y_1	848.49	848.49	0.00
B_1	814.48	814.48	0.00
$B_2 + Y_1$	750.51	750.51	0.00
$B_1 + 2'\varepsilon$	614.31	614.31	0.00

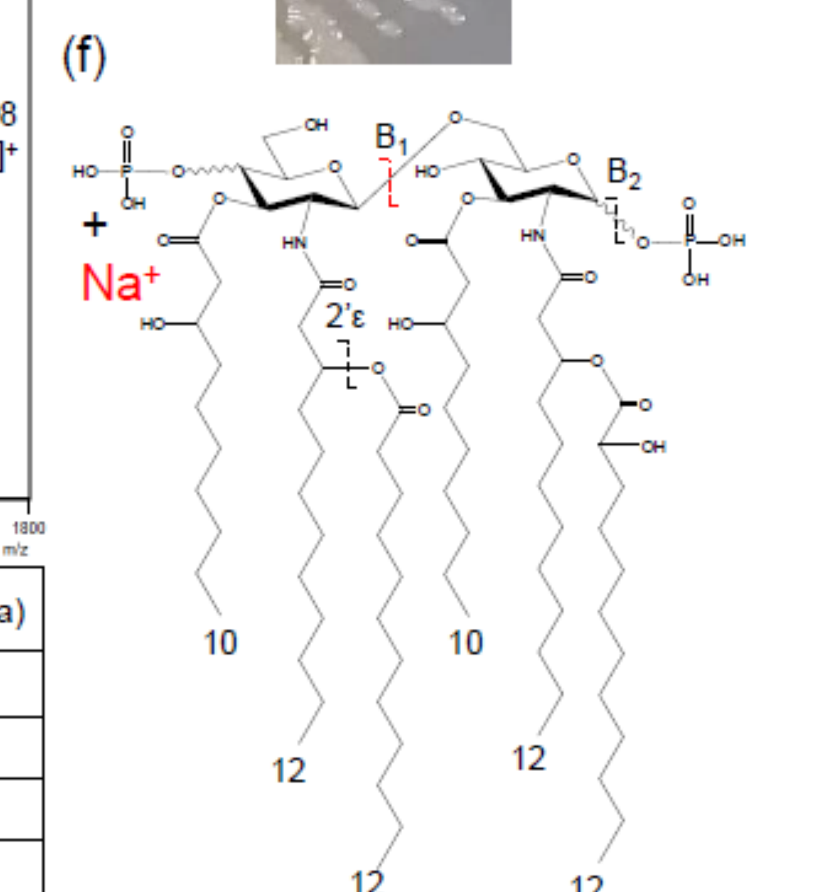


Figure 4. FLAT^{n+} tandem mass spectra of lipid A from a single colony of *P. aeruginosa*. Spectra were collected and analyzed as described in the text. This figure shows (a) FLAT^{n+} tandem mass spectrum of the penta-acylated *P. aeruginosa* lipid A precursor ion at *m/z* 1469.85 as a sodium adduct form of cation, (b) assignments of fragments, and (c) chemical structure of penta-acylated *P. aeruginosa* lipid A in the negative mode. (d) FLAT^{n+} tandem mass spectrum of the hexa-acylated *P. aeruginosa* lipid A precursor at *m/z* 1639.98 as a sodium adduct form of cation, (e) assignments of fragments, and (f) chemical structure of hexa-acylated *P. aeruginosa* lipid A cation in the positive mode. The unset photo indicates the single colony that was used.

Conclusions

We demonstrate the development of a novel analytical approach combining positive-ion and negative-ion MALDI-TOF MS and tandem MS for complete structural characterization of canonical and noncanonical lipid A structures. These capabilities are an adaptation and advancement of the direct-from-biomass lipid characterization system FLAT (Fast Lipid Analysis Technique).

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References

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2. Anal Chem. 2022, 31;94(21):7460-7465; PMID: 35576511