Performance Characterization of Surface Acoustic Wave Nebulization Mass Spectrometry: Studies of Lipid A

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Introduction & Methods

Surface acoustic wave nebulization (SAWN)[1] is a novel ambient sample transfer method that relies on a planar microfluidic chip to transfer analytes from solutions to the gas phase for mass spectrometric analysis. The ease of use and softness[2] make SAWN an ideal method for the analysis of labile lipids from bacterial membranes. A wide range of SAWN applications have been reported recently, but a fundamental study into analytical figures of merit (*e.g.*, limits of detection) important for novel biological assays remains to be investigated. Of particular interest is the MS profiling of glycolipids from bacterial membrane, which can greatly benefit from rapid transfer methods such as SAWN[3]. Here we report a detailed study of analytical performance characterization of SAWN, by studying lipid A, an endotoxin component of bacterial lipopolysaccharides that comprise the bacterial outer membrane. This study focused on defining the limit of detection of (LOD) lipid A by SAWN-MS and presents different methods of characterizing the noise level and signal-to-noise ratio (SNR).

Commercially available monophosphoryl lipid A (MPL) was used as a standard in this study. *Francisella novicida* was grown at 37 °C and lipid A was extracted using a rapid micro-extraction method[4]. The extracts were reconstituted in a mixture of chloroform/methanol/water (12:6:1, v/v/v), serially diluted, and spiked with MPL in each dilution for mass spectrometric analysis. All MS experiments with ESI and SAWN were conducted on a Waters Synapt G2-S. The mass spectrometer was tuned using ESI and optimized for maximum analyte signal and subsequently, used for the SAWN experiments. The LOD was determined based on the signal-to-noise ratio (SNR) of the lipid A signals (SNR ≥ 3). A 20 Da mass window around the lipid A peak (m/z 1744, and 1665 for MPL and *F. novicida* lipid A, respectively) was selected and used for noise amplitude calculation. Three different methods for calculating the noise level and SNR were used: (i) based on standard deviation (SD), (ii) based on the sum of average noise plus SD, and (iii) based on the root mean square (RMS) of the noise.

Surface Acoustic Wave Nebulization:

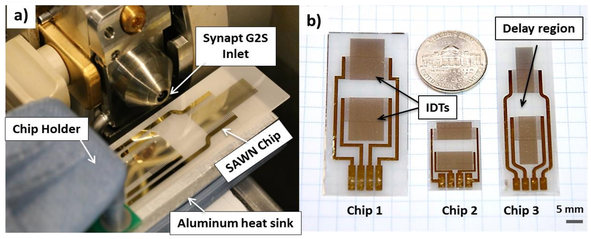

Figure 1. (a) Configuration of the SAWN devices for mass spectrometric analysis on Synapt G2-S. **(b)** Three different geometries of standing wave (SW) SAWN chip designs were selected for testing.

SAWN performance characterization: studies with monophosphoryl lipid A (MPL)

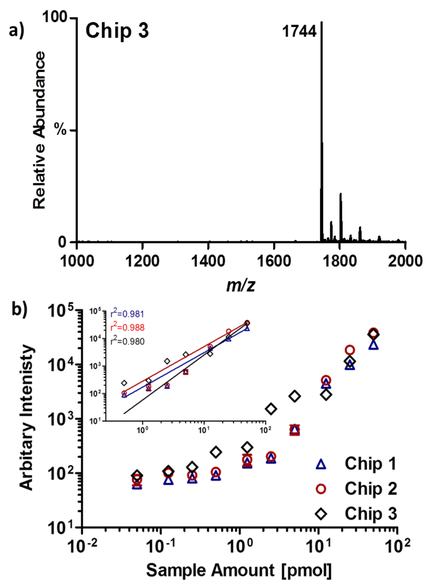

Figure 2. (**a**) Example of SAWN-MS spectrum of MPL acquired with Chip 3. The spectrum shows the precursor ion of lipid A (m/z 1744) acquired in negative ion mode. (**b**) Changes in signal intensity with increasing sample concentration. The inset shows a close-up of the dynamic range (0.5 to 50 µM) of the concentration plot with results from linear regression analysis.

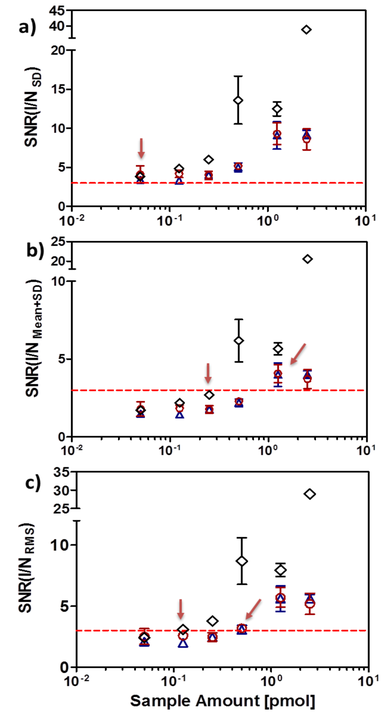

Figure 3. SNR of MPL in the study with three different SAWN chip designs three different methods for estimating the noise were used: (**a**) by SD, (**b**) based on the sum of average noise and SD, and (**c**) based on RMS of the noise. The LOD for each chip is highlighted by the red arrow in the figures above and listed in **Table 1**.

SAWN performance characterization: studies with *F. novicida* lipid A

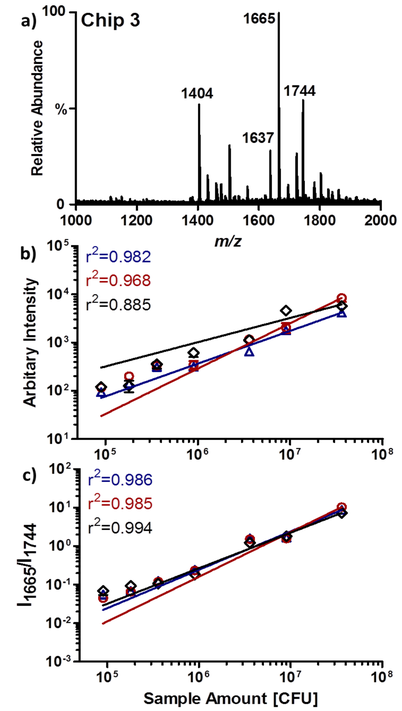

Figure 4. (**a**) Example of SAWN spectrum of lipid A from *F.novicida* acquired with Chip 3. The spectrum shows the precursor ion of lipid A *F.novicida* (m/z 1655) and the internal standard MPL (m/z 1744) were detected in negative ion mode. (**b**) Changes in signal intensity with increasing sample concentration for the three different SAWN chips tested showing the dynamic range of the measurement for lipid A from *F. novicida* and (**c**) normalized to the internal MPL standard.

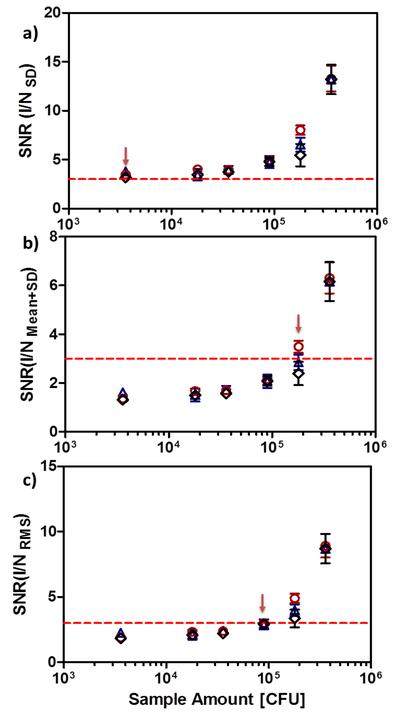

Figure 5. SNR of *F. novicida* extracts in the study with three SAWN chips using three different methods for estimating the noise: **(a)** by SD, **(b)** based on the sum of average noise and SD, and **(c)** based on root mean square of the noise. The LOD for each chip is highlighted by the red arrow in the figure and listed in table 1.

Conclusion & Discussion

Table 1. **Summary of MPL and *F.novicida* lipid A LODs determined by three different methods of SNR calculation for SAWN.**

SAWN Chip	SNR (I/N $_{SD}$)		SNR (I/N $_{Mean+SD}$)		SNR (I/N $_{RMS}$)	
	MPL (fmol)	*F. novicida* lipid A (CFU)	MPL (fmol)	*F. novicida* lipid A (CFU)	MPL (fmol)	*F. novicida* lipid A (CFU)
Chip 1	50	3.6 x 10^3	1250	1.8 x 10^5	500	9 x 10^4
Chip 2	50	3.6 x 10^3	1250	1.8 x 10^5	500	9 x 10^4
Chip 3	50	3.6 x 10^3	250	1.8 x 10^5	125	9 x 10^4

- SAWN-MS enabled the detection of 125 fmol (Chip 3) and 500 fmol (Chip 1 and 2) of the lipid A standard MPL and the method was able to detect lipid A extracted from 9x10^4 CFU (Chip 1, 2 and 3) of *F.novicida* cultures.
- Three methods to define SNR were compared and RMS method was found to be the most reasonable and reliable measure for defining the LOD of lipid A.
- All three SAWN chips could detect a few hundred femtomoles of MPL, which is better than conventional ionization methods (ESI and MALDI), placed on chip and the planar geometry of SAWN provided practical advantages in terms of ease-of-use and rapid ionization.
- The Chip 3 (latest version) has shown better analytical performance in terms of LOD and signal intensities than the other two. Possible explanations are: **a)** thermal images data (not shown) indicates that the rounded electrode corner design reduces the heat development of Chip 3 surface; **b)** the narrower delay region of Chip 3 brings the ionization spot closer to inlet of instrument., which may increase ion flux.

Comparison of signal intensities for the different SAWN-MS studies.

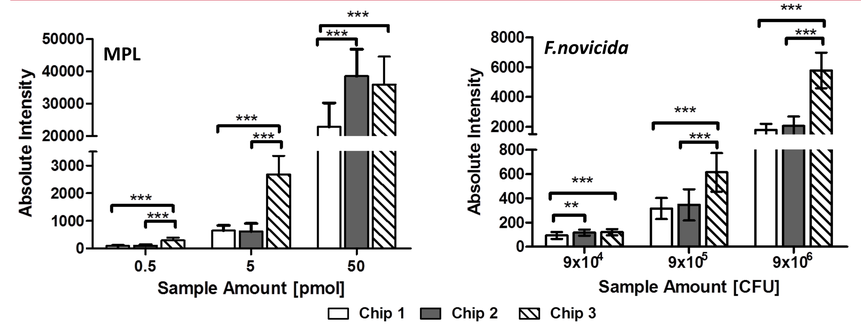

Figure 6. Comparison of signal intensities in SAWN-MS studies with lipid A from MPL (**a**) and *F. novicida* extracts (**b**) at three different concentrations. Shown are the signal intensities at m/z 1744 for MPL **(a)** and m/z 1665 for *F. novicida* **(b)** extracts at low, medium, and high concentrations. Data shown as mean intensities ± SD (n = 3) with one-way ANOVA, 95% confidence interval (** significant at p<0.01; ***, p<0.001).

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References:

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