Supplementary material

Why is NanoSIMS imaging of arsenic in seaweed (*Laminaria digitata*) important for understanding of arsenic biochemistry in addition of speciation information?

E. Ender[1], M.A.Subirana[2], A. Raab[1], E.M. Krupp[1], D. Schaumlöffel[2], J. Feldmann[2]

Analytical method for hydrophilic arsenic speciation using HPLC-ICPMS/MS

Inorganic arsenic was determined in the extracts using anion exchange chromatography on a PRP X 100 Hamilton column (4.2 x 250 mm). A 1290 Agilent HPLC-system was used with a flow rate of 1 mL min^{-1} of ammonium carbonate buffer (30 mM and pH 8.0). The injection volume was 40 µL. Hydrophilic compounds were identified using standard compounds by comparison of retention times and detected using ICPMS/MS (Agilent 8800). Arsenic was determined on m/z 91 as AsO^+ when oxygen was used as a reaction gas.

Analytical method for lipophilic arsenic speciation using HPLC-ICPMS/MS-ESI-qTOFMS

Speciation analysis for arsenolipids was conducted on an ACE Excel C18 column (5 µm, 4.6 × 150 mm) using a linear 20 min gradient from 0 – 100% MeOH (0.1% (v/v) formic acid in water and 0.1% (v/v) formic acid in MeOH) followed by 25 min 100% MeOH, before re-equilibration of the column. Lipophilic compounds were identified by splitting the flow after the column with 75 % infused into an ESI-MS, the remainder into an ICP-MS. A Bruker MaXIS II was used as ESI-MS in positive scan mode with 4.5 kV at the source capillary. An Agilent 8800 ICPMS/MS was used with oxygen as reaction gas and Pt-cones (10 % Ar/O_2 80/20) for speciation of phosphorus (*m/z* 31->47), sulfur (*m/z* 32->48) and arsenic (*m/z* 75->91). Iridium (*m/z* 193) was used throughout as continuous standard added post-column.

Quantification was performed using DMA solutions of different concentrations (3 injections each). To control the variable sensitivity of the ICP-MS depending on the MeOH

concentration, a response curve was recorded. Peak areas were calculated from the corrected intensities and used for quantification. Details are described in *Amayo* et al.[i].

Preparation by High Pressure Freezing and Freeze Substitution for NanoSIMS

• High Pressure Freezing carried out using a Leica EM ICE (Leica Microsystems, Milton Keynes, UK)

• Freeze substitution is carried out using Leica AFS 2 following the programme below:

Table S1: freeze drying protocol

Step	**Start temp (°C)**	**End temp (°C)**	**Time (hours:mins)**	**Reagent**
1	-95	-90	30	2% OsO_4 in acetone
2	-90	-90	10:00	2% OsO_4 in acetone
3	-90	-30	08:00	2% OsO_4 in acetone
4	-30	-10	01:00	Acetone
5	-10	4	01:00	Acetone
6	4	20	01:00	Acetone

• Samples are then removed and placed in 10% Spurr's (TAAB, UK): Acetone for 72 hours

• 30% Spurr's O/N

• 50% Spurr's 8 hours

• 70% Spurr's O/N

• 90% Spurr's 8 hours

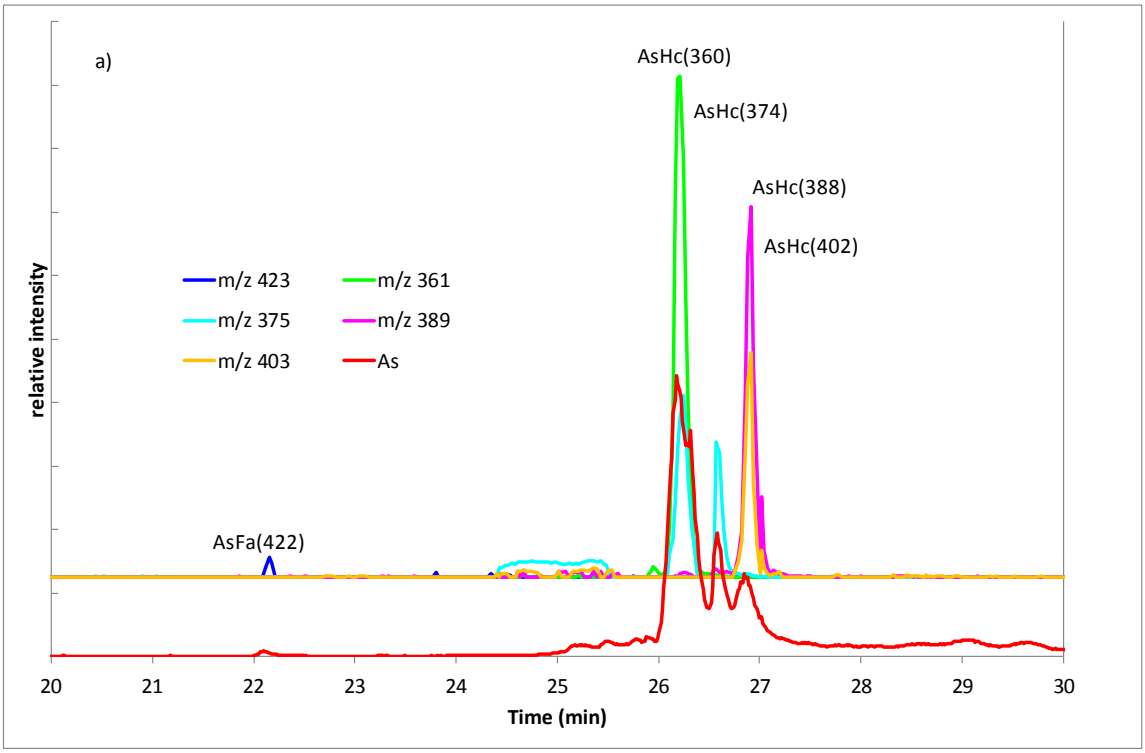
• Embedded in Spurr's resin @60 °C for at least 24 hours

• 90nm sections are prepared using a diamond knife (Diatome Ltd, Switzerland) onto Copper grids (TAAB, UK) using a Leica UC6

• Contrast stained with Uranyl Acetate (replaced with Uranyless (TAAB UK) July 2018) and Lead Citrate in a Leica AC20

• For NanoSIMS 300nm sections are prepared using a diamond knife (Diatome Ltd, Switzerland) onto Silicon wafers

Chromatograms of the arsenolipids



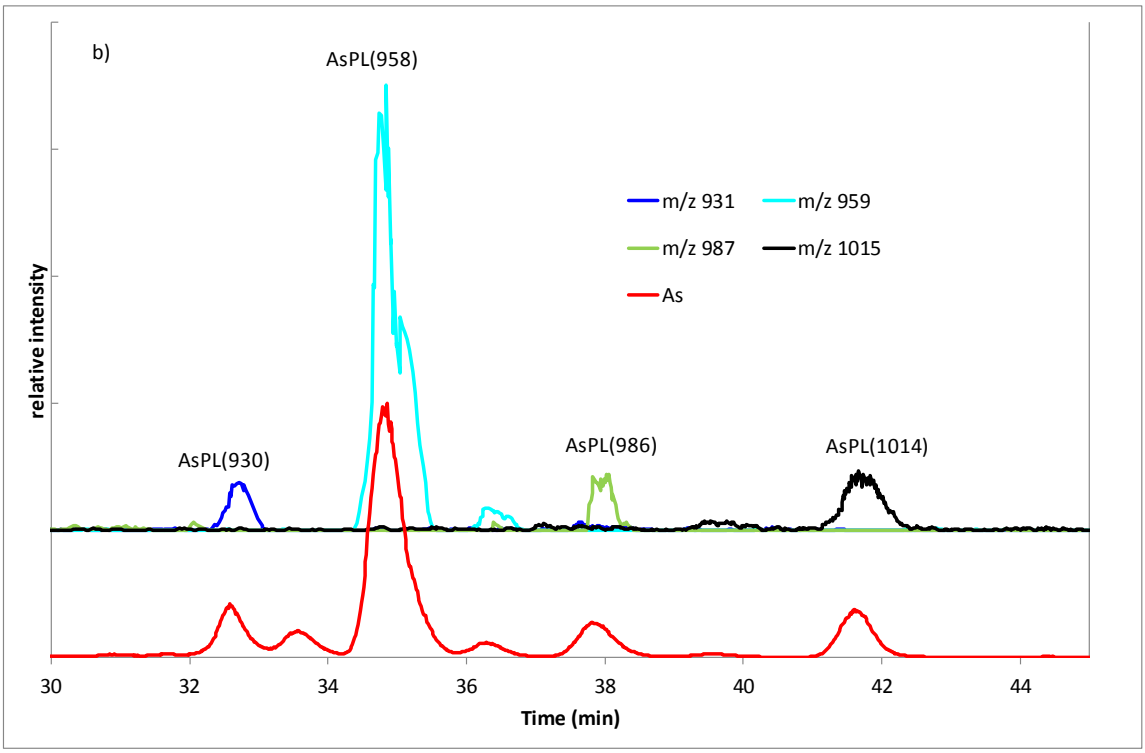


Figure S1a: As-hydrocarbon region of RP-HPLC-ESI-MS separation of lipophilic As compounds, overlay of arsenic trace from ICPMS/MS (measured on mass shift as AsO^+ mode m/z 91) with extracted ion chromatograms (ESI-MS traces) of As fatty acid (AsFA) and identified As-hydrocarbons (AsHC). **1b**: arsenosugar-phospholipid (AsPL) region of RP-separation of lipophilic As compounds, overlay of arsenic trace from ICPMS/MS with extracted ion chromatograms (ESI-MS traces) of major identified AsPL.

[i] K.O. Amayo, K.O, A.H. Petursdottir, C. Newcombe, H. Gunnlaugsdottir, A. Raab, E. Krupp, and J. Feldmann, *Anal. Chem.* 2011, **83**, 3589-3595.