Supporting Information for: Sea spray aerosol structure and composition using cryogenic transmission electron microscopy'

Joseph P. Patterson,[†]* Douglas B. Collins,[†] Jennifer M. Michaud,[†] Jessica L. Axson, [†] Camile M. Sultana,[†] Trevor Moser,[‡] Abigail C. Dommer,[†] Jack Conner,[†] Vicki, H. Grassian,[§] M. Dale Stokes,[‖] Grant B. Deane,[‖] James E. Evans,[‡] Michael D. Burkart,[†] Kimberly A. Prather,[†, ‖] and Nathan C. Gianneschi,[†]

[†]Department of Chemistry & Biochemistry, University of California, San Diego, La Jolla, California, USA, 92093, USA

[‡]Environmental Molecular Science Laboratory, Pacific Northwest National Laboratory, 3335 Innovation Blvd., Richland, WA 99354,

[§]Department of Chemical and Biochemical Engineering and ‖Department of Chemistry, University of Iowa, Iowa City, Iowa 52242, United States

Materials and Methods

STEM/TEM imaging:

TEM and STEM Images were recorded on either an FEI Titan operating at 300 keV with spherical aberration corrected TEM and STEM with a Si(Li) X-ray energy dispersive spectrometer (EDS) with a polymer based windwon and operating with a collection solid angle of <0.1 sr or an FEI Sphera microscope operated at 200 keV. In general the images were recorded at relatively low magnification and electron doses for higher magnification imaging low dose imaging procedures were used in order to prevent beam damage to the particles.

EDS collection:

EDS spectra were collected in STEM mode as either point or line scans. The dwell times were varied (typically 1-10 minutes) depending on sample thickness, but were kept consistence for individual particles, in order to obtain a sufficient signal to noise ratio. EDS spectra typically resulted in localized particle damage, in the form of small holes being drilled through the sample, which could be observed afterwards by imaging in TEM or STEM mode. The post EDS collection images show the localization of the damage and the EDS collection. All spectra are presented as background subtracted, and the peaks are assigned to the relevant element K edges.

Aerosol generation:

Marine aerosol Reference Tank. Details for the procedure of aerosol generation can be found in reference 1.[1]

Waveflume. Details for the procedure of aerosol generation can be found in reference 2.[2]

Synthesis of GOx as described previously [3]

H2SO4 (conc, 170 mL) was added to graphite (5 g) in a 250 mL round bottom flask on ice and stirred for 10 min. KMnO4 (22.5 g) was added over 1 hour and the mixture was stirred for 2 hours on ice. The solution was allowed to warm to room temperature and stirred overnight. The viscous brown liquid was

added to H2SO4 (5 wt%, 500 mL) with continuous stirring for 2 hours. An aqueous solution of H202 (30 wt%, 15 g) was added and stirred for 2 hours. The viscous brown liquid was centrifuged and the supernatant discarded. An aqueous solution (500 mL) of H2SO4 (3 wt%) and H2O2 (0.5 wt%) was added to the precipitate and stirred for 1 min. The centrifugation and washing step was then repeated 5 times with the H2SO4 (3 wt%) and H2O2 (0.5 wt%) solution and 5 times with DI water. After a final centrifuge small portions of GO was stirred in water and freeze dried to obtain brown GO flakes. GO flakes were stirred in a nanopure water over night, sonicated for 30 seconds and left to stand. Any sediment was discarded after one day. Note: the synthesis of graphene oxide involves the use of highly corrosive materials and the appropriate MSDS forms should be consulted.

Preparation of GOx-TEM grids as described previously [3]

Quantifoil R2/2 TEM grids were plasma cleaned using an EMITECH K950X for 3 minutes at 20 mAmp and 0.2 mbar. The grids were immediately transferred to a piece of filter paper and one drop (from a 200 µL pipette) of GO solution (ca. 0.1 mg/mL) was dropped from a height of 1 cm onto the TEM grids (allowing the filter paper to wick away most of the solution). The grids were left to dry in air for at least 30 minutes before use. The GOx solution concentration of 0.1 mg/mL was used to obtain high (generally > 90%) coverage on the TEM grids, as it was noticed that when SSA adhered to areas with holes not covered with GOx that these SSA deformed around the holes (see Figure S5)

Preparation of SSML and bulk samples for GOx-Cryo-TEM

7 µL of sample was added to GOx-TEM grids and left under high humidity for particles to adsorb to the surface for 20-60 minutes. For studies where per mL concentrations of vesicles were determined the waiting time was held at 30 minutes. After the adherence period the grids were manually blotted with filter paper for 25 seconds and rapidly plunged into liquid ethane. The grids were then transferred to liquid nitrogen for storage and transfer to the microscope where they were imaged at < -170 °C. TEM images were recorded on a FEI Sphera microscope operated at 200 keV using a precooled Gatan 626 cryo-transfer holder. Micrographs were recorded on a 2K X 2K Gatan CCD.

Preparation of impinged aerosol samples for GOx-Cryo-TEM

Impinger samples were collected into autoclaved sea water at a flow rate of 1 standard liters per minute over periods of two hours. Multi samples were collected, combined and concentrated approximately 1000x using Amicon Ultra-15 or 0.5 centrifugal filters. Sample were then prepared for GOx-supported cryo-TEM as described above for SSML and bulk samples.

Preparation of Aerosol-Cryo-TEM samples

Either Carbon film or GOx-TEM grids were held with a pair of tweezers and placed into MART tanks either during or after plunging cycles and let for a set period of time 20 minutes-12 hours to allow particles to adhere to the grids. The grids were removed from the MART tanks and either directly plunged into liquid ethane or kept under high humidity until they were plunged into liquid ethane or nitrogen. Note: no blotting step was used in the process.

For the carbon film grids (only used for the images in Figure S4) it was observed qualitatively that far fewer particles were adhered to the grids in comparison with the GOx grids despite having the same collections times. Furthermore, for particles of a similar diameter, the particles on the GOx grids appeared to be much thinner (lower contrast) indicating that the SSA are spreading out more on the GOx grids, which is consistent with GOx being hydrohpilic.[3]

Both liquid nitrogen and liquid ethan can be used of vitrification agents, in this study we did not notice any difference between using the two coolants.

Calculation of SSA thickness at the particle center by EELS

Particle thickness was estimated using electron energy loss spectroscopy and the log ratio technique,[4] where the relative thickness (t/λ) is a function of the integrated area beneath the zero loss peak and the total spectrum.

Absolute thickness was calculated by estimating the inelastic mean free path (λ) of the particles to be ~152.8 nm, assuming the primary components of the aerosol particles to be water and sea salts λ can be calculated from the following equations .

$$\lambda = \frac{106\mathrm{F}.\,\mathrm{E}(0)}{\mathrm{E}(\mathrm{M})\ln(2\beta\mathrm{E}(0)/\mathrm{E}(\mathrm{M}))}$$

E(0) is the accelerating voltage expressed in keV, β is the collection angle in mrad, F is a relativistic factor defined by

$$F = \frac{1 + (E(0)/1022)}{(1 + (E(0)/511))^2}$$

And E(M) is an average energy loss defined by E(M) = 7.6 Z(eff)$^{0.33}$. where Z(eff) is defined by

$$Z(eff) = \frac{\Sigma(f(n)Z(n)^{1.3}}{\Sigma(f(n)Z(n)^{0.3}}$$

Where f is the atomic fraction of the samples and Z is the atomic number. In this case the following atomic fractions were used H (0.110), O (0.883), Na (1.08x10^{-2}), Mg (1.29x10^{-3}), Cl (1.94x10^{-2})

For a measured log ratio of 3.59, a thickness (t) value of approximately 549 nm is determined.

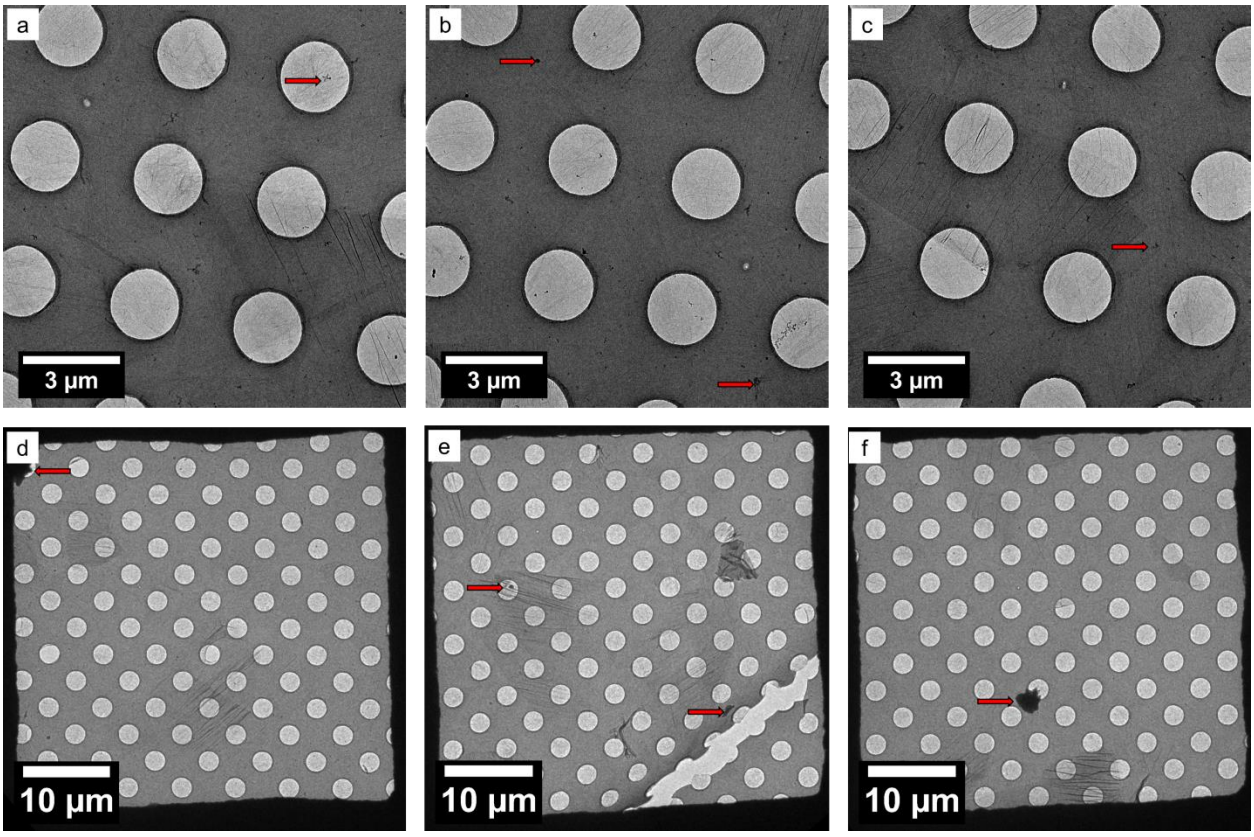

Figure S1. Bright field TEM images for a control sample where the GOx TEM grid was placed inside the MART, but without the plunging mechanism, which generates the aerosols. The red arrows indicate contamination from the cryo-TEM preparation method.

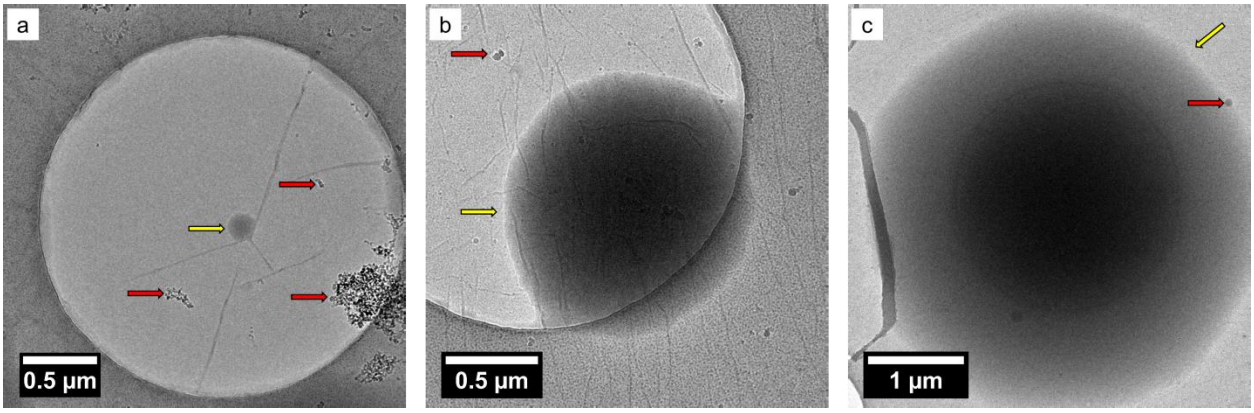

Figure S2. Additional bright field TEM images of SSA collected at stage 1. The yellow arrows indicate the edge of the SSA and the red arrows indicate contamination from the cryo-TEM preparation.

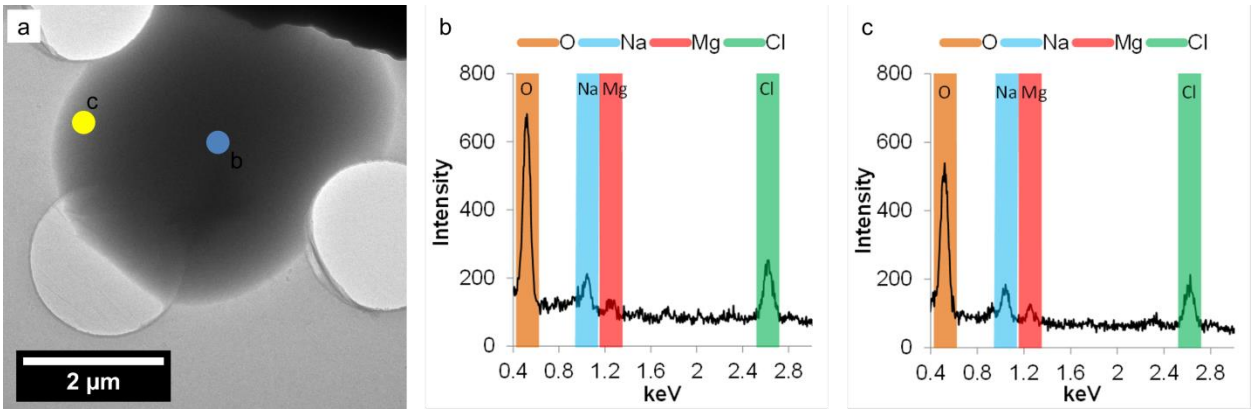

Figure S3. cryo-TEM image and EDS spectra for a particle collected at stage 1. a) bright field TEM image, b) EDS spectrum collected at the center of the particle (blue dot) and c) EDS spectrum collected at the edge of the particle (yellow dot).

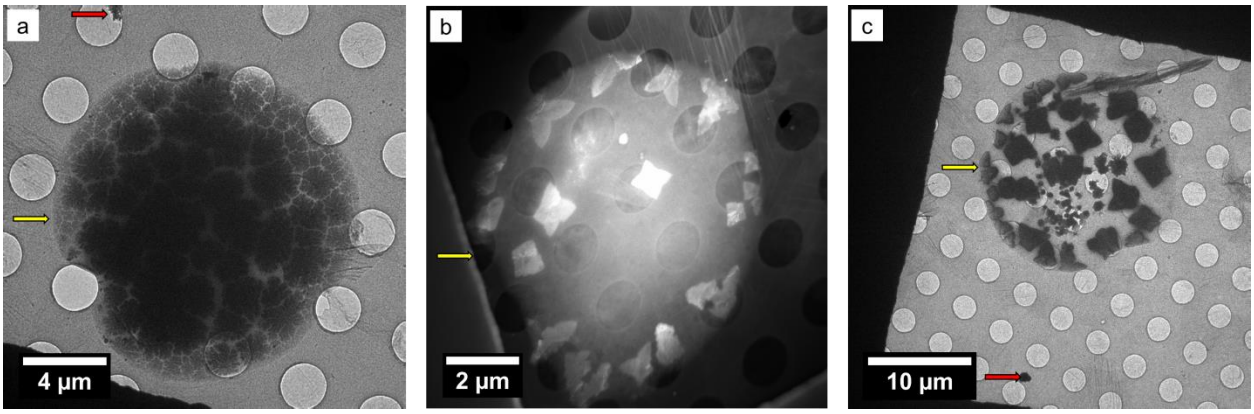

Figure S4. Additional (S)TEM images for SSA collected at stage 2. a) bright field TEM, b) HADDF STEM and c) bright field TEM.

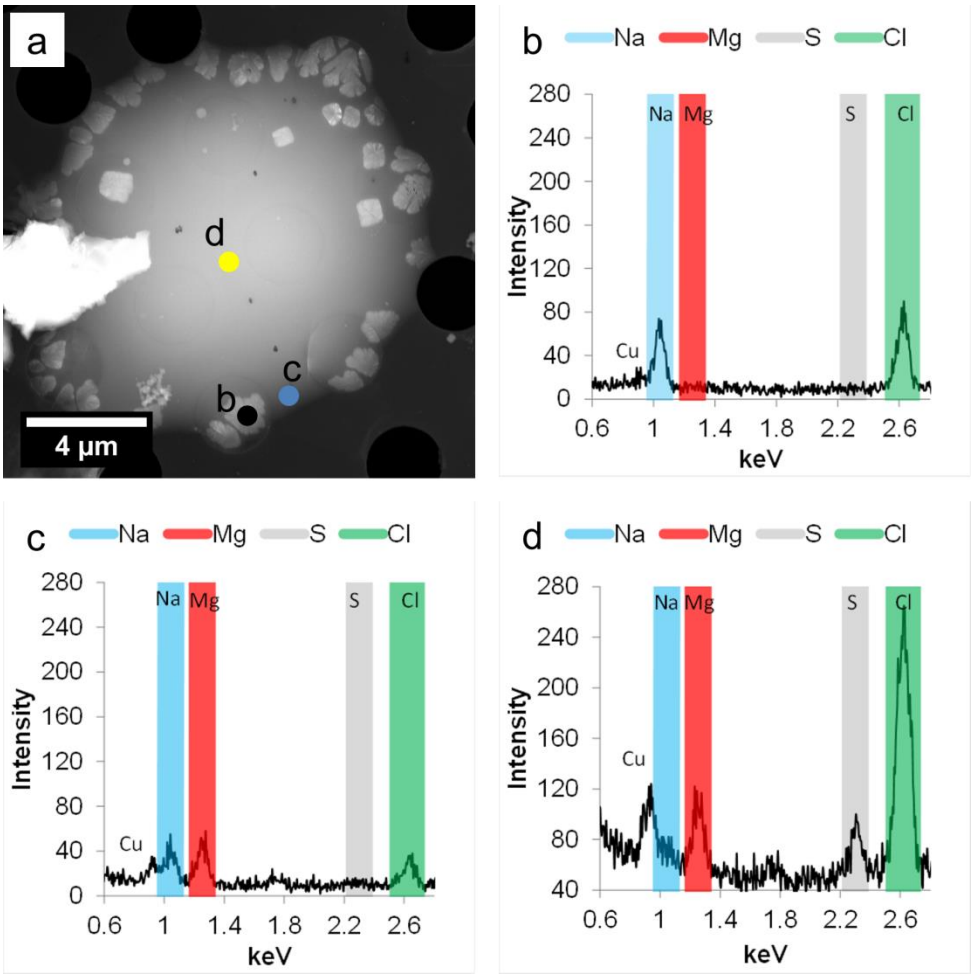

Figure S5. HADDF STEM image and EDS spectra for particles collected at stage 2. a) HADDF STEM images where the colored dots and letter show the location of the EDS spectra, b) An EDS spectrum collected from one of the salt crystals at the edge of the SSA showing its composition is NaCl, c) An EDS spectrum collected from the edge of the SSA, but in the continuous phase (i.e. not on a salt crystal) showing a relatively even composition of Na and Mg and d) EDS collected from the center of the particle showing that a high relative amount of Mg over Na. As discussed in the main text, the general conclusion to this data is that, as the particle dehydrate and effloresce, NaCl crystals form on the particle surface, which leads to a depletion of Na from the core of the SSA. As indicated in the spectra there in a slightly overlapping Cu peak from the substrate, which would interfere with quantitative analysis of the Na and Mg ratio, however qualitatively this does not impact the general conclusion.

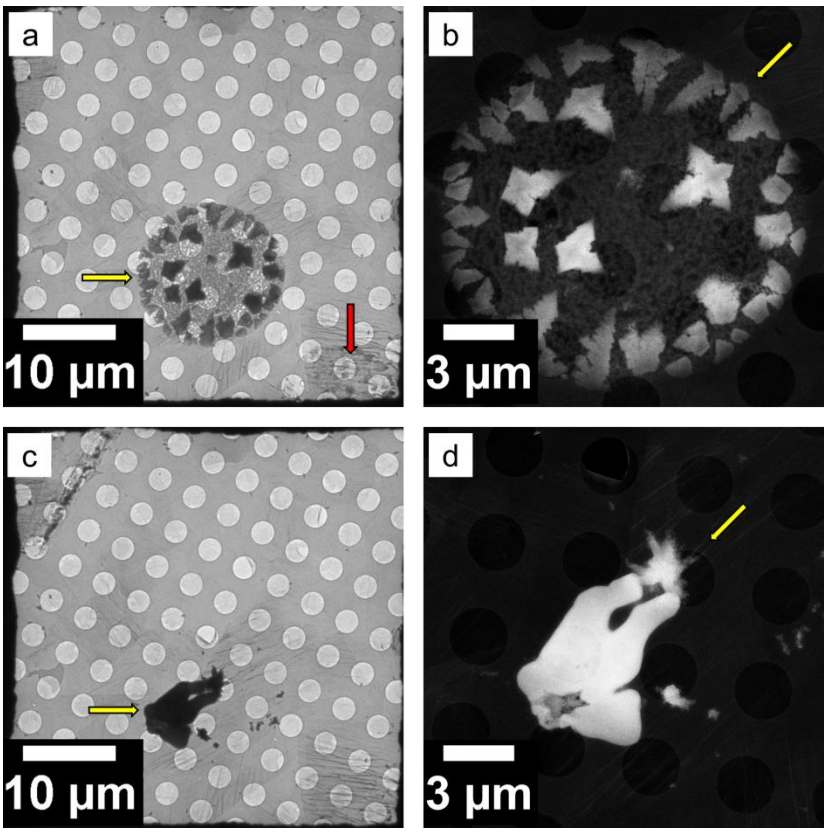

Figure S6. (S)TEM images for the SSA collect by cryo-TEM in Figure 1b, a) and b) bright field TEM and HAADF STEM images respectively after warming inside the microscope to room temperature and c) and d) bright field TEM and HAADF STEM images respectively after removing the sample from the microscope and reinserting into the microscope and thereby exposure the sample to high vacuum.

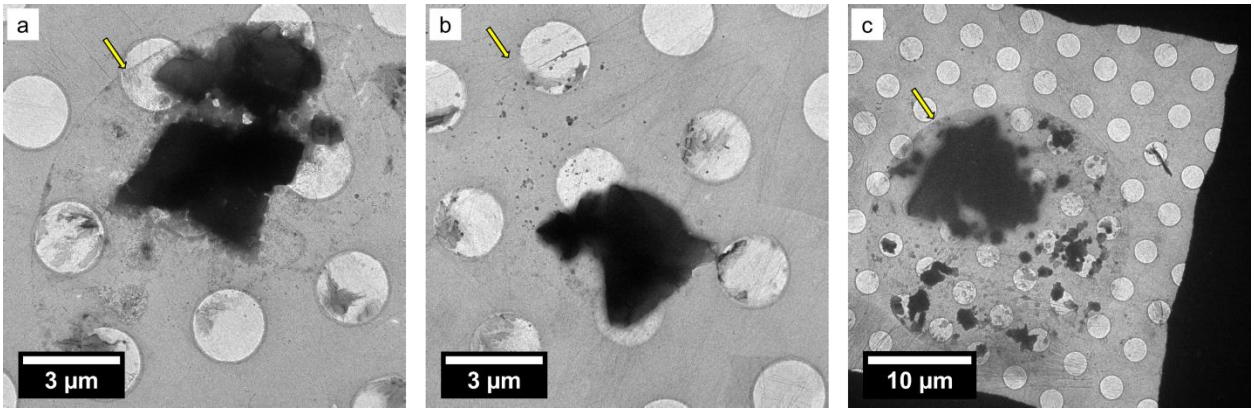

Figure S7. Additional bright field TEM images of SSA collected at stage 3. The yellow arrows indicate the edge of the SSA.

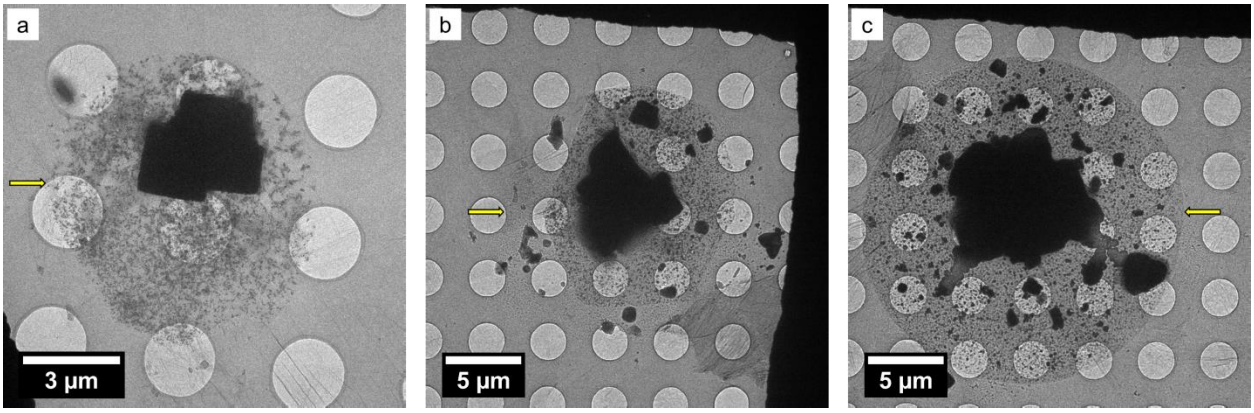

Figure S8. Additional bright field TEM images of SSA collected at stage 4. The yellow arrows indicate the edge of the SSA.

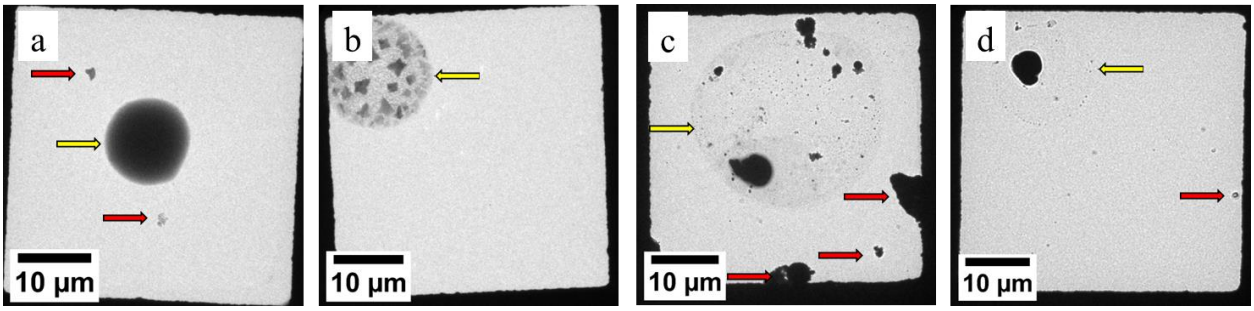

Figure S9. Analogous to Figure 1 in the main text, where SSA particles were collected on hydrophobic amorphous carbon grids after different stages of reorganization and images by bright field TEM. a) stage 1, nascent SSA, b) stage 2, efflorescence, c) stage 3, after laboratory drying and aging and d) stage 4 after exposure to high vacuum. The yellow arrows indicate the edge of the SSA and the red arrows indicate contamination from the cryo-TEM preparation.

Determination of biological structure from the cryo-TEM images:

The cryo-TEM images give a 2D projection of the SSA particles and consequently the assignment of biological structures inside the SSA is based on their size, shape and morphology. The following is a brief description of the biological structures found inside SSA particles, in the SSML or bulk sea water in this study.

Bacterial cells:[5]

Size Approximately 1-2 microns in length

Shape; Rod like

Morphology: Contains a membrane . A membrane is defined as (in a bright field imaging mode) a dark ring around the particles, indicating an increased mass around the exterior of the particle

Virus:[5]

Size <300 nm

Morphology: faceted features.

Membrane vesicle: [6]

Size < 500 nm,

Shape: Largely spherical, although some membrane vesicles in the SSA appeared to be distorted, Figure 3 a) and b),

Morphology: Contains a membrane structure.

Diatom: [7]

Size > 1 micron

Morphology: A silicon containing cell wall. Note: the presence of Si was determined by EDS analysis, shown in Figure S10. Note, no Si was detected in the background spectra collected for the same dwel time.

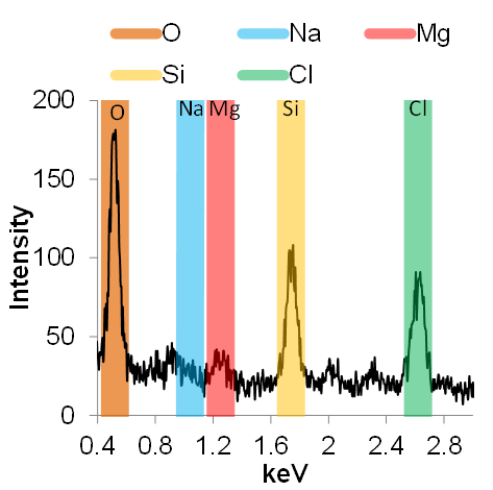

Figure 10. EDS spectra for the particle shown in Figure 2b, strong signal from Si indicated this is likely a diatom. Note, no Si was detected in the background spectra collected for the same dwel time.

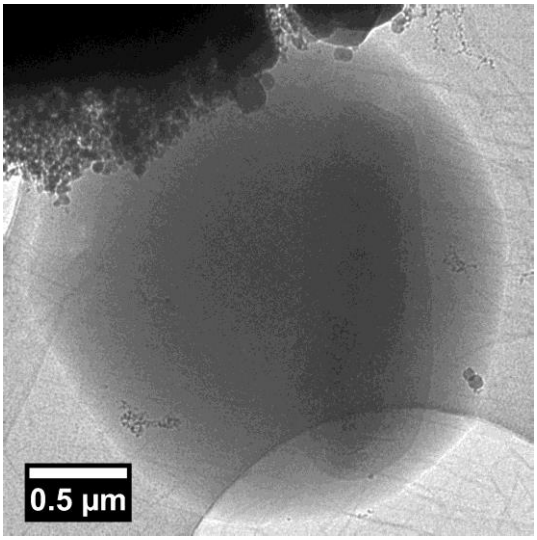

Figure S11. Original unprocessed bright field TEM image corresponding to the processed image in Figure 2a.

Calculation for the number of vesicle per mL by supported cryo-TEM:

The calculation assumes that all vesicles in the 7 µL drop become attached to the GO surface on the carbon film of the TEM grid. Therefore the concentrations of vesicles are likely an underestimation as it is likely that not all vesicles will become attached.

The calculation can then be performed in two ways:

Method 1:

Concentration of vesicles per mL = calculated total number of vesicles on the TEM grid x 1000/7

Calculated total number of vesicles on the TEM grid = the average number of vesicles found per area x the number of areas per grid square (160) x the number of grid squares (400)

OR

Method 2:

Concentration of vesicles per mL = calculated total number of vesicles on the TEM grid x 1000/7

Calculated total number of vesicles on the TEM grid = the average number of vesicles found per area x the number of areas per grid (4*π*15002/7.4) (based on the total size of the 1.5 mm TEM grid and the area of each image which is 7.4 µm2)

Table S1. Summary of vesicle counting studies by GOx-cryo-TEM for the SSML and bulk samples. For each sample the area size was 3 μm^2 and 500 areas were imaged

Sample	Average number of vesicles per area	Vesicles per mL based on method 1	Vesicles per mL based on method 2
SSML run 1	0.152	1.40E+06	8.30E+07
SSML run 2	0.066	6.07E+05	3.60E+07
Bulk run 1	0.03	2.76E+05	1.64E+07
Bulk run 2	0.04	3.68E+05	2.18E+07

As discussed above it is likely that not all vesicles become attached to the grid and therefore both calculations are likely an underestimate and the real value is probably closer to method 2, which we believe is roughly accurate to within an order of magnitude.

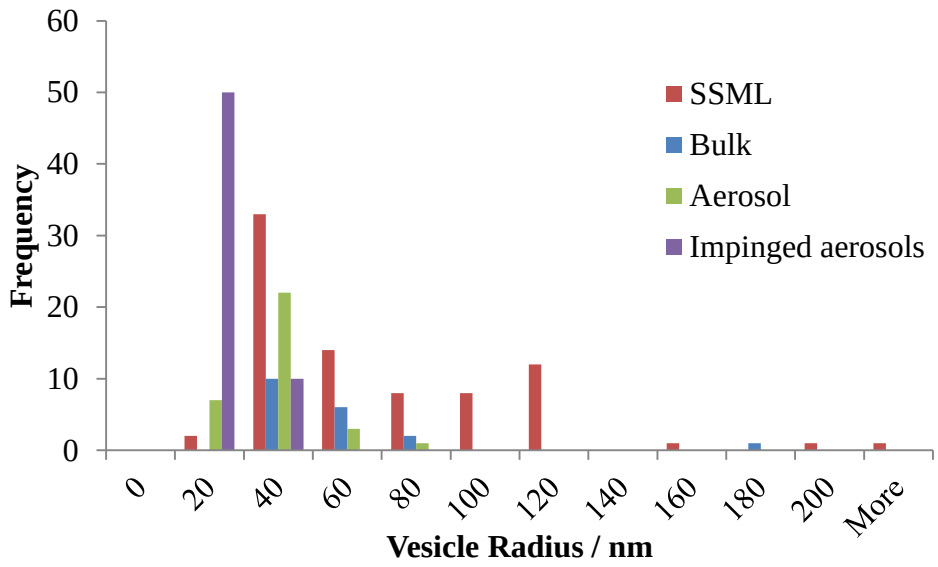

Figure 12. Typical size distribution data for vesicles in the SSML (red), bulk (blue), aerosols collected by capturing by cryo-TEM (green) and impinged aerosols (purple).

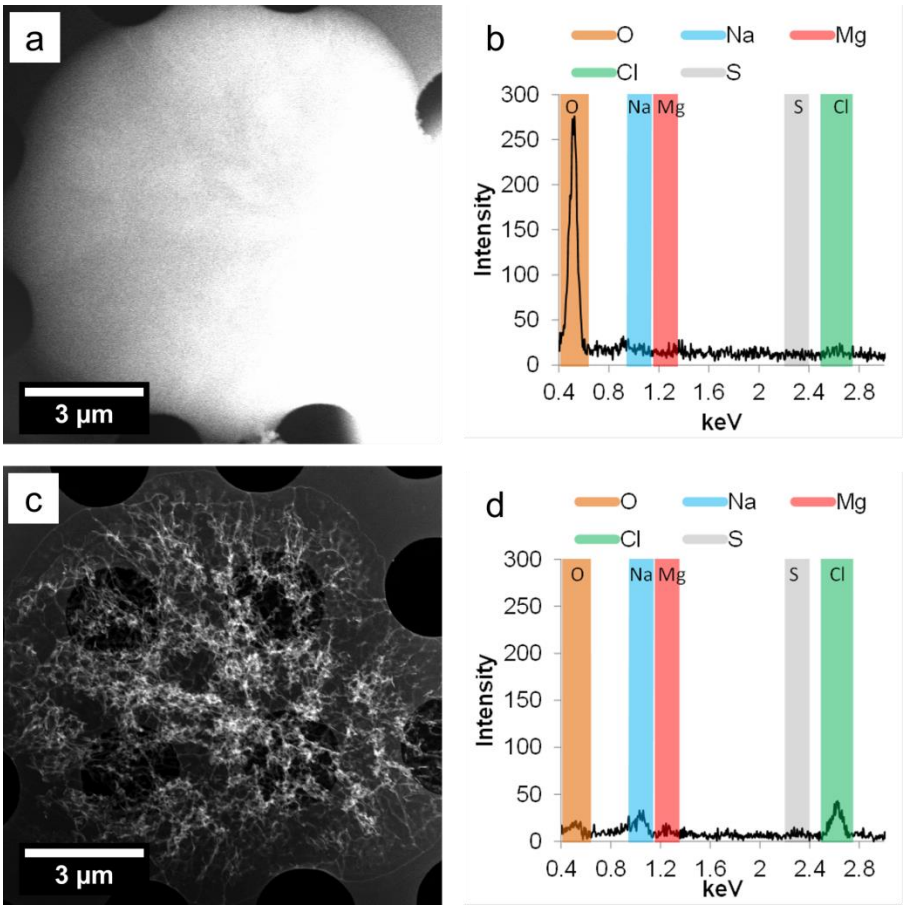

Figure S13. HADDF STEM images and EDS spectra for gel SSA particles. A) cryo-HADDF STEM for a wet particles and b) corresponding EDS spectra taken from the center of the particle, c) HADDF-STEM image for the same particle in a) but after selective dehydration and d) corresponding EDS spectra taken from the center for the particle.

References:

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