

## Cryo-Transmission Electron Microscopy of Sea Spray Aerosols

Joseph P. Patterson,<sup>1</sup> Douglas B. Collins,<sup>1</sup> Jennifer M. Michaud,<sup>1</sup> Jessica L. Axson,<sup>1</sup> Michael D. Burkart<sup>1</sup> Kimberly A. Prather,<sup>1,2</sup> and Nathan C. Gianneschi,<sup>1\*</sup>

<sup>1</sup> Chemistry and Biochemistry, University of California, San Diego, La Jolla, California 92093-0356

<sup>2</sup> Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California 92093

We report the development of the first gas phase cryo-TEM sampling experiments (aerosol-cryo-TEM) allow the detection of hydrated SSA particles by electron microscopy. This unique approach shows the first observation of whole bacteria, phytoplankton, virus particles and marine vesicles within hydrated droplets. Combined with graphene oxide (GOx) supported cryo-TEM of sea surface microlayer (SSML) and bulk seawater, this study reveals the exquisite structural complexity of biogenic marine nanostructures in the ocean and marine atmosphere.

Bacterial marine vesicles are known to be abundant in coastal seawater and the open ocean. The high local concentrations of carbon in the form of DNA, RNA etc. make them a significant source of carbon and information. This study shows that whole intact bacteria and membrane vesicles exist within sea spray aerosol water droplets. While bacteria remain intact, structural differences within membrane vesicles in the SSML and aerosols indicates that the vesicles are formed during the bubble bursting/aerosol production process and therefore exist as a unique structure in the marine environment. Therefore, bubble bursting leading to the ejection of marine vesicles represents a significant mechanism for the transfer of biological nanostructures from the ocean, into the atmosphere and around the planet.

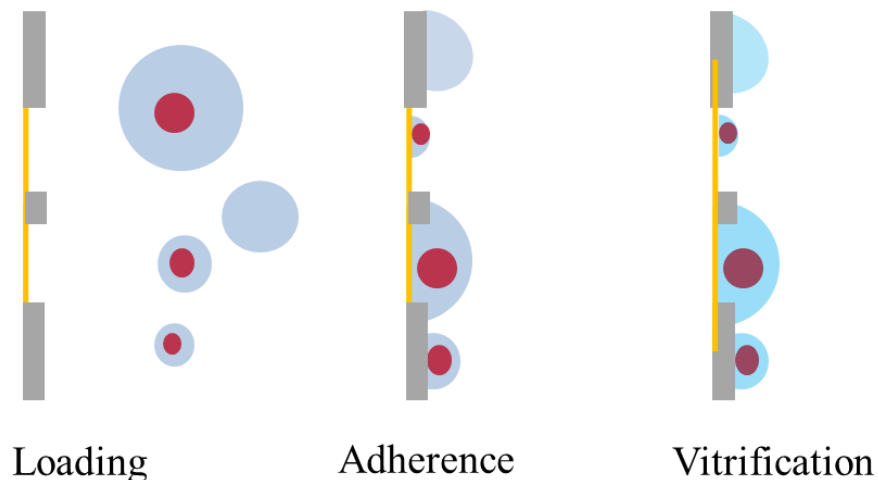
Specifically, the observation of the vesicles in the 50-200 nm size range provides direct evidence for a new mechanism for organic carbon (OC) enrichment in SSA particles, something which has been widely debated in recent literature and previously only ascribed to marine nanogels. This fundamentally changes our knowledge SSA production pathways and their role in cloud formation. While marine vesicles have never been detected in SSA before, lipid-like molecules and vesicles in aerosols have been theorized to play an important role in the evolution of pre-biotic life. This study shows that it is indeed possible for vesicles to be formed and exist in discrete aerosol particles supporting this theory. Thus, this could represent an alternative method for transferring biological species from the ocean and ultimately transported around the planet. We propose this as an alternative to the theorized inverse micelle model for lipids in aerosols. This has implications for how nanostructures comprised of prebiotic species could have resulted in the first single cells.

The ability to observe such nanostructures, as well as their formation pathway at the interface of the ocean, represents a major advance in our understanding of factors influencing the transfer of carbon from the ocean to the atmosphere. We anticipate this study will open a new avenue of analysis for aerosol particles, not only for ocean-derived aerosols, but for those produced over land, in the laboratory, or from other aqueous sources where there is interest in the transfer of organic species.

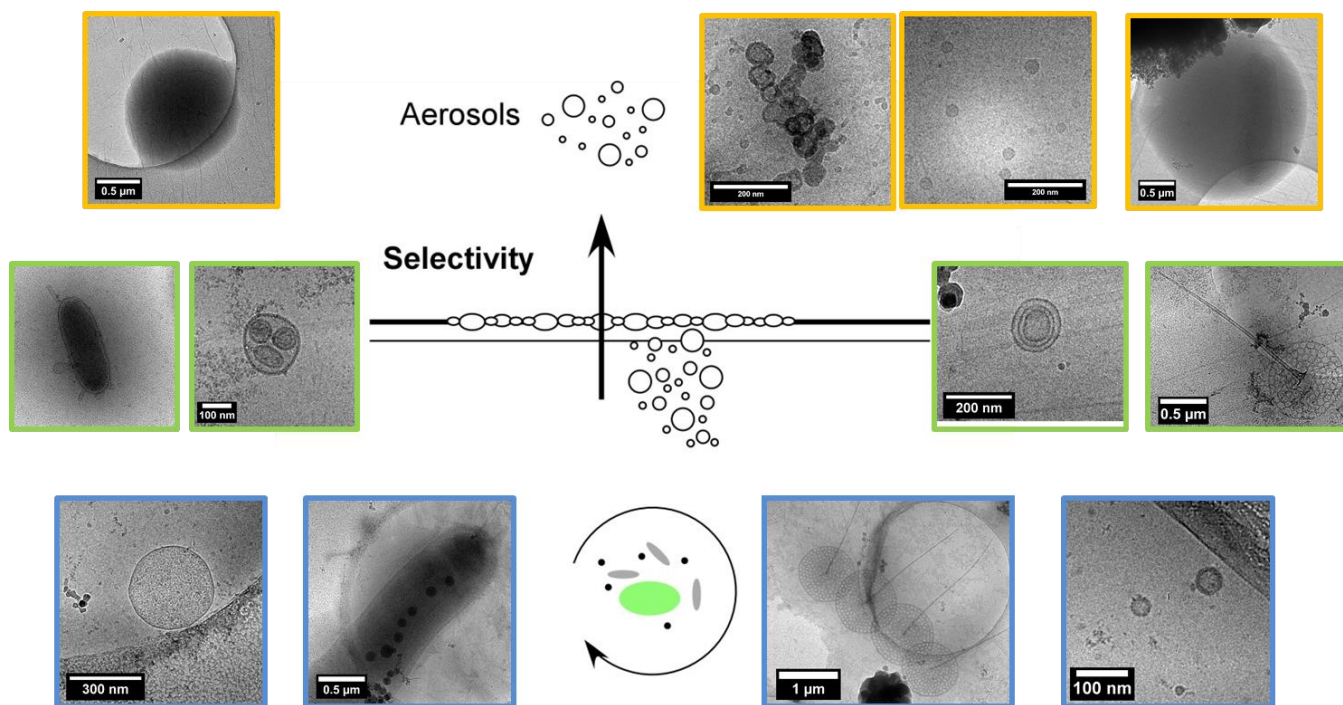
### References

- [1] F Azam, F Malfatti, *Nature Reviews Microbiology*, 5(10), 2007, p. 782.
- [2] SJ Biller et. al, *Science*, 343, 2014, p. 183.

- [3] JP Patterson et. al. *Soft Matter*, 8, 2012, p. 3322.  
 [3] Stokes MD, et al. *Atmospheric Measurement Technology*, 6, 2013, p. 1085.  
 [4] Prather KA, et al. *Proceedings of the National Academy of Sciences*, 110, 2013, p. 7550.



**Figure 1.** Schematic for the preparation of Aerosol-Cryo-TEM grids. Graphene oxide TEM grids are loaded into a humid environment (loading phase). Wet SSA droplets can then passively adhere to the grids (adherence phase). The droplets are then vitrified by rapidly plunging into liquid ethane (vitrification phase).



**Figure 2.** Cryo-TEM images of the marine environment, bulk ocean (blue), sea surface microlayer (green) and sea spray aerosols (orange).