

## EXPERIMENTAL INVESTIGATION OF THE FATE OF MOLECULAR BIOSIGNATURES IN OCEAN WORLD PLUMES: LIQUID-VACUUM TRANSITION.

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**Introduction:** Ocean worlds with plume activity such as Saturn's moon Enceladus and, perhaps, Europa are prime places to search for life without having to access the (sub)surface. Enceladus' plume contains material from subsurface liquid water with organic compounds bearing nitrogen and oxygen, and interacts with a rocky core to provide bioavailable energy, paving the way for a search for signs of life [1].

Yet, plume ejection could affect the potential signatures of life. Several such signatures involve measuring relative abundances of amino acids and lipids [1,2]. In the water plume of Enceladus, vapor (90–99% of plume mass [3]) dominates over ice. This is in part due to ice grains falling back to the surface, but fallout alone may not explain the high vapor enrichment over the  $\approx 1:7$  vapor:ice proportions expected from exposing liquid water at the triple point to a cold vacuum [4]. This suggests a depletion in nonvolatile species in the plume, perhaps by distillation, condensation, and/or recycling between the subsurface water table and the surface vent. This could skew relative abundances of amino acids and lipids of varying volatility or solubility in the plume relative to the ocean.

Here, we investigate experimentally whether, and to what extent, the liquid-vacuum transition can change relative abundances of amino acids and lipids targeted by search-for-life measurements.

**Methods:** The experimental setup at NASA GSFC (Fig. 1) consists of an organic-clean (no elastomer seals), bakeable vacuum chamber in which a solution is injected at 3 to 25 mL/h using a low-flow valve. Liquid passing through the valve turns into vapor and  $\mu\text{m}$  to mm droplets that partially vaporize; the resulting energy loss freezes their core within  $10^{-3}$  to 1 second [5]. The injection line is heated to  $30^\circ\text{C}$  to prevent freeze-clogging and ensure boiling rather than sublimation. The chamber base pressure,  $10^{-6}$  Torr, rises upon injection to  $10^{-2}$ -1 Torr depending on the flow rate, remaining below the 4.6 Torr triple point pressure of  $\text{H}_2\text{O}$  (liquid is not stable). The abundant vapor injected is evacuated by turbo- and fore-pumps, and also condensed on a Stirling-cryocooled copper plate at 175 to 260 K depending on flow rate. A side benefit is the ability to recover condensed vapor for analysis. The chamber is at  $25^\circ\text{C}$ , allowing water in the droplets to be lost and the residue (salt and organic compounds) to be recovered from an aluminum cup facing the injector.

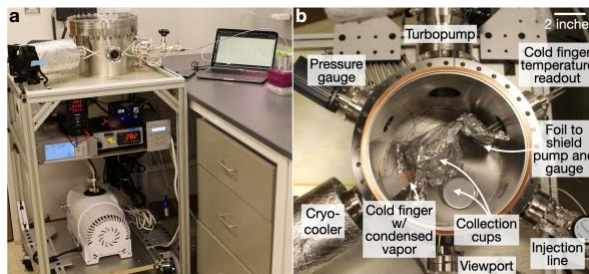


Figure 1. Experimental setup. (a) 3-tier rack with chamber (top), pressure and temperature controllers (middle), and forepump (bottom). The injection line is to the right, in front of the laptop. (b) Top view of the chamber with, inside, a cryocooled copper plate (with trapped frozen vapor) and foil screen.

We injected millimolar ( $\text{mM} = \text{mmol per kg H}_2\text{O}$ ) mixtures of amino acids (phenylalanine, tyrosine, histidine) and lipids (palmitic acid, phenylacetic acid) of diverse size and structure, in 1%  $\text{NaCl}$  solutions with pH adjusted to 9-10 using  $\text{Na}_2\text{CO}_3$ . Salinity and pH are those of Enceladus plume grains [3]. Organics were  $\sim 10^4$  more concentrated than expected at Enceladus [1] to ensure detectability. Palmitic acid was super-saturated, but no solid could be seen in the solution, likely because palmitic acid was suspended as micelles.

Residue and vapor samples were frozen until compositional analysis at Oakland University. Amino acid abundances were determined by high-performance liquid chromatography, after adding 2.0 mL phosphate buffer saline containing 10 mM of phenol (internal standard) and 1.0 mL deionized (DI) water, extraction by vortexing and sonication, neutralization with 0.25 M hydrochloric acid (HCl), and adding DI water to increase the extract volume to 4.0 mL. Solutions were heated to  $70^\circ\text{C}$  to dissolve any leftover solids, and filtered on a  $0.45 \mu\text{m}$  syringe filter. Lipid abundances were determined by gas chromatography after acidification with 2.0 mL of 3.6 M HCl, extraction in 4.0 mL dichloromethane (DCM), evaporation, and redissolution in a 0.5 mL mixture of DCM and decane (internal standard).

**Results:** Most of the white residue coated the injection line from the low-flow valve to, and including, the chamber port (Fig. 2). For these experiments, we analyzed only the organic composition of the smaller fraction of dry residue from the cup in the chamber.

The mass of organic material in this cup was a few percent of that in the parent solution (Fig. 3). Recovery

was higher for more dilute solutions. For each injection, up to a two-fold spread in recovery among organic compounds indicates possible changes in their relative abundances upon injection.

The vapor collected on the cold finger was essentially organic-free, containing < 0.015% and < 0.001% of the injected amino acid and lipid solution, respectively, based on total organic carbon analysis.

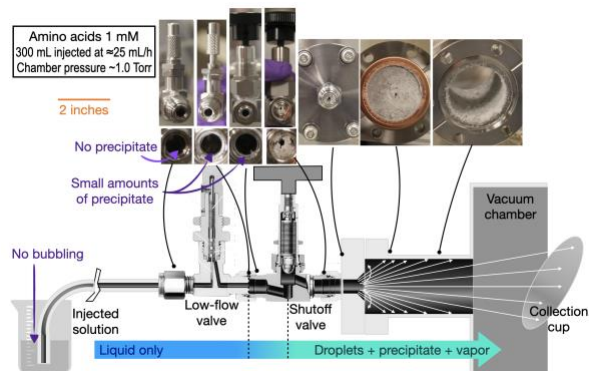


Figure 2. Injection line post-experiment. The amount of white solid deposit increases with increasing distance from the low-flow valve.

**Discussion: Solubility.** The abundant precipitate formed along the injection path, as water is lost to vaporization, suggests that compound solubilities may affect their relative abundances in the cup residue. For each injection, there is indeed a general increasing trend in recovery rate with increasing solubility (Fig. 3a) and with decreasing ratio of parent concentration to solubility (Fig. 3b). Histidine is an outlier, with low recovery despite being the most soluble. The pattern among amino acids is consistent for both injections despite a 20x difference in initial concentrations. The variability in recovery rate across injections suggests that other factors too may change relative abundances.

**Flow rate.** Higher rates of vapor and liquid flow may move more solutes or precipitates along the injection line by limiting the gradual loss of water and, perhaps, by mechanically dislodging prior precipitate.

**Chemical structure and functionality.** Tyrosine, phenylalanine, and phenylacetic acid all comprise a short carbon chain attaching to a benzene ring, yet span nearly the full range of recovered fractions. This points to a possible effect of the amine ( $-NH_2$ ) and phenol ( $-OH$ ) groups that distinguish them on recovery. Likewise, the nitrogen-containing, five-membered ring (imidazole) in histidine may play a role in its relative dearth in the collection cup.

**Volatility.** The lack of organic matter in condensed vapor makes sublimation of these organic compounds an improbable cause of abundance fractionation.

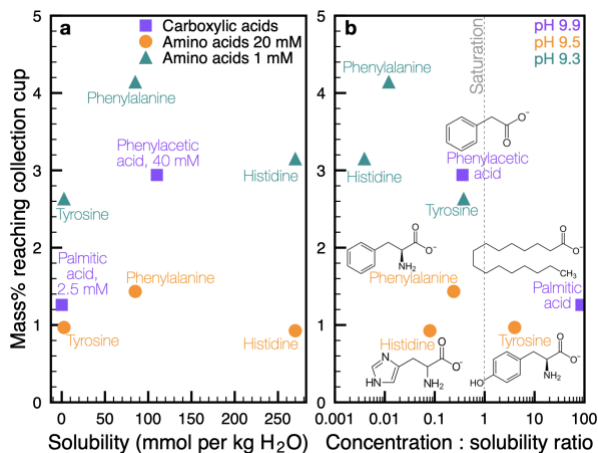


Figure 3. Ratio of mass of compound recovered as residue in the collection cups placed in the chamber to the mass of compound in the injected solution, as a function of solubility (a) and parent concentration-to-solubility ratio (b). Concentrations and pH in the parent solution are shown in the legend and labels.

**Charge.** At the injection pHs, the carboxyl groups are deprotonated ( $COO^-$ ; Fig. 3b) and likely interact with  $Na^+$  in solution. The pH for addition of  $H^+$  to the  $-NH_2$  group (pKa) is about 9.1-9.2 for all three amino acids, close to the pH of the two amino acid solutions injected (9.3-9.5). The resulting partial protonation to  $NH_3^+$  could also affect ionic interactions with salts. Such organic-salt interactions can fractionate relative abundances of organics such as lipids at liquid water-gas interfaces [6], but here charge seems not to be affecting the relative abundance of amino acids with similar pKa.

**Conclusions:** These pilot results point to shifts in relative abundances and distribution of organic compounds targeted in the search for life upon transitioning from liquid water to vacuum environments. They remain to be confirmed, and their causes elucidated, with experiments involving a broader set of compound structures at lower concentrations closer to those expected at Enceladus [3].

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**References:** [1] MacKenzie S. M. et al. (2022) *Astrobiology*, 22, 685–712. [2] Hand K. P. et al. (2022) *PSJ*, 3, 22. [3] Postberg F. et al. (2018) In *Enceladus and the Icy Moons of Saturn*, UA Press, 129–162. [4] Goldstein D. B. et al., *ibid*, 175–194. [5] Stedum R. E. (1971) PhD dissertation, LSU. [6] Cochran R. E. et al. (2016) *J. Phys. Chem. Lett.* 7, 1692–1696.