DEVELOPMENT OF ON-CHIP PURIFICATION OF PROTEINOGENIC AMINO ACIDS, FOR IN SITU EXTRATERRESTRIAL ANALYSES ON OCEAN WORLDS.

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Introduction: Many challenges exist for *in situ* techniques searching for signatures of life in planetary environments. Whether exploring the ocean worlds Europa and Enceladus or the dunes of Mars, collected samples are likely to contain high salinity and are expected to have very low biomass, if present. These environmental factors would act as significant challenges to the instruments/techniques currently used to analyze *in situ* samples for biosignatures (e.g. amino acids and their chirality, or DNA/RNA) and may include mass spectrometers, fluorescent-based optics, and nanopore sequencers [1]. To surmount these challenges, we are developing a robust sample preparation method to desalinate proteinogenic amino acids, independent of type and concentration of salt in the sample on a microfluidic chip. This technique can be used as a single purification tool for multiple downstream biosignature detection analyses. Though similar methods have been used to desalinate meteorite samples on Earth [2], this has yet to be developed as an *in situ* space flight instrument.

Methods: Here we describe the separation achieved using a modular microfluidic chip (Figure 1) that allows for non-destructive sample preparation to remove ionic interference, minimize loss of amino acids, and increase downstream detection sensitivity. Salts and amino acids were chosen as planetary analogs for environments of icy ocean worlds such as Europa and Enceladus [3, 4] and the amino acids used by terrestrial biology [5]. For the experiments, we introduced solutions of one salt (NaCl, $CaCl_2$, or $MgSO_4$) and an aromatic amino acid (phenylalanine).

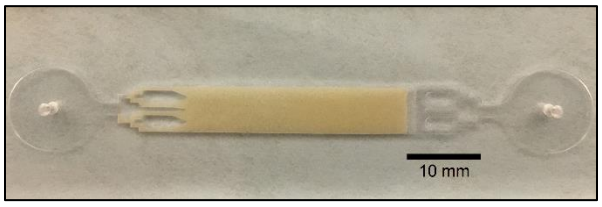

Figure 1. Microfluidic chip (50µL of beads loaded) that allows separation of salts and amino acids.

Results: Phenylalanine has been successfully purified from three salt types: NaCl, $CaCl_2$, and $MgSO_4$, at multiple concentrations.

Figure 2 shows results of on-chip separation of 25µL and 150µL of 5M NaCl with 50 µL of 100mM phenylalanine. The amount of salt-free amino acid collected at the end of the separation decreases as the amount of salt introduced into the chip increases. This is because the salt preferentially binds to the resin, which allows some amino acid to elute during the water wash.

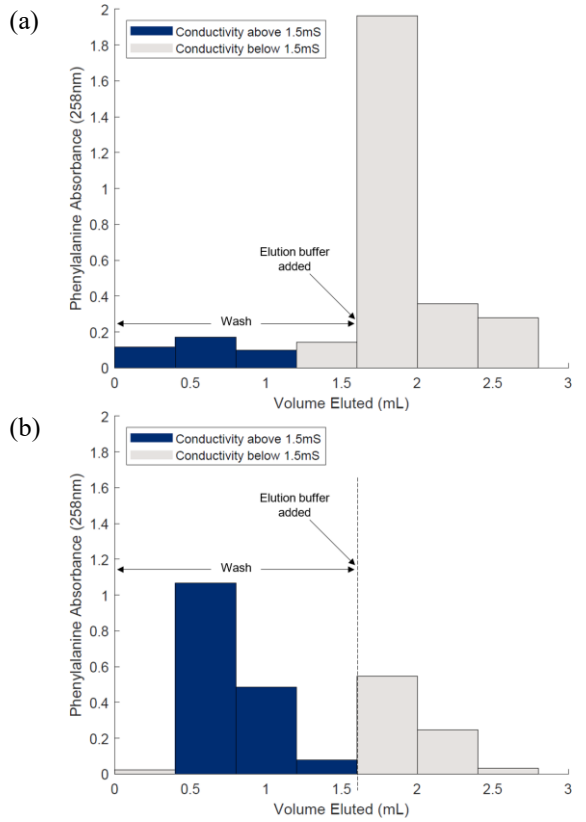

Figure 2. On-chip separation results for (a) 25µL and (b) 150µL of 2.5M MgSO4 with 50µL of 100mM Phe. Vials (~400uL) were collected to determine the conductivity (directly proportional to salt concentration) and UV/Vis absorption (directly proportional to amino acid concentration).

Conclusion: This technology shows promise as a first step for *in-situ* purification of amino acids from salty solutions. Further engineering is underway to improve yields, evaluate different amino acids, optimize performance, and consider space flight design.

References: [1] Stockton et al., 2009, *Astrobiology, 9* [2] Glavin et al. 2010, *Meteor. & Planetary Sci*., *45*; [3] Brown and Hand 2013, *The Astronomical Journal*, *145*(4), 110; [4] Postberg et al. 2009, *Nature*, *459*. [5] McKay 2004, *PLoS Biol. 2*, e302.