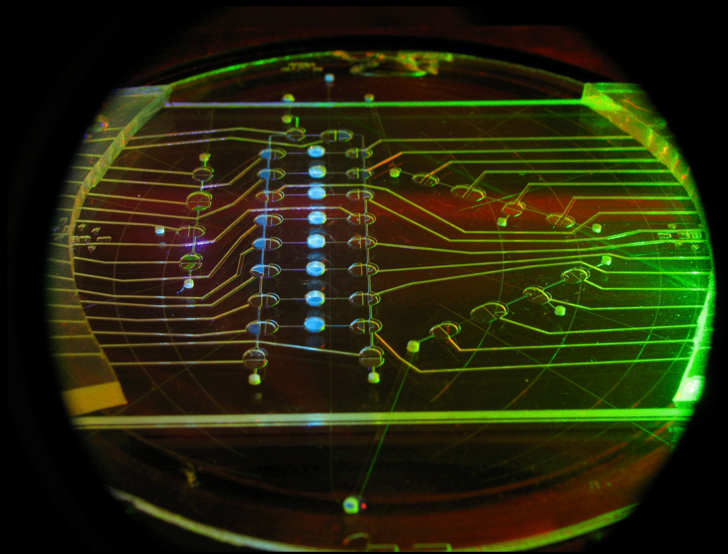
ABSTRACT

We report new microchip non-aqueous capillary electrophoresis (μNACE) methods for the electrophoretic separation and analysis of organic compounds of relevance to astrobiology. We previously reported a protocol for μNACE of primary aliphatic amines in ethanol, and demonstrated separations of short- and long-chain amines down to -20°C. We have optimized this protocol further, and used it to analyze a range of Titan aerosol analogues (tholins). We have also developed a non-aqueous analytical method for analyzing fatty acids utilizing a new custom dye, and tested the method on sedimentary samples collected from a hydrothermal vent system on the ocean floor.

We also report our first field demonstration of our completely portable microfluidic CE apparatus.

Non-Aqueous Microchip Capillary Electrophoresis of Long-Chain Aliphatic Amines in Titan Simulant Material & Fatty Acids in Deep Ocean Sediments



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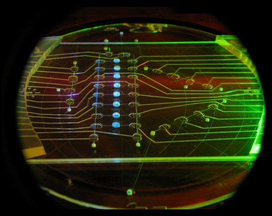
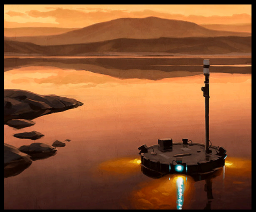
Presentation at MicroTAS 2014, San Antonio, TX
Session 1C1 - Microchip Electrophoresis
10:50-11:10 AM, October 27, 2014

OUTLINE



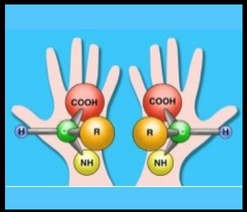
NACE



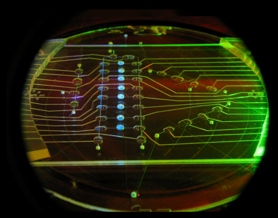
AMINES

FATTY ACIDS

INSTRUMENTATION



SUMMARY & FUTURE OUTLOOK

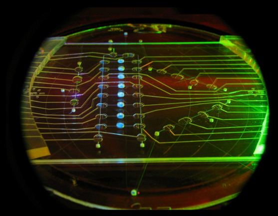


Non-Aqueous Capillary Electrophoresis (NACE) & CHEMICAL EXPLORATION OF SOLAR SYSTEM

- chemical exploration for habitability and life is ***the new frontier*** in exploration beyond Earth

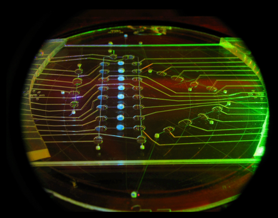
- microfluidic systems would be ideal for this (small, sensitive and fast - see previous talk)

- efforts since 1990’s have focused on aqueous solutions — new efforts utilizing non-aqueous solvents open up a host of technical and scientific avenues, including analysis of organics most likely to survive geological time periods on Mars



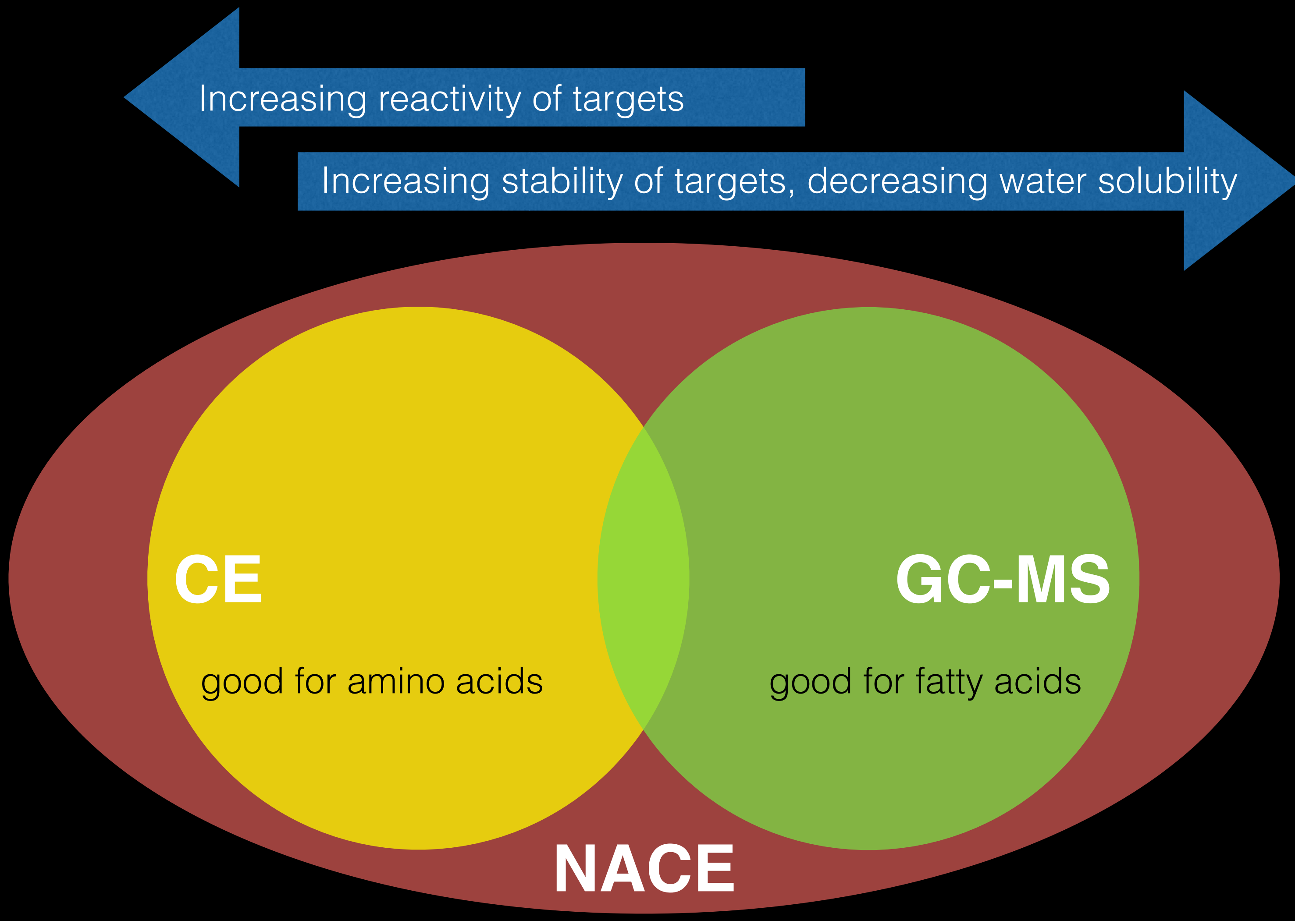
ADVANTAGES OF NACE FOR NASA MISSIONS

- it can dissolve the key organics believed to have highest chance of surviving and existing on Mars today
- it enables a direct correlation with GC-MS measurements

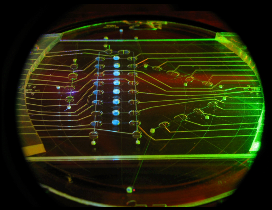


NACE

- different techniques target different organic classes; NACE can target them all

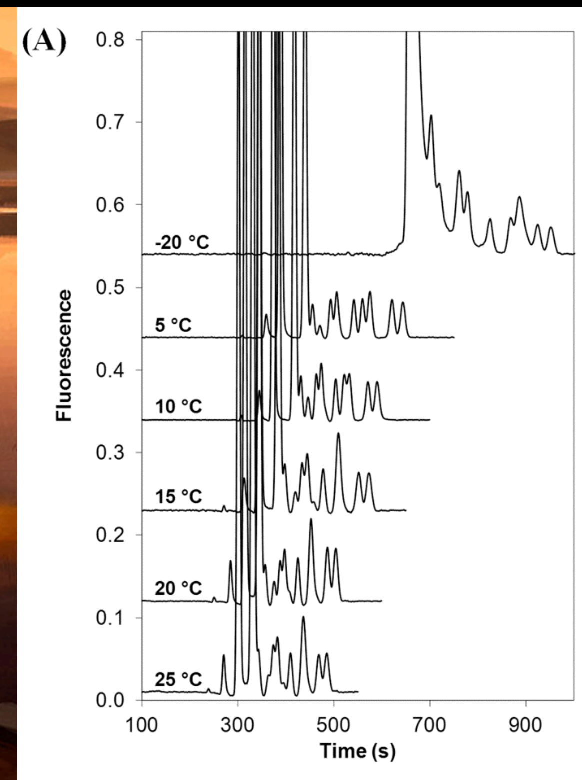


- GC-MS is the NASA “gold standard”



OTHER ADVANTAGES OF NACE FOR NASA MISSIONS

- it greatly alleviates critical thermal constraints on robotic missions - don't need to waste heat because solutions don't freeze at zero Celsius (*extremely important for in situ Titan missions*)

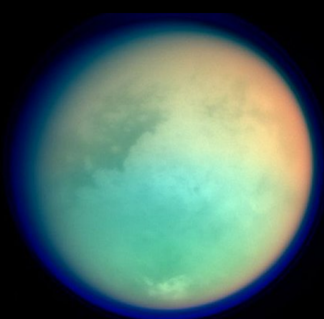


M.L. Cable, P.A. Willis et al., Anal. Chem., 85, 1124-1131 (2013).

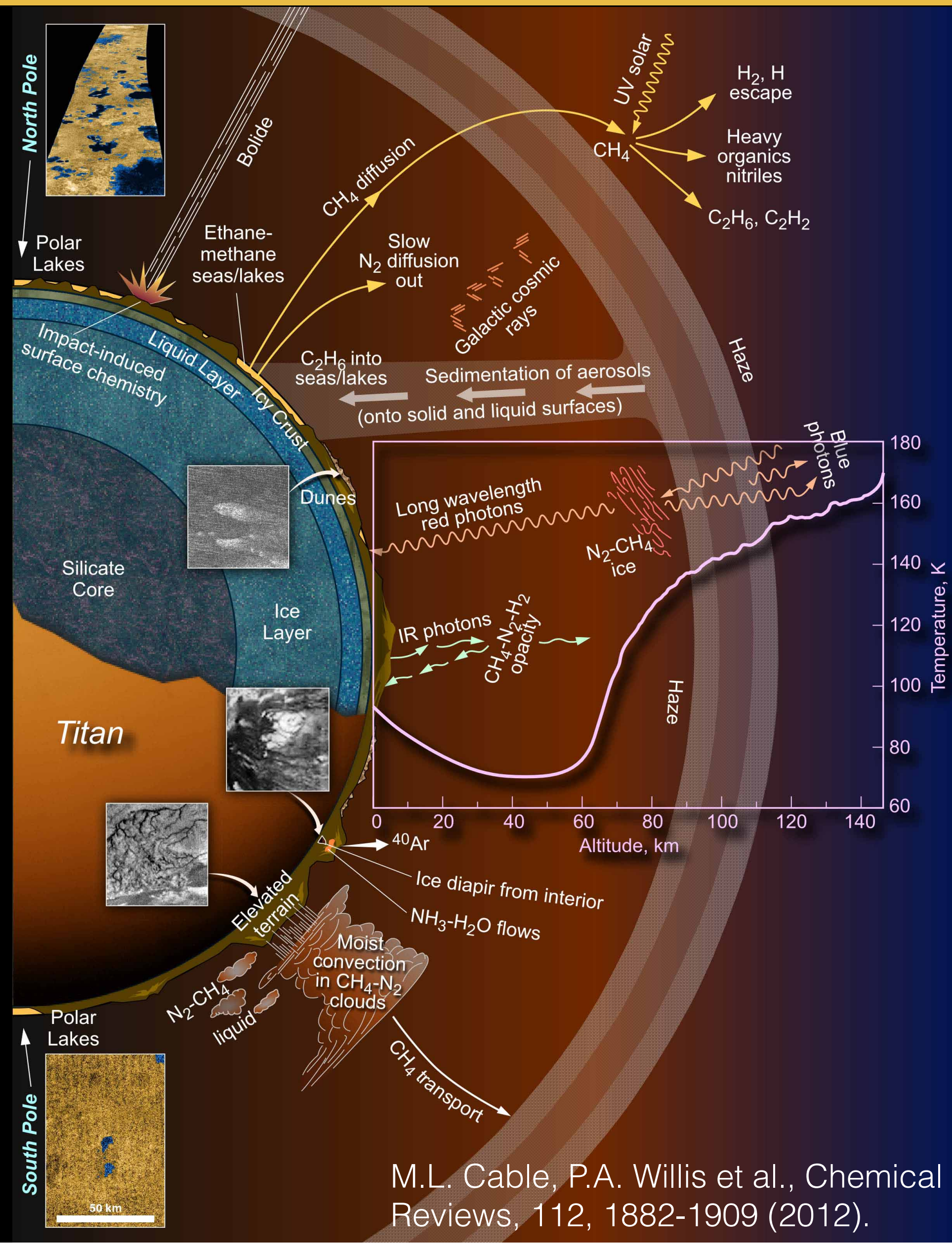
- it has intrinsic planetary protection advantages - microbes grow poorly in solvents

WHY WE CARE ABOUT AMINES ON TITAN: *PREBIOTIC CHEMISTRY*

- Atmospheric modeling suggests amines are present on Titan but they have not been detected yet
- Amines may be exposed to liquid water at meteoritic impact melts or in ammonium/ice cryovolcanoes, possibly creating chemical conditions for the production of proteins

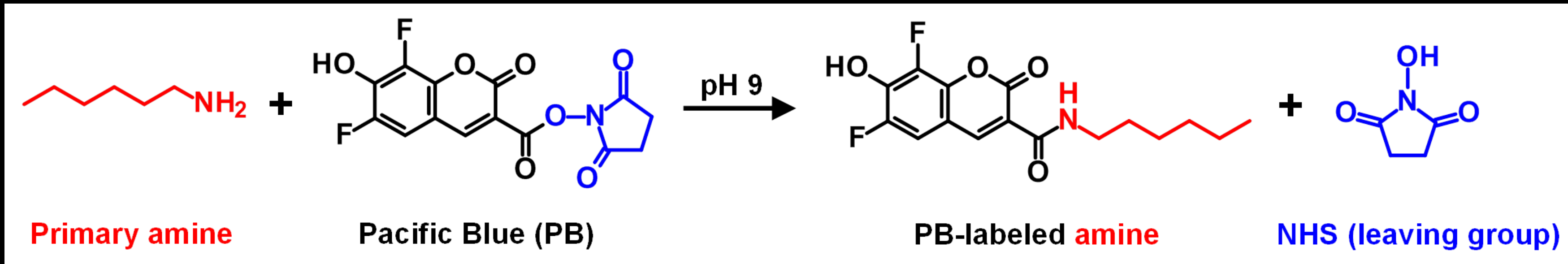


- **Our experiments (described next) show that amines are present in Titan simulation samples.**
- **This begs the question: are cryovolcanoes real?**



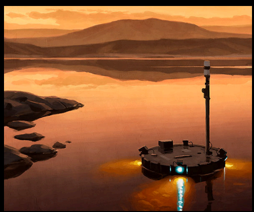
M.L. Cable, P.A. Willis et al., Chemical Reviews, 112, 1882-1909 (2012).

OPTIMIZATION OF ALIPHATIC AMINE ANALYSIS

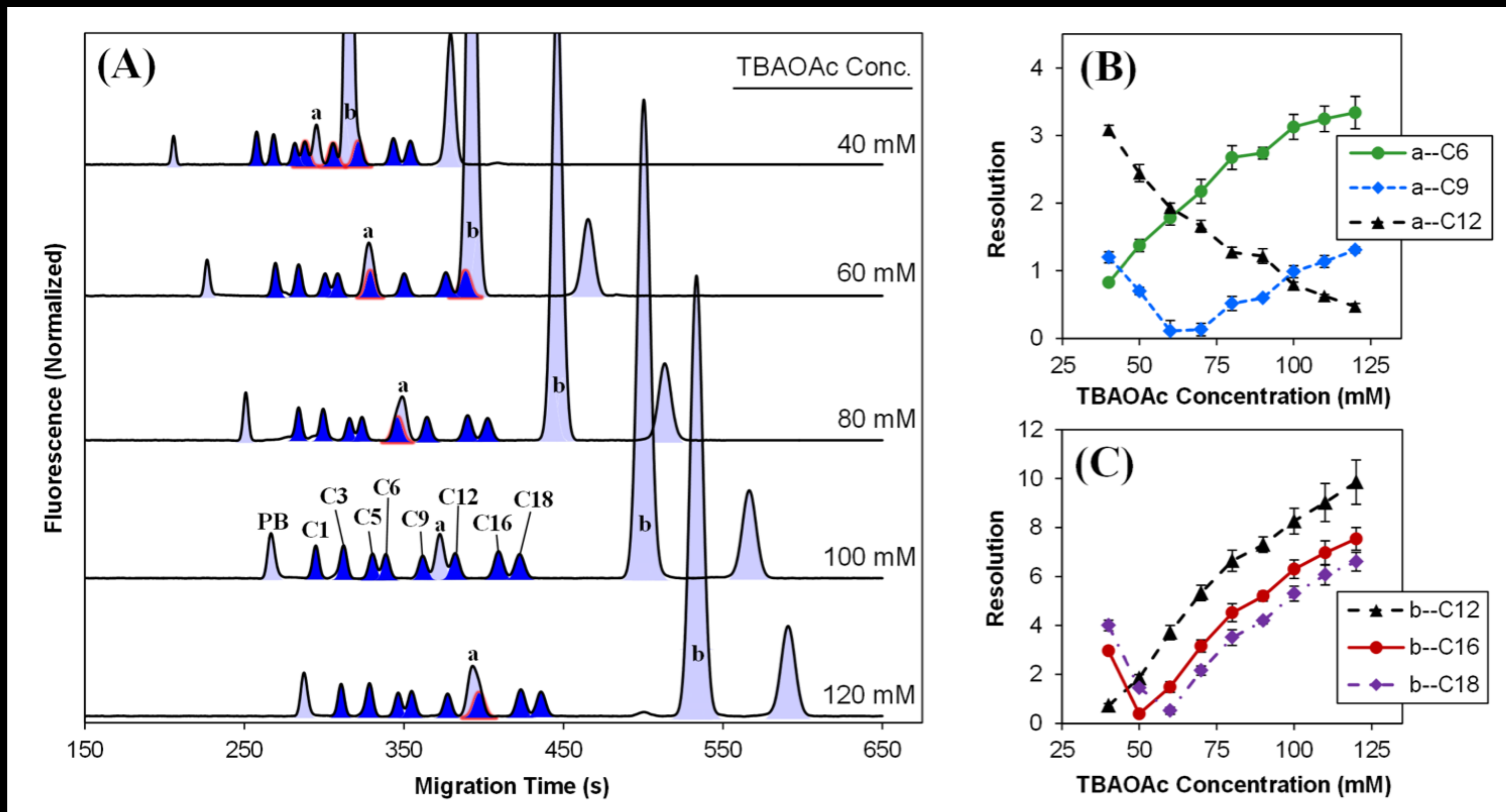


Labeling Reaction of Pacific Blue succinimidyl ester (PB) with a primary amine under basic conditions.

- use of N,N-diisopropylethylamine (DIEA, pKa = 10.5) as the organic base in labeling reactions (in place of Et_3N used in previous work) eliminates C_2 contamination
- use of tetrabutylammonium acetate (TBAOAc) in ethanol buffer system improves results by greatly reducing contamination from ammonia (present in previous work which utilized ammonium acetate in ethanol)



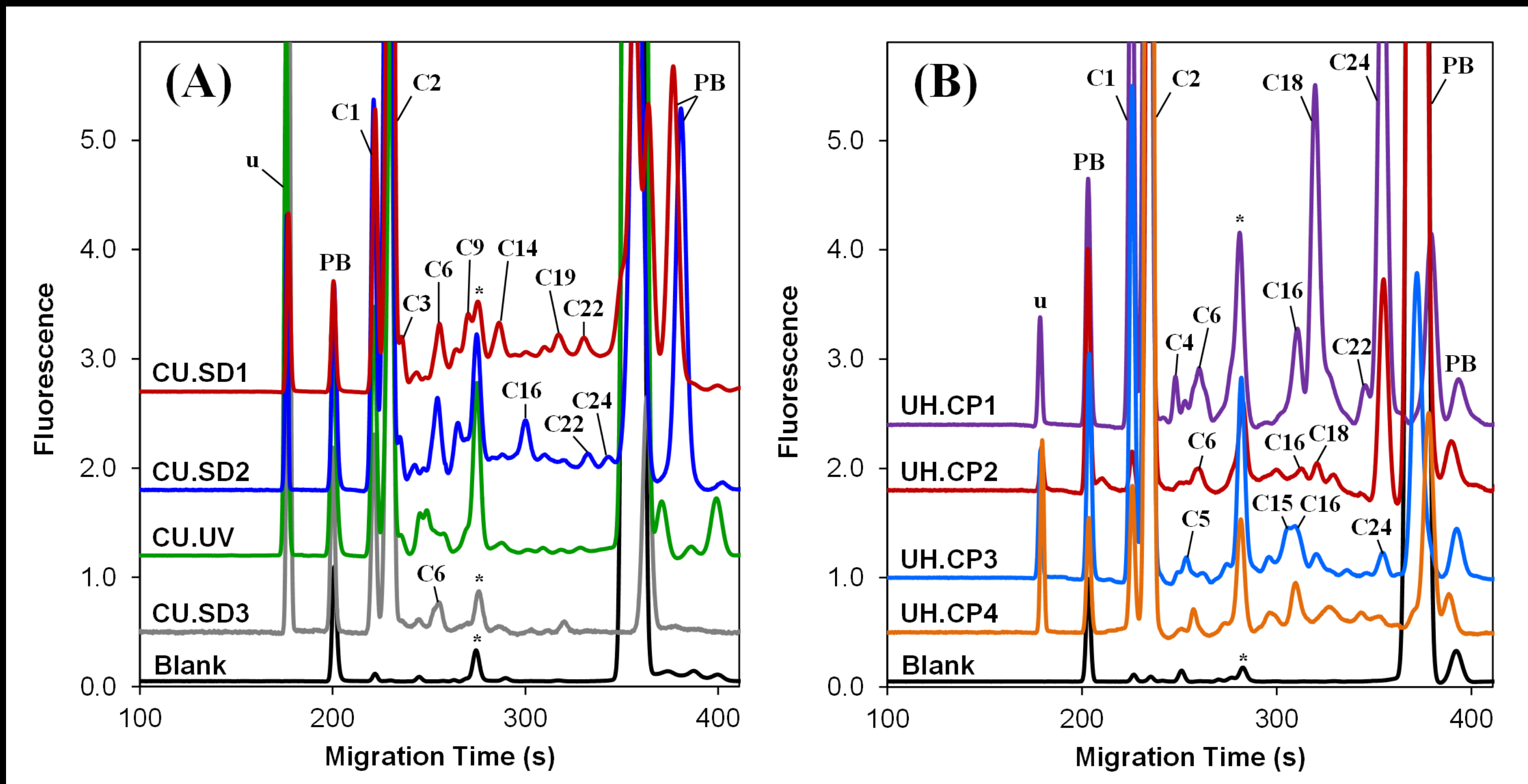
OPTIMIZATION OF ALIPHATIC AMINE ANALYSIS



- Separations of 100 nM C1-C18 amines in ethanol, with various concentrations of TBAOAc. PB and background peaks are shown in light gray; amines in dark blue.

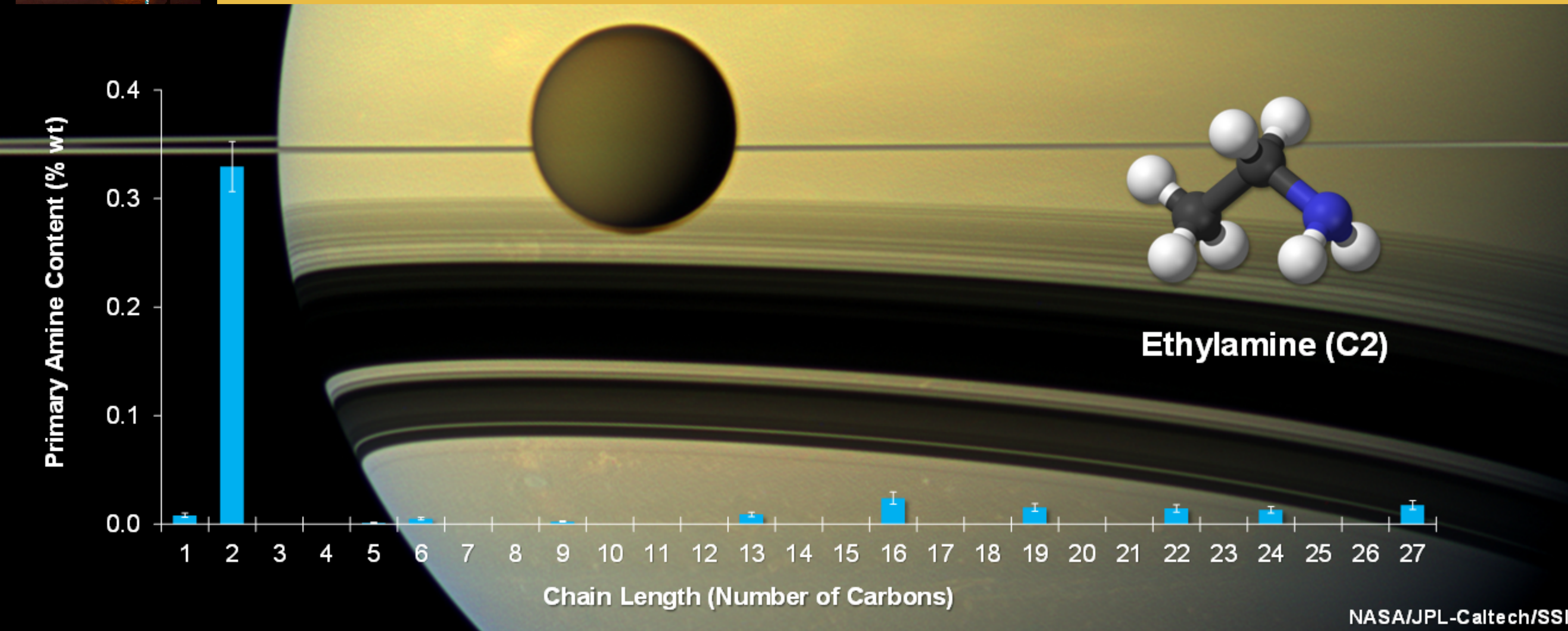
- Optimal run buffer: 100 mM TBAOAc and 1.05 M acetic acid in ethanol

ANALYSIS OF AMINES IN TITAN SIMULANT MATERIALS USING NEW METHODS DEVELOPED



- NACE analysis of 1 mg/mL labeled tholins from (A) University of Colorado at Boulder and (B) University of Houston.
- All samples were labeled at 50 mg/mL with 5 mM Pacific Blue in 25 mM diisopropylethylamine in ethanol.
- **Wide range of amines measured in all samples**

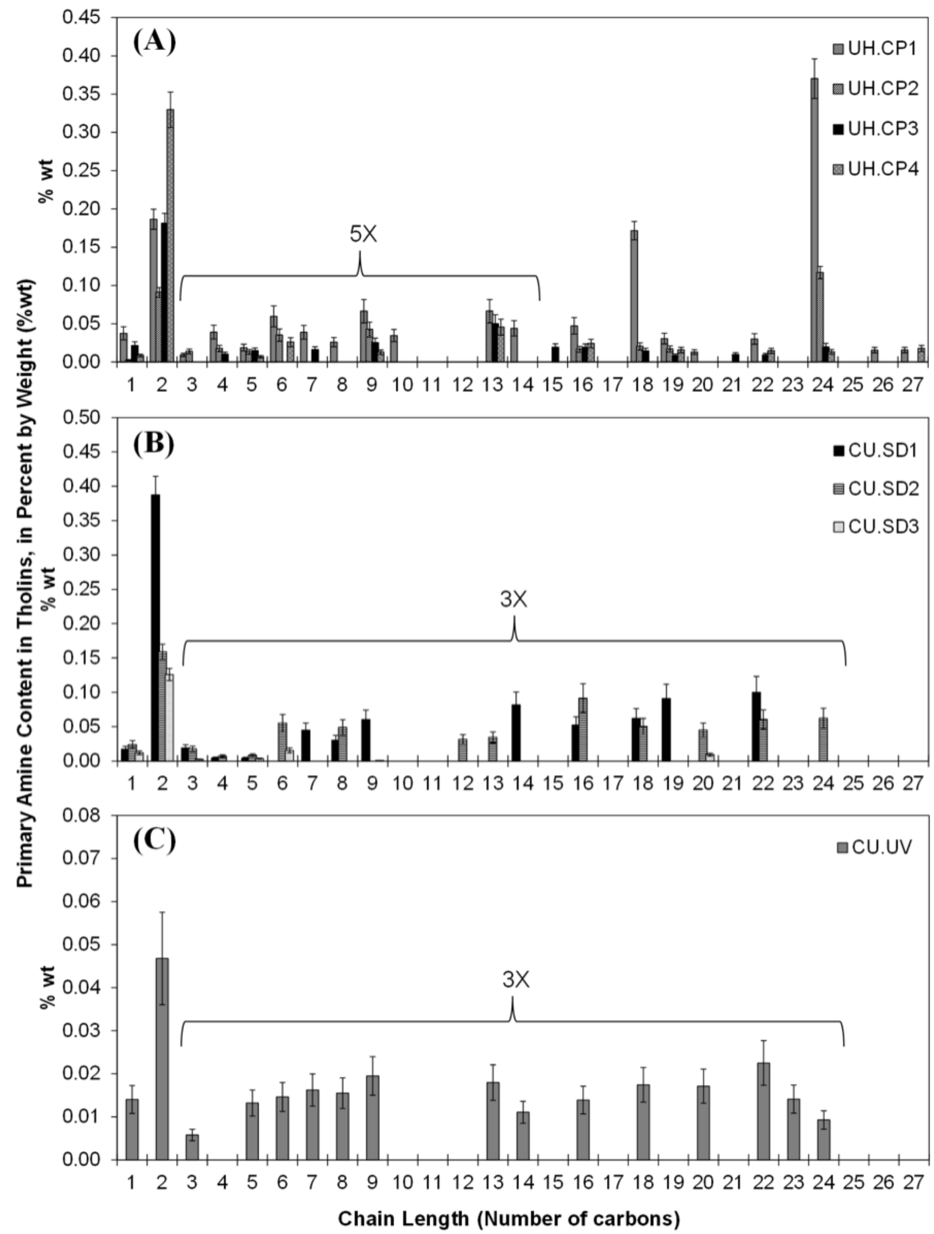
ANALYSIS OF AMINES IN TITAN SIMULANT MATERIALS



- A wide range of amines observed, with ethylamine as major product
- Amino acids not observed

ANALYSIS OF AMINES IN TITAN SIMULANT MATERIALS

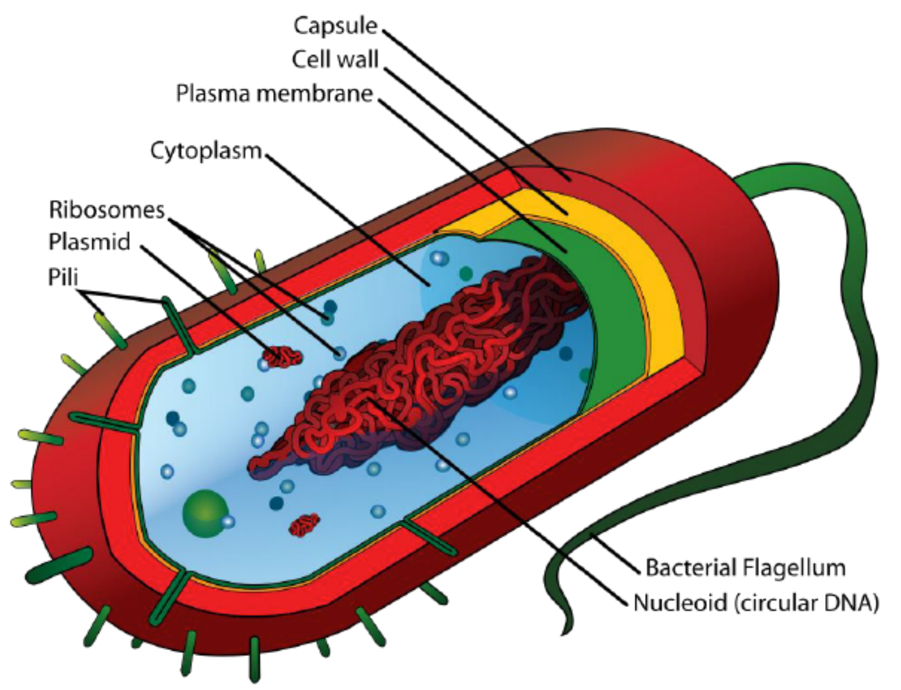
- complex production mechanism proceeds through ethyl amine
- patterns indicate complexity yet little order (more on this later)

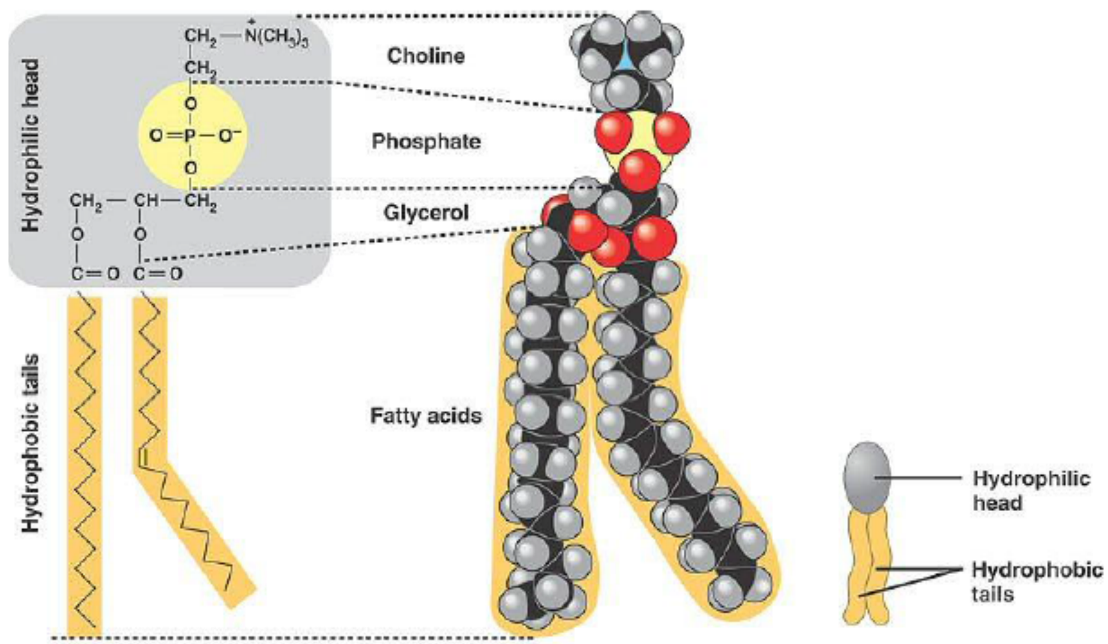


ALIPHATIC AMINES IN TITAN SIMULANT MATERIALS - SUMMARY

- We optimized nonaqueous microfluidic analysis of amines.
- We analyzed simulated Titan samples (tholins) produced under conditions similar to those in Titan's atmosphere.
- Amines are likely to exist on Titan.
- No compelling biosignatures, signs of prebiotic chemistry, or patterns observed in data sets.
- Amino acids were also not observed in samples. However, if amines from Titan's atmosphere react with water in cryovolcanoes, protein formation may be possible.

IMPORTANCE OF FATTY ACIDS TO BIOLOGY

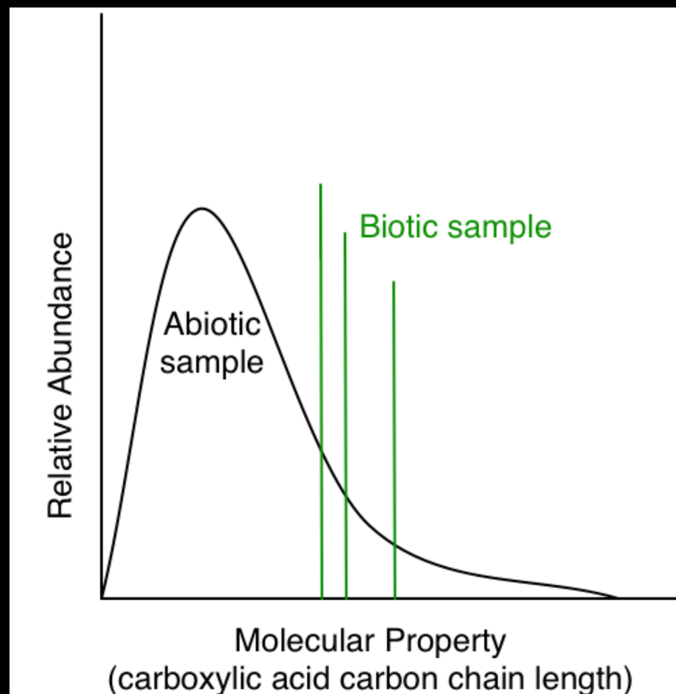




Fatty acid part is extremely chemically durable

FATTY ACIDS: HOW MOLECULAR DISTRIBUTIONS CAN PROVIDE INDICATIONS OF LIFE

The distributions of carbon chain length in Titan tholin samples are an example of an **Abiotic sample**



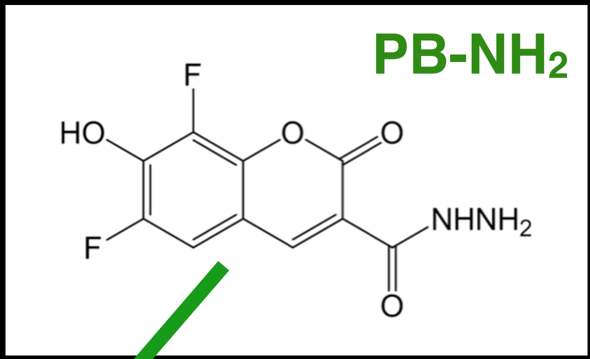
a **Biotic sample** is enriched with a subset of biological “building blocks”

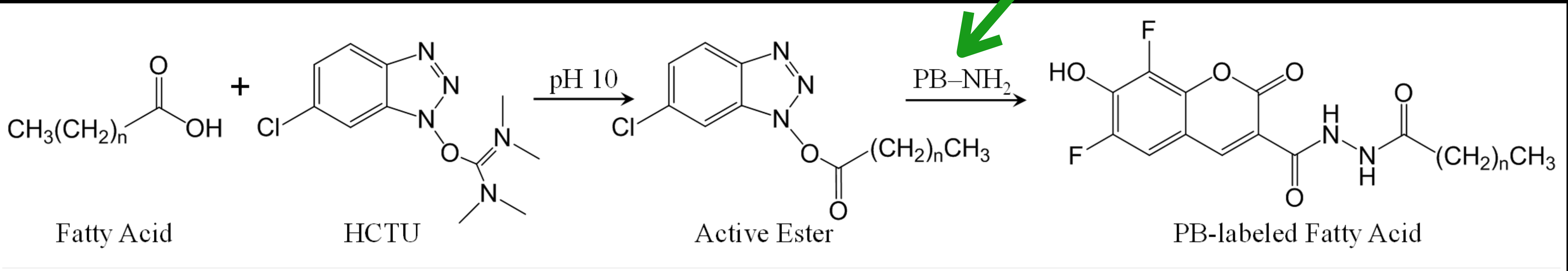
Biological activity is reflected in fatty acid chain length distribution. The size fraction distribution provides means for differentiating between abiotically produced organics (e.g., those found in meteorites) and those produced by biological processes (e.g., those derived from microbes or living organisms).

FATTY ACID ANALYSIS USING A NEW DYE

A new custom-designed fluorescent dye, Pacific Blue hydrazide ($PB-NH_2$)

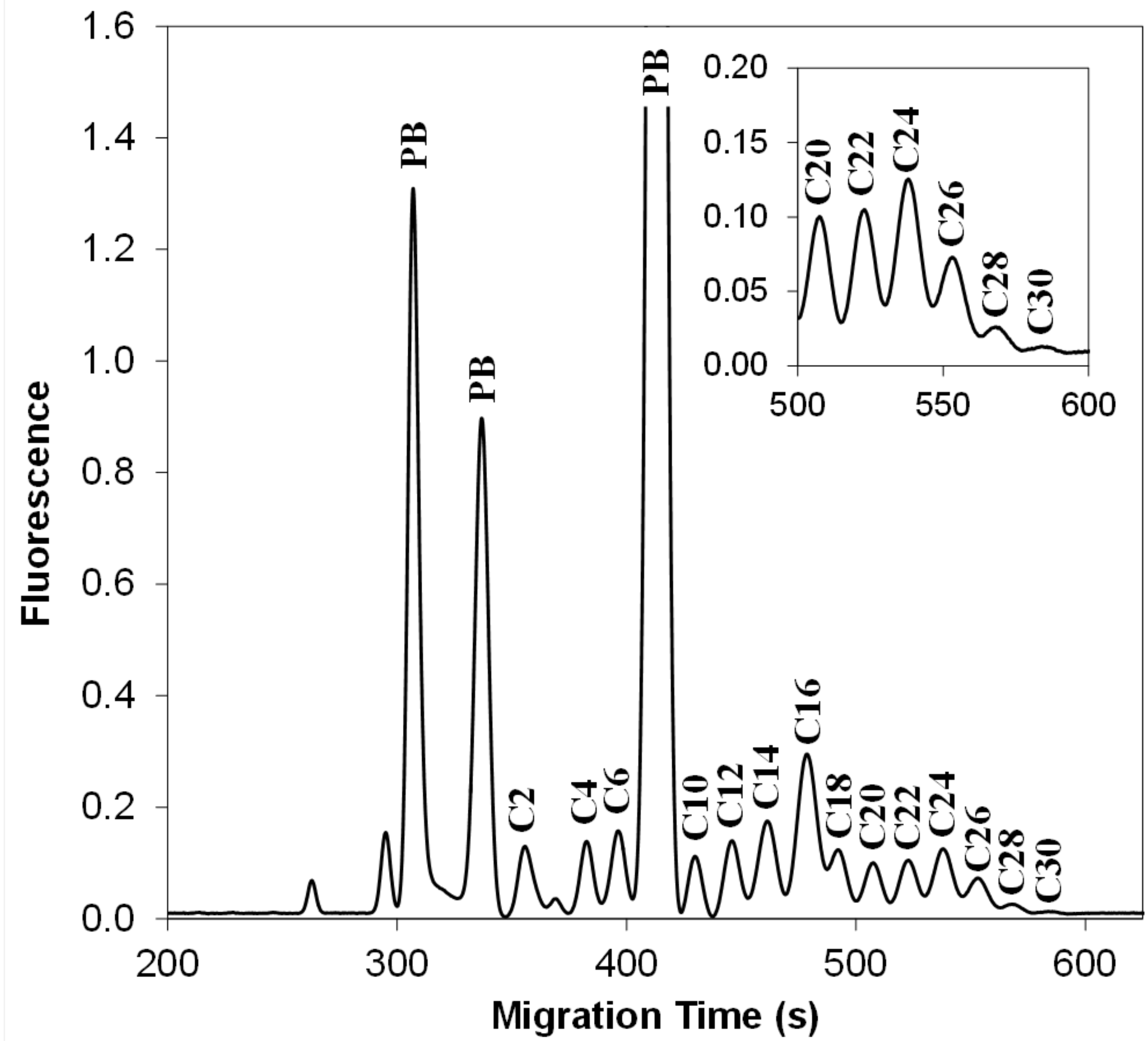
It labels the carboxylic acid in a two-step, one-pot reaction.





Labeling is performed in dimethylformamide (DMF) using 1 mM $PB-NH_2$, 2 mM O-(1H-6-chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyl-uronium hexafluorophosphate (HCTU) coupling agent, and 50 mM DIEA.

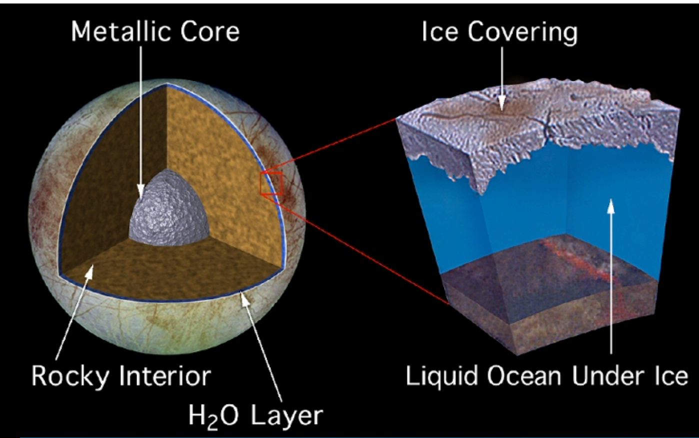
FATTY ACID METHOD DEVELOPMENT



Optimized separation of whole range of fatty acids (2 μM C2-C26, 5 μM C28-C30) in ethanol.

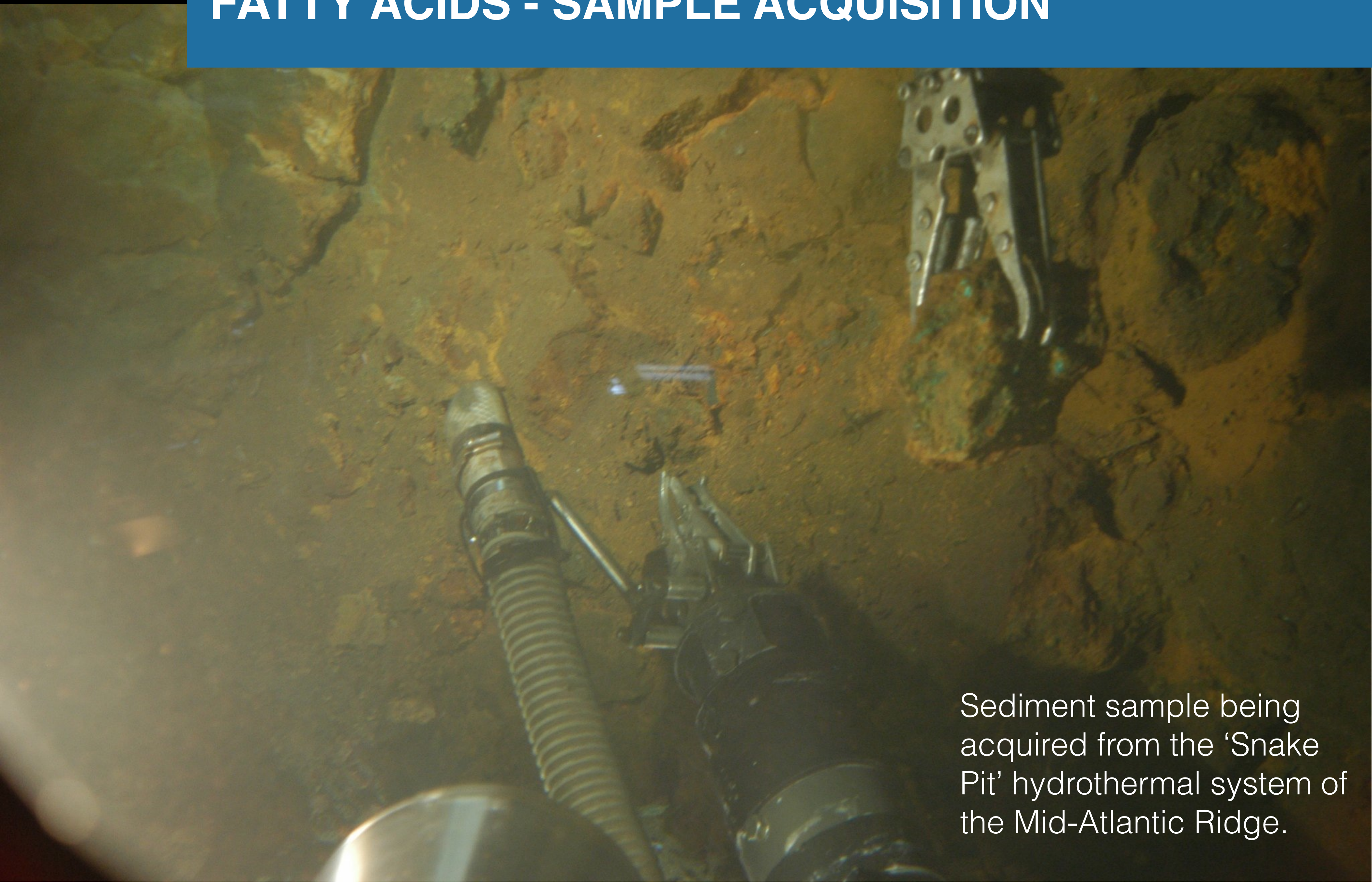
The optimal protocol for analysis of fatty acids allows the separation of acids ranging from 2 to 30 carbons. Limits of detection for C10 to C30 fatty acids ranged from 0.9 to 5.7 μM.

FATTY ACIDS ON THE OCEAN FLOOR?

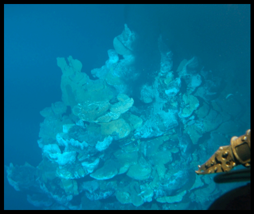


Icy Worlds such as Europa could harbor environments similar to this

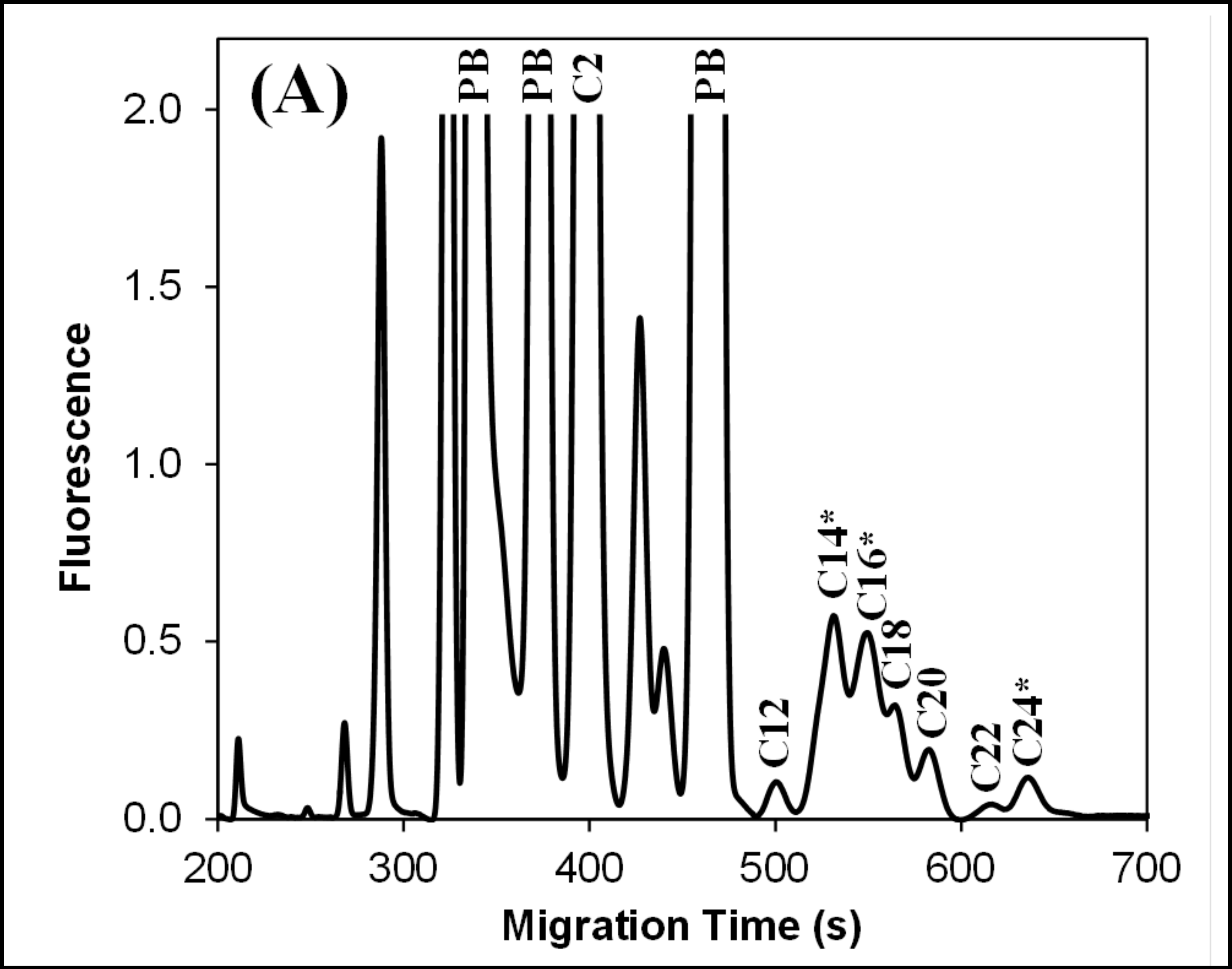
FATTY ACIDS - SAMPLE ACQUISITION



Sediment sample being acquired from the 'Snake Pit' hydrothermal system of the Mid-Atlantic Ridge.



FATTY ACID DATA FROM MID-ATLANTIC RIDGE



Long-chain fatty acids present in sediments collected from the Snake Pit hydrothermal vent system.

FATTY ACID DATA FROM MID-ATLANTIC RIDGE

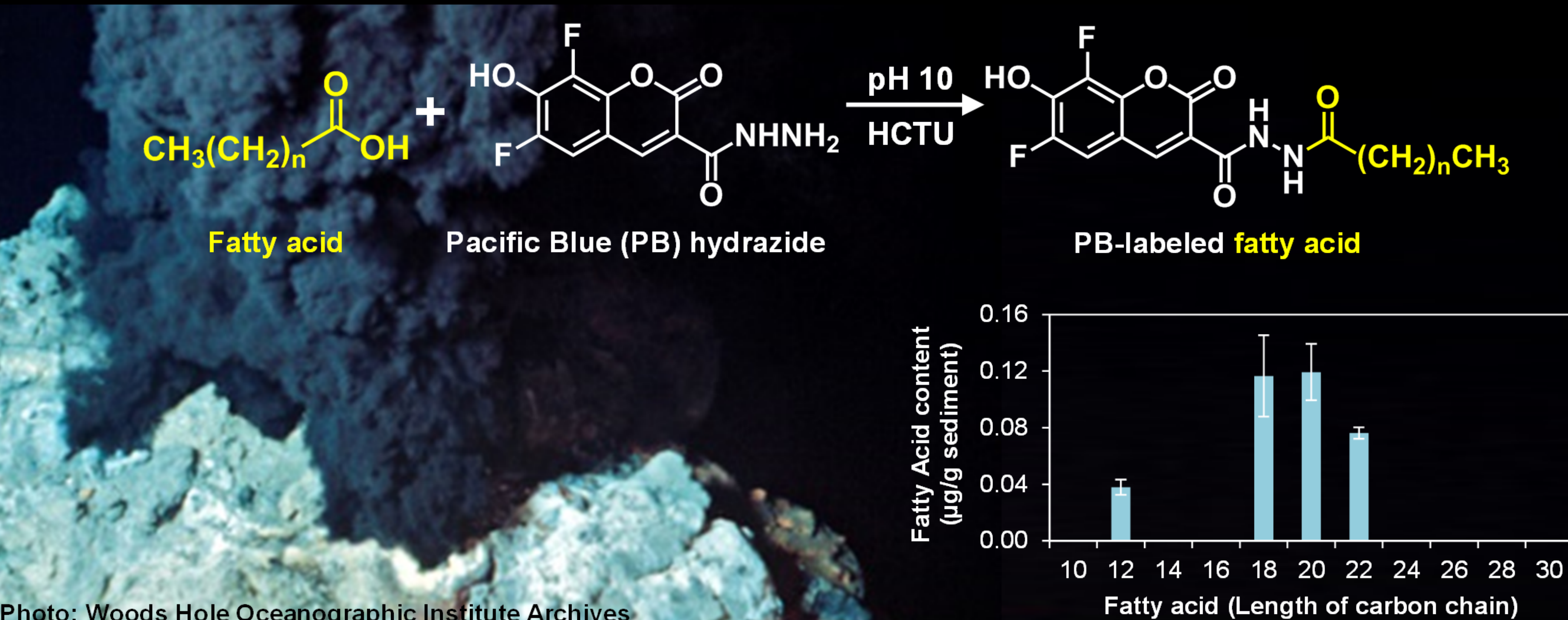


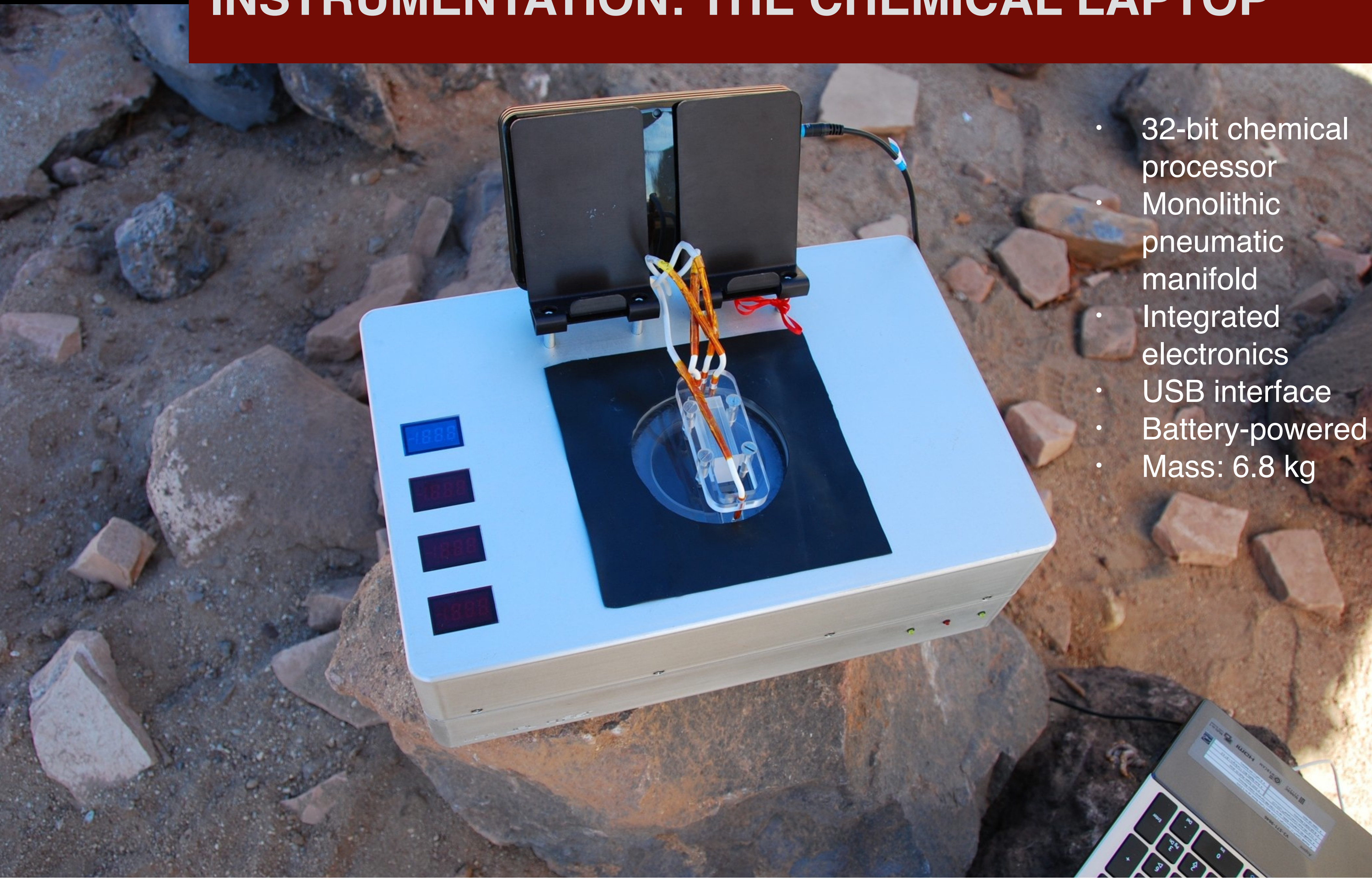
Photo: Woods Hole Oceanographic Institute Archives

- Several long chain fatty acids were detected and quantified.
- These molecules serve as biomarkers of localized microbial ecosystems.

FATTY ACID SUMMARY

- first reported nonaqueous microfluidic method of separating both short and long chain fatty acids (up to 30 carbons long)
- analysis of deep ocean samples using new method developed indicates samples of biological origin
- method **could someday play a key role in the determination of whether or not life exists on other worlds**

INSTRUMENTATION: THE CHEMICAL LAPTOP



- 32-bit chemical processor
- Monolithic pneumatic manifold
- Integrated electronics
- USB interface
- Battery-powered
- Mass: 6.8 kg

MSL AND THE CHEMICAL LAPTOP


EXIT

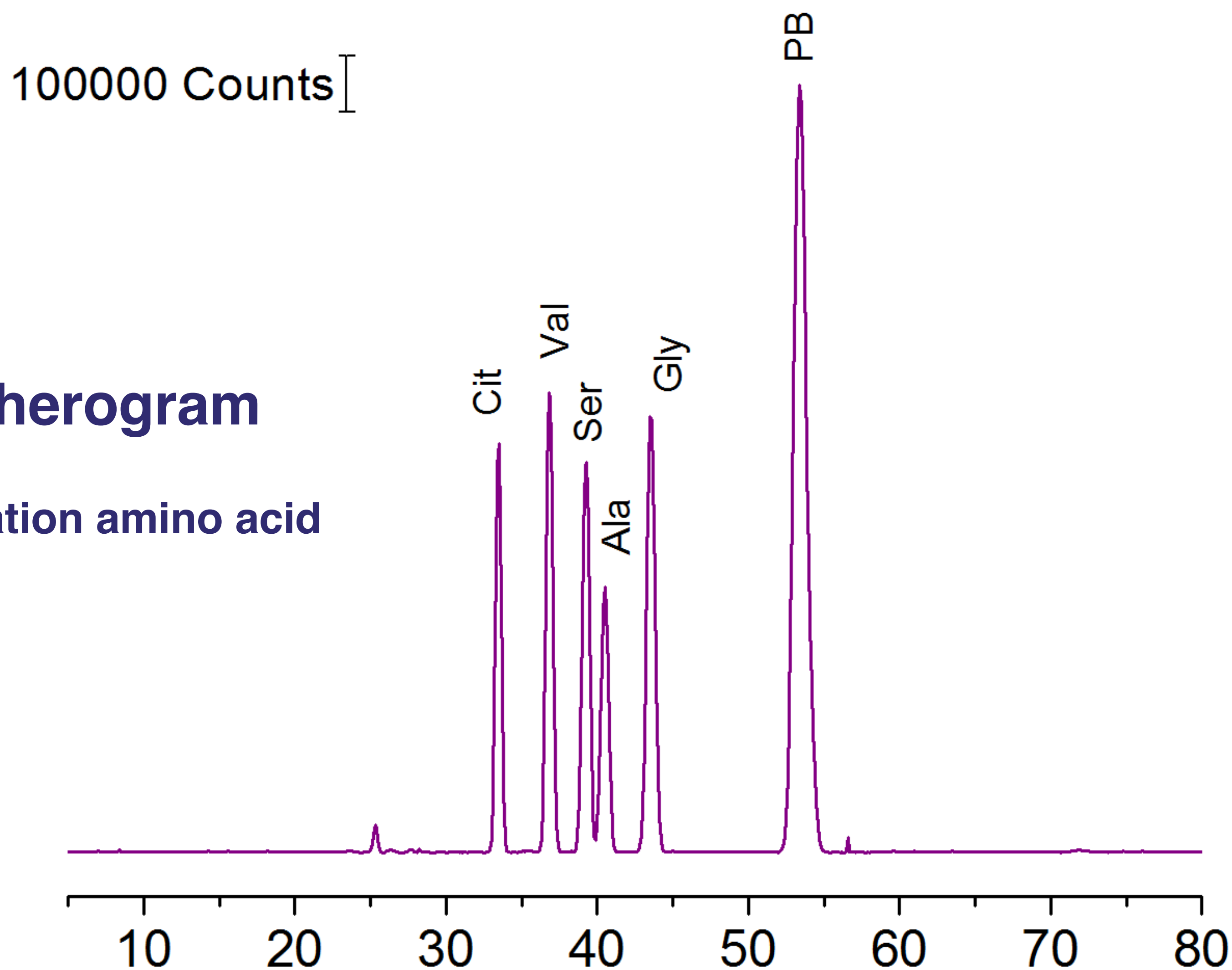
Jet Propulsion Laboratory
California Institute of Technology

Our rover FIDO

NASA JPL
Jet Propulsion Laboratory
California Institute of Technology

INSTRUMENTATION: THE CHEMICAL LAPTOP

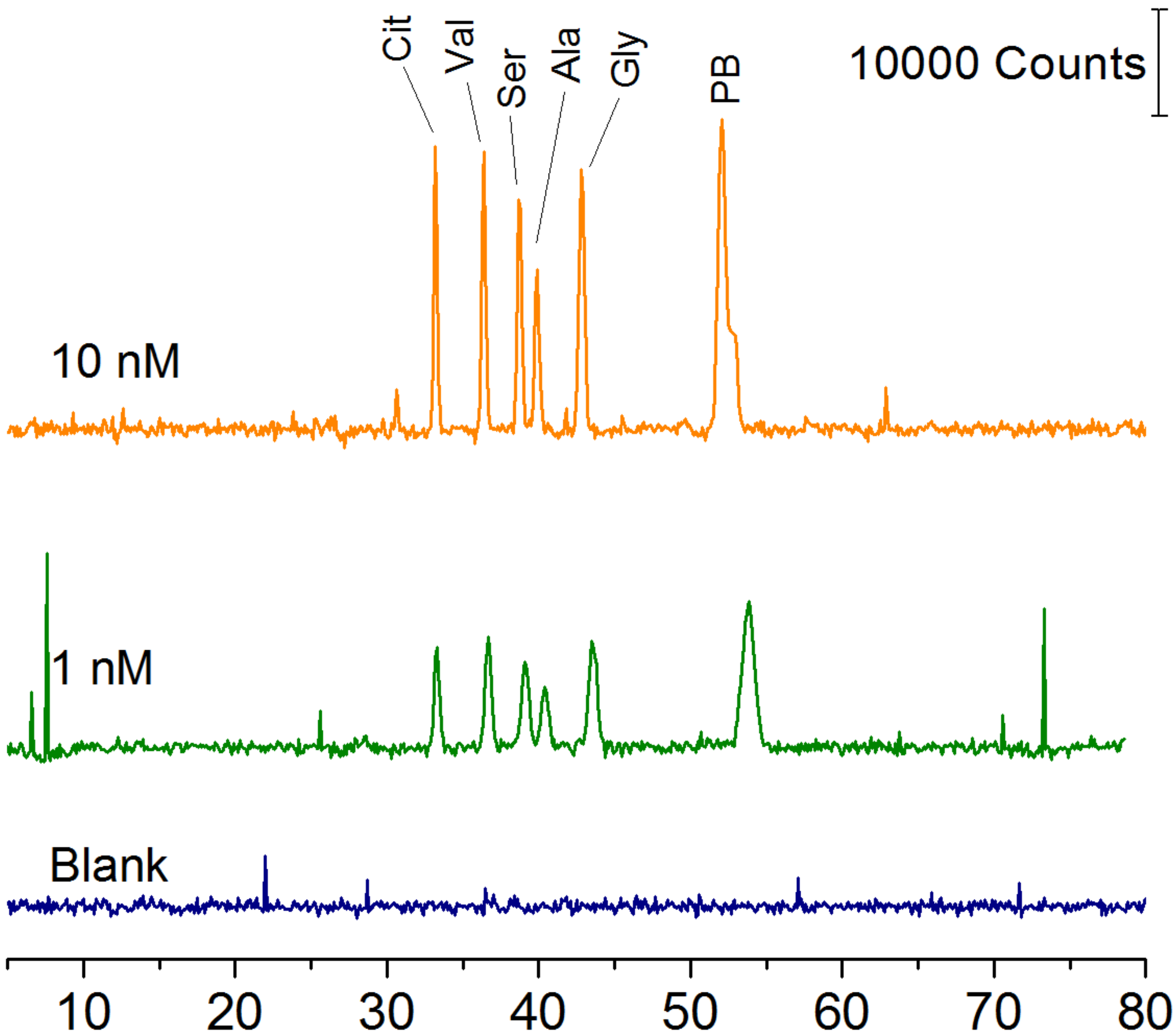
Amino acid standard analyzed with the Chemical Laptop on the Mars Yard at JPL



- **first electropherogram**
- **100 nM concentration amino acid standard**

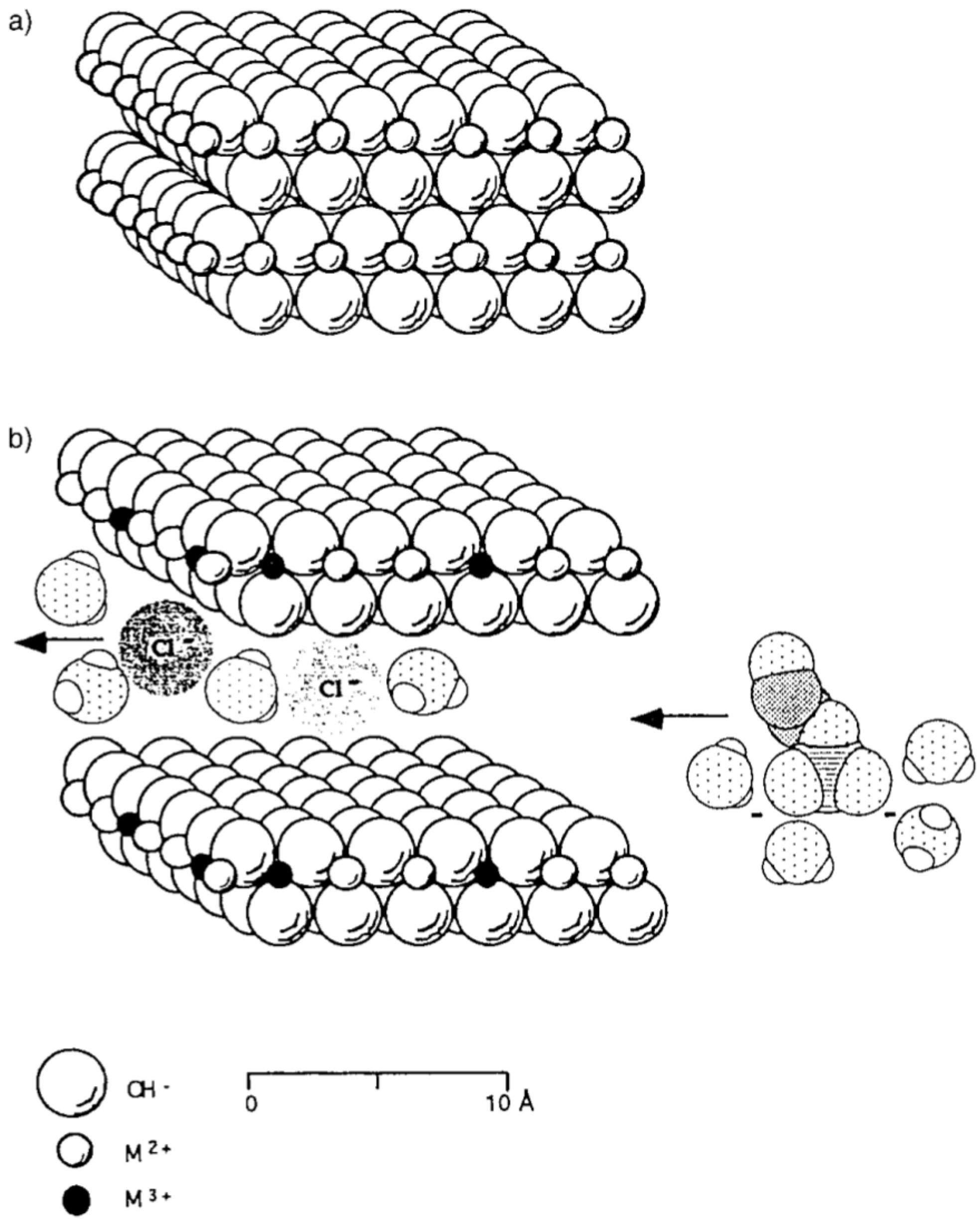
INSTRUMENTATION: THE CHEMICAL LAPTOP

Amino acid standards analyzed with the Chemical Laptop on the Mars Yard at JPL

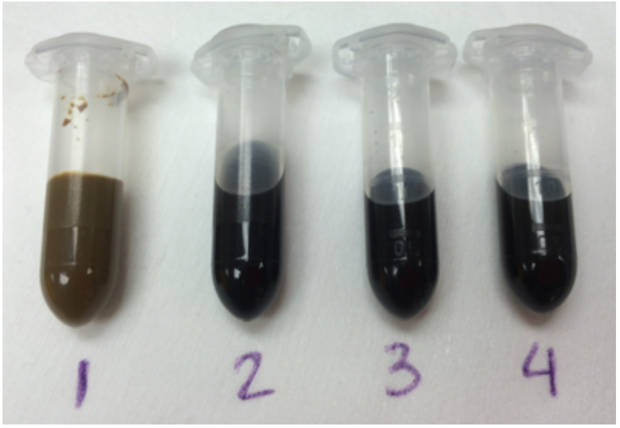


preliminary LOD 500pM

HIDING INSIDE GREEN RUST



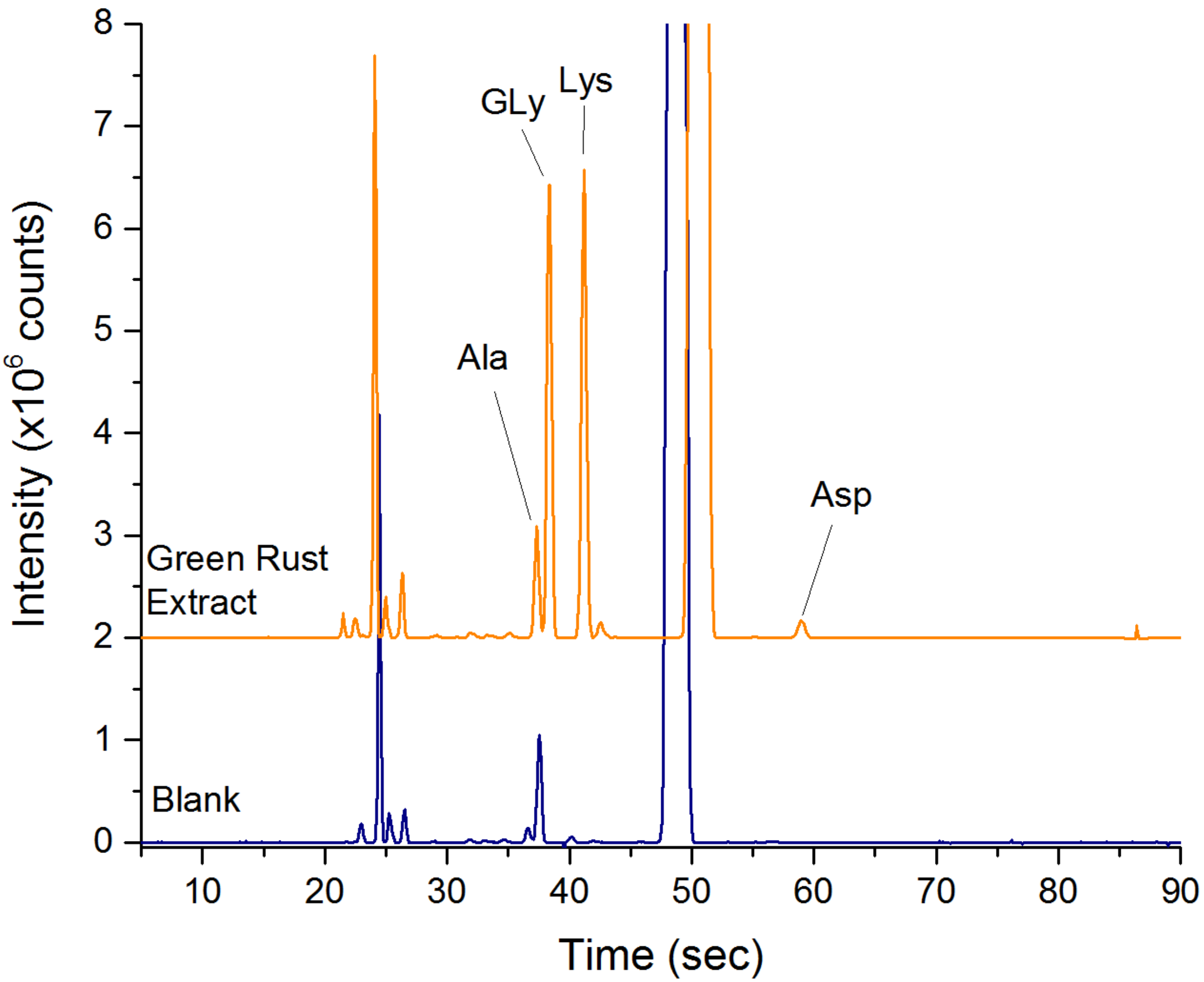
- **Green rust (fougèrite), a double layer hydroxide**



- **sample (1) without amino acid intercalates was oxidized rapidly**
- **samples (2)-(4) contain amino acid intercalates — remain unoxidized**

THE CHEMICAL LAPTOP — MEASURING THE PRESERVATION POTENTIAL OF GREEN RUST

Green Rust extract analyzed with the Chemical Laptop on the Mars Yard at JPL



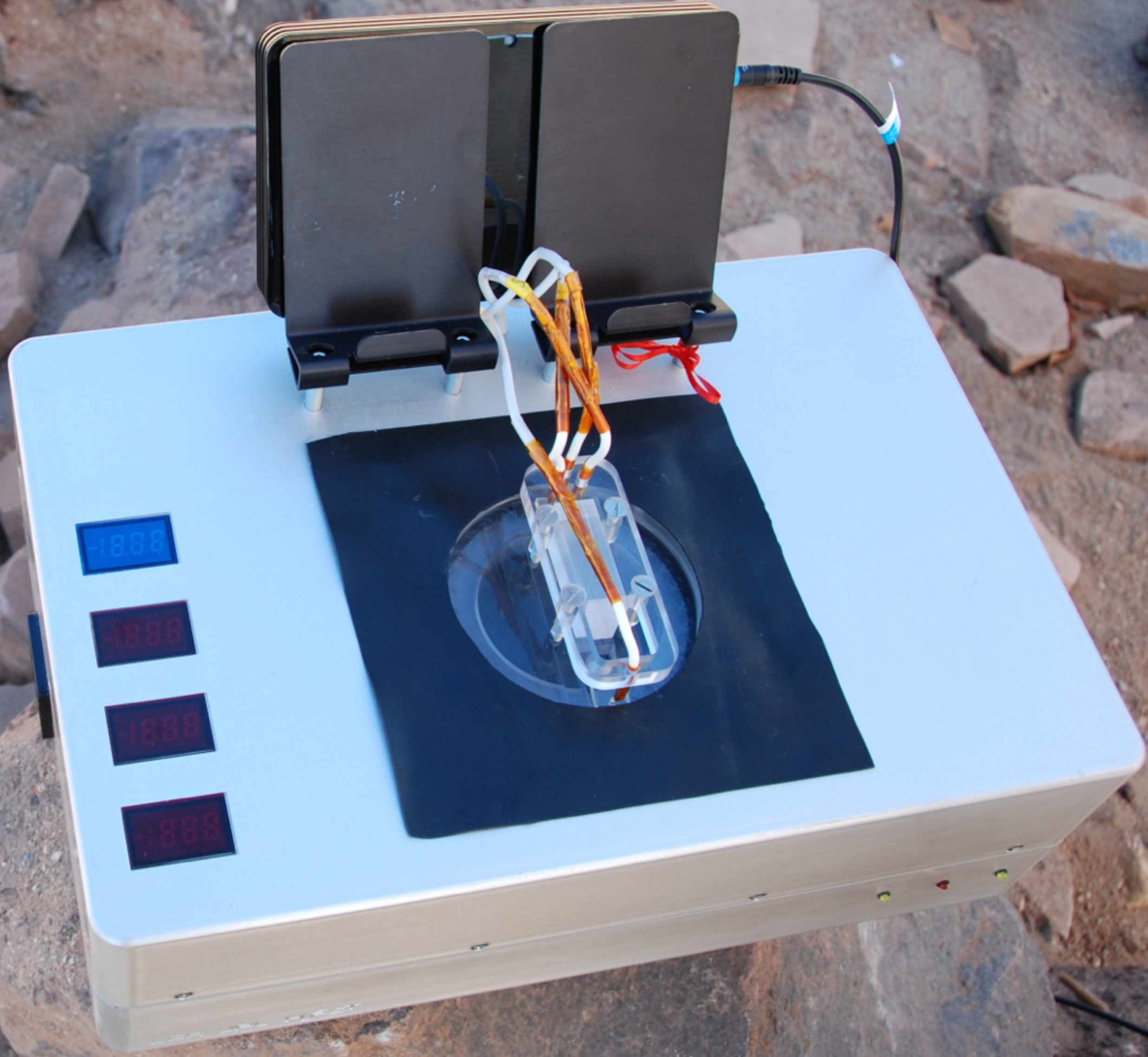
THE CHEMICAL LAPTOP SUMMARY

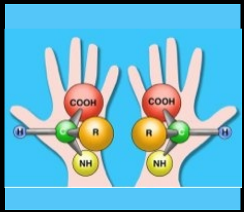
- instrument validated in the field for first time on standards and real samples
- first tests indicate 500 pM sensitivity to amino acids
- Chemical Laptop battery was still ~75% charged after four hours

NEXT STEPS WITH THE CHEMICAL LAPTOP

- further optimize optics to increase sensitivity
- validate on-chip sample handling (CorSolutions manifold)
- reagent storage with Tom Chiesl of Ibis Biosciences
- interface to / hybridize with "coffee maker"

- make it smaller!
- make it easier to use!
- take on field trips! go back to Atacama Desert!

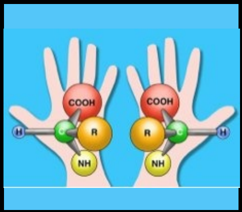




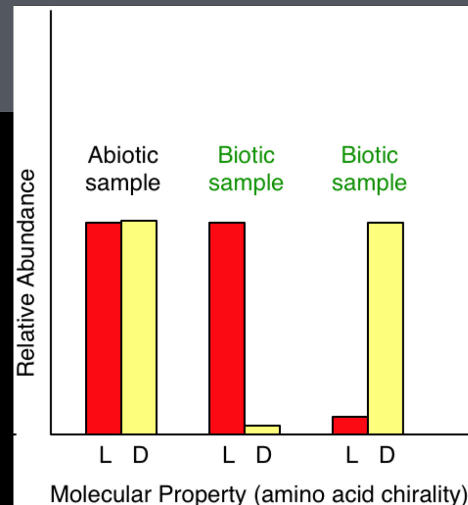
SUMMARY

- Newly developed non-aqueous microchip CE methods greatly extend the possibilities for in situ astrobiology investigations on spaceflight missions.
- This work suggests that amines are present on Titan and that there is a way to a measure them in situ using microfluidics. By extension, if these materials mix with liquid water, prebiotic chemical evolution may be possible through peptide chemistry.
- The non-statistical distribution and abundances of the fatty acids measured in seafloor sediment samples suggests a biogenic source for this material — such information would be invaluable in the search for life beyond Earth.
- Battery-powered portable instrumentation was validated in the field with better than 1nM sensitivity.

Jet Propulsion Laboratory
California Institute of Technology

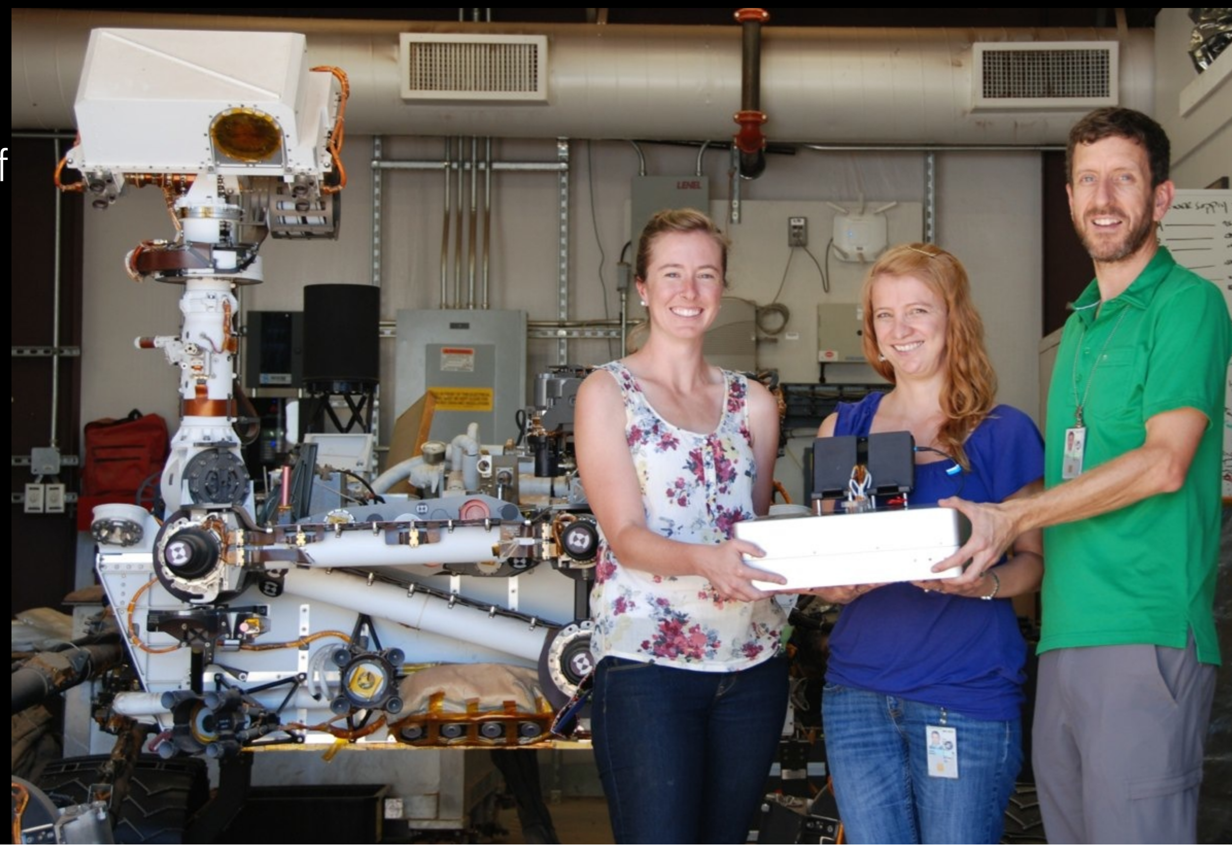


FUTURE OUTLOOK



Biotic samples of amino acids are enriched with either the left- or right-handed versions of these molecules.

- We are in the first month of a three year NASA PICASSO effort entitled “Microfluidic Life Analyzer” — we will focus on chiral amino acid analysis in addition to fatty acid analysis. Jessica Creamer (U.Kansas) began as a NASA Postdoctoral Fellow on Oct 22, 2014.
- We will also investigate biomolecule production and preservation as part of a newly selected NASA Astrobiology Institute (NAI) Node formed at JPL — “Icy Worlds” (2014-2019)
- Commercialization of technology with A-Line Inc. underway now



Acknowledgements

- MLC and AMS were funded through the NASA Postdoctoral Program at JPL, administered by the Oak Ridge Associated Universities through a contract with NASA. SMH was supported by NSF Astronomy and Astrophysics Postdoctoral Fellowship AST-1102827. MAT was funded through NASA Exobiology Grant NNX12AD92G and NASA Planetary Atmospheres NNX11AD82G. CH and MAS were funded through NASA Exobiology Grant NNG05GO58G and the NASA Astrobiology Initiative, through JPL subcontract 1372177. MFM and PAW were funded through the NASA Astrobiology Science and Technology Development (ASTID) Program (Project No. 104320).
- Custom dye for fatty acid analysis developed and synthesized by Life Technologies, Inc.
- Nathan Bramall fabricated the Chemical Laptop unit and designed its custom electronics, optics and software as part of the NASA SBIR Program. Tom Corso of CorSolutions Inc. fabricated the pneumatic manifold.
- Portable Instrumentation Development reported here (by PAW and MFM) is currently funded under the current PICASSO Program “Microfluidic Life Analyzer”.
- Mike Russell produced green rust samples and hypothesis for preservation of amino acids.
- This work was carried out at the Jet Propulsion Laboratory, California Institute of Technology, under contract with NASA.

Key References

1. M.L. Cable, P.A. Willis et al., Anal. Chem., 85, 1124-1131 (2013).

2. M.L. Cable, P.A. Willis et al., Chemical Reviews, 112, 1882-1909 (2012).

3. M.L. Cable, P.A. Willis et al., Earth and Planetary Science Lett., 403, 99-107 (2014).

4. M.L. Cable, P.A. Willis et al., Anal. Methods, Advance Article, DOI: 10.1039/C4AY01243G (2014).

5. M.F. Mora, P.A. Willis et al., Electrophoresis, 33, 2624-2638 (2012).