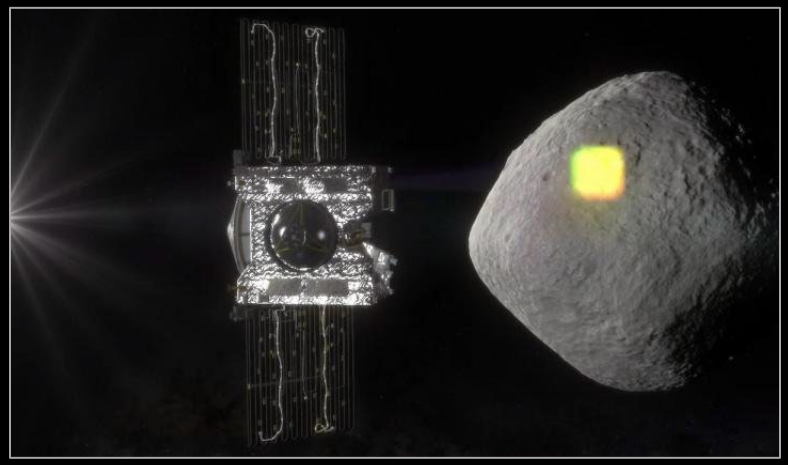
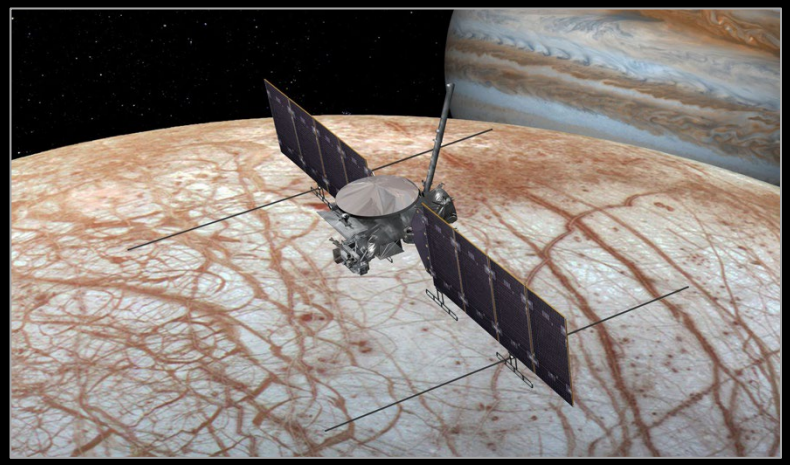
Organic Inventories for Astrobiology

- **Prebiotic Chemistry**
- **Life Detection**

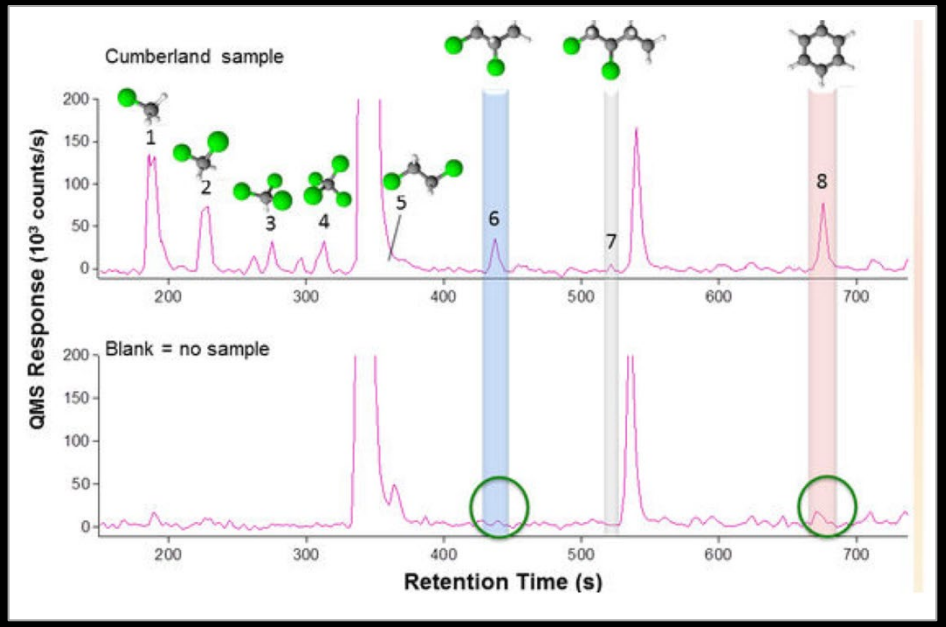




Organic Inventories for Astrobiology

The identification of well-established and widely accepted organic molecules associated with terrestrial life and signatures of biologic processes

- **Particular classes, patterns, and isotopic signatures of organic molecules by mass spectrometry.**



Data from Freissinet et al., 2015 "Organic molecules in the Sheepbed Mudstone, Gale Crater, Mars"

Organic Inventories for Astrobiology

Biosignature and life detection missions require careful attention to terrestrial organic contamination that can be easily convoluted with analytical targets

Contaminant Class	Examples
Nucleic acids	DNA
Spores	Dipicolinic acid
Bacterial and fungal cell walls	N-Acetylglucosamine
Amino acids	Glycine, Alanine
Lipids	Palmitic acid, Squalene
Hydrocarbon biomarkers	Pristane
Halogenated hydrocarbons	Chlorobenzene, Dichloromethane
PAHs	Naphthalene
Nitrogenous compounds	Urea
Short-chain carboxylic acids	Acetic acid
Polyhydroxy compounds	Glycerol
Hydroxy carboxylic acid	Pyruvic acid
Linear hydrocarbons	n-Heptacosane

Organic Inventories for Astrobiology

***CLEAN* means no foreign material is introduced to the sample in an amount that hampers the ability to analyze the chemistry of the sample**

Sample	Total amino acids
Nylon packaged screw	101.13 ng/cm^2
Kimberly Clark 55082 purple nitrile glove	67.3 ng/g
Kimtech G3 tan nitrile glove	646.2 ng/g
TechNitrile blue nitrile glove	150.3 ng/g
Residue from Kimtech glove on foil	0.97 ng/cm^2
Nylon (KNF LB106)	38,000 ng/g
PTFE (KNF LB605)	17 ng/g
Polypropylene (KNF LB3022)	7.8 ng/g
Tyvek	6.3 ng/g
Reynolds aluminum foil	0.015 ng/g

Data from Dworkin et al., 2018 "OSIRIS-REx contamination control strategy and implementation"

Organic Inventories for Astrobiology

***CLEAN* means no foreign material is introduced to the sample in an amount that hampers the ability to analyze the chemistry of the sample**

Sample	Total amino acids
Nylon packaged screw	101.13 ng/cm^2
Kimberly Clark 55082 purple nitrile glove	67.3 ng/g
Kimtech G3 tan nitrile glove	646.2 ng/g
TechNitrile blue nitrile glove	150.3 ng/g
Residue from Kimtech glove on foil	0.97 ng/cm^2
Nylon (KNF LB106)	38,000 ng/g
PTFE (KNF LB605)	17 ng/g
Polypropylene (KNF LB3022)	7.8 ng/g
Tyvek	6.3 ng/g
Reynolds aluminum foil	0.015 ng/g

Chlorobenzene abundance on Mars = ~200ppm
Total amino acid abundance in meteorites = 44 to 6300 ng/g

Organic Inventories for Astrobiology

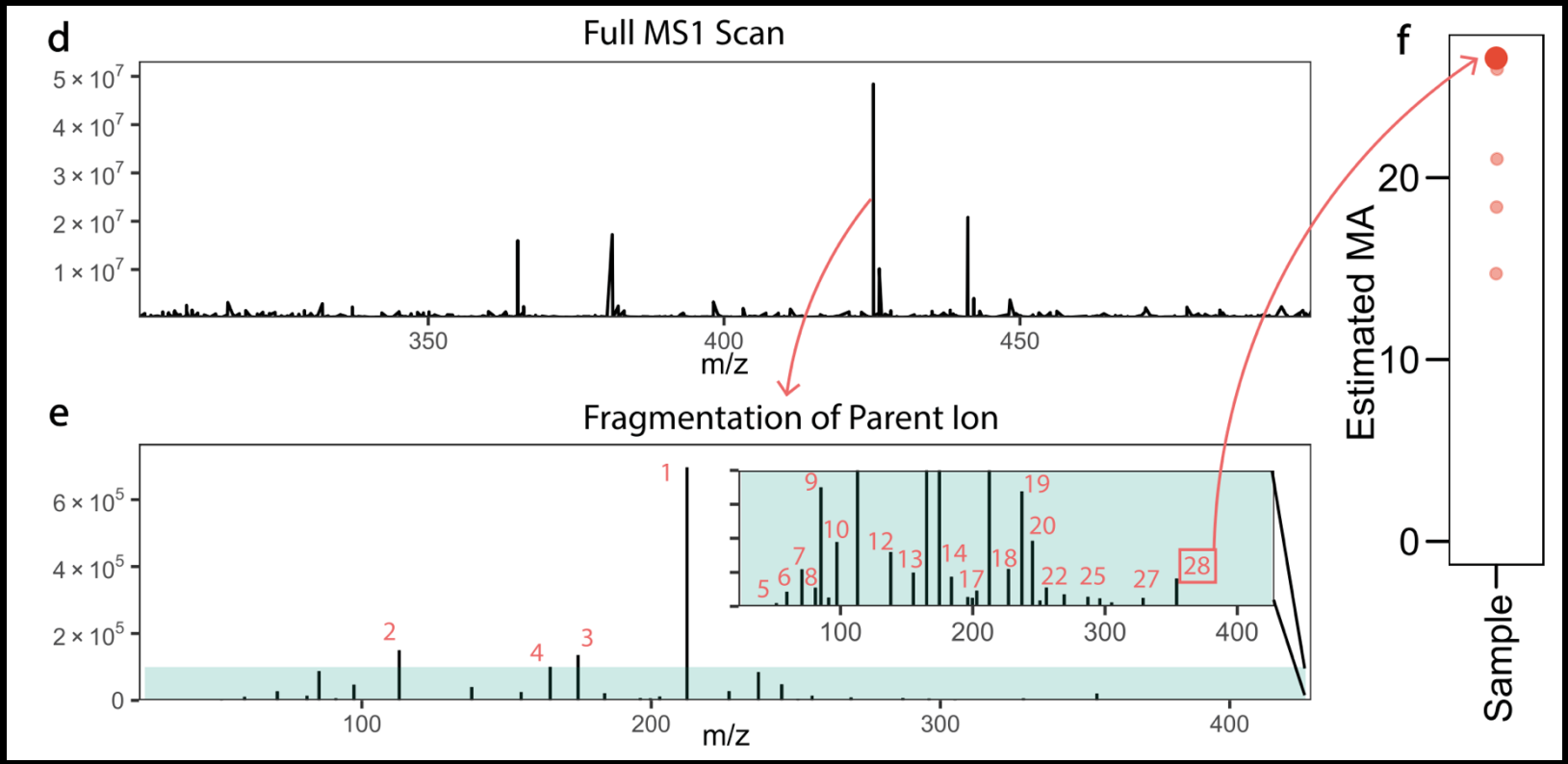
For many astrobiology planetary exploration missions the scientific cleanliness requirements often exceed Planetary Protection bioburden requirements

Sample Species	Allowable abundance ng/g
Aromatic hydrocarbons	8
Carbonyls & hydroxyls	10
Amino acids	1
Amines or amides	2
Aliphatic hydrocarbons	8
DNA	1
Total reduced carbon	40

Data from Mahaffey et al., 2003 "Science priorities related to the organic contamination of martian landers."

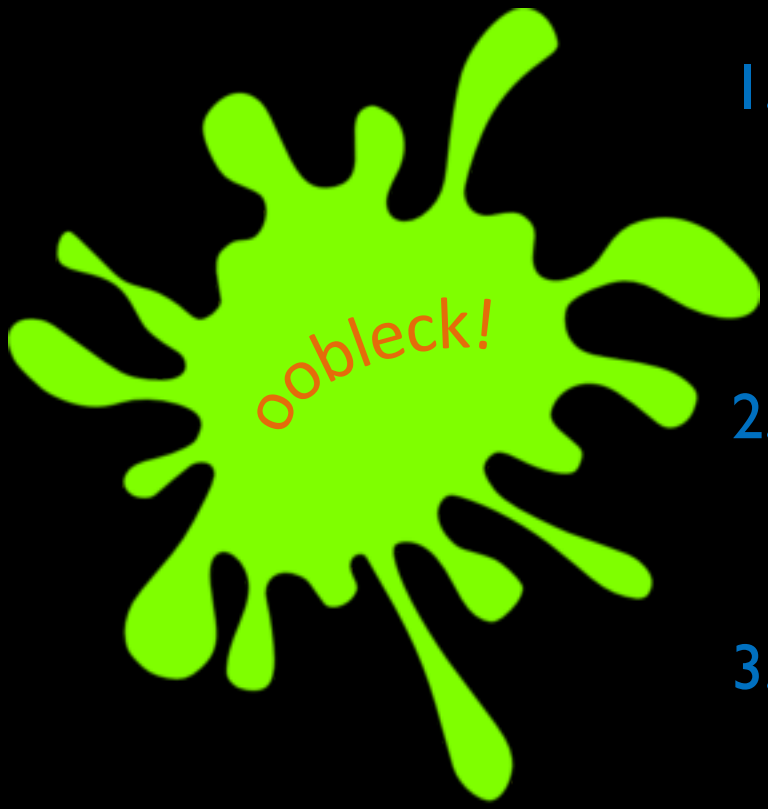
Organic Inventories for Astrobiology

Agnostic life detection methods have an even greater need for organic inventory knowledge since most metrics rely on semi-targetless assemblage analysis



Marshall et al., 2021 "Identifiying molecules as biosignatures with assembly theory and mass spectrometry."

Organic and Organismal Basics for Life Evaluation and Contamination Knowledge



1. A general organic contamination knowledge dataset for sample curation facilities and planetary instrument assembly areas
2. An understanding of the effects of bioburden reduction protocols on organic contamination loads
3. Novel methods to measure bioburden and its contribution to organic backgrounds

Organic Contaminant Knowledge Census

A general organic contamination knowledge dataset for sample curation facilities and spacecraft assemblies

- *Minimum assessments of organic compounds in returned samples is only as good as the standards we hold for our assembly and curation facilities*

Organic Contaminant Knowledge Census

A general organic contamination knowledge dataset for sample curation facilities and spacecraft assemblies

- *Minimum assessments of organic compounds in returned samples is only as good as the standards we hold for our assembly and curation facilities*
  - Organic inventory requirement applies only to Category 3 and 4 destinations and components over 1 kg

Organic Contaminant Knowledge Census

A general organic contamination knowledge dataset for sample curation facilities and spacecraft assemblies

- *Minimum assessments of organic compounds in samples analyzed are only as good as the standards we hold for our assembly and curation facilities*
  - Organic inventory requirement applies only to Category 3 and 4 destinations and components over 1 kg
  - Contamination is addressed primarily through routine cleaning. Monitoring is concentrated on airborne particulates and Total Carbon and FTIR analysis of tools and witness materials

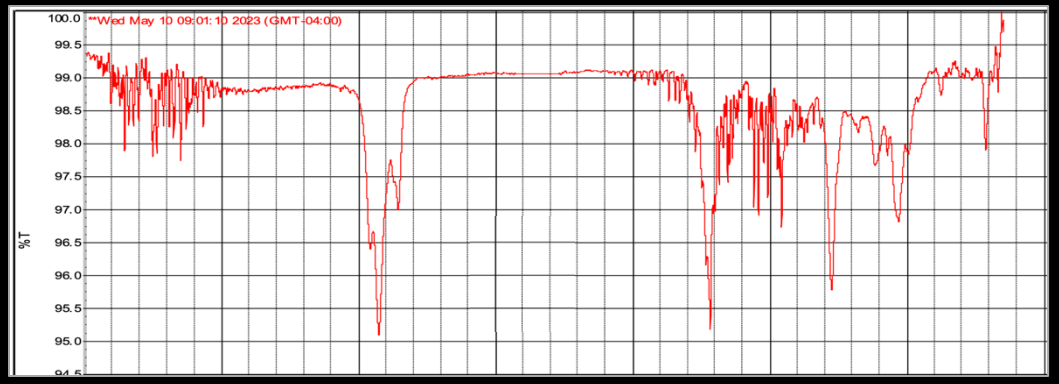
Organic Contaminant Knowledge Census

A general organic contamination knowledge dataset for sample curation facilities and spacecraft assemblies

- *Minimum assessments of organic compounds in returned samples is only as good as the standards we hold for our assembly and curation facilities*
  - Organic inventory requirement applies only to Category 3 and 4 destinations and components over 1 kg
  - Contamination is addressed primarily through routine cleaning. Monitoring is concentrated on airborne particulates and Total Carbon and FTIR analysis of tools and witness materials
  - OOBLECK experimented with new methods for organic reconnaissance cataloging small molecules, volatiles, and hydrocarbons by mass spectrometry.

Organic Contaminant Knowledge Census

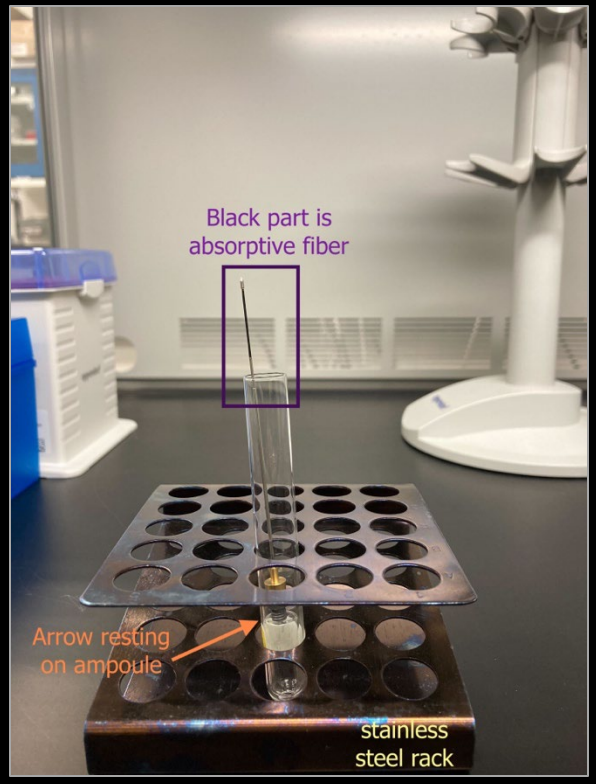
–Contamination is addressed primarily through routine cleaning. Monitoring is concentrated on airborne particulates and Total Carbon and FTIR analysis of tools and witness materials



- Residue amount will indicate need for attention
- Long deployment periods
- Lowest mass detected is ~340 amu
- Methods should be optimized for compound classes
- Analyses identified only nine compounds and three general compound classes

Organic Contaminant Knowledge Census

- *The ability to deconvolve false positives needs the identity and behavior of potential contaminants*



- Passive volatile sampling by absorptive Solid Phase MicroExtraction (SPME) fibers
- Capable of trapping molecules as small as 30 amu
- Coating can be optimized for compound classes
- Deployment time of one week
- Thirty-minute analysis with no solvents needed
- Detecting and identifying up to 40 additional volatile compounds

Evaluating Effects of Bioburden Reduction

An understanding of the effects of bioburden reduction protocols on organic contamination loads

- *The organic contaminant census should also include residues from microbial and molecular cleaning procedures*

Evaluating Effects of Bioburden Reduction

An understanding of the effects of bioburden reduction protocols on organic contamination loads

- *The organic contaminant census should also include residues from microbial and molecular cleaning procedures*
  - Heat Microbial Reduction does not remove many organic molecules or killed microorganisms

Evaluating Effects of Bioburden Reduction

An understanding of the effects of bioburden reduction protocols on organic contamination loads

- *The organic contaminant census should also include residues from microbial and molecular cleaning procedures*
  - Heat Microbial Reduction does not remove many organic molecules or killed microorganisms
  - 70% isopropanol wipes kill vegetative microorganisms but do not kill spores or fungi, has a limited utility for removing apolar organic molecules and may lead to outgassing of surface

Evaluating Effects of Bioburden Reduction

An understanding of the effects of bioburden reduction protocols on organic contamination loads

- *The organic contaminant census should also include residues from microbial and molecular cleaning procedures*
  - Heat Microbial Reduction does not remove many organic molecules or killed microorganisms
  - 70% isopropanol wipes kill vegetative microorganisms but do not kill spores or fungi, has a limited utility for removing apolar organic molecules and may lead to outgassing of surface
  - OOBLECK is characterizing organic molecules left on surfaces cleaned for bioburden reduction

Evaluating Effects of Bioburden Reduction

- *Heat and solvent cleaning incompletely remove organics and necromass, leaving behind potential target compounds and might even select for certain resistant species*

- Inoculate surface of stainless-steel coupon
- Using isolate archive of organisms identified in cleanrooms
- Mixed inoculates of both spores and vegetative cells
- Test for appropriate dilution (standard assay)
- Treat coupons (IPA wipes, ultrasonic cleaning, HMR sterilization, UV irradiation)
- Rinse for molecular identification and quantification

Connecting Bioburden with Contaminants

Novel methods to measure bioburden and its contribution to organic backgrounds

- *Deconvolving the organic background arising from in situ biomass rather than conventional contamination sources*

Connecting Bioburden with Contaminants

Novel methods to measure bioburden and its contribution to organic backgrounds

- *Deconvolving the organic background arising from in situ biomass rather than conventional contamination sources*
  - Current bioburden assays often defined by spore surface density and ATP abundance

Connecting Bioburden with Contaminants

Novel methods to measure bioburden and its contribution to organic backgrounds

- *Deconvolving the organic background arising from in situ biomass rather than conventional contamination sources*
  - Current bioburden assays often defined by spore surface density and ATP abundance
  - Assays are designed to assess resistant bioburden rather than quantifiable viable or residual organisms

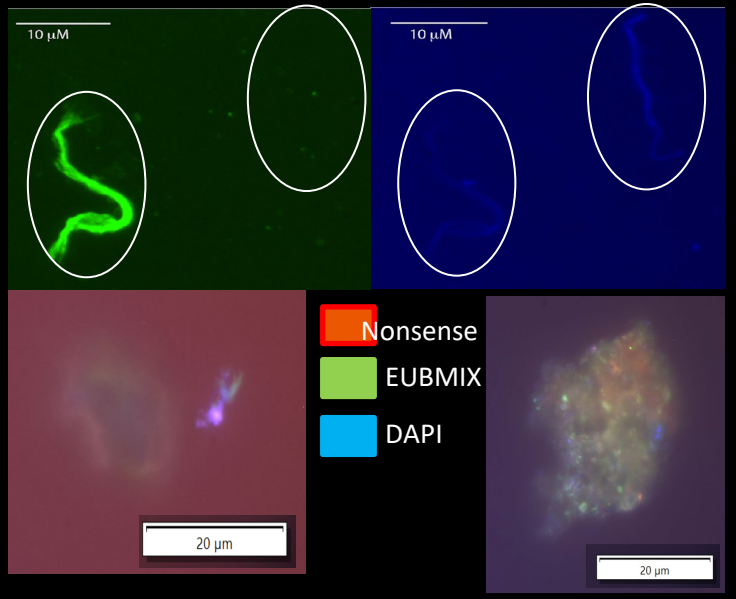
Connecting Bioburden with Contaminants

Novel methods to measure bioburden and its contribution to organic backgrounds

- *Deconvolving the organic background arising from in situ biomass rather than conventional contamination sources*
  - Current bioburden assays defined by spore surface density and ATP abundance
  - Assays are designed to assess resistant bioburden rather than quantifiable viable or residual organisms
  - OOBLECK is working on (more) rapid methods to identify and quantify residual organisms, living or dead

Connecting Bioburden with Contaminants

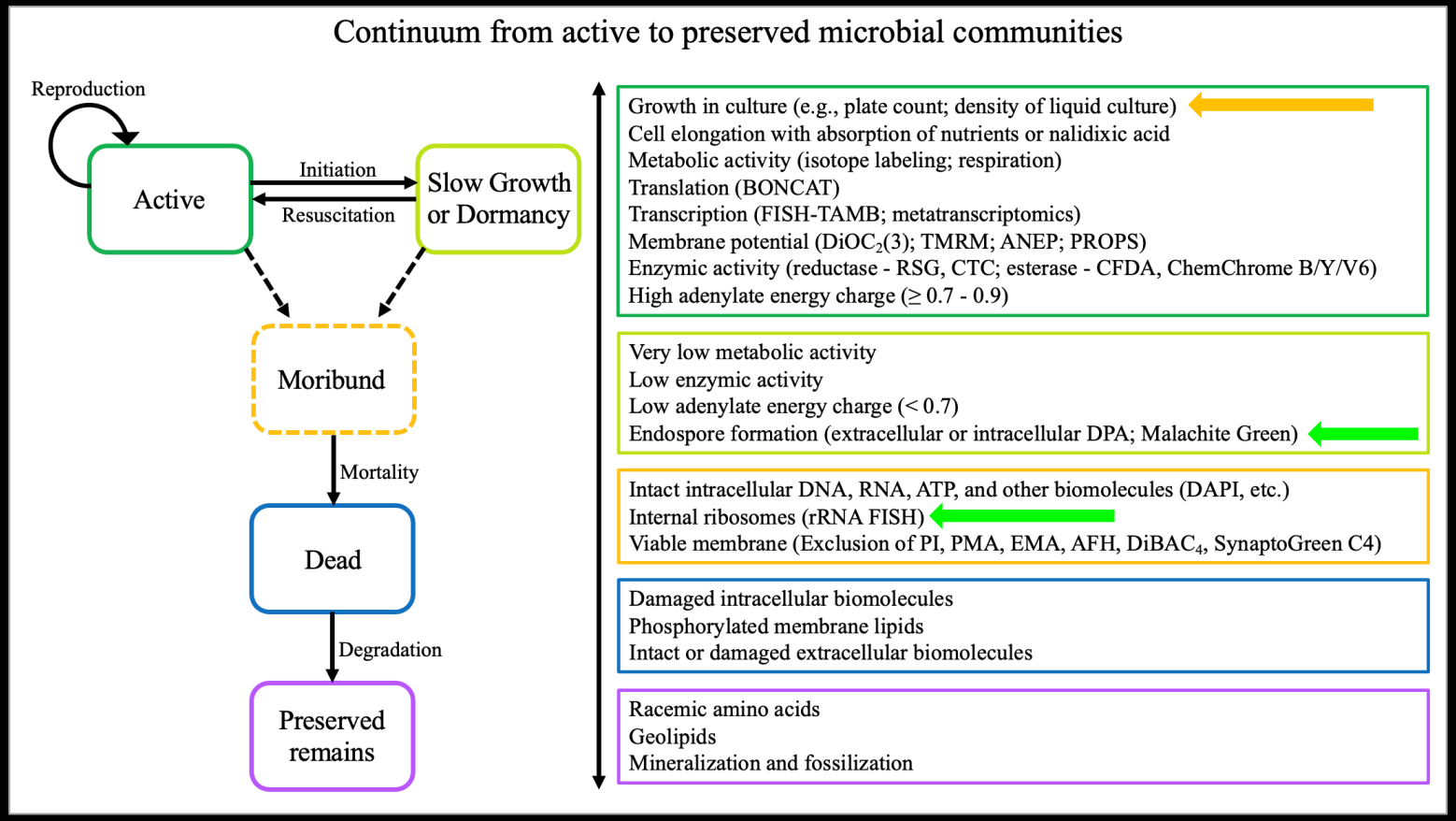
- **FISH (fluorescence in situ hybridization) optimized for low biomass accumulations**
- **Allows quantification**
- **Probes can be tailored for type to give phylogenetic and biodiversity data**
- **FISH can indicate the level of activity of cells detected**



➢ ***The PPIRB encouraged flexible ways to address the intent of Planetary Protection using novel methods and the use of modern molecular biological approaches for analyses of cleanroom samples.***

Some notes about limits of life detection

Many methods are not optimized for microbes in all the many states they may be present

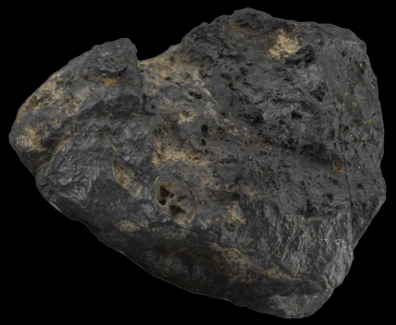


From Ratliff et al., in review "The vacant niche revisited; Using negative results to refine the limits of habitability"

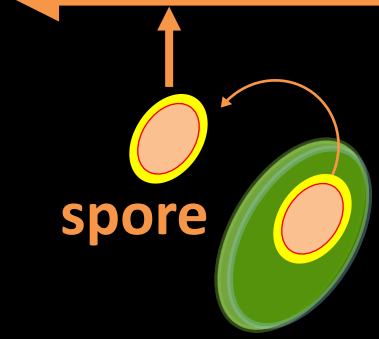
Some notes about limits of life detection

Survivability and the scale of "lifieness"

Cultivation does not revive all spores

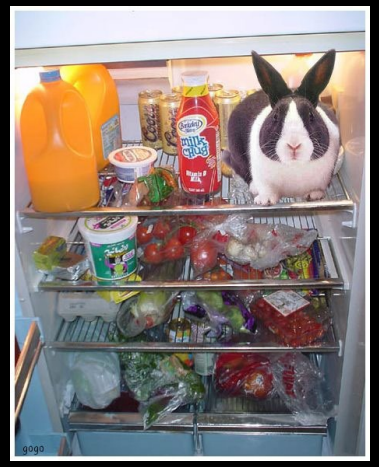


rock



spore

Spores require special treatment for dyes



dorm room fridge

- New methods for expanding the organic reconnaissance cataloging small molecules and volatiles.
- More rapid methods to identify and quantify residual organisms, living or dead
- Characterizing organic molecules left on surfaces cleaned for bioburden reduction

Thanks to my Organic Contamination Knowledge Co-Is

- ***Jason Dworkin/GSFC, OSIRIS-REx Project Scientist***
- ***Erin Lalime, GSFC***
- ***Aaron Regberg, JSC***
- ***Dan Jones/New Mexico Institute of Mining & Technology***