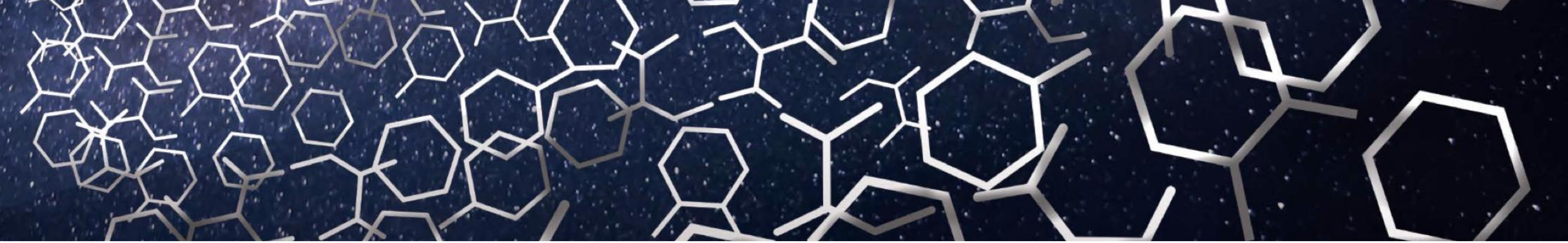


Agnostic Approaches to Life Detection

Presentation to the National Academies Committee on the
Astrobiology Science Strategy for the Search for Life in the Universe

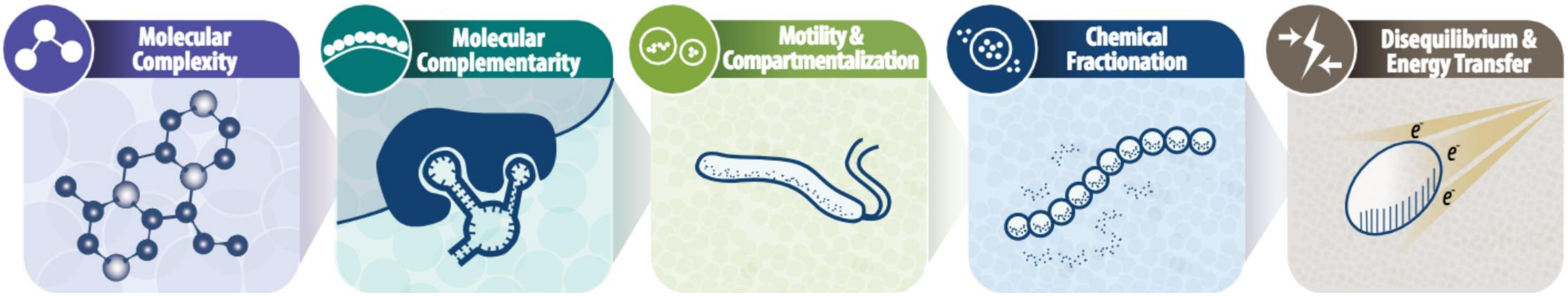
Sarah Stewart Johnson (Georgetown), Heather Graham (NASA), Eric Anslyn (UT), Pamela Conrad (Carnegie), Leroy Cronin (U. Glasgow), Andrew Ellington (UT), Jamie Elsila (NASA), Peter Girguis (Harvard), Christopher Kempes (Santa Fe Inst.), Eric Libby (Santa Fe Inst.), Paul Mahaffy (NASA GSFC), Jay Nadeau (PSU), Robert Rwebangira (Howard), and Andrew Steele (Carnegie)

How do we contend with the truly alien?

How can we search for indicators of life without presupposing a particular molecular framework?

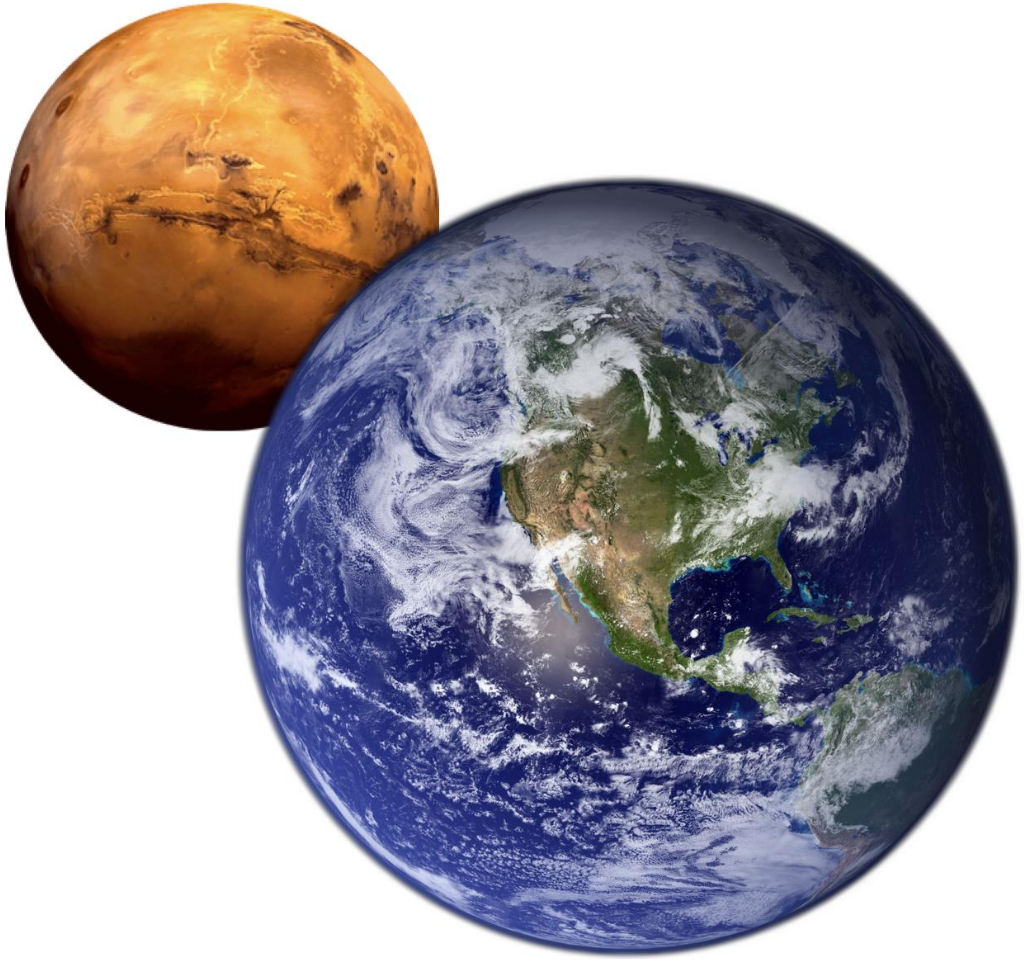
Outline of the Presentation

- Why we need "agnostic" approaches
- Some examples of "agnostic" approaches
  - Some utilizing high heritage hardware
  - Others in nascent stages of development
- Integrative and probabilistic approaches to data analysis



Looking for "Life As We Know It"

- A terrific starting point for Mars
- A nearby planet, with similar composition
- Periodically warm and wet early in its history
- Once protected by a magnetic field



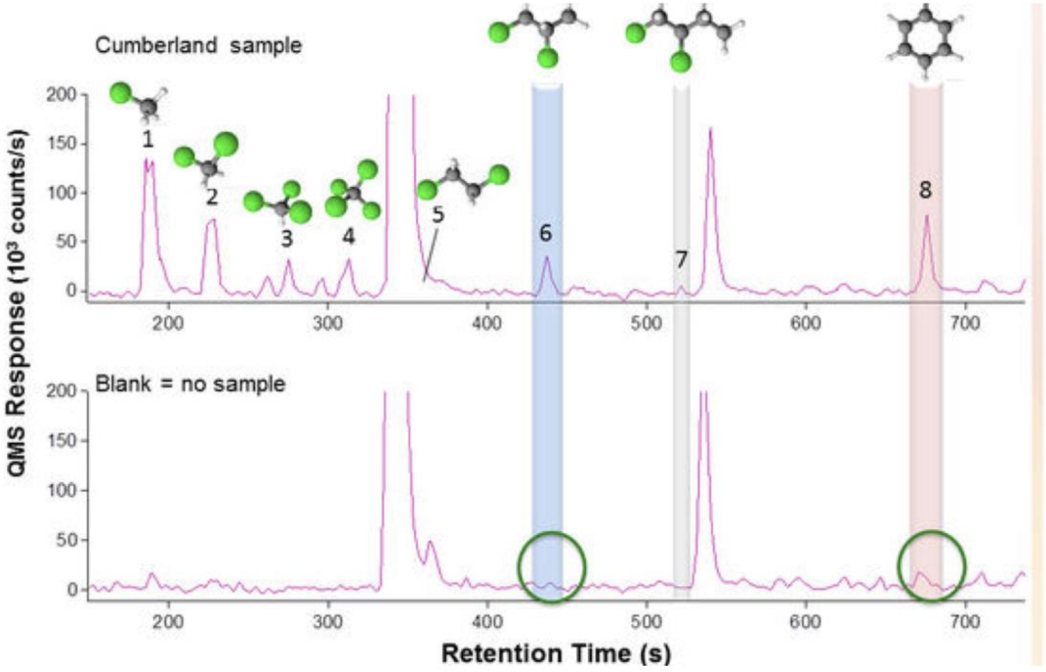
Images: Wikimedia

Possibility that Life on Earth and Mars Share a Common Ancestor

Image: Wikimedia

Common Approaches

- The identification of well-established and widely accepted features associated with terrestrial life and signatures of biologic processes
  - *e.g.*, particular classes of molecules and isotopic signatures
  - patterns within the molecular weights of fatty acids or other lipids, etc.



SAM Team, NASA GSFC

Benefits of these Approaches

- Starting from what we know
- Easier to interpret the data
- Signals may be rich in information
- Stronger understanding of the effects of taphonomic processes from our study of the Earth

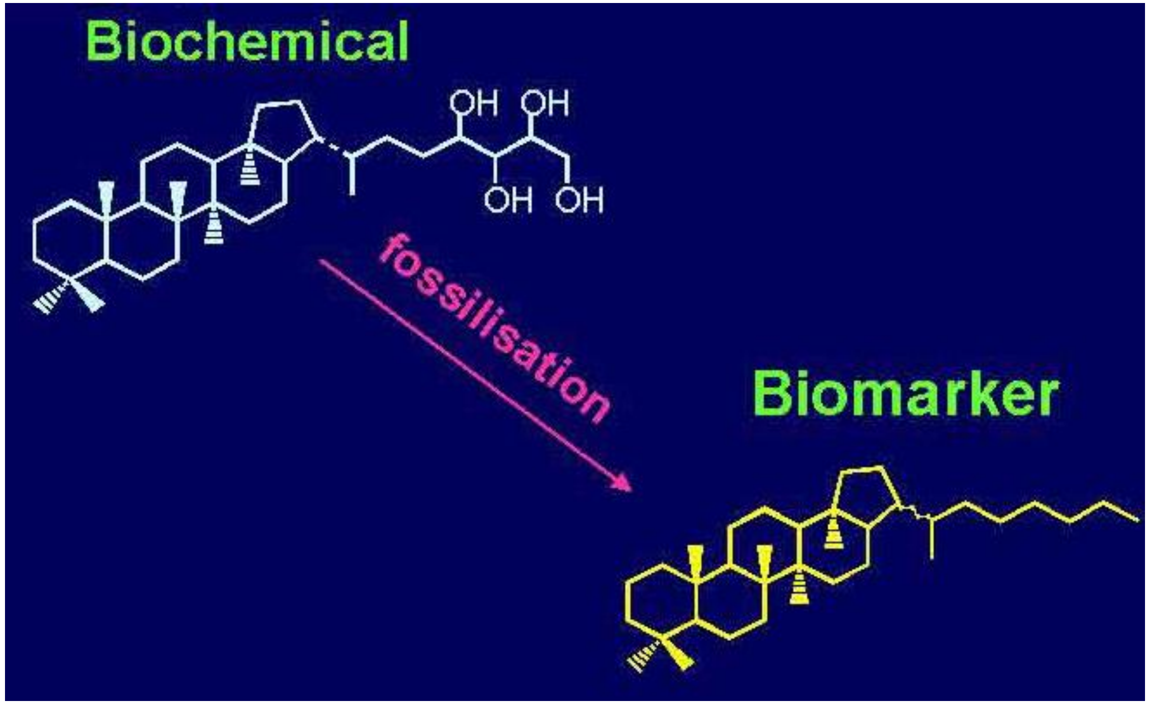


Image: http://www.science.org.au/

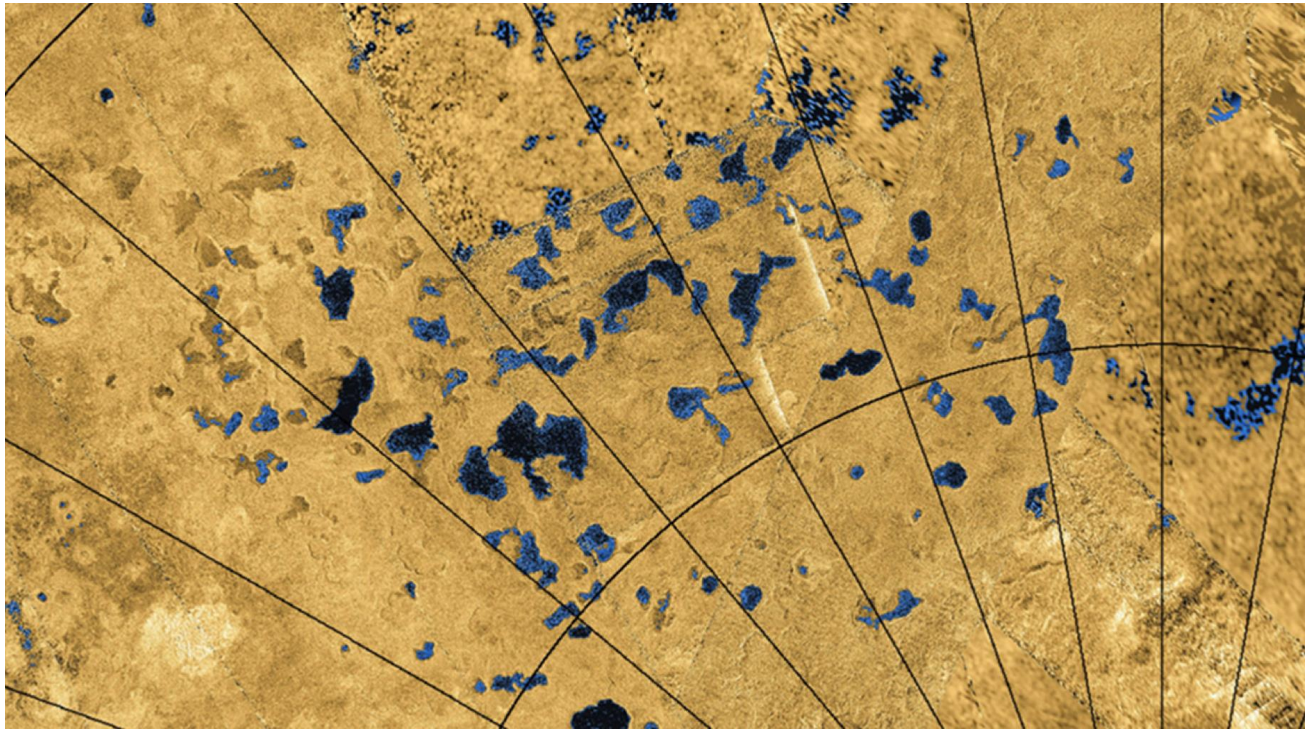
The Limits of Analogy

- Both in terms of where we on the spectrum of understanding life “as we know it”
- For example, we would have designed the Viking experiments differently if we’d designed them today
- As well as the possibilities for life beyond the limits of our current thinking

Image: Campbell’s Soups

New Questions as We Explore Ocean Worlds…

- For example, what might the molecular and polymeric building blocks of life look like on a non-water world like Titan?



Cassini Radar Image, NASA

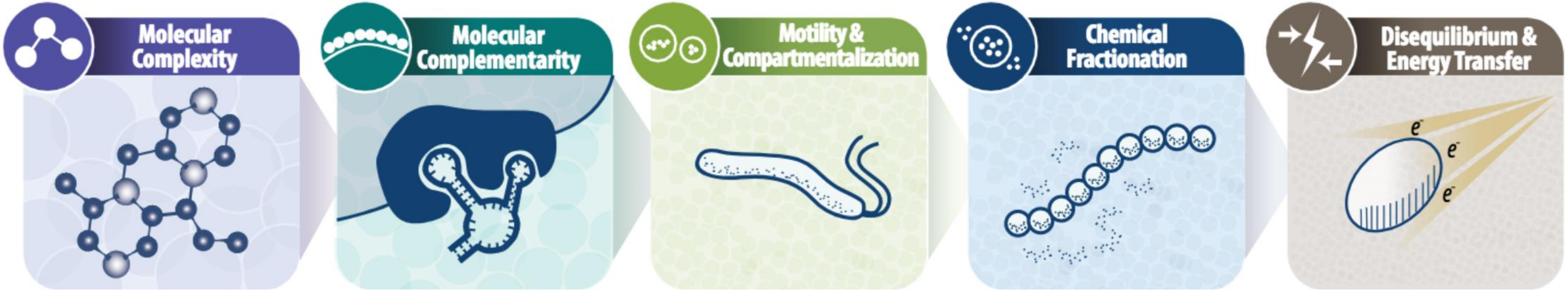
“Ladder of Life”

Ladder Rung	Feature	Measurement	Instrument	Target	Likelihood	Specific to Earth Life vs. Potential for Generic Life	Ambiguity of Feature	Ambiguity of Interpretation	False Positive	False Negative	Detectability
Life (metabolism, growth, reproduction)											
Darwinian Evolution	changes in heritable traits in response to selective pressures	not possible			no	~	~		~		~
Growth and Reproduction	concurrent life stages or identifiable reproductive form [growth and reproduction]	cell(like?) structures in multiple stages	microscope	plume sample	low	Earth	What is a cell? What morphological differences exist?	low	high (not really a cell)	high (don't recognize stages, timing off, sample size low)	hard
Metabolism	isotopes	isotopes indicative of active metabolism	irMS	plume sample	low/med	Earth (can you abstract?)	source, sink, context	low	high	low	easy
	co-located reductant and oxidant (e.g. persistant H_2 +/- CH_4 v. O_2, nitrate, Fe^{3+}, CO_2) [Inferred Persistence]	chemical concentrations of substrates and products involved in redox reactions	spectroscopy	remote detection	med/high	Generic	mixed reactions, large inventory of chemistries	low-med	low-med	med-high	hard (linked to specificity of instrument)
Suspicious biomaterials [not necessarily biogenic]											
	DNA	material produced by extraterrestrial life	spectrographic, immunoassay, PCR, hi-prec MS	plume sample	low	Earth	None	Negligible	high (contamination)	high (technology limited, only terran)	hard (linked to specificity of instrument)

NASA

Ways to Target Unknowable, Unfamiliar Features and Chemistries

- The need for ways to detect signs of processes of *life as-yet unrecognized*
- Building on the work of Conrad and Nealson, 2001, Baross et al., 2007, Hoehler et al., 2007, Schulze-Makuch and Irwin, 2008, Scharf et al., 2015, and many others…



Example 1: Chemical Complexity

- Defining a threshold of chemical complexity that is unlikely or impossible to form spontaneously
- Based on the thesis that the ability of living systems to replicate and evolve allows for the generation of complex molecules, such as metabolites and co-factors, which would be highly unlikely to form in any significant quantity in the absence of biology

Marshall et al., 2017, Philosophical Transactions of the Royal Society B, Images: Wikimedia

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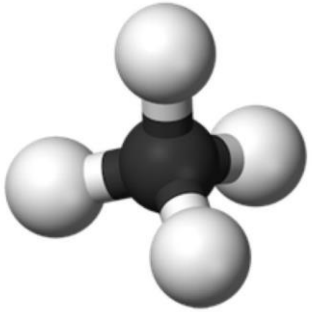

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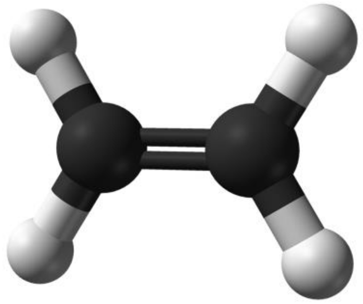

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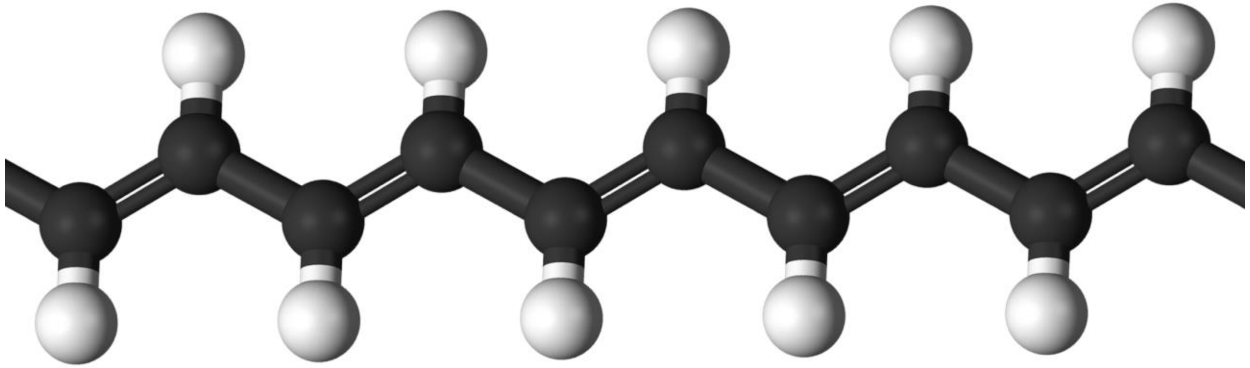
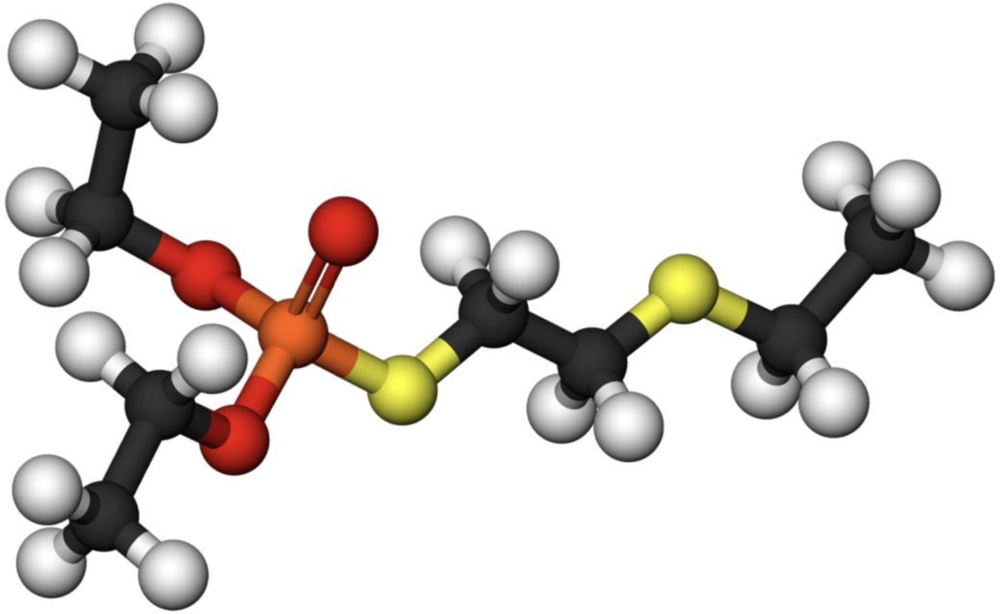


Marshall et al., 2017, Philosophical Transactions of the Royal Society B, Images: Wikimedia

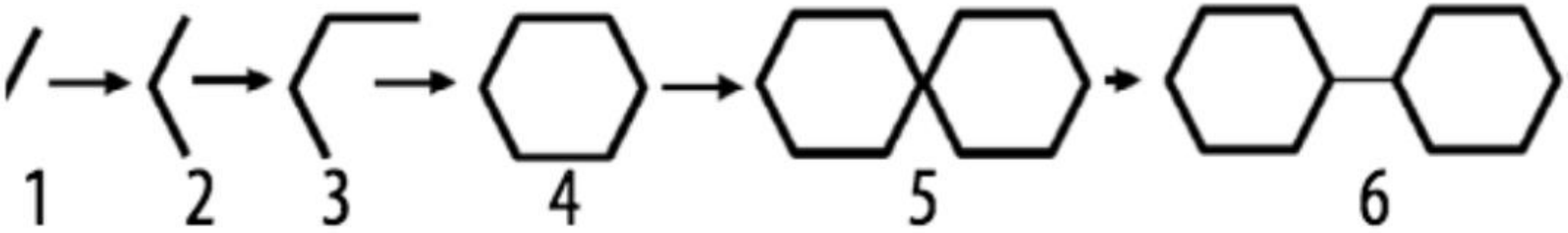
Example 1: Chemical Complexity

- Number of components in a system, the versatility of their interactions
- Structural features, including branching, cyclicity, heteroatoms, etc.



Marshall et al., 2017, Philosophical Transactions of the Royal Society B, Images: Wikimedia

Using Algorithms

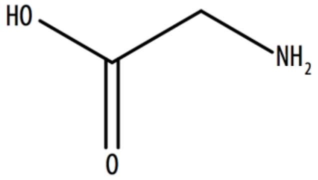


- What is the simplest way to construct a molecule from its parts, accounting for the simplifying feature of duplication?
- An algorithm can be used to break biphenyl into a six-step construction process, therefore assigning a Complexity Index of 6.

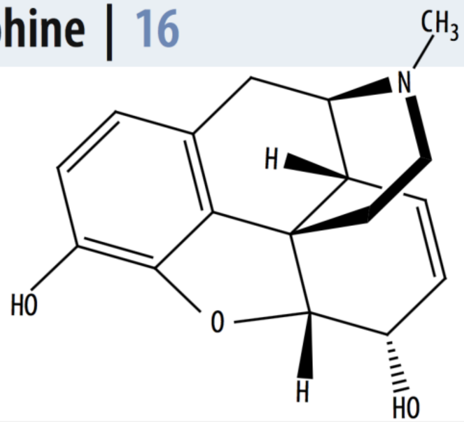
Marshall et al., 2017, Philosophical Transactions of the Royal Society B

Thresholds

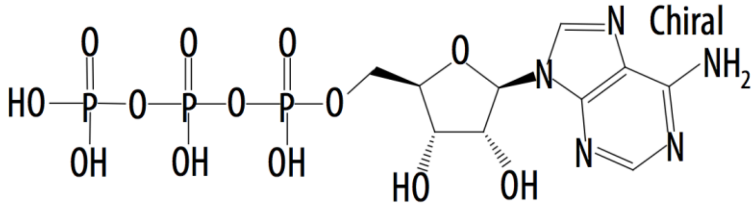
Glycine | 4



Morphine | 16



ATP | 21



- Without making assumptions about the chemical structures of molecules, there appears to be a threshold around 15 beyond which complex molecules are unlikely to form without supporting biological machinery

Marshall et al., 2017, Philosophical Transactions of the Royal Society B

Example 2: Molecular Complementarity

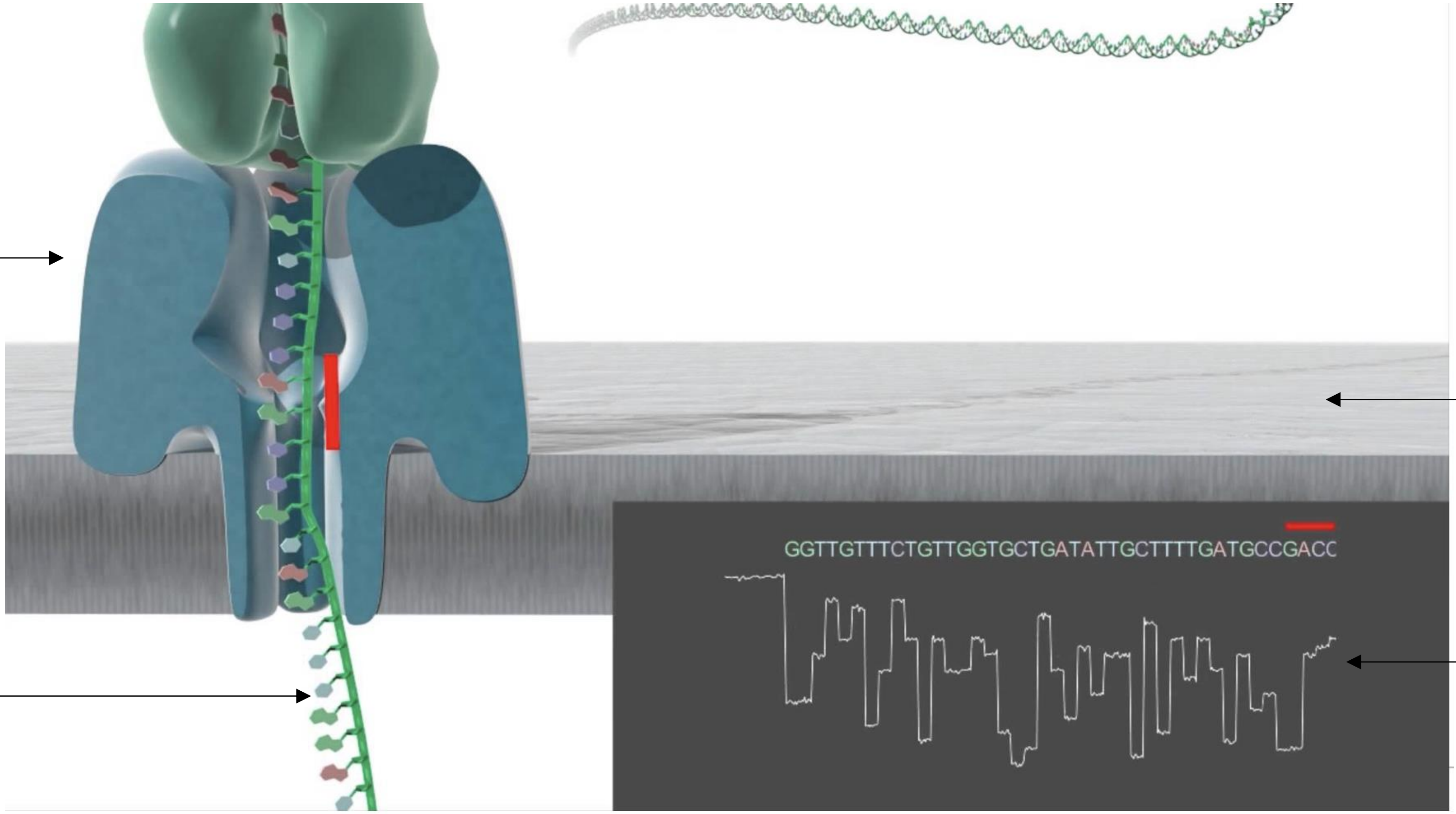
- Rapid advances in miniaturization have led to extremely small nanopore sequencers, like the Oxford Nanopore MinION

Oxford Nanopore Technologies, Johnson et al., 2017, JBT

Nanopore Sequencing

nano-scale protein pore

DNA Strand

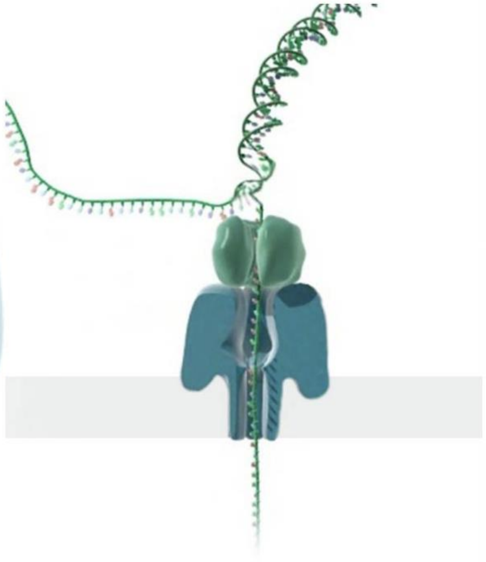


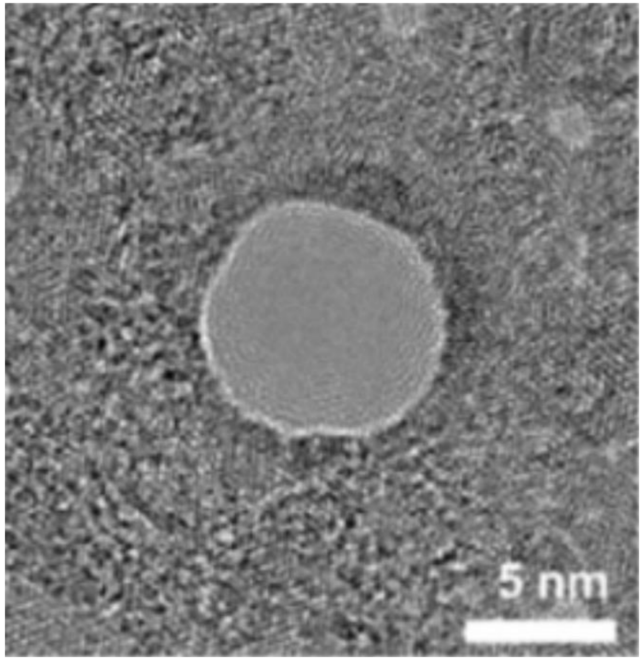
membrane

sequences detected by disruption in applied current

Oxford Nanopore Technologies

Nanopore Sequencing





- Nanopore sequencing technologies are currently being adapted for space by NASA’s MATISSE and COLD-Tech instrument development programs

Oxford Nanopore Technologies, Shi et al., 2015, Microchimica Acta

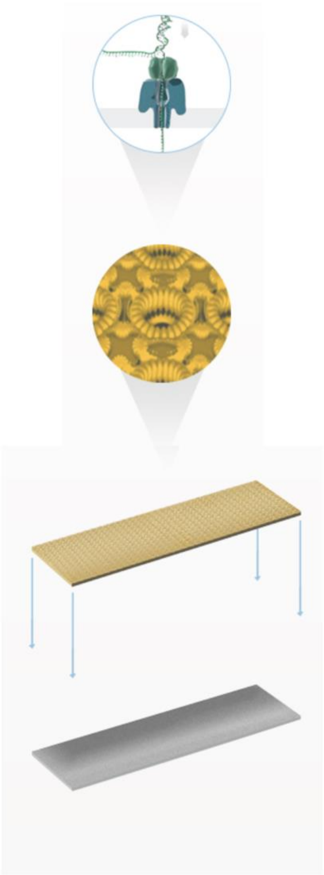
Nanopore Sequencing

nano-scale
protein pore

array of
microscaffolds

sensor chip with
electrode channel array

Application-Specific
Integrated Circuits
(ASICs)



Sutton et al., in prep

Nanopore Sequencing

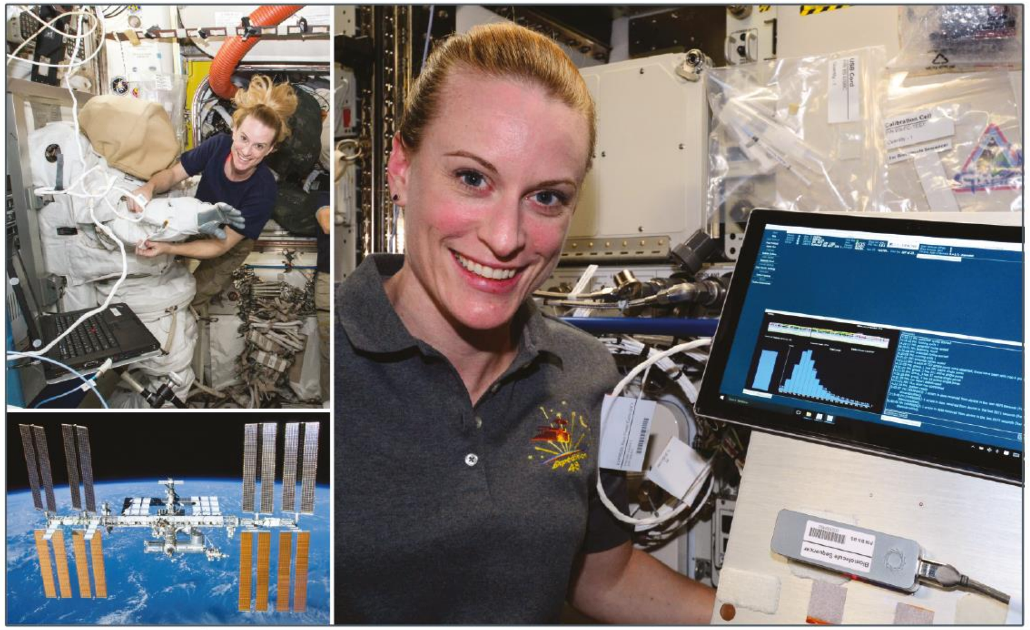
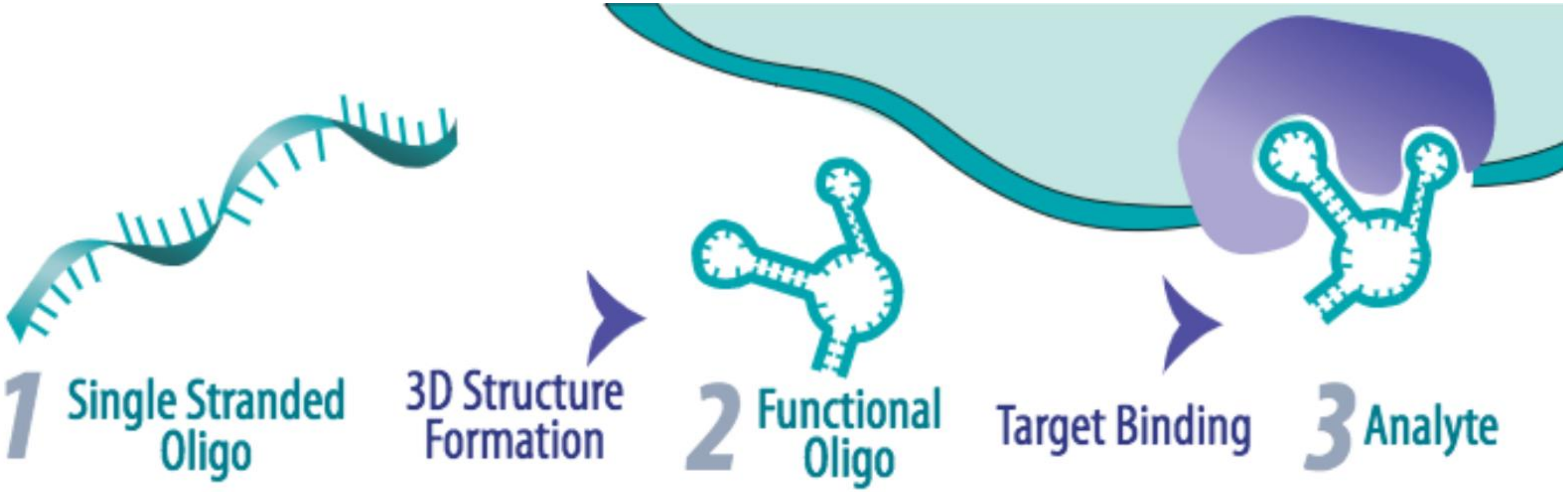


Fig. 1 Astronaut Kate Rubins on the ISS

- Tested in space
- A promising way to look for nucleic acids (including those with nonstandard bases) and monitor contamination (a key challenge for life detection)

Castro-Wallace et al., 2017, Scientific Reports

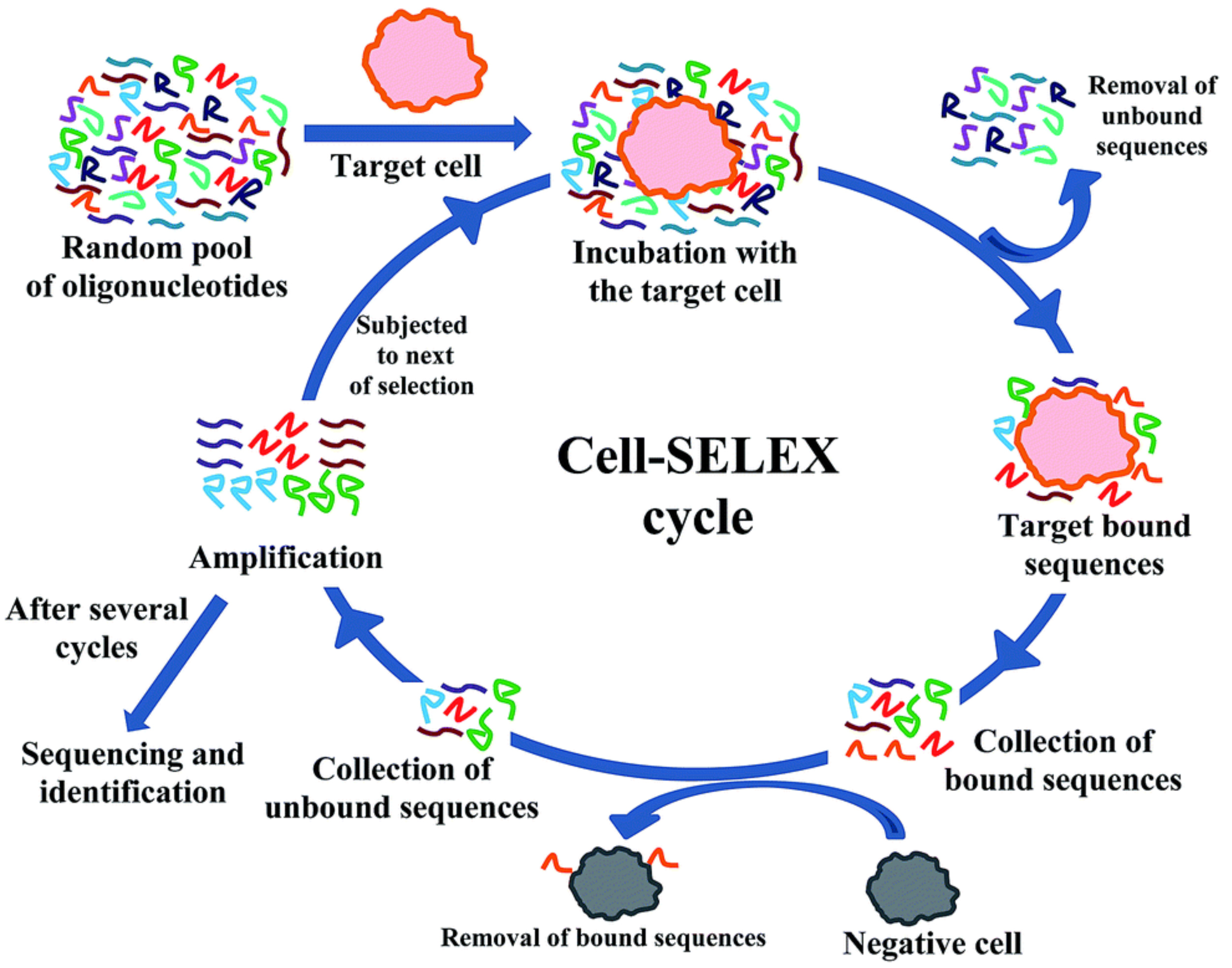
Example 2: Molecular Complementarity



- Harnessing the power of sequencing to explore sample complexity, as measured by molecular complementarity of ligands to short, randomly generated oligonucleotides

Johnson et al., in press, Astrobiology

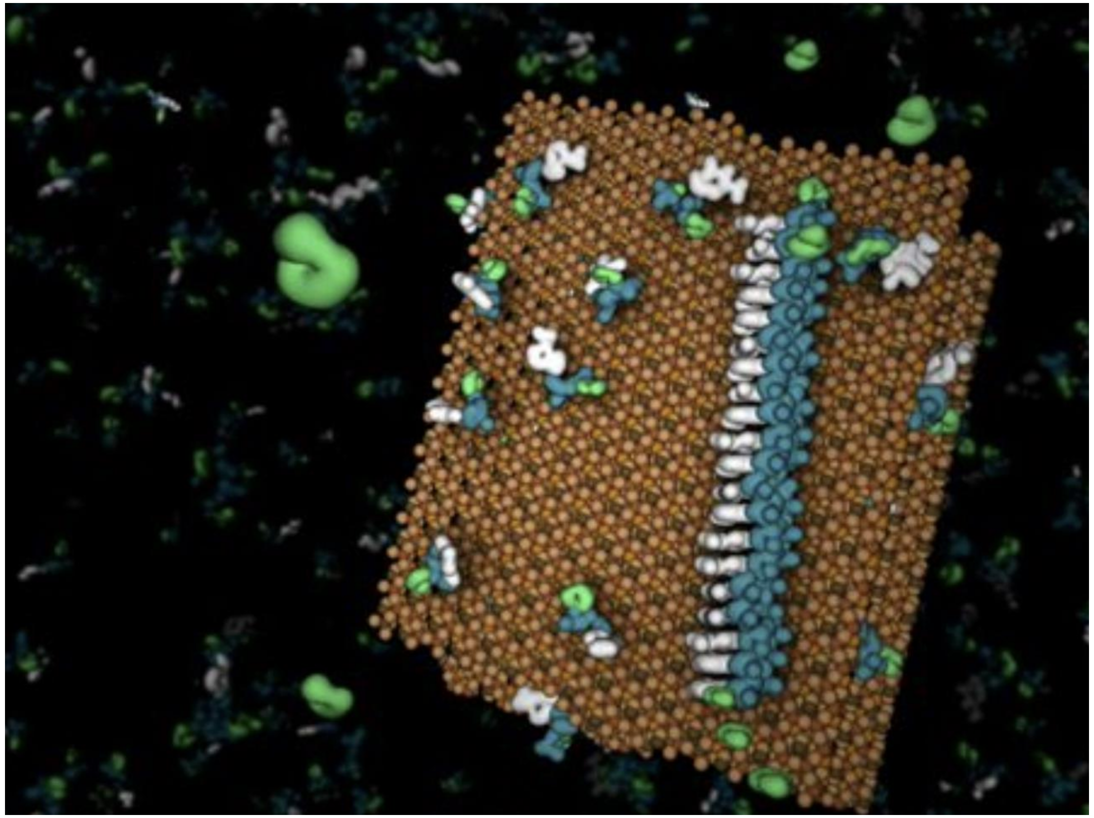
Systematic Evolution of Ligands by Exponential Enrichment (SELEX)



Barman et al., 2015, RSC Advances

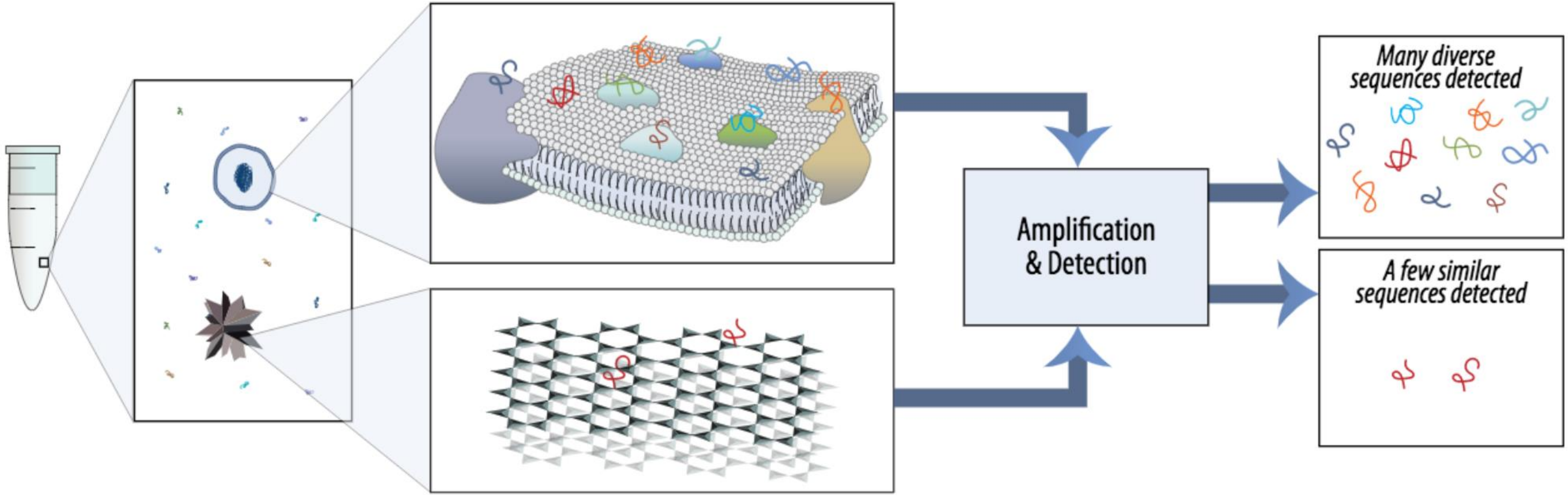
Oligo Binding

- Binding with similar specificities and affinities as antibodies
- Binding to a wide variety of molecules
  - Peptides and proteins (e.g. Sun and Zu, 2015, *Molecules*)
  - Small organic molecules (e.g. Stoltenburg et al., 2007, *Biomolecular Engineering*)
  - Inorganics like mineral surfaces (e.g. Cleaves et al., 2011, *Chemosphere*)
  - Metals (e.g. Ye et al., 2012, *Nanoscale*)



Exploringorigins.org, Ferris Lab

Example 2: Molecular Complementarity



Johnson et al., in press, *Astrobiology*

Low Biomass Targets

- Chemolithotrophic life on Ocean Worlds may have far less biomass
- Because oligos are made from nucleic acid, signal amplification with PCR is possible

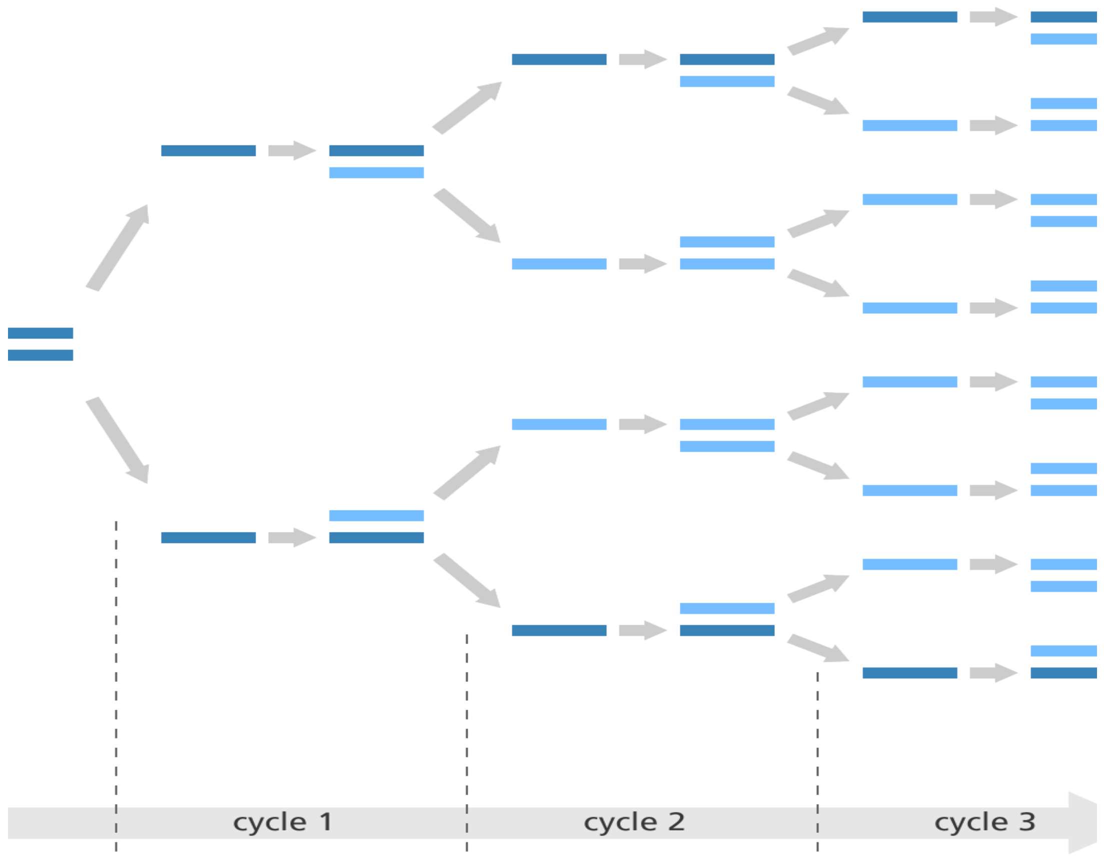
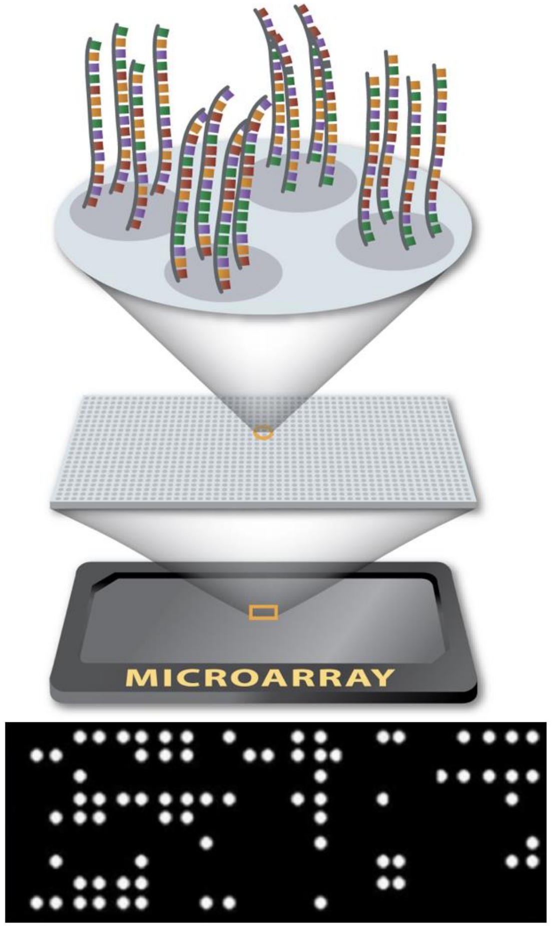


Image: YourGenome

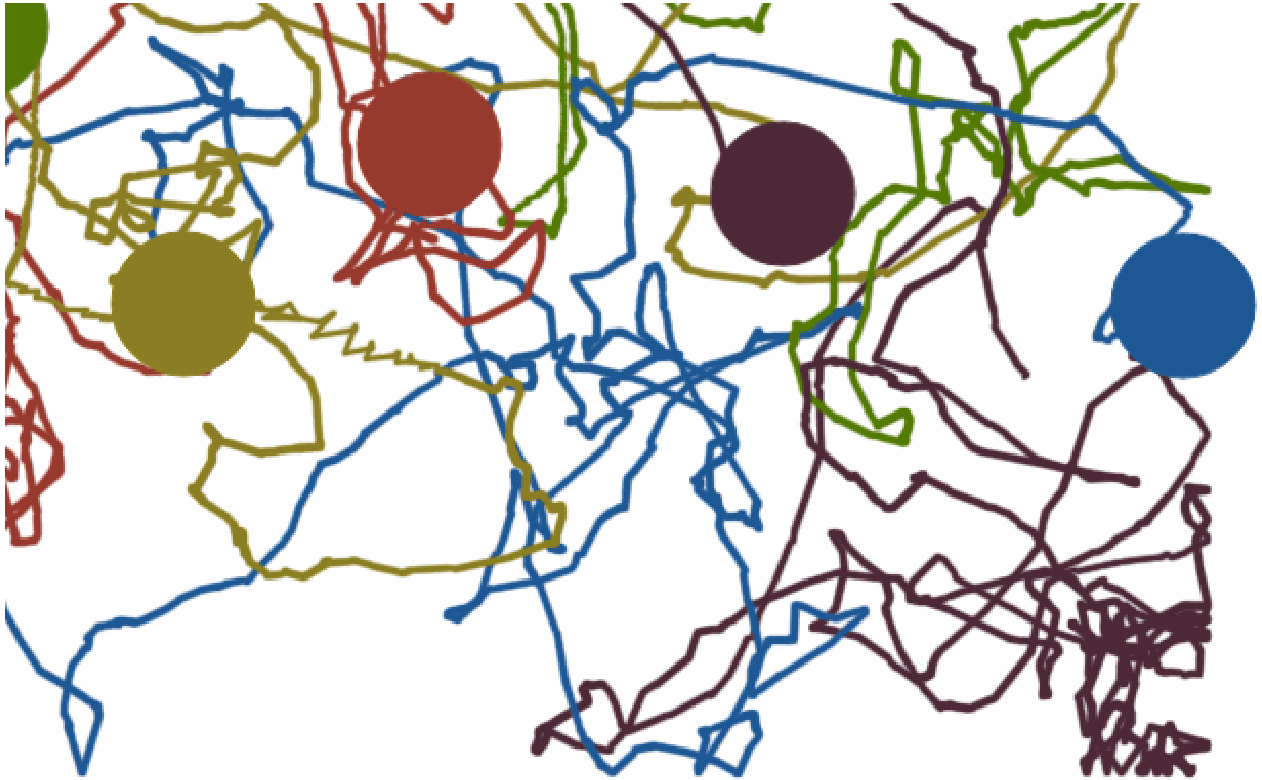
Alternative Detection Possibilities



- Microarrays with spots containing multiple strands of a single DNA sequence
- Thousands to millions can be arrayed in rows on glass the size of a microscope slide, or smaller
- Fluorescent probe released upon binding, which can be simply imaged
- Electrochemical microarrays as well

Image: ISDA

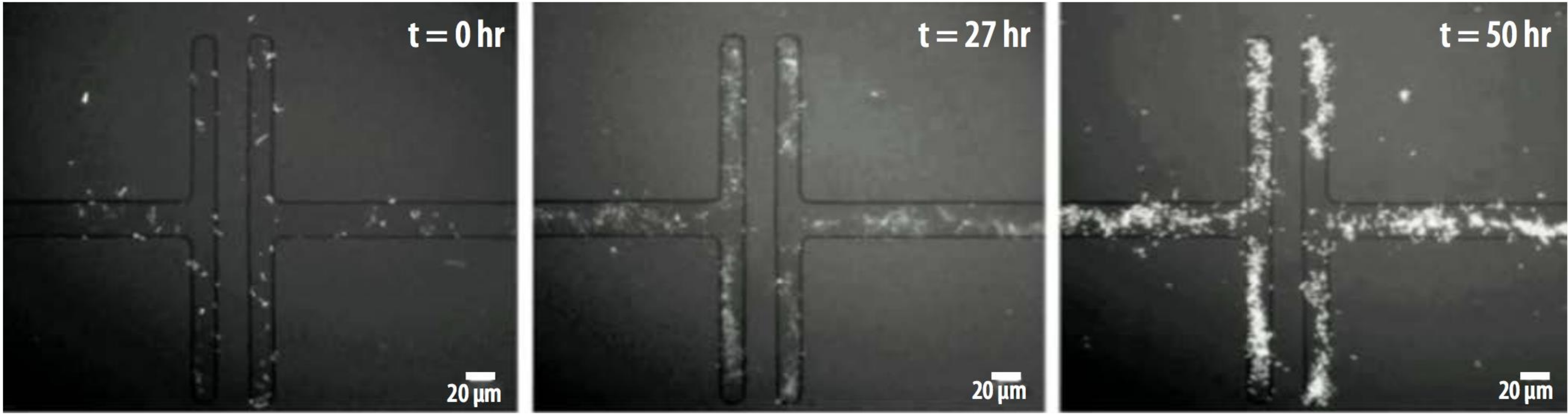
Example 3: Meaningful Motility



- Speeds higher than Brownian motion, patterns inconsistent with fluid drift or currents

Image: Minute Labs

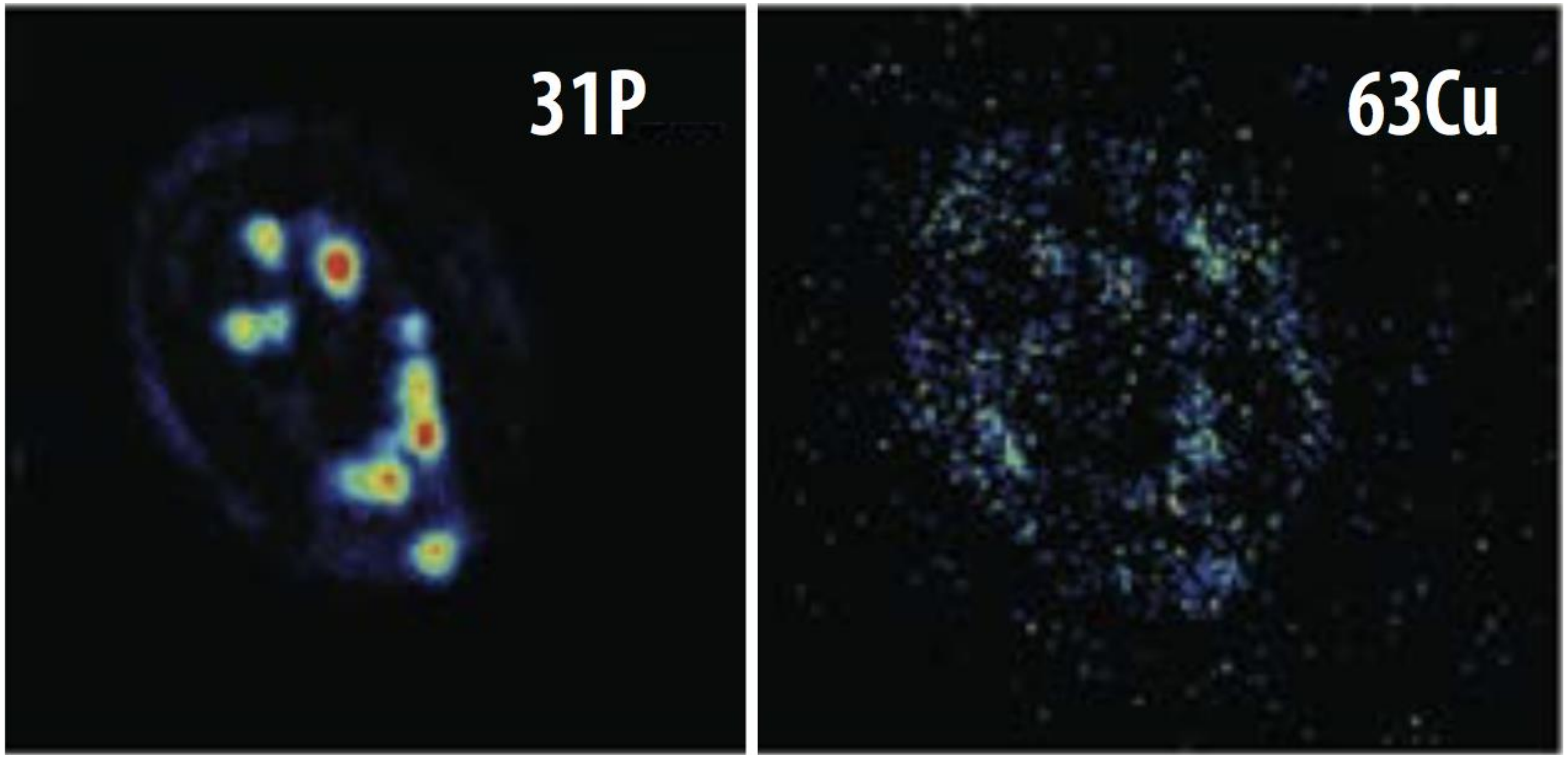
Example 3: Meaningful Motility



- Taxis toward nutrients, oxygen, light, electron acceptors, or other stimuli
- See NASA White Paper by Jay Nadeau and colleagues

Image: Courtesy of Dr. El Naggar, USC

Example 4: Chemical Fractionation



- Exploring elemental and isotopic accumulation in compartments isolated from the environment as a potential biosignature

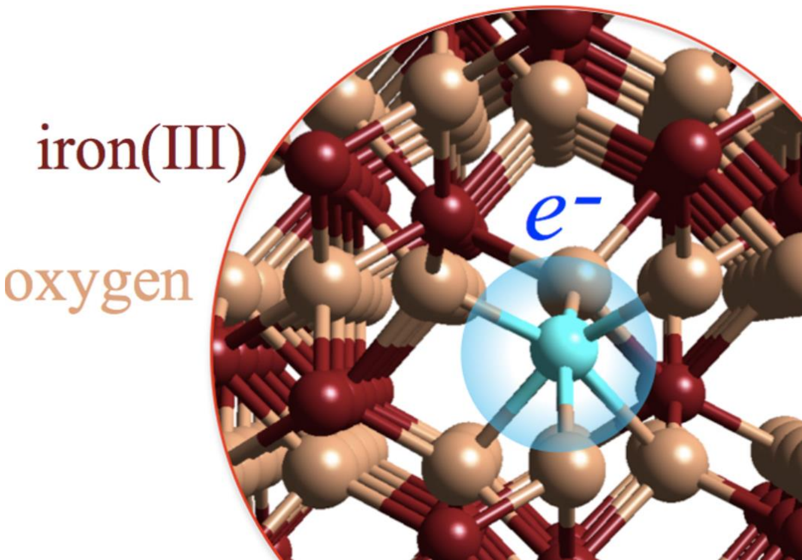
Williams and Da Silva, 2000, Coordination Chemistry Reviews; Slaveykova,, 2009, Analytical and Bioanalytical Chemistry

Example 5: Disequilibrium & Energy Transfer

- *Life is nothing but an electron looking for a place to rest*

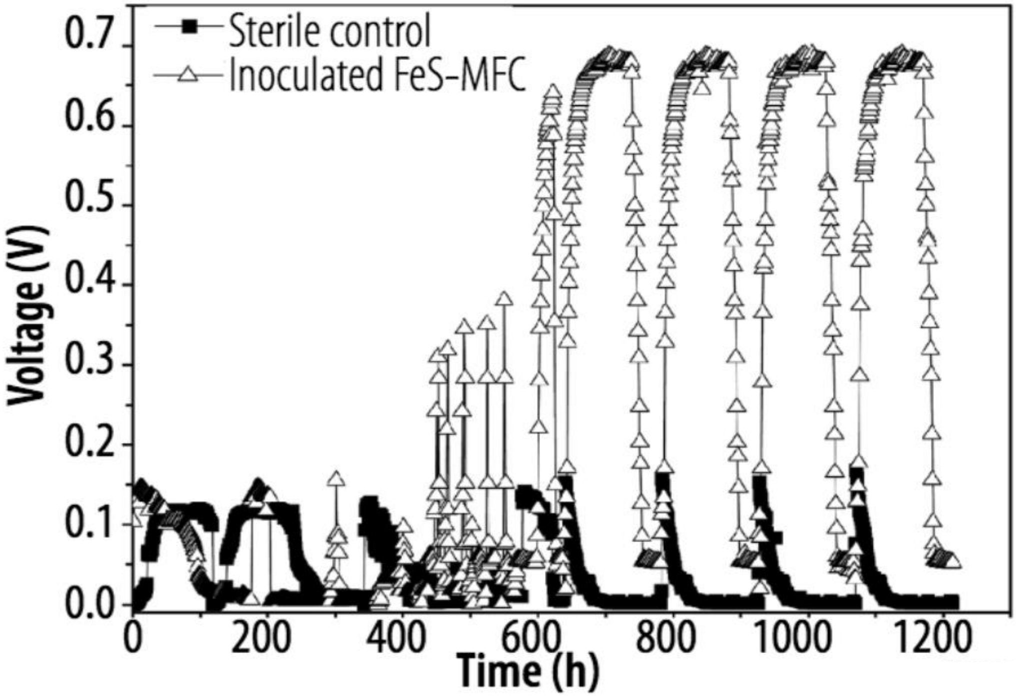
  -Nobel Prize-winning physiologist Albert Szent-Györgyi

- Seeking disequilibrium redox chemistries (inconsistent with abiotic redox reactions) as an indicator of active metabolism…



LBL.gov

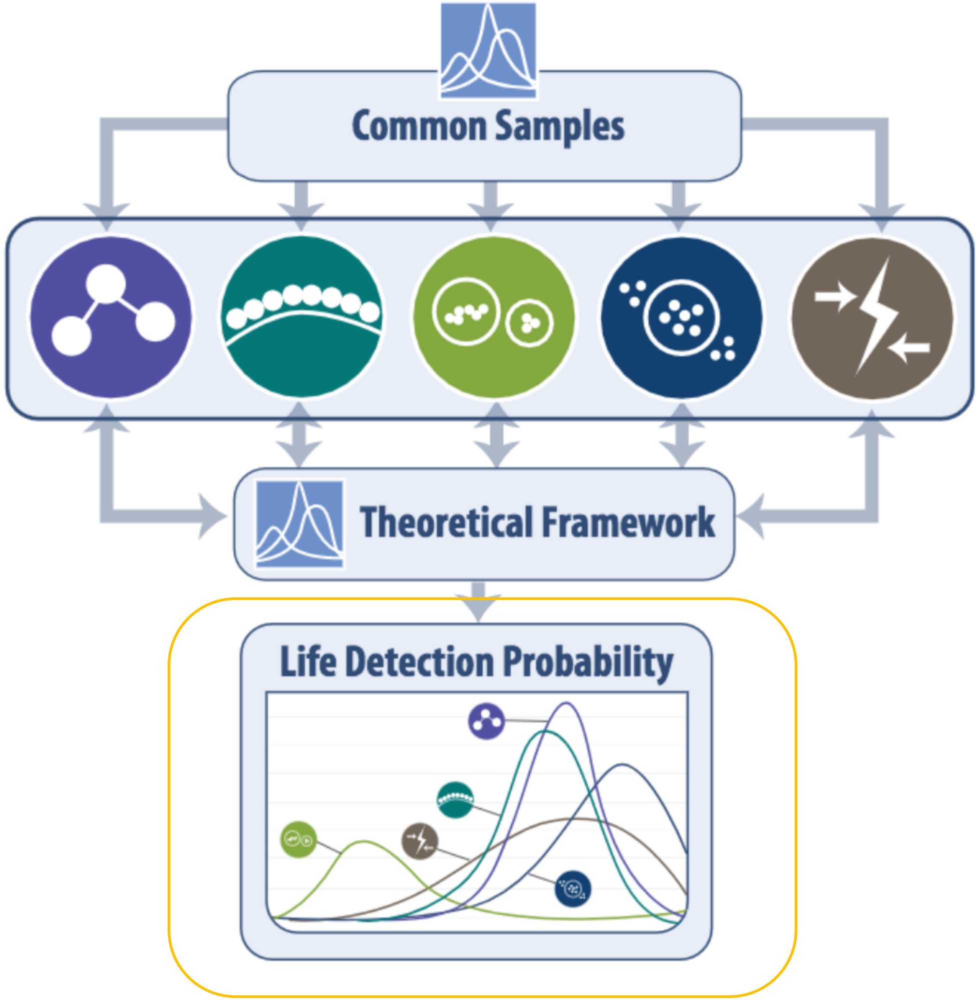
Example 5: Disequilibrium & Energy Transfer



- Abiotic and biological processes distinguishable in simple set-ups
- A marked and sustained increase in voltage in reactors with microbial communities

Nie et al., 2015 RSC Advances

Integrative and Probabilistic Approaches to Data Analysis



- Agnostic methods may trade definitiveness for inclusivity
- A Bayesian Network (for which the output is the probability there is a biosignature given the measurement data) could be utilized, thus converting measurements into likelihoods and thresholds

An Operational Spectrum of Certainty

		Concept 1		Concept 2		Concept 3		Concept 4	
		+	-	+	-	+	-	+	-
2	+	LIVE	INC						
	-	DEAD*	NOT						
3	+	LIVE	INC	LIVE	INC				
	-	SESSILE	NOT	SESSILE	NOT				
4	+	LIVE	INC	LIVE	INC	LIVE	SESSILE		
	-	DEAD*	NOT	DEAD	NOT	INC	NOT		
5	+	LIVE	INC	LIVE	INC	LIVE	SESSILE	LIVE	INC
	-	DEAD	NOT	DEAD	NOT	INC	NOT	DEAD	NOT

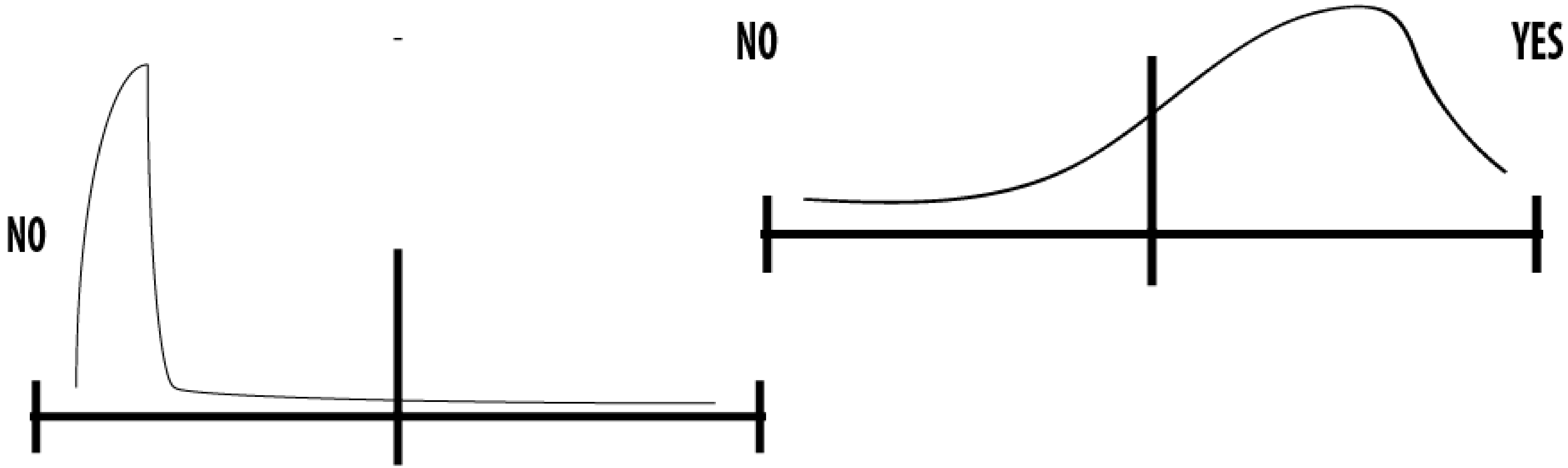
3 Categories of life: LIVE: Life, DEAD: Life, SESSILE: Life, INC: Invonclusive, NOT: No Life.

- Techniques can be combined that offer added interpretive value without redundancy
- A hypothetical pair-wise evaluation can indicate the chance of inconclusive results.

MindfulEcology

A Conceptual Spectrum of Certainty

- From a binary “life” or “no life” to “3σ from what we would expect from abiotic processes”

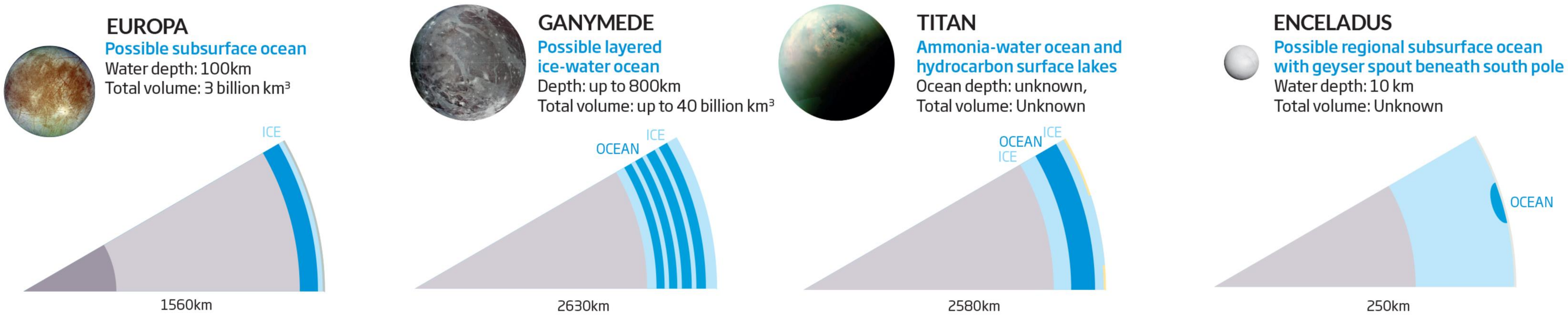


Utility of Computational Approaches

- Build challenging null models
- Explore limits of biology in theoretical foreign environments
- Anticipate trade-offs indicative of alternate life strategies
- Develop threshold values for data interpretation
- Predict outcomes of combinations of approaches
- Predict necessary sample sizes
- Understand detection limits and margins of error
- Provide interpretive strategies based on life detection probabilities and mitigate false positives and negatives

Relevance to the Astrobiology Strategy

- Broader framework for life
- Better understanding of limitations
- Especially important as we expand deeper into the solar system…



New Scientist, NASA