Beyond RNA and DNA

In-Situ Sequencing of Informational Polymers

Christopher E. Carr
Research Scientist, MIT
chrisc@mit.edu
carrlab.org

Co-authors:
Maria T. Zuber, MIT
Gary Ruvkun, MGH

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Overview

- The Search for Extra-Terrestrial Genomes (SETG) Instrument
- The case for nucleic acid based-life beyond Earth (and Mars)
- Prospects for sequencing non-standard nucleic acids

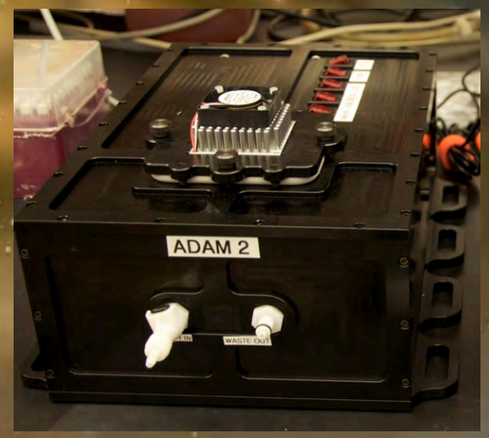
The Search for Extra-Terrestrial Genomes

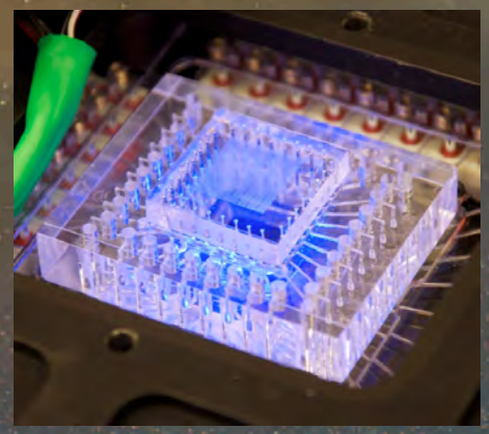
An in-situ detector for life on Mars ancestrally related to life on Earth

Prof. Gary Ruvkun (MGH PI)

Prof. Maria Zuber (MIT PI)

SETG Prototype

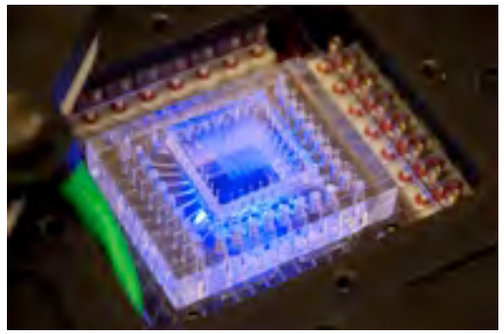




~4 Gya

Life on Mars, if it exists, may be related to life on Earth.

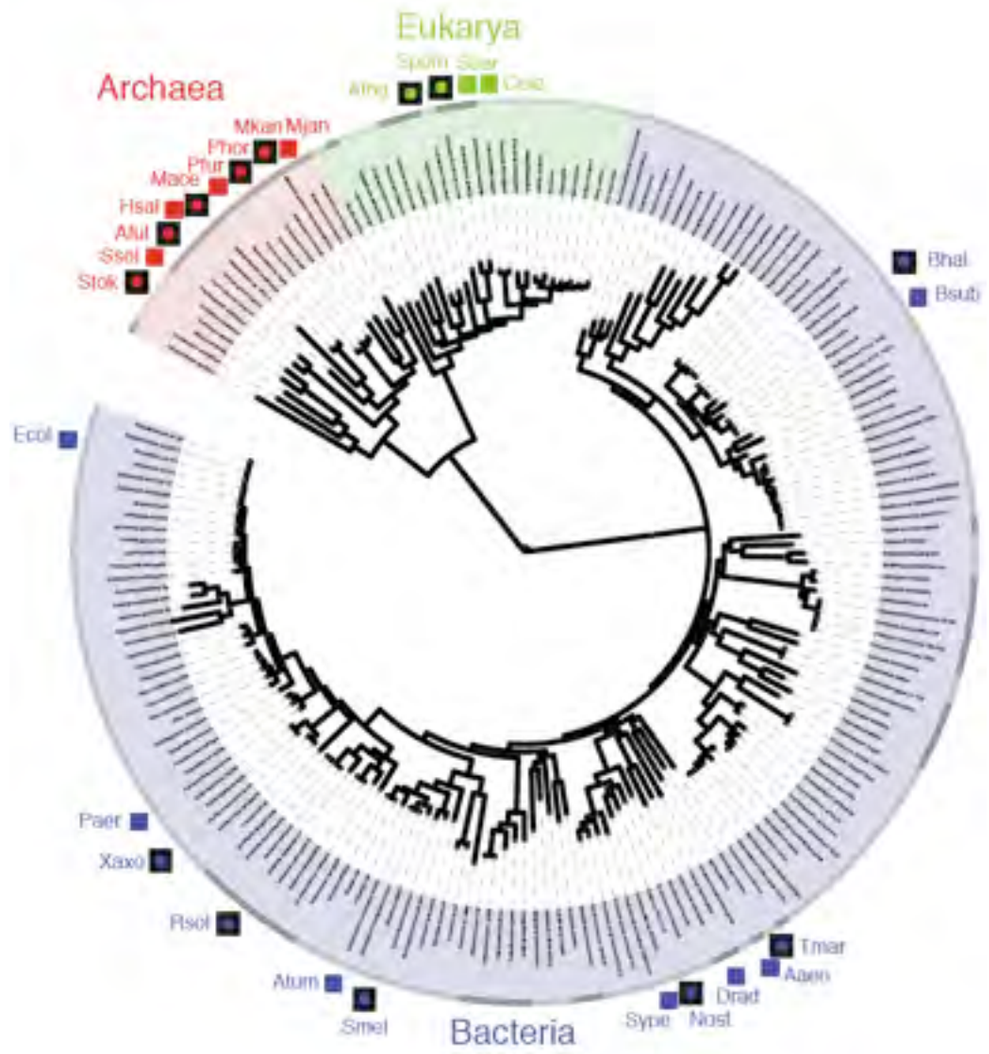
Image Credit: NASA/FUSE/Lynette Cook



2005

2010

Biological protocols
PCR-based approach
Highly conserved sequences

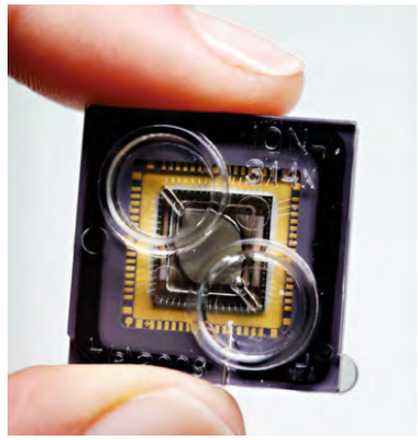


Radiation Sensitivity

First field test

Metagenomics Approach

Miniature sequencer



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If life evolved independently from life on Earth...

- It probably uses an informational polymer to carry information
- It is likely to be based on nucleic acids or a related informational polymer
- It may use a non-standard backbone and/or alternative bases
- We can potentially detect and characterize it using in-situ sequencing

Widespread and Early Synthesis of Complex Organics

“icy grains originating in the outer disk, where temperatures were <30 K, experienced UV irradiation exposures and thermal warming similar to that which has been shown to produce complex organics in laboratory experiments”

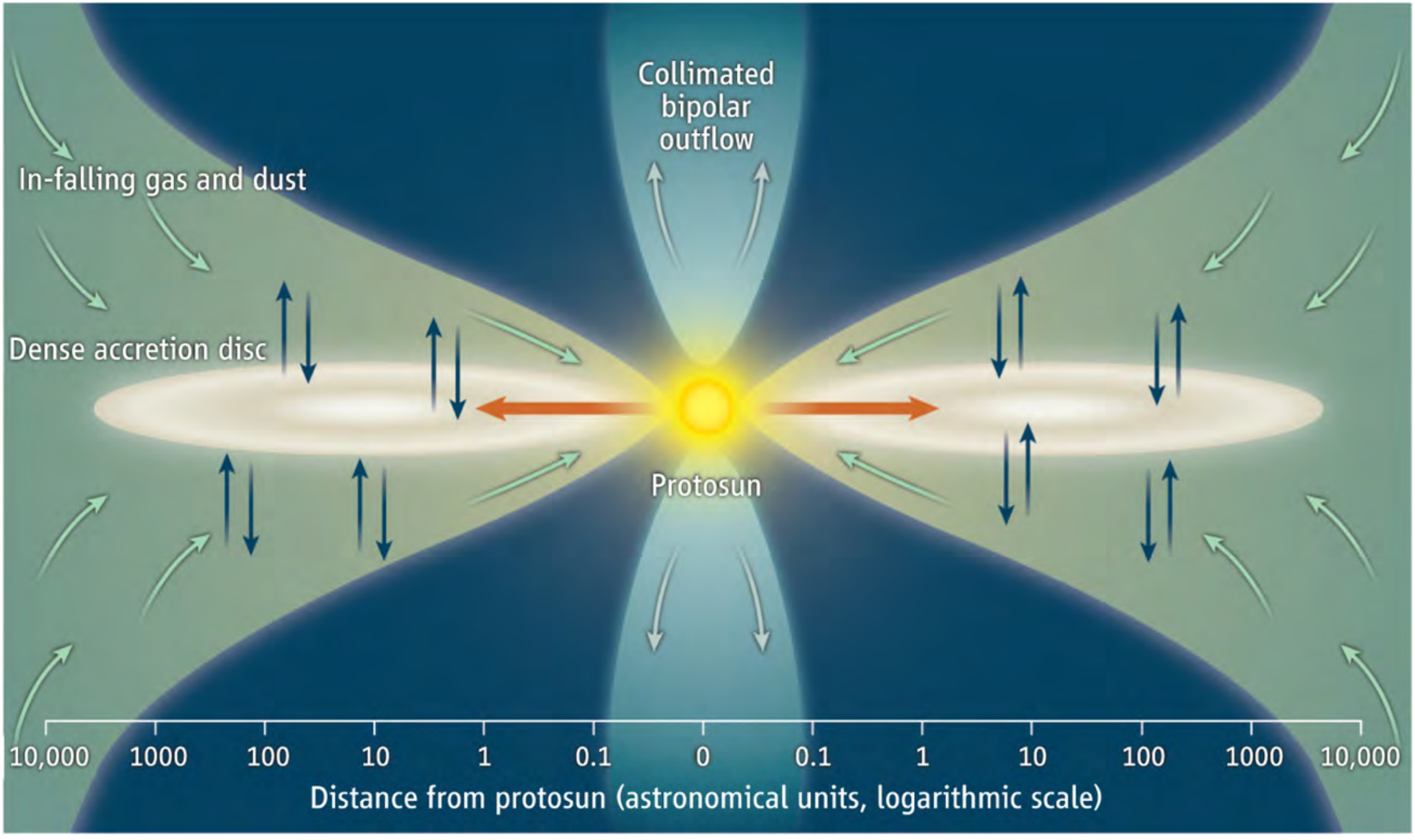
Organic Synthesis via Irradiation and Warming of Ice Grains in the Solar Nebula

Ciesla and Sandford. 2012 Science 336(6080) 452-4. doi: 10.1126/science.1217291

Artist’s impression of the solar nebula

Image credit: NASA

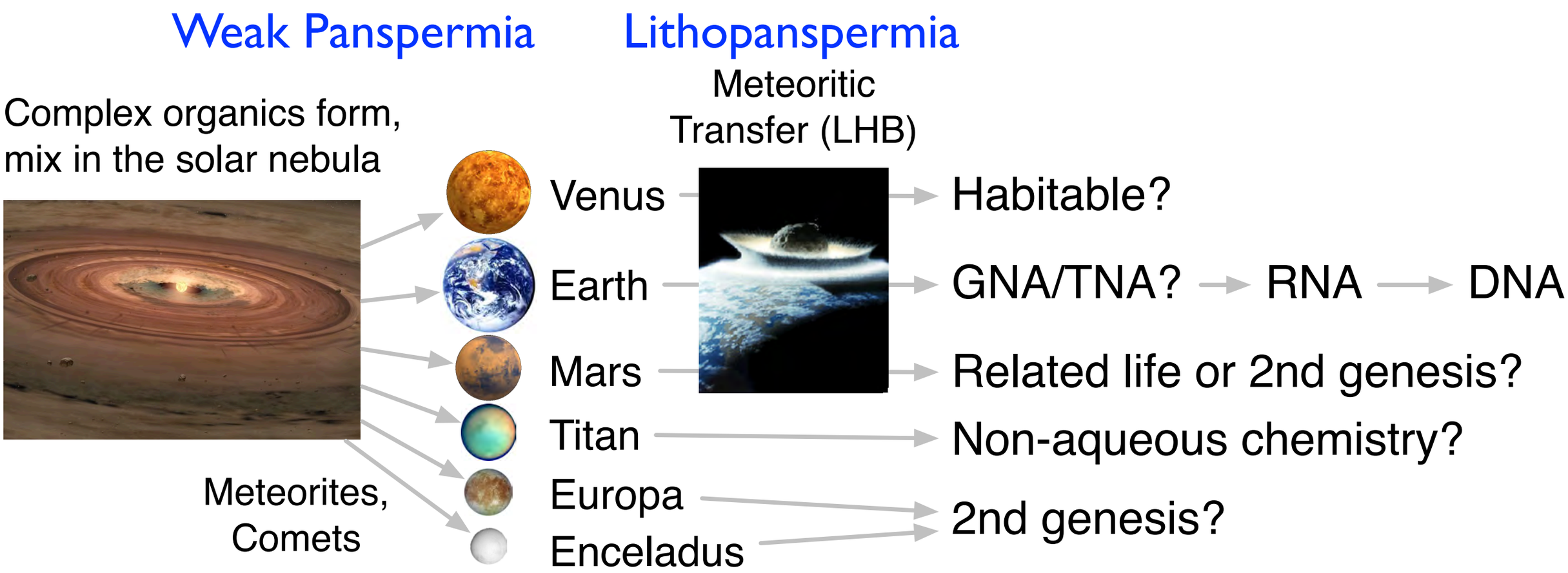
Organics get dispersed throughout the solar nebula



Complex Protostellar Chemistry

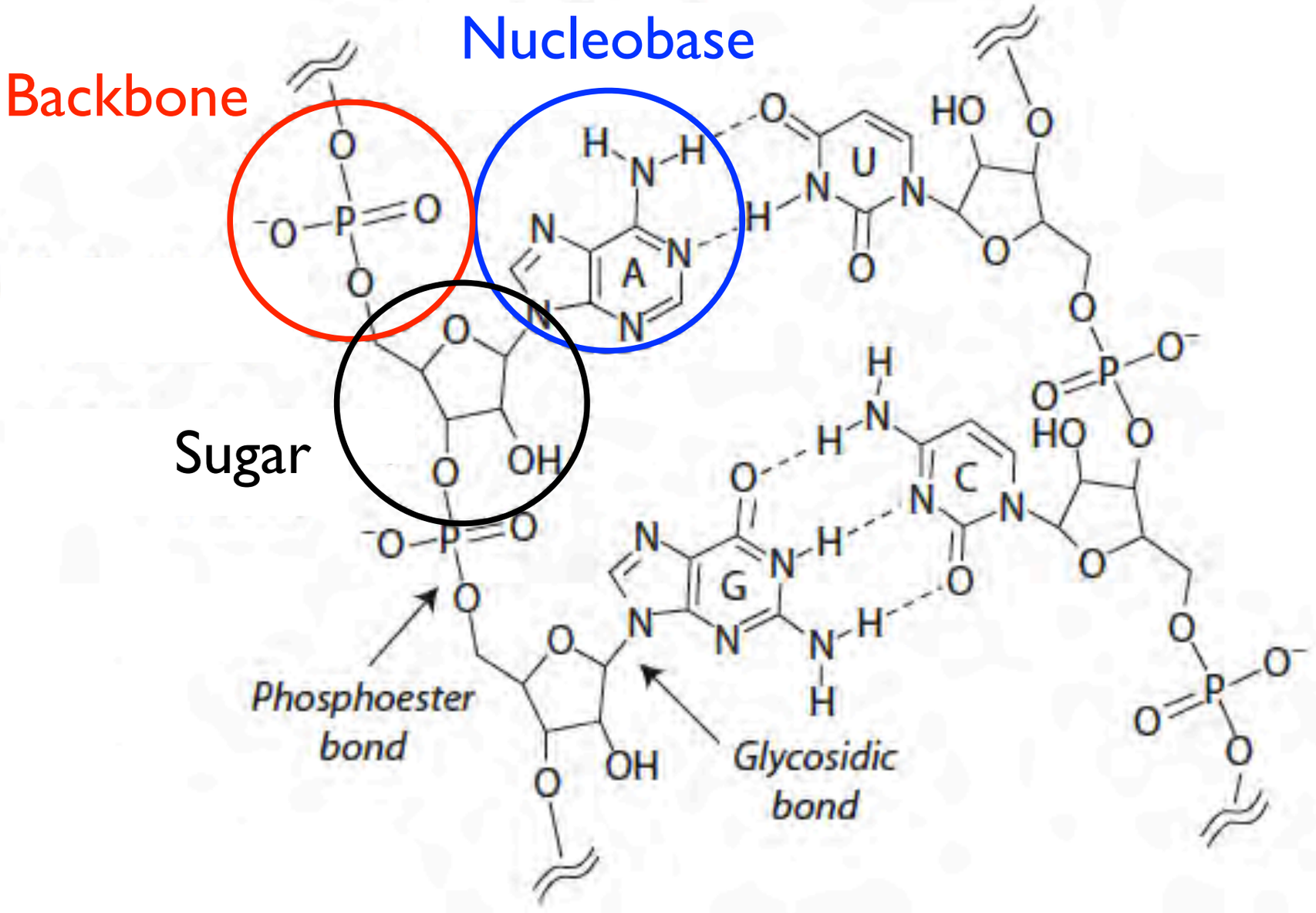
Nuth and Johnson. 2012 Science 336(6080) 424:5 (perspective).
doi: 10.1126/science.1219709

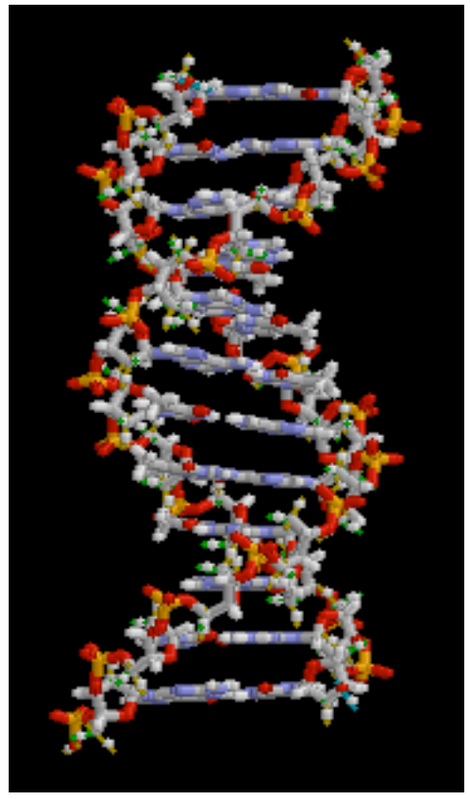
Similar organics delivered to all habitable zones



If life beyond Earth exists, does it use common or different informational polymers?

Nucleic acid informational polymer (IP)



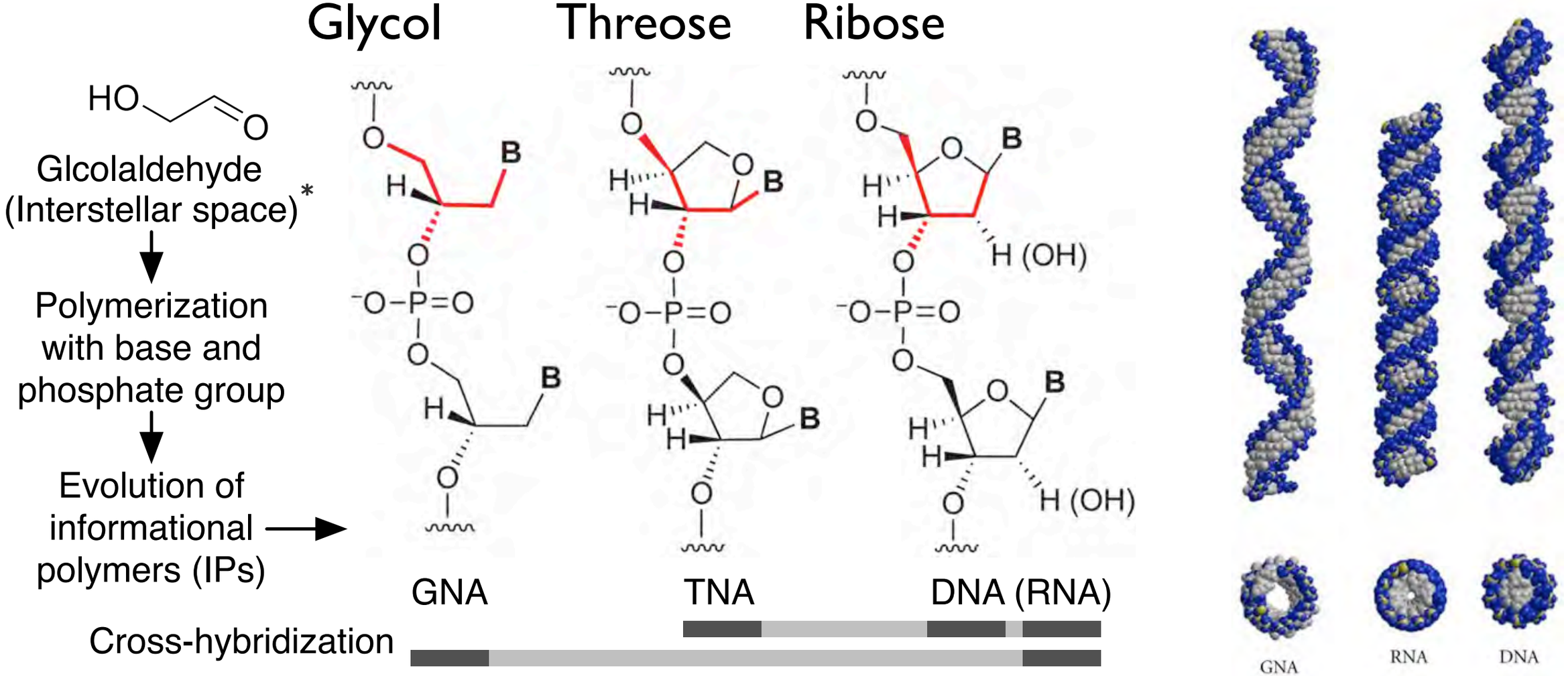


DNA Wikipedia

Primitive Genetic Polymers

Engelhart and Hud. Cold Spring Harb Perspect Biol 2010 doi: 10.1101/cshperspect.a002196

Could life use an alternative sugar?

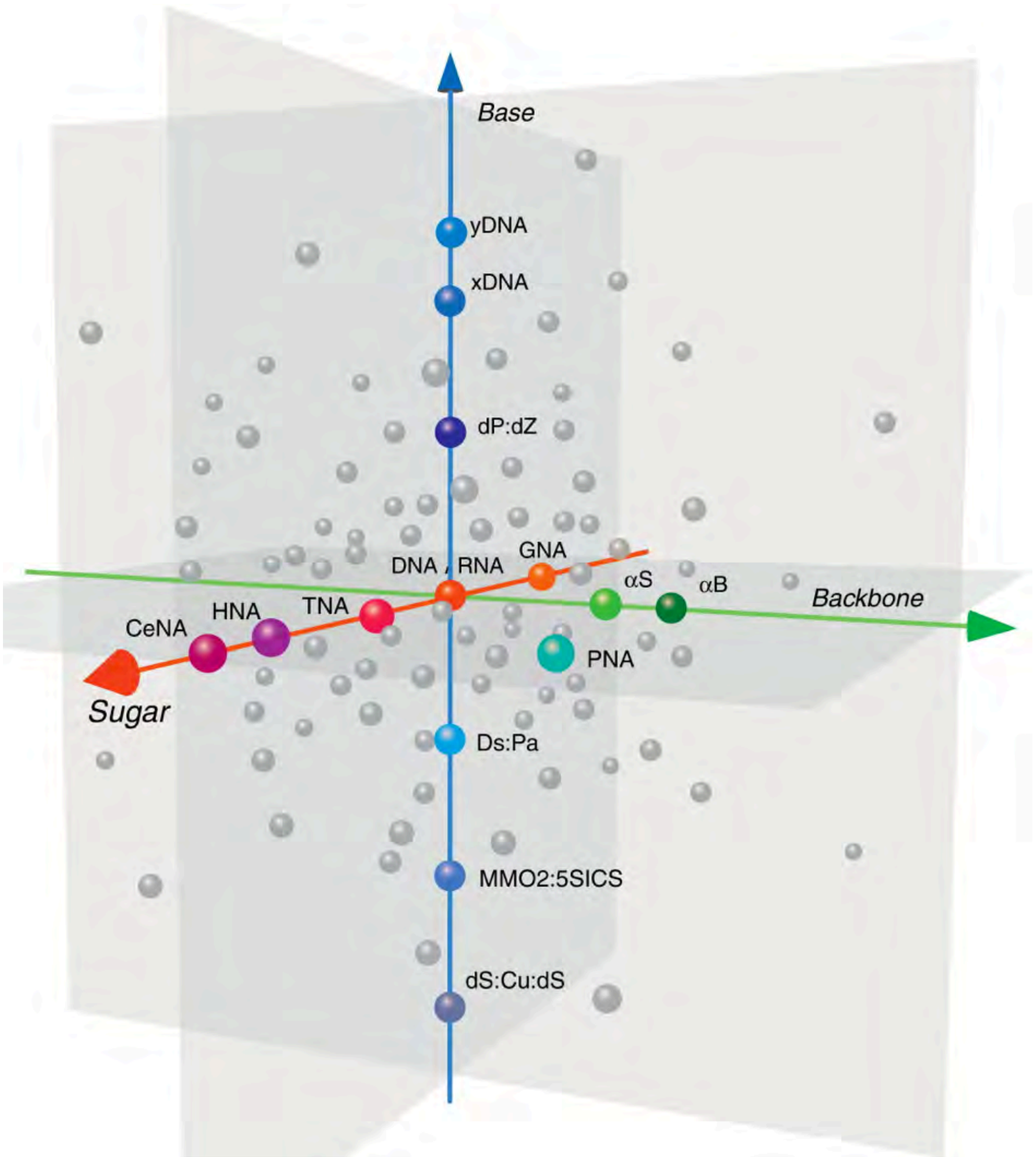


Yes, it's possible life could use other sugars, but there may be reasons to "prefer" DNA

* **Interstellar Glycolaldehyde: The First Sugar** Hollis et al. 2000 ApJ 540 L107 doi:10.1086/312881

Images adapted from: Chen et al. (2009) PLoS ONE 4(3):e4949. doi:10.1371/journal.pone.0004949
Förster et al. (2012) J Nucleic Acids doi: 10.1155/2012/156035

Could life use an alternative backbone?



Reasonable Life Detection Assumptions

Nucleobases are likely to be standard or close relatives.

The sugar may be different than ribose but is probably related.

The charged backbone may be universal in water (Benner, 2004).

In synthetic biology, the possibilities are far more numerous than are likely for the origin(s) of life.

The XNA world Pinheiro and Holliger. Cur Opin Chem Biol 2012, 16:245-252 doi: 10.1016/j.cbpa.2012.05.198

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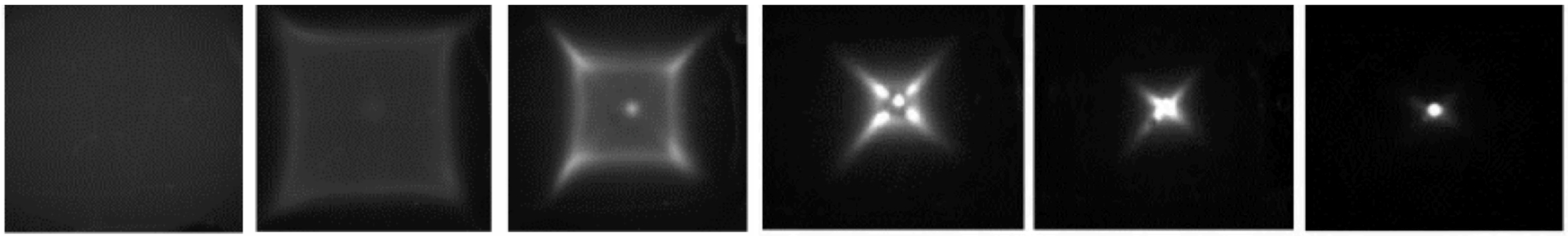
We know how to collect charged polymers

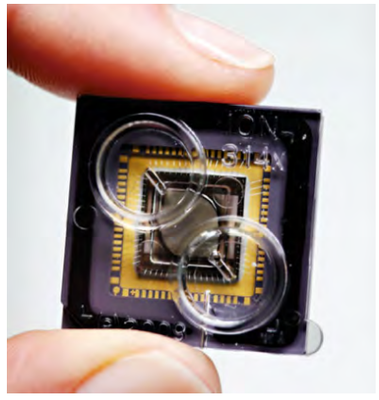
Synchronous Coefficient of Drag Alteration (SCODA)



Extreme contaminant rejection
Sample concentation
Works for RNA, DNA
Should work for any linear charged polymer

BOREAL GENOMICS

Sequencing Progress to Date



Demonstrated

Theoretical

DNA → Sequence DNA → Semiconductor Sequencing

Sequence DNA → Biological Nanopore Sequencing

Sequence DNA → Graphene Nanogap Sequencing

cDNA synthesis

RNA → cDNA synthesis

XNA → cDNA synthesis

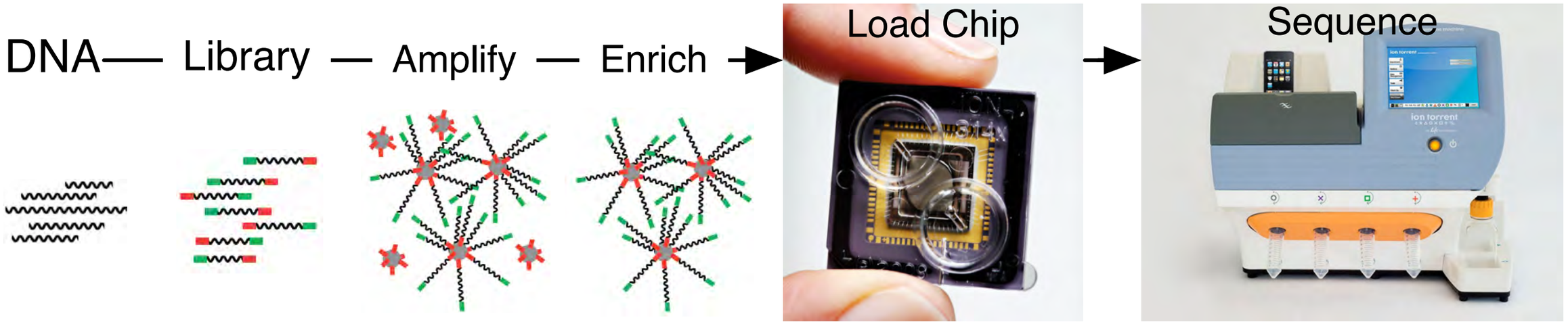
RNA → Graphene Nanogap Sequencing

XNA → Biological Nanopore Sequencing

XNA → Graphene Nanogap Sequencing

Other Informational Polymers → Graphene Nanogap Sequencing

Semiconductor Sequencing



316  6M wells
800 Mb

318  11M wells

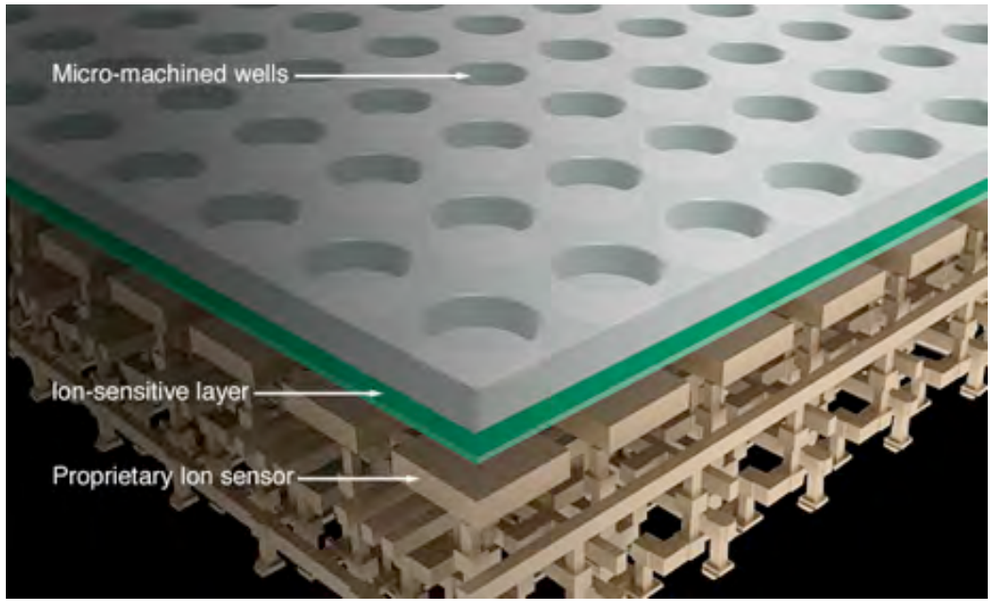
In-situ sequencing
of microbial genome

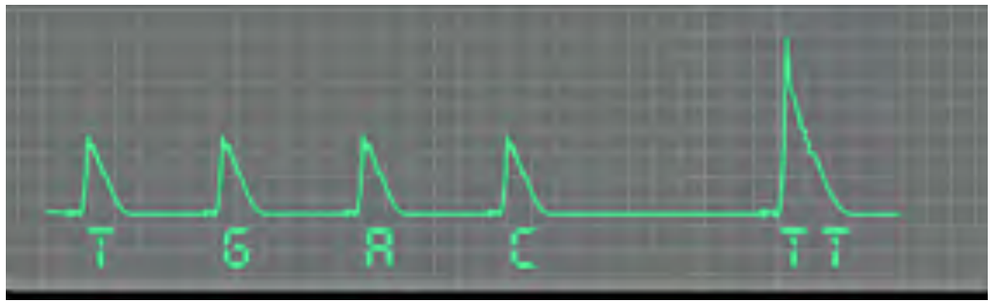
P1, P2  165M, 665M

P3  1.2G wells
250 Gb

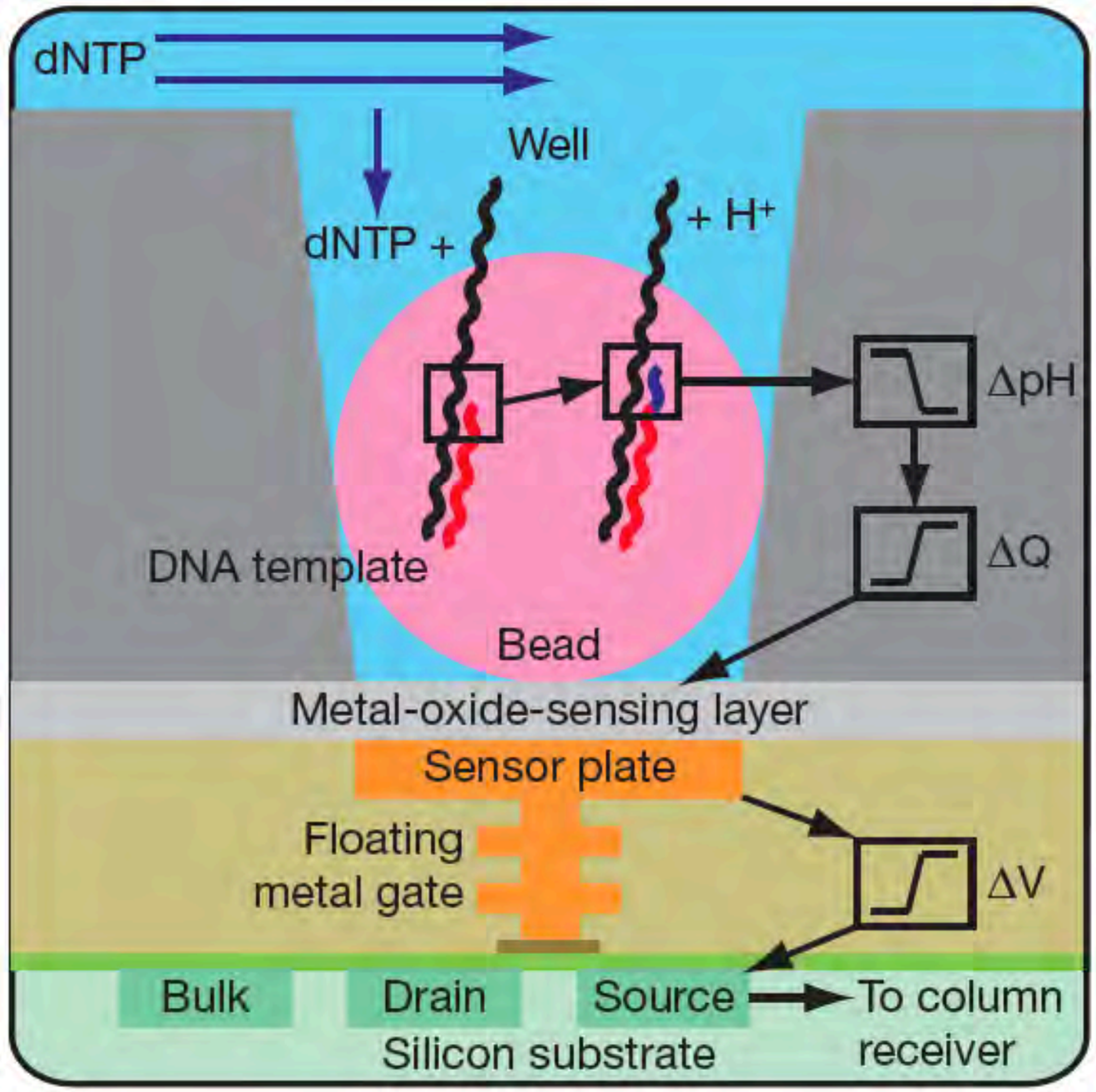
<$1k human genome

Semiconductor Sequencing

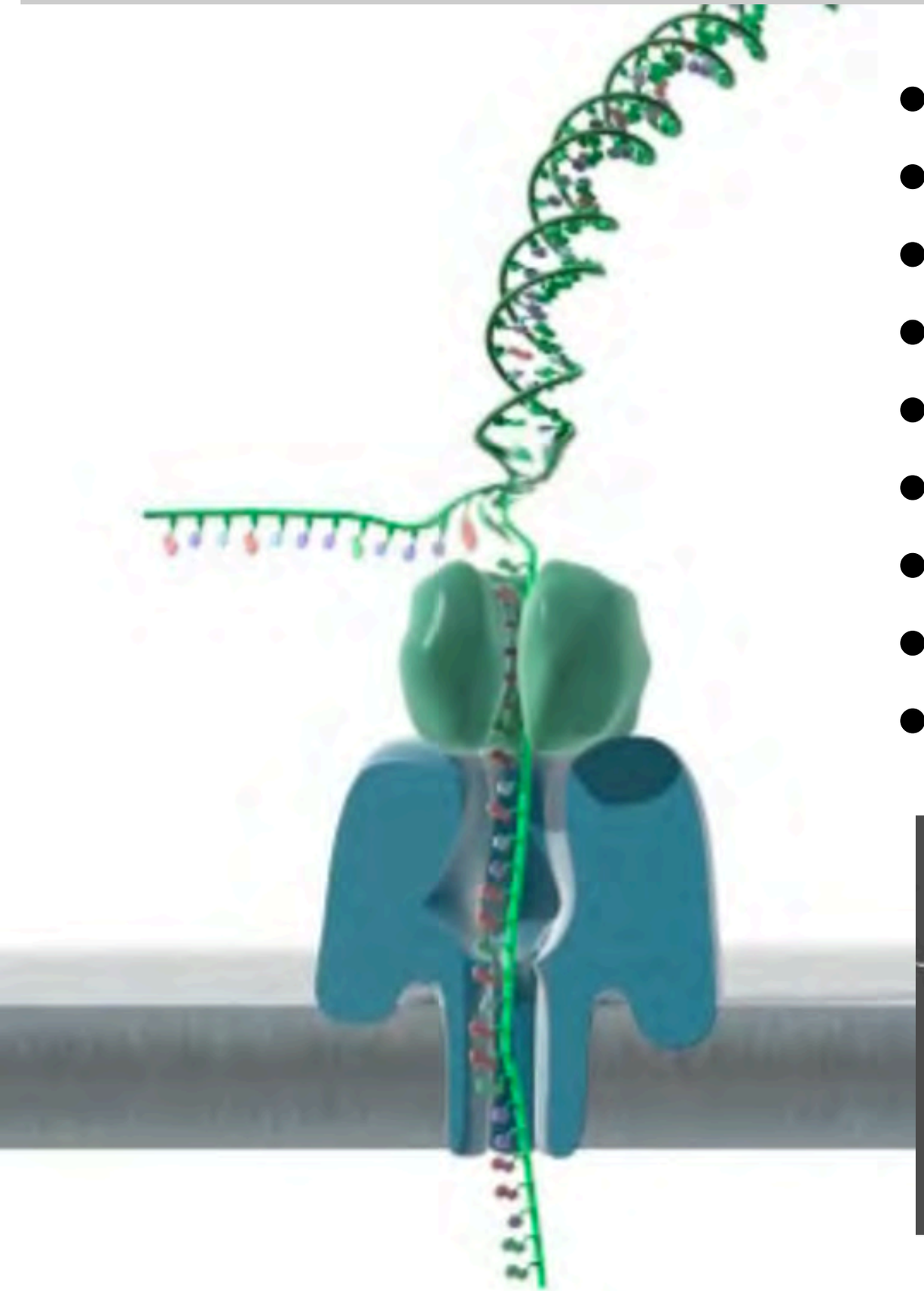




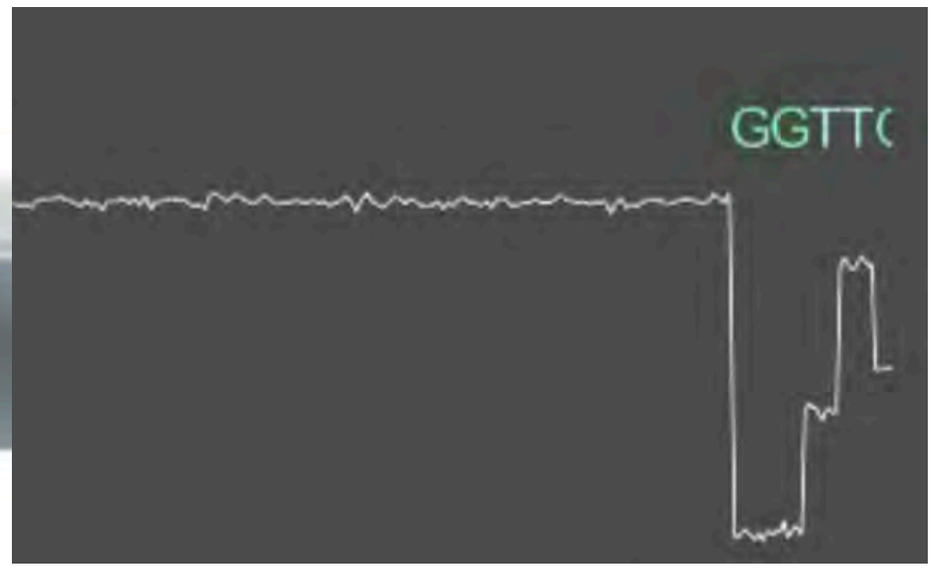
- Eliminates need for optics
- >80 runs over last year
- record run 800 Mb (500 Mb Q20)
- mass challenge: reagents (<0.5L)



Oxford Nanopore Strand Sequencing

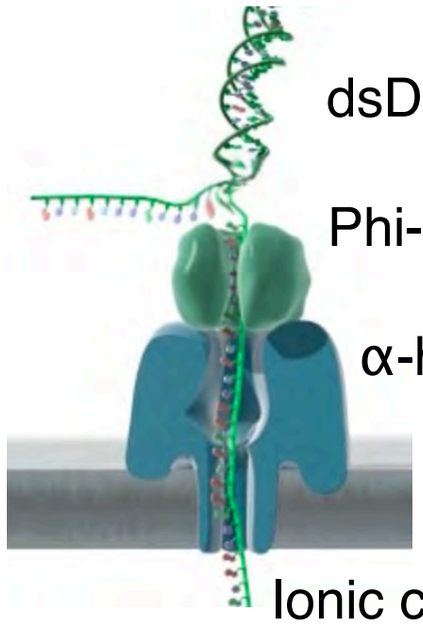


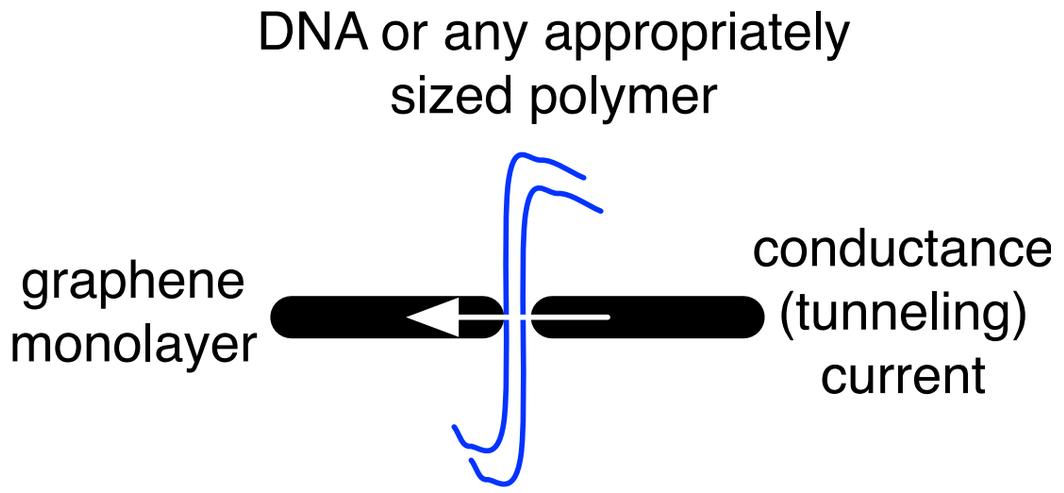
- Direct sequencing from dsDNA
- No amplification
- Minimal sample prep
- Read lengths >> 10kb (assembly!)
- Error constant across read
- Stated high error (4%)
- Very small
- Direct RNA sequencing
- Protein detection via aptamers



http://www.nanoporetech.com/technology/analytes-and-applications-dna-rna-proteins/dna-an-introduction-to-nanopore-sequencing

Nanopore sequencing





Ionic Blockade

end of 2012?

Transconductance

Issues: orientation, adsorption, radiation.

Goals of in-situ sequencing

Assess forward contamination
Rule in/out contamination
Detect informational polymer
Search for ribosomal sequences
Search for conserved genetic code
Search for conserved protein modules

Mars

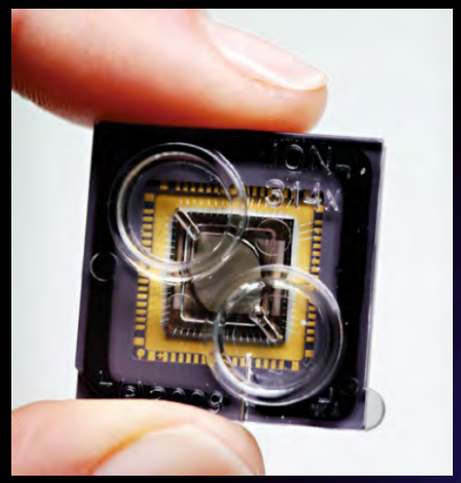
Test for alternative genetic code
Assemble genomes?
Relate to chemical/mineralogical data

If Mars life exists, understand who is there, what they are doing, and how they are related to us.

Mars

"Low" Radiation
RNA/DNA/Other IPs
Near-surface Drill
Soil/Ice/Brine sample
Near-term mission

Semiconductor
Sequencing Chip



Nanopore
Sequencer

Enceladus

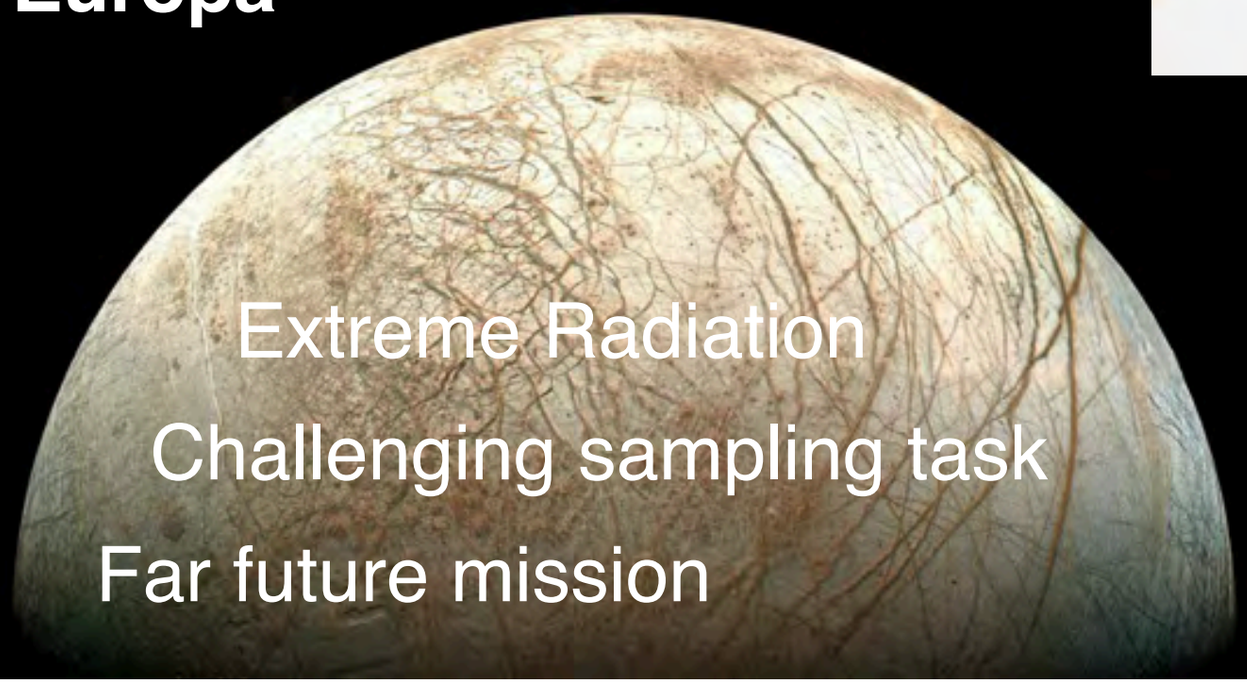
Sample
from
Orbit
or
Flyby

Liquid
Sample

Moderate
Radiation

Mid-term
mission

Europa



Extreme Radiation
Challenging sampling task
Far future mission

Image credits: NASA, Life Technologies, Oxford Nanopore

Enceladus Orbiting Sequencer (EOS)

Challenges

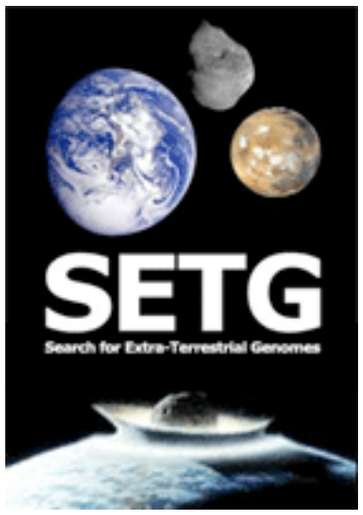
Capture of Ice Grains at high relative velocity (Flyby)

More simple sample prep than Mars

No assumption of shared ancestry

Xeno nucleic acid sequencing

Credit: Cassini Imaging Team (PIA11688)



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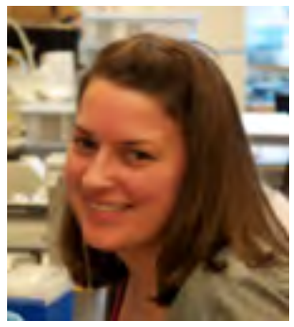

Holli Rowedder

Jessica Sandoval

Stas Tsitkov

And 2 undergrads (photos pending)

Co-Is and Collaborators:
Dr. Michael Finney (Finney Capital)
Prof. George Church (Harvard Medical School)
Prof. Walter Gilbert (Harvard, Emeritus),
Claude Canizares(MIT), William Mayer (MIT, ret.)

Alumni: *High school students:* Sarah Banerji, Aditya Joshi. *MIT undergraduates:* Nisha Vahora, Craig Bielski, Sharon Chou, Eric Connor, Christian Ruiz, Andrea Fabre, Cyrus Vafadari, Kimberly McManus. *Postdocs:* Sarah Johnson, Ting Zhu, Clarissa Lui.

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Astrobiology Instrument Development: NNX09A076G, NNX08AX15G
Field science & instrument testing: NNX09AO76G