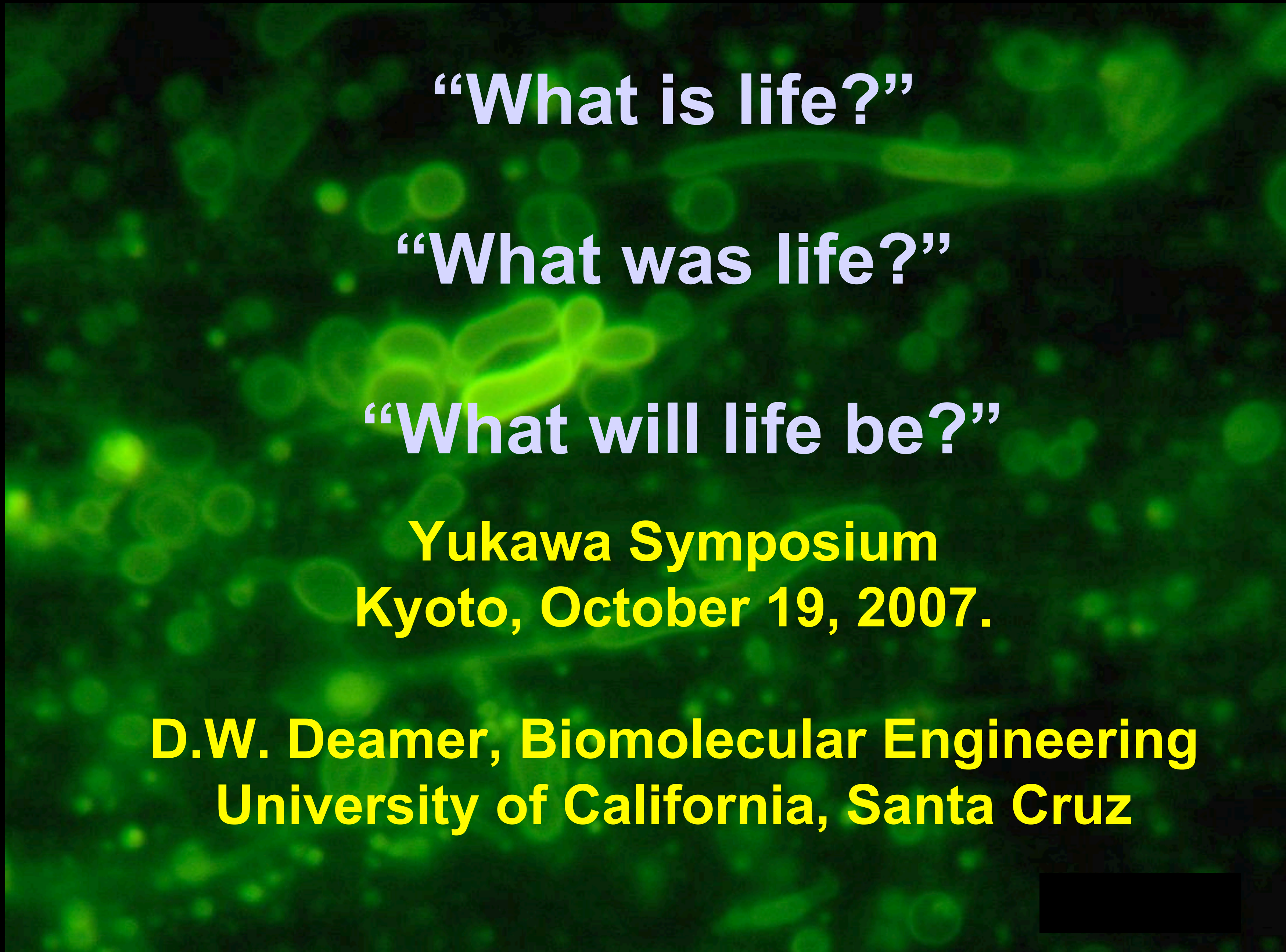

"What is life?"
"What was life?"
"What will life be?"
Yukawa Symposium
Kyoto, October 19, 2007.
D.W. Deamer, Biomolecular Engineering
University of California, Santa Cruz

Defining life is like defining any other complex device.

If the device is complex, so must be the definition.

Example: What is a Toyota? Distinguish between city, person, car.

A Toyota is a four wheeled vehicle used for personal transportation.

BUT this also defines a pony cart drawn by a horse:

A Toyota is a four wheeled vehicle with an engine that is used for personal transportation.

BUT: This could be a pony cart taking an engine to the mechanic for repair.

A Toyota is a motorized four wheeled vehicle used for personal transportation in which the engine causes the wheels to rotate and produce forward motion.

BUT the engine won't run unless it has fuel.

A Toyota is a motorized four wheeled vehicle used for personal transportation in which the engine uses fuel to cause the wheels to rotate and produce forward motion.

BUT: We need to guide the motion.

A Toyota is a motorized four wheeled vehicle with a steering wheel that is used for personal transportation in which the engine uses fuel to cause the wheels to rotate and produce forward motion.

PROBLEM: This definition also fits a Honda.

Also, what is an engine? What is fuel? What is a steering wheel?

MAIN POINT: A Toyota is a complex device and requires a complex definition.

LIFE is also complex, and the answer to "What Is Life" must be complex.

What is life?

All life is cellular. The minimal unit of life is a cell.

What is a cell?

A cell is the unit of life, defined as a microscopic compartment bounded by a lipid bilayer. The compartment contains polymers called proteins and nucleic acids. The proteins are catalysts, and the nucleic acids contain genetic information.

BUT: I can make this in the lab, and it is not alive.

What is missing?

A cell is the unit of life, defined as a microscopic compartment bounded by a lipid bilayer. The compartment contains polymers called proteins and nucleic acids. The proteins are catalysts, and the nucleic acids contain genetic information. The protein catalysts use nutrients and energy in a process called metabolism to synthesize both proteins and nucleic acids by polymerization, a process we call growth. When nucleic acids are synthesized, the genetic information content is replicated, but not precisely. Errors are called mutations. When a cell reaches a certain size, it can divide into two daughter cells which may be identical to the parent cell, but occasionally are different because of mutations. The differences allow populations of cells to evolve by natural selection, thereby adapting to changes in their environment.

This group of sentences absolutely defines the simplest unit of life. Nothing else in the universe fits the definition.

BUT like a Toyota, each of the key words must also be defined: lipid bilayer, protein, nucleic acid, catalyst, nutrient, energy, polymerization, genetic, mutation, evolution, natural selection….

How can we go beyond a simple description, and understand life at a deeper level?

Origin of life: How did life begin on the early Earth?

Synthesize life: Can we produce artificial life in the laboratory?

We start by asking how life can begin:

Life is not just restricted to the Earth, but is best understood in terms of astrobiology: Life is part of a universal process.

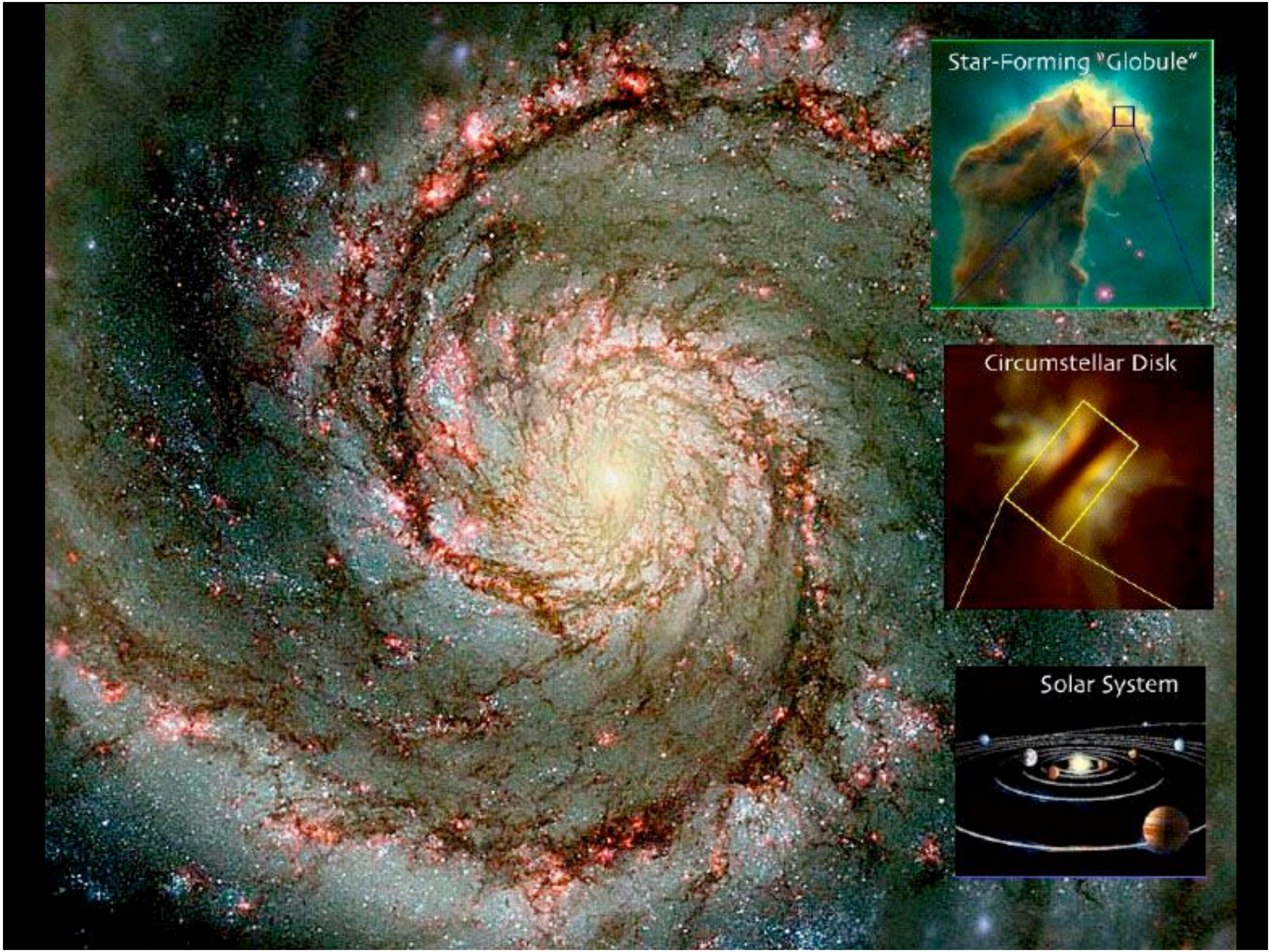
We must first understand the origin of biogenic elements.

The biogenic elements

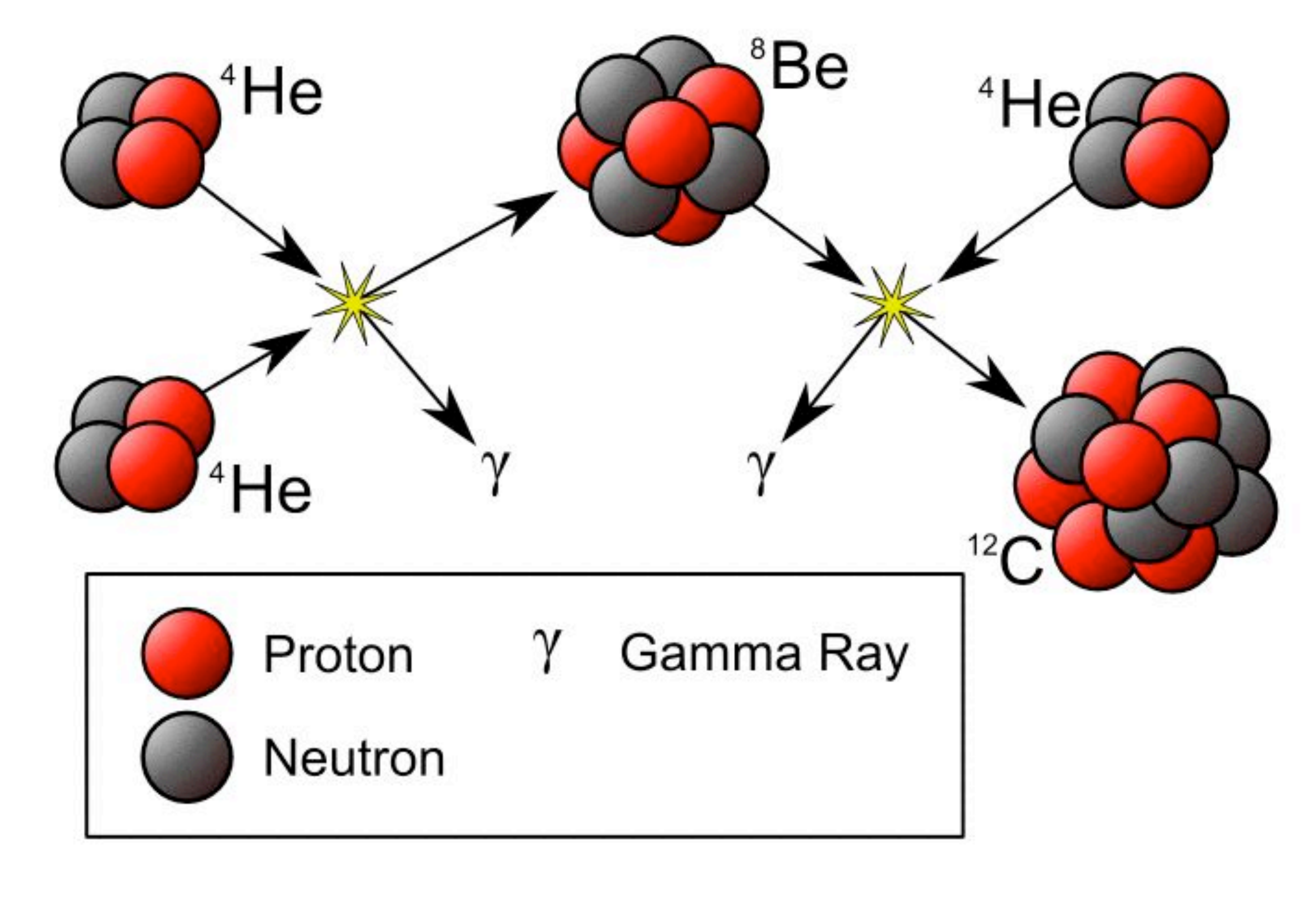
Carbon	C
Hydrogen	H
Oxygen	O
Nitrogen	N
Sulfur	S
Phosphorus	P

Compose >99% of a living cell

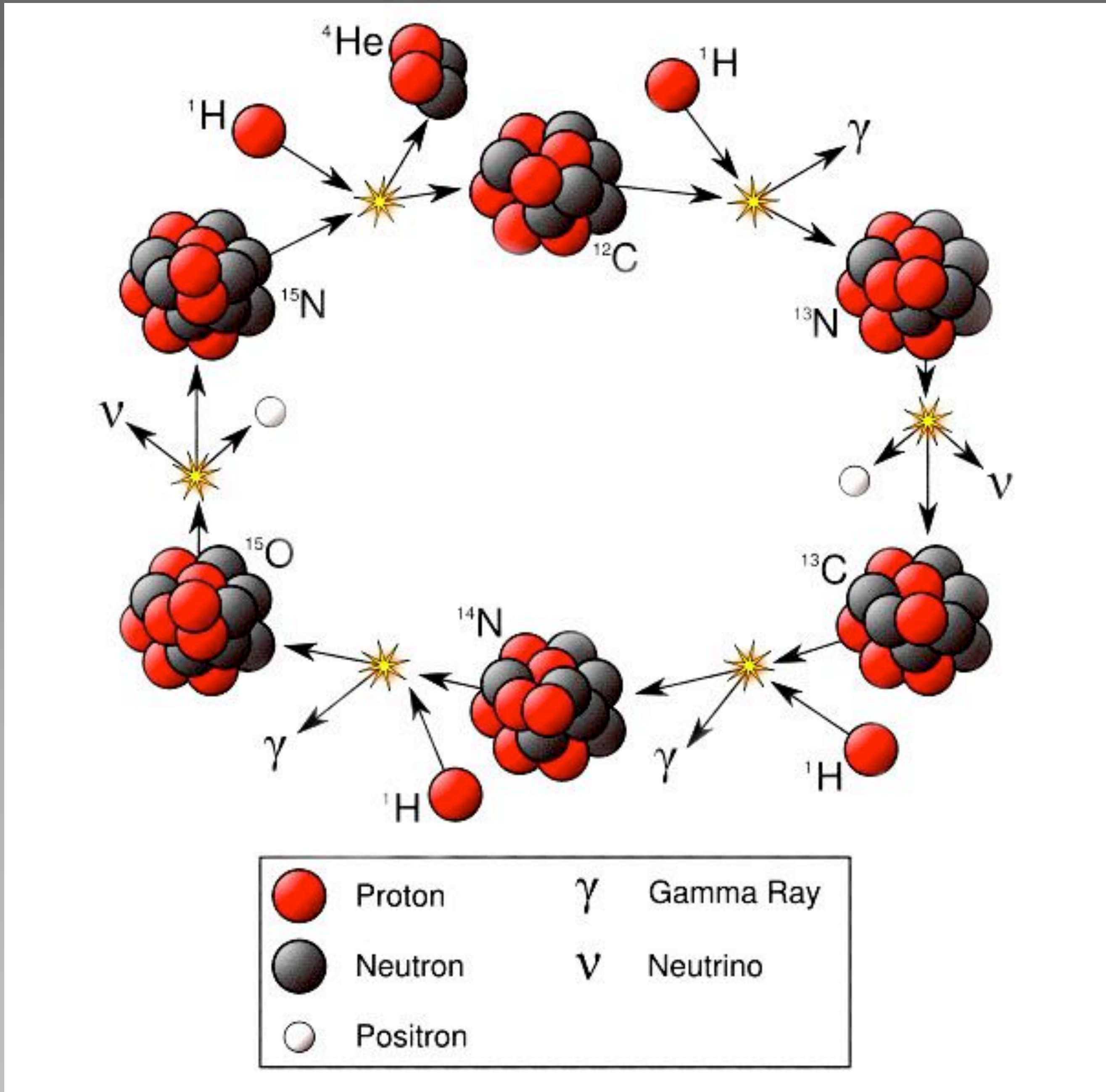
WHERE DO THEY COME FROM?


Star-Forming "Globule"
Circumstellar Disk
Solar System

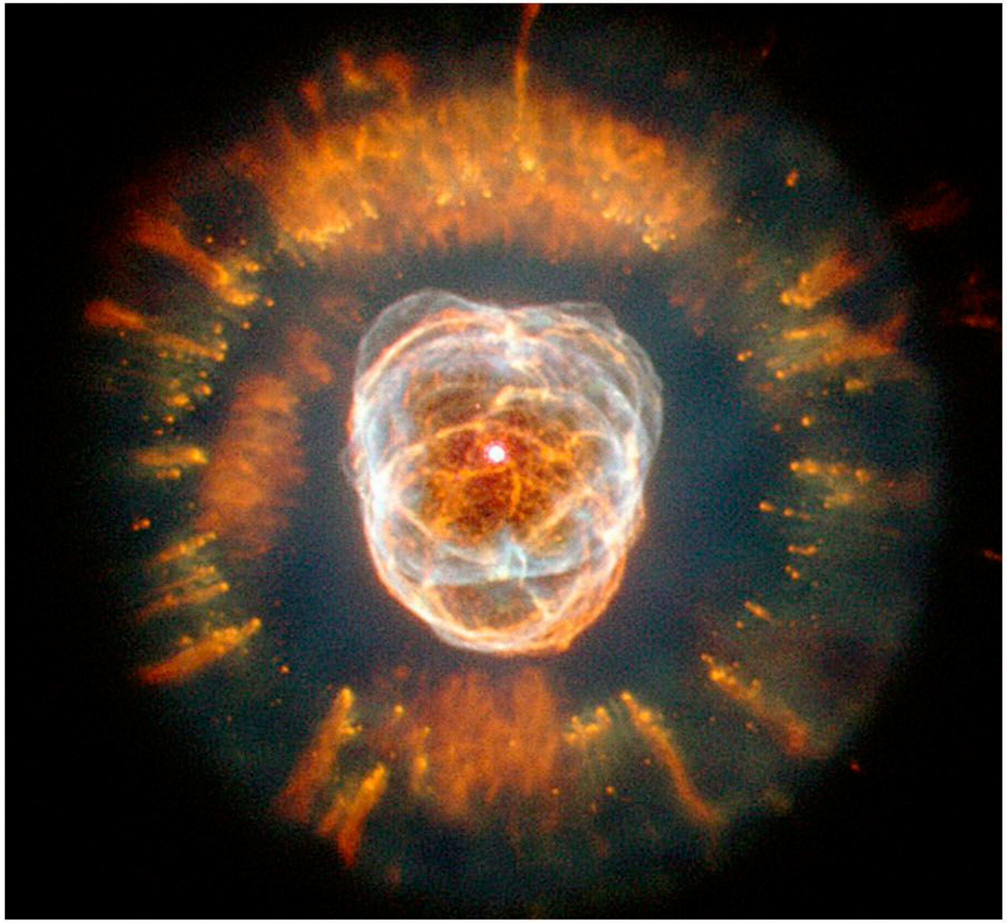
Carbon synthesis: Triple alpha process in stars at 100 million degrees. Fred Hoyle, 1947.



Nitrogen and oxygen synthesis in CON cycle



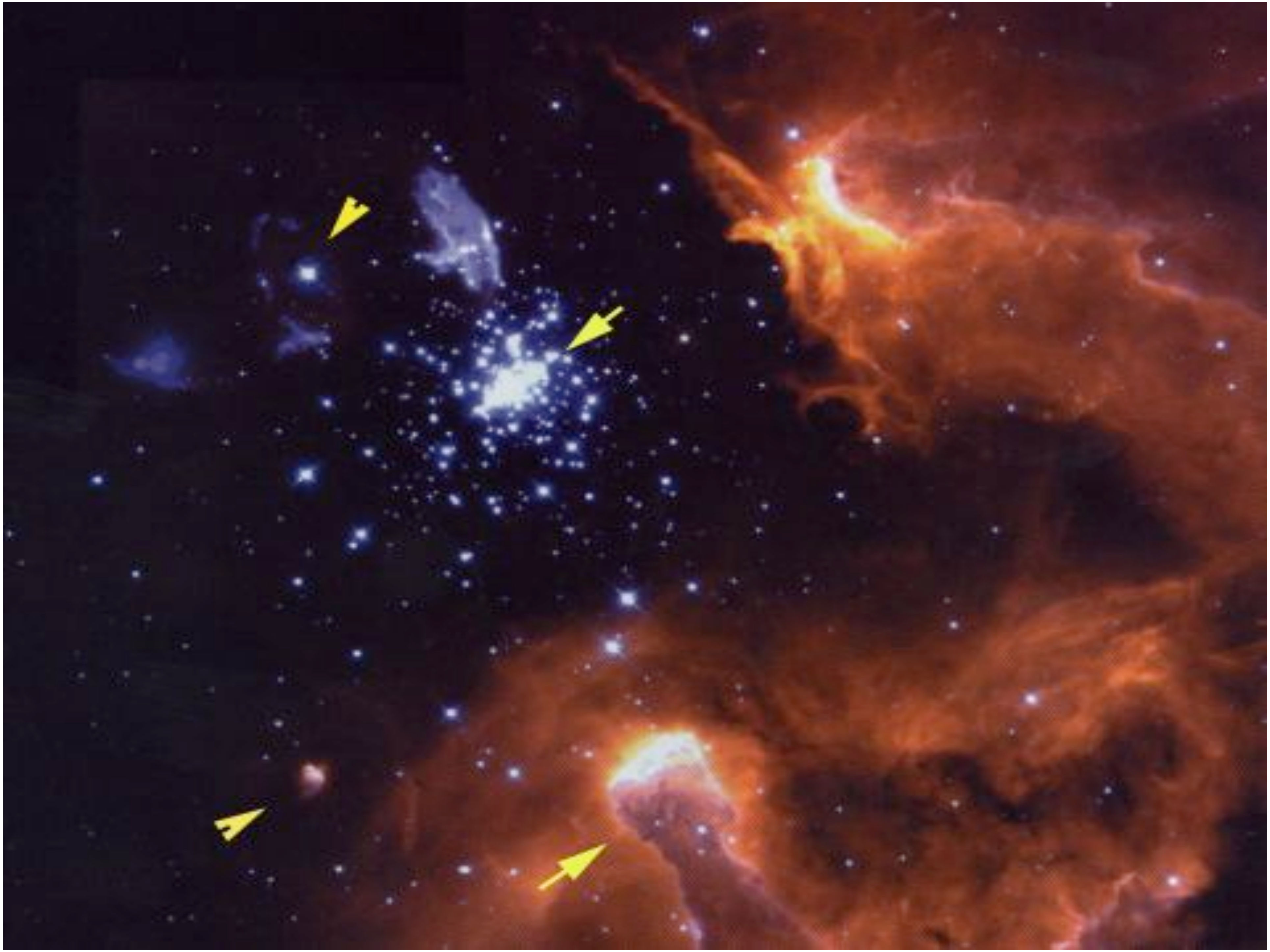
Planetary nebulas around dying stars

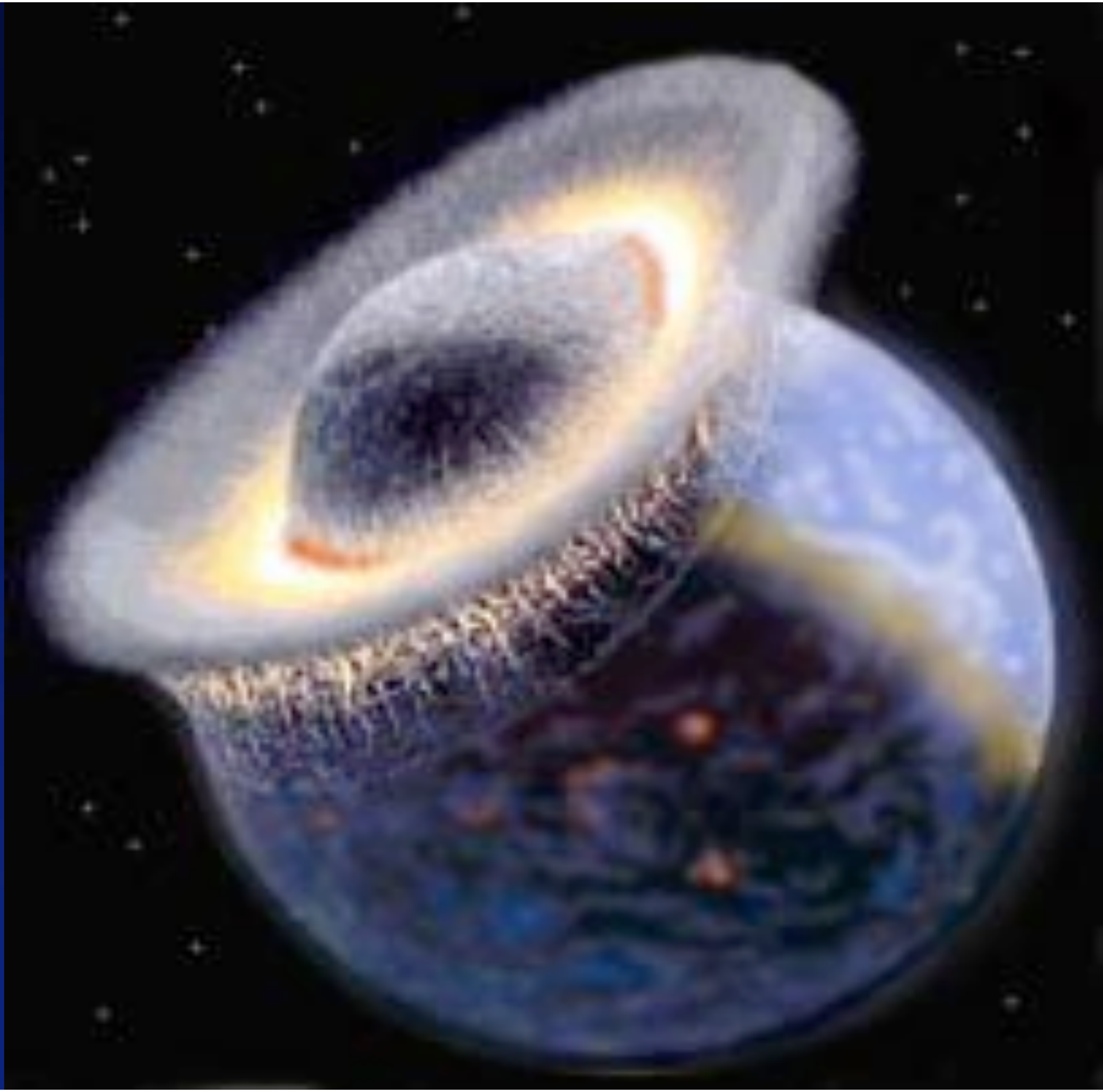


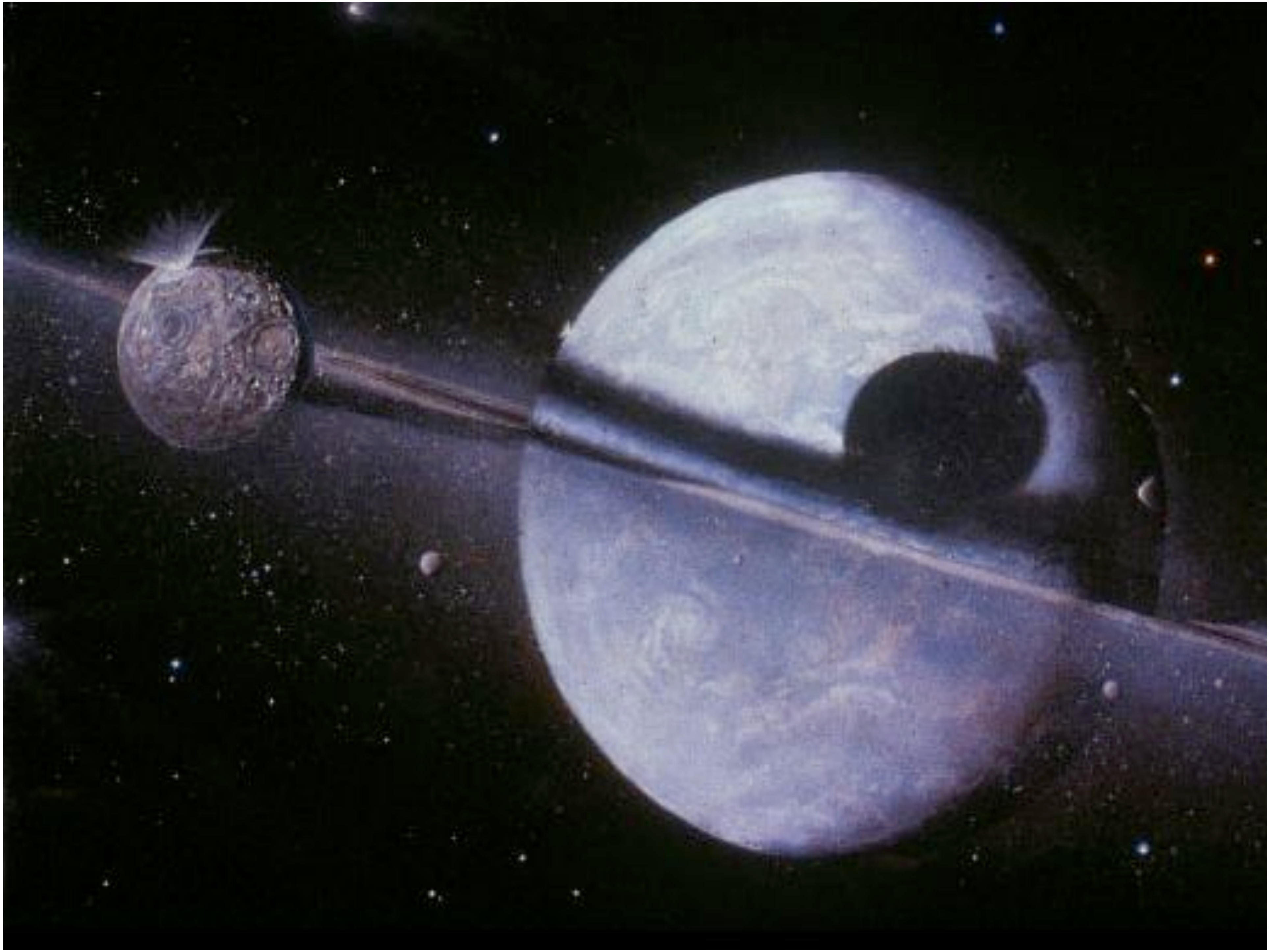
“Eskimo” nebula

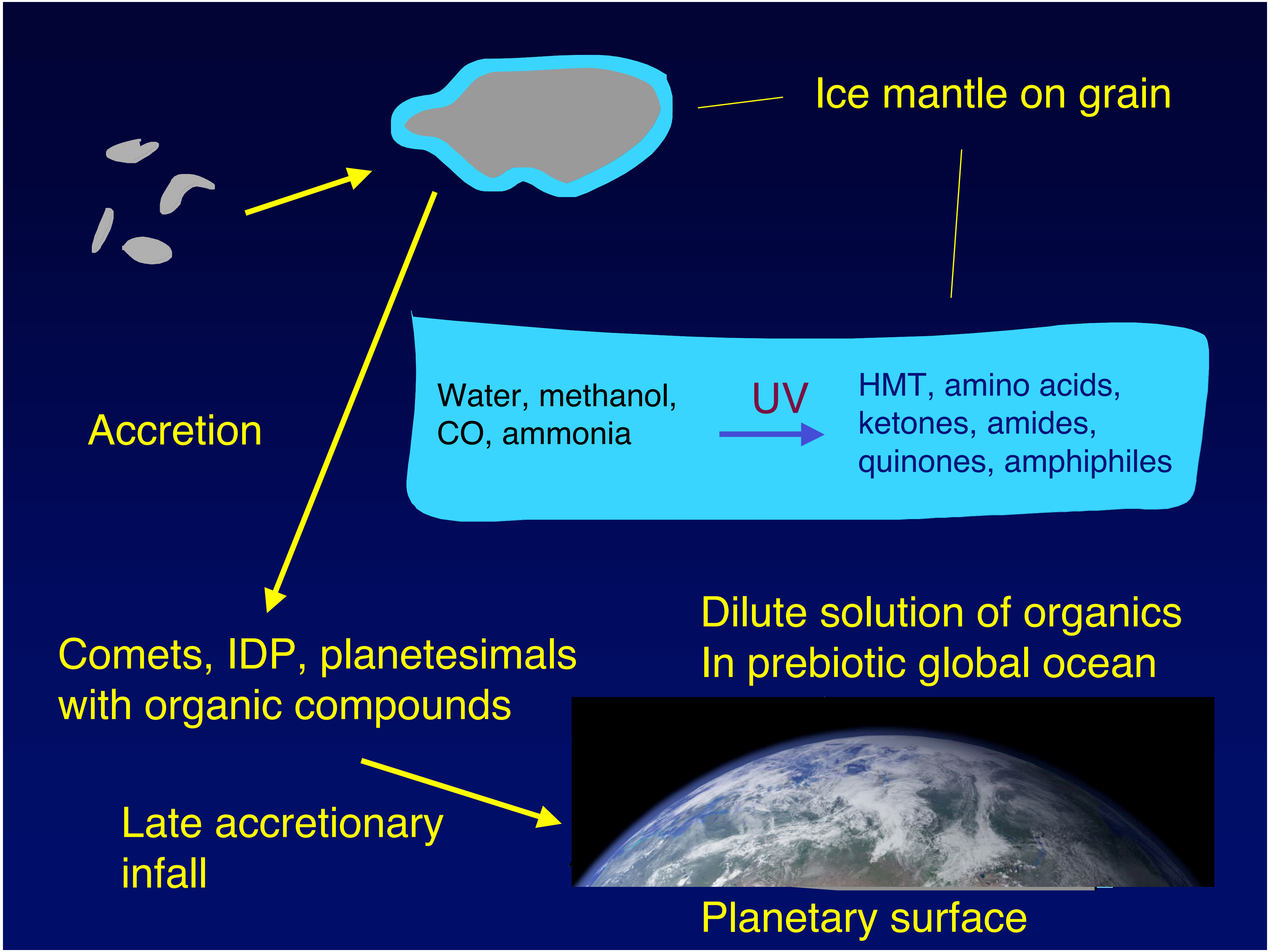
“Cat’s Eye” nebula

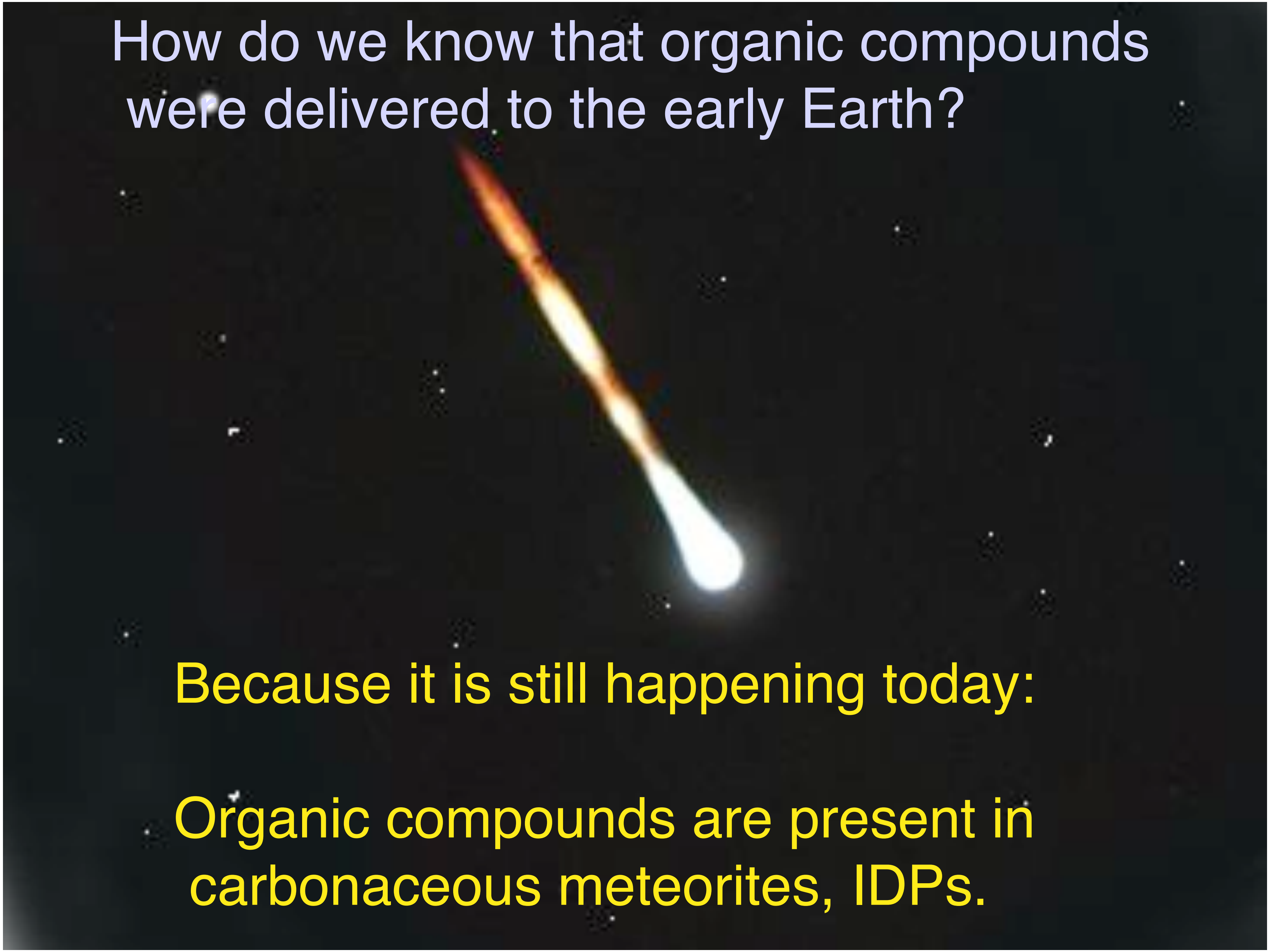
Interplanetary dust particle (IDP)
Micron size range.

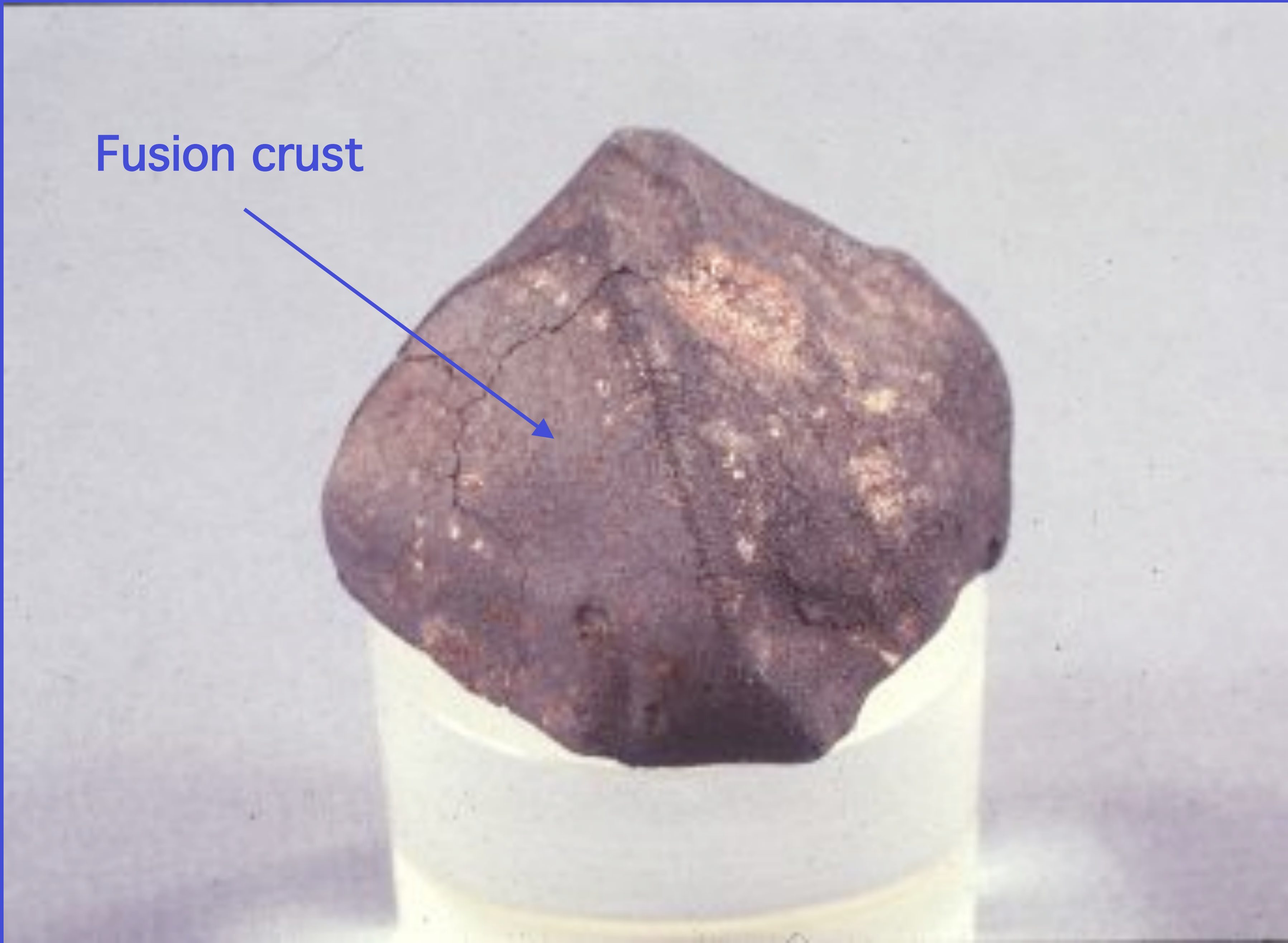


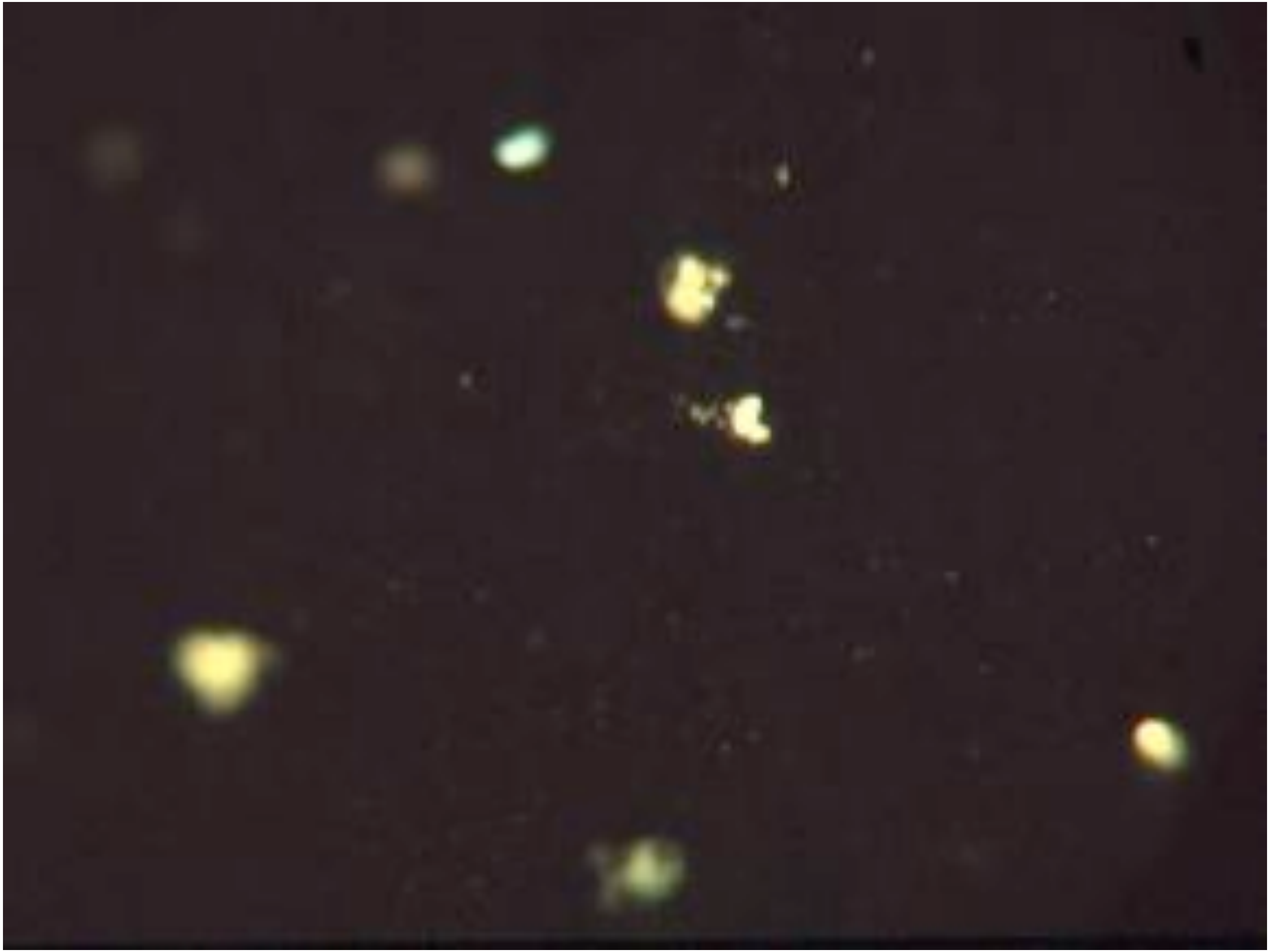




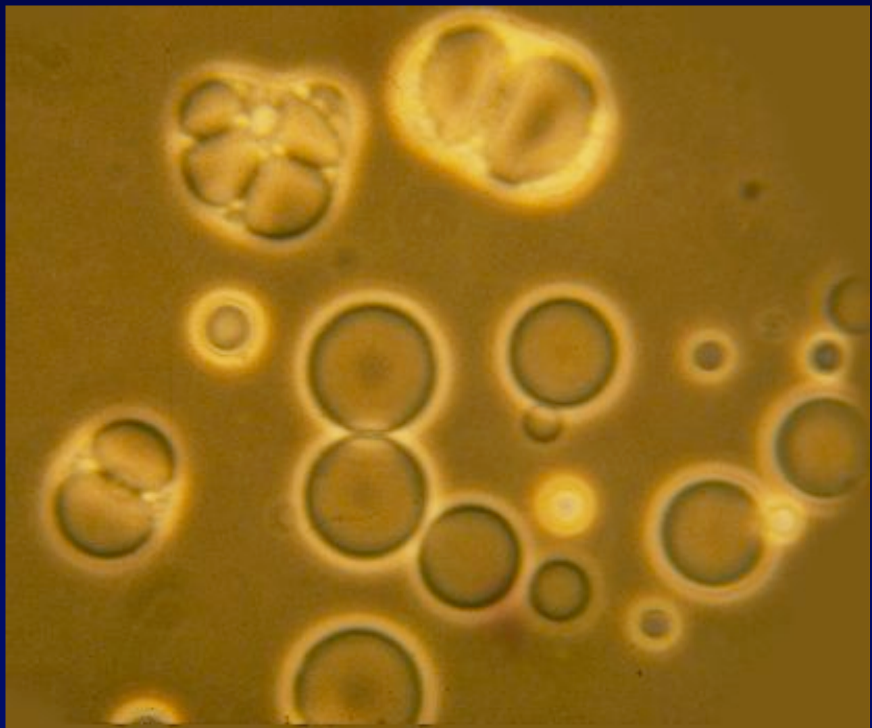

Ice mantle on grain
Accretion
Water, methanol,
CO, ammonia
UV
HMT, amino acids,
ketones, amides,
quinones, amphiphiles
Comets, IDP, planetesimals
with organic compounds
Dilute solution of organics
In prebiotic global ocean
Late accretionary
infall
Planetary surface


How do we know that organic compounds were delivered to the early Earth?
Because it is still happening today:
Organic compounds are present in carbonaceous meteorites, IDPs.

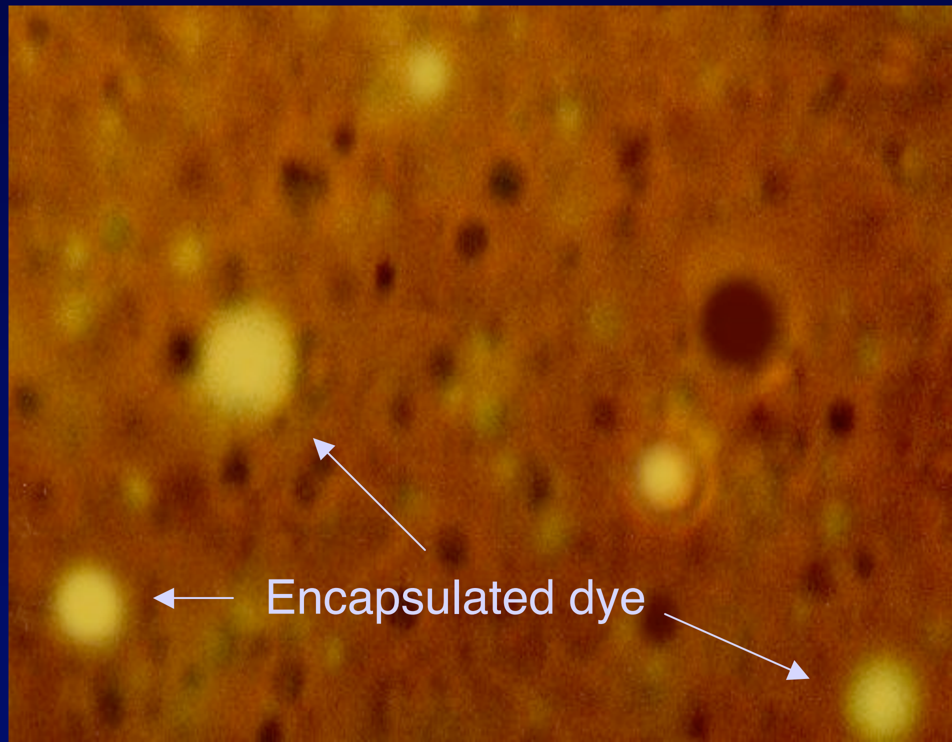

Fusion crust



Question: Can amphiphilic molecules present in carbonaceous meteorites form stable membranes? Answer: YES



Phase image



Fluorescence

THE FIRST CELLS

Cells are molecular systems of boundaries and polymers - <u>both</u> structures are required for the origin of cellular life.

Self-assembly of boundary structures

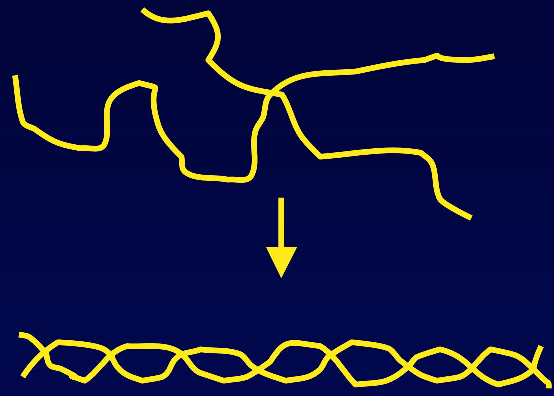
Amphiphilic molecules assemble spontaneously into bilayer structures.

Directed assembly of polymers

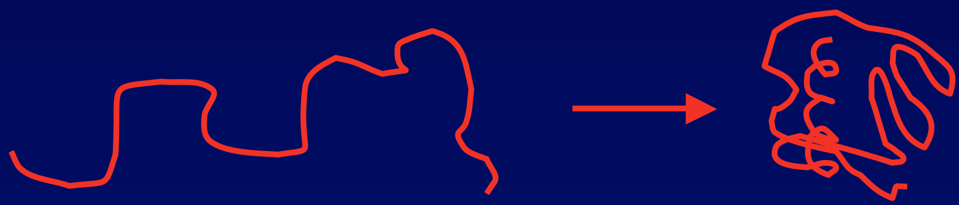
Catalyzed growth and replication involving catalytic polymers and genetic polymers that contain sequence information.

Self Assembly Processes

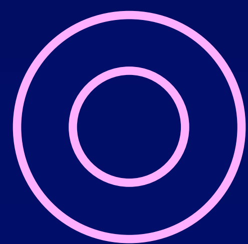
Single DNA strands -->
Double helix



Nascent proteins --> Folded proteins



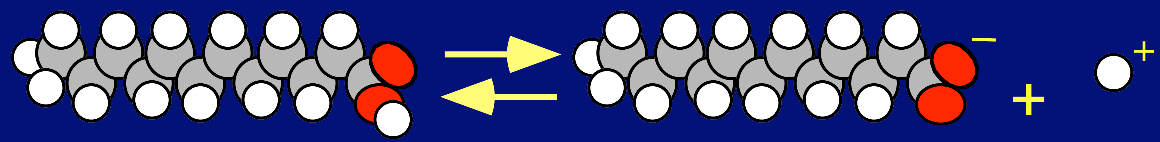
Lipid dispersions --> Membranes

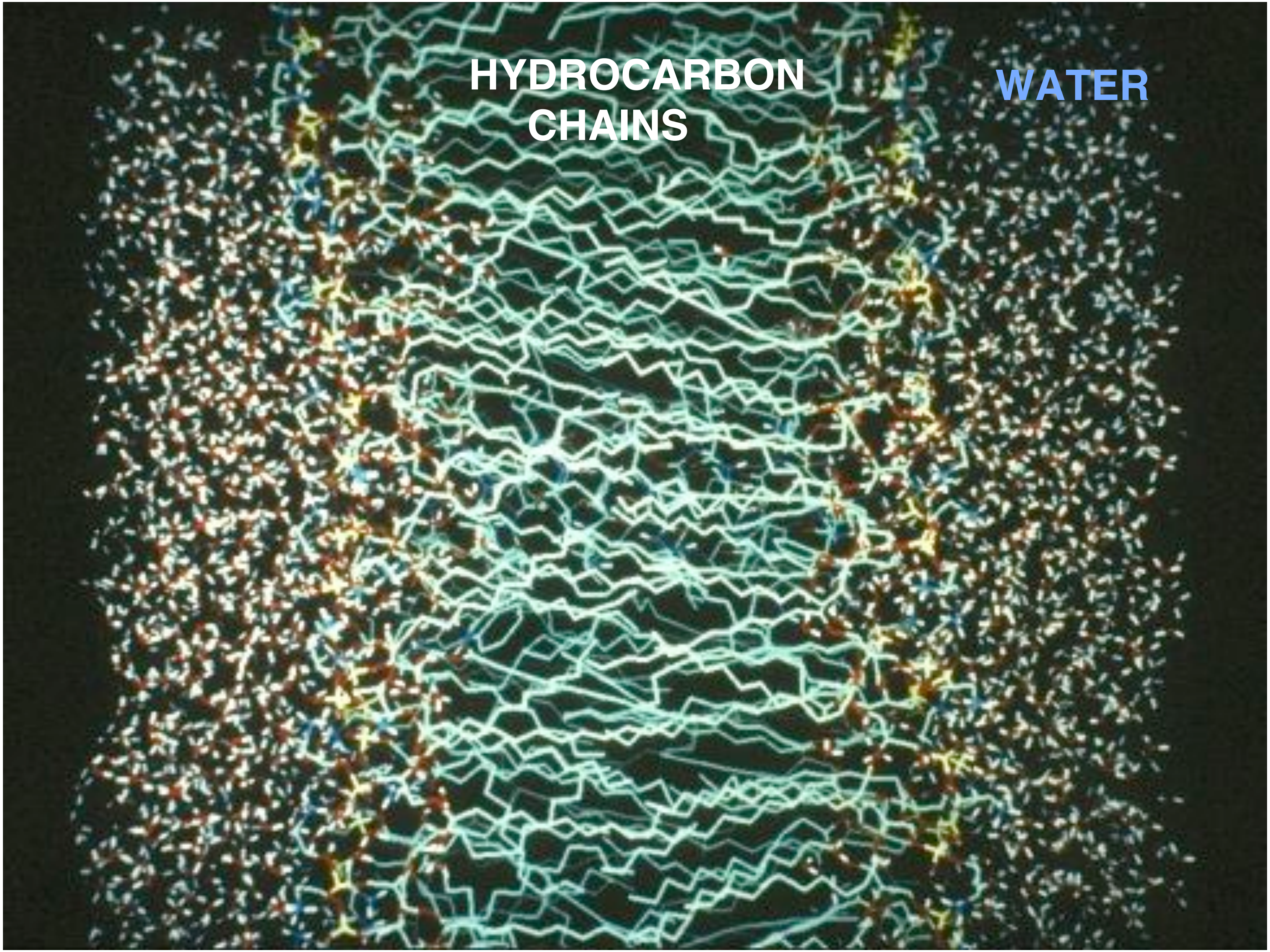


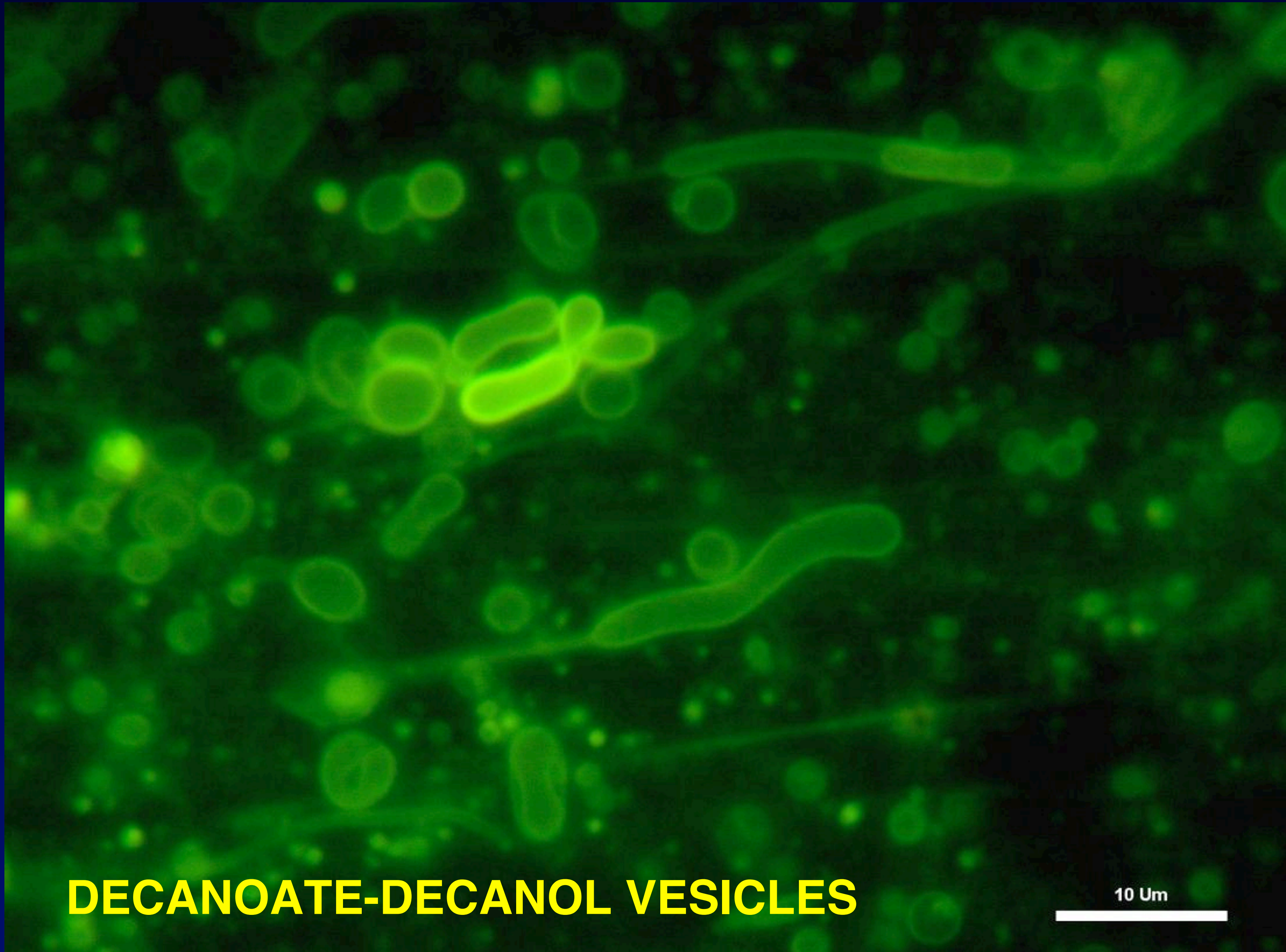
Carbonaceous meteorites provide samples of prebiotic organics delivered to the Earth.

What amphiphilic molecules are present?

Monocarboxylic acids 8 - 12 carbons long.




HYDROCARBON
CHAINS
WATER


DECANOATE-DECANOL VESICLES
10 Um

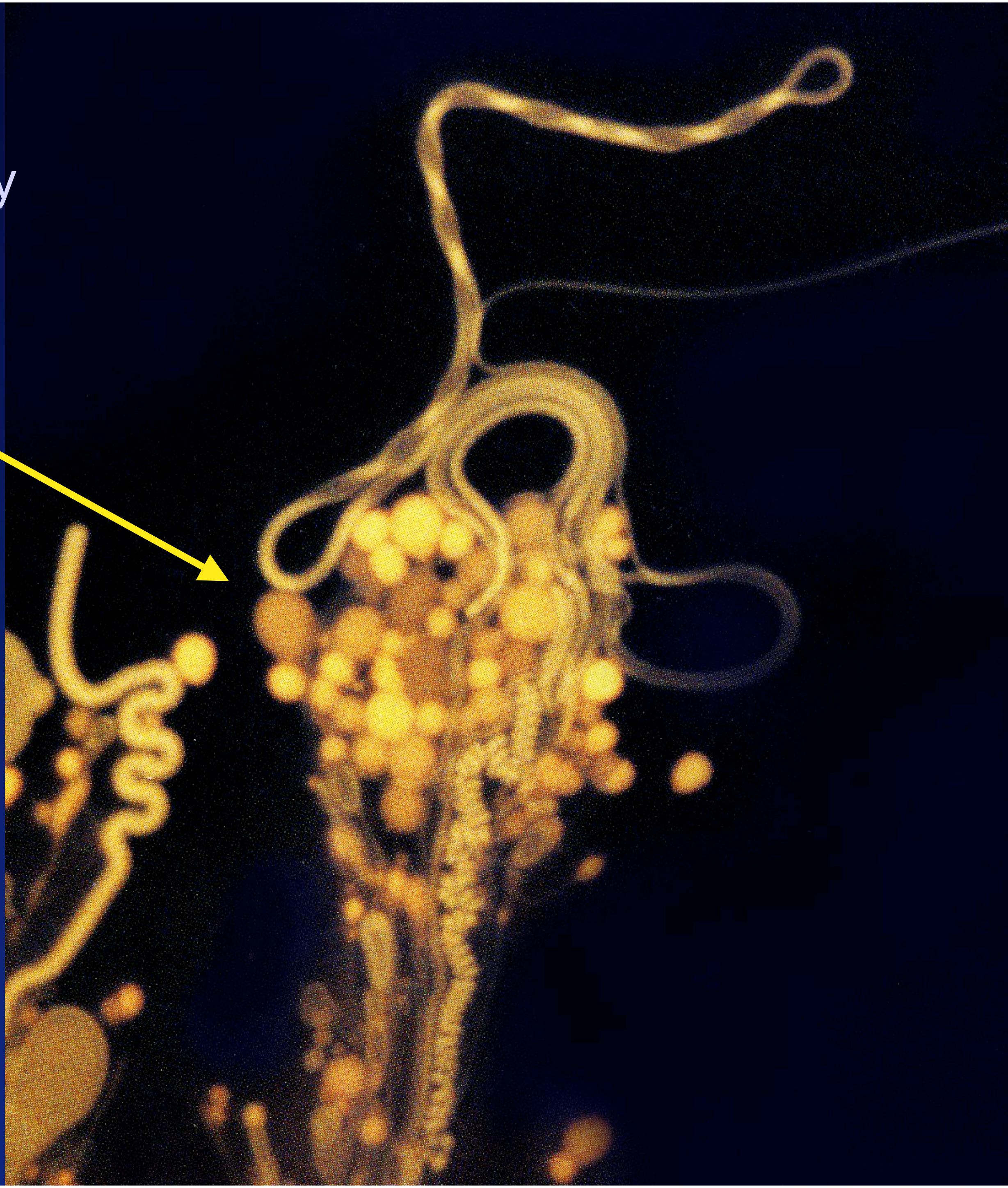
ENCAPSULATION OF MACROMOLECULES

Fact: Lipid vesicles are impermeable to large molecules.

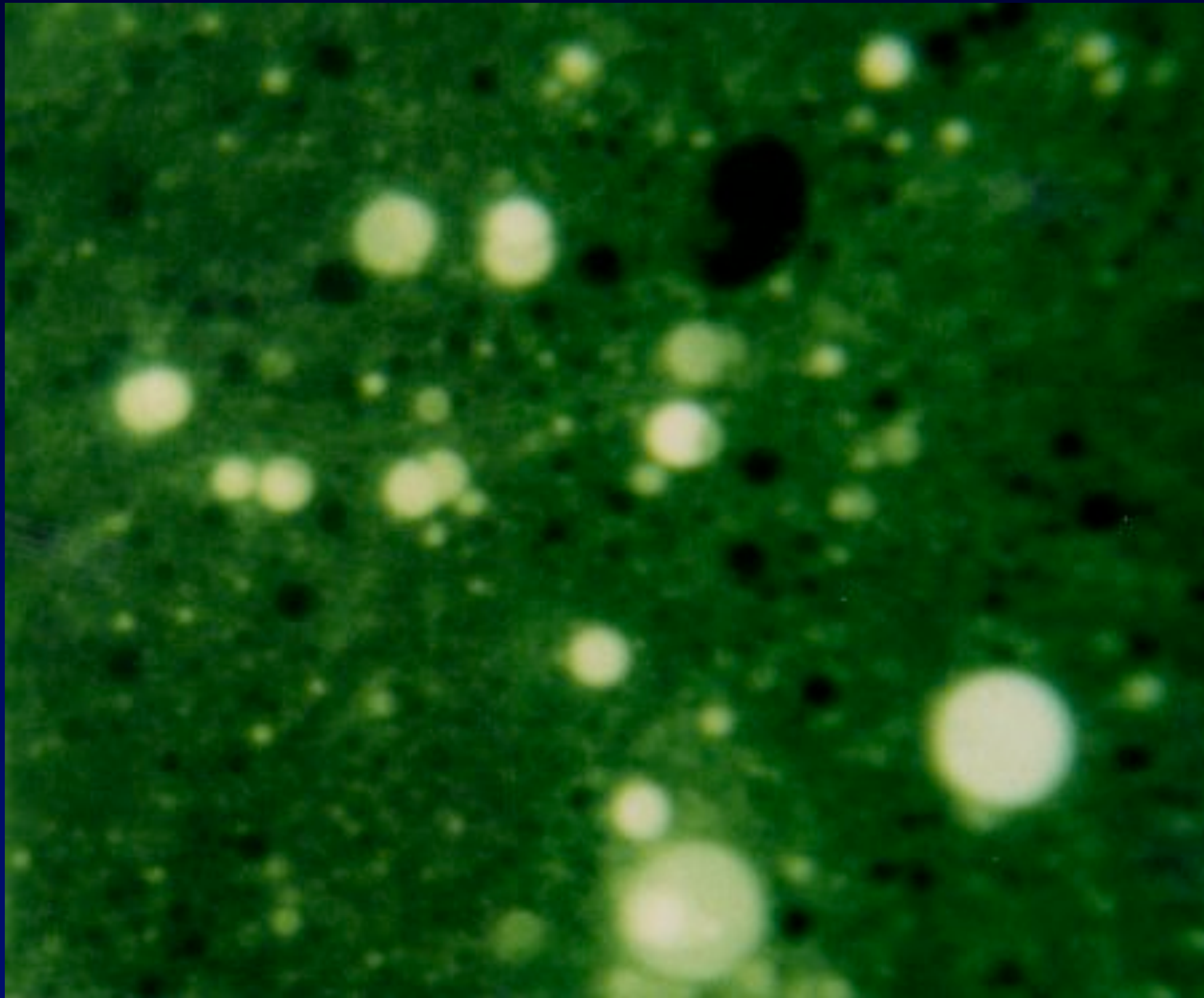
Question: How could large molecules be captured in a membrane-bounded compartment boundary to produce simple protocells?

Dehydration cycles: When an amphiphile is dried in the presence of a macromolecule, then rehydrated, vesicles are produced that contain the macromolecule.

DNA can be easily encapsulated in lipid membranes.



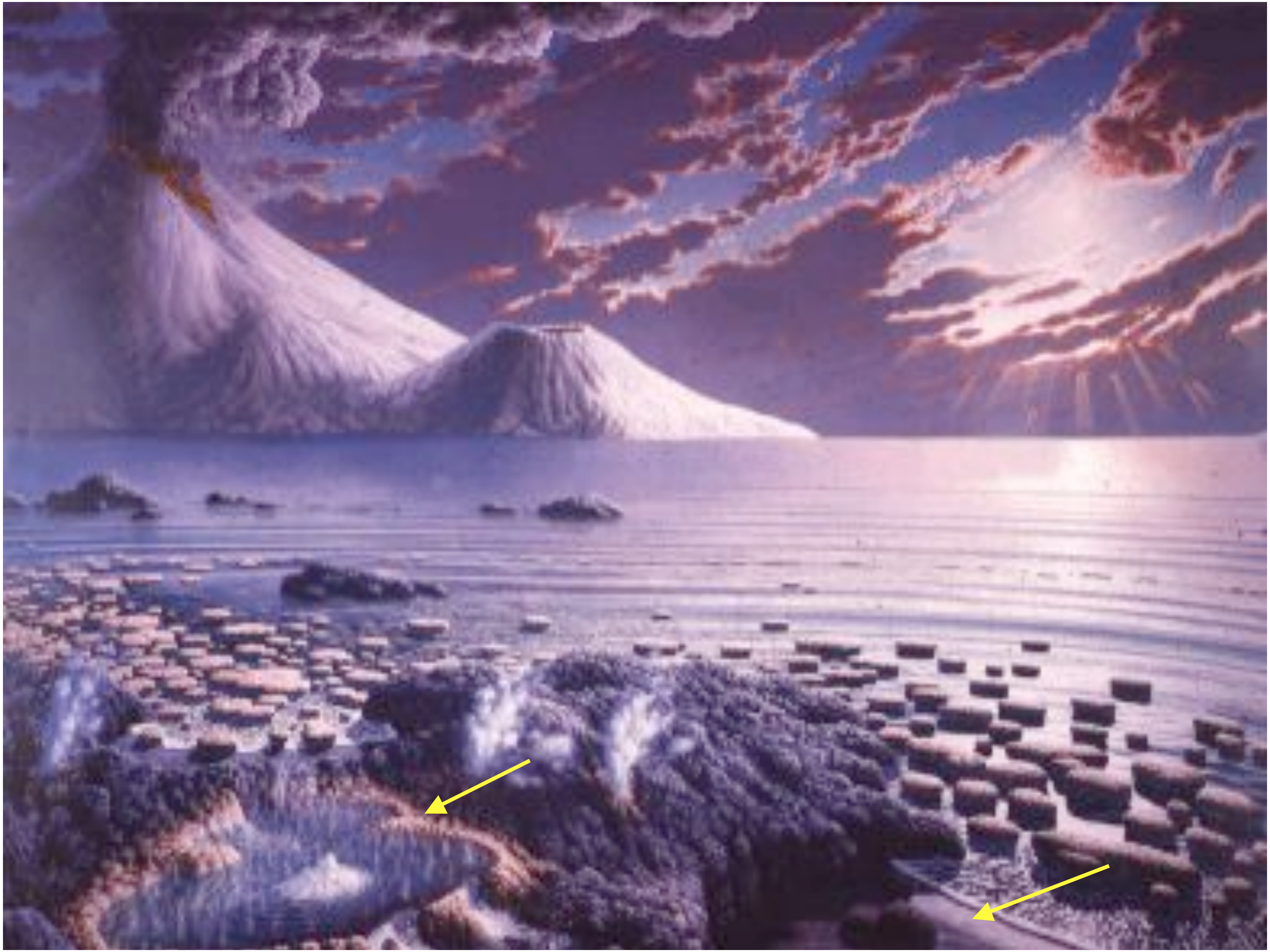
dsDNA (~600 BP) IN DECANOIC ACID VESICLES



WHERE DID LIFE BEGIN?

Can self-assembly occur in environmental models of prebiotic conditions?

Model to be tested: Hydrothermal regions associated with volcanic activity.



Kamchatka geothermal regions in Eastern Russia are plausible models of the primitive Earth environment:

High altitude, high latitude, recent volcanism produce sterile sites for experimental analysis.

Does organic synthesis occur?


Petropavlovsk
Mt. Mutnovsky
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43

The RNA World Hypothesis:

The first forms of life used RNA both as genetic material and as a catalyst (ribozymes)

But how was the first RNA synthesized?

Assume a source of mononucleotides:

How could polymerization reactions be driven?

Use fluctuating wet-dry cycles like the edges of volcanic hydrothermal ponds.

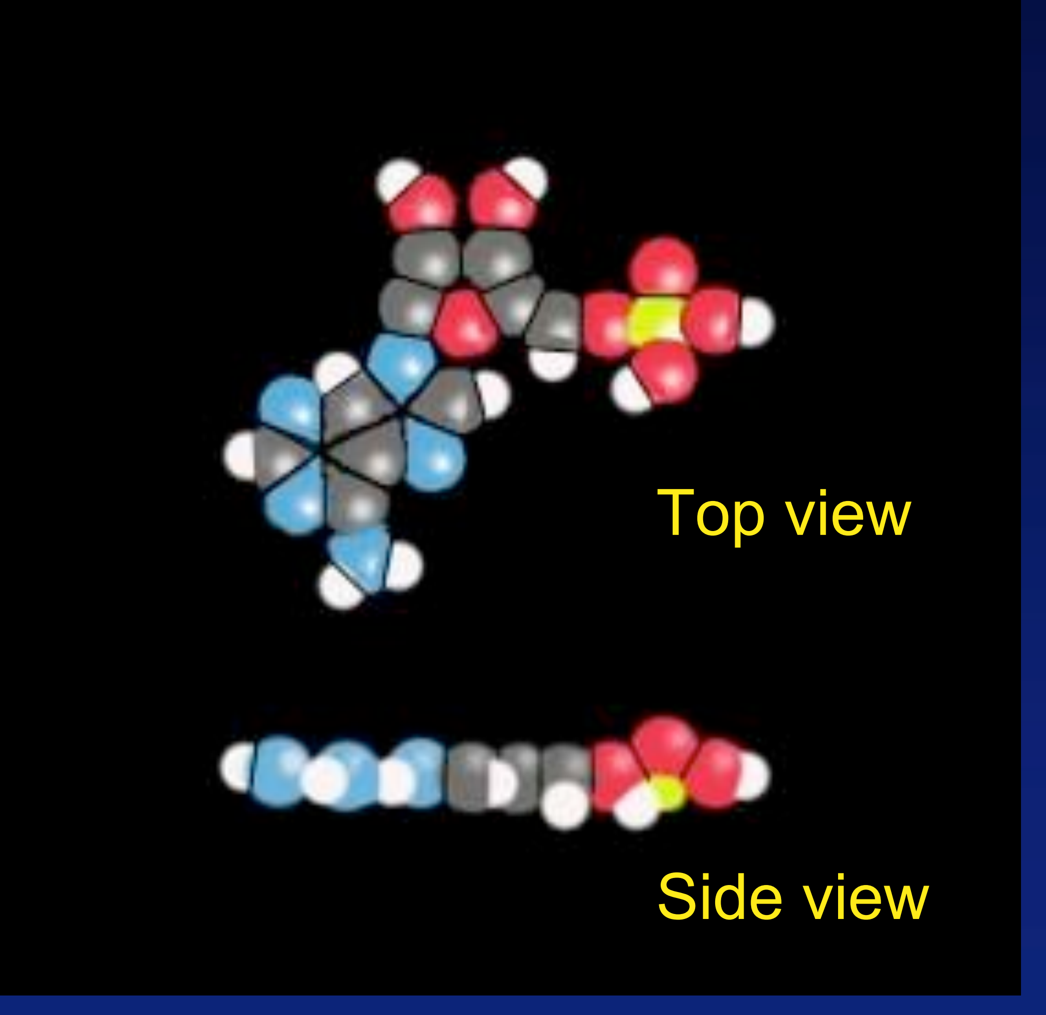
RNA hydrolysis in water

RNA + H_2O → Mononucleotides (AMP, UMP, GMP, CMP)

Condensation when water is removed

RNA ← ($- H_2O$) Mononucleotides (AMP, UMP, GMP, CMP)

AMP



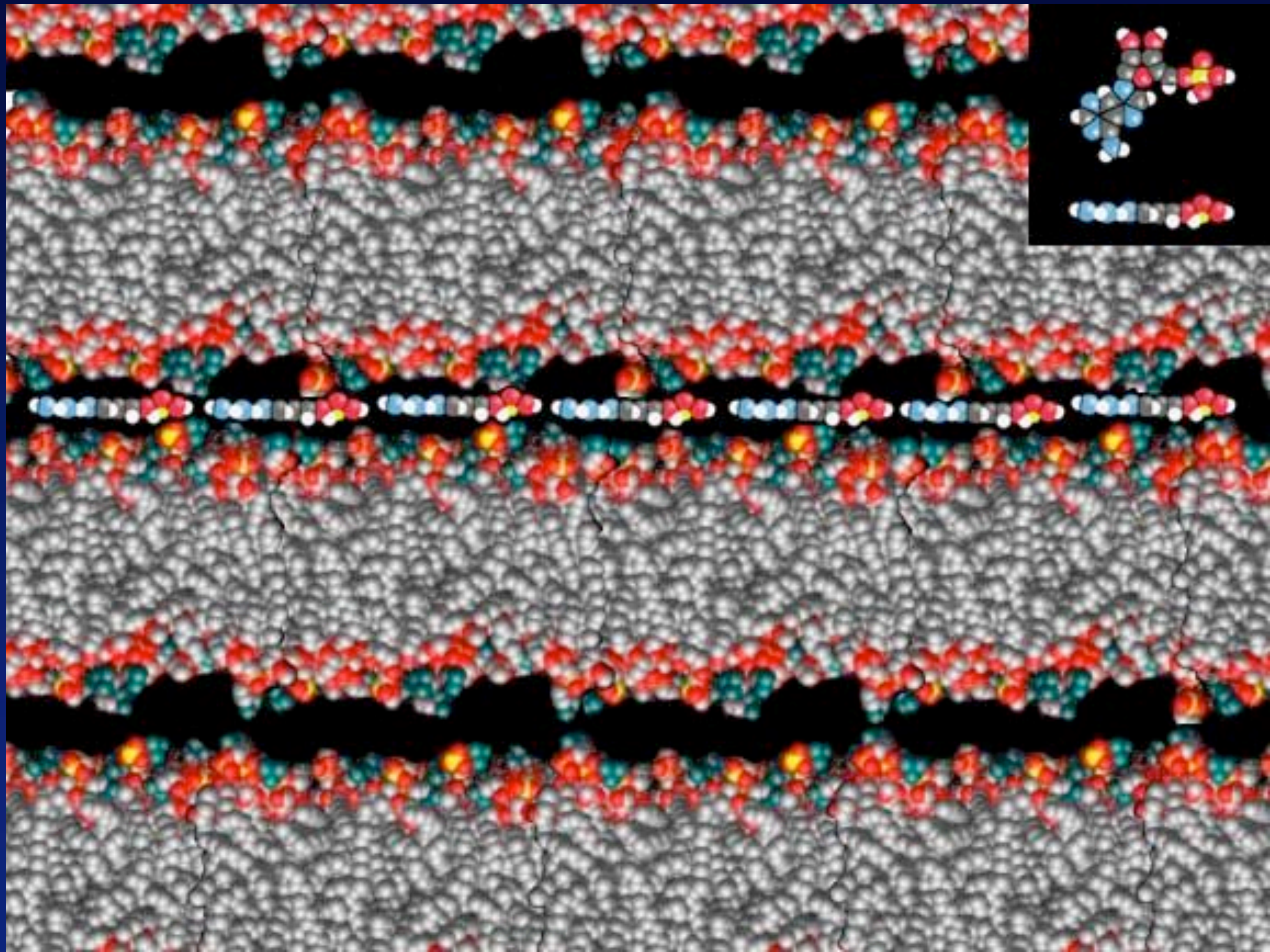
Experimental conditions:

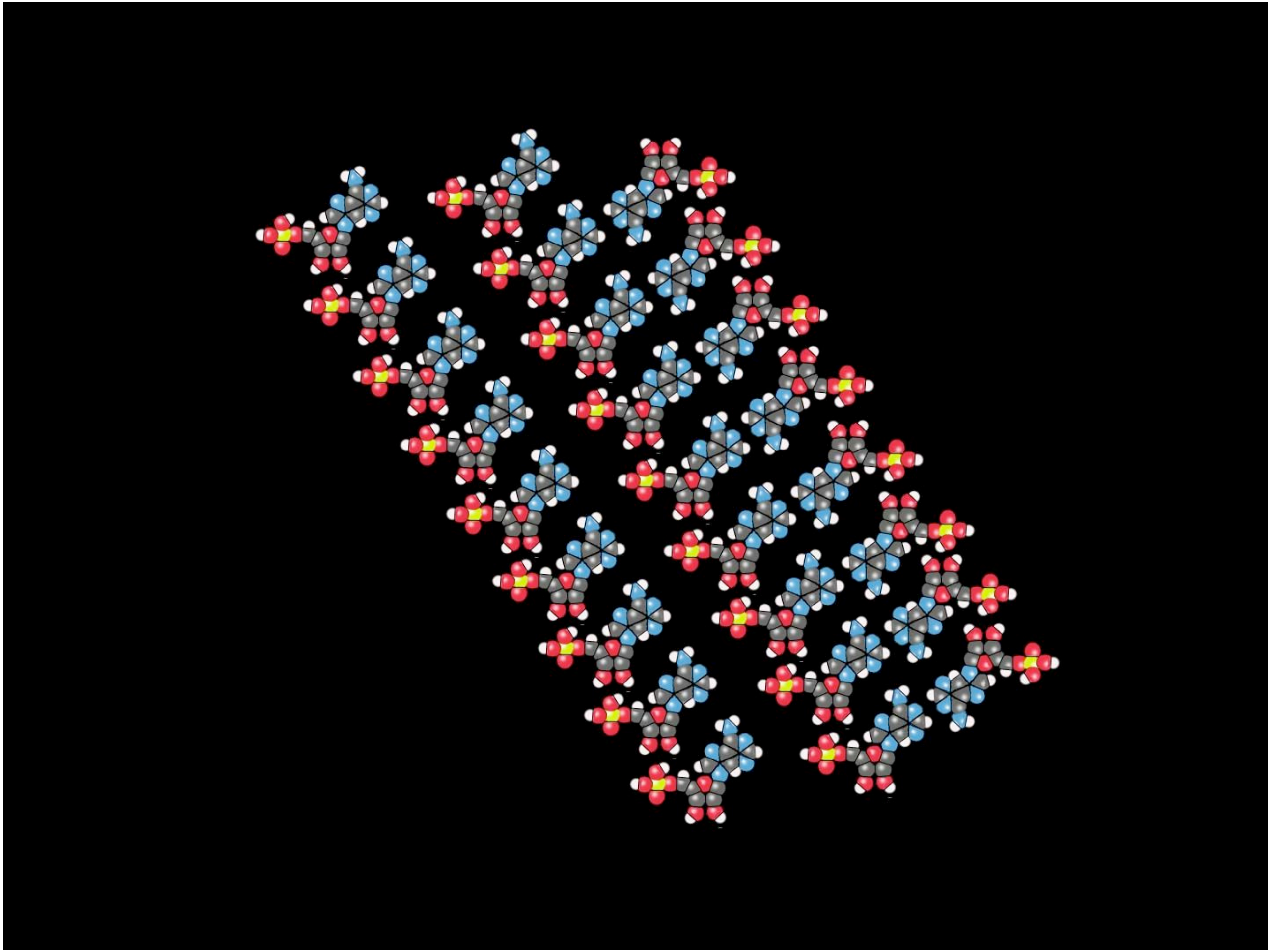
Lipids (prepared as small liposomes) were mixed in varying mole ratios with mononucleotides (5'-AMP or UMP) in 0.5 ml water.

The samples were dried and rehydrated up to seven times. The samples were heated and a stream of carbon dioxide(~1 ml/min) was used to remove water vapor.

Variables tested: lipid species, reaction time (30 - 120 min), temperature (60 - 90 ^{o}C), number of wet-dry cycles.

Polymer synthesis was detected by nanopore analysis, RiboGreen assays, and end-labeling followed by gel elecrophoresis.

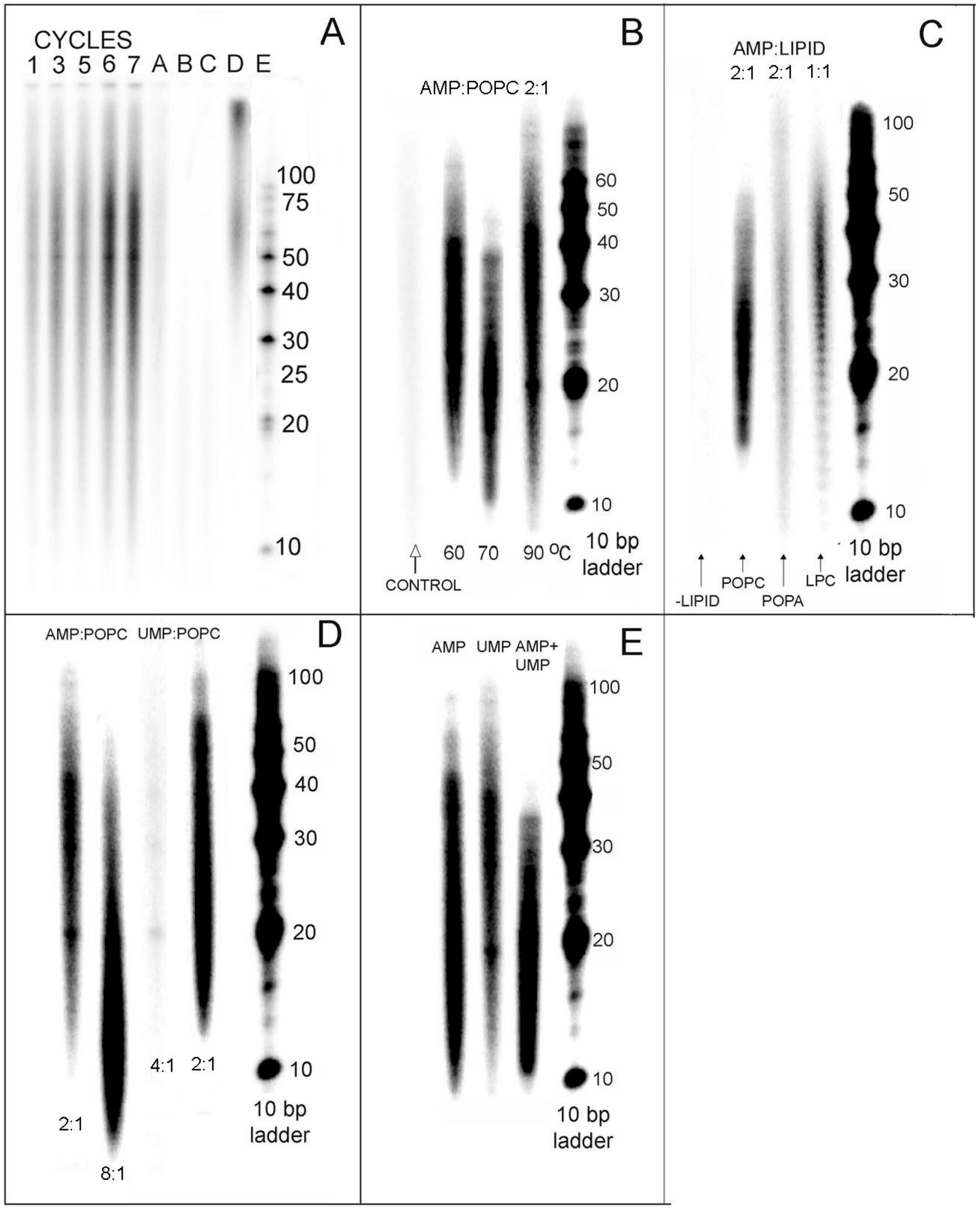




Disordered bulk phase AMP: Cannot undergo extensive polymerization

AMP may polymerize in fluid lipid microenvironment

$\Delta G^o = +5.3$ kcal/mol (Dickson et al. 2000)



RNA-like polymers are detected by end-labeling.

RNA

Alkaline phosphatase, T-4 kinase, $AT^{32}P$

RNA-^{32}P

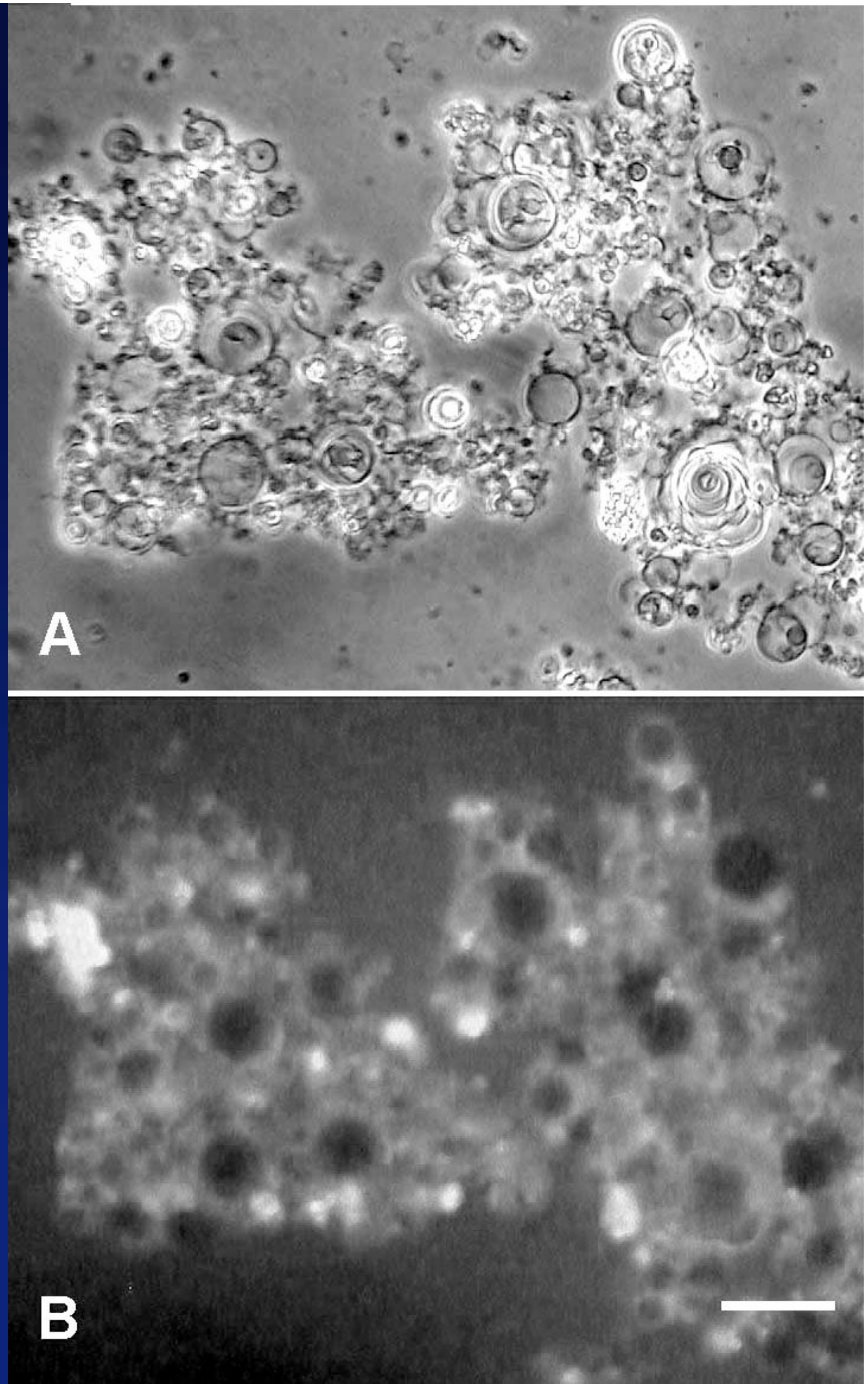
Rajamani et al. 2007

Do the lipid and products survive multiple cycles at elevated temperature (90°C) and low pH?

Examine product after seven cycles.

Phase microscopy

Fluorescence microscopy, Ethidium bromide stain for RNA

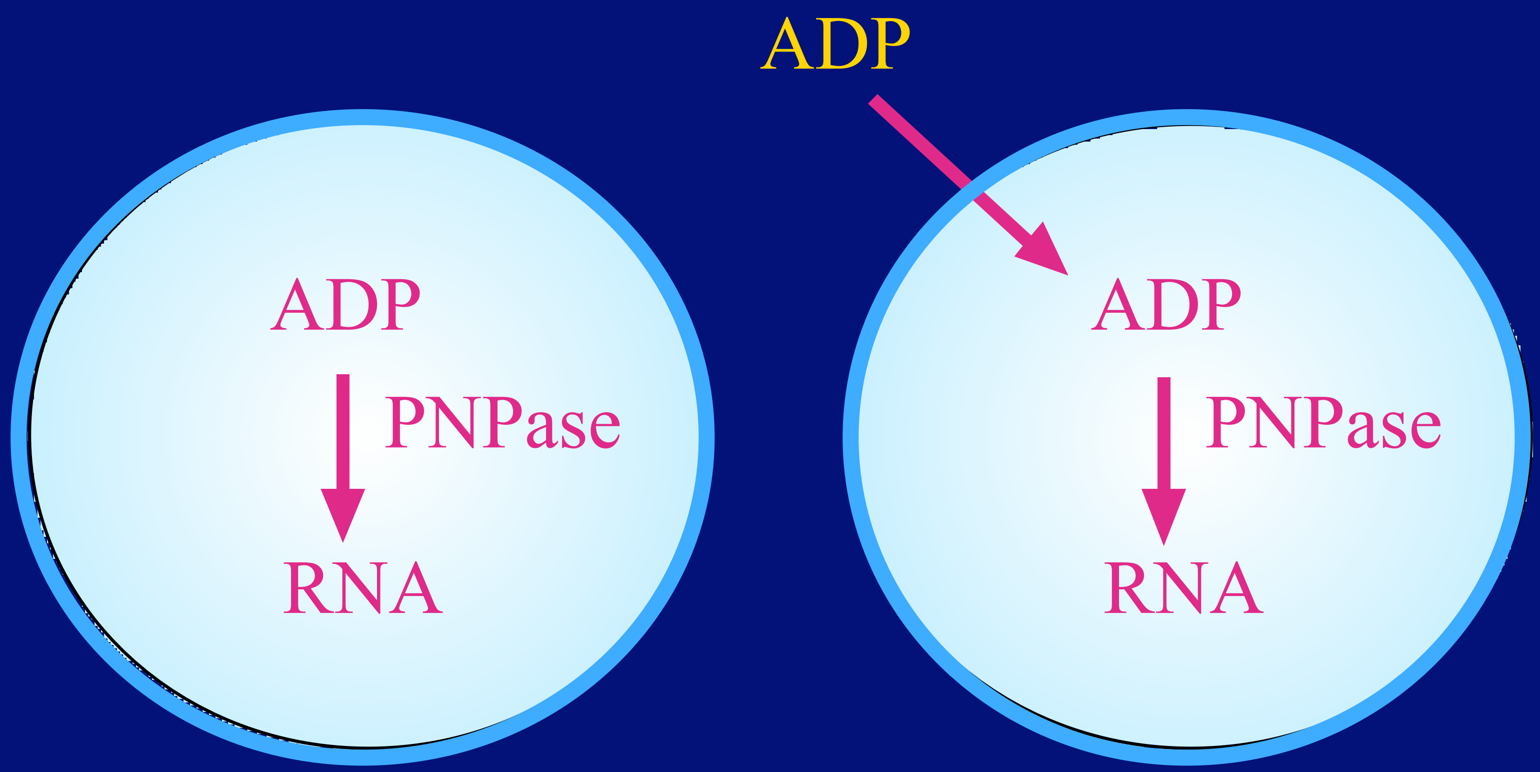


CAN WE PRODUCE ARTIFICIAL LIFE IN THE LABORATORY?

ENCAPSULATED POLYMERIZATION REACTIONS

RNA synthesis in lipid vesicles

Protein syntheis in lipid vesicles



RNA synthesis
Oleic acid vesicles
Walde et al. 1994

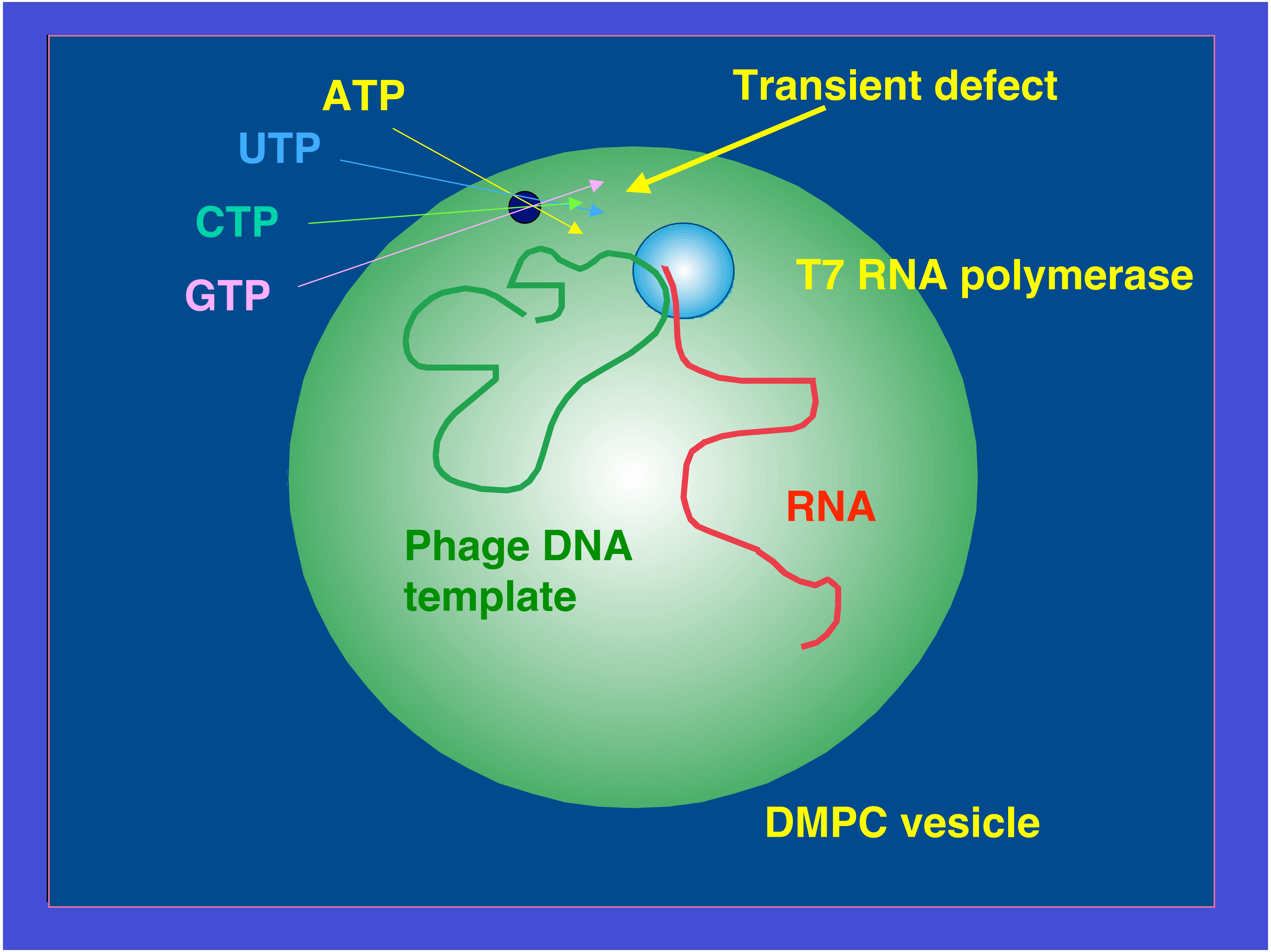
RNA synthesis
Phospholipid vesicles
Chakrabarti et al. 1994

TRANSCRIPTION: DNA --> RNA

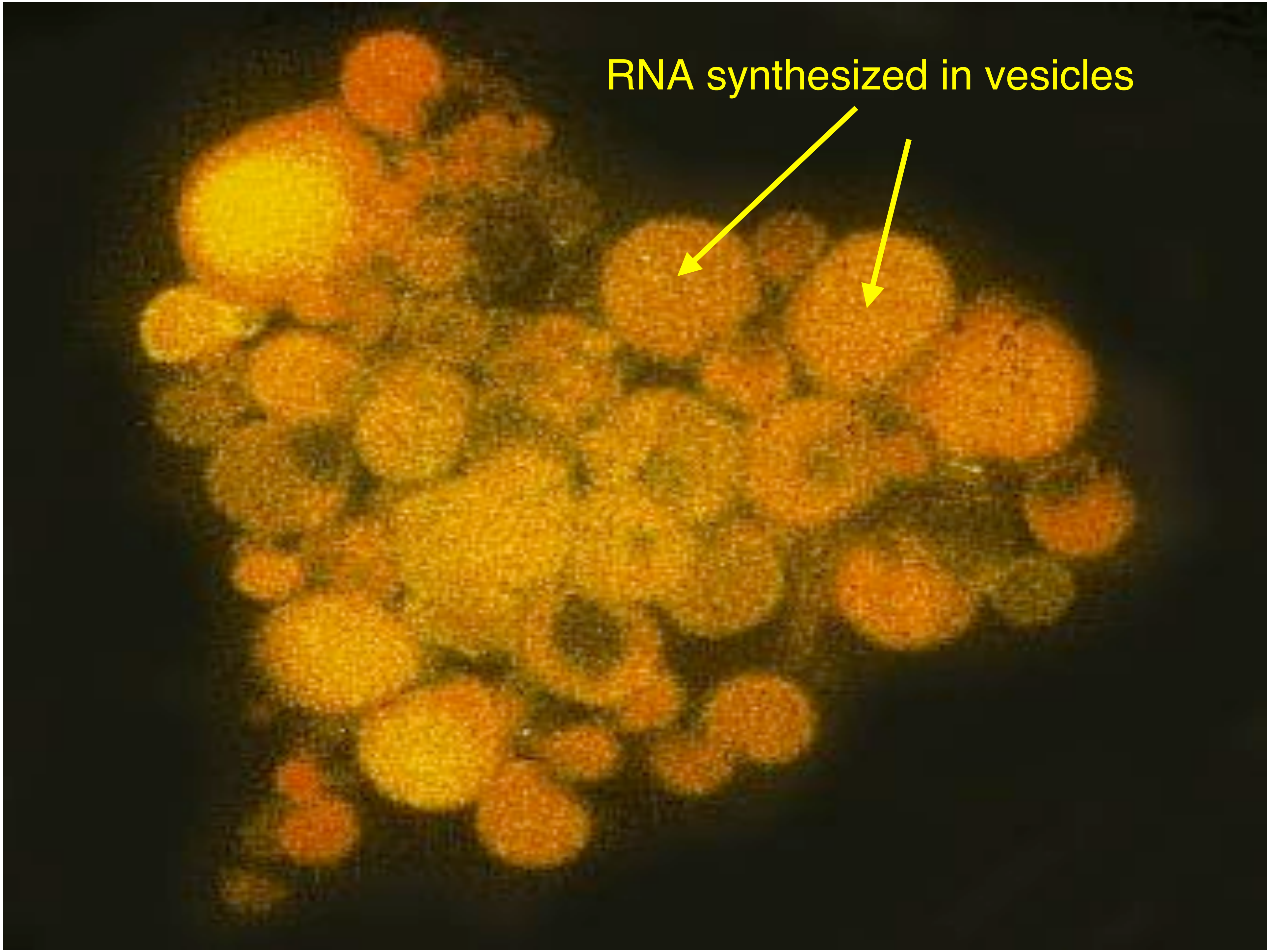
Encapsulated T7 RNA polymerase.

Uses DNA template, NTP substrates to transcribe base sequence from DNA to RNA.

Monnard et al. Phil. Trans. B 2007



ATP
UTP
CTP
GTP
Transient defect
T7 RNA polymerase
RNA
Phage DNA template
DMPC vesicle


RNA synthesized in vesicles

ENCAPSULATED TRANSLATION

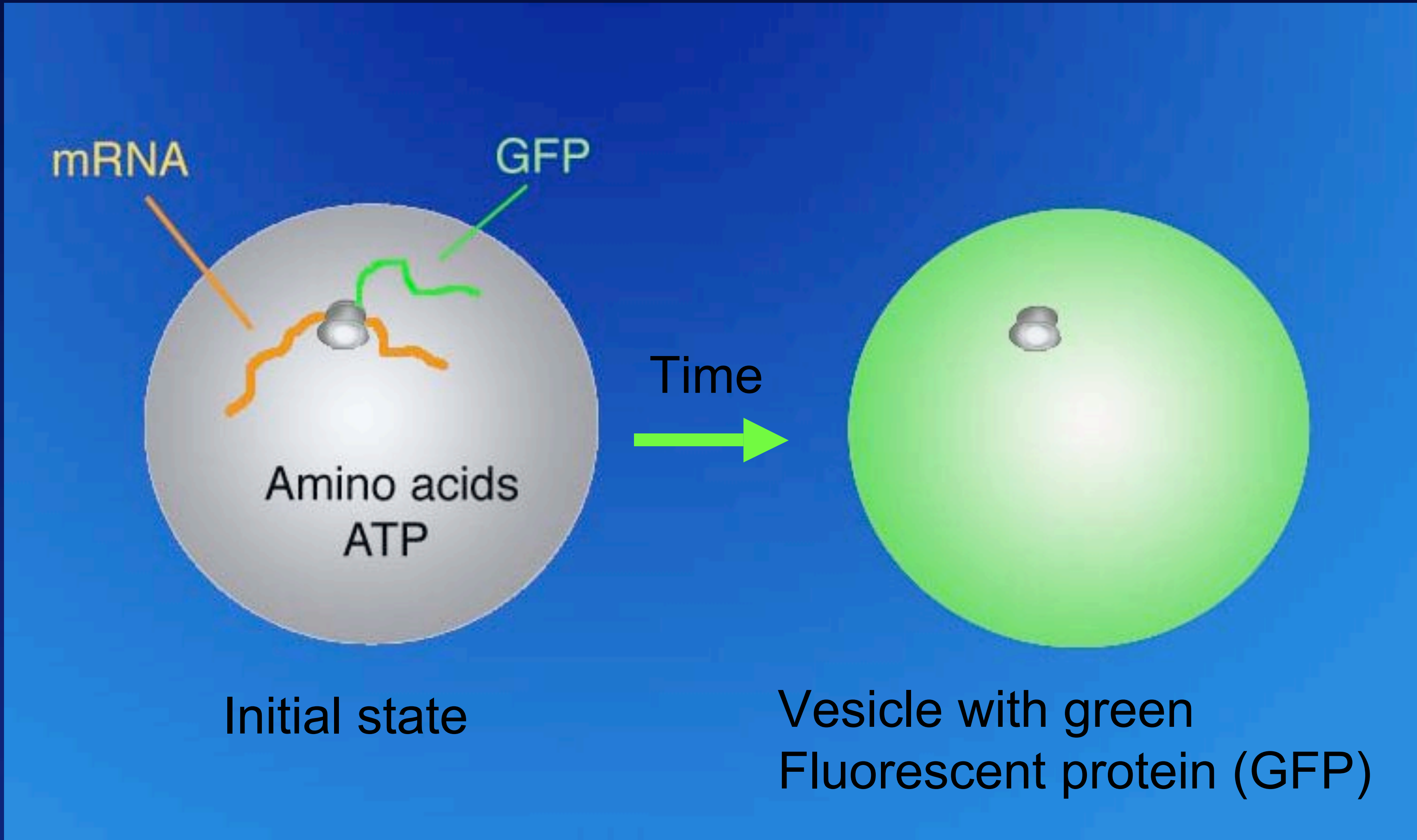
Approach:

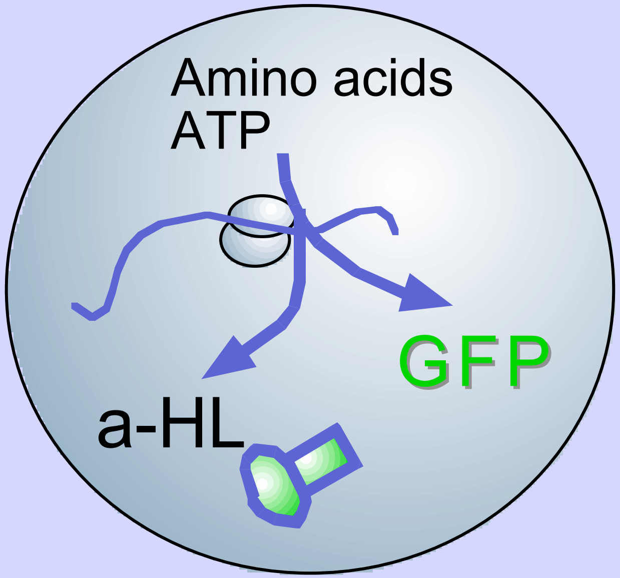
Capture cytosolic components of disrupted E. coli.

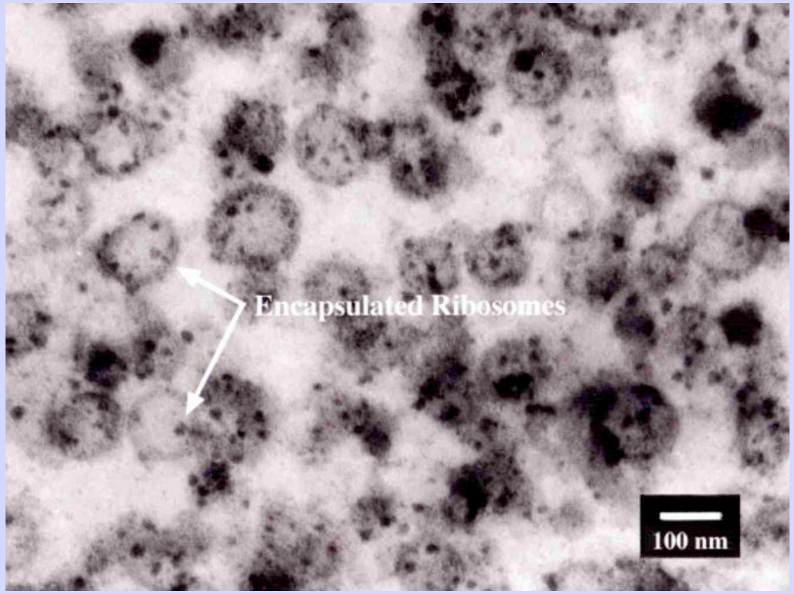
Translation system composed of ribosomes, tRNAs, amino-acyl tRNA synthetases etc.

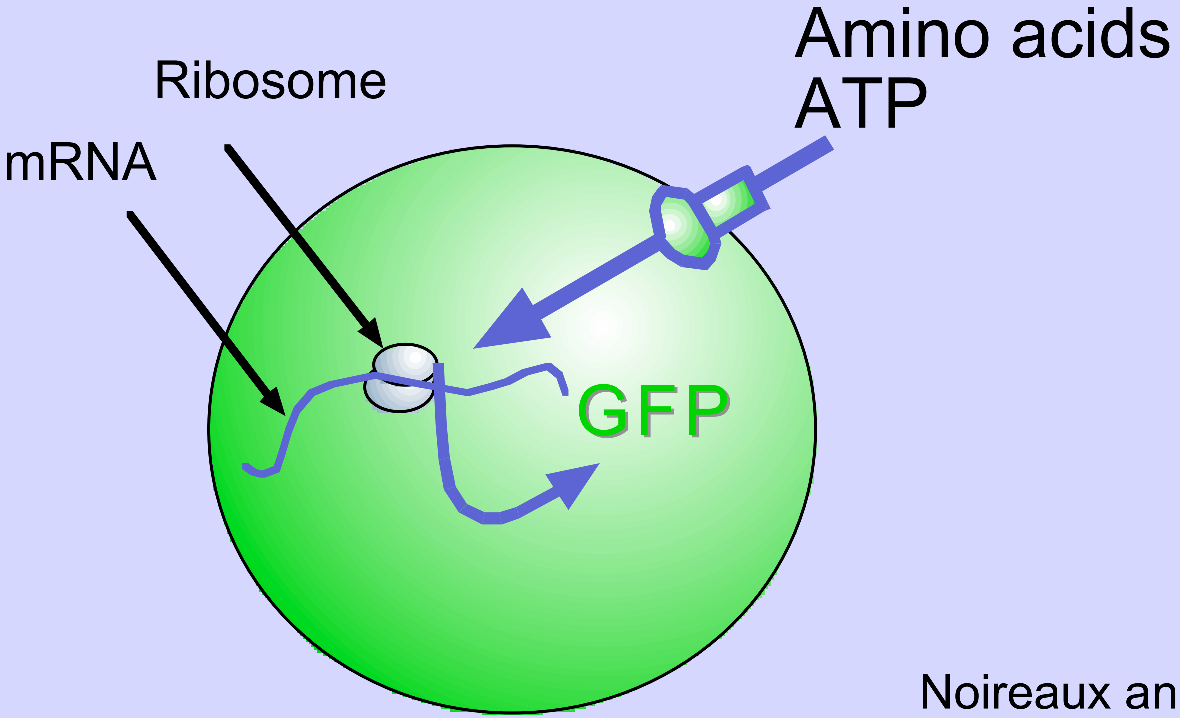
Include desired mRNA, 20 amino acids, ATP

Yu et al. 2001; Nomura et al. 2003


mRNA
GFP
Amino acids
ATP
Time
Initial state
Vesicle with green
Fluorescent protein (GFP)







Noireaux and Libchaber, PNAS 2004

ENCAPSULATED GENETIC CASCADE

Ishikawa et al. 2004, FEBS Letters 576, 387-390.

Plasmid DNA was prepared containing genes for GFP and T7 polymerase, both under control of promoters.

The plasmid was incorporated in large liposomes containing translation system components, amino acids and ATP.

GFP fluorescence was monitored by flow cytometry.

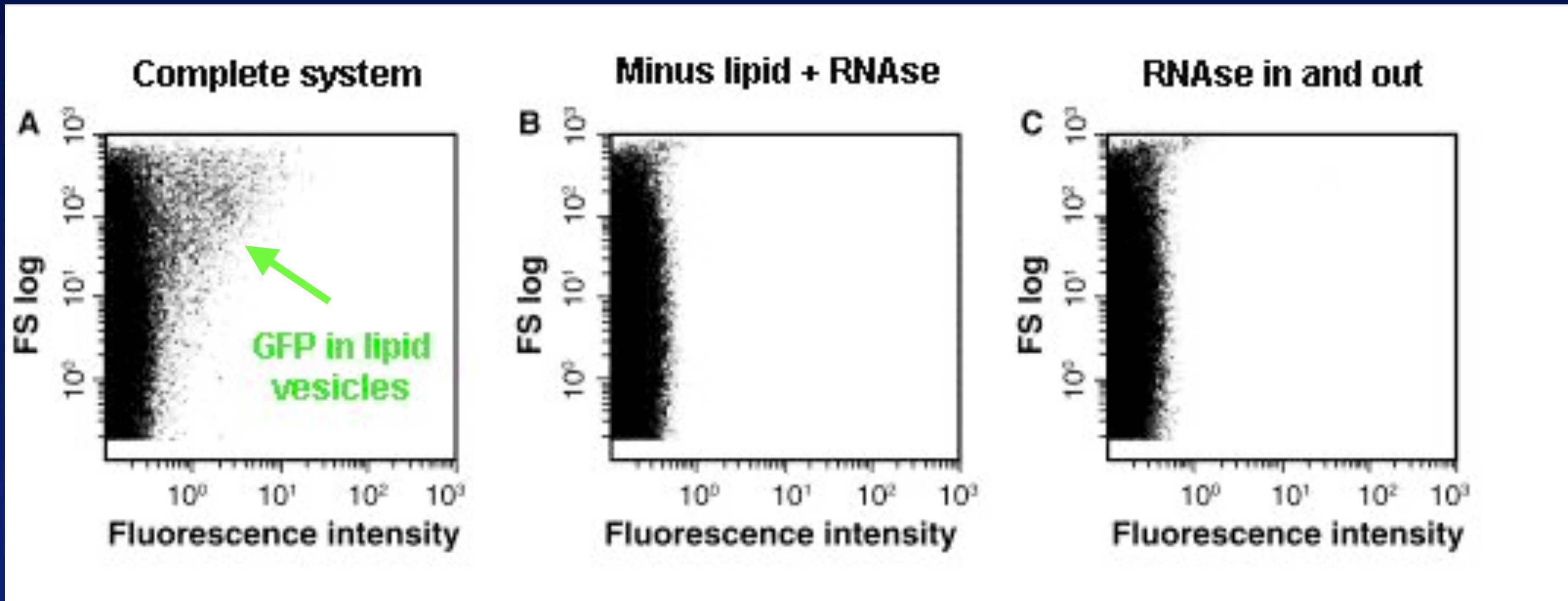
Reaction mixture

Buffer (200 mM Tris acetate), 17 mM Mg(OAc)2, 2.5% PEG8000,
ATP, 7.9 mM, GTP, UTP, and CTP, 0.88 mM
20 amino acids, 0.325 mM each
tRNAs, 0.17 mg/ml
16 nM of template DNA (pTH plasmid, 9000 bp)
8.0 μl of *E. coli* S-30 cell extract.

Encapsulation

10 μl of the reaction mixture added to the lyophilized liposomes
Egg phosphatidylcholine-cholesterol, 1:1
Mixed by pipetting to form large liposomes, 1 - 10 μm diameter
Final lipid concentration 120 mM
RNAse added to inhibit translation external to liposomes

GFP SYNTHESIS, FLOW CYTOMETRY



RESULTS OF THE GENETIC CASCADE

1. T7 RNA polymerase is synthesized from its gene.

2. The polymerase binds to its promoter to transcribe GFP mRNA

3. GFP is translated by the encapsulated ribosomes.

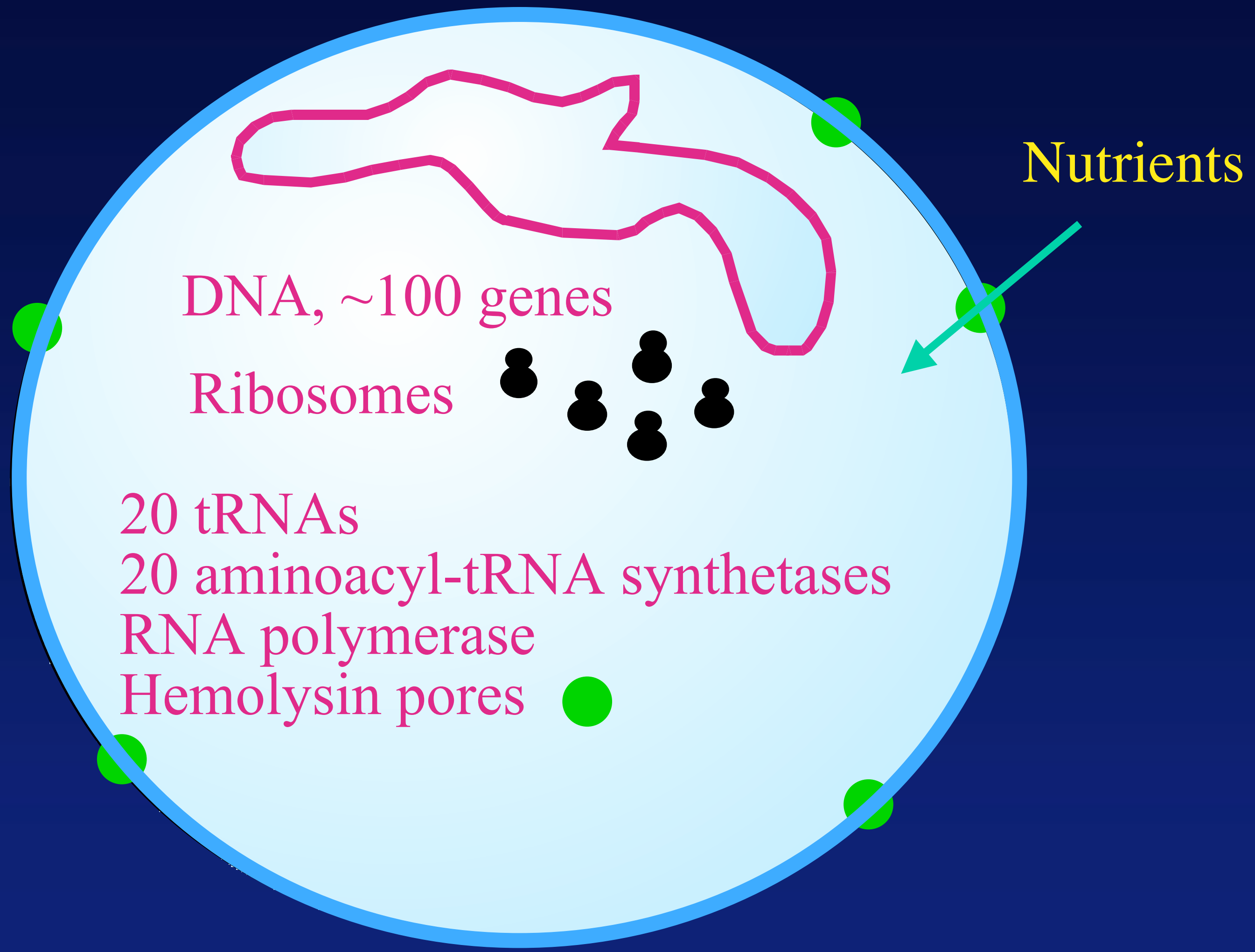
4. Approx. 500 copies of GFP were synthesized per gene.

Is this system “alive”?

NO. Only two proteins are synthesized. Everything else -- ribosomes, enzymes, lipid boundaries -- is left behind.

THE CHALLENGE NOW:

Use self-assembly to reconstitute cellular assemblies that grow and reproduce.



Synthetic cell, initial state

~100 genes required in artificial cell genome

Translation system
- 20 tRNAs
- 3 rRNAs (5S, 16S, 23S)
- 55 ribosomal proteins
- 20 aminoacyl tRNA synthetases

Nucleic acid synthesis
- RNA polymerase
- DNA polymerase

Membrane growth - phospholipid synthesis
- Acyl transferase

Transport - α-Hemolysin channel

NOT VERY PLAUSIBLE IN PREBIOTIC ENVIRONMENTS !

THE FIRST FORMS OF LIFE MUST HAVE BEEN SIMPLER.

REMAINING QUESTIONS:

Initial synthesis of catalytic and replicating polymers?

Assembly of translation system (ribosomes)?

Replication of the transcription and translation systems.

Sources of energy and nutrients?

Membrane growth to accommodate internal growth?

Regulatory feedback between internal polymer growth and growth of the membrane.

Division into smaller systems.

We can be optimistic: The first cellular forms of life spontaneously overcame all of these hurdles!


UC Santa Cruz
NASA Ames
Pierre-Alain Monnard
Charles Apel
Sudha Rajamani
Sara Singaram
Trishool Namani
Lynn Rothschild
Supported by NASA Exobiology program,1985 - present.