FROM THE ORIGIN OF LIFE TO

SYNTHETIC BIOLOGY (MINIMAL CELL)

With an excursus on self-aggregation/emergence

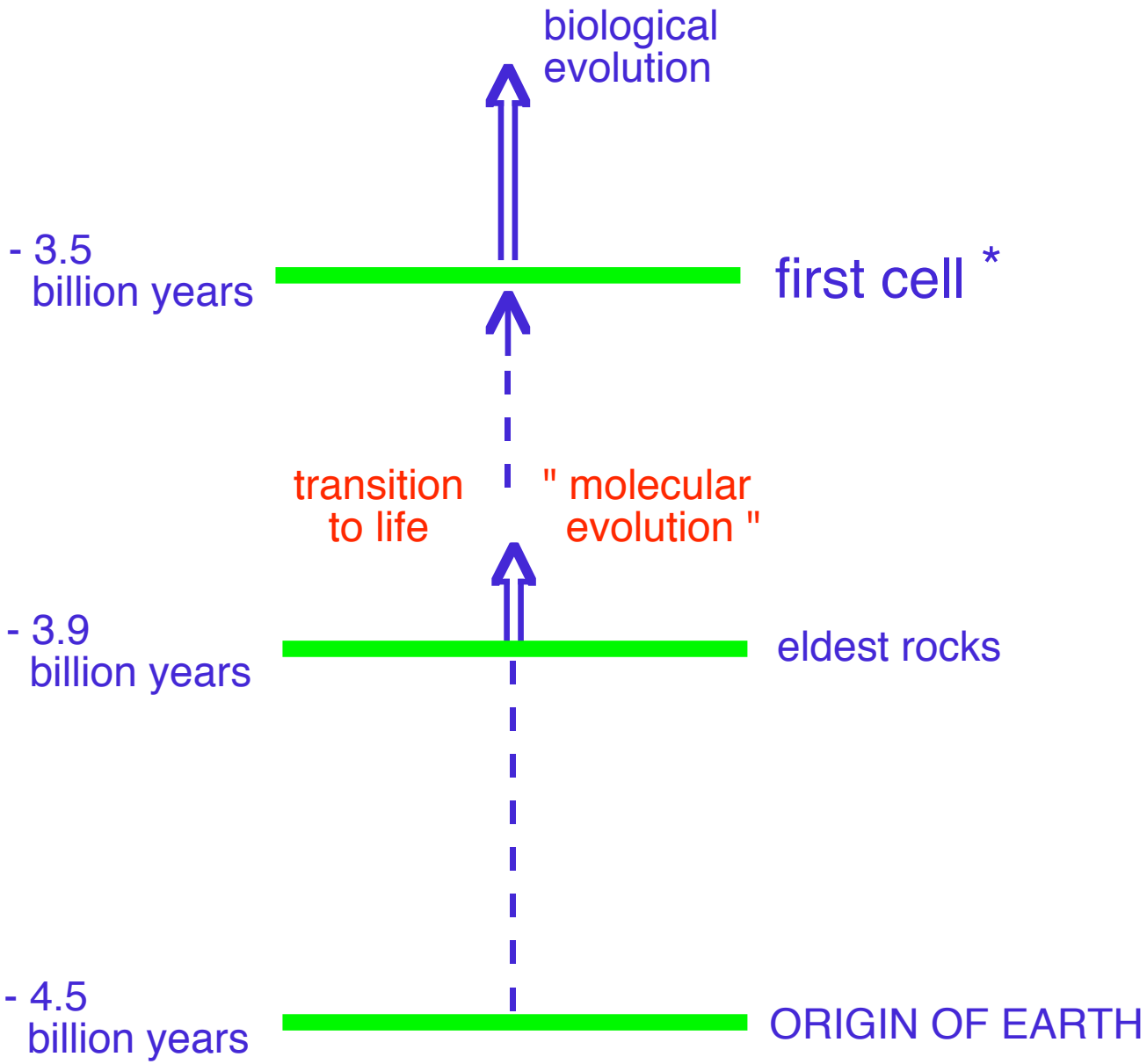
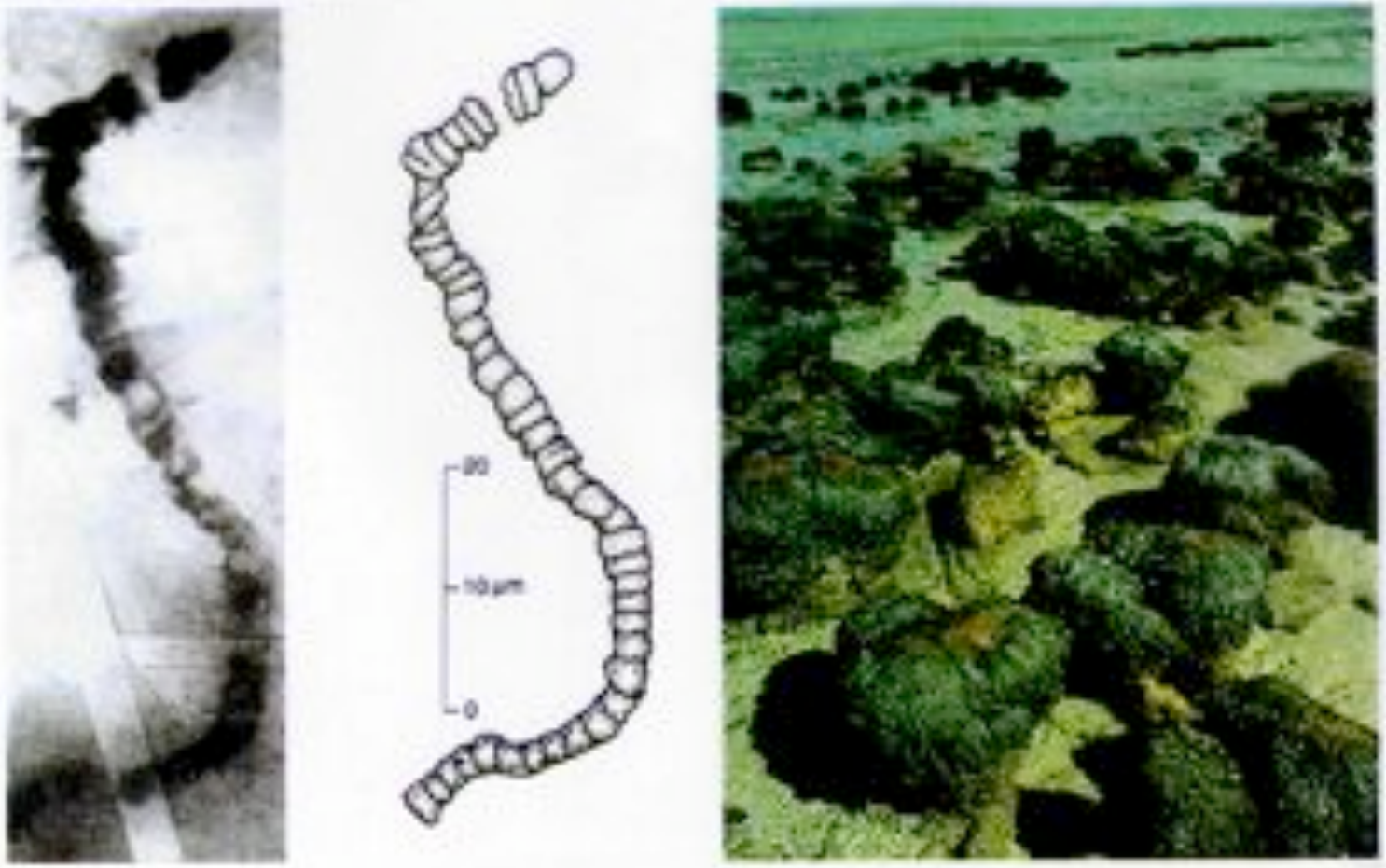

biological
evolution
- 3.5
billion years
first cell *
transition
to life
" molecular
evolution "
- 3.9
billion years
eldest rocks
- 4.5
billion years
ORIGIN OF EARTH

Figure 21.9

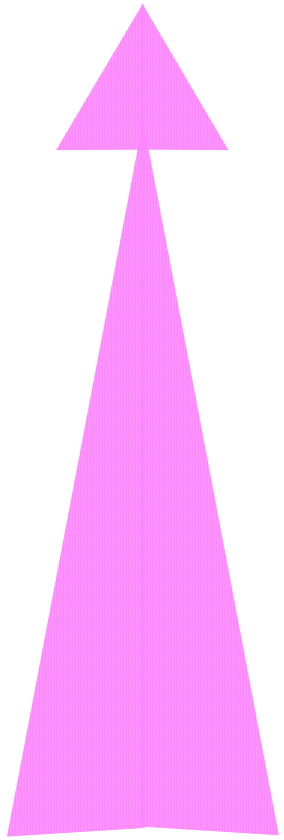
Fossils of prokaryotes dated from about 3.5 billion years ago. **a.** This prokaryote microorganism, *Primaevifilum*, (with interpretive drawing), was found in a fossilized stromatolite. **b.** Living stromatolites are located in shallow waters off the shores of western Australia and also in other tropical seas.



a

b

LIFE



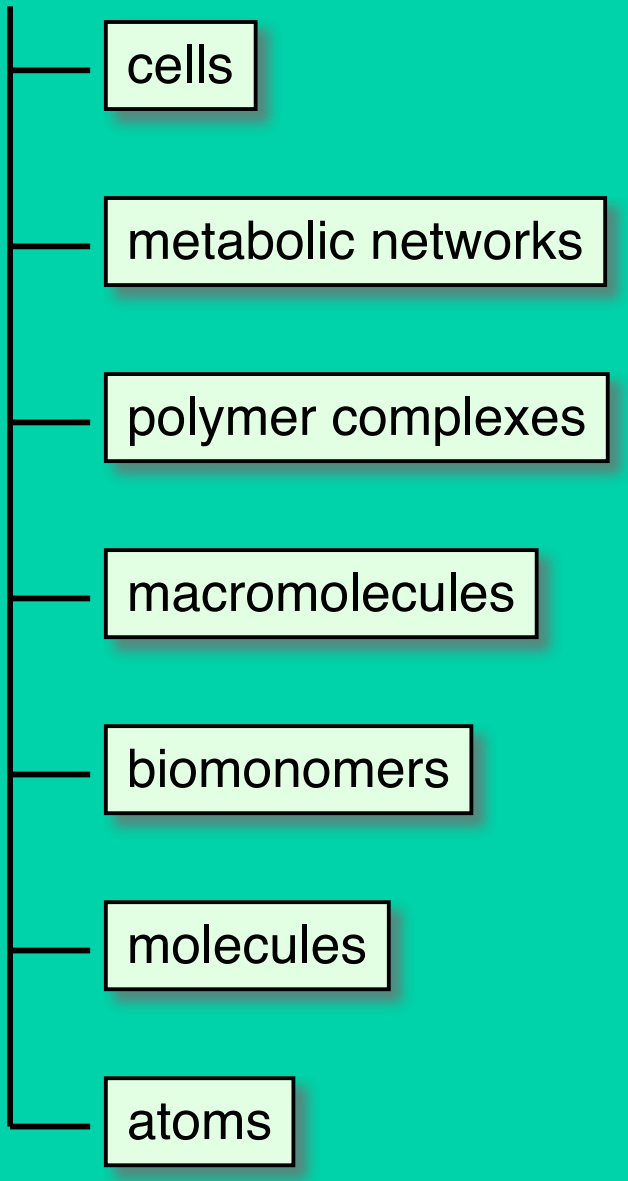
INANIMATE MATTER

(NON - LIFE)

The traditional definition:

Science is
the attempt
to explain the phenomenology
of the world in terms of the laws
of physics and chemistry

..not the only way to explain the world


cells
metabolic networks
polymer complexes
macromolecules
biomonomers
molecules
atoms

Aleksandr Oparin (1894 – 1980)

ПАРОЛЬ ТЕПЛЫЙ ВСЕХ СТРАН, СОЕДИНЯЙТЕСЬ!

А. И. ОПАРИН

ПРОИСХОЖДЕНИЕ ЖИЗНИ

"МОСКОВСКИЙ РАБОЧИЙ"
1924

About the philosophical framework

Determinism vs Contigency in the origin of life

The deterministic view of the origin of life

the “continuity principle”

no unbridgeable gap between inorganic and living matter;
each stage in evolution develops continuously from the previous one,
at each stage there is a continuous path backwards to the prebiotic state and forward to modern organisms

AND:

…given the suitable initial conditions, the emergence of life is highly probable and governed by the laws of chemistry and physics…

Orgel; Morrowitz; de Duve

The science of the origin of life has to adopt the deterministic, continuity view- otherwise it would not be possible to adopt a scientific method of inquiry

Christian de Duve
Harold Morowitz
And others…

as opposite to this, the view by which:

life originated as a entirely chance event
as a highly improbable event
as a “happy accident”
comparable to the assemblage of a 747 Boeing
by a tornado whirling through a junkyard (Hoyle 1981, 1993)

the origin of life as an impenetrable
barrier to science and a residue to all
attempts to reduce biology to chemistry
and physics
(Popper (1972, 1982)

we cannot give a causal explanation of the
origin of biological organization. We have
to do as if the biological organization is
given by an external organizer
Kant, 1790

See Iris Fry, Biol and Phyl. 10 (1995) 389

...we also reject the suggestions of Monod that the origin
requires a series of highly improbable events...
The study of origin of life is useful only if that beginning
took place under probably deterministic conditions,
otherwise ...it becomes a branch of history rather than
natural science...

H. J. Morowitz
Beginning of Cellular Life, Yale Univ. Press, 1992

…”*I favor the view that life was bound to arise under the physical-chemical conditions that surrounded its birth*”

De Duve, 2002

We have no reason to believe that biogenesis was not a series of chemical events subject to all of the laws governing atoms and their interactions.”

Morowitzt, 1991

“..*It is self-evident that the universe was pregnant with life and the biosphere with man. Otherwise, we would not be here. Or else, our presence can be explained only by a miracle…*”

De Duve, 2002

Other „crypto-creationists“????

Anthropic principle

Panspermia

SETI

The anthropic principle can be expressed in different ways but the basic idea is that the universal constants, the geometric parameters, and all things of the universe are so precisely determined– not a 5% deviation would allow life–
They are the way they are in order for life and evolution to develop.

It is the *post hoc* argument that since we are so improbable, our presence must signify a purposeful universe.

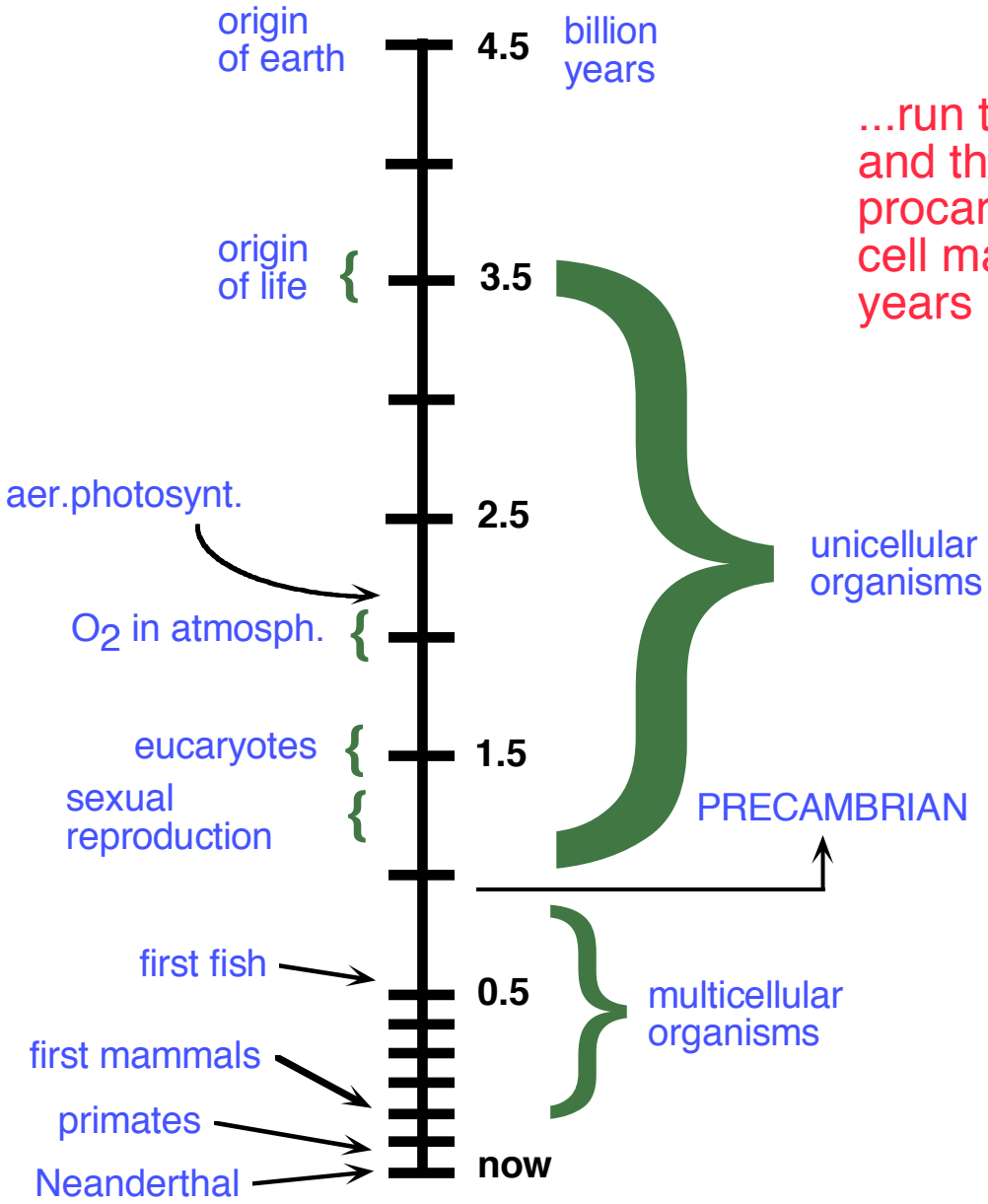
(Davies, 1999; Barrow and Triples, 1988 and 1996; Barrow, 2001; Carr, 2001).

"If life follows from (primordial) soup with
causal dependability, the laws of nature encode a hidden subtext, a cosmic imperative, which tell them:
'Make life! And, through life, its by-products,
mind, knowing, understanding…' ".

Paul Davis, 1991

OPPOSITE TO THAT....

CONTINGENCY



...run the tape again, and the first step from procaryotic to eucaryotic cell may take 12 billion years instead of 2...

Stephen J.Gould
in "Wonderful Life"
1991 Penguin Science

WE WOULD LIKE TO THINK OURSELVES NECESSARY, INEVITABLE, ORDAINED FOR ALL ETERNITY.
ALL RELIGIONS, ALL PHILOSOPHIES, AND EVEN PART OF SCIENCE TESTIFY
TO THE UNWEARYING, HEROIC EFFORT OF MANKIND
DESPERATELY DENYING
ITS OWN CONTINGENCY

J.Monod, Chance and Necessity, 1971

ARE WE ALONE IN THE UNIVERSE??

SOME MAIN ASSUMPTIONS OF PRESENT DAY RESEARCH ON THE ORIGIN OF LIFE

1. Life originated from inanimate matter as a spontaneous and continous increase of molecular complexity. Chemical continuity principle - no transcendental principle.
2. The chemical process(es) to transition to life can be reproduced in the laboratory with the presently available chemical techniques and chemicals.
3. And this can be implemented in a reasonable (hours or max. days) experimental time span - once you know the right combination of prebiotic compounds and theconditions.
4. Since there is no documentation on how things really happened, there is no obligatory research pathway.

reductionism versus holism

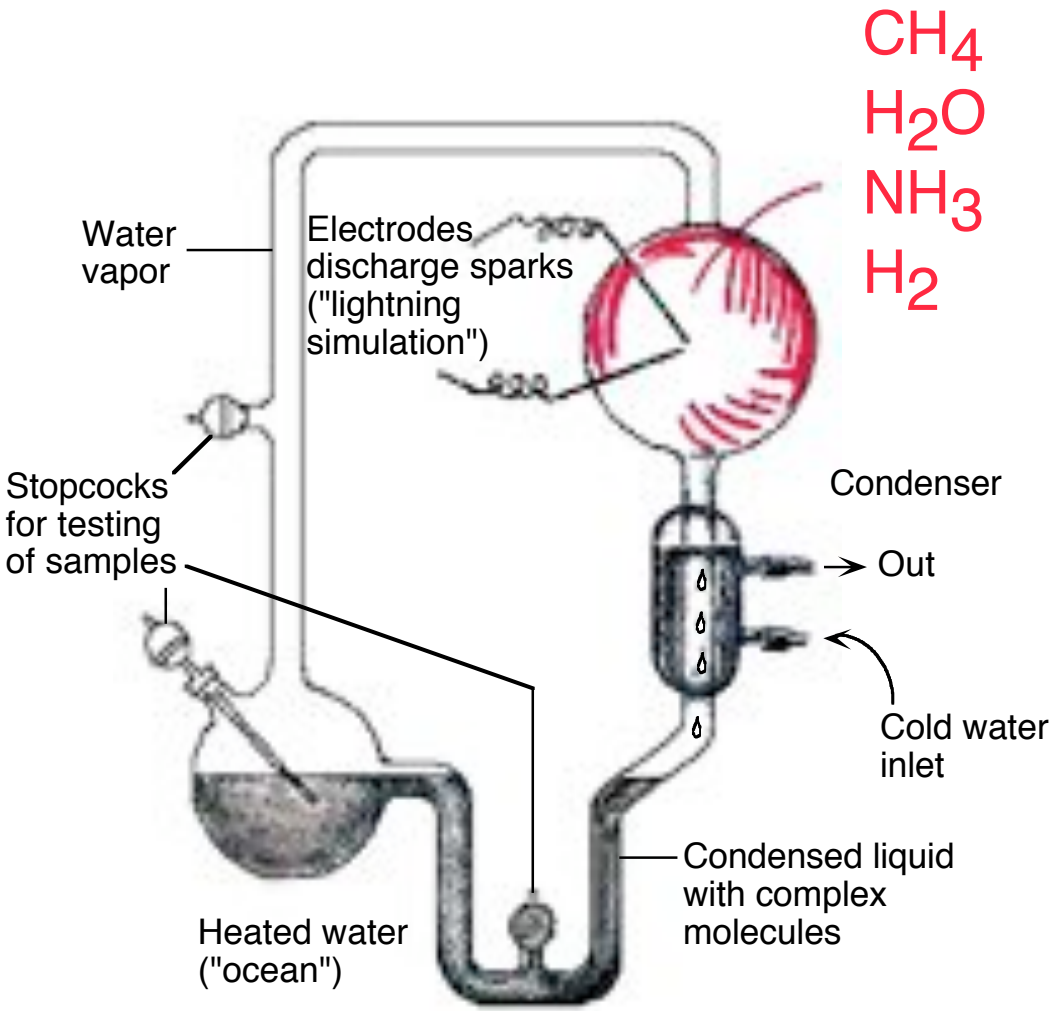
only an apparent dichotomy

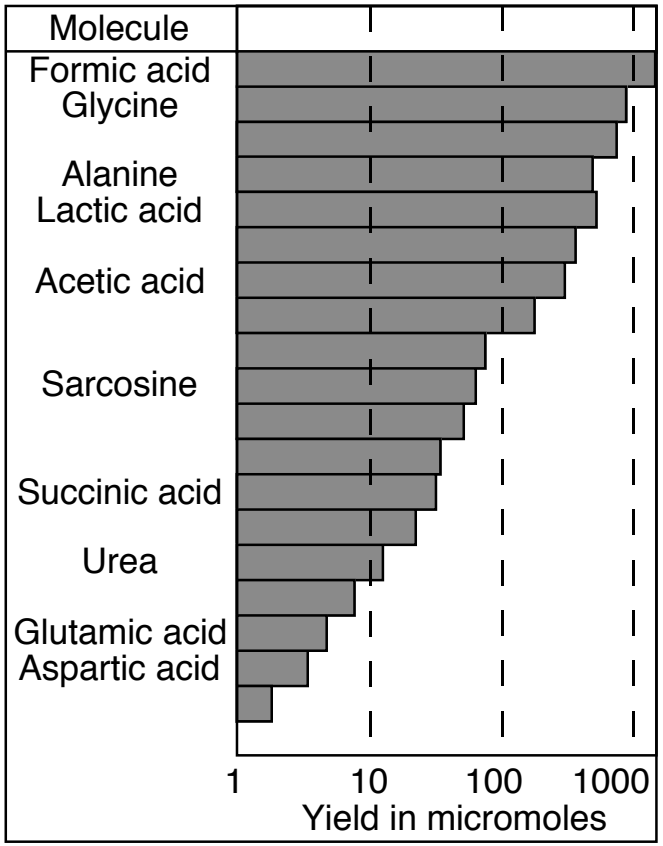
as
the whole is made by the sum of its constituents
but
some of the qualities of the whole cannot be foreseen
or explained from the sum of the qualities
of the components

the notion of emergence

the chemistry of the origin of life as a challenge to create molecular complexity and specificity by using the simplest possible means

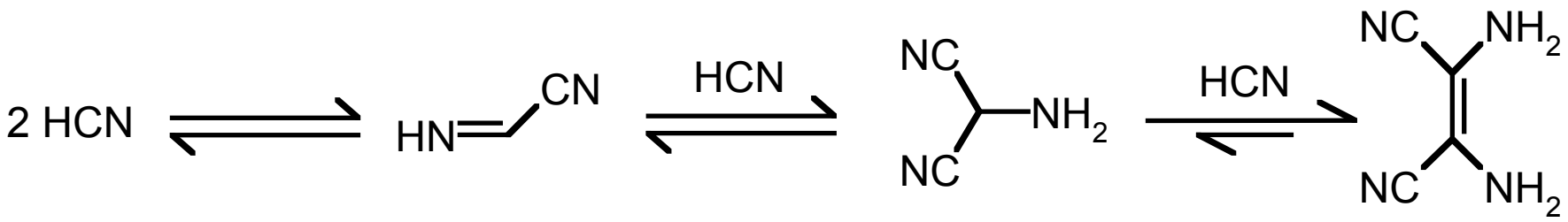
since we do not know how life emerged really, every scientists is free to choose her/his own working path.
Freedom and phantasy
Do it as you wish, just do it


CH_4
H_2O
NH_3
H_2
Water vapor
Electrodes discharge sparks ("lightning simulation")
Stopcocks for testing of samples
Condenser
Out
Cold water inlet
Condensed liquid with complex molecules
Heated water ("ocean")

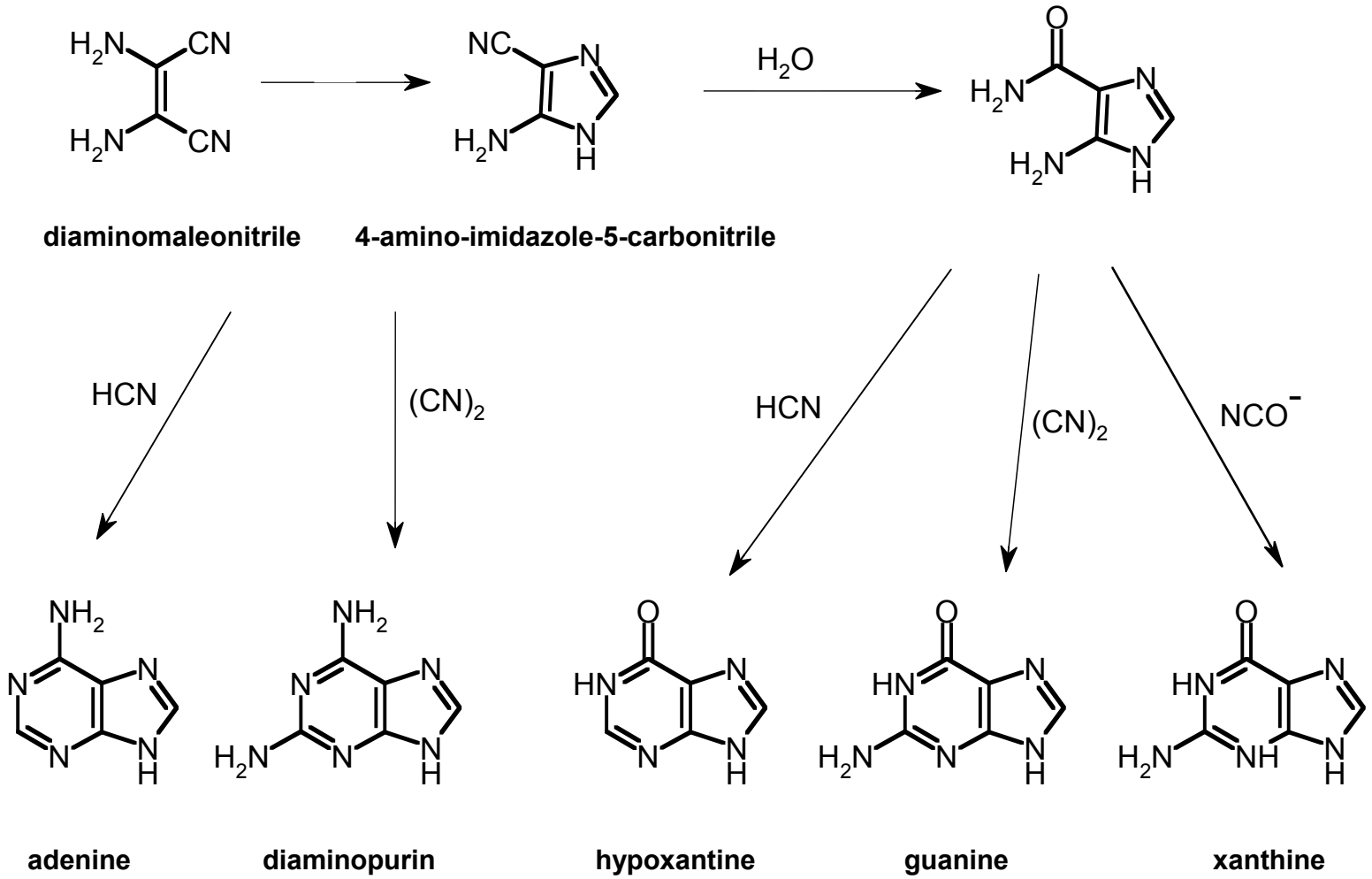

Molecule
Formic acid
Glycine
Alanine
Lactic acid
Acetic acid
Sarcosine
Succinic acid
Urea
Glutamic acid
Aspartic acid
1
10
100
1000
Yield in micromoles

Chemistry of HCN, precursor of purines

Ferris & Orgel 1996, 1967

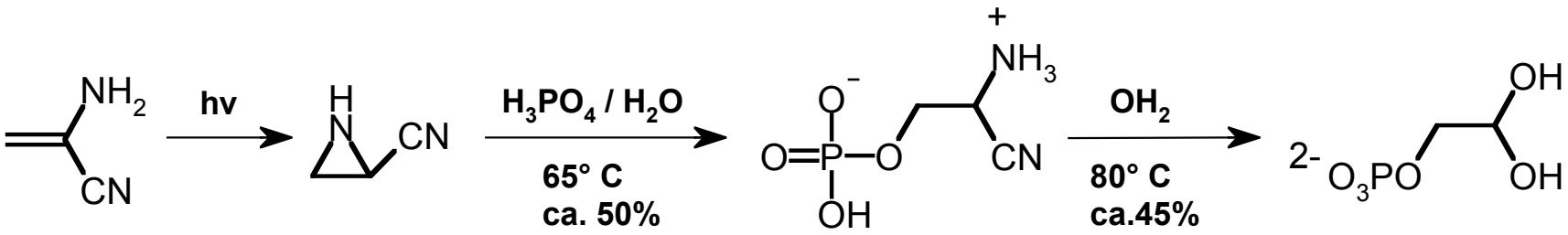


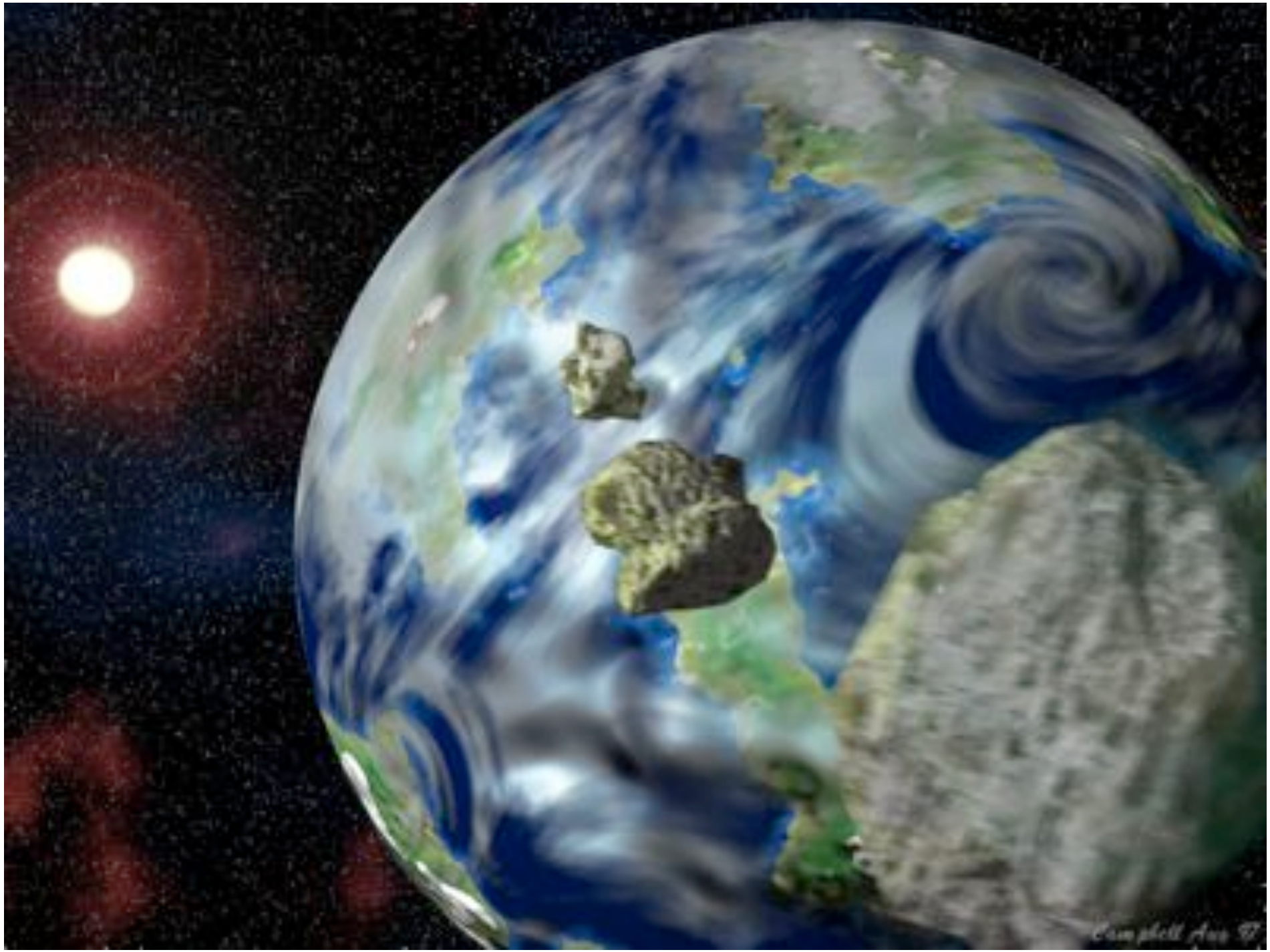
Synthesis of purines
Oró, 1960



Synthesis of sugar phosphates

Eschenmoser 1988, 1990





Every year 10,000 Kg of „cosmic dust“ comes to the Earth from space:
10-15 % of this material are carbonaceous molecules

Meteorites and comets bring additional compounds. Including water.
Aminoacids are formed in meteorites

Why do aminoacid form in Miller‘ experiment?

Why this...and not that?....

They form because there are the most stable of the possible compounds- due to processes under thermodynamic control

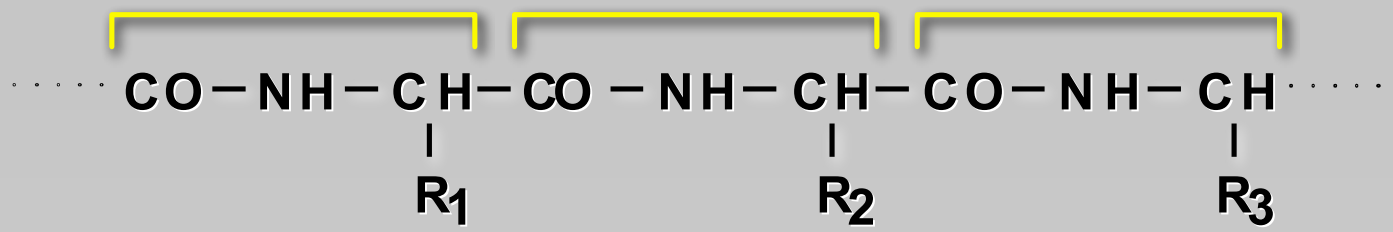
PREBIOTIC CHEMISTRY HAS REACHED CONSIDERABLE SUCCESS ..... BUT

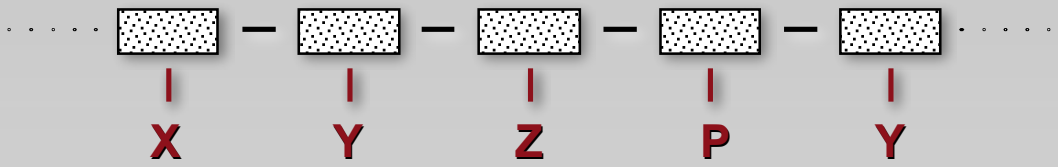
Even if the chemist would have access to all prebiotic bio-monomers

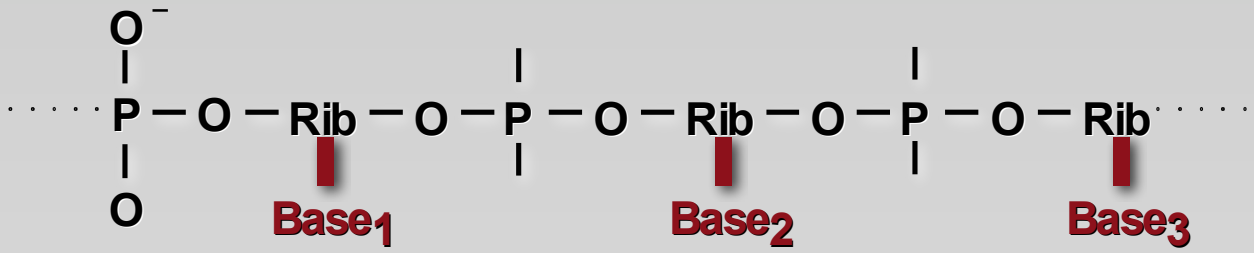
he would NOT have the solution to the origin of life

THE CORE OF THE PROBLEM:

LIFE AS WE KNOW IT IS DETERMINED BY SPECIFIC MACROMOLECULAR SEQUENCES LIKE NUCLEIC ACIDS AND PROTEINS


CO – NH – CH – CO – NH – CH – CO – NH – CH
R1
R2
R3

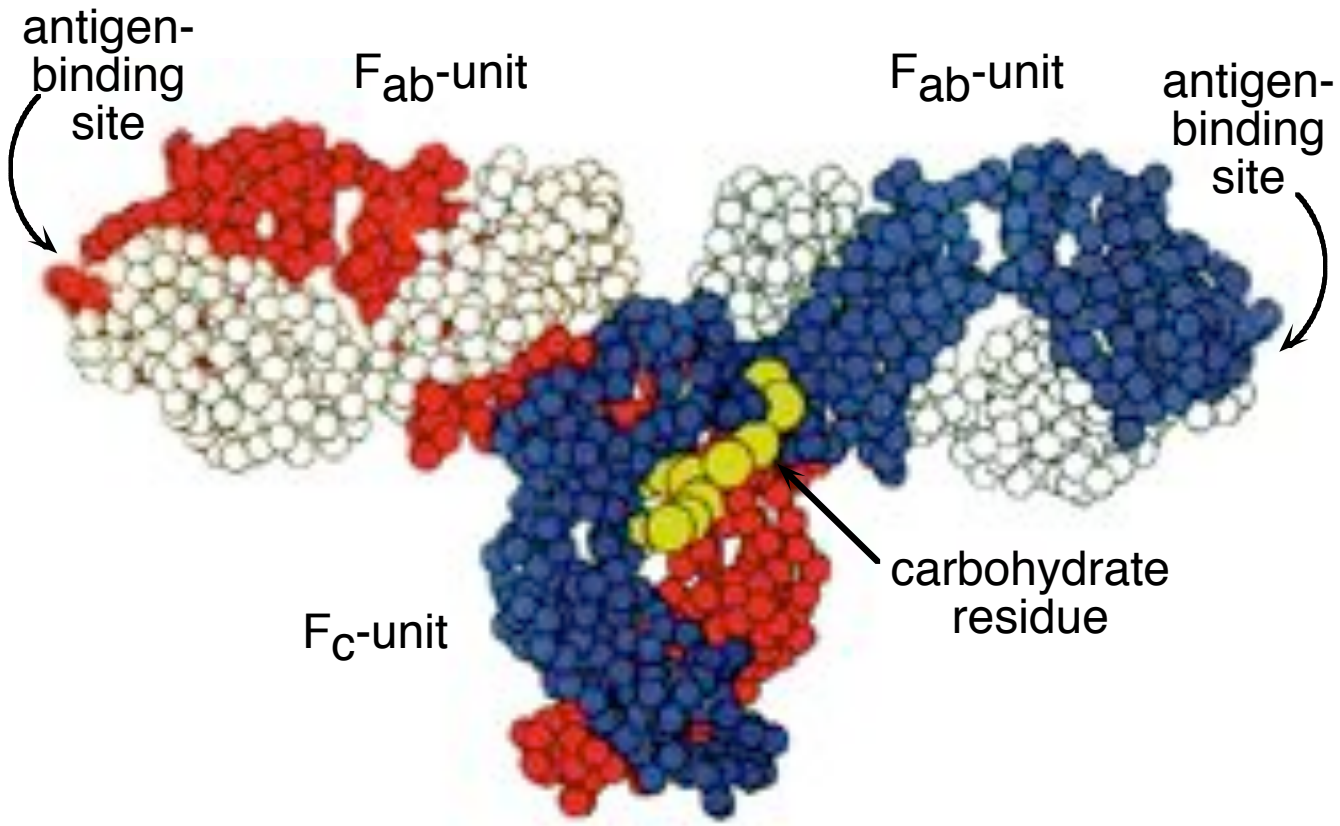

X
Y
Z
P
Y


O⁻
P – O – Rib – O – P – O – Rib – O – P – O – Rib
O
Base1
Base2
Base3

(75)

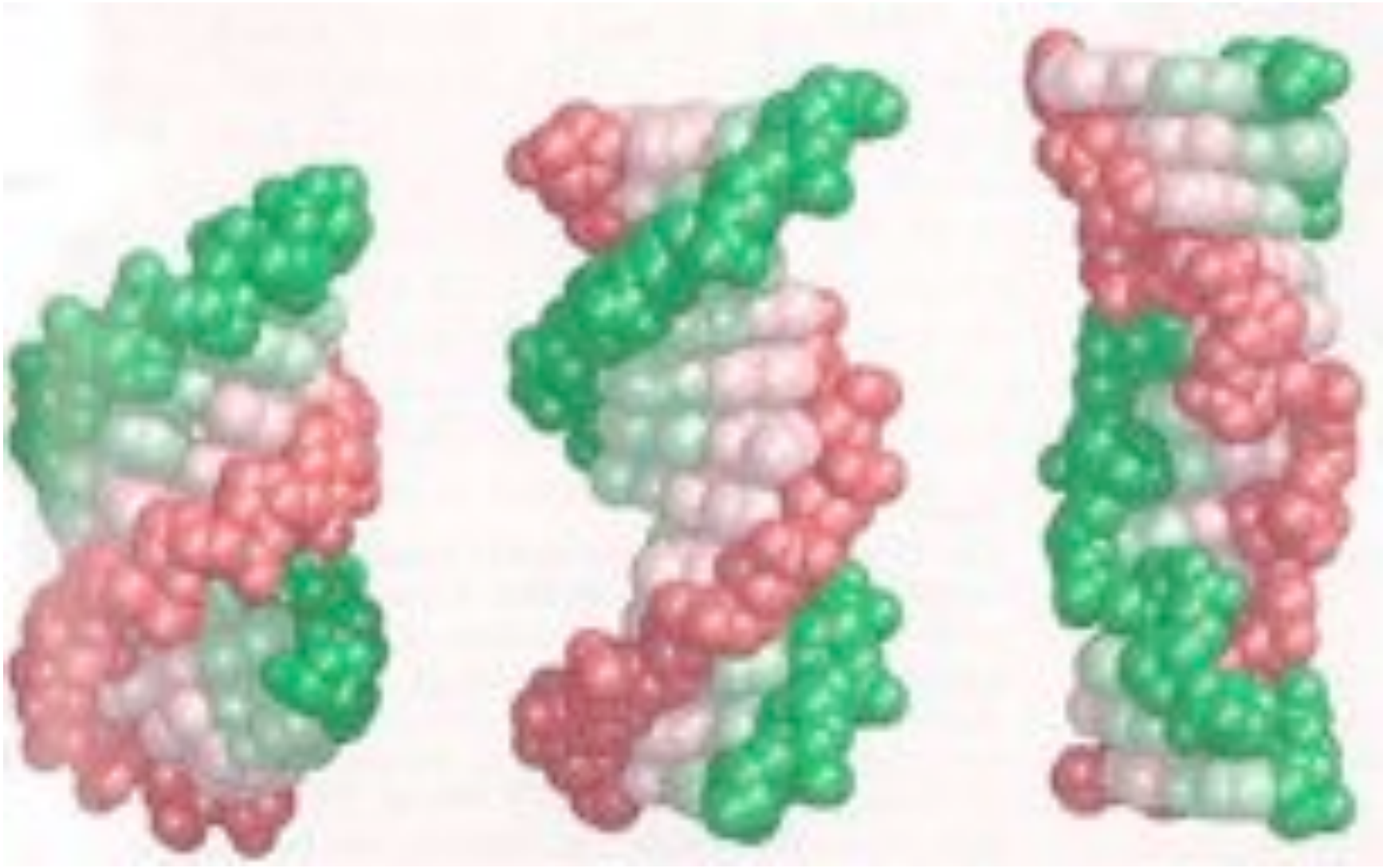
Ein raumfüllendes CPK-Modell von Lysozym. Links: Enzym ohne Substratmolekül; man erkennt das spaltförmige aktive Zentrum. Rechts: Enzym-Substrat-Komplex. Substratmolekül in Farbe

SCHEMATIC REPRESENTATION OF THE THREE-DIMENSIONAL STRUCTURE OF I_gG

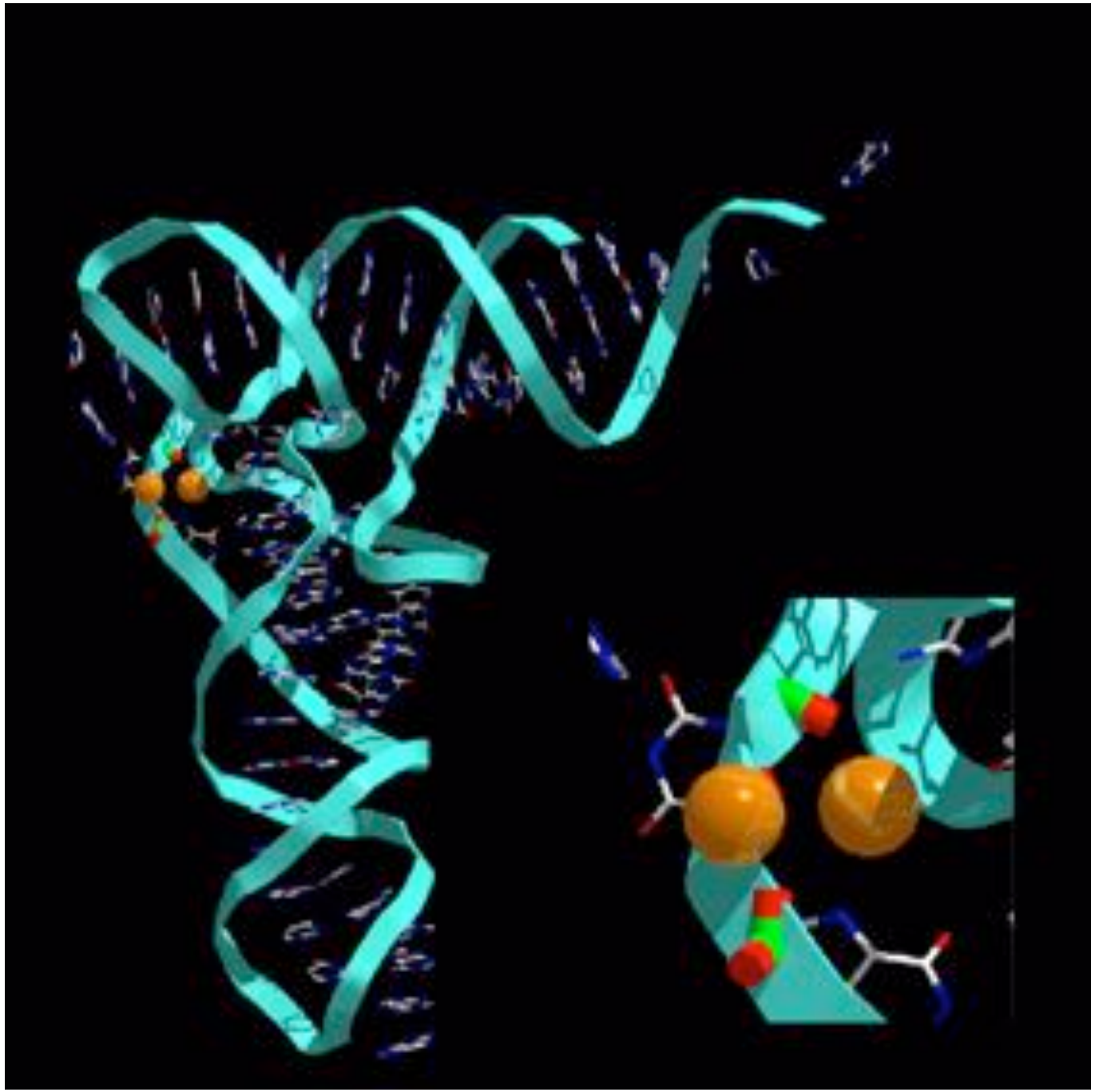


Each amino acid residue is represented by a small circle. The H chains are red and the L chains blue. A carbohydrate residue is yellow.

E. W. Silverton, et al. *Proc. Nat. Acad. Sci. 74 (1977); p. 142.*



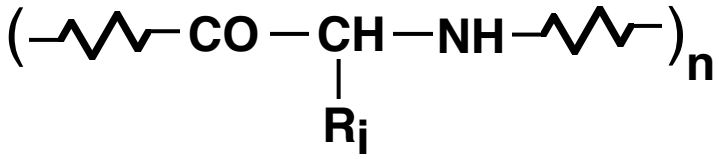
A-DNA B-DNA Z-DNA



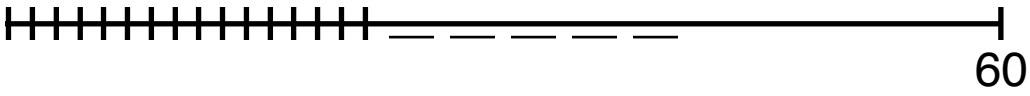
The macromolecules of life
are not there
because they are more stable
than their billions of constitutional isomers

They are the products of statistics and contingency
and
evolution

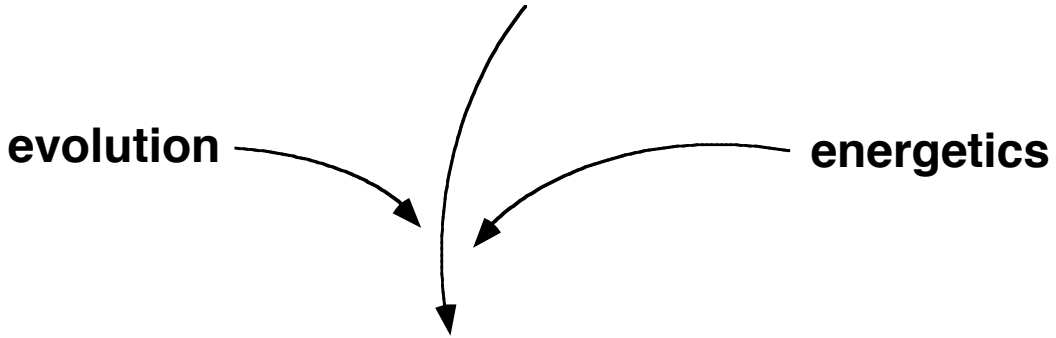
On the importance of being a copolymer



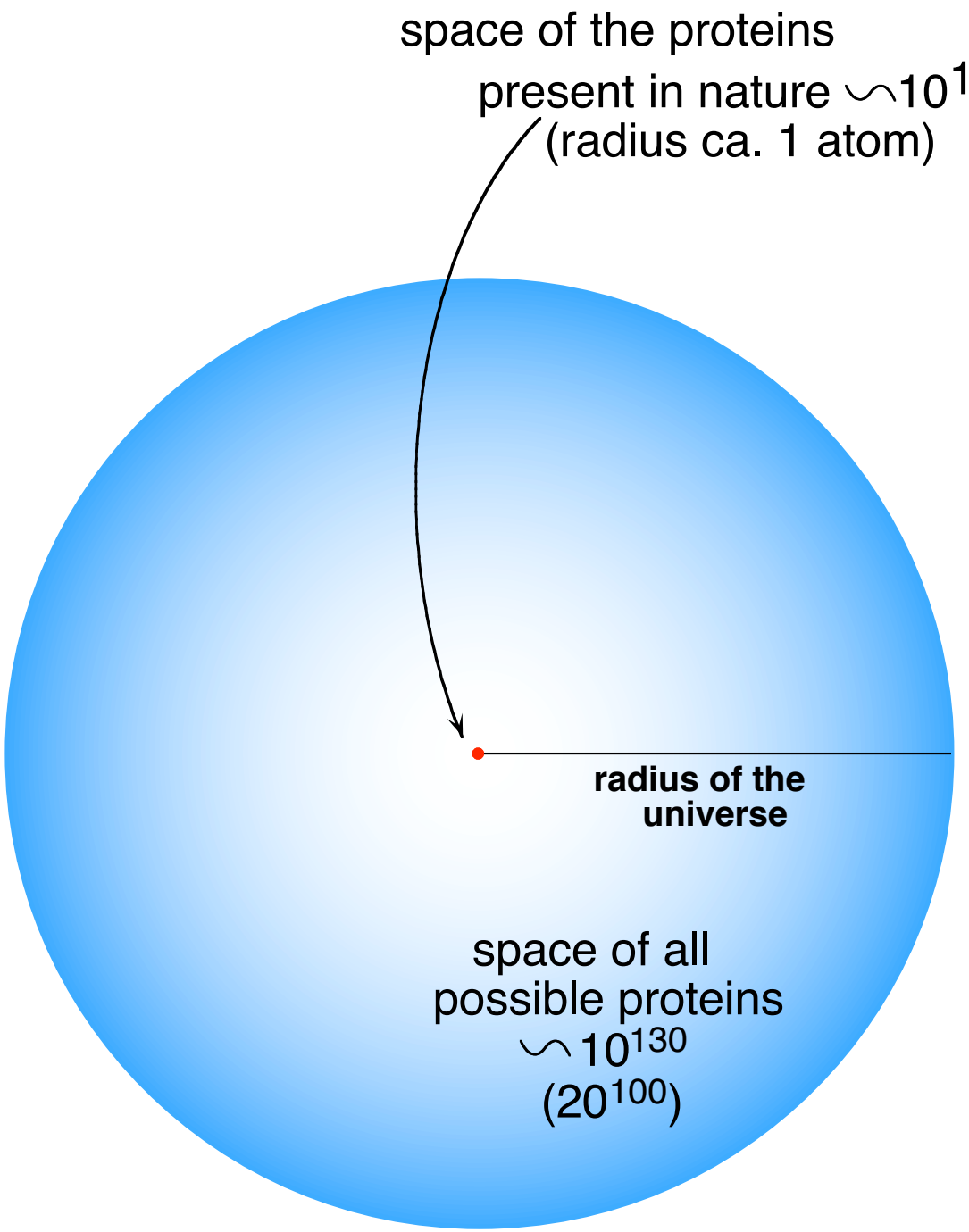
Calculate: How many different macromolecules can you build, when n = 60 and i = 1 - 20



$20 \times 20 \times 20 \times \ldots\ldots \quad N = 20^{60} \simeq 10^{70}$!!!



In nature there are only $10^{12} - 10^{14}$ proteins


space of the proteins
present in nature ~10^{10}
(radius ca. 1 atom)
radius of the
universe
space of all
possible proteins
~10^{130}
(20^{100})

Cast the dice again and.....

And you will get a different set
of macromolecules
that do not necessarily support life

QUESTION:

ARE THE PROTEINS OF LIFE THE ONLY ONES THAT COULD BE FORMED- AND GAVE ORIGIN TO LIFE BY A DETERMINISTIC (OBLIGATORY) SERIES OF EVENTS

OR

ARE THEY THE PRODUCT OF CONTINGENCY(CHANCE) AND LIFE IS ALSO A PRODUCT OF CONTINGENCY?

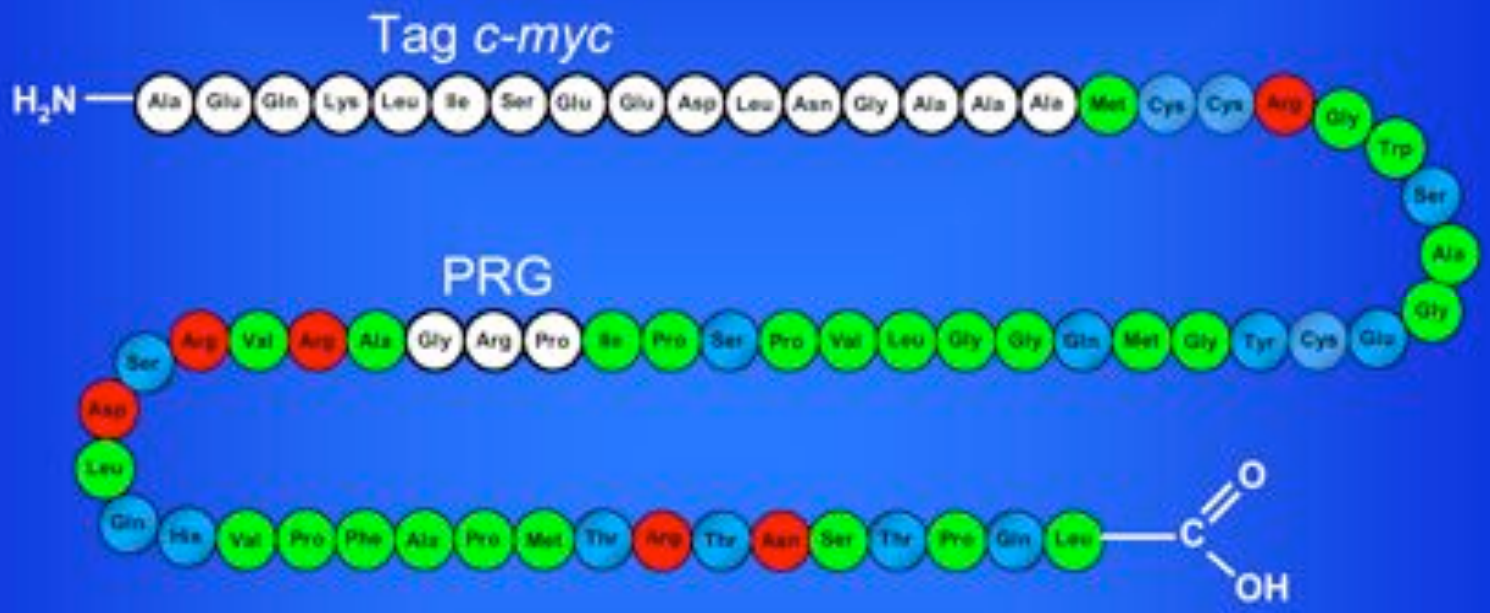
PROJECT RANDOM POLYPEPTIDES:

MAKE A VASTE LIBRARY OF RANDOM DE NOVO PROTEINS

ASK THE QUESTION: HOW MANY OF THEM WILL BE FOLDED?

Folding is the prerequisiste for any intelligent function by proteins

Analisi della sequenza primaria della proteina 1



aa apolari

aa carichi

aa polari

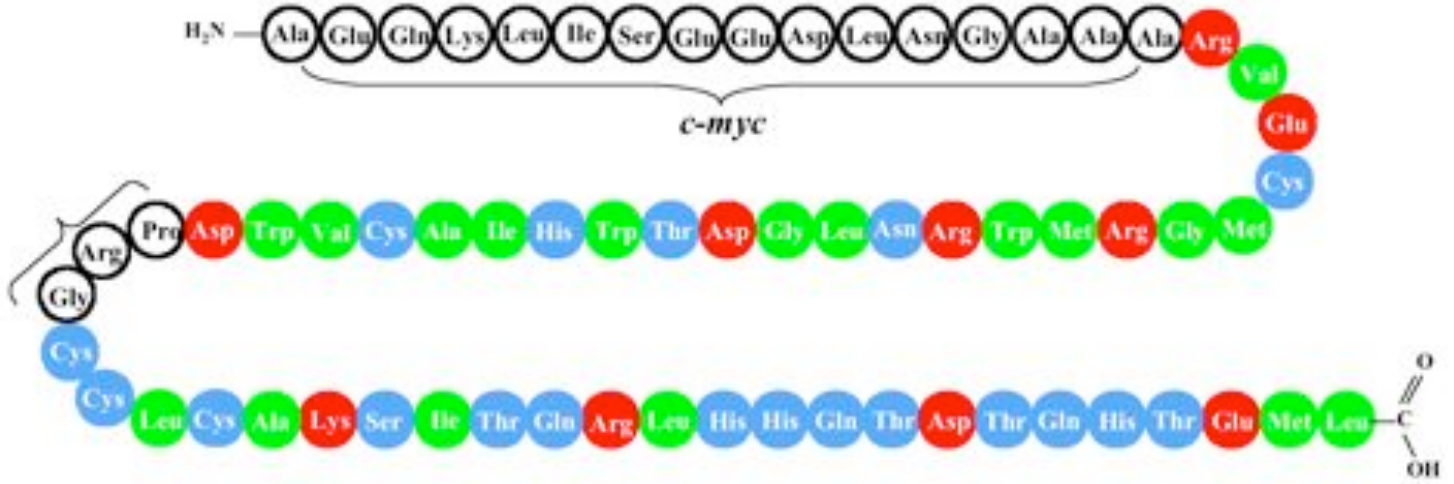
55% amminoacidi apolari

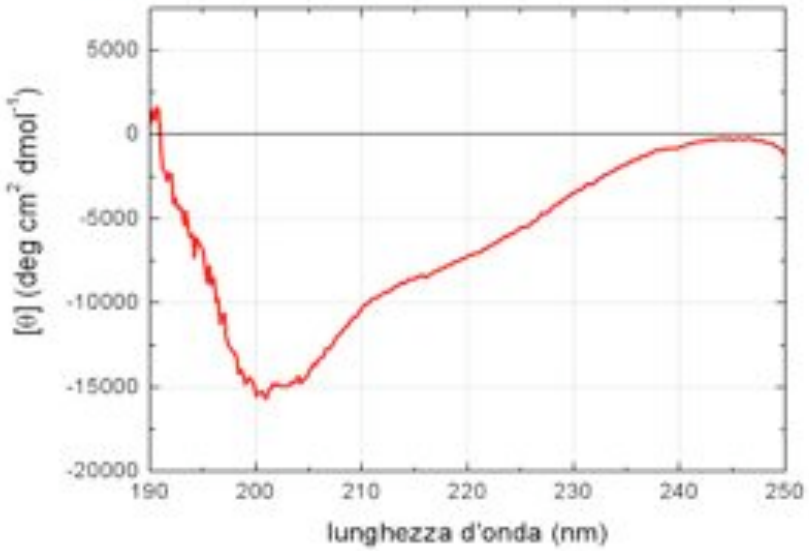
13% amminoacidi carichi

32% amminoacidi polari

Ester Papa

SEQUENZA PRIMARIA DEL POLIPEPTIDE 127

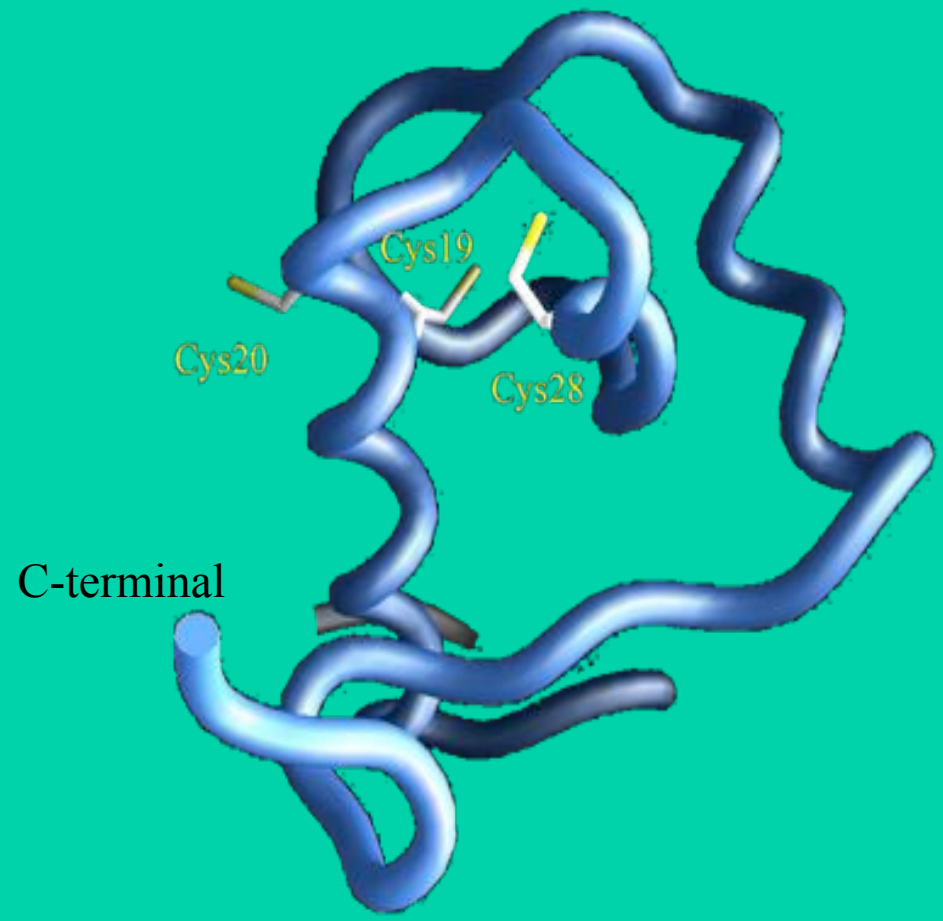




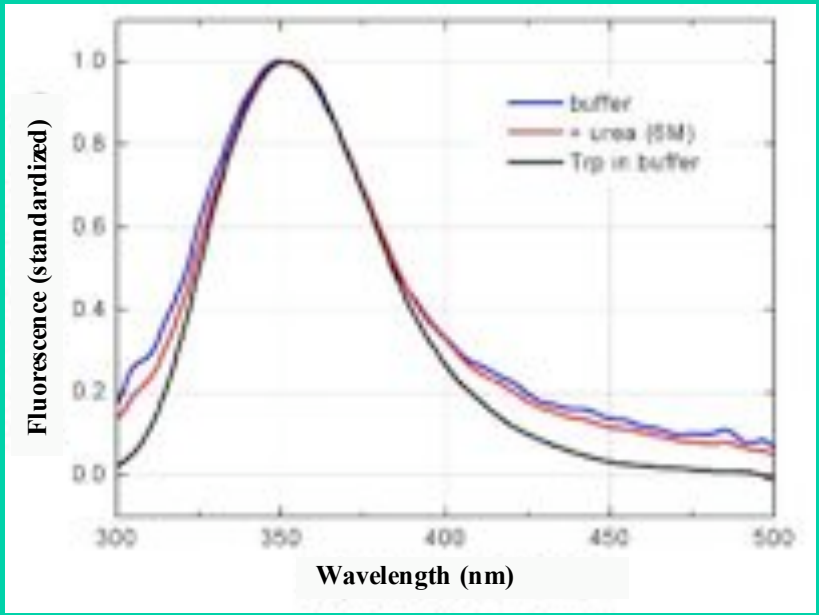
Buffer glicina 5 mM, pH = 2,5

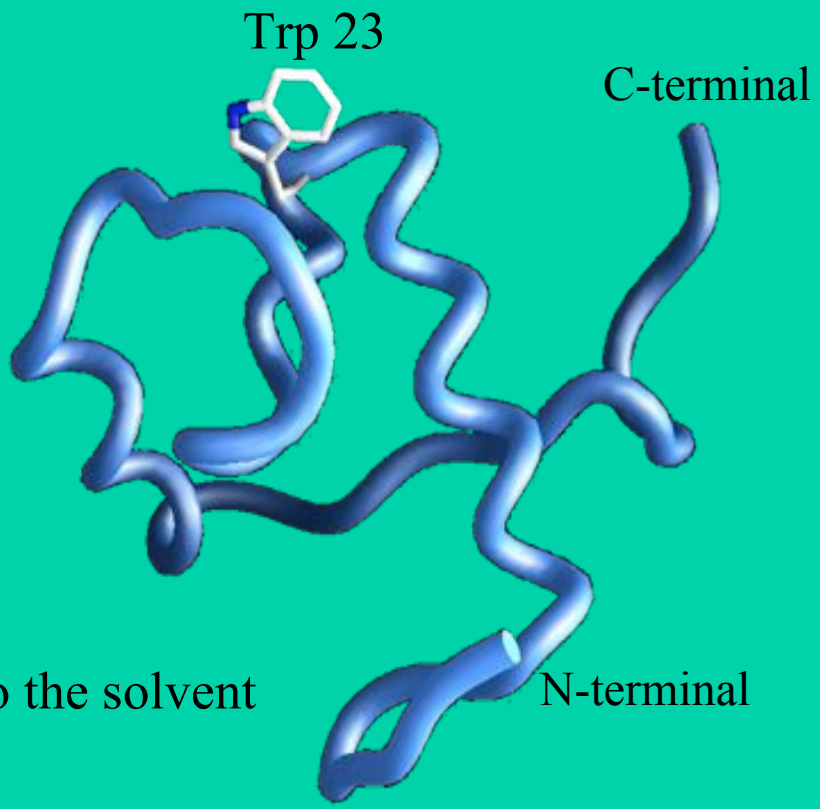
Tiziana Ottone

TRIDIMENTIONAL STRUCTURE PREDICTION
Cys residues



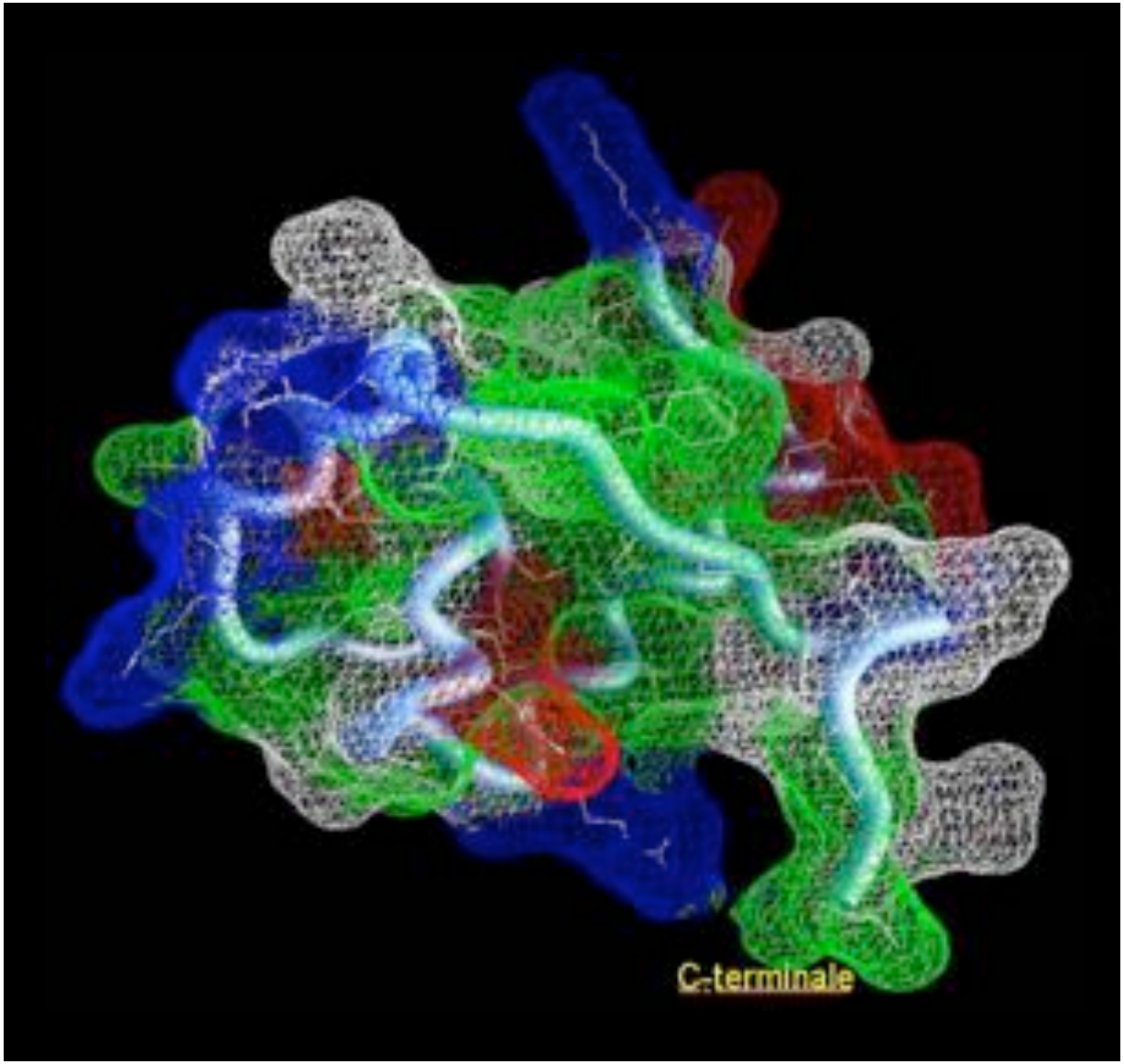
FLUORESCENCE STUDIES





- The Trp residue could be exposed to the solvent

TRIDIMENTIONAL STRUCTURE PREDICTION



Globular Structure:

3 α-Helices:

1. Res. 9 → 22
2. Res. 27 →30
3. Res. 42 → 52 (PRG)

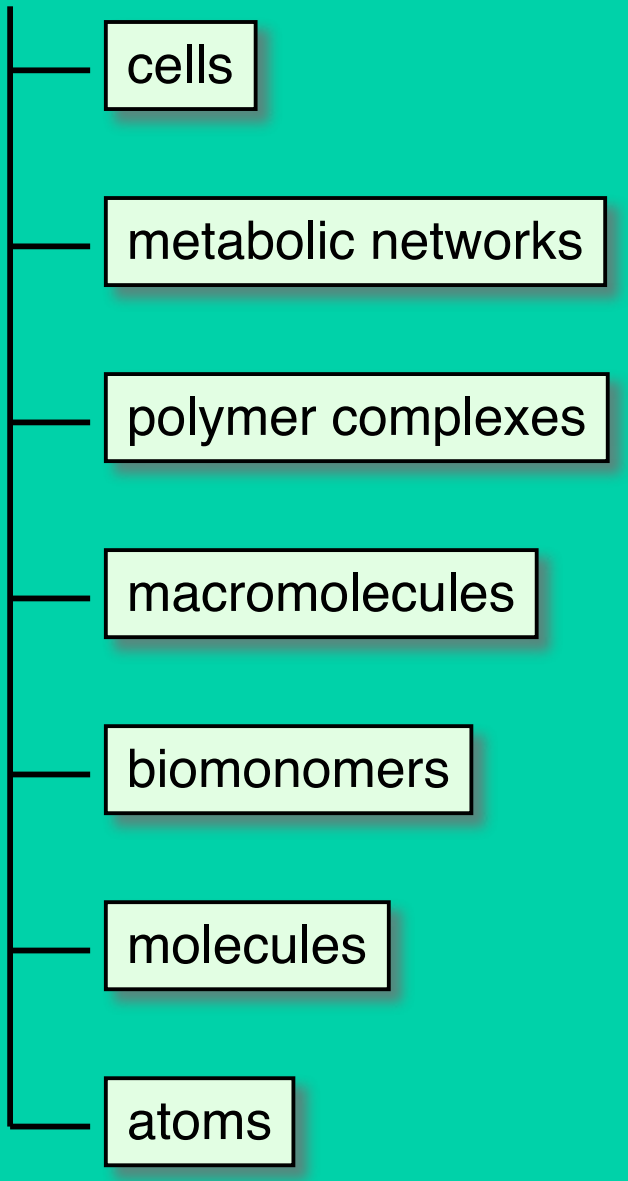
Tot. α-Helix ~ 40%

: no polar zones

: polar zones

: + residues

: - residues


cells
metabolic networks
polymer complexes
macromolecules
biomonomers
molecules
atoms

What is life?

THE INTERACTION WITH BUDDHISM:

THE NOTION OF BEGINNING-LESSNESS

.....and the notion of dependent origination

TWO SCHOOLS OF THOUGHT

....AND TWO DIFFERENT EXPERIMENTAL APPROACHES

1. THE CELLULAR VIEW:

YOU NEED A BOUNDARY (SEMIPERMEABLE MEMBRANE)

IN ORDER TO ACHIEVE THE NECESSARY SPATIAL ORGANIZATION,

LOCAL CONCENTRATIONS, , PROTECTION AND NUTRIENT SELECTION

2. THE MOLECULAR REPLICATION VIEW:

ALL WHAT YOU NEED IS A MOLECULAR SPECIES

(E:G: A RNA QUASI-SPECIES) WHICH IS ABLE TO SELF-REPLICATE

AND MUTATE (EVOLVE) IN THE PROCESS

– – – – – – – – – – – – – – – – – – – -

THESE TWO VIEWS ARE OF COURSE NOT CONTRACDITORY,
ACUALLY THEY ARE COMPLEMENTARY TO EACH OTHER

THE RNA-WORLD

IN THE ORIGIN OF LIFE

Mg-TRANSFER-RNA

THE BASIS OF THE RNA-WORLD

RNA IS THE PRIME MOLECULE
CAME INTO EXISTENCE
BEFORE PROTEINS AND DNA
AND ORIGINATED THE WHOLE
THING

RNA → RIBOZYMES → PROTEIC ENZYMES → DNA

THE BASIS OF THE RNA-WORLD

RNA IS THE PRIME MOLECULE
CAME INTO EXISTENCE BEFORE PROTEINS AND DNA
AND ORIGINATED THE WHOLE THING

RNA ⟶ RIBOZYMES ⟶ PROTEIC ENZYMES ⟶ DNA

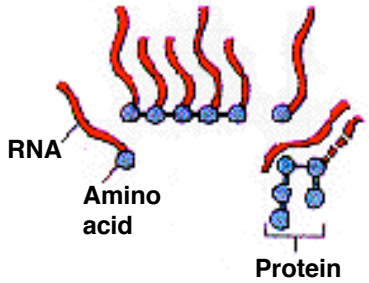
BUT
WHO (WHAT) MADE RNA ?

TO CONVALIDATE THE ABOVE VIEW
ONE SHOULD SHOW THAT RNA (IN PARTICULAR RIBOZYMES)
CAN COME TO EXISTENCE SPONTANEOUSLY
WITHOUT THE ASSISTENCE OF ENZYMES AND
OF PRE-ADDED TEMPLATE RNA

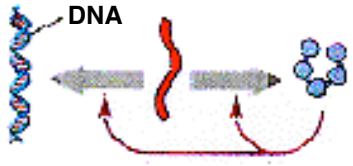
.....THIS HAS NOT BEEN ARCHIVED YET

[A] RNA forms

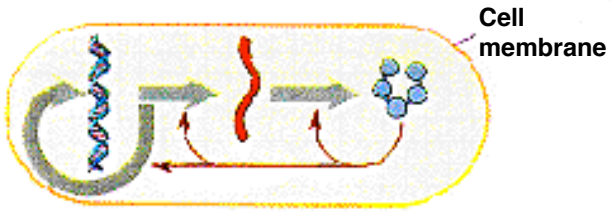
[B] Ribozymes catalyze RNA replication



[C] RNA catalyzes protein synthesis



[D] RNA encodes both DNA and protein



[E] Proteins catalyze cell activities

The Origins of life in a Proposed RNA World

[**A**] Organic subunits could have combined and formed RNA molecules. [**B**] RNA molecules could have acted as ribozymes, catalyzing their own replication. [**C**] RNA molecules could also have catalyzed the synthesis of protein, which in turn stabilized RNA molecules and catalyzed RNA replication. [**D**] DNAs could have been copied from RNA molecules, and at some point, proteins may have begun to catalyze the synthesis of more proteins from information in RNA. [**E**] DNA assumed an information storage role, while RNA continued to be involved in protein synthesis; a cell membrane also appeared.

" The Nature of Life "
Postlethwait J.H., Hopson J.L. (1995)
Mc Graw-Hill Inc. New York

HOW DO YOU PROCEED EXPERIMENTALLY

FOR CONSTRUCTING IN THE LABORATORY

A LIVING CELL MODEL?

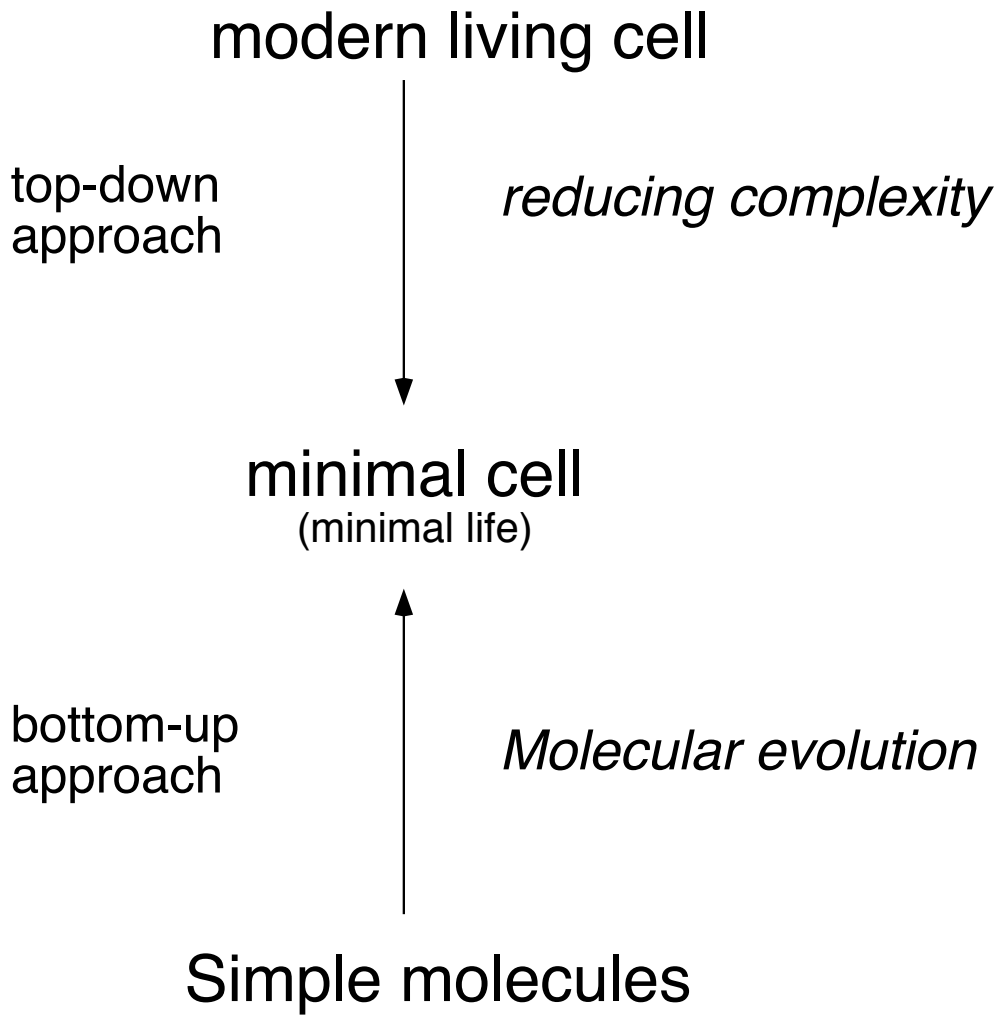
towards an operational definition of minimal life

- at which level of molecular complexity does the quality emerge, which one can call life?

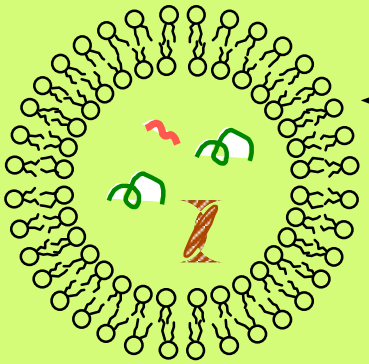
- what are the minimal and sufficient conditions for a physical system to have "life"?

- which is the simplest possible synthetic chemical equivalent of a living cell?

two working directions



the notion of the minimal cell:

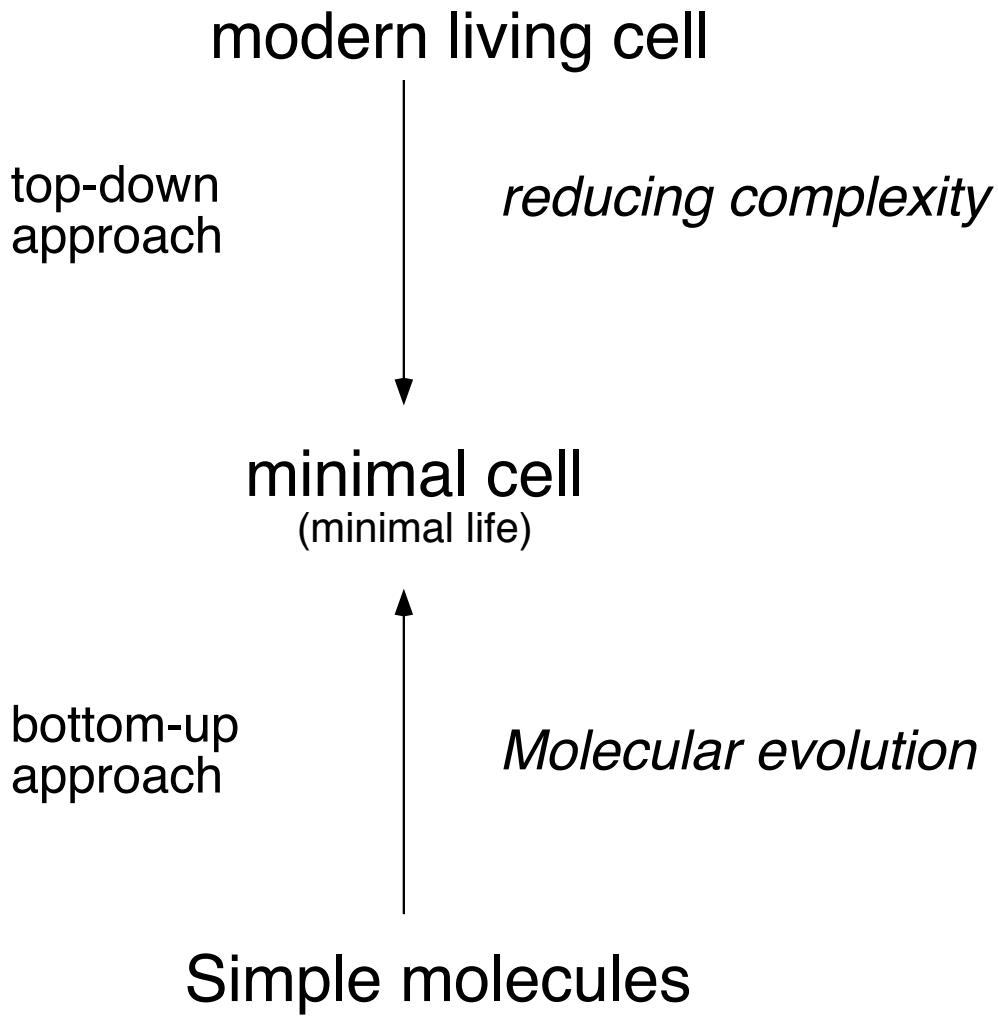


lipidic bilayer

containing the minimal and sufficient number of components to be "alive"

alive → self-maintenance
alive → reproduction
alive → mutation

two working directions



towards an operational definition of minimal life

- at which level of molecular complexity does the quality emerge, which one can call life?

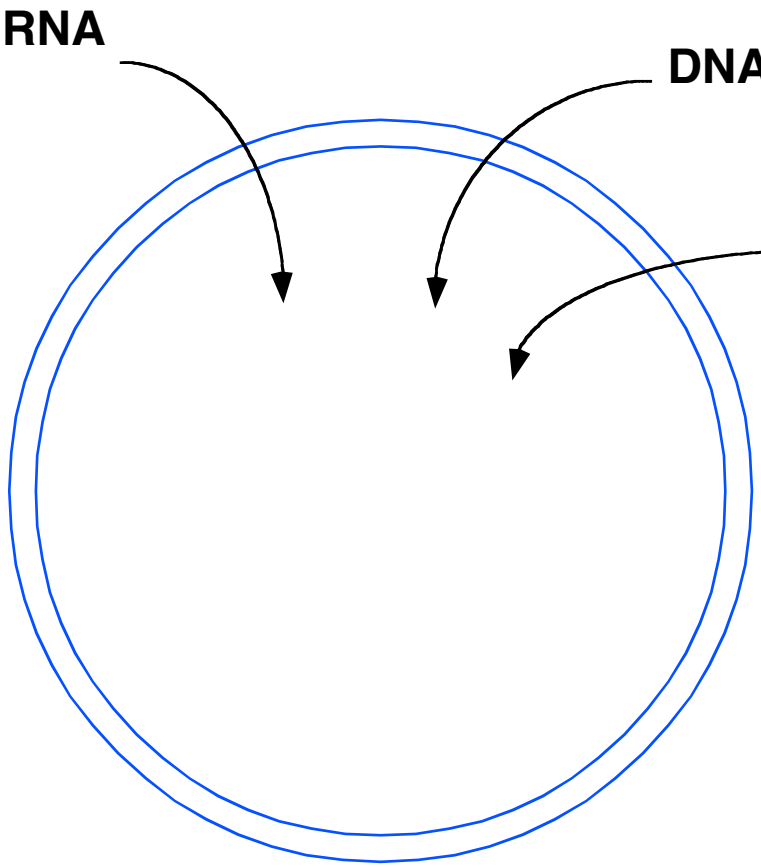
- what are the minimal and sufficient conditions for a physical system to have "life"?

- which is the simplest possible synthetic chemical equivalent of a living cell?

However, the reconstitution of life by the bottom-up approach is made difficult or impossible by the laws of contingency :the problem of re-making our present macromolecular sequences

..you can make different ones, and show that in principle this pathway is possible

TOP-DOWN APPROACH
TO THE MINIMAL CELL



(allowed...)

...the minimal artificial
(semi-artficial?) form of life.

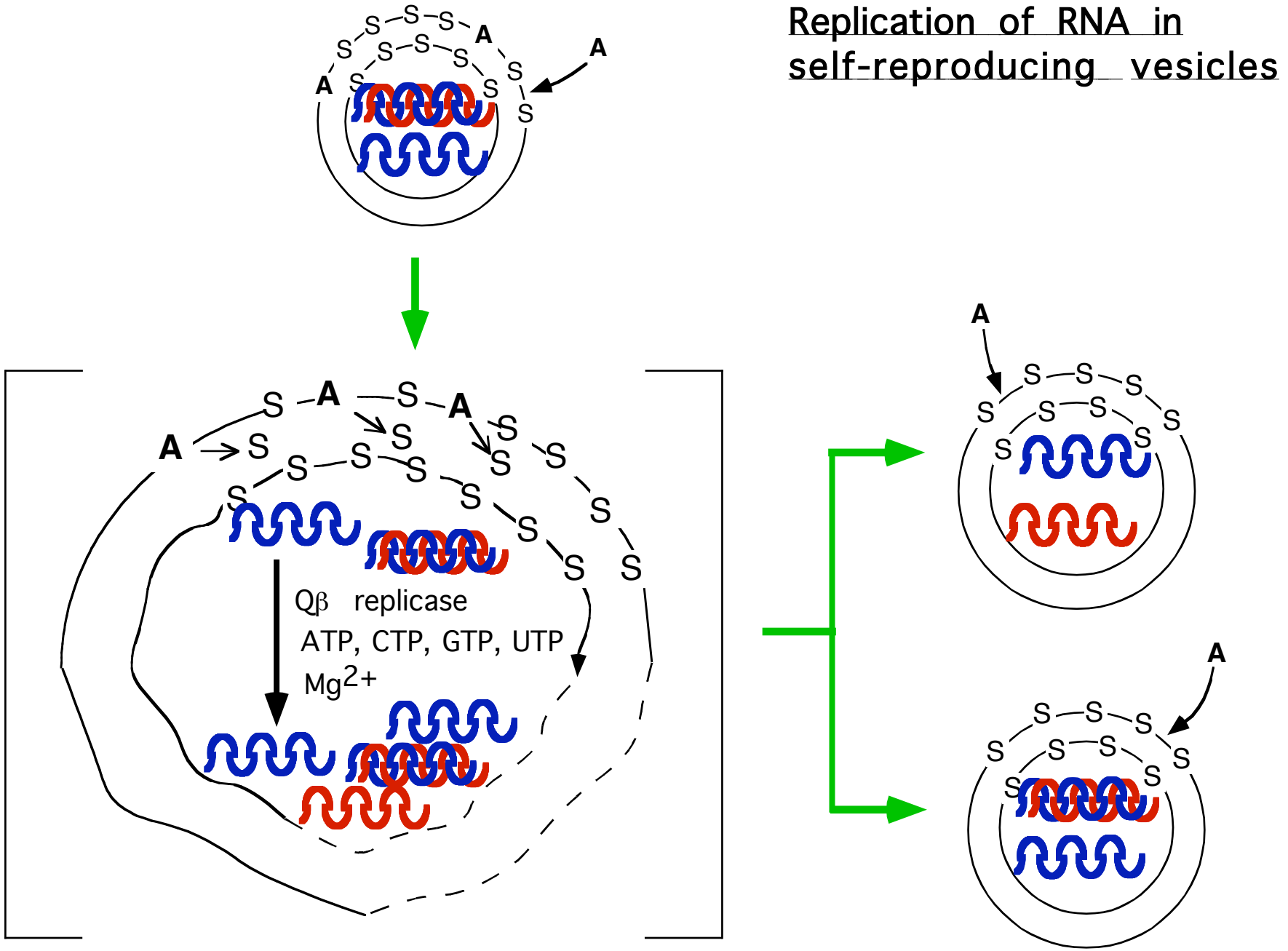
SHELL AND CORE

SELF-REPRODUCTION

SHELL AND CORE

…*UNCOUPLED...*

Replication of RNA in self-reproducing vesicles



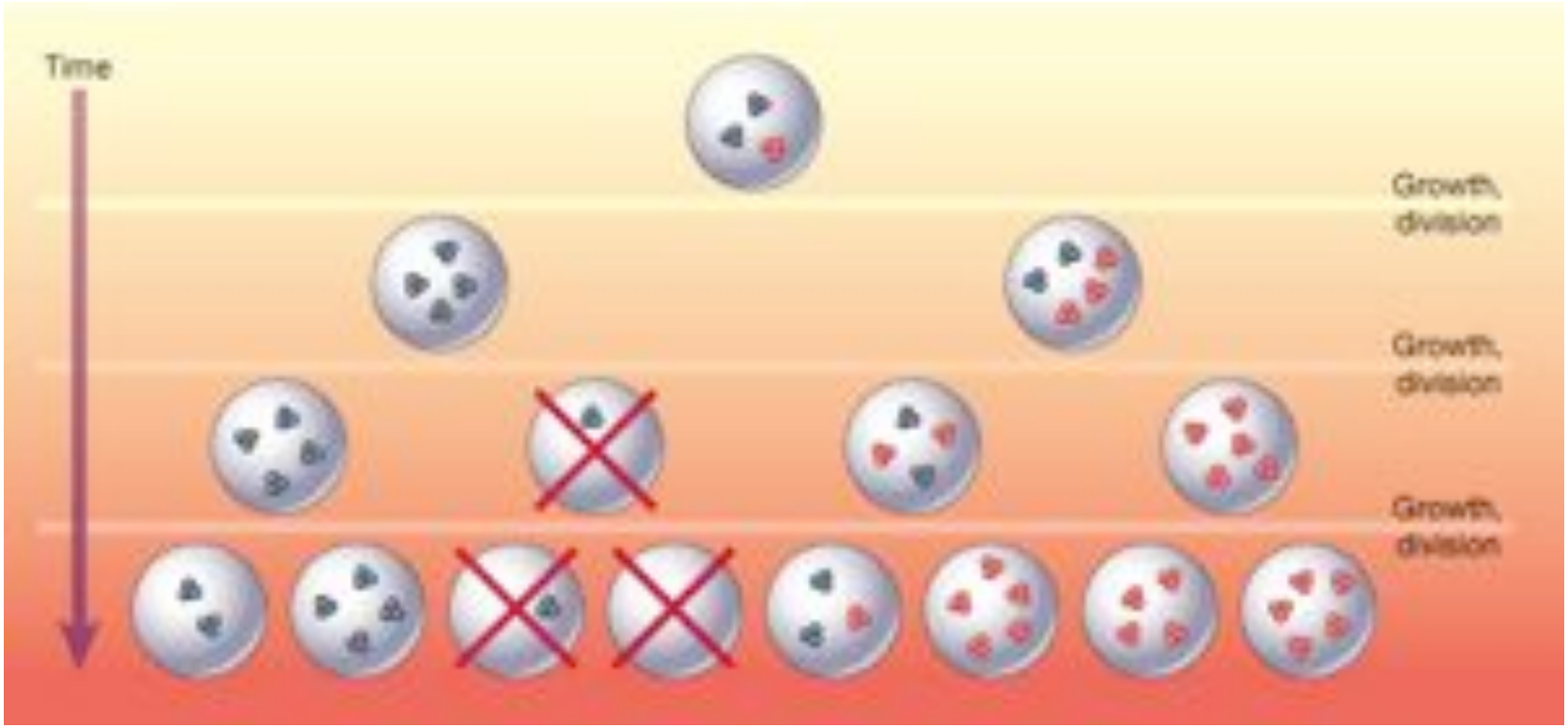
Is this life?

Not really.
With progressive
generations, the active components
are diluted out
because they are not fabricated
by the compartment itself

Nature 409, 387 - 390 (2001)

Synthesizing life

JACK W. SZOSTAK, DAVID P. BARTEL & P. LUIGI LUISI



The work on the minimal living cell
must be accompanied by studies on the
early cells - namely the protocells at
the time before the advent of ribosomes
and before the high selectivity
of modern times —

This would eliminate the 55 ca. genes of the ribosomal proteins
and would reduce the 20 t-RNA genes to a much lower number

→ DOWN TO a final 20-25 GENES??

AS A WAY OF CONCLUSION.1

CONCERNING THE TRANSITION TO LIFE FROM THE INANIMATE MATTER:

1. IT HAS NOT BEEN IMPLEMENTED IN THE LAB YET. THEREFORE, IT REMAINS AN HYPOTHESIS.
AND THE BOTTOM UP APPROACH SEEMS TO BE MADE IMPOSSIBLE BY THE LAWS OF CONTINGENCY- CONCEPTUALLY AND EXPERIMENTALLY

2.THE CONSTRUCTION OF SYNTHETIC LIVING CELLS APPEARS POSSIBLE USING EXTANT MACROMOLECULES.
MOST SCIENTISTS BELIEVE, THAT „SOON“,
THIS WILL BE REALIZED.

THE NEW RESEARCH AREA ON THE MINIMAL CELLS INTERESTS NOW ABOUT ONE DOZEN GROUPS AROUND THE WORLD.

WHY IS THIS RESEARCH RELEVANT?

1. UNDERSTANDING THE CHEMICAL ESSENCE OF LIFE BY RECONSTRUCTING IT IN THE LAB

2. UNDERSTANDING OF THE EARLY CELLS

3.BIOTECHNOLOGICAL RELEVANCE (E.G., PROTEINS SYNTHESIS WITH SIMPLE LIPOSOME SYSTEMS

- How far is it master?

- That is irrelevant. Hold your tongue and walk!

Parole/domande chiave su cui riflettere:

ORIGINE DELLA VITA DALLA MATERIA INANIMATA

DETERMINISMO/CONTINGENZA

VIVIAMO DI UN „ATOMO“ DI PROTEINE

SIAMO SOLI NELL‘UNIVERSO?

ANCHE IL VIVENTE CONSISTE SOLO DI ATOMI

CREAZIONISMO

MOVIMENTO CRIPTO-CREAZIONISTA

COSE PER I PROSSIMI GIORNI:

CHIMICA PREBIOTICA

AUTO-ORGANIZZAZIONE

PROPRIETA‘ EMERGENTI

AUTO-RIPRODUZIONE

VITA ARTIFICIALE

COS‘Ê VITA?

VITA E COGNIZIONE

Questions to the reader chapter one

1. Do you accept the view that life on Earth originated from inanimate matter without any contribution from transcendent power?

2. Do you accept the idea that biological evolution is mostly shaped by contingency? If not, what would you add to this picture?

3. Are you at peace with the idea that mankind might not have existed; and with the idea that we may be alone in the universe?

4. Do you accept the idea that the living is made up only by molecules and nothing else?