

From the emergence of life to the emergence of consciousness

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- 1.: LIFE FROM THE INANIMATE MATTER
- 2.: SOME PHILOSOPHICAL QUESTIONS
- 3.: THE SYNTHETIC BIOLOGY OF NEVER BORN PROTEINS
4. THE SYNTHETIC BIOLOGY OF MINIMAL LIFE
- 5.: FROM CELL BIOLOGY TO COGNITION



The narrative of the BigBang

- 13.7 billion years ago: BB and creation of time, space, heat, laws of nature
- After 1/100 sec.: t ca. 100 million C, density 4 billion more than water density

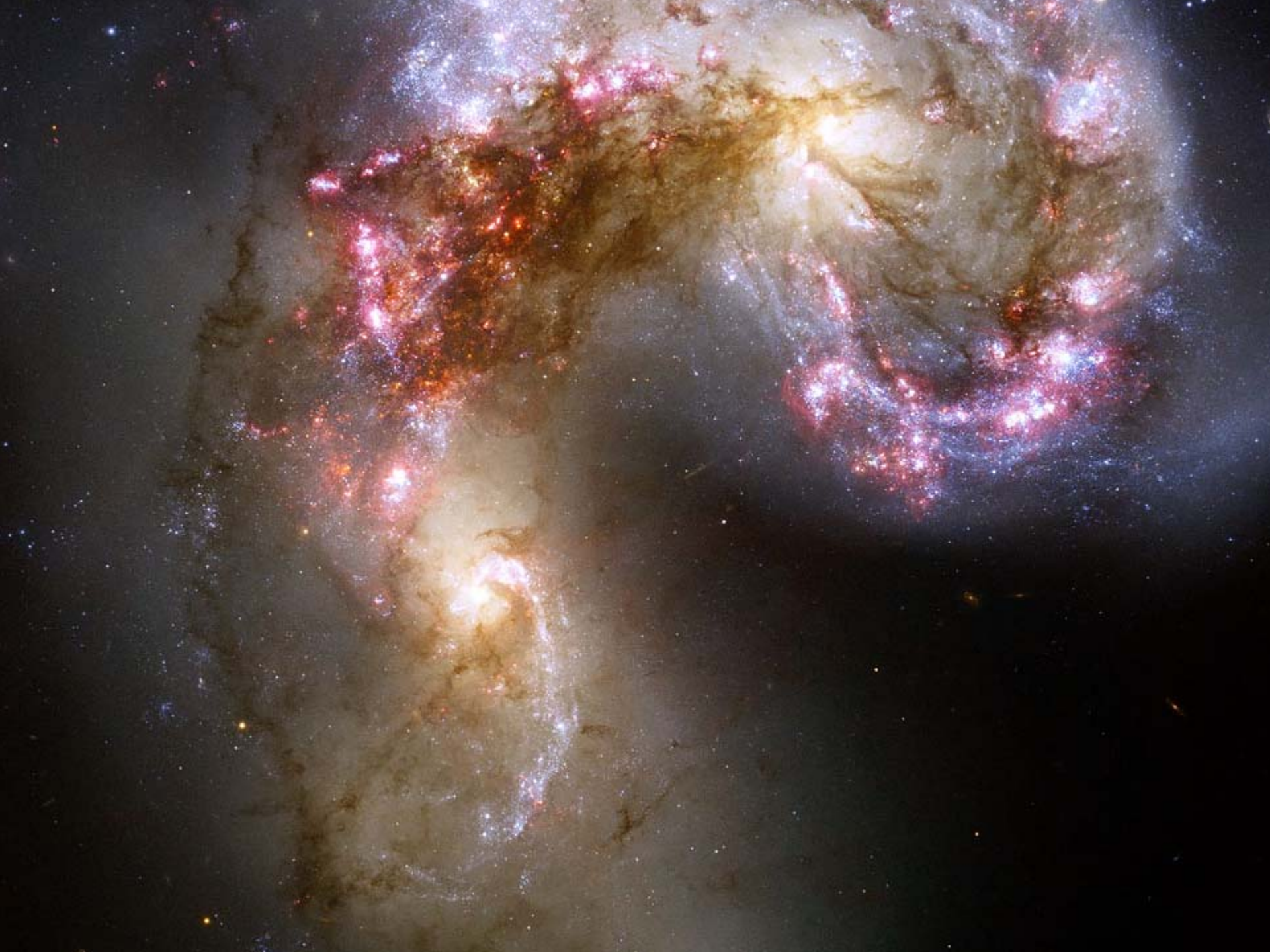
After 3.6 min: $t = 30$ million C, and formation of protons and neutrons and then formation of Hydrogen and Helium atoms, ($\frac{3}{4}$ and $\frac{1}{4}$, as ca. today)

After 300-400 million years: H and He build gigantic cold clouds with high density

As temp. goes down to -170 C, these clouds collapse, forming the first galaxies and stars







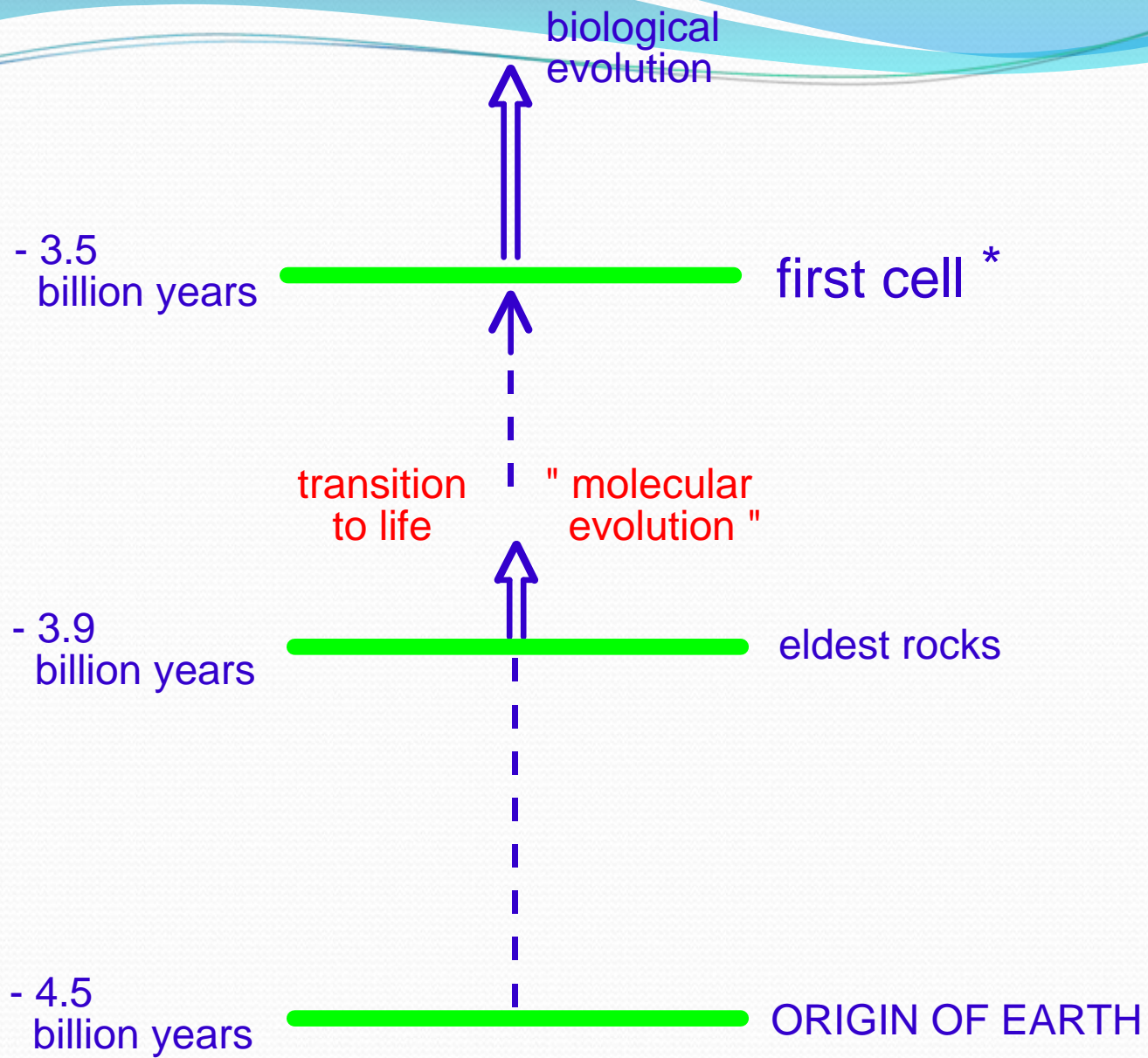
- „„„„„HEIDEGGER
- And his crucial question:
- **Why there is something instead of nothing?**

...the narrative of the BigBang

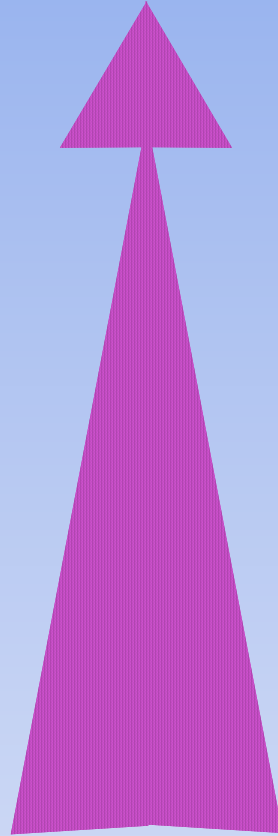
- 5 billions years ago: formation of “our” mother cloud, from which our solar system
- Inside the stars, nuclear fusion of He and H produces the first heavy atoms, C, O, N, Si, Fe
- And then the first molecules, CO, ammonia, water, CS
- From these particles, formation of the “planetesimals”, which eventually forms planets







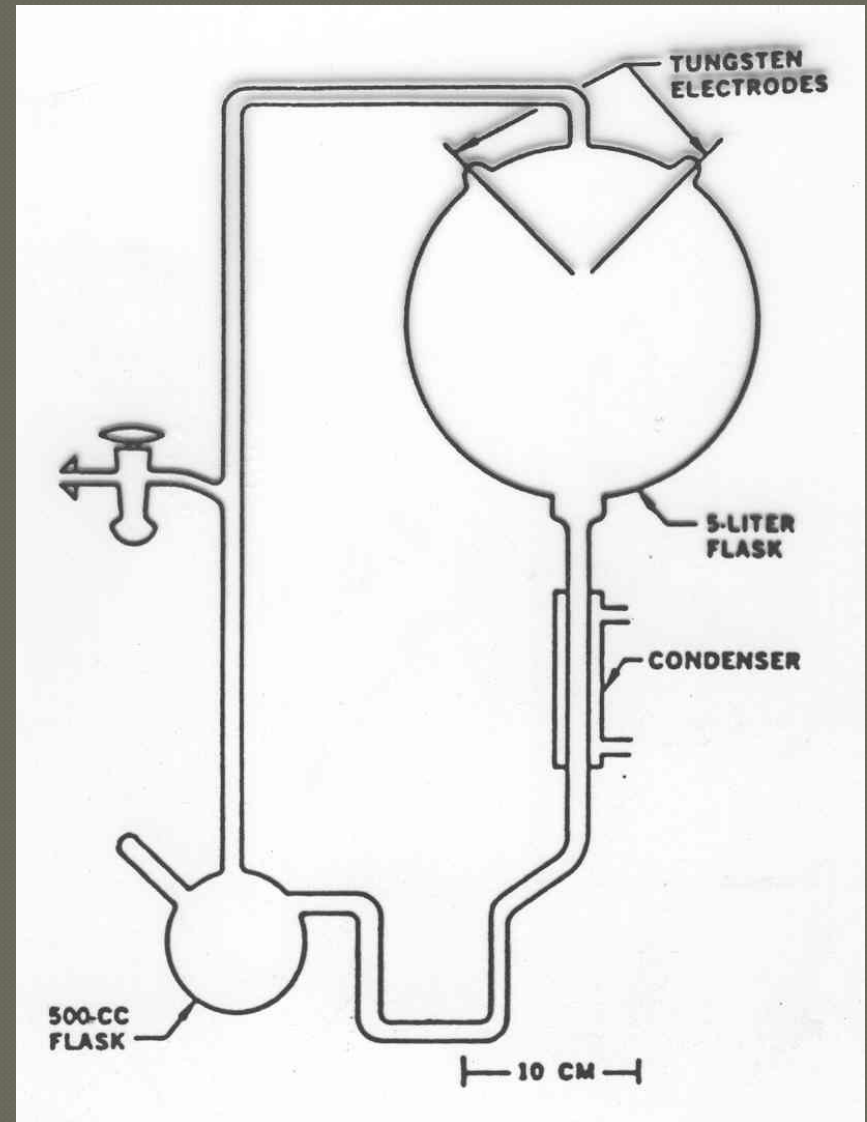
LIFE

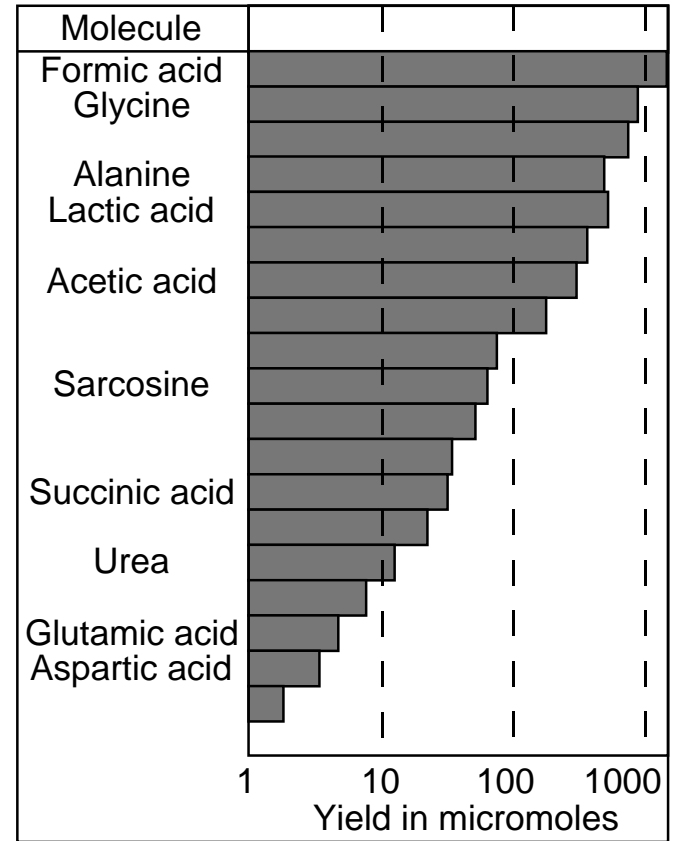
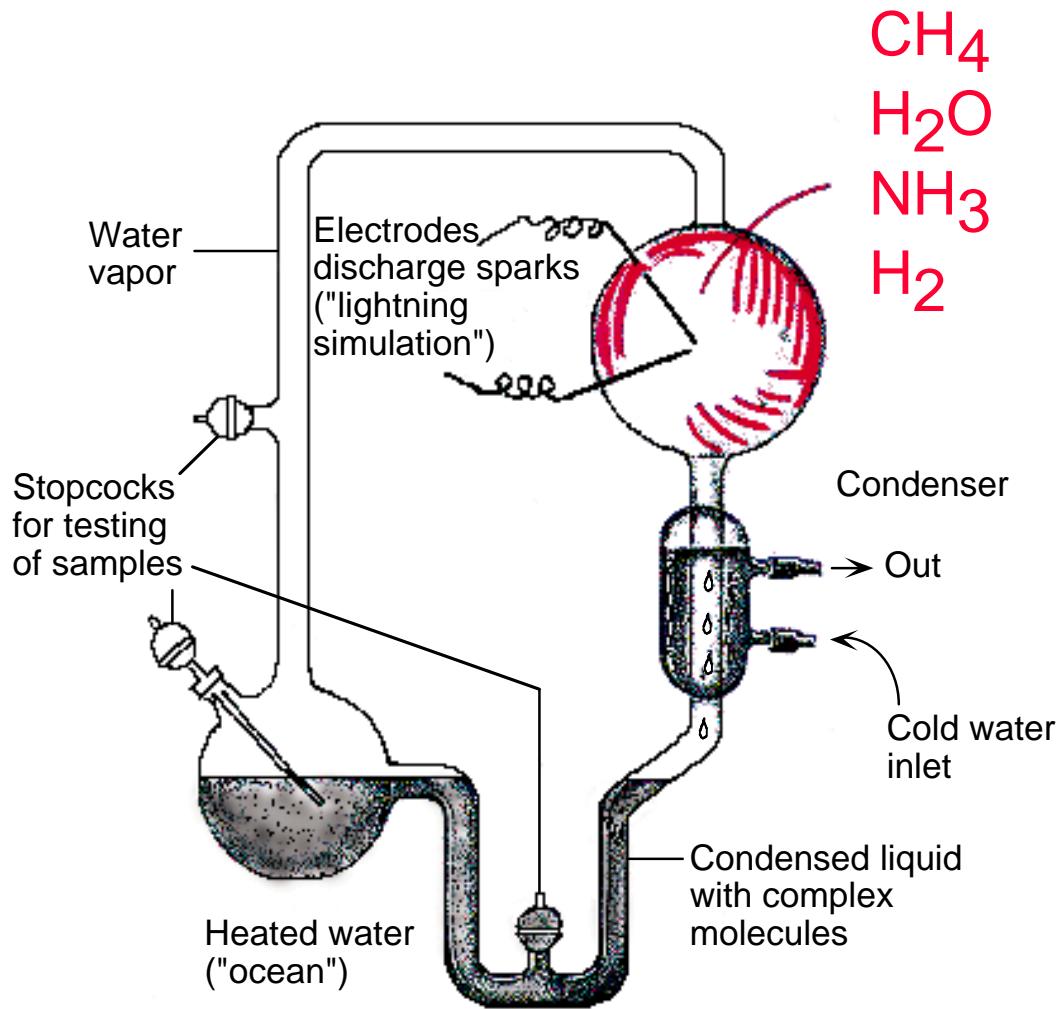


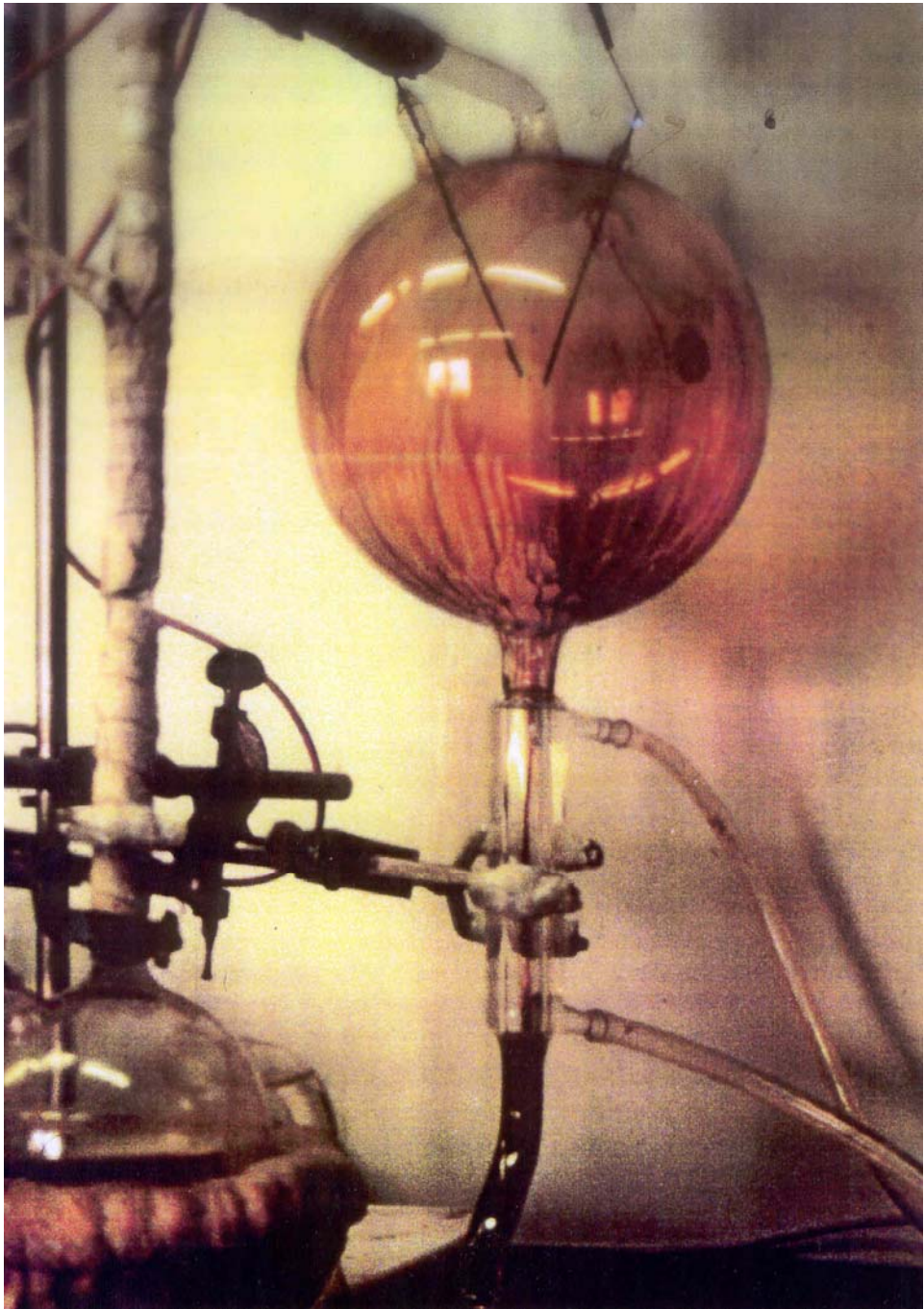
INANIMATE MATTER
(NON - LIFE)

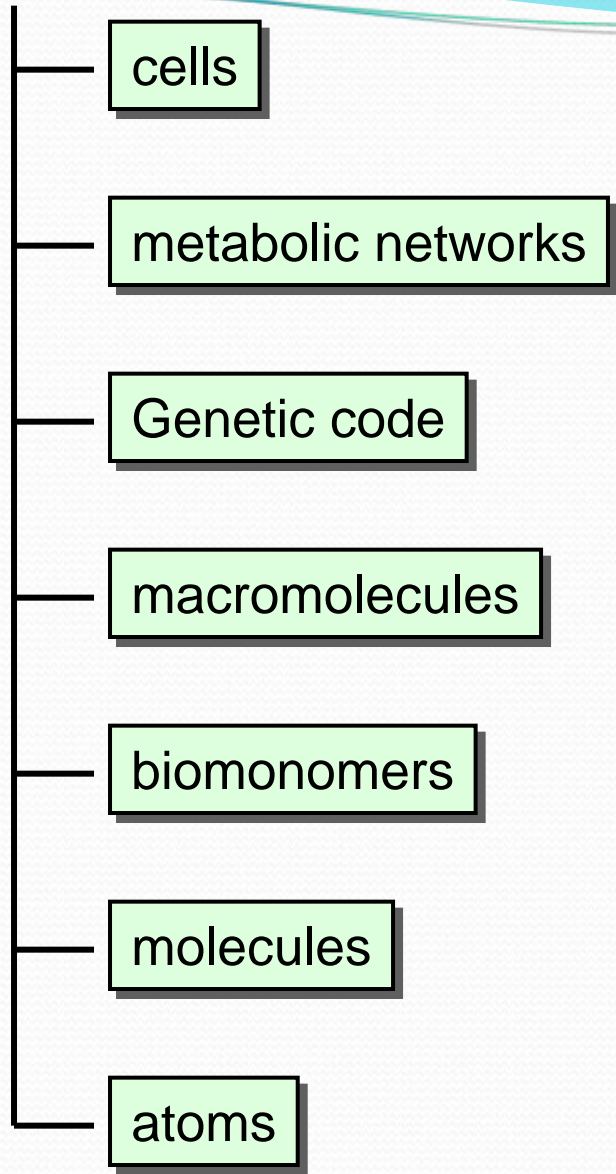


The 1953 Miller experiment







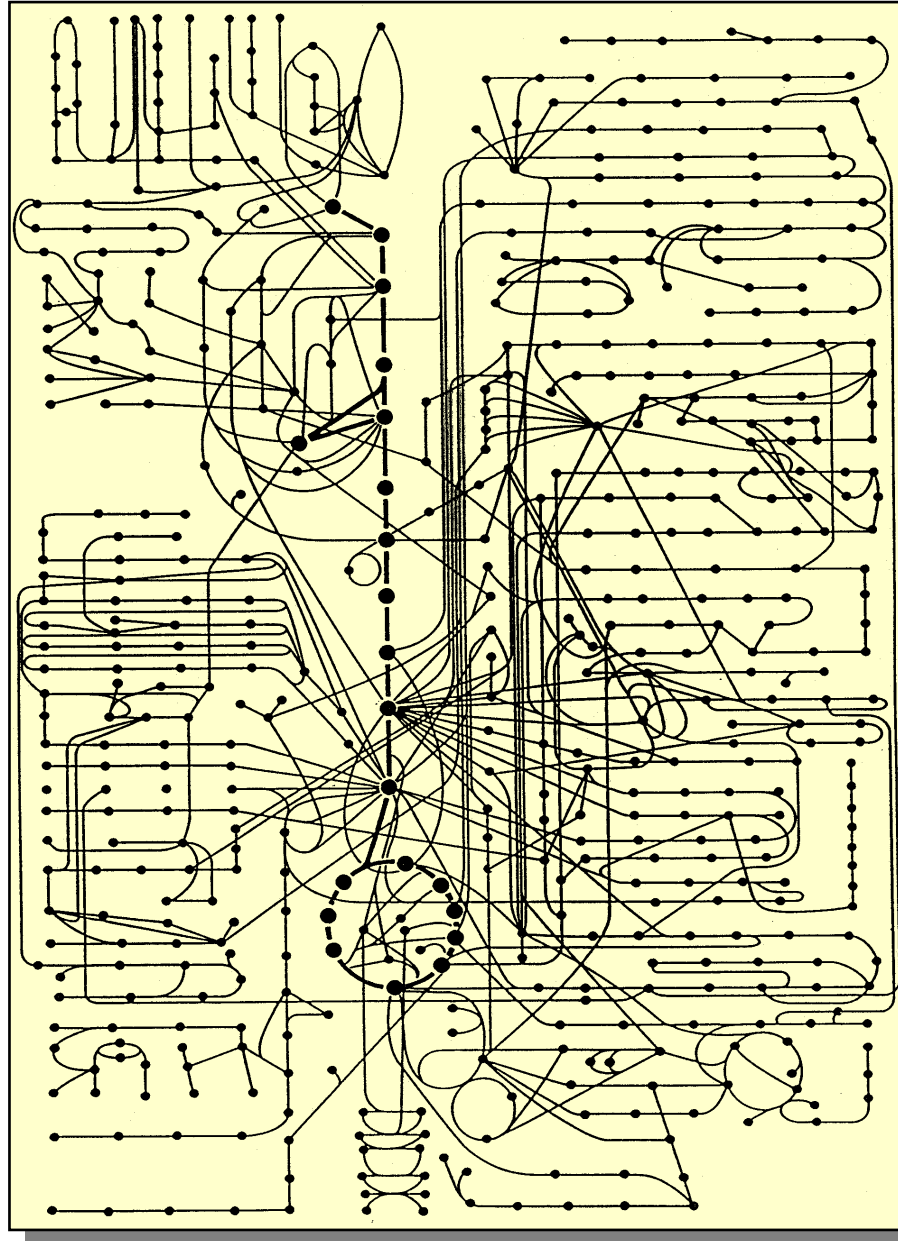


WHAT IS LIFE?

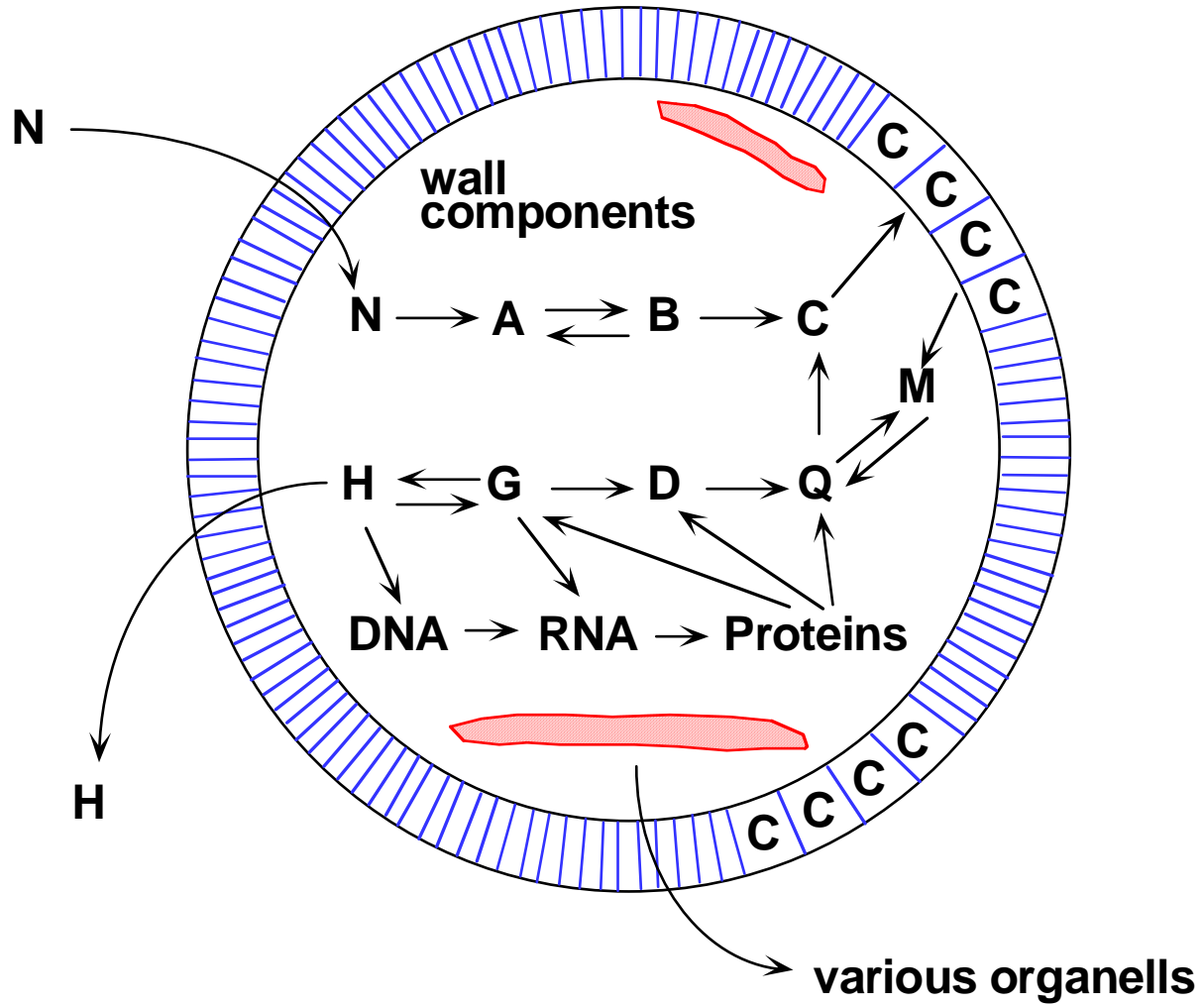
-A PHENOMENOLOGICAL
APPROACH



A maze illustrating the chemical reactions that interconvert small molecules in cells.



NUTRIENTS / ENERGY



First phenomenological observation

THE CELL'S MAIN ACTIVITY IS
TO MAINTAIN ITS OWN IDENTITY
(SELF-MAINTENANCE)
IN FACE OF THE ENORMOUS NUMBER
OF TRANSFORMATIONS;
AND THE CELL DOES SO
THANKS TO A PROCESS OF
RE-GENERATION FROM WITHIN

Second phenomenological observation:

- Cellular life (life in general) is a global, distributed quality. Is not due to any particular “centre”, any particular compound or any particular reaction.

...in other words,

The living cell is defined by its organization, and therefore life must be interpreted in terms of relations among the components rather than in terms of the properties of the components

These concepts are the basis of
the theory of **autopoiesis**
developed by Maturana and Varela
since 1974

..the „Santiago School“

See „The tree of knowledge“ by Maturana and Varela

**LIVING SYSTEMS TRANSFORM INSIDE THEMSELVES
MATTER, IN SUCH A WAY THAT THE PRODUCT IS THEIR
OWN ORGANIZATION**

Maturana and Varela

When you regard a living system you always find a network of processes or molecules that interact in such a way as to produce the very network that produced them and that determine its boundary. Such a network I call autopoietic.

MATURANA, 2004

*Whenever you encounter a network whose operations eventually produce itself as a result, you are facing an autopoietic system. It produces itself. The system is **open** to the input of matter but **closed** with regard to the dynamics of the relations that generate it.”*

MATURANA 2004

Important in autopoiesis:

Relationship between structure and self-organization

Self-organization is the invariant property, the relationship between the processes that produce the components

The **structure** may be contingent, and may vary depending on evolution and other perturbations

.

Note: the two things are inseparable, there cannot be the one without the other in a living system

**self-maintenance from within,
due to a dynamic network of
interactions,
which are defined and
constructed
by the system itself"**

The cellular „definition“ of life:

A system spatially defined by a boundary of its own making- and which is self-sustaining by re-generating the system's components from the inside

SECOND PART:

SOME PHYLOSOPHICAL QUESTIONS

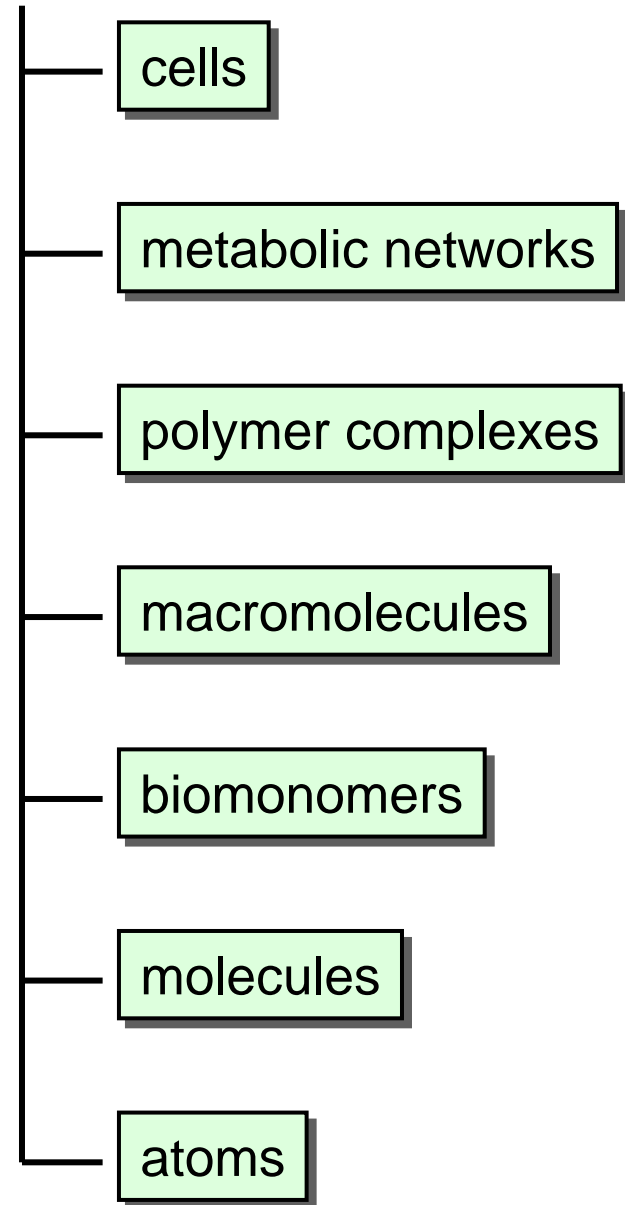
Phylosophical questions:

Is the advent of life on Earth

a)An obligatory process?

b)Or a chance event?

**DETERMINISM VERSUS
CONTINGENCY
IN LIFE AND EVOLUTION**



... "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."

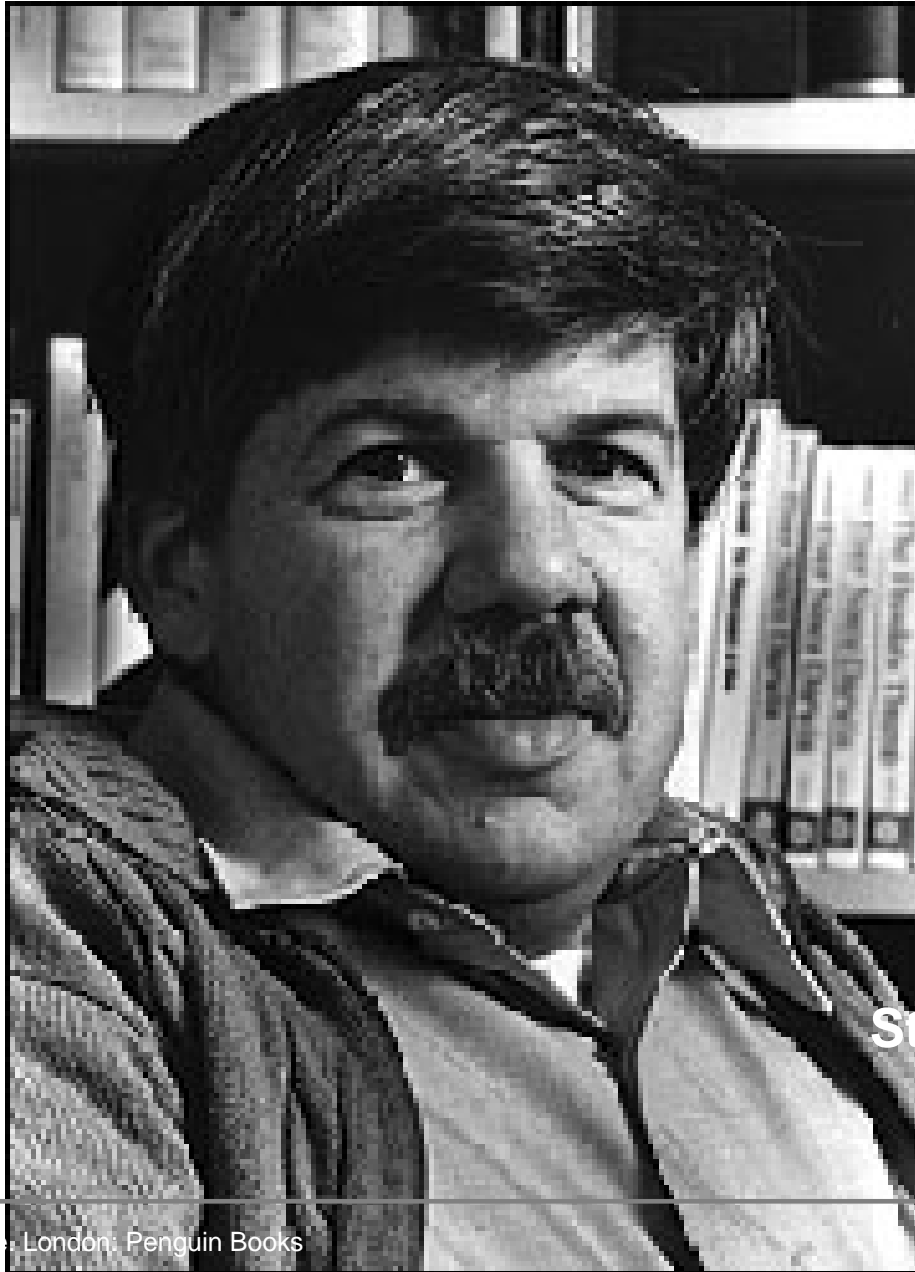
Morowitz, 1991

"..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..."

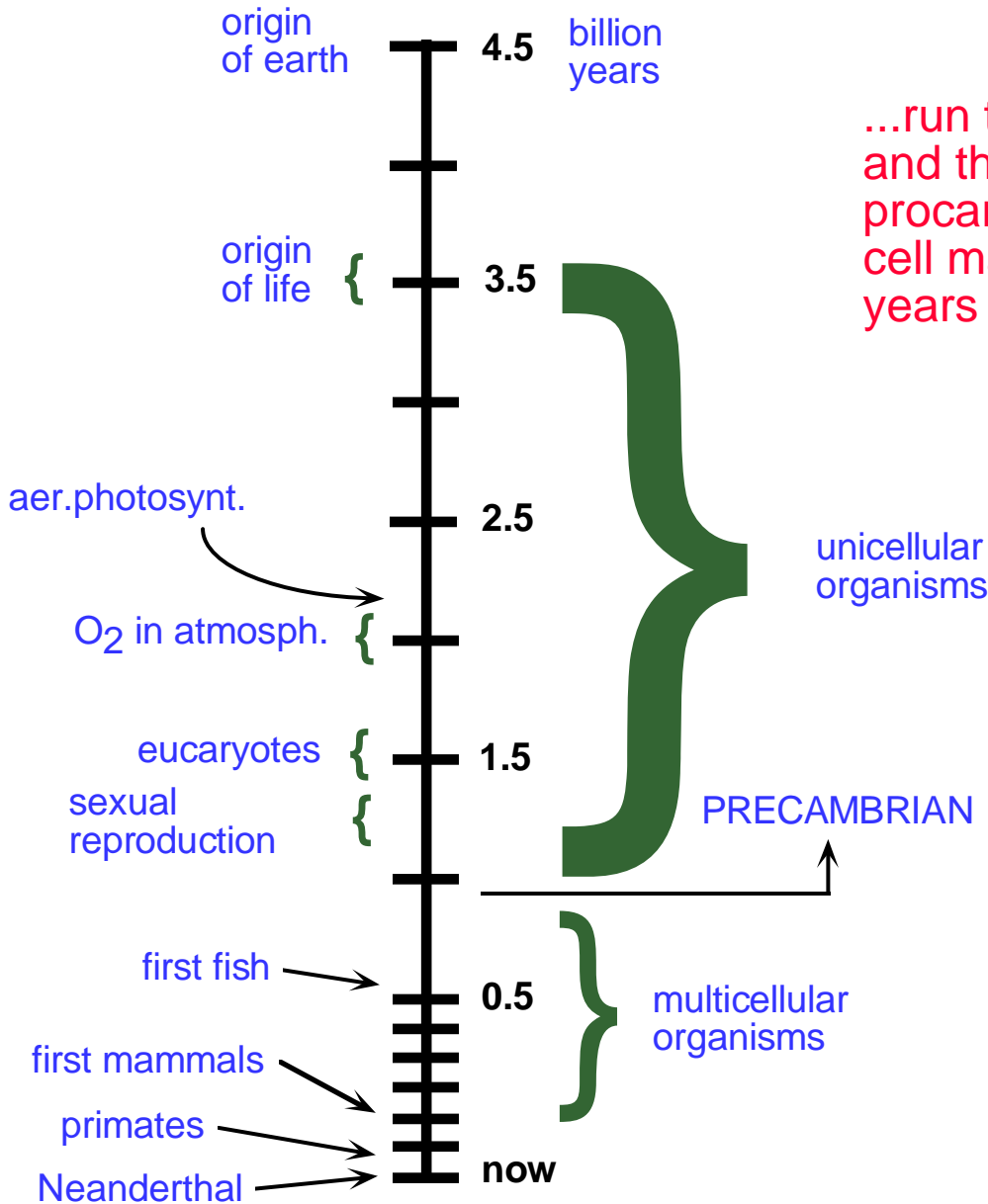
De Duve, 2002

**OPPOSITE TO THE
IDEA OF THE
INEVITABILITY OF
LIFE....**

CONTINGENCY



London: Penguin Books



...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

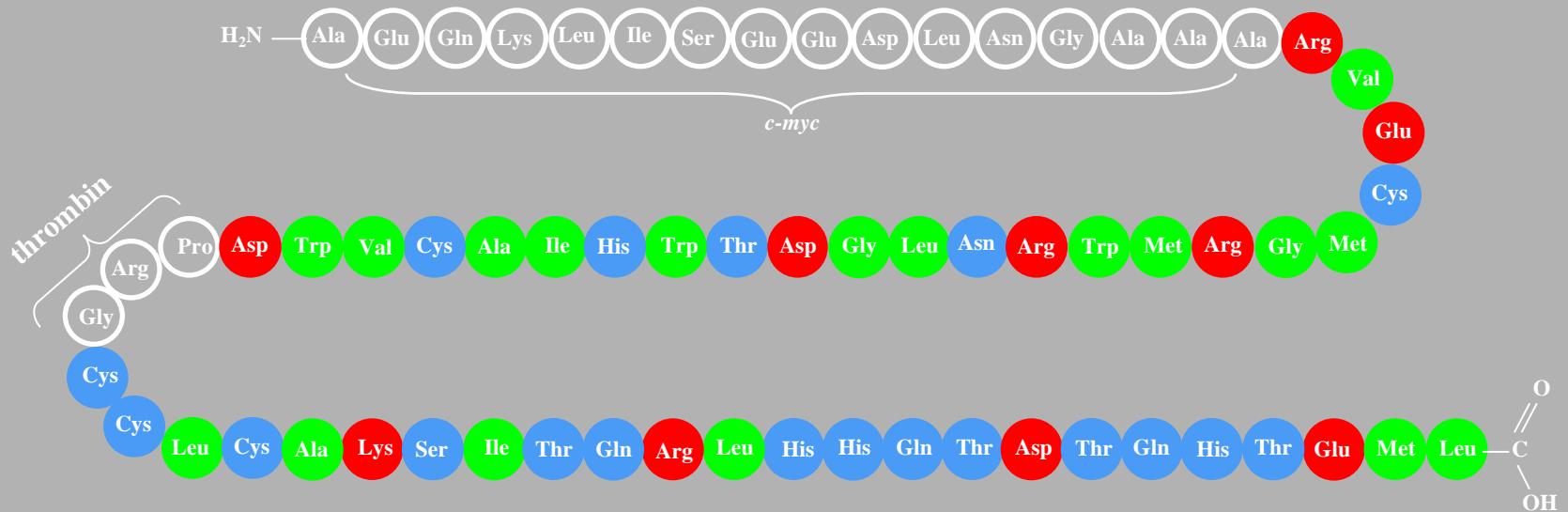
**WHERE SCIENCE AND
PHILOSOPHY MEET:**

**EXPERIMENTAL PROJECTS
OF SYNTHETIC BIOLOGY**

**CAN YOU DO EXPERIMENTAL RESEARCH
ON THESE ITEMS OF
DETERMINISM
VS CONTINGENCY?**

**...CONSIDER THE
PROTEINS OF
LIFE**

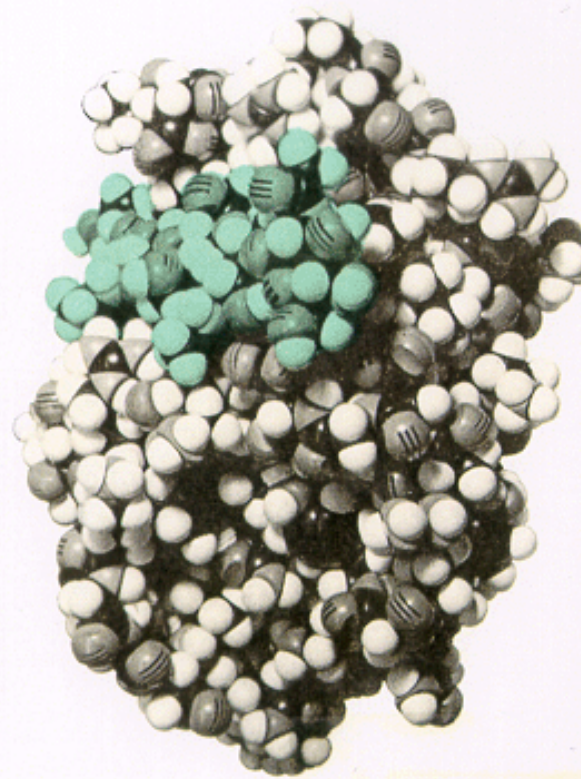
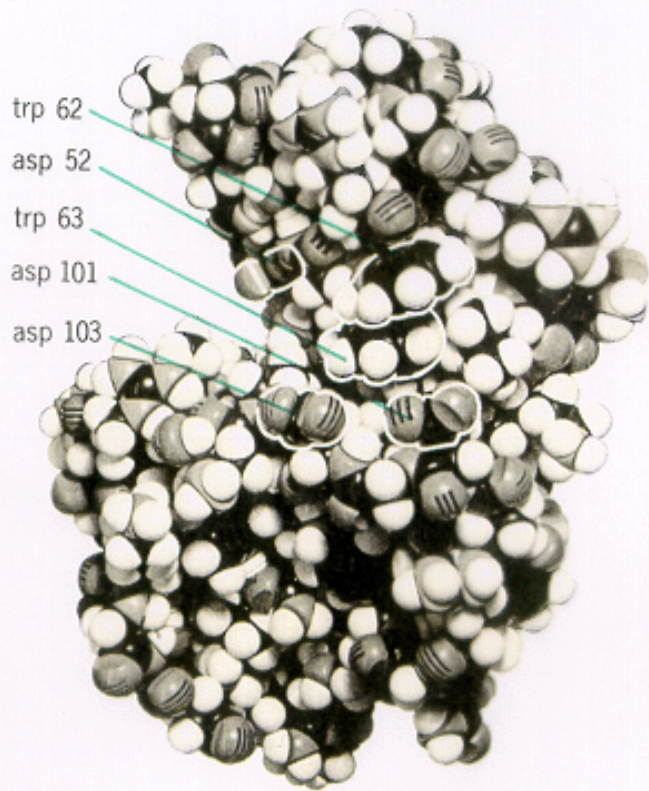
POLYPEPTIDE NBP127 PRIMARY SEQUENCE



 Non polar aa – 39%

 Charged aa – 21%

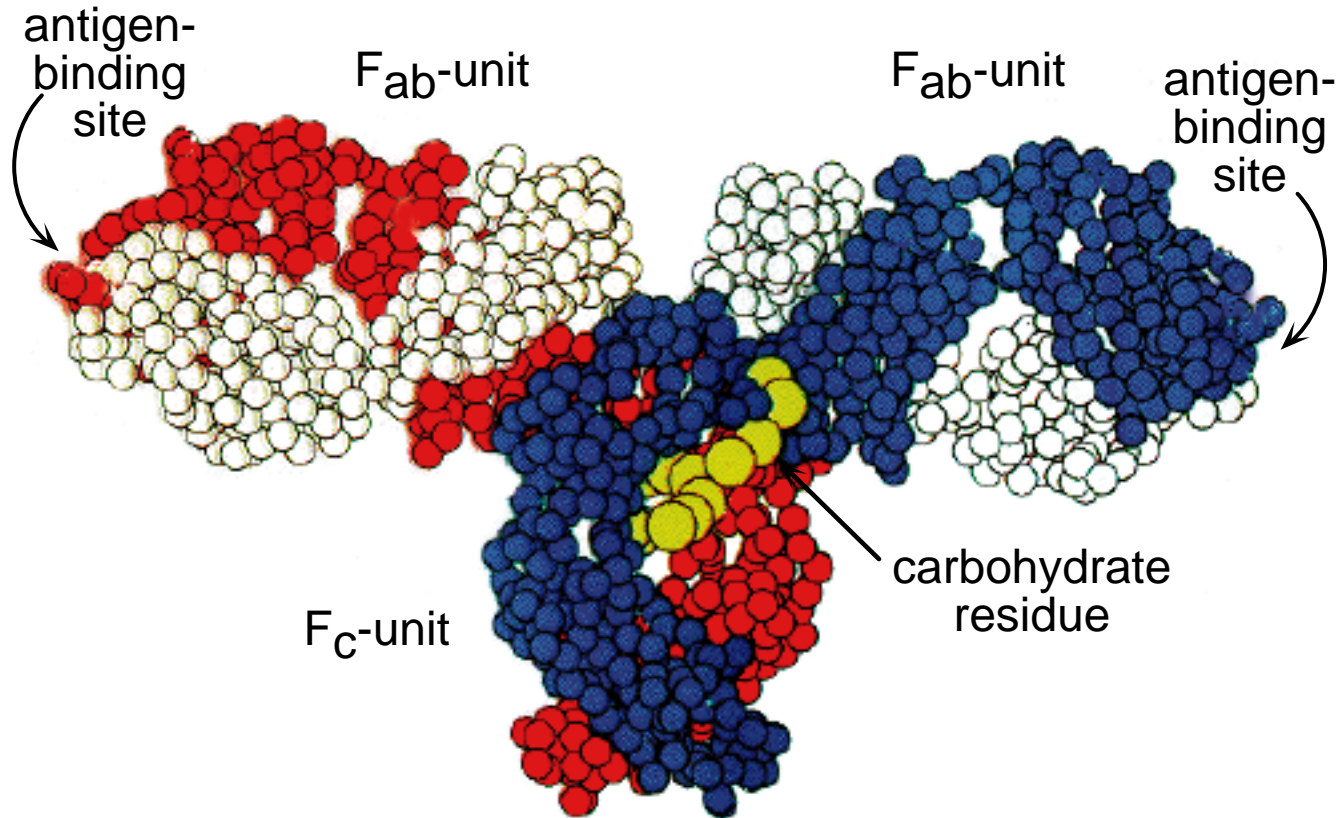
 Polar aa – 40%



(75)

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

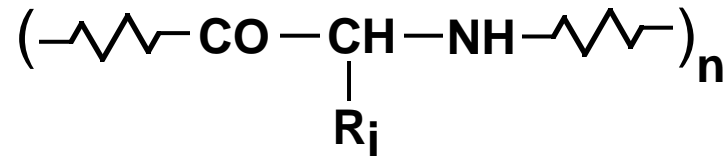
SCHEMATIC REPRESENTATION OF THE THREE-DIMENSIONAL STRUCTURE OF I_gG



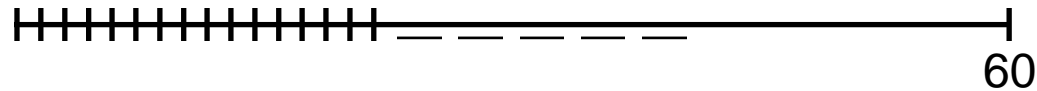
Each amino acid residue is represented by a small circle. The H chains are red and the L chains blue. A carbohydrate residue is yellow.

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

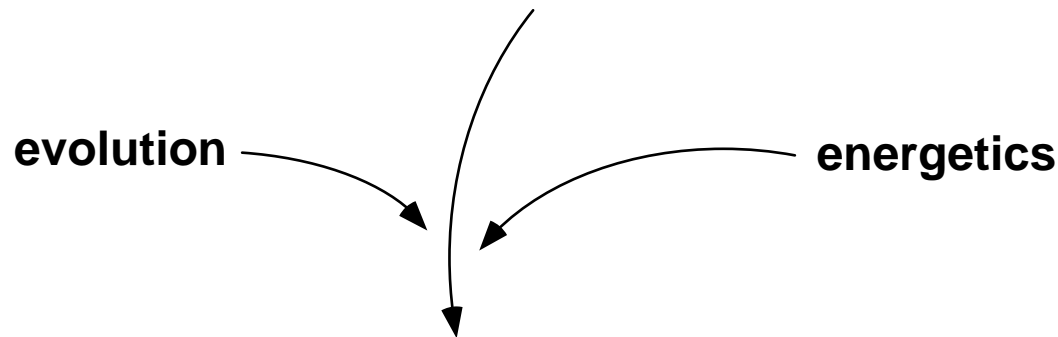
On the importance of being a copolymer



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



$$20 \times 20 \times 20 \times \dots \quad N = 20^{60} \approx 10^{70} \quad !!!$$



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



14
Space of the actual proteins, ca. 10^{14}



- HOW HAVE THESE “FEW” PROTEINS BEEN SELECTED OUT? DO THEY HAVE SPECIAL PHYSICAL OR THERMODYNAMIC PROPERTIES? (SOLUBILITY, STABILITY, FOLDING, HYDRODYN. PROPERTIES....)

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?**

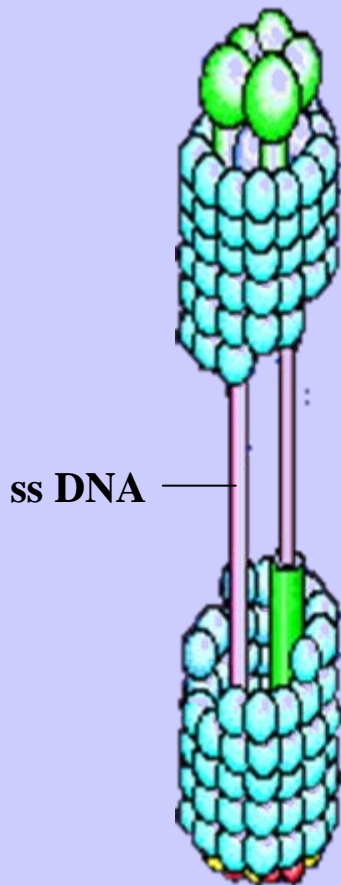
..why this, and not that?

PROJECT „NEVER BORN PROTEINS“

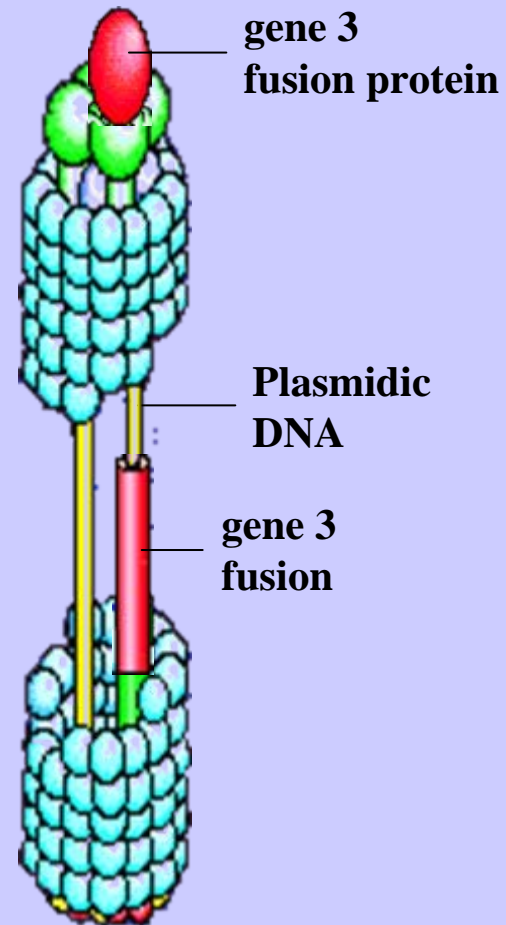
- Synthesize in the lab proteins non existing in nature, i.d., not selected by evolution. Ask the question: how do they compare with „our“ proteins? (stability, folding, solubility....)
- **Cristiano Chiarabelli, Davide de Lucrezia,**
- **Fabrizio Anella, Anna Quintarelli, Alessio Marcozzi, Cecilia Portera**

PHAGEMID VECTOR SYSTEM IN PHAGE DISPLAY

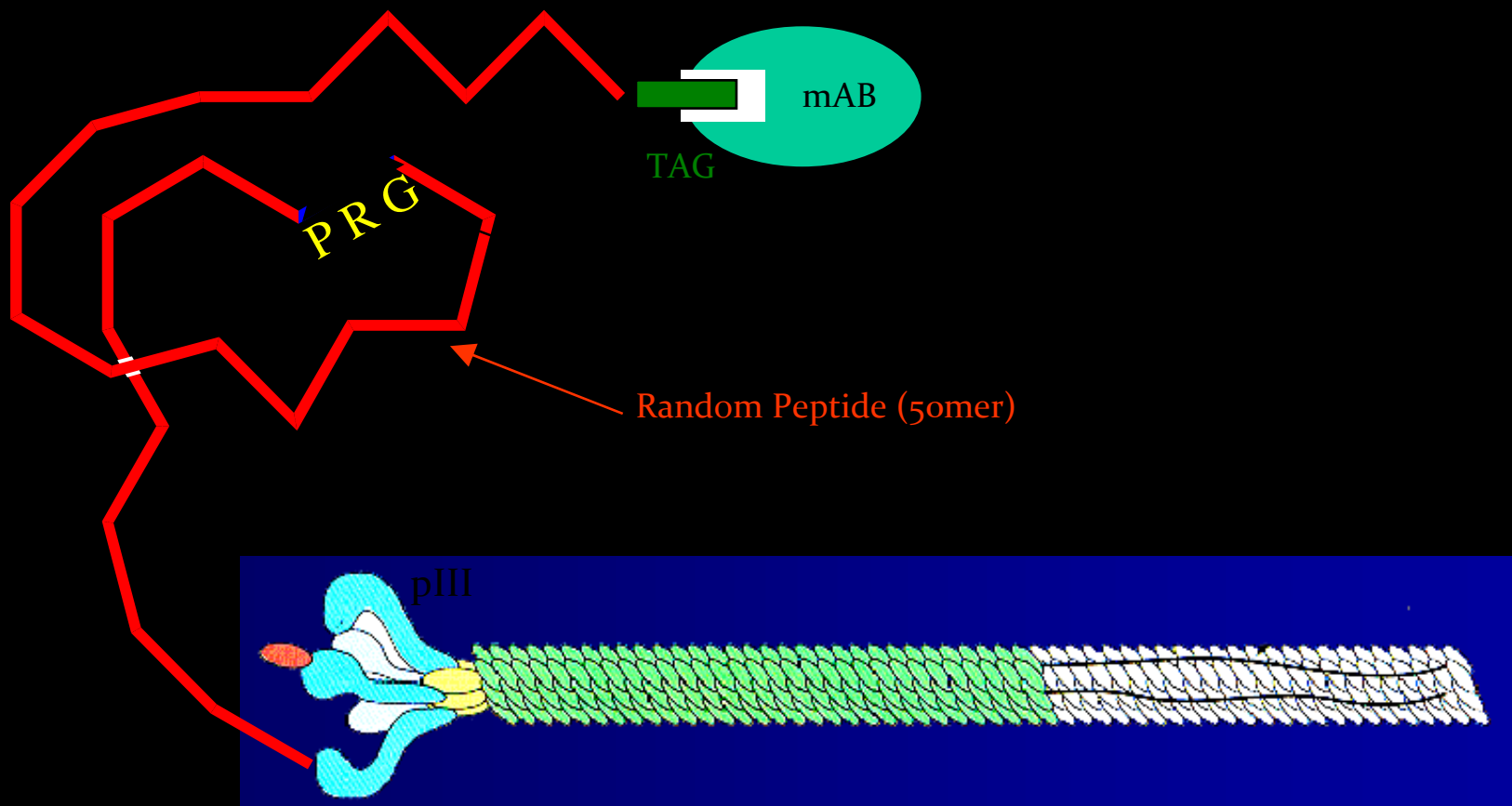
Helper Phage



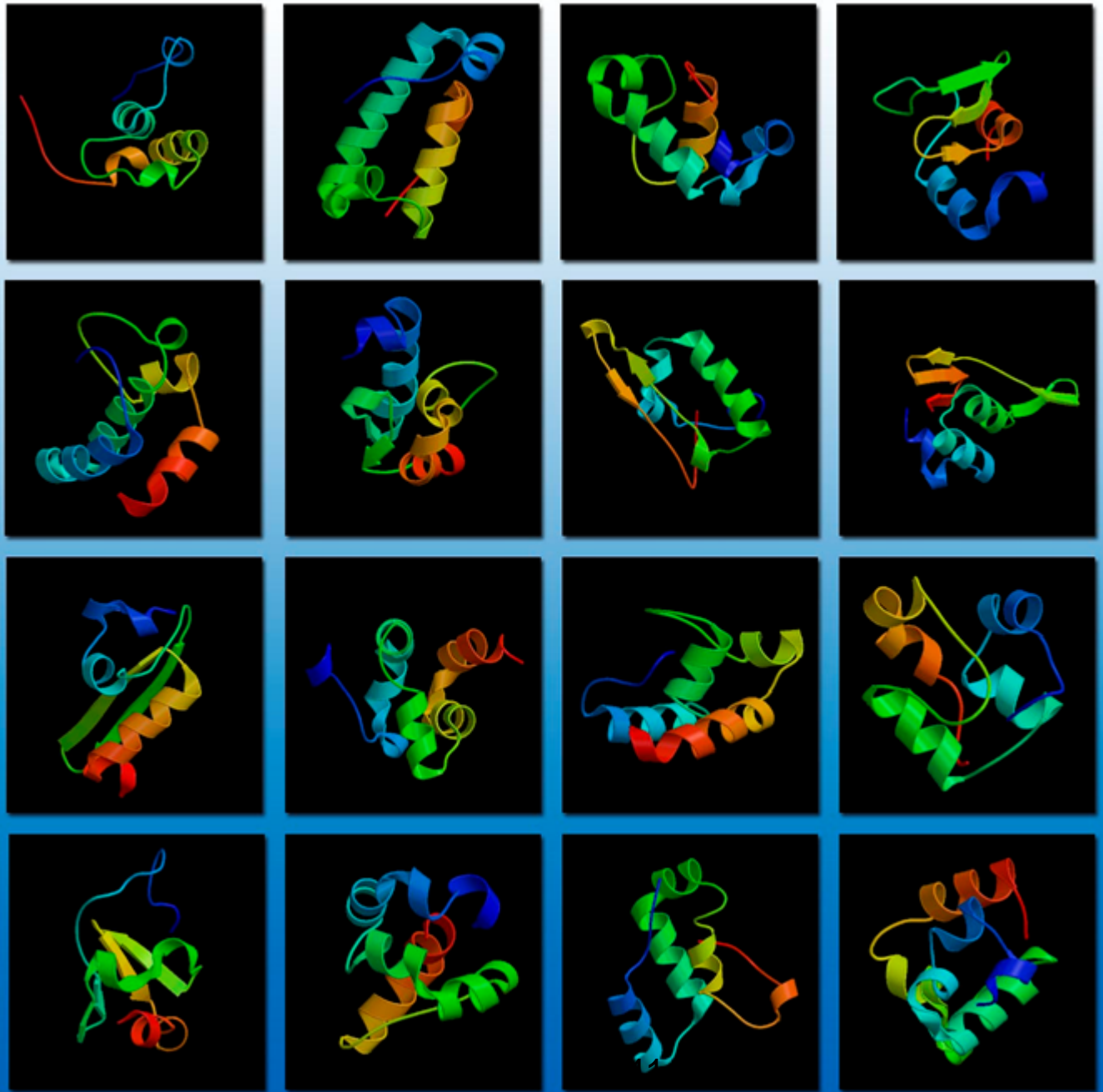
Modified Phage



PHAGE LIBRARY



F.Polticelli
And the Rosetta
method



Temptative conclusions :

**Completely random de novo proteins can be
Stable, folded, soluble in water**

**„Our“ proteins have nothing special from
the
folding or stability point of view**

**Are therefore „our“ proteins simply the
products of contingency?**

ANOTHER PROJECT IN SYNTHETIC BIOLOGY

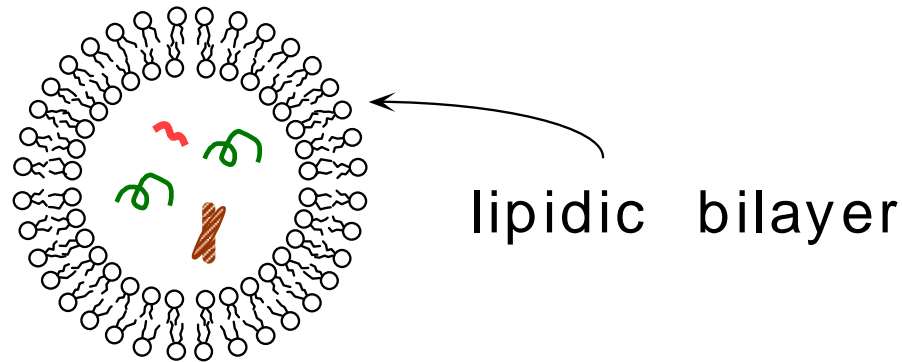
Is it possible to reproduce in the laboratory
cellular life?

Starting from scratch,

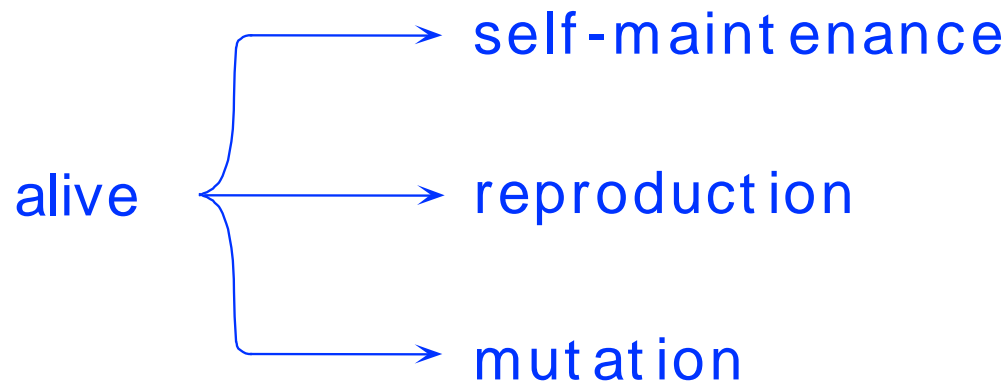
Or...

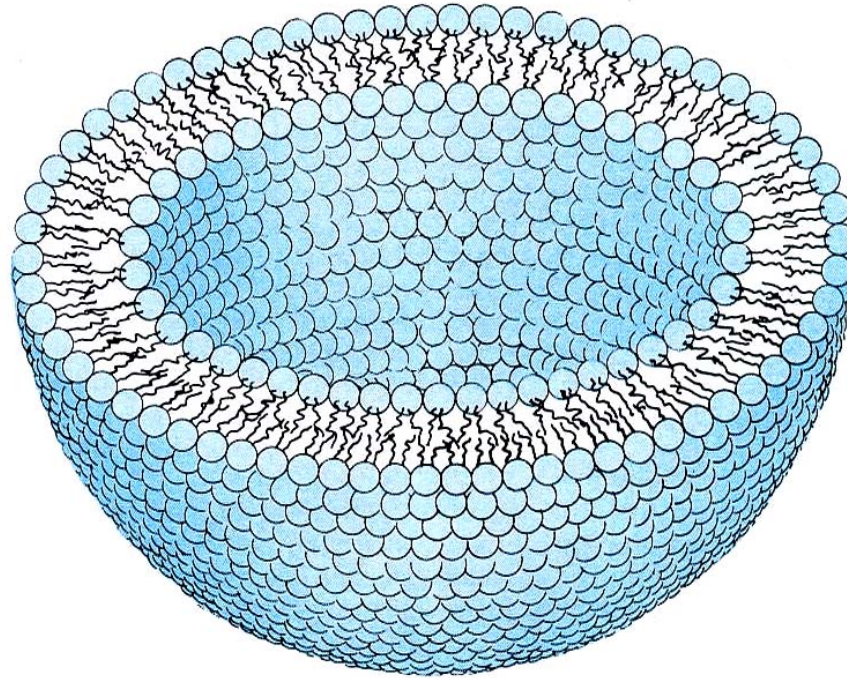
**There are very many different approaches in the
scientific literature**

the notion of the minimal cell:



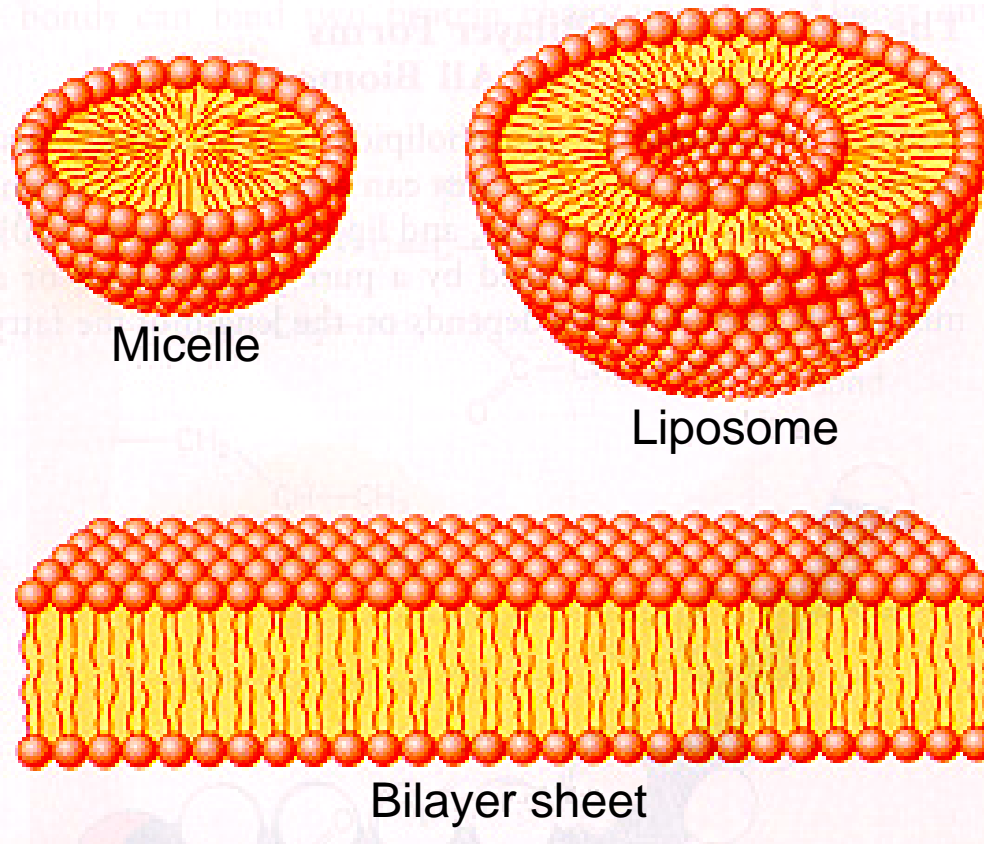
containing the minimal and sufficient number of components to be "alive"





MOLECULAR ARCHITECTURE of the animal-cell membrane is determined primarily by the interactions of phospholipid molecules in water. Phospholipids can minimize their energy in water by forming a bilayer about 40 angstrom units thick. The hydrophobic tails of the molecules sequester themselves on the inside of the bilayer and the hydrophilic heads (*blue*) face the water on both sides of the bilayer. If any edge of the bilayer were open to the water, hydrophobic tails along the edge would be exposed; hence the bilayer closes to form a vesicle, effectively segregating fluid inside the vesicle from fluid surrounding it.

Cross-sectional views of the three structures that can be formed by mechanically dispersing a suspension of phospholipids in aqueous solution



The red circles depict the hydrophilic heads of phospholipids, and the squiggly lines (in the yellow region) the hydrophobic tails.

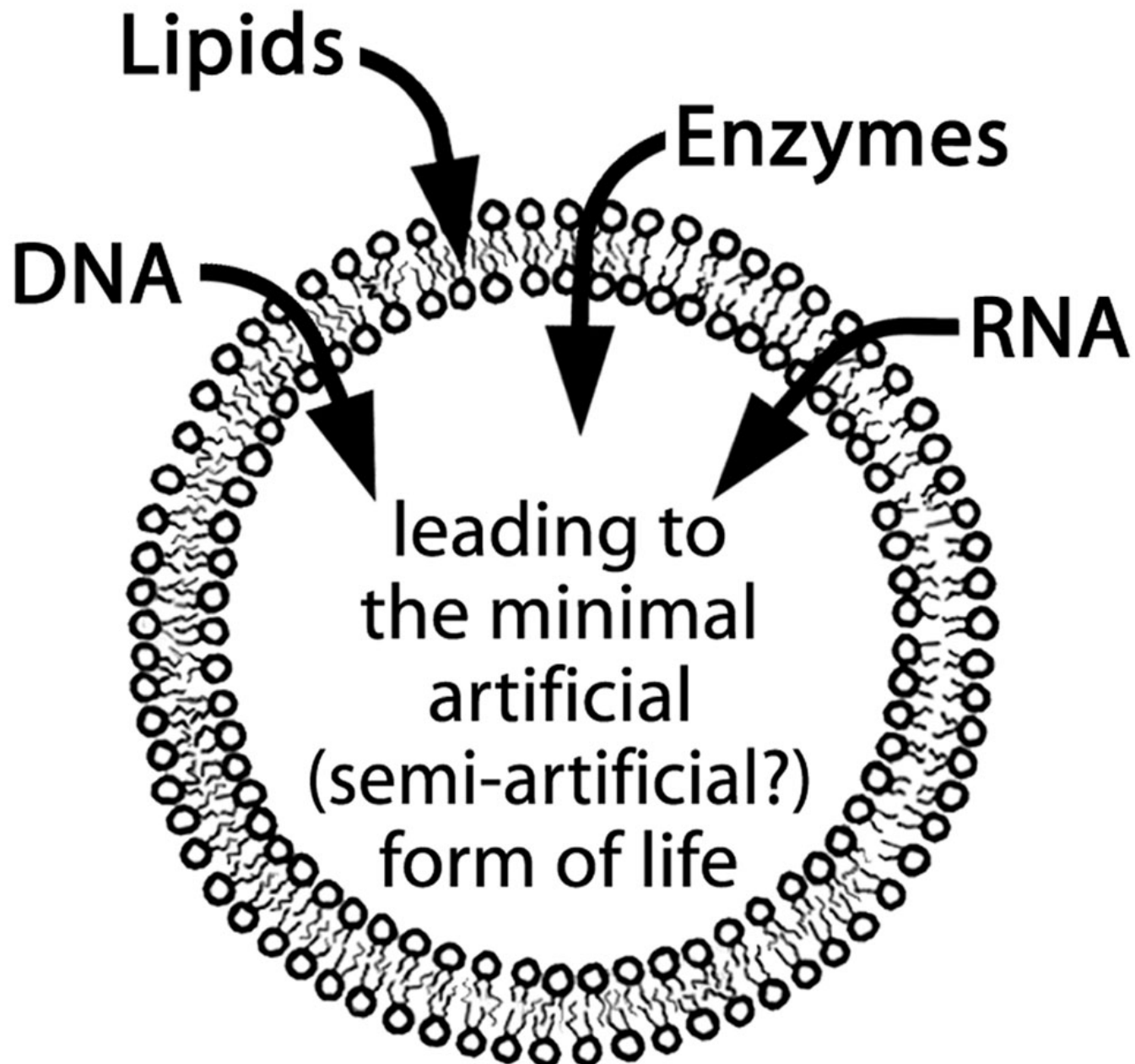
Oleic acid/oleat vesicles

Double Click to play the movie

**NOW, LET US PUT SOMETHING
IMPORTANT INSIDE...**

Project „minimal cell“

**Pasquale Stano, Giovanni Murtas, Yutetsu Kumura
Tereza de Souza,
Paolo Carrara, Valentina Gallo,
Erica De guanno, Katarzyna Adamasala**



Lipids

Enzymes

DNA

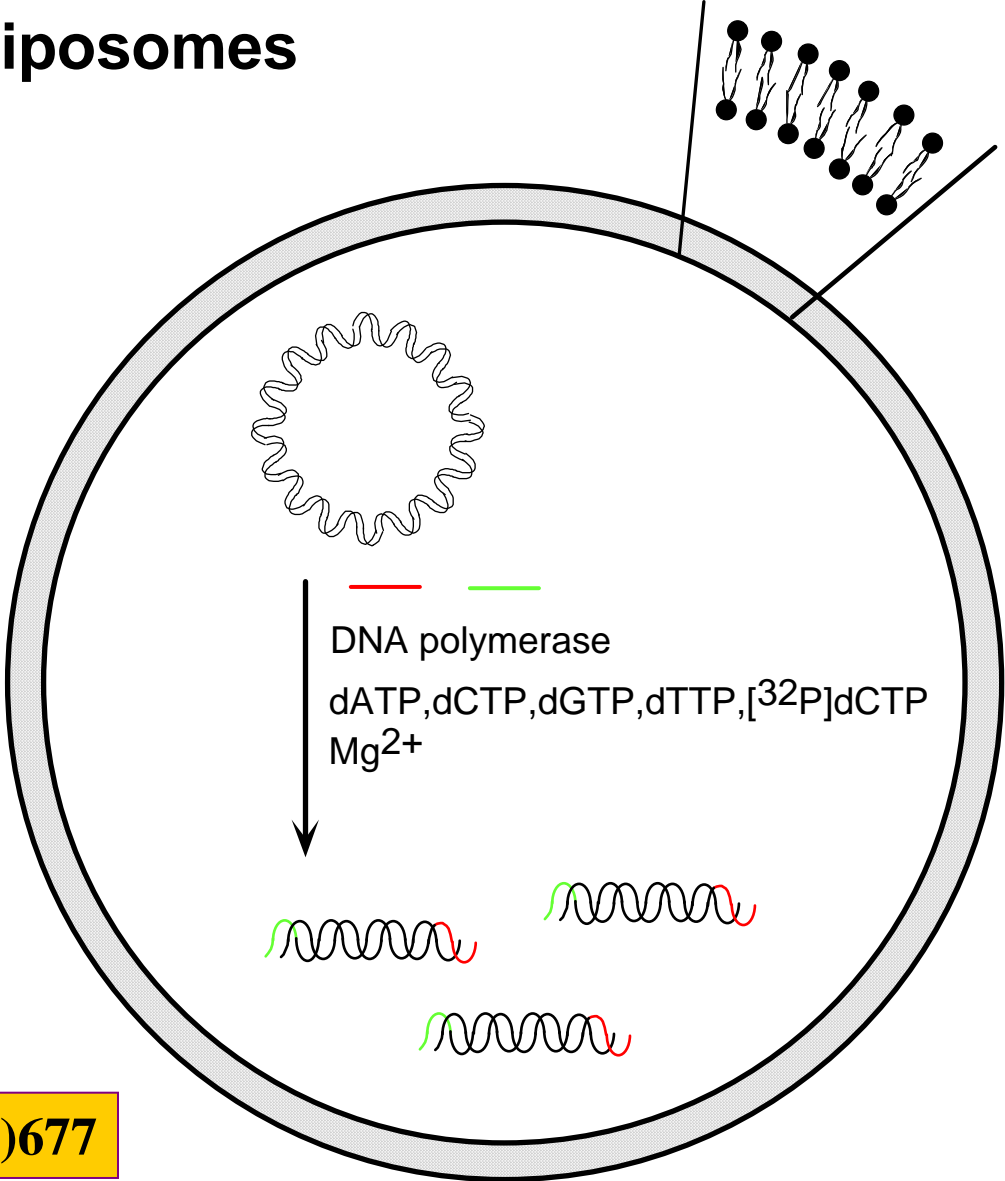
RNA

leading to
the minimal
artificial
(semi-artificial?)
form of life

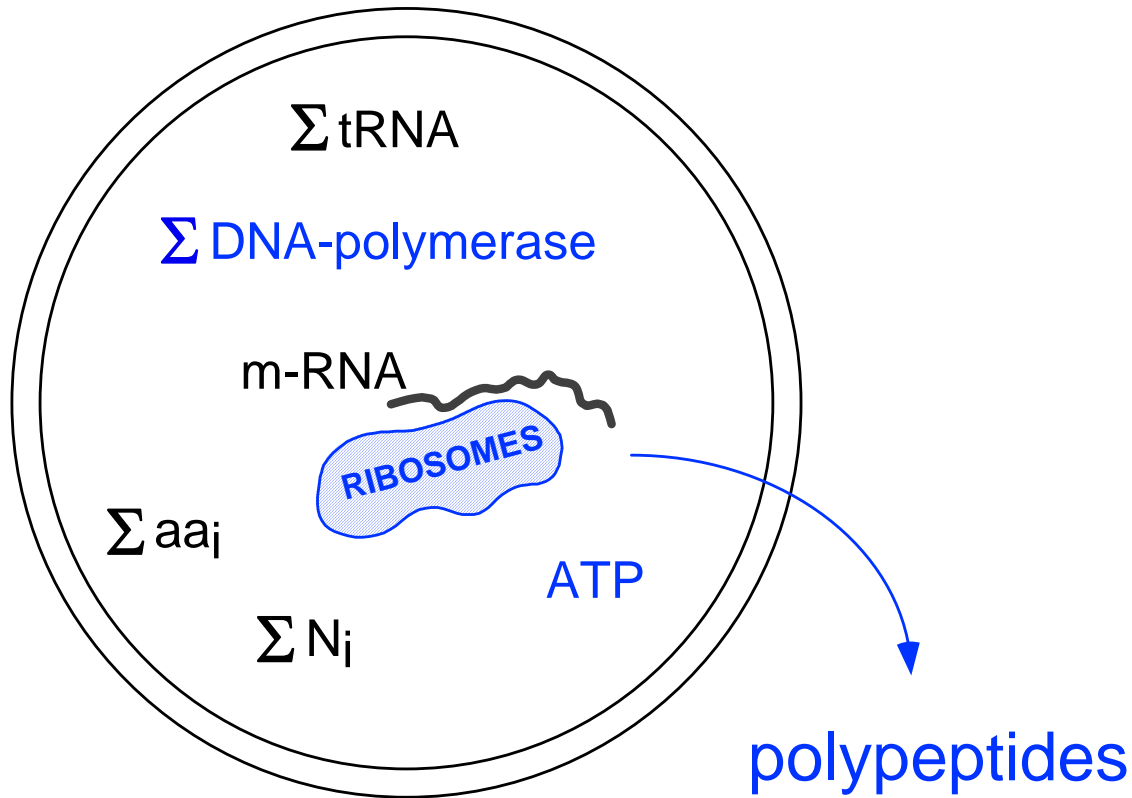
Step one:

Molecular biology inside liposomes

PCR in liposomes



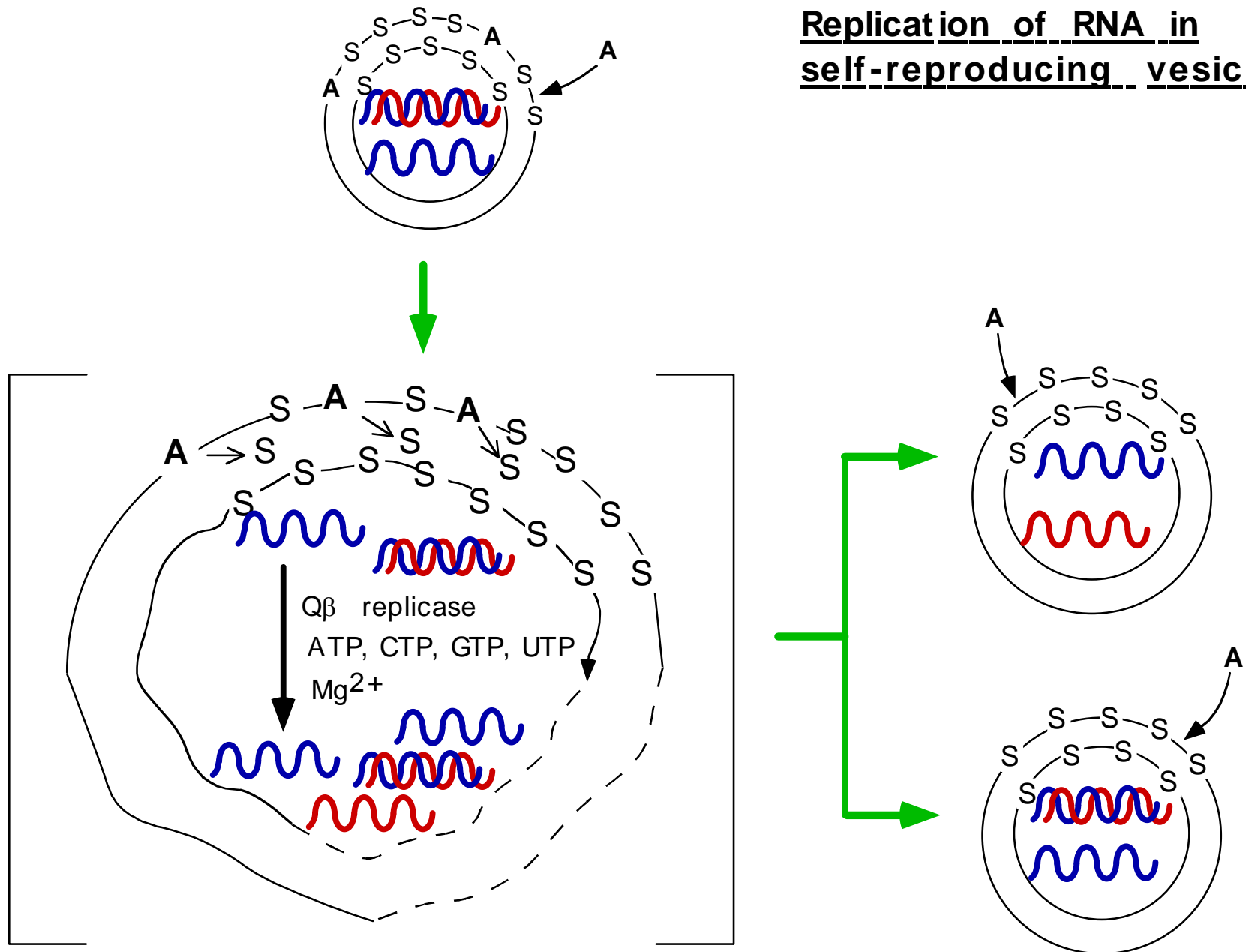
protein biosynthesis in liposomes



Oberholzer et al., 1999
(only poly-phe)

Oberholzer et al, 1995

Replication of RNA in self-reproducing vesicles



IS THIS LIFE?

...death by dilution

Step two:

Protein synthesis inside liposomes

PROTEIN EXPRESSION IN LIPOSOMES (MOSTLY GREEN FLUORESCENCE PROTEIN) HAS BEEN DESCRIBED BY SEVERAL GROUPS:

Oberholzer et al., 1999, 2001

Yomo et al., 2001

Tsumoto et al., 2001

Fischer et al, 2002

Nomura et al., 2003,

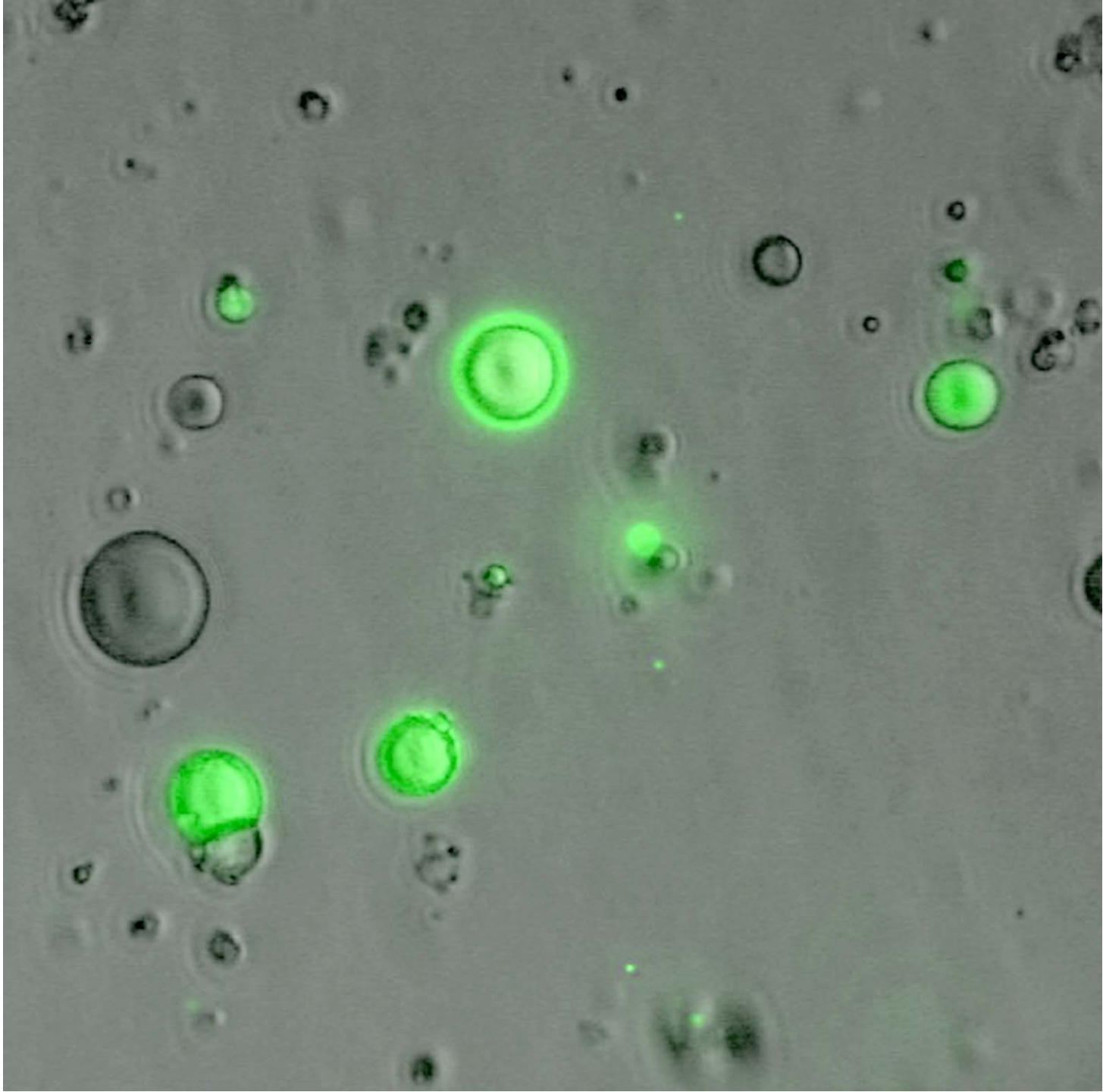
Pietrini et al., 2004

Noireaux et al, 2004

Ishikawa et al, 2004

We are presently working with a kit
of 37 enzymes, plus ribosomes,
tRNAs ,
A total of ca 90 macromolecular
components of known concentration
capable of expressing proteins

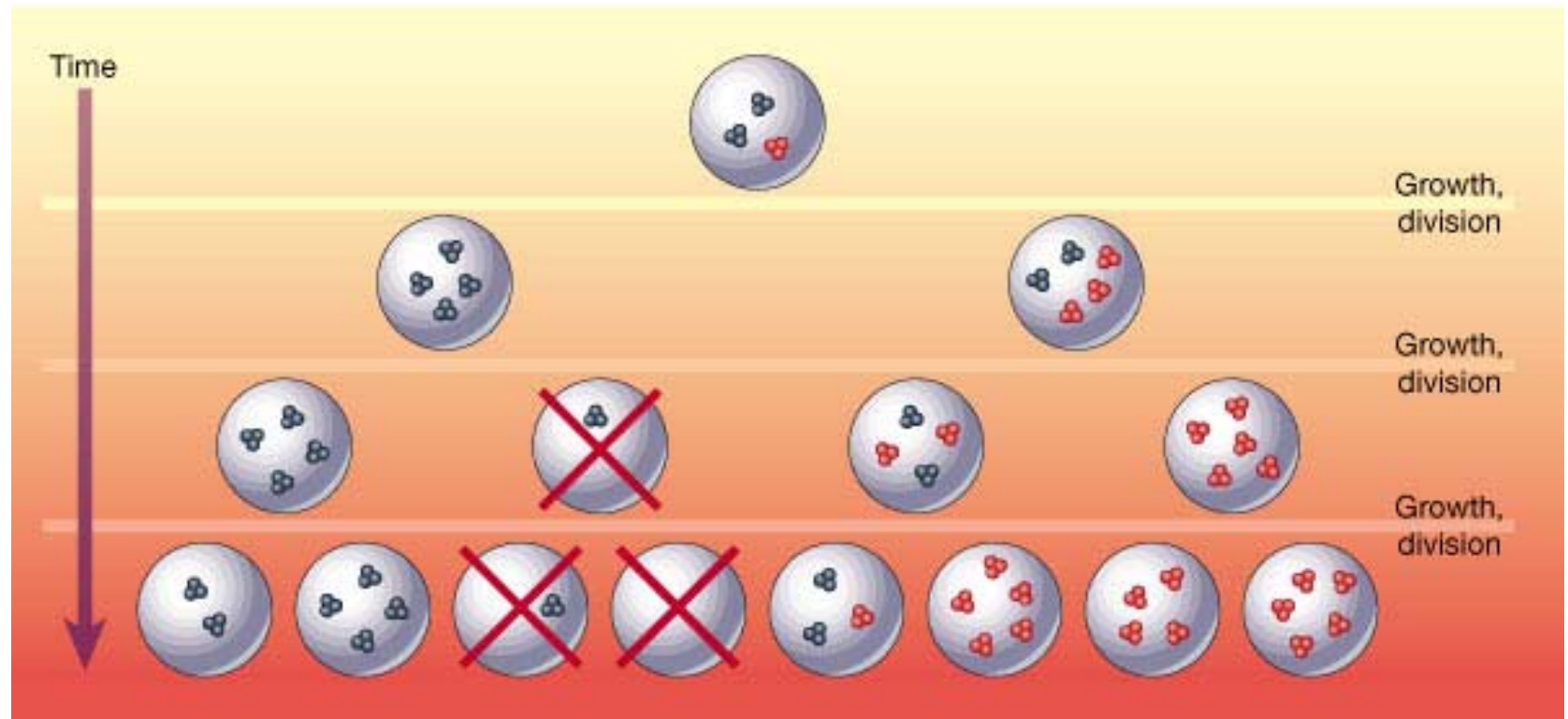
Developed by Ueda and
collaborators (2001)



Nature 409, 387 - 390 (2001)

Synthesizing life

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

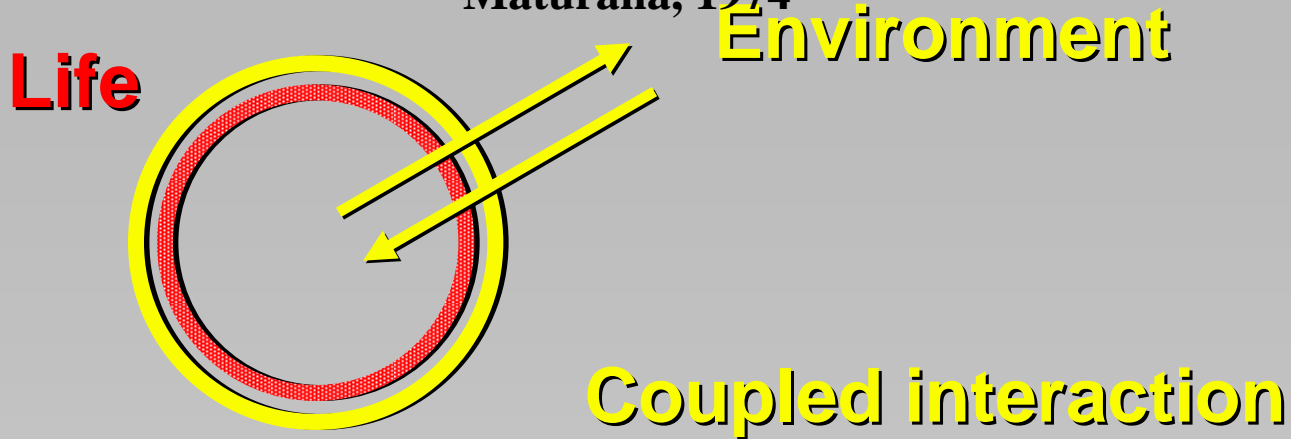


Quarta parte:

**Dalla biologia alle scienze cognitive
E alla coscienza**

**LIVING SYSTEMS ARE COGNITIVE SYSTEMS,
AND LIFE AS A PROCESS IS A PROCESS OF COGNITION**

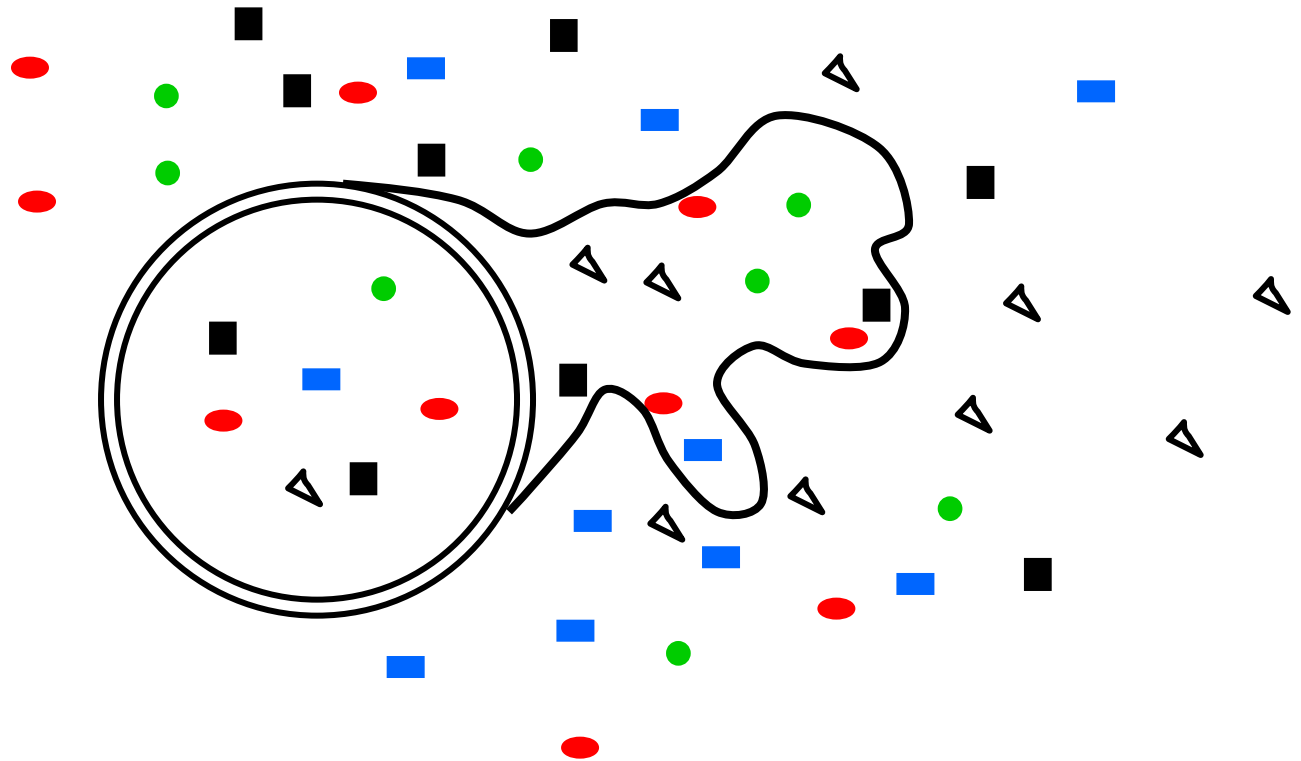
Maturana, 1974



SANTIAGO THEORY :

Life = interaction

with the environment \longrightarrow cognition



the organism creates from the environment
its own world
(→ cognition, → enacting)







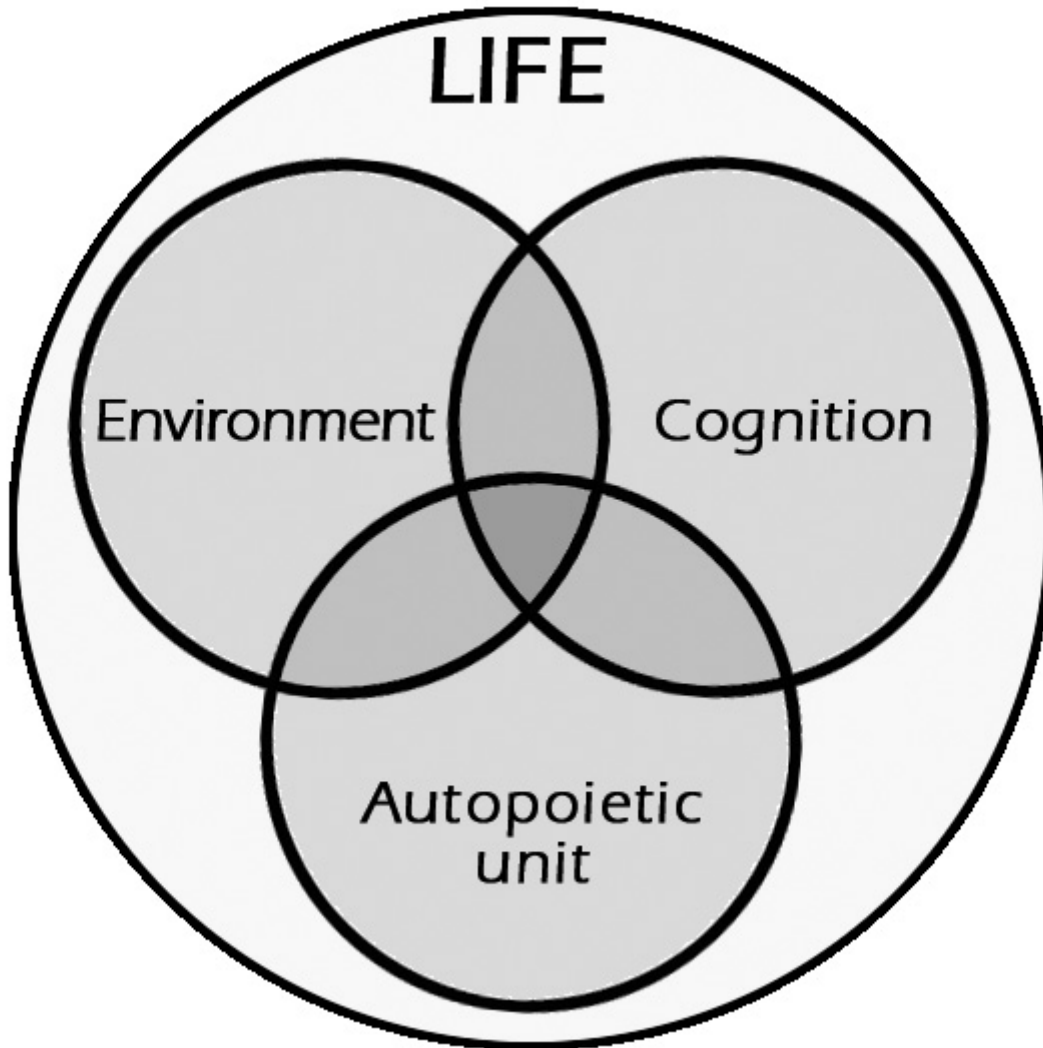
CO-EMERGENCE BETWEEN THE LIVING AND THE ENVIRONMENT:

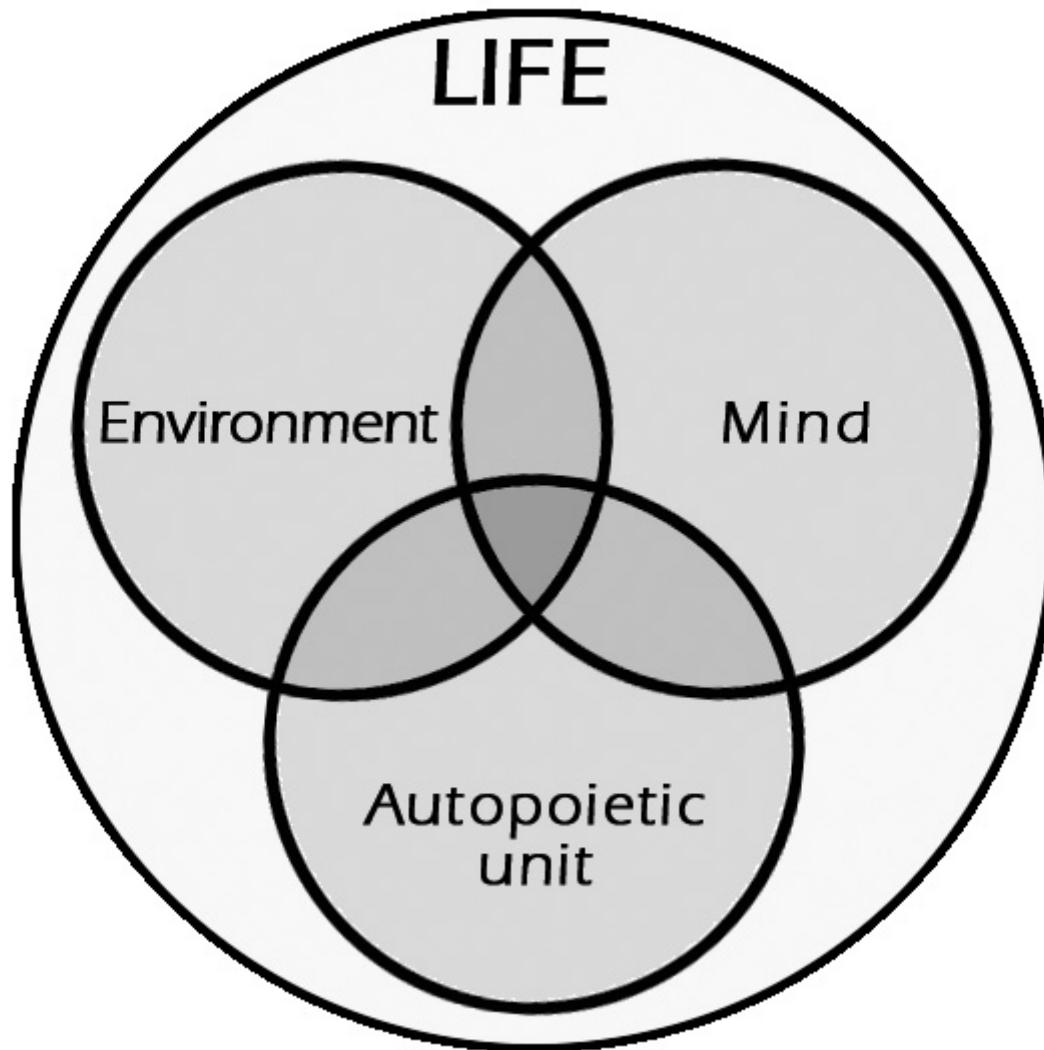
- COGNITION BEING THE FACTOR THAT GOVERNS THE FORMATION OF THIS NOVEL EMERGENT ENTITY

COGNITION AS A CONTINUUM CONCEPT

- At the level of amoeba
- Of insects
- Of simple mammals
- ...of man

**...as the sensorium towards the environment evolves,
„cognition“ becomes more and more sophisticated**



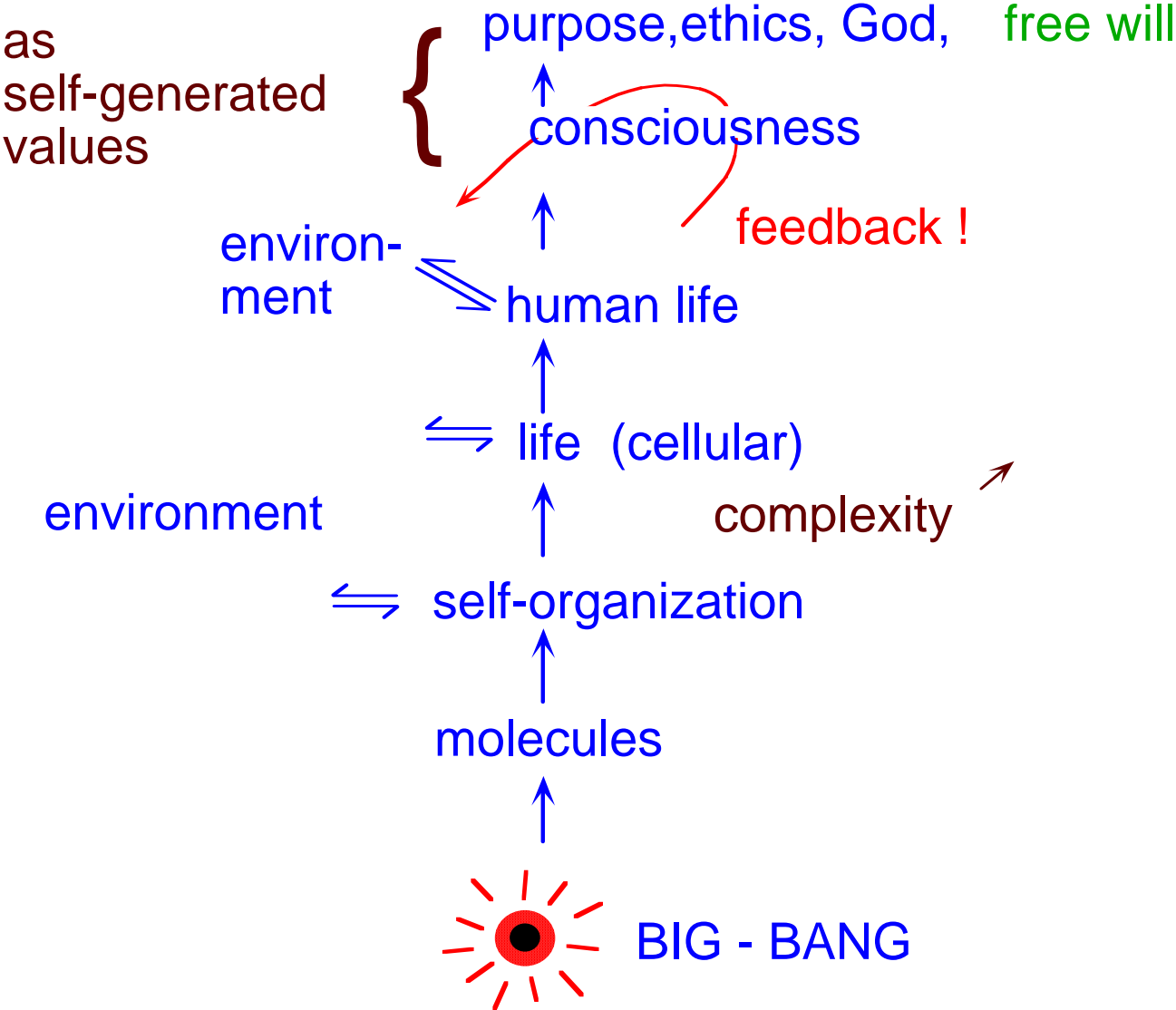


See *The embodied Mind*, Varela et al., 1998

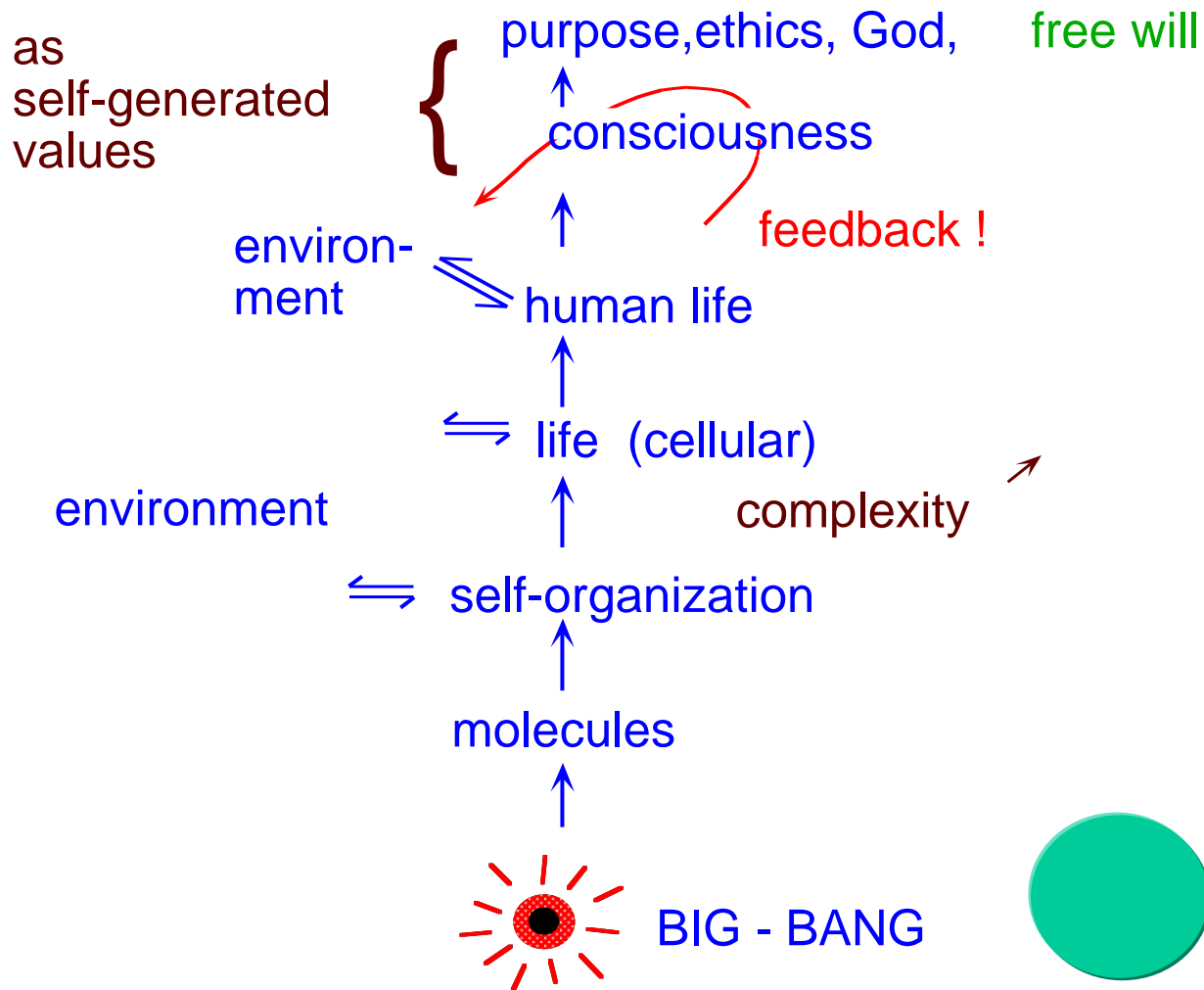
Consciousness is.....

- knowing that you know (being aware of being aware)**
-
- the subjective acknowledgment of experience (such as sensory perceptions, or thought)**
-

THE OVERVIEW



THE OVERVIEW



Cambridge University Press, 2006

The Emergence of Life

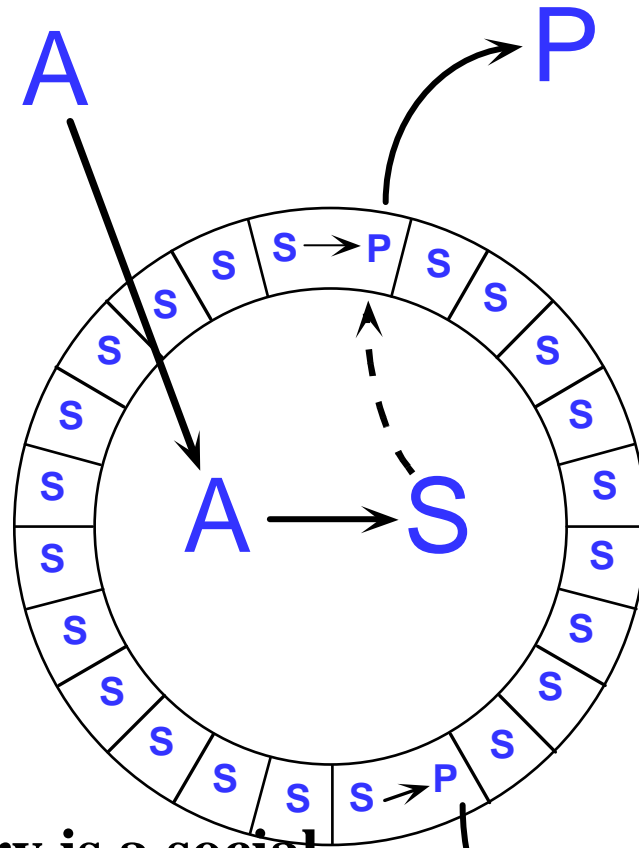
From Chemical Origins to Synthetic Biology

Pier Luigi Luisi

Uniquely combining biology and philosophy, this book offers a systematic course in the emergence of life from inanimate matter. The successive stages, selforganization, emergence, self - reproduction, autopoiesis, synthetic compartments and construction of cellular models are used to demonstrate the spontaneous increment in complexity leading to the first cellular life forms.

luisi@mat.ethz.ch

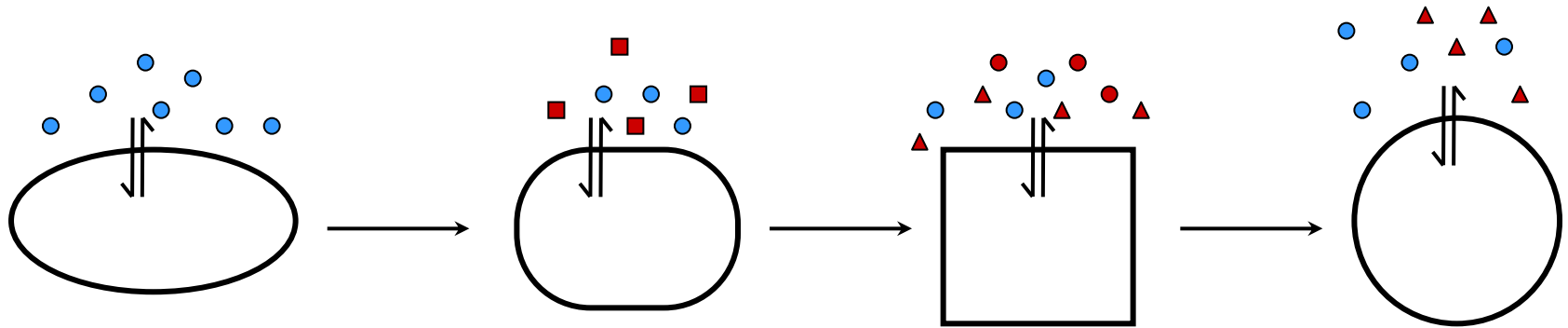
Towards social autopoiesis



If the boundary is a social structure (a city, a party, an hospital...) and **A** a new person who becomes a member (**S**)....

From all the above it is apparent that autopoiesis belongs epistemologically to systems theory, according to which it is the organization of the components that characterizes the quality of the system. Thus, the life of a cell is a global property, and cannot be ascribed to any single component.

evolution:
the history of coupling interactions



a living organism as the
depository of a long history
of adaptive changes

..A HISTORY OF COUPLING INTERACTIONS FROM WHICH THE COGNITIVE DOMAIN IS CONSTITUTED NEITHER INTERNALLY (A SOLIPSISTIC VIEW) NOR EXTERNALLY (THE TRADITIONAL VIEW).

THE ALTERNATIVE THAT ELIMINATES THESE TWO EXTREMES IS BASED ON A CO-DEFINITION BETWEEN THE INTERNAL AND EXTERNAL SYSTEM. THIS IS THE NOTION OF **ENACTION.**

ENACTING MEANS TO MAKE EMERGE

“..the Varela’s school is emphasizing that the external world acts as a mere “kick”, which motivates the system to establish a new equilibrium characterized only by the necessities of self-support. For a biosemiotic approach this means that it is no longer concerned with the constraints of the mind-body-problem. Dualism becomes obsolete by the material circularity of autopoiesis. In a self-referential system, meaning is the “inner side” of the material aspect of the system’s closure.”

(Weber 2001)

the interaction between organisms and their environment is part of the more general scenario of **ecology. It has been in fact stated that living organisms make and continuously change the environment in which they live, and vice versa, so that every act of consumption is also an act of production; also, that we must forget the idea that there is a constant and fixed world – as we are constantly changing it and cannot live without changing it. (Lewontin, 1991). From that, the difficulty of finding a healthy equilibrium that preserves as much as possible the identity of the living.**

**A DISCOVERY: THE CELLULAR
(AUTOPOIETIC)
DEFINITION OF LIFE
ALSO APPLIES**

**TO MULTICELLULAR,
MACROSCOPIC
LIFE**

THE HARD SCIENCE VIEW

(material-based)

consciousness is a property of the mind ...

Mind is an emergent property of the brain

OTHER QUESTIONS ABOUT CONSCIOUSNESS...

-IS THERE ALWAYS A CONTENT IN THE CONSCIOUS EXPERIENCE?

-IS IT ALWAYS “MY” CONSCIOUSNESS? OR THERE IS ALSO A HIGHER DIMENSION?

-LA COSCIENZA HA SEMPRE UN CONTENUTO?

**-SI TRATTA SEMPRE E SOLO DELLA “MIA”
COSCIENZA?**

O C'E' ANCHE UNA DIMENSIONE SUPERIORE?

**A recent discussion panel at the last Genua
Science Festival:**

IS CONSCIOUSNESS MATERIAL-BASED

OR

IS IT PRIMARY

**(DOES NOT DEPEND ON SOMETHING ELSE)
?**

HA LA COSCIENZA UNA BASE MATERIALE

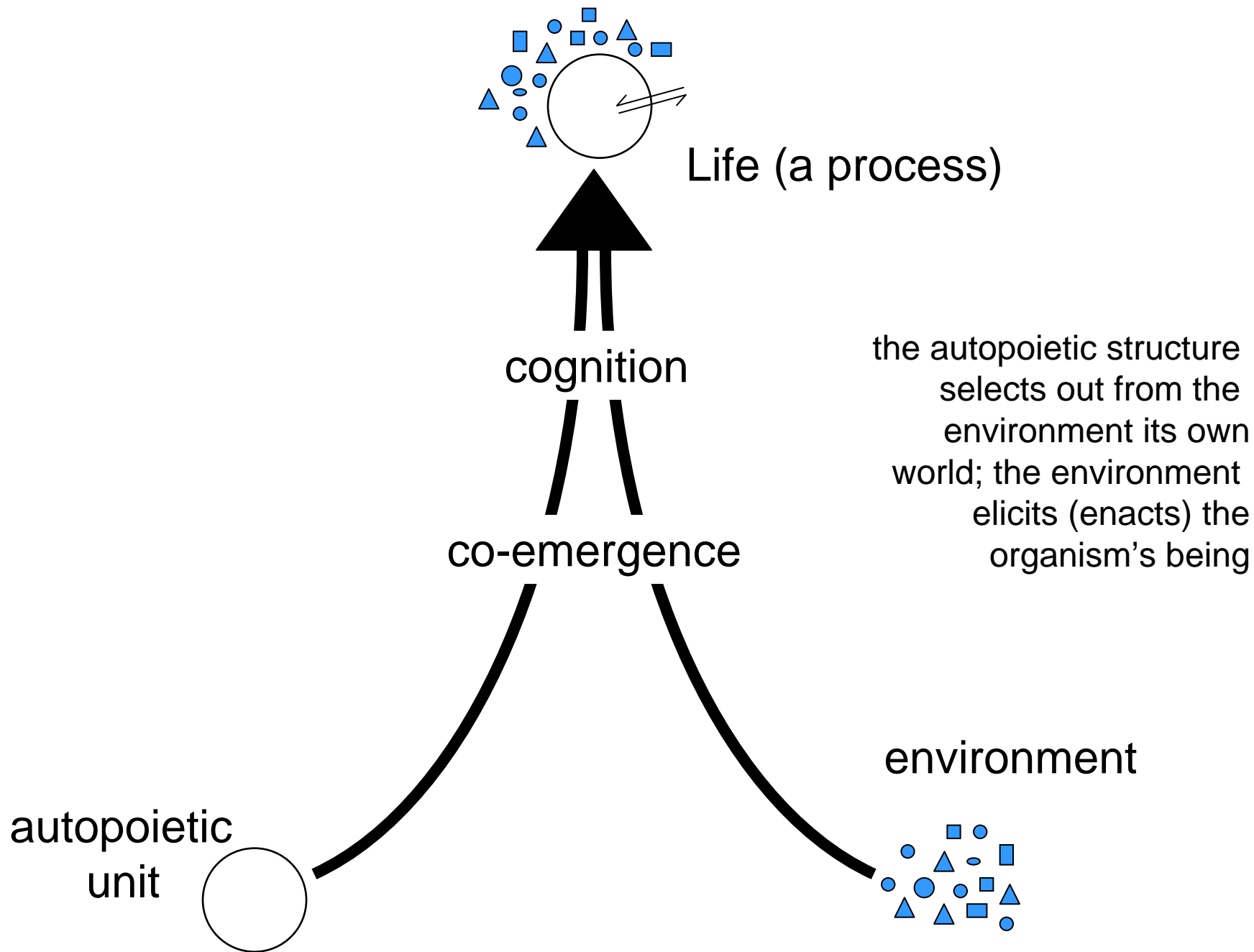
**OPPURE E' PRIMARIA, CIOE' NON
DIPENDE DA UN'ALTRA CAUSA?**

QUESTION:

**ARE THE PROTEINS OF LIFE THE ONLY
ONES THAT COULD BE FORMED-
AND GAVE ORIGIN
TO LIFE BY A DETERMINISTIC
(OBLIGATORY)
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*Whenever you encounter a network whose operations eventually produce itself as a result, you are facing an autopoietic system. It produces itself. The system is **open** to the input of matter but **closed** with regard to the dynamics of the relations that generate it.”*

MATURANA 2004

**Presently: work with THE PURE SYSTEM,
A kit with only 37 enzymes
(plus the ribosomes)
capable of expressing proteins**

**Developed by Ueda and coworkers in Tokyo
in th early 2000**

R. C. Lewontin (Lewontin, 1991) mentions that the atmosphere that we all breathe was not on earth before living organisms and adds:

... there is no “environment” in some independent and abstract sense. Just as there is no organism without an environment, there is no environment without an organism. Organisms do not experience environments. They create them. They construct their own environments out of the bits and pieces of the physical and biological world, and they do so by their own activities.

THE IMPORTANCE OF IMMANENCE

- (THE VIEW FROM WITHIN)

Is it all based on molecules and their interactions?

THE ANTHROPIC PRINCIPLE....

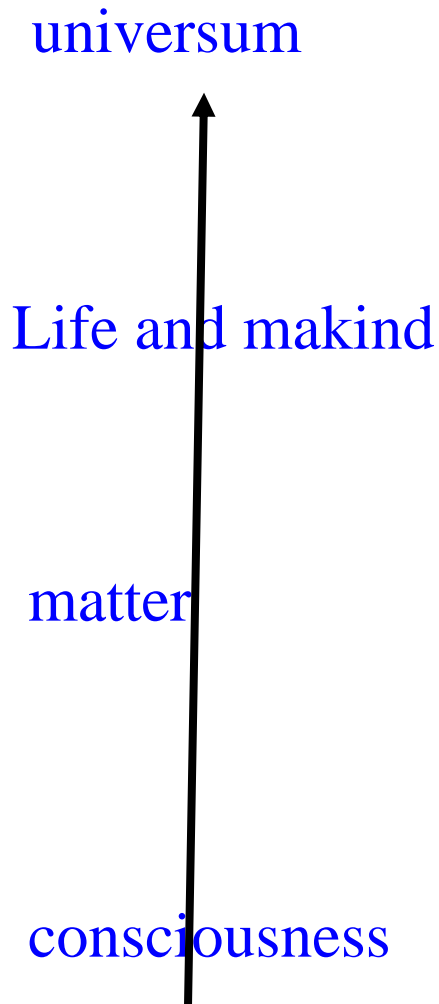
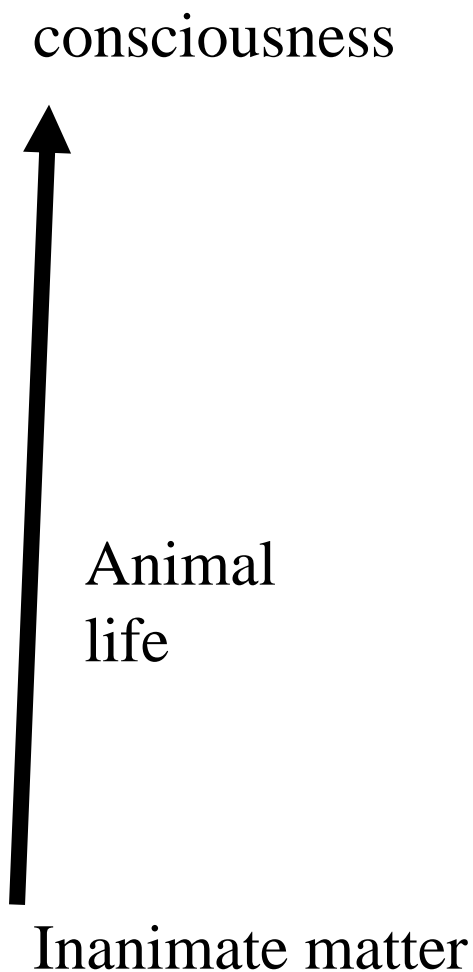
*“If life follows from (primordial) soup with causal dependability, the laws of nature encode a hidden subtext, a cosmic imperative, which tell them:
‘Make life! And, through life, its by-products mind, knowing, understanding...’”*

Paul Davis, 1991

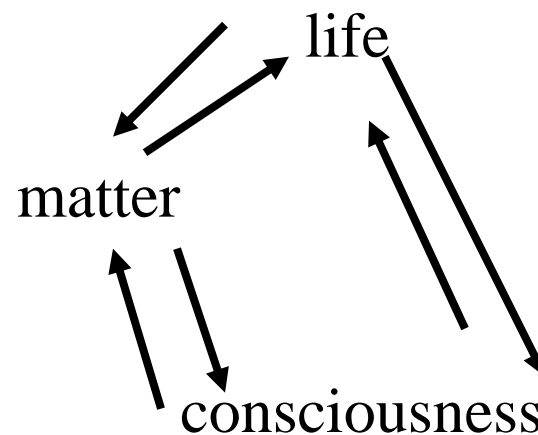
QUESTIONS FOR THE DIALOGUE SCIENCE/RELIGION:

The science view

religion view



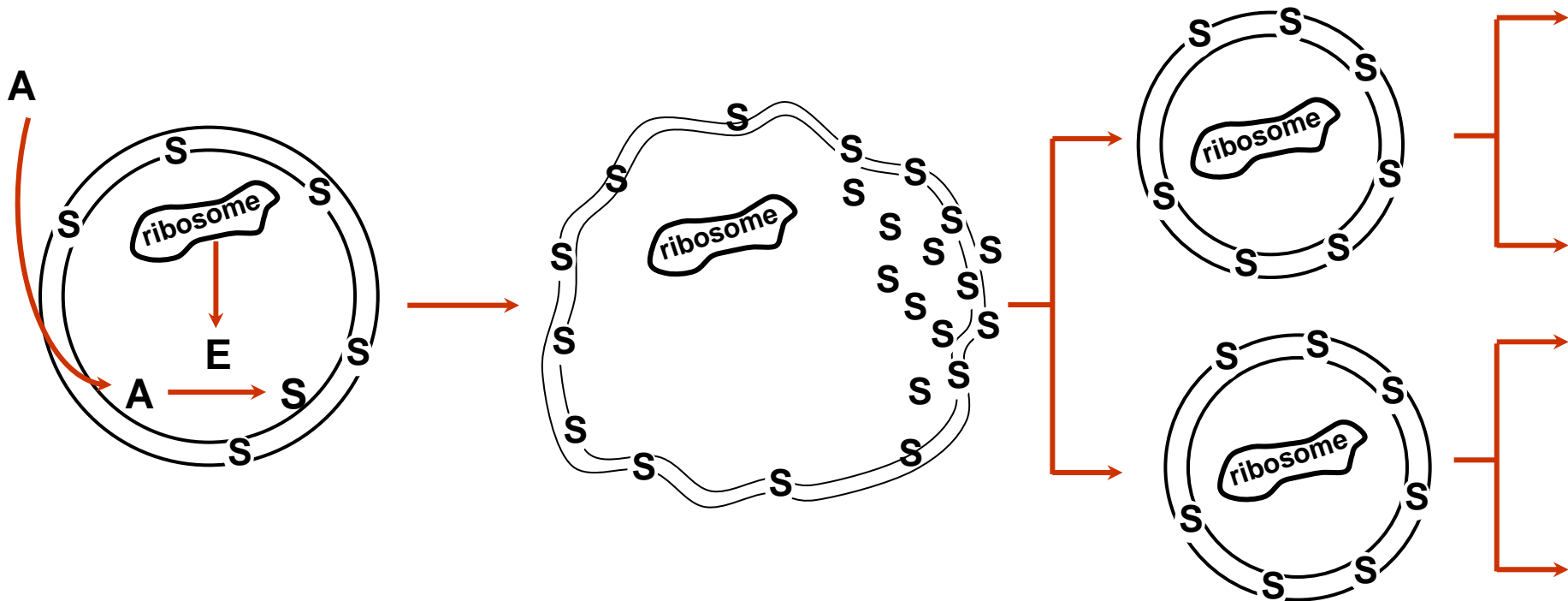
Do we need a Beginning?



Protein expression inside the liposomes and next step (minimal cell)

Working plan

3. Production of the boundary as in 2., and with self-reproduction of the ribosomes and of the DNA-machinery



How many genes are then necessary?

***CRESCITA
E
DIVISIONE***

**Under study-not yet
done**

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)

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

1. Life originated from inanimate matter as a spontaneous and continuous increase of molecular complexity. Chemical continuity principle - no transcendent 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 the conditions.
4. Since there is no documentation on how things really happened, there is no obligatory research pathway.

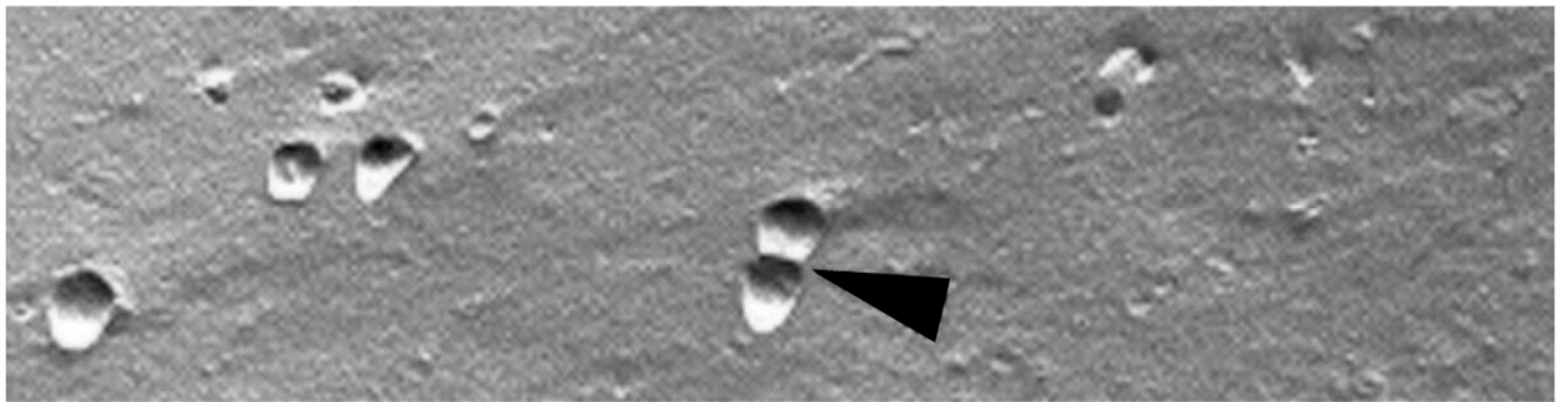
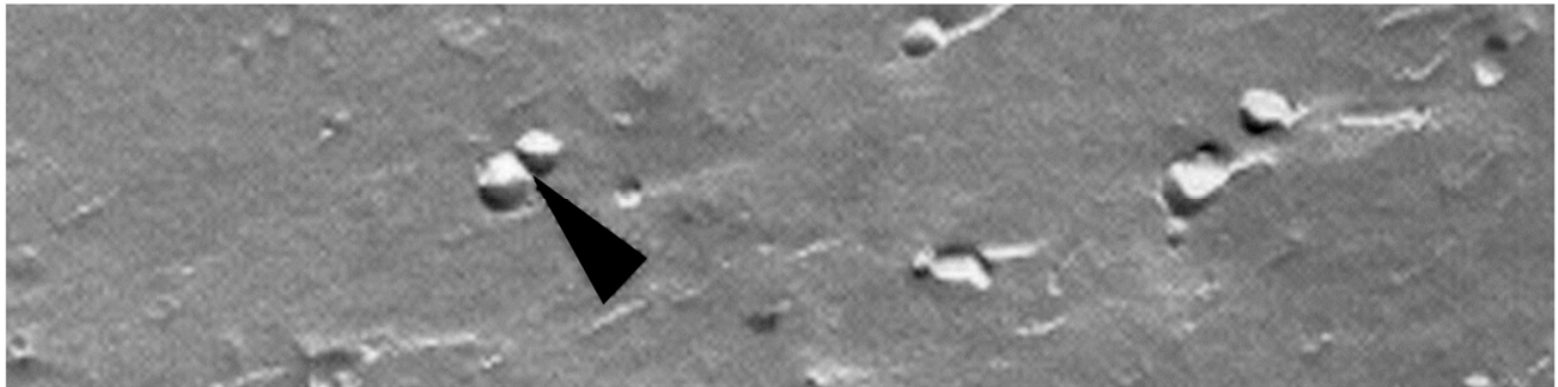
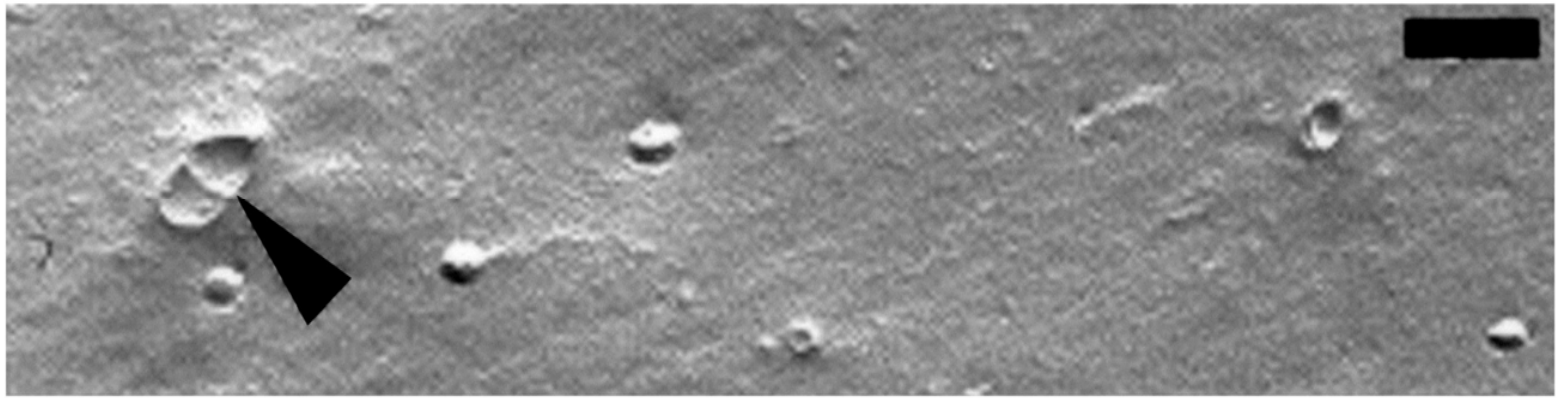
LIFE



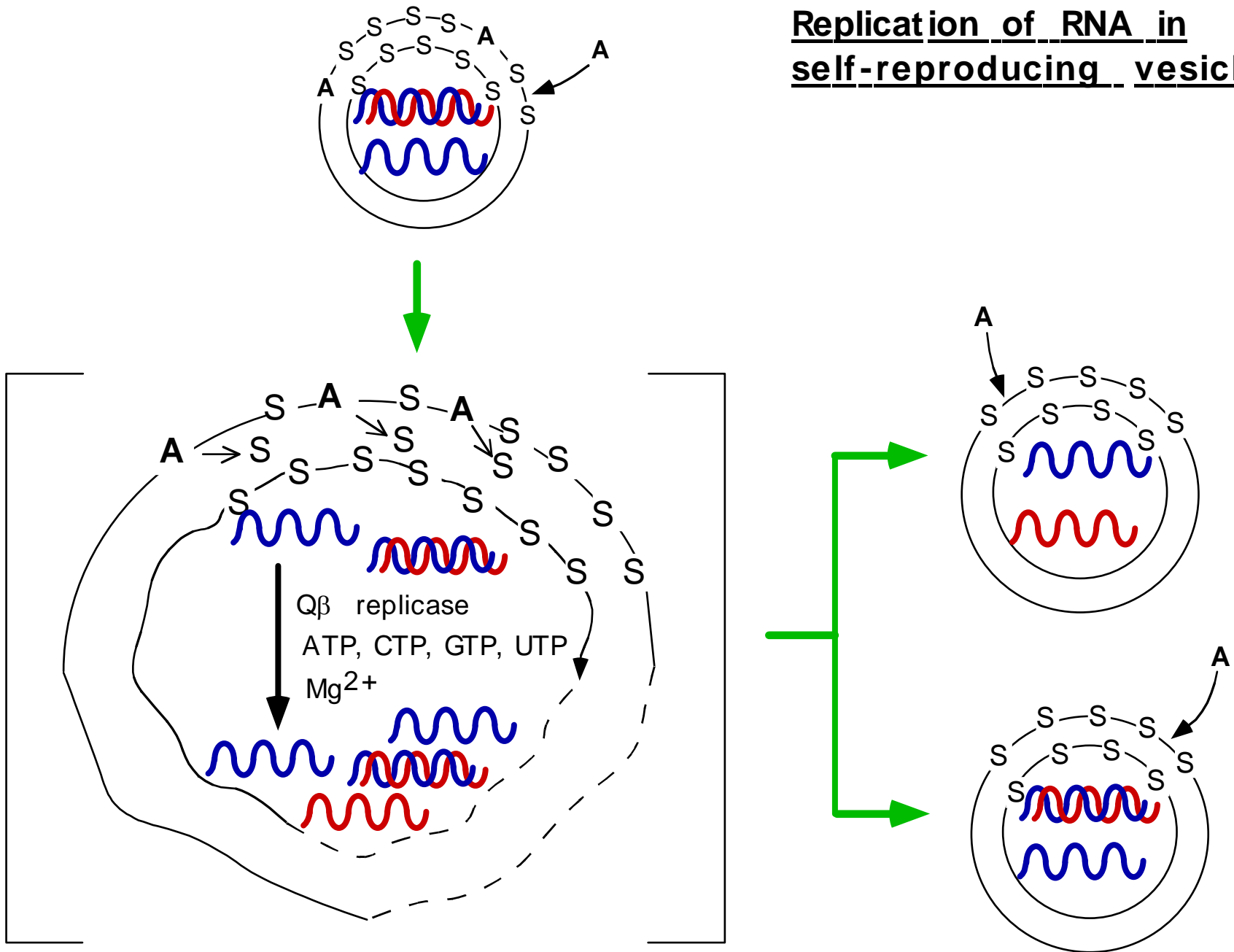
**inanimate
matter**

**To start this project in the laboratory, you need
a „compartment“**

**Which is a good model for the biological cellular
membrane**



Replication of RNA in self-reproducing vesicles



Experimental observation:

„Our“ proteins have nothing special from the folding or stability point of view

Temptative conclusion :

Lacking special features, they are probably the products of contingency

Another conclusion about the reconstruction of life from prebiotic molecules:

The re-making of our present macromolecular sequences is made difficult or impossible by the laws of contingency :

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

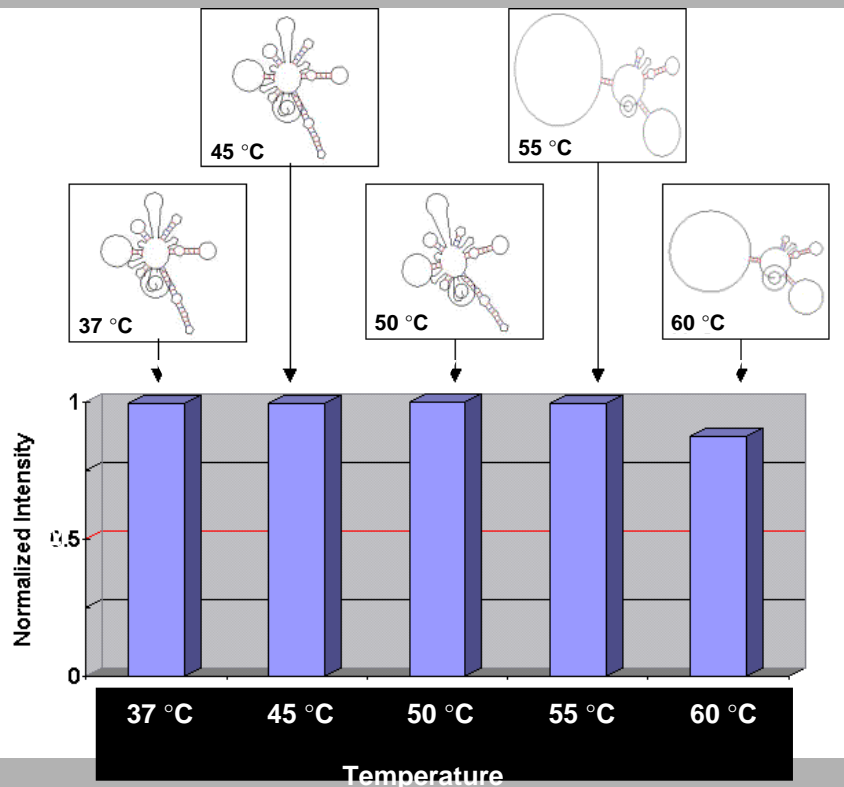
People have done so by taking different approaches

The never born Rnas

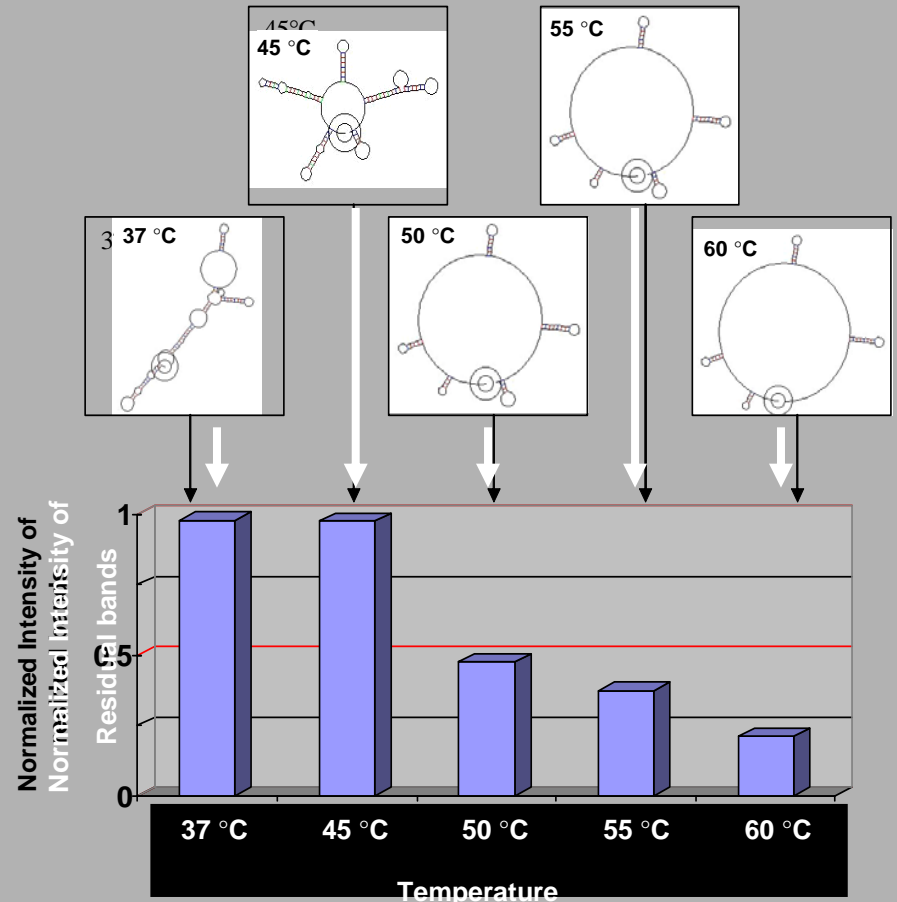
And the study of their
folding/stability

Davide de lucrezia

RNA S1-DIGESTION EXAMPLES



Clone p32

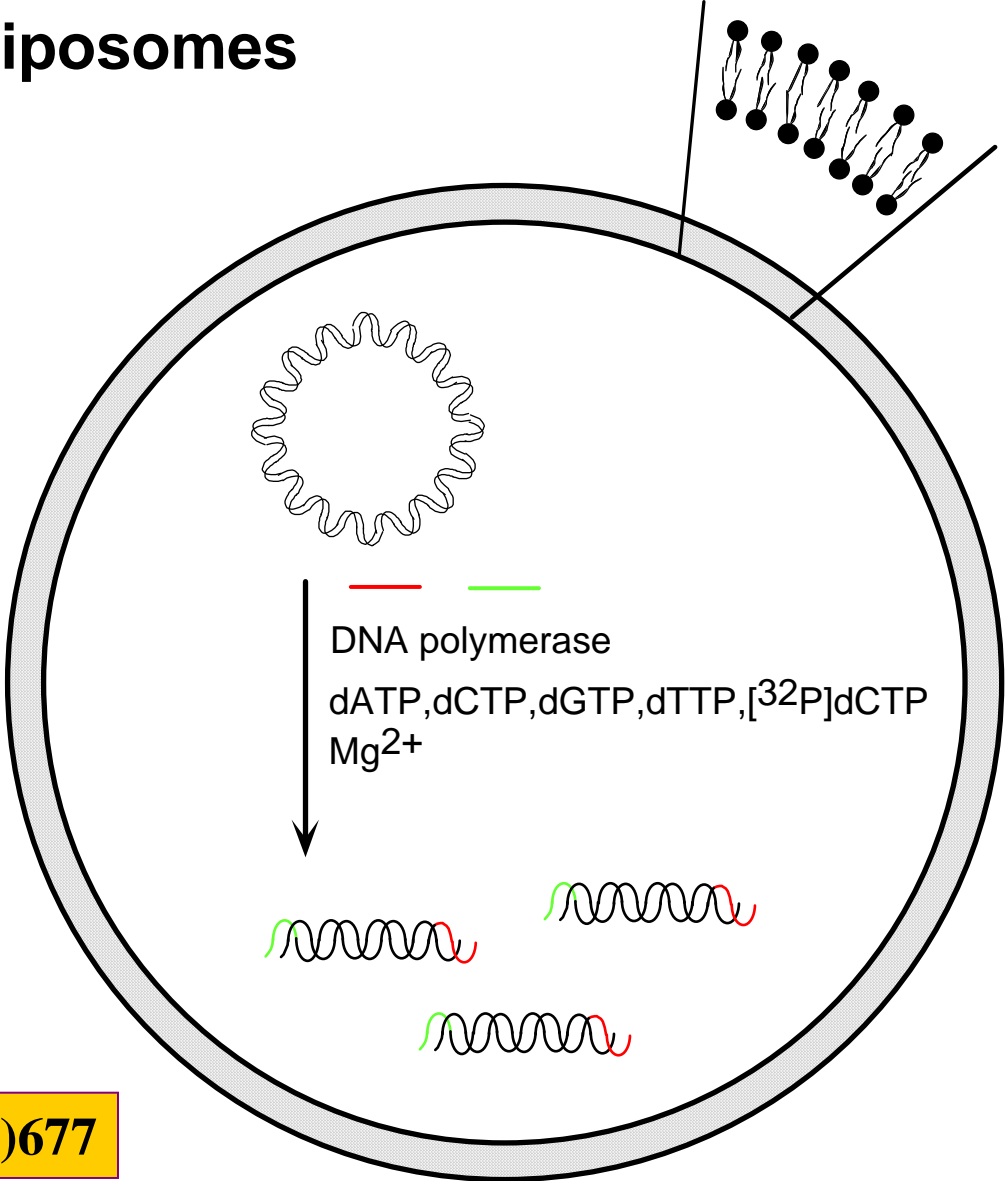


Clone p33

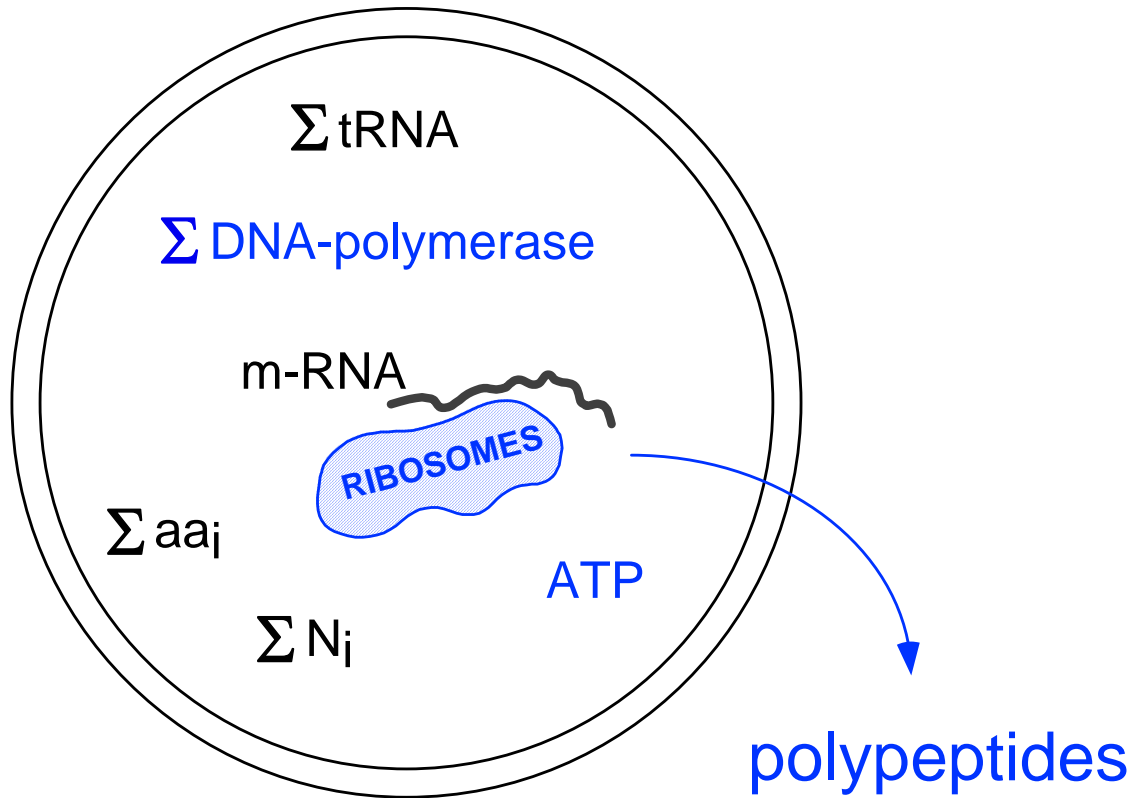
EU PROJECT ON THE MINIMAL CELL, FW 6

- A COLLABORATION BETWEEN
- ALFRED FAHR, JENA
- WOLFANG WEIGAND MUENICH
- PETER NIELSEN DENMARK
- PETER STRAZEWSKI, LYON
- P.L. LUISI, ROME

PCR in liposomes



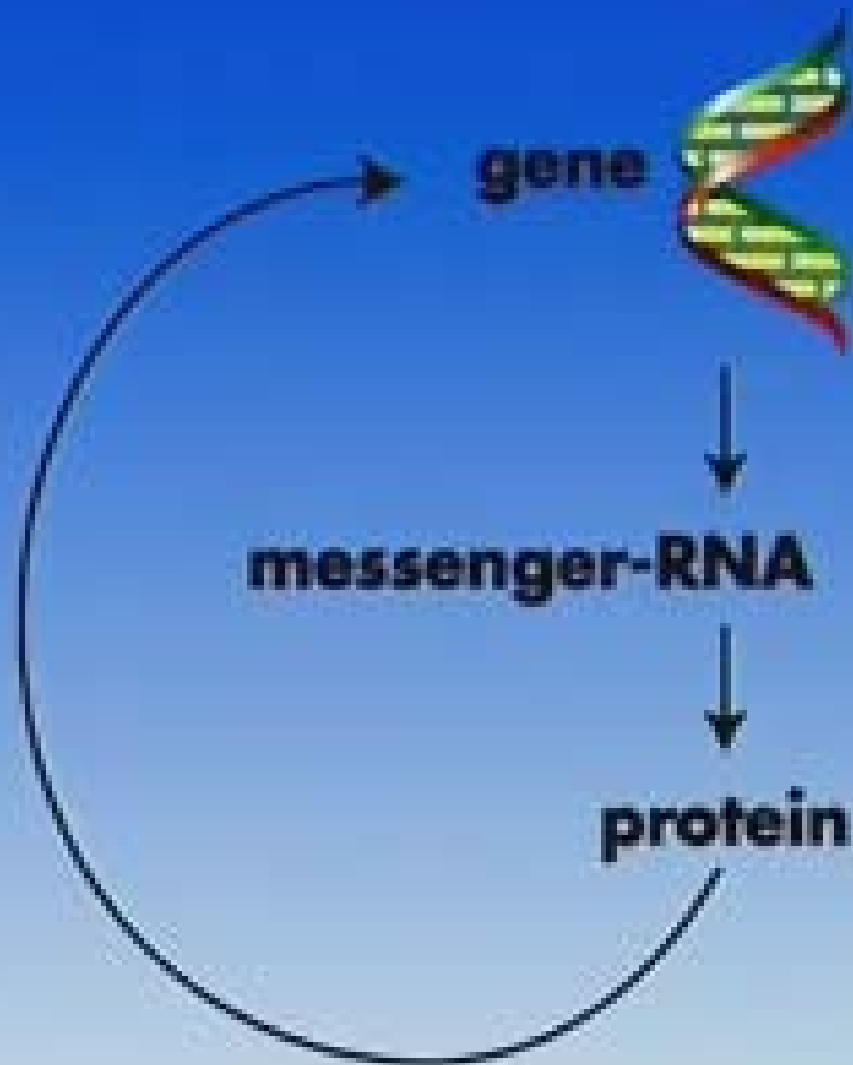
protein biosynthesis in liposomes



Oberholzer et al., 1999
(only poly-phe)

- **How far is it master?**
- **That is irrelevant. Hold your tongue and walk!**





FROM THE PRAGMATIC POINT OF VIEW

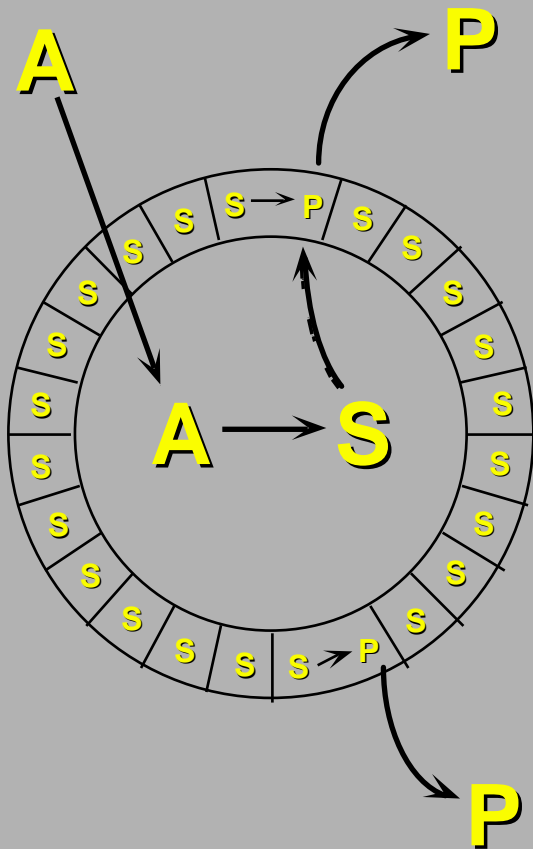
- IT PERMITS TO CONCEIVE AND
CONSTRUCT CHEMICAL
MODELS IN THE LABORATORY

About the philosophical framework

IS THE ORIGIN OF LIFE ON EARTH AN
OBLIGATORY
PATHWAY (IT HAD TO HAPPEN!)
OR
IS IT DUE TO CHANCE?

Determinism vs contingency
in the origin of life

THE MINIMAL AUTOPOIETIC SYSTEM



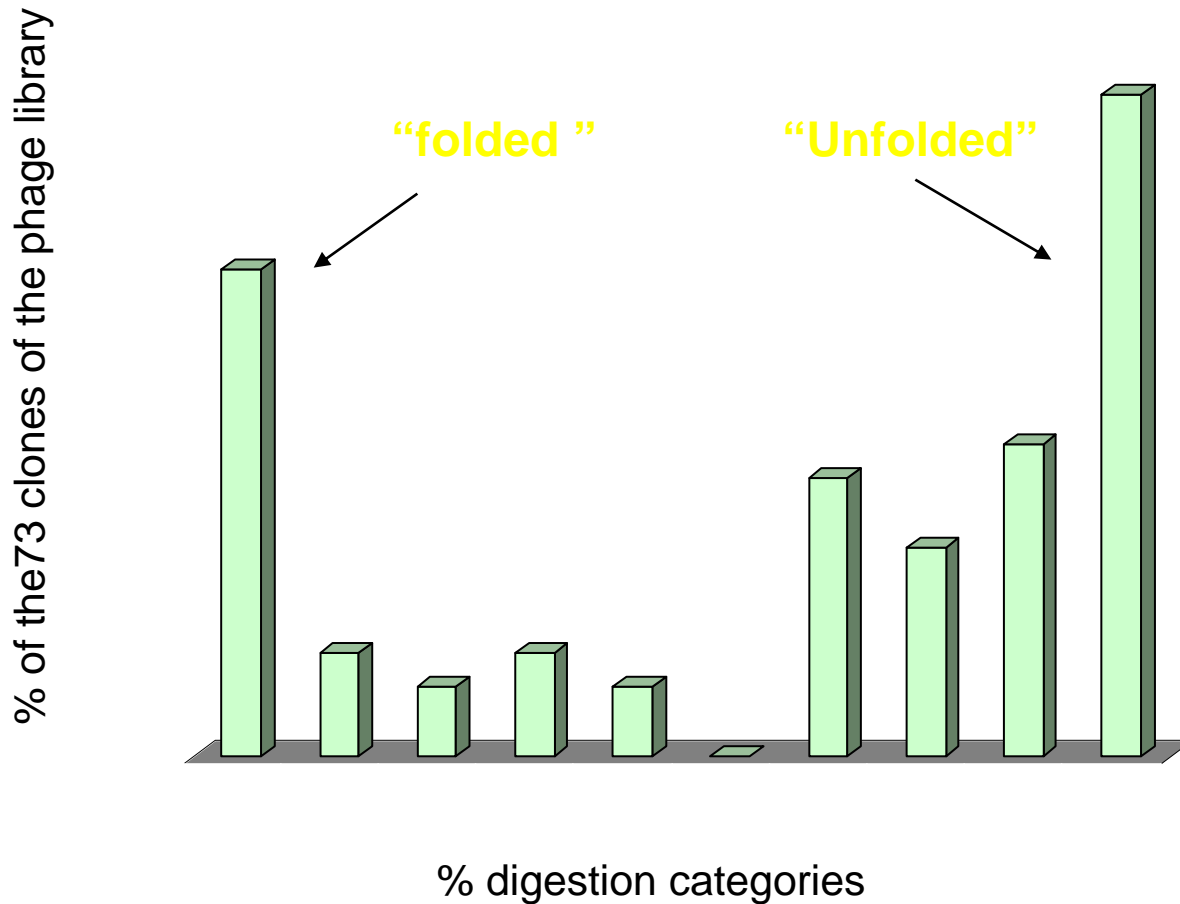
$$v_{\text{gen}} = \frac{d[S]}{dt} \quad ; \quad v_{\text{dec}} = -\frac{d[S]}{dt}$$

if $v_{\text{gen}} = v_{\text{dec}}$ **homoestasis**

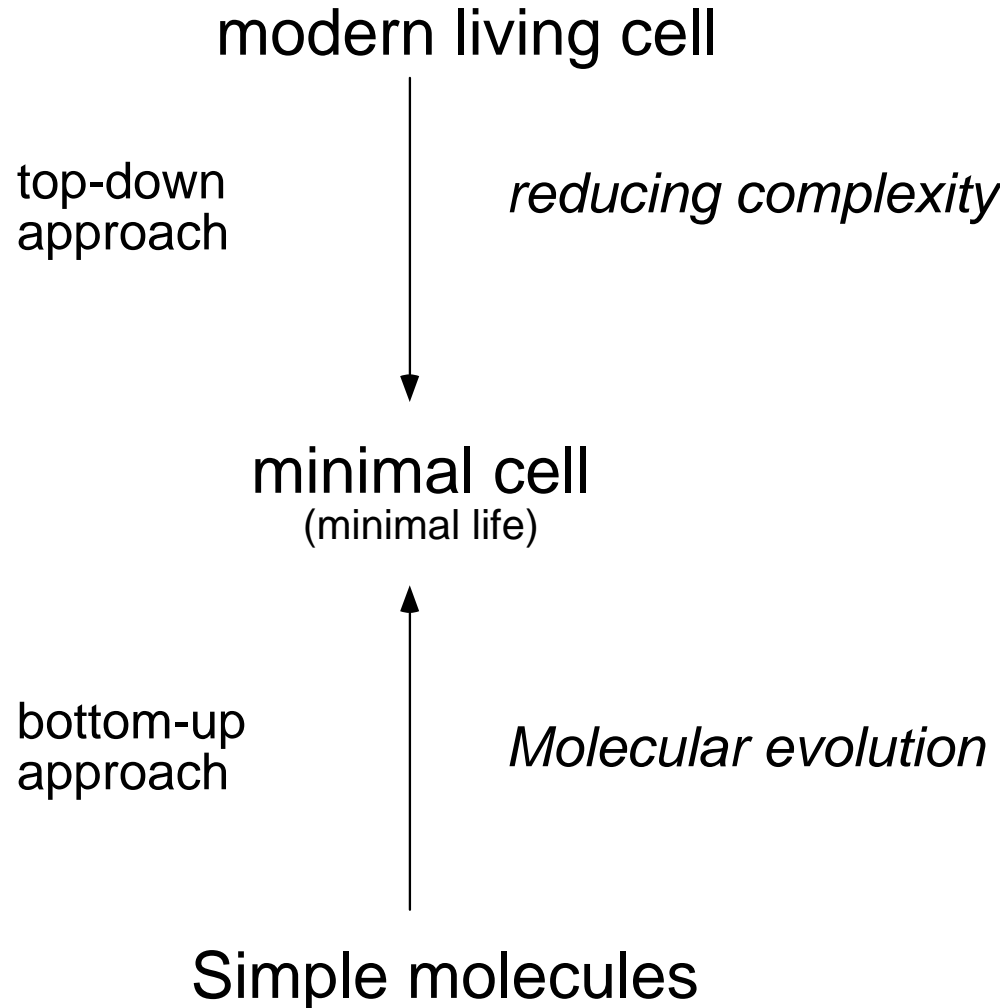
if $v_{\text{gen}} > v_{\text{dec}}$ **self - reproduction**

if v_{gen} less than v_{dec} , death

DISTRIBUTION OF THE PEPTIDE LIBRARY WITH RESPECT TO THROMBIN DIGESTION



two working directions



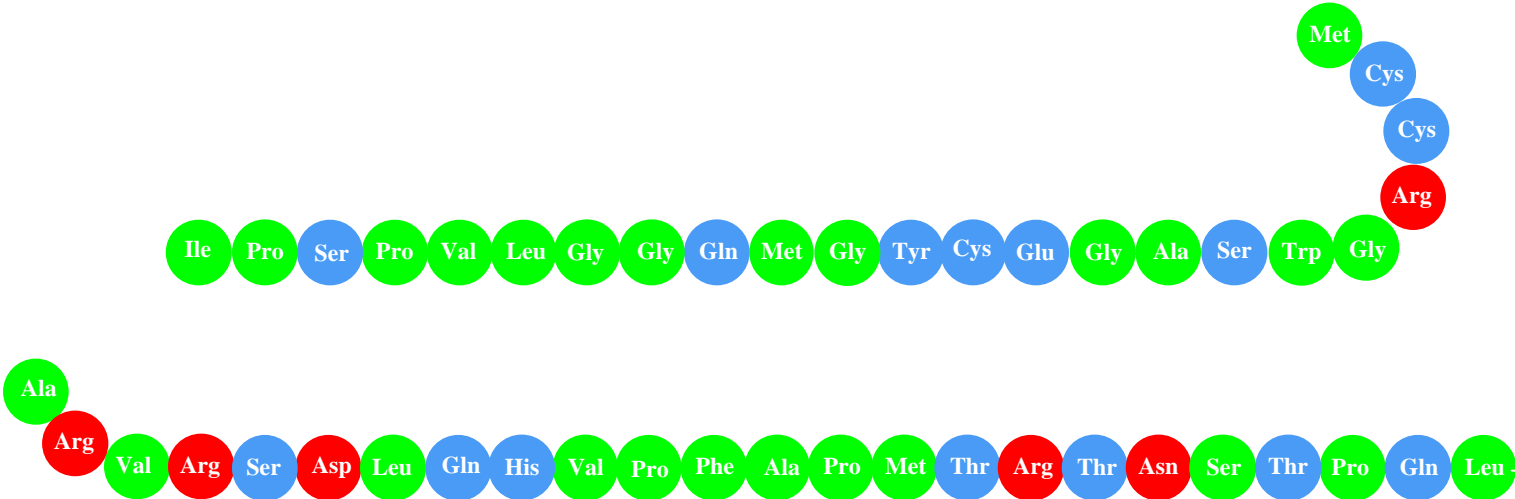
**THE ALTERNATIVE APPROACH
TO THE CONSTRUCTION OF THE
MINIMAL CELL:**

**USE THE EXTANT NUCLEIC ACIDS
AND PROTEINS**

**..this is not the approach to the origin of
life**

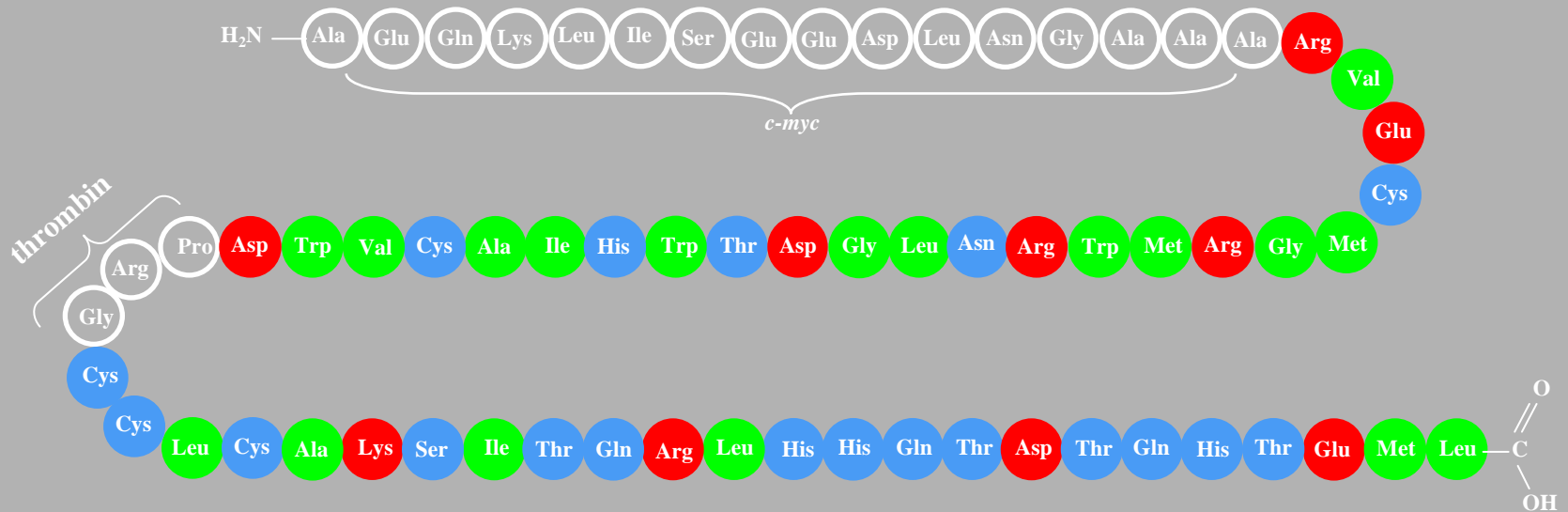
**But an approach to make cellular life
from non-living components**

POLYPEPTIDE NBP1 PRIMARY SEQUENCE

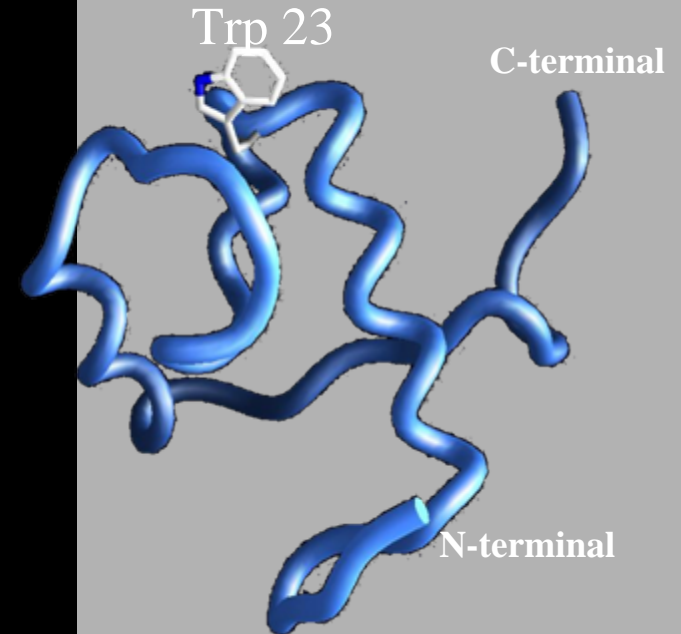
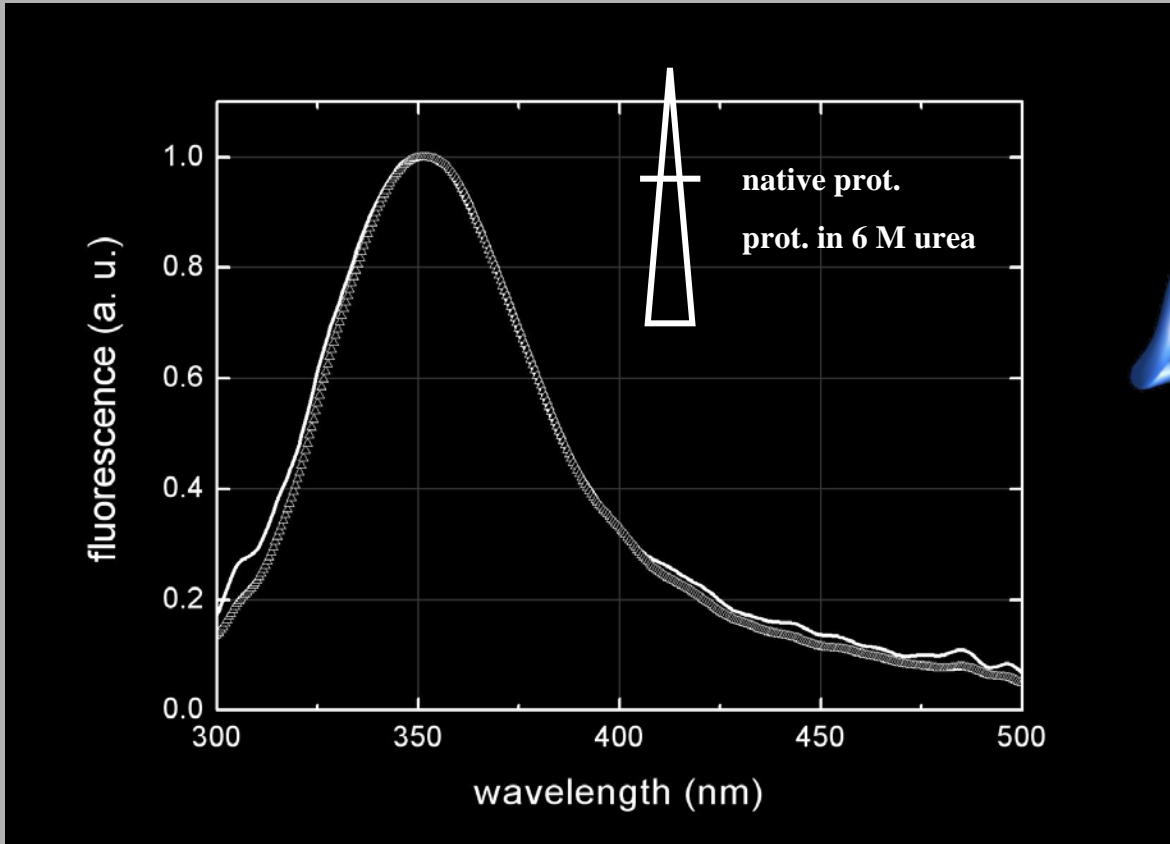


CHIARABELLI ET AL., 2006

POLYPEPTIDE NBP127 PRIMARY SEQUENCE

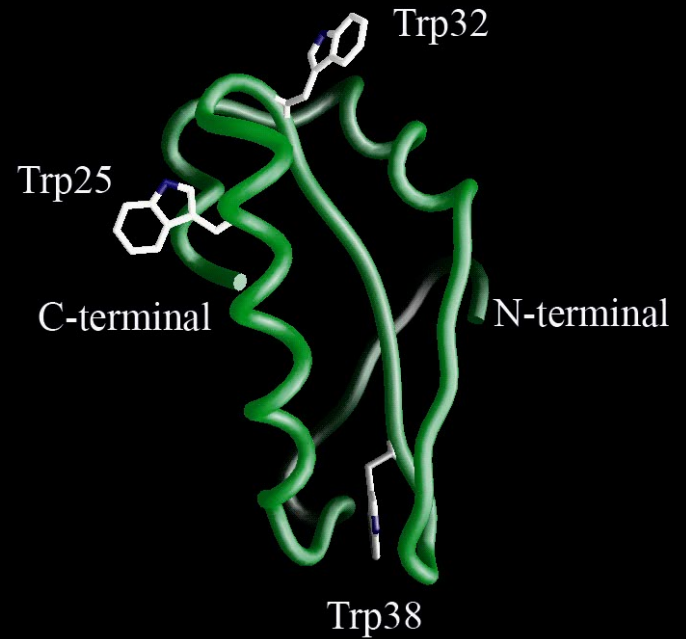
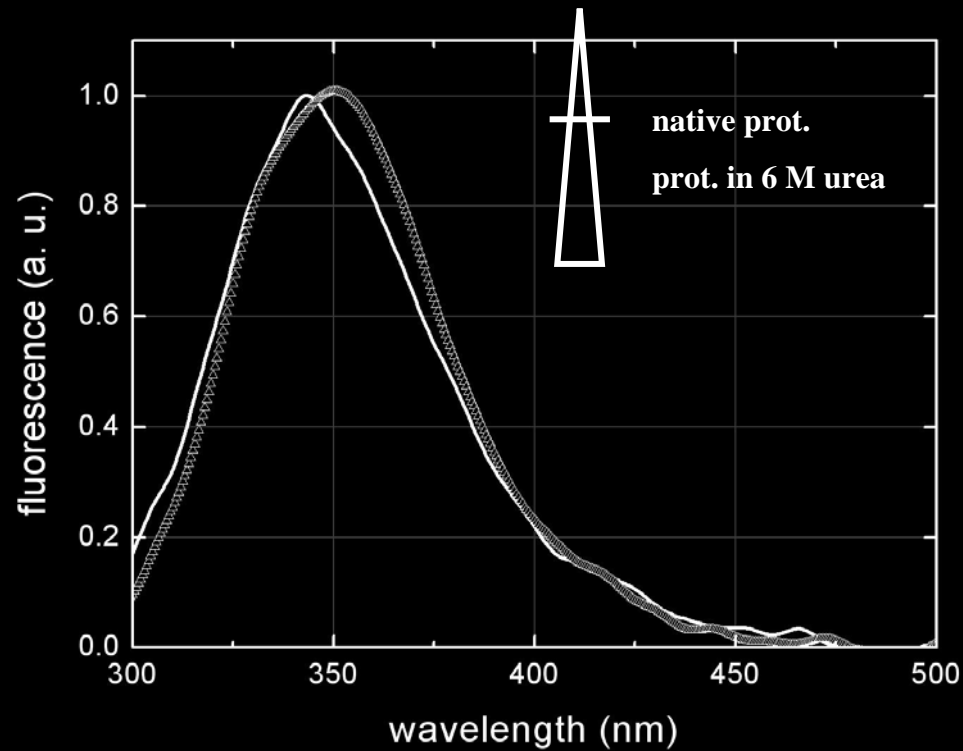


FLUORESCENCE STUDIES NBP1

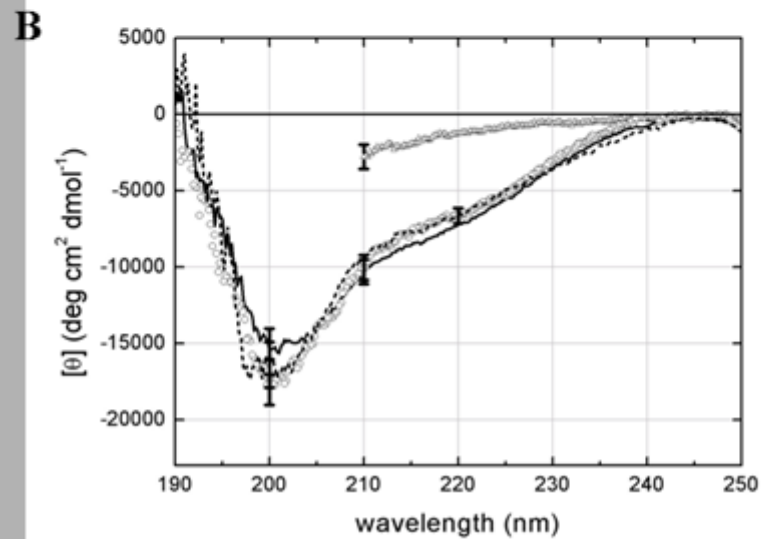
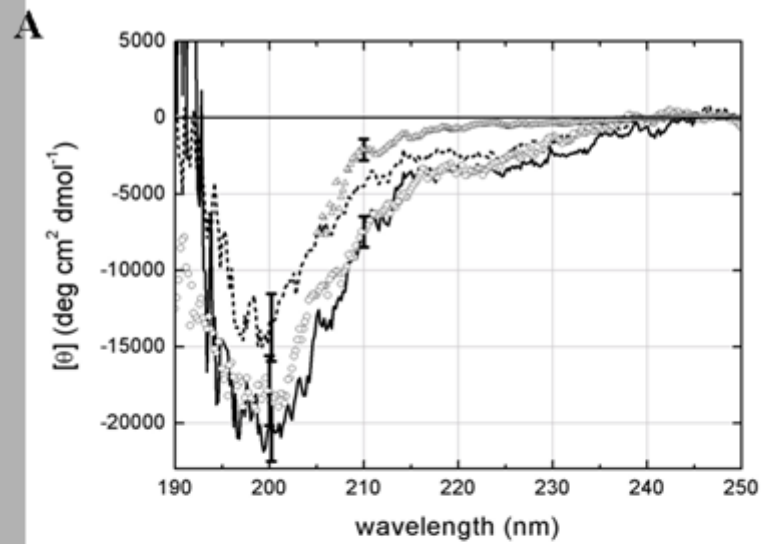


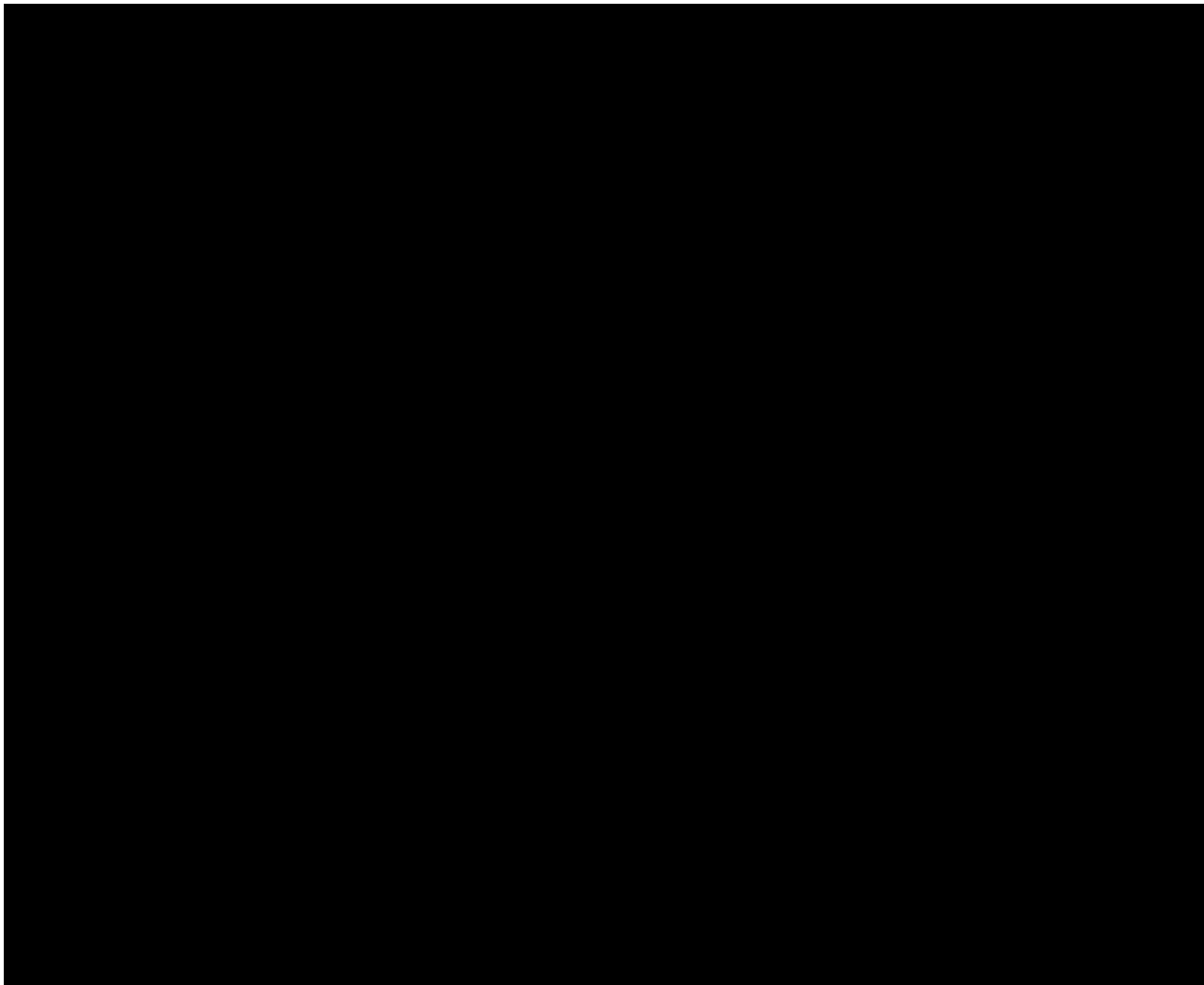
- The Trp residue could be exposed to the solvent

FLUORESCENCE STUDIES NBP127



One Tryptophan residue is localized in a zone less exposed to the solvent





IN PROGRESS

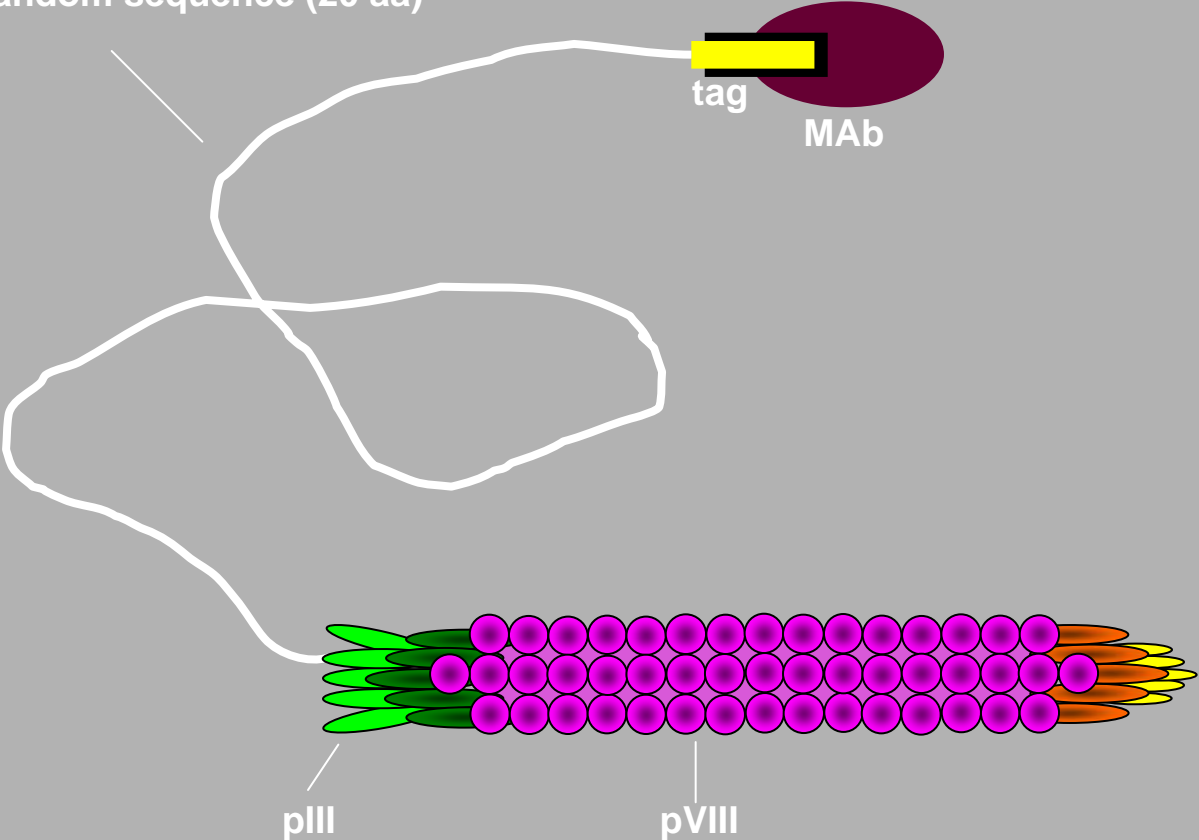
- Library of random peptides with 20 residues
- ...and corresponding library of RNA with 60 residues
- (Davide de Lucrezia and Fabrizio Anella)

The work on the minimal living cell
Is connected with the study of the
early cells - namely the protocells at
the time before the advent of ribosomes
and before the high selectivity
of modern times —



PHAGE DISPLAY

Random sequence (20 aa)



tag

MAb

pIII

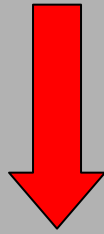
pVIII

(Schematic drawing not to scale)

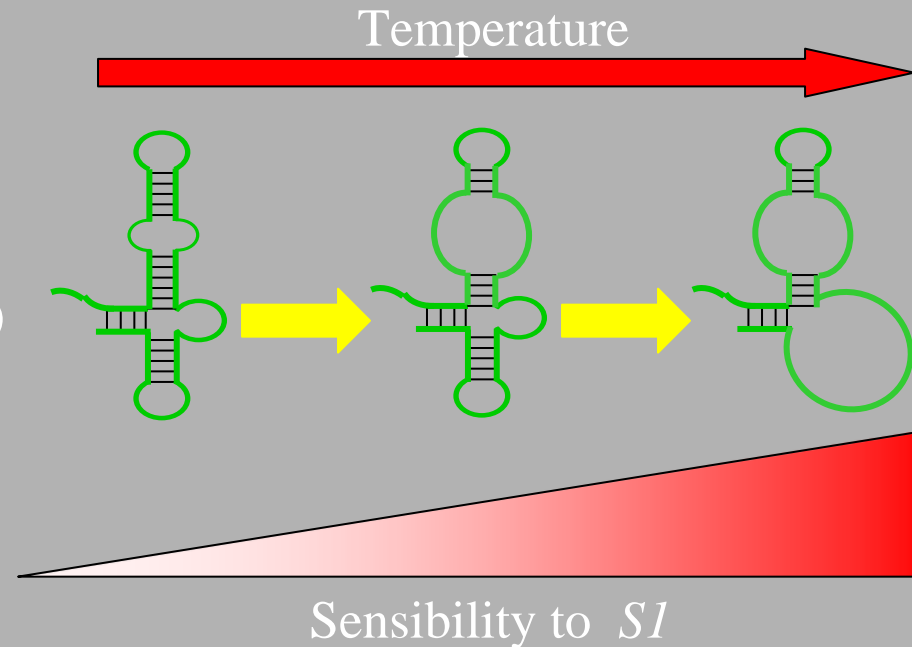
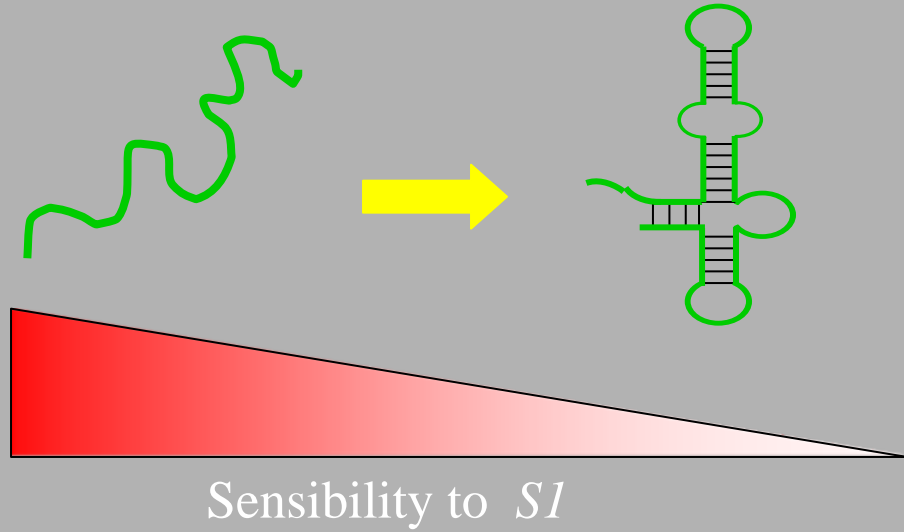
Methodological approach

*Use of RNase S1,
single strand specific*

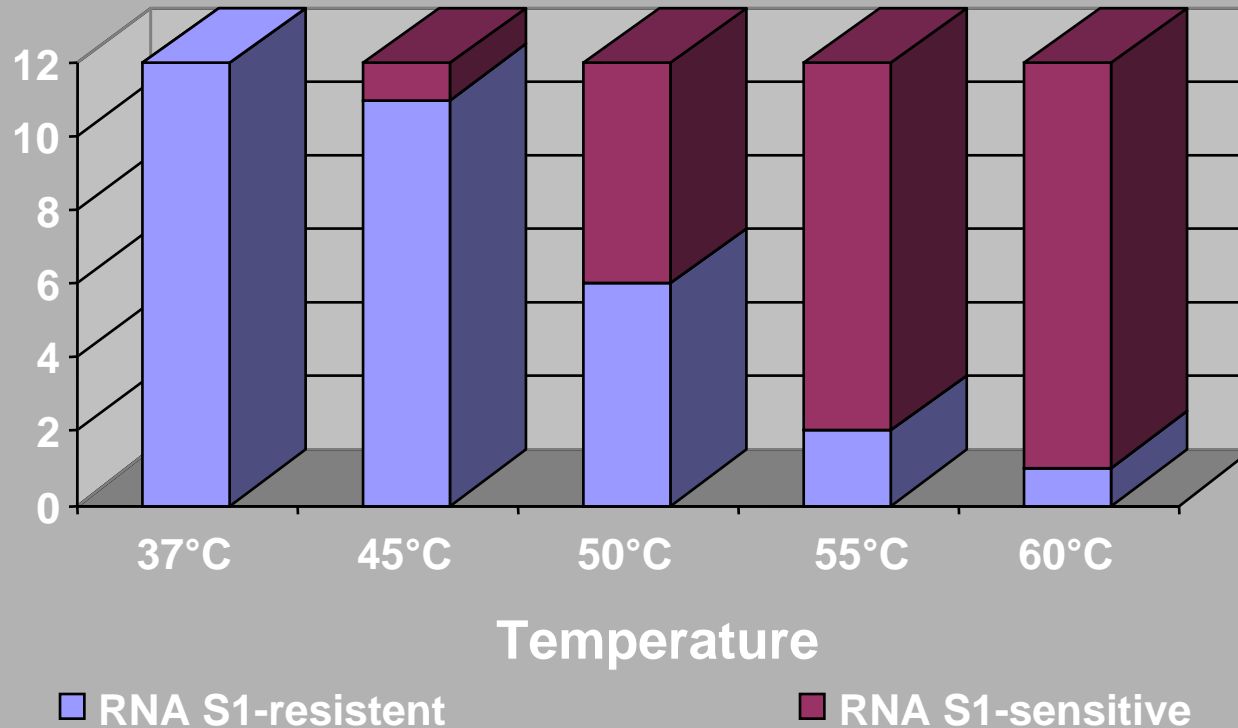
*S1 mapping: analysis of single
strand domains at different
temperatures*



RNA Folding Stability Test (RNA Foster)



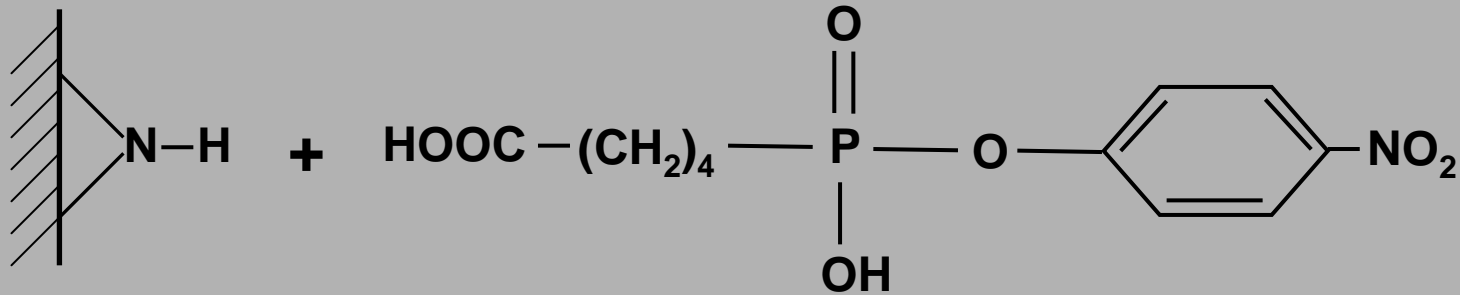
Stability of structural domains



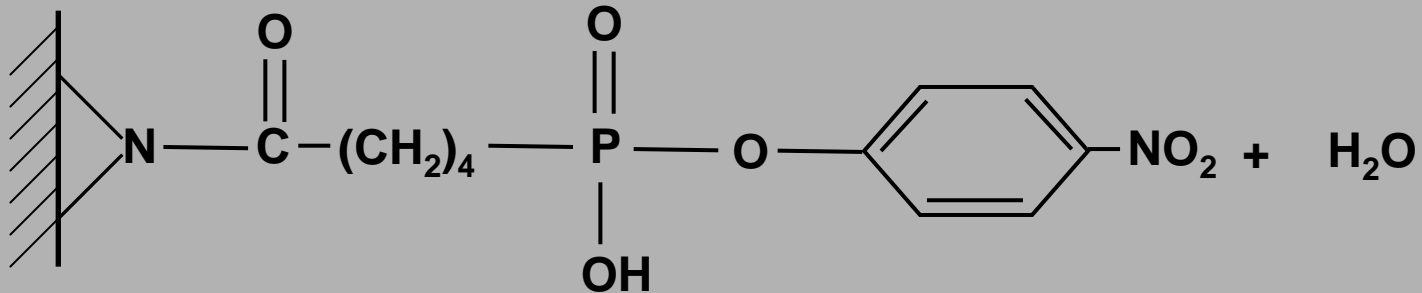
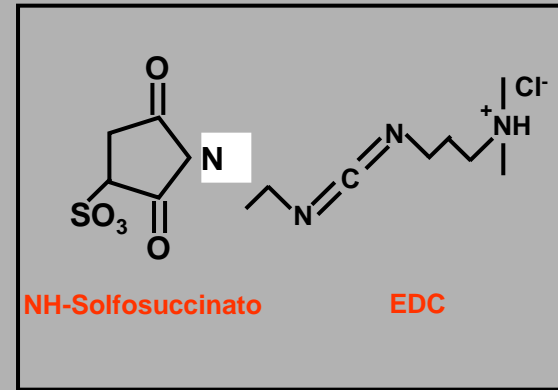
- Tutti gli RNA analizzati mostrano domini strutturali resistenti alla S1 a 37°C
- La T_m media si colloca nell'intervallo 45°C – 50°C
- Presenza di RNA con domini strutturali stabili oltre i 60°C

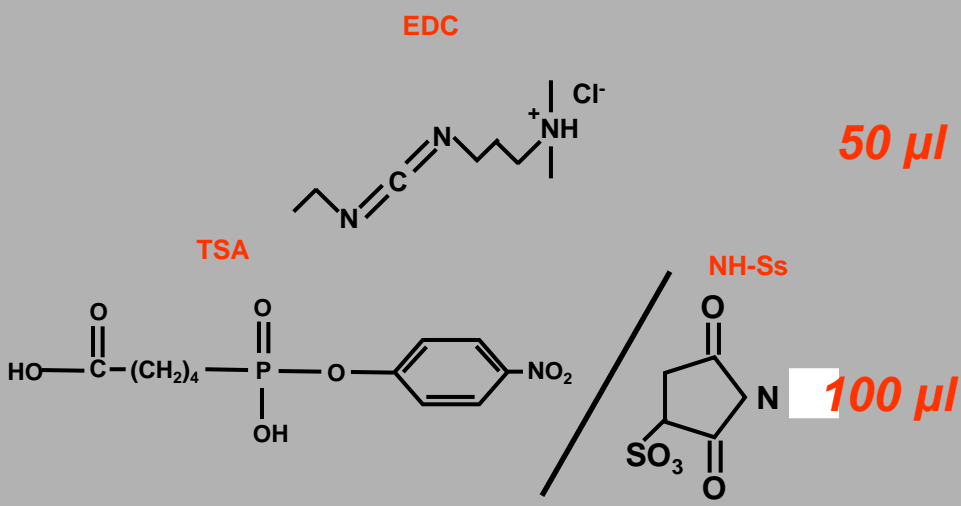
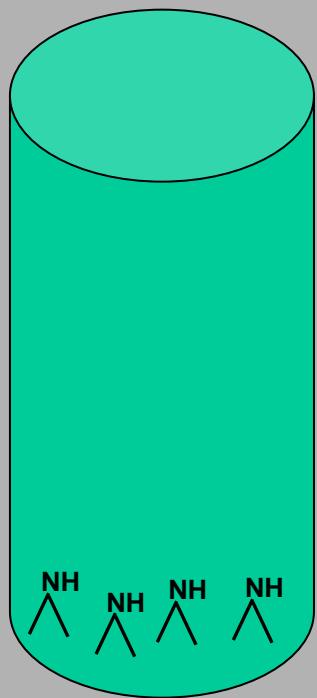
Ammina
secondaria
della piastra

Transition state analoge



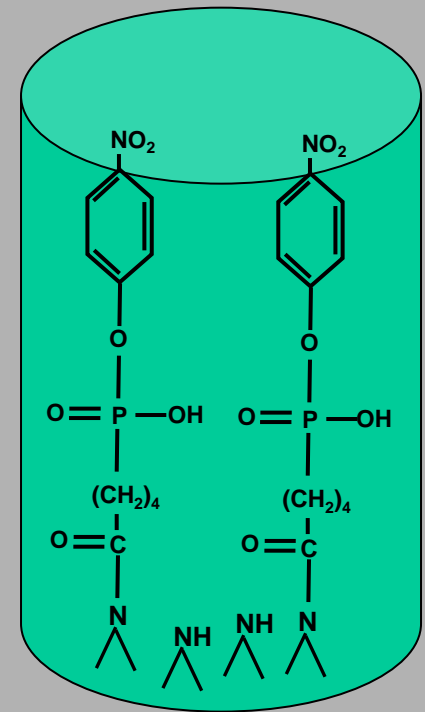
NHSS
EDC
120' r.t.

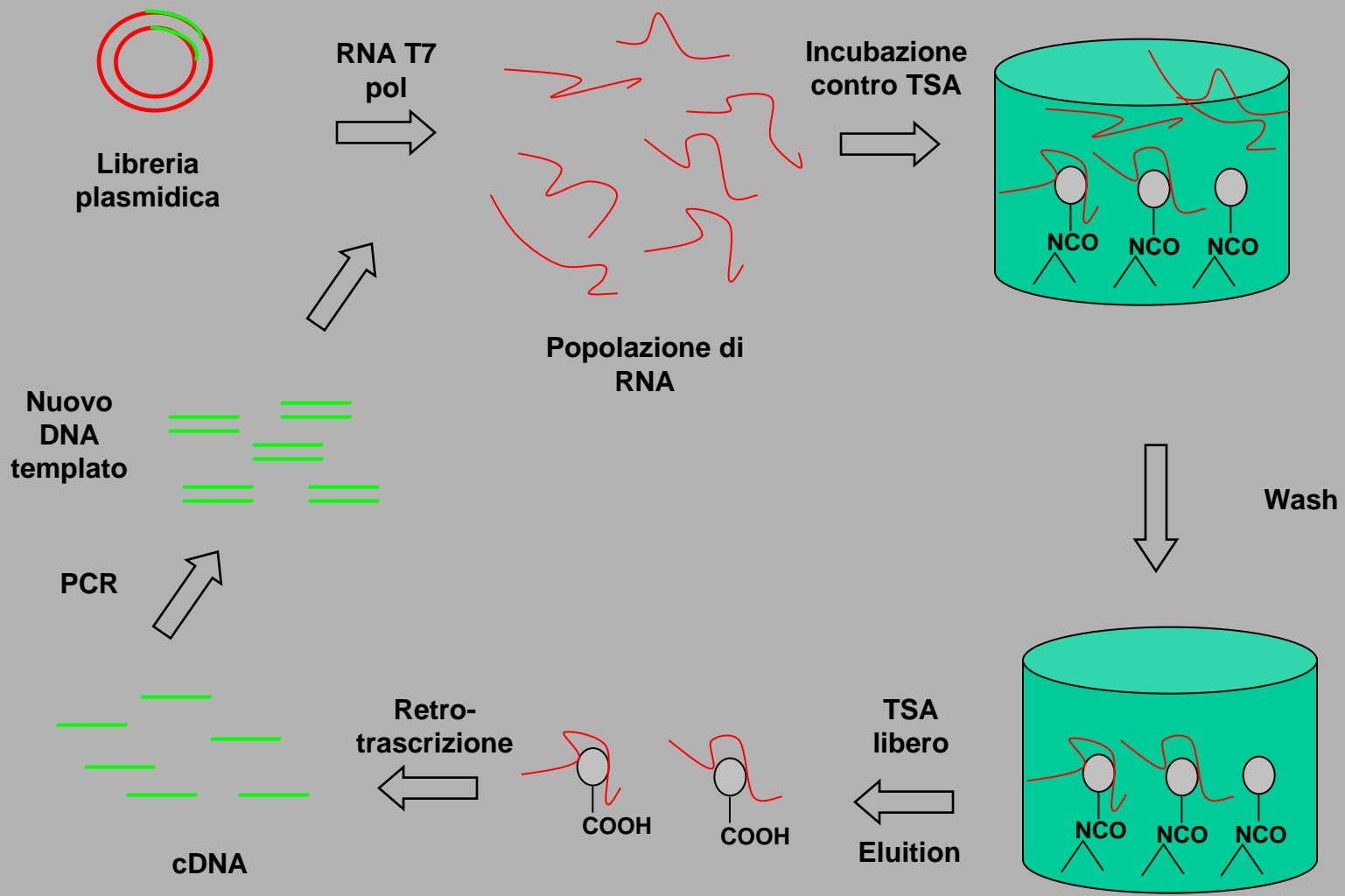


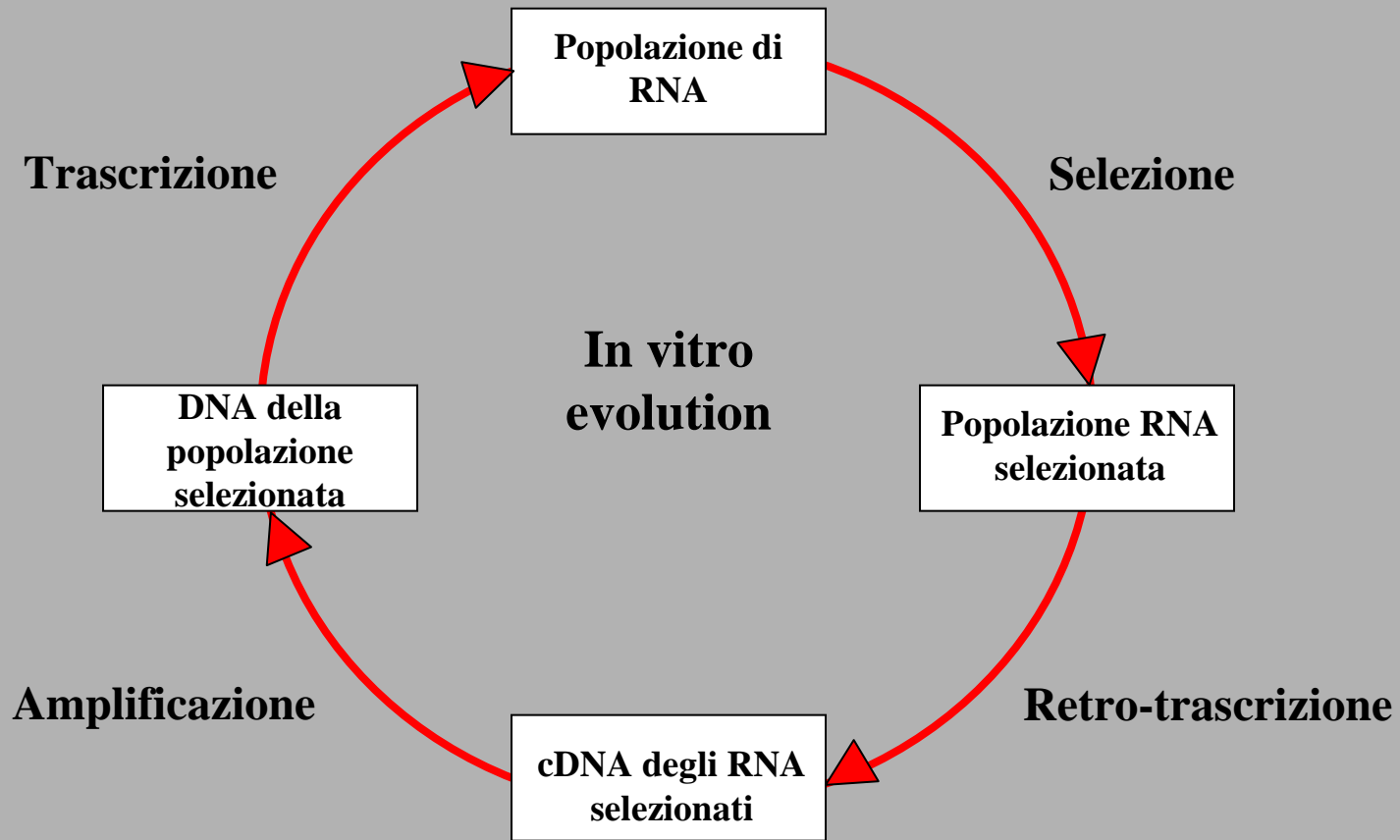


50 μ l DEPC-Water

90' sotto agitazione a temperatura ambiente







THE GAME OF THE TWO LISTS

LIST OF THE LIVING

The fly
The tree
The mule
The baby
The mushroom
The amoeba

LIST OF THE NON-LIVING

The radio
The automobile
The robot
The crystal
The moon
The computer

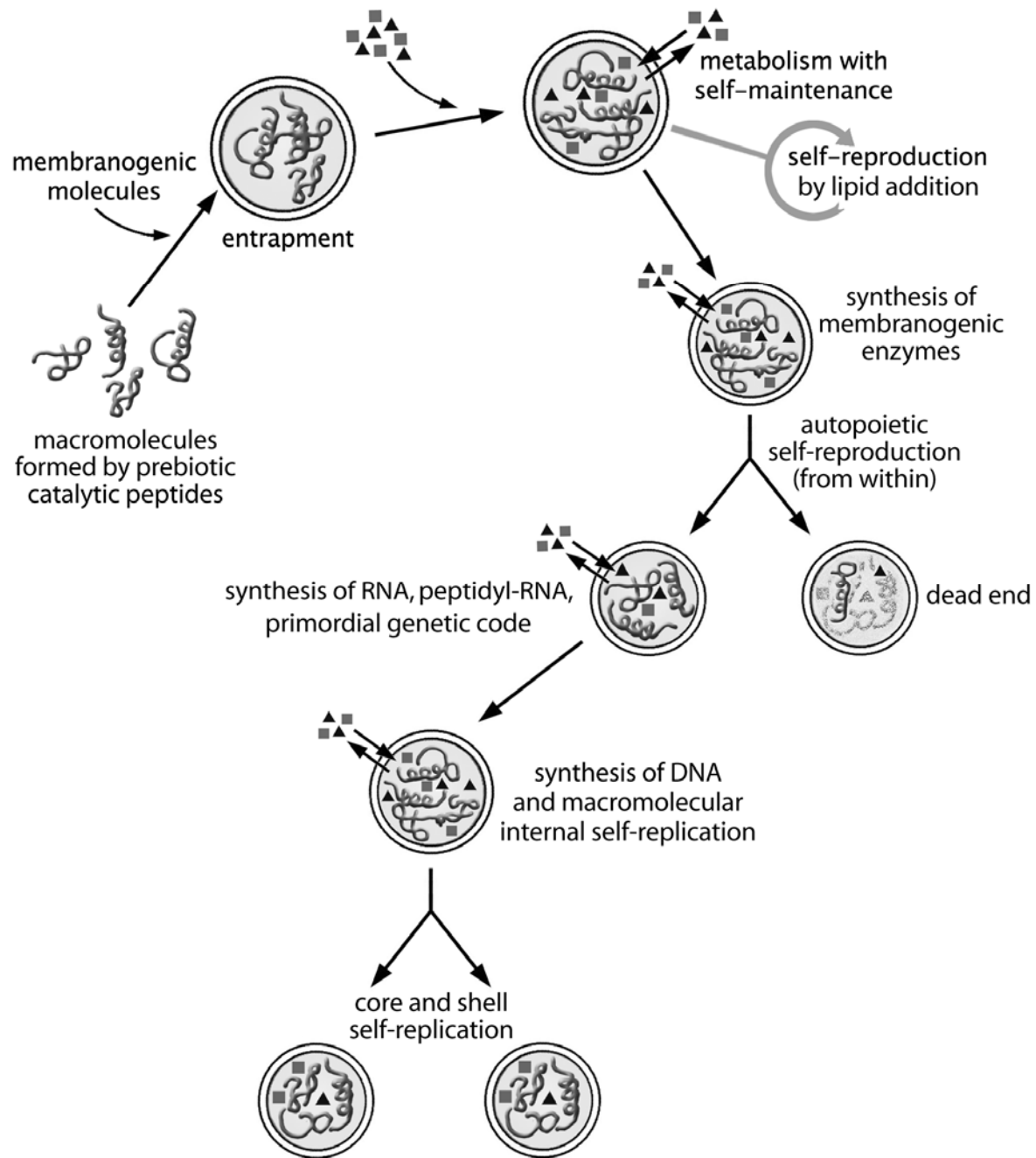
QUESTION: What discriminates the living from the non-living?

IN OTHER WORDS: What is the quality (or qualities) which is present in all members of the "living list" and which is not - and cannot - be present in any of the elements of the "non-living" list?

(movement? growth? reaction to stimuli? reproductivity? processing of energy arriving from outside? etc....)

**THE MAIN ASSUMPTION OF THE SANTIAGO SCHOOL
IS THE EQUIVALENCE BETWEEN THE PROCESS
OF COGNITION
AND THE PROCESS OF LIFE**

**ACCORDING TO THIS VIEW, BRAIN IS NOT
NECESSARY FOR COGNITION: BACTERIA AND
PLANTS DO NOT HAVE A BRAIN,
THEY POSSESS HOWEVER COGNITIVE CAPABILITY**



Is this life?

Not really.

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

..death by dilution....

PROTEIN EXPRESSION IN LIPOSOMES (MOSTLY GREEN FLUORESCENCE PROTEIN) HAS BEEN DESCRIBED BY SEVERAL GROUPS:

Oberholzer et al., 1999, 2001

Yomo et al., 2001

Tsumoto et al., 2001

Fischer et al, 2002

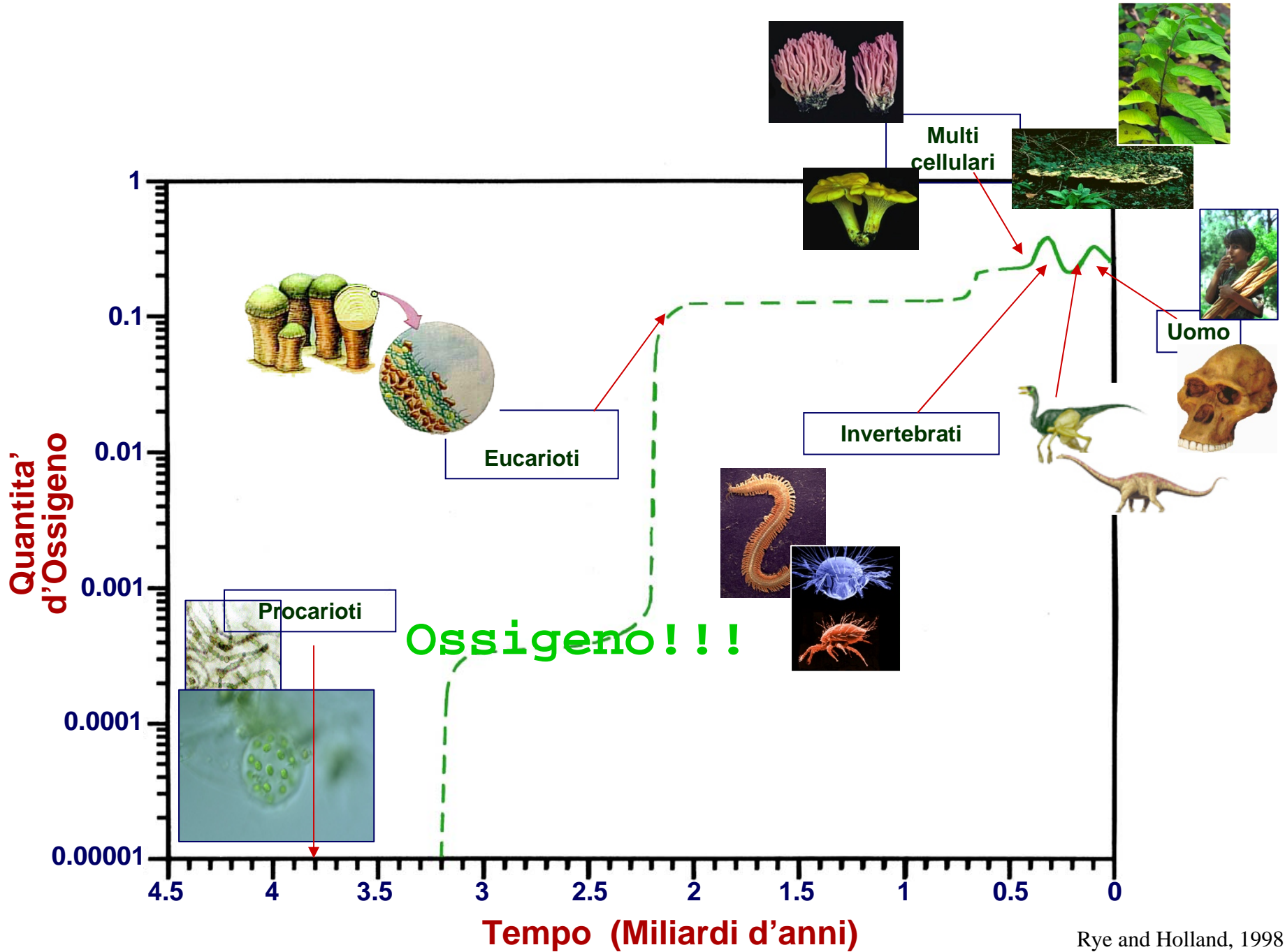
Nomura et al., 2003,

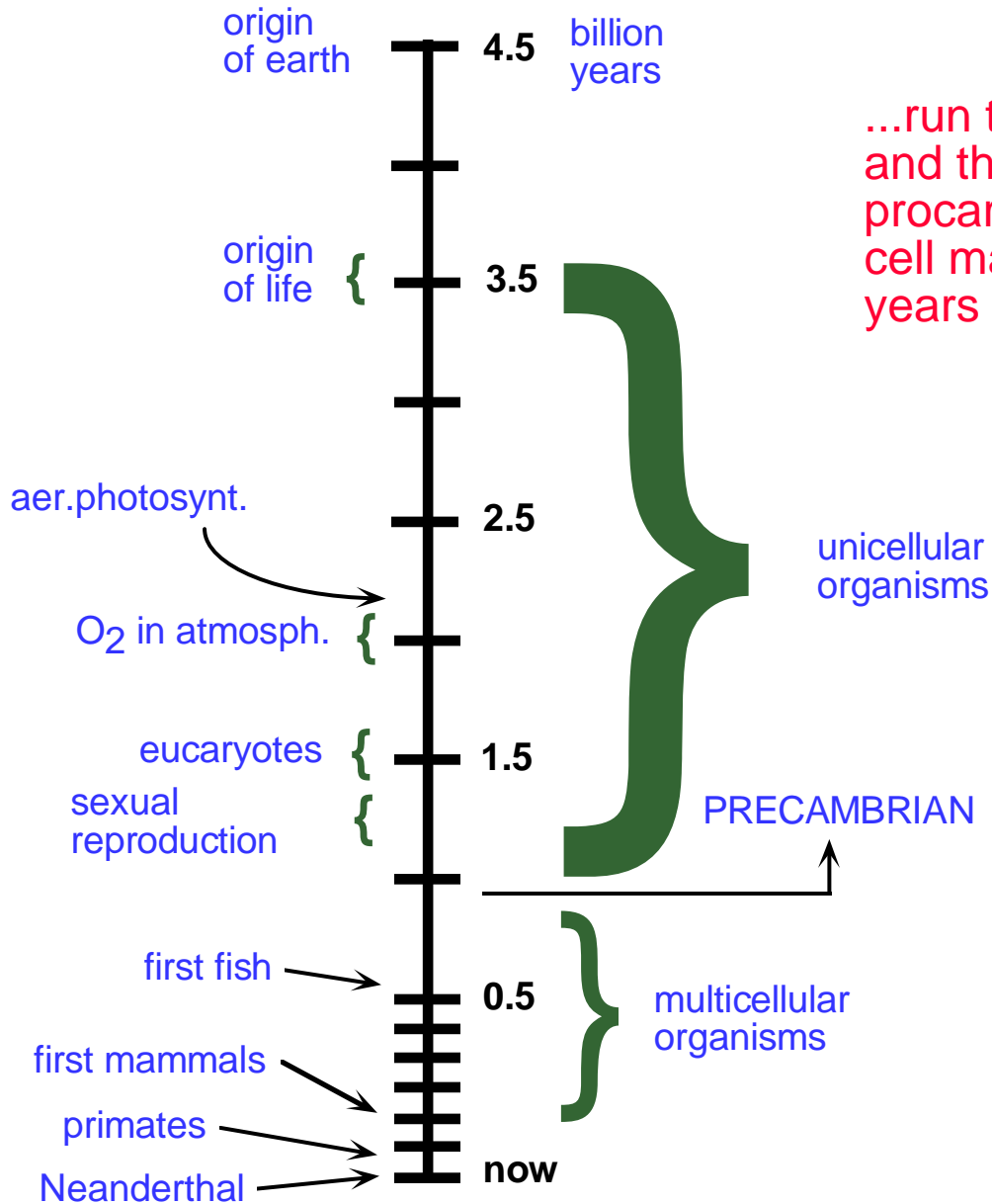
Pietrini et al., 2004

Noireaux et al, 2004

Ishikawa et al, 2004

In Roma3: Pasquale Stano, Giovanni Murtas,
Paolo Carrara e Valentina





...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

..where is the environment in all that?

The contradiction of the living:

Biological autonomy, operational closure

But

dependent from the environment for survival

Maturana and Varela define as „cognition**“ the specific interaction with the environment**

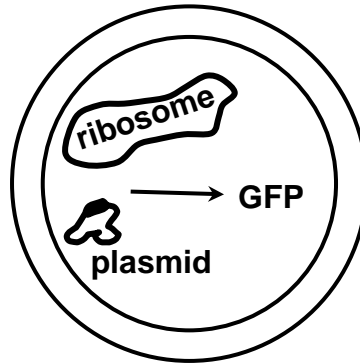
ON THE BASIS OF THE EQUIVALENCE
BETWEEN LIVING STRUCTURE AND
THE ACT OF COGNITION, THE OLD
CARTESIAN
SEPARATION BETWEEN MATTER AND
SPIRIT
IS SUPERSEDED.

THESE TWO CATEGORIES
ARE NO LONGER DISTINCT ENTITIES,
THEY ARE TWO FACES OF THE SAME
PHENOMENON:
LIFE

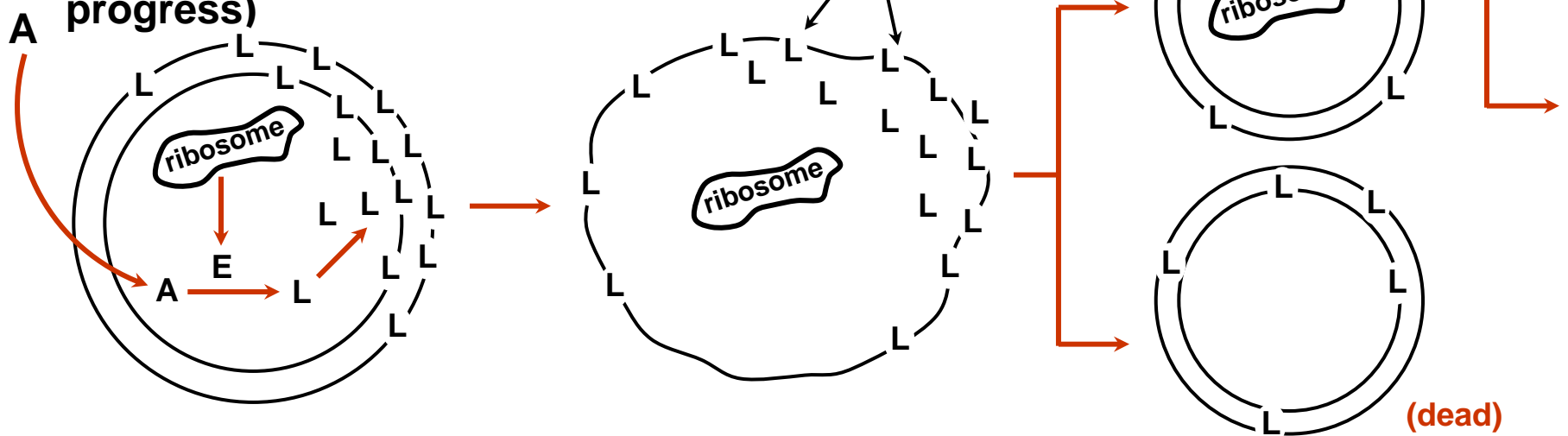
Protein expression inside the liposomes

Working plan

1. Expression of green fluorescent protein (GFP) or any other simple model protein



2. Expression of the enzymes that make the liposome (in progress)



Cast the dice again and.....

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

Important in autopoiesis:

Relationship between structure and self-organization

Self-organization is the invariant property, the relationship between the processes that produce the components

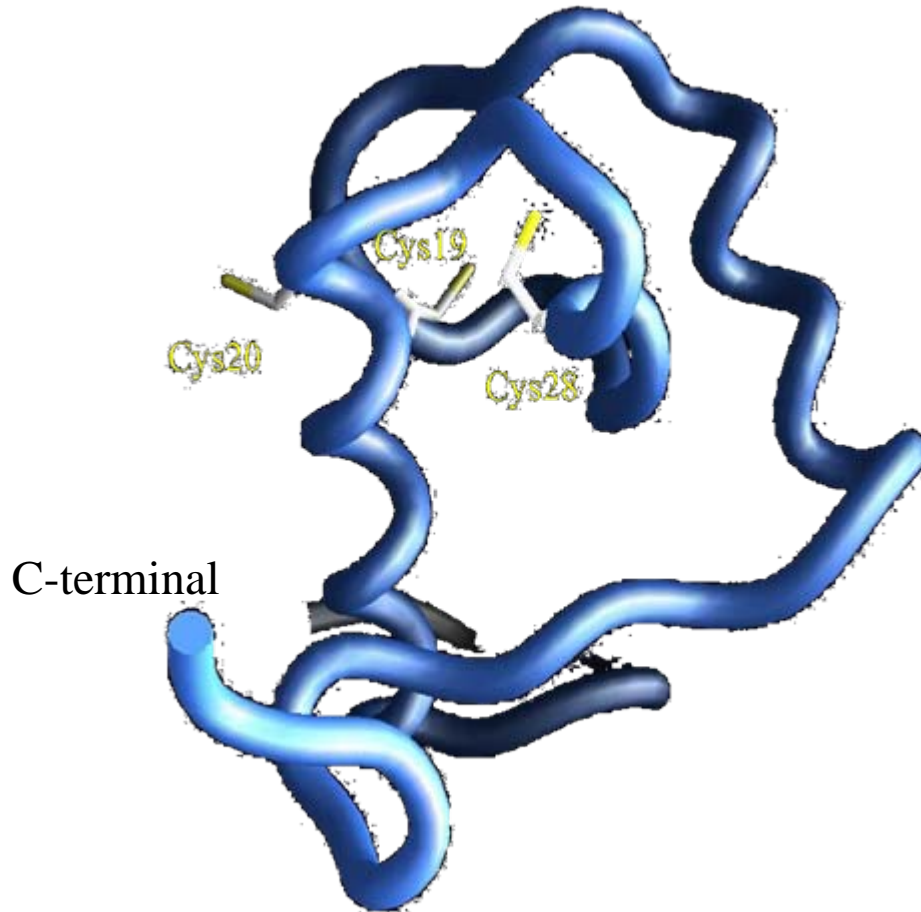
The **structure** may be contingent, and may vary depending on evolution and other perturbations

.

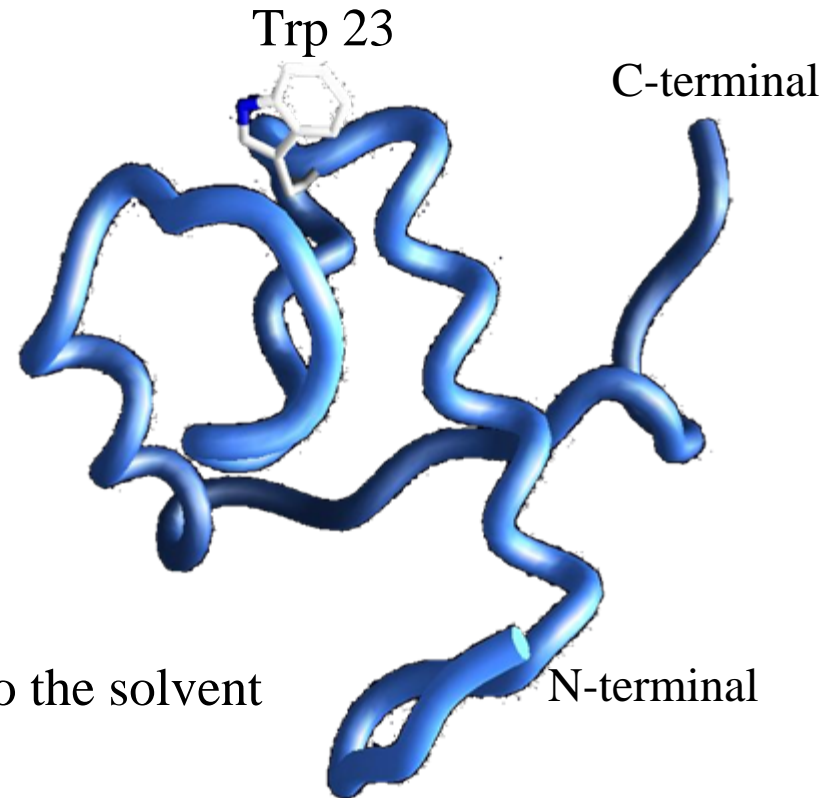
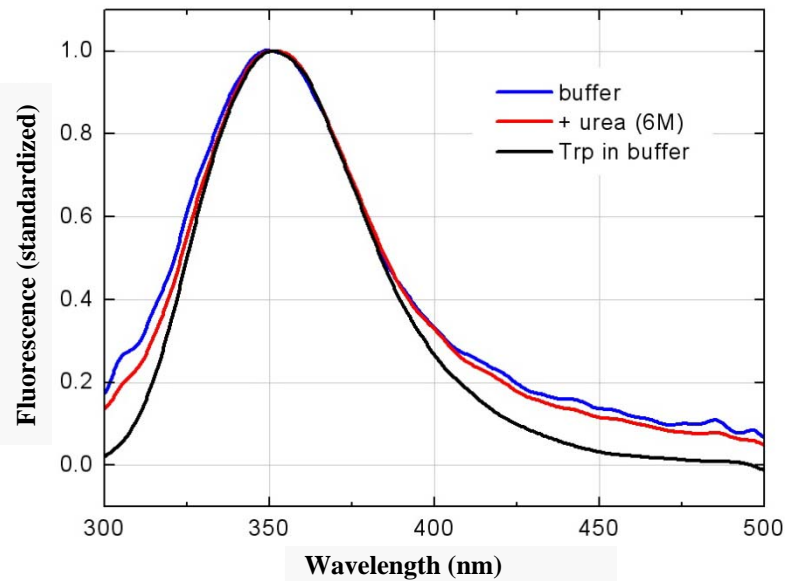
Note: the two things are inseparable, there cannot be the one without the other in a living system

TRIDIMENSIONAL STRUCTURE PREDICTION

Cys residues

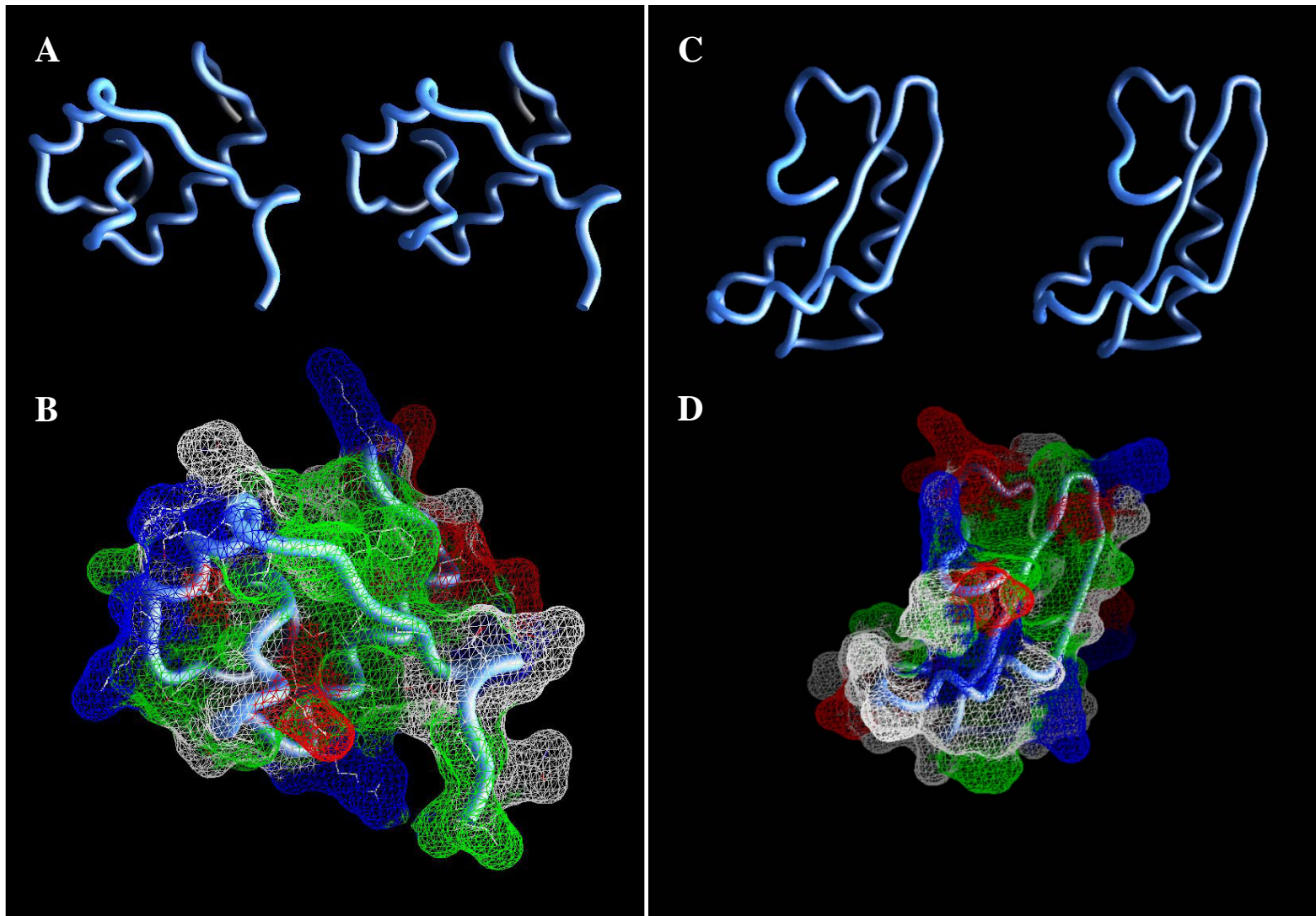


FLUORESCENCE STUDIES



- The Trp residue could be exposed to the solvent

INVESTIGATION OF DE NOVO TOTALLY RANDOM BIOSEQUENCES
II. ON THE FOLDING FREQUENCY IN A TOTALLY RANDOM LIBRARY OF DE NOVO PROTEINS OBTAINED
BY PHAGE DISPLAY
Reg. No CB-070



**LIVING SYSTEMS TRANSFORM MATTER
INSIDE THEMSELVES,**

IN SUCH A WAY

**THAT THE PRODUCT IS THEIR
OWN ORGANIZATION**

Maturana and Varela

VARIOUS HYPOTHESES ON THE ORIGIN OF LIFE ARE STILL NOWADAYS UNDER STUDY

LIFE STARTED FROM

PREBIOTIC SOUP

ON CLAY

RNA FIRST

PROTEINS FIRST

MEMBRANE FIRST

AT VERY HIGH TEMPERATURE

ON ICE.....

ARE WE ALONE IN
THE UNIVERSE??

CONCLUSIONS FROM THIS FIRST PART (WHAT IS LIFE?):

THE BASIC FEATURES OF CELLULAR LIFE (AUTOPOIESIS, COGNITION) CAN BE EXPLAINED IN TERMS OF SELF-ORGANIZATION OF NON-LIVING COMPONENTS.

LIFE AND COGNITION ARE EMERGENT PROPERTIES

THIS APPEARS TO BE TRUE ALSO FOR HIGHER FORMS OF LIFE

**ALL IS „IMMANENT“, COMING FROM WITHIN.
NO TRASCENDENT PRINCIPLE INVOKED.**

Andria, aprile 08
Pier Luigi Luisi
UniRoma3

THE ORIGIN OF LIFE ON EARTH:

WHAT SCIENCE HAS TO SAY

The traditional definition:

Science is

the enterprise

to explain the phenomenology

of the world in terms of the

natural laws

Science is only one part of the human activity

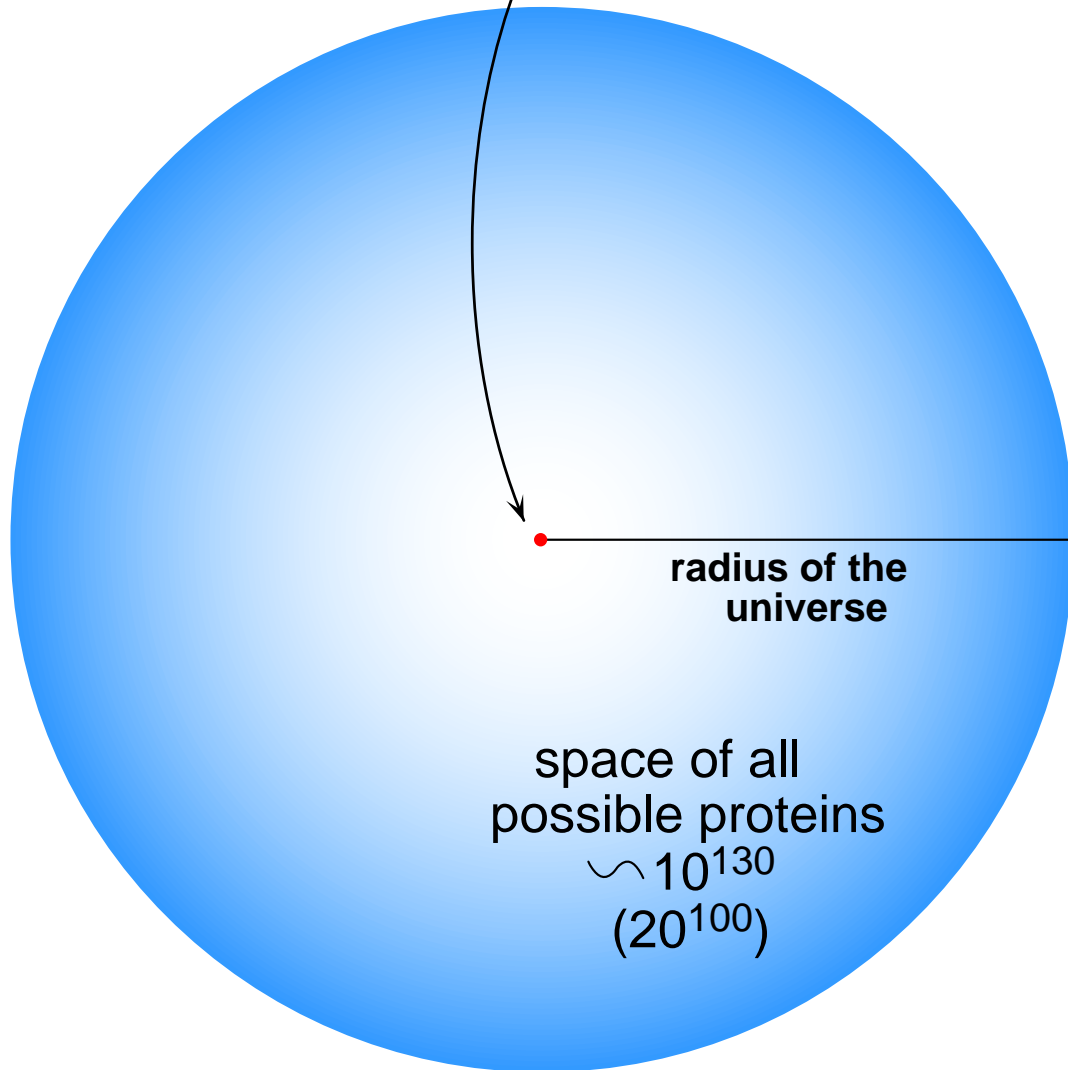
...and not the only way to explain the world

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

Orgel; Morowitz; de Duve

space of the proteins
present in nature $\sim 10^{10}$
(radius ca. 1 atom)



**CAN THIS HYPOTHESIS BE TESTED
WITH EXPERIMENTS?**

(THE BIRTH OF PREBIOTIC CHEMISTRY)

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

1. Life originated from inanimate matter as a spontaneous and continuous 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 the conditions.
4. Since there is no documentation on how things really happened, there is no obligatory research pathway.

LIFE



**inanimate
matter**

**THE NOTION OF
SELF-ORGANIZATION**

**AND THE NOTION OF
EMERGENCE**

**TWO PILLARS OF THE MODERN SCIENTIFIC
VIEW OF COMPLEXITY**

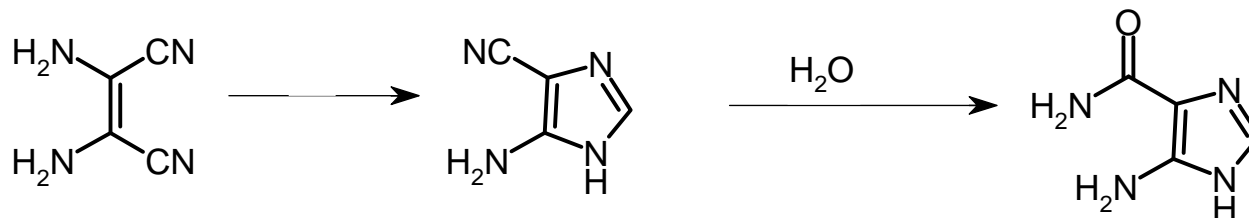
The traditional definition:

**Science is the enterprise
to explain the phenomenology
of the world in terms of the
natural laws &
to discover new phenomena
and new laws of nature**

..not the only way to explain the world

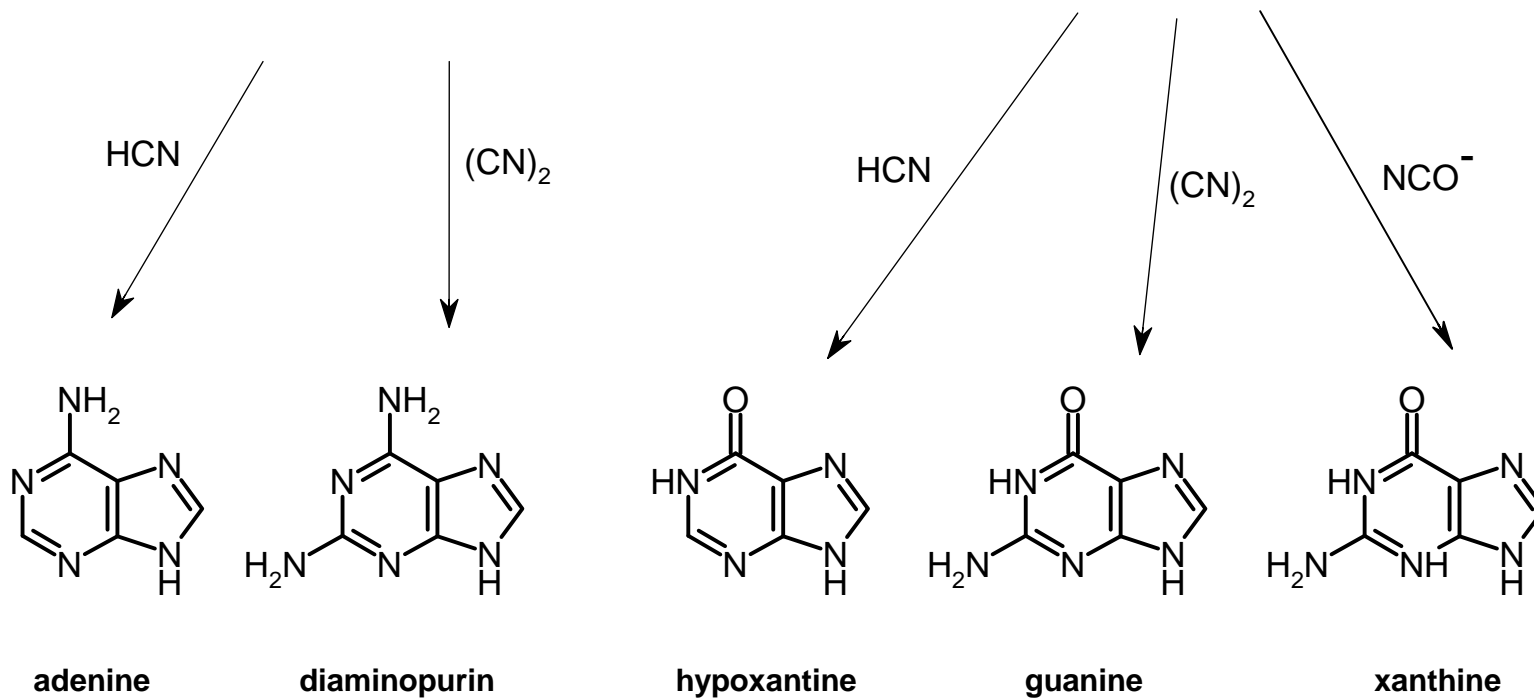
Synthesis of purines

Oró, 1960



diaminomaleonitrile

4-amino-imidazole-5-carbonitrile



adenine

diaminopurin

hypoxanthine

guanine

xanthine

ANALYSIS OF THE PEPTIDE PHAGE LIBRARY

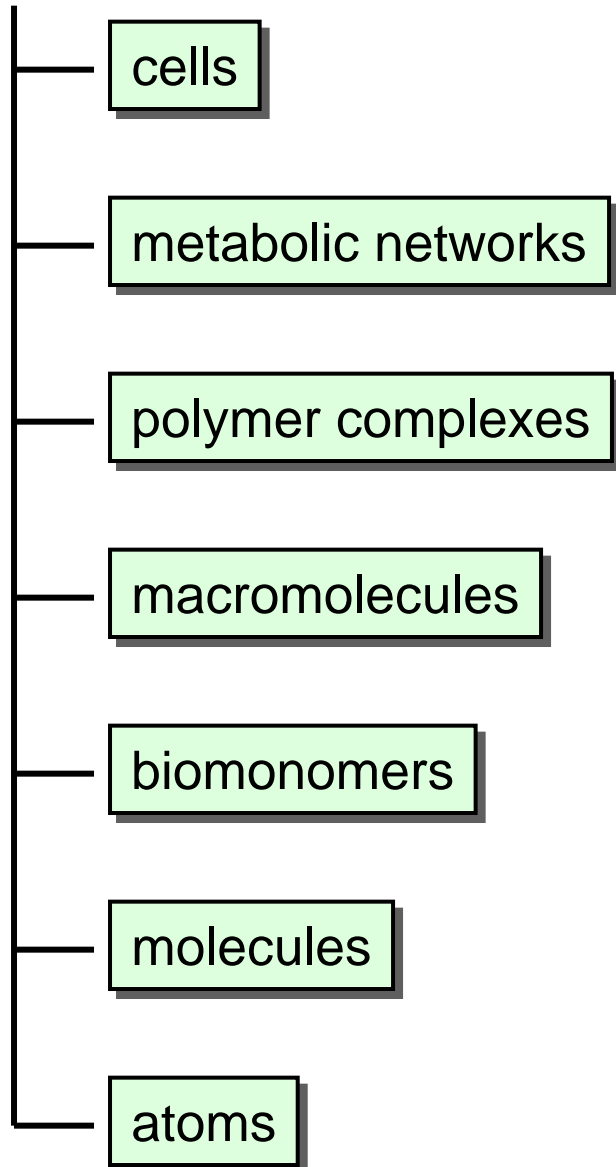
Library
1.98 x 10⁹ clones
180 clones were randomly selected



44% correct insert (79 clones)



73 clones tested for thrombin stability



**THE INCREASE OF COMPLEXITY
TOWARDS THE EMERGENCE OF LIFE
PROCEEDS
VIA THE INTERPLAY BETWEEN
SELF-ORGANIZATION AND
EMERGENCE**

SELF-ORGANIZATION:

THE CAPABILITY OF THE

**SYSTEM TO SPONTANEOUSLY
ASSEMBLE INTO AN ORDERED STATE
(because this is the more stable form)**

**..the duplex of DNA, protein folding,
chromatin, membranes, soap bubbles, the bee
hive, muscle fibers**

Emergence:
the formation of a higher
complexity level brings about
NOVEL properties
that are not present
in the basic components

..the **whole** is more
than the sum of
the parts
...**holism**

REDUCTIONISM & EMERGENCE



**structural reductionism and
novel emergent properties**

**A DISCOVERY: THE CELLULAR
(AUTOPOIETIC)
DEFINITION OF LIFE
ALSO APPLIES**

**TO MULTICELLULAR,
MACROSCOPIC
LIFE**

Life is an emergent property:

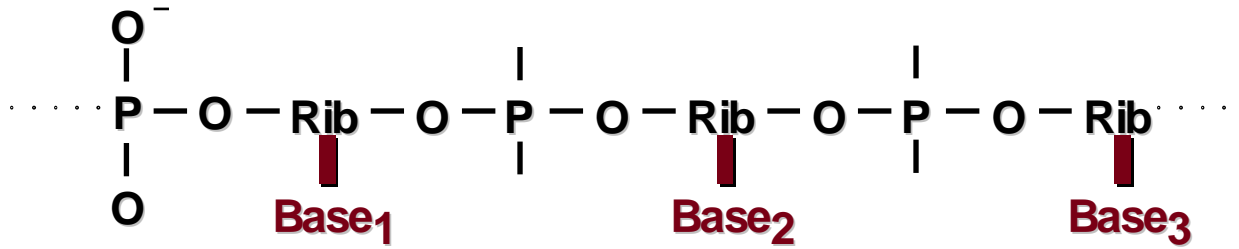
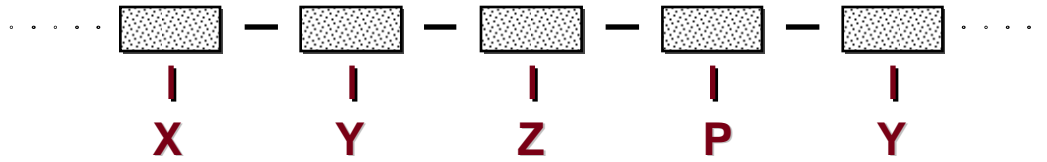
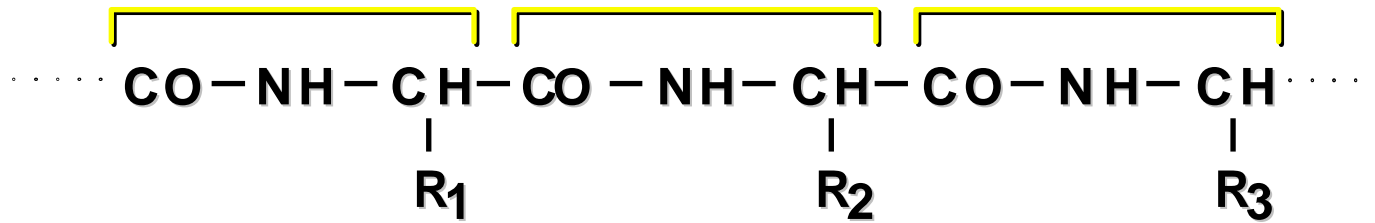
**the components (nucleic acids,
proteins, lipids, sugars etc)**

are per se' not living;

When they are assembled together

in a particular space/time

structure, then life emerges

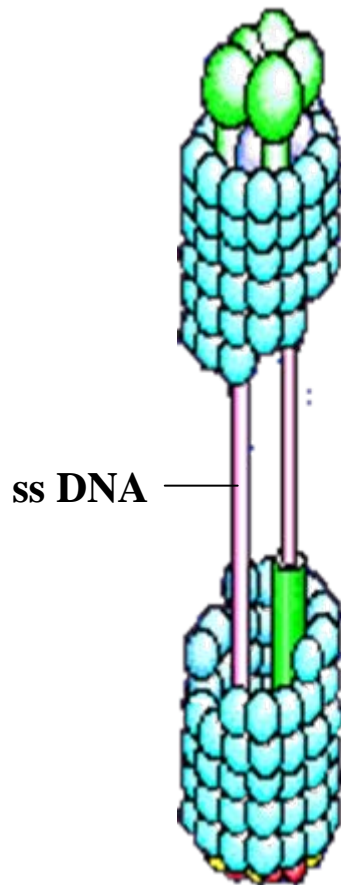


The cellular „definition“ of life:

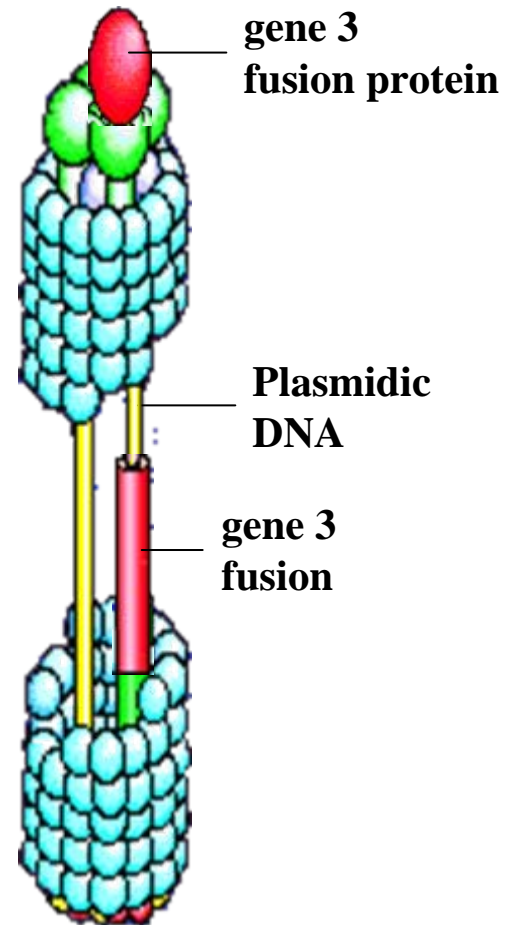
A system spatially defined by a boundary of its own making- and which is self-sustaining by re-generating the system's components from the inside

PHAGEMID VECTOR SYSTEM IN PHAGE DISPLAY

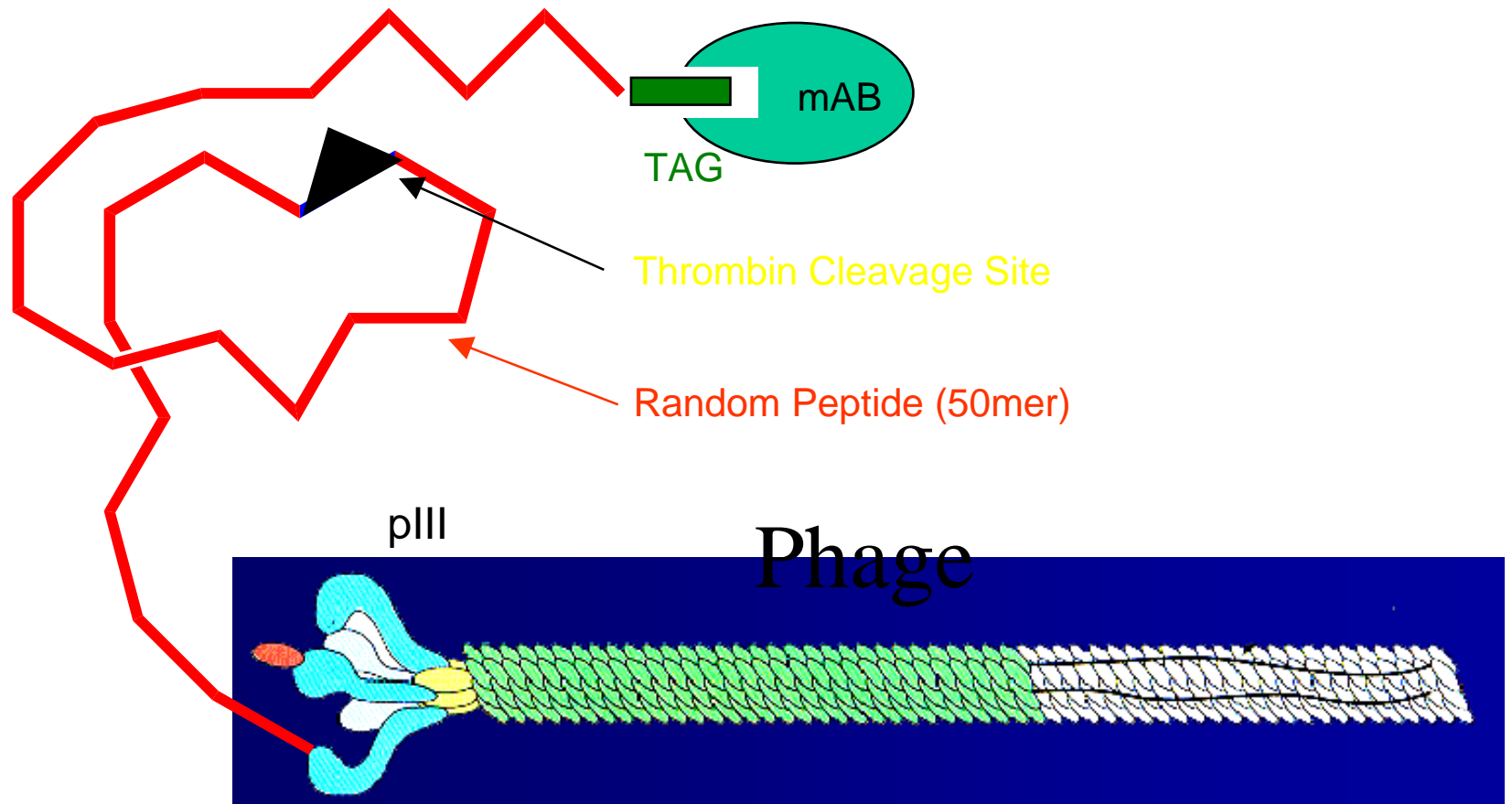
Helper Phage



Modified Phage



STRATEGY FOR THE ISOLATION OF FOLDED PEPTIDES



The narrative of the BigBang

- 13.7 billion years ago: BB and creation of time, space, heat, laws of nature
- After 1/100 sec.: t ca. 100 million C, density 4 billion more than water density

After 3.6 min: $t = 30$ million C, and formation of protons and neutrons and then formation of Hydrogen and Helium atoms, ($\frac{3}{4}$ and $\frac{1}{4}$, as ca. today)

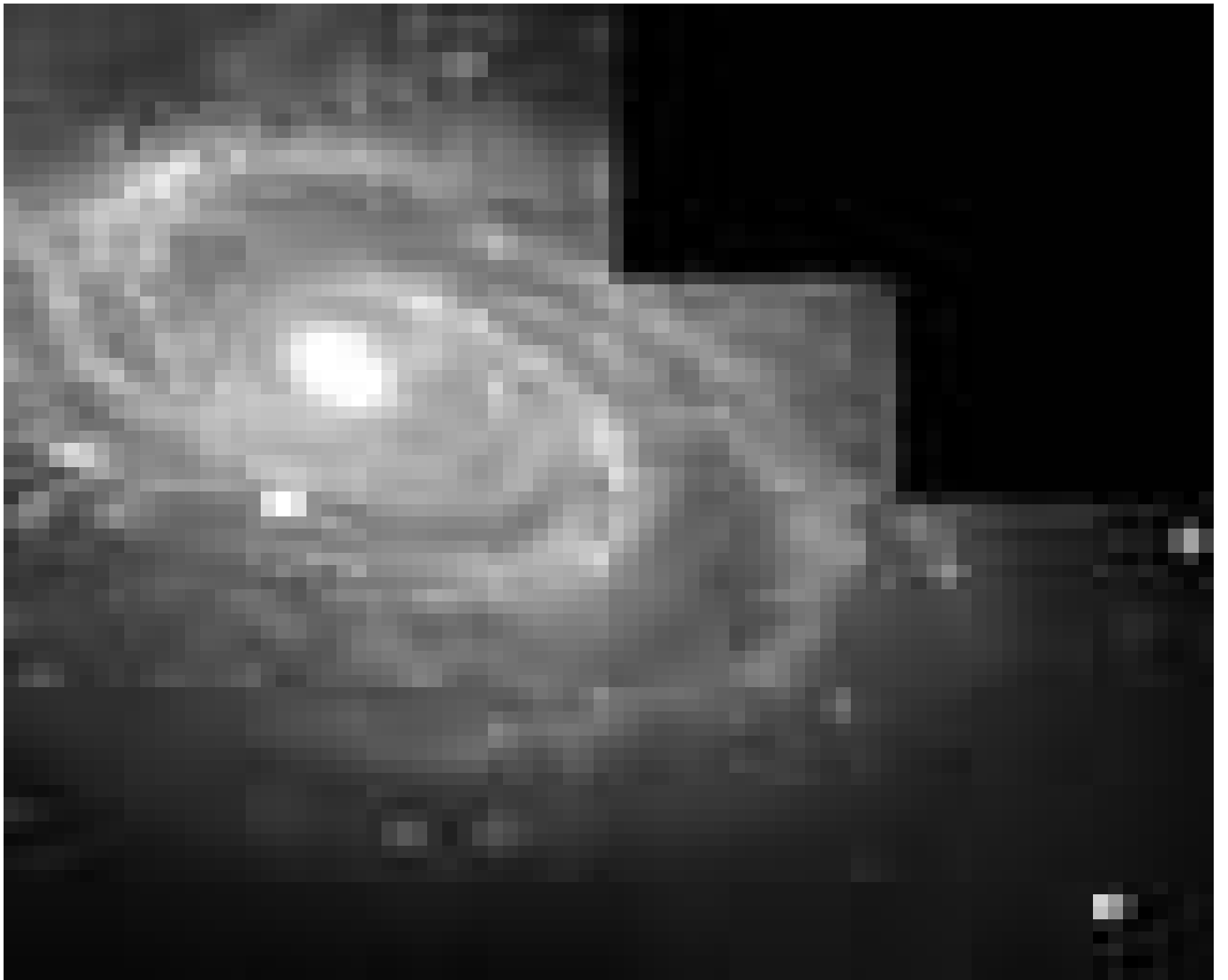
After 300-400 million years: H and He build gigantic cold clouds with high density

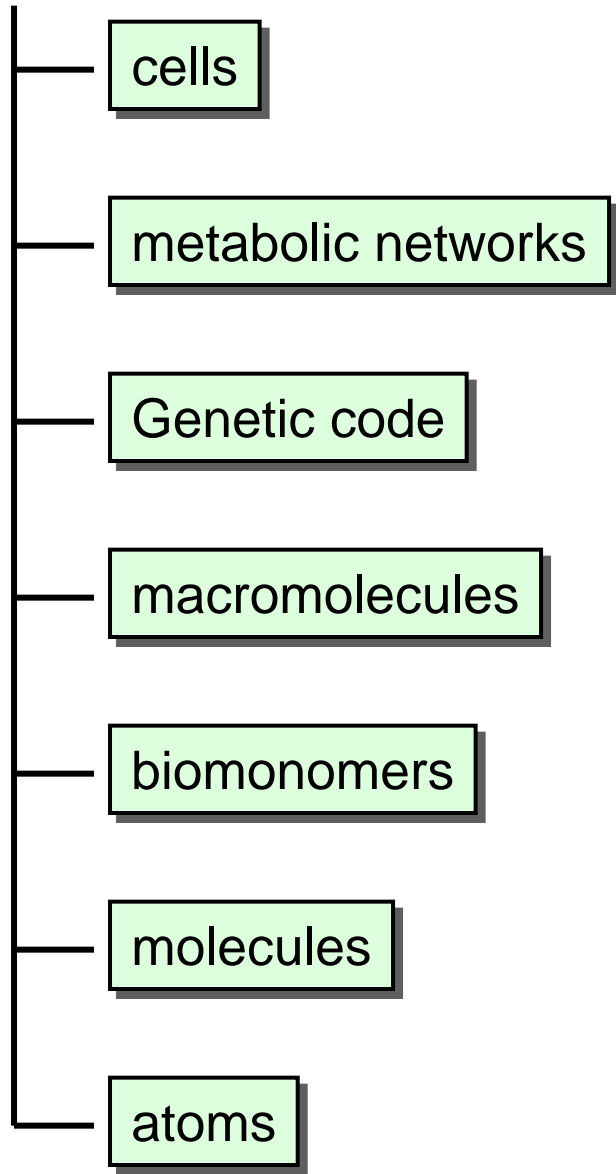
As temp. goes down to -170 C, these clouds collapse, forming the first galaxies and stars

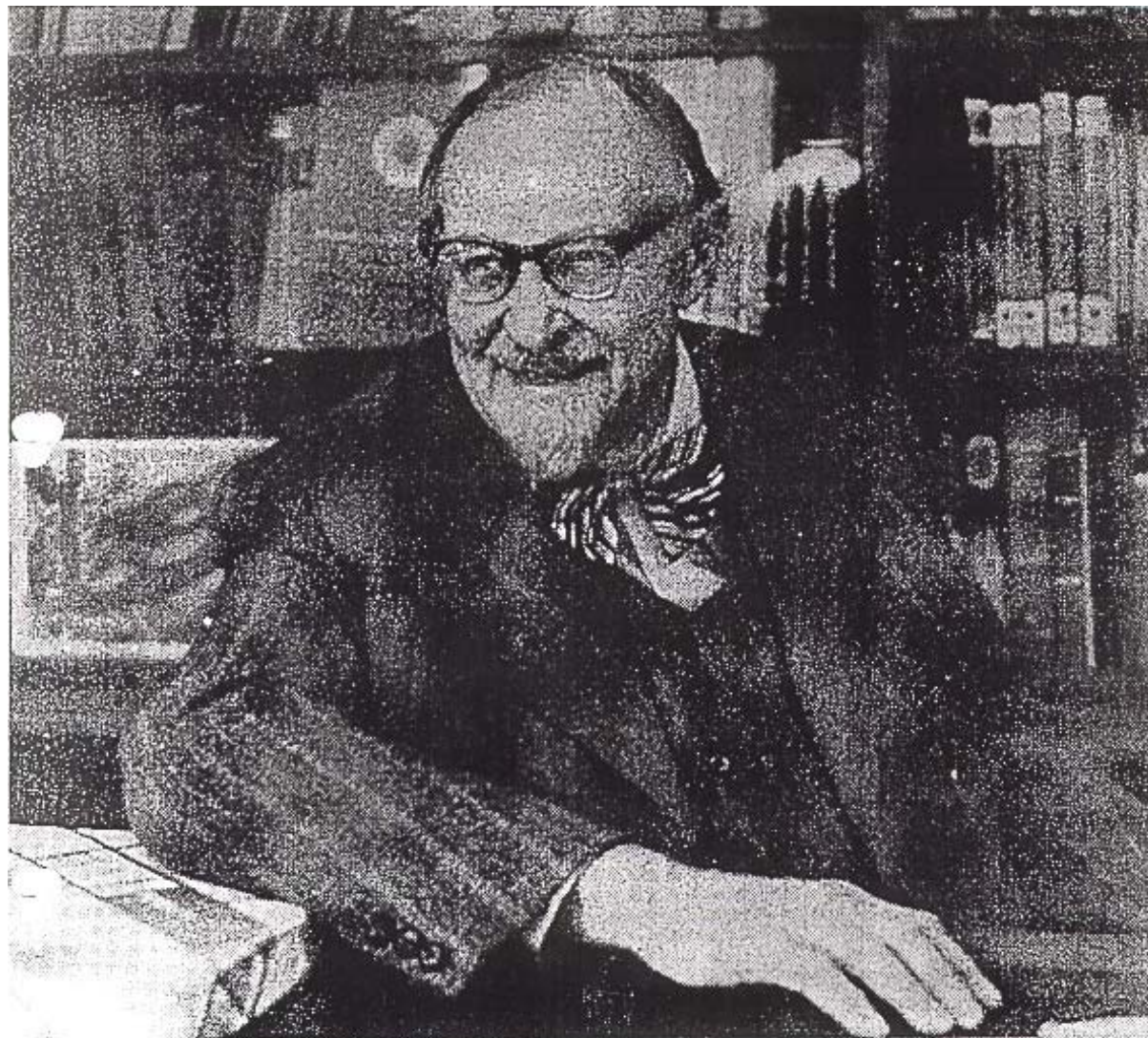
...the narrative of the BigBang

- 5 billions years ago: formation of “our” mother cloud, from which our solar system
- Inside the sstars, nuclear fusion of He and H produces the first heavy atoms, C, O, N, Si, Fe
- And then the first molecules, CO, ammonia, water, CS
- From these particles, formation of the “planetesimals”, which eventually forms planets









Aleksandr Oparin (1894 – 1980)

autopoietic unity = minimal life

" an autopoietic unity is able to self-generate owing to a reaction network taking place within its own boundary "

(the reaction network makes all components of the unit, including the boundary.)

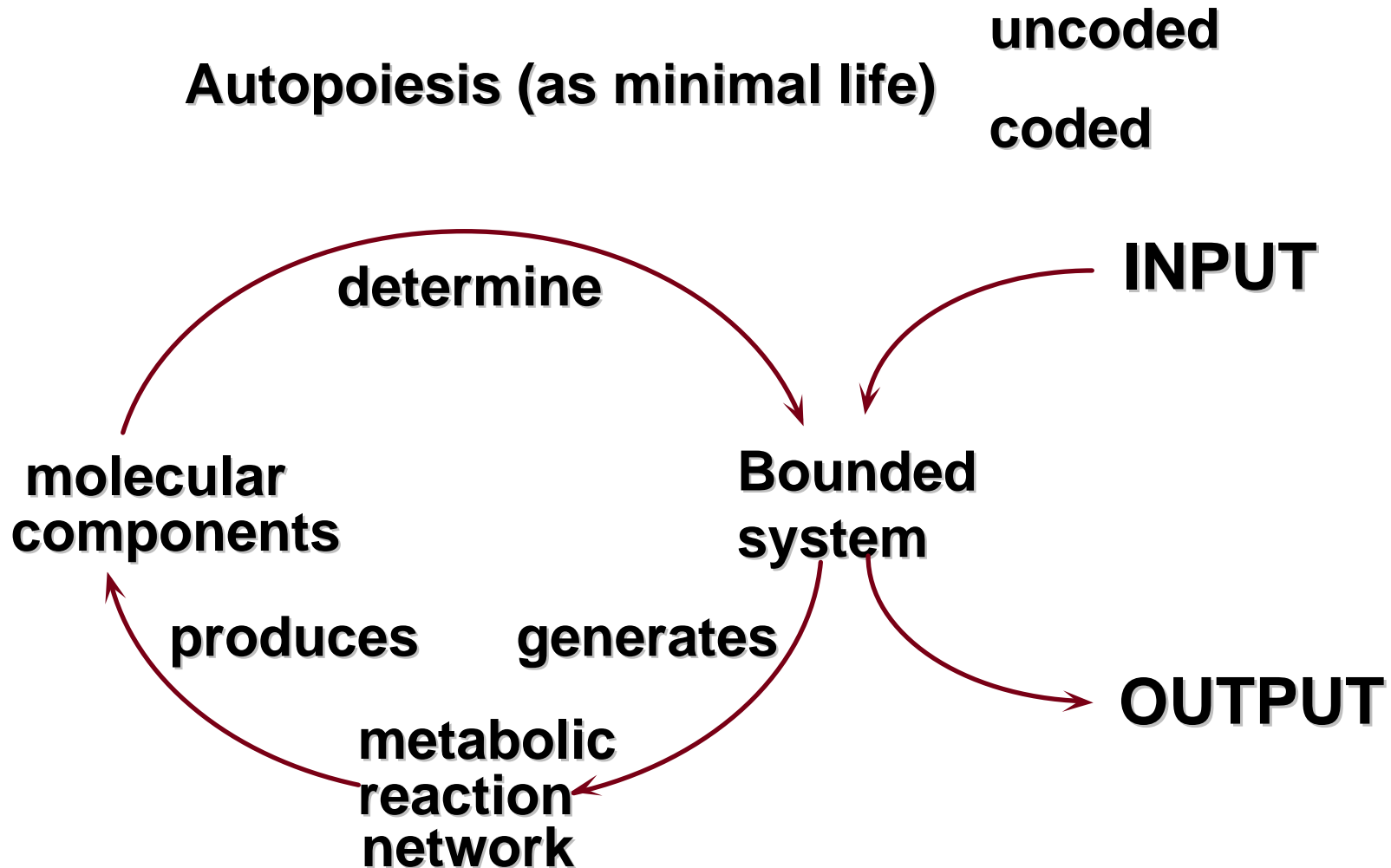
Varela et al., Biosystems, 5 (1974) 187

" self-generate" — [self-maintenance
or
self-reproduction

in general

self-reproduction ≠ self-replication

Autopoiesis is the most general pattern of minimal life. It does not specify the actual structures and their mechanistic processes.



...in other words,

The living system is defined by its organization, and therefore must be interpreted in terms of relations among the components

And not in terms of the properties of the components

Life is an emergent property:

**the components (nucleic acids,
proteins, lipids, sugars etc)**

are per se' not living;

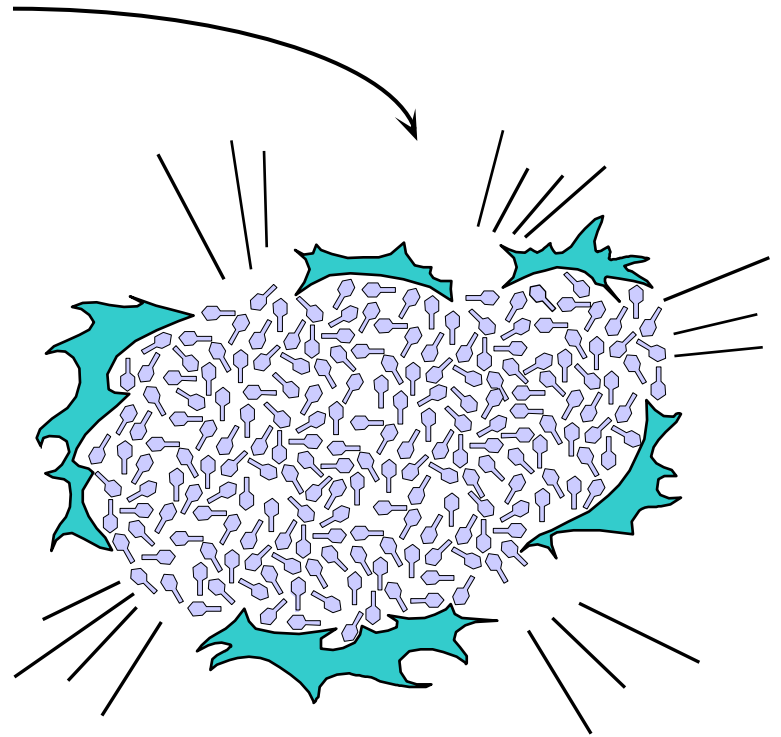
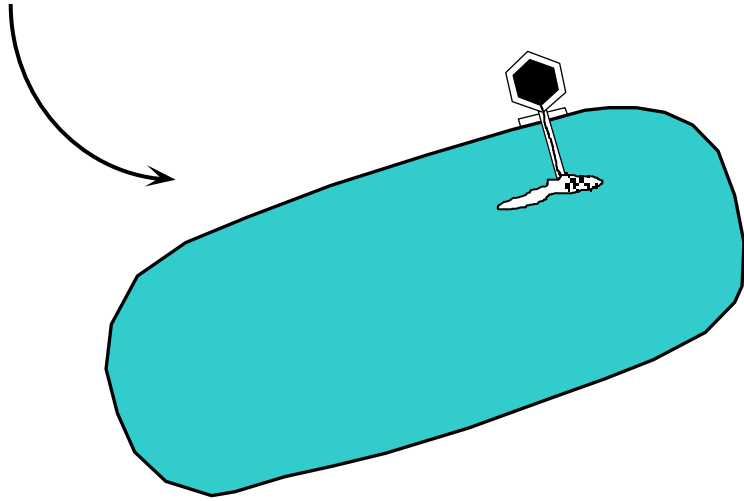
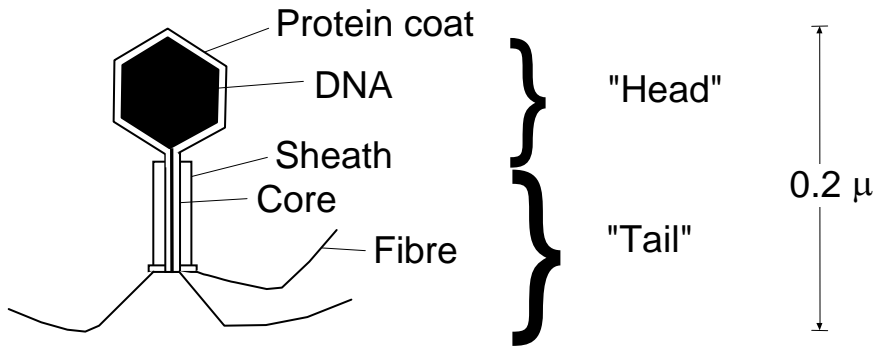
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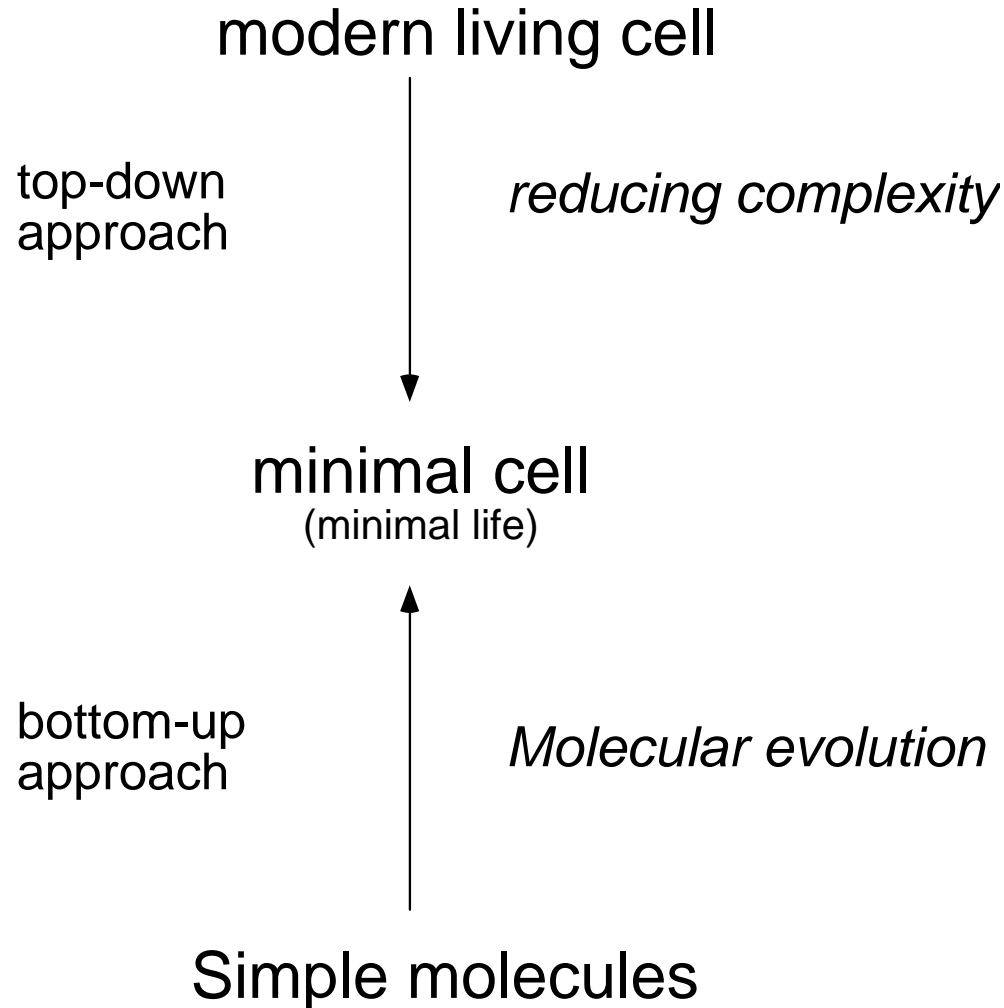
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Emergence, emergent properties:
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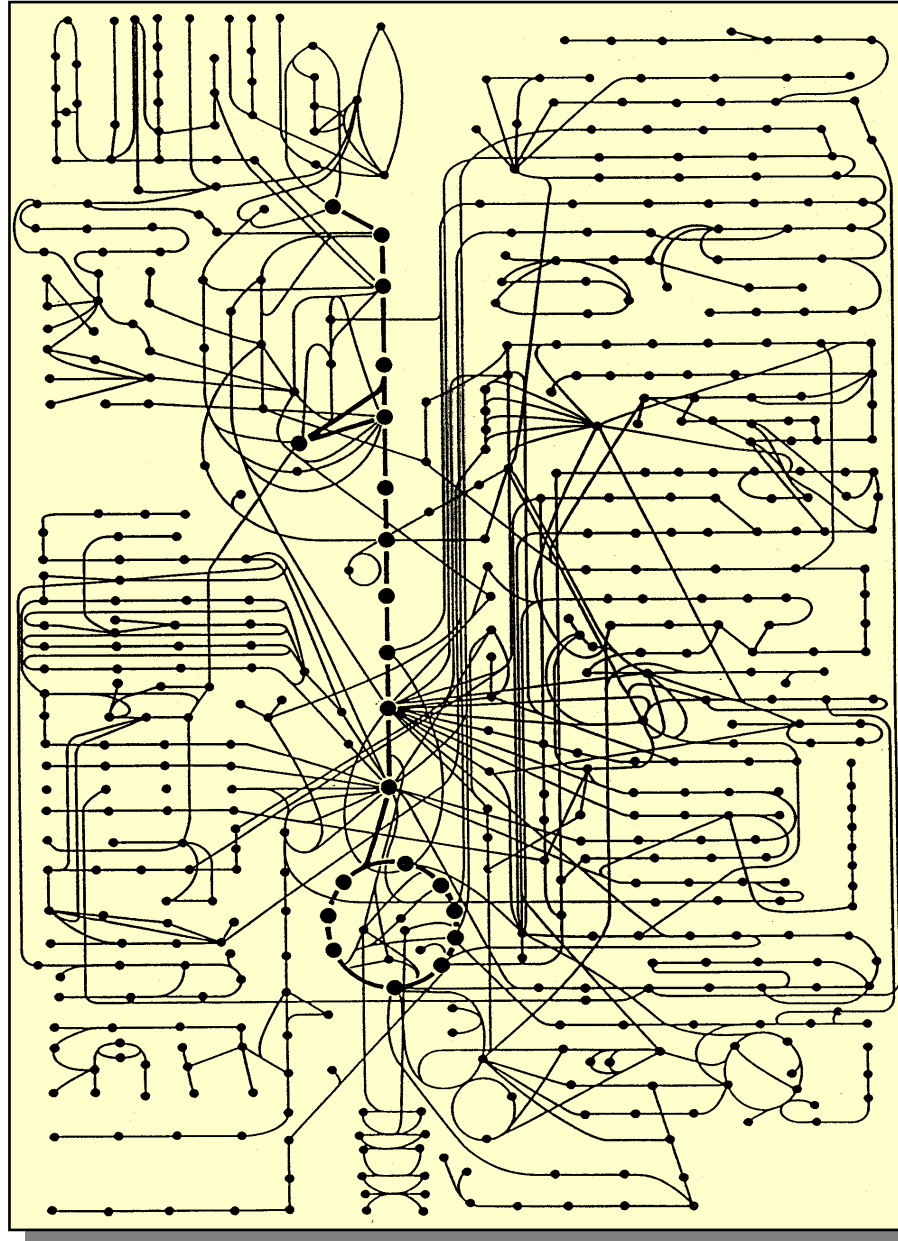
..the **whole** is more
than the sum of
the parts
...**holism**



two working directions



A maze illustrating the chemical reactions that interconvert small molecules in cells.



Is this complexity really necessary for minimal cellular Life?

...recognizing that early cells could not have started with such complexity

What about the early minimal cells?

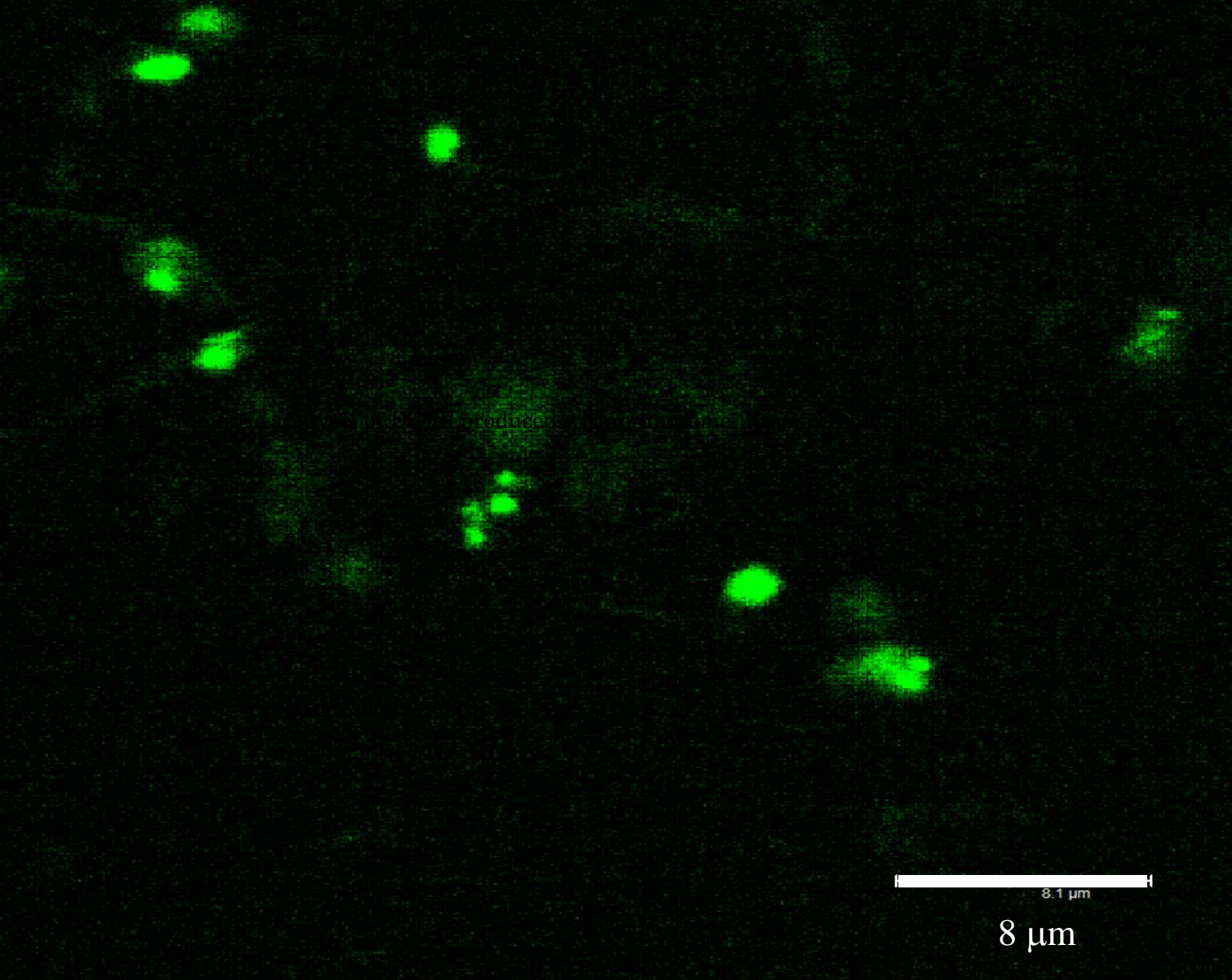


Figure 4. Confocal images of EGFP fluorescent liposomes. From ref. [36].

**What can be the minimal size
of a living cell?**

**We could see protein
expression inside liposomes
with 100 nm radius**

**Bacteria can in principle exist
with this size**

Consciousness is.....

-knowing that you know (being aware of being aware)

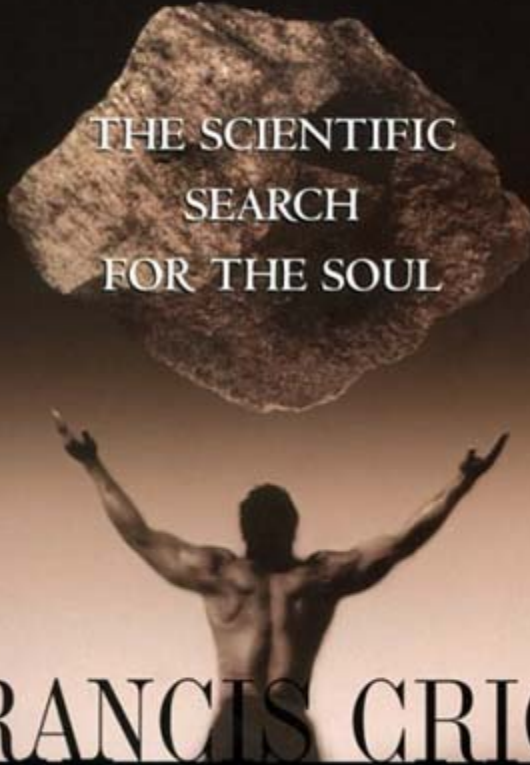
-Sapere di sapere (essere consapevoli della propria consapevolezza)

-the subjective acknowledgment of experience (such as sensory perceptions, or thought)

-il riconoscimento soggettivo dell'esperienza (come la percezione sensoriale, o di un pensiero)

-

The
Astonishing Hypothesis



THE SCIENTIFIC
SEARCH
FOR THE SOUL

FRANCIS CRICK

NOBEL LAUREATE

"A fascinating, lucid portrait of a great scientific search."—Stephen Compton, *San Francisco Chronicle*