

SHANG-AI LECTURE ON CHEMICAL SYNTHETIC BIOLOGY

Prof. Pier luigi Luisi

Univ, Roma3, roma

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- **Synthetic biology**: constructing in the laboratory life forms, or biological functional structures, which do not exist in nature (utilizing nature's biochemistry-no AL).
- **The two souls of SB:**
- 1. Constructing by **genetic manipulation** novel bacterial forms which can be useful for energy production (Hydrogen, methane...), for drugs, as industrial catalysts. A teleological, engineering approach. See Craig Venter and many others.
- **2. Chemical SB**: no genetic manipulation, mostly chemistry, with the question: why this, and not that, in nature?
- ...and using SB to synthetize «that»- the alternative form that nature has not chosen (why?)

WHY NUCLEIC ACIDS HAVE RIBOSE INSTEAD OF GLUCOSE?

WHY PROTEINS HAVE 20 AMINOACID RESIDUES INSTEAD OF –SAY- 15 OR 35?

WHY JUST OUR TINY SET OF PROTEINS, OUT OF AN ASTRONOMICAL NUMBER OF POSSIBILITIES (THE” NEVER BORN PROTEINS”)?

WHY THE FOUR BASES A, T, G, C AND NOT SOME OTHER STRUCTURES-WHY THESE HAVE BEEN CHOSEN?

WHY SHOULD CELLS BRE COMPLEX, WITH THOUSAND OF GENES AND MANY THOUSAND OF OTHER MACROMOLUAR COMPONENTS?

Why this...and not that in nature?

APPROACH THESE QUESTIONS THANKS TO chemical SB

**MOST OF THE IMPORTANT BIO-
STRUCTURES ARE NOT THE
PRODUCTS OF THERMODYNAMIC
CONTROL:**

**LYSOZYME IS NOT WITH US
BECAUSE IT IS THE MOST STABLE
POLYPEPTIDE CHAIN WITH 121
RESIDUES...**

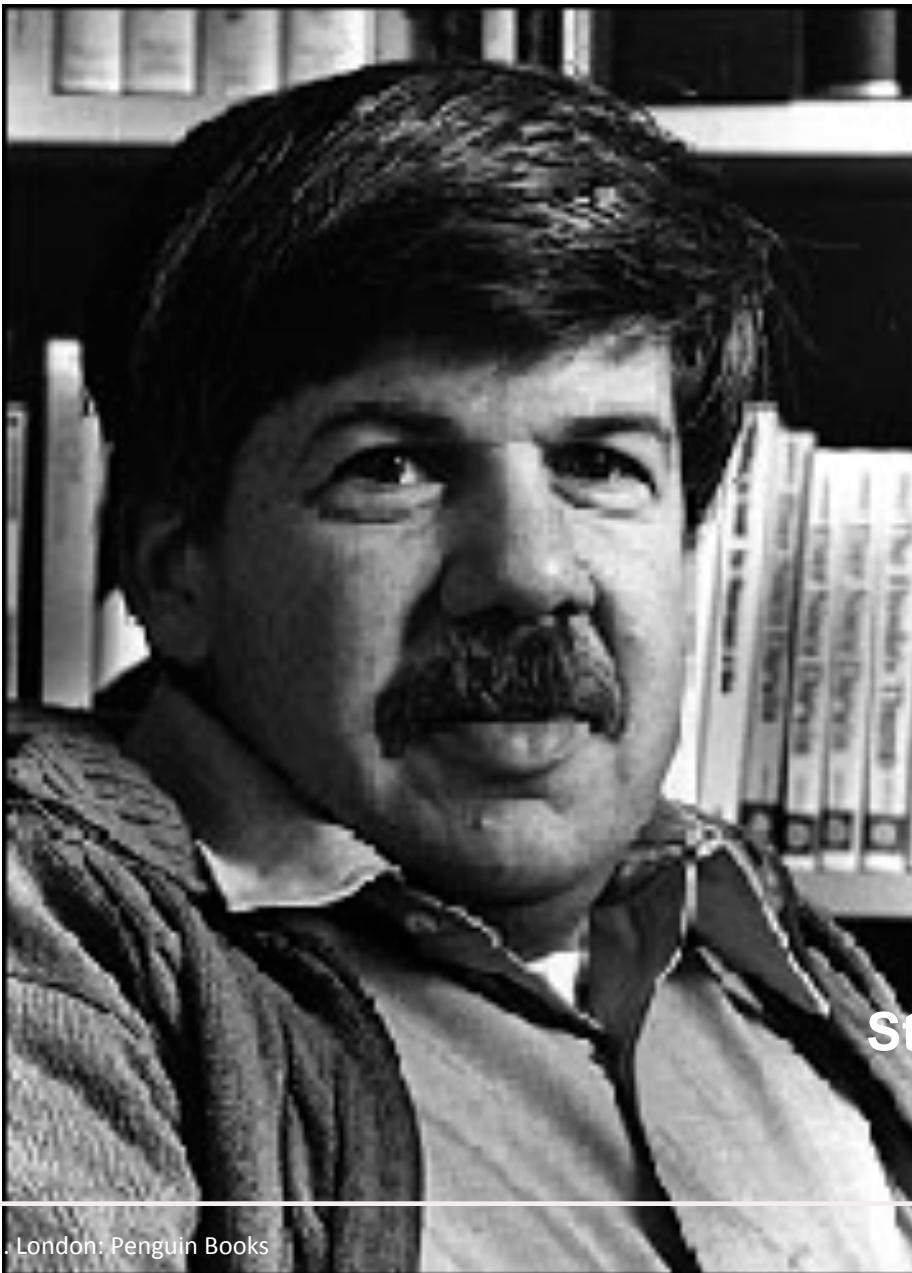
**THE SAME FOR ALL OTHER
PROTEINS, ORDERED RNA, DNA
CHAINS....**

**THEY ARE THE PRODUCTS OF
MOLECULAR EVOLUTION
**EVOLUTION WORKS WITH THE
PRINCIPLE OF CONTINGENCY****

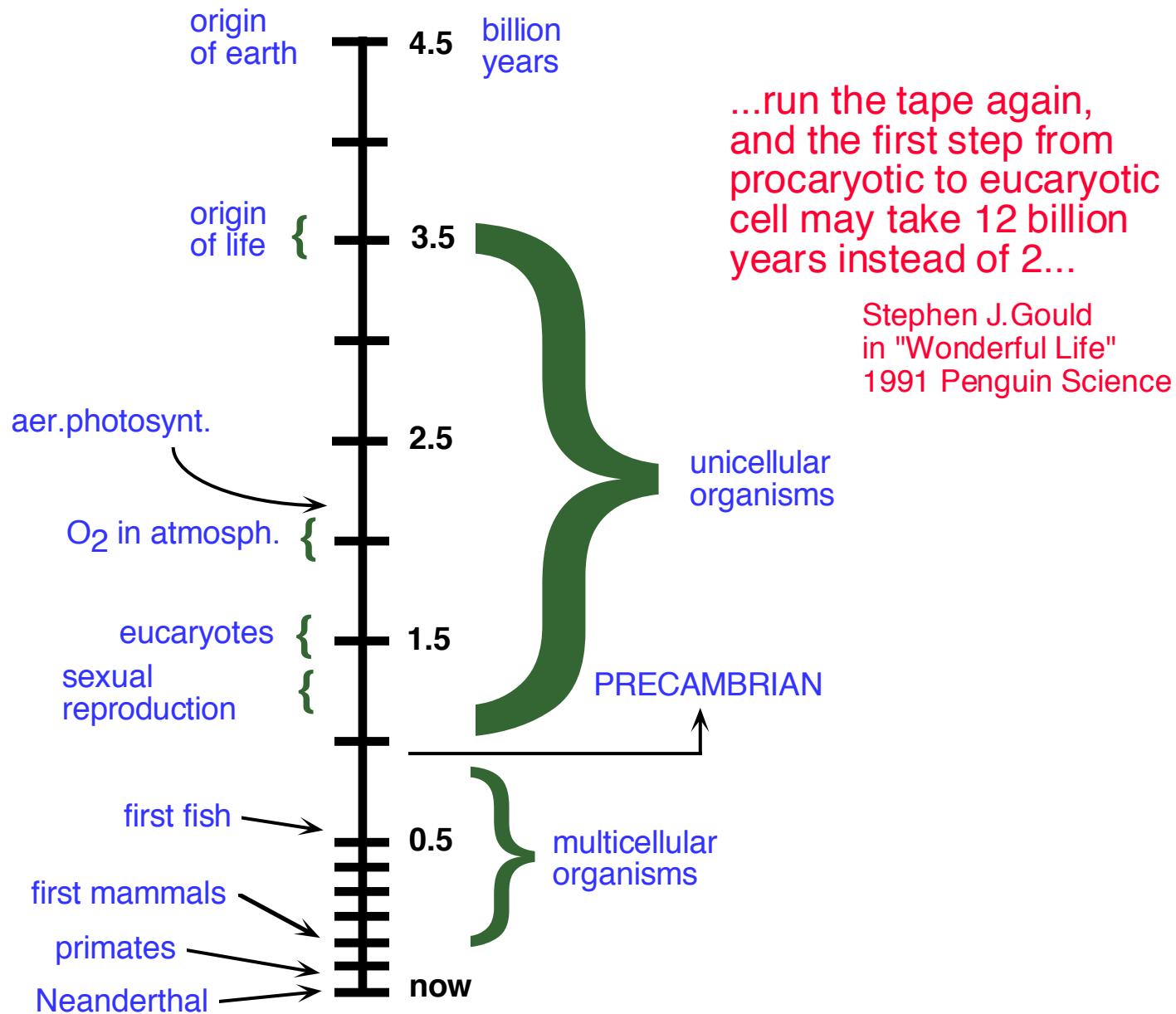
CONTINGENCY

COINCIDENCE OF SEVERAL PARAMETERS
INDEPENDENT FROM EACH OTHER
WHICH TAKE PLACE SIMULTANEOUSLY IN
A GIVEN SITUATION

LIKE, IN CHEMICAL EVOLUTION,
CONCENTRATION, SALINITY, pH,
TEMPERATURE, PRESSURE, RAIN, THE
PRESENCE OF WIND, RAIN, DUST, LIGHT,
OF A CATALYST....



. London: Penguin Books



FEATURES OF CONTINGENCY

**1. THE TYRANNY OF ONE PARAMETER:
CHANGE ONE SINGLE PARAMETER, AND YOU
MAY GET
A QUITE DIFFERENT
OUTCOME---OR NOTHING AT ALL**

**WHY THIS AND NOT THAT IN
NATURE?**

**CHEMICAL SYNTHETIC
BIOLOGY
CAN HELP US
BY SYNTHETIZING “THAT”
THE STRUCTURE(S) THAT
NATURE DID NOT MAKE**

QUESTION:

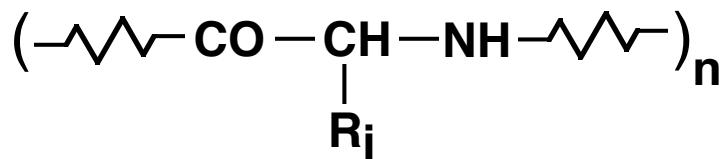
**ARE THE PROTEINS OF LIFE THE ONLY
ONES THAT COULD BE FORMED-
AND GAVE ORIGIN
TO LIFE BY A DETERMINISTIC
(OBLIGATORY)
SERIES OF EVENTS**

OR

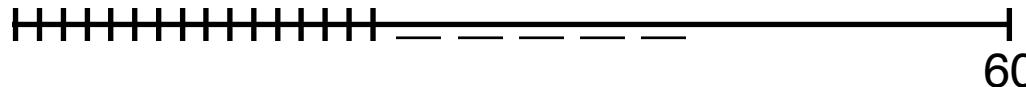
**ARE THEY THE PRODUCT OF
CONTINGENCY(CHANCE)
AND LIFE IS ALSO A PRODUCT OF
CONTINGENCY?**

..why this, and not that?

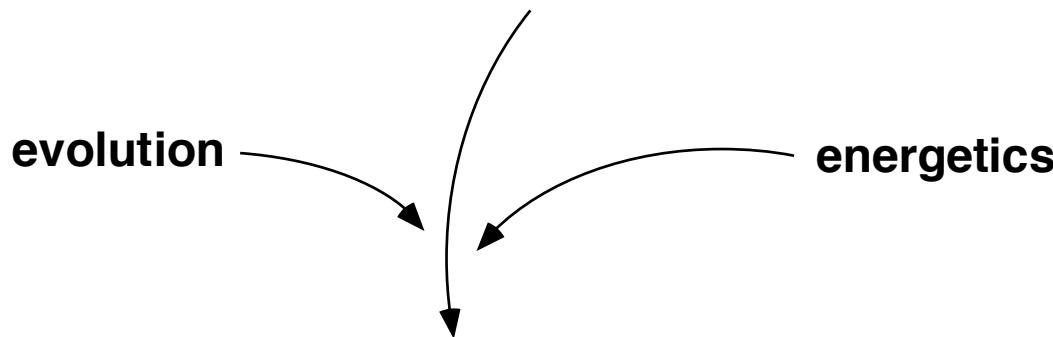
On the importance of being a copolymer



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



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



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

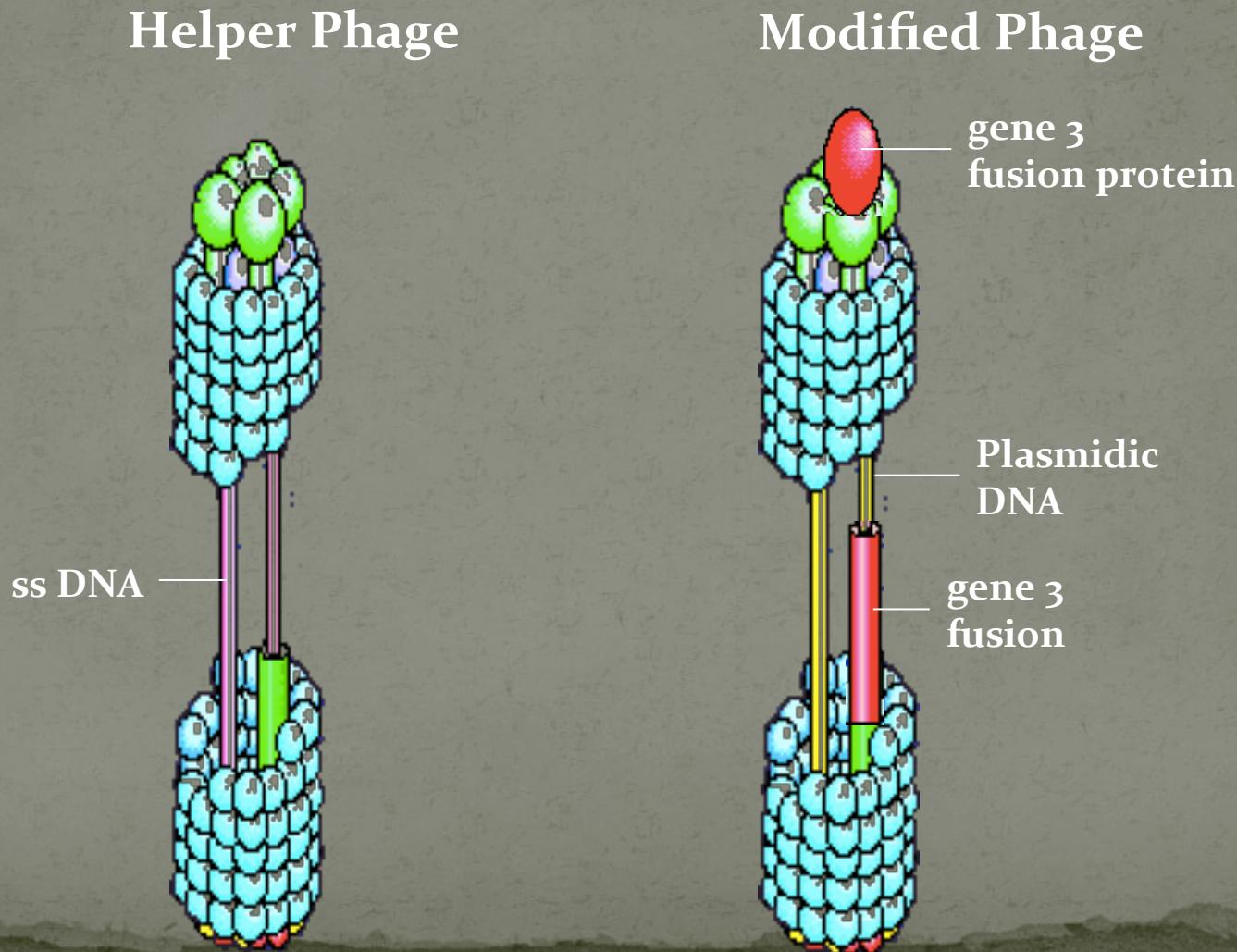


¹⁴
Space of the actual proteins, ca. 10



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

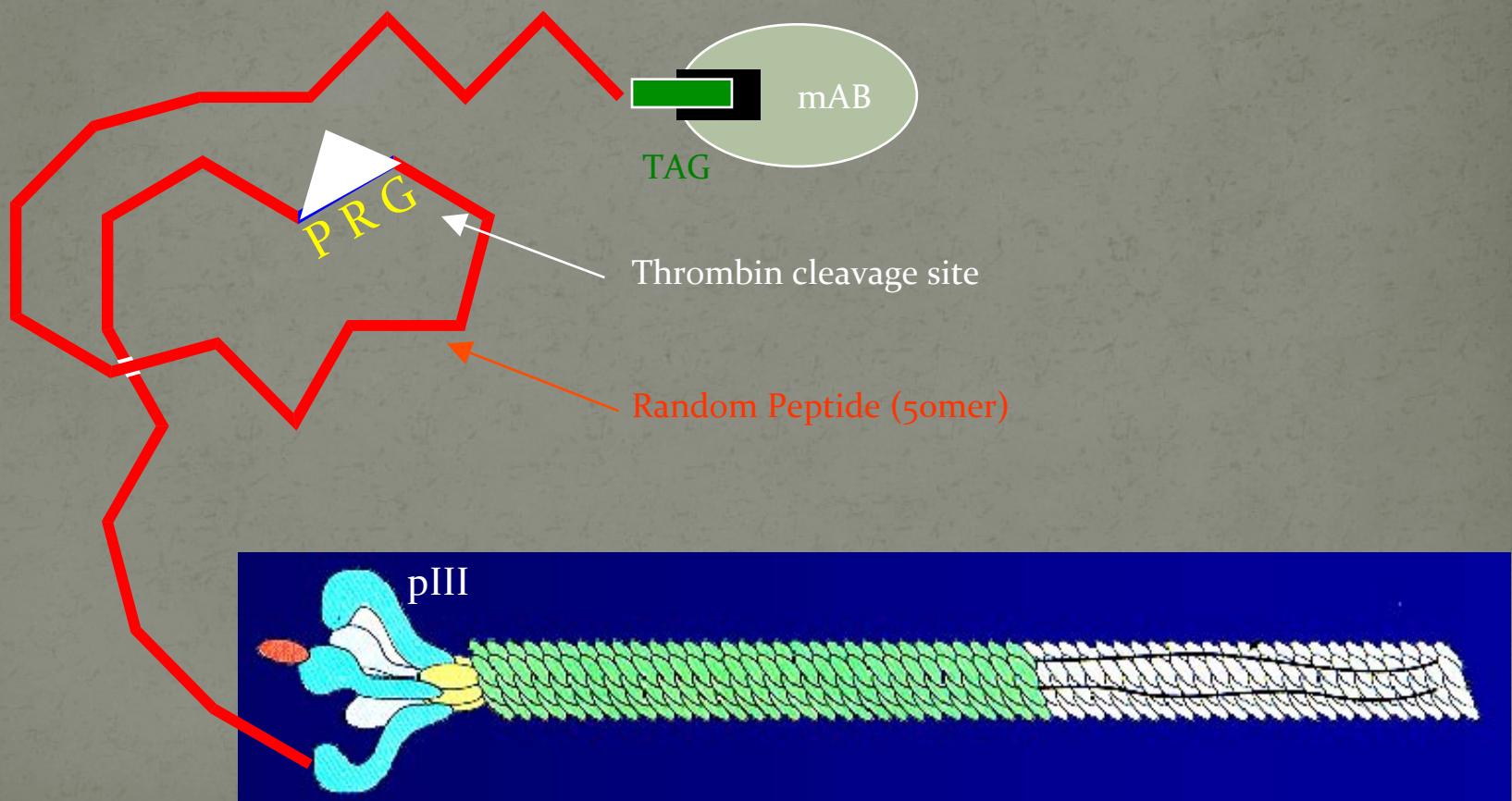
PHAGEMID VECTOR SYSTEM IN PHAGE DISPLAY



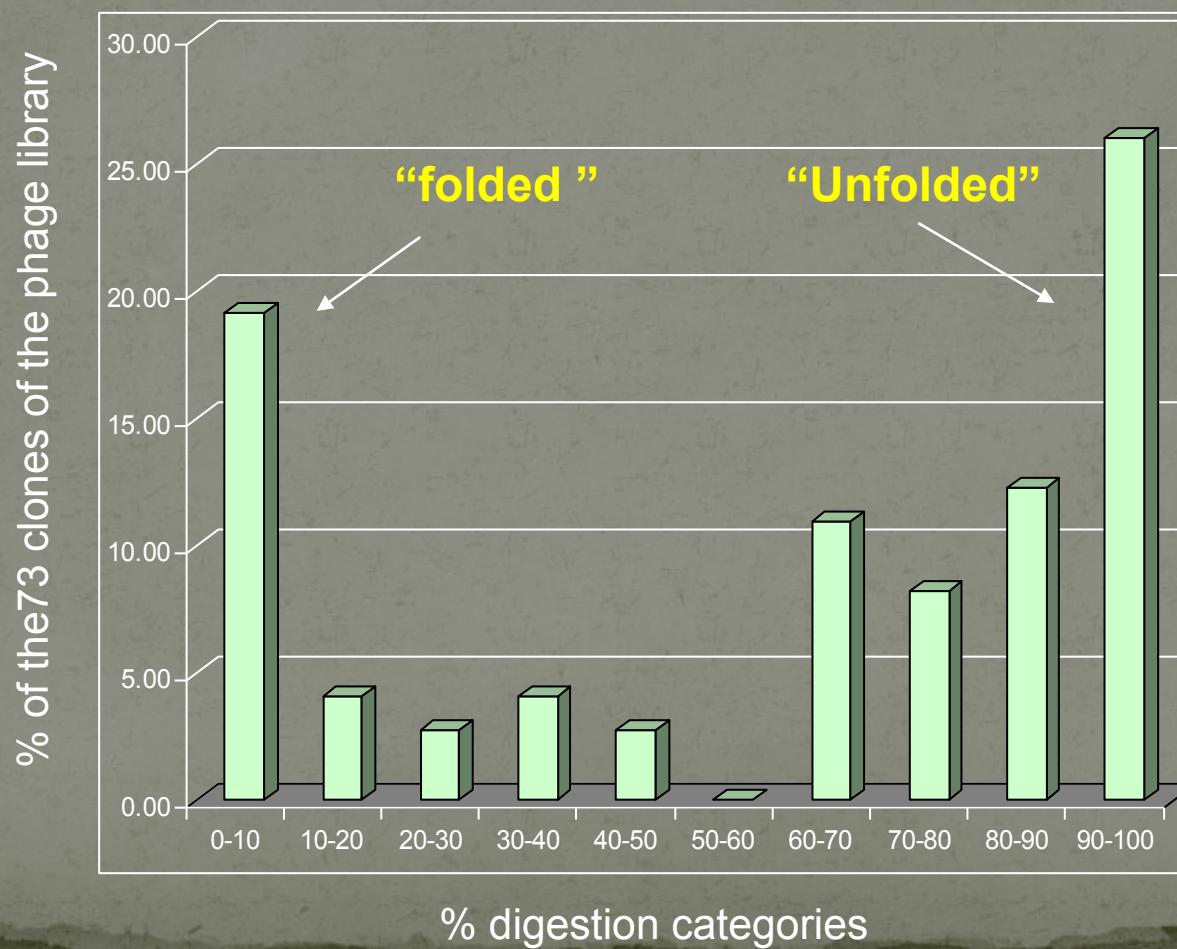
Cristiano chiarabelli
Davide de lucrezia

PHAGE LIBRARY

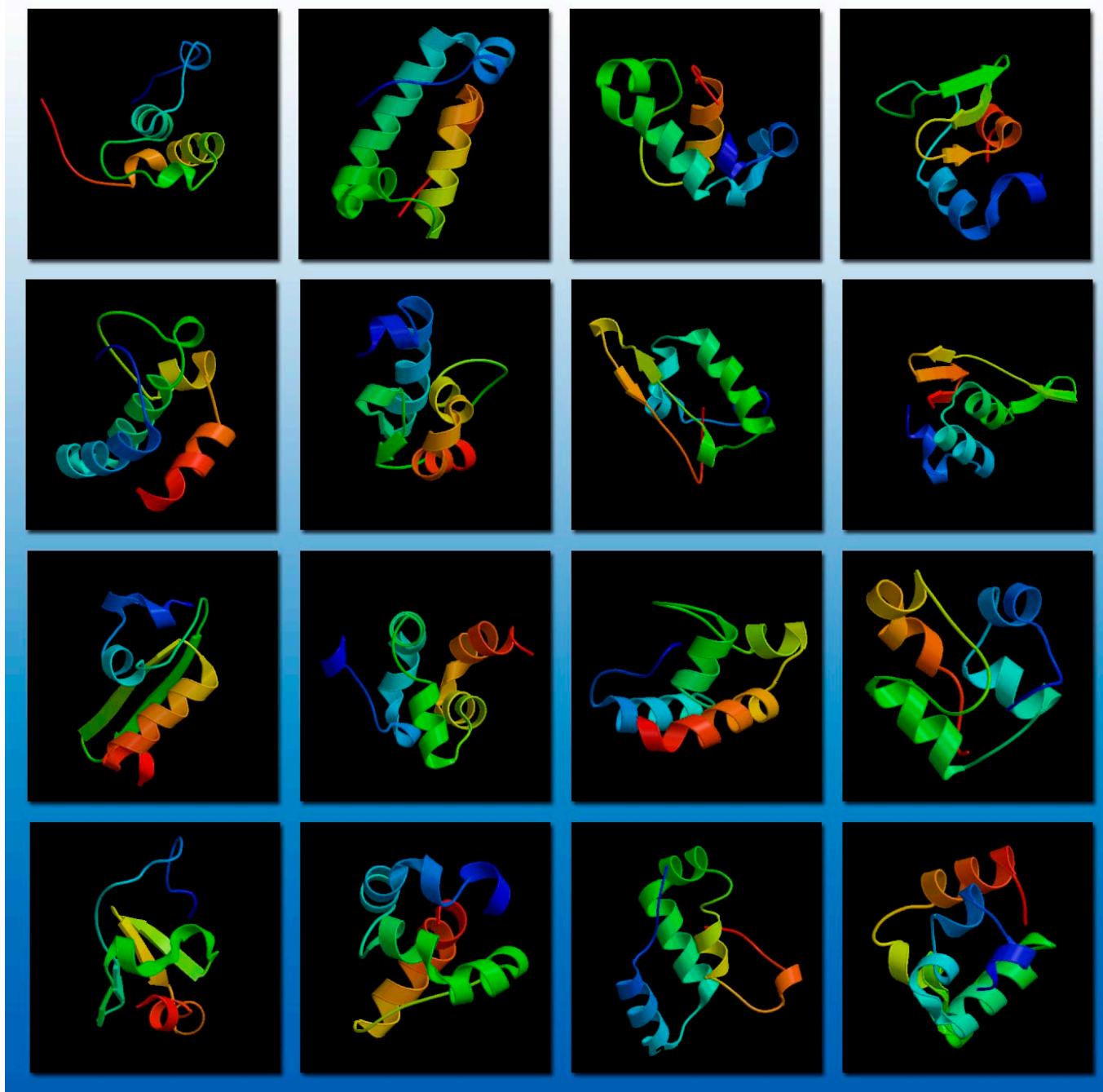
They made a library of ca, 100 million
«never born proteins»



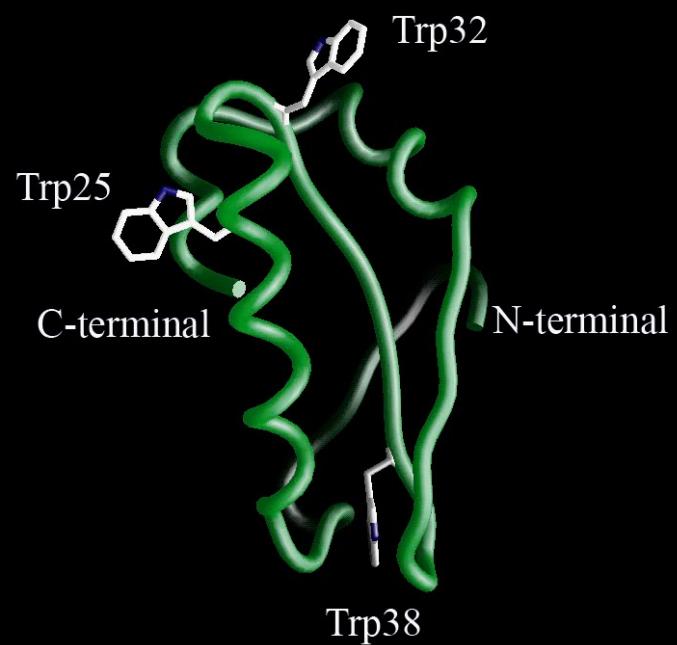
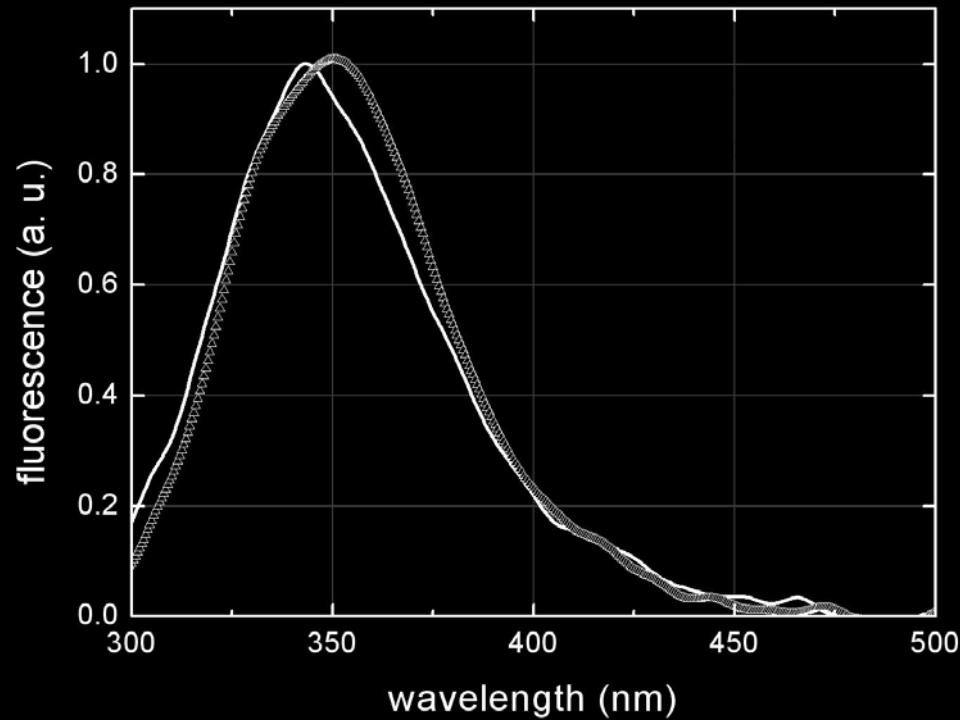
DISTRIBUTION OF THE PEPTIDE LIBRARY WITH RESPECT TO THROMBIN DIGESTION



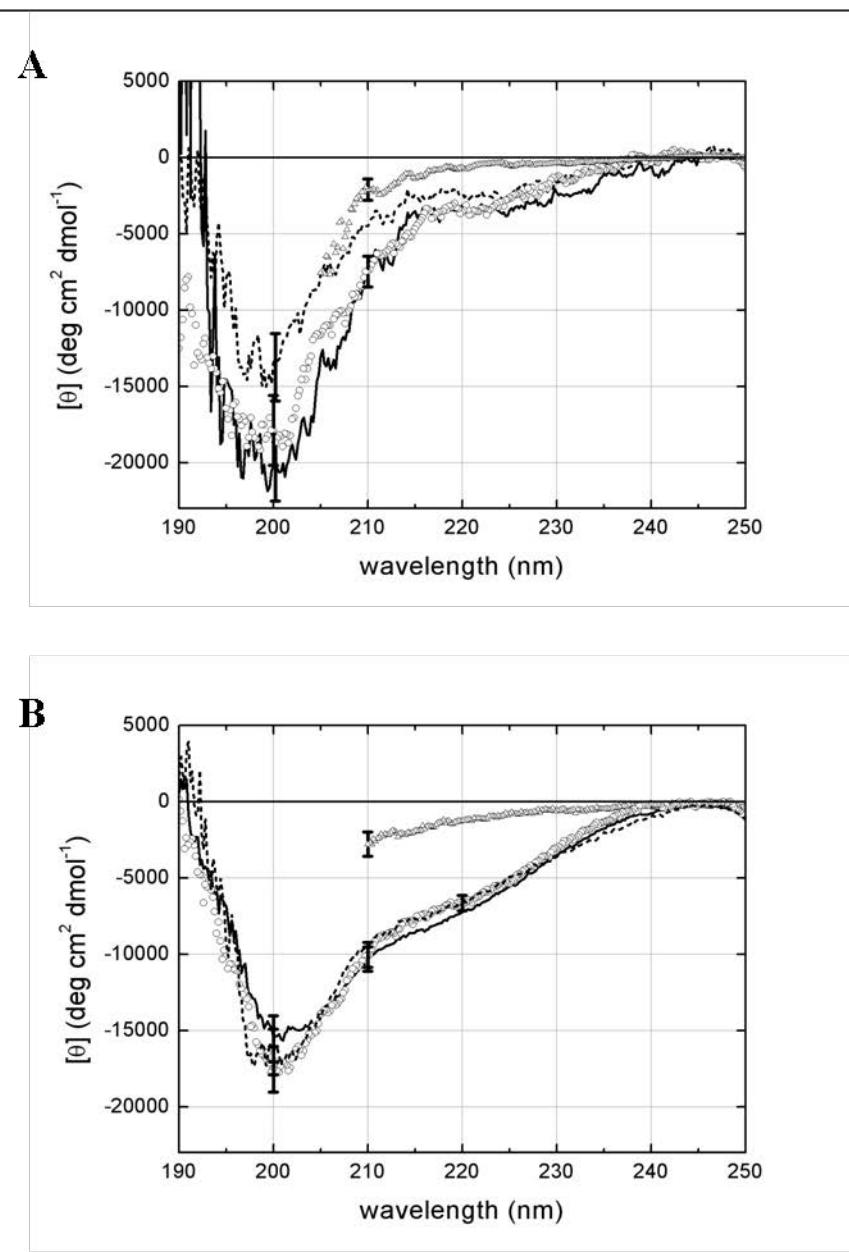
F.Polticelli
And the Rosetta
method



FLUORESCENCE STUDIES NBP127



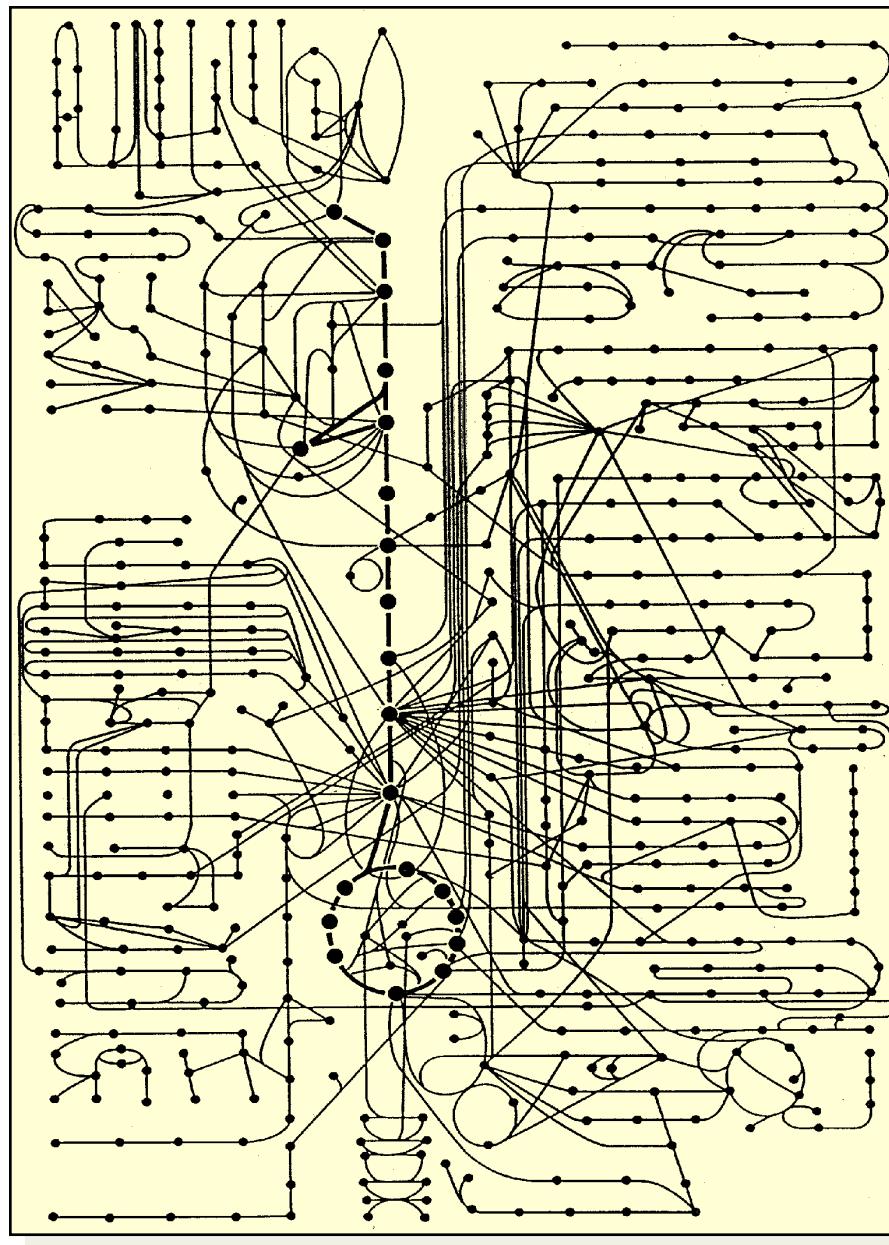
One Tryptophan residue is localized in a zone less exposed to the solvent



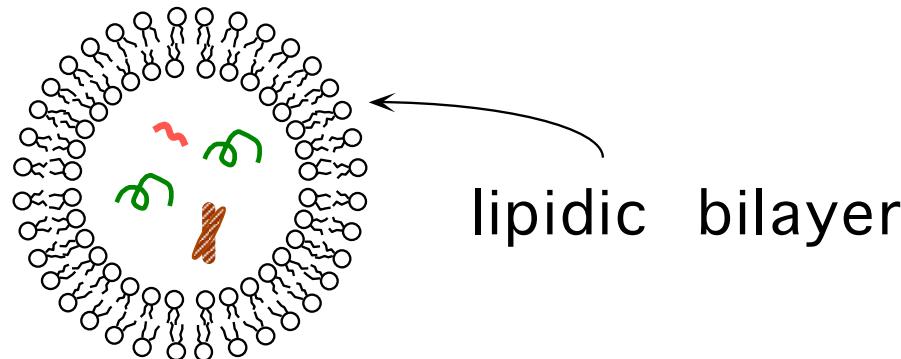
Another exercise in chemical synthetic biology

Modern cells are extremely complex.
Can you build in the laboratory very simple ones,
Corresponding to the „minimal cell“
Or „minimal life“?

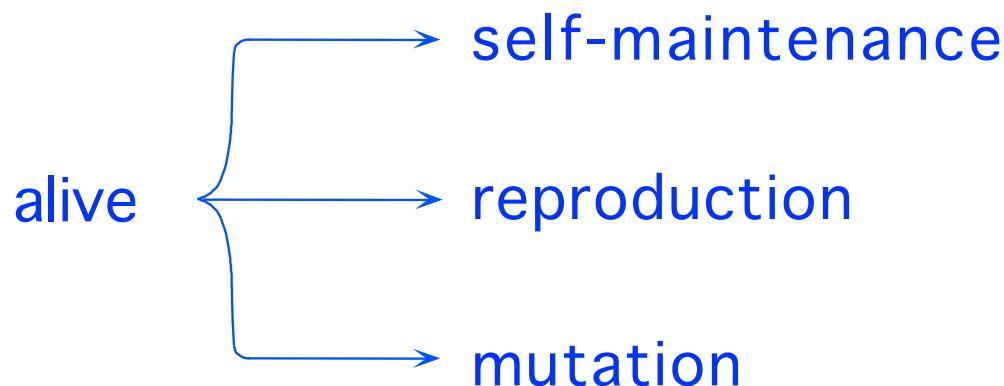
A maze illustrating the chemical reactions that interconvert small molecules in cells.

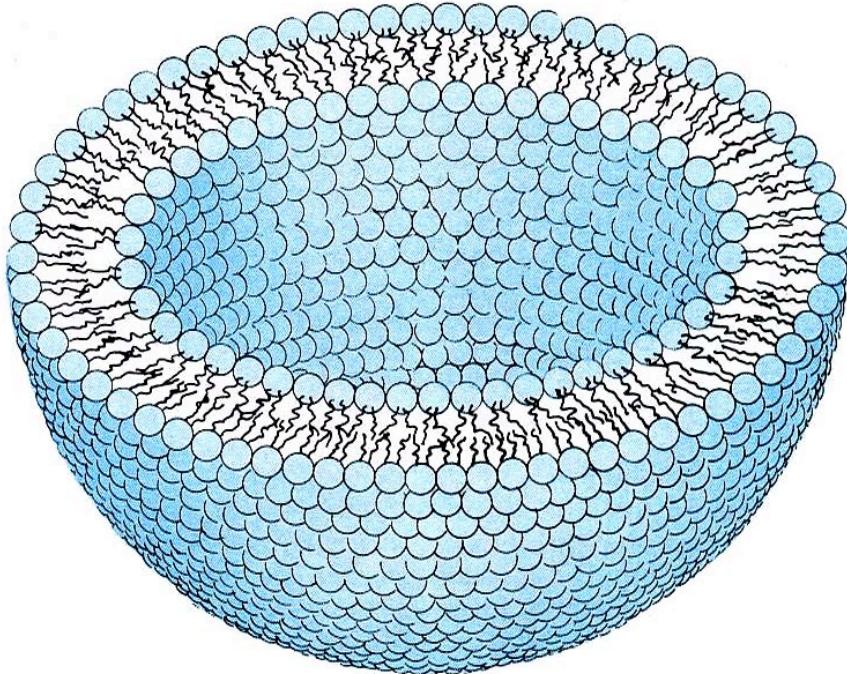


the notion of the minimal cell:



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



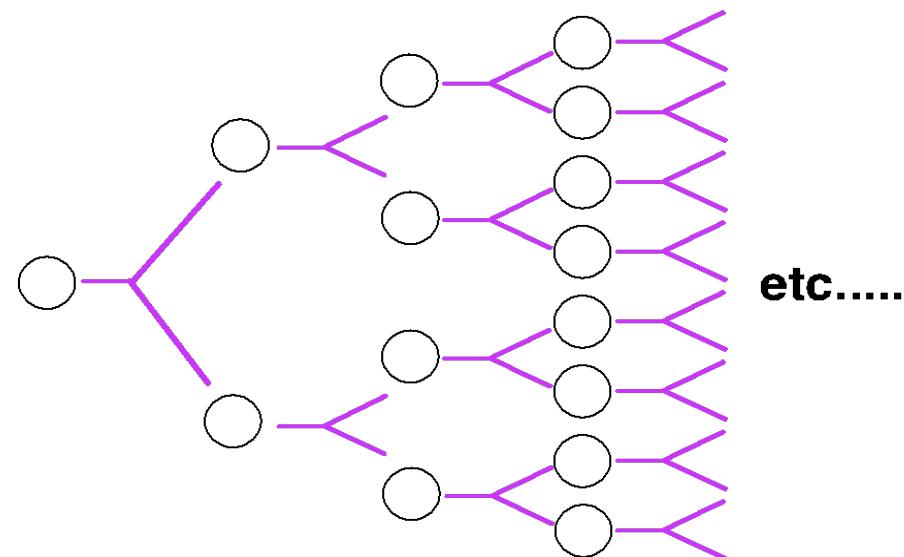


MOLECULAR ARCHITECTURE of the animal-cell membrane is determined primarily by the interactions of phospholipid molecules in water.

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.

**FIRST PREREQUISITE:
CELL-LIKE COMPARTMENTS CAPABLE
OF SELF-REPRODUCTION**

**SELF-REPRODUCING MICELLES, LIPOSOMES
& CHEMICAL AUTOPOIESIS**



caprylate



or

oleate



form micelles at alkaline pH



(Deamer, 1976)

vesicles at pH=7-8
($\text{pH} \approx \text{pk}$)



precursors (water insoluble!)

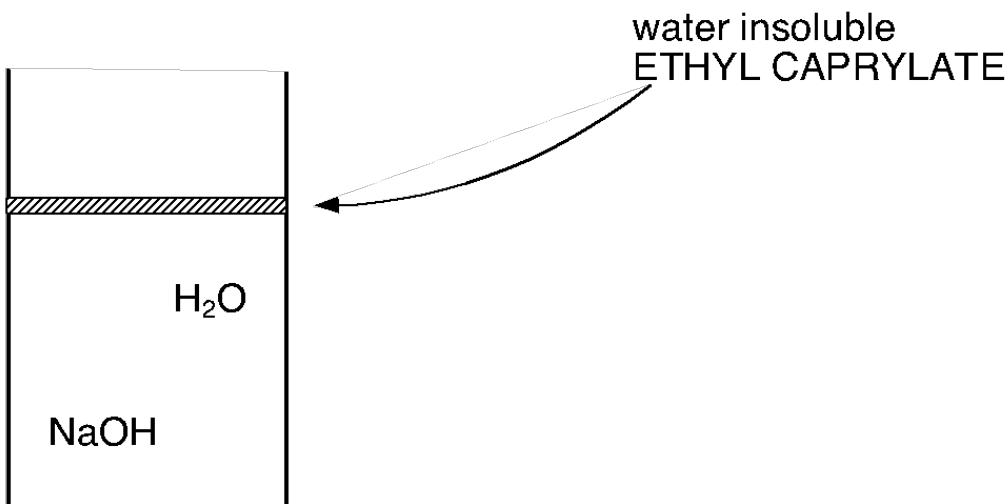


or

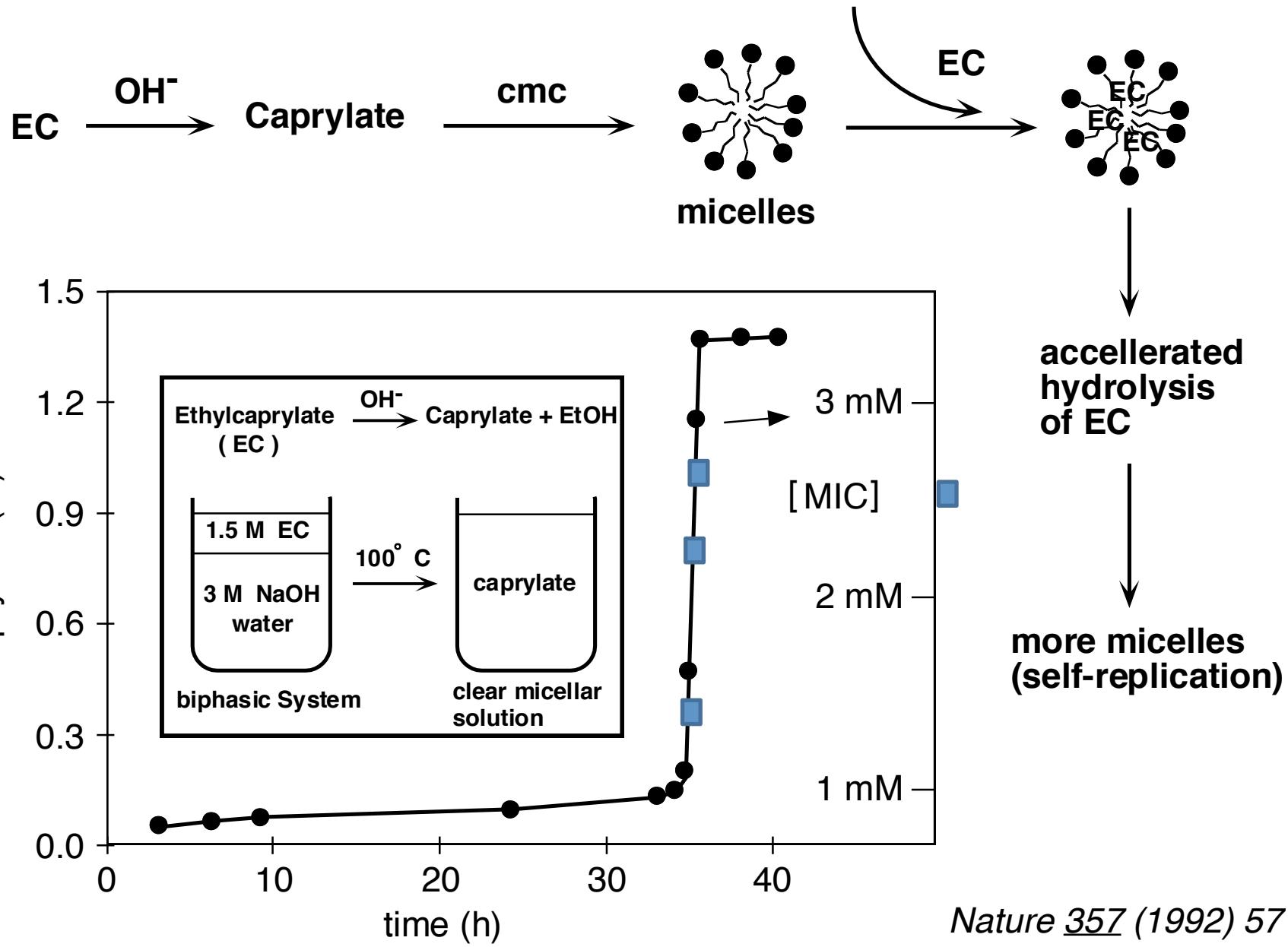


{ micelles or
R COO⁻
vesicles

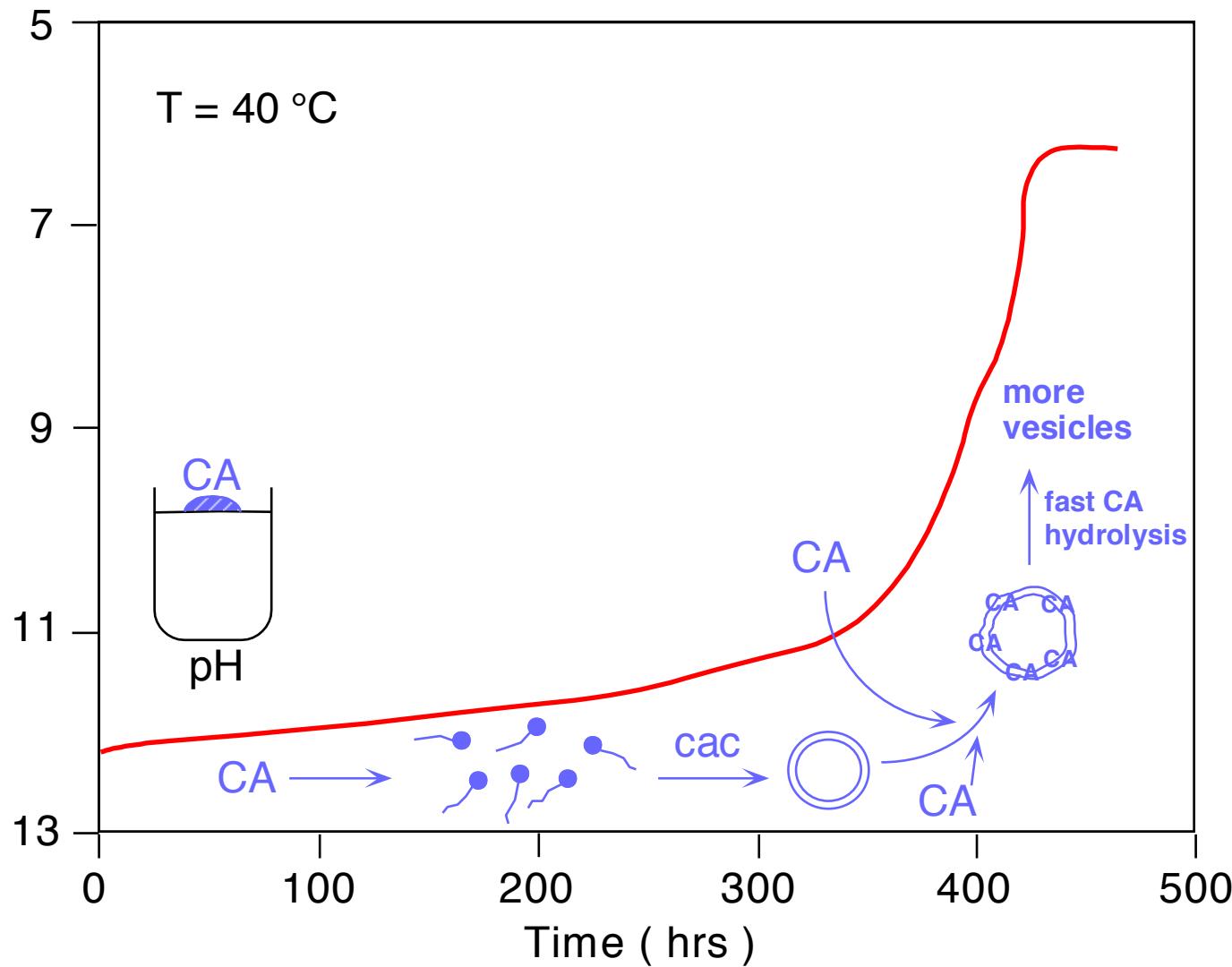
... a reaction which creates its own
microenvironment for self-replication....

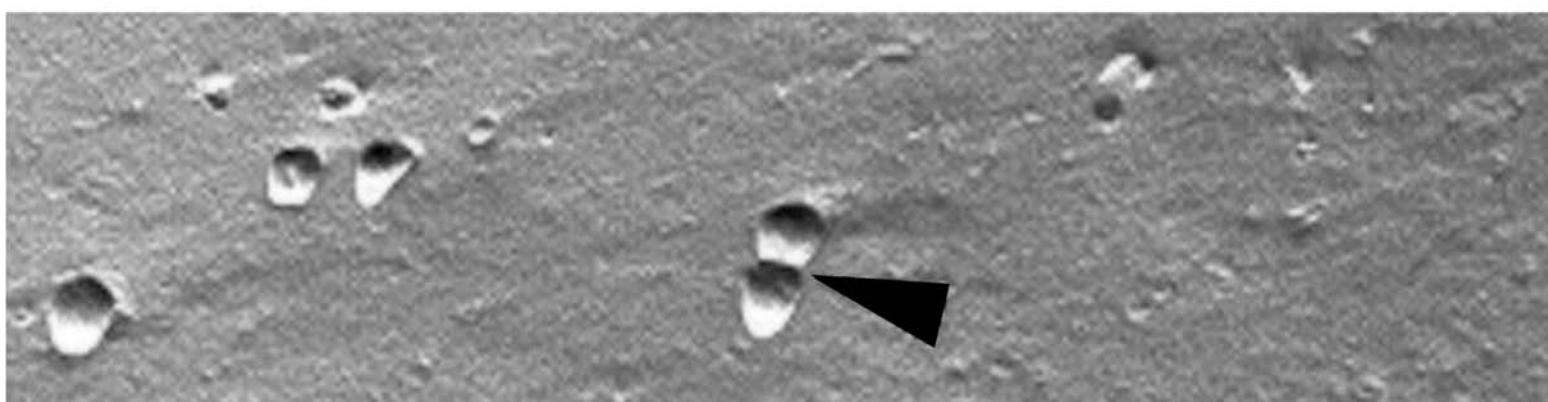
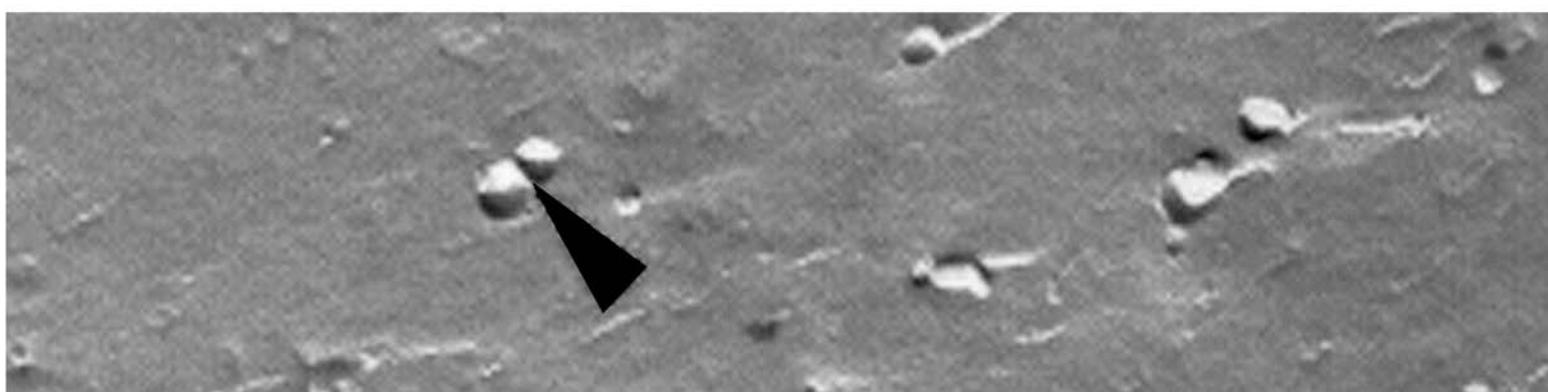
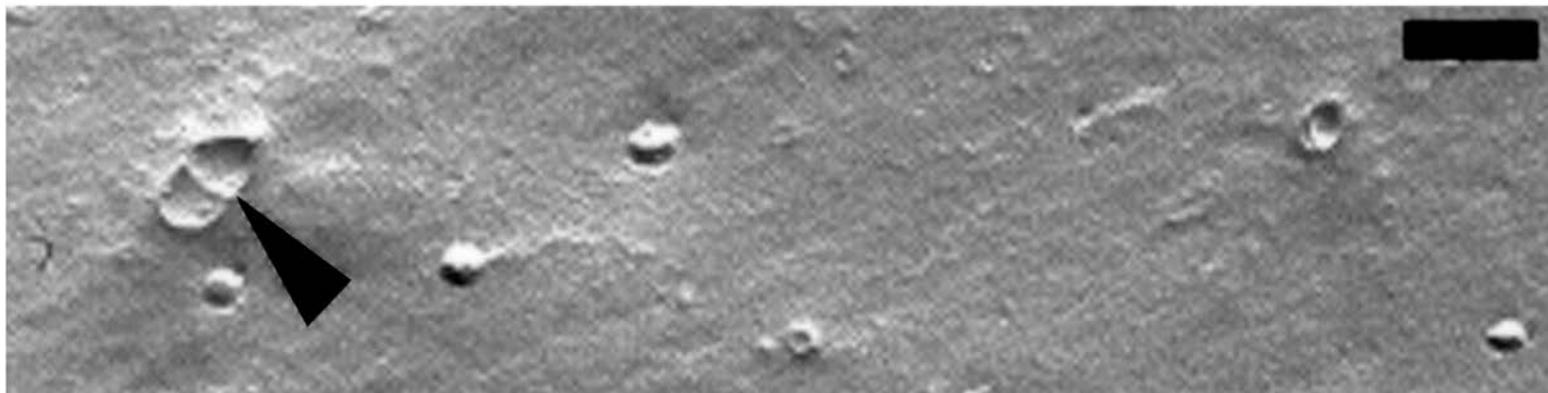


or vesicles (pH ~ 7 - 8)
(start with anhydride)

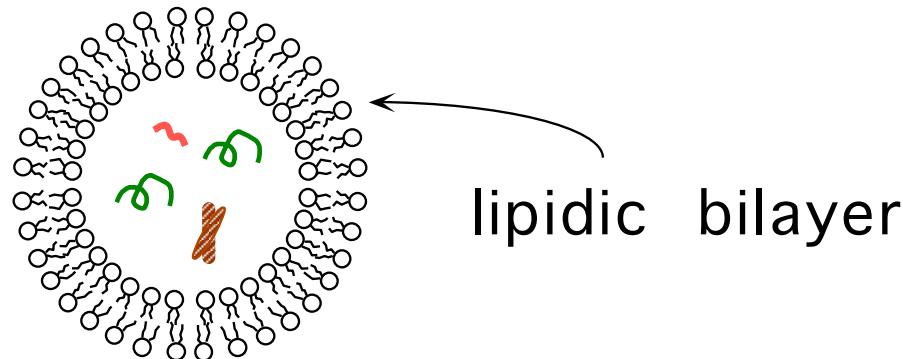


Hydrolysis of Caprylic anhydride



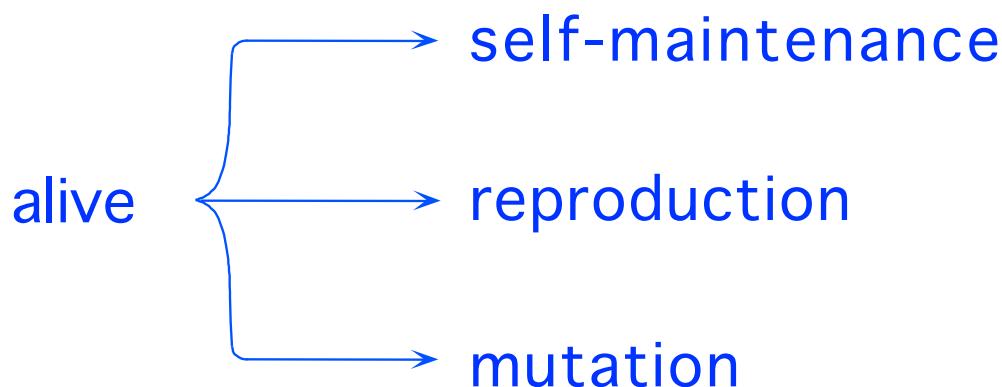


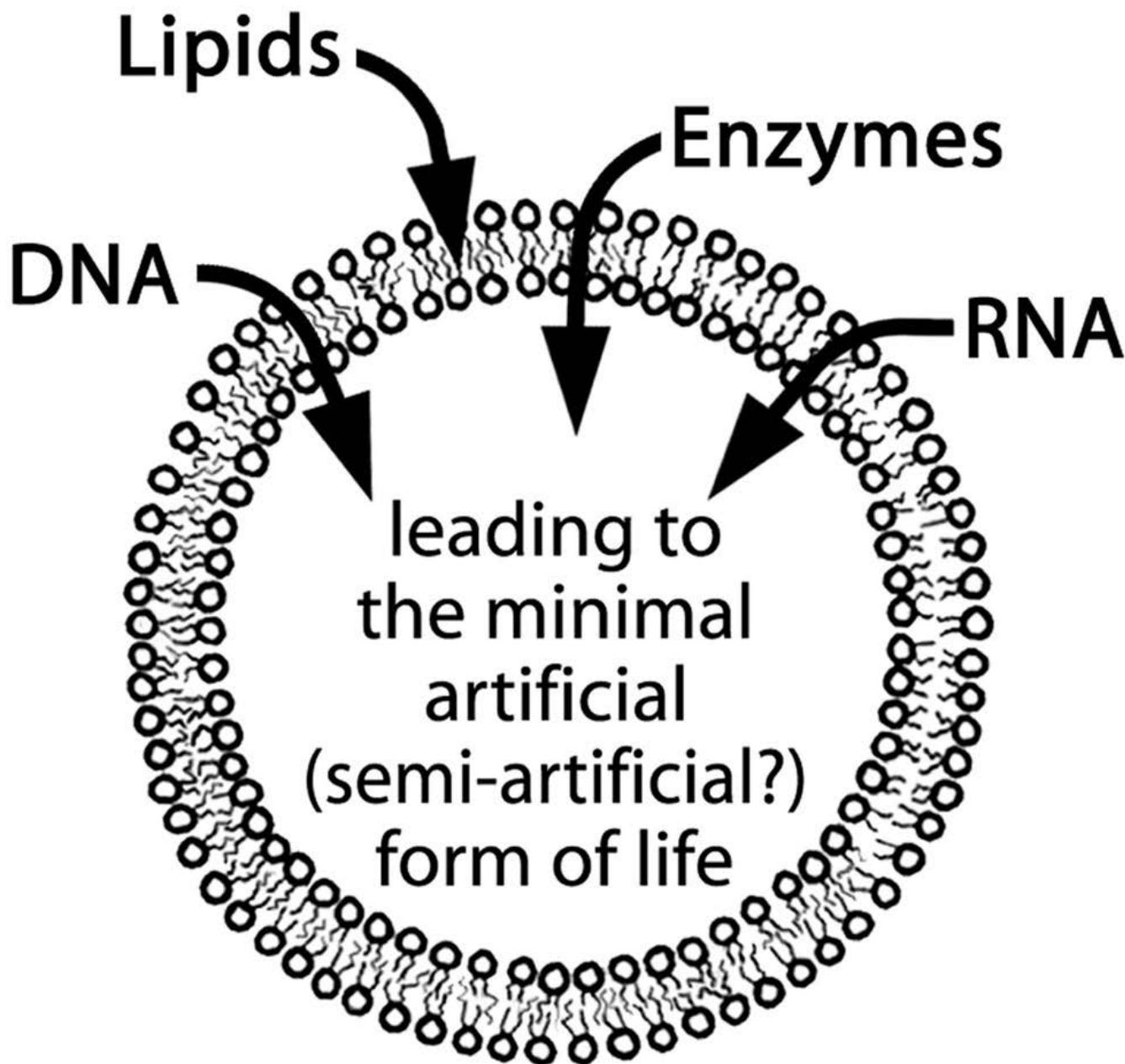
the notion of the minimal cell:



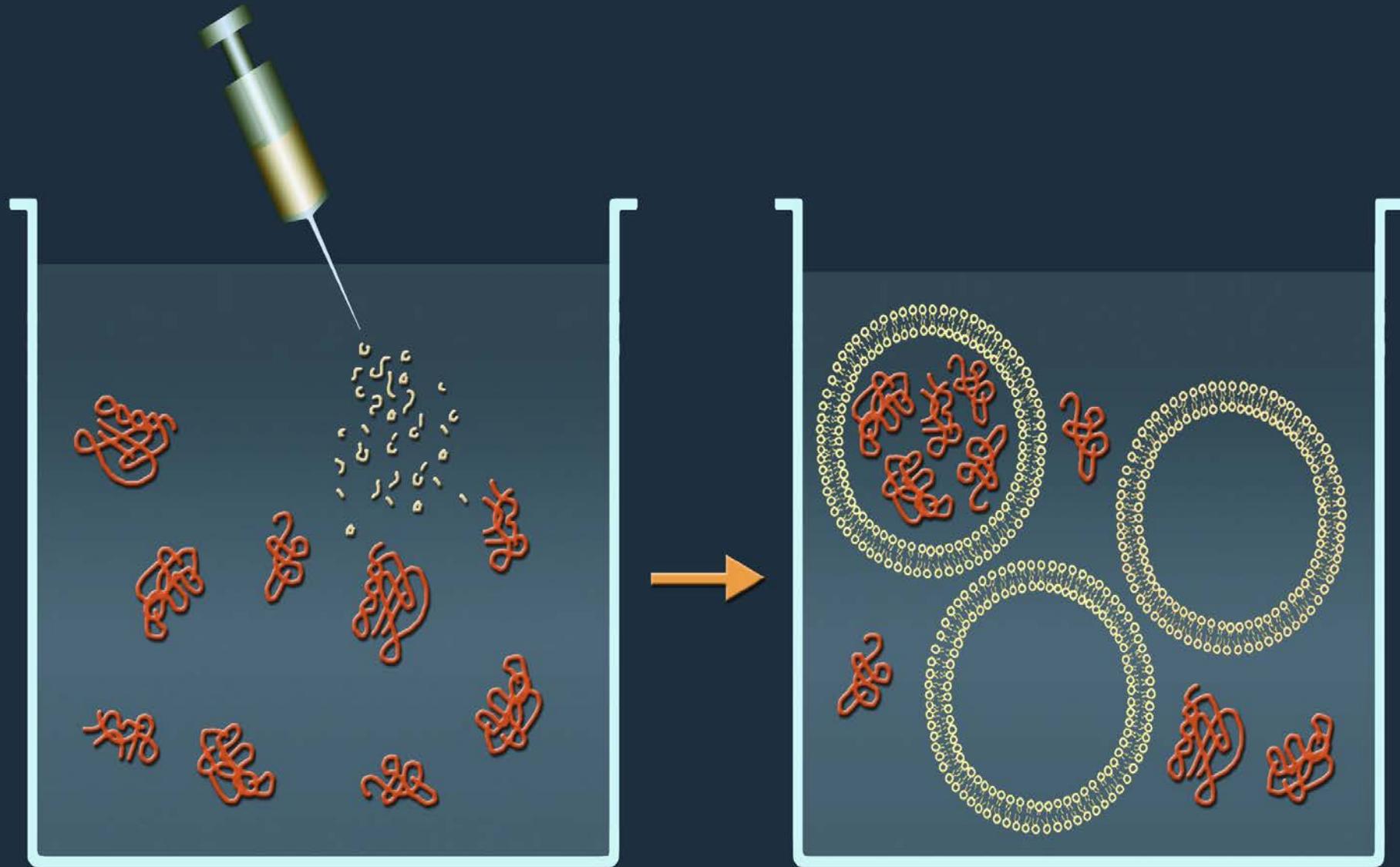
containing the minimal and sufficient number of components to be "alive"

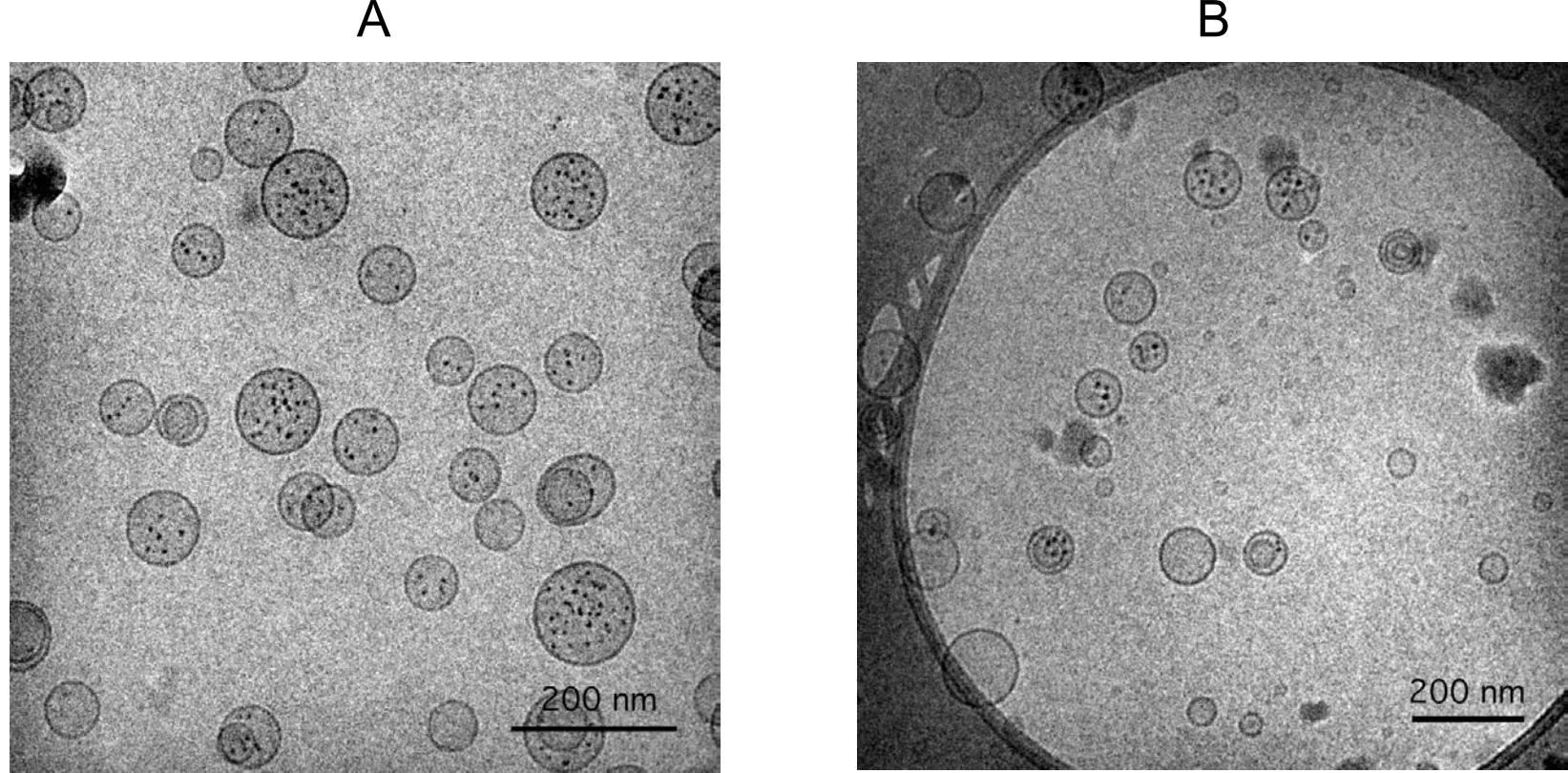
Pasquale Stano
Teresa Pereira de Souza





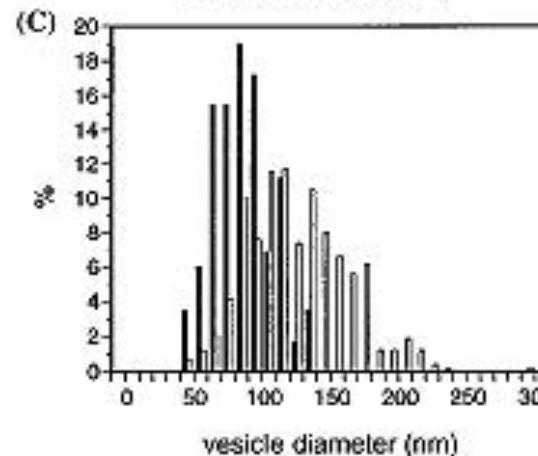
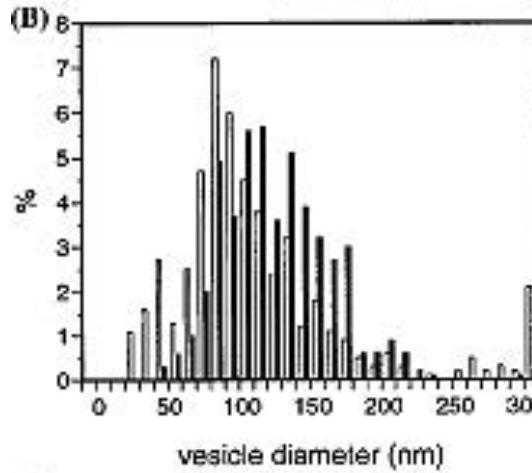
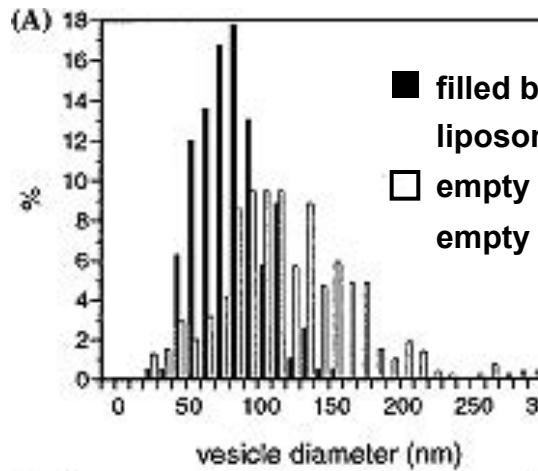
LIPIDS





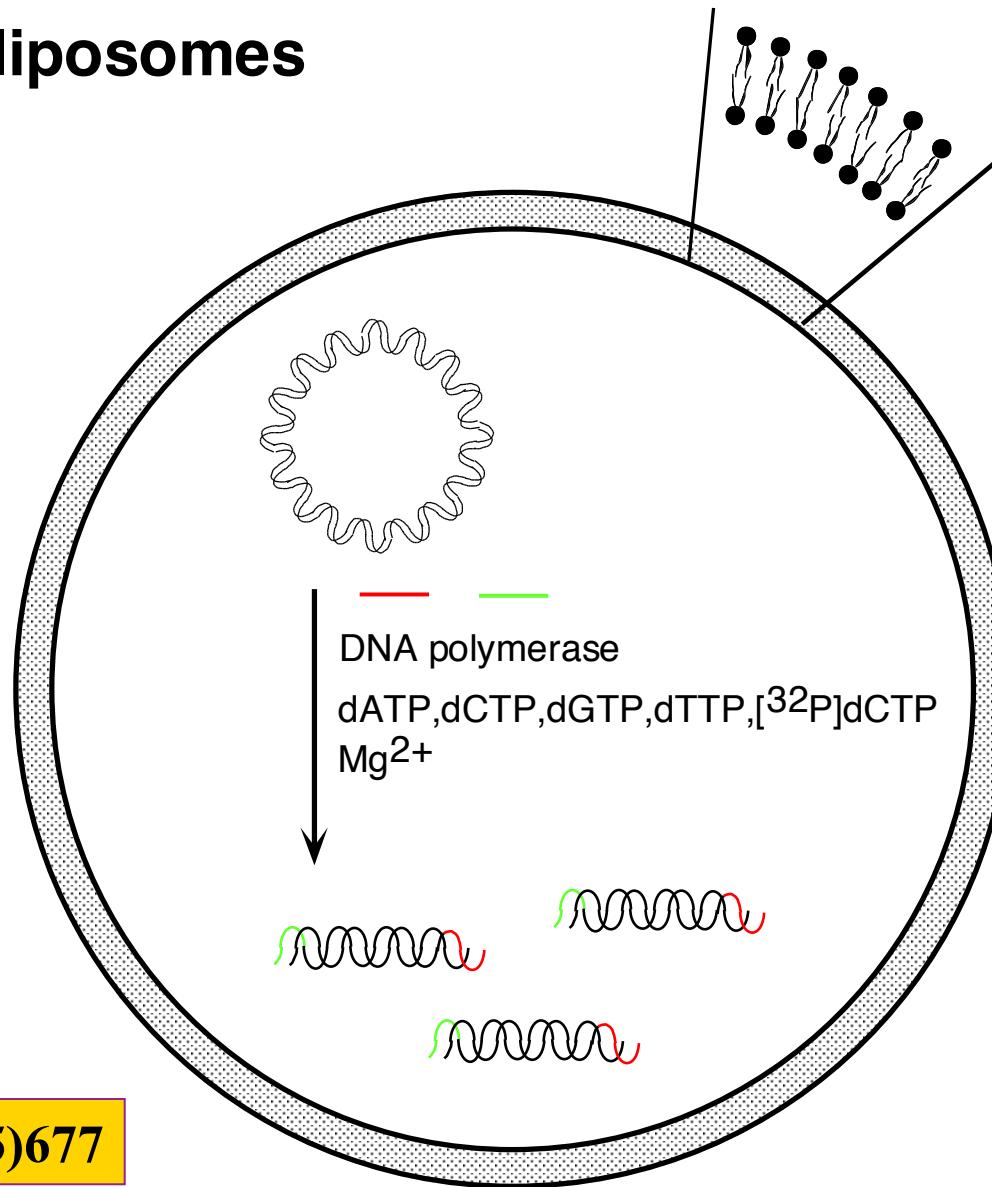
Cryo-TEM micrographs of

- (A) ferritin -containing POPC liposomes prepared using the reverse phase evaporation method, followed by a sizing down by extrusion through polycarbonate membranes with 100 nm pore diameters ($[POPC] = 6.1 \text{ mM}$); and of
- (B) the vesicle suspension obtained after addition of oleate to preformed POPC liposomes ($[POPC] = 3 \text{ mM}$, $[\text{oleic acid} + \text{oleate}] = 3 \text{ mM}$).

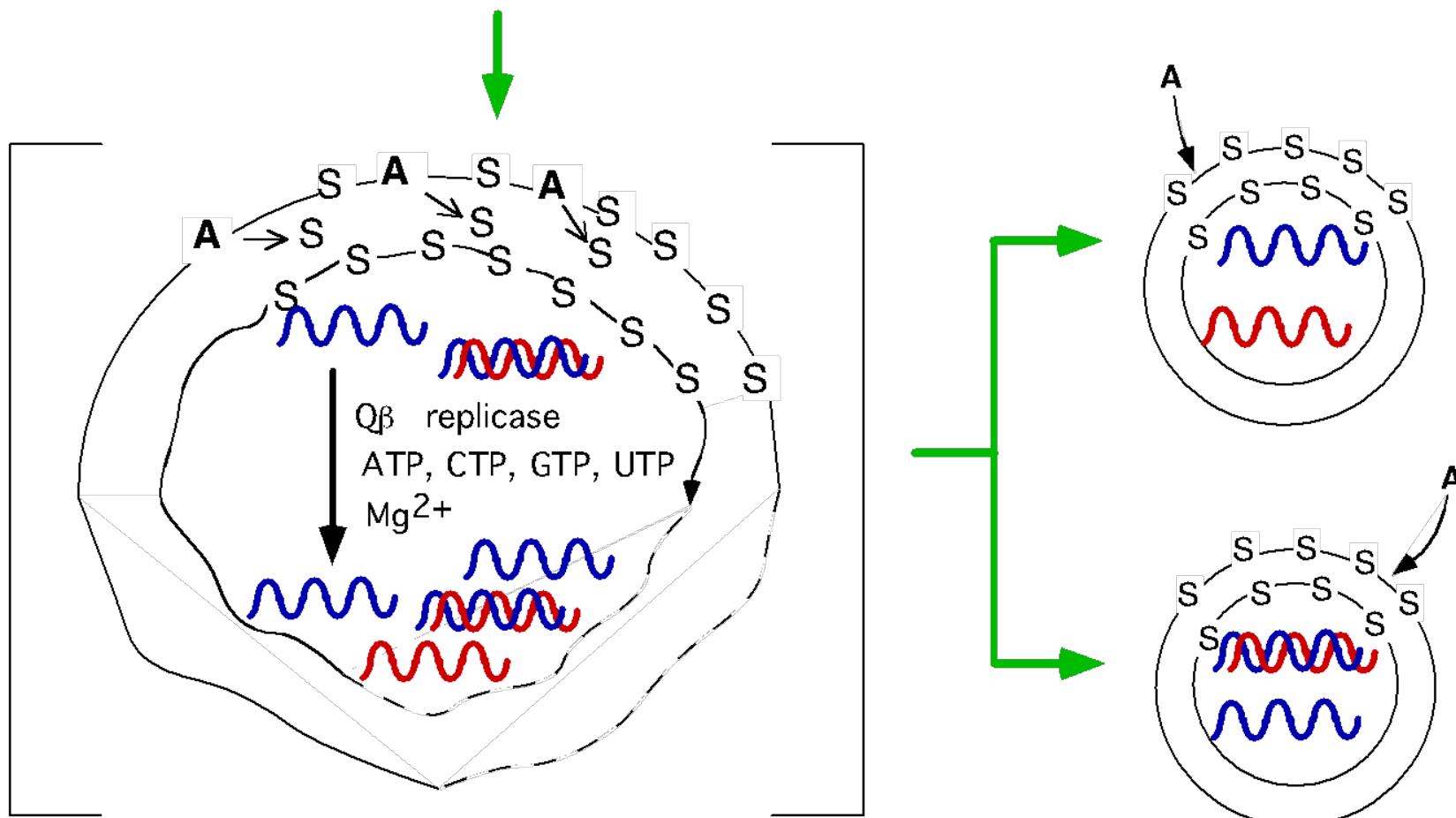


- (A) Number-weighted size distribution as obtained by cryo-TEM for the oleic acid vesicle suspension analyzed before hydrolysis (filled bars) and after oleic anhydride hydrolysis (empty bars).
- (B) Number-weighted size distribution as obtained by cryo-TEM for the oleic acid vesicle suspension examined after oleic anhydride hydrolysis. Empty (empty bars) and ferritin-containing (filled bars) vesicles are represented individually.
- (C) Comparison of the number-weighted size distribution of the filled oleic acid vesicles obtained before (filled bars) and after (empty bars) oleic anhydride hydrolysis. The total of all ferritin-containing vesicles was set to 100%. The last bar of the histogram in the three figures corresponds to all the vesicles larger than 300 nm.

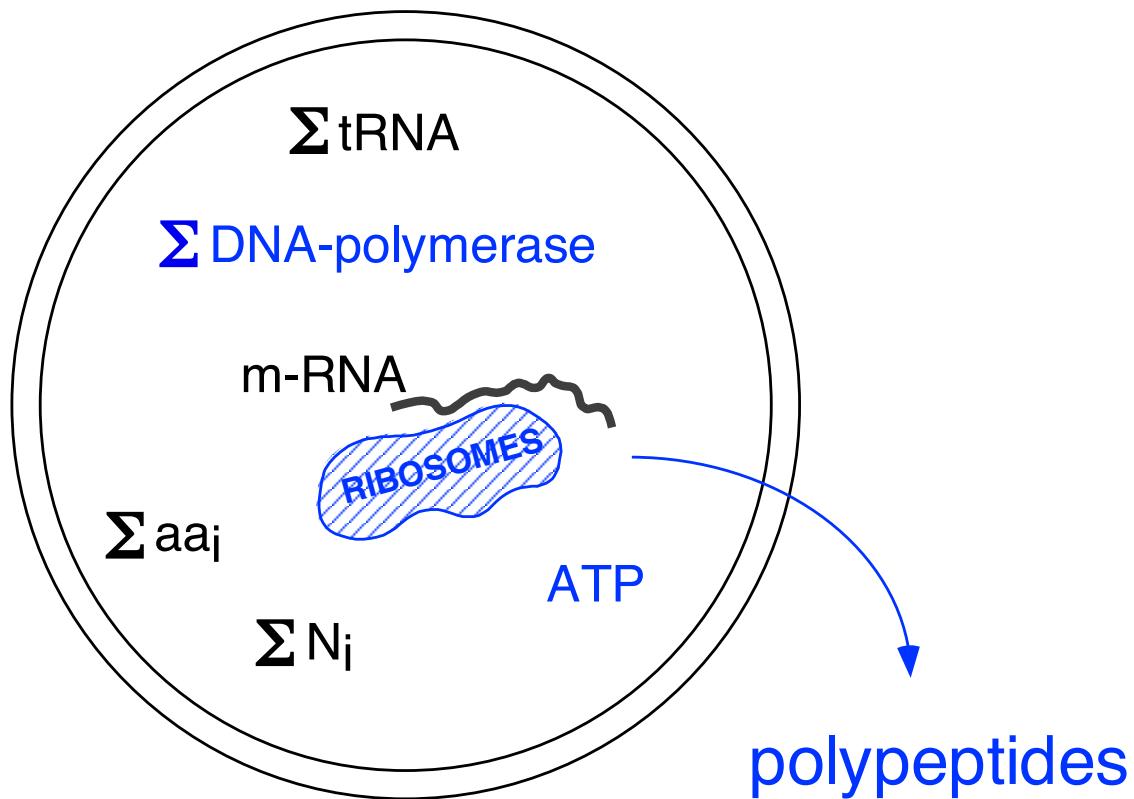
PCR in liposomes



Replication of RNA in
self-reproducing vesicles



protein biosynthesis in liposomes



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

IN THE LAST FEW YEARS, PROTEIN EXPRESSION

**INSIDE LIPOSOMES HAS BEEN ACHIEVED
BY SEVERAL GROUPS ALL AROUND THE WORLD**

**YOMO ET AL, IN JAPAN,
ISHIKAWA ALSO IN JAPAN,
NOIREAX AND LIEBCHABER IN USA
NAKATANI IN STRASBOURG
LUISI' GROUP IN ZÜRICH AND ROME**

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

The «PureSystem» developed by Ueda and collaborators (2001)

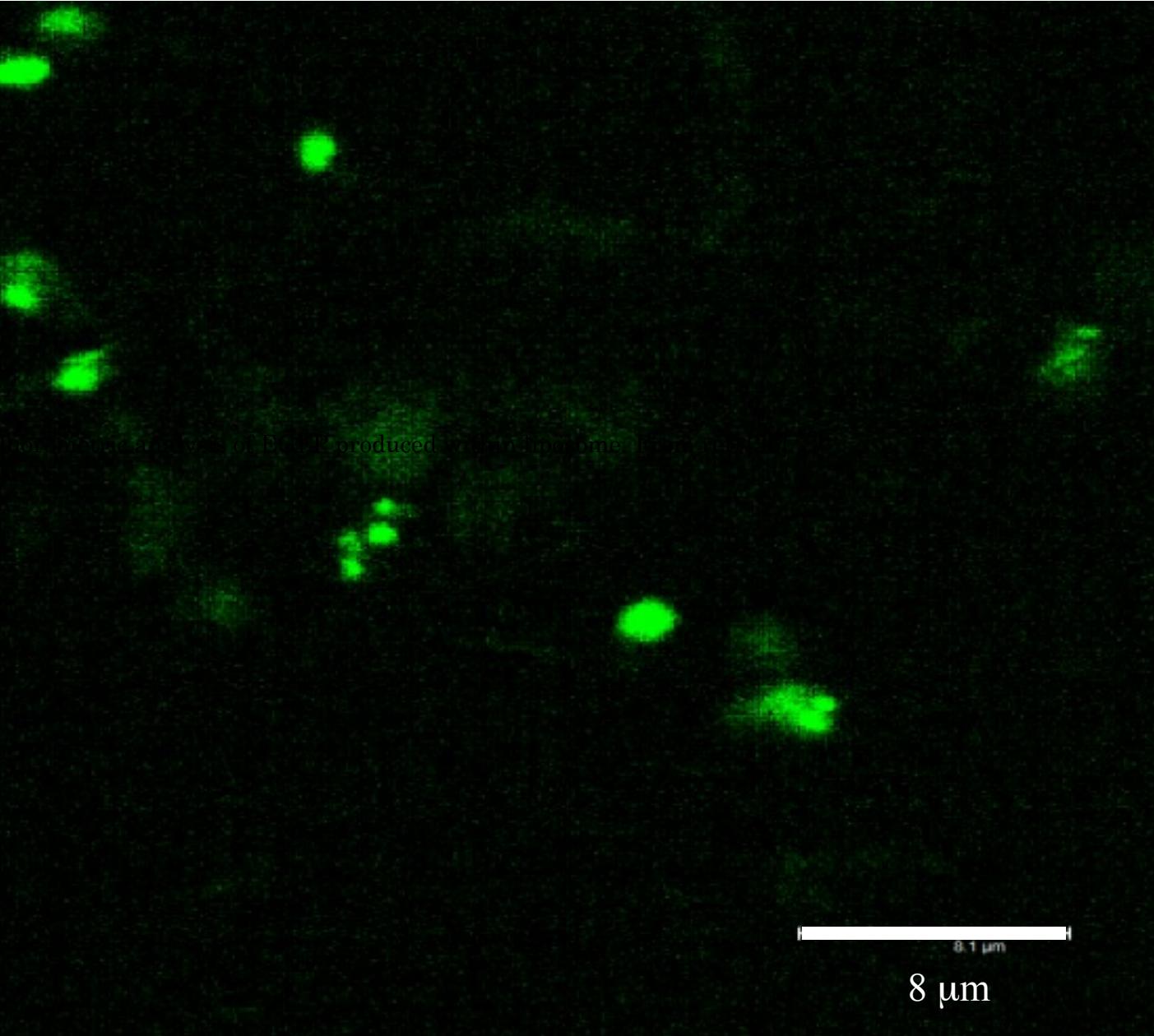
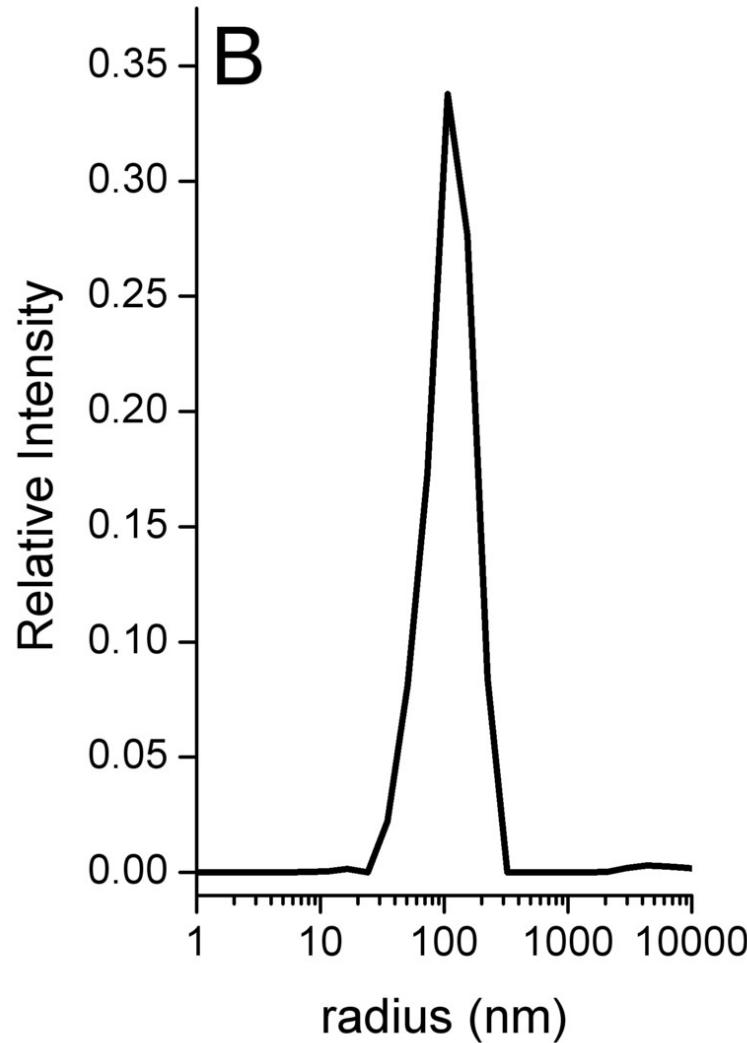
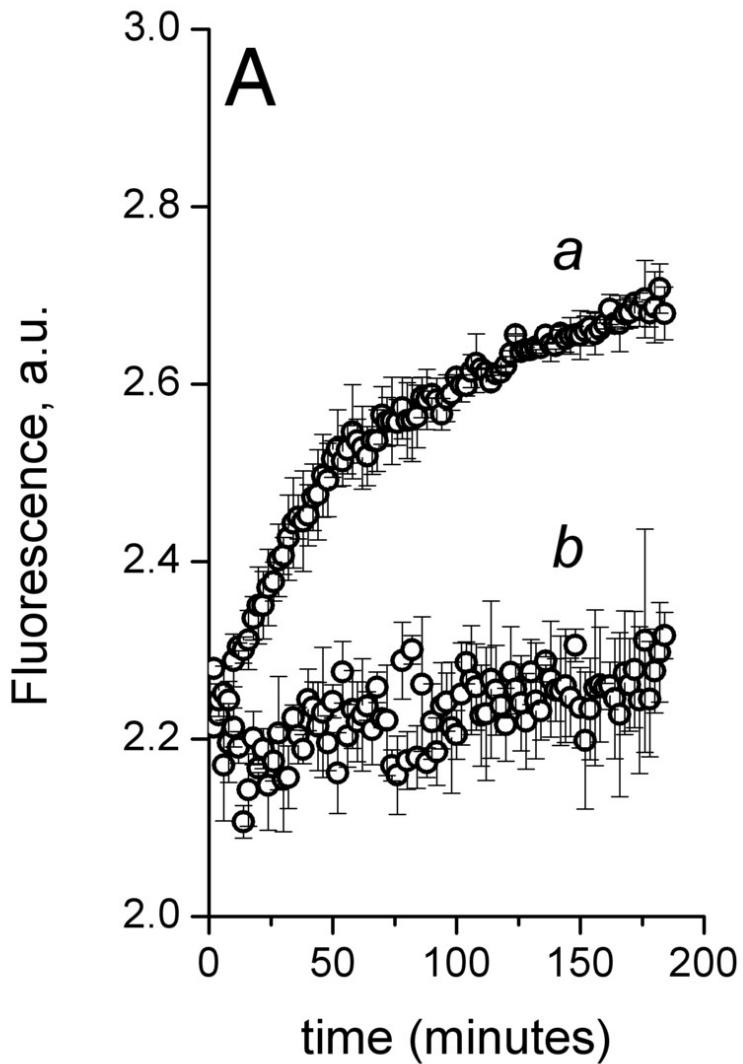


Figure 4. Confocal images of EGFP fluorescent liposomes. From ref. [36].

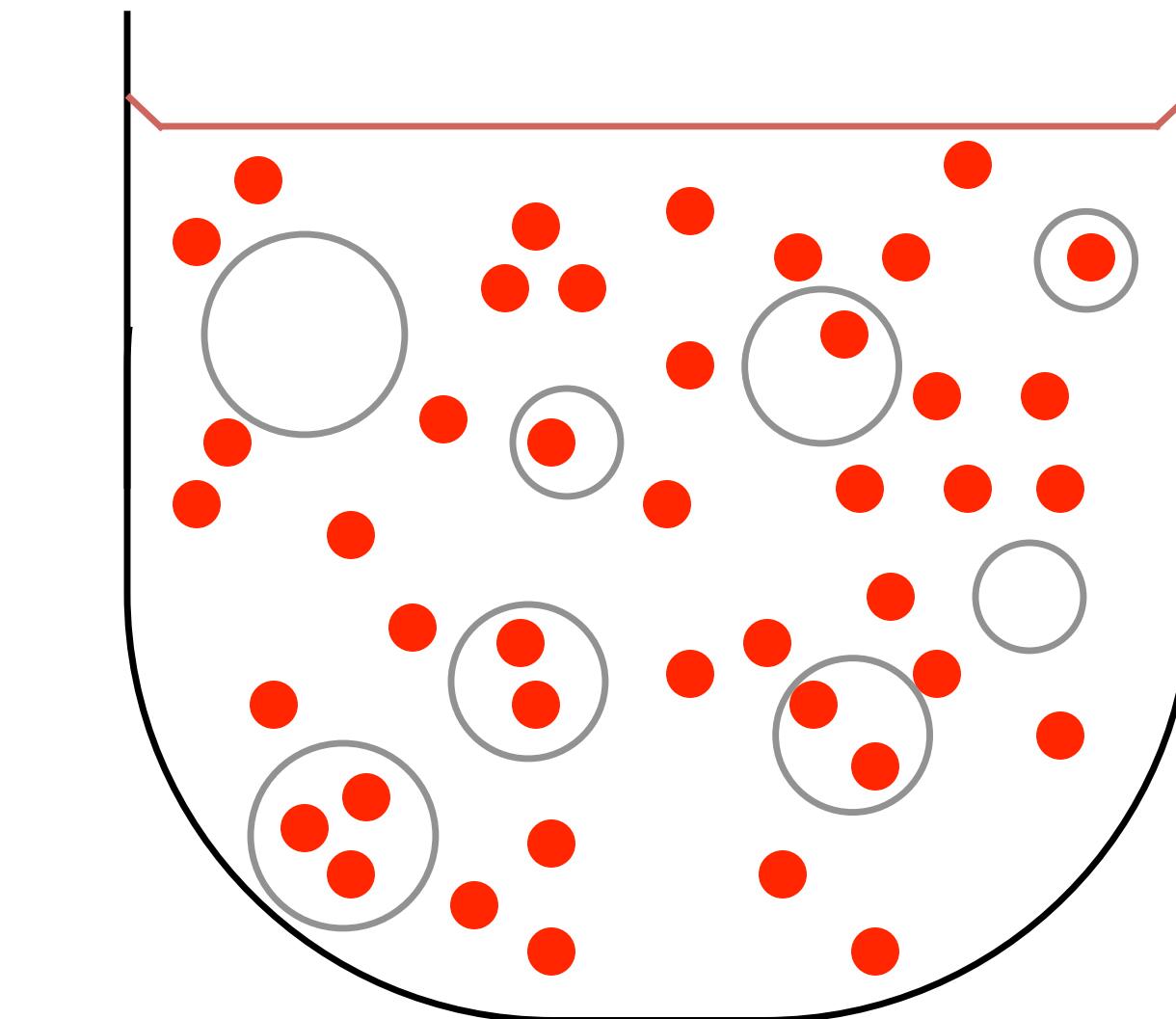
SMALL VESICLES PREPARED BY INJECTION METHOD

Tereza Souza



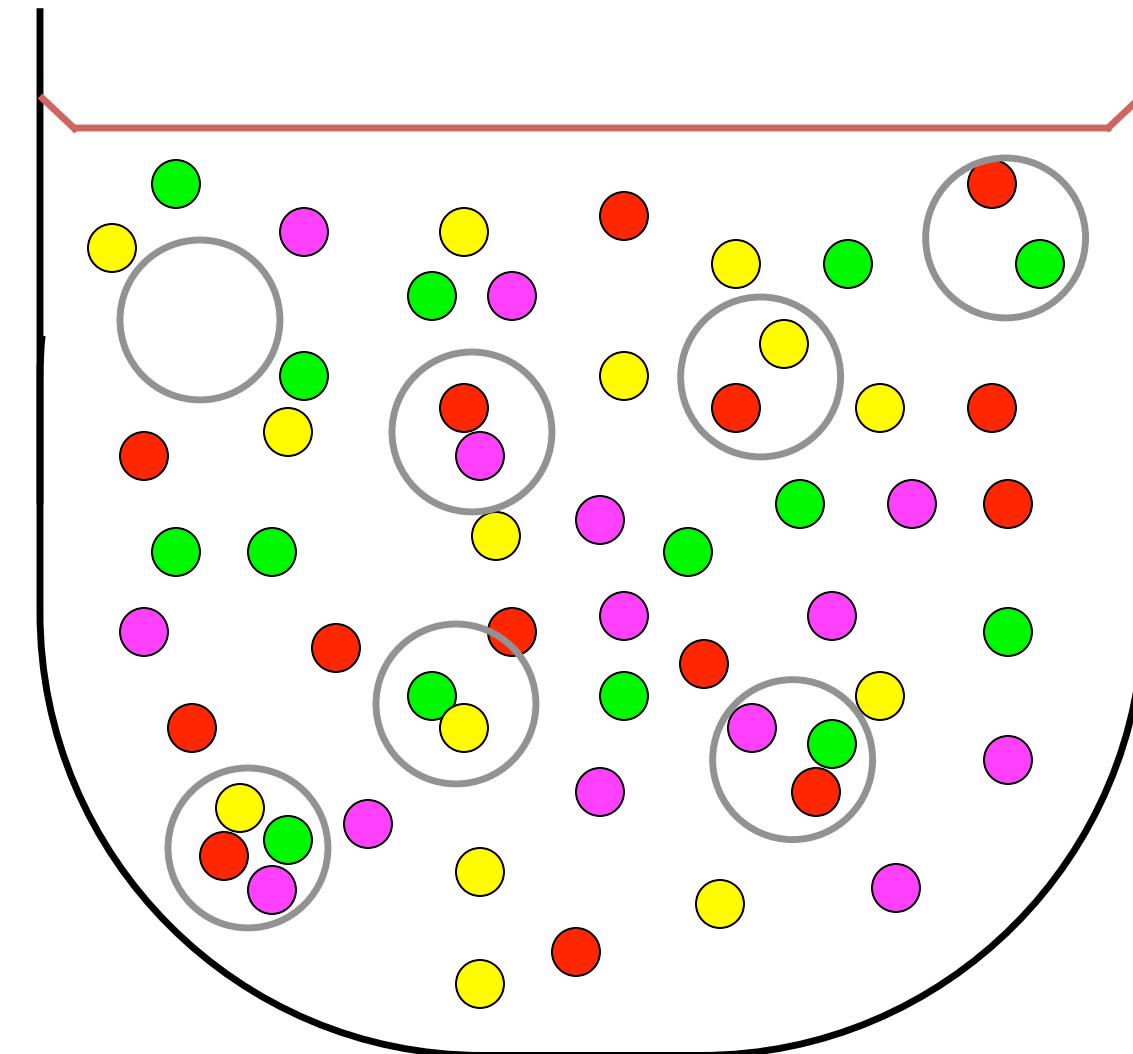
Statistics of entrapment

POISSON DISTRIBUTION



Statistics of entrapment

POISSON DISTRIBUTION



POISSON DISTRIBUTION

Probability to find a vesicle with **at least** 1 molecule
of the k -th species inside

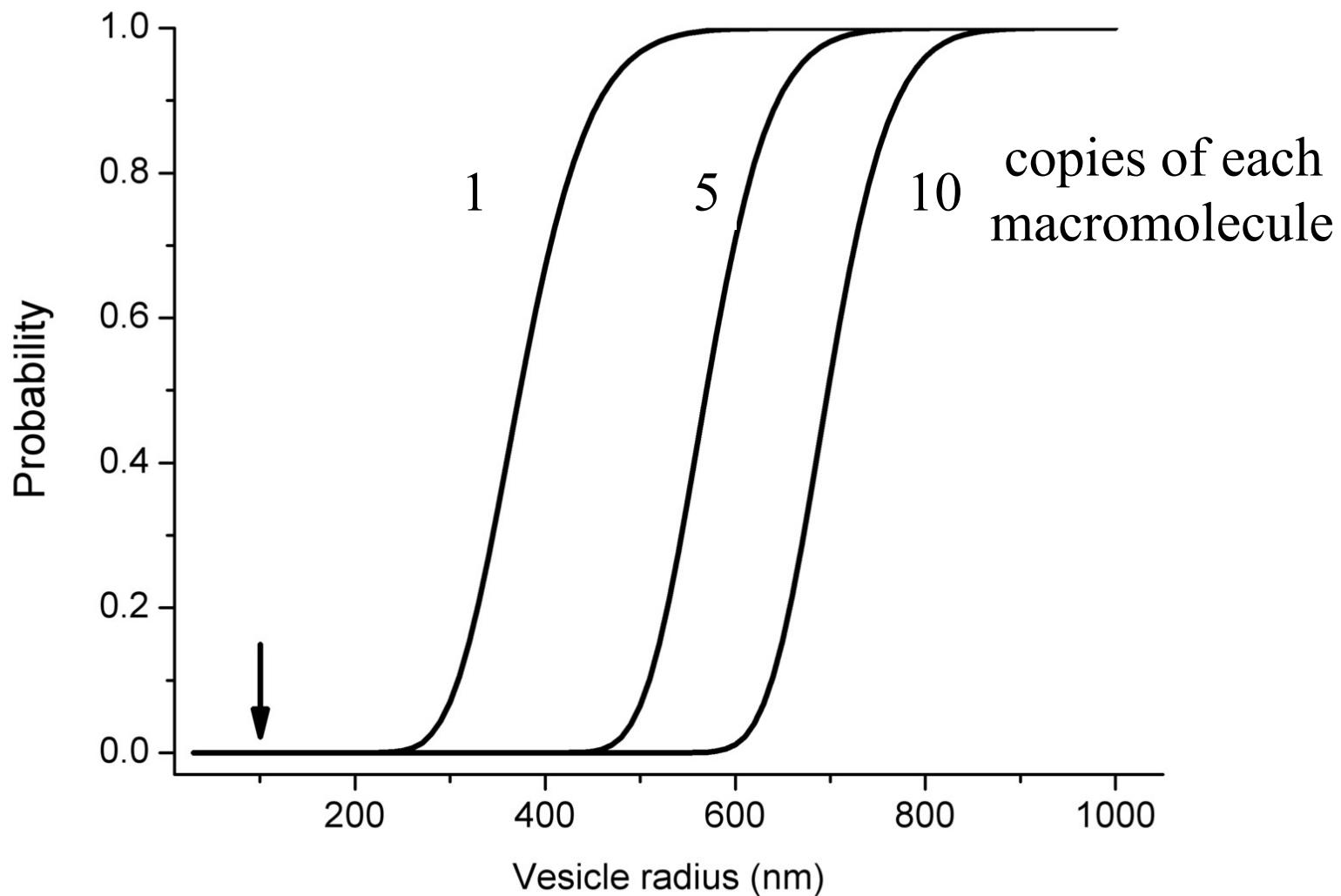
$$\wp(a_k, n_k \geq 1) = 1 - e^{-a_k}$$

Probability to find a vesicle with **at least** 1 molecule
of each of the N species inside ($N = 82$)

$$\wp_c = \prod_{k=1}^N \left(1 - e^{-a_k}\right)$$

ENTRAPMENT EVENTS ARE CONSIDERED INDEPENDENT

PROBABILITY TO FIND A VESICLE WITH ALL TRANSCRIPTION-TRANSLATION COMPONENTS INSIDE



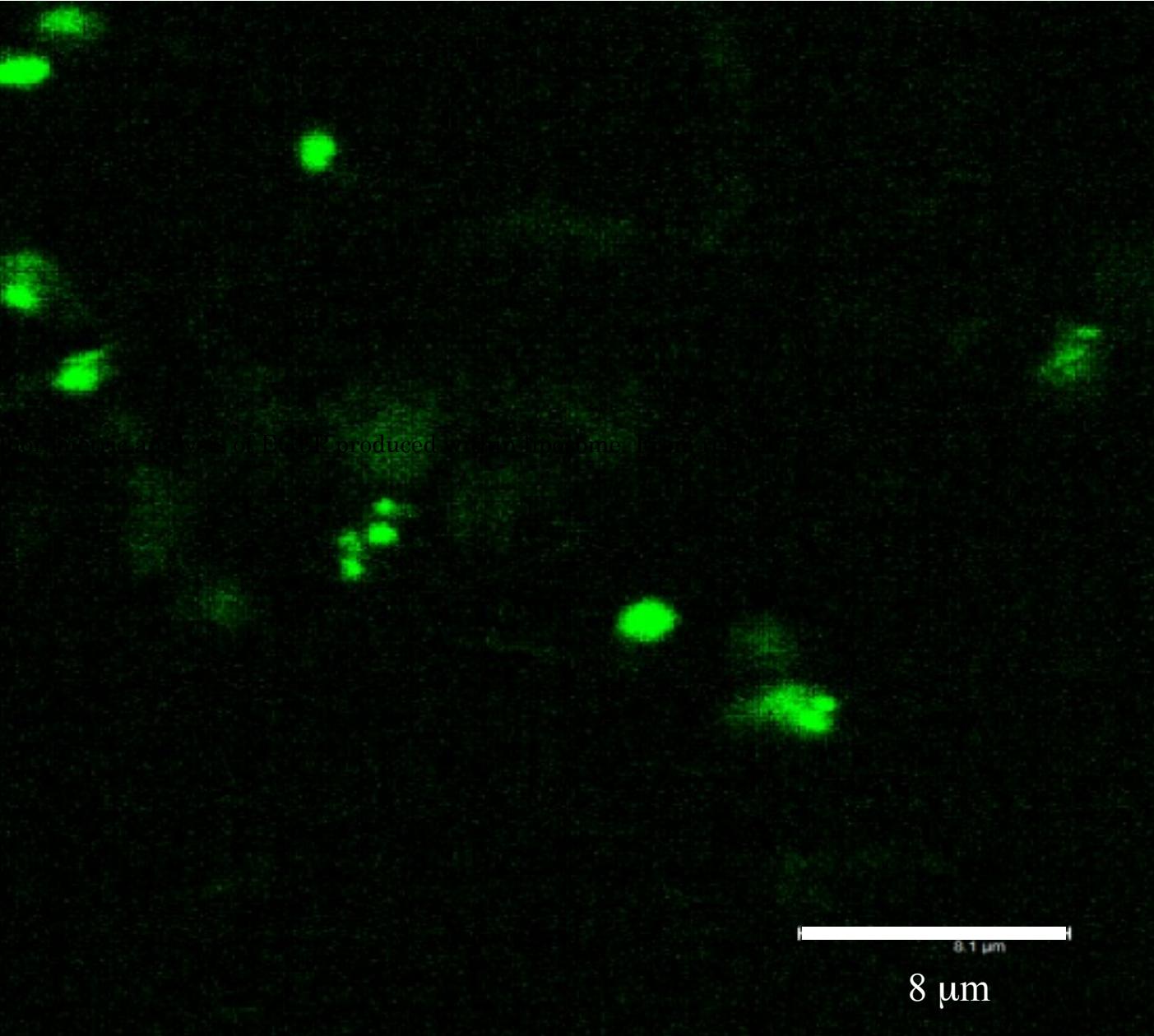
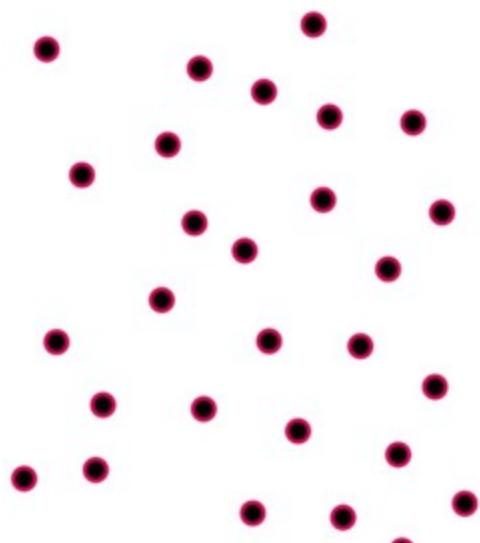


Figure 4. Confocal images of EGFP fluorescent liposomes. From ref. [36].

THEORY AGAINST THE EXPERIMENTS

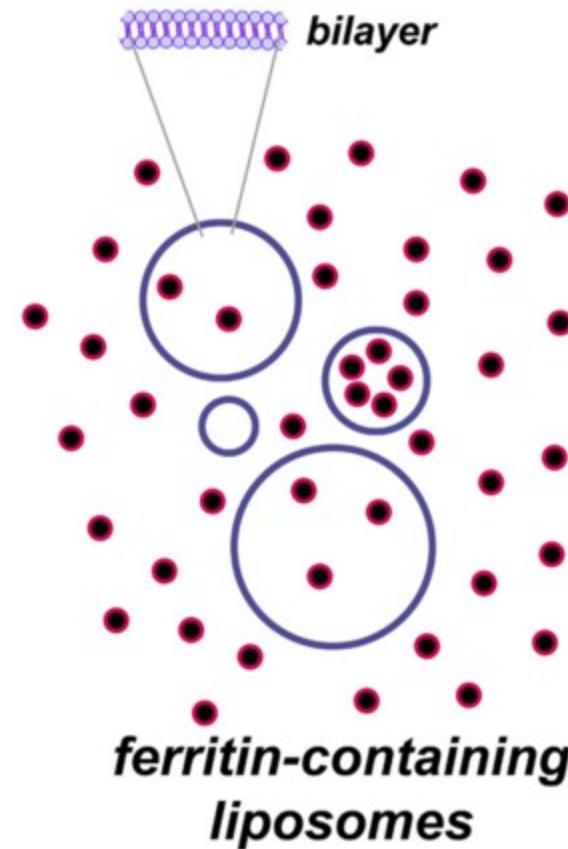
- ...of course experiments –once free of artifacts- are always right
- The theory is then not correct? In our case: is the Poisson distribution not applicable? Let's us see with a single component, ferritin

a

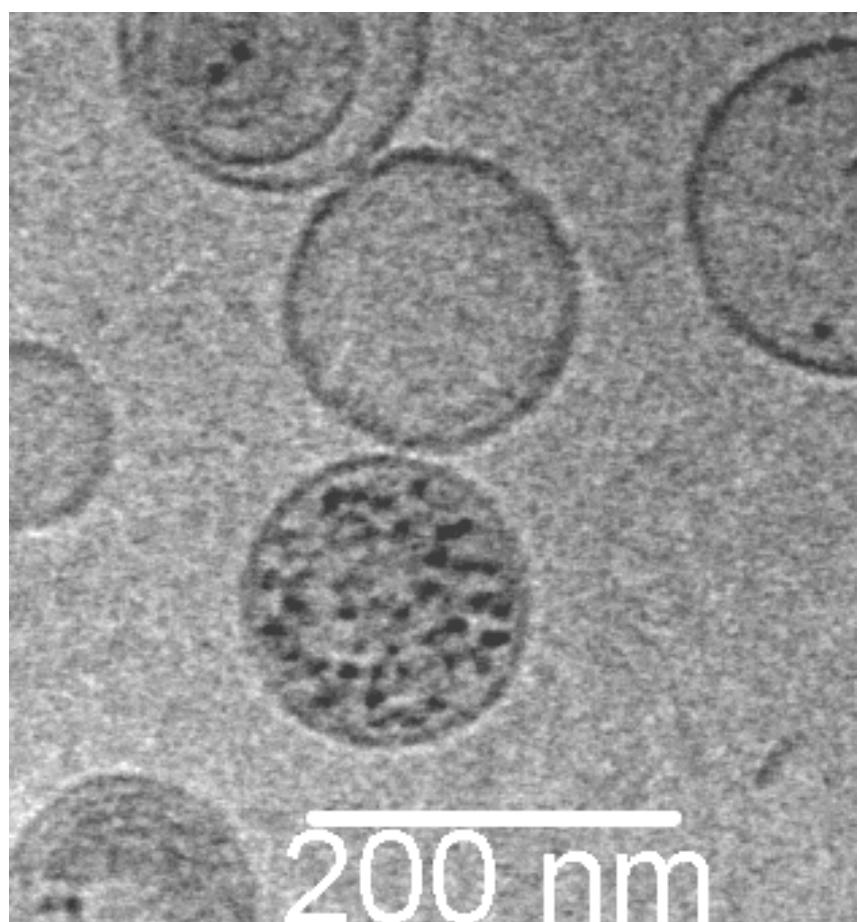
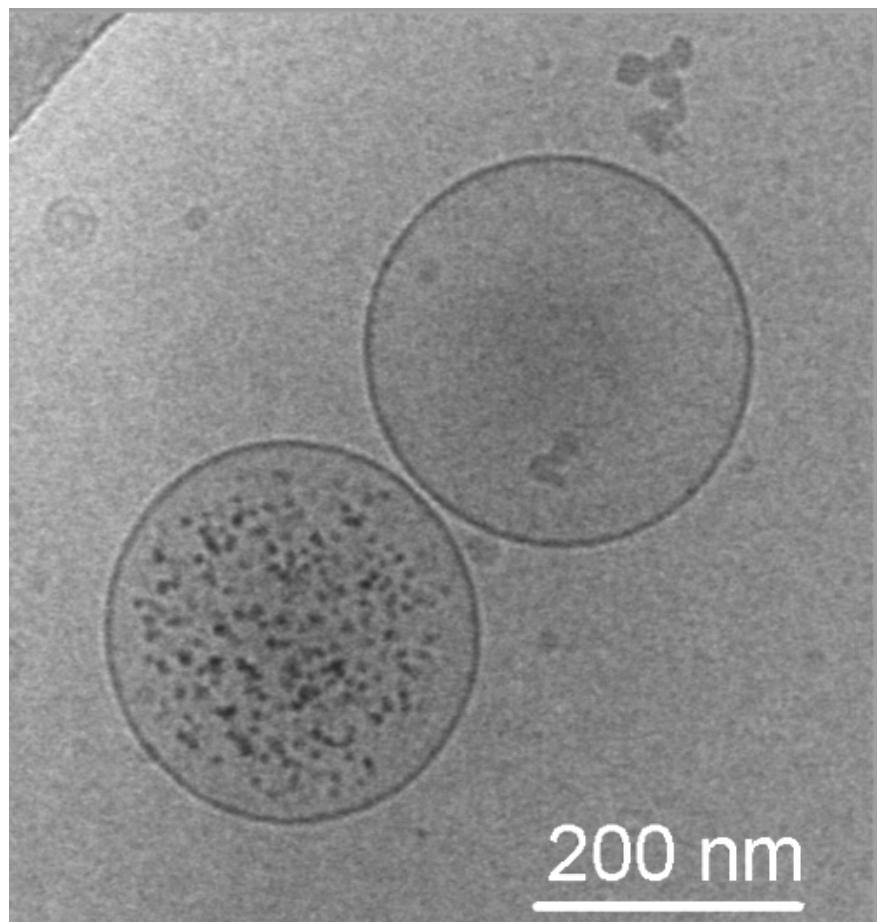


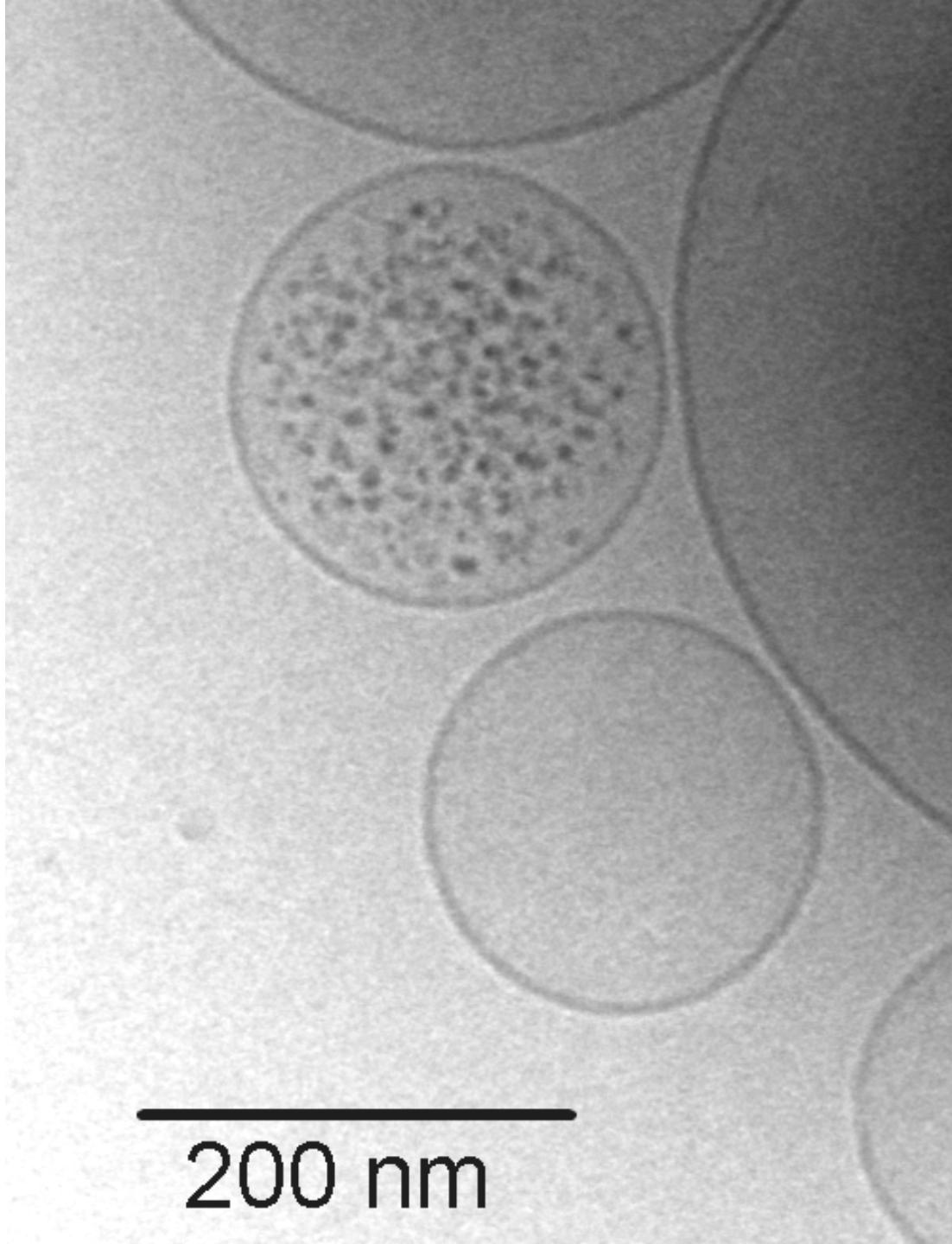
ferritin solution

+ *lipids*
→

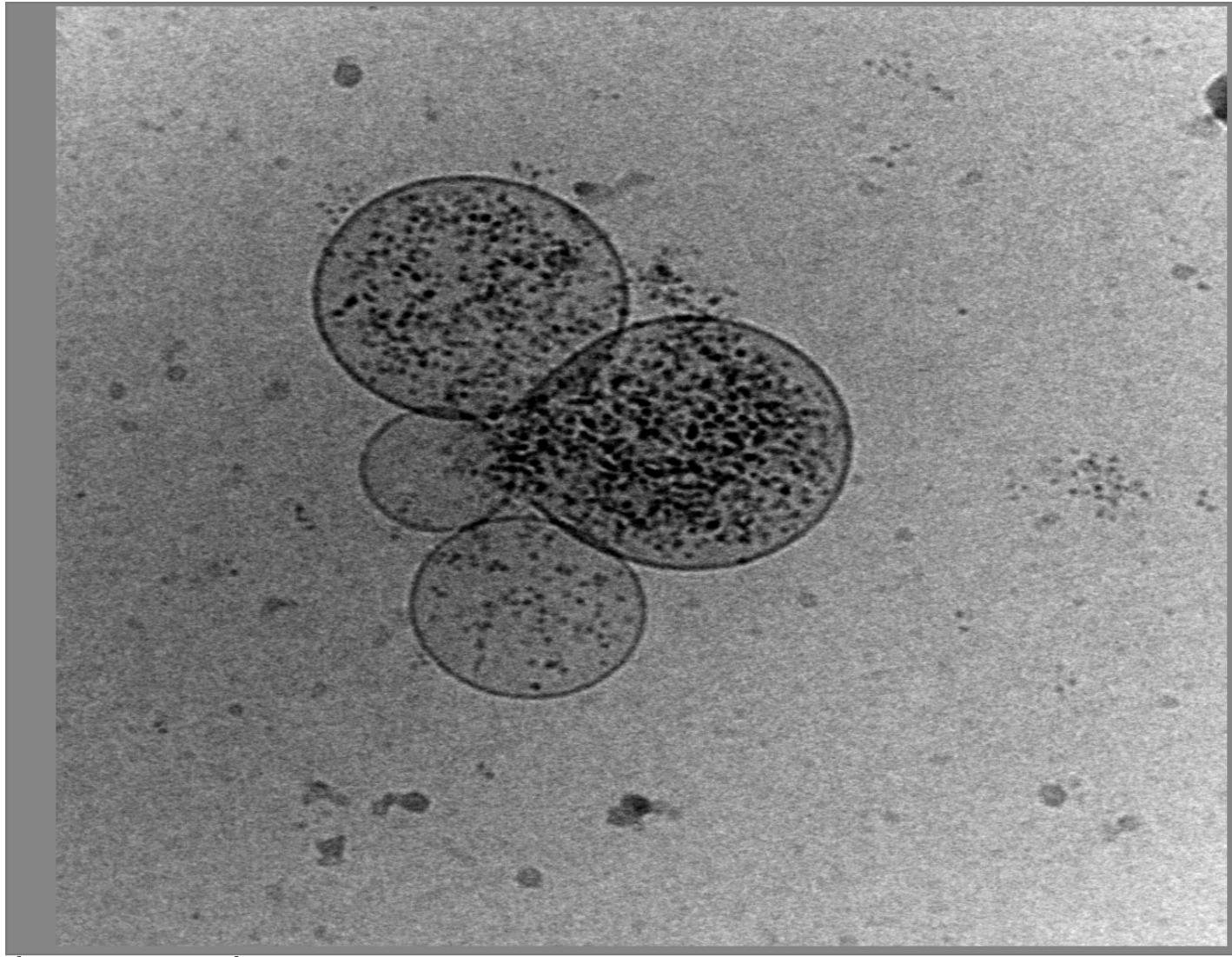


*ferritin-containing
liposomes*





200 nm



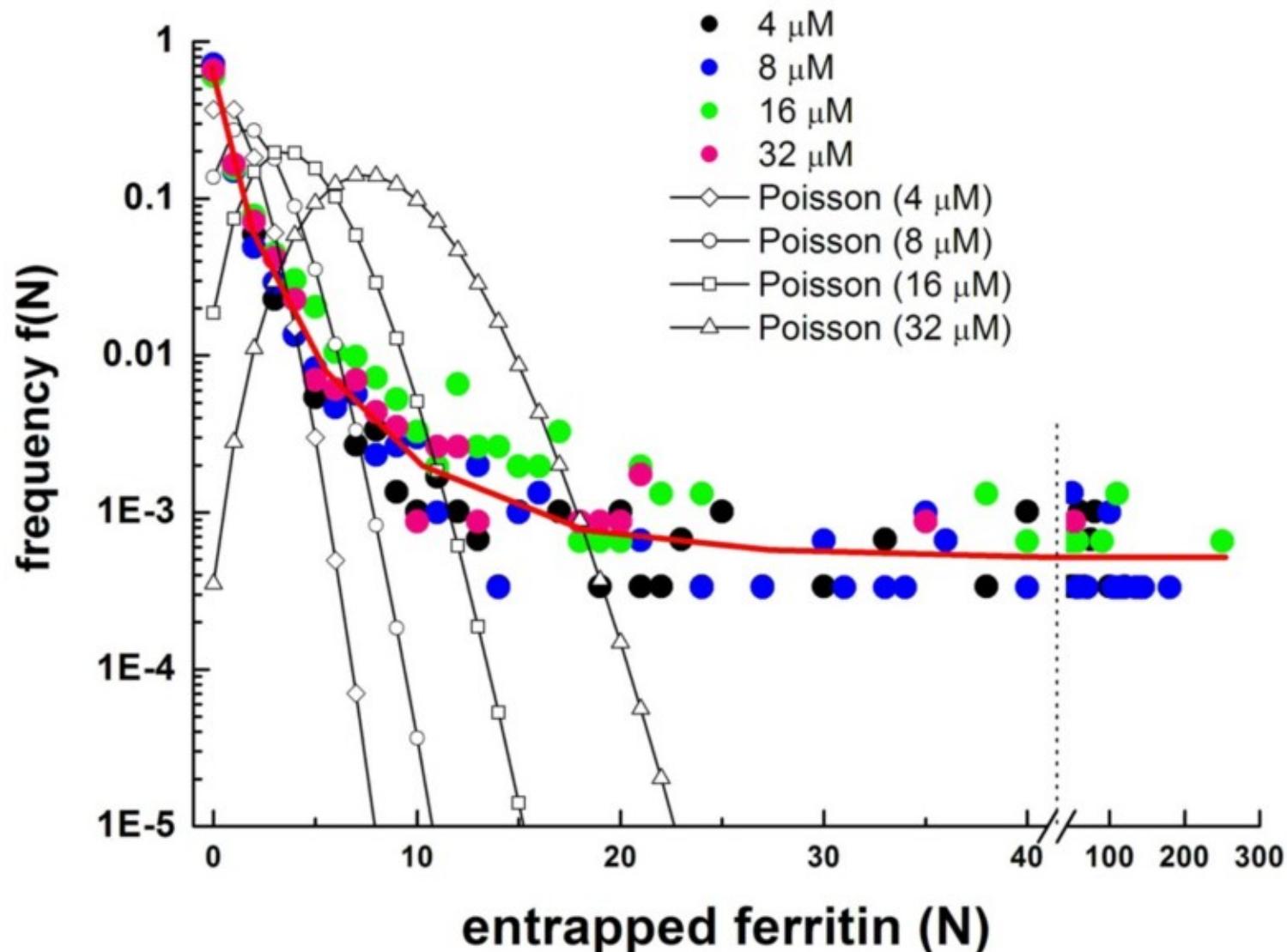
200nm

Mag (k) = 10.0
Spe ang = -0.0
Mean = 144.5

Luisi et al., ChemBioChem 2010

De Souza et al., ChemBioChem 2011

Stano et al., Angew. Chemie, 2013



A new law

- of cellular biochemistry?

Perhaps the origin of metabolism

in the origin of life?

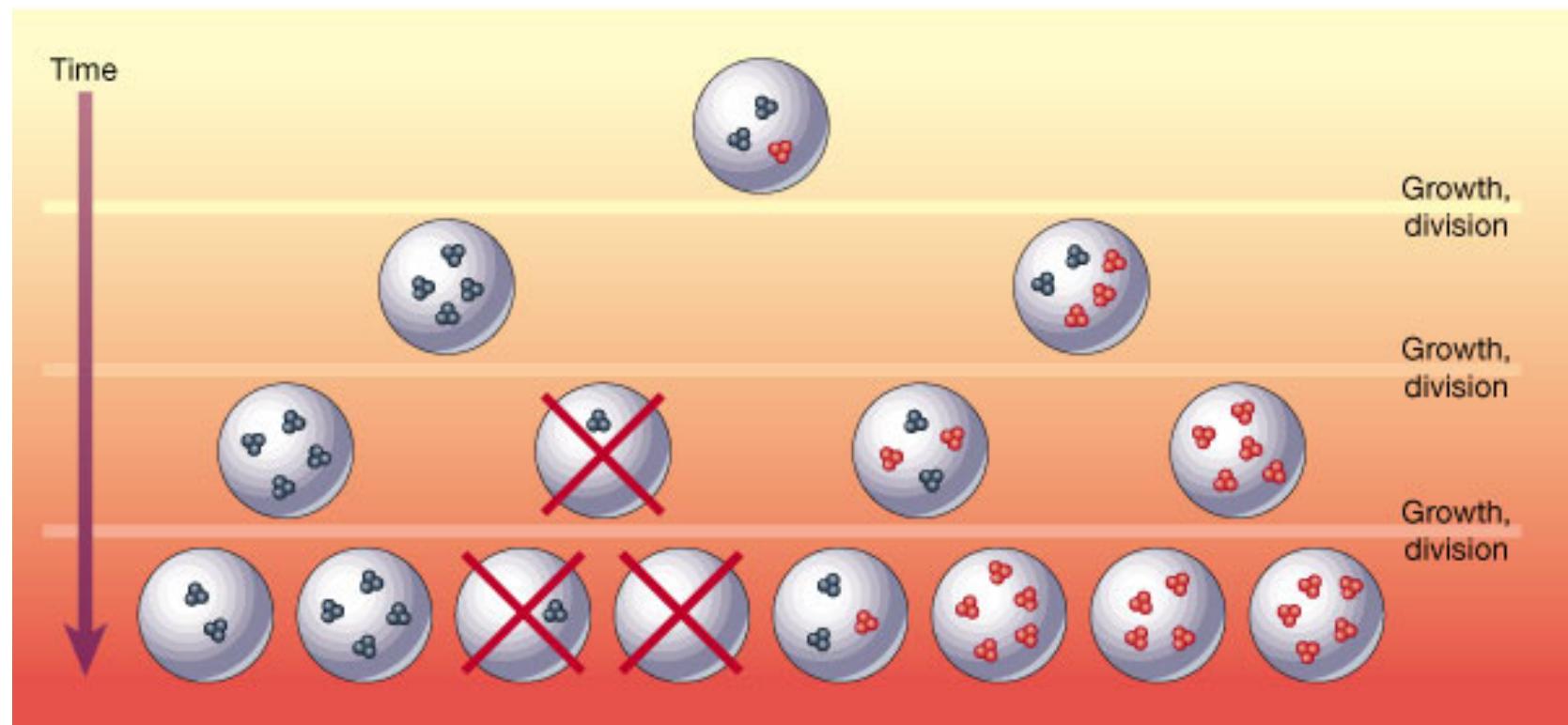
NOT AN AUTOPOIETIC CELL

- The Cell which produces proteins is not a living cell
- It does not make itself from within...(not autopoietic)
- It is just a first, important, step

Nature 409, 387 - 390 (2001)

Synthesizing life

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



- How far is it master?
- It depends on contingency. Hold your tongue and walk!

