Dept. of Logic and Philosophy of Science (IAS Research Group)
Biophysics Research Unit (CSIC-UPV/EHU)

'Bottom-up' strategies towards the synthesis of minimal (living?) cells

Kepa Ruiz-Mirazo

Palacio Miramar, Donostia-San Sebastián
23 June 2010

OUTLINE

- *INTRO: A GENERAL VIEW (synthesis vs. analysis/bottom-up vs. top-down)*
- *REVIEW OF VARIOUS PROTOCELL APPROACHES (in vitro/in silico)*
- *SEVERAL OPEN PROBLEMS*
- *TAKING THINGS STRICTLY 'BOTTOM-UP': THE 'LIPID-PEPTIDE' SCENARIO*
- *SOME SIMULATION RESULTS AND IDEAS TO EXPLORE*
- *FINAL REMARKS*

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TWO PRELIMINARY COMMENTS ON SYNTHETIC BIOLOGY:

- *'DESCRIBING' IS DIFFERENT THAN 'FABRICATING/PUTTING TOGETHER'*

((POTENTIAL OF THE APPROACH))

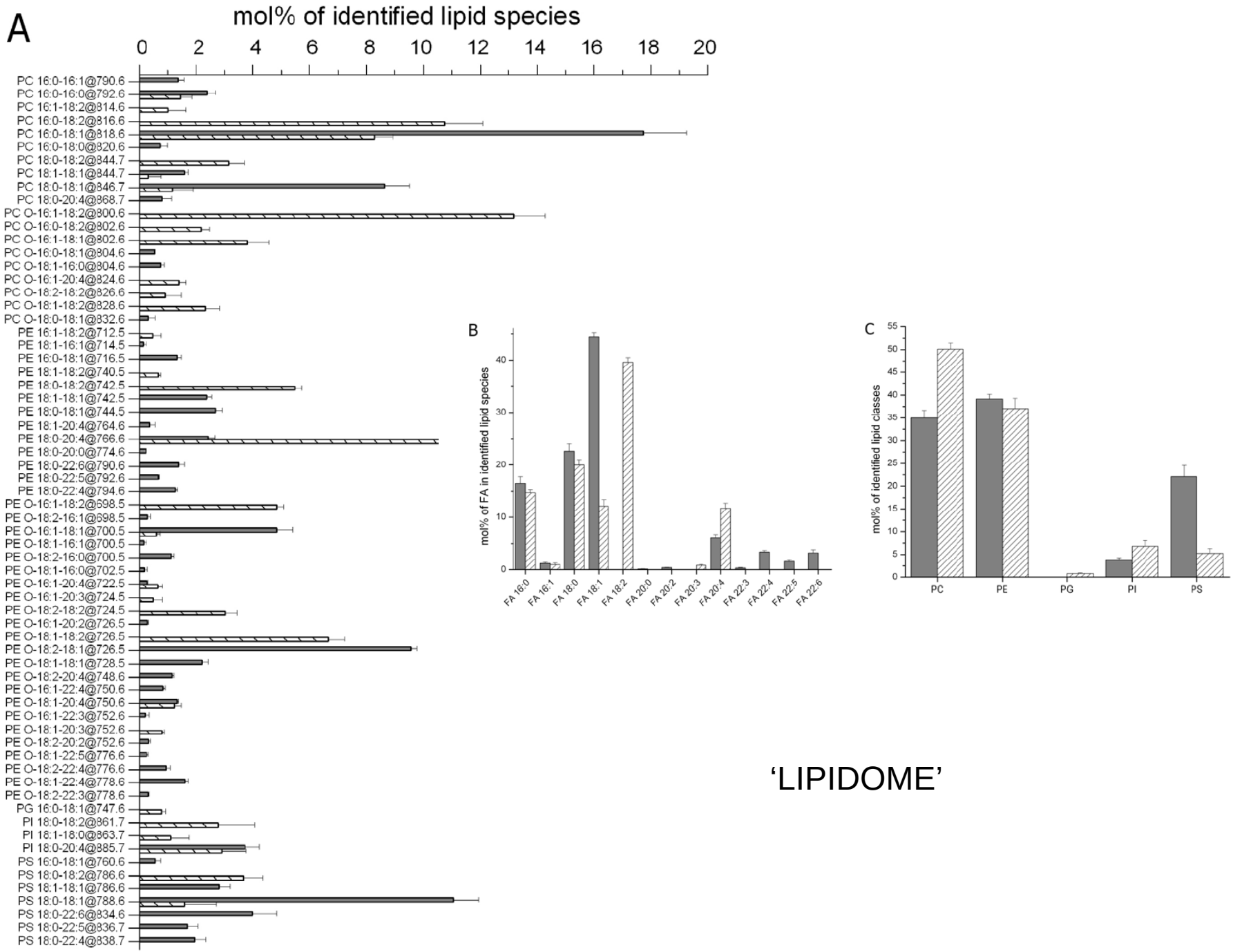
- *IF WE TRY TO SYNTHESIZE A LIVING SYSTEM or ITS MAIN COMP. PARTS*

  WE ARE INTERFERRING IN ITS INTRINSIC ACTIVITY/NATURE

((LIMITATIONS OF THE APPROACH))

«Synthesis drives discovery and understanding in ways that analysis cannot»

[Benner & Sismour 2005]


A
mol% of identified lipid species
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PC 16:0-18:2@816.6
PC 16:0-18:1@818.6
PC 16:0-18:0@820.6
PC 18:0-18:2@844.7
PC 18:1-18:1@844.7
PC 18:0-18:1@846.7
PC 18:0-20:4@868.7
PC O-16:1-18:2@800.6
PC O-16:0-18:2@802.6
PC O-16:1-18:1@802.6
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PC O-18:1-16:0@804.6
PC O-16:1-20:4@824.6
PC O-18:2-18:2@826.6
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PC O-18:0-18:1@832.6
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PE 18:0-20:0@774.6
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PE O-16:1-22:3@752.6
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PS 18:0-18:1@788.6
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PS 18:0-22:5@836.7
PS 18:0-22:4@838.7
B
mol% of FA in identified lipid species
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C
mol% of identified lipid classes
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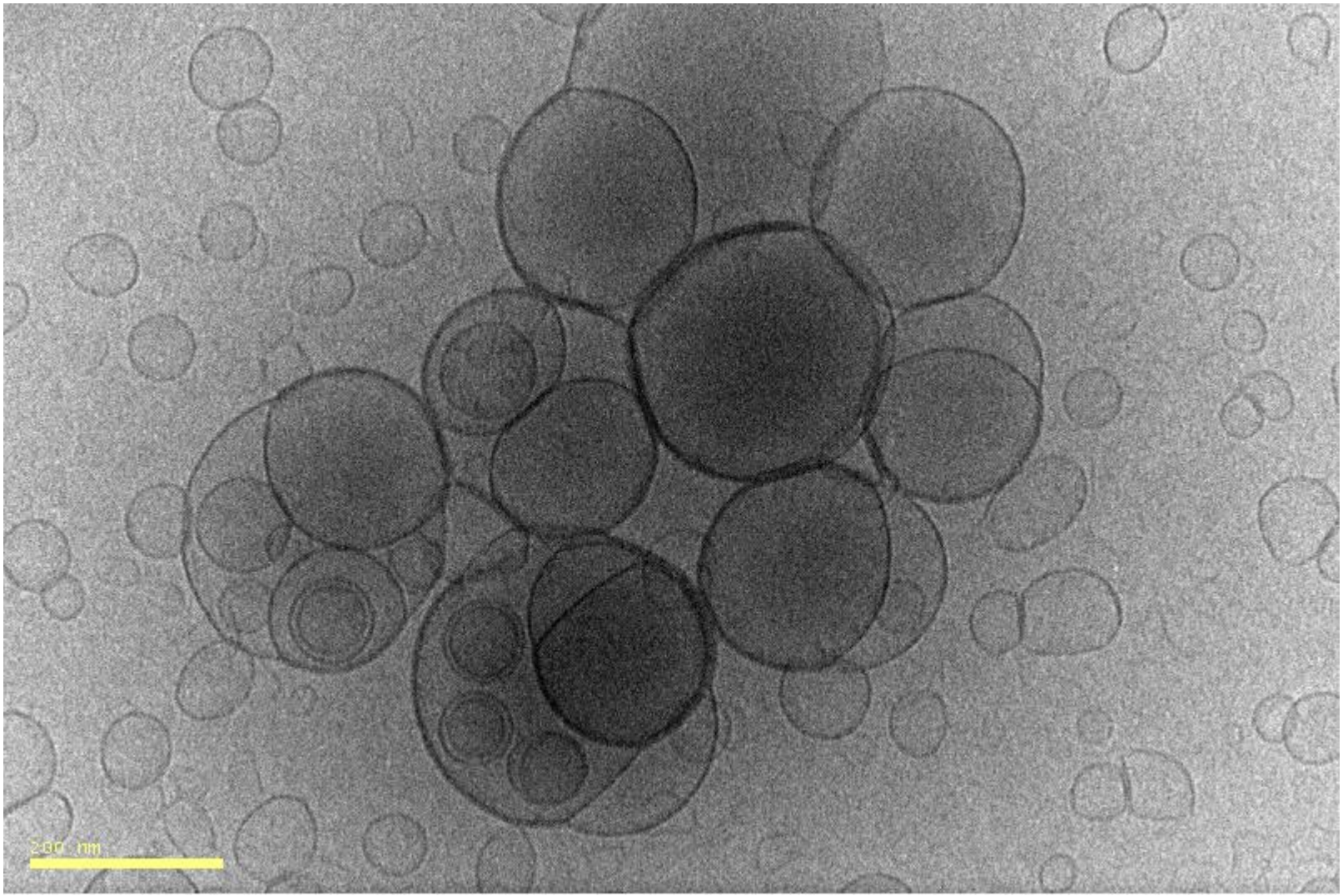
PC PE PG PI PS

‘LIPIDOME’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doi:10.1038/nature07018 NATURE 2008 Jul 3; 454:122-5.

Template-directed synthesis of a genetic polymer in a model protocell

Sheref S. Mansy[1], Jason P. Schrum[1], Mathangi Krishnamurthy[1], Sylvia Tobé[1], Douglas A. Treco[1] & Jack W. Szostak[1]

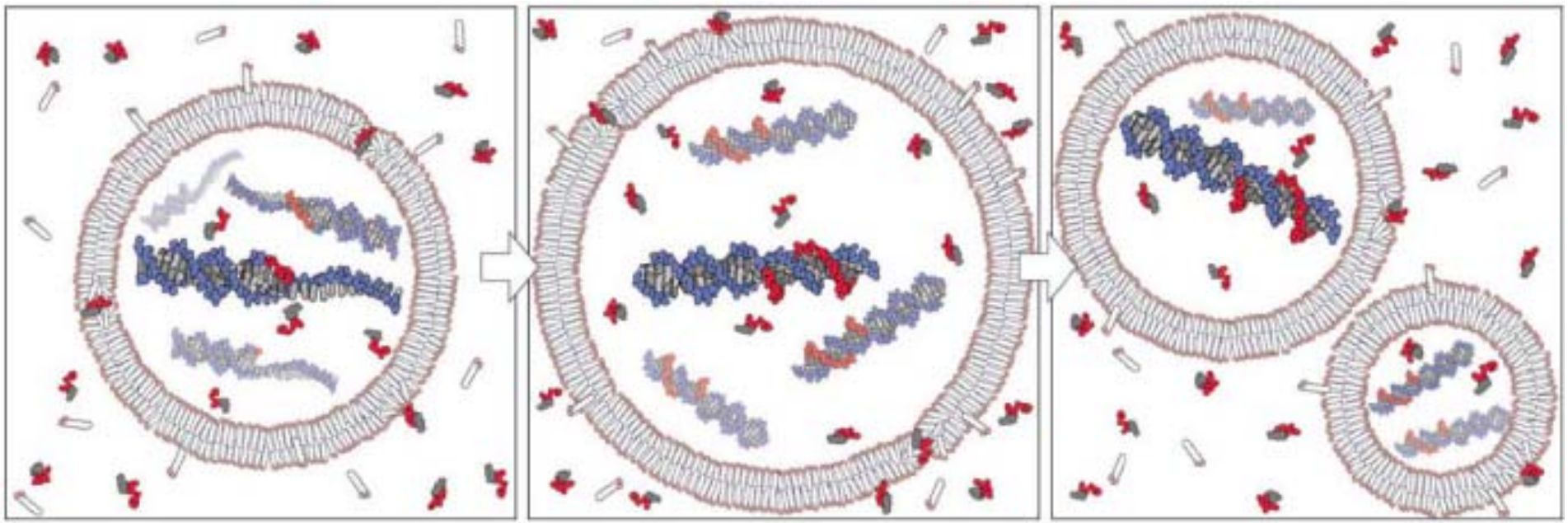

Figure 1 | Conceptual model of a heterotrophic protocell. Growth of the protocell membrane results from the incorporation of environmentally supplied amphiphiles, whereas division may be driven by intrinsic or extrinsic physical forces. Externally supplied activated nucleotides permeate across the protocell membrane and act as substrates for the non-enzymatic copying of internal templates. Complete template replication followed by random segregation of the replicated genetic material leads to the formation of daughter protocells.

A vesicle bioreactor as a step toward an artificial cell assembly

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Communicated by Paul M. Chaikin, Princeton University, Princeton, NJ, November 4, 2004 (received for review June 18, 2004)

An *Escherichia coli* cell-free expression system is encapsulated in a phospholipid vesicle to build a cell-like bioreactor. Large unilamellar vesicles containing extracts are produced in an oil–extract emulsion. To form a bilayer the vesicles are transferred into a feeding solution that contains ribonucleotides and amino acids. Transcription–translation of plasmid genes is isolated in the vesicles. Whereas in bulk solution expression of enhanced GFP stops after 2 h, inside the vesicle permeability of the membrane to the feeding solution prolongs the expression for up to 5 h. To solve the energy and material limitations and increase the capacity of the reactor, the α-hemolysin pore protein from *Staphylococcus aureus* is expressed inside the vesicle to create a selective permeability for nutrients. The reactor can then sustain expression for up to 4 days with a protein production of 30 μM after 4 days. Oxygen diffusion and osmotic pressure are critical parameters to maintain expression and avoid vesicle burst.

α-hemolysin | cell-free protein expression | membrane-anchoring polypeptide

In his logical theory of automata, J. von Neumann (1) compared computing machines and living organisms. The self reproduction of automata was discussed and linked to a Turing-like principle (2). In parallel, the biological sciences raised the question of how to engineer a minimal self-reproducing cell (3). Building a protocell gives clues to how self-replicating systems emerge but also may help researchers to engineer artificial self-replicating machines. Although theoretical models have been proposed (4–6), approaches to reducing the problem complexity are not straightforward. Based on the concept of minimal cell and one possible definition of life (7, 8), a critical step in building an artificial cell is the construction of an enclosed space displaying exchange and use of external energy/nutrients through a semipermeable membrane. In fact, the simple encapsulation of active ingredients into a phospholipid bilayer can be considered as an important transition (9) and a major step in making an artificial cell.

Two complementary approaches are in general considered to build an artificial cell. The bottom-up approach starts with the construction of a minimal cell from the molecular level, the RNA world being one of the main models (10). With the top-down approach, scientists try to reach a minimal cell by reducing the genome of bacteria to a minimum set of genes or proteins (7, 11, 12). In this paper, an approach is presented where the first step consists in assembling a mesoscopic bioreactor by encapsulation of a cell-free expression extract in phospholipid vesicles.

To express proteins *in vitro*, transcription-translation cell-free systems of wheat germ and *Escherichia coli* are usually used. Expression stops after a few hours because of energy and nutrients consumption (13, 14). Accumulation of the hydrolyzed forms of ATP and GTP is the main factor of this limitation (15). To solve this problem, large-scale continuous systems have been constructed where a buffered solution containing the nutrients for energy and materials feeds the reaction compartment through a dialysis polymeric membrane (16). The extract itself cannot be used as a feeding solution because of the presence of proteins that hydrolyze ATP and GTP.

In this work, an *E. coli* extract has been used to carry out *in vitro* transcription and translation of plasmid genes. Outside of the nonaffordable reconstitution of Shimizu and coworkers (17), an extract is the best available system to express protein *in vitro*. We chose DNA as the substrate of genetic information instead of RNA because it gives more possibilities to program and develop functions inside the cell-like bioreactor. Furthermore, it is important to show that the two universal steps of genetic expression, transcription and translation, can be performed in an artificial compartment. To go from a homogeneous to a heterogeneous system displaying two distinct aqueous phases, our first challenge was to encapsulate the extract into a vesicle composed of a phospholipid bilayer in nondenaturing conditions for the extract. Phospholipids are the main constituents of biological membranes (18). No other biological or synthetic barrier provides as many possibilities or as much flexibility to functionalize and establish controllable exchanges between the two phases. We present a method to encapsulate the extract efficiently and carry out *in vitro* transcription and translation inside large unilamellar vesicles made of L-α-lecithin. It is striking to see that formation of vesicles is still possible with a solution as complex and as dense as a cell-free extract, full of proteins that interfere with phospholipids for the formation of interfaces. Such vesicles are transferred into a feeding solution composed of a buffer with the nutrients (mainly ribonucleotides and amino acids). Composition of both phases is the same except for the high protein concentration of the extract. This asymmetry causes a high osmotic pressure that reduces considerably the yield of vesicle formation and their stability in time. To overcome these difficulties, a fine-tuning was found between the extract and the feeding mixture. In such a configuration, expression stops after 5 h.

Our next goal was to establish a more reliable exchange between the two phases. We used the internal expression of a protein to improve the capacity and lifetime of the bioreactors. In large-scale continuous systems (16), extracts and feeding solution are both stirred. For our vesicular reactor, exchange of nutrients is realized by diffusion; the main problem was then to find a protein that makes a nonspecific pore into the phospholipids bilayer without perturbing or lysing the membrane and that has a compatible channel size. We show that the expressed α-hemolysin toxin is functional and create such a selective leak. Expression of the toxin boosts the expression up to 4 days at the level of 1 mg/ml synthesized proteins. For expression, effective diffusion of oxygen is essential. The vesicles have thus to be within 1 mm from the feeding solution–air interface.

Lastly, we indicate how one can further functionalize the membrane by incorporating a small polypeptide that can act as an anchor for other proteins. Such an anchor can be used to bind biopolymers to the membrane that can induce mechanical stress, a step toward possible fission of the vesicles. Such a long-lived bioreactor that sustains expression for 4 days is a first step toward

Abbreviations: eCFP, enhanced cyan fluorescent protein; eGFP, enhanced GFP; eYFP, enhanced yellow fluorescent protein; RITC, rhodamine isothiocyanate.

*To whom correspondence should be addressed. E-mail: axvette@mail.rockefeller.edu.

LIFE : { very complex molecules (DNA, RNA, proteins, sugars, lipids,...) ; very complex organization ('genetically-instructed' metabolisms) }

A universal definition of 'minimal life'

[Ruiz-Mirazo et al. (2004) OLEB 34: 323-346]

'autonomy' ⟷ **CAPACITY FOR 'SELF-CONSTRUCTION' (metabolism)**

'open-ended evolution' ⟷ **POTENTIAL FOR 'INDEFINITE GROWTH OF COMPLEXITY' ('Darwinian' evolution)**

LIFE: SYSTEM PROPERTY ! → ORIGINS + ARTIFICIAL IMPLEM.: SYSTEMIC !!

ORIGINS OF LIFE

[Ruiz-Mirazo et al. (2004) OLEB 34: 323-346]

INCREASE IN MOLECULAR AND ORGANIZATIONAL COMPLEXITY

minimal living systems
(autonomy + open-ended evolution):
'TWO/THREE-POLYMER WORLD'
(RNA-protein/DNA-RNA-protein)

!

third major bottleneck:
phenotype-genotype decoupling
(catalysis /// template activity)
'translation' mechanisms
and genetic code

'hereditary autonomous' systems
'ONE-POLYMER (RNA) WORLD'

!

second major 'bottleneck':
'template-replication' mechanisms

'basic autonomous' systems
'OLIGOMER (peptides) WORLD'

!

first major bottleneck: 'proto-bioenergetic' mechanisms

ORIGINS OF LIFE

[Ruiz-Mirazo et al. (2004) OLEB 34: 323-346]

INFORMATION

INCREASE IN MOLECULAR AND ORGANIZATIONAL COMPLEXITY

FUNCTION

minimal living systems
(autonomy + open-ended evolution):
'TWO/THREE-POLYMER WORLD'
(RNA-protein/DNA-RNA-protein)

!

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'OLIGOMER (peptides) WORLD'

!

first major bottleneck: 'proto-bioenergetic' mechanisms

‘FOOD FOR THINKING’
ON A GENERAL THEORY OF BIOLOGY

AUTONOMY ⇔ FUNCTION (and AGENCY)

Towards a theory autonomy (basic biological organization) well rooted in physics-chemistry: in particular, developing a naturalized concept of function that includes material-thermodynamic aspects (work-constraint cycle, endo-exergonic couplings!)

[Kauffman 2000, 2003]

[Ruiz-Mirazo & Moreno (1998, 2000, 2004): Basic autonomy as a fundamental step in the synthesis of life -- Artificial Life 10: 235–259]

OPEN-ENDED EVOLUTION ⇔ INFORMATION

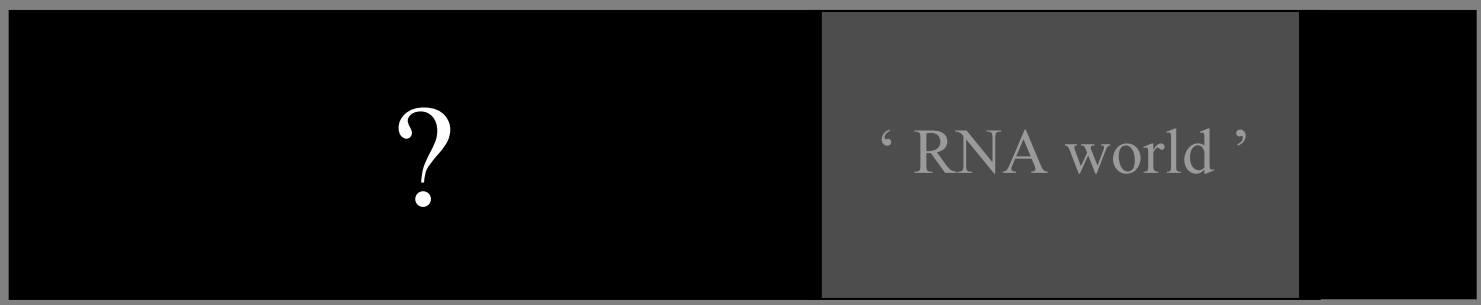
Naturalization of the ‘Shannon & Weaver’ (syntactic) conception of information by embedding it in a new theoretical framework that takes into account organizational (semantic) aspects

[Ruiz-Mirazo, Umerez & Moreno (2008): Enabling conditions for open-ended evolution -- Biology &Philosophy 23(1): 67-85]

ORIGINS OF LIFE (i) :

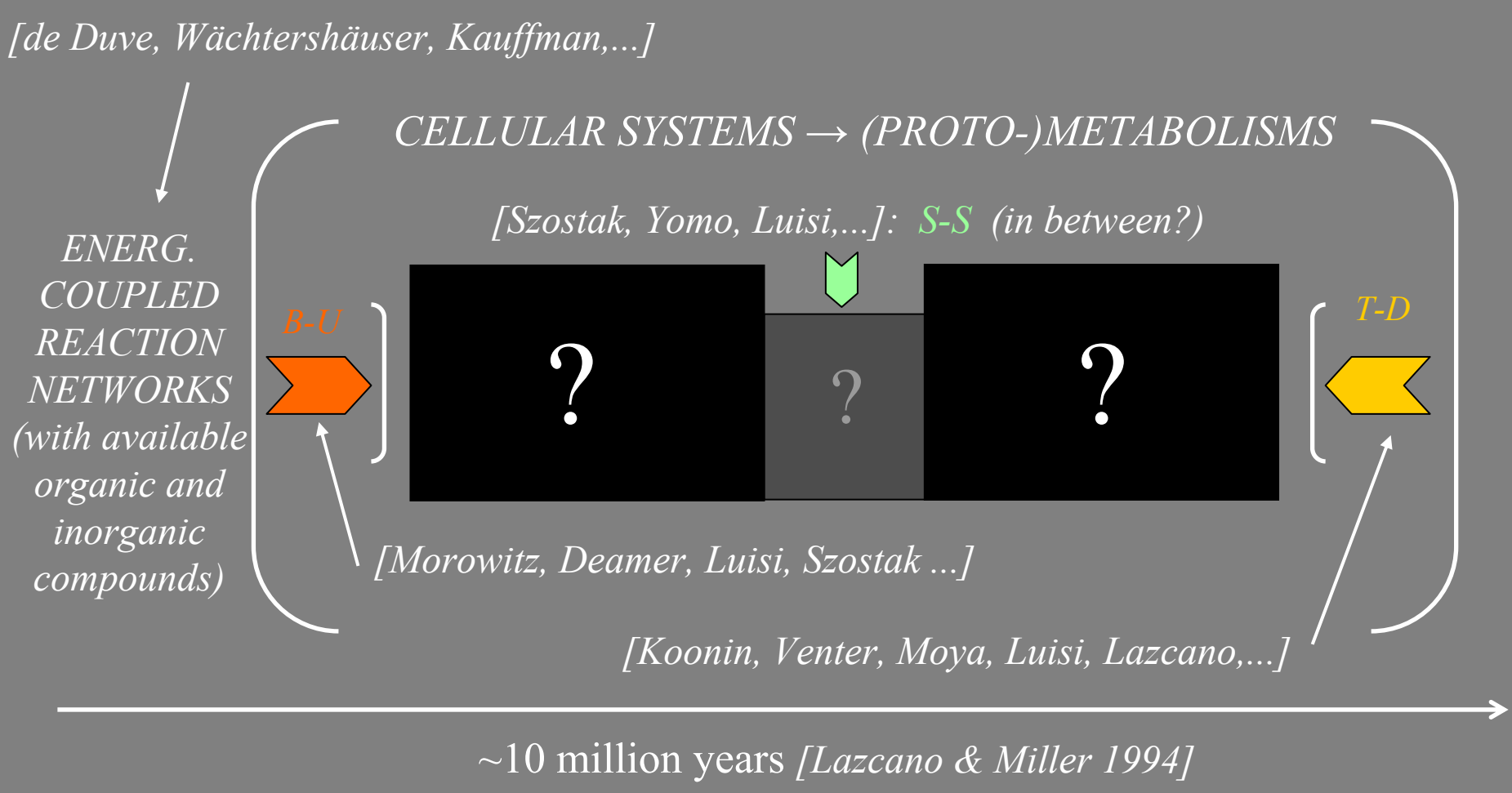
Sequence of transitions
that lead to living systems?

PREBIOTIC MOLECULAR EVOLUTION → *MACROMOLECULAR STRUCTURES* → *CELLULAR METABOLISMS*



~10 million years *[Lazcano & Miller 1994]*

ORIGINS OF LIFE (ii) : Sequence of transitions that lead to self-constructing systems with potential for open-ended evolution?



SEARCH FOR THE 'MINIMAL CELL': 'BOTTOM-UP/TOP-DOWN' APPROACHES

[most typically, a mixture of the two: 'semi-synthetic' app., like the 'ribocell']

modern (complex) living cells

'TOP-DOWN'

(evolutionary or direct gene 'knock-down' process)

progressive reduction of organizational complexity

HOW MANY COMPONENTS?

minimal life/cell (?)

HOW MANY, BUT ALSO: WHAT KIND OF COMPONENTS?

progressive increase both in molecular and organizational complexity

'BOTTOM-UP'

(process of 'self-organization' and 'self-maintenance')

simple molecules

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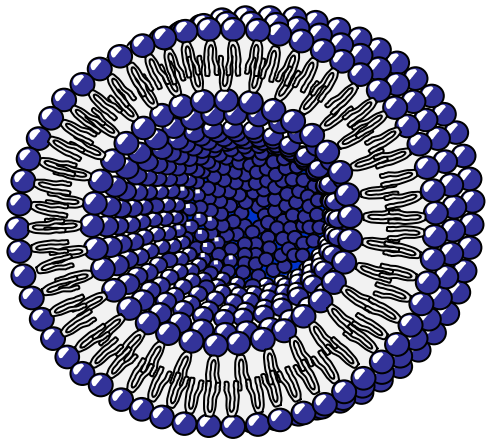
‘BOTTOM-UP’ APPROACH

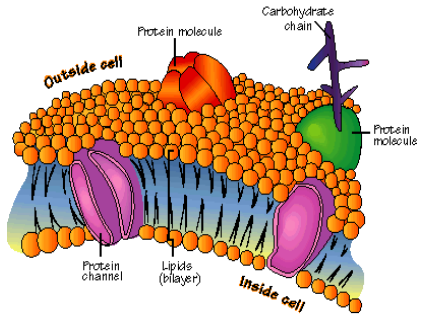
COMPARTMENTS:

(not just ‘hosts’ or ‘containers’ but)
SEMIPERMEBLE SUPRAMOLECULAR STRUCTURES
THAT DEFINE THE BOUNDARIES OF THE SYSTEM AND ALLOW
ACTIVE CONTROL OF MATTER-ENERGY FLOW THROUGH IT
(TRANSPORT + ENERGY TRANSDUCING MECHANISMS)

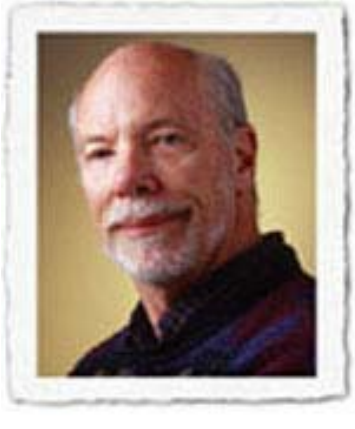


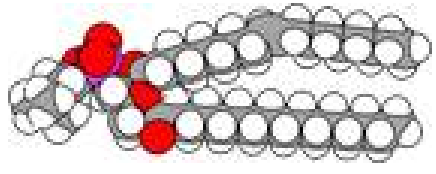
‘co-evolution’

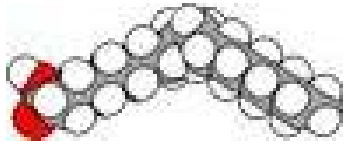
between boundary
(scaffolding)
and protometabolic
reactions



‘COMPARTIMENTALIST VIEW’: Morowitz, Deamer, Luisi,...







Liposomes from Ionic, Single-Chain Amphiphiles†

William R. Hargreaves* and David W. Deamer‡

MICROBIOLOGY AND MOLECULAR BIOLOGY REVIEWS, June 1997, p. 239–261 Vol. 61, No. 2
1092-2172/97/$04.00+0

The First Living Systems: a Bioenergetic Perspective

DAVID W. DEAMER*

Department of Chemistry and Biochemistry, University of California, Santa Cruz, California 95064

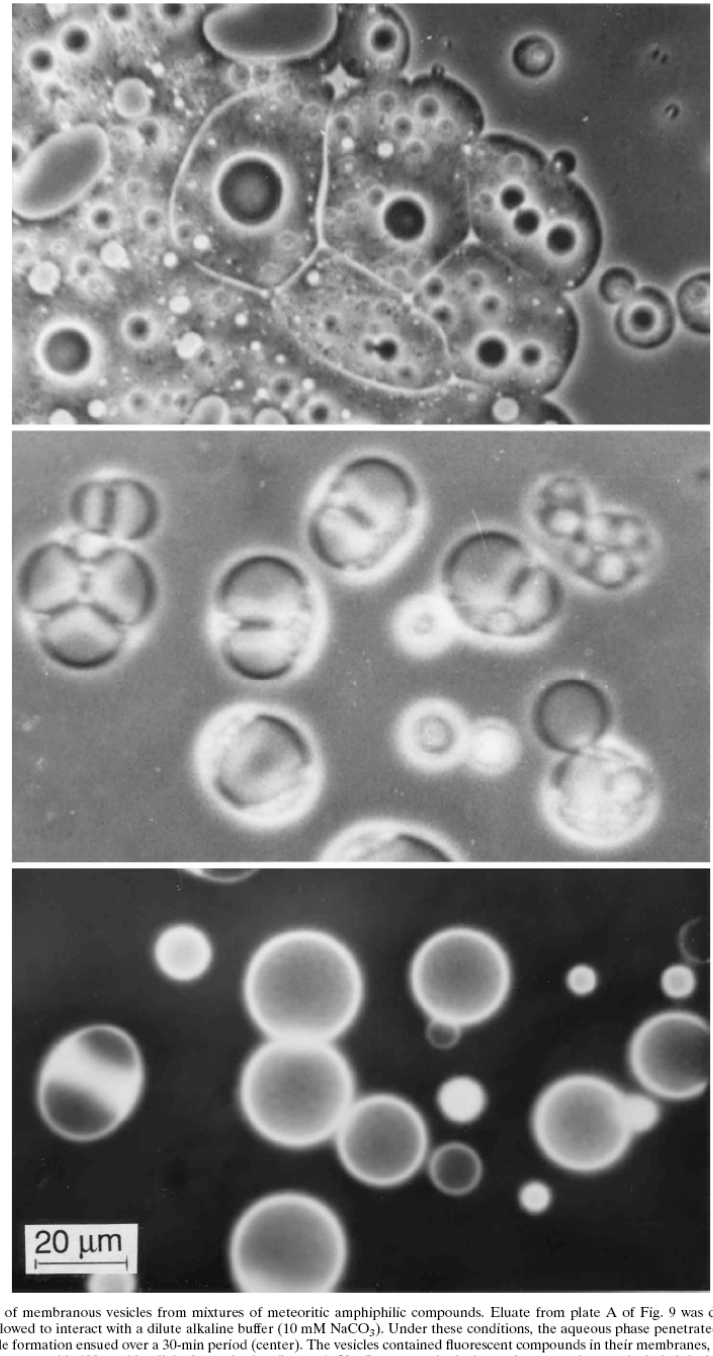


FIG. 10. Formation of membranous vesicles from mixtures of meteoritic amphiphilic compounds. Eluate from plate A of Fig. 9 was dried on a standard gla microscope slide and allowed to interact with a dilute alkaline buffer (10 mM $NaCO_3$). Under these conditions, the aqueous phase penetrated the dried extract with minutes (top) and vesicle formation ensued over a 30-min period (center). The vesicles contained fluorescent compounds in their membranes, which could be visualiz by epifluorescence microscopy with 400-nm blue light for excitation (bottom). If a fluorescent ionic dye such as pyranine was included during the initial hydration, was captured by the vesicles (not shown).

‘Autopoietic vesicles’ (Pier Luigi Luisi)

Luisi (2003) “Autopoiesis: a review and a reappraisal” Naturwissenschaften 90:49-59

THE MINIMAL AUTOPOIETIC SYSTEM

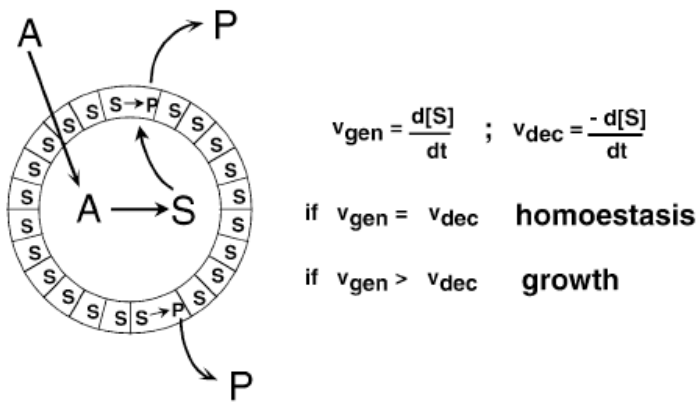


Fig. 4 The minimal autopoietic system. This system is characterized by two competitive reactions, one that builds the component of the boundary, and another one that destroys it. According to the relative value of these two velocity constants, the system can be in homeostasis, or grow, or die

a simple experimental model of chemical homeostasis

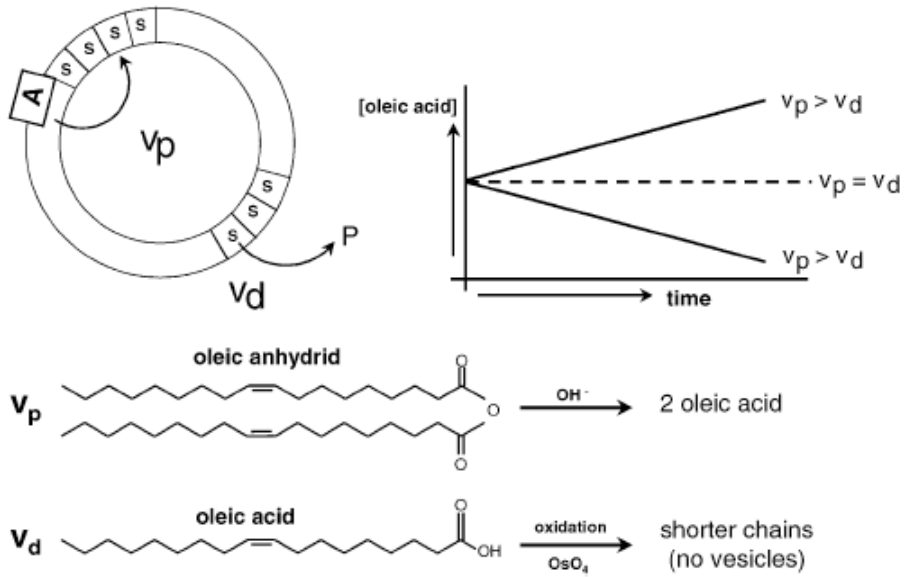
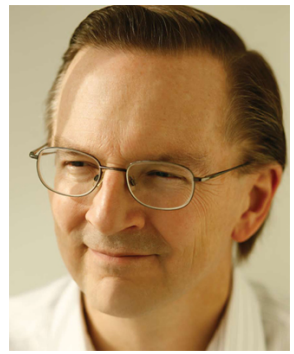


Fig. 8 The experimental implementation of the autopoietic model of Fig. 3 with two competitive reactions. Here, one reaction forms new oleate surfactant from the hydrolysis of the anhydride, and one reaction destroys oleate via oxidation of the double bond. Depending upon whether the two velocities are equal or not, different pathways for the systems are obtained–homeostasis (which corresponds to an autopoietic self-maintenance system), growth and self-reproduction, or decay and death (Zepik et al. 2001)

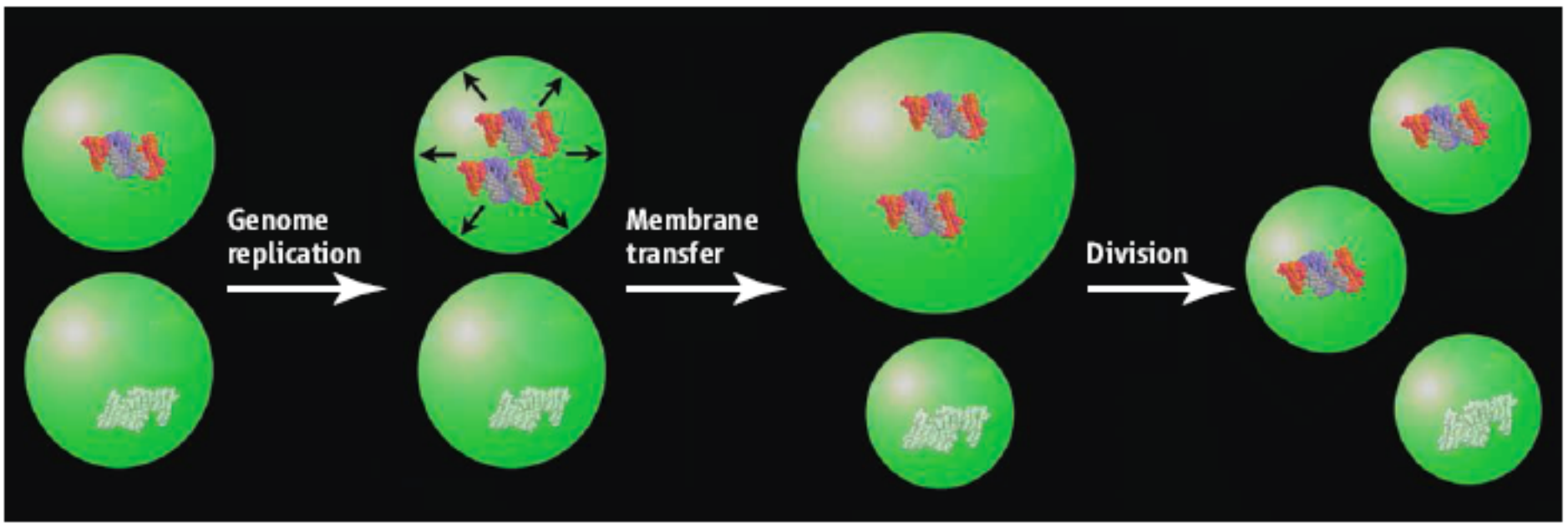
3 SEPTEMBER 2004 VOL 305 SCIENCE

The Emergence of Competition Between Model Protocells

Irene A. Chen,[1,2] Richard W. Roberts,[3] Jack W. Szostak[1]*



The transition from independent molecular entities to cellular structures with integrated behaviors was a crucial aspect of the origin of life. We show that simple physical principles can mediate a coordinated interaction between genome and compartment boundary, independent of any genomic functions beyond self-replication. RNA, encapsulated in fatty acid vesicles, exerts an osmotic pressure on the vesicle membrane that drives the uptake of additional membrane components, leading to membrane growth at the expense of relaxed vesicles, which shrink. Thus, more efficient RNA replication could cause faster cell growth, leading to the emergence of Darwinian evolution at the cellular level.



The emergence of cellular behavior. Competition emerges as protocells containing replicating genomes steal membrane from protocells containing inactive molecules.

J|A|C|S
ARTICLES

Published on Web 03/26/2009

Coupled Growth and Division of Model Protocell Membranes

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Received February 5, 2009; E-mail: szostak@molbio.mgh.harvard.edu

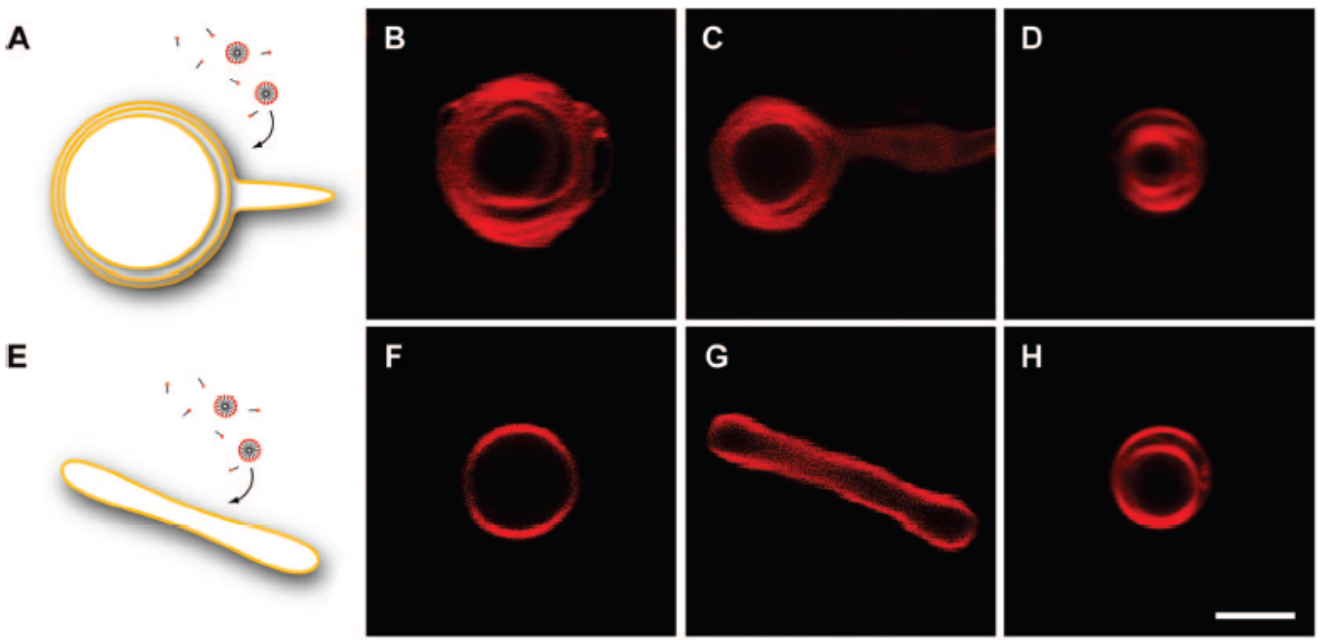

Figure 5. Growth of multilamellar versus unilamellar vesicles (A) Schematic diagram of incorporation of micelles into a multilamellar vesicle: the outermost membrane grows faster than the inner membrane layers. (B, C) Confocal images of multilamellar oleate vesicle (0.2 mol % Rh-DHPE, in 0.2 M Na-bicine, pH 8.5, ~1 mM initial oleic acid) before and 10 min after the addition of 1 equiv of oleate micelles, respectively. (D) Confocal image of multilamellar vesicle after division. (E) Schematic diagram of incorporation of micelles into a unilamellar vesicle. (F, G) Confocal images of unilamellar oleate vesicle (conditions as above) before and 10 min after the addition of 1 equiv of oleate micelles, respectively. (H) Confocal image of a multilamellar vesicle formed after the agitation of elongated unilamellar vesicles. Scale bar for B−D, F−H; 2 μm.

doi:10.1038/nature07018 NATURE 2008 Jul 3; 454:122-5.

Template-directed synthesis of a genetic polymer in a model protocell

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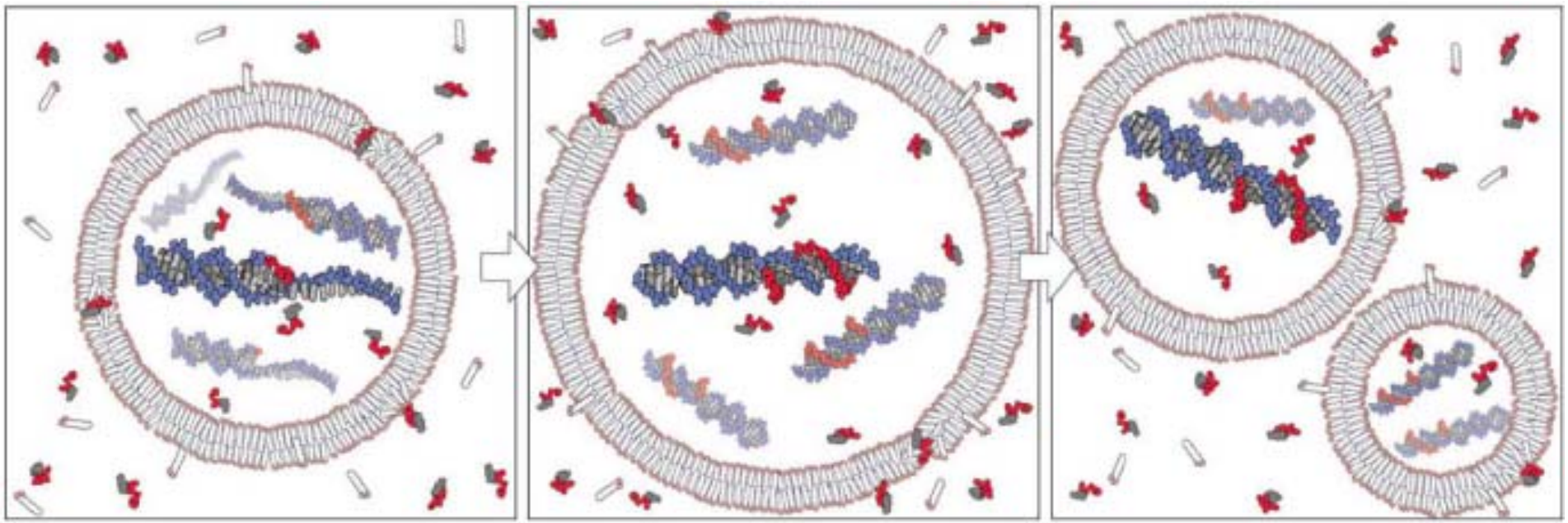
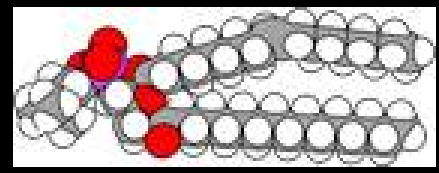


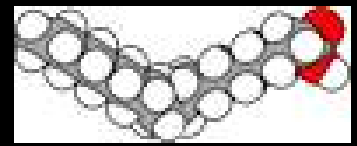
Figure 1 | Conceptual model of a heterotrophic protocell. Growth of the protocell membrane results from the incorporation of environmentally supplied amphiphiles, whereas division may be driven by intrinsic or extrinsic physical forces. Externally supplied activated nucleotides permeate across the protocell membrane and act as substrates for the non-enzymatic copying of internal templates. Complete template replication followed by random segregation of the replicated genetic material leads to the formation of daughter protocells.

Comparison between contemporary biomembrane models and vesicles made of hypothetical prebiotic amphiphiles



standard biomembrane bilayers:

a) double-chained phospholipids
b) low solubility of monomers in H_2O (very low cac: ~ 10^{-10} M)
c) high stability of the aggregate (temperature, salts,…)
d) relatively low permeability
e) pH-insensitive (zwitterionic)
f) relat. high smotic burst tension (25 dyn/cm --10 atm)
g) not possible to grow and reproduce *in vitro*



vesicles made of prebiotic amphiphiles (ffaa):

a) single chain lipids (saturated/non-saturated)
b) relatively high monomer solubility (relatively high cac: ~ 1mM)
c) lower stability (higher rate of exchange with monomer)
d) relatively high permeability
e) high pH-sensitivity
f) lower but still significant osmotic burst tension (10 dyn/cm -- 4 atm)
g) possible to grow and reproduce *in vitro*

OUTLINE

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- *REVIEW OF VARIOUS PROTOCELL APPROACHES (in vitro/in silico)*
- ***SEVERAL OPEN PROBLEMS***
- *TAKING THINGS STRICTLY 'BOTTOM-UP':*
  THE 'LIPID-PEPTIDE' SCENARIO
- *SOME SIMULATION RESULTS AND IDEAS TO EXPLORE*
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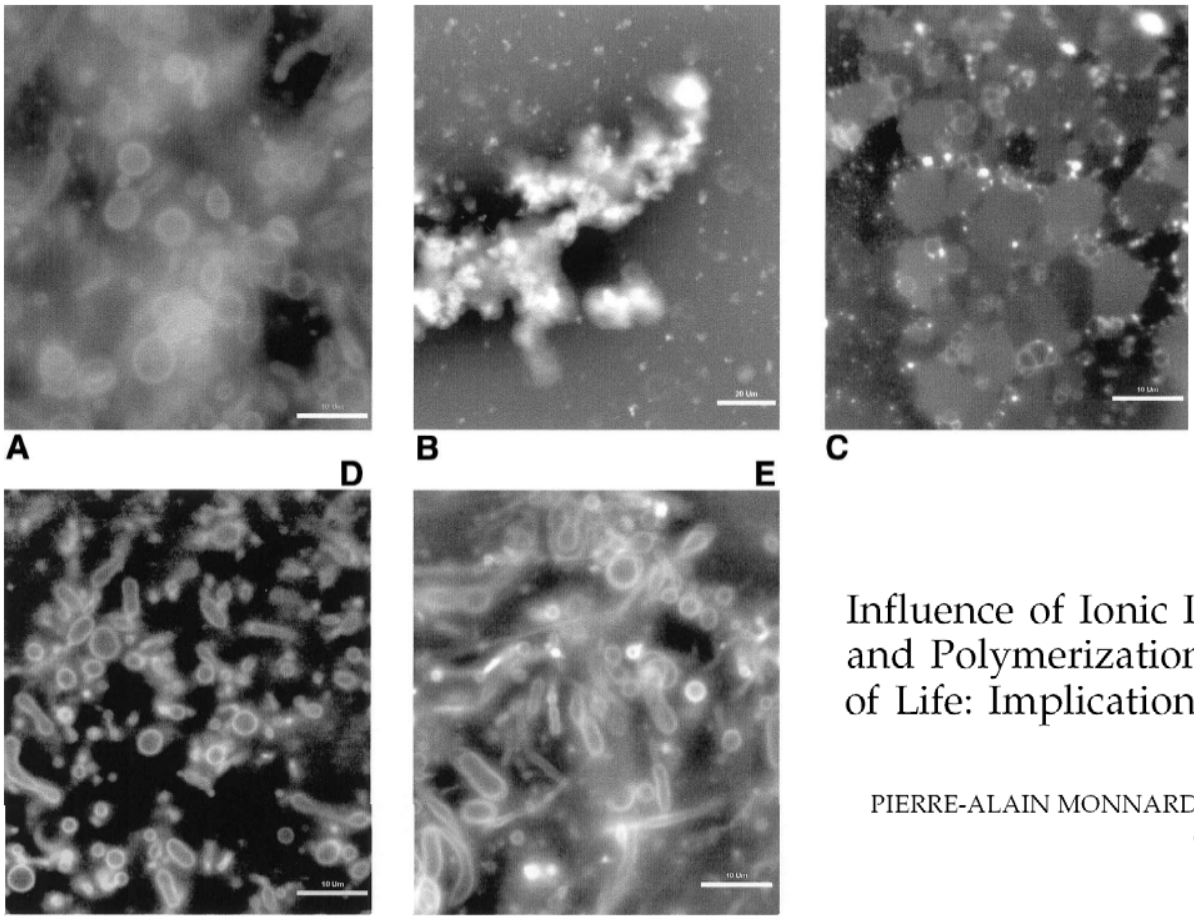

ASTROBIOLOGY
Volume 2, Number 2, 2002

Research Paper

Influence of Ionic Inorganic Solutes on Self-Assembly and Polymerization Processes Related to Early Forms of Life: Implications for a Prebiotic Aqueous Medium

PIERRE-ALAIN MONNARD, CHARLES L. APEL, ANASTASSIA KANAVARIOTI, and DAVID W. DEAMER

FIG. 1. Sodium chloride influence on DA-based vesicles. Vesicles were stained with rhodamine 6G. Photomicrographs of (**A**) DA vesicles (Na^+:DA molar ratio of 0:1), (**B**) macroscopic crystals of carboxylic acid and NaCl produced after addition of NaCl to DA stock solution (Na^+:DA molar ratio 7:1), and (**C**) collapsed vesicles and lipid sheets at a Na^+:DA molar ratio of 10:1. Photomicrographs of (**D**) stock solution of GMD:DA (7.5:15) mixed vesicles and (**E**) GMD:DA mixed vesicles after addition of NaCl (Na^+:DA molar ratio 33:1). The bar scales represent 10 μm in **A** and **C–E** and 20 μm in **B**.

Orig Life Evol Biosph (2007) 37:267–285
DOI 10.1007/s11084-007-9065-6

The Influence of Environmental Conditions, Lipid Composition, and Phase Behavior on the Origin of Cell Membranes

Jacquelyn A. Thomas • F. R. Rana

OPEN QUESTIONS (difficulties):

- high cac?

- pH dependence?

- sensitivity to salts?

- capacity to hold gradients?

- fatty acids or isoprenoids?

ELSEVIER

Biochimica et Biophysica Acta 1559 (2002) 1–9

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

www.bba-direct.com

Self-assembled vesicles of monocarboxylic acids and alcohols: conditions for stability and for the encapsulation of biopolymers

Charles L. Apel [a,*], David W. Deamer [a], Michael N. Mautner [b,c]

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[b] *Soil, Plant and Ecological Sciences Division, Lincoln University, Canterbury, New Zealand*
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Received 22 June 2001; received in revised form 14 August 2001; accepted 14 August 2001

Abstract

We tested the ability of saturated *n*-monocarboxylic acids ranging from eight to 12 carbons in length to self-assemble into vesicles, and determined the minimal concentrations and chain lengths necessary to form stable bilayer membranes. Under defined conditions of pH and concentrations exceeding 150 mM, an unbranched monocarboxylic acid as short as eight carbons in length (*n*-octanoic acid) assembled into vesicular structures. Nonanoic acid (85 mM) formed stable vesicles at pH 7.0, the p*K* of the acid in bilayers, and was chosen for further testing. At pH 6 and below, the vesicles were unstable and the acid was present as droplets. At pH ranges of 8 and above clear solutions of micelles formed. However, addition of small amounts of an alcohol (nonanol) markedly stabilized the bilayers, and vesicles were present at significantly lower concentrations ($\sim$20 mM) at pH ranges up to 11. The formation of vesicles near the pK_a of the acids can be explained by the formation of stable $RCOO^-$...HOOCR hydrogen bond networks in the presence of both ionized and neutral acid functions. Similarly, the effects of alcohols at high pH suggests the formation of stable $RCOO^-$...HOR hydrogen bond networks when neutral RCOOH groups are absent. The vesicles provided a selective permeability barrier, as indicated by osmotic activity and ionic dye capture, and could encapsulate macromolecules such as DNA and a protein. When catalase was encapsulated in vesicles of decanoic acid and decanol, the enzyme was protected from degradation by protease, and could act as a catalyst for its substrate, hydrogen peroxide, which readily diffused across the membrane. We conclude that membranous vesicles produced by mixed short chain monocarboxylic acids and alcohols are useful models for testing the limits of stabilizing hydrophobic effects in membranes and for prebiotic membrane formation.

Keywords: Self-assembly; Lipid vesicle; Encapsulation

Orig Life Evol Biosph (2008) 38:329–341
DOI 10.1007/s11084-008-9131-8

Stability of Model Membranes in Extreme Environments

Trishool Namani • David W. Deamer

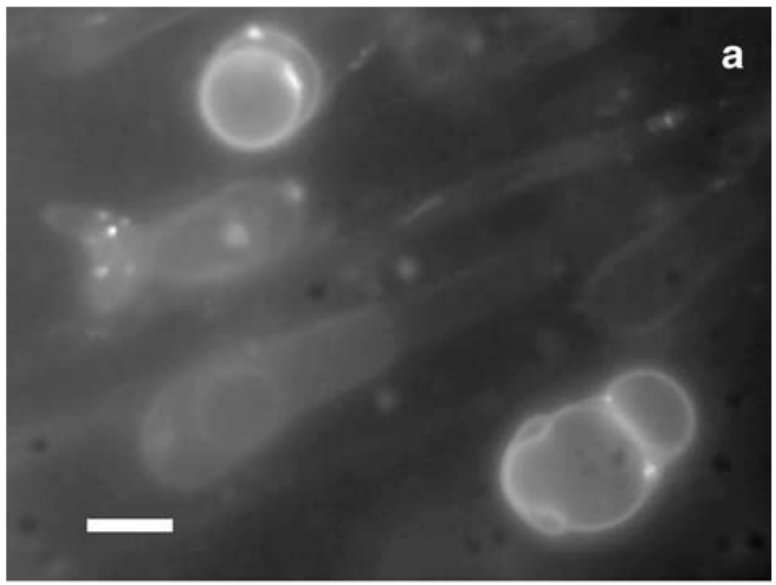


Fig. 5 Epifluorescence micrograph of decylamine/decanoic acid (25 mM each) vesicles in presence of **a** 0.1 M $MgCl_2$, **b** 0.1 M $CaCl_2$ and **c** sea salts 40 g/l. All the vesicles were prepared in 0.1 M borate at pH 2. The scale bars indicate 5 μm

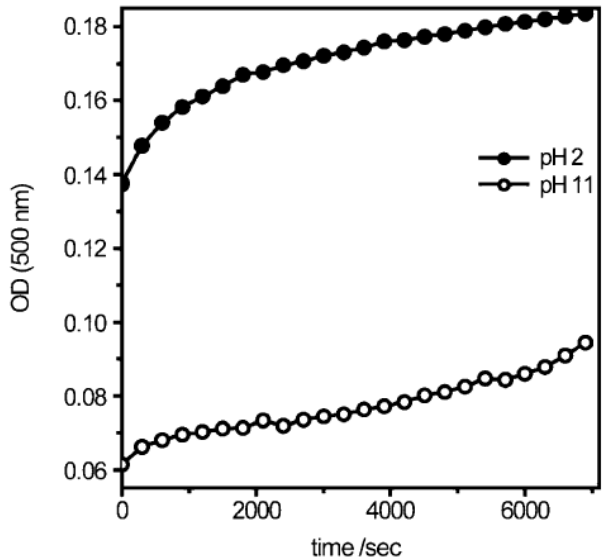


Fig. 7 The stability of decylamine (10 mM) and decanoic (10 mM) acid vesicles at pH 11 and pH 3 was monitored as turbidity changes over time, using absorbance at 500 nm to measure turbidity. Open circles correspond to vesicles at pH 11 and the closed circles correspond to pH 2. The vesicles were sonicated for 15 min in a bath sonicator before measuring the absorbance

mixtures: permeability!

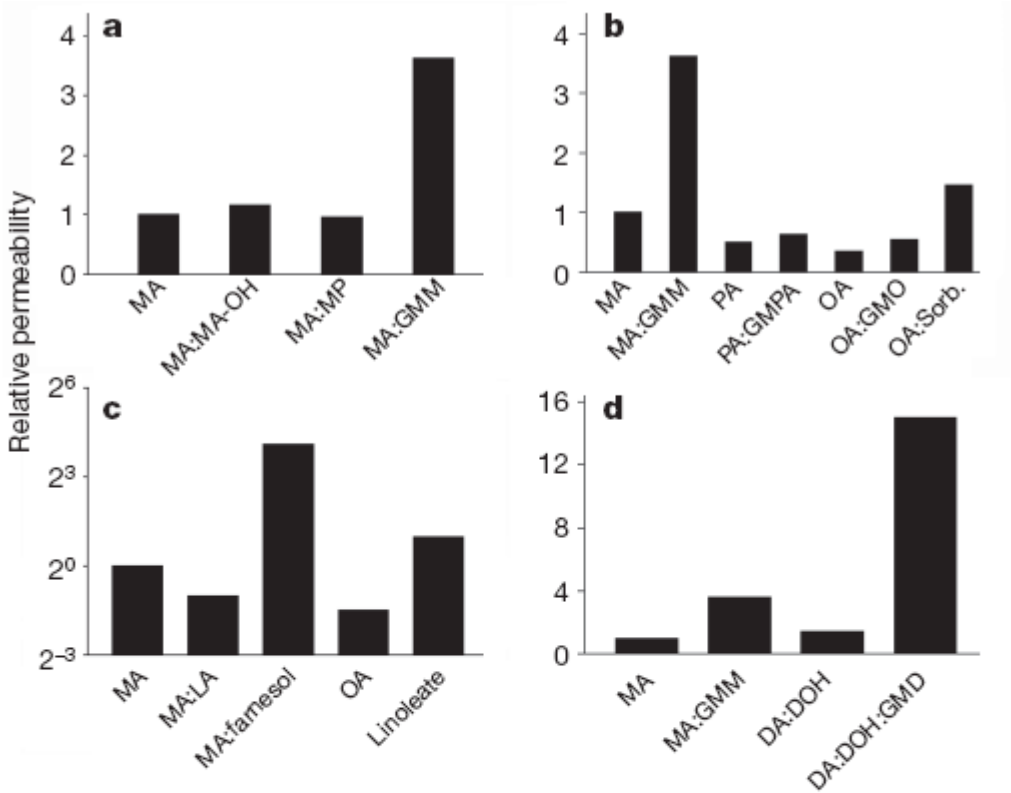


Figure 2 | Ribose permeability of fatty acid based membranes. **a–c**, Influence of head group charge (**a**), head group size (**b**) and membrane fluidity (**c**). **d**, Comparison of decanoic-acid-based membranes with myristoleic acid based membranes. All binary lipid mixtures were 2:1 molar ratios of fatty acid:additive; a 4:1:1 ratio of decanoic acid:DOH:GMD was used. Ribose permeabilities are relative to that of myristoleic acid membranes. DA, decanoic acid; DOH, decanol; GMD, glycerol monoester of decanoic acid; GMM, glycerol monoester of myristoleate; GMO, glycerol monoester of oleate; GMPA, glycerol monoester of palmitoleate; LA, lauric acid; MA, myristoleic acid; MA-OH, myristoleoyl alcohol; MP, myristoleoyl phosphate; OA, oleate; PA, palmitoleic acid; Sorb., sorbitan monooleate.

charge density?
irregular geometries?
saturation degree?
packing density?

[Mansy et al. 2008]

Table 2. Encapsulation Trends of CF or tRNA in Mixed and Pure Systems

System	*CF*	*tRNA*
8A	−	−
GM8/8A	−	−
10A	−	−
GM10/10A	−/+	+
12A	−/+	+
GM12/12A	+	+
18A	+	+
GM18/18A	+	+

(−) indicates no encapsulation; (−/+) indicates a leakage from the vesicles within 1 h; (+) indicates encapsulation was detectable after 1 h. All experiments were performed at room temperature, except for 12A (lauric acid), which was at 32°C.

ASTROBIOLOGY
Volume 9, Number 10, 2009

DOI: 10.1089/ast.2009.0384

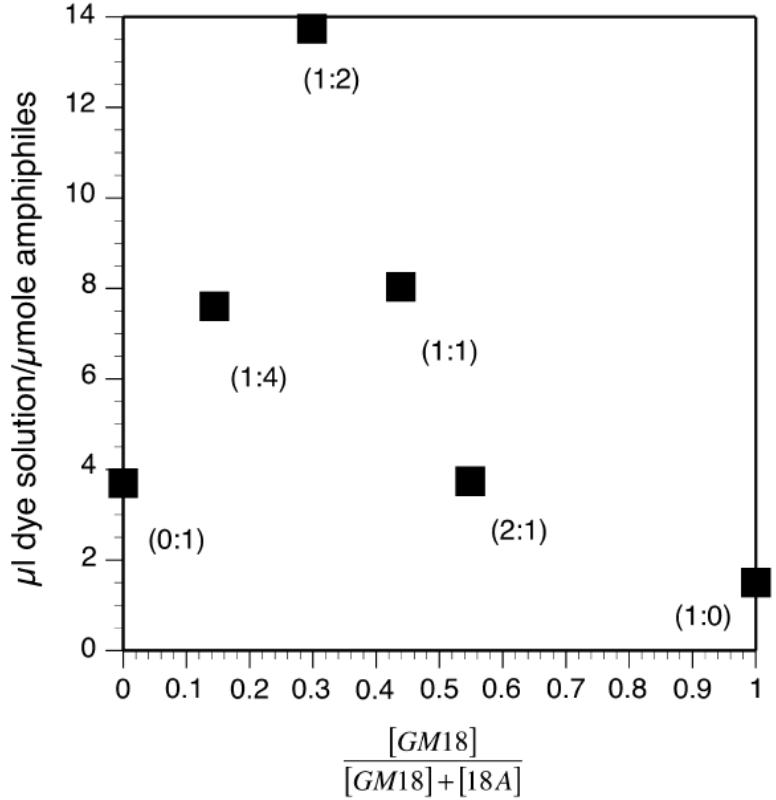


FIG. 2. Effect of the mixed bilayer composition (ratio of GM18 to 18A) on the encapsulation of pyranine. Ratio of [GM18] to [18A] is shown in parentheses; pH = 9.0.

Chemical Evolution of Amphiphiles: Glycerol Monoacyl Derivatives Stabilize Plausible Prebiotic Membranes

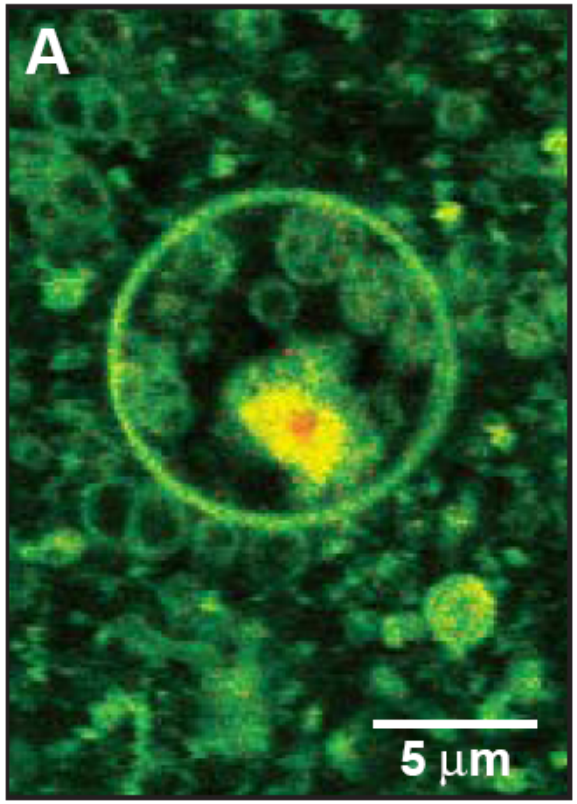
S.E. Maurer,[1,2,3] D.W. Deamer,[2] J.M. Boncella,[3] and P.-A. Monnard[1,3]

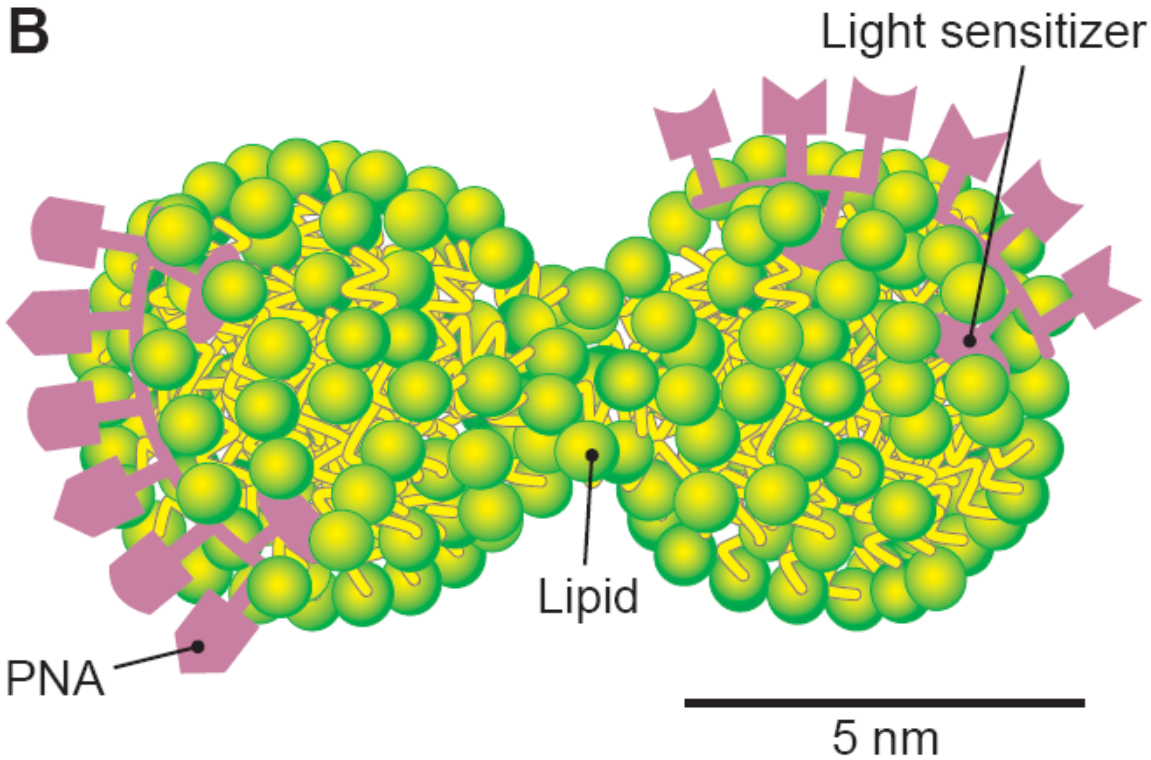
MAIN SOLUTION to the problems:

continue experimental work

1) using MIXTURES!
(of ffaa, alcohols, other surfactants, etc.)

2) trying different CHEMISTRIES (reaction networks)
in those ‘messy’, heterogeneous conditions





When is a molecular aggregate alive?

Transitions from Nonliving to Living Matter

Steen Rasmussen, Liaohai Chen, David Deamer, David C. Krakauer, Norman H. Packard, Peter F. Stadler, Mark A. Bedau

www.sciencemag.org SCIENCE VOL 303 13 FEBRUARY 2004

OUTLINE

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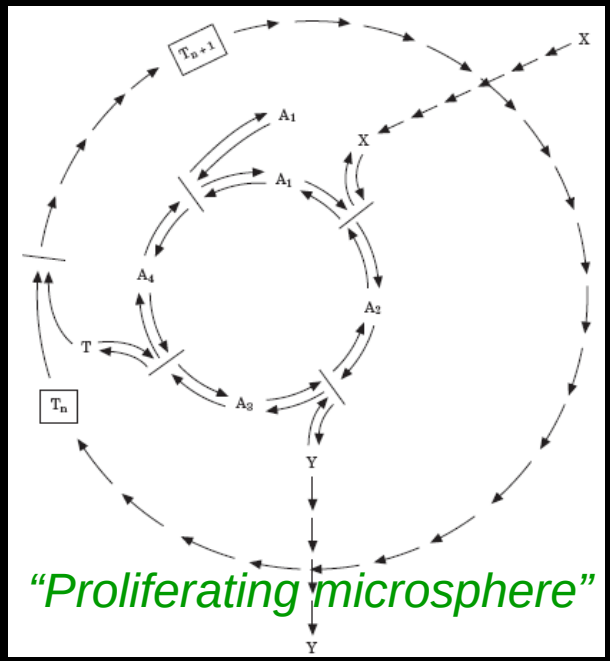
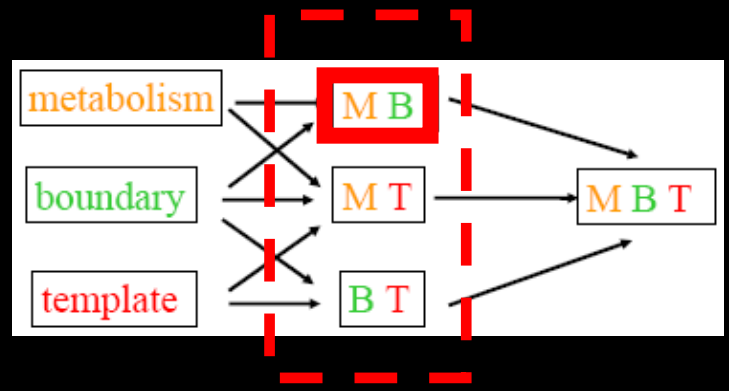
FORGET (temporarily) ABOUT COMPLEX BIOMOLECULES (e.g.: DNA, RNA, PROTEINS,…):

CAN WE DO INTERESTING 'SYSTEMS CHEMISTRY' WITH MUCH SIMPLER (and prebiotically plausible) MOLECULES ?

(e.g., fatty acids, amphiphiles/surfactants, alcohols, aminoacids,…)



(Gánti 1975; 2002)



Infrabiological systems

(Szathmáry et al., 2005)

BOTTOM-UP APROACH: MINIMAL 'lipid-peptide' CELL

(Pre-biopolymer) scenario with:

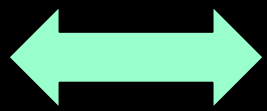
- SELF-ASSEMBLING VESICLES
  made of fatty acids, amphiphiles/surfactants, alcohols, mixtures,...
  evidence from: (a) external sources [Deamer 1986, 1997; Dworkin et al. 2001]
  (b) abiotic (Fischer-Tropsch) synthesis [Nooner et al. 1976; Rushdi & Simoneit 2001]

- SHORT PEPTIDE CHAINS (rudimentary channels/carriers and catalysts)
  made of: Ala, Gly, Asp, Glu, Ser, Val…
  evidence from: (a) external sources [Pizzarello et al. 2006; Bernstein et al. 2002]
  (b) abiotic (Strecker, SIPF,…) synthesis [Miller 1953; Rode 1999]

\+

- VARIOUS 'COENZYME-LIKE' COMPOUNDS (e^- carriers, pigments...)
- PAHs: PHOTOCHEMICALLY ACTIVE and MEMBRANE STABILIZING!
- PRIMITIVE ENERGY TRANSDUCTION MECHANISMS ?
  ('chemical and chemiosmotic' -- energy currency precursors)

DEVELOPMENT OF LIPIDIC COMPARTMENTS



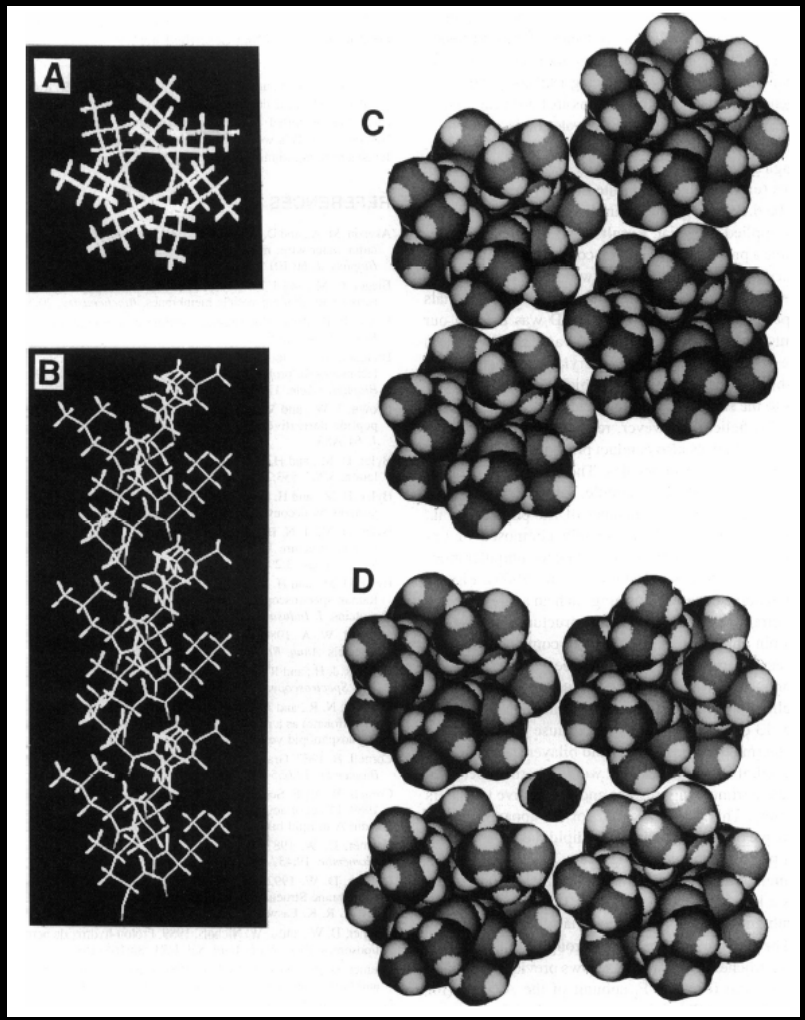
PRODUCTION OF MOLEC. COMPLEXITY (e.g., POLYPEPTIDES)

- avoid diffusion
- adequate scaffolding to anchor transduction mechanisms
- catalytic effect (hydrophobic phase)

- osmotic control/regulation
- accessibility of simple molecules
- constructive use of conc. gradients

[Skulachev, V.P. 1992; Harold F. M., 1986]

Why postpone the appearance of compartments when they seem to be pivotal for the material-energetic implementation of a complex reaction system ??
(+later on: only makes integration problems worse!)



1364 Biophysical Journal Volume 66 May 1994 1364–1379

α-Helical Hydrophobic Polypeptides Form Proton-Selective Channels in Lipid Bilayers

A. E. Oliver and D. W. Deamer
Section of Molecular and Cellular Biology, University of California-Davis, Davis, California 95616 USA

ABSTRACT Proton translocation is important in membrane-mediated processes such as ATP-dependent proton pumps, ATP synthesis, bacteriorhodopsin, and cytochrome oxidase function. The fundamental mechanism, however, is poorly understood. To test the theoretical possibility that bundles of hydrophobic α-helices could provide a low energy pathway for ion translocation through the lipid bilayer, polyamino acids were incorporated into extruded liposomes and planar lipid membranes, and proton translocation was measured. Liposomes with incorporated long-chain poly-L-alanine or poly-L-leucine were found to have proton permeability coefficients 5 to 7 times greater than control liposomes, whereas short-chain polyamino acids had relatively little effect. Potassium permeability was not increased markedly by any of the polyamino acids tested. Analytical thin layer chro-

FIGURE 13 Computer-generated α-helical aggregates. Polyleucine α-helices (20 residues) were produced with MacImdad software (Molecular Applications Group, Stanford University, Stanford CA). The backbone structure is shown from above in 13 *A*, and in side view in 13 *B*. CPK space-filling versions of the top view were then fitted to show the 3a structure (13 *C*), described by Furois-Corbin and Pullman (1986), which excludes water, and the 4a structure (13 *D*), which may include a chain of water molecules. Certain pentameric aggregates (not shown) have ample room to provide an aqueous channel. The bundles of α-helices shown here are for illustration only. No attempt was made to find energy-optimized structures.

ASTROBIOLOGY
Volume 5, Number 1, 2005

Review

The Origin and Early Evolution of Membrane Channels

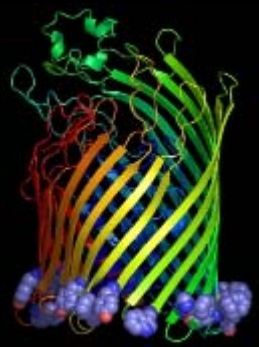
ANDREW POHORILLE,[1,2] KARL SCHWEIGHOFER,[1,3] and MICHAEL A. WILSON[1,2]

ABSTRACT

The origin and early evolution of ion channels are considered from the point of view that the transmembrane segments of membrane proteins are structurally quite simple and do not require specific sequences to fold. We argue that the transport of solute species, especially ions, required an early evolution of efficient transport mechanisms, and that the emergence of simple ion channels was protobiologically plausible. We also argue that, despite their simple structure, such channels could possess properties that, at the first sight, appear to require markedly greater complexity. These properties can be subtly modulated by local modifications to the sequence rather than global changes in molecular architecture. In order to address the evolution and development of ion channels, we focus on identifying those protein domains that are commonly associated with ion channel proteins and are conserved throughout the three main domains of life (Eukarya, Bacteria, and Archaea). We discuss the potassium-sodium-calcium superfamily of voltage-gated ion channels, mechanosensitive channels, porins, and ABC-transporters and argue that these families of membrane channels have sufficiently universal architectures that they can readily adapt to the diverse functional demands arising during evolution. Key Words: Ion channels–Ion transport–Folding of membrane proteins–Protocells. Astrobiology 5, 1–17.

Aromatic residues of actual membrane proteins:

typically located in the transition zone between the low dielectric lipid interior and the polar lipid head groups



www.dur.ac.uk/.../science/peptide_lipid/pl.html

Proc. Nat. Acad. Sci. USA
Vol. 72, No. 5, pp. 1909–1912, May 1975

A Co-Evolution Theory of the Genetic Code

(amino-acid biosynthesis/prebiotic pathways/tRNA)

J. TZE-FEI WONG

Department of Biochemistry, University of Toronto, Toronto M5S 1A8, Canada

Communicated by J. Tuzo Wilson, March 5, 1975

ABSTRACT **The theory is proposed that the structure of the genetic code was determined by the sequence of evolutionary emergence of new amino acids within the primordial biochemical system.**

The genetic code for protein molecules is a triplet code, consisting of the 64 triplets of the four bases adenine, guanine, cytosine and uracil (1, 2). The cracking of the code was a monumental achievement, but it posed in turn what Monod (3) regards as one of the challenges of biology, namely the "riddle of the code's origin." Crick (4) has discussed two different theories which have been proposed regarding this origin. The Stereochemical Theory postulates that each

more primitive form of Thr, is even nearer still to Met). Although Ser and Cys can enter into the Met-biosynthetic pathway subsequent to the entry of Thr, neither Ser nor Cys is a straightforward precursor of Met. Ser is not the only possible contributor of a one-carbon group to Met, and Cys is not the only possible contributor of sulfur (10). α-Transaminations, because of their relative nonspecificity, are not regarded as useful criteria for the tracing of precursor–product relationships. Aside from the above precursor–product relationships, Glu, Asp, and Ala are known to be interconvertible via the tricarboxylate cycle, and Ala, Ser, and Gly via the metabolism of pyruvate, glycerate, and glyoxylate (6).

Glu → Gln	Asp → Lys	Ser → Trp
Glu → Pro	Gln → His	Ser → Cys
Glu → Arg	Thr → Ile	Val → Leu
Asp → Asn	Thr → Met	Phe → Tyr
Asp → Thr		

Ala, Gly, Asp, Glu & Ser (!)

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General features of our simulation platform

1.- A flexible (object-oriented/C++) computational environment to simulate the dynamics of *chemically reacting* cellular systems (e.g., minimal proto-metabolic cells, biological cells,…)

2.- *Stochastic kinetics (Gillespie method)*: tool to explore all possible dynamic behaviours (including critical transitions at low population numbers, role of noise, fluctuations,…)

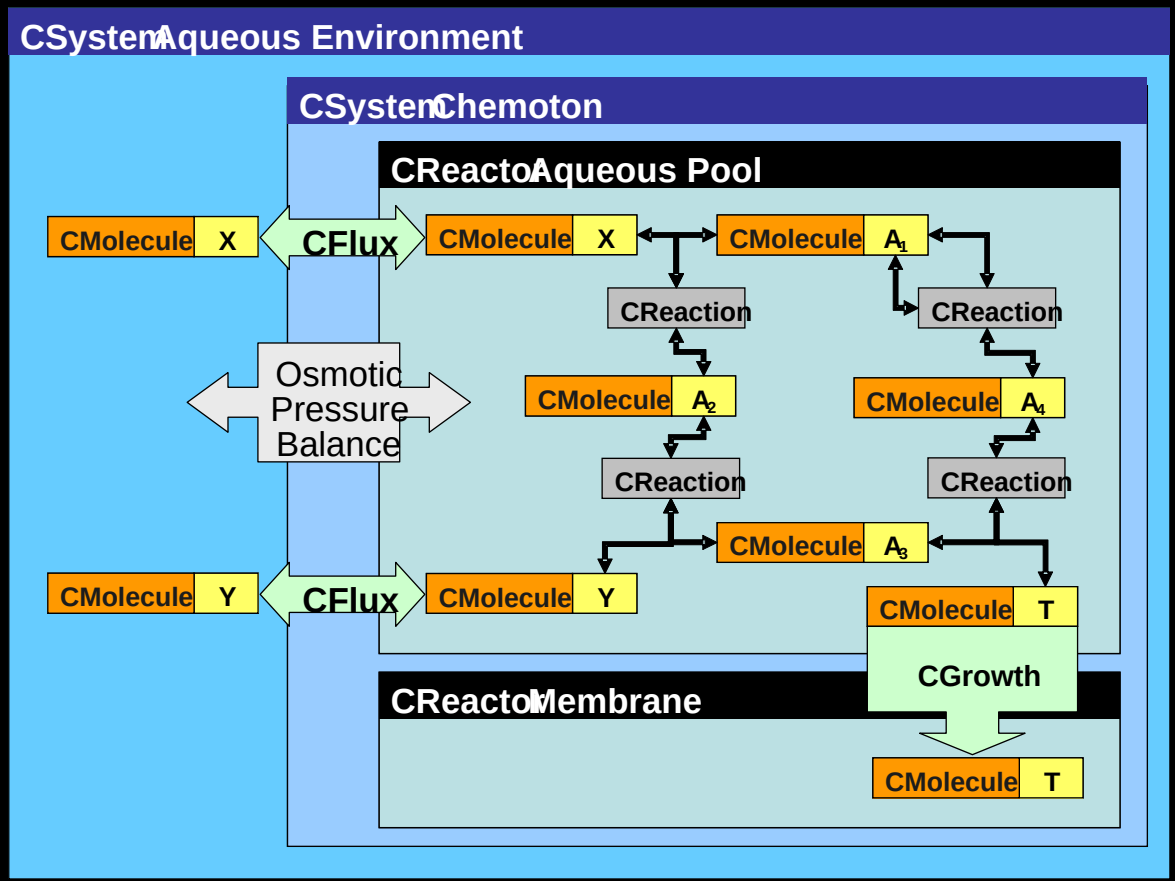
3.- *Realistic* but not aiming to mimic nature (goal: to inform/complement *in vitro* models)
- 3a. Not spatially-explicit, but vol/surf constraints calculated from molec. prop.
- 3b. Water molecules not included, but buffering or osmotic effects into account

4.- *Heterogeneous* conditions (beyond the 'well-stirred tank flow reactor' hypothesis)
- 4a. Various reactive domains/phases
- 4b. Cell population dynamics
- 4c. Transport processes between the different reactive domains ('molecular diffusion', 'gradient diffusion', 'aggregation process',…)

[crucial point: coupling between transport and internal –or boundary– reaction proc.]

A new object-oriented computational environment to simulate complex chemically reacting systems (minimal proto-metabolic cells)

The object-oriented paradigm allows a one-to-one correspondence between objects in the real world and the abstract objects -or classes- in the code. In this case, the C++ classes *CSystem*, *CReactor*, *CMolecule* *CFlux* and *CReaction* cooperate all together to perform the stochastic time evolution of a reacting system (minimal cell model) according to the master equation.



MOLECULAR_DIFFUSION

Membrane Release Density Probability

$$X_{Mem} \xrightarrow{k_{re}} X_{aq} \qquad p_{MR} = k_{re} N_{X_{Mem}}$$

$N_{X_{mem}}$ ≡ Number of X molecules in membrane [molecules]
k_{re} ≡ release constant [s^{-1}]

MEMBRANE_MOLECULAR_UPTAKE

Membrane Uptake Density Probability

$$X_{aq} \xrightarrow{k_{up}} X_{Mem} \qquad p_{MU} = k_{up} S_{Mem} \left[X_{aq} \right]$$

S ≡ Membrane area [dm^2]
$[X_{aq}]$ ≡ aqueous concentration of X molecules [$mole/dm^3$]
k_{up} ≡ uptake constant [$mole^{-1}s^{-1}dm$]

$$w^{J}_{L/C \to \mu} = k_{in} \left[x_{i,E/C} \right] S_{\mu} \exp\left(\frac{1-\phi}{\phi} \right)$$

MEMBRANE_MOLECULAR_EXCHANGE

Reaction Density Probability

$$X_{aq} \underset{k_{re}}{\overset{k_{up}}{\rightleftharpoons}} X_{Mem} \qquad p_{MEX} = \left| p_{MD} - p_{MMU} \right|$$

$$p_{MMU} = k_{up} S_{Mem} \left[X_{aq} \right]$$

$$p_{MD} = k_{re} N_{X_{Mem}}$$

The flux direction depends on the relative values of p_{MD} and p_{MMU}.

S ≡ Membrane area [dm^2]
$[X_{aq}]$ ≡ aqueous concentration of X molecules [$mole/dm^3$]
$N_{X_{Mem}}$ ≡ Number of X molecules in the membrane [molecules]

MEMBRANE_TRANSPORT

Reaction Density Probability

$$X_{aq1} \underset{D}{\Leftarrow} \left| \text{Mem} \right| \overset{D}{\Rightarrow} X_{aq2} \qquad p_T = D S_{Mem} \left| \frac{\Delta \left[X_{aq} \right]}{\lambda} \right|$$

$$\Delta \left[X_{aq} \right] = \left[X_{aq1} \right] - \left[X_{aq2} \right]$$

S ≡ Membrane area [dm^2]
$[X_{aq}]$ ≡ aqueous concentration of X species [$mole/dm^3$]
λ ≡ Membrane thickness is fixed [4.0e-8 dm]
D ≡ Diffusion constant [$mole^{-1}s^{-1}dm^2$]

Our protocell model: main features/assumptions

[Mavelli & Ruiz-Mirazo: Phil Trans. B (2007)]

1) Realistic diffusion processes (passive transport) across the membrane, considering free flow of water

*Overall **isotonic condition**:*

$$C_{Total} = \frac{\sum_{i}^{Internal\ species} n_i}{N_A V_{Core}} = \frac{\sum_{j}^{External\ species} n_j}{N_A V_{Env}}$$

so:

$$V = \frac{\sum_{i}^{internal\ species} n_i}{\sum_{j}^{external\ species} n_j} V_{Env}$$

$$S = \frac{1}{2} \sum_{i}^{membrane\ species} \alpha_i n_i$$

2) Conditions for division or an eventual 'osmotic crisis'

$$\sqrt[3]{36\pi V^2} = S_{sphere} < S < 2S_{sphere(V/2)} = 2\sqrt[3]{9\pi V^2}$$

Initially spherical

pre-division state

- *If:* $S \le \sqrt[3]{36\pi V^2}$ *then:* ***OSMOTIC BURST!***
- *When (or before):* $S = 2\sqrt[3]{9\pi V^2}$ *then:* ***DIVISION!***

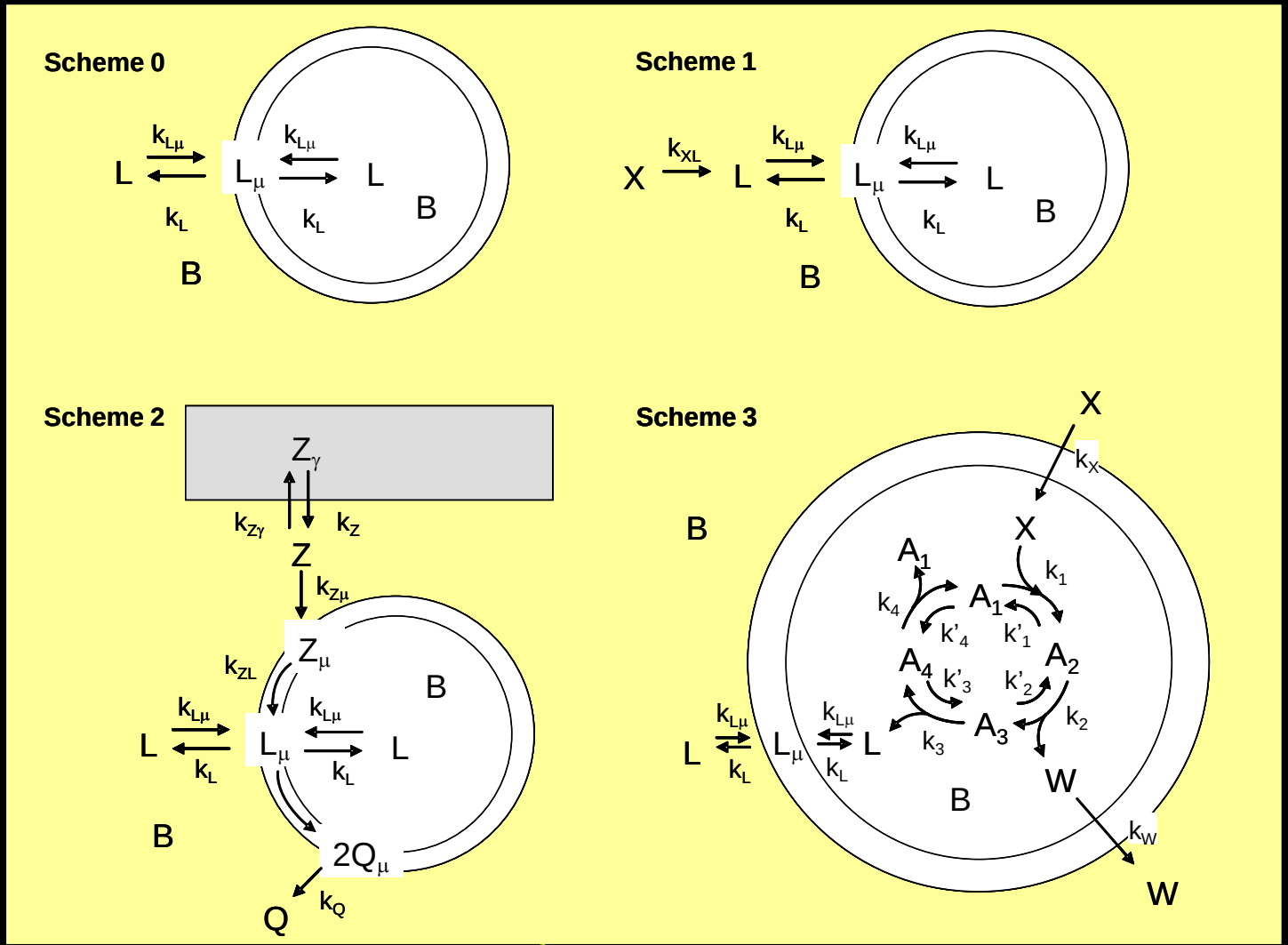
$$\Phi = S / \sqrt[3]{36\pi V^2} \qquad 1 \le \Phi \le \sqrt[3]{2}$$

$$1 - \varepsilon \le \Phi \le (1 + \eta)\sqrt[3]{2}$$

$$(\varepsilon = \eta = 0.1) \qquad 0.9 \le \Phi \le 1.386$$

Minimal cell models

[Mavelli & Ruiz-Mirazo: Phil Trans. B (2007)]



Eq. condition: $k_{L\mu}[L]_{aq} S = k_L n_{L,mem}$

$$S \approx \alpha_L n_{L,mem} / 2$$

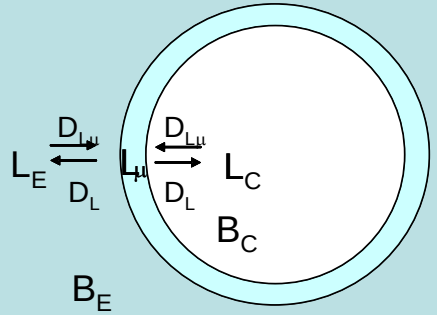
$$[L]_{aq} = \frac{k_L}{k_{L\mu}} \frac{2}{\alpha_L}$$

$k_{L\mu} = 1.0\ M^{-1}t^{-1}$; $k_L = 0.001 t^{-1}$

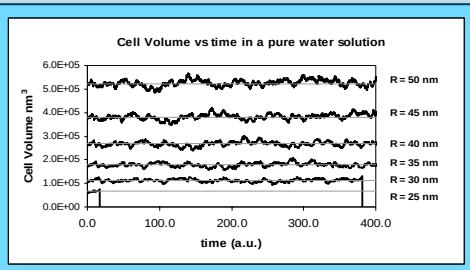
$[\mathrm{L}]_{\mathrm{eq}} = 0.004\ M$

‘Empty cell’ dynamics

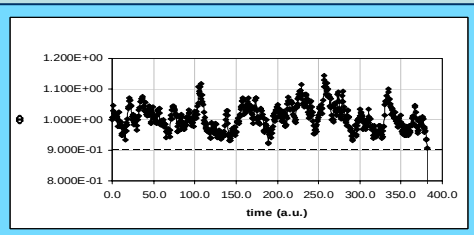
Spherical membranes with different radius (R) in a pure water solution continuously exchanging lipids L with the internal core and the external environment. As a consequence, the volume fluctuates around the initial spherical value $4/3\pi R^3$. In fact, these fluctuations bring small structures ($R \leq 30$) to collapse due to an osmotic crisis.



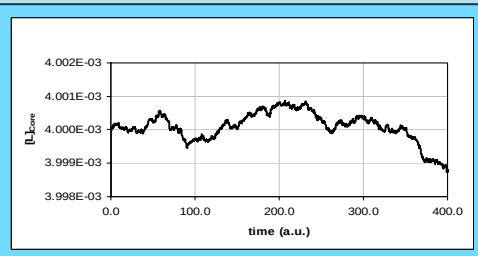
$a_L = 0.5\ nm^2$ $D_{L,\mu} = 1.0 M^{-1}t^{-1}$
$v_L = 1.0\ nm^3$ $D_L = 0.001 M^{-1}t^{-1}$



Stability of spherical membranes with different radius (R) in a pure water solution

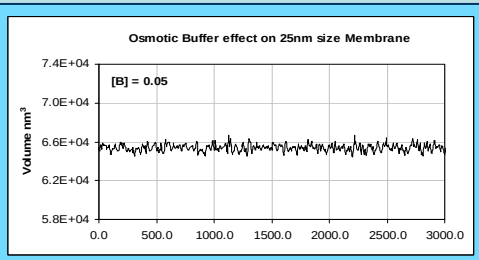


Φ stability factor reported against time for an initial spherical membrane of 30 nm radius. Φ fluctuates around 1. 0 (i.e., the value of a perfect sphere).

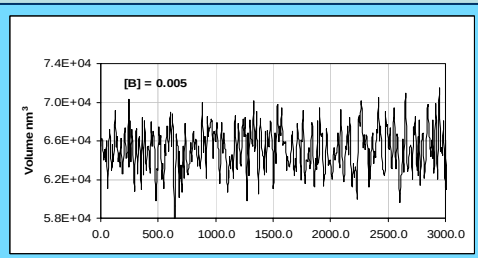


Cell water pool lipid concentration against time for a 50 nm radius membrane: $[L]_C$ fluctuates around the equilibrium value 0.004M.

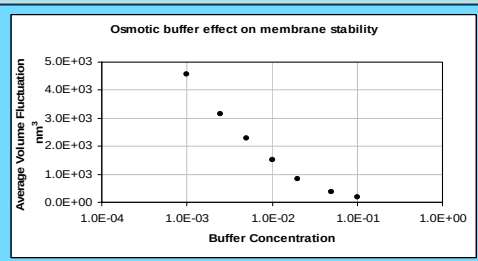
In the presence of an osmotic buffer B, the fluctuations of the core volume decrease as the buffer concentration increases and this enlarges size range for cell stability.



Osmotic buffer effect on the core volume fluctuations: $[B]_E = [B]_C = 0.05M$.



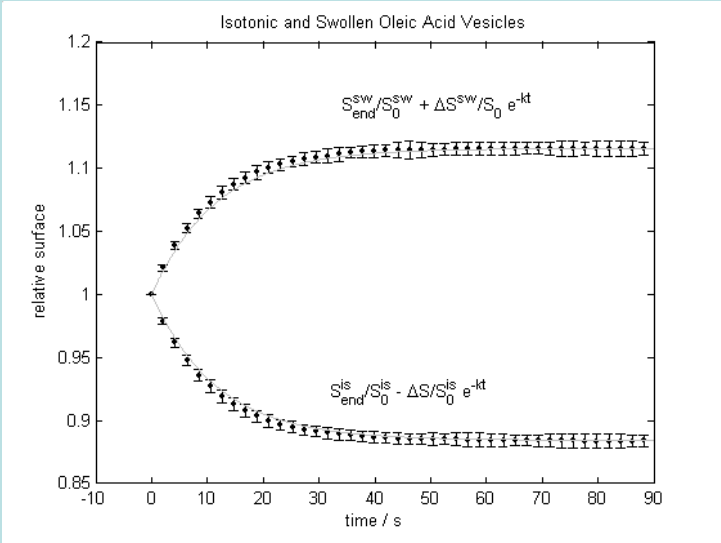
Osmotic buffer effect on the core volume fluctuations: $[B]_E = [B]_C = 0.005M$.



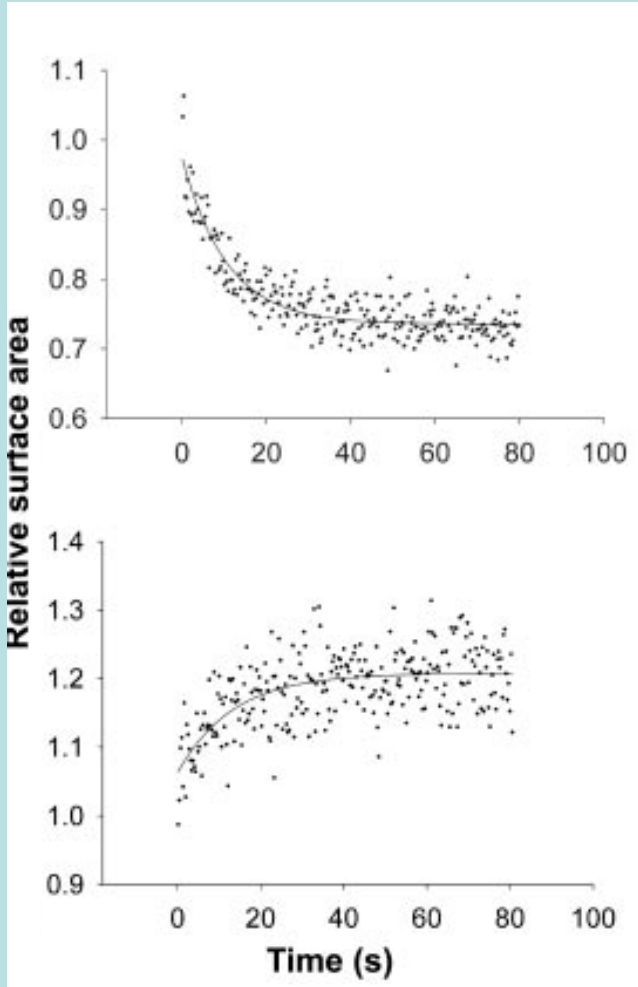
The average volume fluctuations are reported against buffer concentration

Reproducing real experimental data: swollen vs. deflated protocell competition dynamics

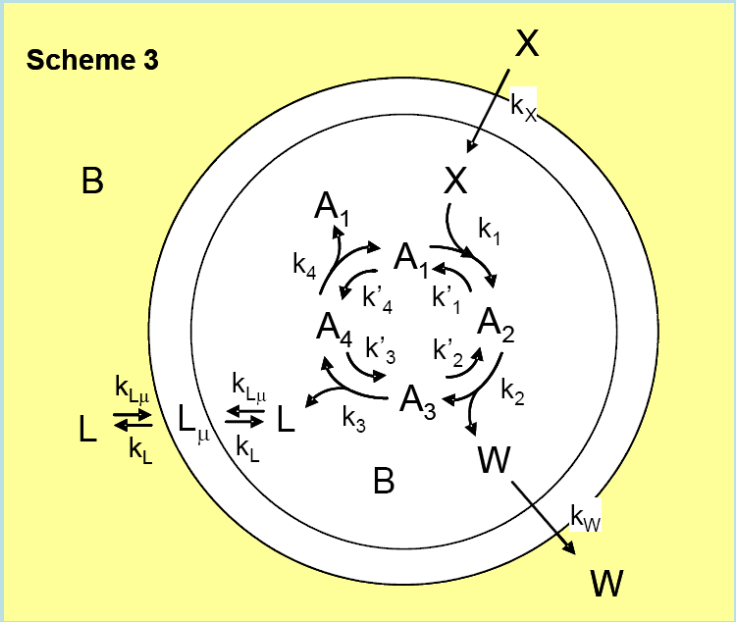
[Mavelli & Ruiz-Mirazo (2008): BIOCOMP'08 Proceedings]



Parameters	Oleic Acid	POPC
k_{in}	7.6 $10^3 s^{-1}M^{-1}nm^{-2}$	7.6 $10^3 s^{-1}M^{-1}nm^{-2}$
k_{out}	7.6 10^{-2} s^{-1}	7.6 10^{-7} s^{-1}
$[L]_{Eq}$ (ϕ=1)	6.667 10^{-5}M	2.857 10^{-10}M
α	0.3 nm^2	0.7 nm^2
ν	0.6 nm^3	1.3 nm^3
ε	0.21	0.59



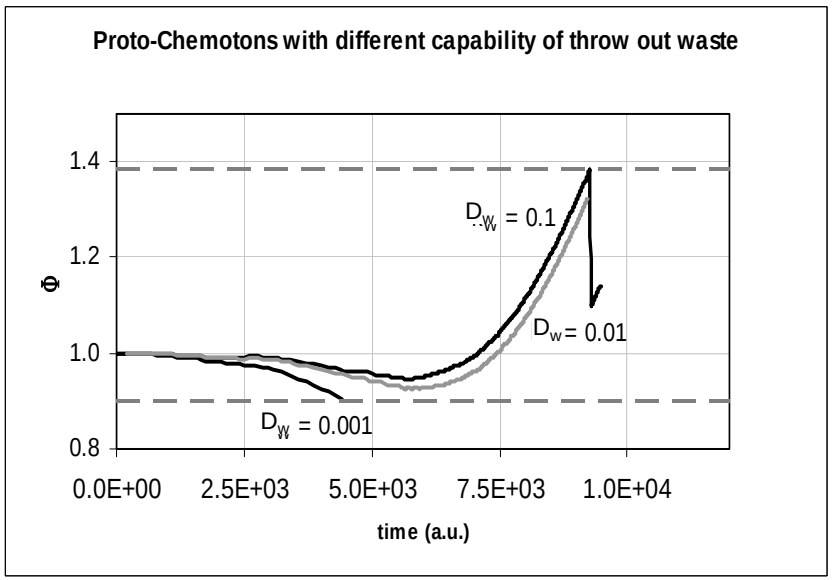
[Chen et al. (2004): Science 305]



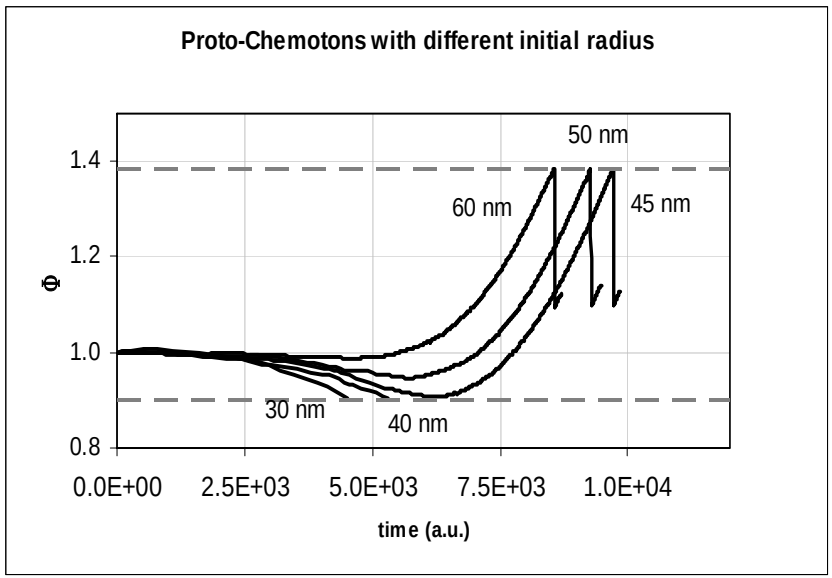
A 'proliferating microsphere'?

[Ganti T. 1975; 2002]

[Mavelli & Ruiz-Mirazo: Phil Trans. B (2007)]

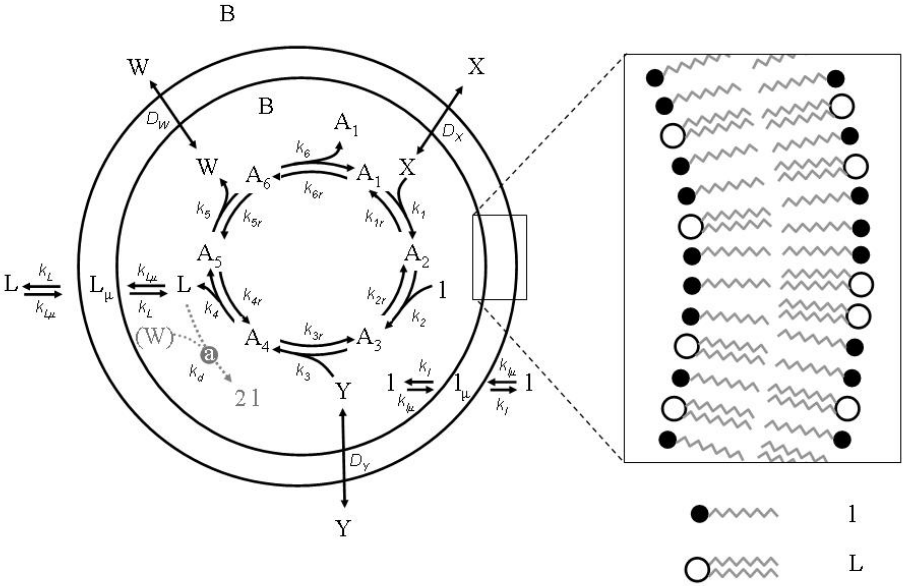


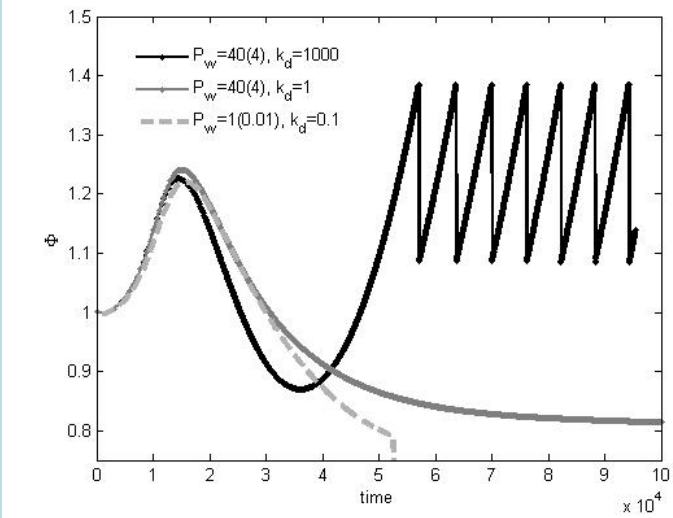
The permeability coefficient to waste results to be a fundamental parameter to guarantee the stability of the cell.

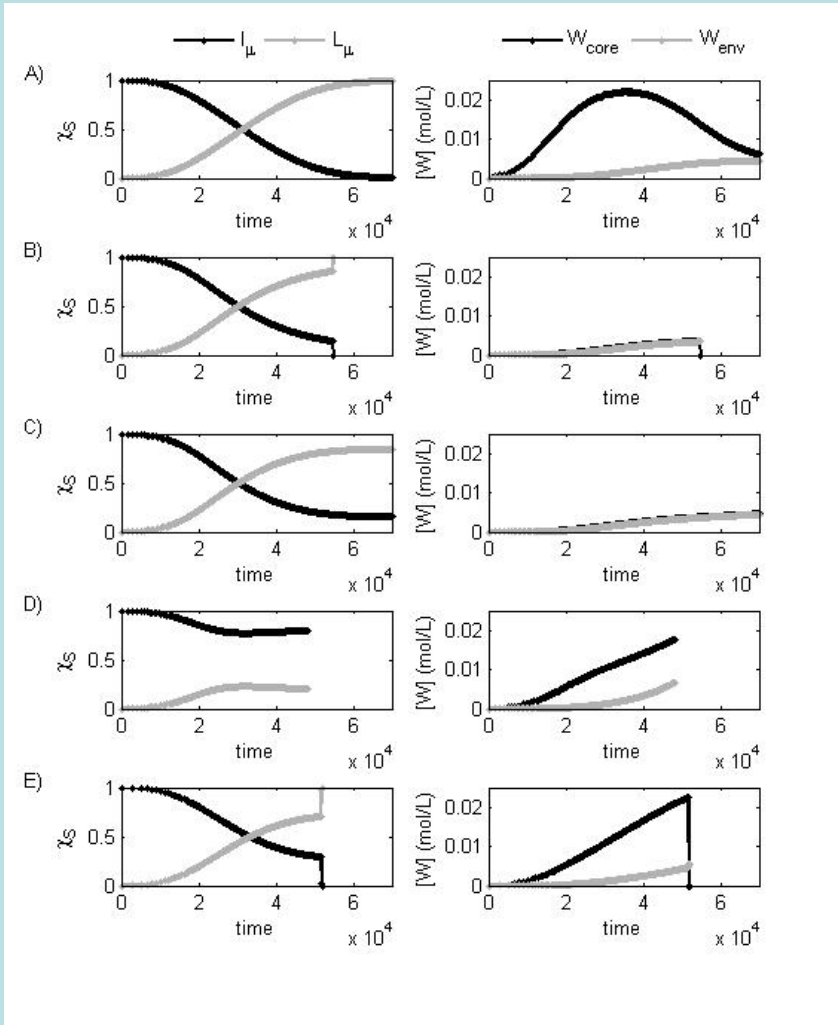


A critical size was found to overcome an eventual osmotic crisis. As much bigger is the size as higher are the stability and growth rate

Two-lipid membranes: FROM 'SELF-ASSEMBLY' TO 'SELF-PRODUCTION'

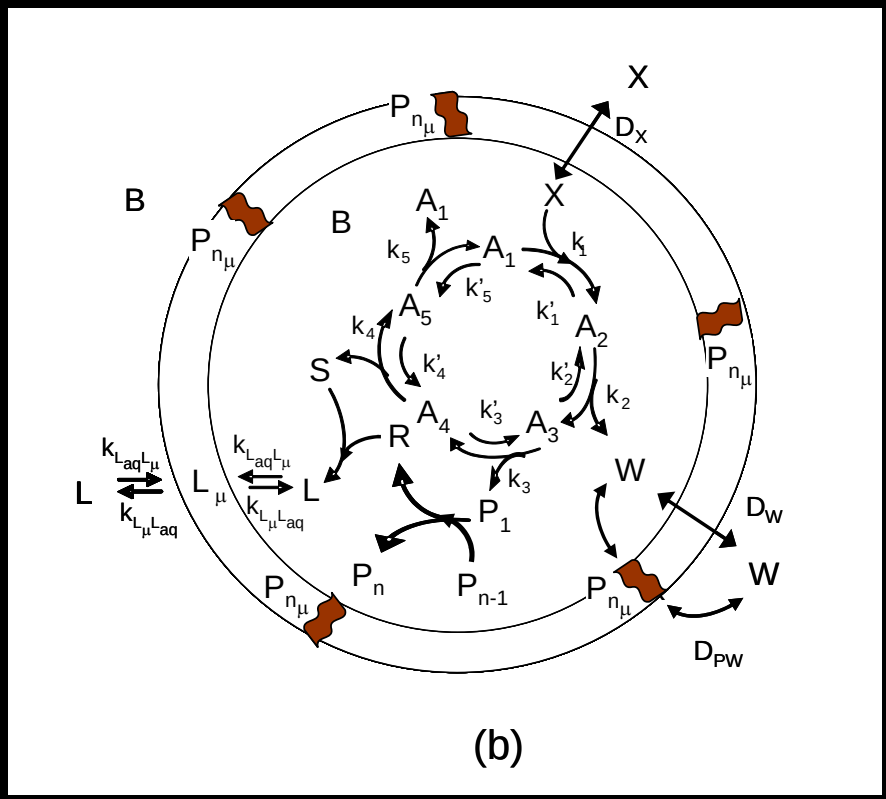






[Piedrafita et al. 2009 ECAL Proc.]

Minimal 'lipid-peptide' protocell(*)



(*) **Ruiz-Mirazo K., Mavelli F., *BioSystems 91*,2008, 374**

Cell Metabolic Network

$$A_1 + X \underset{k_{2,1}}{\overset{k_{1,2}}{\rightleftarrows}} A_2$$

$$A_2 \underset{k_{3,2}}{\overset{k_{2,3}}{\rightleftarrows}} A_3 + W$$

$$A_3 \underset{k_{4,3}}{\overset{k_{3,4}}{\rightleftarrows}} A_4 + P$$

$$A_4 \underset{k_{5,4}}{\overset{k_{4,5}}{\rightleftarrows}} A_5 + S$$

$$A_5 \underset{k_{1,5}}{\overset{k_{5,1}}{\rightleftarrows}} 2A_1$$

$$S + R \xrightarrow{k_{SR}} L$$

$$P_n + P_m \xrightarrow{k(n,m)=k_P} P_{n+m} + R$$

$$k_{2,3} = k_{3,4} = k_{4,5} = k_{5,1} = 1.0t^{-1}$$

$$k_{1,2} = 1.0(Mt)^{-1}$$

$$k_{3,2} = k_{4,3} = k_{5,4} = k_{1,5} = 10^{-3}(Mt)^{-1}$$

$$k_{2,1} = 10^{-3}t^{-1}$$

$$k_{SR} = k_P = 1.0(Mt)^{-1}$$

Membrane Molecular Uptake and Release

$$(L)_{Env} \underset{k_{L_\mu L_{aq}}}{\overset{k_{L_{aq}L_\mu}}{\rightleftarrows}} (L)_\mu \underset{k_{L_{aq}L_\mu}}{\overset{k_{L_\mu L_{aq}}}{\rightleftarrows}} (L)_{Core}$$

$$(P_n)_{Core} \xrightarrow{k_{P_w P_\mu}} (P_n)_\mu$$

$$n \geq 20$$

$$k_{L_{aq}L_\mu} = 1.0(Mt)^{-1}\, nm^{-2}$$

$$k_{L_\mu L_{aq}} = 10^{-3}t^{-1}$$

$$k_{P_{aq}P_\mu} = 1.0(Mt)^{-1}\, nm^{-2}$$

Molecular Transport Processes

$$(X)_{Env} \underset{}{\overset{D_X}{\rightleftarrows}} (X)_{Core}$$

$$(W)_{Env} \underset{}{\overset{D_W}{\rightleftarrows}} (W)_{Core}$$

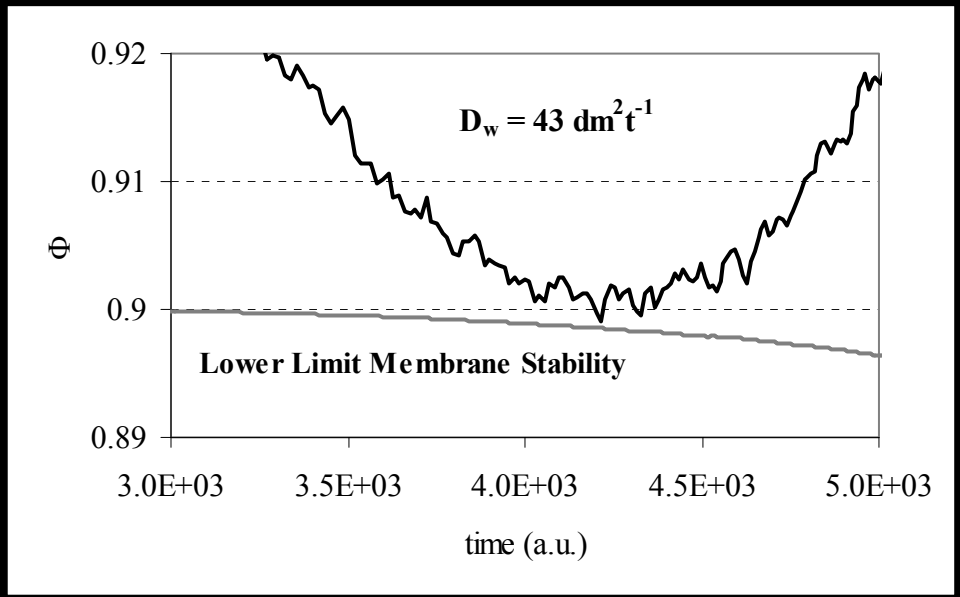
$$(W)_{Env} \underset{}{\overset{D_{WP}(P_{20})_\mu}{\rightleftarrows}} (W)_{Core}$$

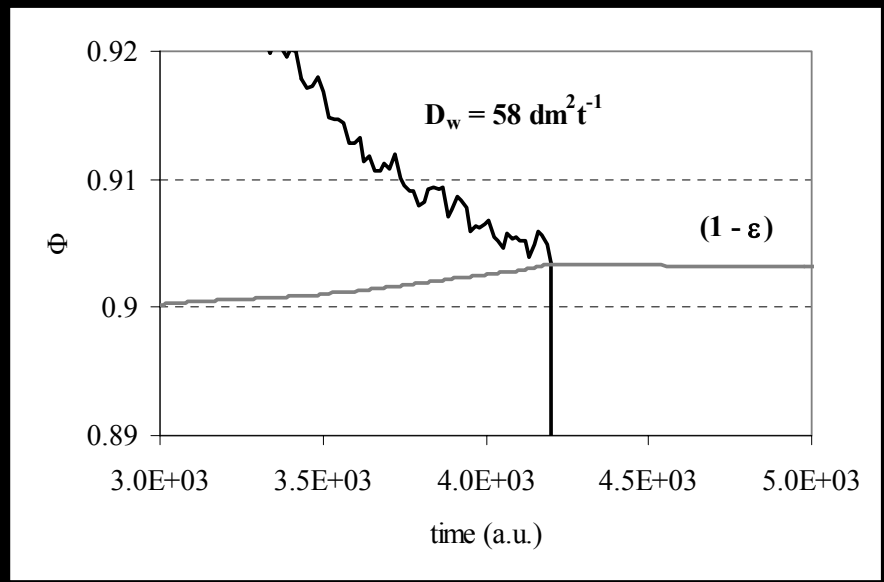
$$D_X = 40dm^2t^{-1}$$

$$p_X = 10^{-7}\, cm \cdot t^{-1}$$

‘Tolerance effect’

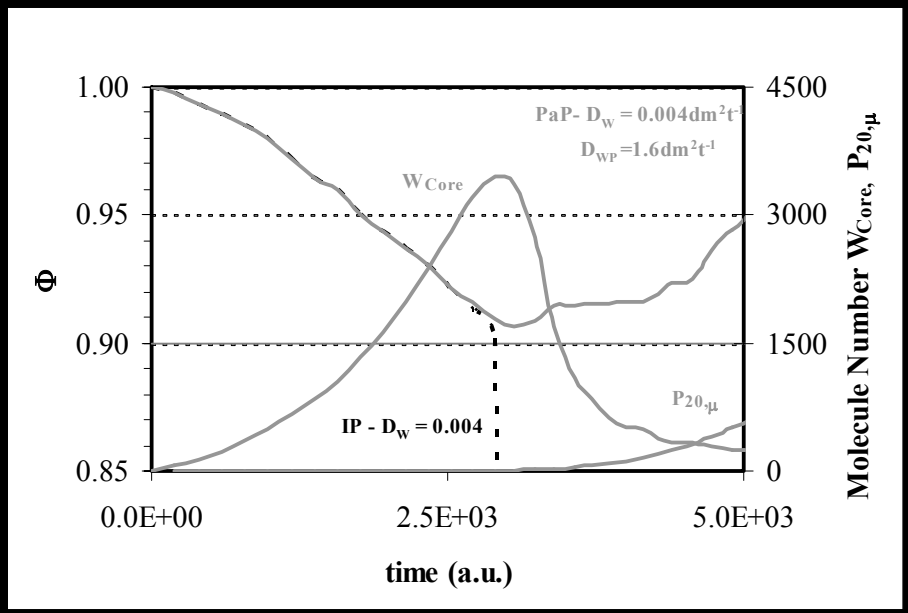
The polypeptides inserted in the lipid bilayer have an effect in its elastic properties: they can increase (ε_{Pn}= η_{Pn}= +1) or decrease (ε_{Pn}= η_{Pn}= -2) the flexibility of the membrane (i.e, its ‘tolerance’).

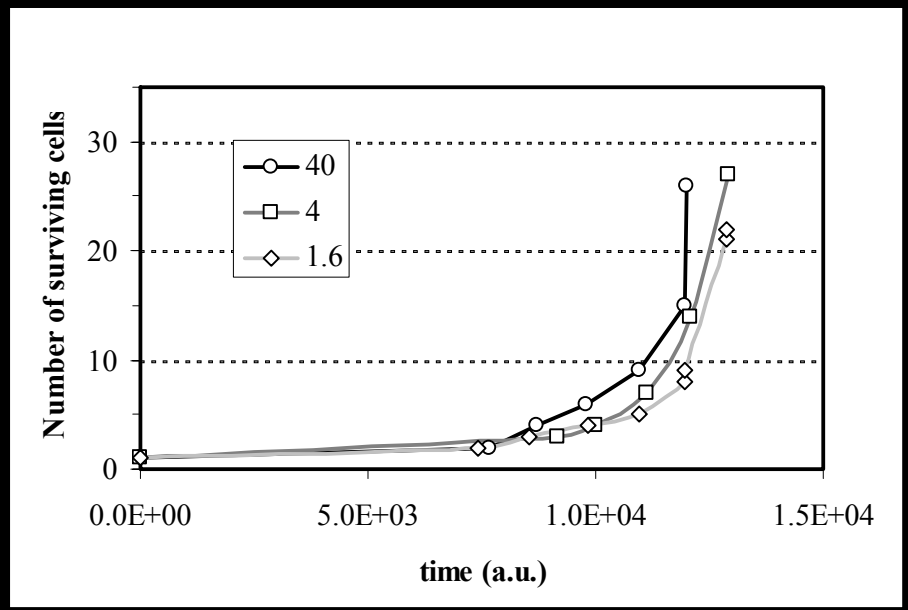




‘Permeability effect’

As soon as alternative W transport pathways are formed in the membrane, the amount of waste in the cell aqueous core rapidly decreases





The model revisited:

- peptides are formed in the membrane
- a conformational equilibrium between peptides is assumed
- the mediated transport of the waste is activated by the membrane elastic tension, i.e. only if the cell is swollen

Cell Metabolic Network

$$A_1 + X \underset{k_{2,1}}{\overset{k_{1,2}}{\rightleftharpoons}} A_2$$

$$A_2 \underset{k_{3,2}}{\overset{k_{2,3}}{\rightleftharpoons}} A_3 + W$$

$$A_3 \underset{k_{4,3}}{\overset{k_{3,4}}{\rightleftharpoons}} A_4 + P$$

$$A_4 \underset{k_{5,4}}{\overset{k_{4,5}}{\rightleftharpoons}} A_5 + L$$

$$A_5 \underset{k_{1,5}}{\overset{k_{5,1}}{\rightleftharpoons}} 2A_1$$

$$k_{2,3} = k_{3,4} = k_{4,5} = k_{5,1} = 1.0$$
$$k_{3,2} = k_{4,3} = k_{5,4} = k_{1,5} = 0.1$$
$$k_{2,1} = 0.1$$
$$k_{1,2} = 1.0$$

Membrane Molecular Uptake and Release

$$(P_1)_{in} \underset{k_{P_\mu P_{aq}}}{\overset{k_{P_{aq} P_\mu}}{\rightleftharpoons}} (P_1)_\mu \underset{k_{P_{aq} P_\mu}}{\overset{k_{P_\mu P_{aq}}}{\rightleftharpoons}} (P_1)_{out}$$

$$(L)_{in} \underset{k_{L_\mu L_{aq}}}{\overset{k_{L_{aq} L_\mu}}{\rightleftharpoons}} (L)_\mu \underset{k_{L_{aq} L_\mu}}{\overset{k_{L_\mu L_{aq}}}{\rightleftharpoons}} (L)_{out}$$

$$k_{L_{aq} L_\mu} = 1.0 \qquad k_{L_\mu L_{aq}} = 10^{-3}$$
$$k_{P_{aq} P_\mu} = 1.0 \qquad k_{P_\mu P_{aq}} = 10^{-4}$$

Membrane Reactions

$$P_n + P_m \underset{k_B(n,m)}{\overset{k_G(n,m)}{\rightleftharpoons}} P_{n+m}$$

$$k_{G/B}(n,m) = \begin{cases} 0 & \text{if } n+m > 20 \\ k_{G/B} & \text{if } n+m \le 20 \end{cases}$$
$$k_G = 100 \qquad k_B = 0.1$$

$$P_{20} \underset{k_{\Pi P}}{\overset{k_{P\Pi}}{\rightleftharpoons}} \Pi$$

$$k_{P\Pi} = 100 \qquad k_{\Pi P} = 50$$

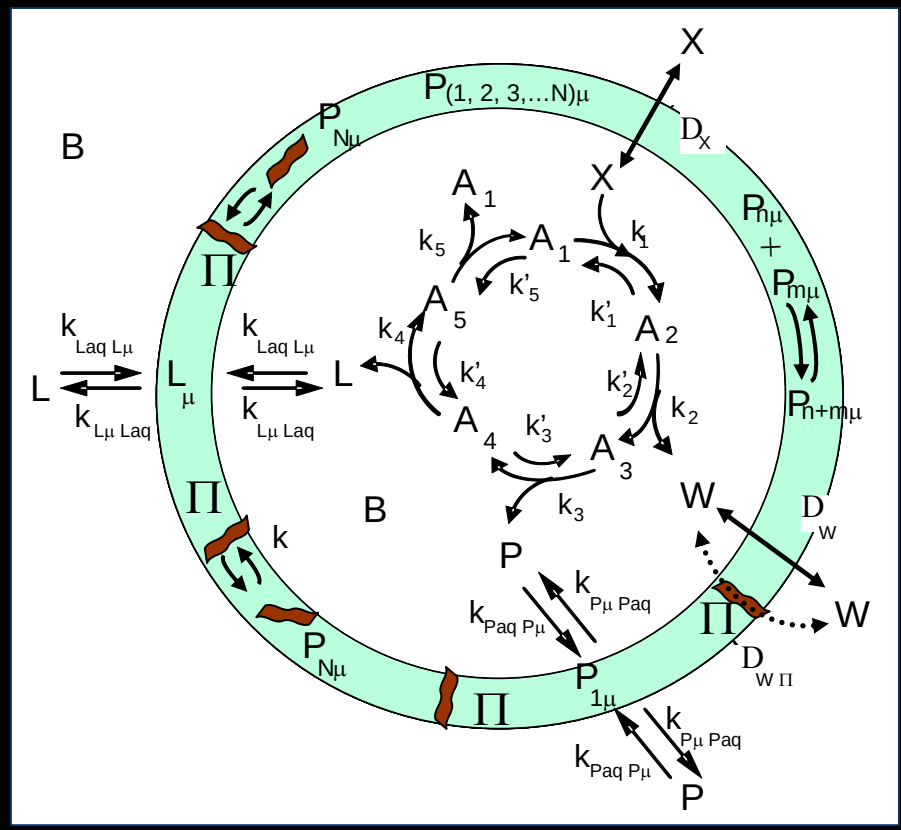
Molecular Transport Processes

$$(X)_{Env} \underset{}{\overset{D_X}{\rightleftharpoons}} (X)_{Core}$$

$$(W)_{Env} \underset{}{\overset{D_W}{\rightleftharpoons}} (W)_{Core}$$

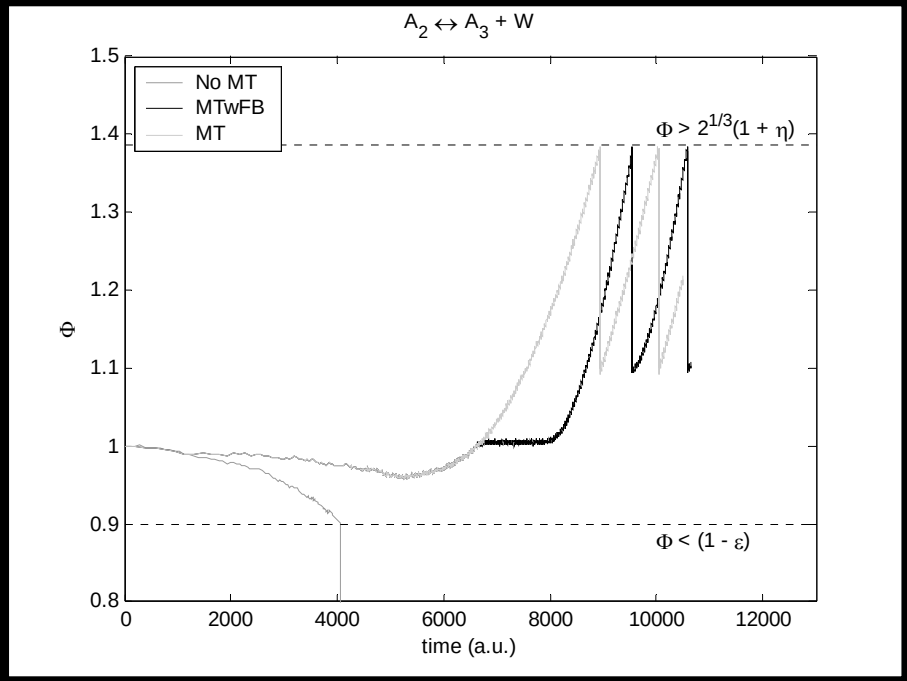
$$(W)_{Env} \underset{(\Phi<1)}{\overset{D_{W\Pi}(\Pi)_\mu}{\rightleftharpoons}} (W)_{Core}$$

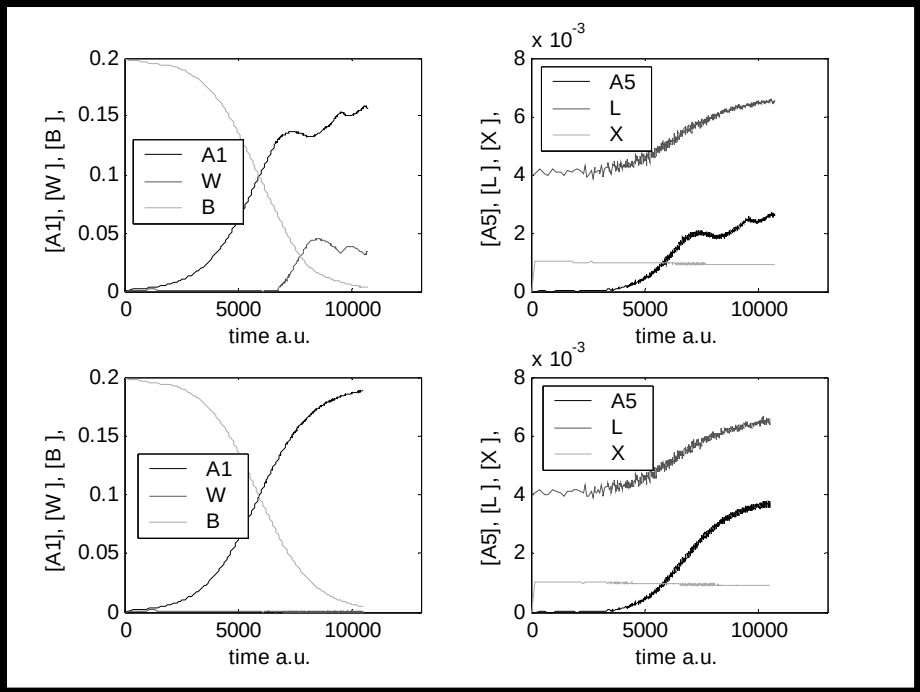
$$D_X = 40$$
$$D_W = 4$$
$$D_{W\Pi} = 400$$

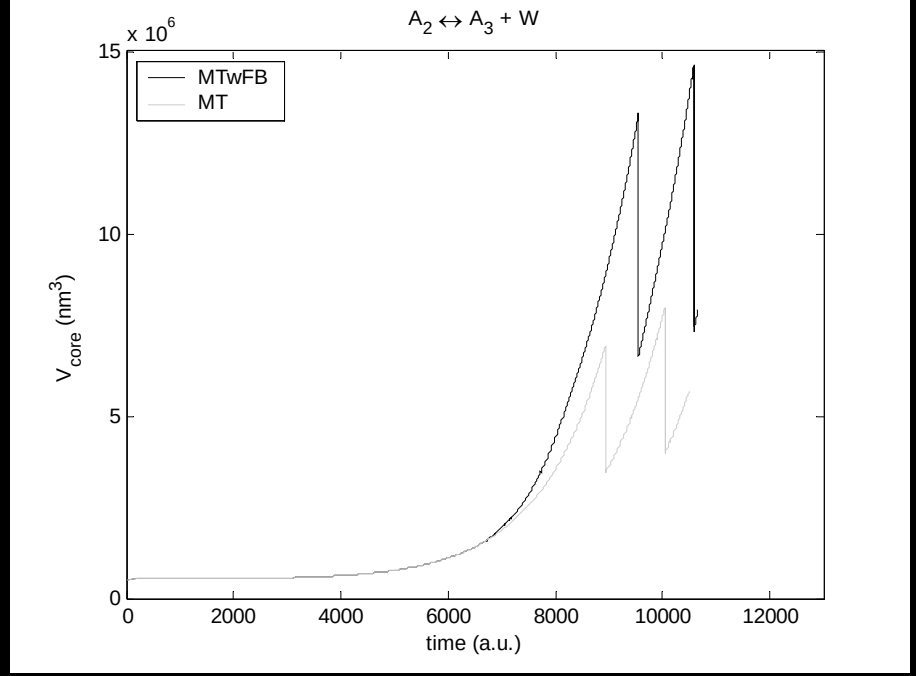


Feedback mechanism: ‘self-regulation’ of waste release/transport

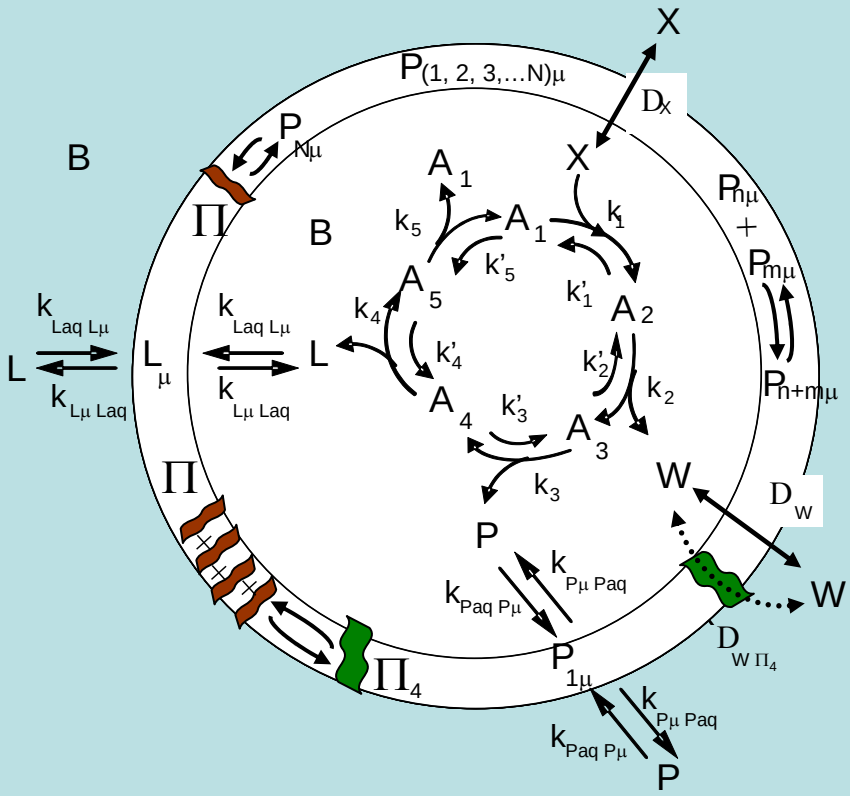
Ruiz-Mirazo K. & Mavelli F. (2007) – ECAL Proc.





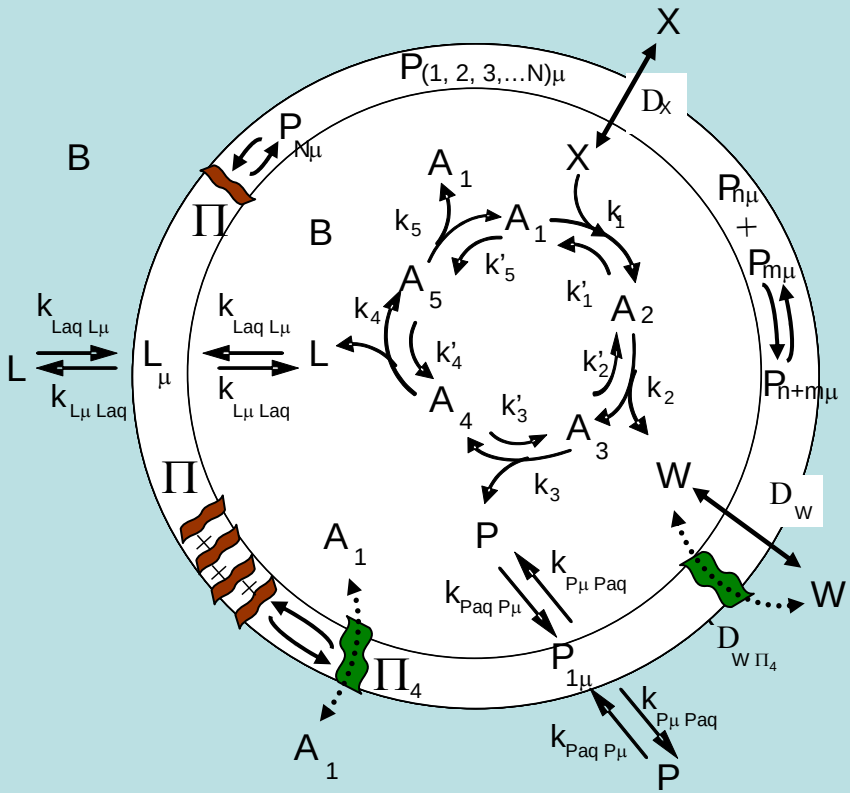


New scheme we are trying:

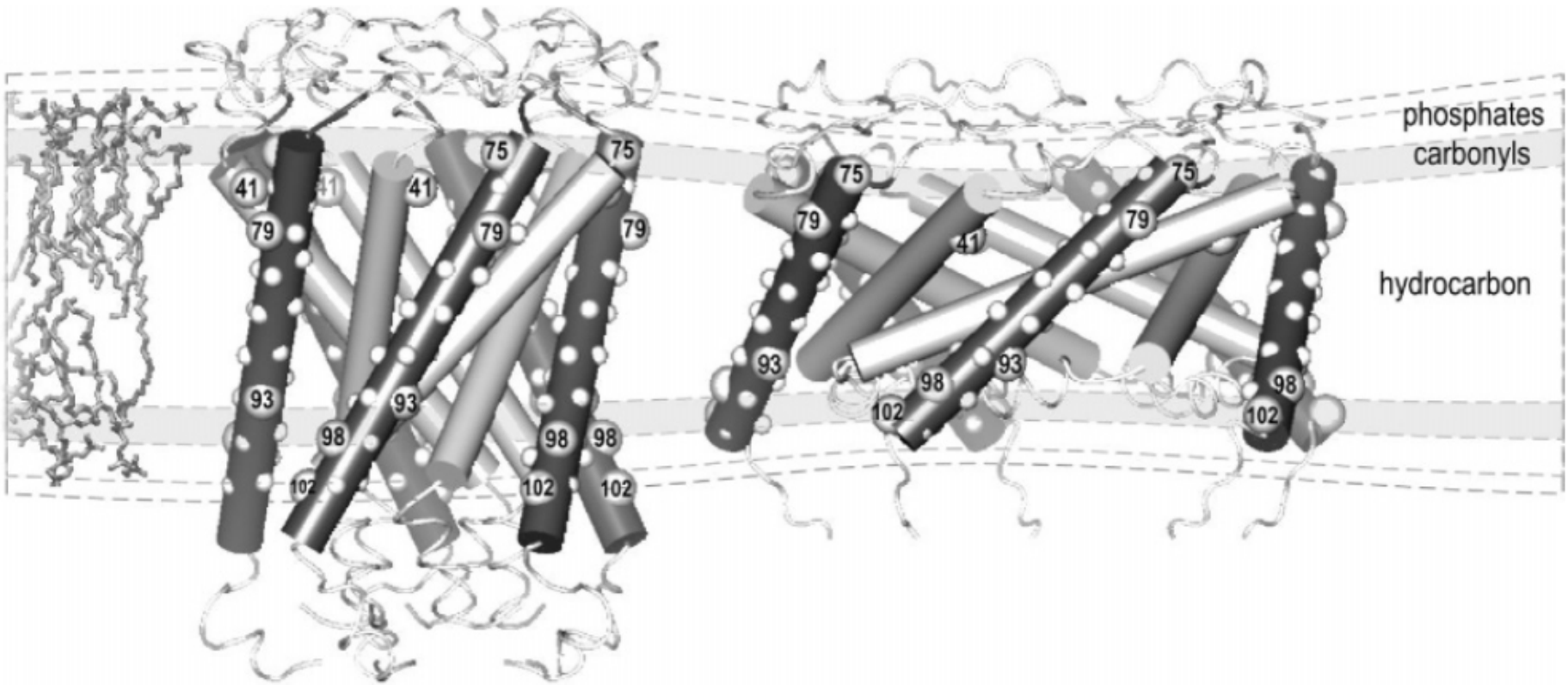


Shirt-Ediss et al. (2010) – ALIFE XII -- *Forthcoming*

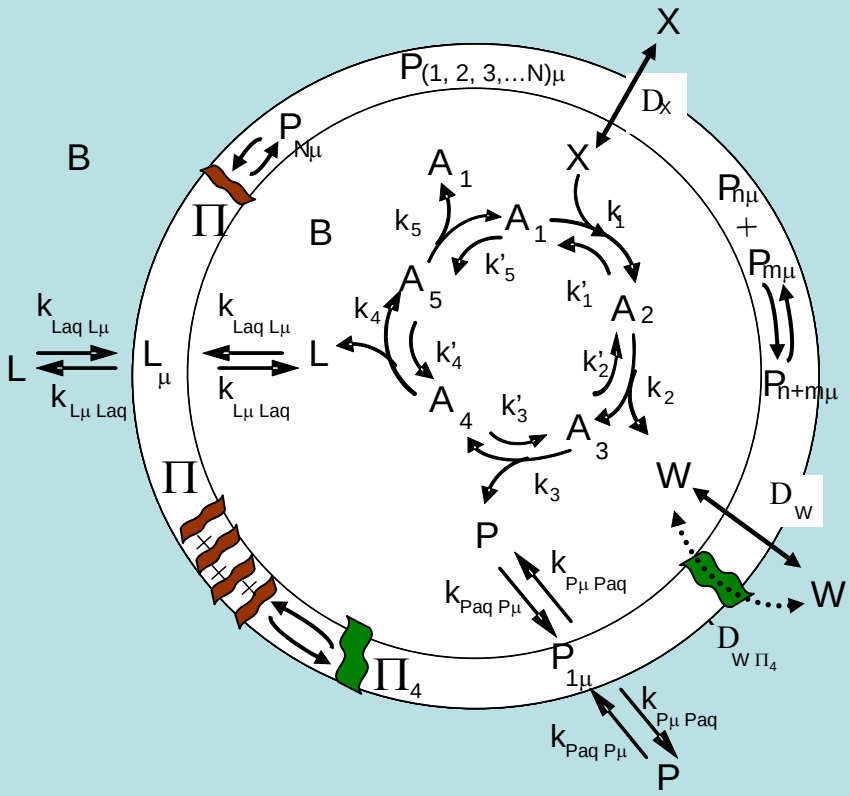
New scheme we are trying:



Shirt-Ediss et al. (2010) – ALIFE XII -- *Forthcoming*



Chiang et al. 2003



Then: active transport mechanisms!

OUTLINE

- *INTRO: A GENERAL VIEW (synthesis vs. analysis/bottom-up vs. top-down)*
- *REVIEW OF VARIOUS PROTOCELL APPROACHES (in vitro/in silico)*
- *SEVERAL OPEN PROBLEMS*
- *TAKING THINGS STRICTLY 'BOTTOM-UP':*
  THE 'LIPID-PEPTIDE' SCENARIO
- *SOME SIMULATION RESULTS AND IDEAS TO EXPLORE*
- ***FINAL REMARKS***

A giant step towards artificial life?

David Deamer

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Step by step, the components of an artificial form of cellular life are being assembled by researchers. Lipid vesicles the size of small bacteria can be prepared and under certain conditions are able to grow and divide, then grow again. Polymerase enzymes encapsulated in the vesicles can synthesize RNA from externally added substrates. Most recently, the entire translation apparatus, including ribosomes, has been captured in vesicles. Substantial amounts of proteins were produced, including green fluorescent protein used as a marker for protein synthesis. Can we now assemble a living cell? Not quite yet because no one has produced a polymerase that can be reproduced along with growth of the other molecular components required by life. But we are closer than ever before.

Introduction

FINE, BUT HOW CLOSE REALLY?

ORIGINS OF LIFE

[Ruiz-Mirazo et al. (2004) OLEB 34: 323-346]

INFORMATION

INCREASE IN MOLECULAR AND ORGANIZATIONAL COMPLEXITY

FUNCTION

minimal living systems
(autonomy + open-ended evolution):
'TWO/THREE-POLYMER WORLD'
(RNA-protein/DNA-RNA-protein)

!

third major bottleneck:
phenotype-genotype decoupling
(catalysis /// template activity)
'translation' mechanisms
and genetic code

'hereditary autonomous' systems
'ONE-POLYMER (RNA) WORLD'

!

second major 'bottleneck':
'template-replication' mechanisms

'basic autonomous' systems
'OLIGOMER (peptides) WORLD'

!

first major bottleneck: 'proto-bioenergetic' mechanisms

FINAL REMARKS

NEED TO DEVELOP
PROTOCELL CHEMISTRY RESEARCH (*in silico* + *in vitro*)
TO GAIN BETTER UNDERSTANDING OF:

- *ORIGINS OF INCREASINGLY COMPLEX PROTO-METABOLIC NETWORKS*

- *ROLE OF THE MEMBRANE AS A SUPRAMOLECULAR STRUCTURE THAT CAN HAVE VARIOUS FUNCTIONS: AVOID DIFFUSION, CONTROL ON MATTER-ENERGY FLOW THROUGH THE SYSTEM, CATALYSIS,…*

- *'PRE-DARWINIAN' EVOLUTIONARY DYNAMICS: IN WHICH STABILITY/ROBUSTNESS OF THE SYSTEM IS JUST AS IMPORTANT AS (OR MORE IMPORTANT THAN) REPRODUCTIVE SUCCESS*

"Cellular organization, far from an afterthought, must have been from the beginning part and parcel of the origin of life. . . Therefore a believable biopoetic scheme is one that creates mounting levels of biological order naturally, by providing the means to convert the flux of energy into the organization of matter. This seems to me inconceivable without compartments."

Franklin Harold, 1986

chemistry + topology

vectorial metabolism, bioenergetics, combination of reactions and gradients,…

CRITICAL not only to understand cellular life but, e.g.: morphogenesis!

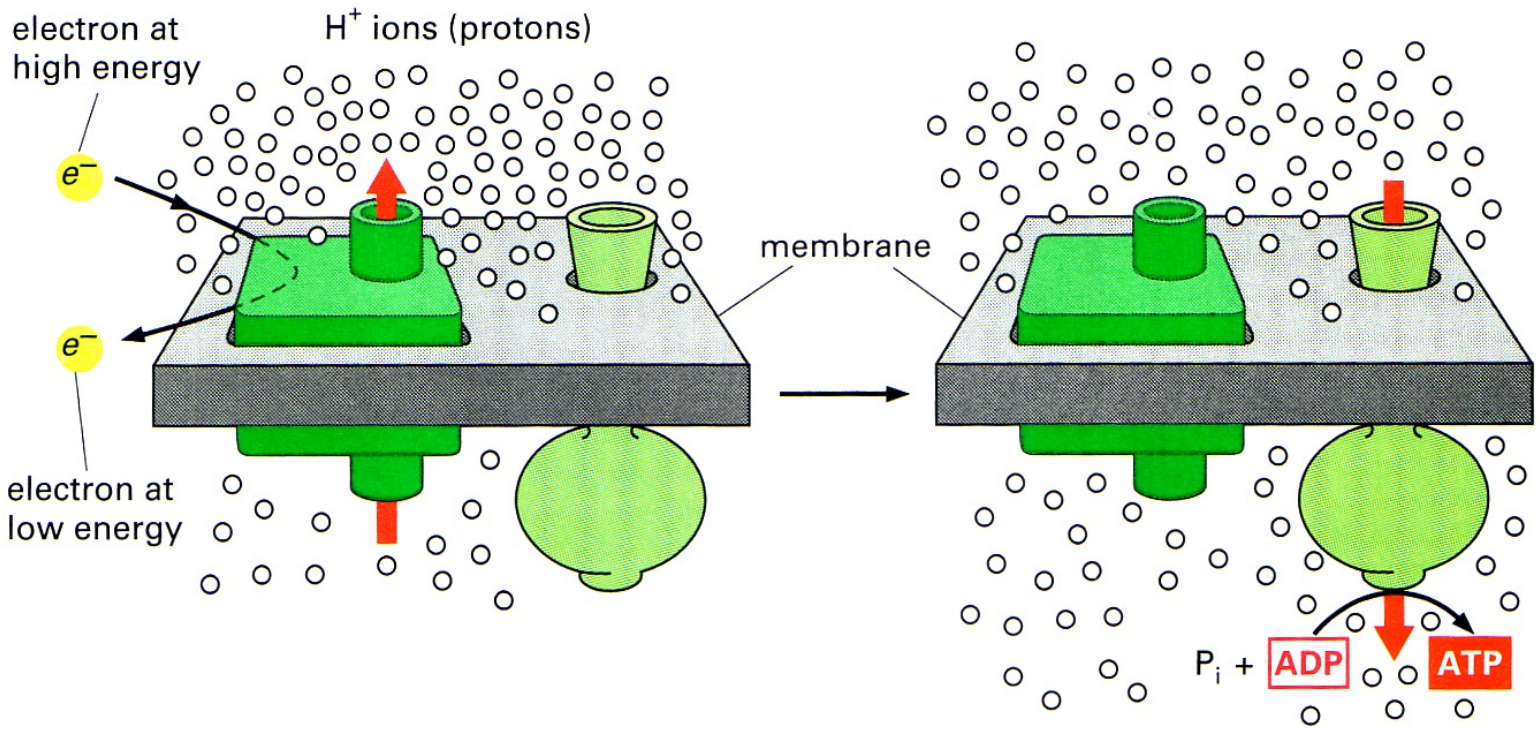
Thank you!

Acknowledgements:

IAS Research Group (ALVARO MORENO)

and FABIO MAVELLI

BIOENERGETIC MECHANISMS: CHEMIOSMOSIS



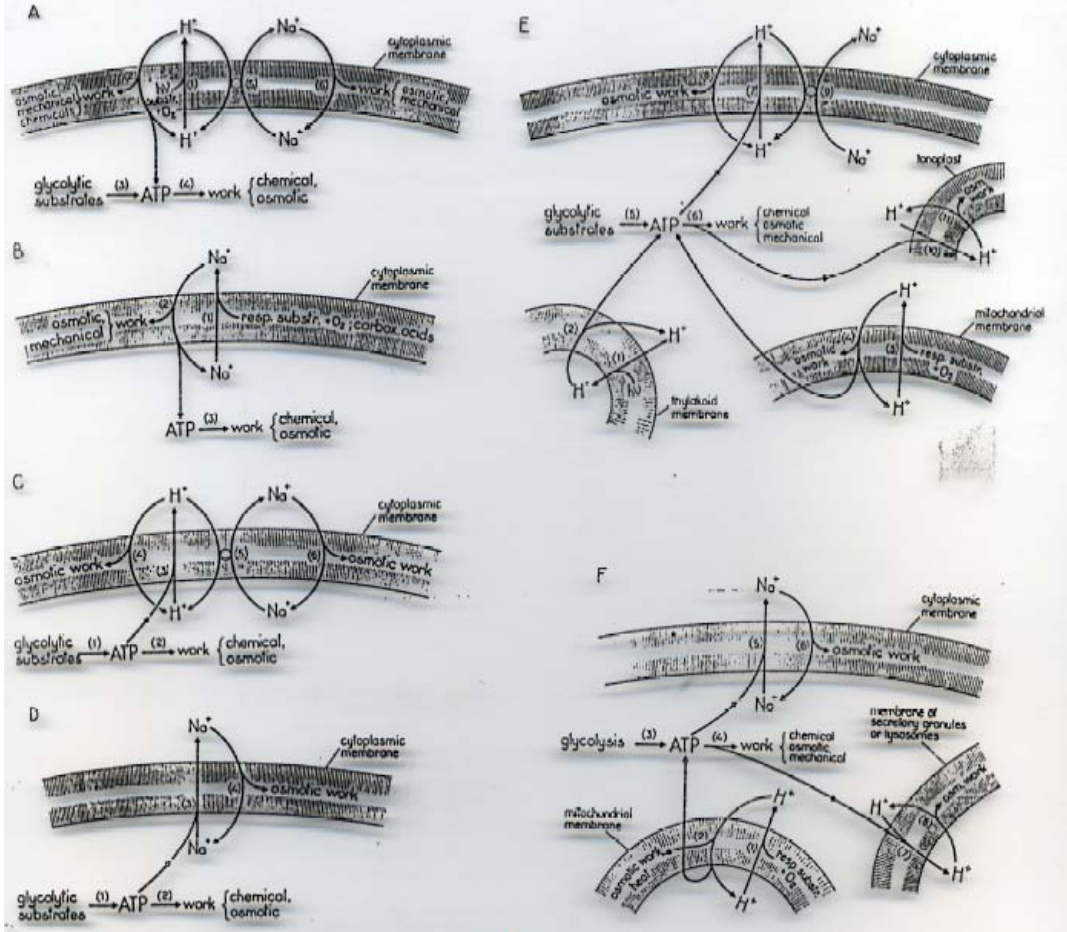


Fig. 1. Main pathways of energy transductions in living cells. (A) Respiratory and photosynthetic bacteria employing the H^+ cycle. (1) Respiratory or photosynthetic redox chains (in halobacteria, bacteriorhodopsin) pump H^+ from the cell at the expense of light or respiratory energy. (2) H^+ moves downhill from the medium to the cell interior via membrane proteins performing different types of useful work, i.e. 'osmotic' (uphill import of solutes by H^+/solute symporters), mechanical (rotation of flagellum) or chemical (ATP synthesis or reverse transfer of electrons). (3) ATP is synthesized by glycolytic and other substrate-level phosphorylations. (4) ATP is utilized to perform chemical (biosyntheses) or 'osmotic' work. (5) Na^+ is extruded from the cell by Na^+/H^+ antiporter. (6) Na^+ moves downhill to the cell via Na^+/solute symporters ('osmotic' work) or Na^+-driven flagellar motor (mechanical work). (B) Respiring or decarboxylating bacteria employing the Na^+ cycle. (1) Na^+ is pumped from the cell by Na^+-motive respiratory chain enzymes or decarboxylases. (2) Na^+ comes back downhill, performing thereby 'osmotic' and mechanical work or synthesizing ATP. (3) ATP is used to support chemical or 'osmotic' work. (C) Anaerobic bacteria employing (a) glycolysis (or other substrate-level phosphorylations) as the only energy source and (b) H^+-ATPase as the mechanism of membrane energization. (1) Glycolytic ATP formation. (2) ATP supports performance of the chemical or 'osmotic' work. (3) H^+-ATPase pumps H^+ from the cell at the expense of ATP energy. (4) H^+ returns to the cell, performing 'osmotic' work. (5) Na^+ is exported via Na^+/H^+ antiporter. (6) Na^+ influx drives 'osmotic' work. (D) As C but Na^+-ATPase substitutes for H^+-ATPase. (E) The plant cell. (1) Photoredox chain pumps H^+ to the thylakoid interior. (2) H^+-ATP synthase forms ATP which is coupled to the downhill H^+ efflux from thylakoid. (3) H^+ is pumped from the mitochondrial matrix space by the respiratory chain. (4) Downhill H^+ influx to matrix is coupled to ATP synthesis or to performance of 'osmotic' work (e.g., uptake of solutes by mitochondria via H^+/solute symporters). (5) ATP is formed by glycolysis. (6) ATP is utilized to perform chemical, 'osmotic', and mechanical work. (7) ATP is hydrolyzed by the plasma membrane H^+-ATPase which pumps H^+ from the cell. (8) Downhill H^+ movement supports 'osmotic' work of the outer cell membrane. (9) Na^+ is pumped from the cell by Na^+/H^+ antiporter. (10) H^+ is pumped to vacuole by the tonoplast H^+-ATPase. (11) Downhill H^+ efflux from vacuole supports 'osmotic' work. (F) The animal cell. (1) Respiratory chain pumps H^+ from mitochondria. (2) H^+ comes back performing chemical work (ATP synthesis) or osmotic work (uphill transport of metabolites). (3) ATP is formed by glycolysis. (4) Chemical, 'osmotic', and mechanical work is driven by ATP hydrolysis. (5) Na^+ is extruded from the cell by Na^+/K^+-ATPase. (6) Na^+ comes into the cell via Na^+/solute symporters of the outer cell membrane. (7) H^+ is pumped to the secretory granules, lysosomes, etc. by H^+-ATPase of vacuolar type. (8) The H^+ efflux from these vesicles supports 'osmotic' work.

[Lipmann 1941]
[Mitchel 1961]
[Harold 1986]

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[Skulachev 1992]

IN VITRO (I): THE 'GARD' MODEL

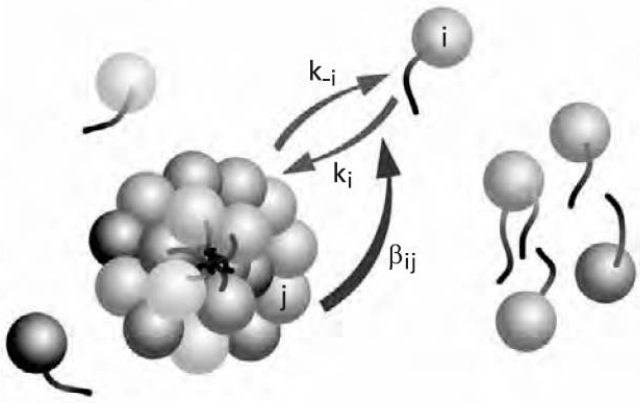


Figure 11.3
GARD: the Graded Autocatalysis Replication Domain model. The main reaction step in the simplest amphiphile joining GARD formulation, is the reversible exchange of an amphiphilic molecule A_i between the environment and an assembly (black arrows, representing k_i and k_{-i}, respectively the forward and reverse basal rate constants). A key aspect in reaching a kinetic homeostasis is the dependence of the reaction rates on the current composition of the assembly, through mutual catalysis. The matrix β_{ij} signifies the mutual rate enhancement parameters for the catalysis exerted by species A_j on the joining and leaving reactions of A_i (bottom arrow). The β matrix elements are drawn from a probability distribution generated through the Receptor Affinity Distribution model (Lancet et al., 1993; Rosenwald, Kafri, and Lancet, 2002; Segré and Lancet, 1999).

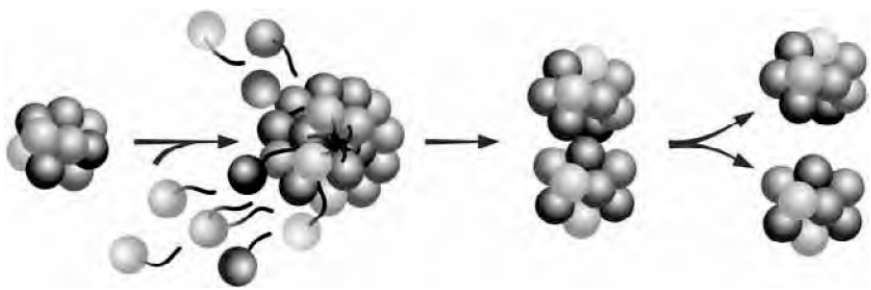
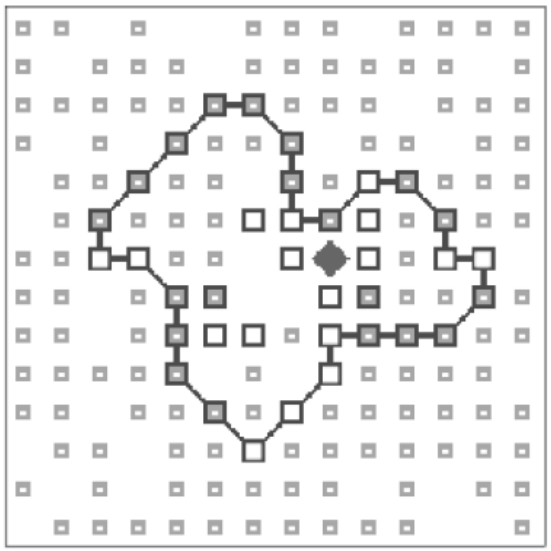
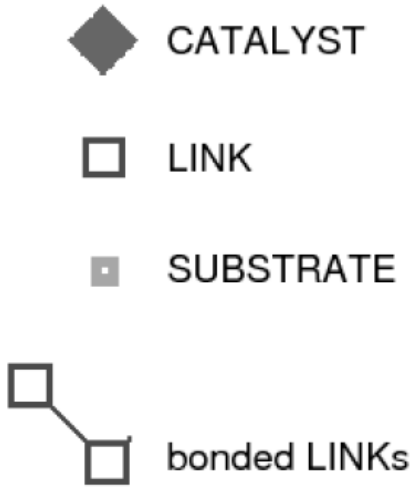


Figure 11.4
The life cycle of a GARD assembly. A GARD assembly undergoes growth through selective joining of molecules from the external environment. As the assembly accretes more and more molecules, it may lose structural stability and "split" into two progenies, as described in Tanaka, Yamashita, and Yamazaki (2004). Some assemblies with distinct compositions manifest homeostatic growth, that is, there is no change in the ratio of the molecular counts within the assembly. Their daughter assemblies are similes of the original assembly with comparable composition (Segré et al., 2000; Segré, Shenhav, et al., 2001), and are likely to undergo homeostatic growth. Thus, a series of "faithful" transmissions of the composition from parent assembly to daughter assembly is generated. Such self replicating compositions are termed *composomes.*





IN VITRO (II): AUTOPOIETIC TESELLATION MODEL