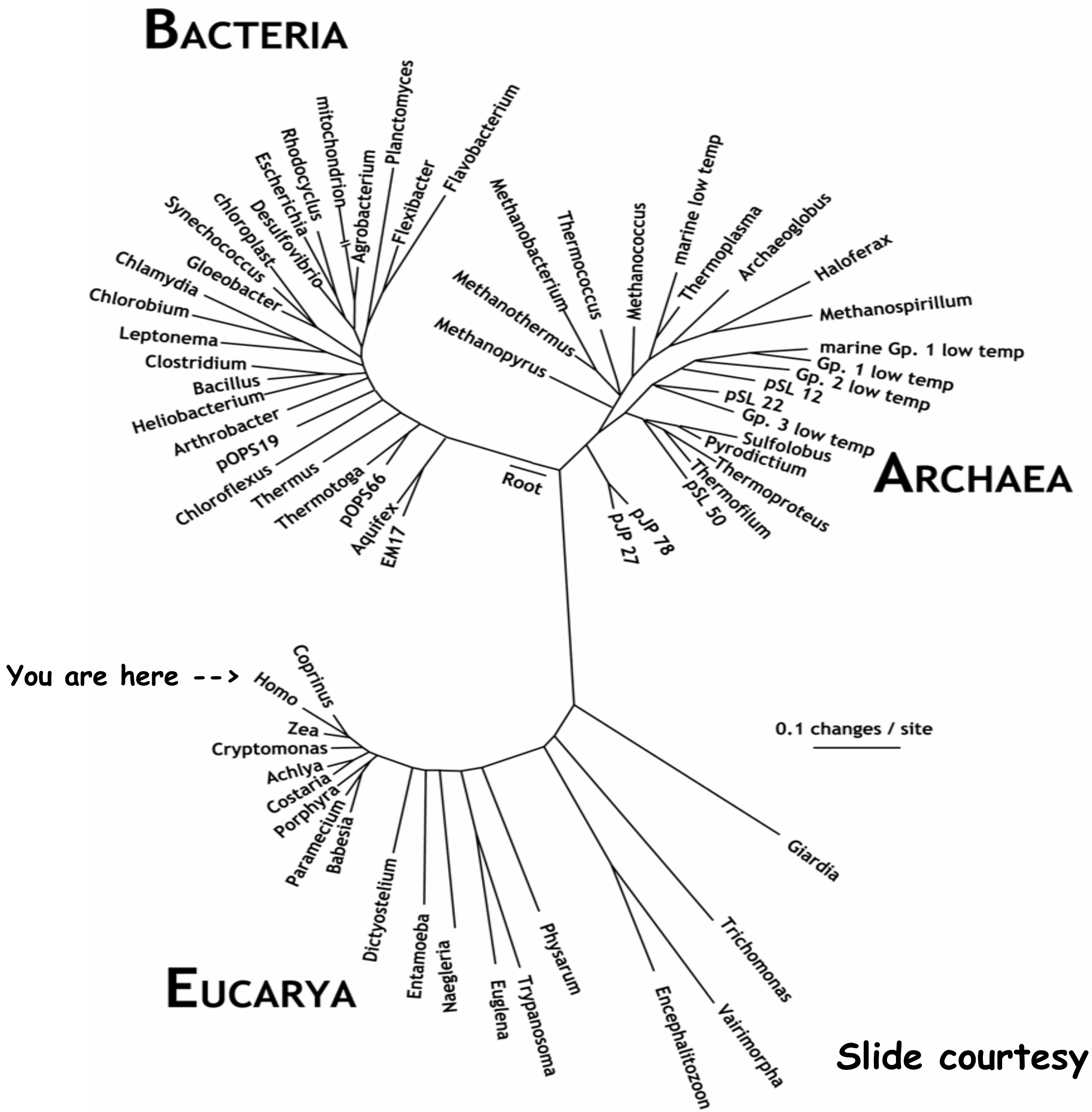
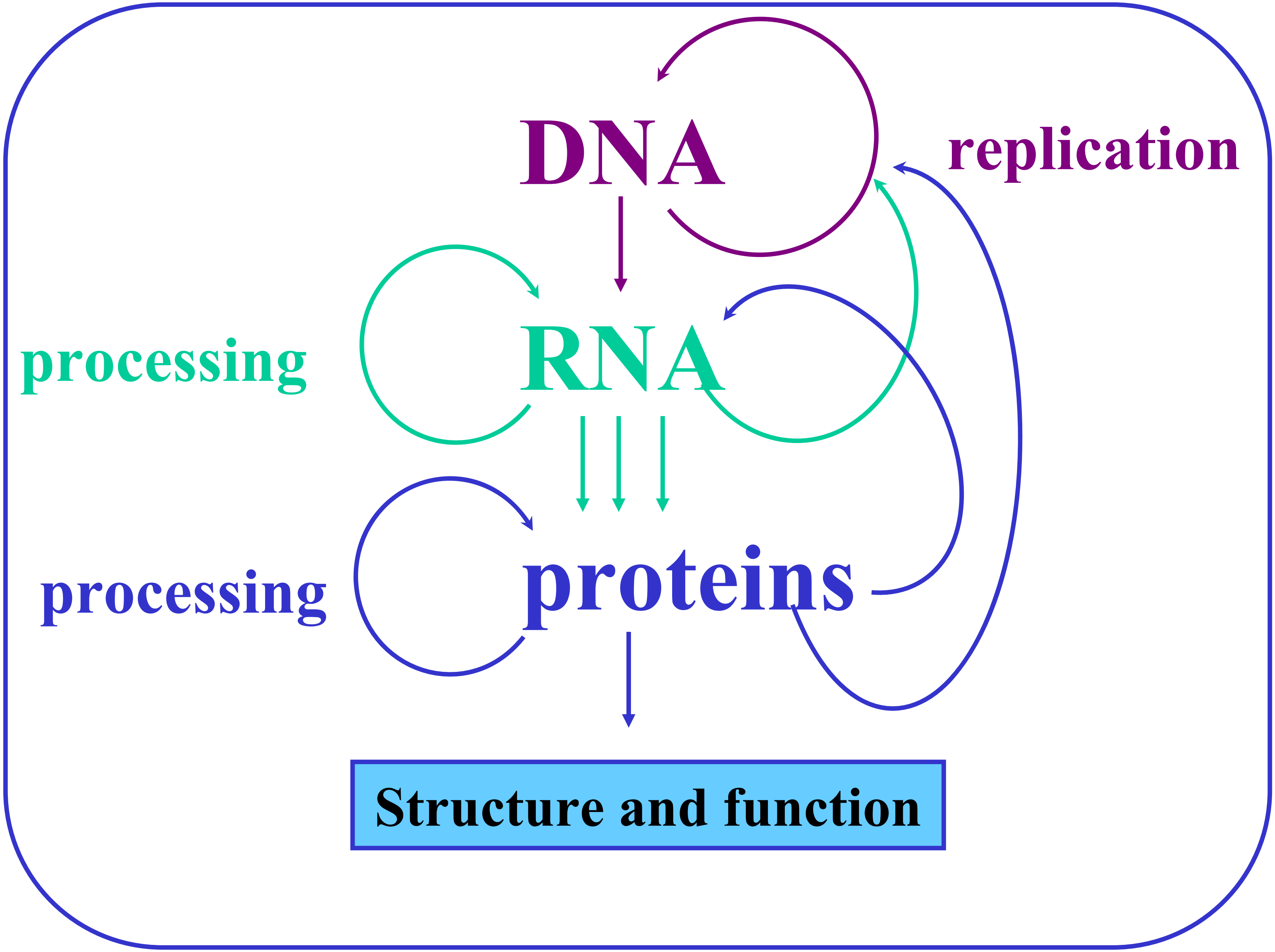
The Origin of Life
and the
Emergence of Darwinian Evolution

Jack W. Szostak
Howard Hughes Medical Institute
Massachusetts General Hospital
Harvard Medical School

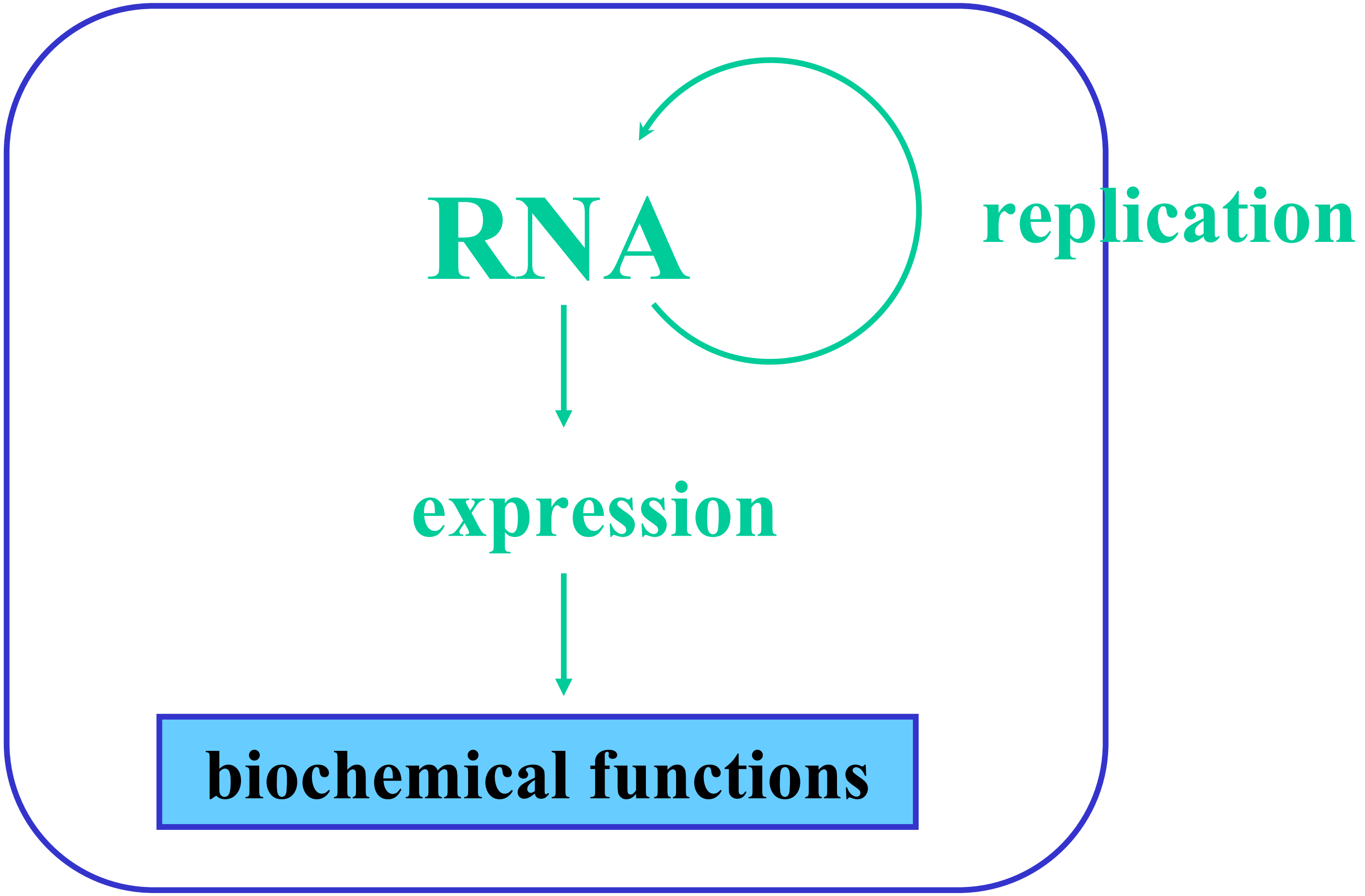


Slide courtesy of Norm Pace

Organizational Complexity of Modern Life



A great simplification:



How did we get to the RNA World?

small molecules (CO, H_2, H_20, NH_3, CH_4…) + energy

cofactors

lipids + sugars + nucleobases + high-energy compounds + amino acids

activated nucleotides

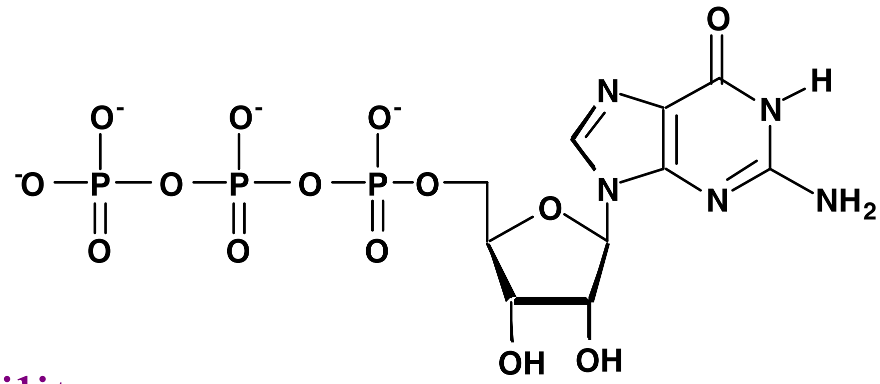
peptides

Self-assembly into vesicles

Assembly into genetic polymer

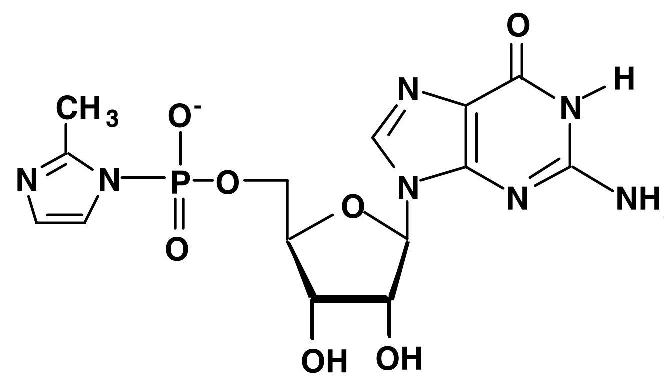
protocell?

Nucleoside triphosphates



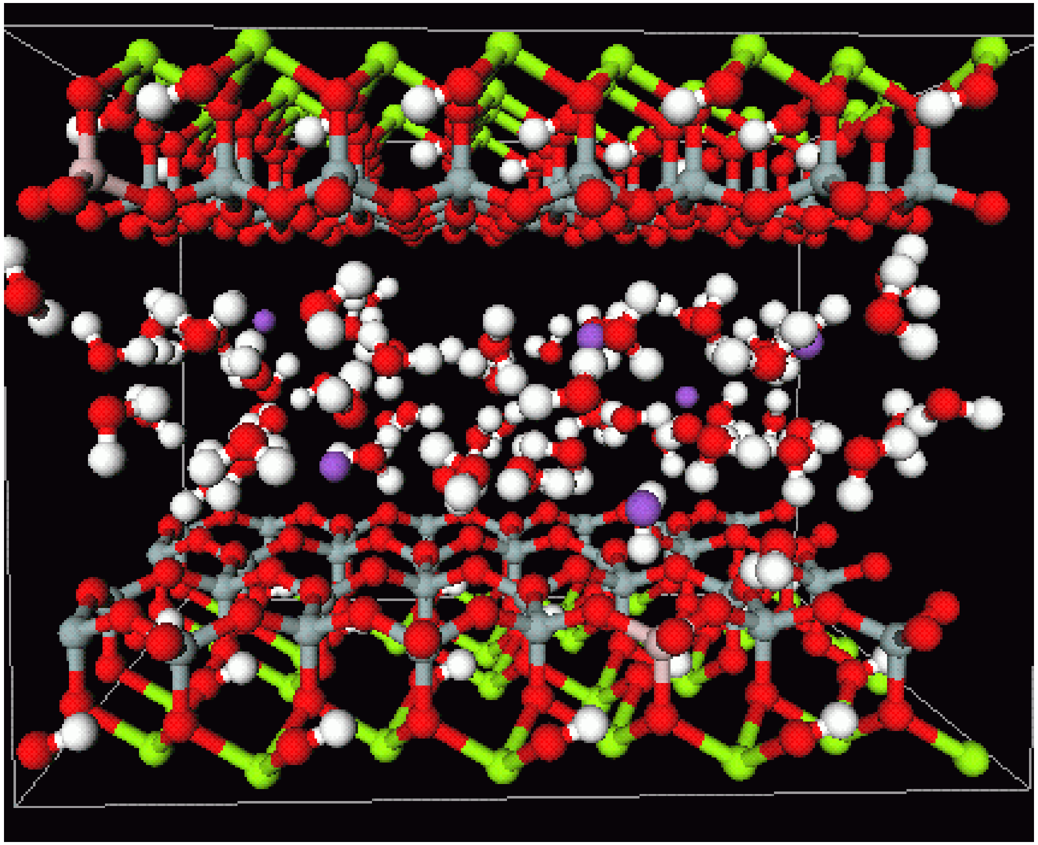
Very polar
Low membrane permeability
Low chemical reactivity

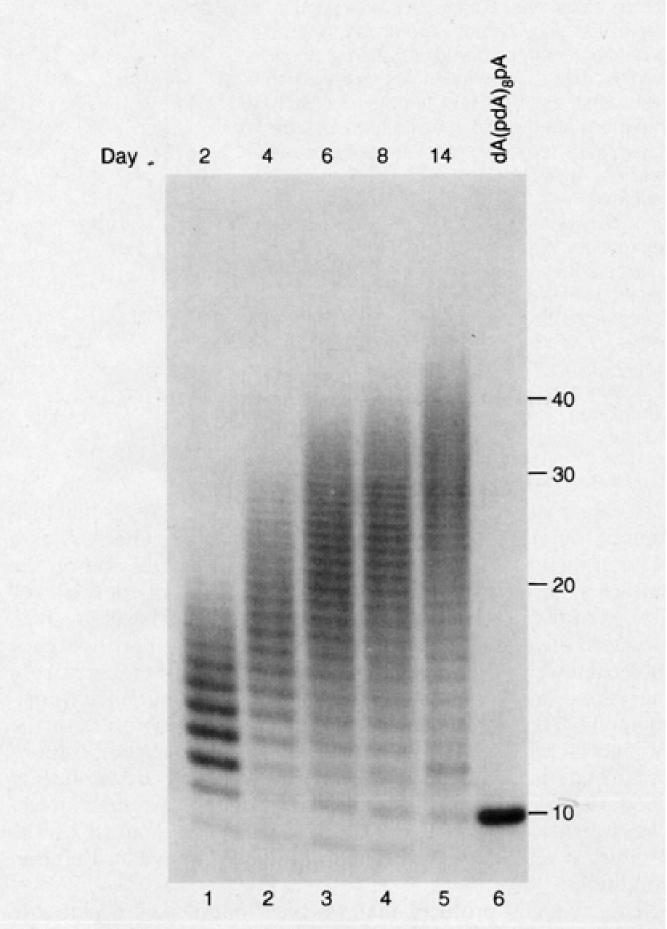
Nucleoside phosphorimidazolides



Less polar
High membrane permeability
High chemical reactivity
(faster polymerization, hydrolysis and cyclization)

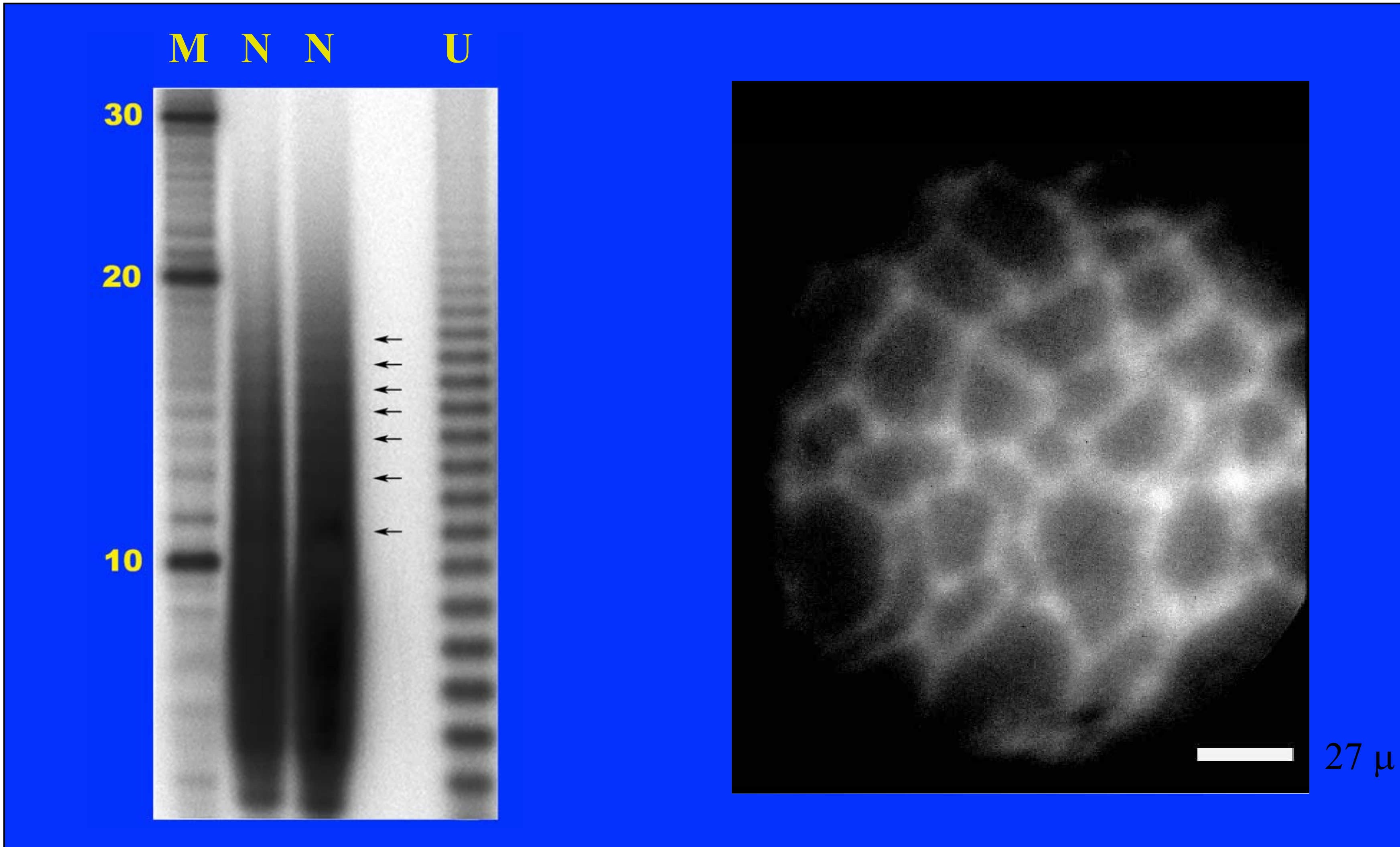
Polymerization of ImpNs on Clay





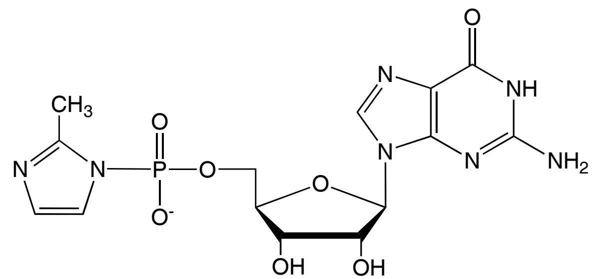
Ferris JP, Hill AR Jr, Liu R, Orgel LE. Synthesis of long prebiotic oligomers on mineral surfaces. Nature. 1996 May 2;381(6577):59-61.

Polymerization of ImpNs in an Ice Eutectic Phase



Kanavarioti A, Monnard PA, Deamer DW. Astrobiology. 2001;1(3):271-81.

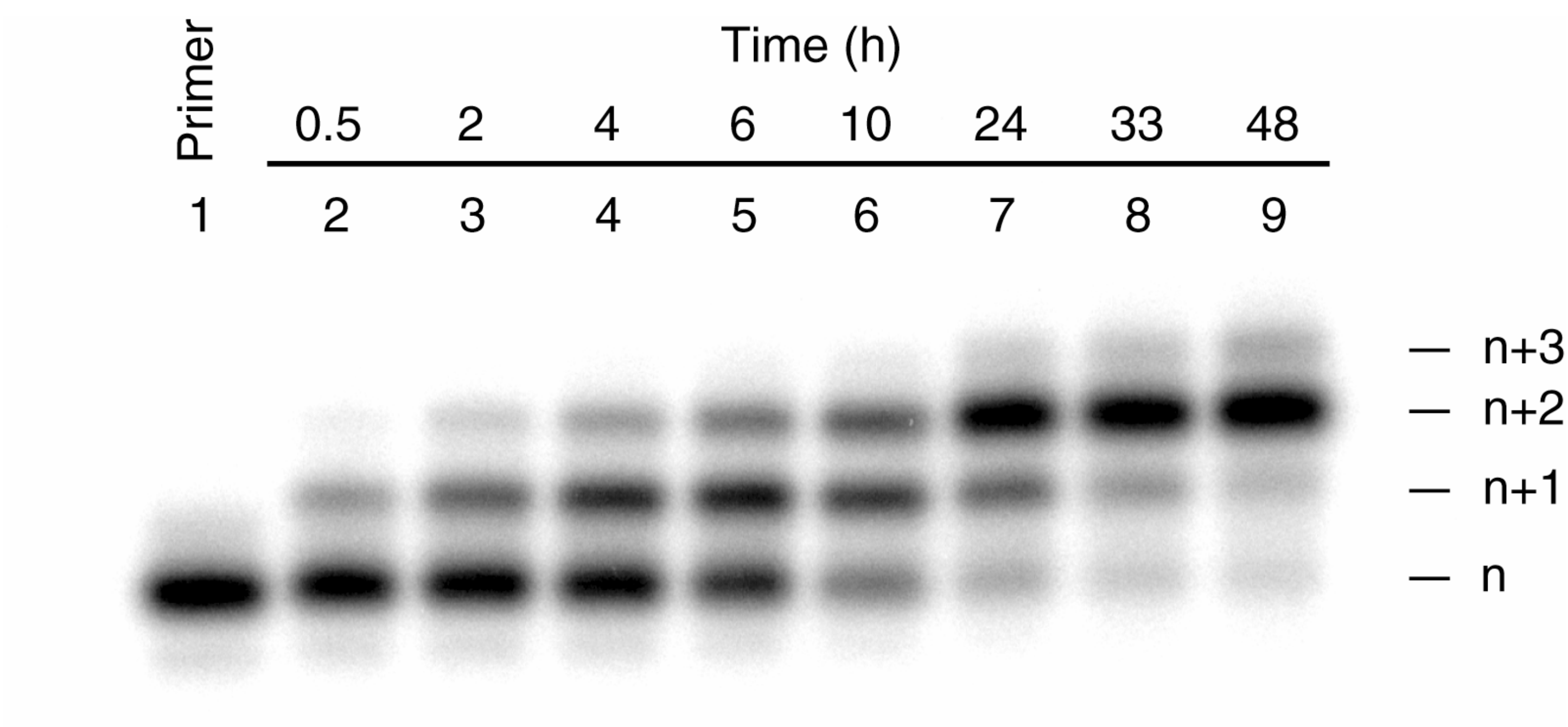
Spontaneous primer-extension



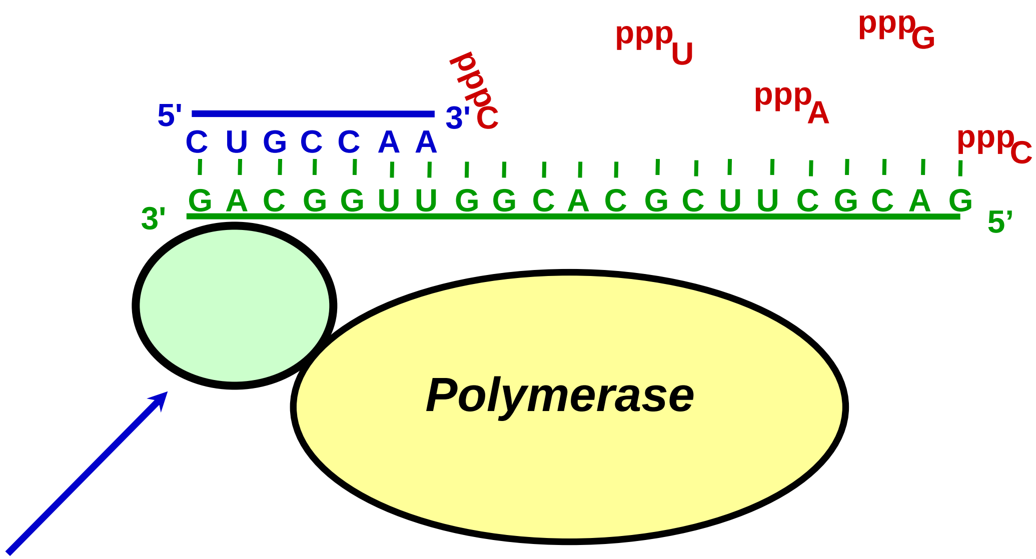
5'-r-GCUGCCAGUG
3'-d-CGACGGTCAC-C-CCTTGAG

2-MeImpG → $MgCl_2$

5'-r-GCUGCCAGUG-**GGG**
3'-d-CGACGGTCAC-C-CCTTGAG



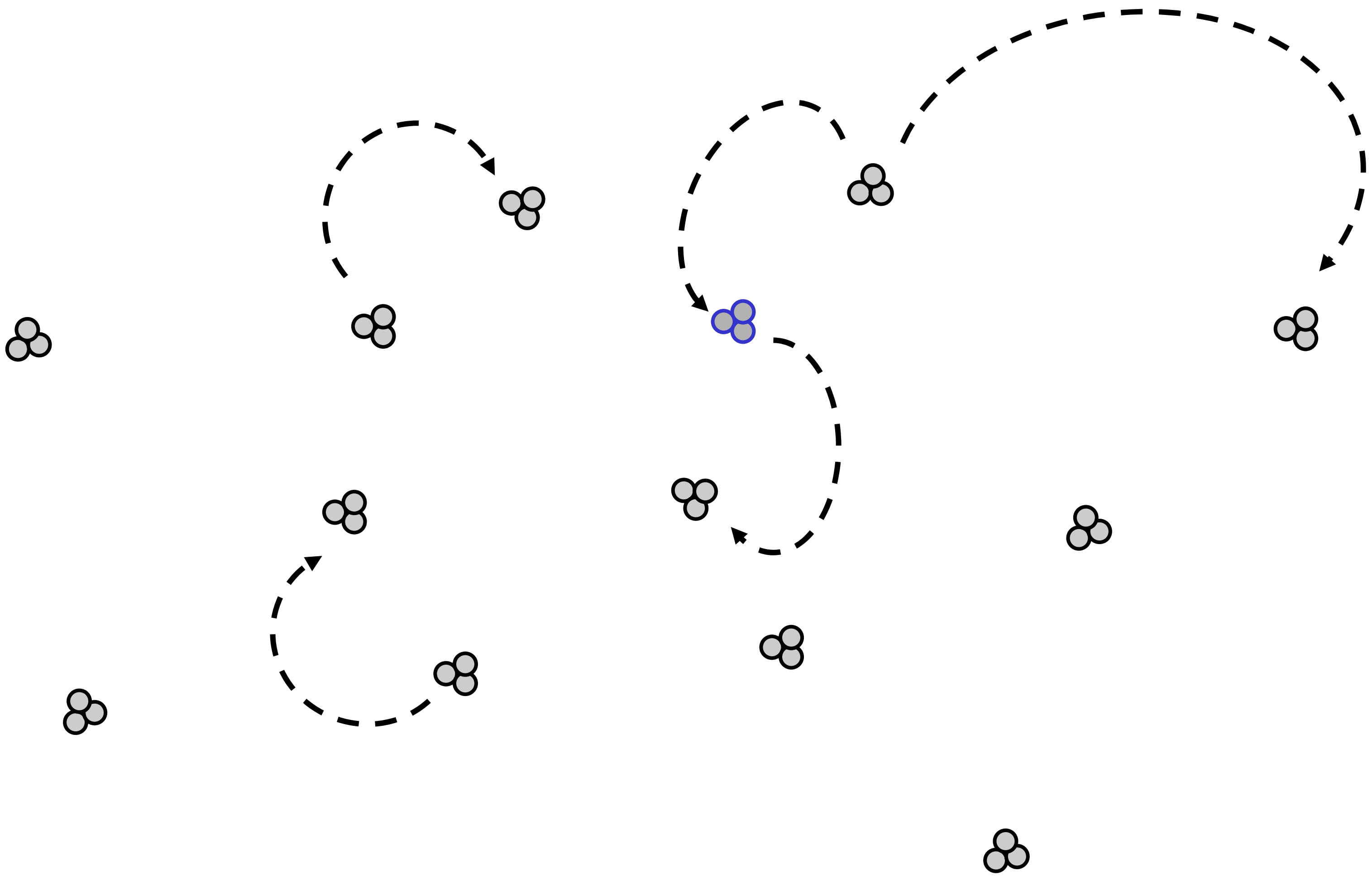
Current state-of-the-art in RNA-catalyzed RNA replication: A 2-domain ribozyme polymerase from the Bartel lab.



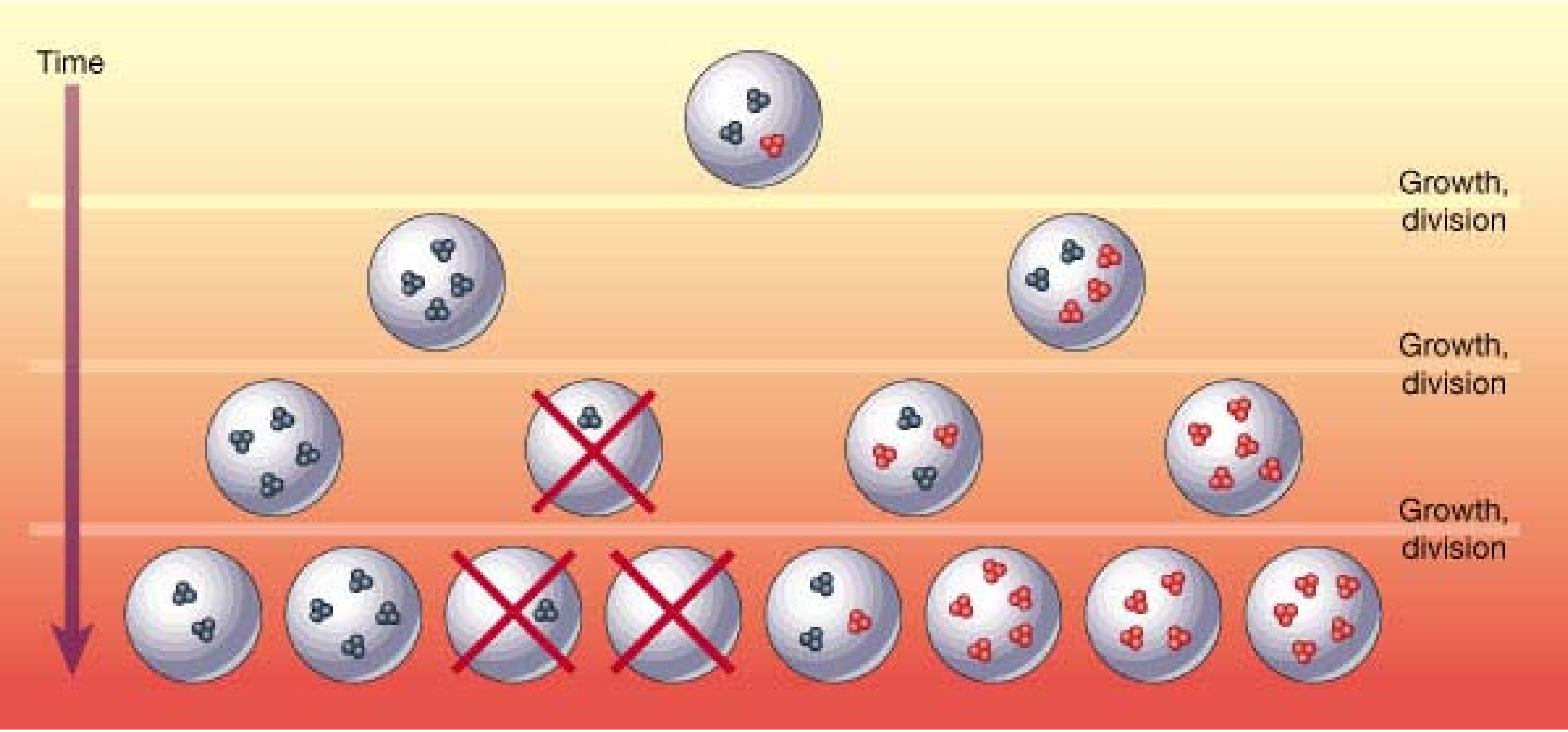
RNA-binding domain

W. K. Johnston, P. J. Unrau, M. S. Lawrence, M. E. Glasner, D. P. Bartel. RNA-Catalyzed RNA Polymerization: Accurate and General RNA-Templated Primer Extension. Science, 2001. 292-1319.

Replicases copying random templates in solution



Random segregation within compartments

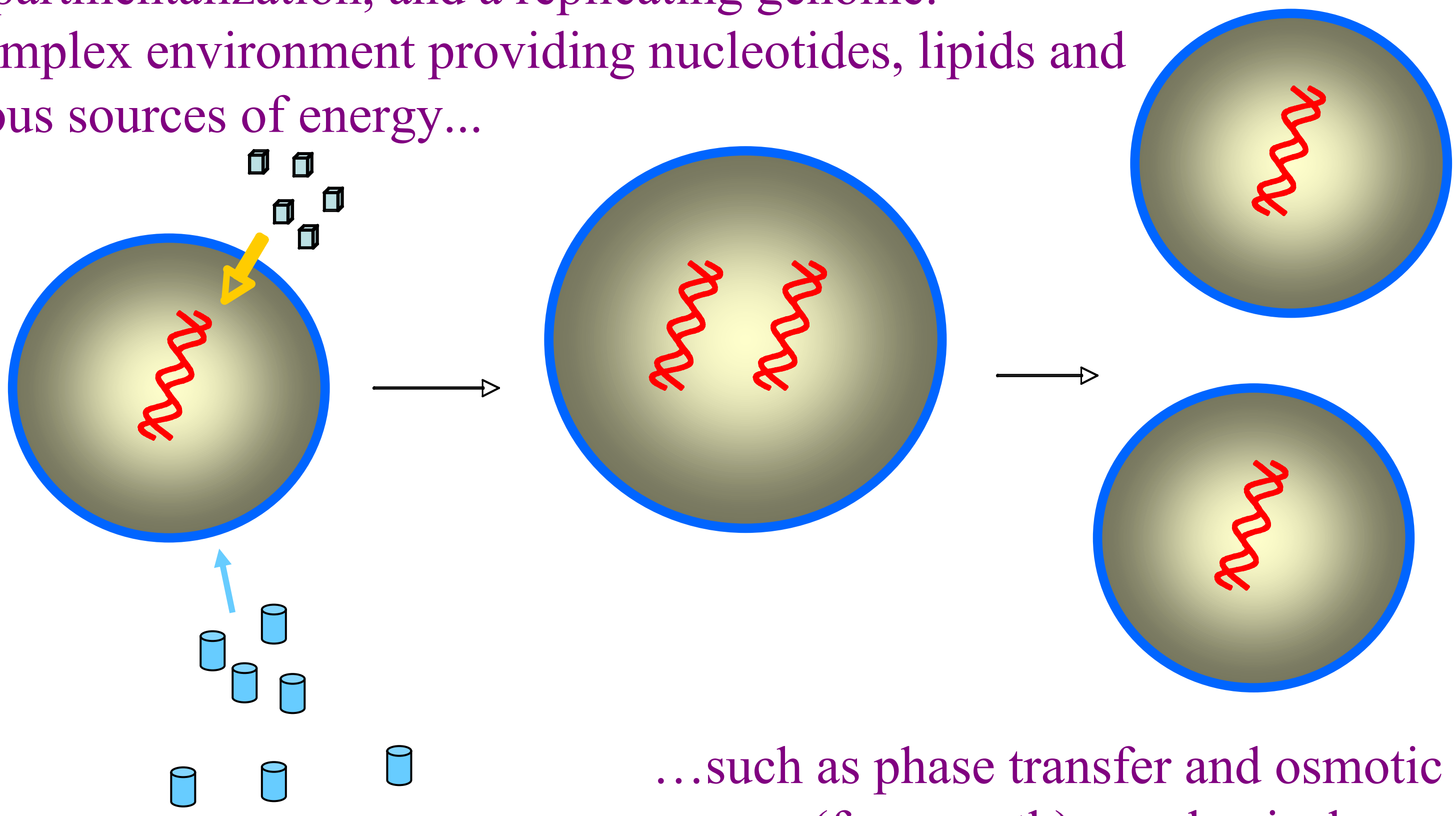


from Szostak, Bartel, and Luisi. Synthesizing life. Nature, 2001; 409:387

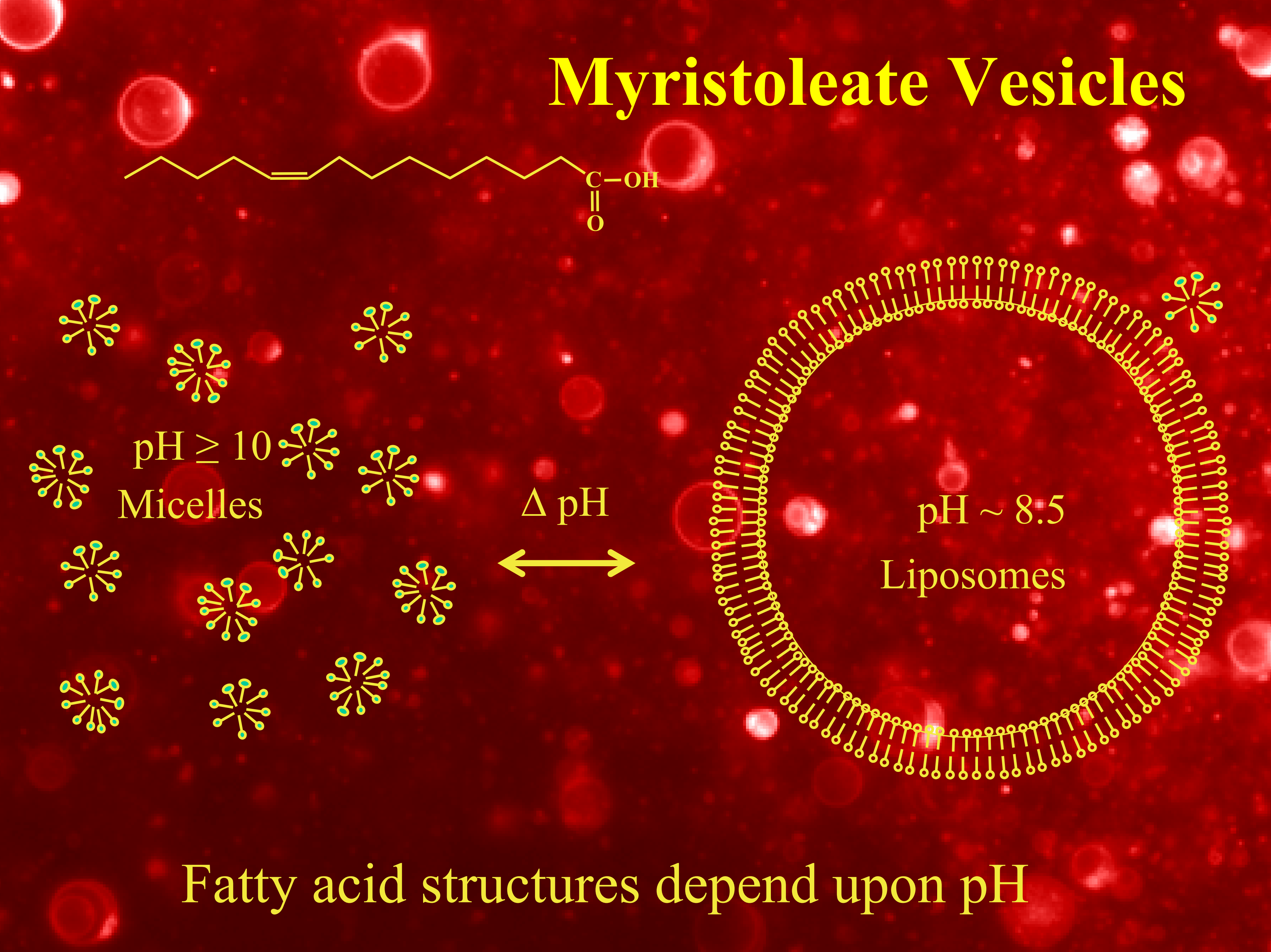
Model of Synthetic Protocell

Growth and Division; Matter and Energy Fluxes

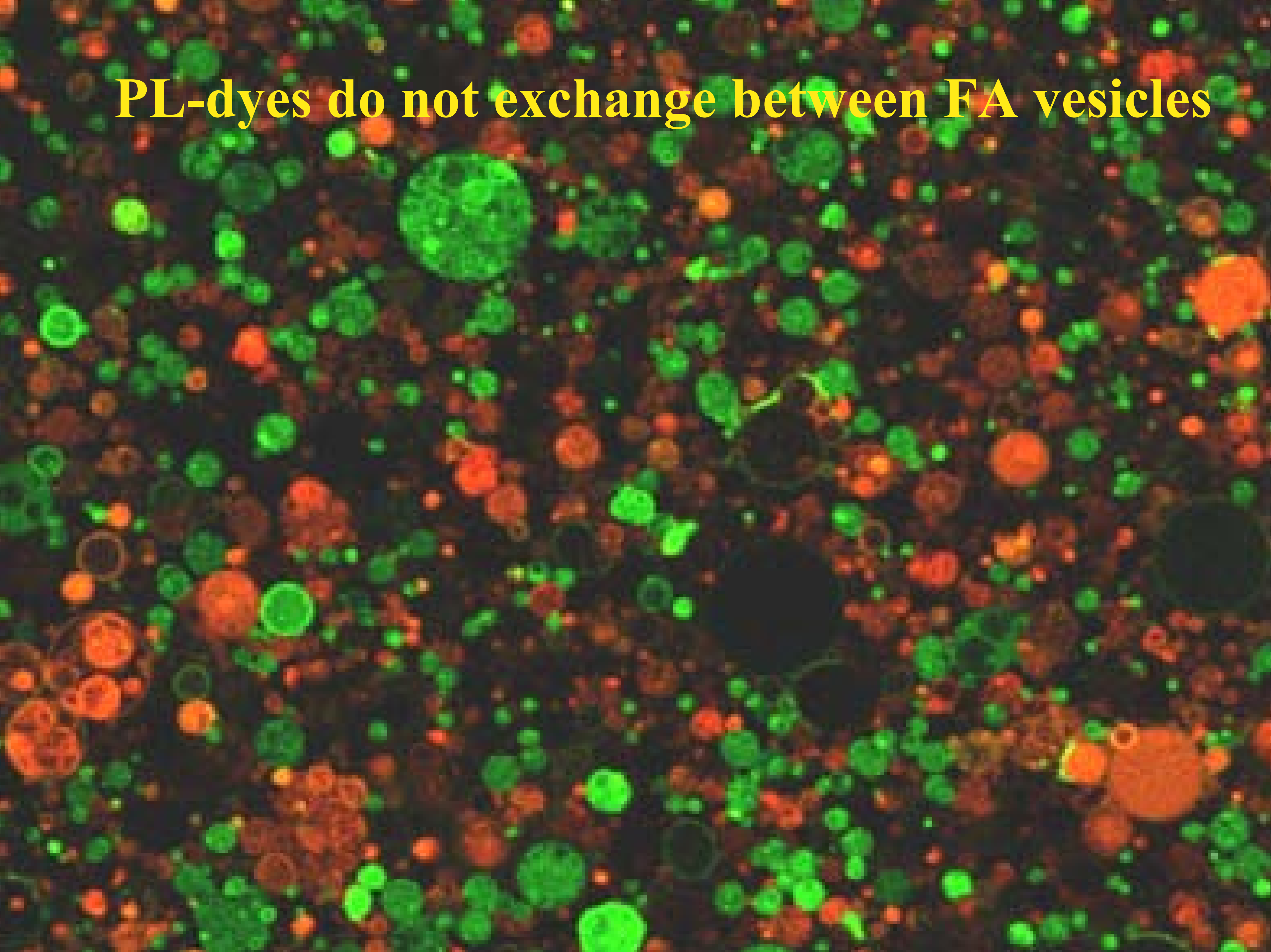
A simple cell based on a replicating vesicle for compartmentalization, and a replicating genome.
A complex environment providing nucleotides, lipids and various sources of energy...



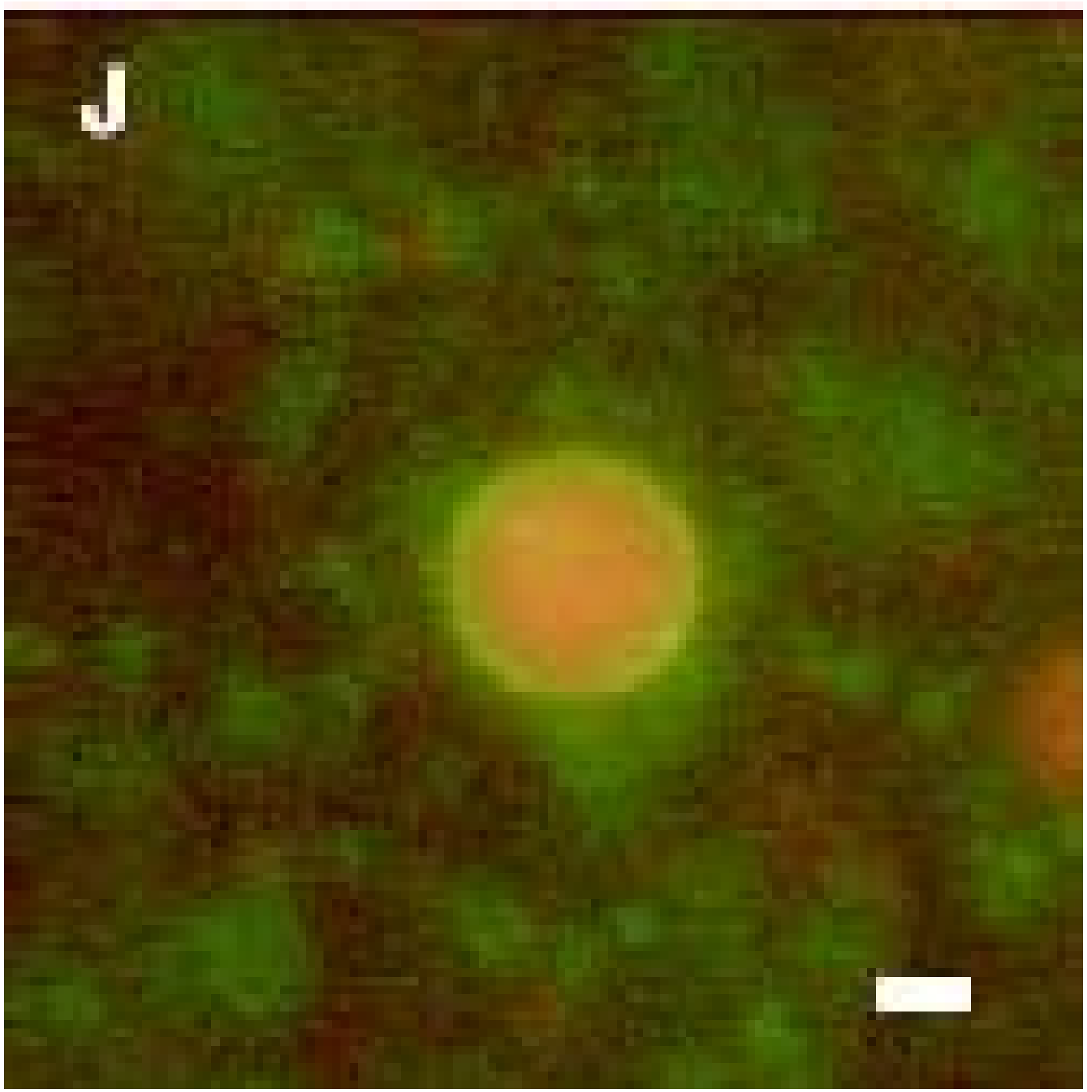
…such as phase transfer and osmotic gradient energy (for growth), mechanical energy and ion gradients (for division), and chemical energy (for nucleotide activation).


Myristoleate Vesicles
C—OH
O
pH ≥ 10
Micelles
Δ pH
pH ~ 8.5
Liposomes
Fatty acid structures depend upon pH

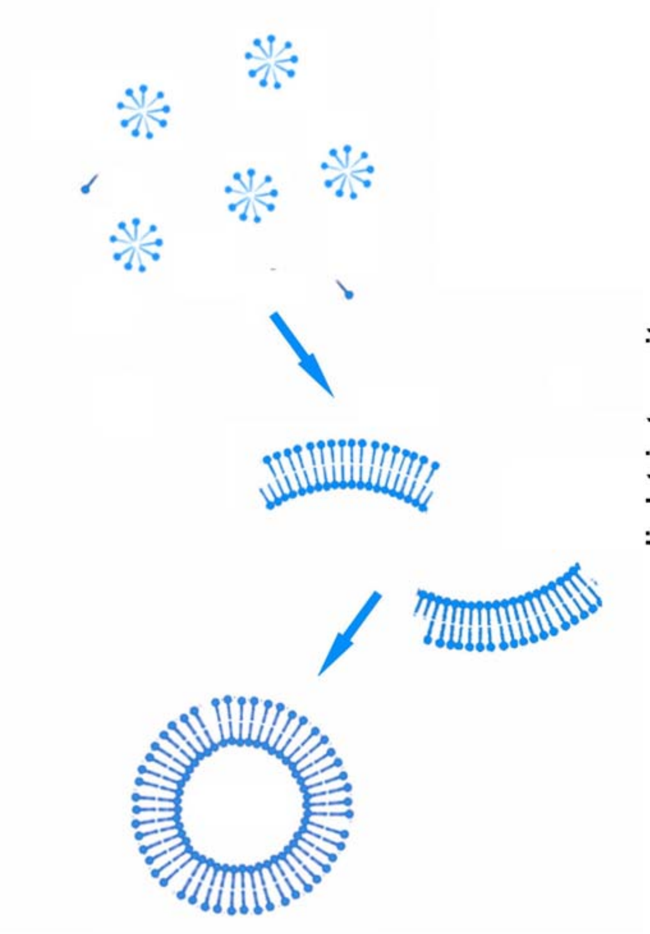
PL-dyes do not exchange between FA vesicles

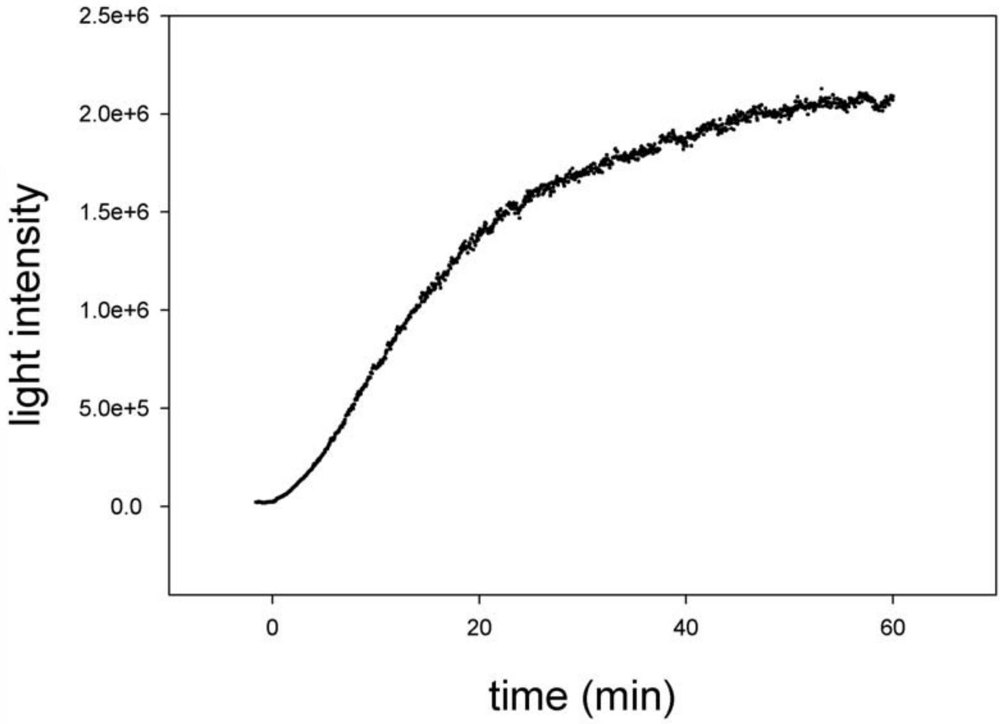


Myristoleate Vesicles Retain Encapsulated RNA



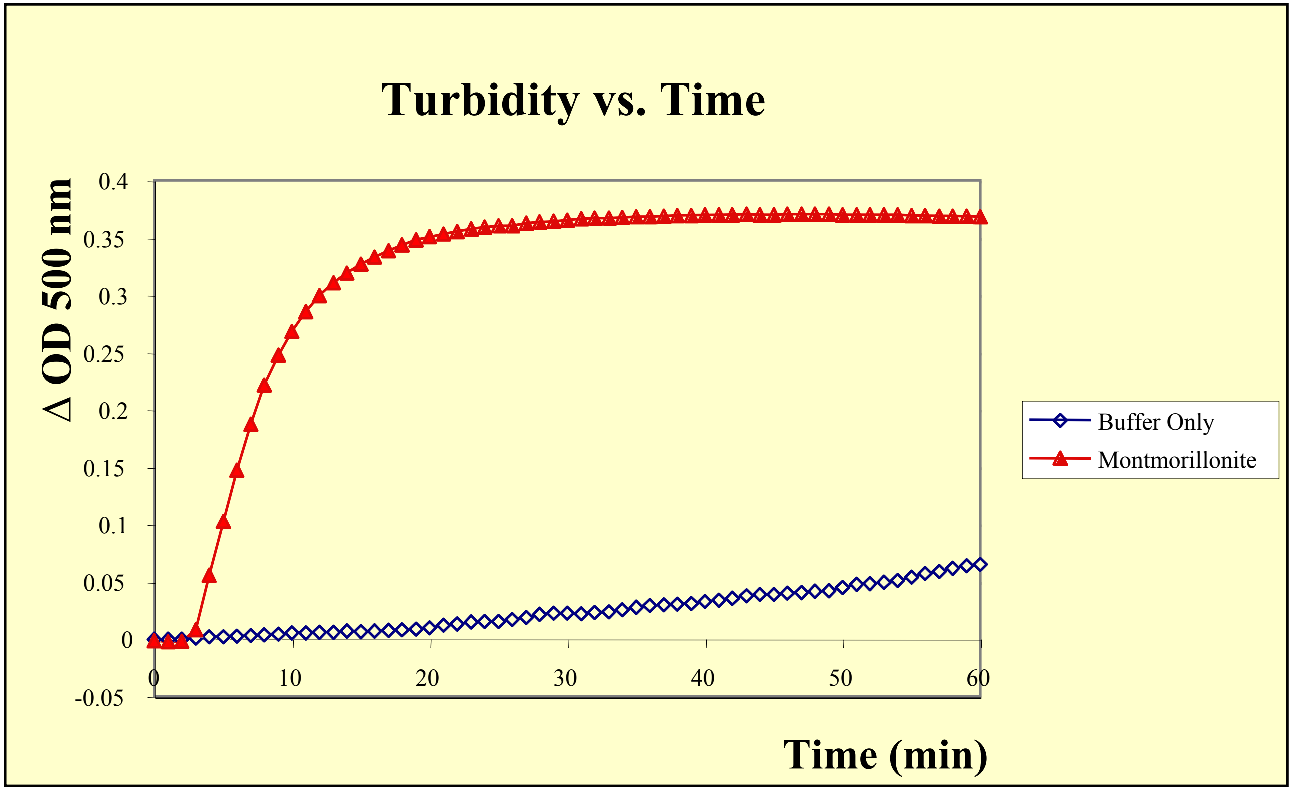
Spontaneous Assembly of Oleate Vesicles





Montmorillonite Clay Accelerates Membrane Formation from Micelles

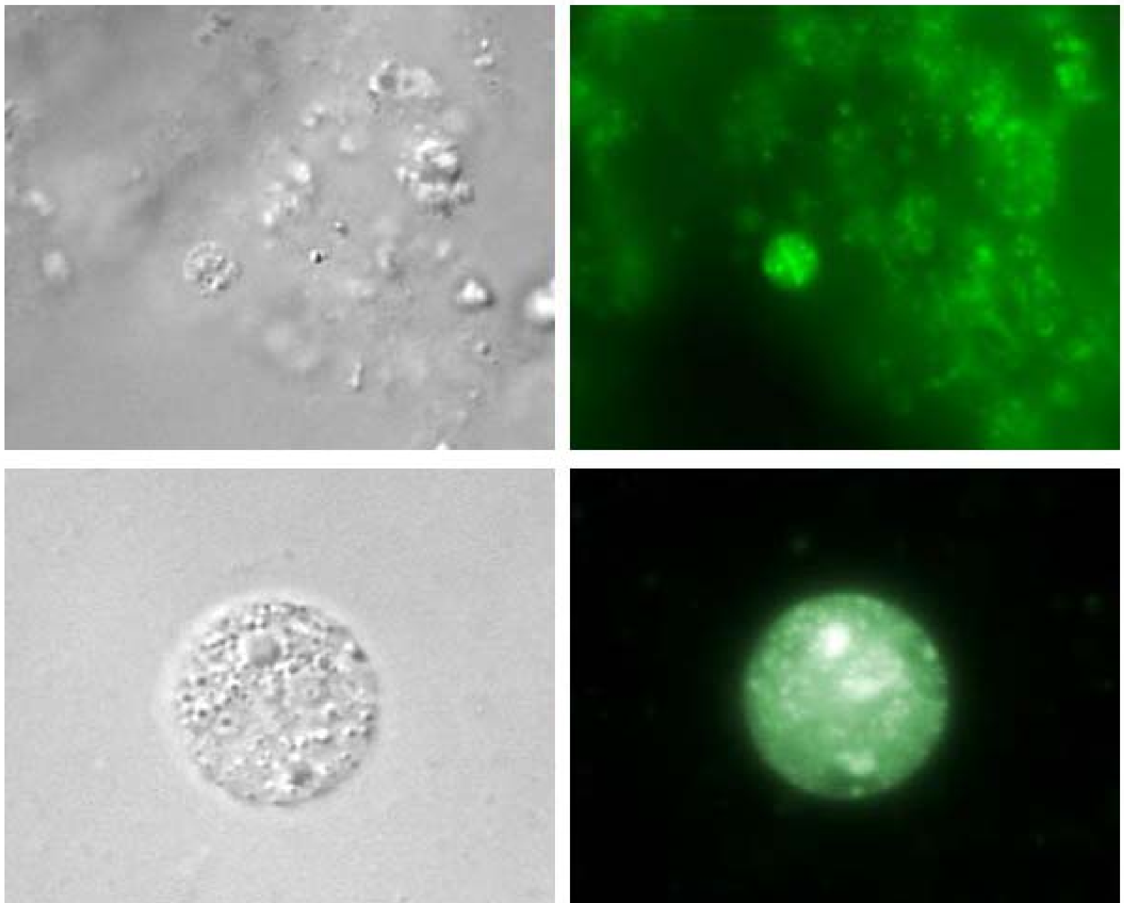
Since oligonucleotides can be synthesized on clay surfaces, clay-catalyzed vesicle assembly provides a direct path for the incorporation of oligos into vesicles



0.2M Na^+-bicine, pH 8.5, 25^{o}C; +/- 0.05mg/mL Na^+-montmorillonite; 10mM myristoleate added after 2mins.

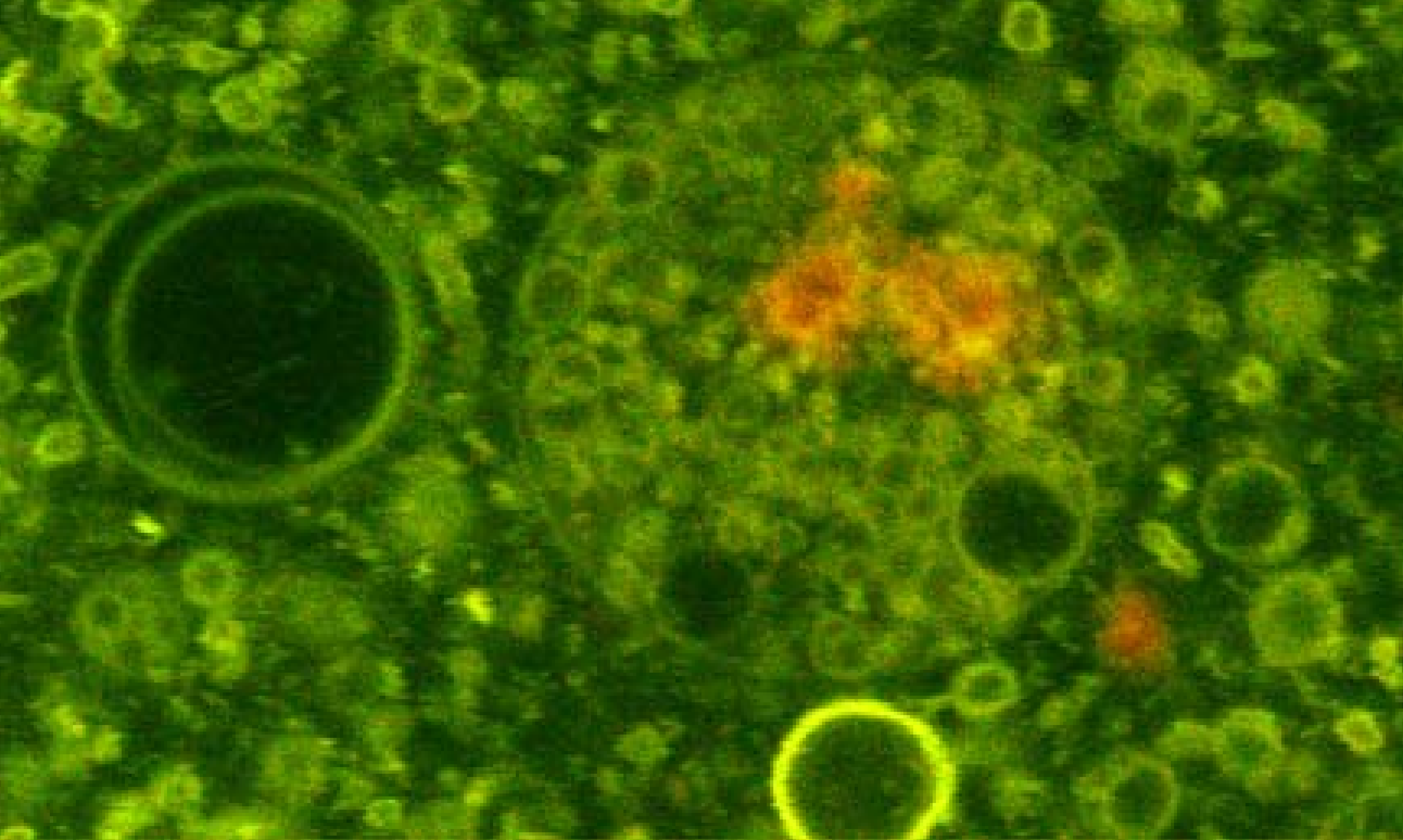
Montmorillonite become Encapsulated in Liposomes

Clay:Nomarski Optics

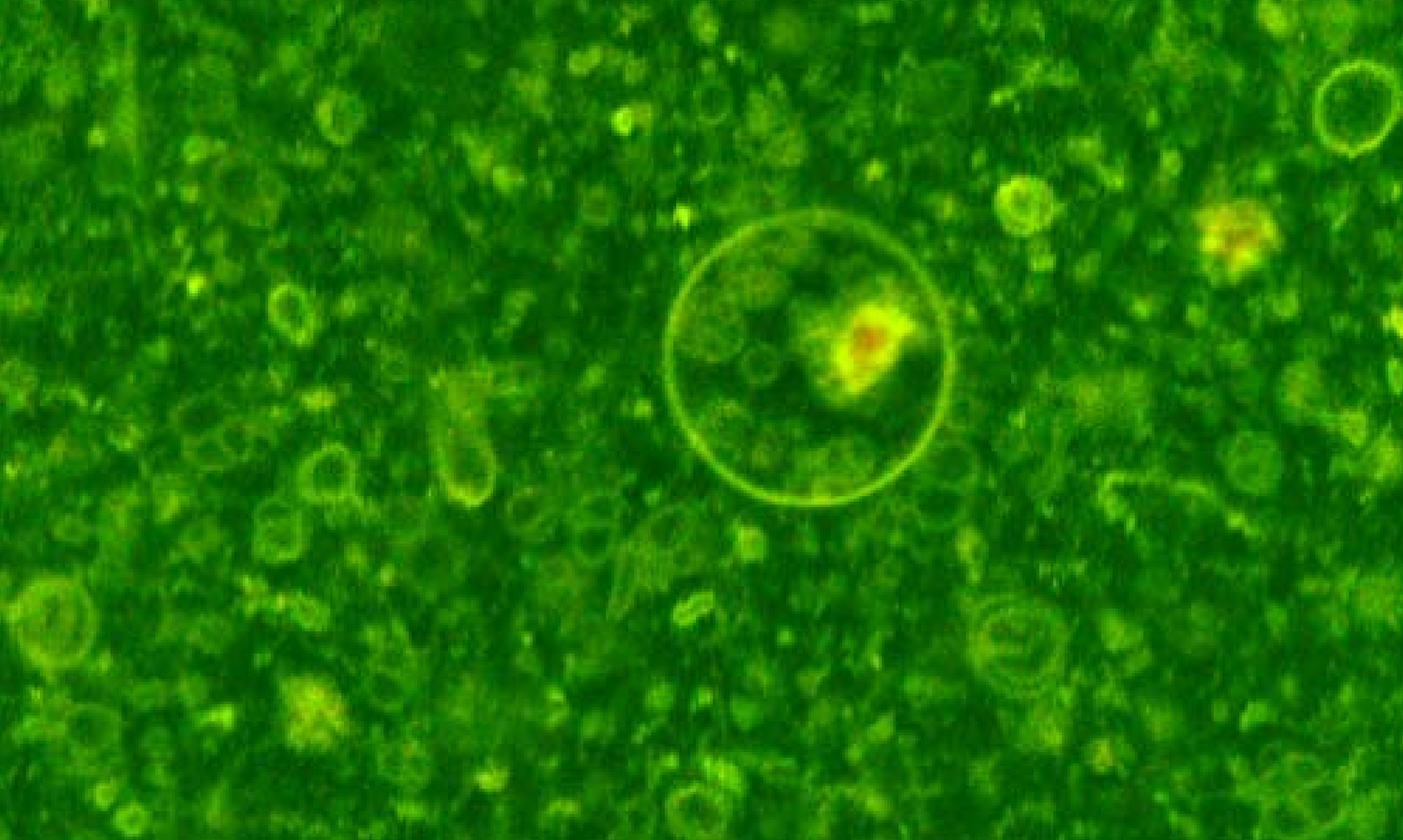


Vesicles; Fluorescence Filter

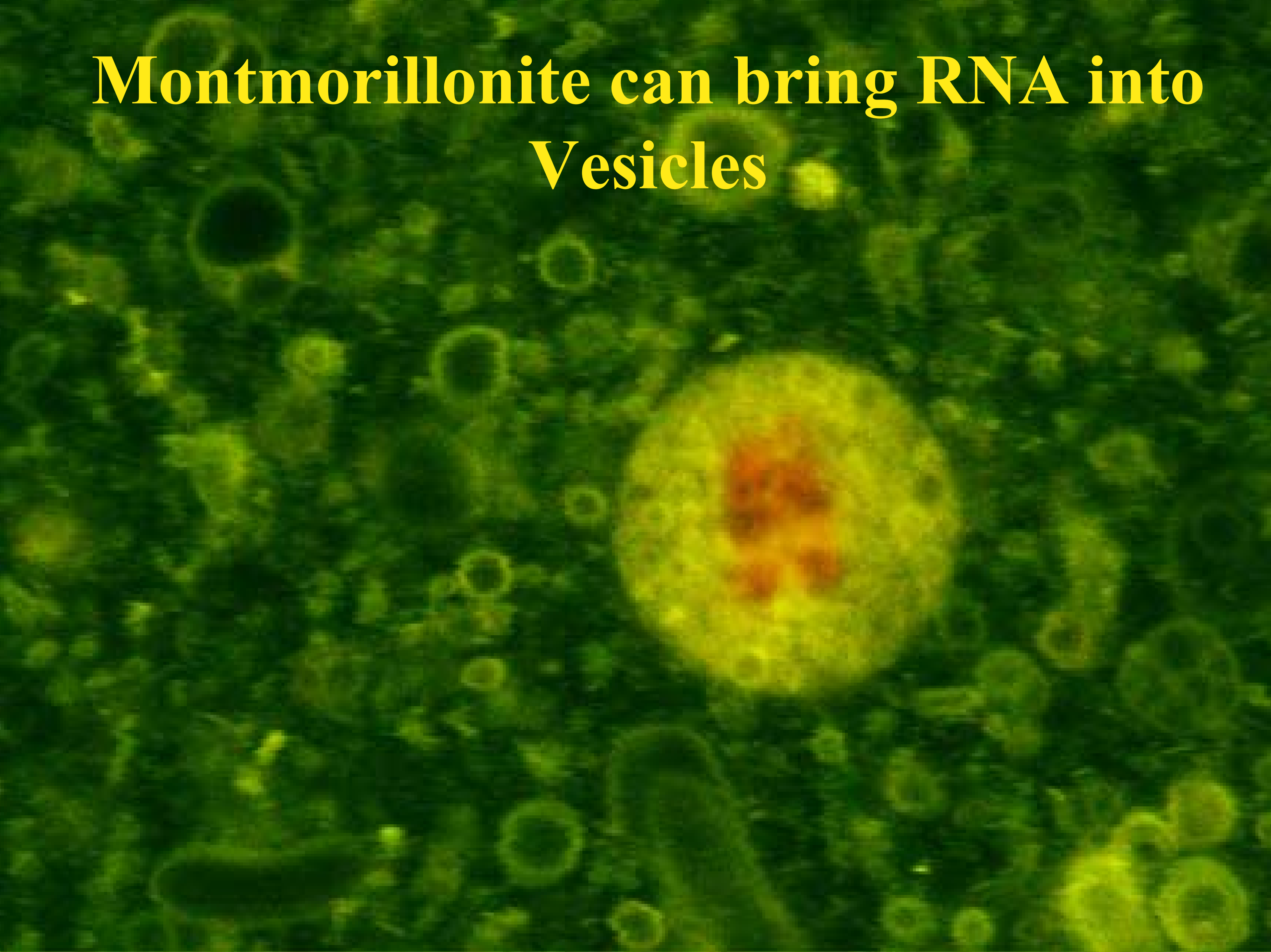
Montmorillonite can bring RNA into Vesicles



Montmorillonite can bring RNA into Vesicles



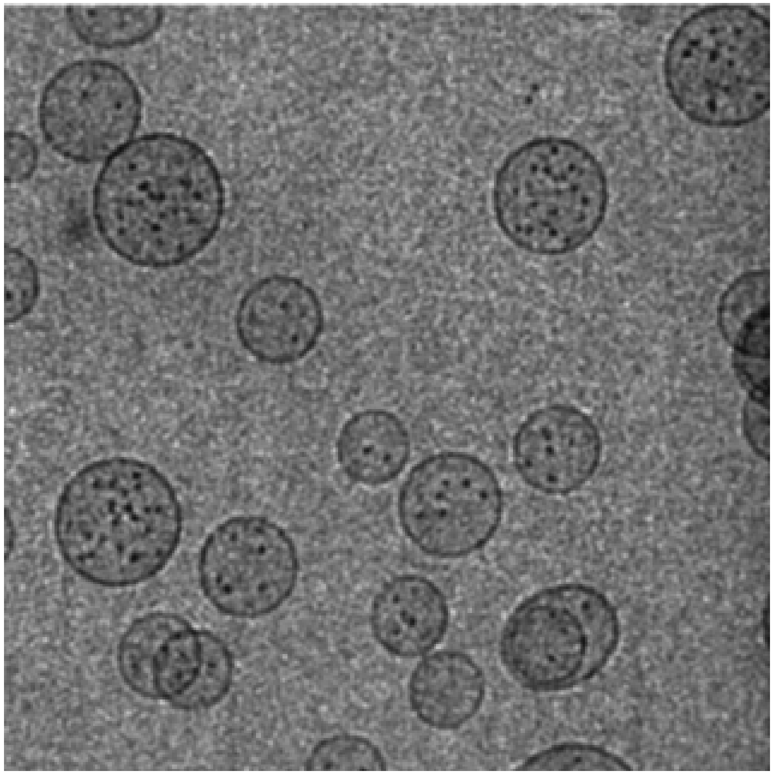
Montmorillonite can bring RNA into Vesicles

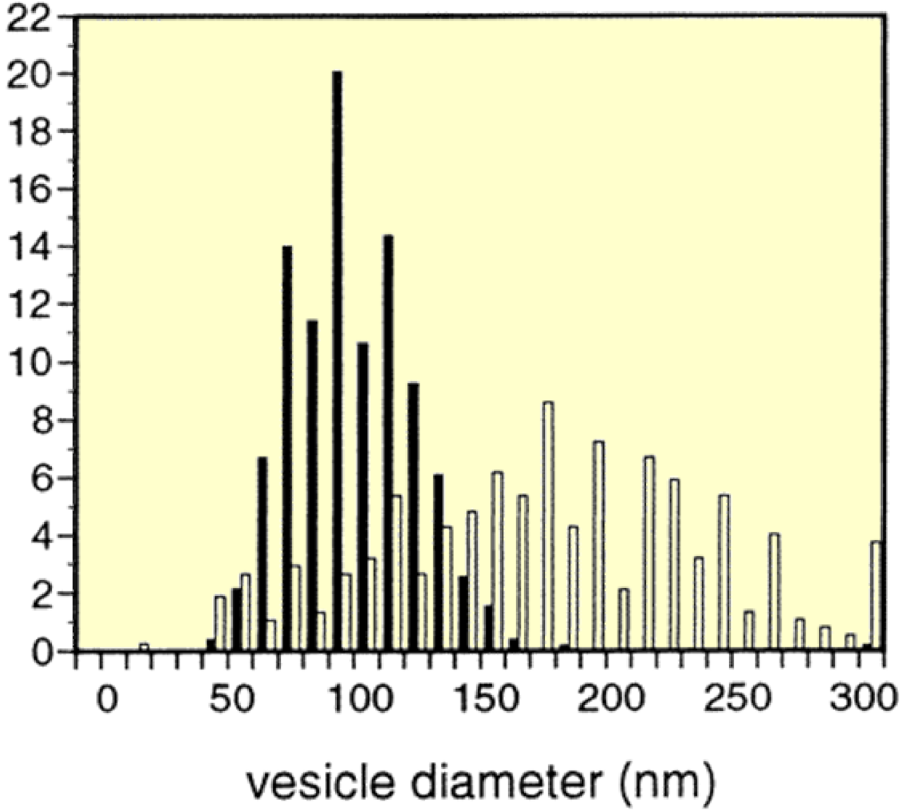


Spontaneous Vesicle Growth

was first demonstrated by the Luisi lab in the ETH, Zurich, Switzerland

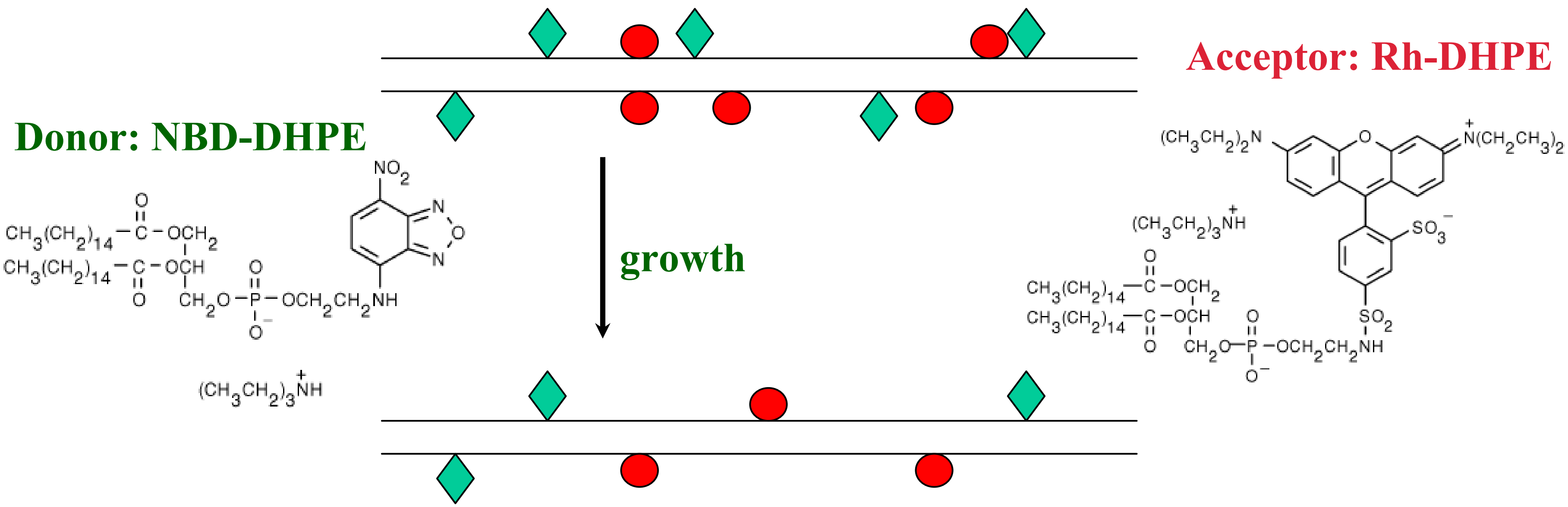
Addition of 5 mM oleate to 0.2 mM POPC vesicles





Berclaz, Blochliger, Walde and Luisi, 2001, J. Phys. Chem. B 105:1056-1064.

A FRET-based assay for lipid incorporation

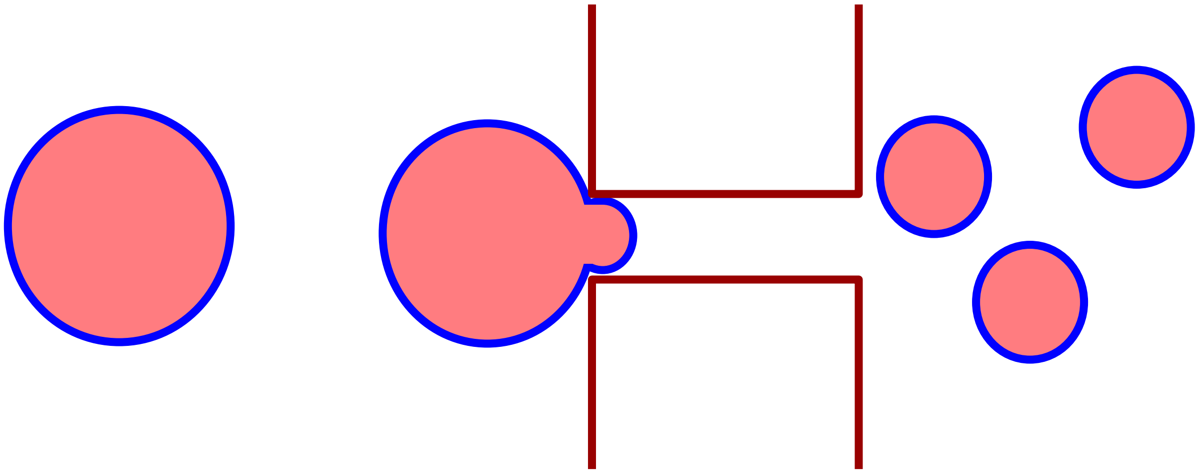


Addition of 1 equivalent of micelles over 4 hours

	F_{don}/F_{acc} (norm)	mol % dye	relative area
control	0.1243	0.093	1.0
grown/divided	0.1725	0.055	1.88

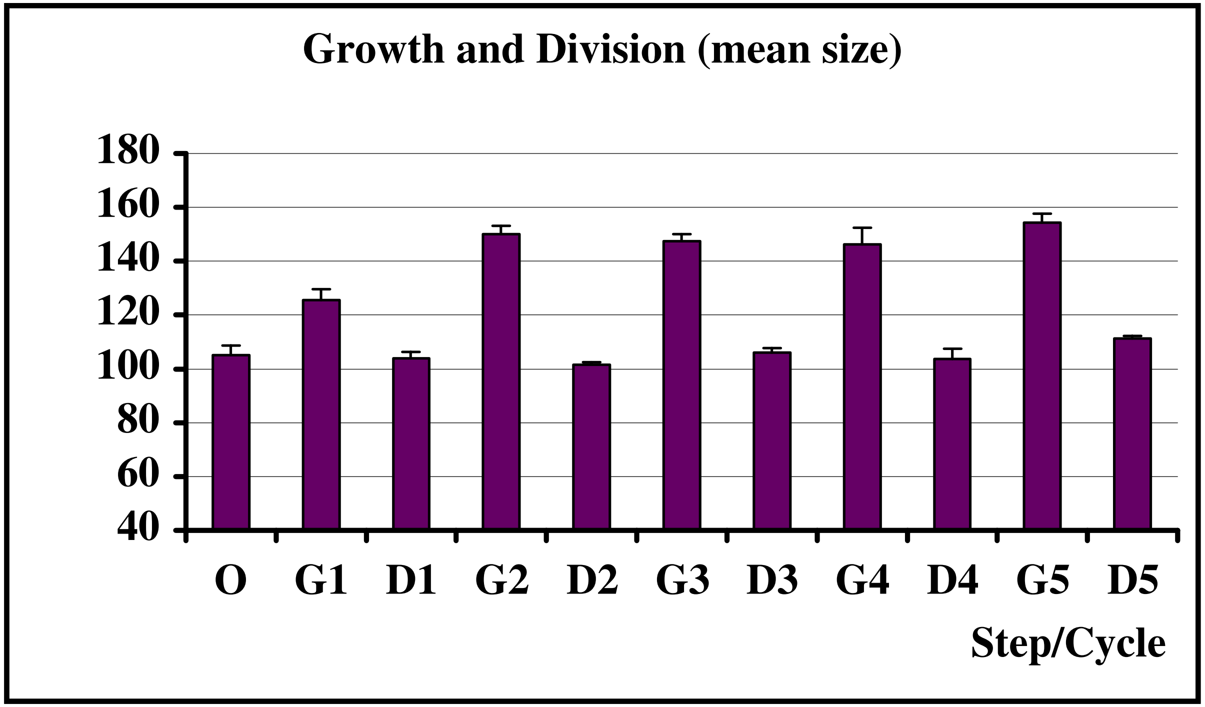
Division by Extrusion

Input of mechanical energy by pore-extrusion causes division



Some contents are lost - but there is little if any dilution

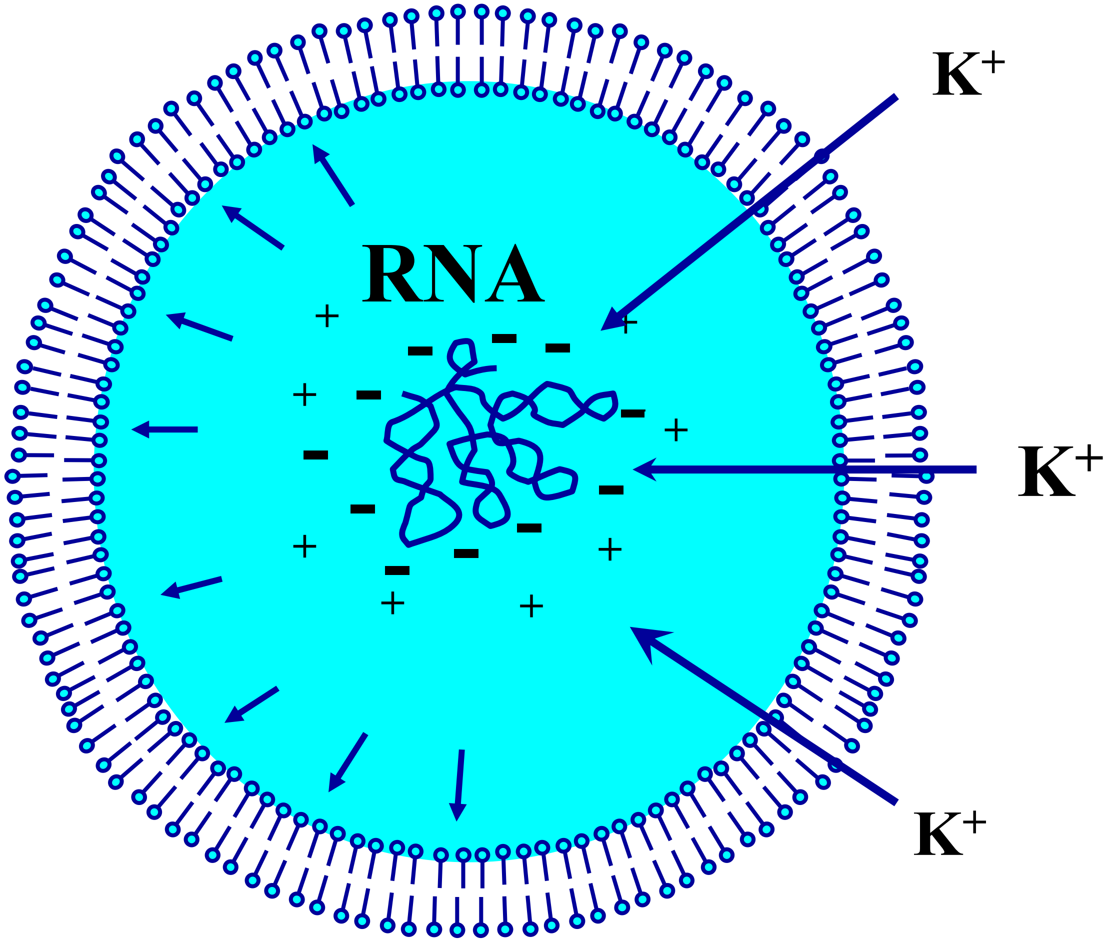
Cycles of growth and division



Darwinian evolution at the cellular level: Vesicle Competition

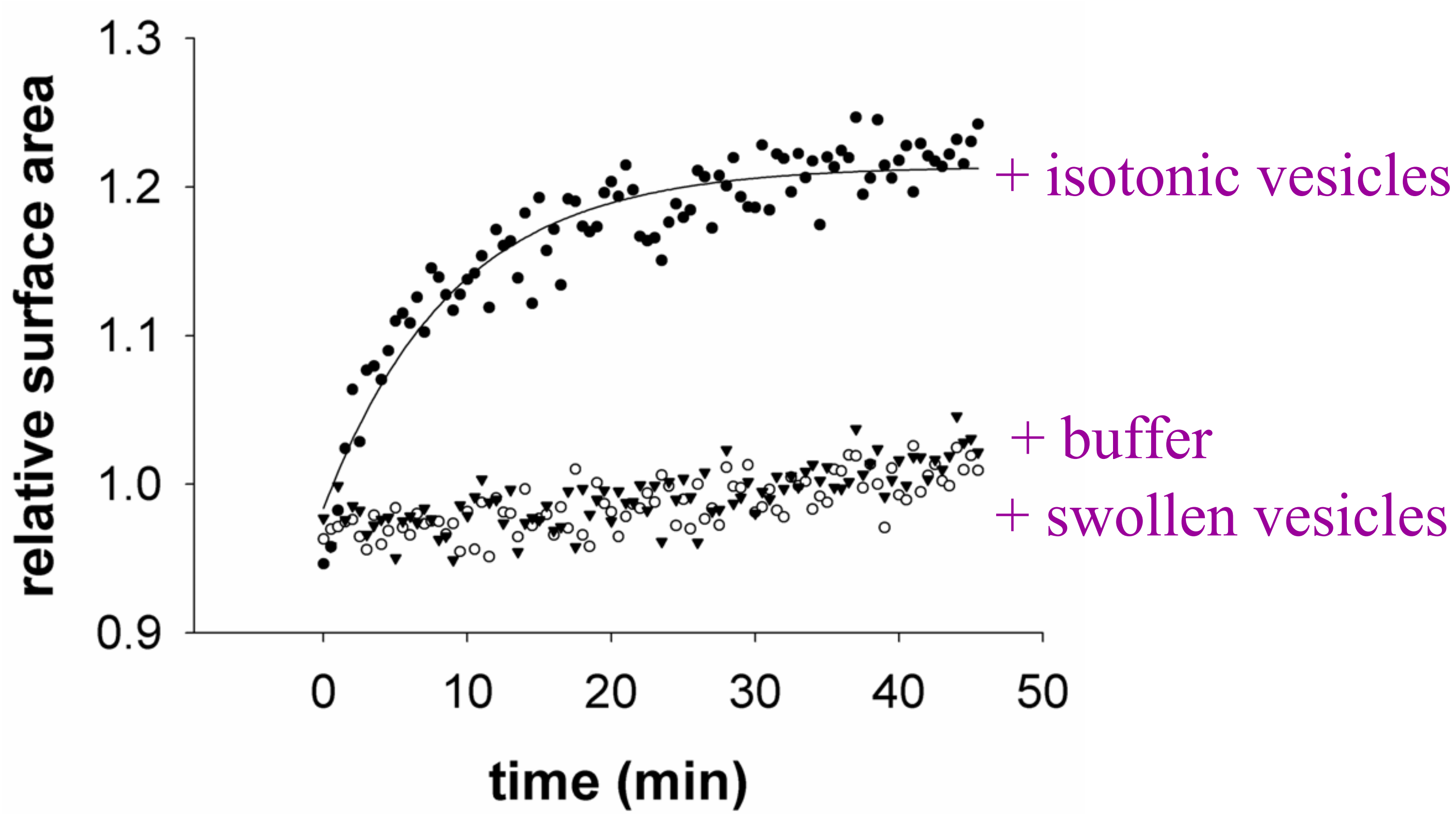
Osmotic Pressure: A link between genome replication and vesicle growth?

Internal osmotic pressure tensions and stretches the membrane, exposing hydrophobic surface area, and favoring the capture of new lipid molecules.



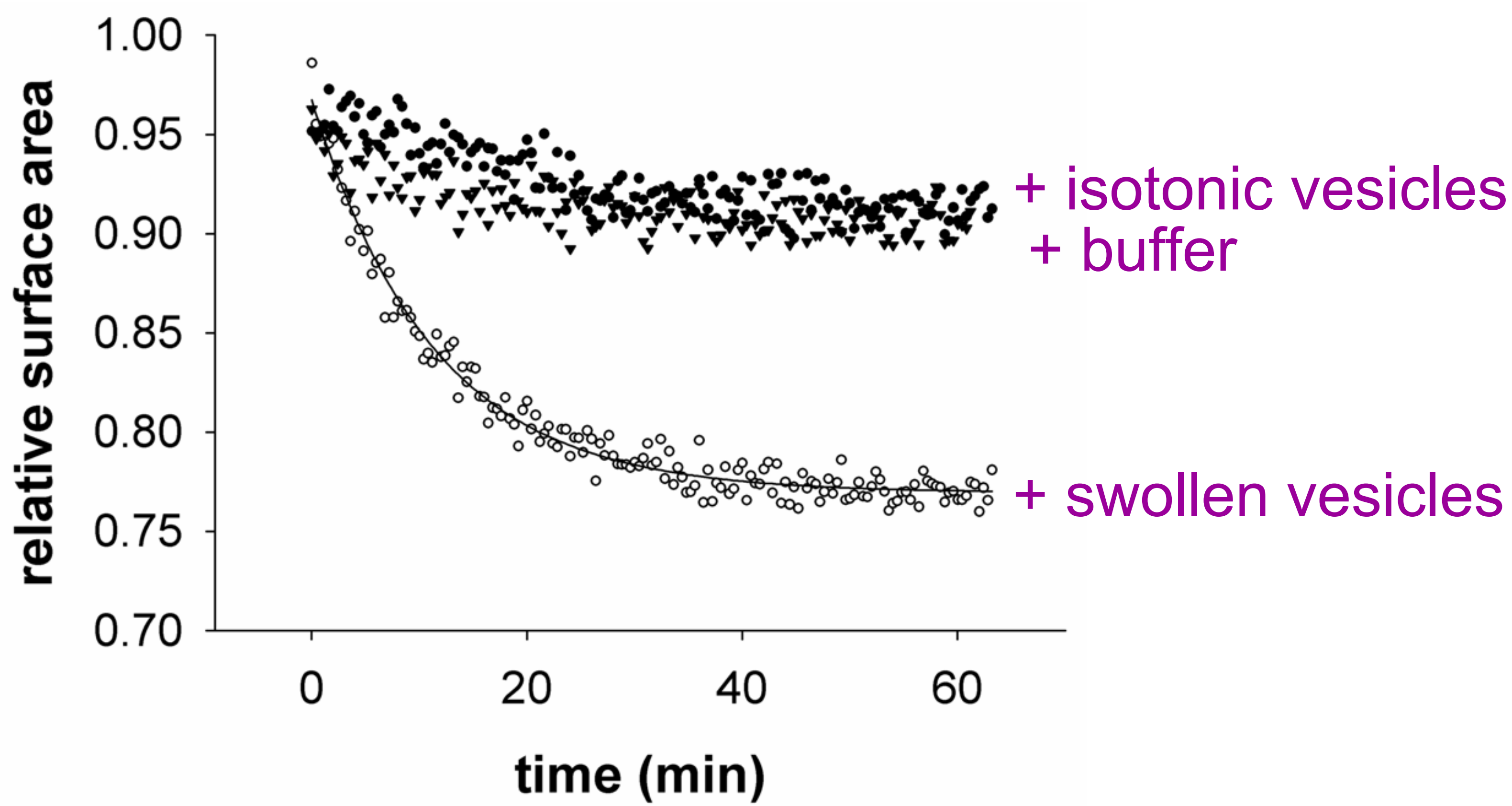
Vesicle competition

Swollen vesicles labeled



Osmolyte: tRNA membrane: MA-GMM.

Vesicle competition
Isotonic vesicles labeled



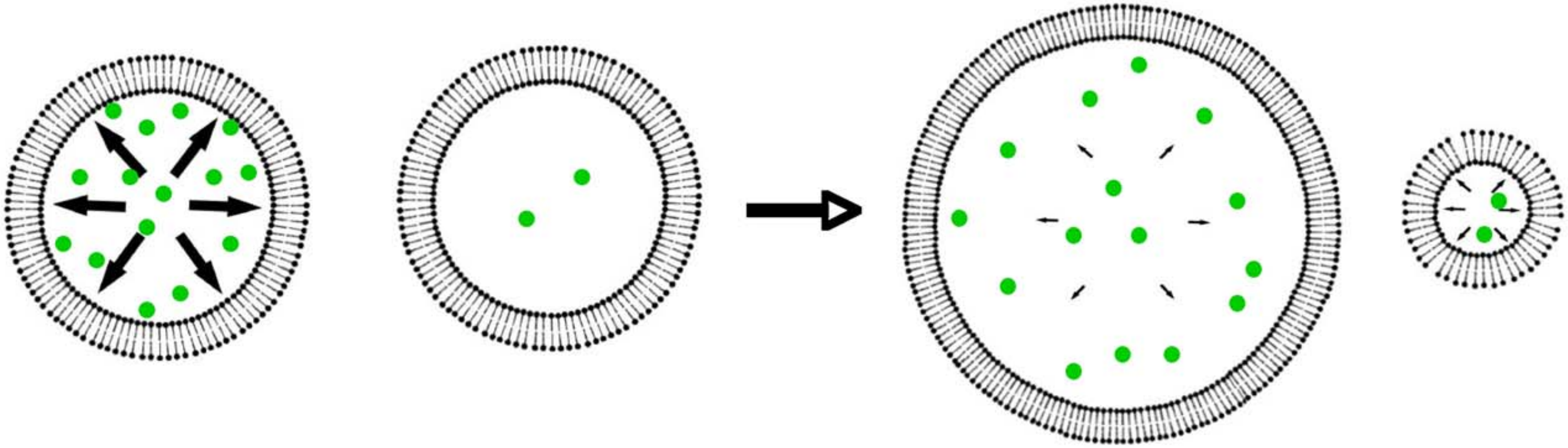
Osmolyte: tRNA membrane: MA-GMM.

Osmotically Driven Membrane Growth

High Internal Concentration of Nucleic Acid

↓

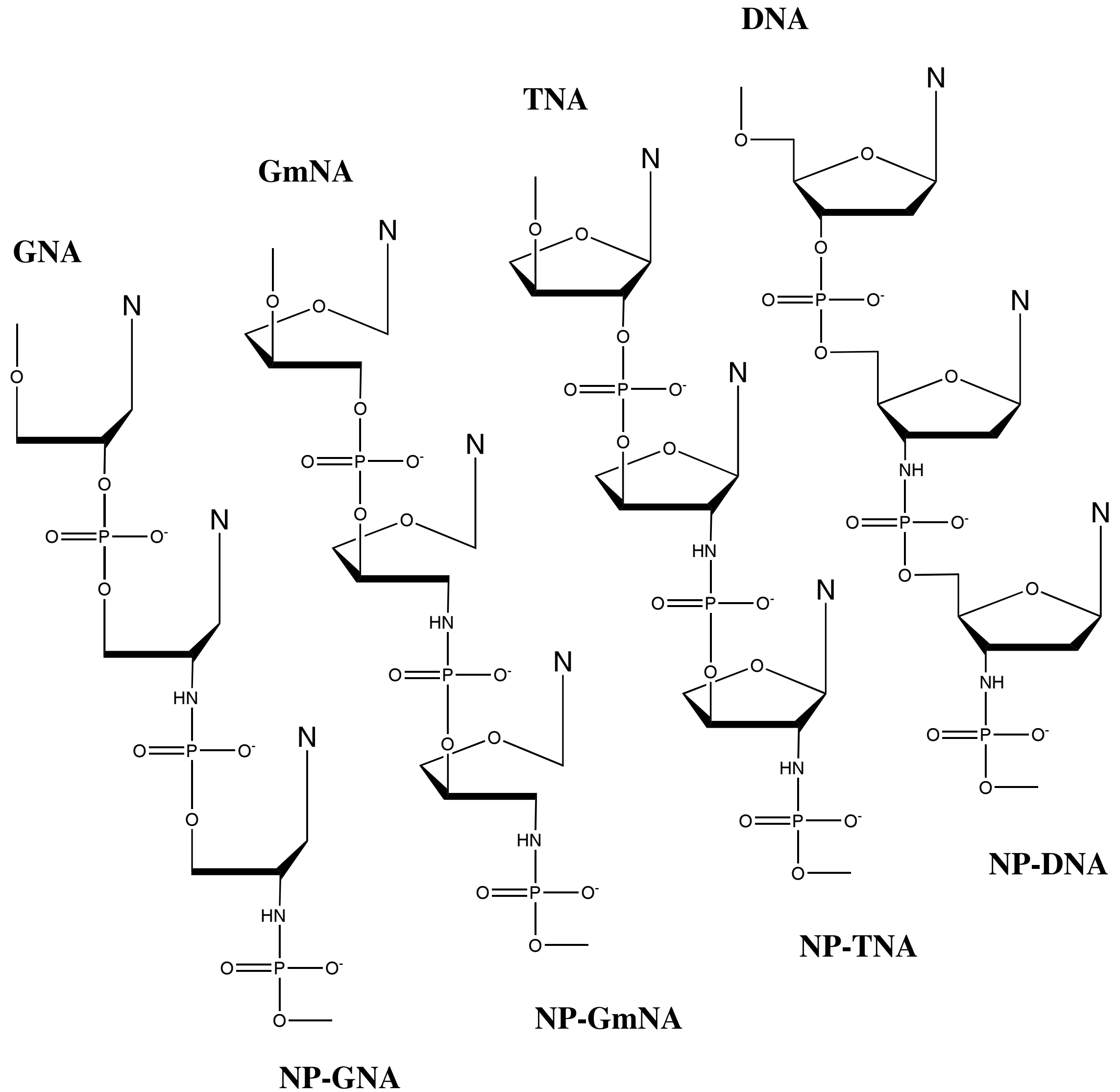
Faster Membrane Growth



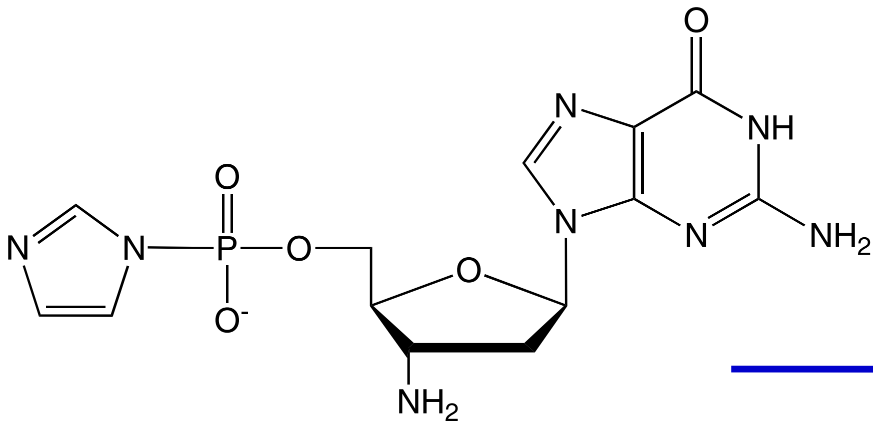
Osmotic pressure creates membrane tension…

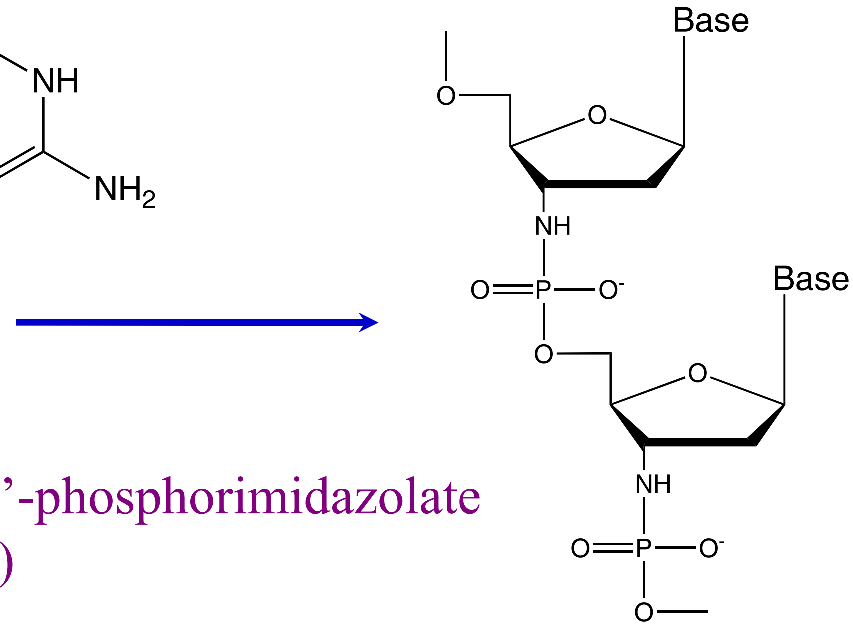
…which relaxes as the membrane grows.

Alternative Nucleic Acid Backbones



Phosphoramidate DNA





3’-amino-2’,3’-dideoxy-guanosine-5’-phosphorimidazolate
($3'NH_2$ImpdG)

- RNA-like structure: 3′ endo sugar, A-type helix
- Forms duplexes with complementary DNA, RNA and NP-DNA.
- Greater nucleophilicity of the 3’-amino vs. 3’-hydroxyl leads to faster spontaneous polymerization.
- Phosphoramidate bonds may also link other backbones (e.g. threose, glycerol, methoxyglycerol).

Summary of Progress

- Multiple pathways for vesicle growth and division
- Nucleotides can diffuse into vesicles
- Ribozymes active inside vesicles
- Chemical approach to nucleic acid replication looks promising, bypassing need for complex ribozyme

What have we learned that is relevant to the Origin of Life?

- Simple but unexpected physical phenomena may play a variety of important roles
  - Selective sugar permeability favoring ribose
  - Osmotic pressure driving vesicle growth
- Mineral particles may help to bring components together
- Simple membranes are surprisingly robust
- Energy dissipated in interesting ways
  - Chemical energy in activated nucleotides does osmotic work
  - Phase transition energy in membrane growth leads to pH gradients

Acknowledgements

Vesicle Replication

Sheref Mansy
Raphael Bruckner
Ting Zhu
M. Heymann
Shelly Fujikawa
Martin Hanczyc
Irene Chen
Michael Sacerdote

Support

HHMI
NASA
NSF

Nucleic Acids

Doug Treco
M. Krishnamurthy
Sylvia Tobé
Mui Sam
Jesse Chen
A. Ricardo
David Horning
John Chaput

Larry McLaughlin
Allen Horhota
Meena