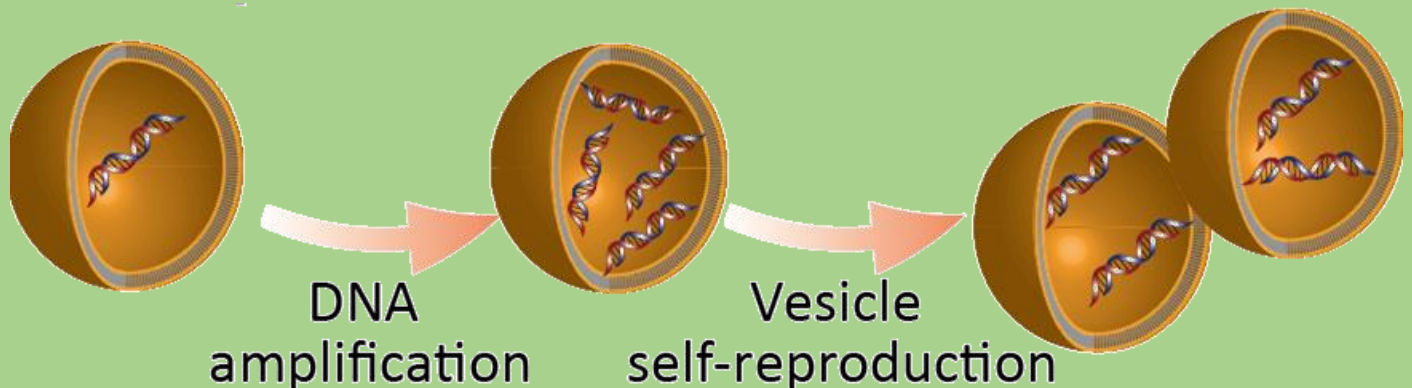
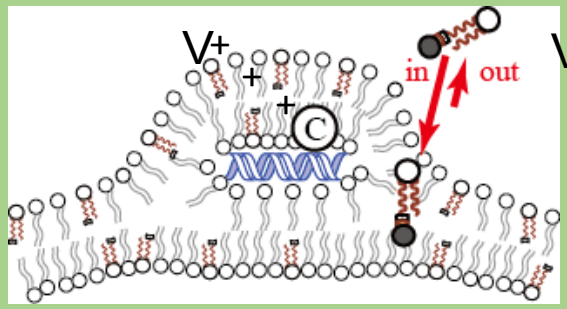
辻川郁二先生没10年記念講演会
H29年4月15日京都大学 理学研究科 セミナーハウス

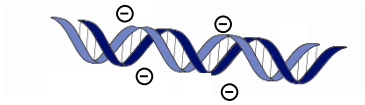
人工細胞から見えてくる生命を解く鍵





C@DNA

dsDNA



神奈川大理学部化学科

菅原正

Dynamics in Molecular Systems

Molecular Crystal

Crystallization of components

Phase transition

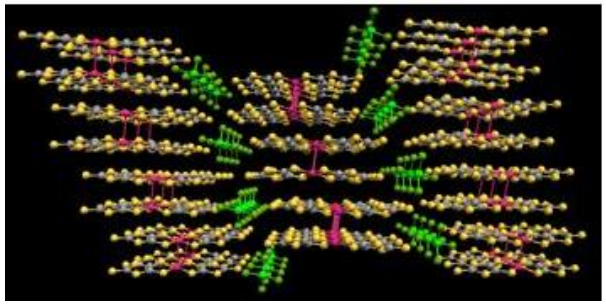
Libration [Dielectrics*]

Carrier transfer [Conductivity]

Spin ordering [Magnetism]

Topochemical reaction

* *Ionic, orientational polarization*



Bio-system

Dynamic arrangement of components in a compartment

Morphological change

Molecular Motor [Energy, Movement]

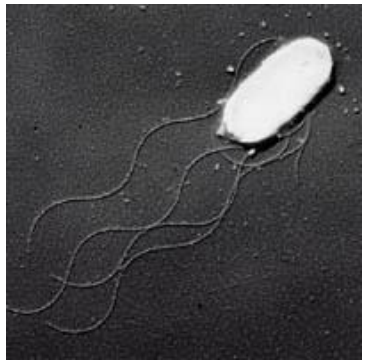
Transport of membrane voltage, Proton, Chemicals [Energy, Information]

Reaction network, Replication

★ Self-proliferation, Self-propelling,

★ Evolution

★ *Characteristic to life system*

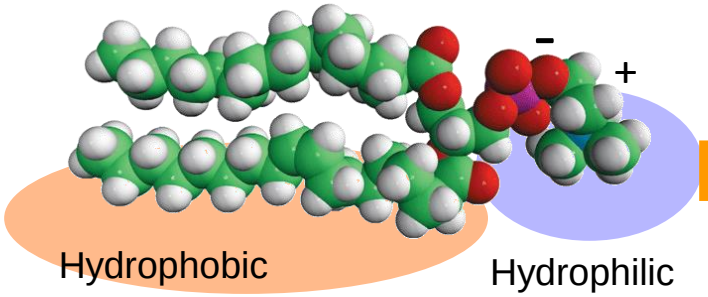


Non-equilibrium open system

Formation of Giant Vesicles (GVs) from Amphiphile

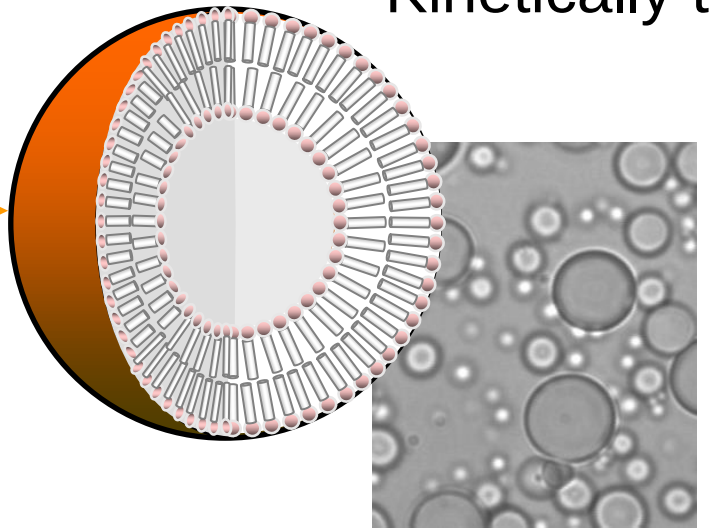
Soft matter *connects inaminate and animate matters*

Amphiphile
(Phospholipid)

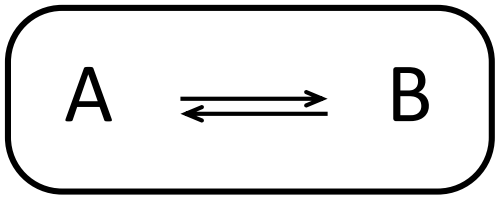


Spherical GV

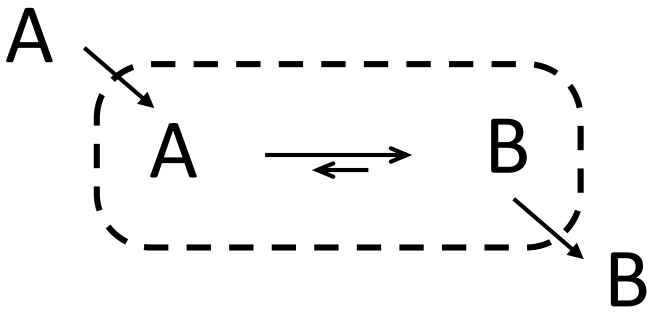
Kinetically trapped



Chemical Reactions in GV

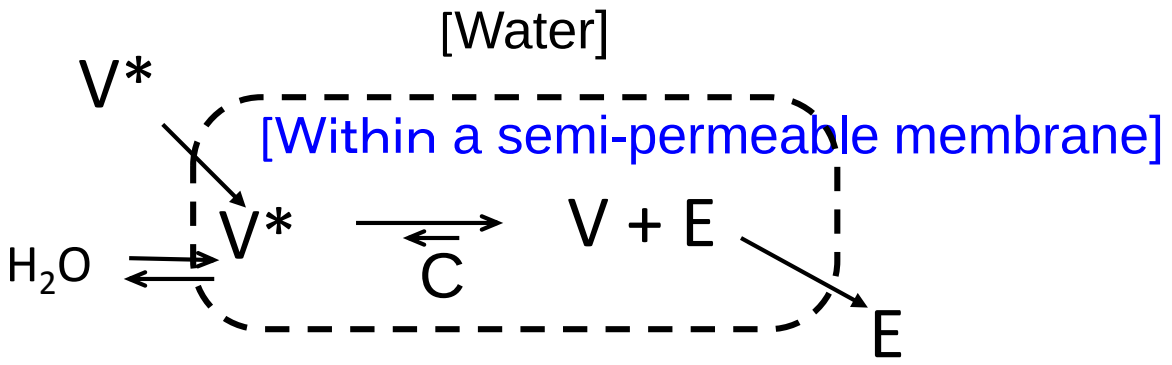


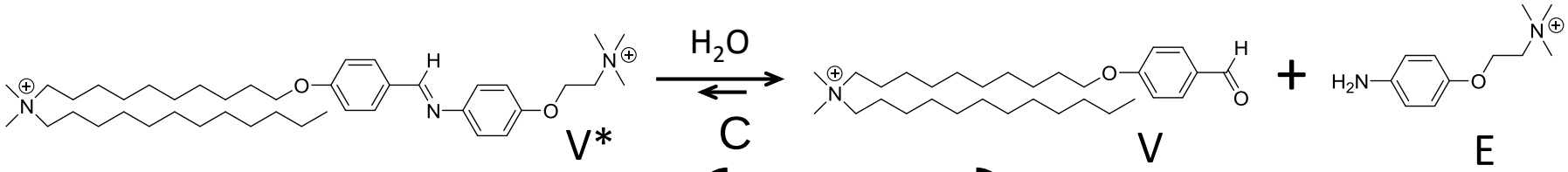
Equilibrium System

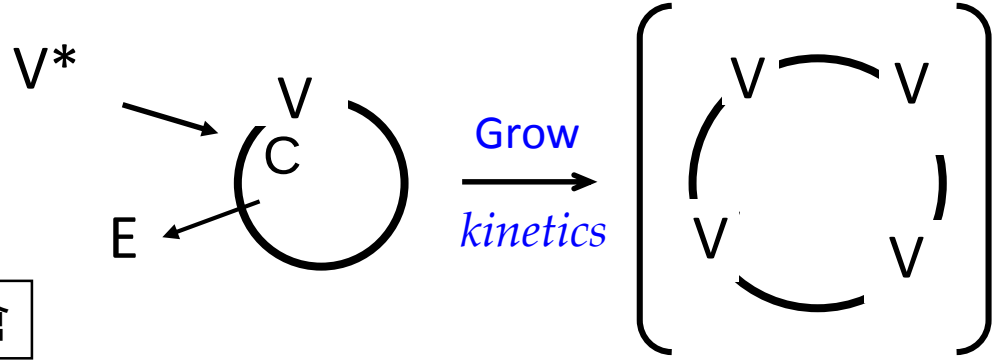


Non-equilibrium Open System

Self-reproduction of Vesicles



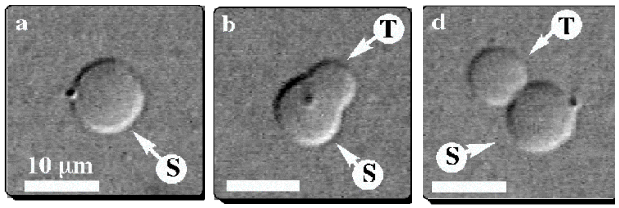




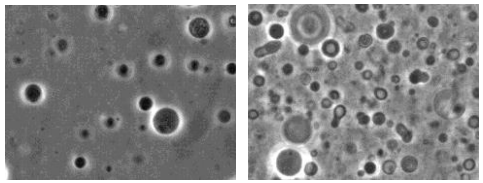
Divide

Cooperative Dynamics

高倉



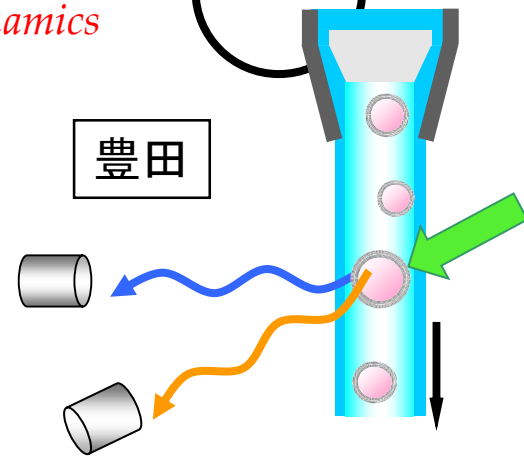
0 min 5 min 20 min



Before After

Addition of V*

豊田



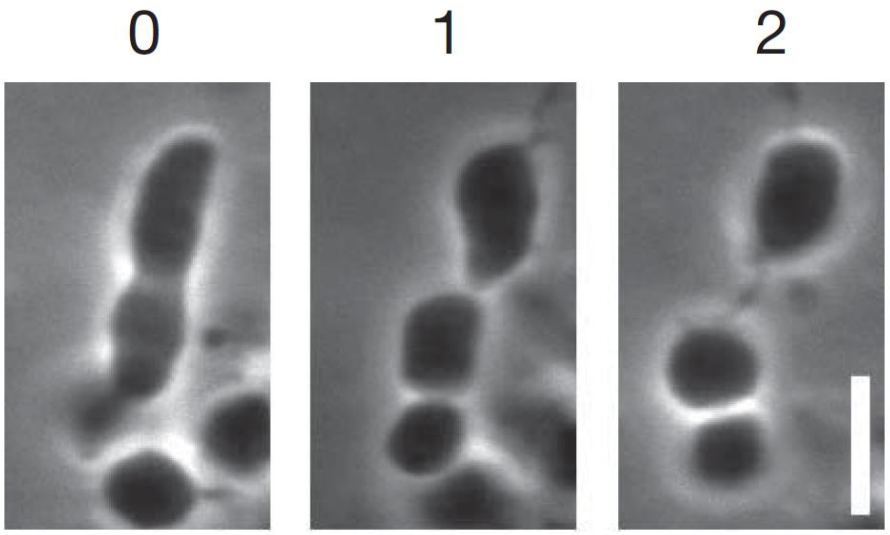
K. Takakura and T. S., *Langmuir* **2004**, *20*, 3832-3834

Our self-proliferation Model vs. L-form bacteria

1) Many modern bacteria retain the ability to switch into a wall-free state called L-form.

2) L-form proliferation is independent of the complicated division machinery based on FtsZ.

3) It occurs by increase in the surface area to volume ratio by producing membrane lipids, then the long tubulated cell divides and proliferates.

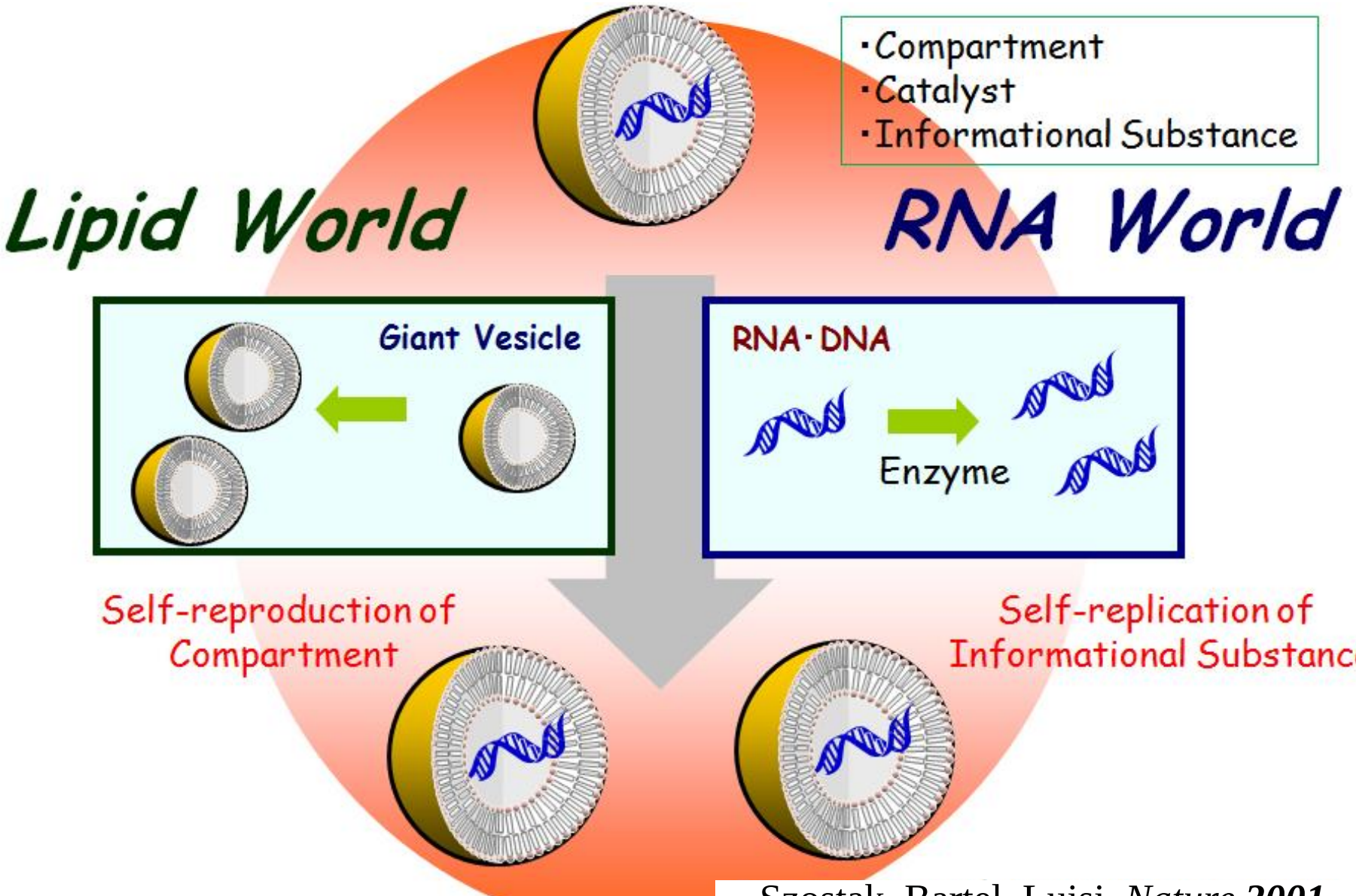


This result indicates that simple physical processes could have supported proliferation for the primitive cell having much simpler molecular system than modern bacteria.

M. Leaver, *et al.*, "Life without a wall or division machine in Bacillus subtilis", *Nature*, **457**, 849-854, 2009.

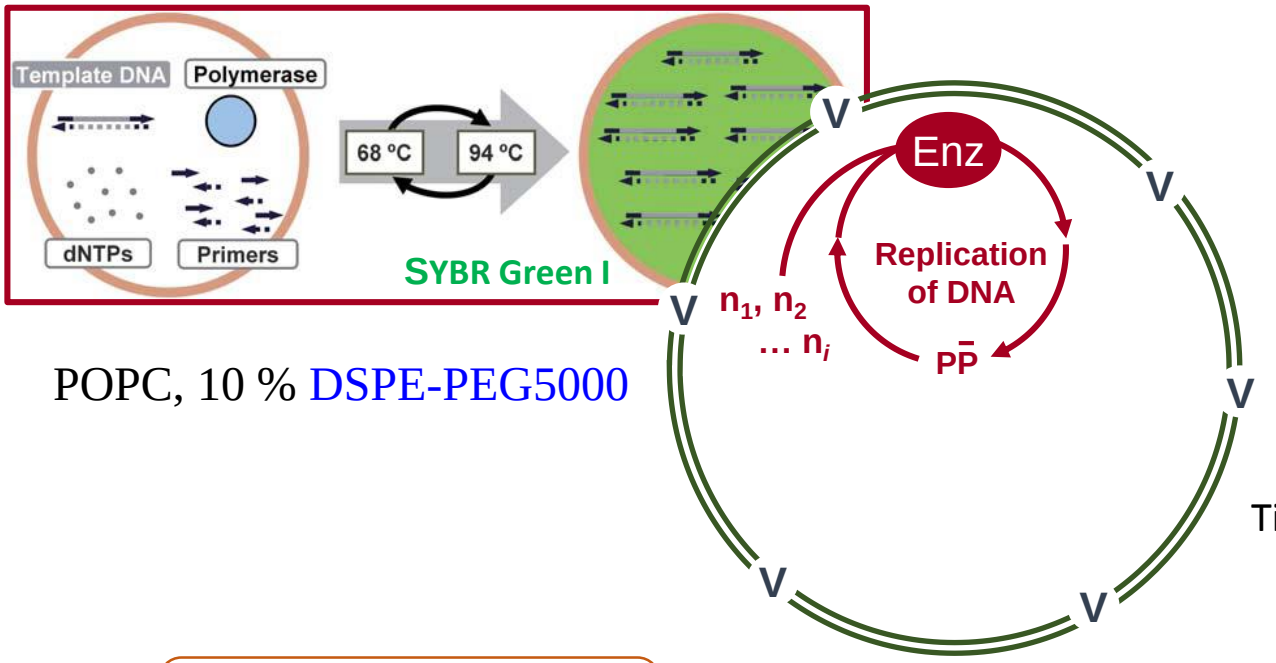
Jeff Errington, *et al.*, "Excess membrane synthesis drives a primitive model of cell proliferation.", *Cell*, **152**, 997, 2013.

Giant Vesicle-based Protocell

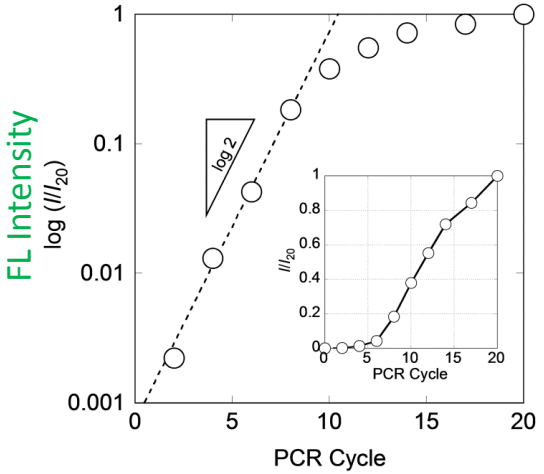


Szostak. Bartel, Luisi, *Nature* ***2001***

How about DNA Replication in Robust GV?



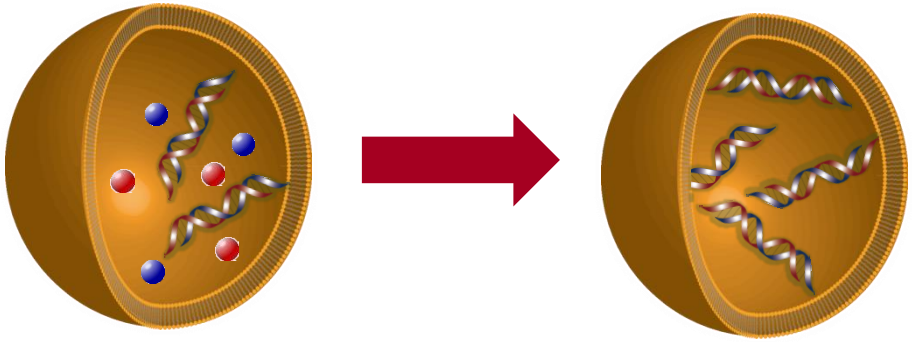
POPC, 10 % DSPE-PEG5000

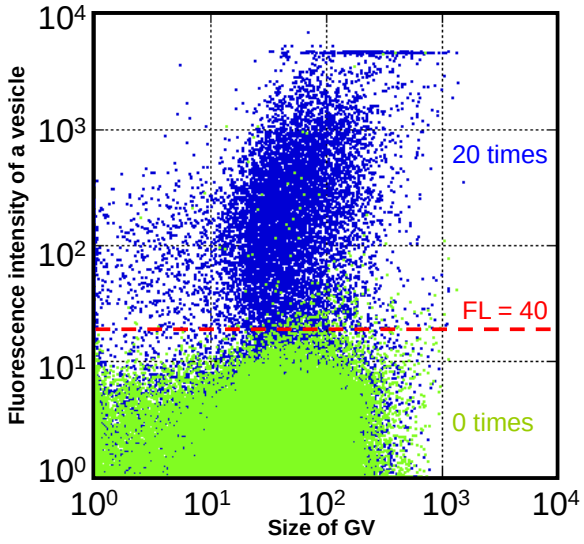


Time-dependence of FL intensity

$$\Sigma n_i \xrightarrow[\bar{P}\bar{P}]{\text{Enz}} P\bar{P}$$

庄田·田村





K. Shohda, M. Tamura *et al.*, *Soft Matter* **7**, 3750 (2011)

Flow cytometry

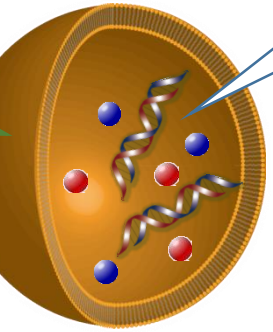
Amplification of DNA in Self-reproductive GV

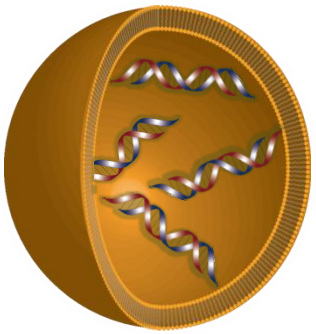
POPC	65
PEG--grafted DSPE	5
Cholesterol	30

Template DNA
dNTP / Primer
DNA polymerase
SYBR Green I

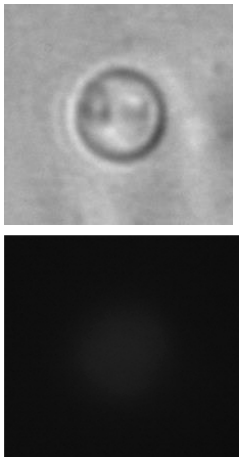
POPC	6
POPG	2
V	2
C	1

Membrane Lipids
and Catalyst for
GV self-reproduction





Membrane
Precursor, V*



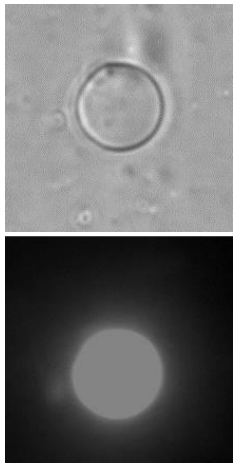
Initial GV

Thermal Cycle

98 °C: Denature

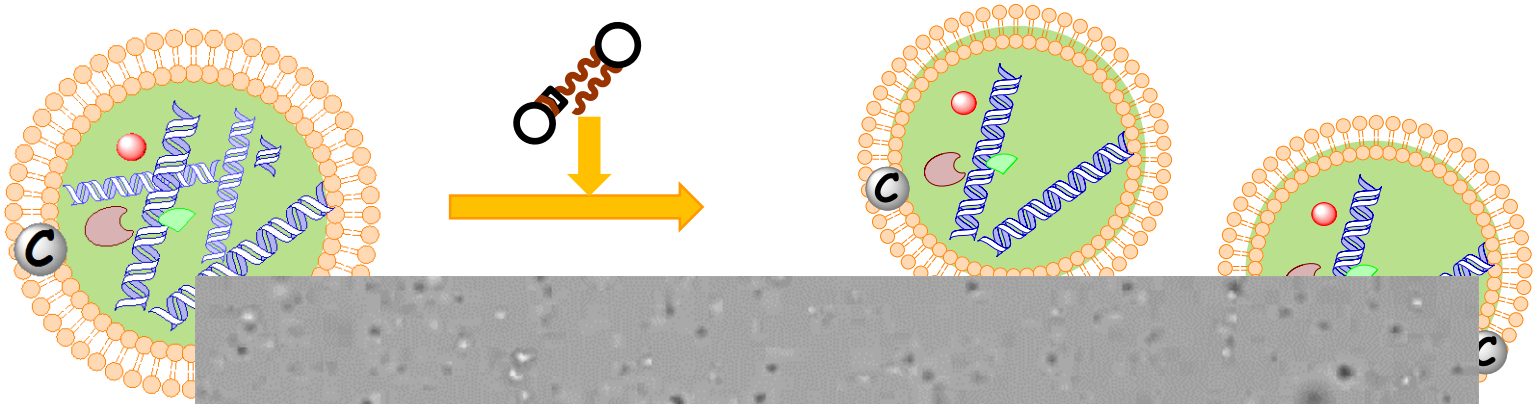
20 times

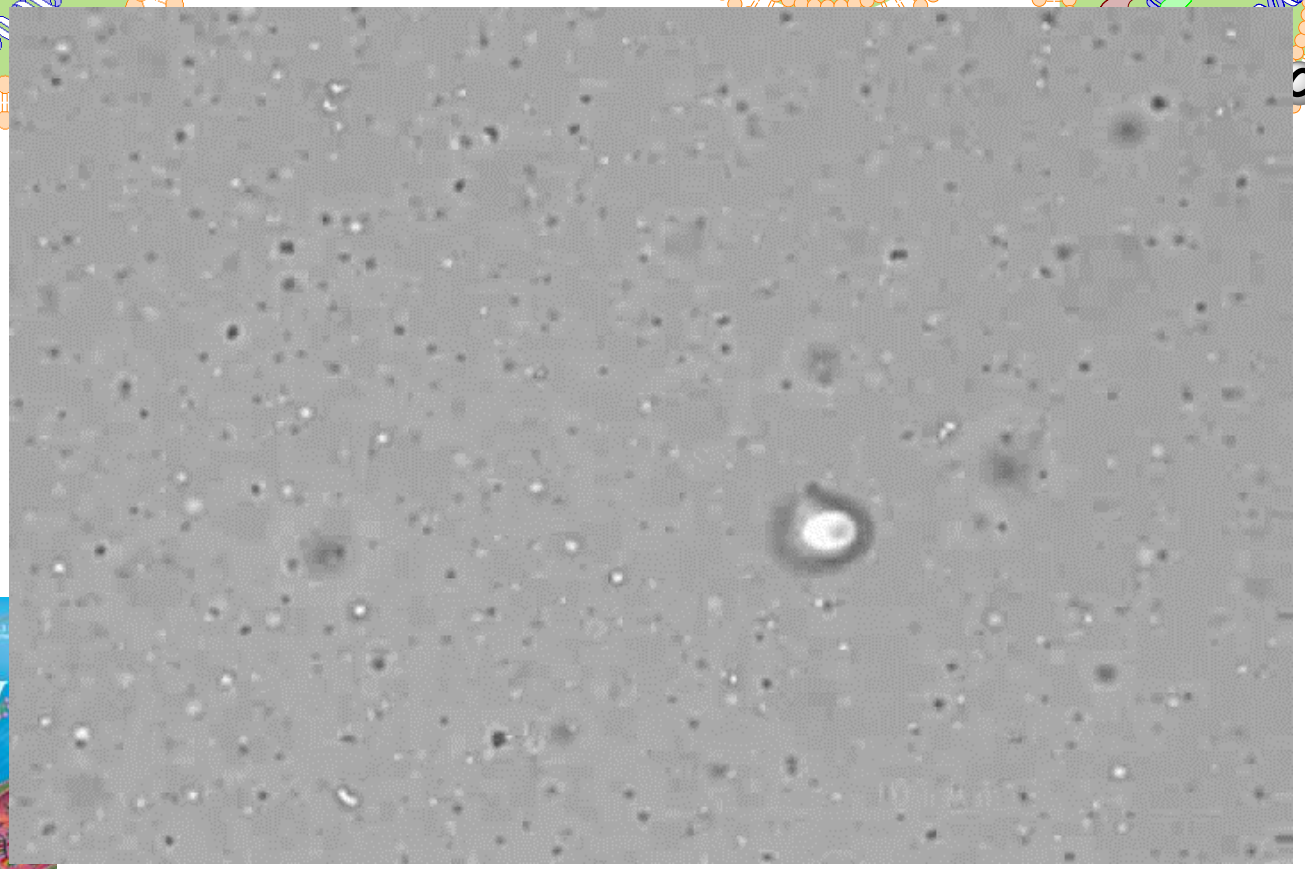
68 °C: Elongation

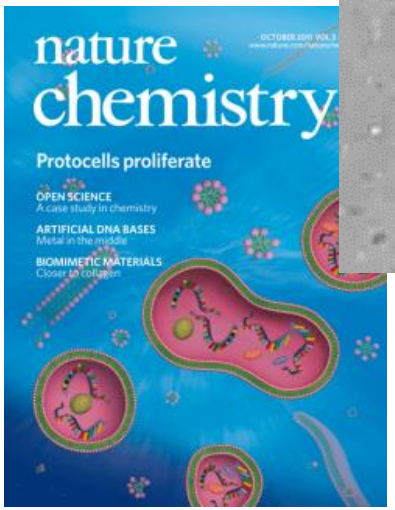


After DNA Amplification

栗原

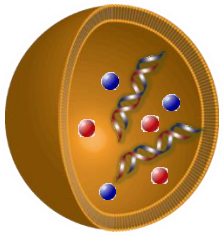




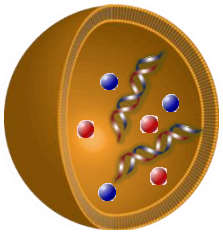


Kurihara *et al.*, *Nature Chem.* **3**, 775 (2011)

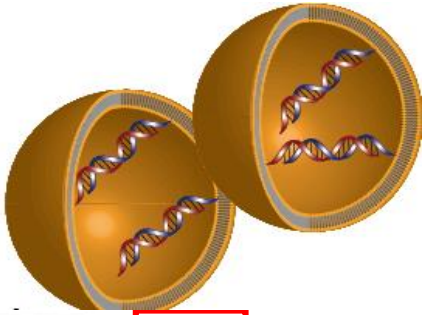
Linked Proliferation

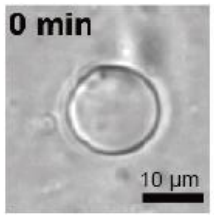


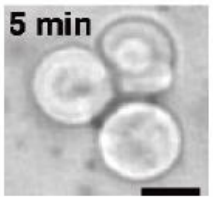
thermal cycle

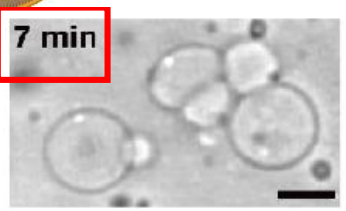


Addition of V*

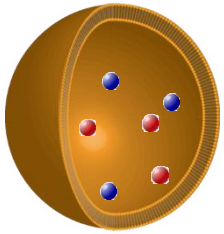




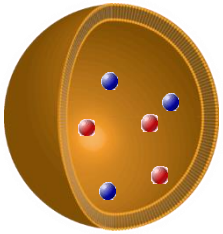




GV with amplified DNA divides within 3 m



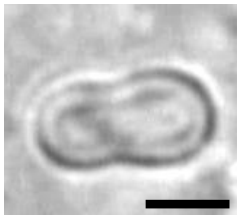
Thermal cycle 20 times

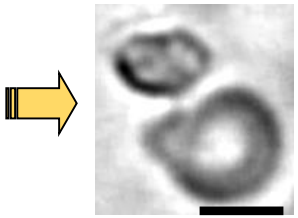


Addition of V*

Slow & rare

No template DNA



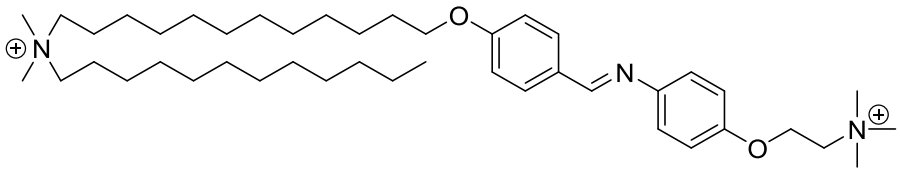


30 min After

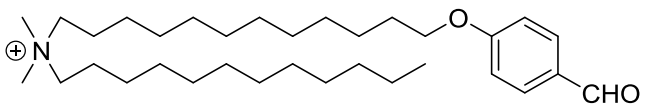
120 min After

Amplified DNA accelerates the growth and division of GV when V* is added !

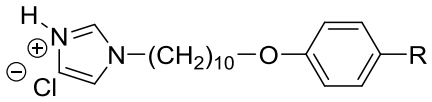
DNA Complex-assisted Budding & Division



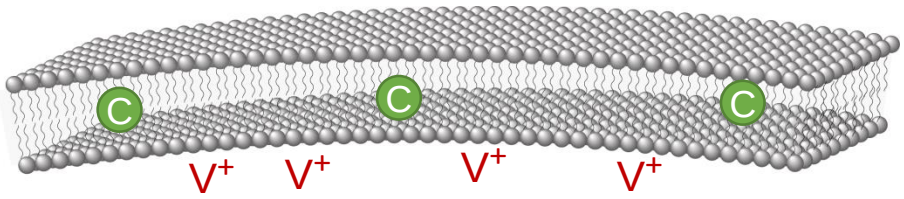
Precursor of membrane lipid

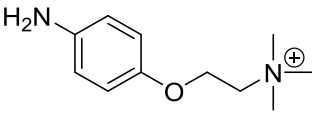


Membrane Lipid

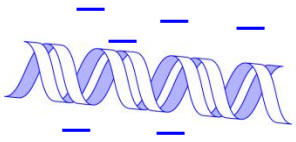


Catalyst





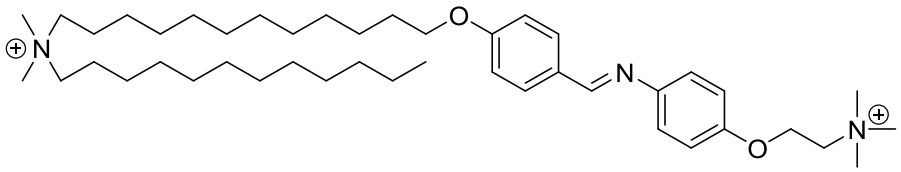
Electrolyte



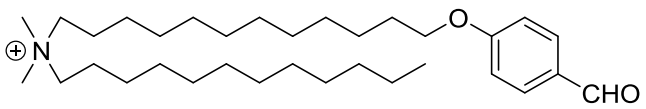
DNA is amplified

Membrane lipid increases more in outer than in inner leaflet → Δa → Budding deformation & division

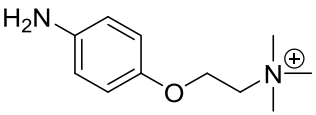
DNA Complex-assisted Budding & Division



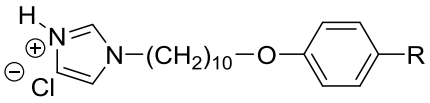
Precursor of membrane lipid



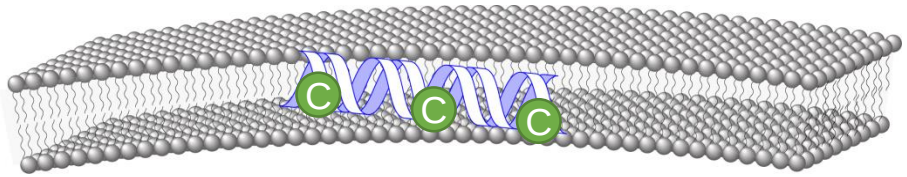
Membrane Lipid



Electrolyte



Catalyst

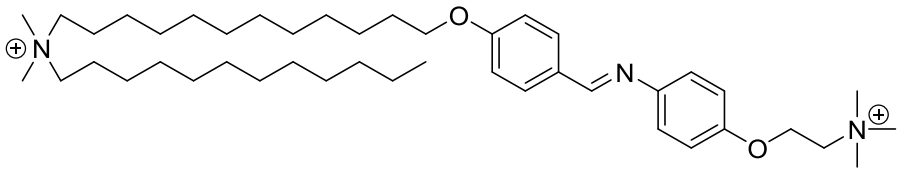


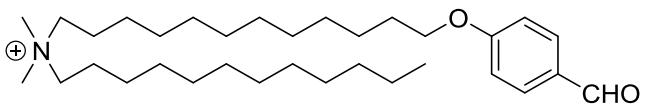
DNA is amplified

Catalyst

Membrane lipid increases more
In the outer leaflet than in the inner → Δa → Budding Deformation & division

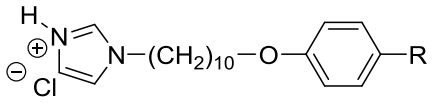
DNA Complex-assisted Budding & Division



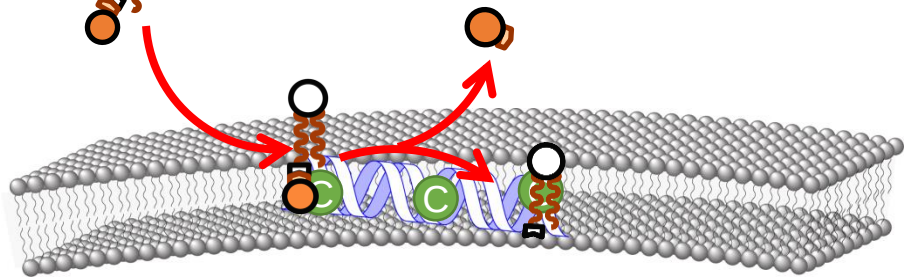


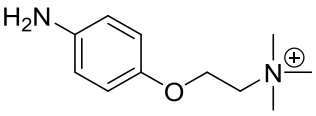
Membrane Lipid

Precursor of membrane lipid



Catalyst



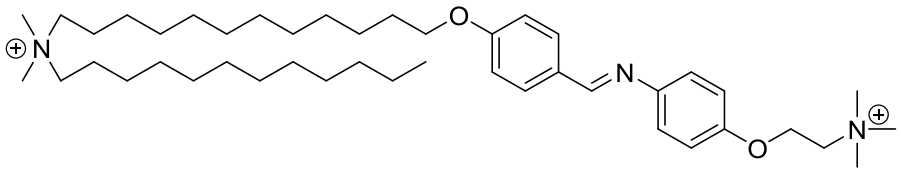


Electrolyte

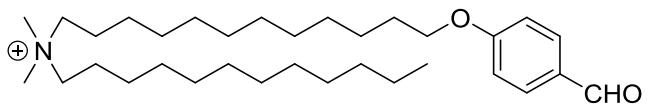
DNA is amplified

Membrane lipid increases more
In the outer leaflet than in the inner → Δa → Budding Deformation & division

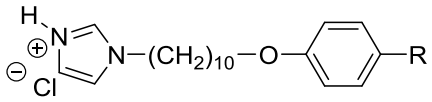
DNA Complex-assisted Budding & Division



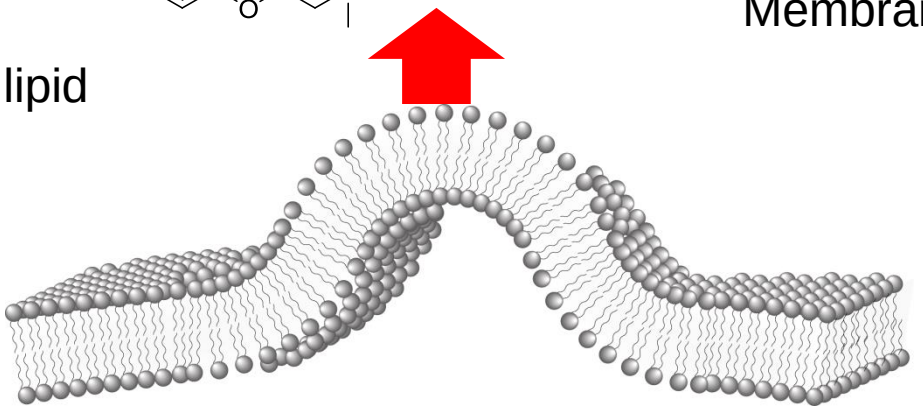
Precursor of membrane lipid

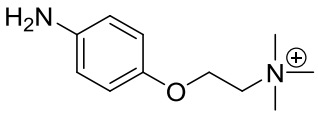


Membrane Lipid



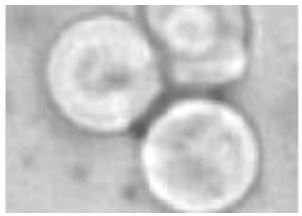
Catalyst





Electrolyte

DNA is amplified



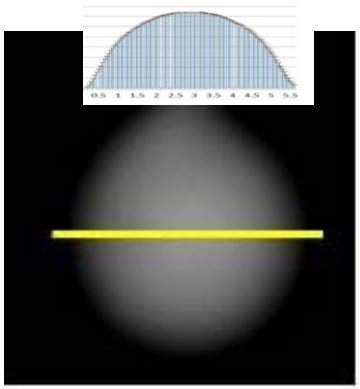
Local production of membrane lipids around C@DNA in the membrane breaks symmetry and determines a mode of deformation.

Membrane lipid increases more In the outer leaflet than in the inner ➡ Δa ➡ Budding Deformation & division

Where is “C@DNA” formed？

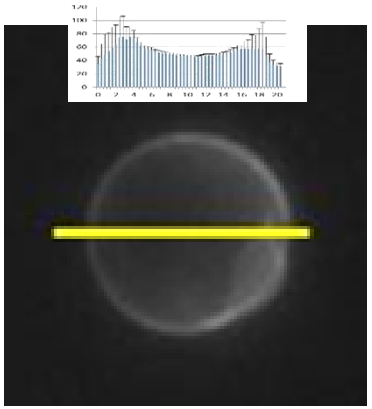
Interaction between amplified DNA and cationic membrane

Distribution of fluorescence intensity of **ds DNA & @SYBR Green** complex



Absence of
cationic membrane lipid **V**

Inner water phase



Presence of
cationic membrane lipid **V**

within GV membrane
or on the membrane

松尾

#1. Detection of FET from Catalyst to DNA
#2. Addition of water-soluble Quencher

Primer with **FL** → **DNA** with **FL**

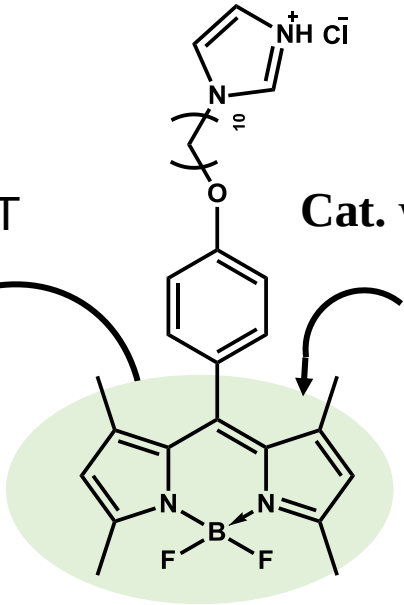
3’ 5’
CTGTCGTAGCGGTCAGTGAT—TexasRed

Primer

FRET

Cat. with **FL**

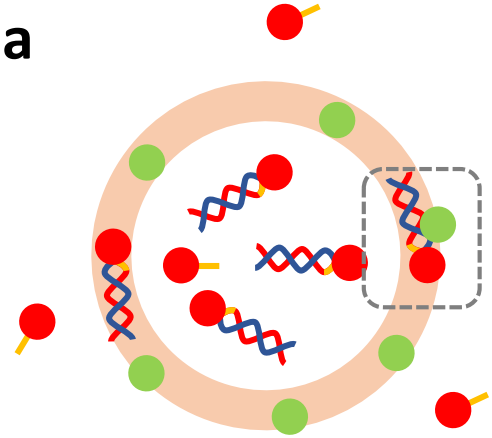
$h\nu$



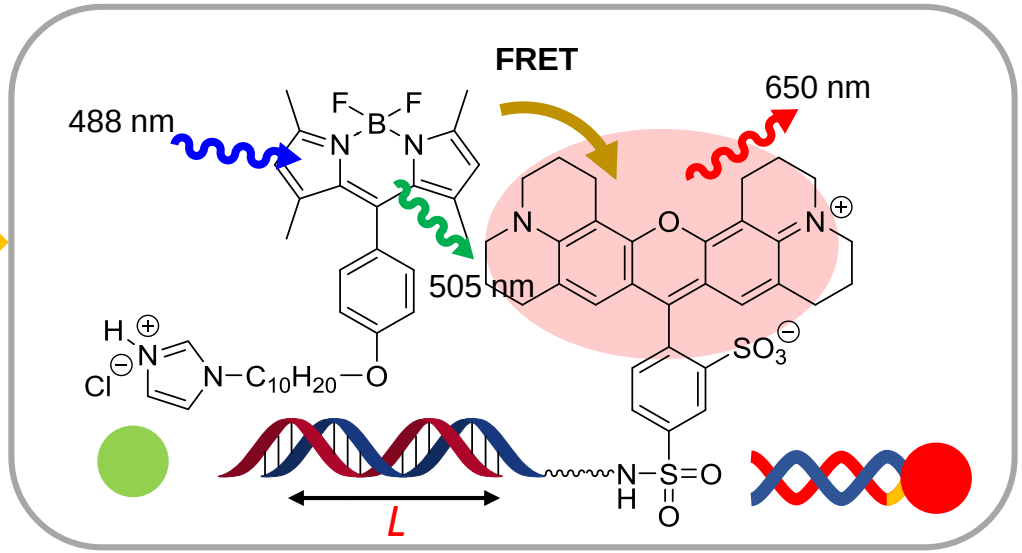
PC:PG:V:C:Chol = 35:39:12:9:5

C@DNA is Located in GV Membrane

a



C@DNA exists in membrane



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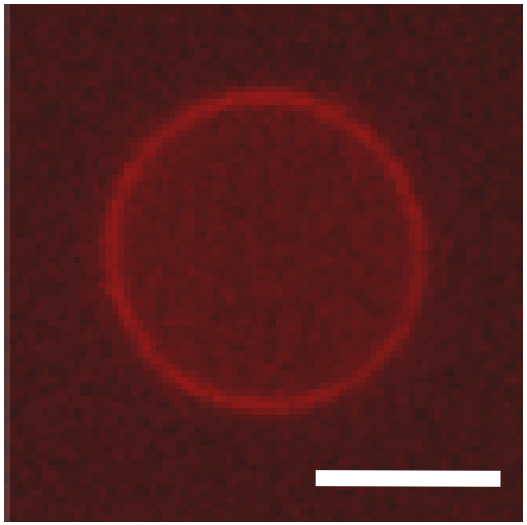
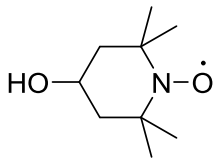


Excitation with 488 nm

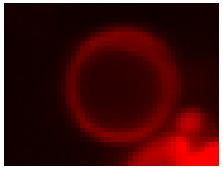
Forster Resonance Energy Transfer

c

Inner water pool

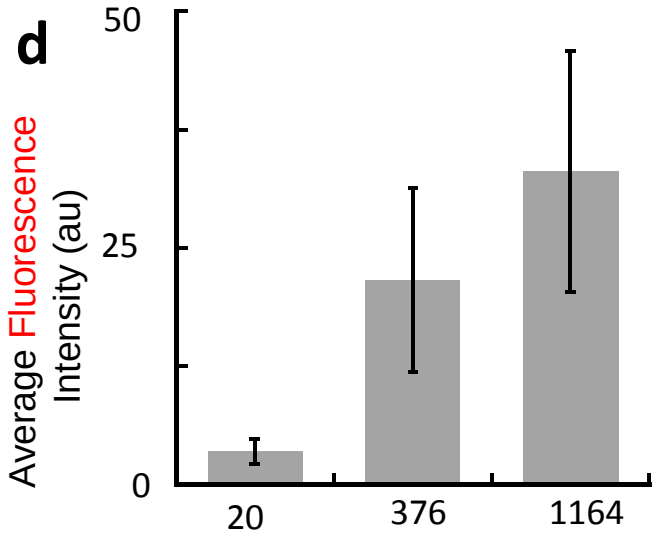


water soluble quencher



Not quenched

d

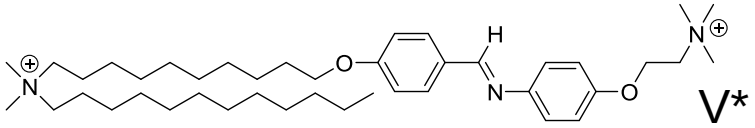


FL intensity depends on the length of DNA

Trace of Hydrolysis of V* in the Presence of C@DNA

Membrane Lipids (PC:PG:V:C = 6:2:2:1)

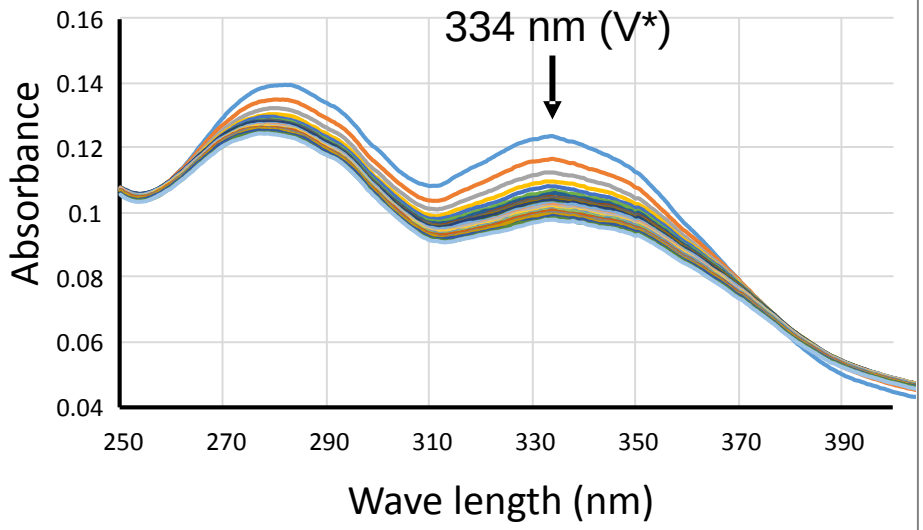
in KOD-plus-buffer (78 μM) + (C +DNA)

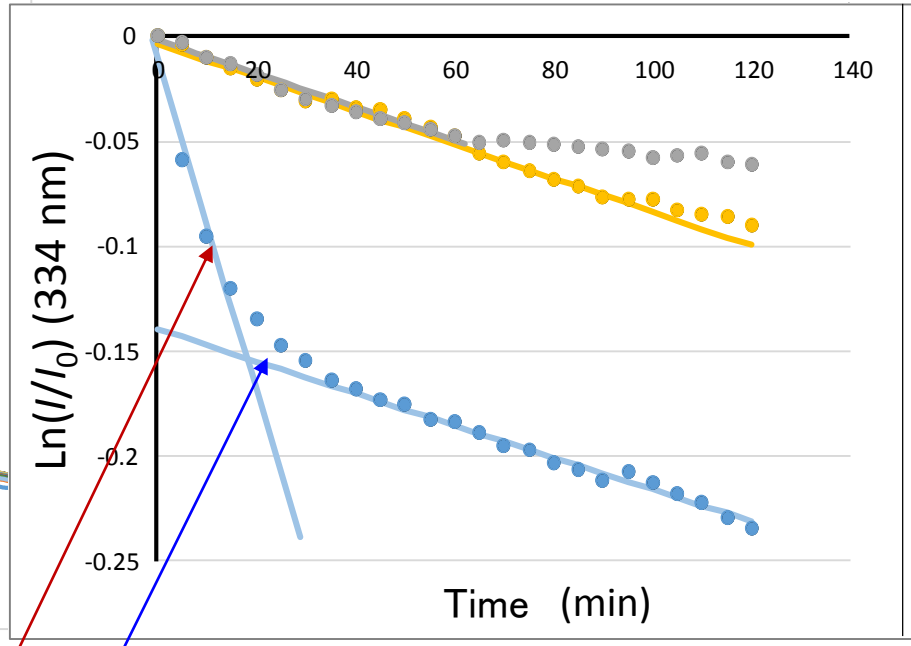


⟶ V + E

東大　松尾
KU　鈴木遼

Hydrolysis of V* in the presence of **C** and **DNA**

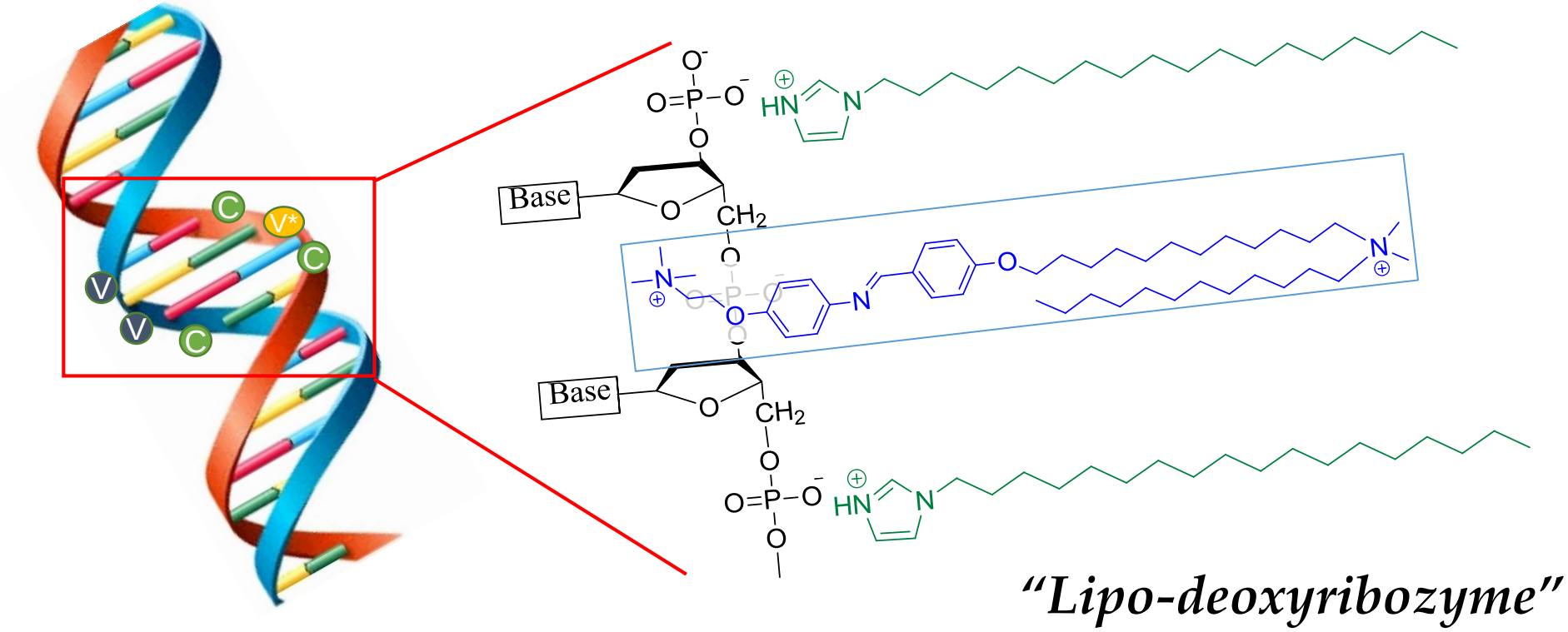




Synergetic effect of **C** and **DNA** on hydrolysis

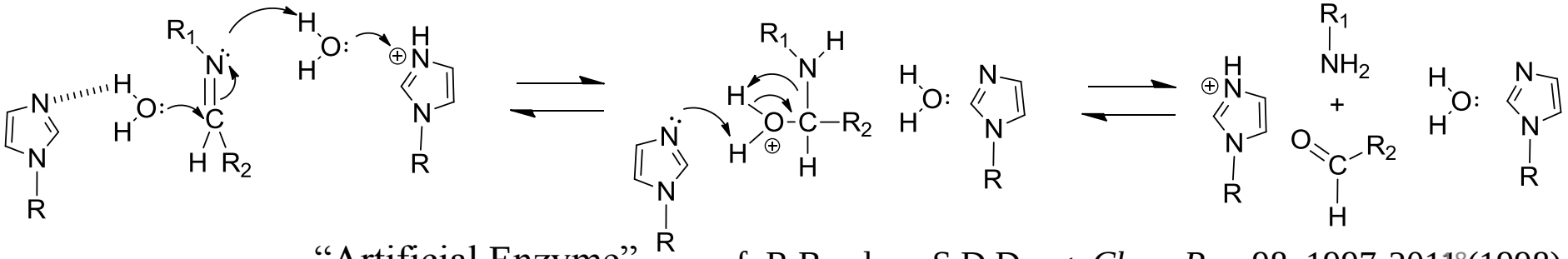
Decay rate suppressed ca.20 min after initiation

Synergetic Effect between Catalyst and DNA



"Lipo-deoxyribozyme"

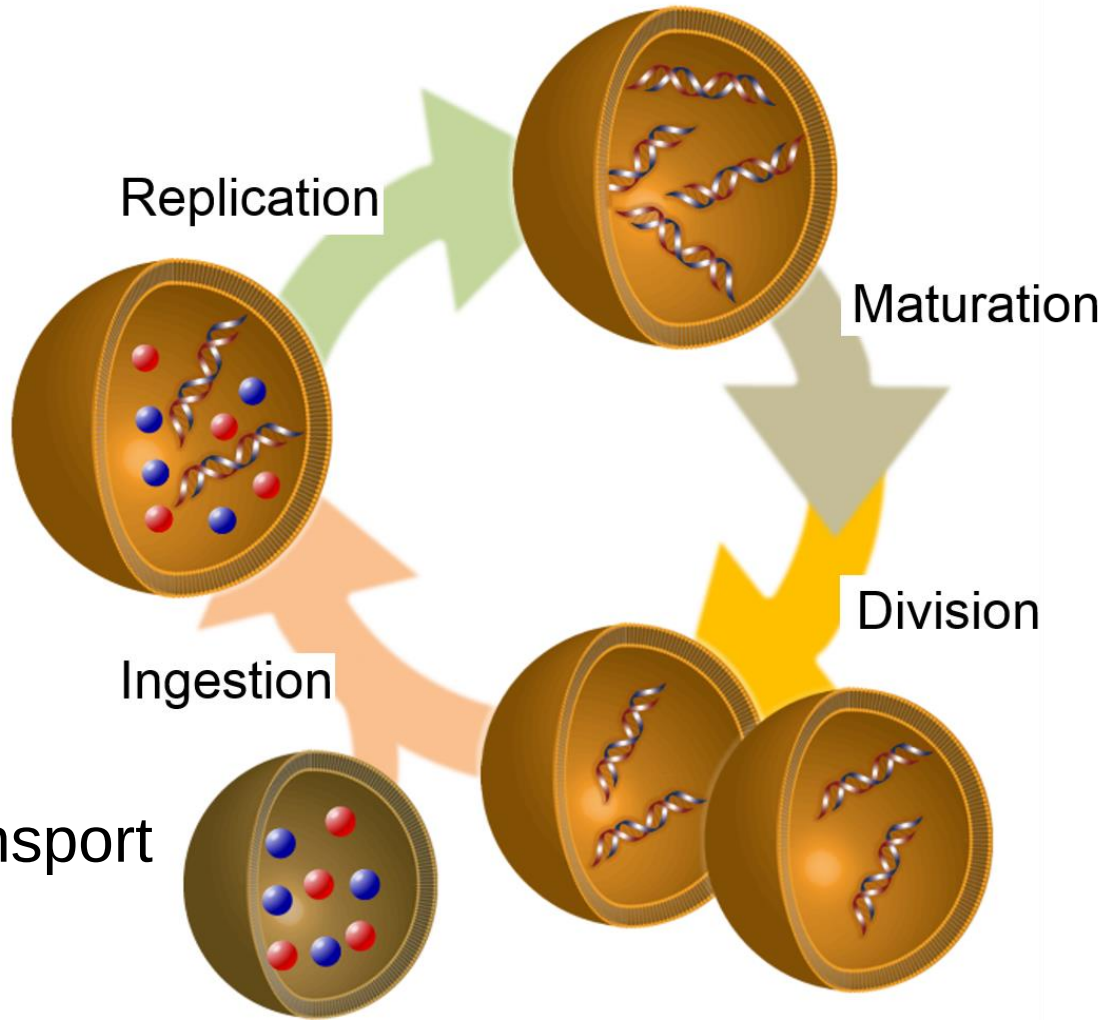
Hydrolysis of "imine" by imidazole and imidazolium salt



"Artificial Enzyme"

cf. R.Breslow, S.D.Dong, *Chem.Rev.* 98, 1997-2011 (1998)

Recursive Proliferation

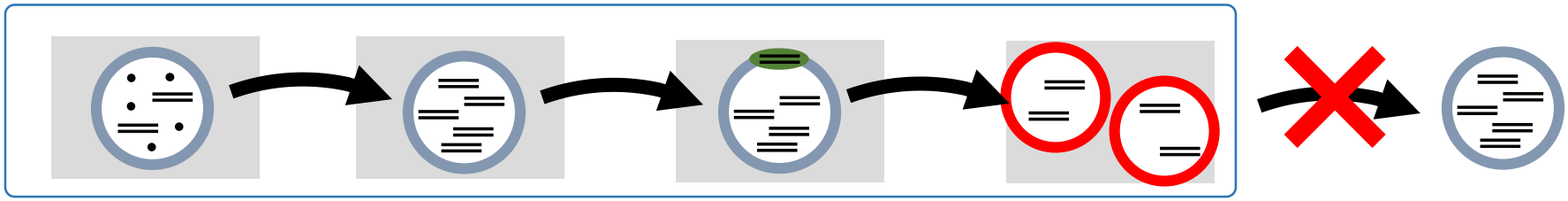


Vesicular Transport

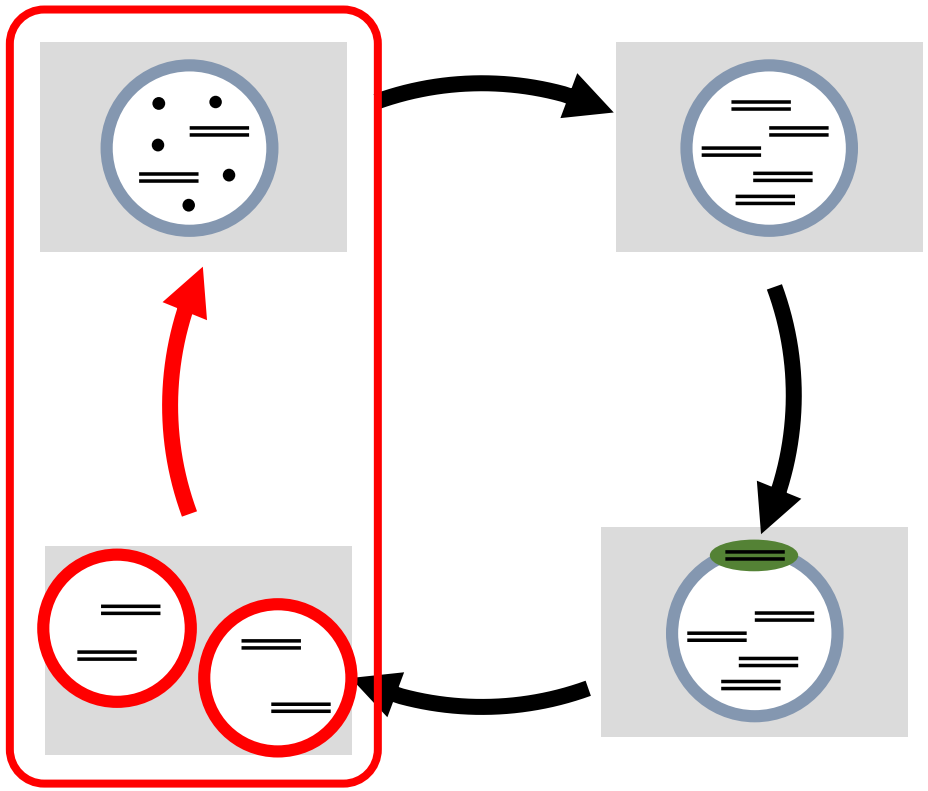
全員

Fusion with a conveyer vesicle containing dNTP (deoxyribonucleoside triphosphate)

Recursive Self-proliferation : from Cascade to Loop

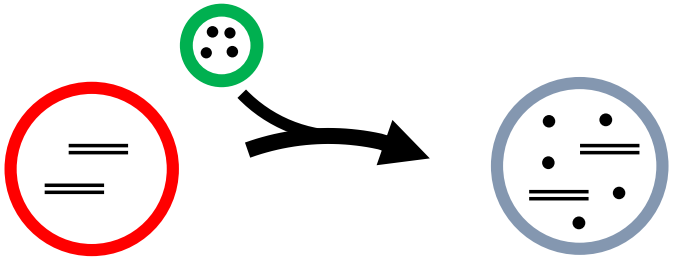


Newly born GV of the 2nd generation cannot amplify DNA
because it has no dNTP inside.



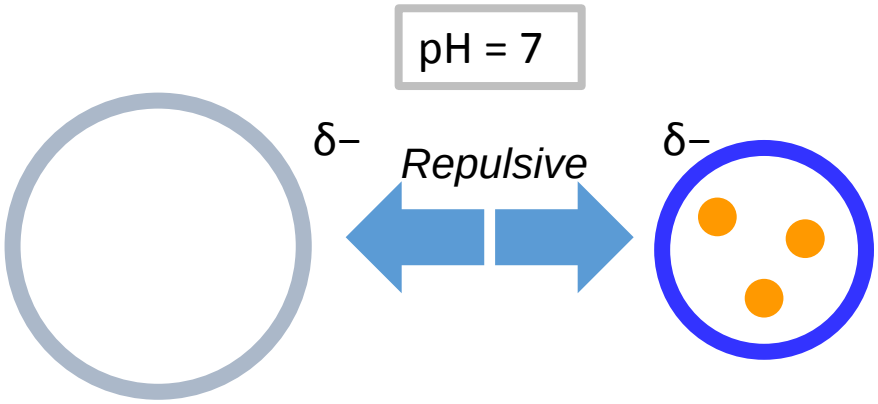
鈴木

Intra-vesicular Transportation



without dNTP

with dNTP



Target GV

9 POPC ⊕⊖
1 POPG ⊖

9 cations ⊕⊕⊕⊕⊕⊕⊕⊕⊕
10 anions ⊖⊖⊖⊖⊖⊖⊖⊖⊖⊖

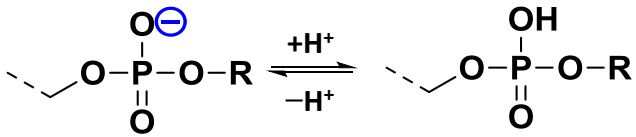
Negative Charge

Conveyer GV

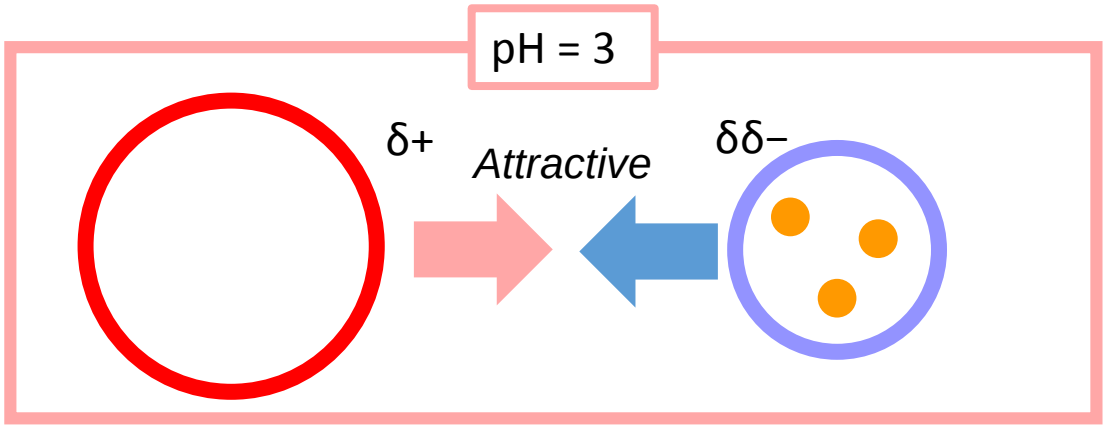
5 POPG ⊖

⊖⊖⊖⊖⊖ 5 anions

Negative Charge



Dissociation Equilibrium of Phospholipid



Target GV

9 POPC ⊕⊖
1 POPG ⊖

9 cations ⊕⊕⊕⊕⊕⊕⊕⊕⊕
5 anions ⊖⊖⊖⊖⊖

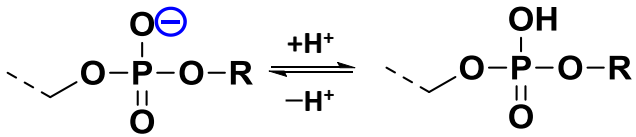
Positive Charge

Conveyer GV

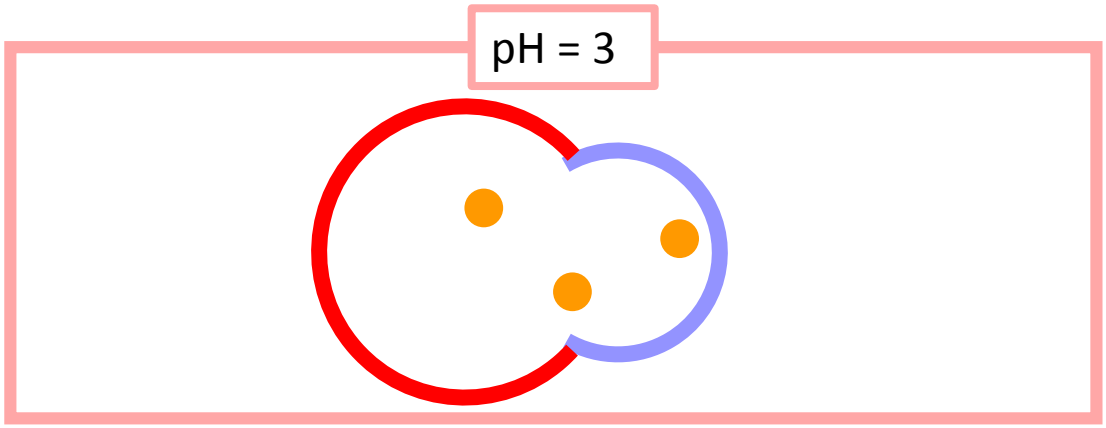
5 POPG ⊖

⊖⊖⊖ 3 anions

Negative Charge

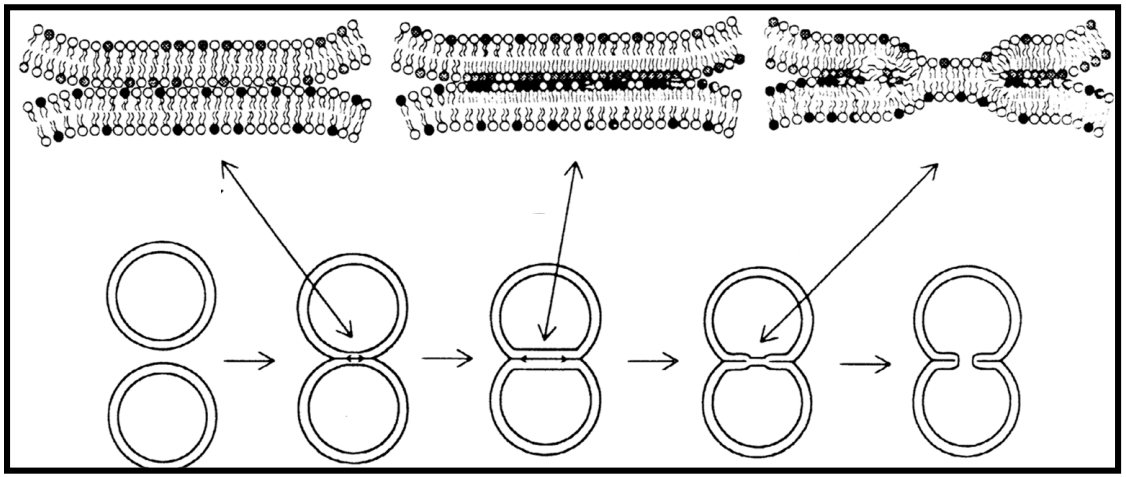


Dissociation Equilibrium of Phospholipid

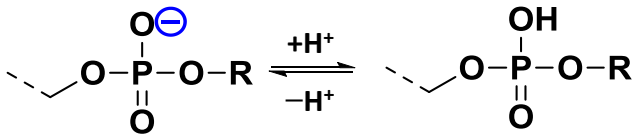


Target GV **Conveyer GV**

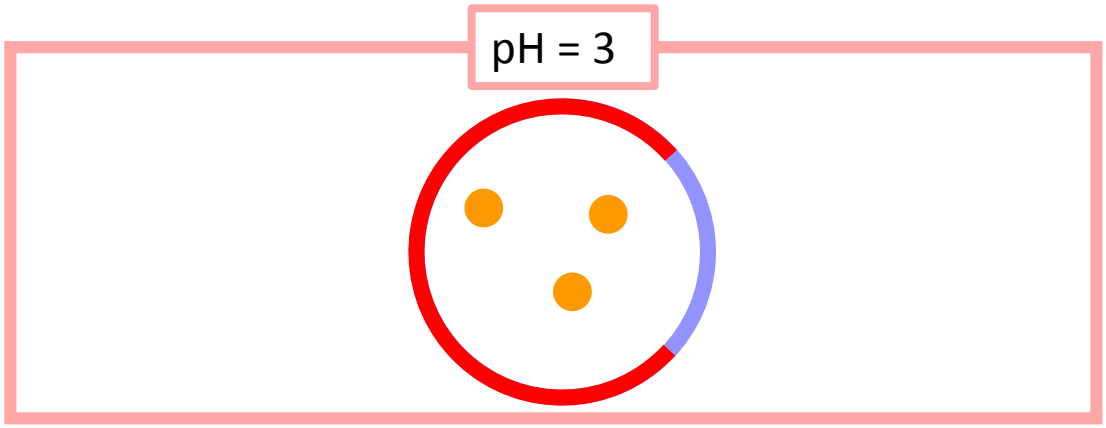
9 cations
5 anions



3 anions



Dissociation Equilibrium of Phospholipid



Target GV

9 POPC ⊕⊖
1 POPG ⊖

9 cations ⊕⊕⊕⊕⊕⊕⊕⊕⊕
5 anions ⊖⊖⊖⊖⊖

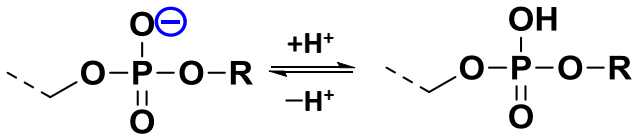
Positive Charge

Conveyer GV

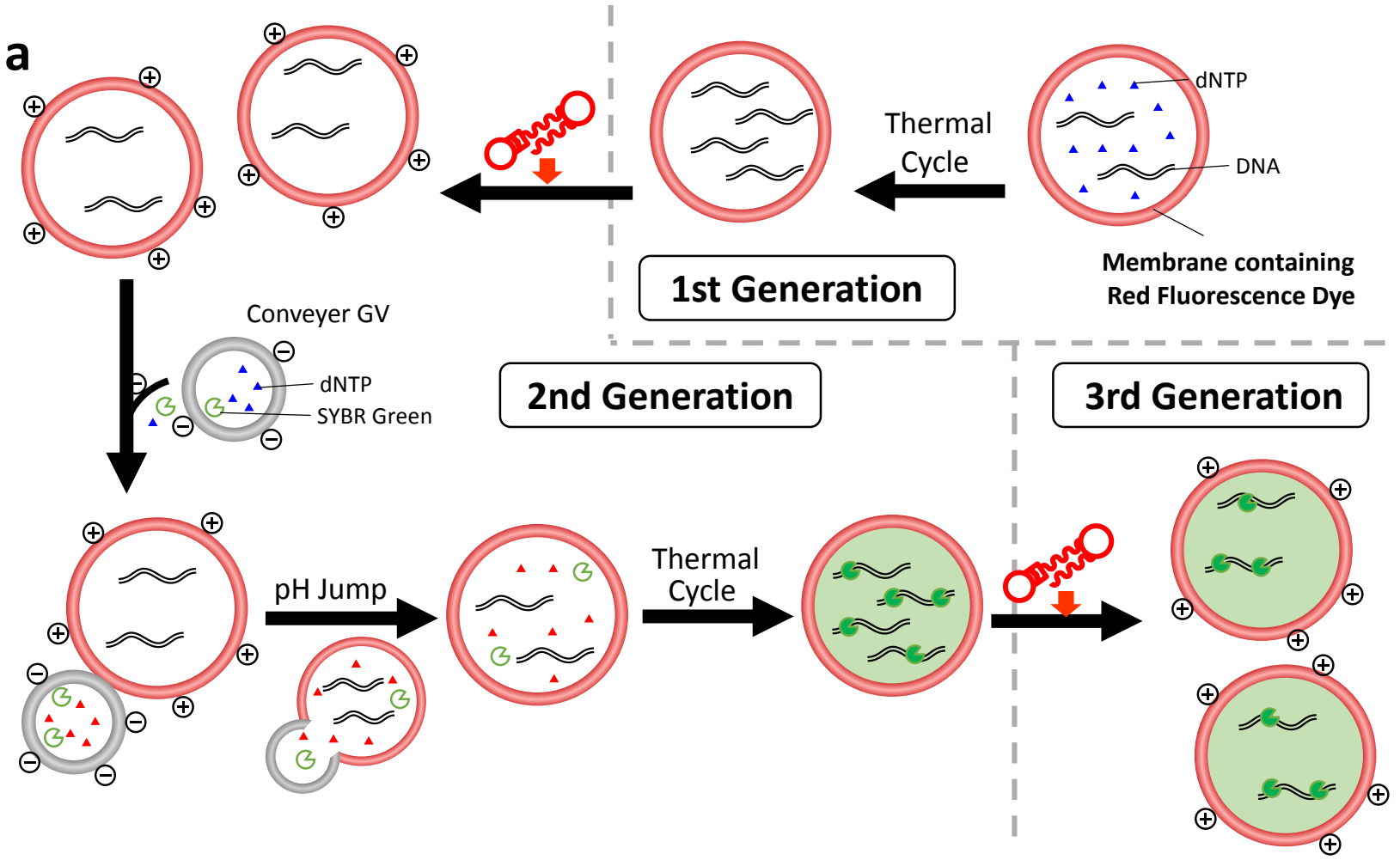
5 POPG ⊖

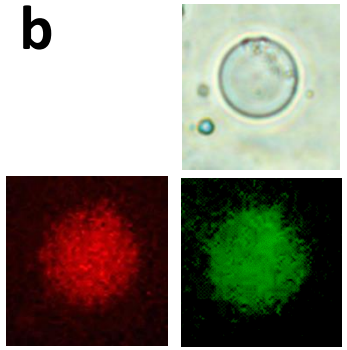
⊖⊖⊖ 3 anions

Negative Charge



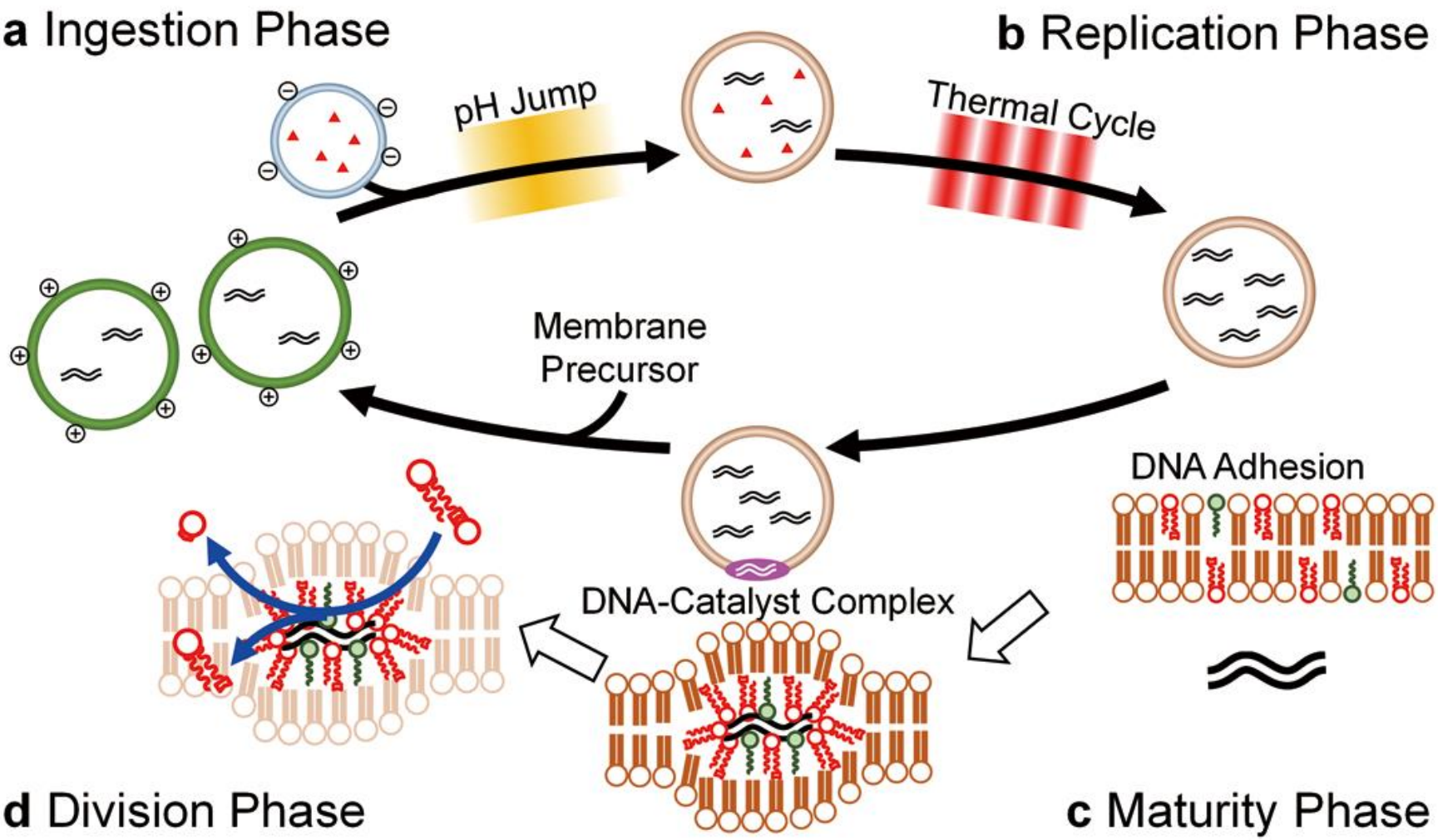
Dissociation Equilibrium of Phospholipid


a
dNTP
DNA
Thermal Cycle
Membrane containing Red Fluorescence Dye
1st Generation
Conveyer GV
dNTP
SYBR Green
2nd Generation
3rd Generation
pH Jump
Thermal Cycle


b

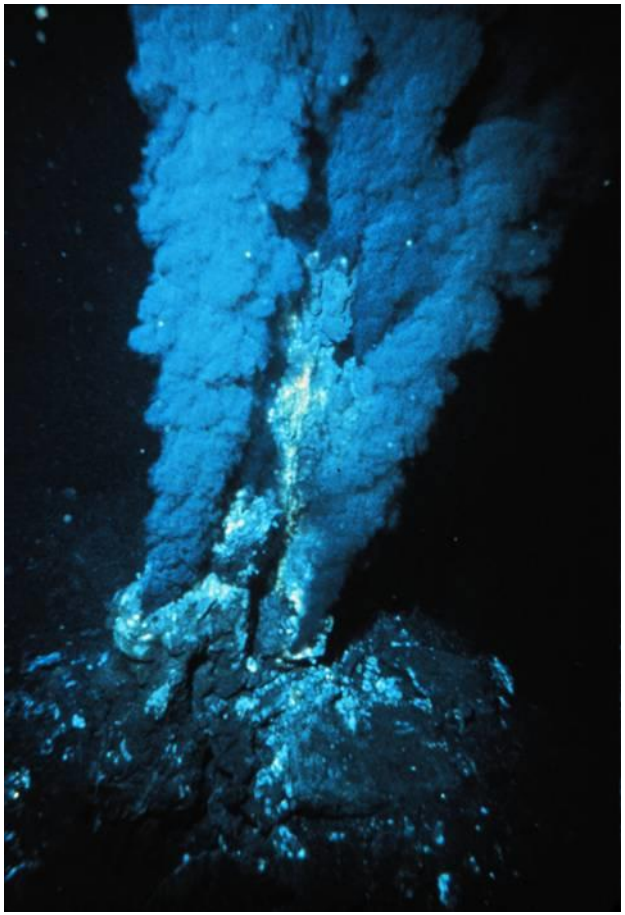

c
6 min
7.5 min
8 min
9 min
14.5 min
20 μm

Vesicle-based Protocell with Primitive Cell Cycles

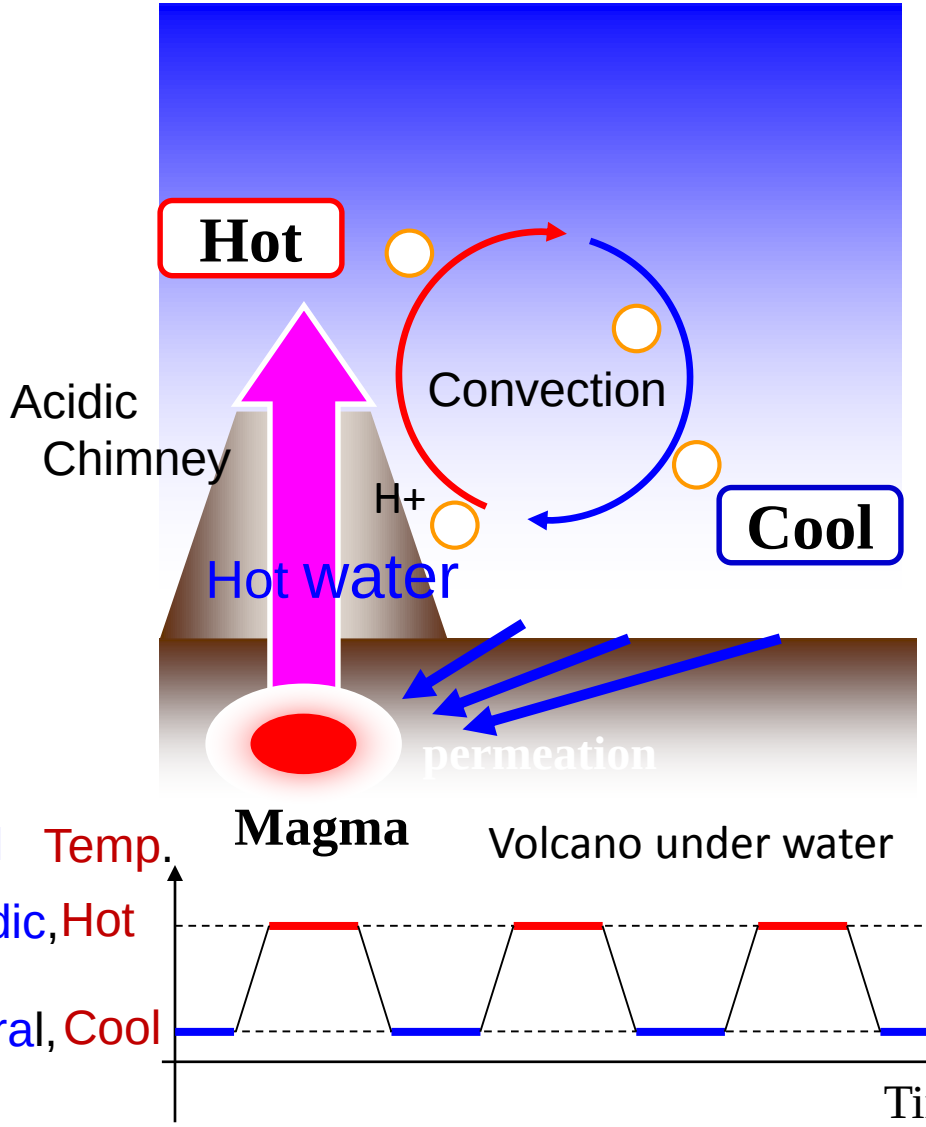


K. Kurihara, T. Sugawara, et al., *Nature Comm*. 6, 8352 (2015)

Thermal Cycle of Hydrothermal Vent



Hydrothermal vent

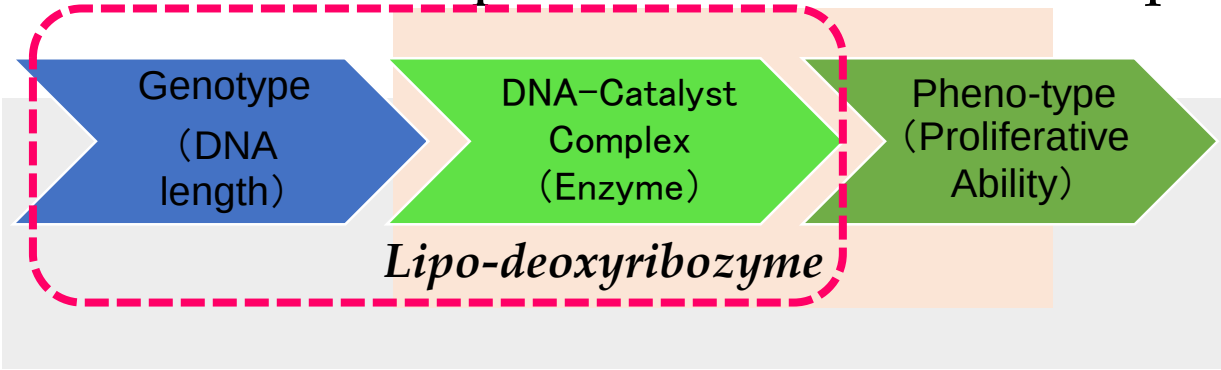


Geno-type and Pheno-type Correlation in Protocell

Mechanism of Gene-expression of a contemporary living cell

Central Dogma

Mechanism of Gene-expression of a GV-based protocell



菅・松尾

Biological distance between *geno-type* and *pheno-type* is close in our GV-based protocell

Origin of Life : Biomolecule-based View

Lipid

RNA･DNA

Protein

Time

Contemporary Life

Evolution

Origin of Life :Hierarchical Dynamics

Self-reproduction

Linked Proliferation

Recursive Proliferation

DNA-amplification

GV Delivery System

Complexity of System

参考資料

SCIENCE CHANNEL
サイエンス チャンネル

インタビュー動画「自己増殖する人工細胞　生命誕生の謎に迫る」

https://sciencechannel.jst.go.jp

研究室ホームページ

http://www.chem.kanagawa-u.ac.jp/~sugawara/

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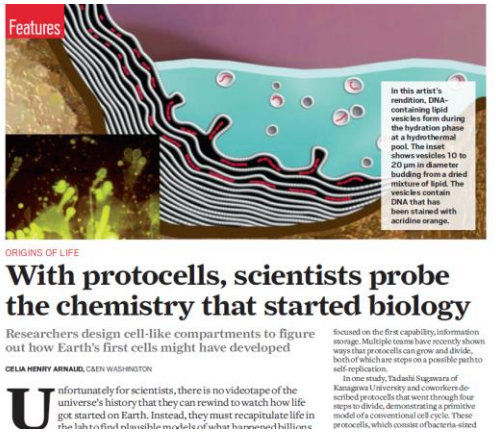
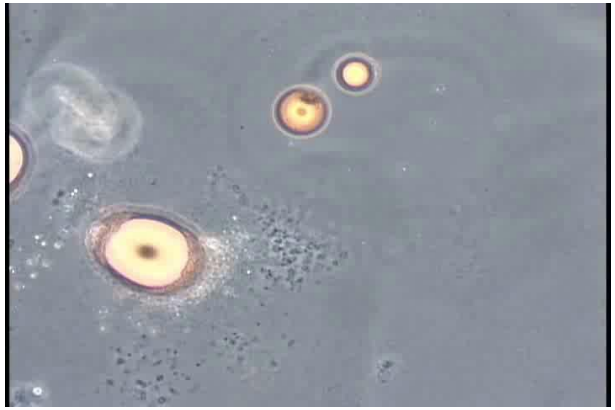
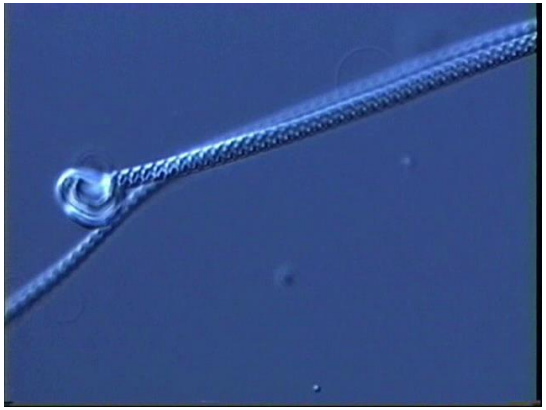
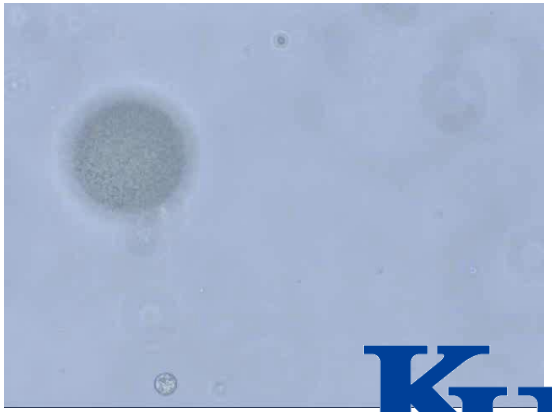


Self-propelling (Active Soft Matter)







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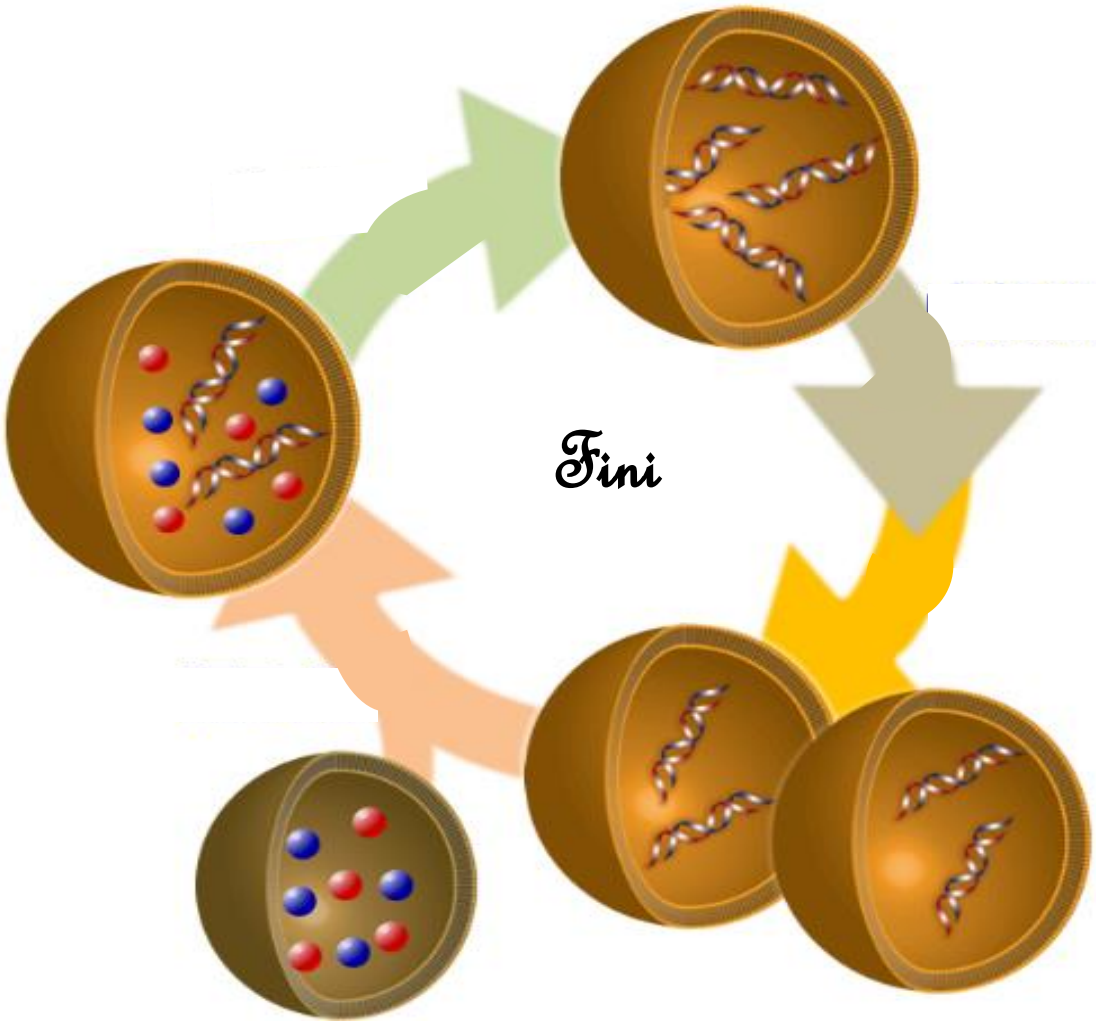
*Okazaki Institute for Integrative Biology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