1. **Pick up** Name Folder

- Pick up name folder and set it up at seat.

2. **Sit** with your lab group.

- laptops almost closed (avoid distracting)

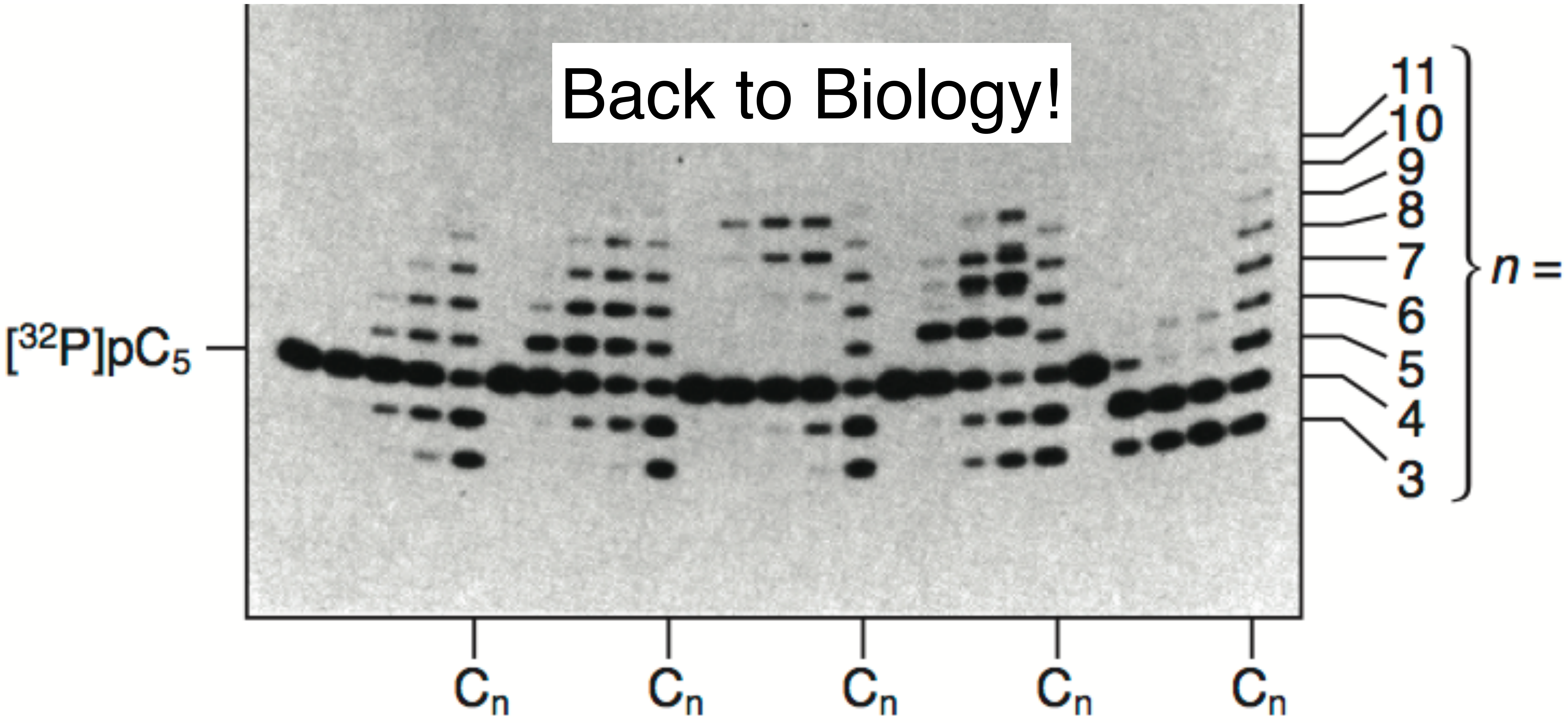
3. **Clicker** Attendance

- Launch your Top Hat, and get ready to click.


. Laptops closed (unless TopHat)
(open if need 4 TopHat, don’t distract others)

Announcements

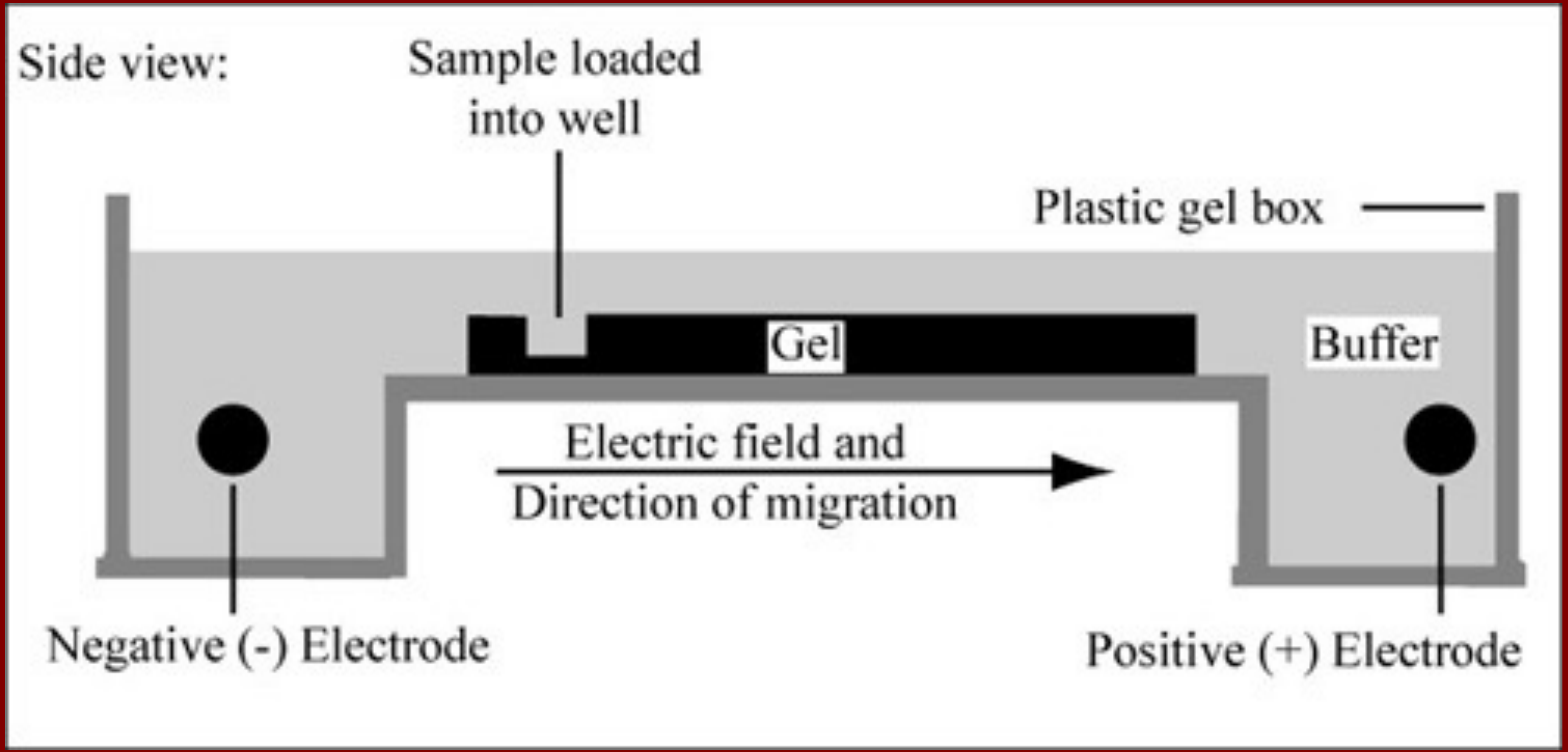
1. Pink **Contract:** signed and submitted was due by Friday 5pm
2. **Thanks to those who completed the TopHat homework for today** and FYI use the Discussion Forum if your have a question which might be shared by others.
3. **Office Hours**- M/W 2-3pm and appts + both espresso and fireplace!
4. **Clickers** and attendance points (if we have 10 clicker Qs, need ?? correct for 4.0 grade, use pen/pencil to keep laptop awake while closed)

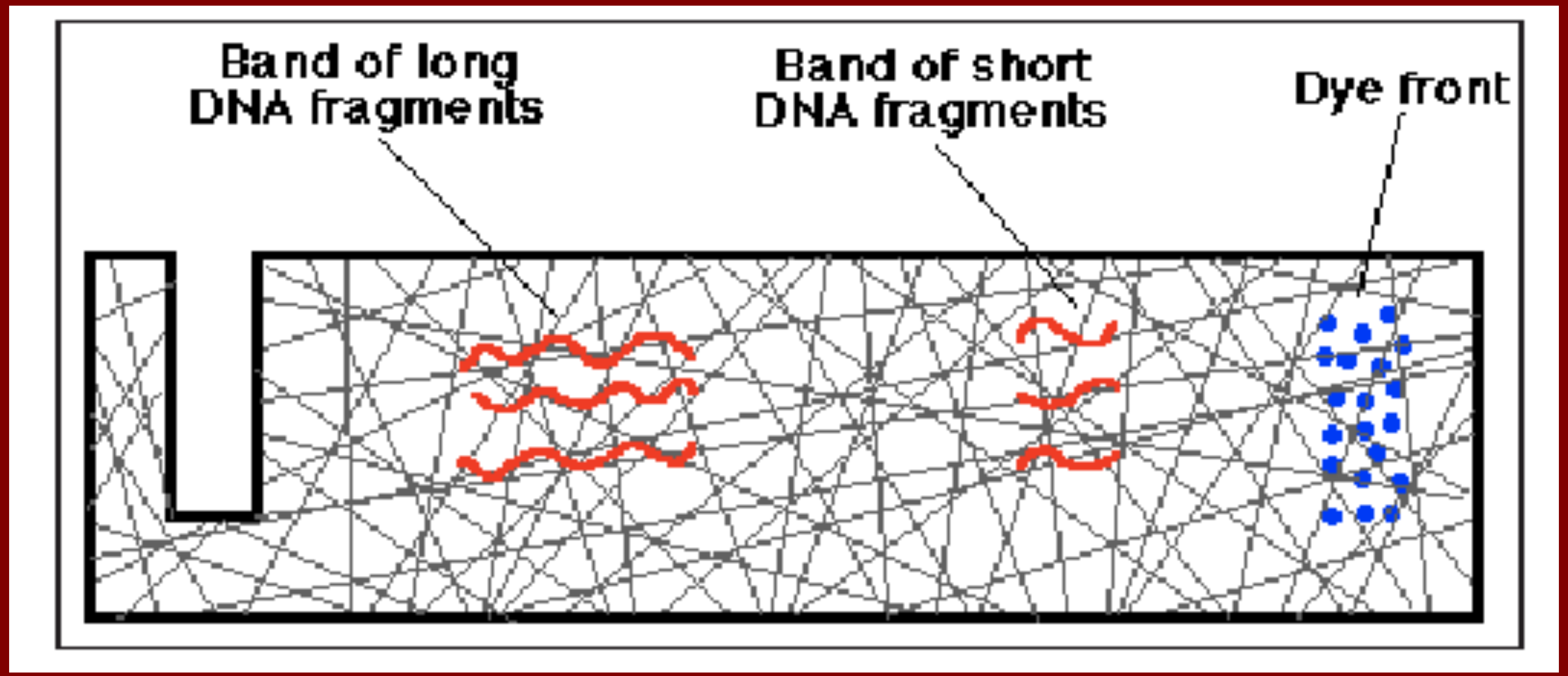


Back to Biology!
[32P]pC5
11
10
9
8
7
6
5
4
3
n =
Cn
Cn
Cn
Cn
Cn

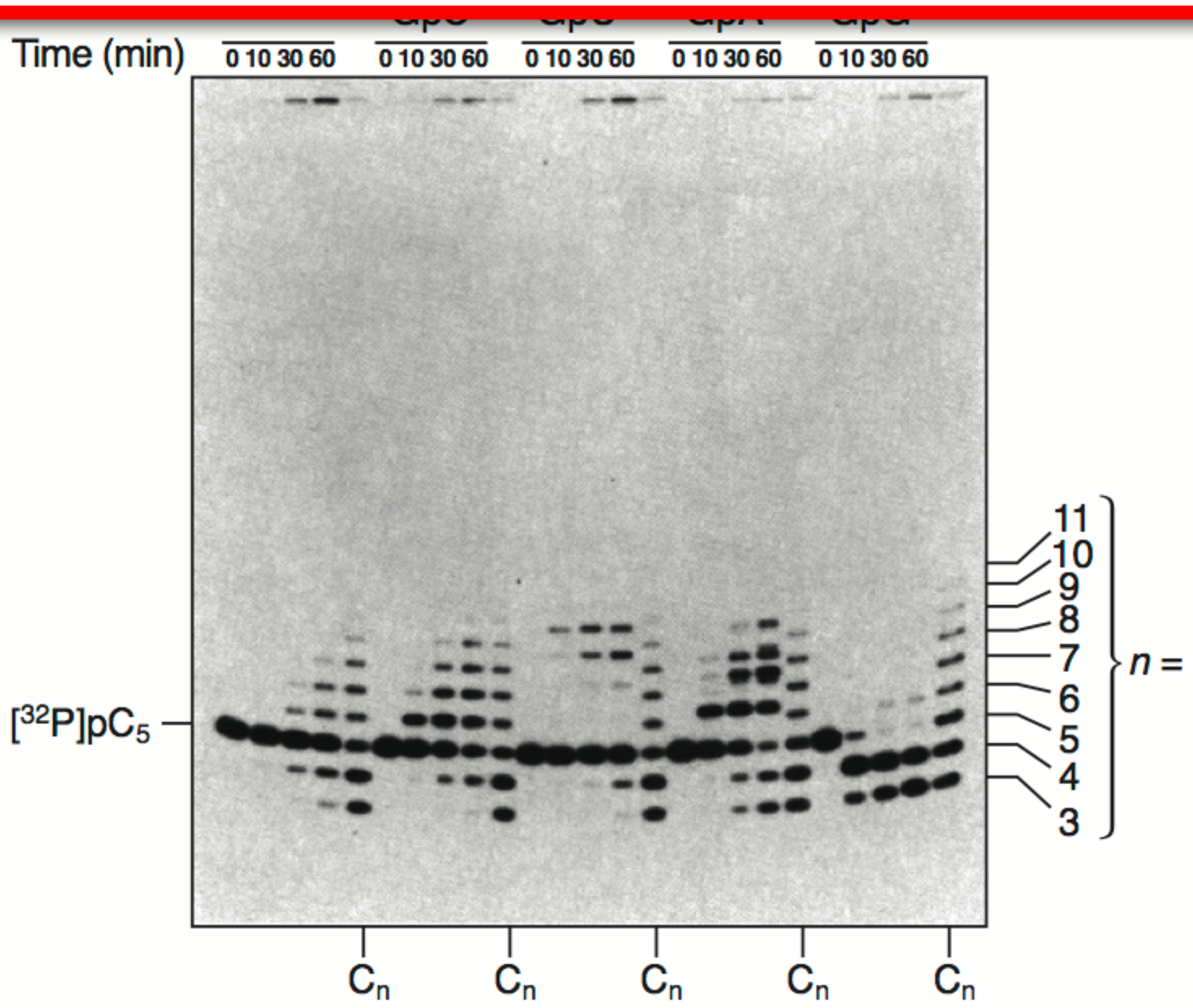
How Does a Gel Work?

- Check a PCR sample by gel electrophoresis.





1. How do they visualize the molecules of interest?
2. How do they separate them in an orderly fashion?



modified from Been and Cech. 1998

Functional Ribozyme

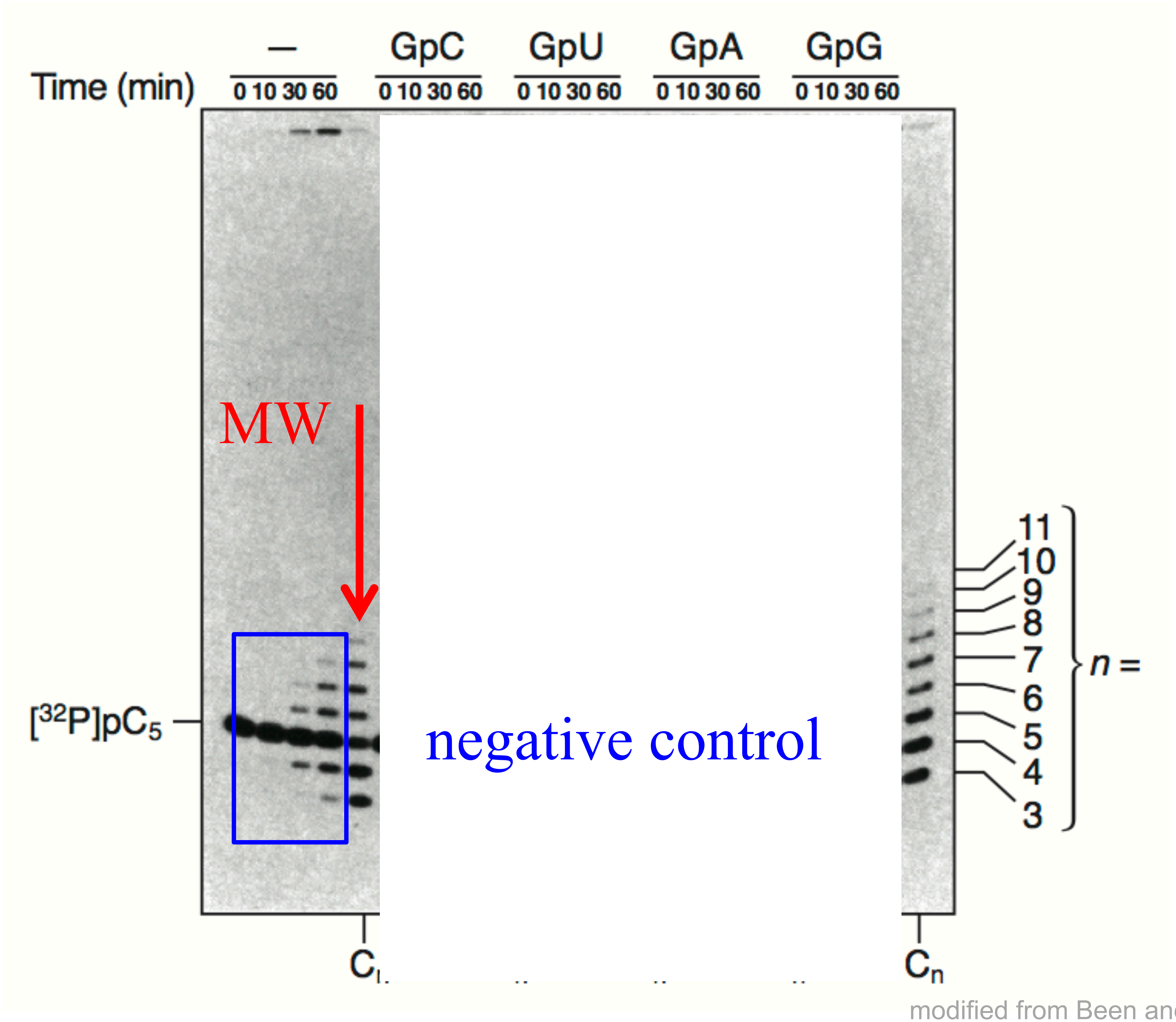


Fig. 4.8

modified from Been and Cech. 1998

Functional Ribozyme

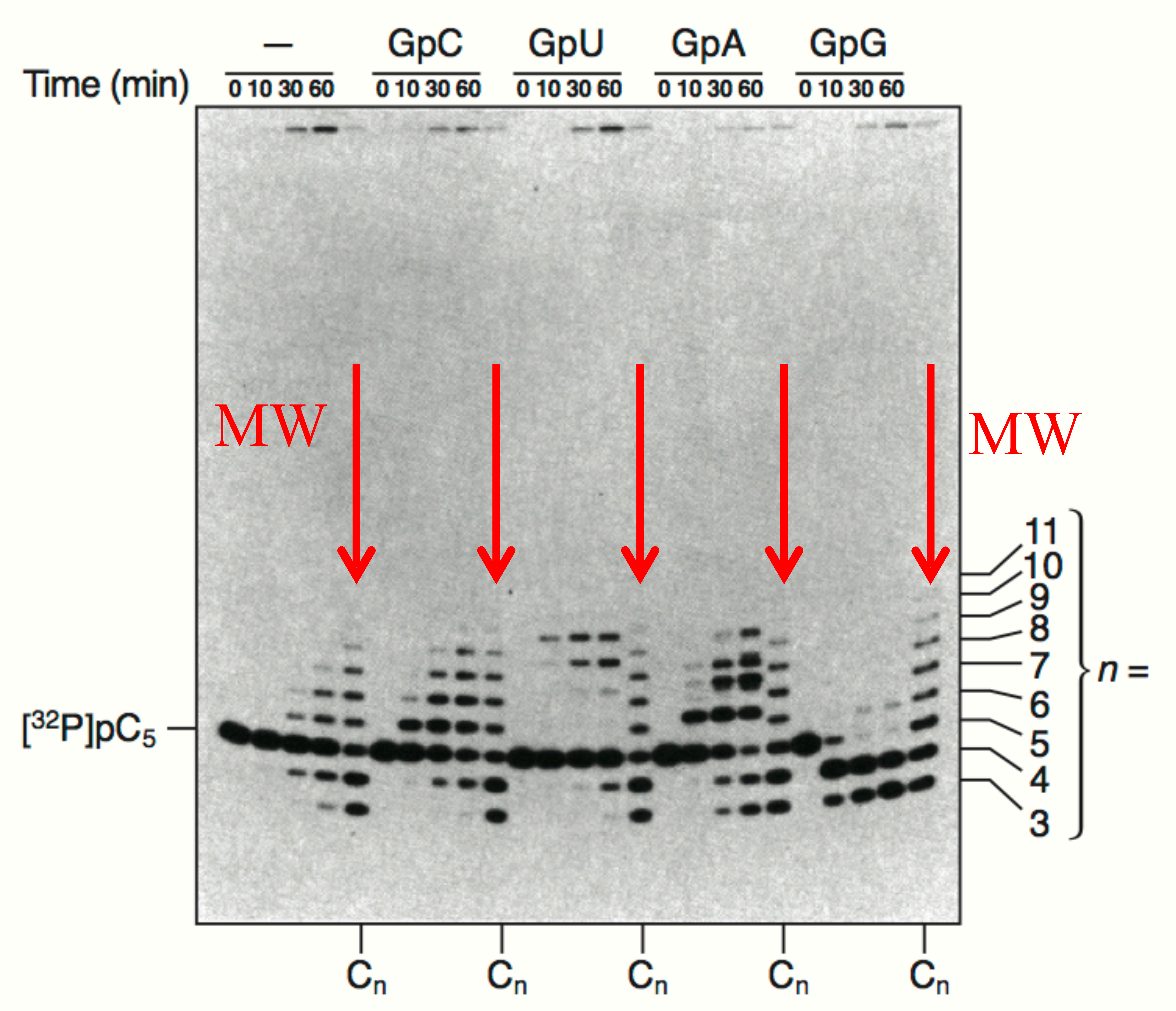


Fig. 4.8

modified from Been and Cech. 1998

Functional Ribozyme

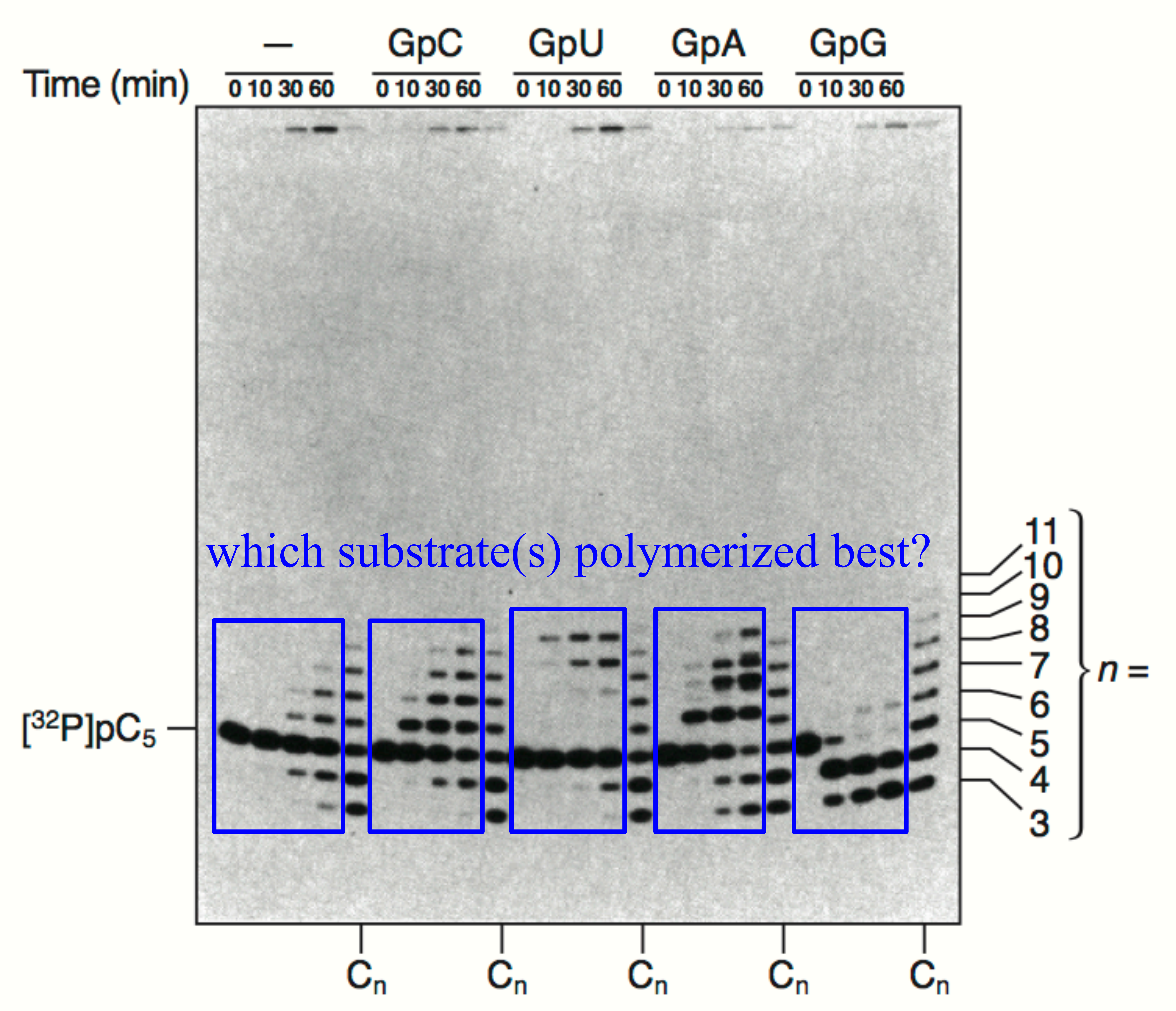


Fig. 4.8

modified from Been and Cech. 1998

4.3 Can non-living objects compete and grow?

- Context: Abiotic processes seem devoid of qualities associated with life such as growth and competition for limited resources.
- Major themes: The origin of living systems occurred by natural processes, and life continues to evolve within a changing environment; organisms can be linked by lines of descent from common ancestry; and natural selection is a mechanism of evolution that accounts for adaptation.
- Bottom line: Abiotic vesicles can grow, reproduce, and compete for limited resources.

Biology Learning Objectives

- Illustrate how natural selection works by giving a real example.
- Discuss how abiotic vesicles can grow and compete.
- Illustrate how abiotic structures exhibit dynamic and competitive behaviors.

Perhaps the most challenging aspect to understand about the origin of life is how a cell membrane could form before cells existed. You have already learned about abiotic production of biologically important organic molecules when you read about organic molecules found on meteors and in Miller's primitive earth experiment. Yet it might seem implausible for abiotic forces to organize lipids into a sphere surrounding some cargo and allow these spheres to grow and produce more spheres. Could one or more lipids self-organize and replicate solely based on the chemical properties determined by the composition of the molecules? If such lipids exist, are they capable of encapsulating cargo such as self-replicating ribozymes? If a vesicle possessed all of these properties, you might be able to imagine how life evolved over a long

Week 3

(Preparing for) **Monday's lecture:**

Budgeting homework time (70 min): In Ch. 4, the first half of section 4.3 is 2000 words in length and section 4.4 is 1500 words, totaling 3500. This should take 17 minutes if you just read it. But when done properly, when you pause to review quite a few figures, read and think about a few of the Integrating Questions, and take careful notes, this homework assignment should take you more like 70 minutes (if you are focused). **Special Allowance**: Your group can divide up the Trifectas for this lecture.

1. _______ **For Monday's lecture, read Chapter 4's** section 4.3(1st half) in the ICB textbook, and then section 4.4. For section 4.3 "Can non-living objects compete and grow?" you only need to carefully read and take notes on items up to and including information related to Figure 4.13. Then stop taking notes and just read the rest to learn about research on vesicles competing with each other. Explore Bio-Math Exploration 4.2 if you find it interesting. Then read and take notes on all of the short reading in section 4.4 "Can non-living objects harvest and store energy?". Be sure to take handwritten notes.

2. _______ **Try to answer some Integrating Question and Review Questions.** As you read the ICB textbook always attempt to test yourself a little, answer at least one of each set.

3. _______ (Trifecta): **Prepare to explain (aloud) Figures 4.11, 4.12, 4.13 and 4.17 in class**.
   Special Allowance today: If you wish your group can designate who will be responsible for each figure and thus split up the responsibility and reduce the load (Purpose, Methods, Findings).

* Revisit large gel from last time?

1-23-23 Ch. 4 4.3 (½) + 4.4

4.3 Can non-living objects compete and grow? Q why ask this question?

Q: What non-living life-like forms particles killed millions last year?

L.O.s
- Illustrate natural selection with real example
- Discuss how abiotic vesicles can grow + compete
- Analyze data to confirm

--> Was challenging to understand how cell membrane could form BEFORE cell. Might seem unlikely for abiotic forces to organize lipids into sphere w/cargo AND allow spheres to grow + reproduce?!

Q: Can lipids self-assemble and form vesicles with cargo?!
↳ what evidence supports it?

Self-organizing vesicles

As described, amphiphilic/pathic lipids have been created on meteors, NASA + even deep sea vents. YET how could they get close together, concentrated? Q:?

Fig 4.11 - fatty acid - myristoleate - congregates on clay - catalyst (example crumb of food dropped into soda)

can spontaneous form structures called micelle or vesicle (even flat planes)

Purpose - test whether different substrates can facilitate micelle formation in a sol'n

Methods - use light spectrophotometer to track light absorption as micelles form (Or vesicles). Vesicles block light.
Mix microspheres or - or - charged clay (montmorillonite) particles with myristoleate in aqueous (water) solution w/ buffer. Perform dose response curve for "clay".

Findings - clay (montmorillonite) works well, negative charged ceramic microspheres too. Dose response shows initial slope/interest

IQs 10: H_2O go thru mems? 11. What is best in 4.11C, charge?, conc of clay best? (use Fig to support claim)
12: Predict what if... add more clay 4.11D? or more lipid?

Fig 4.12 cargo trapped?

↓

1/23/23

...g? Q: characteristic of life: ?

...trapped inside vesicles when form

...abeled green-fatty acids mixed ... ceramic microspheres + viewed via ... RNA molecules in sol'n - red fluore...

...go w/ other vesicles inside and k...

...d reproduction

...ics of life: grow + reproduce

..."grow" and/or "reproduce"?

... ⊙ ← ○

...sicle size over time as add fatty ...ate population of similar size ... filter. Then "fed" them micelles.

...ries? work? push thru filter ... micelles, filter again, ...uch 300% equal

...s surface area as grows vs shrinks ... + volume = to ? children?

...ymes leak out during reproduction ...hanism to form first cell. ← how

...tition" Fig 4.14

...s from characteristics of life to ...lection. And tests vesicles for possible ability to compete. -> Evolution

Section 4.4 1-23-23 (cont) Ch. 4

4.4 Can non-living objects harvest and store energy?

L.O.s
- Use evidence to support possibility life evolved from abiotic...
- Discuss how vesicles could store energy

--> Life requires energy, could abiotic vesicles do that? Make + store?
Idea tested could liquid inside vesicle hoard H+s inside = store

Figure 4.17 + diagram 4.16

Purpose - test possibility prototype primitive cell could hold H+s like (energy) mito does

Methods - Idea/Hypothesis add lipids to existing vesicles, to the outer layer of the membrane, which had negatively charged group that could bind H+s, they likely would flip inward to opposite side and release H+s. -> proton gradient (pmf)
Method squirt in micelles, amount lipid equal to amount in vesicles then and track pH inside vesicles (somehow). start 8.2 (alkaline)
Also measured surface area of vesicles (somehow)

Q: lacking actual Methods?

Findings when adding lipids via micelles pH does drop inside vesicle's lumen, AND if you add more micelles it drops more quickly. AND the surface area of the vesicles grows in same time.
Q: Why checking how fast + surface area change?
life needs fast change, + needs to store energy long time + multi lines of evidence

IQ: explain exp, and predict what happen if vesicles divided/reproduced to H+s trapped inside lumen

All Student Responses

25/29 86%

M3: Predictions for Figure 4.11

Grade Responses

IQ12: Predict what would happen if you added more clay in Figure 4.11D after 30 minutes of reaction. Predict what would happen in Figure 4.11D if you added more lipid to the highest clay condition.

Responses

All Student Responses

25/29 86%

M3: pH gradients?

Grade Responses

IQ20: Based on your previous biology knowledge, can a pH gradient across membranes be used to produce new covalent bonds? (for example?)

Responses

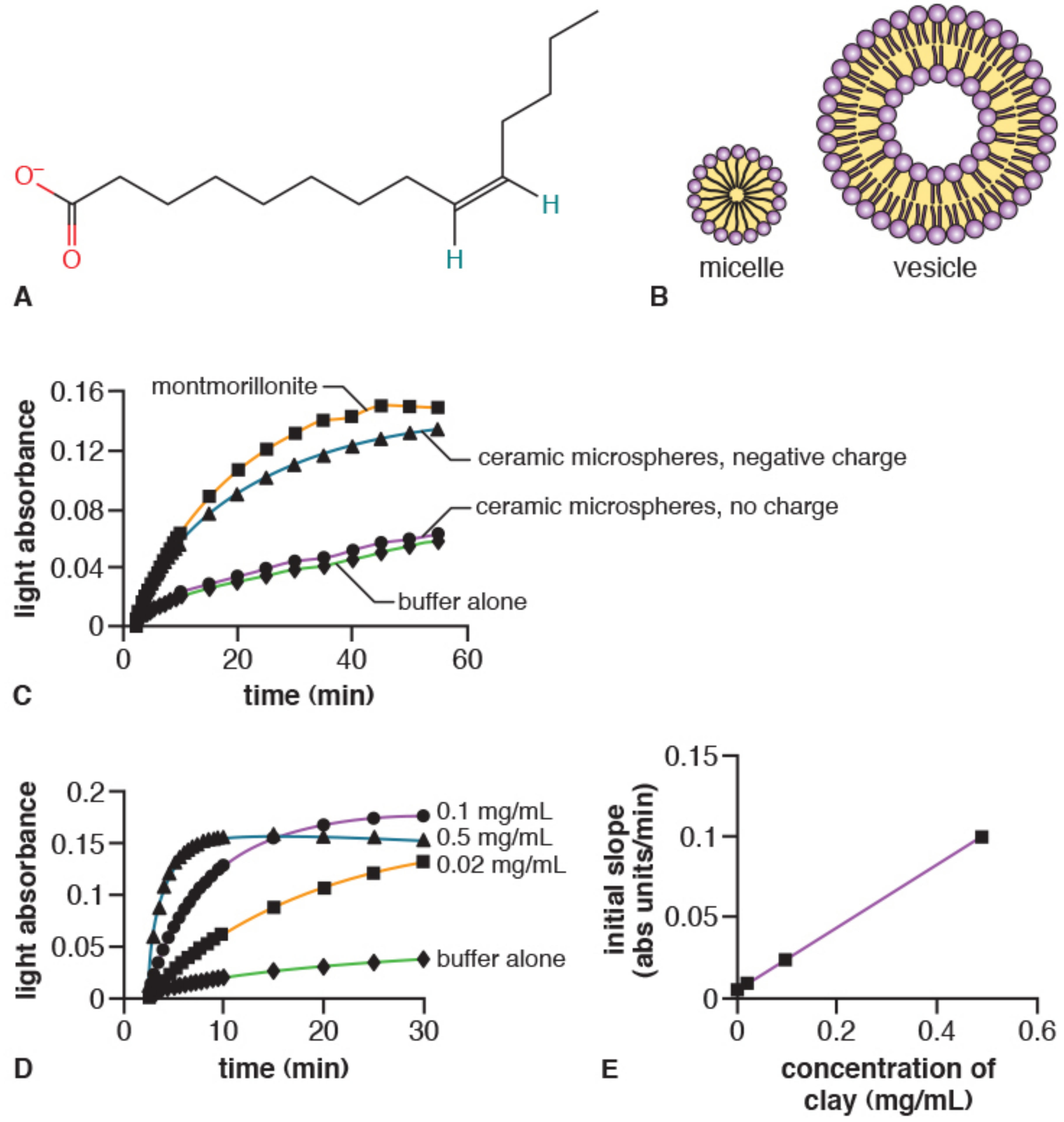
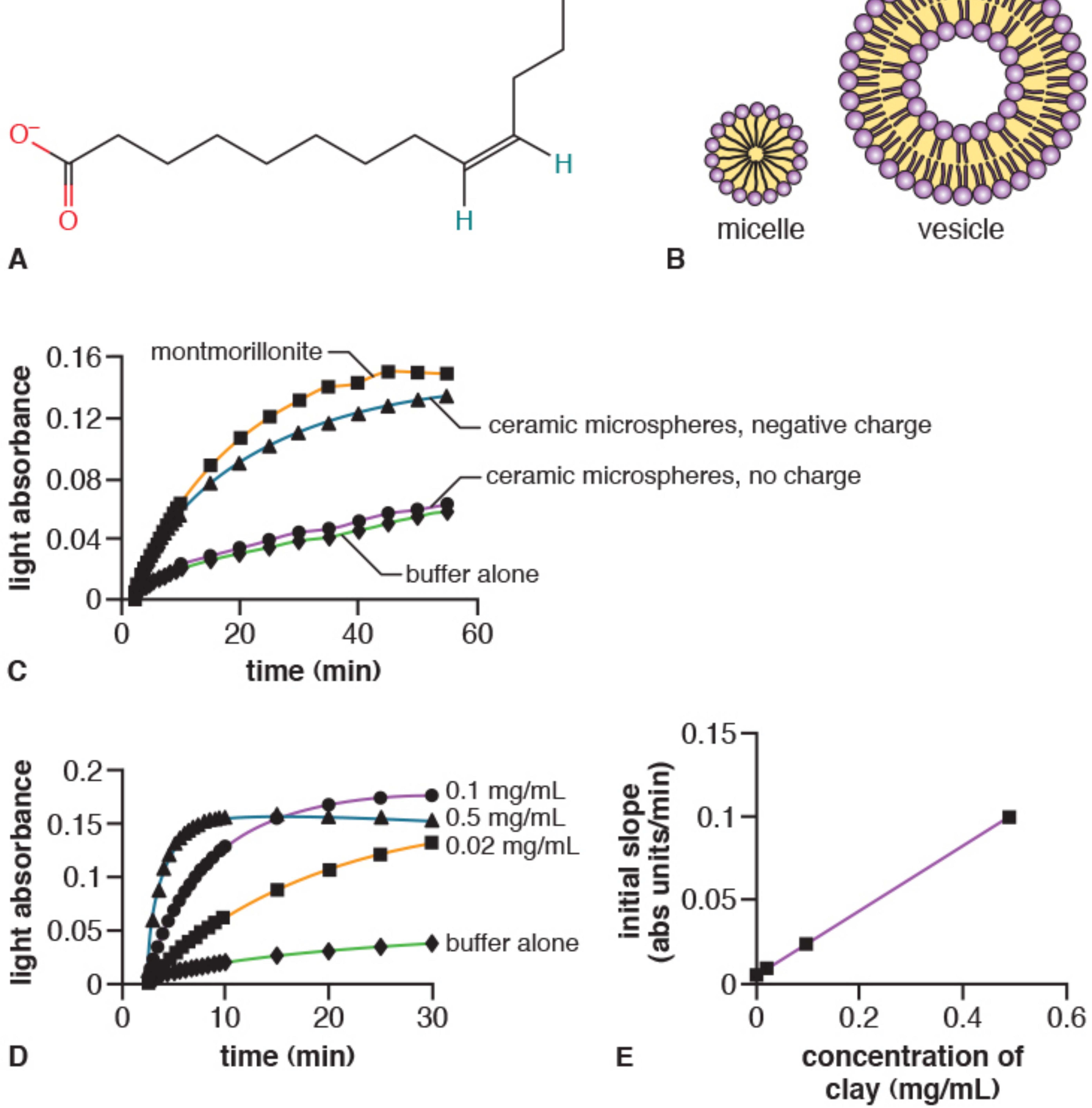


Figure 4.11 Fatty acid structures and vesicle formation rates. **A,** Myristoleate chemical structure. **B,** Fatty acid monolayer forming a micelle and a bilayer forming a membrane vesicle. **C,** Effects of different solid surfaces for vesicle formation. **D,** Effects of different concentrations of clay on vesicle formation. **E,** Initial rates of vesicle formation based on clay concentrations. A. from http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=5461014&loc=ec_rcs B.general knowledge. C, D, E. From Hanczyc *et al.*, 2003. Figure 1D, 1B, and 1E. Martin M. Hanczyc, Shelly M. Fujikawa, Jack W. Szostak. 2003. Experimental Models of Primitive Cellular Compartments: Encapsulation, Growth, and Division. Science. 302: 618 – 622. Reprinted with permission from AAAS.

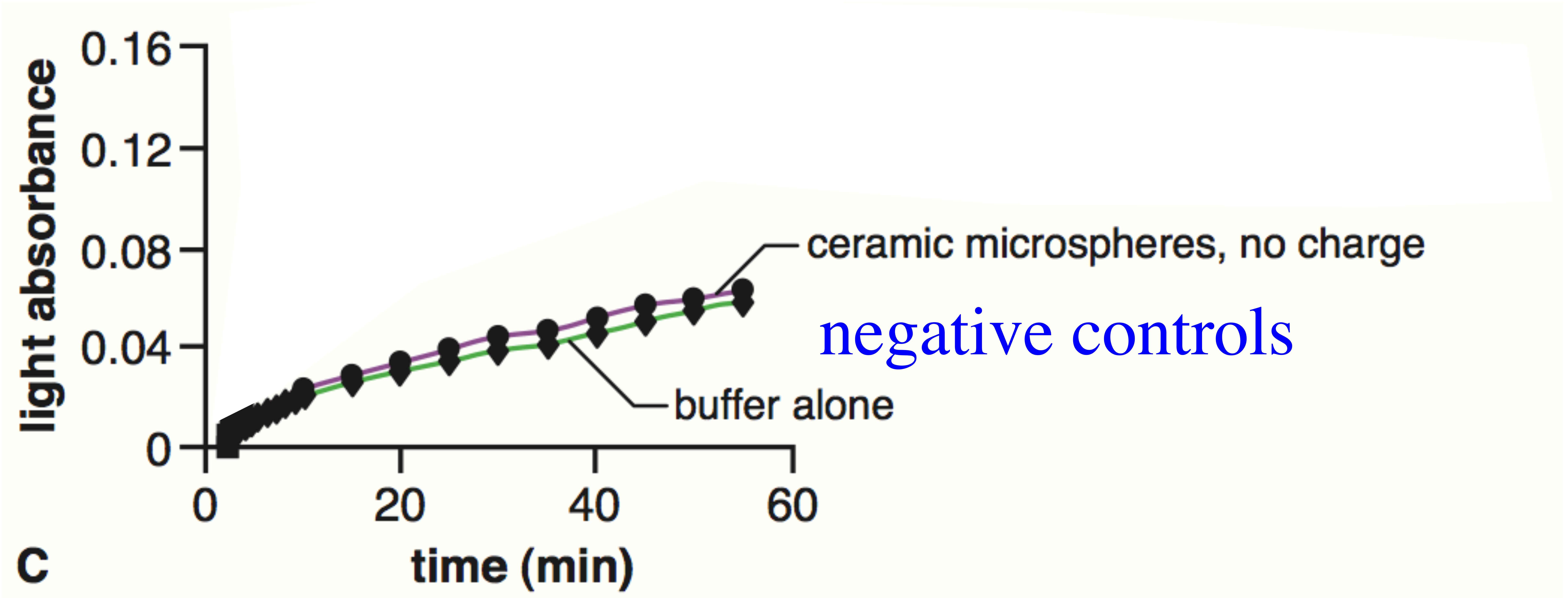
(Trifecta)



O⁻
O
H
H
A
micelle
vesicle
B
montmorillonite
ceramic microspheres, negative charge
ceramic microspheres, no charge
buffer alone
light absorbance
0.16
0.12
0.08
0.04
0
0
20
40
60
time (min)
C
0.1 mg/mL
0.5 mg/mL
0.02 mg/mL
buffer alone
light absorbance
0.2
0.15
0.1
0.05
0
0
10
20
30
time (min)
D
initial slope (abs units/min)
0.15
0.1
0.05
0
0
0.2
0.4
0.6
concentration of clay (mg/mL)
E

Unable to form Vesicles Quickly

measure of
vesicle formation



negative controls

Fig. 4.11

C, D, E. modified from Hanczyc *et al.*, 2003.

Catalytic Charged Surfaces

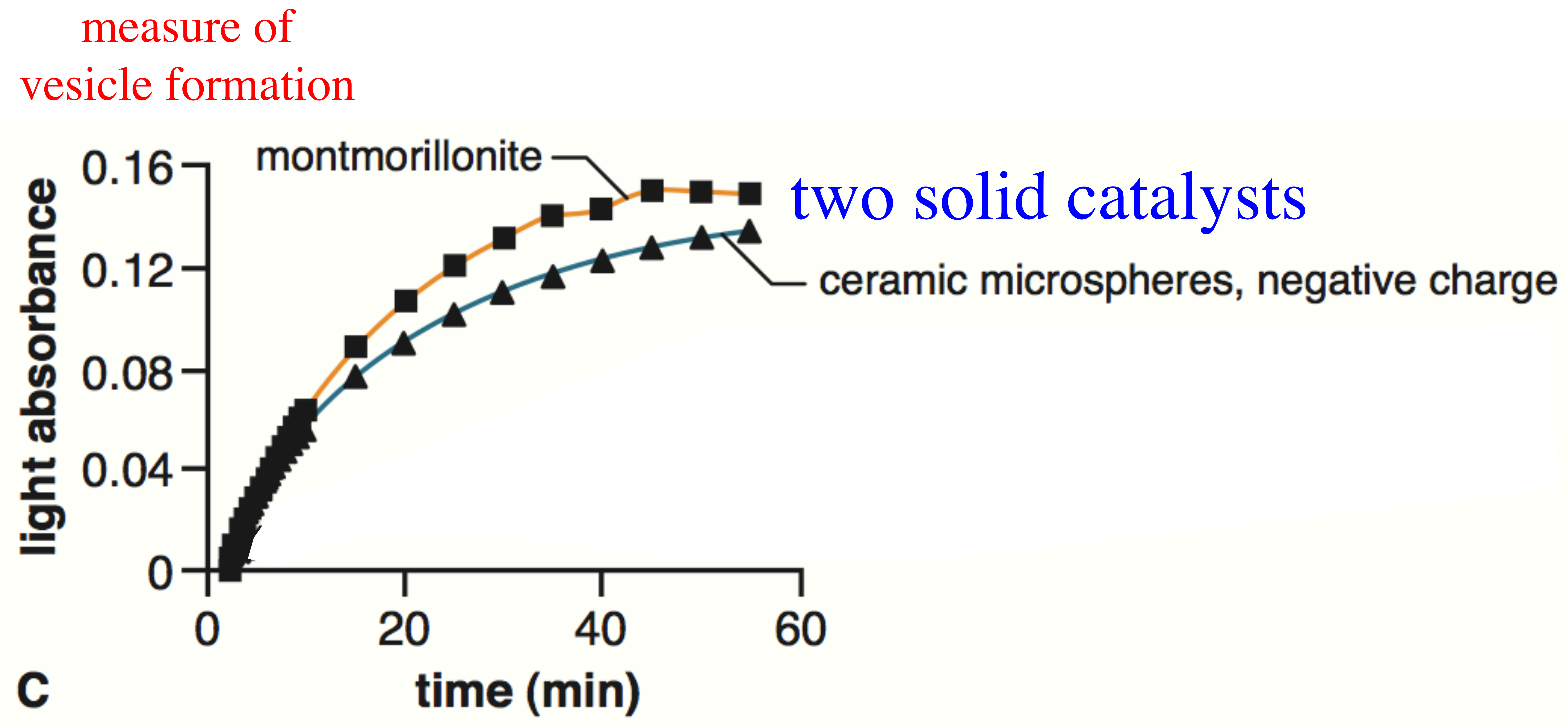


Fig. 4.11

C, D, E. modified from Hanczyc *et al.*, 2003.

Catalytic Charged Surfaces

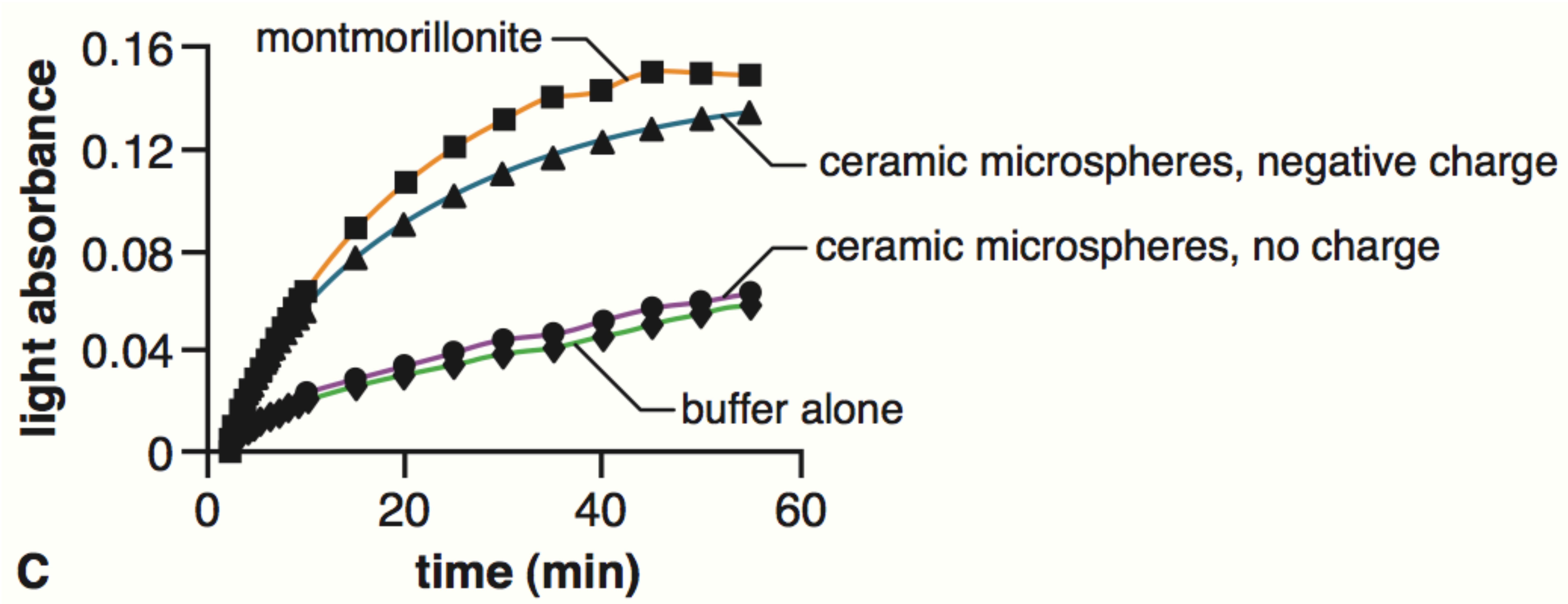


Fig. 4.11

C, D, E. modified from Hanczyc *et al.*, 2003.

Clay Concentration Effects

measure of
vesicle formation

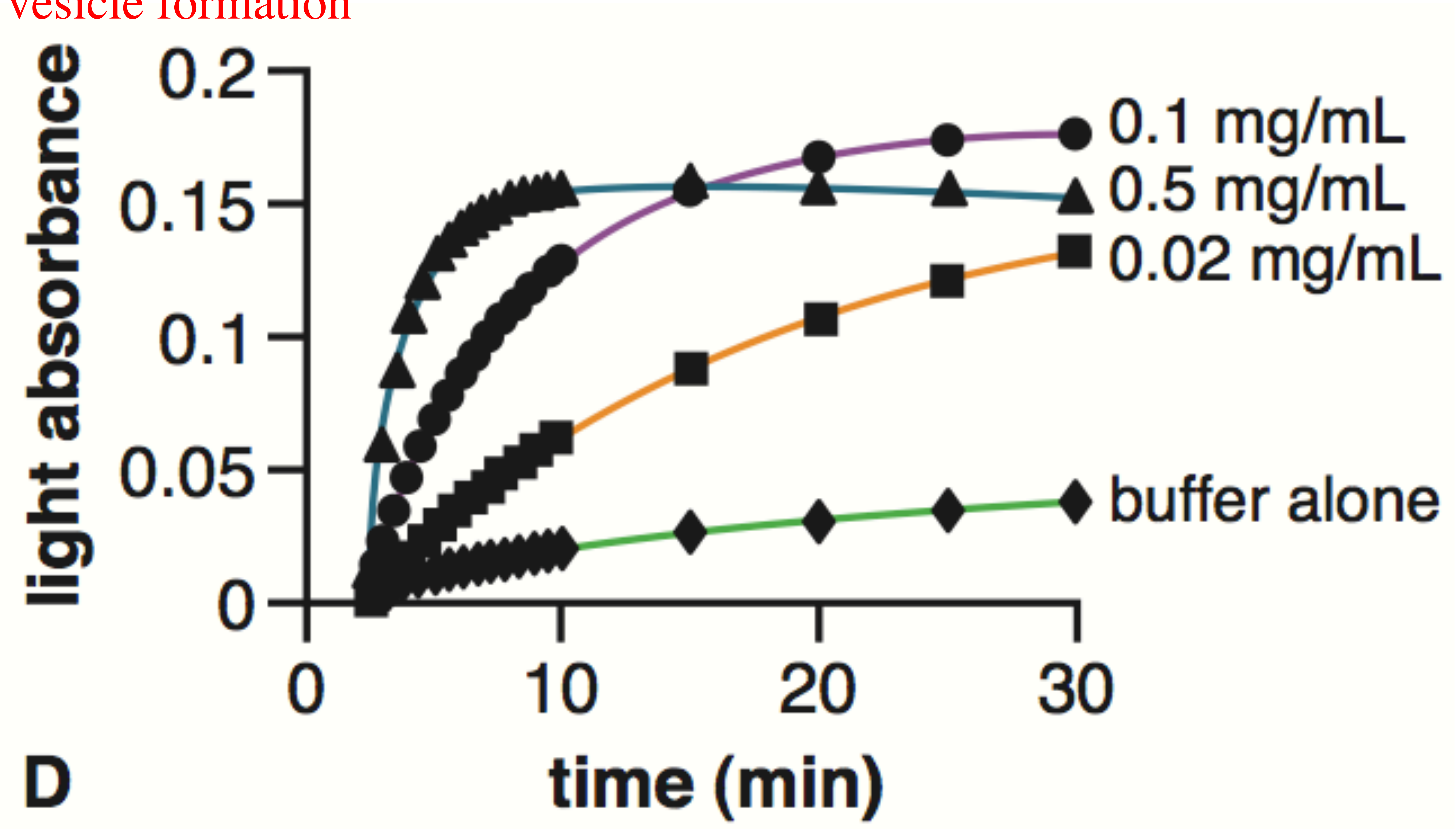


Fig. 4.11

C, D, E. modified from Hanczyc *et al.*, 2003.

Clay Concentration Effects

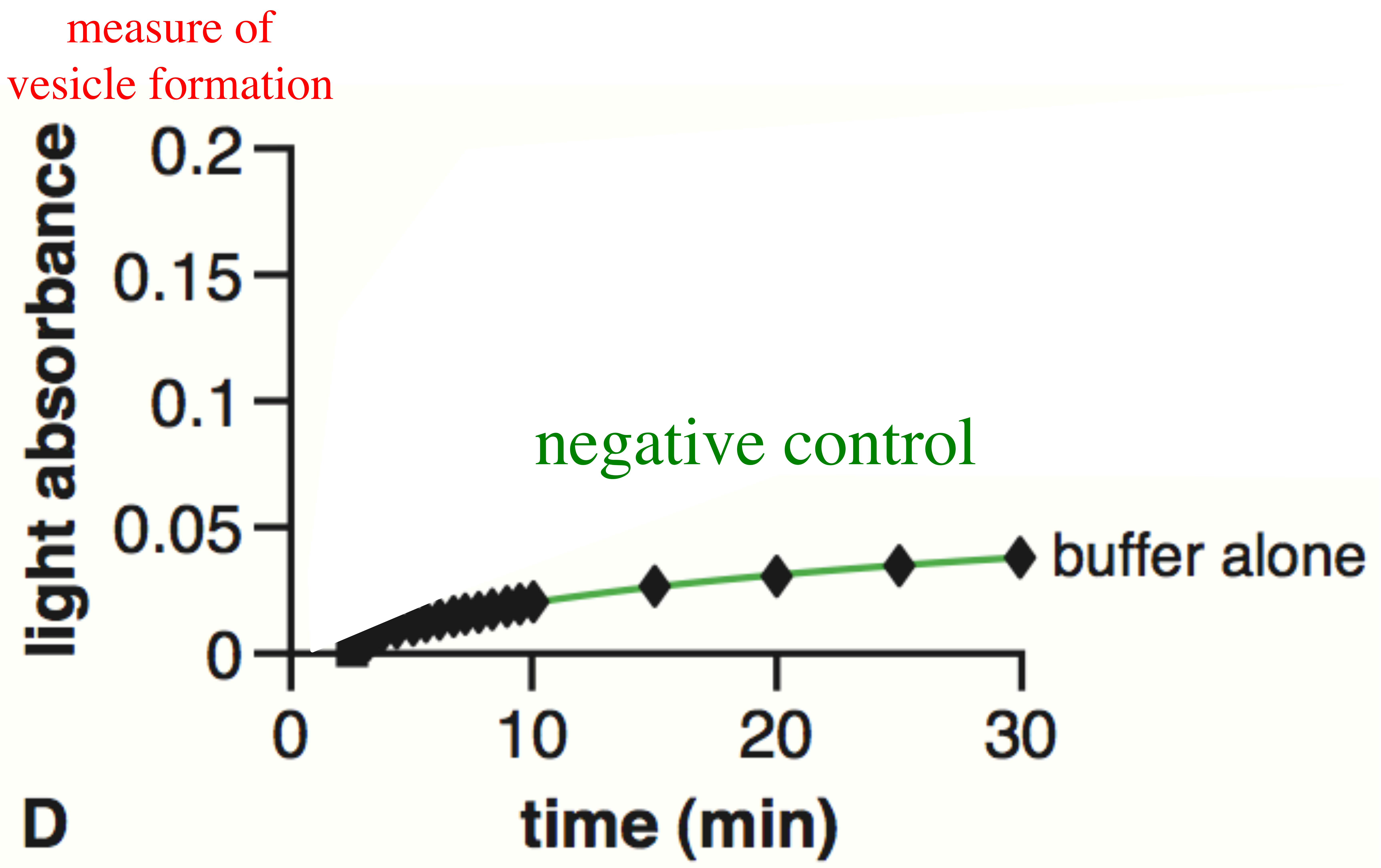


Fig. 4.11

C, D, E. modified from Hanczyc *et al.*, 2003.

Clay Concentration Effects

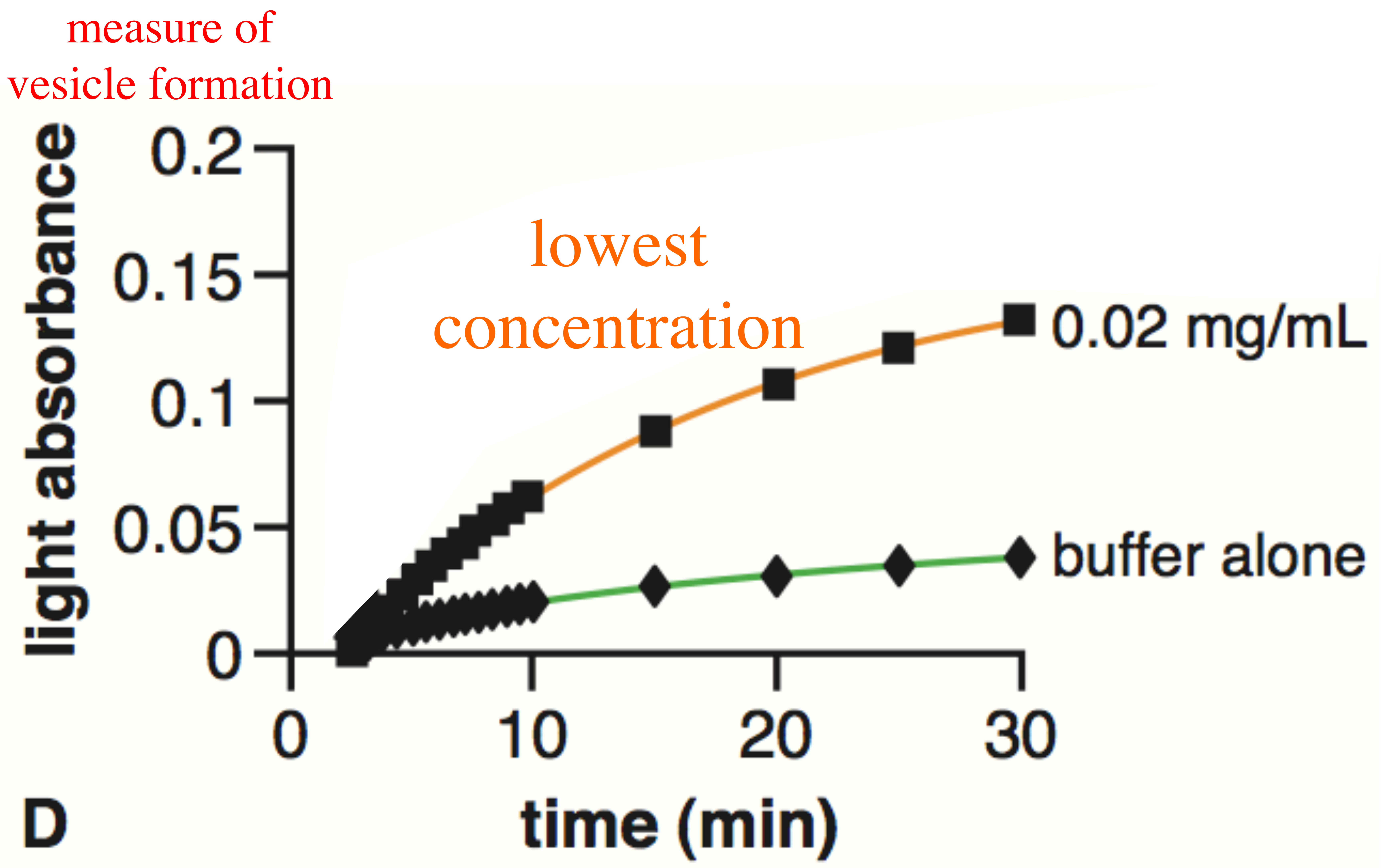


Fig. 4.11

C, D, E. modified from Hanczyc *et al.*, 2003.

Clay Concentration Effects

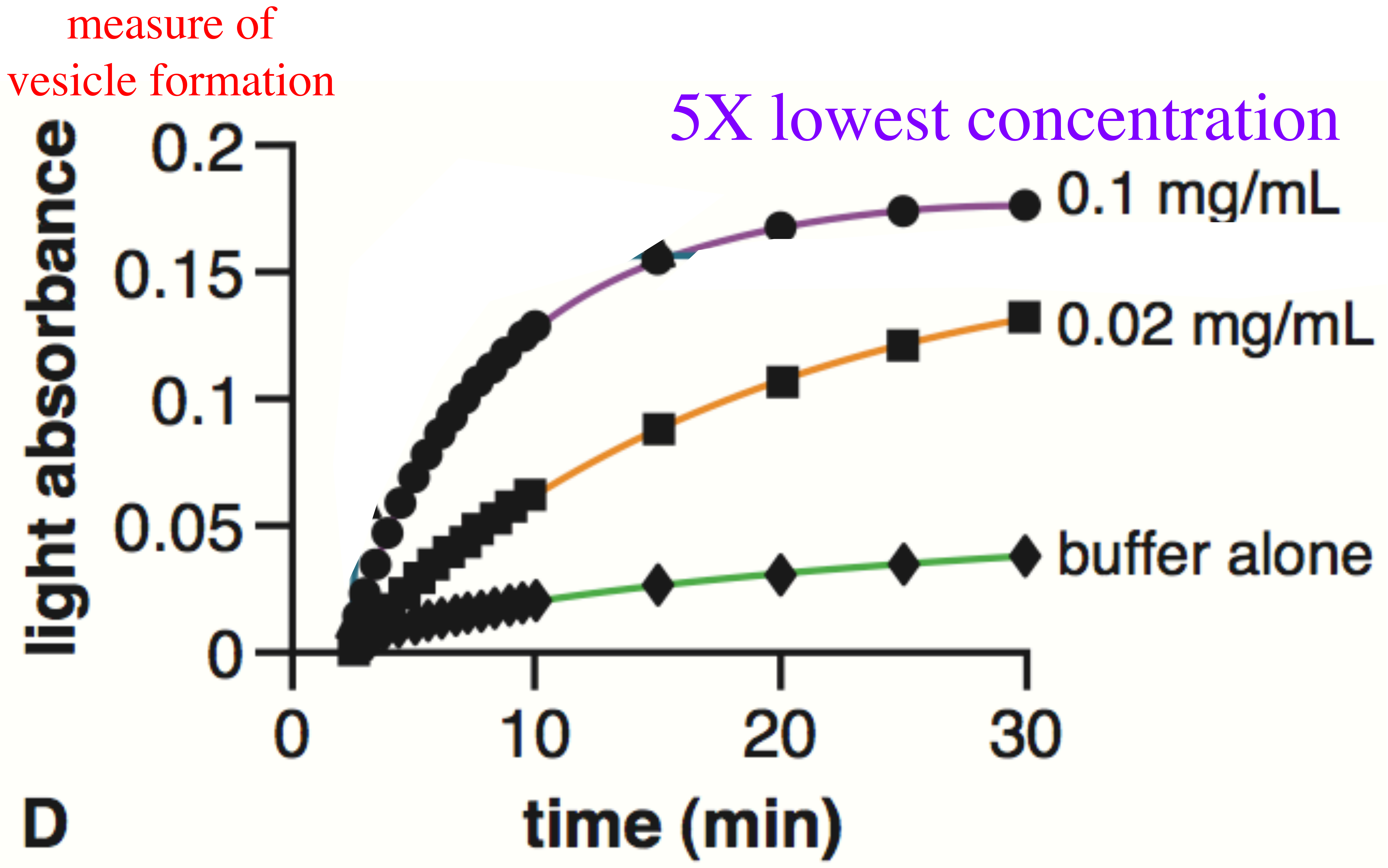


Fig. 4.11

C, D, E. modified from Hanczyc *et al.*, 2003.

Clay Concentration Effects

measure of vesicle formation

25X lowest concentration

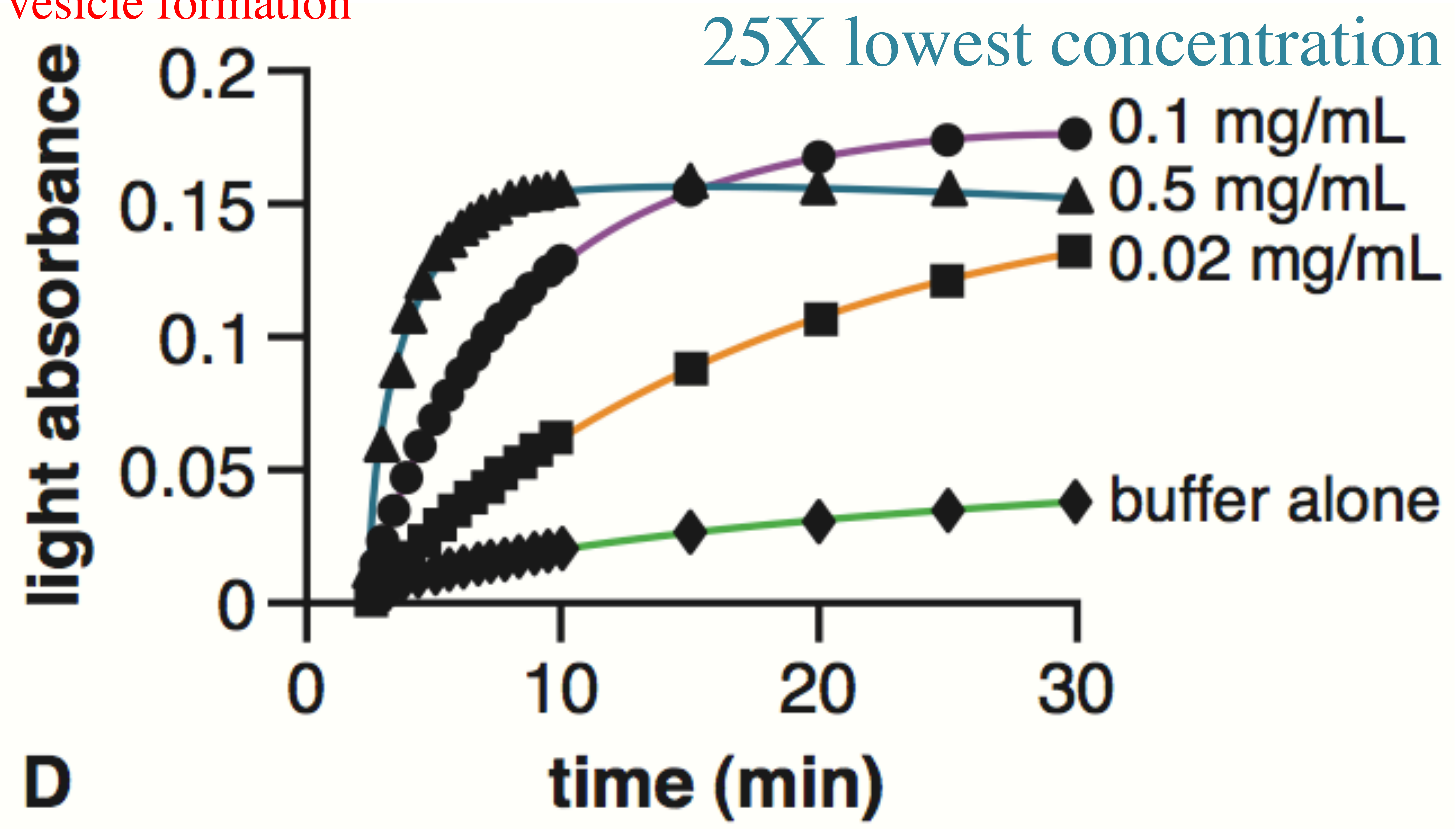


Fig. 4.11

C, D, E. modified from Hanczyc *et al.*, 2003.

Clay Concentration Effects

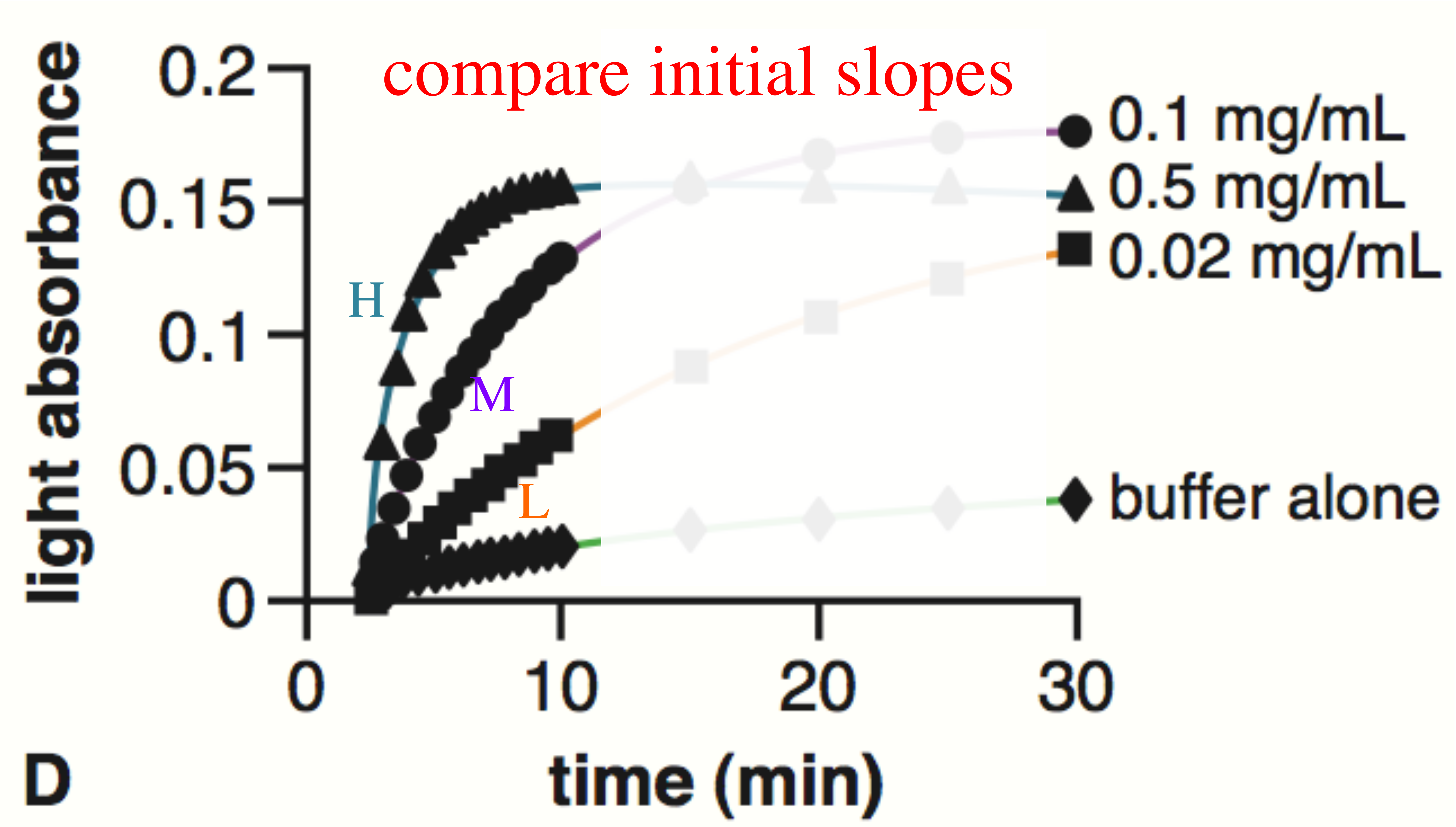


Fig. 4.11

C, D, E. modified from Hanczyc *et al.*, 2003.

Clay Concentration Effects

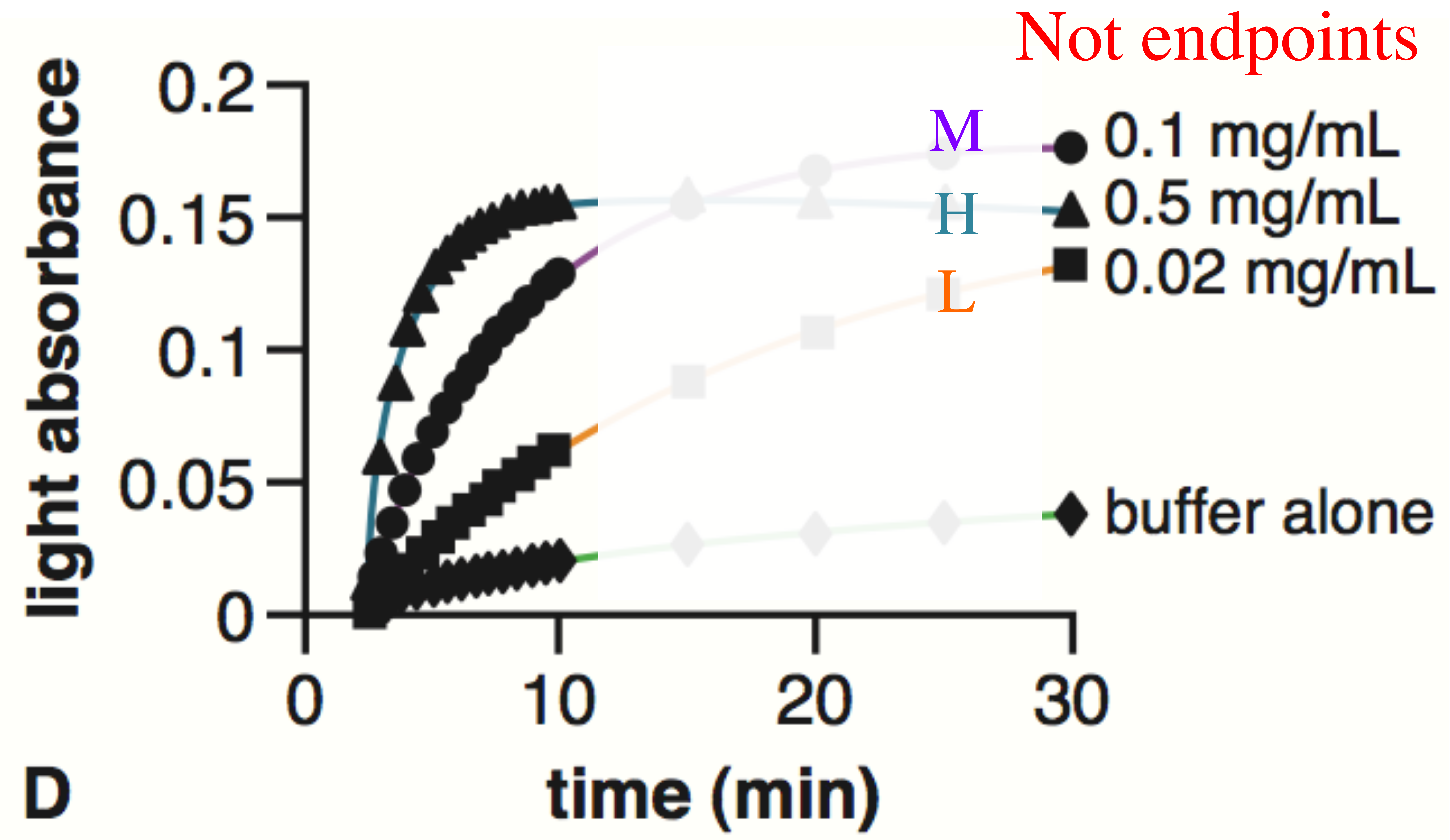


Fig. 4.11

C, D, E. modified from Hanczyc *et al.*, 2003.

Clay Concentration Effects

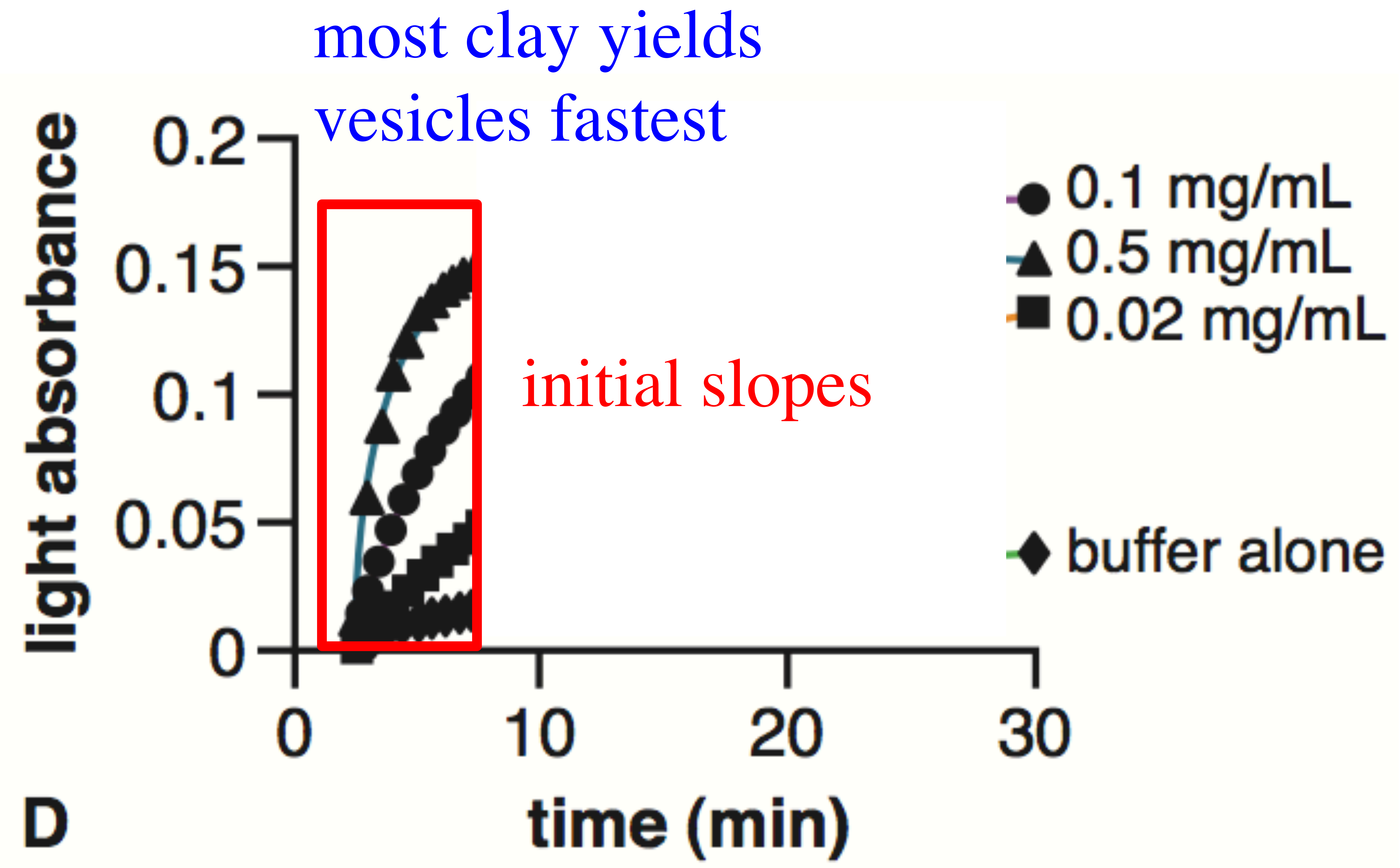


Fig. 4.11

C, D, E. modified from Hanczyc *et al.*, 2003.

Clay Concentration Effects

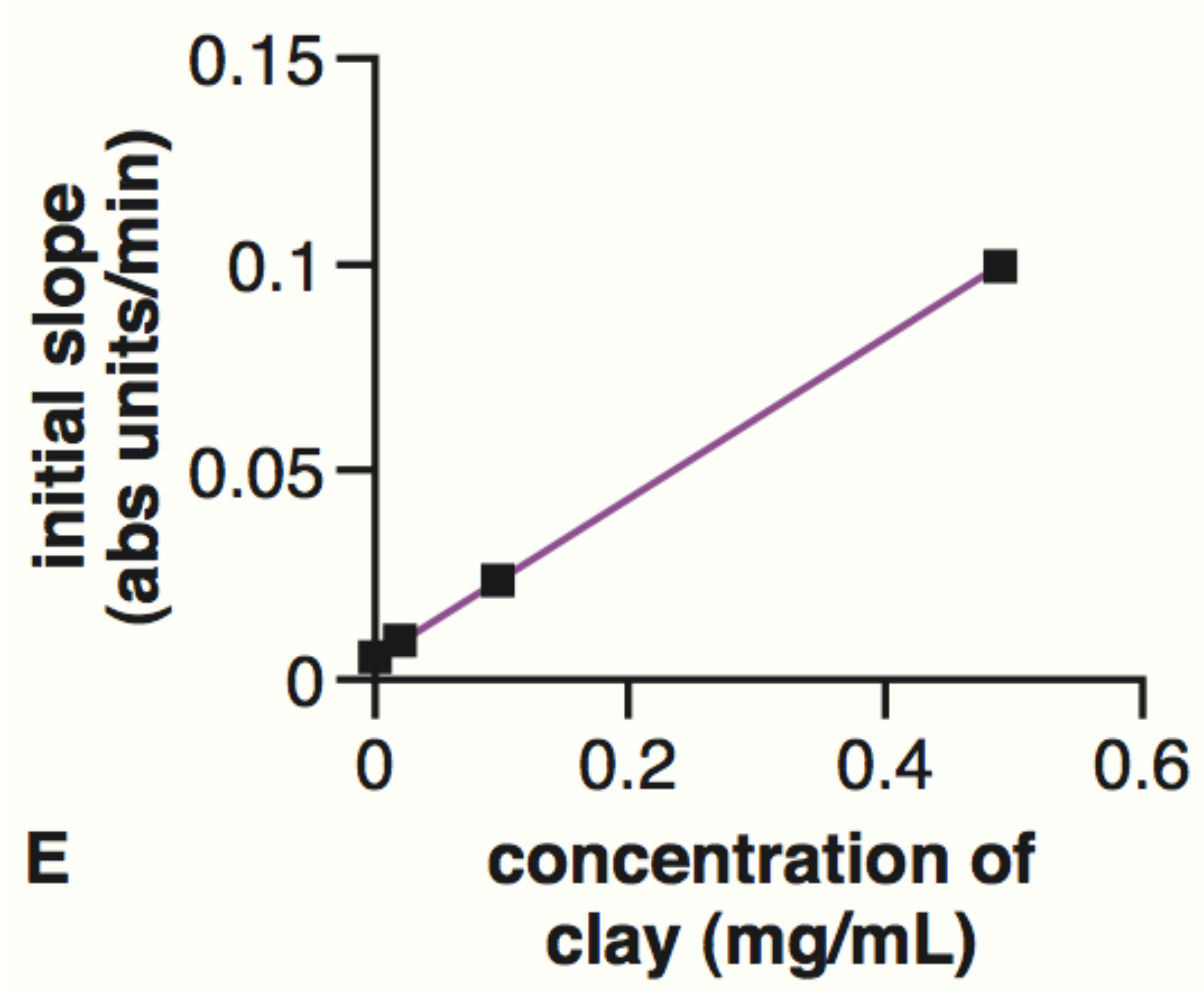
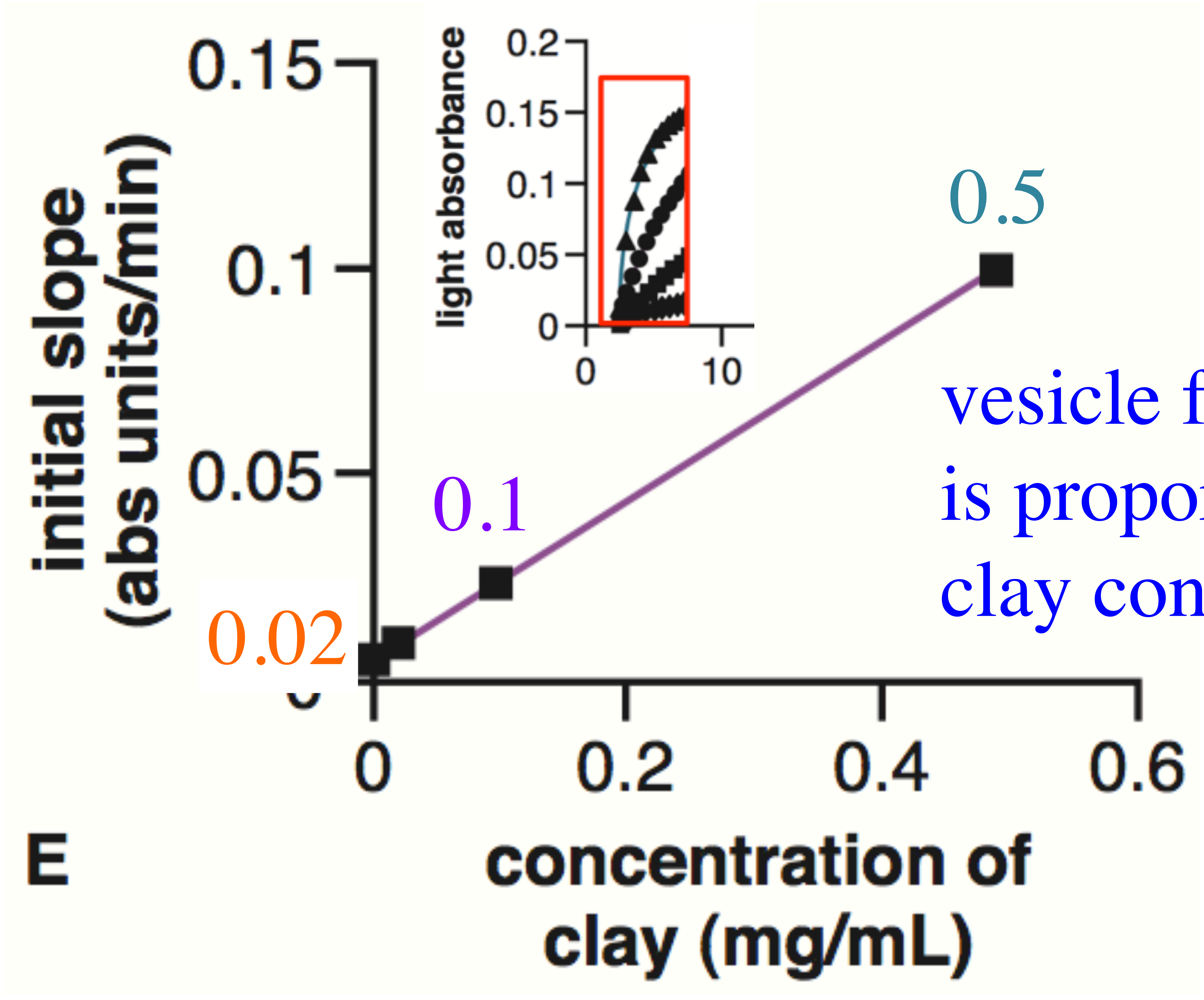


Fig. 4.11

C, D, E. modified from Hanczyc *et al.*, 2003.

Clay Concentration Effects



vesicle formation is proportional to clay concentration

Fig. 4.11

C, D, E. modified from Hanczyc *et al.*, 2003.

(Trifecta)

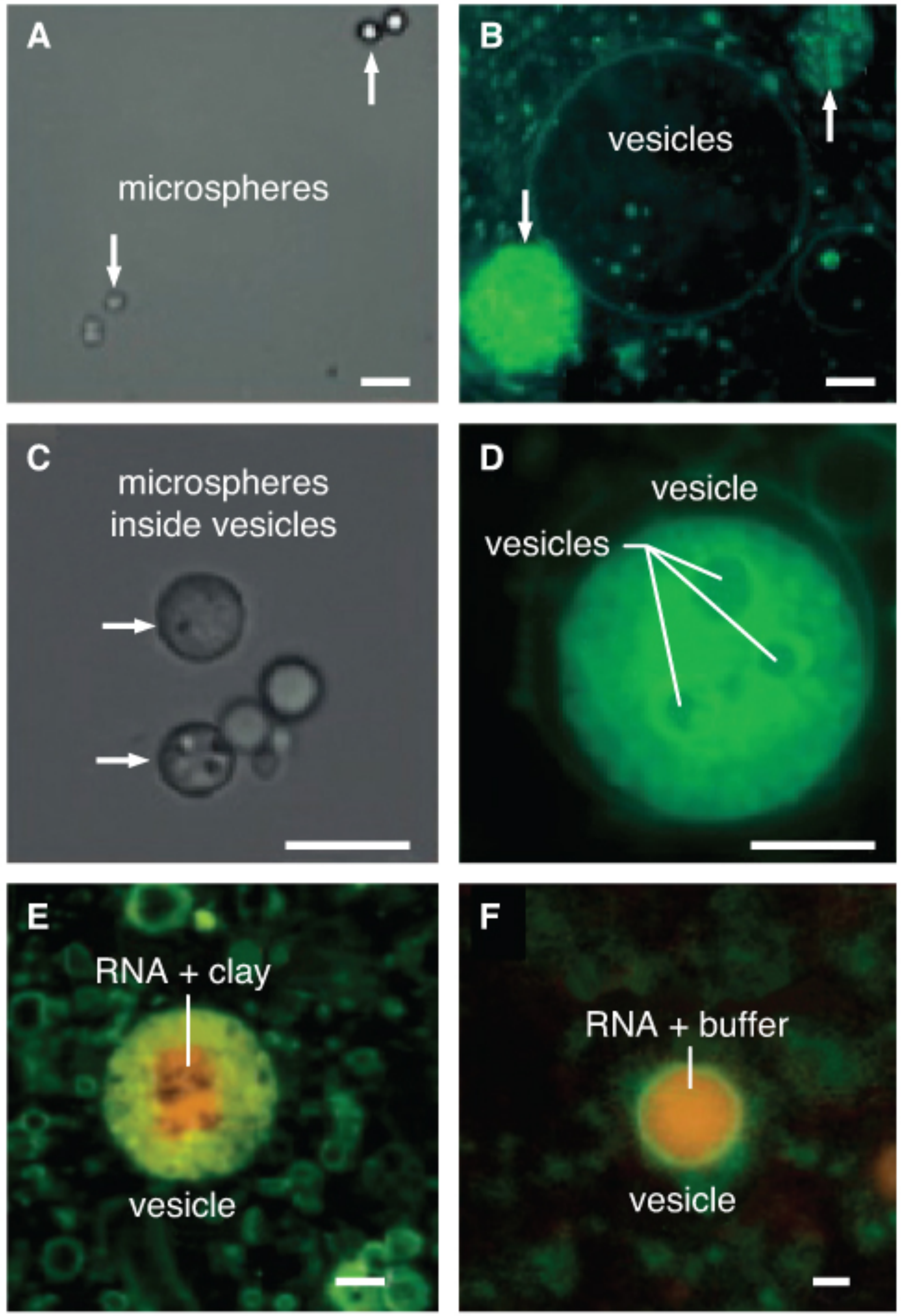
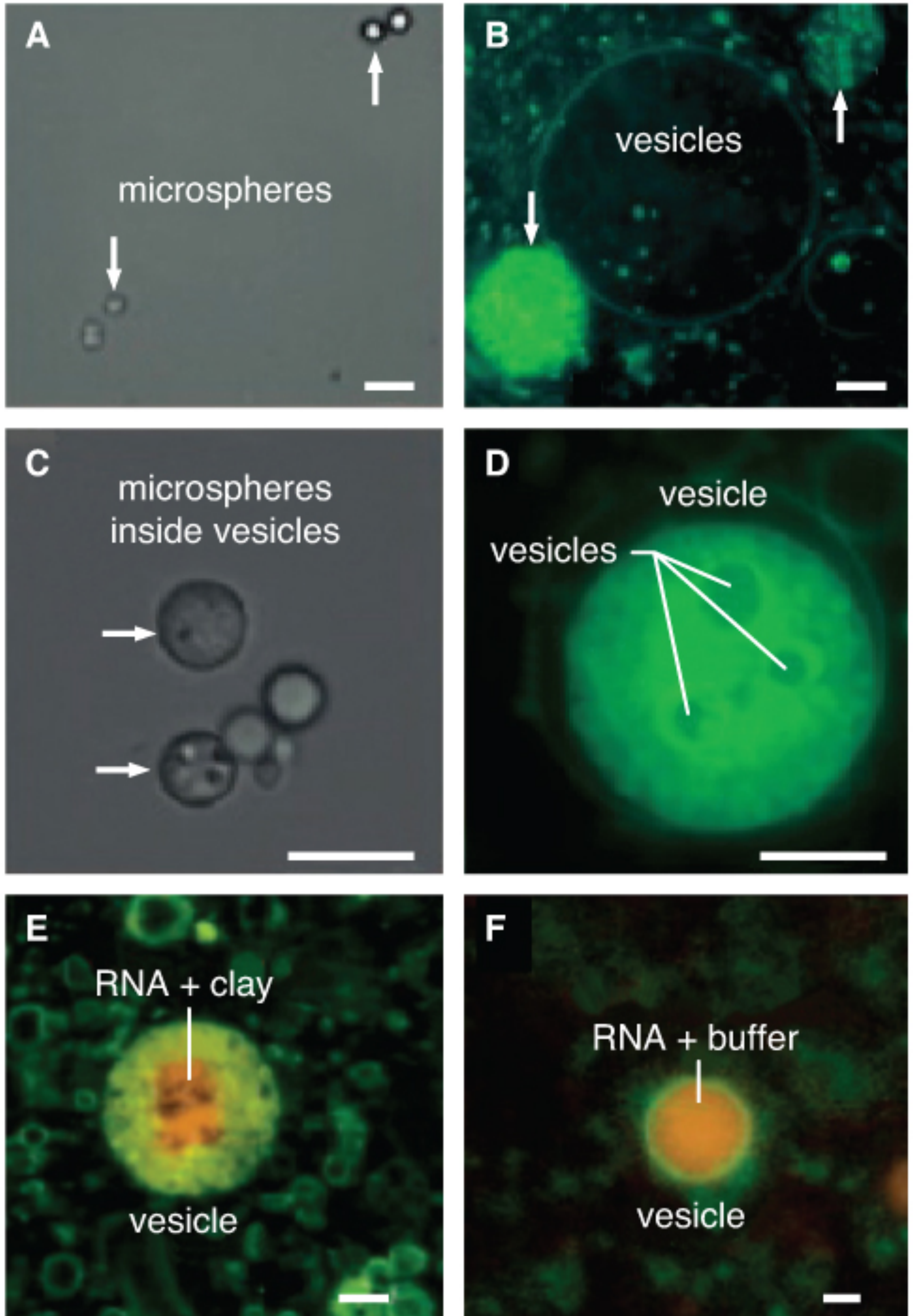
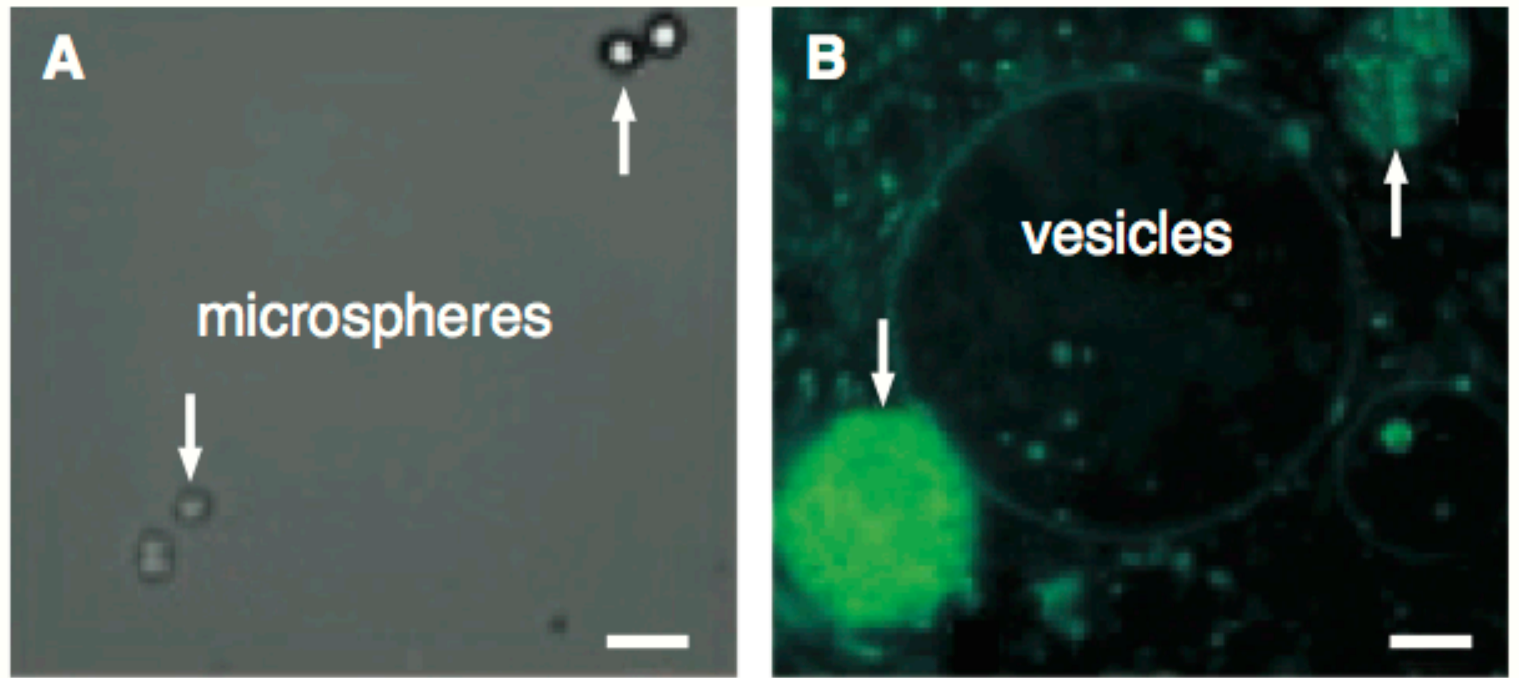


Figure 4.12 Microscopy of spontaneous vesicles with cargo. Green-stained fatty acids were mixed with negatively charged ceramic microspheres. White light in panels **(A)** and **(C)** to emphasize the microspheres. Green fluorescence in panels **(B)** and **(D)** to highlight the vesicles. Red-stained RNA attached to clay **(E)** or in solution **(F)** trapped inside vesicles. Scale bars are 5 μm in **A-D** and 1 μm in **E** and **F.** From Hanczyc et al., 2003. Figure 2A-D, I and J. Martin M. Hanczyc, Shelly M. Fujikawa, Jack W. Szostak. 2003. Experimental Models of Primitive Cellular Compartments: Encapsulation, Growth, and Division. Science. 302: 618 – 622. Reprinted with permission from AAAS.


A
microspheres
B
vesicles
C
microspheres
inside vesicles
D
vesicle
vesicles
E
RNA + clay
vesicle
F
RNA + buffer
vesicle

Microspheres Inside Vesicles



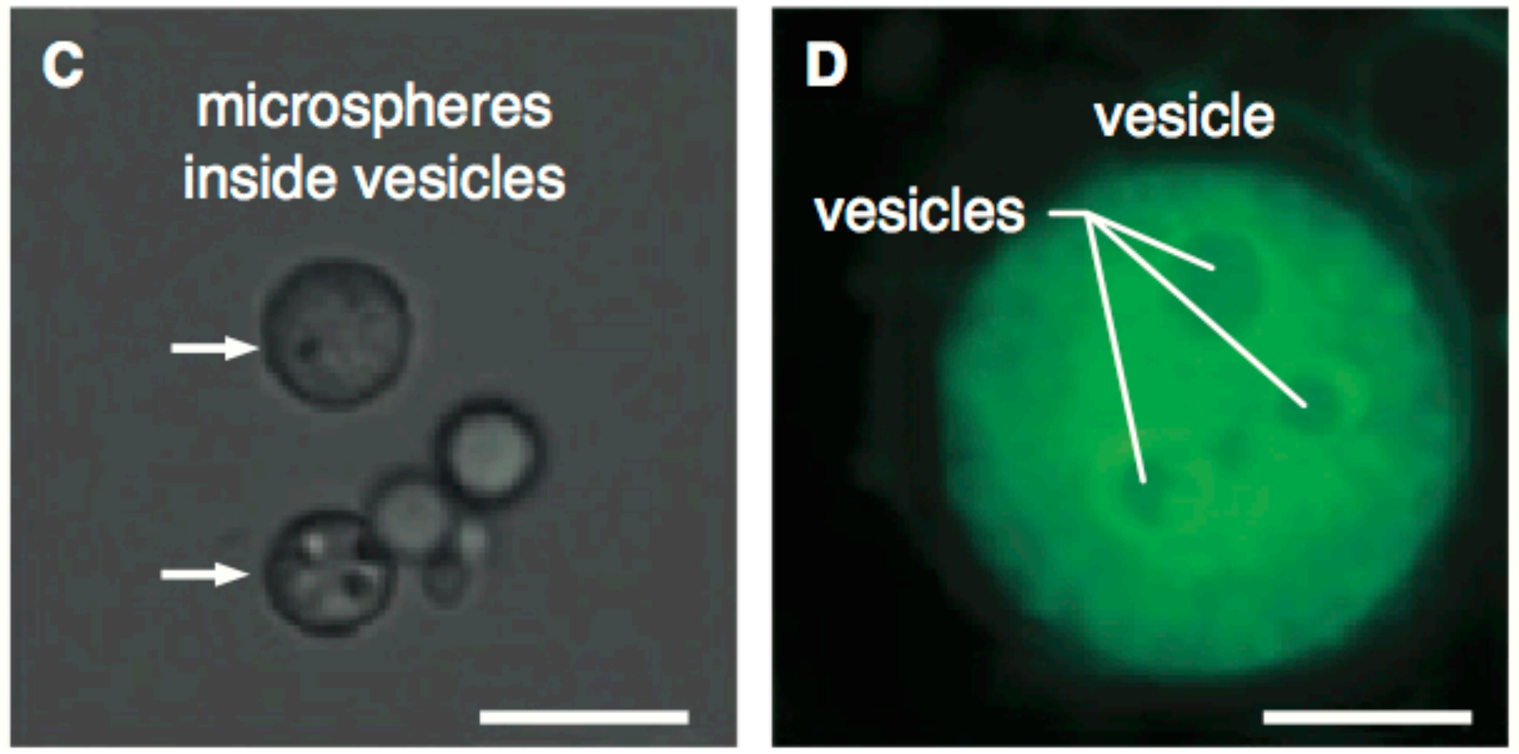
beads visualized lipids visualized

Fig. 4.12

A – D: modified from Hanczyc *et al.*, 2003; E, F: modified from Hanczyc, *et al.*, 2003

Abiotic Vesicles Capture Cargo

beads inside
vesicles



vesicles inside
vesicles

Fig. 4.12

A – D: modified from Hanczyc *et al.*, 2003; E, F: modified from Hanczyc, *et al.*, 2003

RNA Cargo in Vesicles

RNA inside vesicles

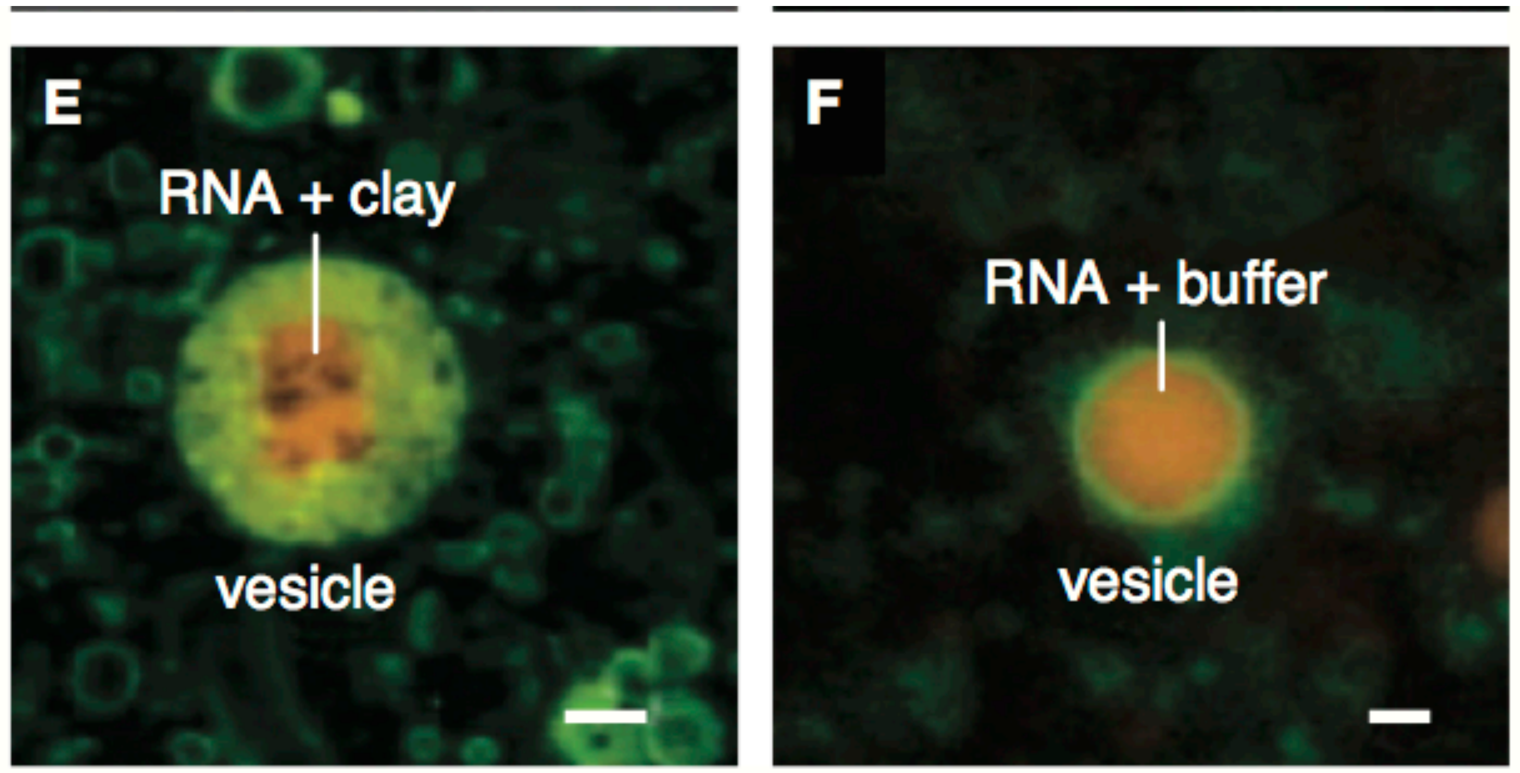


Fig. 4.12

A – D: modified from Hanczyc *et al.*, 2003; E, F: modified from Hanczyc, *et al.*, 2003

RNA Cargo in Vesicles

What chemical property of RNA could enhance its capacity to catalyze the formation of lipid vesicles?

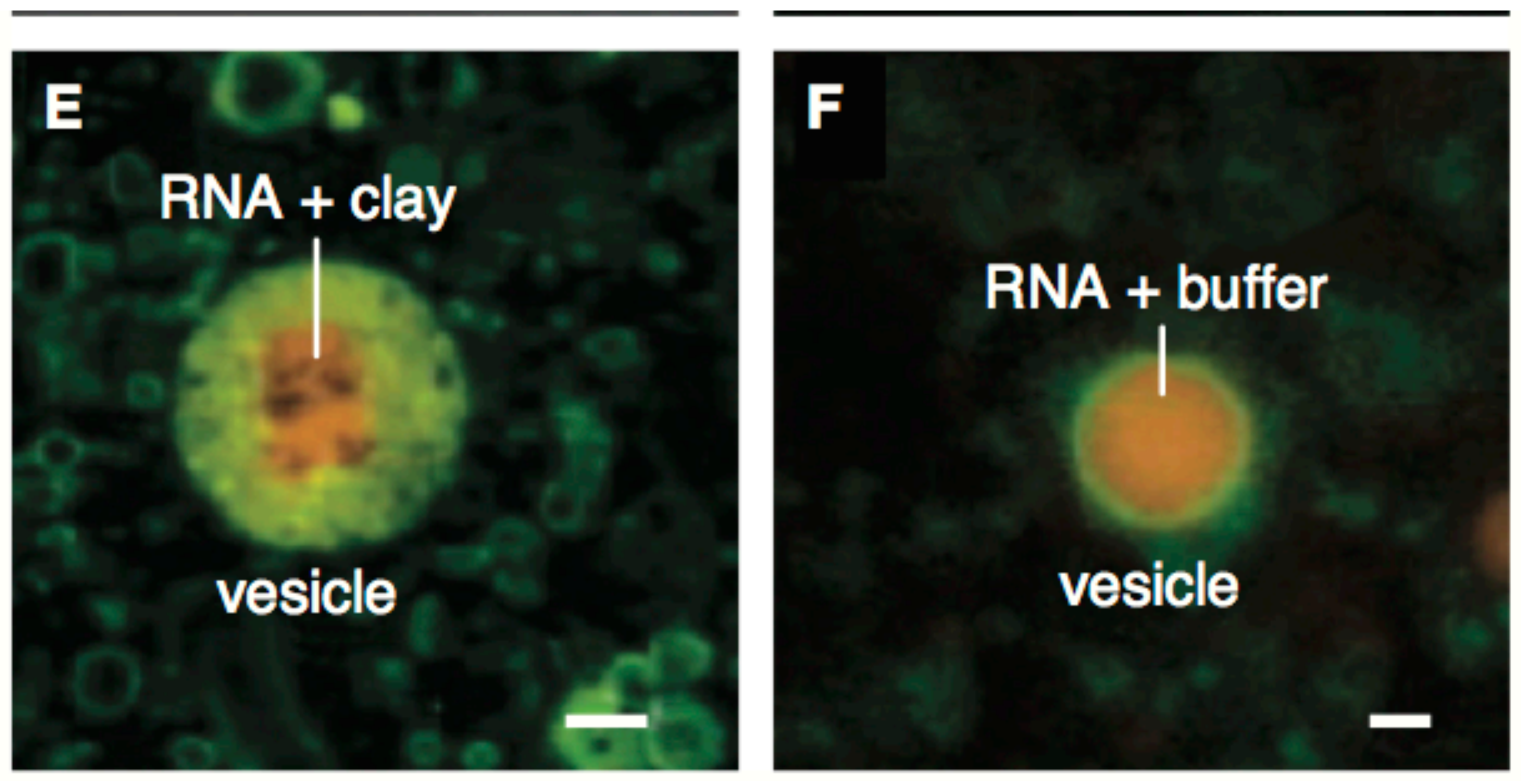


Fig. 4.12

A – D: modified from Hanczyc *et al.*, 2003; E, F: modified from Hanczyc, *et al.*, 2003

RNA Cargo in Vesicles

What chemical property of RNA could enhance its capacity to catalyze the formation of lipid vesicles?

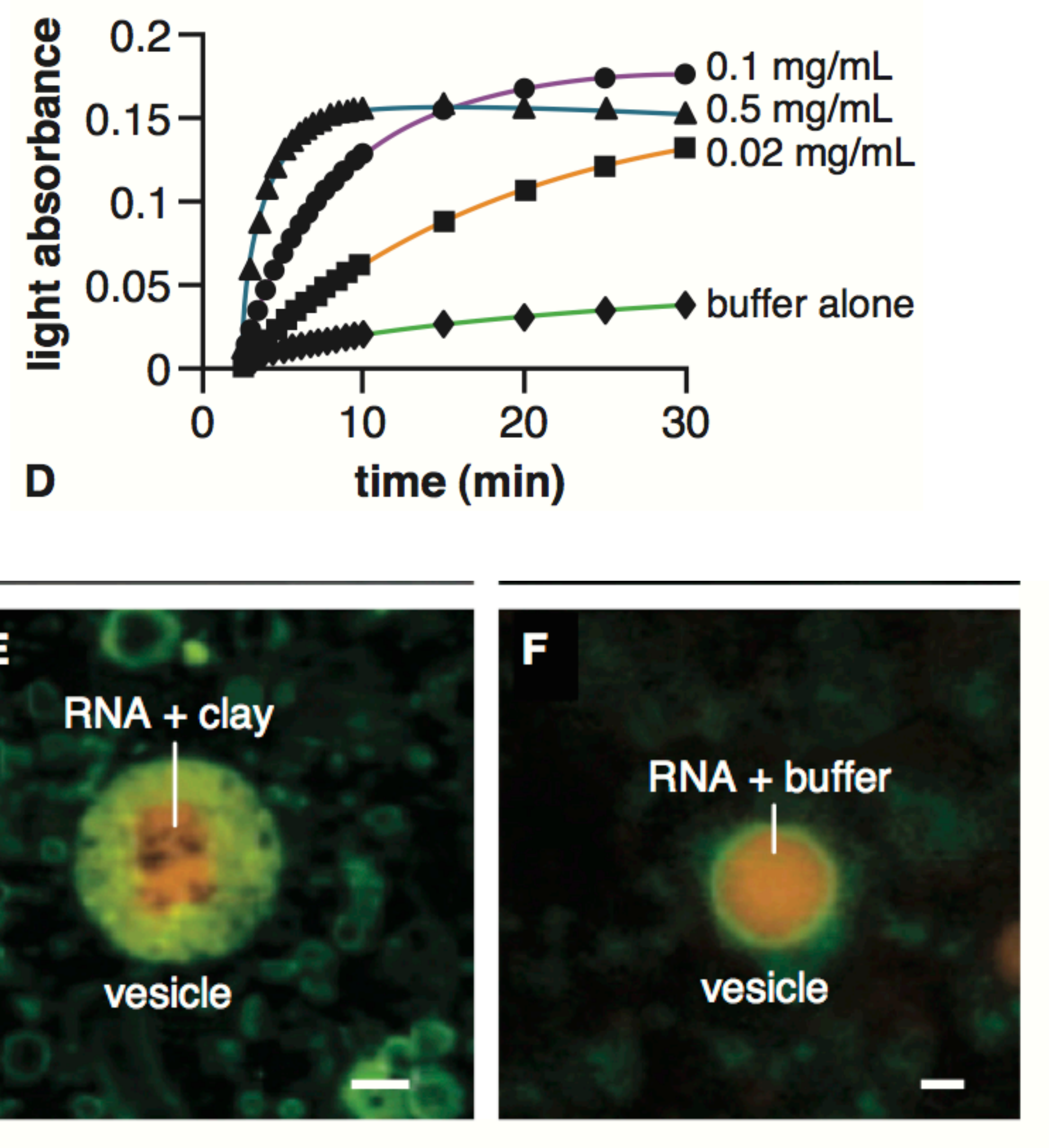


Fig. 4.12

A – D: modified from Hanczyc *et al.*, 2003; E, F: modified from Hanczyc, *et al.*, 2003

RNA Cargo in Vesicles

RNA can function as enzymes inside lipid bilayer

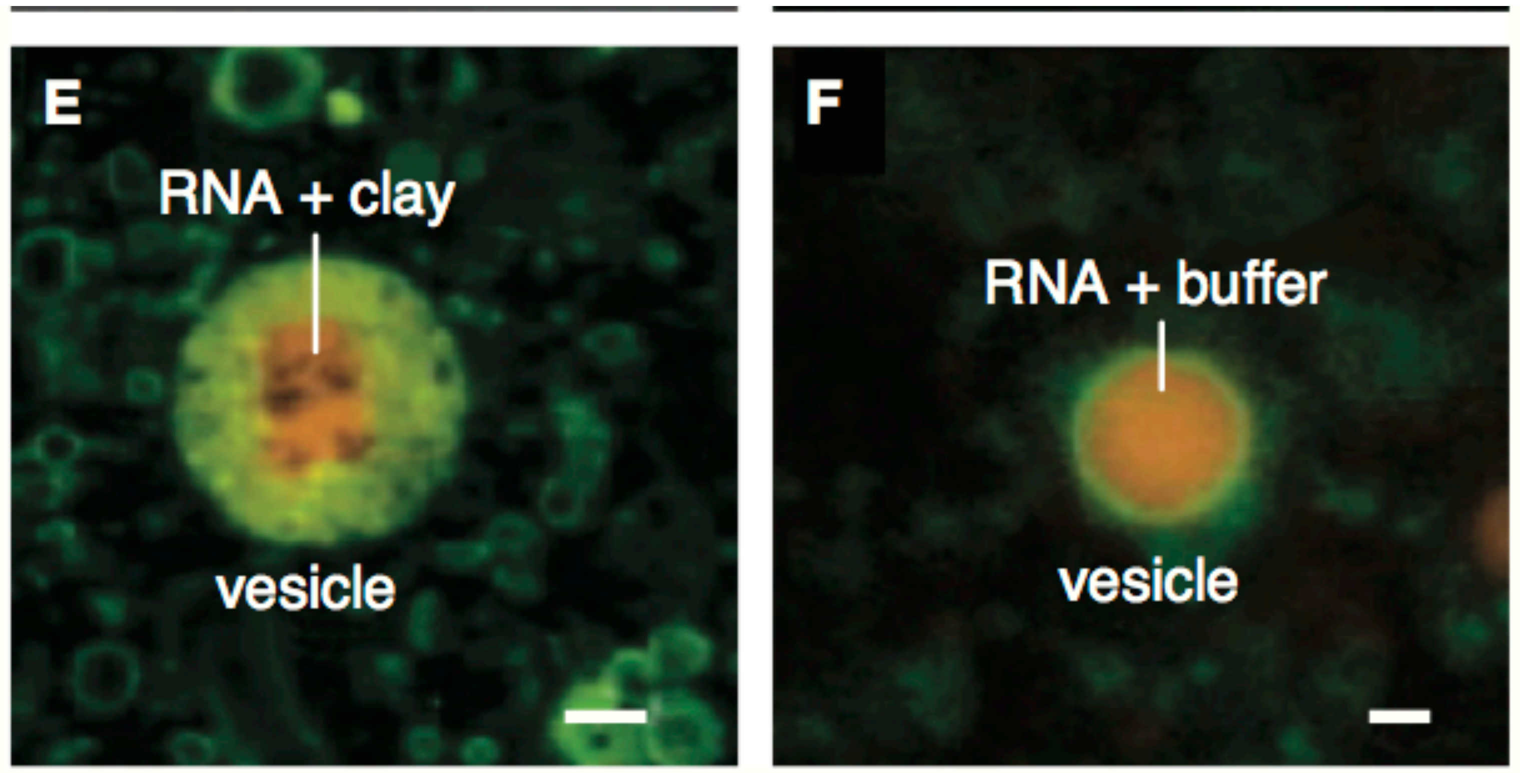


Fig. 4.12

A – D: modified from Hanczyc *et al.*, 2003; E, F: modified from Hanczyc, *et al.*, 2003

Why did they feed vesicles micelles or fatty acids to help induce growth? Think about the structural components of a vesicle?

(Trifecta)

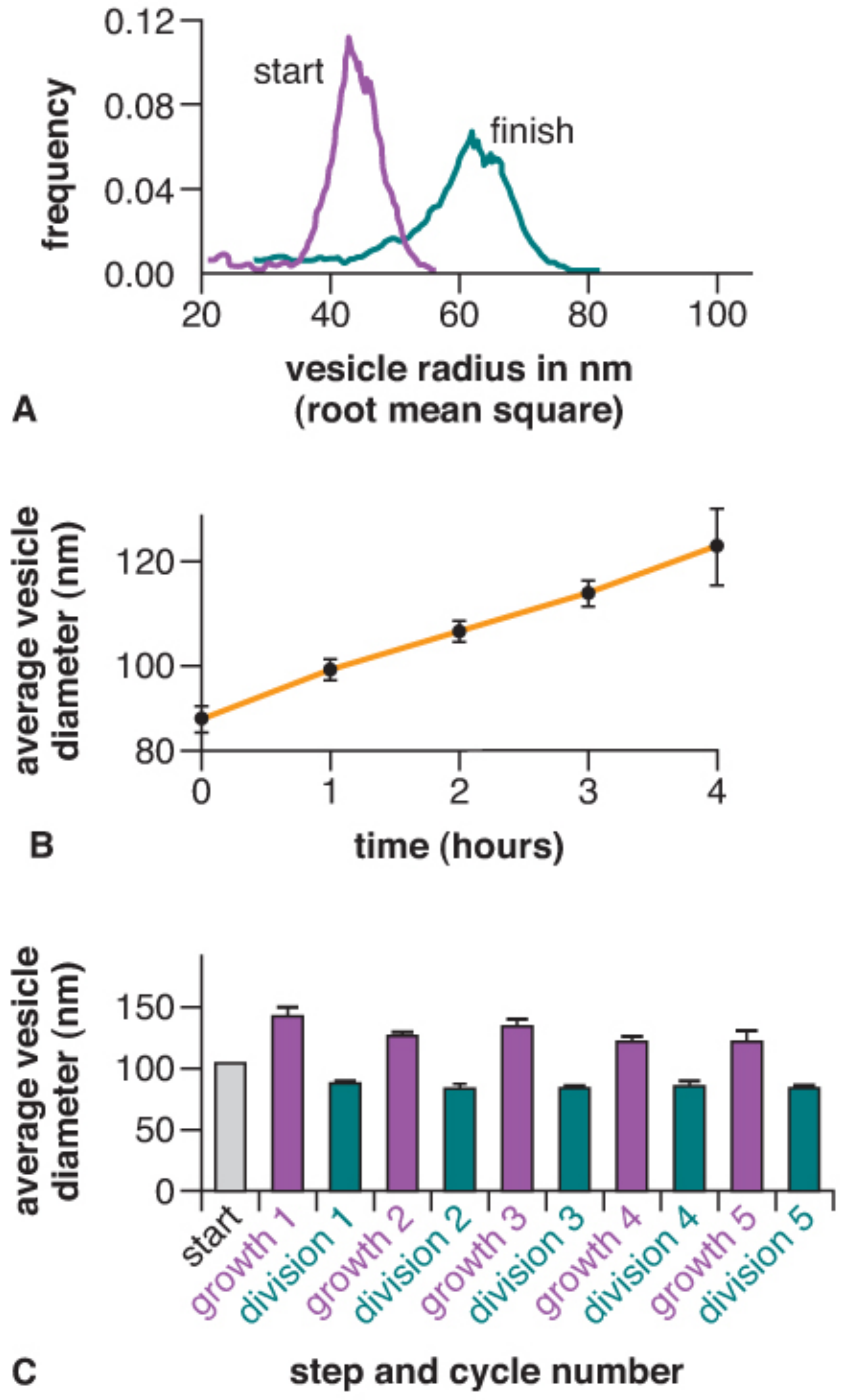
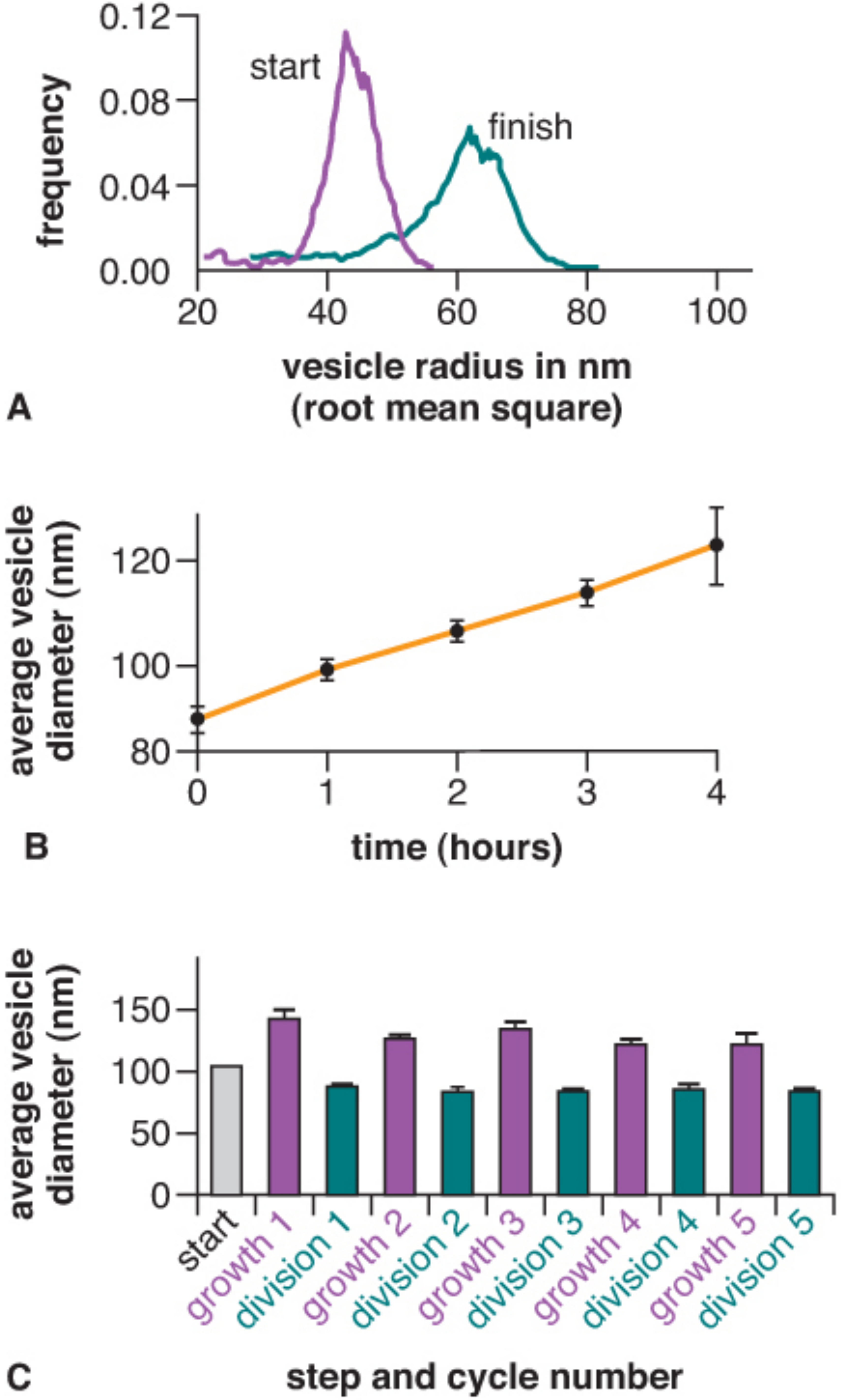


Figure 4.13 Changes in vesicle sizes. **A,** Small starting vesicles grew larger after the addition of micelles. **B,** Vesicles grew over time when the experiment was sampled periodically. **C,** Mean diameter plus standard deviation of four measurements demonstrates reproducibility of vesicle growth. From Hanczyc et al., 2003. a-b) Figure 3 and c) Figure 6A. Martin M. Hanczyc, Shelly M. Fujikawa, Jack W. Szostak. 2003. Experimental Models of Primitive Cellular Compartments: Encapsulation, Growth, and Division. Science. 302: 618 – 622. Reprinted with permission from AAAS.



0.12
0.08
0.04
0.00
frequency
start
finish
20
40
60
80
100
vesicle radius in nm
(root mean square)
A
average vesicle
diameter (nm)
120
100
80
0
1
2
3
4
time (hours)
B
average vesicle
diameter (nm)
150
100
50
0
start
growth 1
division 1
growth 2
division 2
growth 3
division 3
growth 4
division 4
growth 5
division 5
C
step and cycle number

4.4 Can non-living objects harvest and store energy?

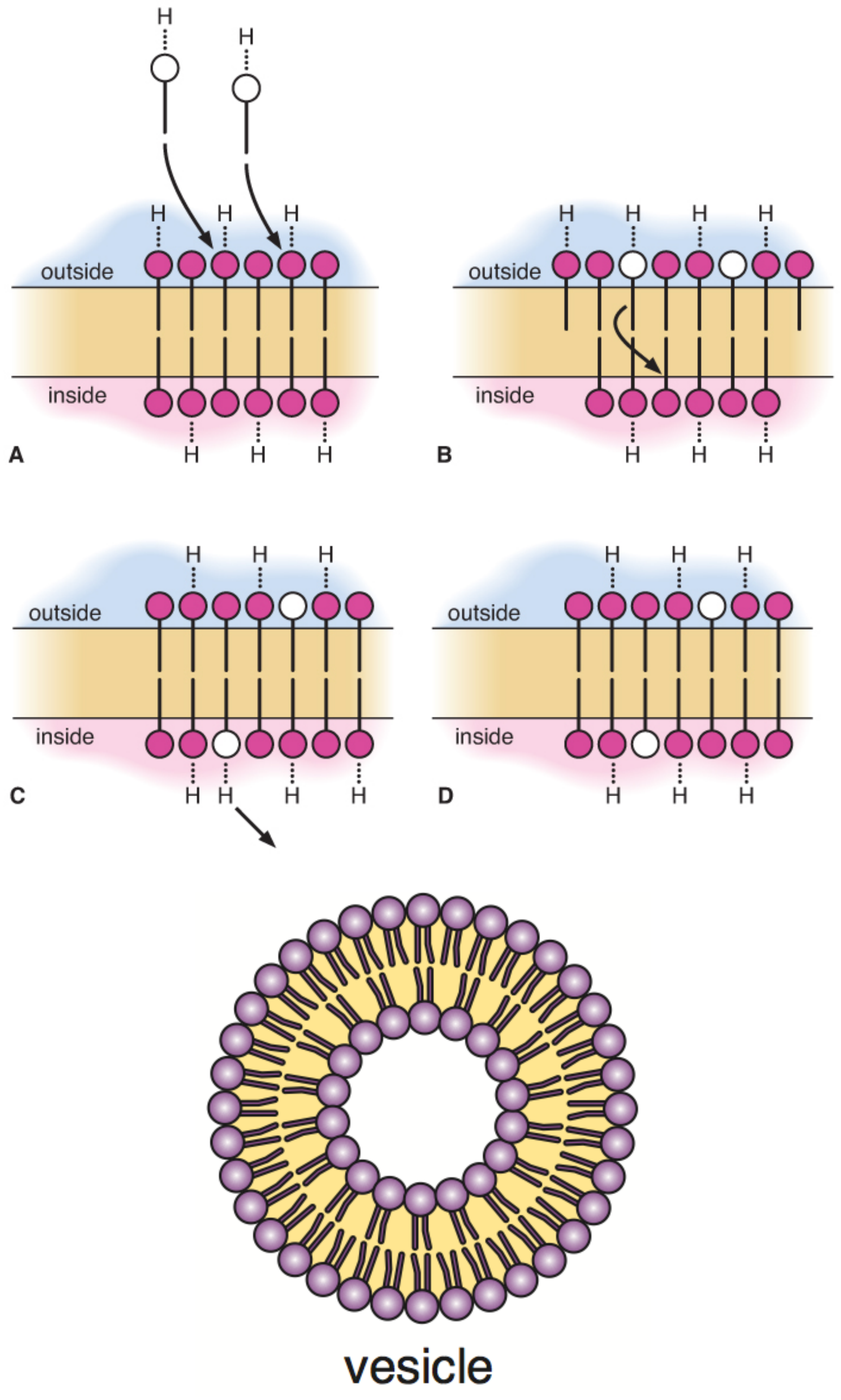
- Context: Life consumes energy, and the first cells would need an abiotic way to harvest and store energy.
- Major theme: The origin of living systems occurred by natural processes, and life continues to evolve within a changing environment; organisms can be linked by lines of descent from common ancestry.
- Bottom line: Nonliving vesicles can accumulate energy in the form of a pH gradient.

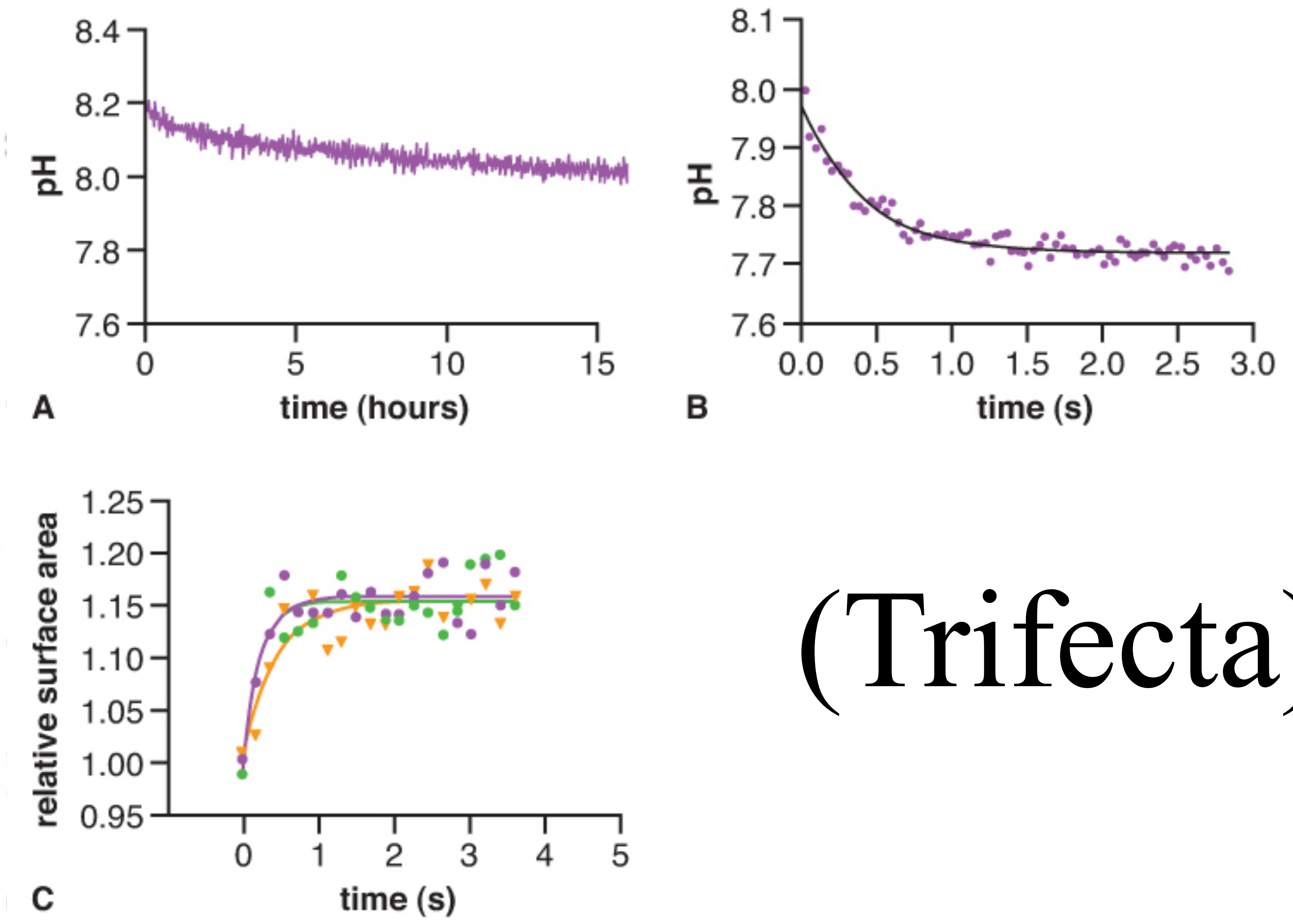
Biology Learning Objectives

- Use evidence to support the scientific understanding that life evolved from abiotic forces and phenomenon.
- Discuss how vesicles can grow, compete, *and store energy*.
- Illustrate how abiotic structures exhibit dynamic and competitive behaviors.

In Section 4.2, you saw RNA molecules function as RNA polymerases. In Section 4.3, you saw RNA molecules become entrapped inside abiotic vesicles and produce osmotic pressure to out-compete relaxed vesicles for lipids. Life also requires energy, and so far you have not seen any data indicating that these primitive cells could harvest or store energy. Is it possible for primitive, abiotic cells to store energy in an abiotic world? Could growing vesicles sequester energy that could be used to do work at a later time? You will analyze published data that will help you determine if energy storage is possible in an abiotic world.

How does conducting this experiment relate to the RNA world Hypothesis?





(Trifecta)

Figure 4.17 Vesicle pH after adding micelles. **A,** pH drops quickly at first, and then slowly. **B,** Reduced pH immediately after adding micelles. **C,** Change of surface area for vesicles in **B.** Three trials are shown with each line representing an exponential curve fit. From Chen and Jack W. Szostak. 2004a. Figure 3. Irene A. Chen and Jack W. Szostak. 2004a. Membrane growth can generate a transmembrane pH gradient in fatty acid vesicles. PNAS. 101 (21): 7965 – 7970. Copyright (2004) National Academy of Sciences, U.S.A.

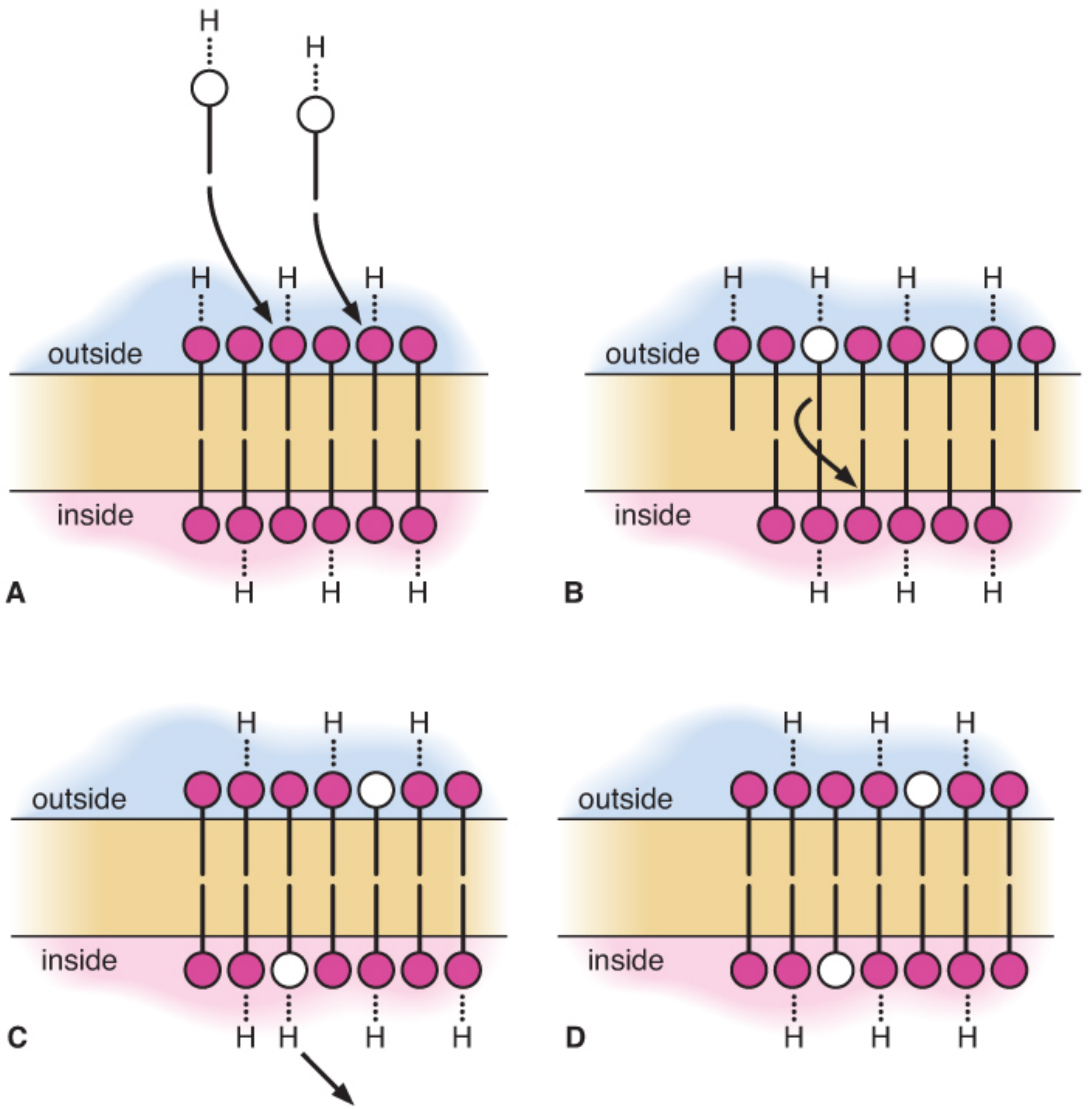
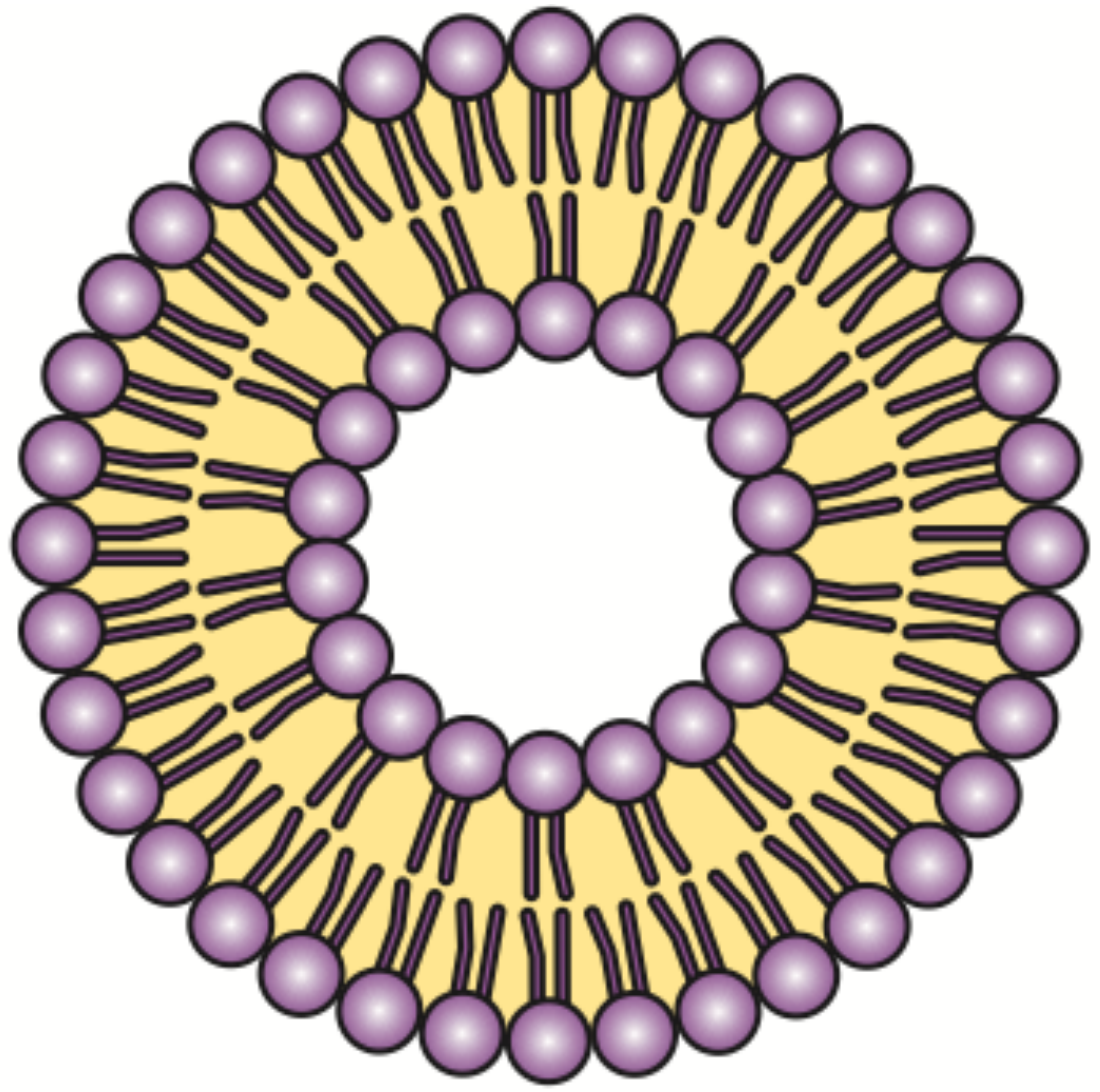
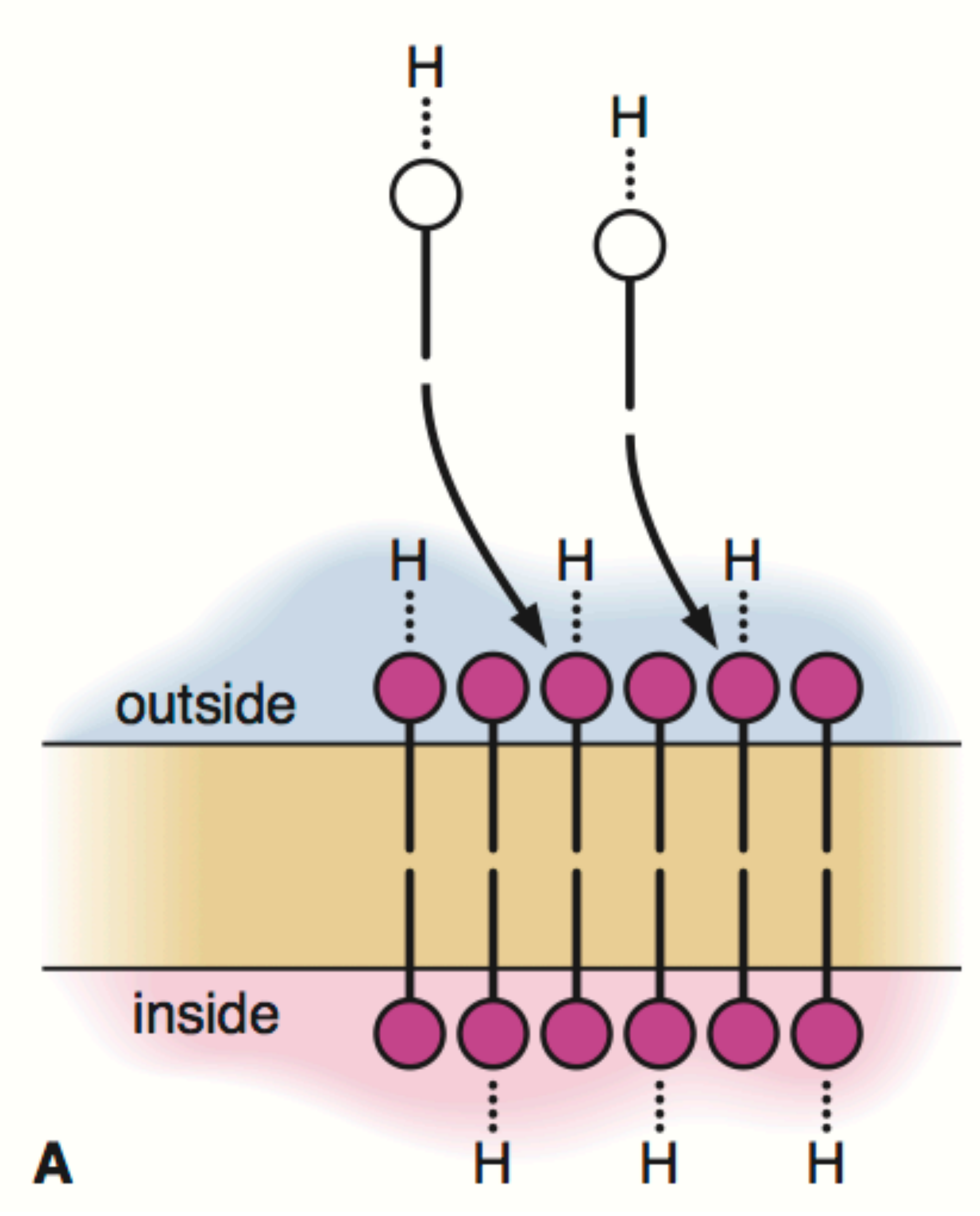


Figure 4.16 Proposed mechanism for accumulation of a pH gradient inside the vesicle lumen. The process proceeds from **(A)** through **(D)** over time to gradually accumulate H^+ ions inside vesicles, which lowers internal pH. Lipids can flip from one side of the bilayer to the other. From Chen and Jack W. Szostak. 2004a. Figure 2. Irene A. Chen and Jack W. Szostak. 2004a. Membrane growth can generate a transmembrane pH gradient in fatty acid vesicles. PNAS. 101 (21): 7965 – 7970. Copyright (2004) National Academy of Sciences, U.S.A.



vesicle

Model of pH Gradient Production

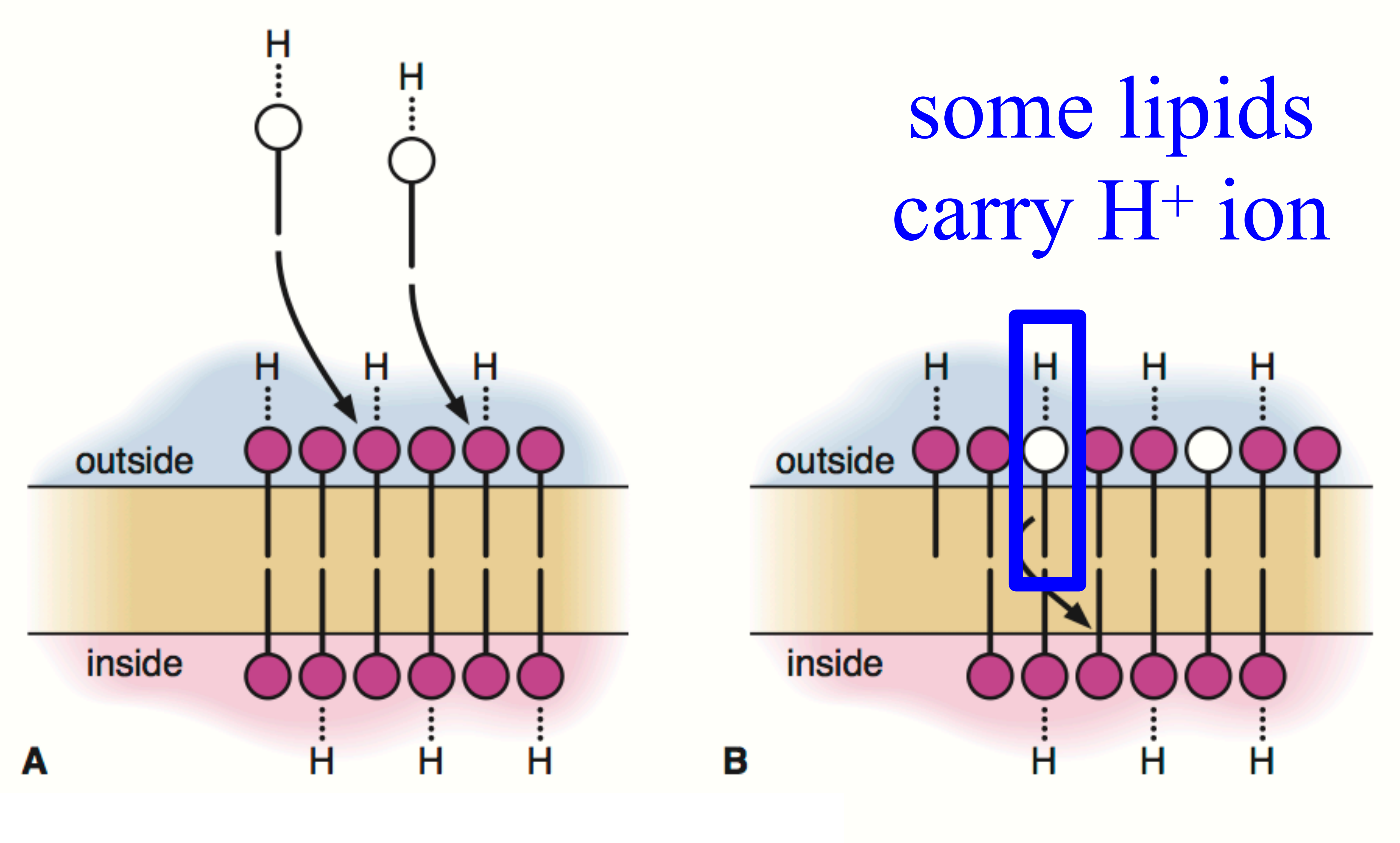


new lipids fuse
with outer layer

Fig. 4.16

modified from Chen and Jack W. Szostak. 2004a

Model of pH Gradient Production



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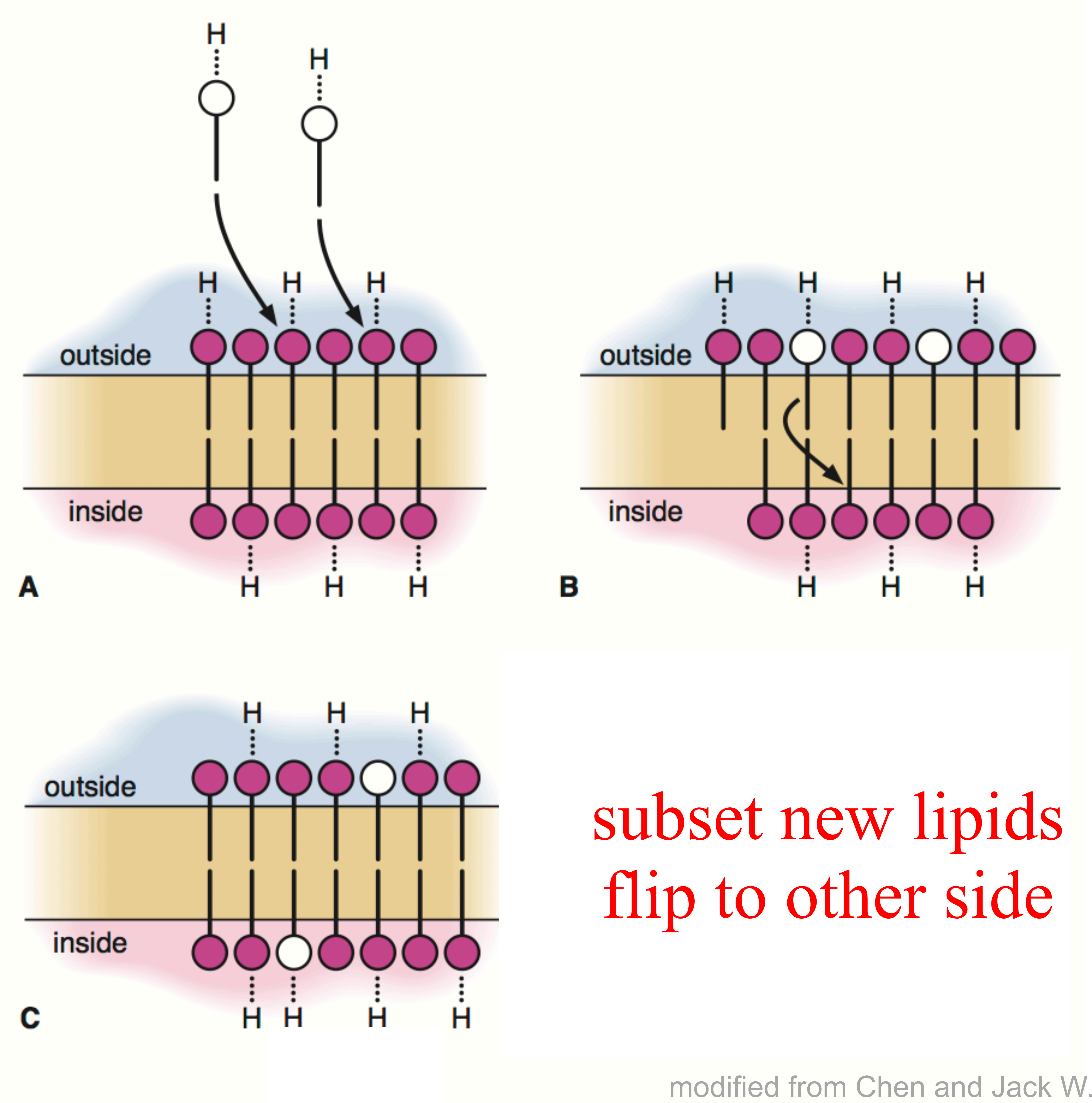
Model of pH Gradient Production

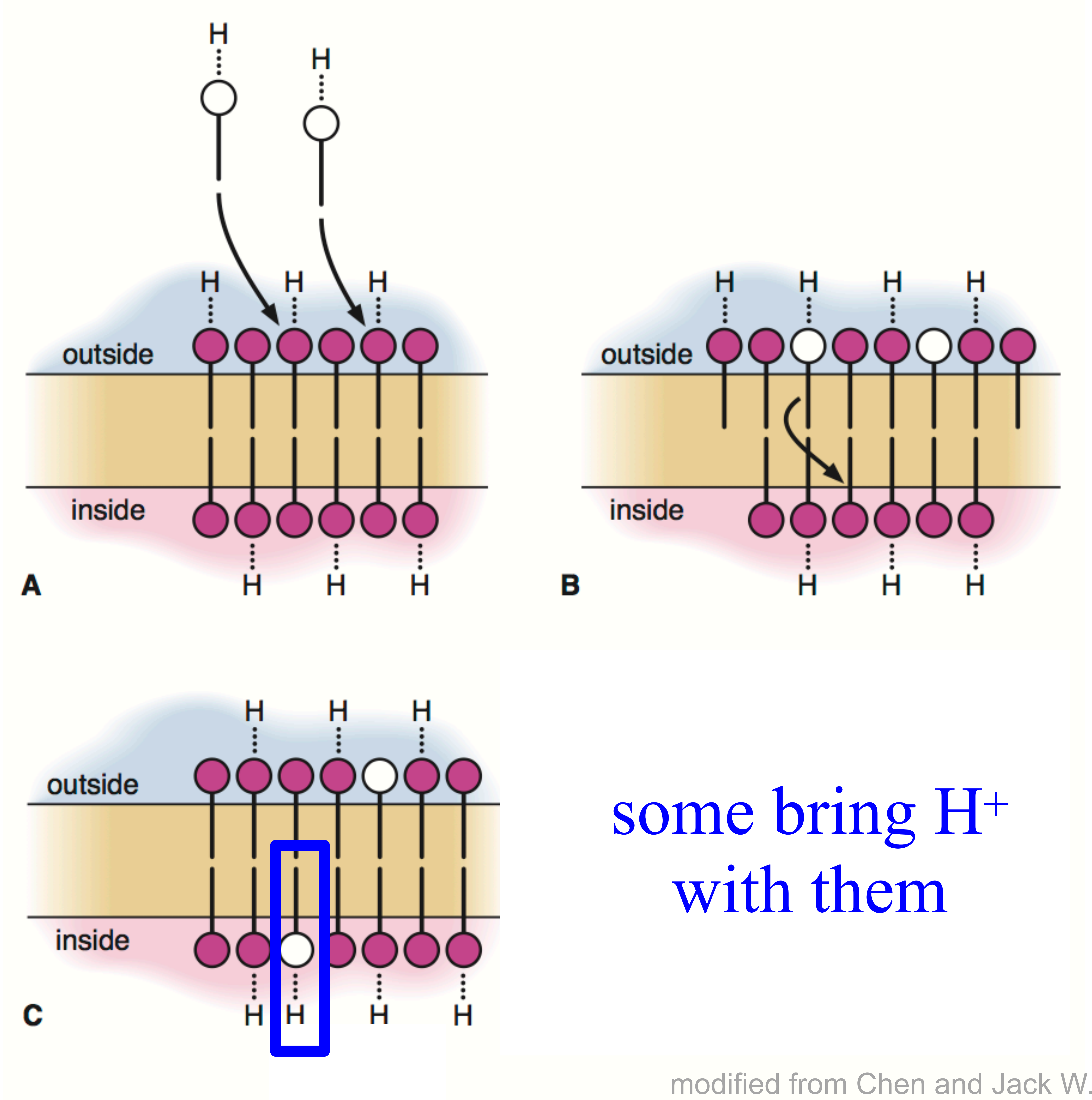


subset new lipids flip to other side

Fig. 4.16

modified from Chen and Jack W. Szostak. 2004a

Model of pH Gradient Production

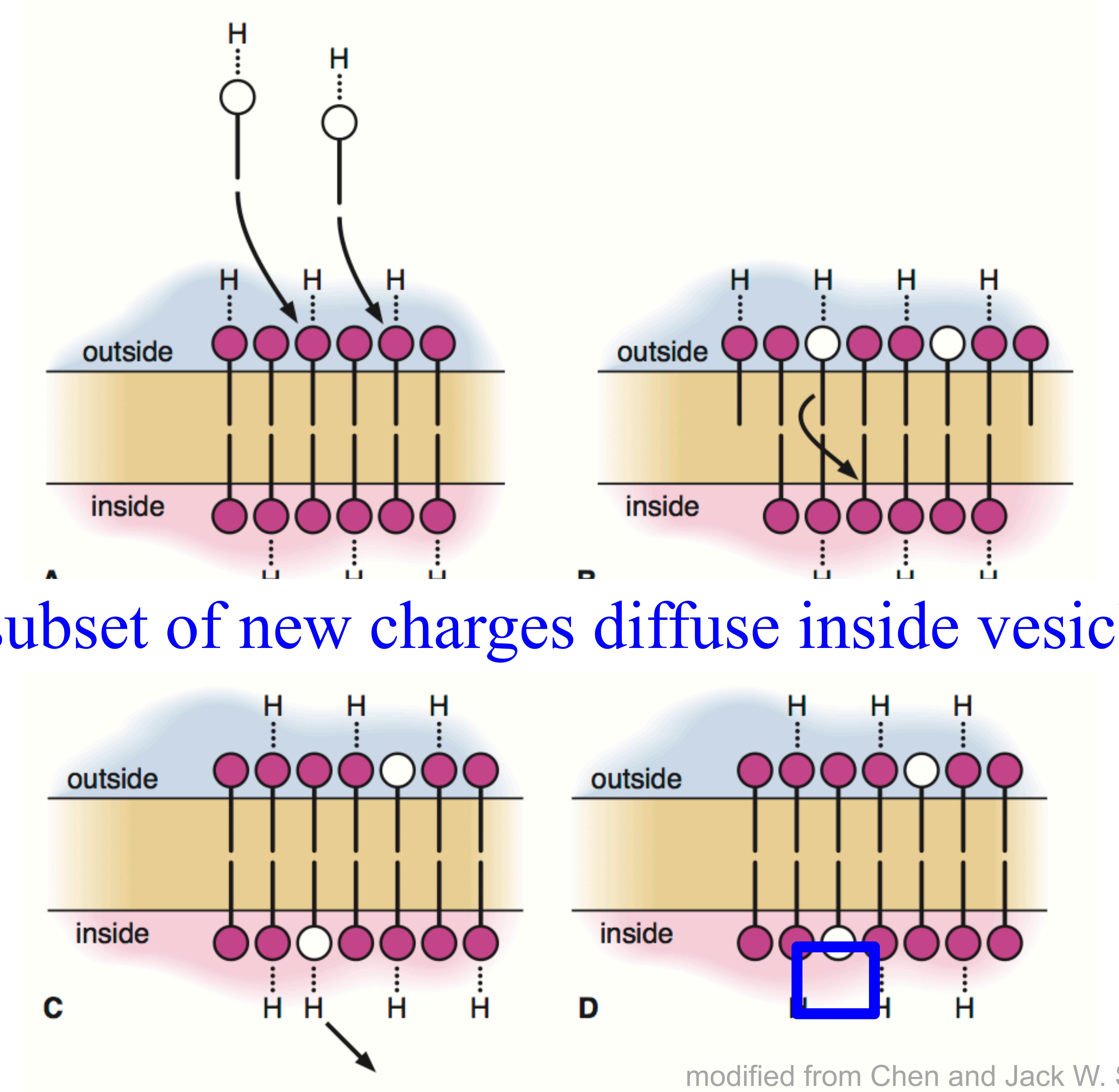


some bring H^+
with them

Fig. 4.16

modified from Chen and Jack W. Szostak. 2004a

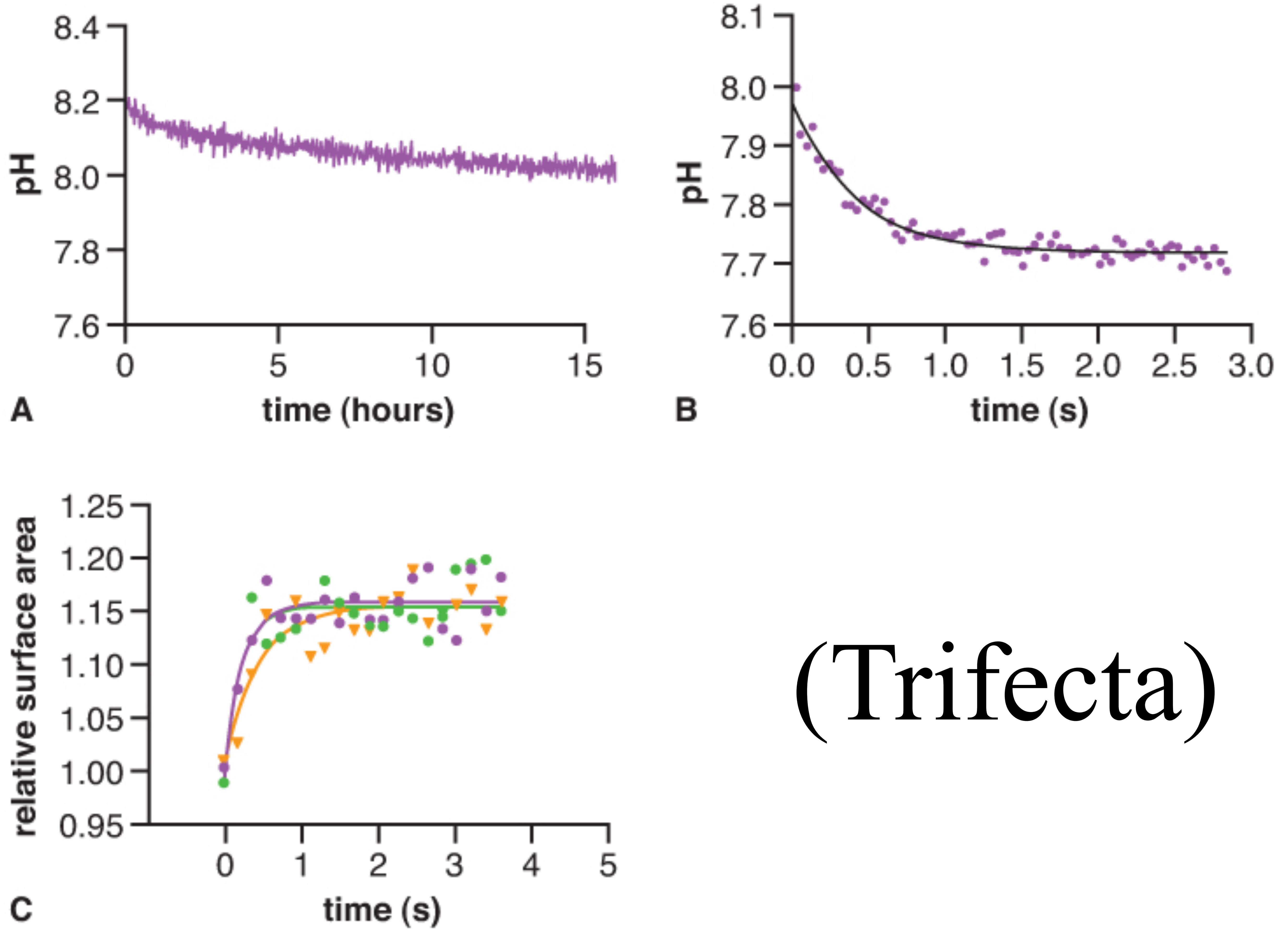
Model of pH Gradient Production



subset of new charges diffuse inside vesicle

Fig. 4.16

modified from Chen and Jack W. Szostak. 2004a



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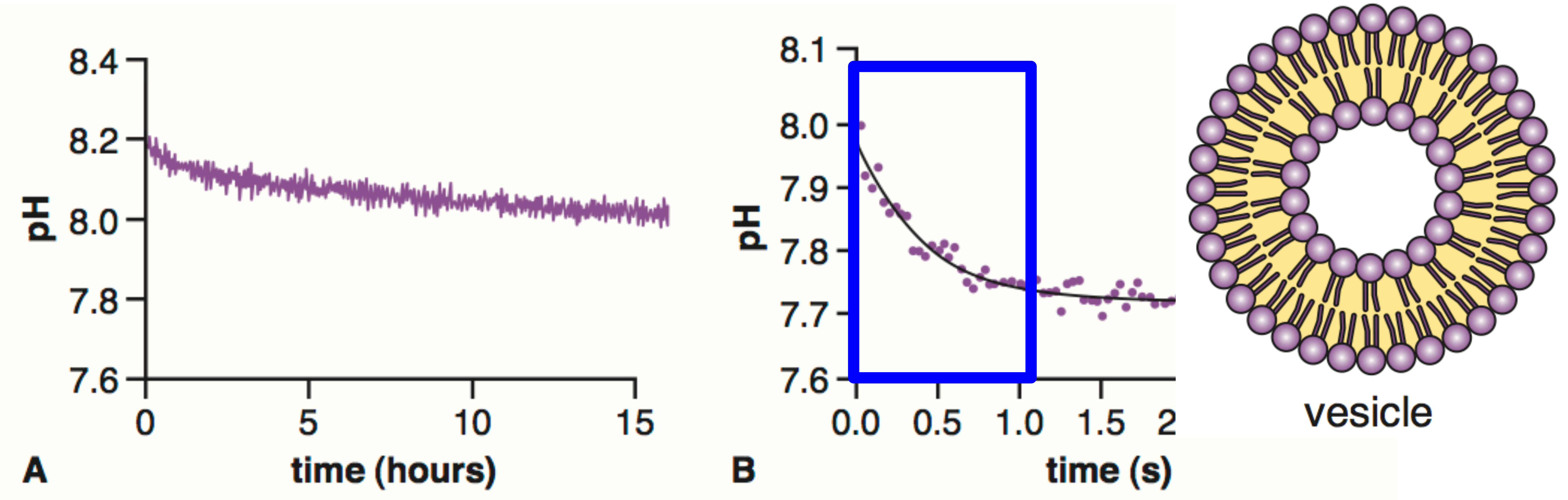
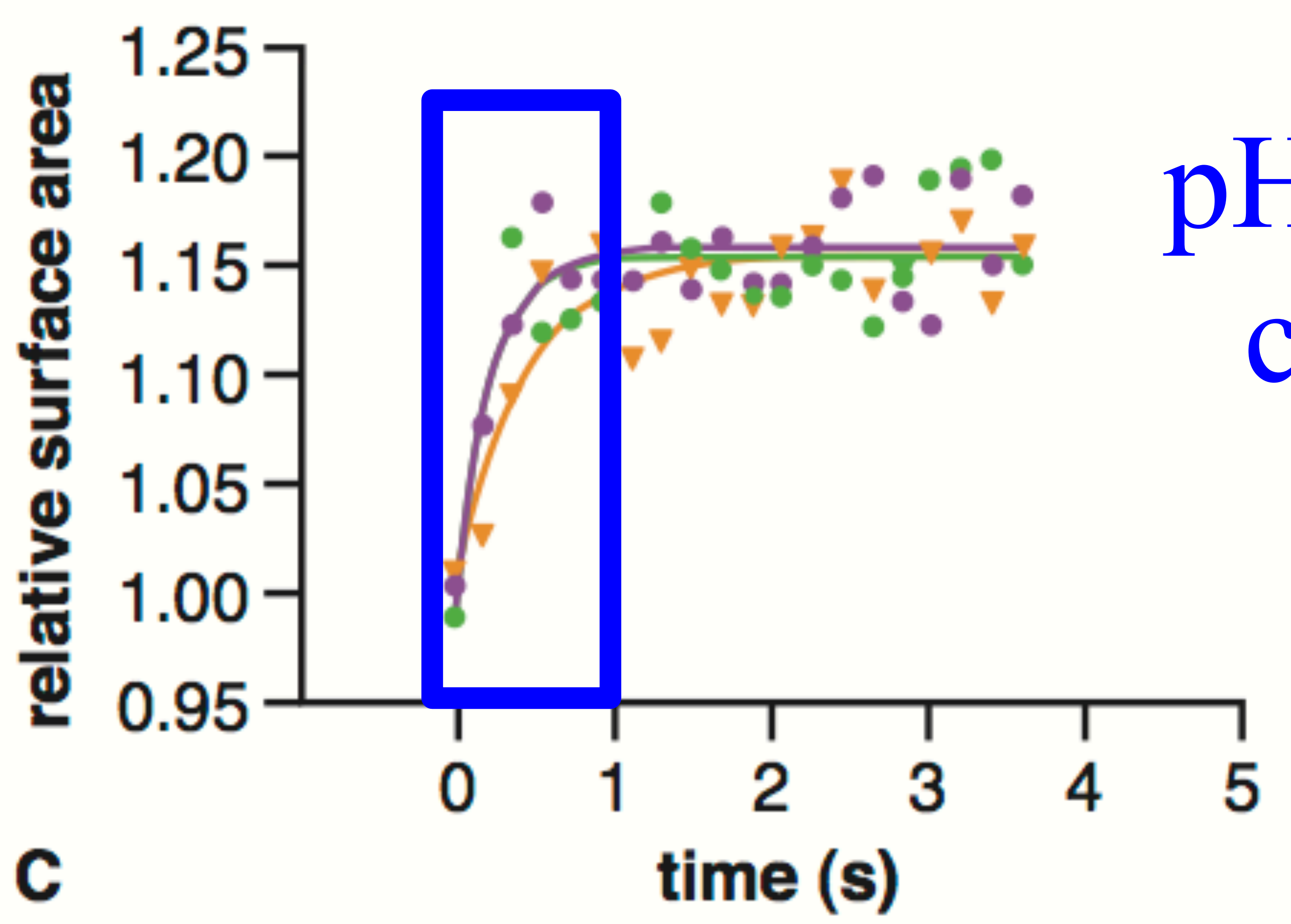




pH and surface area changes on same time scale

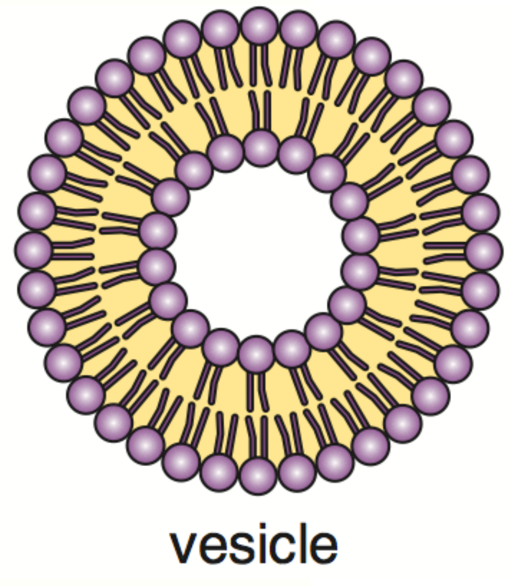


Fig. 4.17

modified from Chen and Jack W. Szostak. 2004a

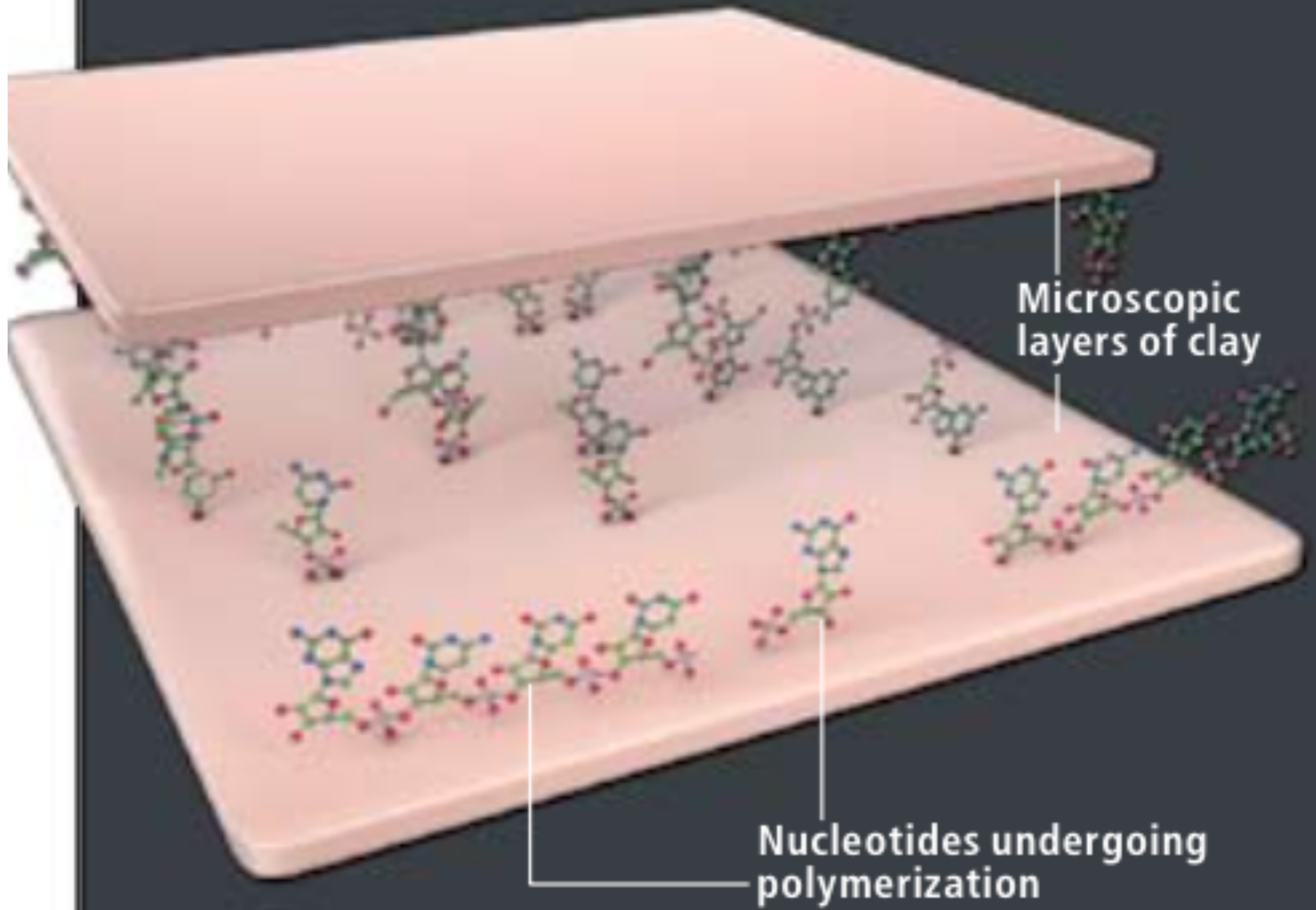
If it looks like a cell, and acts like a cell…?

- Lipids can form through abiotic mechanisms.
- Abiotic vesicles can form spontaneously.
- Abiotic vesicles can trap RNA inside them.
- RNA ribozymes can polymerize RNA molecules.
- Abiotic vesicles can grow by consuming additional lipids.
- Abiotic vesicles can compete against each other for lipids.
- Abiotic vesicles can divide and leak their RNA cargo with each division.
- Abiotic vesicles with RNA inside can outcompete vesicles lacking RNA inside.

[FROM MOLECULES TO ORGANISMS]

ON THE WAY TO LIFE

After chemical reactions created the first genetic building blocks and other organic molecules, geophysical processes brought them to new environments and concentrated them. The chemicals assembled into more complex molecules and then into primitive cells. And some 3.7 billion years ago geophysics may have also nudged these "protocells" to reproduce.



RNA BREEDING GROUNDS

In the water solutions in which they formed, nucleotides would have had little chance of combining into long strands able to store genetic information. But under the right conditions—for example, if molecular adhesion forces brought them close together between microscopic layers of clay (*above*)—nucleotides might link up into single strands similar to modern RNA.

LIFE ON EARTH

BY ALONSO RICARDO AND JACK W. SZOSTAK

Fresh clues hint at how the first living organisms arose from inanimate matter

Every living cell, even the simplest bacterium, teems with molecular contraptions that would be the envy of any nanotechnologist. As they incessantly shake or spin or crawl around the cell, these machines cut, paste and copy genetic molecules, shuttle nutrients around or turn them into energy, build and repair cellular membranes, relay mechanical, chemical or electrical messages—the list goes on and on, and new discoveries add to it all the time.

It is virtually impossible to imagine how a cell's machines, which are mostly protein-based catalysts called enzymes, could have formed

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