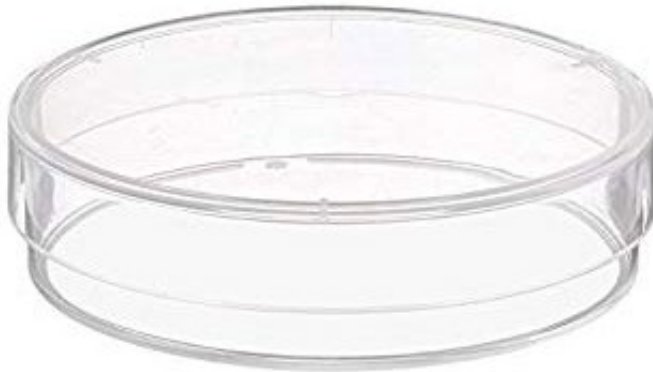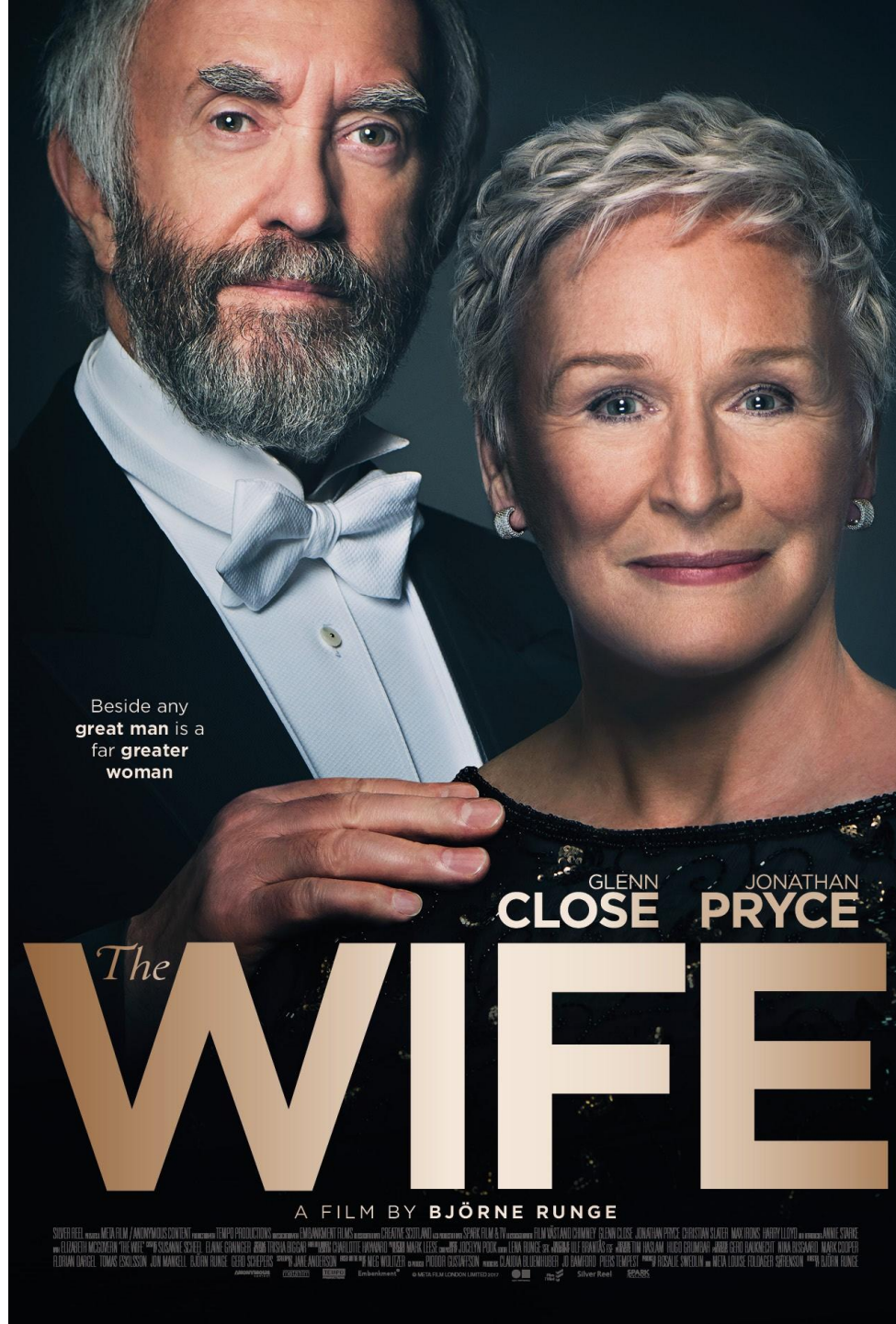# Phage Display:
Simple Evolution in a Petri Dish

George P. Smith

Division of Biological Sciences

University of Missouri

Nobel Prize Lecture in Chemistry

December 8, 2018

Beside any **great man** is a far **greater woman**

GLENN **CLOSE** JONATHAN **PRYCE**

The **WIFE**

A FILM BY BJÖRNE RUNGE

Margie did not invent phage display

My science community

- Molecular biologists
- Evolutionary biologists
- Immunologists
- Protein chemists
- Phage biologists
- Mathematicians
- Philosophers of science
- Bayesian statisticians

→ Phage display

Our science community

- Molecular biologists
- Evolutionary biologists
- Immunologists
- Protein chemists
- Phage biologists
- Mathematicians
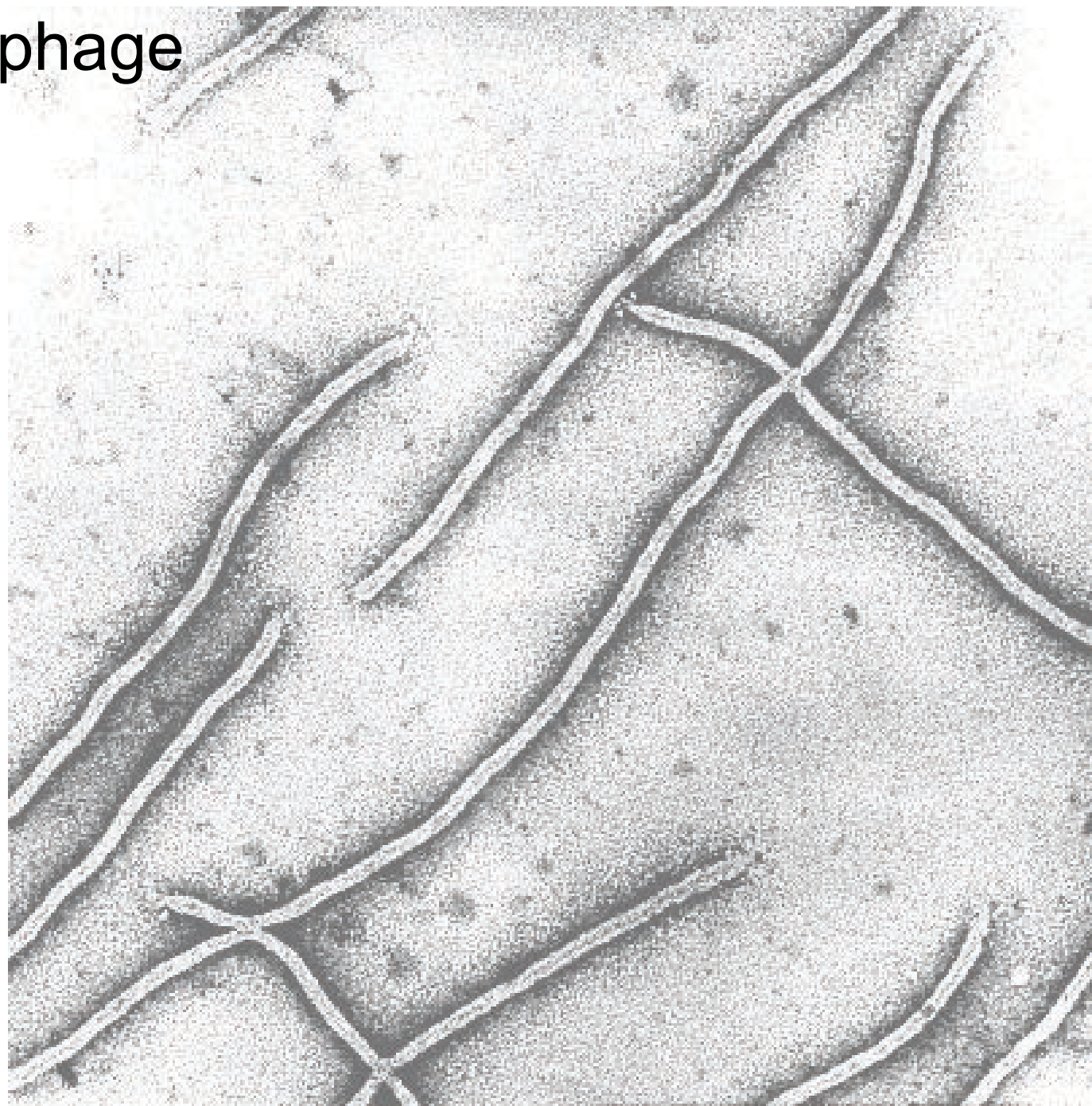- Philosophers of science
- Bayesian statisticians

→ Phage display

Phage = virus that infects bacteria
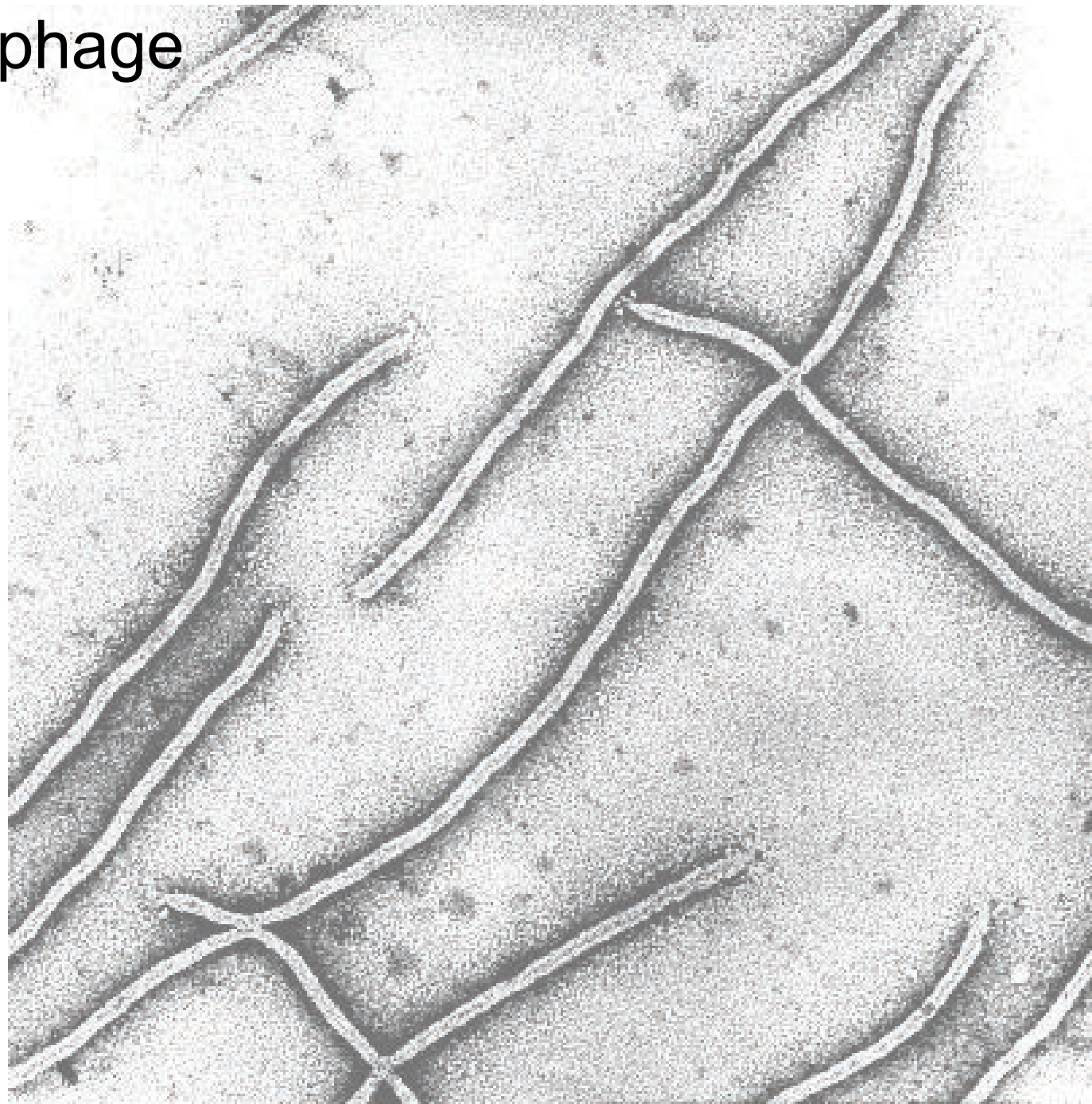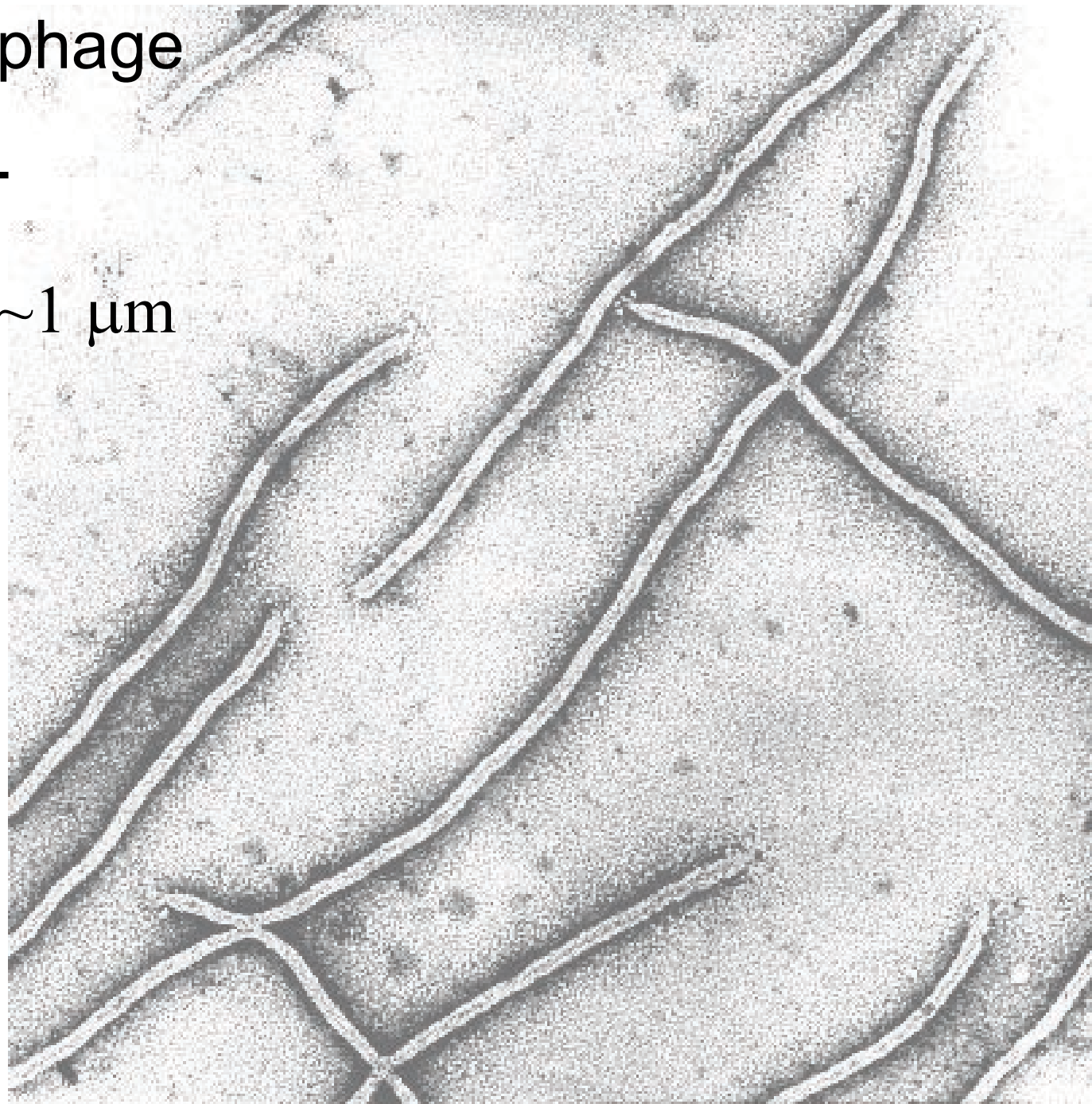
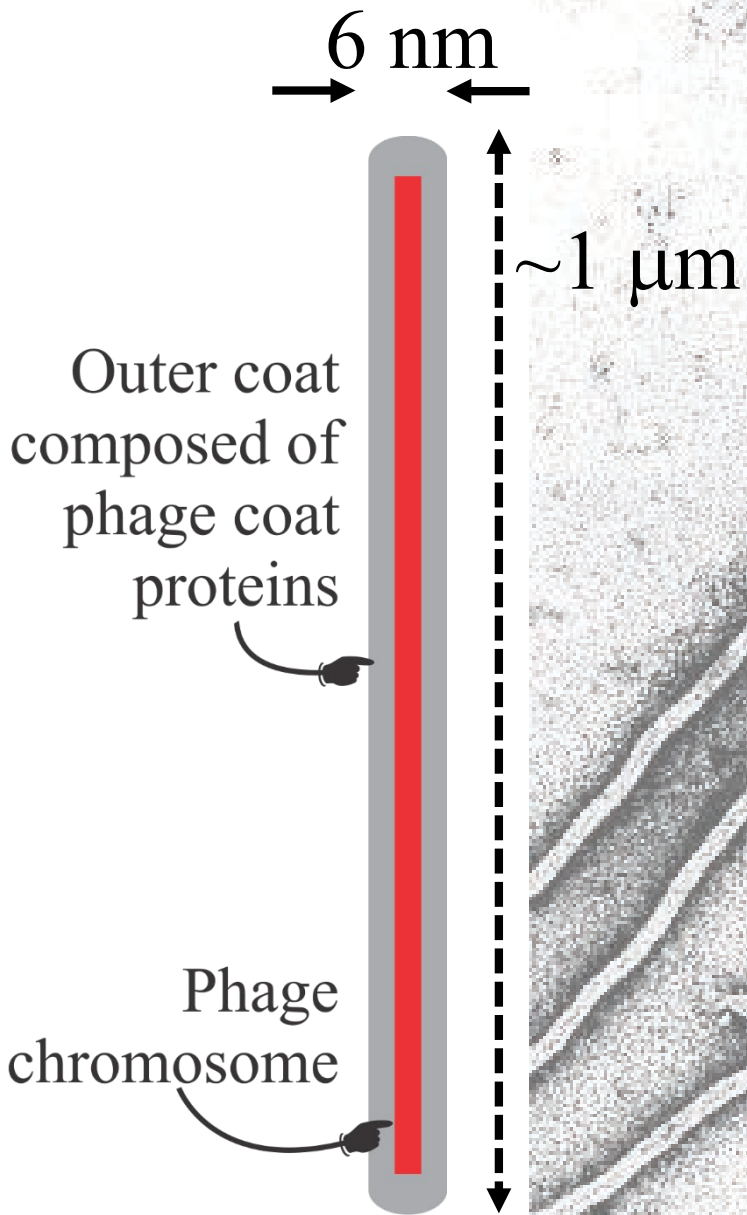# Filamentous phage

Filamentous phage

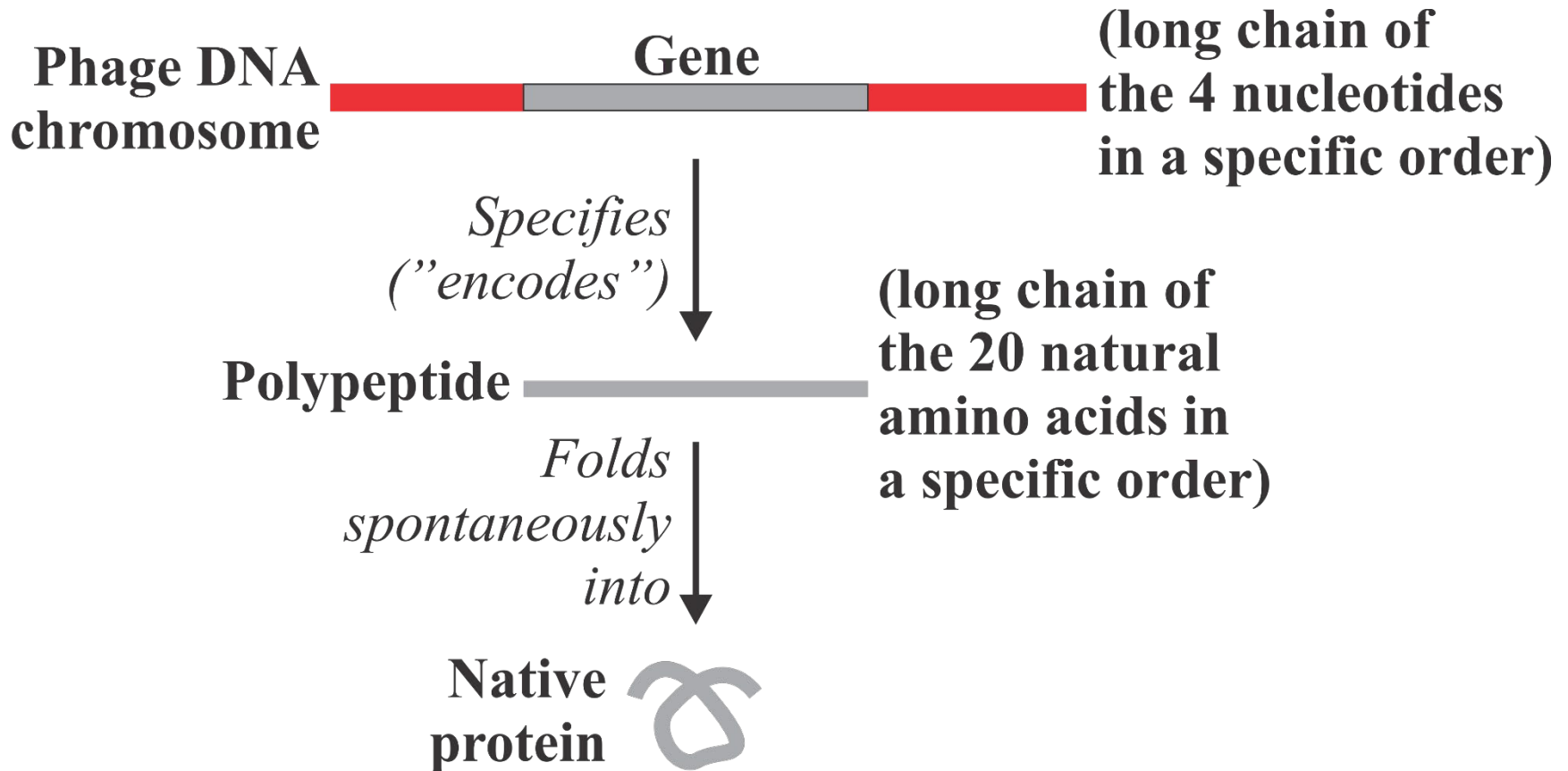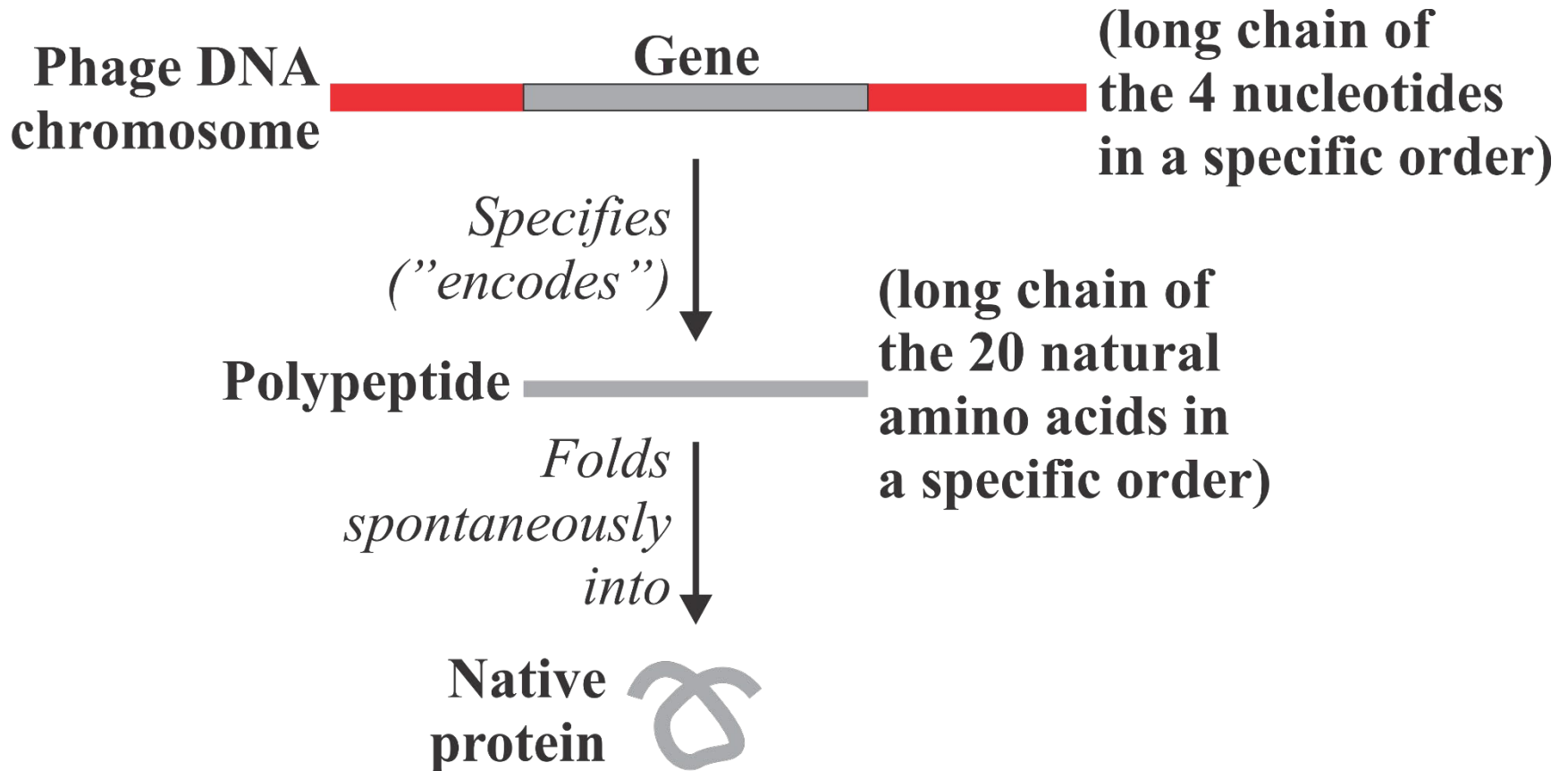Outer coat composed of phage coat proteins

Phage chromosome

Filamentous phage

6 nm

~1 μm

Outer coat composed of phage coat proteins

Phage chromosome

Crash course in molecular biology

Crash course in molecular biology

Phage DNA chromosome — **Gene** — (long chain of the 4 nucleotides in a specific order)

Specifies ("encodes")

Polypeptide (long chain of the 20 natural amino acids in a specific order)
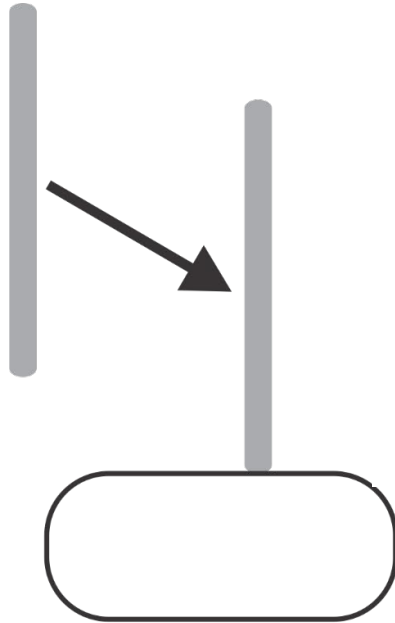
Folds spontaneously into

Native protein

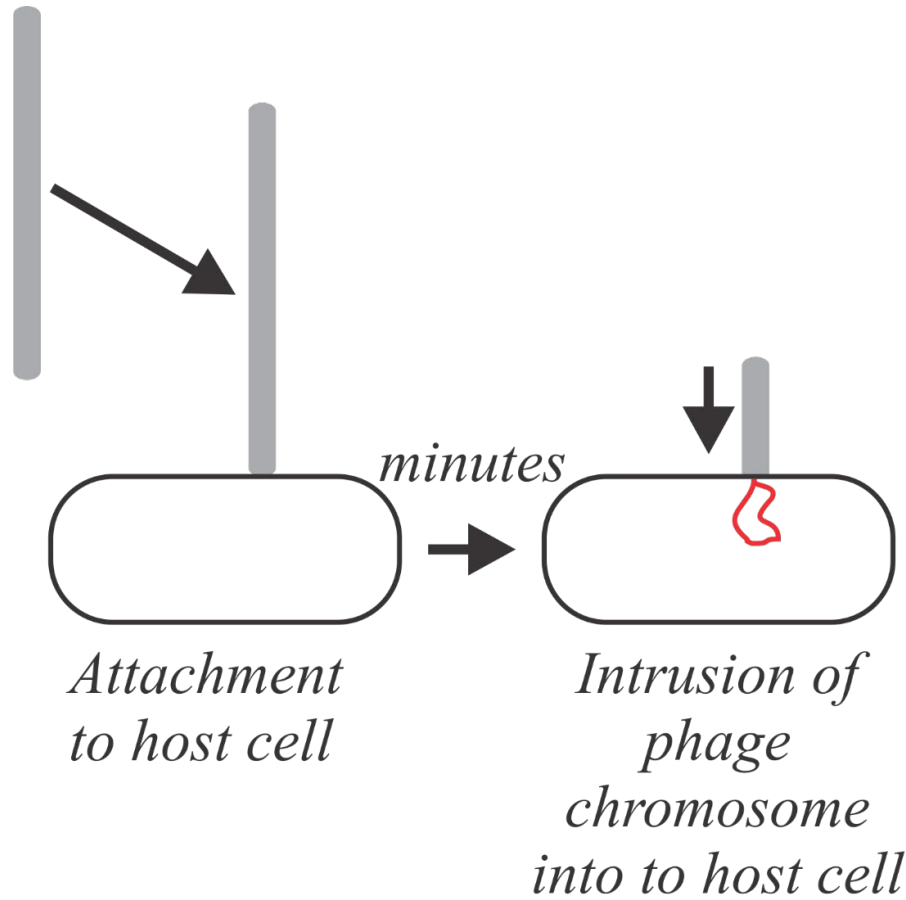**Peptide** — (short chain of amino acids—e.g., encoded by a gene fragment)

Filamentous phage infection cycle (simplified)

Bacterial cell

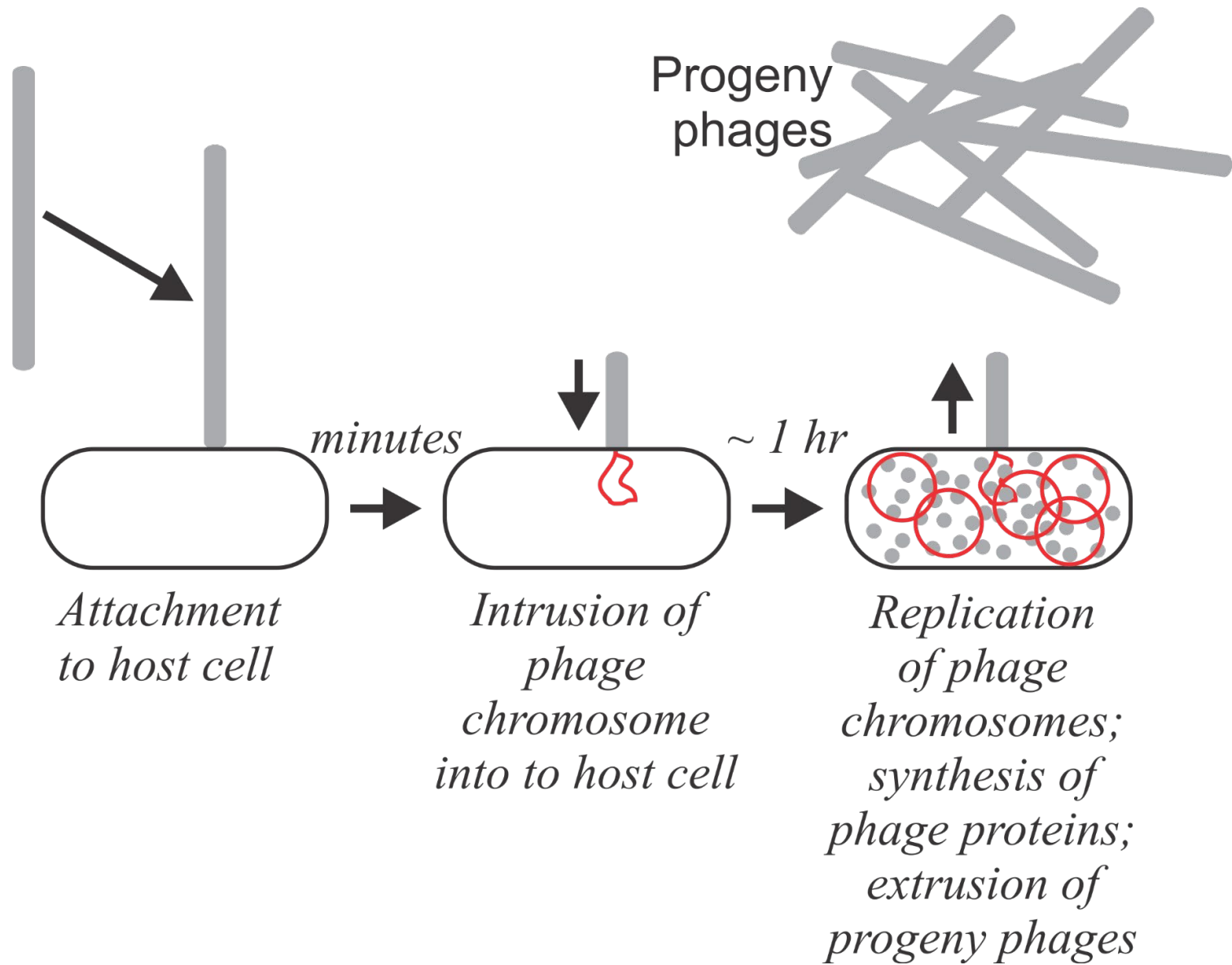Attachment
to host cell

minutes

Attachment
to host cell

Intrusion of
phage
chromosome
into to host cell

Progeny phages

minutes

~ 1 hr

Attachment
to host cell

Intrusion of
phage
chromosome
into to host cell

Replication
of phage
chromosomes;
synthesis of
phage proteins;
extrusion of
progeny phages

shake overnight at 37°C
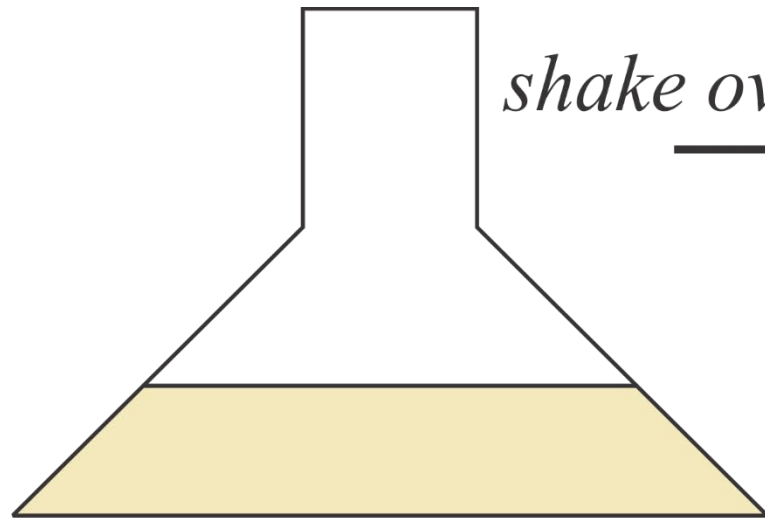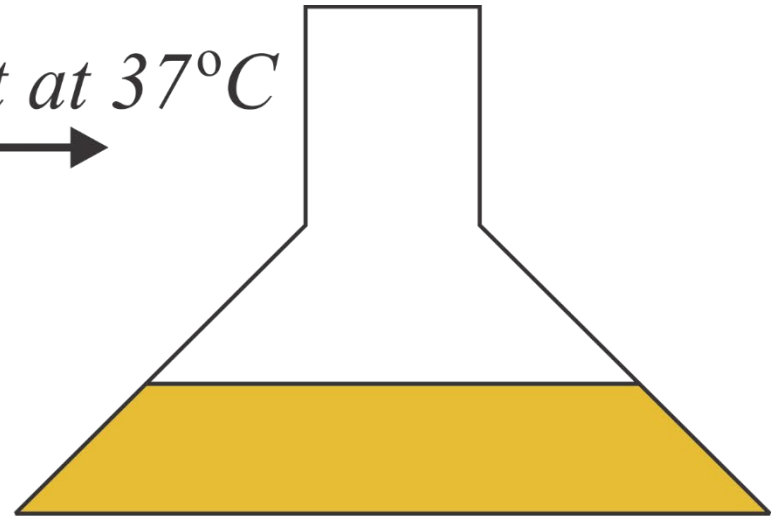
1 phage
10^{11} cells
in 1 liter medium

10^{15} phages
5×10^{12} cells
in 1 liter medium

Bob Webster, Department of Biochemistry, Duke University (now retired to North Carolina coast)

This coat protein gene encodes a coat protein that's partly exposed at one tip of the phage
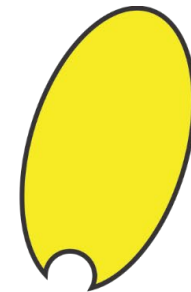
Paul Modrich
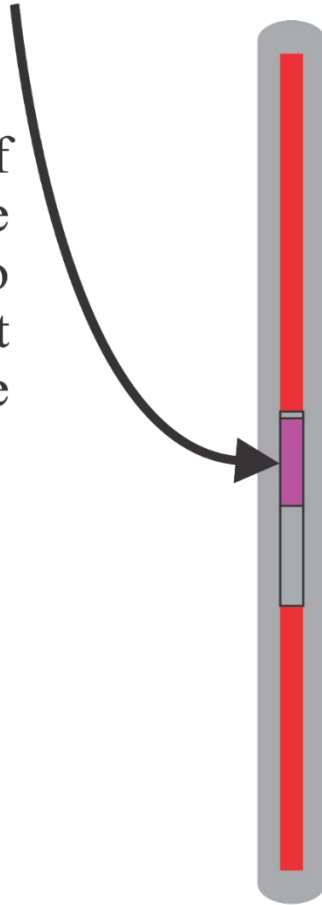Nobel Prize in Chemistry, 2015

*Eco*RI gene

anti-*Eco*RI
antibody

*Eco*RI gene

Fragment of *Eco*RI gene inserted into phage coat protein gene

test phage

*Eco*RI gene

Fragment of *Eco*RI gene inserted into phage coat protein gene

Fragment of *Eco*RI protein displayed on phage surface

test phage

*Eco*RI gene

Fragment of *Eco*RI gene inserted into phage coat protein gene
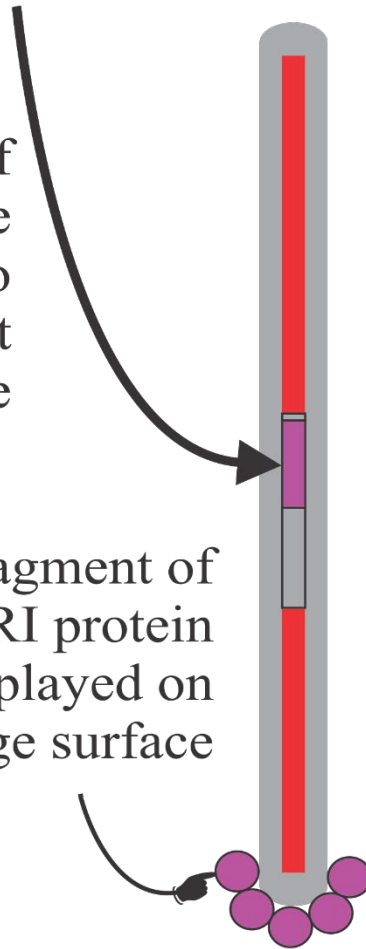
Fragment of *Eco*RI protein displayed on phage surface

*Eco*RI gene

Fragment of
*Eco*RI gene
inserted into
phage coat
protein gene

Fragment of
*Eco*RI protein
displayed on
phage surface

test phage

+

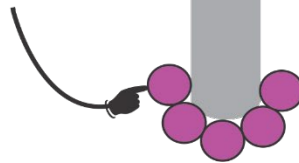anti-*Eco*RI
antibody

Infectivity
blocked

*Eco*RI gene

Fragment of
*Eco*RI gene
inserted into
phage coat
protein gene

Fragment of
*Eco*RI protein
displayed on
phage surface

test phage

+

anti-*Eco*RI
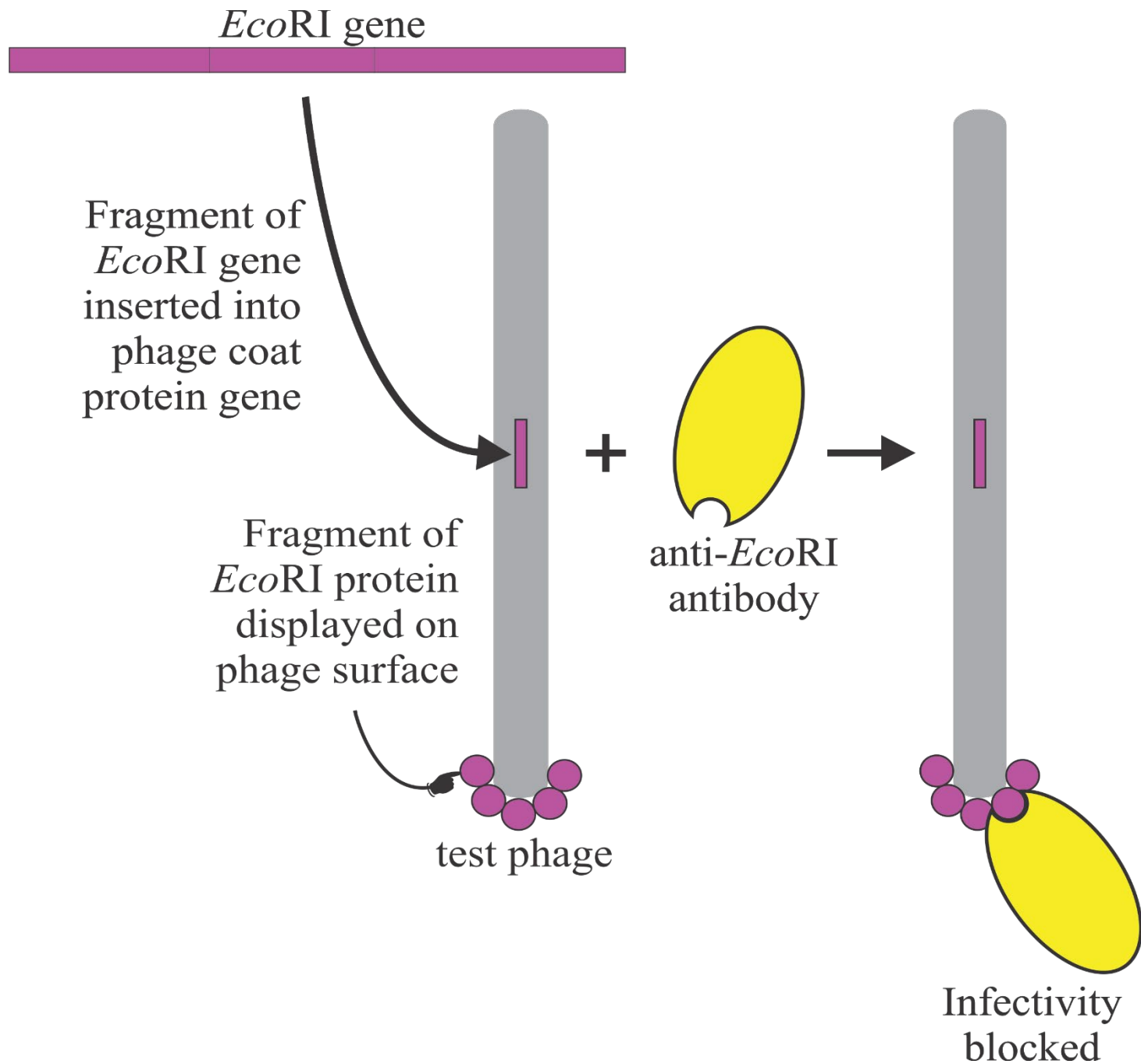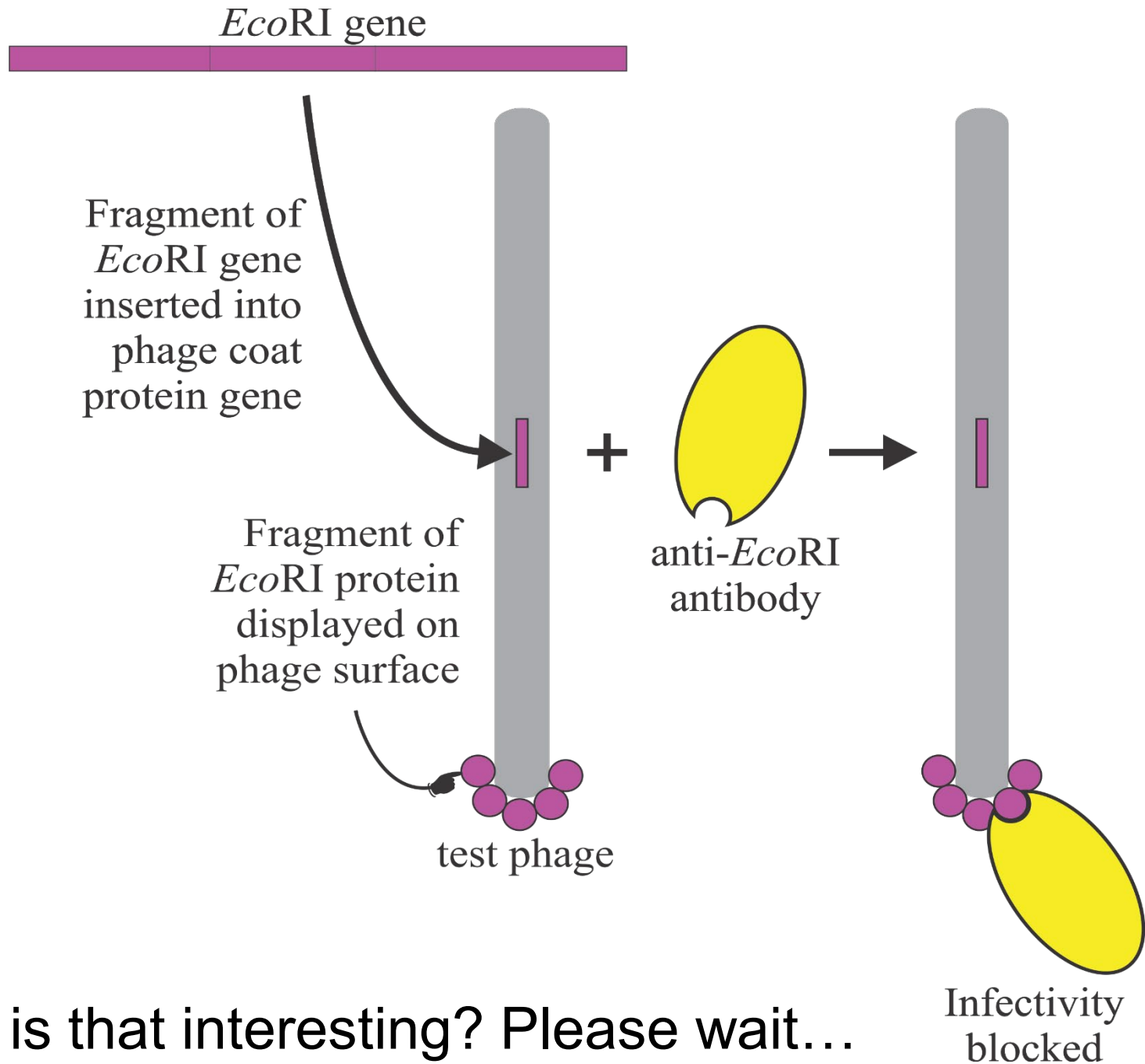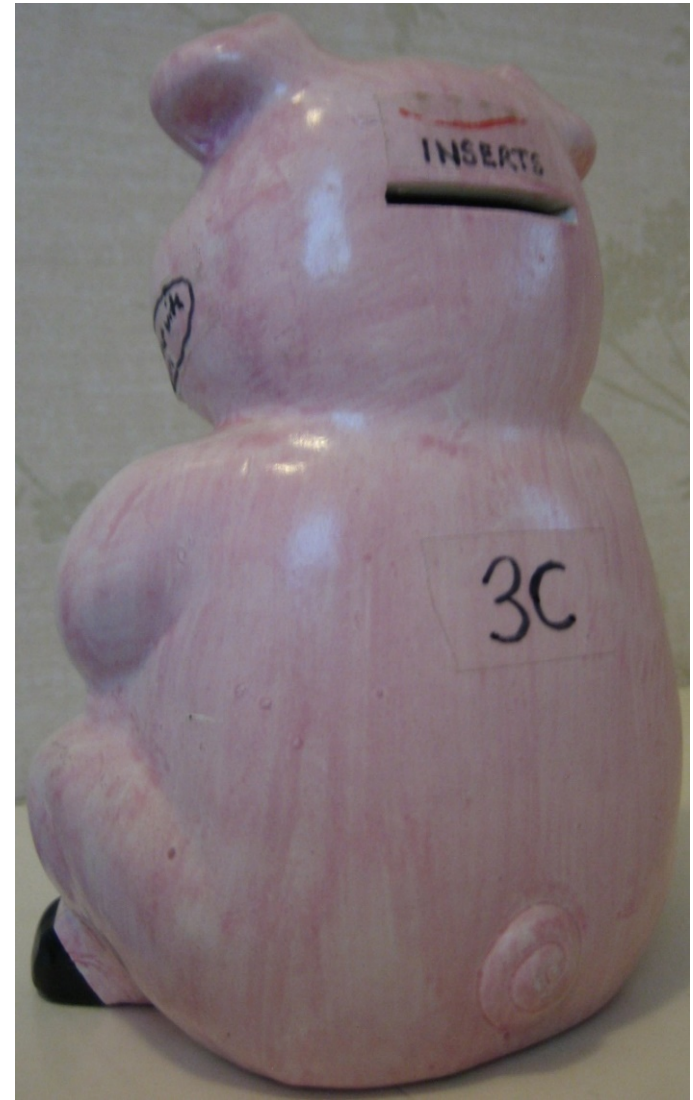antibody

→

Infectivity
blocked

Why is that interesting? Please wait…

Darn good colleague
Steve Parmley

Steve developed a practical phage display vector
(after some false starts like pIG3C) and affinity
selection as grad student, 1985-1988.

Darn good colleague Jamie Scott

Jamie Scott first demonstrated affinity selection of peptides from large random peptide libraries as postdoc, 1988-1991.
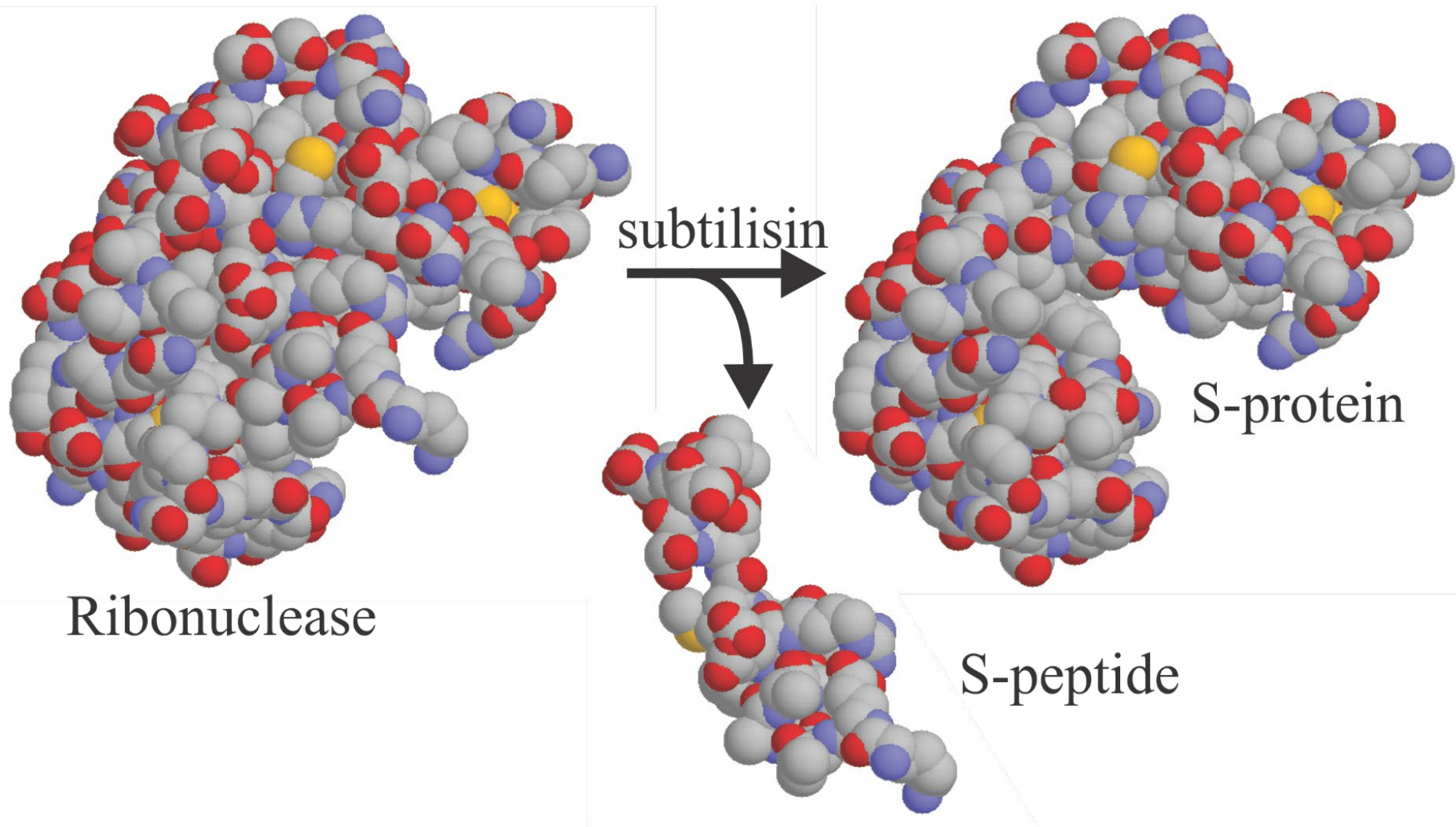
Darn good
colleague
Robert Davis

Robert Davis came to the lab as chief manager and technician in the summer of 1989. We calculate that he sequenced a million DNA bases using old-fashioned radioactive technology.
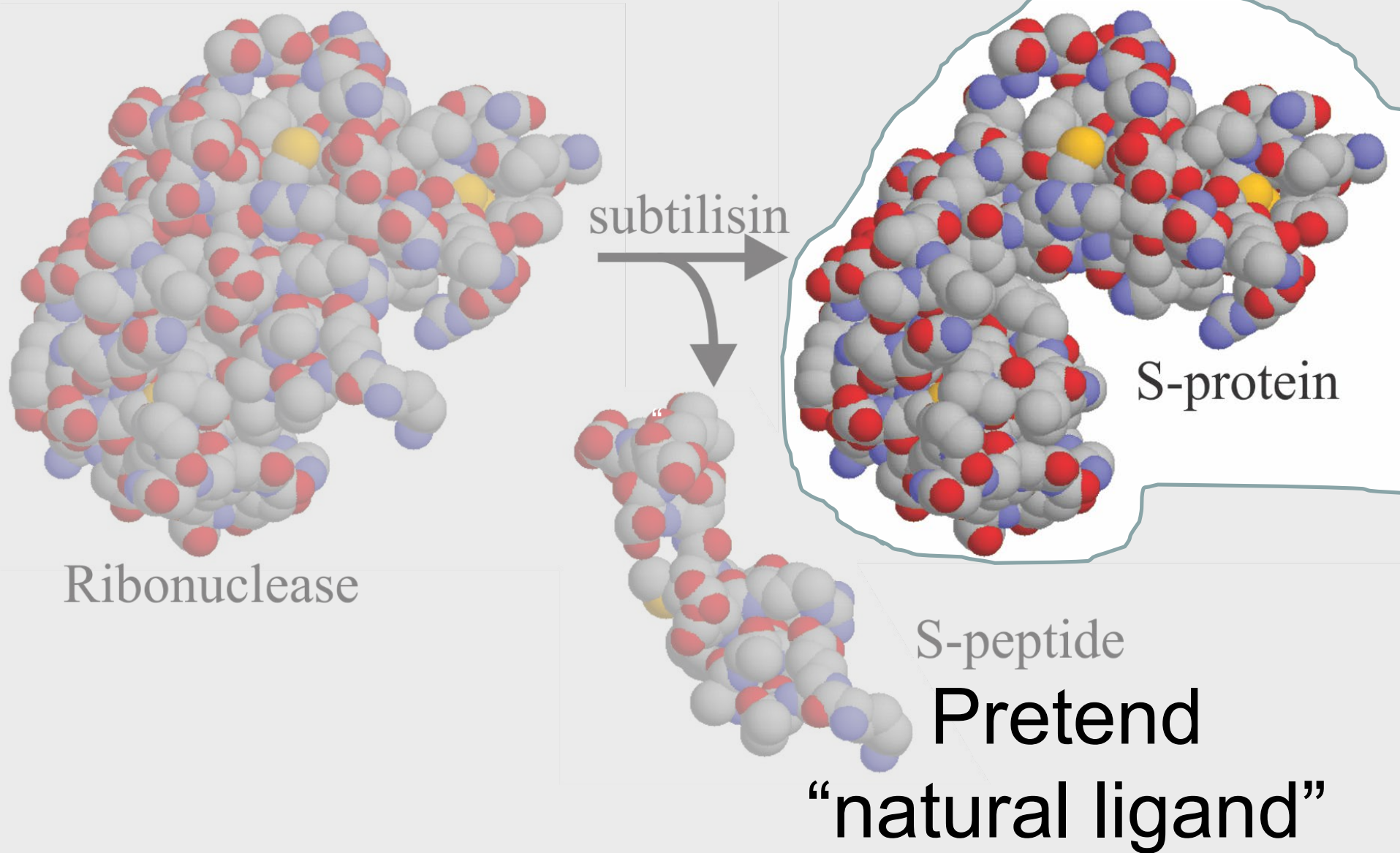
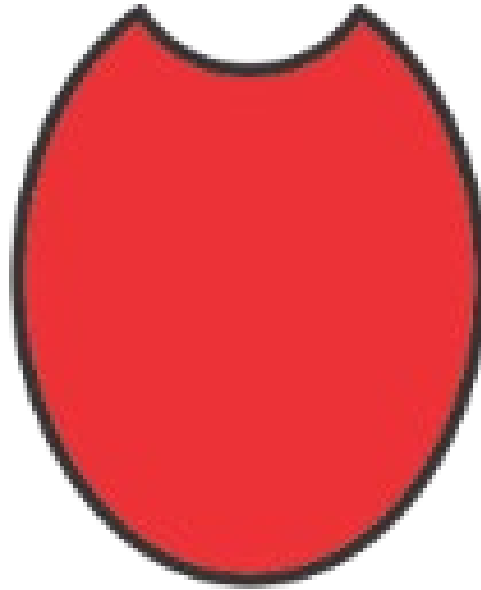# The S-protein system

subtilisin

Ribonuclease

S-protein

S-peptide

Fred Richards at Yale, late 50's; brought to our lab by John Ladbury and David Schultz

Pretend "receptor"

subtilisin

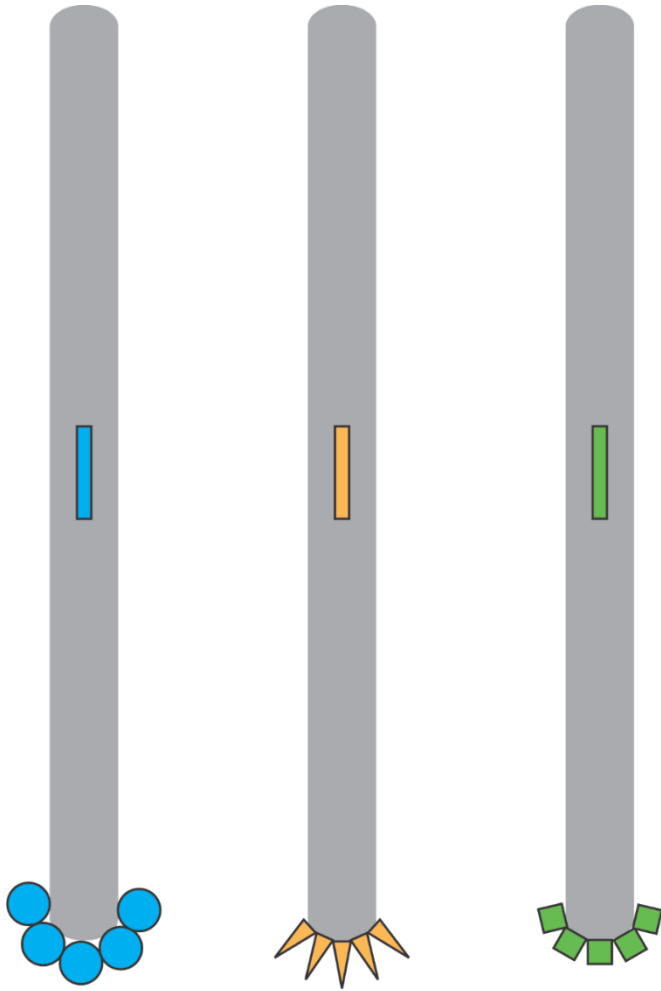S-protein

Ribonuclease

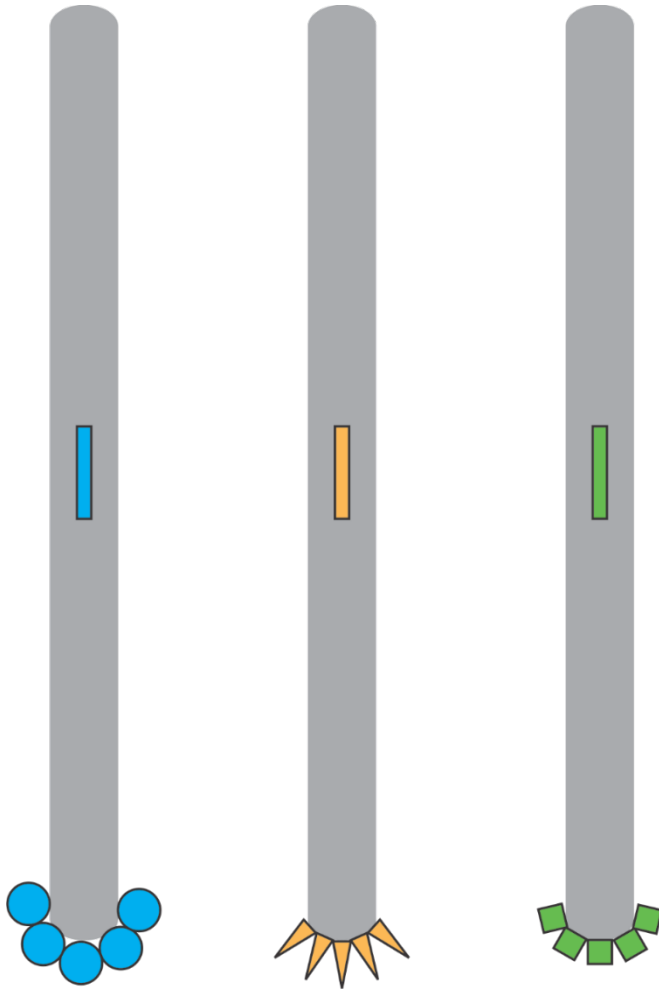S-peptide

Pretend "natural ligand"

S-protein
"receptor"

Jinan Yu, now a researcher at Hainan University, China.

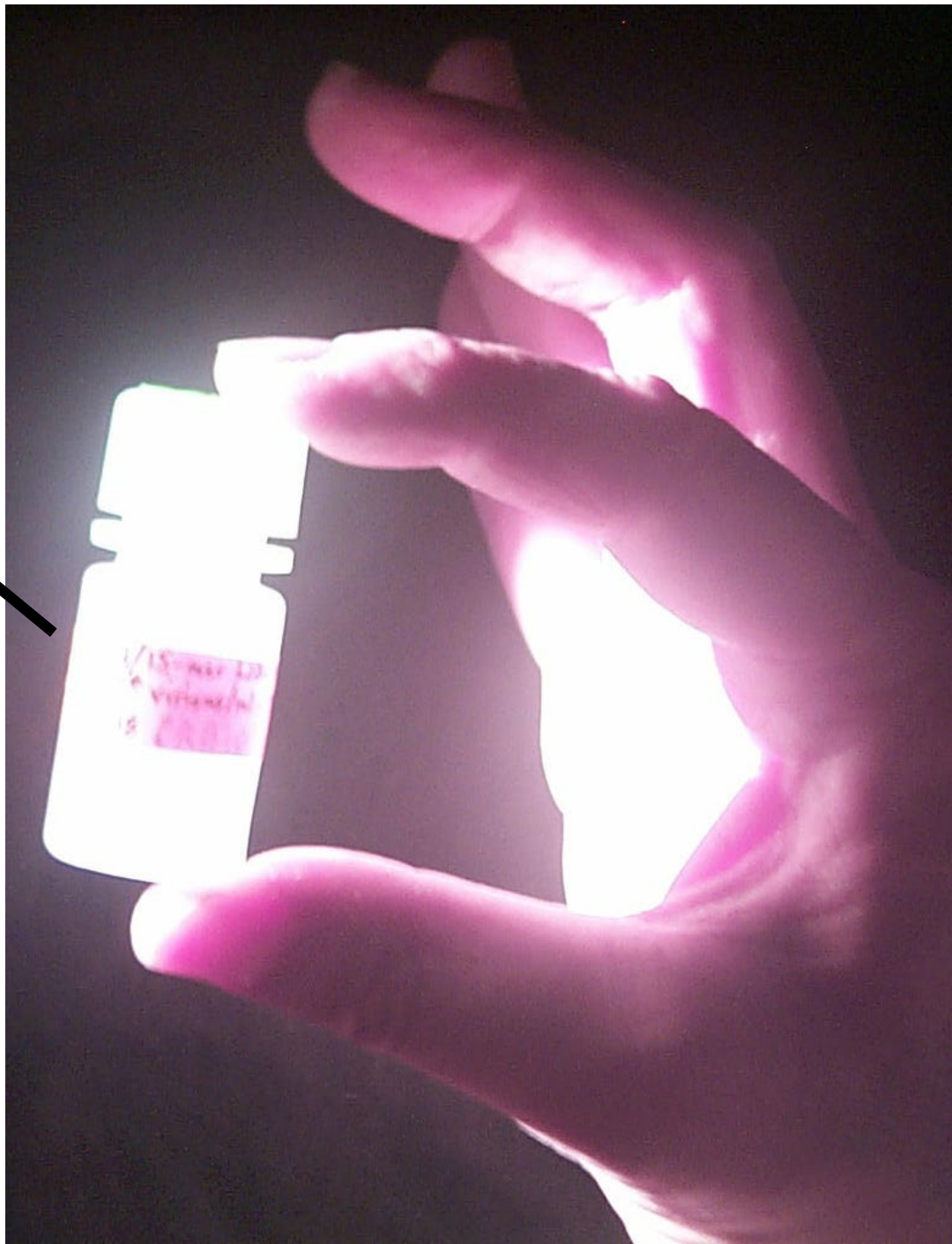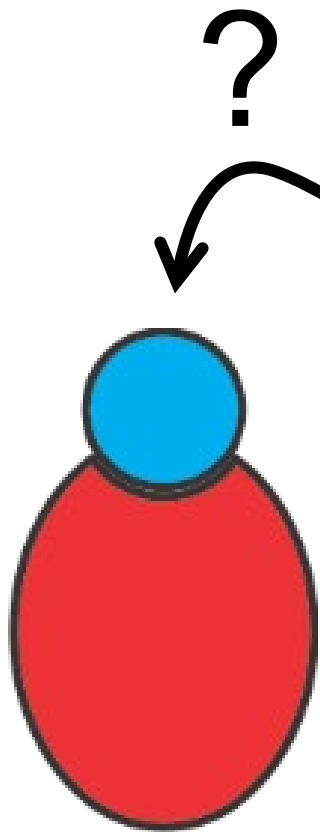# Random peptide library

Random peptide library

10^{15} phages representing 250 million phage clones, each clone displaying a different 15-amino acid guest peptide. [T. Nishi et al., FEBS Letters 399, 237–240 (1996)]

?

Affinity selection

Immobilized
S-protein
"receptor"

Petri dish or other surface

Random peptide library

Immobilized
S-protein
"receptor"

Petri dish or other surface

Random peptide library

**Receptor binds
one of the guest
peptides,
capturing the
attached phage
on surface**

Immobilized
S-protein
"receptor"

Petri dish or other surface

Random peptide library

Receptor binds
one of the guest
peptides,
capturing the
attached phage
on surface

Immobilized
S-protein
"receptor"

Petri dish or other surface

Unbound
phages
washed
away

Random peptide library

Receptor binds one of the guest peptides, capturing the attached phage on surface

Immobilized S-protein "receptor"

Petri dish or other surface

Unbound phages washed away

Captured phages released

Random peptide library

Receptor binds one of the guest peptides, capturing the attached phage on surface

Immobilized S-protein "receptor"

Petri dish or other surface

Unbound phages washed away

Released phages propagated by infecting fresh cells

Captured phages released

Trillions of phages, greatly enriched for clones whose guest peptides bind the S-protein "receptor"
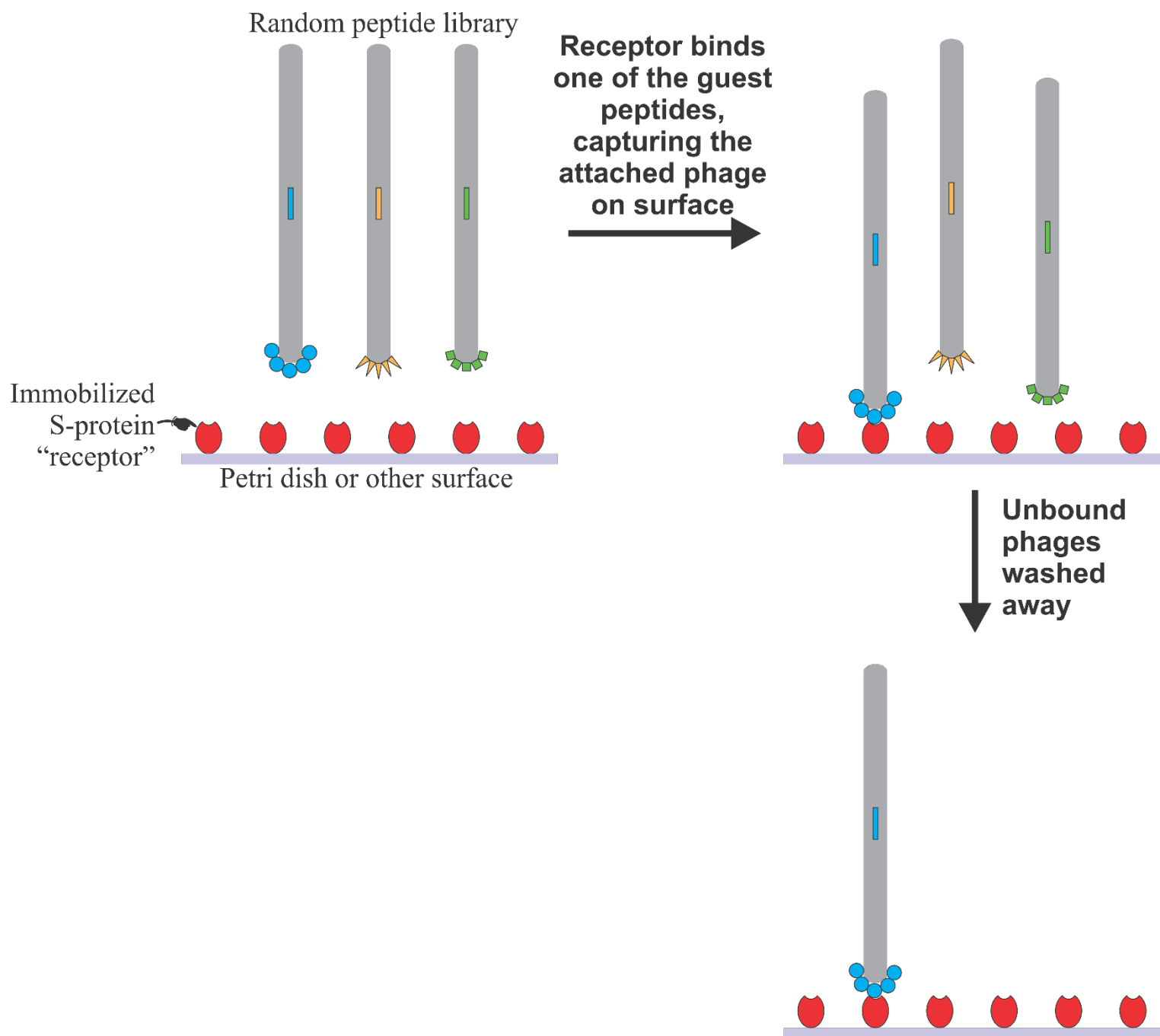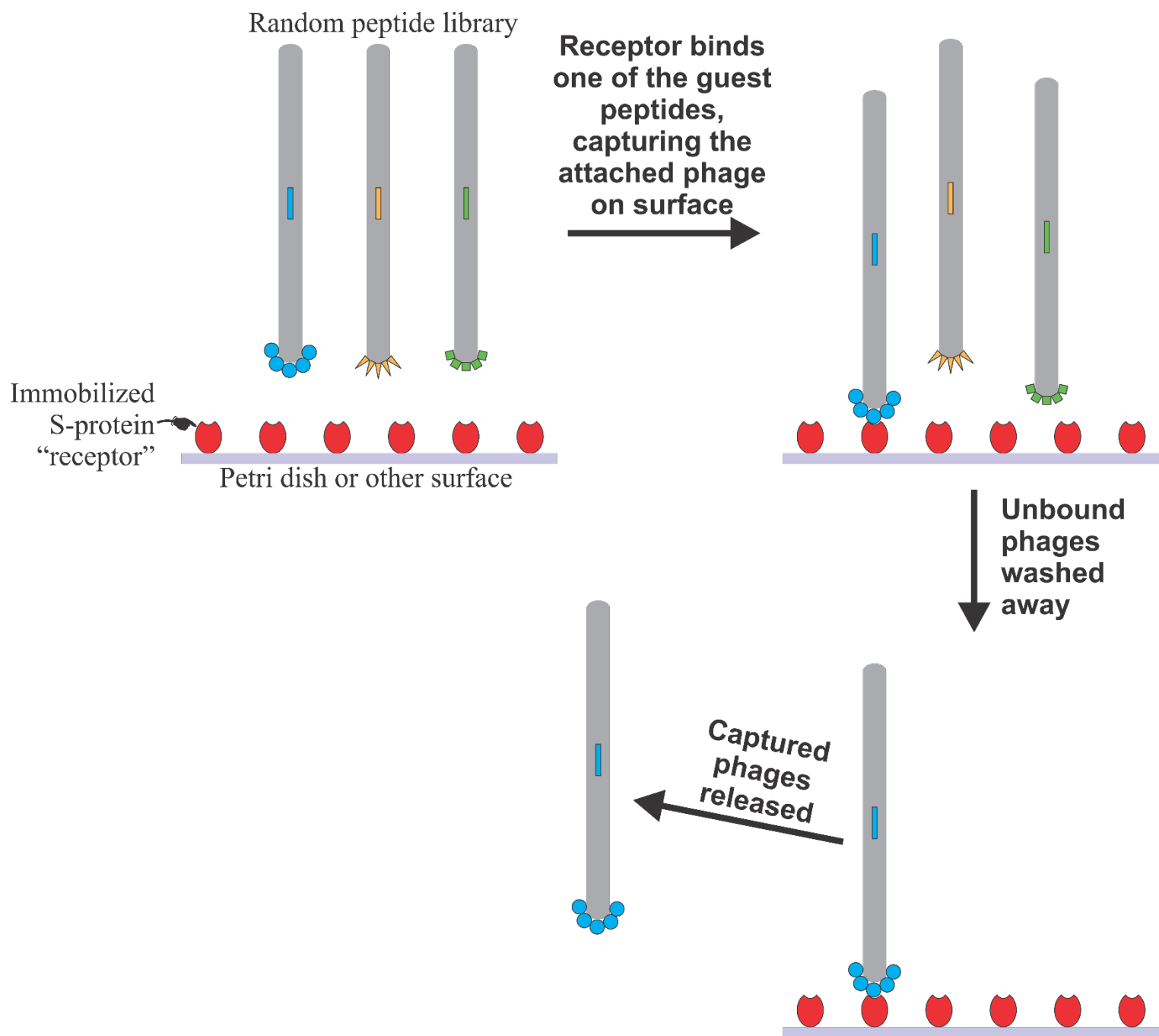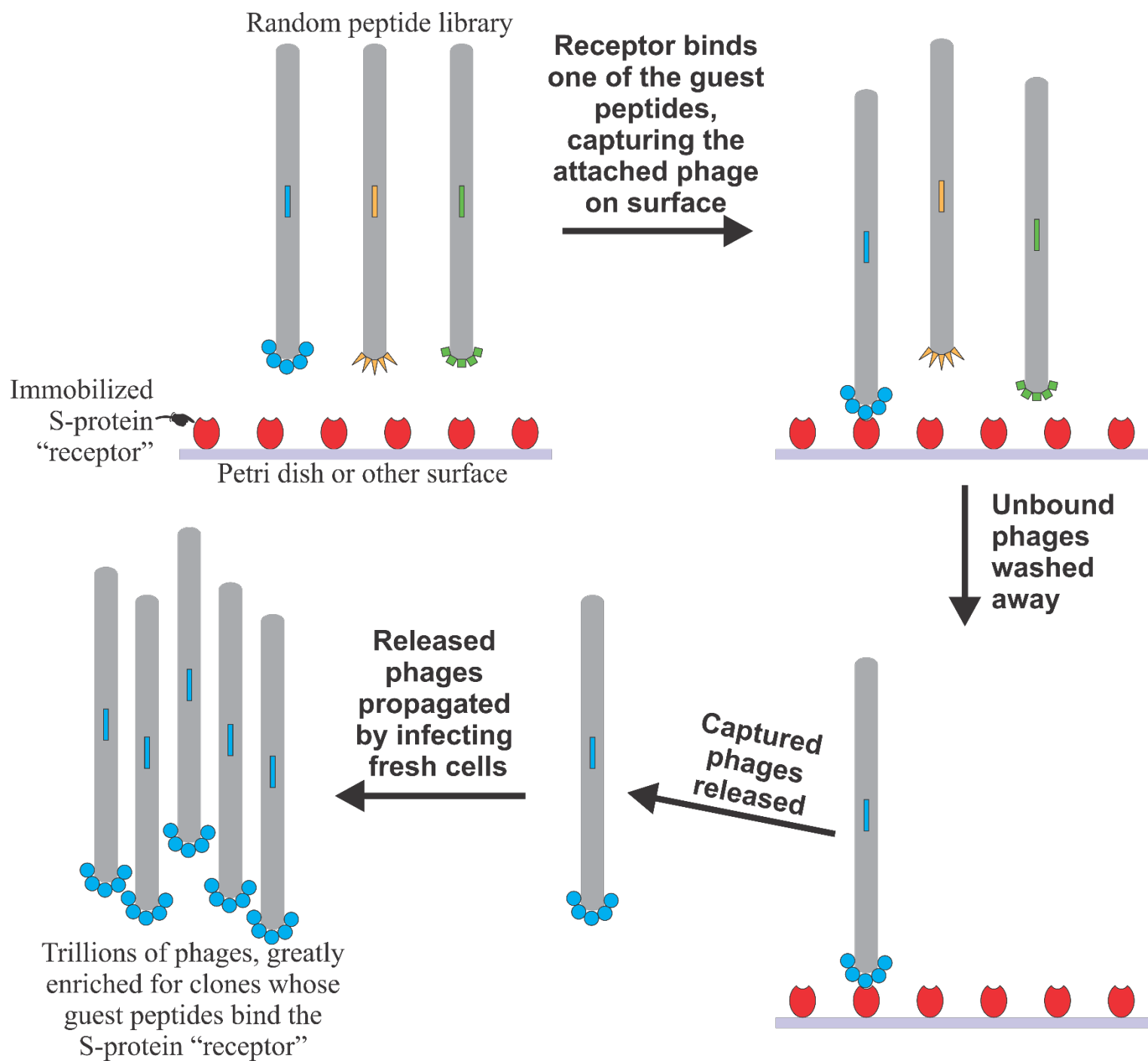
Dominant sequence among selected peptides

Selected peptide **NRAWSEFLWQHLAPV**

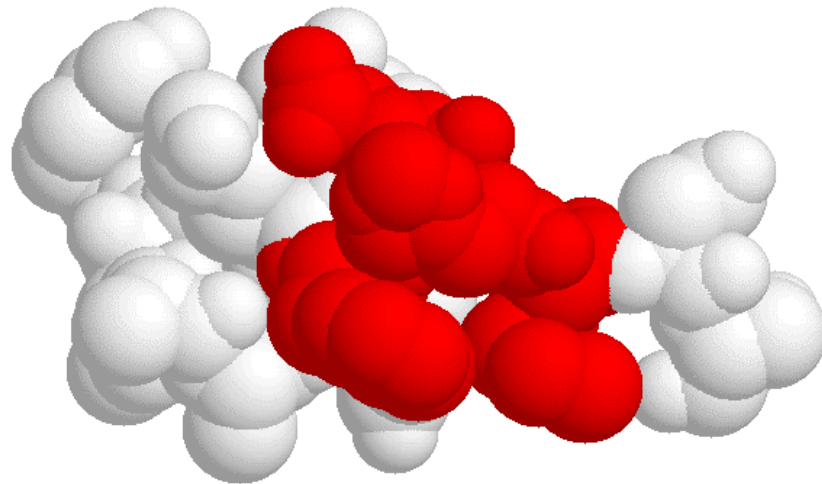(one-letter abbreviations for amino acids)

Dominant sequence among selected peptides aligns with S-peptide "natural ligand"

Selected peptide **NRAWSEFLWQHLAPV**

S-peptide **KETA****A****AAK****F****ER****QHM****DSSTSAA**

Buried amino acids

Artificial evolution in the petri dish

Evolution in the living world

- Diversification

- Natural selection

- Adaptation

Affinity-selection from random peptide libraries

- Construction of library

- Affinity selection

- Peptide with desired activity
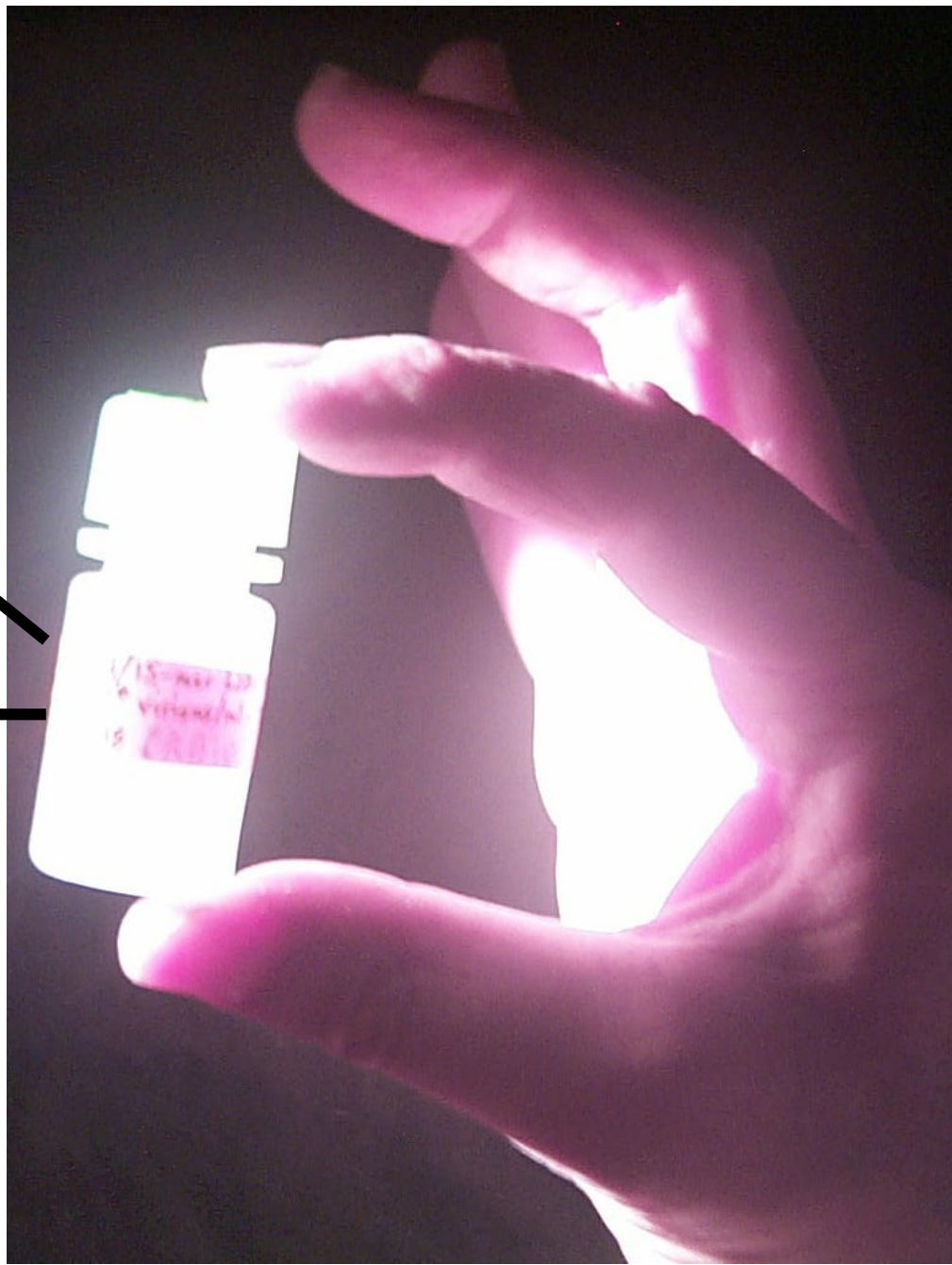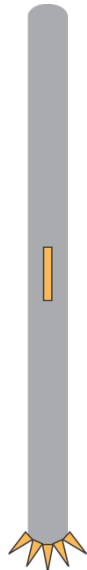
Cure for S-protein disease

Selection scheme 1

Cure for S-protein disease

Selection scheme 1

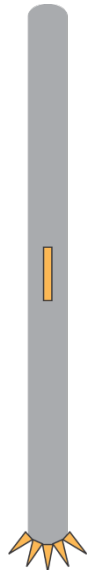*Selection scheme 2*

Marvel 2

Cure for S-protein disease

Selection scheme 1

Selection scheme 2

Marvel 2

Selection scheme 3

Marvel 3