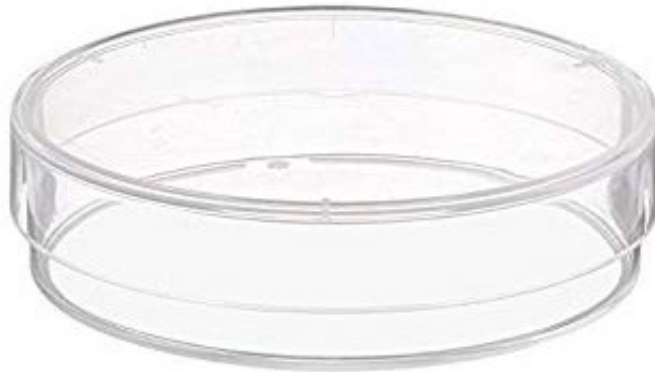


Phage Display: Simple Evolution in a Petri Dish



George P. Smith
Division of Biological Sciences
University of Missouri
Nobel Prize Lecture in Chemistry
December 8, 2018

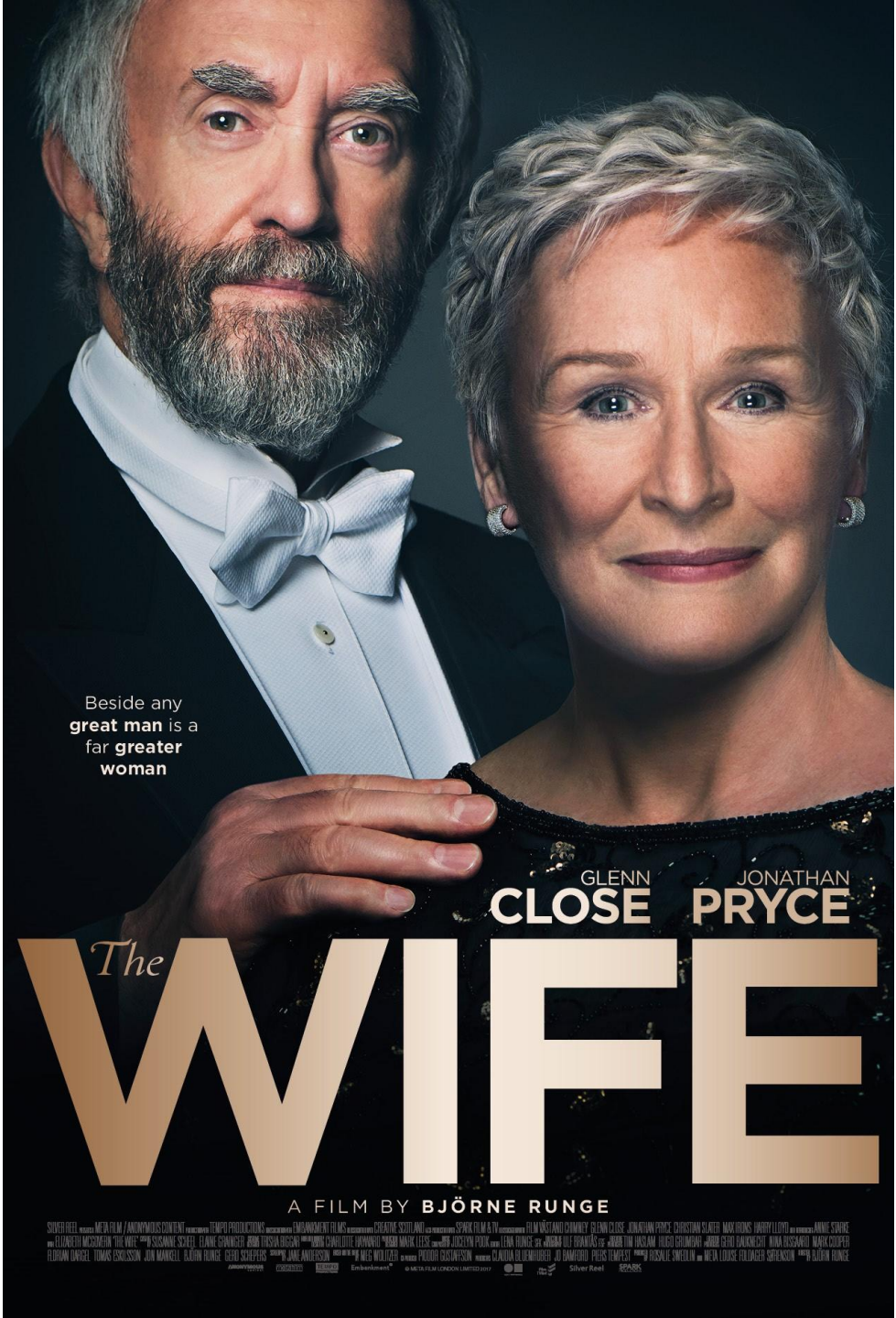
Beside any
great man is a
far greater
woman

GLENN
CLOSE JONATHAN
PRYCE

The
WIFE

A FILM BY BJÖRNE RUNGE

STORY BY METE HILMI / ANONYMOUS COMMENT EXECUTIVE PRODUCERS TEMPO PRODUCTIONS PRODUCED BY EMANUELLE FILMS DIRECTED BY BJÖRNE RUNGE CASTING BY SCOTT AND COSTUME DESIGNER SPARK FILM & TV EXECUTIVE PRODUCERS FILM VÄSTRAUT ÖRN GLENN CLOSE JONATHAN PRYCE CHRISTIAN SLATER MARK FRANKS SHERRY LLOYD PRODUCED BY ANNE SCHWABE
AND ELLENORH MACCARTHEA THE WIFE WITH SUSANNE SCHWABE LEAHNE SPANINGER JONAS THUNDA PRESIDENT JONAS SCHWILDT THOMAS MARIAN MARIE LEISE AND JUDITHEN POK WITH LEENA RUNGE AS JONAS HILJE RENATAS AS ANDREW TIM HASANAH JULIO SCHIMMEL JONAS ESTER HALLMANN ANNA HUSSAR MARIK SCHULTZ
FLORIAN DÜRR TOMAS EKLUND JON MARKELL OLIVIA RUNGE GREGG SCHEPERS PRODUCED BY JANE ANDERSSON AND WITH MARG WOLFFEN PRODUCED BY PRODUKT CASTING CLAUDIA RAJ JOHANNES JOHANNES PHILIP TEMPEL PRODUCED BY ROSALIE SWEDEN WITH LEENA FOLDA SUNDEN BY BJÖRNE RUNGE



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

GLENN
CLOSE JONATHAN
PRYCE

The
WIFE

A FILM BY **BJÖRNE RUNGE**

STORY BY METTA FOLM / ANONYMOUS COMMENT EXECUTIVE PRODUCERS TEMPO PRODUCTIONS PRODUCED BY EMERALD ENTERTAINMENT FILMS EXECUTIVE PRODUCERS SCOTT AND EXECUTIVE PRODUCERS SPARK FILM & TV EXECUTIVE PRODUCERS FILM NÅST ANTI CHAMNEY GLENN CLOSE JONATHAN PRYCE CHRISTIAN SLATER MAX FRODO SHERRY LLOYD PRODUCED BY ANNIE SCHWAB
AND ELLENREITH MACCARTHEA THE WIFE WITH SUSANNE SCHWAB LEAHNE SCHWABER JOMA THUSIA PRESENT WITH CHARLOTTE HORNQUIST MARGA MARIE LEIS AND JUDITHEN PERLIN WITH LEENA RINNE AND JOHANNA HILJE GRIFFITHS ANDER TIM HASANAH JORNA ESTER HALLMANN ANNA ROSSARDO MARGA SCHWAB
FLORIAN DUNICEL TOMAS EKLUND JON MARKELL BLIGH RINNE GREGG SCHEPERS WRITTEN BY JANE ANGLERSON DIRECTED BY BJÖRNE RUNGE PRODUCED BY CLAUDIA RAJ LINDHOLM JOHANNES PETER TEMPLER PRODUCED BY ROSALIE SWEDLUN WITH LEENA RINNE FOLDOREN SURENDRAN WITH BLIGH RINNE

My science community

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



Phage
display

Our science community

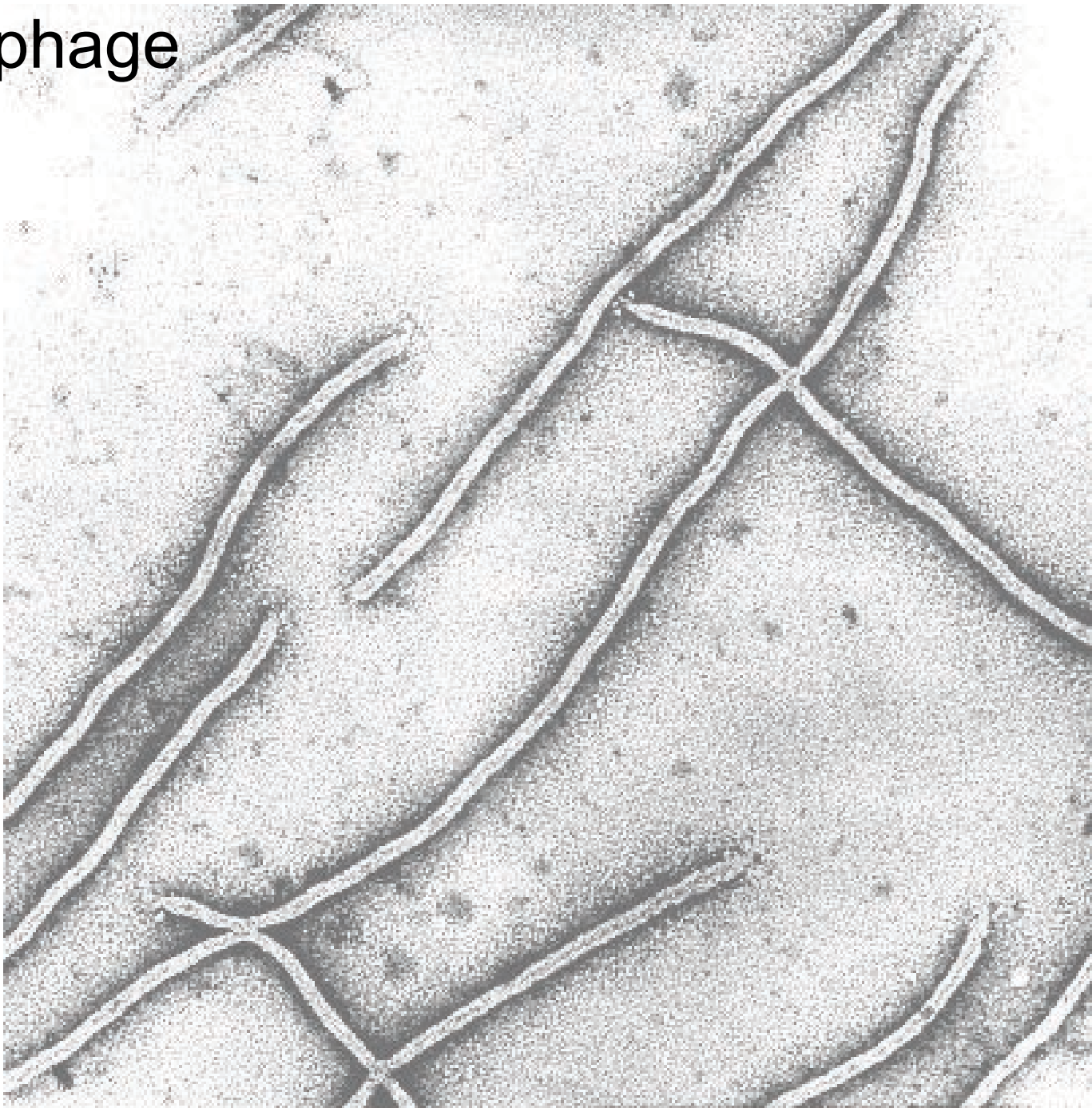
- Molecular biologists
- Evolutionary biologists
- Immunologists
- Protein chemists
- Phage biologists
- Mathematicians
- Philosophers of science
- Bayesian statisticians



Phage
display

Phage = virus that infects bacteria

Filamentous phage

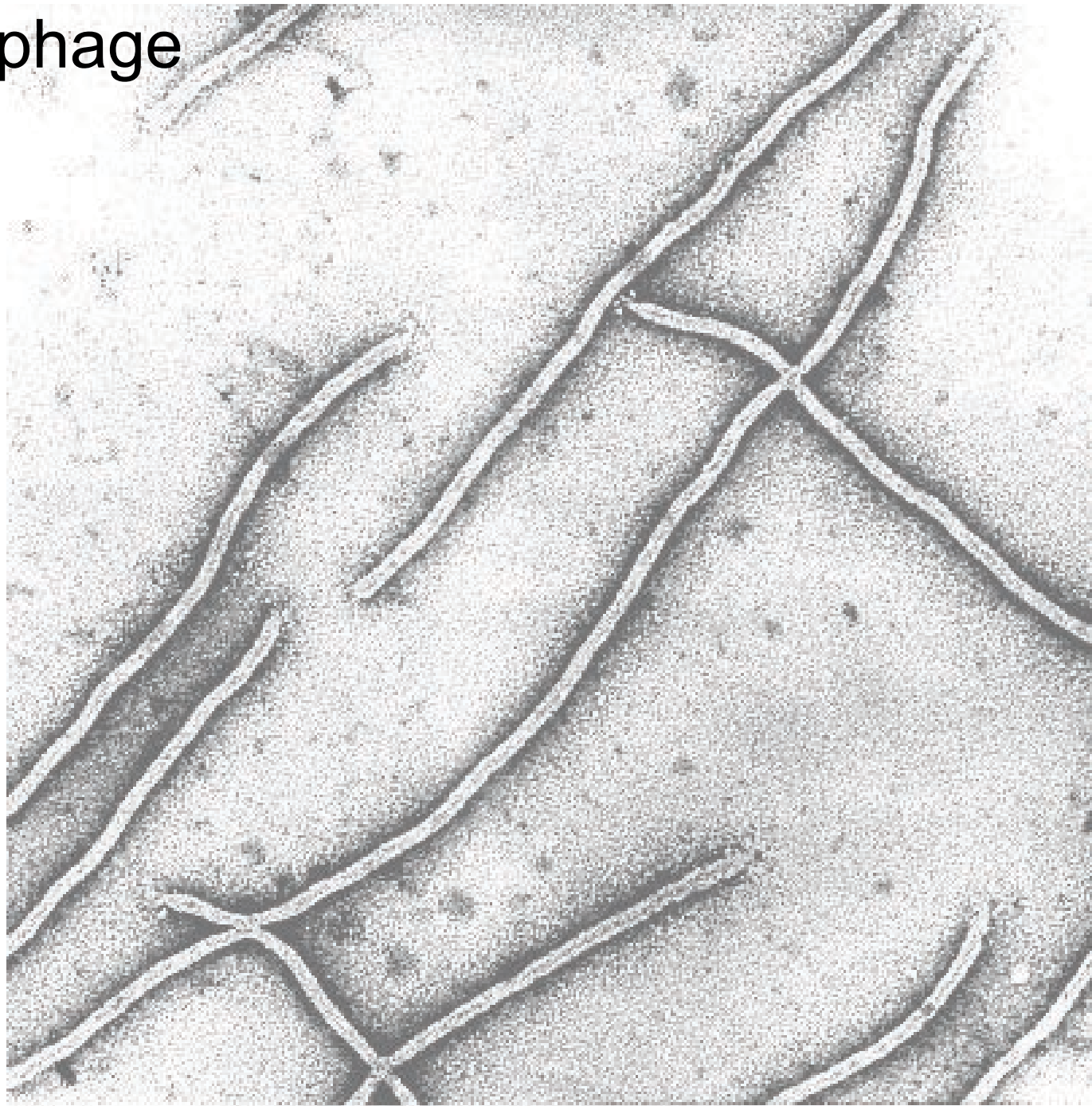


Filamentous phage

Outer coat
composed of
phage coat
proteins



Phage
chromosome



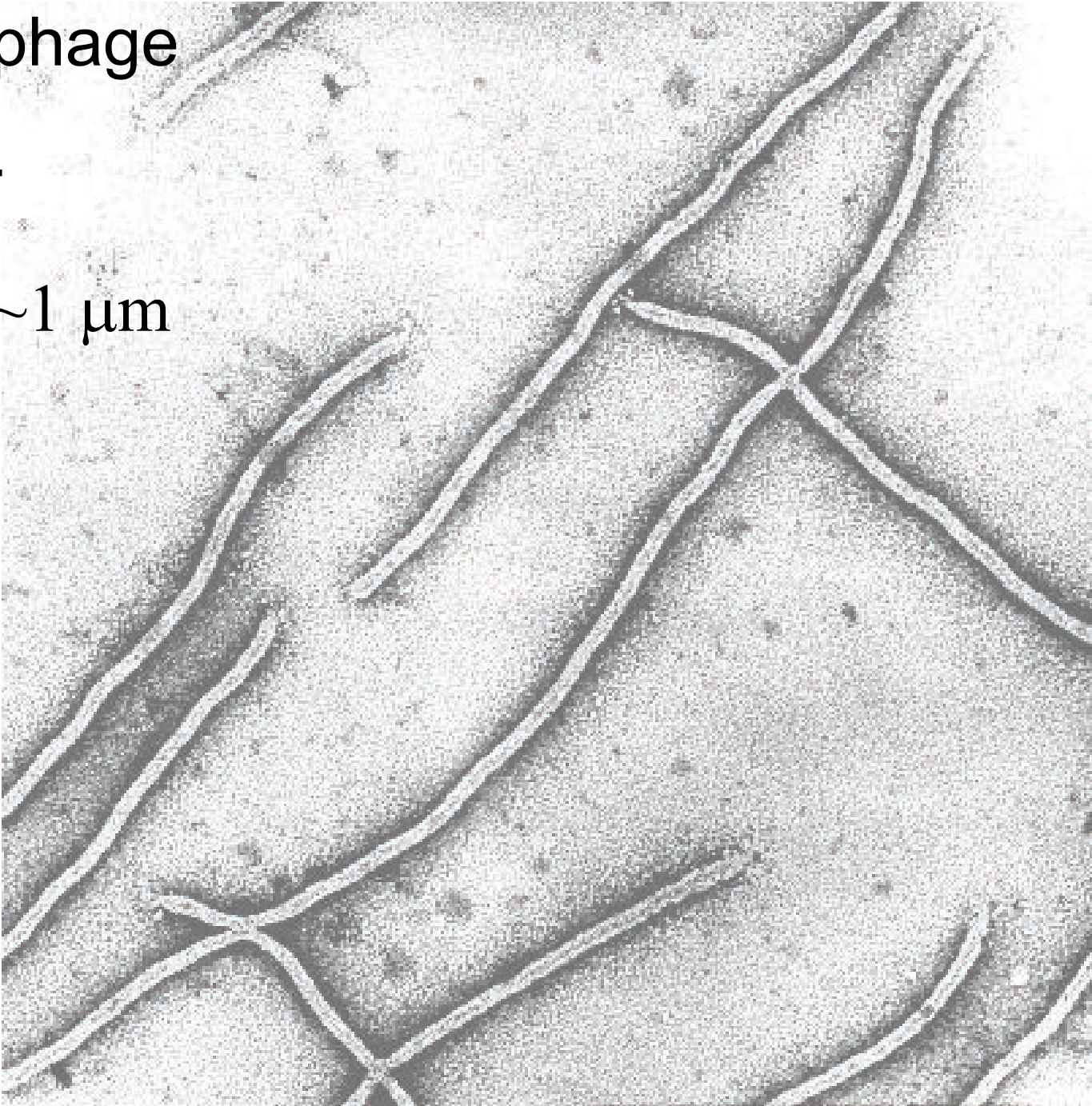
Filamentous phage

6 nm

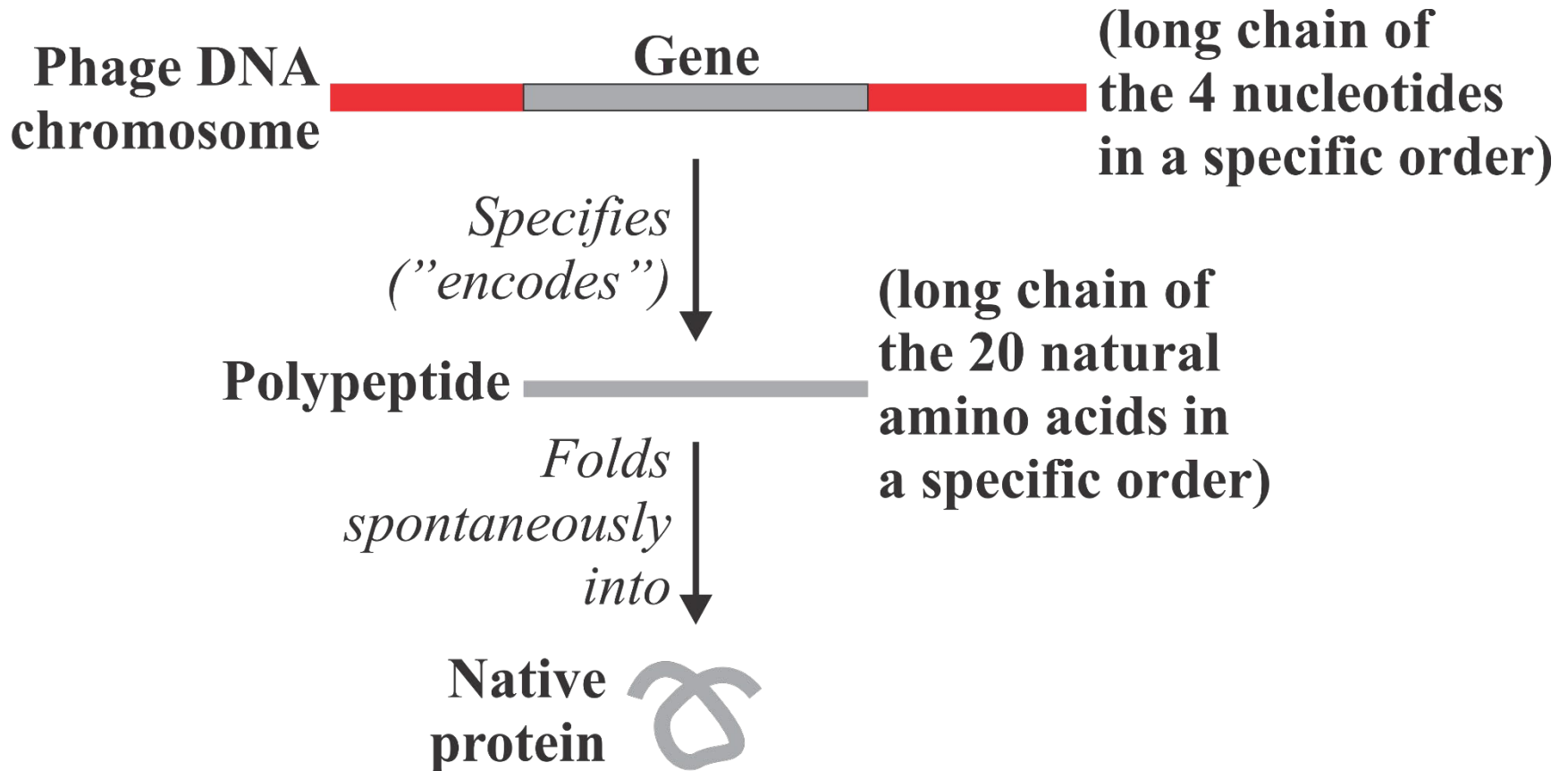
~1 μm

Outer coat
composed of
phage coat
proteins

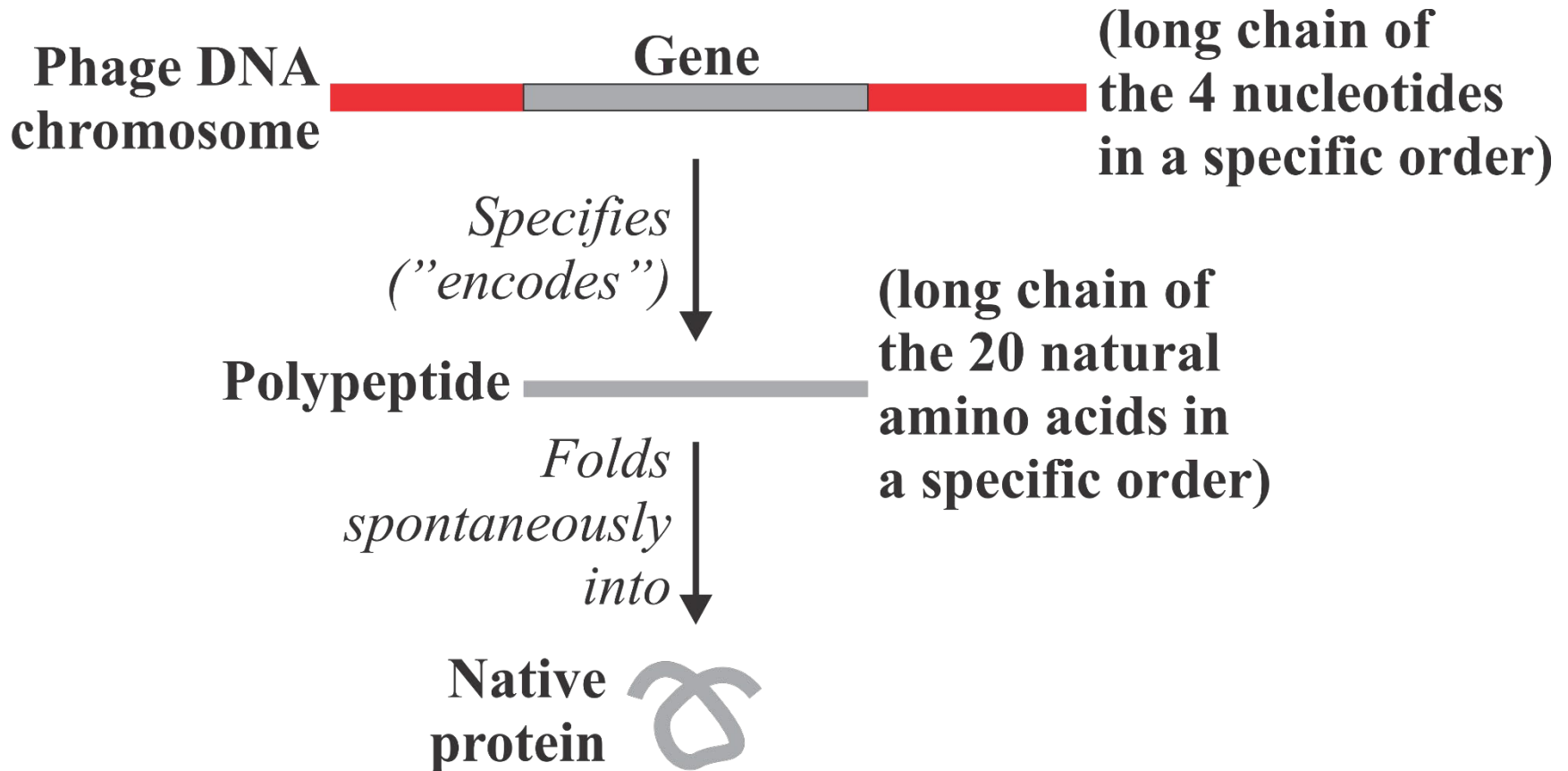
Phage
chromosome



Crash course in molecular biology



Crash course in molecular biology

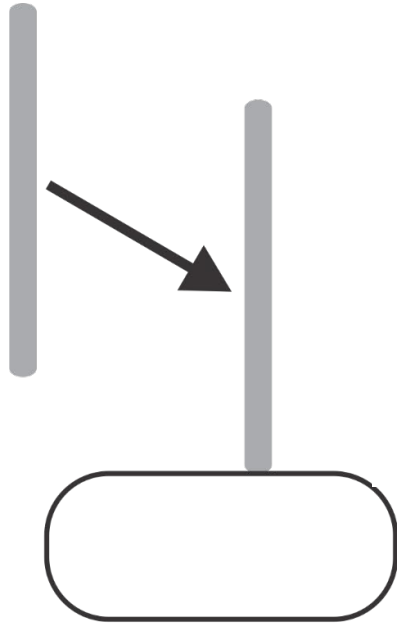


Peptide — (short chain of amino acids—e.g., encoded by a gene fragment)

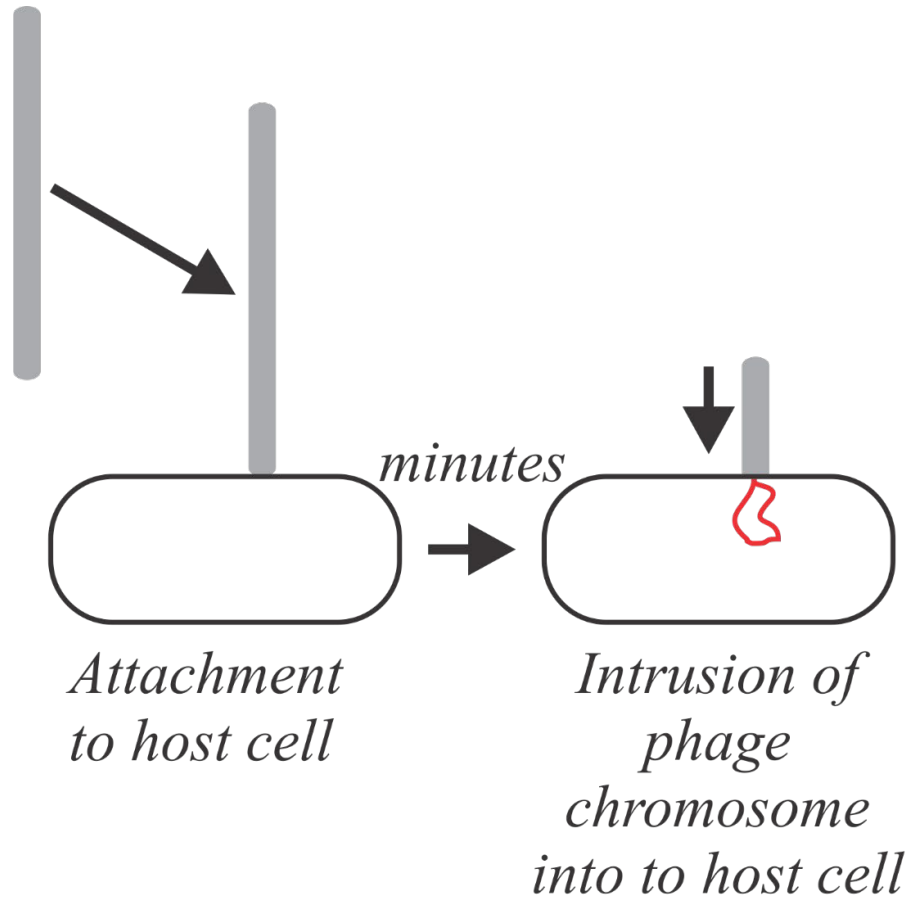
Filamentous phage infection cycle (simplified)

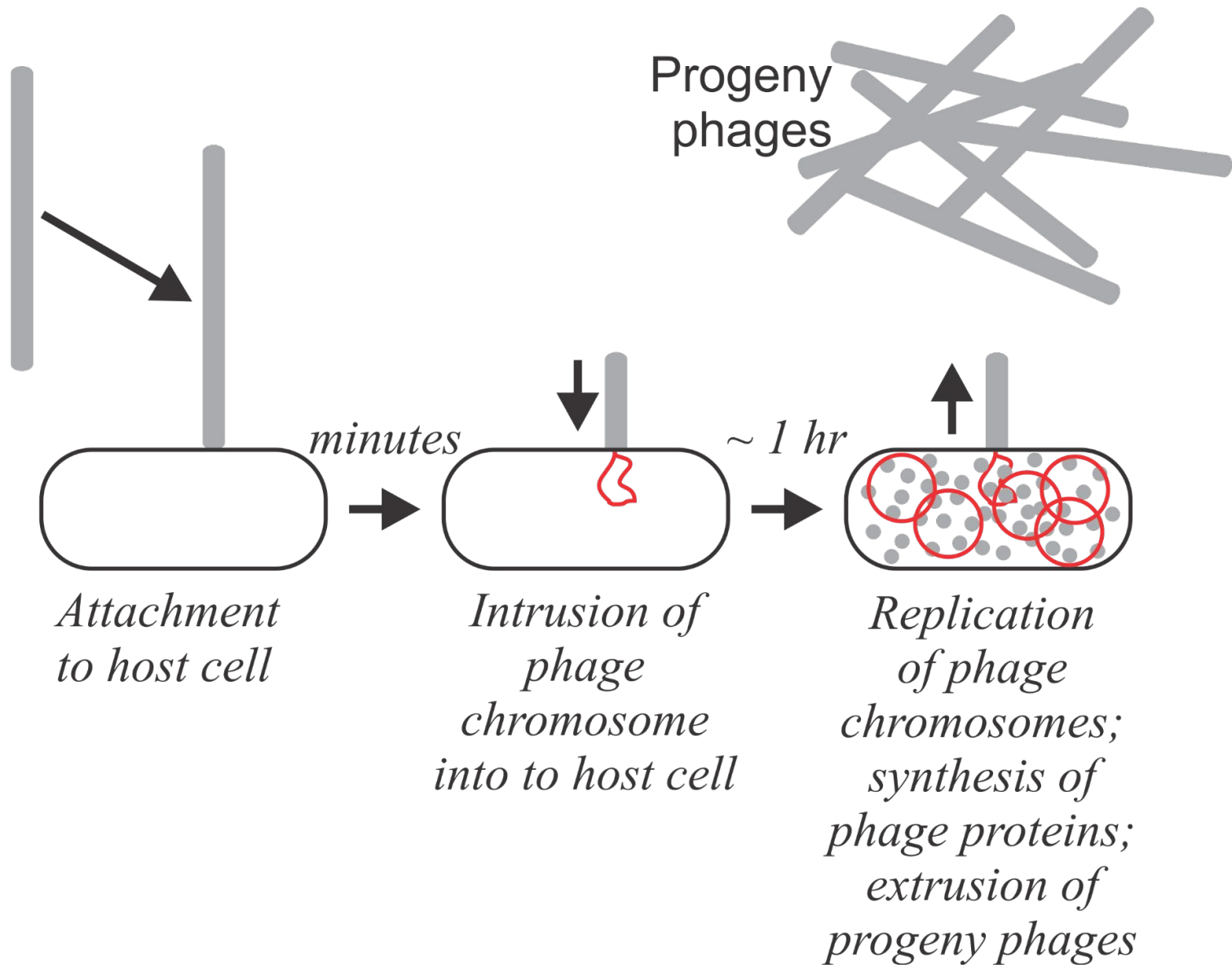


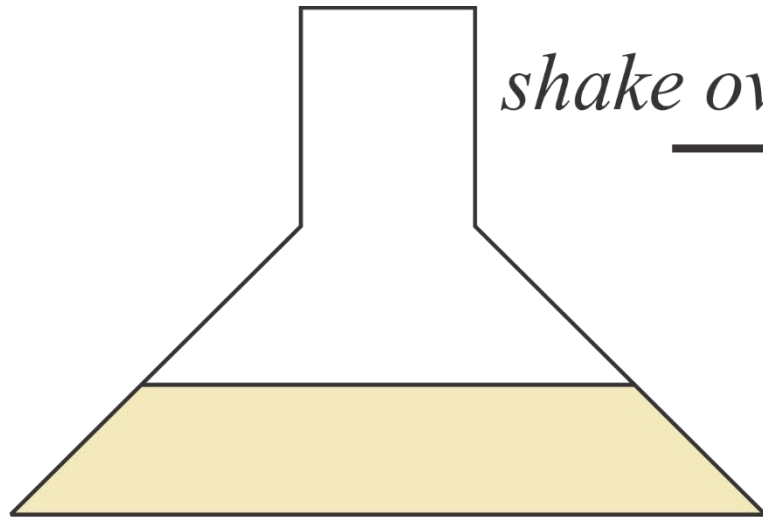
Bacterial
cell



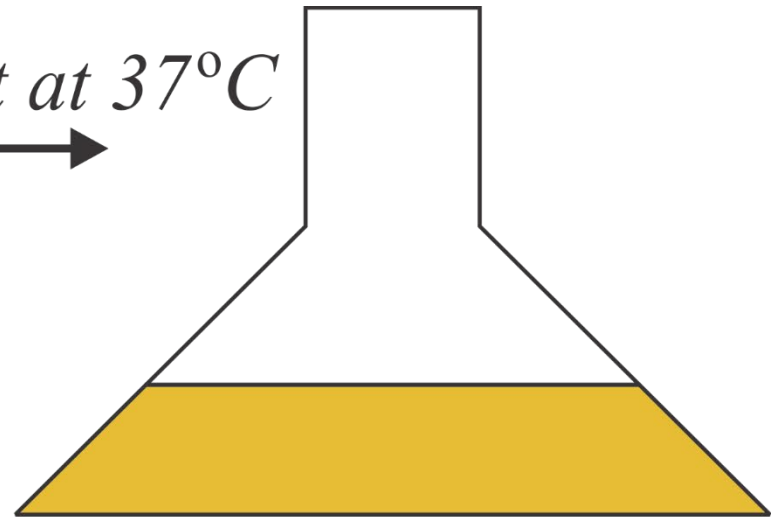
*Attachment
to host cell*







shake overnight at 37°C



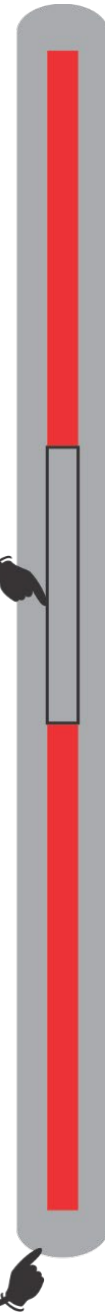
1 phage
 10^{11} cells
in 1 liter medium

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

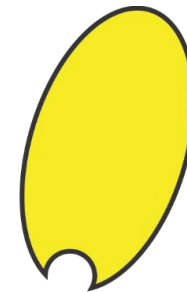




Paul Modrich
Nobel Prize in Chemistry, 2015



EcoRI gene

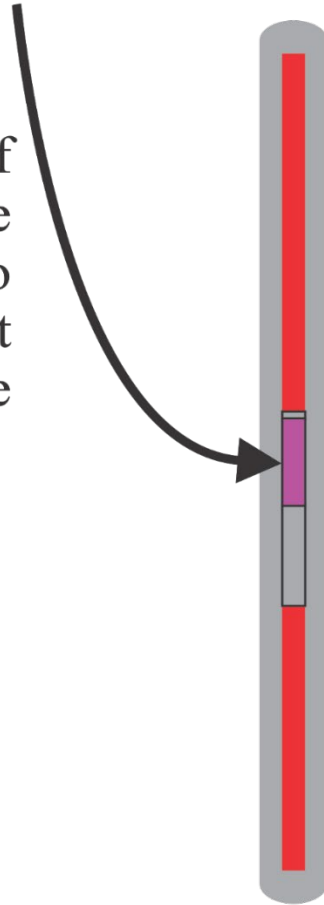


anti-*EcoRI*
antibody

EcoRI gene



Fragment of
EcoRI gene
inserted into
phage coat
protein gene

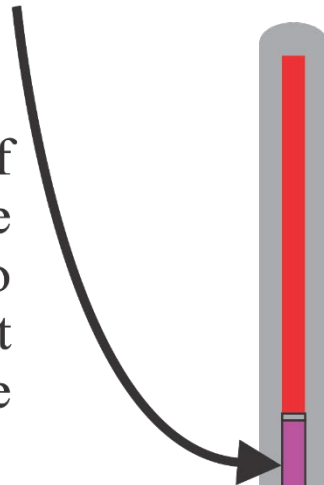


test phage

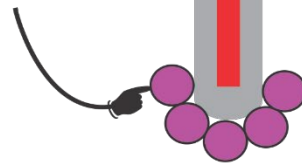
EcoRI gene



Fragment of
EcoRI gene
inserted into
phage coat
protein gene



Fragment of
EcoRI protein
displayed on
phage surface

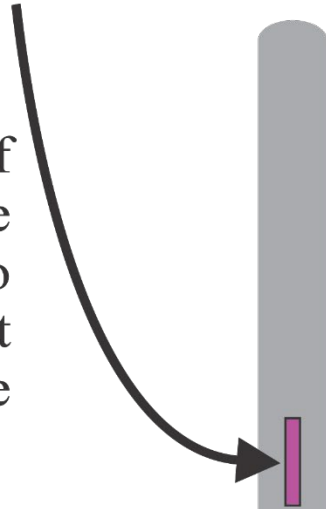


test phage

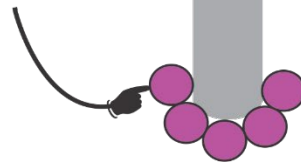
EcoRI gene

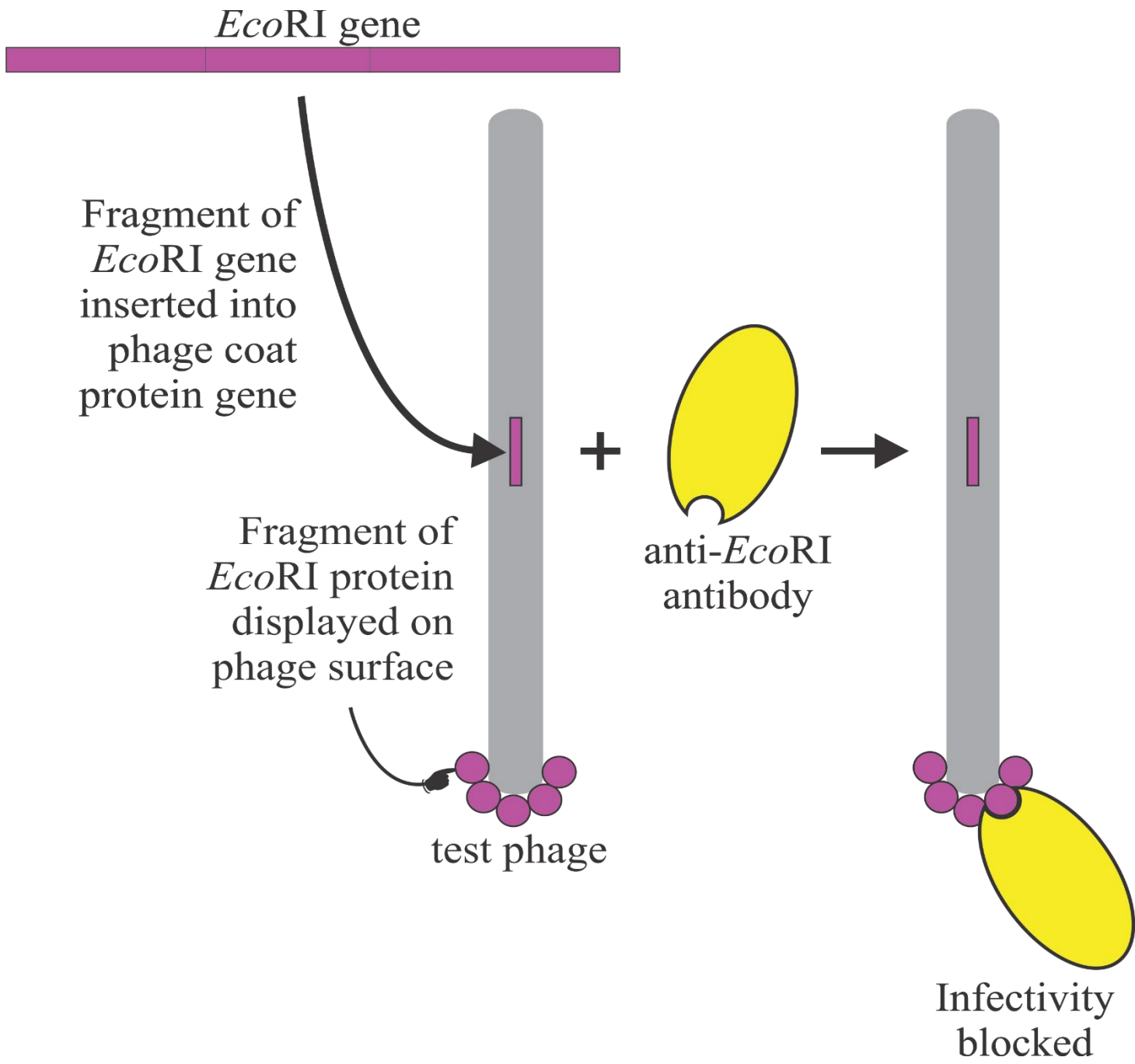


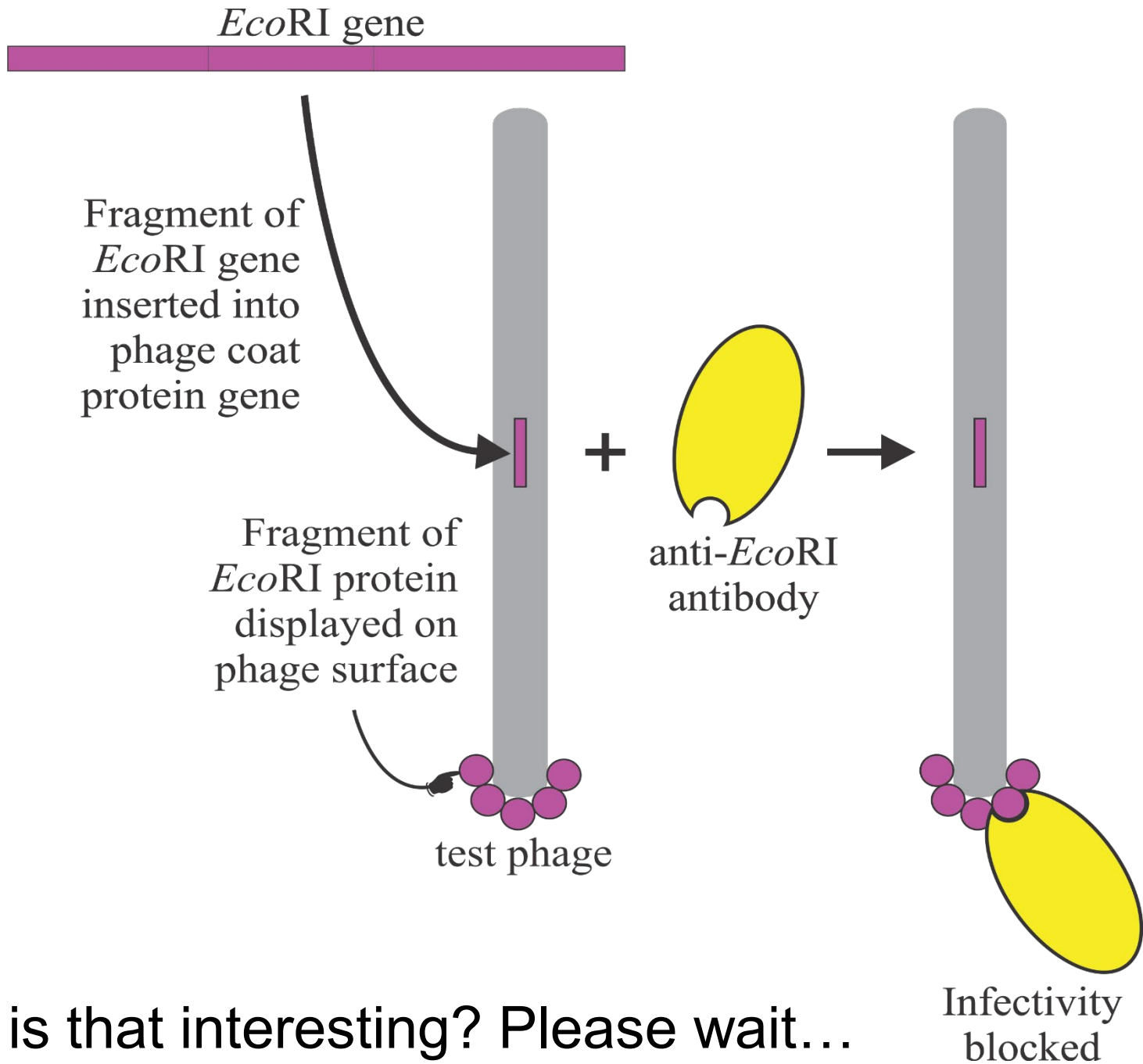
Fragment of
EcoRI gene
inserted into
phage coat
protein gene



Fragment of
EcoRI protein
displayed on
phage surface

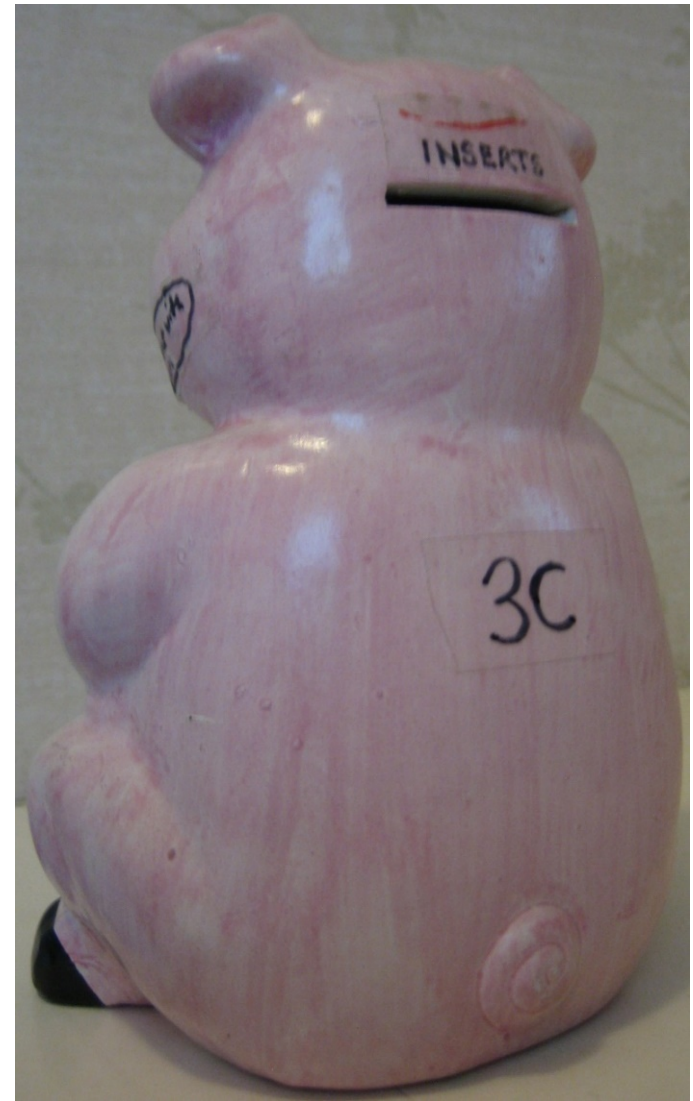






Why is that interesting? Please wait...

**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.

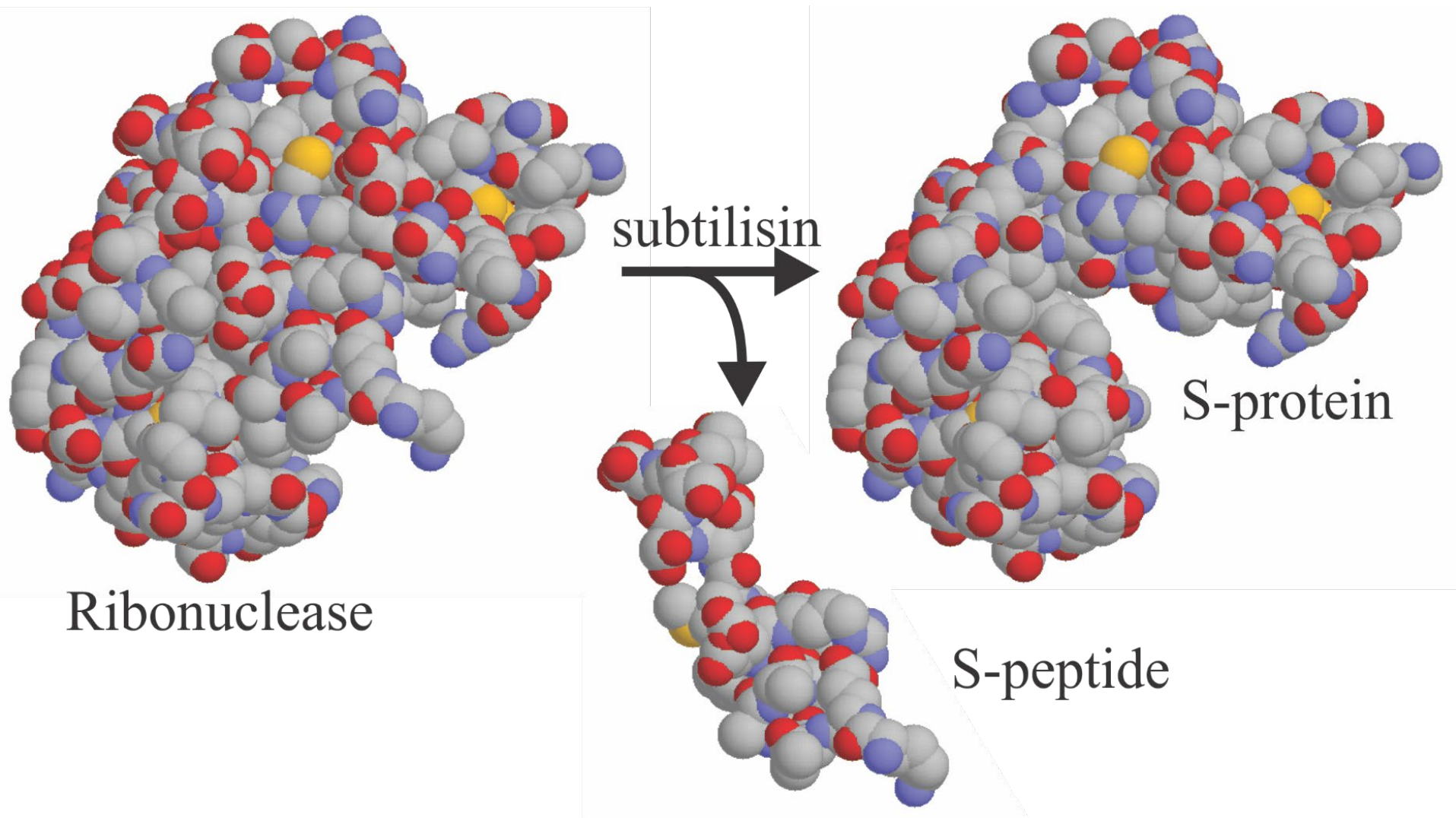




**Darn good
colleague
Robert Davis**

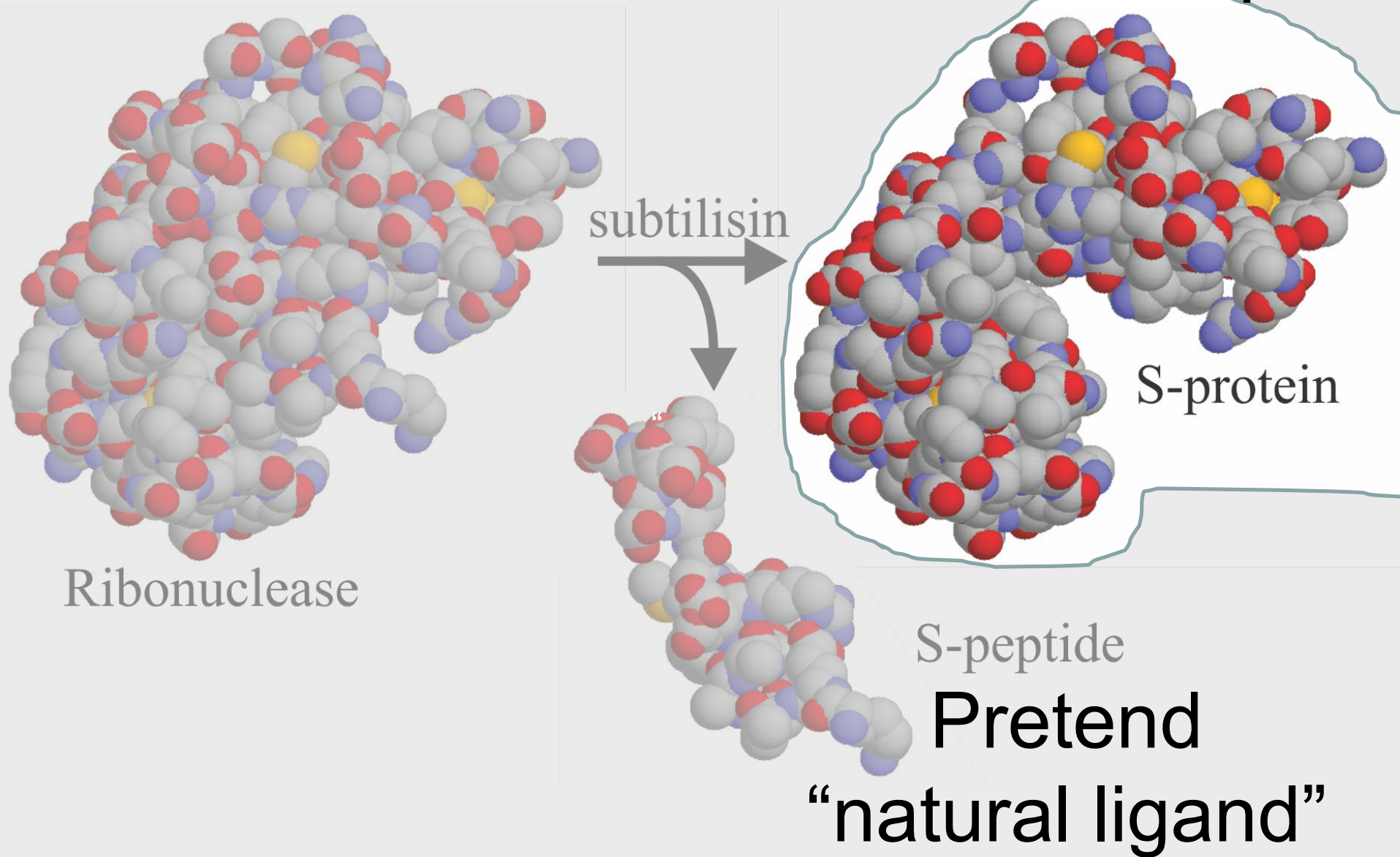
Robert Davis came to the lab as chief manager and technician in the summer of 1989. We calculate that he sequenced a million DNA bases using old-fashioned radioactive technology.

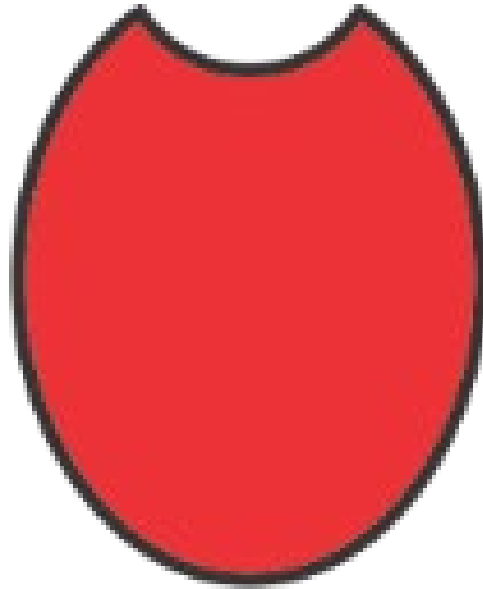
The S-protein system



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

Pretend “receptor”



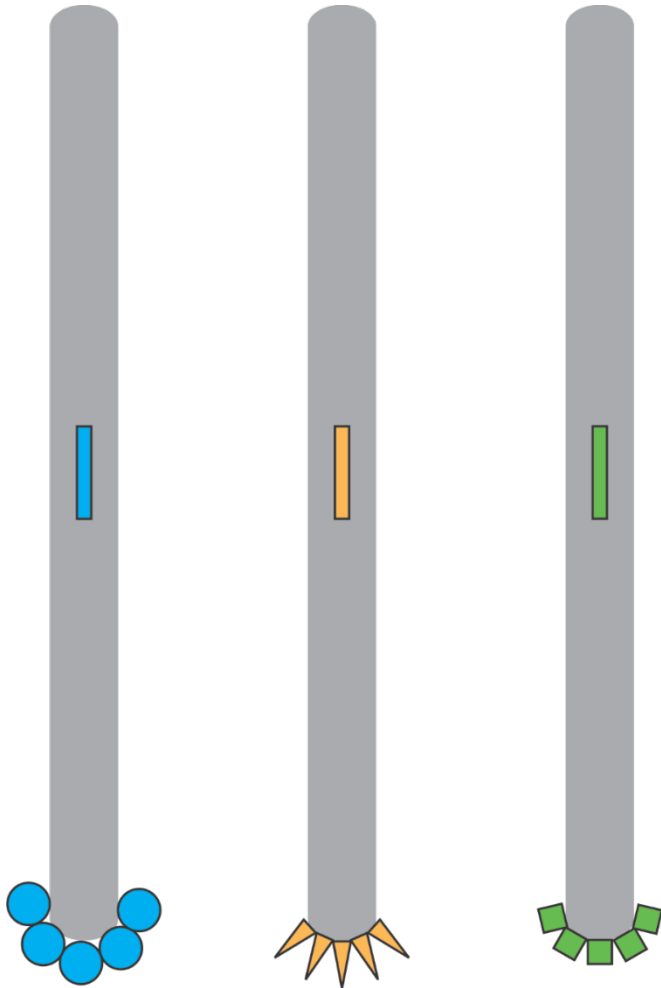


S-protein
“receptor”

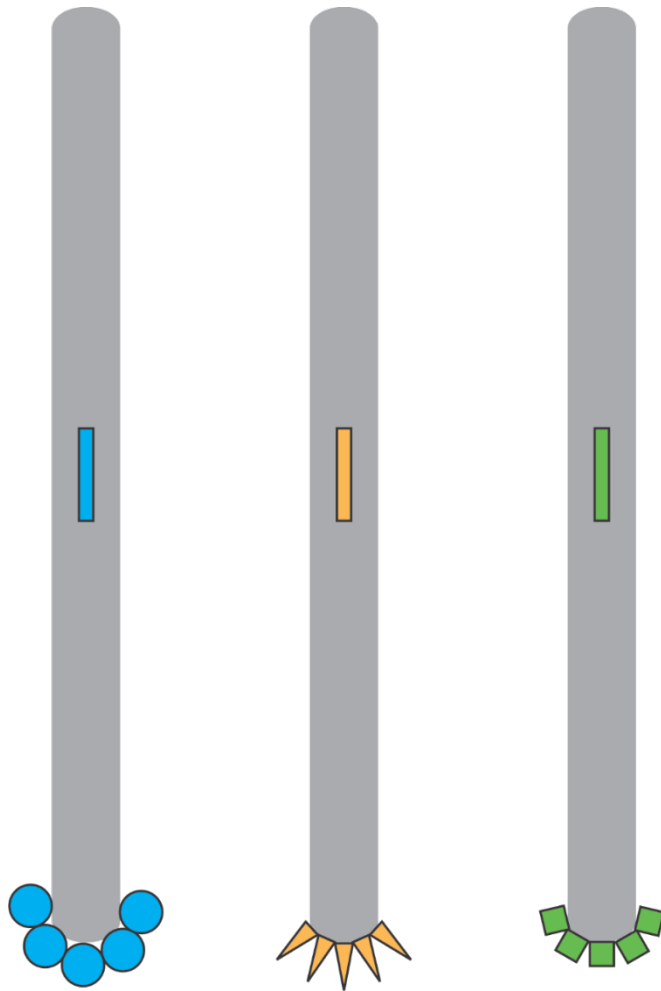


Jinan Yu, now a researcher at Hainan University, China.

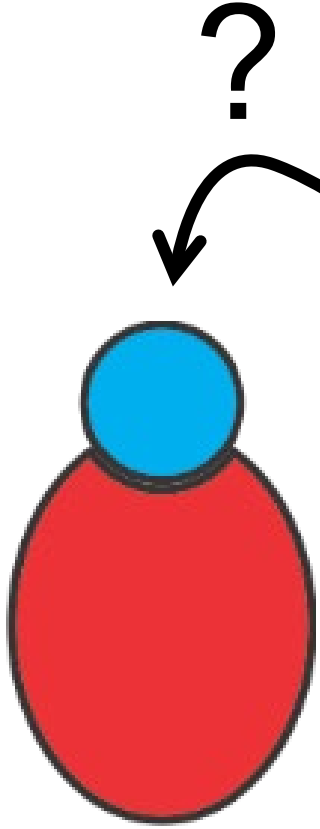
Random peptide library



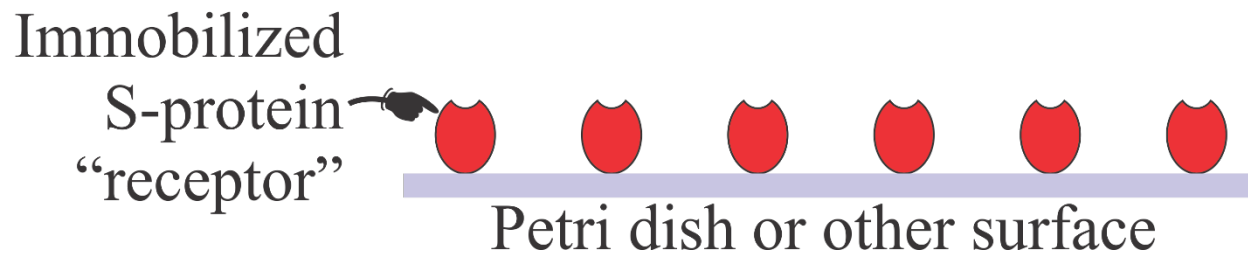
Random peptide library



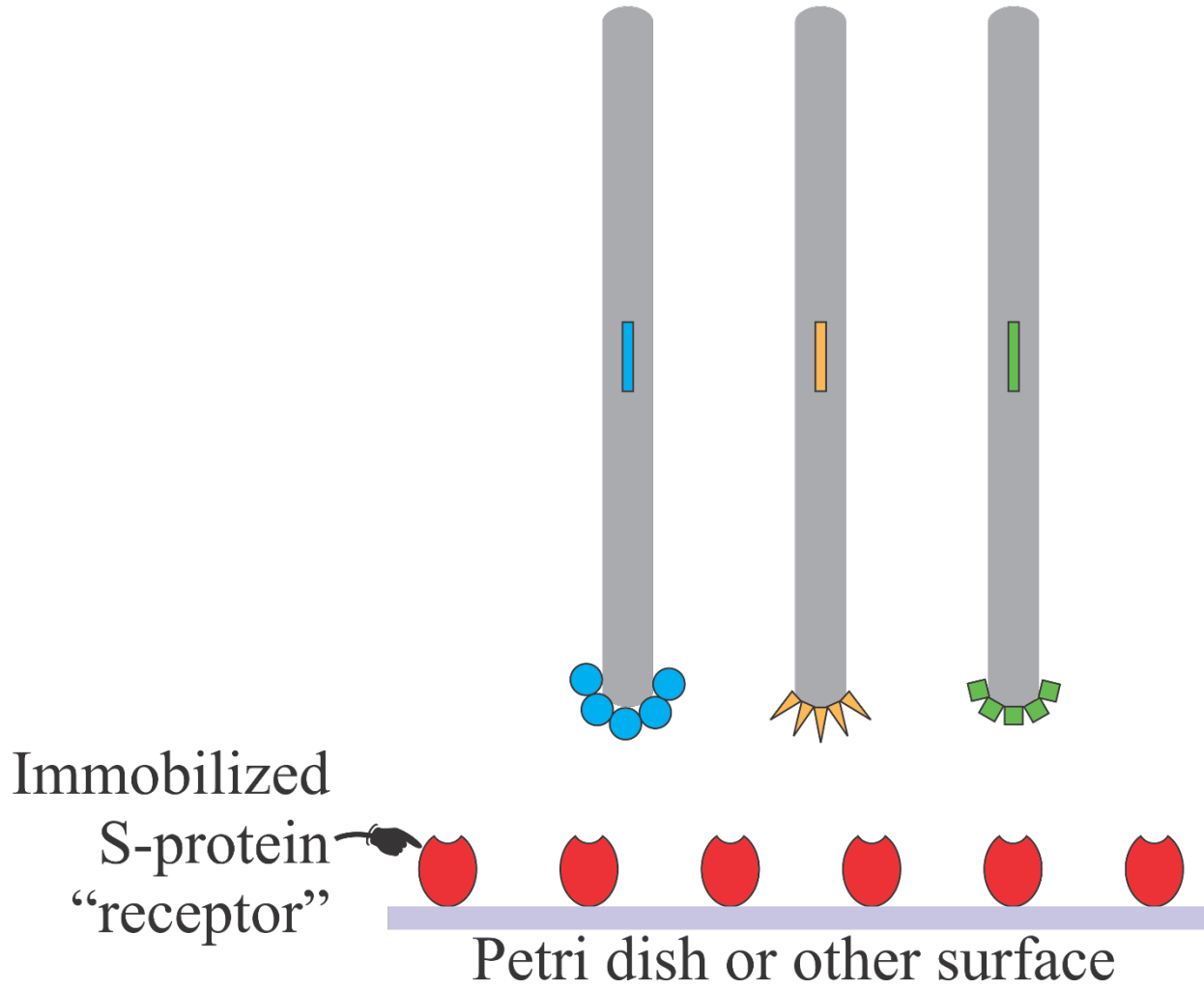
10^{15} phages representing 250 million phage clones, each clone displaying a different 15-amino acid guest peptide. [T. Nishi et al., FEBS Letters 399, 237–240 (1996)]

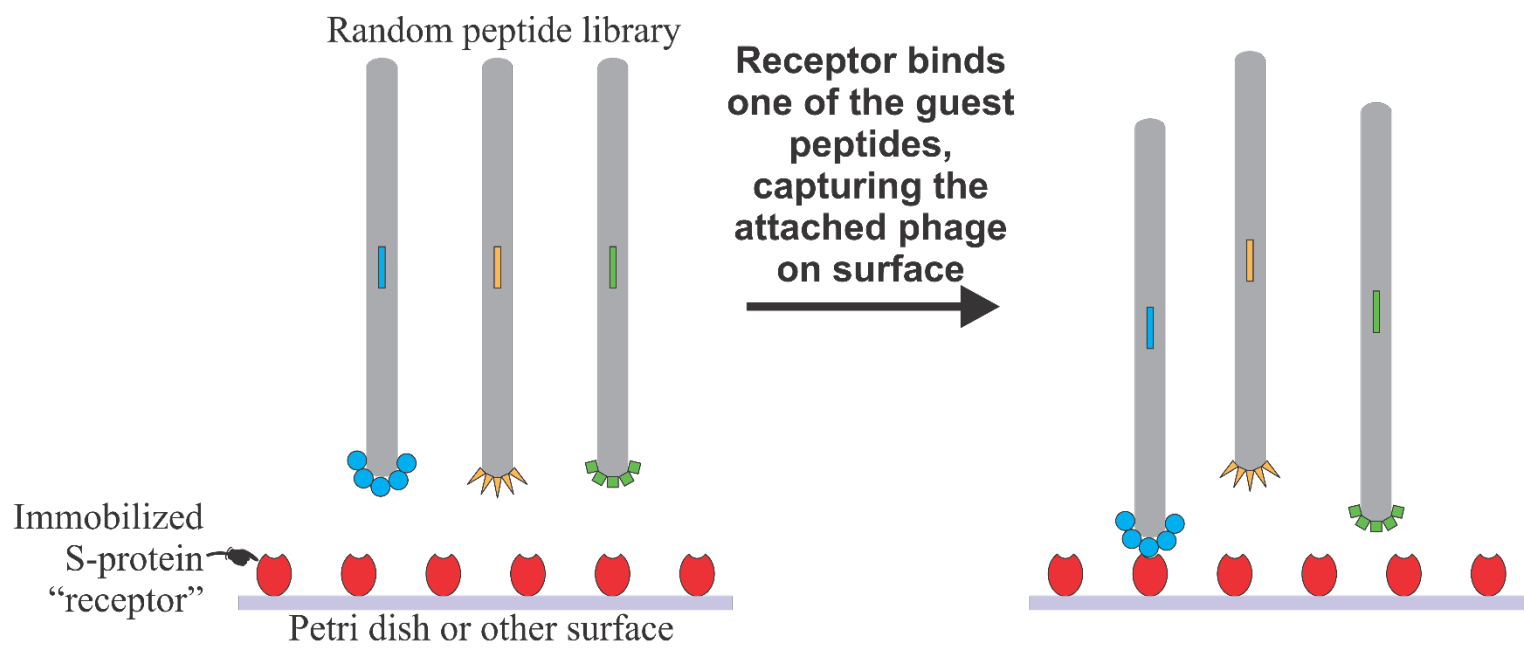


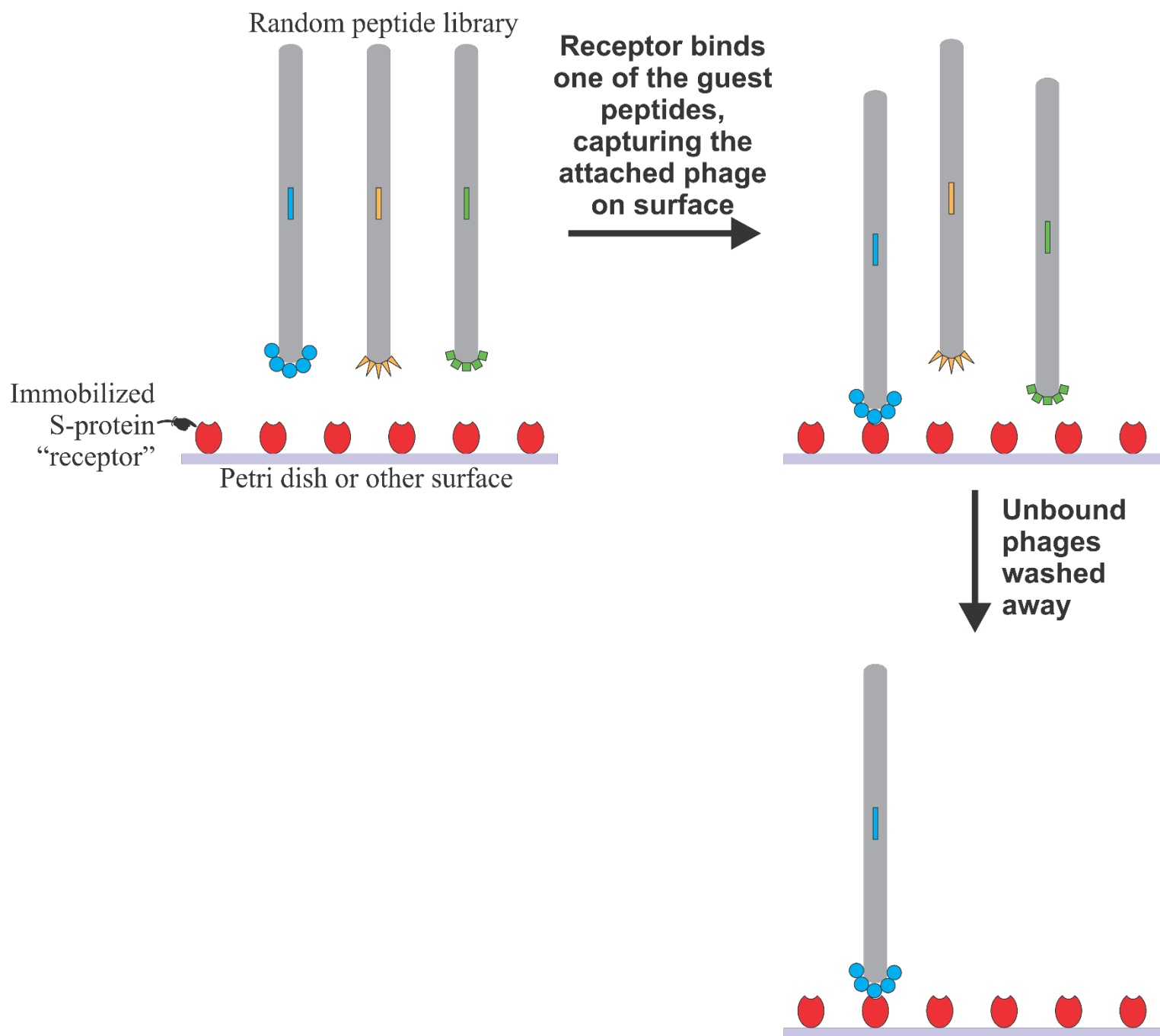
Affinity selection

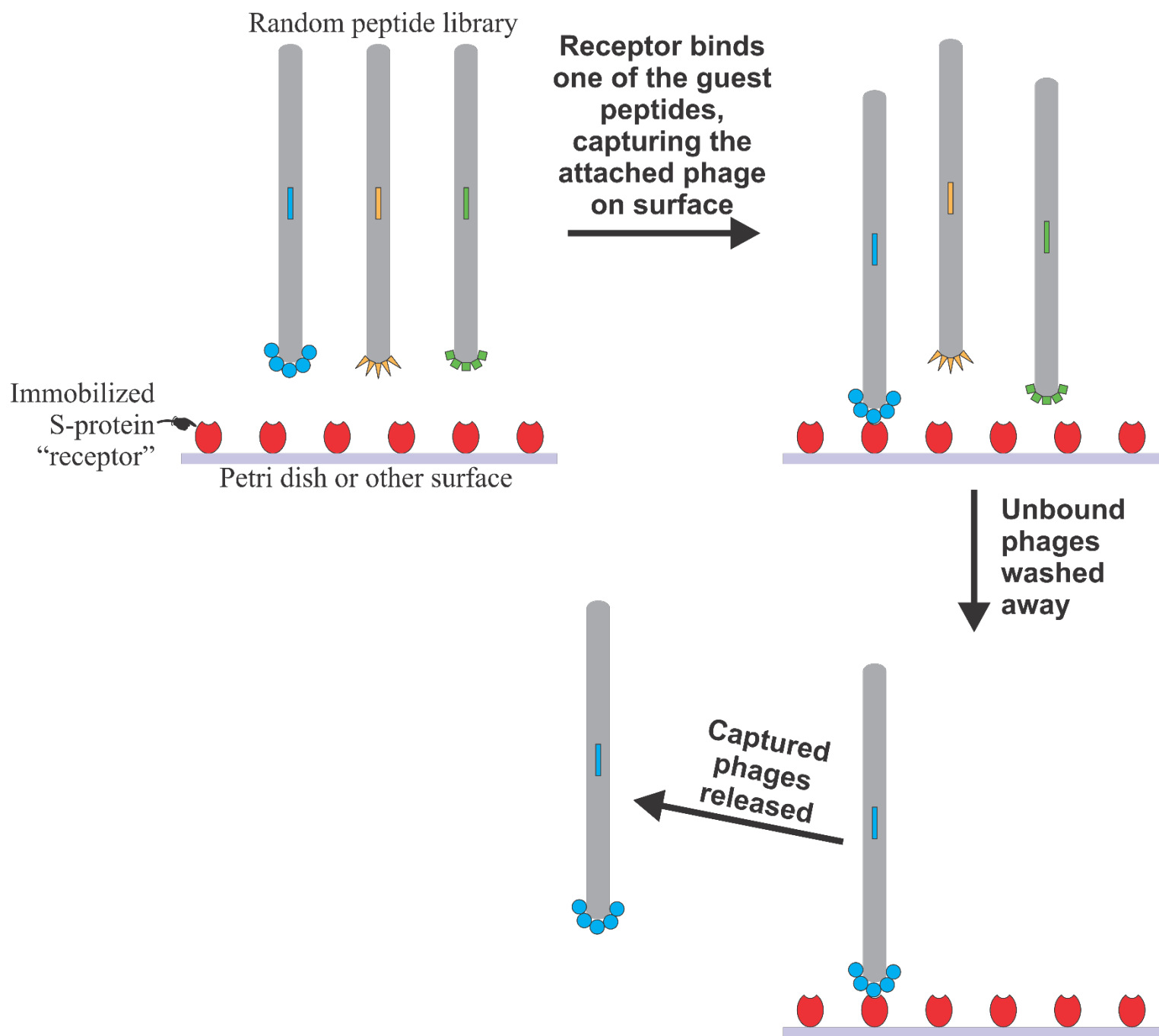


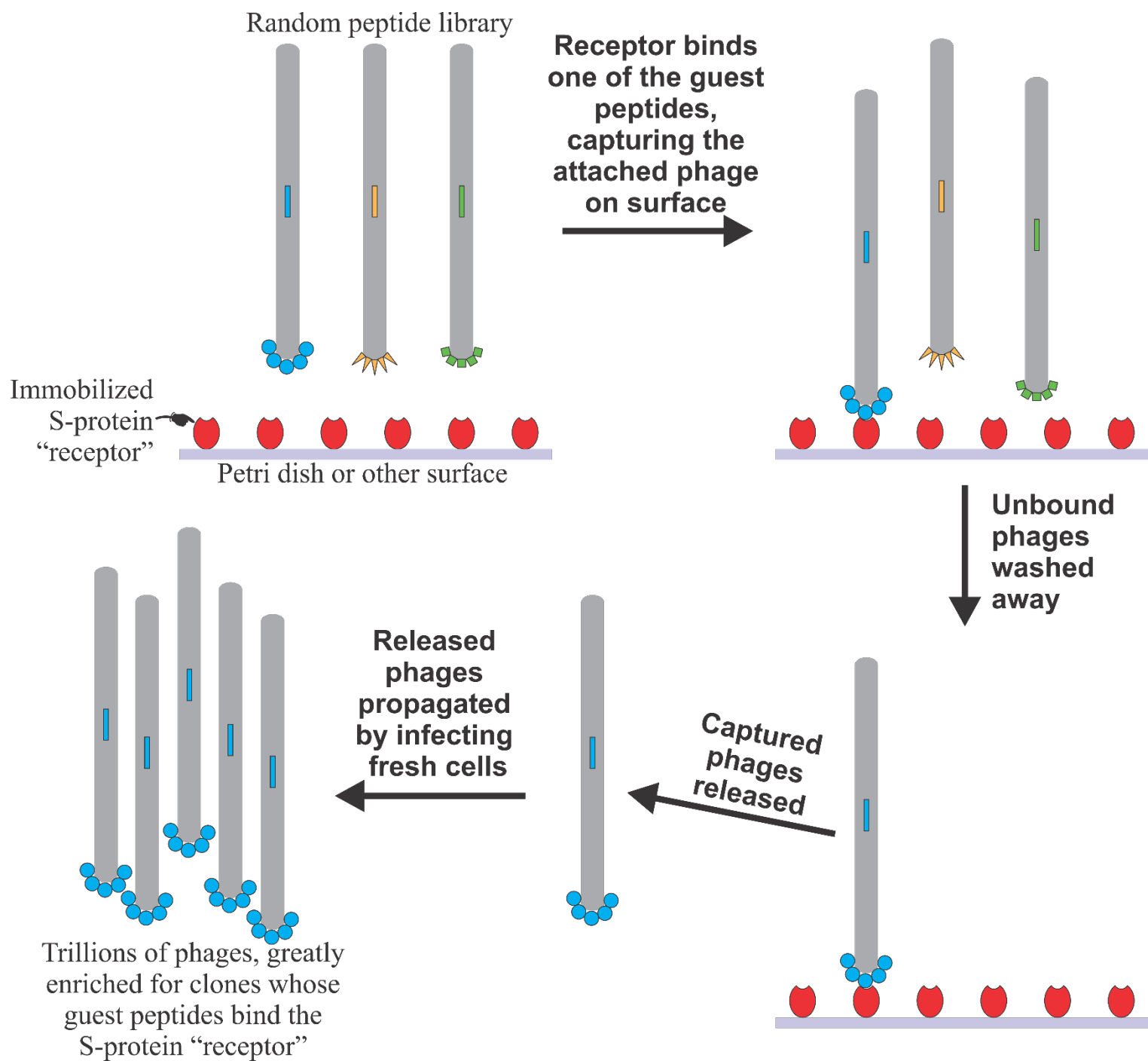
Random peptide library











Dominant sequence among selected peptides

Selected peptide **NRAWSEFLWQHLPV**

(one-letter abbreviations for amino acids)

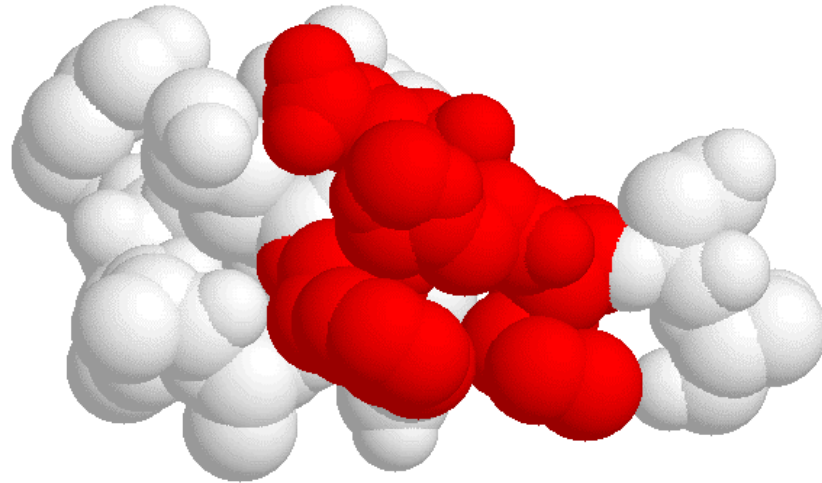
Dominant sequence among selected peptides
aligns with S-peptide “natural ligand”

Selected peptide **NRAWSEFLWQHLPV**

S-peptide **KETA**AAAK**FERQH****MDSSTSA**A

| | | |

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



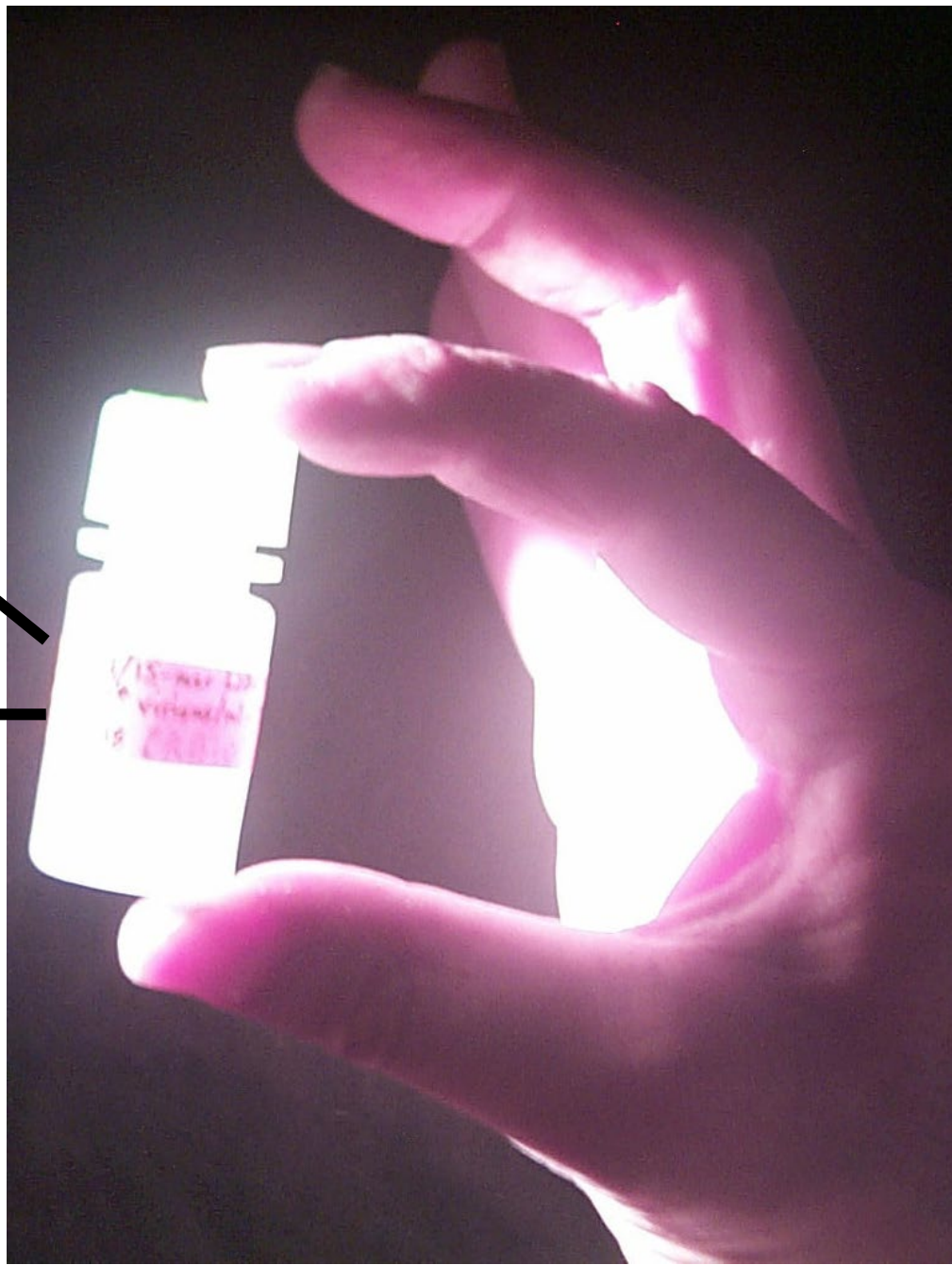
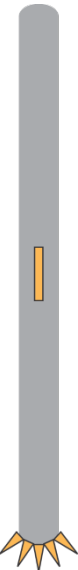
Cure
for S-
protein
disease



Selection scheme 1

Selection scheme 2

Marvel
2



Cure
for S-
protein
disease

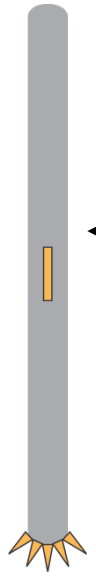


Selection scheme 1

Selection scheme 2

Selection scheme 3

Marvel
2



Marvel
3

