

The Use of a Selenium-Peptide to Specifically Inactivate *Yersinia pestis*

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The Use of a Selenium-Peptide to Specifically Inactivate *Yersinia pestis*

Joe A. Fralick, Ph.D.

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Phat Tran, B.A.

Overview of project

- Develop an antibiotic that will selectively kill *Y. pestis* without killing other bacteria

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- Use a killing mechanism for which the bacteria can not develop a resistance

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- b. The F1 protein on the surface of *Y. pestis* is known to function as a good vaccine for the bacteria.
- c. Peptides are known to be very selective in their binding.
- d. Peptides are known to have high affinity binding.
- e. Peptides are less expensive to produce, more stable and are easier to deliver to a target in vivo than antibodies.

Experimental Approach:

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- b. Libraries of peptides are available which contain large numbers (10^9) of different peptides.
- c. We need to isolate a single peptide from the library that will bind to the F1 antigen.

The problem:

[illegible]

The Solution:

PHAGE DISPLAY!

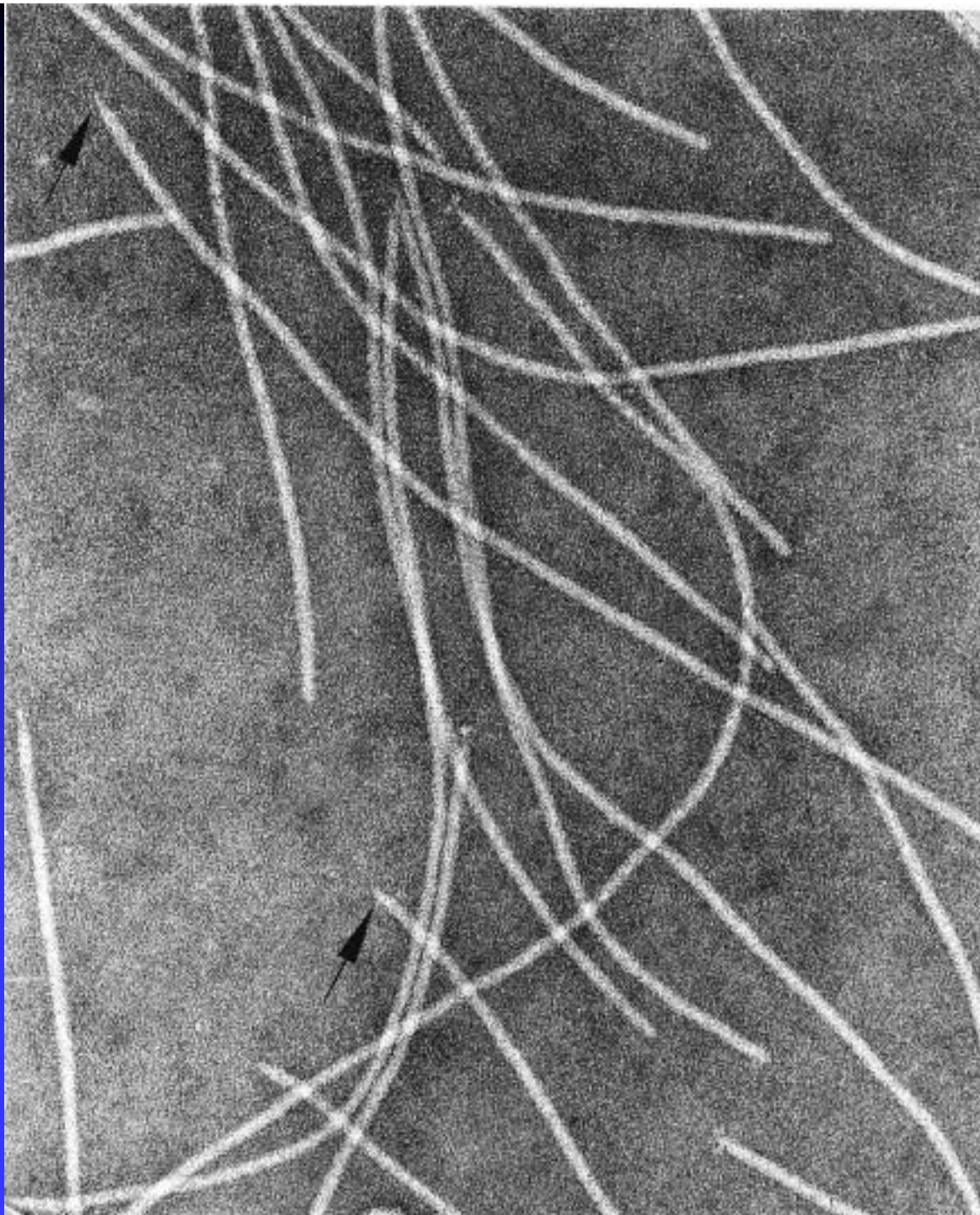
The power of phage display lies in the fact that it creates a physical linkage between a selectable function (the displayed peptide sequence) and the DNA encoding that function.

The Solution:

PHAGE DISPLAY!

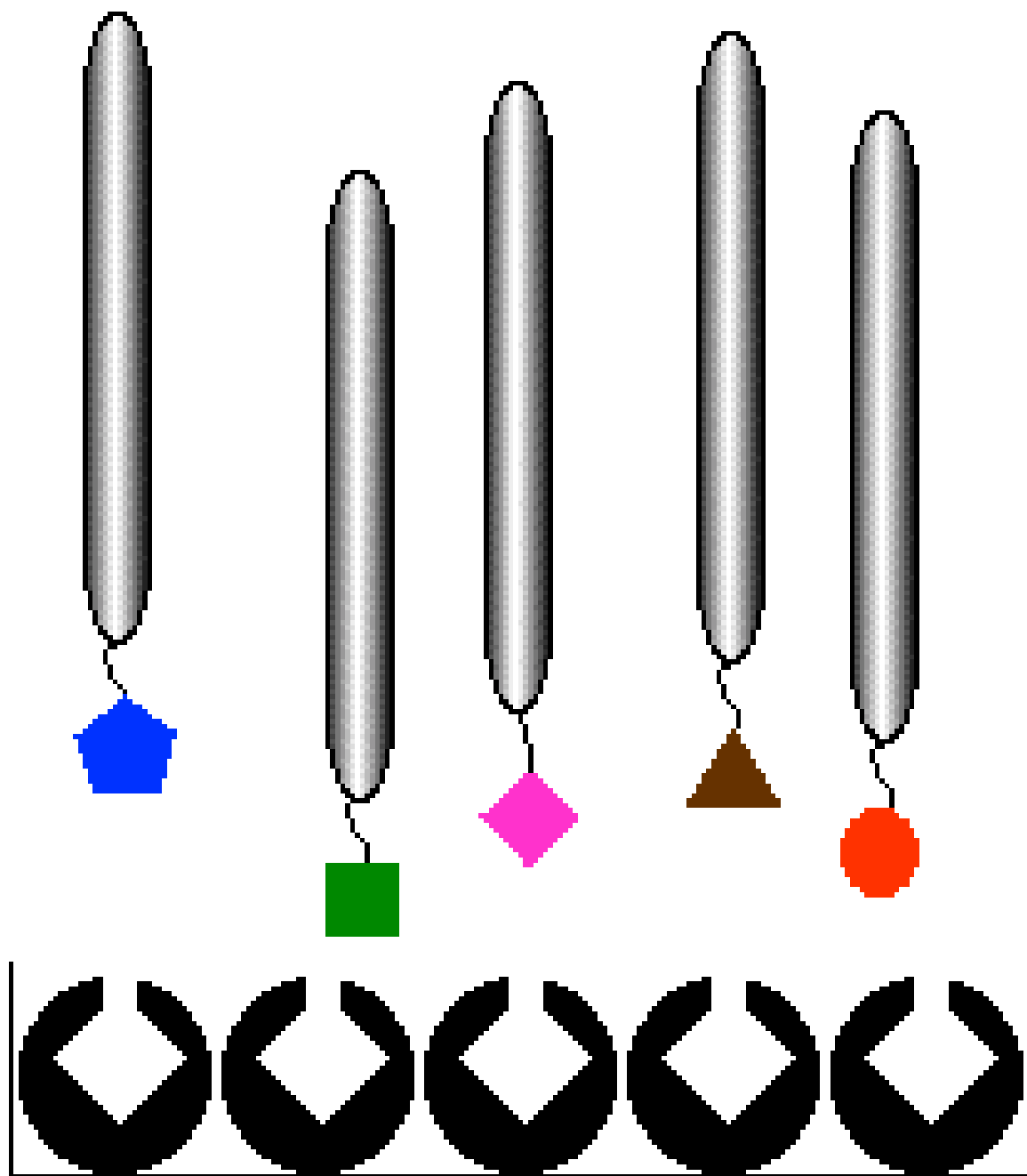
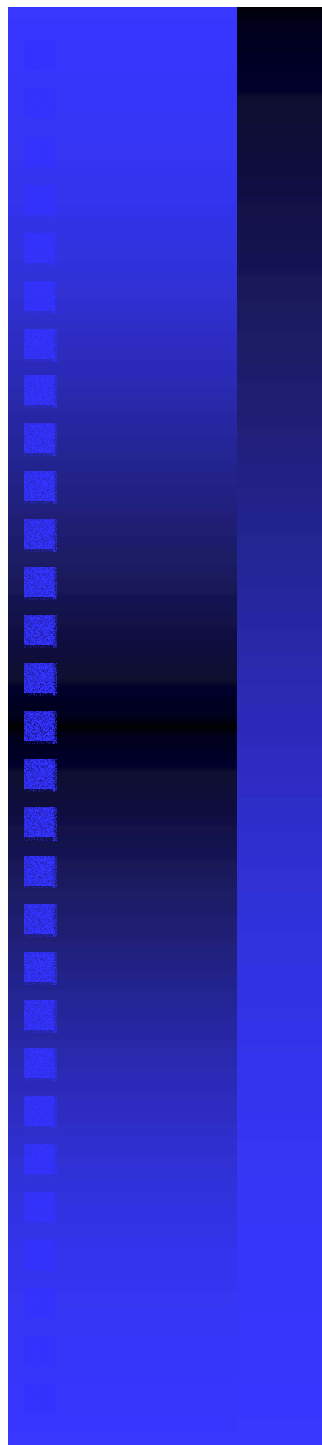
The power of phage display lies in the fact that it creates a physical linkage between a selectable function (the displayed peptide sequence) and the DNA encoding that function.

In other words, the peptide comes with its own built in message that tells us its sequence. A message that we can determine from a single isolated phage.



Filamentous Bacteriophage

QuickTime™ and a
Photo - JPEG decompressor
are needed to see this picture.



Affinity
Selection or
Biopanning:

10^{11} phage + target



Wash off unbound phage



Harvest bound phage



Amplify selected phage



Sequence Phage DNA

Stringency

The stringency of affinity selection is controllable in some degree by the choice of conditions used in biopanning experiments.

Low Stringency



Presence of detergents,
soluble vs fixed target,
temperature, competition
agents, etc.

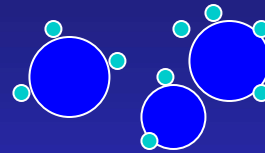
High Stringency

Biopanning:

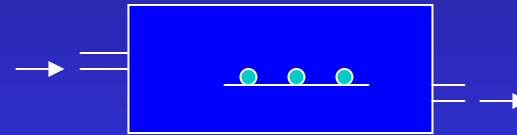
Microtiter plates



Beads



BiaCore

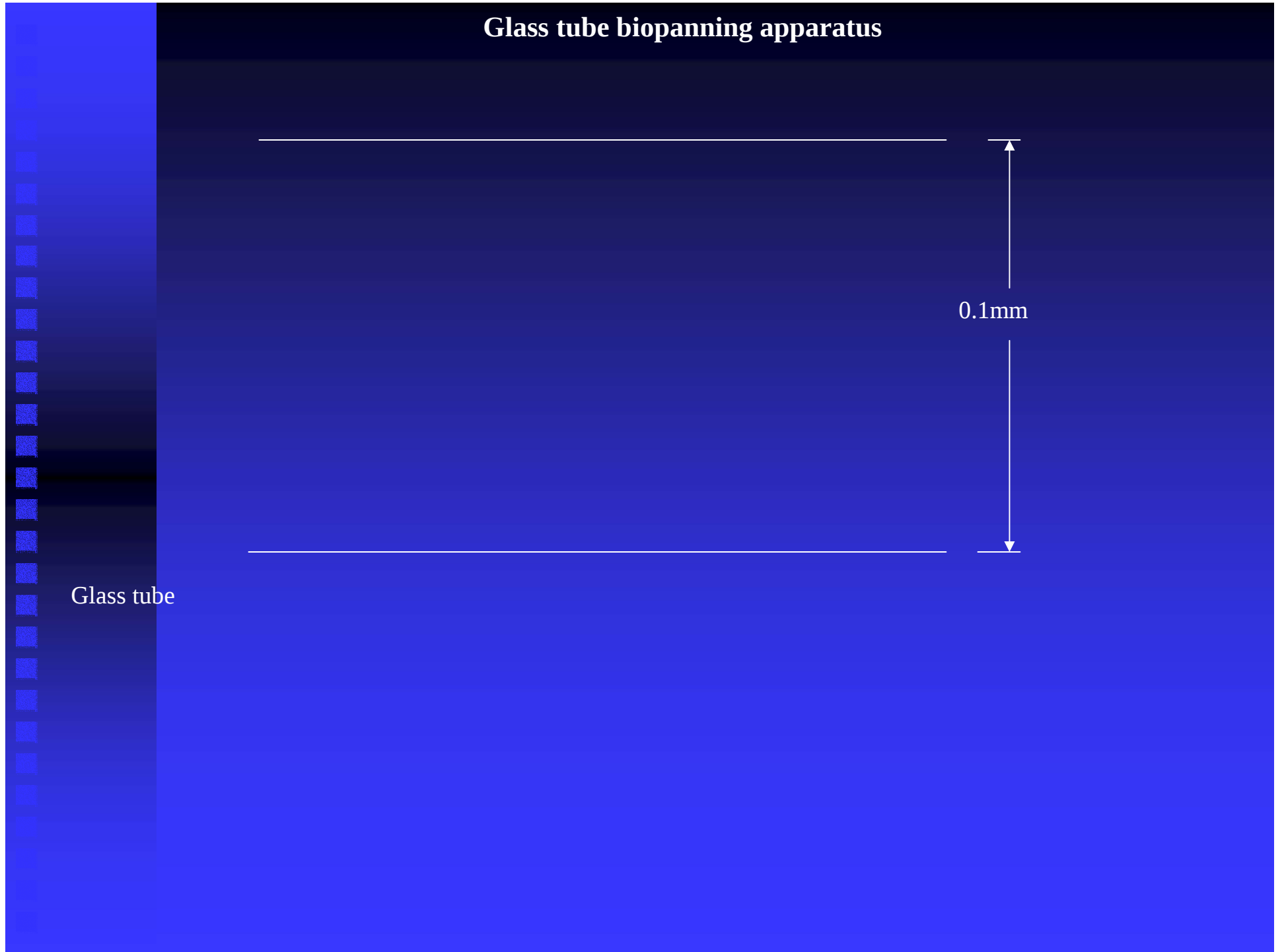


>\$100,000

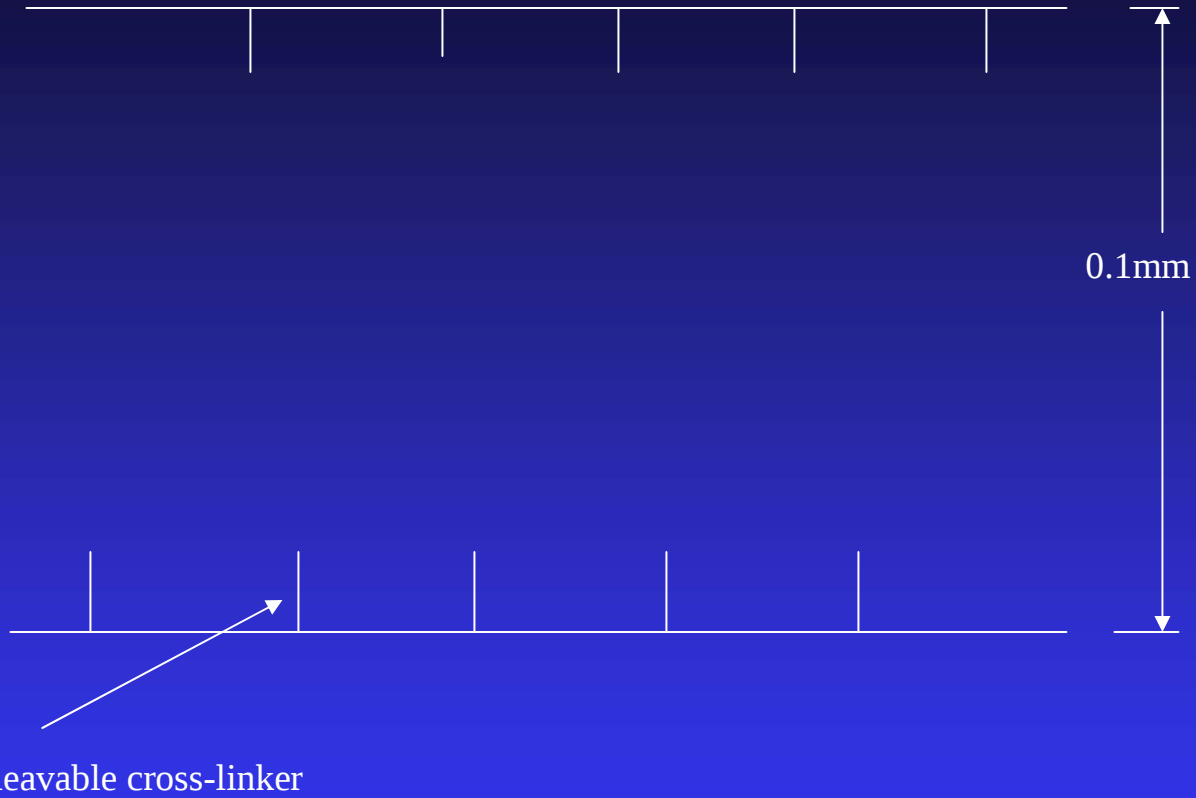
Glass tube biopanning apparatus

Glass tube

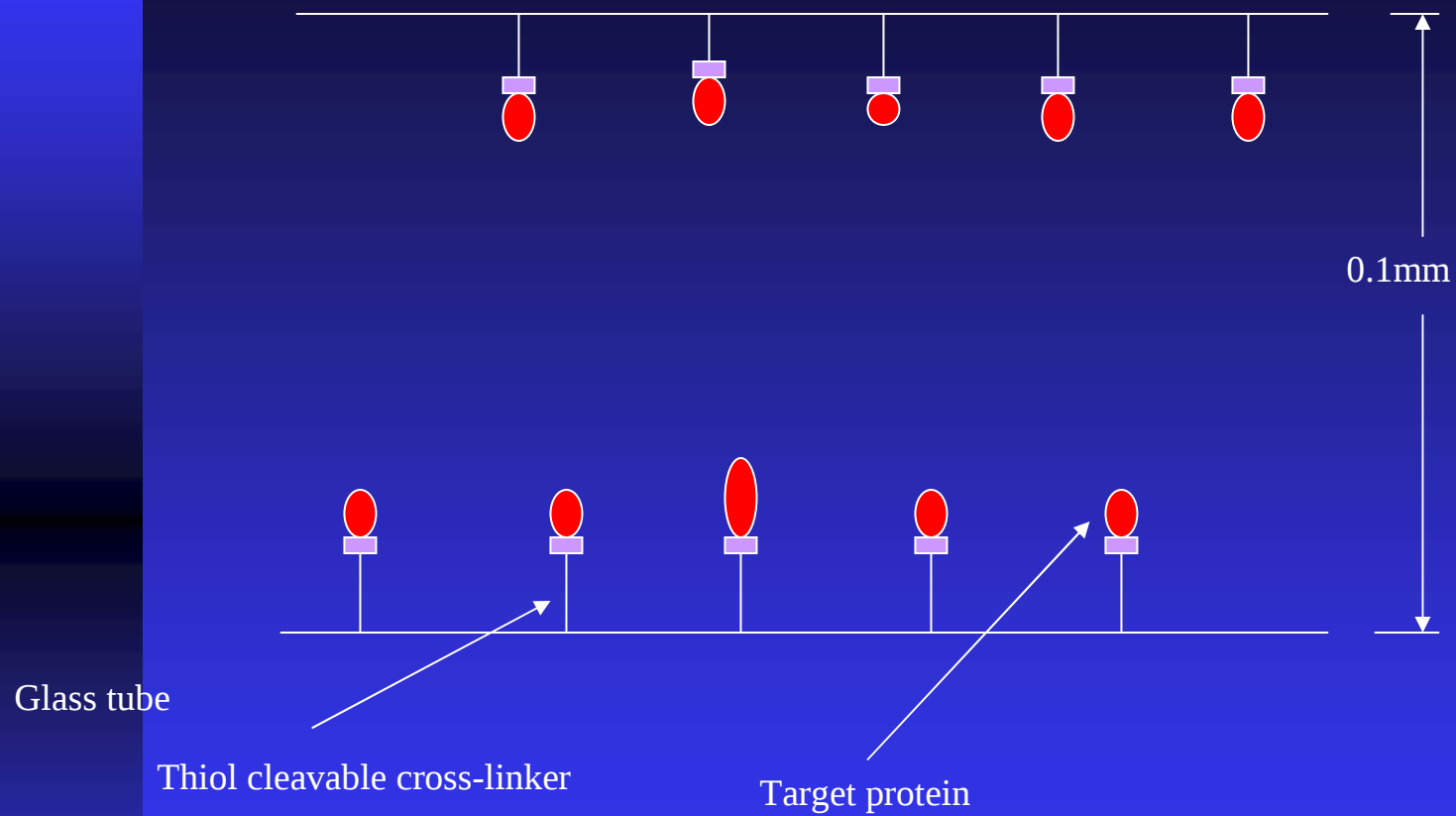
0.1mm



Glass tube biopanning apparatus



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Peptide display phage

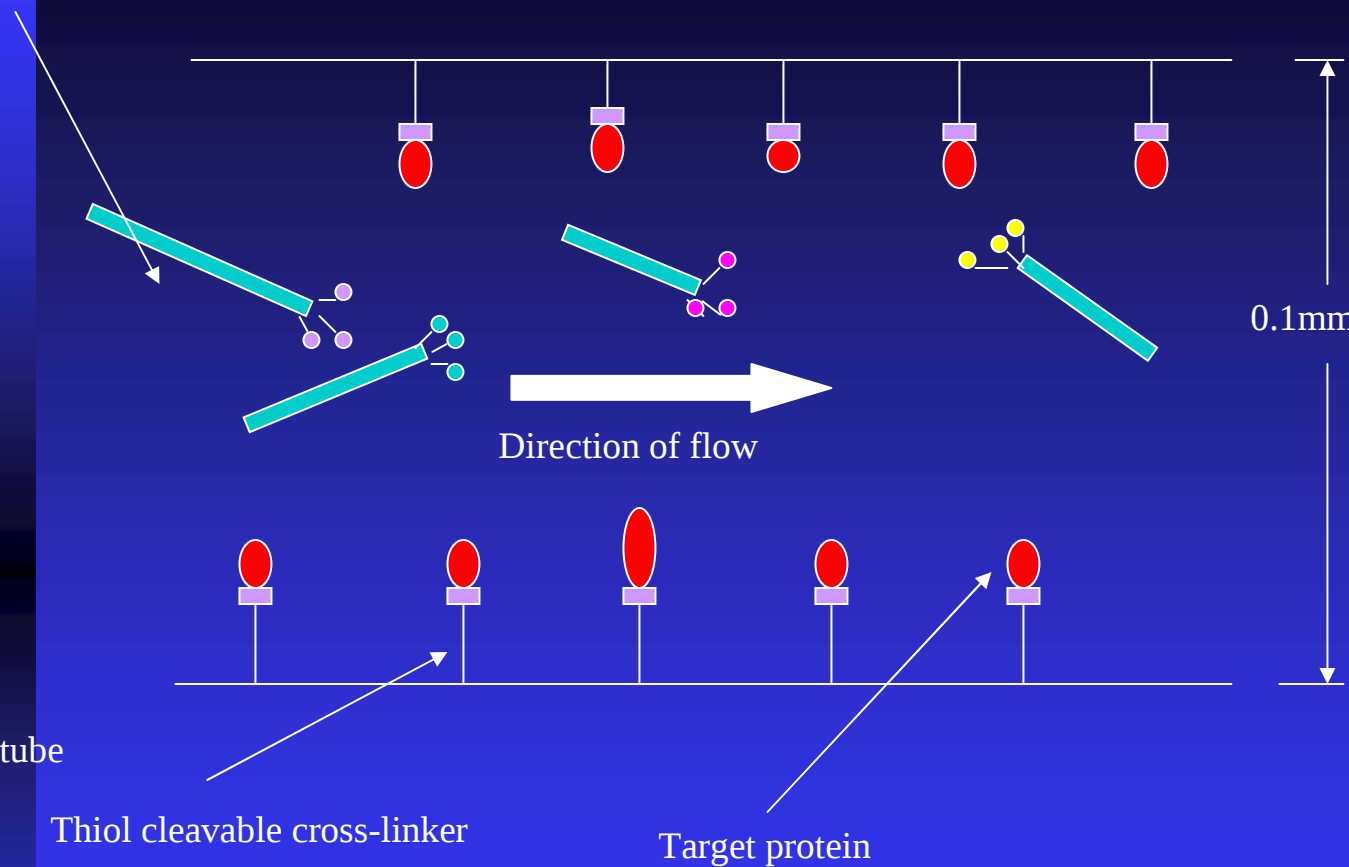
0.1mm

Direction of flow

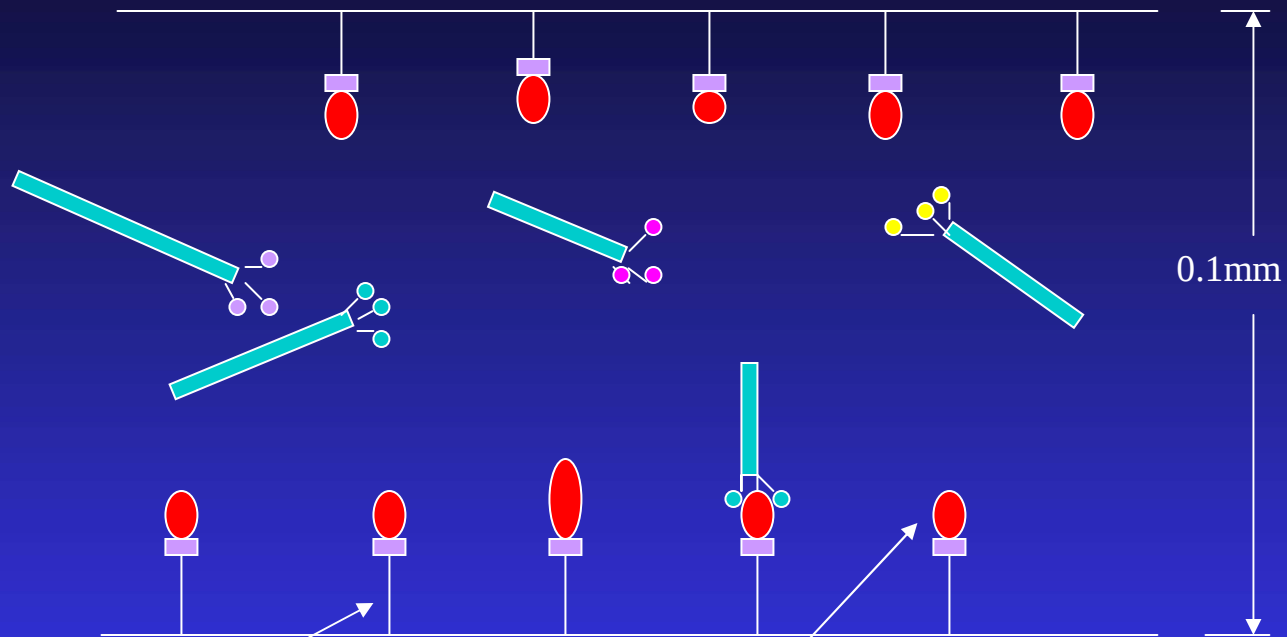
Glass tube

Thiol cleavable cross-linker

Target protein



Glass tube biopanning apparatus

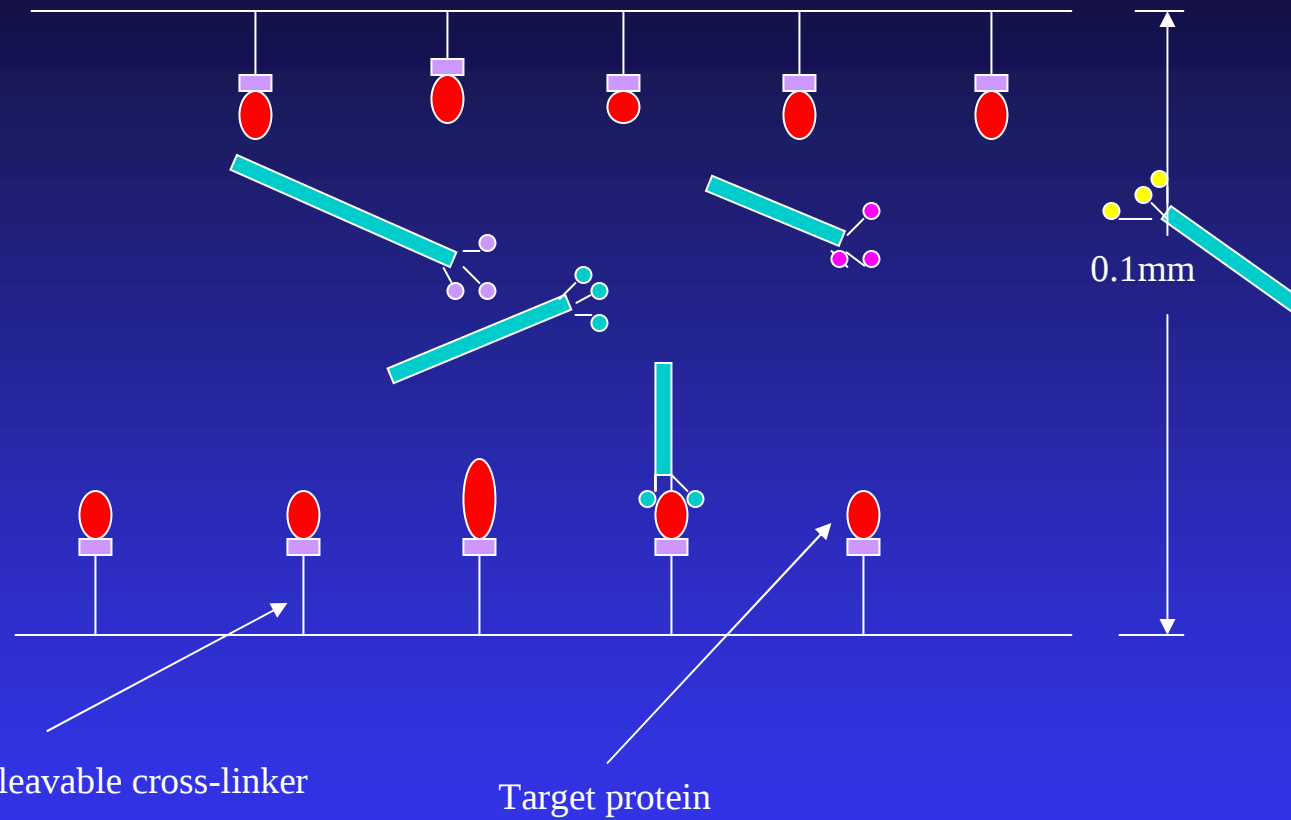


Glass tube

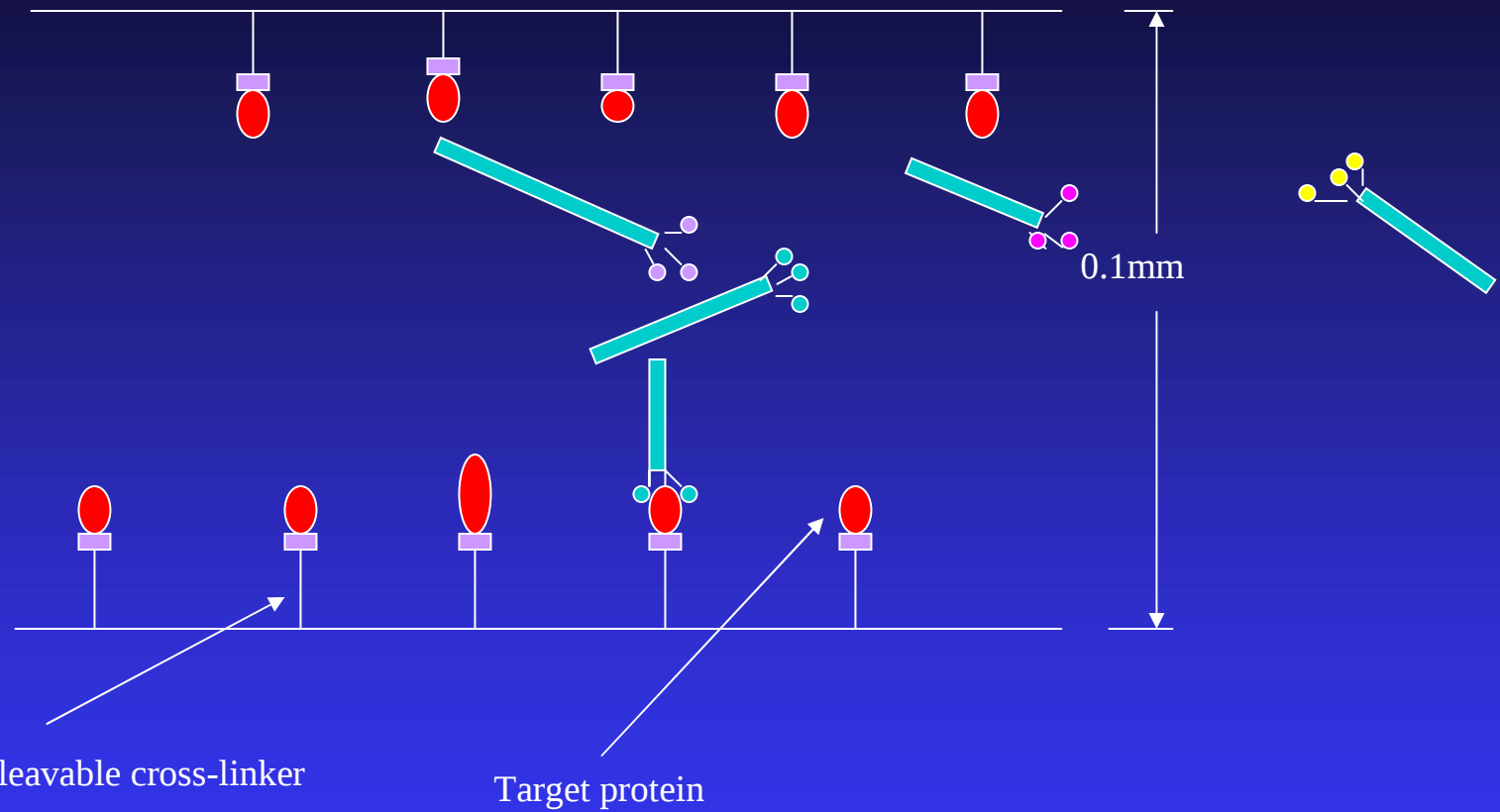
Thiol cleavable cross-linker

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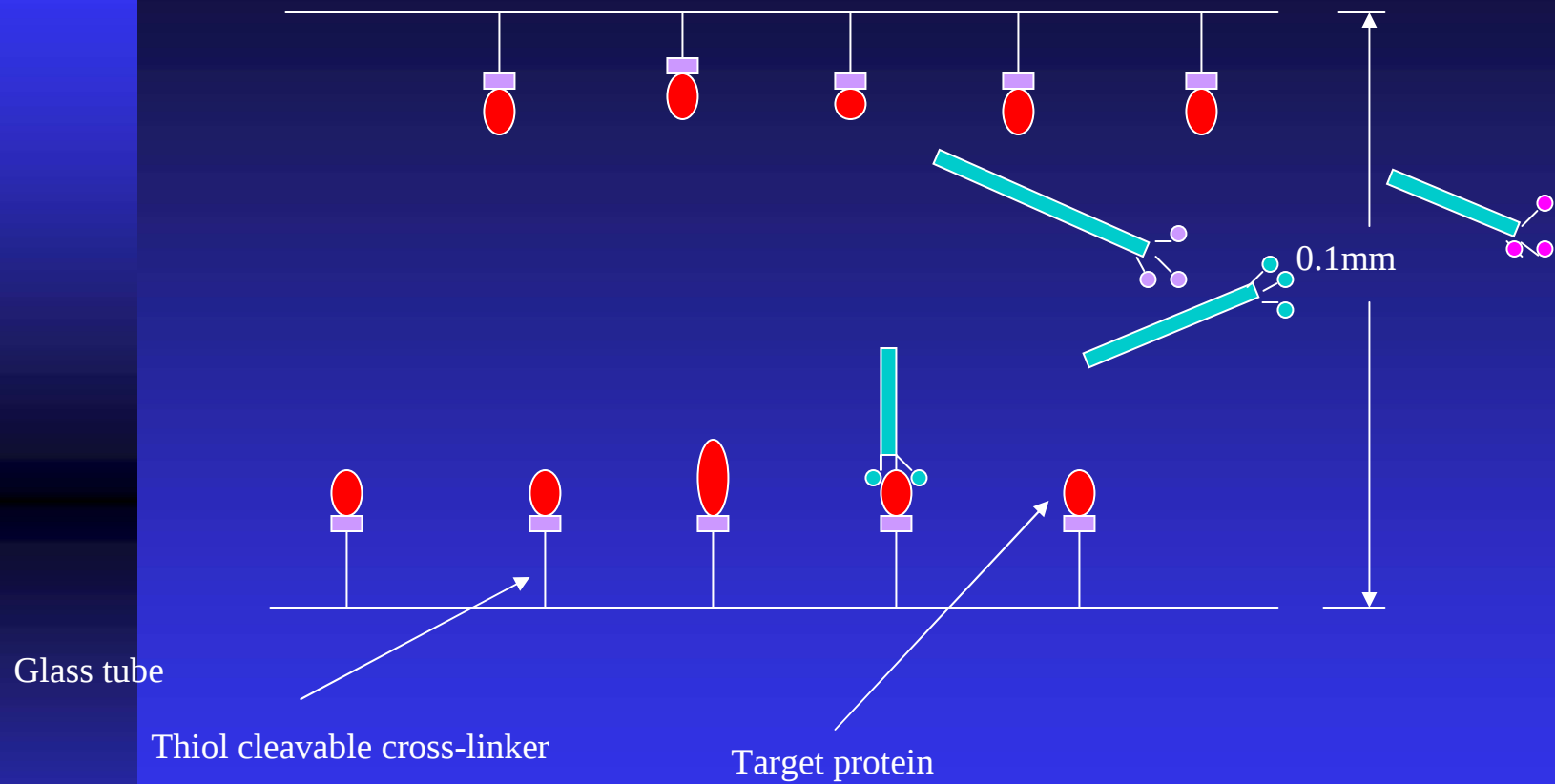
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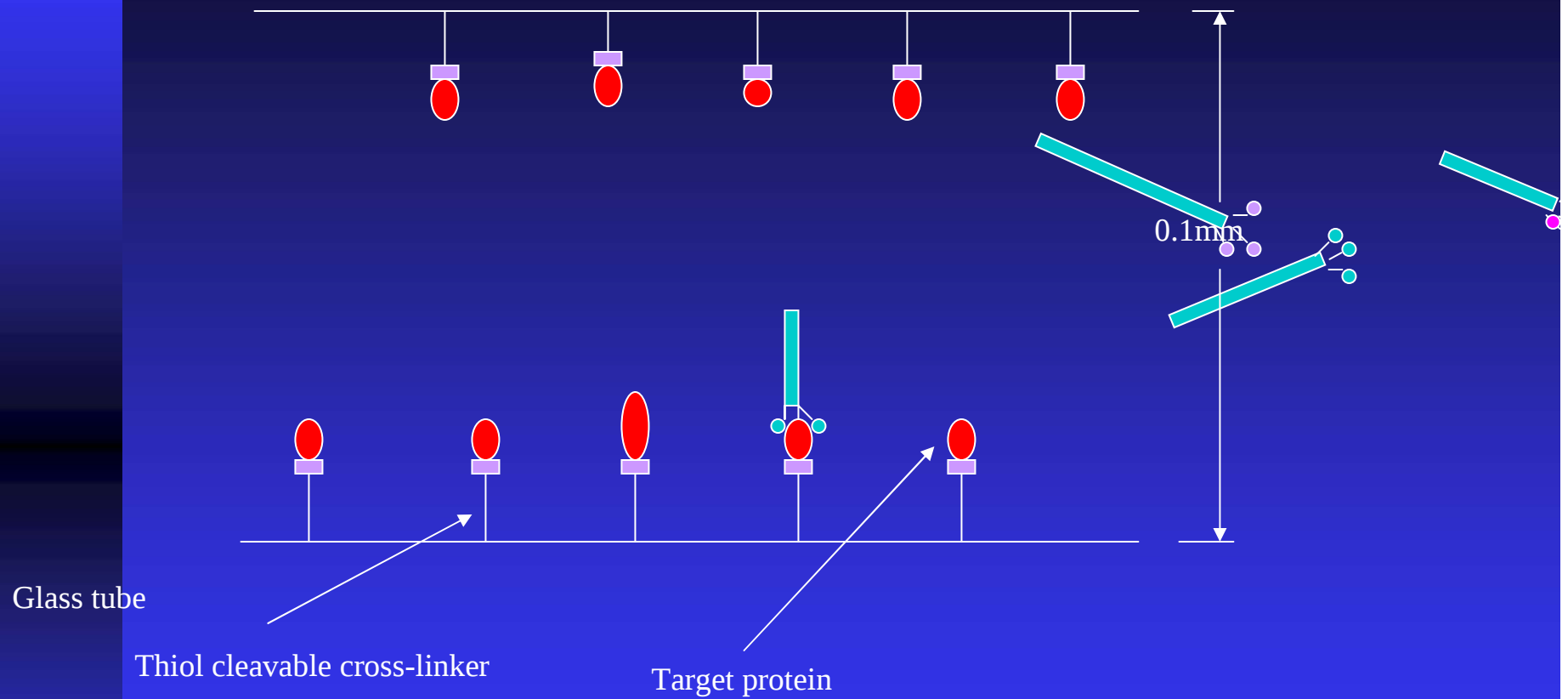
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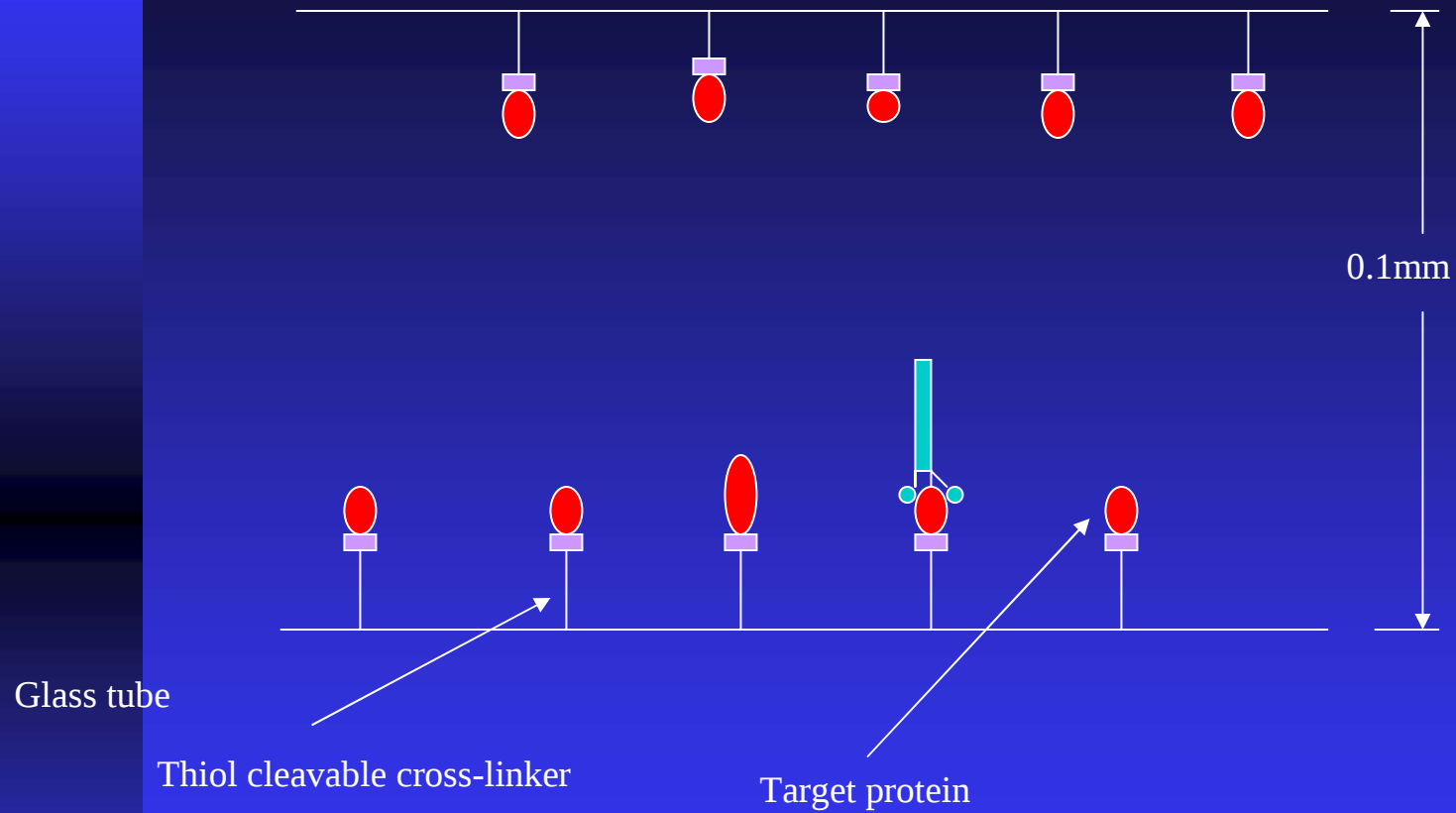
Glass tube biopanning apparatus



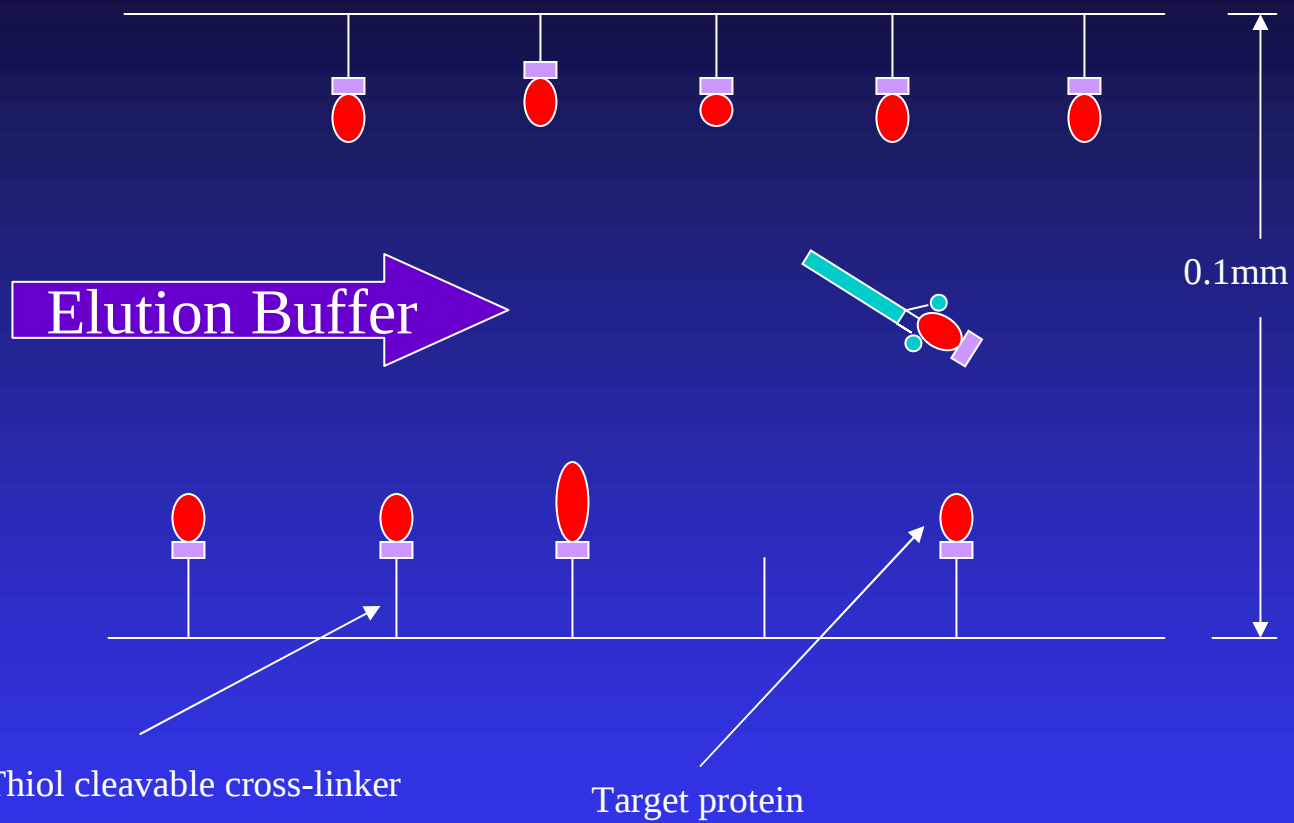
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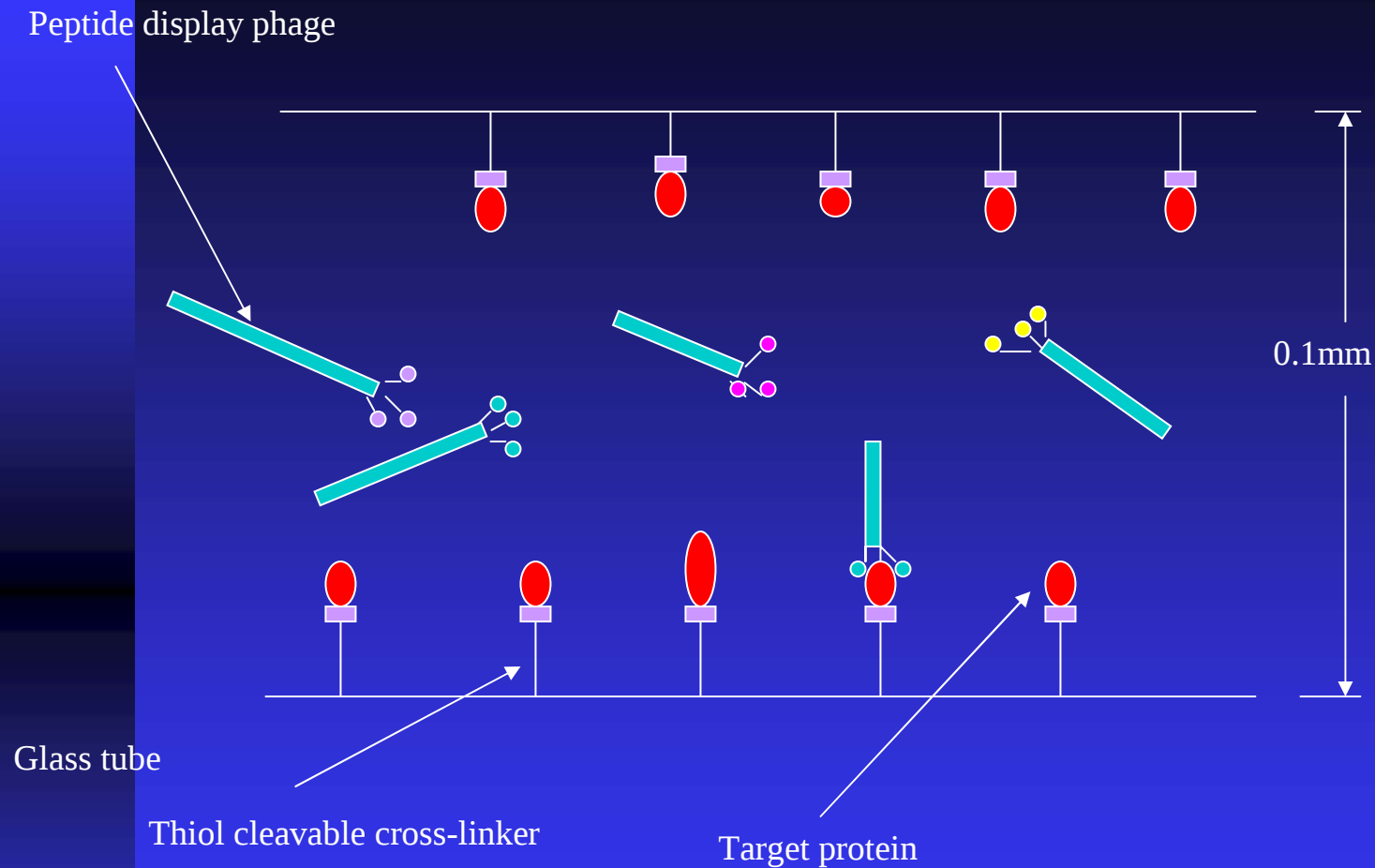
Glass tube biopanning apparatus



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Advantages:

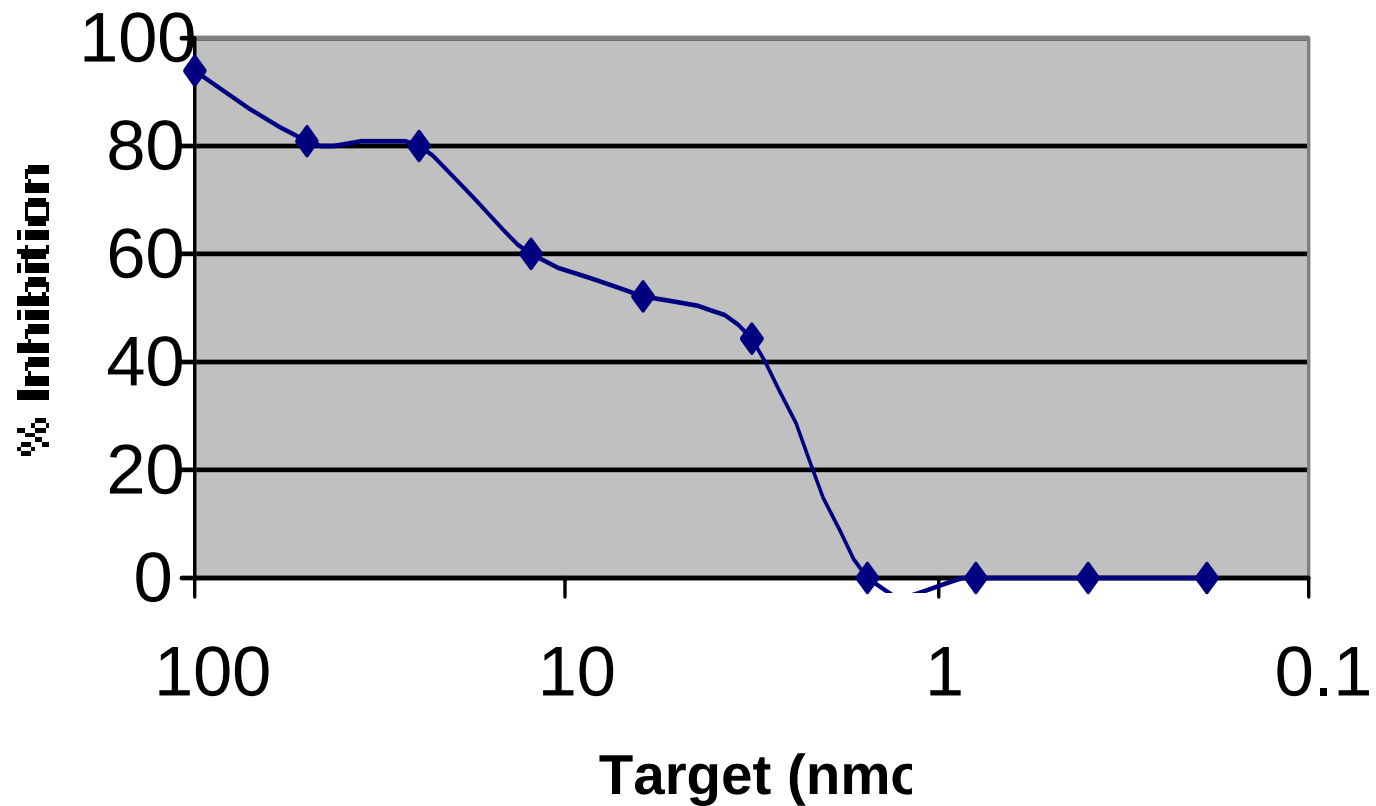
1. Can adjust flow rate = rigor of washing
2. Can remove all targets = cleavable cross-linkage agent
3. Can be automated for high-throughput
4. Can be adapted to small volumes (<5 ul)

Sequences for peptides which bind to the Y. pestis F1 antigen

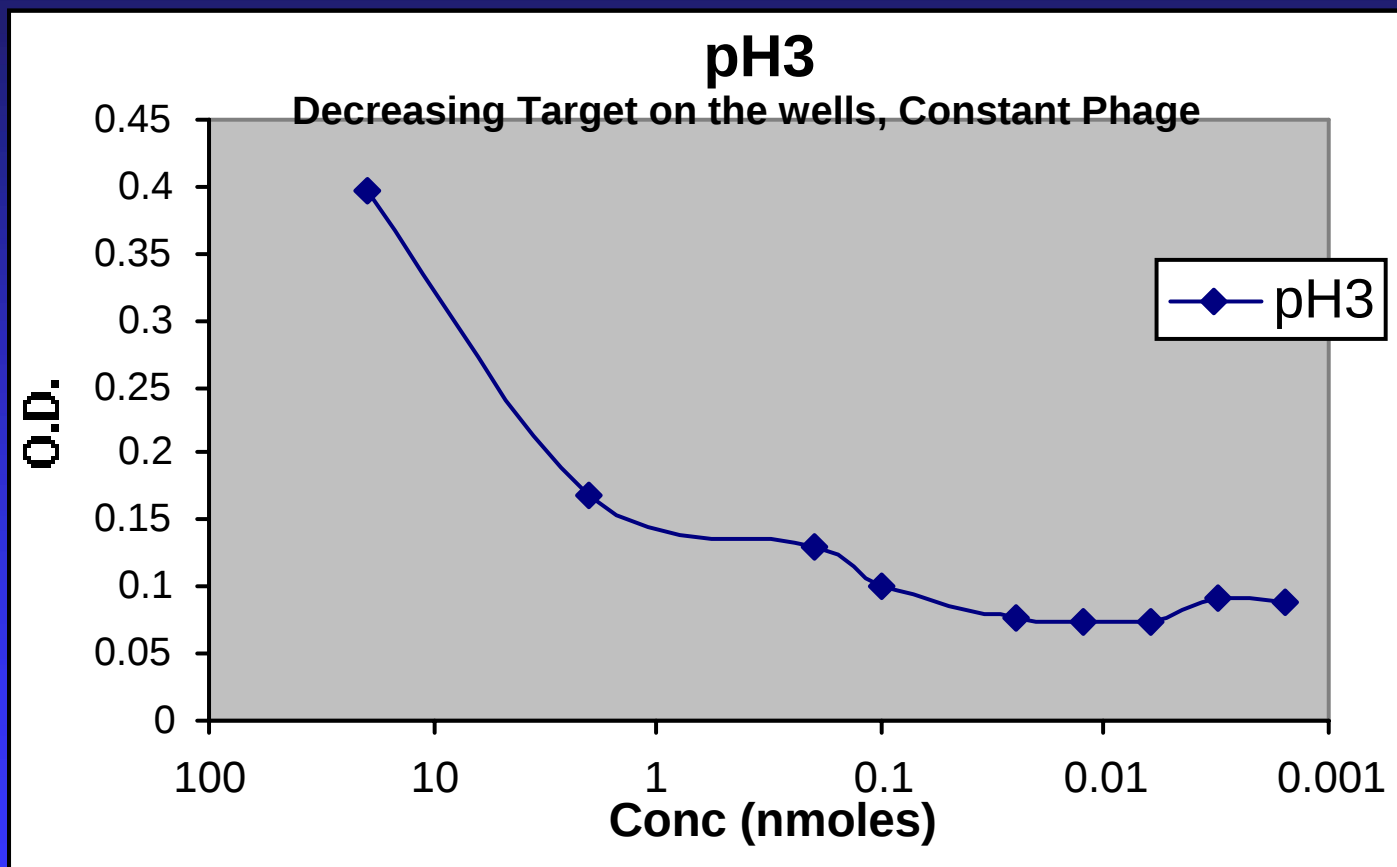
#1 SFSLKPHASLIR
#2 GPNKFSLMHLFS
#3 SFSLSSYSALLW
#4 KFSLSPTHAWFL
#5 KLSLNPHFMFQS
#6 FSLKNPTIANTM
#7 LISVEPASLSAH
#8 SSLTLAPFSWSL
#9 GPWFSLRHLSPPQ
#10 SHSWFRVNTLHL
#11 GWFSTPLKWRMQ
#12 SNFTLPFLKTER
#13 SWFTLHNLPNRP
#14 NFSINPRMMWPV
#21 FSIKHPPWFFLP
#28 FSLKLPPYWQRTE

Use of free F1 antigen to compete with phage
in its binding to F1 attached to a plate

Competitive Inhibiti



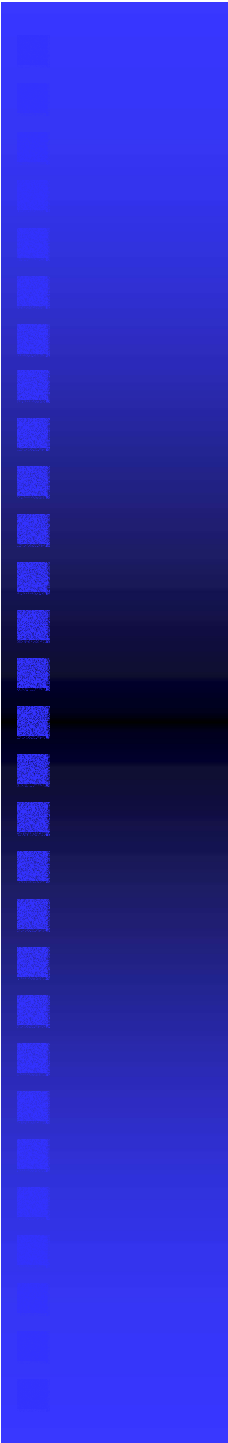
Effect of decreasing the amount of F1-antigen on the plate



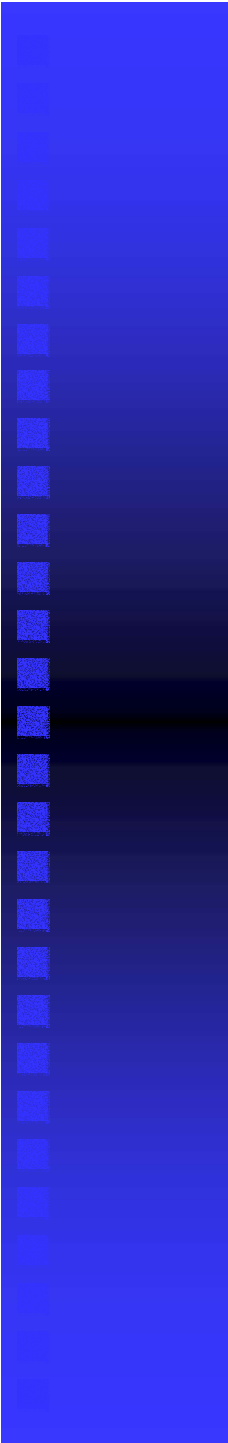
ORGANO-SELENIUM

Selenium Chemistry is a lot like that of Sulfur

1 H 1.008 Hydrogen		硒 SELENIUM																2 He 4.003 Helium																																	
3 Li 6.941 Lithium		4 Be 9.012 Beryllium																		5 B 10.82 Boron		6 C 12.011 Carbon		7 N 14.008 Nitrogen		8 O 16.000 Oxygen		9 F 18.998 Fluorine		10 Ne 20.183 Neon																					
11 Na 22.991 Sodium		12 Mg 24.32 Magnesium																		13 Al 26.98 Aluminum		14 Si 28.29 Silicon		15 P 30.973 Phosphorus		16 S 32.06 Sulfur		17 Cl 35.453 Chlorine		18 Ar 39.944 Argon																					
19 K 39.100 Potassium		20 Ca 40.08 Calcium		21 Sc 44.96 Scandium		22 Ti 47.88 Titanium		23 V 50.94 Vanadium		24 Cr 52.01 Chromium		25 Mn 54.94 Manganese		26 Fe 55.85 Iron		27 Co 58.94 Cobalt		28 Ni 58.71 Nickel		29 Cu 63.54 Copper		30 Zn 65.38 Zinc		31 Ga 69.72 Gallium		32 Ge 72.60 Germanium		33 As 74.91 Arsenic		34 Se 78.96 Selenium		35 Br 79.90 Bromine		36 Kr 83.80 Krypton																	
37 Rb 85.48 Rubidium		38 Sr 87.63 Strontium		39 Y 88.91 Yttrium		40 Zr 91.22 Zirconium		41 Nb 92.91 Niobium		42 Mo 95.95 Molybdenum		43 Tc 99 Technetium		44 Ru 101.1 Ruthenium		45 Rh 102.91 Rhodium		46 Pd 106.4 Palladium		47 Ag 107.866 Silver		48 Cd 112.41 Cadmium		49 In 114.82 Indium		50 Sn 118.70 Tin		51 Sb 121.76 Antimony		52 Te 127.61 Tellurium		53 I 126.91 Iodine		54 Xe 131.30 Xenon																	
55 Cs 132.91 Cesium		56 Ba 137.34 Barium		57 La 138.91 Lanthanum		72 Hf 178.50 Hafnium		73 Ta 180.95 Tantalum		74 W 183.84 Tungsten		75 Re 186.21 Rhenium		76 Os 190.2 Osmium		77 Ir 192.2 Iridium		78 Pt 195.08 Platinum		79 Au 197.0 Gold		80 Hg 200.59 Mercury		81 Tl 204.38 Thallium		82 Pb 207.2 Lead		83 Bi 208.98 Bismuth		84 Po 210 Polonium		85 At 210 Astatine		86 Rn 222 Radon																	
87 Fr (223) Francium		88 Ra (226) Radium		89 Ac (227) Actinium		104 (Rutherfordium) Rutherfordium																		58 Ce 140.12 Cerium		59 Pr 140.91 Praseodymium		60 Nd 144.24 Neodymium		61 Pm (147) Promethium		62 Sm 150.36 Samarium		63 Eu 151.96 Europium		64 Gd 157.25 Gadolinium		65 Tb 158.93 Terbium		66 Dy 162.50 Dysprosium		67 Ho 164.93 Holmium		68 Er 167.26 Erbium		69 Tm 168.93 Thulium		70 Yb 173.04 Ytterbium		71 Lu 174.97 Lutetium	
90 Th (232) Thorium		91 Pa (231) Protactinium		92 U 238.03 Uranium		93 Np (237) Neptunium		94 Pu (242) Plutonium		95 Am (243) Americium		96 Cm (247) Curium		97 Bk (247) Berkelium		98 Cf (251) Californium		99 Es (254) Einsteinium		100 Fm (253) Fermium		101 Md (258) Mendelevium		102 No (259) Nobelium		103 Lr (262) Lawrencium																									



Selenium has a Good Side and a Dark Side



Selenium is essential for your
diet

Selenium is essential for your diet

- You need approximately 200 ug/day.

Selenium is essential for your diet

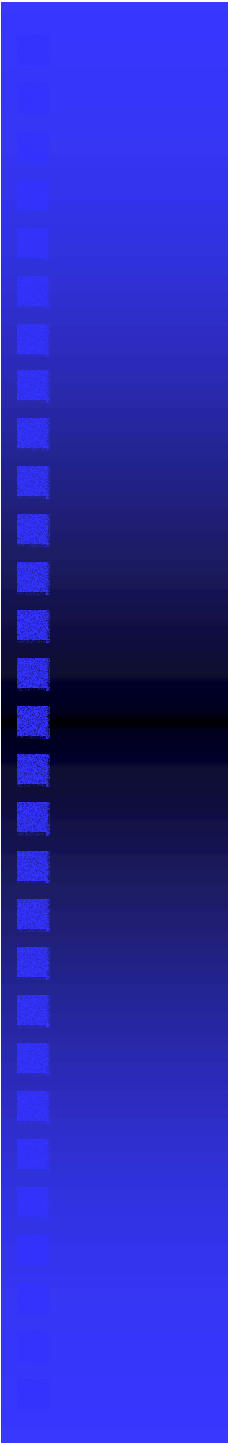
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- From the sequencing of the human genome it is found that selenium is incorporated into 25 different proteins.

Selenium is essential for your diet

- You need approximately 200 ug/day.
- A selenium amino acid has its own genetic code.
- From the sequencing of the human genome it is found that selenium is incorporated into 25 different proteins.
- Many of these selenium containing proteins function to destroy oxygen radicals.



The Dark Side: If you eat too
much Selenium it will kill you

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- 3 mg/day will make you sick

The Dark Side: If you eat too much Selenium it will kill you

- 3 mg/day will make you sick
- 30 mg/day will kill you



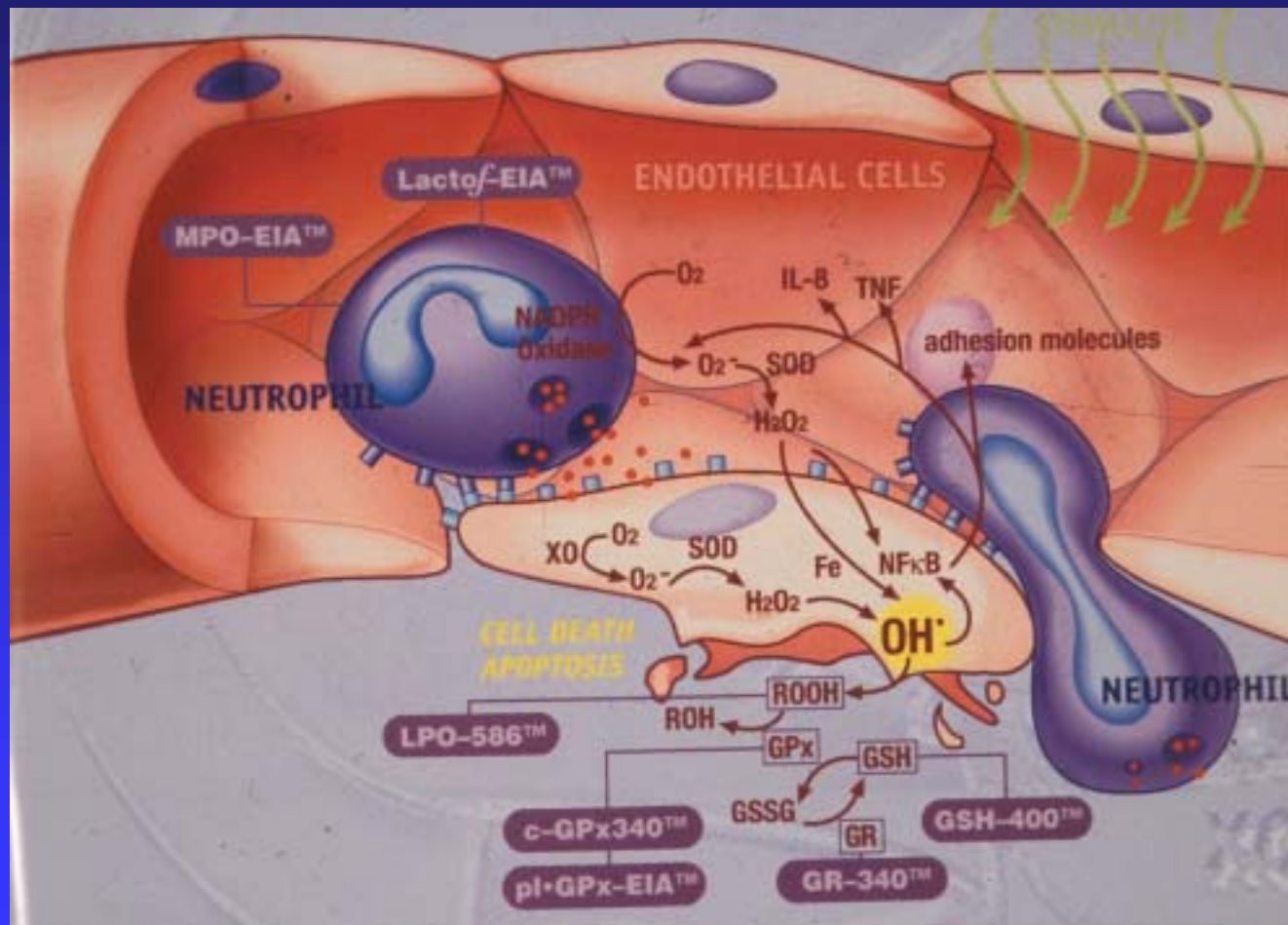
We Work on the Dark Side

Selenium can catalyze the
formation of superoxide radicals

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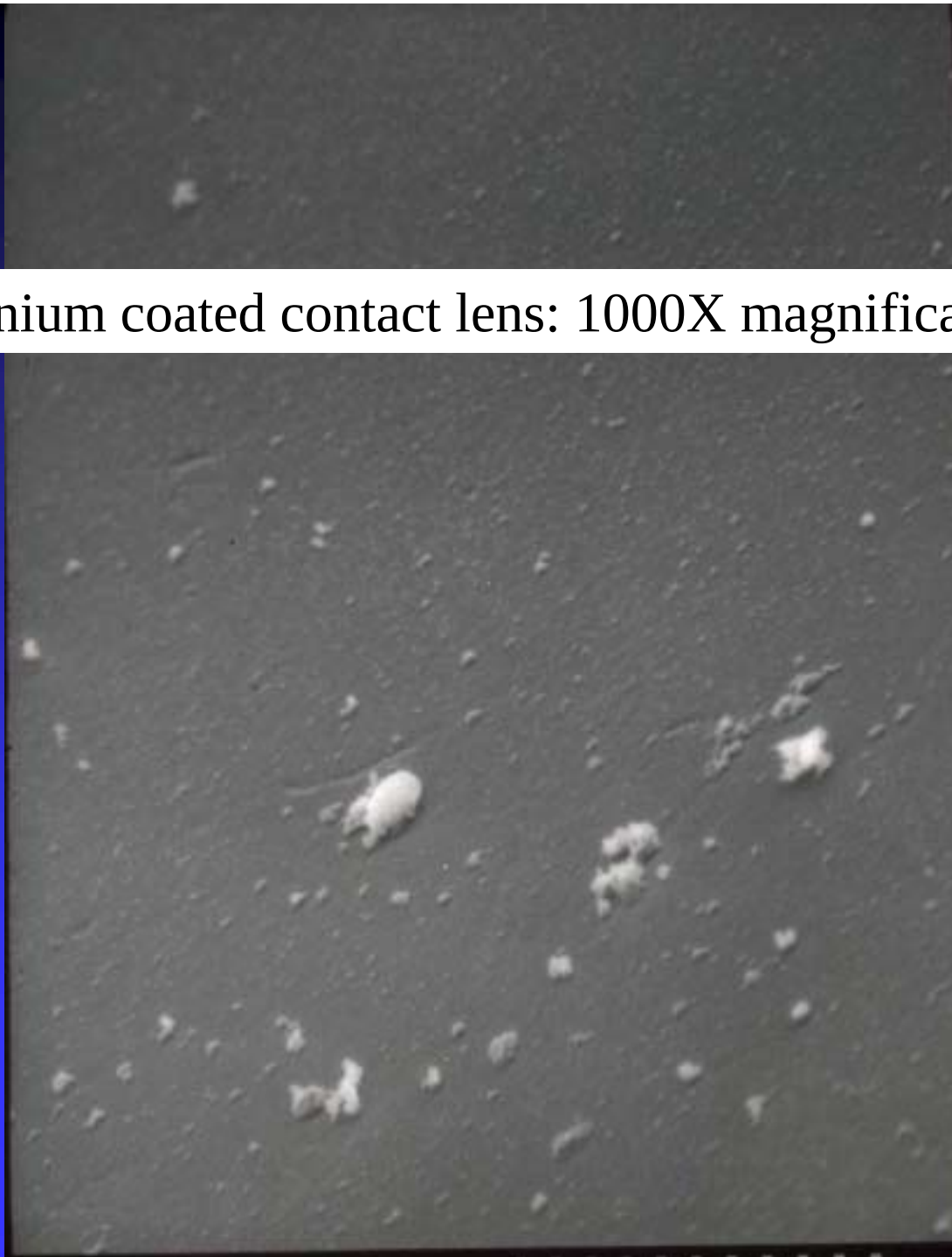
Superoxide killing mechanism



Selenium covalently attached to a contact lens.

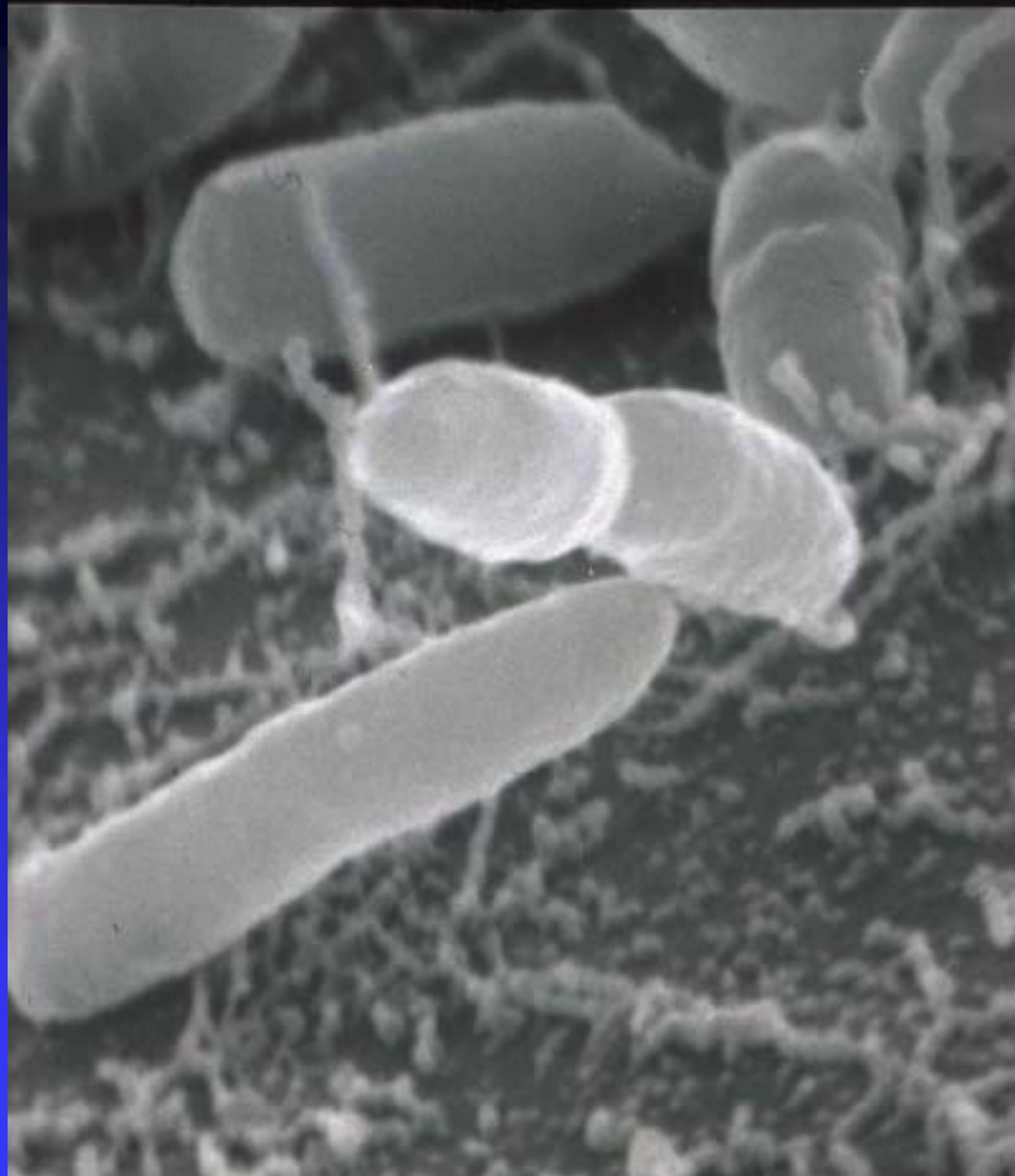
Contact lens placed in broth with
Pseudomonas aeruginosa for
4 days

Selenium coated contact lens: 1000X magnification





Uncoated contact lens: 1000X magnification



Uncoated contact lens: 25,000X magnification

Hypothesis

Selenium labeled peptides and selenium labeled bacterial viruses (phage) can be produced that can selectively bind to the surface of a pathogenic bacteria and inactivate them through the generation of superoxide radicals on their surface.

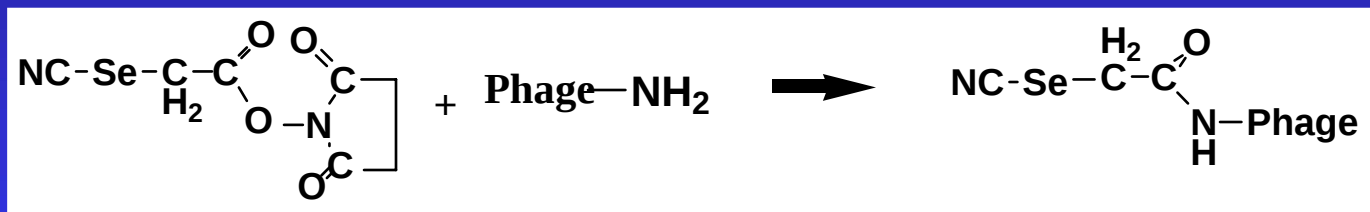
Initial experiments

- Initial experiments were done with labeled bacterial viruses

Filamentous Bacteriophage

QuickTime™ and a
Photo - JPEG decompressor
are needed to see this picture.

Attachment of Selenium to Phage



Bacteria with F1 protein expressed on their surface

Use of phage which show F1 specific binding

Bacteria with F1 protein expressed on their surface

Use of phage which show F1 specific binding

Attach selenium to the specific phage

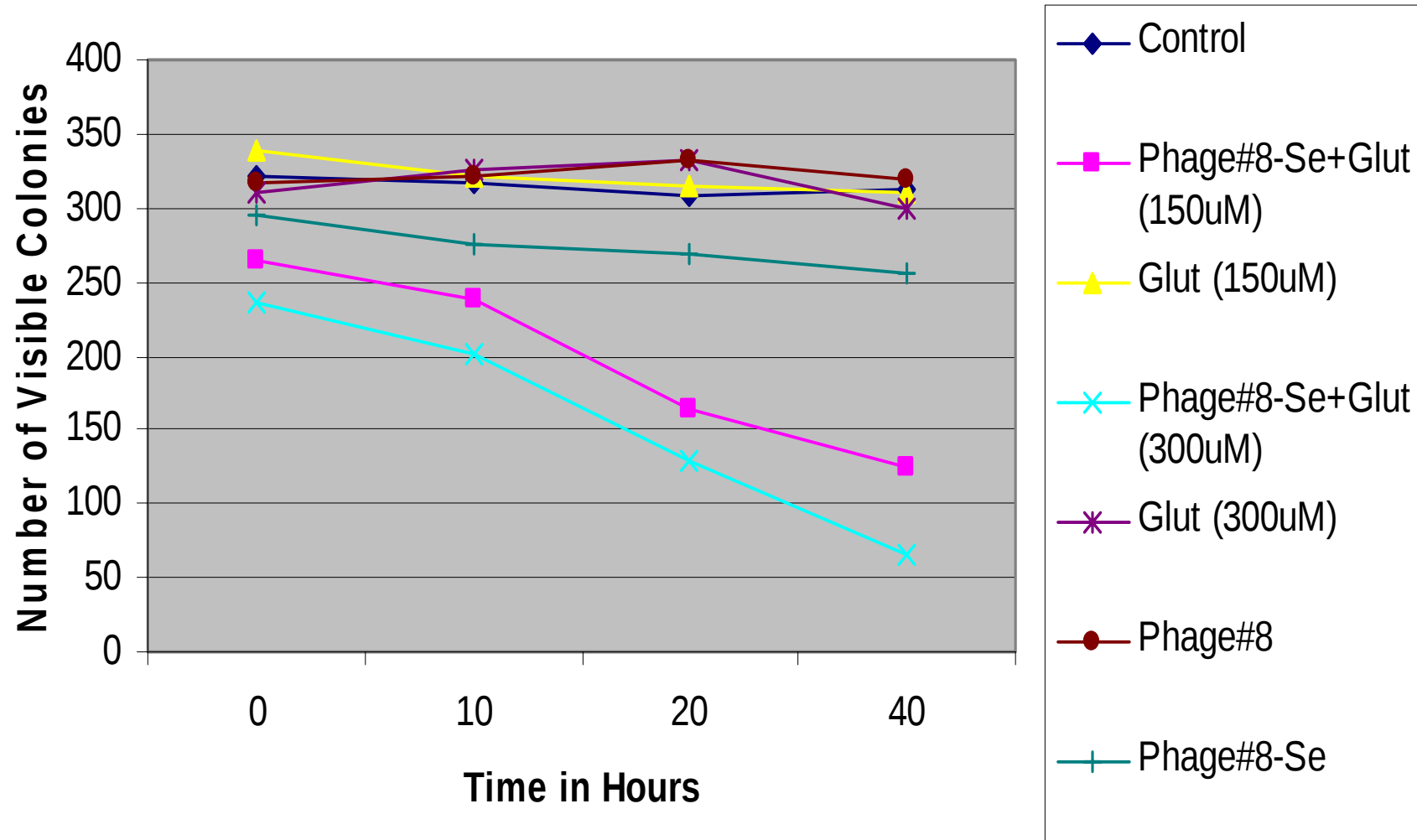
Bacteria with F1 protein expressed on their surface

Use of phage which show F1 specific binding

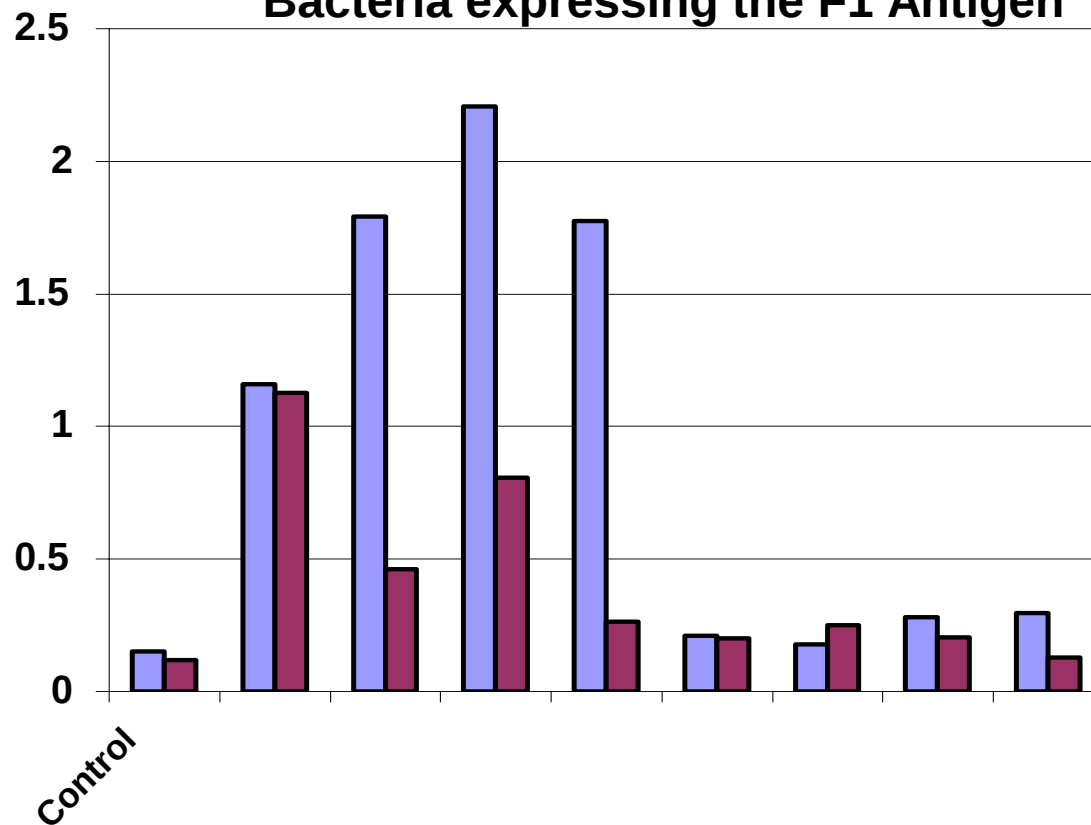
Attach selenium to the specific phage

Test selenium labeled phage with and with out
an external source of sulfur (glutathione) by
mixing with bacteria and then plating to
determine number of live bacteria

Phage #8 Labeled with Selenium Kill F1 Antigen Expressed PYPR1b Strain in the Presence of Glutathione



**Specific F1 YP Phage #8 (10¹¹) Inhibit F1 Mouse
Monoclonal Antibody in Competition Binding Assay with
Bacteria expressing the F1 Antigen**



Peptide #8 From the Phage Binding Studies

Ser-Ser-Leu-Thr-Leu-Ala-Pro-Phe-Ser-Trp-Ser-Leu

Peptide #8 From the Phage Binding Studies

Ser-Ser-Leu-Thr-Leu-Ala-Pro-Phe-Ser-Trp-Ser-Leu

Selenium was covalently attached to this peptide

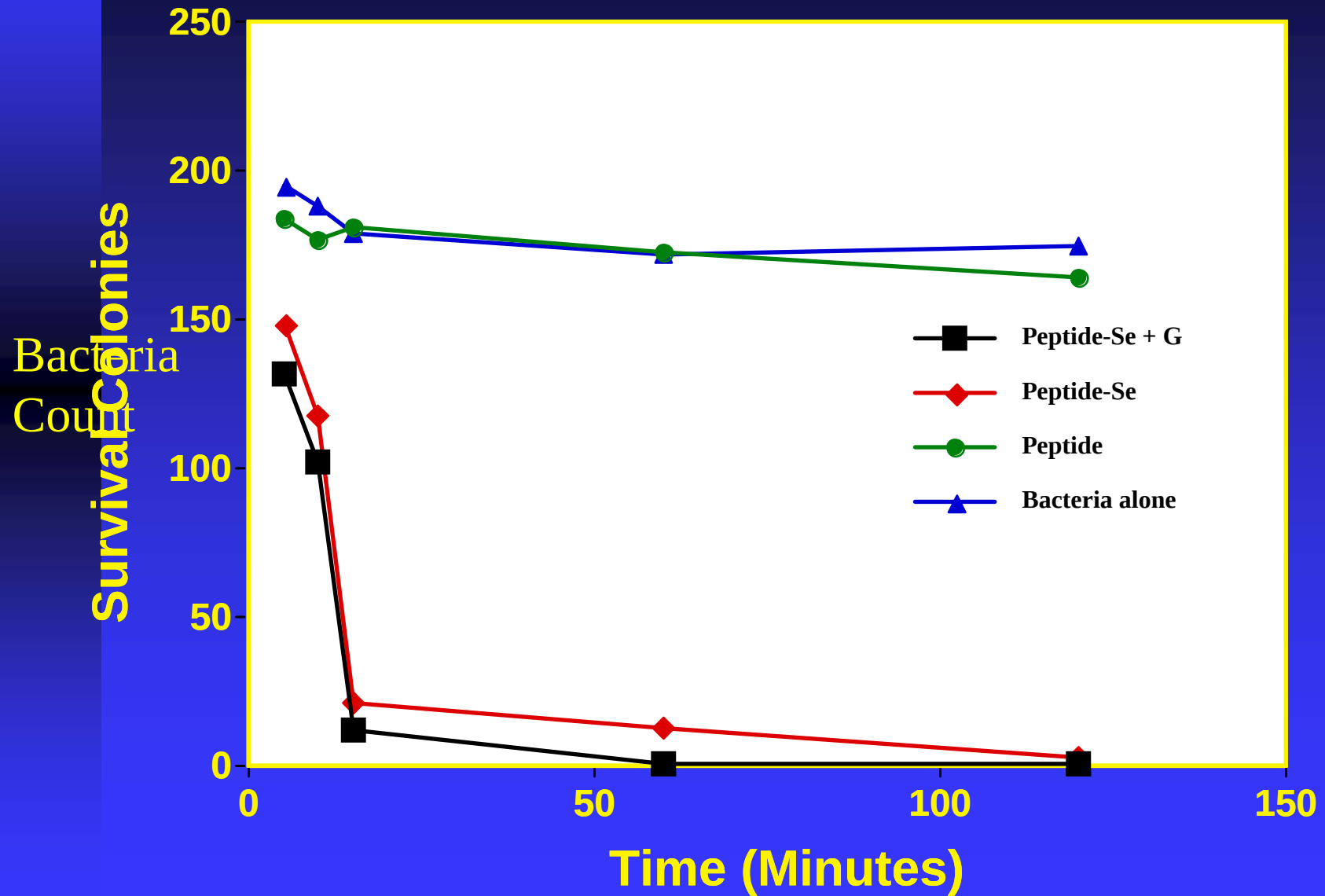
Peptide #8 From the Phage Binding Studies

Ser-Ser-Leu-Thr-Leu-Ala-Pro-Phe-Ser-Trp-Ser-Leu

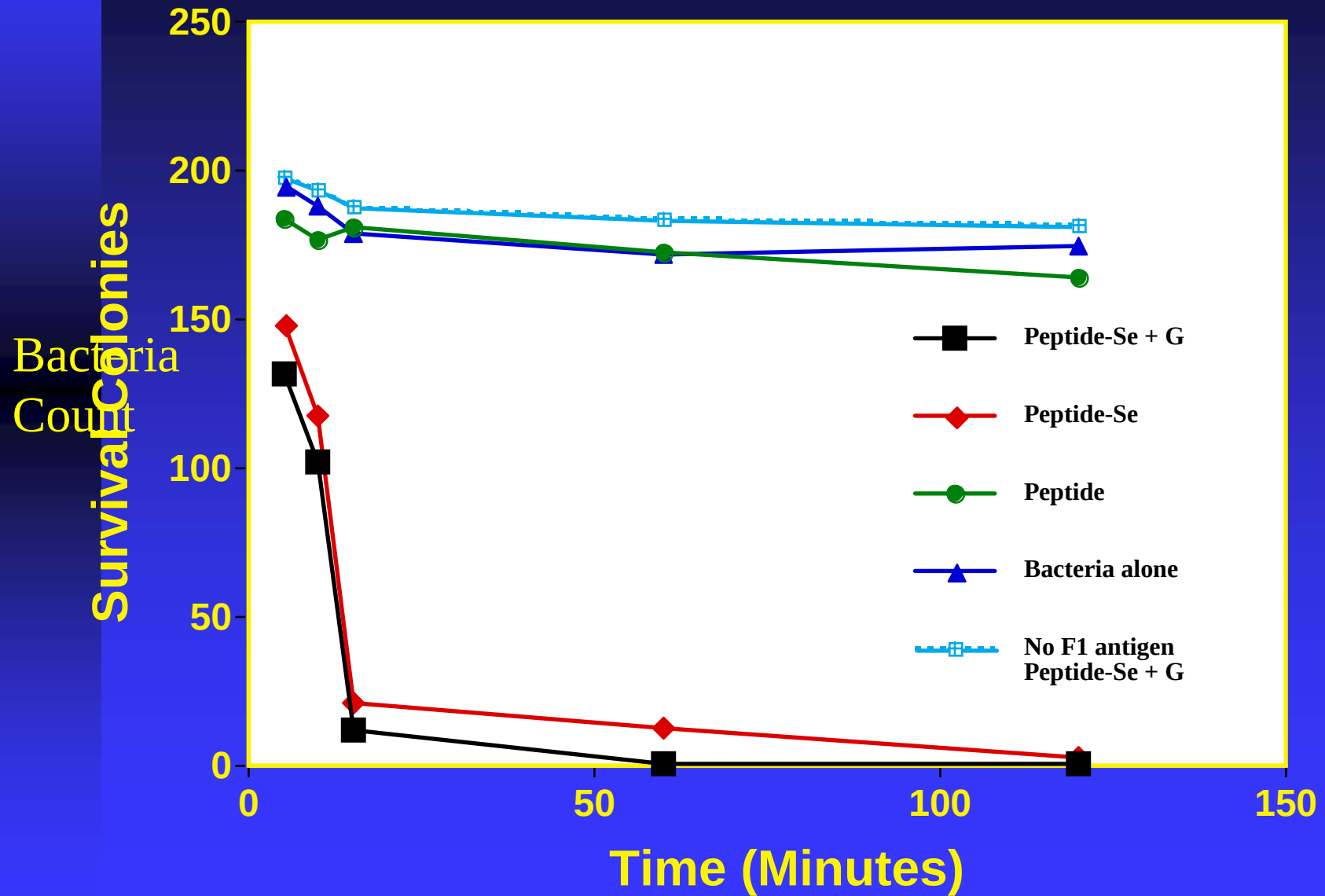
Selenium was covalently attached to this peptide

The seleno-peptide was tested at 1 μM

Survival of Bacteria expressing F1 antigen In the presence of Seleno-peptide



Survival of Bacteria expressing F1 antigen In the presence of Seleno-peptide



Conclusions

- Selenium can be attached to a bacterial virus which can target and bind to a specific bacteria.

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- A selenium labeled virus targeted for a specific bacteria can kill the bacteria.
- The selenium labeled virus killing of the bacteria is promoted by glutathione.
- Bacterial killing with a selenium labeled phage takes about 40-60 hours using 10^{11} phage.

Conclusions - Peptide

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- A seleno-peptide can kill 95% of a specific bacteria in 15 minutes and all of the bacteria in less than 1 hour.

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Conclusions - Peptide

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- A seleno-peptide can kill 95% of a specific bacteria in 15 minutes and all of the bacteria in less than 1 hour.
- The seleno-peptide can kill at 1 micromolar concentration.
- The seleno-peptide will not kill bacteria that do not express the required binding protein.

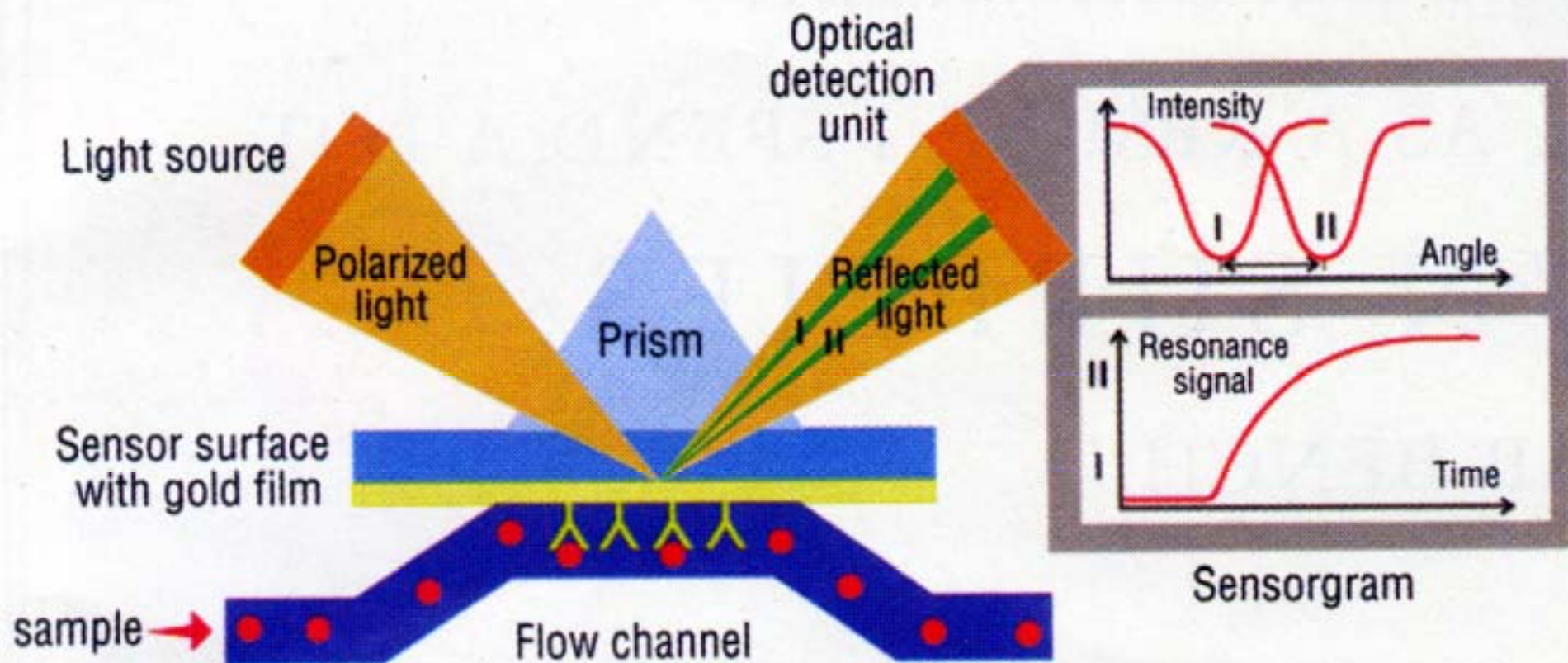
Future Studies

- Bacterial killing studies in vivo

Future Studies

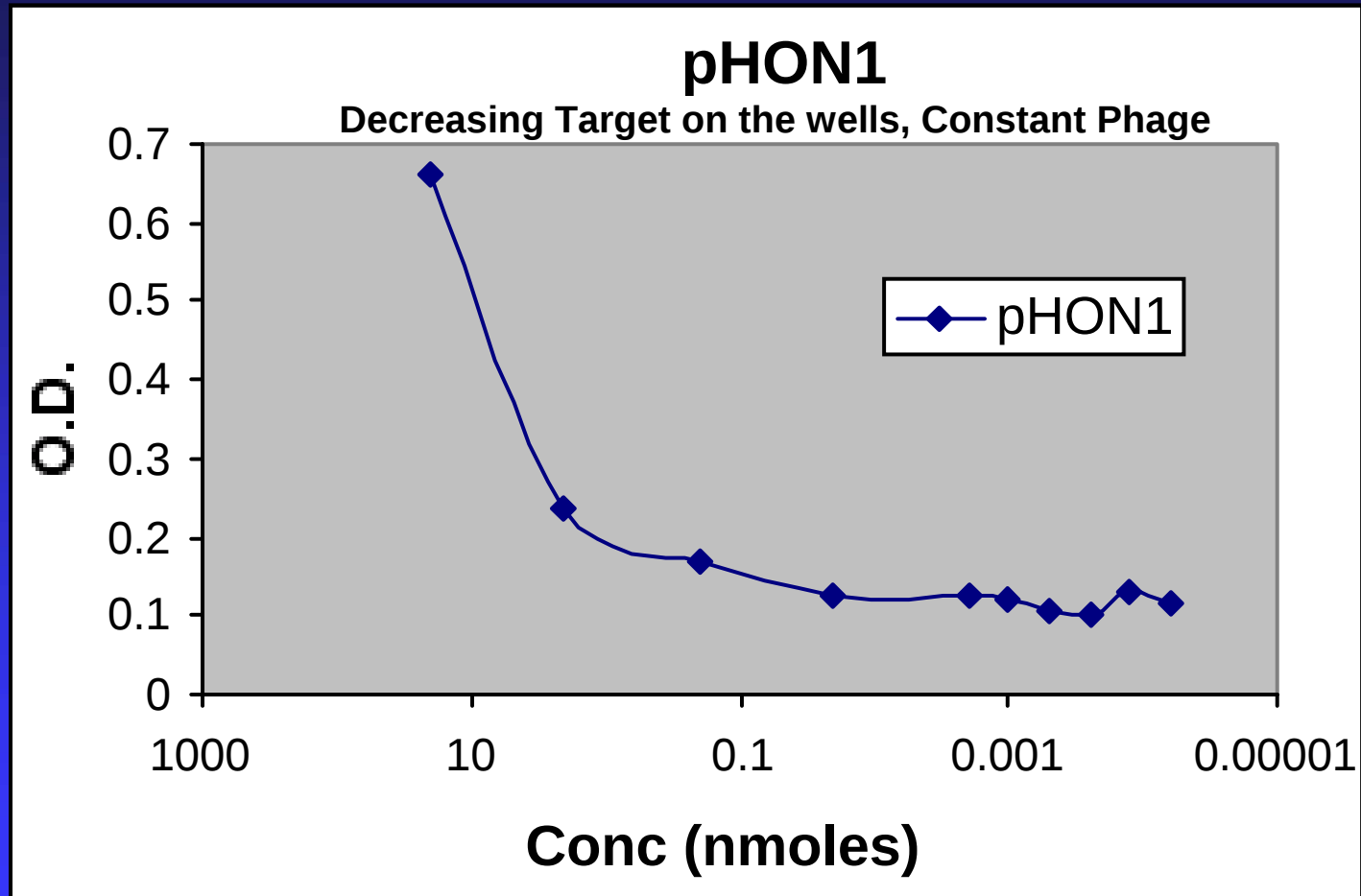
- Bacterial killing studies in vivo.
- Testing and design of new seleno-peptides which bind to other targets (new bacteria).



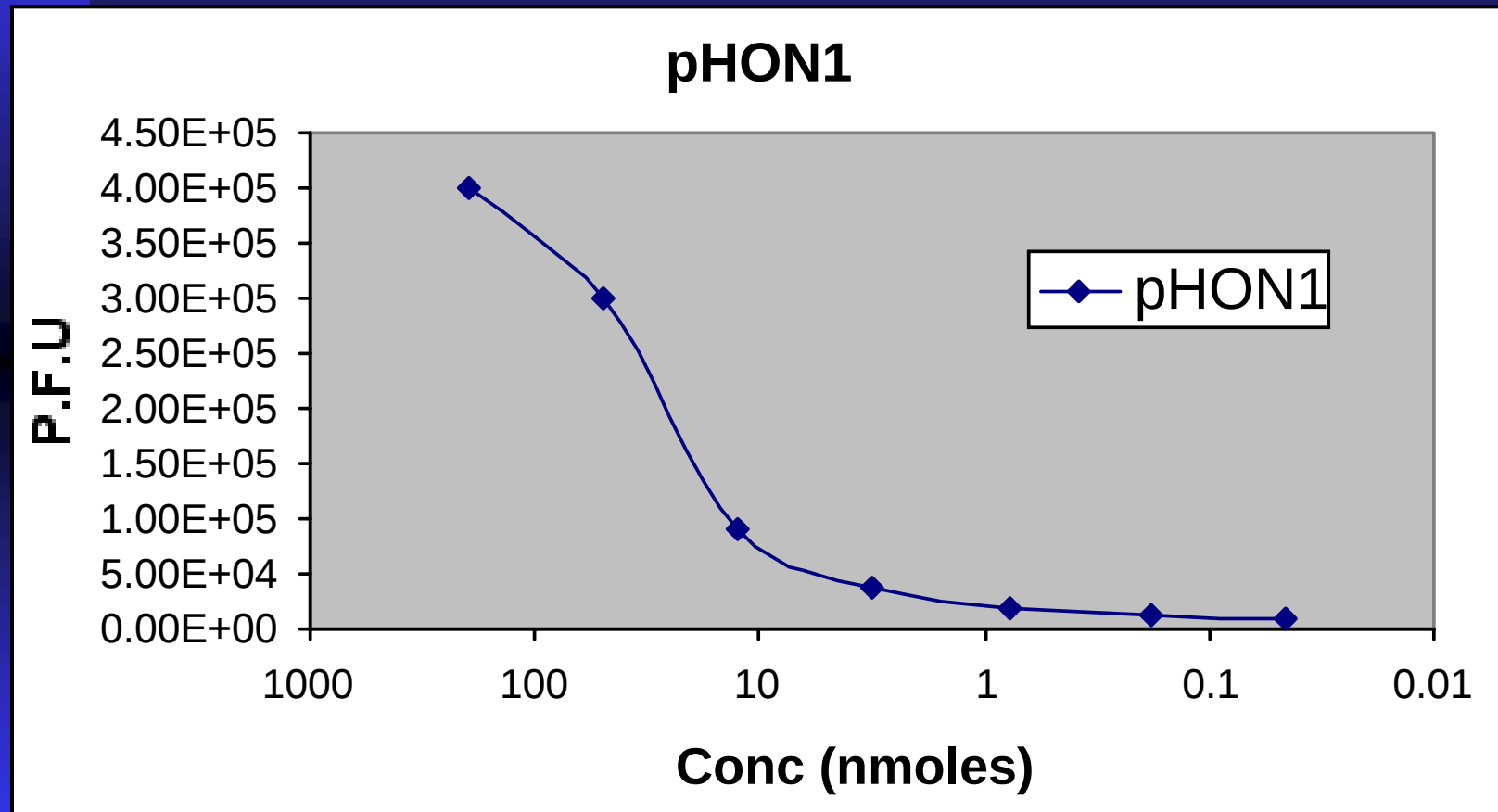


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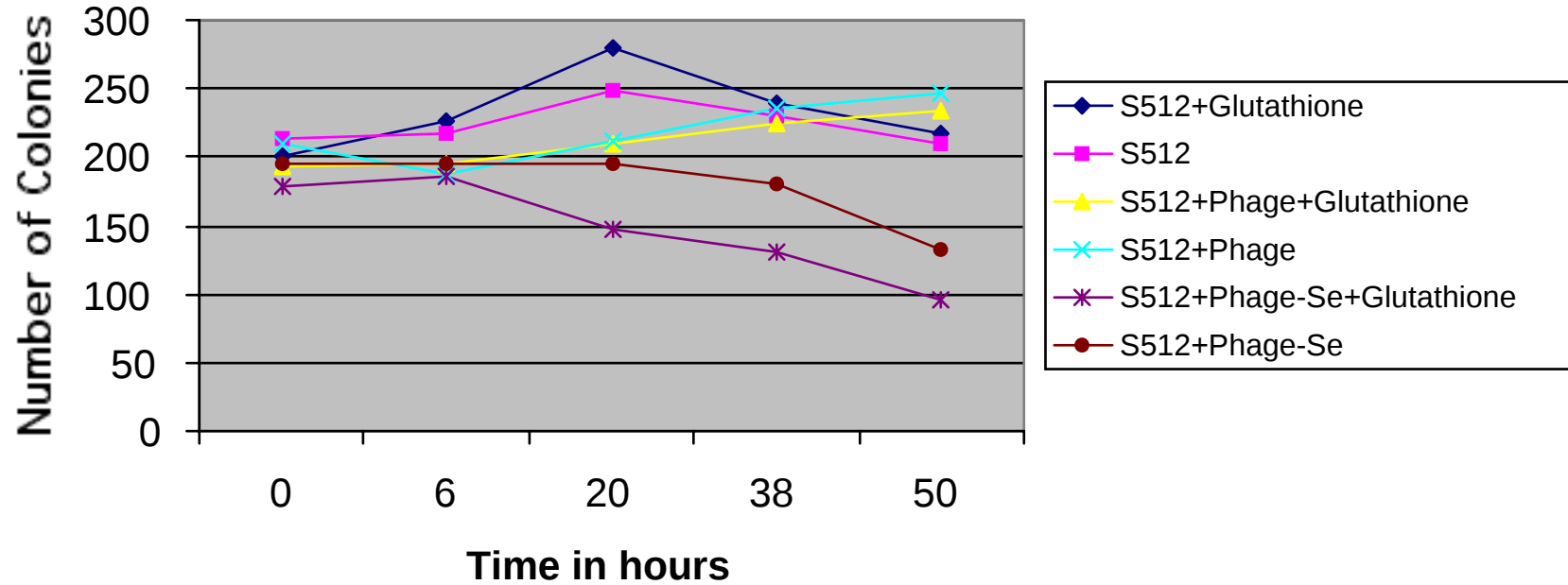
Effect of decreasing the amount of F1-antigen on the plate



Competition between phage and free F1 antigen for antigen attached to the plate



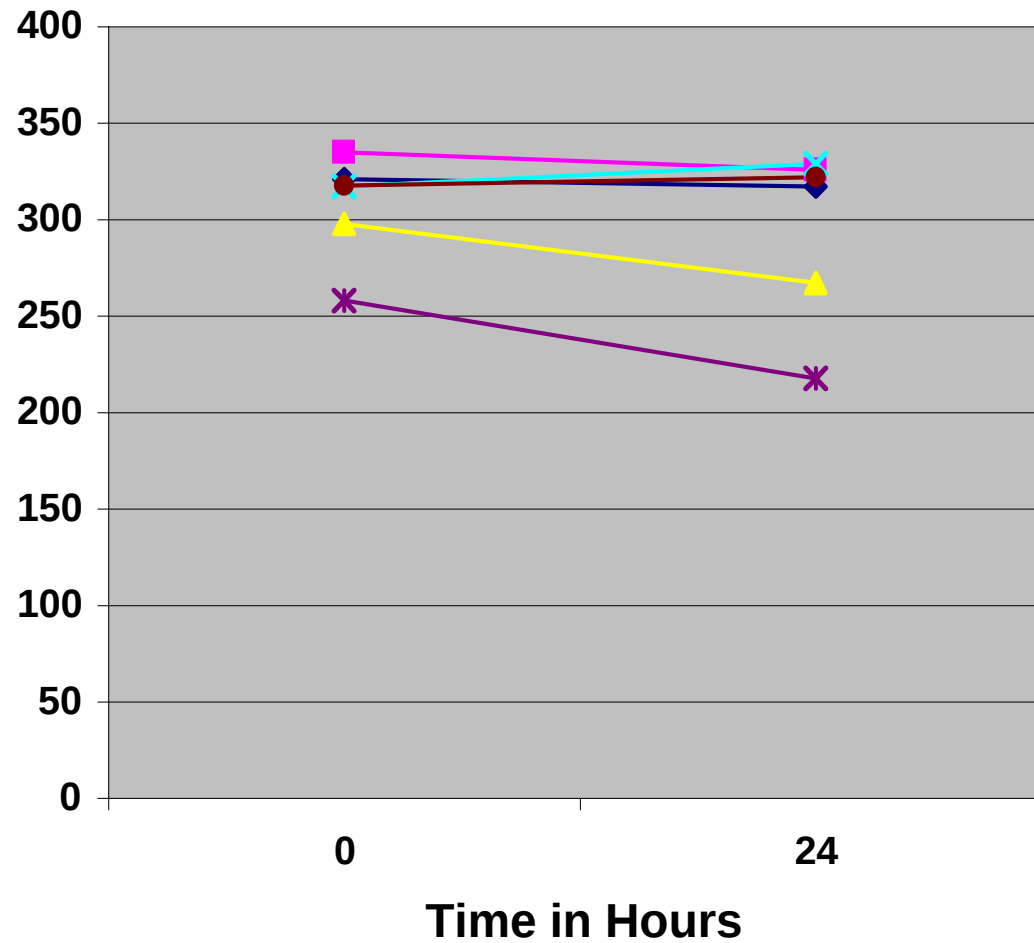
S512 Cells treated with Phage and Phage-Se with and without Glutathione



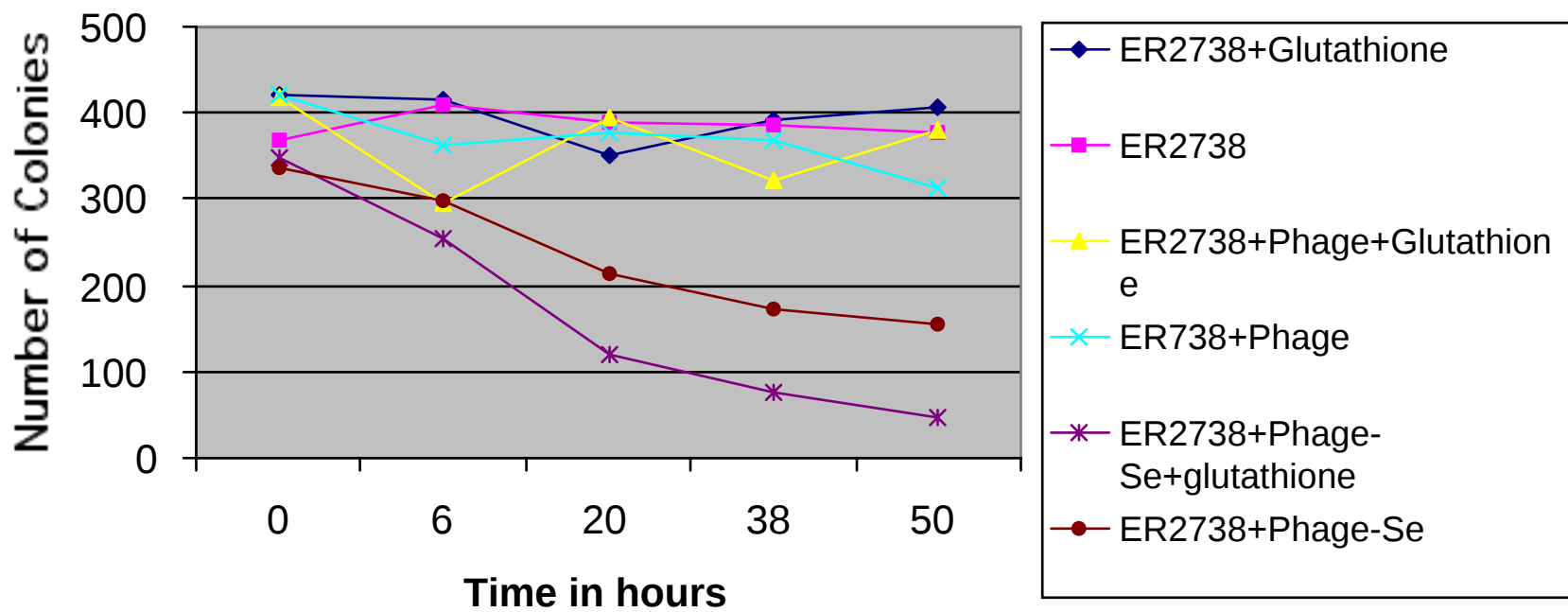




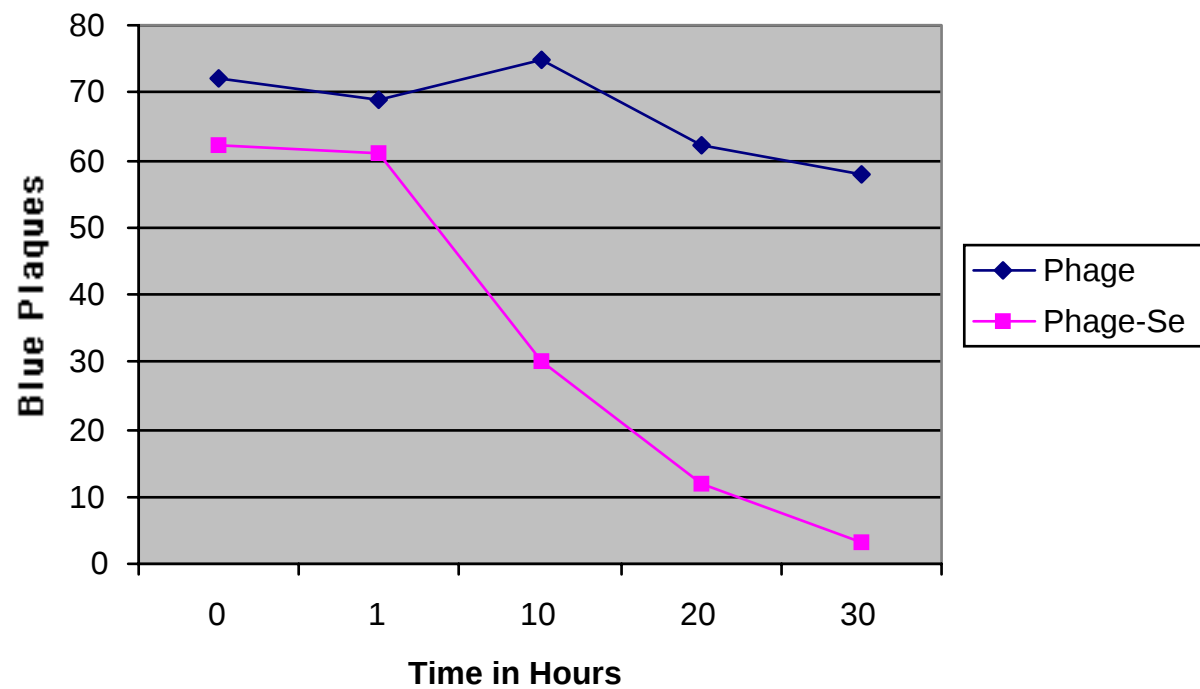
Survival Number of PYPR-1b Bacteria After Treatment with Phage-6 Labeled Selenium



ER2738 Treated with Phage and Phage-Se with and without Glutathione



Kiling Curve Between Phage and Phage-Se



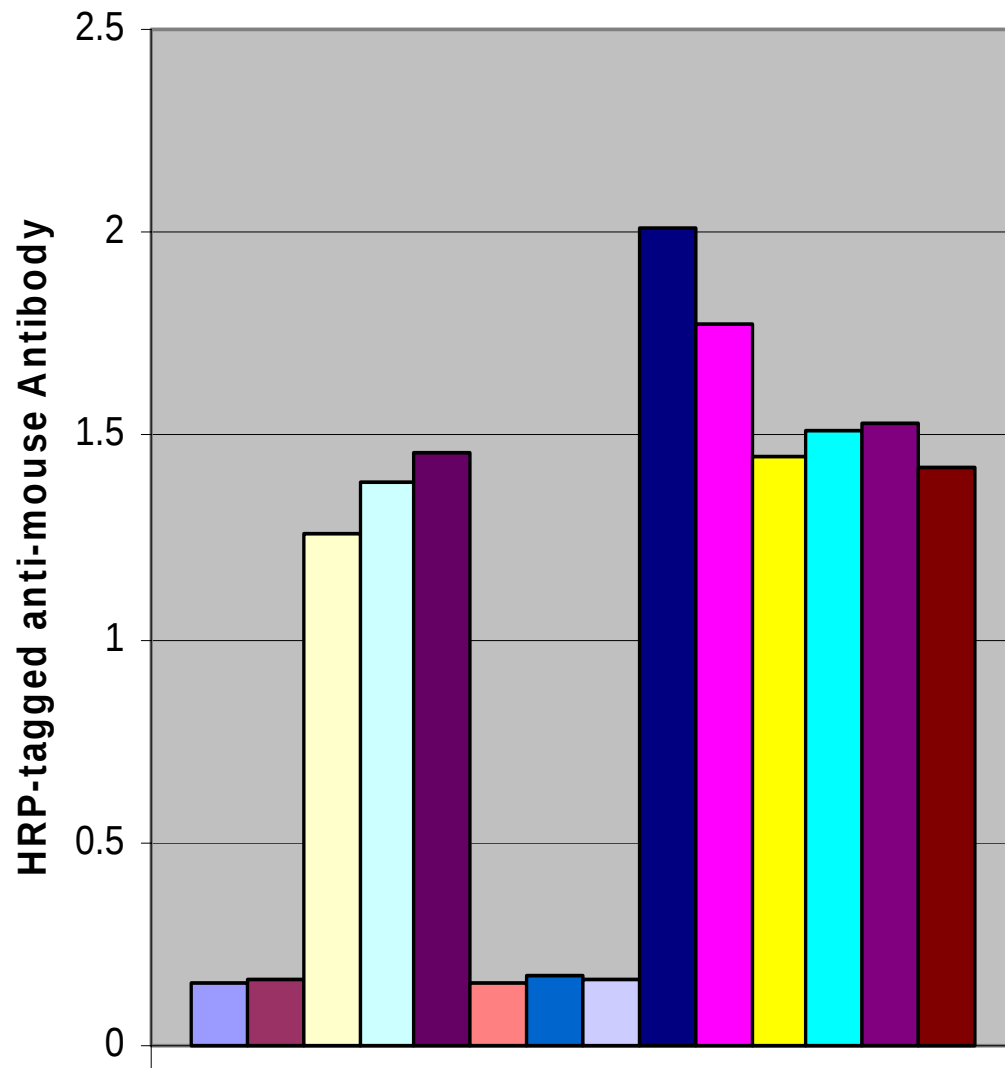
Killing curves for bacteria using selenium labeled phage with binding specificity for those bacteria

Phage mixed with bacteria for different periods of time under different reaction conditions. Bacteria then plated and counted.



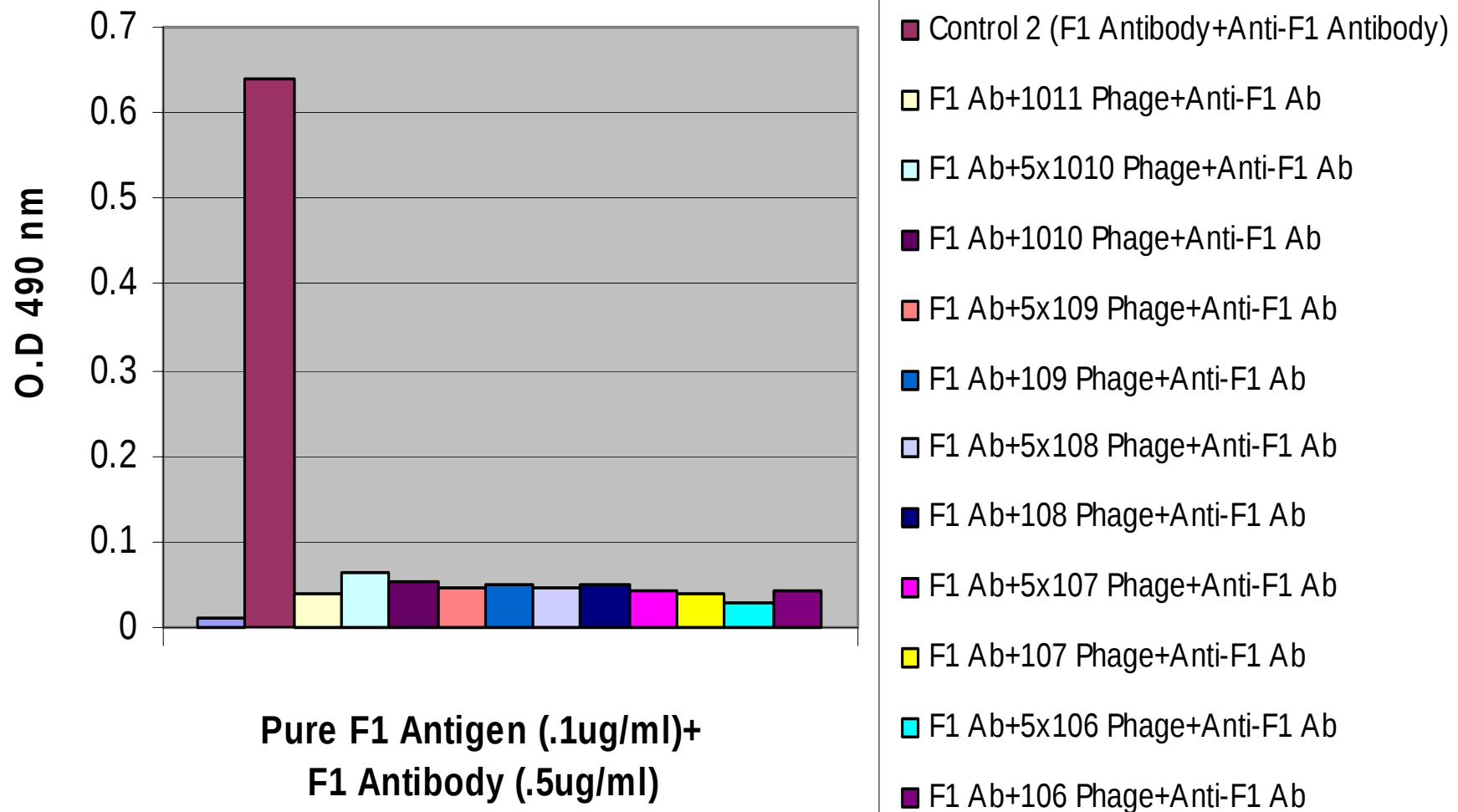


Determine The Presence of F1 Antigen on The Surface of *E.coli* PYPR-1b Strain by ELISA Assay and The knock-out of F1 Antibody by Phage

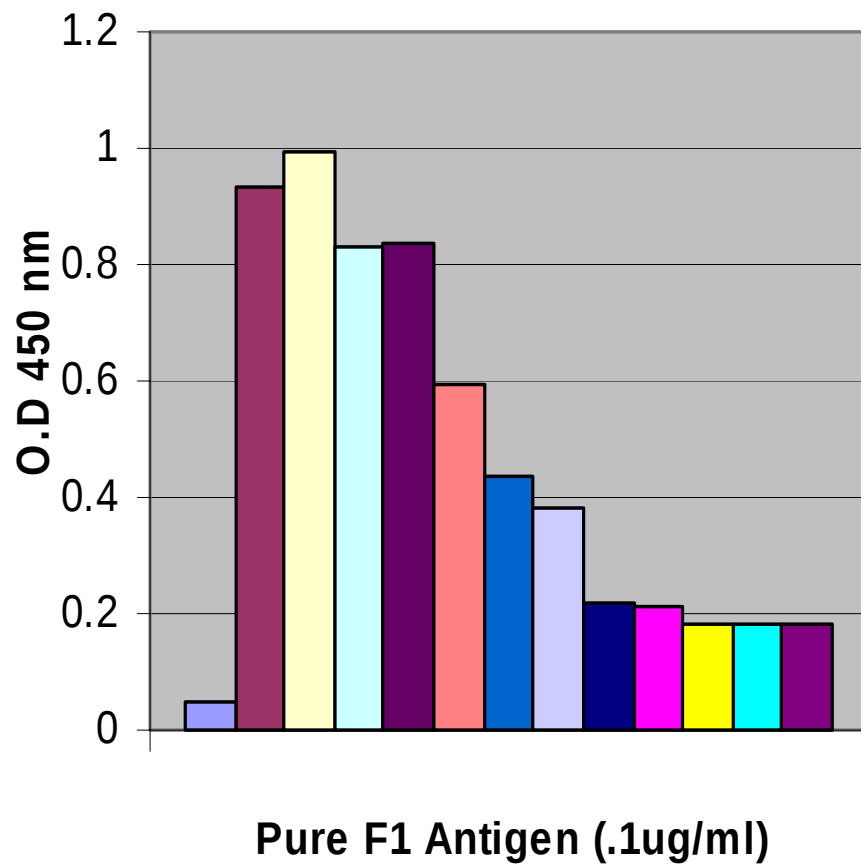


- PY PR-1b(Positive Control)
- XLI-Blue(Negative Control)
- PY PR-1b+F1 Antibody (5ug/ml)
- PY PR-1b+F1 Antibody (1ug/ml)
- PY PR-1b+F1 Antibody (.5ug/ml)
- XLI-blue+F1 Antibody (5ug/ml)
- XLI-blue+F1 Antibody (1ug/ml)
- XLI-blue+F1 Antibody (.5ug/ml)
- PY PR-1b+P#8+F1 Antibody (5ug/ml)
- PY PR-1b+P#8+F1 Antibody (1ug/ml)
- PY PR-1b+F1 Antibody (5ug/ml)+P#8
- PY PR-1b+F1 Antibody (1ug/ml)+P#8
- PY PR-1b+P#6+F1 Antibody (1ug/ml)
- PY PR-1b+F1 Antibody (1ug/ml)+P#6

Binding Competition of Phage #8 with F1 Antibody to Pure F1 Antigen Attached to Plastic Wells



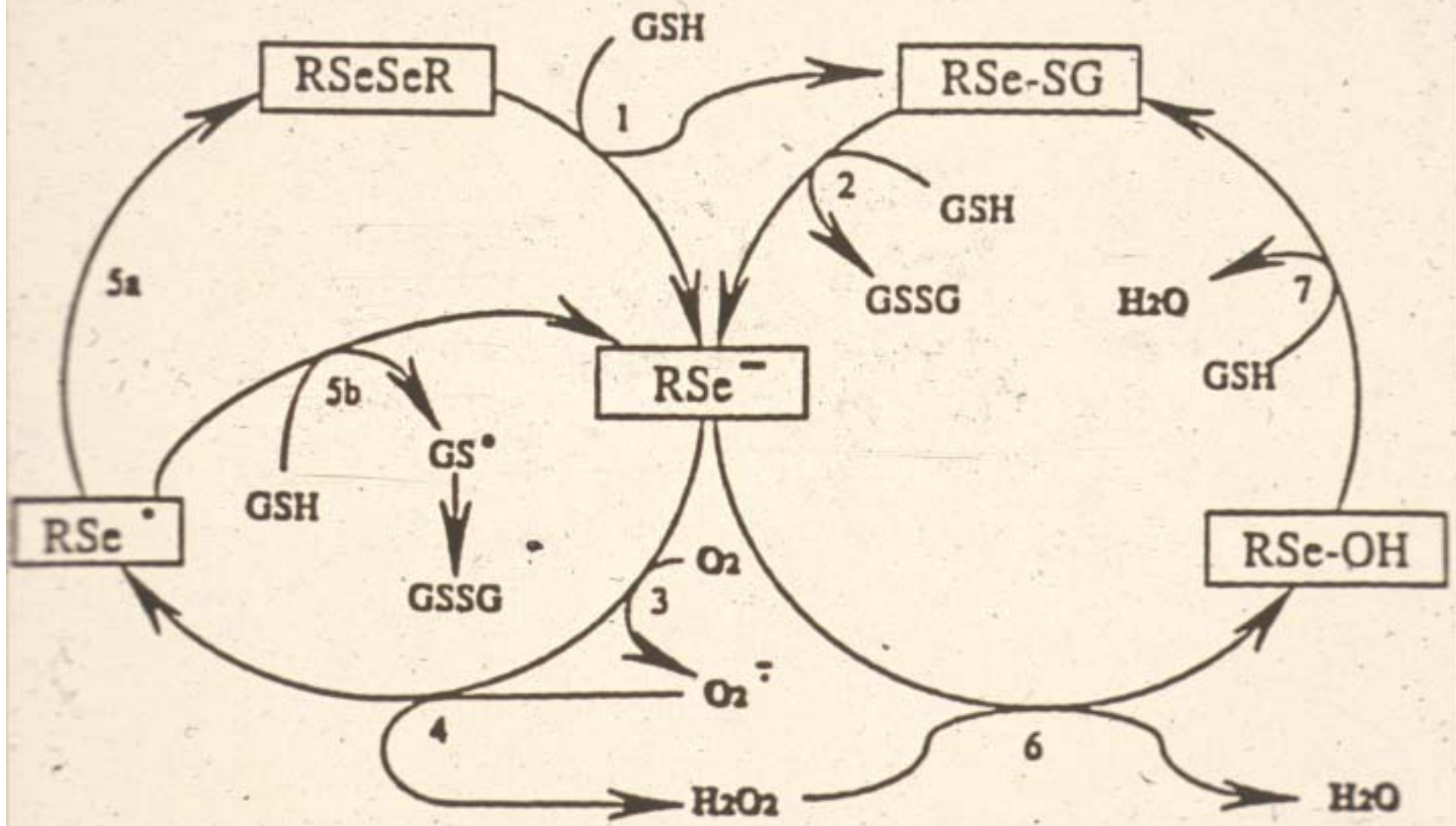
Binding Competition of Phage #8 with Different Concentration of F1 Antibody to Pure F1 Antigen Attached to Plastic Wells



- Control 1 (Anti-F1 Antibody)
- Control 2 (F1 Antibody+Anti-F1 Antibody)
- 2ug/ml F1 Ab+1011 Phage+Anti-F1 Ab
- 1ug/ml F1 Ab+1011 Phage+Anti-F1 Antibody
- .5ug/ml F1 Ab+1011 Phage+Anti-F1 Ab
- .25ug/ml F1 Ab+1011 Phage+Anti-F1 Ab
- .125ug/ml F1 Ab+1011 Phage+Anti-F1 Ab
- .0625ug/ml F1 Ab+1011 Phage+Anti-F1 Ab
- .03125ug/ml F1 Ab+1011 Phage+Anti-F1 Ab
- .0156ug/ml F1 Ab+1011 Phage+Anti-F1 Ab
- .0078ug/ml F1 Ab+1011 Phage+Anti-F1 Ab
- .0039ug/ml F1 Ab+1011 Phage+Anti-F1 Ab
- .00195ug/ml F1 Ab+1011 Phage+Anti-F1 Ab

Conclusions

- Selenium can be attached to a bacterial virus which can still target and bind to the bacteria.
- A selenium labeled virus targeted for a specific bacteria can kill the bacteria.
- The killing of the bacteria is promoted by glutathione.



QuickTime™ and a
GIF decompressor
are needed to see this picture.

