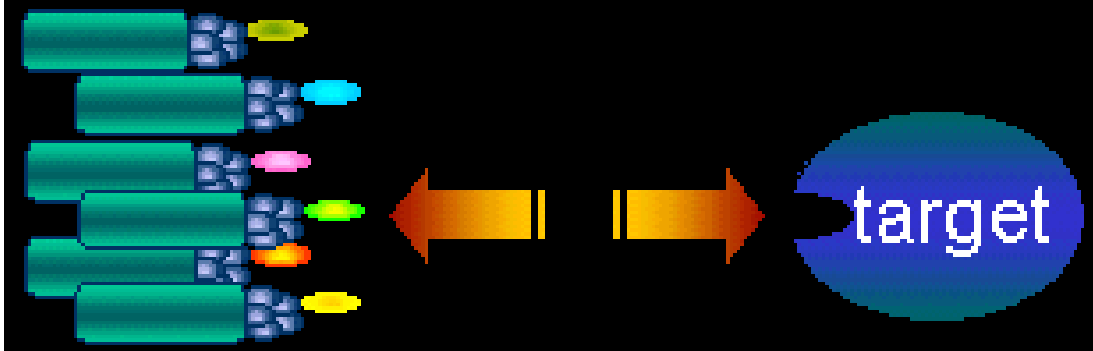
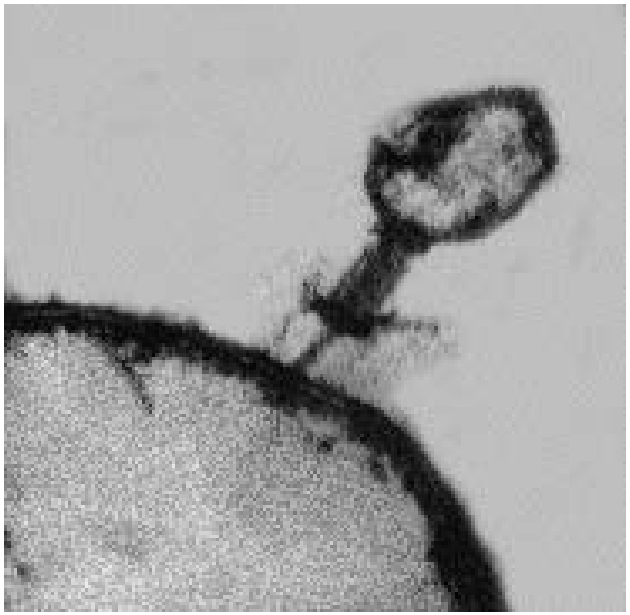
Phage Display Technology



What is phage display?

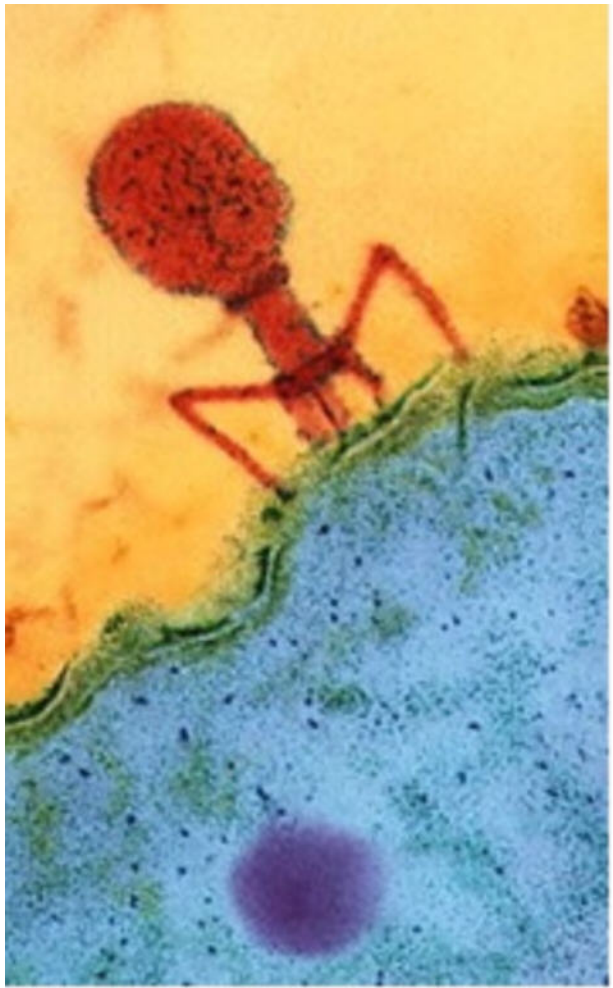
An in vitro selection technique using a peptide or protein genetically fused to the coat protein of a bacteriophage.



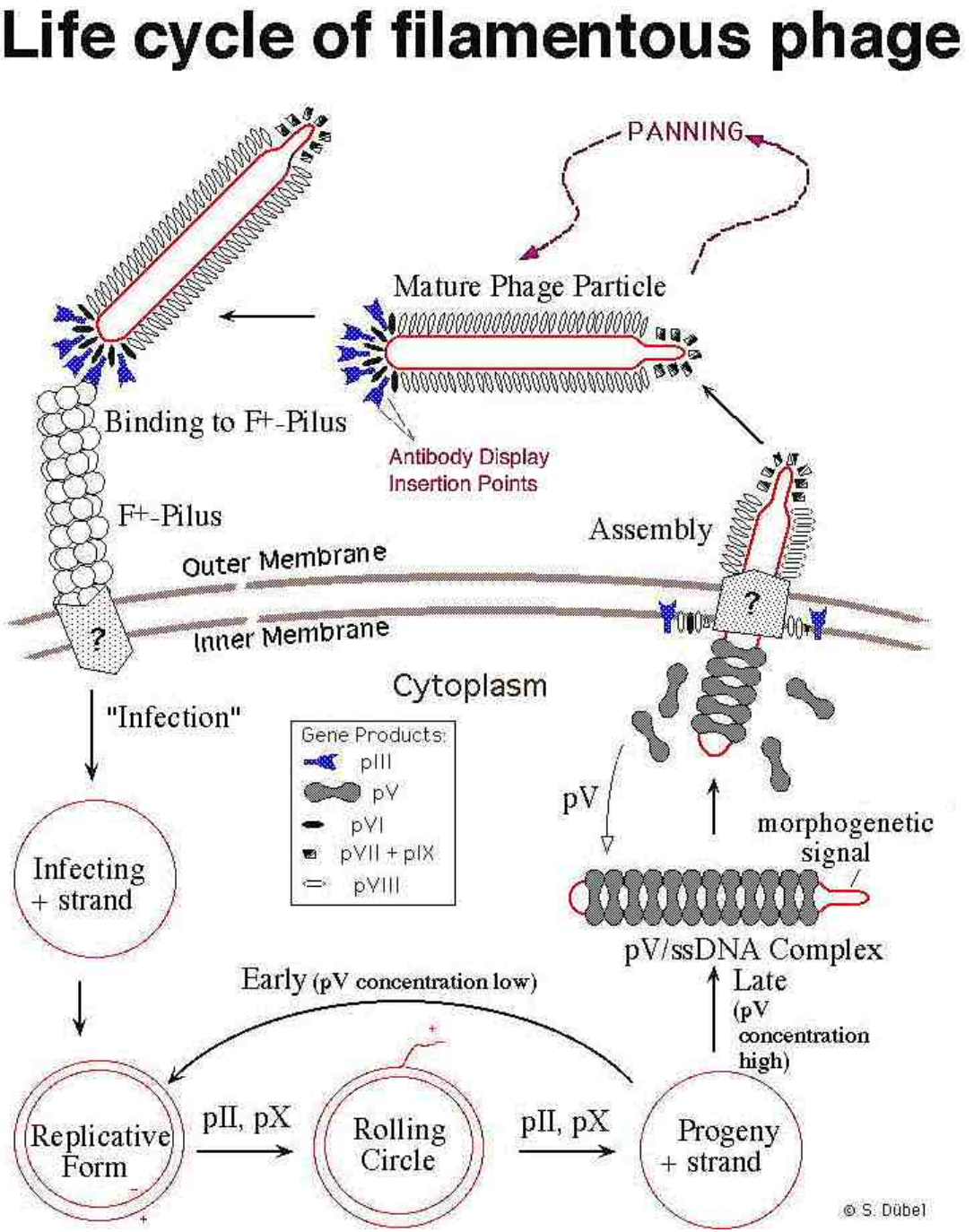
Advantages of Coat Protein Display for Selection

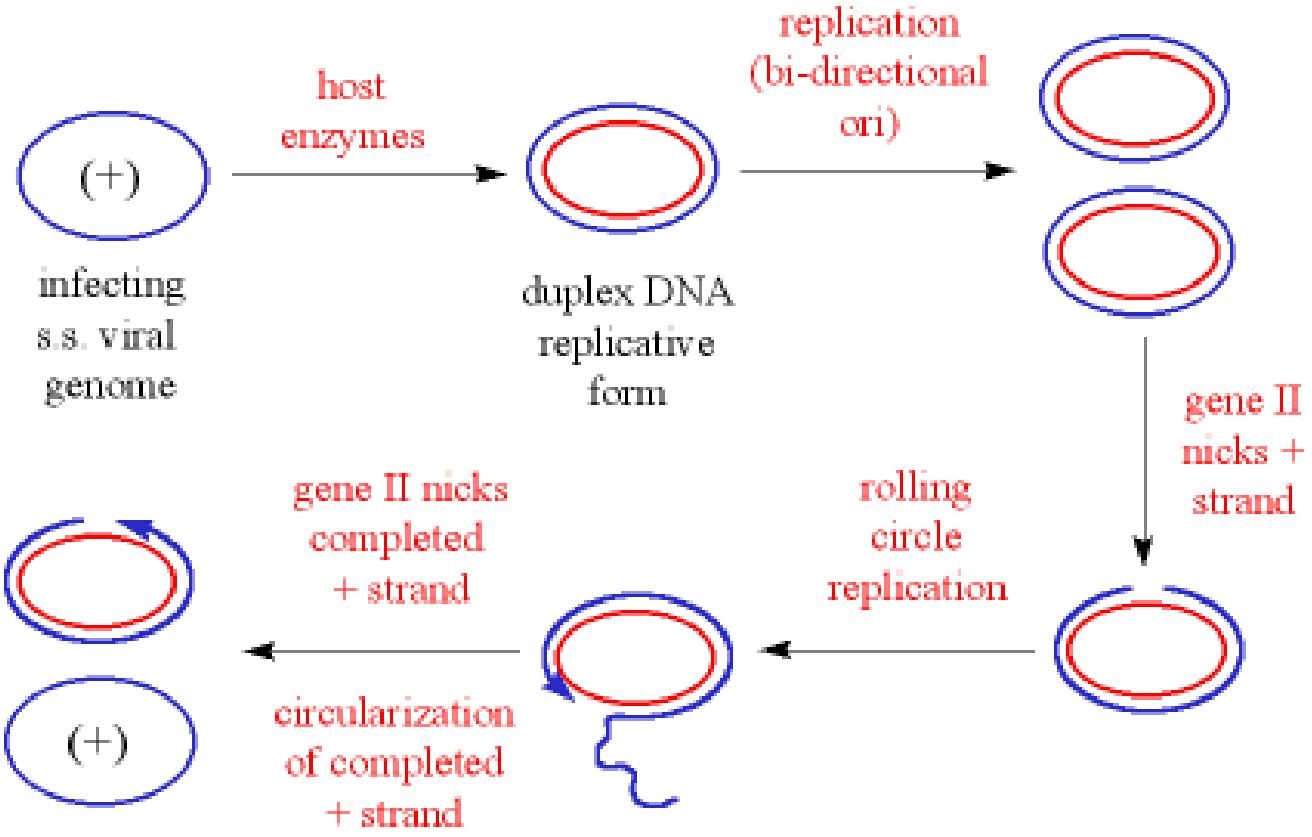
- Allows for a direct link between the DNA sequence and protein.

- Exposed to solvent so the protein can retain its affinities and functions.

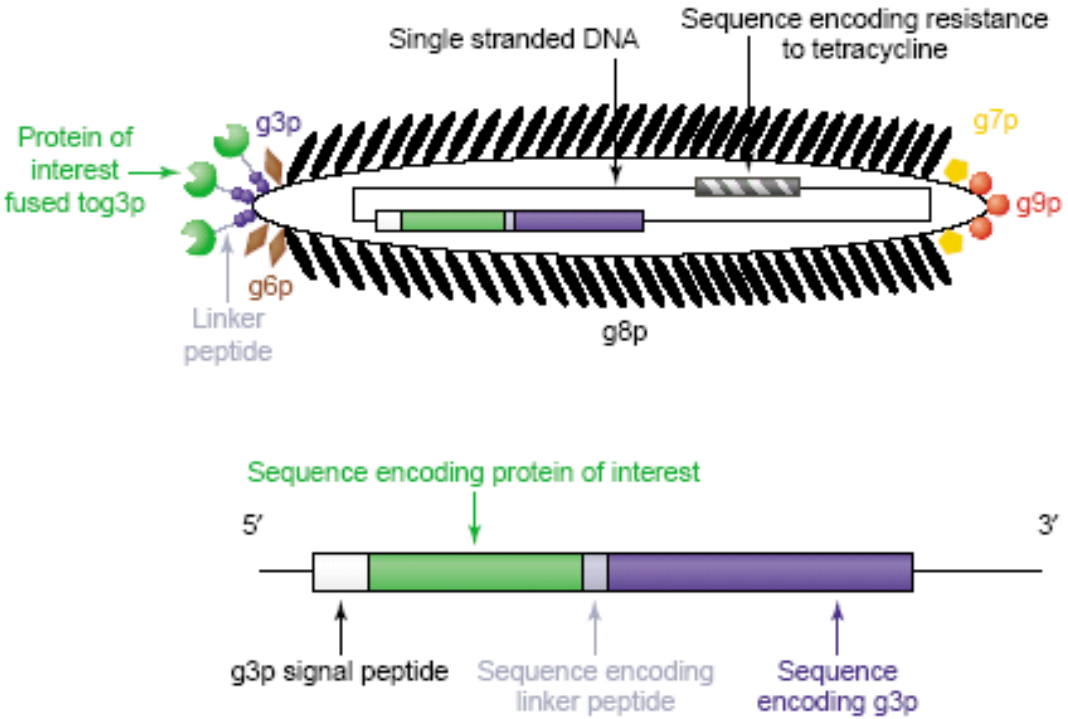


Life cycle of phage




(+)
infecting
s.s. viral
genome
host
enzymes
duplex DNA
replicative
form
replication
(bi-directional
ori)
gene II
nicks +
strand
rolling
circle
replication
gene II nicks
completed
+ strand
circularization
of completed
+ strand
(+)

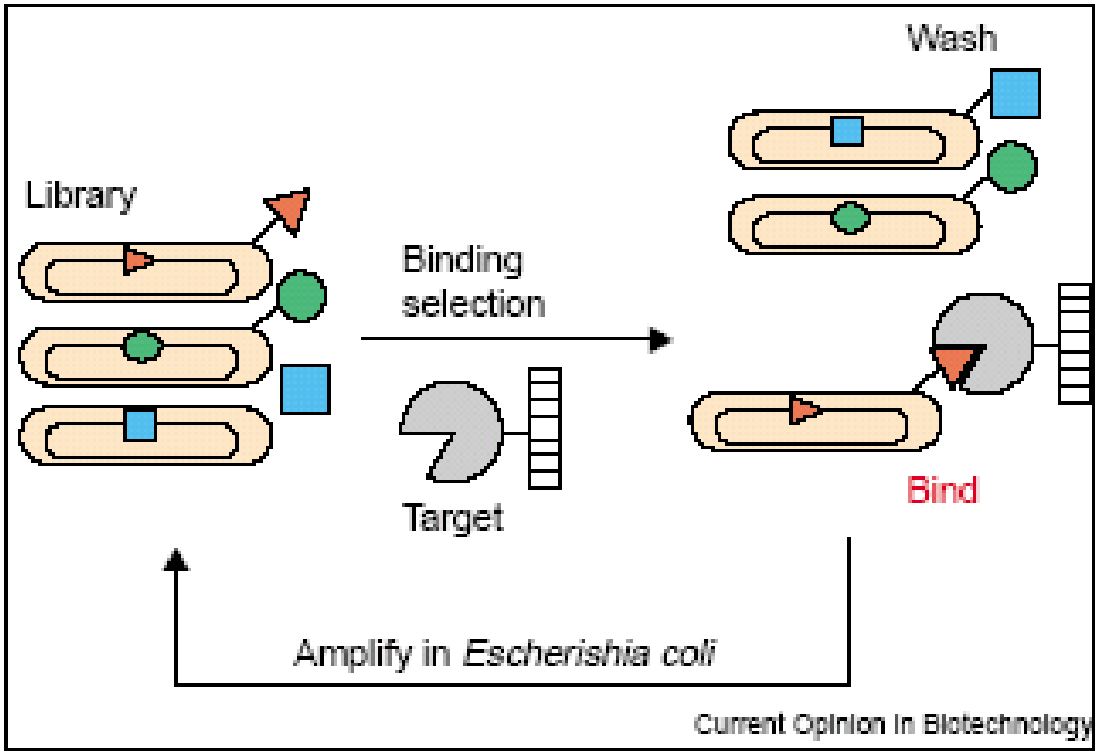
PHAGE DISPLAY



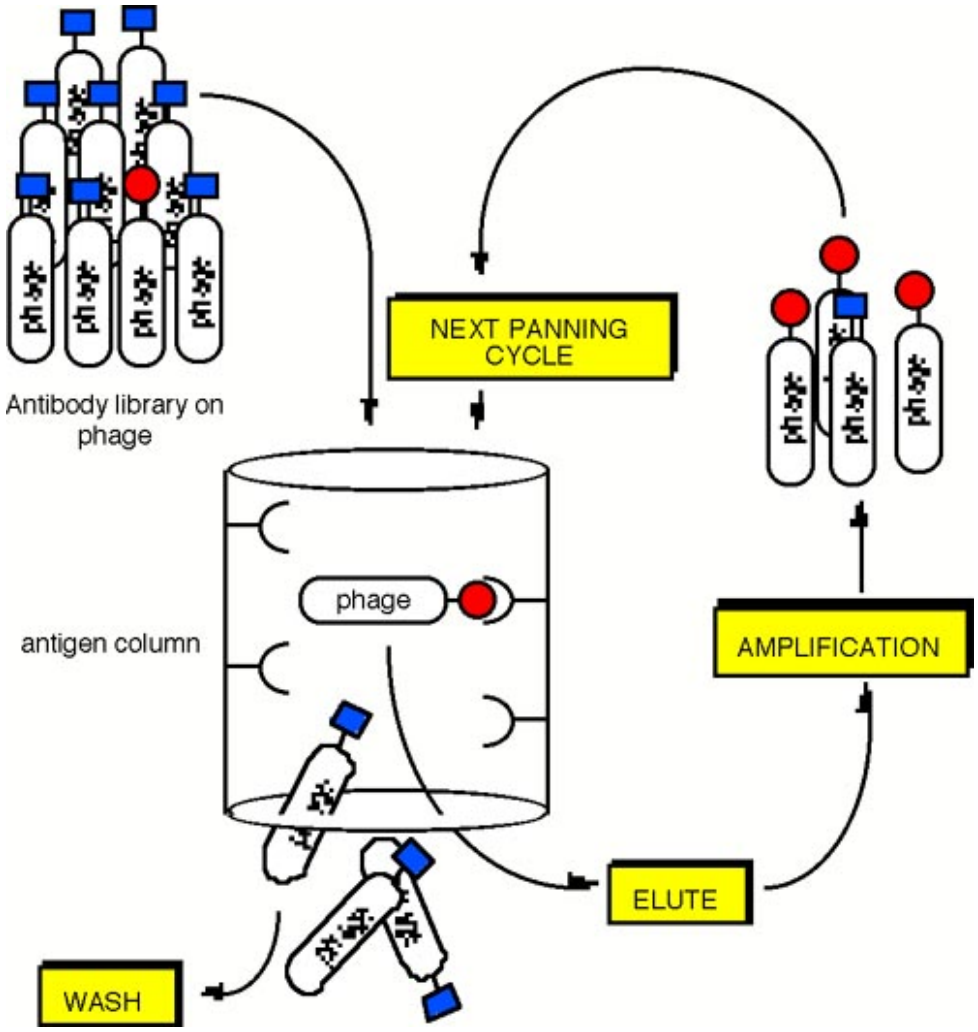
Steps Involved In Using Phage Display

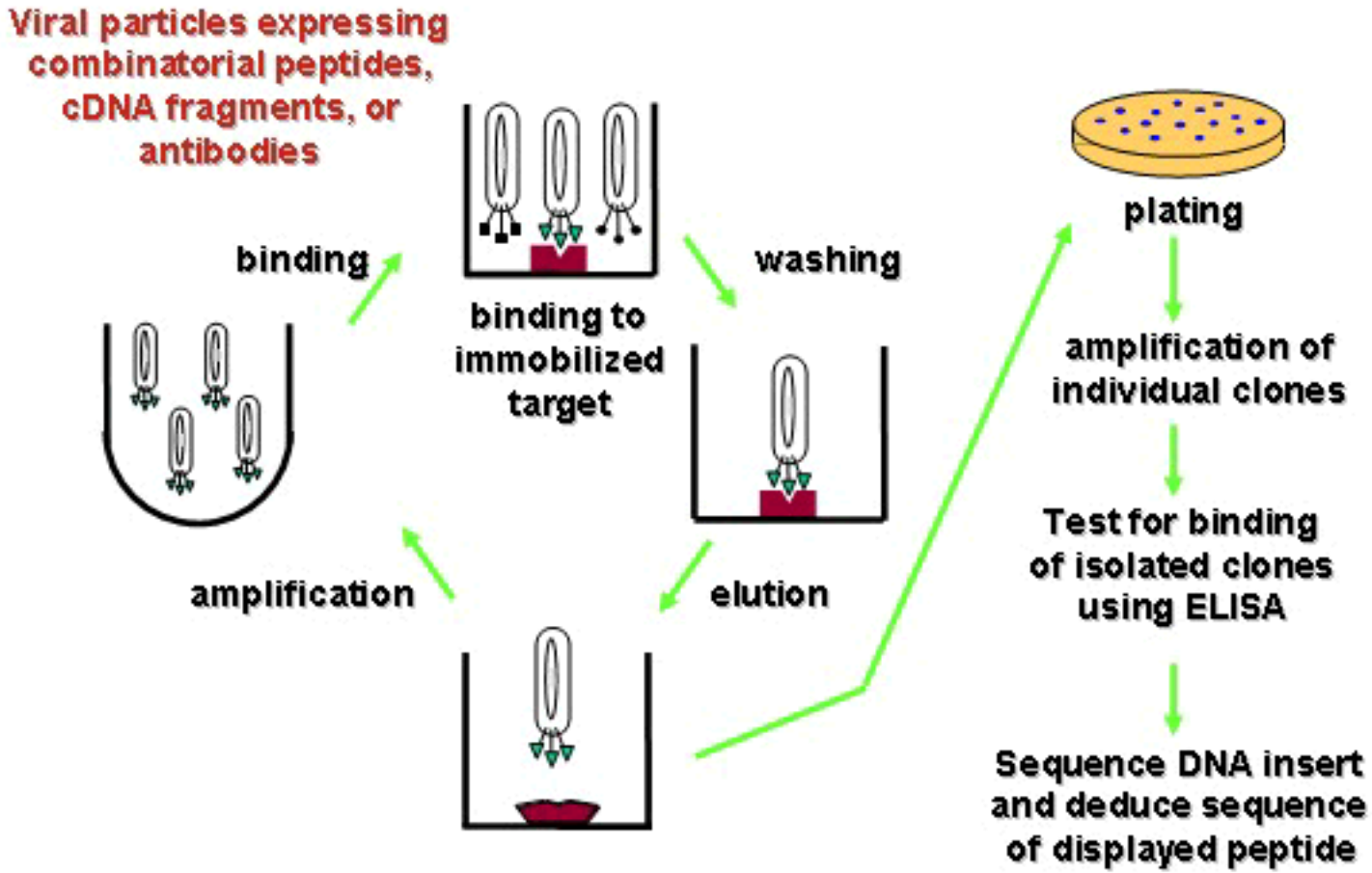
- Creation of vector
- Binding/Selection
- Wash
- Elution
- Amplification

Figure 1



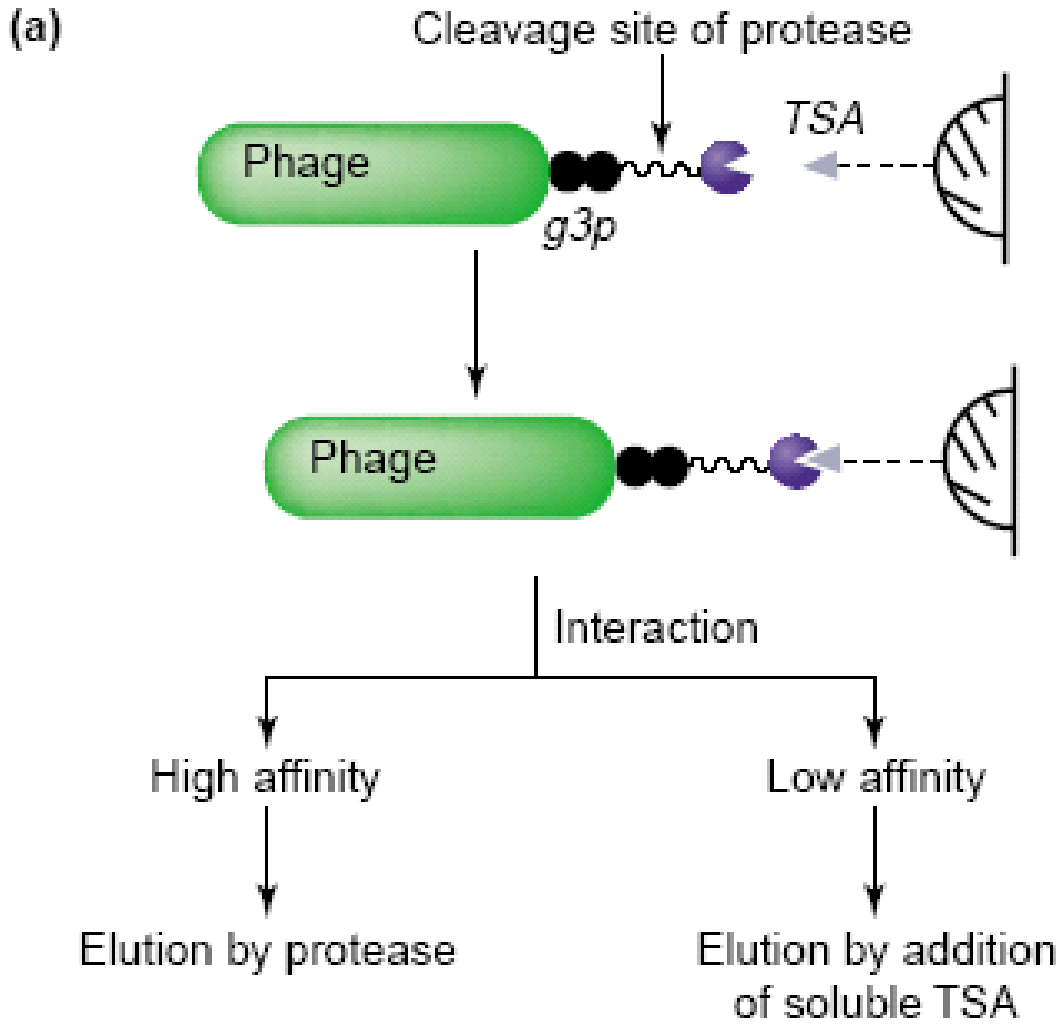
In vitro selection with phage display. Proteins are displayed on the surfaces of phage particles (orange ovals) that also encapsulate the encoding DNA. The phage-displayed library is exposed to an immobilized target; non-binding phage are removed by washing while bound phage are retained. Bound phage are then eluted and amplified by infection of an *Escherichia coli* host. Pools of amplified phage can be used in additional rounds of selection to further enrich for binding clones or, alternatively, the sequences of displayed proteins can be determined by sequencing the encapsulated DNA. Other *in vitro* display methods follow similar principals of binding selections followed by clonal amplification.

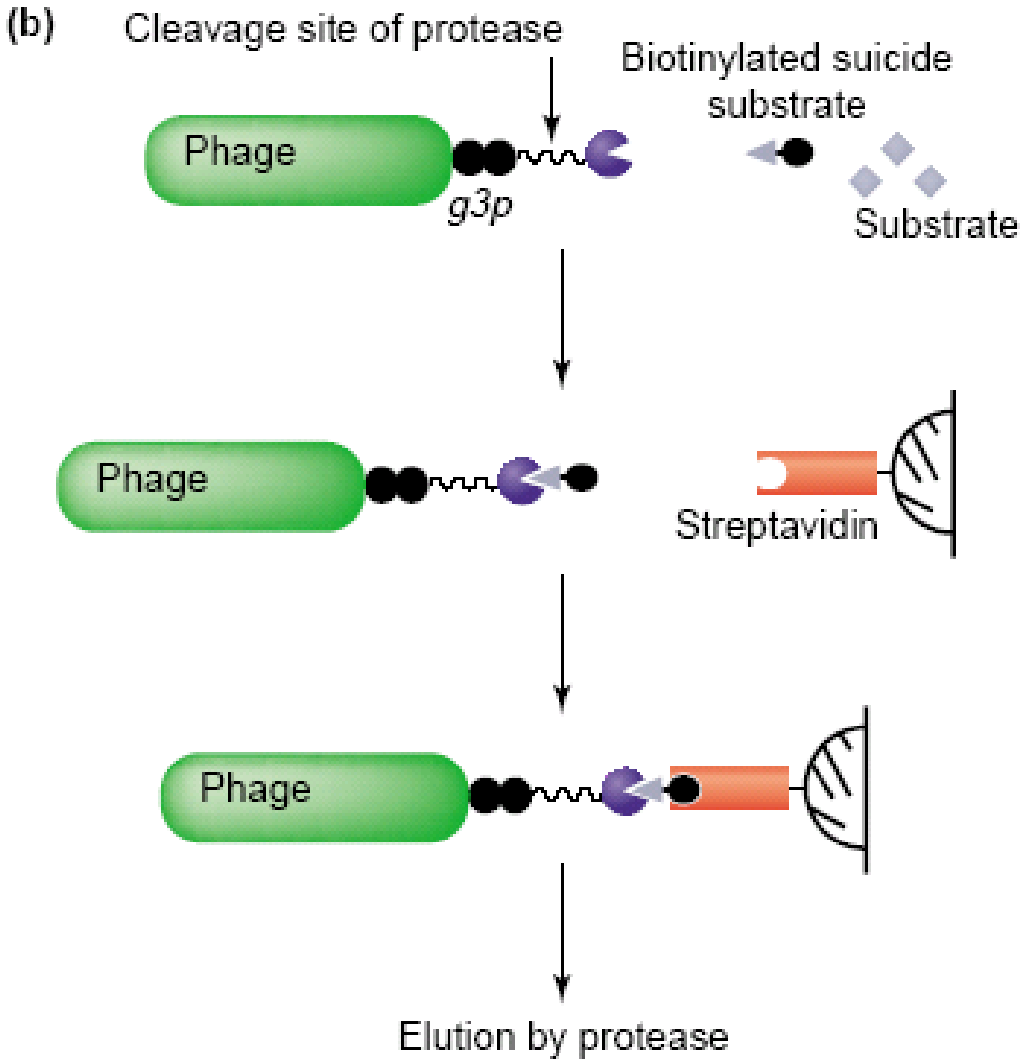

Antibody library on phage
NEXT PANNING CYCLE
phage
antigen column
AMPLIFICATION
ELUTE
WASH


Viral particles expressing combinatorial peptides, cDNA fragments, or antibodies
binding
binding to immobilized target
washing
elution
amplification
plating
amplification of individual clones
Test for binding of isolated clones using ELISA
Sequence DNA insert and deduce sequence of displayed peptide

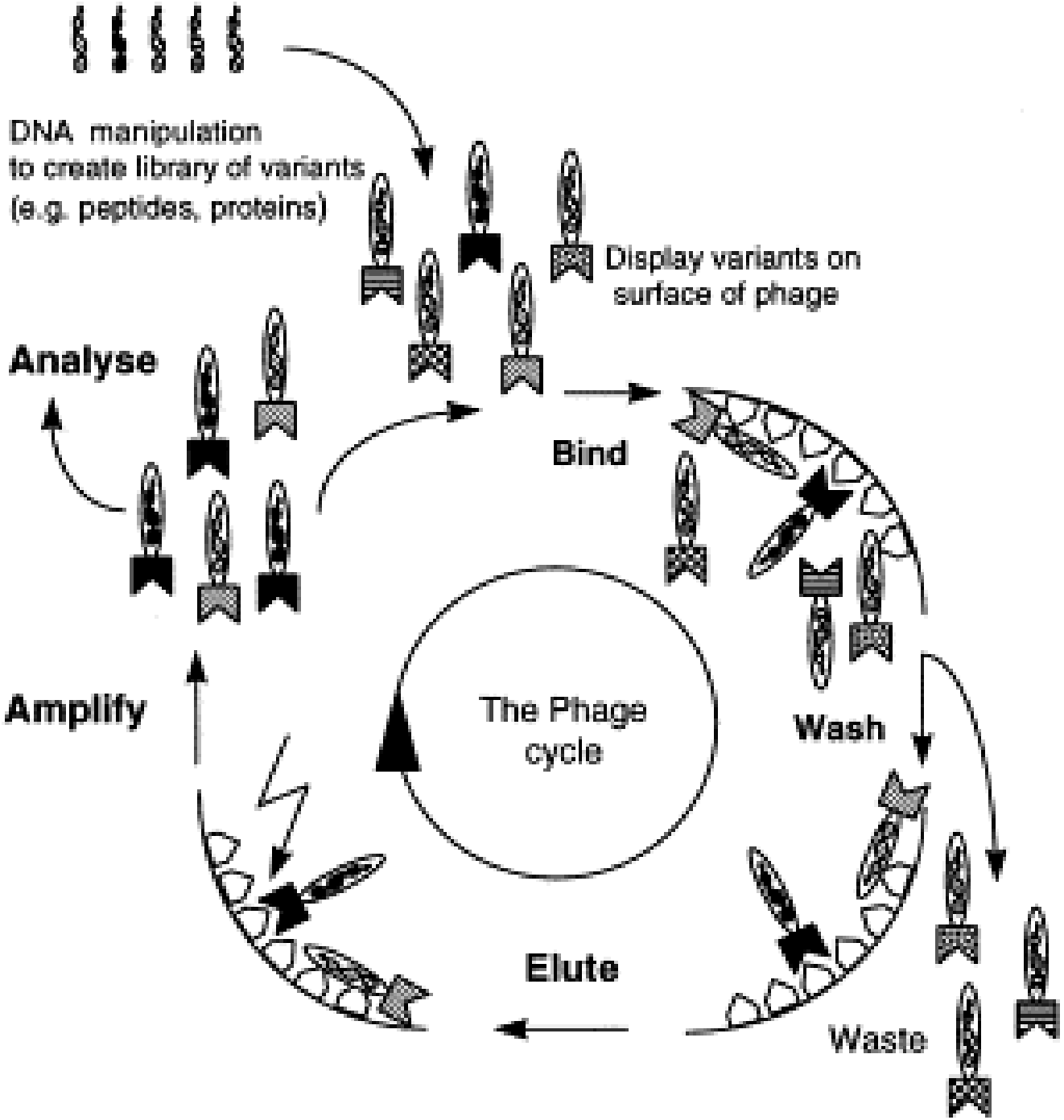
However, selection of interesting mutants based on catalytic activity is far more demanding than selection of protein variants based on affinity for a specific target

Selection based on binding to substrate product or transition state analogous (TSAs)



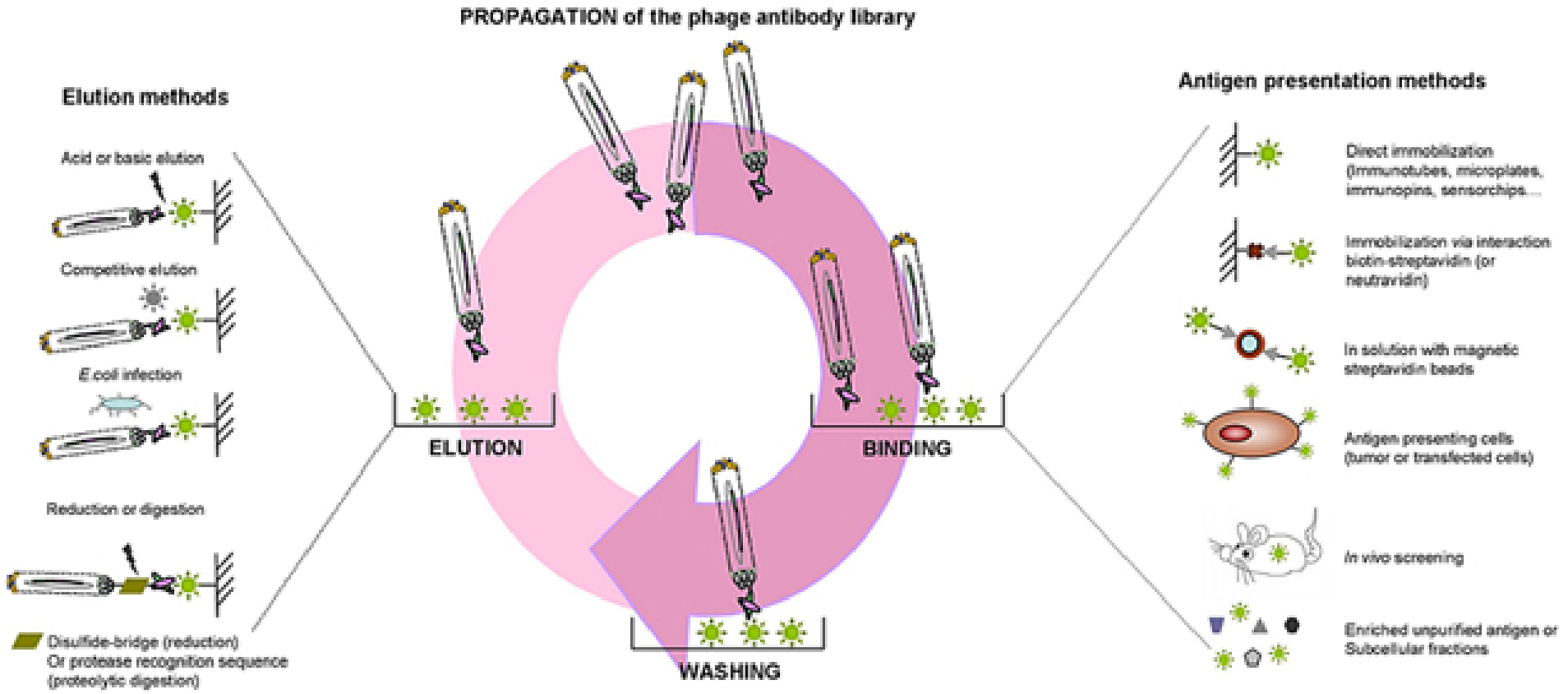

(b)
Cleavage site of protease
Biotinylated suicide substrate
Phage
g3p
Substrate
Phage
Streptavidin
Phage
Elution by protease

Phage Display cycle



Phage Display Overview:

Propagate, Bind, Wash, Elute, Repeat



Applications

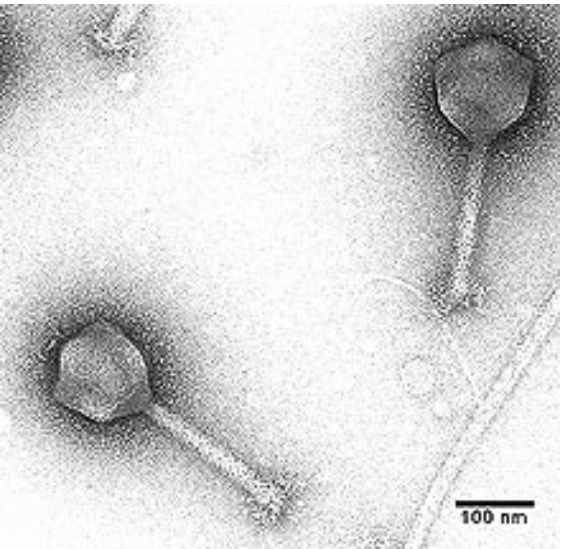
- Epitope mapping and mimicking
- Identification of new receptors & ligands
- Drug discovery
- Epitope discovery – new vaccines
- Creation of antibody libraries
- Organ targeting

Epitope mapping and mimicking

- Use random libraries to determine if it is continuous
- Compare phage sequence motif to amino acid sequence of natural ligands
- Map critical binding sites of epitope/ligands

Identification of new receptor ligands

- Can identify new receptors that bind the same ligand.
- Can use to study signal proteins and pathways – link
- Match receptor with unknown ligand



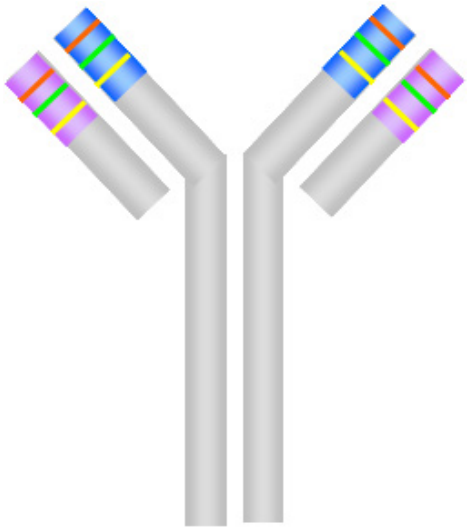
http://www.apsnet.org/online/feature/phages/image/phage4sm.jpg

Drug Discovery

- Test receptors as targets of drugs
- Peptides can act as antagonists, agonists, or modulators
- Large scale search but might not have good pharmacological properties

Epitope Discovery

- Use antibodies as a receptor to select peptide that is an antigen mimic.
- Use mimic to immunize or elicit antibody increase (immunogenic mimic)
- Can bypass animal immunization by mimicking immune selection.



Organ Targeting

- Help Identification of endothelial cell selective markers that target cells to help get drugs to selected tissue.
- Inject phage into mouse then extract phages from different organs.
- Identify common motifs possibly involved with localization.

Advantages of Phage Display

- Easy to screen large # of clones $>10^9$
- Easy to amplify selected phages in E. coli
- Selection process easy and already in use in various forms.
- Can create Phage library variation by inducing mutations, using error prone PCR, etc.

Disadvantages

- Might not have long enough peptide insert so critical folding can be disrupted.
- Could lose phage variations if first bind/wash step too stringent.
- Affinities or binding that results during selection might not work in vivo.

Other approaches to DNA encoded libraries is provided by cell surface display in which polypeptide are displayed directly on bacterial or yeast cells.

Thus, these libraries can be used in selections analogous to those with phage display.

Using cell display strategies, library diversity is limited by the fact that library construction involves a transformation step in which recombinant DNA is introduced into host cells ($<10^{9-}$ 10^{10} members).