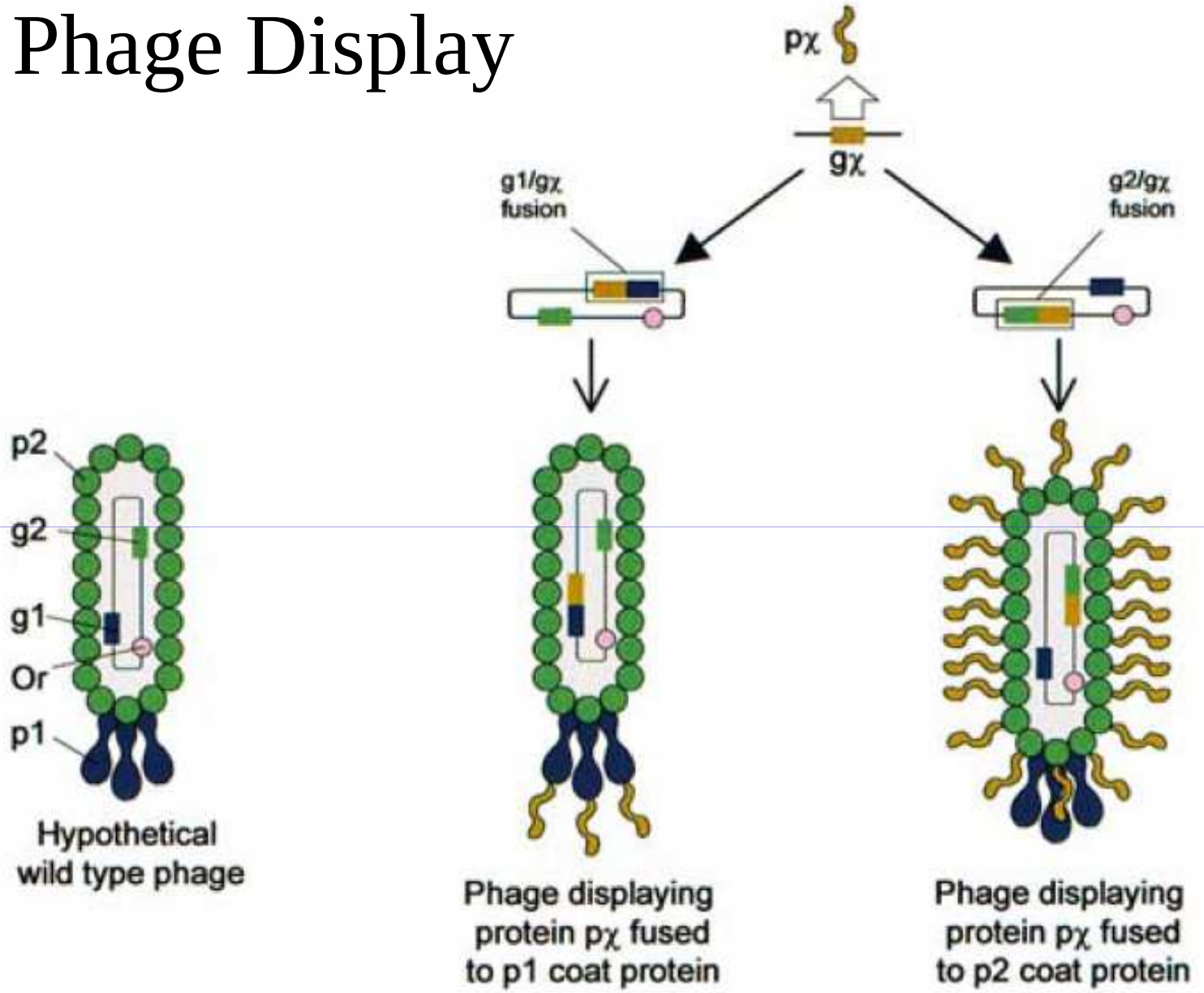
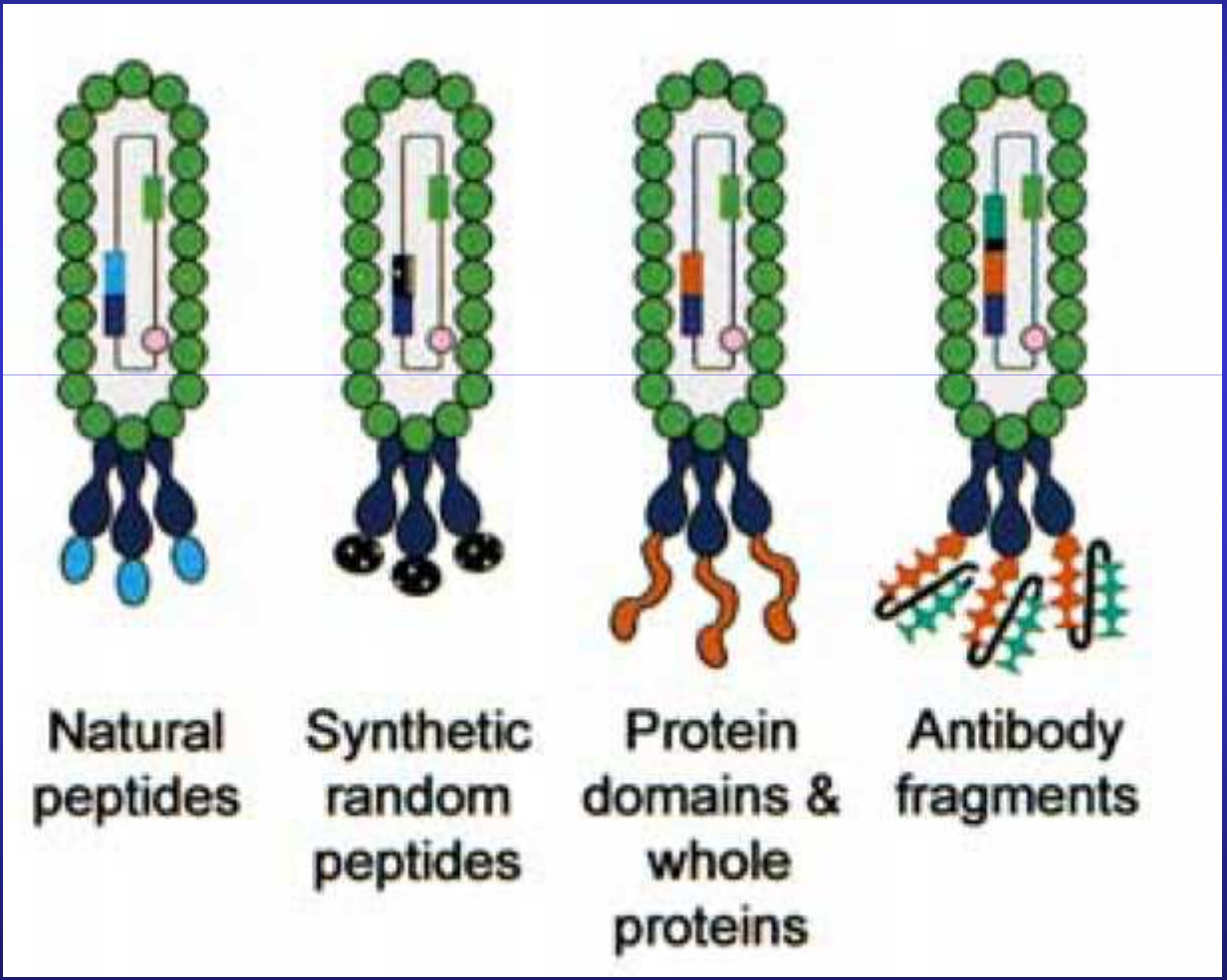
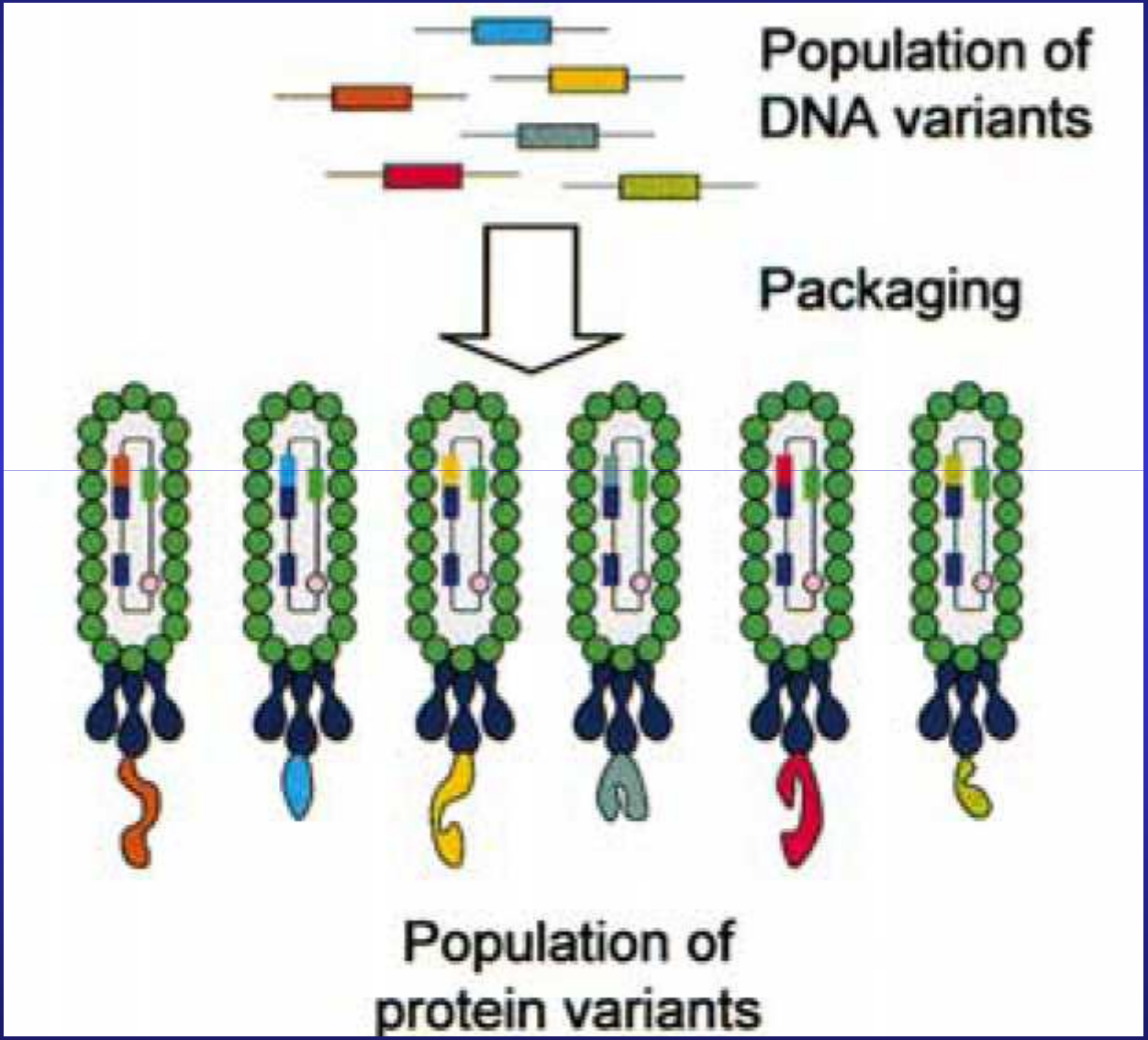
Phage Display



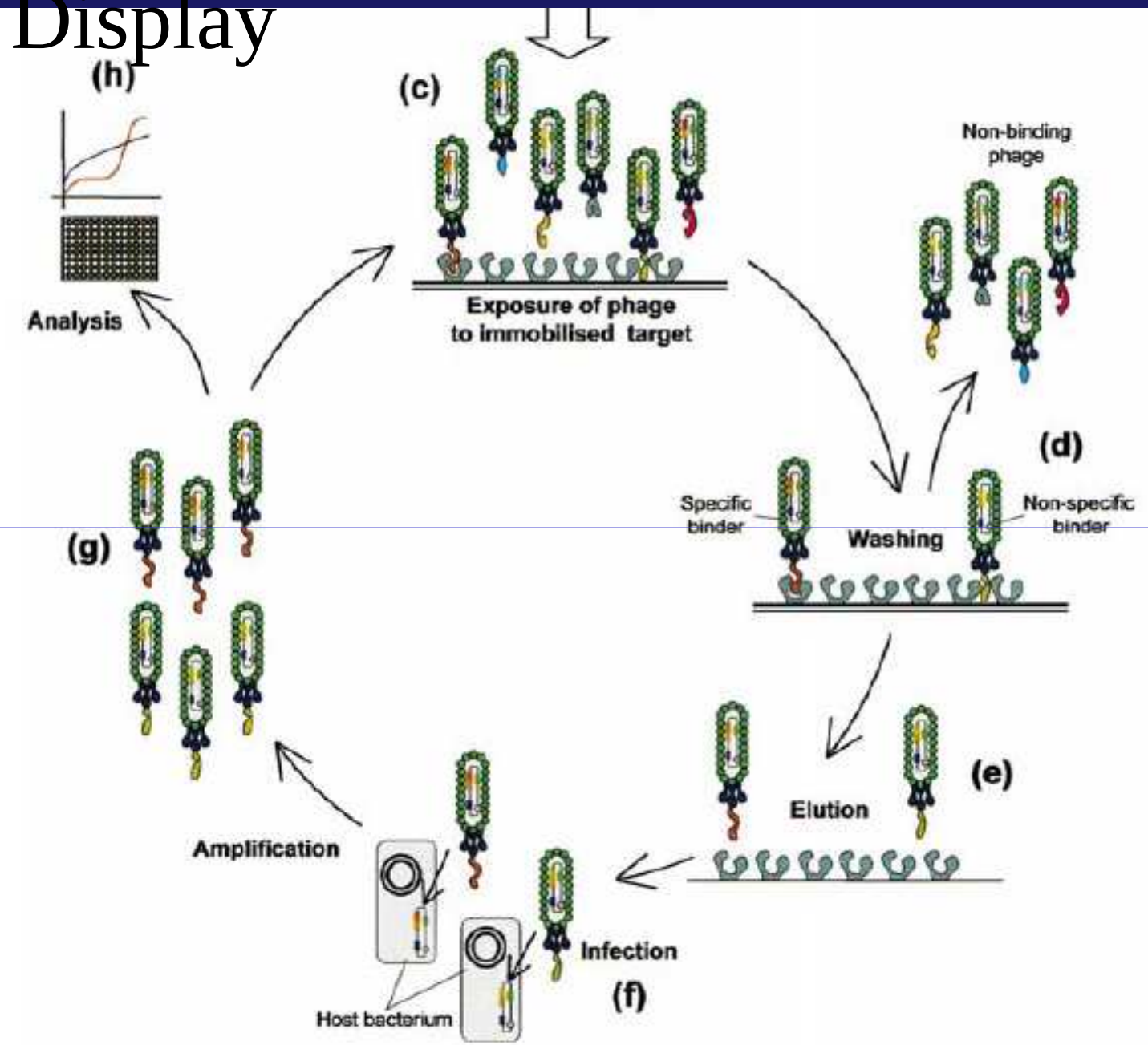
Phage Display

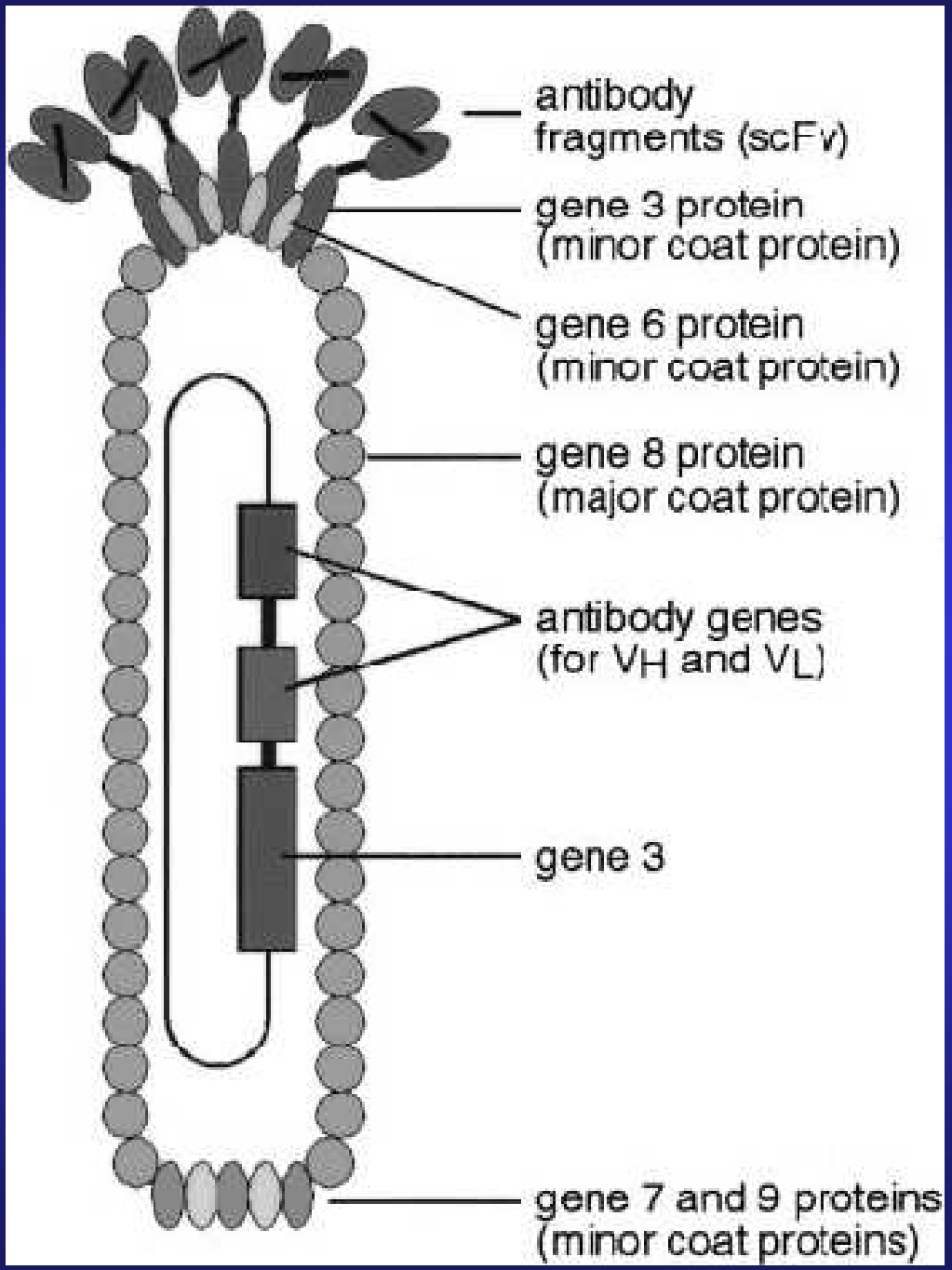


Phage Display

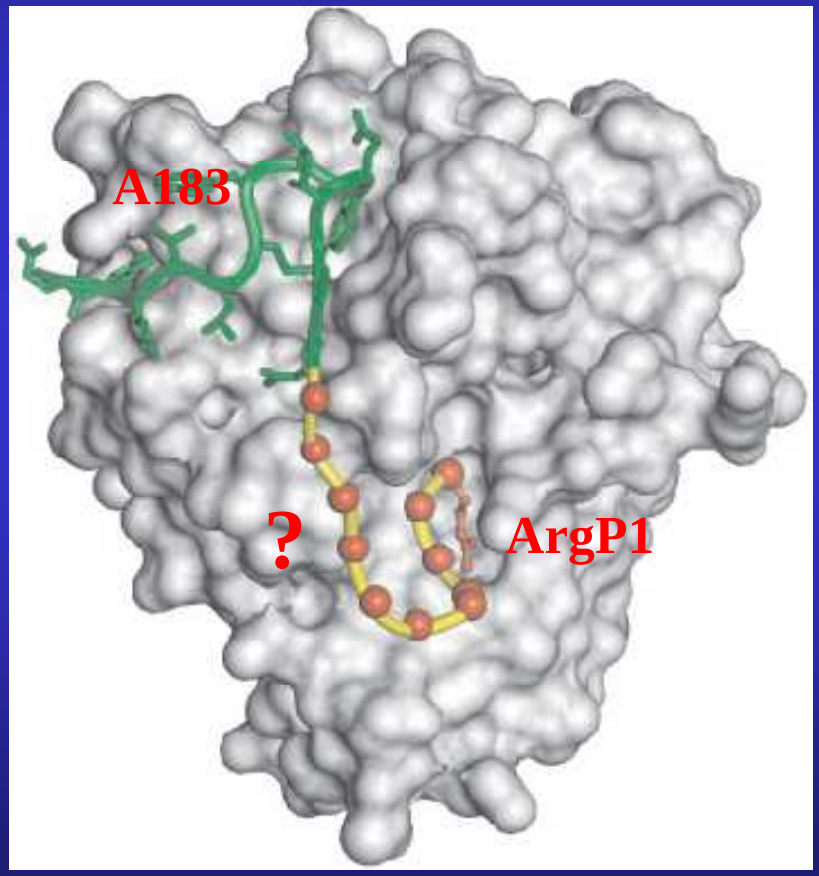


Phage Display




antibody
fragments (scFv)
gene 3 protein
(minor coat protein)
gene 6 protein
(minor coat protein)
gene 8 protein
(major coat protein)
antibody genes
(for V_H and V_L)
gene 3
gene 7 and 9 proteins
(minor coat proteins)

Model of FVIIa protease domain with A-183 extension peptide



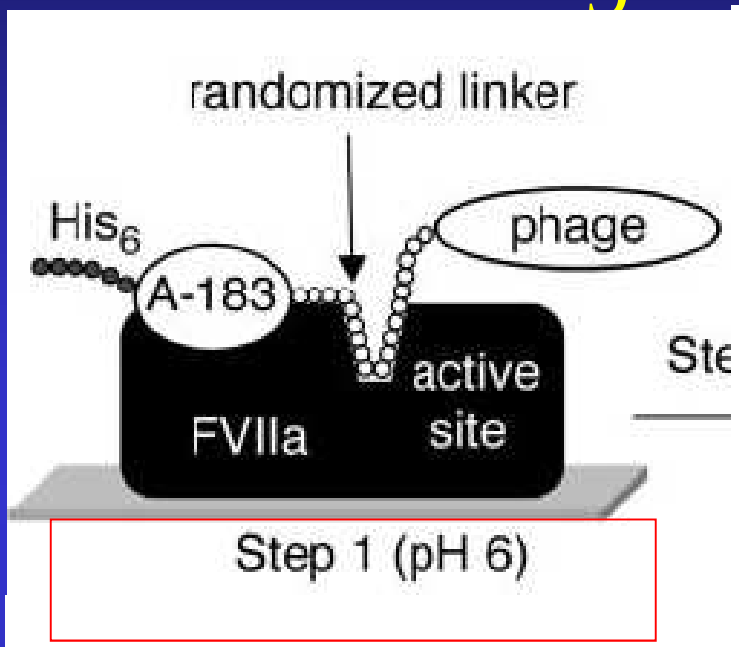
Inhibitors of Factor VIIa

?

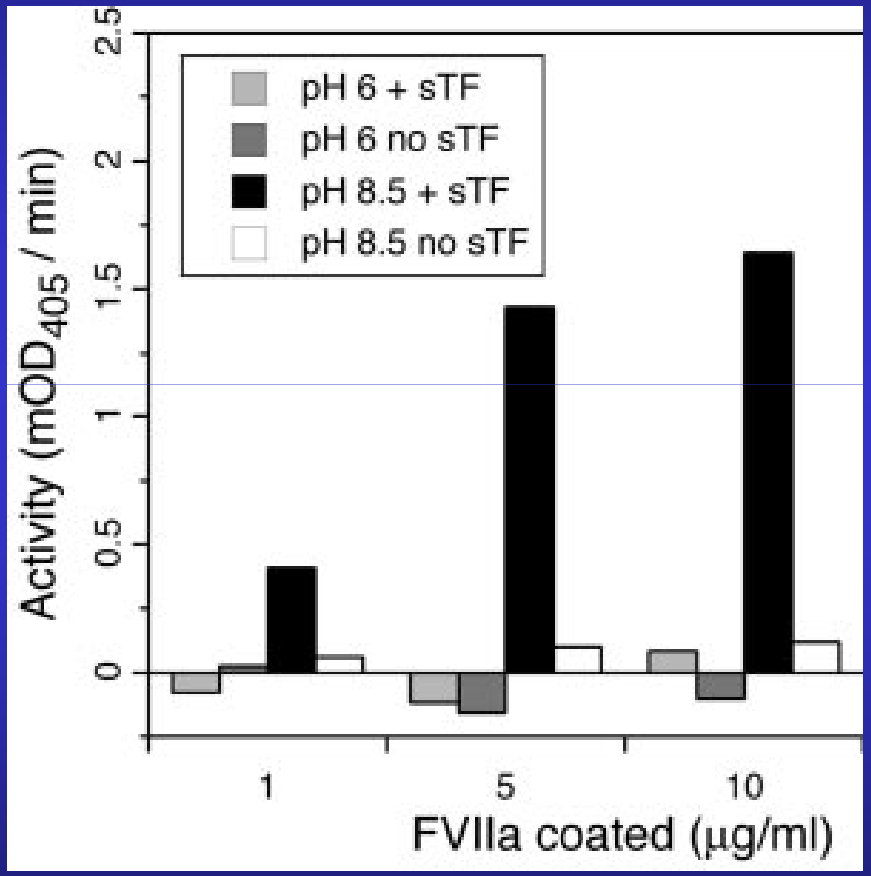
Position / Library	Anchor	Linker library positions 1	2	3	4	5	6	7	8	9	10	12	14	16	Spacer	Phage coat protein
A	A-183	X	X	X	X	X	X	X	*a*	**N**	**L**	**T**	**R**	**I**	protease resistant spacer	p3
B		X	X	X	X	X	X	X	*b*	**L**	**T**	**R**	**I**	**V**		
C		X	X	X	X	X	X	X	*c*	**T**	**R**	**I**	**V**	**G**		
D		**G**	**G**	**S**	**G**	**G**	**S**	**G**	X	X	X	X	X	X		

a= S,N,K,R; *b*= N,K; *c* = L,Q

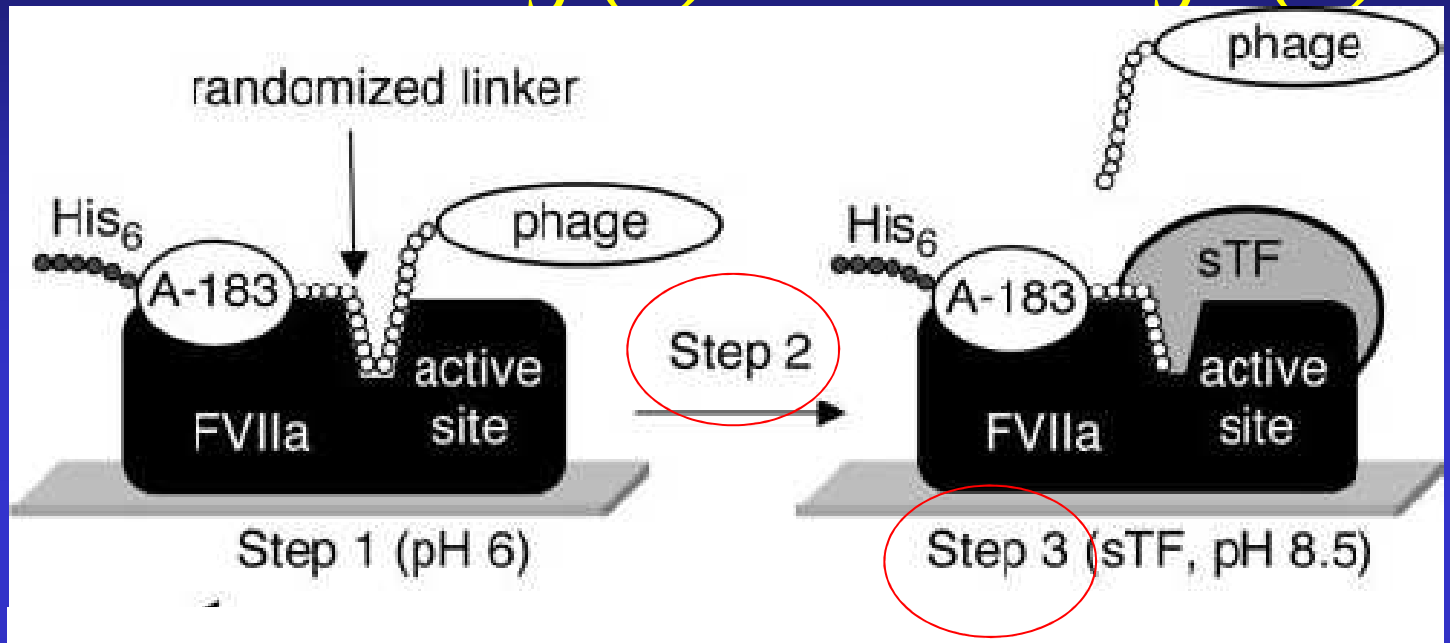
Peptide Inhibitors of Factor VIIa: Phage binding



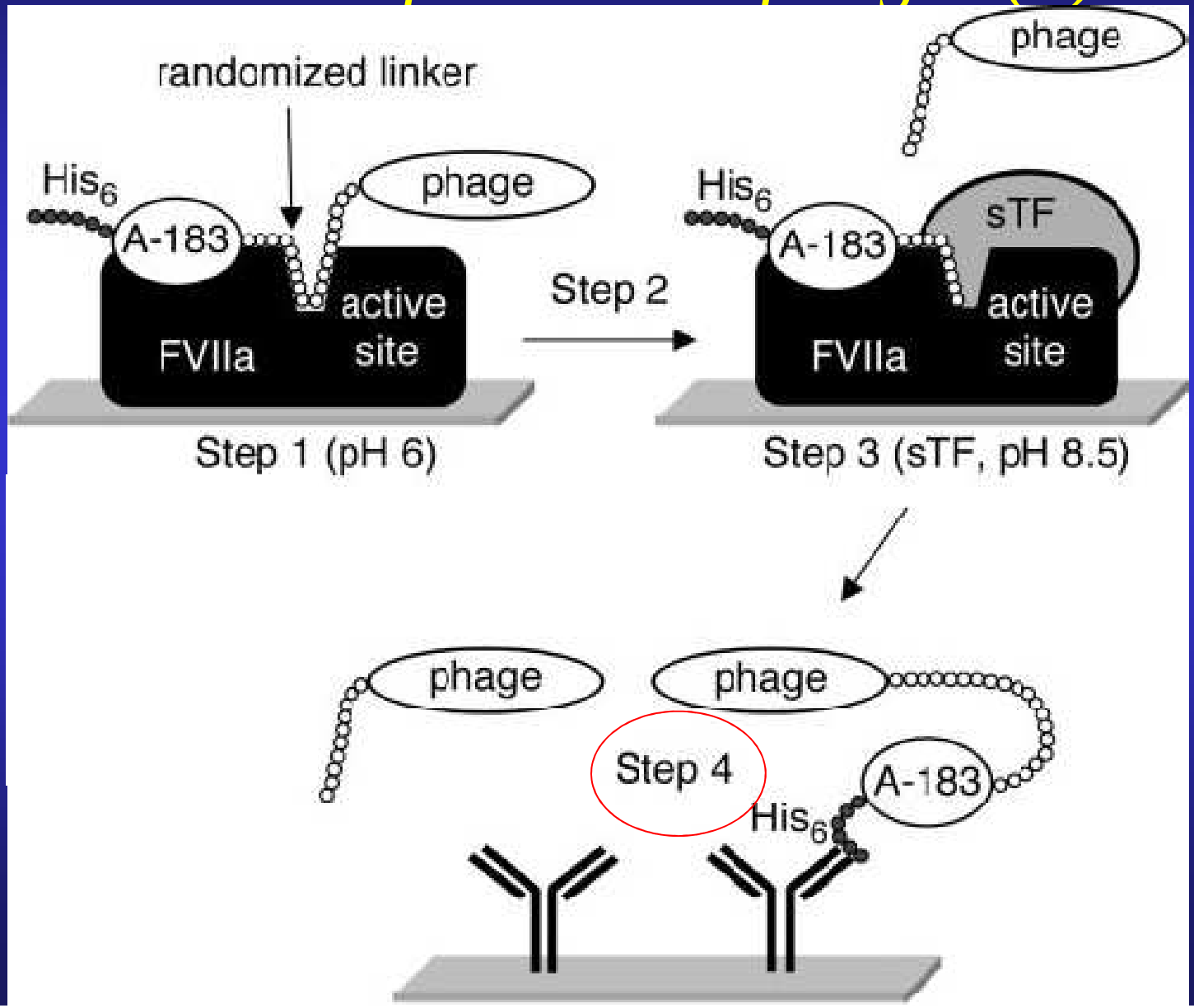
FVIIa Cleavage conditions



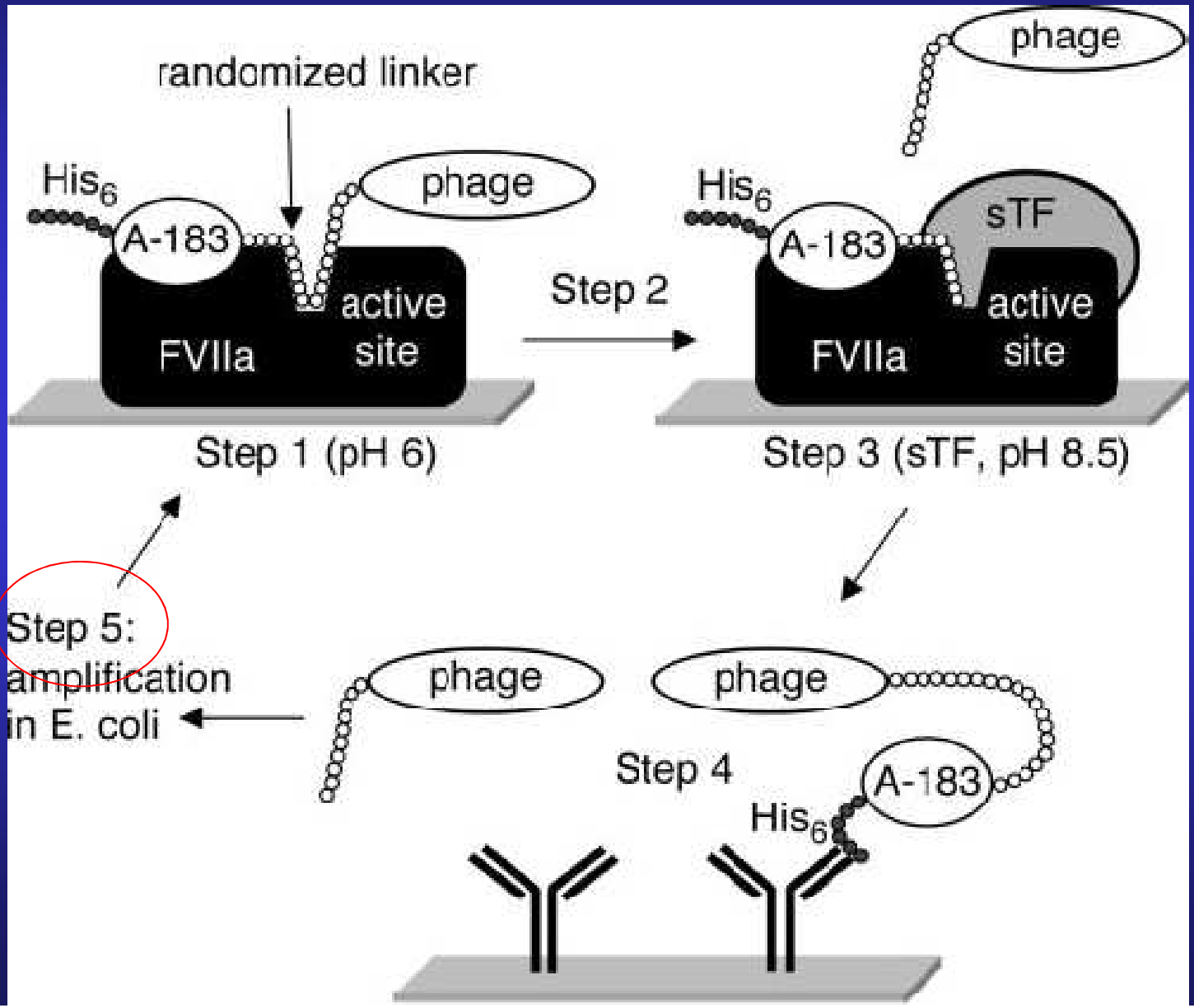
Peptide Inhibitors of Factor VIIa:washing (2) and cleavage (3)



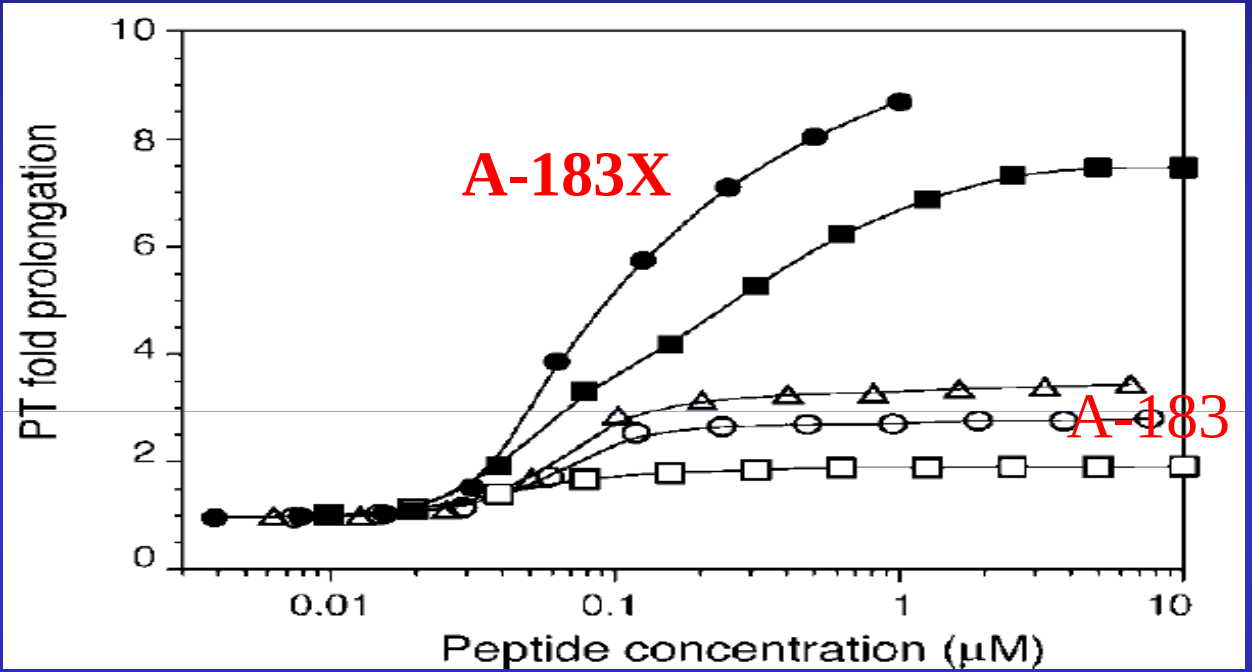
Peptide Inhibitors of Factor VIIa: removal of unbound phages (4)



Peptide Inhibitors of Factor VIIa: propagation of selected phages and new rounds (5)



Prolongation of TF-dependent clotting times



X=EEWEVLCWTWETCERGEGVEEELWEWR

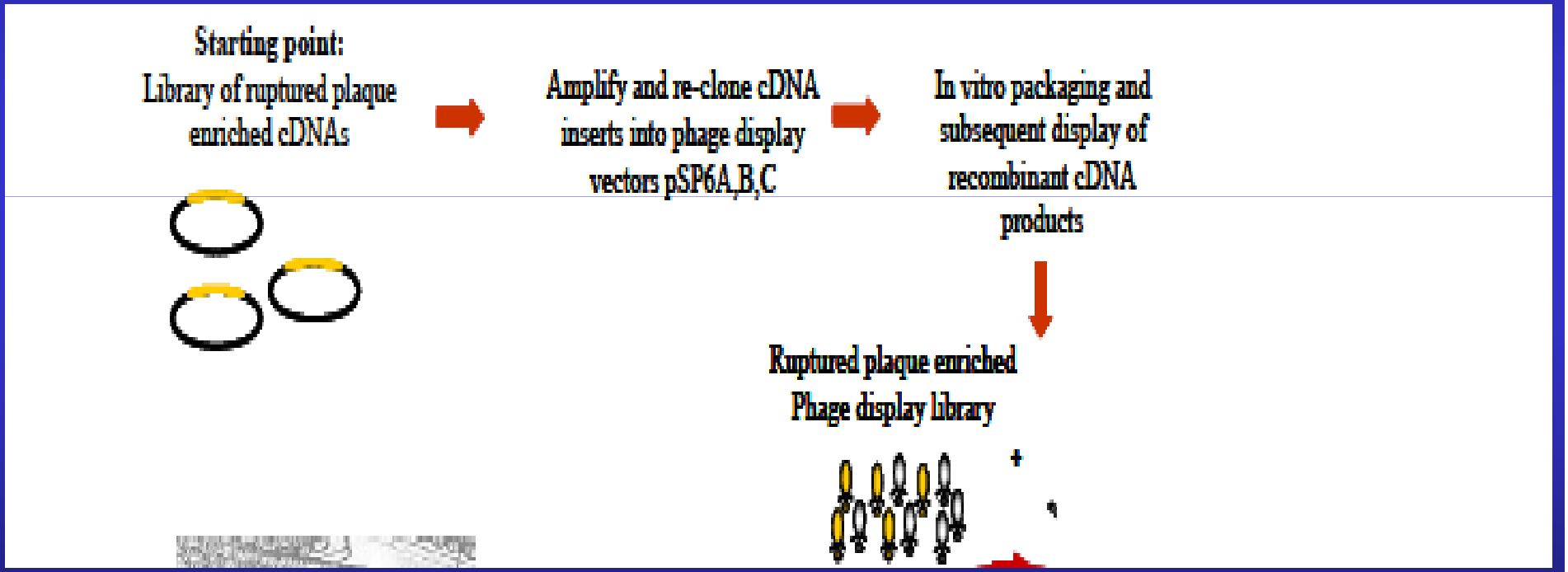
A-183X was a potent and complete inhibitor of FX activation, having a maximal extent of inhibition of 99% with an IC50 of 230 pM *versus* A-183 which maximally inhibited to 74% with an IC50 of 1.5 nM.

A-183X also had a maximal prolongation of the prothrombin time of 7.6- *versus* 1.9- fold for A-183, making it a more effective anticoagulant

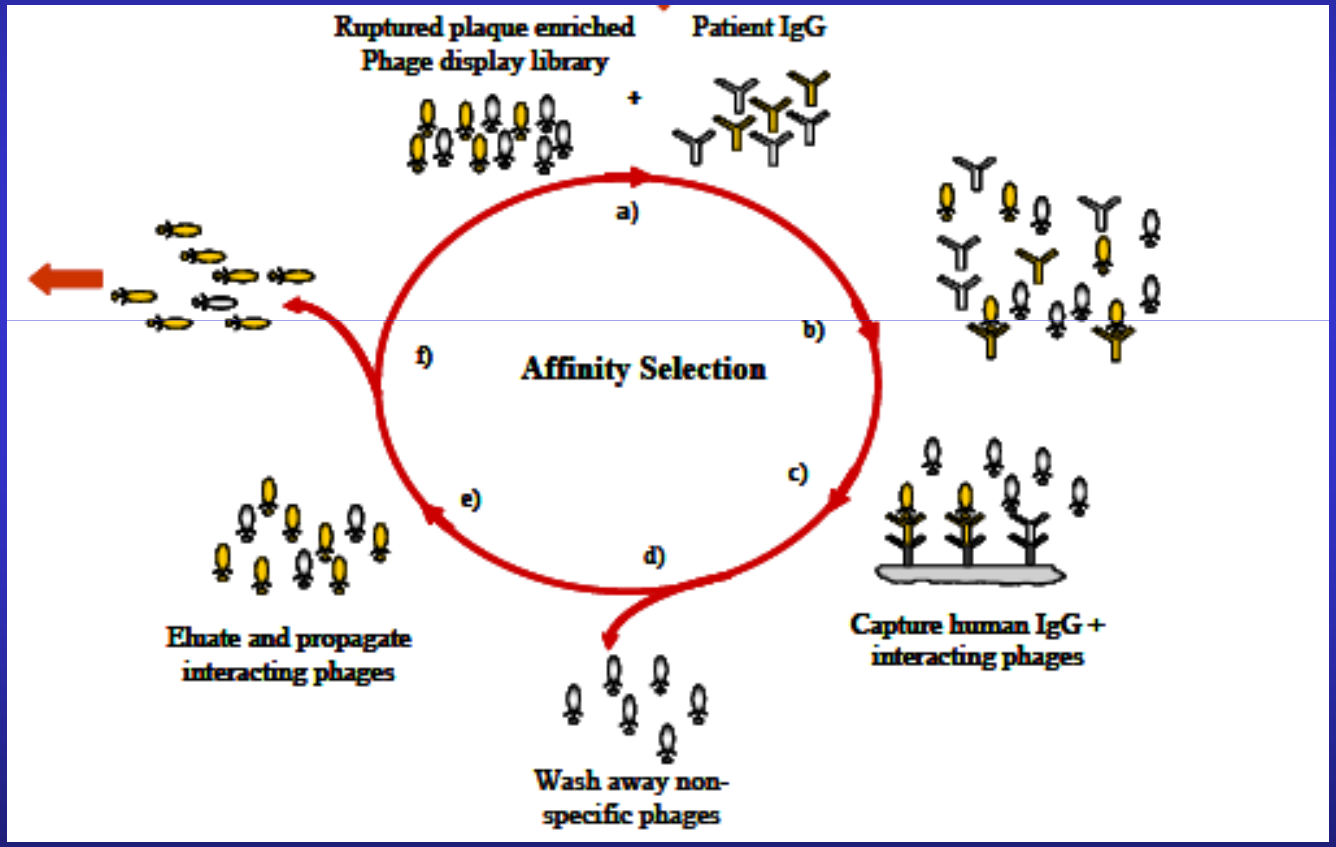
Noninvasive diagnosis of ruptured peripheral atherosclerotic lesions and myocardial infarction by antibody profiling

- Novel biomarkers, such as circulating (auto)antibody, may improve early detection and treatment of ruptured atherosclerotic lesions and accompanying cardiovascular events, such as myocardial infarction.
- Using a phage-display library derived from cDNAs preferentially expressed in ruptured peripheral human atherosclerotic plaques, we performed serological antigen selection to isolate displayed cDNA products specifically interacting with antibodies in sera from patients with proven ruptured peripheral atherosclerotic lesions.

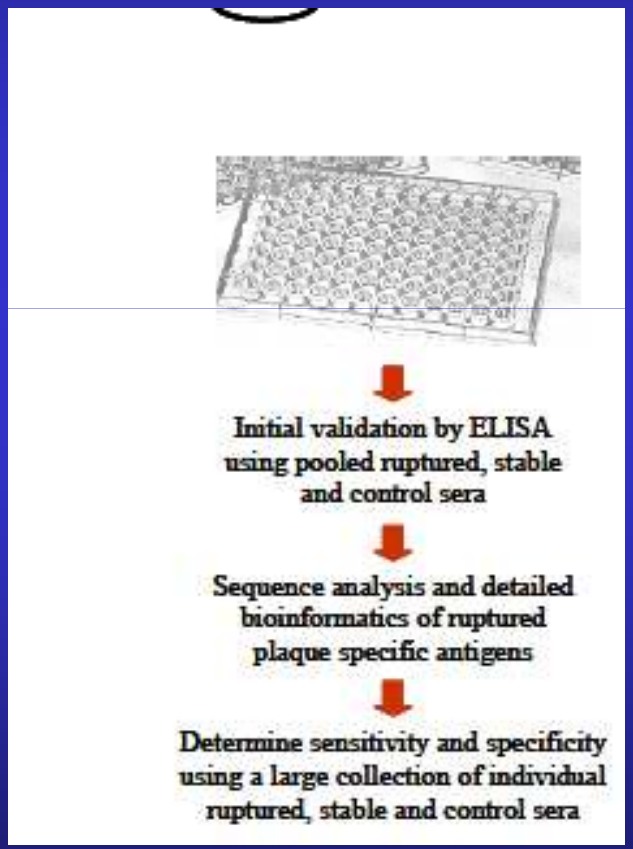
identification of ruptured plaque specific antigens



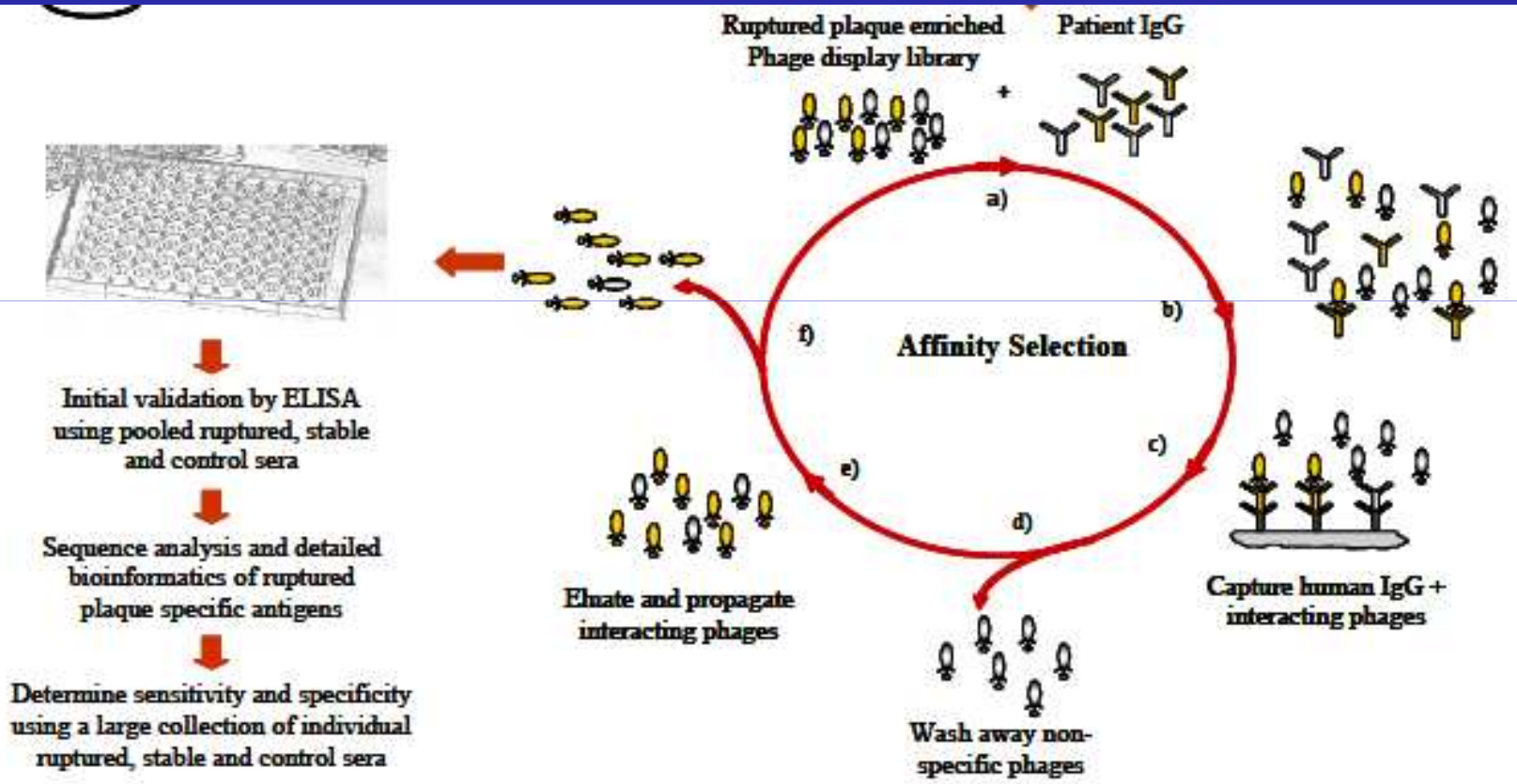
identification of ruptured plaque specific antigens



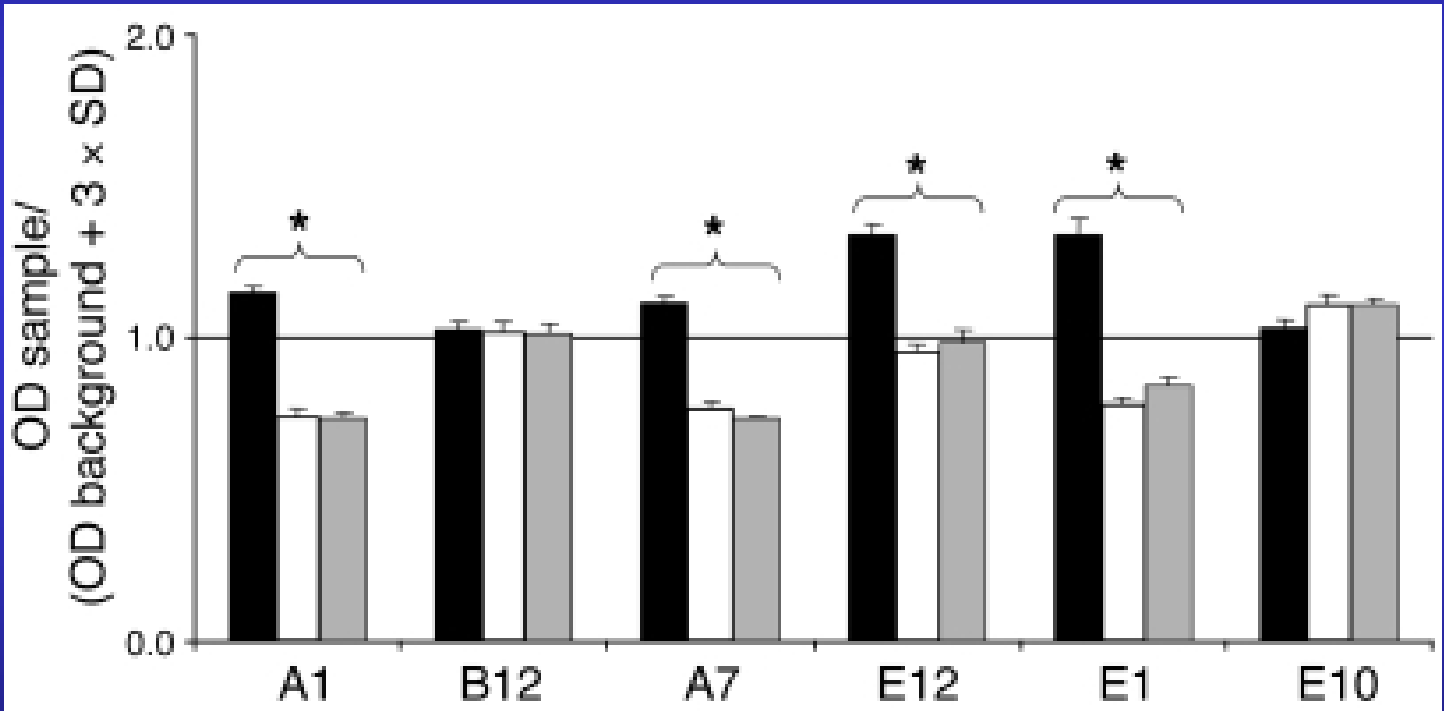
identification of ruptured plaque specific antigens



identification of ruptured plaque specific antigens



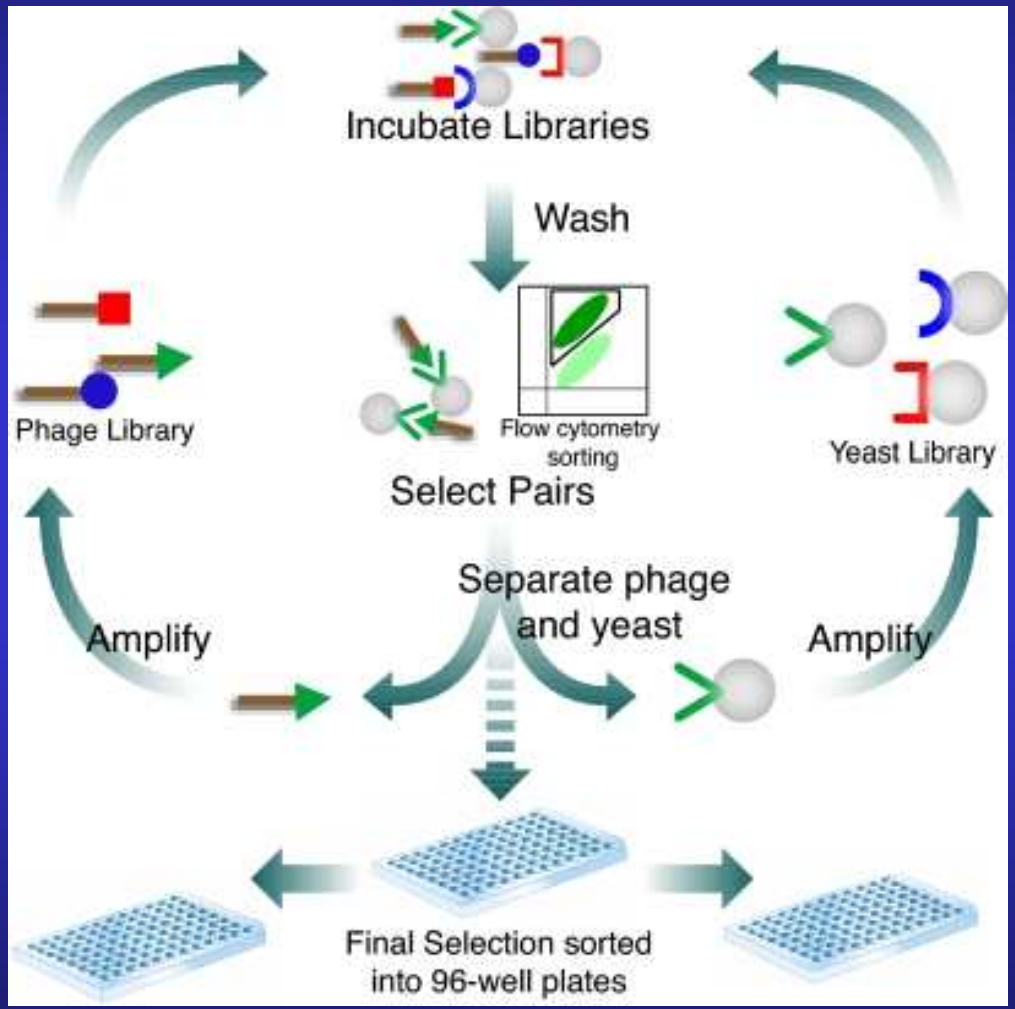
phage-displayed peptides in pooled sera of patients with proven peripheral ruptured (black bars) or stable (white bars) lesions and in pooled control sera (gray bars).



Reactivity is represented as the ratio of OD450 sample/(mean OD450 + 3SD) for empty phage.

Libraries against libraries for combinatorial selection

PNAS09



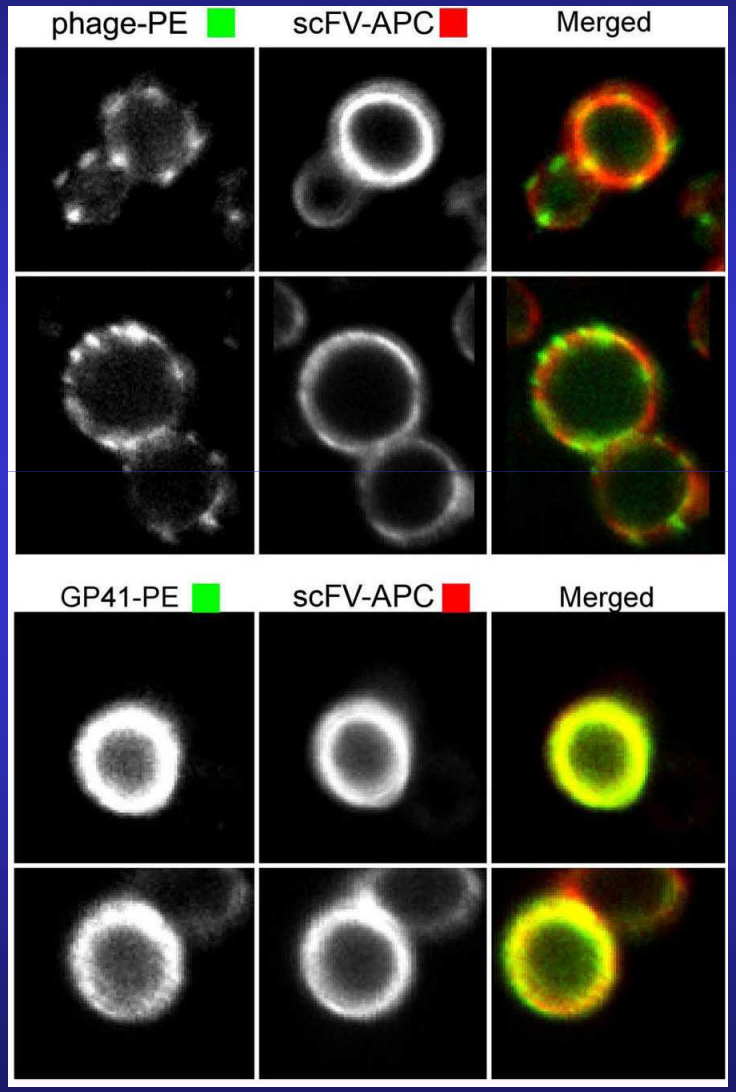
Libraries against libraries for combinatorial selection

PNAS09

- Dual-display for the identification of antibody–antigen pairs by library-against-library selection. A library of antigens (or antibodies) is displayed on phage, and a library of antibodies (or antigens) is displayed on yeast.
- The two libraries are mixed, and phage that are not bound to yeast cells are washed away.
- Phage that are bound to yeast cells are labeled with a fluorescencereagent, and flow cytometry sorting is used to select yeast cells bound to phage. The yeast and phage are separated for amplification, and the selection round is repeated until significant enrichment of pairs has been achieved.
- During the final round of selection, single cells of phage-positive yeast are sorted into 96-well plates. By eluting the phage from a single yeast cell, the information link between the platforms is maintained, and clonal pairs of antigens and antibodies are isolated.

Libraries against libraries for combinatorial selection

PNAS09



Libraries against libraries for combinatorial selection

PNAS09

Confocal microscopy imaging of the yeast–phage interaction.
Yeast cells displaying were stained with anti-c-myc-Alexa Fluor 647 to visualize the presence of scFv on the cell surface (scFv-APC red).
Binding of TJ1D phage to yeast cells was visualized by using an anti-phage antibody and Zenon-PE (phage-PE, green).