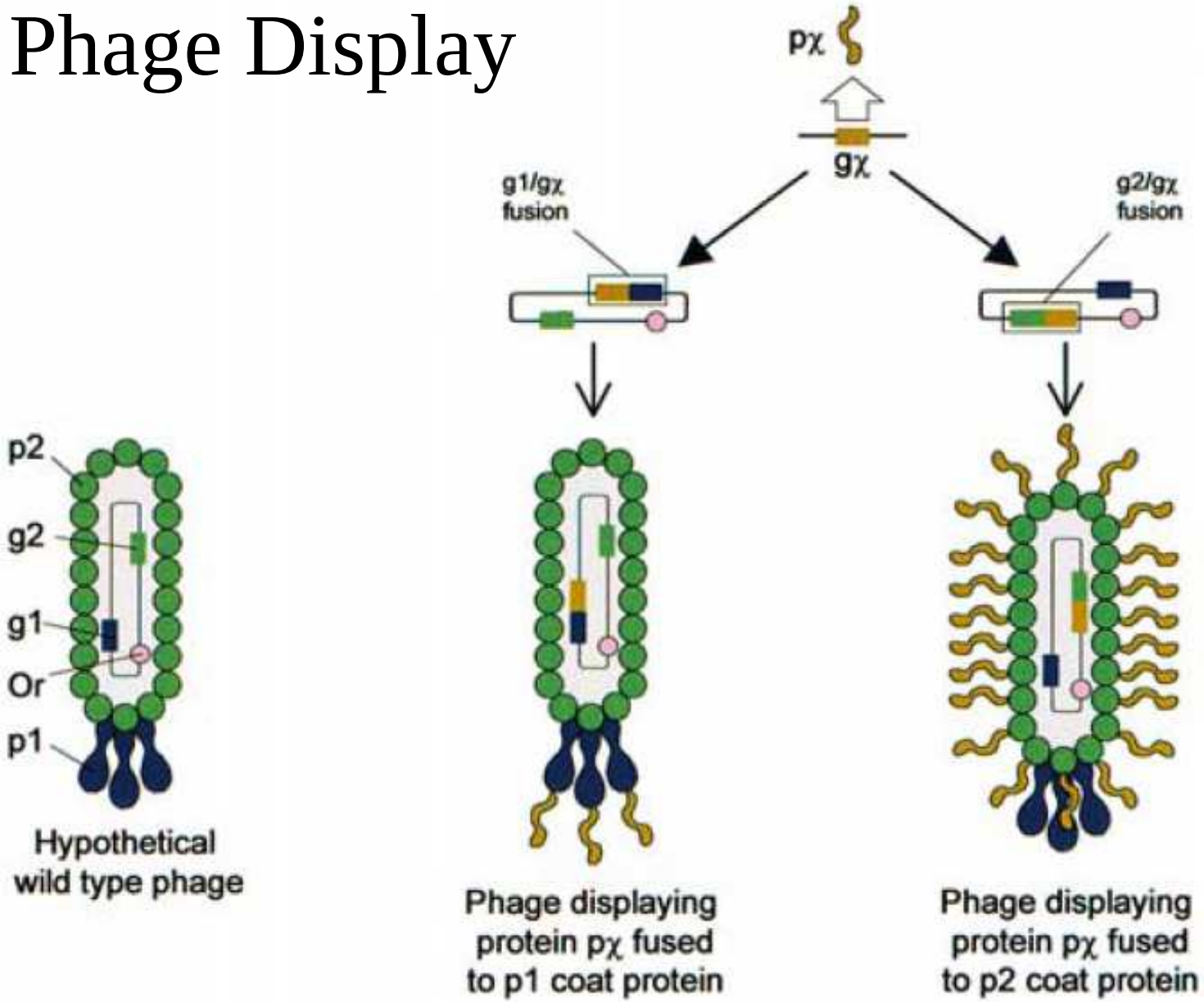
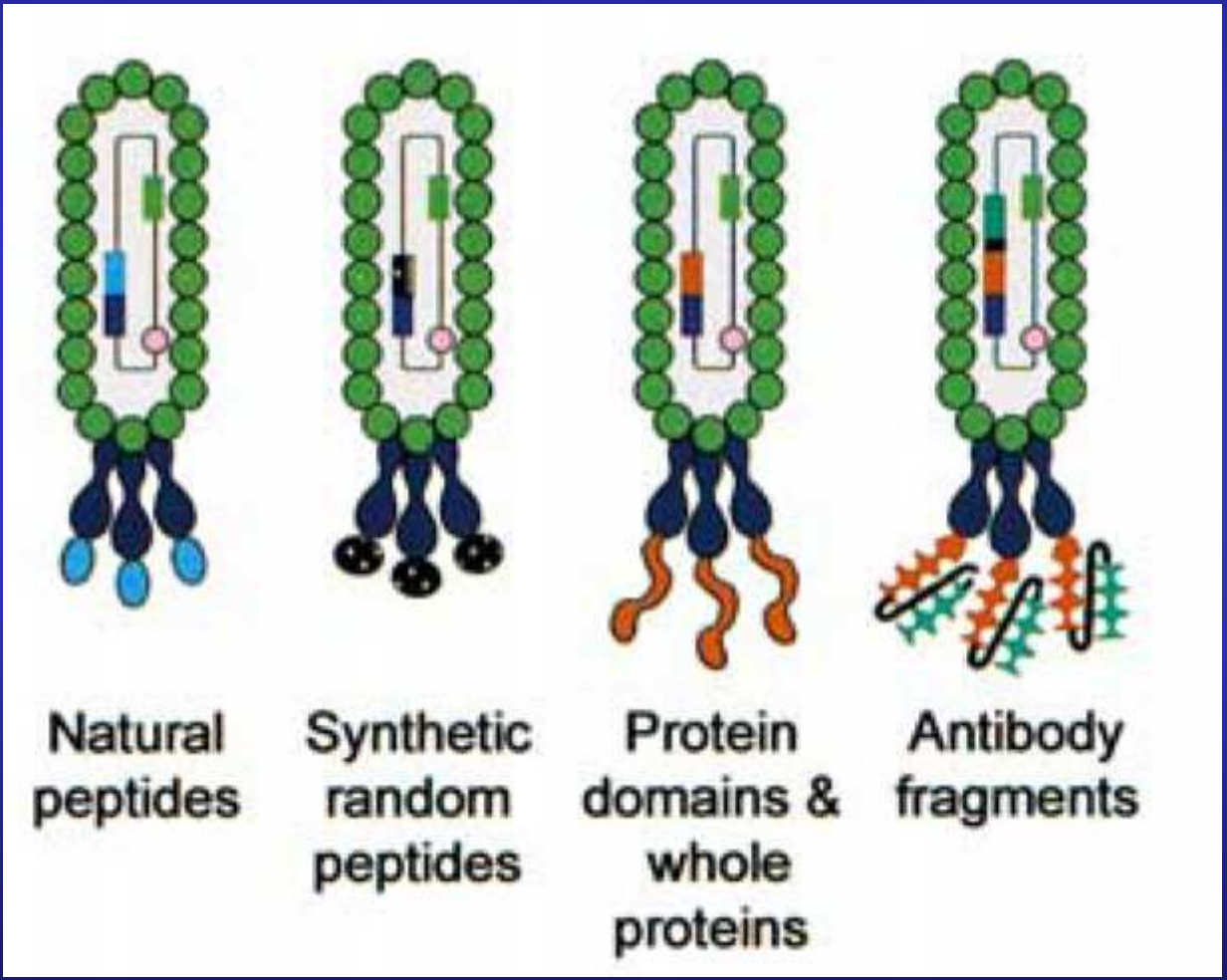
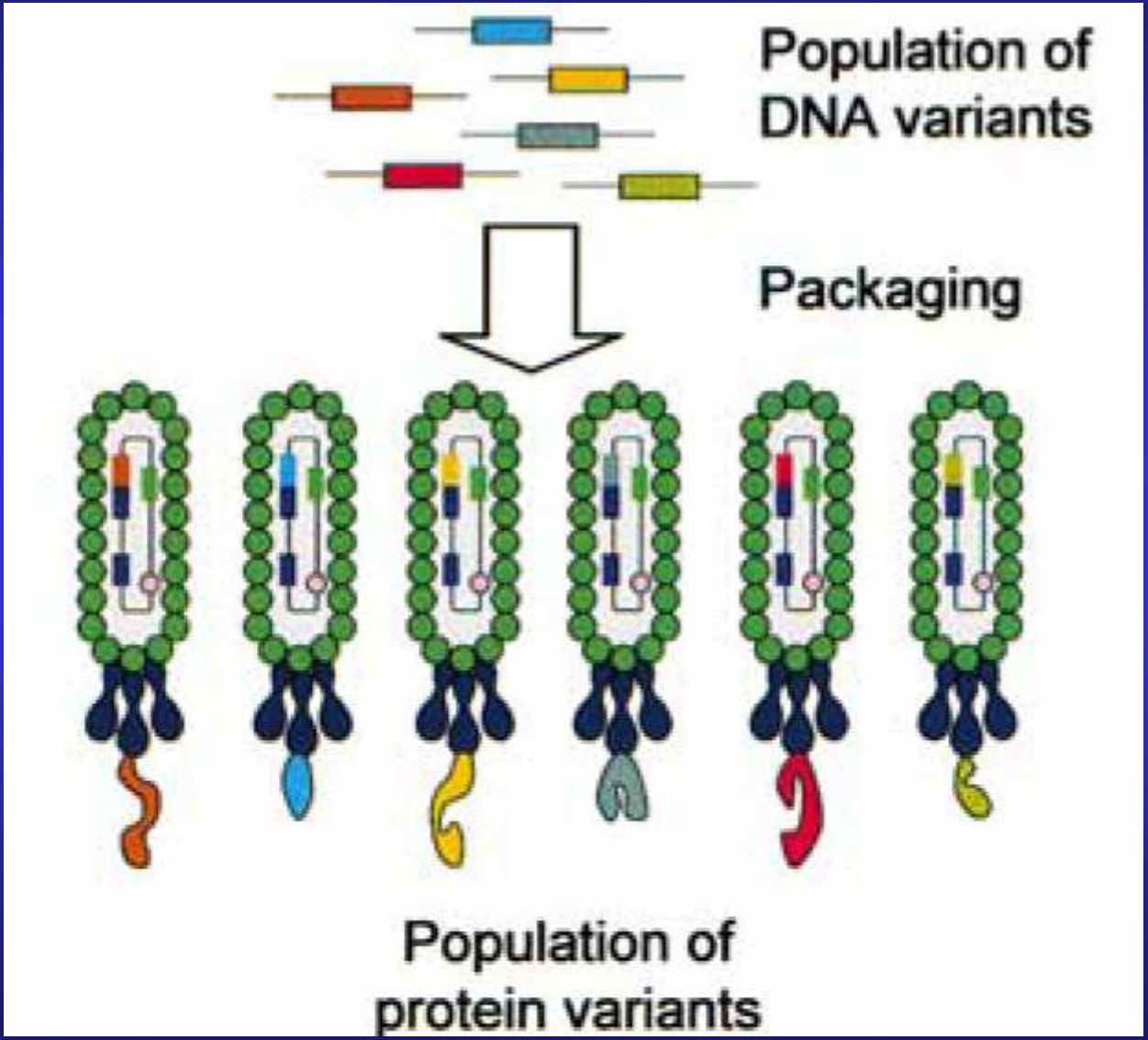
Phage Display



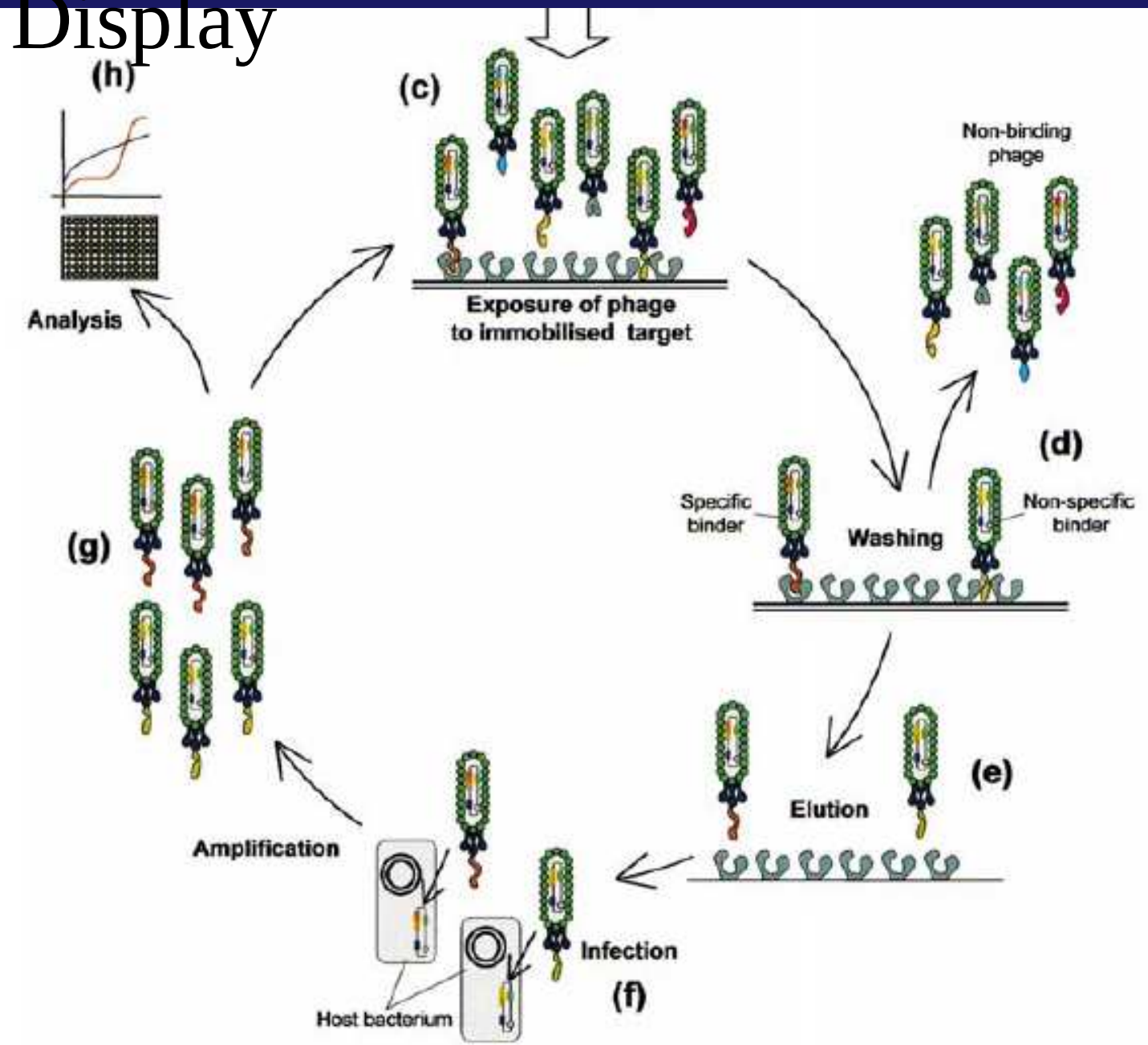
Phage Display

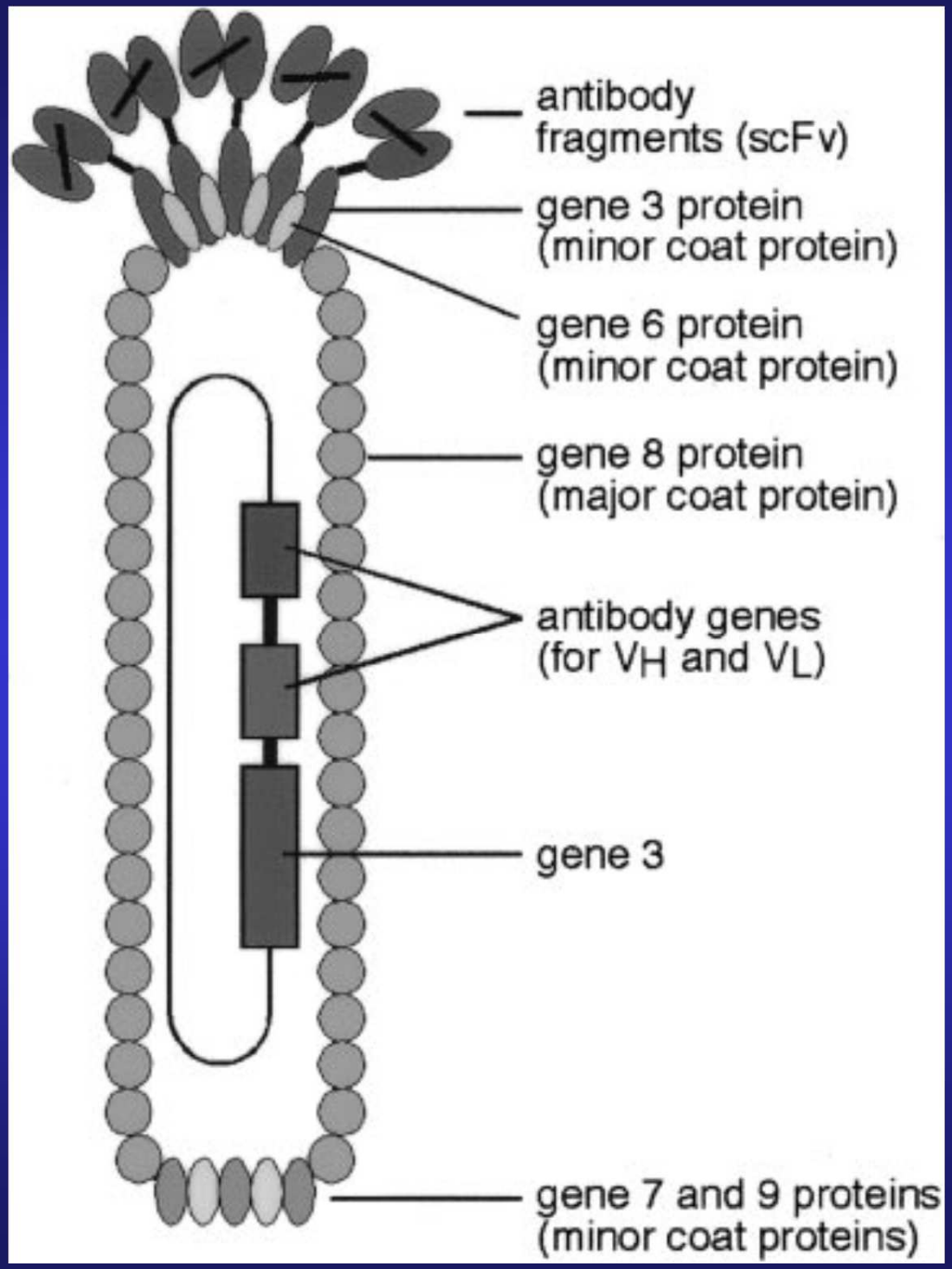


Phage Display



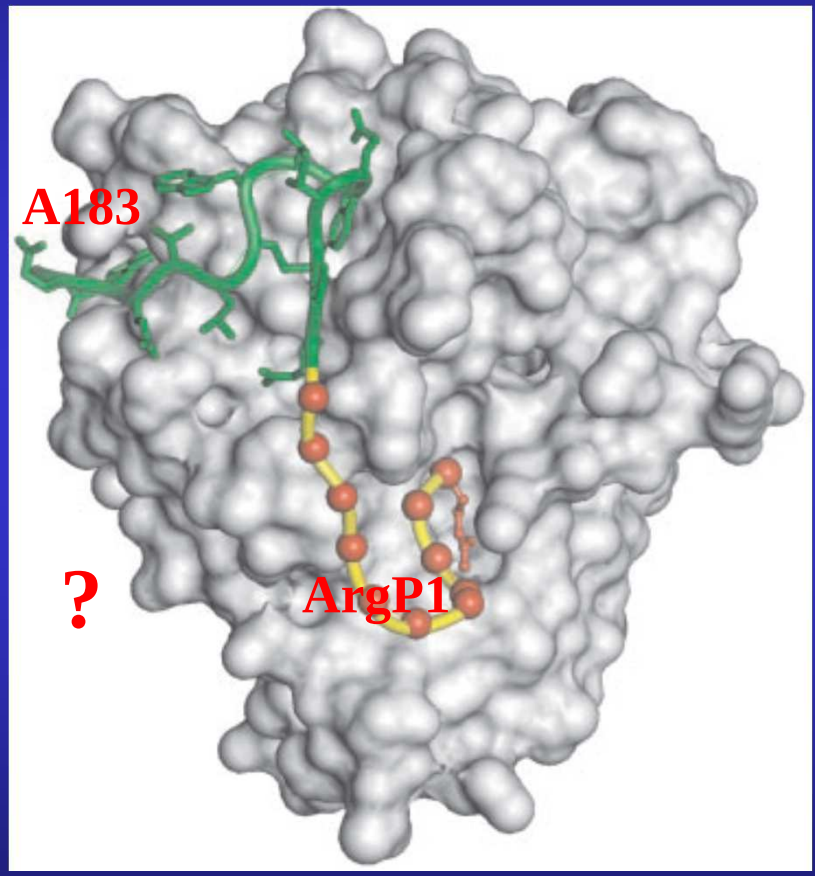
Phage Display




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 X ?

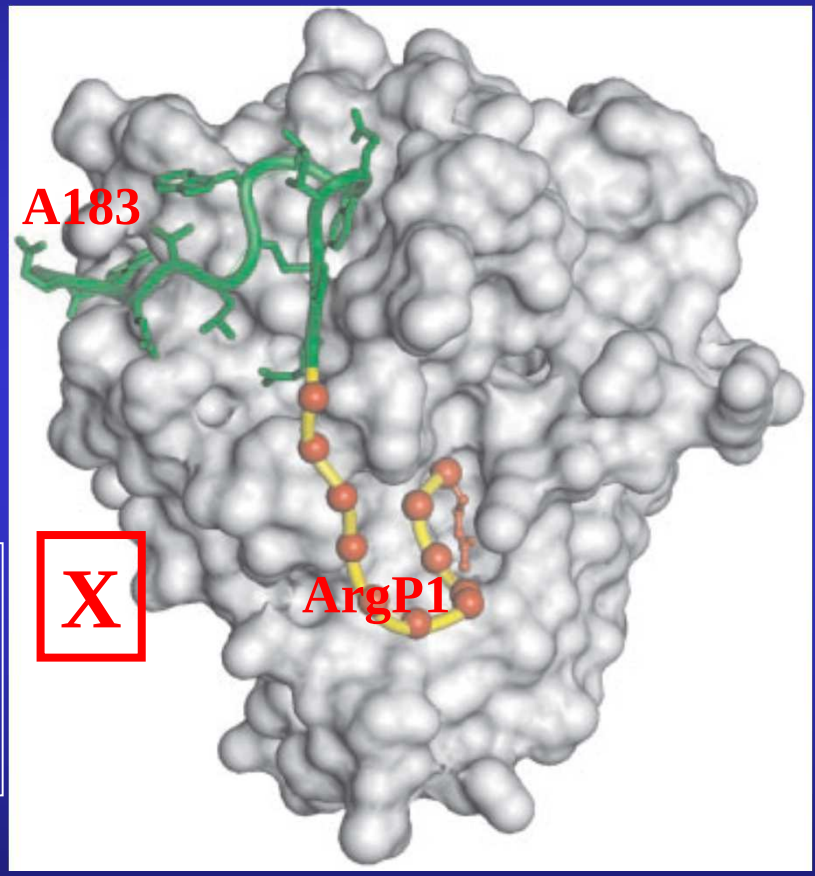
A183, 15mer,
EEWEVLCWTWETCER
exosite interactions



Model of FVIIa protease domain with A-183 + extension peptide X ?

A183, 15mer,
EEWEVLCWTWETCER
exosite interactions

A chimeric peptide with a high degree of specificity and potency



+ greater steric hindrance in the substrate binding cleft
+ higher affinity due to a more extensive binding surface

determinanti di specificità di proteasi

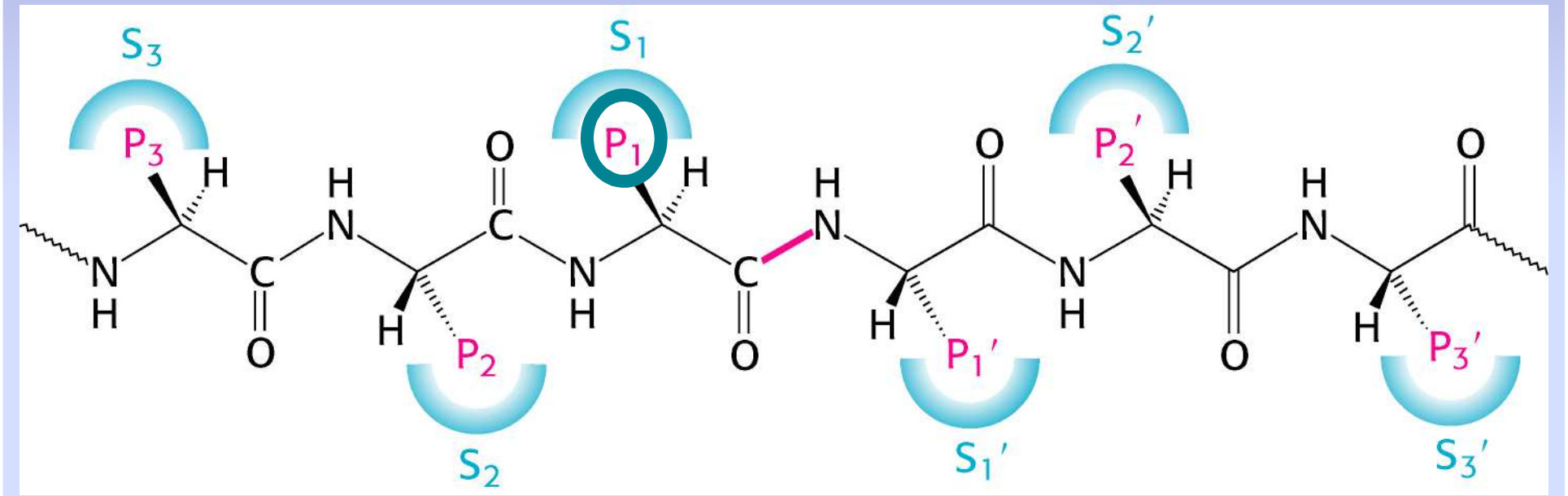
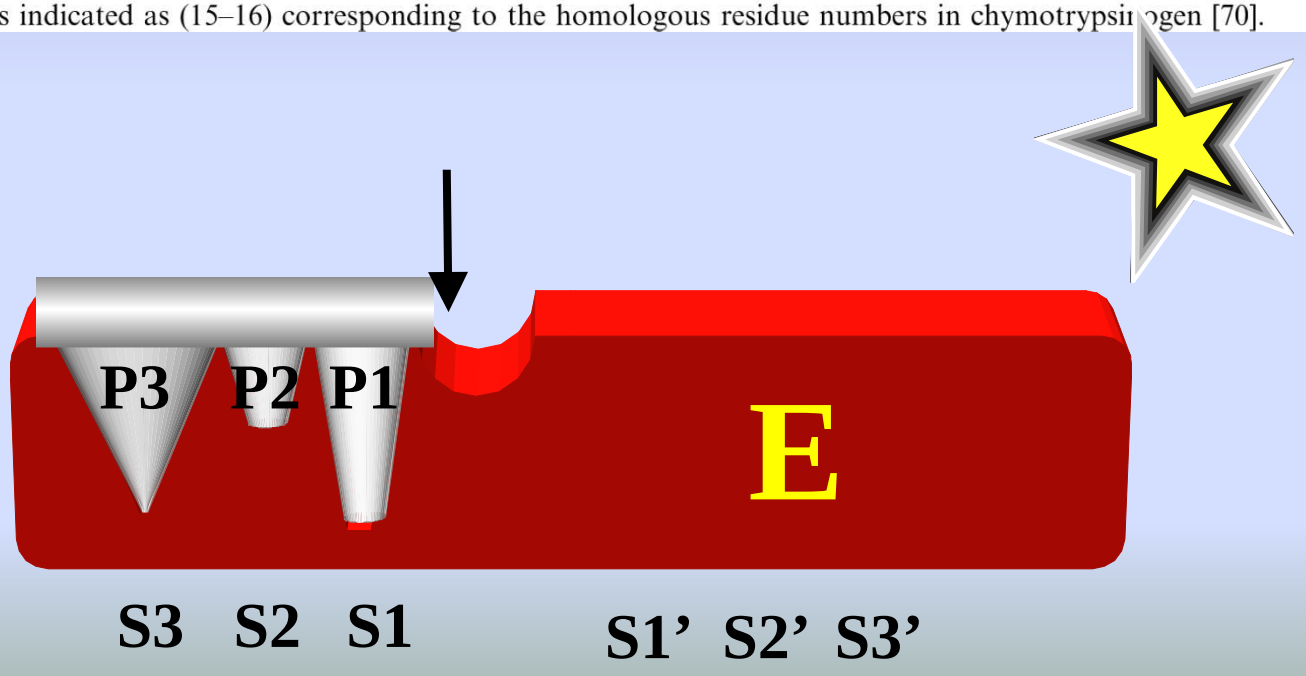
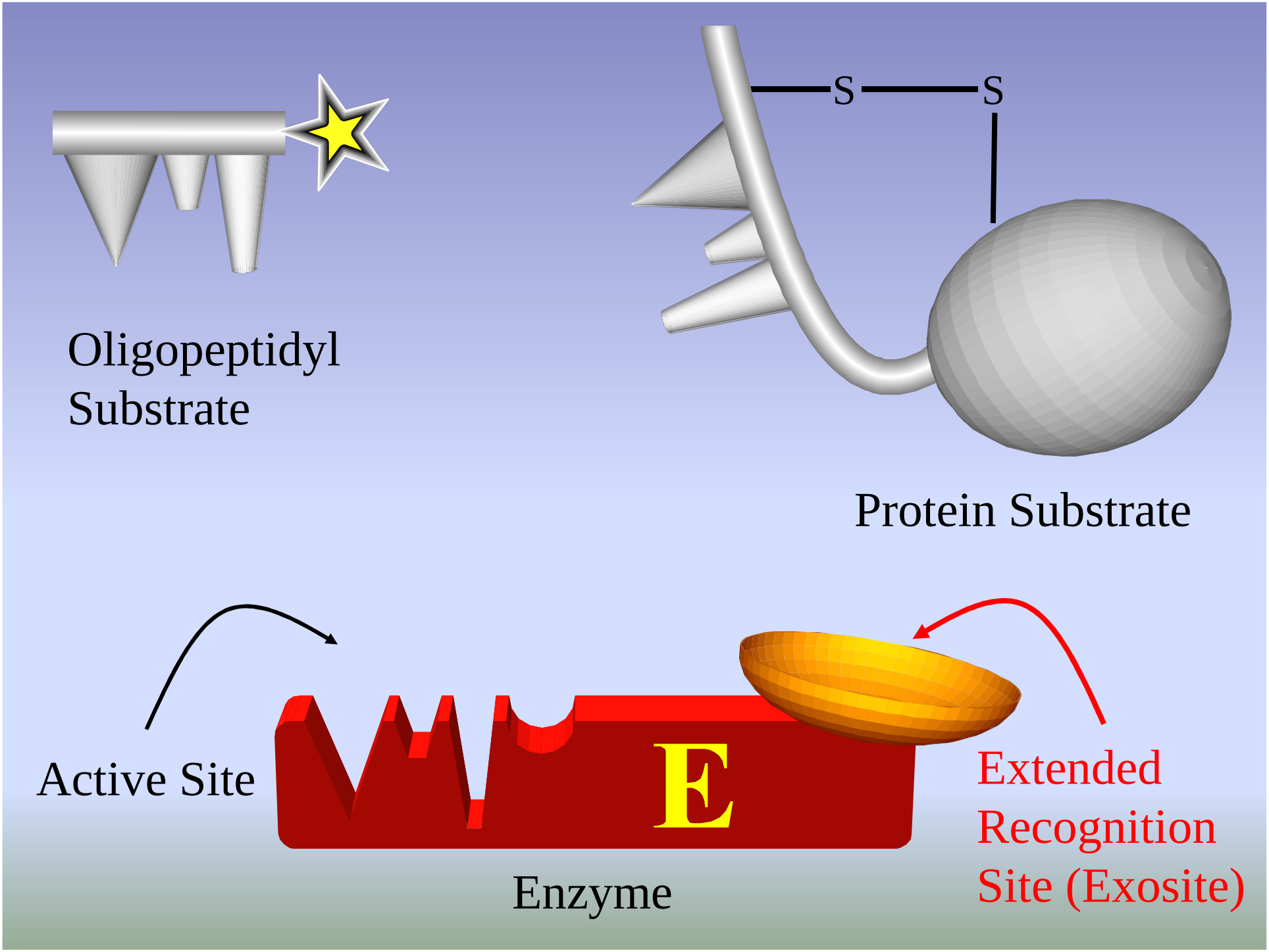


Table 1 Sites of cleavage in the human vitamin K-dependent zymogens*

Enzyme	Substrate†	P_4	P_3	P_2	P_1	↓	$P_{1'}$	$P_{2'}$	$P_{3'}$	$P_{4'}$
Xa/Va	II	I	E	G	R		T	A	T	S
	$II_{(15–16)}$	I	D	G	R		I	V	E	G
VIIa/TF, IXa/VIIIa	$X_{(15–16)}$	N	L	T	R		I	V	G	G
VIIa/TF, XIa	IX	K	L	T	R		A	E	A	V
	$IX_{(15–16)}$	D	F	T	R		V	V	G	G
VIIa/TF, Xa	$VII_{(15–16)}$	P	Q	G	R		I	V	G	G
IIa/TM	$PC_{(15–16)}$	V	D	P	R		L	I	D	G

*Sequences flanking cleavage sites relevant to the activation of the vitamin K-dependent zymogens are presented along with the relevant enzymes that catalyze these reactions. The site of bond cleavage is denoted by the arrow. †The site, in each substrate, at which cleavage is required to produce the serine proteinase is indicated as (15–16) corresponding to the homologous residue numbers in chymotrypsinogen [70].




S
S
Oligopeptidyl
Substrate
Protein Substrate
Active Site
E
Enzyme
Extended
Recognition
Site (Exosite)

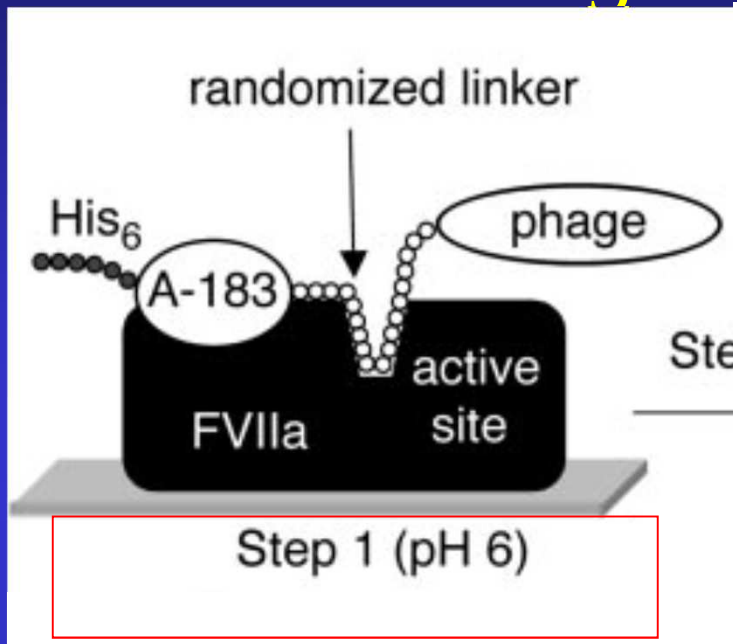
- Libraries A–D designed to determine the length and sequence of the extension to reach into the active site

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 V G G** –	protease resistant spacer	p3
B		X X X X ? X X X *b* **L T R I V G G** – –		
C		X X X X X X X *c* **T R I V G G** – – –		
D		**G G S G G S G** X X X X X X X **G G** –		

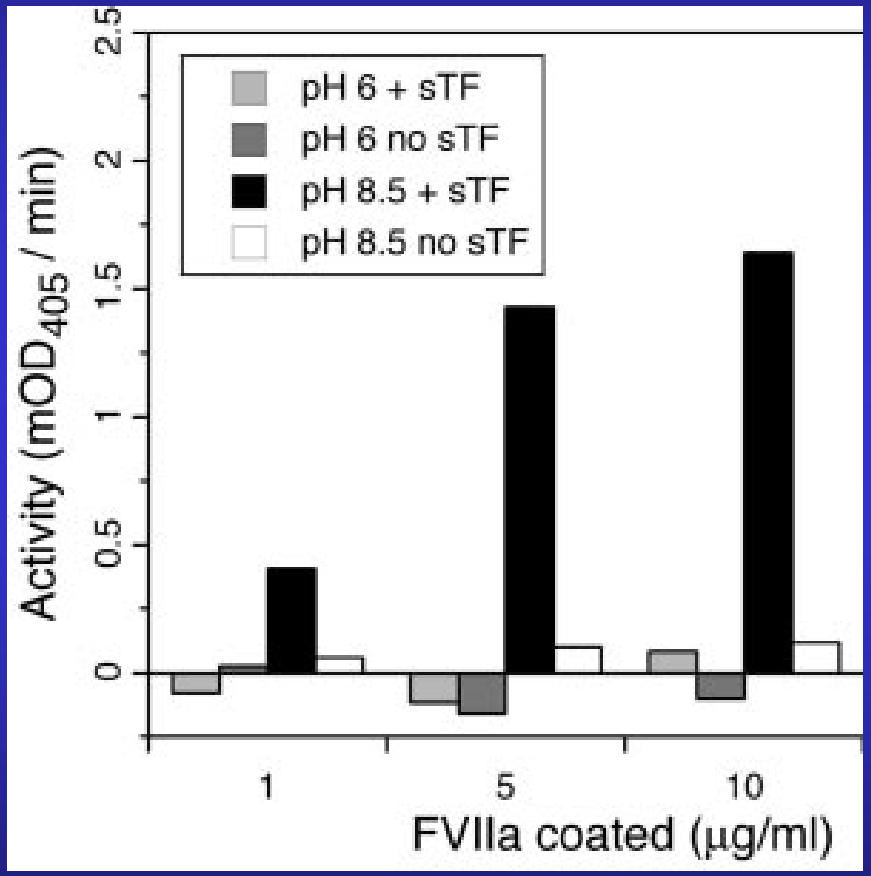
a= S,N,K,R; *b*= N,K; *c* = L,Q

Peptide Inhibitors of Factor VIIa: Phage binding

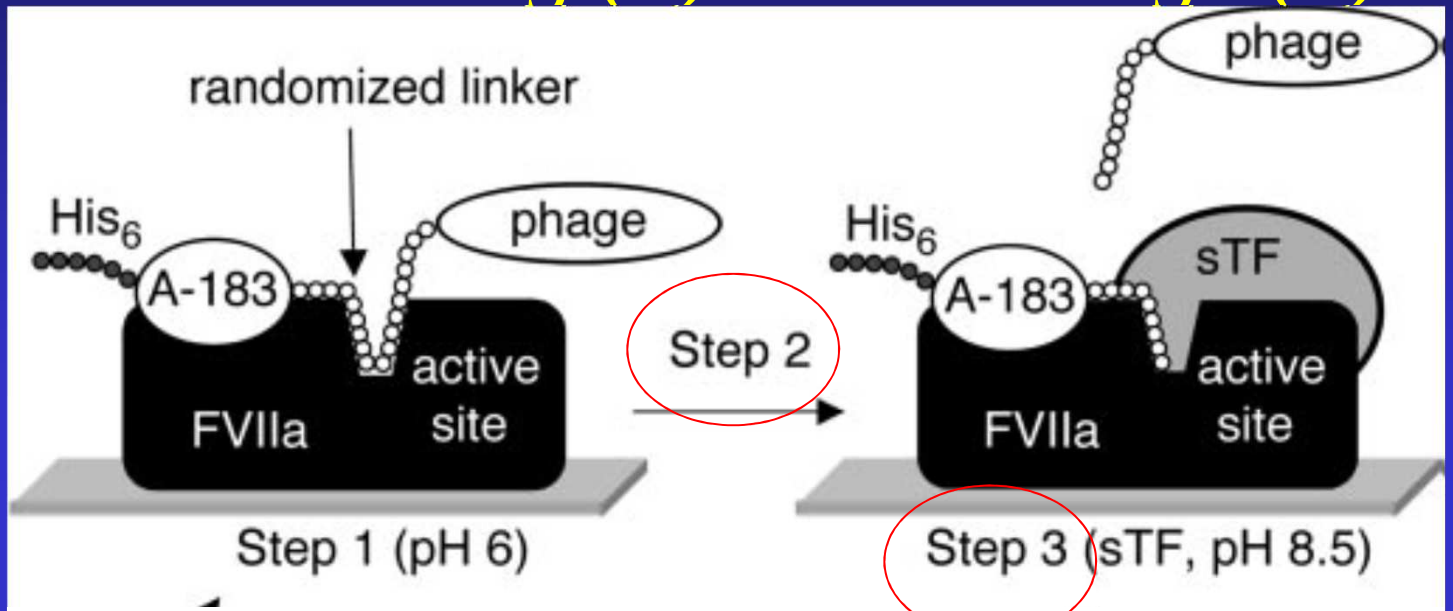


Unbound phage were removed by repetitive washing with binding buffer (step 2).

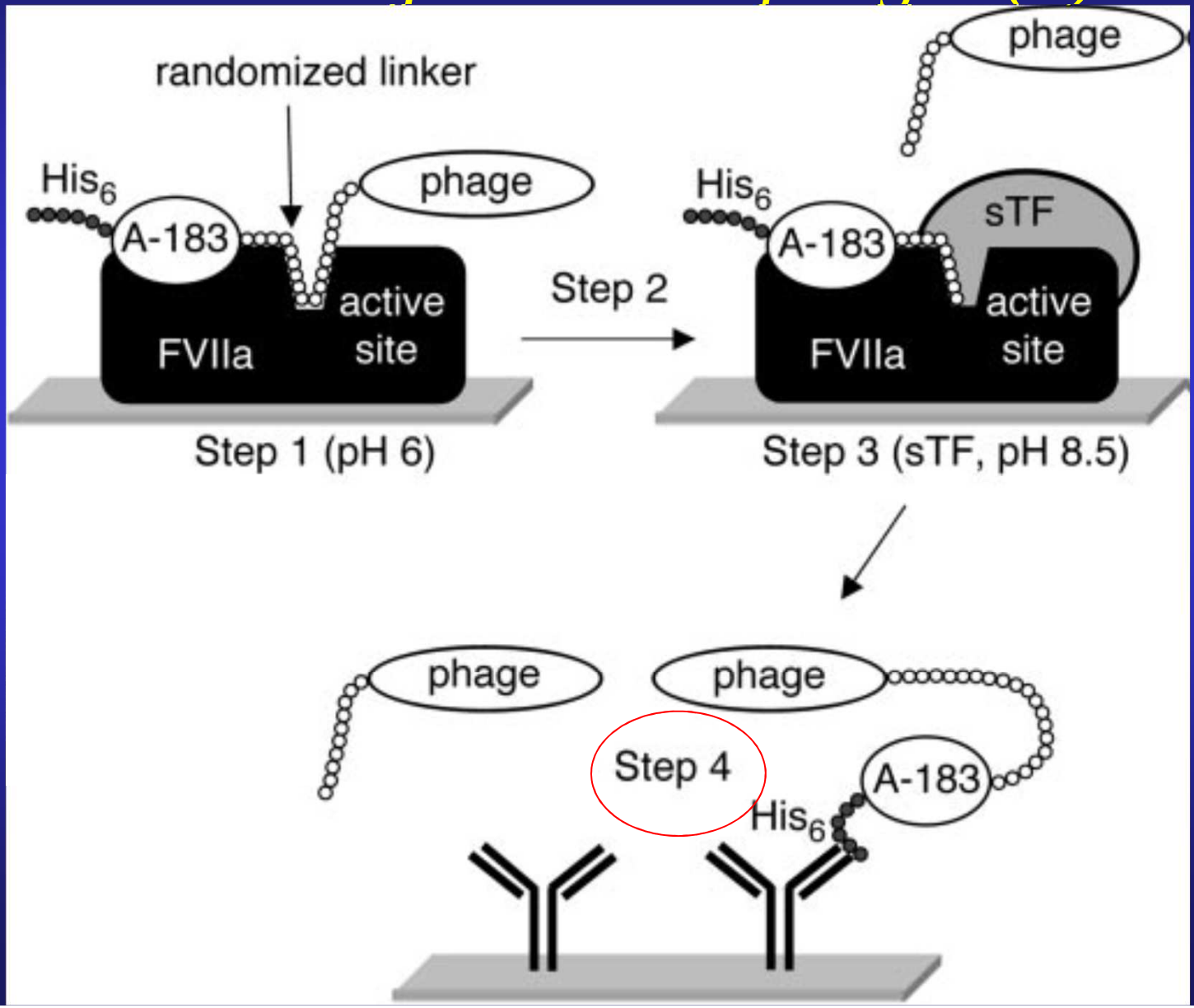
FVIIa Cleavage conditions



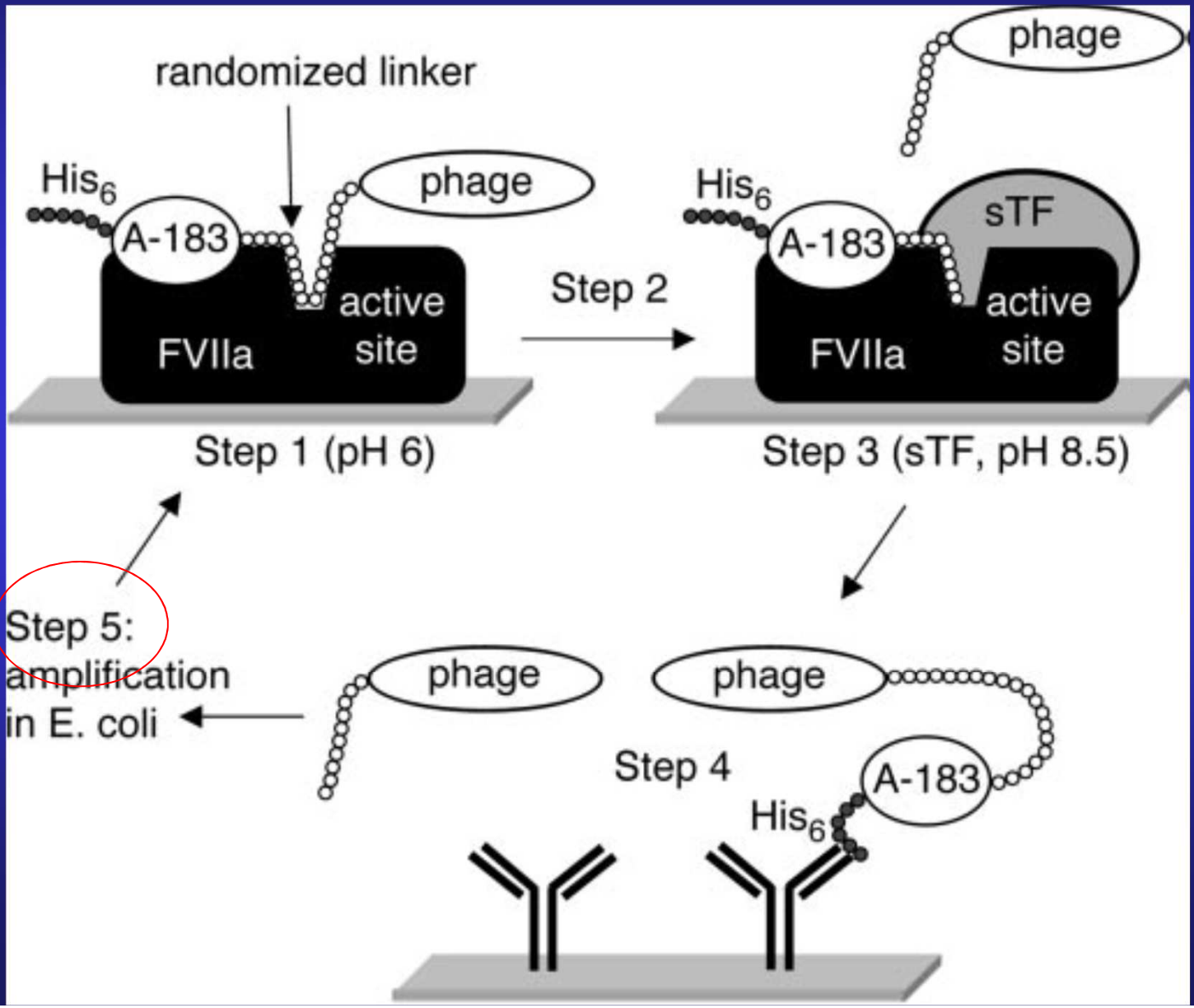
Peptide Inhibitors of Factor VIIa:washing (2) and cleavage (3)



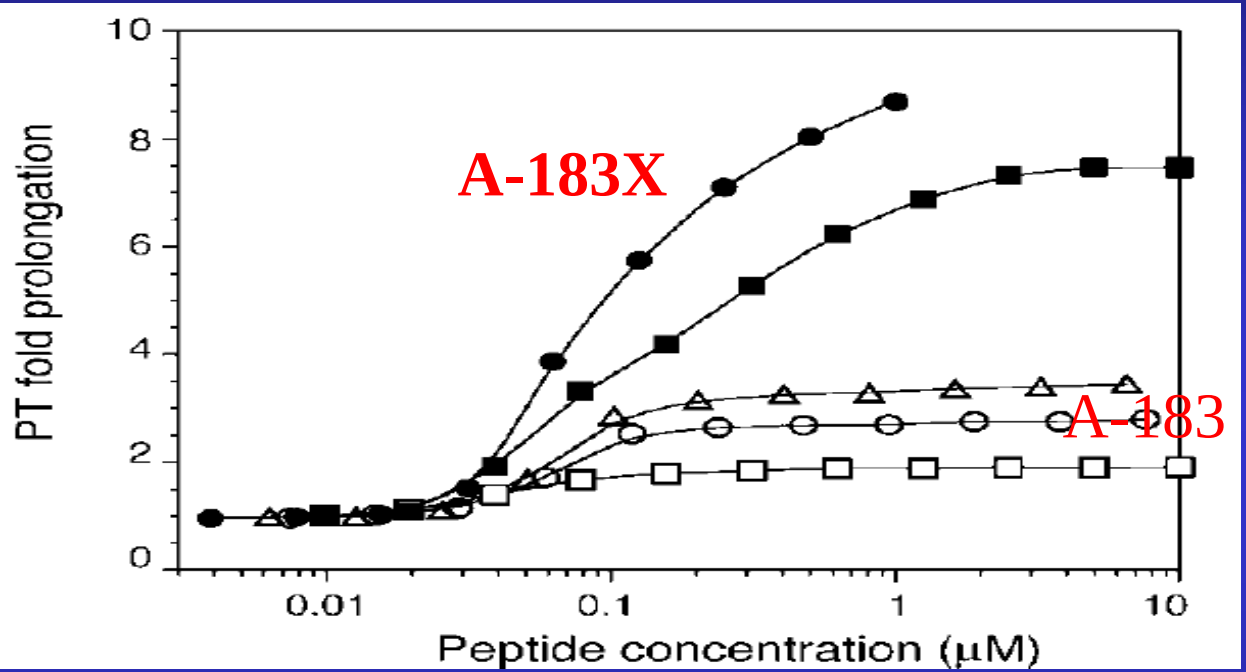
Peptide Inhibitors of Factor VIIa: removal of unbound phages (4)



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



Prolongation of TF-dependent clotting times



A-183X was a potent and complete inhibitor of FX activation
maximal extent of inhibition of 99% with an IC50 of 230 pM
(A-183, 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


In vitro selection milestones
Milestones in genetics & genomics
A quarter century of findings through in vitro selection celebrated
2015
In vitro evolution of a cross-chiral RNA polymerase ribozyme
First synthetic yeast chromosome revealed
In vitro selection combined with rational design of an RNA aptamer
CRISPR system developed as a genome editing tool
XNAs and XNA aptamers in vitro selection
Next generation sequencing used with in vitro selection
2010
Creation of a bacterial cell with a synthetic genome
In silico selection of RNA aptamers
Research on stem cells & genetic modification of crops
P. patens genome release
C. reinhardtii genome release
Cell-SELEX for molecular imaging
In vitro selection of a ribozyme from an entire genome
Genome-wide maps of DNA methylation
An in vitro selection system for TNA
2005
The next generation sequencing arrives
In vitro selection of DNA aptamers using capillary electrophoresis
A. thaliana genome released
Magnetic bead SELEX & SELEX-SAGE
UCSC genome browser started
Natural riboswitches discovered
mRNA display for protein in vitro selection
In vitro selection of a hammerhead ribozyme from a random unbiased RNA pool
2000
Sequencing of Drosophila genome completed
Human genome projects completed
The first application of synthetic biology
In vitro selection of cell binding aptamers (Cell SELEX)
Discovery of RNA interference
Ribosome display for protein in vitro selection
mRNA peptide fusions for in vitro selection of proteins
The first cloning of a mammal (Dolly) performed
The yeast genome completed
1995
The microarray revolution
First genome of free living organism sequenced (H. influenzae)
Deoxyribozymes isolated using in vitro selection
Polysome display for protein in vitro selection
GFP: the green revolution
In vitro selection of ligating ribozymes
In vitro selection of DNA ligands
First expressed sequence tags (EST)
In vitro selection of organic dye binding RNA molecules
1990
The development of in vitro selection & SELEX
The Nobel Prize in Chemistry:
"Discovery of catalytic properties of RNA"
Nucleosomes regulation of gene expression revealed
Generation of random oligonucleotide pools
Automated DNA sequencing & Invention of PCR
1985