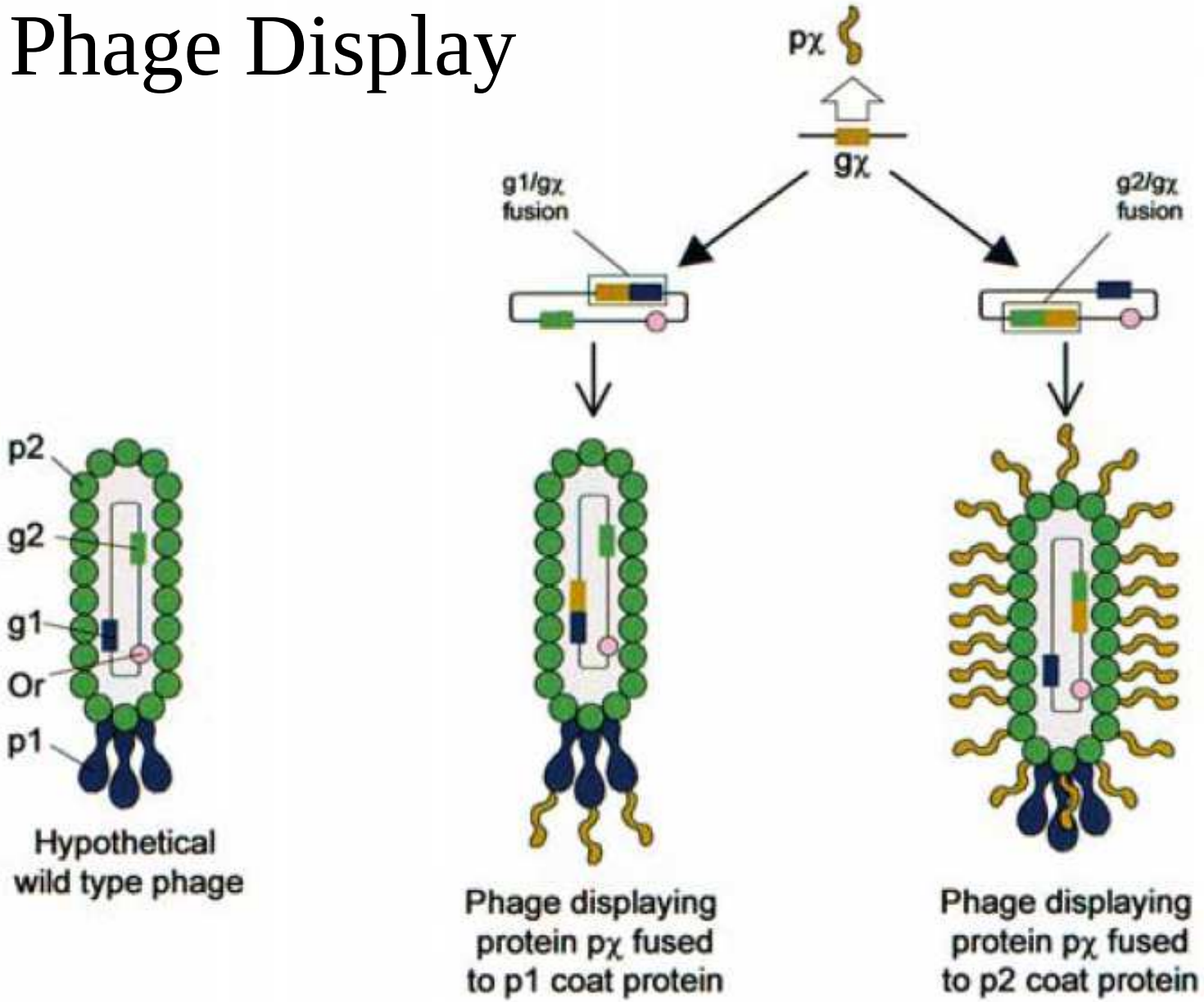
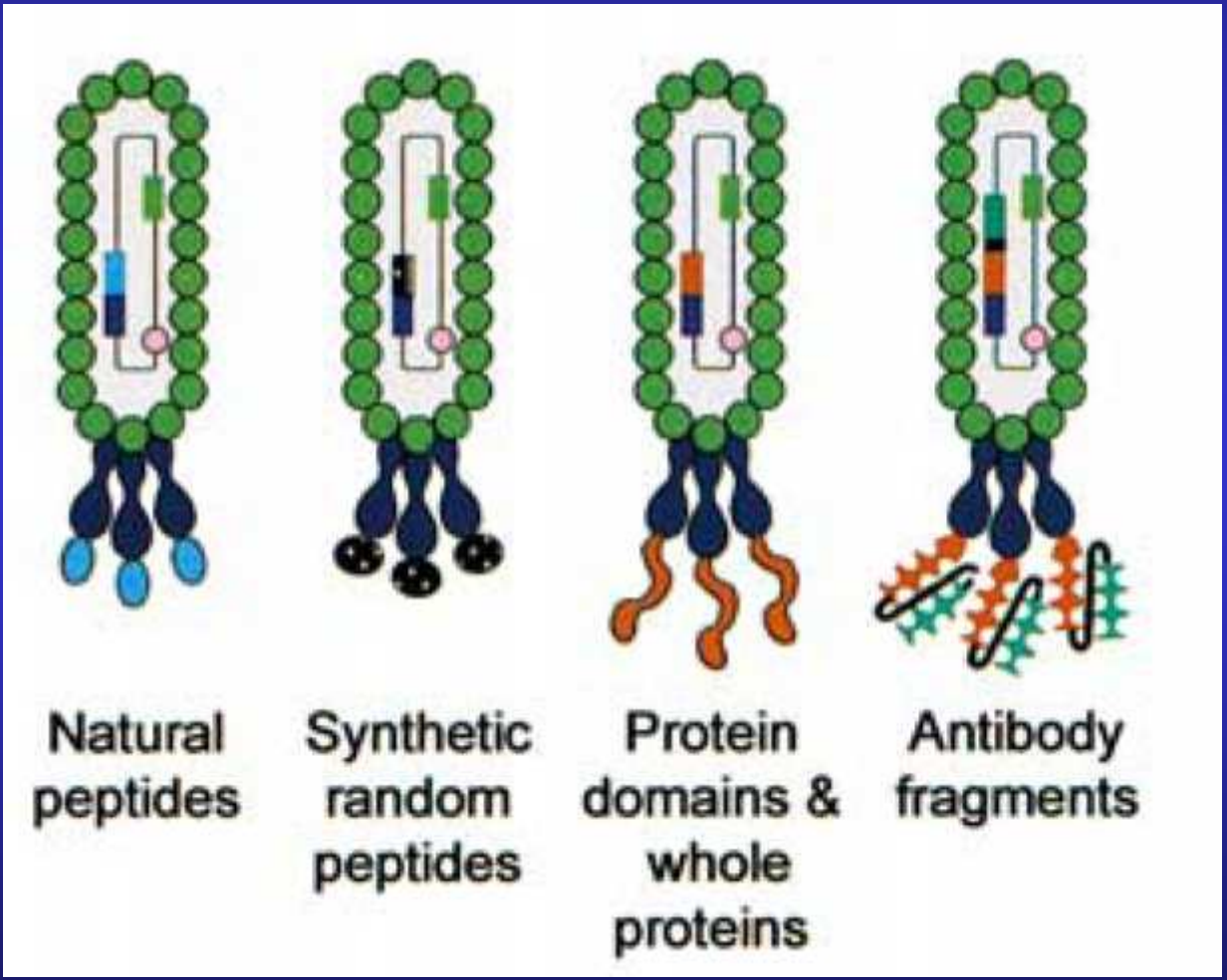
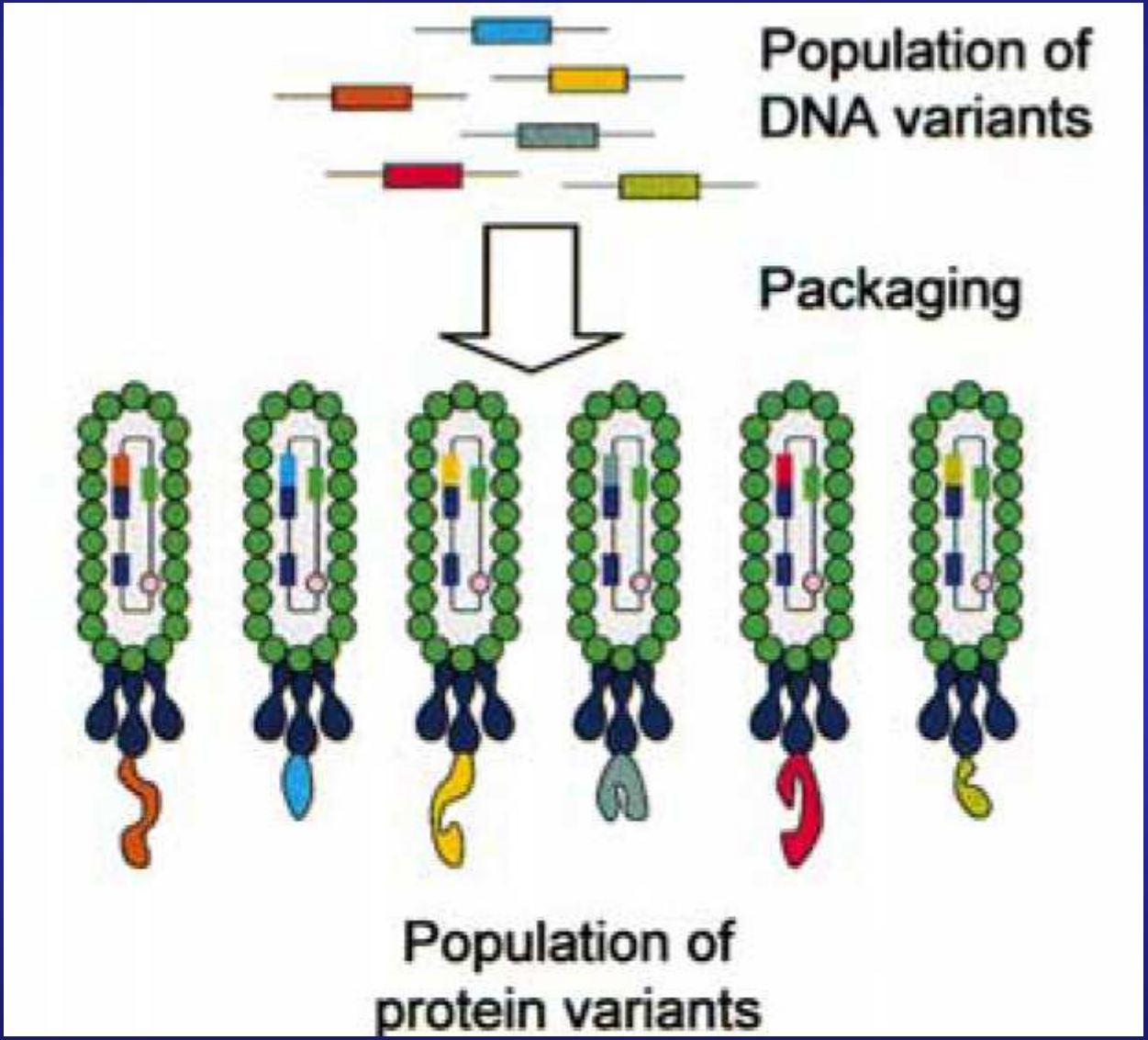
Phage Display



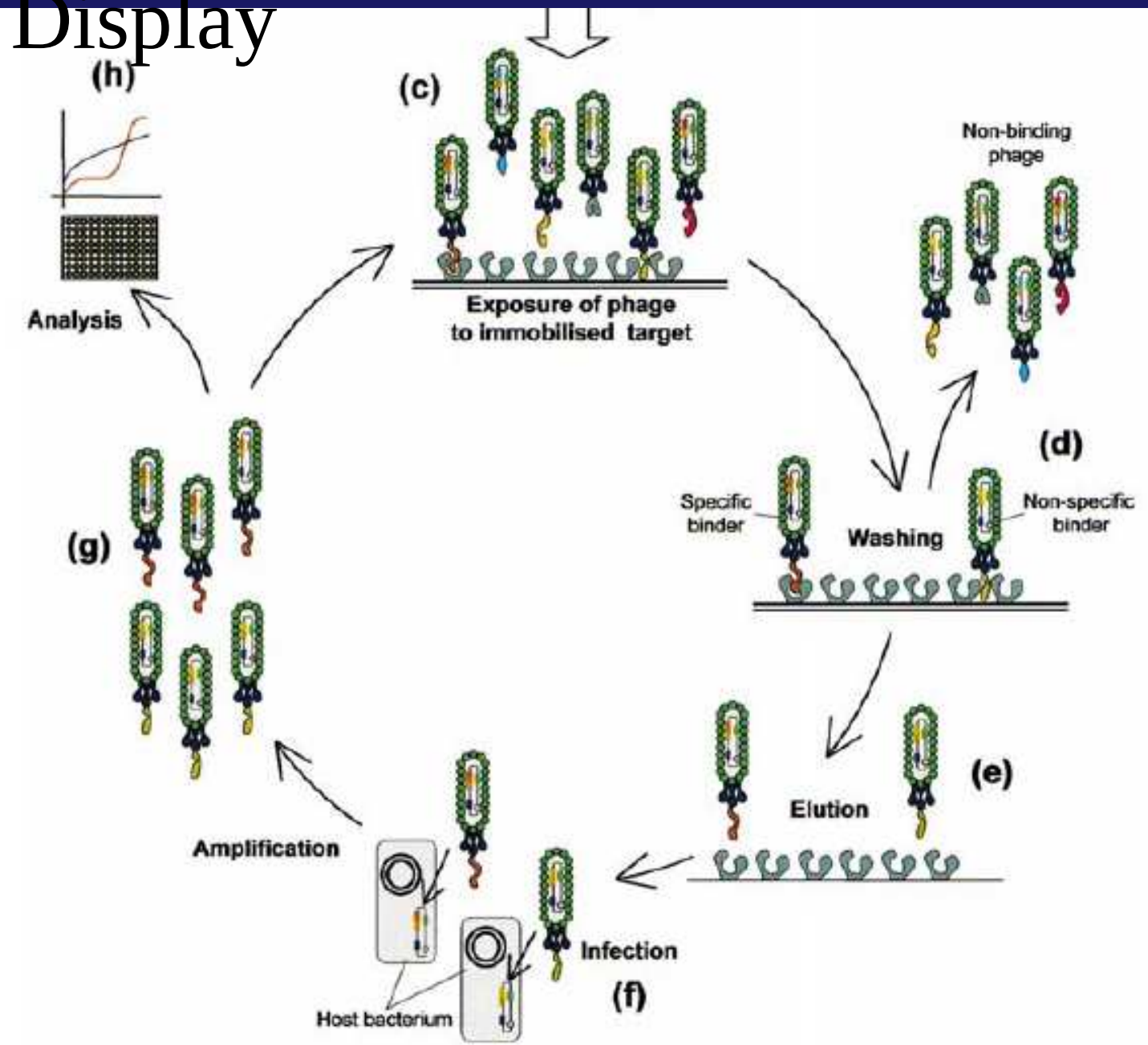
Phage Display

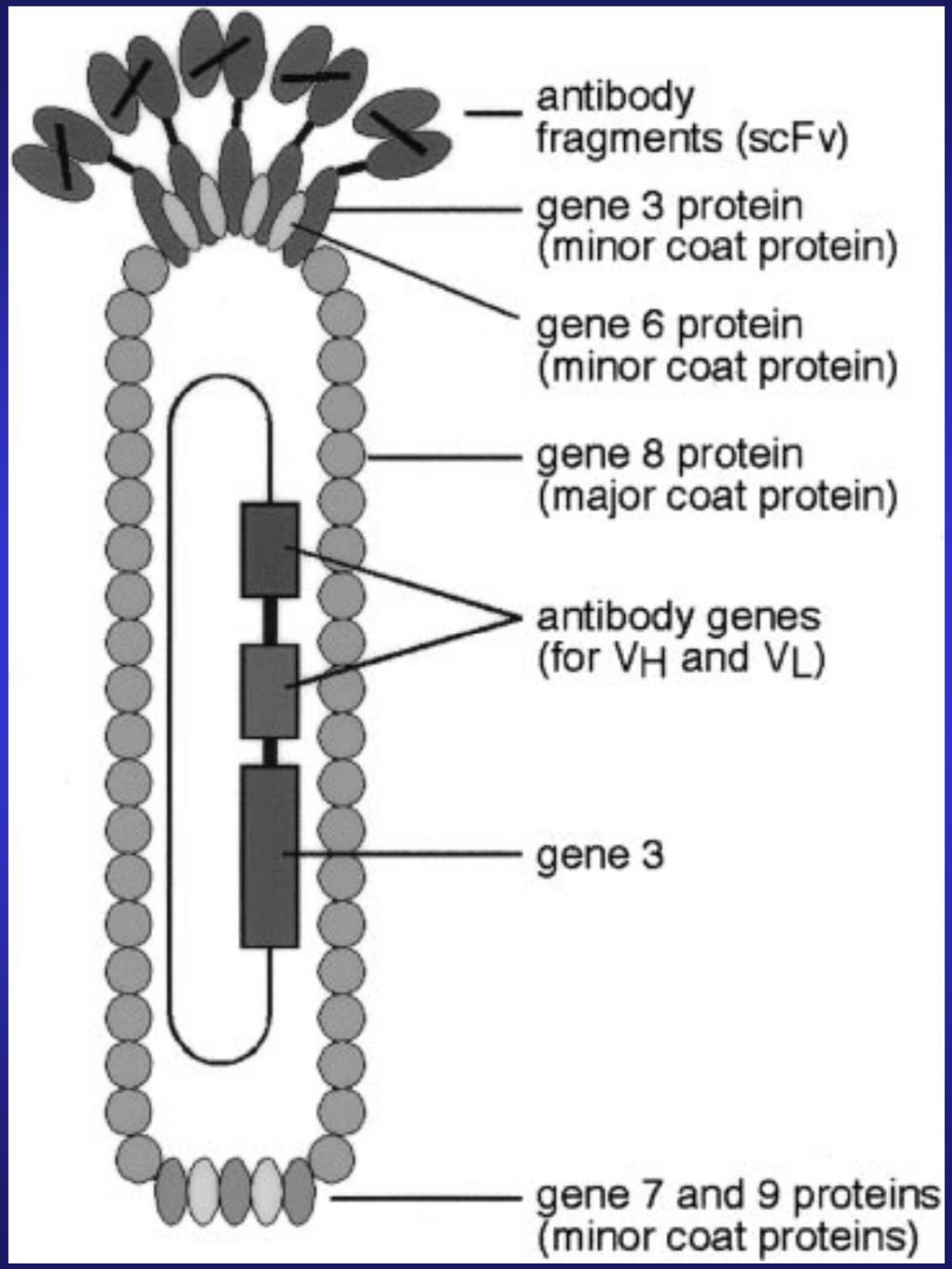


Phage Display

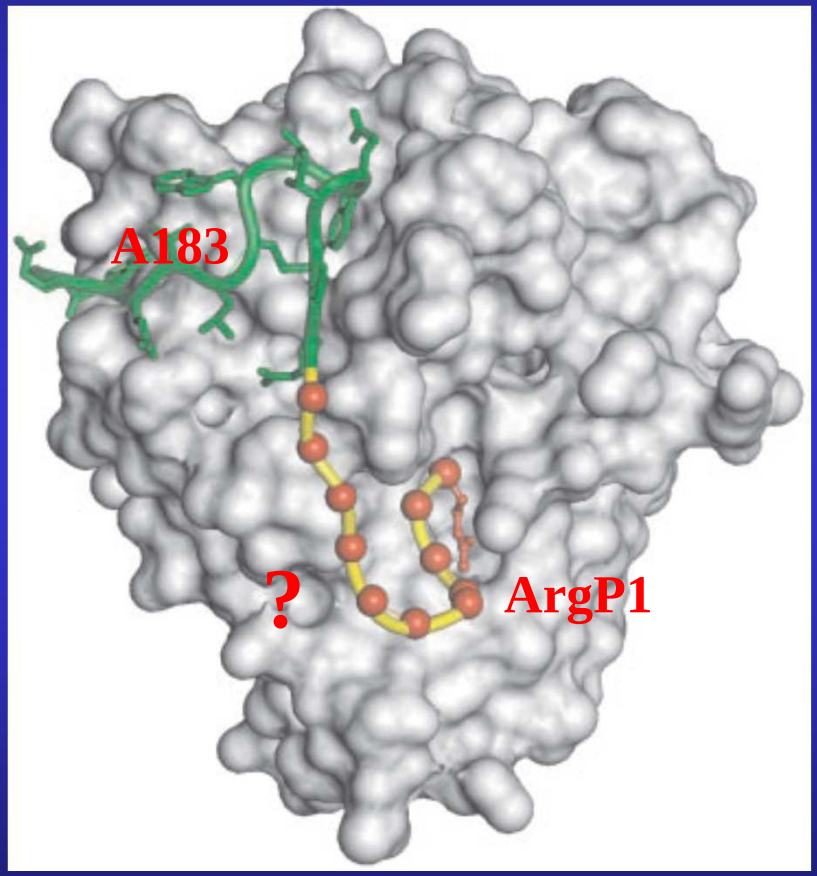


Phage Display




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



a chimeric peptide with a high degree of specificity and potency:
exosite interactions + greater steric hindrance in the substrate binding cleft + higher affinity due to a more extensive binding surface

determinanti di specificità

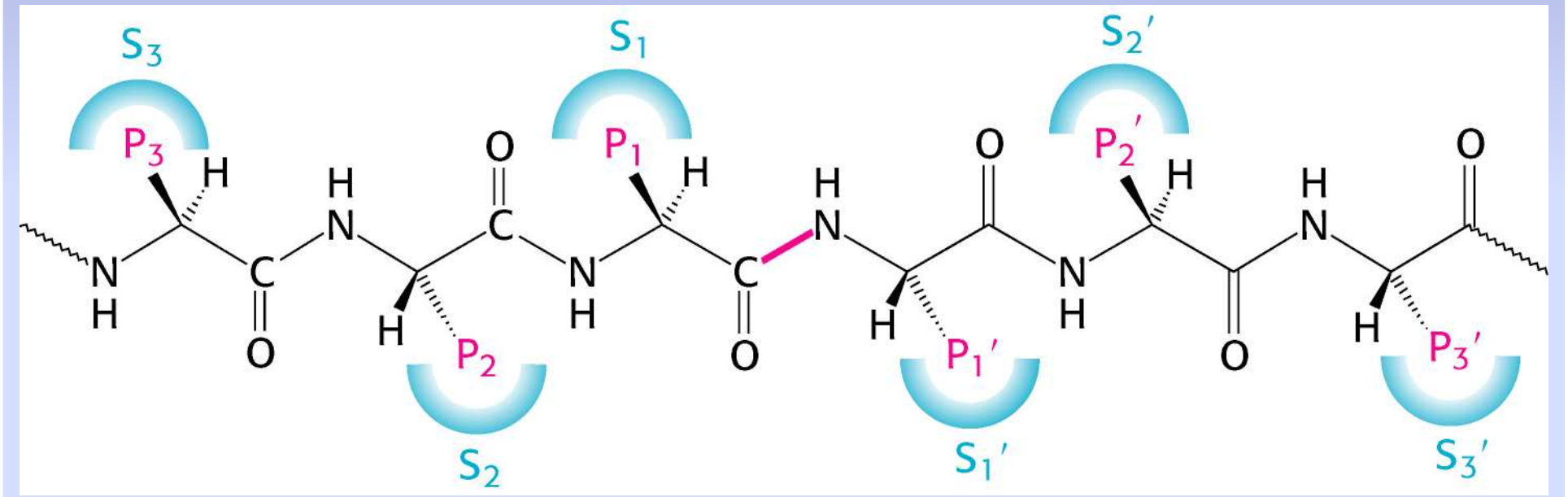
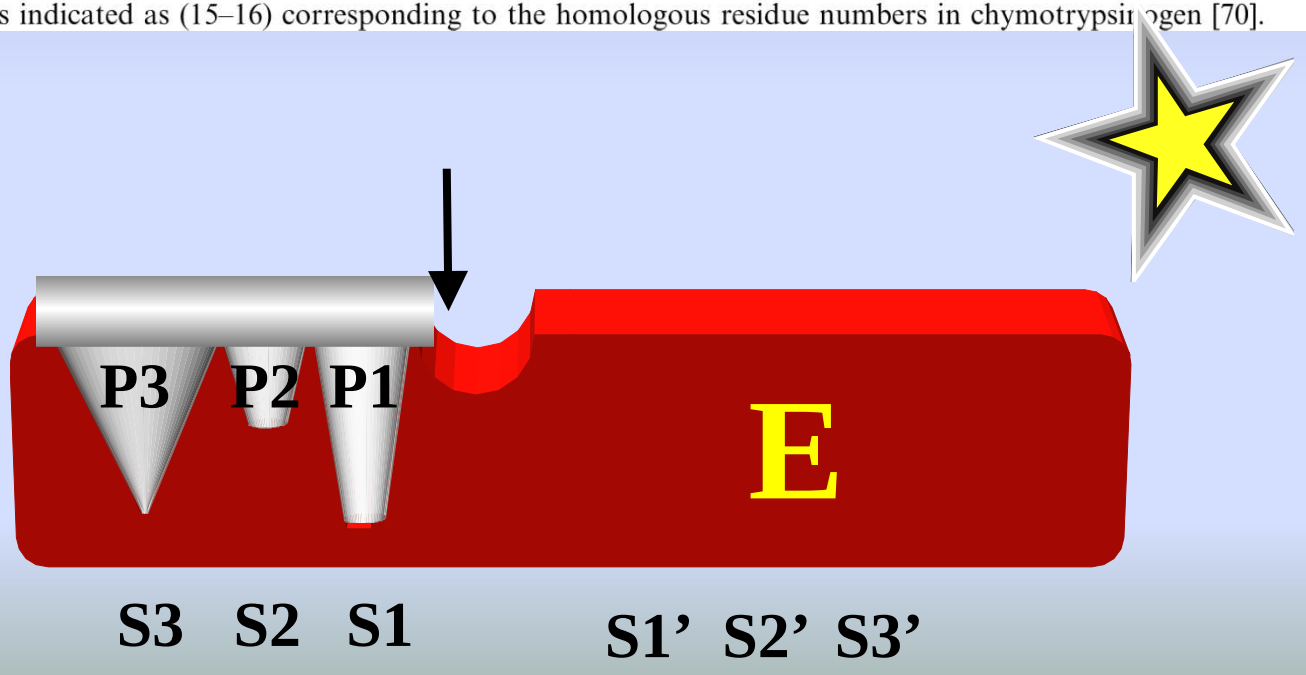
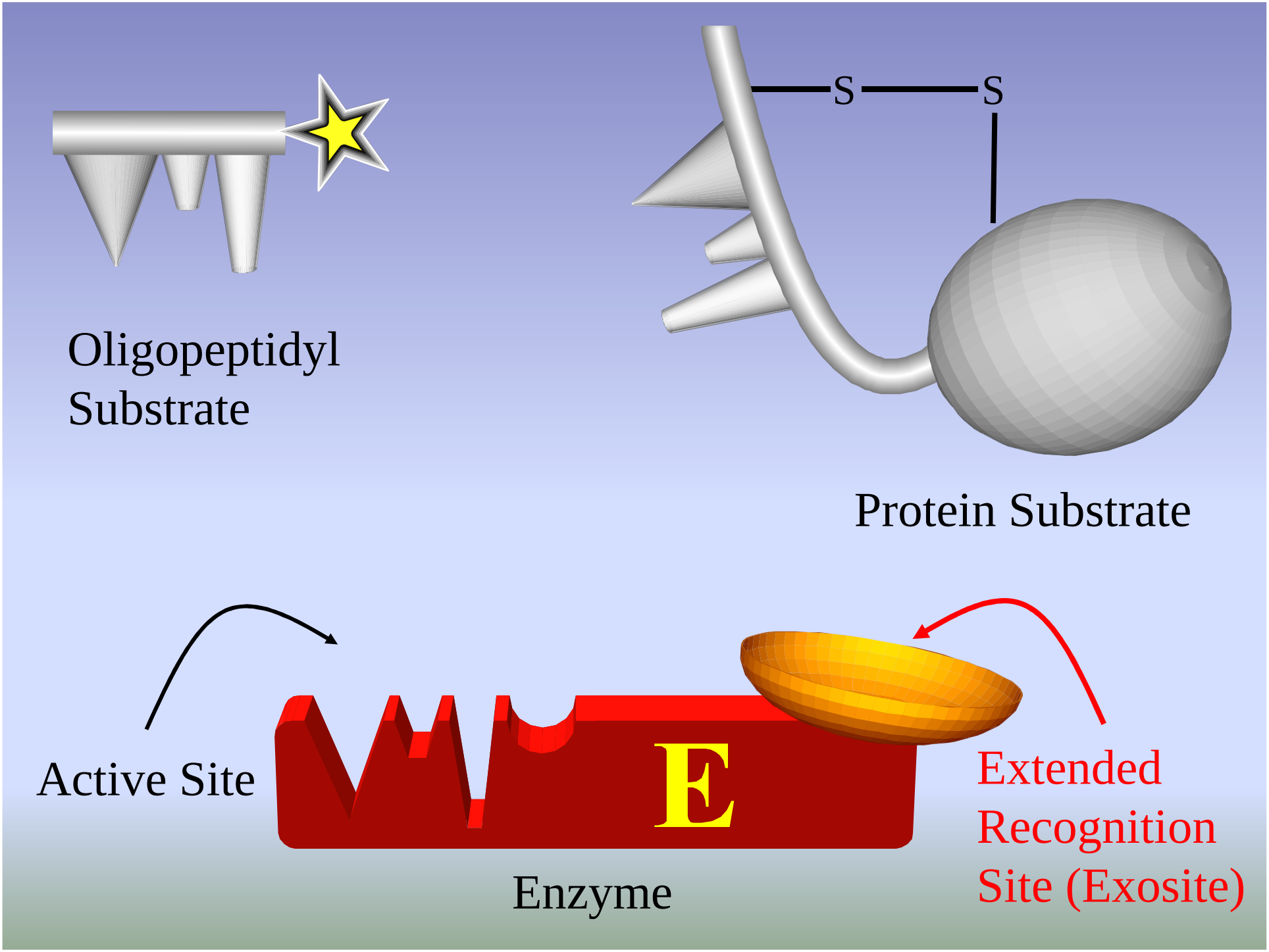


Table 1 Sites of cleavage in the human vitamin K-dependent zymogens*

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)

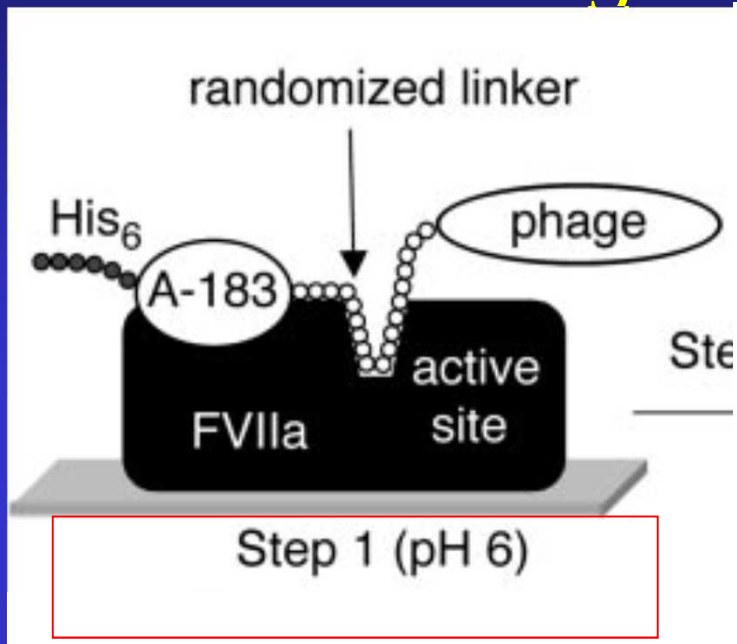
Inhibitors of Factor VIIa

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**	–		

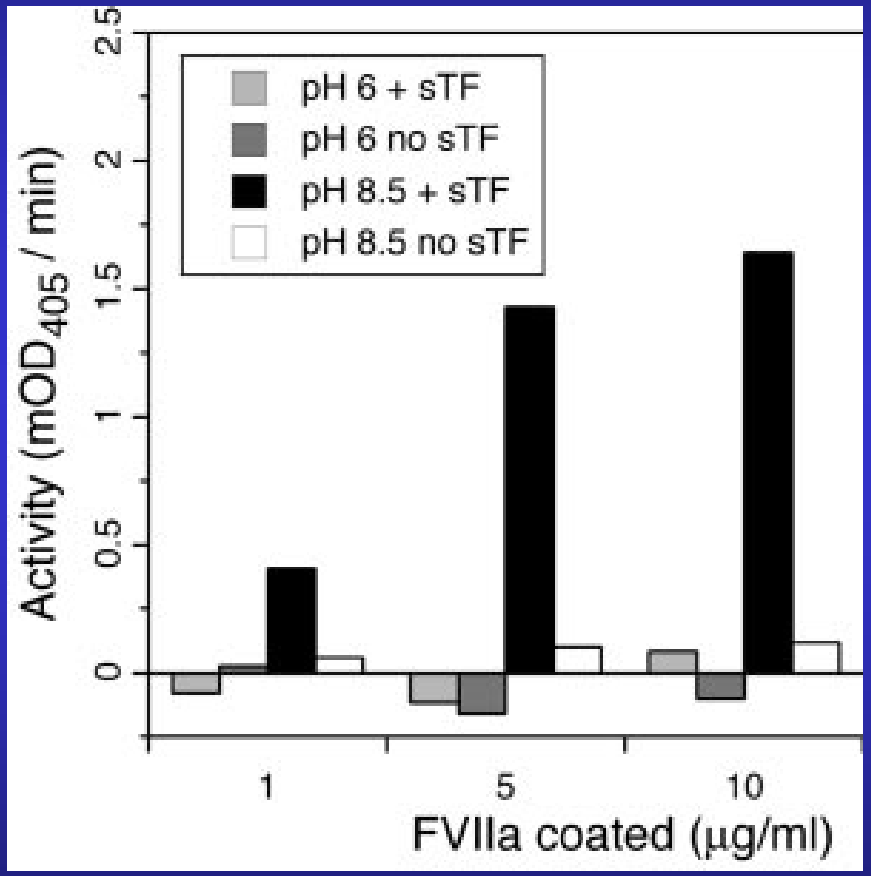
?

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

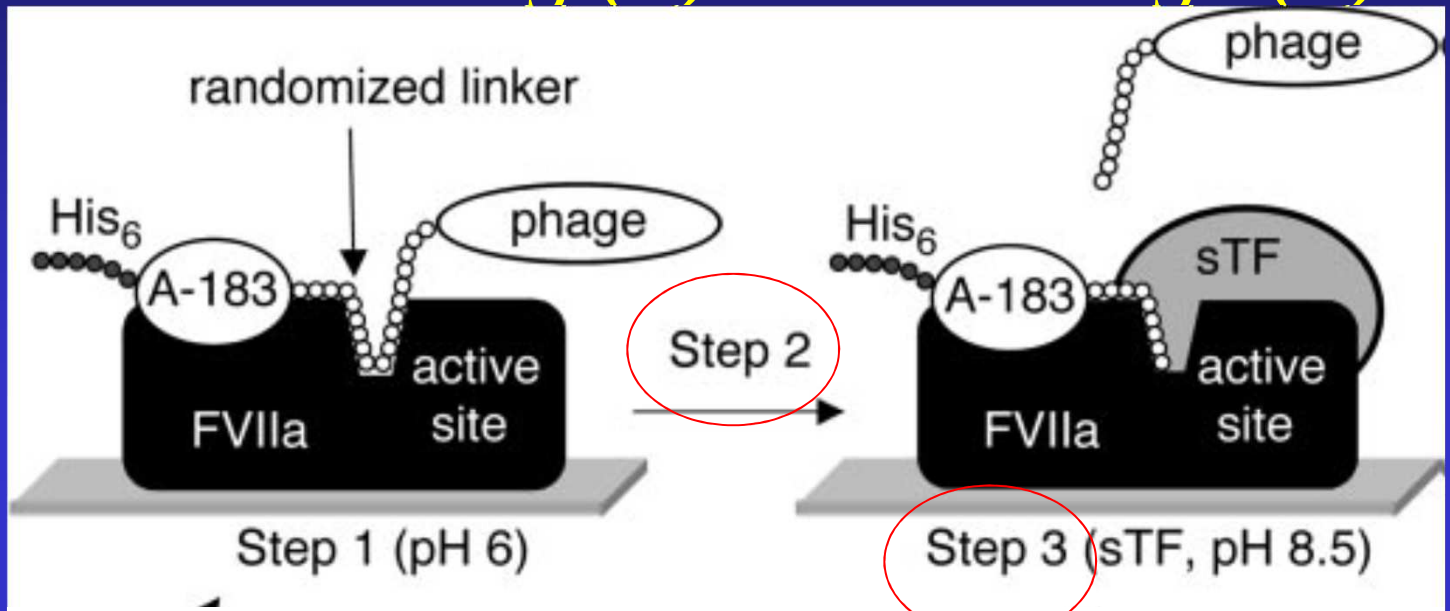
Peptide Inhibitors of Factor VIIa: Phage binding



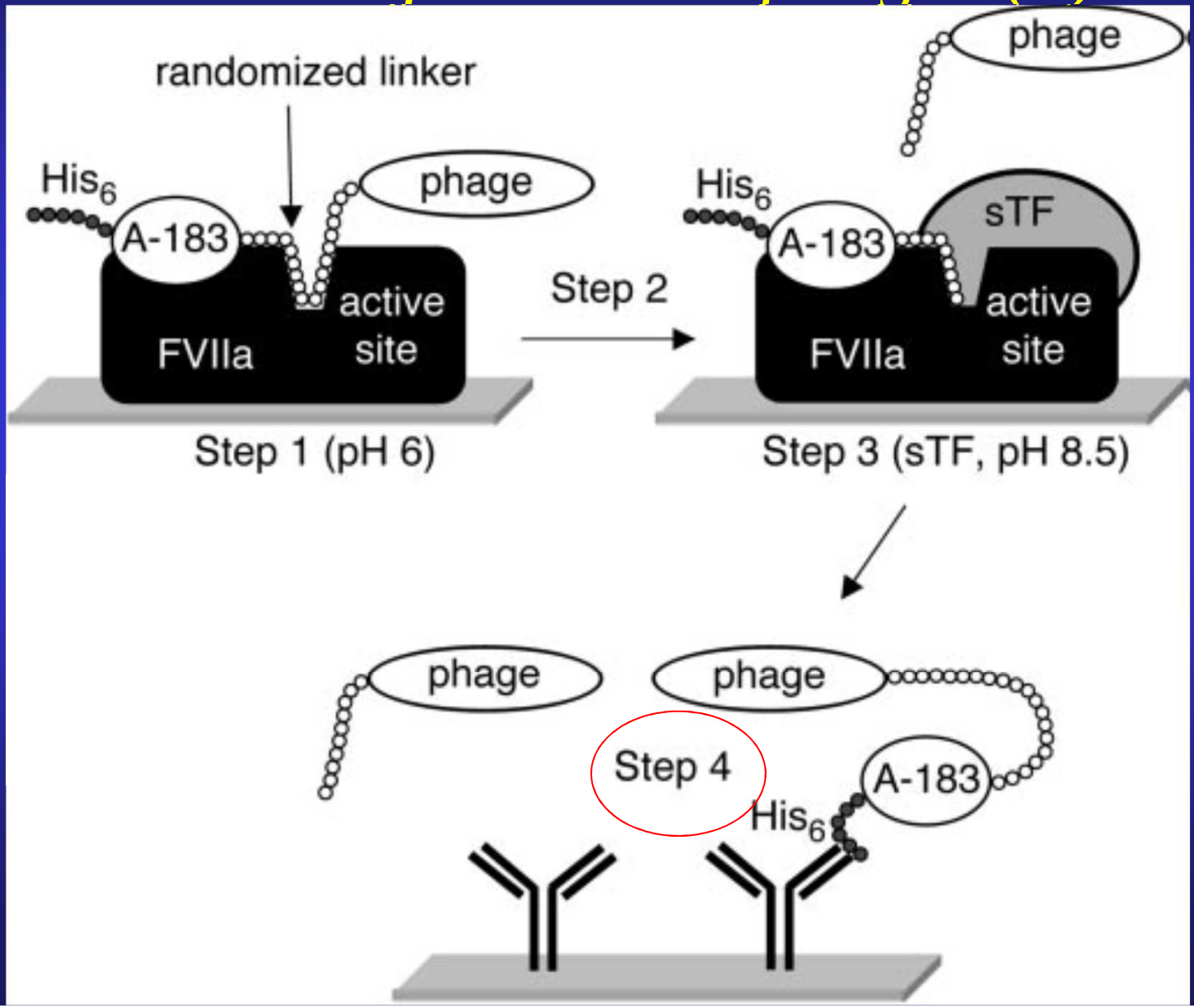
FVIIa Cleavage conditions



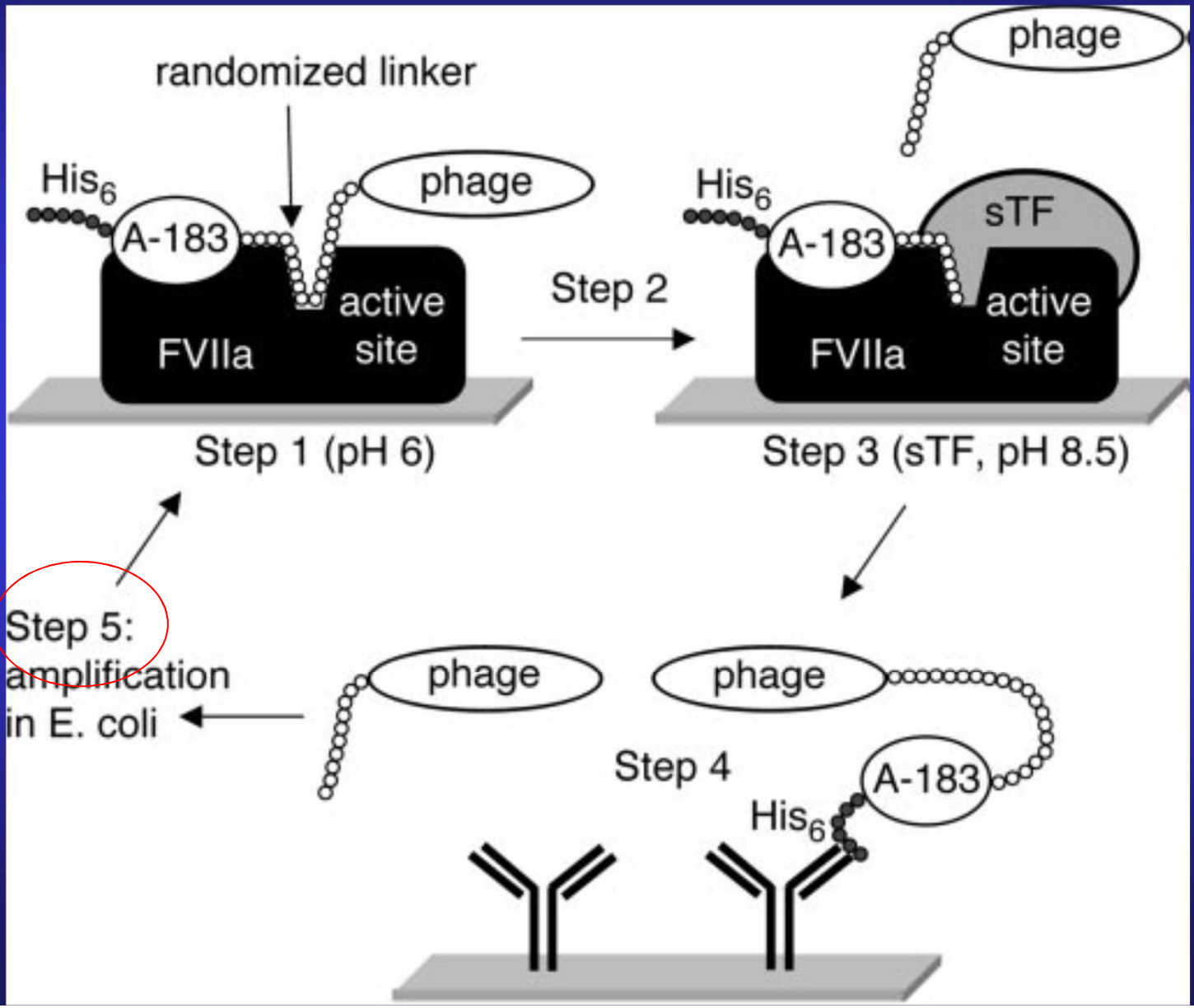
Peptide Inhibitors of Factor VIIa:washing (2) and cleavage (3)



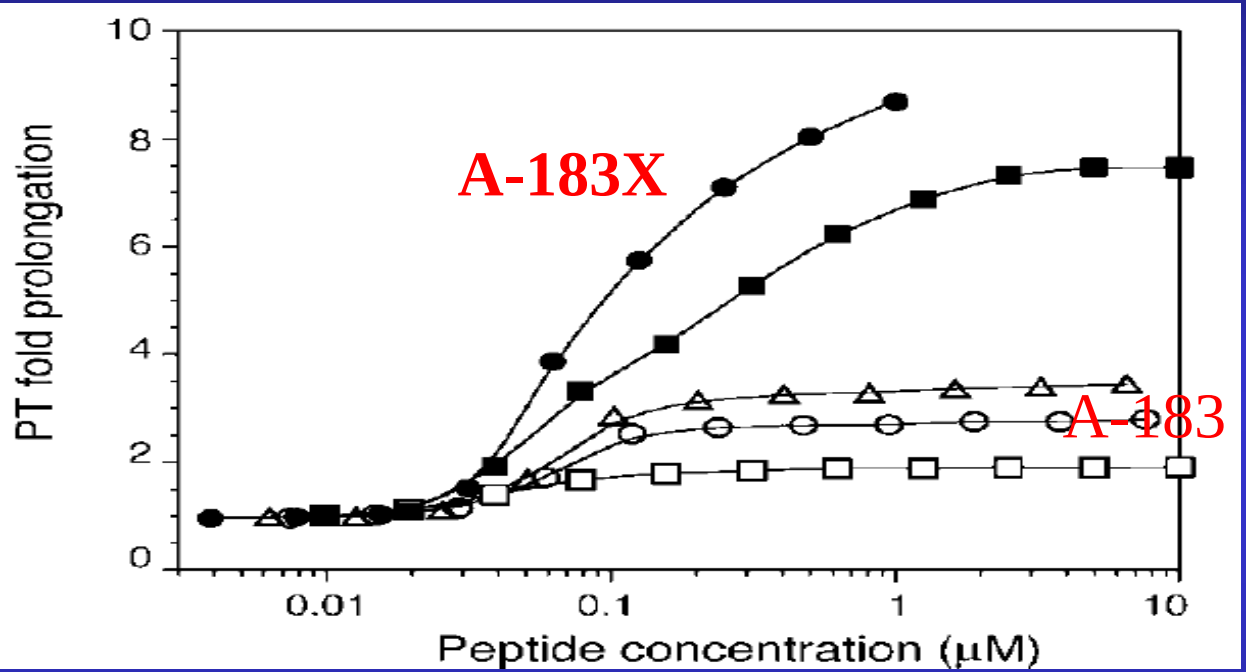
Peptide Inhibitors of Factor VIIa: removal of unbound phages (4)



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