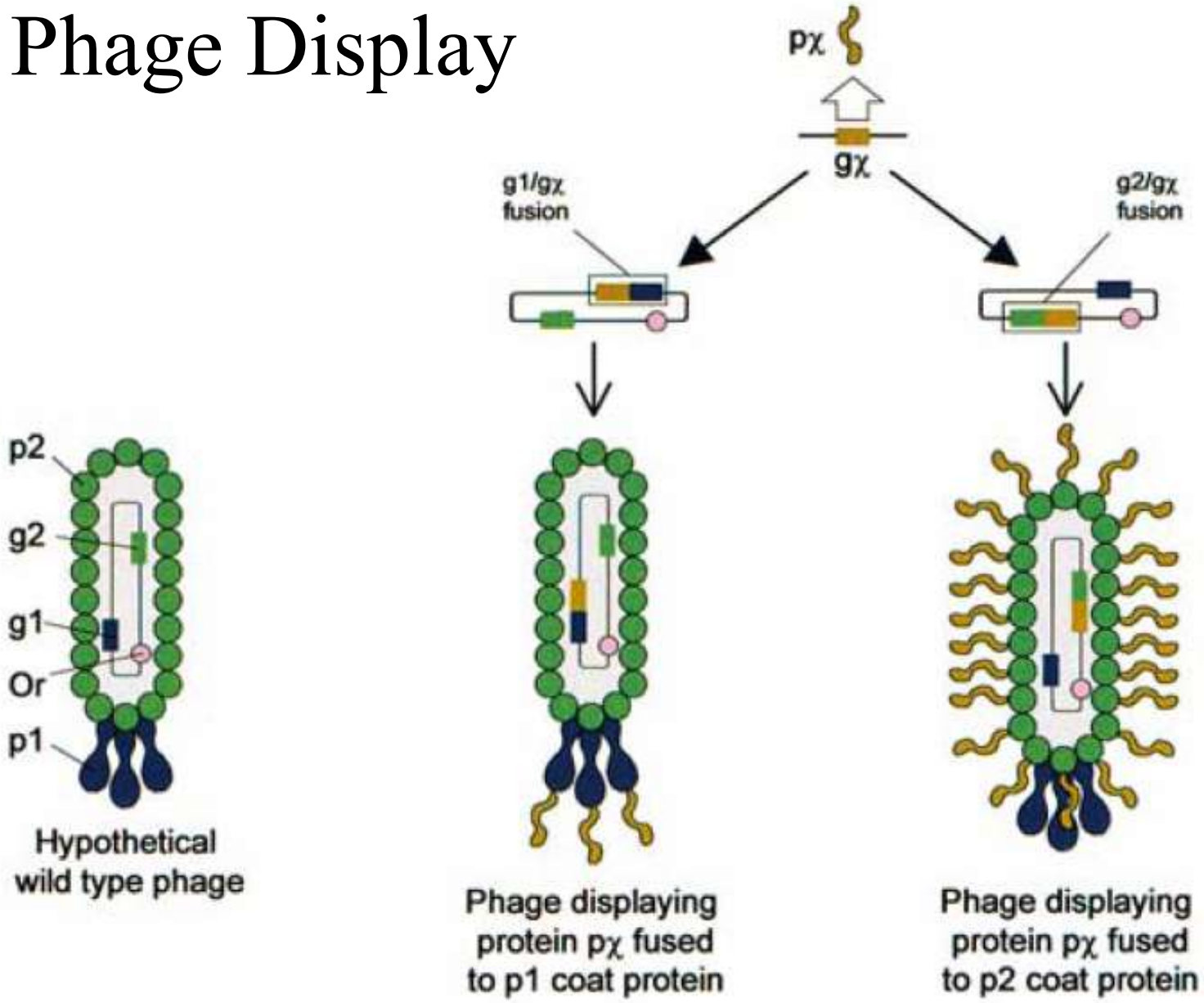
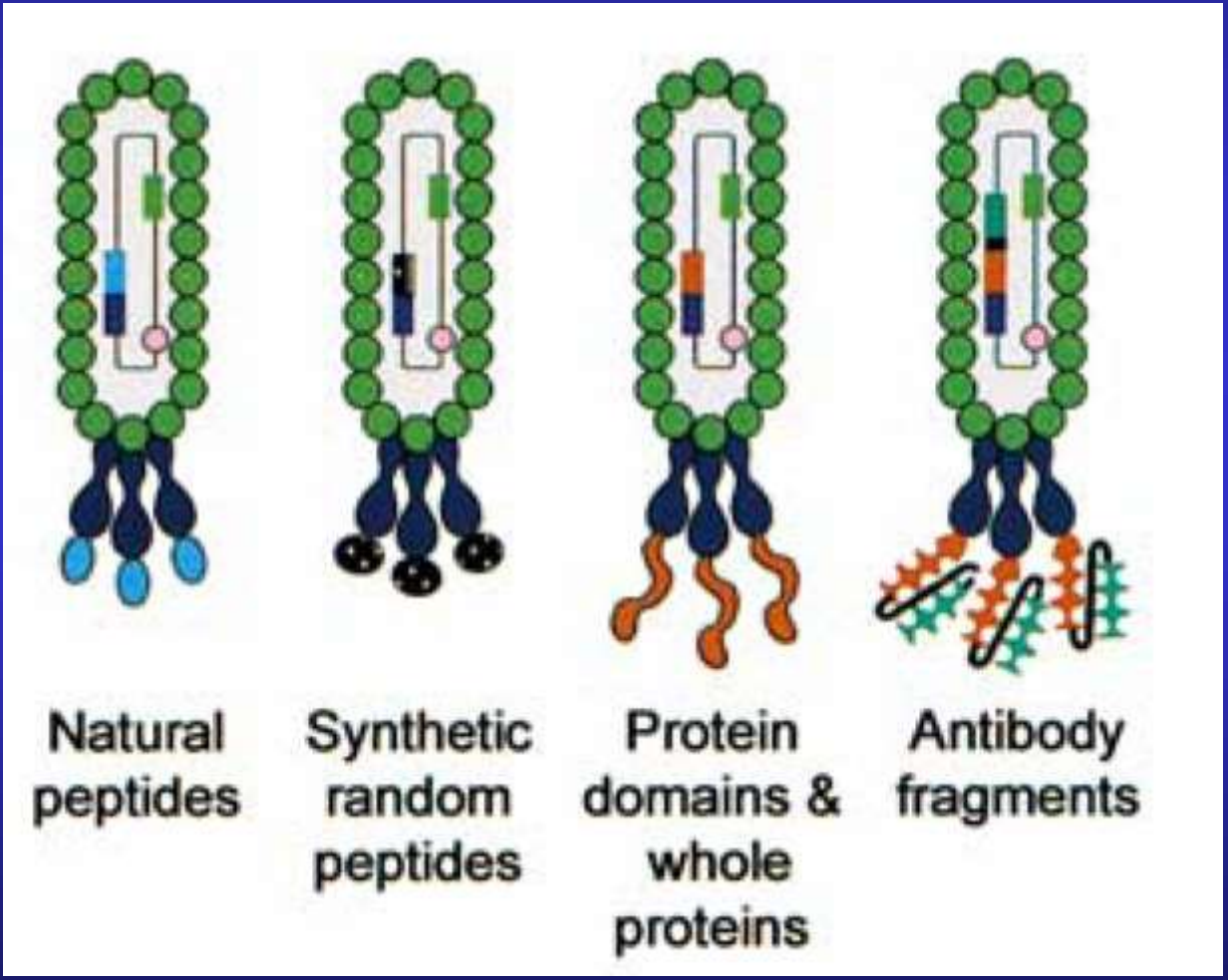
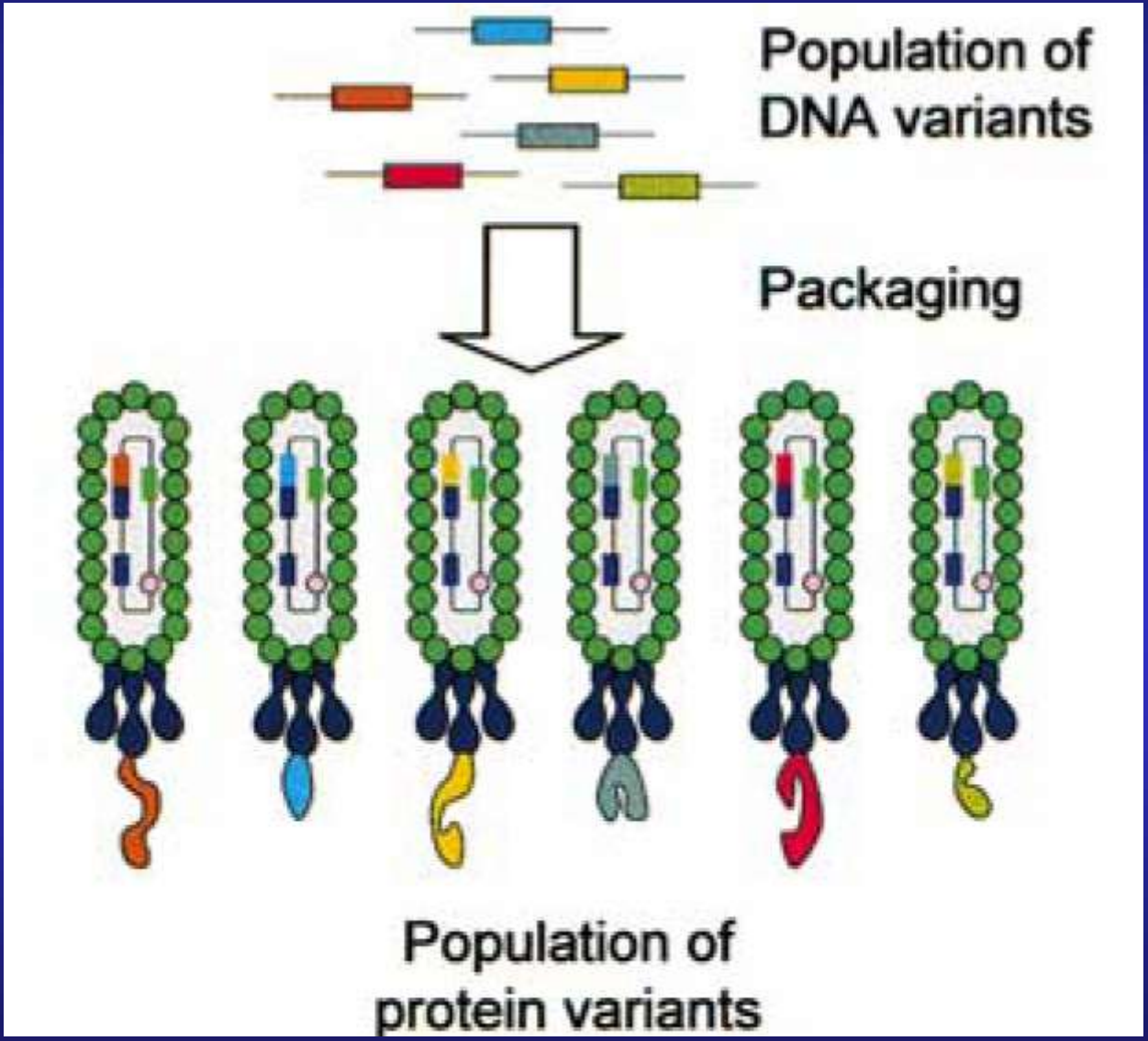
Phage Display



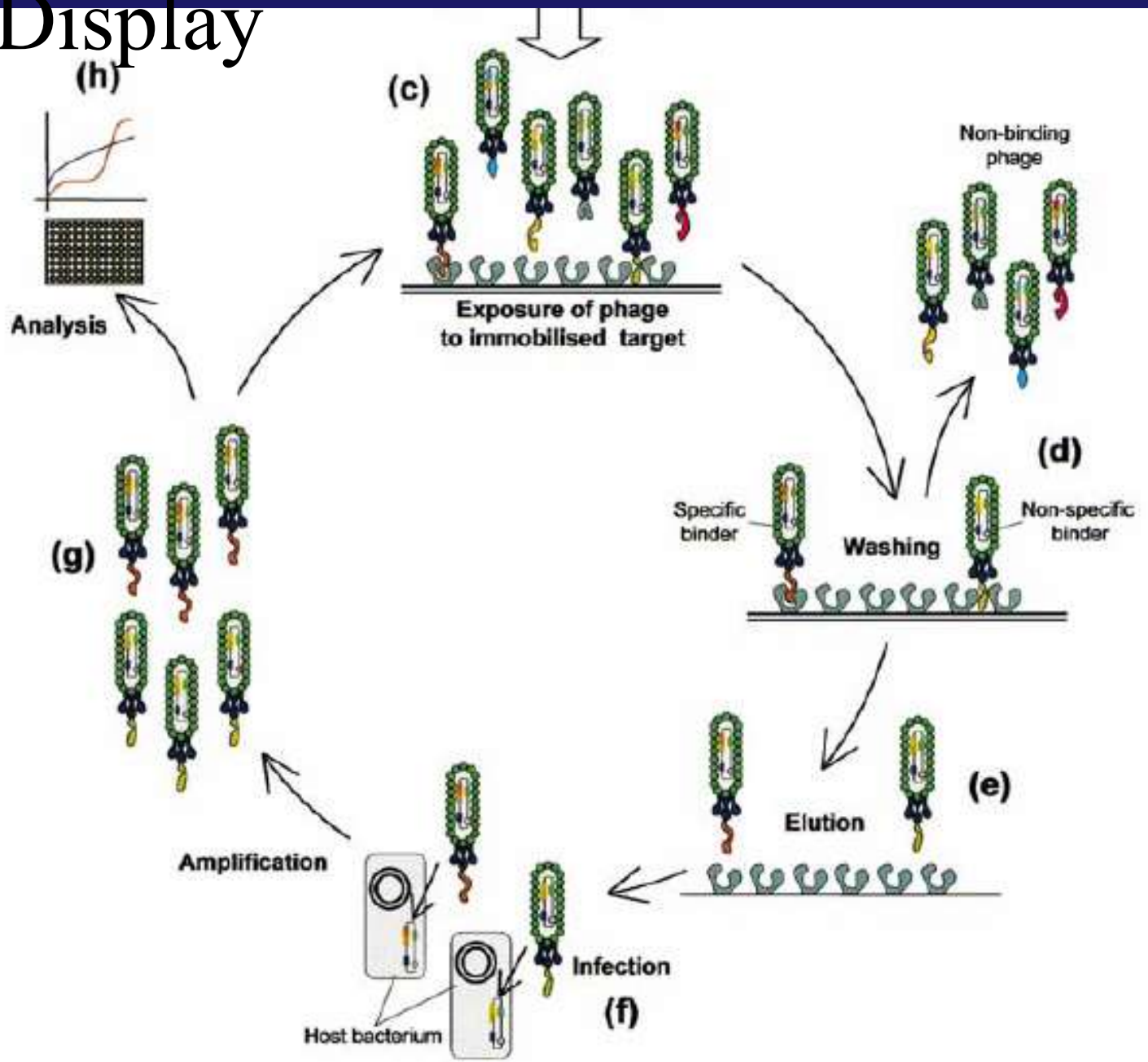
Phage Display

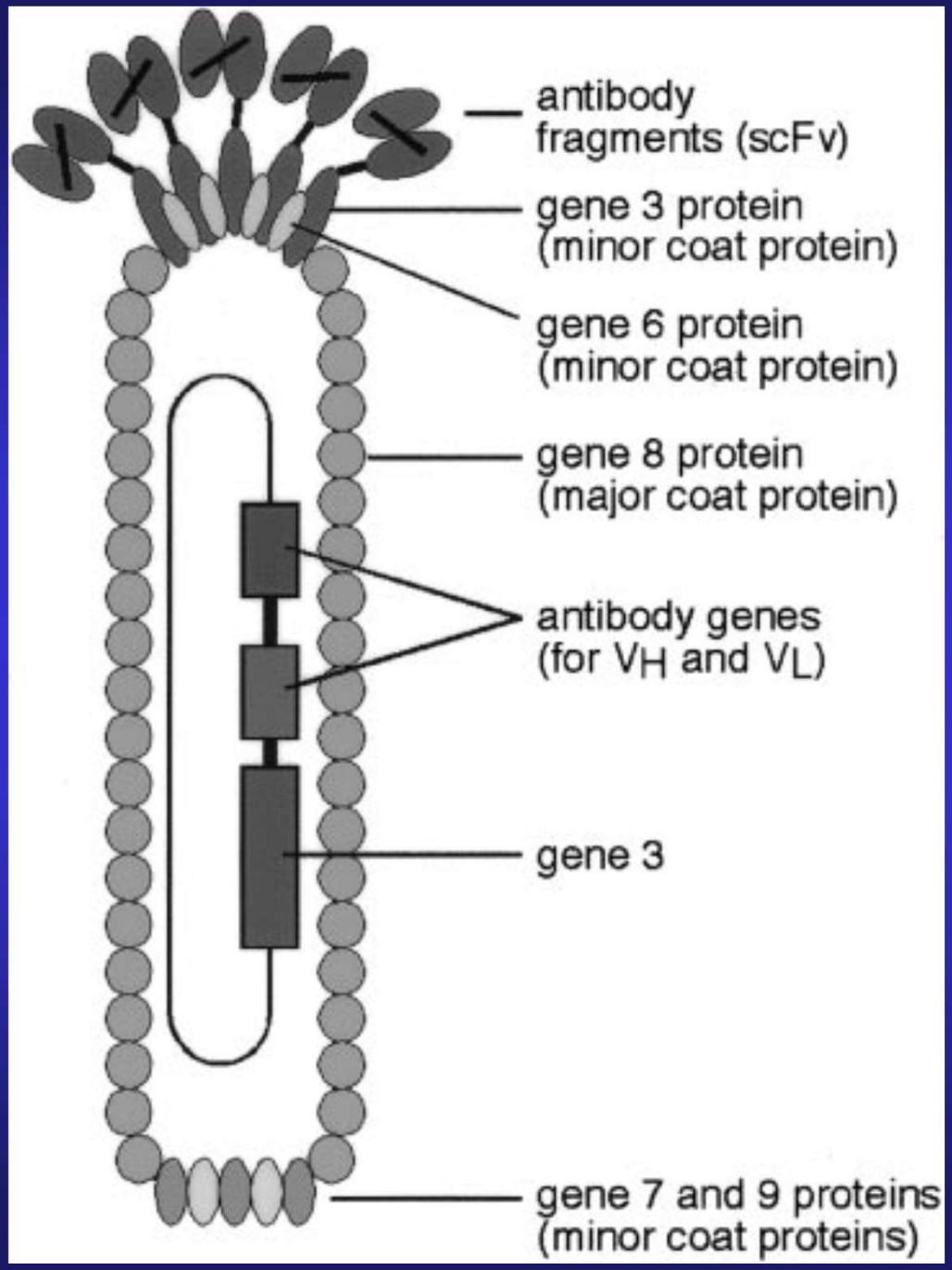


Phage Display



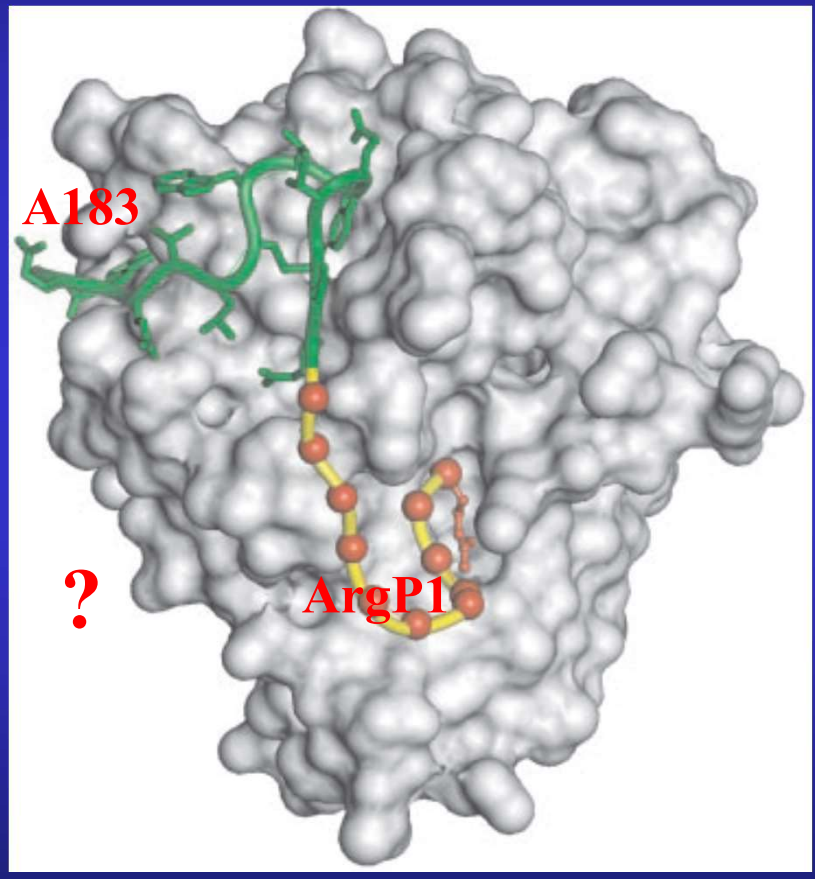
Phage Display




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 ?

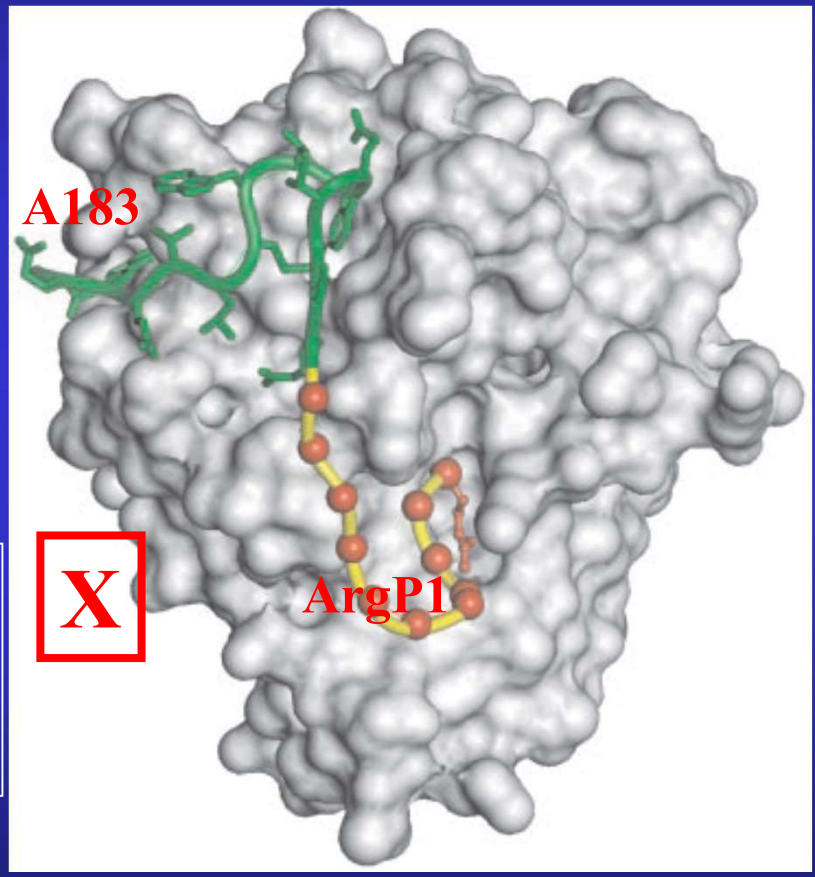
A183, 15mer,
EEWEVLCWTWETCER
exosite interactions



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

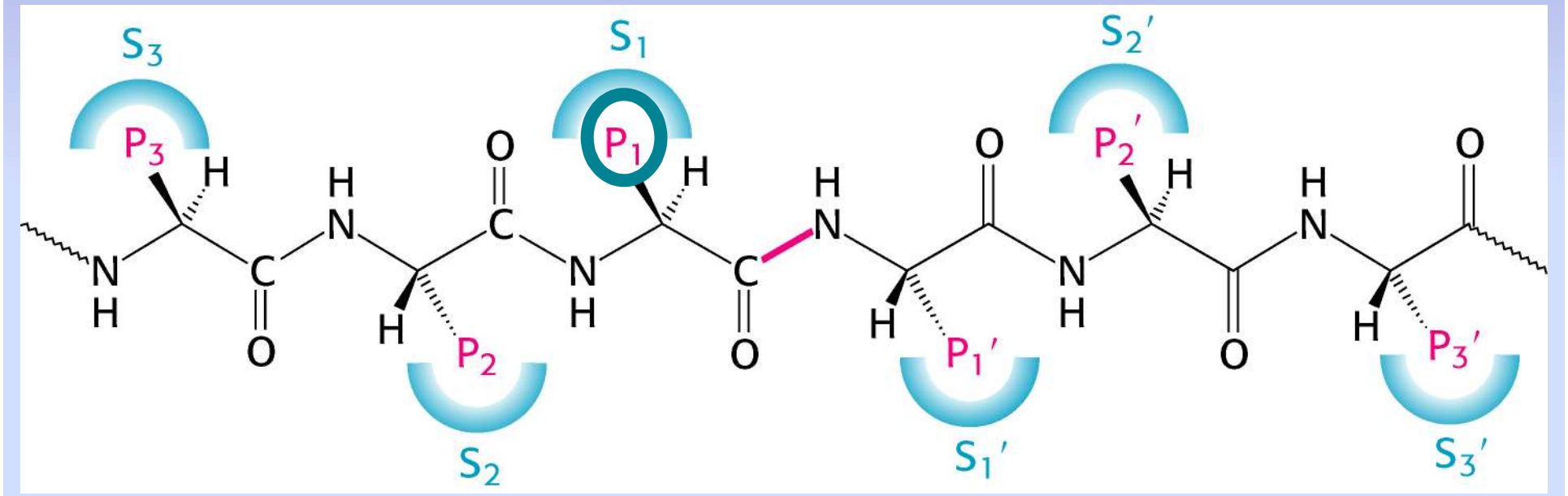
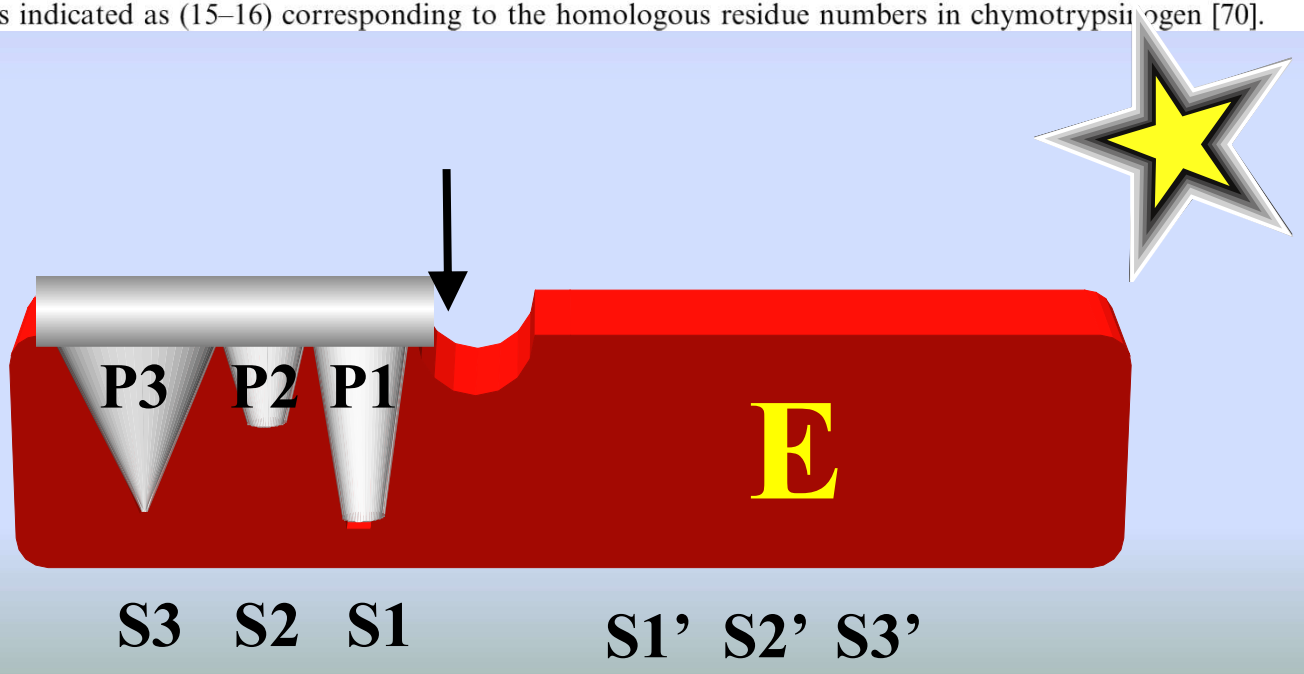
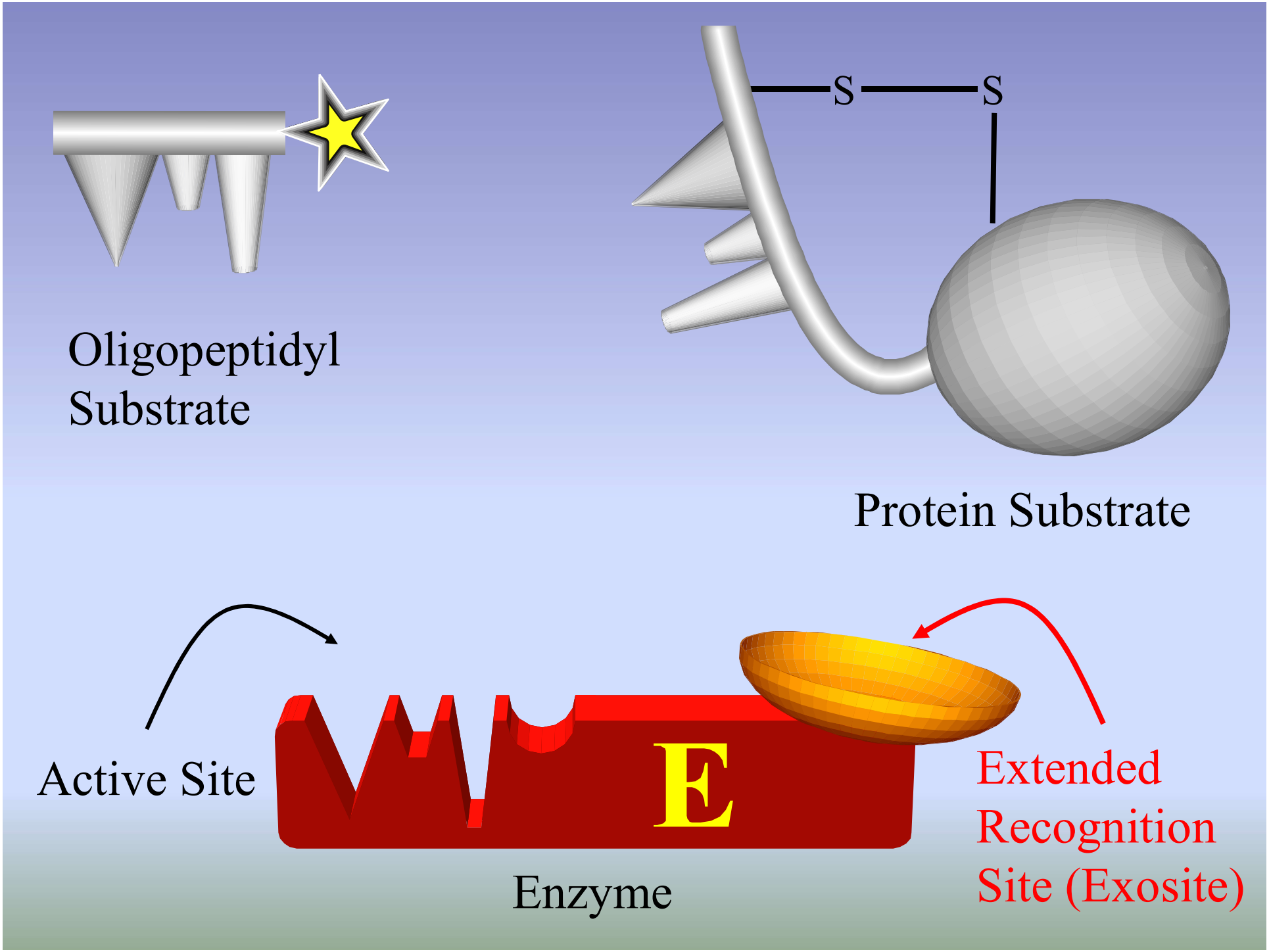


Table 1 Sites of cleavage in the human vitamin K-dependent zymogens*

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].



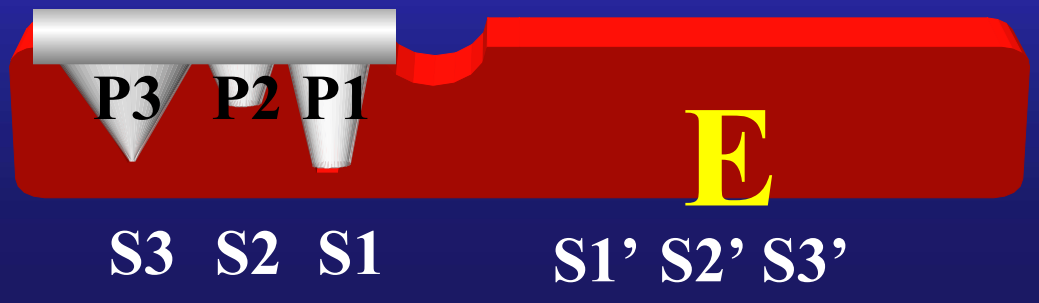

S
S
Oligopeptidyl
Substrate
Protein Substrate
Active Site
E
Enzyme
Extended
Recognition
Site (Exosite)

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

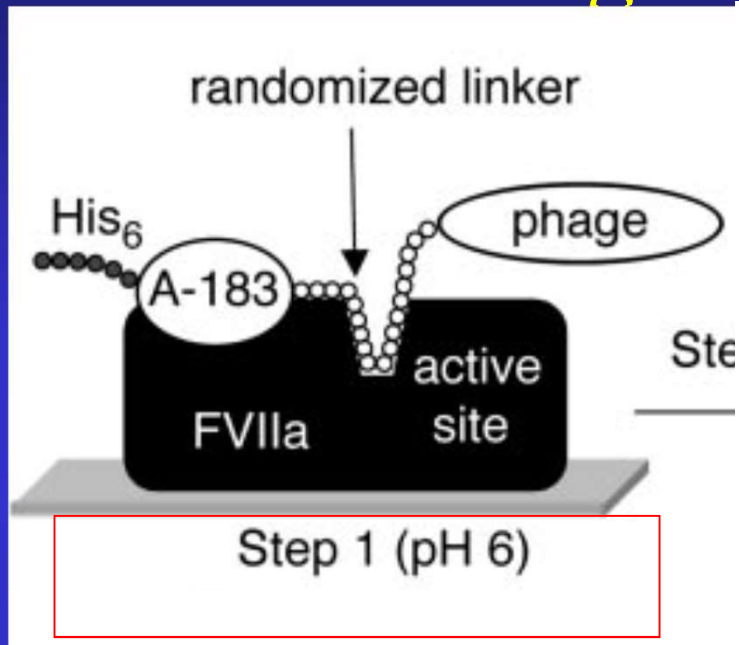
Inhibitors of Factor VIIa

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

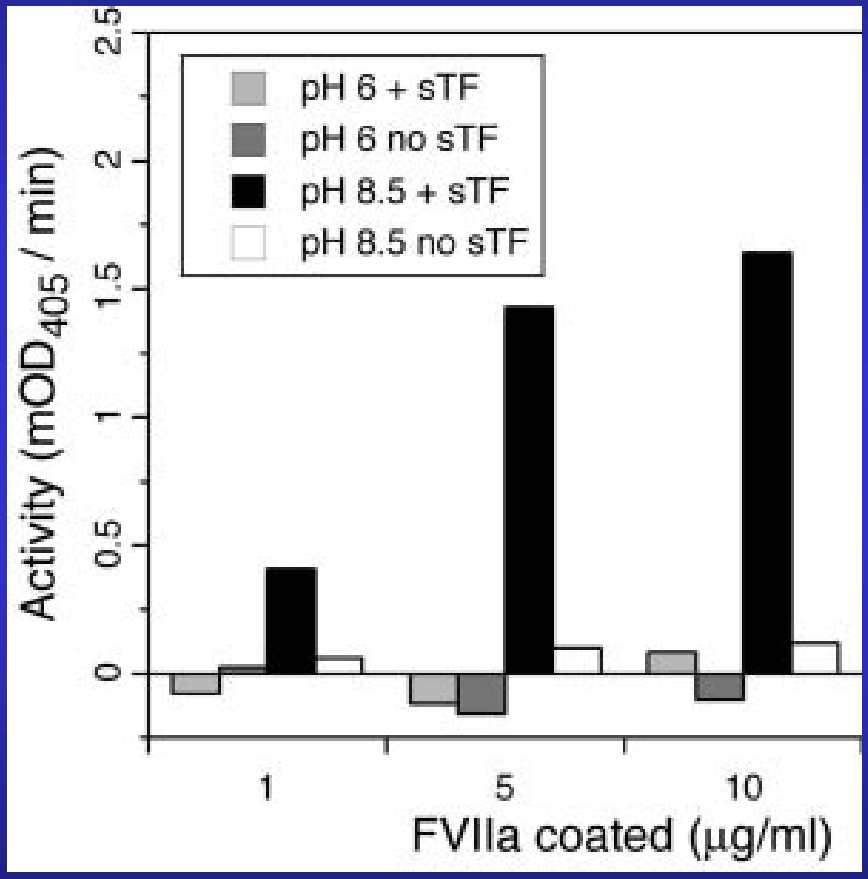


Peptide Inhibitors of Factor VIIa: Phage binding

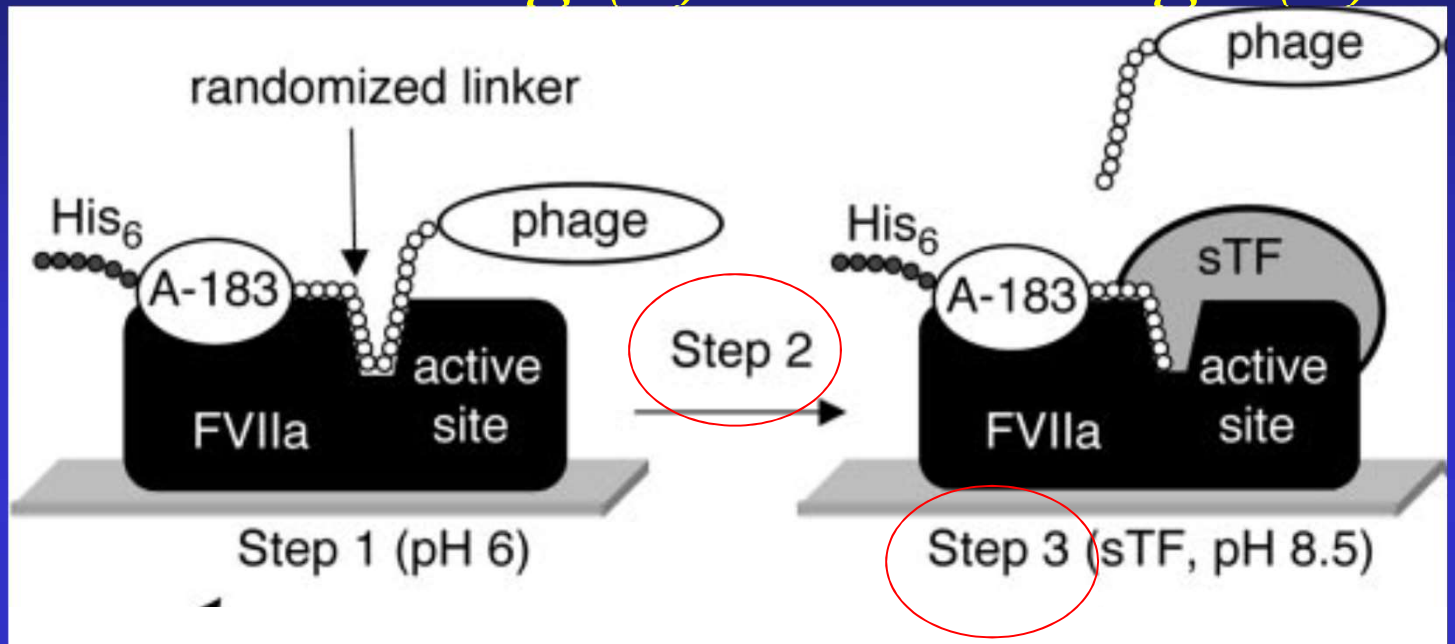


Unbound phage were removed by repetitive washing with binding buffer (step 2).

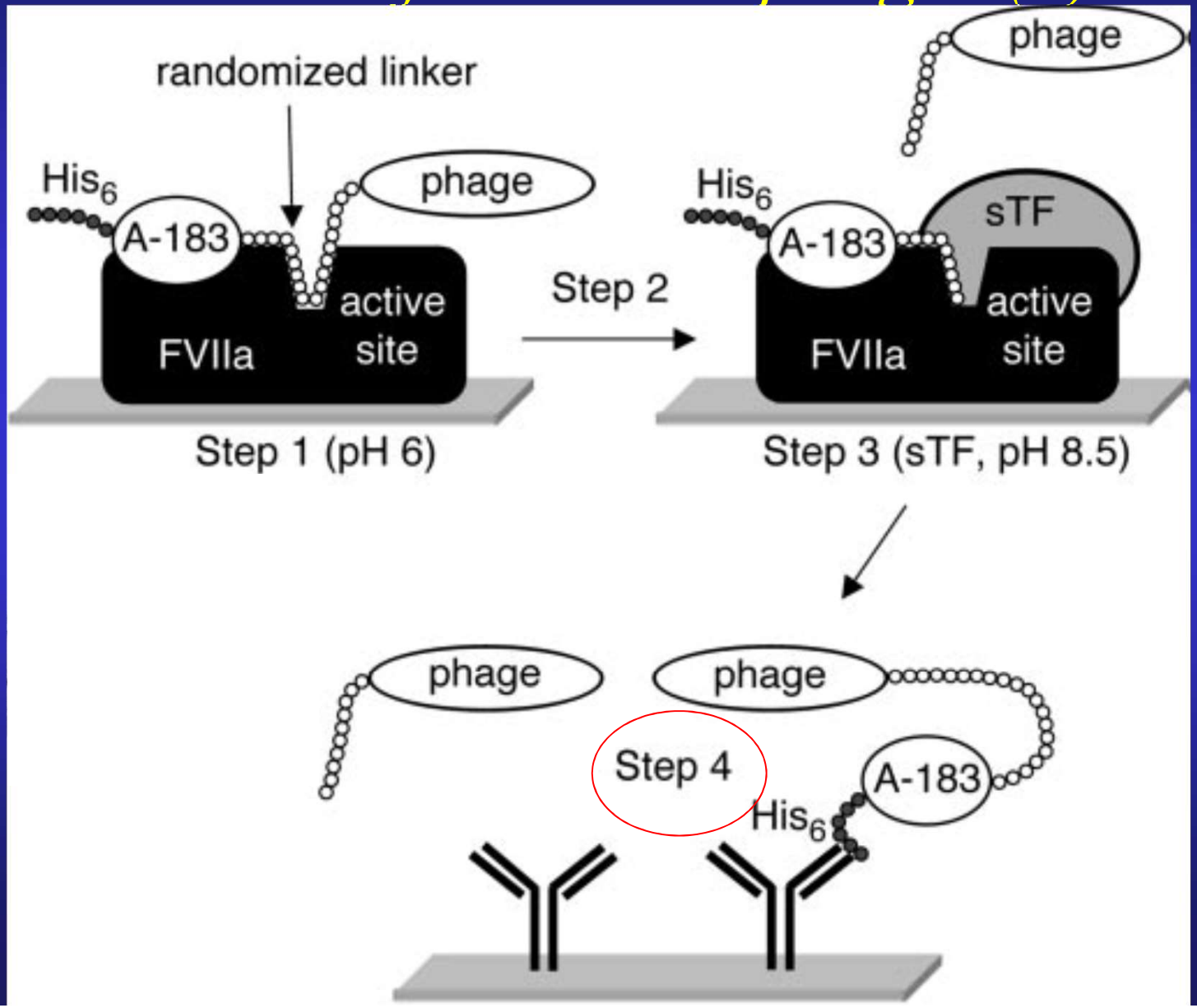
FVIIa Cleavage conditions



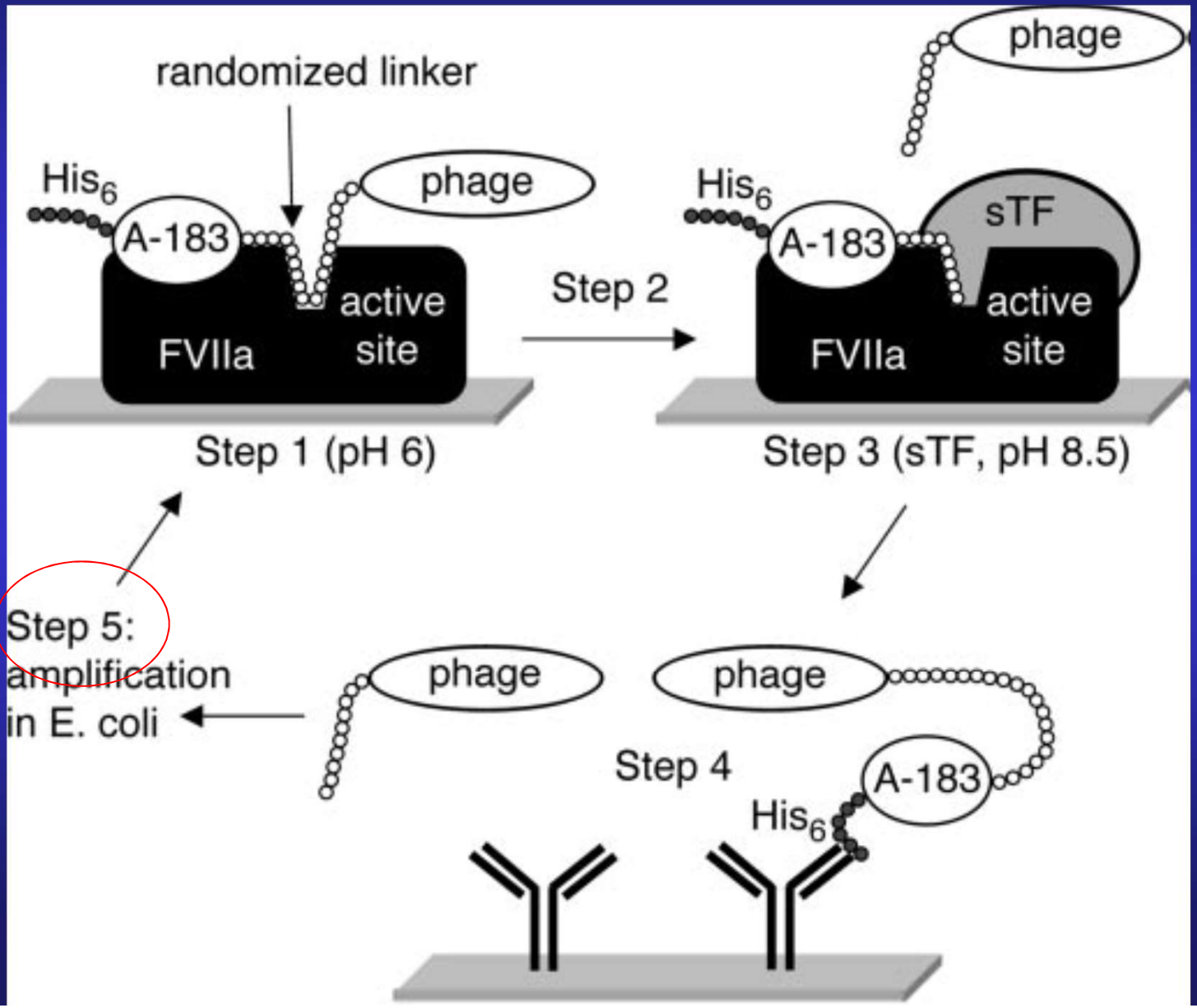
Peptide Inhibitors of Factor VIIa:washing (2) and cleavage (3)



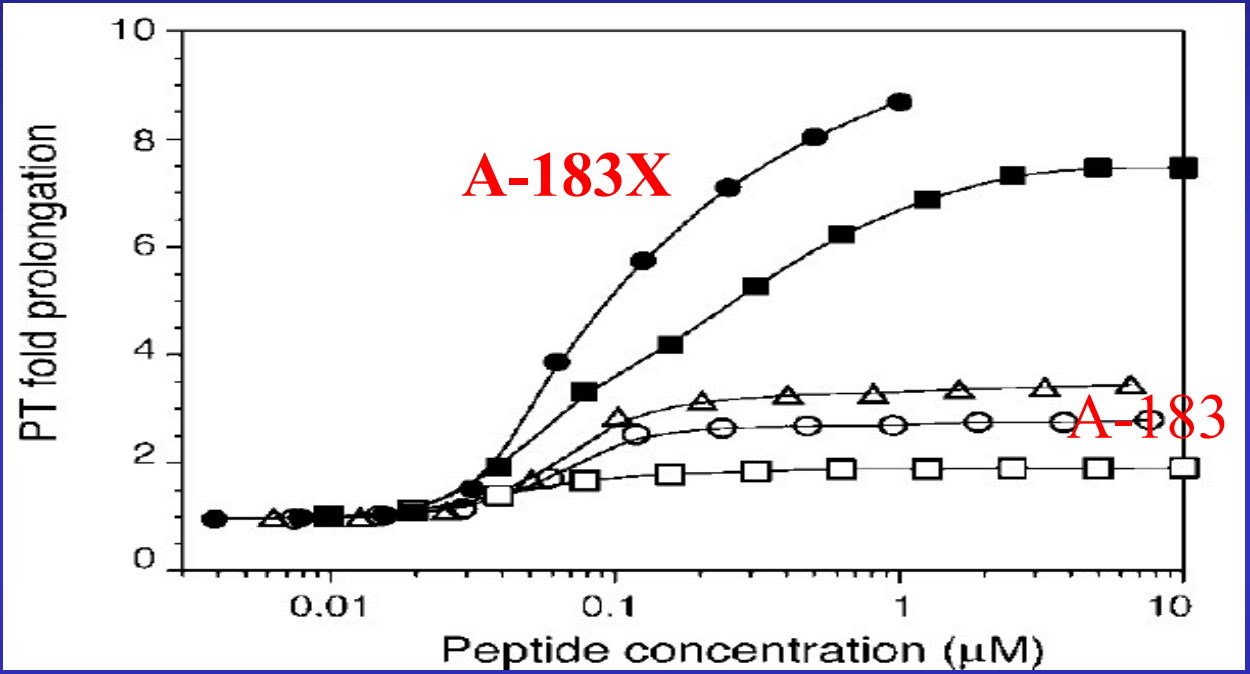
Peptide Inhibitors of Factor VIIa: removal of unbound phages (4)



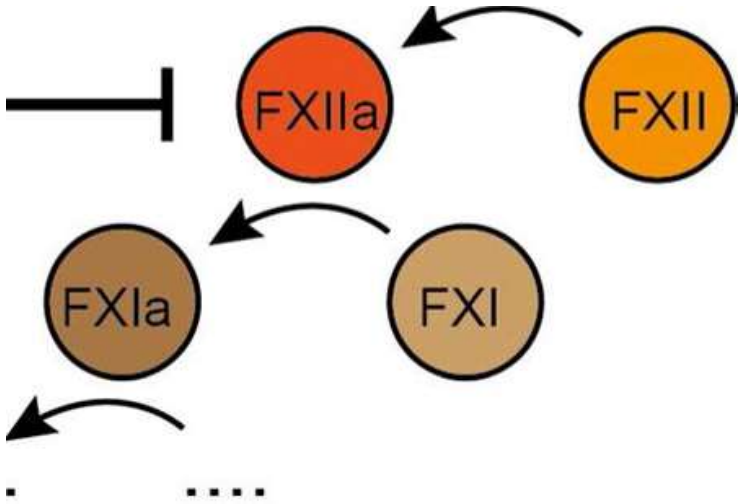
Peptide Inhibitors of Factor VIIa: propagation of selected phages and new rounds (5)



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



Published in: Vanessa Baeriswyl; Sara Calzavarini; Christiane Gerschheimer; Philippe Diderich; Anne Angelillo-Scherrer; Christian Heinis; *J. Med. Chem.* **2013,** 56, 3742-3746.

A

4X4 library

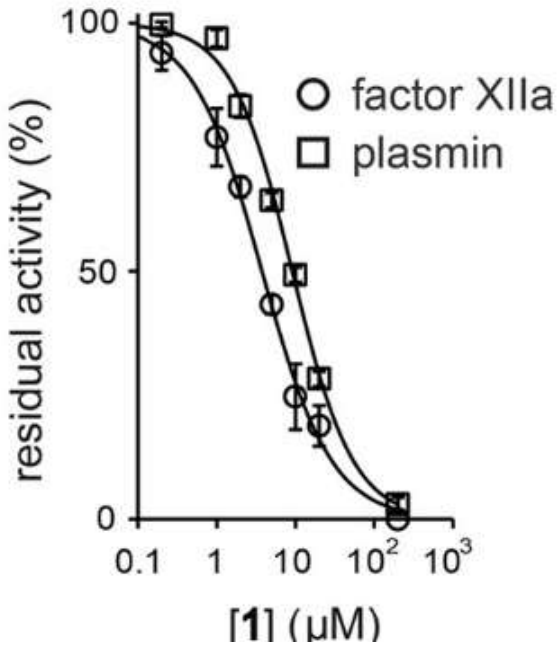
peptide:	sequence:	abundance:	K_i for FXIIa (μM):
FXII301	A C D A R P C P Q T Y C L	28	20.5 +/- 5.2
FXII302	Q C N A R P C P S S Y C R	2	4.7 +/- 1.5
FXII303	G C M G R P C P V S Y C F	2	5.0 +/- 1.3
FXII304 (**1**)	S C G G R P C P P A Y C K	22	3.1 +/- 0.5
FXII305	G C L G R P C P M A Y C S	13	5.0 +/- 1.5
FXII306	G C W A R P C P L A L C Q	1	10.2 +/- 4.6
FXII307	G C A A R P C P L T A C W	1	33.5 +/- 5.9
FXII308	G C H G R P C P L Q Y C K	1	11.2 +/- 4.4
FXII309	R C Y A N P C P I S Y C R	1	
FXII310	S C S G R R C P P S Y C K	1	7.8 +/- 3.2
FXII311	V C V Q K F C W R G W C P	4	> 75
FXII312	A C Q Q Q F C W R G W C P	2	
FXII313	L C E Y T L C W R G W C P	2	
FXII314	H C R Y V F C W R G W C P	2	
FXII315	N C V N R Y C W R G W C S	1	

Development of peptide inhibitor of FXII. All indicated inhibitory constants (*K*i) are averages of at least three measurements. Standard deviations are indicated. Amino acid similarities are highlighted in Rasmol color code.

(A)Peptide sequences isolated after three rounds of phage panning against β-FXIIa from peptide libraries

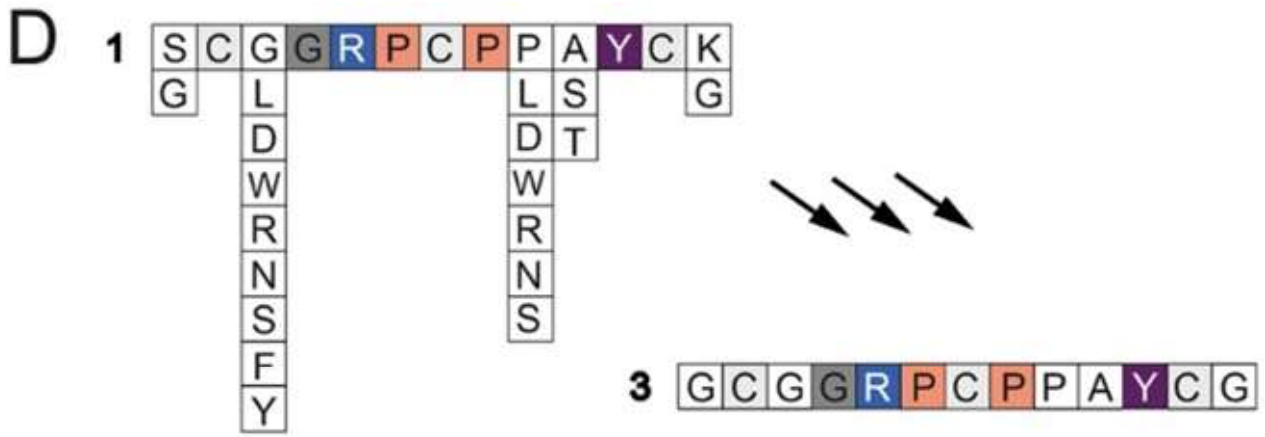
B peptide **1**

Protease	K_i (μM)
factor XIIa	3.1 +/- 0.5
tPA	> 120
uPA	> 120
factor XIa	> 120
PK	> 120
thrombin	> 120
plasmin	8.3 +/- 2.2
trypsin	> 120



Development of a peptide inhibitor of FXII.

Inhibitory activity of peptide 1 (clone FXII304) toward FXIIa and a panel of human proteases.
Inhibition of plasmin by FXII inhibitors

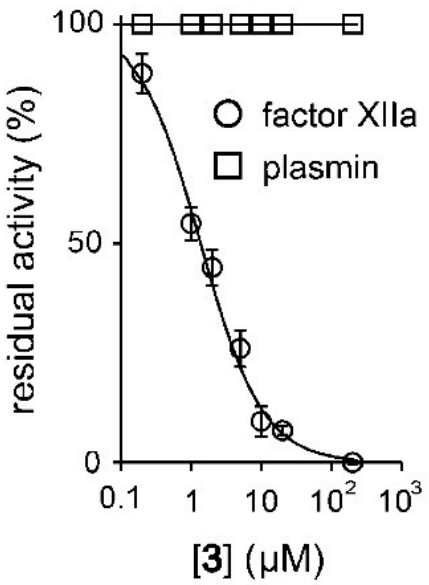


Affinity maturation of peptide 1. A range of peptides with amino acid substitutions in the indicated positions were synthesized and tested and led to peptide 3 (clone FXII402) having a 3-fold improved Ki

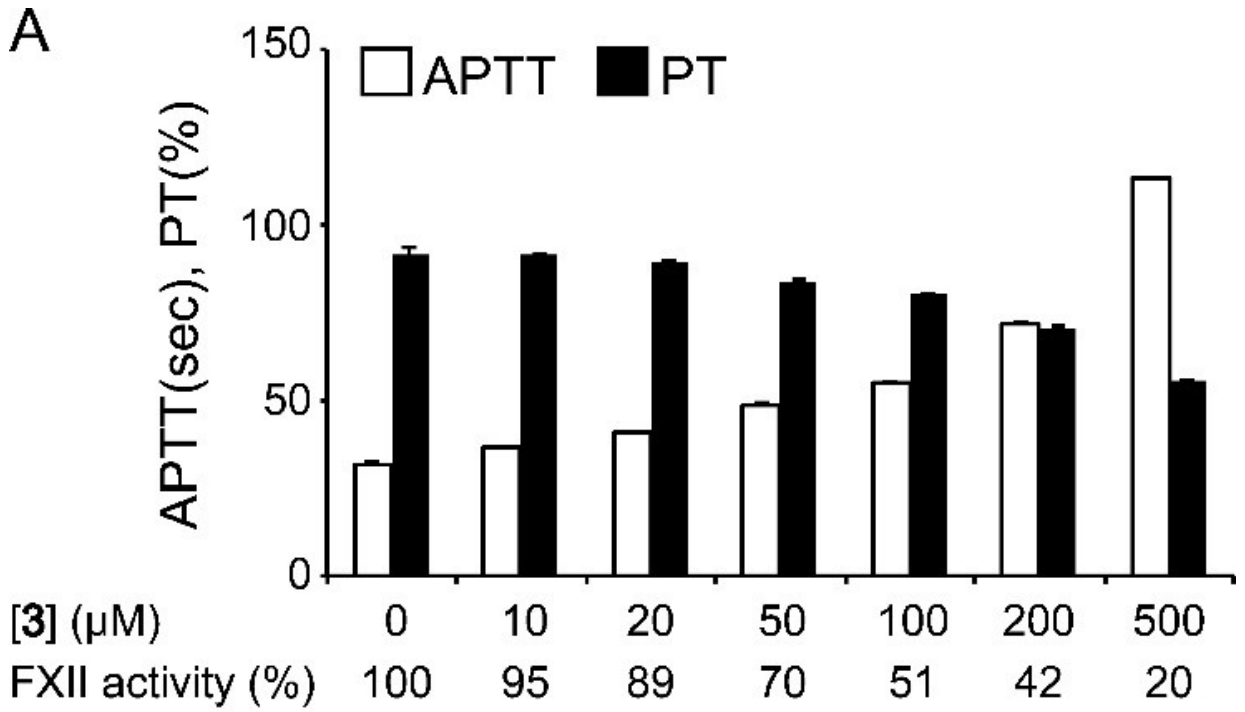
A

peptide **3**

Protease	K_i (μM)
factor XIIa	1.2 +/- 0.2
tPA	> 120
uPA	> 120
factor XIa	> 120
PK	> 120
thrombin	> 120
plasmin	> 120
trypsin	> 120



Inhibitory activity of peptide 3 toward FXIIa and a panel of human proteases



Coagulation times (aPTT- intrinsic FXII dependent, PT – extrinsic Tissue Factor dependent) and FXII activity in the presence of the engineered FXII inhibitor peptide **3**