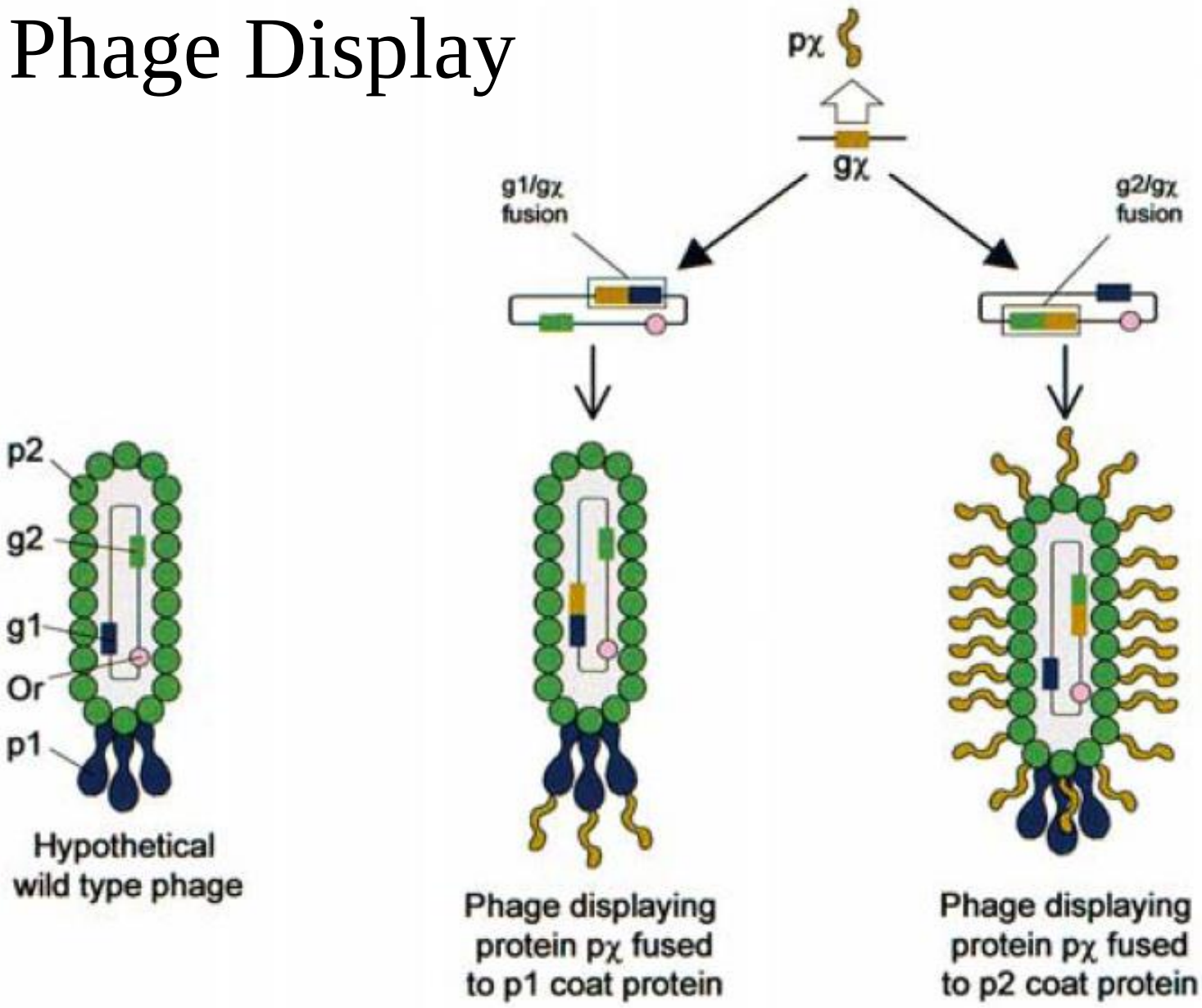
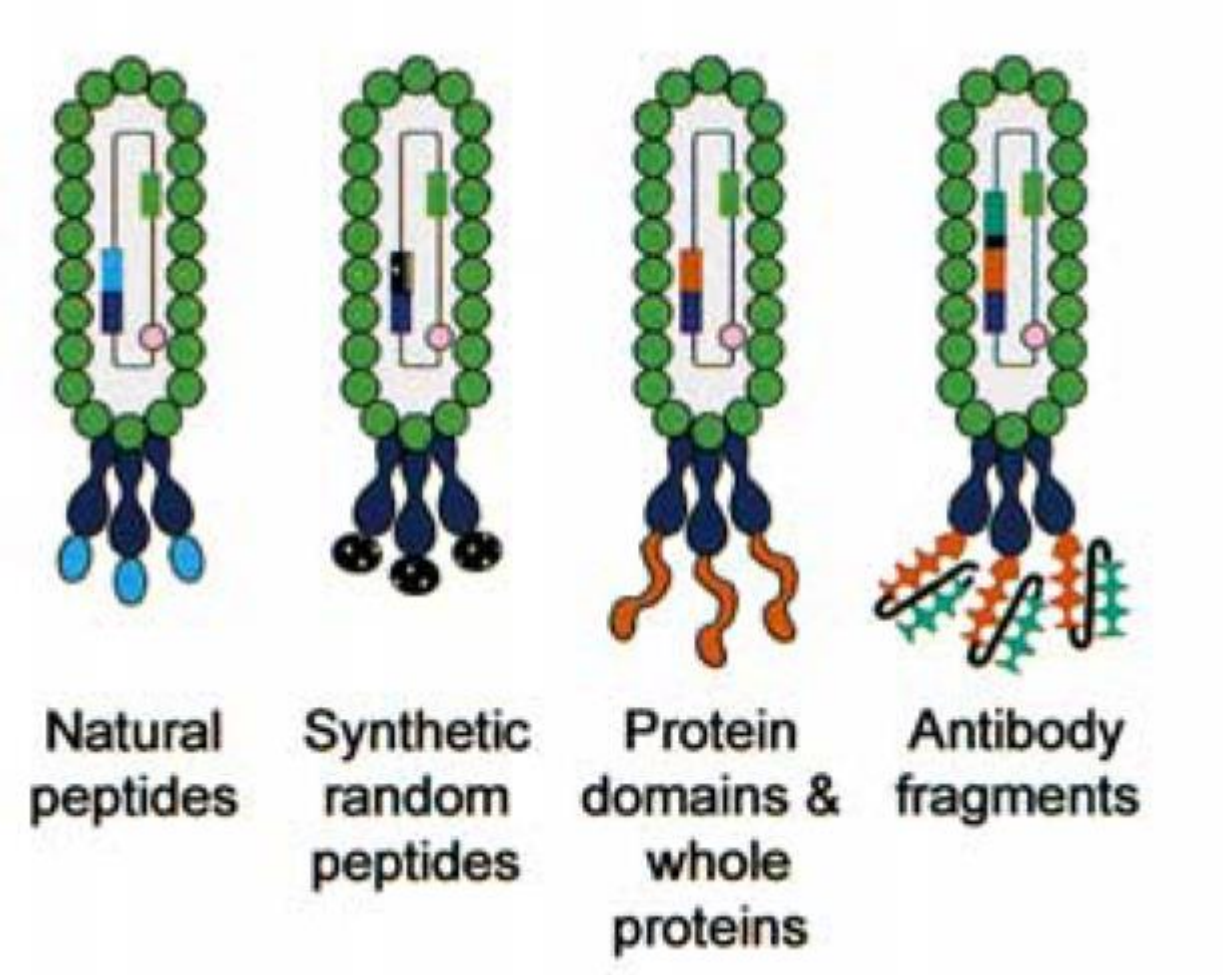
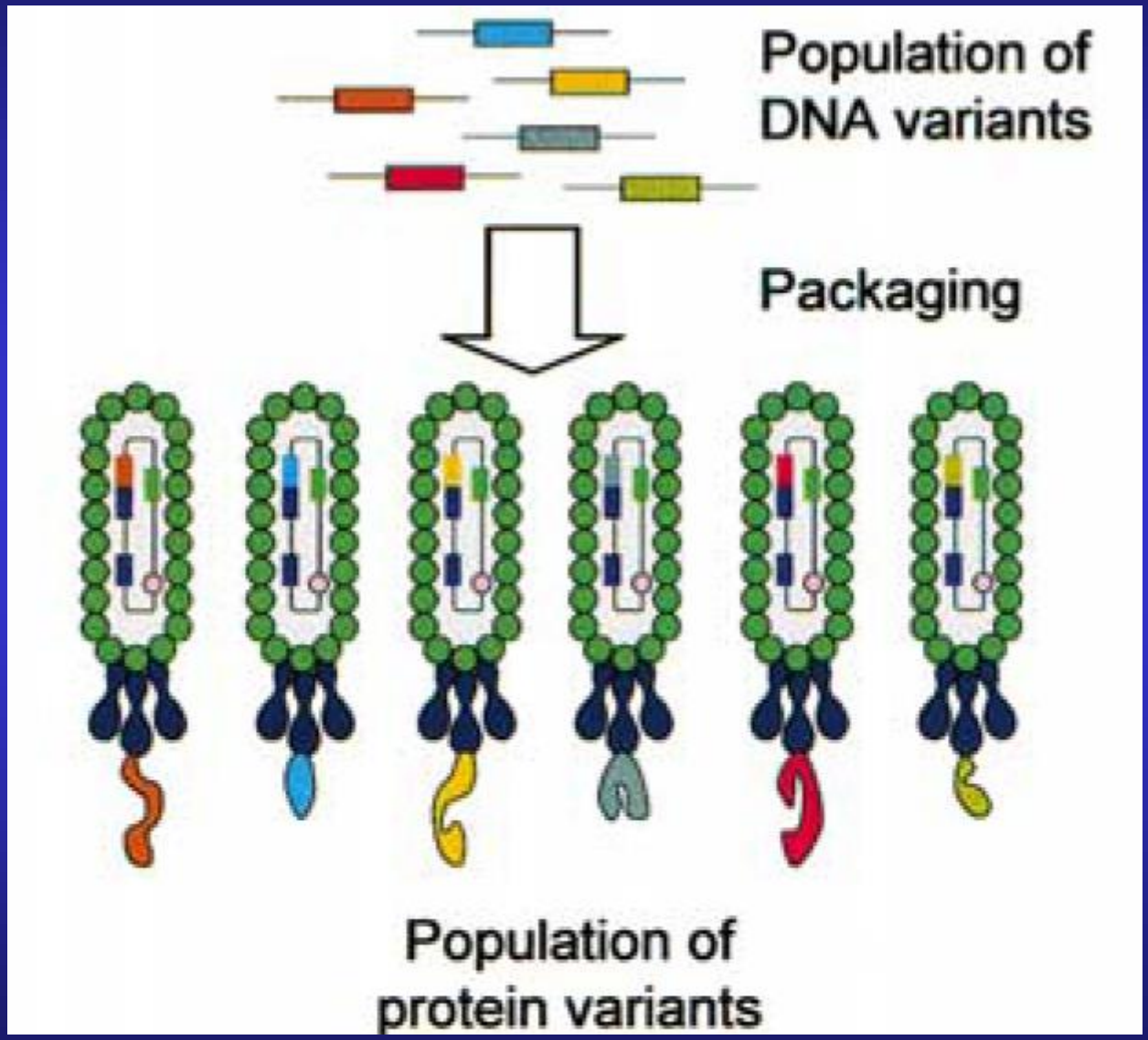
Phage Display



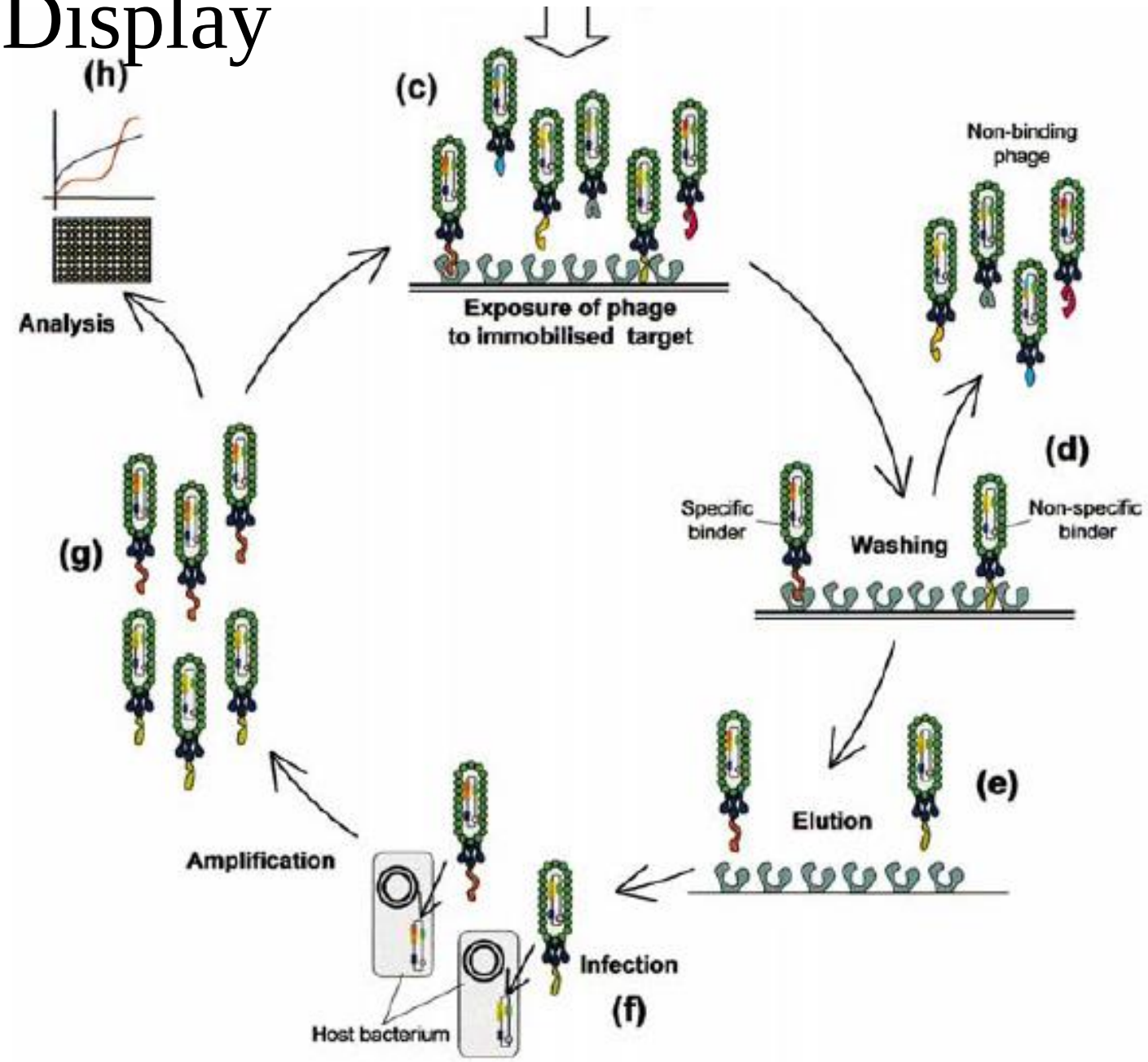
Phage Display

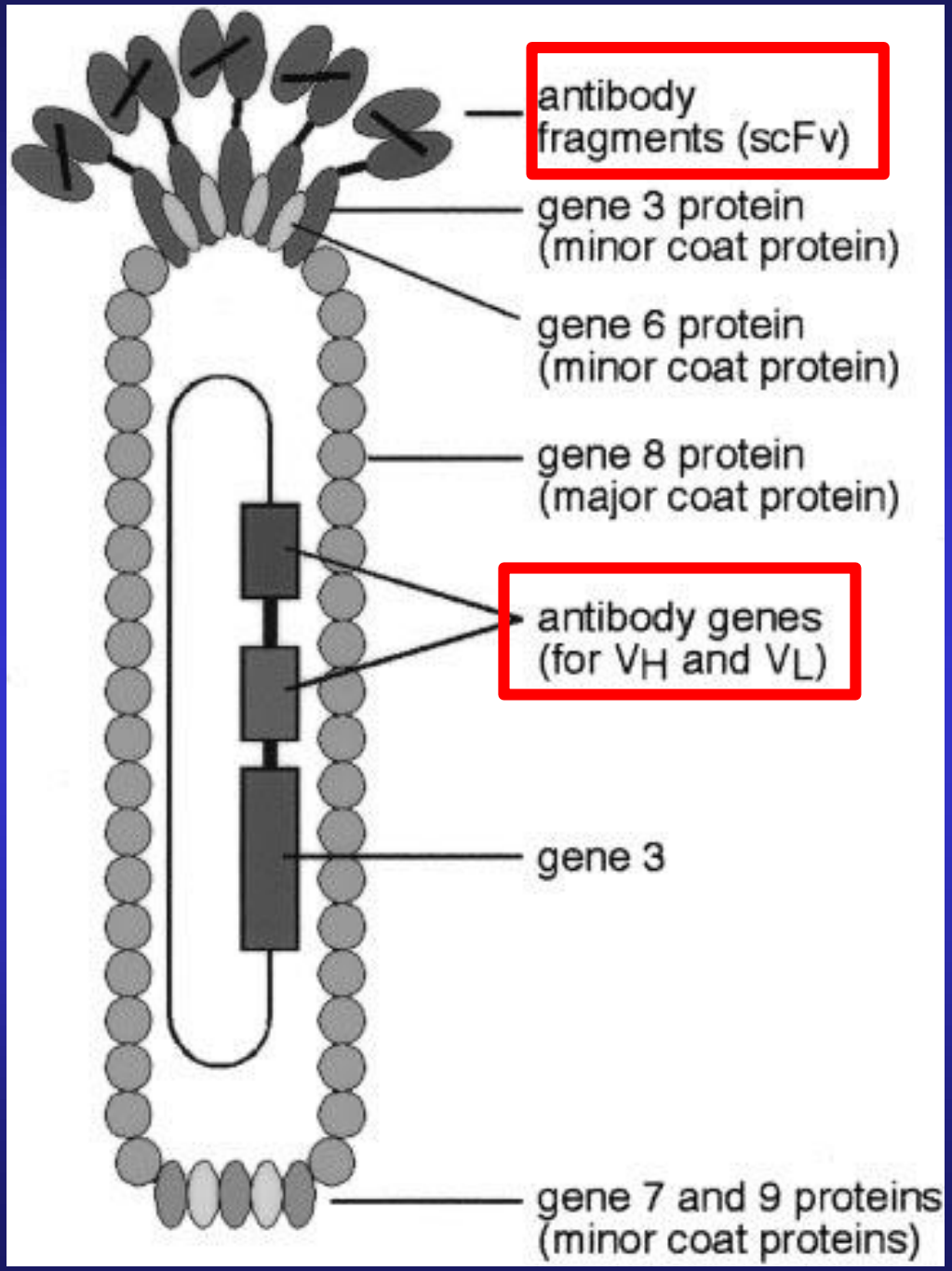


Phage Display



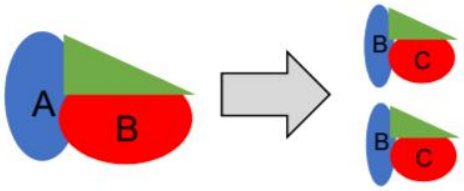
Phage Display

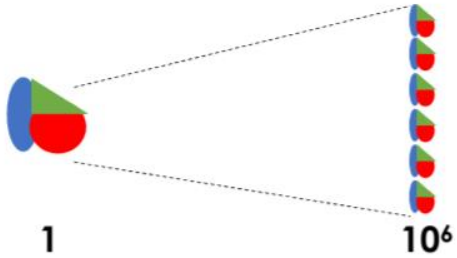



antibody fragments (scFv)
gene 3 protein (minor coat protein)
gene 6 protein (minor coat protein)
gene 8 protein (major coat protein)
antibody genes (for V_H and V_L)
gene 3
gene 7 and 9 proteins (minor coat proteins)

The cascade organization

Consequential enzymatic conversions of zymogens to activated enzymes

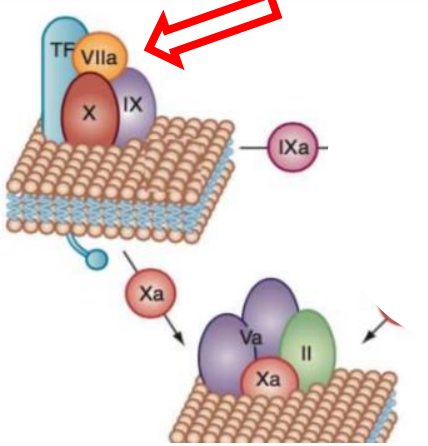




Huge Signal Amplification!

It takes place on ***macromolecular complex***:

Complex name	Enzyme (active)	Cofactor	Substrate (zymogen)	Catalytic Efficiency
Extrinsic Tenase	FVIIa	TF	FX	>15 x 10^6

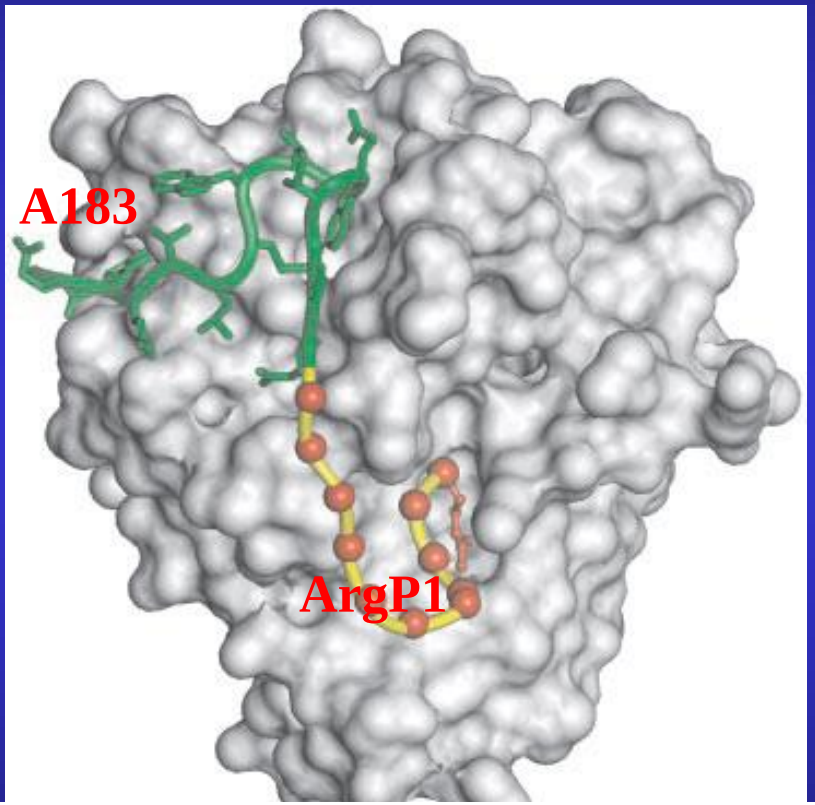


Increased catalytic efficiency

Products Channeling

Model of FVIIa protease domain with A-183 inhibitor

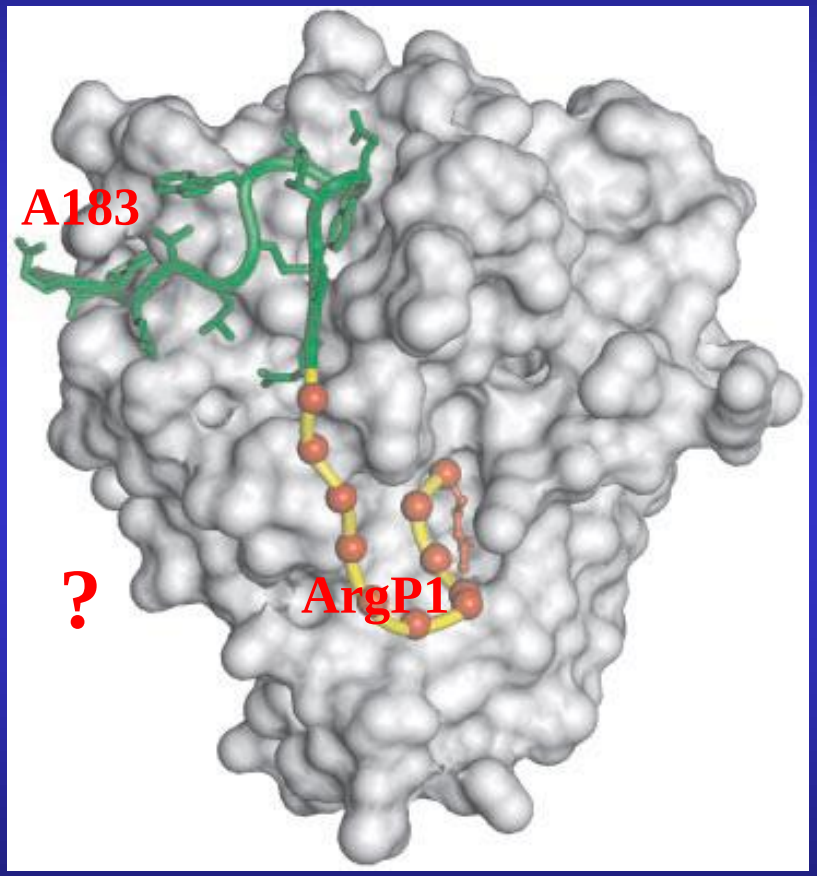
A183, 15mer,
EEWEVLCWTWETCER
exosite interactions



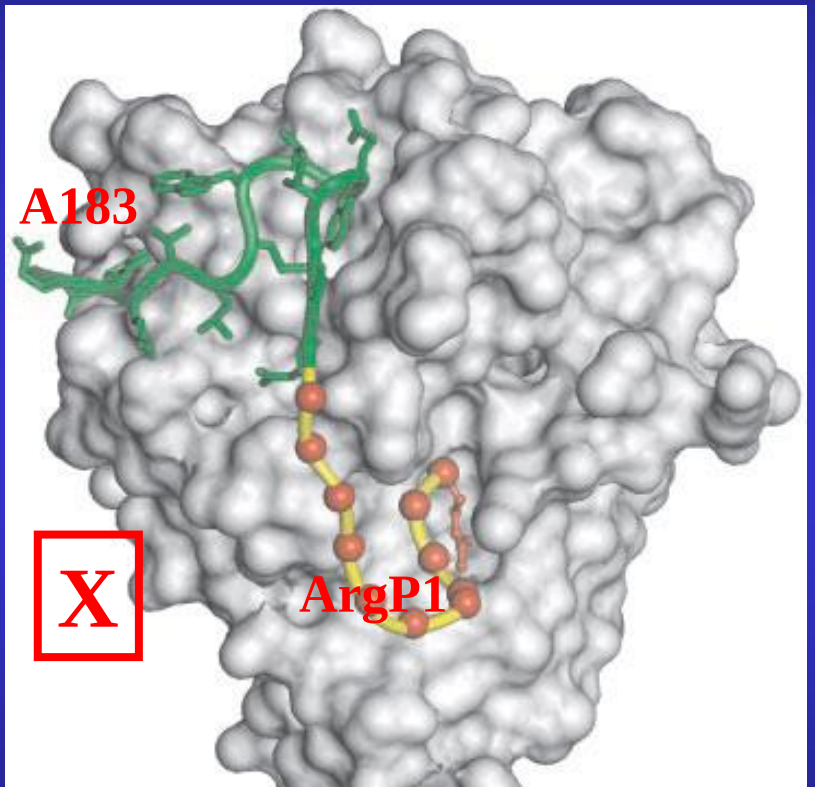
A-183 potent inhibitor of TF-FVIIa - inhibition was incomplete. At saturating concentrations A-183 showed a maximal extent of inhibition of FX activation of 78%

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

A183, 15mer,
EEWEVLCWTWETCER
exosite interactions
+ active site interactions
steric hindrance with the substrate



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



A chimeric peptide with a high degree of specificity and potency

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

determinanti di specificità di proteasi

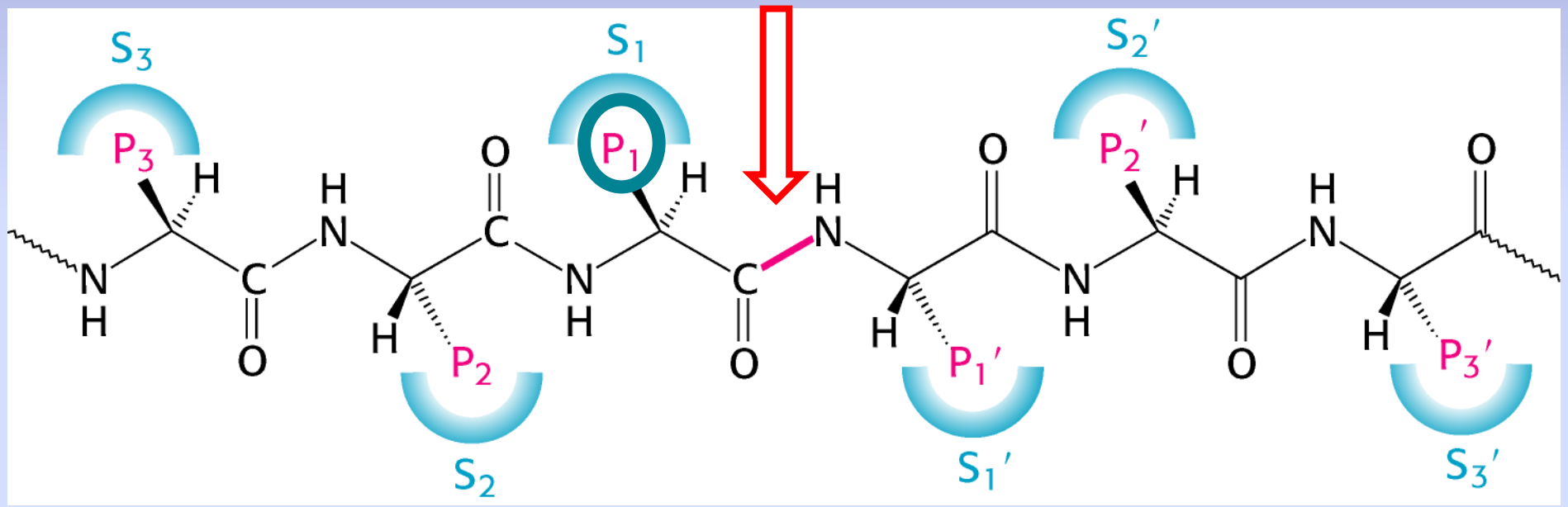
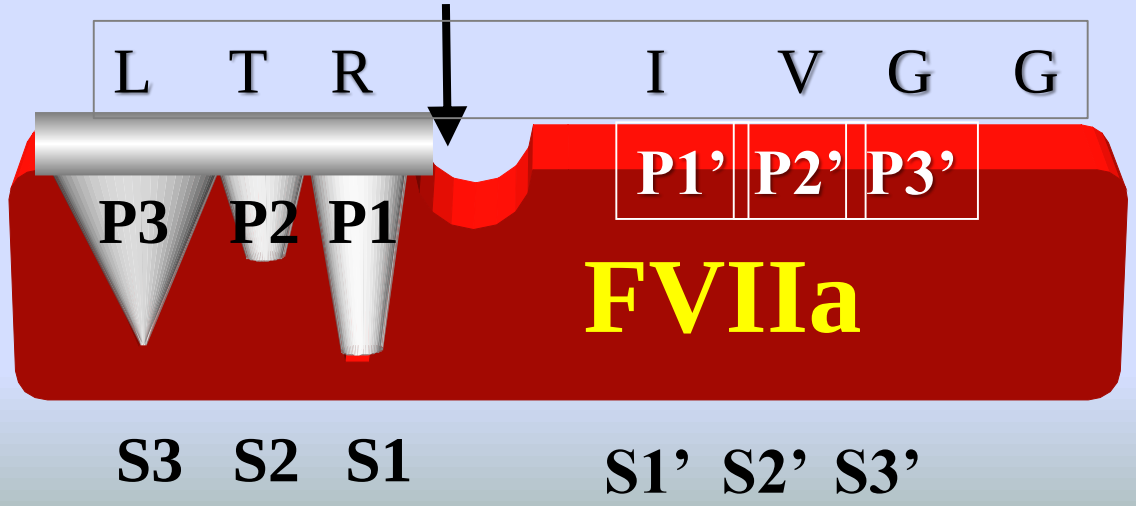


Table 1 Sites of cleavage in the human vitamin K-dependent zymogens*

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



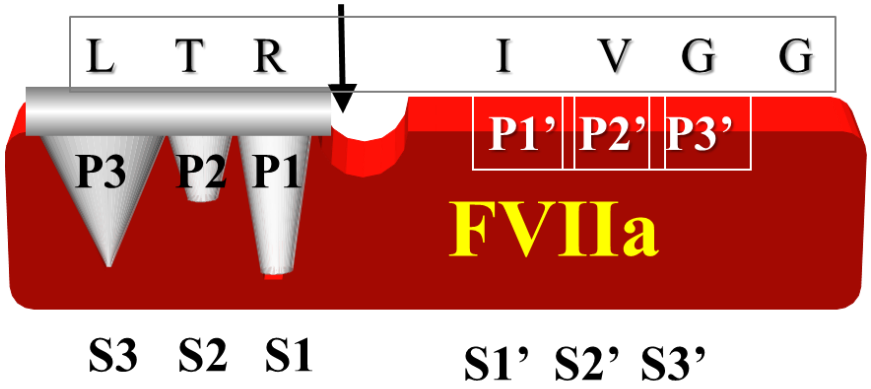
Library **A** designed to determine the length and sequence of the extension to reach into the active site

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

EEWEVLCWTWETCER

a= S,N,K,R;



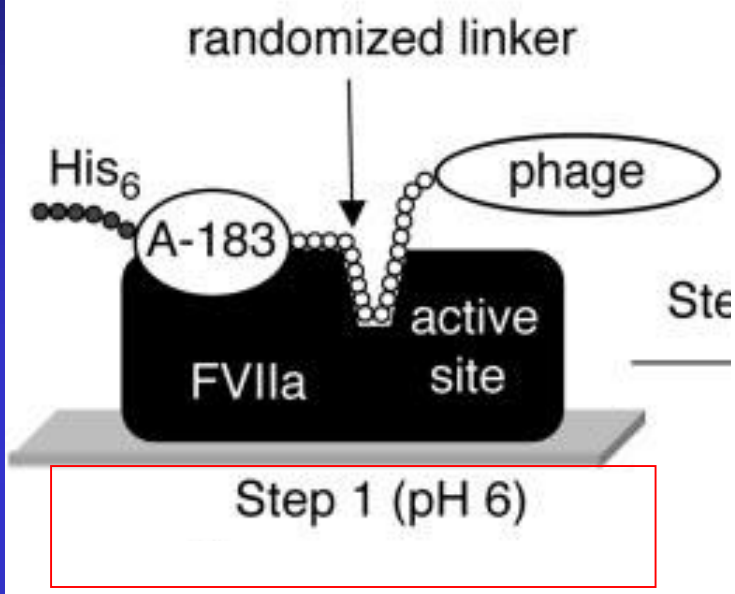
Library **A-D** designed to determine the length and sequence of the extension to reach into the active site

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 –		

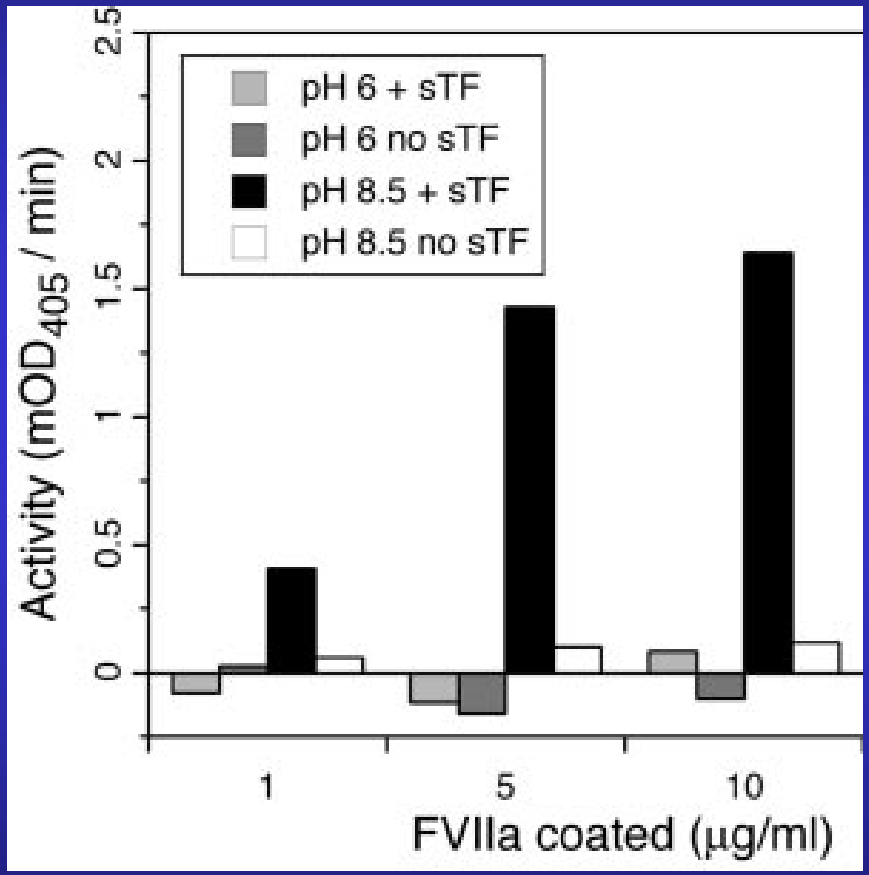
a= S,N,K,R; *b*= N,K; *c* = L,Q

Peptide Inhibitors of Factor VIIa: Phage binding

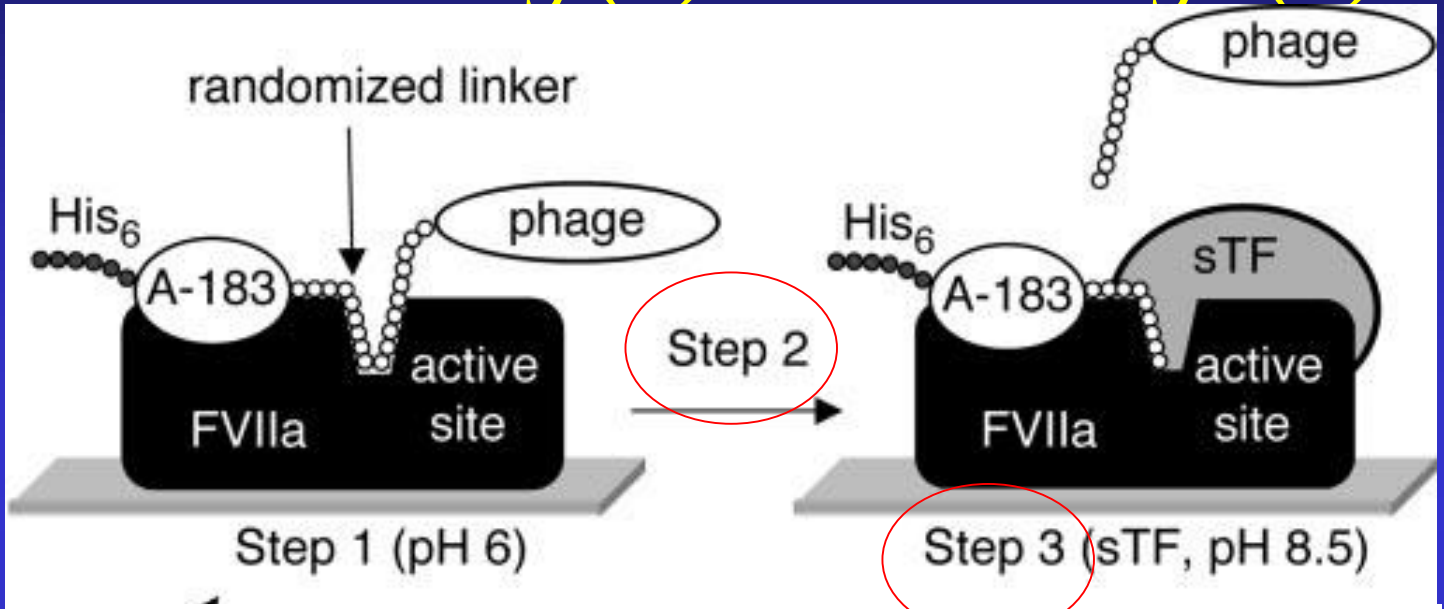


Unbound phage were removed by repetitive washing with binding buffer (step 2).

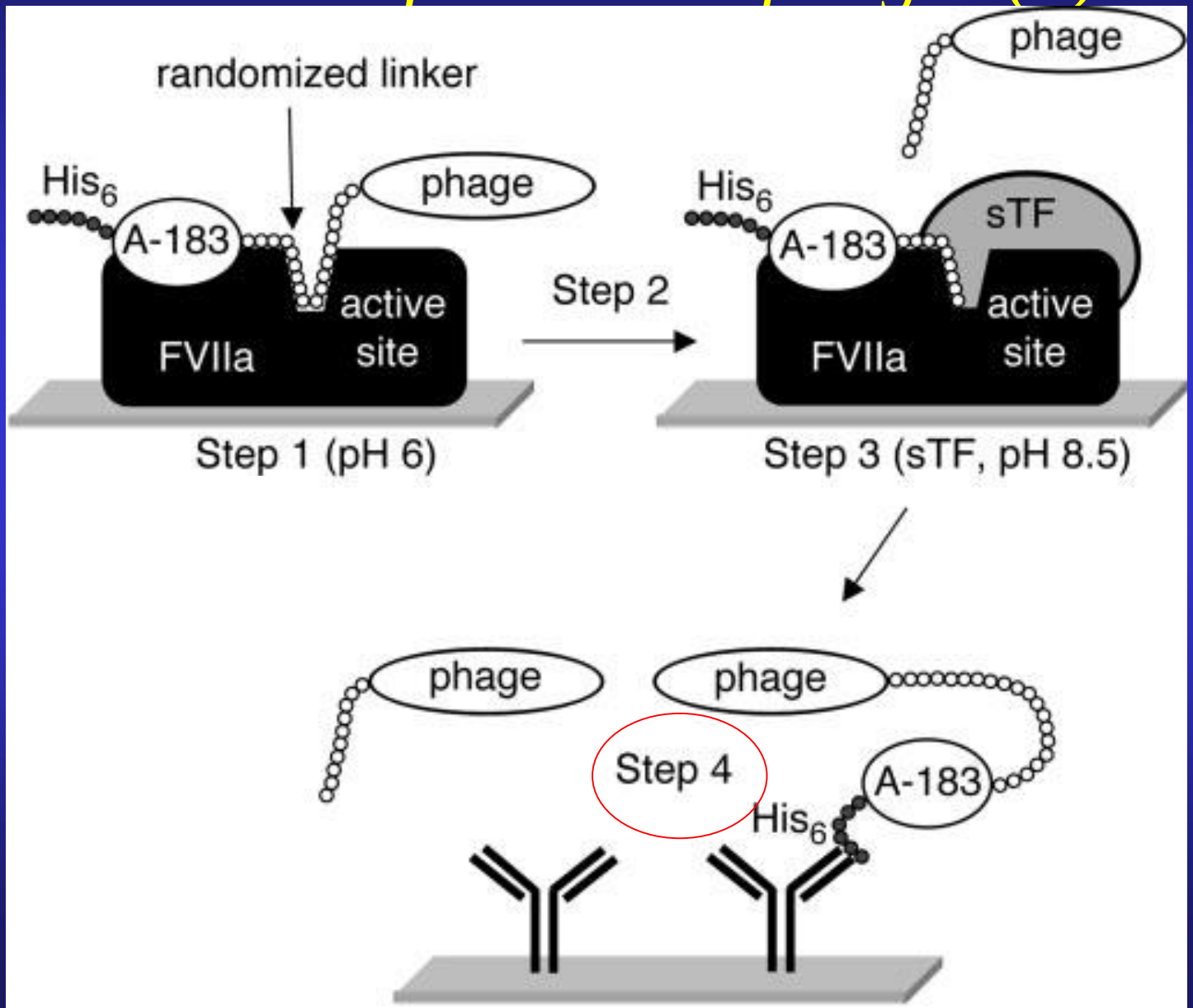
FVIIa Cleavage conditions



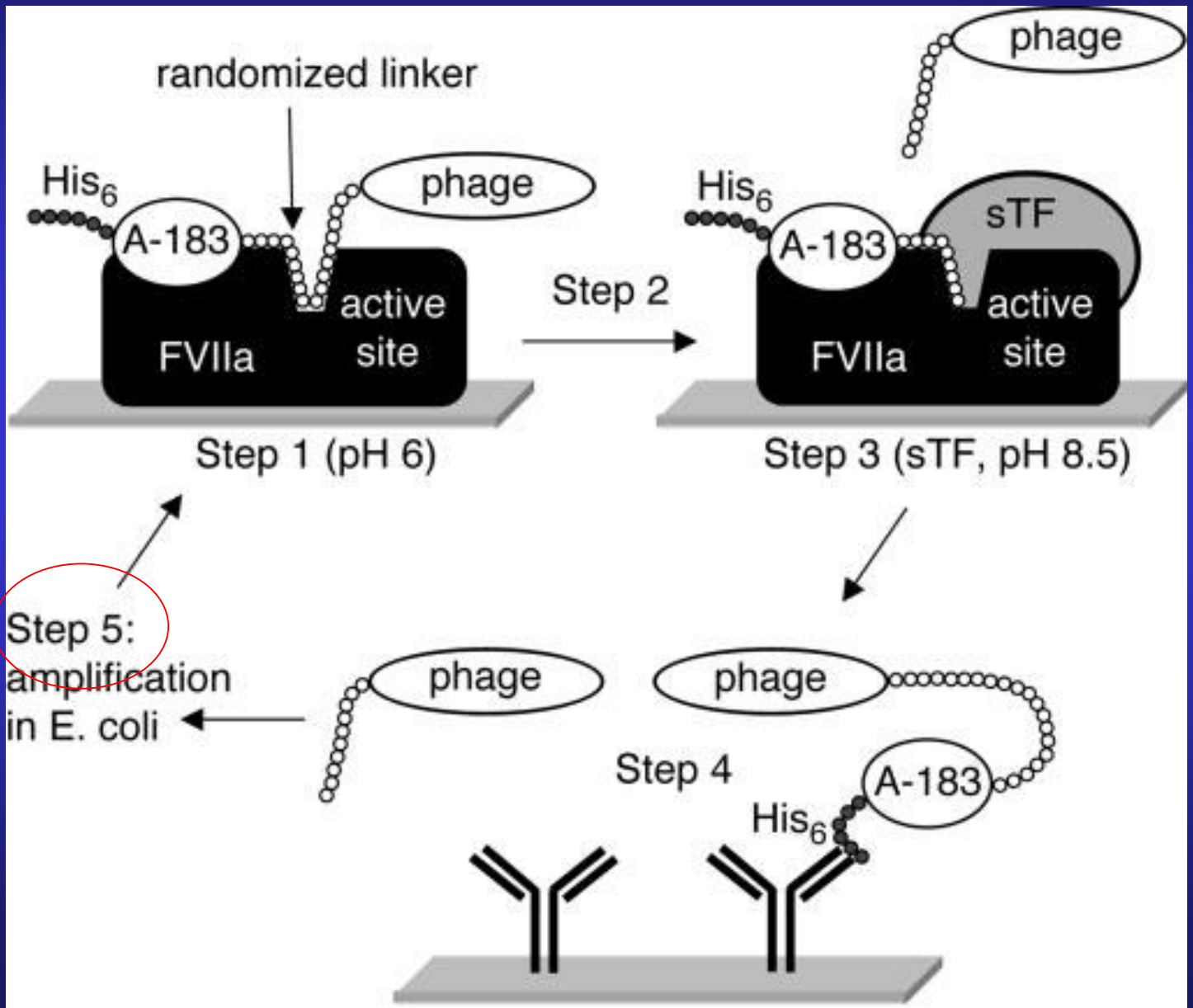
Peptide Inhibitors of Factor VIIa:washing (2) and cleavage (3)



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



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



A 183 X - sequence of the extension

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

G E G V E E E L W E W **R**

A 183 X

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

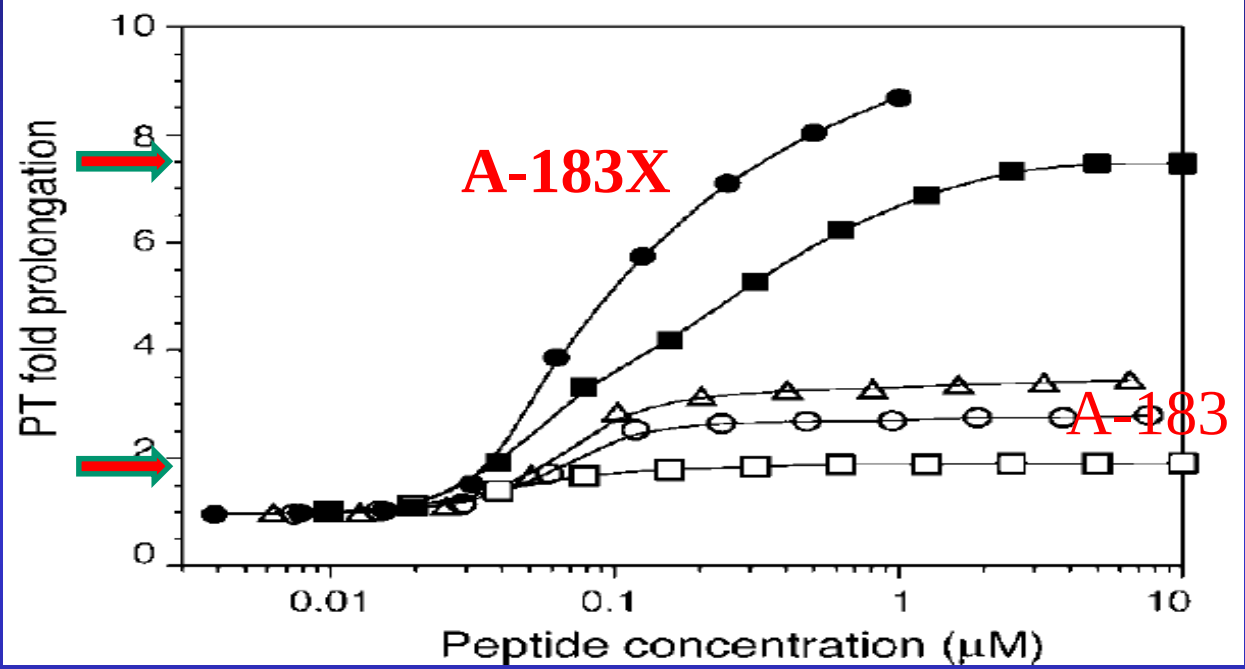
Inhibition of TF-dependent FX activation

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%**	**IC50 of 1.5 nM**

Prolongation of TF-dependent clotting times

A-183X is a more effective anticoagulant

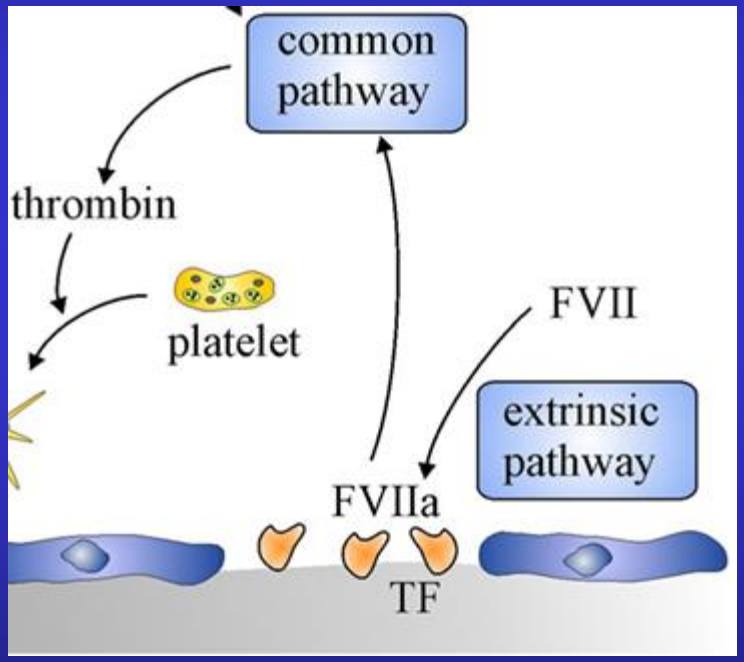


A-183X had a maximal prolongation of the prothrombin time of 7.6 fold

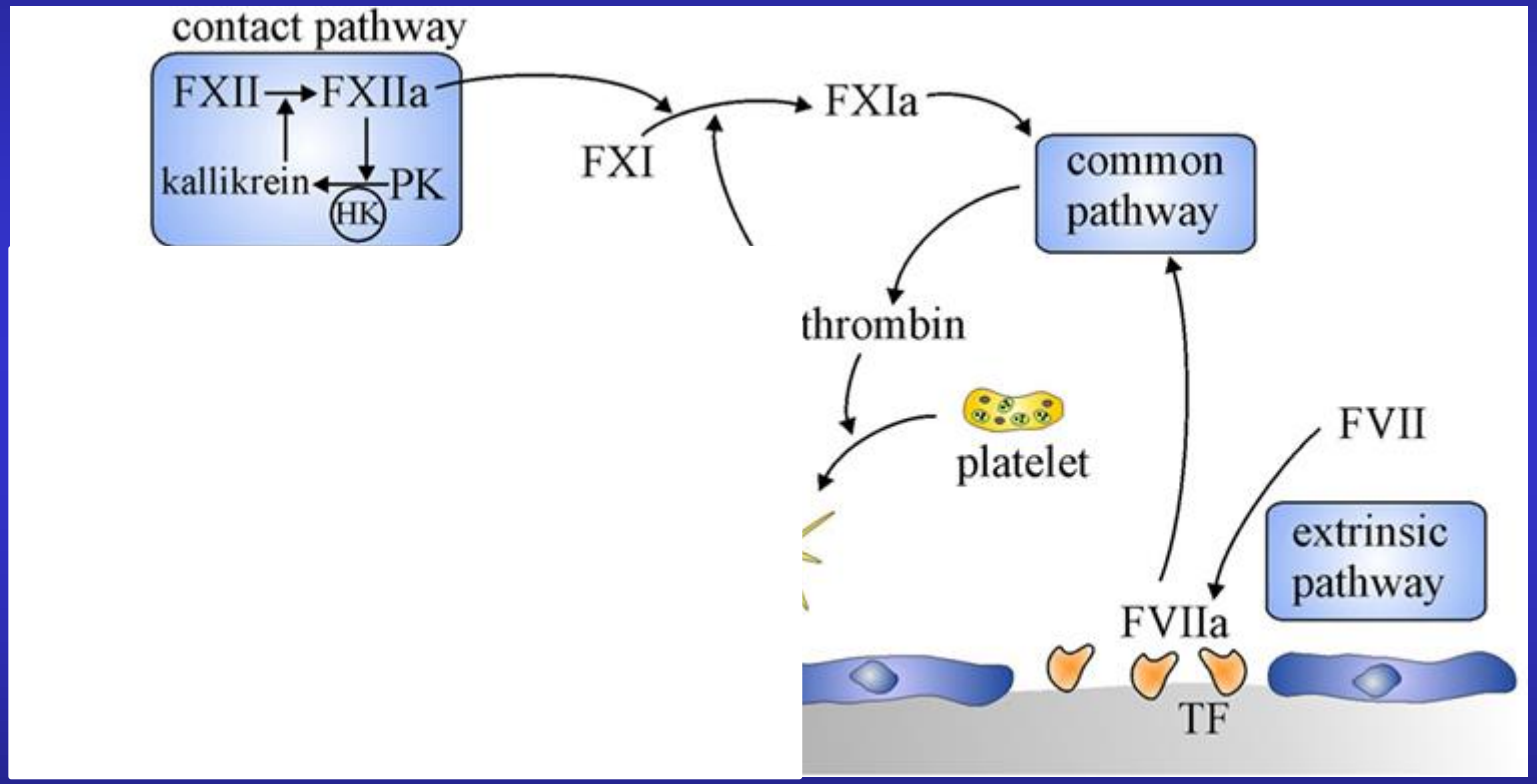
A-183 1.9- fold

II Modello Phage Display

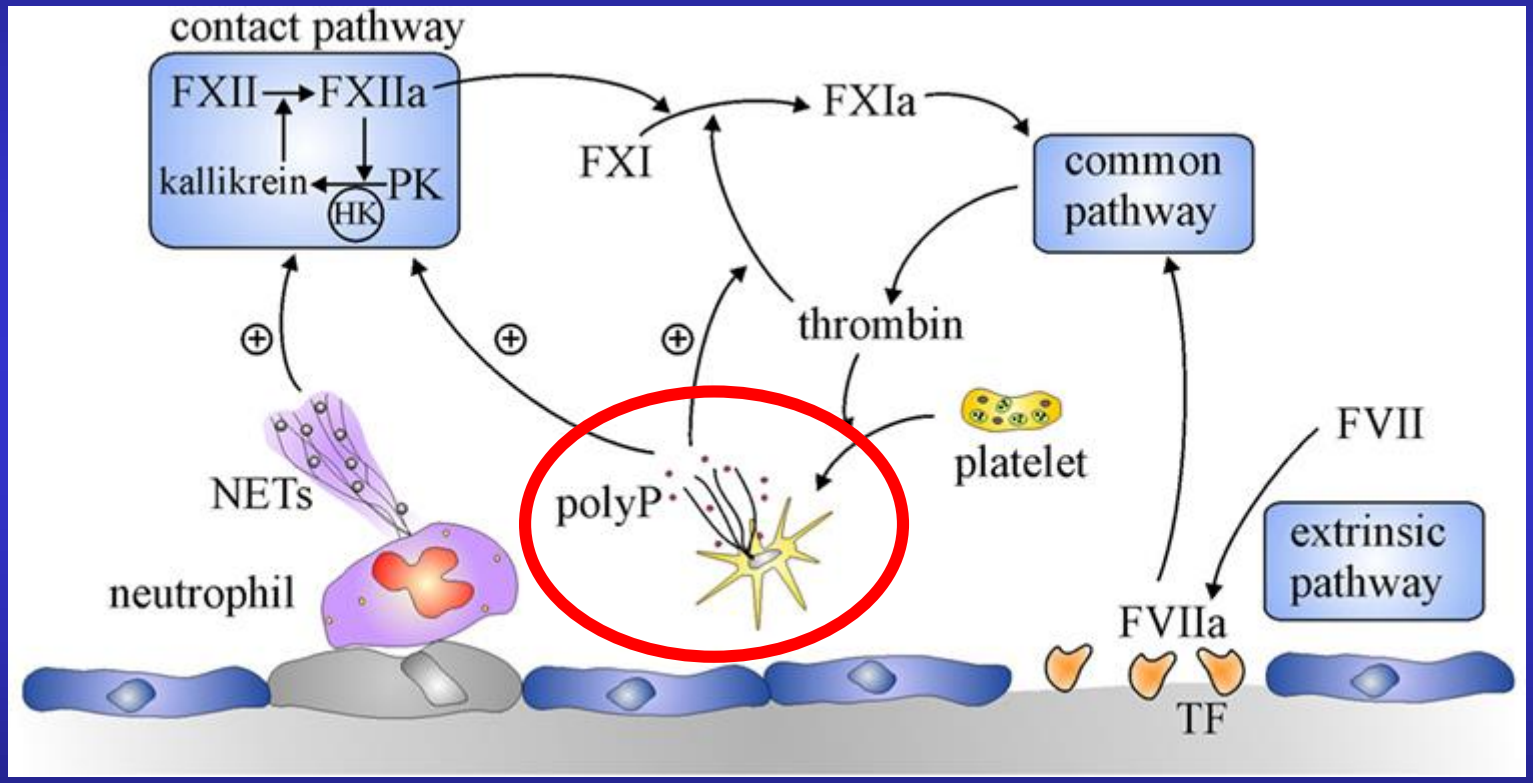
Coagulazione iniziata da Tissue Factor (TF)



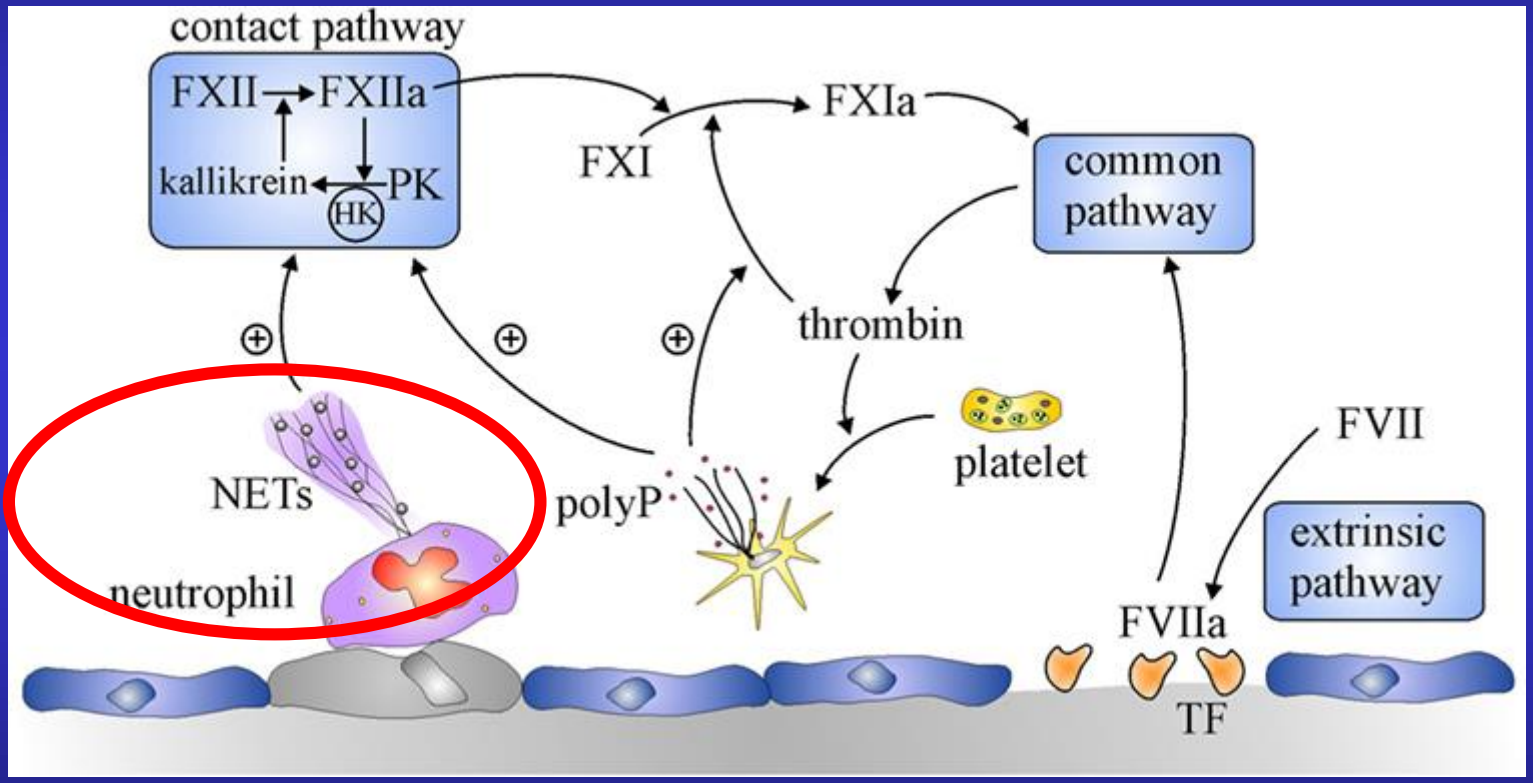
…e potenziata dalla via di contatto



additional activation of coagulation occurs when thrombin-activated platelets release polyphosphate (polyP)

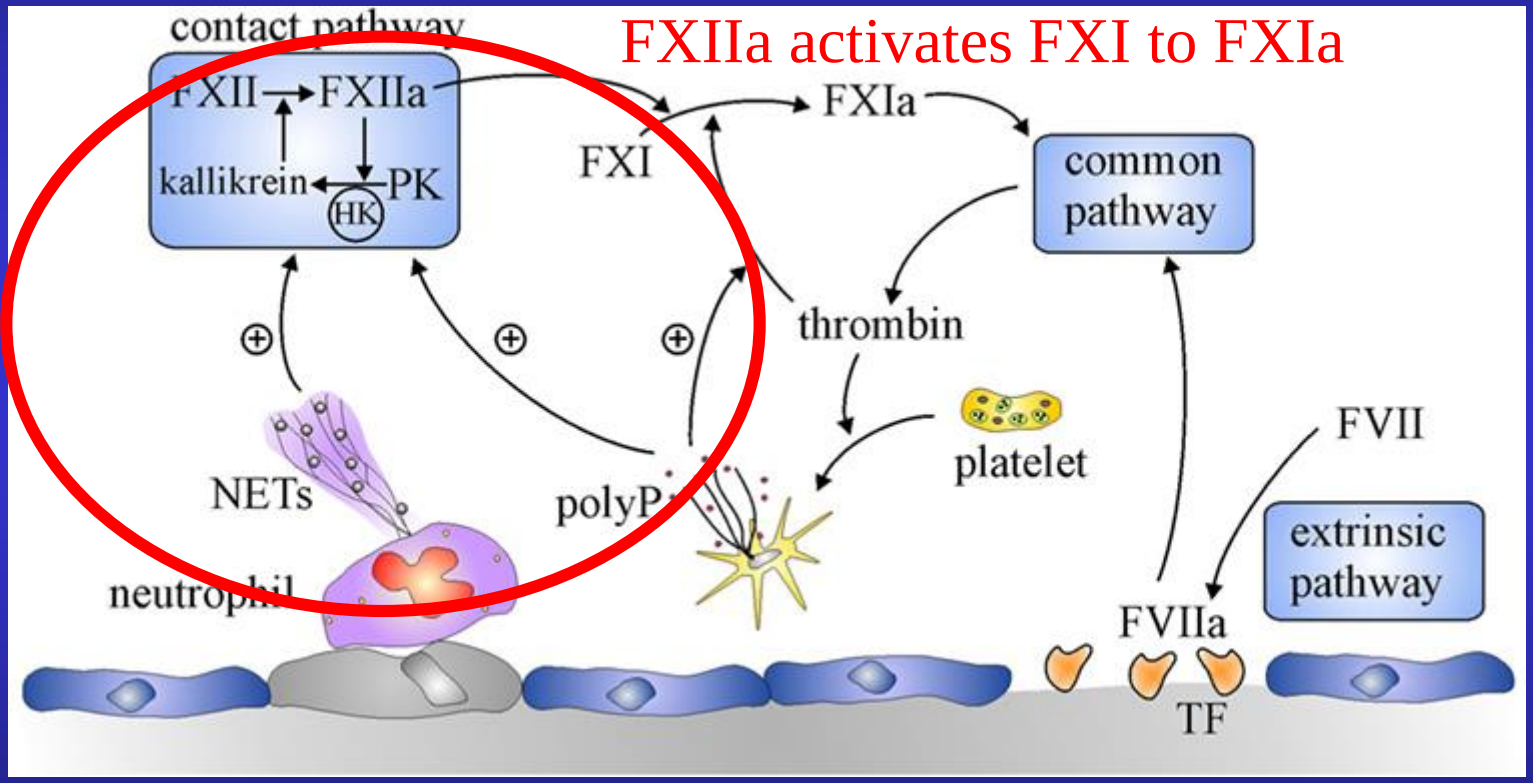


…and activated neutrophils extrude DNA and histones to form neutrophil extracellular traps (NETs)



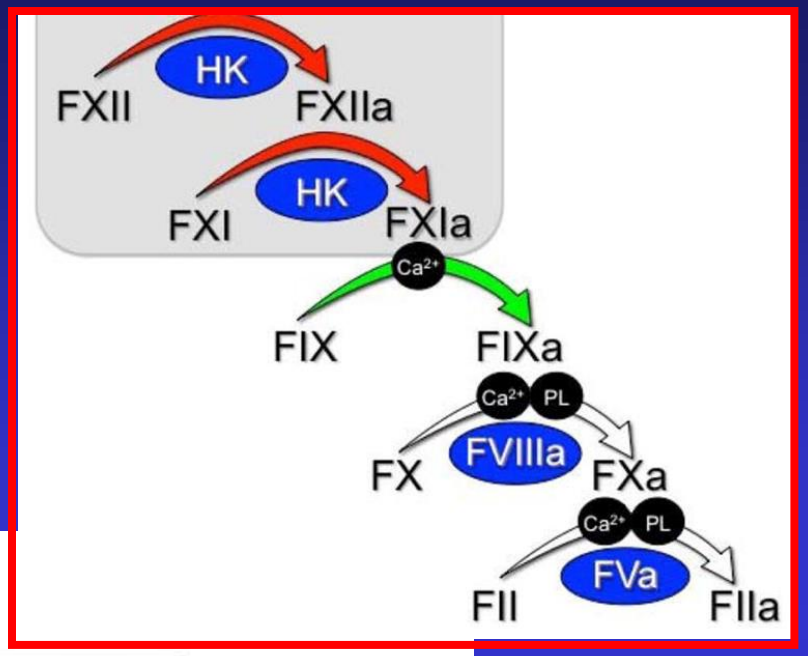
NETs and polyP activate the contact pathway

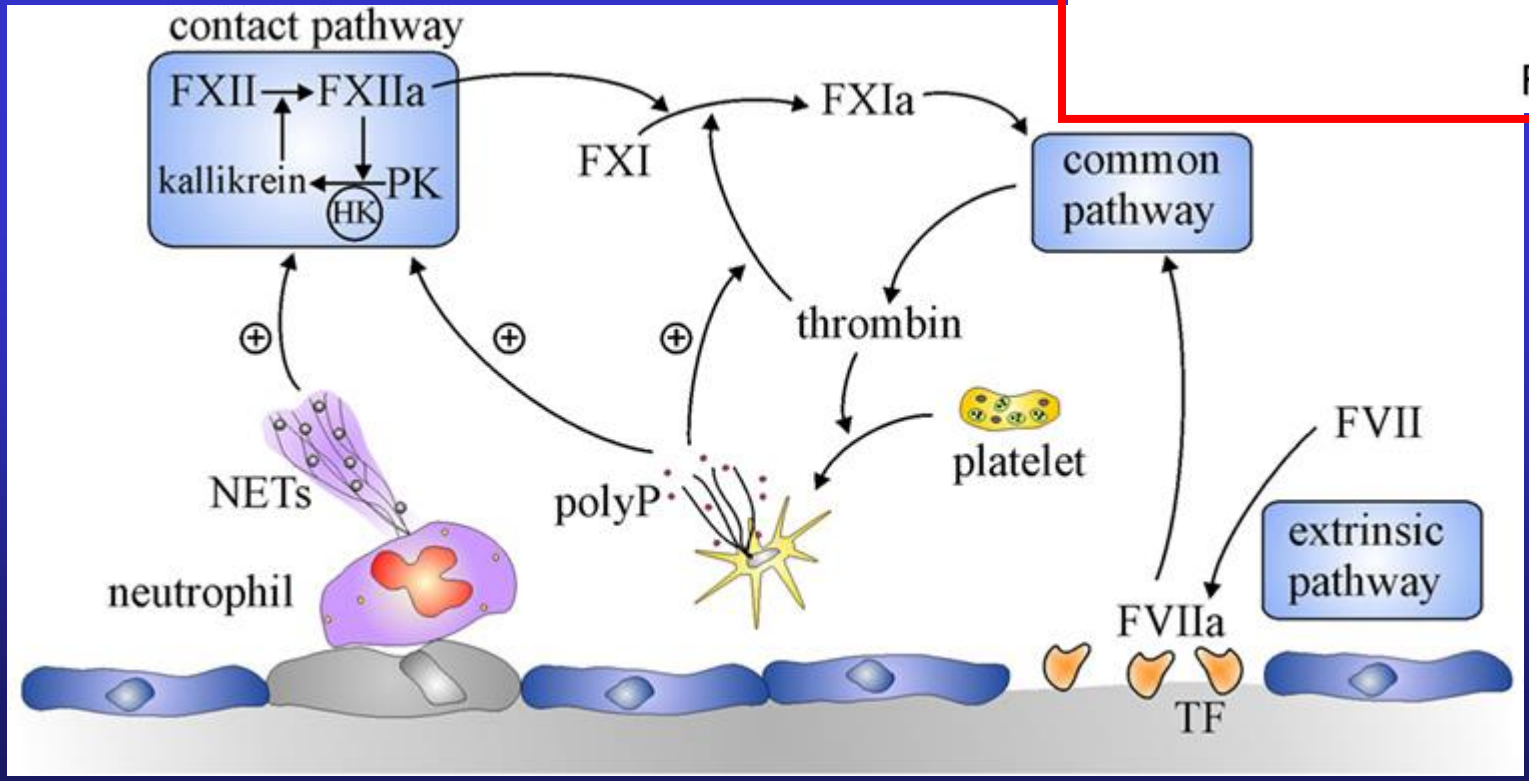
.



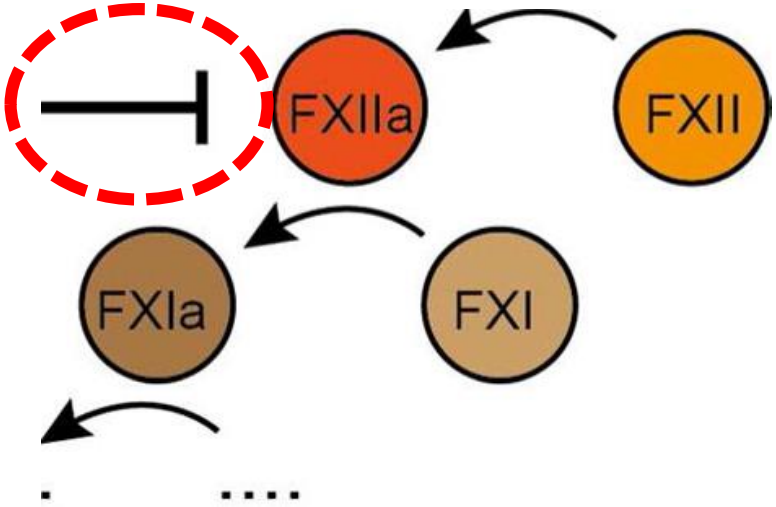
FXII and prekallikrein (PK) reciprocally activate each other to generate FXIIa and kallikrein, respectively

Coagulazione potenziata dalla via di contatto





Inibitori del FXII come antitrombotici



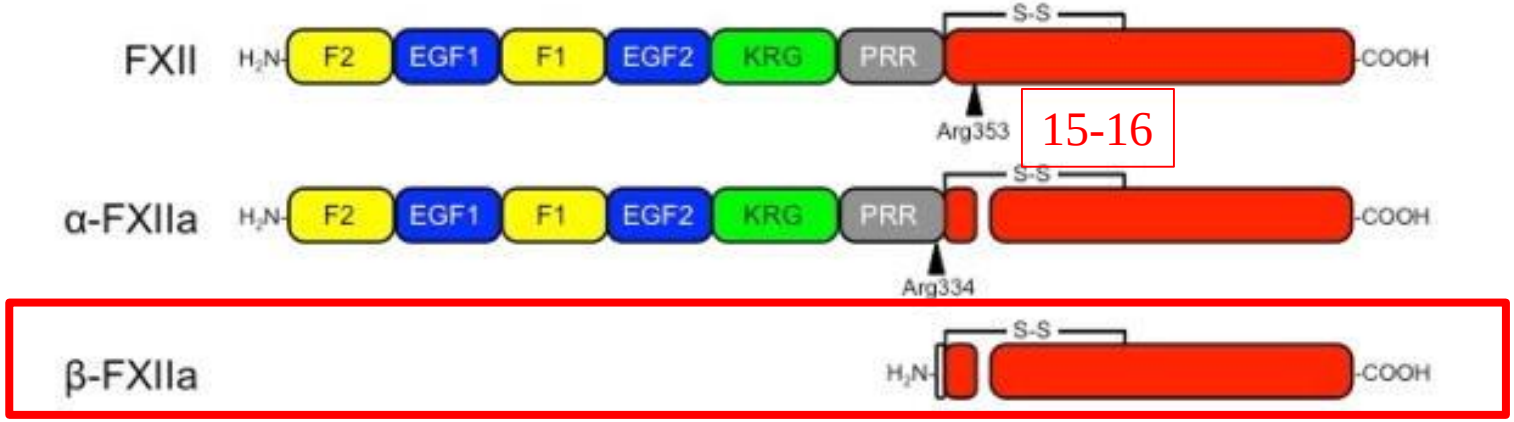
Using phage display combined to rational design, we developed a potent inhibitor of FXII with more than 100-fold selectivity over related proteases.

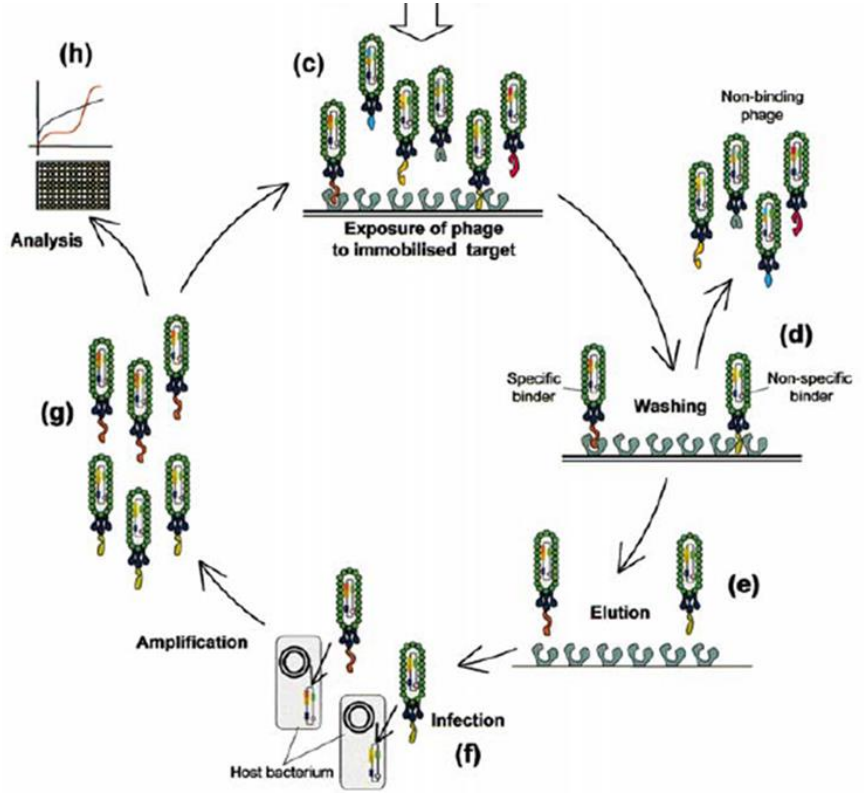
Published in: Vanessa Baeriswyl; **Sara Calzavarini**; Christiane Gerschheimer; Philippe Diderich; Anne Angelillo-Scherrer; Christian Heinis; *J. Med. Chem.* **2013,** 56, 3742-3746.

II Modello Phage Display

- Con modificazione chimica e ciclizzazione dei peptidi esposti

Three rounds of phage panning against β-FXIIa





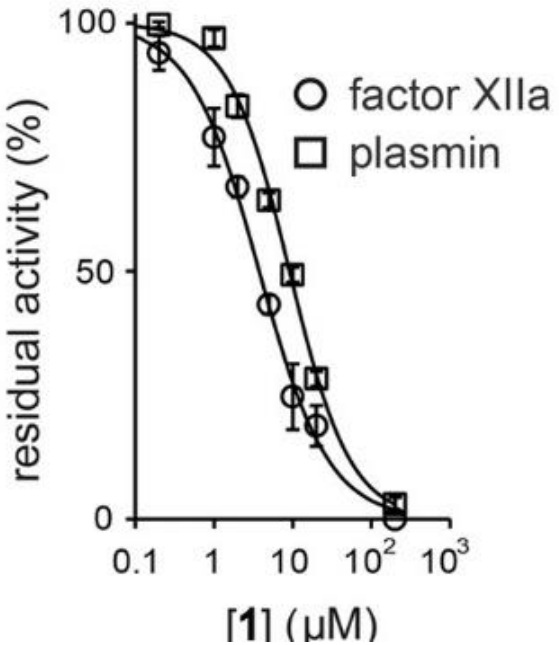
Peptide sequences isolated after three rounds of phage panning against β-FXIIa

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

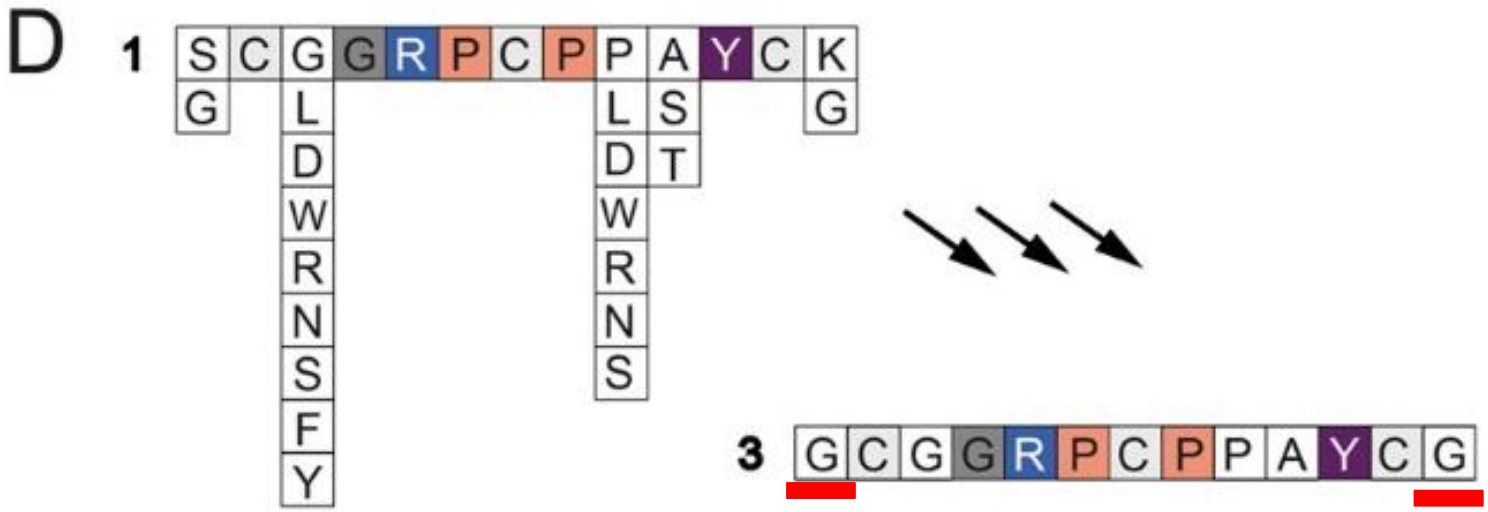
Development of a peptide inhibitor of FXII.

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



Affinity maturation of peptide 1.



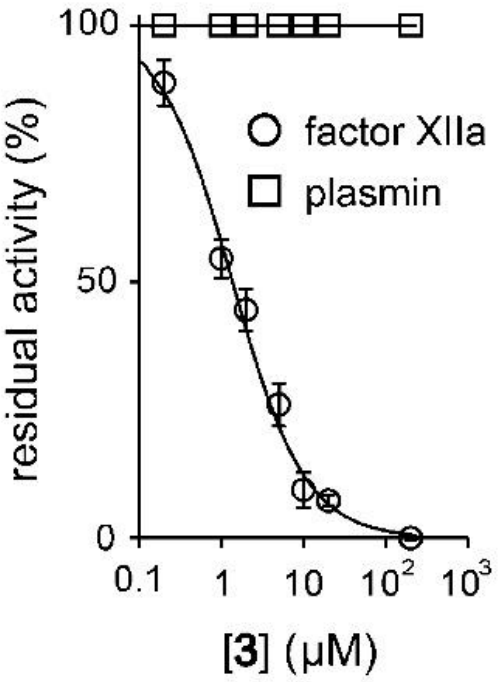
Peptides with amino acid substitutions in the indicated positions were synthesized and tested and led to peptide 3 (clone FXII402)

Inhibitory activity of peptide 3

A

peptide **3**

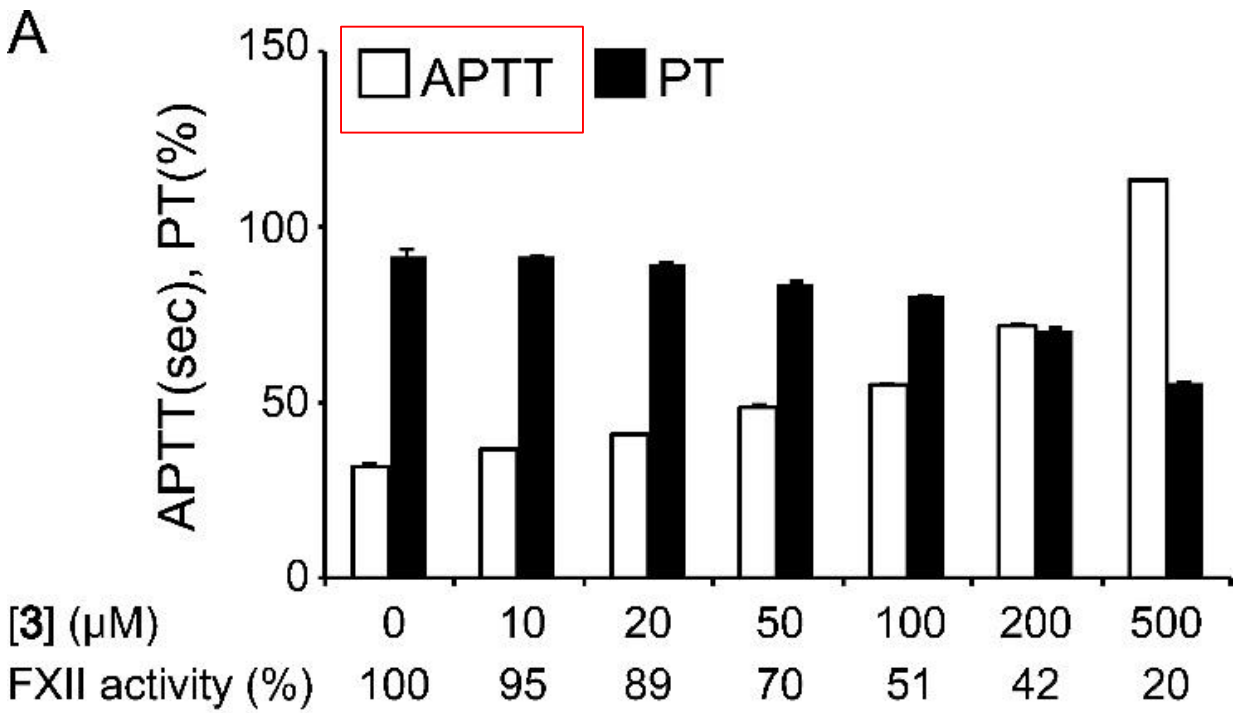
Protease	K_i (μM)
factor XIIa	1.2 +/- 0.2
tPA	> 120
uPA	> 120
factor XIa	> 120
PK	> 120
thrombin	> 120
plasmin	> 120
trypsin	> 120



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

Coagulation times in the presence of the FXII inhibitor peptide 3



Coagulation times

aPTT- intrinsic FXII dependent

PT – extrinsic Tissue Factor dependent

The highly selective peptide is candidate for antithrombotic therapy.