Corso di laurea in Scienze Biologiche
Corso di laurea magistrale in Scienze Biomolecolari e dell'Evoluzione

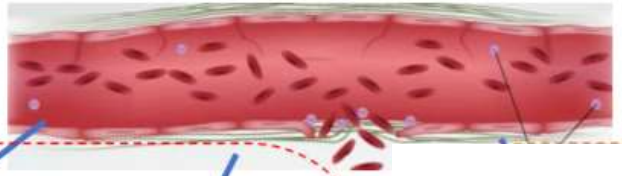
Materiale didattico di supporto

Tutto il materiale fornito a supporto delle lezioni e reperibile nel minisito dell'insegnamento o sulla piattaforma online UniFE deve essere inteso come traccia degli argomenti svolti e non sostituisce il libro di testo.

Coagulation Cascade
Activation and specificity of Thrombin

Da Giulia Pavani
Sara Calzavarini

Hemostatic process: an overview



Vascular Injury

Collagen
vWF

Vasoconstriction

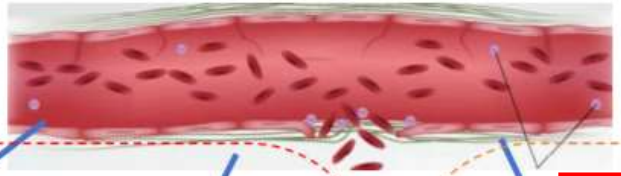
Platelet activation

vWF
Fibrinogen

Platelets plug

Primary Hemostasis

Hemostatic process: an overview



Vascular Injury

Collagen
vWF

Tissue factor

Vasoconstriction

Platelet activation

Coagulation cascade

vWF
Fibrinogen

Thrombin

Fibrinogen → Fibrin

Platelets plug

Plasmin

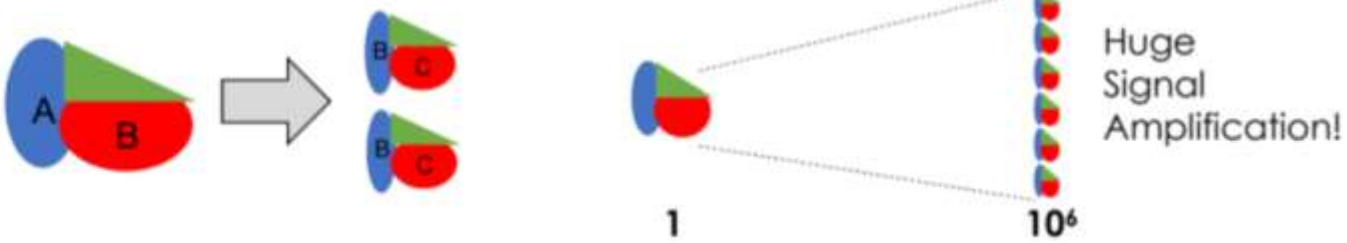
Fibrinolysis
&
Clot degradation

Primary Hemostasis

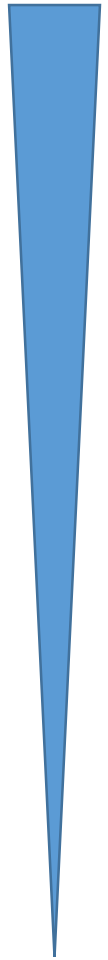
Secondary Hemostasis

The cascade organization

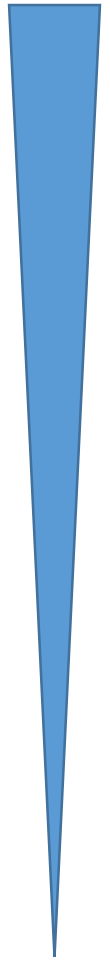
Consequential enzymatic conversions of zymogens to activated enzymes



Clotting factor number	Clotting factor name	Plasma concentration (mg/L)
I	Fibrinogen	3000
II	Prothrombin	100
III	TF **Tissue Factor**	-
IV	Calcium	-
V	Proacclerin, labile factor	10
VII	Stable factor, proconvertin	0.5
VIII	Antihaemophilic factor A	0.1
IX	Antihaemophilic factor B or Christmas factor	5

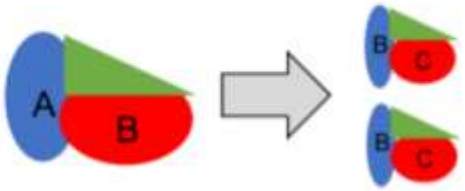


Clotting factor number	Clotting factor name	Plasma half-life (h)	Plasma concentration (mg/L)
I	Fibrinogen	90	3000
II	Prothrombin	65	100
III	TF **Tissue Factor**	-	-
		-	-
V	Proacclerin, labile factor	15	10
VII	Stable factor, proconvertin	5	0.5
VIII	Antihaemophilic factor A	10	0.1
IX	Antihaemophilic factor B or Christmas factor	25	5

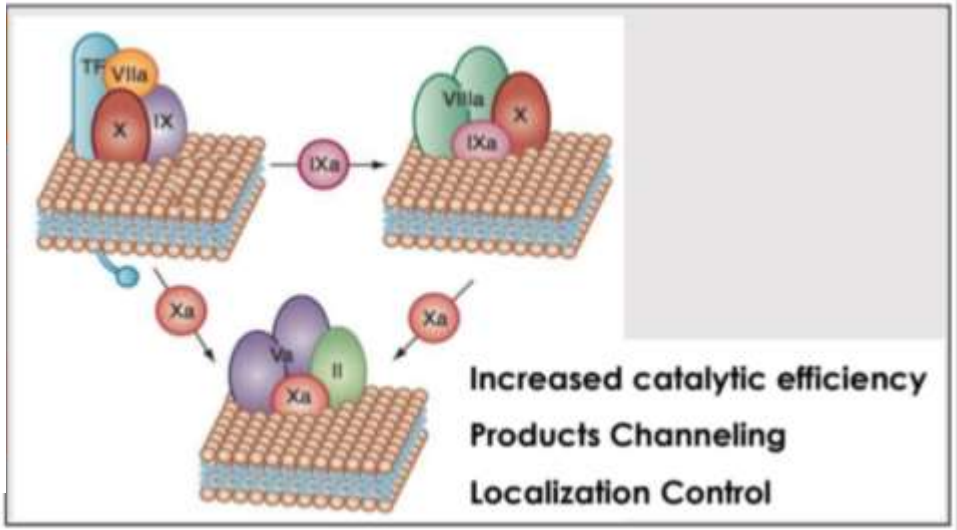


The cascade organization

Consequential enzymatic conversions of zymogens to activated enzymes

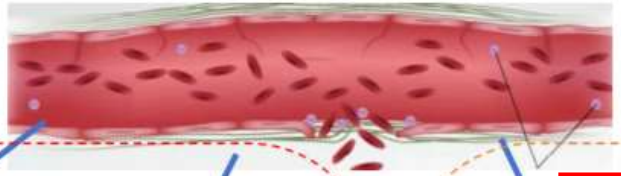


Huge
Signal
Amplification!



Attivazione del Fibrinogeno a Fibrina
Il collante del Coagulo

Hemostatic process: an overview



Vascular Injury

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Platelet activation

Coagulation cascade

vWF
Fibrinogen

Thrombin

Fibrinogen → Fibrin

Platelets plug

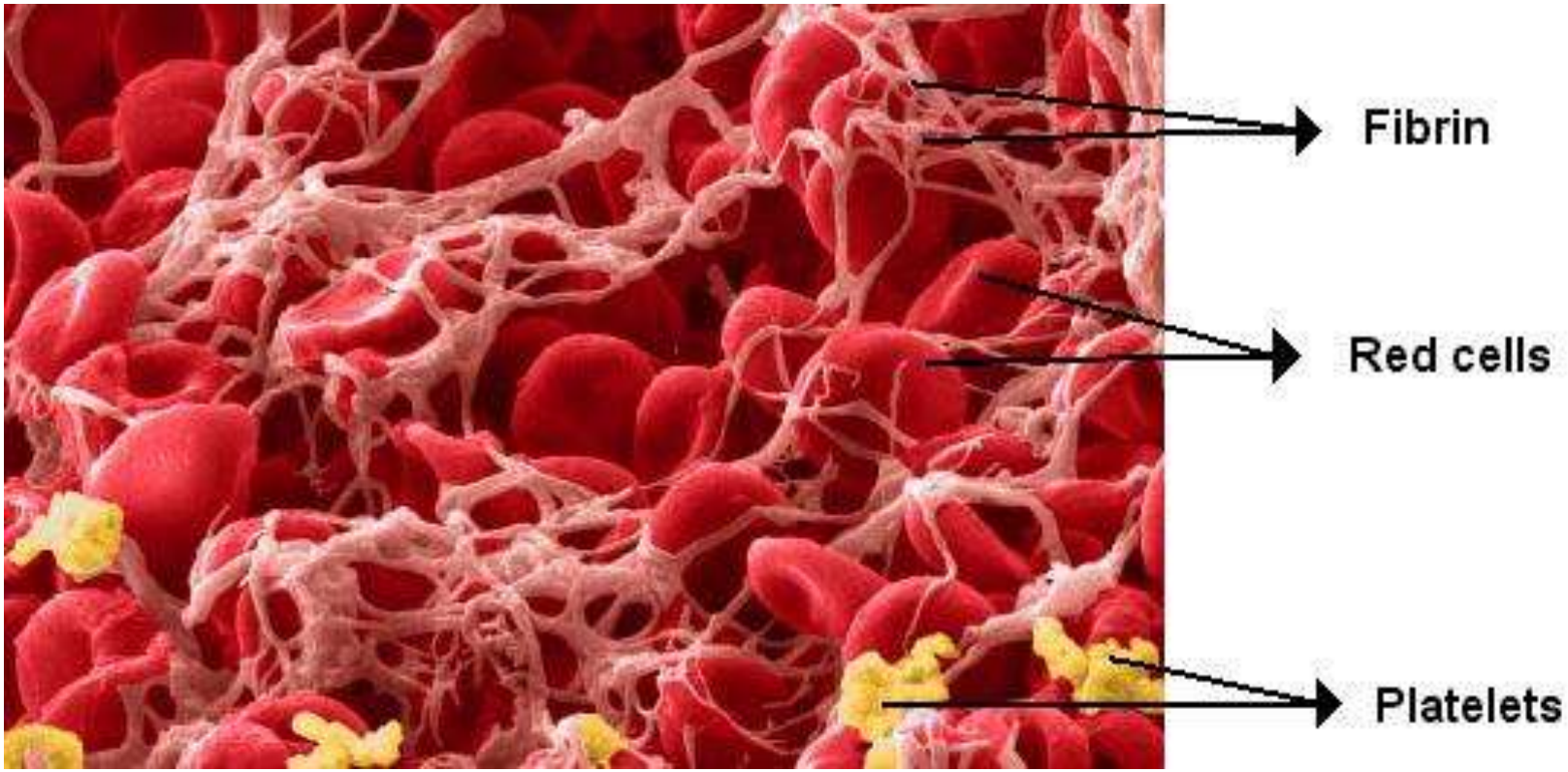
Plasmin

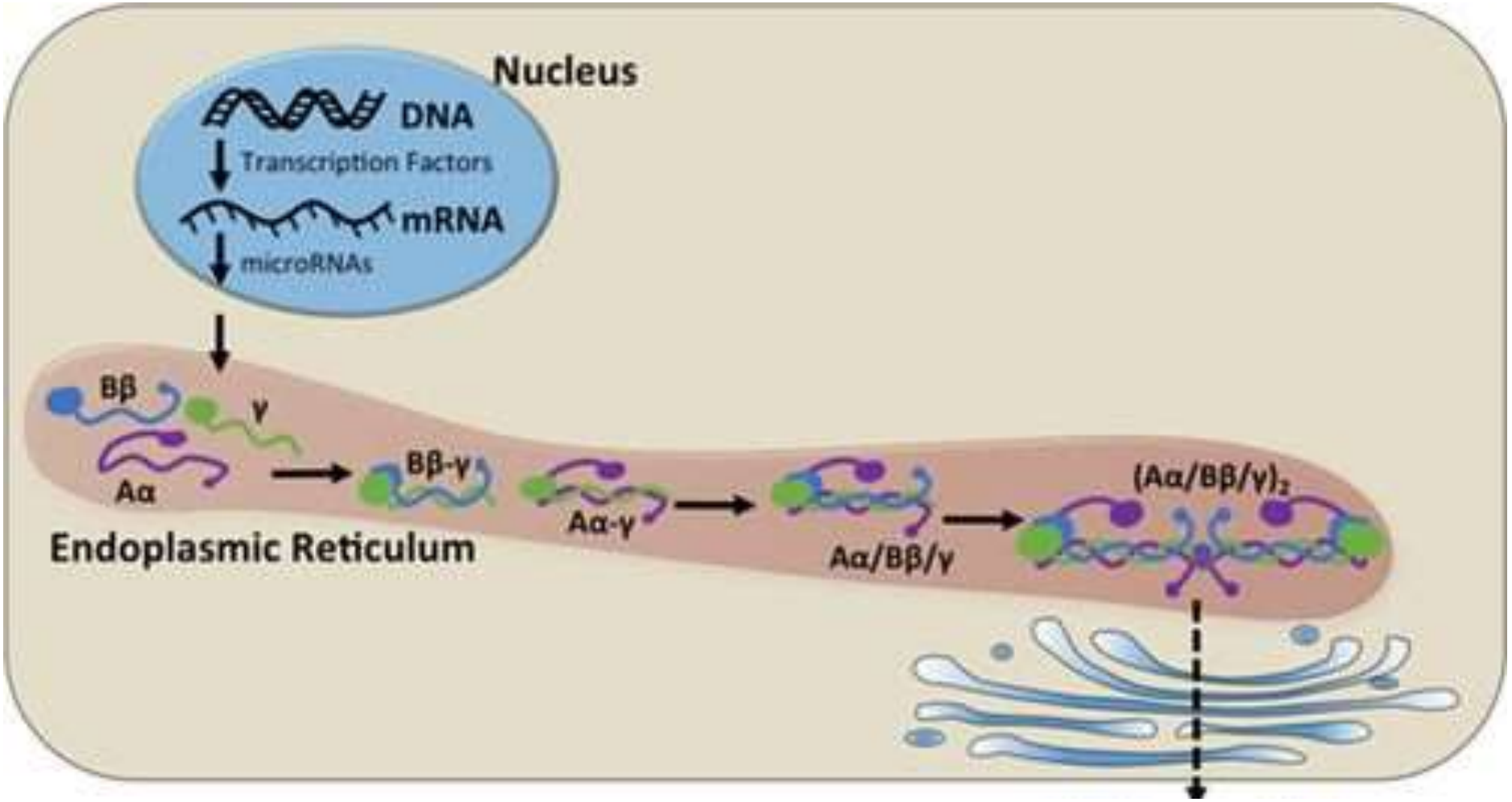
Fibrinolysis
&
Clot degradation

Primary Hemostasis

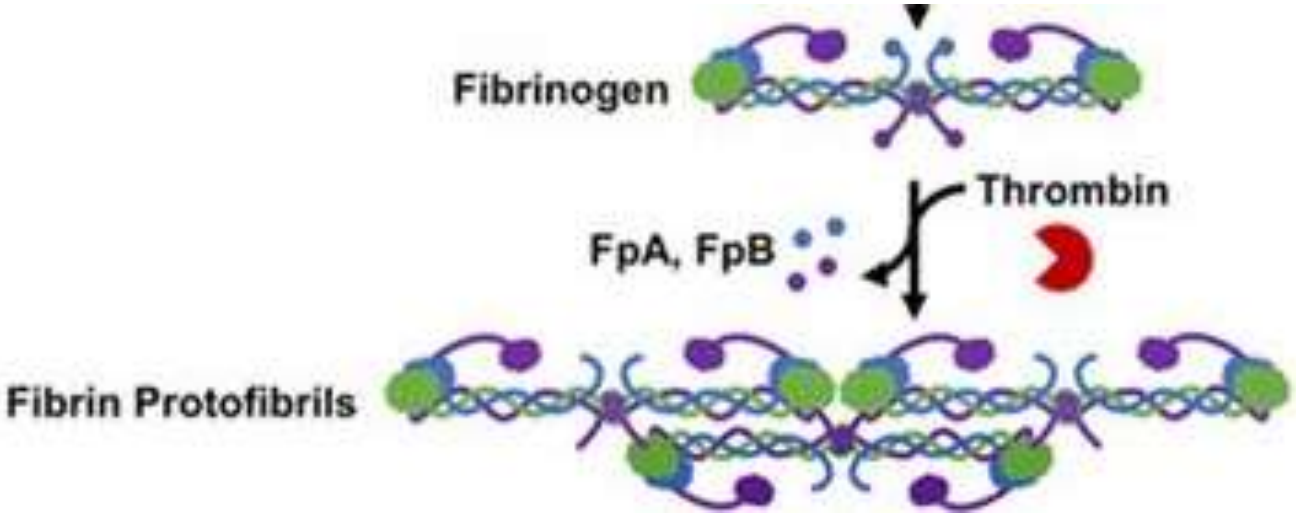
Secondary Hemostasis

Thrombin cleavage of the plasma protein fibrinogen





Sravya Kattula. Arteriosclerosis, Thrombosis, and Vascular Biology. Fibrinogen and Fibrin in Hemostasis and Thrombosis, Volume: 37, Issue: 3, Pages: e13-e21, DOI: (10.1161/ATVBAHA.117.308564)



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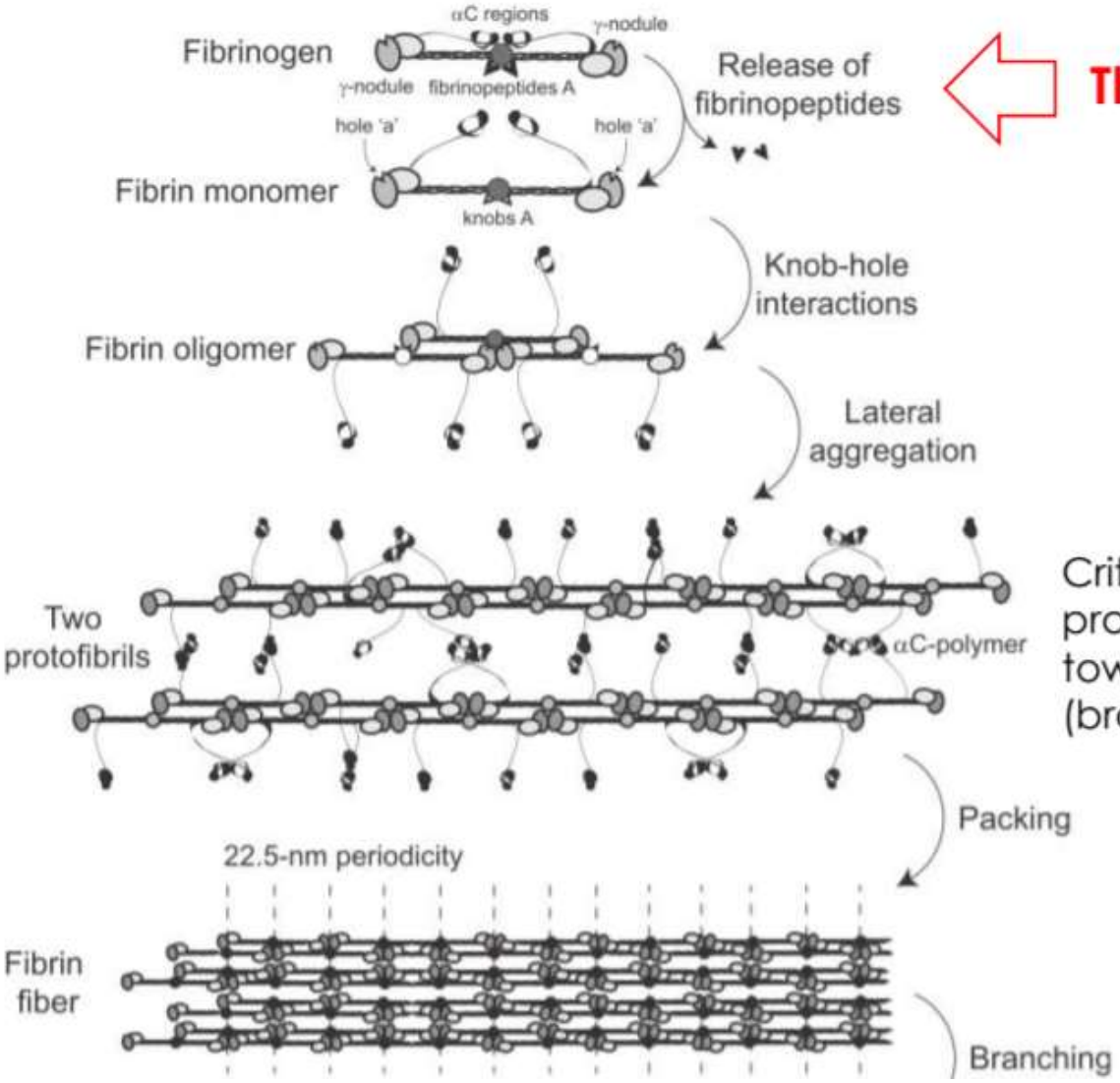
Fibrinogen to fibrin



Thr

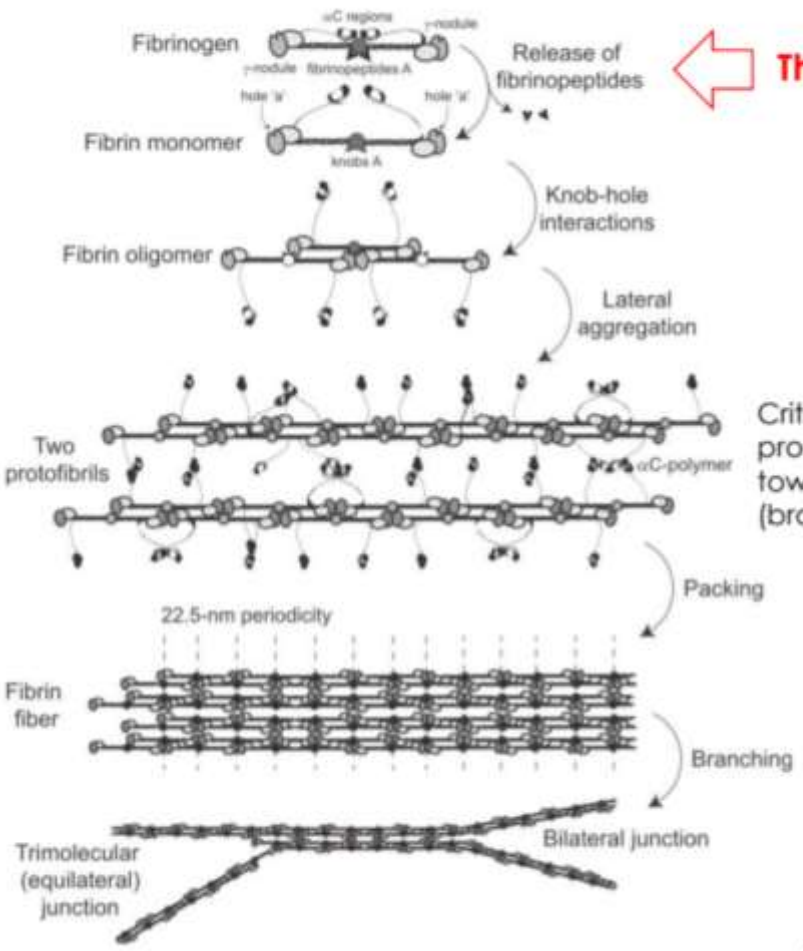
SOLUBLE

It occurs when only about 15–20 % of the fibrinogen is converted to fibrin!

INSOLUBLE

Critically important intermediate product, capable to undergo toward lateral aggregation (branching) -> 3D network

Fibrinogen to fibrin



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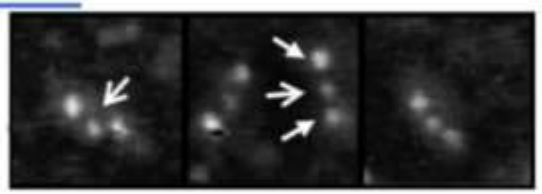
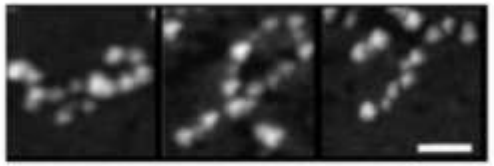


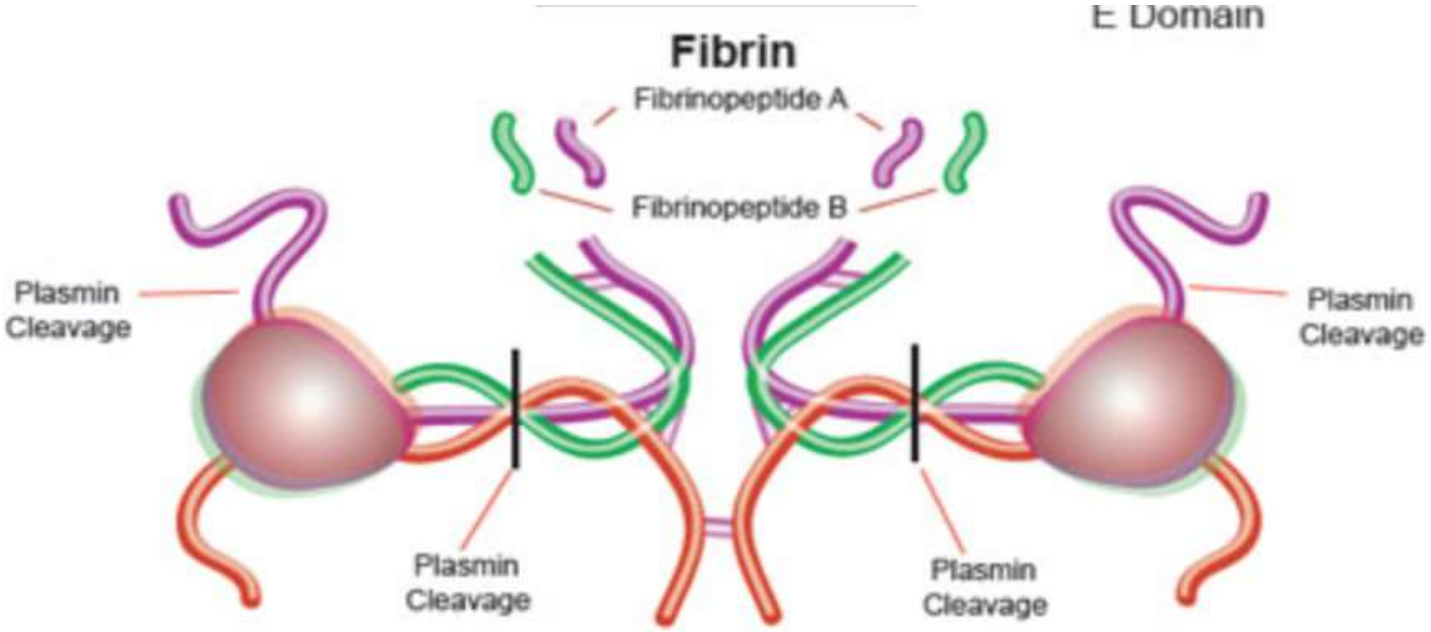


protofibril **50 nm**

Fibrin fiber **1 μm**

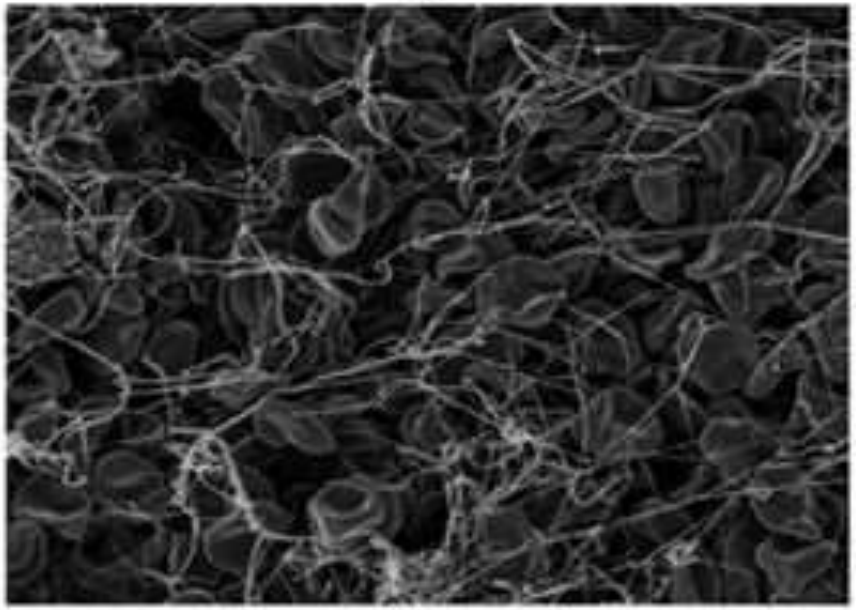
Weisel JW and Litinov RI Blood 20013

Thrombin /Plasmin cleavage of the plasma protein fibrinogen/fibrin



Modifiers of Fibrin Clot Formation, Structure, and Stability

- Concentrations of:
  - Procoagulants
  - Anticoagulants
  - Fibrinogen variants
- Metal ions
  - pH
  - Temperature
- Blood cells
  - Vascular cells
  - Cellular vesicles
- Polyphosphates
  - DNA & histones
  - Heparin
  - Protamine
- Post-translational modification
  - Blood flow
  - Others?

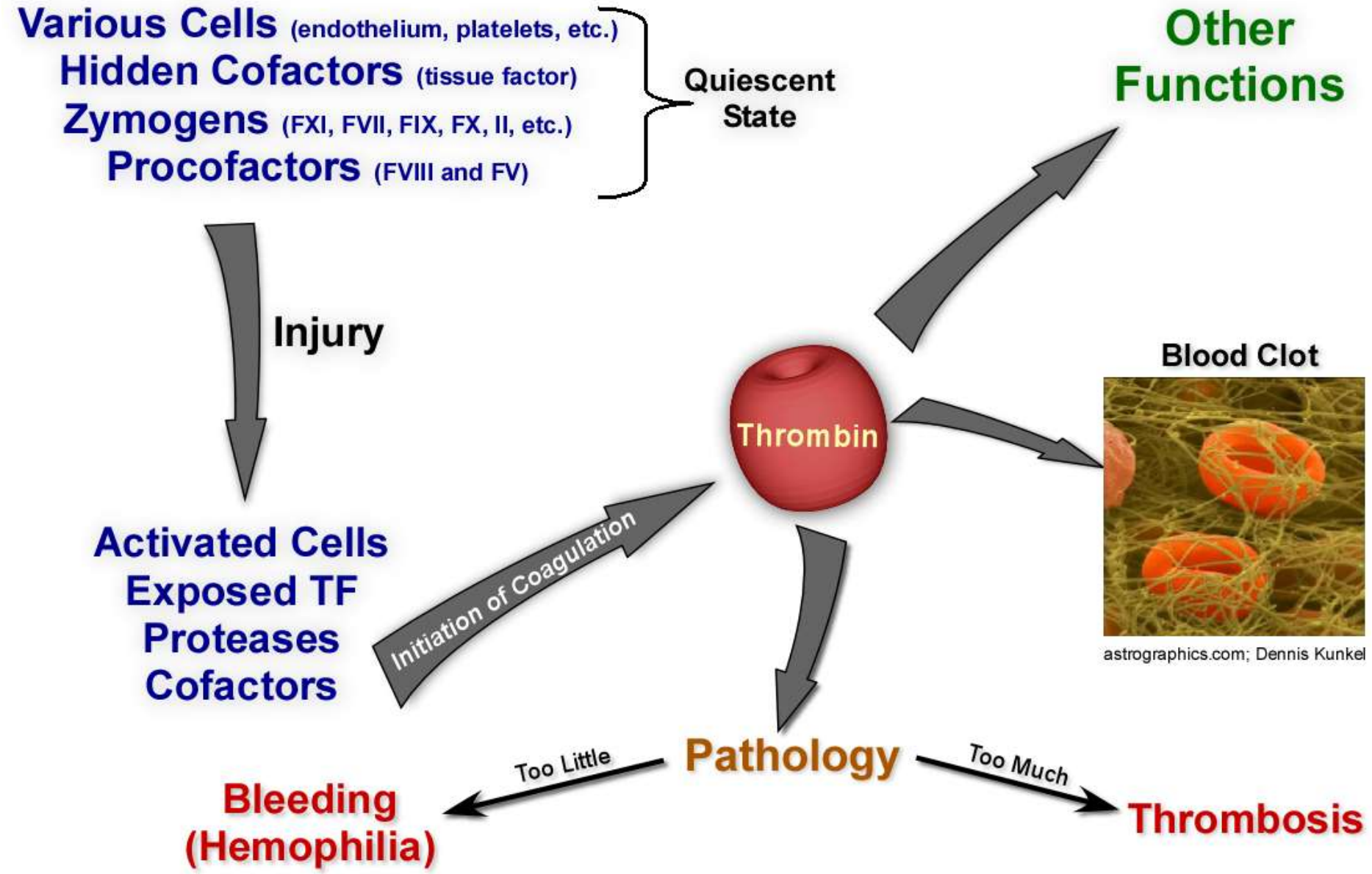


Diseases Associated with Abnormal Fibrin(ogen) Structure and Stability

- Coronary Artery Disease
- Myocardial Infarction
- Ischemic Stroke
- Venous Thromboembolism
- Abdominal Aortic Aneurysm
- Smoking
- Chronic Kidney Disease
- In-stent Thrombosis
- Cirrhosis
- Hemophilia
- Others?

Sravya Kattula. Arteriosclerosis, Thrombosis, and Vascular Biology. Fibrinogen and Fibrin in Hemostasis and Thrombosis, Volume: 37, Issue: 3, Pages: e13-e21, DOI:

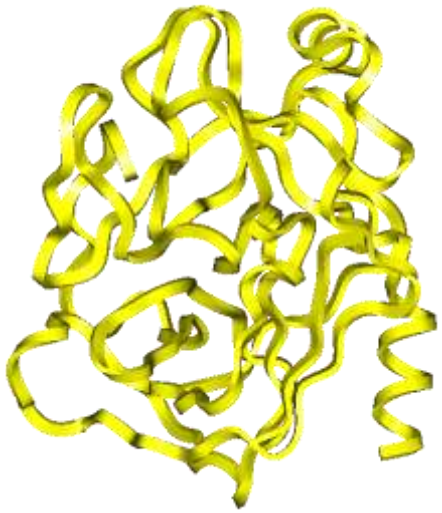
The Blood Coagulation Response:



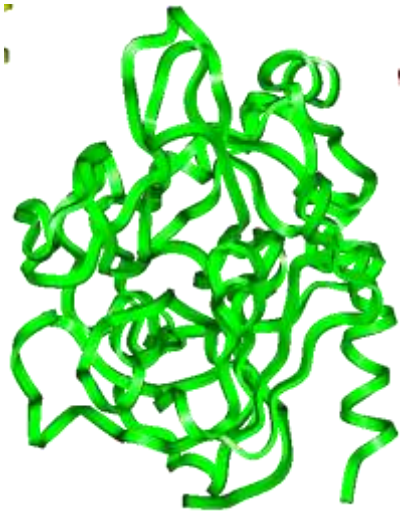
SPECIFICITA E MOLTEPLICITA SUBSTRATI

Trypsin and Thrombin have similar structures

Trypsin



Thrombin



Serina proteasi

classe di enzimi proteolitici il cui meccanismo catalitico è basato sulla presenza di un residuo di serina nel sito attivo.

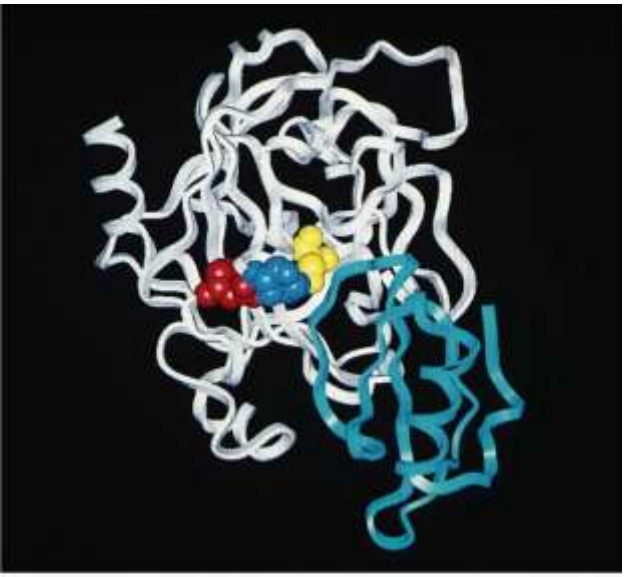
-tripsina
-chimotripsina
-elastasi

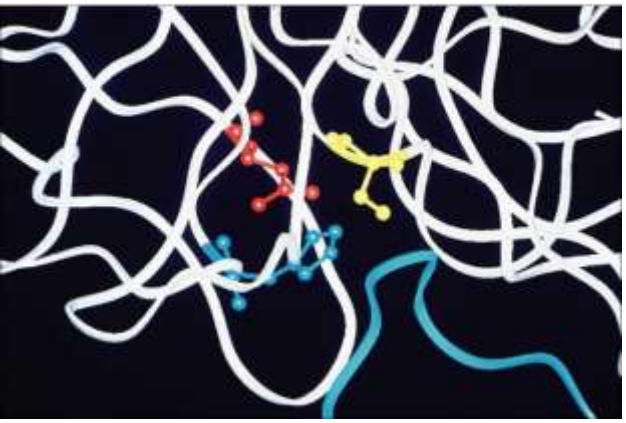
} enzimi digestivi sintetizzati nel pancreas e secreti nell'apparato digerente come *proenzimi inattivi o zimogeni*

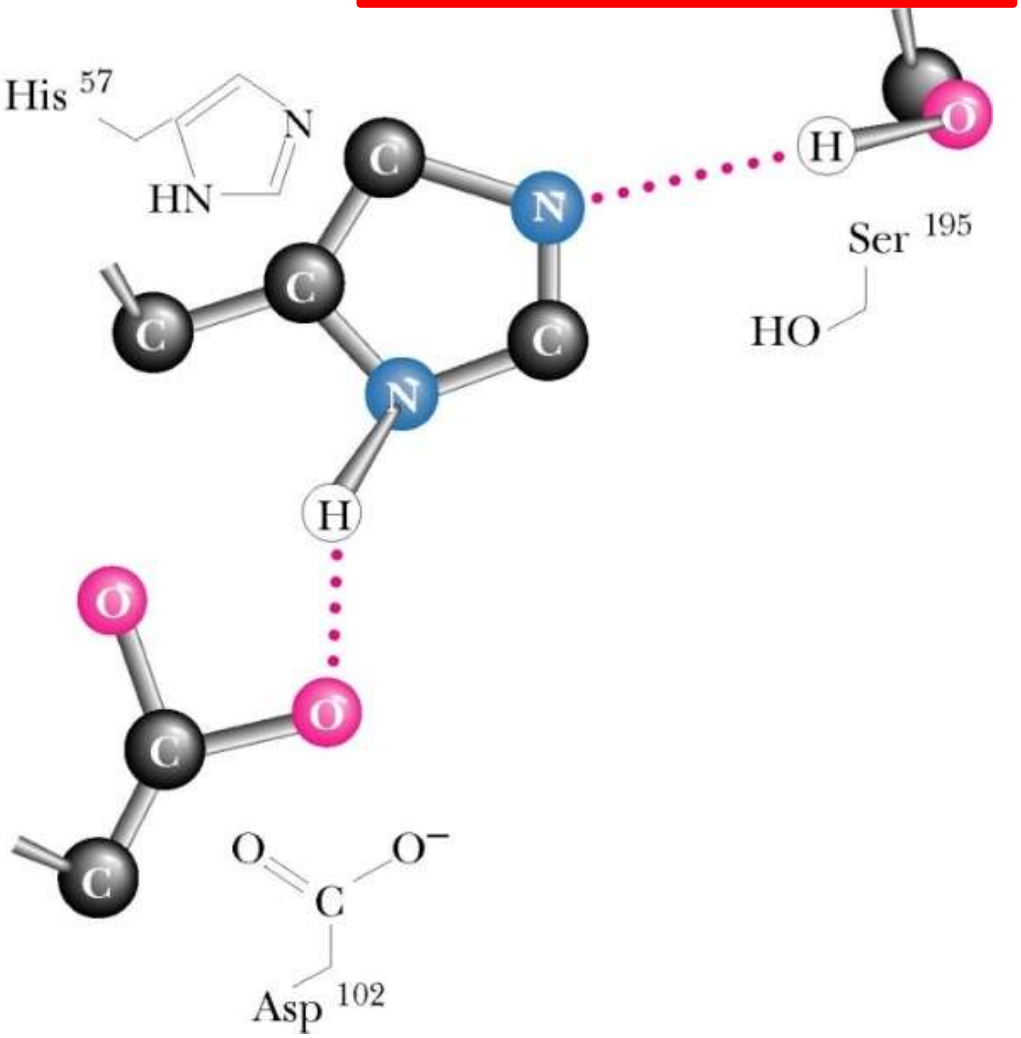
-trombina
-plasmina
-attivatore tissutale del plasminogeno

-acetil colinesterasi= non è una proteasi ma una serina esterasi il cui meccanismo d'azione è correlabile a quello delle serina proteasi. Essa idrolizza il neurotrasmettitore acetilcolina nello spazio sinaptico interneuronico.

Serin-Proteasi

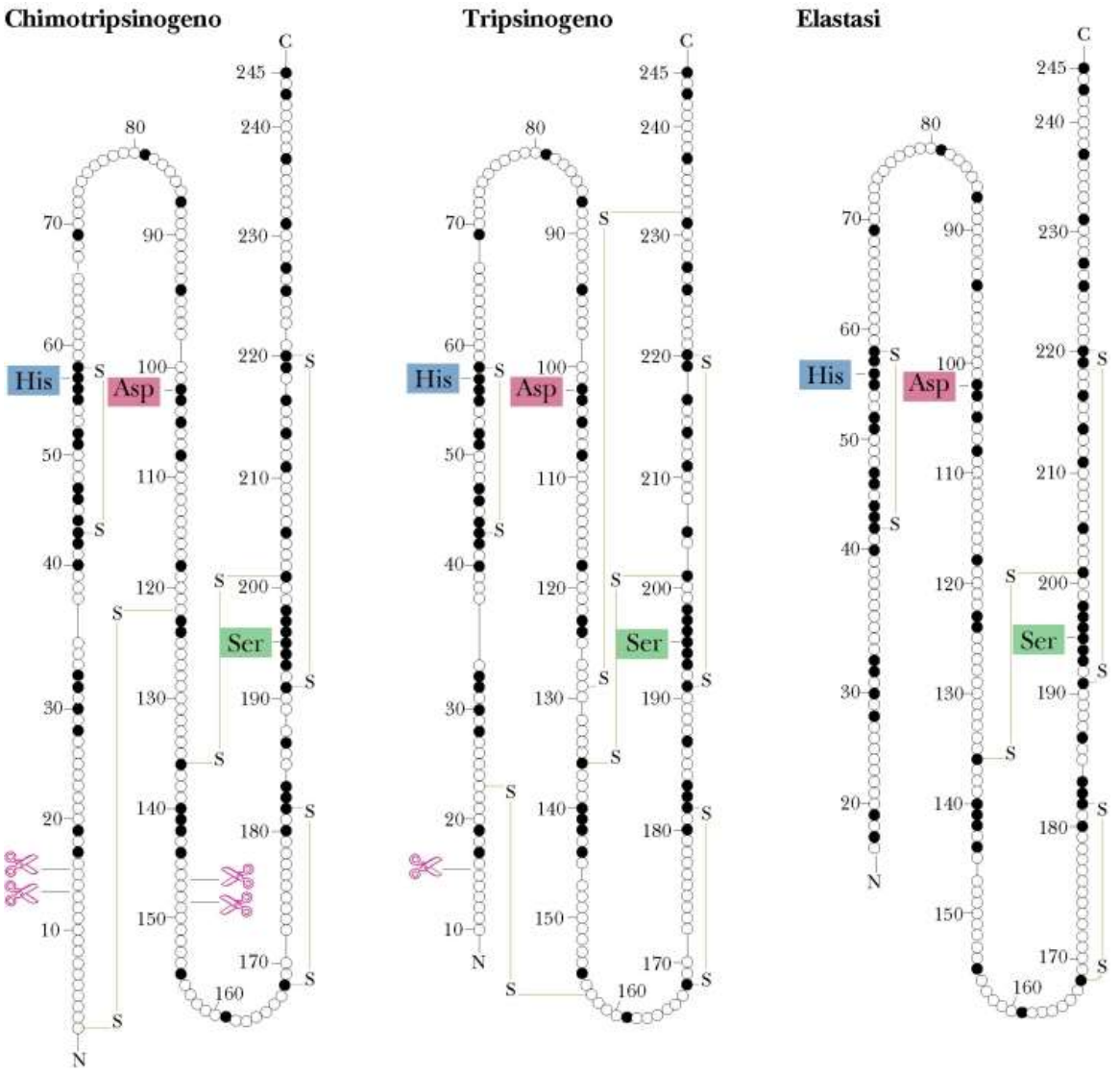






Tre residui polari (His [57], Asp[102], Ser[195]) formano la cosiddetta *triade catalitica* in corrispondenza del sito attivo il quale è costituito da una depressione sulla superficie dell'enzima.

Tripsina, chimotripsina ed elastasi catalizzano tutti la stessa reazione: la scissione di una catena polipeptidica.



Tripsina: agisce su aa basici (arginina, lisina)

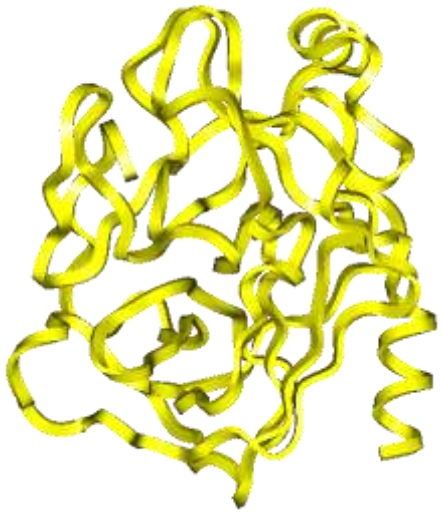
Chimotripsina: agisce su aa aromatici

Elastasi: agisce su piccoli residui di aa neutri

Trypsin and Thrombin have similar structures

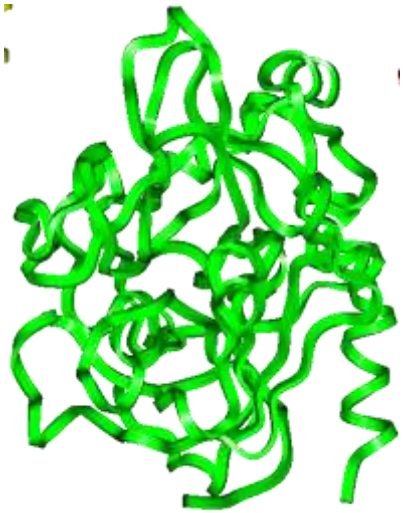
Trypsin

- Cleaves peptides on the C-term of Lys and Arg amino acid residues



Thrombin

- Cleaves peptides at Arg (Pro, Arg, Ser/Ala/Gly/Thr, not acidic)

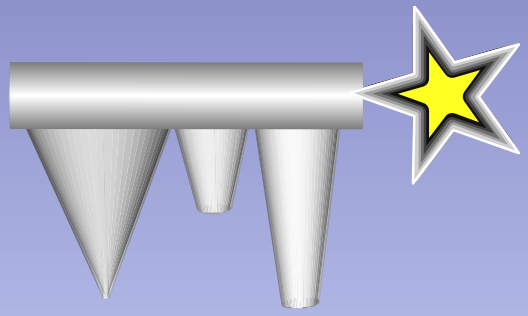


Thrombin cleaves different substrates

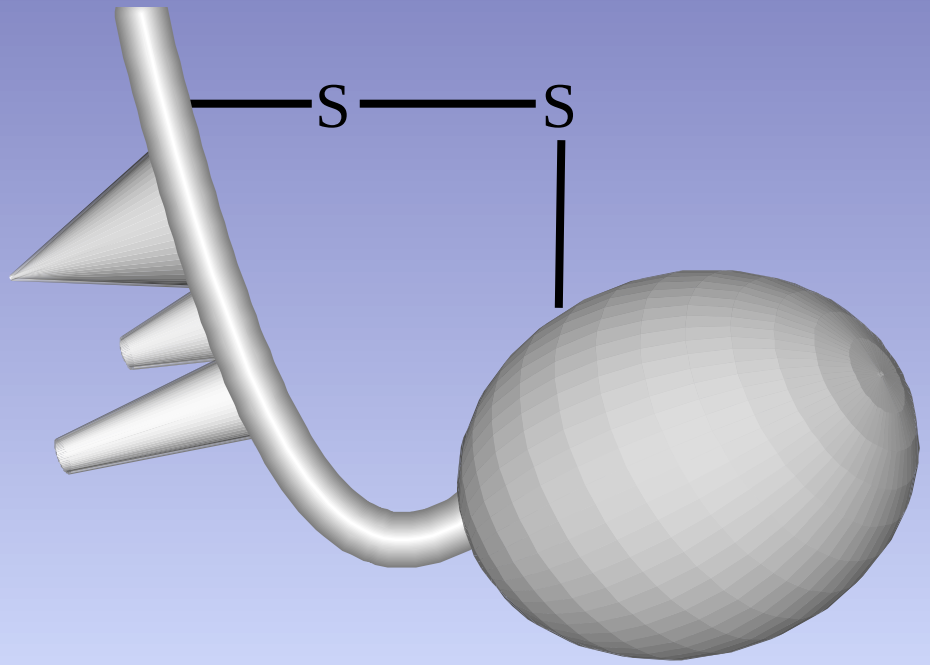
- Thrombin cleaves after Arg residues

Cleavage Sites for Natural Thrombin Substrates

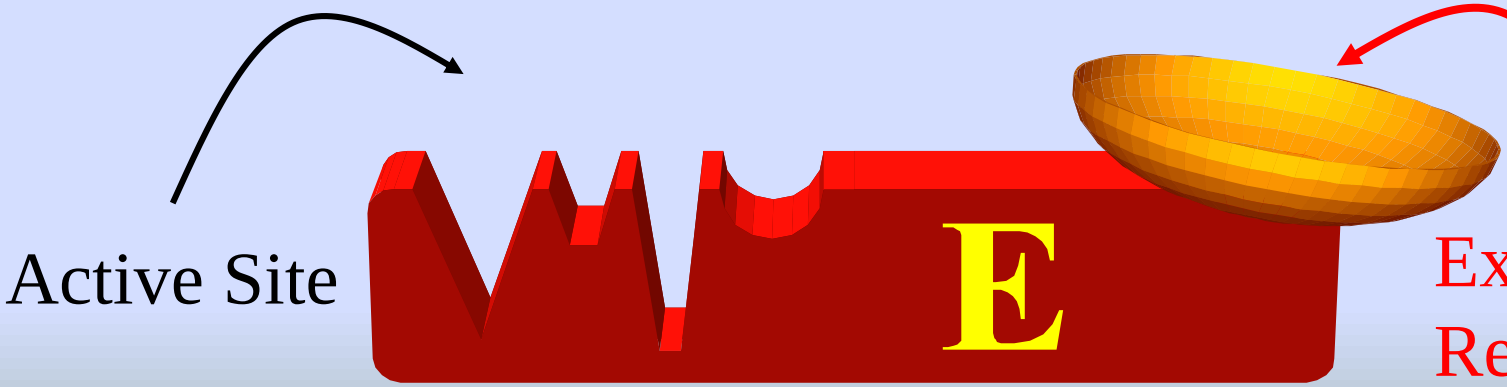
	P4	P3	P2	P1	P1′	P2′	P3′
Fibrinogen (A)	Gly	Gly	Val	Arg	Gly	Pro	Arg
Fibrinogen (B)	Phe	Ser	Ala	Arg	Gly	His	Arg
FV (709)	Leu	Gly	Ile	Arg	Ser	Phe	Arg
FV (1018)	Leu	Ser	Pro	Arg	Thr	Phe	His
FV (1545)	Trp	Tyr	Leu	Arg	Ser	Asn	Asn
FVIII (372)	Ile	Gln	Ile	Arg	Ser	Val	Ala
FVIII (740)	Ile	Glu	Pro	Arg	Ser	Phe	Ser
FVIII (1689)	Gln	Ser	Pro	Arg	Ser	Phe	Gln
FXIII	Gly	Val	Pro	Arg	Gly	Val	Asn
PAR1	Leu	Asp	Pro	Arg	Ser	Phe	Leu
PAR4	Pro	Ala	Pro	Arg	Gly	Tyr	Pro
FXI	Ile	Lys	Pro	Arg	Ile	Val	Gly
PC	Val	Asp	Pro	Arg	Leu	Ile	Asp
TAFI	Val	Ser	Pro	Arg	Ala	Ser	Ala
AT	Ile	Ala	Gly	Arg	Ser	Leu	Asn



Oligopeptidyl Substrate



Protein Substrate

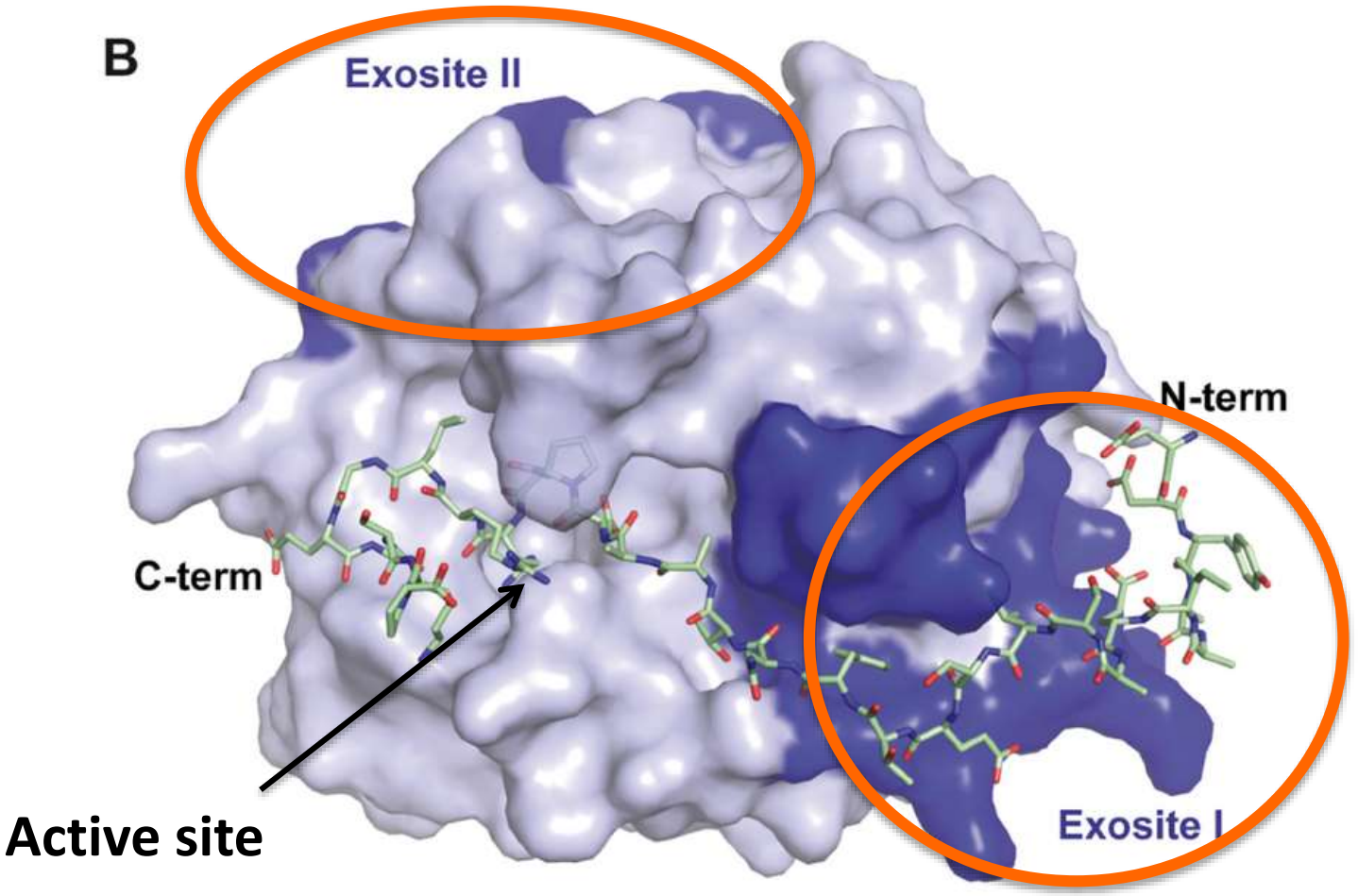


Active Site

Enzyme

Extended Recognition Site (Exosite)

Thrombin X-ray structure

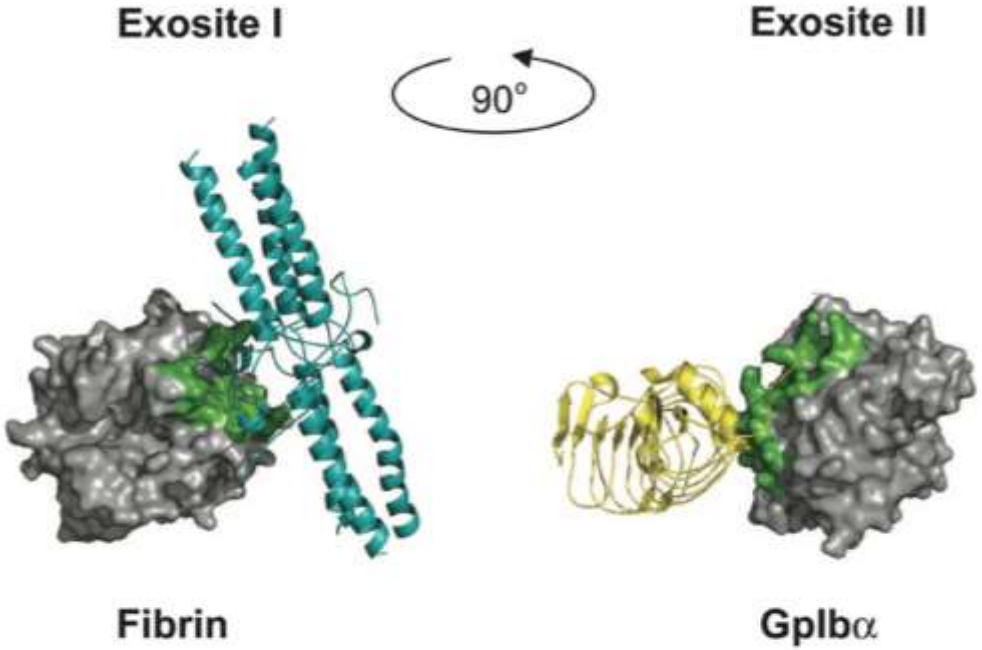


Exosite binding determines substrate specificity

- Thrombin targets are restricted due to specific interactions between the protein substrate and residues outside the catalytic cleft termed Exosite

- Extended interactions at exosites drive substrate affinity and contribute to substrate specificity.

Clot stabilization

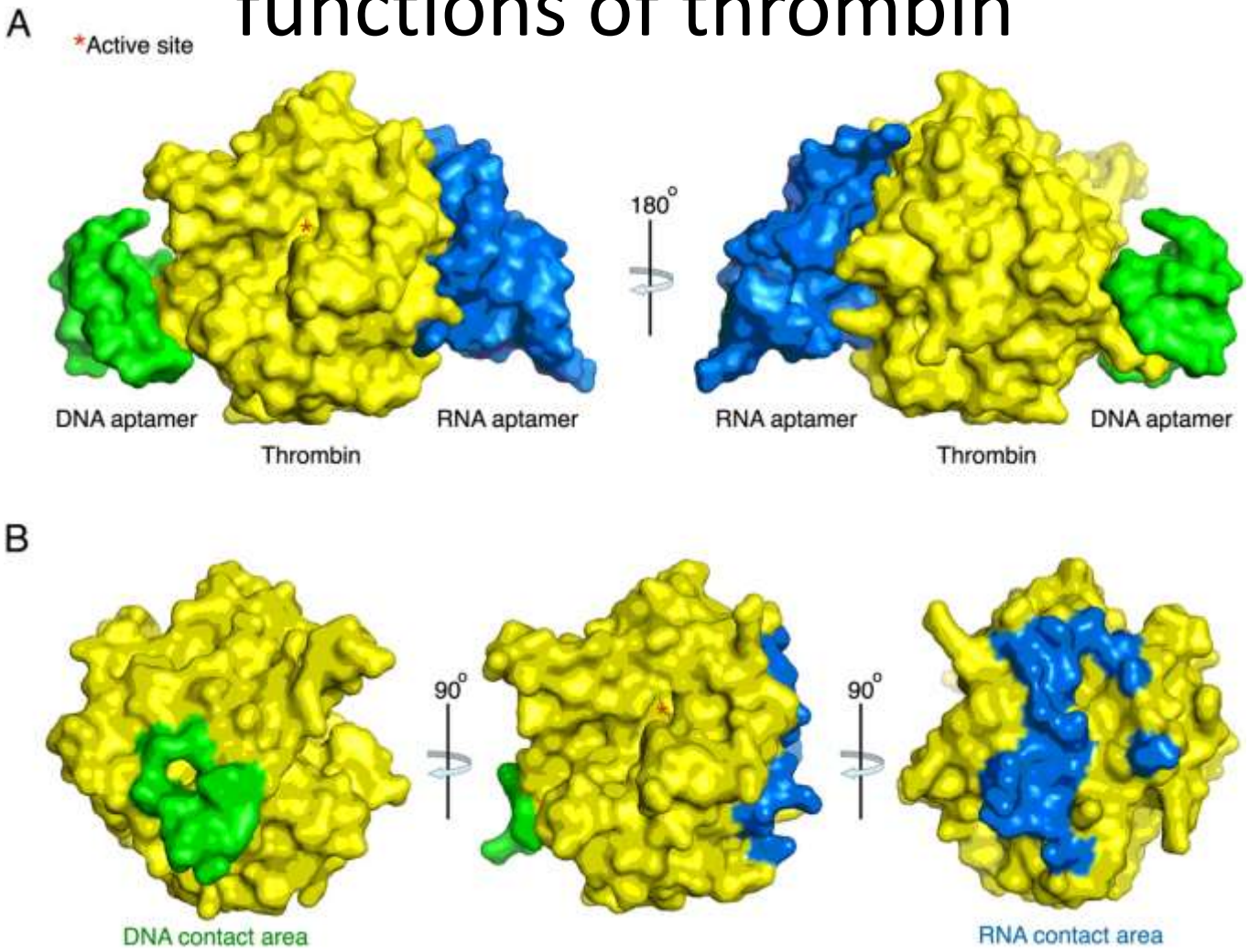


Platelet activation

Activation of the anticoagulant pathway

Inhibition

Exosites are good targets to inhibit specific functions of thrombin

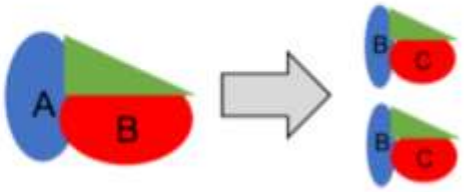


Nimjee S M et al. RNA 2009;15:2105-2111

ATTIVAZIONE della Trombina

The cascade organization

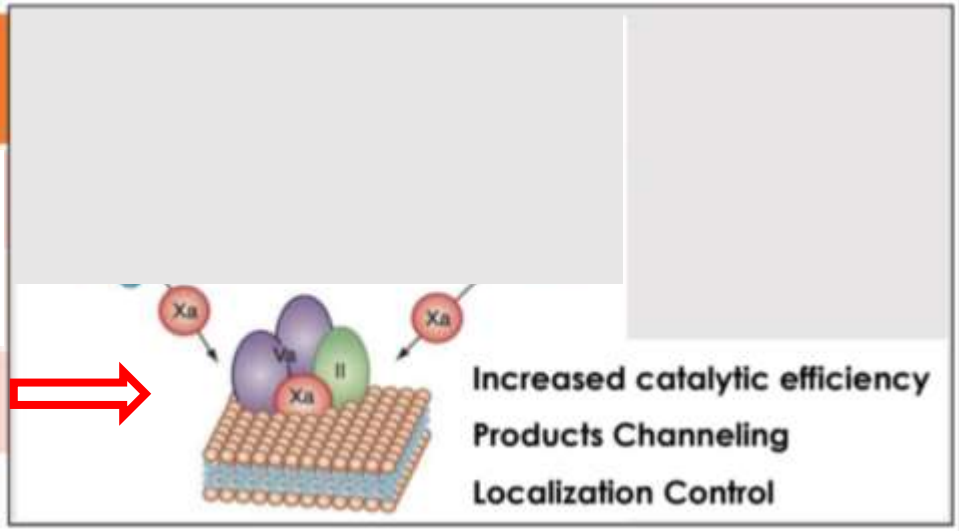
Consequential enzymatic conversions of zymogens to activated enzymes



Huge Signal Amplification!

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

Complex name	Enzyme (active)	Cofactor	Substrate (zymogen)	Catalytic Efficiency
Prothrombinase	FXa	FVa	Prothrombin	>3 x 10^5

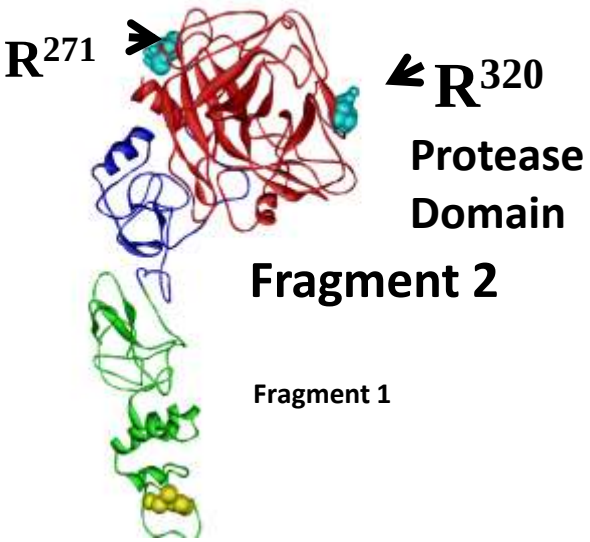


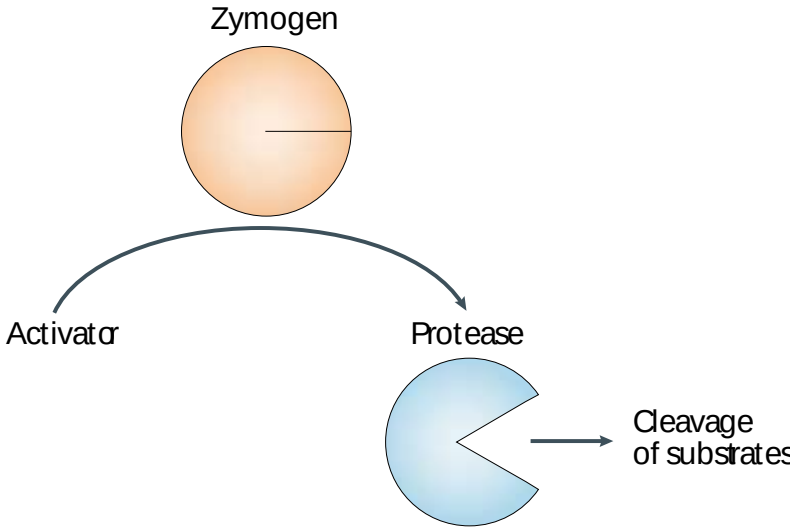
Thrombin is synthesized as a Zymogen: Prothrombin

Zymogen:

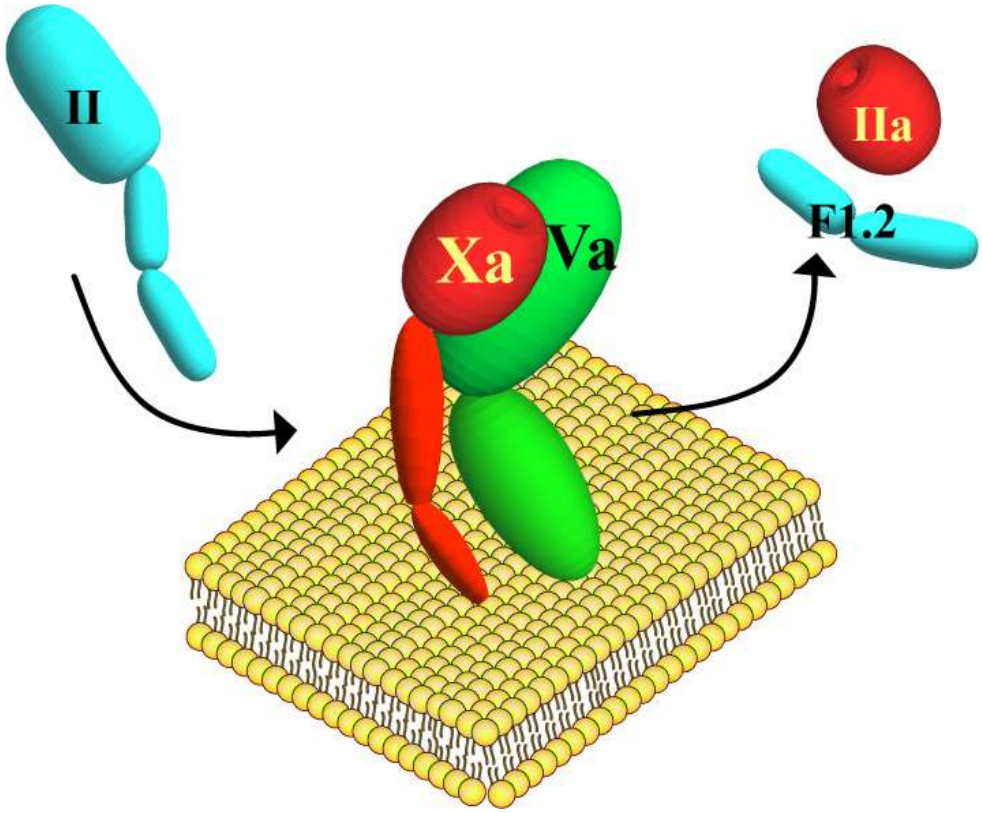
A proenzyme or inactive enzyme. It requires a biochemical change to reveal the active site for it to become an active enzyme.

Zymogens lack the structural attributes required for formation of the enzyme-substrate complex.





Prothrombin is activated to thrombin by two proteolytic cleavages

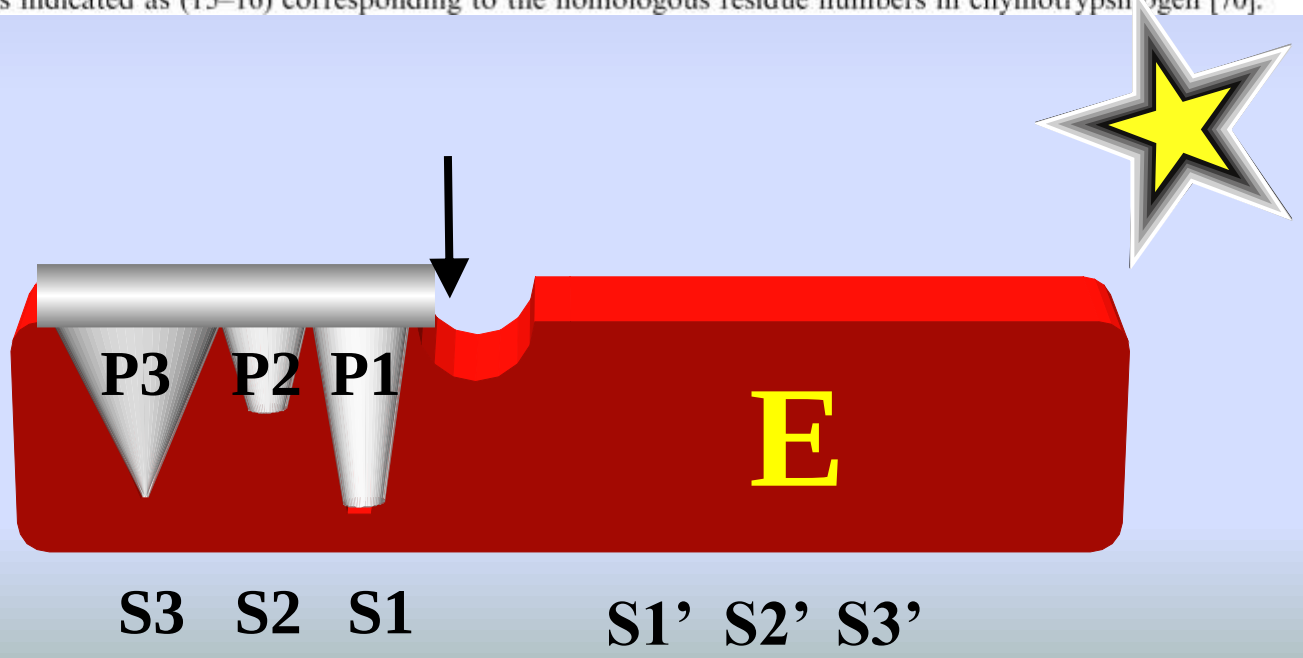


Prothrombinase

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].



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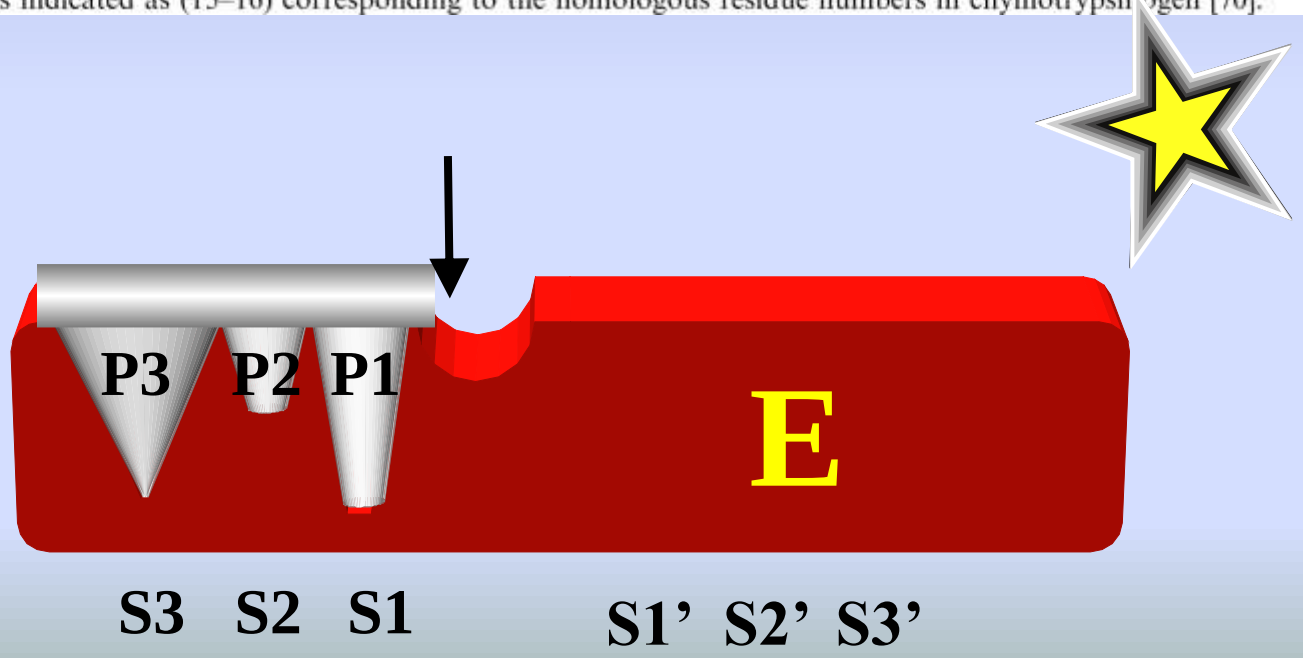
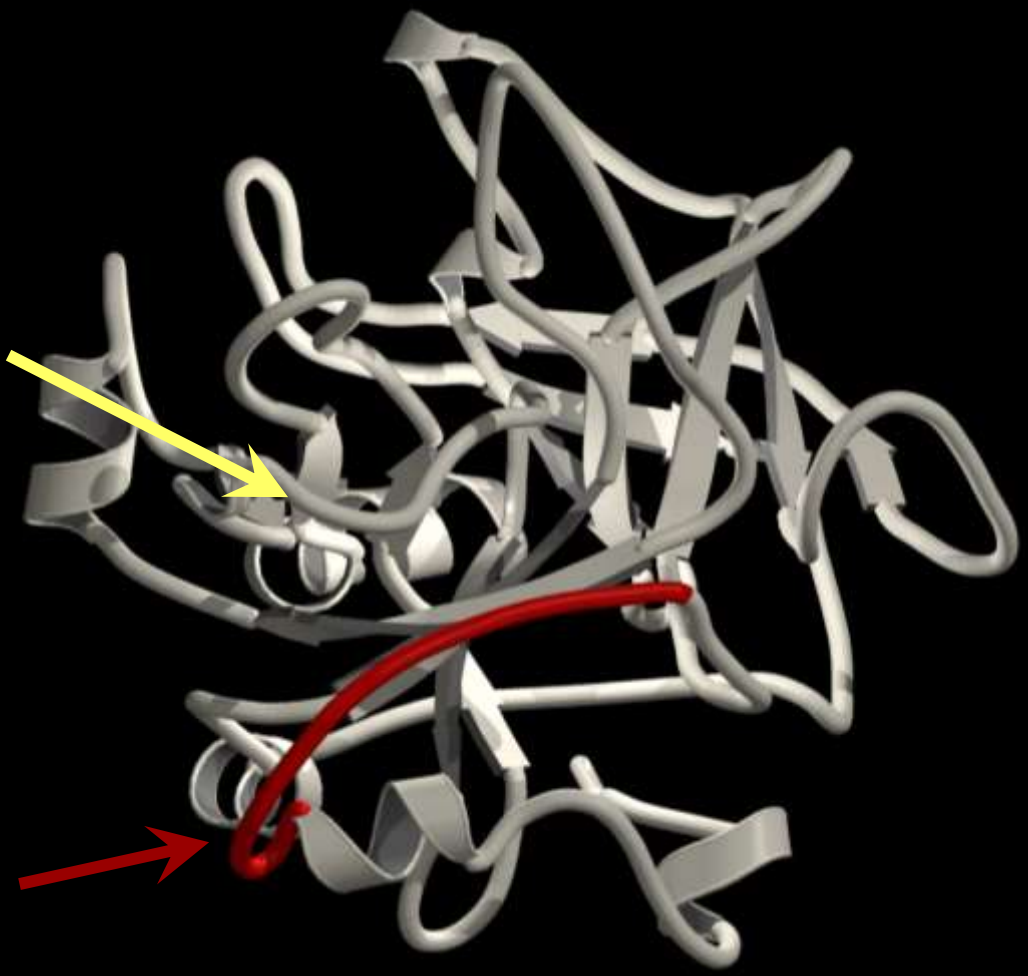
Enzyme	Substrate†	P_4	P_3	P_2	P_1	↓	$P_{1'}$	$P_{2'}$	$P_{3'}$	$P_{4'}$
Xa/Va	II	I	E	G	R		T	A	T	S
	$II_{(15-16)}$	I	D	G	R		I	V	E	G
VIIa/TF, IXa/VIIIa	$X_{(15-16)}$	N	L	T	R		I	V	G	G
VIIa/TF, XIa	IX	K	L	T	R		A	E	A	V
	$IX_{(15-16)}$	D	F	T	R		V	V	G	G
VIIa/TF, Xa	$VII_{(15-16)}$	P	Q	G	R		I	V	G	G
IIa/TM	$PC_{(15-16)}$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



Serine Proteases: Conversion Pathway

- Cleavage between Arg^{15}-Ile^{16} → Exposure of new N-terminus
- New N-terminus (IleVal) forms salt bridge with Asp^{194}
- N-terminal insertion leads to a conformational change in the "activation domain"



Courtesy of W. Bode, Max Planck Institute of Biochemistry

Prossima

MECCANISMO ATTIVAZIONE BATTERICA

Protrombina - Trombina