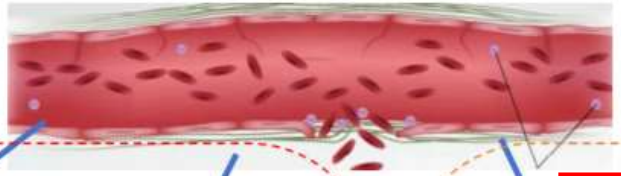
Hemostatic process: an overview



Vascular Injury

Collagen
vWF

Tissue factor

Vasoconstriction

Platelet activation

Coagulation cascade

vWF
Fibrinogen

Thrombin

Fibrinogen → Fibrin

Platelets plug

Plasmin

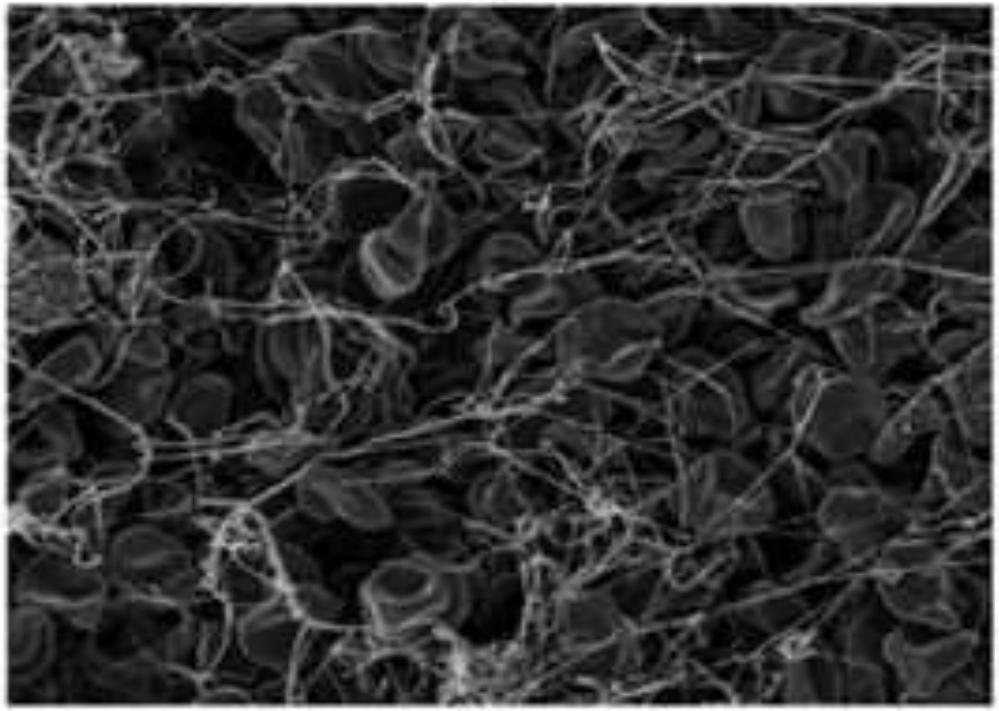
Fibrinolysis
&
Clot degradation

Primary Hemostasis

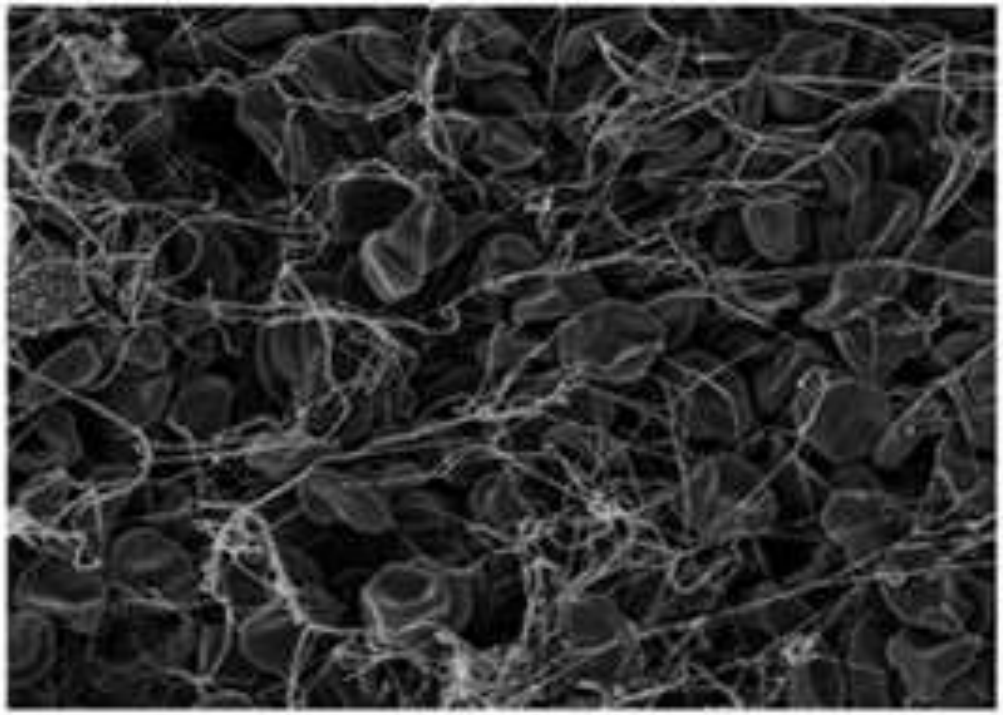
Secondary Hemostasis

Modifiers of Fibrin Clot Formation, Structure, and Stability

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



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)

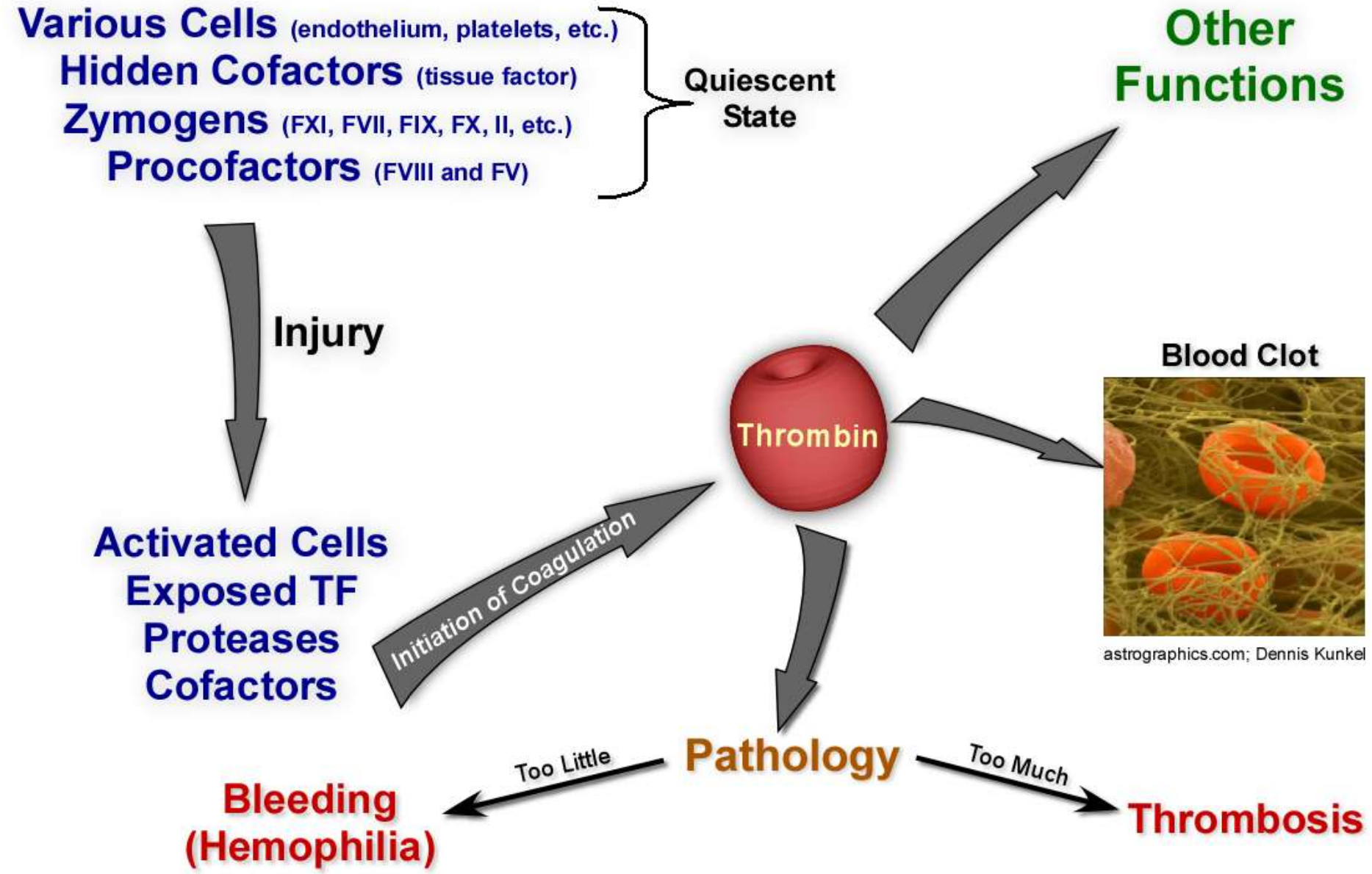


Diseases Associated with Abnormal Fibrin(ogen) Structure and Stability

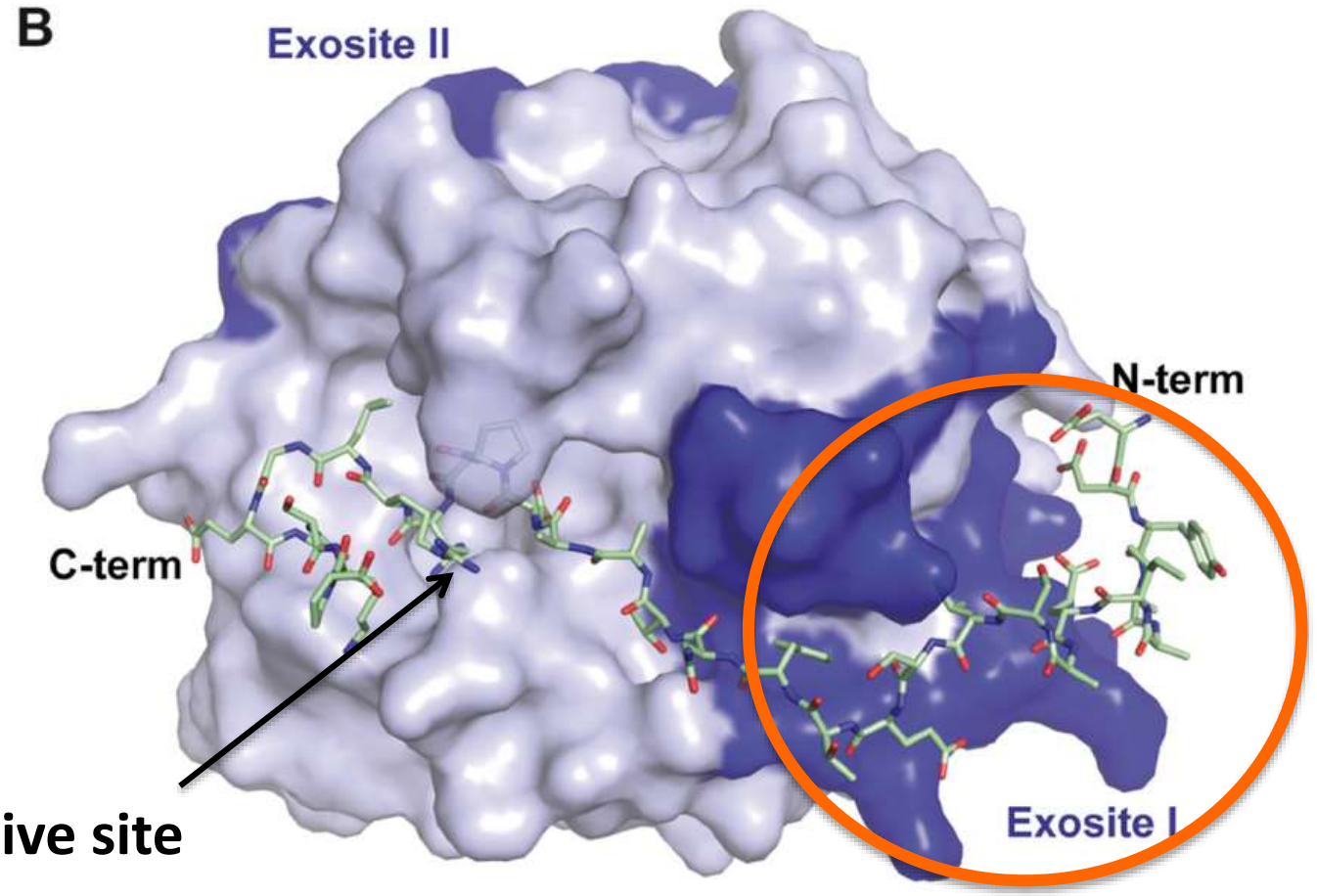
Coronary Artery Disease
Myocardial Infarction
Ischemic Stroke
Venous Thromboembolism
Abdominal Aortic Aneurysm
Smoking
Chronic Kidney Disease
In-stent Thrombosis
Cirrhosis
Hemophilia
Others?

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

The Blood Coagulation Response:



Thrombin X-ray structure

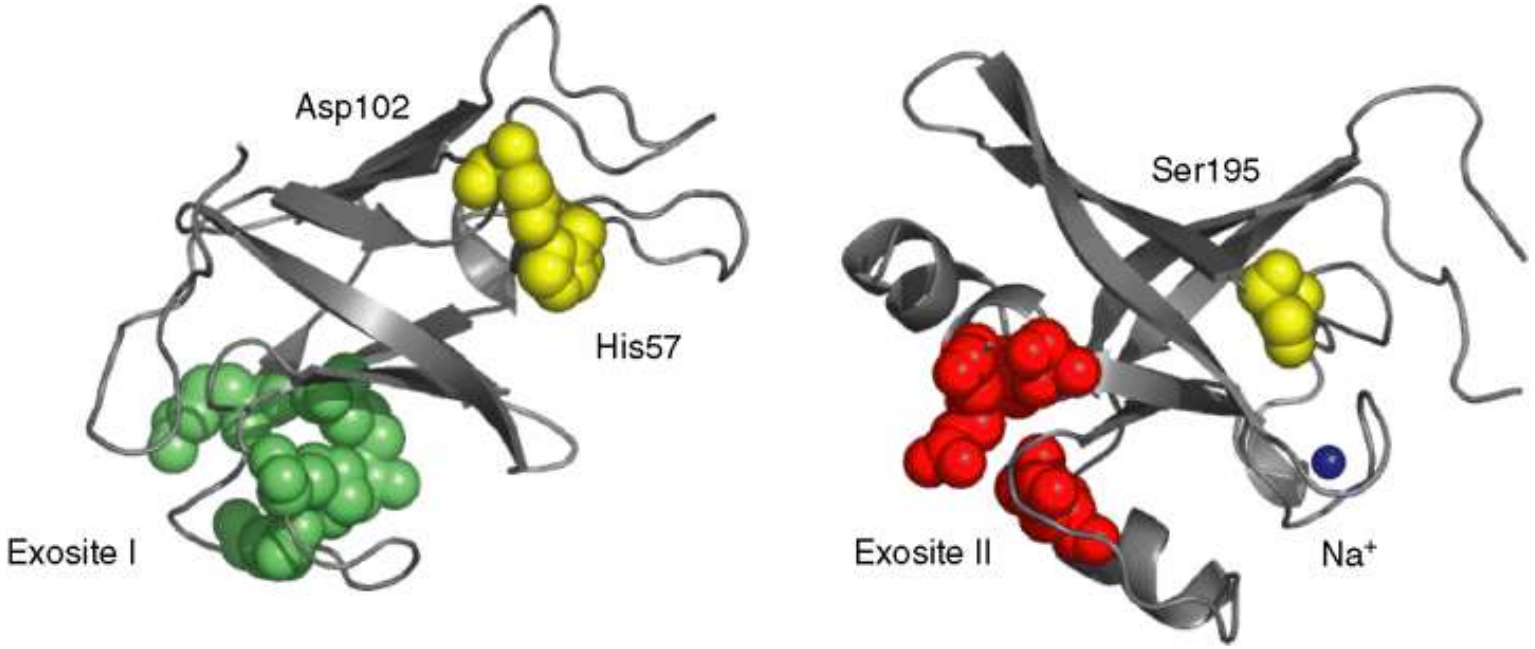


Exosite binding determines substrate specificity

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

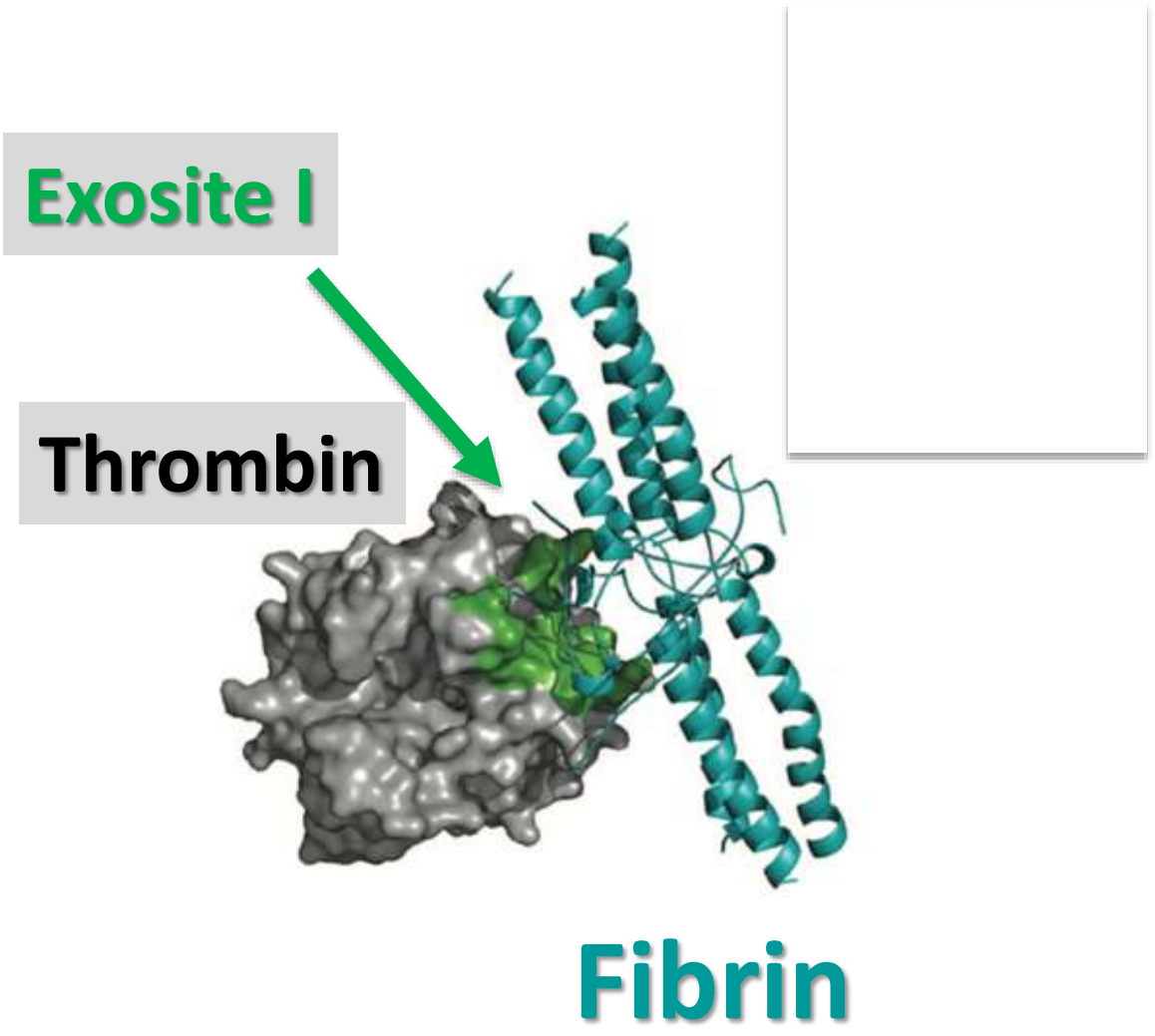
- Extended interactions at exosites drive substrate affinity and contribute to substrate specificity.

Determinants of specificity in coagulation proteases



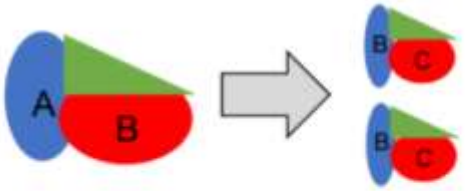
Journal of Thrombosis and Haemostasis, Volume: 3, Issue: 11, Pages: 2401-2408, First published: 14 October 2005, DOI: (10.1111/j.1538-7836.2005.01456.x)

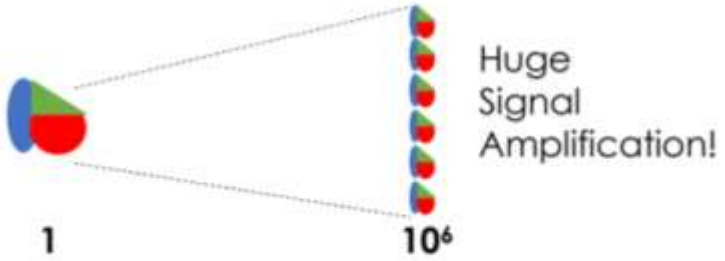
Clot formation



ATTIVAZIONE della Trombina

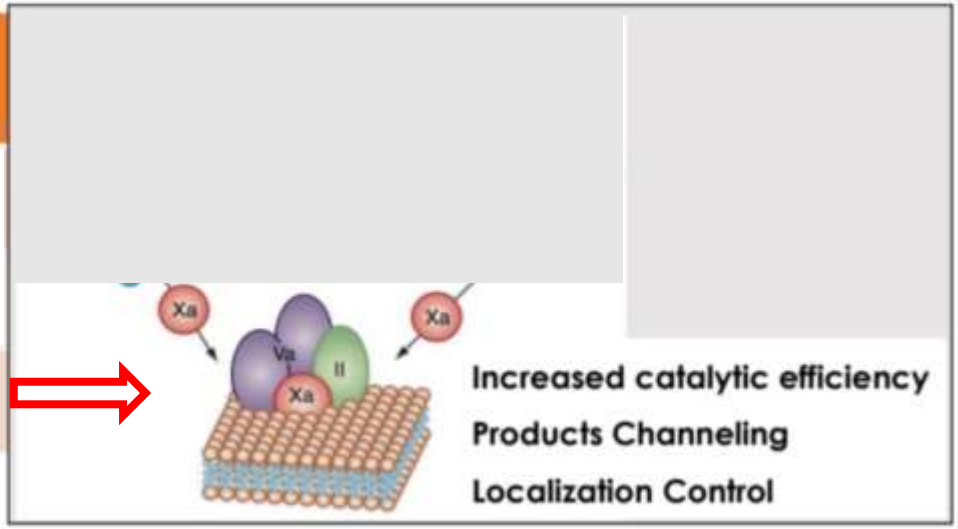
ATTIVAZIONE della Trombina



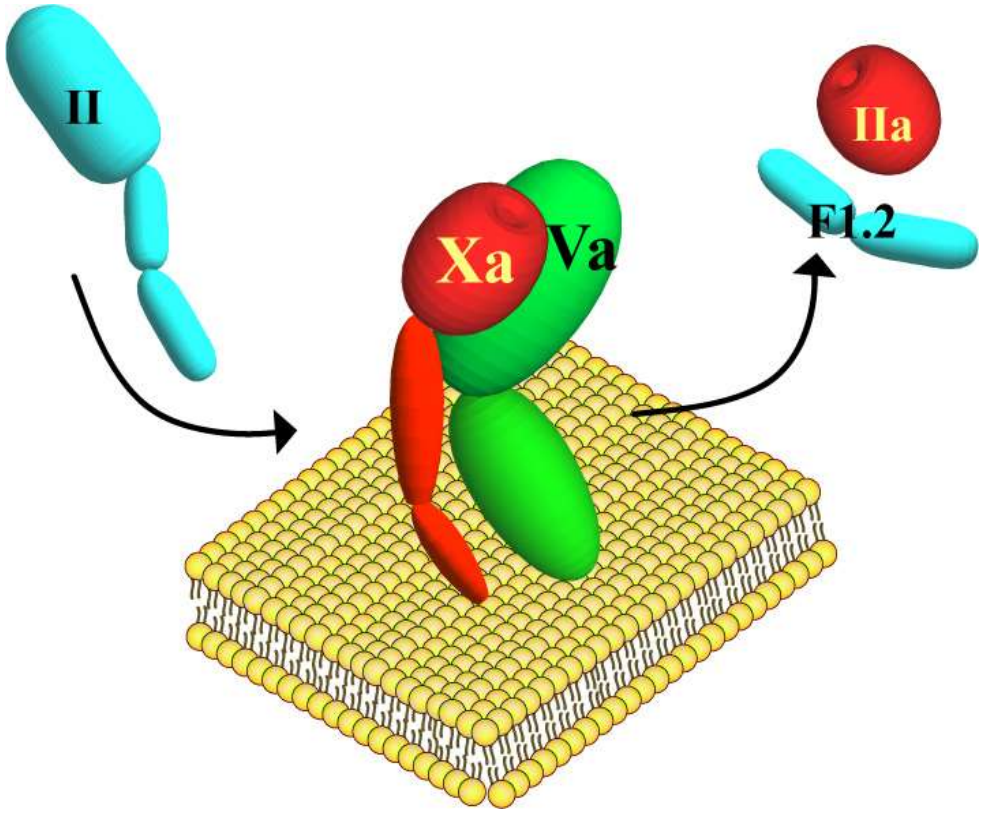


It takes place on ***macromolecular complex***:

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

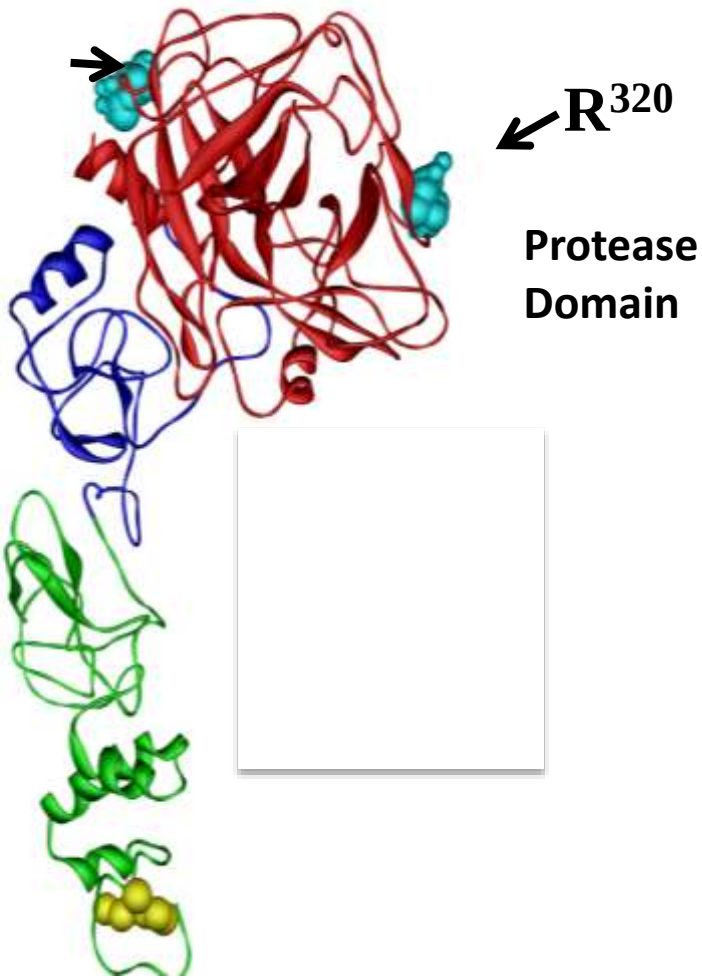


Prothrombin is activated to thrombin by two proteolytic cleavages

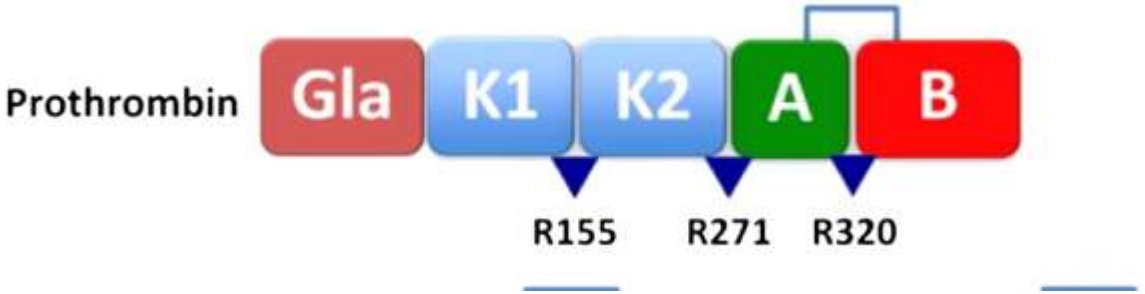


Prothrombinase

Prothrombin



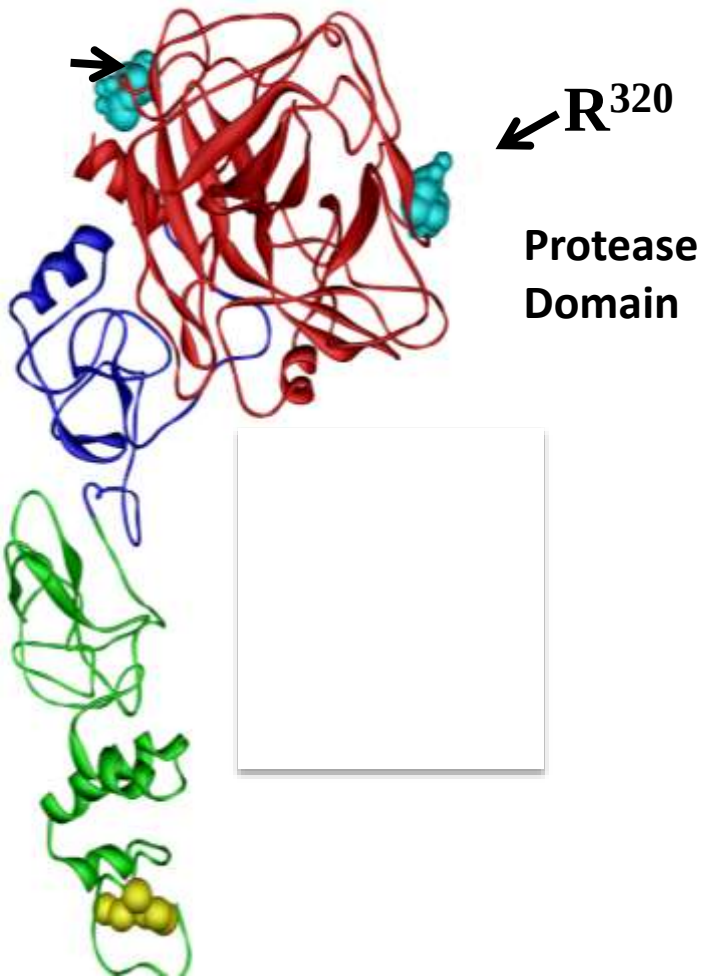
I SITI DI TAGLIO SONO TRE!

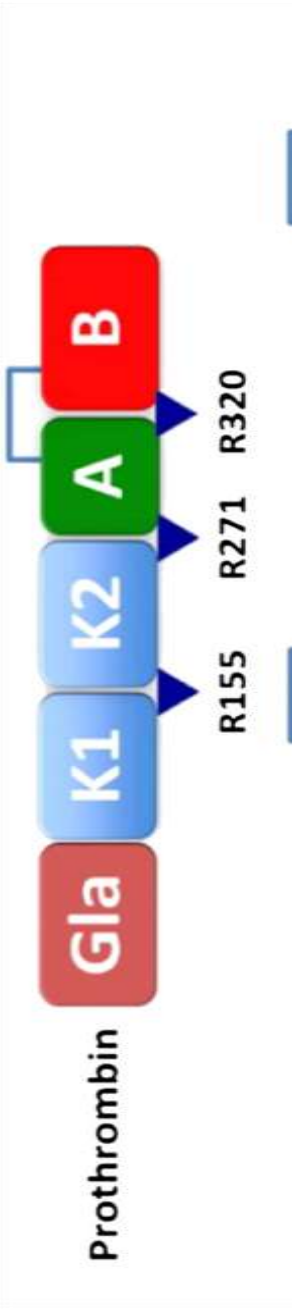


Prothrombin contains a Gla domain, two kringles (K1 and K2), and a protease domain composed of the A and B chains.

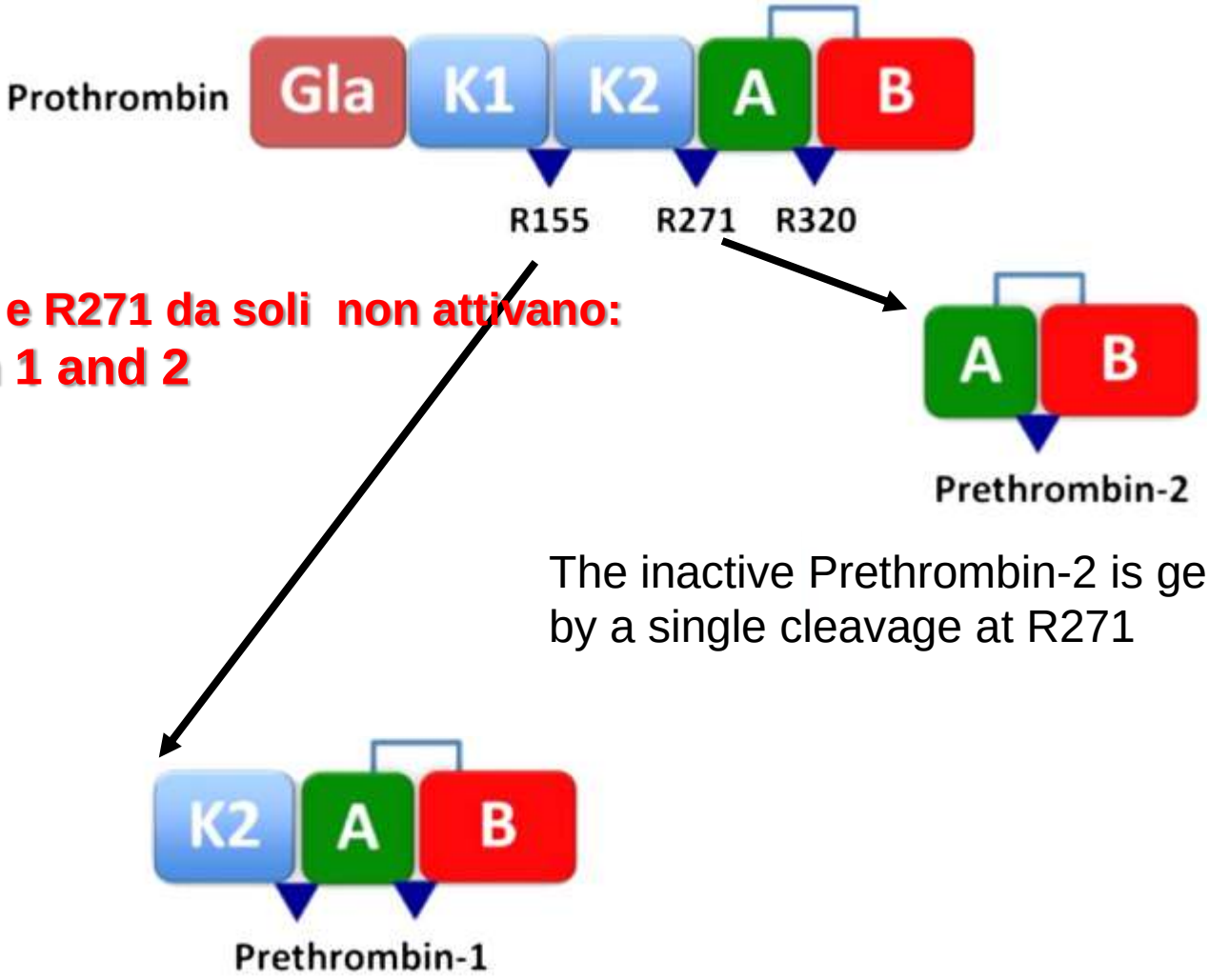
Zhiwei Chen et al. PNAS 2010;107:45:19278-19283

Prothrombin





Schematic representation of prothrombin activation.



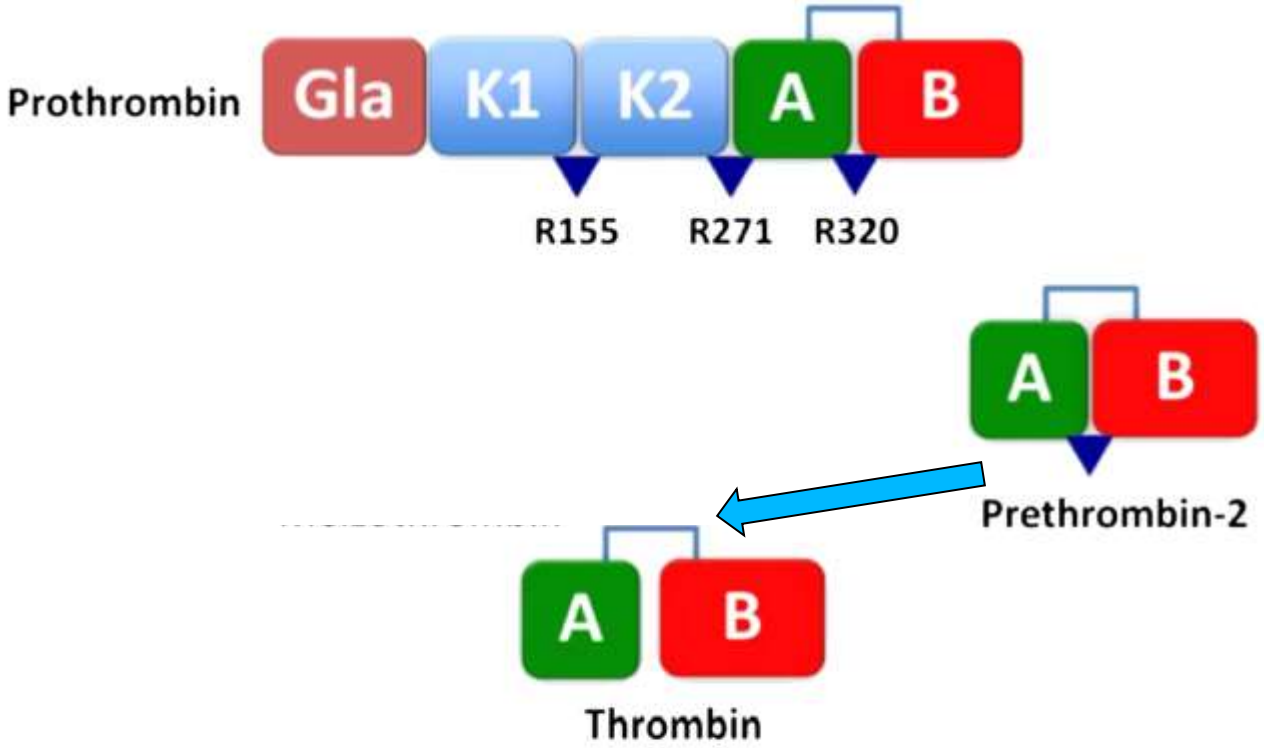
I tagli in R155 e R271 da soli non attivano: Prethrombin 1 and 2

The inactive Prethrombin-2 is generated by a single cleavage at R271

Zhiwei Chen et al. PNAS 2010;107:45:19278-19283

PNAS

Cleavage at R271 and R320 produce Thrombin



Cleavage at R320 separates the A and B chains and generates an active protease.

The inactive Prethrombin-2 is generated by a single cleavage at R271

Zhiwei Chen et al. PNAS 2010;107:45:19278-19283

Prothrombin is activated to thrombin by two proteolytic cleavages

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

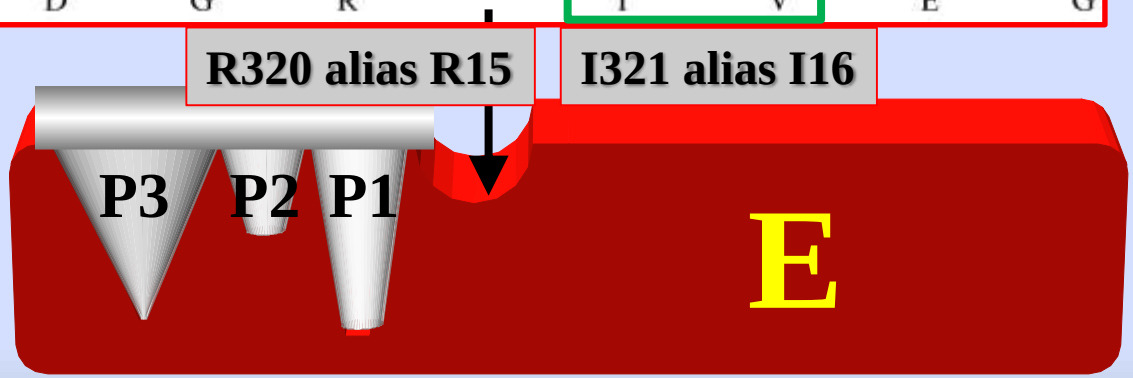
Enzyme	Substrate†	P_4	P_3	P_2	P_1	↓	$P_{1'}$	$P_{2'}$	$P_{3'}$	$P_{4'}$
Xa/Va	II	I	E	G	R		T	A	T	S
	$II_{(15–16)}$	I	D	G	R		I	V	E	G

R271

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

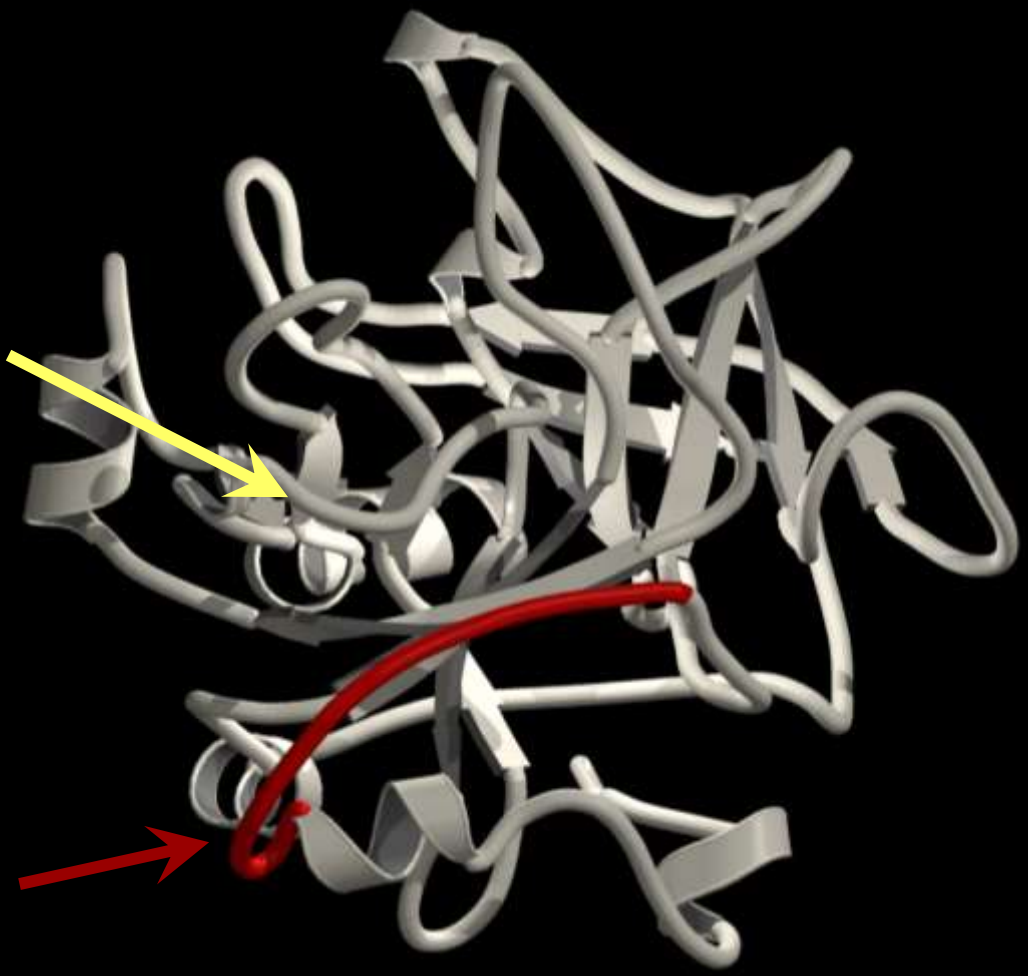
R320 alias R15

I321 alias I16



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