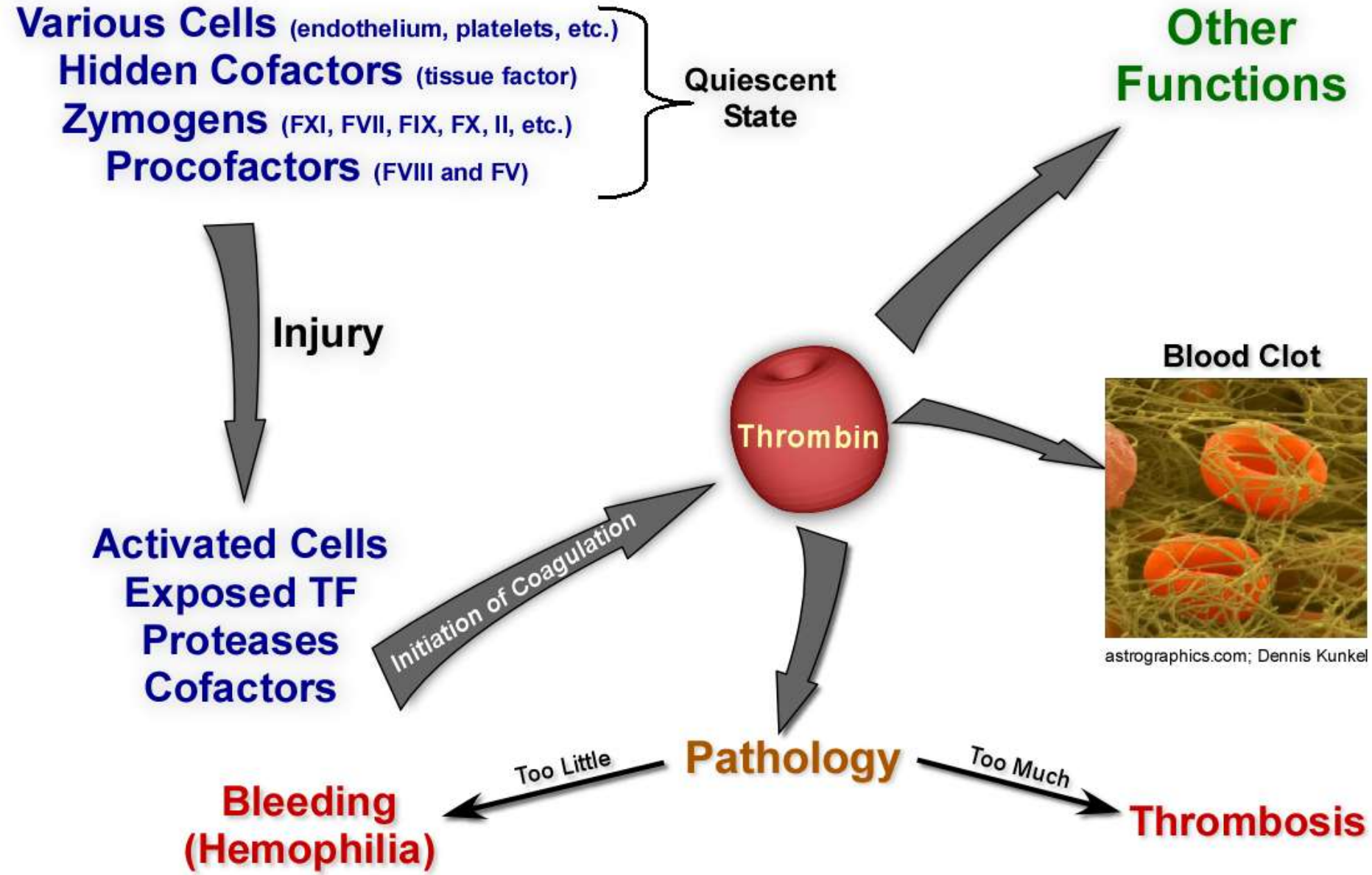
Activation and specificity of Thrombin

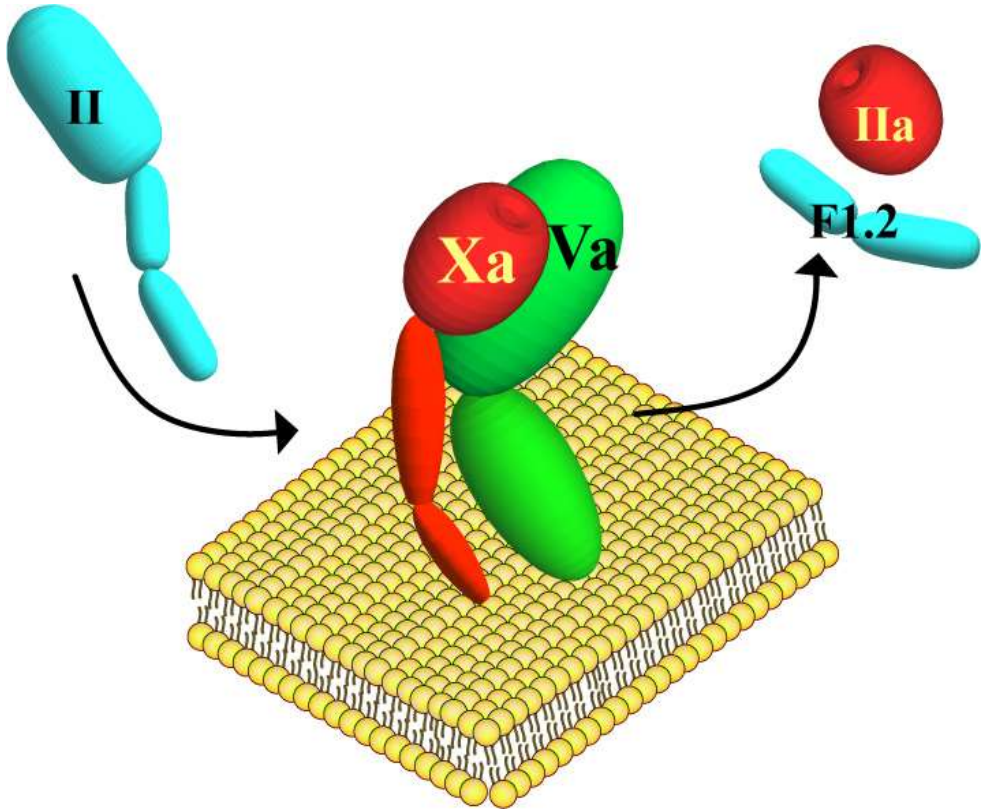
Da Giulia Pavani

The Blood Coagulation Response:



ATTIVAZIONE

Prothrombin is activated to thrombin by two proteolytic cleavages



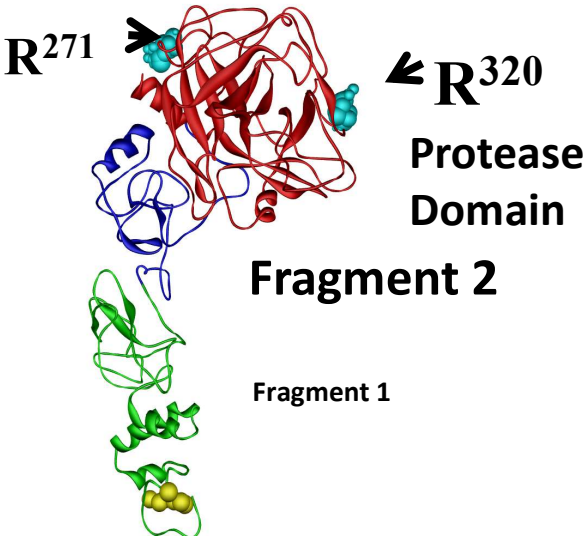
Prothrombinase

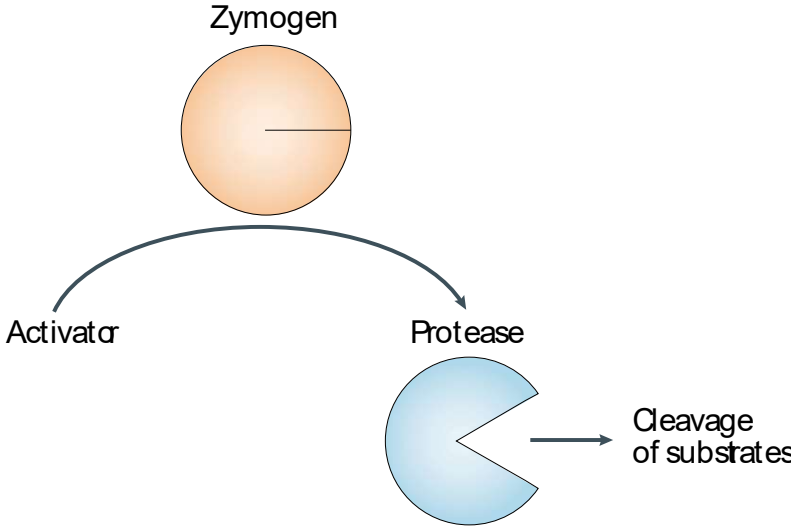
Thrombin is synthesized as a Zymogen: Prothrombin

Zymogen:

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

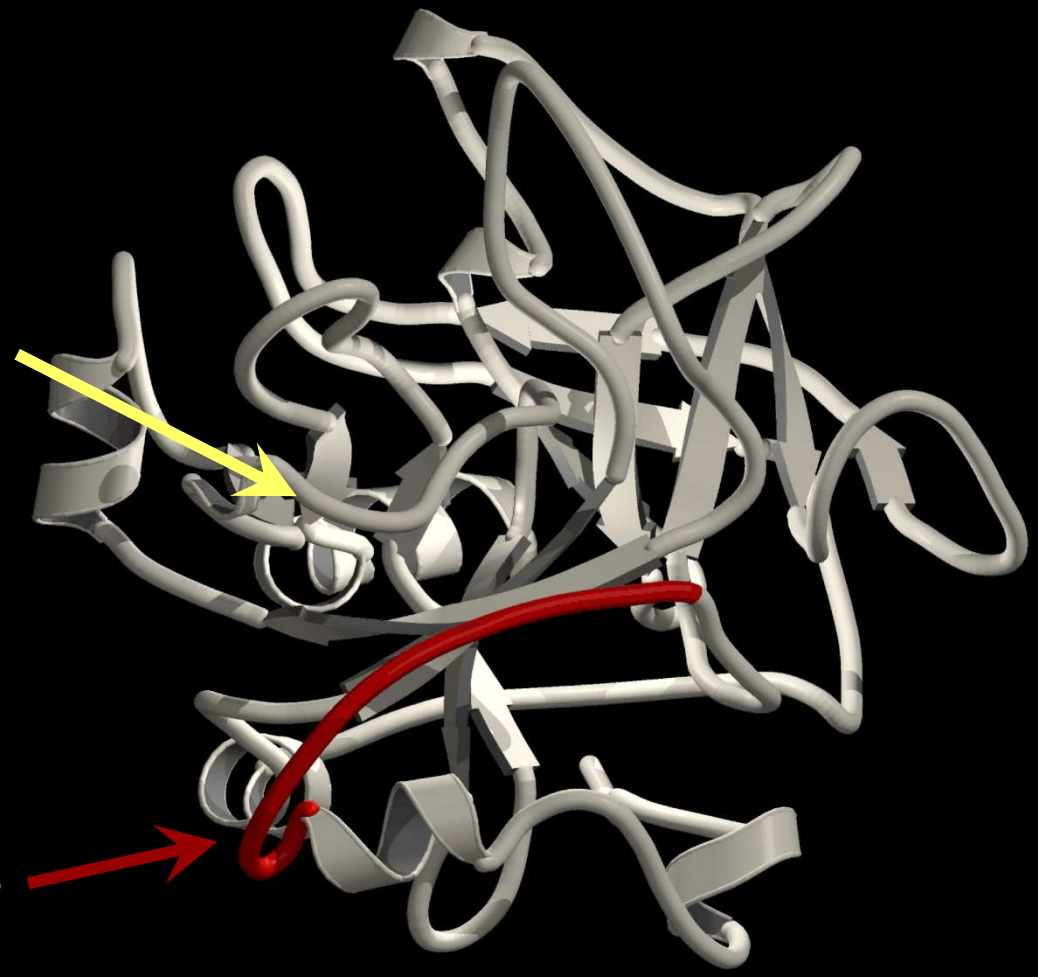
Zymogens lack the structural attributes required for formation of the enzyme-substrate complex.





Serine Proteases: Conversion Pathway

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

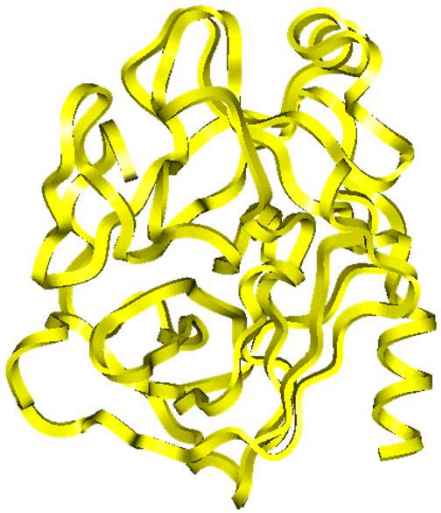


Courtesy of W. Bode,
Max Planck
Institute of Biochemistry

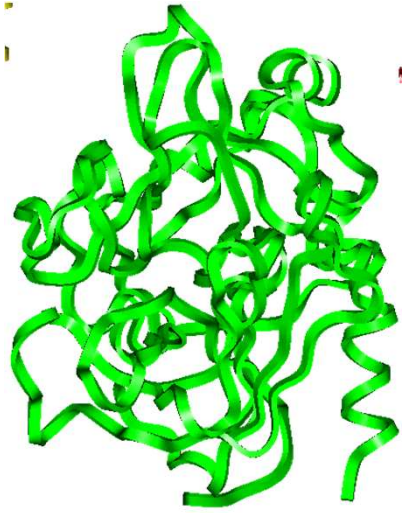
SPECIFICITA E MOLTEPLICITA SUBSTRATI

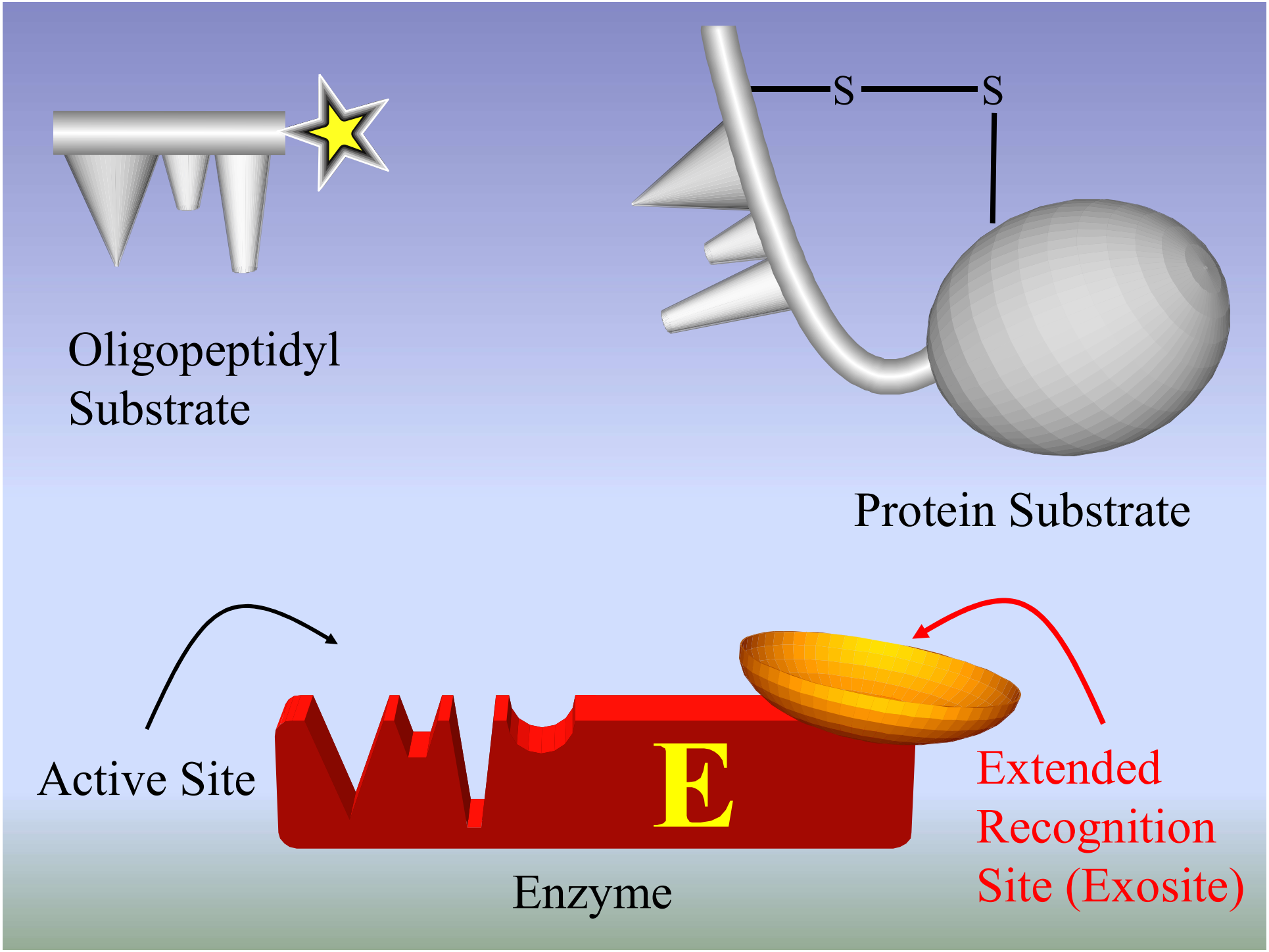
Trypsin and Thrombin have similar structures

Trypsin

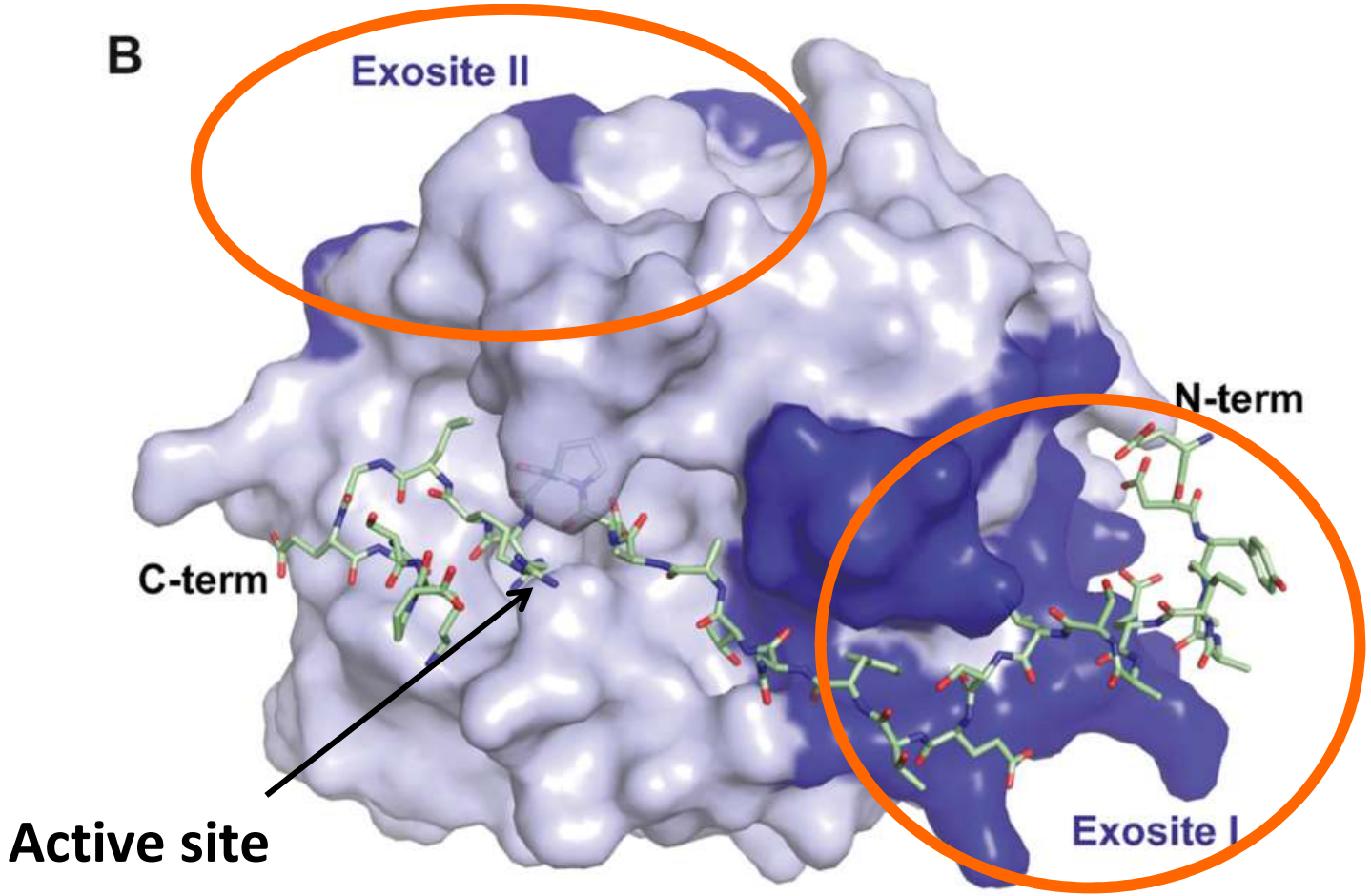


Thrombin




S
S
Oligopeptidyl
Substrate
Protein Substrate
Active Site
E
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

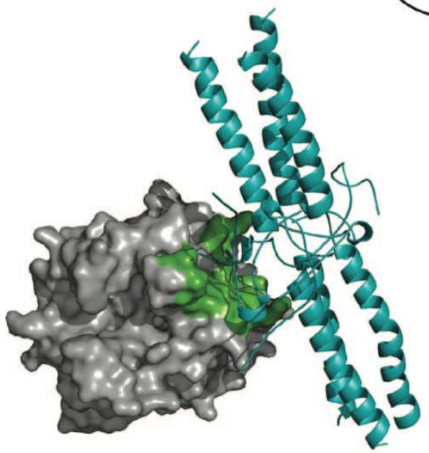
- Extended interactions at exosites drive substrate affinity and contribute to substrate specificity.

Exosite I

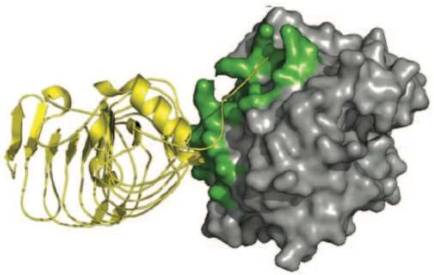
Exosite II

90°

Clot stabilization



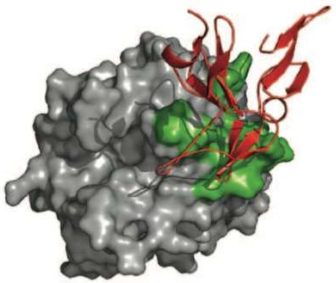
Fibrin



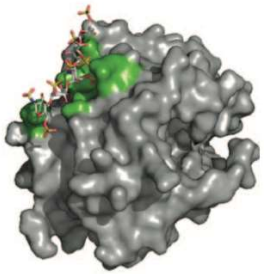
GpIbα

Platelet activation

Activation of the anticoagulant pathway



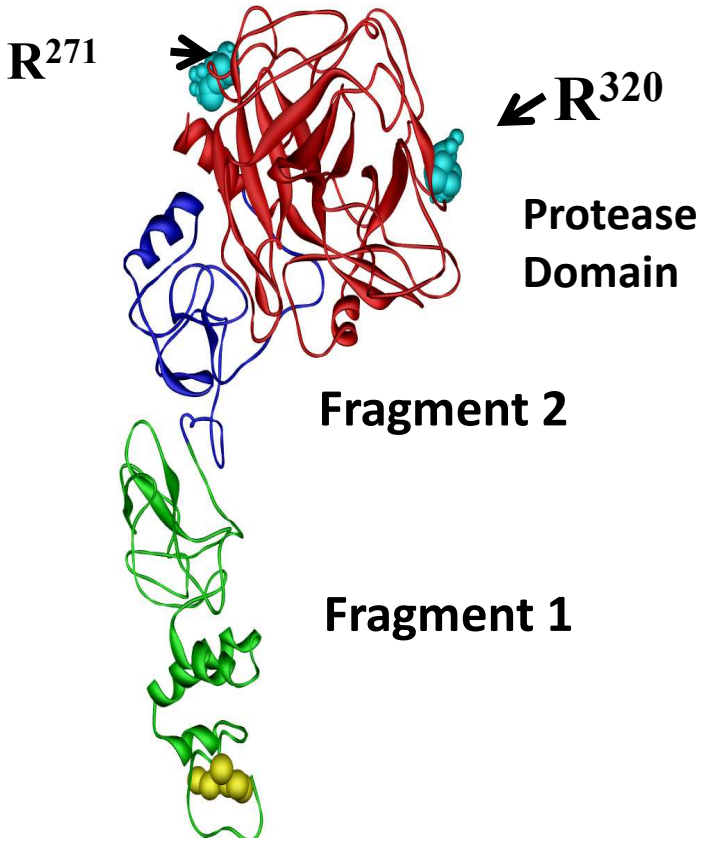
TM

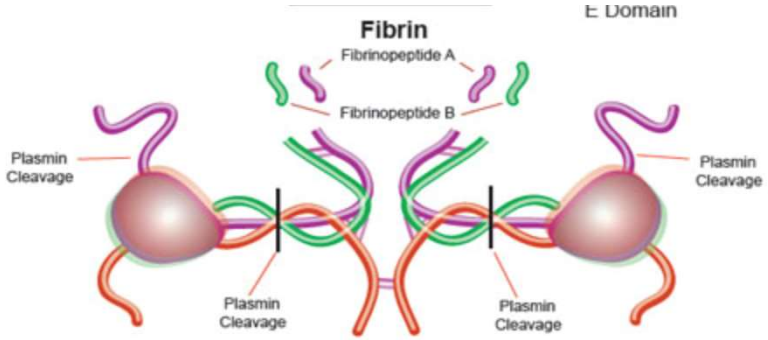


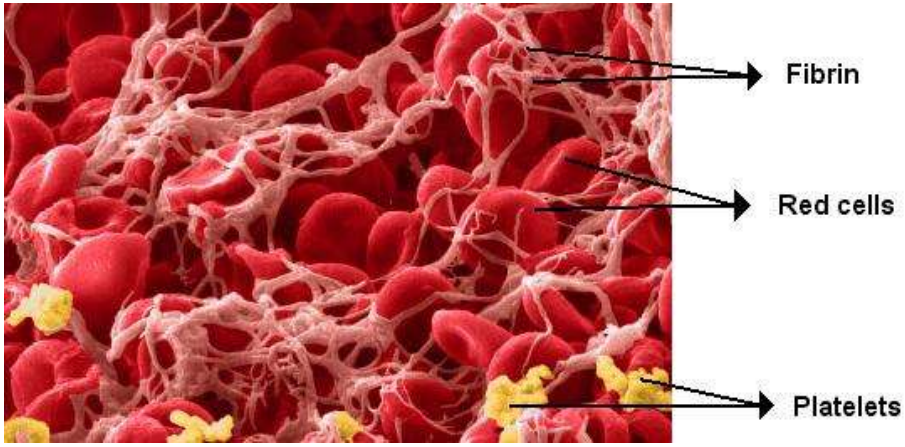
Heparin

Inhibition

Thrombin cleavage of the plasma protein fibrinogen







Thrombin cleaves different substrates

- Thrombin cleaves after Arg residues

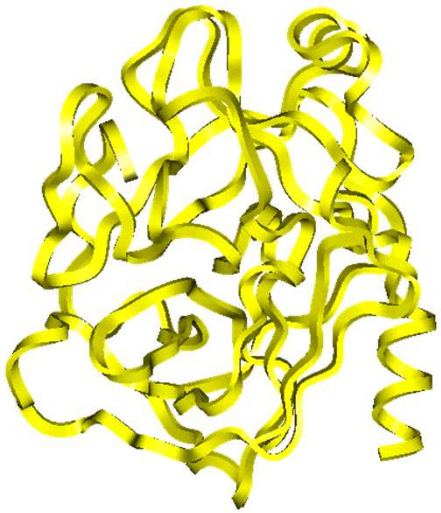
Cleavage Sites for Natural Thrombin Substrates

	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

Trypsin and Thrombin have similar structures

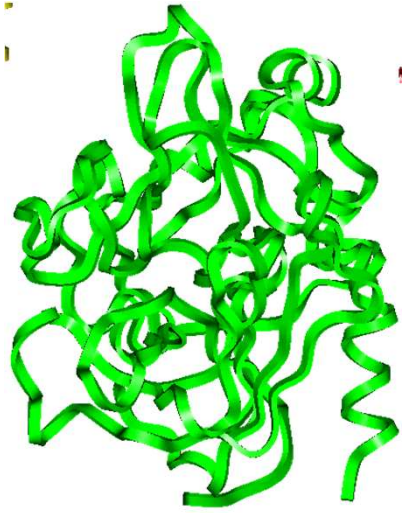
Trypsin

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

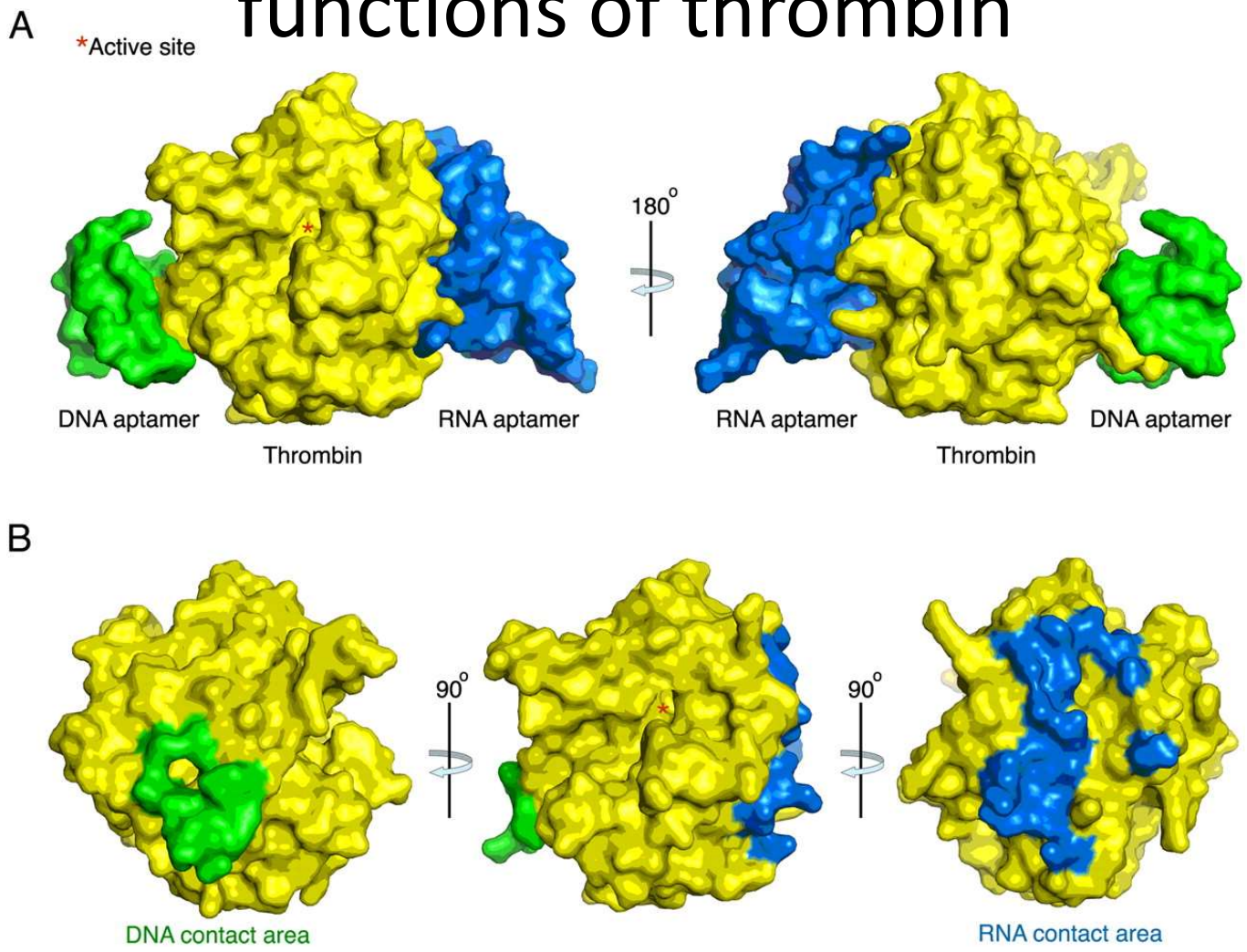


Thrombin

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

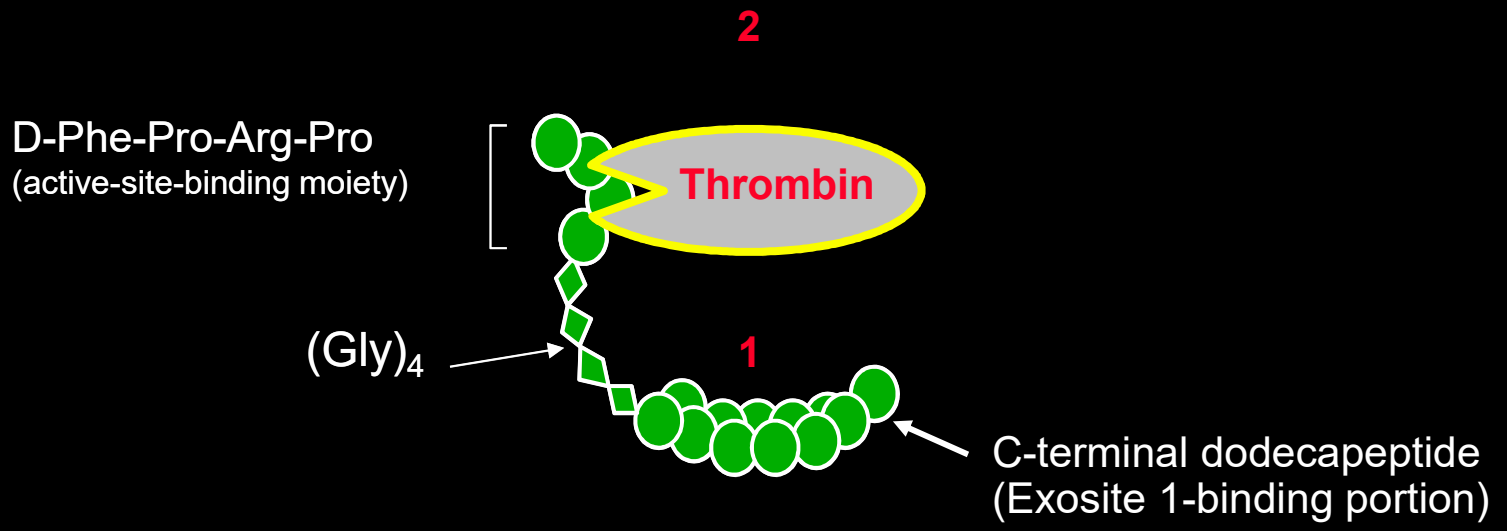


Exosites are good targets to inhibit specific functions of thrombin



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

Bivalirudin binds bivalently to thrombin's active site and exosite 1.



Bivalirudin is a synthetic molecule, designed as a bivalent direct thrombin inhibitor.

Molecule consists of an N-terminal Gly-Pro-Arg-Pro sequence that binds to the active site of thrombin, linked via a four glycine residue spacer to a dodecapeptide analog of the C-terminal of hirudin (an anticoagulant protein isolated from the medicinal leech).

Binds specifically to thrombin and directly to both the active catalytic site and the anion-binding exosite 1 with high affinity. Bivalirudin inhibits both circulating and fibrin-bound thrombin.

MECCANISMO ATTIVAZIONE BATTERICA

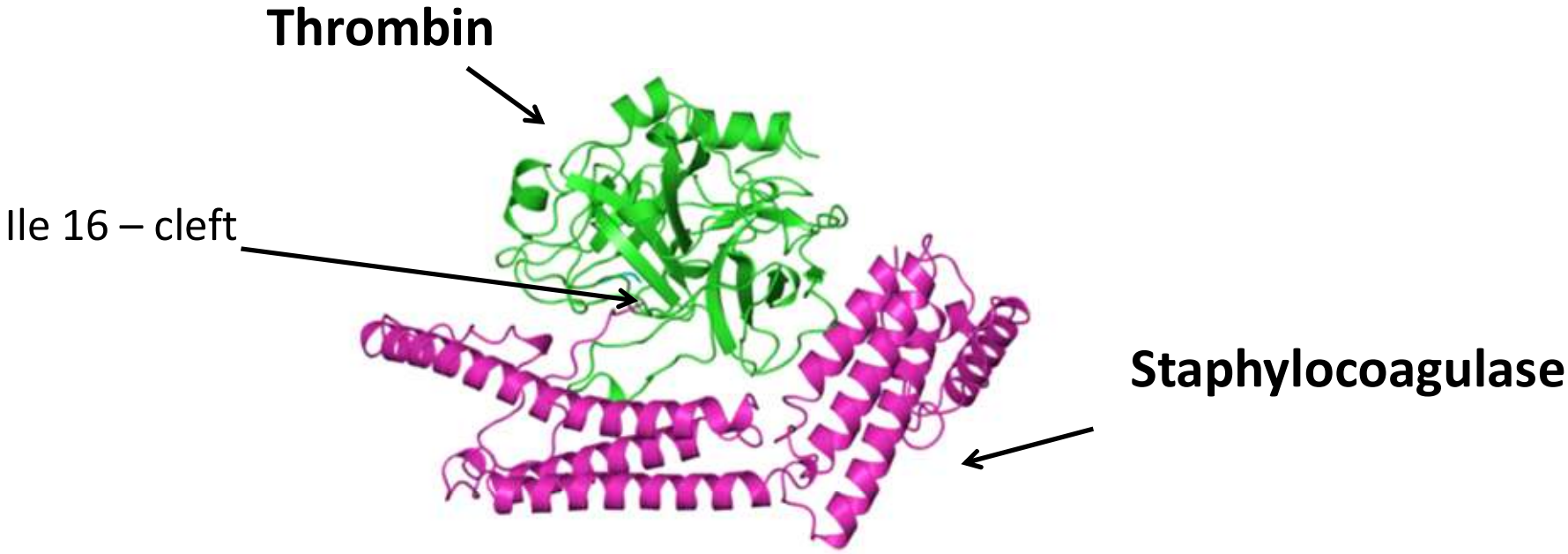
be briefly stated as follows: The staphylococcus pyogenes aureus has a specific influence in causing coagulation of the blood. Bouillon cultures of the staphylococcus were much more potent than any one of the other organisms. The

- Certain strains of *Staphylococcus Aureus* trigger coagulation (1903)
- Isolation of a bacterial agent that specifically activates thrombin: Staphylocoagulase (1970)
- SC does not cleave thrombin, No cleavage between Arg^{15}-Ile^{16}

How is that possible???

Staphylocoagulase (SC) X ray-structure

In 2003 crystal structure of (Pre2)Thrombin-bound Staphylocoagulase was published (Friedrich, et al. *Nature*, 2003)

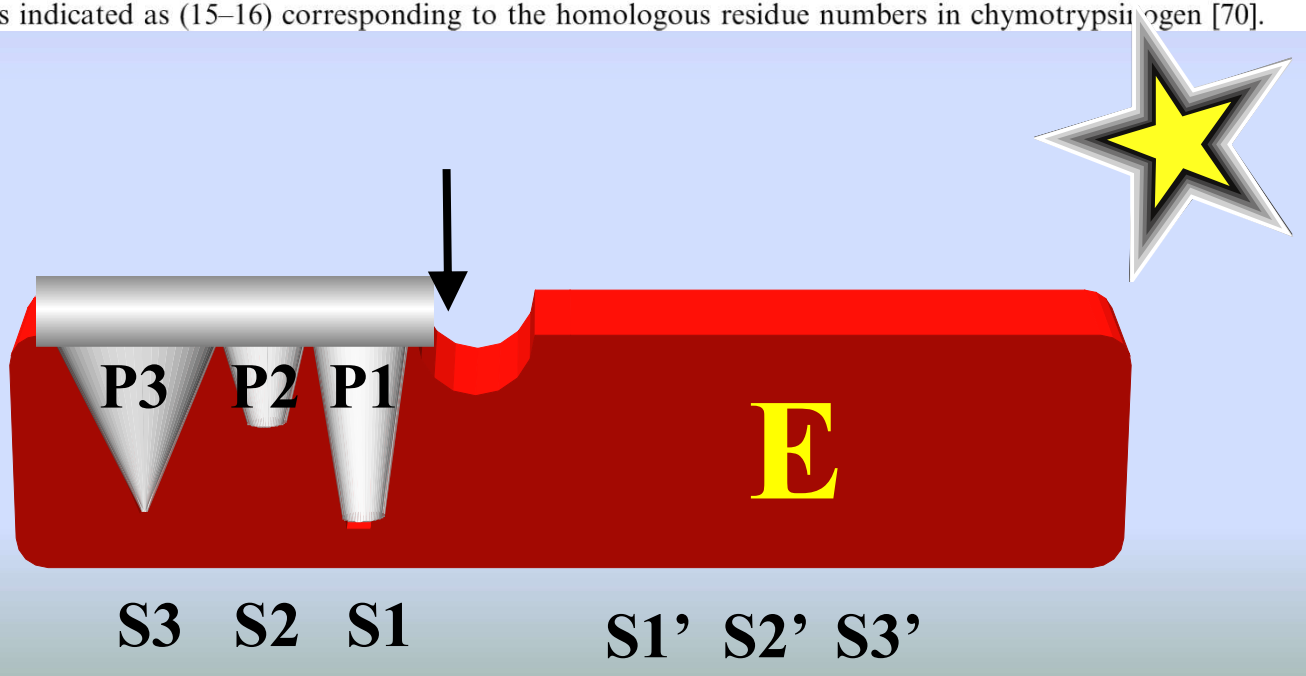


Non-Proteolytic Activation of Prothrombin by Staphylocoagulase support for the “Molecular Sexuality” Hypothesis

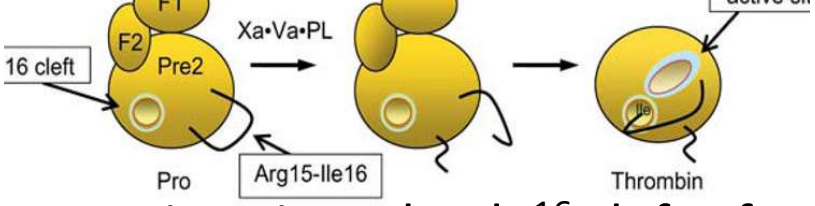
Table 1 Sites of cleavage in the human vitamin K-dependent zymogens*

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

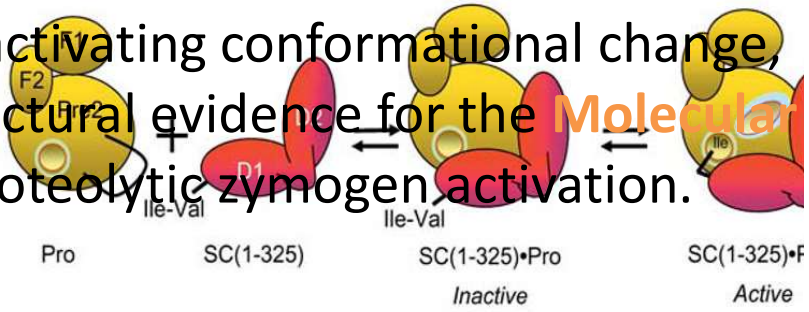
*Sequences flanking cleavage sites relevant to the activation of the vitamin K-dependent zymogens are presented along with the relevant enzymes that catalyze these reactions. The site of bond cleavage is denoted by the arrow. †The site, in each substrate, at which cleavage is required to produce the serine proteinase is indicated as (15–16) corresponding to the homologous residue numbers in chymotrypsinogen [70].

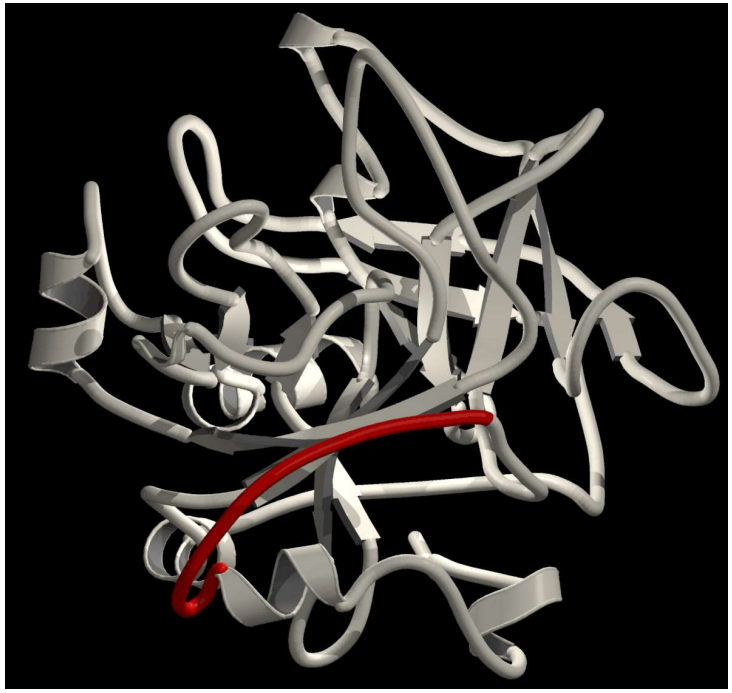


Non-Proteolytic Activation of Prothrombin by Staphylocoagulase support for the “Molecular Sexuality” Hypothesis



The observed insertion of the SC N-terminus into the Ile^{16} cleft of prethrombin 2, which triggers the activating conformational change, provided the first unambiguous structural evidence for the **Molecular Sexuality** mechanism of non-proteolytic zymogen activation.



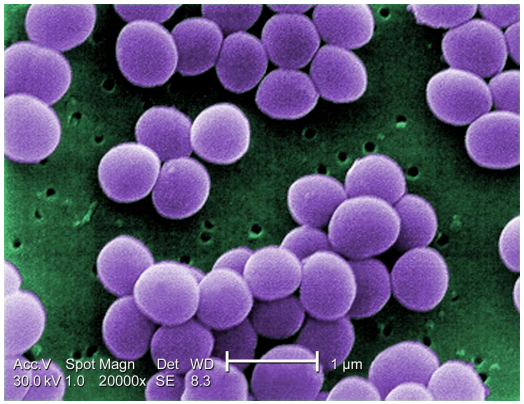


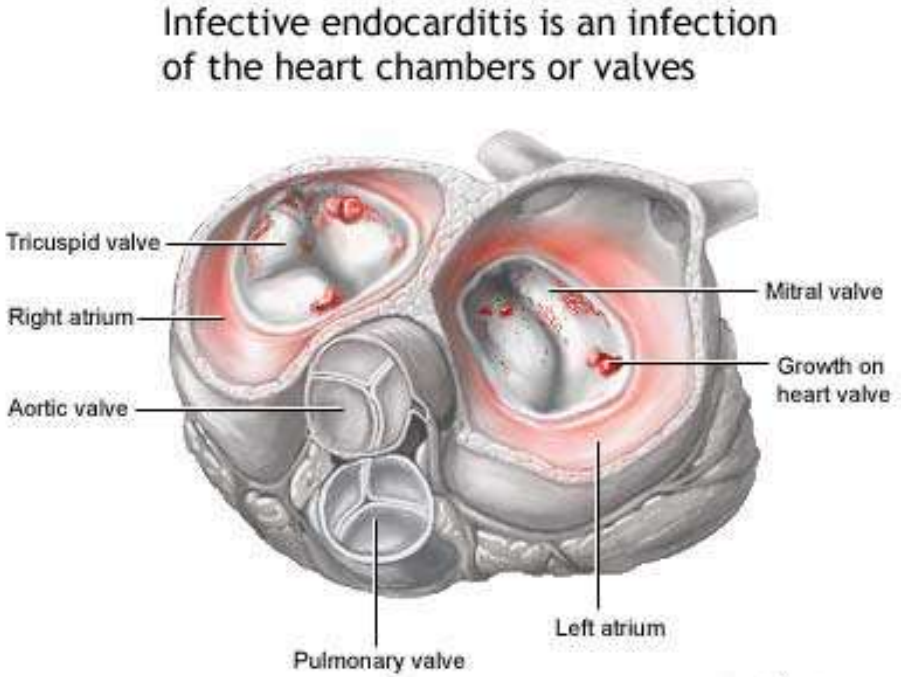
S. Aureus causes Endocarditis

- Severe infection of the heart valves
- More than 50% of patients dies within days or weeks despite treatment
- Difficult diagnosis
  - new heart murmur, fever and the detection of circulating bacteria in blood cultures
- Coagulase-positive S. aureus causes 40–50% of neonatal endocarditis and 30–40% of endocarditis in adults

Acute bacterial endocarditis is characterized by vegetations on heart valves consisting of bacteria, platelets and fibrin

S. Aureus





- Growth and fortification of the vegetation by SC-induced fibrin deposition protects the bacteria in the vegetation from clearance by leukocytes and macrophages

- Heart valves are not easily accessible to the immune system

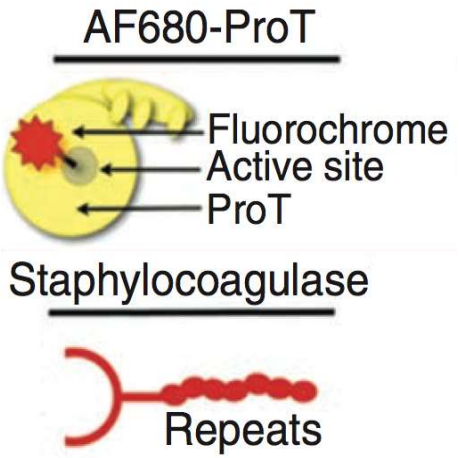
nature medicine

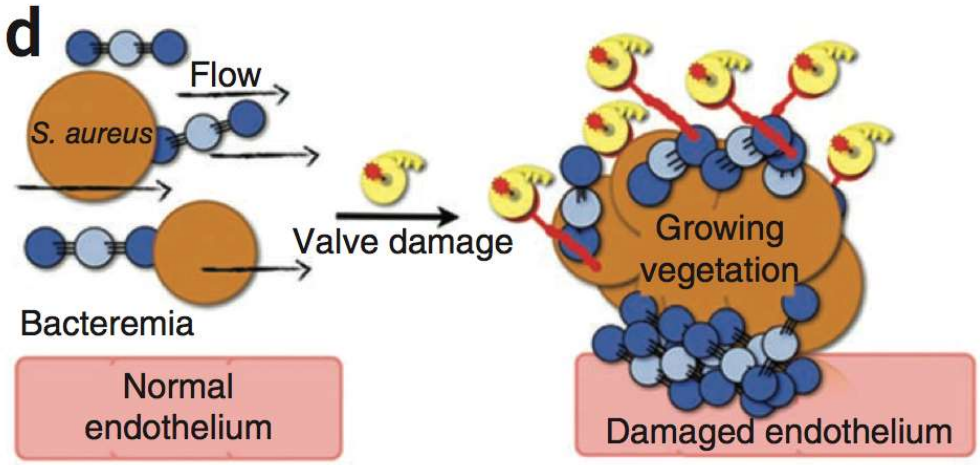
In vivo detection of *Staphylococcus aureus* endocarditis by targeting pathogen-specific prothrombin activation

Peter Panizzi[1,2,9], Matthias Nahrendorf[1,9], Jose-Luiz Figueiredo[1], Jennifer Panizzi[3], Brett Marinelli[1], Yoshiko Iwamoto[1], Edmund Keliher[1], Ashoka A Maddur[4], Peter Waterman[1], Heather K Kroh[4], Florian Leuschner[1], Elena Aikawa[1], Filip K Swirski[1], Mikael J Pittet[1], Tilman M Hackeng[5], Pablo Fuentes-Prior[6], Olaf Schneewind[7], Paul E Bock[4] & Ralph Weissleder[1,8]

SC Prothrombin as a probe for *S. Aureus*

- SC binds prothrombin with high affinity and activates it through a conformation change
- SC-Prothrombin complex clots fibrinogen but is impervious to physiologic thrombin inhibitors.
- SC-Prothrombin is present in the vegetation
- Labeled Prothrombin can be used as a probe to detect bacterial vegetation in the heart

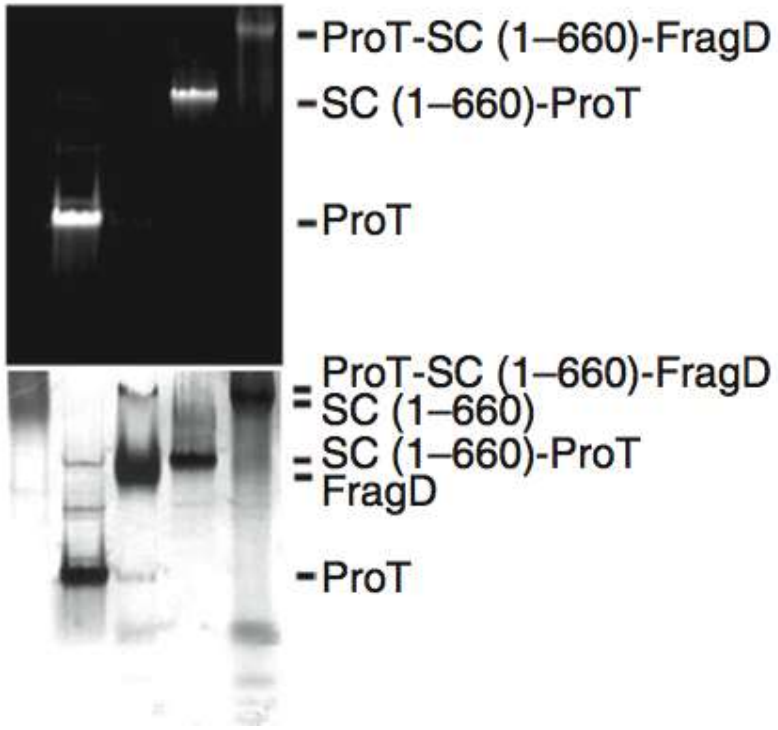




Visualisation of *S. Aureus* in vivo using Near Infrared Imaging

The PROBE

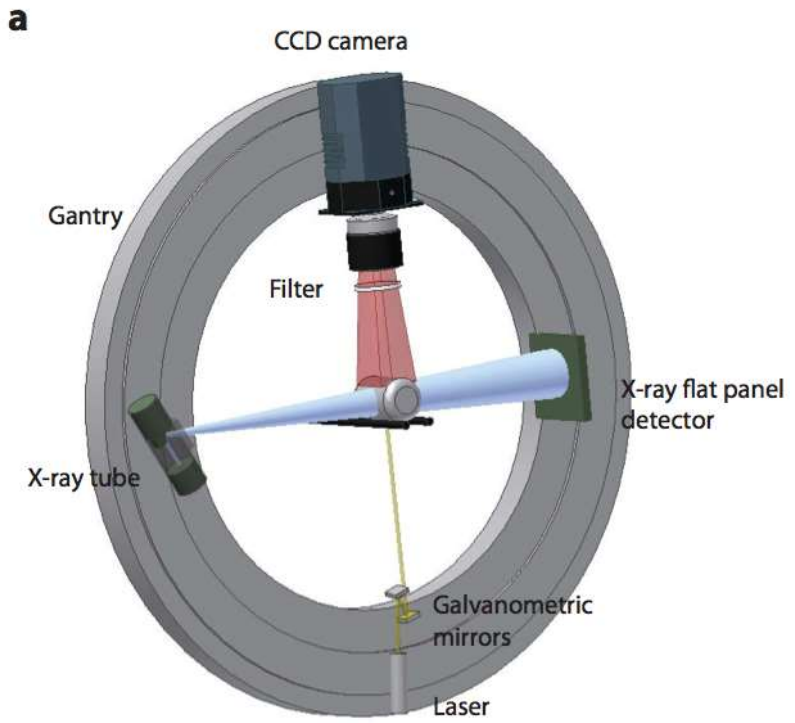
AF680- Prothrombin



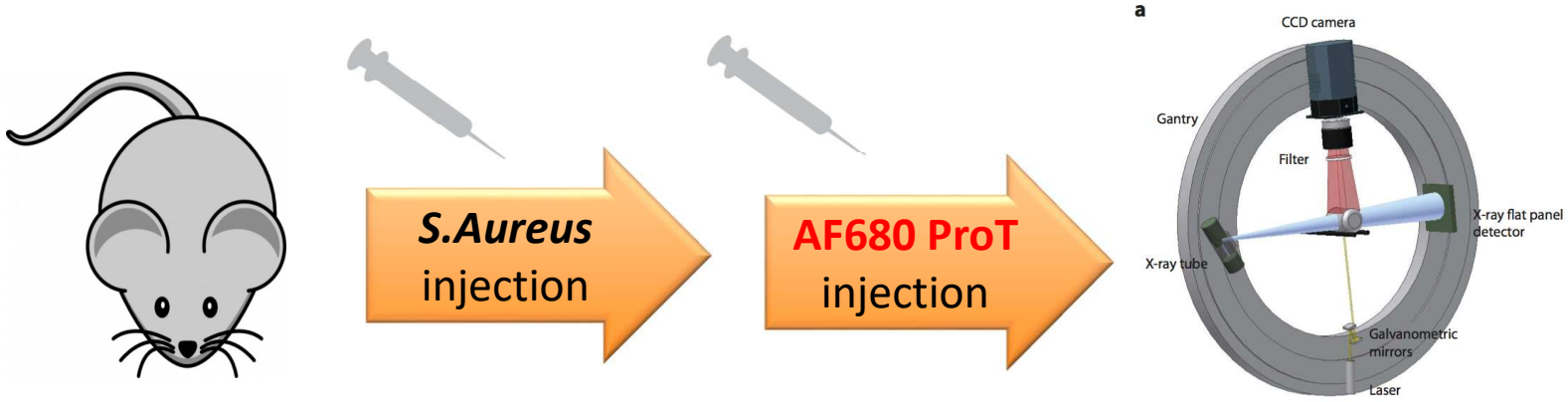
The DETECTOR

Fluorescence molecular tomography

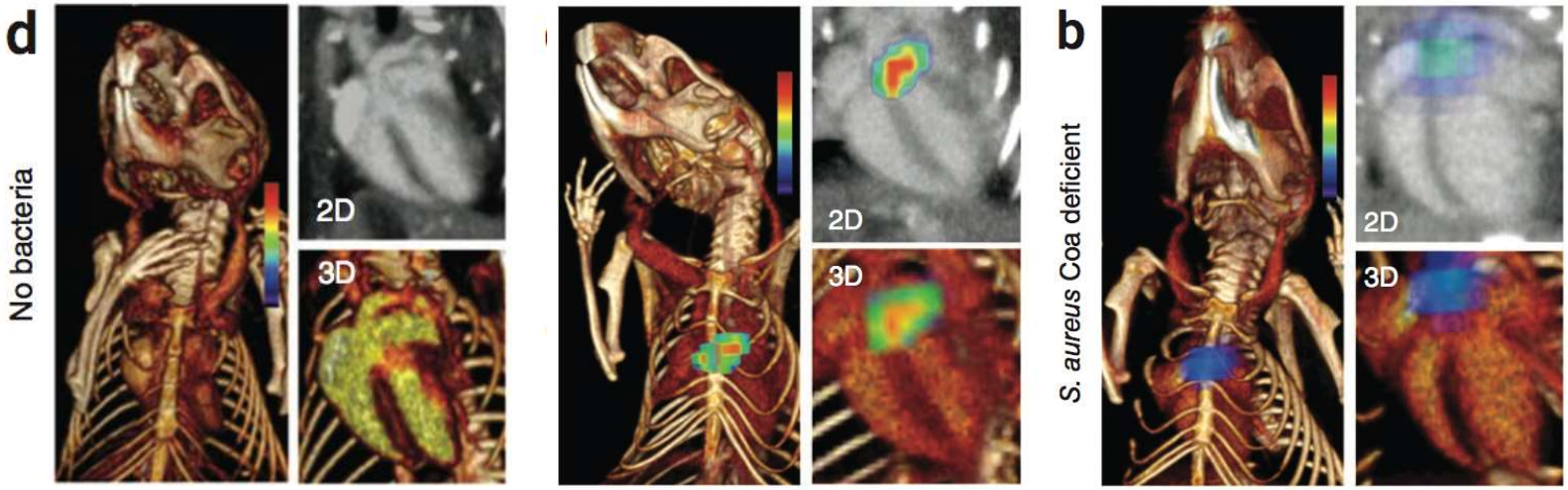
- Computer Tomography



Fluorescent prothrombin co-localise with SC positive bacteria



No Bacteria *S. Aureus wt* *S. Aureus SC deficient*



Conclusion

- Zymogen activation requires conformational changes and maturation of the active site. This can be achieved even in the absence of canonical proteolysis.
- Exosite-Substrate interactions determine enzyme specificity.
- AF680ProT detects S.Aureus in vivo and can be used as a diagnostic tool to determine site, bacterial load and activity of the infection.

Bibliography

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