# Activation and specificity of Thrombin

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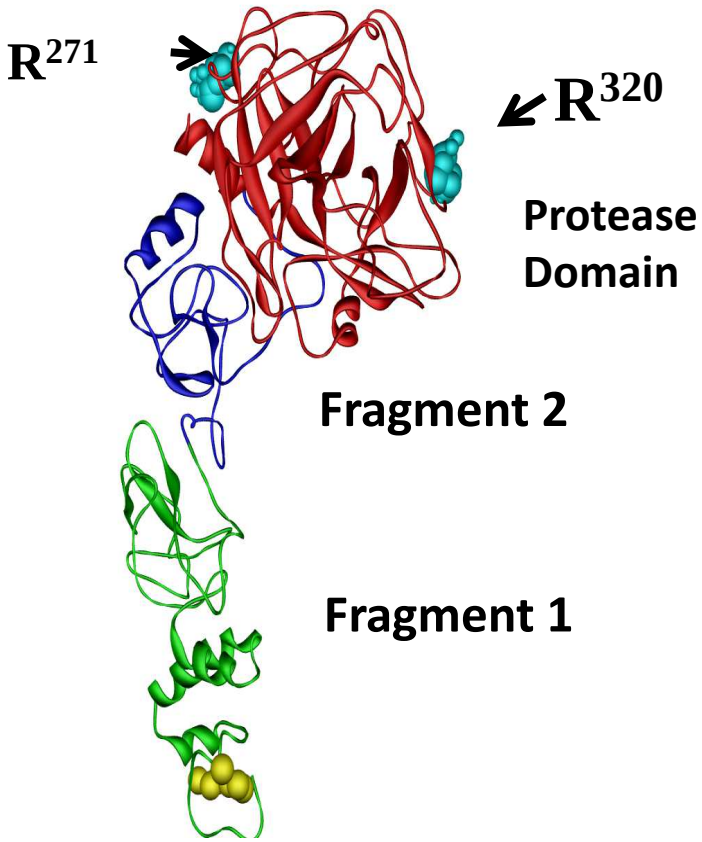
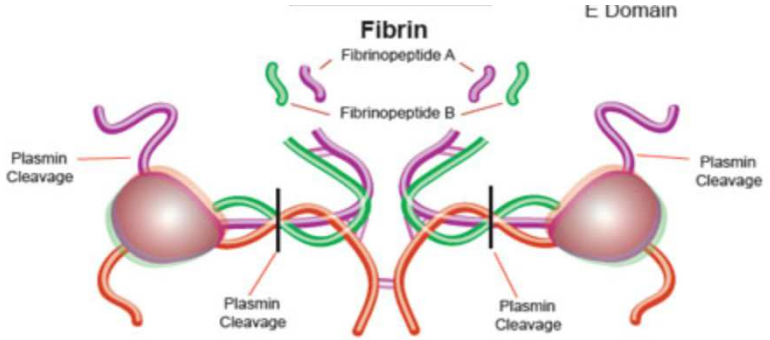
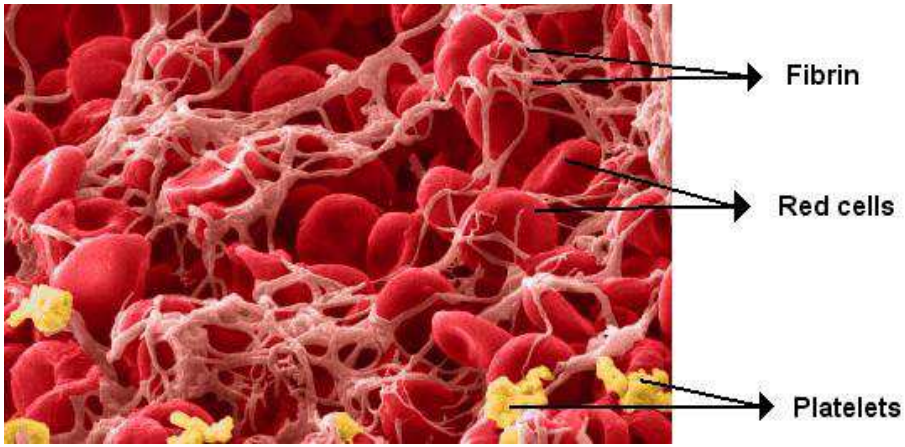
# Summary

- Regulation of a Serine Protease: Thrombin
  - Zymogen □ Enzyme □ Substrate Specificity
- Staphylocoagulase
  - Bacteria know how a protease works (much more than we do…)
- Diagnostic applications
  - Imaging of Staphylococcus vegetations in the hearth

# Thrombin is a Serine Protease involved in blood coagulation



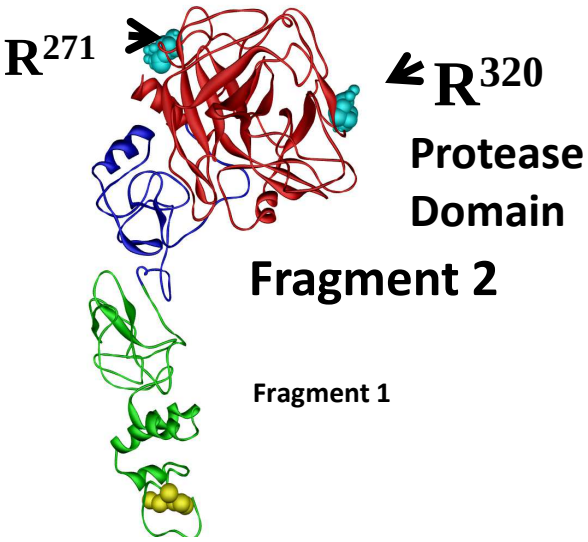



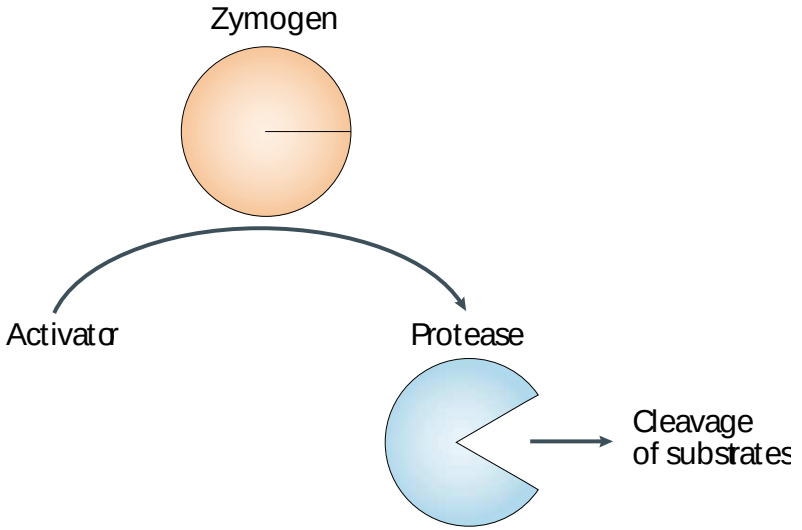
# Thrombin is synthesized as a Zymogen: Prothrombin

## Zymogen:

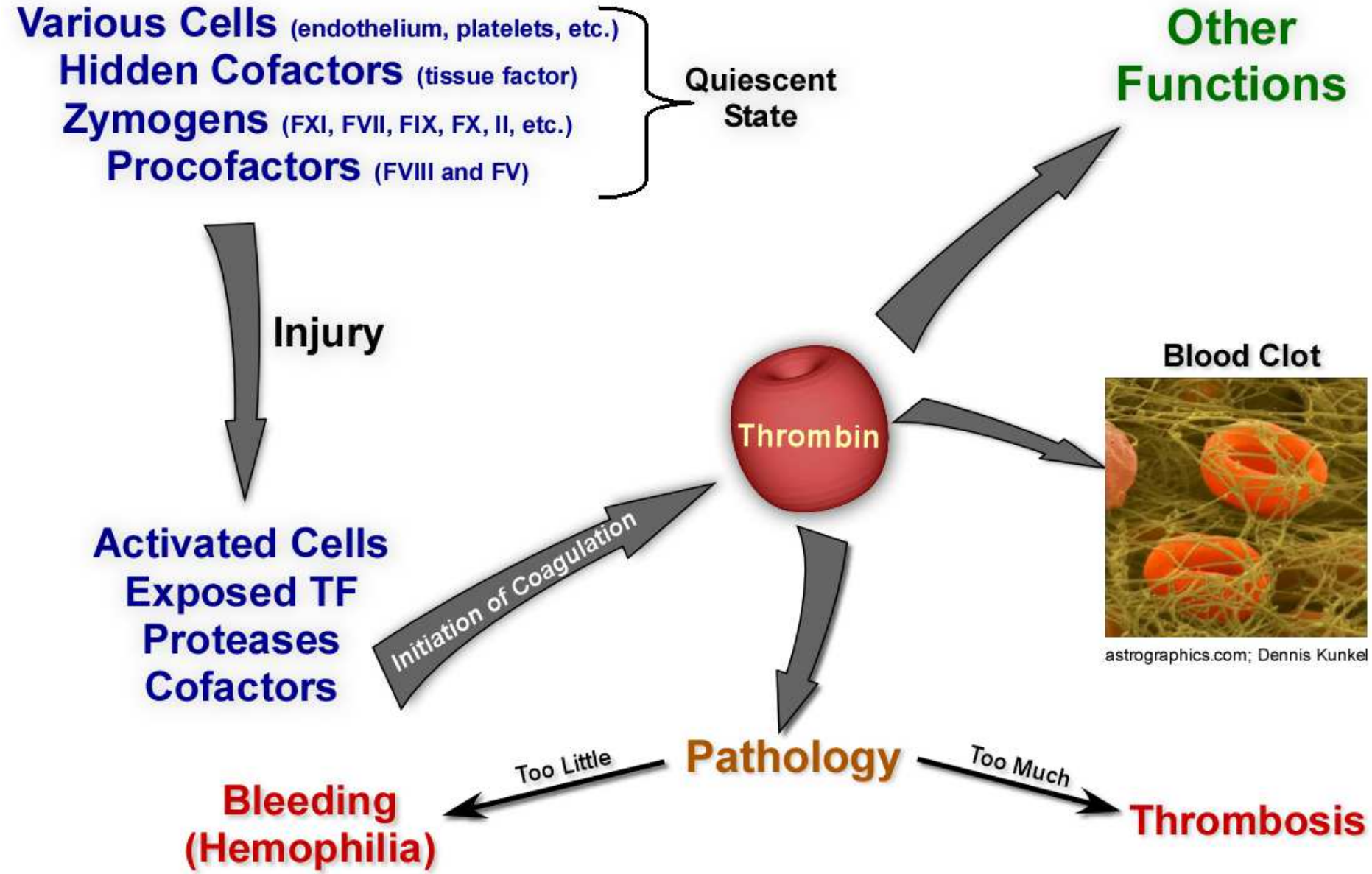
A proenzyme or inactive enzyme. It requires a biochemical change to reveal the active site for it to become an active enzyme.

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

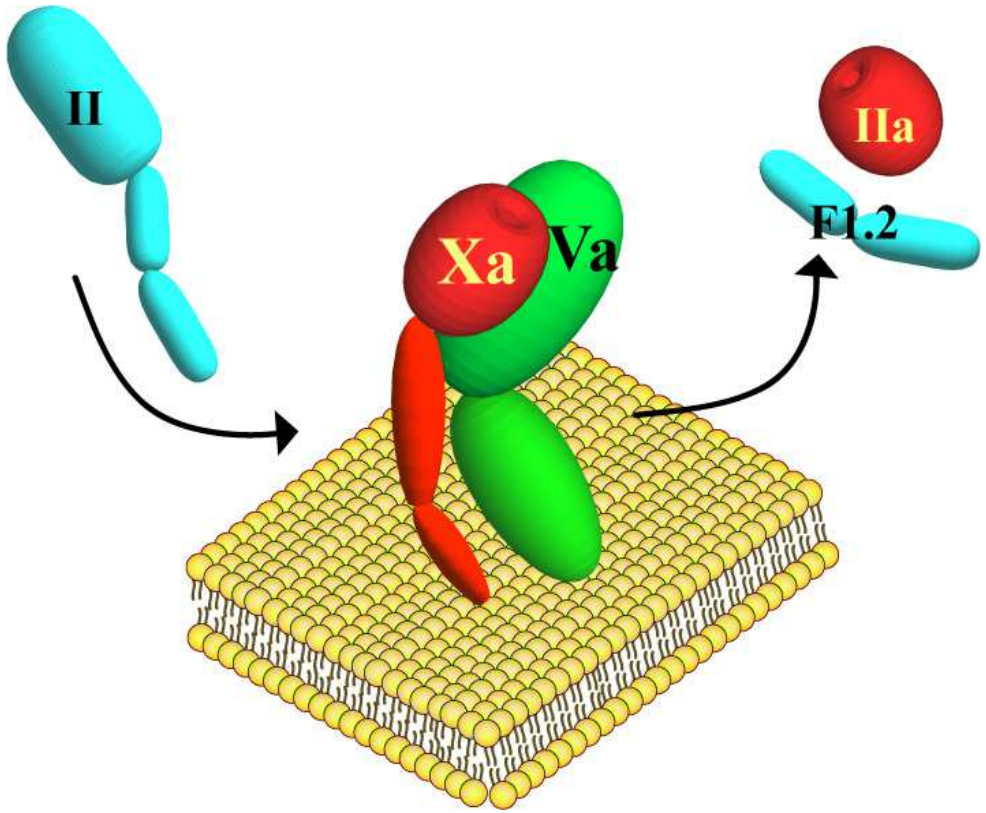


# The Blood Coagulation Response:



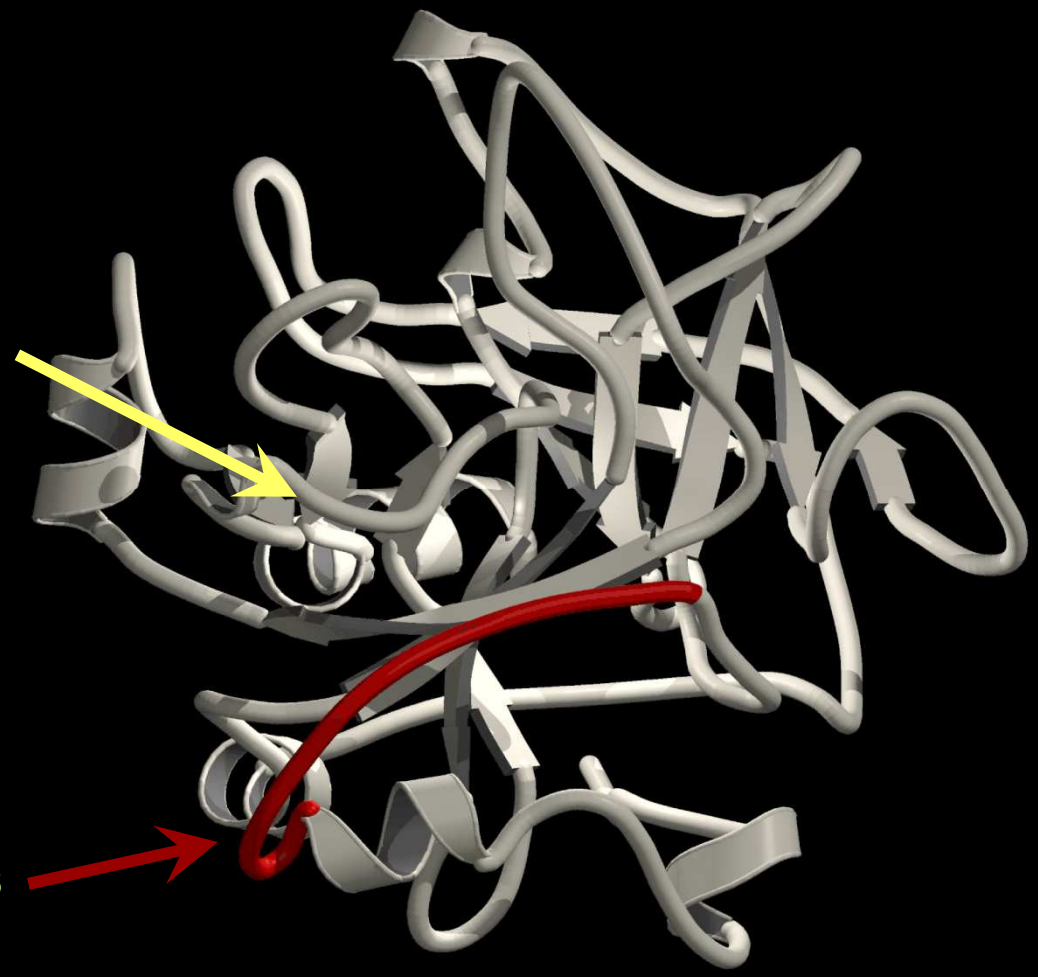
# Prothrombin is activated to thrombin by two proteolytic cleavages




Prothrombinase

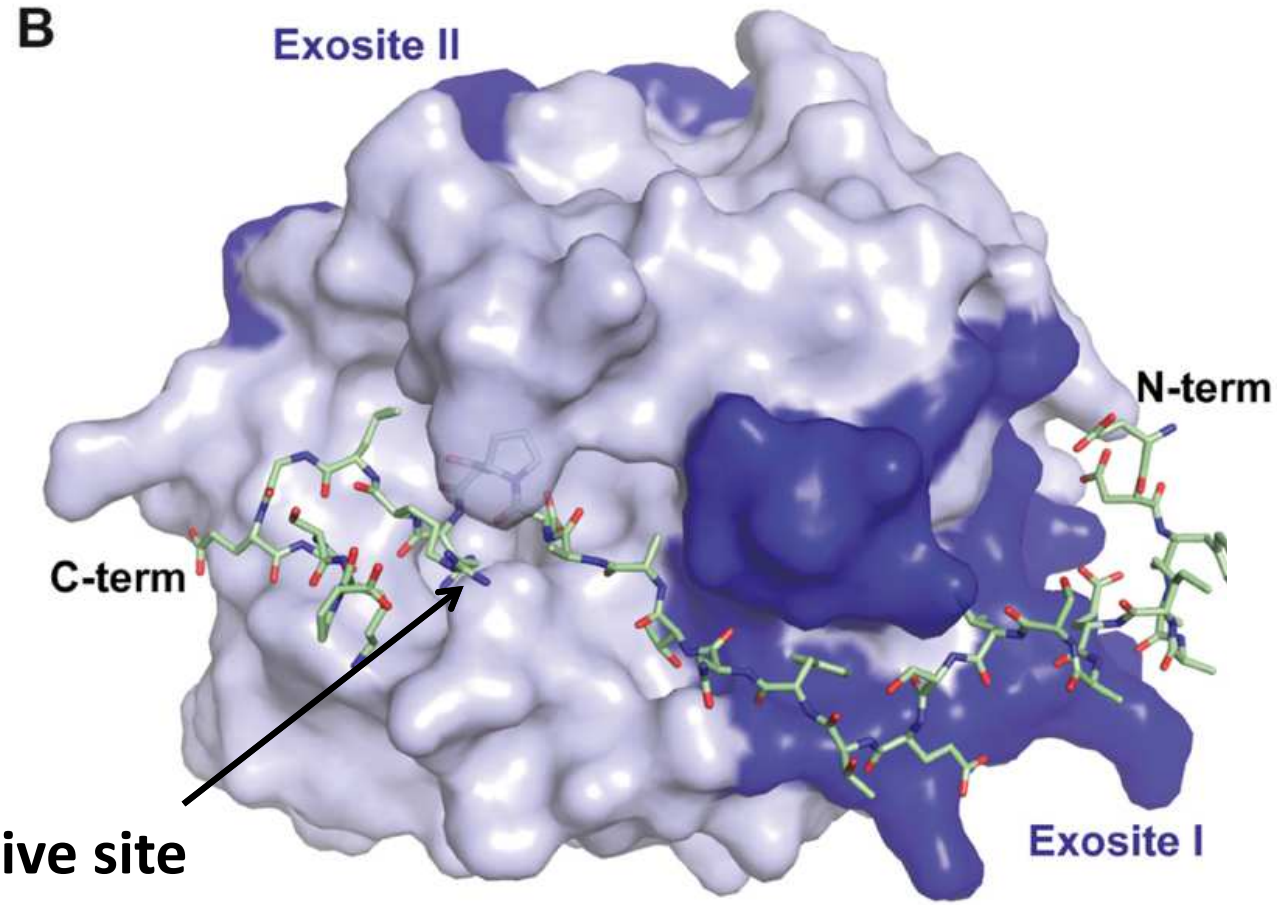
# Serine Proteases: Conversion Pathway

- 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*

# Thrombin X-ray structure

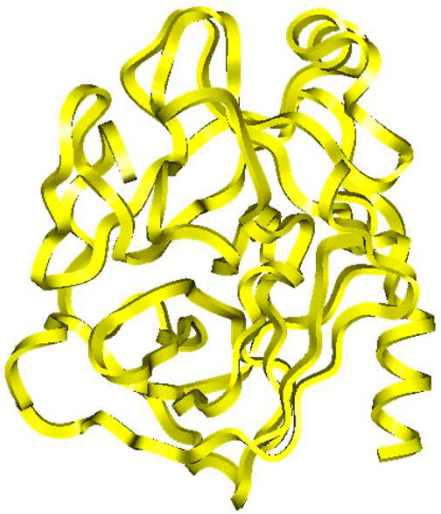



Substrate Pocket

# Trypsin and Thrombin have similar structures

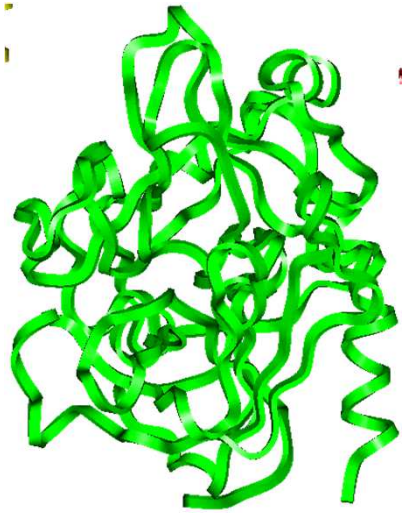
**Trypsin**

- 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, Arg)



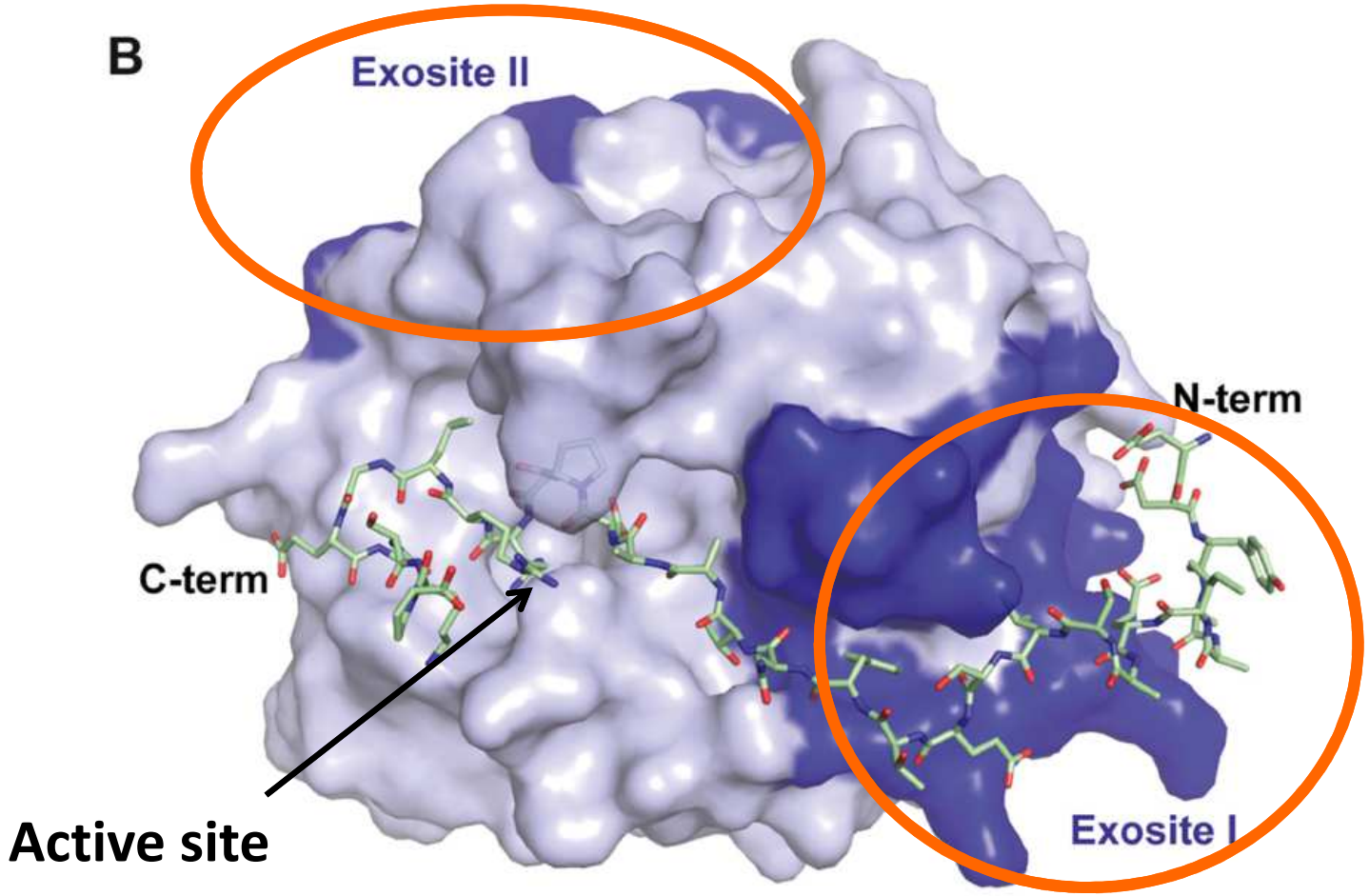
# Thrombin cleaves different substrates

- 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 |

# 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

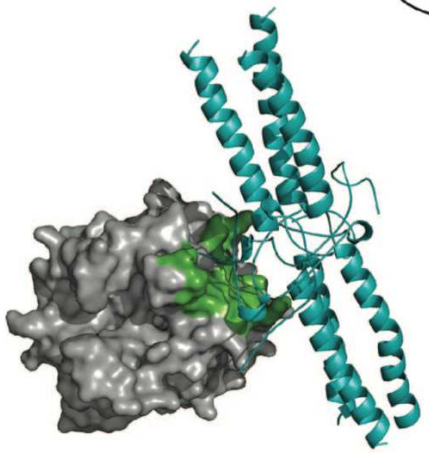
- Extended interactions at exosites drive substrate affinity and contribute to substrate specificity.

Exosite I

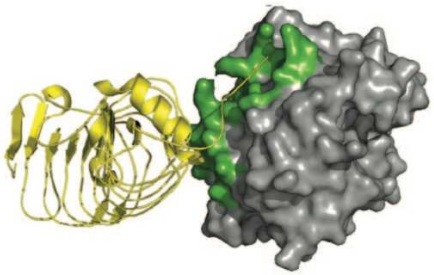
Exosite II

90°

**Clot stabilization**



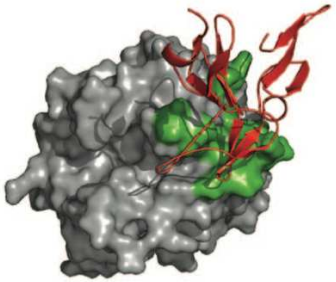
Fibrin



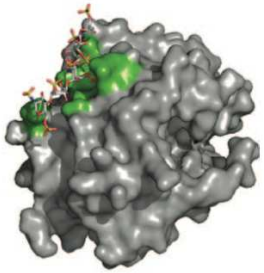
Gplbα

**Platelet activation**

**Activation of the anticoagulant pathway**



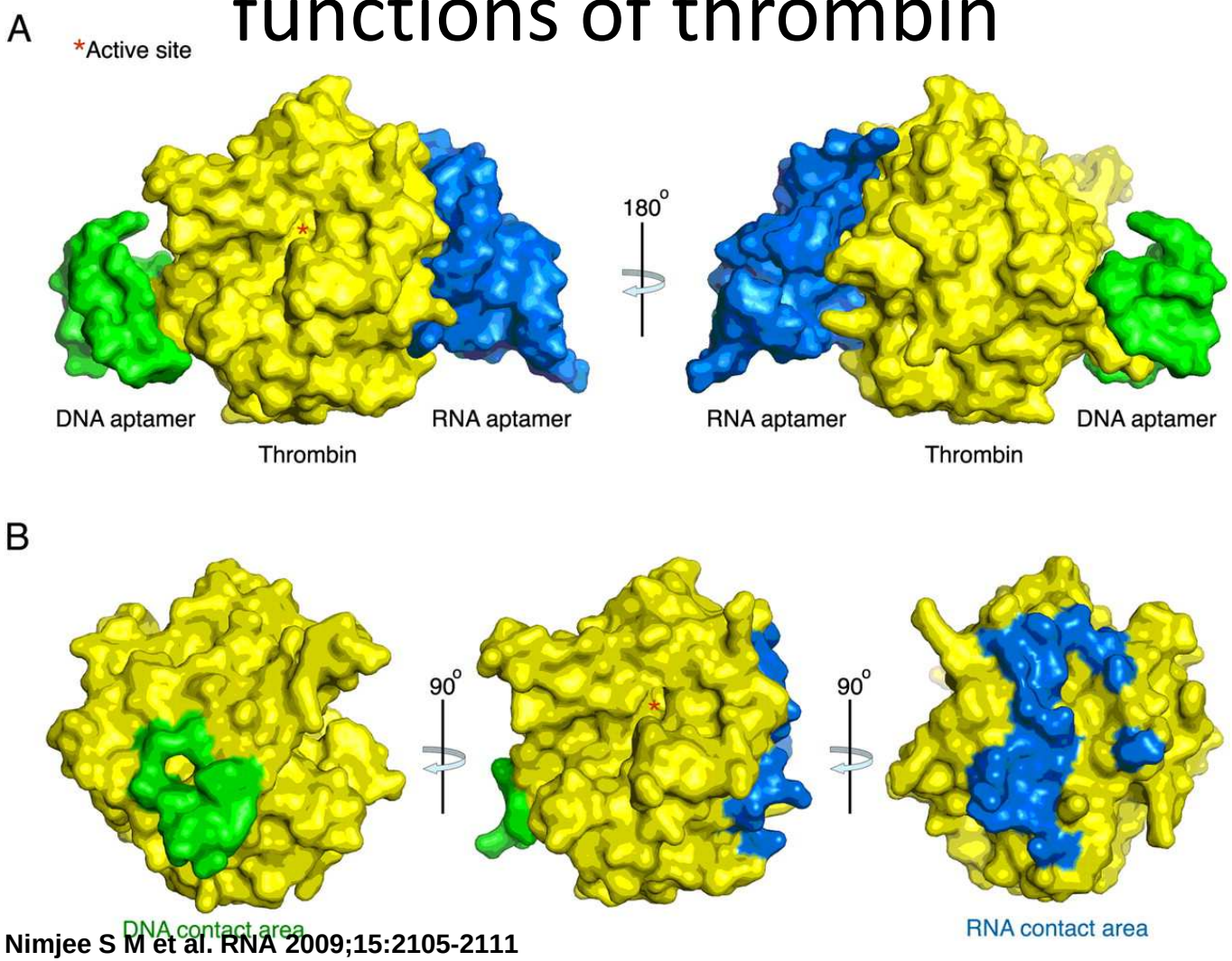
TM



Heparin

**Inhibition**

# Exosites are good targets to inhibit specific functions of thrombin




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

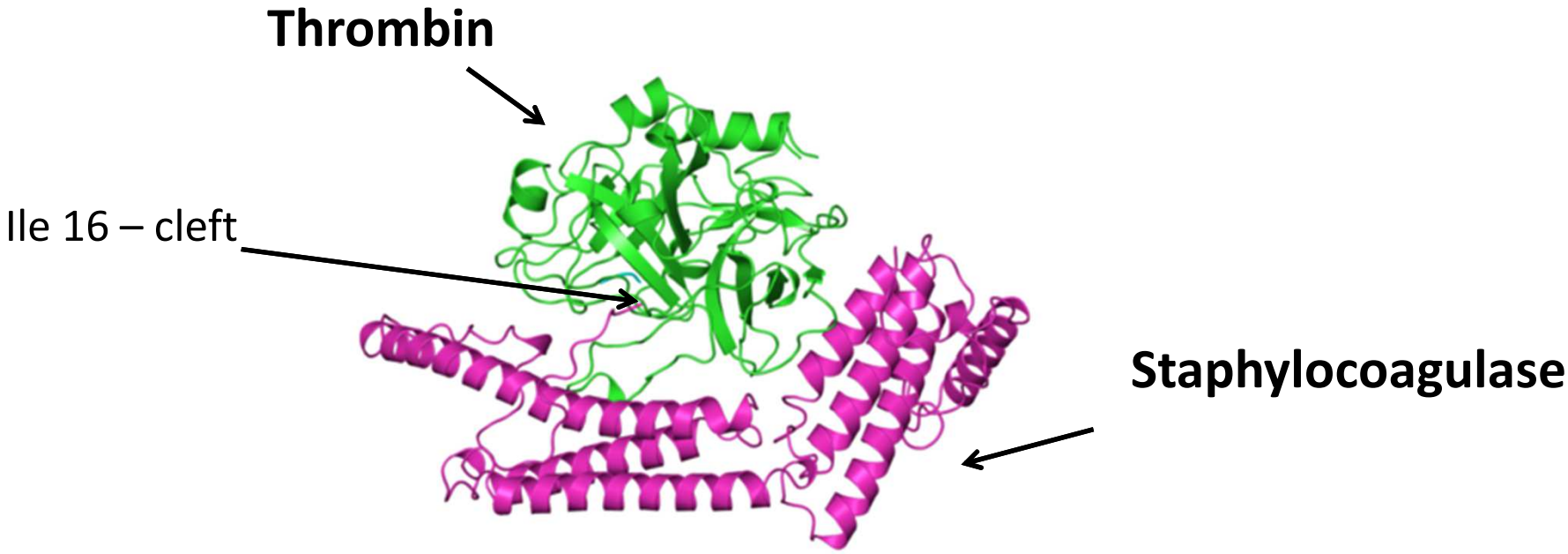
be briefly stated as follows: The staphylococcus pyogenes aureus has a specific influence in causing coagulation of the blood. Bouillon cultures of the staphylococcus were much more potent than any one of the other organisms. The

- 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 $Arg^{15}$-$Ile^{16}$

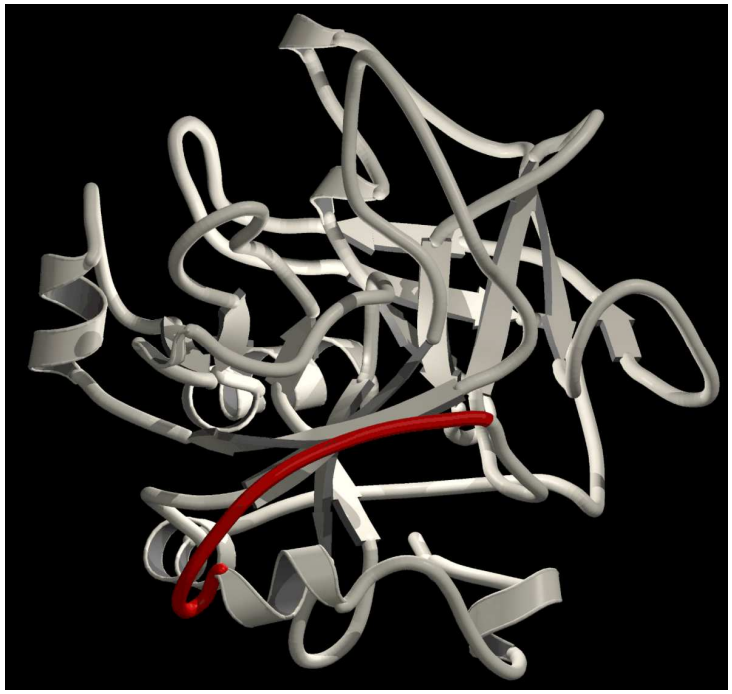
*How is that possible???*

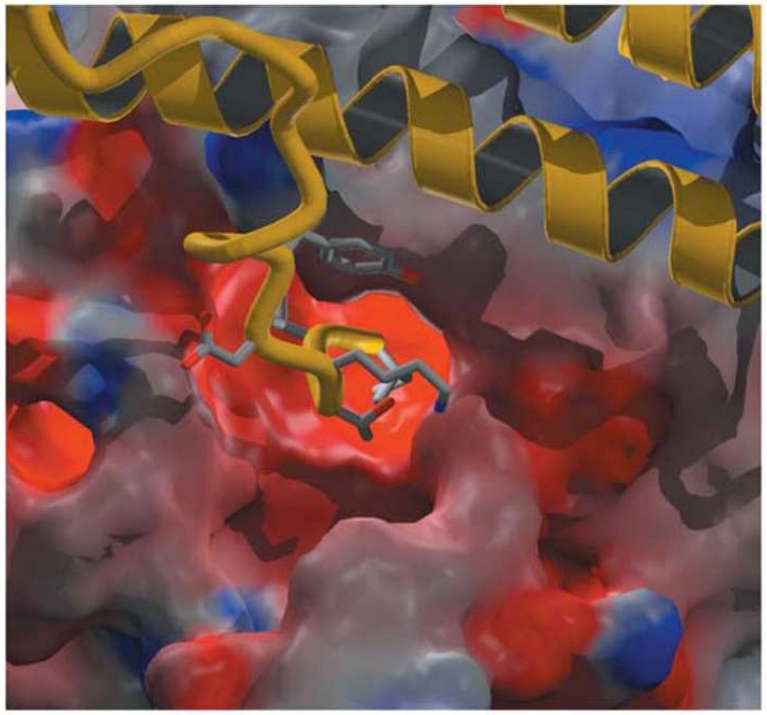
# Staphylocoagulase (SC) X ray-structure

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

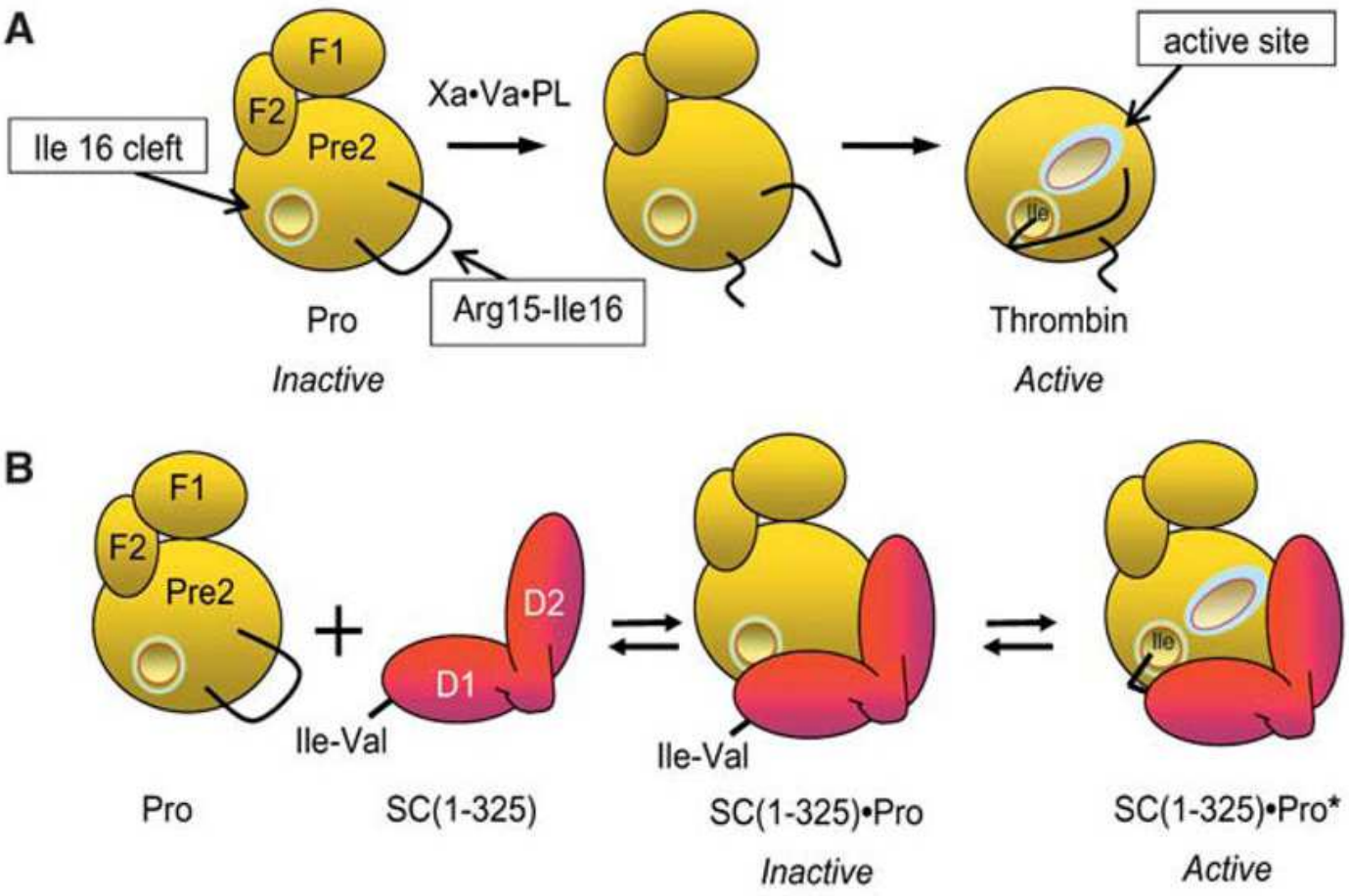


The observed insertion of the SC N-terminus into the $Ile^{16}$ cleft of prethrombin 2, which triggers the activating conformational change, provided the first unambiguous structural evidence for the **Molecular Sexuality** mechanism of non-proteolytic zymogen activation.





# Non-Proteolytic Activation of Prothrombin by Staphylocoagulase support for the "Molecular Sexuality" Hypothesis

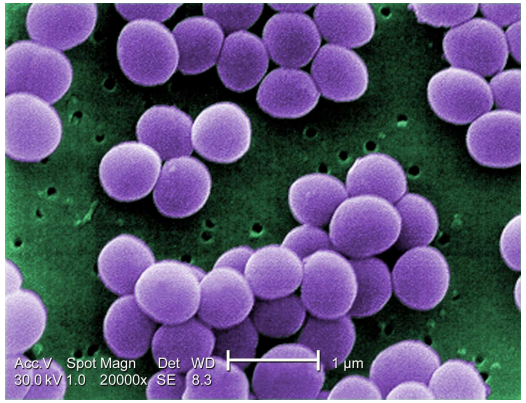


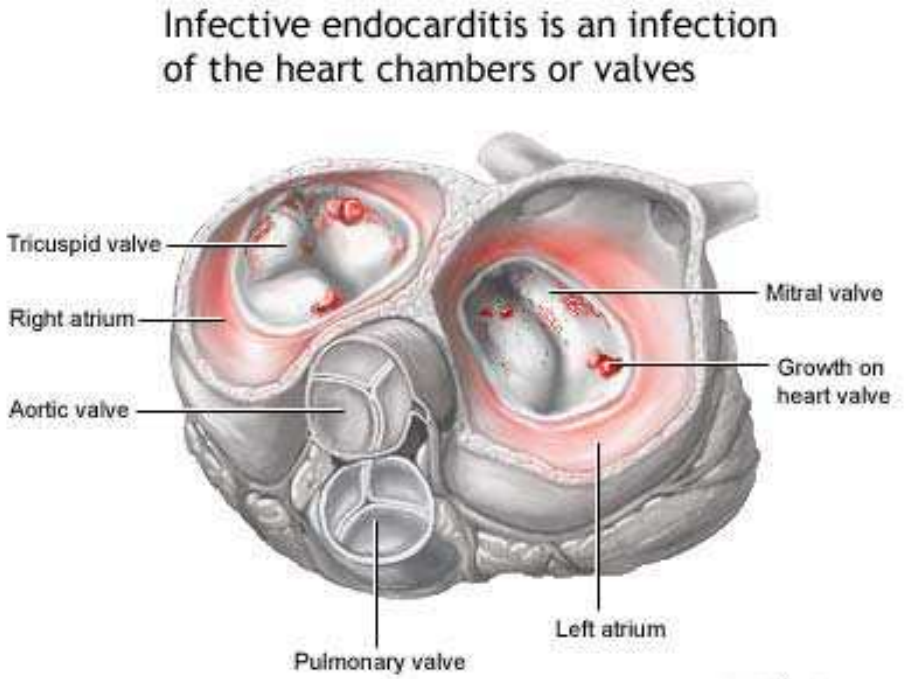
# *S. Aureus* causes Endocarditis

- 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

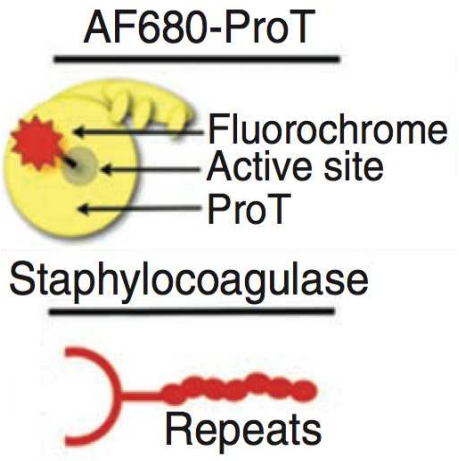
nature medicine

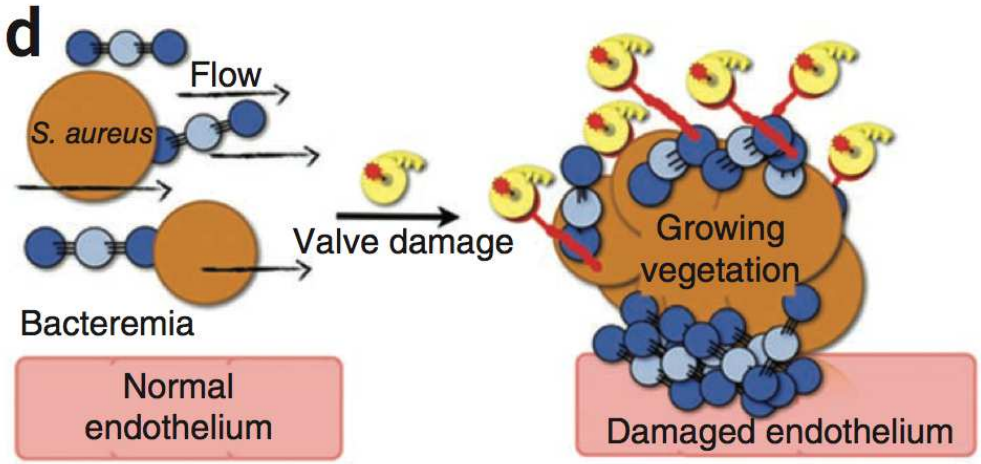
# *In vivo* detection of *Staphylococcus aureus* endocarditis by targeting pathogen-specific prothrombin activation

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

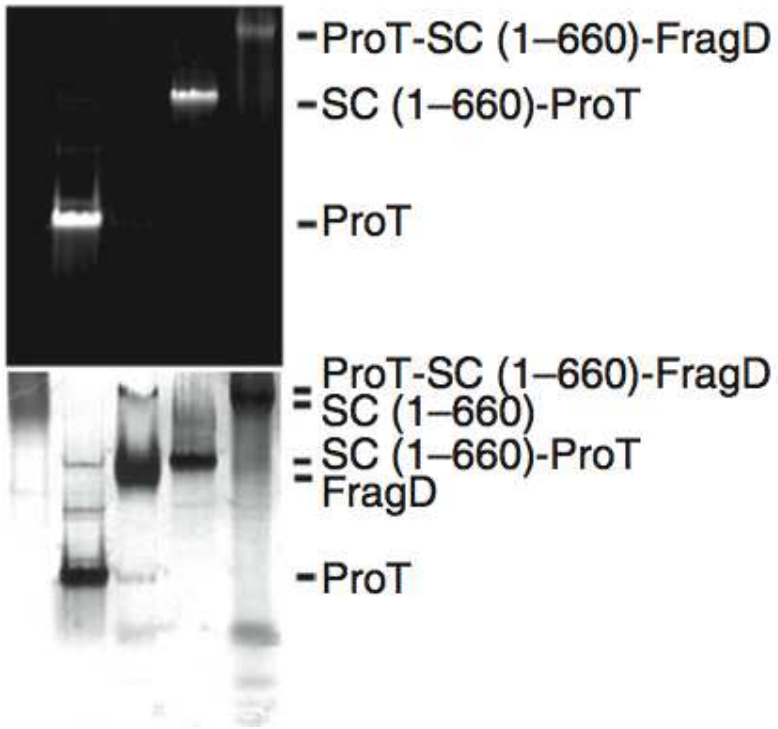


# Visualisation of *S. Aureus* in vivo using Near Infrared Imaging

## The PROBE

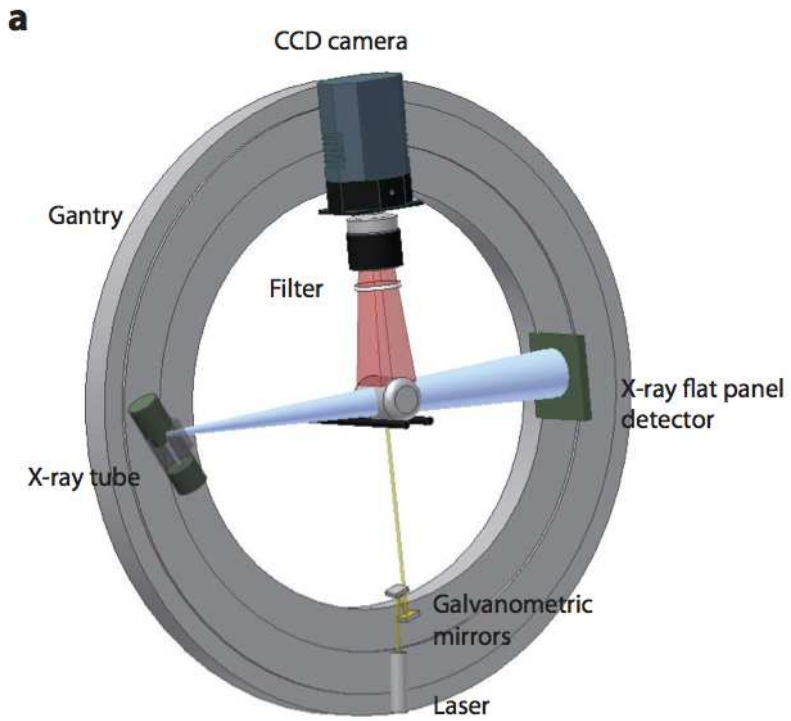
**AF680- Prothrombin**



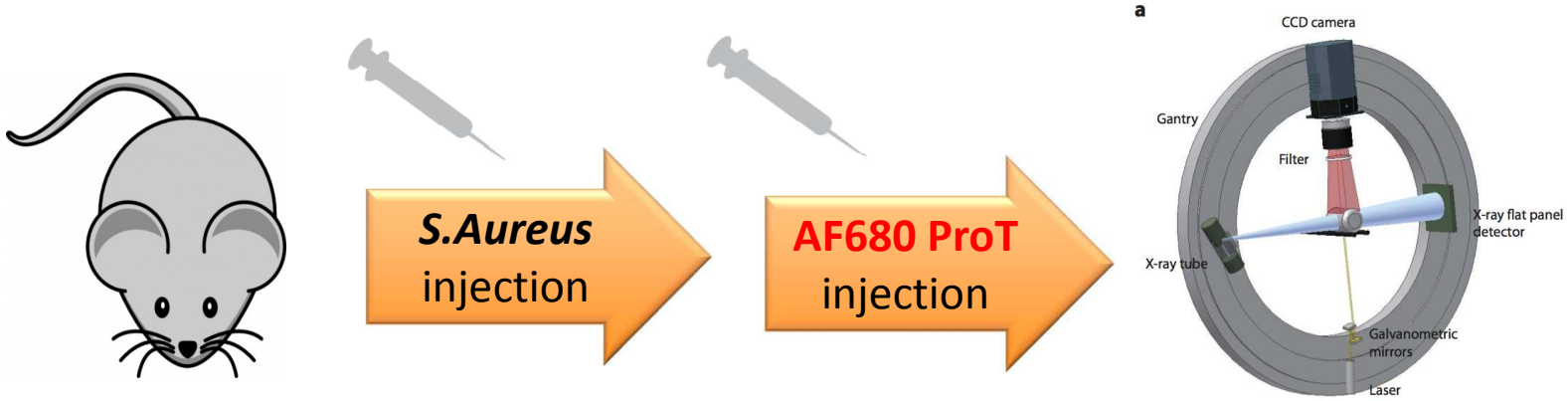

## The DETECTOR

**Fluorescence molecular tomography**

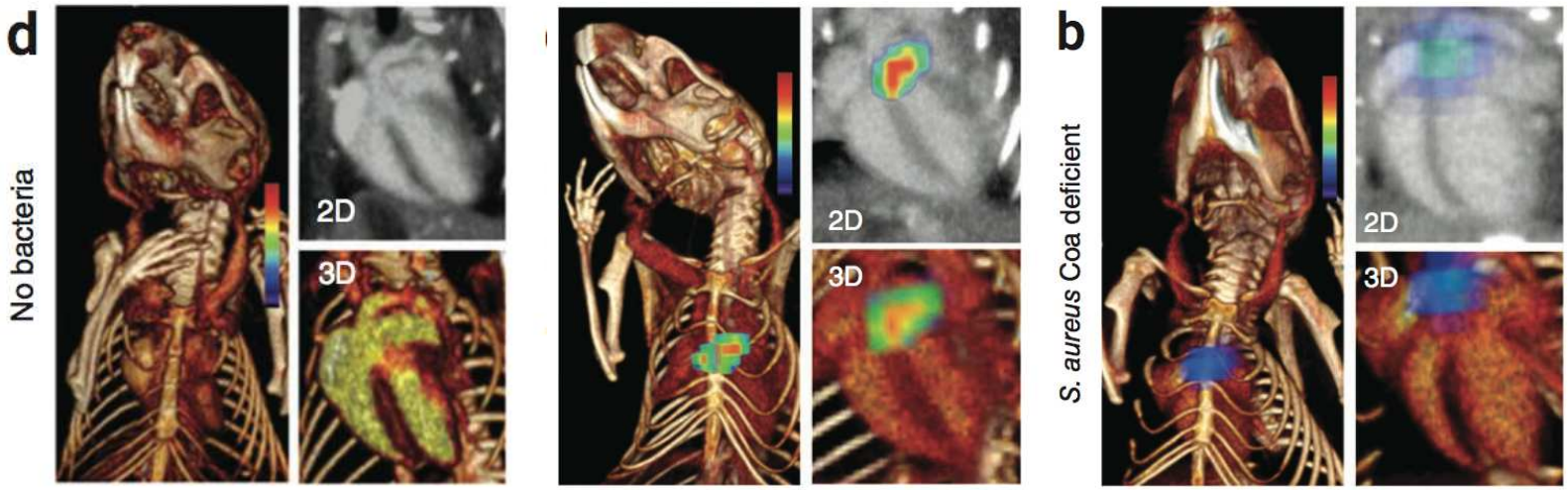
**- Computer Tomography**



# *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


- Adams, T. E. (2006). Thrombin-Cofactor Interactions: Structural Insights Into Regulatory Mechanisms. Arteriosclerosis, Thrombosis, and Vascular Biology, 26(8), 1738–1745. doi:10.1161/01.ATV.0000228844.65168.d1
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- Panizzi, P., Nahrendorf, M., Figueiredo, J.-L., Panizzi, J., Marinelli, B., Iwamoto, Y., et al. (2011). In vivo detection of Staphylococcus aureus endocarditis by targeting pathogen-specific prothrombin activation. Nature Medicine, 1–6. doi:10.1038/nm.2423