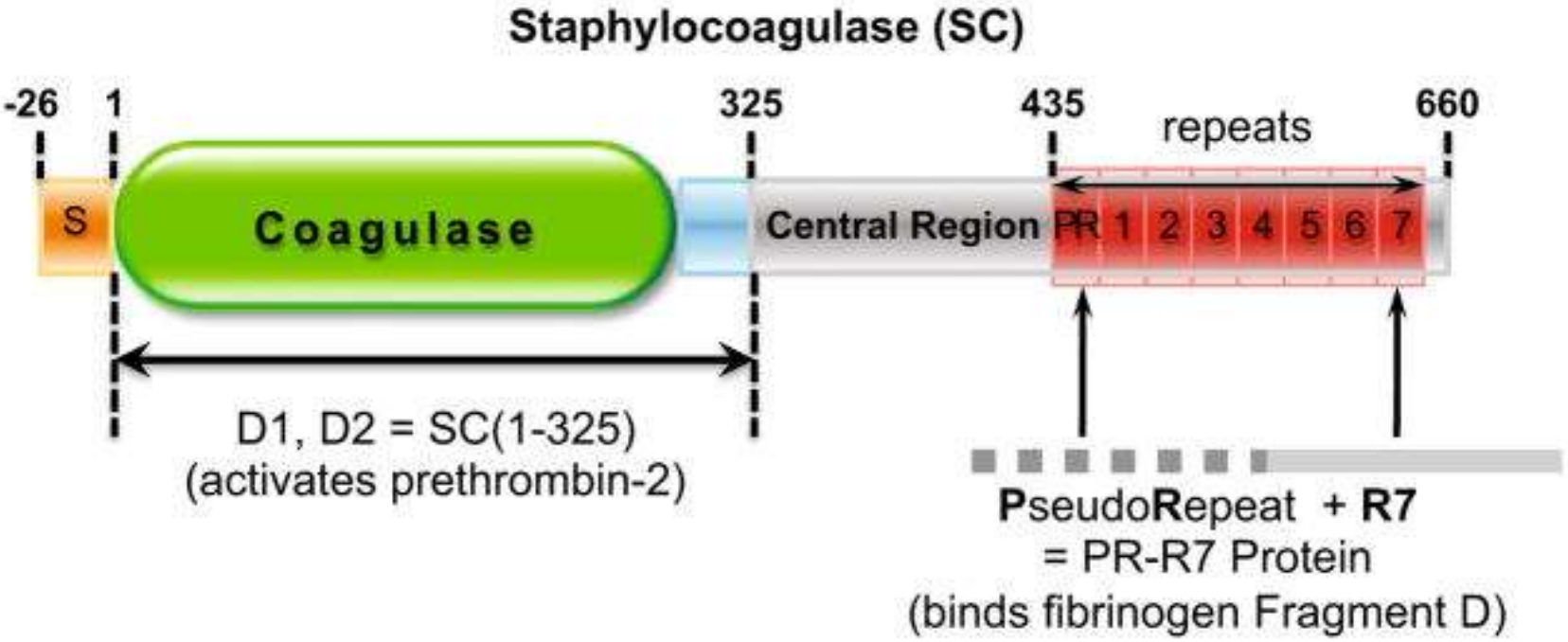
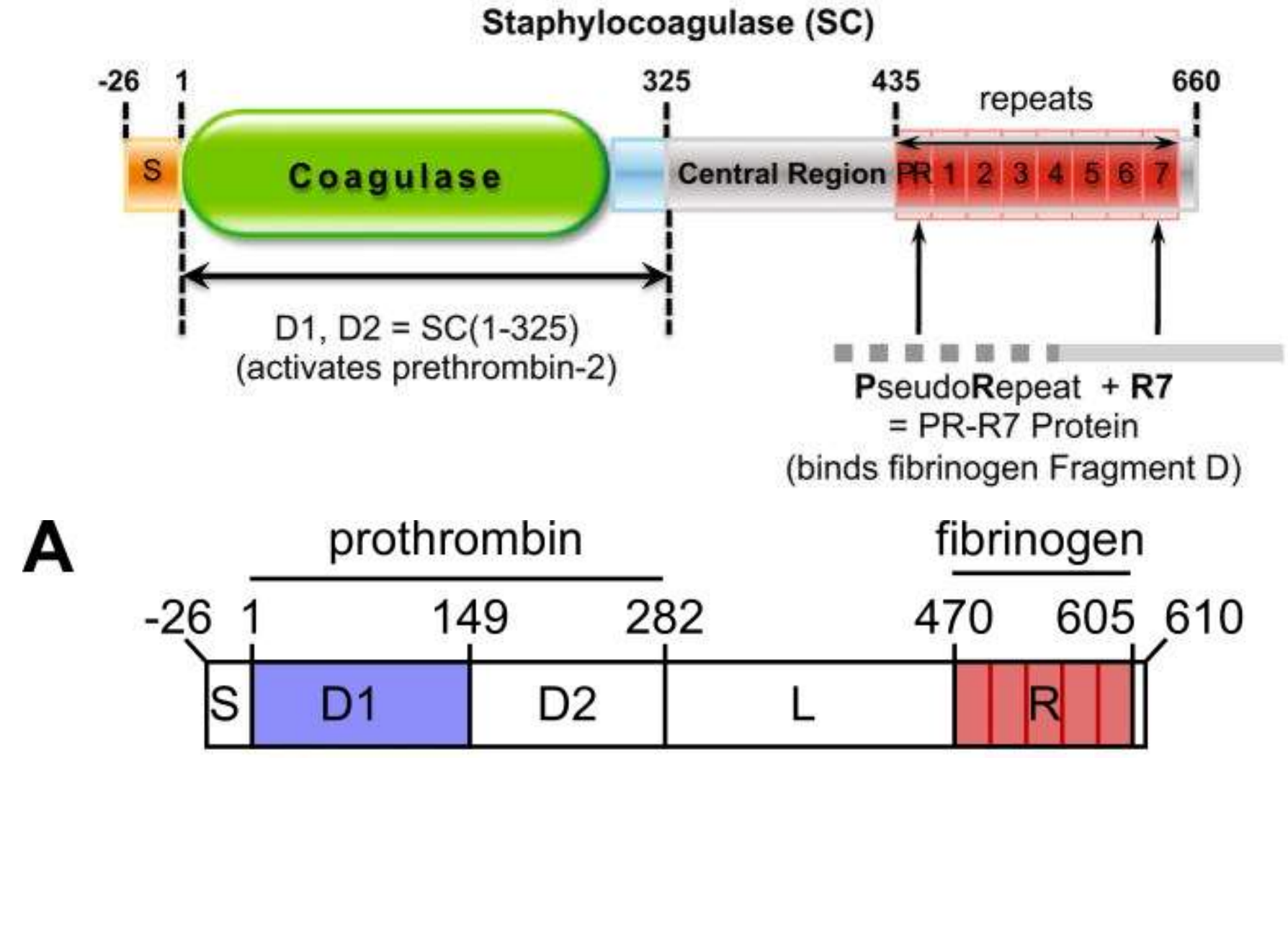
MECCANISMO ATTIVAZIONE BATTERICA
Protrombina - Trombina

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)
-26
1
325
435
repeats
660
S
Coagulase
Central Region
PR 1 2 3 4 5 6 7
D1, D2 = SC(1-325)
(activates prethrombin-2)
PseudoRepeat + R7
= PR-R7 Protein
(binds fibrinogen Fragment D)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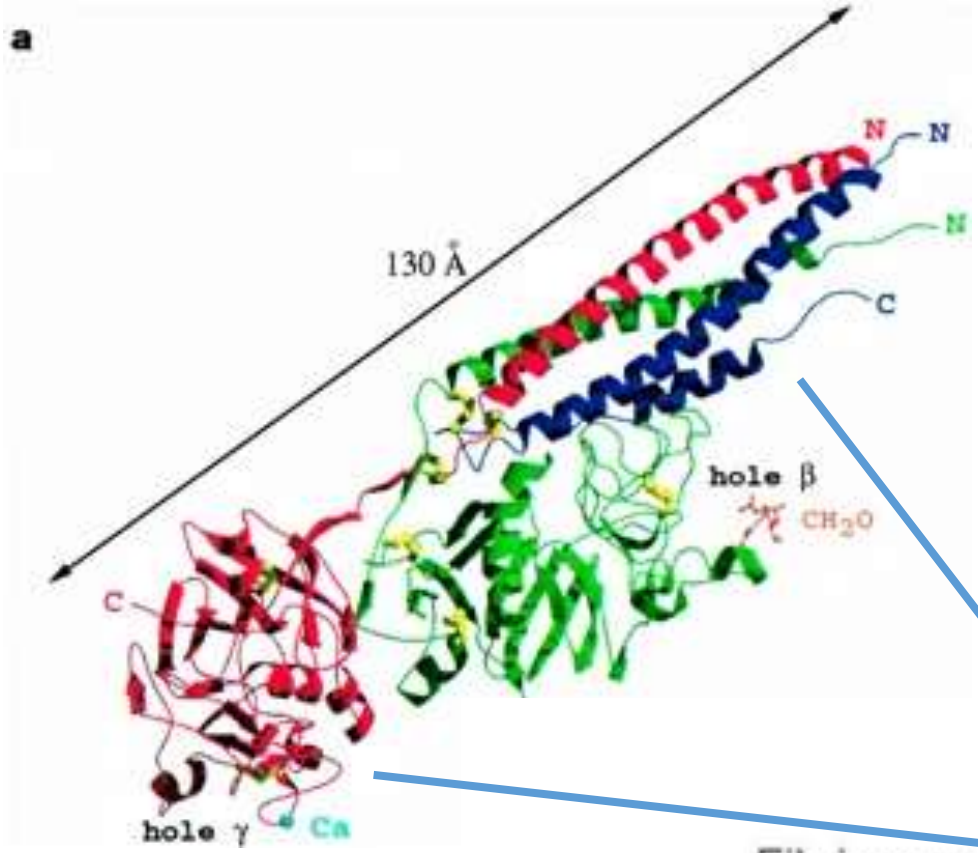
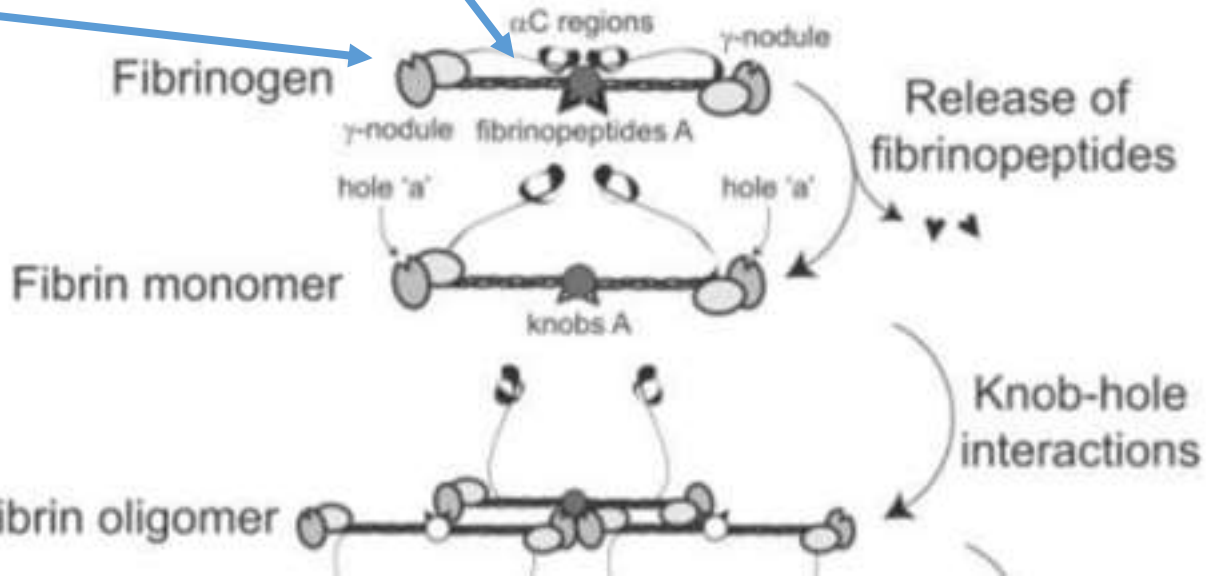
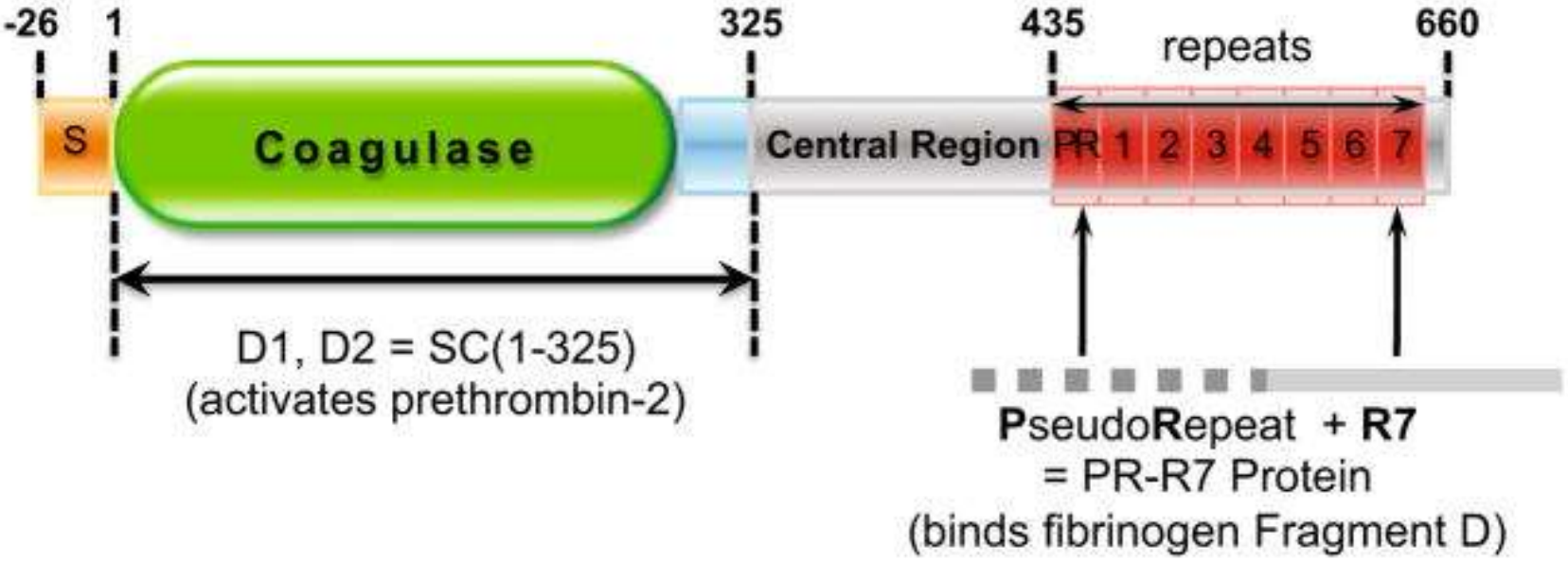
PR 1 2 3 4 5 6 7
D1, D2 = SC(1-325)
(activates prethrombin-2)
PseudoRepeat + R7
= PR-R7 Protein
(binds fibrinogen Fragment D)
A
prothrombin
fibrinogen
-26
1
149
282
470
605
610
S
D1
D2
L
R

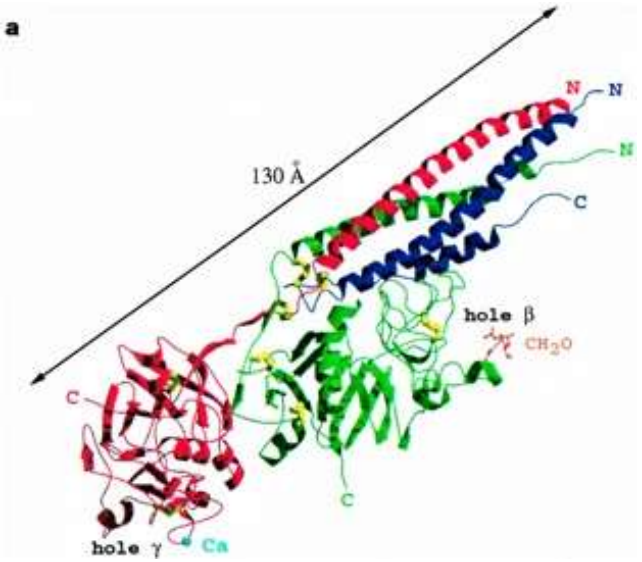
Fibrinogen fragment D, showing the region of coiled coils and the globular β and γ domains.

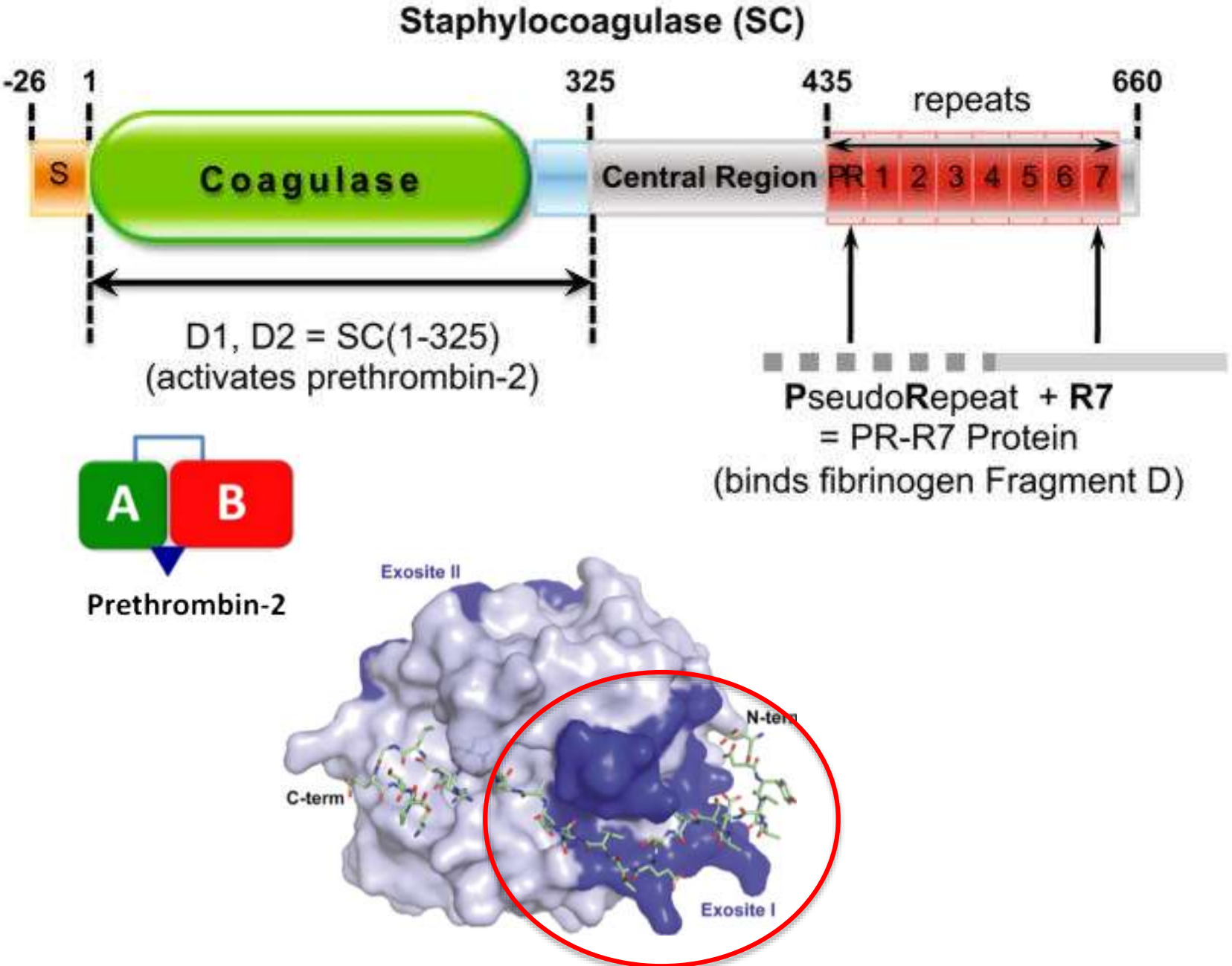




Staphylocoagulase (SC)



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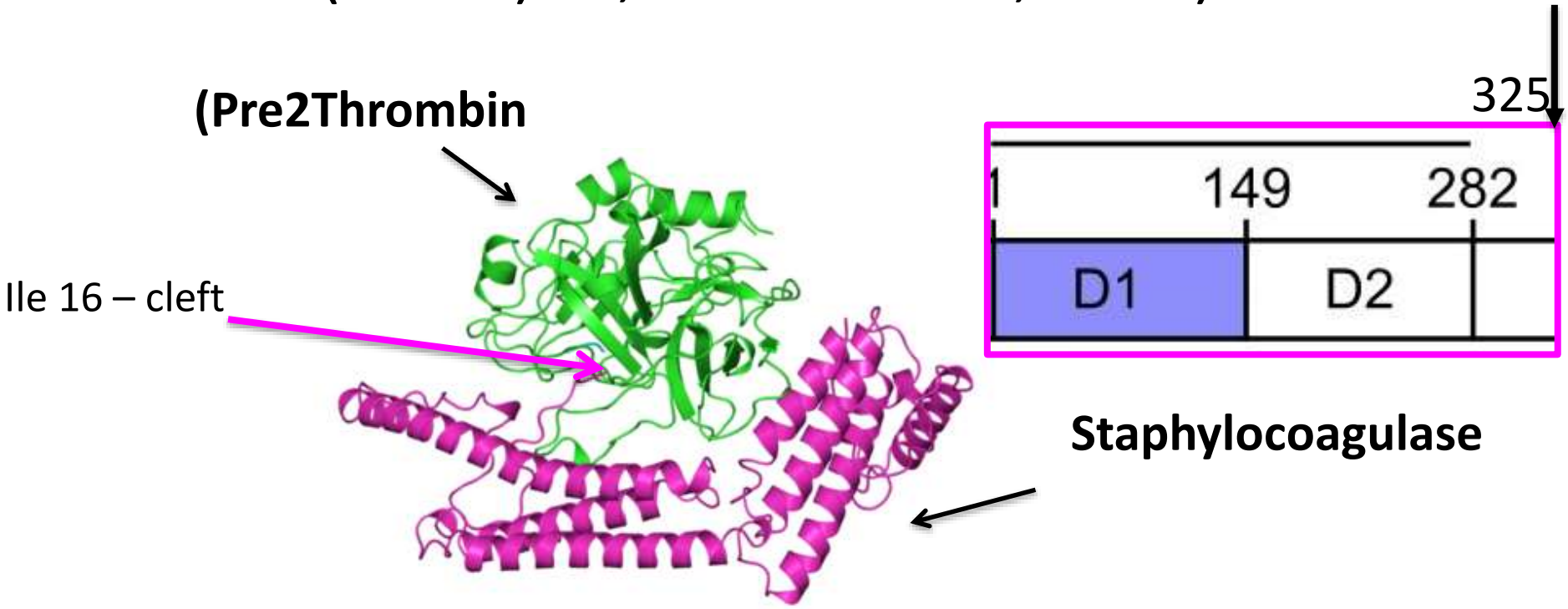
(activates prethrombin-2)
PseudoRepeat + R7
= PR-R7 Protein
(binds fibrinogen Fragment D)
A
B
Prethrombin-2
Exosite II
N-term
C-term
Exosite I

Staphylocoagulase (SC) X ray-structure

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

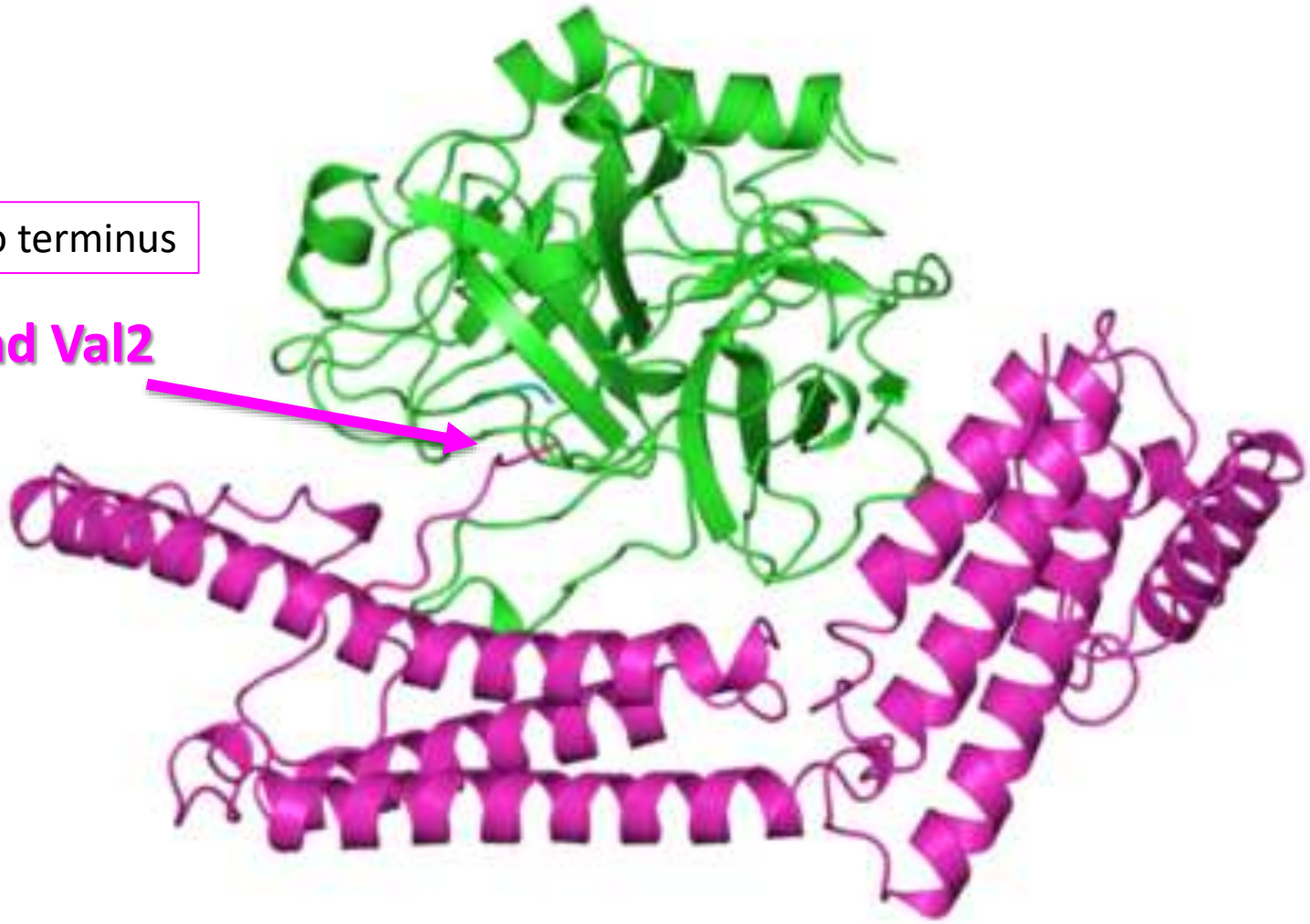


Staphylocoagulase (SC) X ray-structure

(Friedrich, et al. *Nature*, 2003)

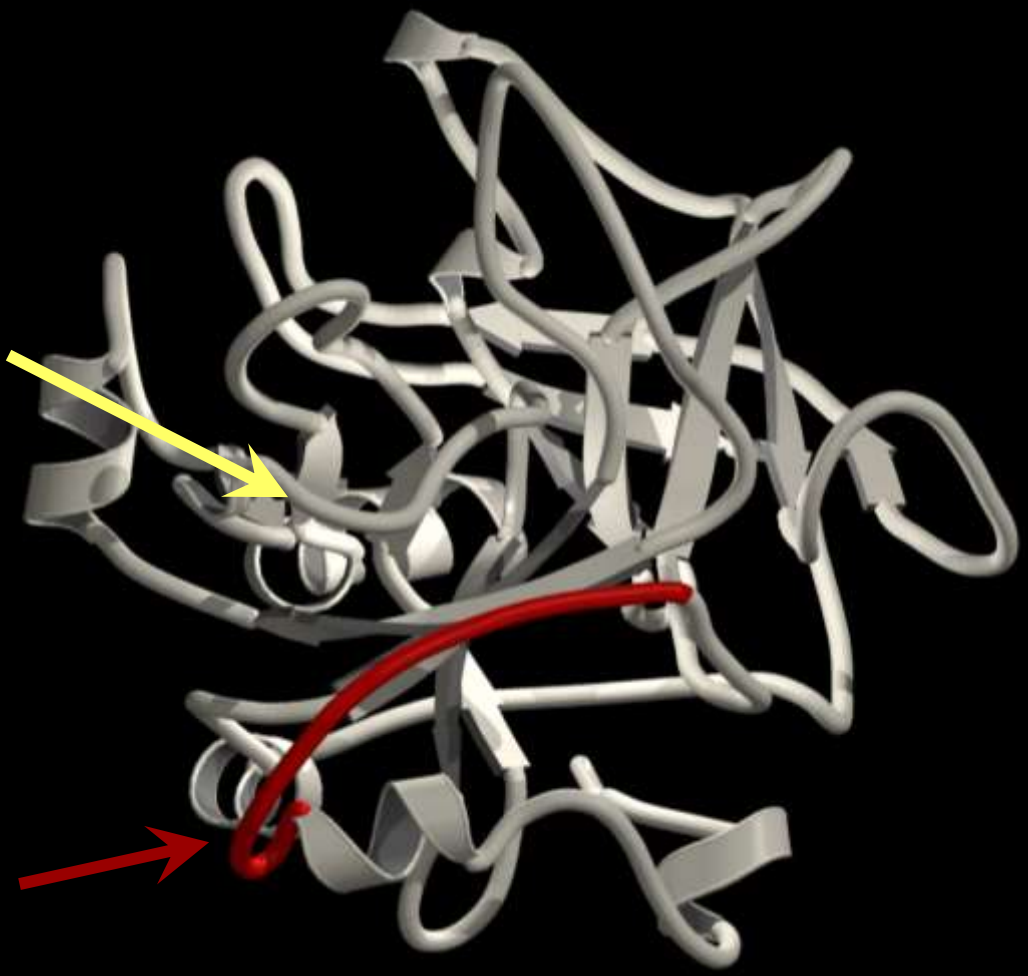
The amino terminus

Ile 1 and Val2



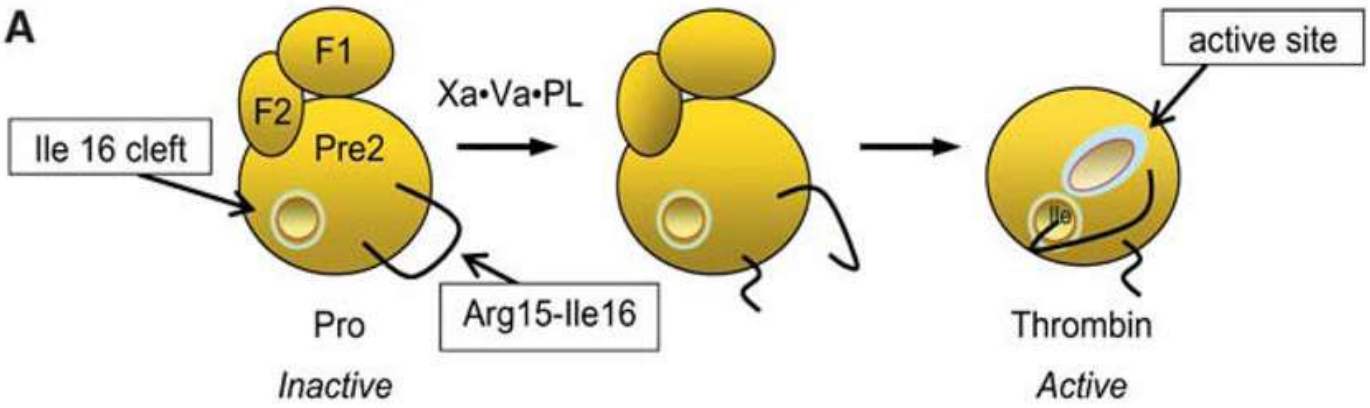
Serine Proteases: Conversion Pathway

- 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"

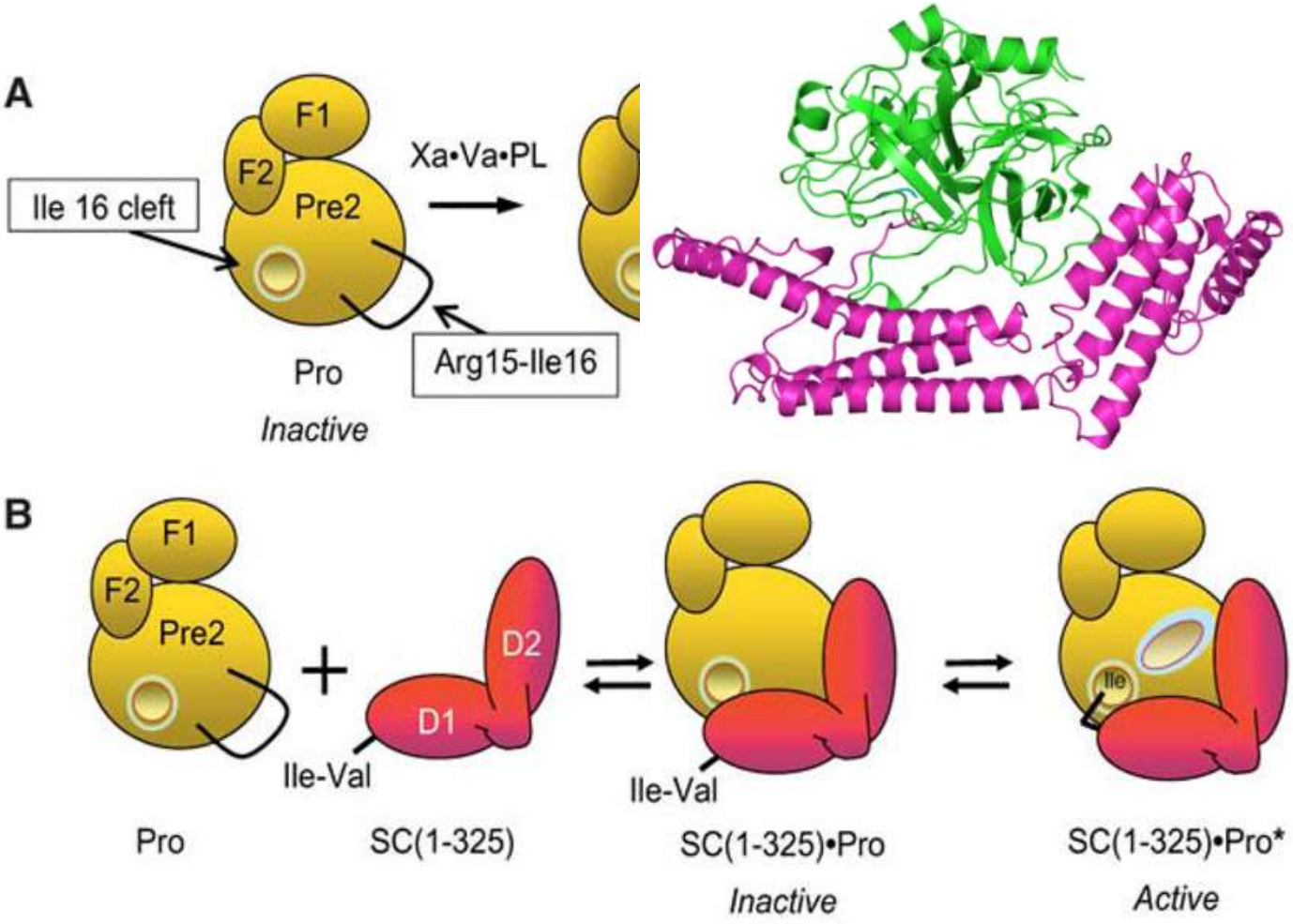


Courtesy of W. Bode, Max Planck Institute of Biochemistry

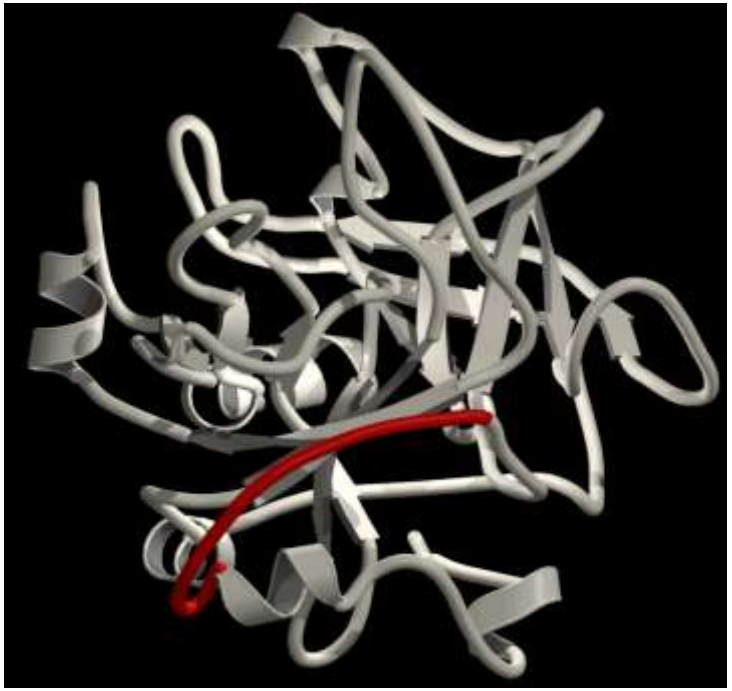
Proteolytic Activation of Prothrombin

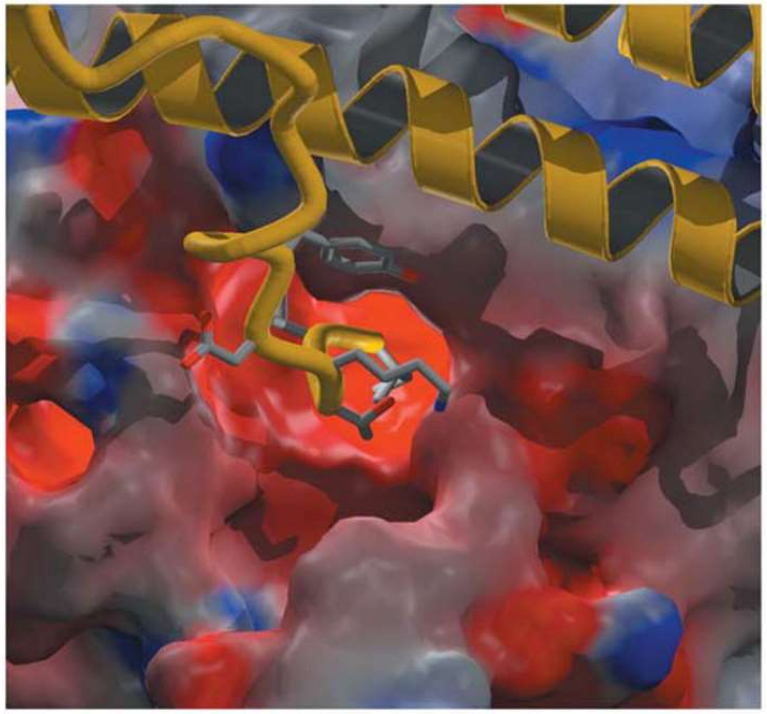


Non-Proteolytic Activation of Prothrombin by Staphylocoagulase support for the "Molecular Sexuality" Hypothesis



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.





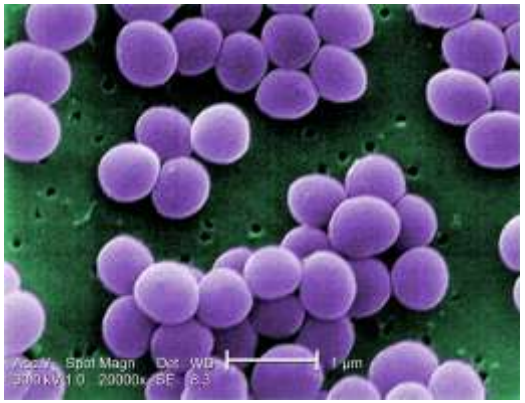
- Zymogen activation requires conformational changes and maturation of the active site.

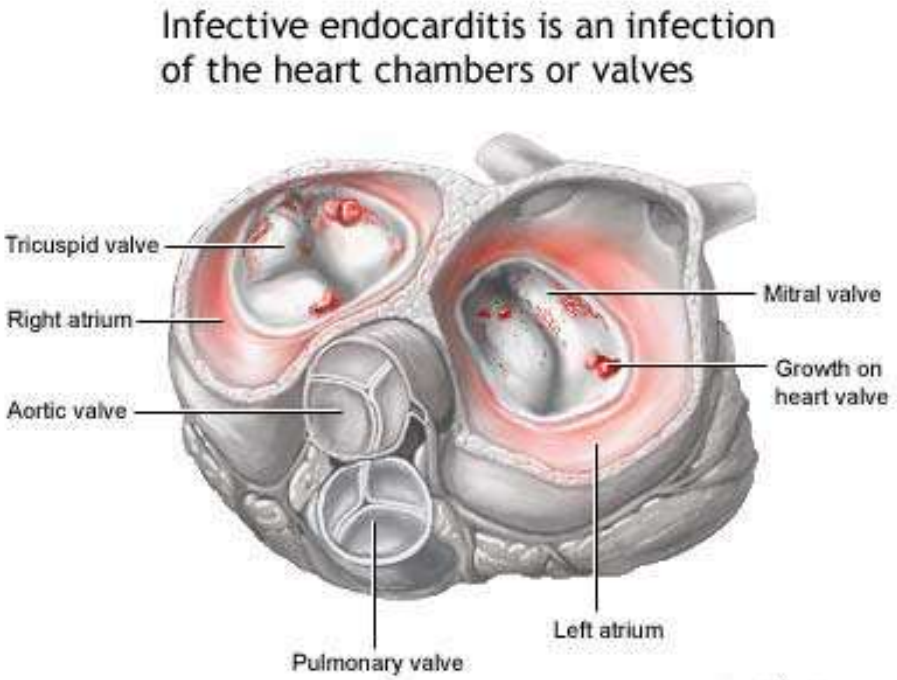
This can be achieved even in the absence of canonical proteolysis.

- Snake venom thrombin-like enzymes (SVTLEs) constitute the major portion (10–24%) of snake venom

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

S. Aureus





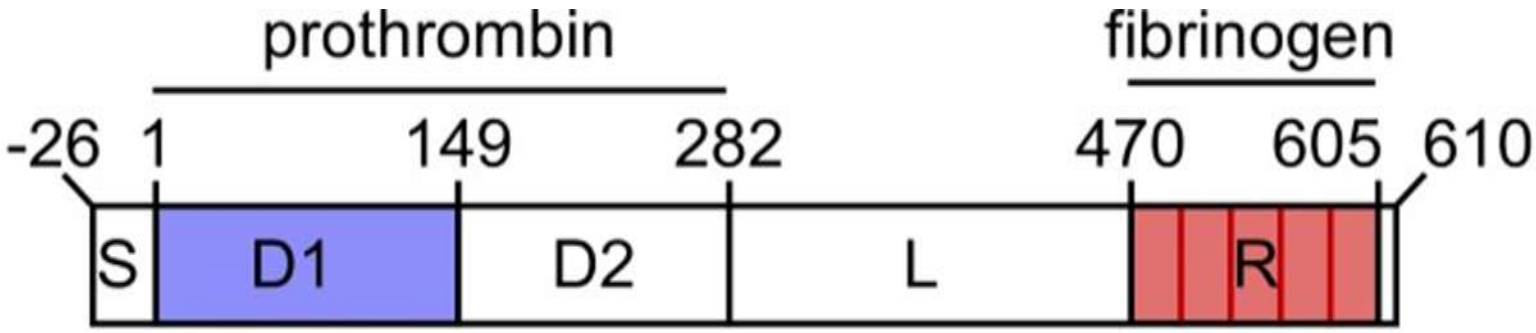
- Growth and fortification of the vegetation by SC-induced fibrin deposition protects the bacteria in the vegetation from clearance by leukocytes and macrophages

- Heart valves are not easily accessible to the immune system

Staphylococcus aureus coagulase R domain, a new evasion mechanism and vaccine target

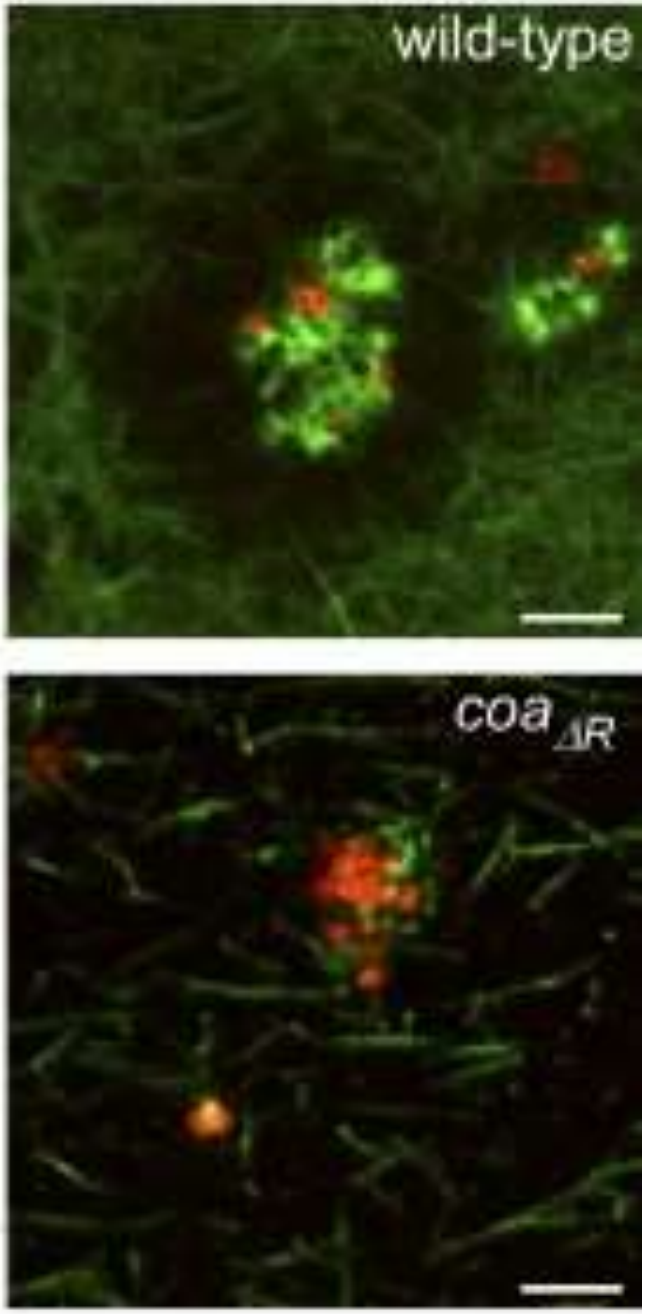
C. Pozzi et al J Exp Med. 2016; 213: 292.

A

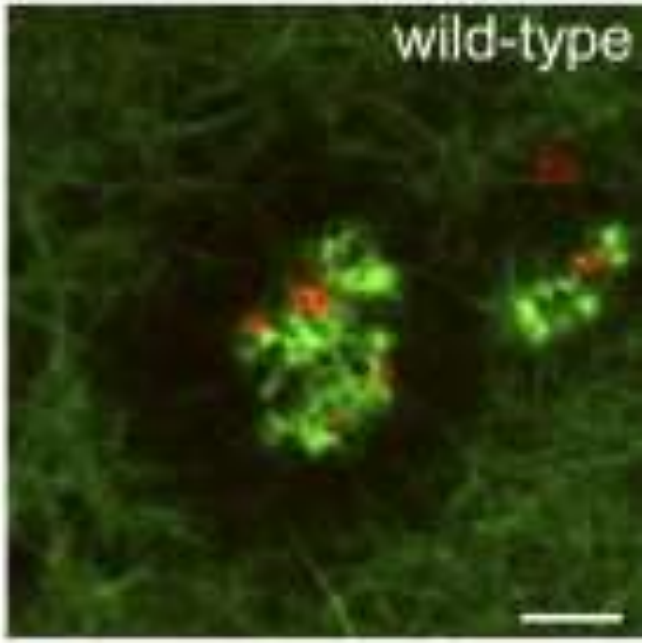


The fibrinogen C-terminal repeats region is well conserved across S. aureus strains

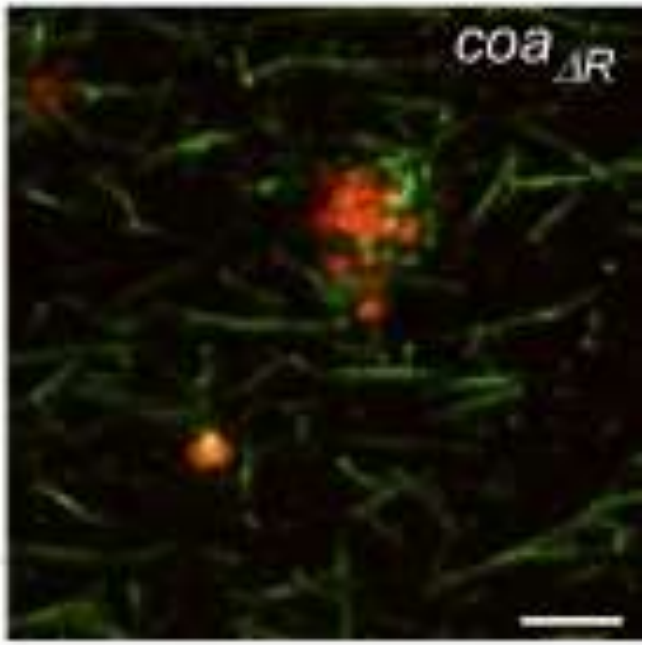
S. aureus (Red) wild-type or deficient in Staphylocoagulase R domain (coaΔR) were incubated with human plasma and flurescent human fibrinogen (Green)



Wild-type staphylococci generated large fibrin deposits (**Green**) in the vicinity of bacteria (**Red**)



The *coa*$_{\Delta R}$ mutant produced long fibrin strands that were only loosely associated with the pathogen

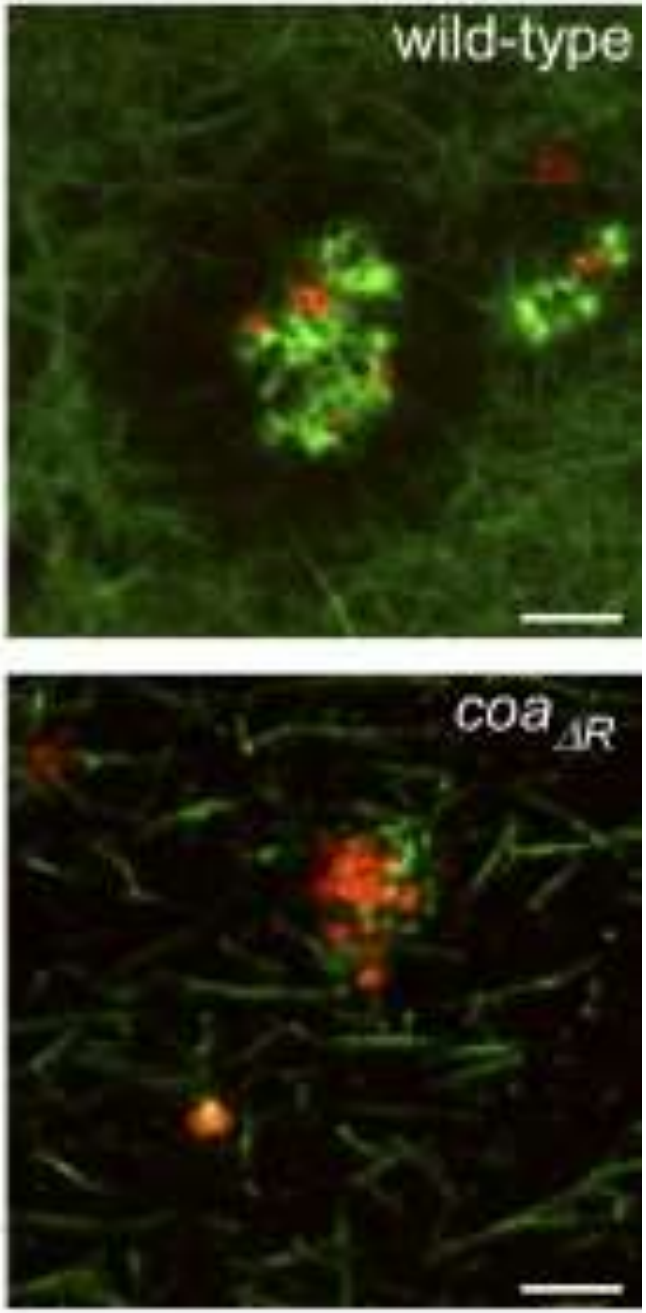


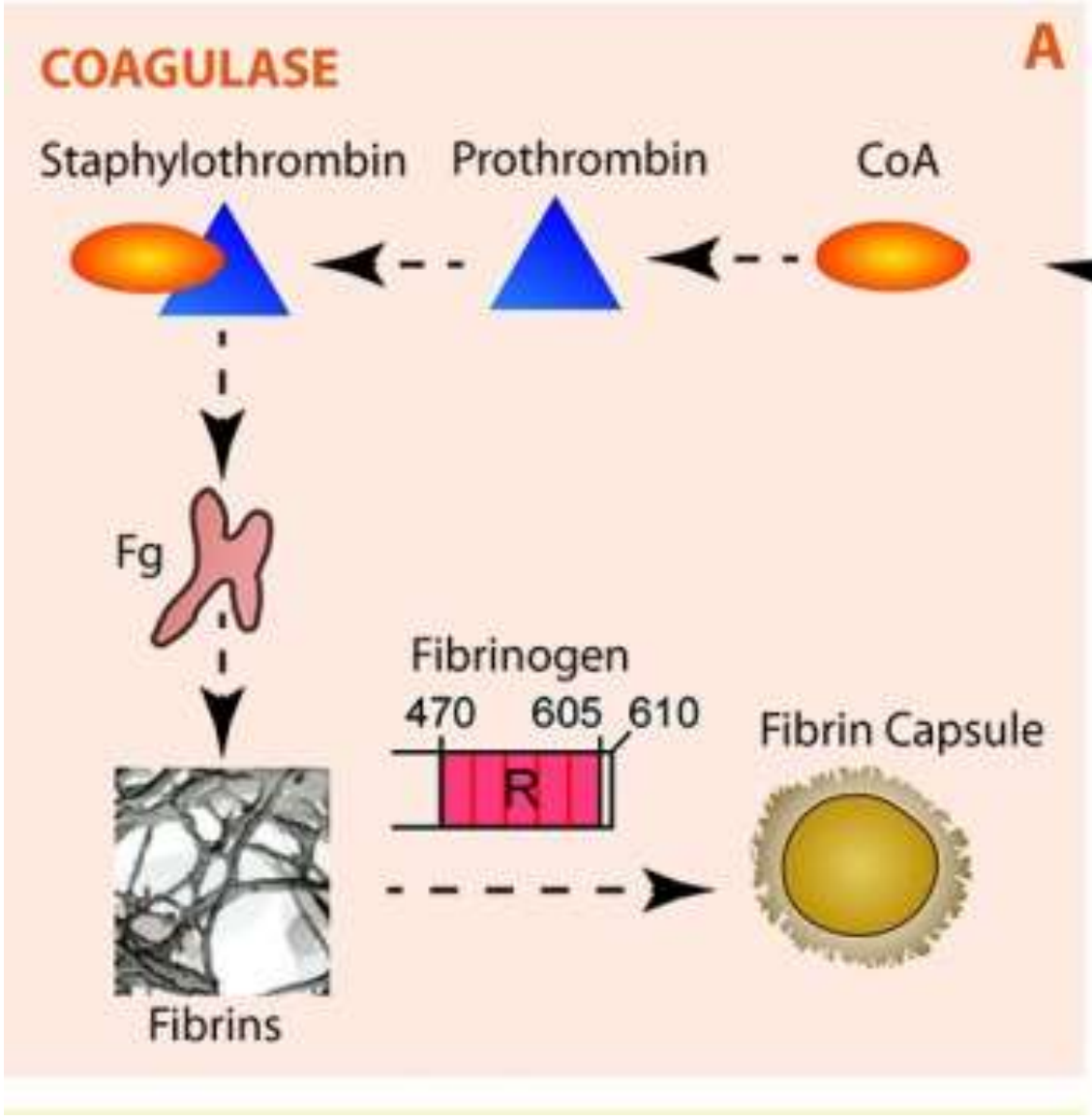
Conclusioni

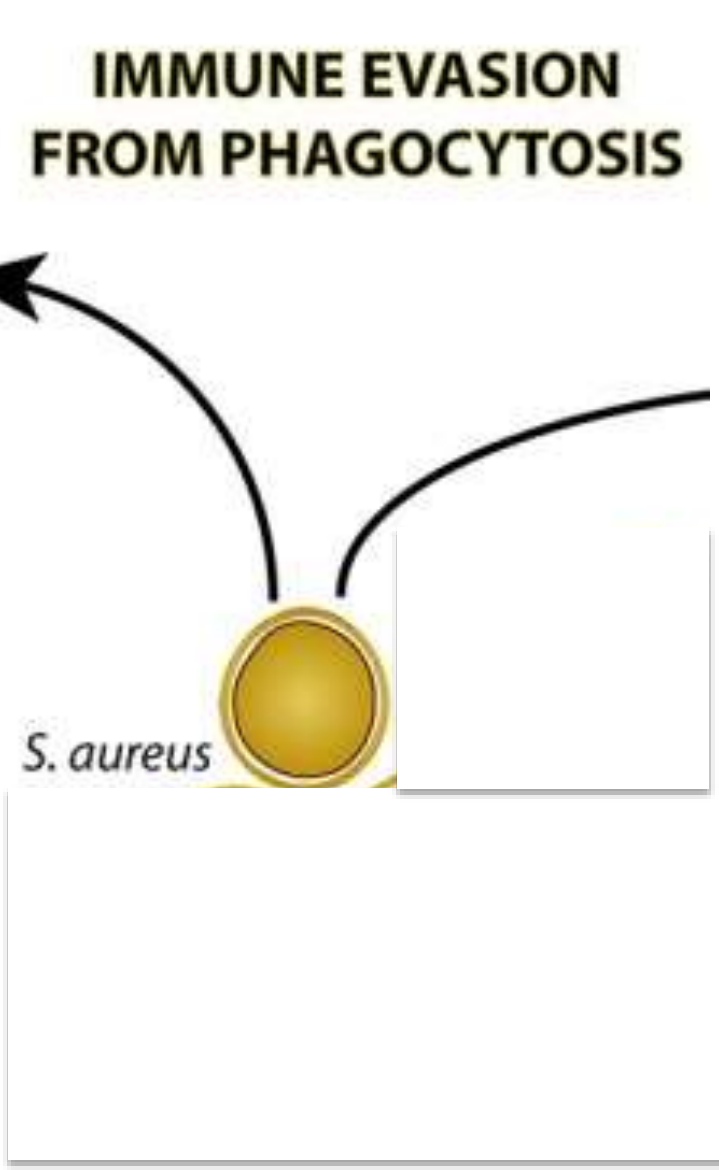
By augmenting the recruitment of soluble fibrinogen, the C-terminal repeats favor Coagulase-induced fibrin clots

This limit diffusion of Coagulase away from staphylococci

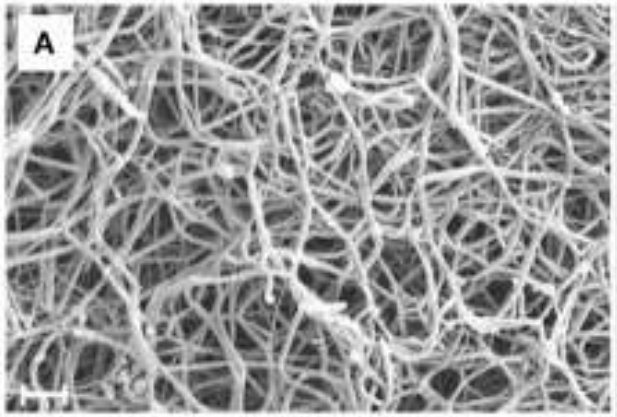
The C-terminal repeats localize the staphylothrombin-generated fibrin shield in the immediate vicinity of the bacteria.



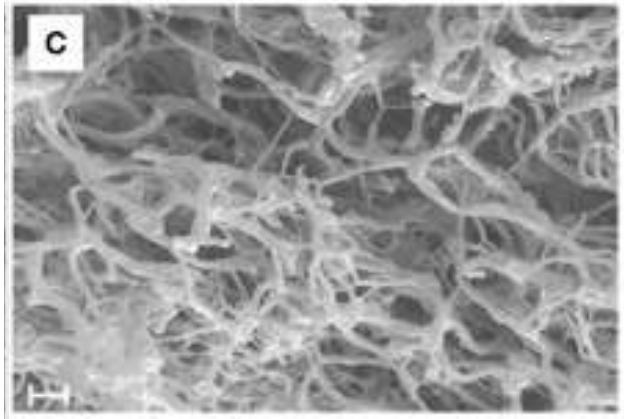

COAGULASE
A
Staphylothrombin
Prothrombin
CoA
Fg
Fibrinogen
470
605
610
R
Fibrin Capsule
Fibrins


IMMUNE EVASION
FROM PHAGOCYTOSIS
S. aureus

Structure, Mechanical, and Lytic Stability of Fibrin and Plasma Coagulum Generated by Staphylocoagulase From *Staphylococcus aureus*



human thrombin

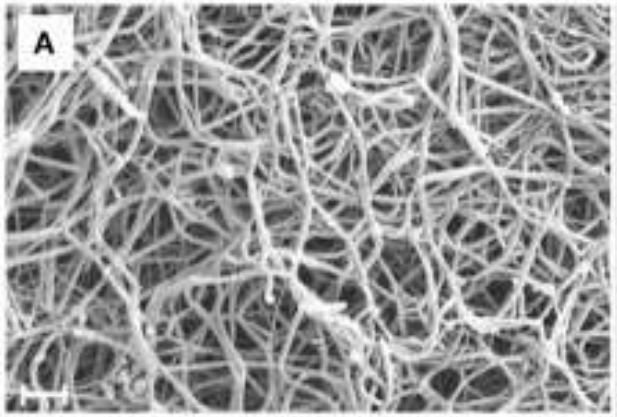


staphylocoagulase-prothrombin

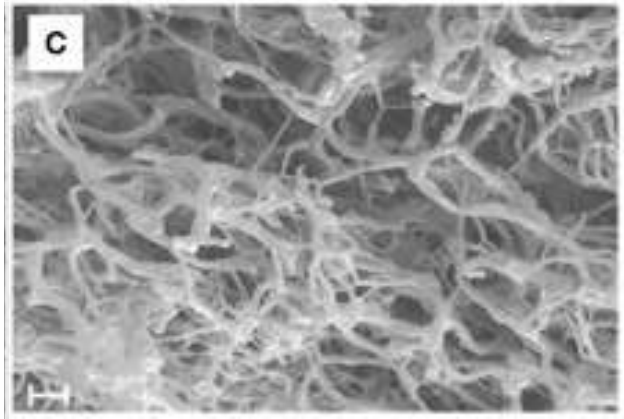
Scanning electron microscopy of fibrin clots

Farkas et al Front Immunol. 2019; 10: 2967

Structure, Mechanical, and Lytic Stability of Fibrin and Plasma Coagulum Generated by Staphylocoagulase From *Staphylococcus aureus*



human thrombin



staphylocoagulase-prothrombin

Staphylocoagulase generates a thrombus with reduced mechanical stability and increased lytic susceptibility.
This proneness to clot disintegration could favor the embolism from endocardial bacterial vegetation

Farkas et al Front Immunol. 2019; 10: 2967

Azione mirata della Stafilocoagulase

- Staphylothrombin does not cut other endogenous substrates of thrombin
- Staphylothrombin polymerizes fibrin but does not activate other clotting and inflammatory factors

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

nature medicine

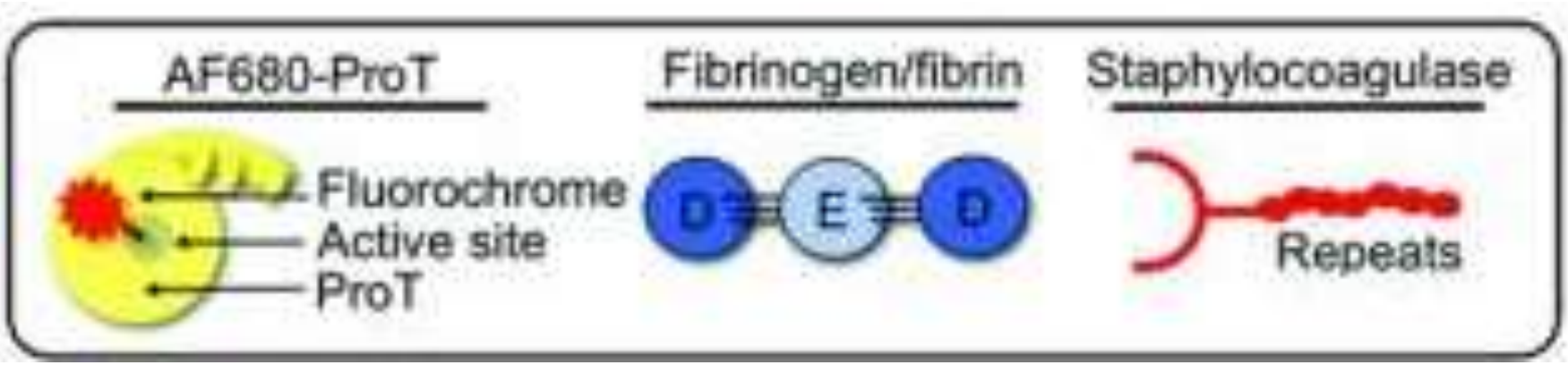
In vivo detection of *Staphylococcus aureus* endocarditis by targeting pathogen-specific prothrombin activation

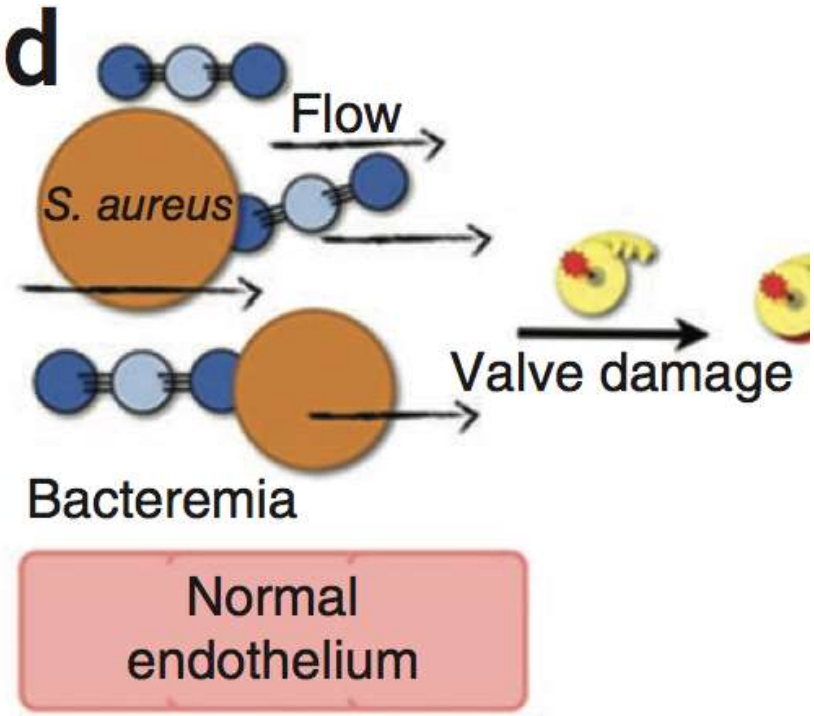
Peter Panizzi[1,2,9], Matthias Nahrendorf[1,9], Jose-Luiz Figueiredo[1], Jennifer Panizzi[3], Brett Marinelli[1], Yoshiko Iwamoto[1], Edmund Keliher[1], Ashoka A Maddur[4], Peter Waterman[1], Heather K Kroh[4], Florian Leuschner[1], Elena Aikawa[1], Filip K Swirski[1], Mikael J Pittet[1], Tilman M Hackeng[5],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

Prothrombin as a probe for *S. Aureus*

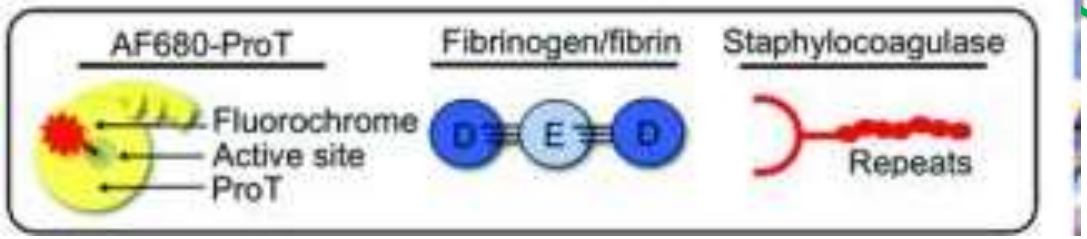
- SC binds prothrombin with high affinity and activates it through a conformation change
- SC-Prothrombin complex clots fibrinogen but is impervious to physiologic thrombin inhibitors.
- SC-Prothrombin is present in the vegetation
- Labeled Prothrombin can be used as a probe to detect bacterial vegetation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

Prothrombin as a probe for *S. Aureus*

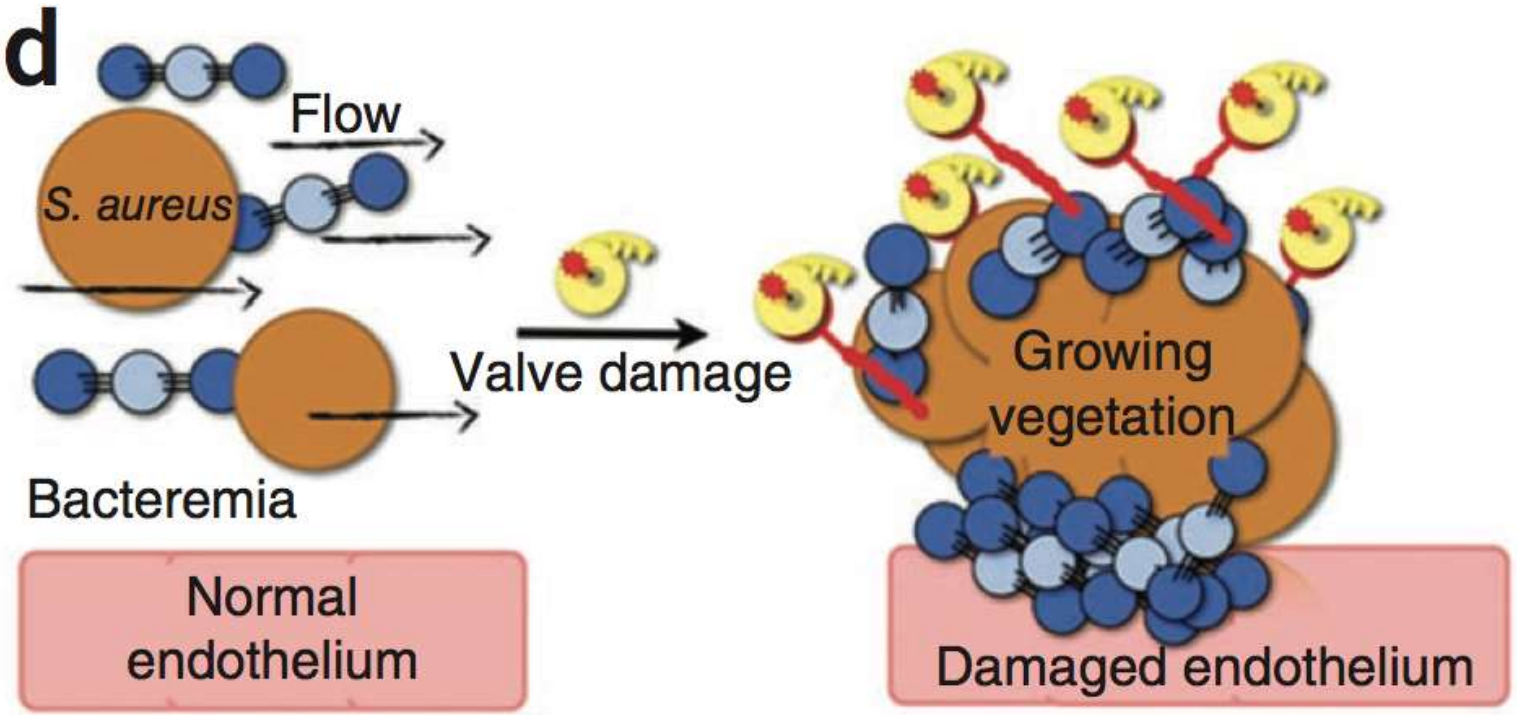




Prothrombin as a probe for *S. Aureus*

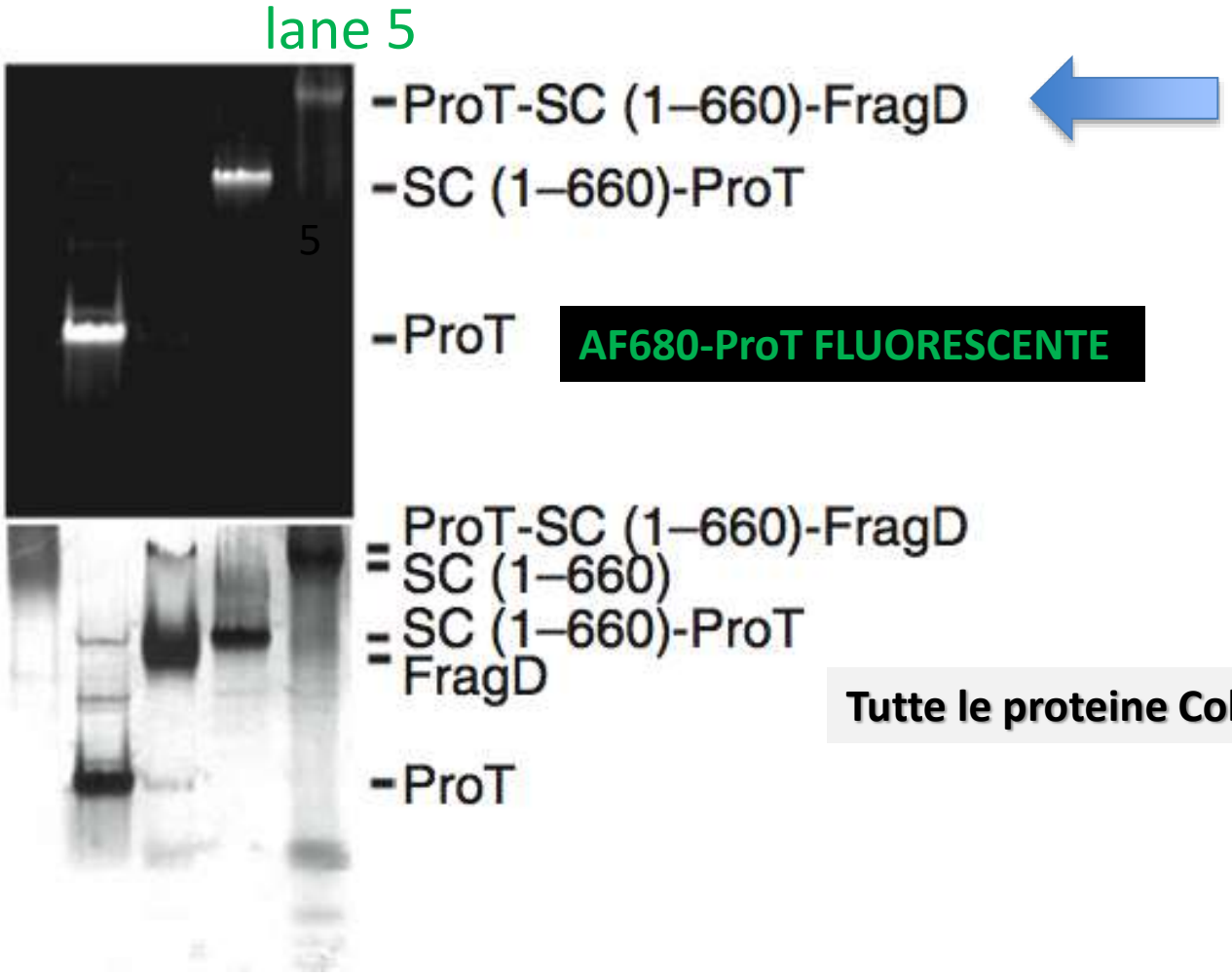


Staphylocoagulase (SC) is shown tethering AF680-ProT to fibrinogen/fibrin



Ternary complex

Full-length SC (SC(1-660)) forms a ternary complex (lane 5) with prothrombin (ProT) and fibrinogen/fibrin(FragD)



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

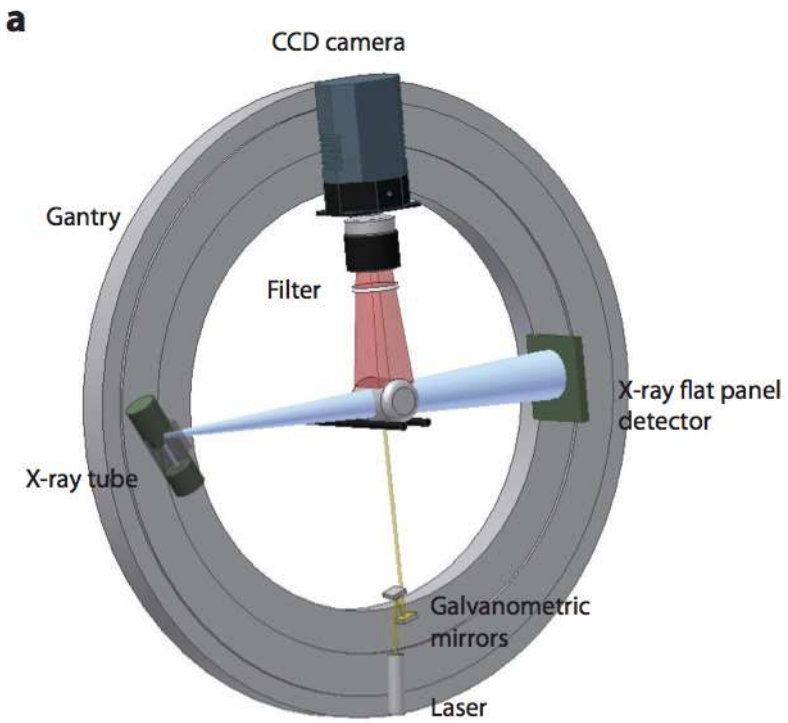
The PROBE

AF680- Prothrombin

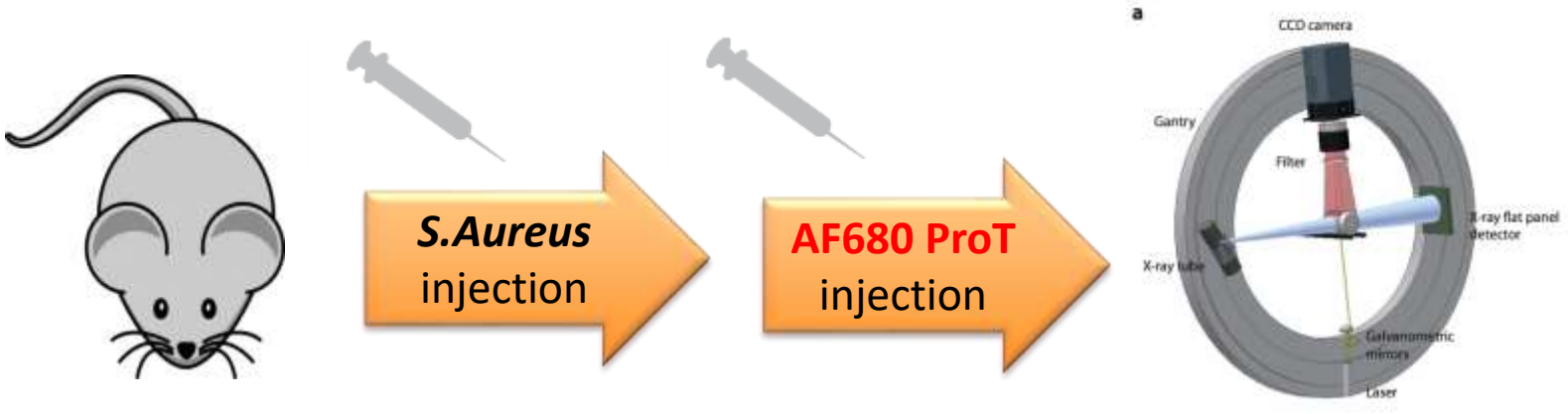
The DETECTOR

Fluorescence molecular tomography

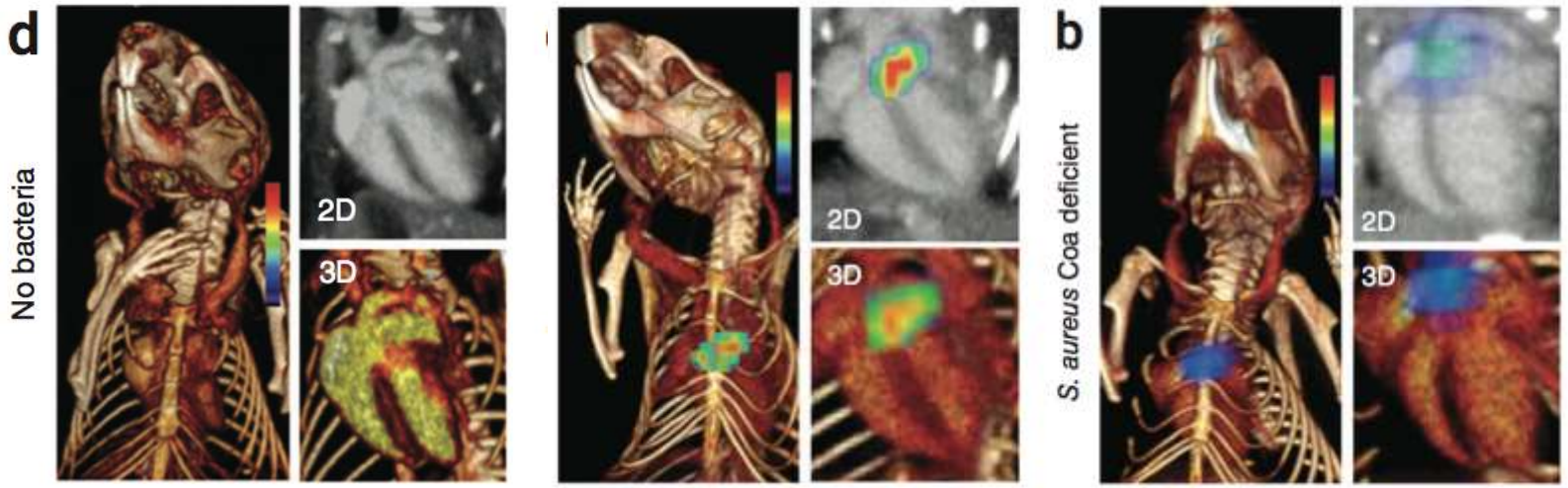
- Computer Tomography



Fluorescent prothrombin co-localise with SC positive bacteria

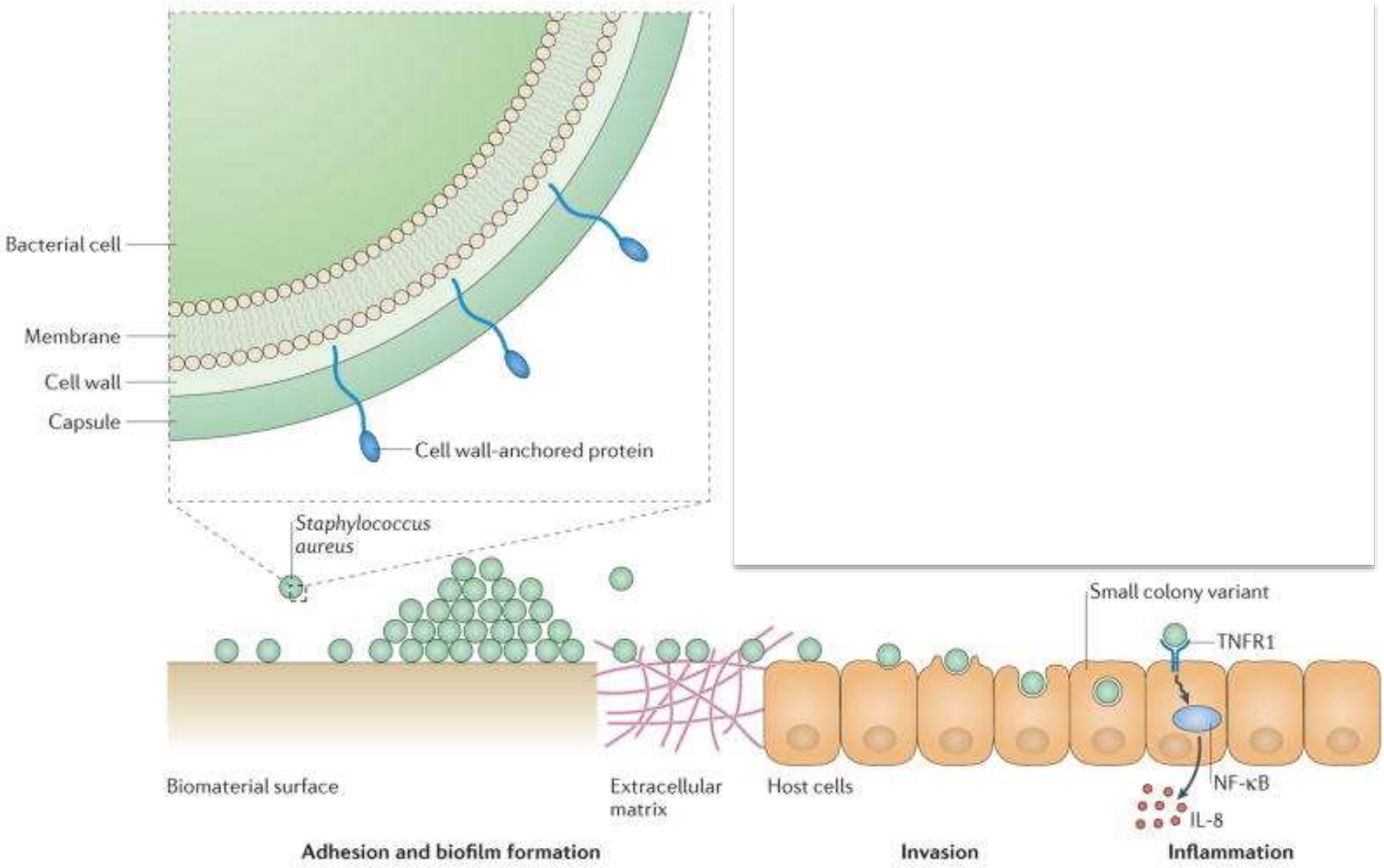


No Bacteria *S. Aureus wt* *S. Aureus SC deficient*



- AF680ProT detects S.Aureus in vivo and can be used as a diagnostic tool to determine site, bacterial load and activity of the infection.

Adhesion, invasion and evasion: the many functions of the surface proteins of Staphylococcus aureus



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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