Hemostasis : Overview

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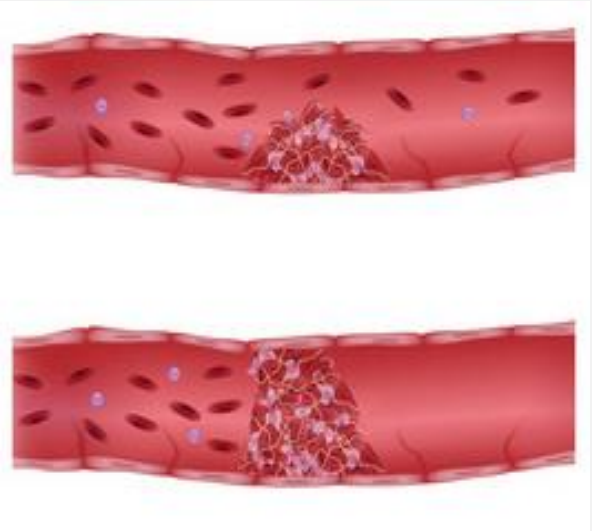
Hemostasis

Hemostasis : Processes involved in the maintain of the blood flow.

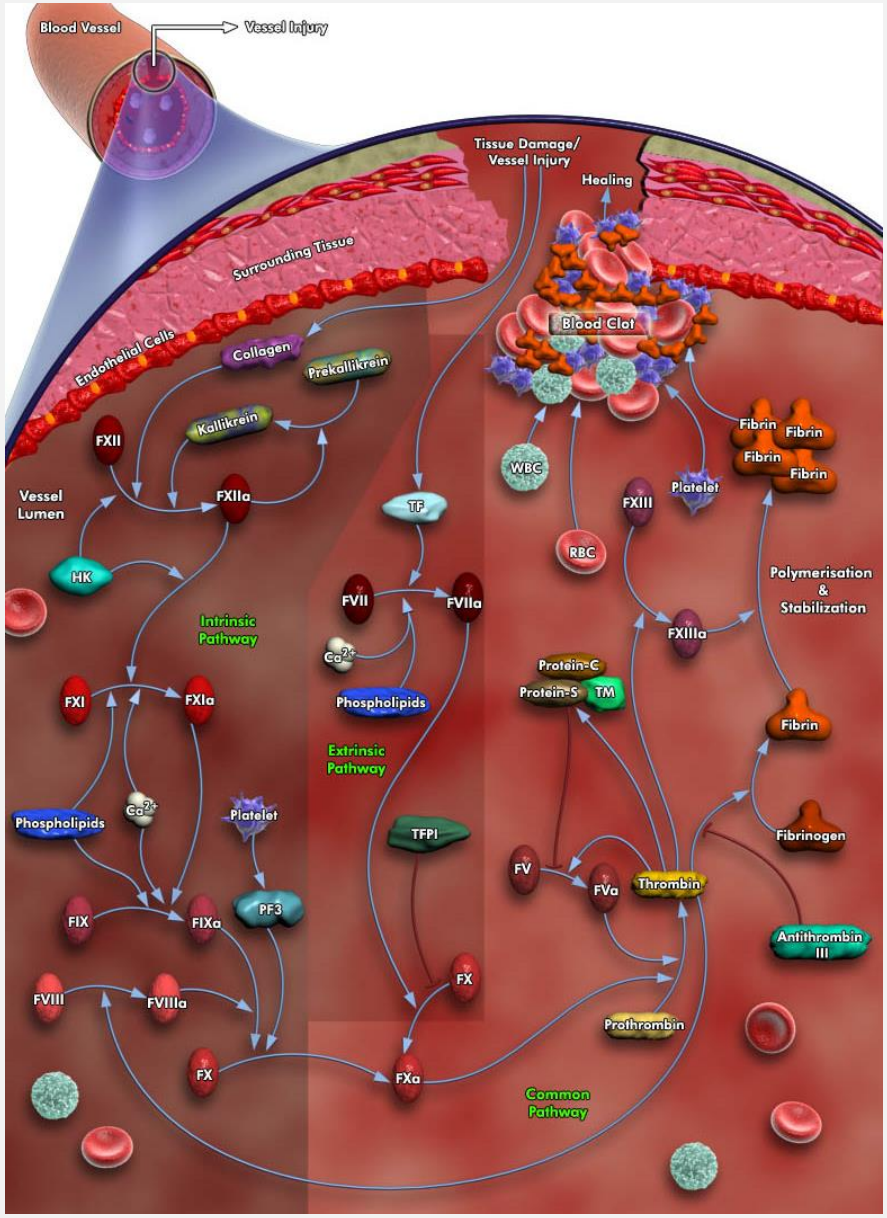
Finely regulated to prevent (i) blood loss and (ii) vessel occlusion.



Bleeding

Thrombosis

Hemostasis: an irreducible complexity



Hemostasis: sequential events perfectly synchronized **in time and in space**

Players in hemostasis:

- **Vessel wall** (endothelial cells and sub-endothelial space)
- **Blood cells** (platelets)
- **Plasma proteins** (coagulation factors)

hemostasis

- **Primary hemostasis**

3 - 5 min

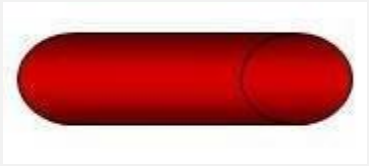
Platelets activation/aggregation → Platelet plug formation

- **Coagulation**

5 - 10 min

Cascade of enzymatic reactions → Fibrine formation

- **Fibrinolysis**



48 - 72 hrs

Cascade of enzymatic reactions → Fibrine degradation

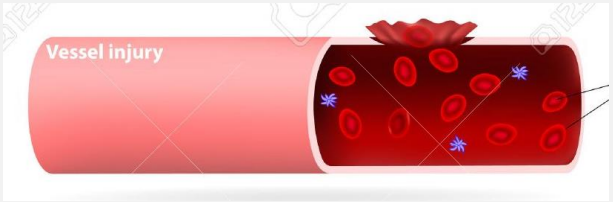
Primary hemostasis

WHAT: platelet plug formation, stable enouth to stop blood loss.

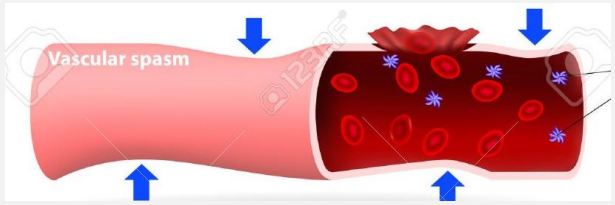
WHERE: On the site of injury, in the vessel lumen, anchored to the vessel wall

WHO: Vessel wall, adhesive proteins in the extracellular matrix, plasma proteins, platelets

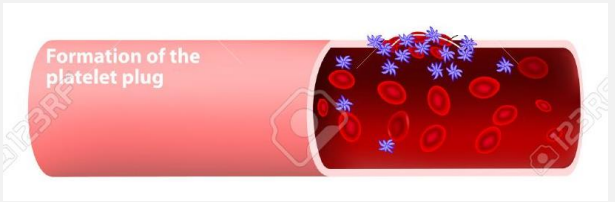
HOW: …



Wound/lesion



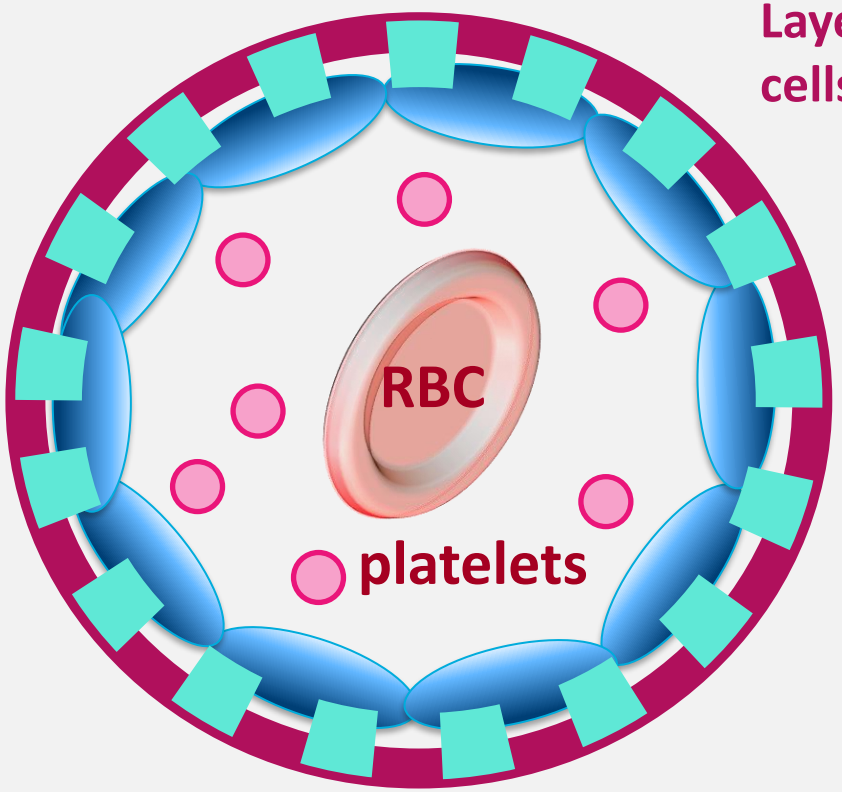
Vasoconstriction and platelet adhesion



Platelet activation and aggregation

Primary hemostasis

Intact vessel wall



Layer of smooth muscle cells

Extracellular matrix (collagen fibers)

Endothelium (endothelial cells)

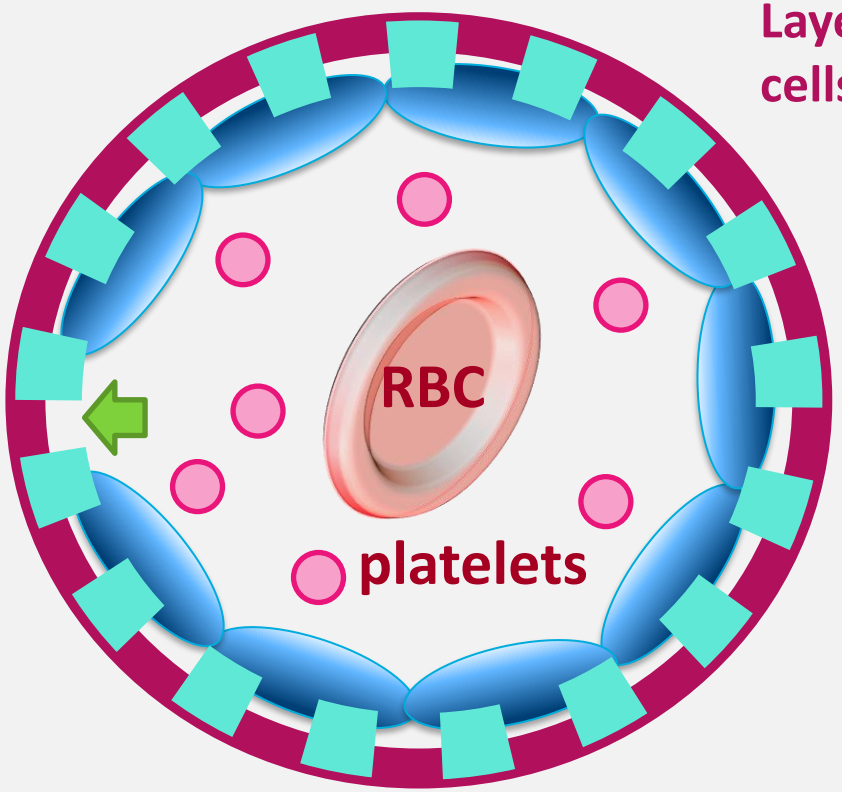
Prostacyclin and nitric oxide release: inhibit platelet activation

Primary hemostasis

Vascular injury

Break in endothelium continuity

Contact between blood and collagen



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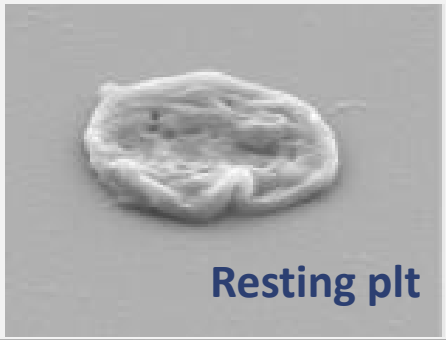

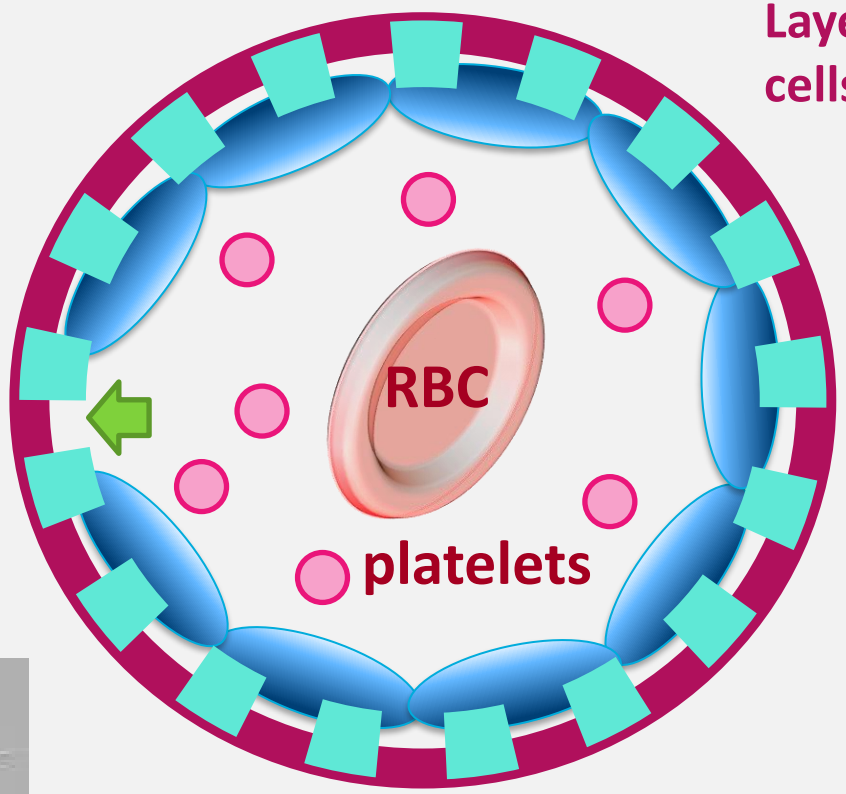

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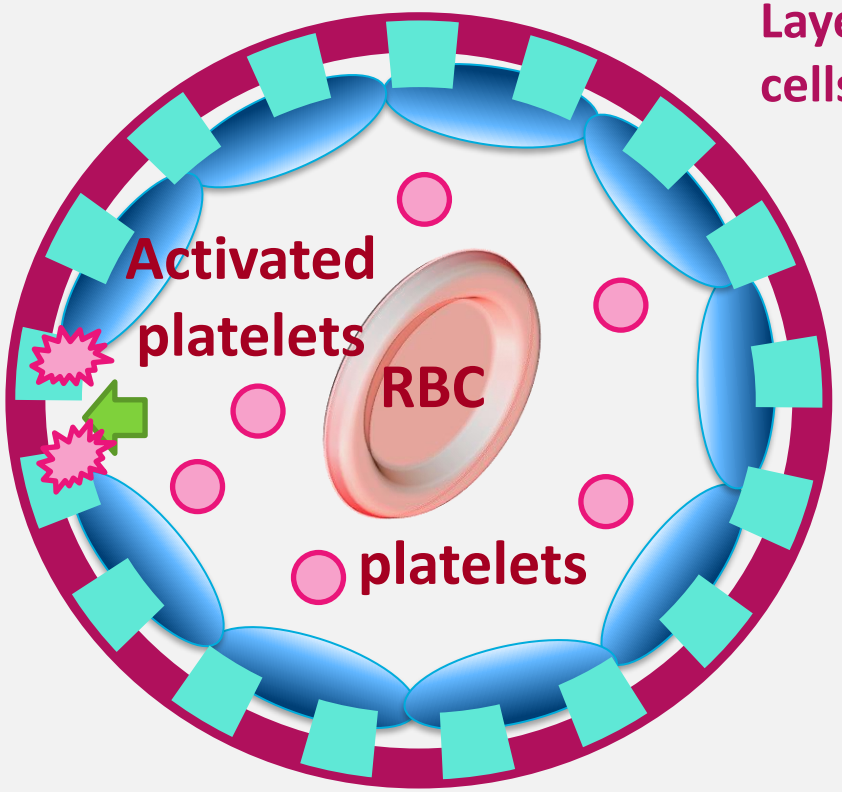
Contact between blood and collagen

Platelet activation

Von Willebrand Factor ⇨ Platelet adhesion to the vessel wall



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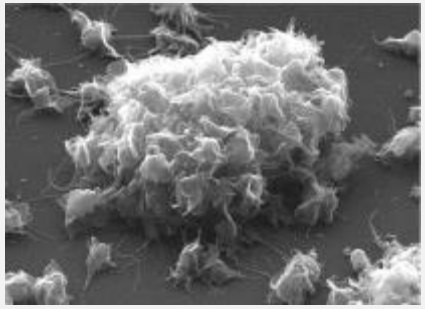
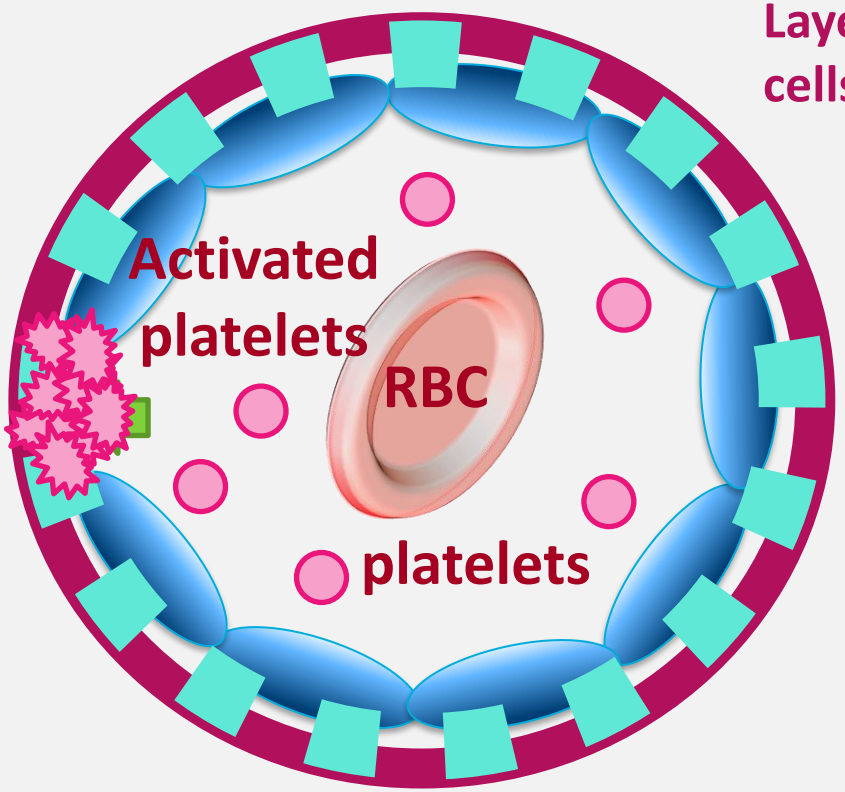


Platelet plug formation



Intact vessel wall

Layer of smooth muscle cells

Extracellular matrix (collagen fibers)

Endothelium (endothelial cells)

Prostacyclin and nitric oxide release: inhibit platelet activation

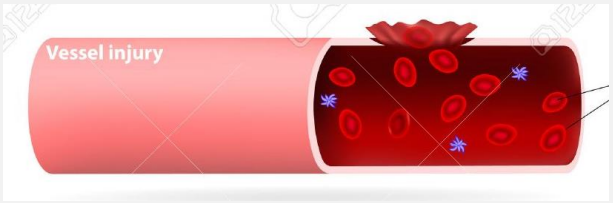
Coagulation

WHAT: Fibrine network formation (polymerized fibrine forms a mesh that stabilizes platelet aggregate)

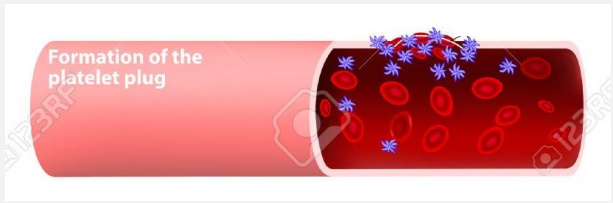
WHERE: On the site of injury, in the vessel lumen, **on the surface of activated platelets**

WHO: Coagulation factors, vessel wall, platelets

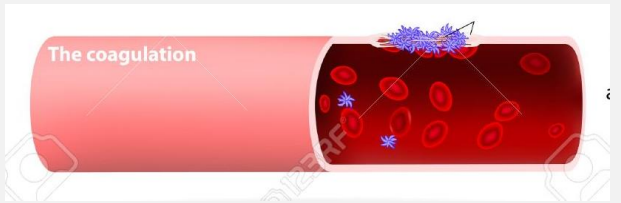
HOW: …



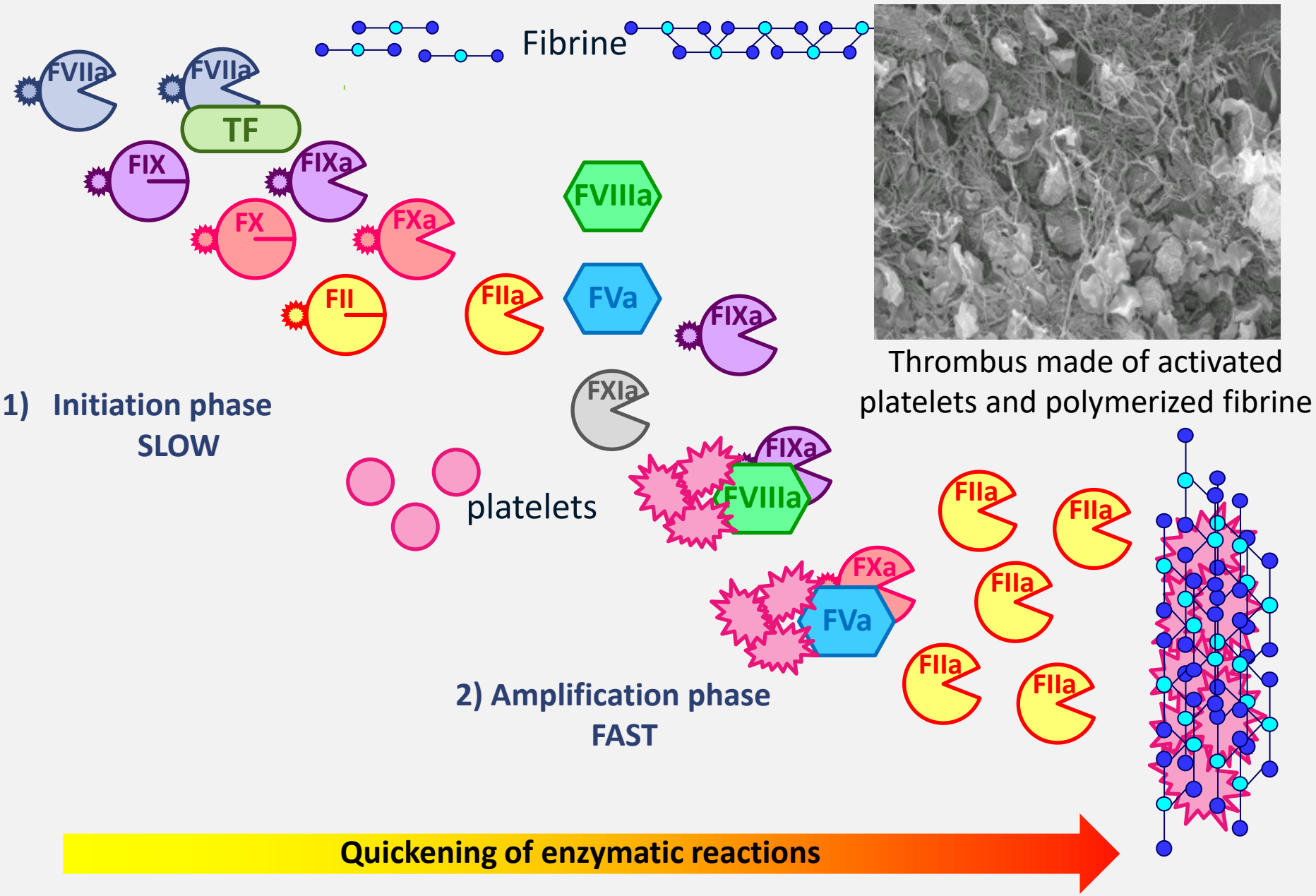
Injuried endothelium



Coagulation cascade activation

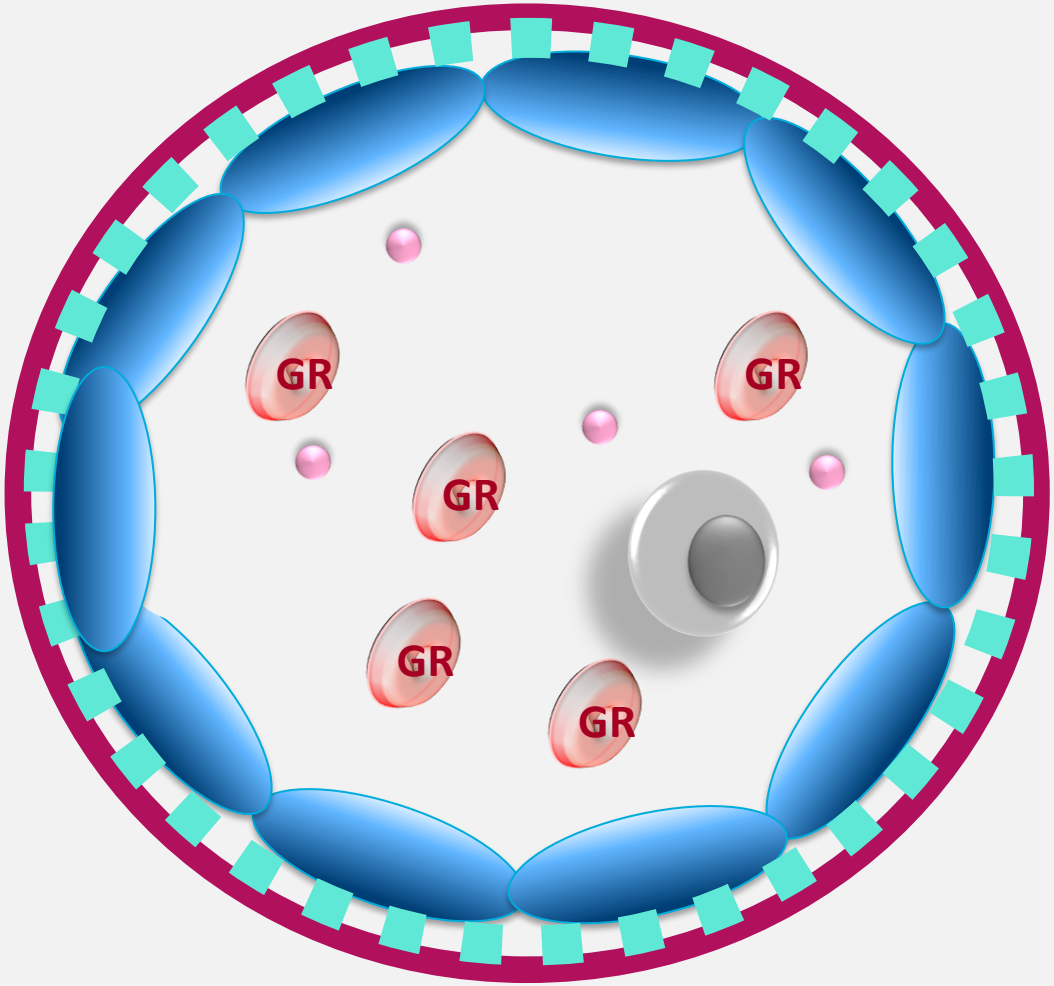


Thrombus formation


Fibrine
FVIIa
FVIIa
TF
FIX
FIXa
FX
FXa
FII
FIIa
FVIIIa
FVa
FIXa
FXIa
platelets
FIXa
FVIIIa
FXa
FVa
FIIa
FIIa
FIIa
FIIa
FIIa
Thrombus made of activated platelets and polymerized fibrine
1) Initiation phase
SLOW
2) Amplification phase
FAST
Quickening of enzymatic reactions

Coagulation

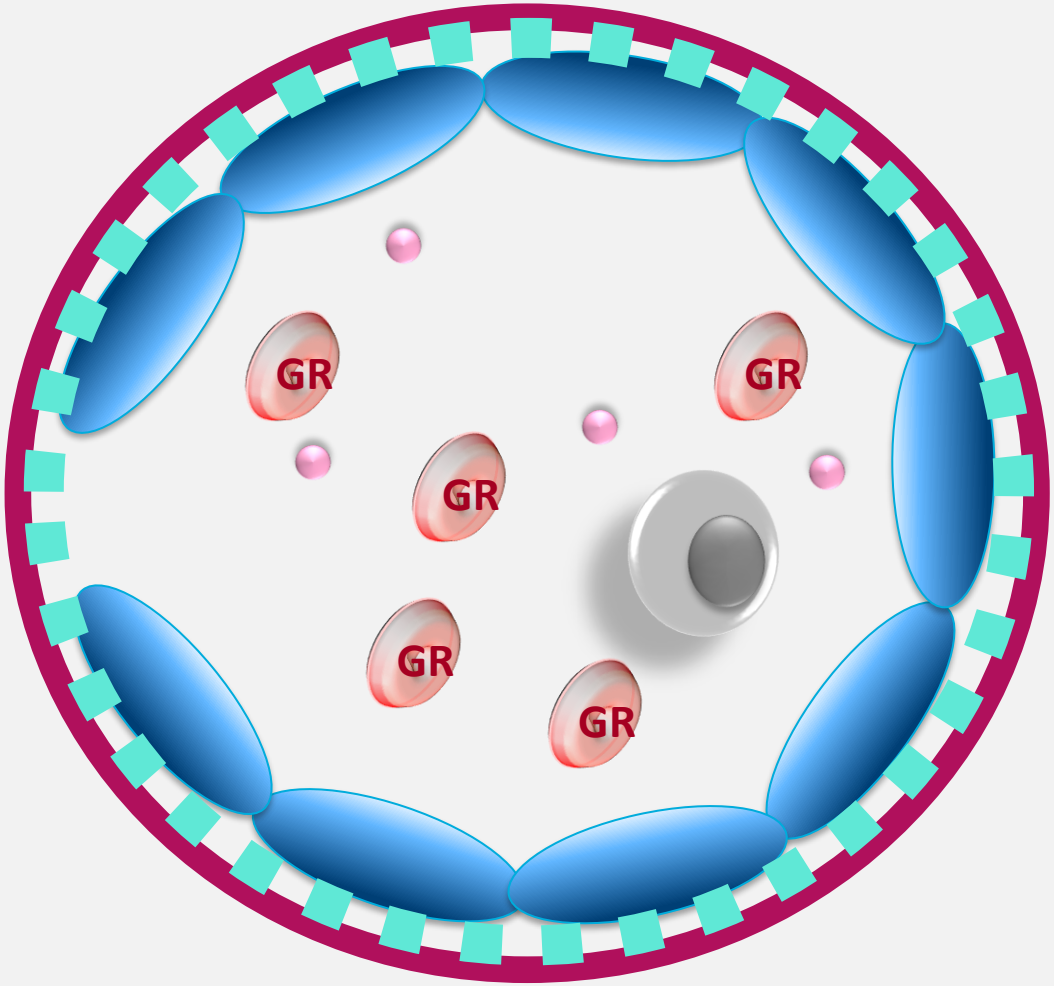
1) Initiation



Coagulation

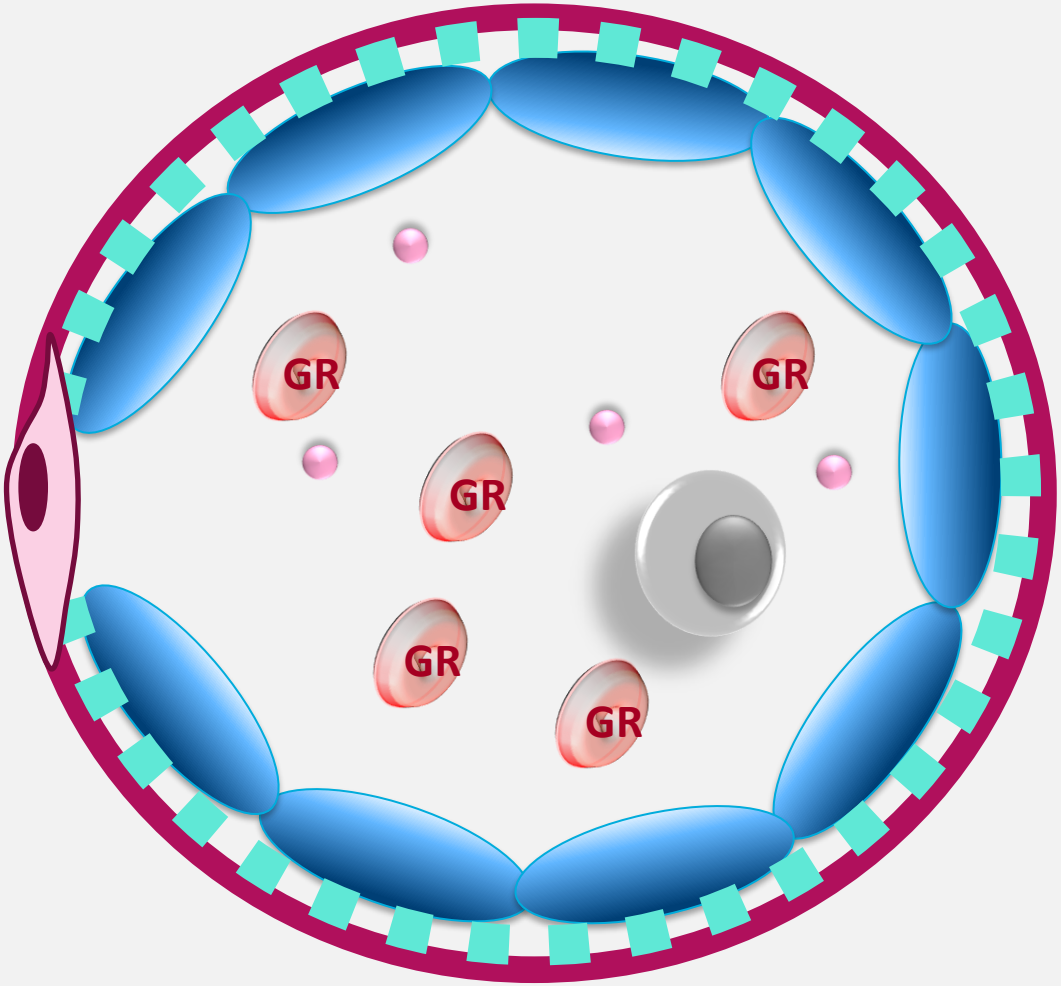
1) Initiation

Break in endothelium continuity



Coagulation

1) Initiation

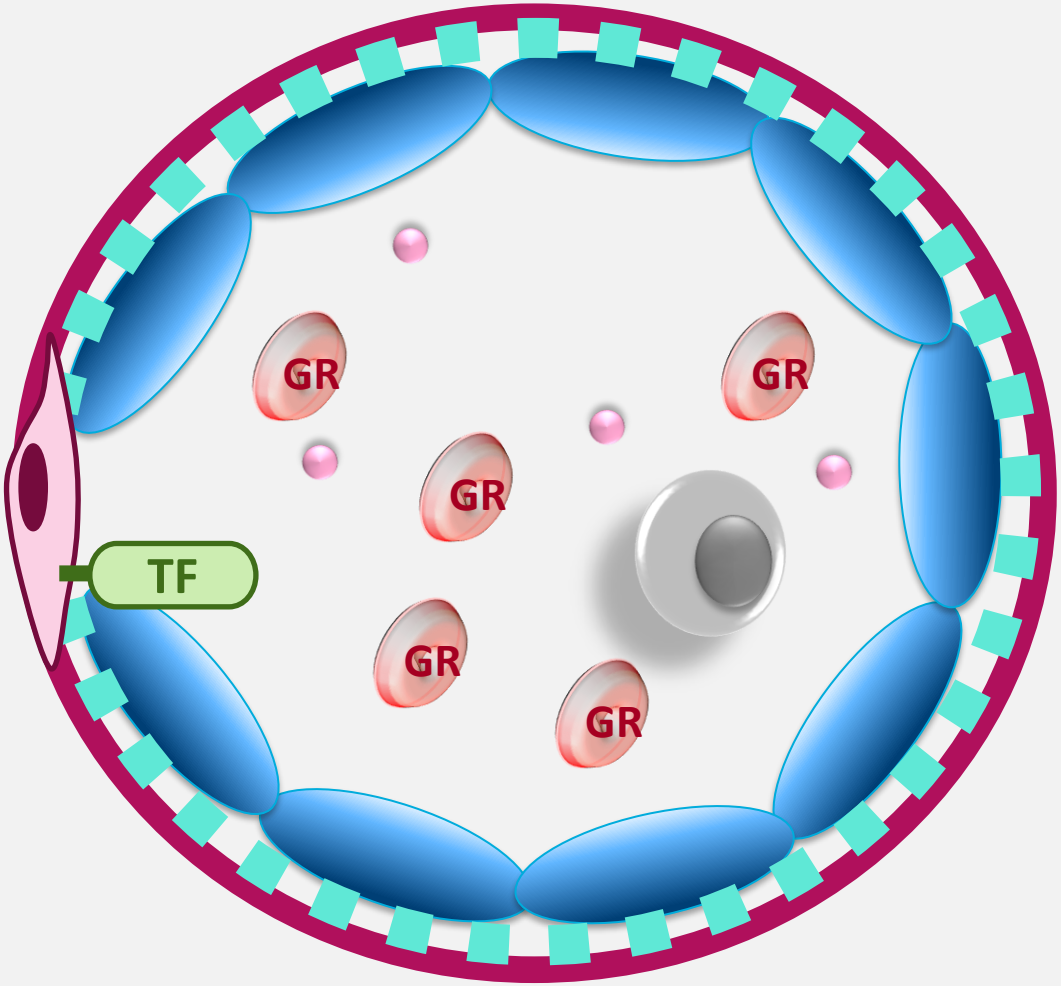


Break in endothelium continuity

Exposure of sub-endothelial cells to the blood flow

Coagulation

1) Initiation



Break in endothelium continuity

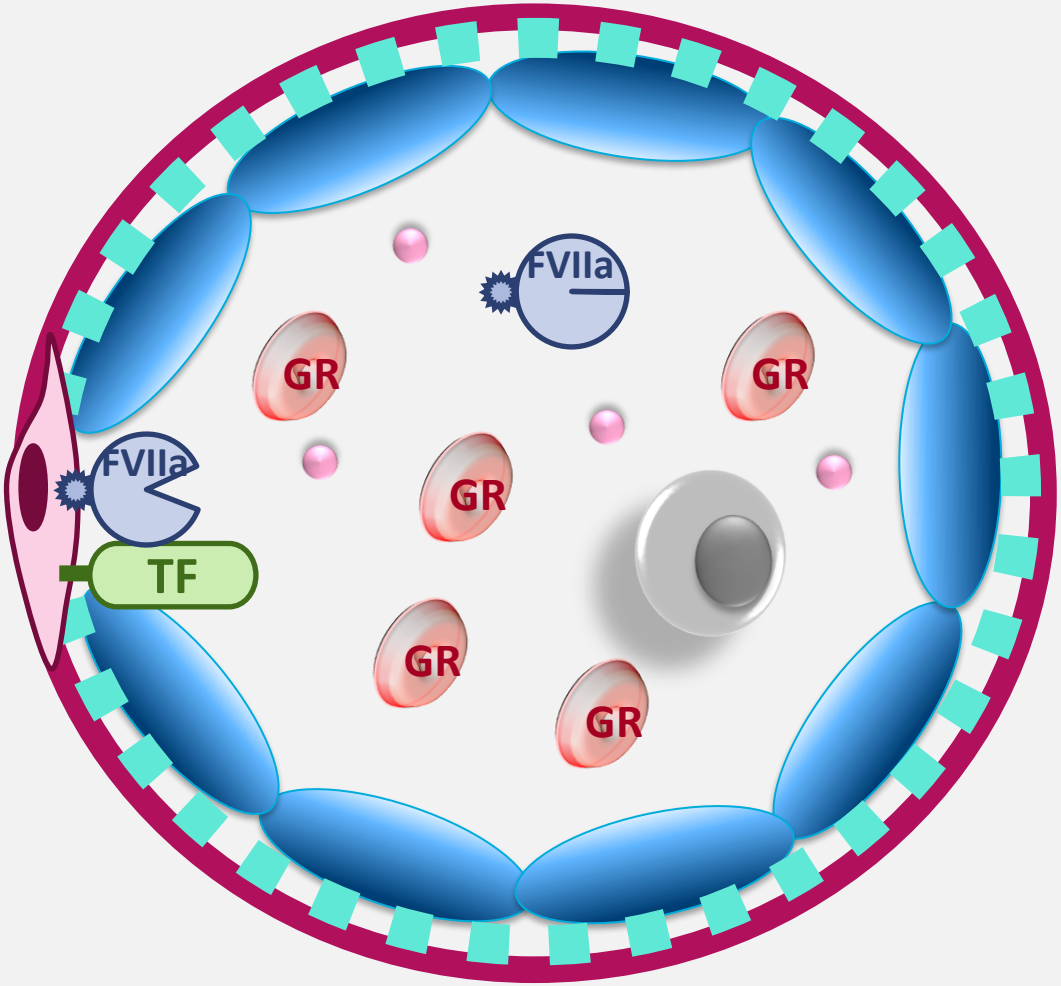
Exposure of sub-endothelial cells to the blood flow

Exposure of Tissue-Factor to the blood flow

Coagulation

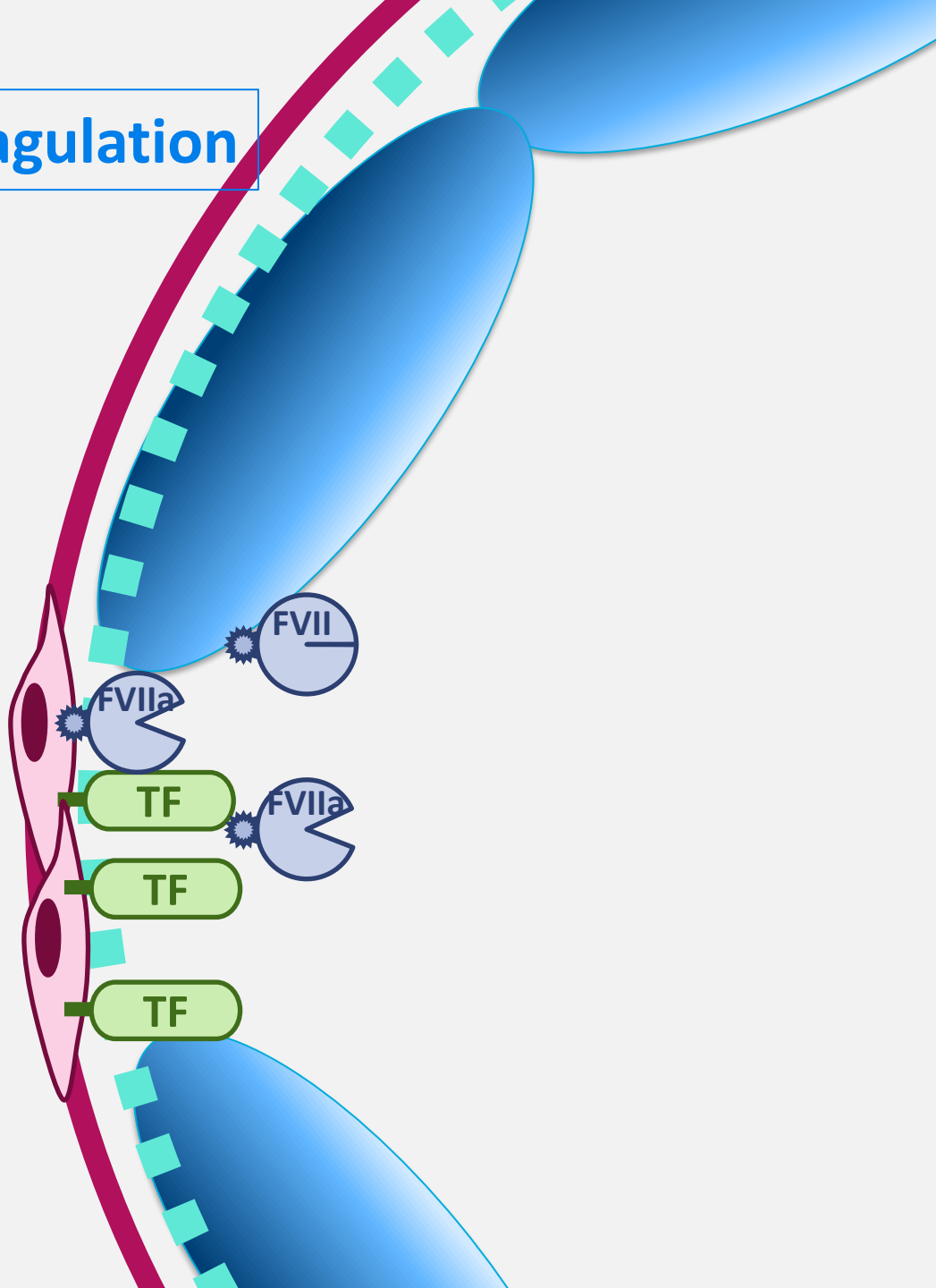
1) Initiation

Binding of FVIIa to TF triggers coagulation cascade.



Coagulation

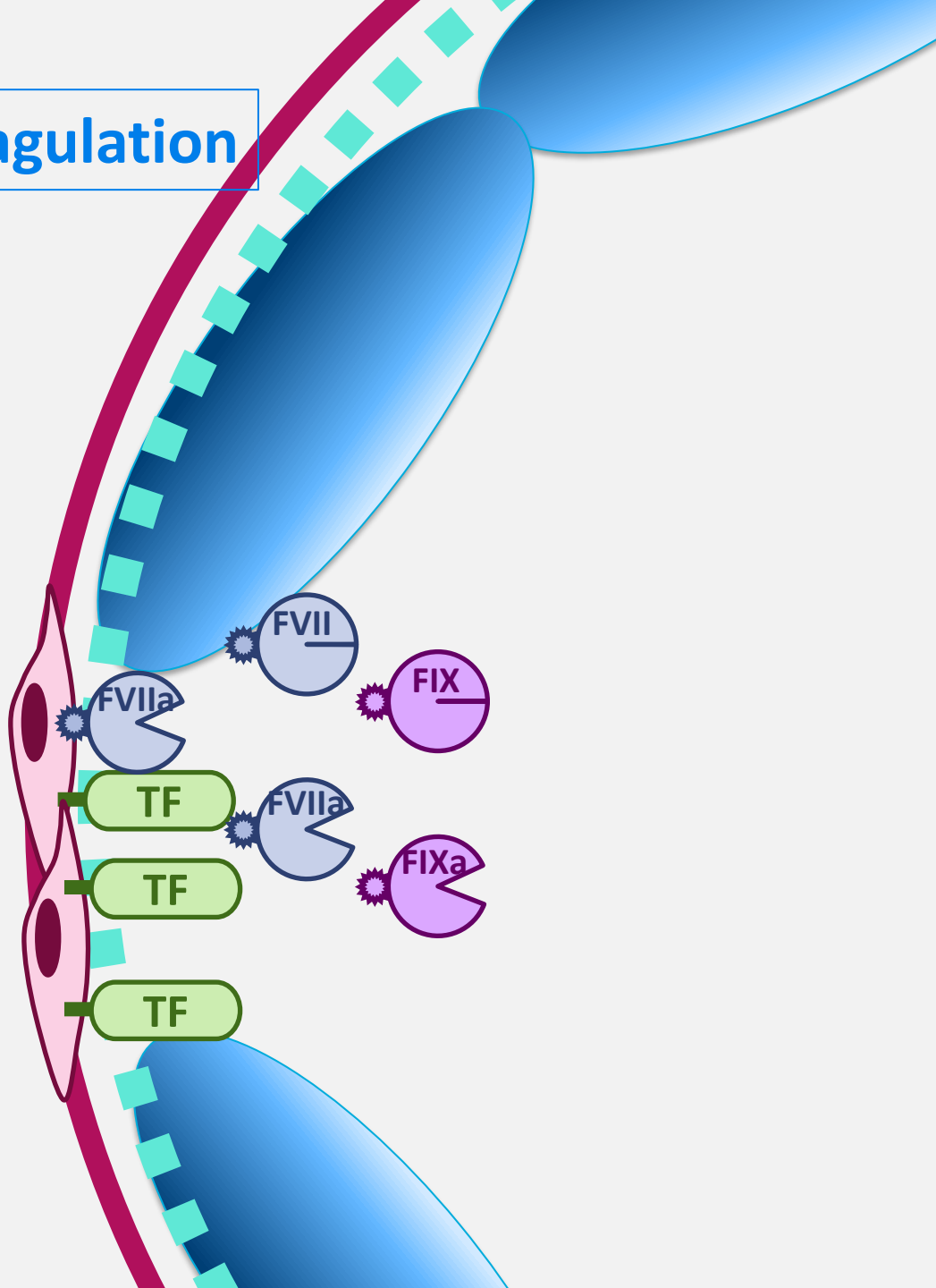
1) Initiation



⇨activation of FVII

Coagulation

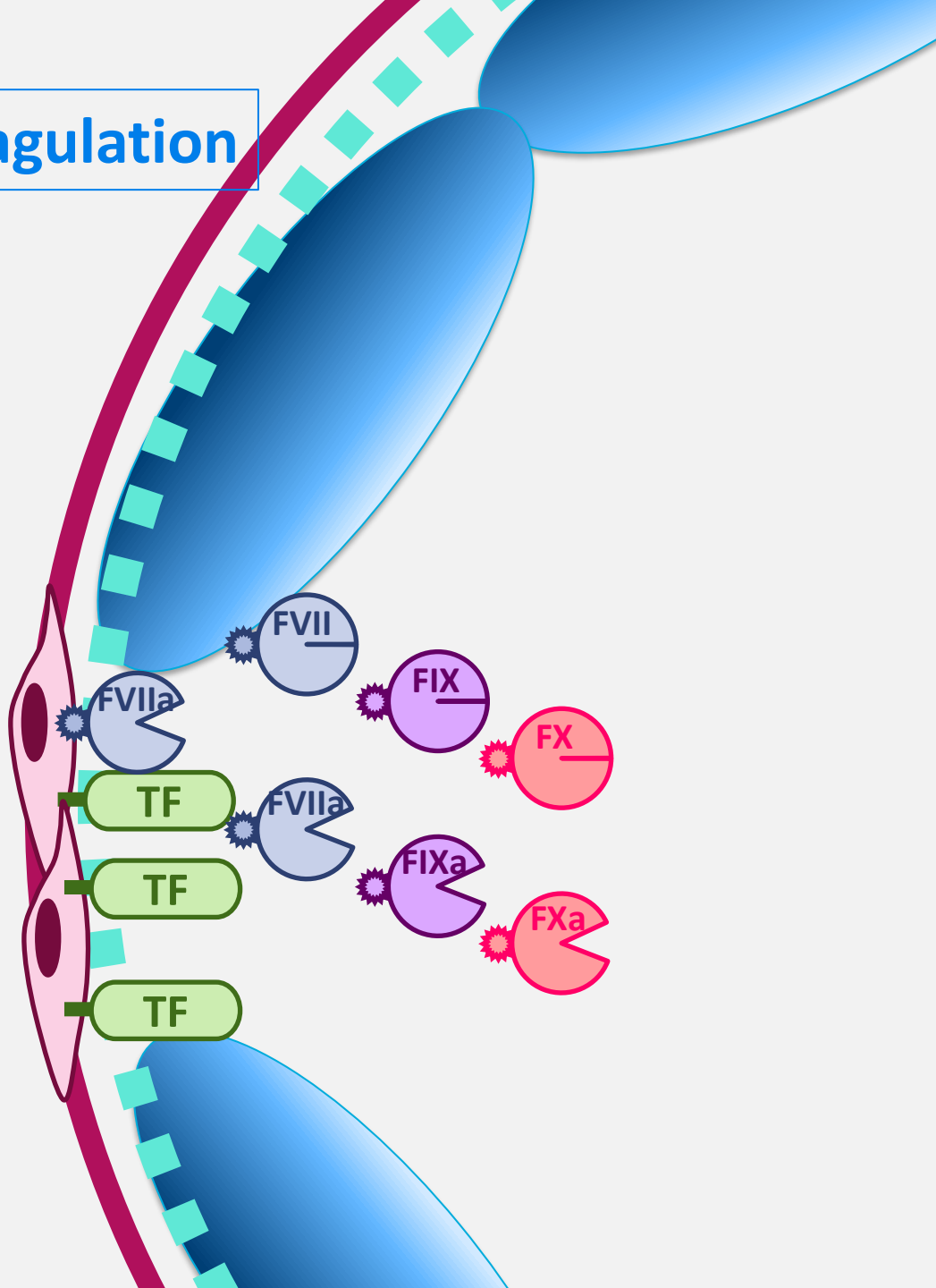
1) Initiation



⇨activation of FIX

Coagulation

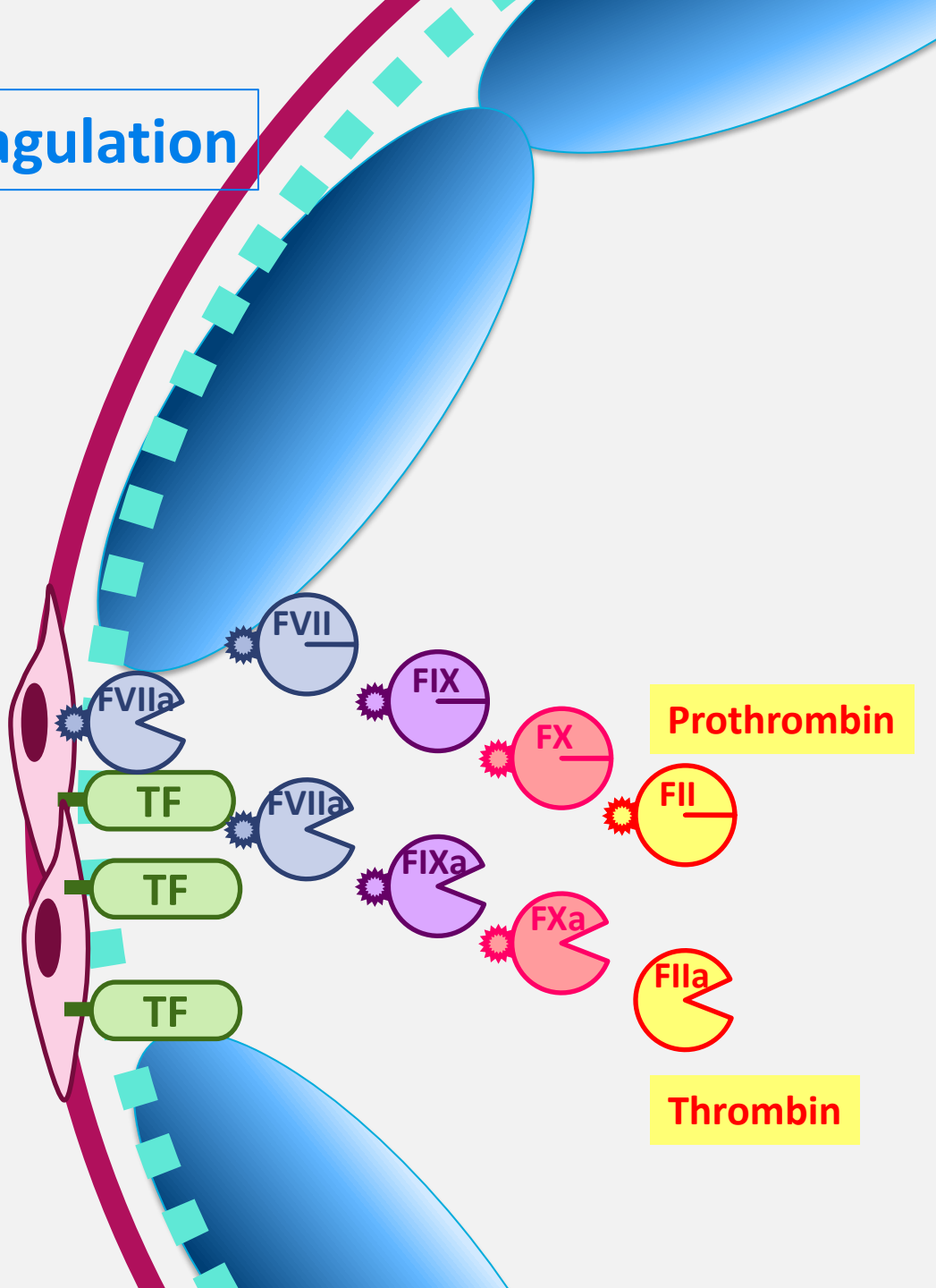
1) Initiation



⇨activation of FX

Coagulation

1) Initiation

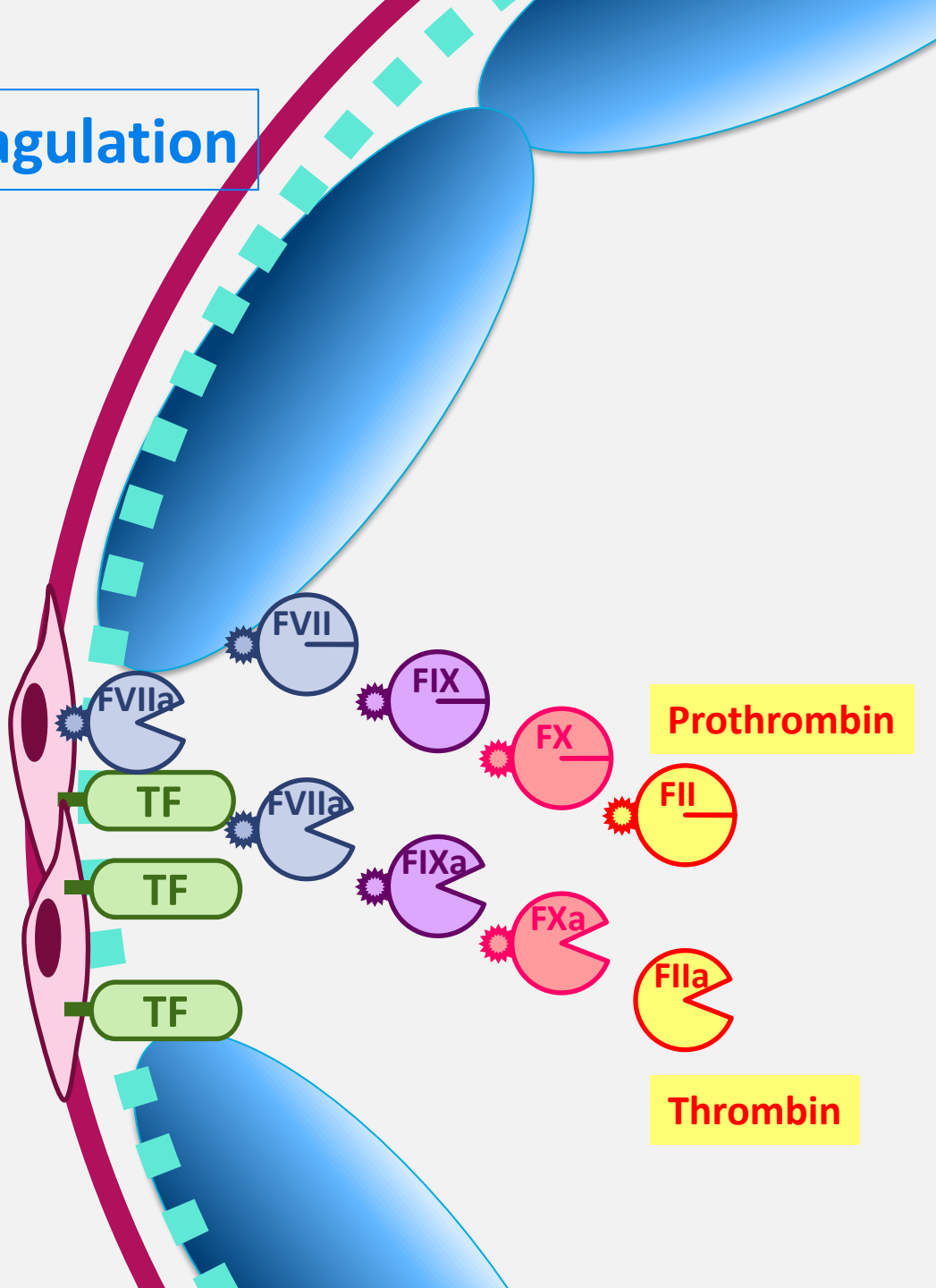


⇨activation of FII

Coagulation

1) Initiation

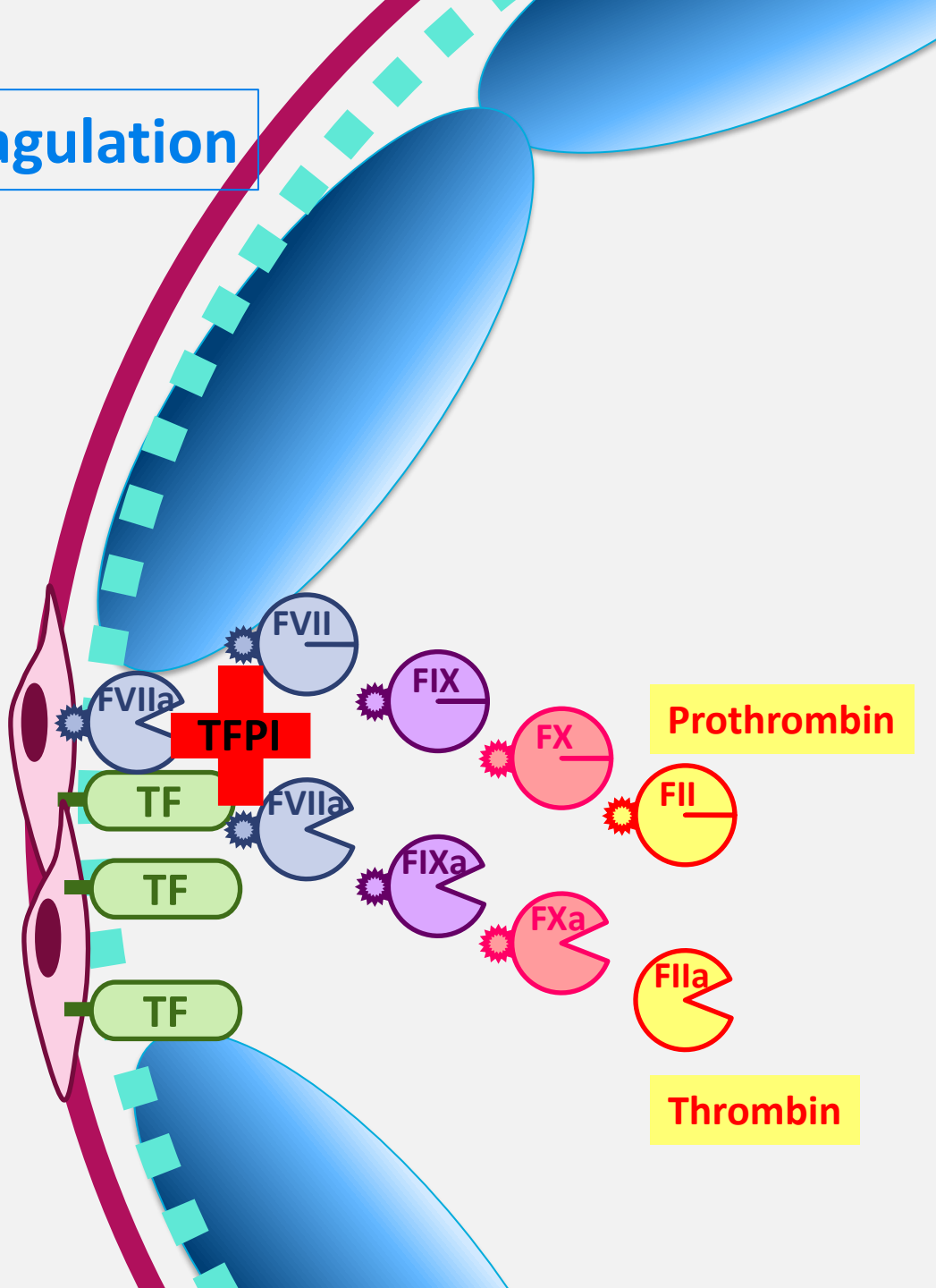
- Enzymatic reactions are slow
- Slow zymogens-enzymes conversion
- Enzymatic reactions are localized on the vicinity of TF exposure
- The first trace amounts of thrombin are produced !



Coagulation

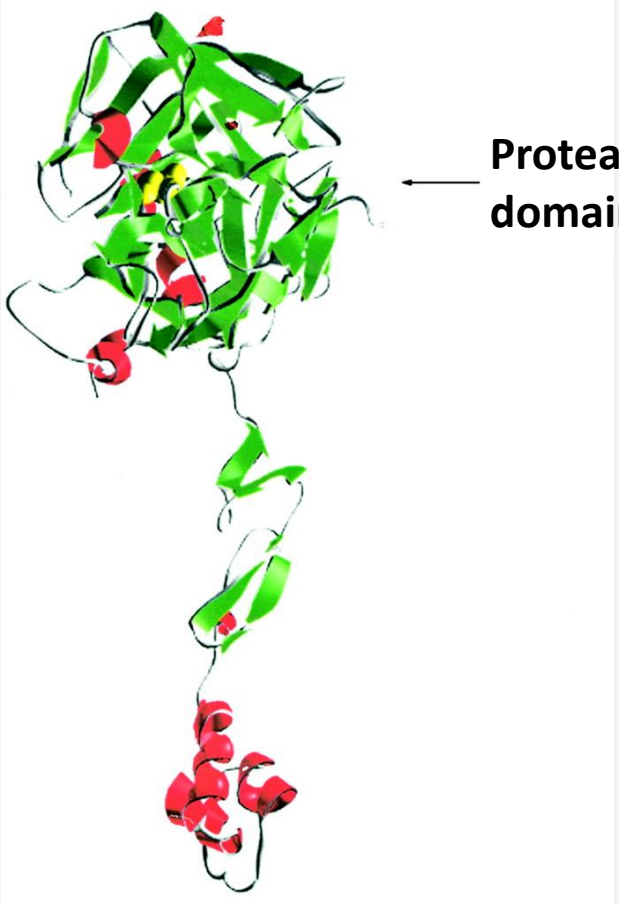
1) Initiation

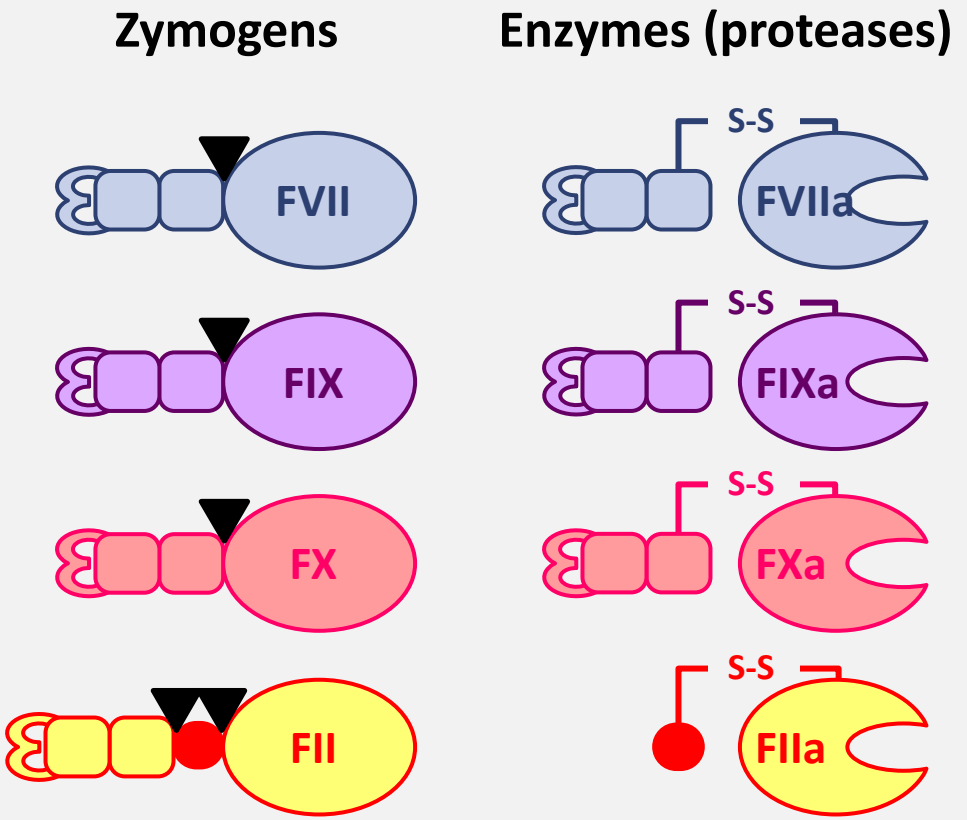
- Enzymatic reactions are slow
- Slow zymogens-enzymes conversion
- Enzymatic reactions are localized on the vicinity of TF exposure
- **The first trace amounts of thrombin are produced !**
- **Regulation by TFPI, slows down initiation.**



Coagulation enzymes

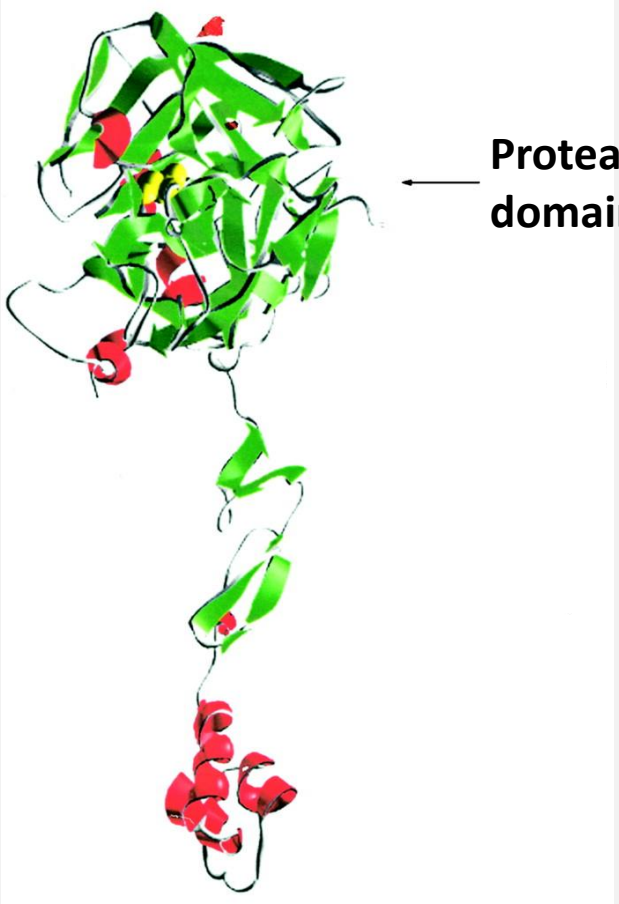
- All synthetized by hepatocytes as inactive precursors (= Zymogens)

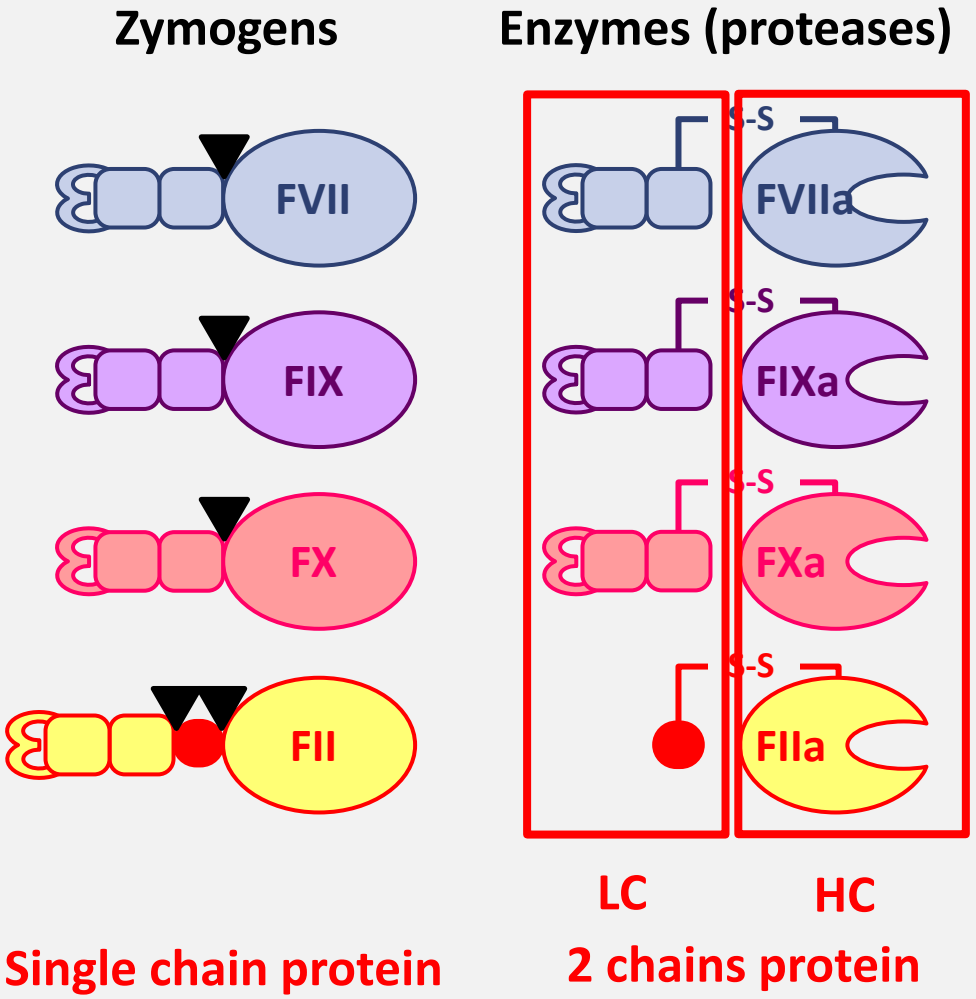




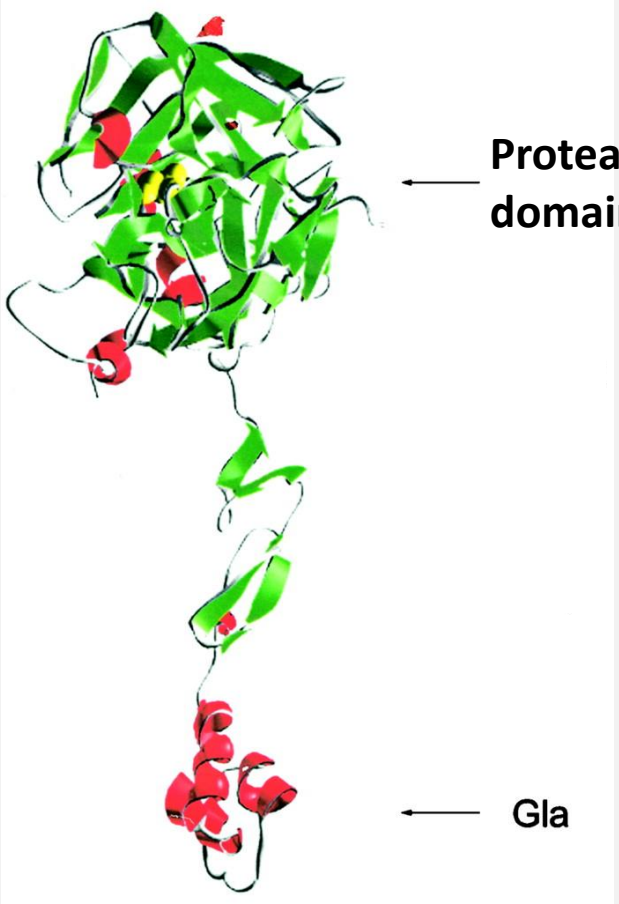
- Converted into enzyme by proteolytic cleavage
- All have similar structural organization

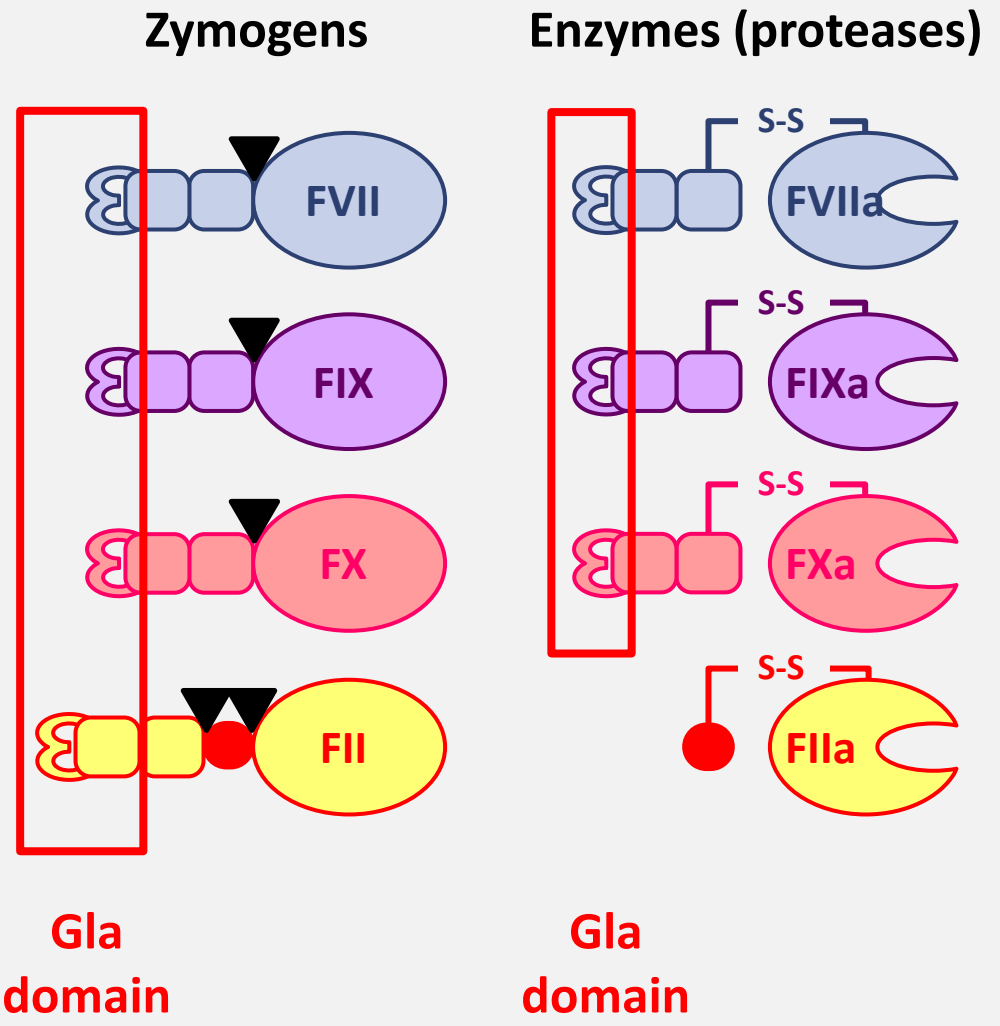
Coagulation enzymes





Coagulation enzymes



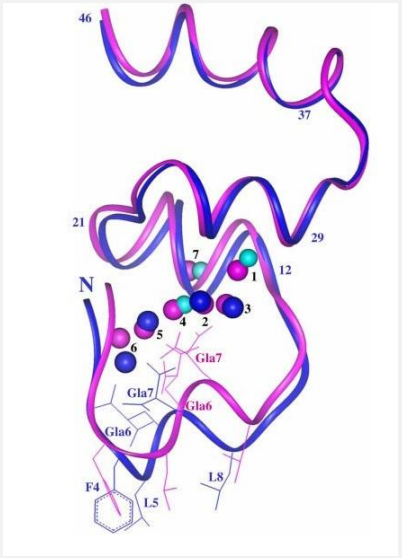


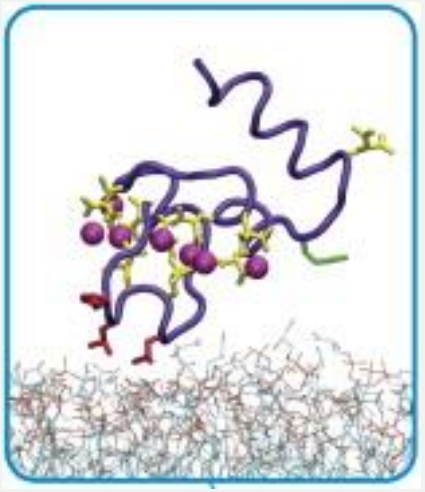
Gla domain

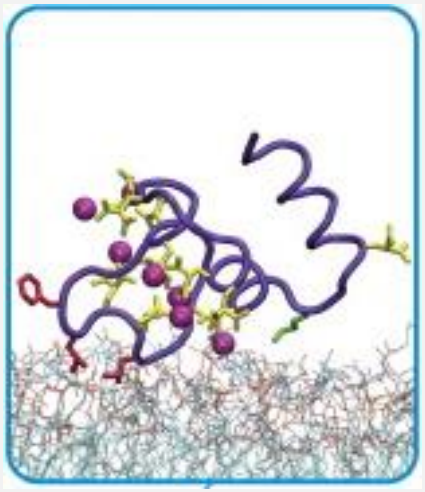
- Post-translational modification of **Glu** residues into **Gla** residues (gamma-carboxy glutamic acid)
- Post-translational modification requires the presence of **vitamin K (vitamin K-dependent factors)**

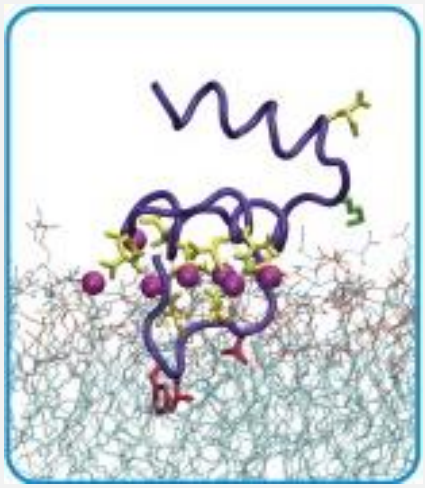
Target of AVK

- **Ω-loop conformation** upon folding in the presence of **Ca^{2+} ions** ⇨ **hydrophobic** residues exposure
- **Hight affinity** for **lipid bilayer** of the cell membrane surface
- **Gla domain allows clotting factors anchoring on the cell membrane surface**

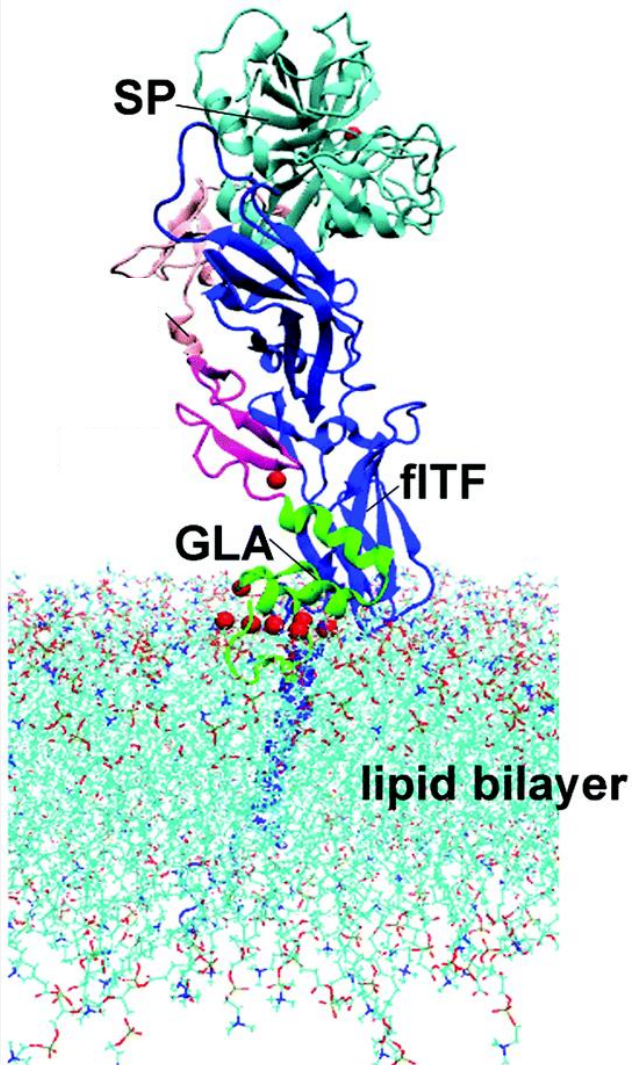








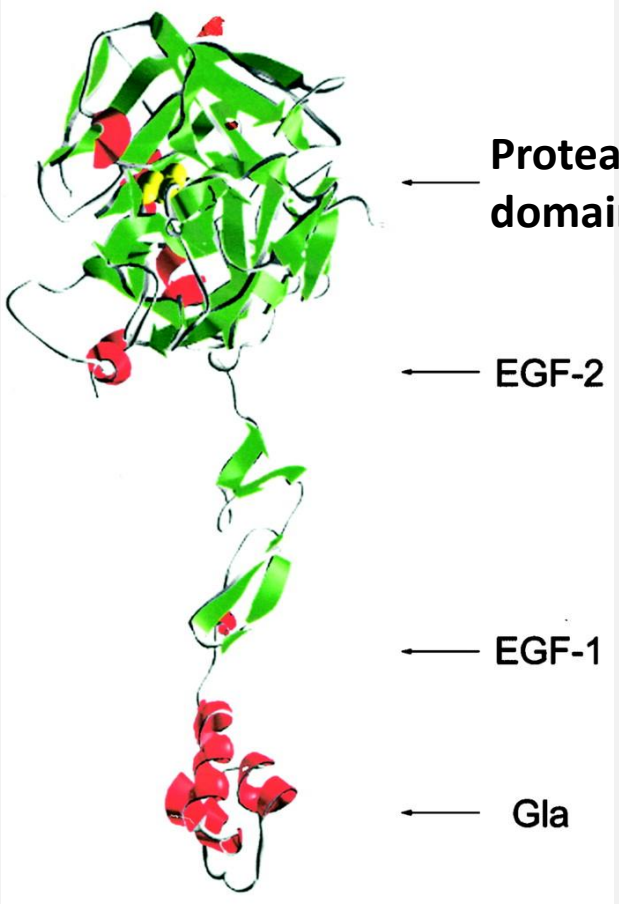
Gla domain

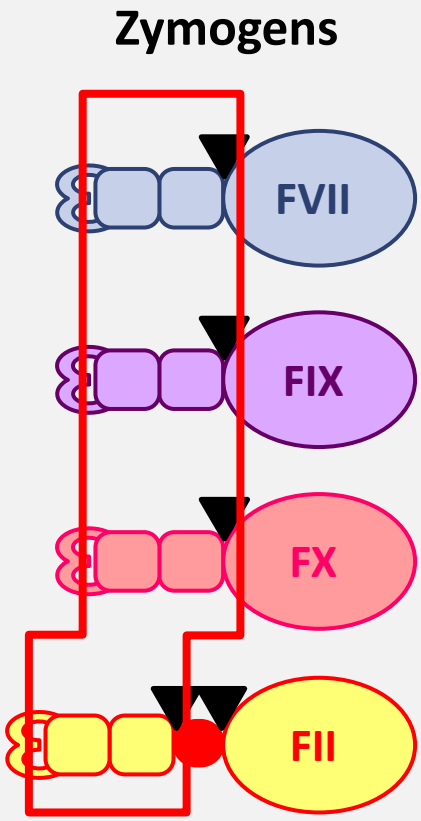


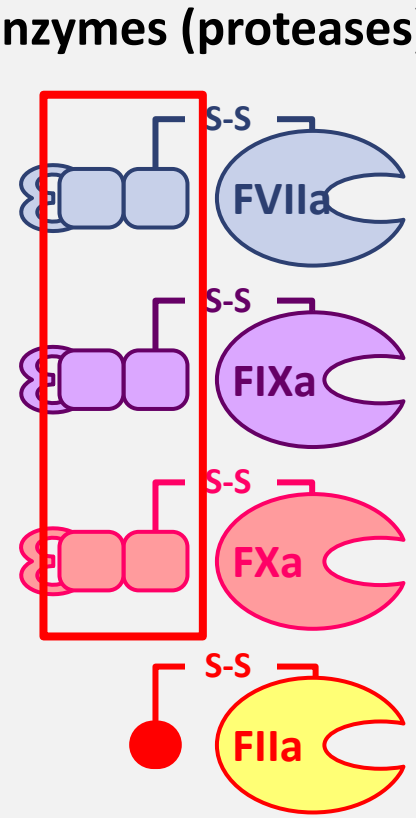
Exemple of TF/FVIIa complex

- ⇨ Tight Binding of clotting factor to the cell mbr surfaces
- ⇨ Repartition of clotting factors in a 2D space (mbr surface) instead of in a 3D space (plasma)
- ⇨ Increase local concentrations
- ⇨ Increase enzymatic reactions rate
- ⇨ Circumscribe enzymatic activity on the vicinity of mbr surface (prevent dissemination)

Coagulation enzymes



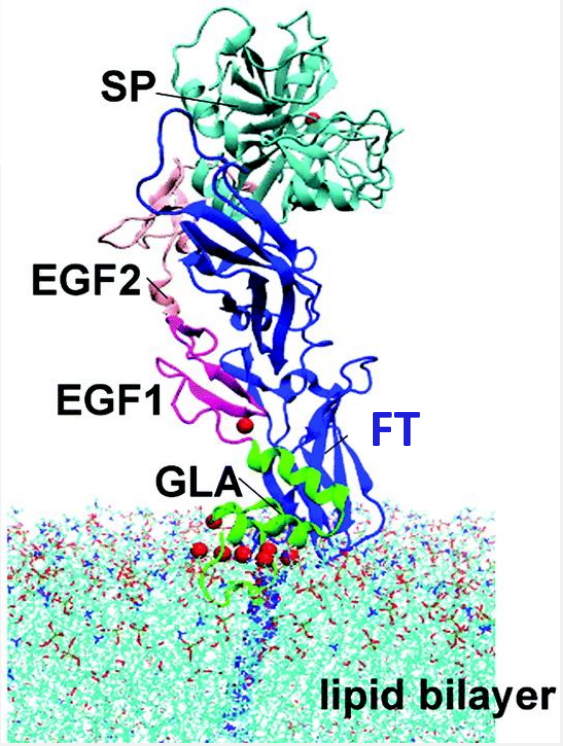




EGF-like domains

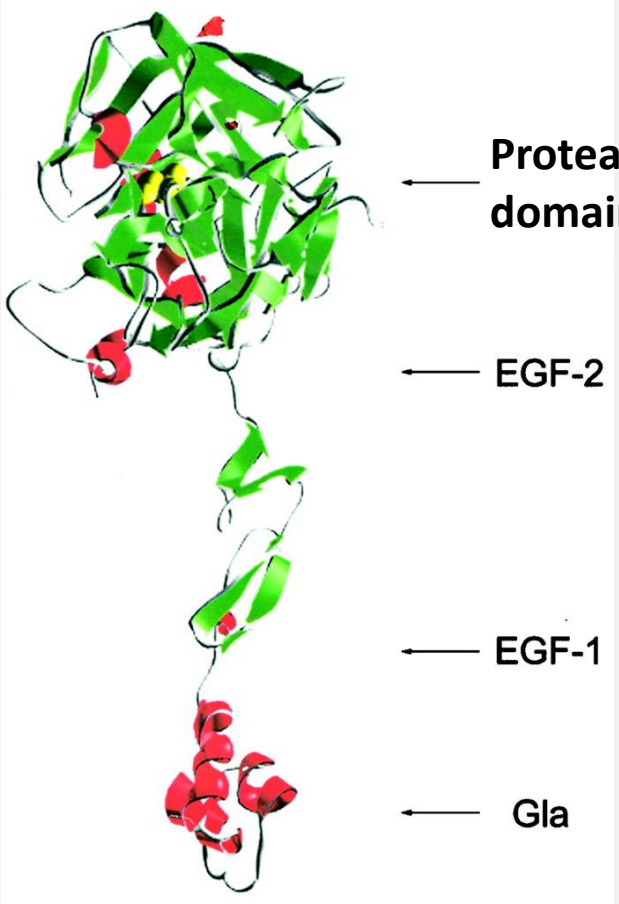
Flexible N- and C-term regions

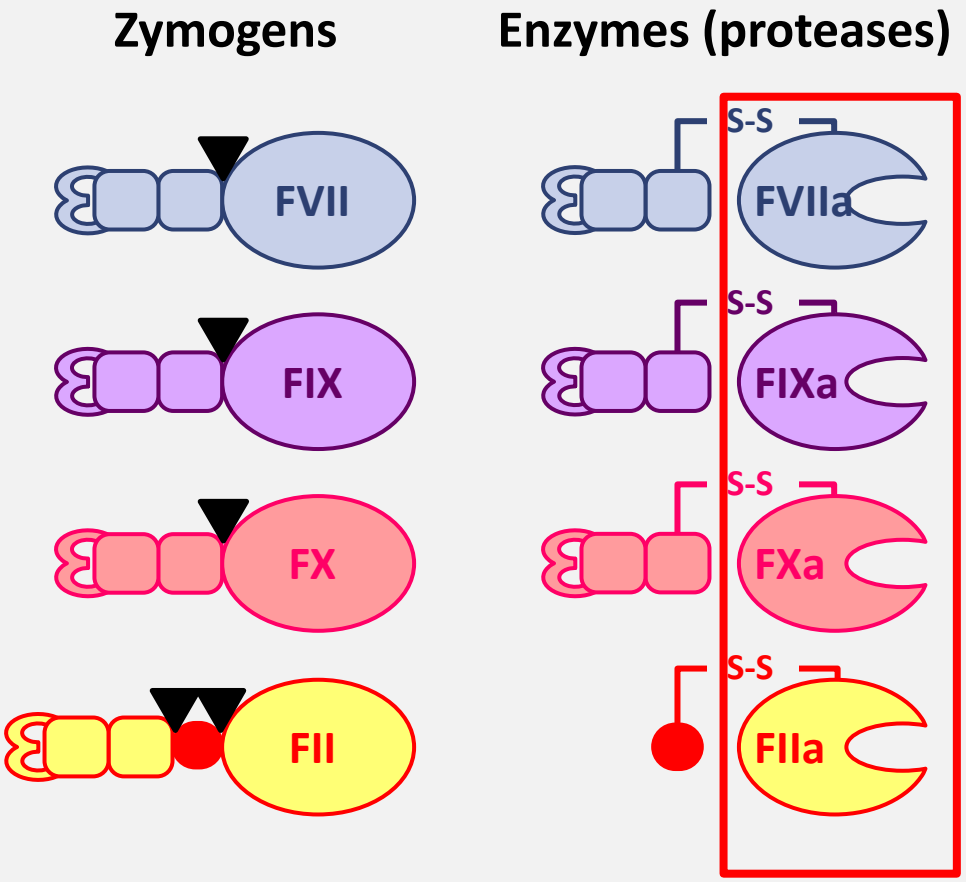
Close-packed core



- By homology with Epidermal Growth Factor (EGF)
- **Spacer** between **Gla** domain and **catalytic** domain
- **β-sheet** folding with **flexible** N- and C-term regions
- **Allow optimal exposure of the catalytic domain, distant from the cell mbr surface**

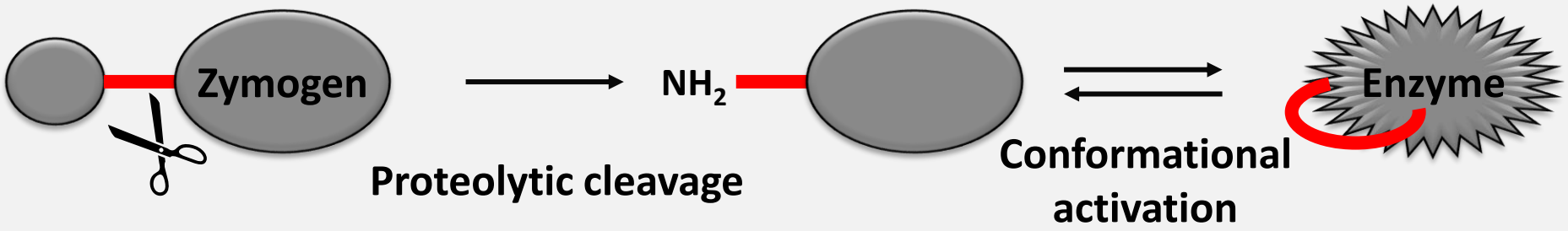
Coagulation enzymes

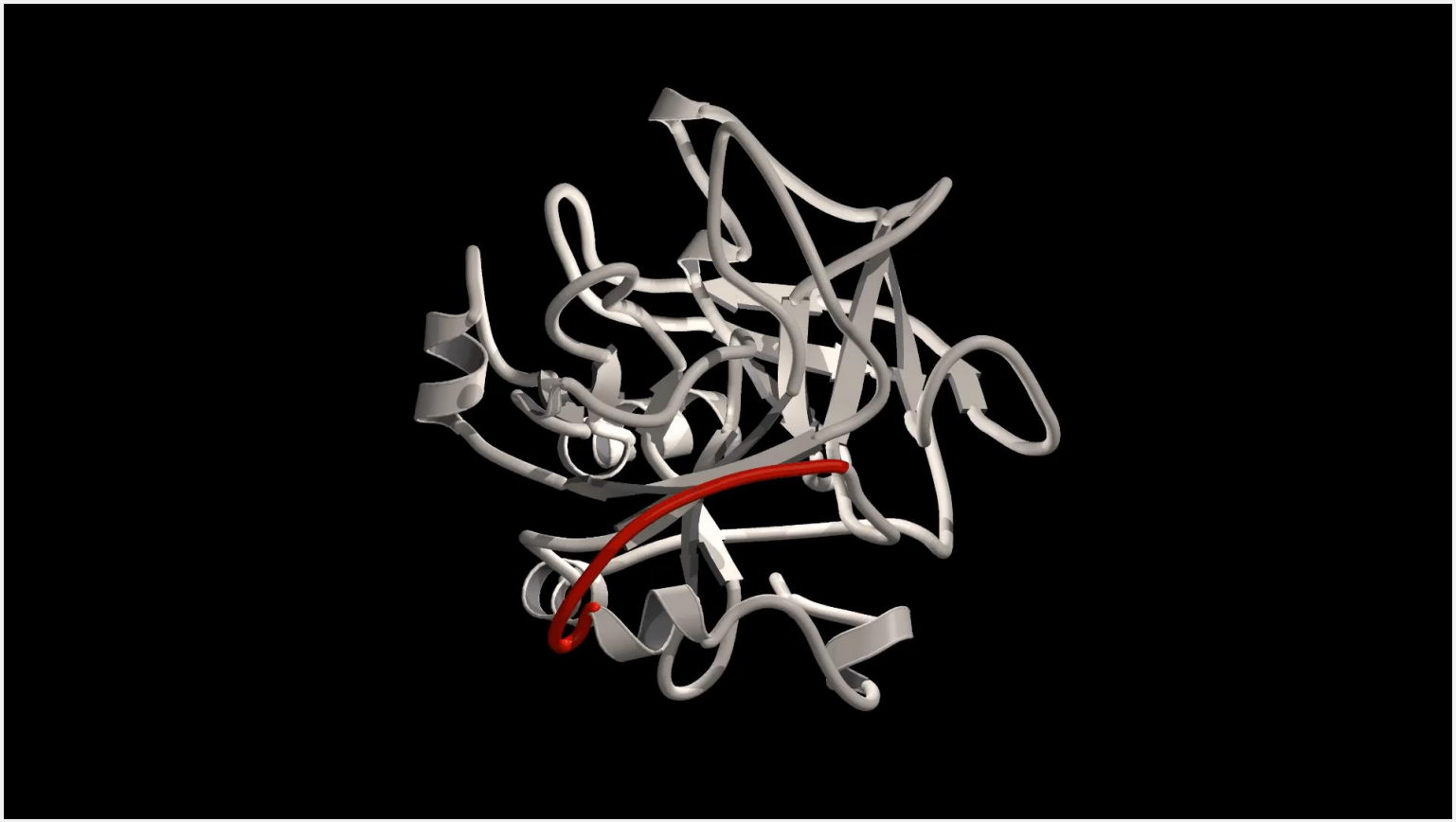




Heavy chain = catalytic domain

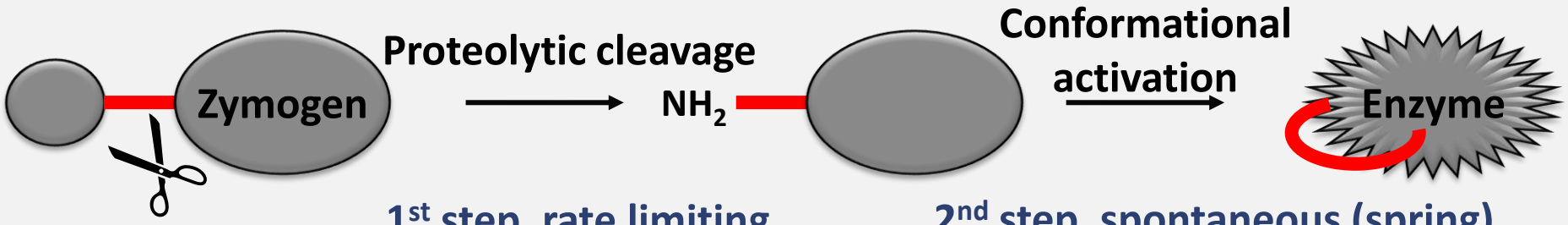
Molecular mechanism of Catalytic domain activation





Molecular mechanism of FVII activation

General mechanism

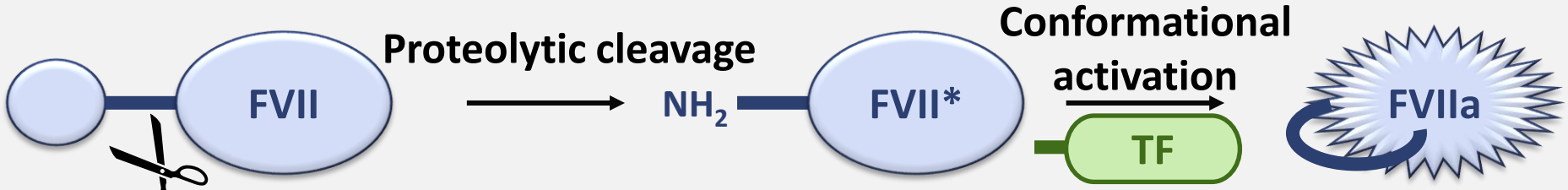


1st step, rate limiting
Requires the presence of a catalyst

2nd step, spontaneous (spring)

Factor VII activation mechanism

Forme circulante
« Préactivée-Inactive »



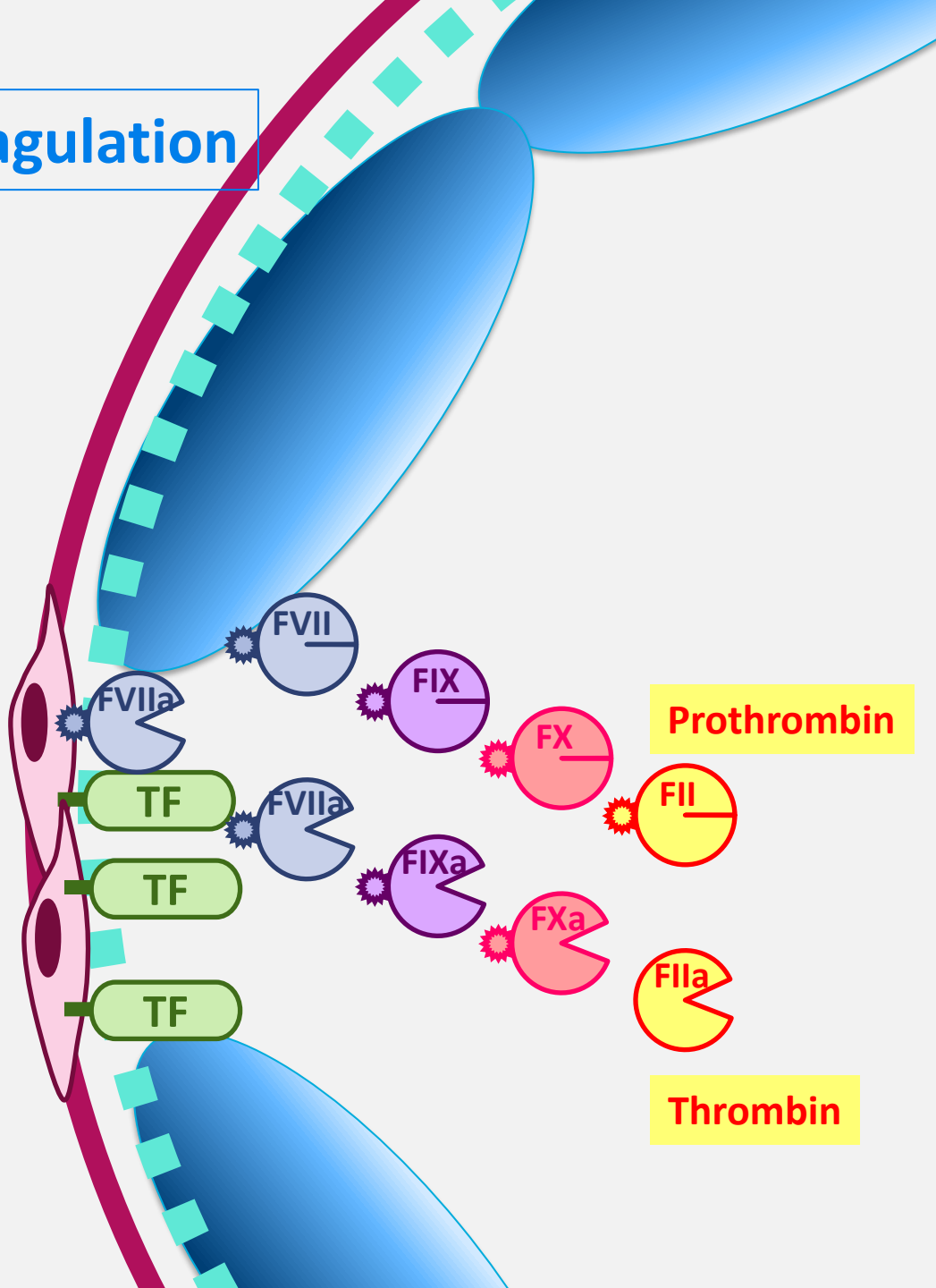
1st step, occurs spontaneously
"auto-activation"

2nd step, rate limiting
Requires the presence of the cofactor

Coagulation

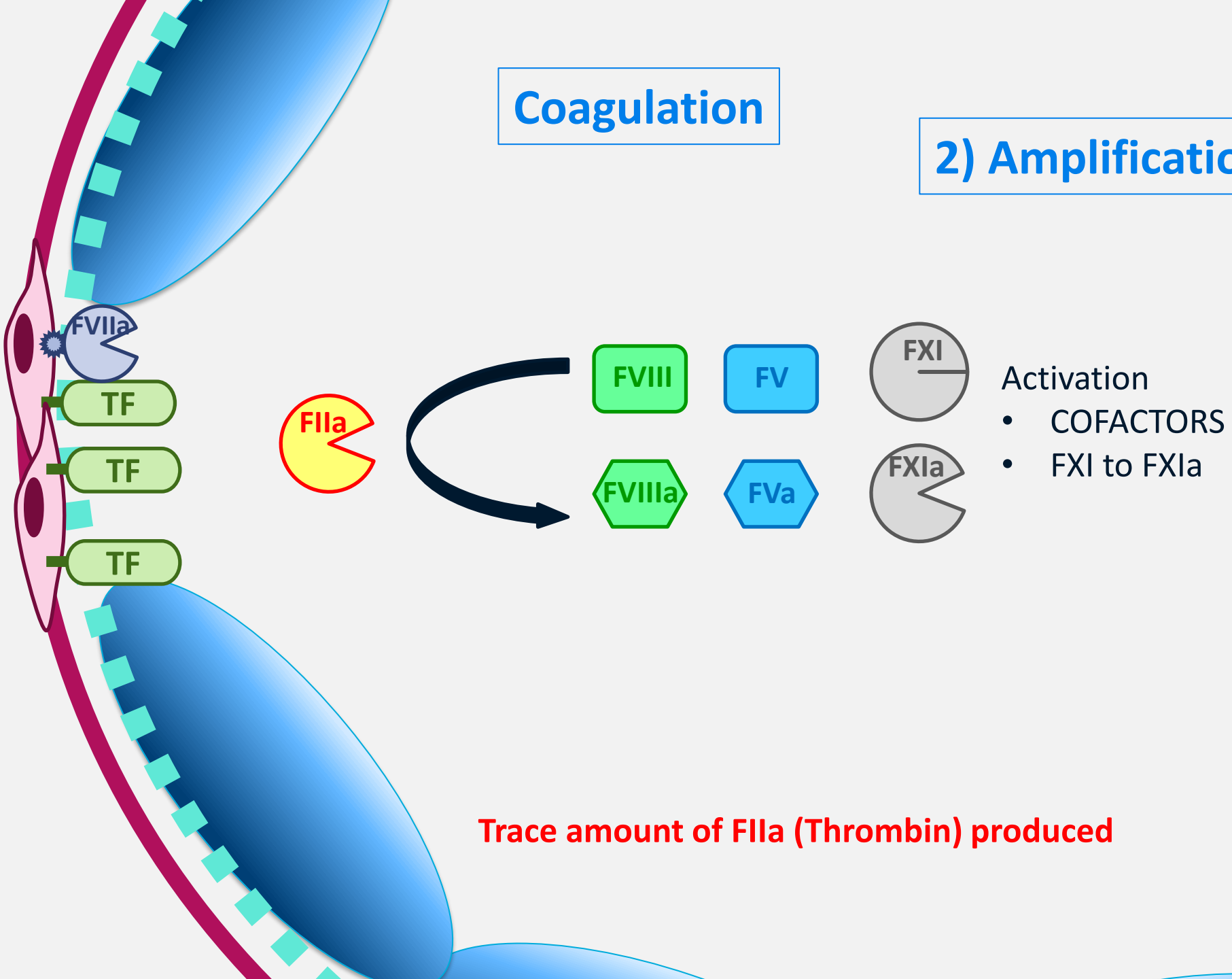
1) Initiation

- Enzymatic reactions are slow
- Slow zymogens-enzymes conversion
- Enzymatic reactions are localized on the vicinity of TF exposure
- The first trace amounts of thrombin are produced !



Coagulation

2) Amplification



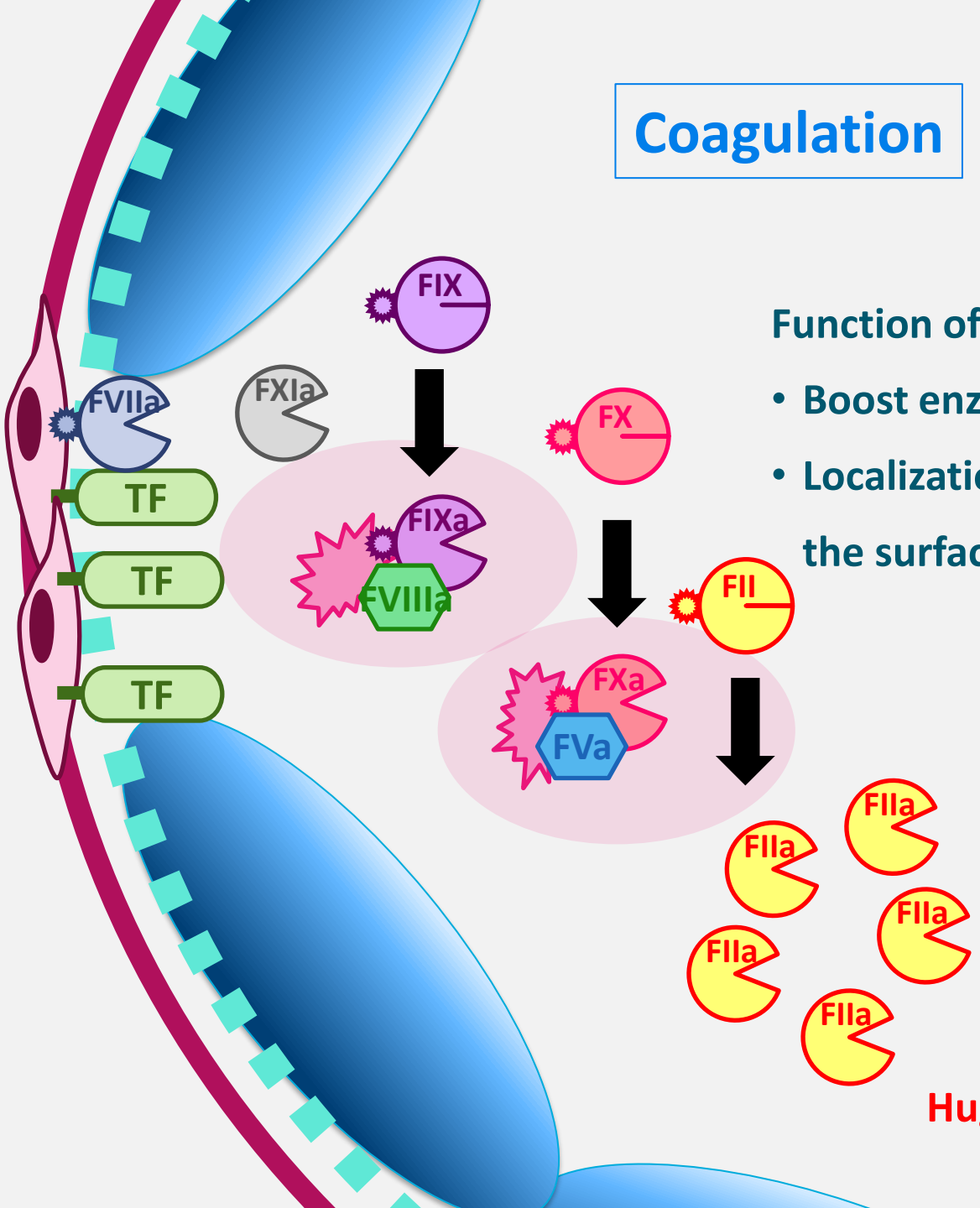
Activation

- COFACTORS
- FXI to FXIa

Trace amount of FIIa (Thrombin) produced

Coagulation

2) Amplification

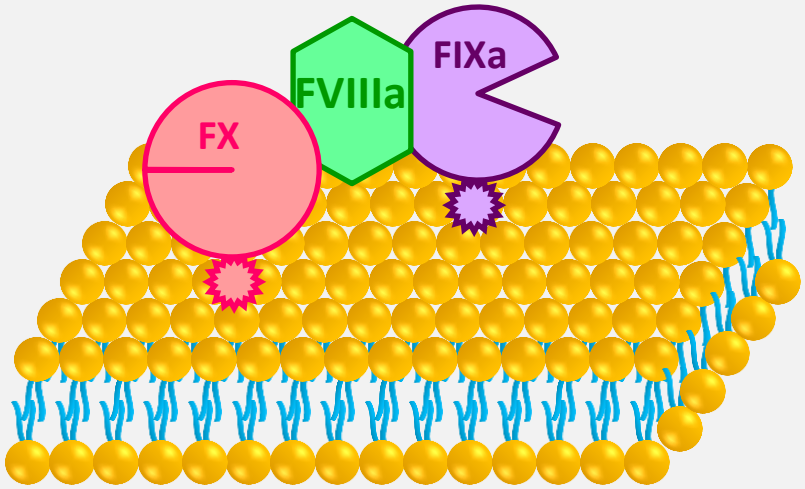


Function of enzyme-cofactor complexes

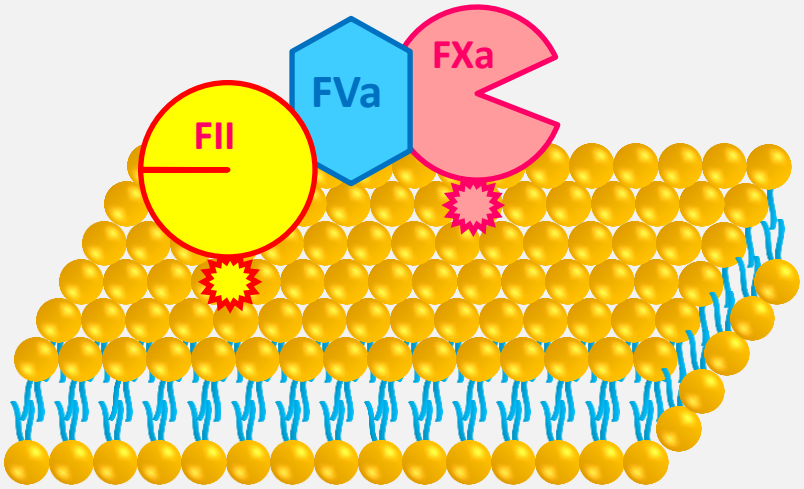
- **Boost enzyme reactivity**
- **Localization of procoagulant activity on the surface of activated platelets**

Huge amount of FIIa produced

Enzyme-cofactor complexes



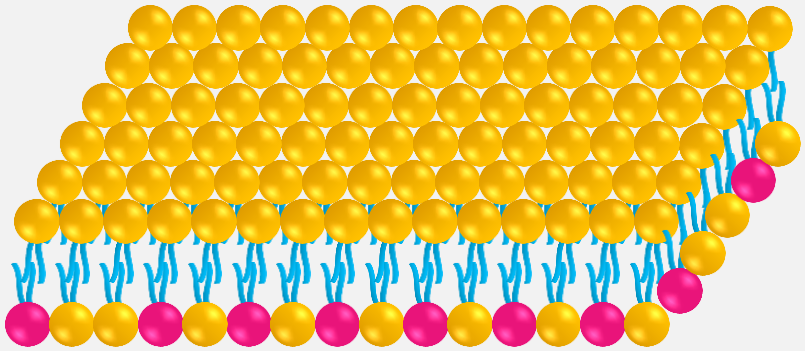
"TENASE" complex



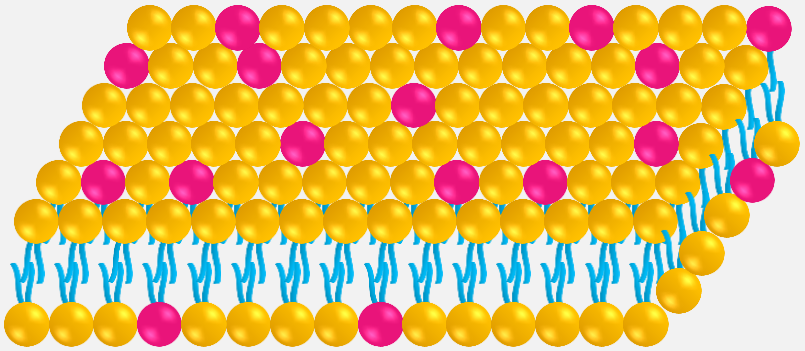
"PROTHROMBINASE" complex

- **Assembling on the cell mbr surface (Gla domain, requires Ca^{2+})**
- **Repartition of clotting factors in a 2D space (mbr surface) instead of in a 3D space (plasma)**
- **Increase local concentrations ⇨ Increase enzymatic reactions rate**
- **Circumscribe enzymatic activity on the vicinity of mbr surface (prevent dissemination)**

Membrane composition



Resting cells
PC in outer layer
PS in inner layer
Energy consuming process



Activated or damaged cells
Randomized repartition of PS and PC

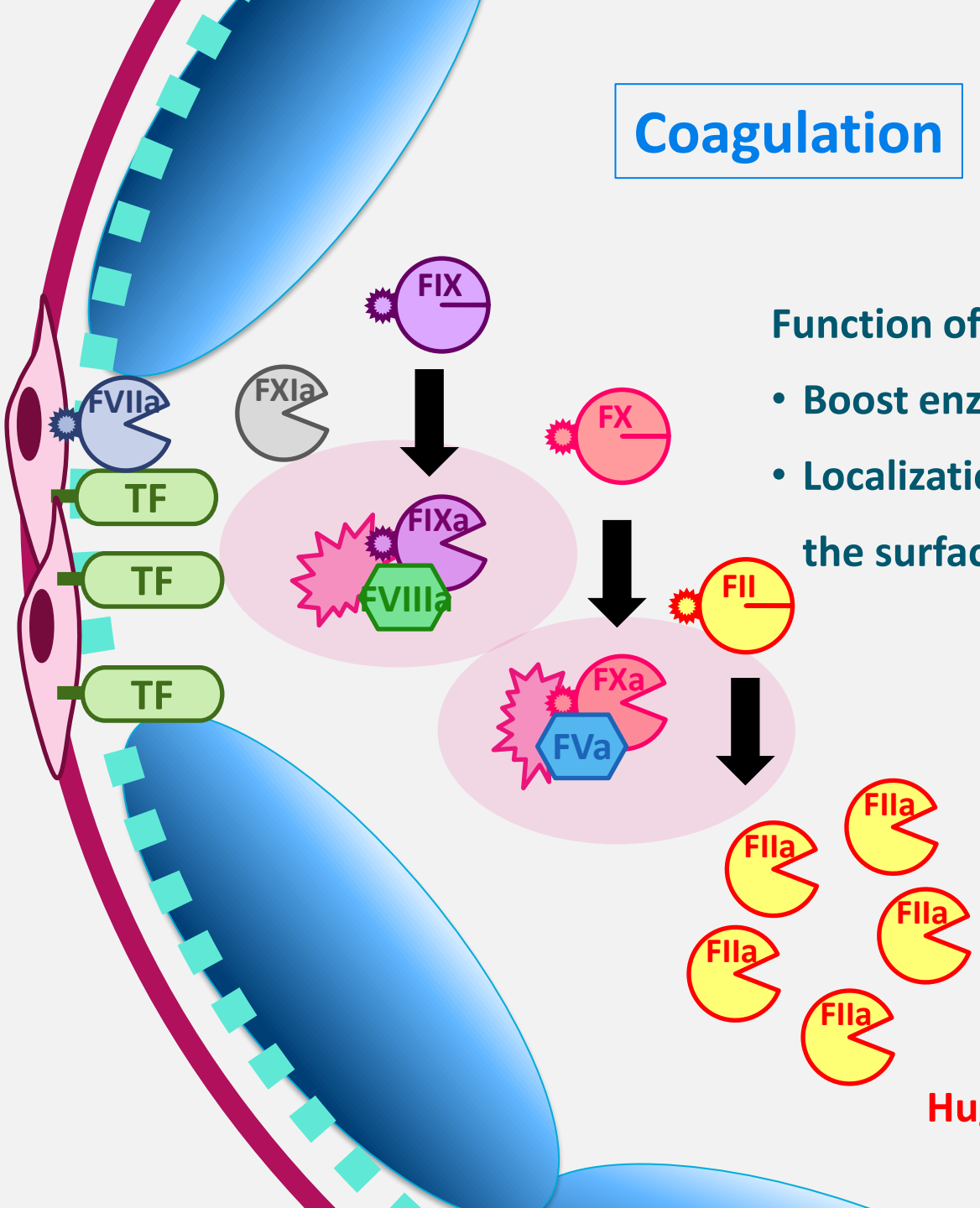
- **PS exposure on the extracellular face is mandatory for enzyme-cofactor complexes formation**
- **Complexes formation only on activated platelets or on damaged endothelial cells**

Phosphatidylcholine = **Neutral PL**

Phosphatidylserine = **Acidic PL**

Coagulation

2) Amplification



Function of enzyme-cofactor complexes

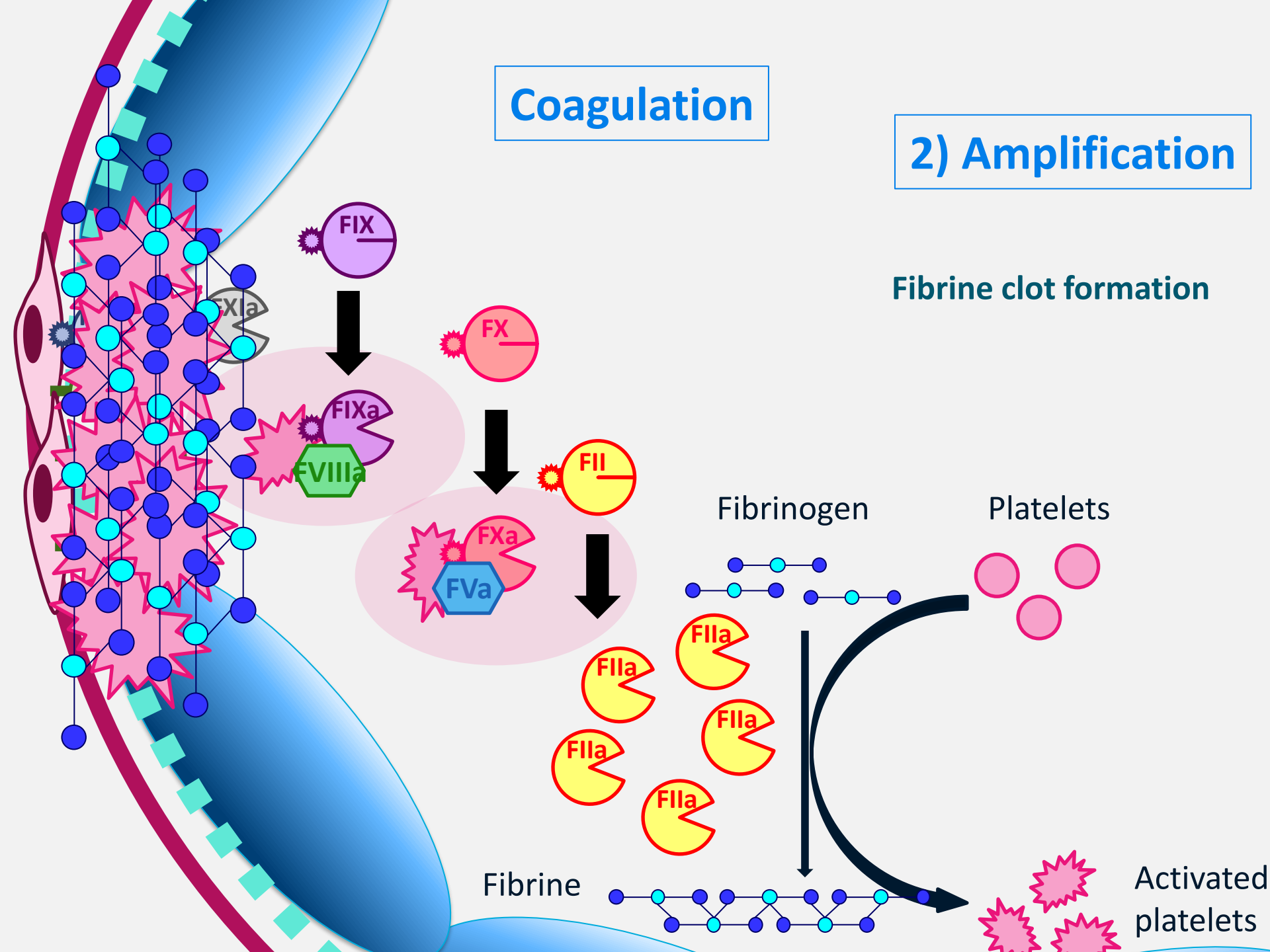
- **Boost enzyme reactivity**
- **Localization of procoagulant activity on the surface of activated platelets**

Huge amount of FIIa produced

Coagulation

2) Amplification

Fibrine clot formation

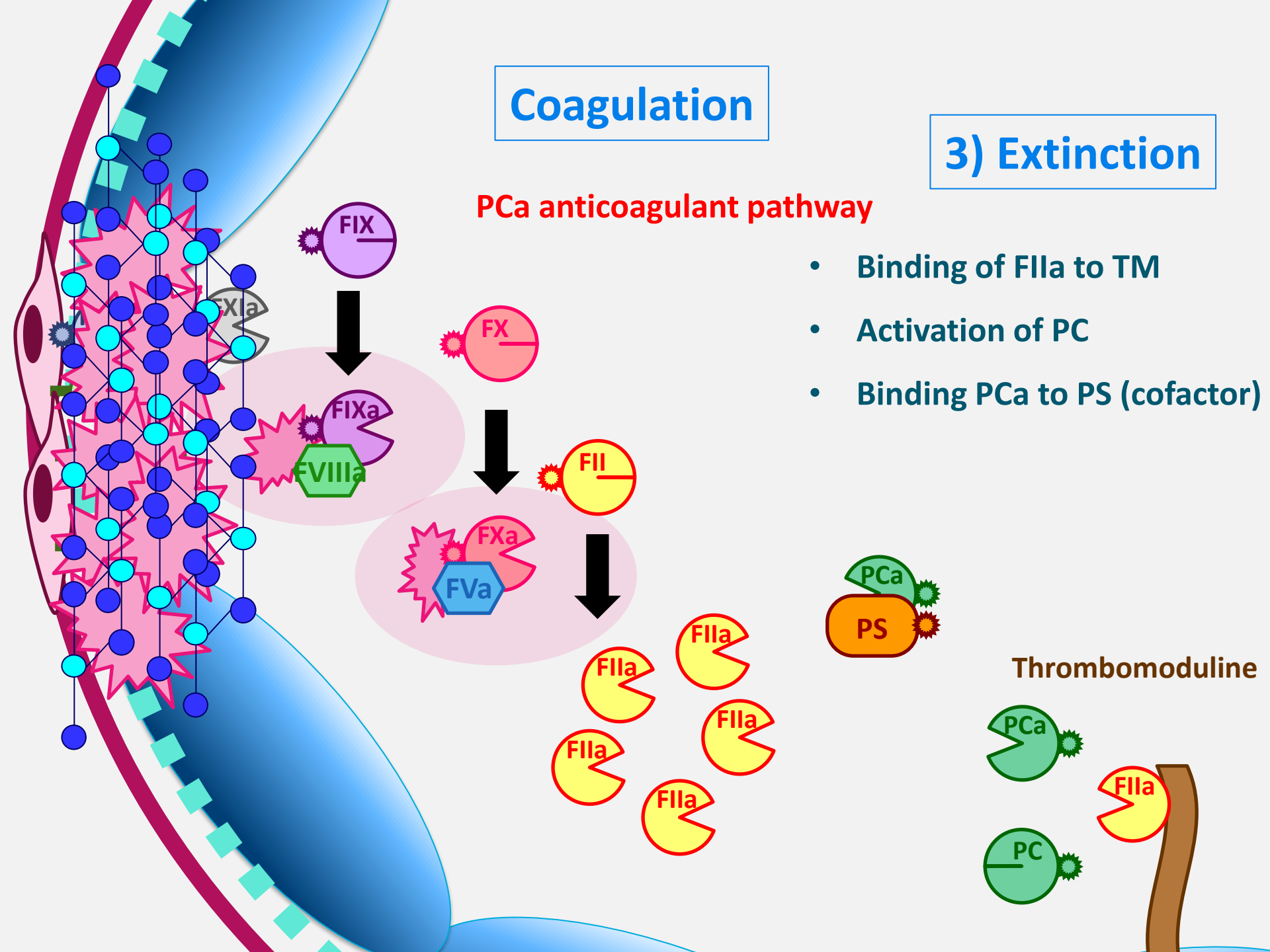


Coagulation

3) Extinction

PCa anticoagulant pathway

- Binding of FIIa to TM
- Activation of PC
- Binding PCa to PS (cofactor)

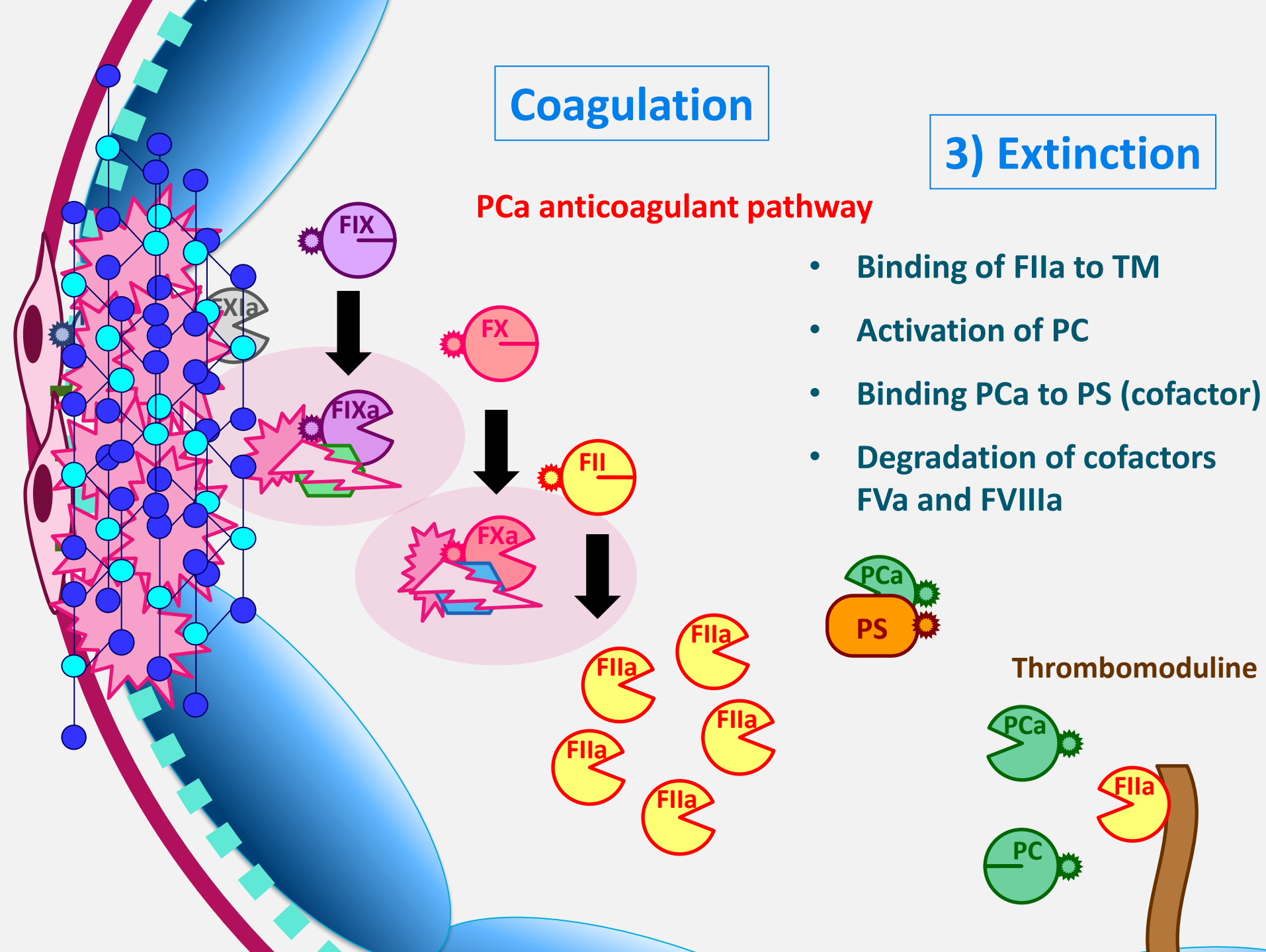


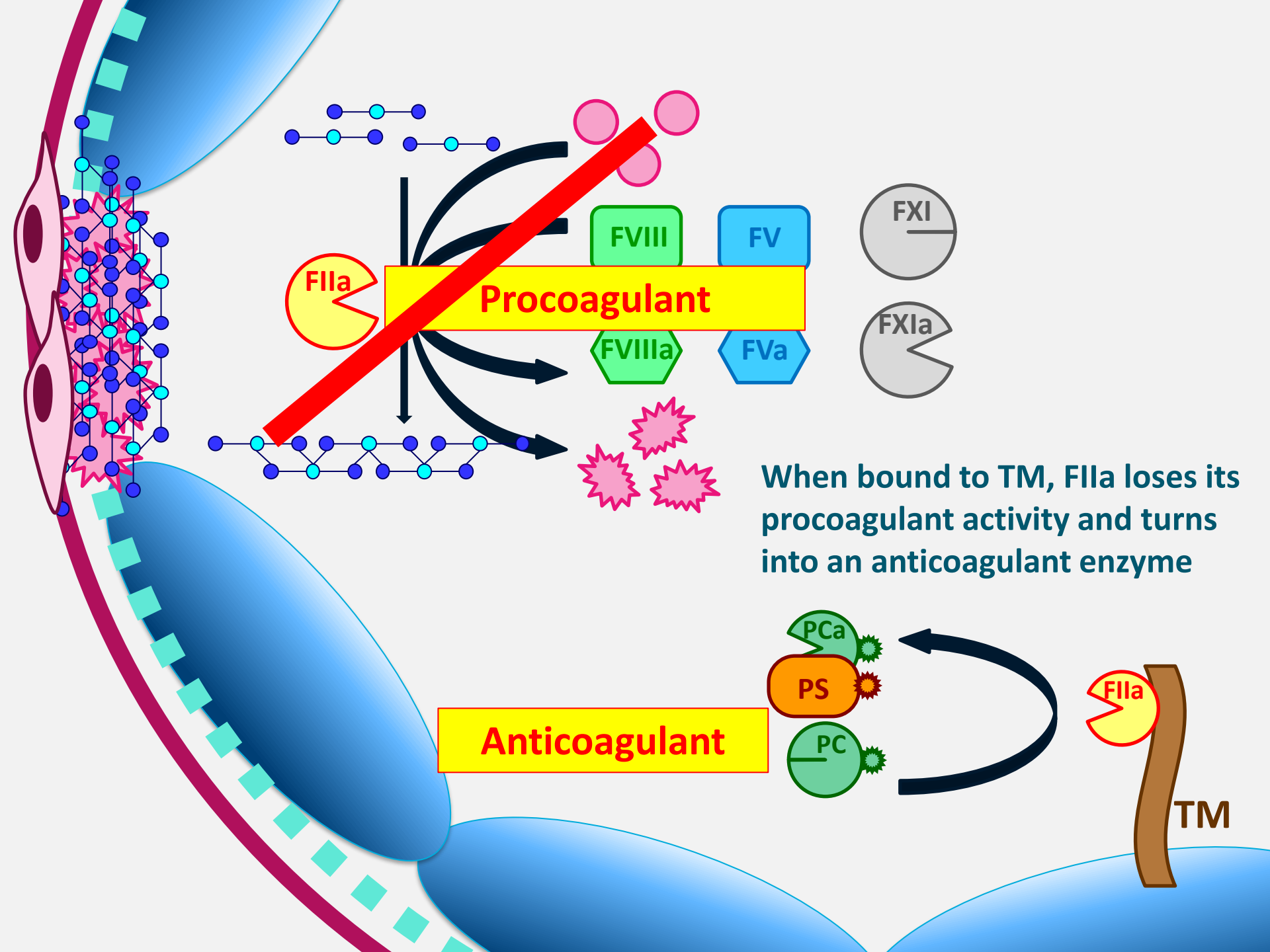
Coagulation

3) Extinction

PCa anticoagulant pathway

- Binding of FIIa to TM
- Activation of PC
- Binding PCa to PS (cofactor)
- Degradation of cofactors FVa and FVIIIa



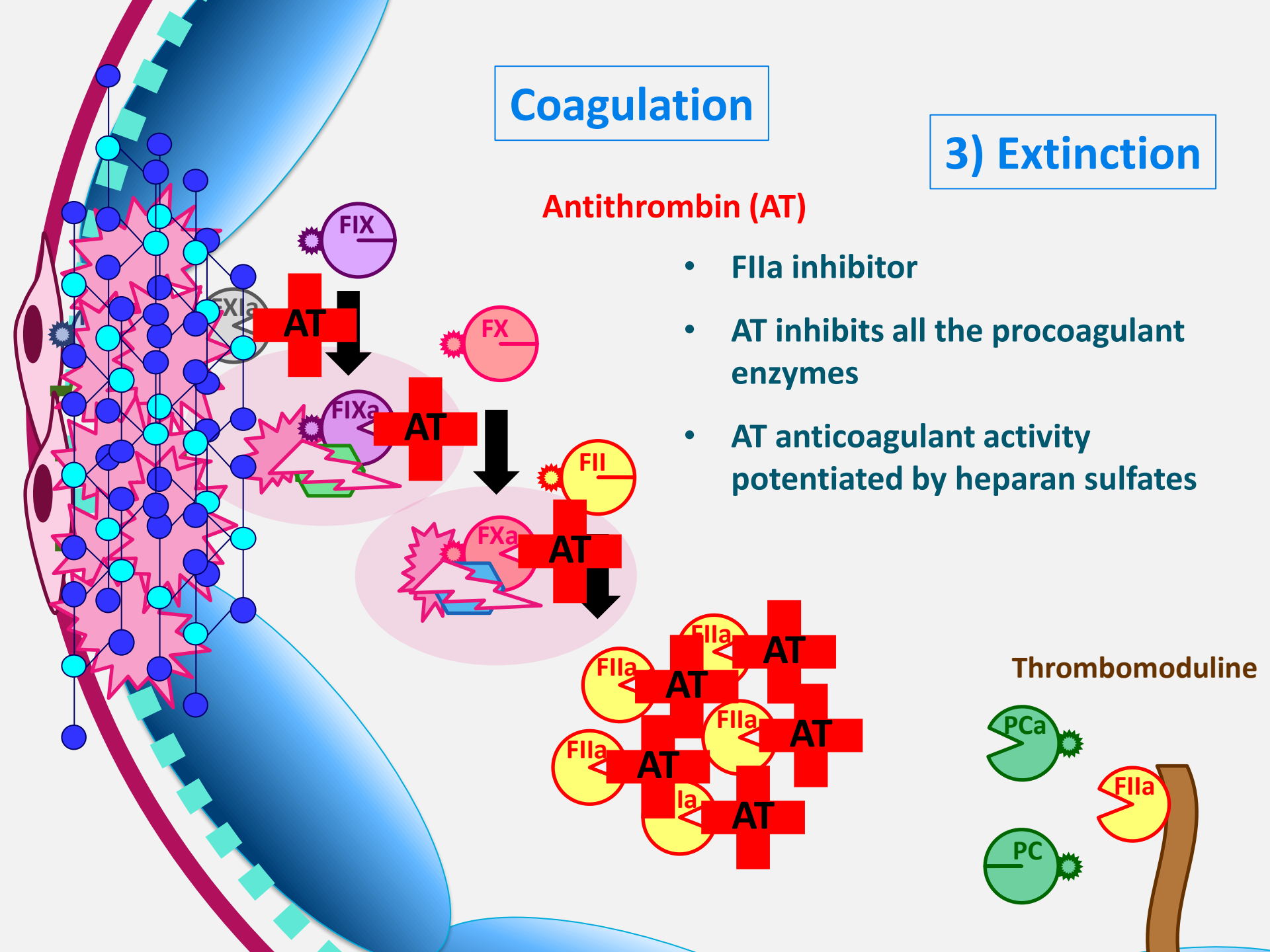

FVIII
FV
FXI
FIIa
Procoagulant
FXIa
FVIIIa
FVa
When bound to TM, FIIa loses its procoagulant activity and turns into an anticoagulant enzyme
PCa
PS
PC
FIIa
Anticoagulant
TM

Coagulation

3) Extinction

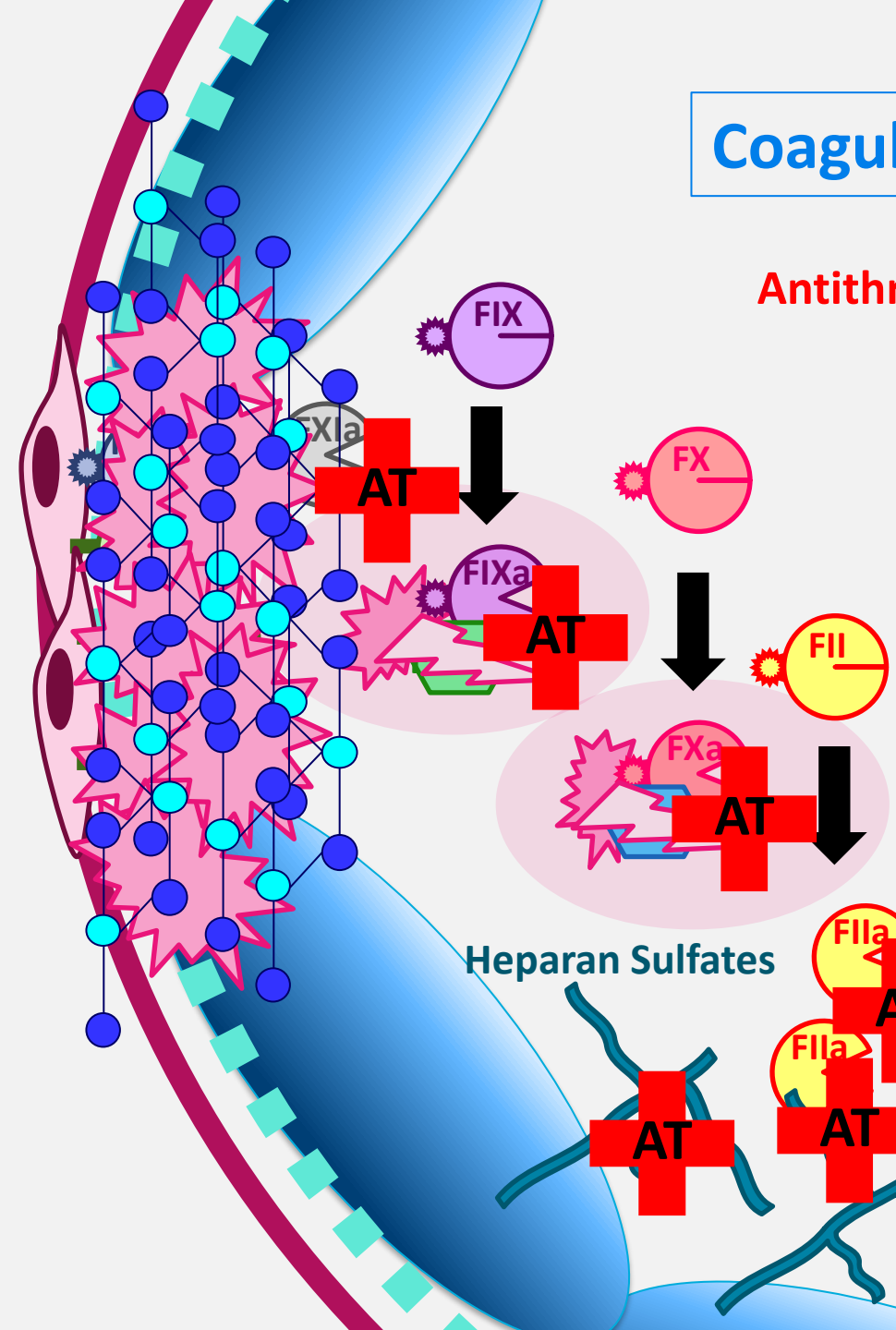
Antithrombin (AT)

- FIIa inhibitor
- AT inhibits all the procoagulant enzymes
- AT anticoagulant activity potentiated by heparan sulfates



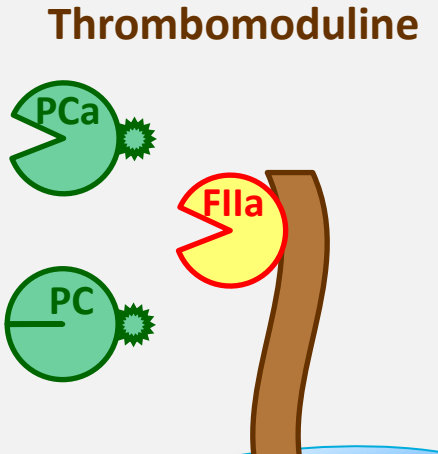
Coagulation

3) Extinction



Antithrombin (AT)

- FIIa inhibitor
- AT inhibits all the procoagulant enzymes
- AT binds tightly to heparan sulfates
- Heparan sulfates potentiate its anticoagulant activity



Coagulation

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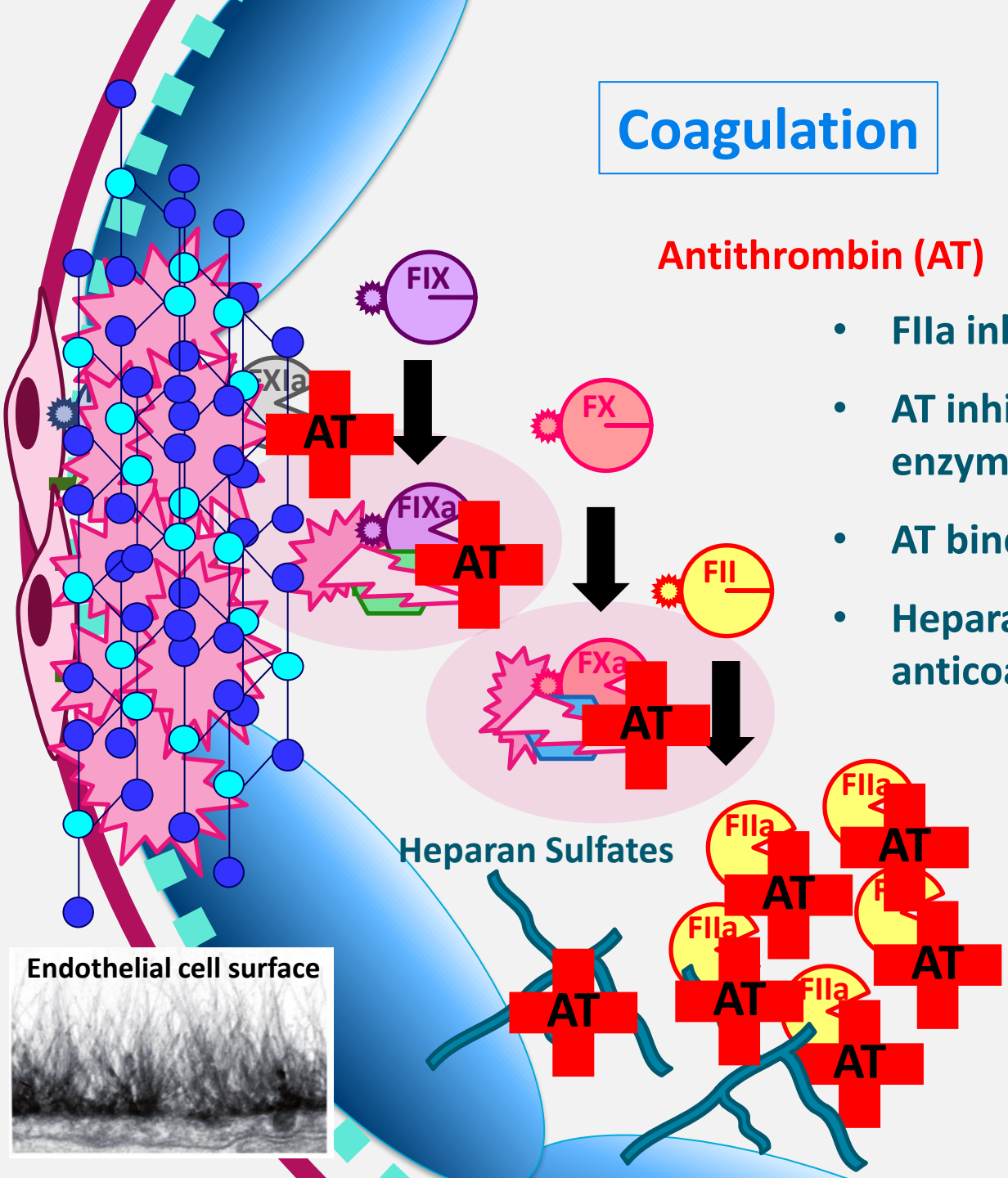
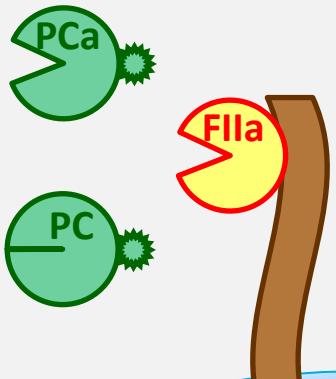
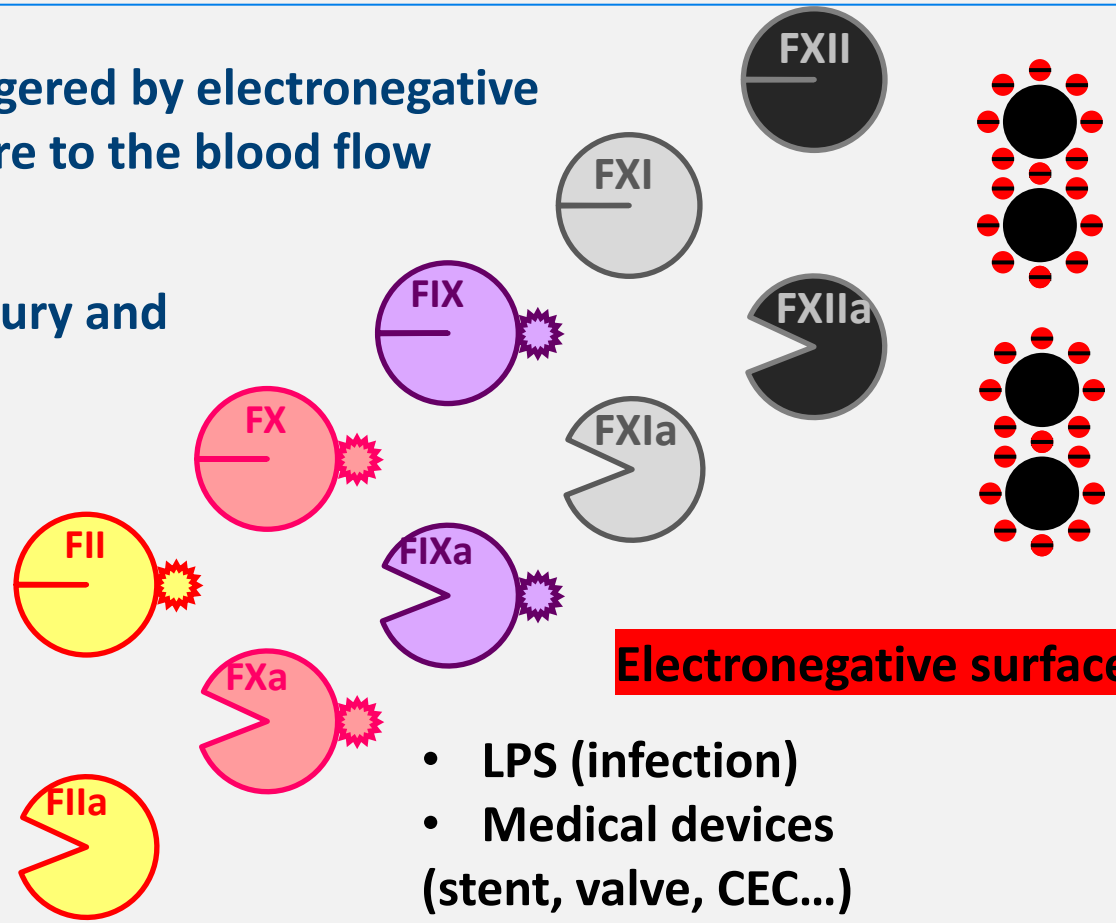




Activation of coagulation by the "contact system pathway"

Coagulation triggered by electronegative surfaces exposure to the blood flow

Independent on injury and TF exposure



Electronegative surfaces ?

- LPS (infection)
- Medical devices (stent, valve, CEC…)
- Silica, kaolin (in-vitro use of reagents to induce coagulation)

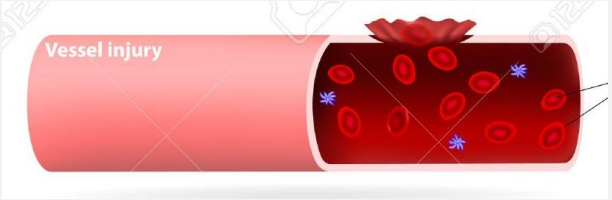
Fibrinolysis

WHAT: Degradation of fibrin clot to prevent blood flow obstruction

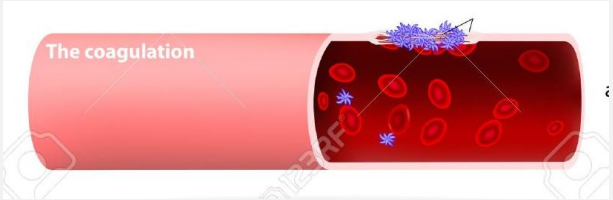
WHERE: On the thrombus, catalyzed by **Fibrine** itself

WHO: Plasma proteins and proteins secreted by endothelial cells

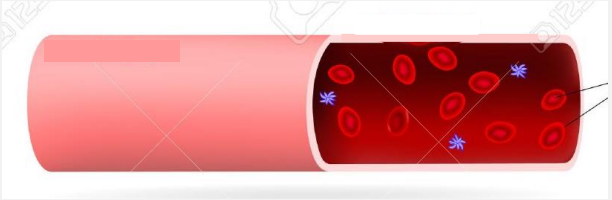
HOW: …



Injury

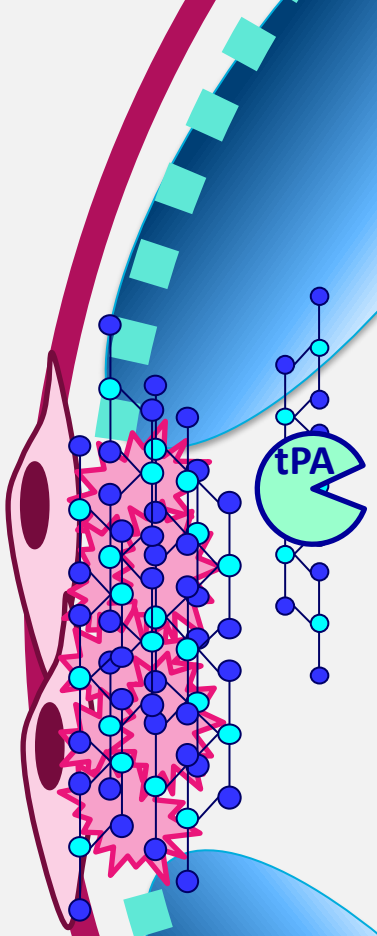


Thrombus



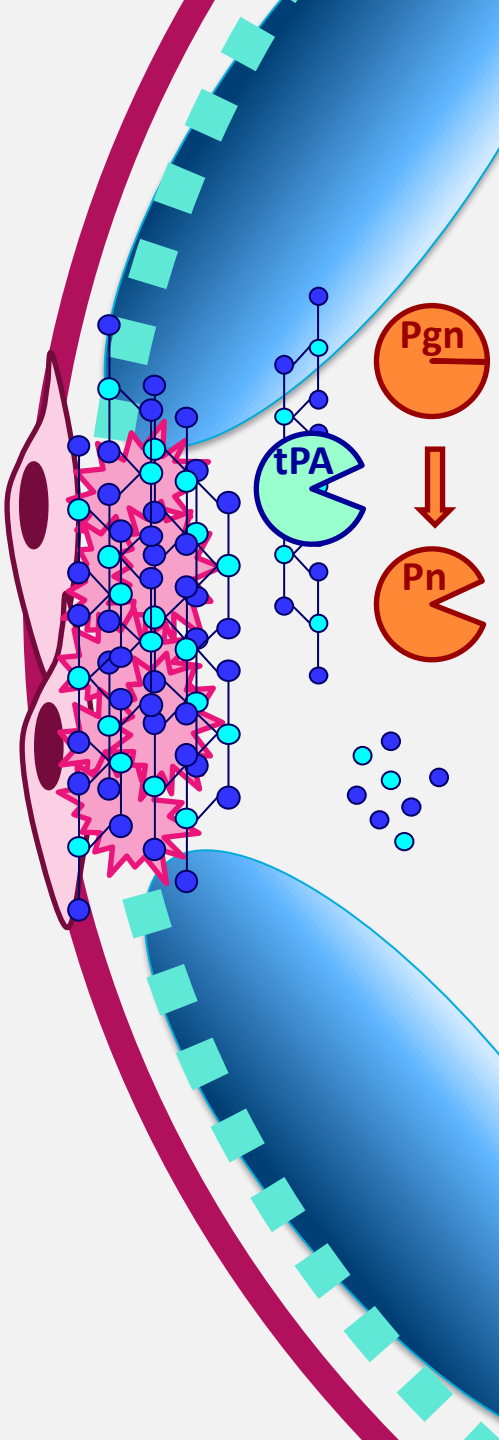
Fibrine lysis

Fibrinolysis



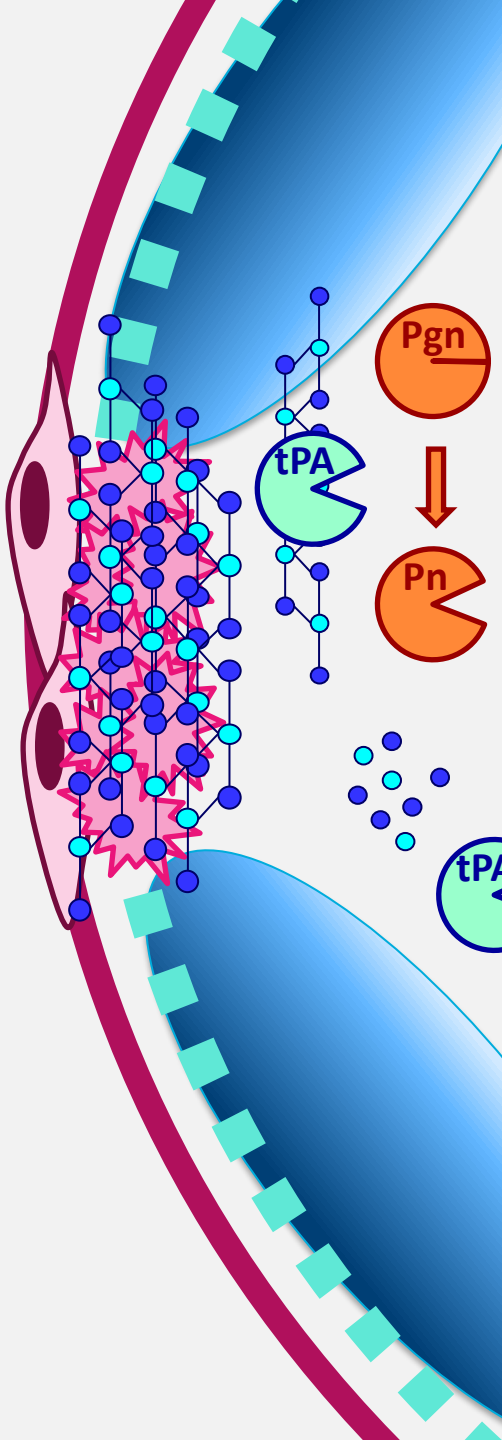
- Fibrinolysis is initiated by tPA
- tPA requires the presence of fibrine to acquire its enzymatic activity

Fibrinolysis



- Fibrinolysis is initiated by tPA
- tPA requires the presence of fibrine to acquire its enzymatic activity
- tPA then activates plasminogen into plasmin
- Plasmin converts fibrine into soluble fibrine degradation products

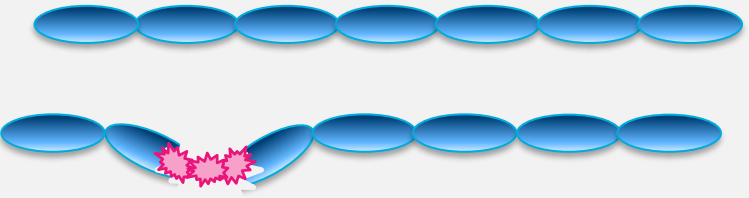
Fibrinolysis



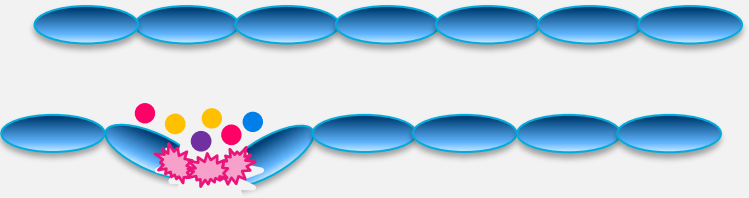
- Fibrinolysis is initiated by tPA
- tPA requires the presence of fibrine to acquire its enzymatic activity
- tPA then activates plasminogen into plasmin
- Plasmin converts fibrine into soluble fibrine degradation products
- Fibrinolysis is modulated by enzyme inhibitors: PAI-1 and α2-AP

Everything starts and ends with fibrine !

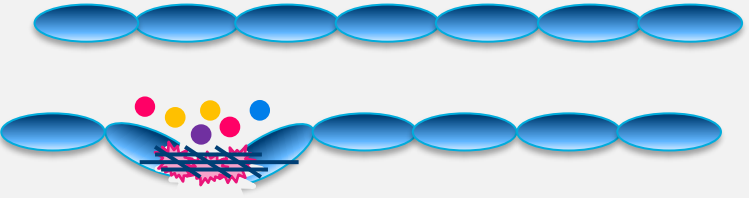
summary



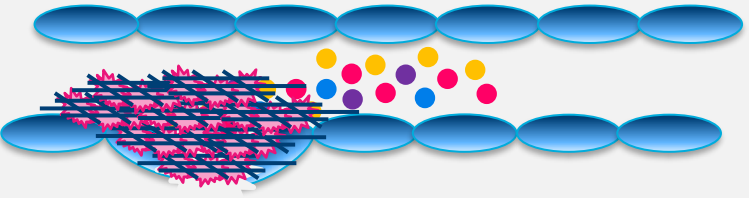
Primary hemostasis = platelet aggregation



Coagulation, 1) initiation = clotting factors activation

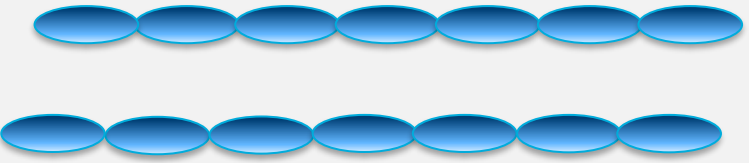


Coagulation, 2) amplification = thrombus formation



Coagulation, 3) extinction =

- Prevents thrombus propagation (obstruction)
- Prevents clotting factors dissemination (DIC)



Fibrinolysis = thrombus resorption, wound healing

Summary

Procoagulant factors	Anticoagulant factors
Antifibrinolytic factors	Profibrinolytic factors

Deficit → **bleeding**

Deficit → **Thrombosis**

Summary

Procoagulant factors

Antifibrinolytic factors

Anticoagulant factors

Profibrinolytic factors