

# TU03: Vascular Functions

## Endothelial quiescence and activation

François Saller

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Thursday, November 14, 2024



Hemostasis, Inflammation  
& Thrombosis  
UMR-S1176  
Dir: Dr Cécile Denis

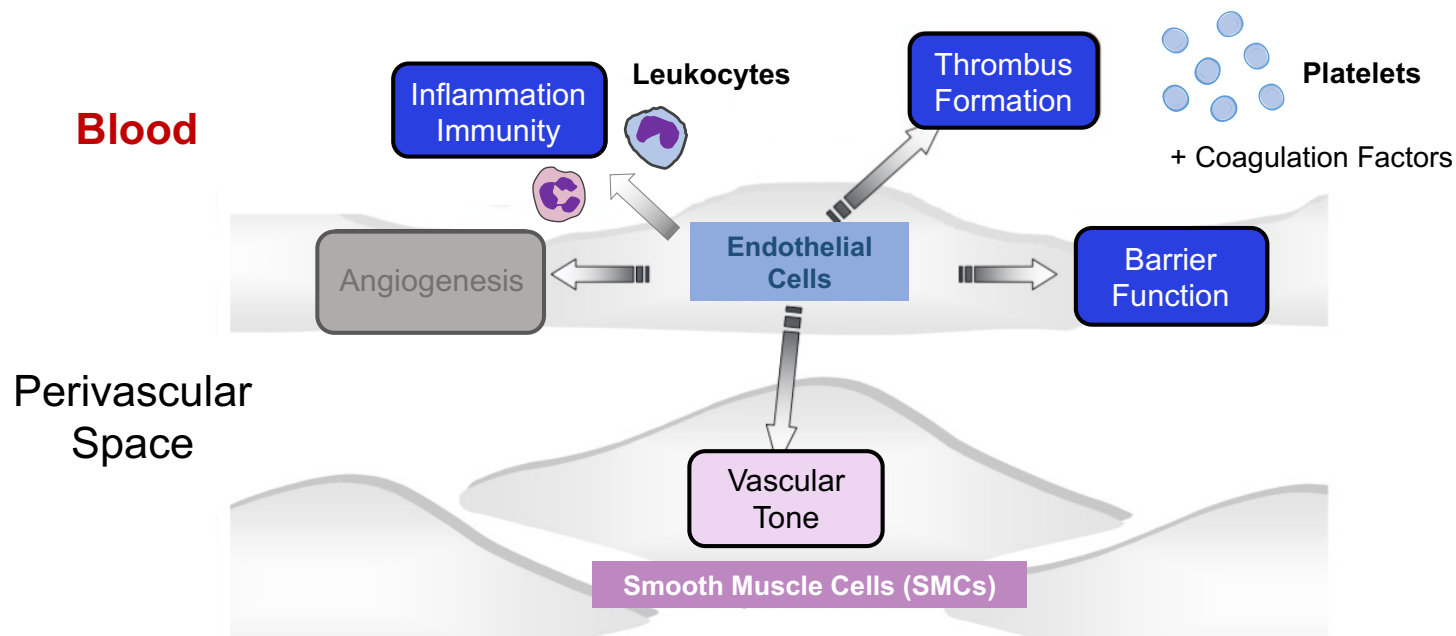
Hôpital de Bicêtre, Bâtiment Pincus (Le Kremlin-Bicêtre)



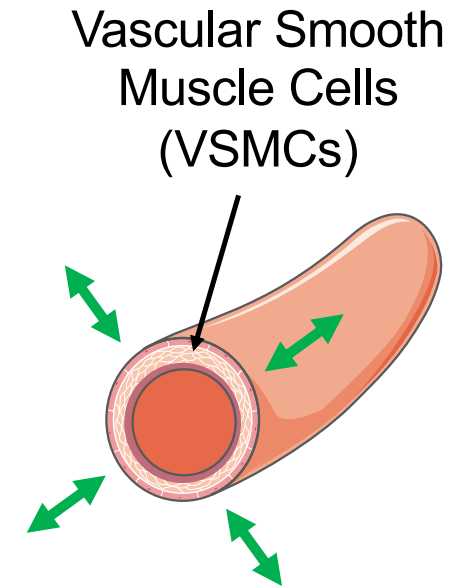
**Inserm**

Institut national  
de la santé et de la recherche médicale

# The vascular endothelium regulates the vascular tone



Adapted from Dr Boris Manoury, UMR-S1180, School of Pharmacy, Paris-Saclay



Vasoconstriction

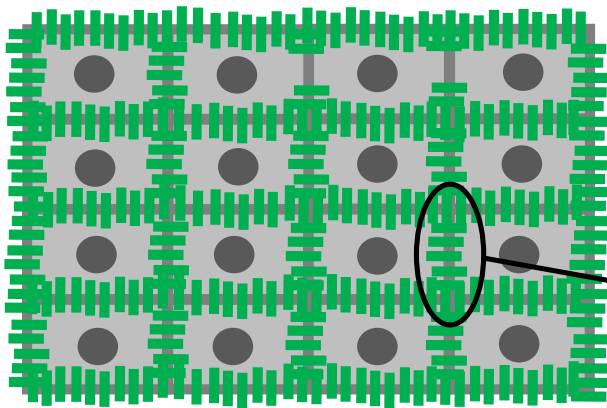
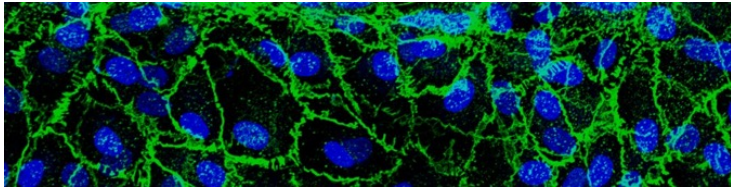
Vasodilation

**= Vascular tone**

**Boris Manoury**  
**TU03 - S1**

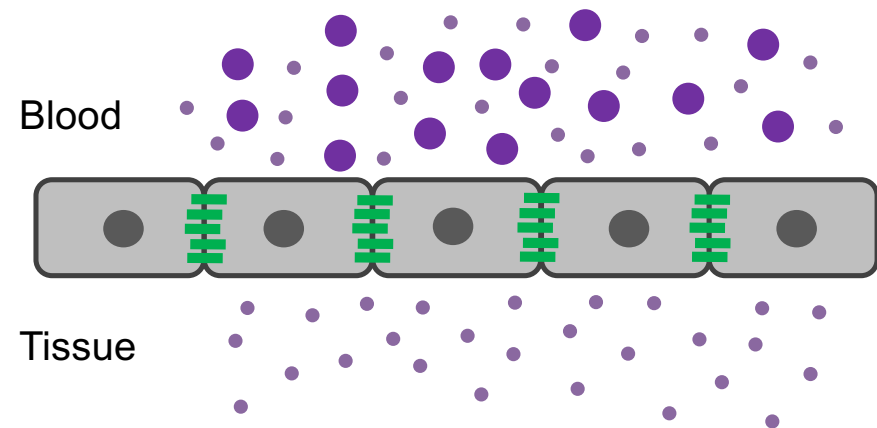
# The vascular endothelium is a physiological **barrier**

Endothelial cell  
Monolayers (*in vitro*)

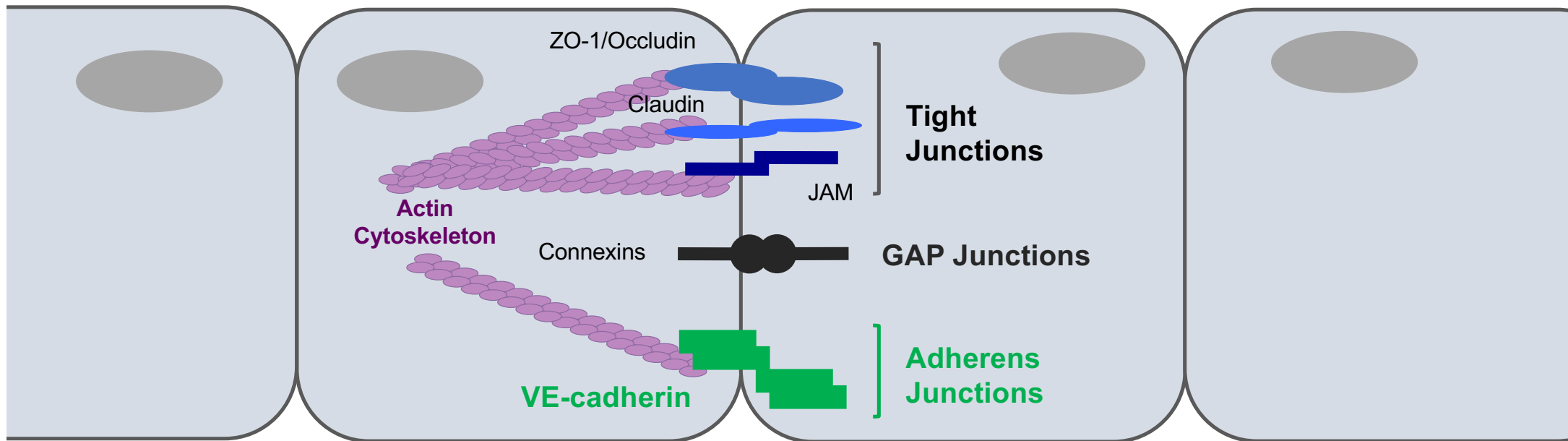
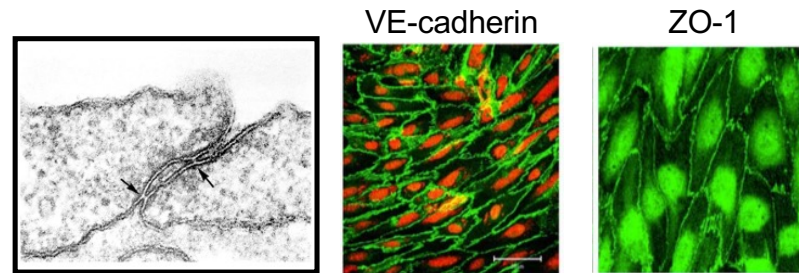


Inter-Endothelial  
"Junctions"

Size-dependent permeability



# Inter-endothelial junctions

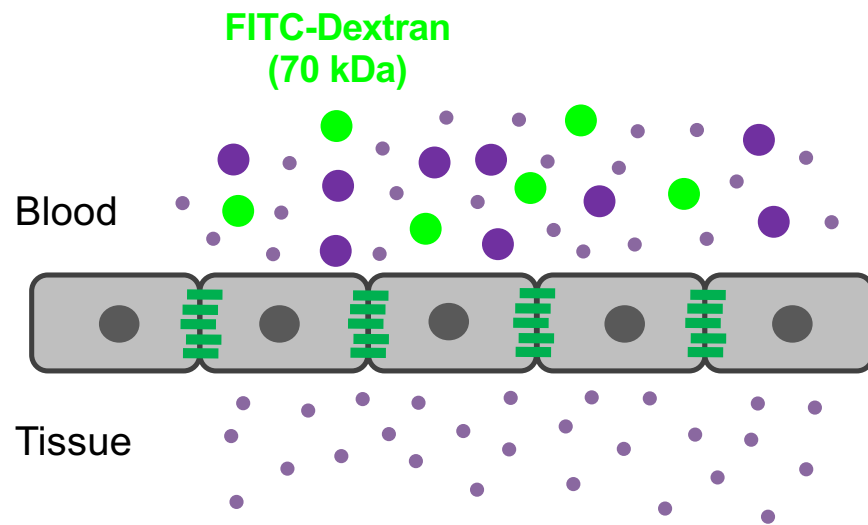
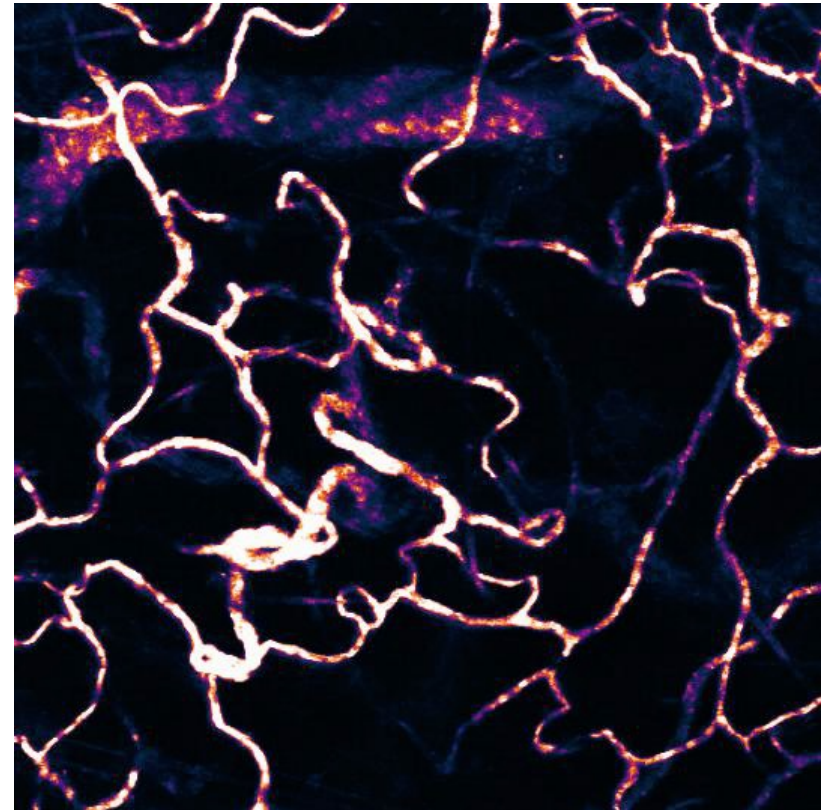
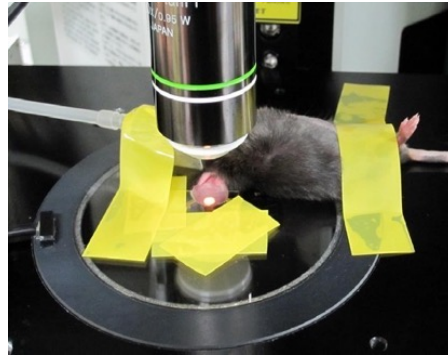


# Visualization of vascular permeability *in vivo*

Intravital microscopy

Skin **Microvessels** (Mouse ears)

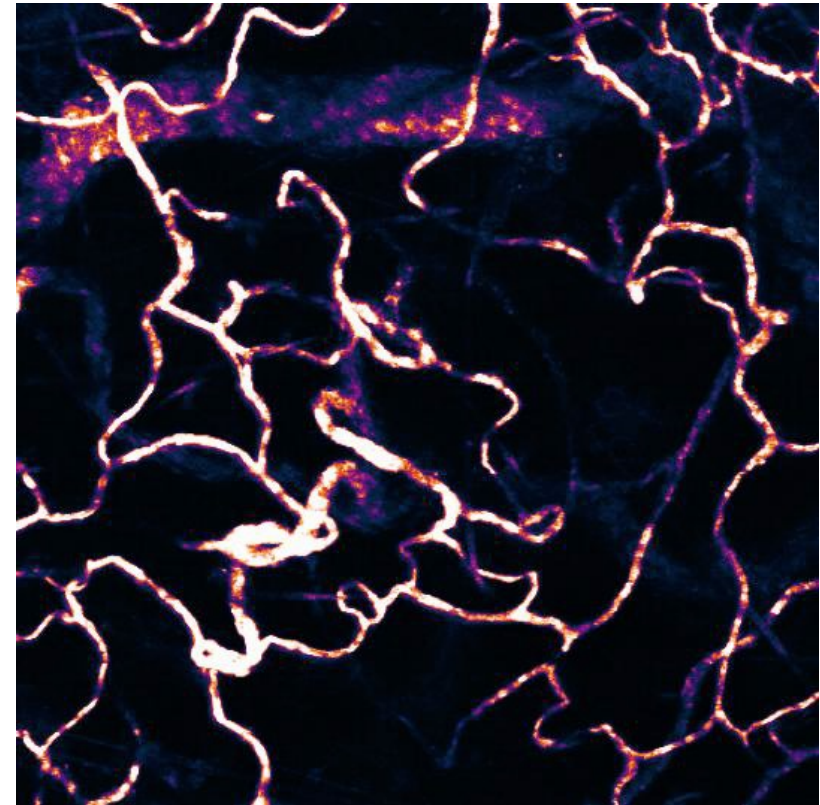
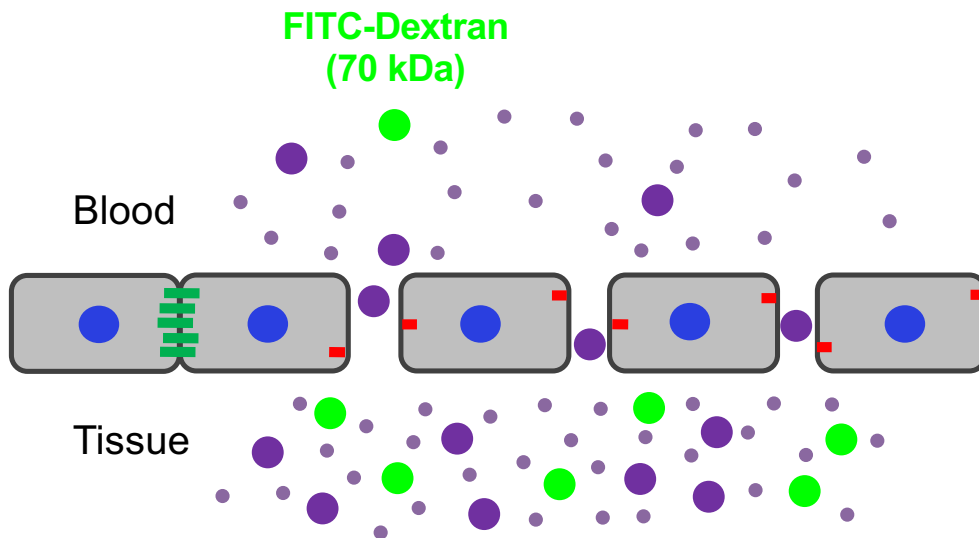
Injection of a **fluorescent tracer** into the circulation (FITC-Dextran)



# Visualization of vascular permeability *in vivo*

+ Histamine

Vascular permeability mainly due to  
**disruption of inter-endothelial junctions**

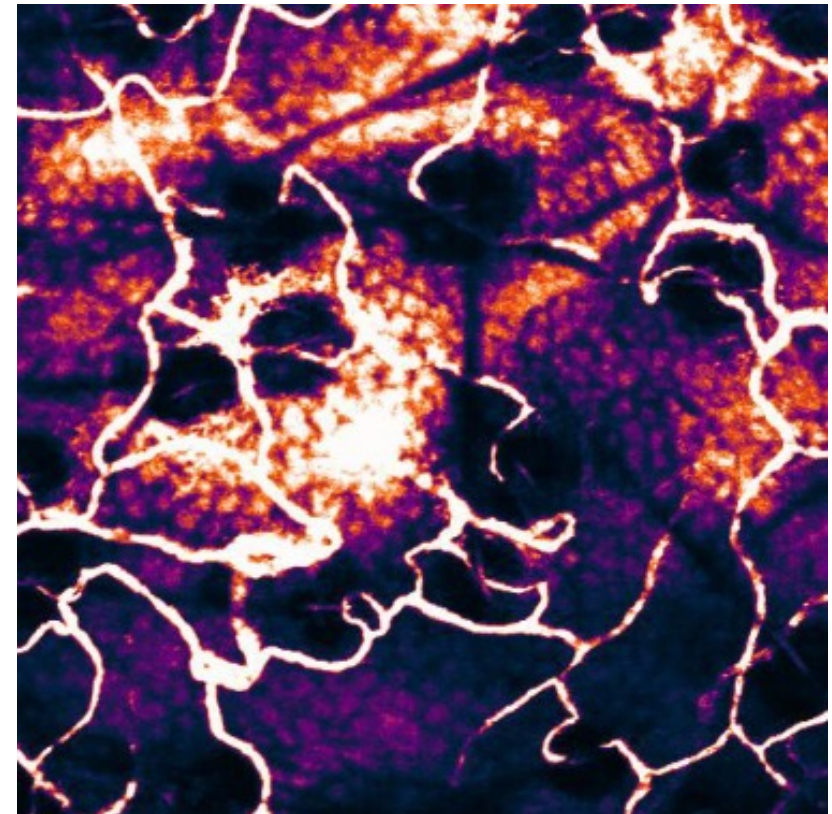
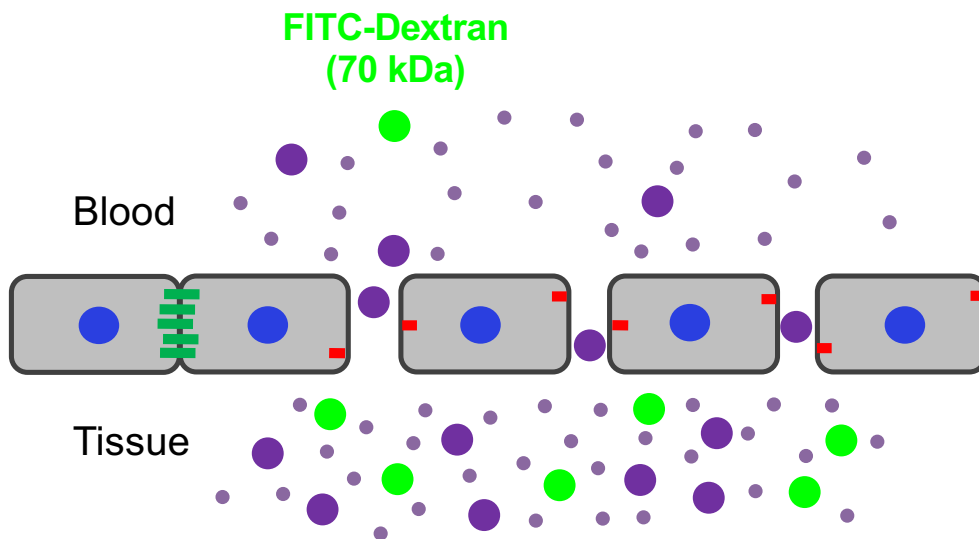




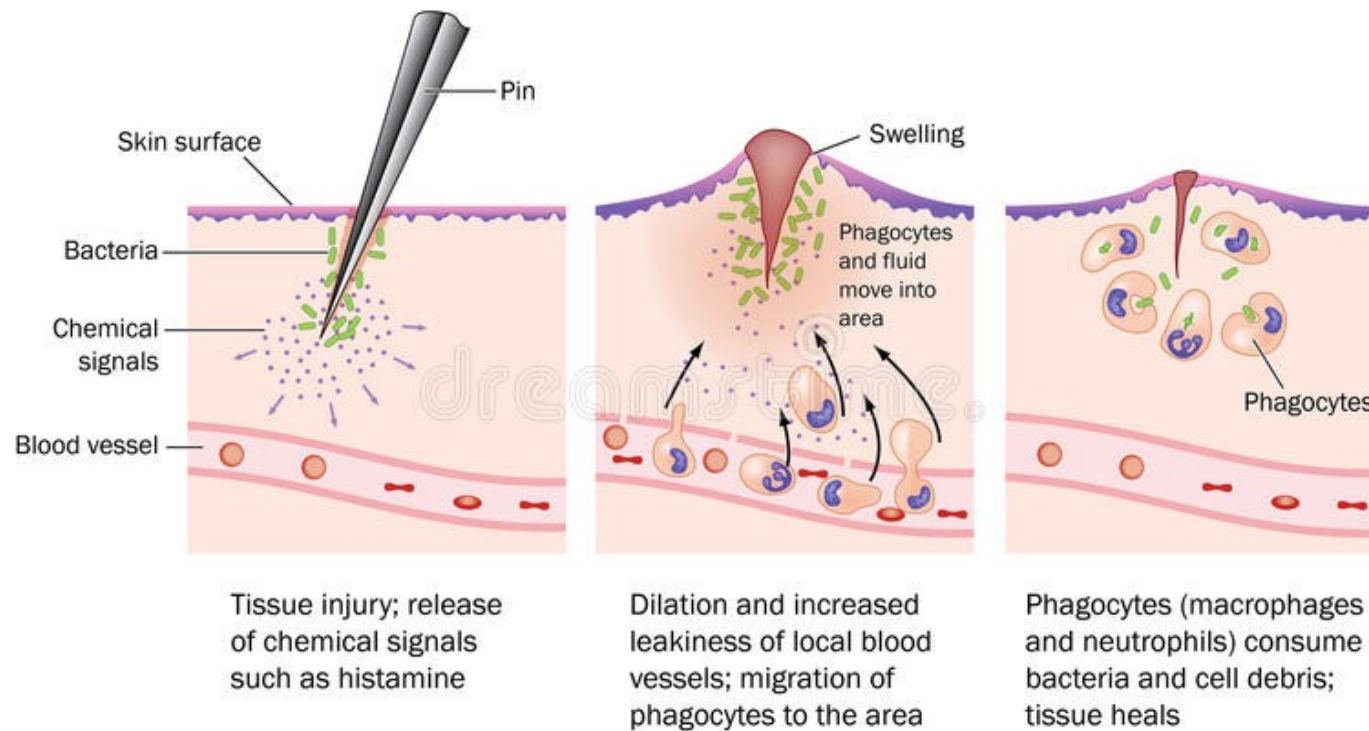
# Visualization of vascular permeability *in vivo*

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Vascular permeability mainly due to  
**disruption of inter-endothelial junctions**



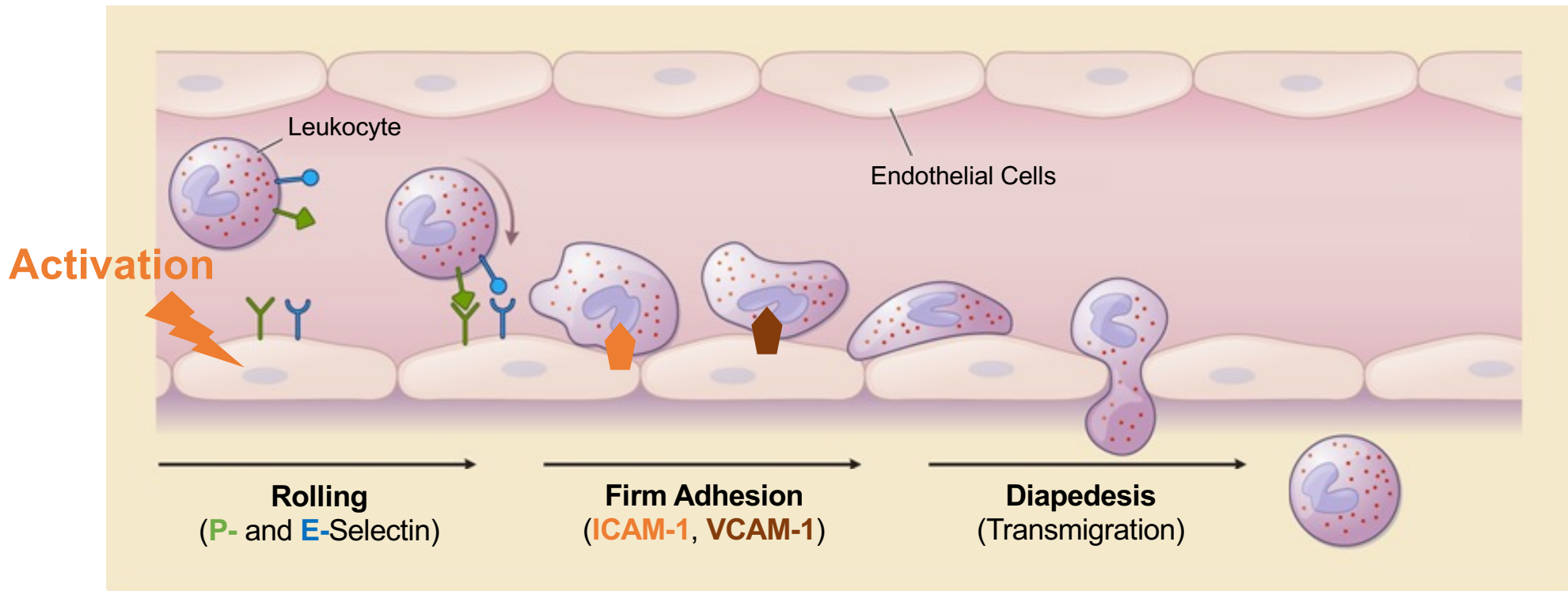
# The vascular endothelium has a central role in inflammation



Leukocyte adhesion and recruitment is a **physiological response** in the context of inflammation, if these processes are **transient** and **self-limited**



# Leukocyte recruitment by an **activated** endothelium



Source: Goldsmith LA, Katz SI, Gilchrist BA, Paller AS, Leffell DJ, Wolff K: *Fitzpatrick's Dermatology in General Medicine, 8th Edition*: [www.accessmedicine.com](http://www.accessmedicine.com)

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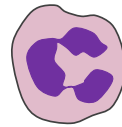
P- and E-Selectin

ICAM-1, VCAM-1

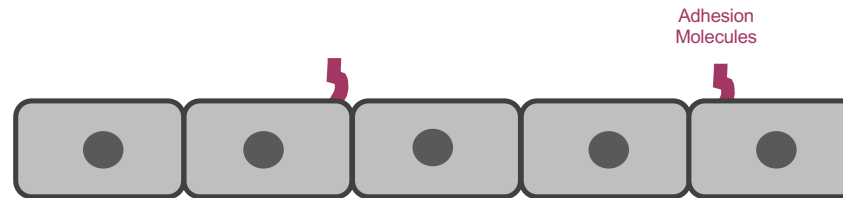
= « Adhesion Molecules »

# Endothelial adhesion molecules

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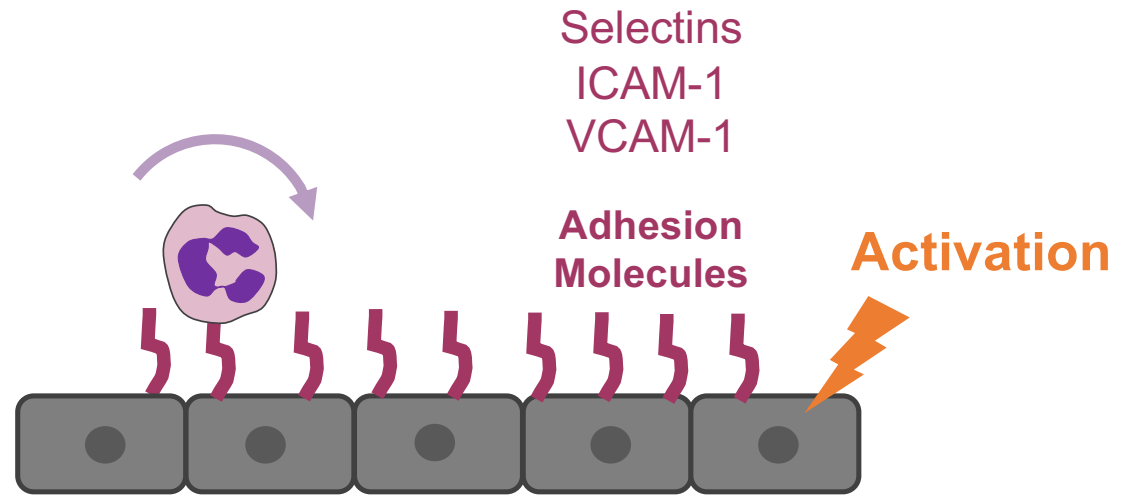
No or very little expression of **adhesion molecules** by a quiescent (*i.e.* non activated) endothelium



# Endothelial adhesion molecules

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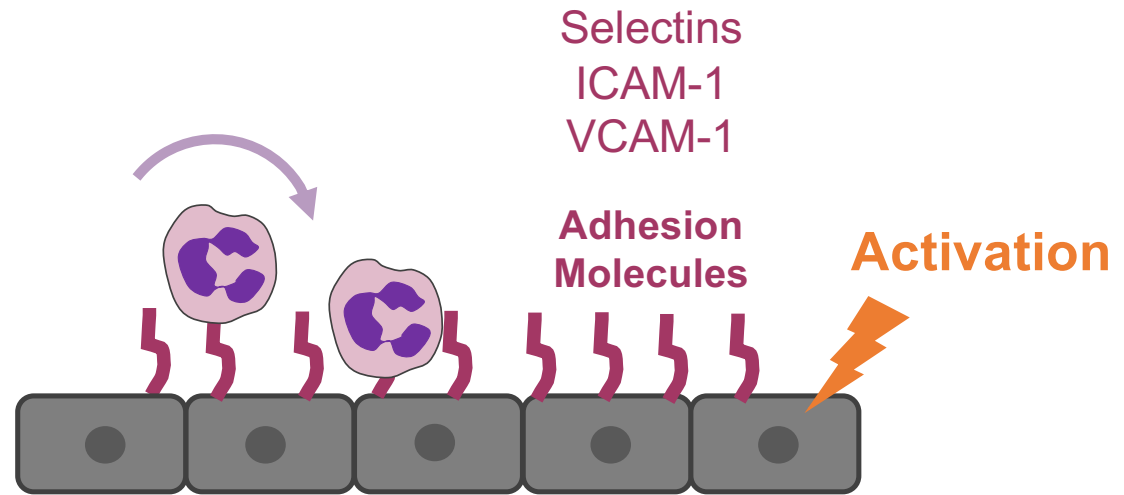
Strong expression of **adhesion molecules** by an activated endothelium



# Endothelial adhesion molecules

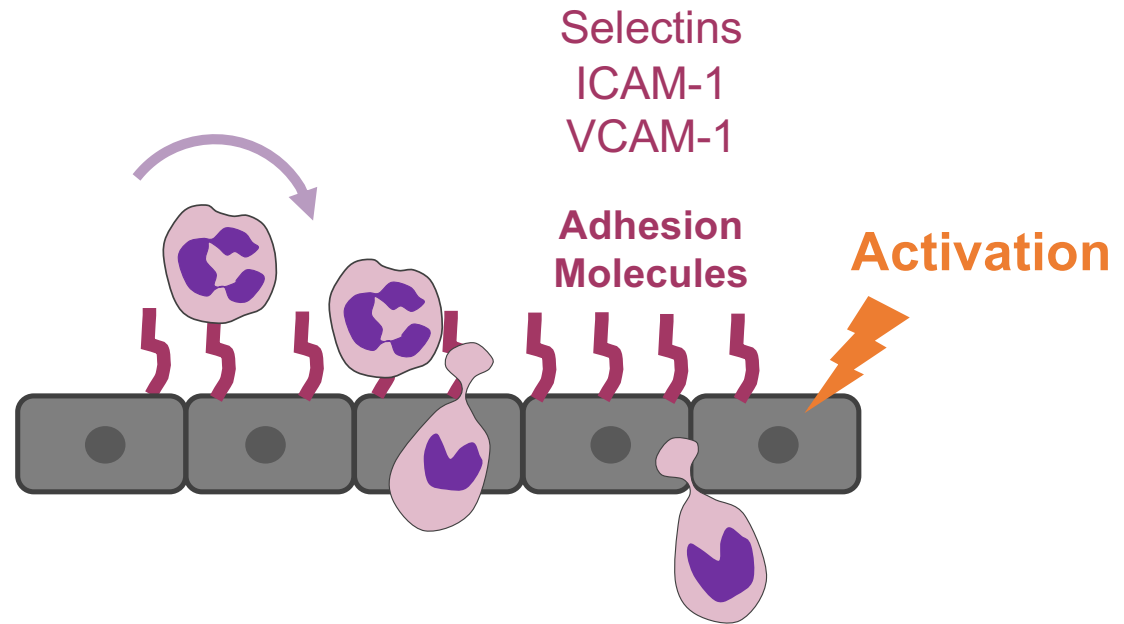
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Strong expression of  
**adhesion molecules** by  
an activated endothelium



# Endothelial adhesion molecules

Strong expression of **adhesion molecules** by an activated endothelium





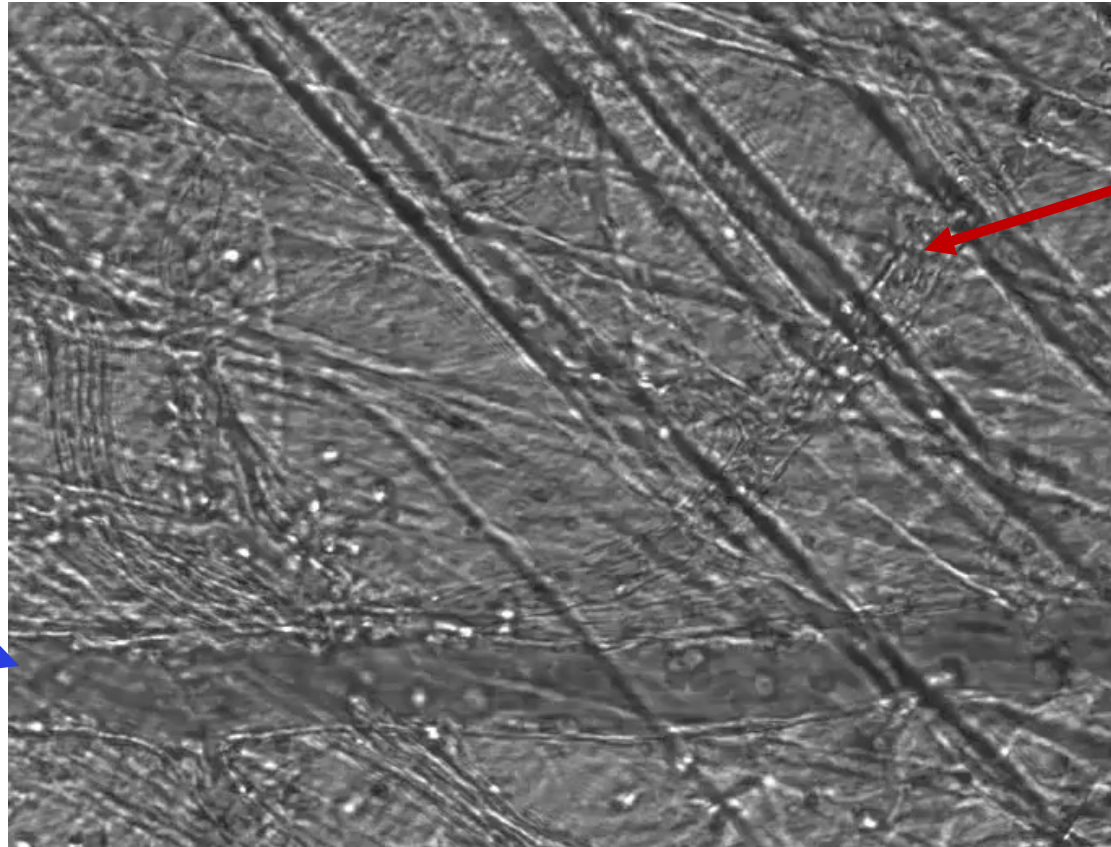
# Leukocyte recruitment by an activated endothelium

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Cremaster muscle  
microvessels (mouse)

Intravital microscopy

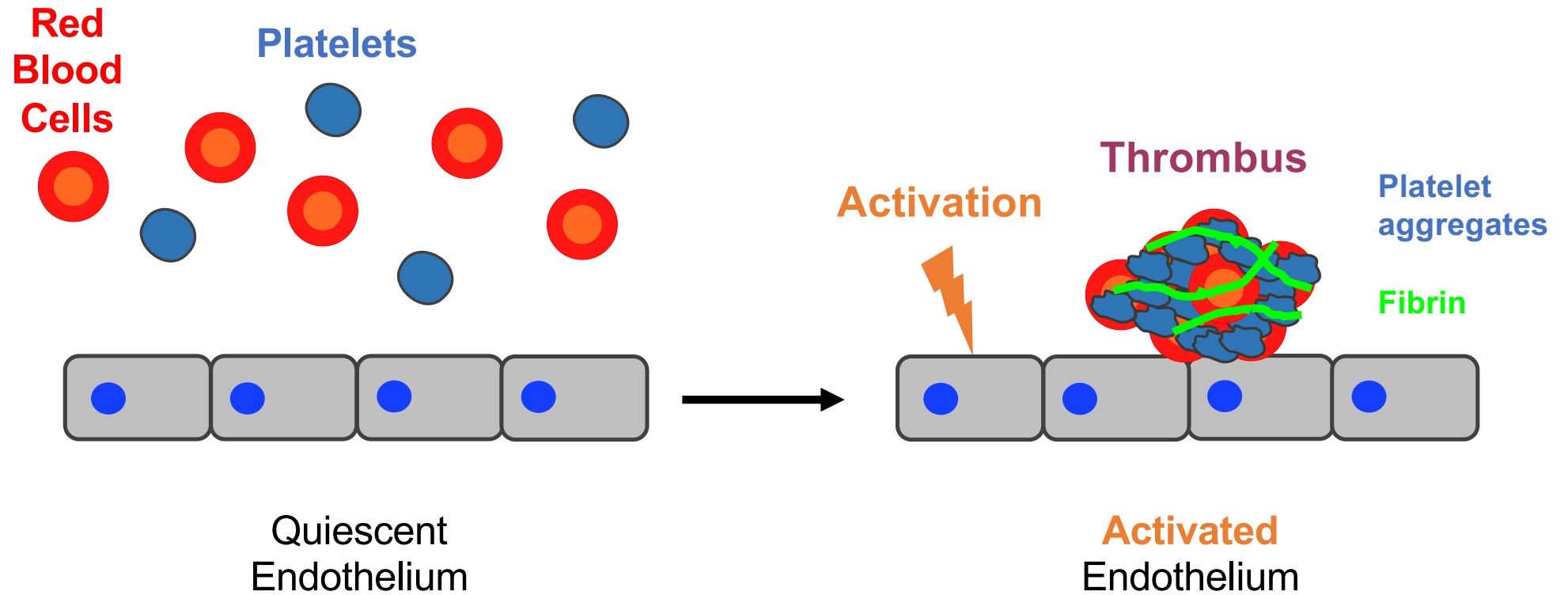
Venule



Arteriole

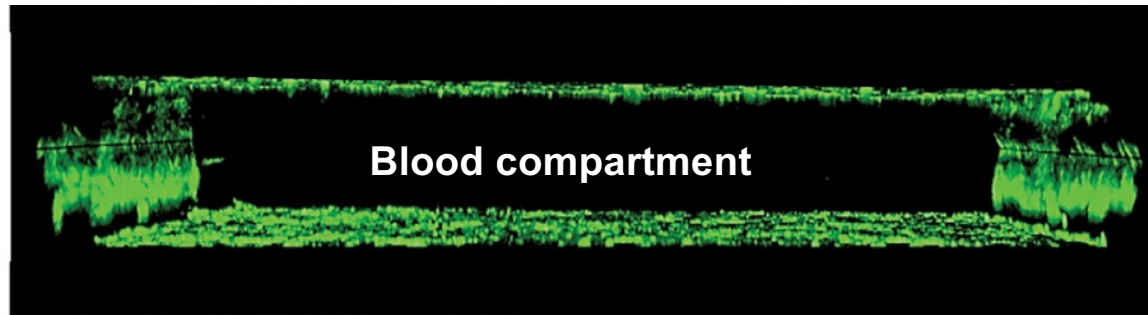
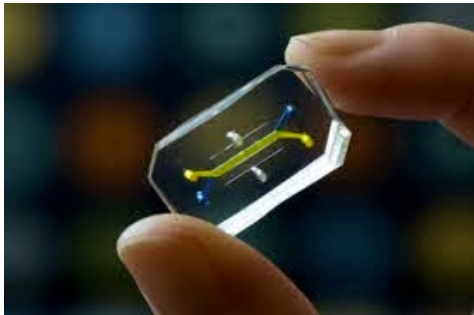
Mainly in the **post-capillary venules** in most organs

# The vascular endothelium can mount **thrombotic responses**



# Microfluidic model of a blood microvessel

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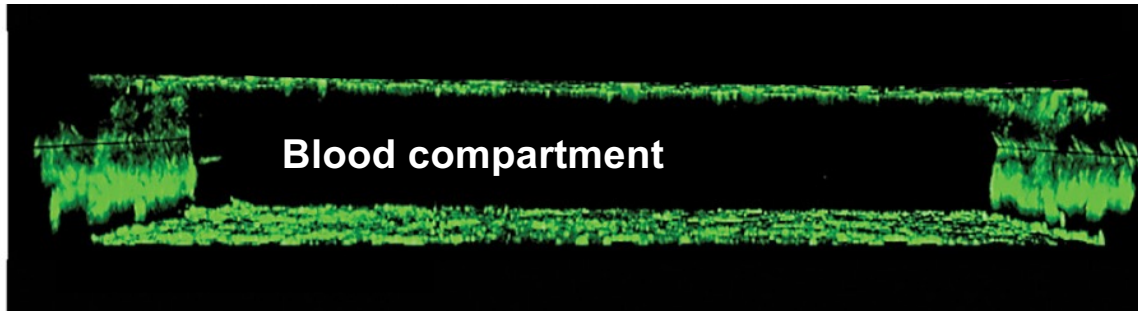
Human endothelial cells cultured **under flow**

« **Physiological** » conditions

Endothelial cells acquire a **quiescent** phenotype

# Microfluidic model of a blood microvessel

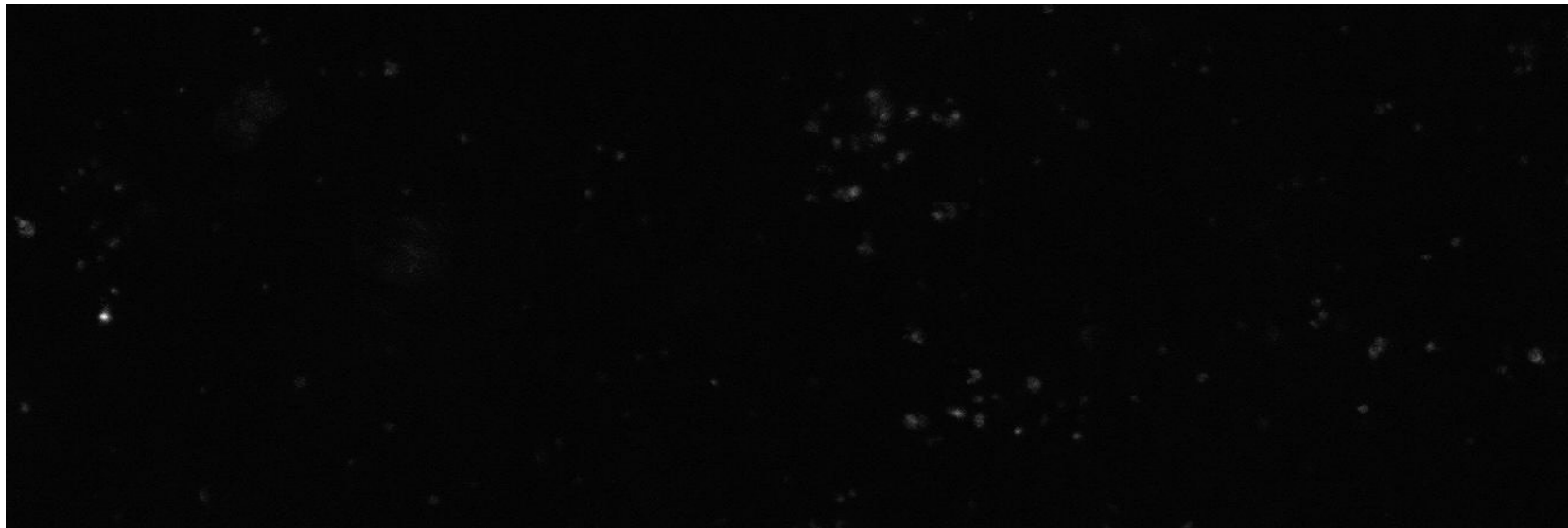
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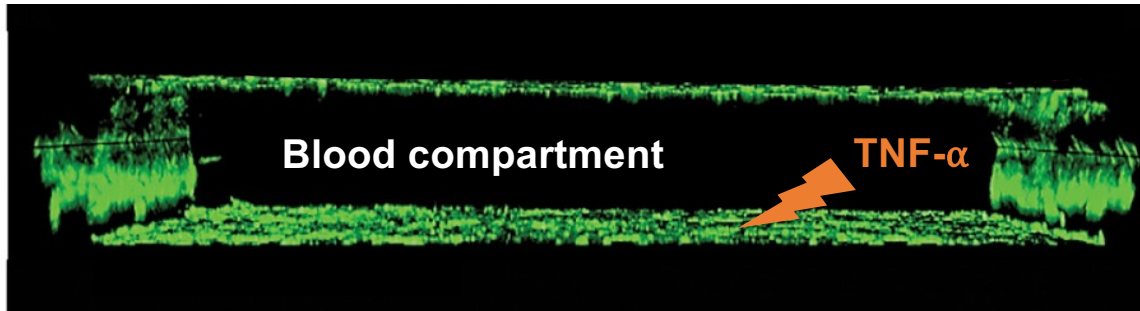
**Untreated** microvessels

**Whole blood** perfusion

Staining of **platelets** in blood with Anti-CD41-PE



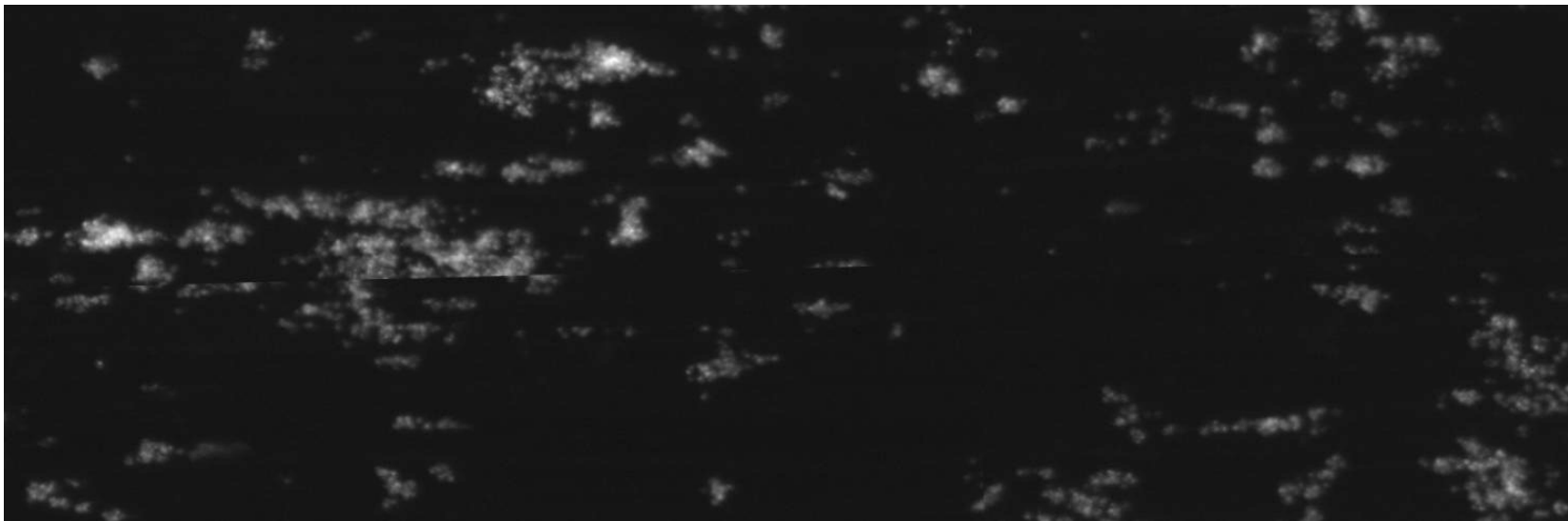
# Microfluidic model of a blood microvessel



Microvessels **treated with TNF- $\alpha$**  (4 h)

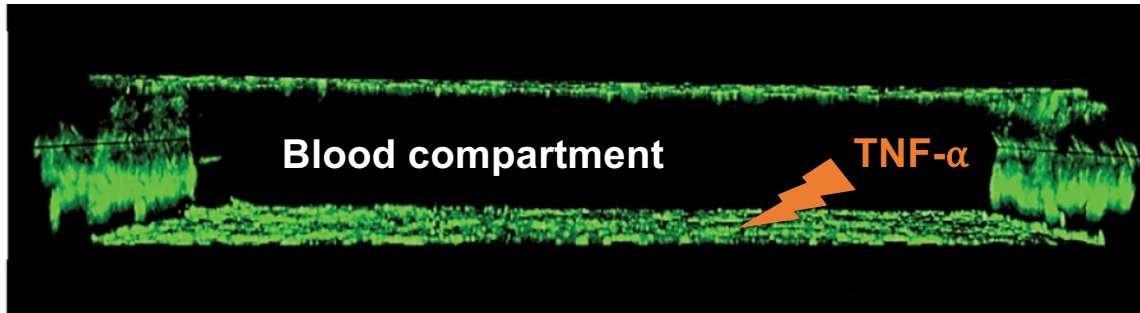
**Whole blood** perfusion

Staining of **platelets** in blood with Anti-CD41-PE





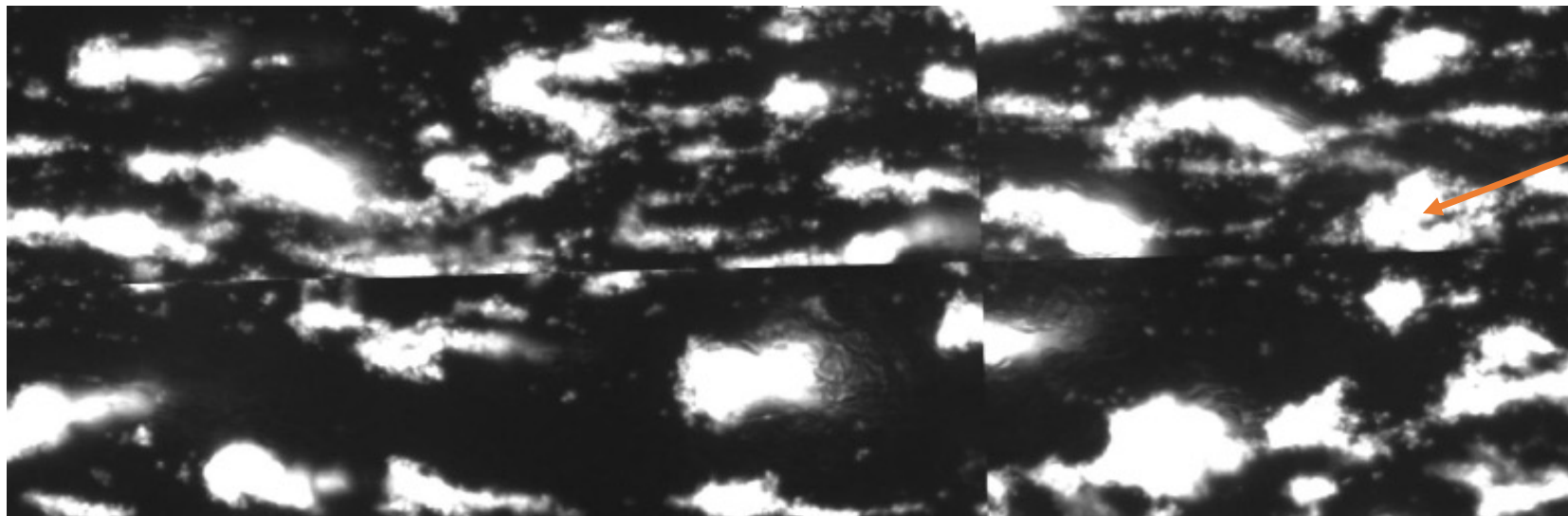
# Microfluidic model of a blood microvessel



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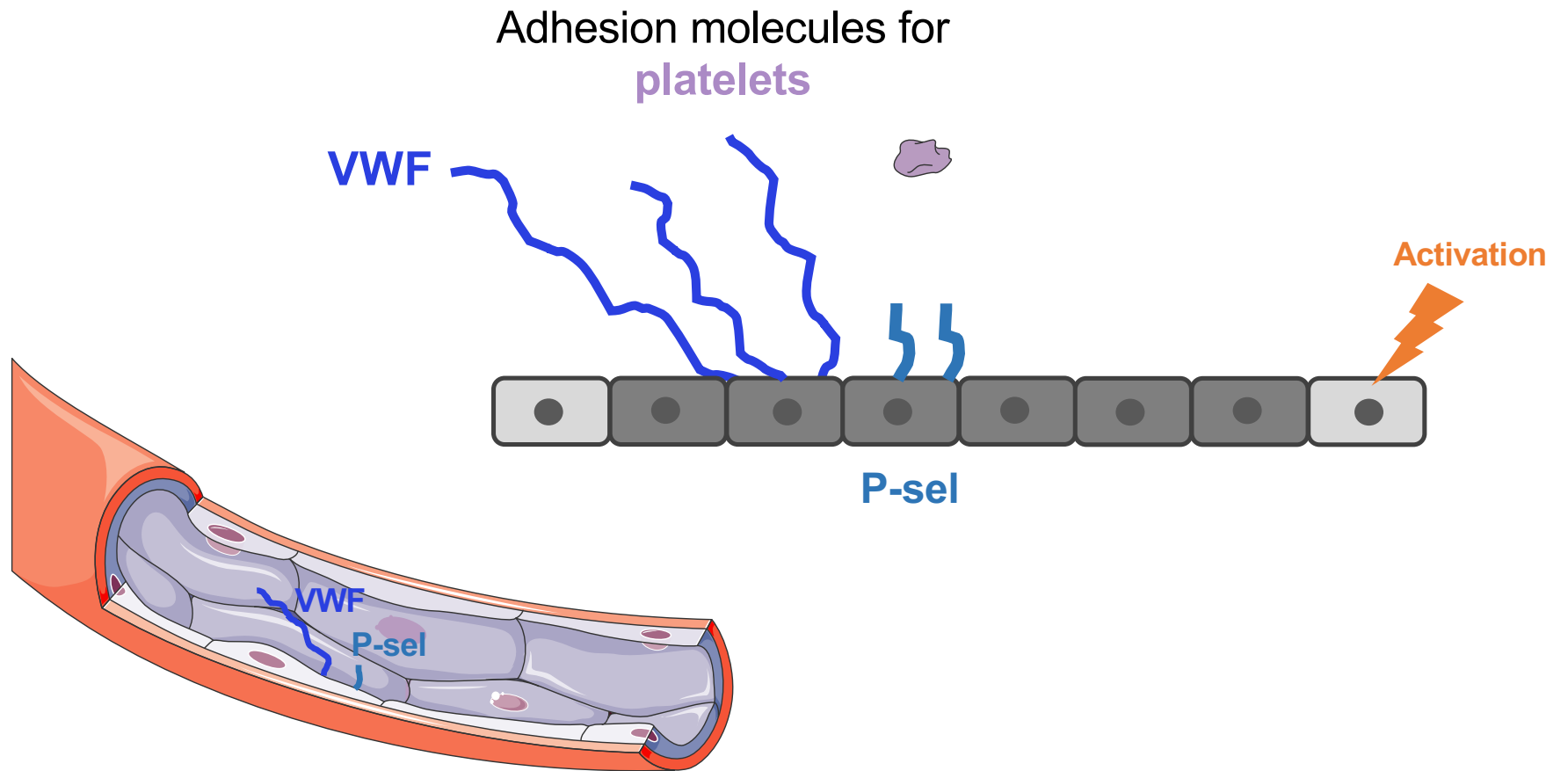
**Whole blood** perfusion

Staining of **platelets** in blood with Anti-CD41-PE

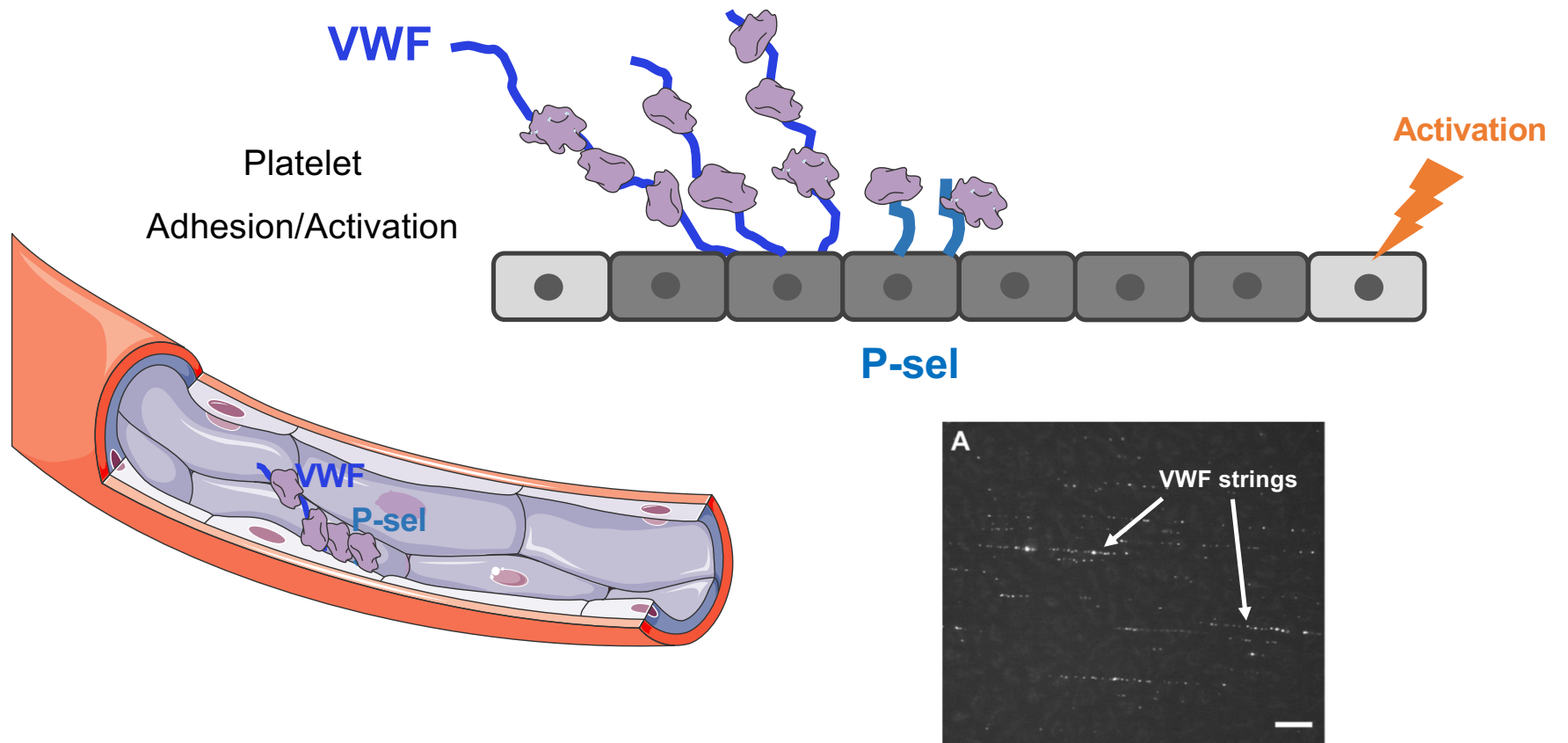


Platelet  
Thrombi

# Thrombus formation on the endothelial surface



# Thrombus formation on the endothelial surface



# Thrombus formation on the endothelial surface

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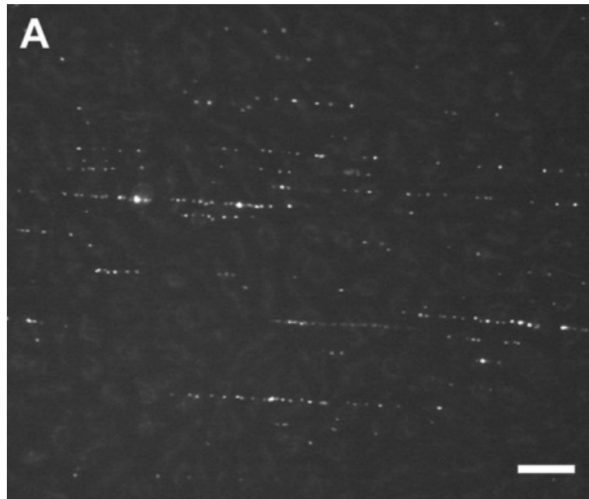
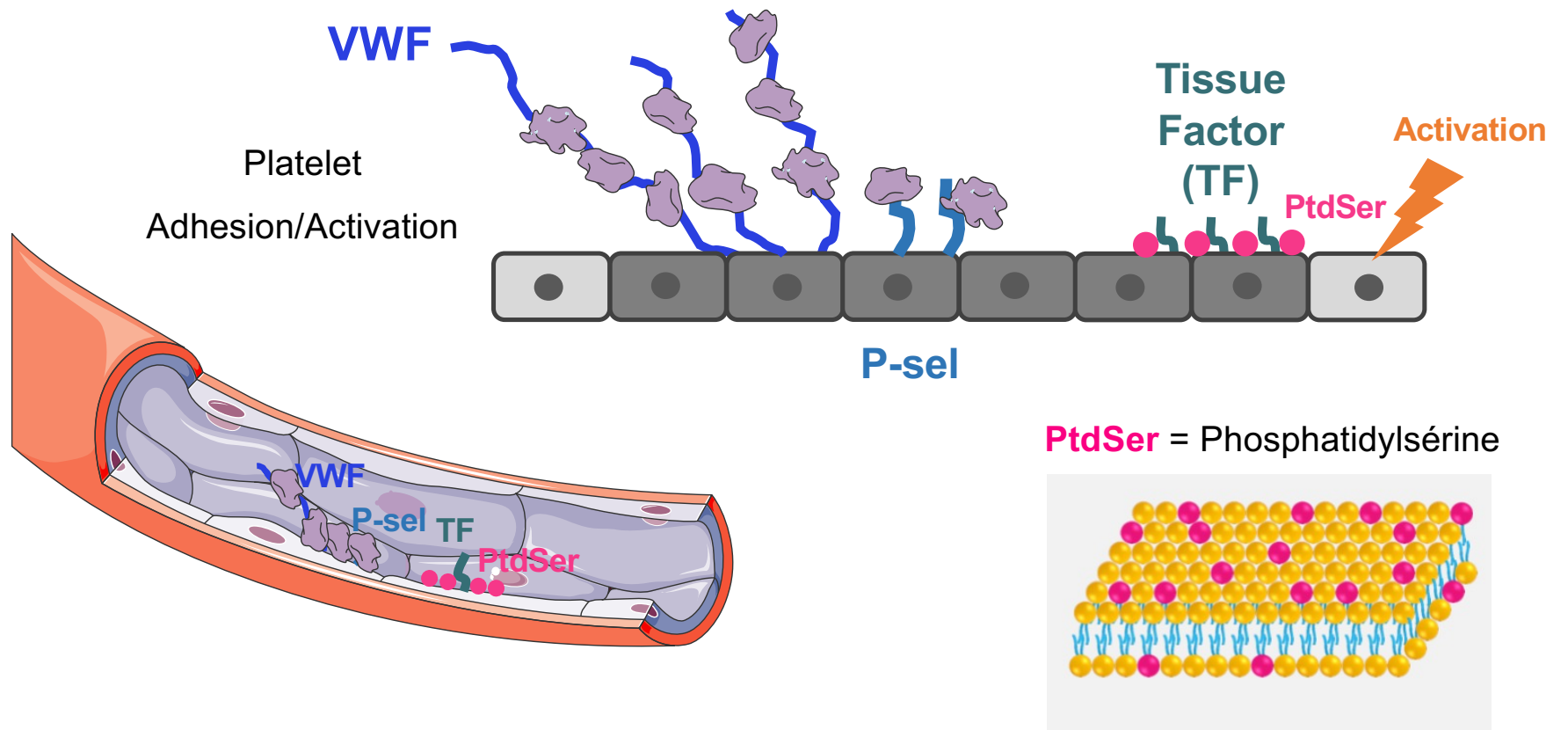


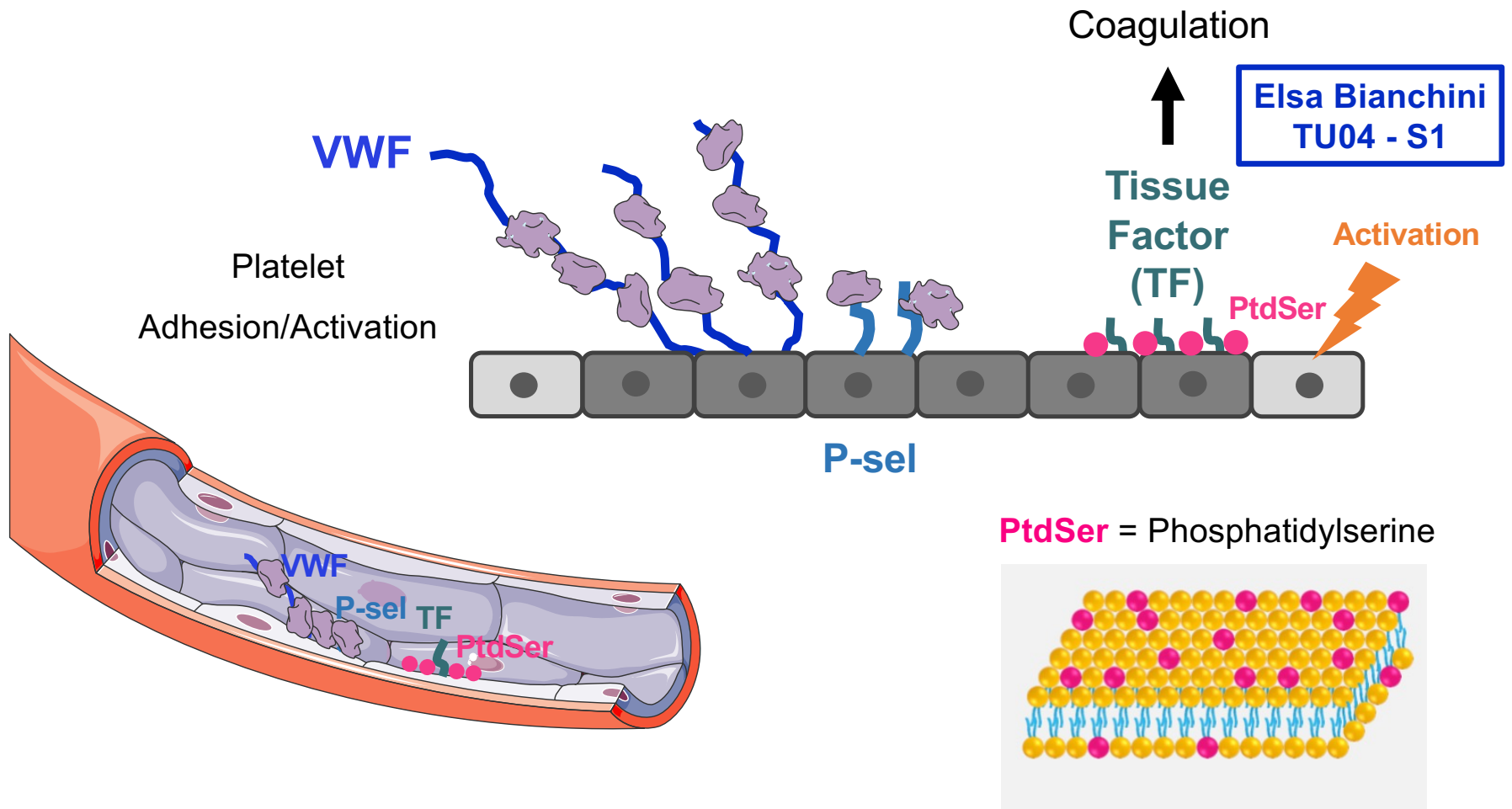
FIGURE 1. **VWF strings on the surface of BOECs are removed by ADAMTS13.** A, platelet-decorated VWF strings are visualized by fluorescence microscopy on the surface of BOECs perfused with washed DIOC6-labeled platelets at a shear rate of  $250 \text{ s}^{-1}$ . Scale bar corresponds to  $50 \mu\text{m}$ .

# Thrombus formation on the endothelial surface



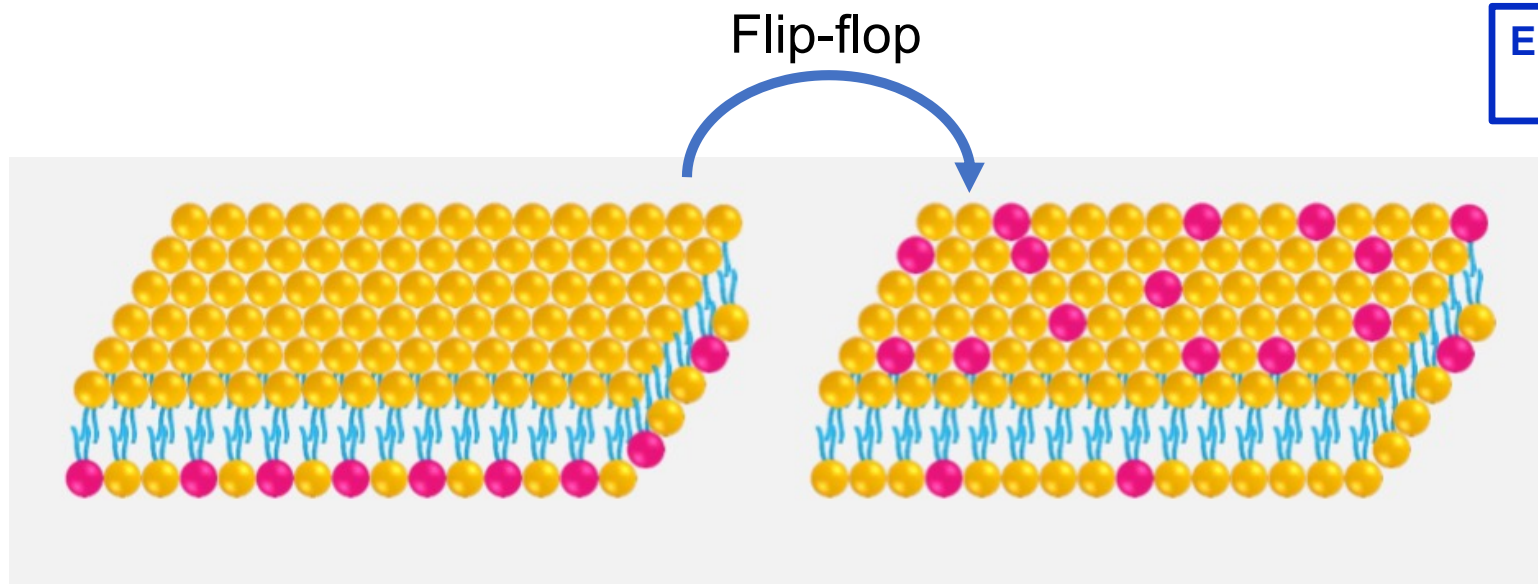


# Thrombus formation on the endothelial surface



# Phosphatidylserine (PtdSer) exposition by activated ECs

Elsa Bianchini  
TU04 - S1



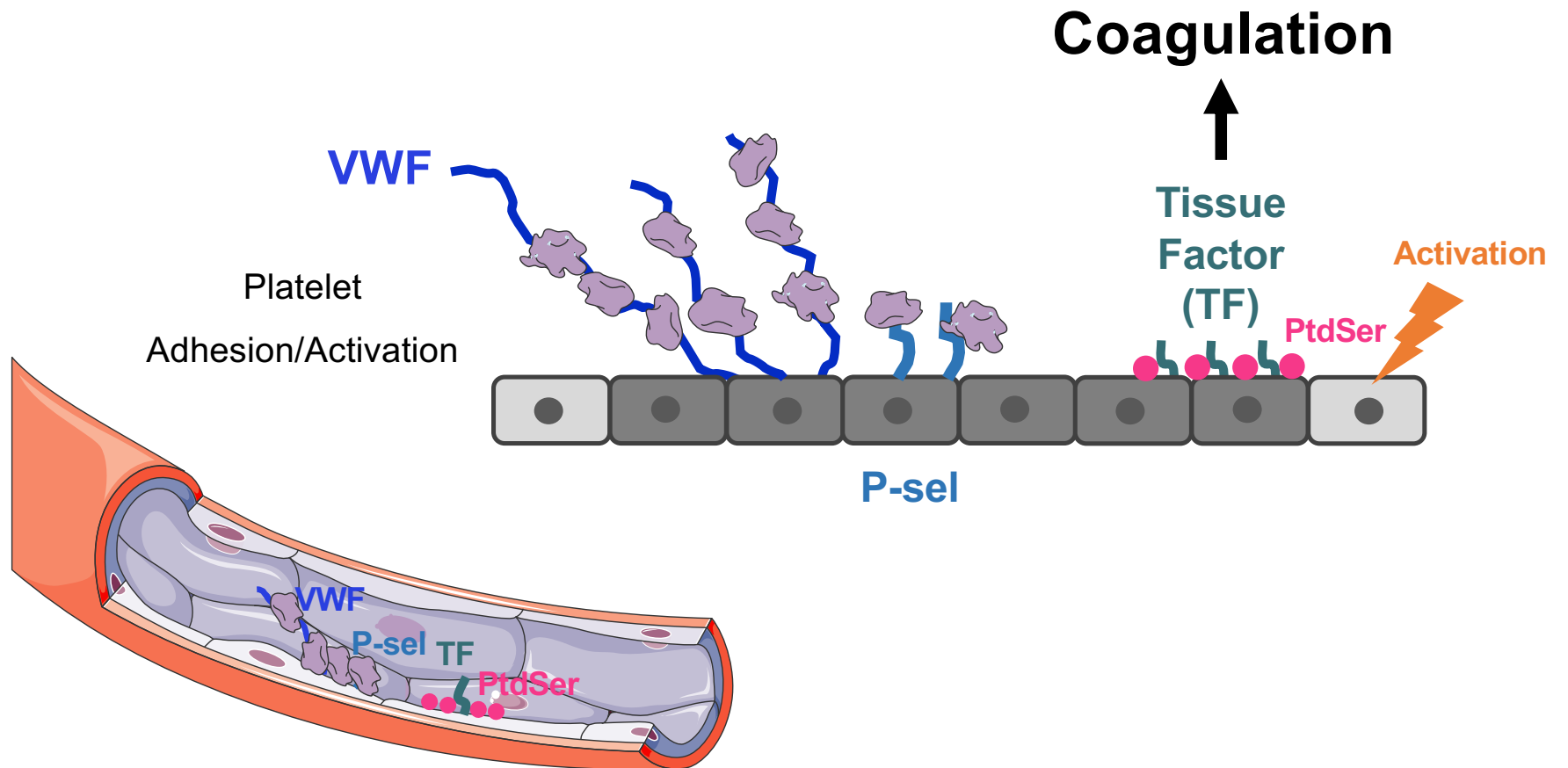
PtdCholine : **neutral** phospholipid

PtdSerine : **negatively charged** phospholipid

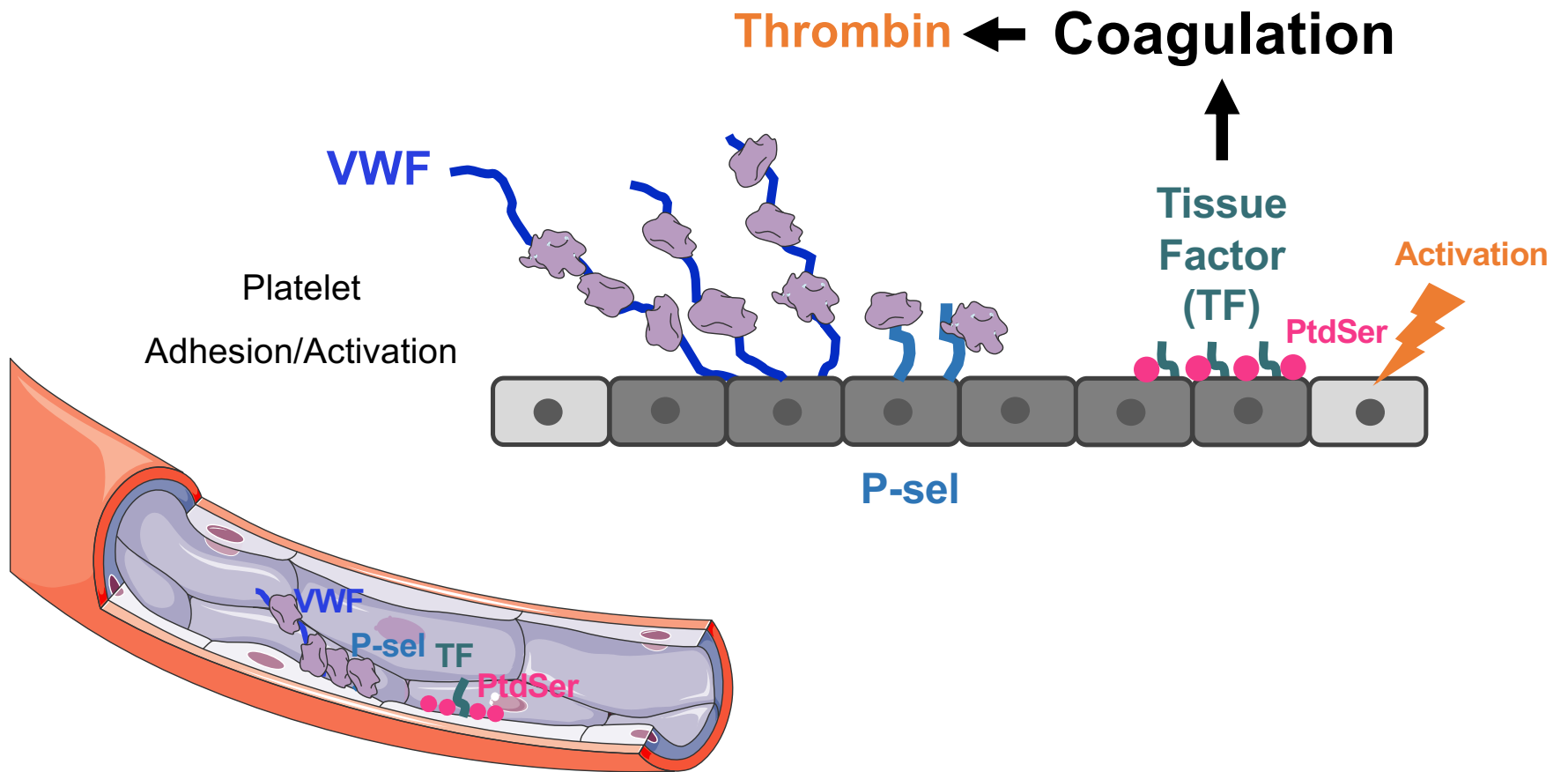
Analogy with the exposition of PtdSer on the surface of **apoptotic cells**



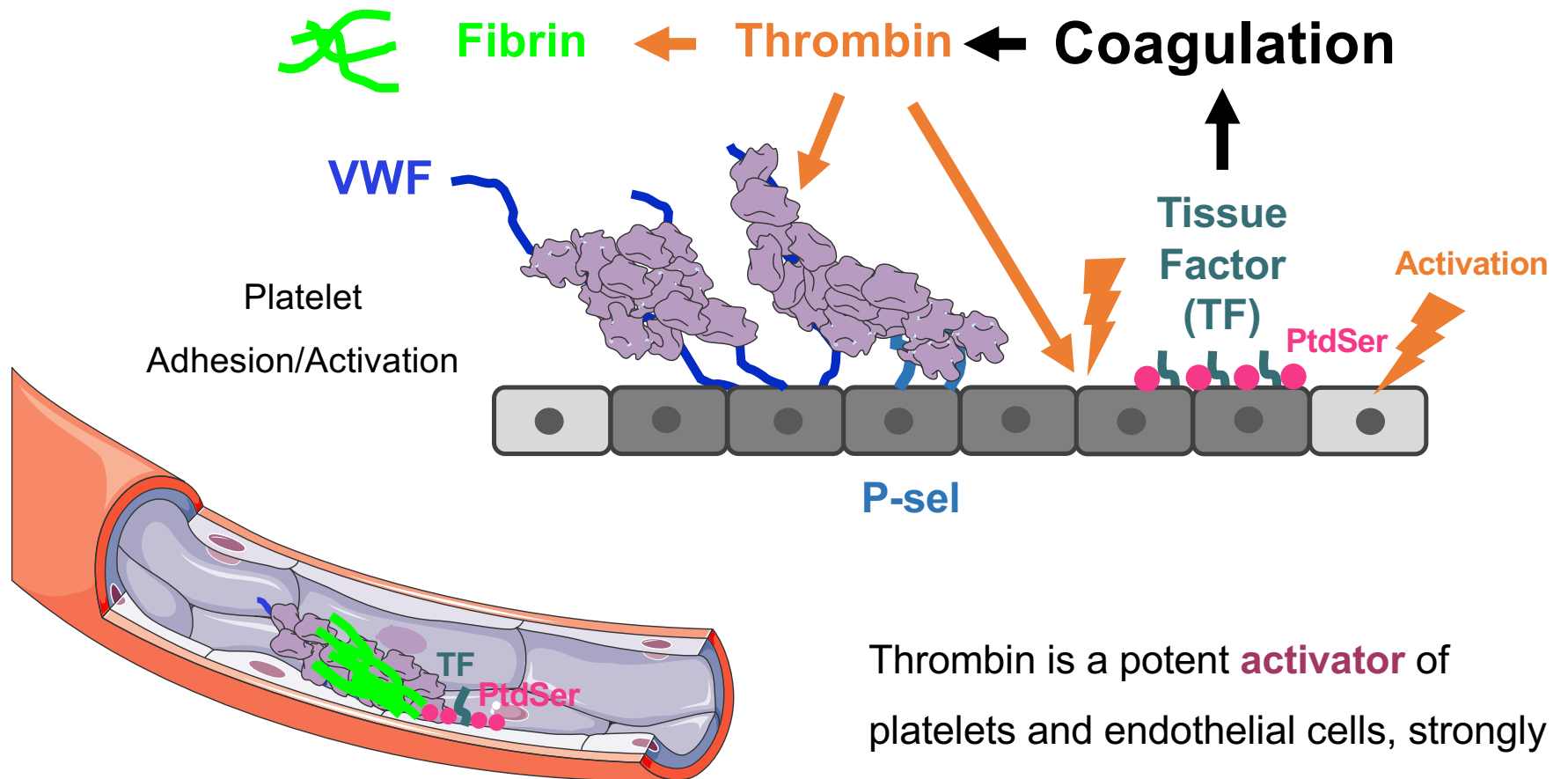
# Thrombus formation on the endothelial surface



# Thrombus formation on the endothelial surface

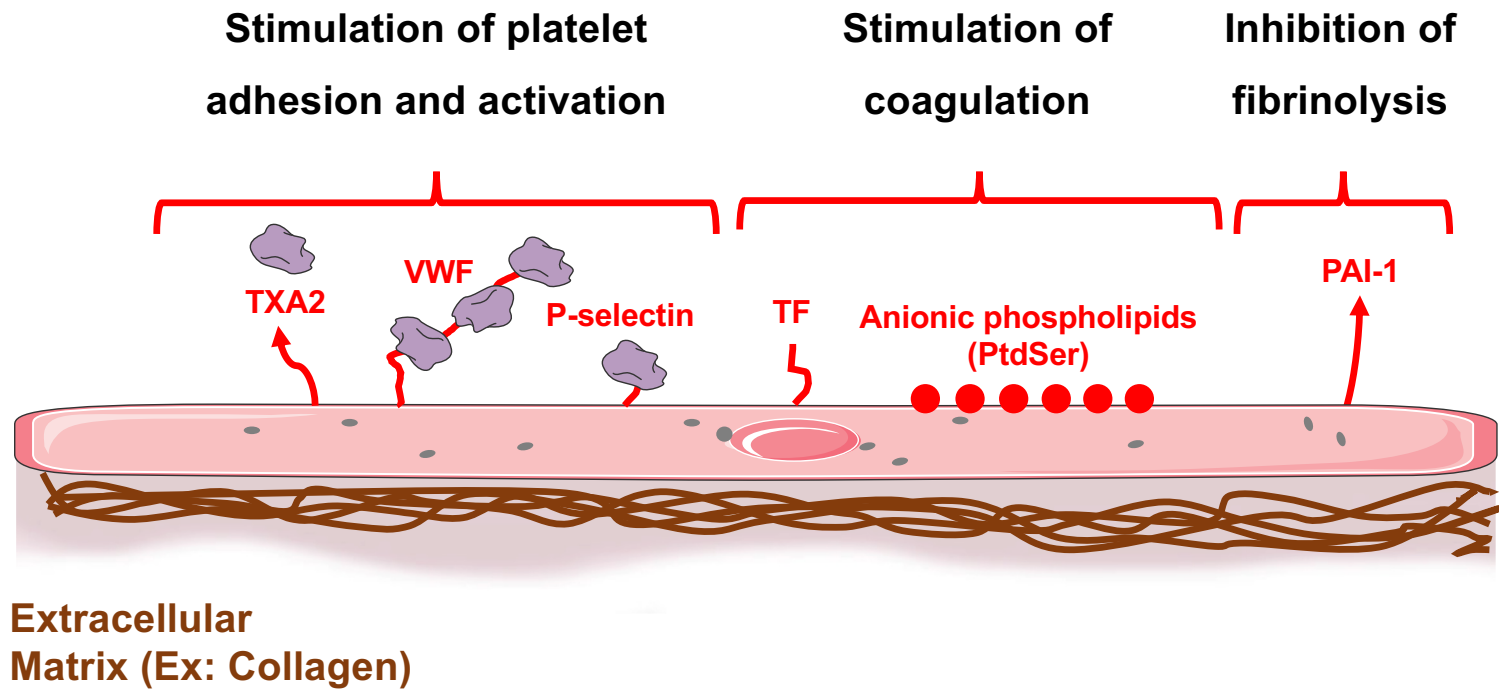


# Thrombus formation on the endothelial surface



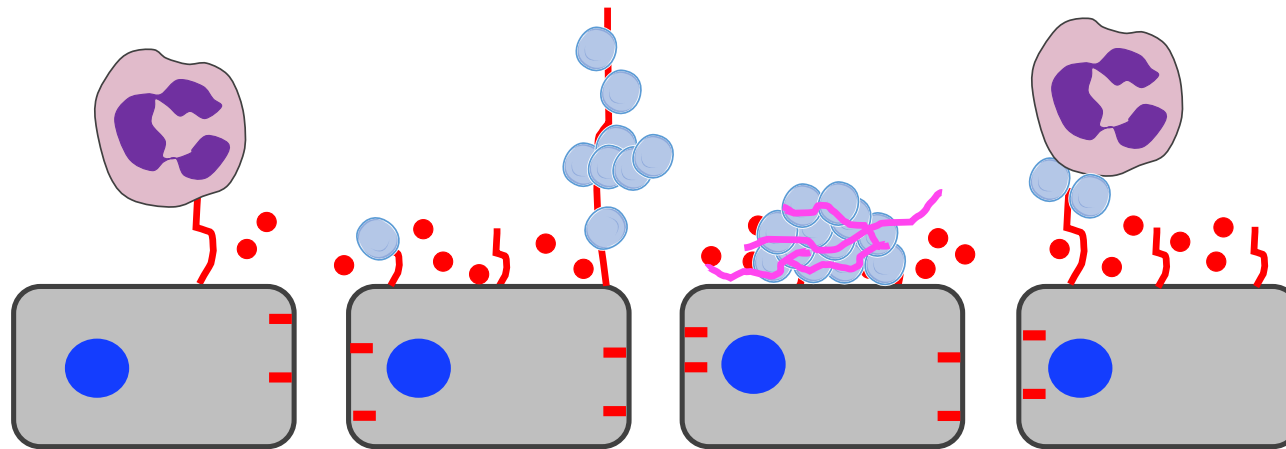
Thrombin is a potent **activator** of platelets and endothelial cells, strongly **fueling thrombo-inflammation**

# The **activated** endothelium is **thrombogenic**





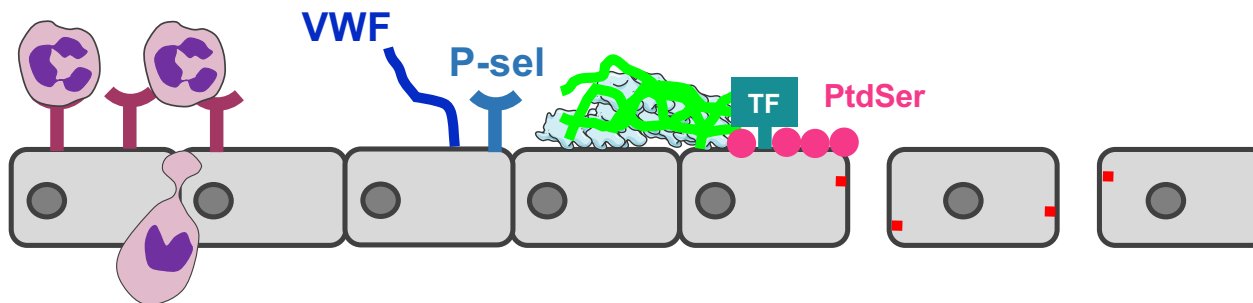
# Cardinal features of endothelial activation



An **activated** endothelium is

**Pro-Inflammatory**  
**Prothrombotic**  
**Permeable**

**Adhesion Molecules**  
E-selectin  
ICAM-1  
VCAM-1

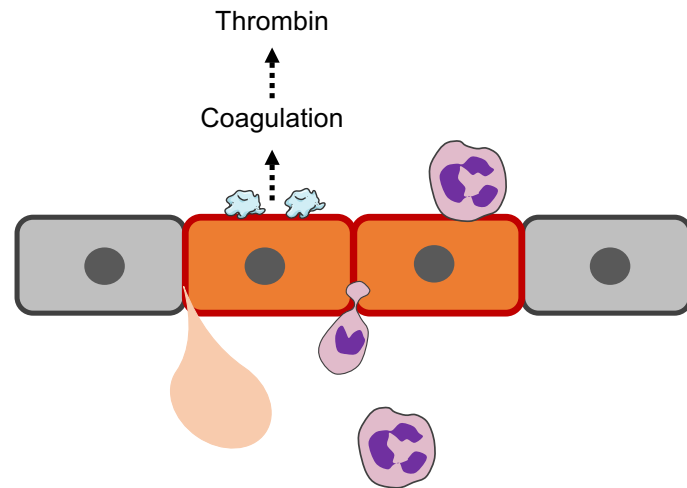


**Inflammation**

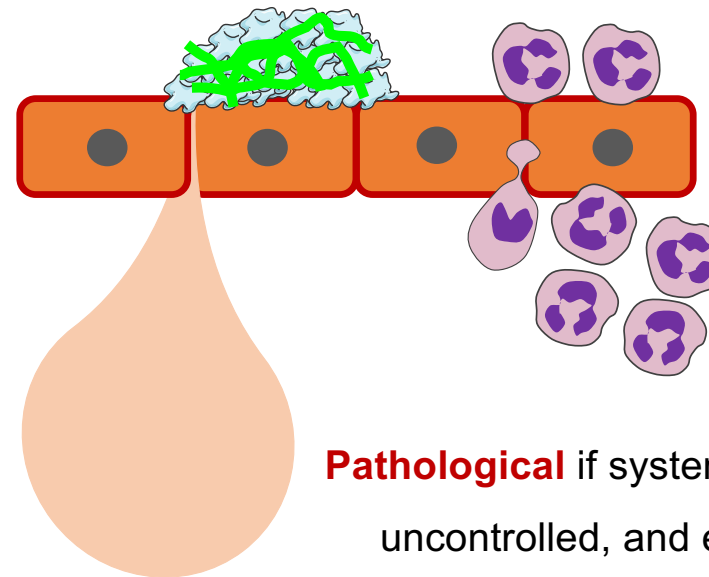
**Thrombosis**

**Vascular Permeability**

# Endothelial activation is physiological... but can be pathological



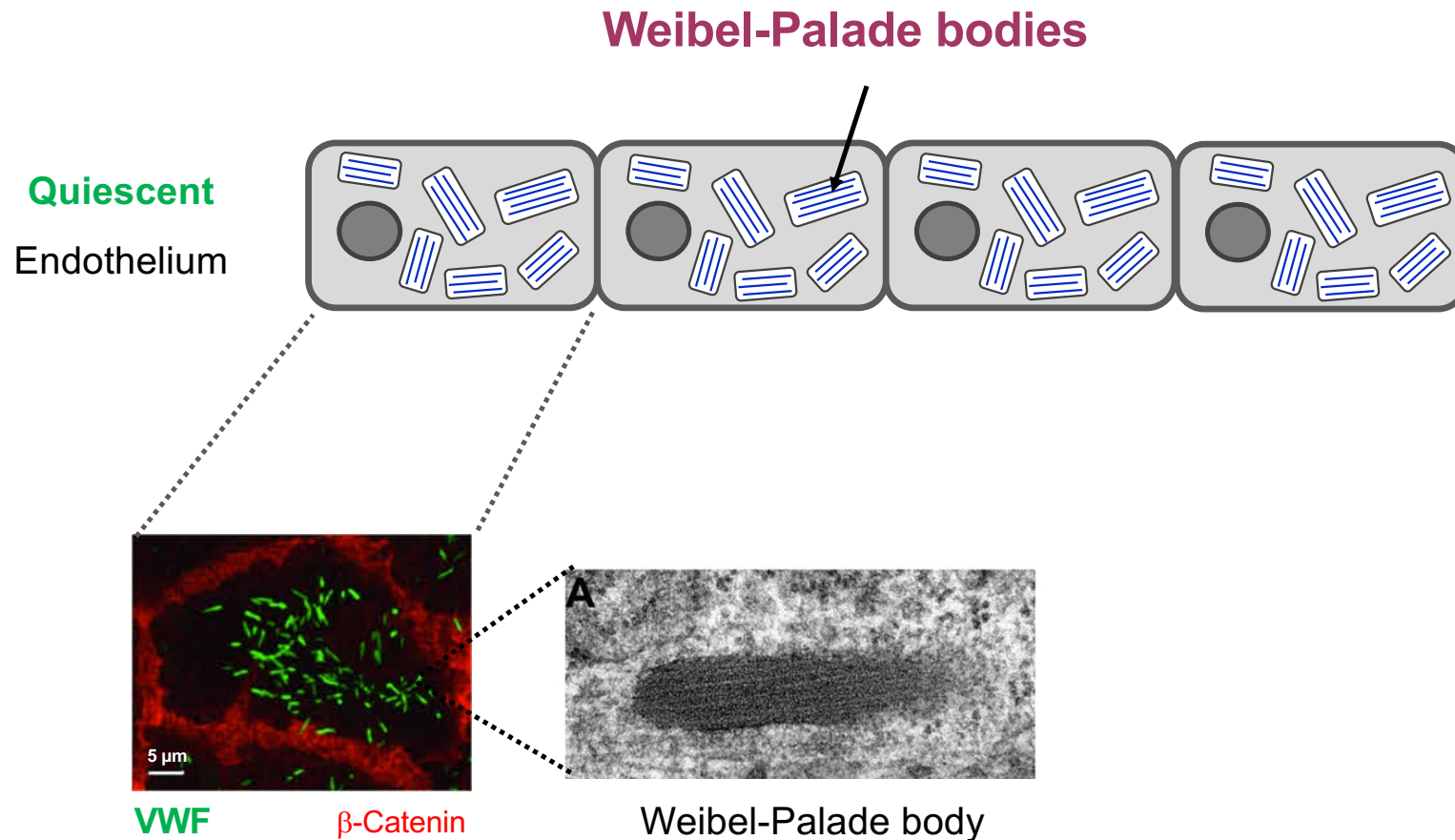
Physiological but must be  
**transient** and **self-limited**



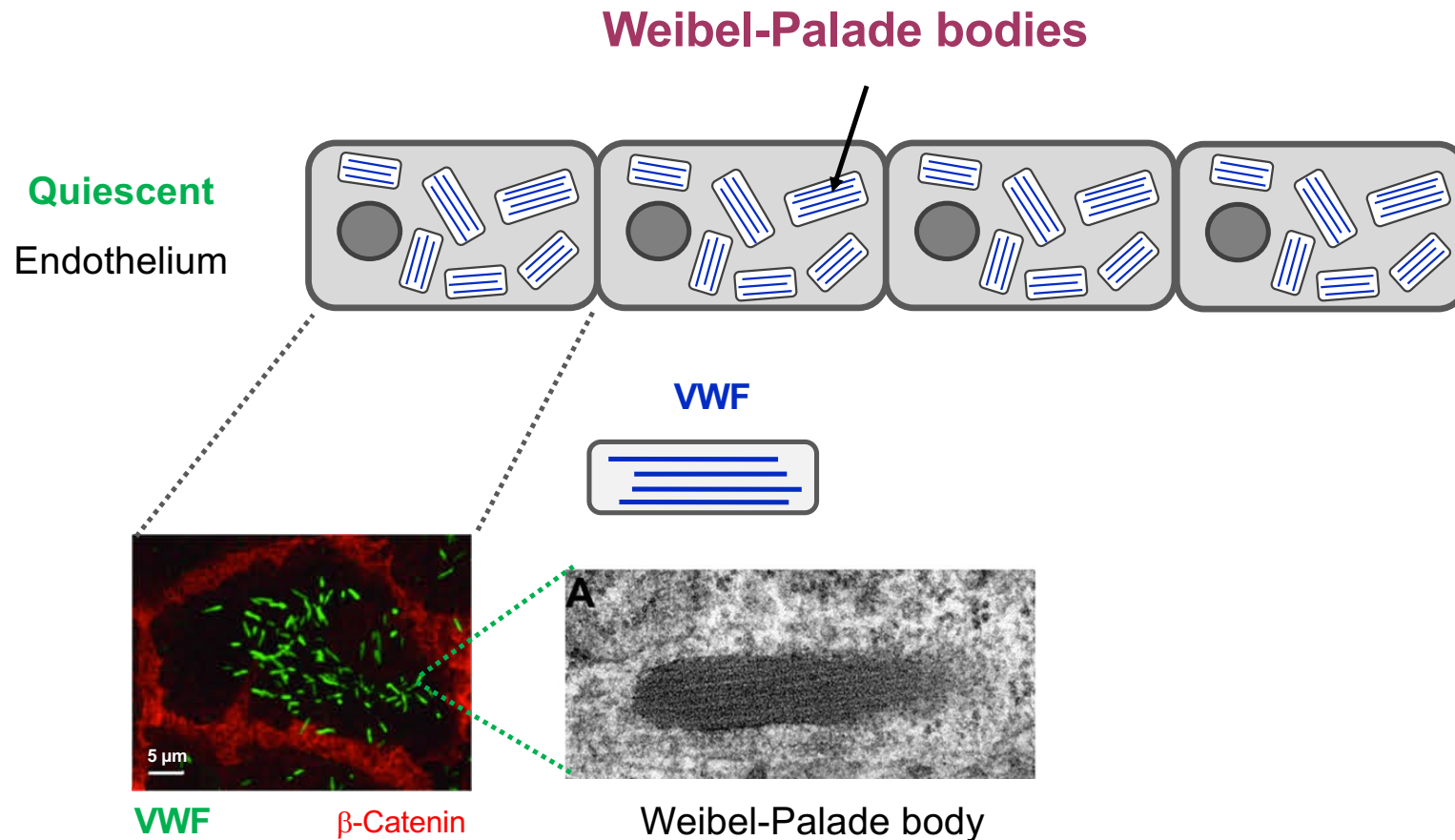
**Pathological** if systemic, prolonged,  
uncontrolled, and exacerbated

**Thrombo-  
Inflammation**

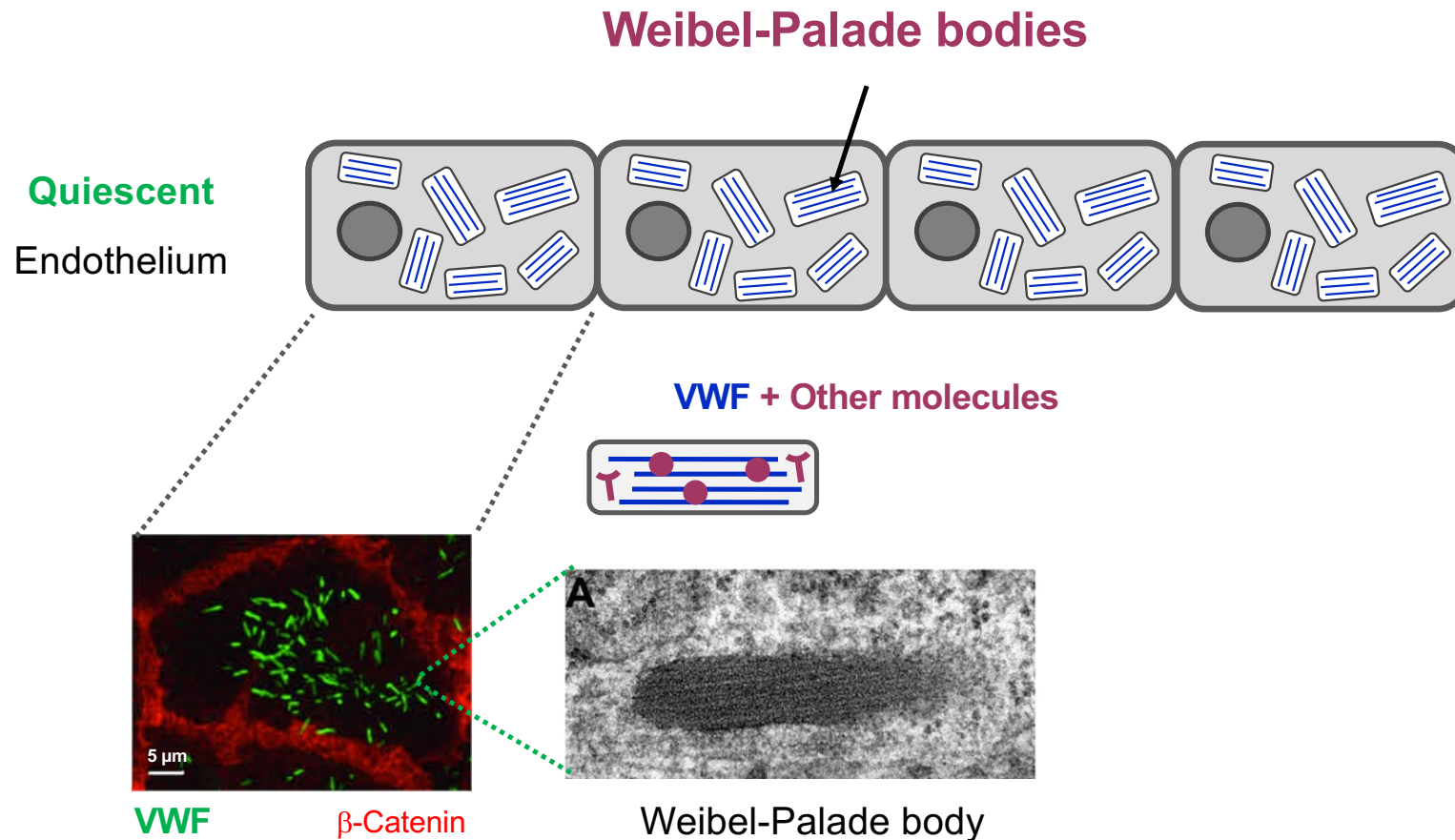
# Strong reactivity of the vascular endothelium



# Strong reactivity of the vascular endothelium

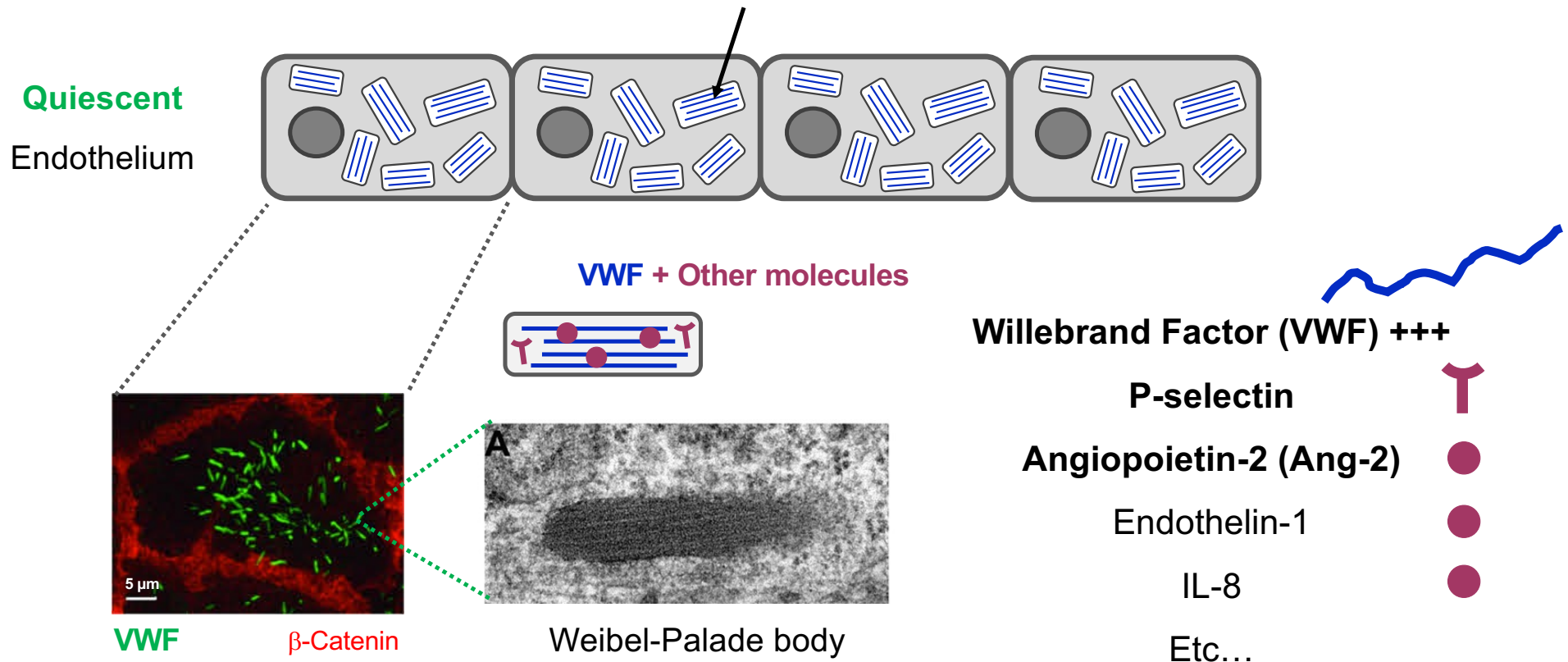


# Strong reactivity of the vascular endothelium

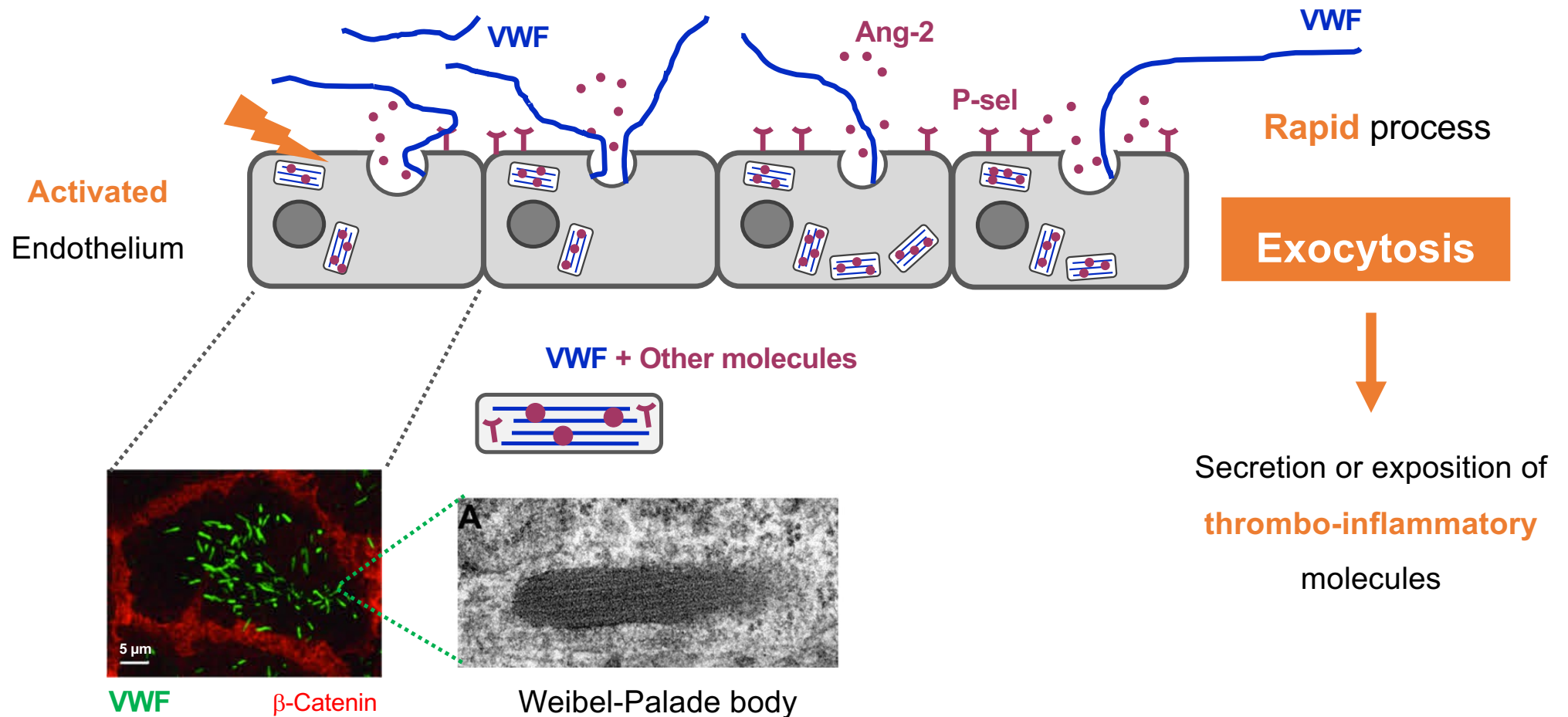


# Strong reactivity of the vascular endothelium

**Weibel-Palade bodies** = **secretion** granules specific for ECs

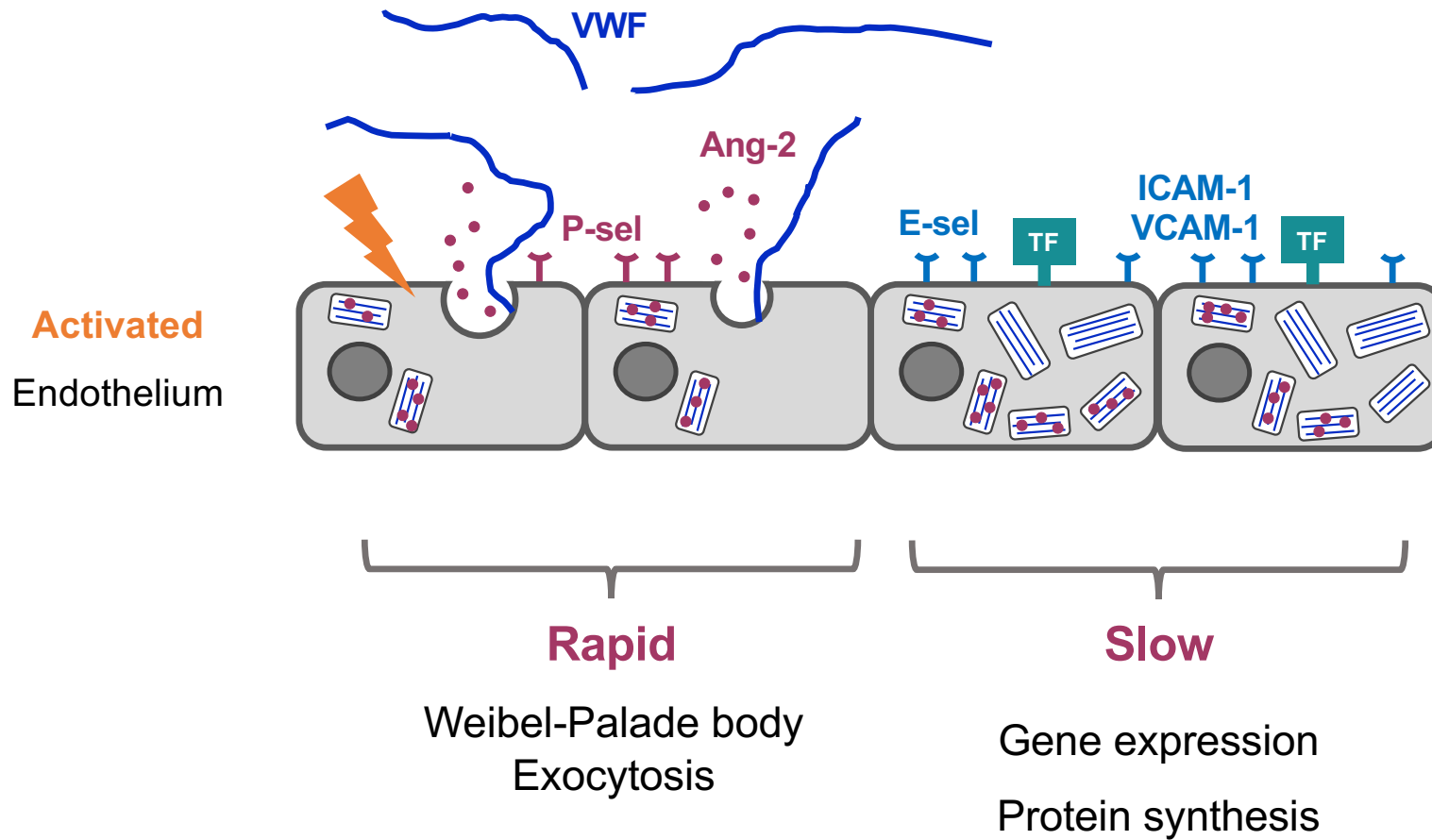


# Strong reactivity of the vascular endothelium



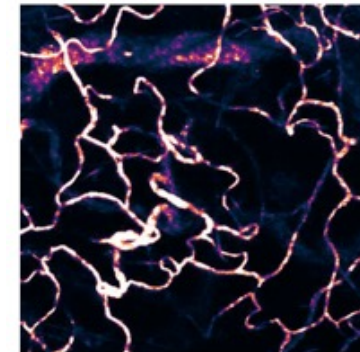
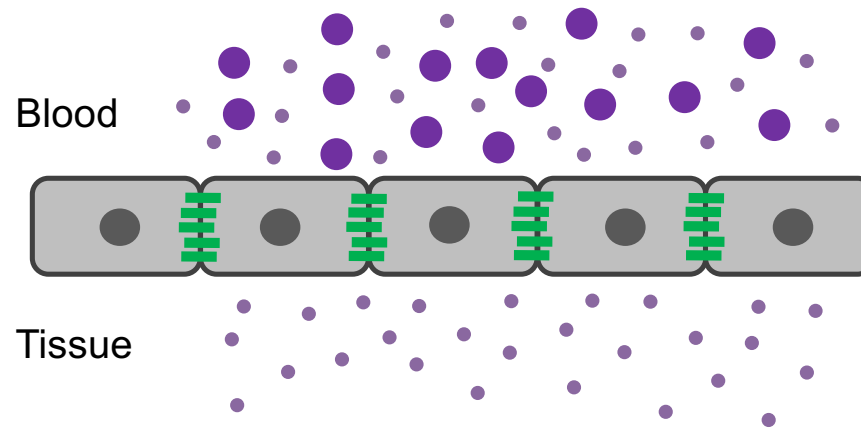
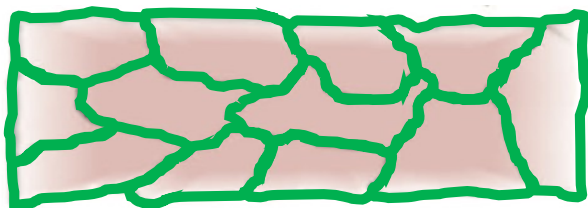


# Endothelial activation



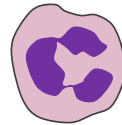
# Barrier function of the **quiescent** endothelium

**Expression and maintenance**  
of inter-endothelial junctions

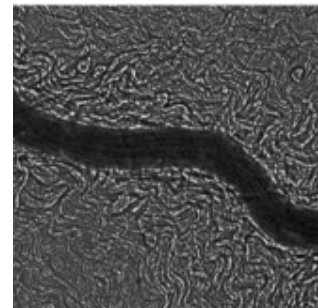
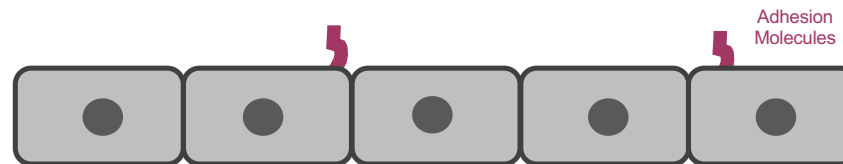


# The **quiescent** endothelium is naturally **anti-inflammatory**

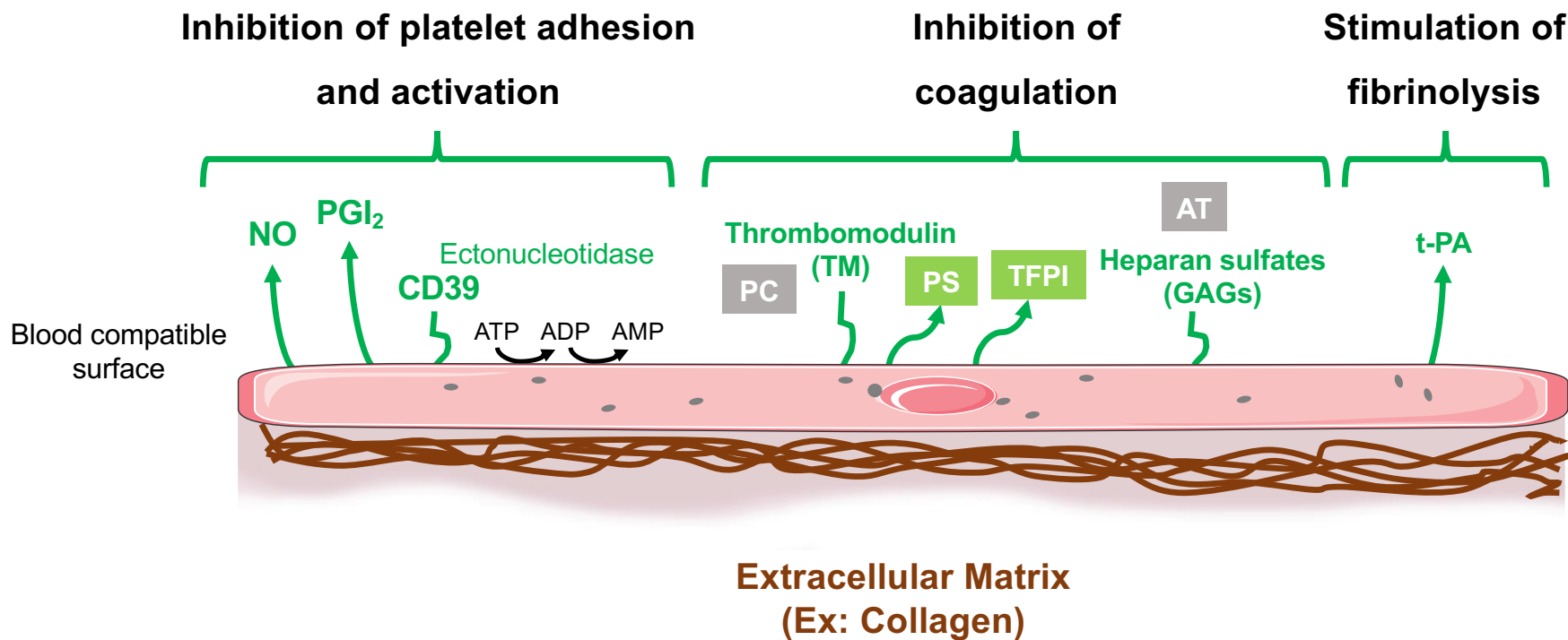
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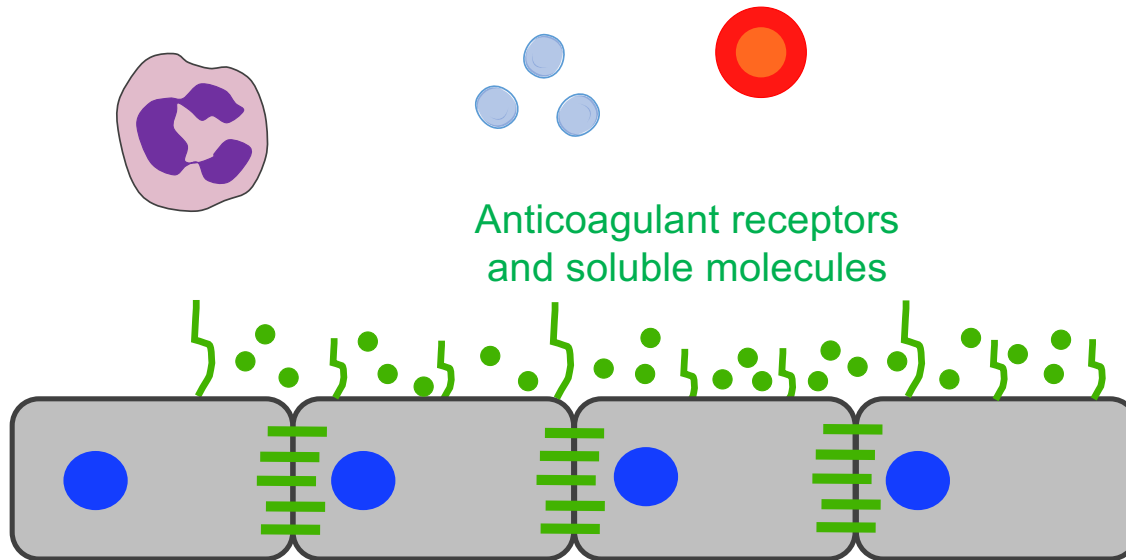
No or very little expression of **adhesion molecules** by a quiescent endothelium



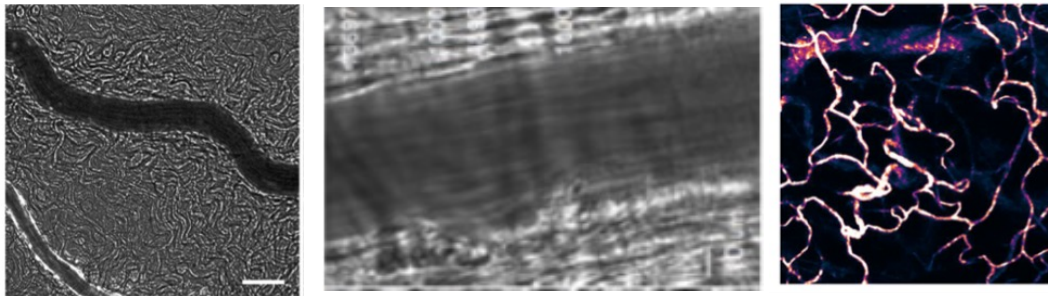
# The **quiescent** endothelium is naturally **antithrombotic**



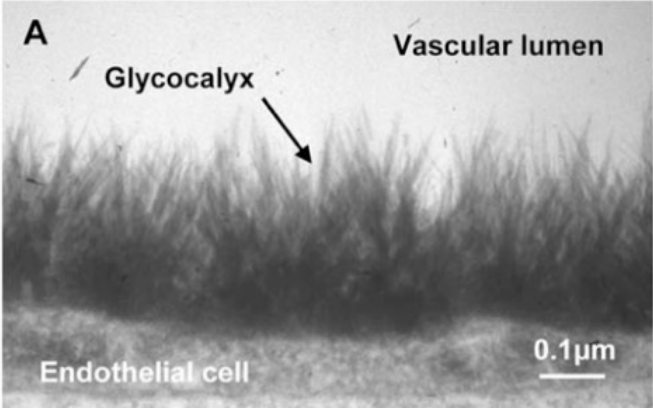
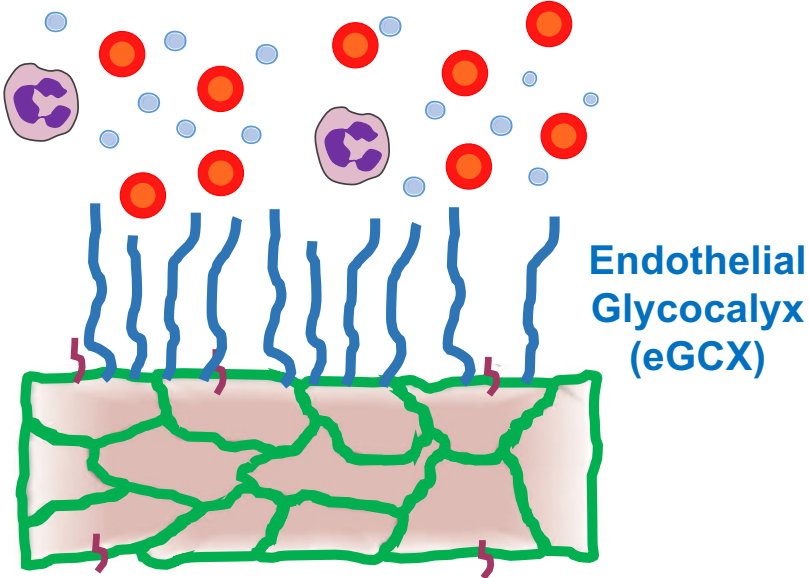
# « Quiescent » endothelium



**Impermeable**  
**Anti-Inflammatory**  
**Antithrombotic**



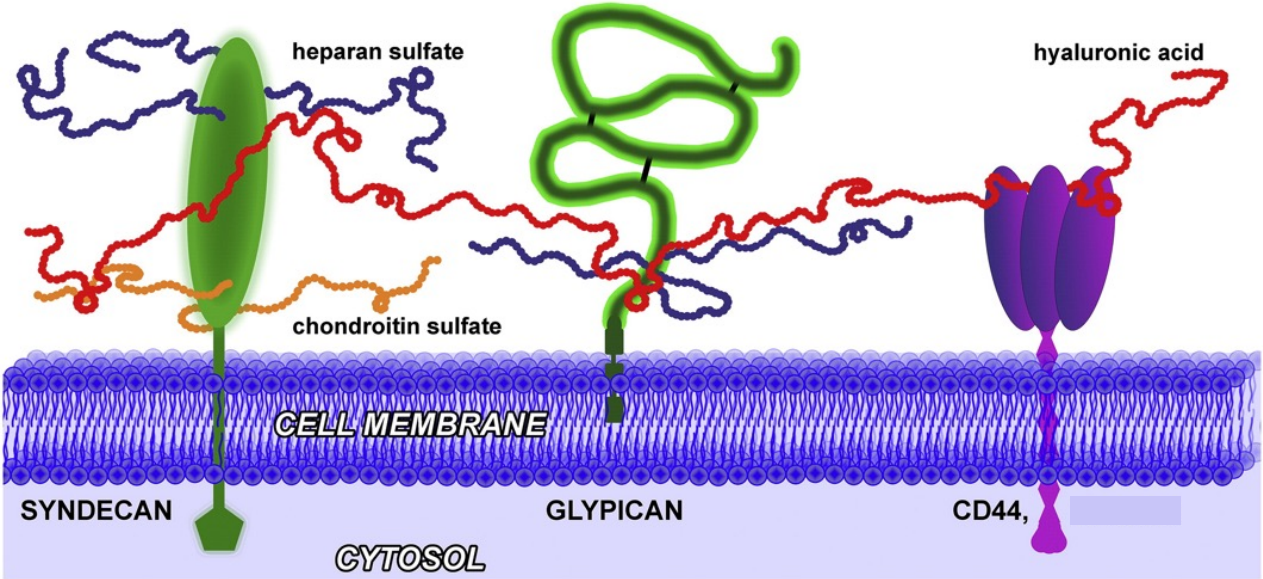
# These endothelial responses are modulated by the **glycocalyx**



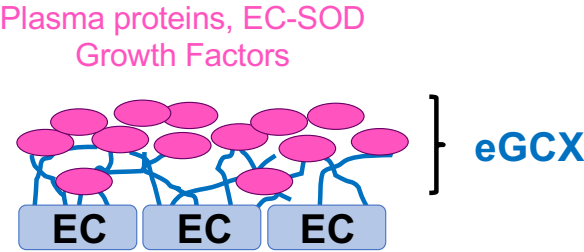
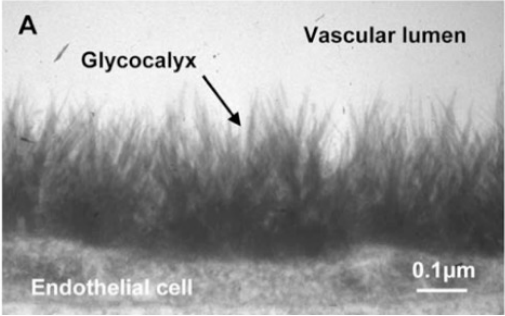
**GAGs**  
Glycosaminoglycans

Chappell *et al.* Cardiovasc Res 2009

# The endothelial **glycocalyx** (eGCX)

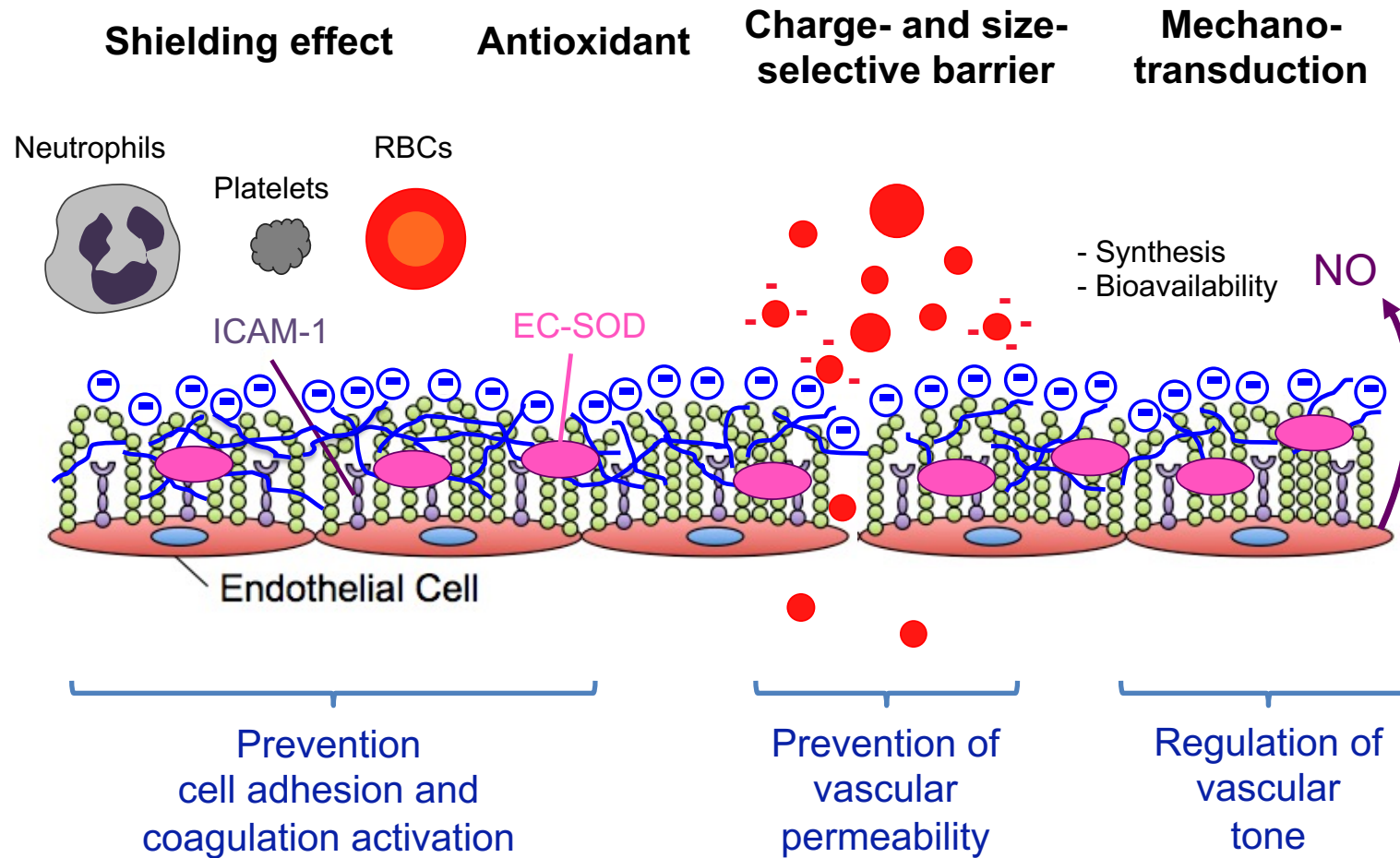


**Proteoglycans = Proteins + GAGs**

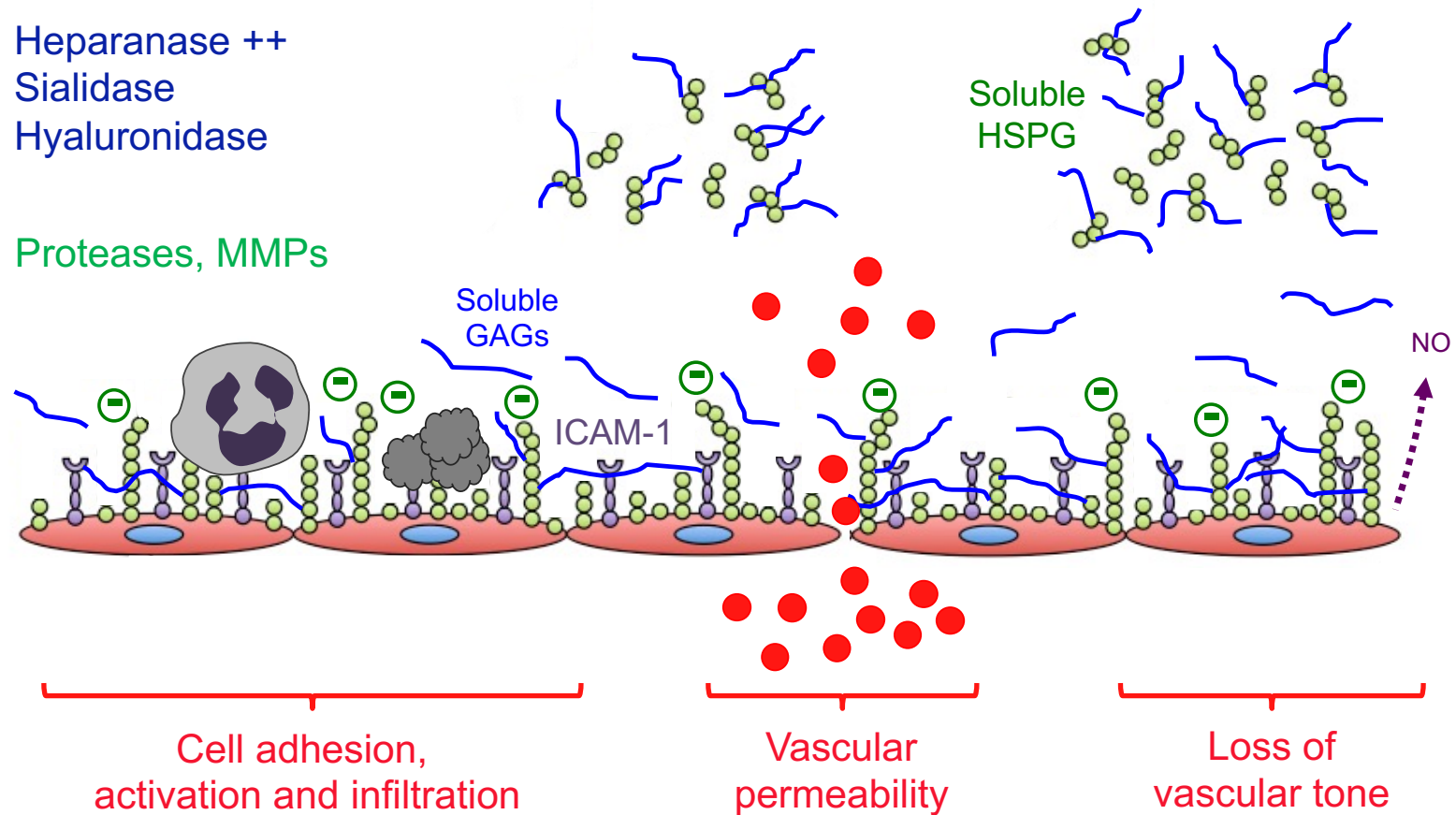




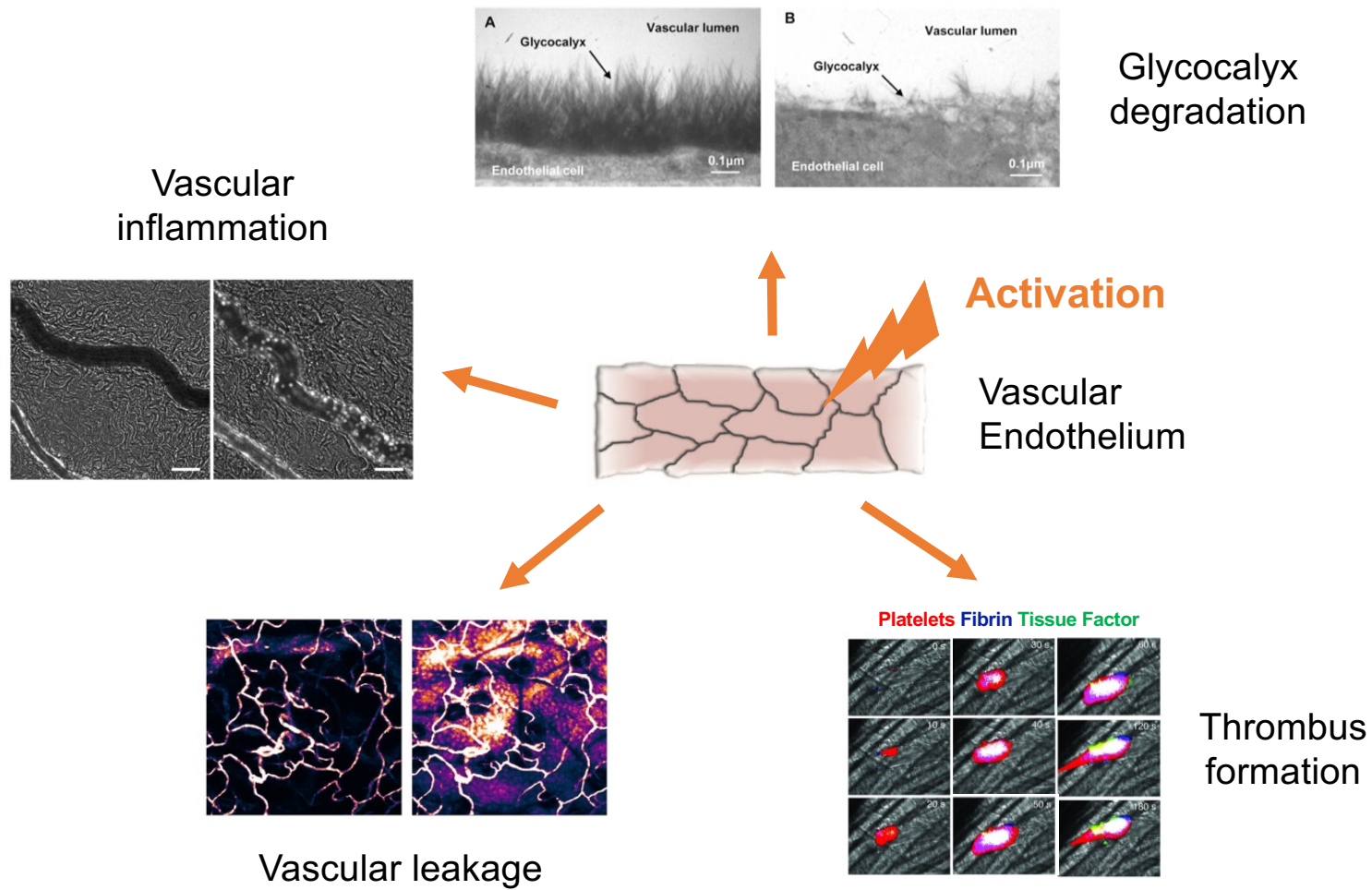
# Physiological functions of the endothelial glycocalyx



# Endothelial glycocalyx degradation

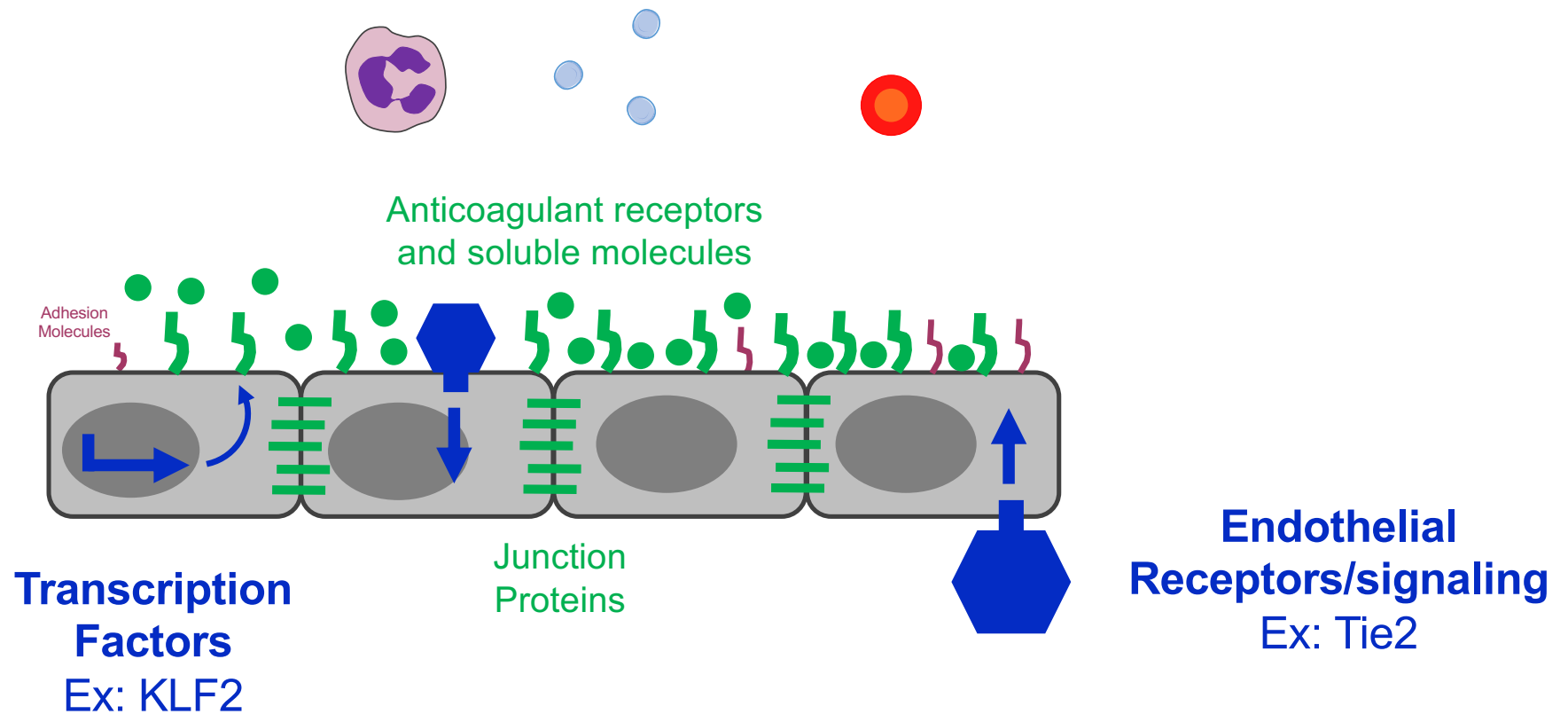


# Cardinal features of endothelial activation

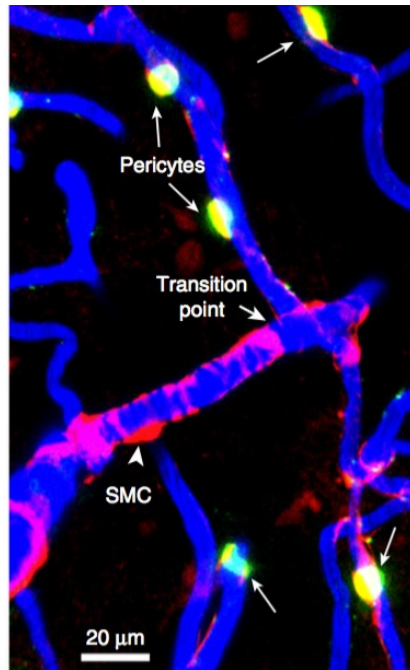


# How to maintain a quiescent endothelium ?

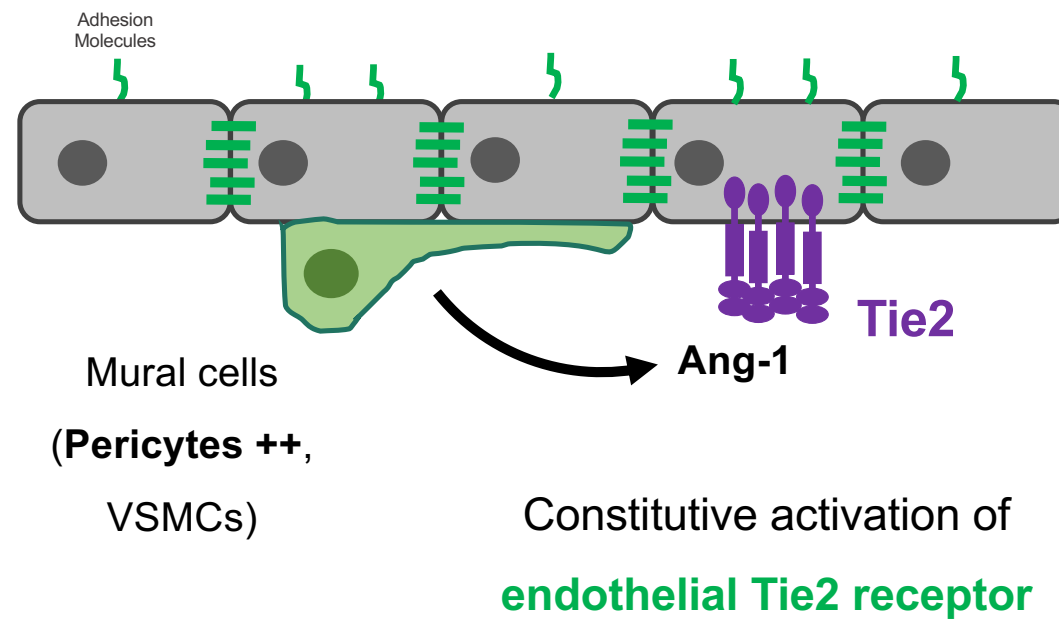
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# How to maintain a quiescent endothelium ?

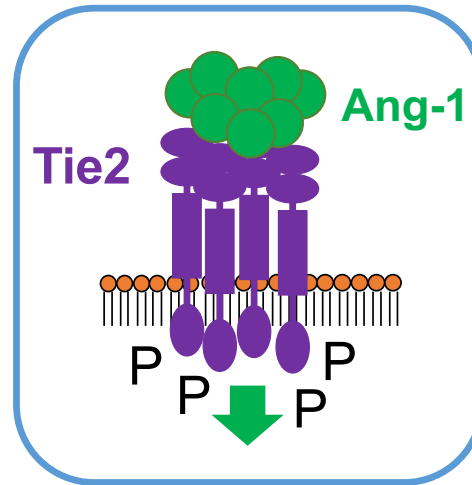


One example of **receptor/signaling** ensuring endothelial quiescence



# Tie2 signaling and endothelial quiescence

Tie2 = Receptor Tyrosine Kinase

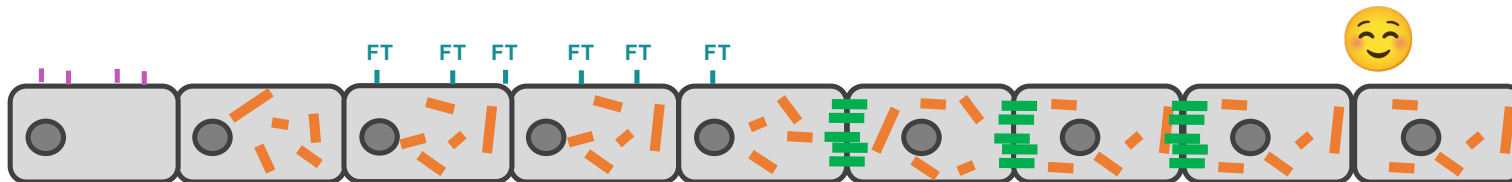


Anti-inflammatory  
Effects

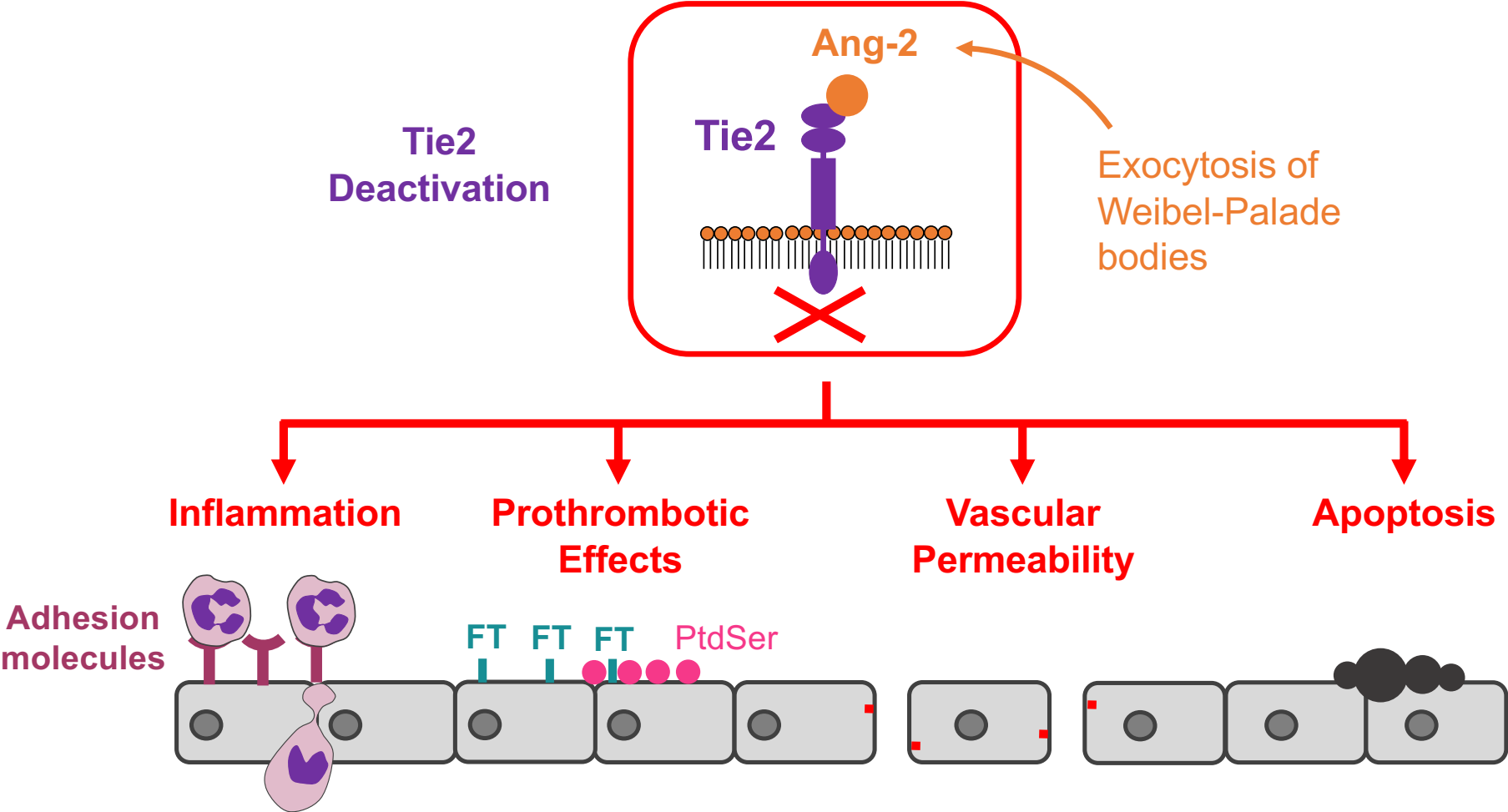
Antithrombotic  
Effects

Endothelial barrier  
protective effects

Survival



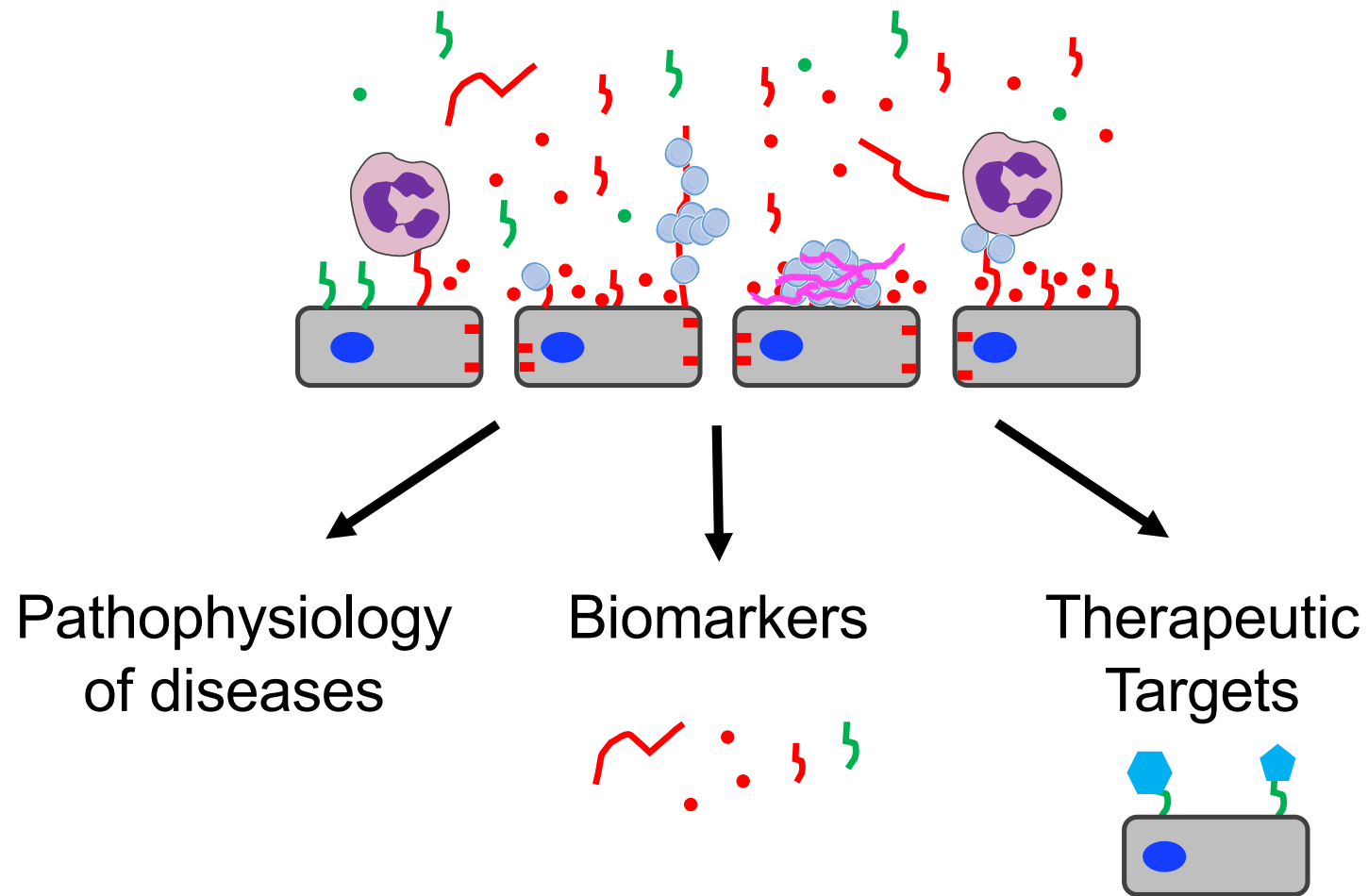
# Dysregulation of Tie2 signaling by Ang-2



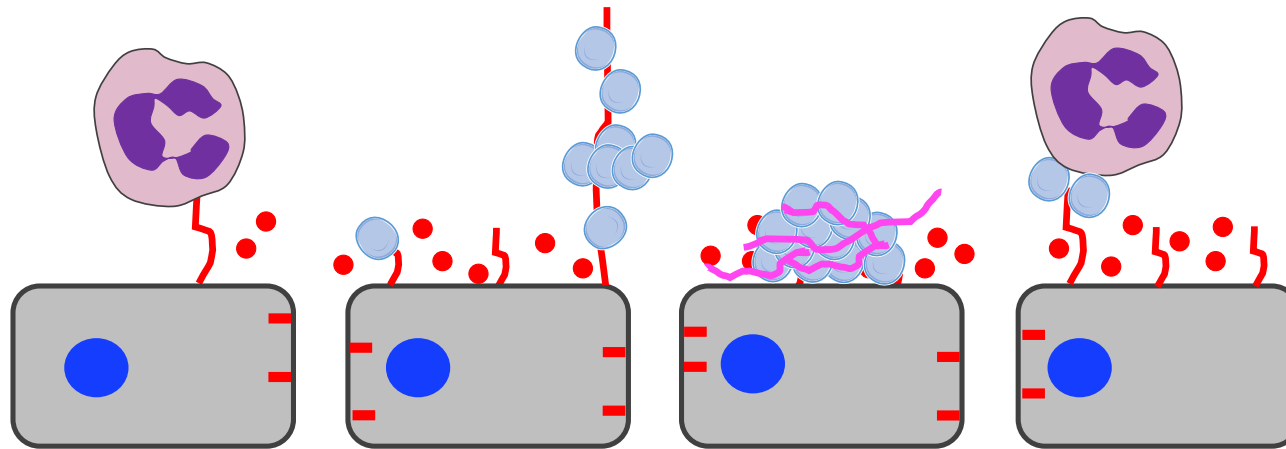


# Endothelial dysfunction in development of drugs & health products

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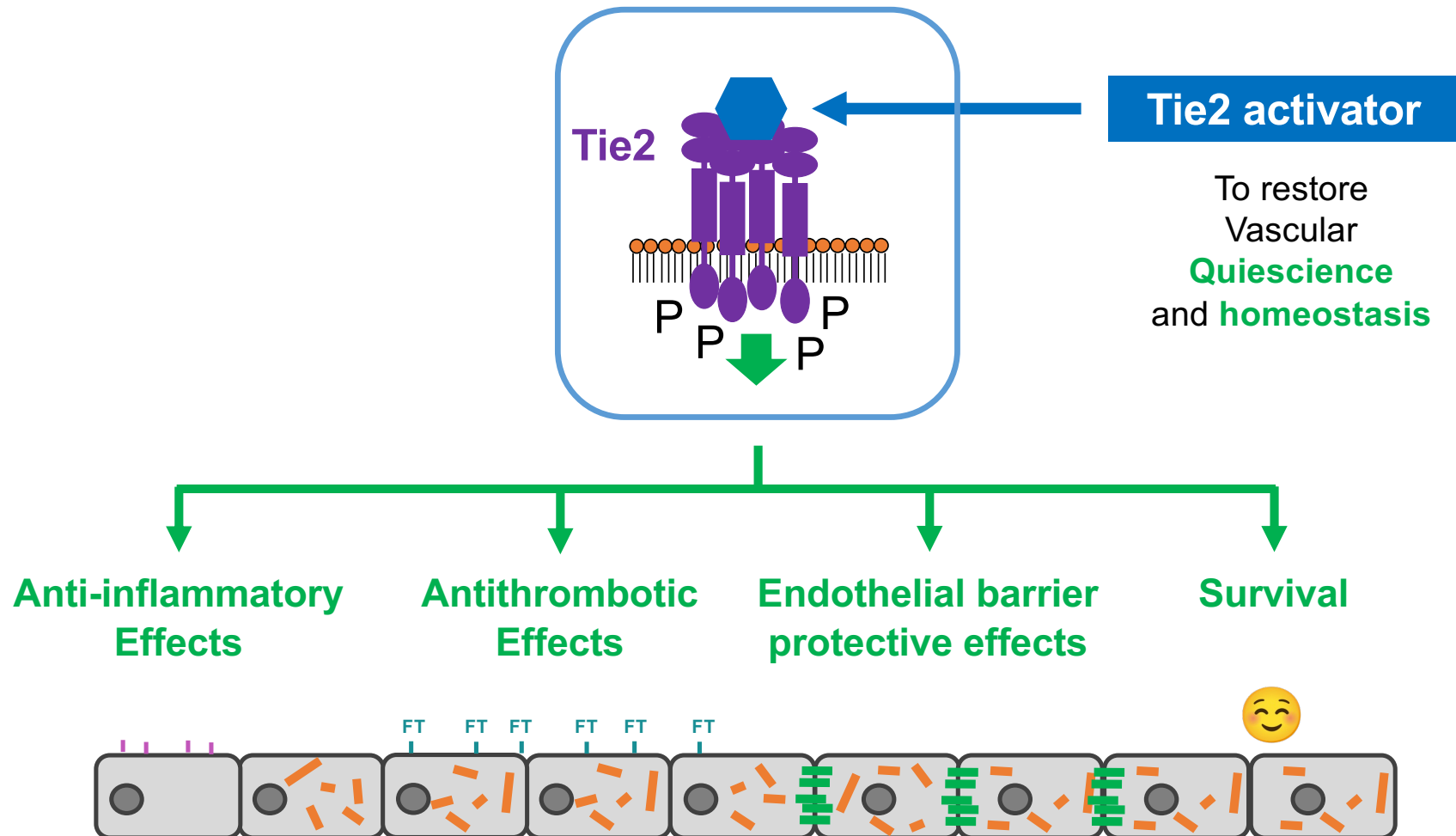
# Pathophysiological role of endothelial dysfunction



Drives and aggravates various **diseases**

Sepsis, Ischemic Stroke  
Sickle Cell Disease  
Atherosclerosis, Hypertension  
COVID and Long COVID  
+ Many others !

# Pharmacological activation of endothelial Tie2 receptor



# Questions and key messages

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- 1) What are the cardinal features of endothelial activation ? Briefly describe how endothelial cells drive each of these features.
- 2) Enumerate the so-called “endothelial adhesion molecules” and specify their role in endothelial functions.
- 3) What are the so-called Weibel-Palade bodies found in endothelial cells ? Enumerate some molecules contained within these bodies. How is called the process resulting in the release of their content and what are the effects of this release on the endothelial responses ?
- 4) What is the endothelial glycocalyx ? How does the endothelial glycocalyx modulate the physiological functions of the vascular endothelium ?
- 5) Describe one endothelial receptor implicated in the maintenance of endothelial quiescence, and the resulting functional effects of its activation on the vascular endothelium. Specify the nature and the cellular origin of its agonistic and antagonistic ligands.