# Platelet Function and Biology

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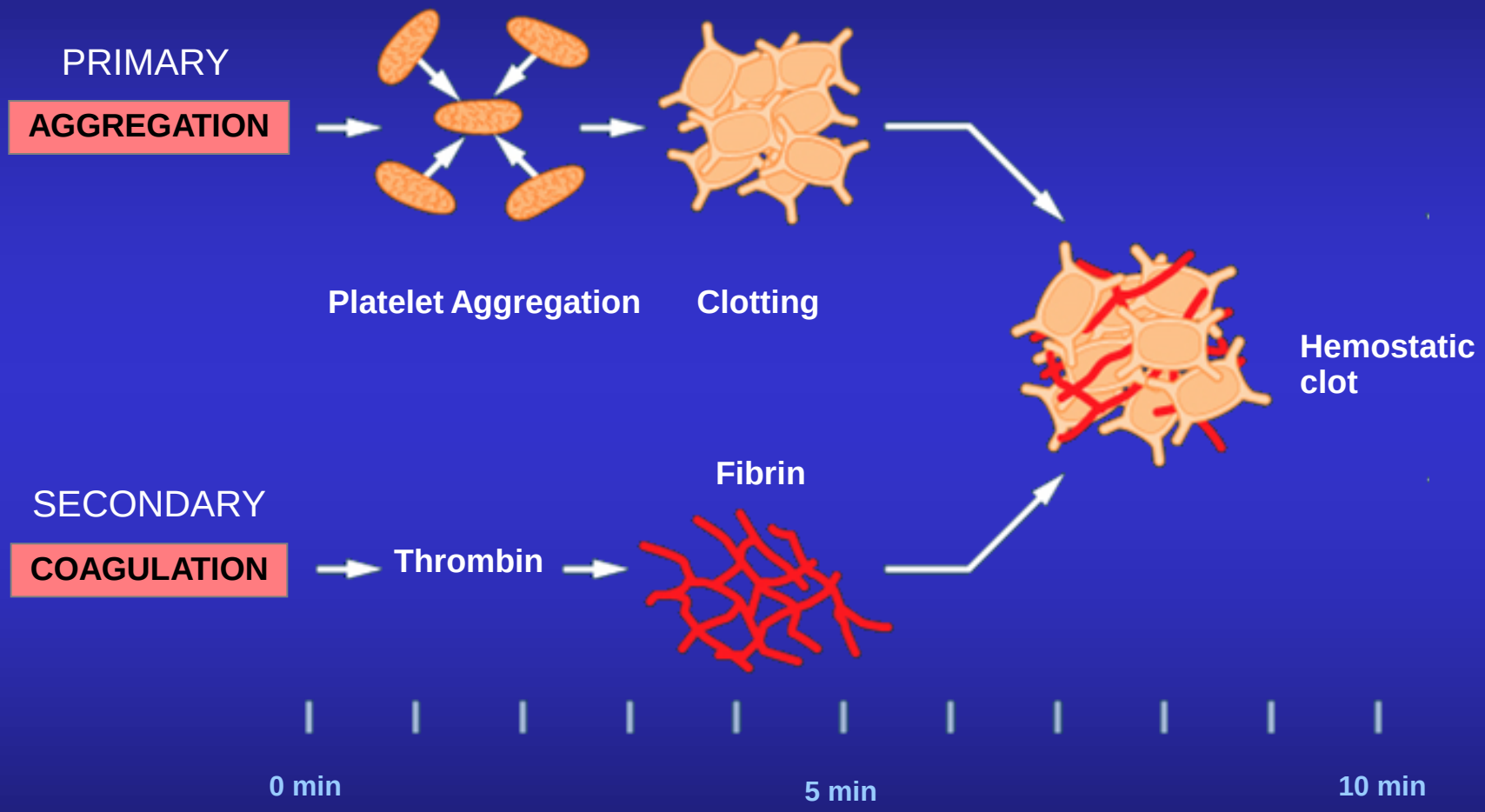
# Hemostatic Plug Formation



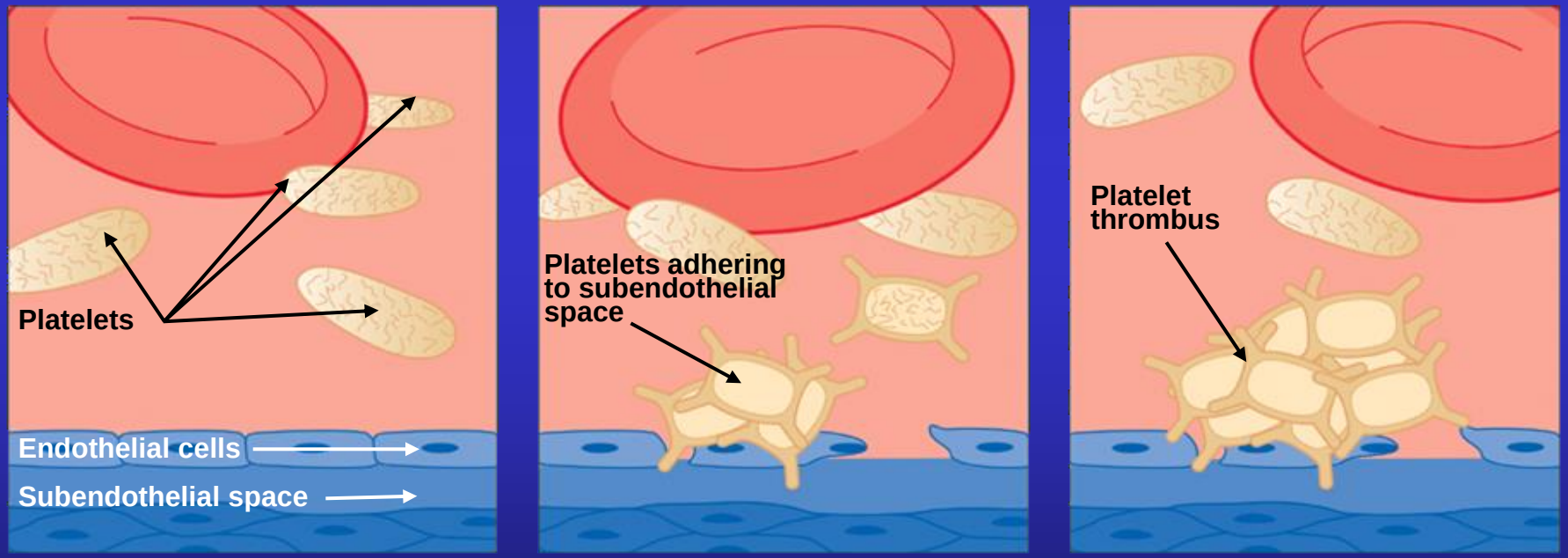

Adapted from: Ferguson JJ. *The Physiology of Normal Platelet Function*. In: Ferguson JJ, Chronos N, Harrington RA (Eds). *Antiplatelet Therapy in Clinical Practice*. London: Martin Dunitz; 2000: pp.15–35.

# Platelet Adhesion, Activation and Aggregation

Normal platelets in flowing blood

Platelets adhering to damaged endothelium and undergoing activation

Aggregation of platelets into a thrombus




NO, $PGI_2$, Ectonucleotidase

Adapted from: Ferguson JJ. *The Physiology of Normal Platelet Function*. In: Ferguson JJ, Chronos N, Harrington RA (Eds). *Antiplatelet Therapy in Clinical Practice*. London: Martin Dunitz; 2000: pp.15–35.

# Platelet Biology and Function

1. Platelet adhesion
2. Platelet activation
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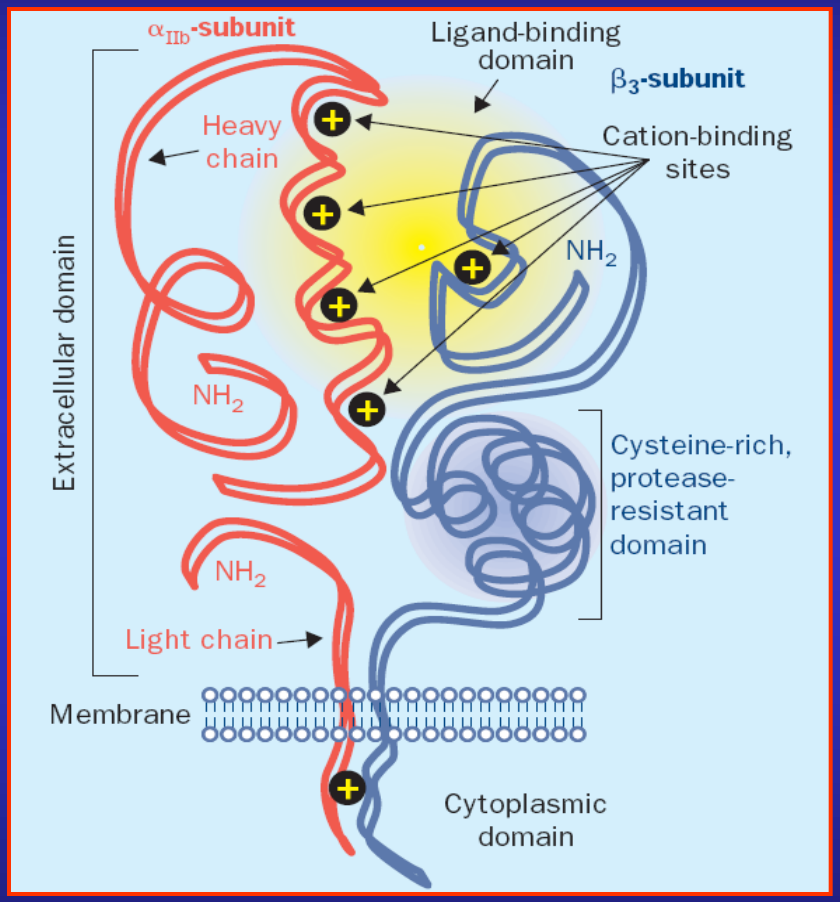
# Integrin

- α and β subunits
- Active and inactive state
- L-arginyl-L-glycyl-L-aspartate (RGD)
- “Inside out” signaling
- “Outside in” signaling




Integrin αIIbβ3

*Topol et al, Lancet 1999;353:227-31*

# Integrins in Cardiovascular System

- Platelets

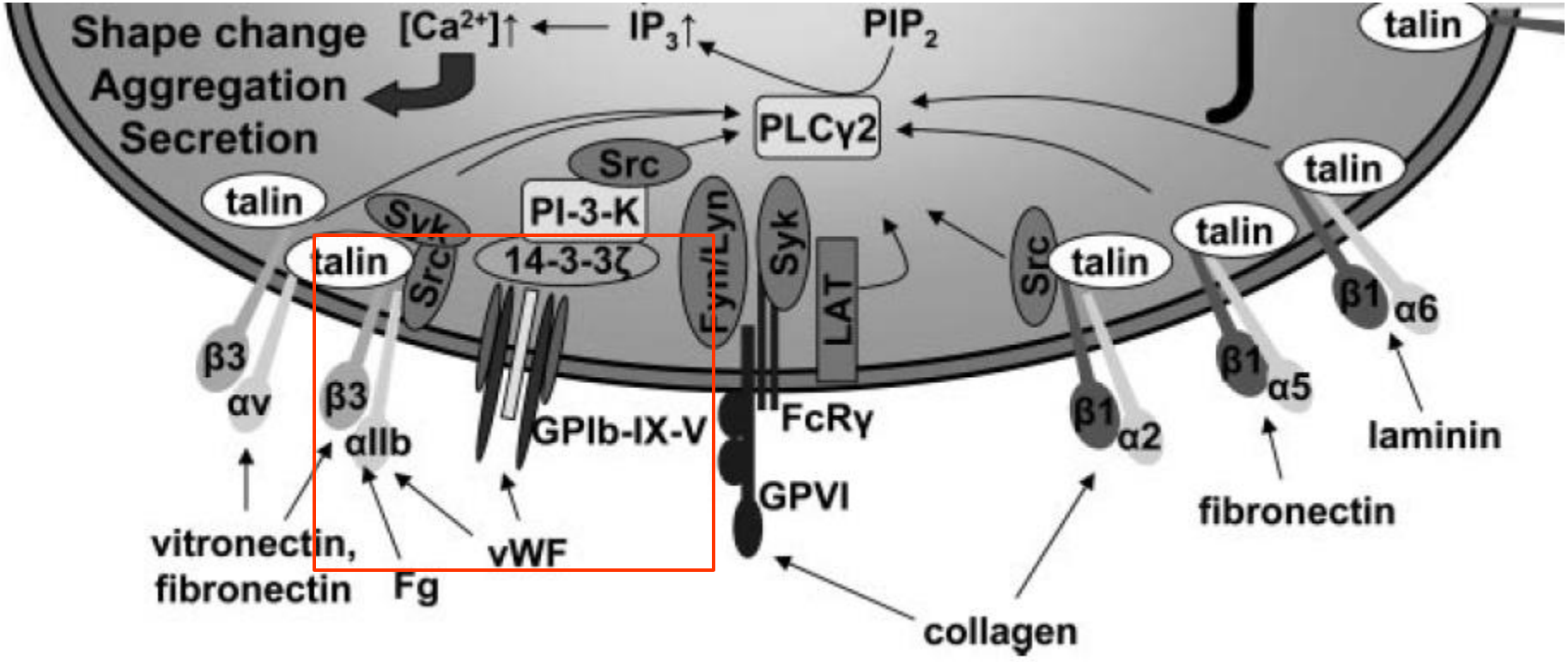
  $\alpha_{IIb}\beta_3$, $\alpha_v\beta_3$, $\alpha_2\beta_1$, $\alpha_5\beta_1$, $\alpha_6\beta_1$
- Endothelial cells

  $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_2\beta_1$, $\alpha_3\beta_1$, $\alpha_5\beta_1$, $\alpha_1\beta_1$, $\alpha_6\beta_1$
- Smooth muscle cells

  $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_2\beta_1$, $\alpha_3\beta_1$, $\alpha_5\beta_1$
- Leukocyte

  $\alpha_M\beta_2$

# Platelet Adhesion Receptors




vWF ↔ GPIb-IX-V

*Varga-Szabo D, et al. Arterioscler Thromb Vasc Biol 2008;28:403*

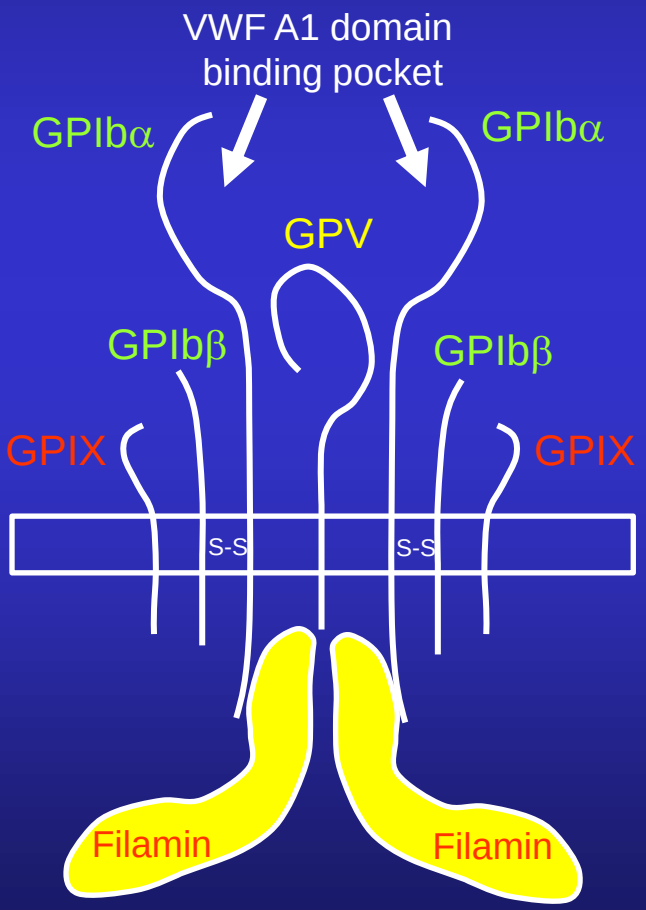
# Von Willebrand Factor (vWF)

- Found in the Weibel-Palade bodies of endothelial cells, in the $\alpha$-granules of platelets, and in the plasma
- A large polymer of disulfide-linked subunits, each comprising 2050 amino acid residues and up to 22 carbohydrate chains
- Platelet receptors; GPIb and integrin $\alpha$IIb$\beta$3
- No significant interactions with GPIb-V-IX under normal conditions.
- Conformational changes because of high shear forces and the immobilization on a surface
- A strong adhesive substrate when immobilized on exposed collagen at sites of injury

# GP Ib-V-IX Complex

- Four different genes encode the receptor complex
  - α-subunits of GP Ib (135 kDa); the major functional subunit
  - β-subunits of GP Ib (25 kDa)
  - GP IX (22kDa)
  - GP V (88 kDa)

- Bernard-Soulier syndrome
  - Lack or dysfunction of GP Ib-V-IX
  - A congenital bleeding disorder characterized by mild thrombocytopenia, giant platelets, and inability of the cells to aggregate in response to ristocetin



# Platelet Biology and Function

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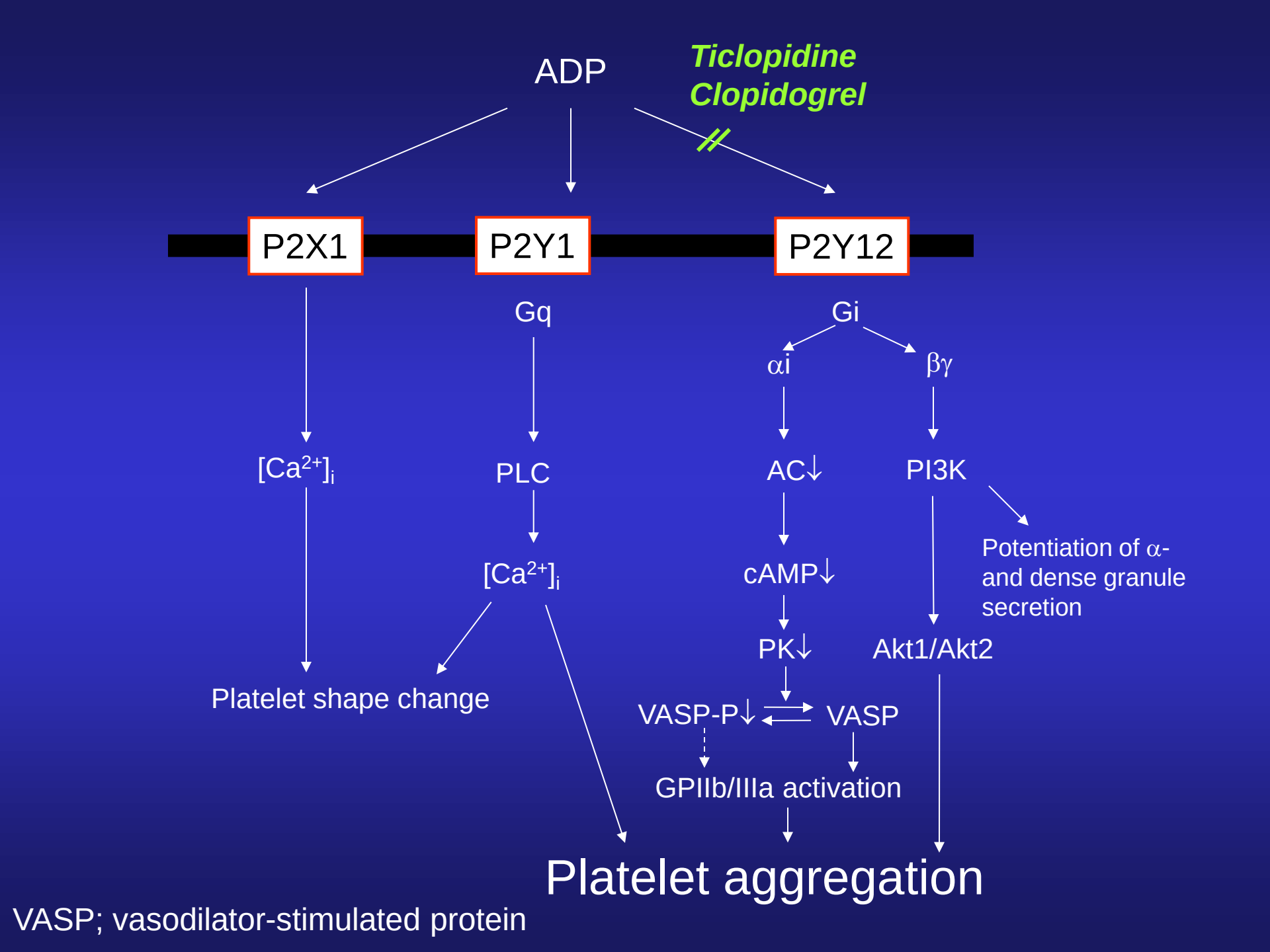
# Platelet Activation

- Rapid changes in platelet morphology
  - From smooth disks into irregular spheroids
  - Extrusion of filopodia, which not only enhance adhesion but also are rich in GP IIb/IIIa receptors
- Granule secretion (ADP), and generation of thromboxane $A_2$
- Involvement of the cell surface in coagulation reactions; thrombin generation
- Platelet aggregation

# ADP

- Stored at high concentrations in dense granules of platelets, and released on platelet activation.
- Released ADP strongly activates platelets in an autocrine and paracrine fashion.
- It can also be released from damaged cells at places of vascular injury.
- Platelet activation by ADP is mediated by 2 G protein-coupled receptors, P2Y1 ($G_q$) and P2Y12 ($G_{i2}$).



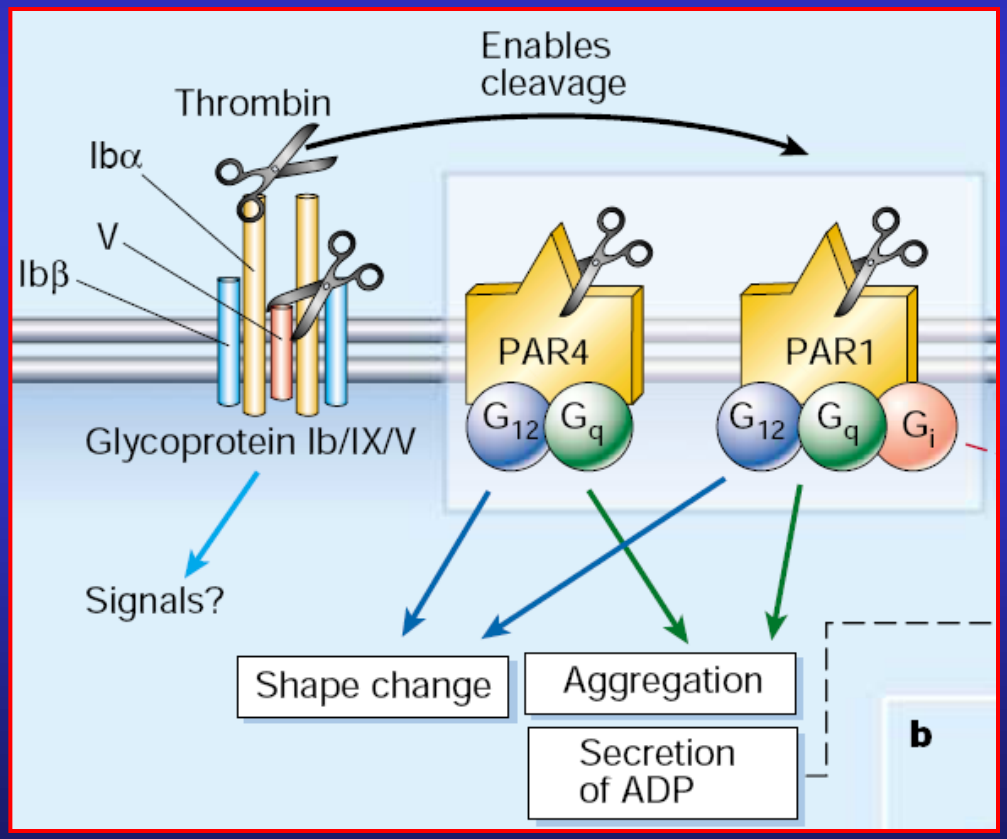

VASP; vasodilator-stimulated protein

# Thrombin

- Thrombin formation after disruption of the vascular endothelium. Thrombin formation takes place on cellular surfaces including that of activated platelets.
- Protease-activated receptors (PARs)
  - G protein-coupled receptors
  - PAR1 and PAR4 on human platelets
  - PAR1; at low thrombin concentrations
  - PAR4; only at high thrombin concentrations
- SCH 530348
  - an oral reversible PAR1 antagonist

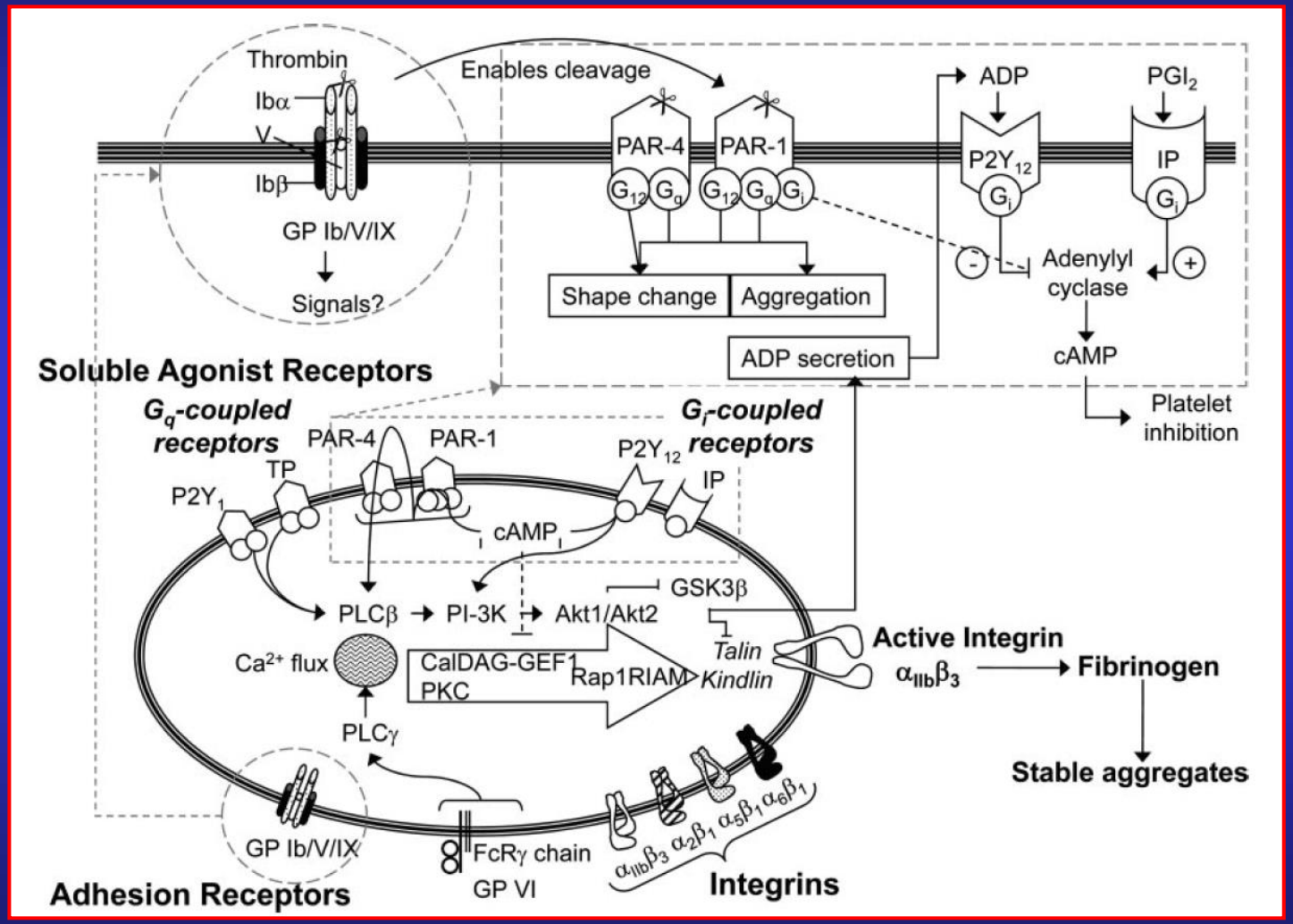
# Thrombin; signaling

- Thrombin mediated cleavage of the extracellular domain of the receptor and exposure of a “tethered ligand” at the new end of the receptor
- Signal transduction
  - Activation of PLC and PKC
  - Autoamplification through the production of $TXA_2$, the release of ADP, and generation of more thrombin on the platelet surface




*Brass S. Nature 2001;413:26*

# Role of G protein–coupled Receptors in the Thrombotic Process




CalDAG-GEF1, calcium and diacylglcerol-regulated guanine-nucleotide exchange factor 1
RIAM, Rap1-GTP–interacting adapter molecule

*2008 Platelet Colloquium Participants, ATVB 2009;29:449-457*

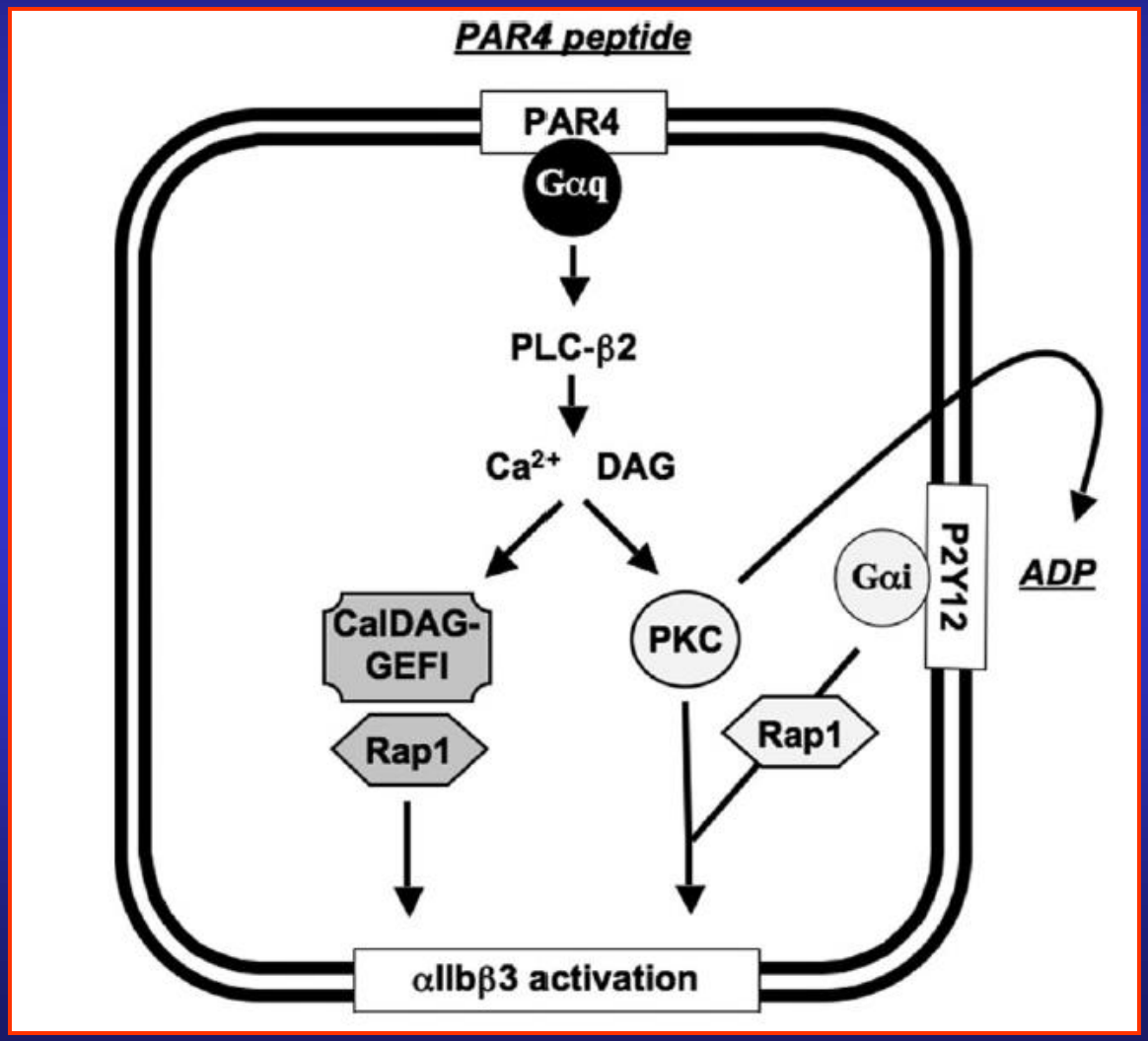
# Rap1b/CalDAG-GEFI

- A small GTP binding protein of the Ras family
- Deficiency of Rap1b in platelets leads to defective $\alpha IIb\beta 3$ activation, prolonged bleeding times, and protection against arterial thrombosis.
- Activation of Rap1b is controlled by
  1. CalDAG-GEFI; rapid but reversible Rap1 activation
  2. Protein kinase C (PKC); sustained Rap1 activation
  - ❖ Cal-DAG-GEFI deficiency
    - impaired platelet aggregation responses to ADP or TxA2 ex vivo
    - prolonged bleeding times and protection from arterial thrombosis in vivo.

CalDAG-GEFI; $Ca^{2+}$ and diacylglycerol-regulated guanine-nucleotide-exchange factor I

*Nieswandt B et al, J Thromb Haemost, 2009;7(Suppl 1): 206*

# Schematic representation of the CalDAG-GEFI-dependent and PKC-dependent signaling pathways leading to αIIβ3 activation in mouse platelets




*Cifuni SM et al, Blood 2008;112:1696-1703*

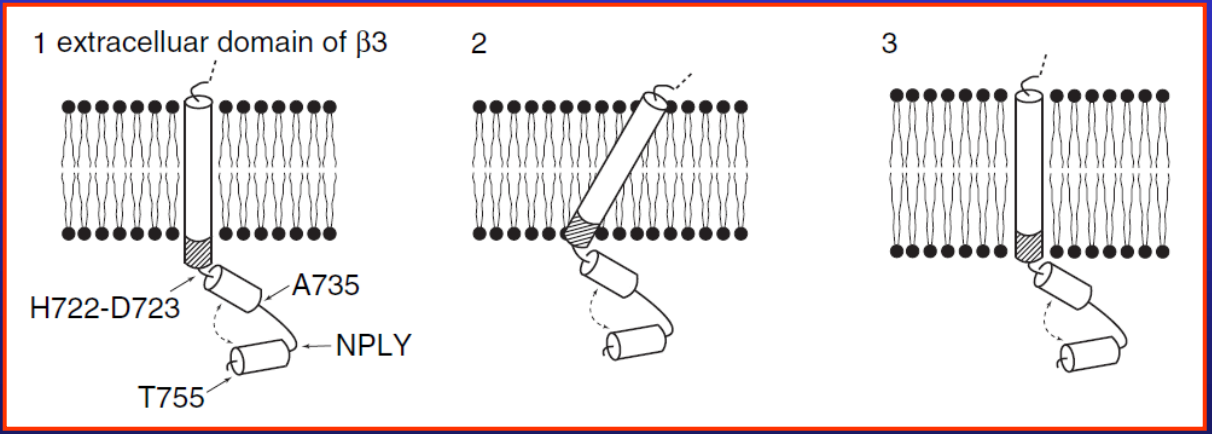
# Protein Kinase Akt

- A principal target for PI-3K signaling
- Both Akt1 and Akt2 isoforms in platelets.
- Both Akt1 and Akt2 are required for thrombus formation in mice
- Glycogen synthase kinase (GSK)-3 suppresses platelet function and thrombosis in mice
- Akt mediated phosphorylation of GSK-3 inhibits the kinase activity of the enzyme, and with it, its suppression of platelet function

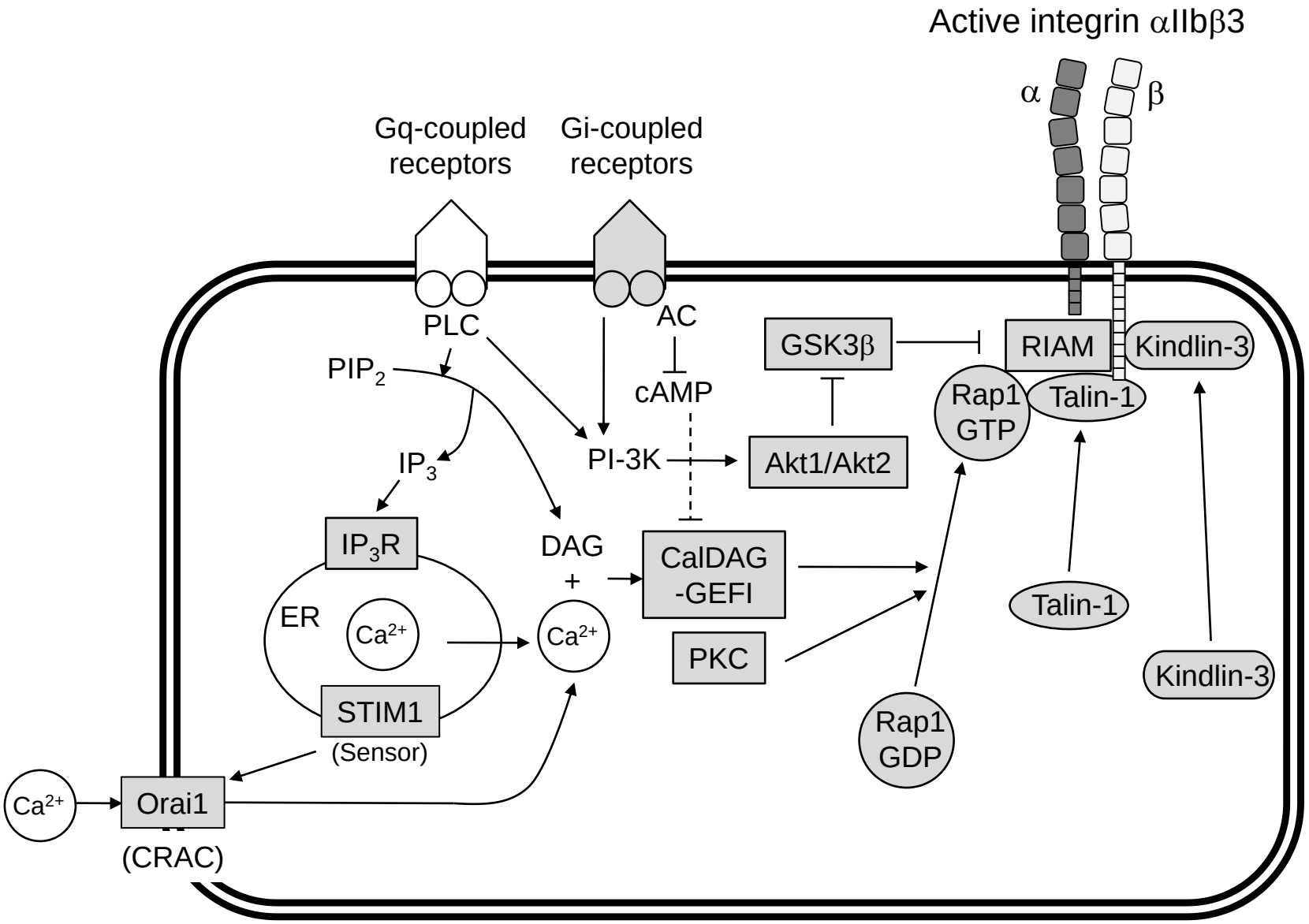
*Smyth SS et al, ATVB 2009;29:449*

# β3 Cytoplasmic Tail

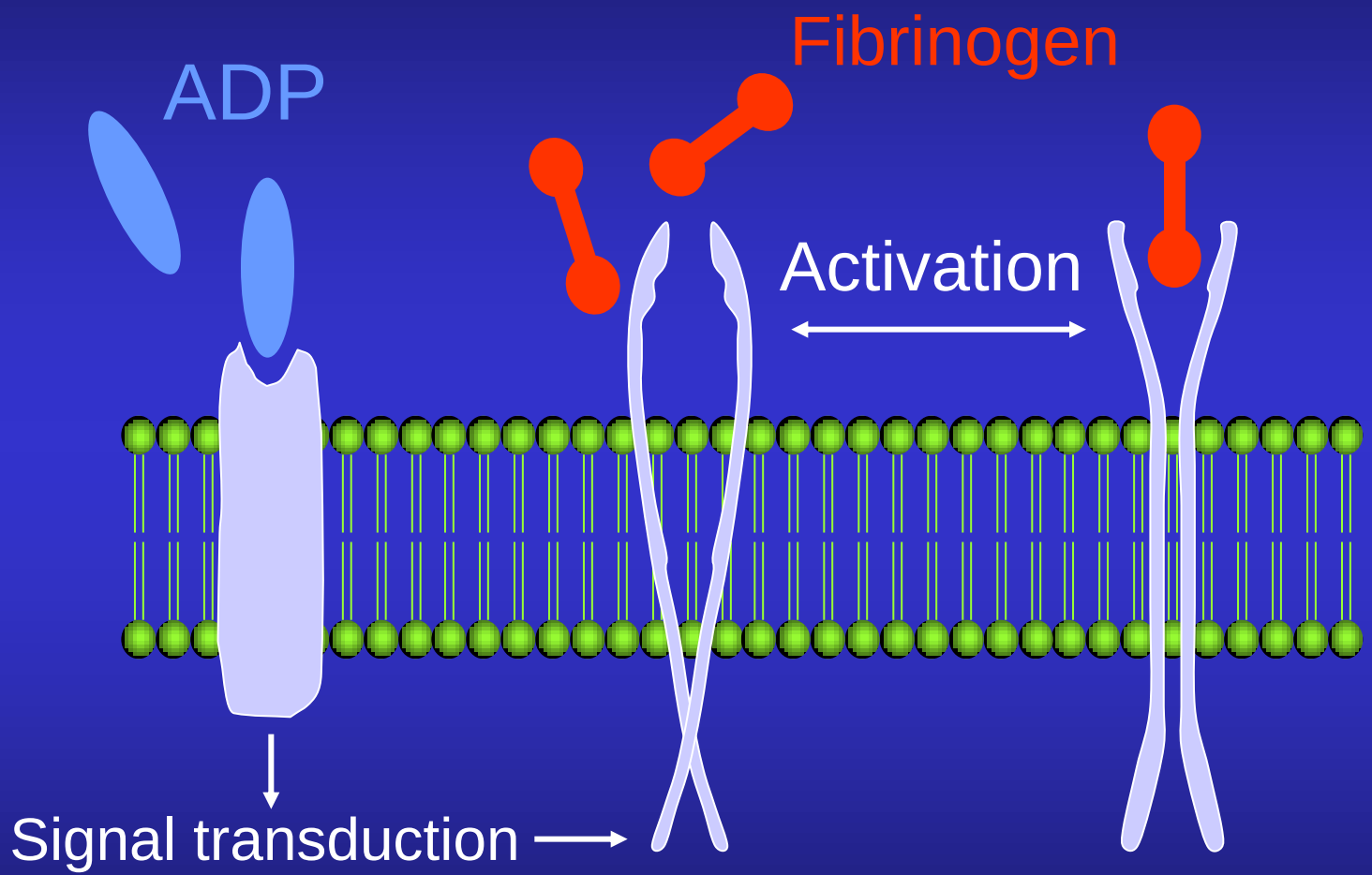
- β3 TM and cytoplasmic domain
- β3 TM helix - hinge - 2nd helix - hinge - NPLY motif - 3rd helix - NITY motif
  - NPLY motif (residues 744-747); talin FERM domain
  - NITY motif (residues 756-759); kindlin-3 FERM domain
- Interaction with large number of cytosolic protein, but identified functional significance in a few proteins
  - Talin-1, Kindlin-3, Rap1b/CalDAG-GEFI, RIAM




*Bennett JS et al, Journal of Thrombosis and Haemostasis, 2009;7:200–205*

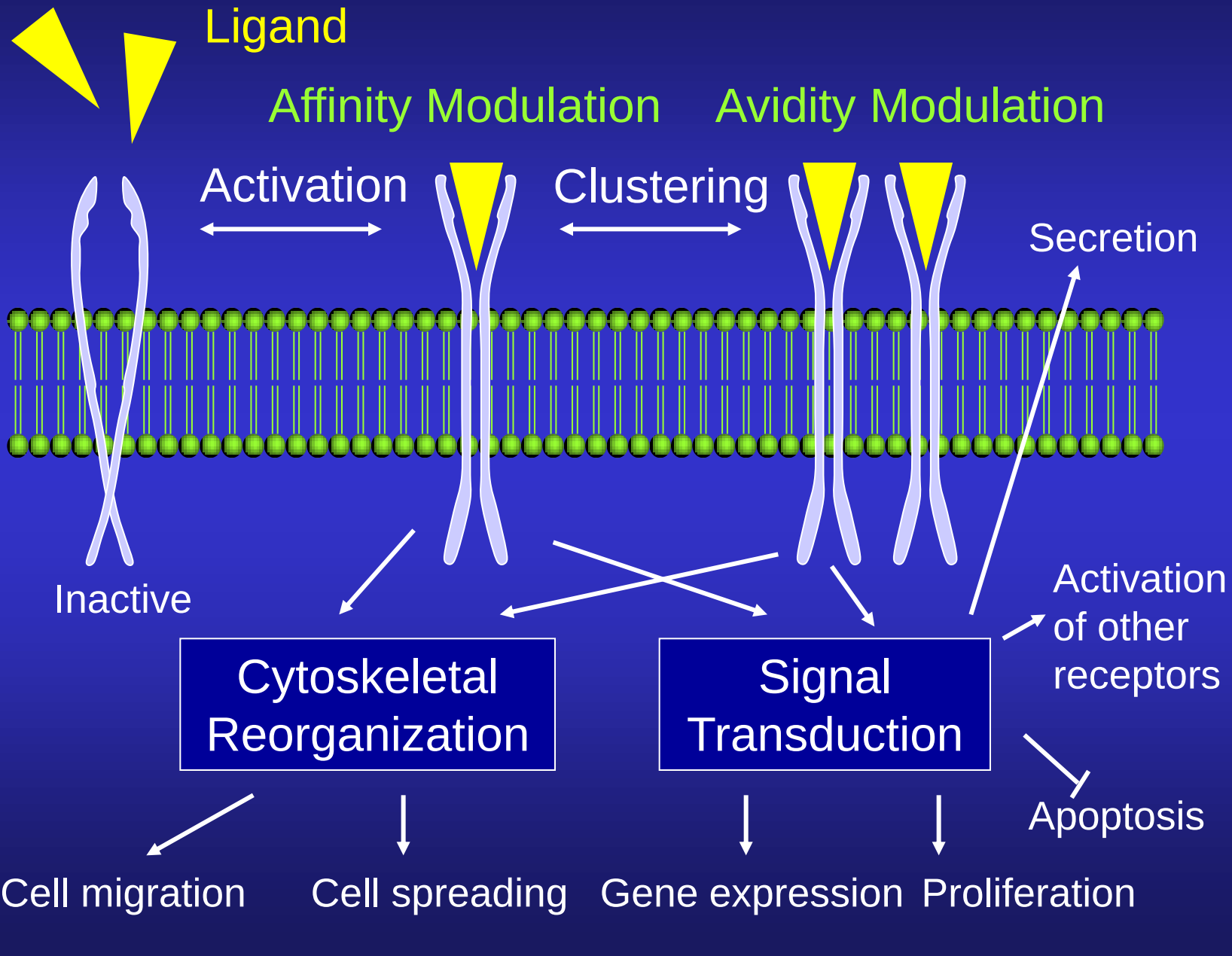

Active integrin αIIbβ3
α
β
Gq-coupled receptors
Gi-coupled receptors
PLC
AC
PIP2
cAMP
GSK3β
RIAM
Kindlin-3
Rap1 GTP
Talin-1
IP3
PI-3K
Akt1/Akt2
IP3R
DAG
+
CalDAG -GEFI
ER
Ca2+
Ca2+
PKC
Talin-1
Kindlin-3
STIM1
(Sensor)
Rap1 GDP
Ca2+
Orai1
(CRAC)

# Integrin; “Inside out” signaling




Inactive $\alpha_{IIb}\beta_3$ Active $\alpha_{IIb}\beta_3$

# Integrin; “Outside in” signaling



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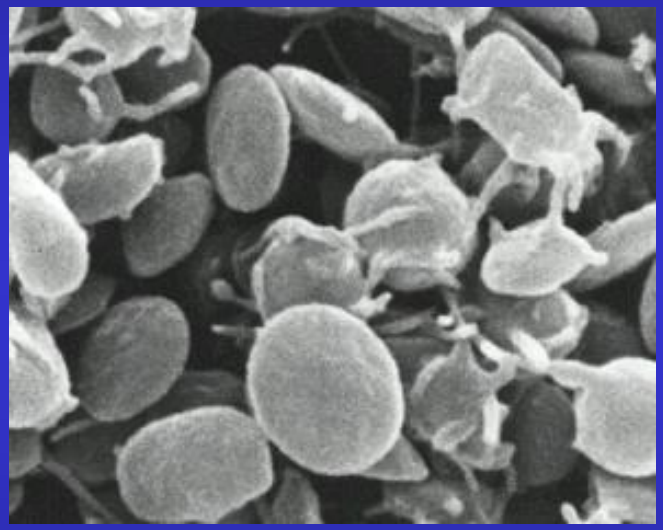
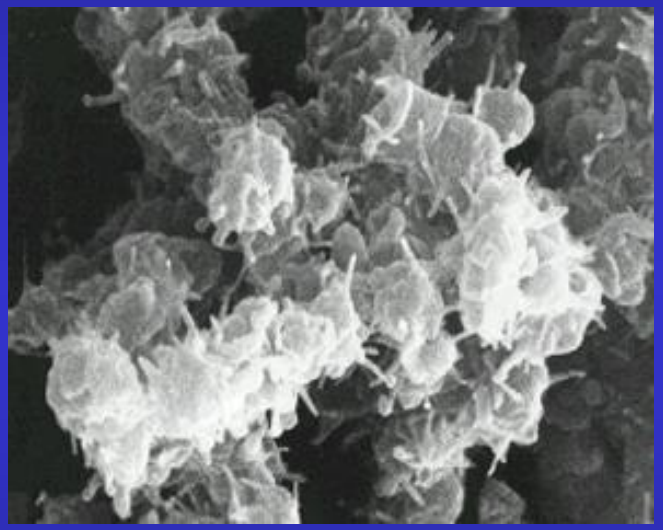
# Platelet Aggregation

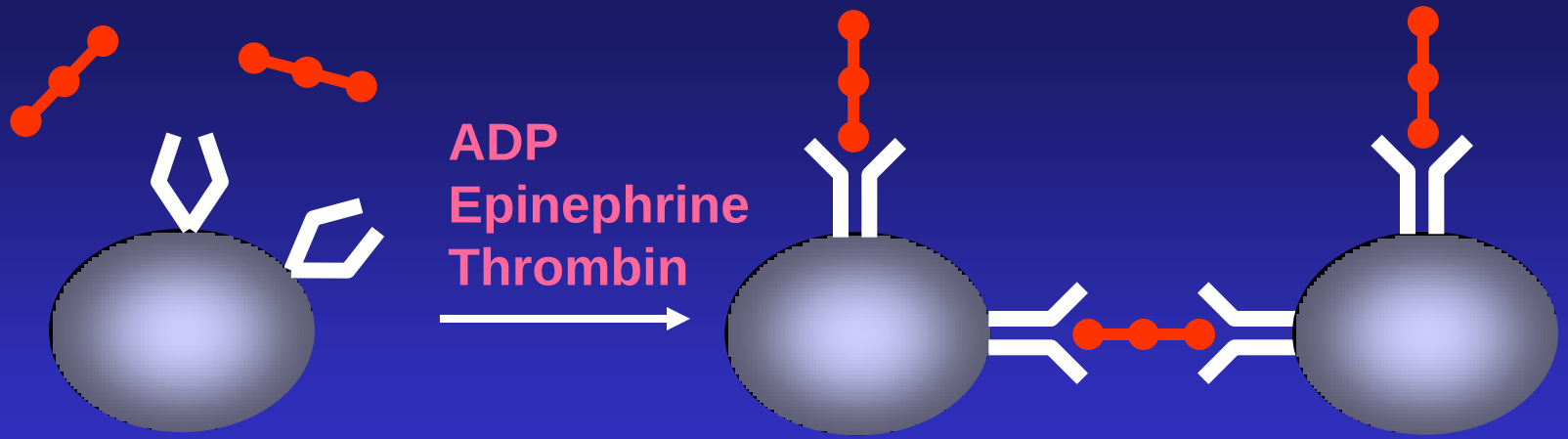


Scanning electron micrograph of discoid, dormant platelets



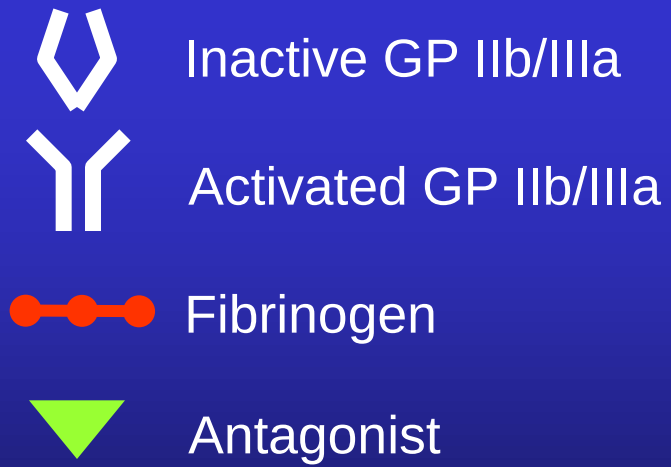
Activated, aggregating platelets illustrating fibrin strands

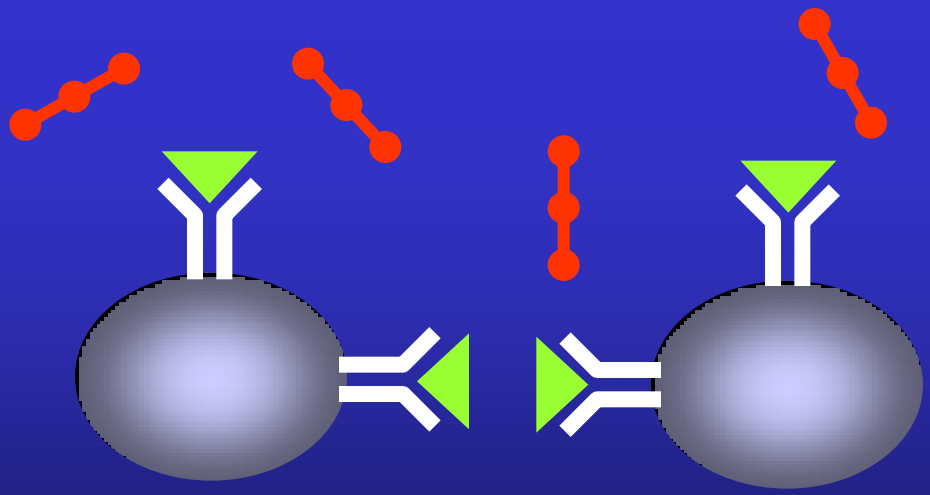
*Kuwahara M et al. Arterioscler Thromb Vasc Biol 2002;22:329*




Inactive platelets

Activated platelets

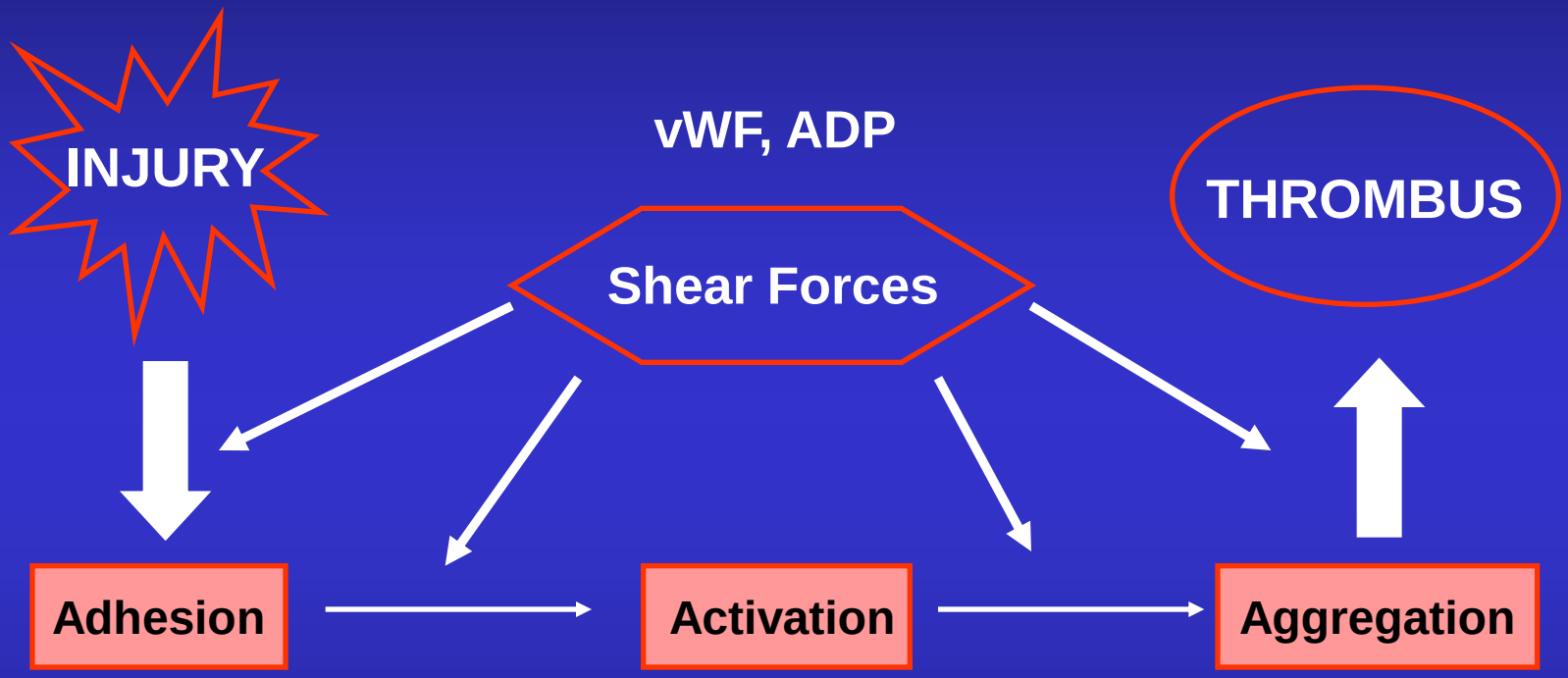


# Antagonist to Glycoprotein IIb/IIIa

- Abciximab
  - Chimeric monoclonal antibody
- Eptifibatide
  - Peptide inhibitor of KGD sequence
- Tirofiban, Lamifiban
  - Nonpeptide inhibitor of RGD sequence

# Key Mediators in Platelet Adhesion, Activation and Aggregation




- vWF
- Collagen
- GP Ib-V-IX

- Membrane changes
- Granule secretion
- GPIIb/IIIa expression
- Multiple agonists; ADP, Thrombin, $TXA_2$
- Positive feedback loops

- GPIIb/IIIa
- Fibrinogen