ERN-EuroBloodNet Topic on Focus on Inherited Platelet Function Disorders (IPFD)

webinar

HEALTH PROFESSIONALS

Platelet Activation Mechanisms

Loredana BURY
University of Perugia, Department of Medicine and Surgery, Section of Internal and Cardiovascular Medicine

24 September 2025

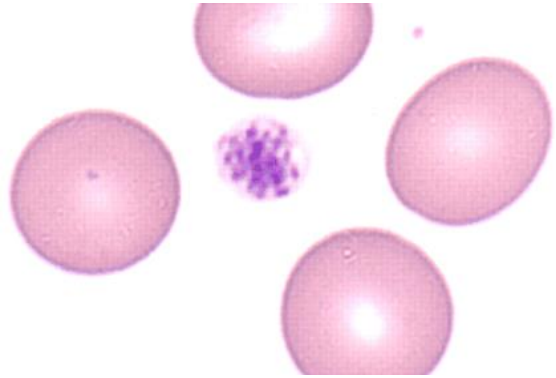
CRPP Centre de Référence Pathologies Plaquettaires
MHEMO La Filière des maladies rares de l'hémostase
ebn ERN | EuroBloodNet

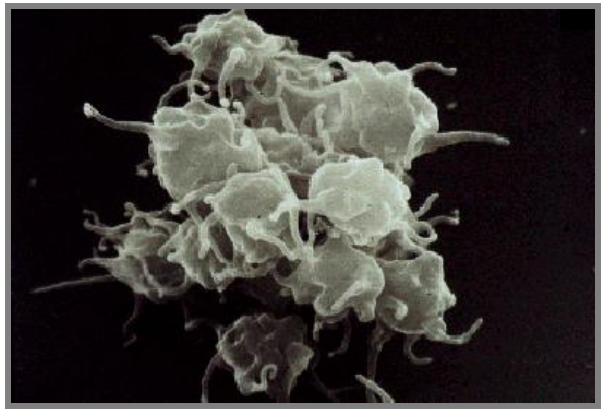
Disclosure for conflict of interest

No conflicts of interest

Platelets

- Anucleate cells circulating in blood
- Normal count: 150-400 x 10^9/L
- Diameter: 1.5–3 µm
- Life: 8-10 days
- Primary function in hemostasis

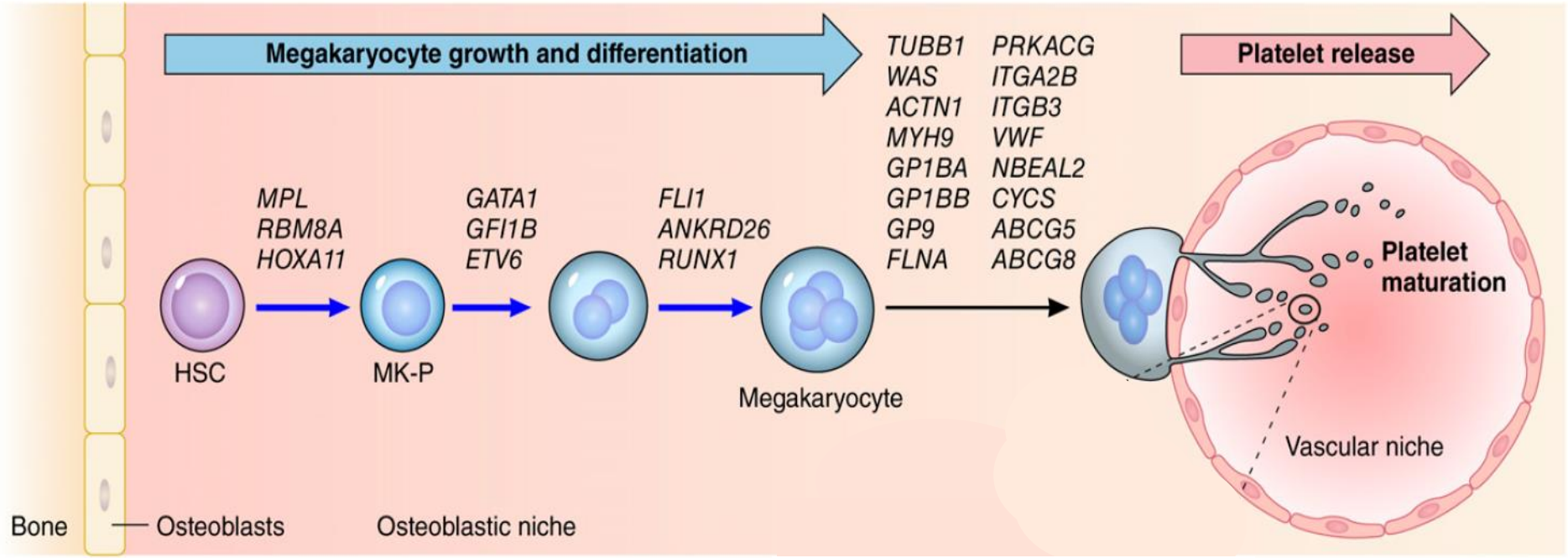




Platelets

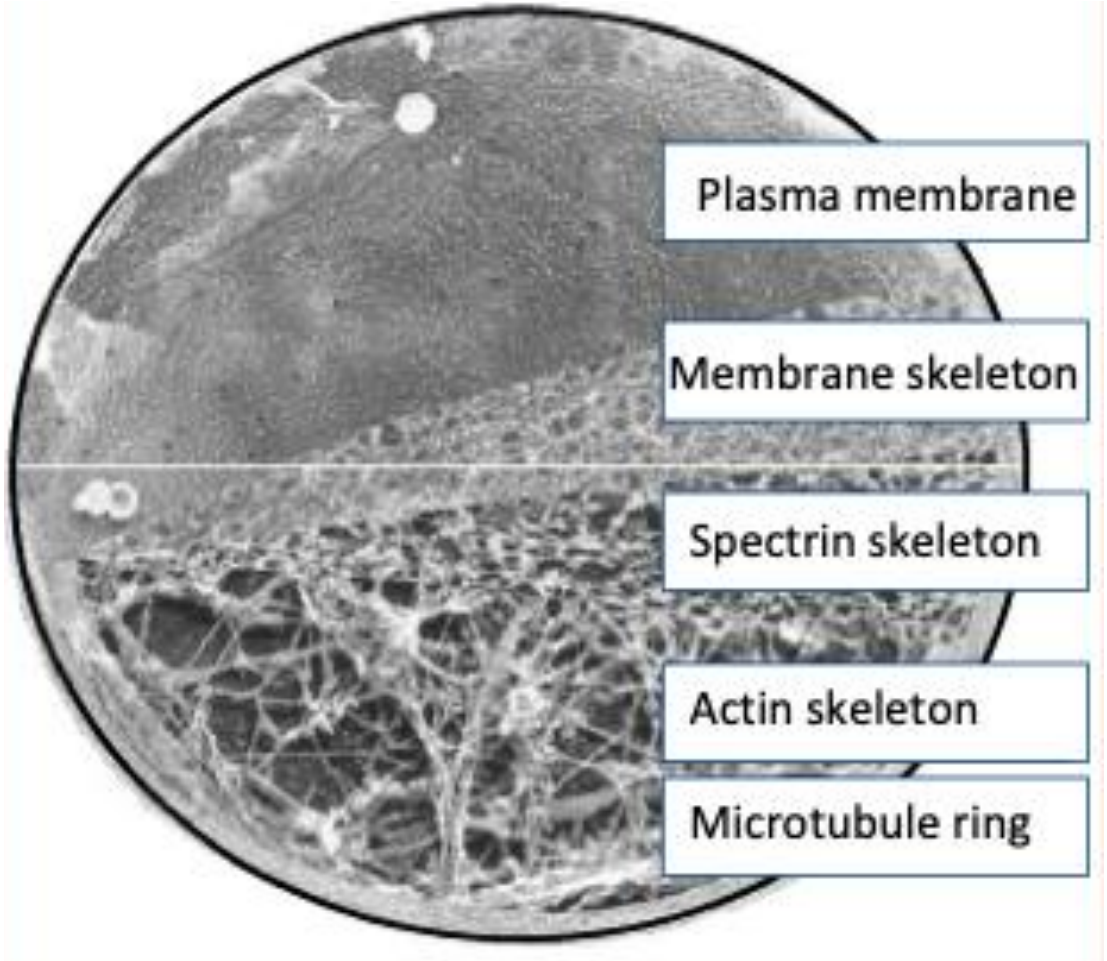
- Total platelet mass: 10^{12}

- 10^{11} platelets are released each day from their bone marrow precursors megakaryocytes to maintain a normal circulating platelet count



Junt T et al. Science 2007, 317:1767

Platelet structure



-Plasma membrane rich of receptors

-Cytoskeleton that supports the cell structure, but also platelet adhesion and activation, mainly composed of actin and spectrin

-Peripheral ring of microtubules essential for maintaining the discoid shape of the platelet

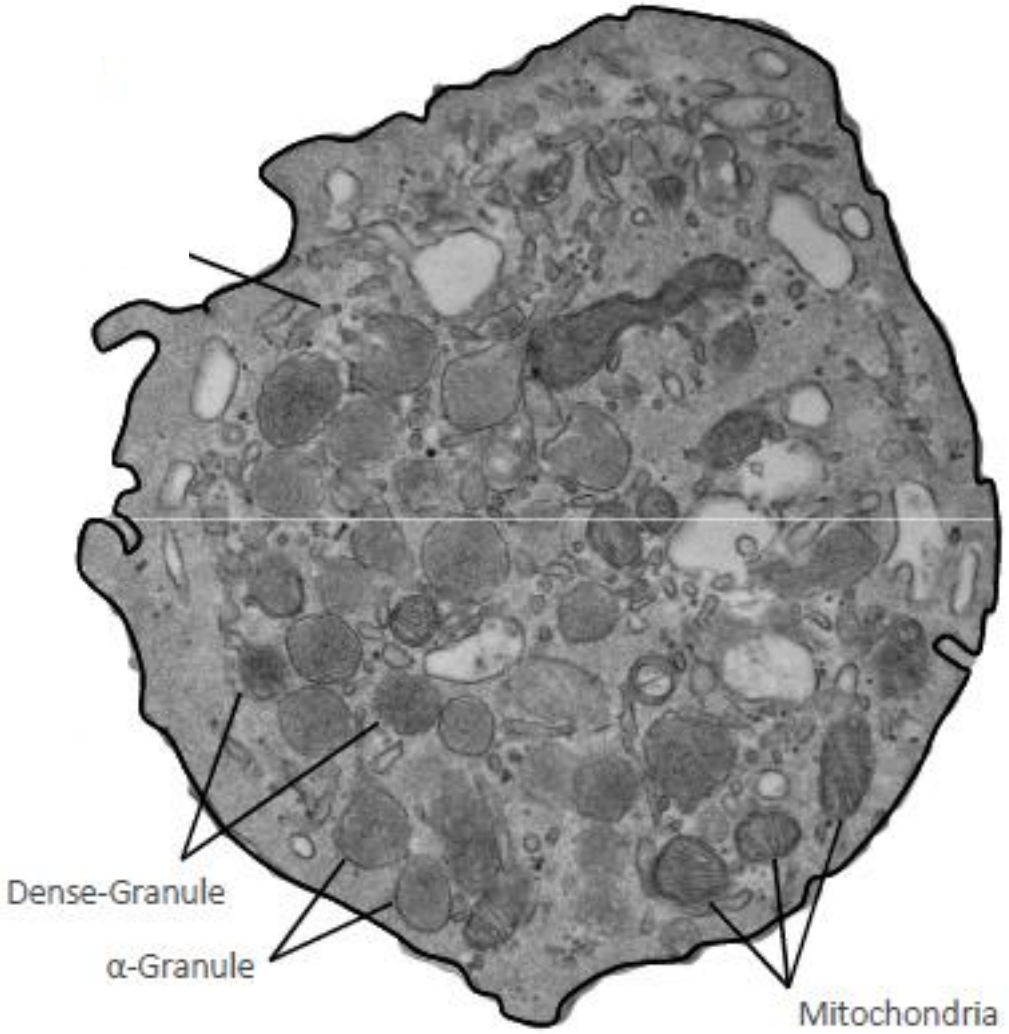
Thon JN & Italiano JE in Gresele P et al. Handb Exp Pharmacol. 2012; 3:3-22

Platelet structure: granules and organelles

- Mitochondria, lysosomes, peroxisomes, ribosomes, endoplasmic reticulum, Golgi apparatus

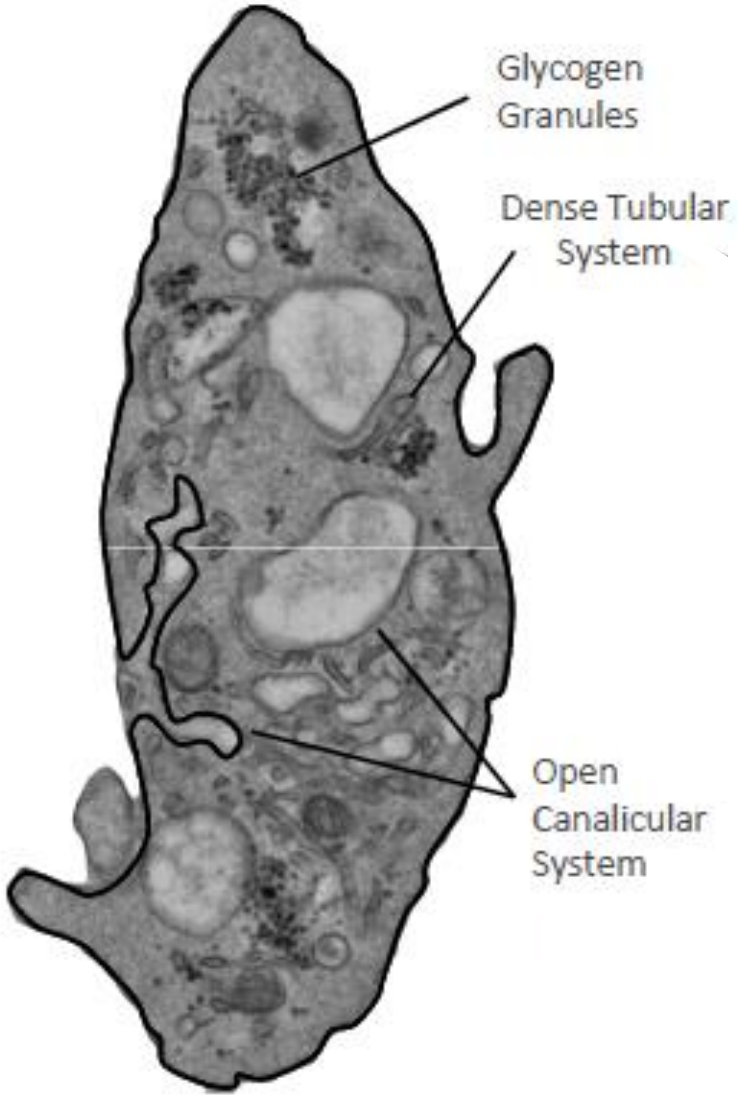
– α-granules: store proteins that are released upon platelet activation

– δ-granules (or dense granules): store nuleotides (ADP and ATP), serotonin and calcium.



Thon JN & Italiano JE in Gresele P et al. Handb Exp Pharmacol. 2012; 3:3-22

Platelet structure: membrane systems

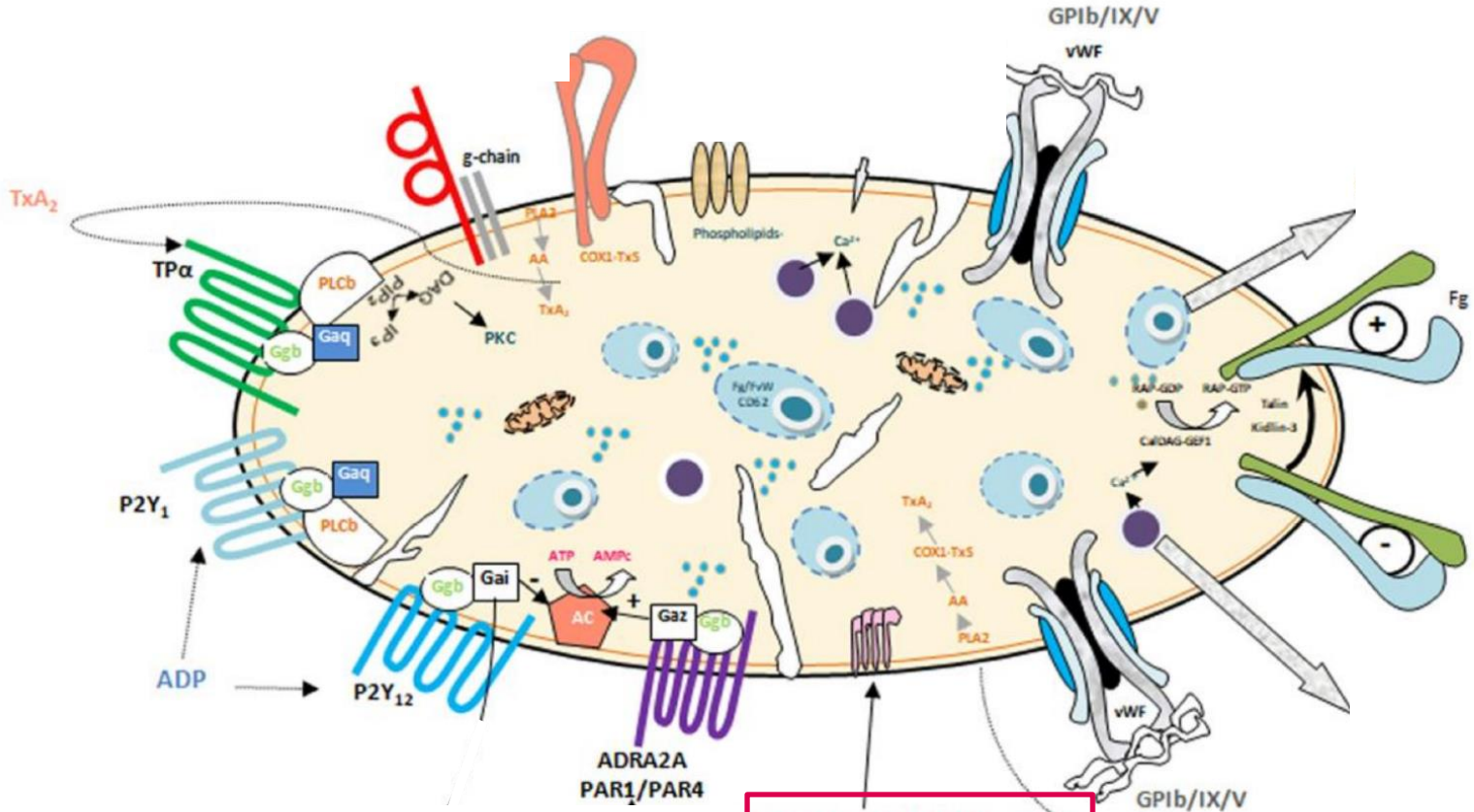


- Open canalicular system (OCS): surface-connected, crucial for granule secretion. Membrane storage to increase platelet surface during activation.

- Dense tubular system (DTS): an internal smooth endoplasmic reticulum membrane system that regulates platelet activation by sequestering or releasing calcium

Thon JN & Italiano JE in Gresele P et al. Handb Exp Pharmacol. 2012; 3:3-22

Defects of transcription factors and cytoskeletal proteins



Defects in cytoskeletal proteins
NMM-IIA (MYH9)
Filamin-A
Tubulin β1
Actinin-1
DIAPH1
WASP
TPM4
ACTB

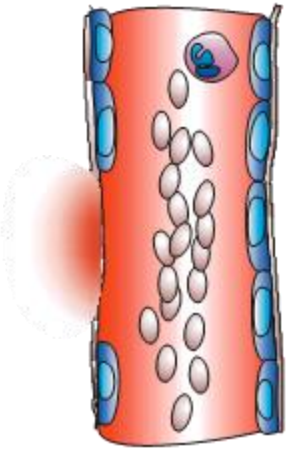
Transcription factors (ANKRD26, ETV6, FLI1, GATA1, GFI1B, RUNX1)
Reduced aggregation response
Defects in signaling pathways

Modified from: Palma-Barqueros V, et al. Int J Mol Sci. 2021;22(9):4521

Platelets in hemostasis

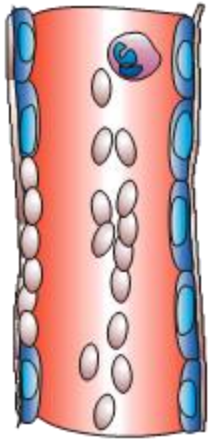
VASCULAR PHASE

Lesion and vasoconstriction

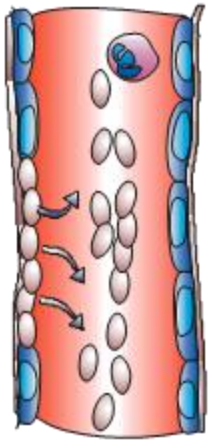


PLATELET PHASE

Adhesion

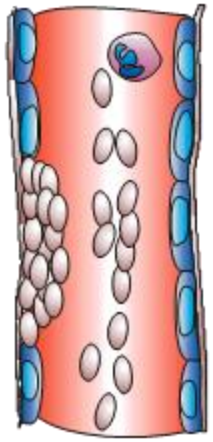


Platelet release reaction



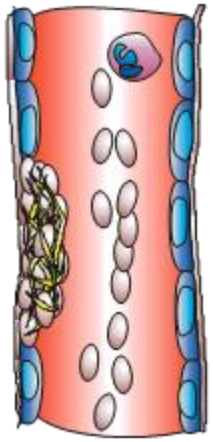
PLATELET PHASE

Aggregation



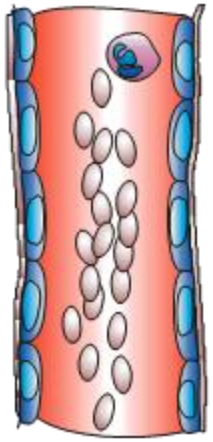
COAGULATION PHASE

Coagulation

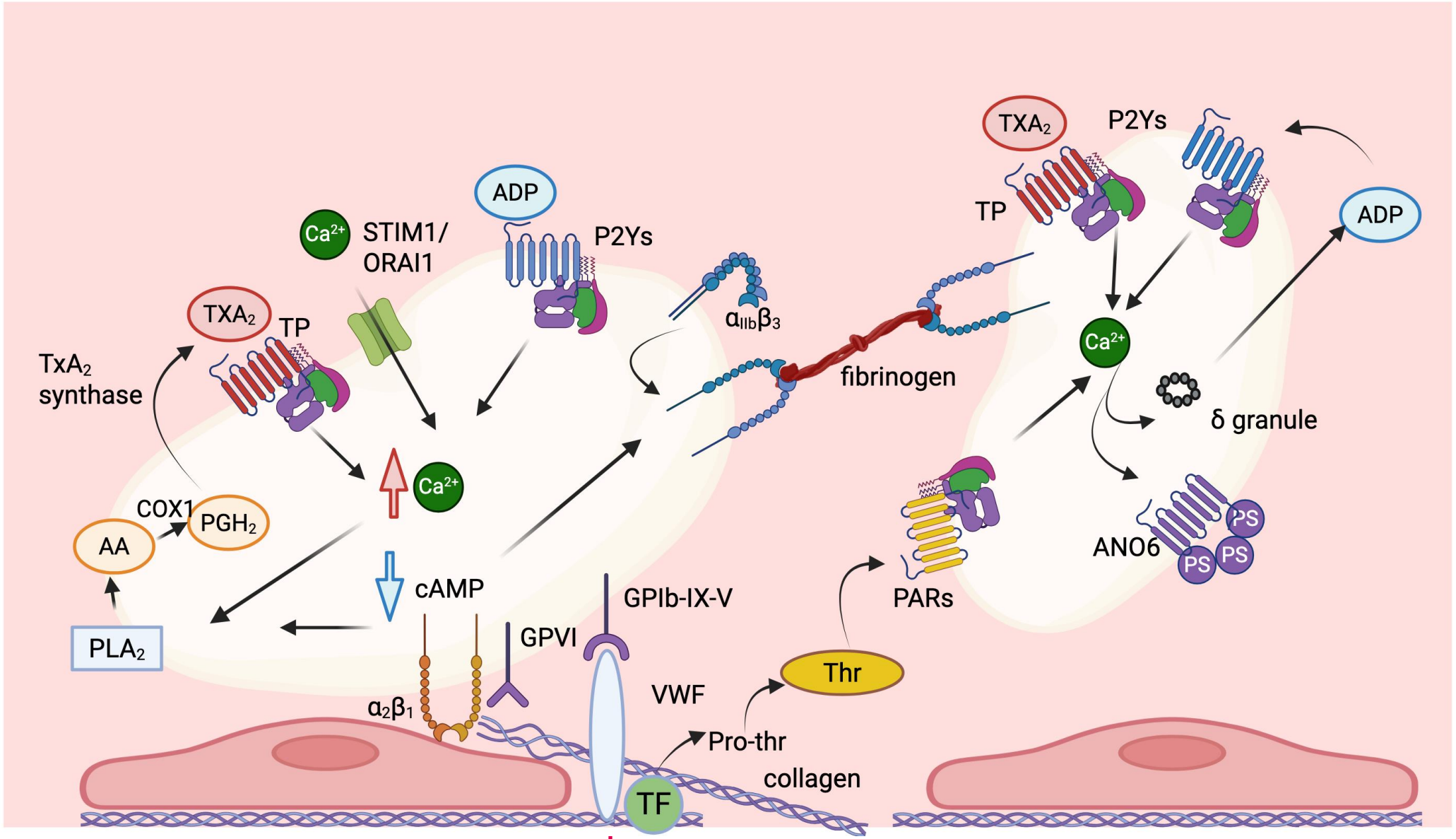


FIBRINOLYTIC PHASE

Fibrinolysis

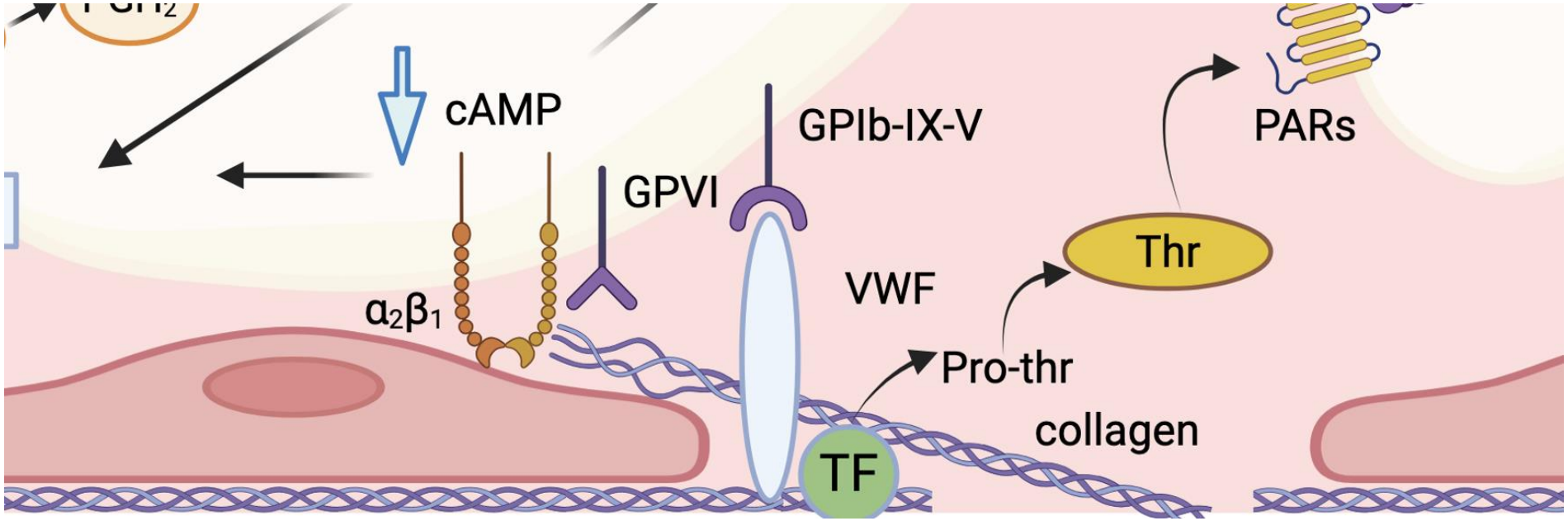


Adhesion-activation/release-aggregation



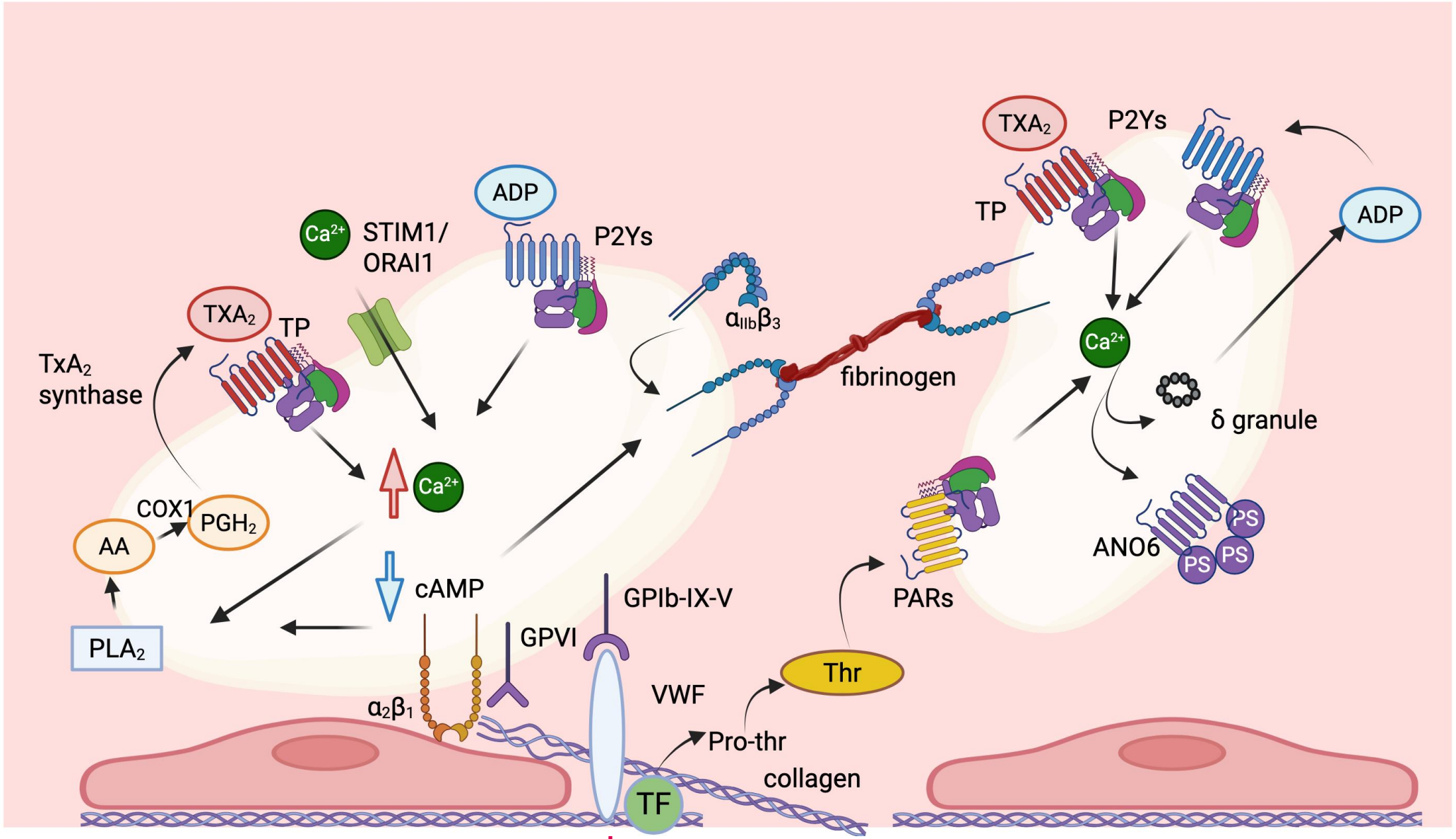
Loredana Bury, Emanuela Falcinelli and Paolo Gresele.
"Platelet structure and function" WILLIAMS HEMATOLOGY 11TH EDITION. 2025.

Adhesion-activation/release-aggregation
Platelet adhesion



- VWF interacts with collagen and with GPIb-IX-V
- GPVI and $\alpha_2\beta_1$ interact with collagen

Adhesion-activation/release-aggregation



Loredana Bury, Emanuela Falcinelli and Paolo Gresele.
"Platelet structure and function" WILLIAMS HEMATOLOGY 11TH EDITION. 2025.

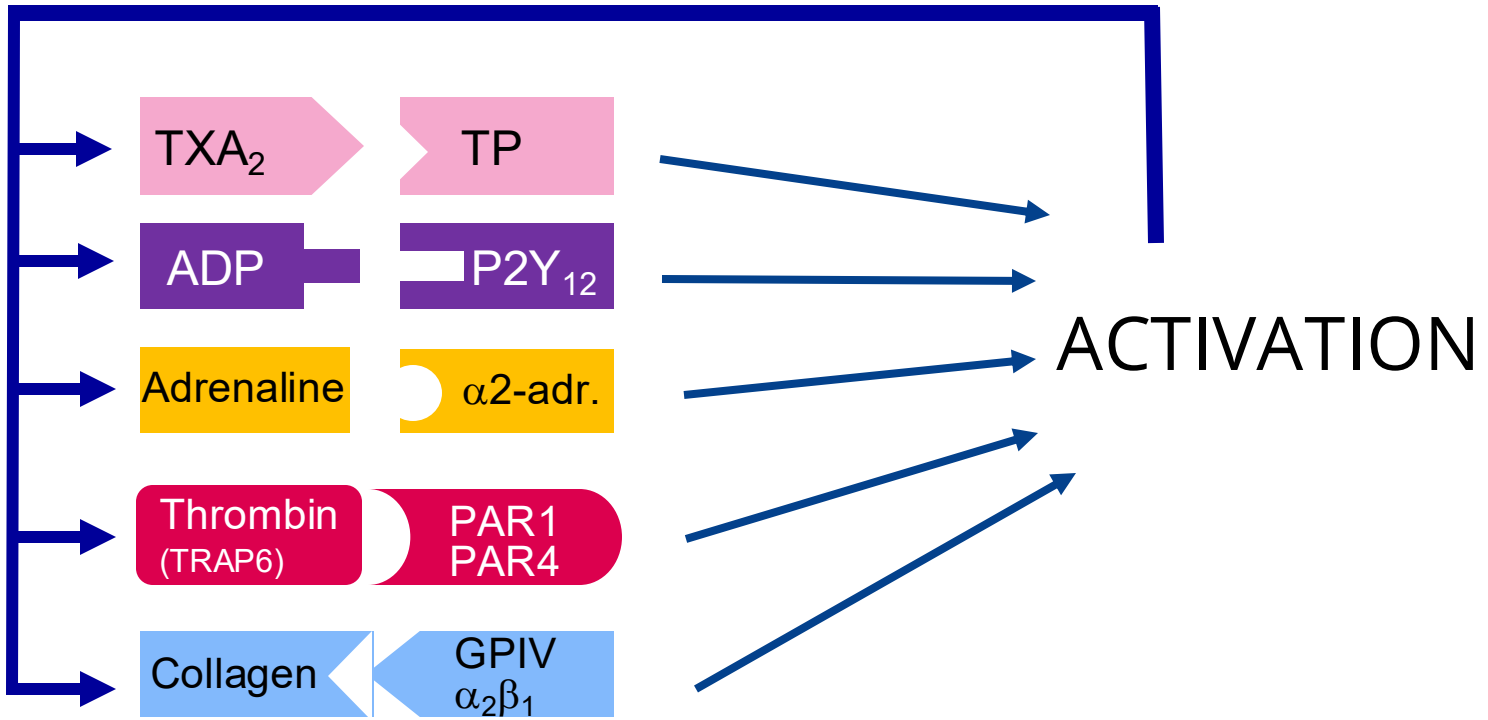
Main signaling pathways for platelet activation

1. ITAM-mediated signaling pathways
   (collagen, von Willebrand Factor)

2. G-protein-mediated signaling pathways
   (thrombin, ADP, TxA_2, epinephrine,...)

Platelet agonists

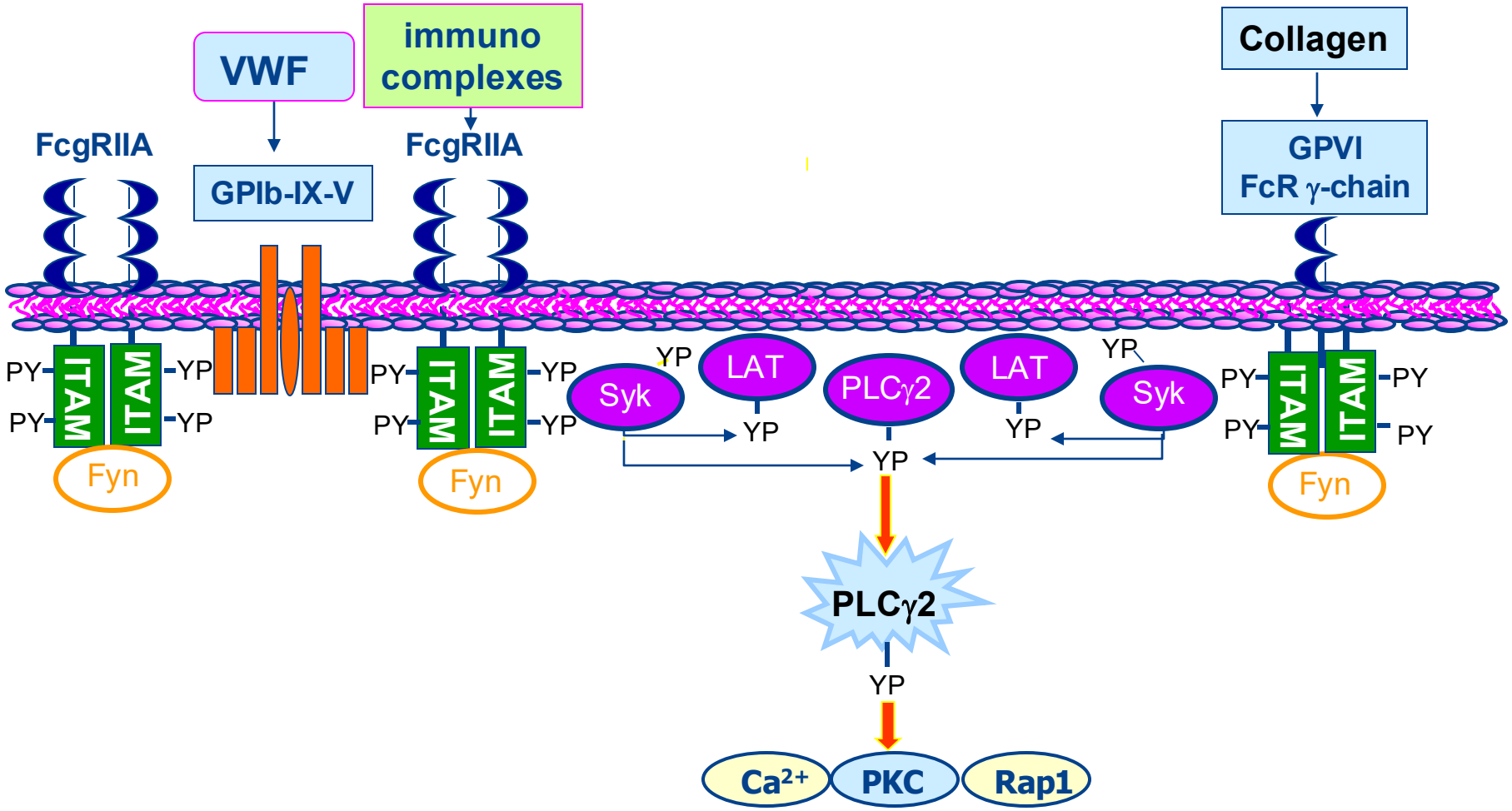
Agonists are released by granules (**ADP**), produced by the coagulation cascade (**thrombin**), produced by platelet phospolipids (**thromboxane TXA_2**) or present on the damaged endothelium (**collagen**) or in plasma (**epinephrine**).



ITAM-mediated activatory signalling

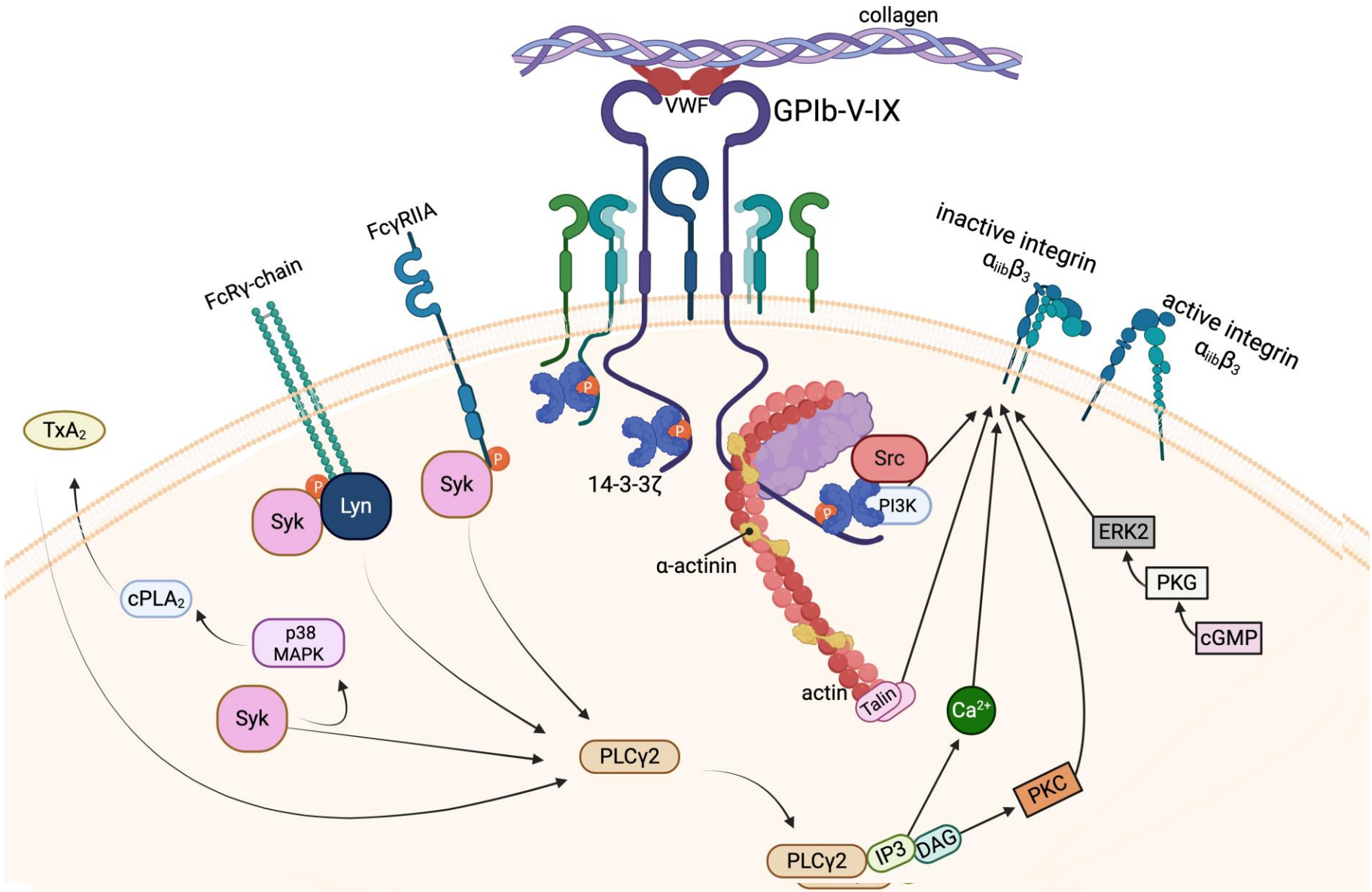
receptors containing an intracellular sequence called ITAM

(**Immunoreceptor Tyrosine-Based Activation Motif**)



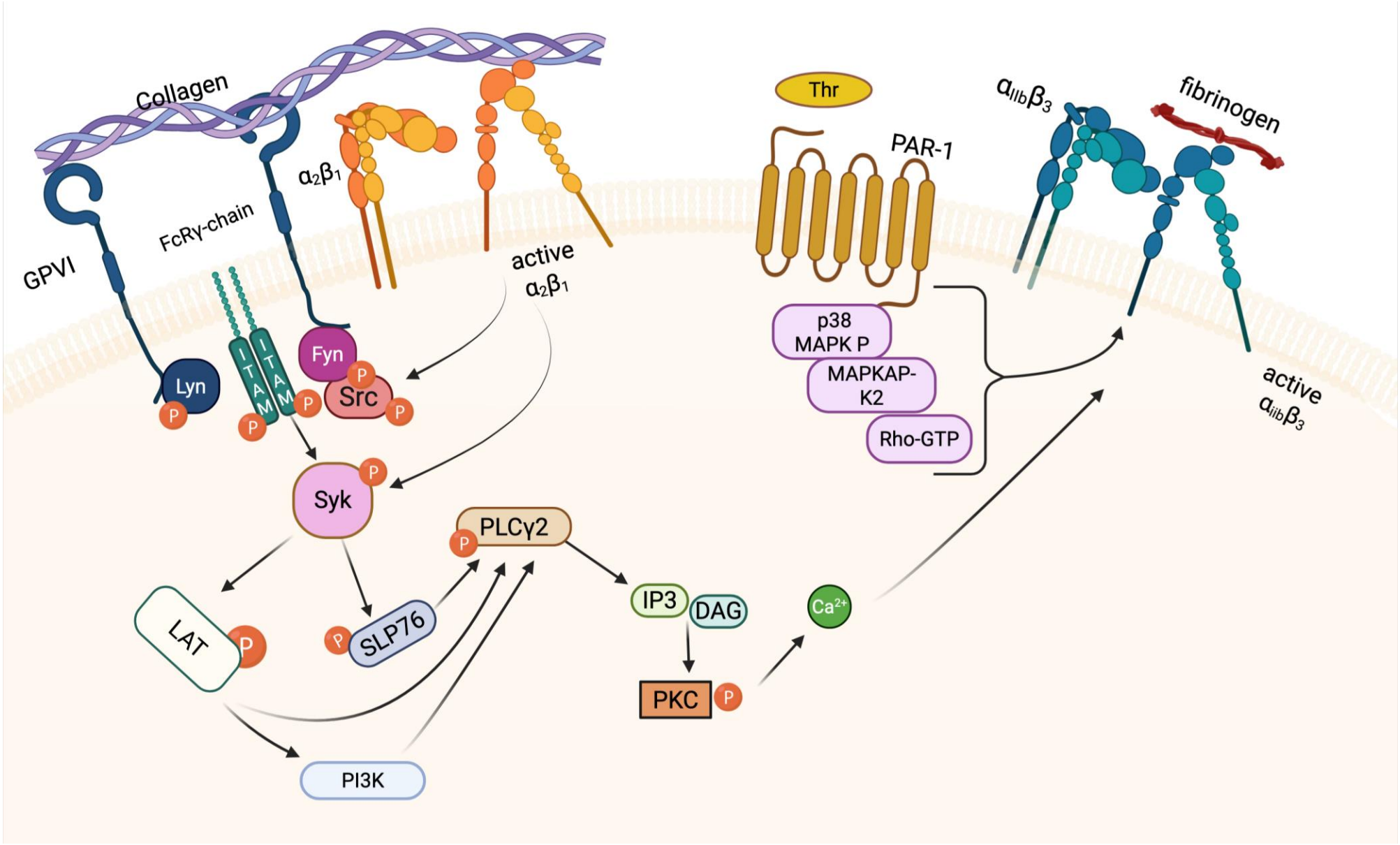
Integrin $\alpha_{IIb}\beta_3$ activation and platelet aggregation

GPIb-IX-V mediated signaling



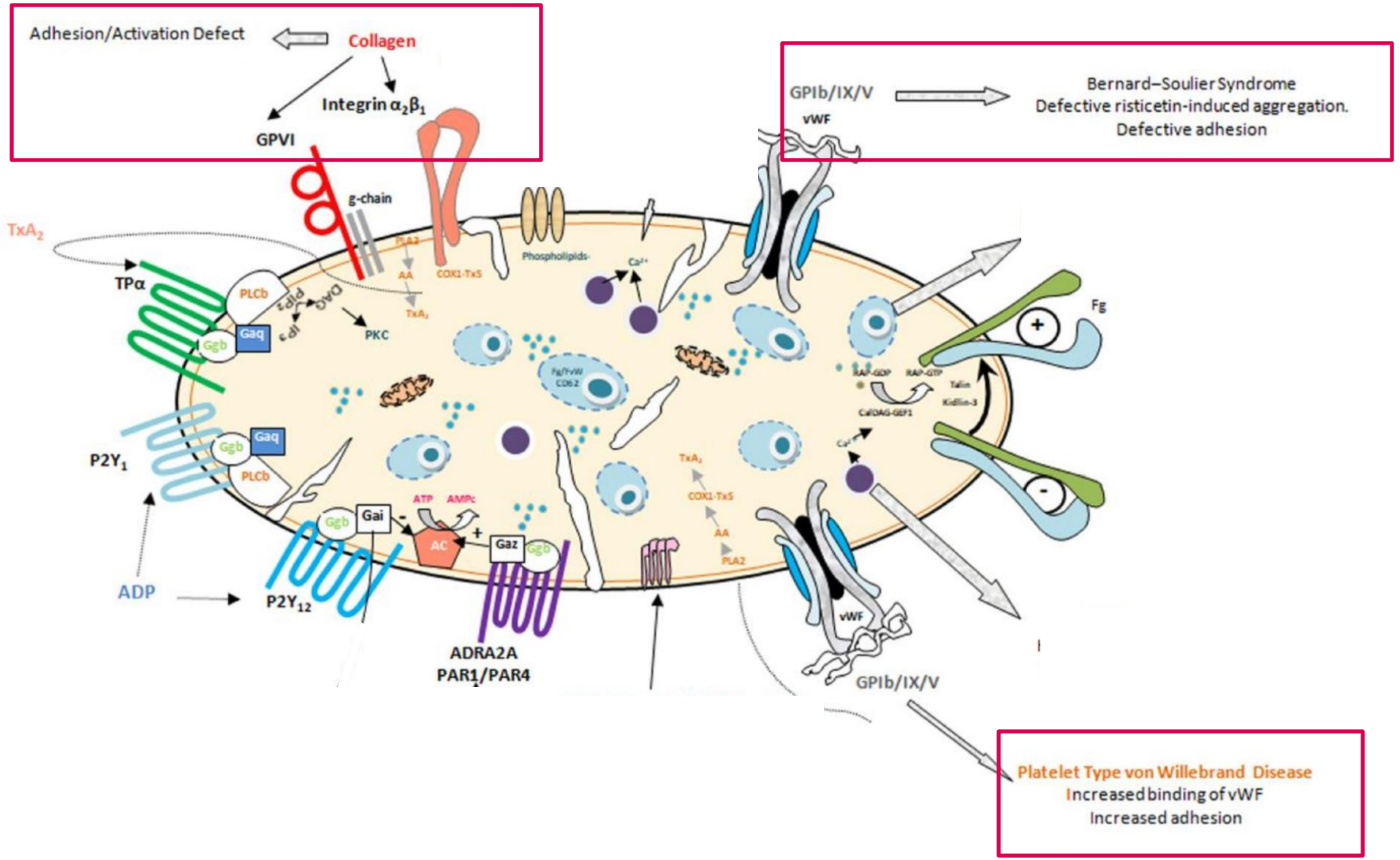
Loredana Bury, Emanuela Falcinelli and Paolo Gresele. "Platelet structure and function" WILLIAMS HEMATOLOGY 11TH EDITION. 2025.

Collagen receptors mediated signaling



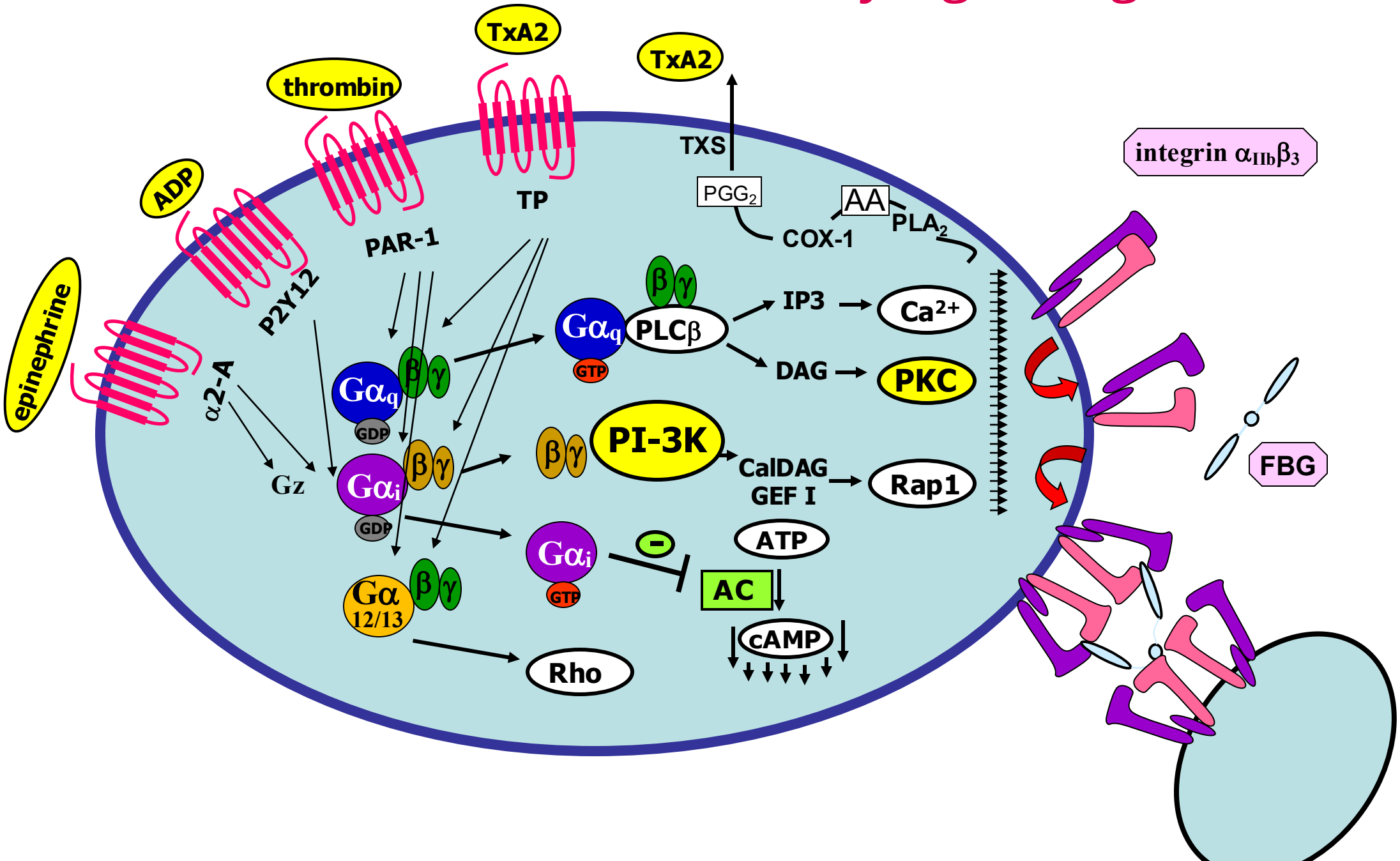
Loredana Bury, Emanuela Falcinelli and Paolo Gresele. "Platelet structure and function" WILLIAMS HEMATOLOGY 11TH EDITION. 2025.

Defects of GPIb/IX/V and collagen receptors

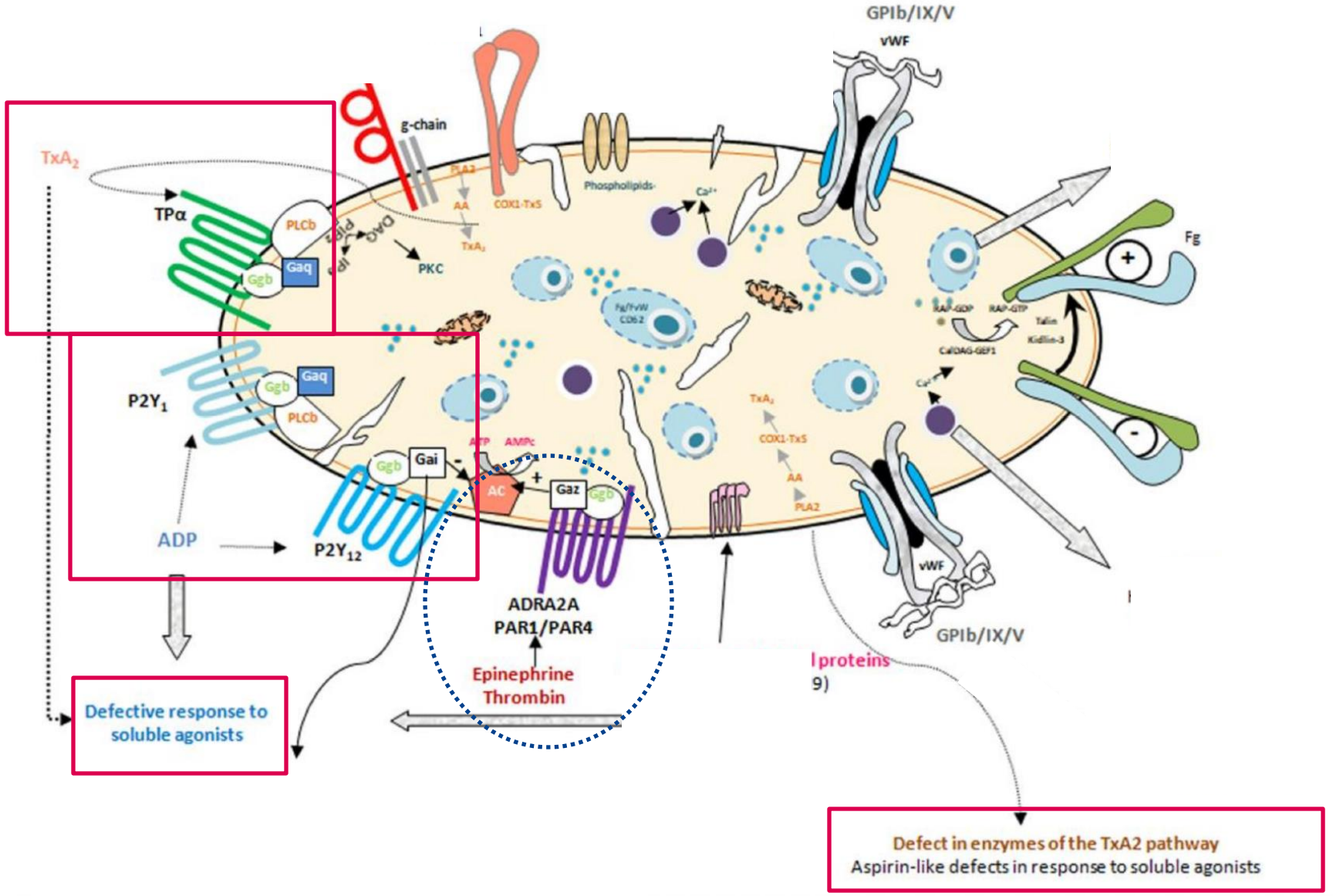


Modified from: Palma-Barqueros V, et al. Int J Mol Sci. 2021;22(9):4521

GPCR-mediated activatory signalling

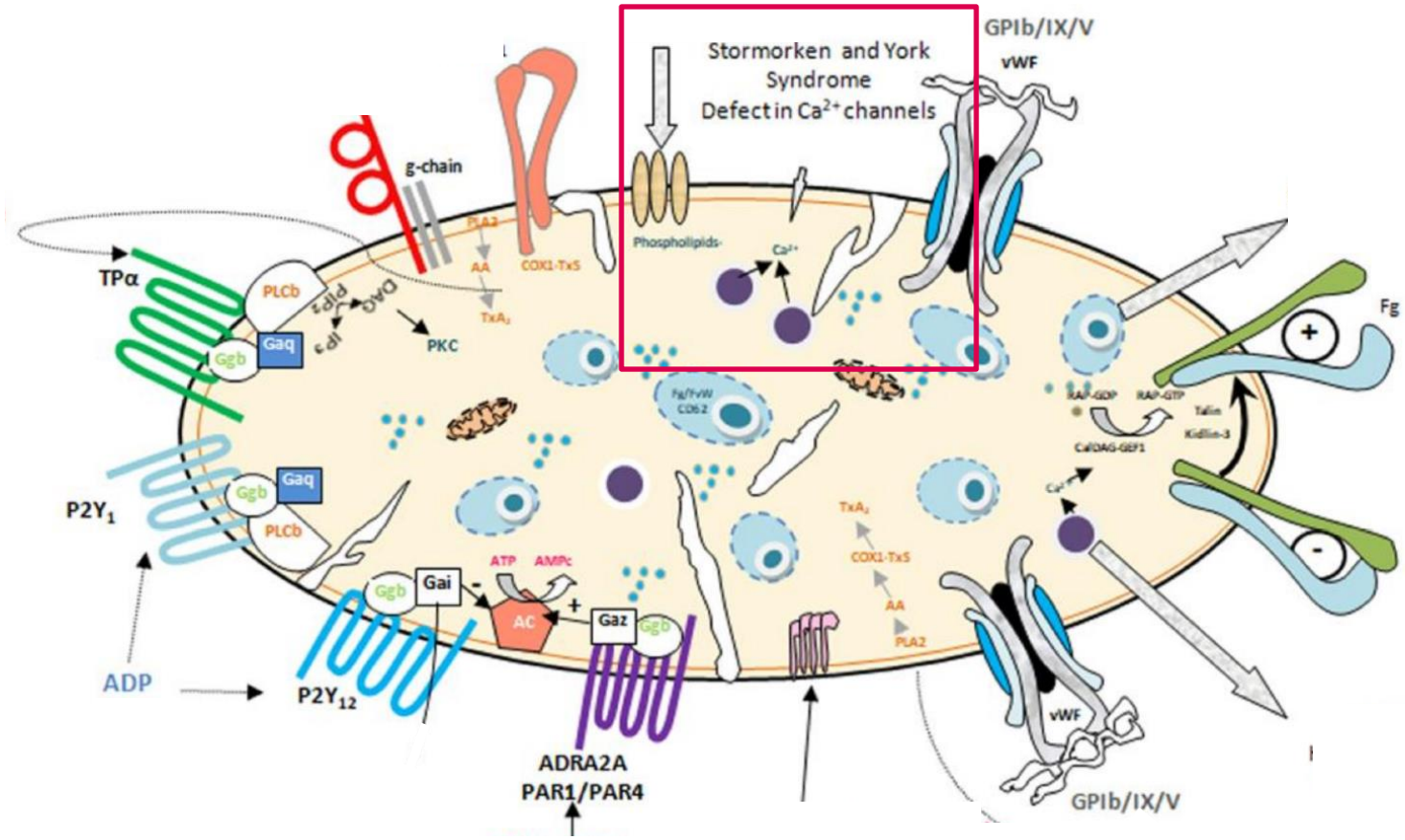


Disorders of G-protein coupled receptors



Modified from: Palma-Barqueros V, et al. Int J Mol Sci. 2021;22(9):4521

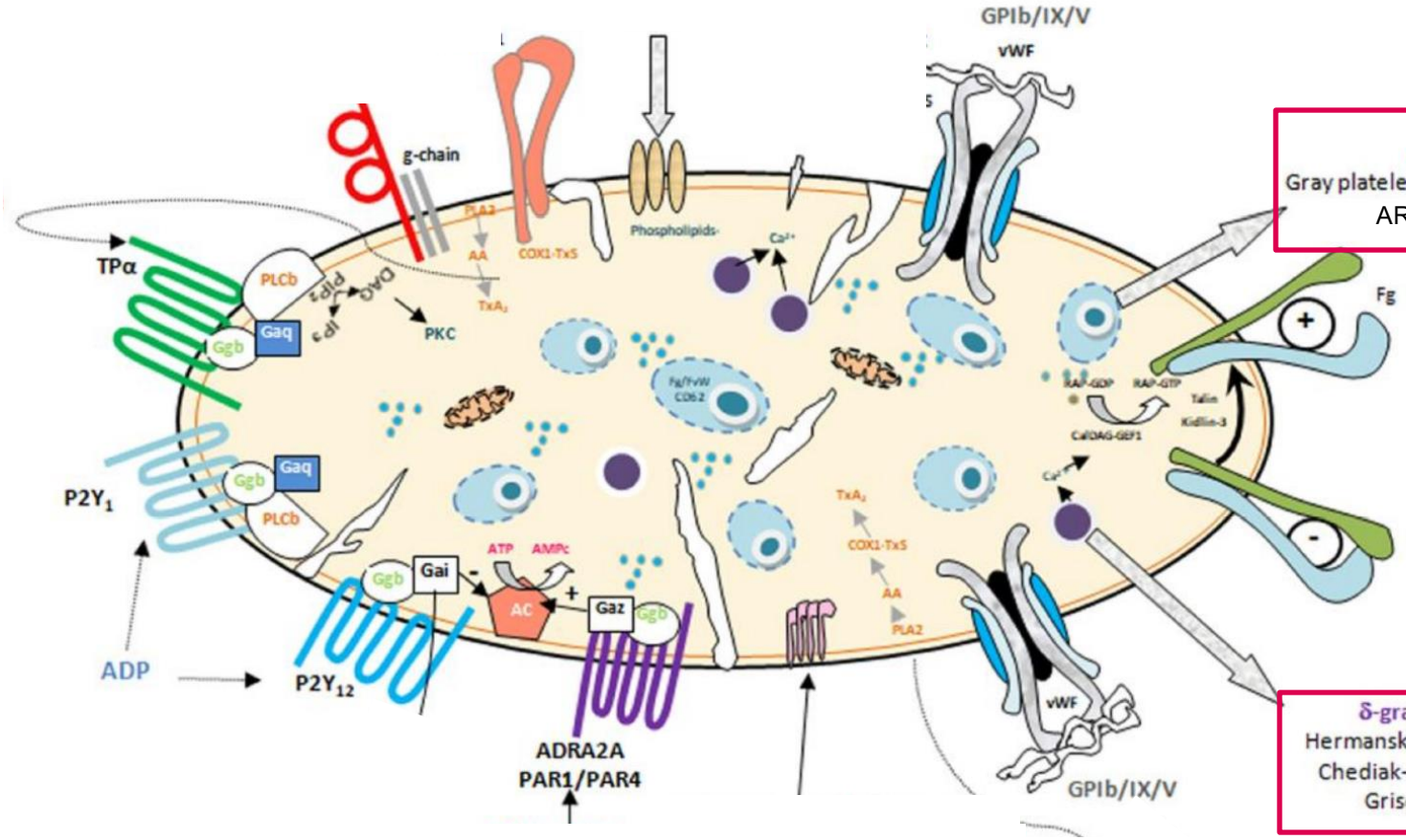
Defects of signaling proteins



Defects in G-proteins (GNAS, GNAQ, GNAZ, GNAI1)
Signaling proteins (CYCS, KDSR, ABCG5, ABCG8, TRPM7)

Modified from: Palma-Barqueros V, et al. Int J Mol Sci. 2021;22(9):4521

Disorders of granules



α-granules defects
Gray platelet Syndrome, Quebec Syndrome
ARC,Paris-Trousseau syndrome

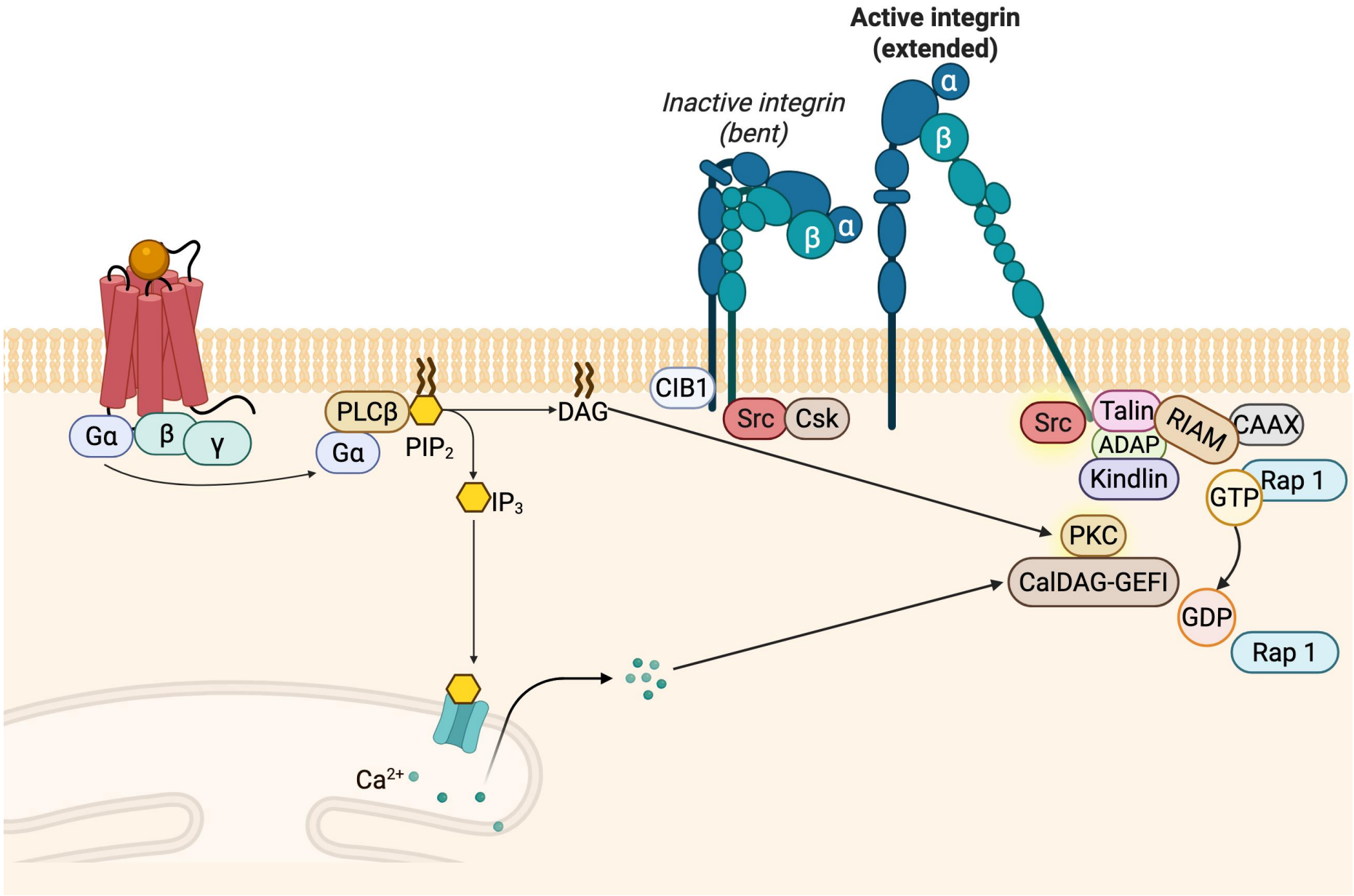
α/δ-storage pool disease

δ-granules defects
Hermansky-Pudlak Syndrome
Chediak-Higashi Syndrome
Griselli Syndrome

δ-storage pool disease
SLFN14-RT
NBEA-RT

Modified from: Palma-Barqueros V, et al. Int J Mol Sci. 2021;22(9):4521

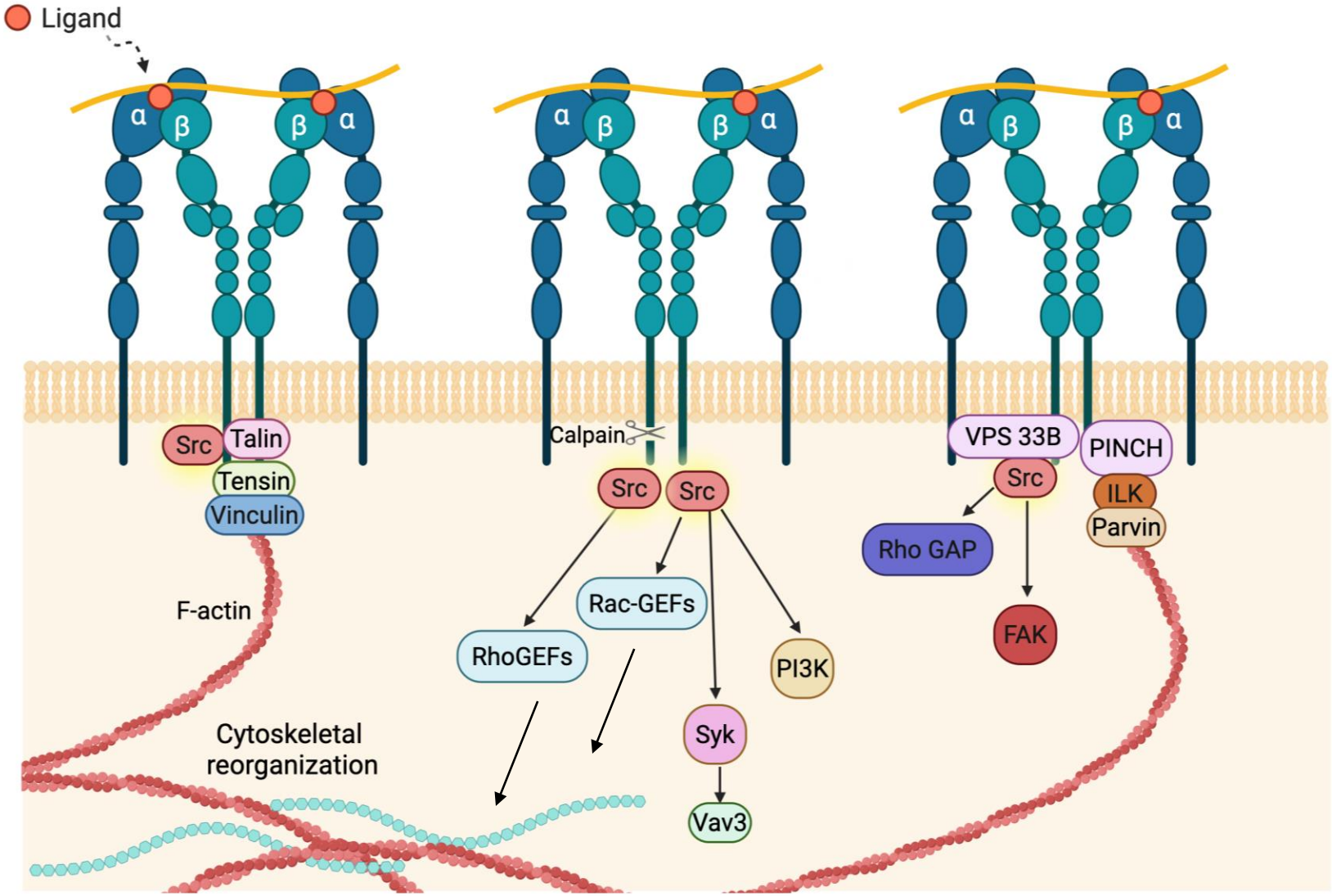
$\alpha_{IIb}\beta_3$ inside out signaling



Loredana Bury, Emanuela Falcinelli and Paolo Gresele. "Platelet structure and function" WILLIAMS HEMATOLOGY 11TH EDITION. 2025.

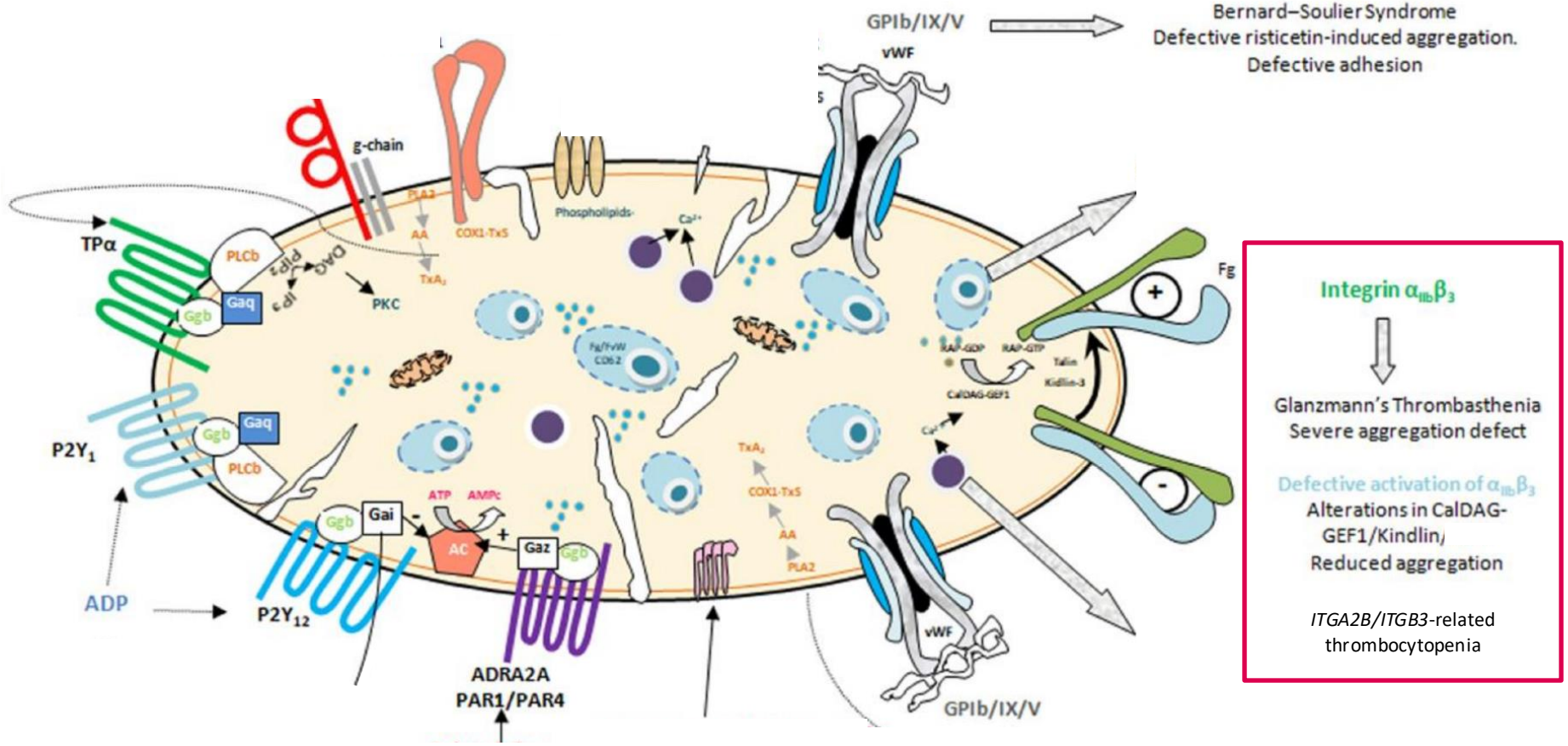
$\alpha_{IIb}\beta_3$ outside in signaling

Fibrinogen binding to active $\alpha_{IIb}\beta_3$ triggers a signal that is called outside-in signaling that leads to actin polymerization and cytoskeletal reorganization. This contributes to clot retraction and stabilization.



Loredana Bury, Emanuela Falcinelli and Paolo Gresele. "Platelet structure and function" WILLIAMS HEMATOLOGY 11TH EDITION. 2025.

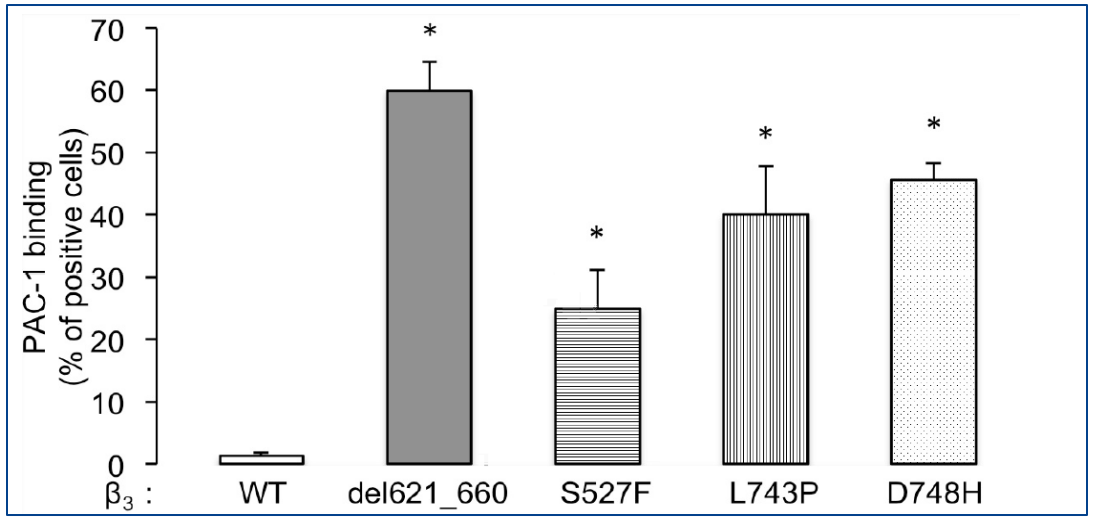
Glanzmann thrombasthenia and disorders of the $\alpha_{IIb}\beta_3$ signaling



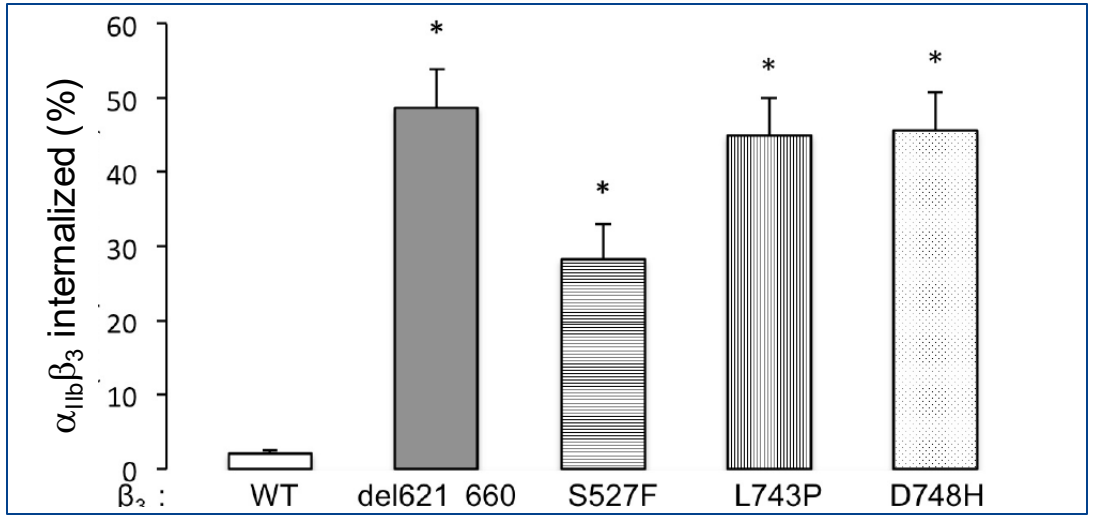
Modified from: Palma-Barqueros V, et al. Int J Mol Sci. 2021;22(9):4521

ITGA2B/ITGB3-related thrombocytopenia

Increased $\alpha_{IIb}\beta_3$ activation (PAC-1 binding)

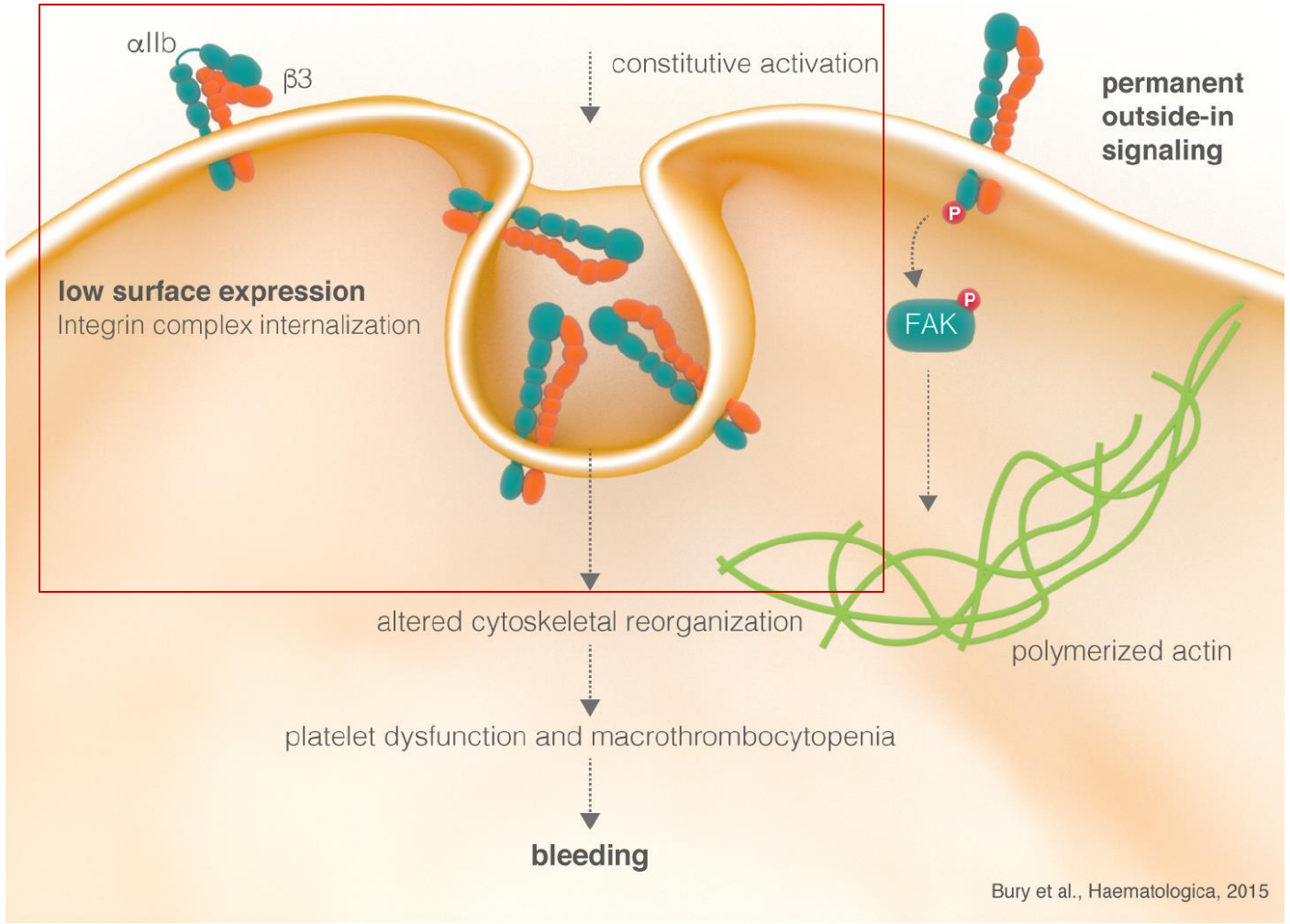


Increased $\alpha_{IIb}\beta_3$ internalization



Cytoskeletal perturbation leads to platelet dysfunction and thrombocytopenia in variant forms of Glanzmann thrombasthenia

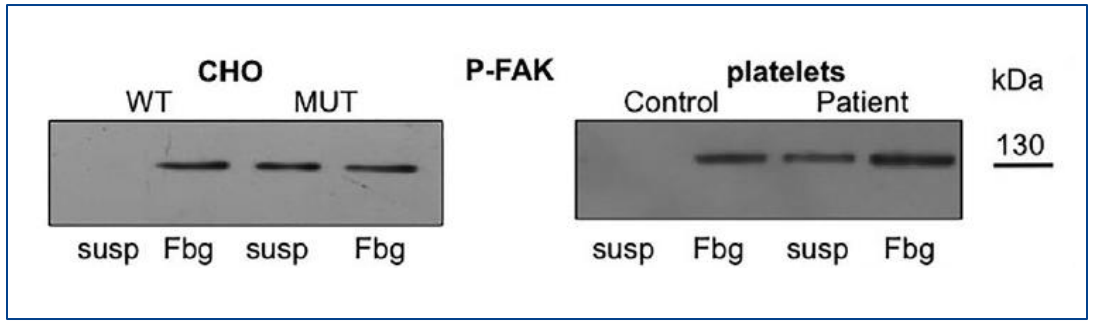
haematologica



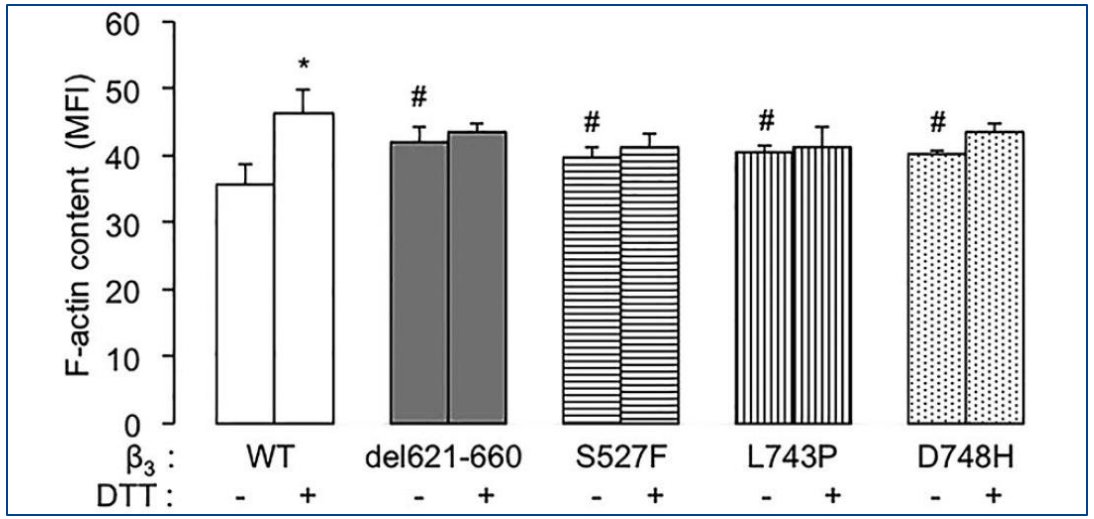
Bury L. et al. Haematologica 2016; 101:46-56

ITGA2B/ITGB3-related thrombocytopenia

Increased Focal adhesion kinase (FAK) phosphorylation

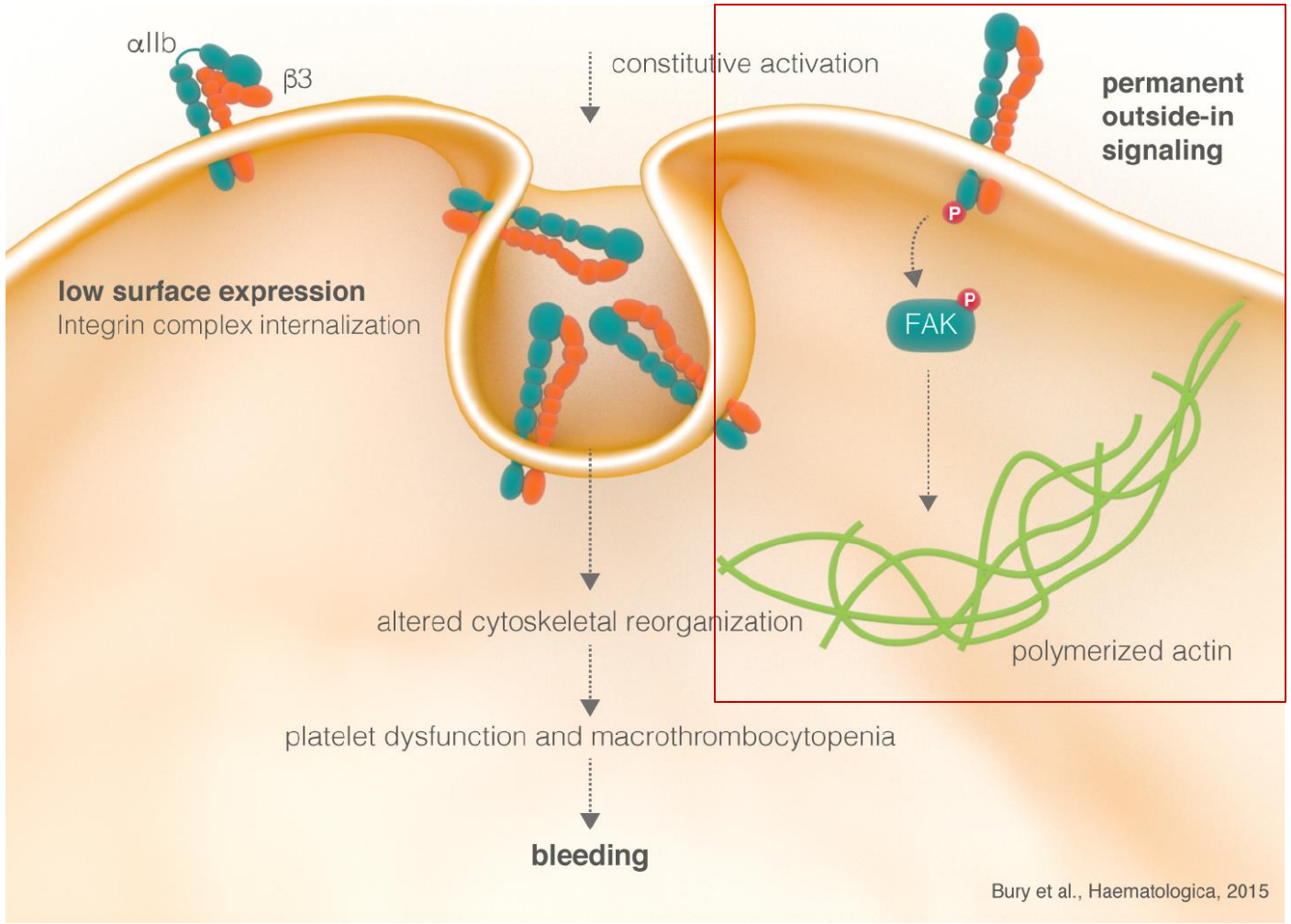


Increased actin polymerization



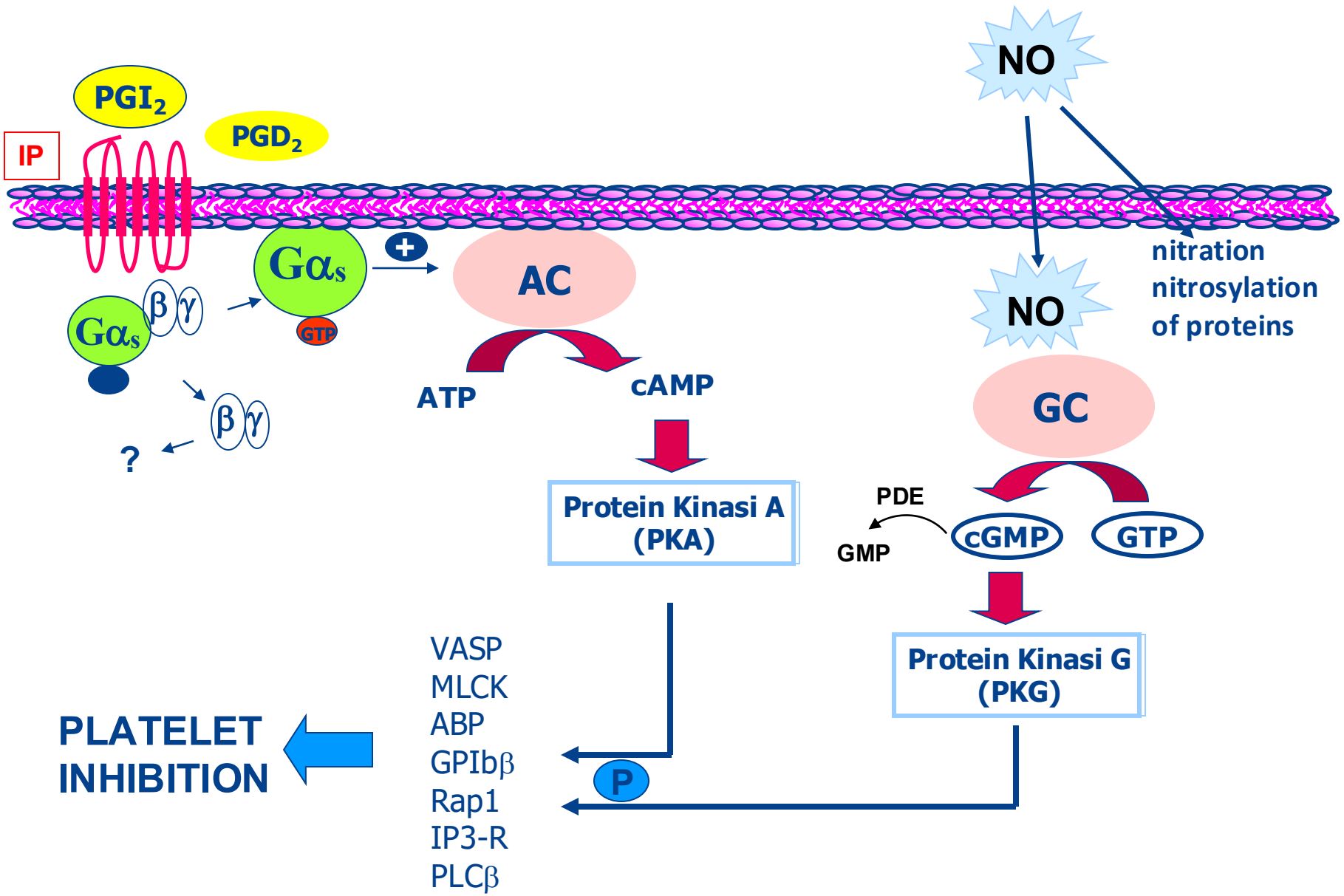
Cytoskeletal perturbation leads to platelet dysfunction and thrombocytopenia in variant forms of Glanzmann thrombasthenia

haematologica

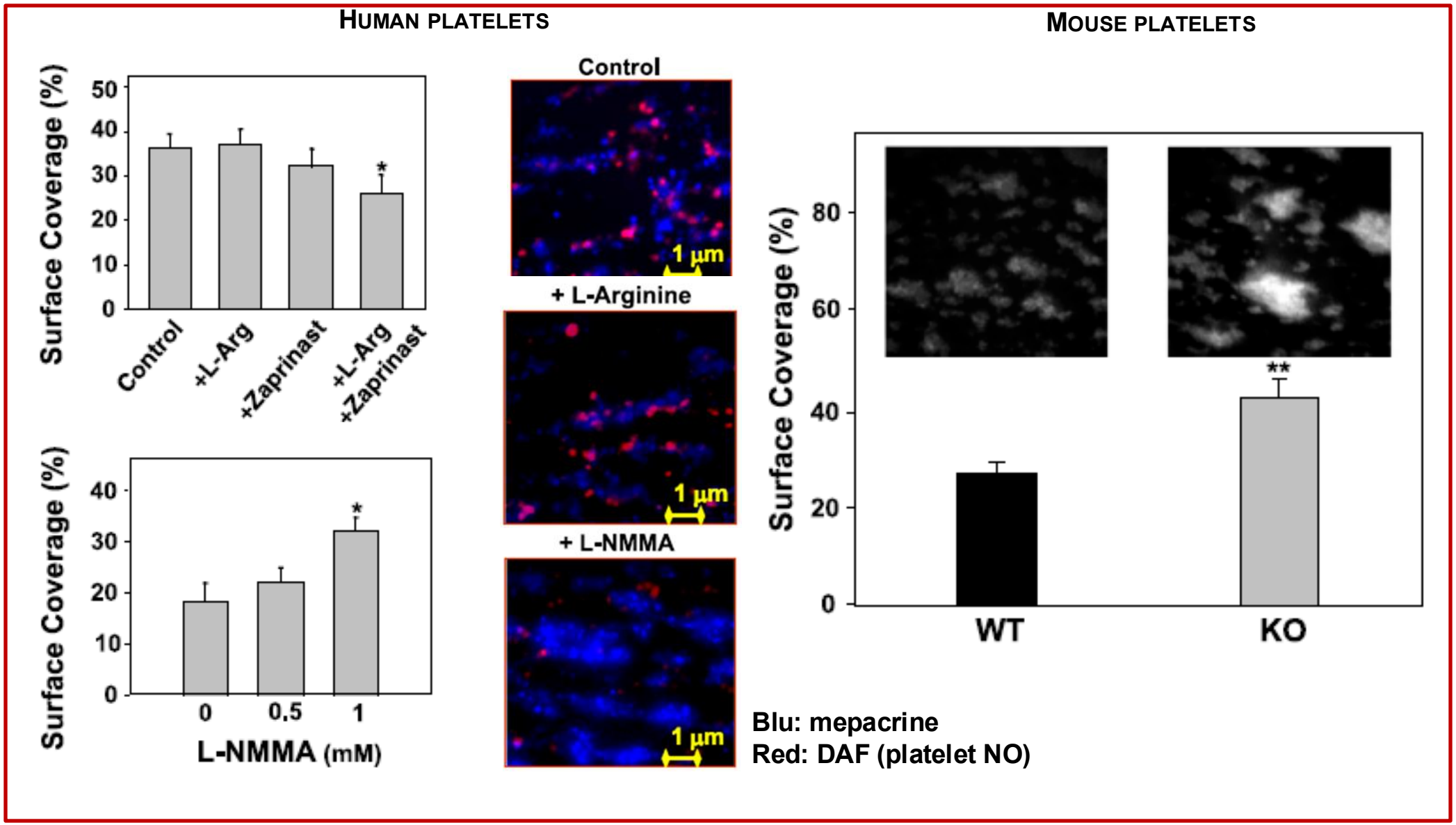


Bury L. et al. Haematologica 2016; 101:46-56

Platelet Inhibitory Signaling mediated by G proteins



Platelet-derived NO inhibits platelet deposition on collagen under flow



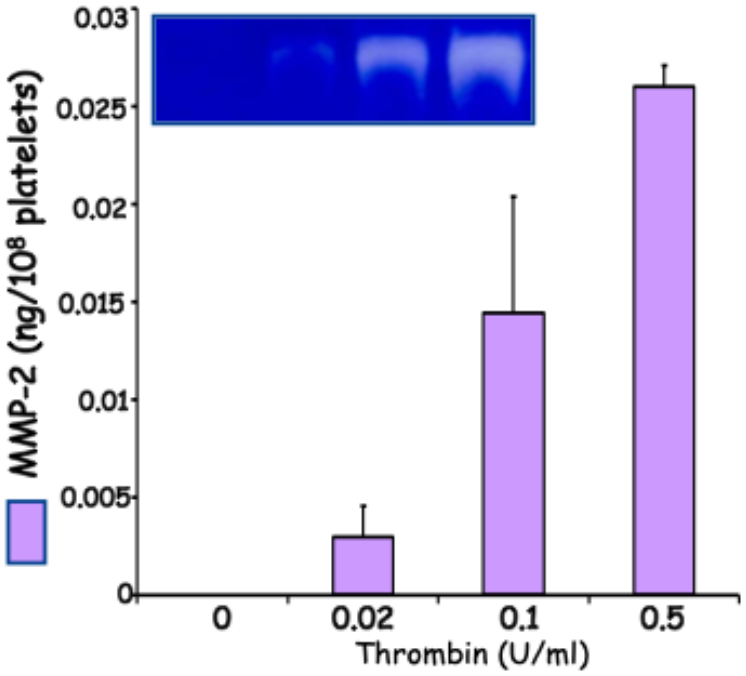
paolo.gresele@unipg.it

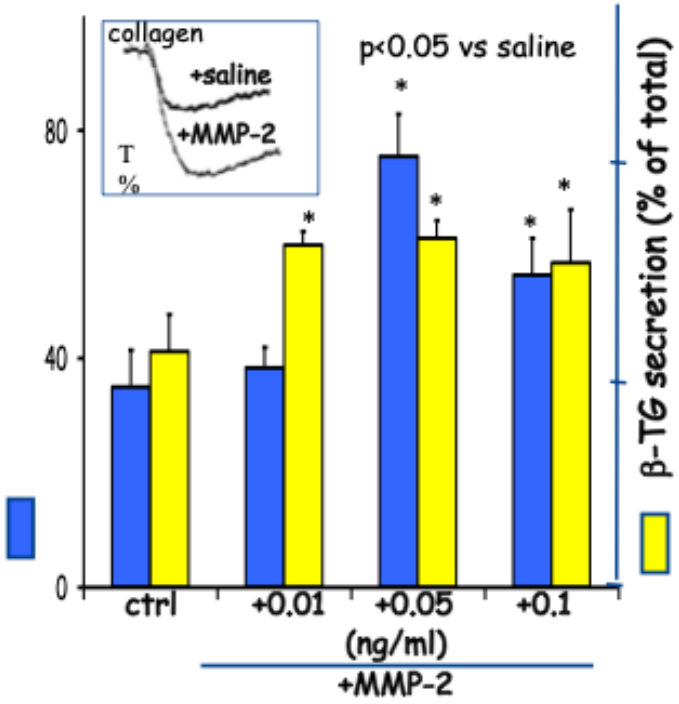
Cozzi M et al., Blood 2015; 125:697

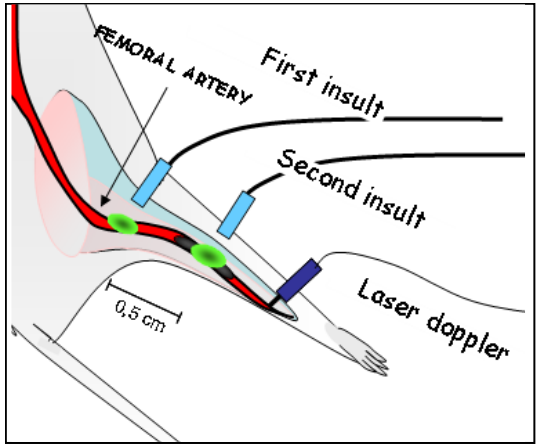
Platelet Priming

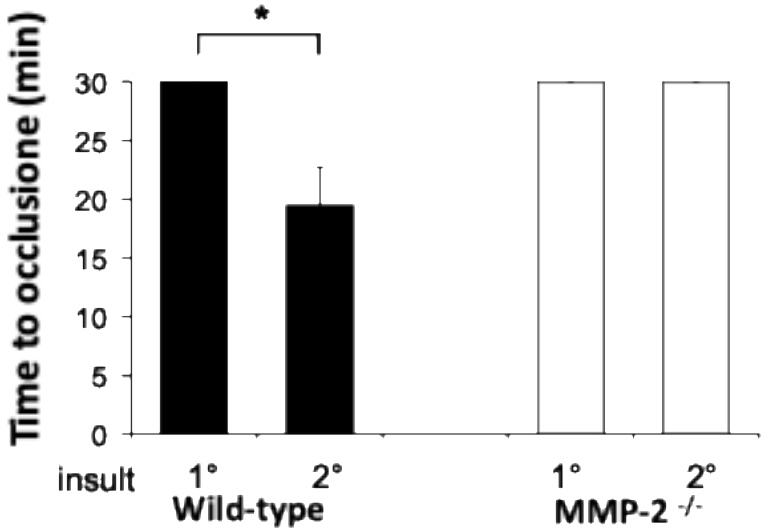
- A regulatory mechanism controlling the response of excitable cells to stimuli: when the prior exposure to a given mediator predisposes a cell to a more effective response to a subsequent stimulus.
- Several physiologic substances potentiate the activation response to primary agonists without eliciting themselves platelet aggregation

Release of MMP-2 by activated platelets and effects on aggregation





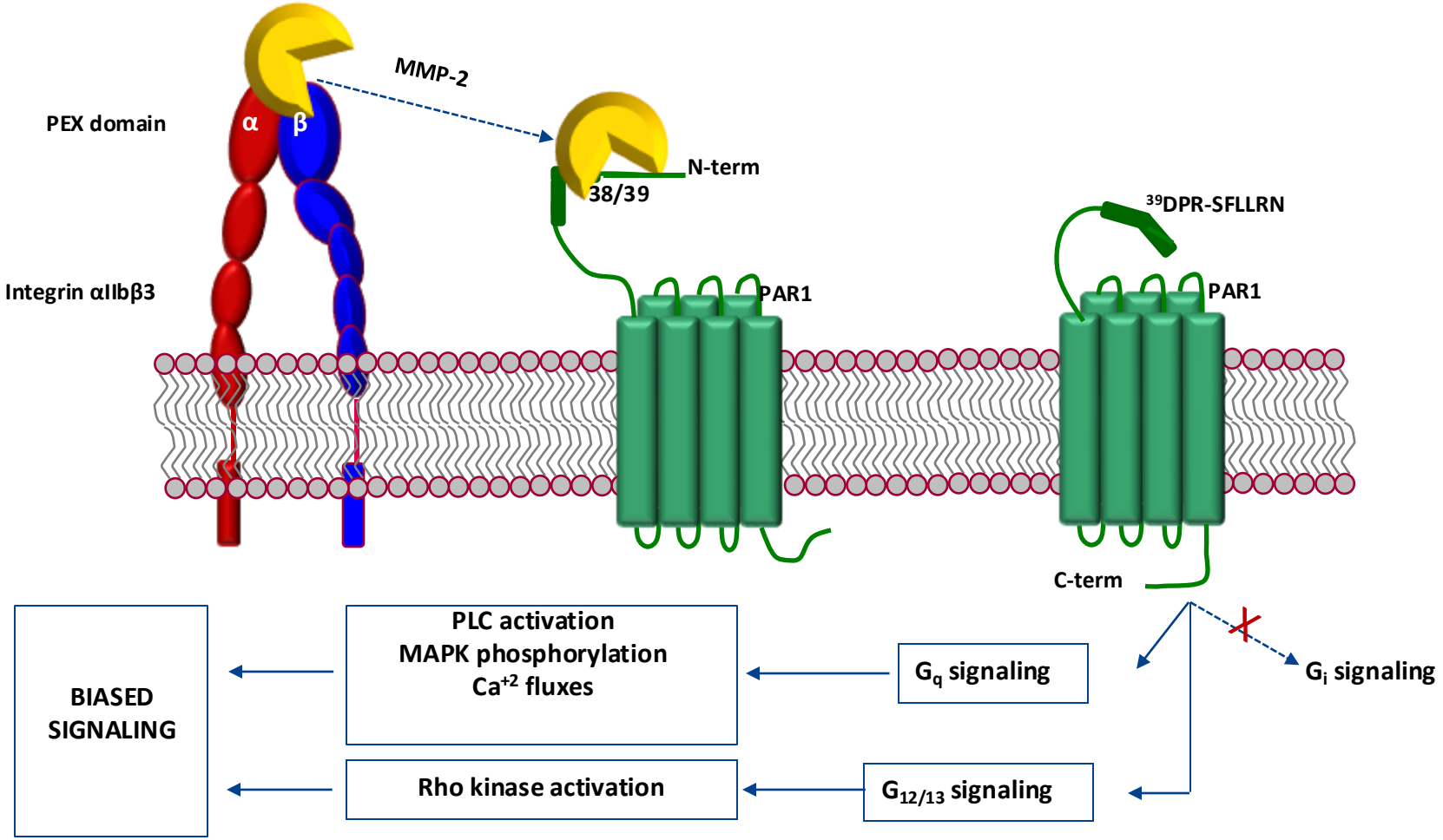




Falcinelli E et al., J Thromb Haemost 2005, 3: 2526.

Momi S et al., J Exp Med 2009, 206: 2365-2379.

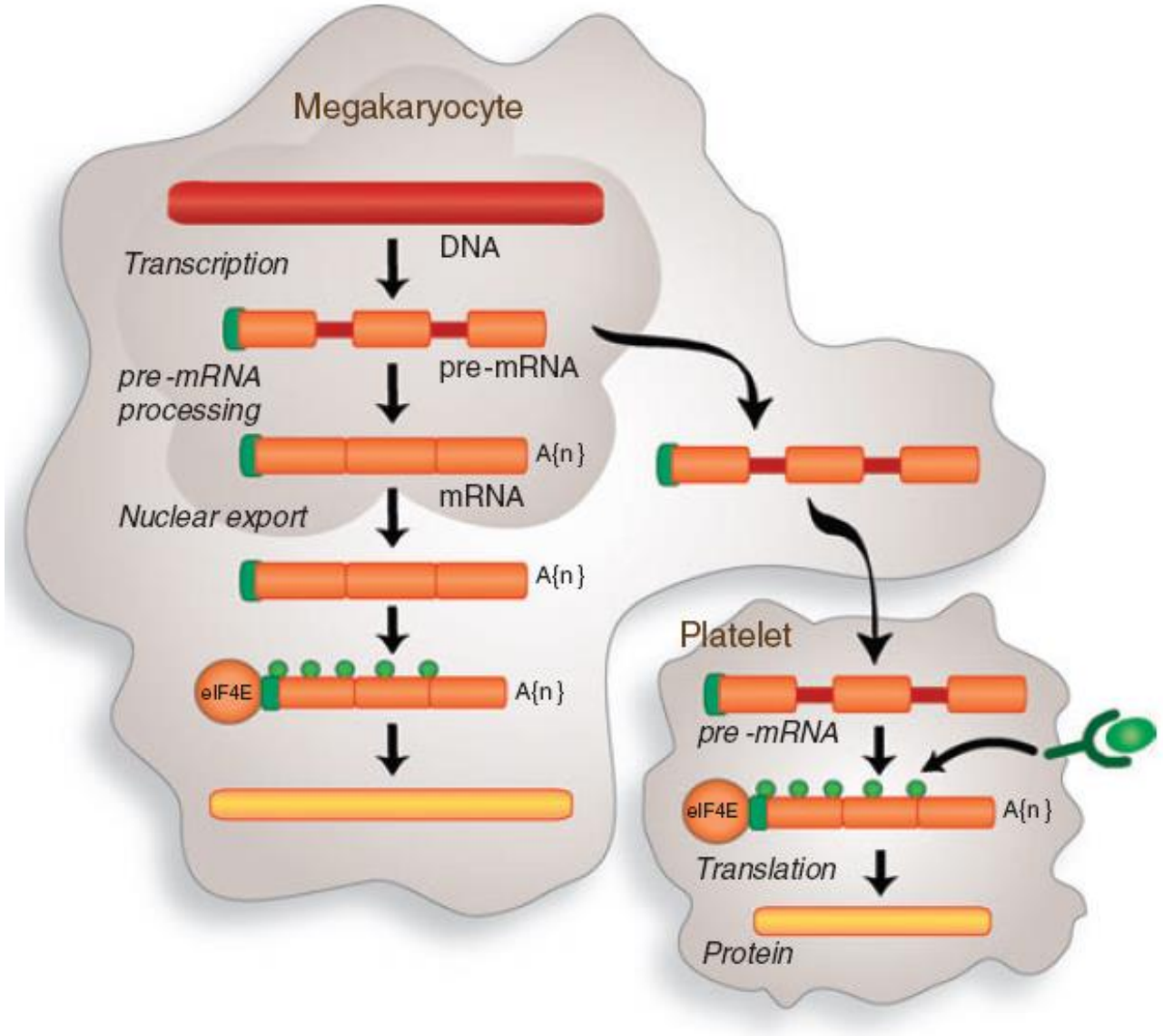
Mechanism of the modulation of platelet signaling by MMP-2



Sebastiano M et al., Blood 2017, 129: 883

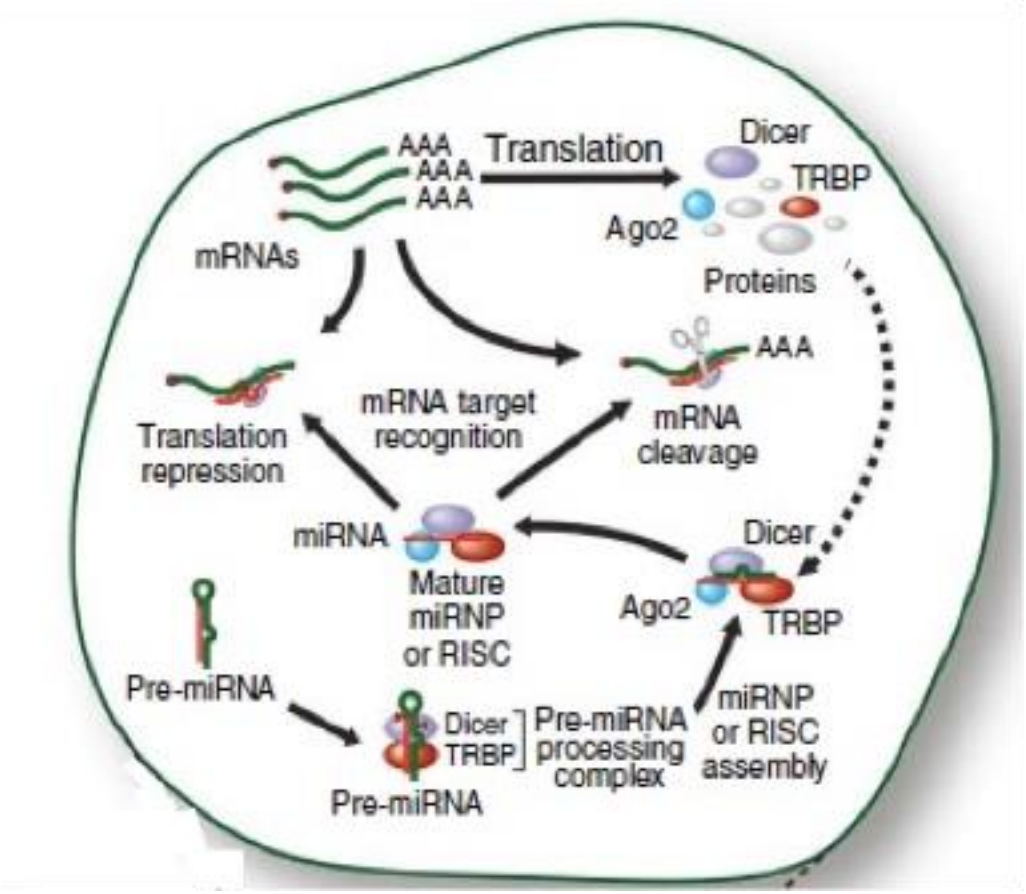
Platelet synthesize new proteins

Examples:
- BCL-3
- COX-1
- COX-2
- DICER1
- IL-1β
- PAI-1
- TF
- TIMP-2



Weyrich A et al., J Thromb Haemost 2009, 7:241

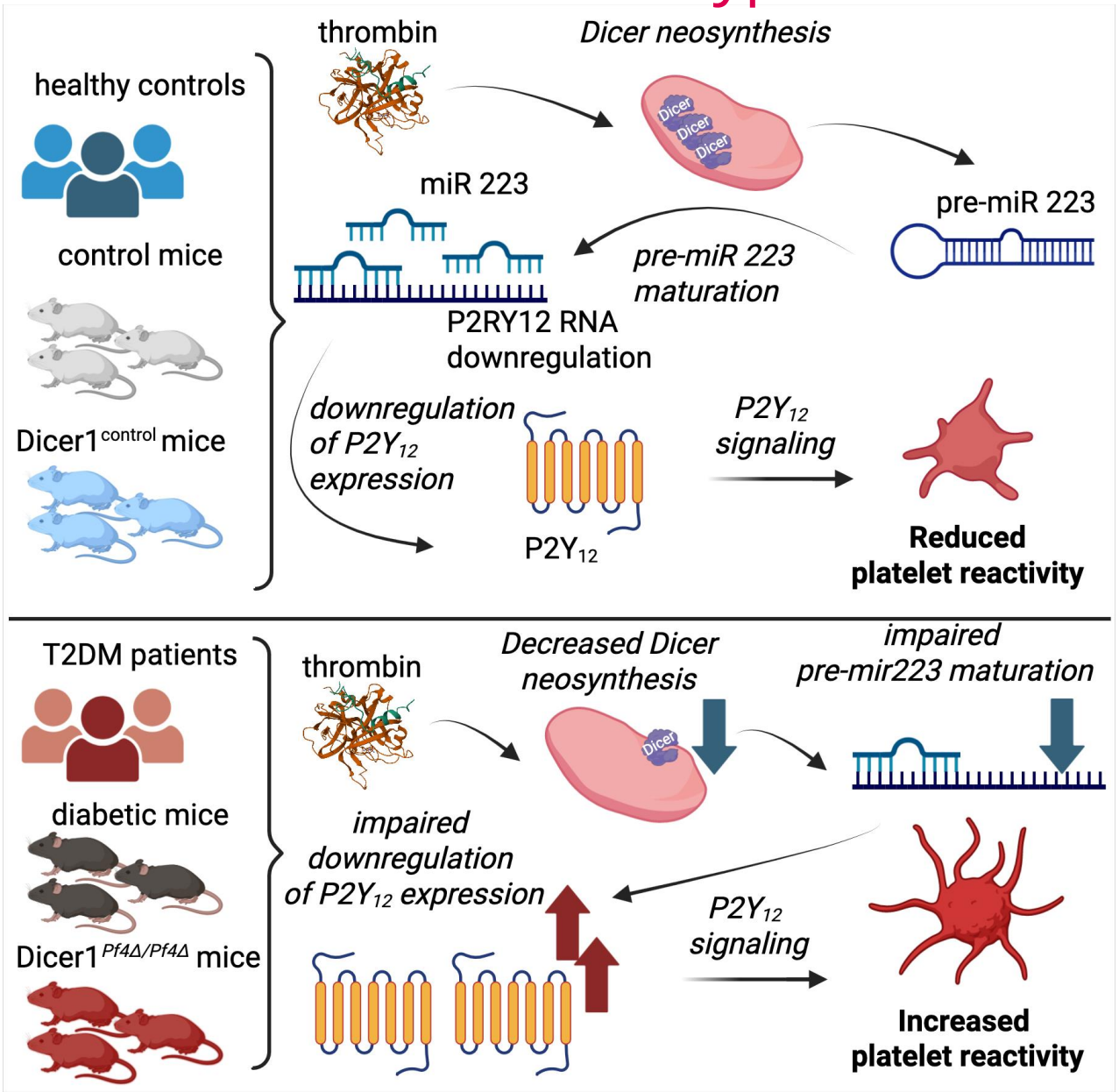
miRNAs regulate protein synthesis in platelets



- Platelets contains miRNAs and the RNA interference machinery (Dicer, Ago2, TRBP)
- miRNa regulates gene expression in anucleate platelets

Landry P et al. Nature 2009

Dicer neosynthesis regulates platelet reactivity: a mechanism altered in type 2 diabetes



Bury L, et al. Circ Res. 2025;137(6):882-898.

Conclusions

- Platelet activation is orchestrated by ITAM-mediated receptors (GPVI, FcγRIIA), G-protein–coupled receptors (thrombin, ADP, TxA_2, epinephrine), and integrin $\alpha_{IIb}\beta_3$ signaling.

- These canonical pathways regulate adhesion, secretion and aggregation, ensuring effective hemostasis.

- However, platelets also exploit alternative regulatory mechanisms, including priming, neosynthesis of proteins and regulation by miRNAs.

- Many of these mechanisms may be dysregulated in disease.

THANK YOU

CRPP Centre de Référence Pathologies Plaquettaires

MHEMO La Filière des maladies rares de l'hémostase

ebn ERN | EuroBloodNet

Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Health and Digital Executive Agency (HaDEA). Neither the European Union nor the granting authority can be held responsible for them.

Funded by the European Union

CRPP
Centre de Référence
Pathologies Plaquettaires

https://maladies-plaquettes.fr/

https://x.com/crpp12

https://www.linkedin.com/company/fili%C3%A8re-de-sant%C3%A9-maladies-rares-mhemo/

MHEMO
La Filière des maladies hémorragiques constitutionnelles

https://mhemo.fr/

https://x.com/filiereMHEMO

https://www.linkedin.com/company/fili%C3%A8re-de-sant%C3%A9-maladies-rares-mhemo/

Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Health and Digital Executive Agency (HaDEA). Neither the European Union nor the granting authority can be held responsible for them.

Funded by
the European Union

webinar

www.eurobloodnet.eu

@ERNEuroBloodNet

eurobloodnet-european-reference-network-on-rare-hematological-diseases

Eurobloodnet - European Reference Network on Rare Hematological Diseases

ERN-EuroBloodNet's EDUcational Youtube channel

Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Health and Digital Executive Agency (HaDEA). Neither the European Union nor the granting authority can be held responsible for them.

Funded by the European Union