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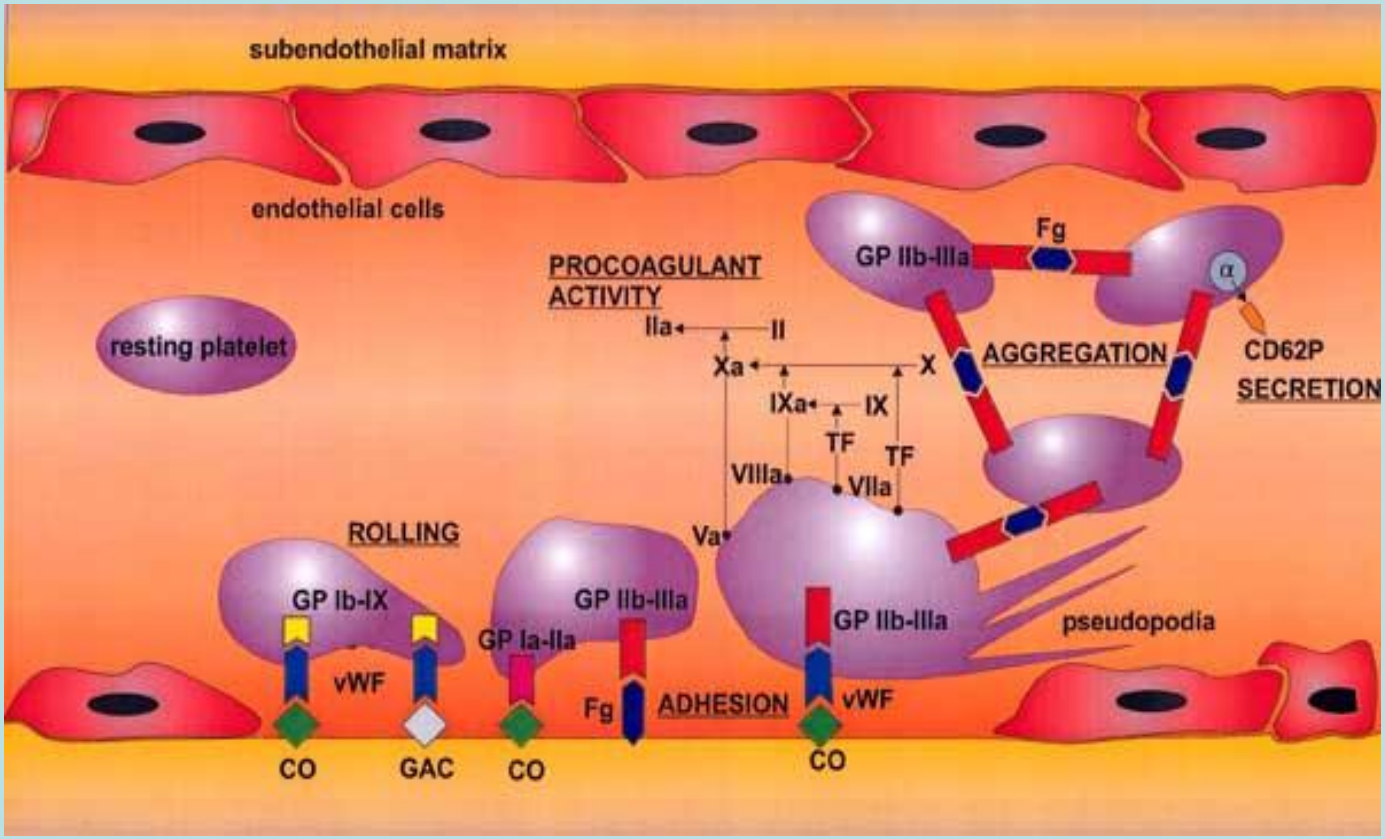
Integrin αIIbβ3: The role of peptides derived from its extra-cellular and intracellular domain on outside-in and inside-out platelet activation

D.C.Tsoukatos

University of Ioannina, Department of Chemistry, Ioannina - 45110, Greece

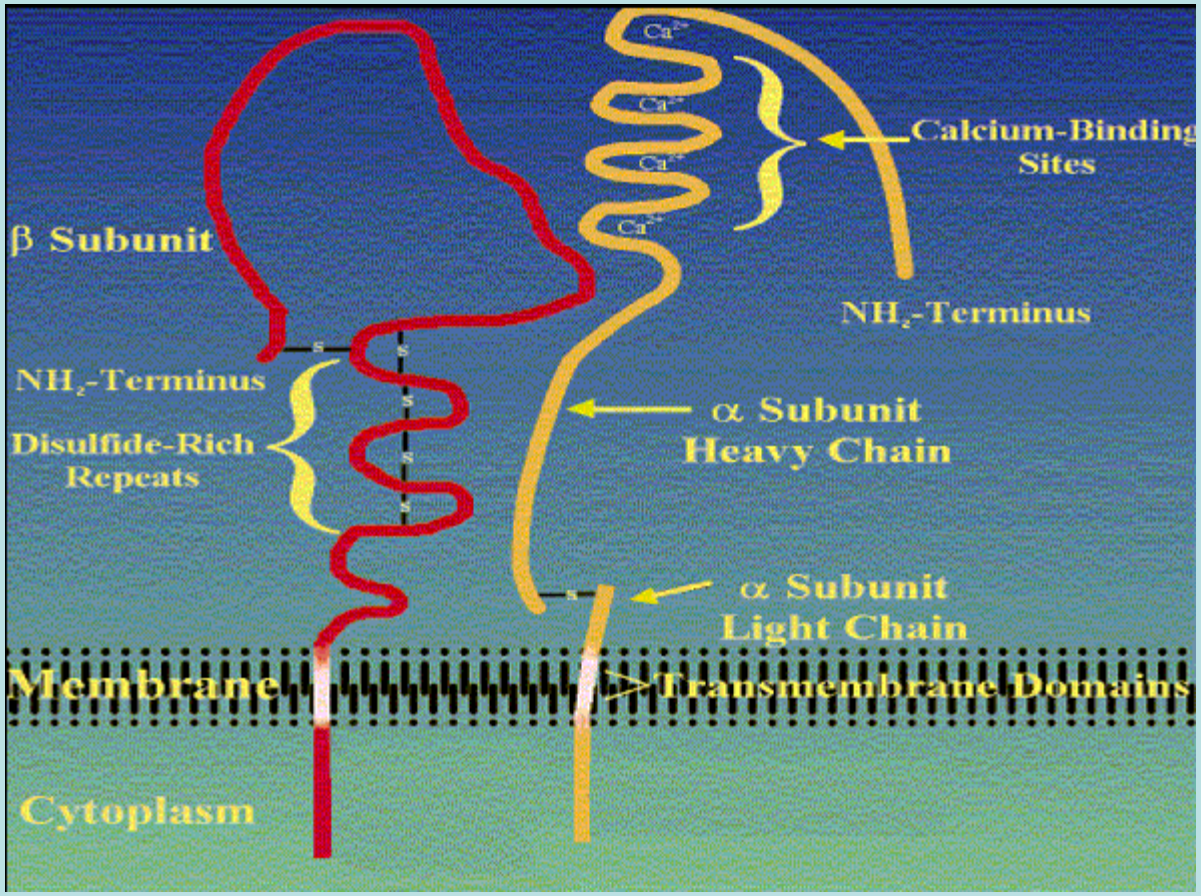
Tel: +0030 2651 008368, Fax: +0030 2651 047832, e-mail: dtsoykat@cc.uoi,gr

Platelet integrin αIIbβ3 (GPIIb-IIIa) the Fg receptor



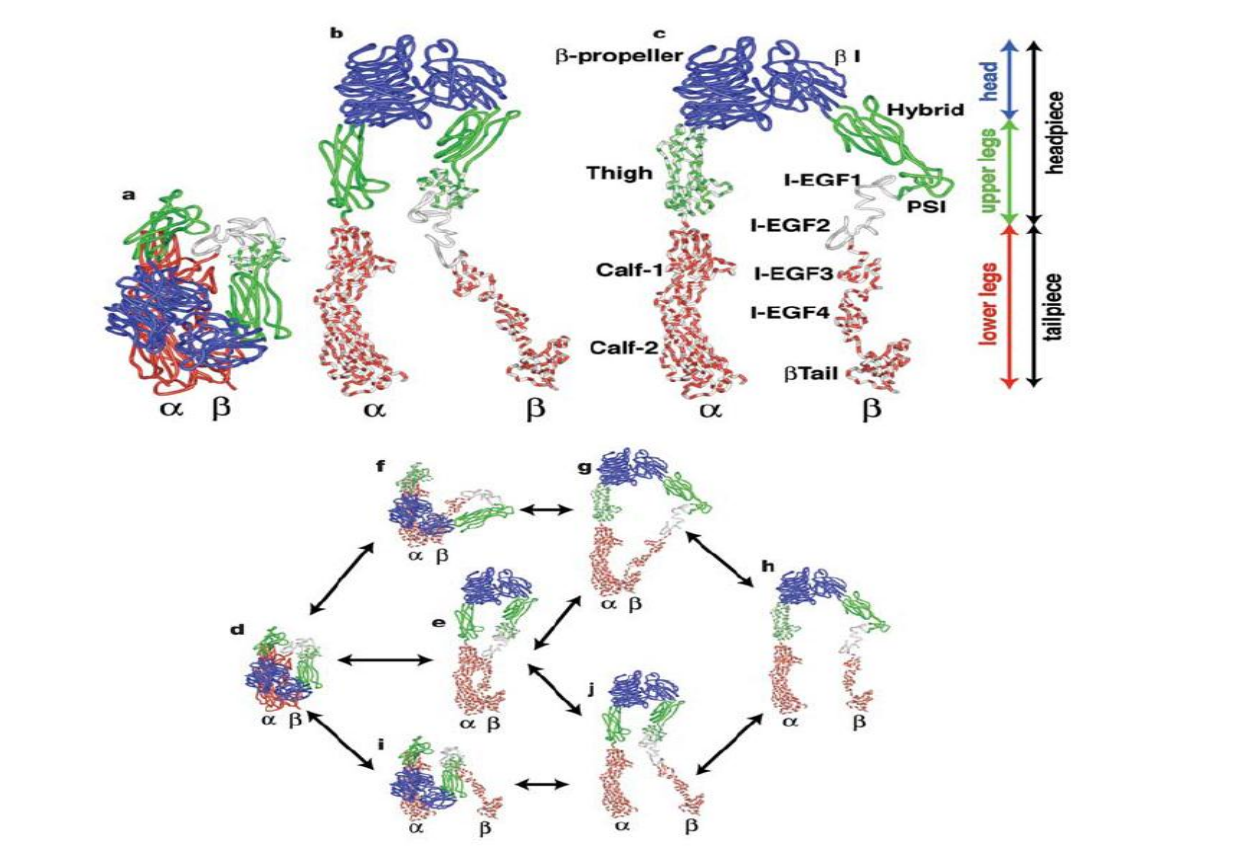
αIIbβ3 plays an important role in platelet adhesion and is responsible for platelet aggregation

Platelet integrin αIIbβ3 (GPIIb-IIIa)



- Integrin αIIbβ3 is a (α/β) dimer
- Each subunit contains an extracellular, a transmembrane and a cytoplasmic domain.

Integrin αIIbβ3 is strictly temporary and locally activated

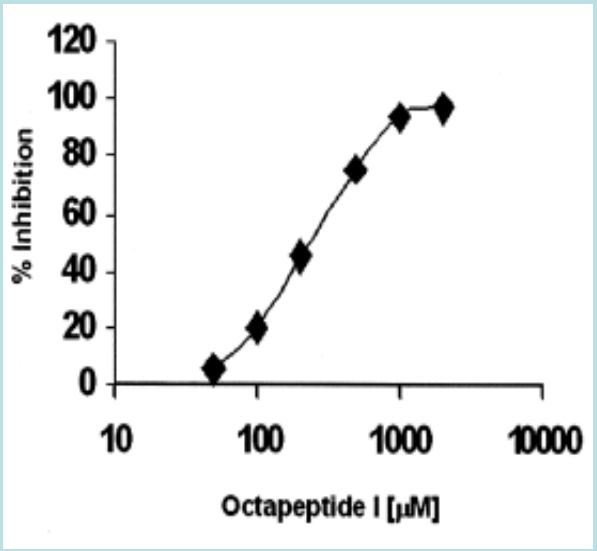


- In resting platelets integrin has a low-affinity (inactive), bent conformation.
- Upon platelet activation it takes an intermediate affinity conformation.
- Upon Fibrinogen binding it takes the high-affinity, extended form with an ‘open’ headpiece.
- The molecular rearrangement leading from the bent to the open structure is a multi step procedure.
- It is activated from the out-side via ligand binding and from the in-side via separation of α and β subunits.

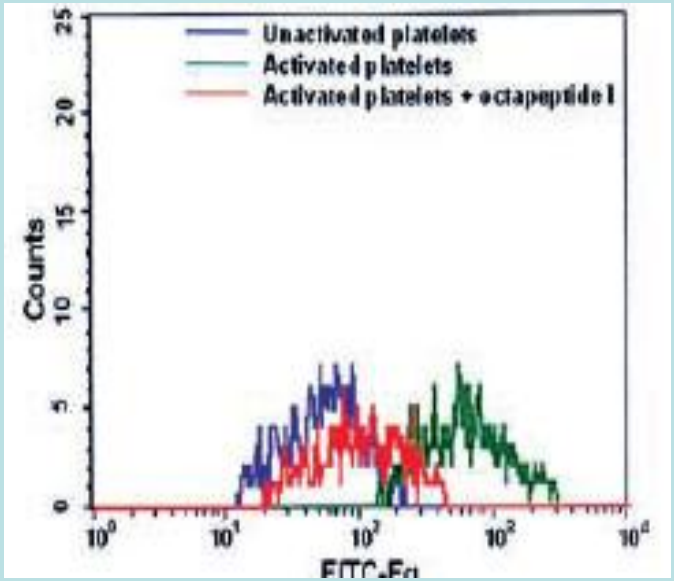
Xiao, T., et al 2004, Nature, 432. 59-67

Inhibition of platelet aggregation and fibrinogen binding to activated platelets by the $_{313}$YMESRADR$_{320}$

A



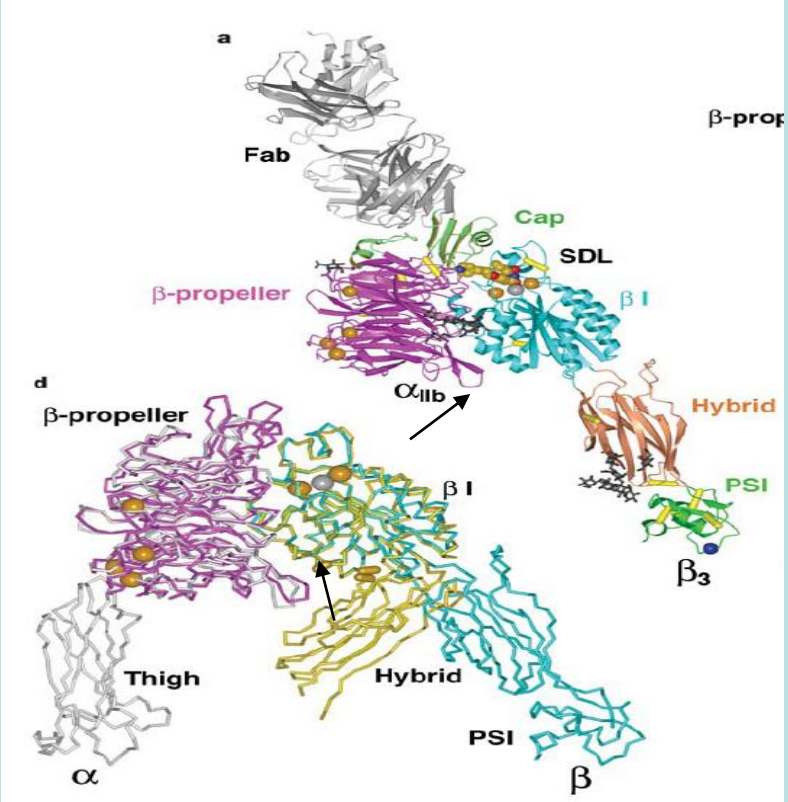
B



- A. Dose response curve for octapeptide YMESRADR demonstrating the inhibition of platelet aggregation induced by ADP.
- B. Representative histograms obtained by FACS analysis illustrating the inhibition by YMESRADR of Fibrinogen binding to ADP activated platelets

Mitsios JVet al 2004 Eur J Biochem 271: 855-862

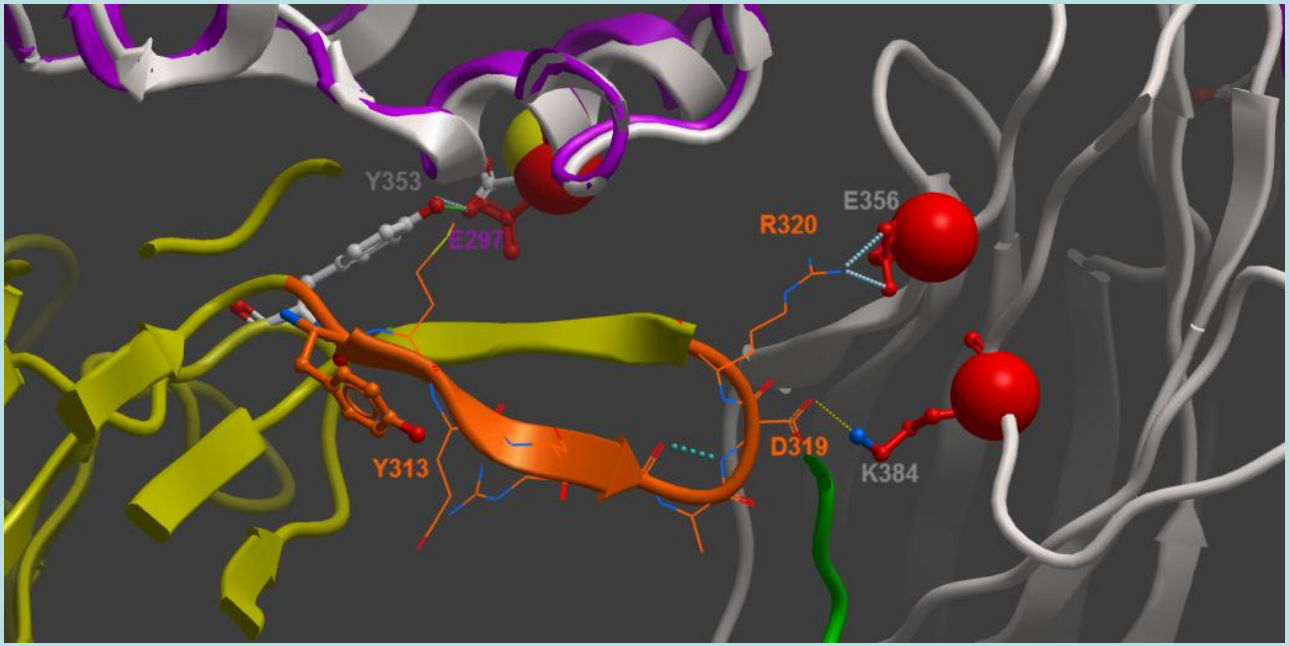
The YMESRADR sequence in the propeller motif of αIIb



Xiao, T., et al 2004, Nature, 432. 59-67

- The YMESRADR sequence is located in the beta-propeller structural motif of αIIb and consists a hairpin.
- It may keep the αIIb in contact with β3 in the bent inactive conformation.
- It can inhibit the opening of the angle between βI and hybrid domain of β3

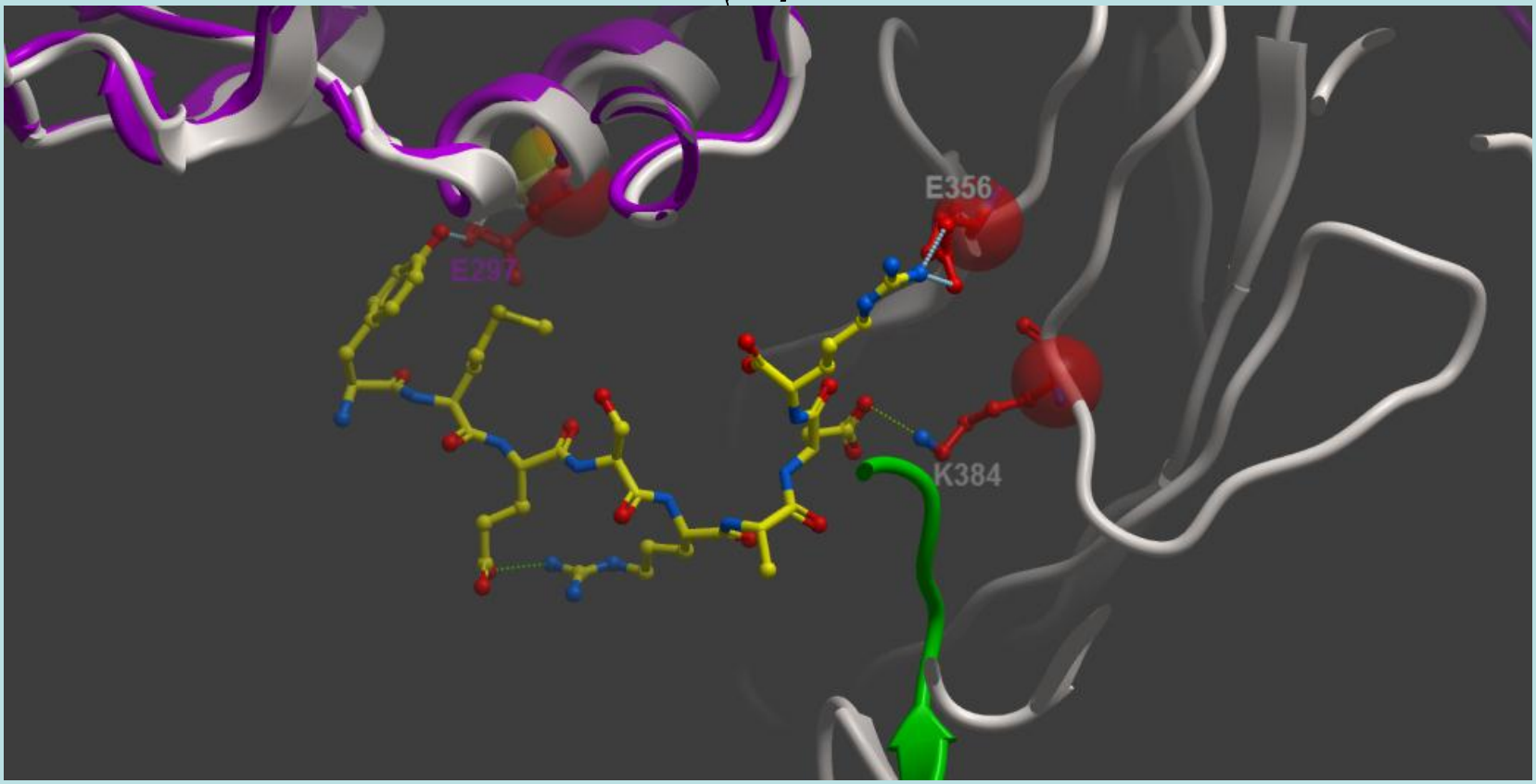
Detail of the hairpin in action



Wen Hwa Lee et al 2012 under preparation

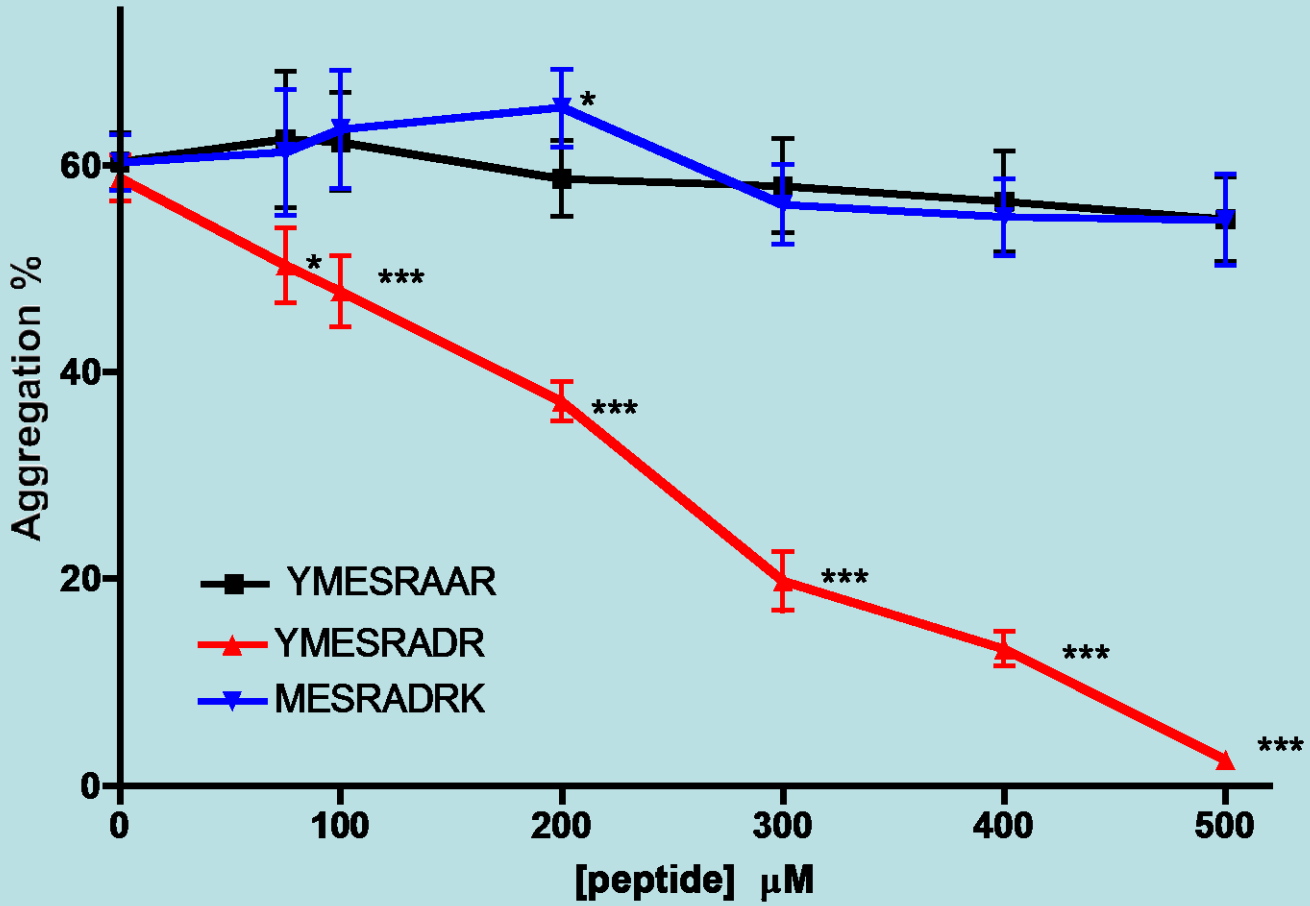
- YMESRADR sequence (orange) is engaged in two salt bridges with the hybrid domain (right side of pic) whereas the αIIb β-propeller is interacting with the β3 βI (Y353 is one of such interactions and it is shown interacting with E297).

A possible conformation adopted by the YMESRADR peptide, bridging across βI and the hybrid domains of β3.

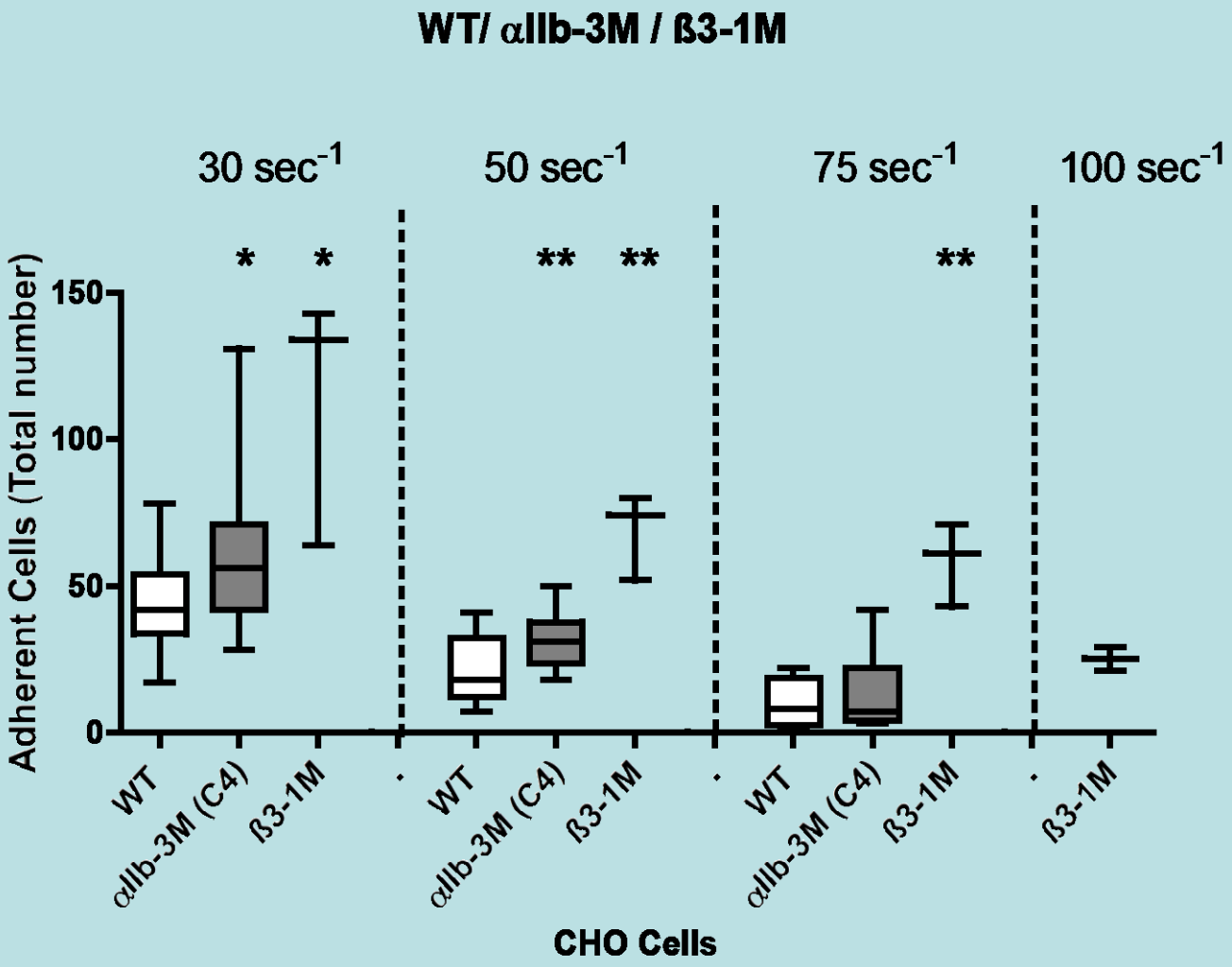


- Y313 now replaces the Y353 in the bridging role

Inhibitory effects of octapeptides (YMESRADR (red); substituted YMESRAAR (black) or MESRADRK (blue)) on thrombin-induced human washed platelet aggregation

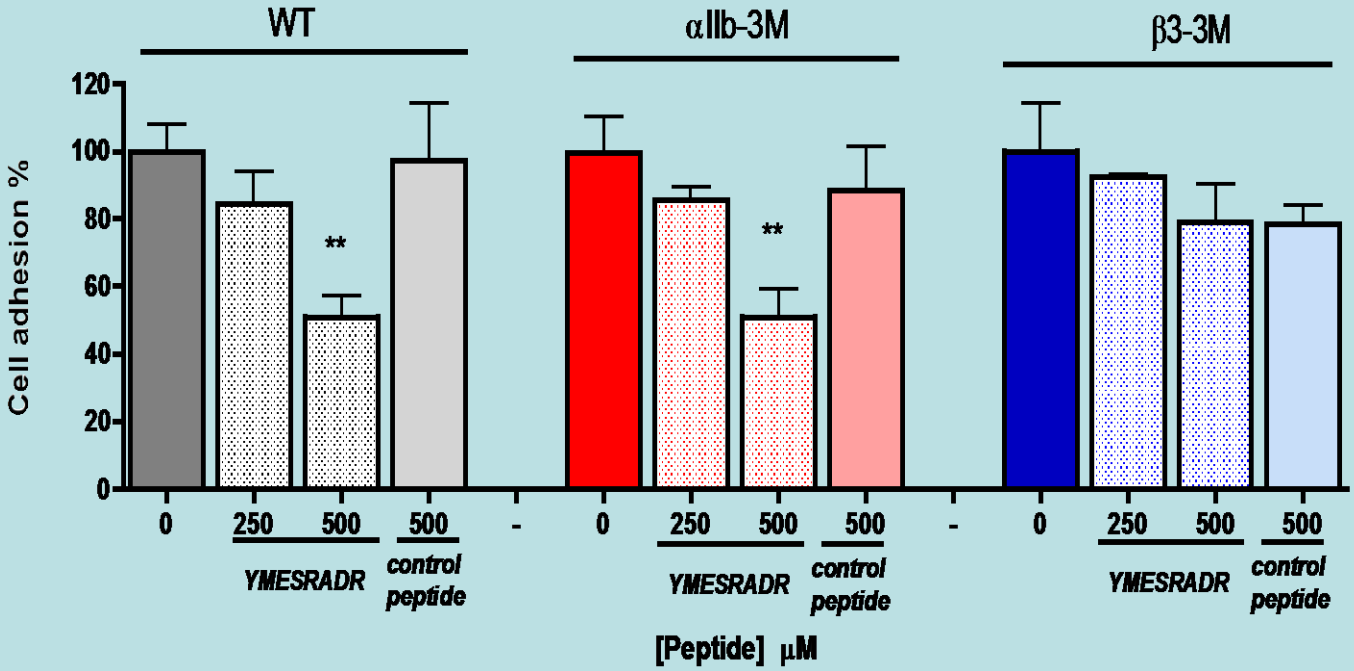


Cell adhesion under shear



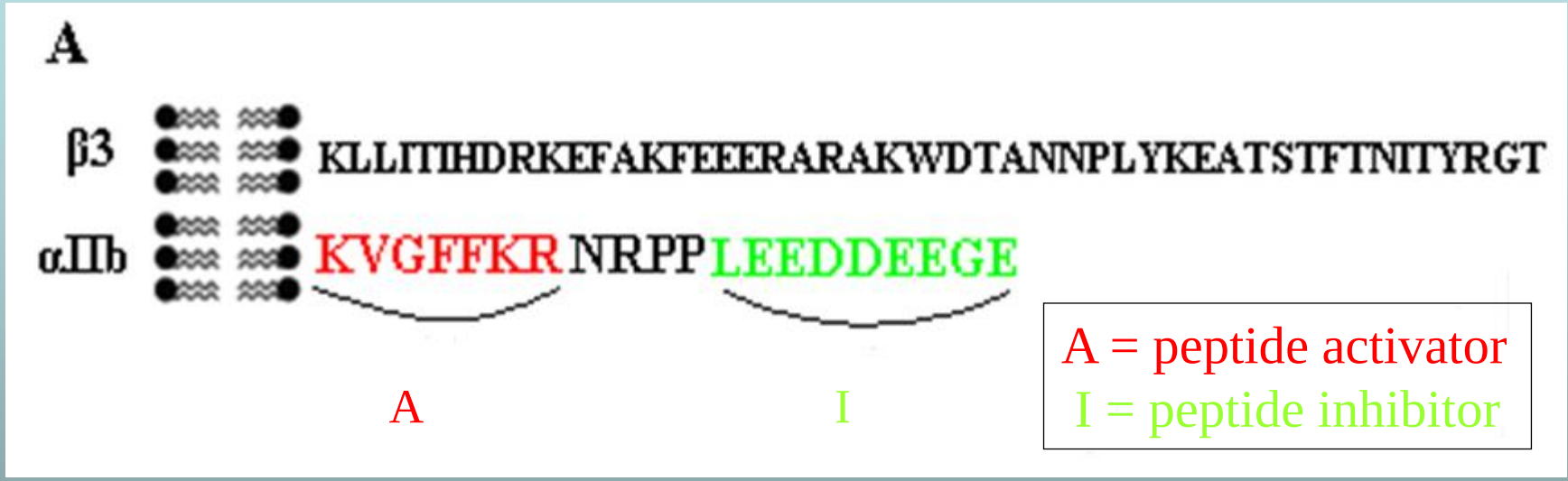
WT, aIIb-3M (R317A.D319A.R320A), b3-1M (K384A)

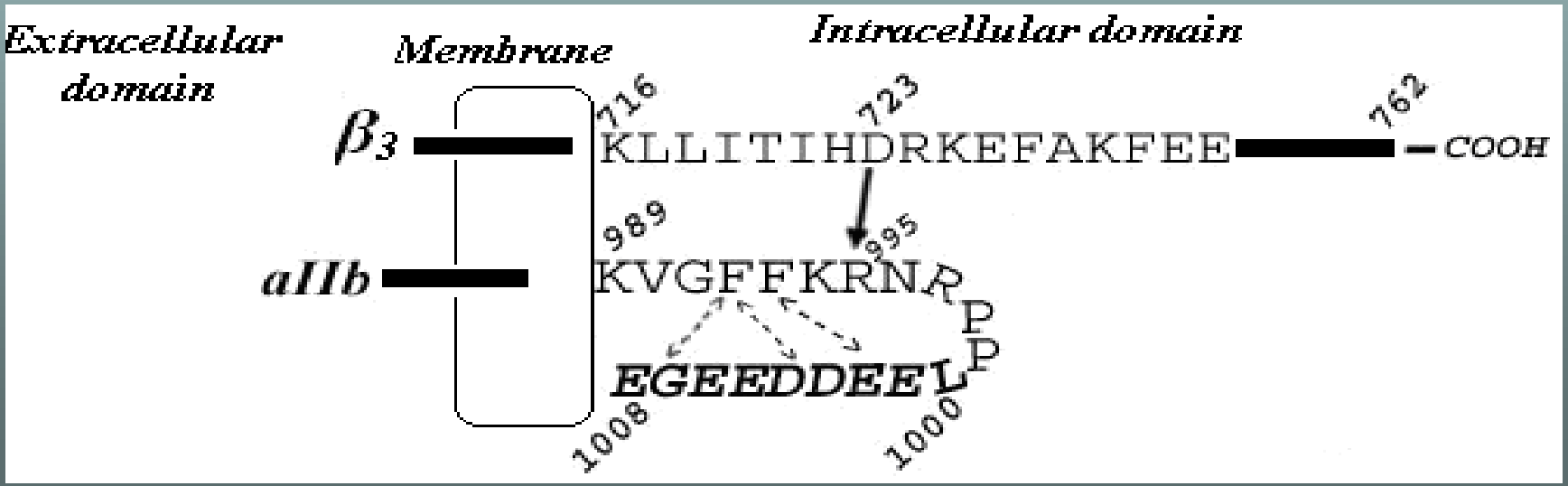
Effect of octapeptide on CHO cells adhesion under shear



WT, (aIIb-3M) R317A-D319A-R320A, (b3-3M) E297A-E356A-K384A

The α_{IIb} intracellular tail

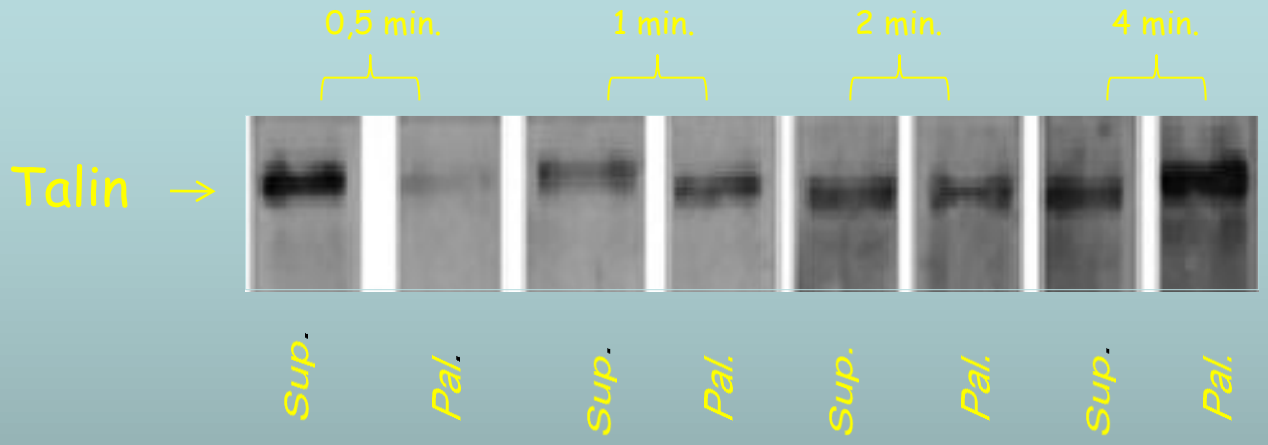




Koloka V et al Platelets 19: 502-511 2008

Talin is a cytoskeletor protein

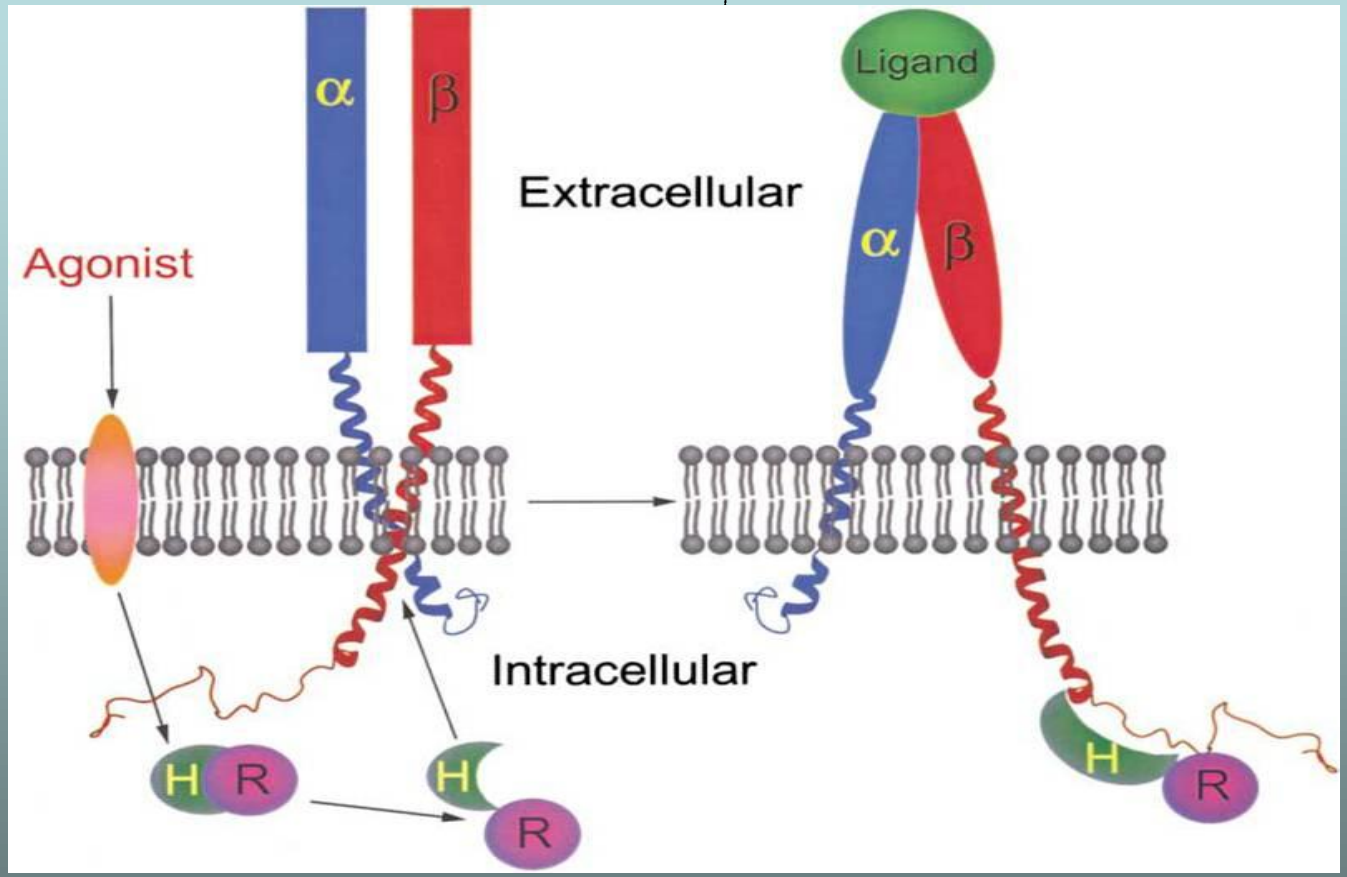
W.B : anti-talin



- During platelet aggregation by thrombin, talin translocates to the cytoskeletal sendiment.

AV Gourogianni, et al 2009-2010 Pathophysiol Haemost Thromb 37(suppl 1) p A7

The role of Talin in the in-side activation of the receptor



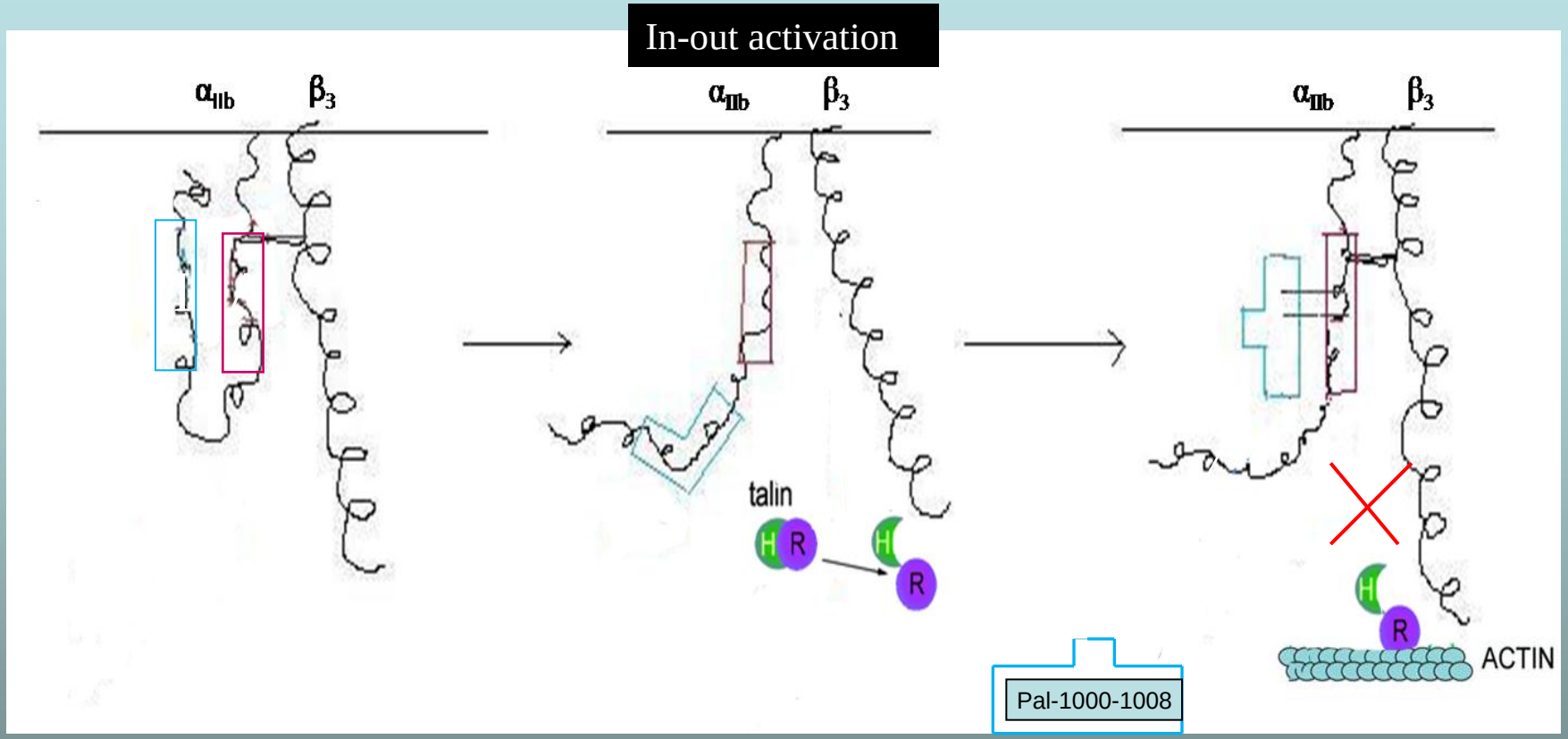
Vinogradova O et al 2002 cell 110:587-597

Coprecipitation of talin and aIIbβ3

I.P : anti-aIIbβ3
W.B : anti-talin

- Talin coprecipitates with integrin αIIbβ3 the first minute of platelet aggregation in the presence of Pal-100-1008 .
- Pal-1000-1008 inhibits this coprecipitation from the second to the fourth minute of incubation.

AV Gourogianni, et al 2009-2010 Pathophysiol Haemost Thromb 37(suppl 1) p A7



❖ Pal-LEEDDEEGE can act as a molecular switch, by blocking the binding of Talin to αIIbβ3 and reconstructing the clasp betweem αIIb and β3 membrane proximal tails.

Conclusions

- The present work, provides intracellular and extra-cellular peptide inhibitors of αIIbβ3.
- In combination the peptides could inhibit both outside-in and inside-out activation of the receptor.
- The inhibition by a non competitive to Fg , non RGD like mechanism of action, could provide an alternative anti-thrombotic pharmacological approach.

CONTRIBUTORS OF THE STUDY

- Department of Chemistry University of Ioannina

Laboratory of Biochemistry

Prof DC Tsoukatos, Prof M. Sakarellos Daitsiotis, Assoc.Prof E. Panou-Pomonis

Post Graduate students: A Gourogianni, K. Kiouptsi

Laboratory of Organic Chemistry

Prof V. Tsikaris,Dr V Moussis

- INSERM U765 Faculte des Sciences Pharmaceutiques et Biologiques University of Paris 5

Dr C. Bachelot-Loza, Dr Kelly Aylward, Dr Dominique Baruch, Marion Egot

- University of Oxford

Dr Wen Hwa Lee

- Laboratoire de Biologie et Physiologie Integrée, University of Luxembourg

Prof Nelly Kieffer, Dr Elisabeth Schaffner-Reckinger

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JOINT RESEARCH AND TECHNOLOGY PROGRAMMES