XVI Congreso Chileno de Hematología
VI Congreso de Medicina Transfusional
Coquimbo, Septiembre 2008.

Hemostasia y Trombosis. ¿Hacia un Modelo Integrador?

Diego Mezzano
Depto. Hematología-Oncología
P. Universidad Católica de Chile

Figure 1 Cascade & Waterfall Hypothesis of Coagulation

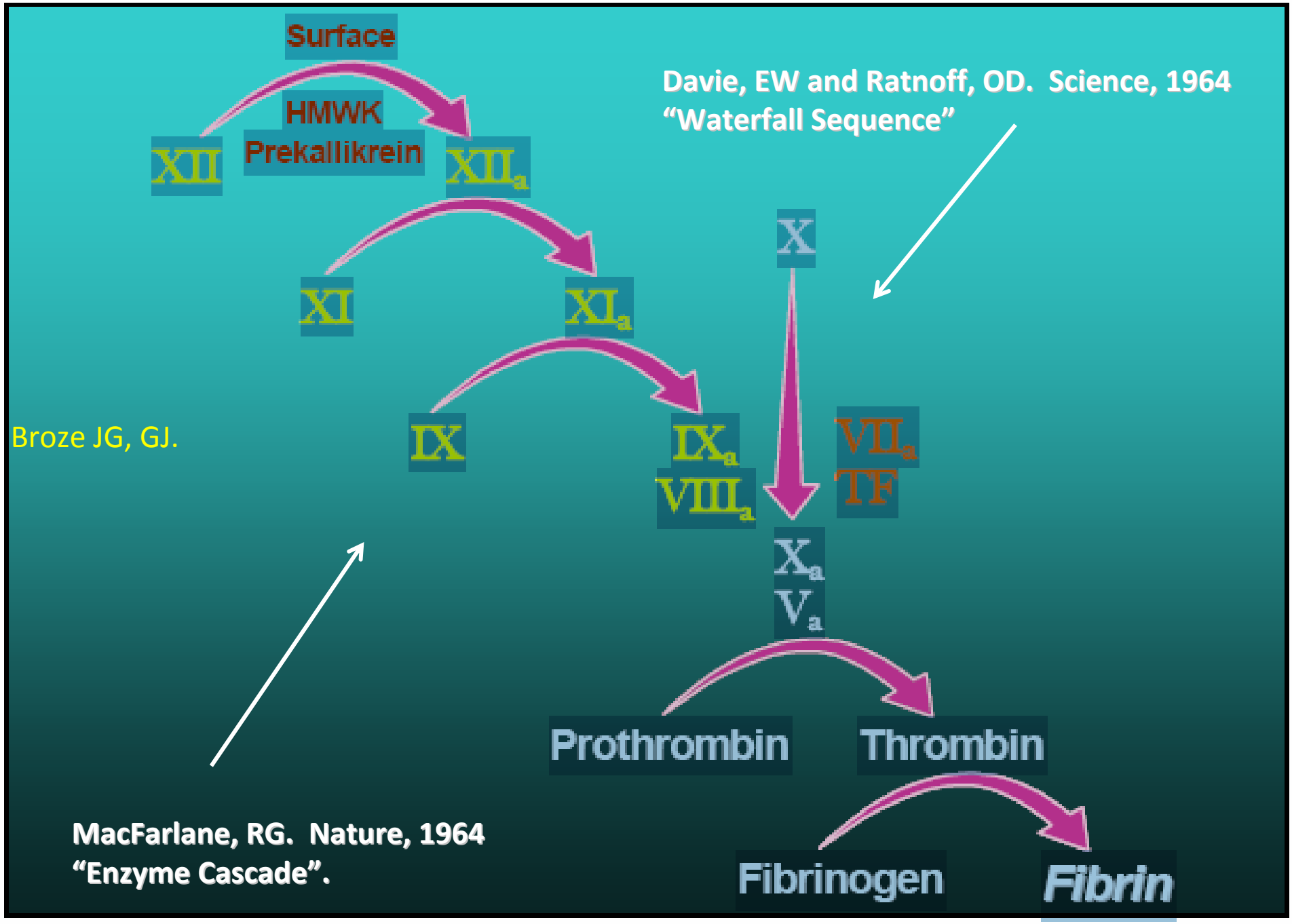
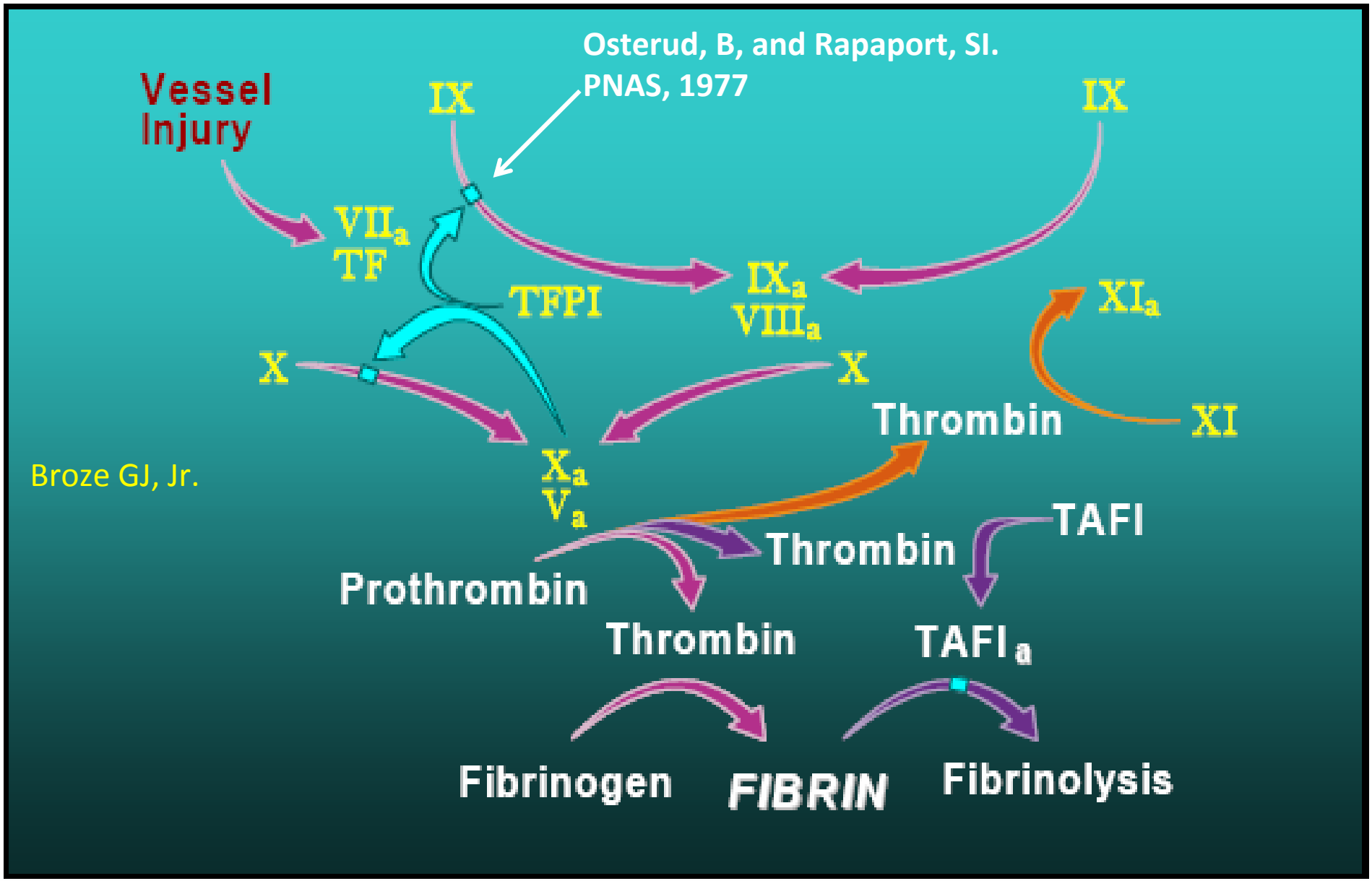


Figure 2 Tissue Factor Pathway of Coagulation



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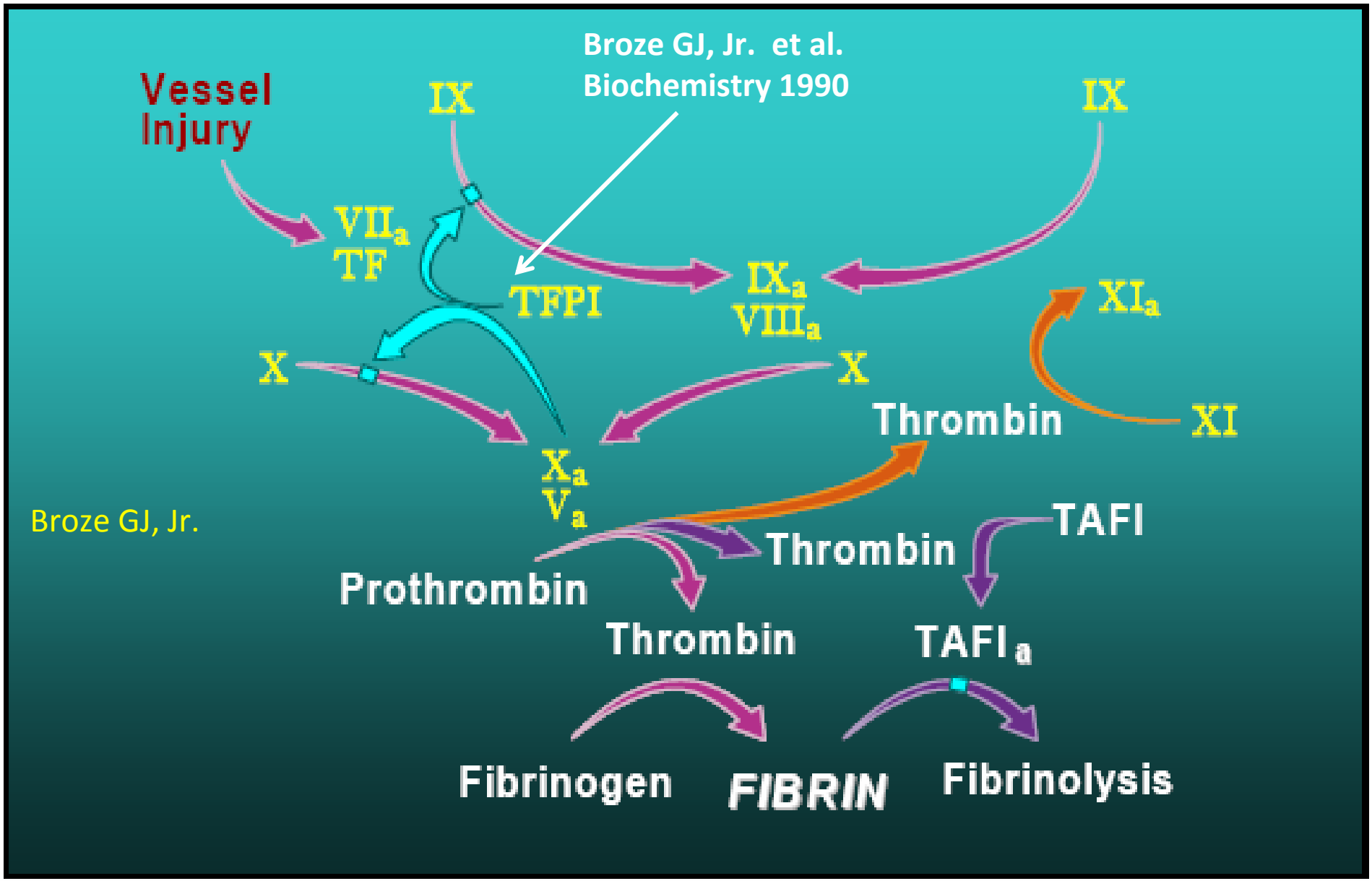
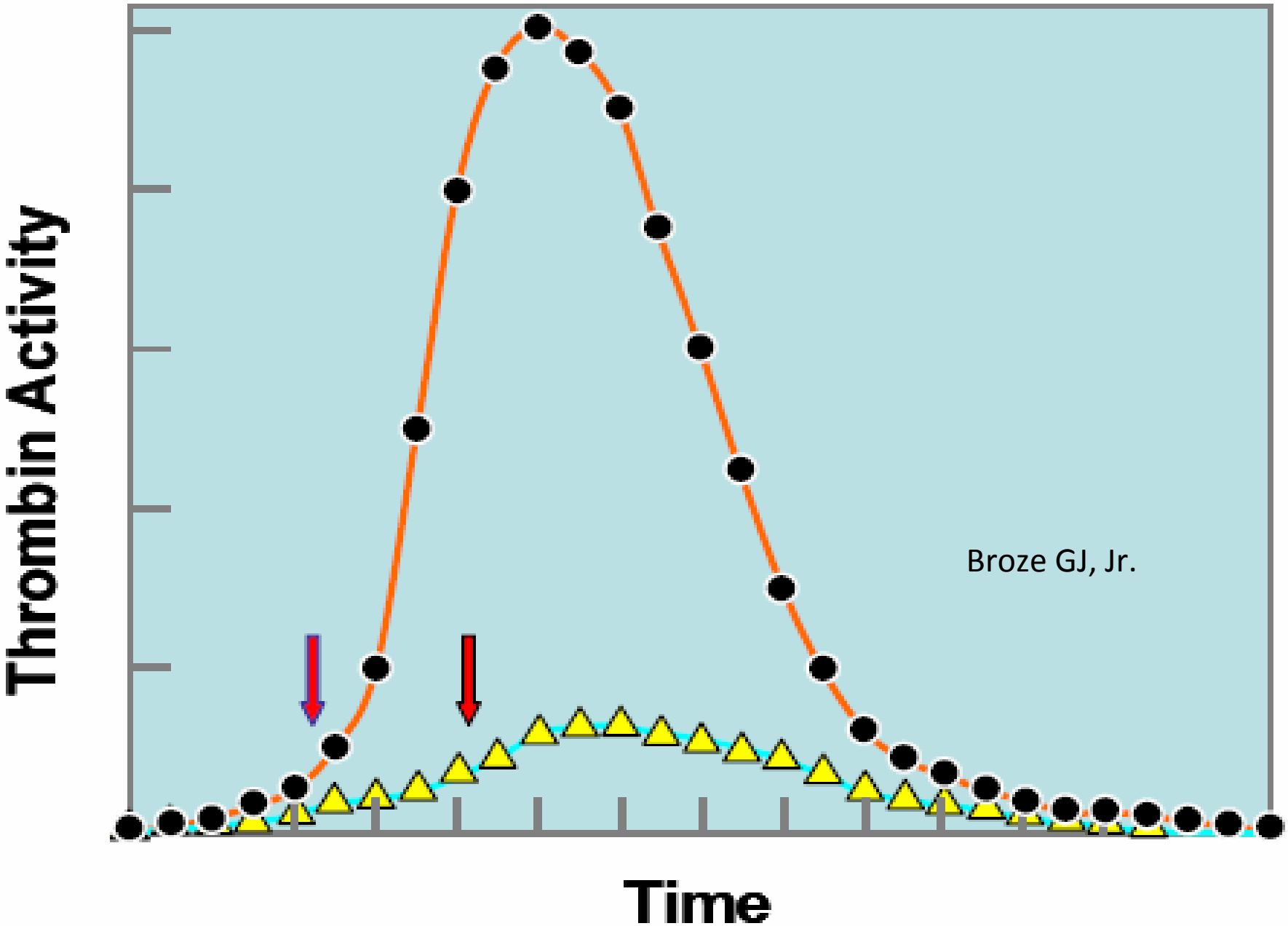


Figure 3 Initiation & Amplification Phases of Coagulation



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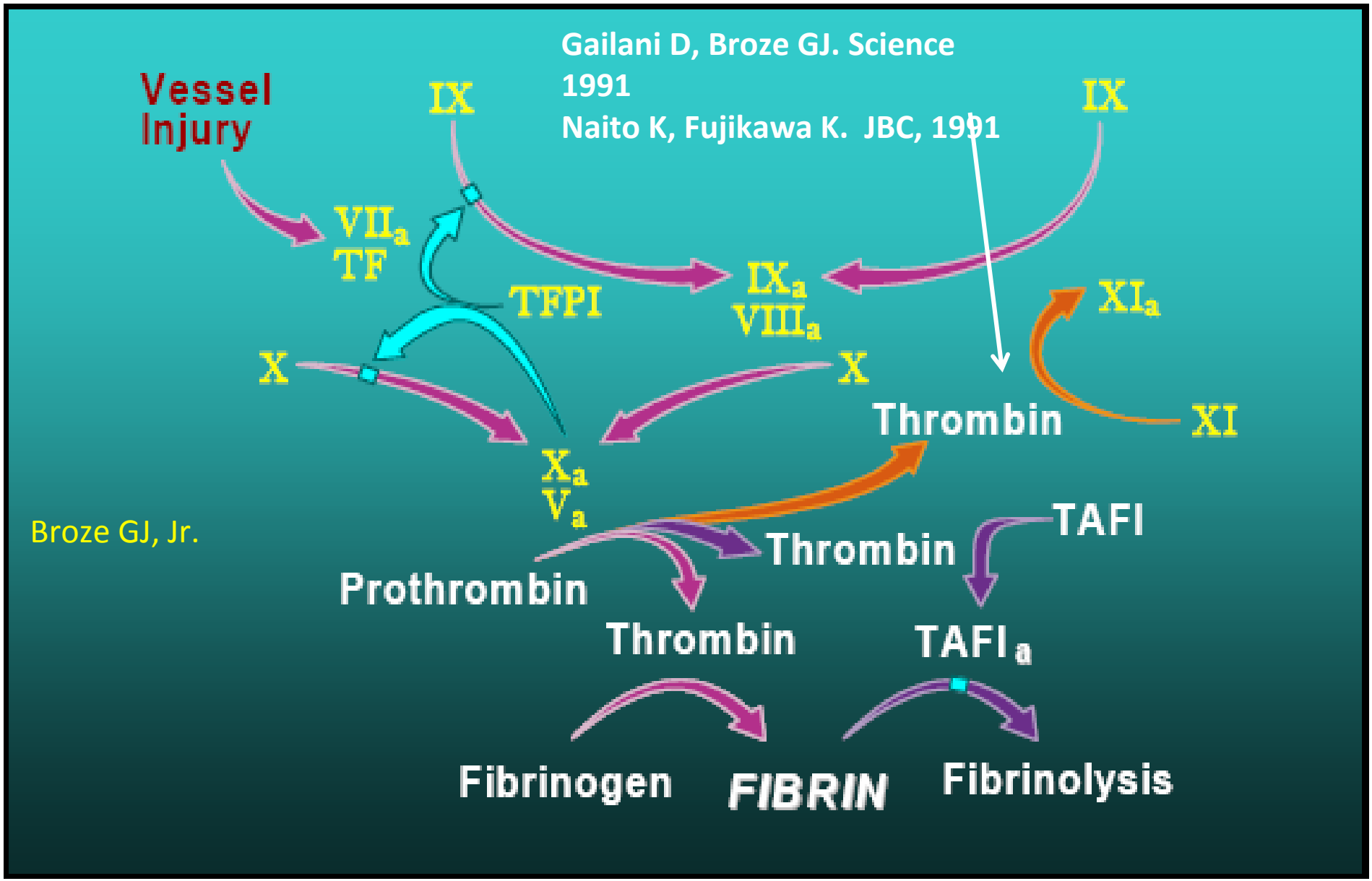
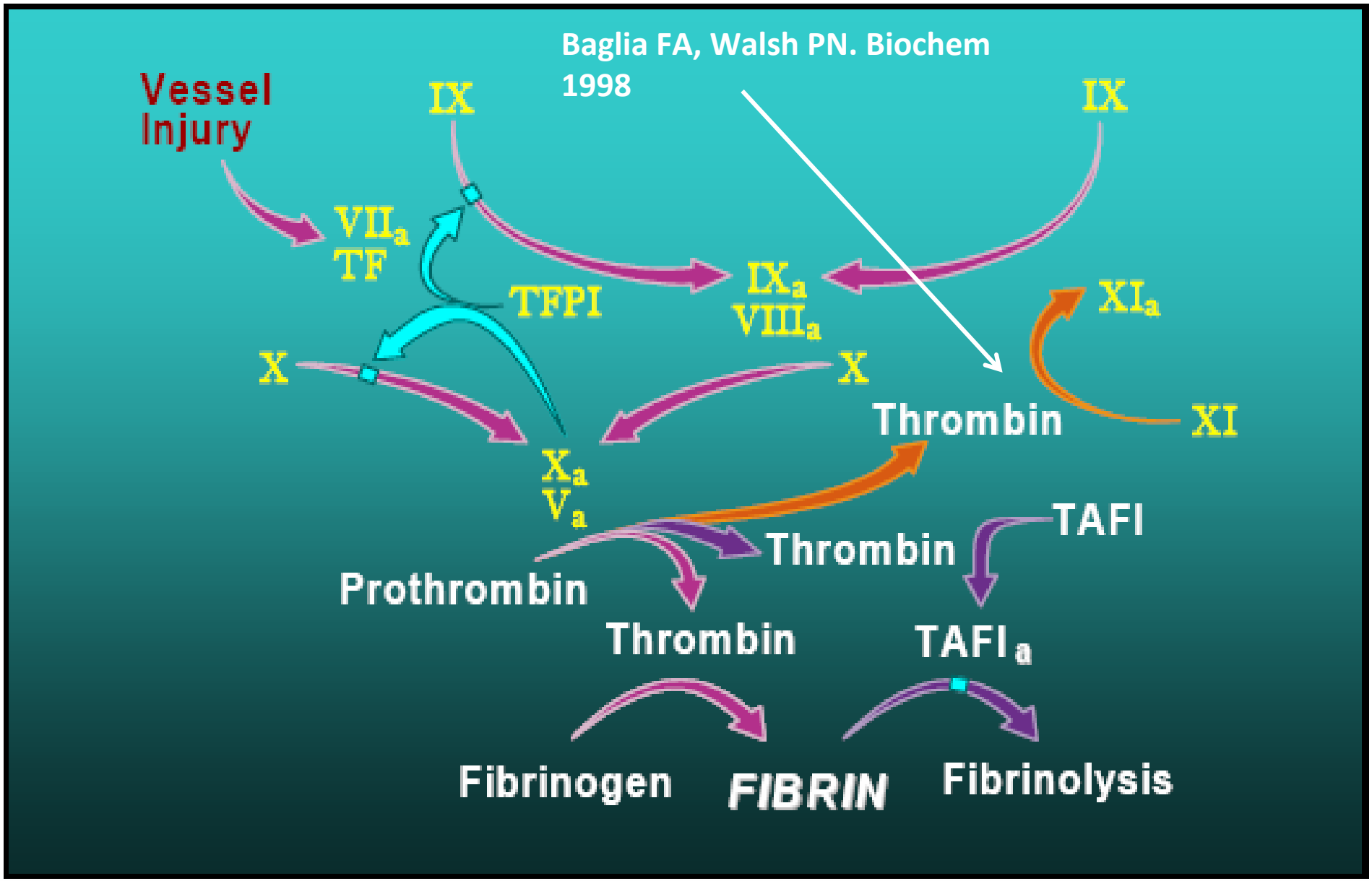


Figure 2 Tissue Factor Pathway of Coagulation

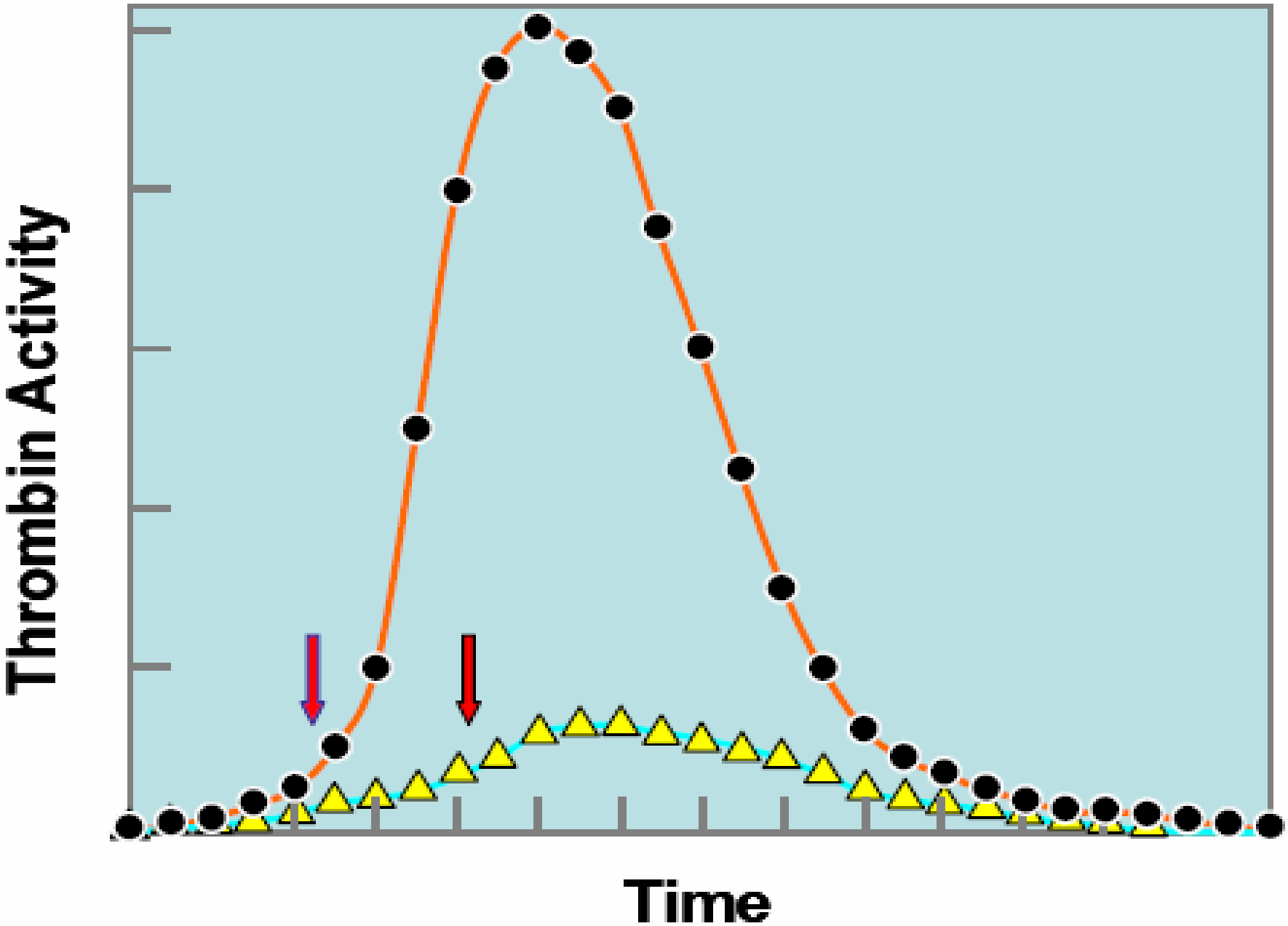


Insuficiencias del Modelo

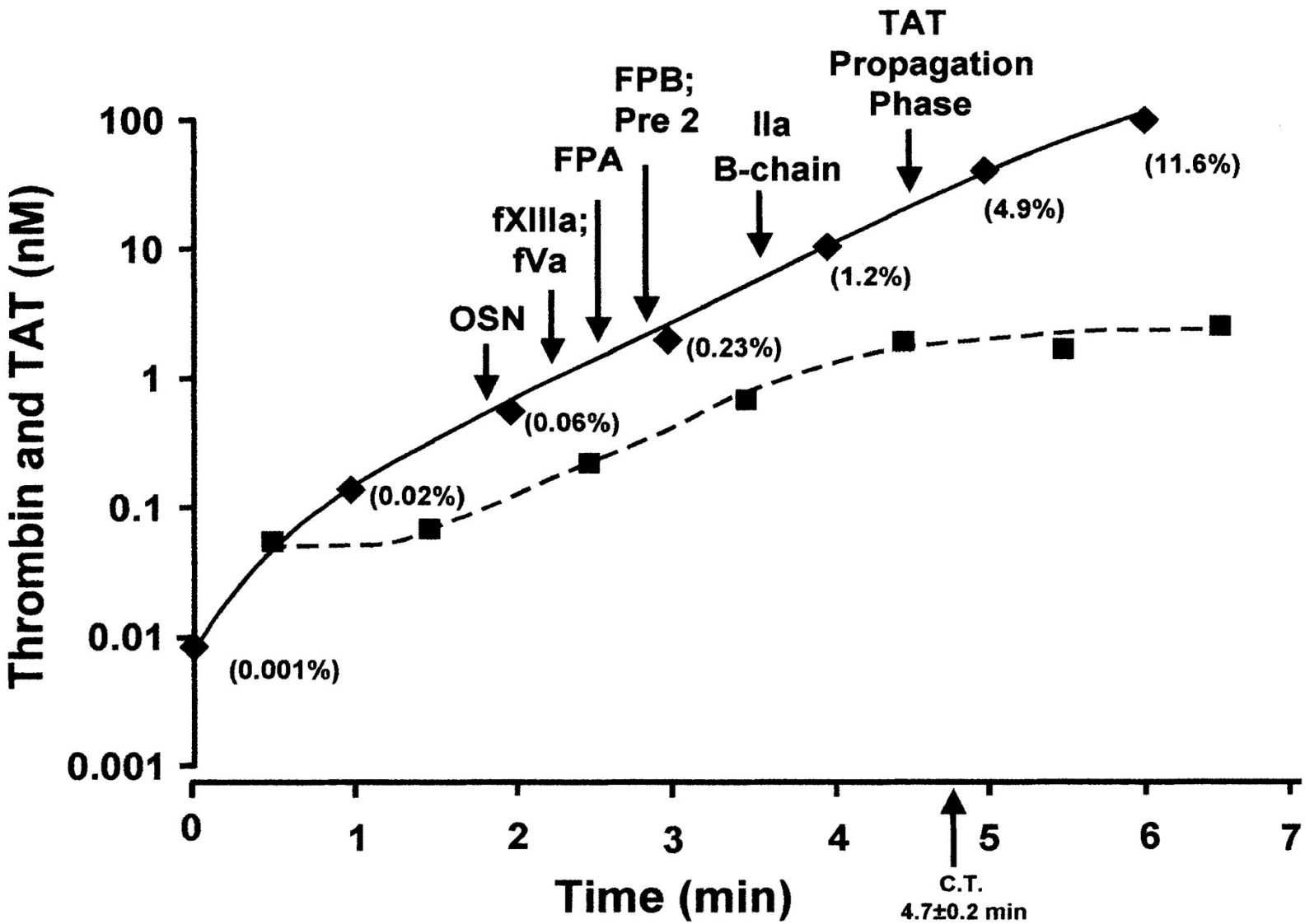
Experimentos in vitro usan fosfolípidos, que no replican resultados obtenidos con membranas celulares, ricas en factores adicionales.

Las plaquetas tienen una membrana rica en receptores y factores de la coagulación, superficie óptima para el ensamble de los complejos multimoleculares de la coagulación

Figure 3 Initiation & Amplification Phases of Coagulation

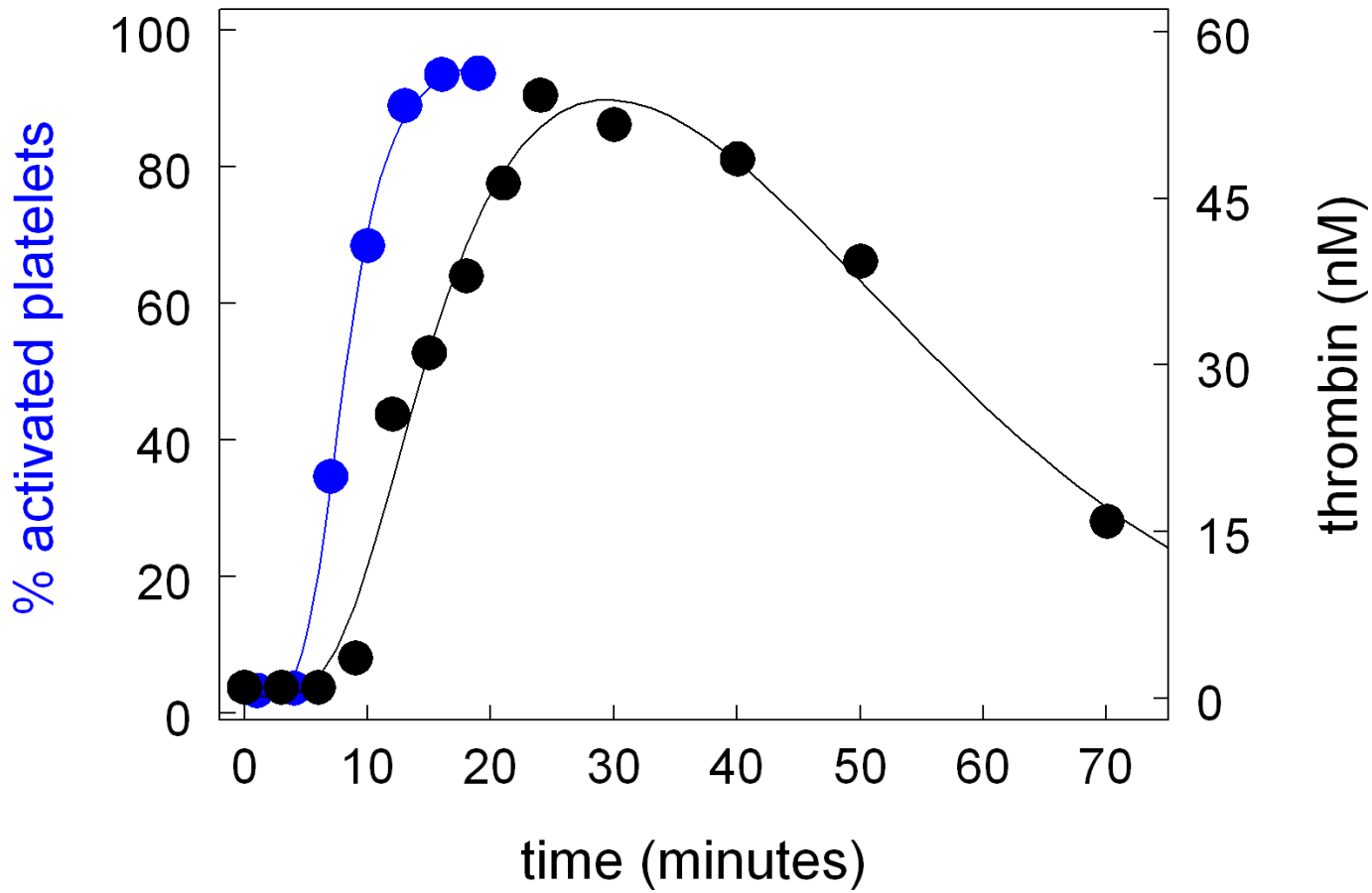


blood
JOURNAL OF THE AMERICAN SOCIETY OF HEMATOLOGY



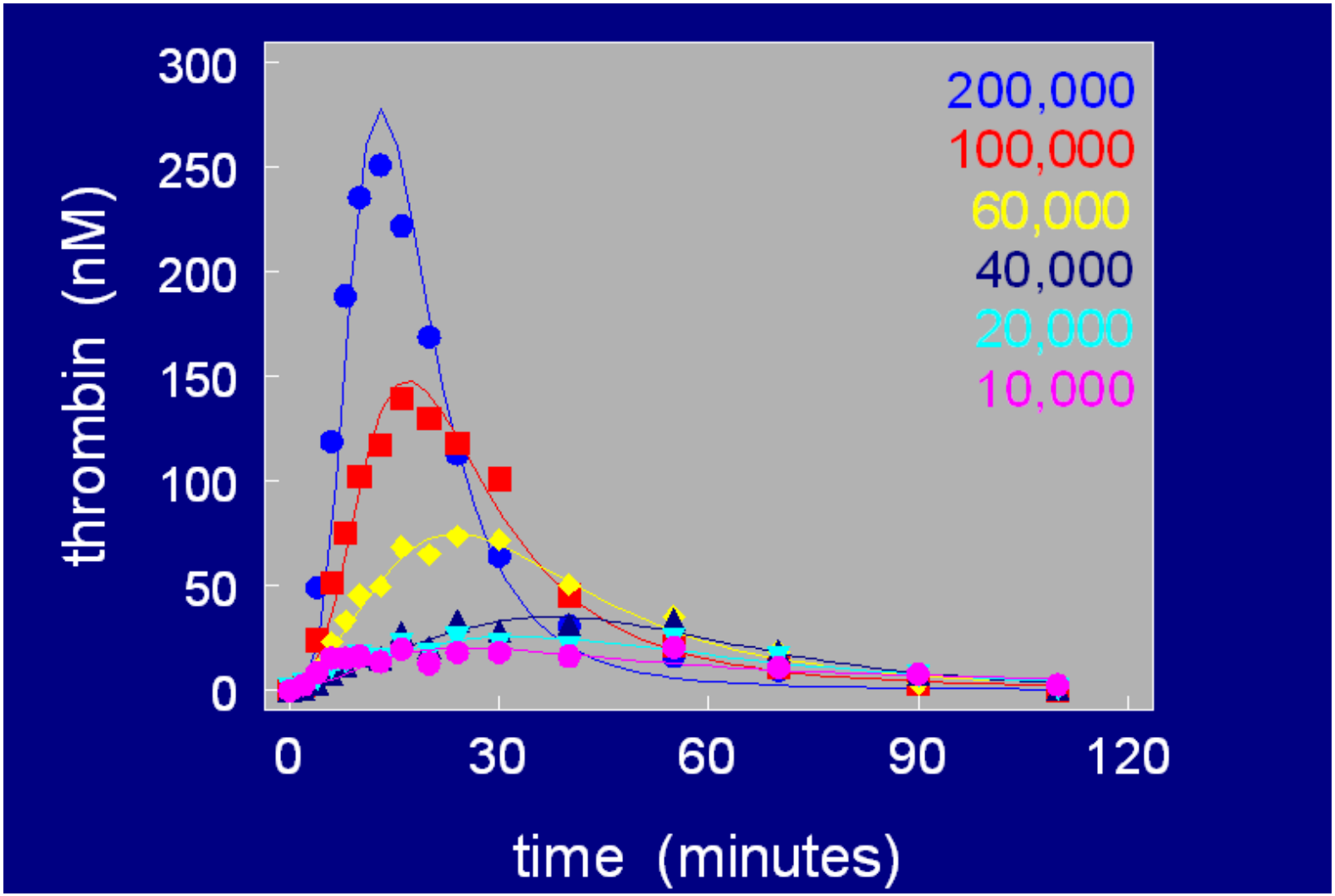
Brummel, K. E. et al. Blood 2002;100:148-152

Platelets are activated prior to the burst of thrombin generation



Roberts HR et al.

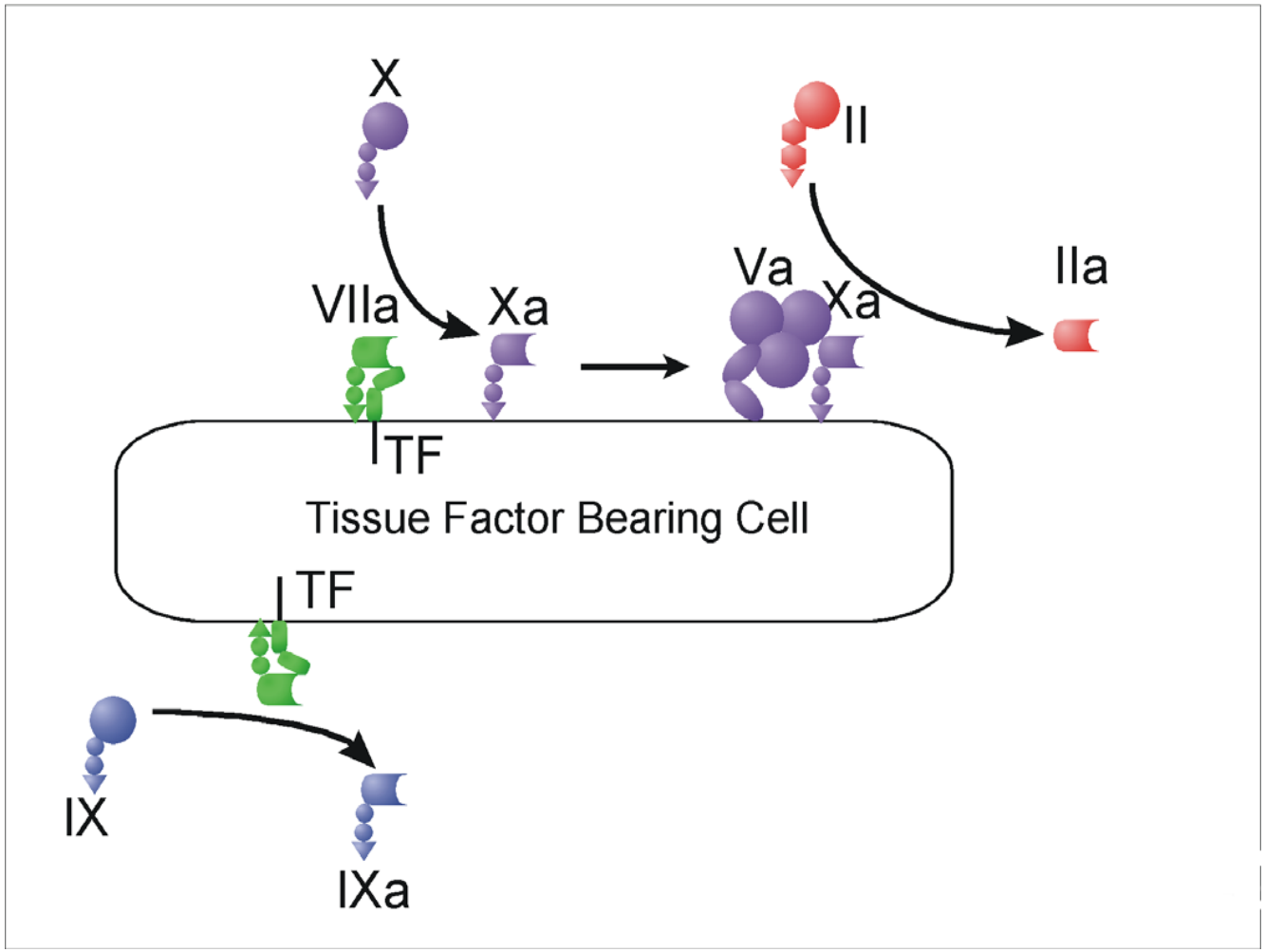
Platelets are required for thrombin generation in the model system



Monroe DM, et al. ATVB 2002

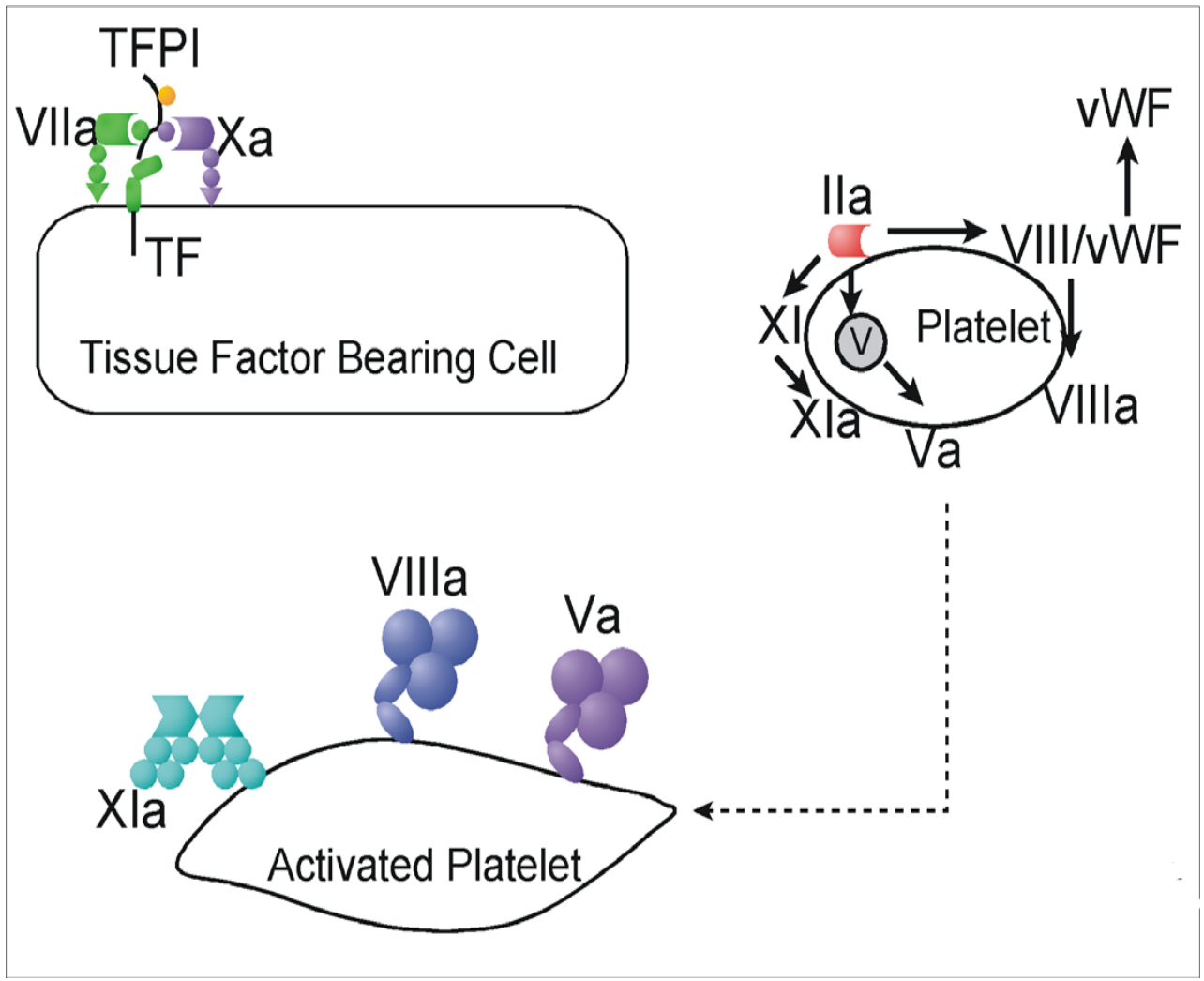
Cell-based Model of Thrombin Generation
Cell-based Model of Hemostasis

Initiation



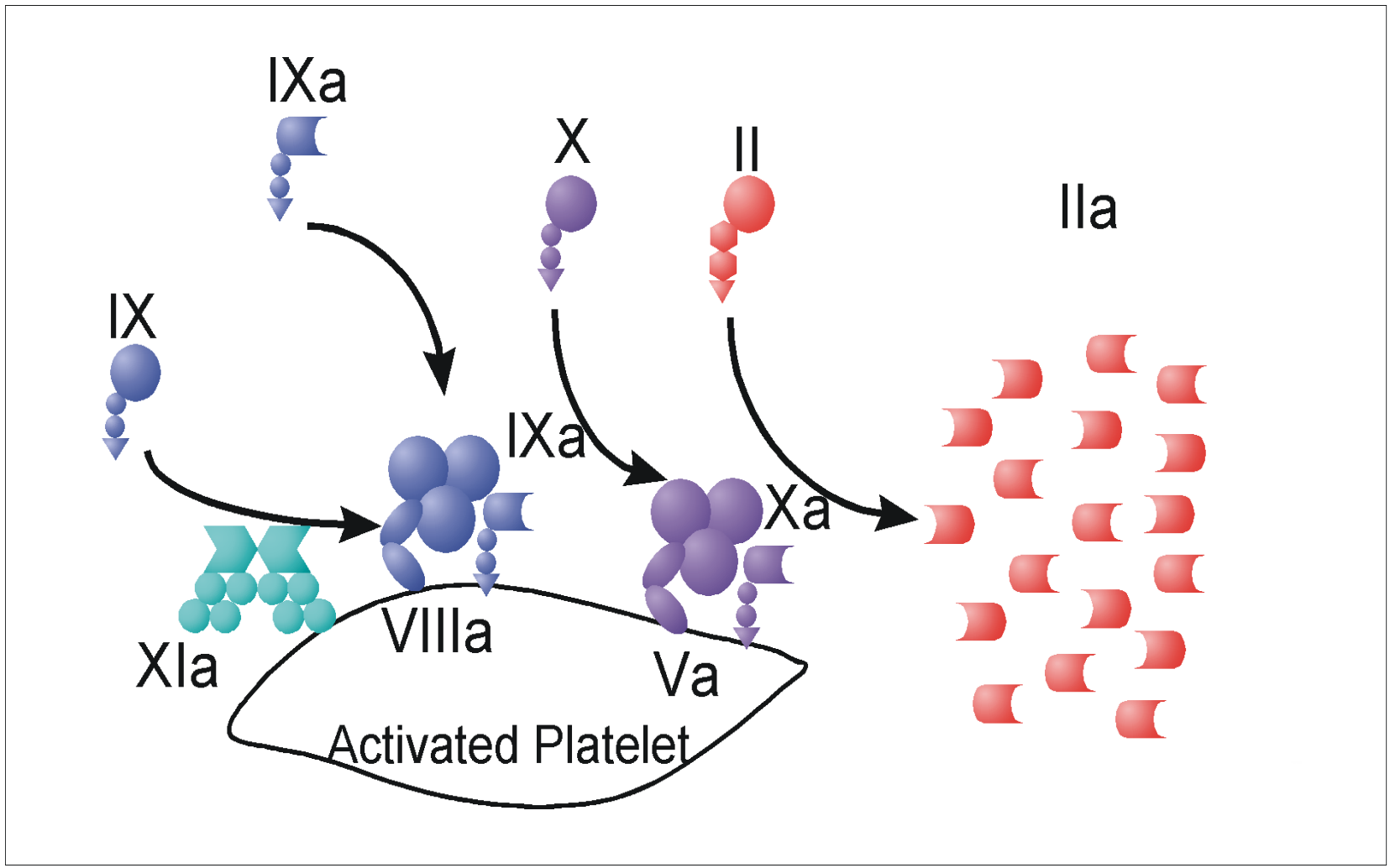
A cell-based model of thrombin generation. Roberts HR, et al. Sem Thromb Haemost 2006

Amplification



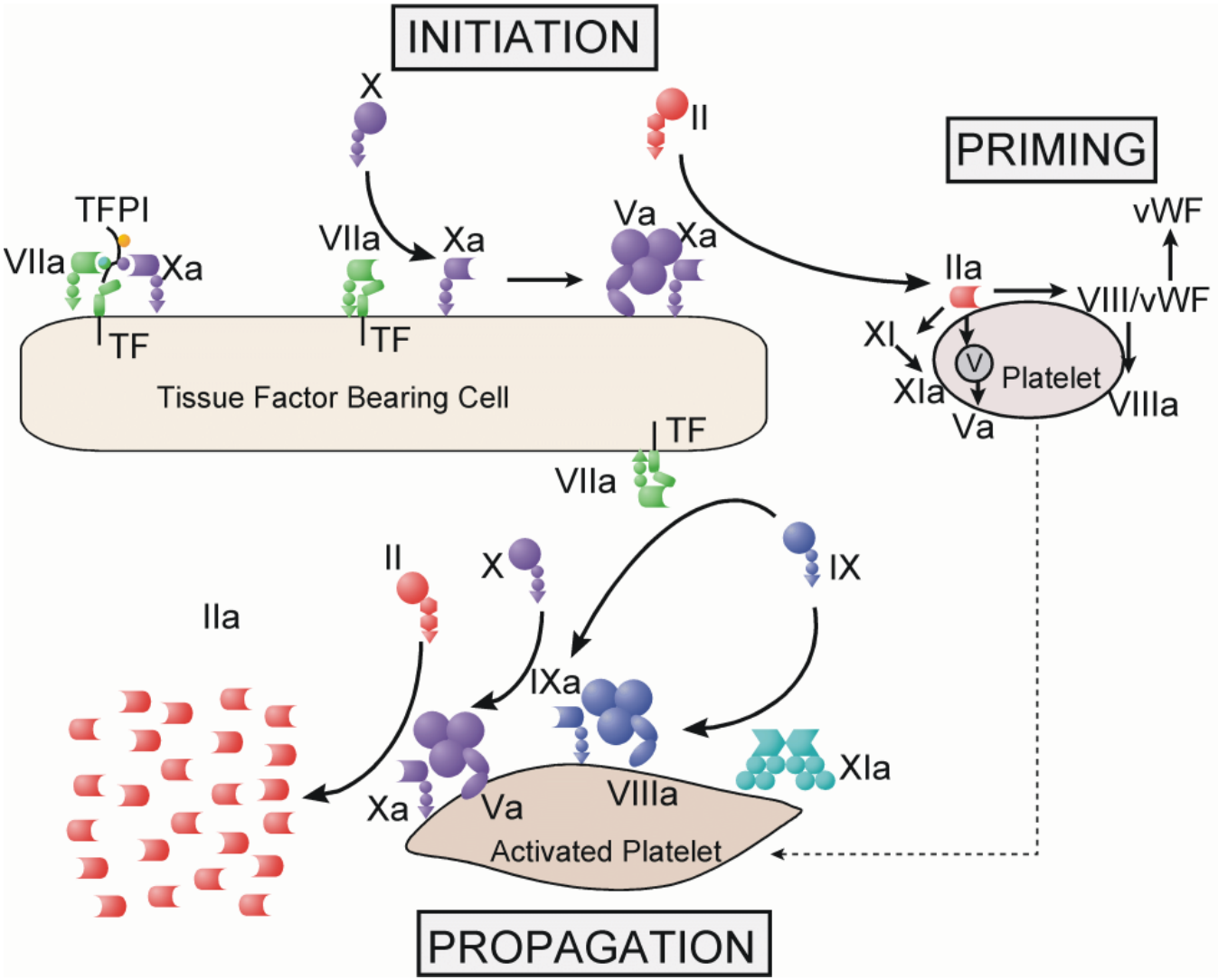
A cell-based model of thrombin generation. Roberts HR, et al. Sem Thromb Haemost 2006

Propagation



A cell-based model of thrombin generation. Roberts HR, et al. Sem Thromb Haemost 2006

Coagulation



A cell-based model of thrombin generation. Roberts HR, et al. Sem Thromb Haemost 2006

Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 2311–2315, March 1999
Medical Sciences

Blood-borne tissue factor: Another view of thrombosis

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*Division of Thrombosis Research, Department of Medicine, ‡Cardiovascular Institute, and §Department of Pathology, Mount Sinai School of Medicine, New York, NY 10029; and †F. Hoffmann La Roche Ltd., Pharma Division, Preclinical Research, CH-4070, Basel, Switzerland

Giesen PLA, et al. PNAS 1999

ABSTRACT Arterial thrombosis is considered to arise from the interaction of tissue factor (TF) in the vascular wall with platelets and coagulation factors in circulating blood. According to this paradigm, coagulation is initiated after a vessel is damaged and blood is exposed to vessel-wall TF. We have examined thrombus formation on pig arterial media (which contains no stainable TF) and on collagen-coated glass slides (which are devoid of TF) exposed to flowing native human blood. In both systems the thrombi that formed during a 5-min perfusion stained intensely for TF, much of which was not associated with cells. Antibodies against TF caused '70% reduction in the amount of thrombus formed on the pig arterial media and also reduced thrombi on the collagencoated glass slides. TF deposited on the slides was active, as there was abundant fibrin in the thrombi. Factor VIIai, a potent inhibitor of TF, essentially abolished fibrin production and markedly reduced the mass of the thrombi. Immunoelectron microscopy revealed TF-positive membrane vesicles that we frequently observed in large clusters near the surface of platelets. TF, measured by factor Xa formation, was extracted from whole blood and plasma of healthy subjects. By using immunostaining, TF-containing neutrophils and monocytes were identified in peripheral blood; our data raise the possibility that leukocytes are the main source of blood TF. We suggest that blood-borne TF is inherently thrombogenic and may be involved in thrombus propagation at the site of vascular injury.

Blood-borne TF: ¿de dónde viene?

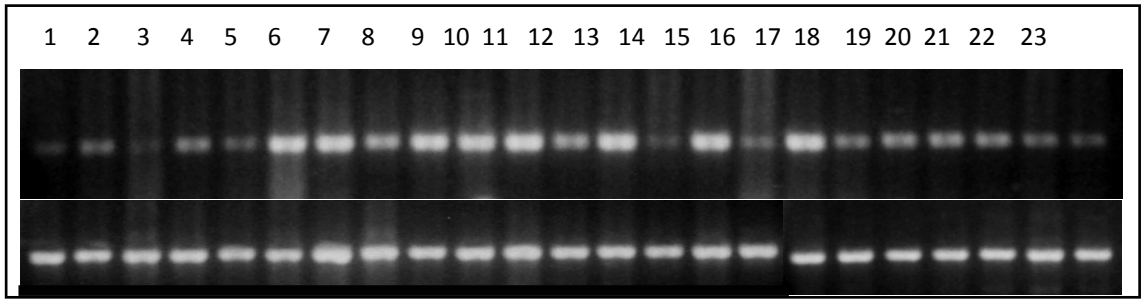
- Monocitos : √
- Granulocitos : ??
- Endotelio: ???
- Micropartículas : √
- Plaquetas : √-?

Table 3. Plasma markers of oxidative stress, endothelial activation/dysfunction and haemostatic activation in patients with chronic renal failure and healthy controls. (Kidney Int 2001; 60:1844-50)

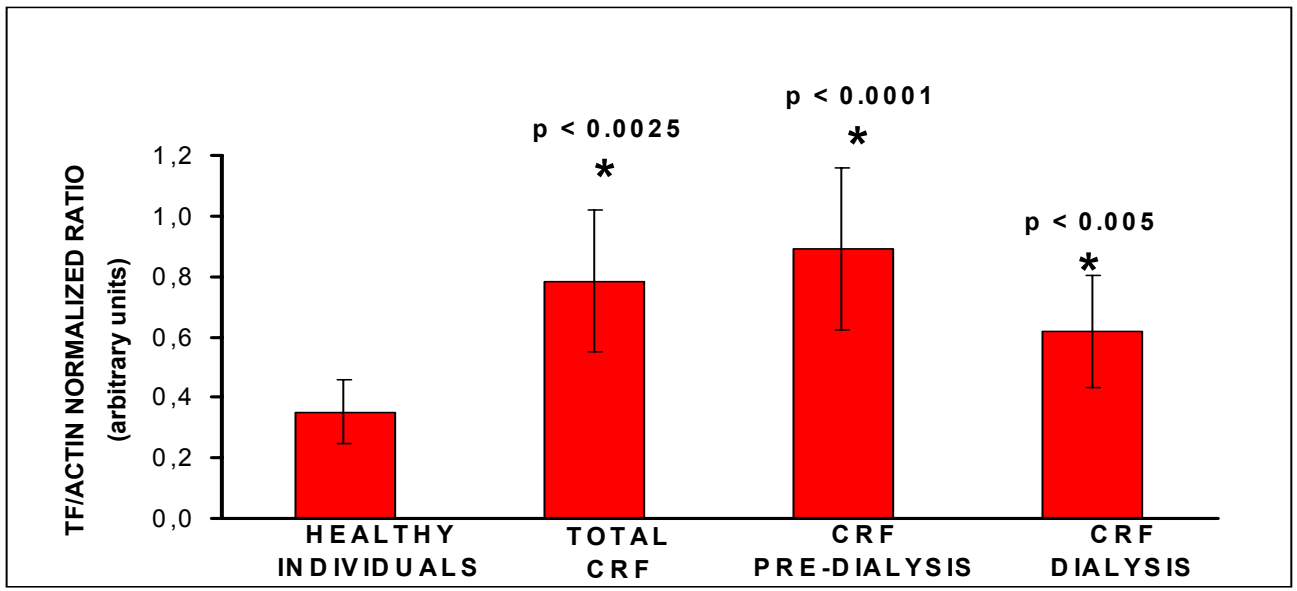
Plasma concentration of:	Patients (n = 64)	Controls (n = 16 – 40)	p=
TBARS (μmol/L)	1.98 ± 0.48	1.55 ± 0.39	0.009
AOPP (mmol, eq. chloramine T)	281 (45-915)	121 (14-414)	0.0001
von Willebrand factor (%)	182 ± 78	116 ± 46	0.0001
Soluble thrombomodulin (ng/mL)	15.7 ± 2.1	5.7 ± 0.45	0.0001
Soluble ICAM-1 (ng/mL)	301 (174-508)	233 (179-275)	0.0001
TAT (μg/L)	3.3 (0.94-14.2)	2.1 (0.84-4.7)	0.03
PF_{1+2} (nmol/L)	3.0 ± 1.1	1.8 ± 0.8	0.0001
PAP (μg/L)	874 (146-2302)	475 (321-805)	0.0001
FnDP (ngFE/mL)	675 (118-3622)	173 (9-543)	0.0001
FgDP (ngFE/mL)	391 (20-5875)	89 (20-236)	0.0001

BASAL MONOCYTE TF EXPRESSION IS INCREASED IN CRF PATIENTS COMPARED WITH HEALTHY INDIVIDUALS

A



B



Platelet-associated tissue factor contributes to the collagen-triggered activation of blood coagulation. Zillmann A, Luther T, Müller I, Kotzsch M, Spannagl M, Kauke T, Oelschlägel U, Zahler S, Engelmann B.
Biochem Biophys Res Commun. 2001 Feb 23;281(2):603-9.

Platelet activation induces cell-surface immunoreactive tissue factor expression, which is modulated differently by antiplatelet drugs. Camera M, Frigerio M, Toschi V, Brambilla M, Rossi F, Cottell DC, Maderna P, Parolari A, Bonzi R, De Vincenti O, Tremoli E.
Arterioscler Thromb Vasc Biol. 2003 Sep 1;23(9):1690-6.

Escaping the Nuclear Confines: Signal-Dependent Pre-mRNA Splicing in Anucleate Platelets .

Cell , Volume 122 , Issue 3 , Pages 379 - 391

M . Denis , N . Tolley , M . Bunting , H . Schwertz , H . Jiang , S . Lindemann , C . Yost , F . Rubner , K . Albertine , K . Swoboda

Este artículo dio sustento científico a otros previos que reportaban síntesis de IL1β, bcl-2 y PAI-1 por las plaquetas.

OUTLINE

1. Evidence of TF synthesis by human platelets

2. Is TF present in non-stimulated, circulating platelets?

3. TF-dependent pro-coagulant activity of human platelets.

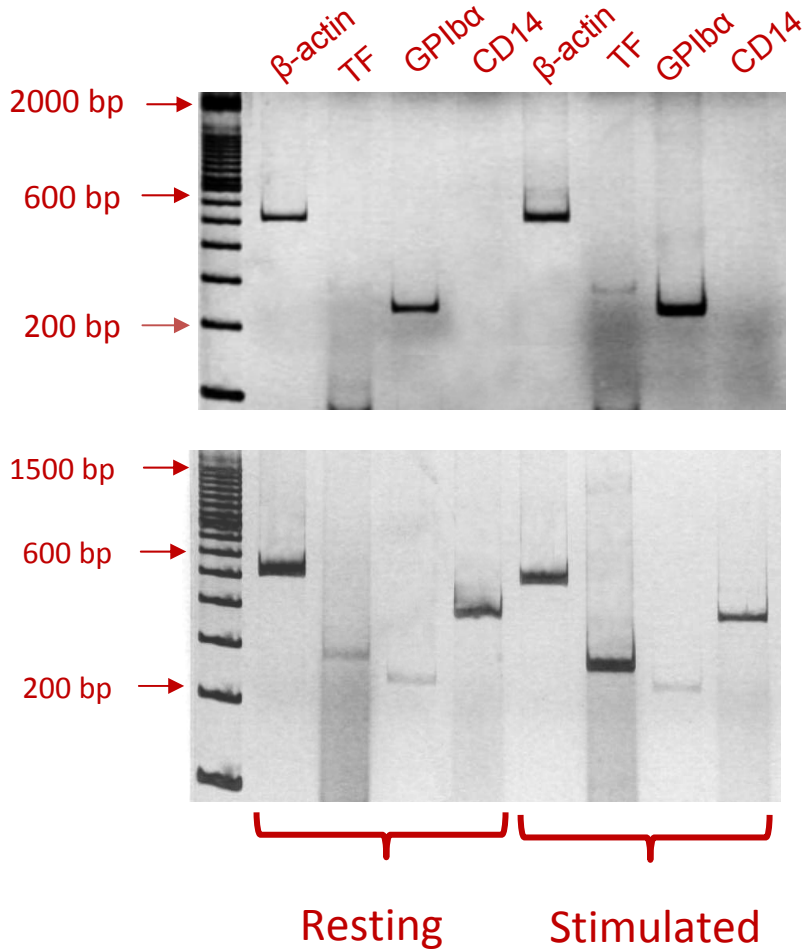
   Possible mechanisms of TF activation.

4. Is this TF hemostatically relevant?

Classical RT-PCR reveals that human platelets express TF-mRNA

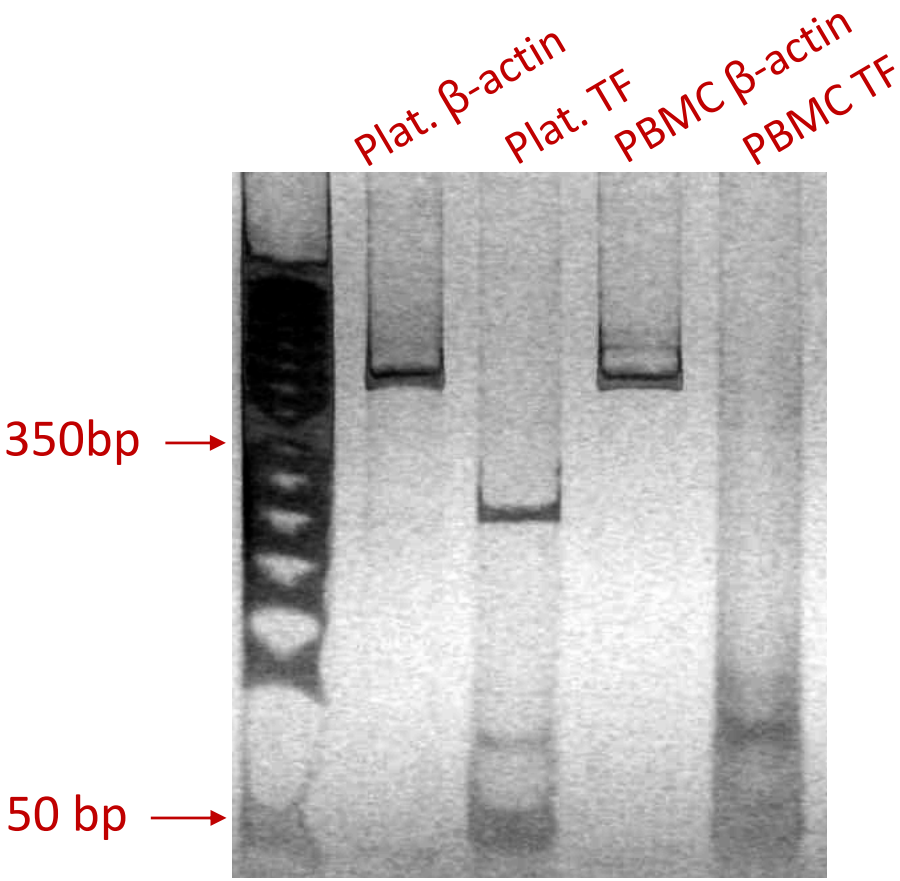
Stimulated (5mM TRAP, 15 min) human platelets free of monocytes, express TF-mRNA after activation, and frequently,……. even without stimulation

LPS-stimulated PBMC (2 h) increase dramatically TF-mRNA expression, but also contain GPIbα-mRNA, denoting contamination with platelets.

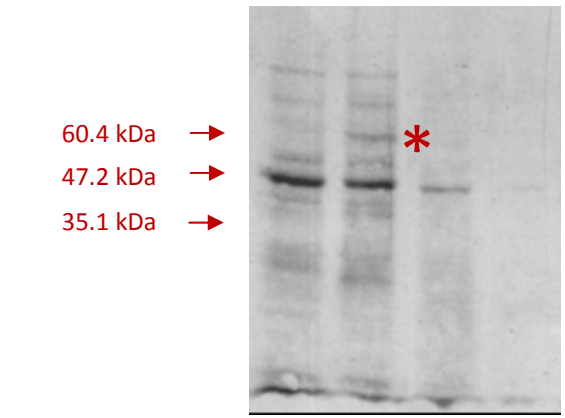


(J Exp Med 2006; 203:2433. Blood 2007; 109:5242).

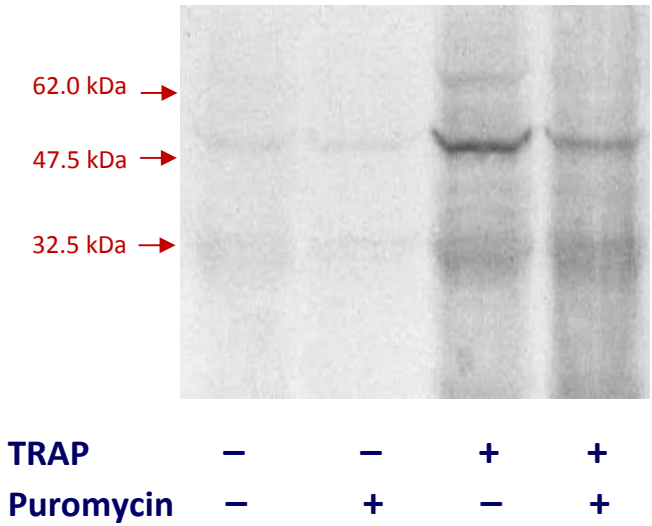
RT-PCR: TRAP-activated platelets (15 min), but not PBMC of one individual express TF-mRNA



Metabolic radio-labeling with ^{35}S-methionine demonstrates TF neo-synthesis by platelets.



IP: polyclonal α-TF



IP: MoAb α-TF
(Am. Diag 4509)

TF synthesis (≈47 kDa) by non stimulated platelets is enhanced with activation (band of ≈60kDa*), and inhibited by puromycin.

(Blood 2007; 109:5242)

1. Conclusions

Isolated human platelets express TF-mRNA and neo-synthesize the protein. These phenomena were observed in non-stimulated conditions, but mainly after activation.

OUTLINE

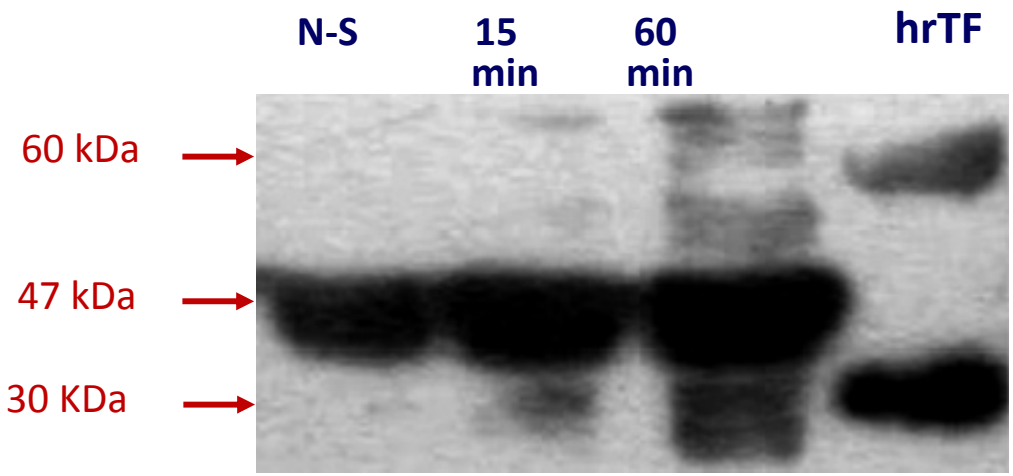
1. Evidence of TF synthesis by human platelets.

2. Is TF present in non-stimulated, circulating platelets?

3. TF-dependent pro-coagulant activity of human platelets. Possible mechanisms of TF activation.

4. Is this TF hemostatically relevant?

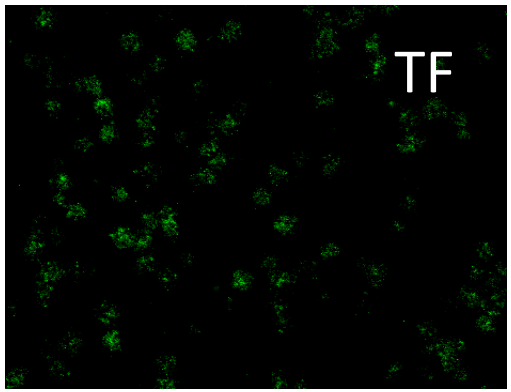
Western Blot of membrane fractions of non-stimulated (N-S) and TRAP-activated platelets.

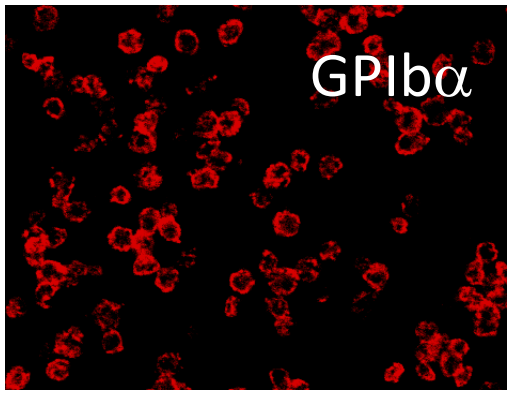


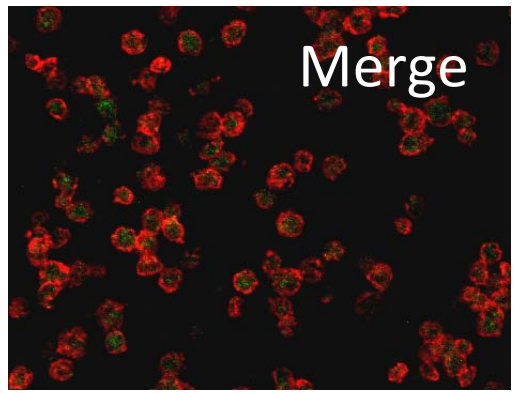
Consistently, TF protein was present in non-stimulated platelets.

(Blood 2007; 109:5242)

Non-stimulated, non-permeabilized human platelets express TF protein, which appears centralized in relation to GPIbα

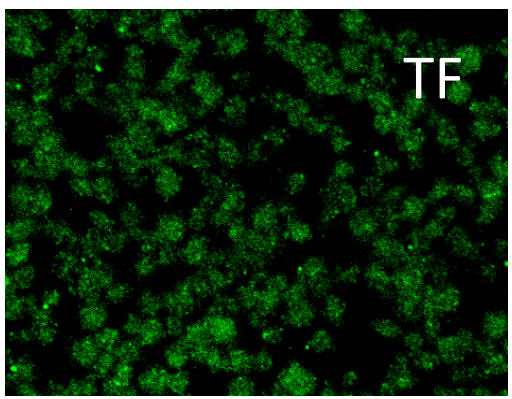


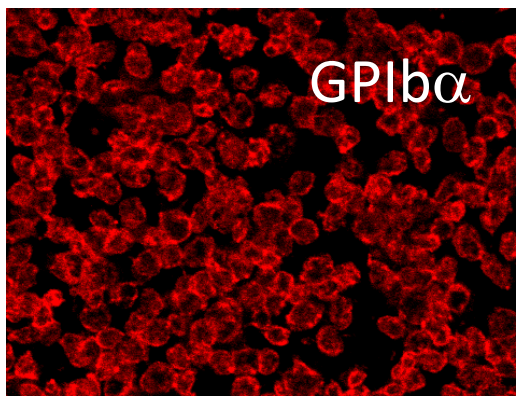


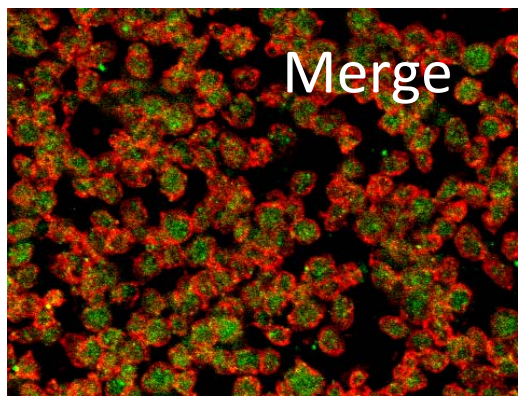


Pearson Coeff. : 0.1855

TRAP-stimulation strikingly enhances the immuno-reactivity of TF and its co-localization with GPIbα





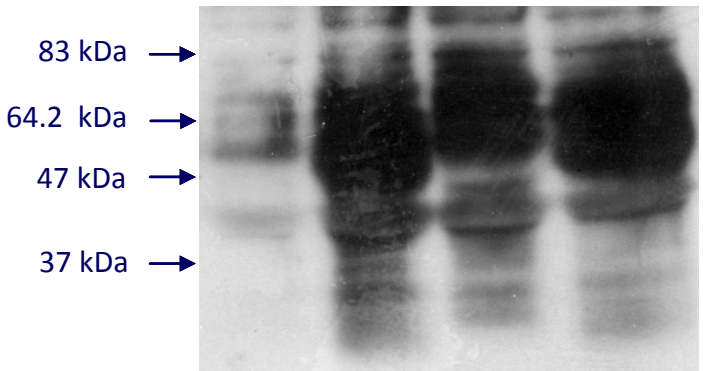


Pearson Coeff. : 0.3188

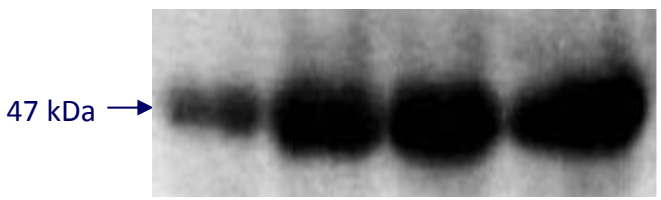
This apparent externalization of TF is also observed after membrane biotinilation and TF immunoprecipitation.

IP: polyclonal-αTF

WB: Streptavidin-HRP



WB: MoAb α-TF 4509



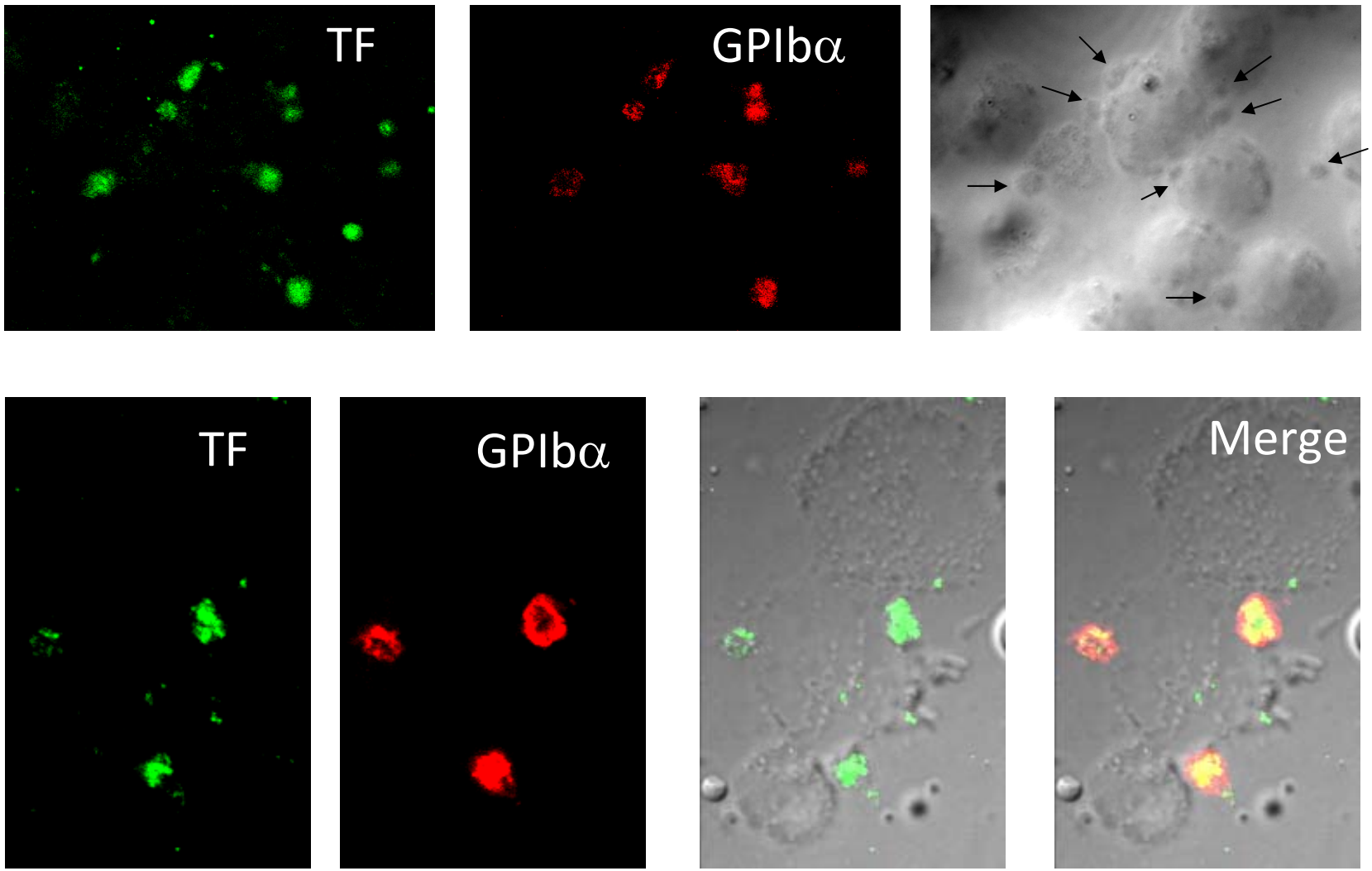
N-S 30 min 60 min 90 min

TF is transferred to plasma membrane after TRAP stimulation

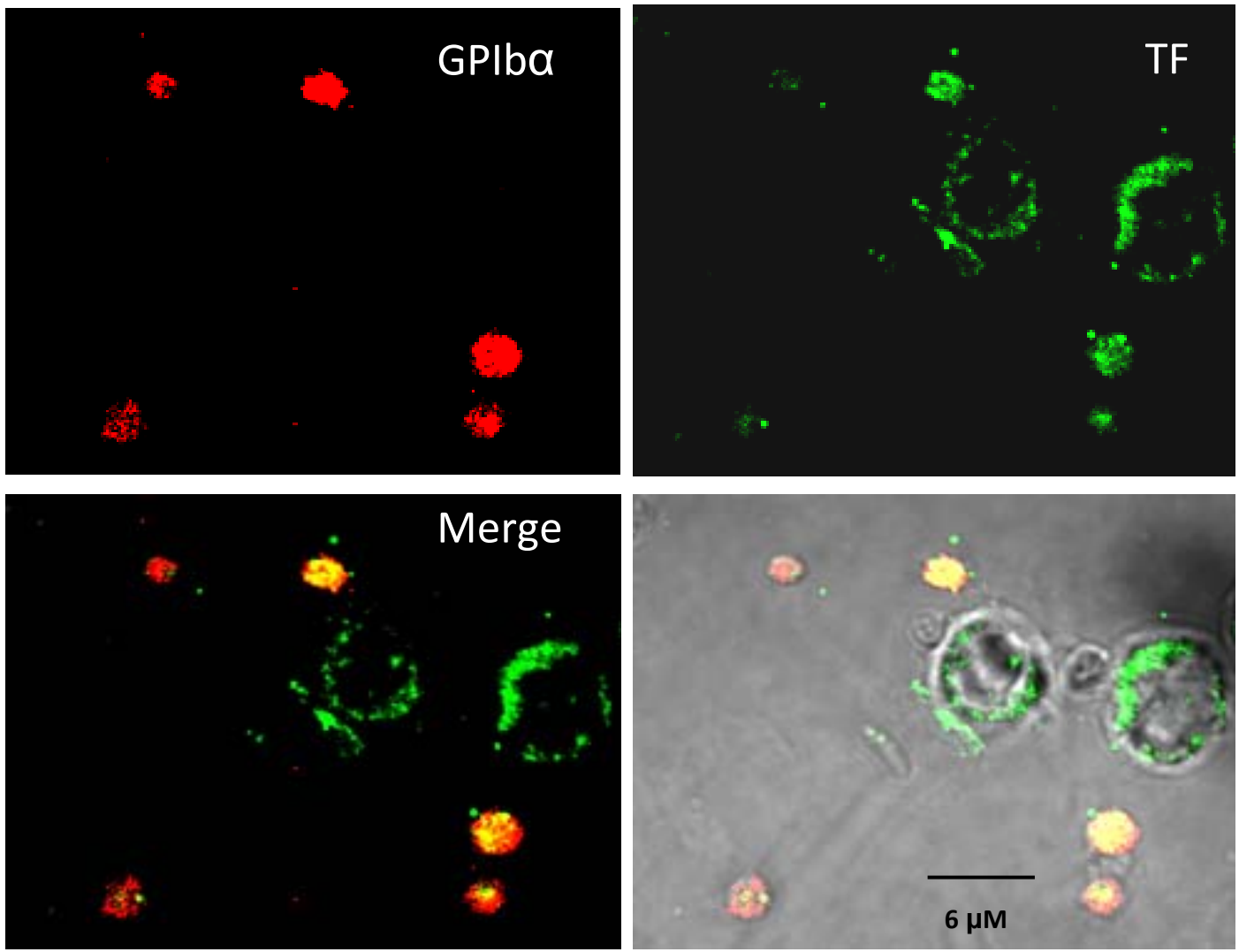
Again, TF is present in non-stimulated platelets

(Blood 2007; 109:5242)
(J Thromb Haemost 2007;5(Suppl 2): O-W-074)

In contrast, resting, non-permeabilized PBMC do not exhibit membrane TF. Platelets, always present in these preparations, contain TF co-localized with GPIbα

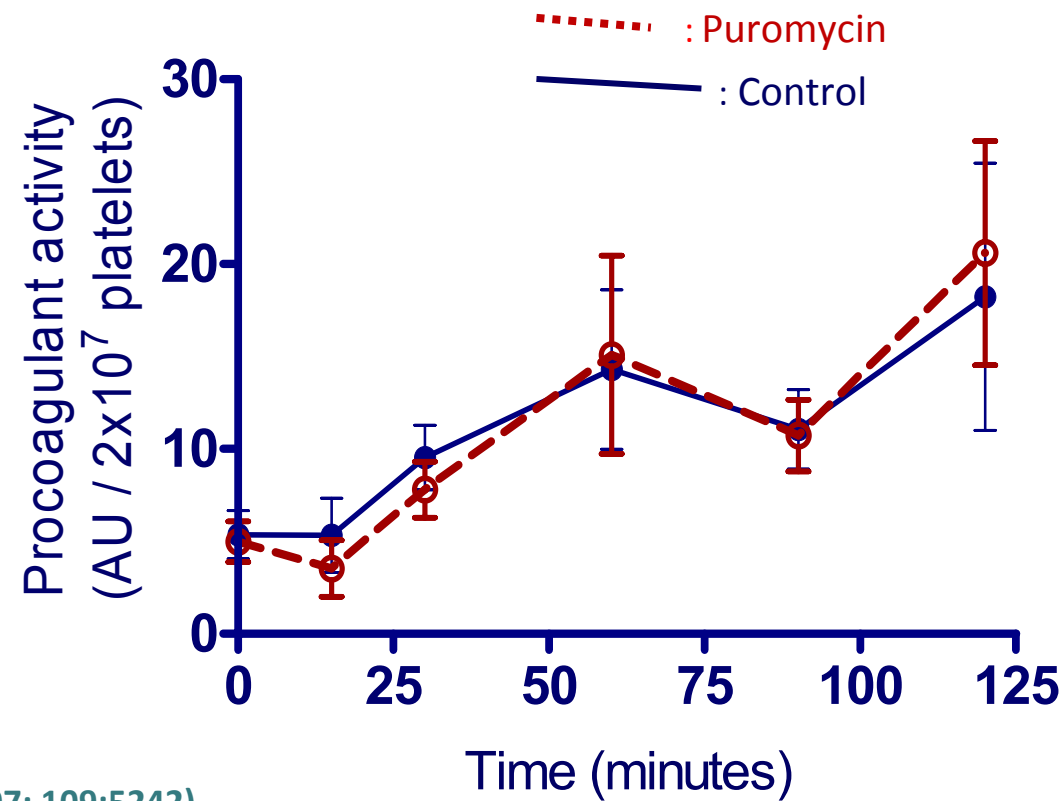


However, TF protein appears in LPS-stimulated PBMC (2 h). Again, high expression of TF-GPIbα is observed in "contaminating" platelets.



Blood 2007; 109:5242

TF-related pro-coagulant activity (PCA) is not inhibited by puromycin during a 2-h period, denoting that PCA depends on already stored TF in platelets.



(Blood 2007; 109:5242)

2. Conclusions

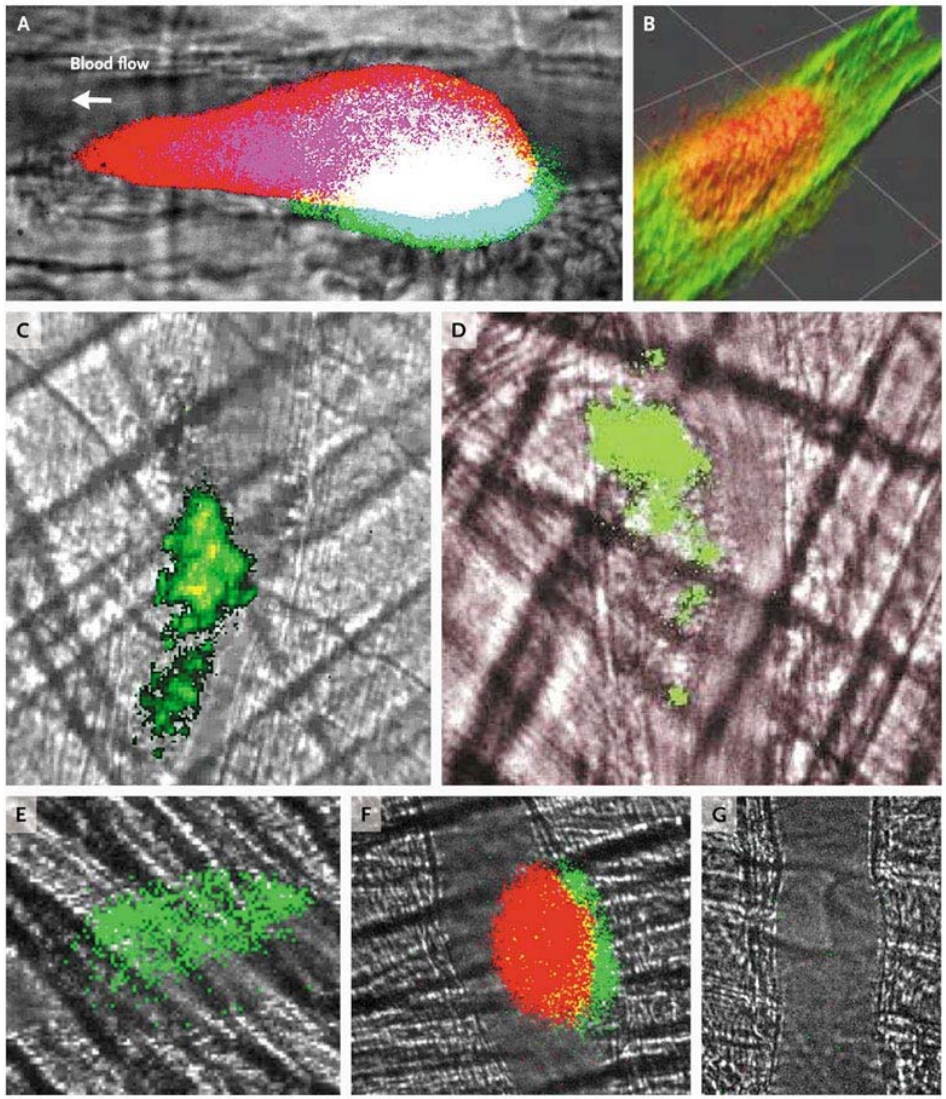
a. TF is demonstrated in N-S platelets by western blotting, membrane biotinilation with immunoprecipitation and immunofluorescence-confocal microscopy.

b. In contrast, surface exposure of TF in PBMC is detected only after 2-hour stimulation with LPS.

c. TF activity is not inhibited by simultaneous incubation with puromycin, suggesting that TF is already stored in platelets.

d. These findings support the idea that human platelets contain stored TF, likely synthesized by circulating platelets or, alternatively, derived from megakaryocytes.

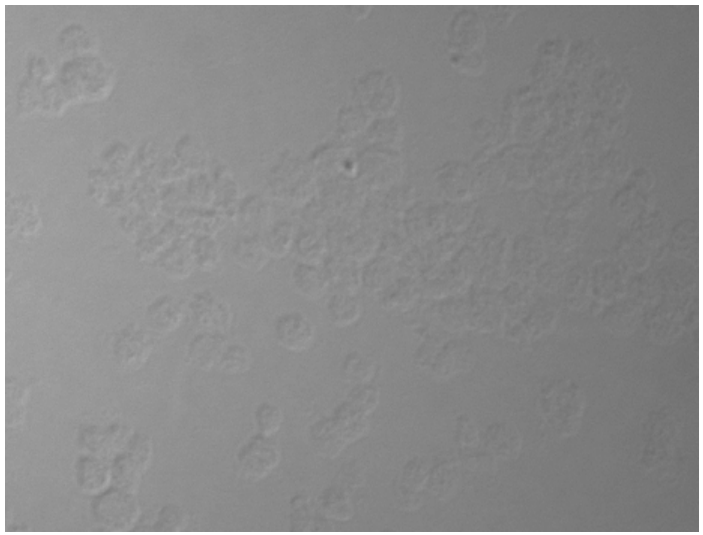
Thrombus Formation In Vivo

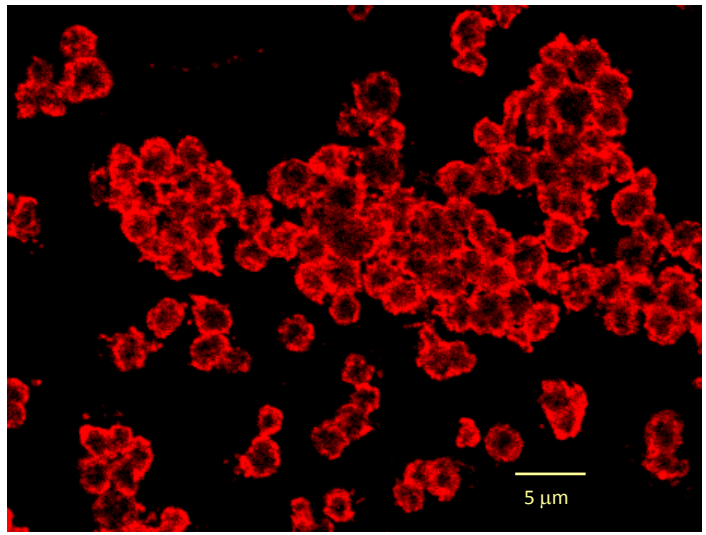


Furie B and Furie B. N Engl J Med 2008;359:938-949

The NEW ENGLAND JOURNAL of MEDICINE

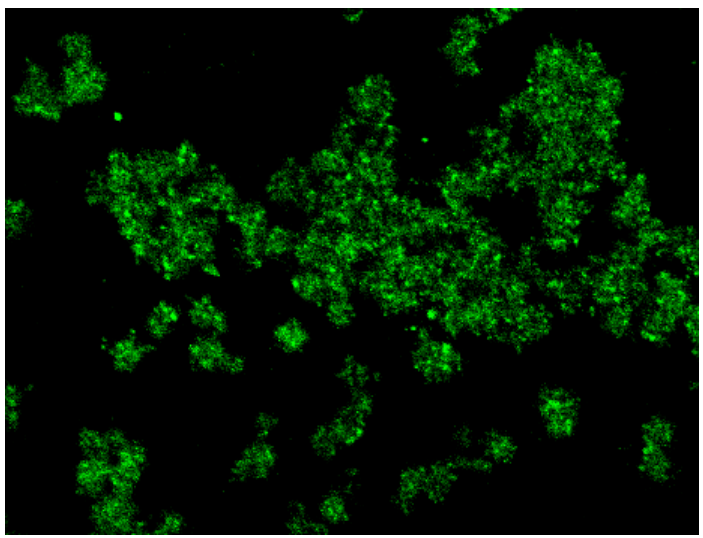
Is this co-localization of TF and GPIbα in activated platelets functionally relevant?

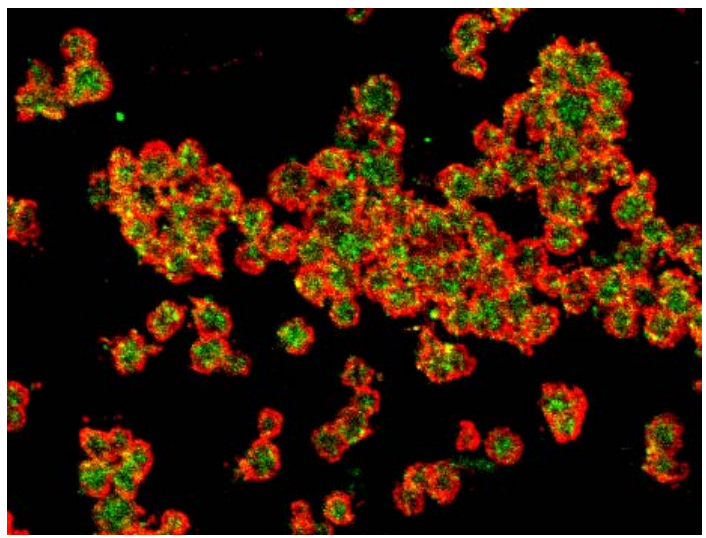




GPIbα

TF

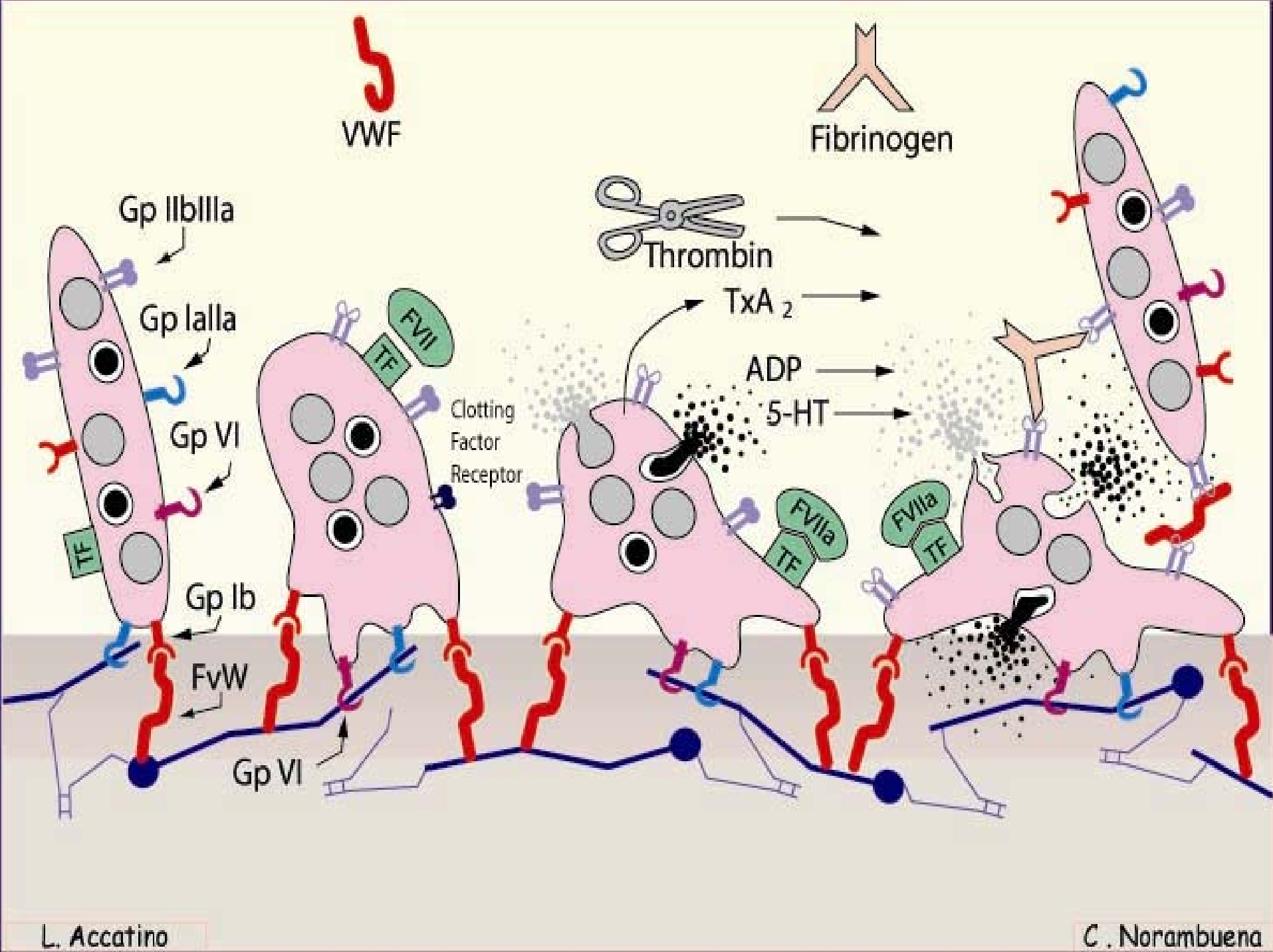


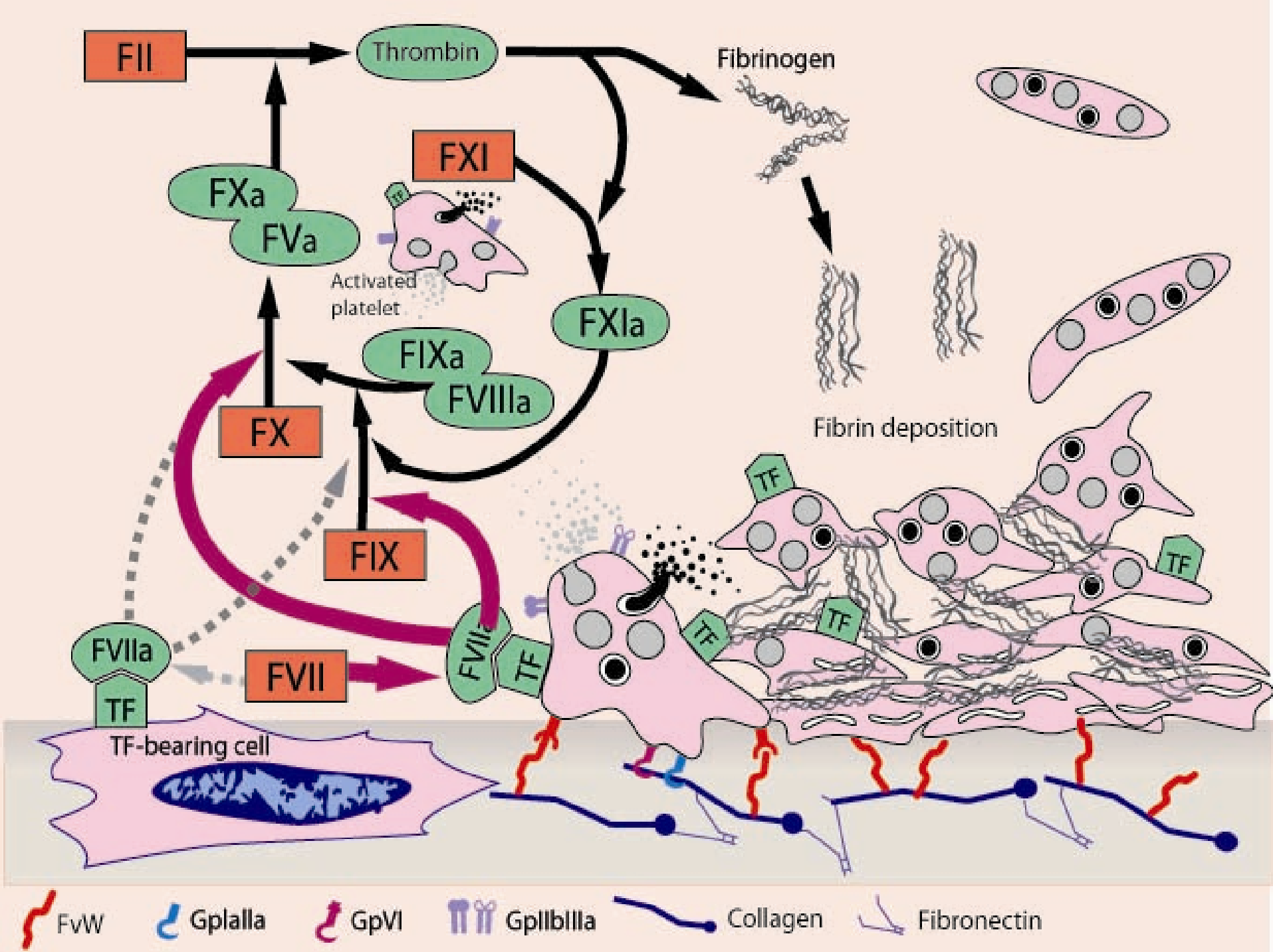


Merge

Pearson Coeff. : 0,57

PLATELET-BASED MODEL OF HEMOSTASIS: A PROPOSAL.


VWF
Fibrinogen
Gp IIbIIIa
Thrombin
Gp Ialla
TxA 2
FVII
TF
ADP
5-HT
Gp VI
Clotting
Factor
Receptor
TF
FVIIa
FVIIa
TF
TF
Gp Ib
FvW
Gp VI
L. Accatino
C. Norambuena


FII
Thrombin
Fibrinogen
FXI
FXa
FVa
Activated platelet
FXIa
FIXa
FVIIIa
FX
FIX
Fibrin deposition
TF
FVIIa
FVII
TF-bearing cell
FvW
GpIaIIa
GpVI
GpIIbIIIa
Collagen
Fibronectin

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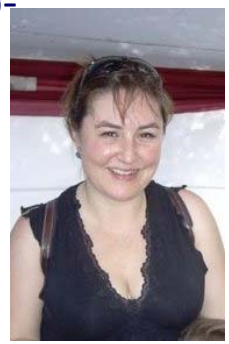
Olga Panes, conceived the study as main author, performed the radiolabeling assays, WB´s, IP´s, biotinilation and pro-coagulant assays.



Valeria Matus, PhD: developed all the molecular biology studies.

Claudia Sáez, PhD: performed all the imaging studies.

Paula Ibarra, BQ thesist, did the lipid raft studies.

Jaime Pereira , MD: contributed in the design of the study, discussion and critical interpretation of data and provided original ideas along the study.

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