מערכת הקרישה והמוסטזיס בקרדיולוגיה

ד"ר אלי לב
המערך הקרדיולוגי
מרכז רפואי רבין

3 Major systems involved

- **Vessel wall**
  - **Endothelium**
- **Platelets**
- **Coagulation cascade**

Anti-platelet Properties of the Endothelium

- Covers highly thrombogenic basement membrane (type IV collagen, TF)

  Uninjured endothelium does not bind platelets

- NO from uninjured endothelium inhibits platelet aggregation and adhesion, PGI2 (prostacyclin) inhibits platelet aggregation

- ADPase counters the platelet aggregating effects of ADP

Anti-coagulant Properties of the Endothelium

-Endothelial cells produce **t- PA** which activates fibrinolysis via plasminogen to plasmin

- Heparin-like molecules (proteoglycans), which activate anti-thrombin III (inactivates thrombin, other clotting factors)

- Thrombomodulin – transmembrane proteoglycan binds thrombin – activates protein C (by cleavage) - process occurs on thrombomod. (protein C , inactivates Va & VIIIa)

- TFPI – tissue factor pathway inhibitor – released from endothelial cells (and from platelets), inhibits TF-VIIa & Xa

3 Major systems involved

- **Vessel wall**
  - **Endothelium**
- **Platelets**
- **Coagulation cascade**

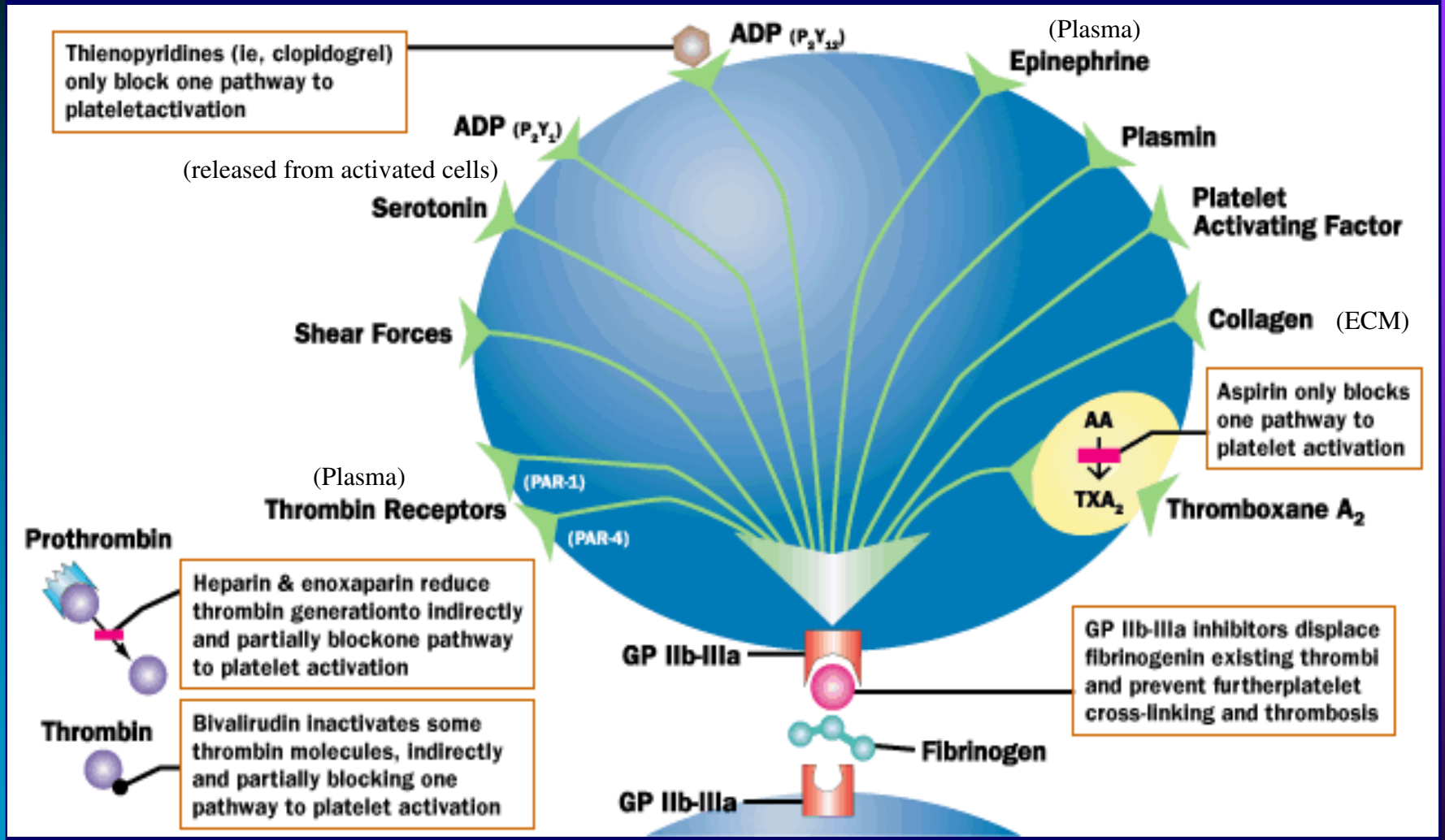
Platelets

- Adhesion
- Activation
- Aggregation

Platelet Adhesion

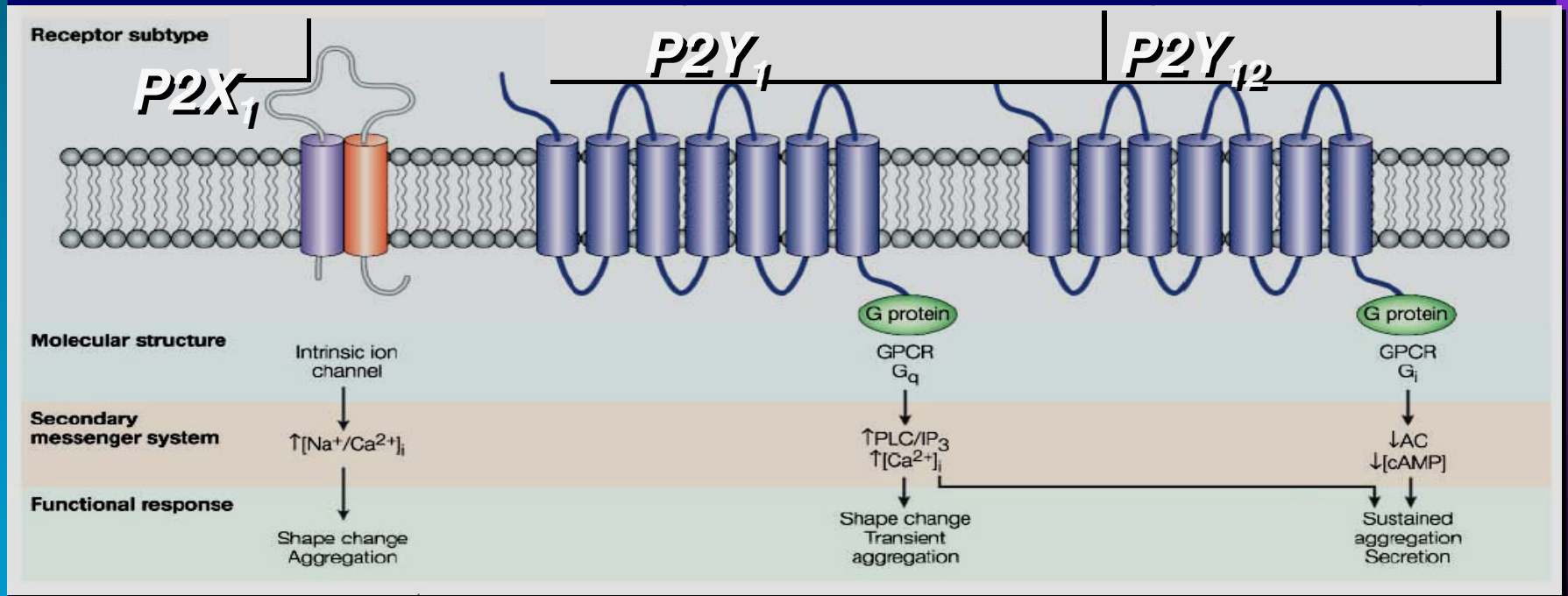
- Platelets are the first cells to thether and adhere to injured vascular wall (subendothelium)
- Adhesion is mediated by vWF – a mutimetric protein synthesized by both endothelial cells and megakaryocytes (stored in α granules) – present in plasma and ECM – serves as "an anchor"
- Platelet receptor – GPIb (part of the GP Ib/IX-V complex)
- Binding occurs only under high shear stress conditions !

Platelet Activation



Platelet Purinergic Receptors

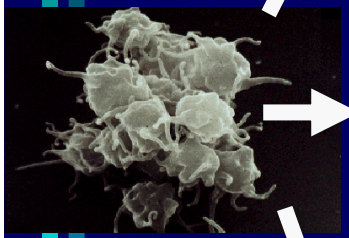
Active Metabolite of Clopidogrel



Platelet Activation

- Release from alpha and dense granules
- Dense granules: ADP, serotonin
- Alpha granules: vWF, finbrinogen, fibronectin, growth factors (PDGF), PF4, factor V

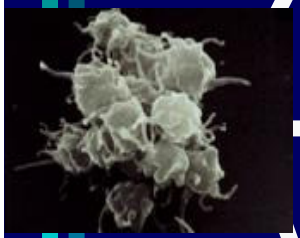
- Activated platelets also synthesize (denovo) TxA2 from arachidonic acid



UD16_MOUSE UDP glucuronosyltransferase 1-6 precursor
TRA1_MOUSE TNF receptor associated factor
TLR5_HUMAN Toll/interleukin-1 receptor-like protein 3
TAST_HUMAN Trophinin-associated protein
SEP6_MOUSE Septin 6
SACS_HUMAN Sacsin
Q9Z2V7 Lymphocyte specific formin related protein
Q9Y6V0 Piccolo protein [Fragments)
Q9HCV9 HSPC164/HSPC169
Q9H233 BCL-6 corepressor
Q9BZG3 Acid phospahatase variant
Q96QE3 ATPbinding protein
Q96PH3 Proliferation potential-related protein
Q925P2 CEA related cell adhesion molecule 2
Q91ZT8 Ankyrin repeat and SOCS box containing protein 9
Q91W89 Alpha-mannosidase 2C1
Q8TDN5 Retinoblastoma-associated factor 600
Q8TDL7 Spermatogenesis associated factor
Q8TCH0 Nebulin-related anchoring protein
Q8R099 Similar to compliment component 1
Q14393 Growth arrest specific protein, Gas 6
PSD2_HUMAN 26S proteasome subunit p97
MS1P_HUMAN Site-1-protease
MGD2_HUMAN Melanoma-associated antigen D2
MAP2_HUMAN Microtubule-associated protein 2
MAGB_HUMAN Melanoma-associated antigen 11
HPS3_HUMAN Hermansky-Pudlak syndrome 3 protein)
FCGA_HUMAN CD32
ECEL_MOUSE Endothelin-converting enzyme-like 1
COTR_MOUSE Serpin
CFAH_HUMAN Compliment H
ACRO_HUMAN Acrosin
SNX2_MOUSE Nexin
S23A_HUMAN Protein transport protein Sec 23A
Q9QXA1 Cysteine and histidine-rich protein
Q9EPX2 Papilin
Q9DBX8 Vacuolar protein sorting 11
MM02_HUMAN MMP2, metalloproteinase
IC1_MOUSE Plasma protease C1 inhibitor

ATS7_HUMAN ADAMS TS 7
CATW_HUMAN Cathepsin W
HS9A_HUMAN Heat shock protein HSP 90-alpha
TAC2_MOUSE Transforming acidic coiled-coil-containing protein 2
SG2_HUMAN Secretogranin II
Q9DC90 Proprotein convertase subtilisin/kexin type 4
Q9D7C0 Transcript expressed during hematopoiesis 1
Q925U0 Ooocyte secreted factor
CANS_MOUSE Calcium-dependent protease, small subunit
EMBP_HUMAN Proteoglycan 2, bone marrow
GILT_HUMAN Gamma-interferon-inducible protein IP-30)
IBA4_HUMAN ITBA4 protein
TPIS_MOUSE Triosephosphate isomerase
MHYB_MOUSE Myosin heavy chain
P97315 Cysteine rich protein
NP25_MOUSE Neuronal protein NP25
CD63_MOUSE CD63
WDNM_MOUSE protease inhibitor
TNF8_HUMAN Tumor necrosis factor ligand superfamily member 8.
MABC_HUMAN Mannose Binding Protein
KLK5_MOUSE Kallikrein
IL13_MOUSE Interleukin 13
ABP_HUMAN Diamine oxidase
OXDD_HUMAN D-aspartate oxidase
O00391 Quiescin
Q9JHQ5 Leucine zipper transcription factor-like
Q9DCA5 Ribosome biogenesis protein Brix
Q9BWF3 RNA binding protein motif
Q920Q2 Deoxyribonucleotidyl transferase
CAZ1_MOUSE F-actin capping protein
SPCB_MOUSE Spectrin
PKP4_HUMAN Plakophilin 4
MOES_MOUSE Moesin
CADH_HUMAN Cadherin-17
CTA4_MOUSE Cell recognition molecule Casp4

Coppinger JA. Blood 2004;103:2096-2104



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Q9NW13 Hypothetical
Q9NVI6 Hypothetical
Q9NRD6 Hypothetical
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Q9JK52 Hypothetical
Q9HAE6 Hypothetical
Q9H9F1 Hypothetical
Q9H8M2 Hypothetical
Q9H7T0 Hypothetical
Q9H0H6 Hypothetical
Q9H0C4 Hypothetical
Q9GZR2 Hypothetical
Q9ESE1 Hypothetical
Q9ERR6 Hypothetical
Q9DCD5 Hypothetical
Q9DBG7 Hypothetical
Q9DB87 Hypothetical
Q9D9U1 Hypothetical
Q9D5S9 Hypothetical
Q9D310 Hypothetical
Q9D2F5 Hypothetical
Q9CZI9 Hypothetical
Q9CYB7 Hypothetical
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Q9C099 Hypothetical
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Q99J78 Hypothetical

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Q8R3C5 Hypothetical
Q8R1E4 Hypothetical
Q15042 Hypothetical
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Q9HCL6 Hypothetical
Q9H911 Hypothetical
Q9H625 Hypothetical
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Q96IN9 Hypothetical
Q8R0R1 Hypothetical
Q8R0R1 Hypothetical

Q9CW15 Hypothetical
O60331 Hypothetical
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Q8TBY3 Hypothetical
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Q99620 Hypothetical
Q9DBC3 Hypothetical
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Q9P0R0 Hypothetical
Q9UKN4 Hypothetical
Q9Y2J3 Hypothetical
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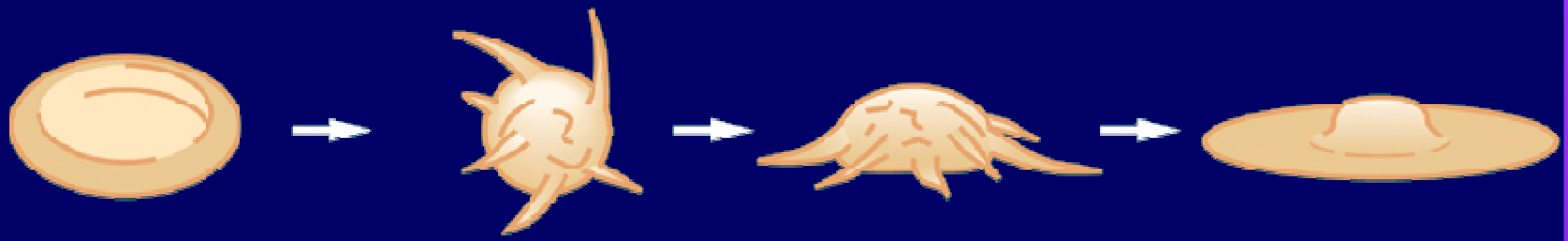
Q8VCF4 Hypothetical
O60311 Hypothetical

Coppinger JA. Blood 2004;103:2096-2104

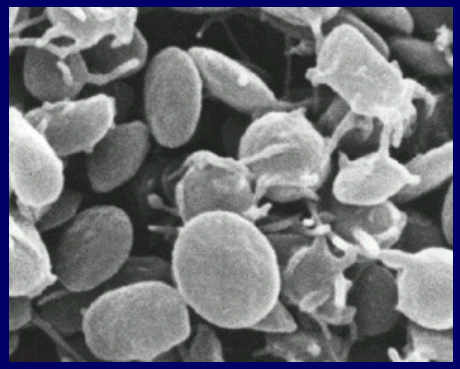
Platelet Aggregation



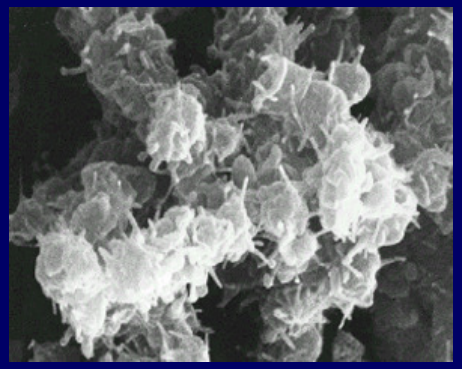
Flowing disc-shaped platelet → Rolling ball-shaped platelet → Hemisphere-shaped platelet → Spreading platelet

FIRM, BUT REVERSIBLE ADHESION

IRREVERSIBLE ADHESION



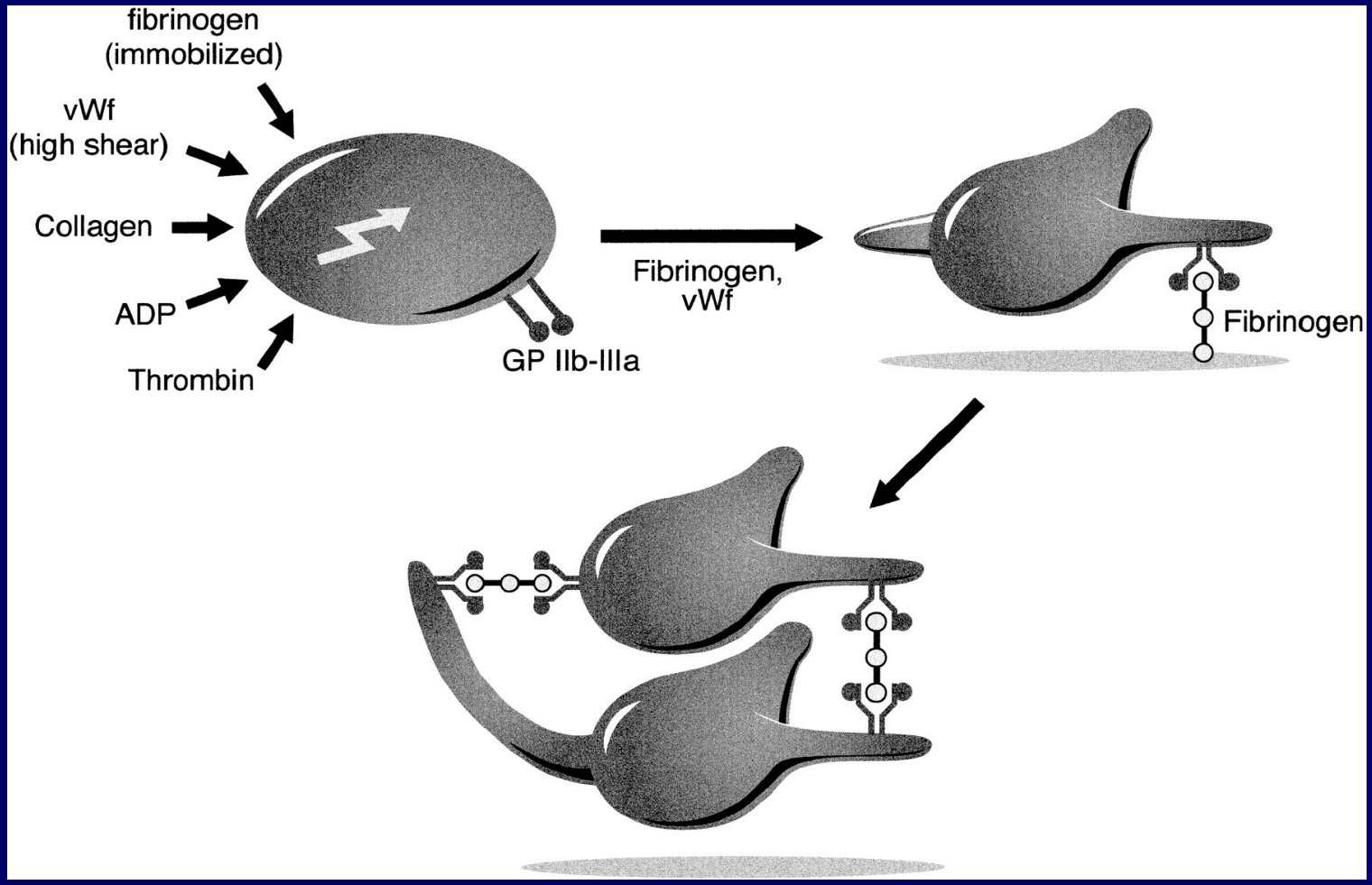
Scanning electron micrograph of discoid, dormant platelets



Activated, aggregating platelets illustrating fibrin strands

1. Kuwahara M *et al. Arterioscler Thromb Vasc Biol* 2002; 22: 329–34.

Platelet Aggregation



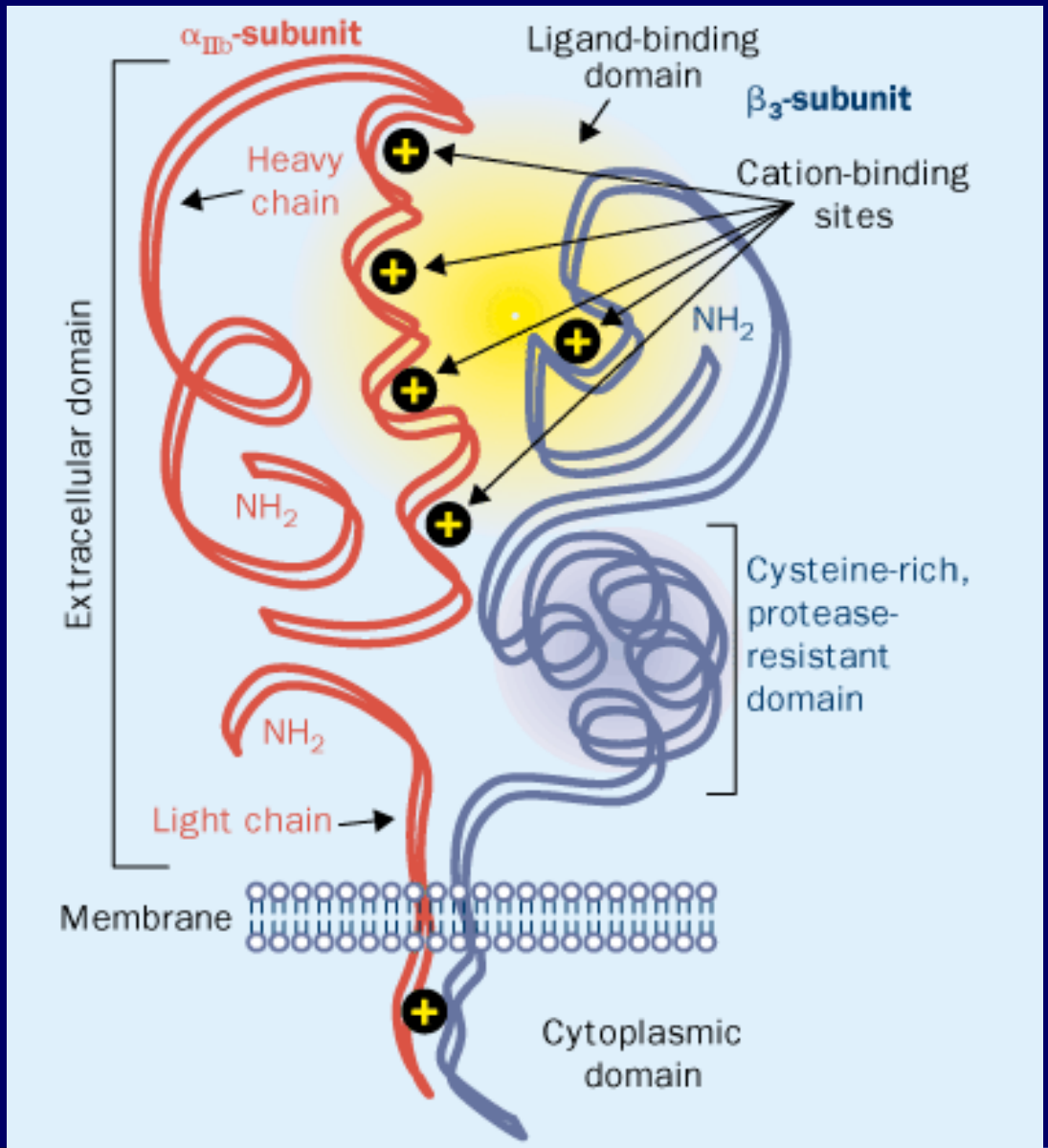
GP IIb/IIIa Receptor

- Mediates platelet aggregation
- Member of the integrin receptor family – can interact with both extracellular and cytoskeletal molecules
- One of the most abundant cell surface receptors (50-80,000 receptors per *resting* platelet, 15% of surface protein)
- Ca^{+} ions are critical for maintenance of both structure and function
- In the resting platelet the receptor has minimal binding affinity for ligands – fibrinogen and vWF

GP IIb/IIIa Receptor – cont.

- Upon activation of the platelet, conform. change of the receptor → high affinity ligand binding state + clustering of receptors on platelet surface
- *Biderctional signaling* occurs (→ initiate numerous cellular responses)
- All ligands are characterized by the arginine-glycine-aspartate (RGD) sequence → implicated as the binding sites to the GP IIb/IIIa receptor
- Fibrinogen is a divalent ligand – each molecule can bind simultaneously to two GP IIb/IIIa receptors on adjacent platelets → cross-linking

Schematic depiction of integrin $\alpha_{IIb}\beta_3$

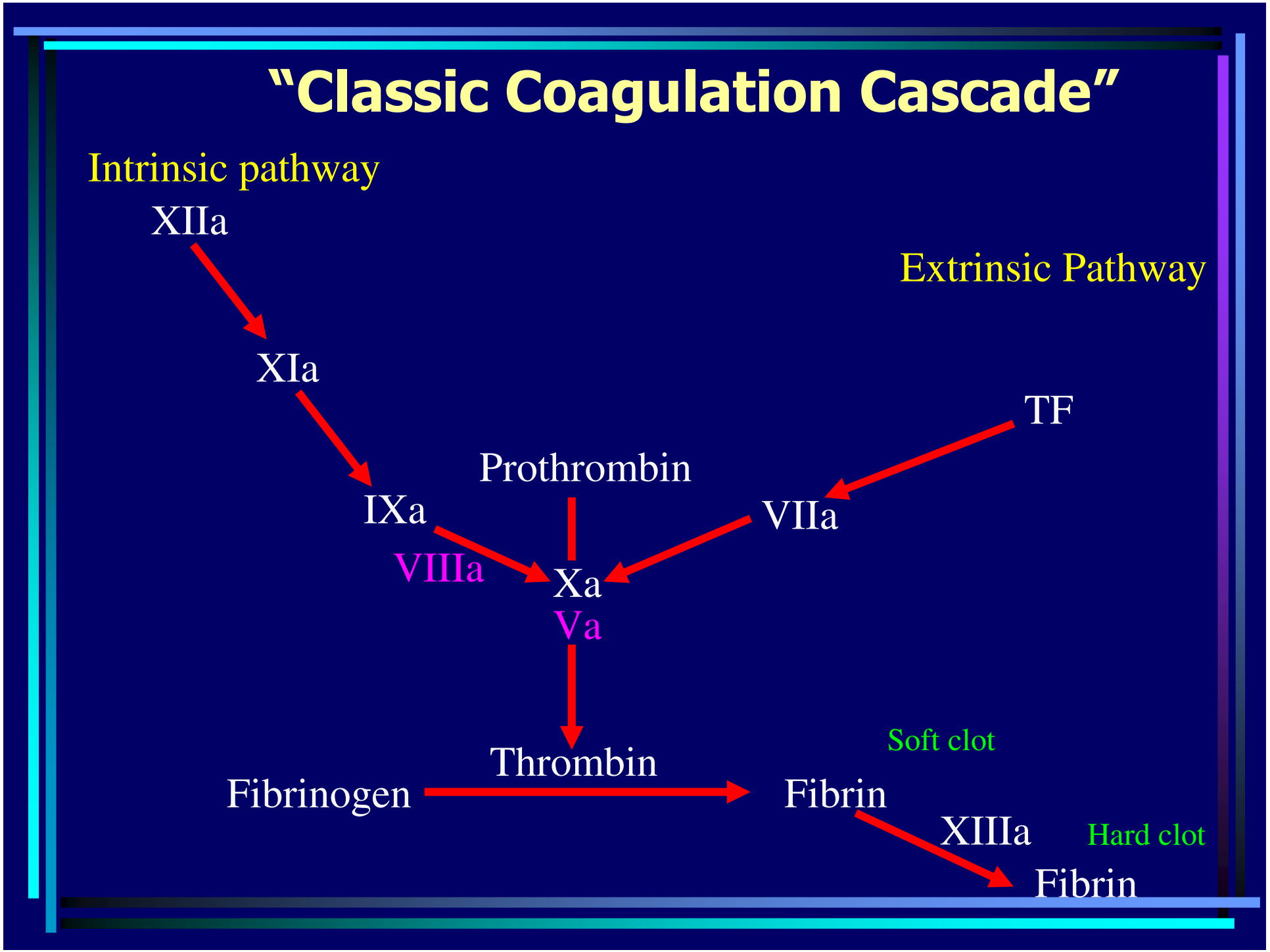


Both subunits composed of a short cytoplasmatic tail, a single transmembrane domain and a large extra-cell. domain that consists of a series of linked domains

Both subunits are a product of a single gene located on chrom. 17

3 Major systems involved

- **Vessel wall**
  - **Endothelium**
- **Platelets**
- **Coagulation cascade**

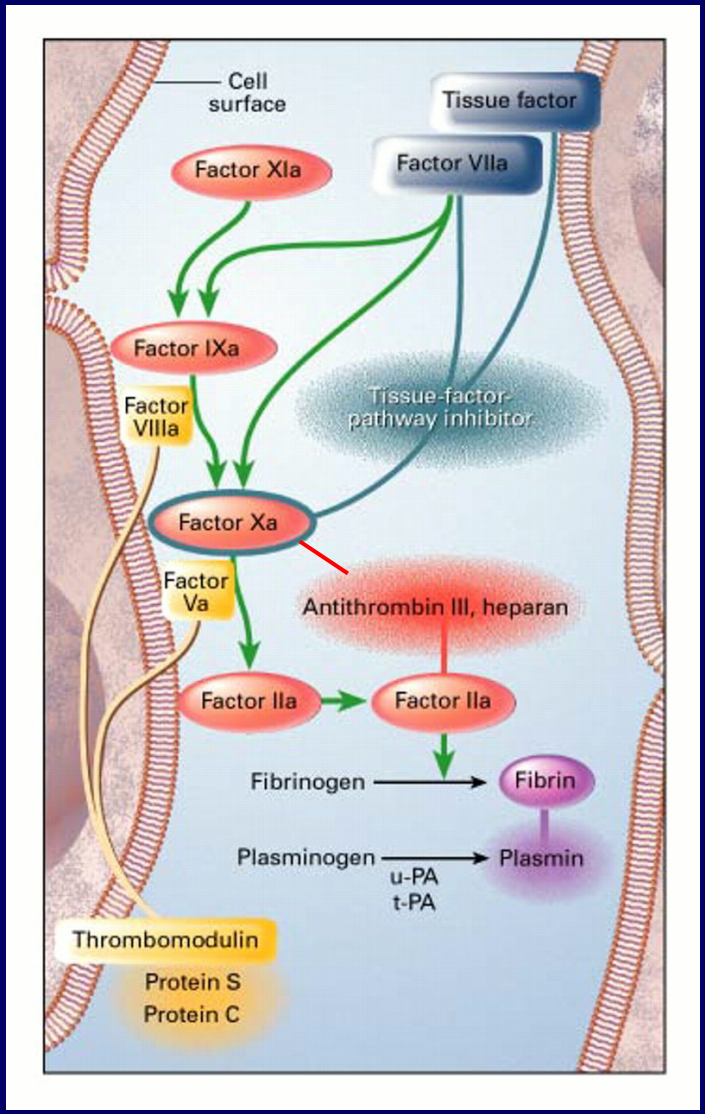

"Classic Coagulation Cascade"
Intrinsic pathway
XIIa
XIa
IXa
VIIIa
Extrinsic Pathway
TF
Prothrombin
VIIa
Xa
Va
Thrombin
Fibrinogen
Fibrin
Soft clot
XIIIa
Hard clot
Fibrin

"Classic Coagulation Cascade"

- **Enzymatic cascade** (amplification)
- **Several serine protease complexes**
  - Each complex consists of serine protease enzyme, its zymogen substrate and a cofactor
  - Produced by liver (most)
  - Several require Vit K (IIa, VIIa, IXa, Xa)
- **3 protein cofactors (not enzymes) – Va, VIIIa, TF**
- **Requires Ca^{2+}**
- **Localized to site of injury**
- **Reversible (via production of plasmin)**

"Classic Coagulation Cascade"

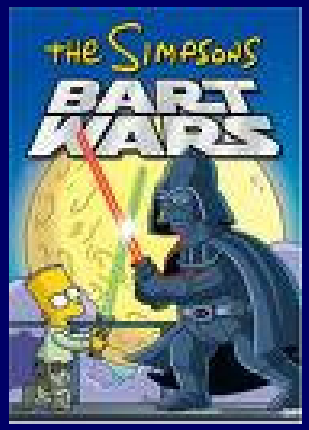
Localization to sites of vascular injury. Protease complexes assemble on PL membranes of activated platelets, endothelial cells and monocytes.
The coagulation cascade occurs very slowly in fluid phase plasma and with resting cells



4 major Anti-thrombotic Pathways (TFPI, Prot C/S, ATIII, Plasmin)

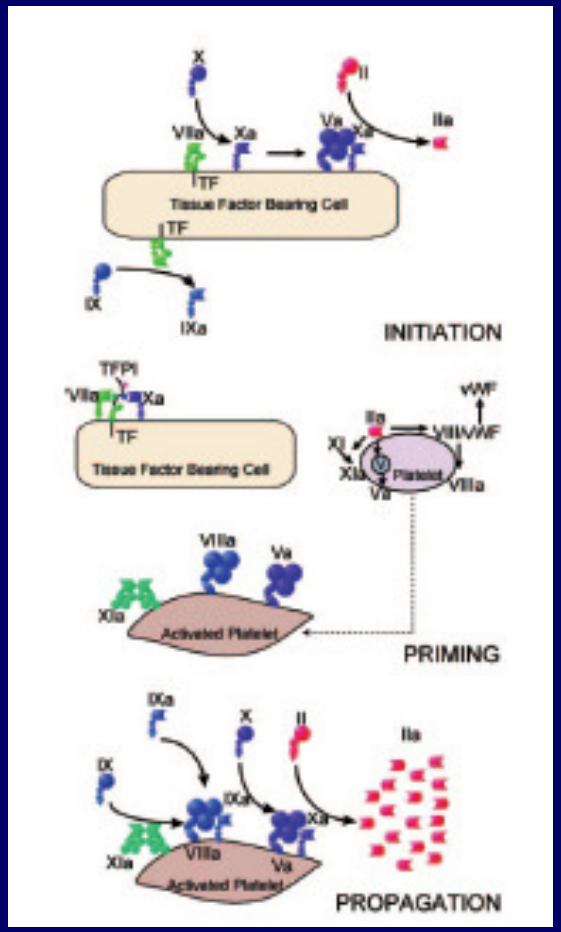
Rosenberg et al NEJM 1999

From “Classic” to Current View

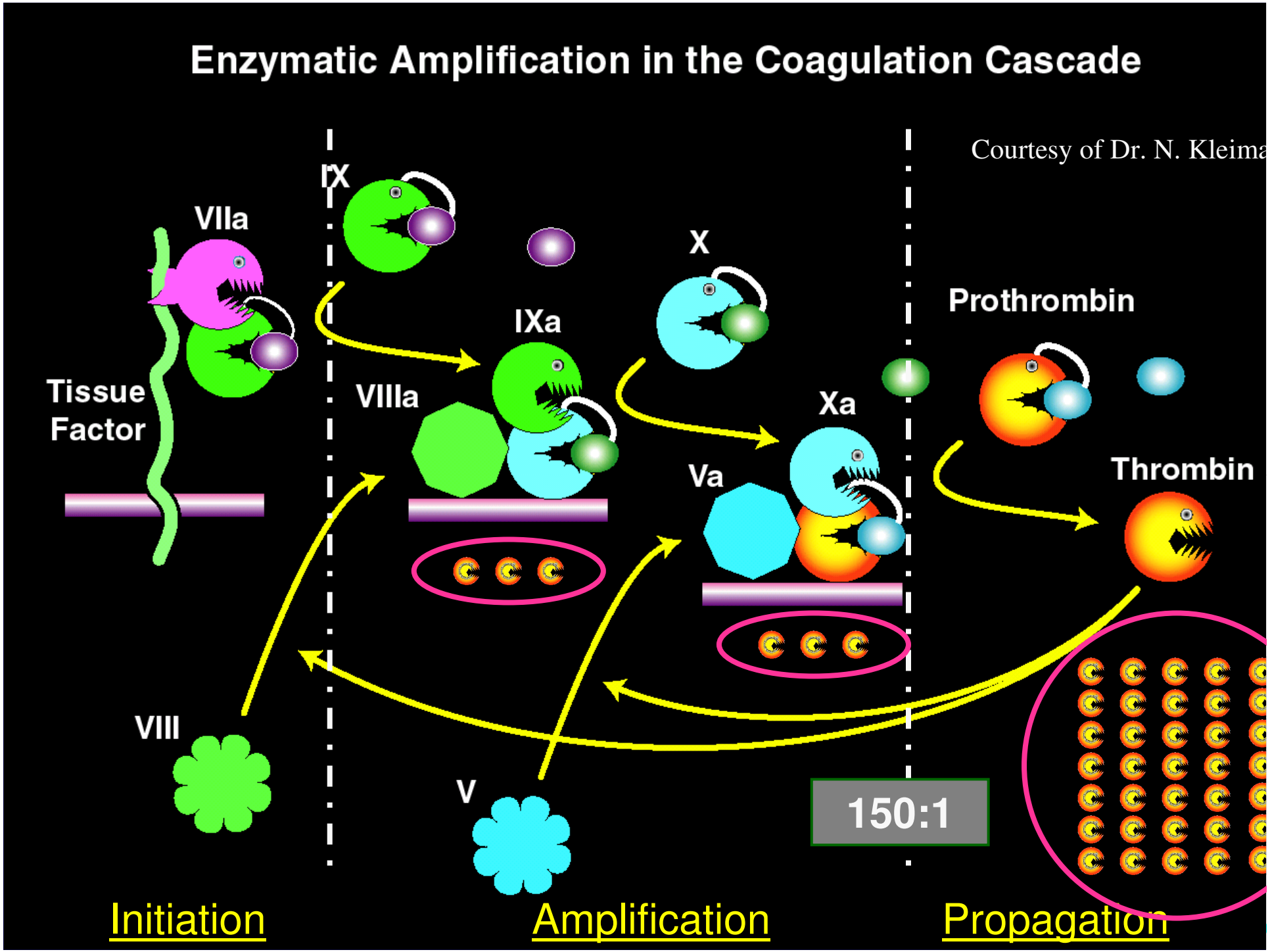


Current View of the Coagulation System

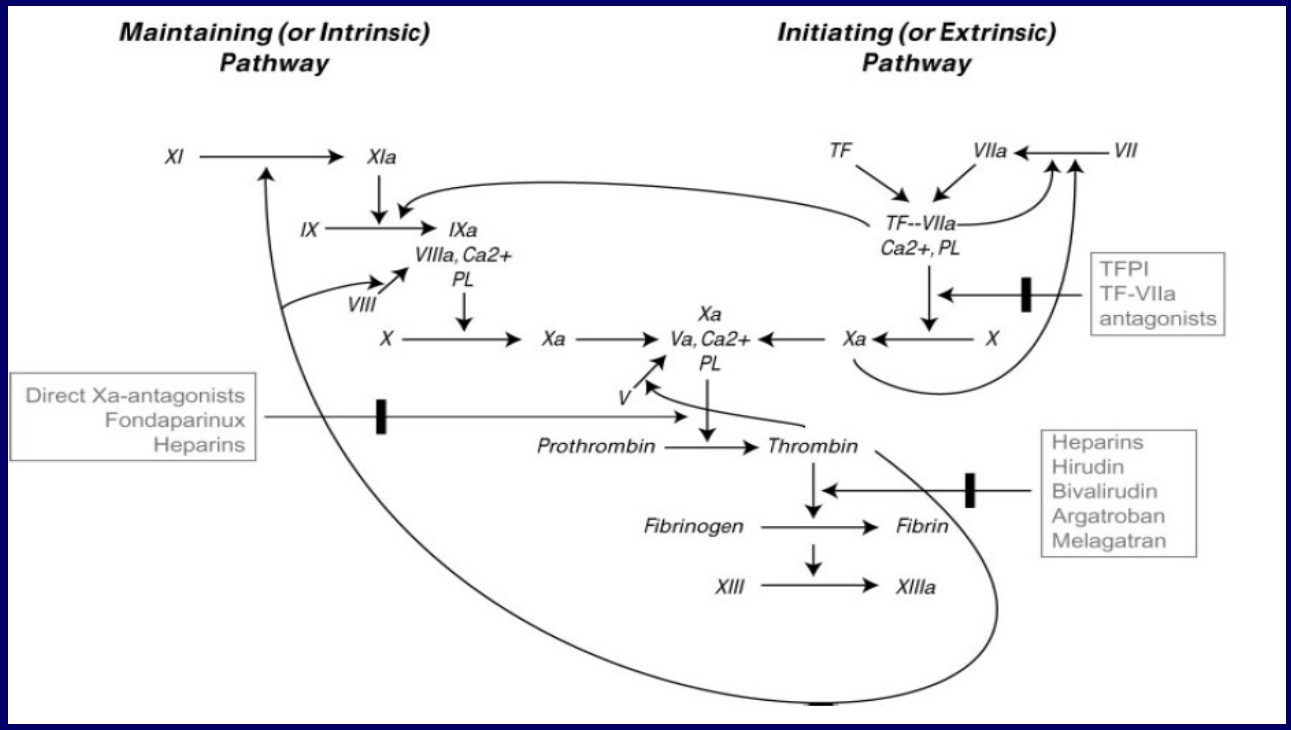
- **Initiation** by vessel wall injury which exposes blood to cells with **TF** on their surface → TF/FVIIa activates FX → Xa + Va cleaves II → small amounts of **IIa (thrombin)**
- Minute amounts of thrombin produced initially than lead to a marked increase in activation of FXI, FIX, FVIII, FV and marked generation of thrombin.
- **Priming** invloves adherence and activation of **platelets**.The small amounts of initial thrombin activates platelets → release of FV + PL surface for protease activation
- **Propagation** – an explosive increase in thrombin generation mediated by the classic "intrinsic system" → FXI, FIX → Fxa/VIIIa/Va on activated platelets → IIa + fibrin formation



Schneider D et al, Circulation 2007


Enzymatic Amplification in the Coagulation Cascade
Courtesy of Dr. N. Kleima
rX
VIIa
X
IXa
Prothrombin
Tissue Factor
VIIIa
Xa
Va
Thrombin
VIII
V
150:1
Initiation
Amplification
Propagation

Current View of the Coagulation System

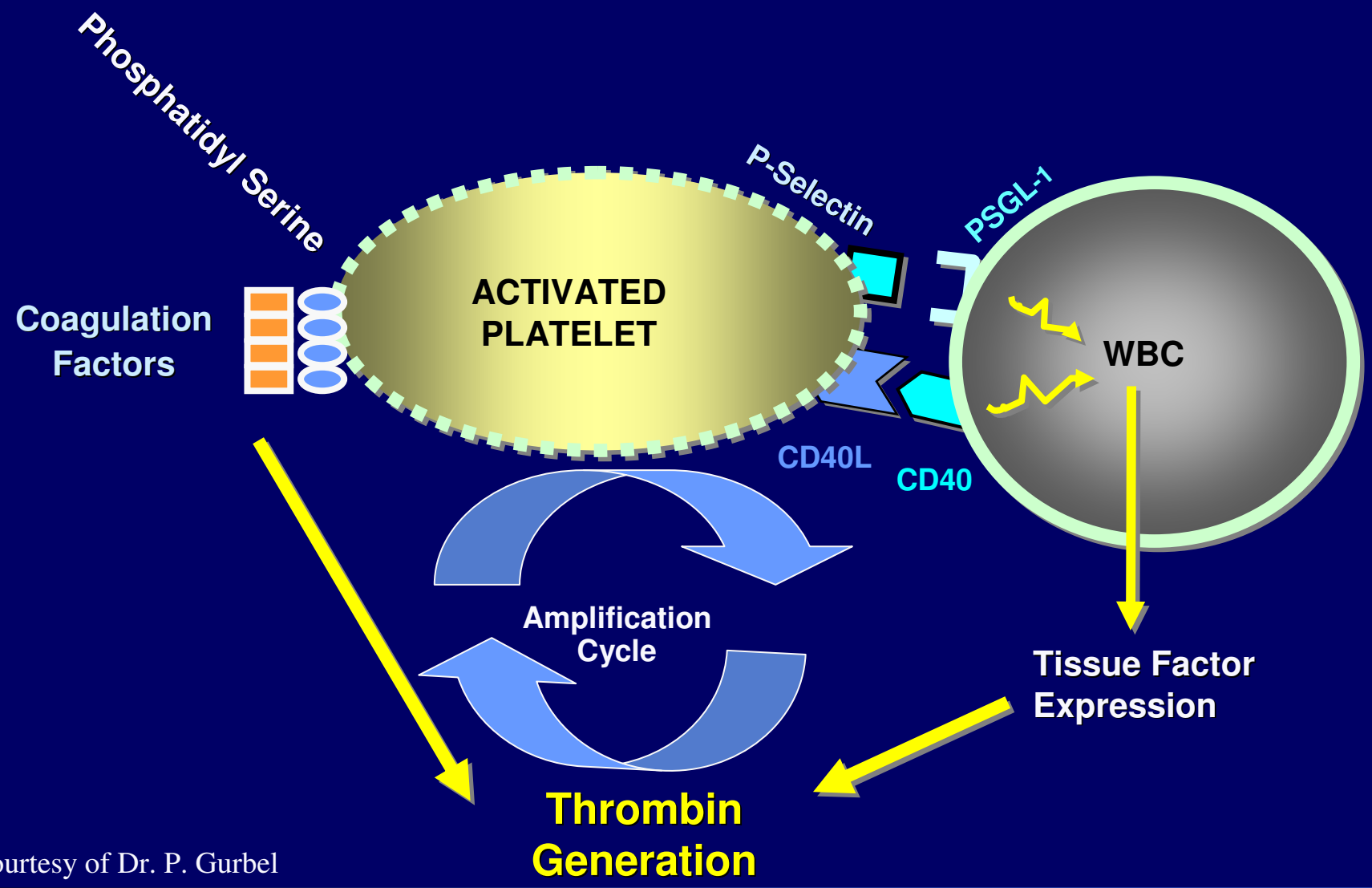


Del Conde et al CCI, 2003

Role of Platelets in Current View of the Coagulation System

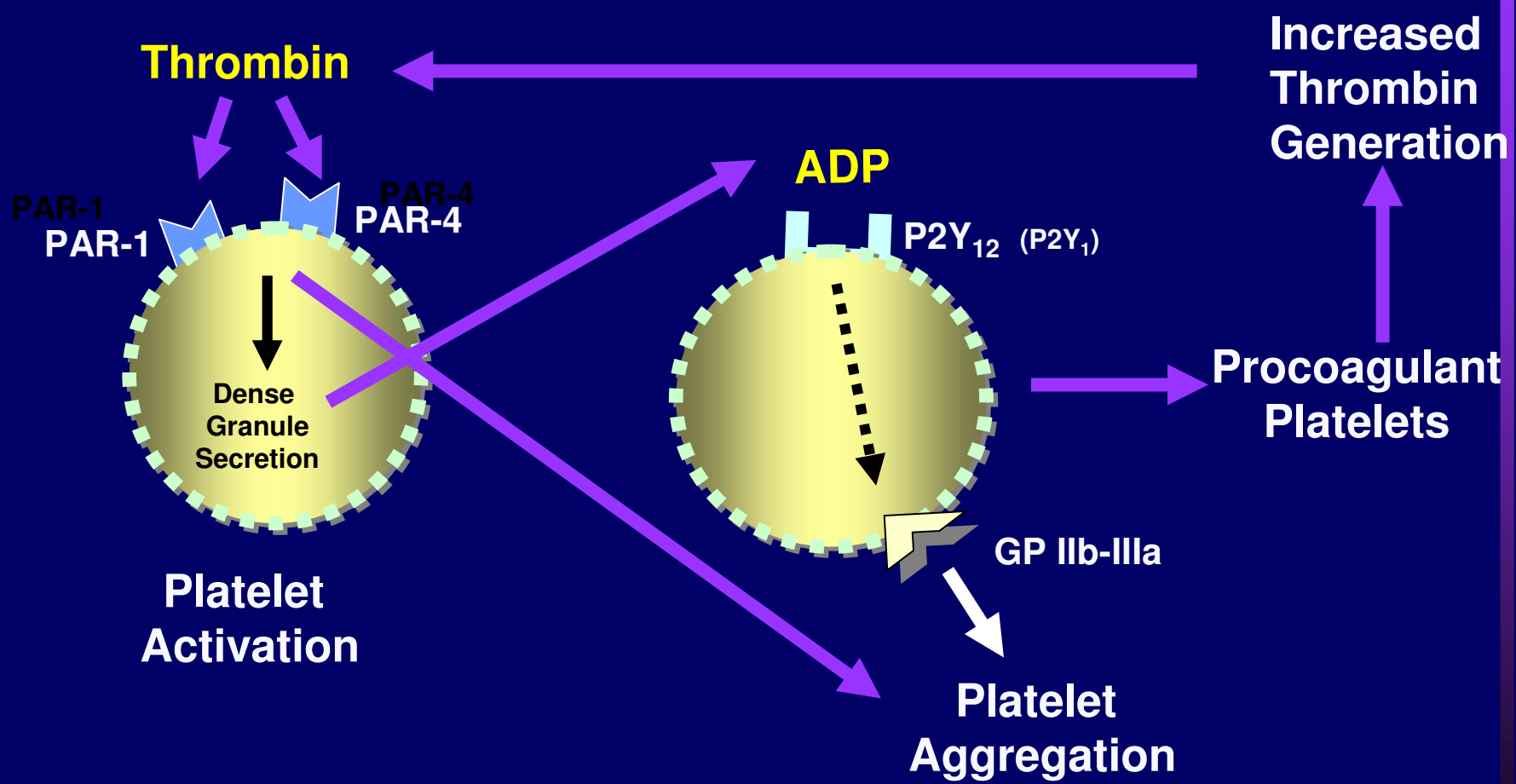
1. Adherence after vascular injury
2. Formation of platelet-platelet aggregates (GP IIb/IIIa) and platelet-WBC aggregates (P-selectin)
3. Release of platelet granule products – Ca, FV, fibrinogen
4. Recruitment of additional activated platelets (ADP, TXA2)
5. Stimulation of vasoconstriction (serotonin)
6. Promotion of formation of **thrombin** by PL surface on which the coagulation complexes form (priming + proagation)
7. Change in shape with pseudopod extension

The Platelet as a Mediator of Coagulation



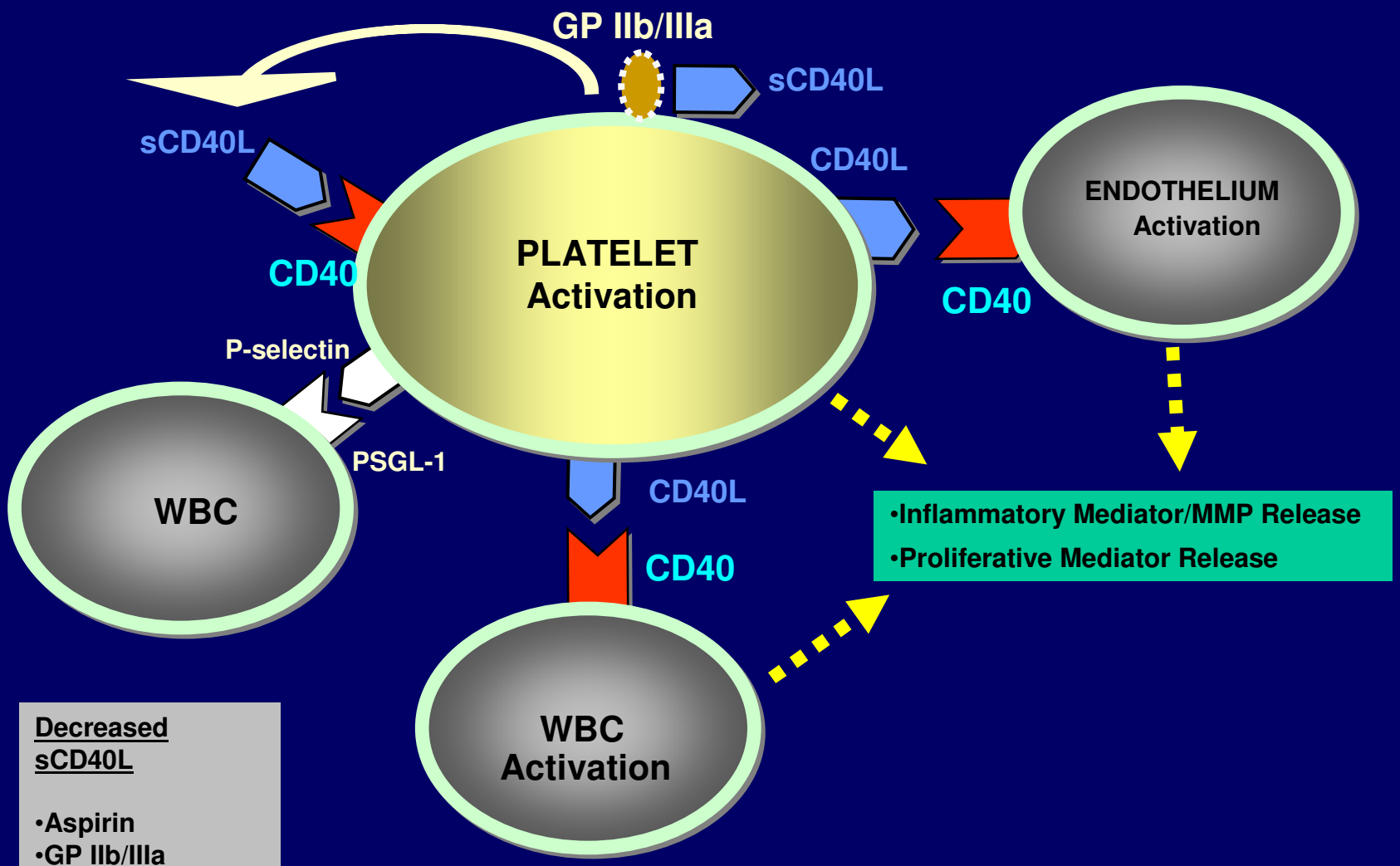
Courtesy of Dr. P. Gurbel

Central Role of ADP and Thrombin Crosstalk: a "Viscous" Cycle



Courtesy of Dr. P. Gurbel

The Platelet as a Mediator of Inflammation

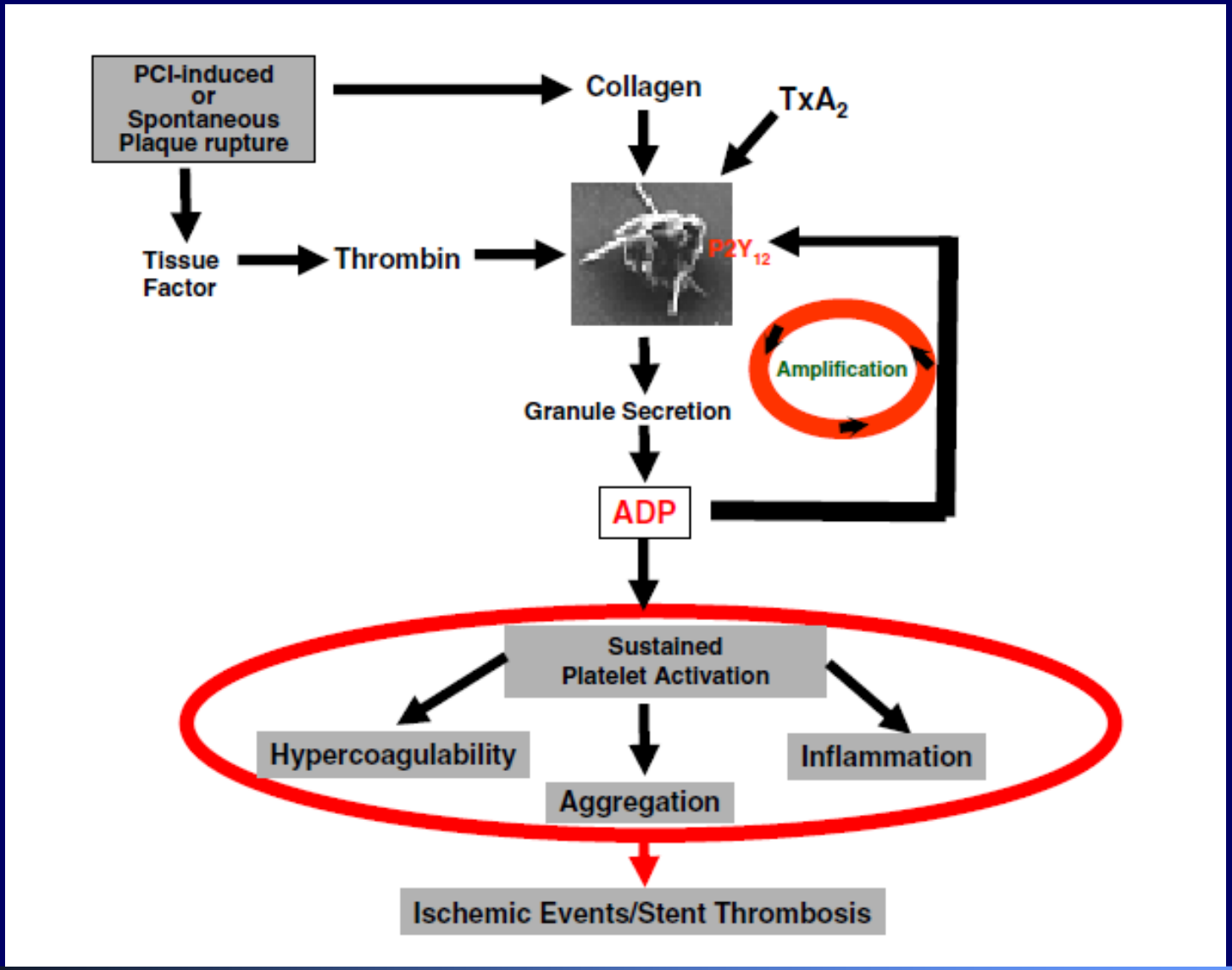


Courtesy of Dr. P. Gurbel

Main Culprits:

- TF
- Thrombin (+ Xa)
- Activated Platelets

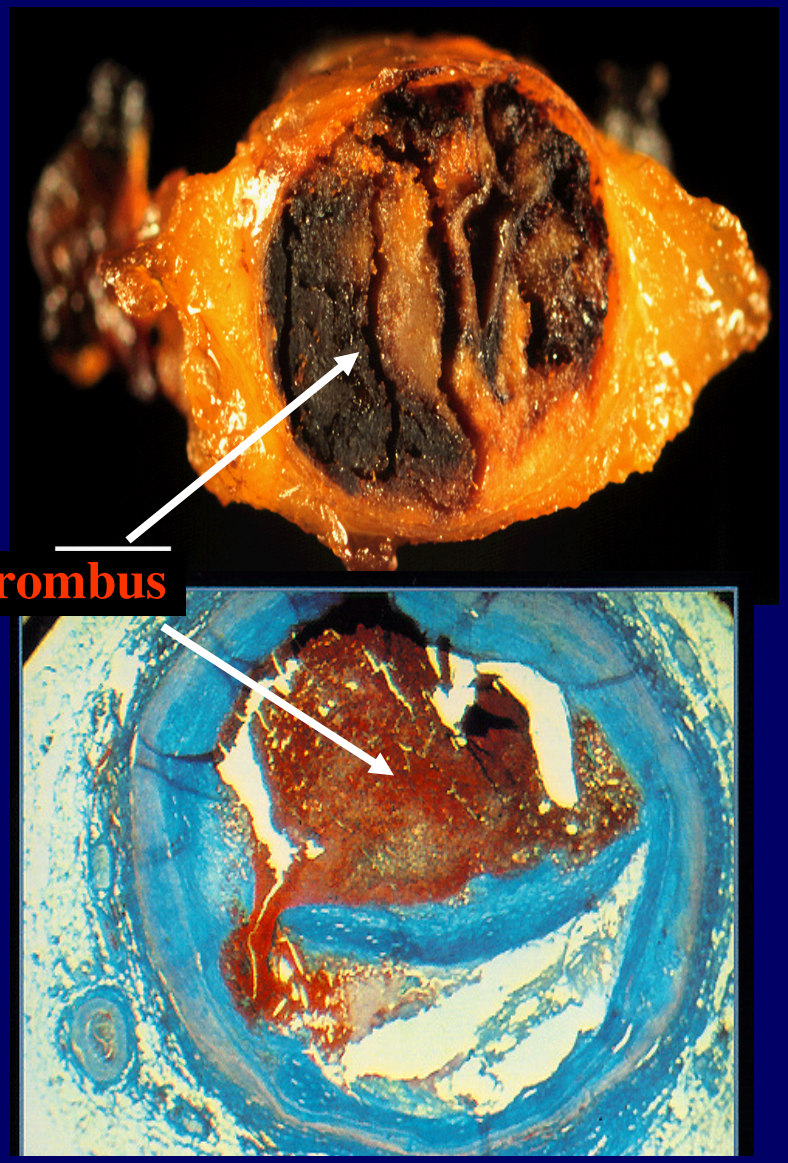
In the context of PCI or spontaneous plaque rupture



Bonello et al. JACC 2010

Plaque Rupture and Platelet-Thrombus Formation:

A common substrate for acute coronary syndromes



- Courtesy of Dr. Dan Simon

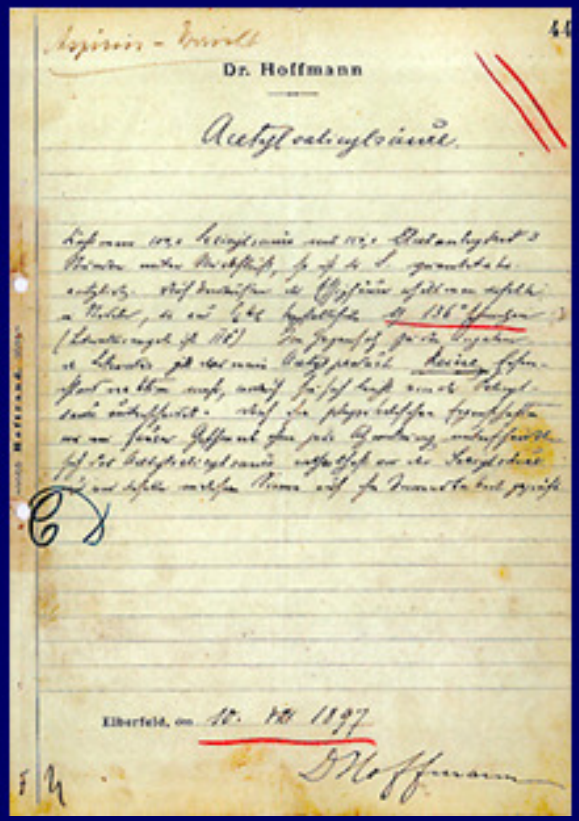
Anti-thrombotic Treatment for CVD

- Anti-platelet Drugs
  - Aspirin
  - Clopidogrel
  - GP IIb/IIIa inhibitors
  - Thrombin receptor antagonists
- Anti-thrombin drugs
  - Unfractionated heparin
  - LMWH (enoxaparin)
  - Direct thrombin inhibitors (bivalirudin)
- Anti- factor Xa drugs
  - Fondaparinux

Anti-Platelet Medications

Aspirin

(From the German acetylspirsaure + chemical suffix – in)



First synthesized in pure form by Felix Hoffman of Friedr. Bayer & Co. in 1897.

UK

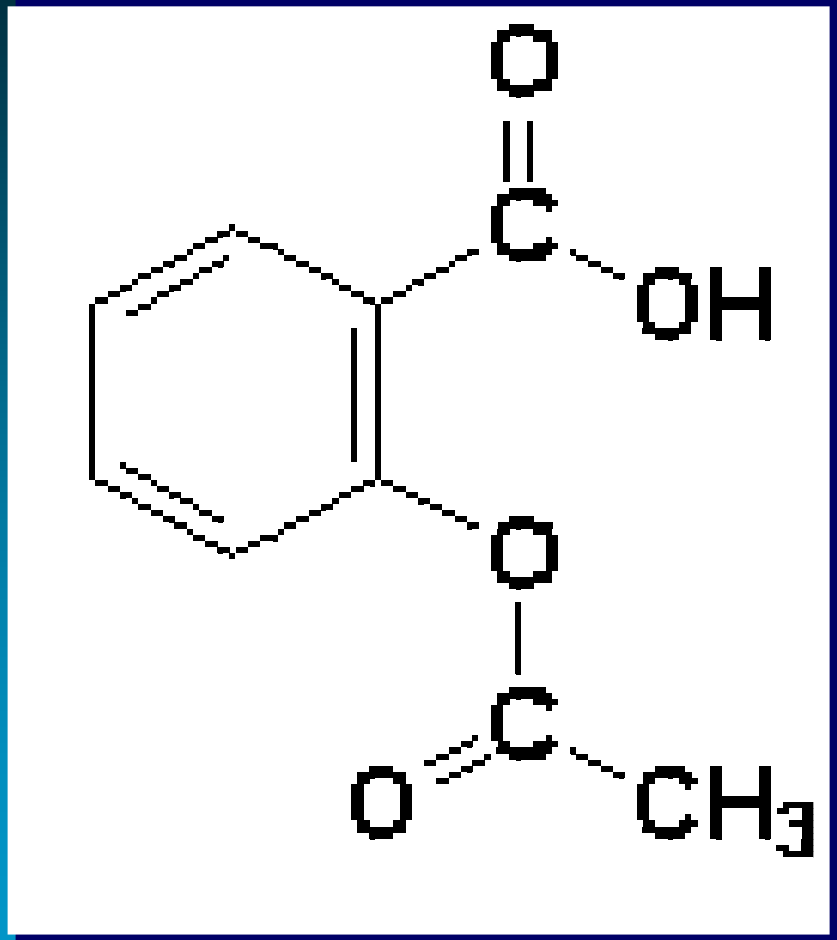
Courtesy of Dr S. Steinhubl, U. Kentucky

An original package of Bayer Aspirin sold in the United States from 1909.

Each pill is 5 grains, or ~ 325mg.

Developed by Felix Hoffrman, Bayer Co. , 1897

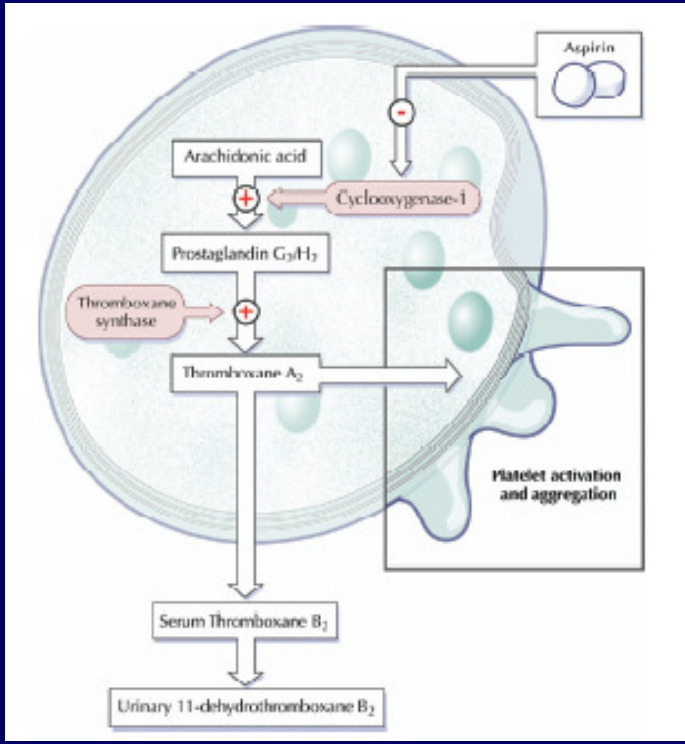
Aspirin



1. **1897: Felix Hoffrman, Bayer Develop Aspirin**
2. Irreversivle inhibitor of cyclooxygenase-1
3. In platelets inhibits conversion of AA to TxA2 $\rightarrow$ platelet activator
4. May exert other effects by acetylation
5. **Now, 1 in 5 Americans Take an Aspirin each Day !! (26 million Americans – for cardioprotection)**

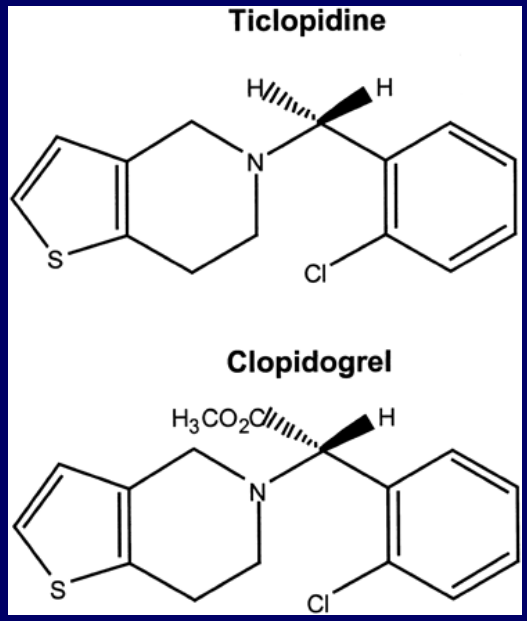
Aspirin

- ASA modifies both COX-1 and COX-2, although its affinity for COX-1 is 50-100 times that for COX-2.
- ASA acetylates a serine hydroxyl group
- Platelets are anucleated cytoplasts and largely lack transcriptional activity. Therefore, ASA induces an irreversible defect in TX synthesis, which persists for the platelet lifespan (8-10 dys)
- Despite the short $t_{1/2}$ of aspirin (15-20 min), low-dose aspirin can fully inhibit platelet COX-1 on repeat daily dosing.
- This apparently dose-independent effect on platelet function contrasts with the clearly dose-dependent aspirin-induced GI toxicity



CLOPIDOGREL (PLAVIX)

- A thienopyridine , inhibits ADP induced platelet aggregation
- The specific target of inhibition is the $P2Y_{12}$ receptor
- Fewer side effects than ticlopidine



Thienopyridines

Ticlopidine
(Ticlid)

Clopidogrel
(Plavix)

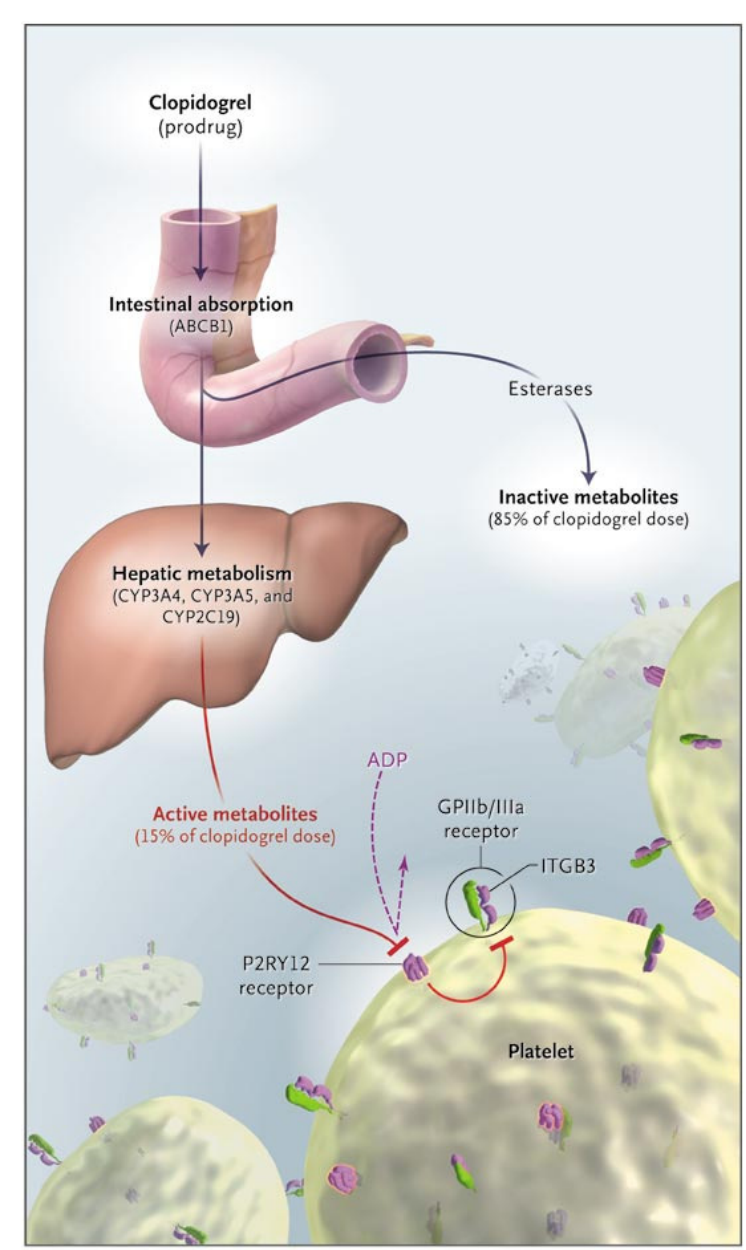
Pharmacokinetic properties

- Approximately 85% of the pro-drug is hydrolyzed by esterases in the blood to an inactive carboxylic acid derivative
- Only 15% of the pro-drug is metabolized by cytochrome P450 (CYP3A4, 2C19) enzyme system to generate the active metabolite → irreversibly inhibits P2Y12 (lifespan of the platelet)
- After hepatic metabolism, peak plasma metabolite concentrations occur at 1 hour bioavailability is unaffected by food .

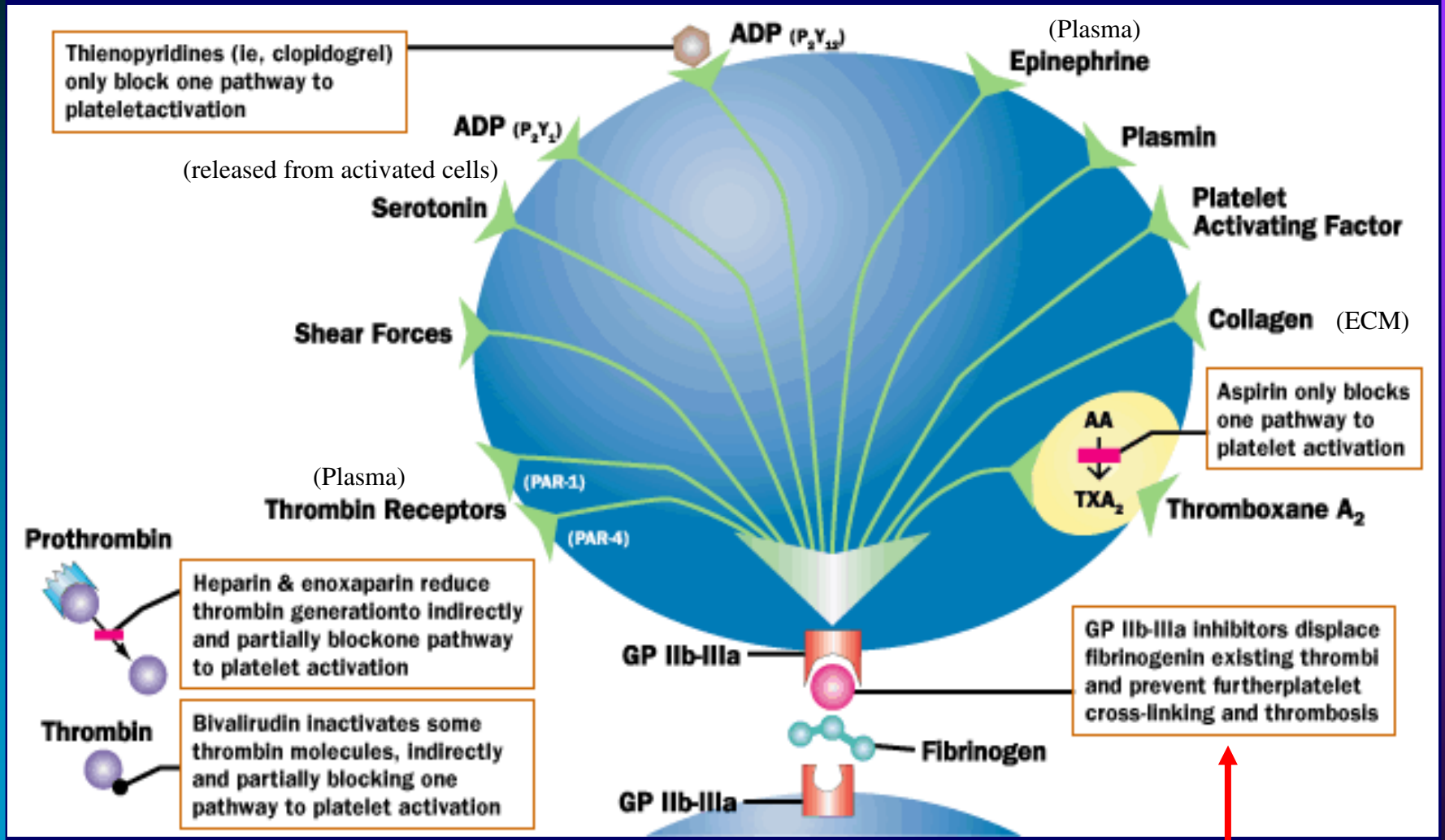
Pharmacodynamic Properties

- Platelet function recovers about 5 days after drug withdrawal.
- With a loading dose of 300 mg clopidogrel, maximum inhibition of platelet aggregation occurs within 6 hours.
- With a loading dose of 600 mg maximum platelet imhibition is attained approximately after 2 hours

Clopidogrel Metabolism



Platelet Activation



GP IIb/IIIA Inhibitors

- **Abciximab (ReoPro®)** – the first inhibitor developed and approved for clinical use.

  Chimeric monoclonal antibody – 7E3, the murine constant region was replaced by its human counterpart. Not specific for GP IIb/IIIa receptor

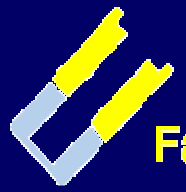
- **Eptifibatide (Integrilin®)** – synthetic cyclic hepta-peptide derived from a sequence found in the venom of the southeastern pygmy rattlesnake

- **Tirofiban (Aggrastat®)** – synthetic small molecule with structure similar to that of the RGD sequence of the snake venom echistatin

GP IIbIIIa inhibitors

Antibody

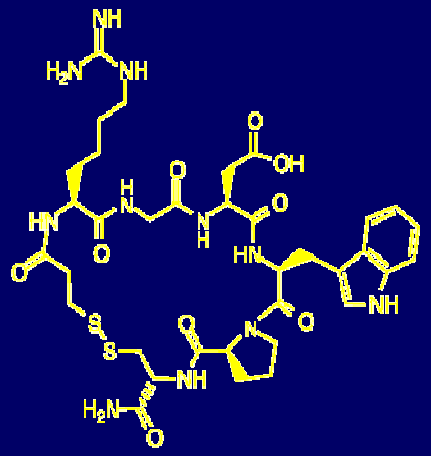
- abciximab



■ Murine variable region
■ Human constant region

Cyclic peptide

- eptifibatide



Nonpeptide

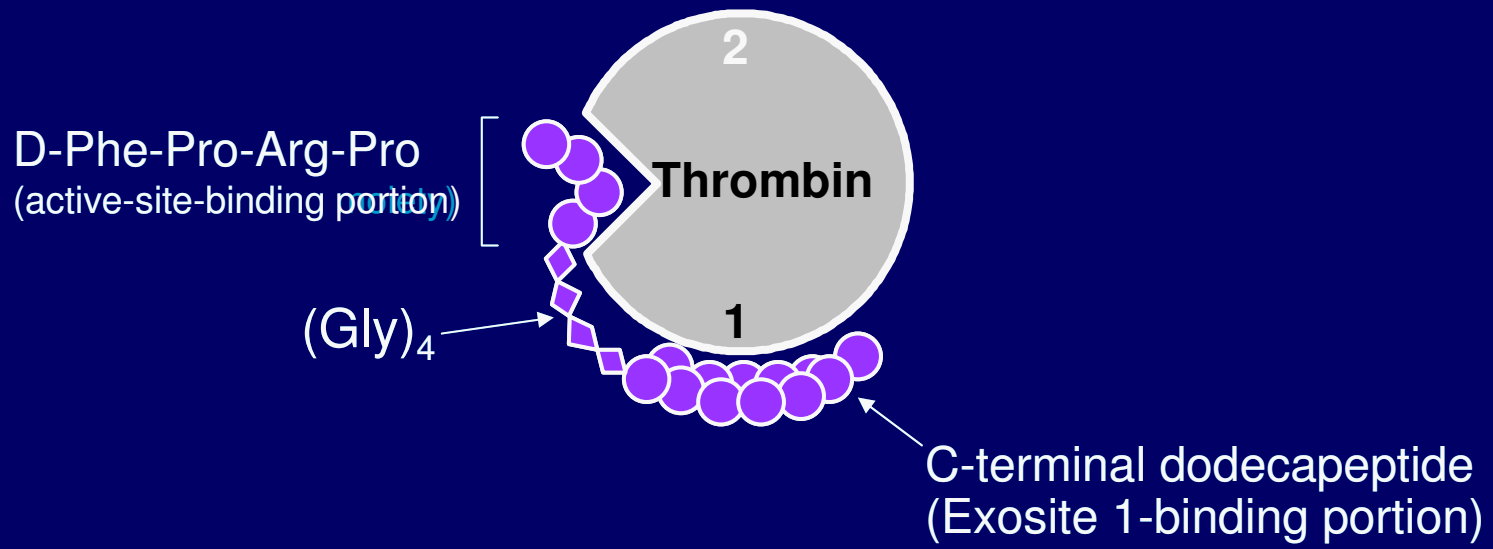
- tirofiban HCl
  (Aggrastat®, Merck)

Glycoprotein IIb/IIIa Receptor Antagonists

	Abciximab	Tirofiban	Eptifibatide
Pharma	Fab portion of chimeric monoclonal antibody	Synthetic non-peptide	Cyclic heptapeptide
Plasma ½ life	30 minutes	1.8 hours	2.5 hours
Specificity	Not specific	Highly specific	Highly specific
Dose	0.25 mcg/kg bolus followed by 0.125 mcg/kg/min drip (max 10 mcg/min) for 12-24 hours	0.4 mcg/kg/min for 30 minutes followed by 0.1 mcg/kg/min drip for 48-96 hours	180 mcg/kg bolus (x2) followed by 2.0 mcg/kg/min drip for 18-24 hours

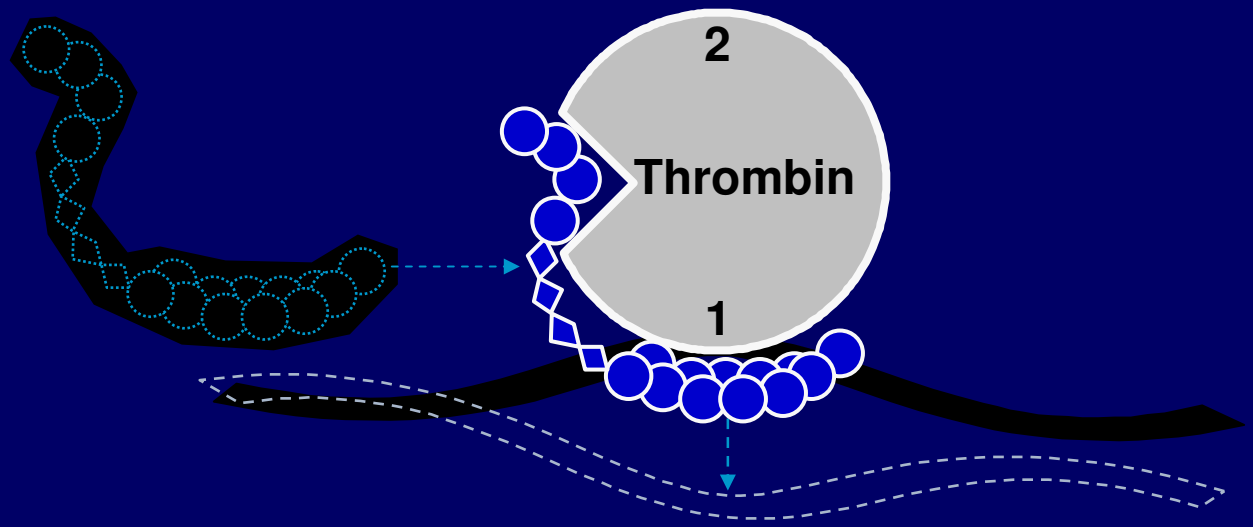
Bivalirudin - Angiomax

Bivalirudin – a direct thrombin inhibitor - binds bivalently and with high affinity to thrombin's active site



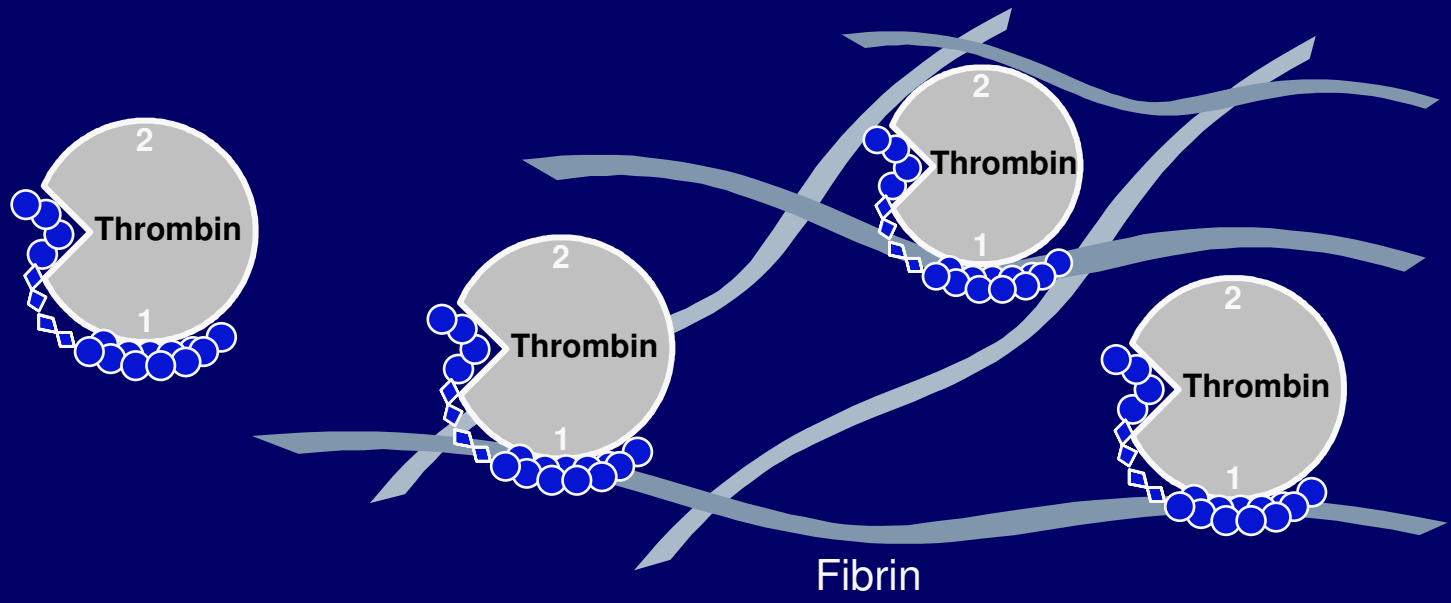
Bivalirudin

Bivalirudin can displace fibrin bound to thrombin—
Bivalirudin has high specificity for thrombin.



Bivalirudin

Bivalirudin inhibits both fibrin-bound and circulating thrombin.



Bivalirudin

- Bivalirudin is cleared from plasma by a combination of renal mecahnisms and proteolytic cleavage
- Plasma half life = 25 minutes (norm renal funct)
- Mod. renal impairment, half life = 34 minutes (dose reduced)
- Almost immediate prolongation of ACT. aPTT
- Coagulation times return to normal after about 1 hour following drug d/c

Question 1

- Which of the following is not an anti-thrombotic property of the endothelium?

1. TFPI
2. NO
3. Prostacyclin
4. vWF
5. tPA

Question 2

- What is true about platelet activation?

1. Thrombin is synthesized inside the activated platelet
2. Activated platelets synthesize TxA2 from arachidonic acid
3. In the resting platelet there are almost no surface GP IIb/IIIa receptors
4. Activated platelets secrete collagen from granules

Question 3

- What is true about the coagulation cascade?

1. Occurs at the same rate on phopholipid membranes and in fluid phase plasma
2. Initiation of the coagulation process occurs on platelet PL surfaces
3. Thrombin is a key molecule by positive feedback activation of several factors and cofactors of the cascade
4. Inhibition of the coagulation process occurs only at the terminal sites (thrombin, fibrin)

Question 4

- What is untrue about the role of platelets in the coagulation scheme?

1. Recruitment of additional activated platelets by ADP, TxA2
2. Interact with WBC via P-selectin and CD40L mediation
3. Release of platelet granule products – Ca, ADP, FV, fibrinogen
4. Produce Vit K dependent factors
5. Adhere after vascular injury only at high shear rate

Question 5

- True statements about aspirin (may be more than one):

1. Modifies COX-1 and COX-2 enzymes to the same degree
2. Requires metabolism by cytochrome P450 to the active metabolite
3. Induces an irreversible defect in TX synthesis, which persists for the platelet lifespan
4. Clear dose-dependent effect on platelet function
5. May cause GI side-effects + increased bleeding risk