Methods of Biomaterials Testing

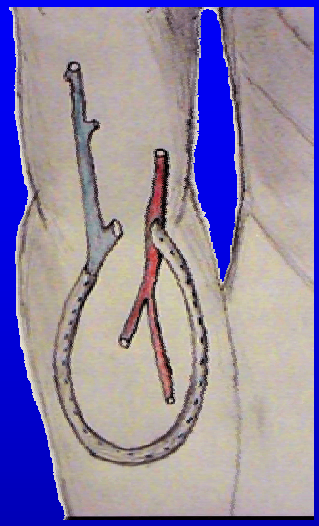
Special Biology: Blood & Vessel

Blood Compatibility

Mainly: Inhibition of blood clot formation

Materials in Bloodflow

Venous catheter

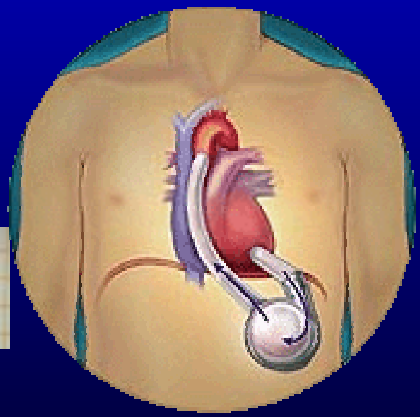


Dialysis shunt

Dialysis filter

Heart Valves

Vascular Stents

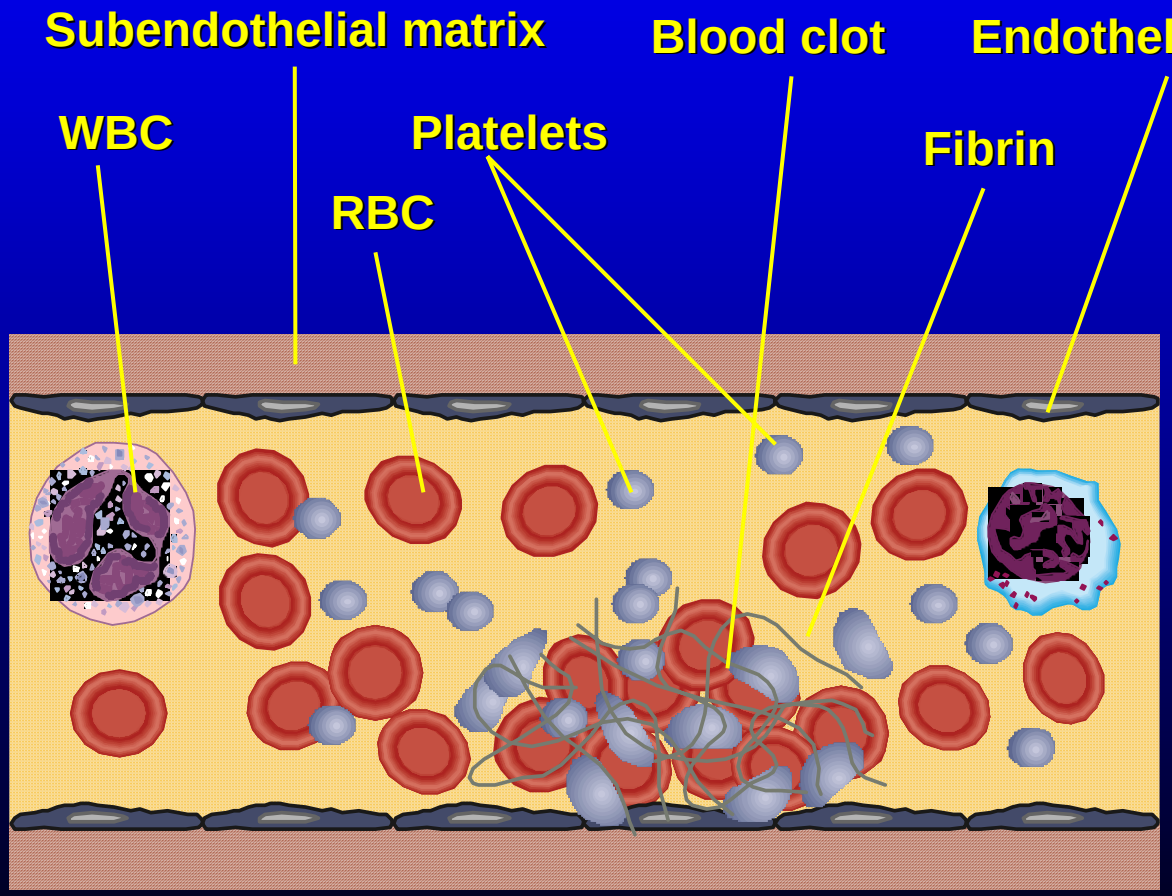


Cardiac Assist Devices

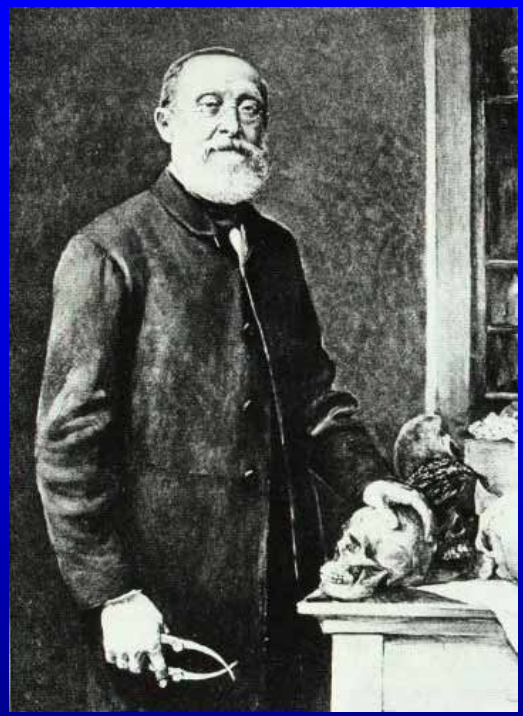
Artificial Hearts

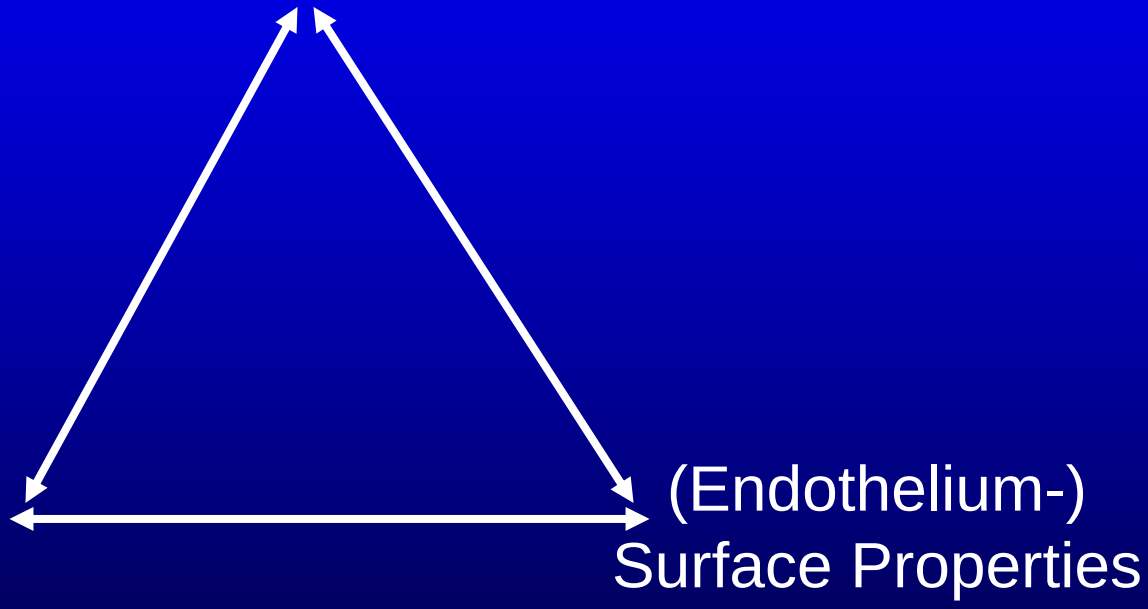
Blood Compatibility

Cells and proteins come in contact with foreign materials



The Triade of Virchow





Red Blood Cells

Hemolysis

- Reasons
  - Important for polymers (plastiziser)
  - Result of corrosion of metals
  - Mechanically at heart valves
- Measurement
  - Absorption of Hemoglobin
  - Reaction with cyanide and measurement of the absorption of cyan-methemiglobin λ = 546nm
- Consequences
  - Activation of blood platelets and clotting cascade

White Blood Cells

Activation of Phagocytes

- Release of reactive oxygen species
  - $2\ O_2 + NADPH \rightarrow 2\ O_2^- + NADP^+ + H^+$
  - $O_2^{\cdot -} + 2H^+ \rightarrow H_2O_2 + O_2$
  - $H_2O_2 + Cl^- \rightarrow HOCl + H_2O$
- Release of inflammatory cytokines
  - Interleukin 1
  - Interleukin 6
  - TNF-α

Consequences

- Activation of endothelium cells
- Proliferation of vascular smooth muscle cells
- Recrutation and activation of other cells

Activation of Lymphocytes

- So far not described for implants in the blood

Reactive Oxygen Species

Because of short lifetime (<< 1 second) direct measurement is often difficult

- Hydrogen Peroxide
  - Direct measurement

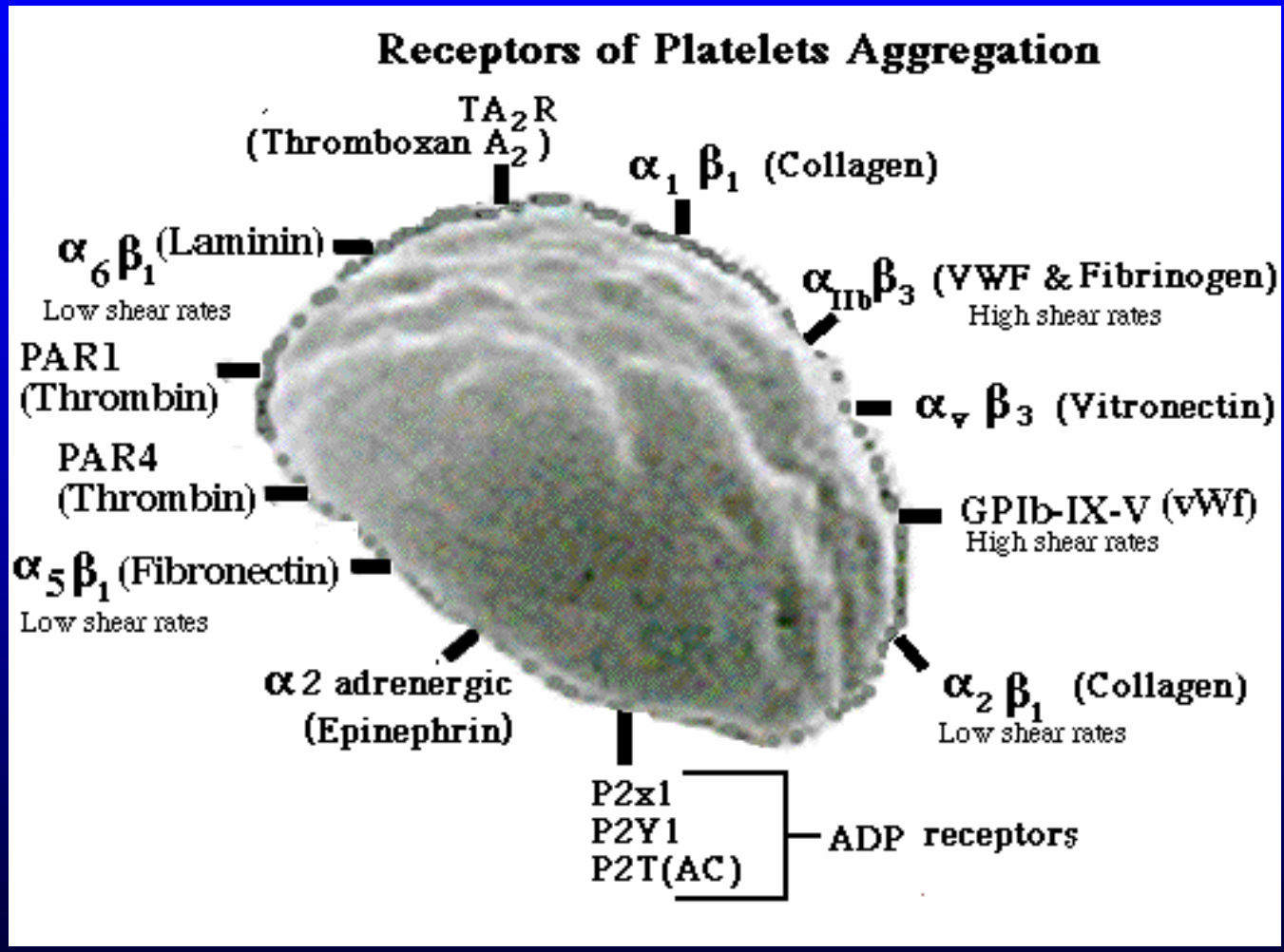
- Other free radicals
  - Detection with luminescent probes (luminol or lucigenin) in real-time
  - Chromogenic or fluorogenic substrates
- Detection of free radicals effects
  - Lipide peroxidation: Malondialdehyde (MDA) formation
    - Direct detection with HPLC
    - Detection with Tiobarbituric acid (pink product)
  - Consumption of scavengers (radical antagonists)
    - Ascorbic acid
    - SH groups (glutathione)

Thrombocyte Activation

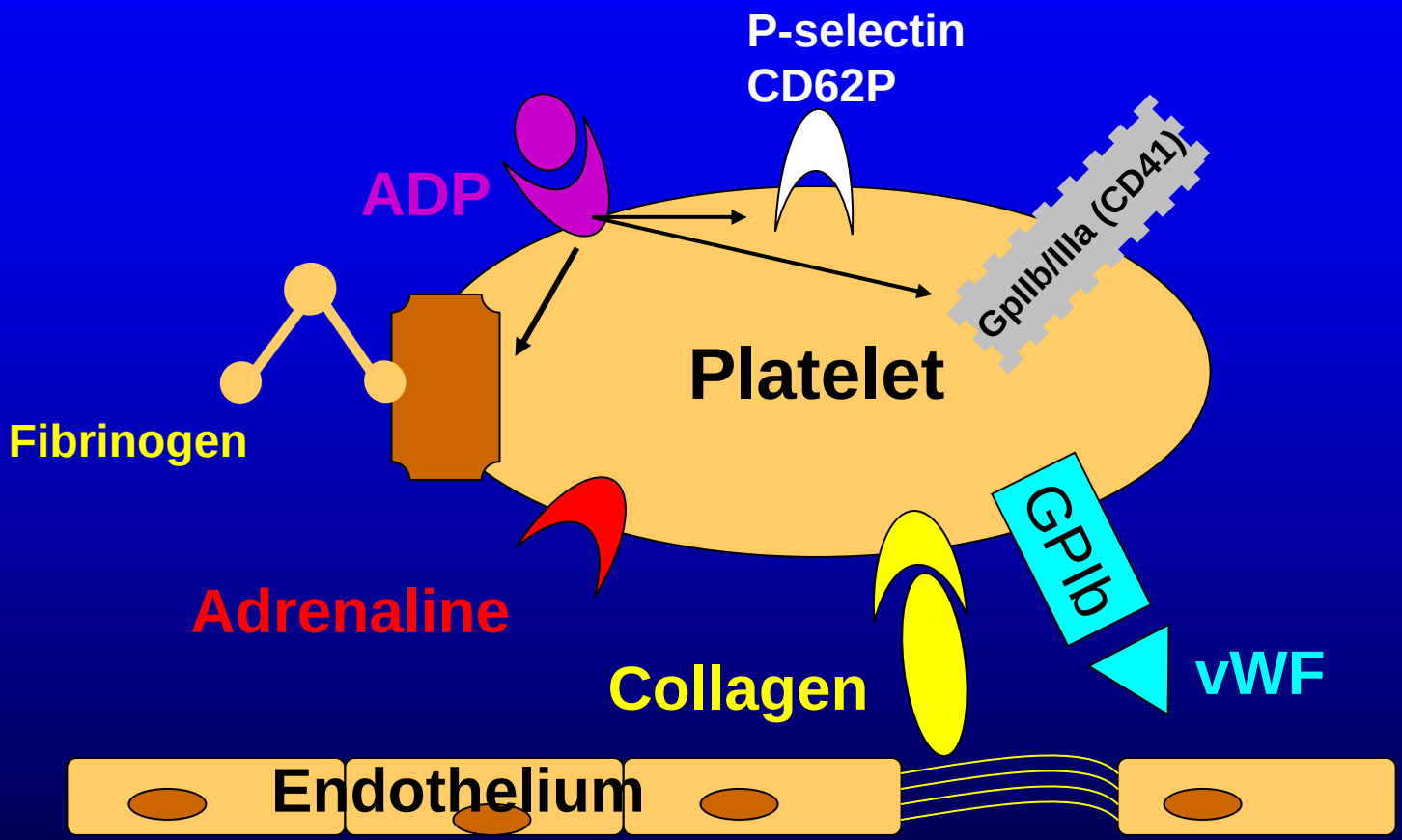
Reasons

- Activated endothelium cells (expression of vWF)
- Defect of the endothelium cell layer
  - Uncovered collagen, fibronectin und laminin
  - Negatively charged surfaces
- Fibrin and activated clotting factors
- Denatured proteins
- Mediators
  - Noradrenalin
  - ADP
- Decreased blood flow

Platelet Activation Pathways



Platelet Activation Pathways



Thrombocyte Activation

Consequences

- Aggregation
- Surface adhesion
  - Change in morphology/ spreading
- Release of Serotonin
  - → Vasodilatation
- Release of Platelet factor 3
  - → Activation of the clotting cascade
- Release of Platelet factor 4
  - → Heparin-Inactivation
- Release of growth factors (PDGF, FGF)
  - → Proliferation of vascular smooth muscle cells
- Release of RANTES
  - → Monocyte recruitment to endothelial cells

Detection

Morphology

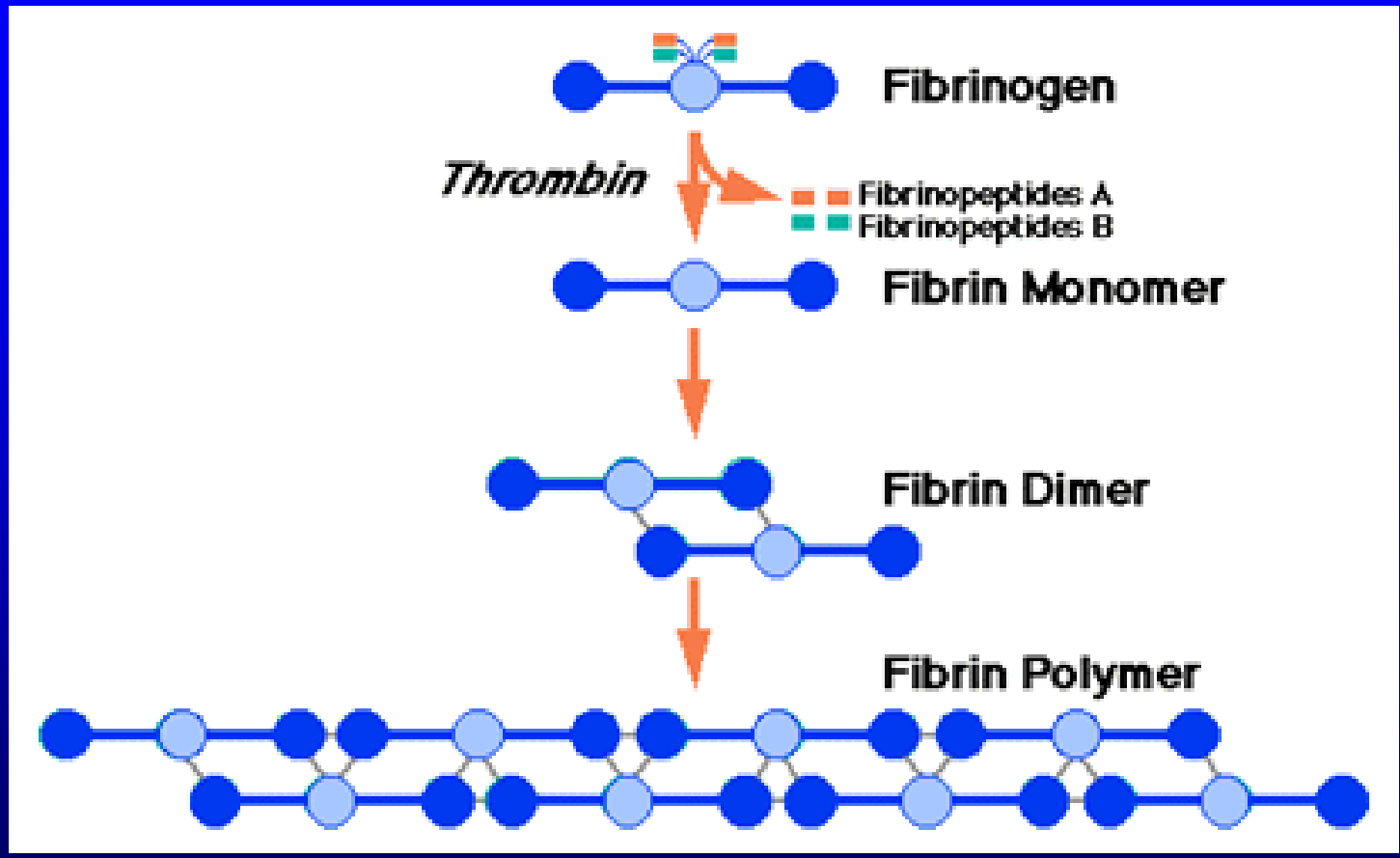
ELISA

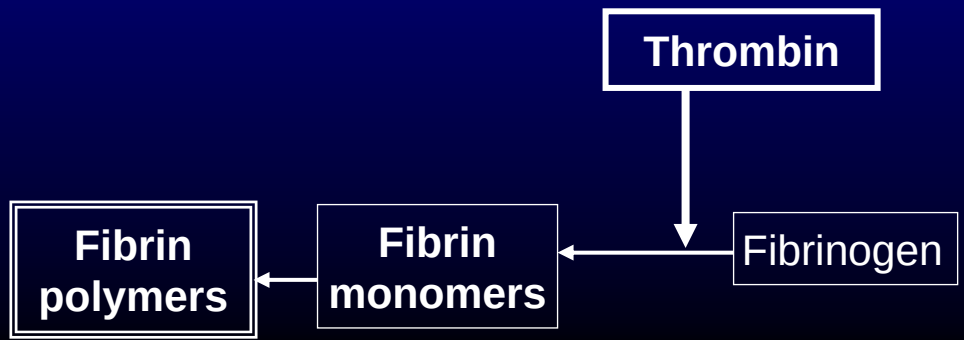
Protein Adsorption

- Albumin and fibrinogen show competitive behaviour
  - *In vitro* dependent from the salt concentration and buffer system
  - Albumin binds more to hydrophobic surface
  - Fibrinogen binds more to hydrophilic surfaces
- Different behaviour of free and adsorbed fibrinogen
  - Adsorbed fibrinogen activates blood platelets
  - Increased effect by parallel adsorption of a phospholipide (lecithin)
- Threshold Surface Fibrinogen Concentration for Platelet Activation
  - 30 ng/cm^2

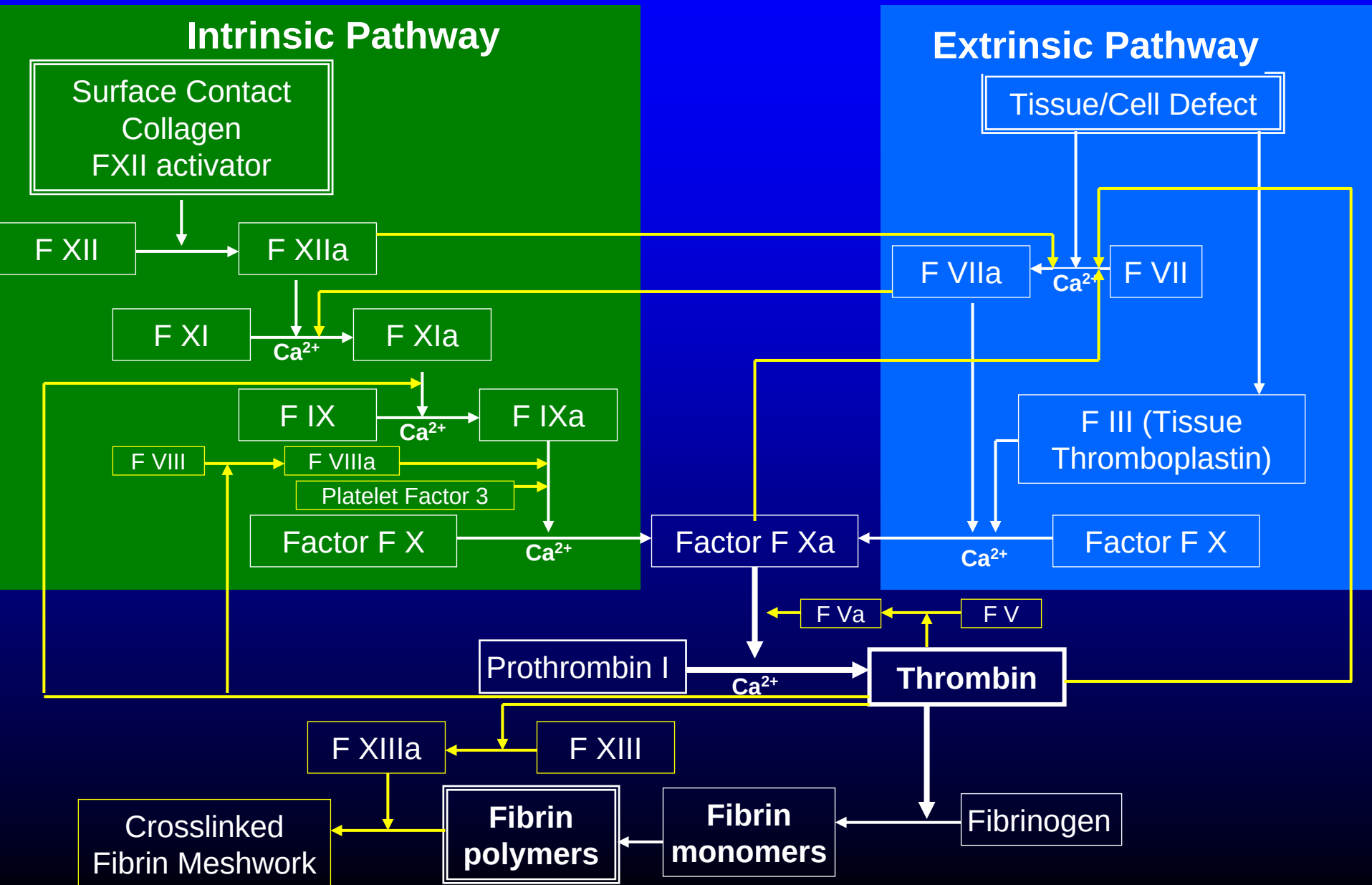
The Blood Clotting Cascade

The Clotting Cascade

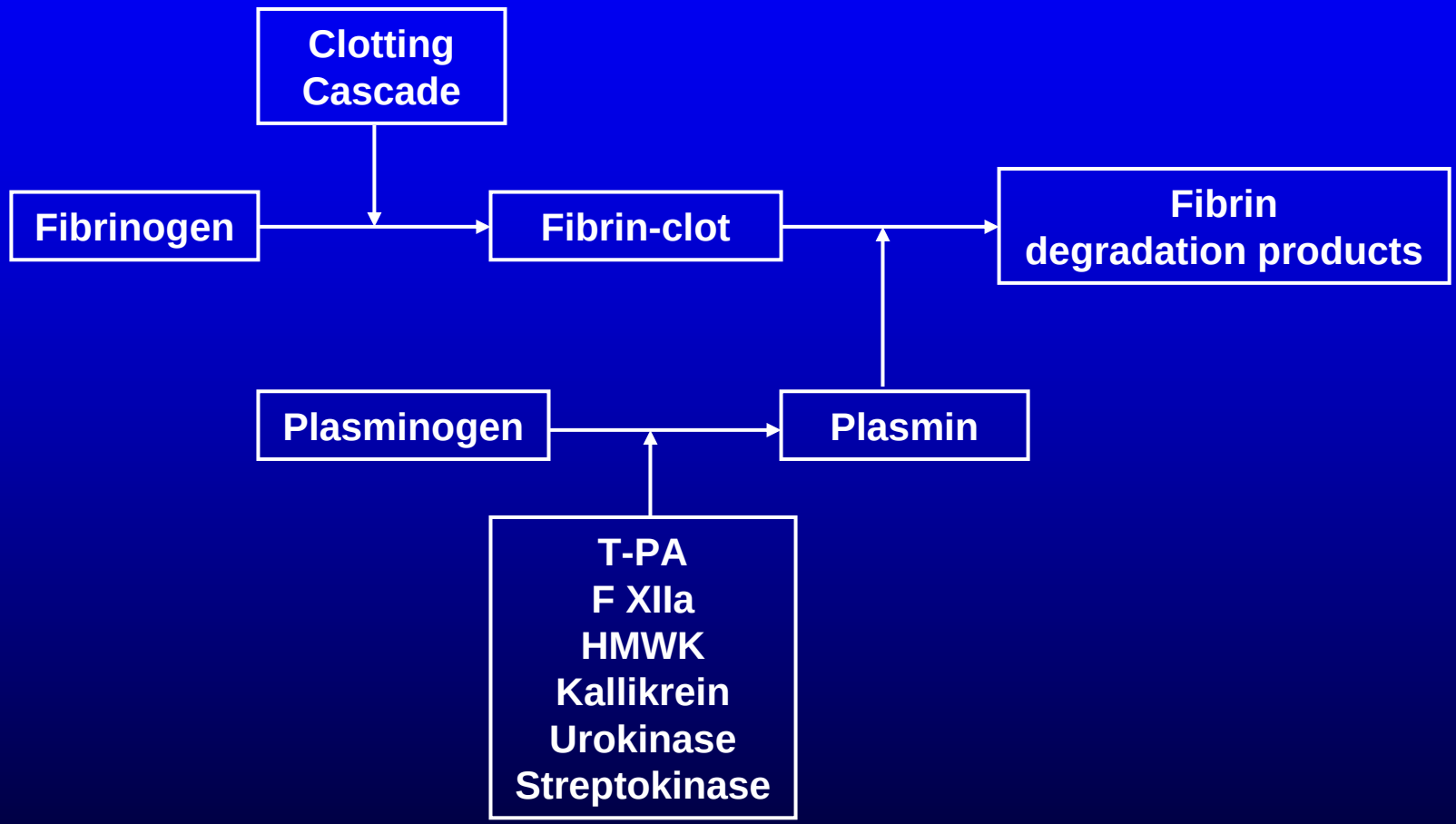




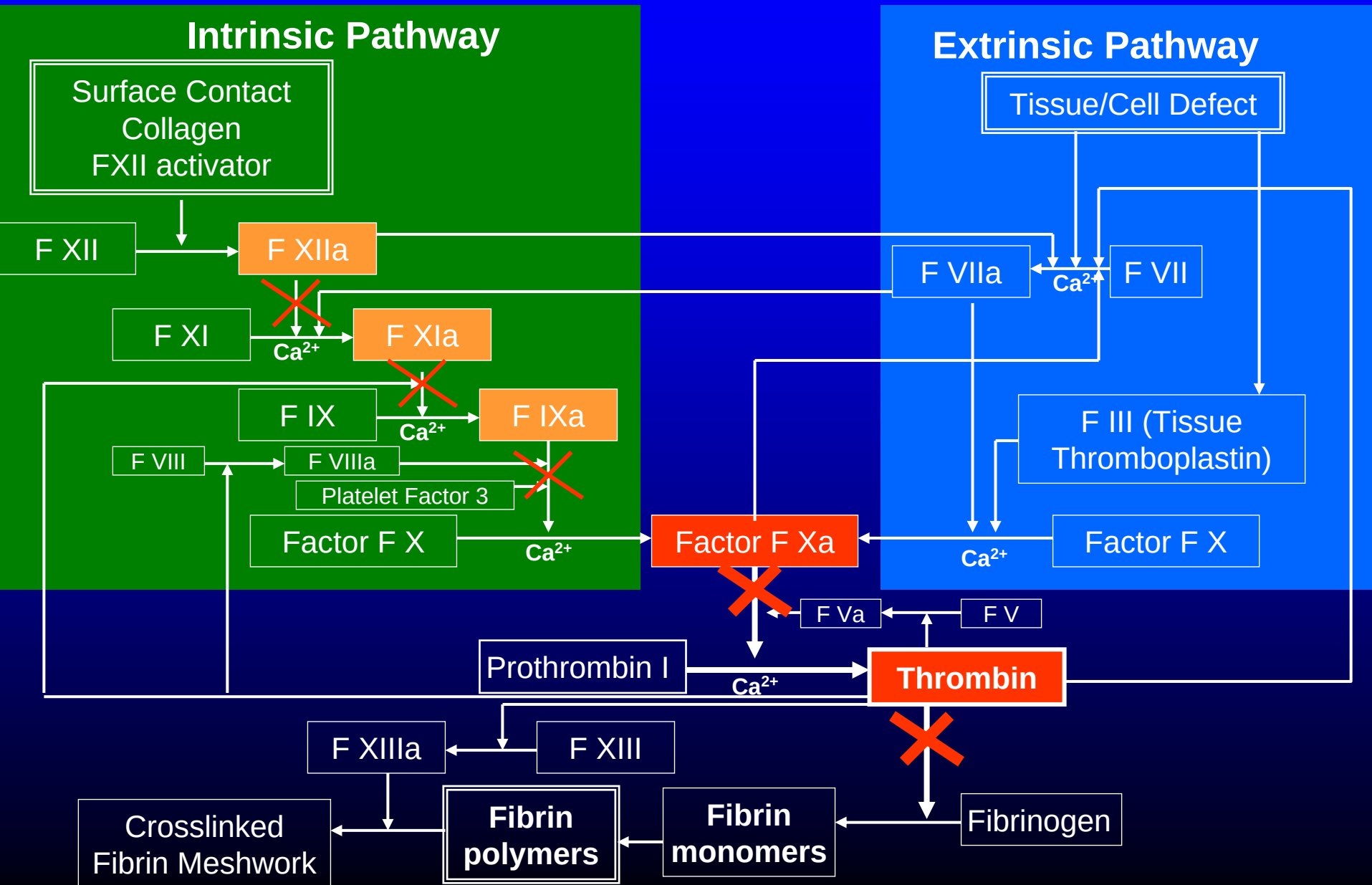
The Clotting Cascade



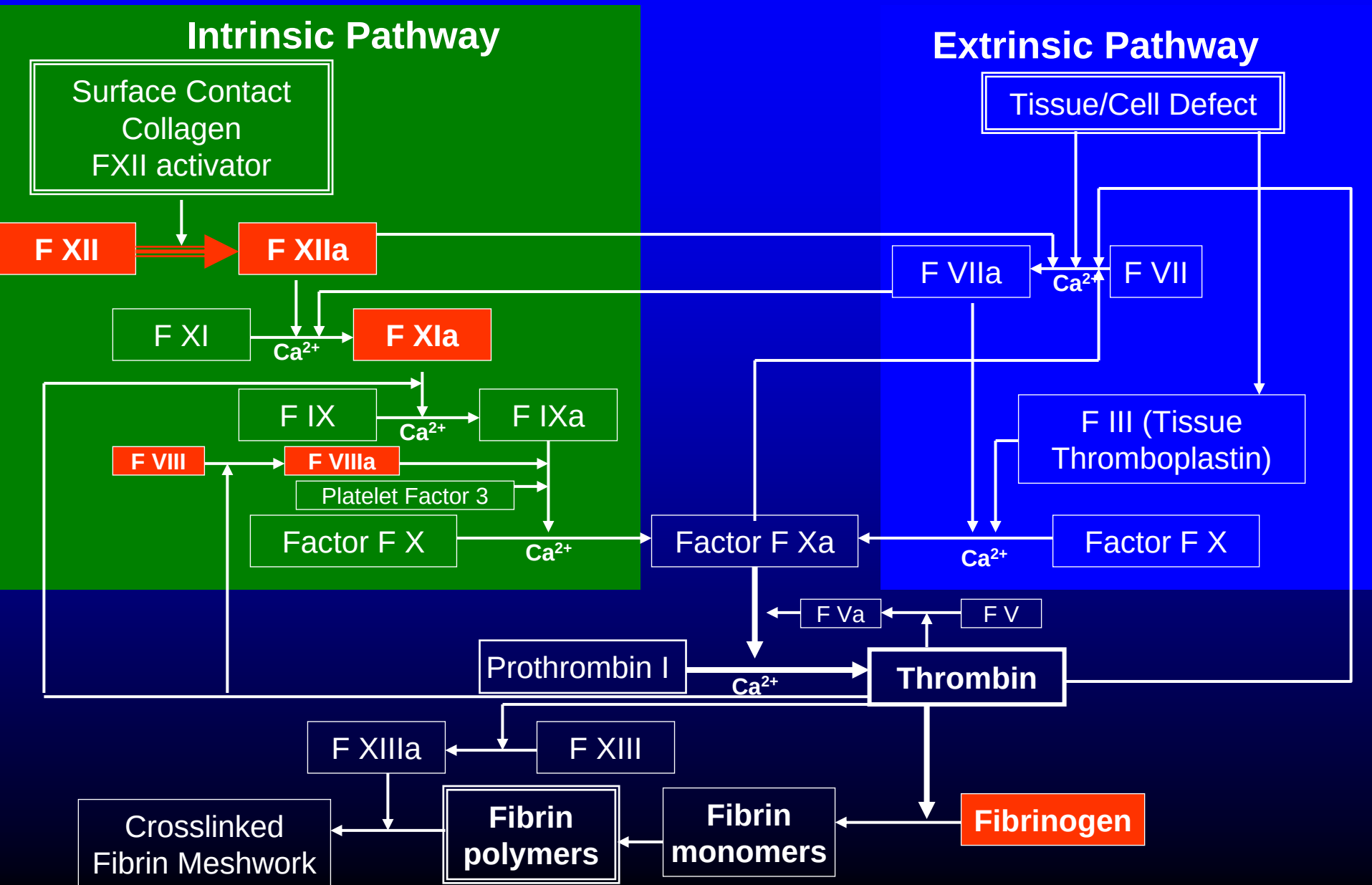
The Fibrinolytic System



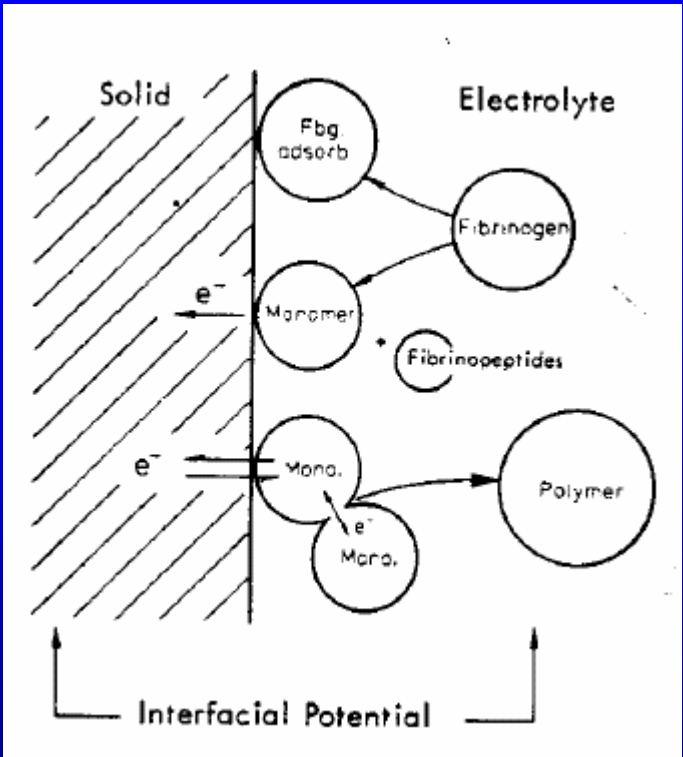
Effect of Antithrombin III/Heparin

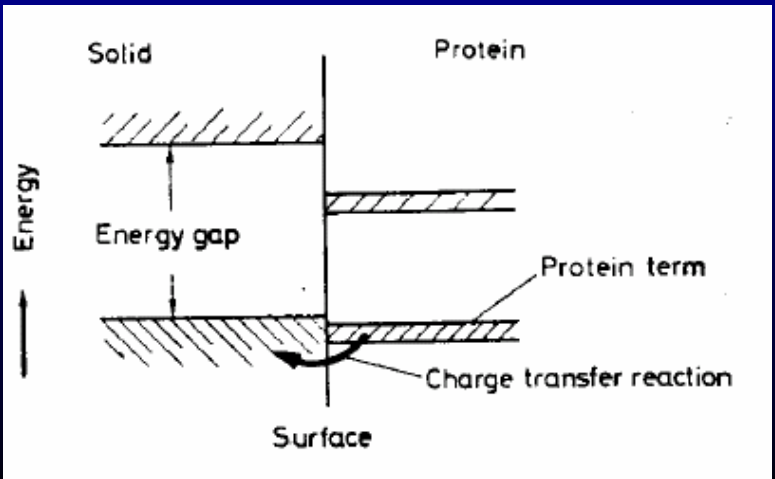


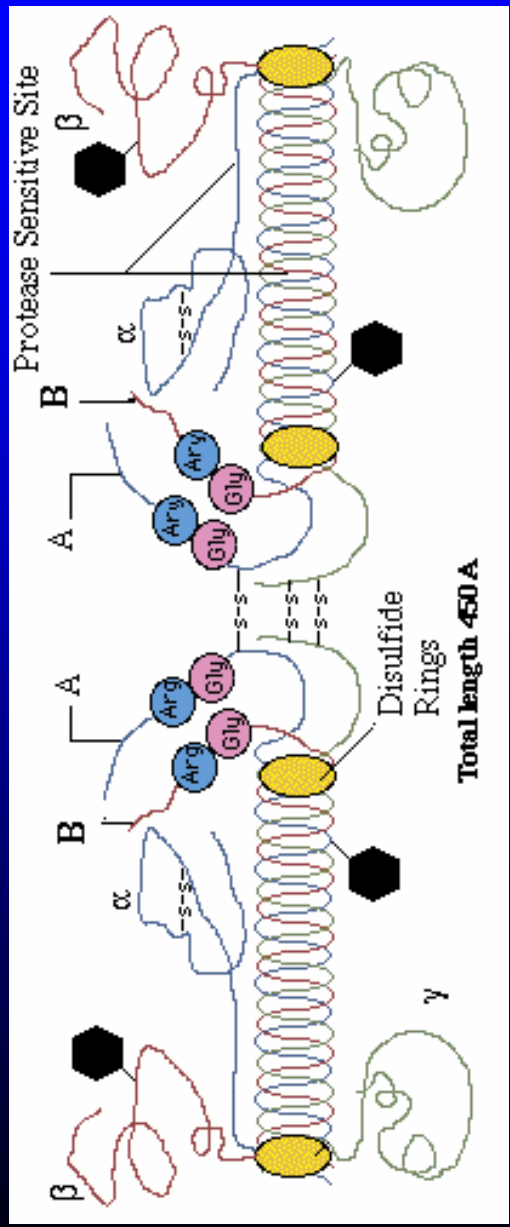
Surface Sensitive Steps



Physical Model of Fibrinogen Activation

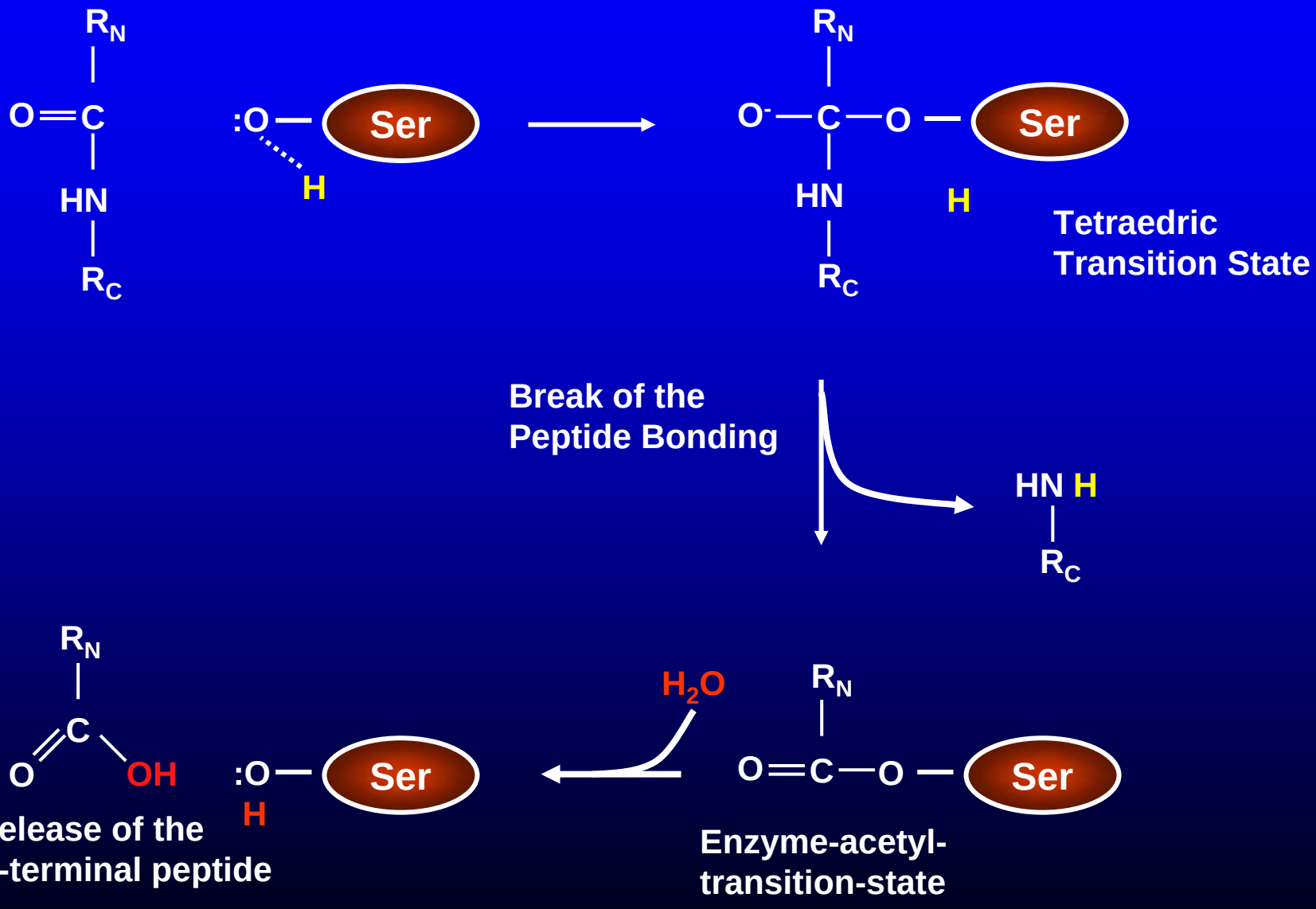






Source: Baurschmidt & Schaldach, *Med Biol Eng Comput* **18**: 496-502 (1980)

Serine Proteases

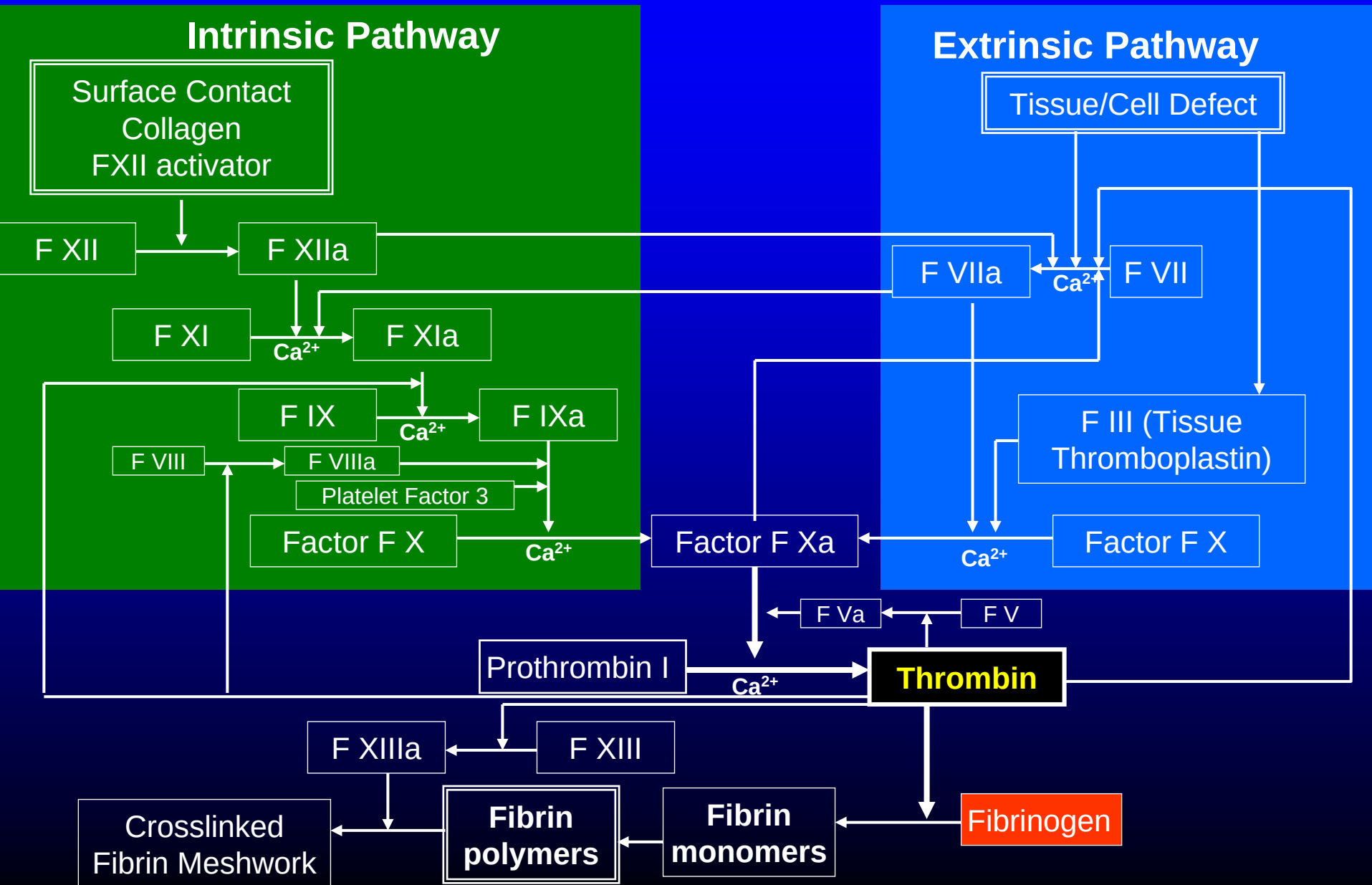


Testing of the Clotting Cascade

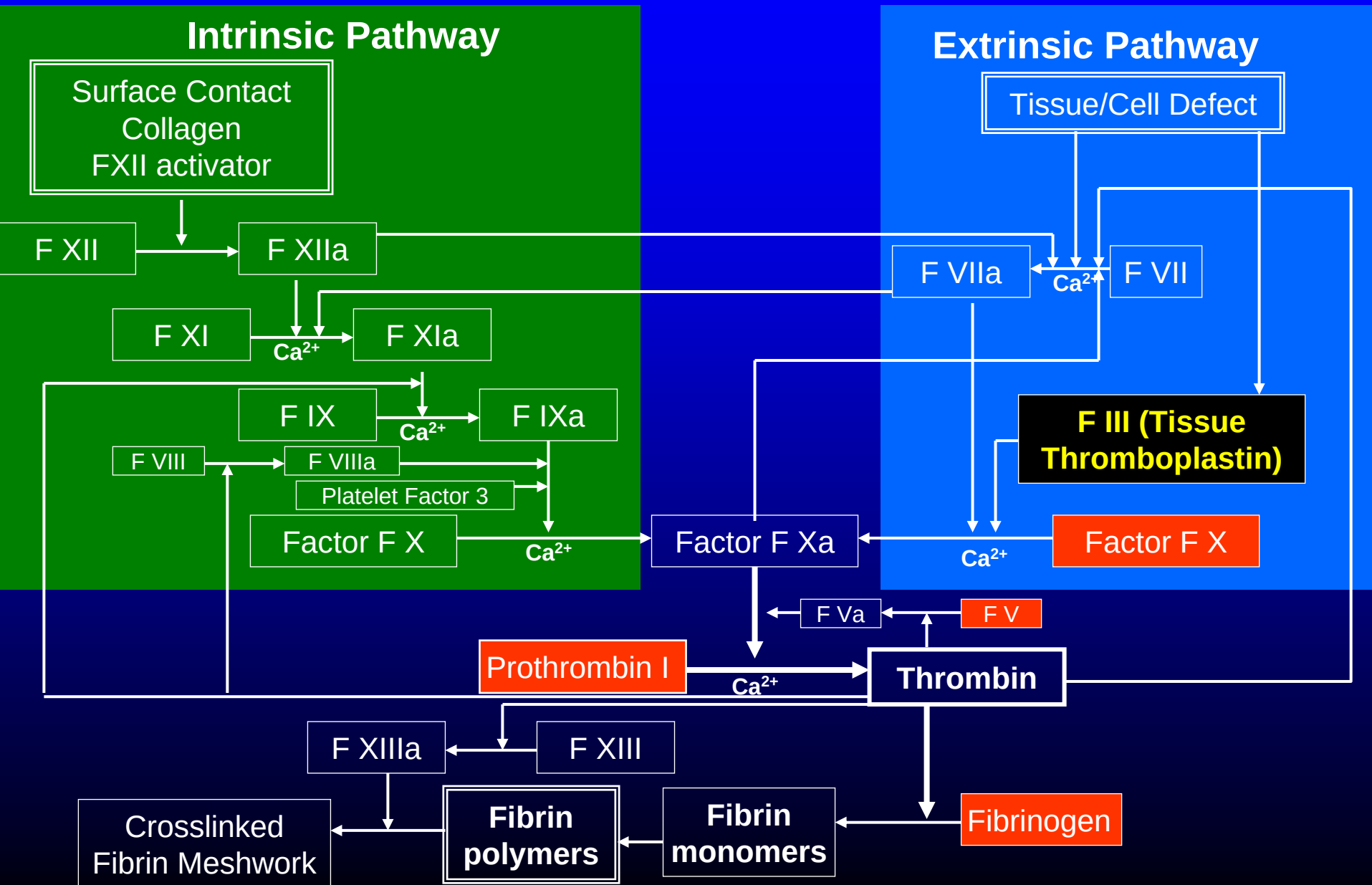
Tests of the Clotting Cascade

- Global Test: Clotting Time
  - Start the clotting cascade by addition of $CaCl_2$
  - Stir the sample and measure the time until a fibrin clot is formed (visually, change of viscosity, turbidimetry (change of transparence)
  - $\Rightarrow$ Can be performed with the plasma on the test material or after some incubation of the plasma with the sample
- Modified Global Tests
  - F1+2 fragment: Fragment of prothrombin, which is cleaved off during activation
  - TAT (Thrombin-antithrombin) complex: Further downstream indicator of activated thrombin
- Amount/ activity of individual (pro-)factors in the plasma
  - Mix plasma with factor deficient plasma and determine clotting time
- Activity of some activated factors
  - Chromogenic substrates are available for some factors, e.g. FXIIa: Z-Lys-Phe-Arg-pNA; Thrombin: S-2238
- Clinical clotting tests (TT, PT, aPTT)
  - Mainly test some systems

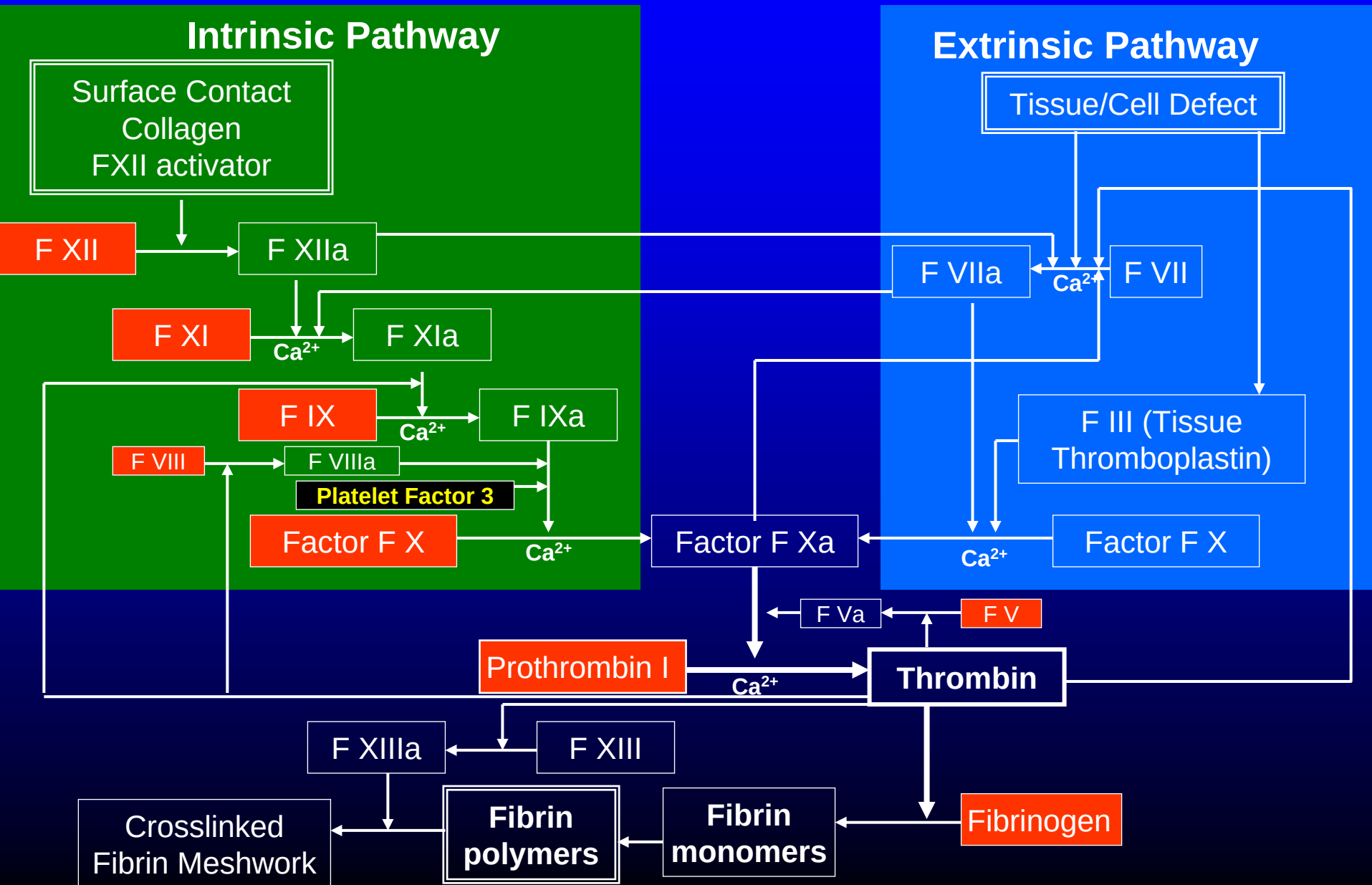
Thrombin Time (TT)



The Quick (PT)-Test



Partial Thromboplastin Time [(a)PTT]



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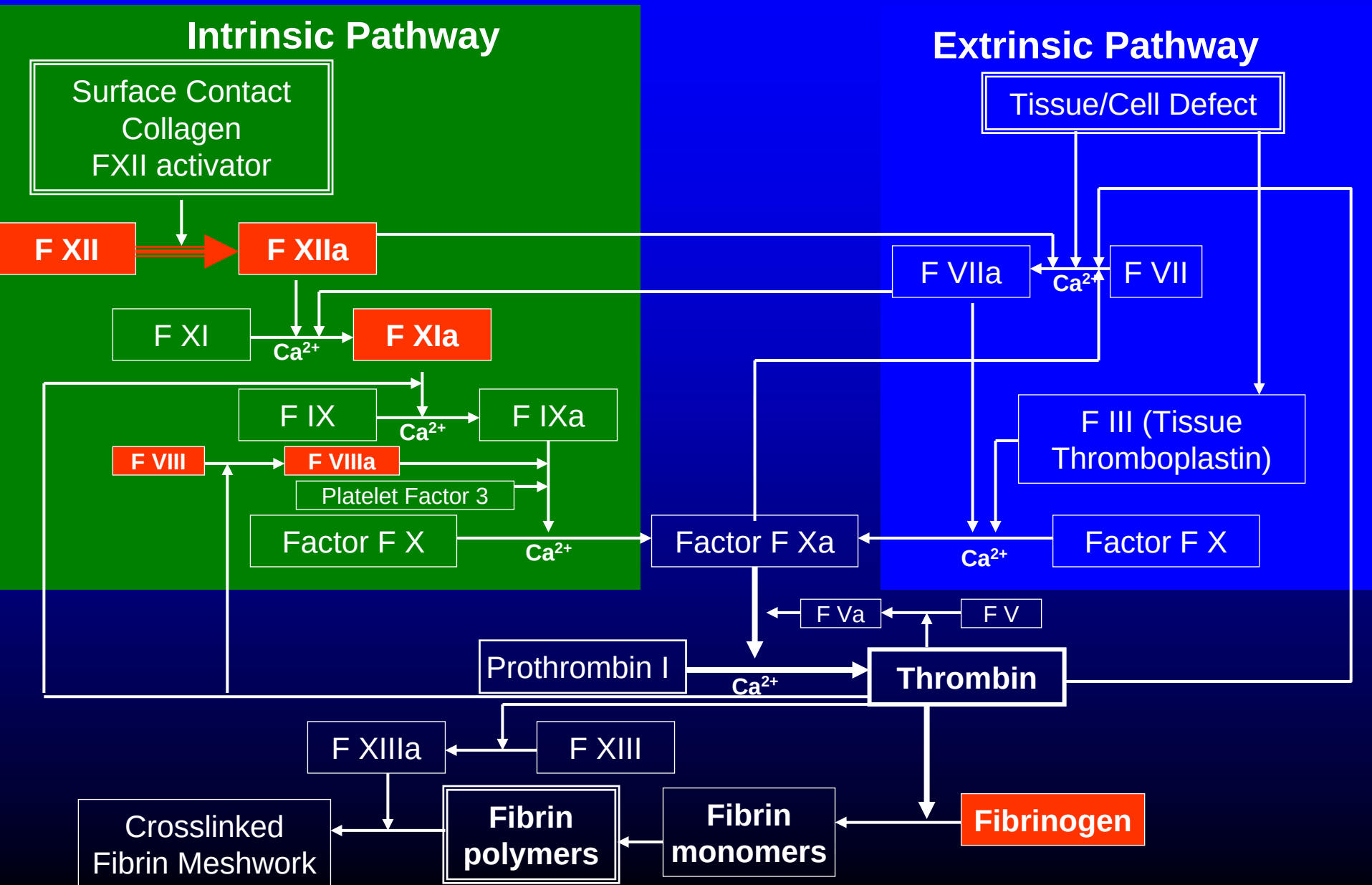
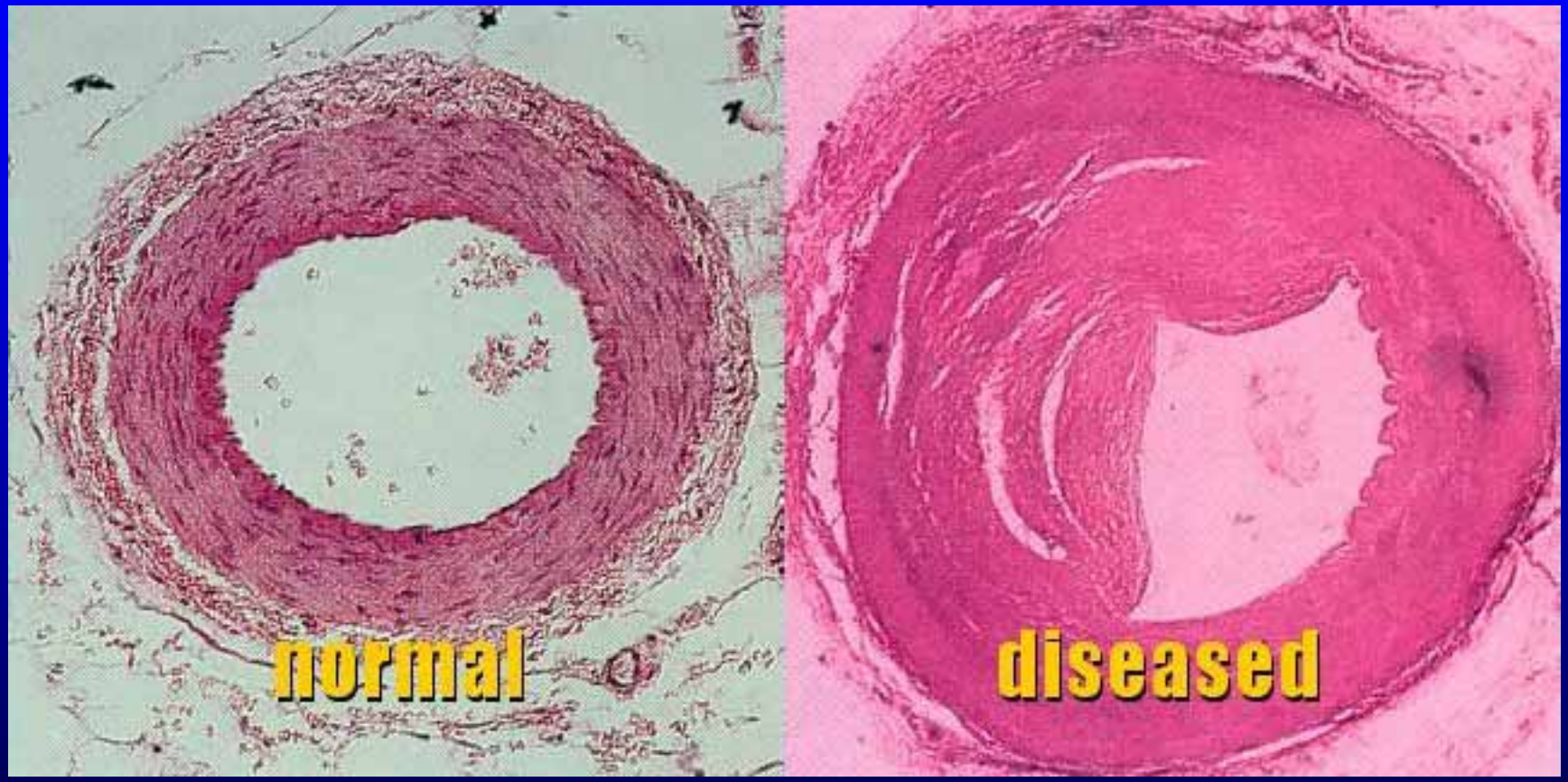


Reaction of the Vessel Wall

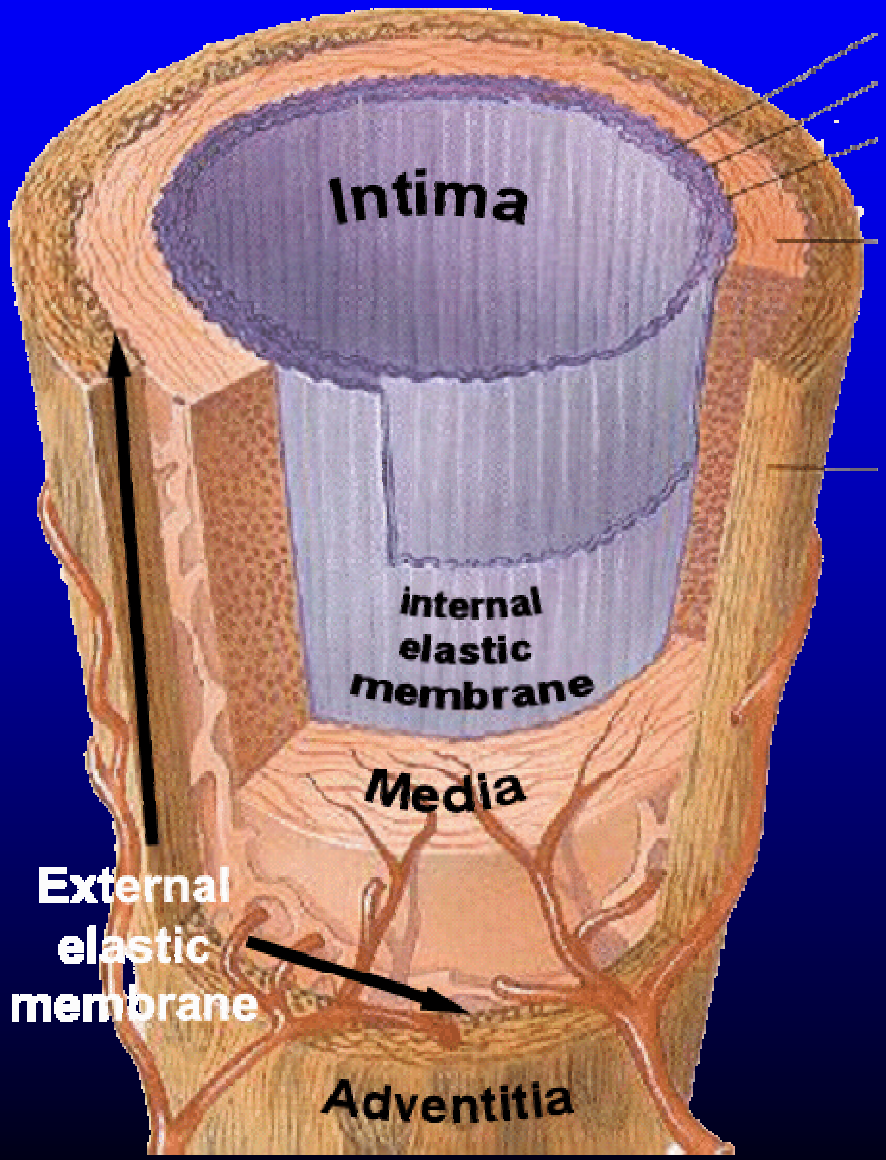
Blood Vessel Cells

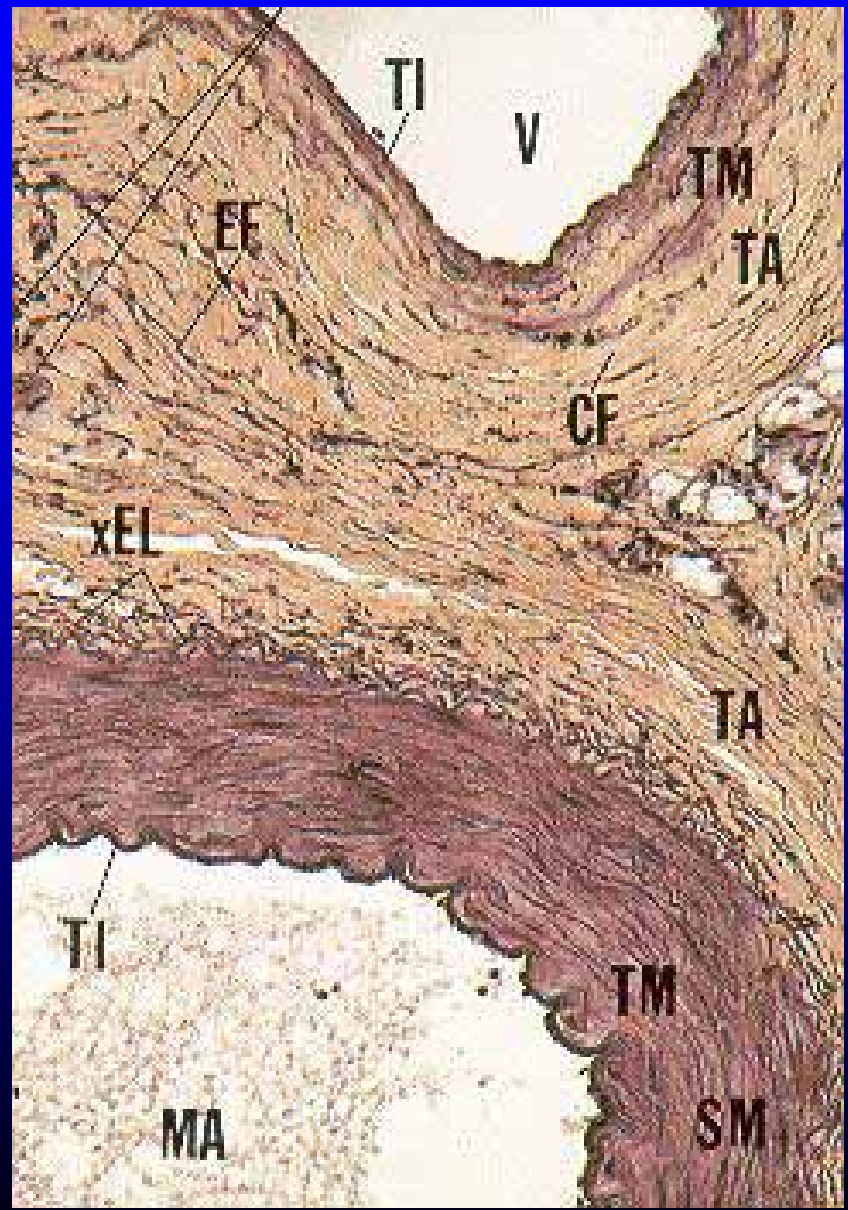


Neo-Intima

Smooth Muscle Cell-Proliferation

Blood Vessel Anatomy



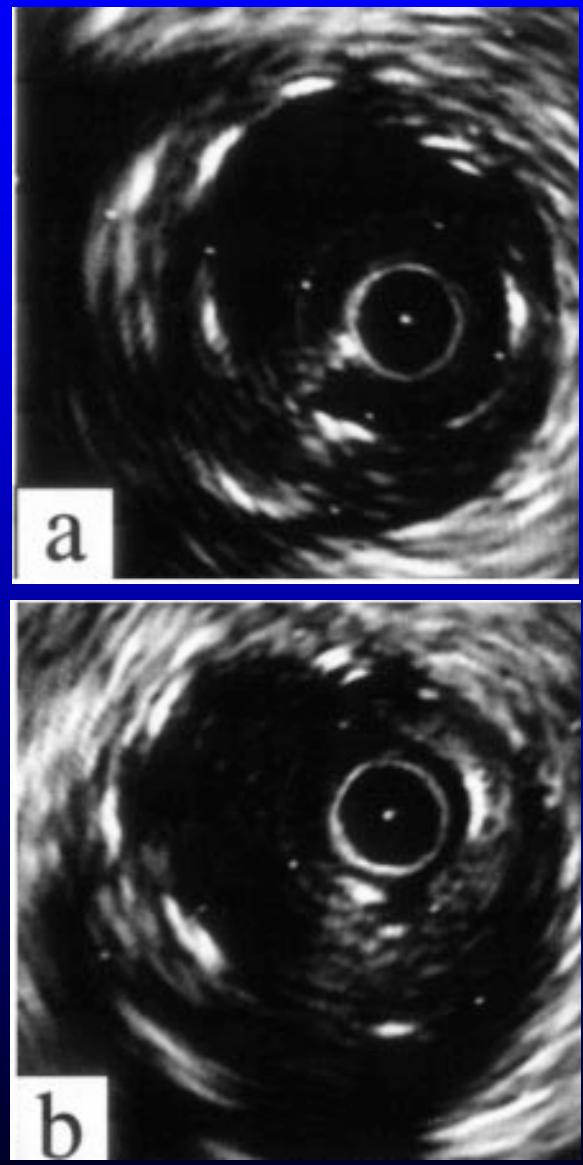


(Re-)Stenosis Evaluation

- Angiography: X-Ray with contrast media
  - Length and extent of the stenosis
- Intravascular ultrasound
- Histology

(Re-)Stenosis Evaluation

- Angiography: X-Ray with contrast media
- Intravascular ultrasound
  - Wall thickness
  - (Length of stenosis)
- Histology



(Re-)Stenosis Evaluation

- Angiography: X-Ray with contrast media
- Intravascular ultrasound
- Histology
  - Wall thickness
  - Measurement of intima and media area
  - Scores for injury or stenosis
  - Inflammatory cells
  - Fibrin deposition
  - Immunochemistry
    - Cell types, proliferation markers, various cytokines

Endothelial Cells

Resting State

- Phospholipids on the surface → optimum blood compatible surface

Stimuli for Activation

- Inflammatory cytokines (IL-1, IL-6, TNF etc)
- Thrombin (Proteinase Activated Receptor PAR-1)
- Low Density Lipoprotein (LDL-Cholesterol)
- Mechanically (?)

Consequences of Activation

- Expression of E-selectins
  - Leukocyte rolling and adhesion and penetration
- Expression of von Willebrand Factor (vWF)
  - Platelet adhesion and activation
- Production of NO
  - Relaxation of vascular smooth muscle cells

Antibody techniques

Nitric Oxide

- NO is a free radical with extremely short lifetime
- NO has very many signaling functions
- NO is produced by Nitric Oxide Synthase (NOS) in an oxidation of L-arginine to L-citrulline
  - Neuronal NOS: nNOS
  - Inducible NOS: iNOS
  - Endothelial NOS: eNOS
- **Detection**
  - NO sensitive electrodes
  - Measurement of nitrate as stable end product of NO
    - Griess Reaction -> photometrical detection
    - 4,5-diaminofluorescein -> fluorescent detection
  - Molecular biology, RNA-methods for iNOS

Smooth Muscle Cells

- Usual cells in the Tunica Media
- At inflammatory processes (arteriosclerosis, mechanical irritation) they proliferate forming a neo-*intima*
- Restenosis tissue is 30% smooth muscle cells, the rest are inflammatory cells
- Analysis
  - Characteristic protein: smooth muscle cell actin
  - Proliferation markers
  - Inflammatory cytokines
  - Growth factors
    - Transforming growth factor beta (TGF-β) provokes SMC growth
    - Vascular endothelial growth factor (VEGF) induces endothelialization and inhibits SMC growth