



Verworven stollingsinhibitoren

Marc Jacquemin

Erfelijke stollingsziekten

Routine stollingstesten

Genetische analyse

<https://doi.org/10.1016/j.jtha.2022.12.007>

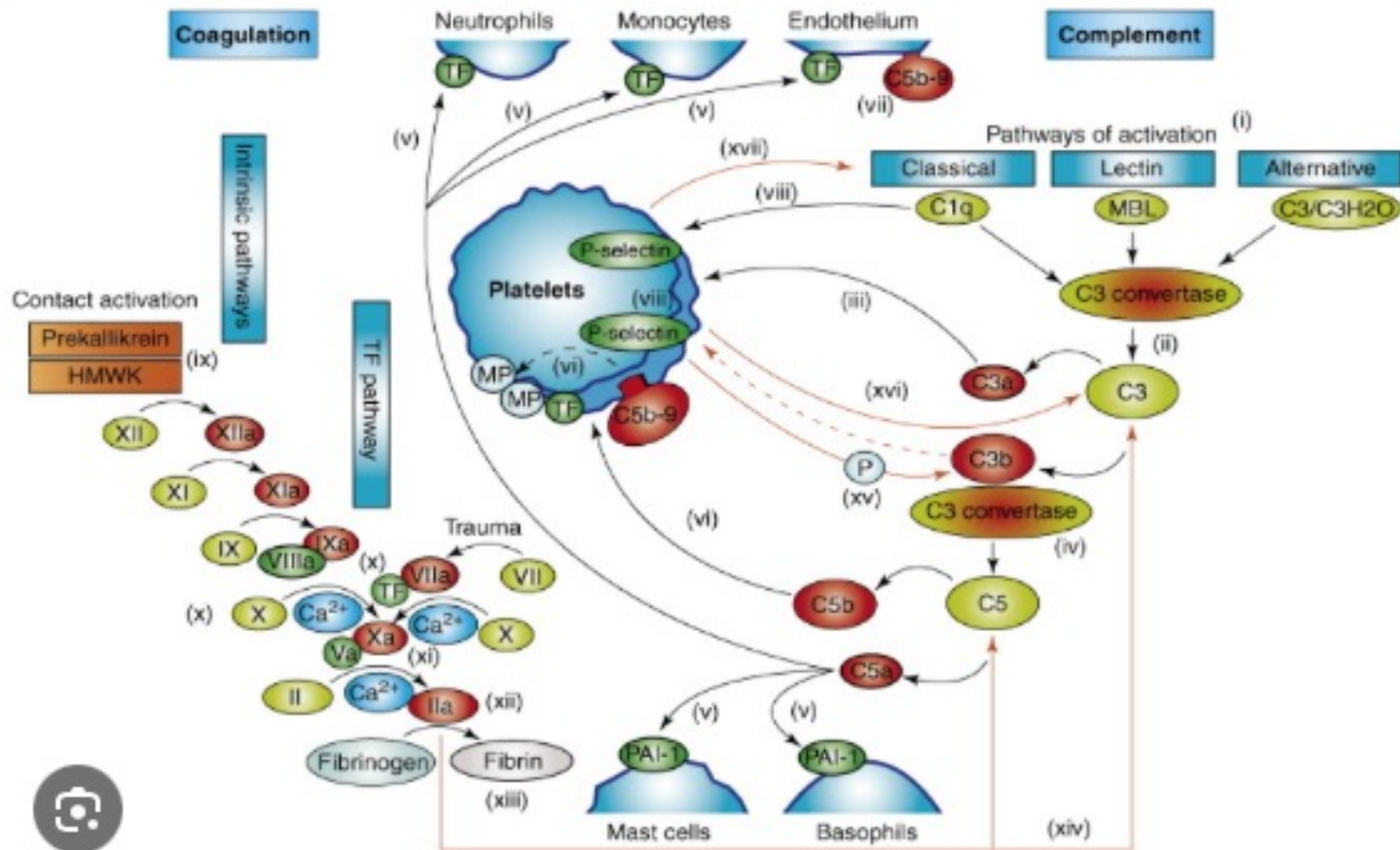
BRIEF REPORT

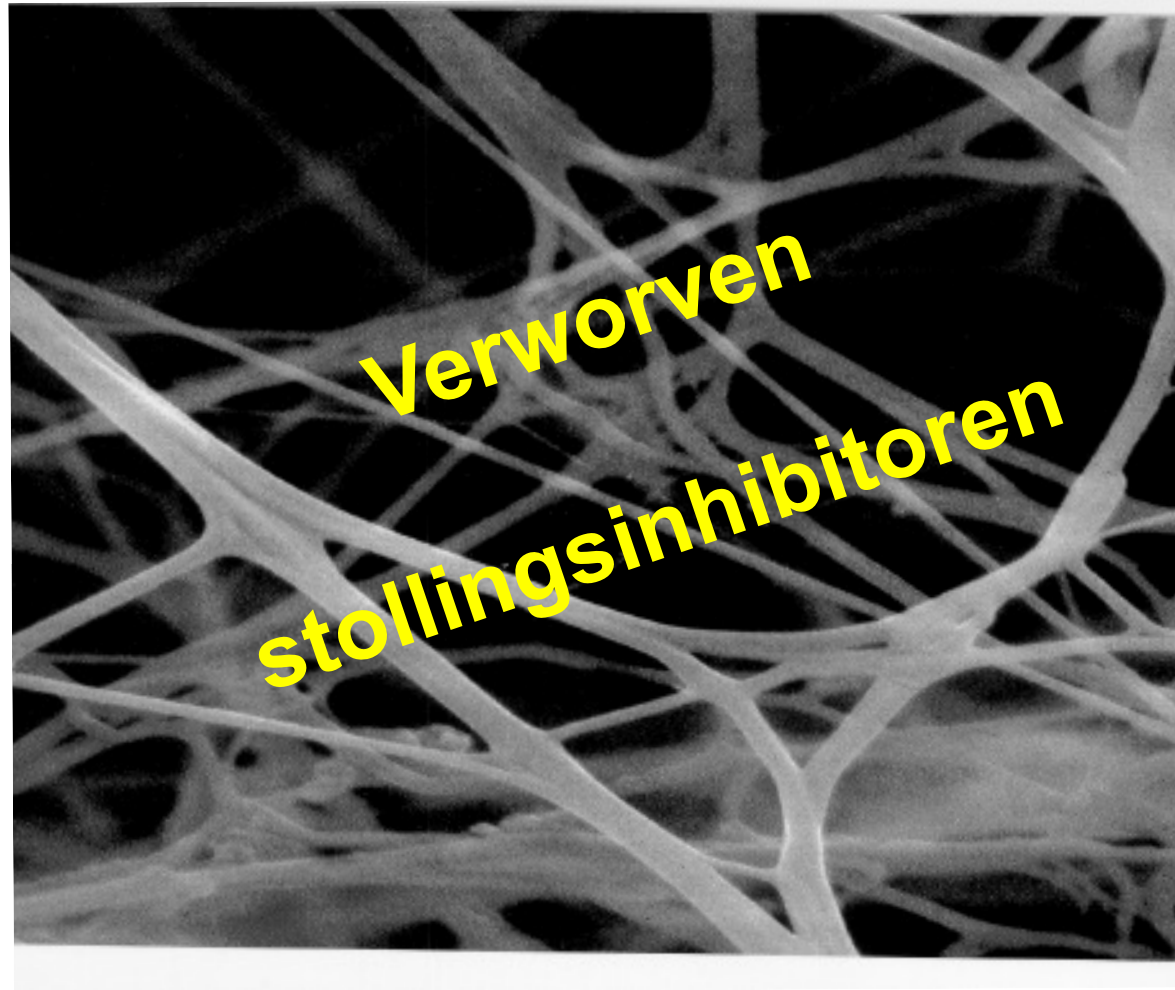
Clinical application of multigene panel testing for bleeding, thrombotic, and platelet disorders: a 3-year Belgian experience

jth

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Verworven stollingsziekten







- **hemofilie A**
- **behandeld met FVIII**
- **antistoffen tegen FVIII**



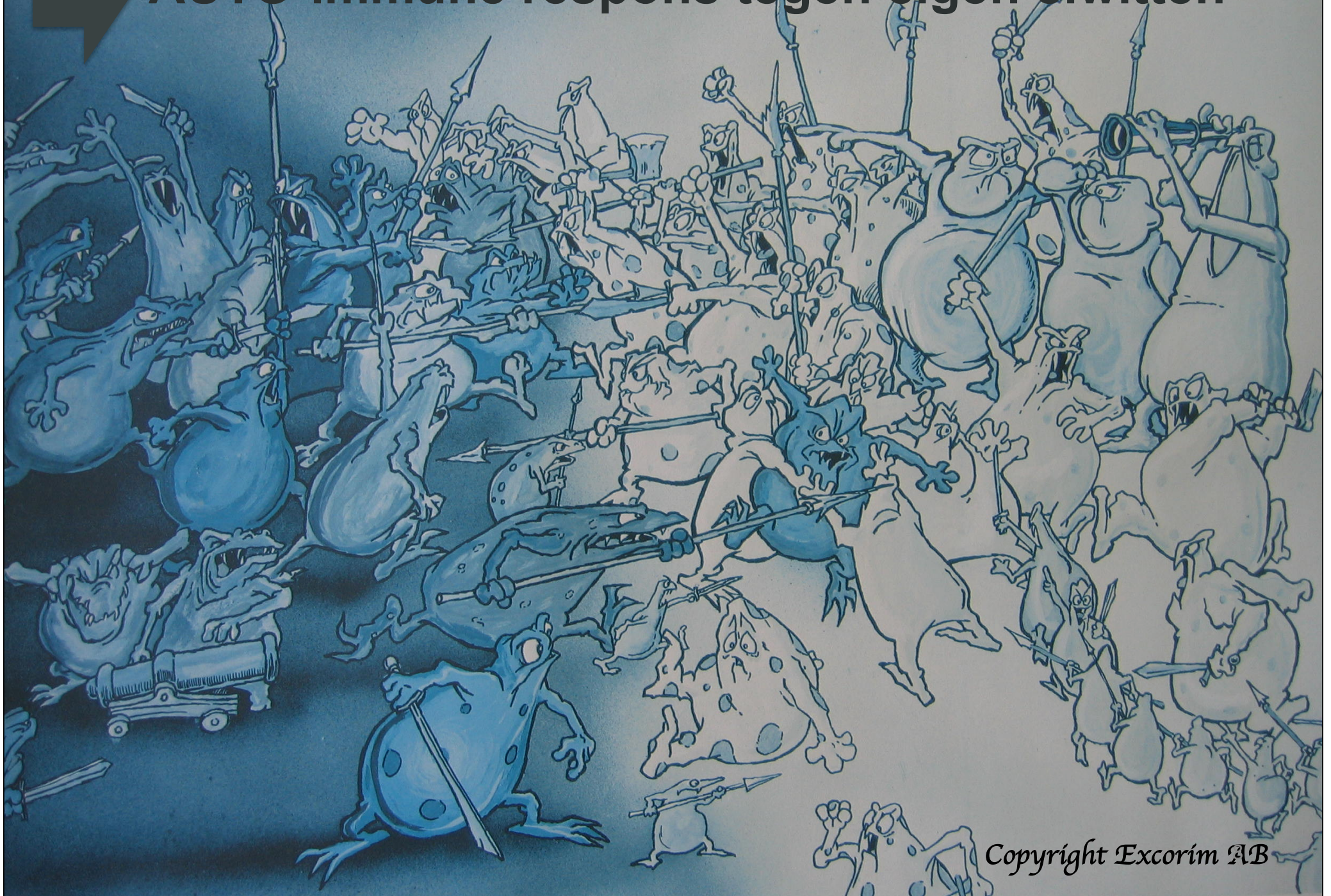
**ALLO-immune respons tegen
toegediende stollingsfactoren**





**GEEN mutatie
in de genen
van stollingsfactoren**

AUTO-immune respons tegen eigen eiwitten



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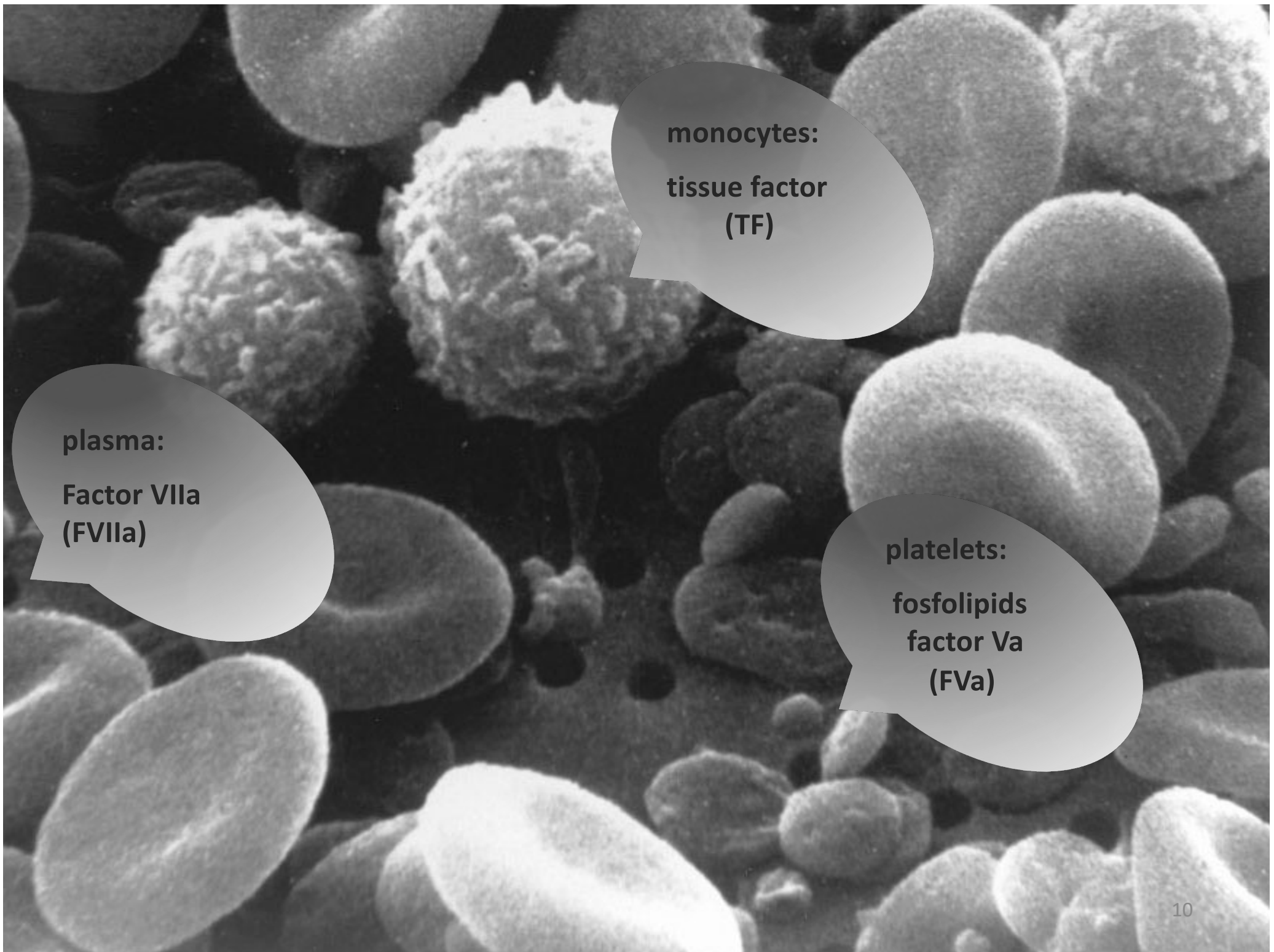
AUTO-immune respons tegen eigen eiwitten



antistoffen tegen stollingsfactoren



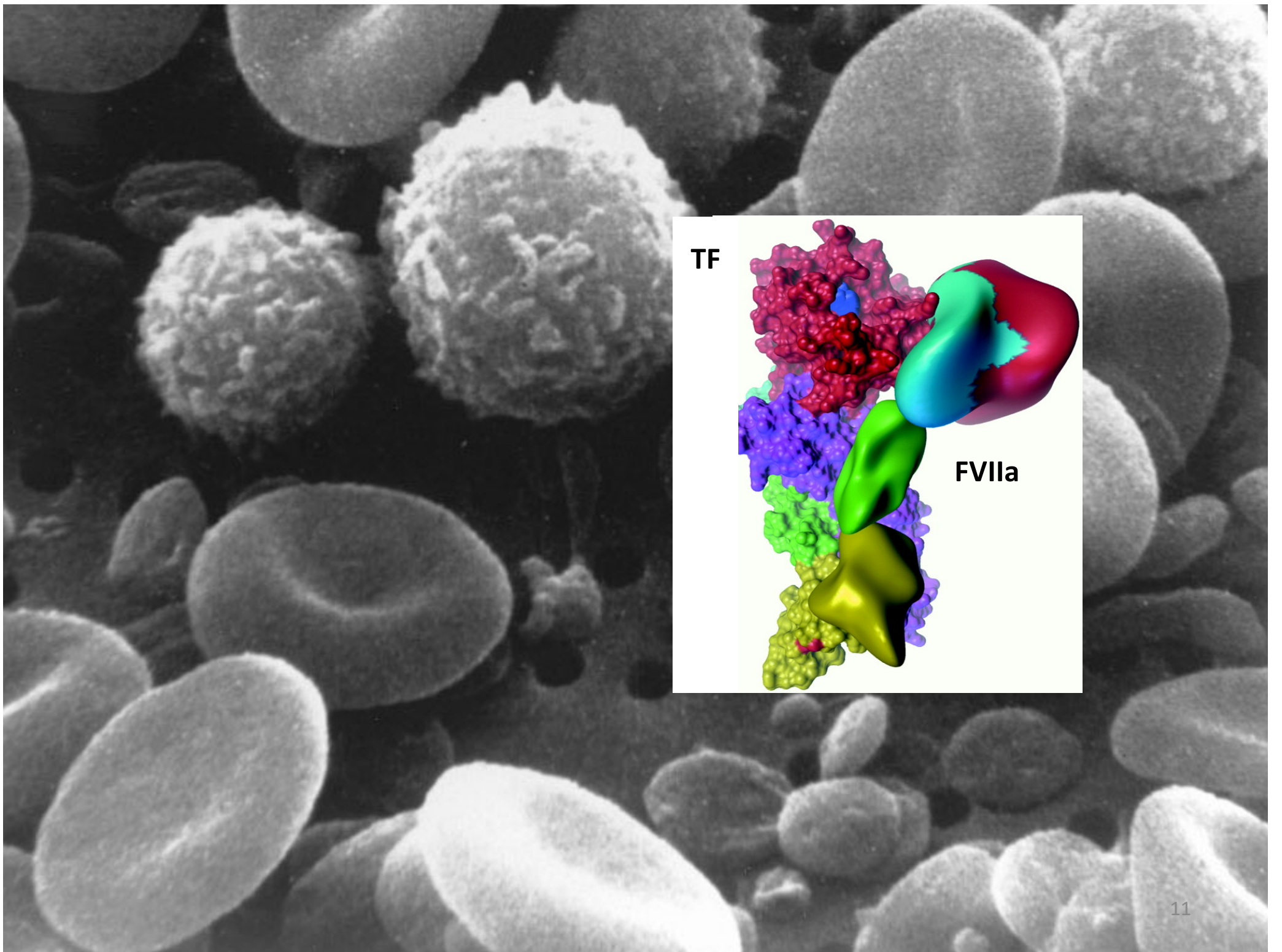
Lupus anticoagulans



**monocytes:
tissue factor
(TF)**

**plasma:
Factor VIIa
(FVIIa)**

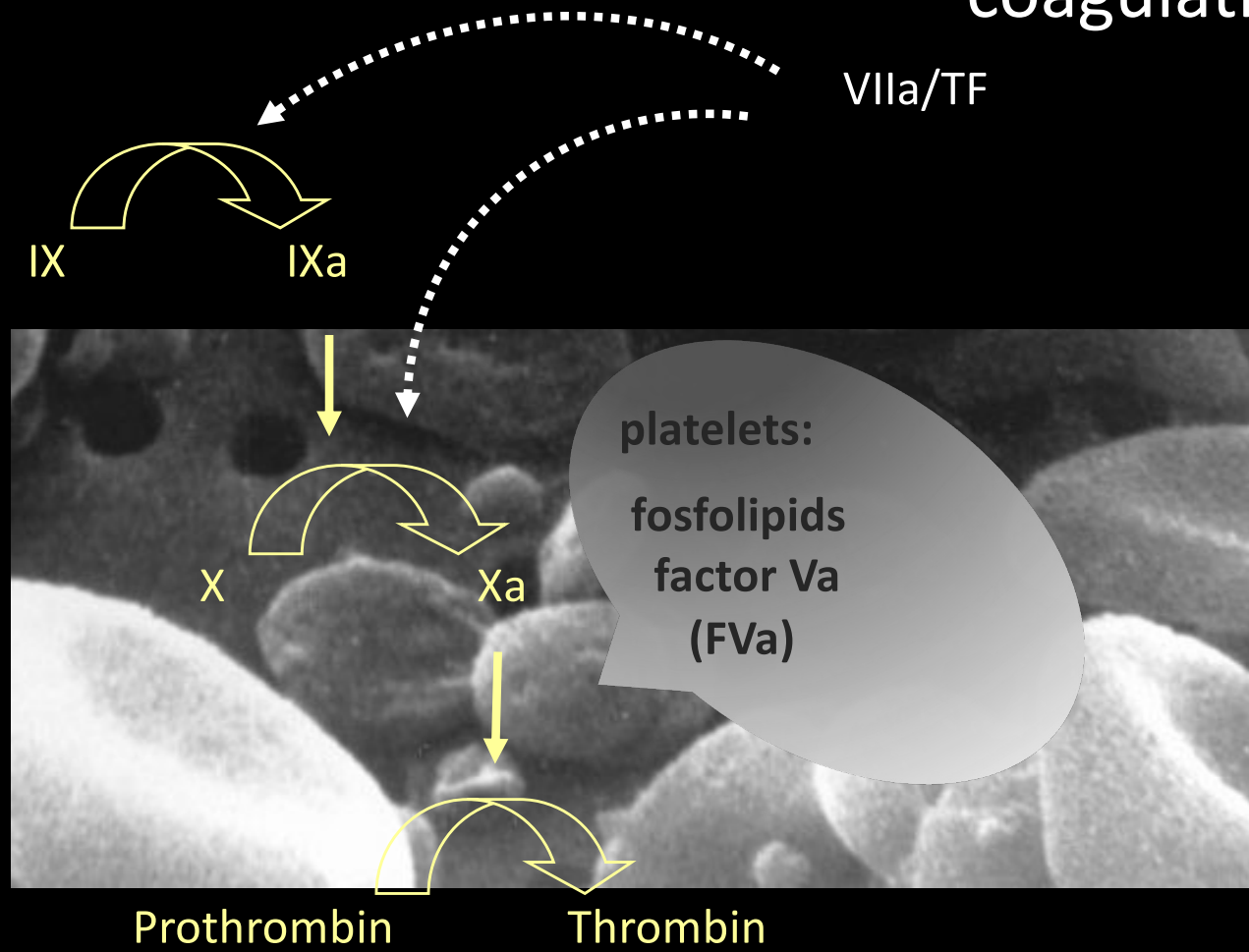
**platelets:
fosfolipids
factor Va
(FVa)**



TF

FVIIa

Initiation of coagulation



Initiation of coagulation

VIIa/TF

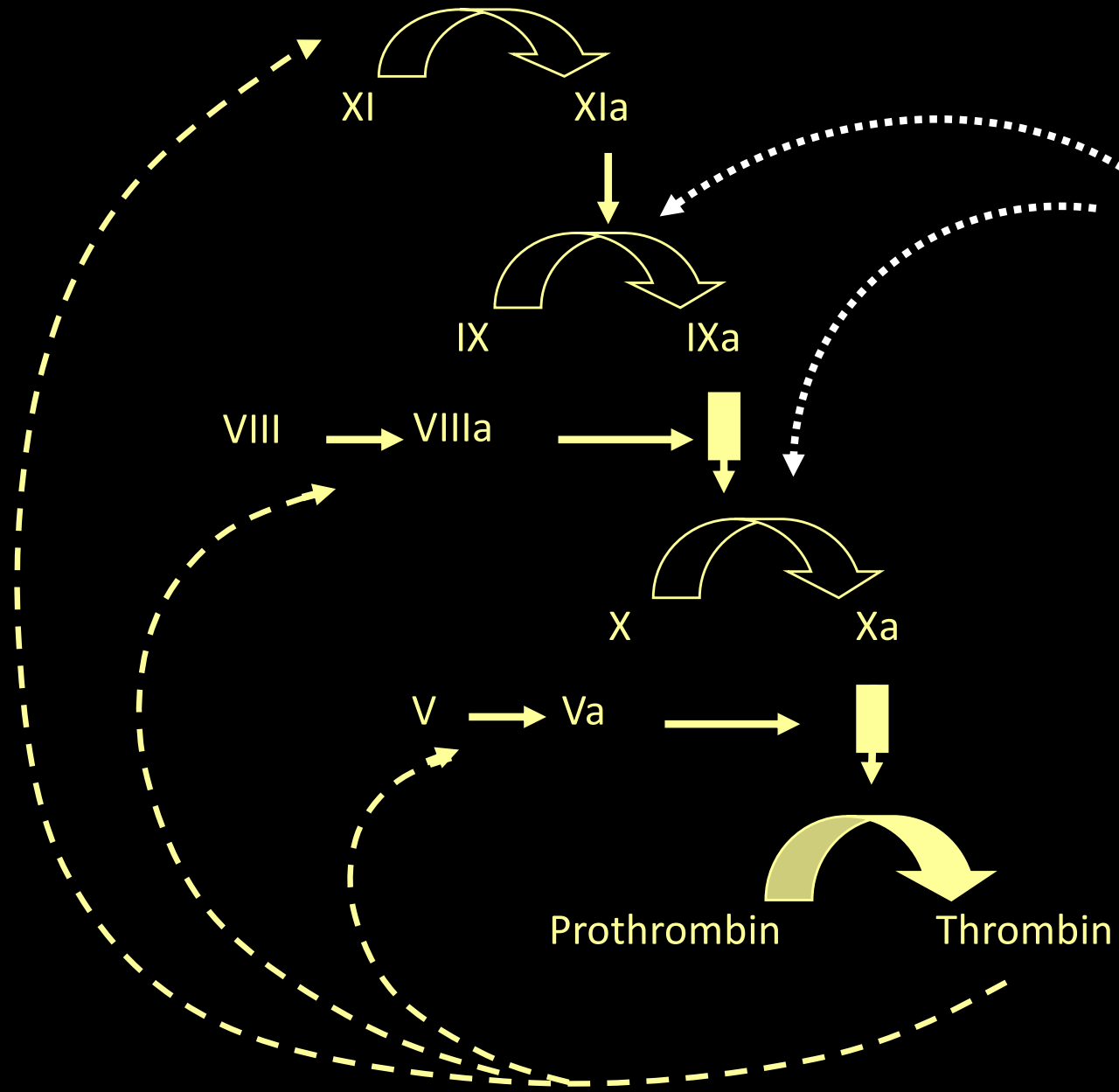
Sustained hemostasis

FXIII

Fibrinogen

Fibrin

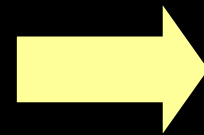
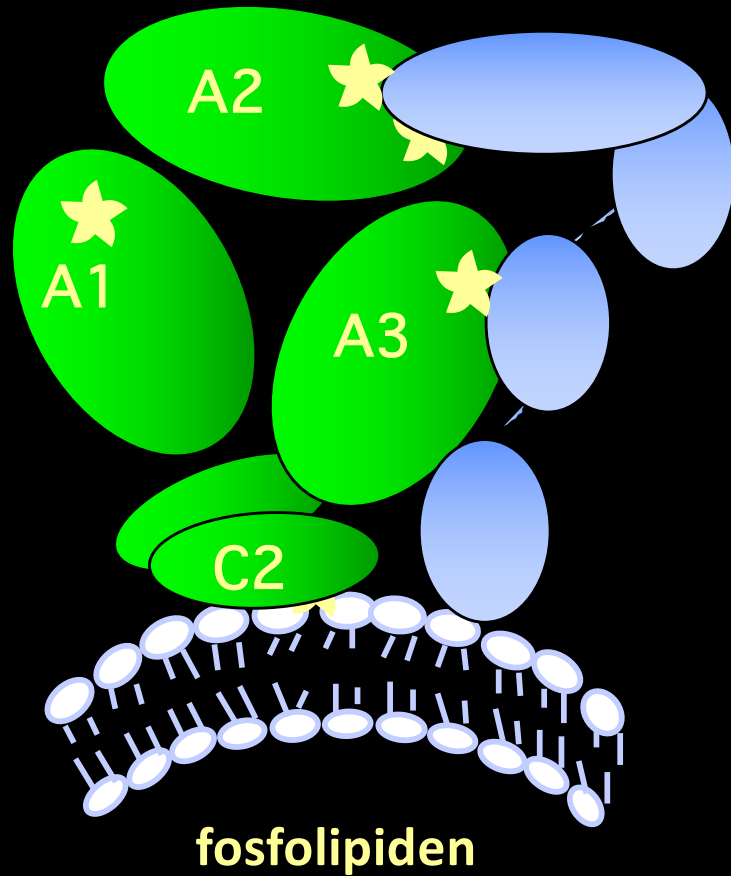
Amplification



Geactiveerde Factor VIII als cofactor van Factor IXa

Factor VIIIa

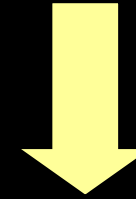
Factor IXa



Factor X



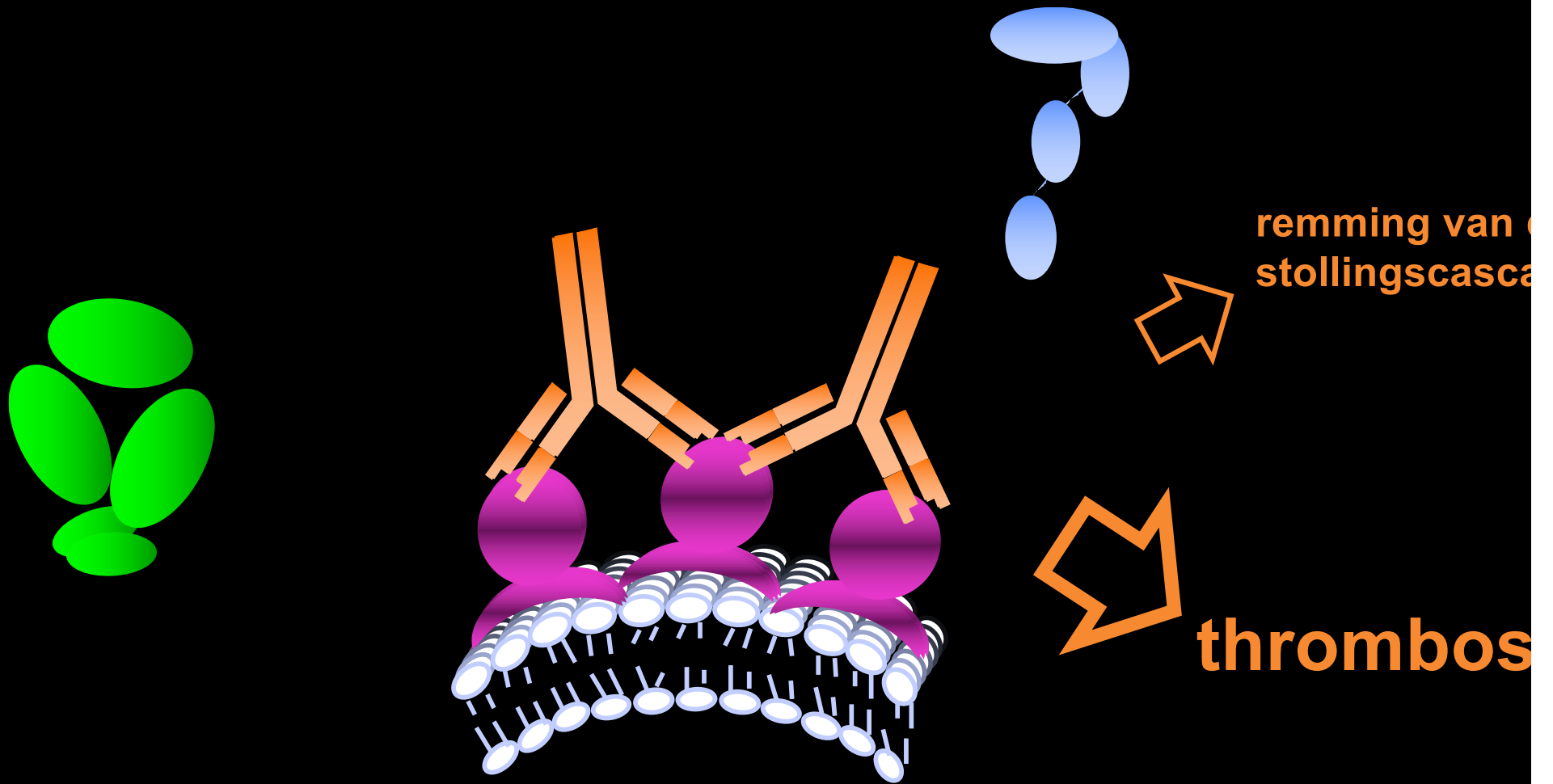
Factor Xa



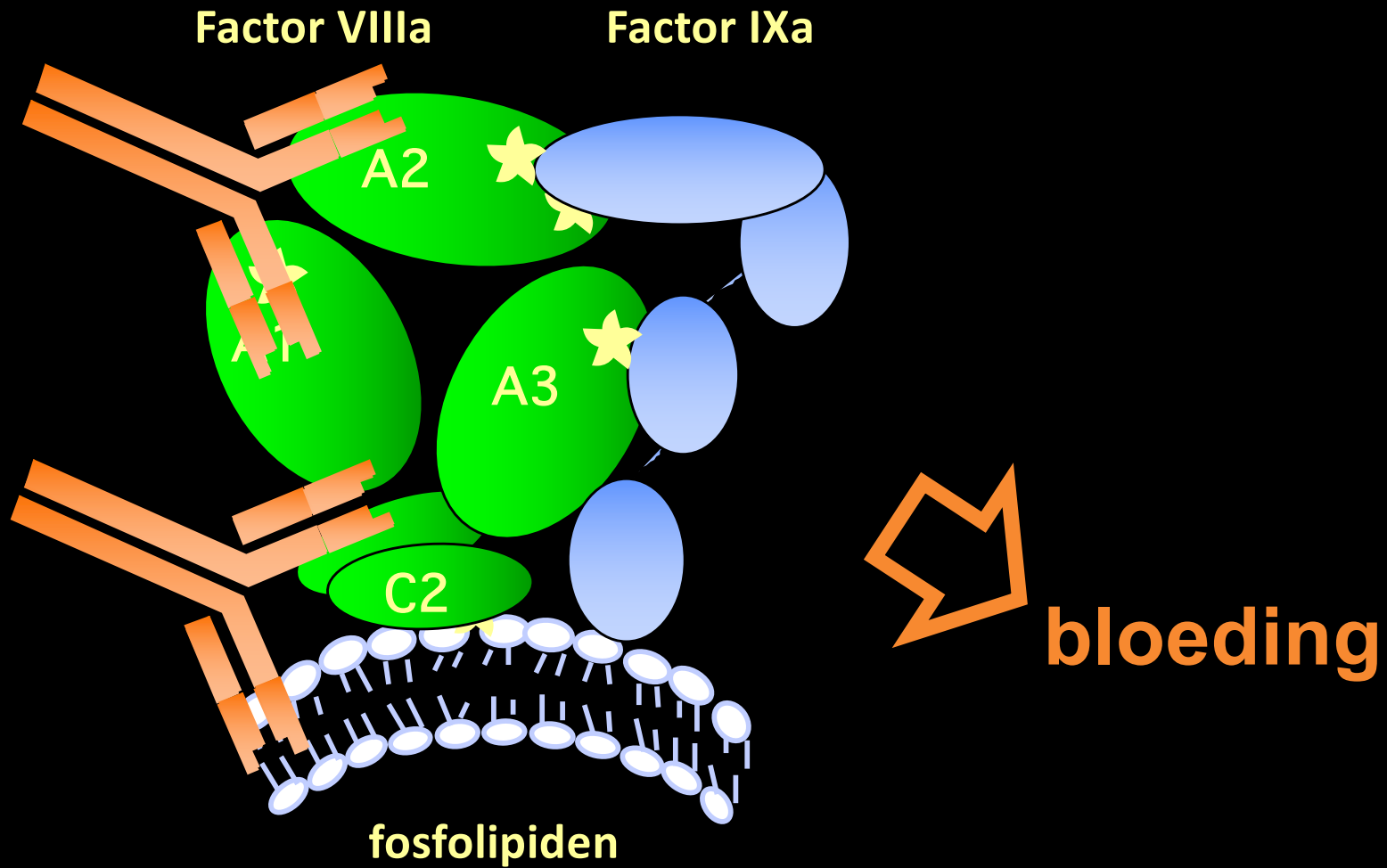
Hemostase

X 100.000 door Factor VIII

Anti-phospholipid antibodies



Verworven FVIII inhibitor





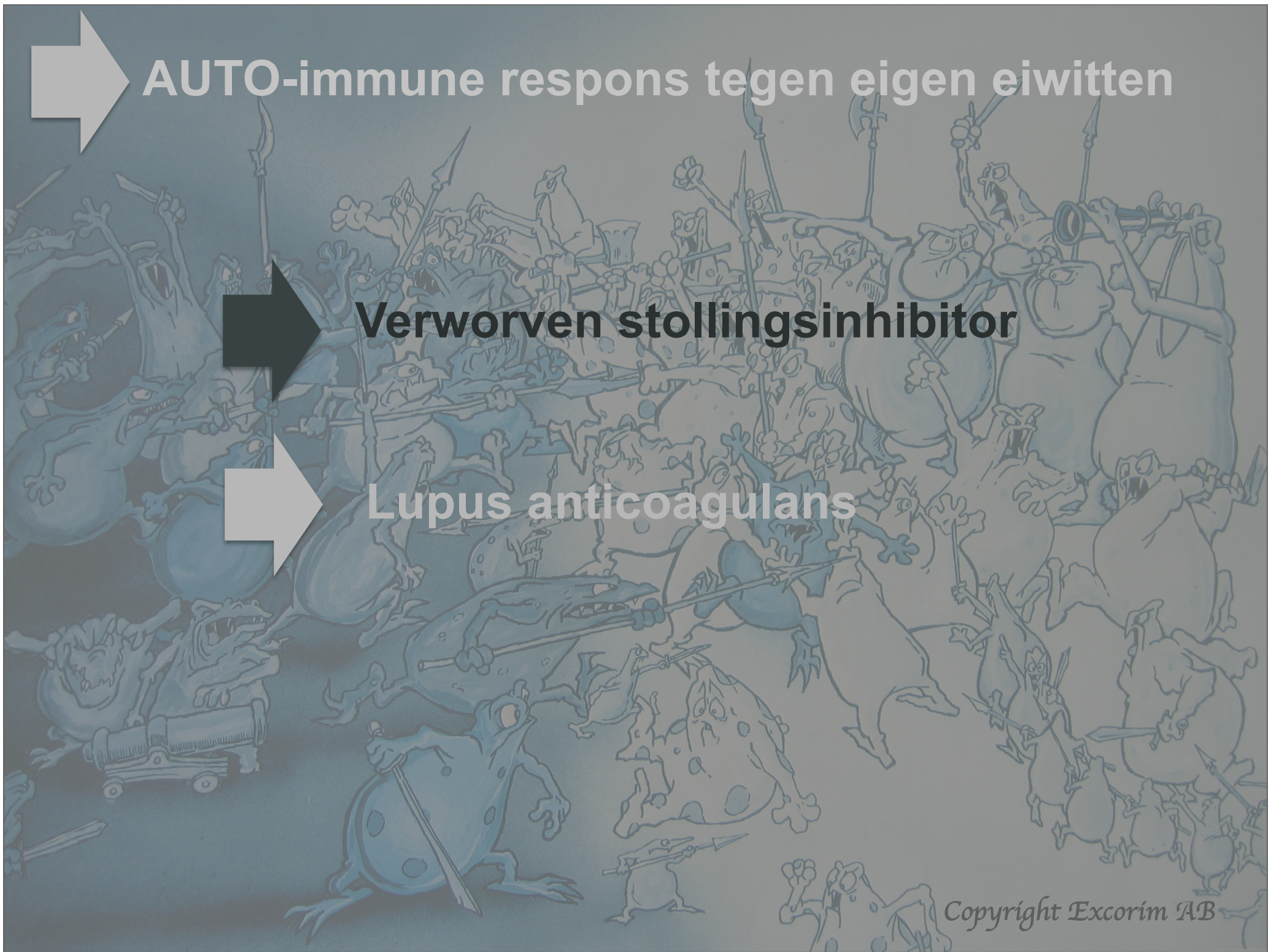
AUTO-immune respons tegen eigen eiwitten



Verworven stollingsinhibitor



Lupus anticoagulans



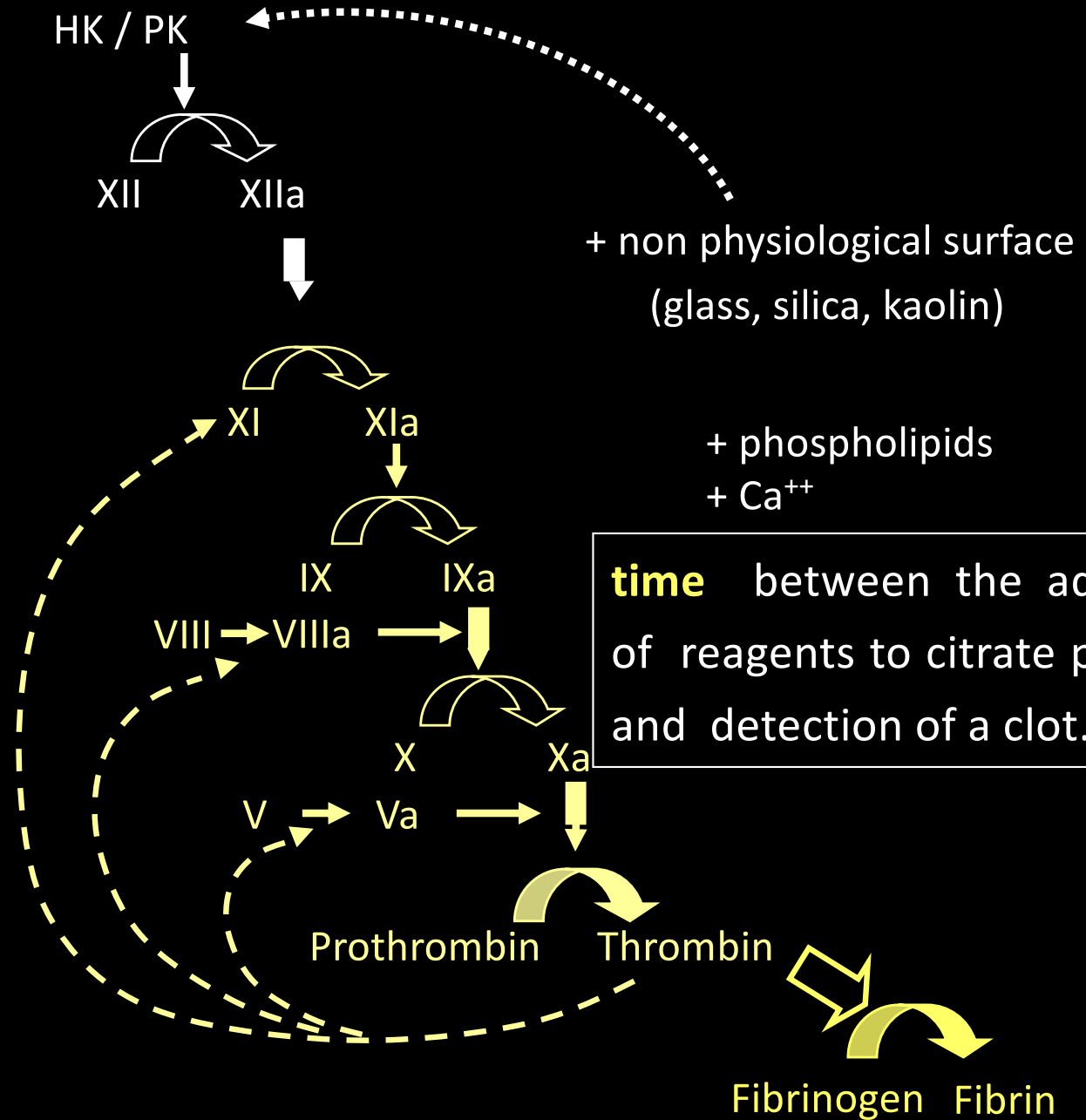


- **Senior**
- **uitgebreide hematomen op de rug en de benen sinds 10 dagen**
- **Hb: 10,4**
- **verlengde APTT**
- **normale PT**

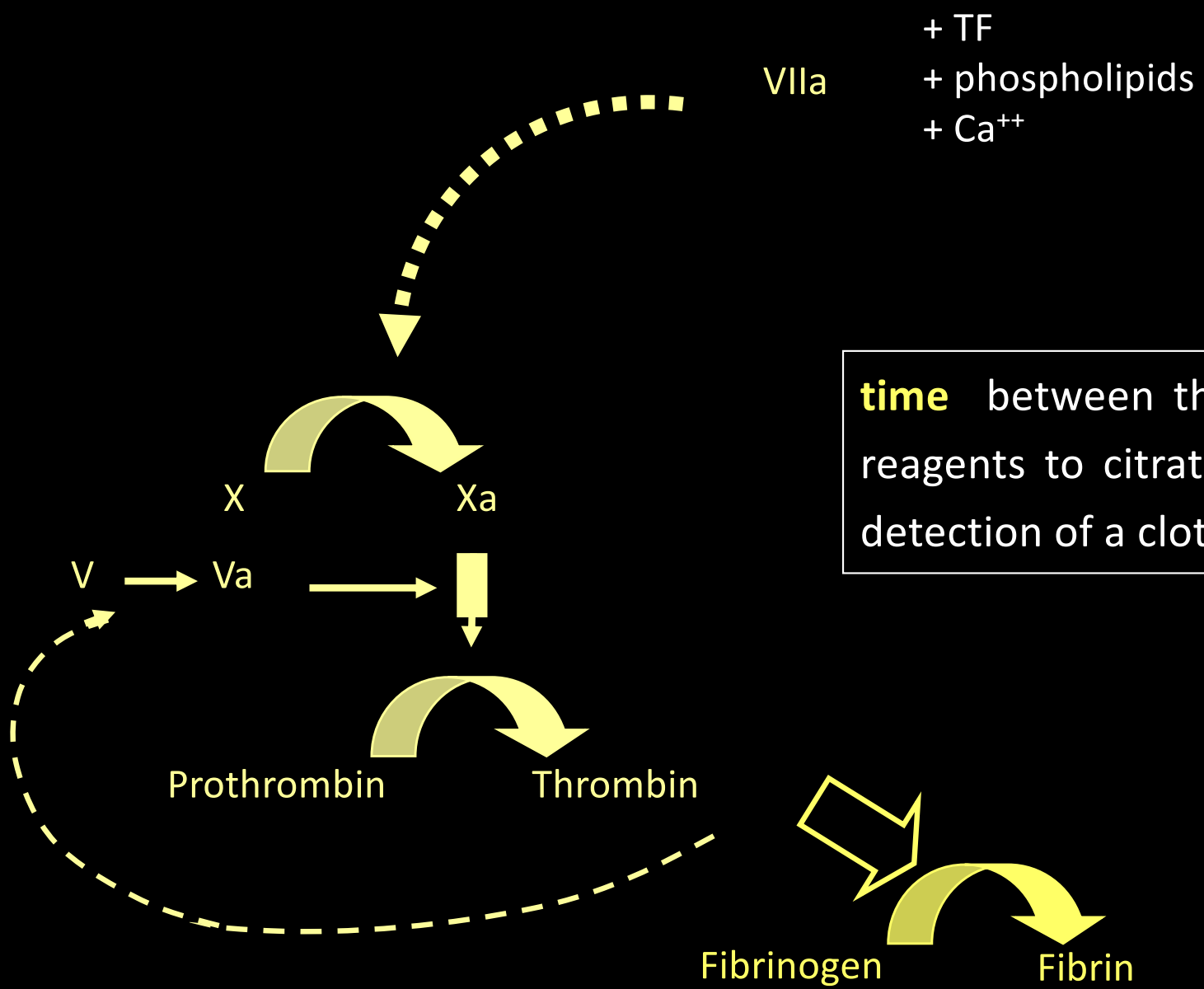
➡ **Oorzaken van een geïsoleerde verlengde APTT?**

APTT (Activated Partial Thromboplastin Time)

Contact
Activation
pathway



Prothrombin time (PT)



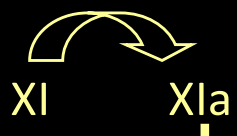
time between the addition of reagents to citrate plasma and detection of a clot.

Prolonged isolated APTT

APTT >

PT nl

HMWK / PK



Prothrombin Thrombin (IIa)

Fibrinogen Fibrin



Prothrombin Thrombin (IIa)

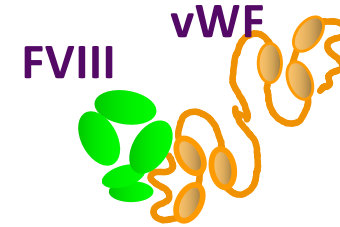
Fibrinogen Fibrin

VIIa

Isolated prolonged APTT

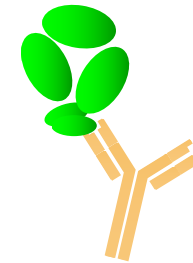
constitutive

- Von Willebrand disease
- hemophilia A and B (FVIII, FIX)
- FXI
- FXII (contact activation pathway) *



acquired

- inhibitor antibody (FVIII)
- « anti-phospholipid » antibody *
- heparine / dabigatran



* not bleeding disorders





- **Senior**
- **uitgebreide hematomen op de rug en de benen sinds 10 dagen**
- **Hb: 10,4**
- **verlengde APTT**
- **normale PT**

tekort aan stollingsfactor?



antistoffen tegen stollingsfactor?

Meng test

mengsel van plasma van de patient met normaal plasma



correctie van de stollingstijd:

geen correctie van de test :

LAGE FACTOR

ANTISTOF

lage titer of slow acting antistof

Factors?

inhibitor antistof?

anti-fosfolipiden antistof?



- **Senior**
- **uitgebreide hematomen op de rug en de benen sinds 10 dagen**
- **Hb: 10,4**
- **verlengde APTT**
- **normale PT**

Geen correctie van de APTT in de meng test: inhibitor

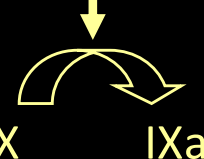
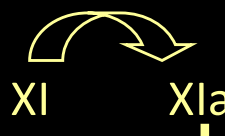
=> antistoffen tegen welke factor?

Prolonged isolated APTT

APTT >

PT nl

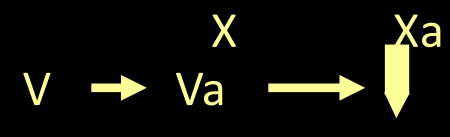
HMWK / PK



Prothrombin → Thrombin (IIa)

Fibrinogen → Fibrin

VIIa



Prothrombin → Thrombin (IIa)

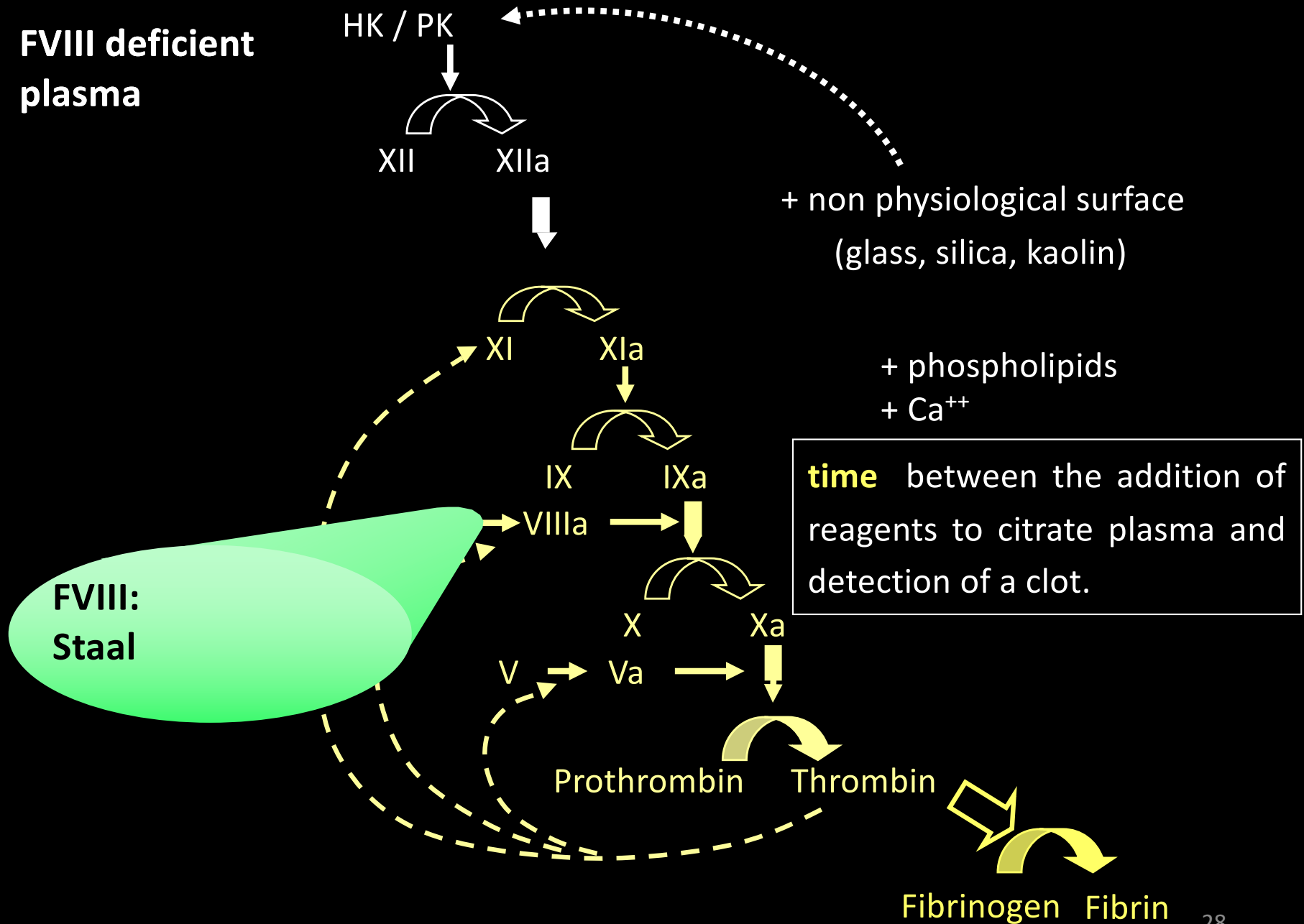
Fibrinogen → Fibrin

Kwantitatieve meting van FVIII activiteit:

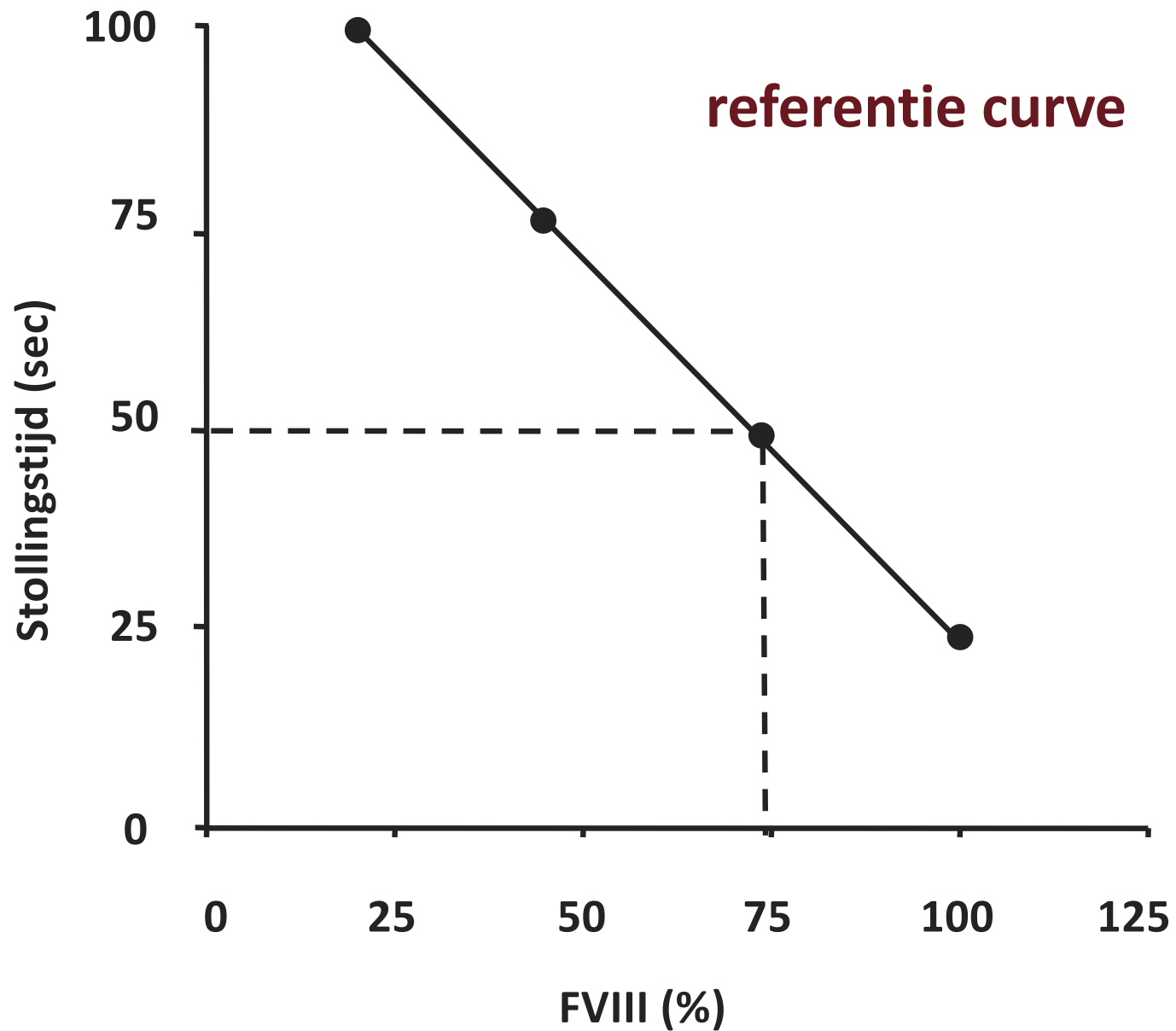
FVIII:C test gebaseerd op een APTT

Meting van FVIII met een APTT gebaseerde test

FVIII deficient plasma



Meting van FVIII met een APTT gebaseerde test





- **Senior**
- **uitgebreide hematomen op de rug en de benen sinds 10 dagen**
- **Hb: 10,4**
- **verlengde APTT**
- **normale PT**

Geen correctie van de APTT in de meng test: inhibitor

FVIII: 20%

andere factoren: normaal

=> antistoffen tegen FVIII (verworven hemofilie A)



**24 jaar oud
1 week na bevalling,
Belangrijke vaginale
bloedingen**

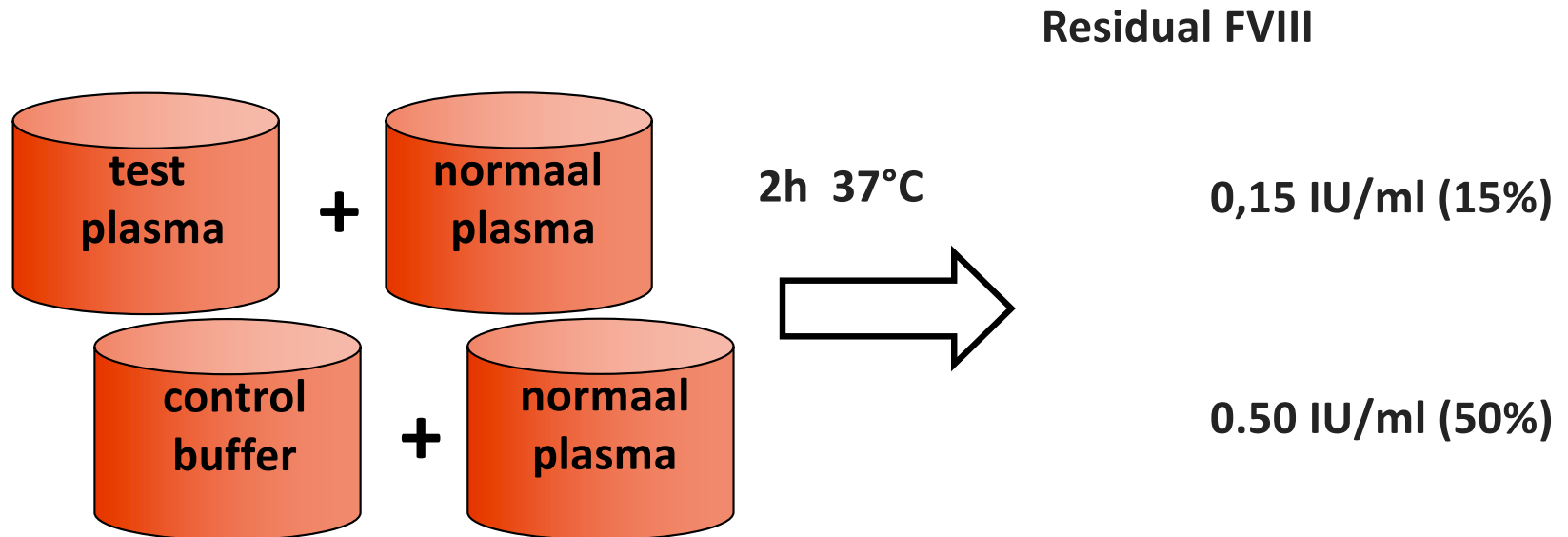
**Normale PT
Verlengde APTT**

Meng test: APTT blijft verlengd

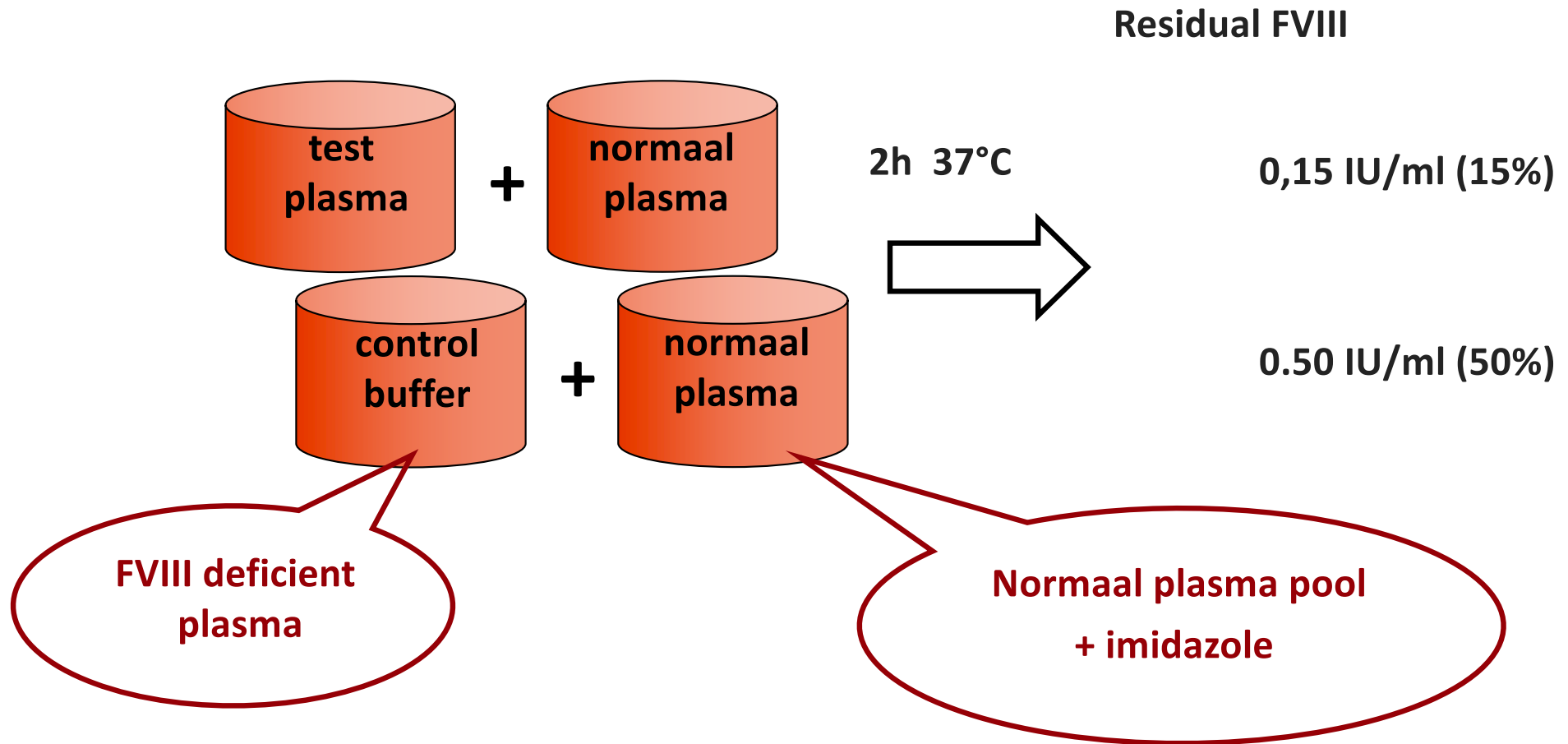
7% FVIII

➔ Antistof tegen FVIII?

Bethesda assay

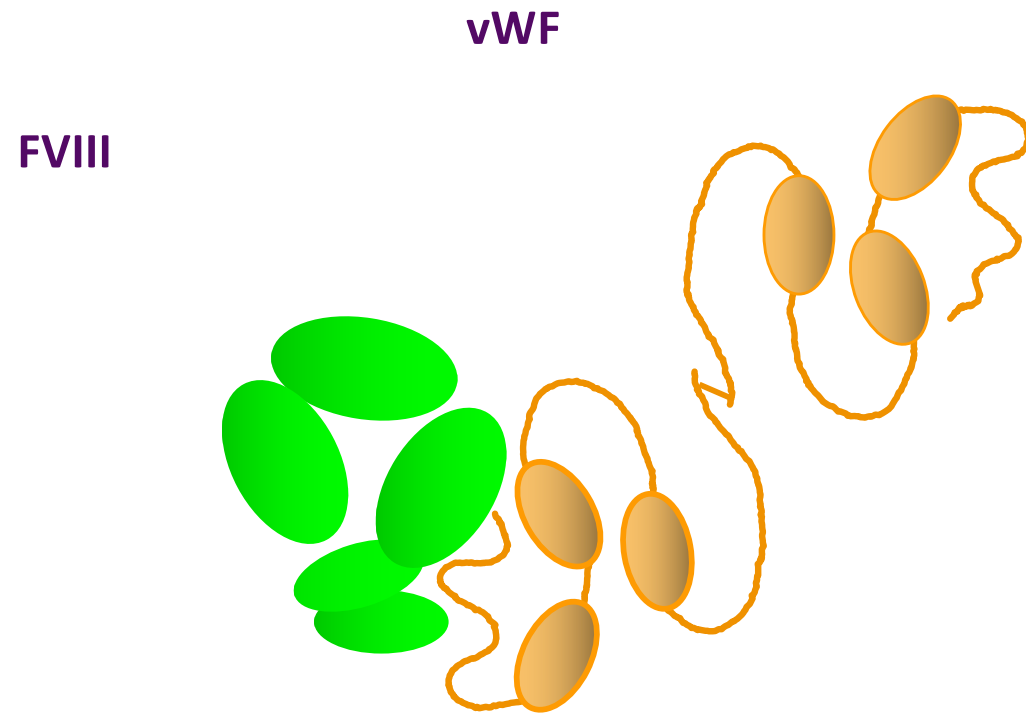


Nijmegen wijziging van de Bethesda assay



Verbruggen et al., Thromb Haemost 1995

Waarom 2u incubatie?





**24 jaar oud
1 week na bevalling,
Belangrijke vaginale
bloedingen**

**Normale PT
Verlengde APTT**

**Meng test: APTT blijft
verlengd**

7% FVIII

➡ **Antistof tegen FVIII +**
➡ **Titer?**

Kwantificatie van een FVIII inhibitor

Bethesda method

(test uitgevoerd met de Nijmegen wijziging van de Bethesda test)

The Bethesda graph

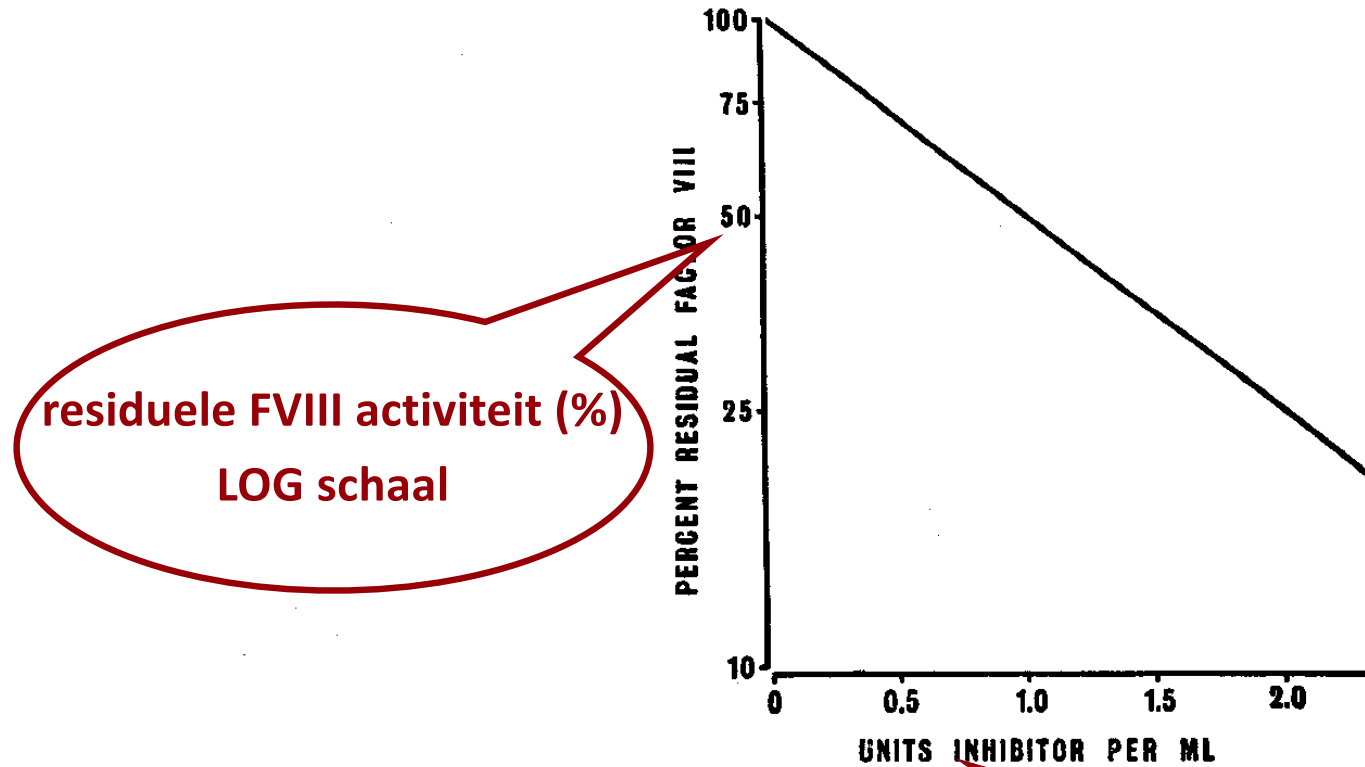


Fig. 1. The percent residual Factor VIII is related to the number of Bethesda units per ml of test plasma.

hoeveelheid inhibitor
LINEAIRE schaal

Kasper et al., Thromb Diathes

Bethesda unit (BU):

**Een test plasma die 50% residuele FVIII activiteit
in de Bethesda test laat overblijven
bevat één BU inhibitor per ml**

Kasper et al., Thromb Diathes Haemorrh 1975

The Bethesda graph

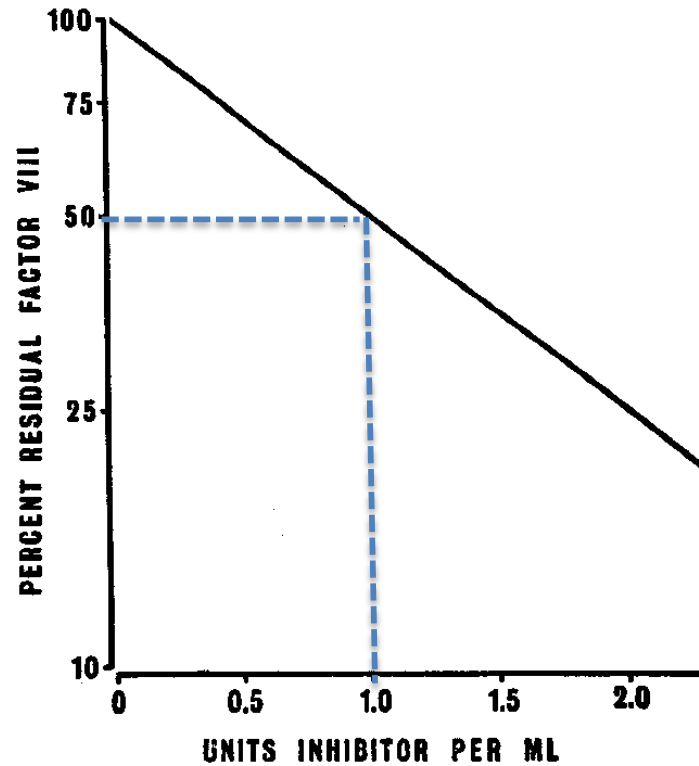


Fig. 1. The percent residual Factor VIII is related to the number of Bethesda units per ml of test plasma.

Kasper et al., Thromb Diathes Haemorrh, 1975

The Bethesda graph

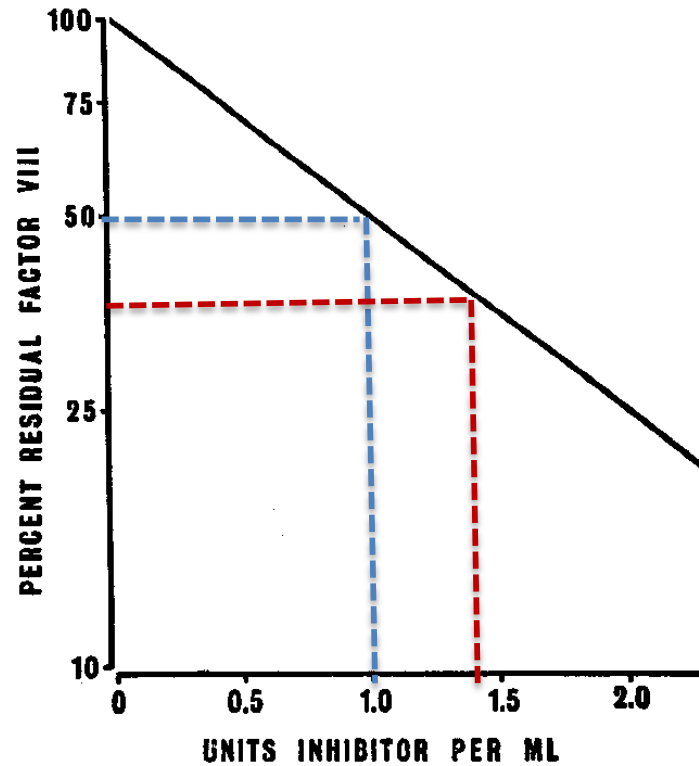


Fig. 1. The percent residual Factor VIII is related to the number of Bethesda units per ml of test plasma.

Kasper et al., Thromb Diathes Haemorrh, 1975



**24 jaar oud
1 week na bevalling,
Belangrijke vaginale bloedingen**

**Normale PT
Verlengde APTT**

Meng test: APTT blijft verlengd

7% FVIII

anti-FVIII antistof (1.3 BU)

➔ Verworven hemofilie A

Auto-immune antistoffen tegen FVIII (verworven hemofilie A)

symptomen: bloedingen

labo: verlengde APTT; normale PT
meng test APTT: verlengd
FVIII: laag tot onmeetbaar
Bethesda test: vaak type II anti-FVIII antistof

etiologie:

- **senior (idiopatisch)**
- **antibiotica**
- **auto-immune aandoening**
- **post partum**





- **chirurgische ingreep een week geleden**
- **geen probleem nadien**
- **verlengde APTT en PT**
- **meng test: verlenging**
- **FV: 3%**
- **Bethesda test: 5 BU (FV)**

Antibodies to FV

etiology

- antibiotics
- cancer
- autoimmune disorders
- surgery (historical: local use of BOVINE thrombin)

symptoms: asymptomatic to severe bleedings

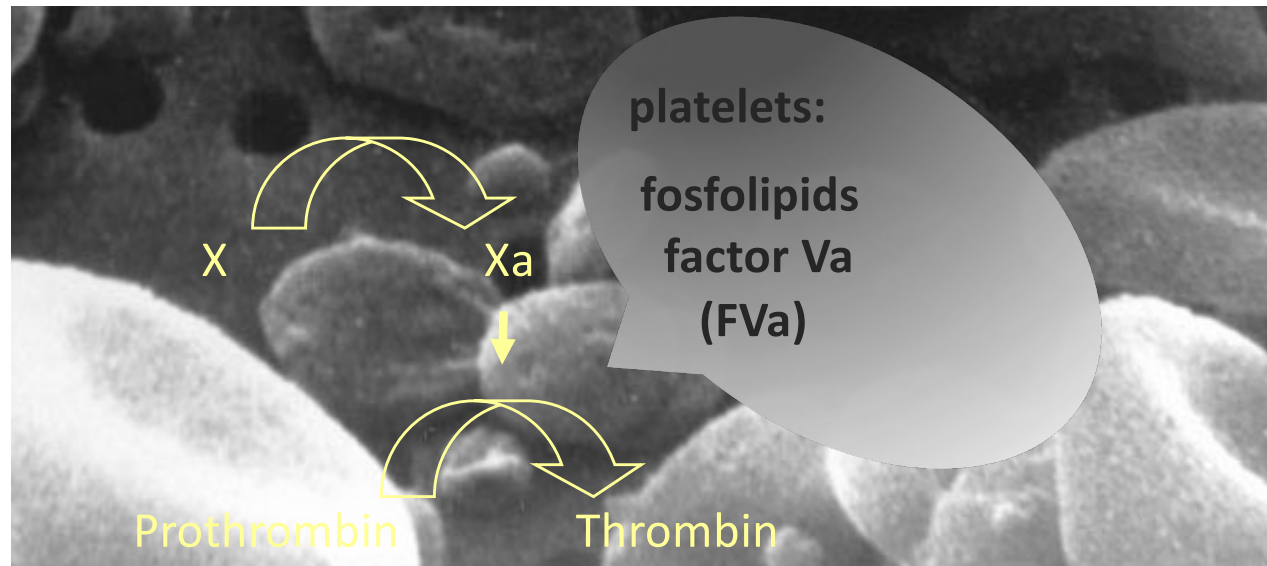
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Antibodies to FV

etiology

- antibiotics
- cancer
- autoimmune disorders
- surgery (historical: local use of BOVINE thrombin)

symptoms: asymptomatic to severe bleedings
(FV in platelets)



Antibodies to FV

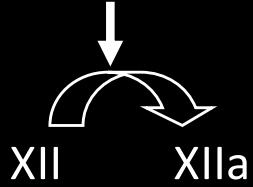
etiology

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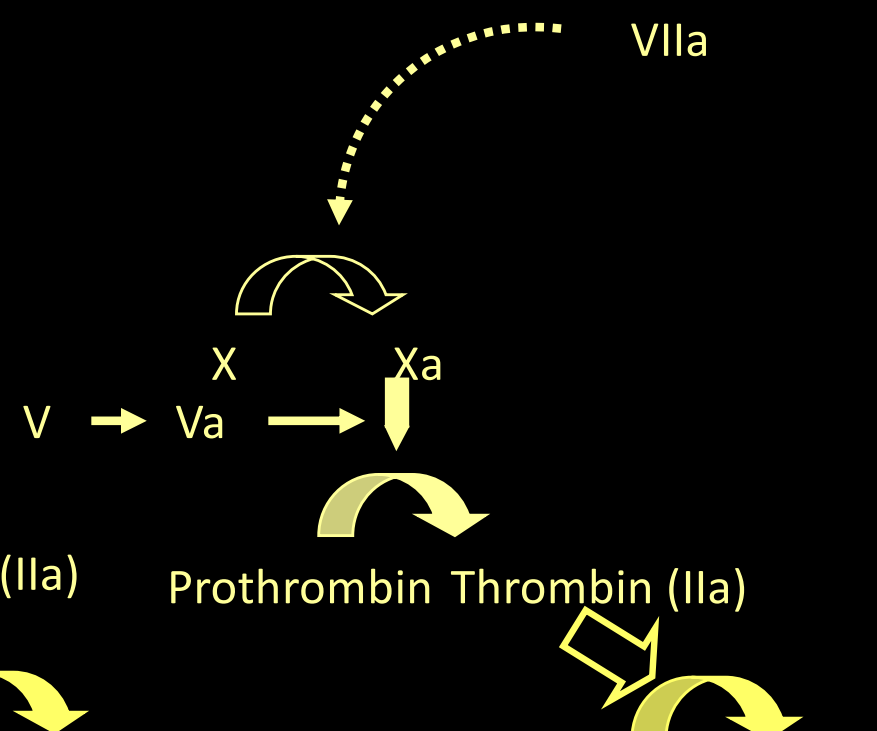
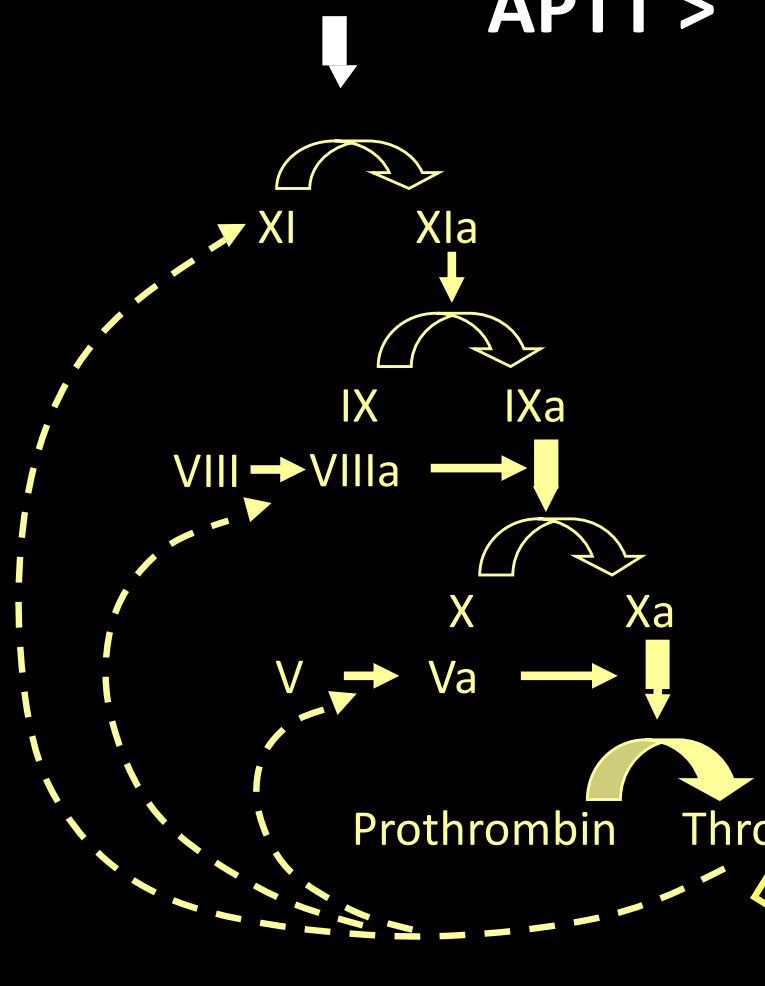
labo: prolonged APTT en PT

HMWK / PK



APTT >

PT >



Fibrinogen Fibrin

Fibrinogen Fibrin



**Antibodies to
FXI, FIX, FX,
FVII, FII, FXIII**

Antibodies to FXIII

Initiation of coagulation

VIIa/TF

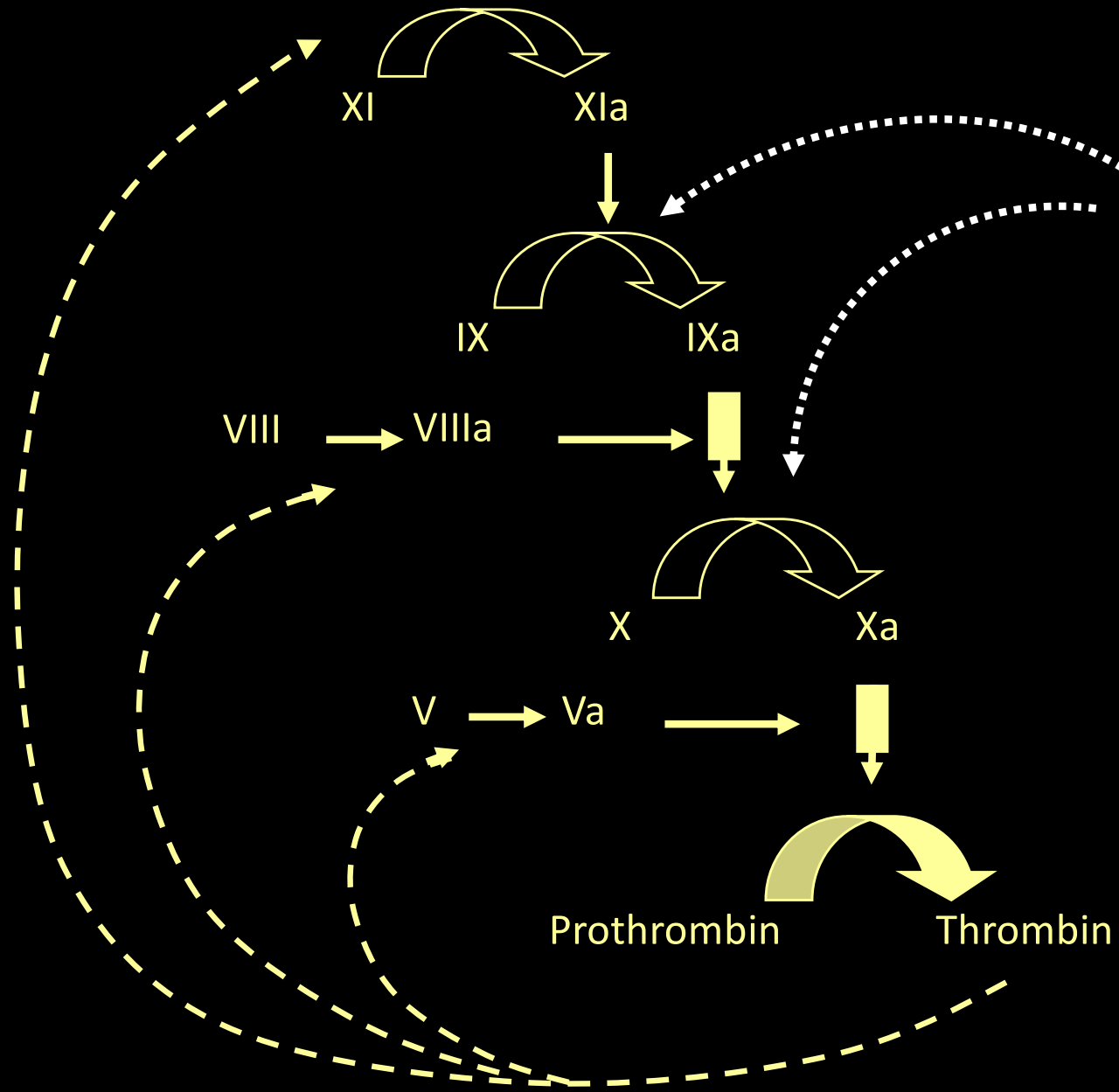
Sustained hemostasis

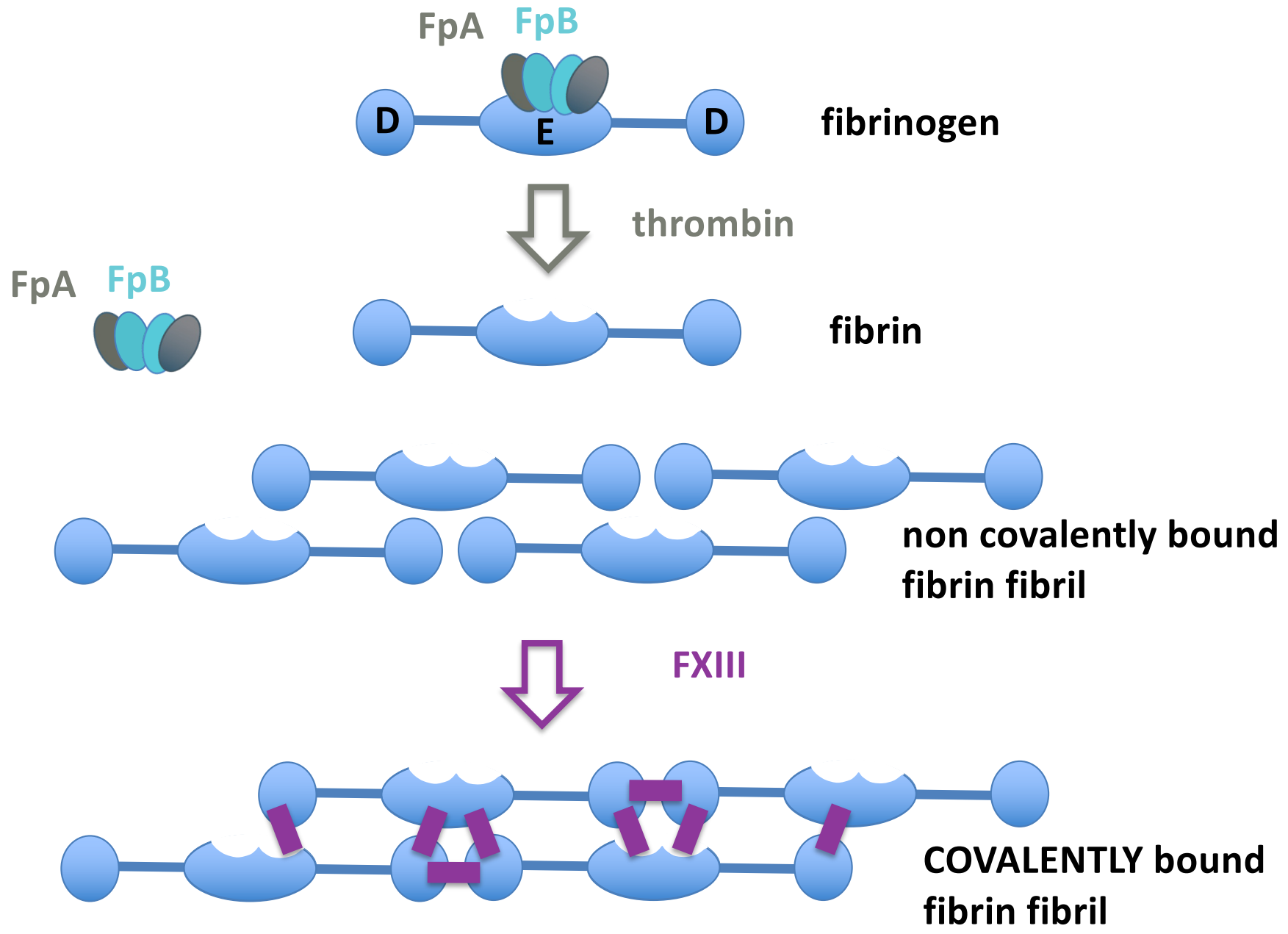
FXIII

Fibrinogen

Fibrin

Amplification





Antibodies to FXIII

Frequency: extremely rare

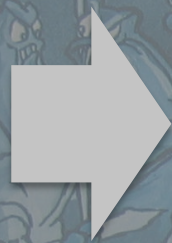
Etiology:

symptoms: severe bleedings

labo: NORMAL APTT en NORMAL PT



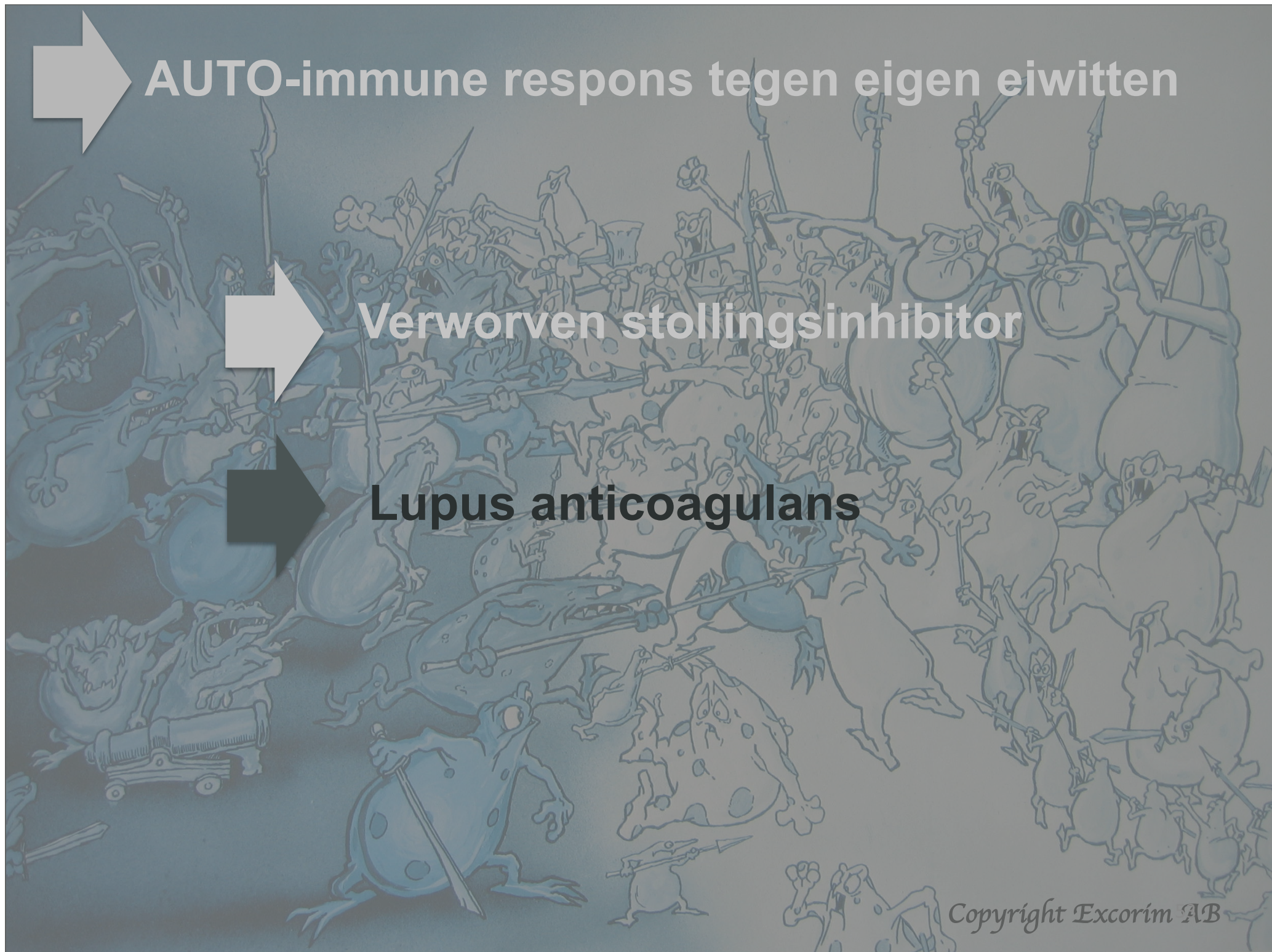
AUTO-immune respons tegen eigen eiwitten



Verworven stollingsinhibitor



Lupus anticoagulans





- **37 jaar oud**
- **meerdere miskramen**
- **één kindje**
- **ischemische cerebrale trombose**

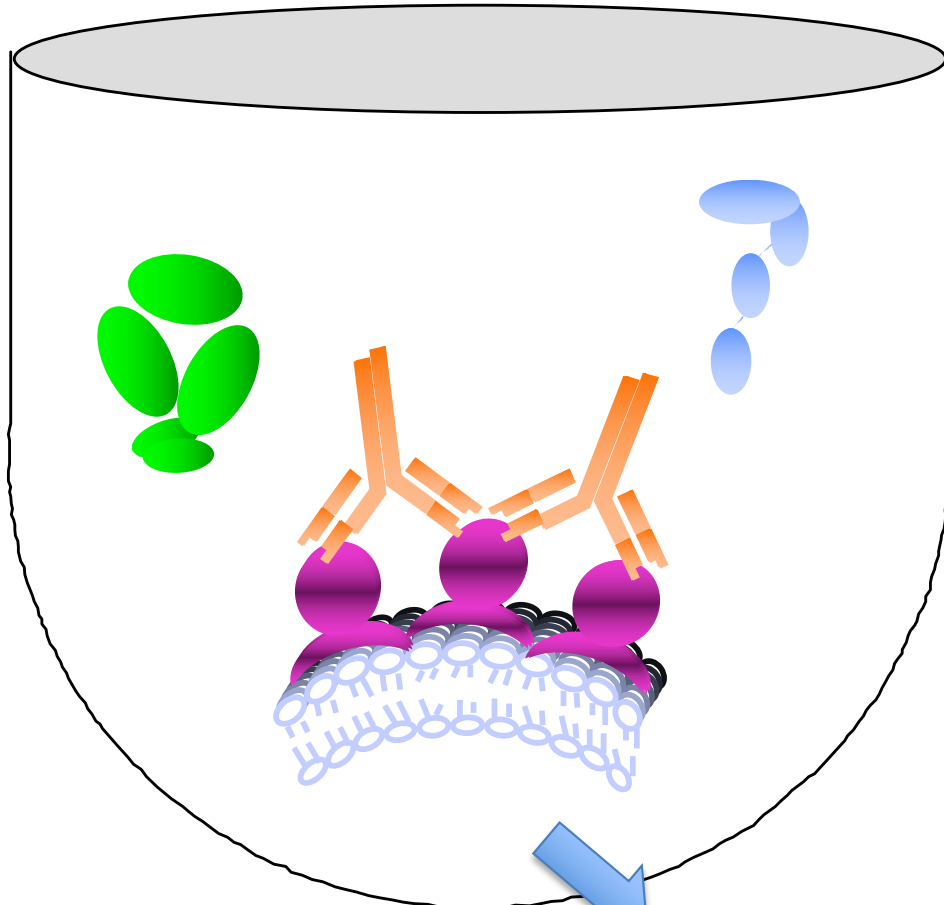
- **Verlengde APTT en PT**
- **Laag FXI, FVIII, FIX, FV, FX en FVII**

➔ **Lupus anticoagulans?**

Lupus anticoagulans test: APTT reagenten

Screen

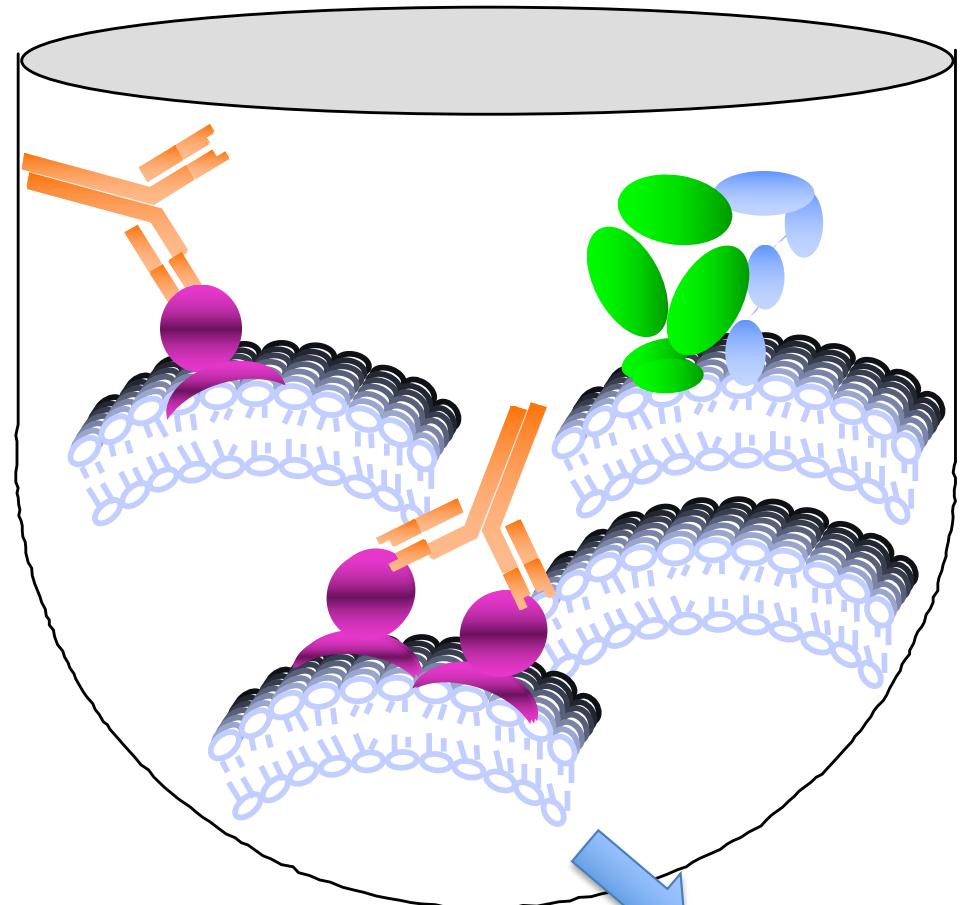
LAGE concentratie fosfolipiden



Geen clotting

Confirm

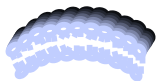
HOGE concentratie fosfolipiden



clotting



stollingsfactoren



fosfolipiden



antistof



b2-glycoproteine I

Detectie van anti-fosfolipiden antistoffen in ELISA

- **“anti-cardiolipine” antistoffen**
- **“Anti- β 2 glycoproteine I” antistoffen**



- **37 jaar oud**
- **meerdere miskramen**
- **één kindje**
- **ischemische cerebrale trombose**

- **Verlengde APTT en PT**
- **Laag FXI, FVIII, FIX, FV, FX en FVII**

➔ **Lupus anticoagulans ++**

! Controle testen: trombine tijd, meng test

**(bias bij lage concentratie factoren (anti-vit K behandeling),
heparine, LMWH, anti-Xa (Rivaroxaban), anti-IIa (Dabigatran))**

! twee verschillende LA testen

- dRVVT (dilute Russel's viper venom time)**
- dilute APTT**

! twee bloedafnames:

! Gedetailleerde guidelines voor de detectie van "lupus anticoagulans syndrome"

! Meting van factoren bij lupus anticoagulans positief staal:

- lupus anticoagulans ongevoelige reagenten**
- meer verdunning van het staal**

Cave!

vals positief LA testen bij factor tekort of inhibitor

guidelines:

altijd de aanwezigheid van een factor tekort uit te sluiten voor te concluderen dat een LA aanwezig is

!!! Belangrijke differentiele diagnose



antistoffen tegen stollingsfactoren

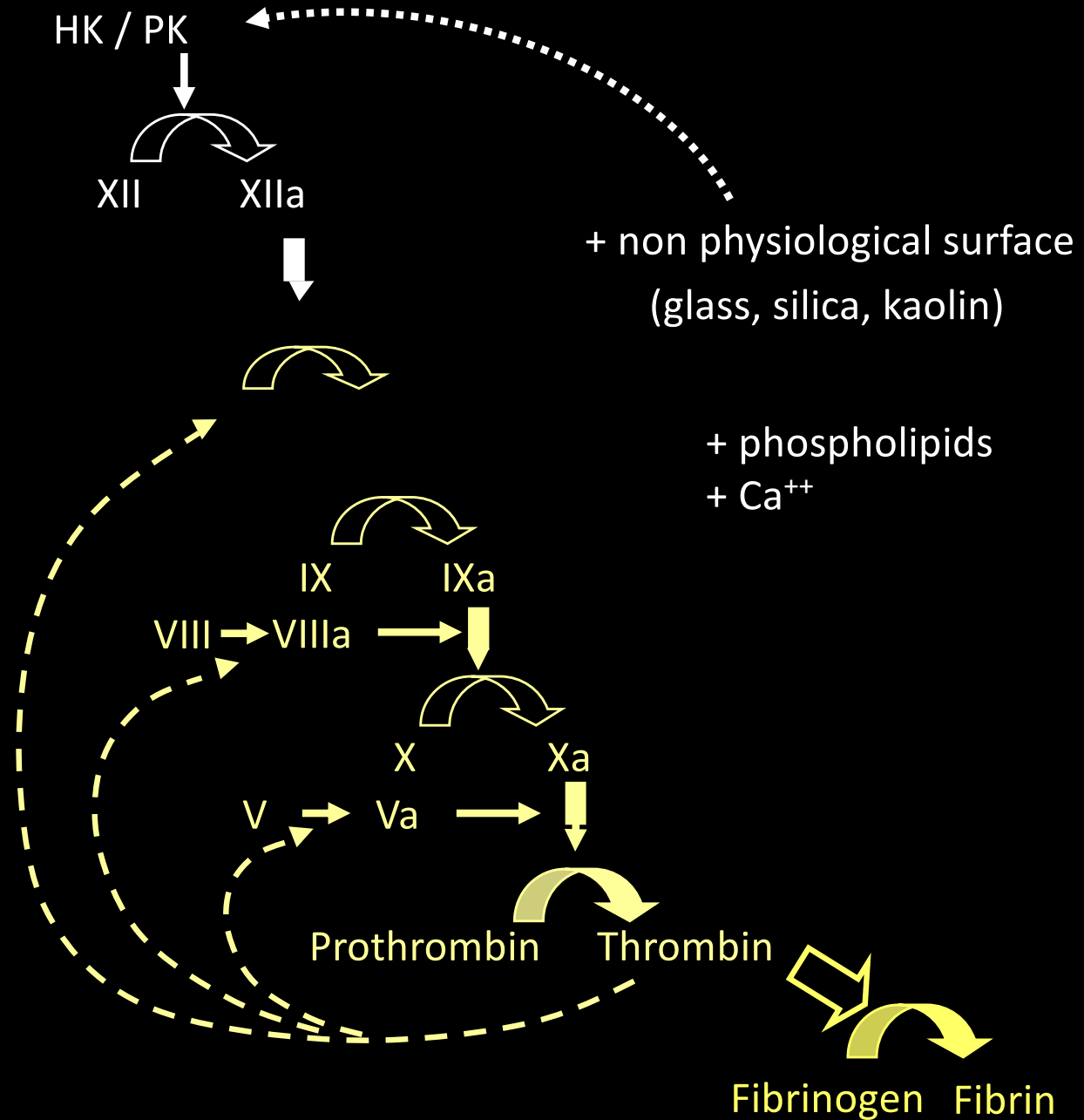


Lupus anticoagulans

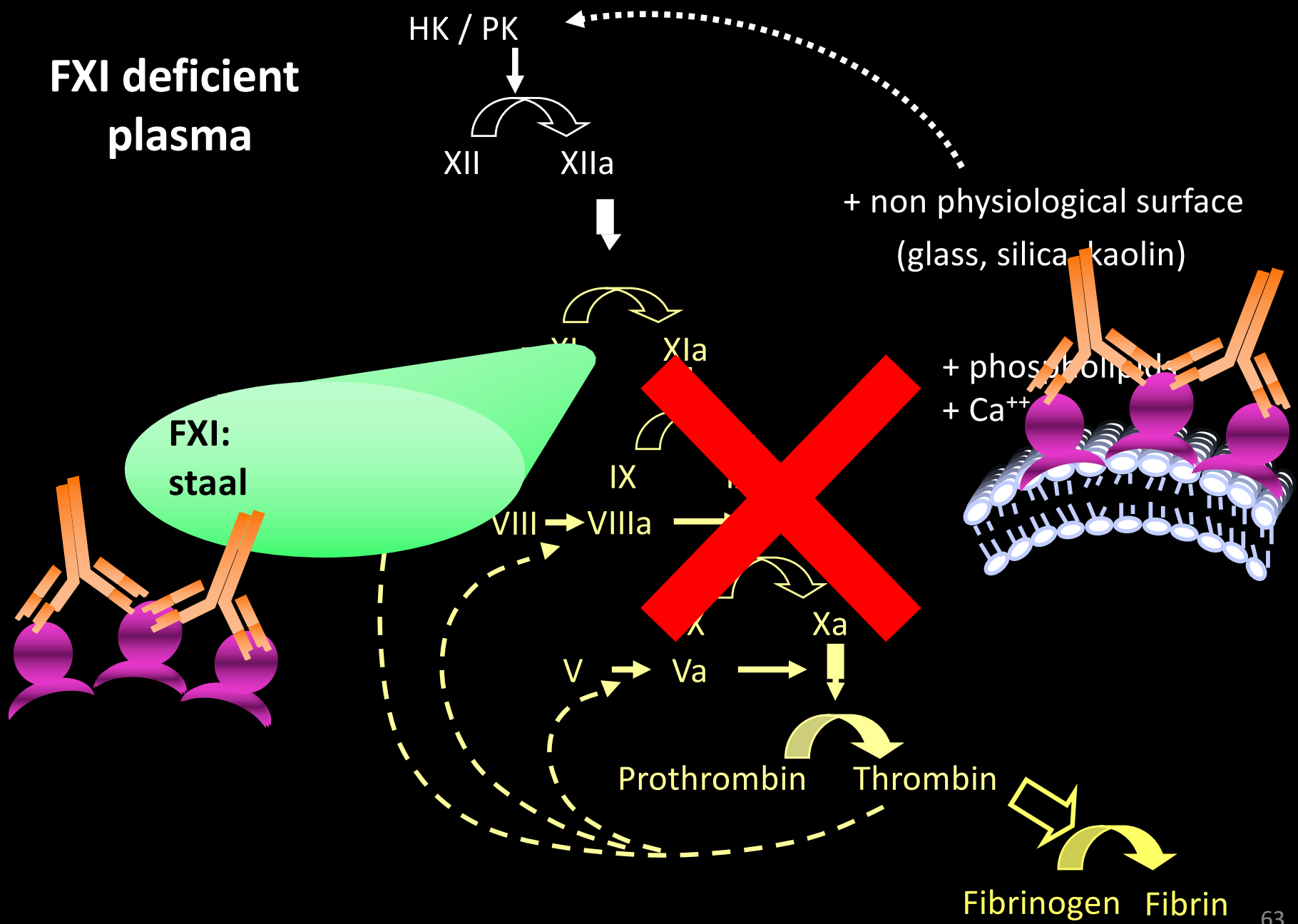
Meting van stollingsfactoren in aanwezigheid van een lupus anticoagulans

Meting van FXI met een APTT gebaseerde test

FXI deficient plasma

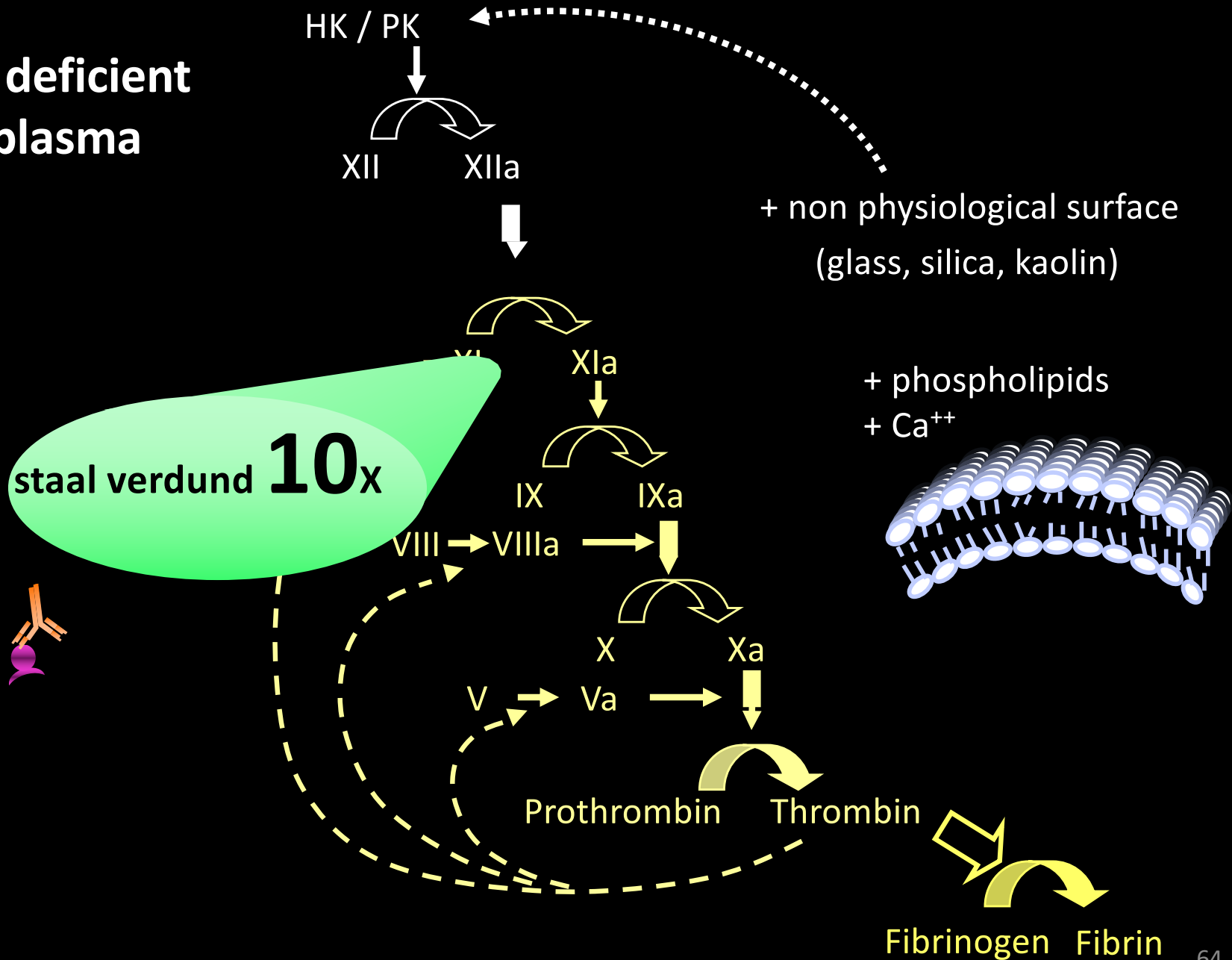


Meting van FXI met een APTT gebaseerde test



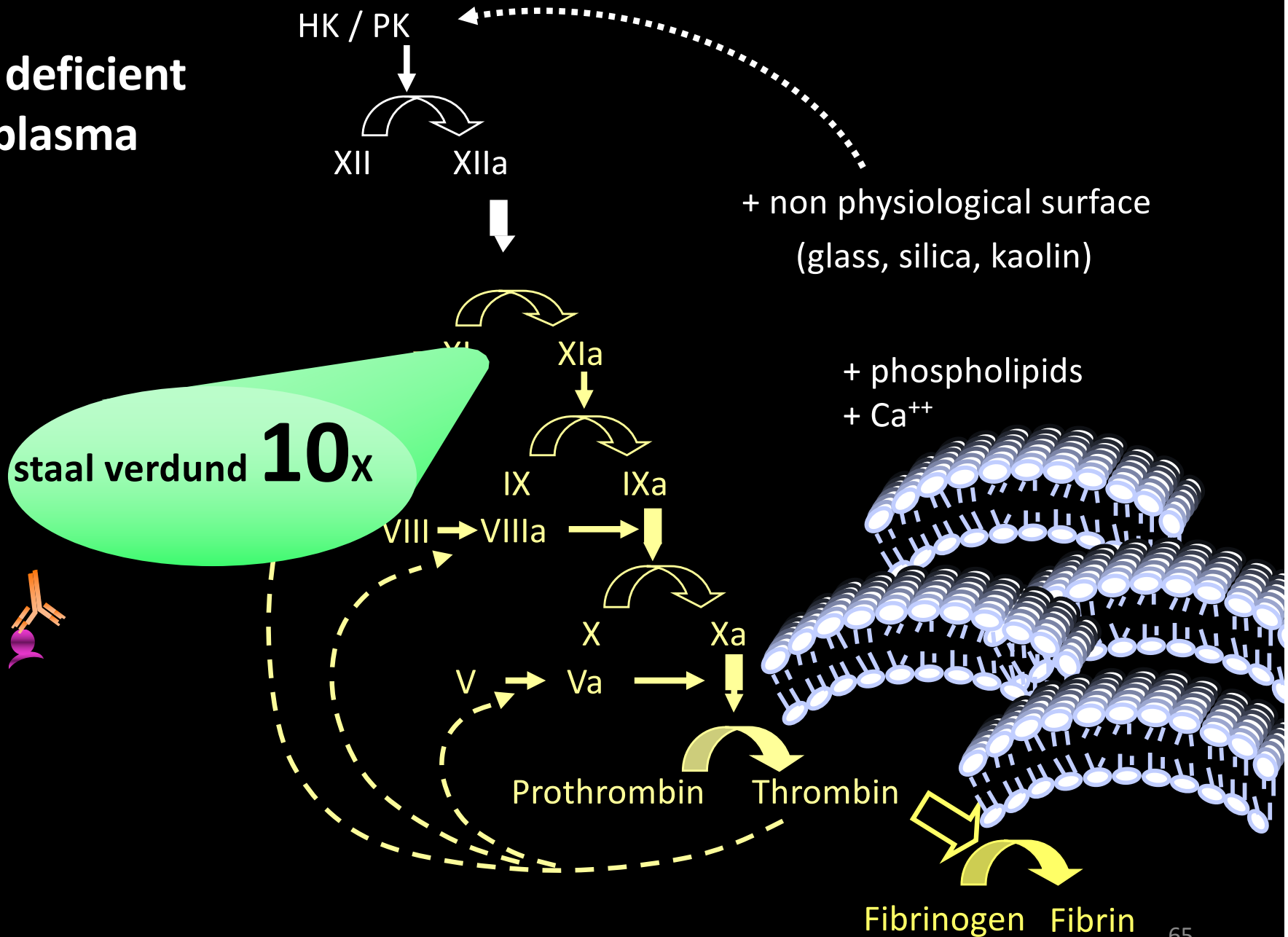
Meting van FXI met een APTT gebaseerde test

FXI deficient plasma



Meting van FXI met een APTT gebaseerde test

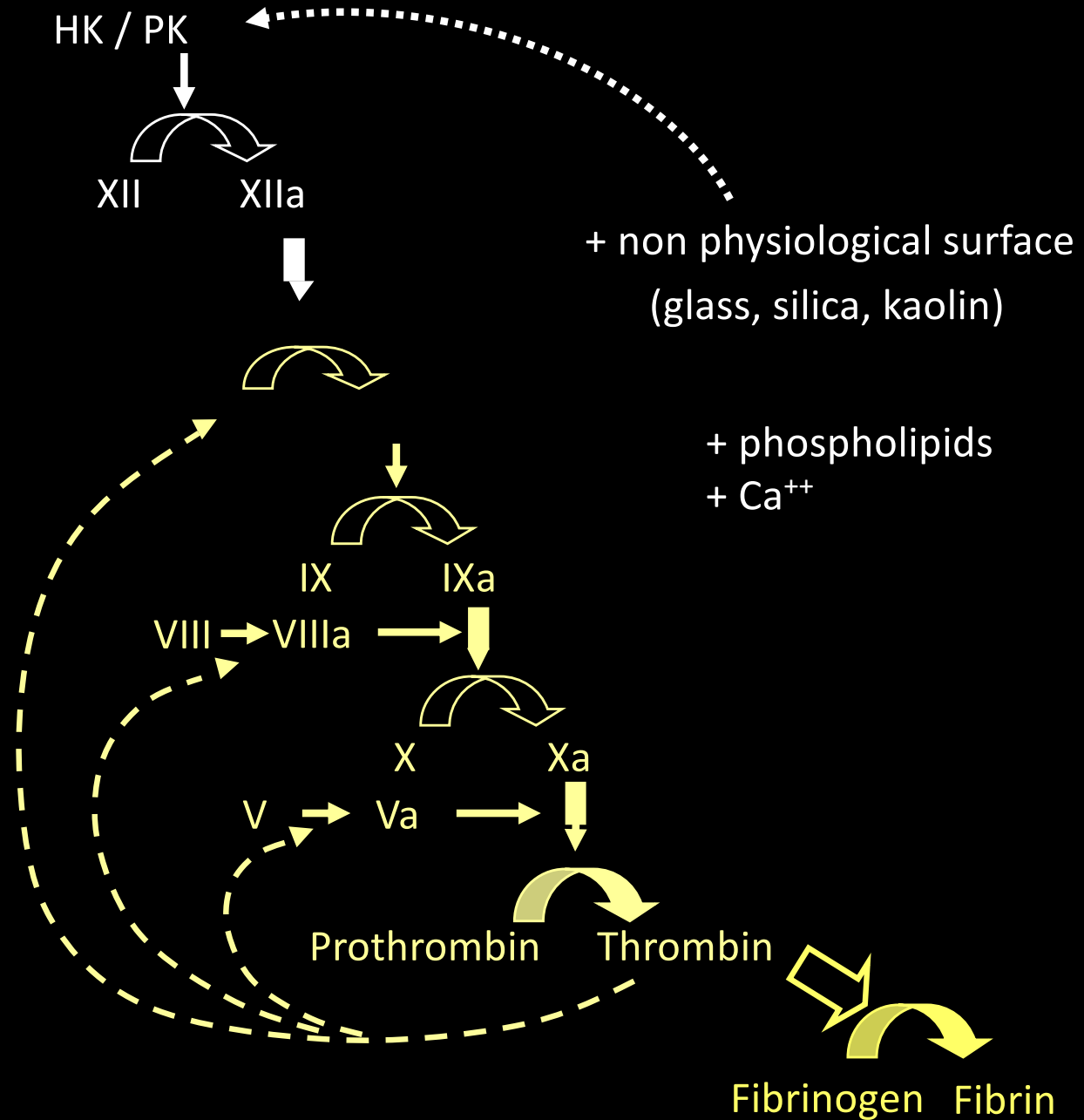
FXI deficient plasma



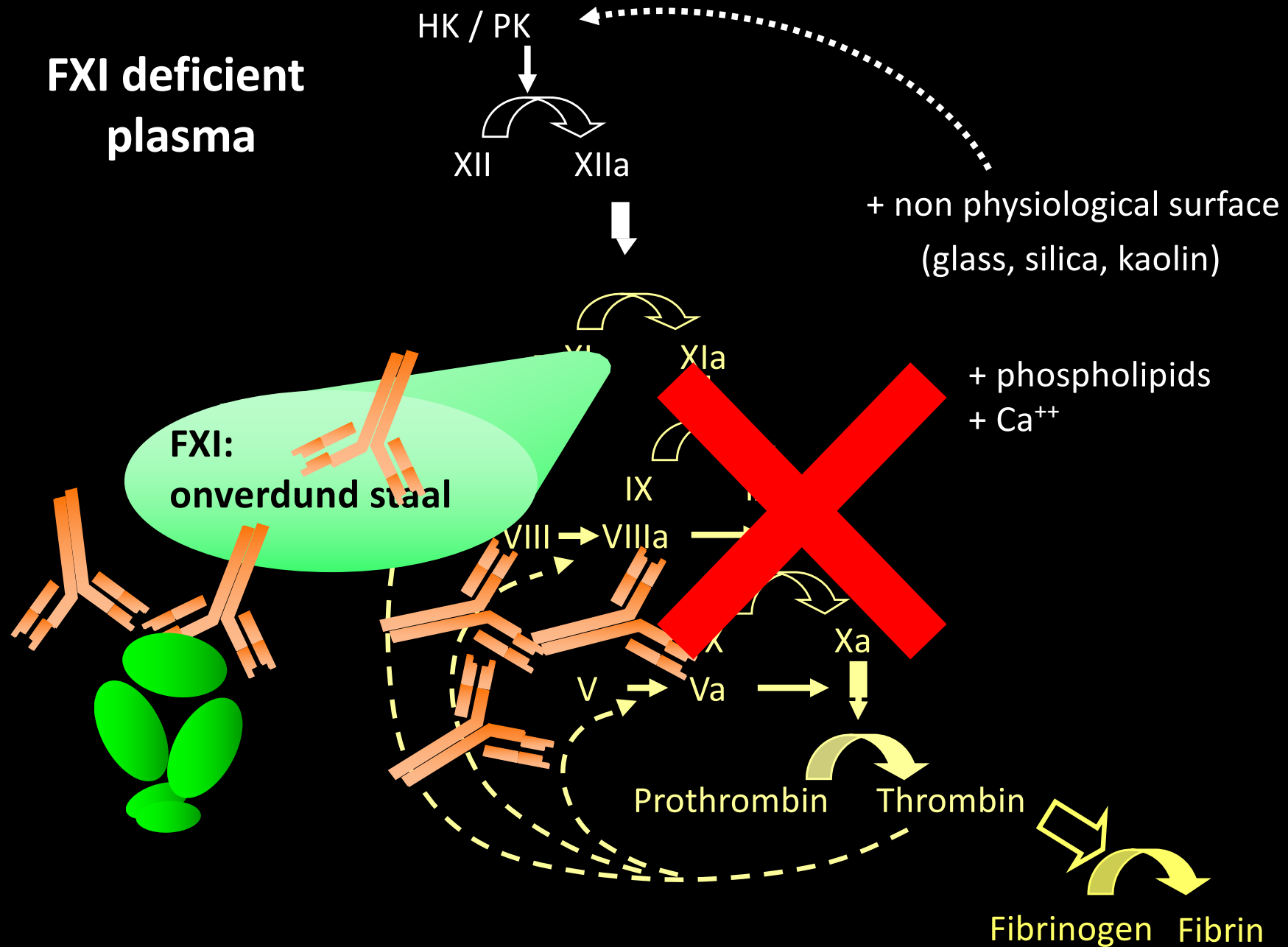
Meting van stollingsfactoren in aanwezigheid van een hoog titer anti-FVIII antistof

Meting van FXI met een APTT gebaseerde test

FXI deficient plasma

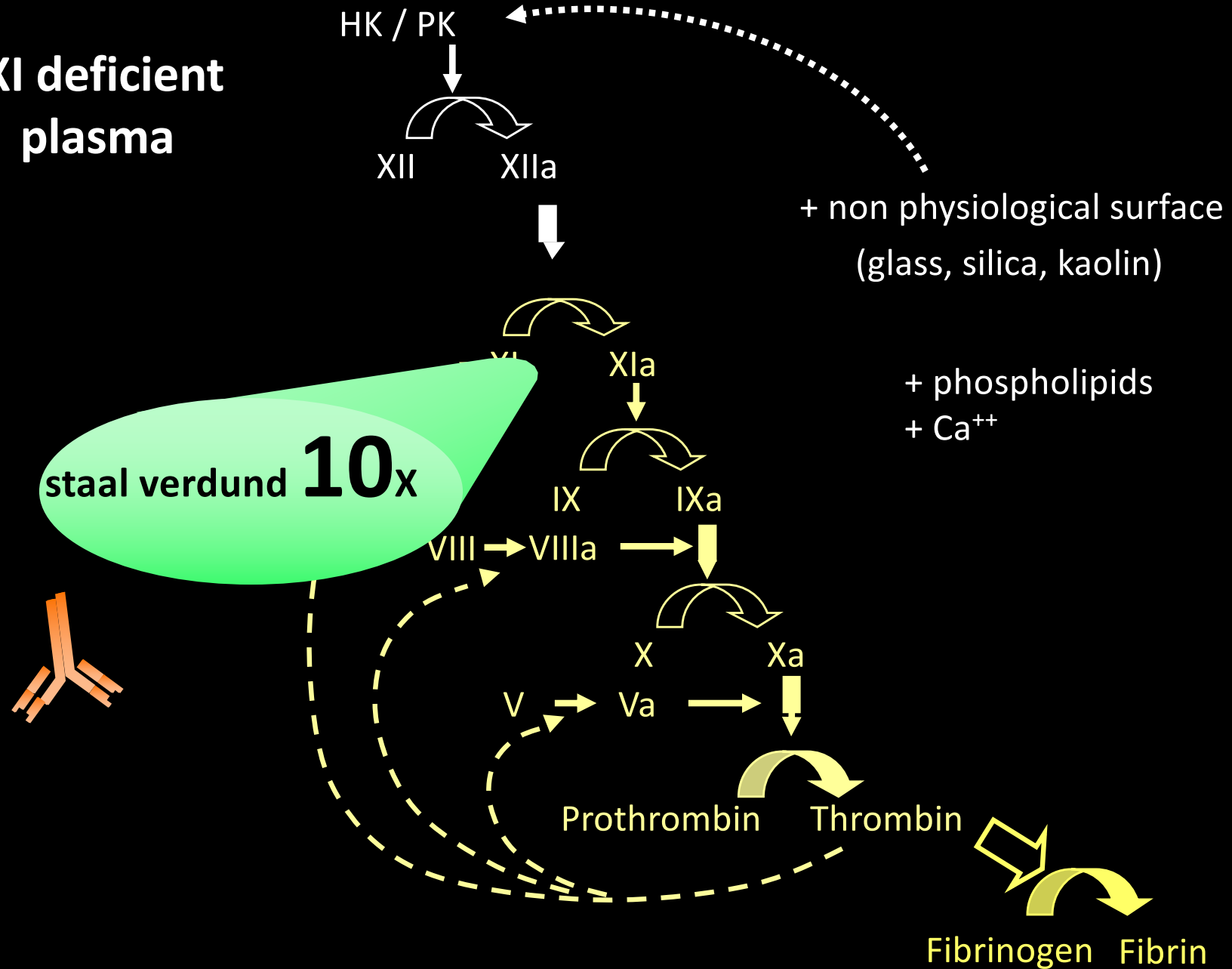


Meting van FXI met een APTT gebaseerde test



Meting van FXI met een APTT gebaseerde test

FXI deficient plasma



Bethesda test met meerdere verdunningen van het staal

Nog inhibitie van FVIII

Normalisatie van FXI!

casus 1



- 7 dagen oud
- APTT: **46,8 sec**
- PT: 11,7

APTT van 46,8 sec?

Wat doen we om dit uit te leggen?



Reference values for coagulation tests in the healthy full-term infant

Table 2. Reference Values for Coagulation Tests in the Healthy Full-term Infant During the First 6 Months of Life

Tests	Day 1 (n)	Day 5 (n)	Day 30 (n)	Day 90 (n)	Day 180 (n)	Adult (n)
PT (s)	13.0 ± 1.43 (61)*	12.4 ± 1.46 (77)*†	11.8 ± 1.25 (67)*†	11.9 ± 1.15 (62)*	12.3 ± 0.79 (47)*	12.4 ± 0.78 (29)
APTT (s)	42.9 ± 5.80 (61)	42.6 ± 8.62 (76)	40.4 ± 7.42 (67)	37.1 ± 6.52 (62)*	35.5 ± 3.71 (47)*	33.5 ± 3.44 (29)

Day 5

PT (s): 12.4 ± 1.5
 APTT (s): 42.6 ± 8,6

Adult

PT (s): 12.4 ± 0,8
 APTT (s): 33.5 ± 3.4

casus 2

Klinisch verhaal

- mevrouw, 50 jaar, reumatoïde artritis
- klachten van melena en soms van petechiae
- verlengde APTT VANAF JUNI (normale PT)

14.11.2014 PT: 12,3 APTT: 31,6

10.05.2015 PT: 12 APTT: 30

11.06.2015 PT: 13 APTT: 66

15.7.2015 PT: 12,3 APTT: 119,7

Fg: 2.93 g/L

Plaatjes: 179-204.000/ μ l

Hb: 10,5-11,6



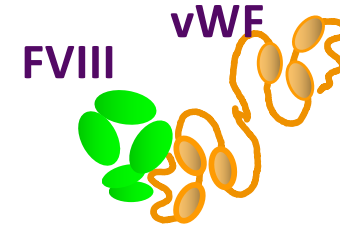
**Mogelijke oorzaken van deze
verworven verlengde APTT?**



Isolated prolonged APTT

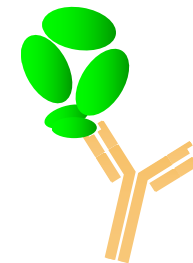
constitutive

- Von Willebrand disease
- hemophilia A and B (FVIII, FIX)
- FXI
- FXII (contact activation pathway) *



acquired

- acquired Von Willebrand disease
- inhibitor antibody (FVIII)
- « anti-phospholipid » antibody *
- heparine / dabigatran



* not bleeding disorders

Heparine?

volgens ASO, telefonisch bereikt,
geen heparine, geen cathether

ook geen dabigatran



volgens andere collega,
Port-a-cath voor recombinant CTLA4
(Orencia) voor reumatoïde artritis
=> heparine in Port-a-cath

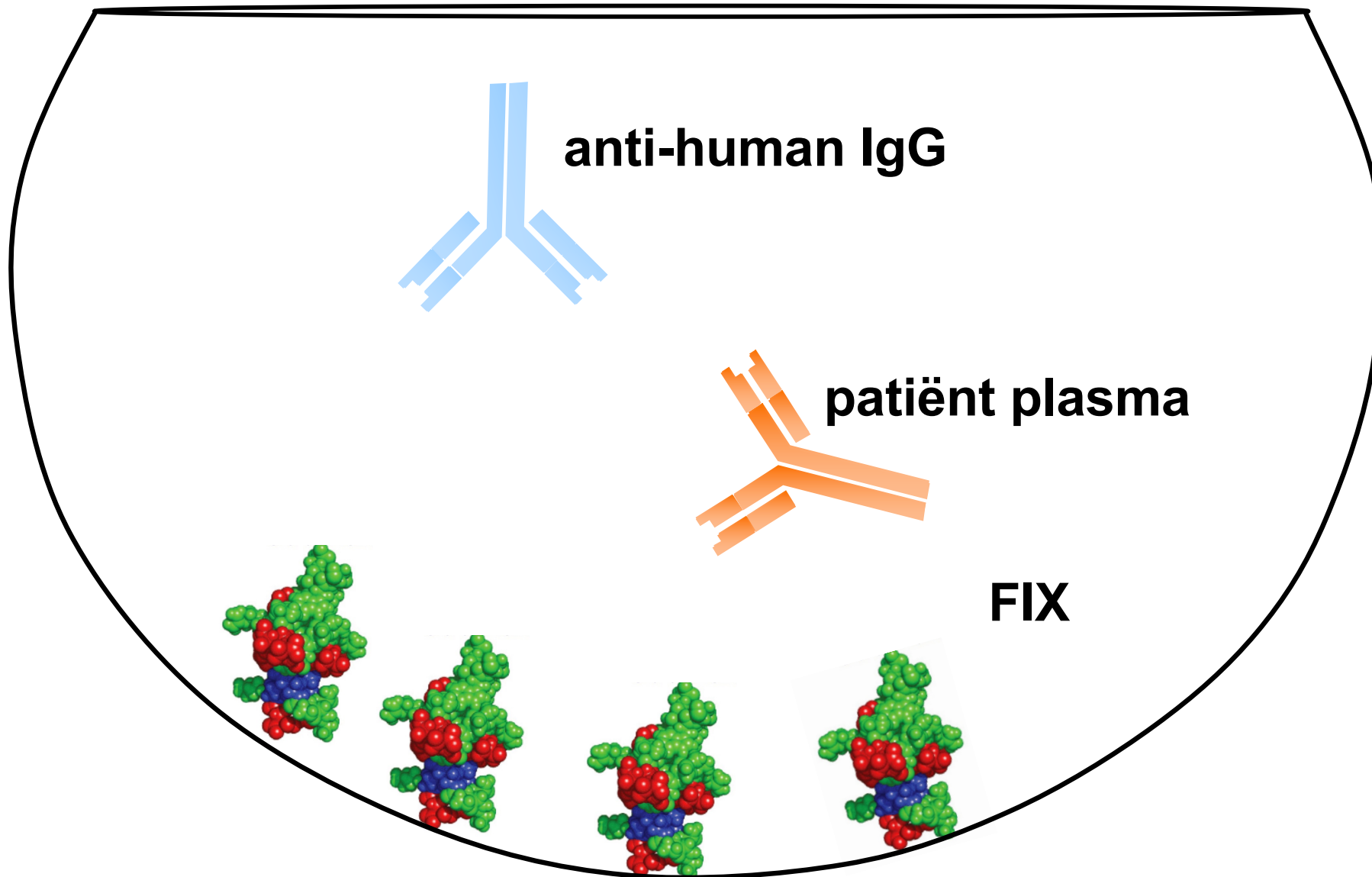


casus 3



- **hemofilie B**
- **behandeld met FIX**
- **slecht “FIX recovery”**
- **antistoffen tegen FIX?**
- **Bethesda test: 0 BU !!!**

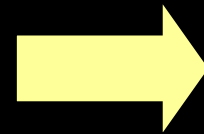
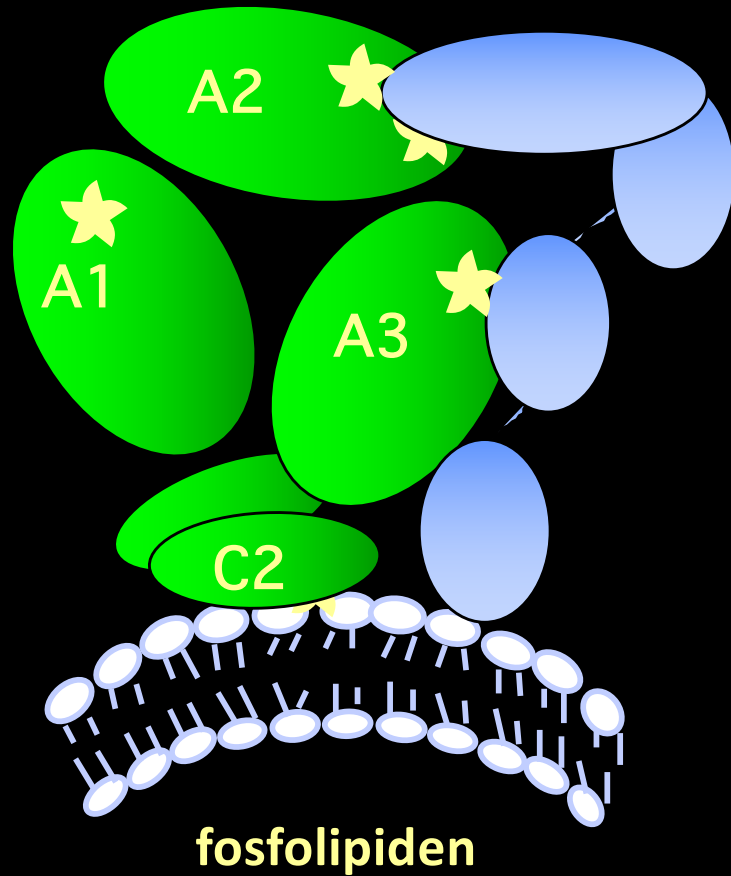
Detection of anti-FIX antibody in ELISA



Geactiveerde Factor VIII als cofactor van Factor IXa

Factor VIIIa

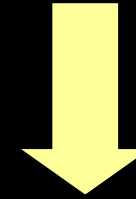
Factor IXa



Factor X



Factor Xa

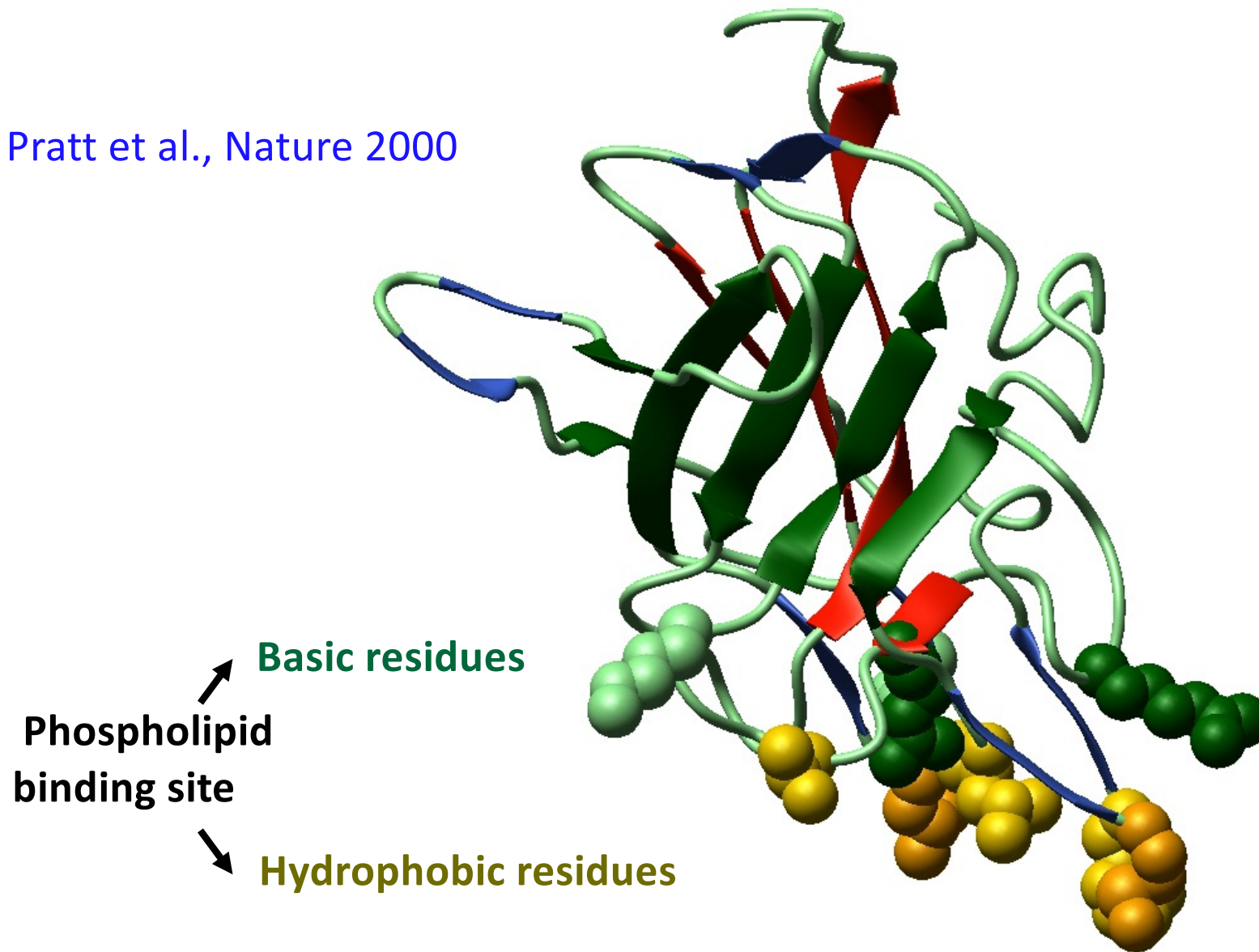


Hemostase

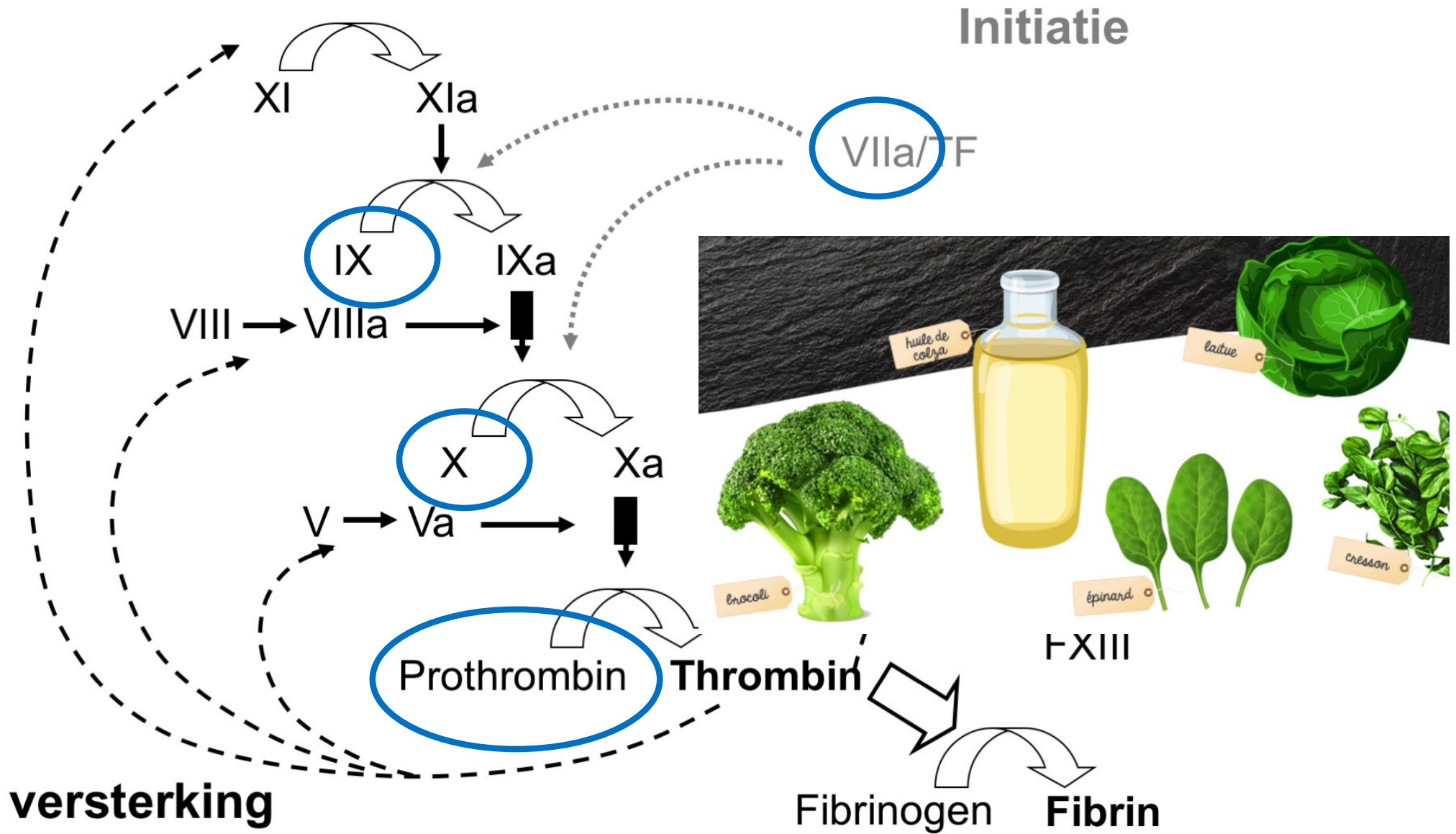
X 100.000 door Factor VIII

FV and FVIII binding to phospholipids

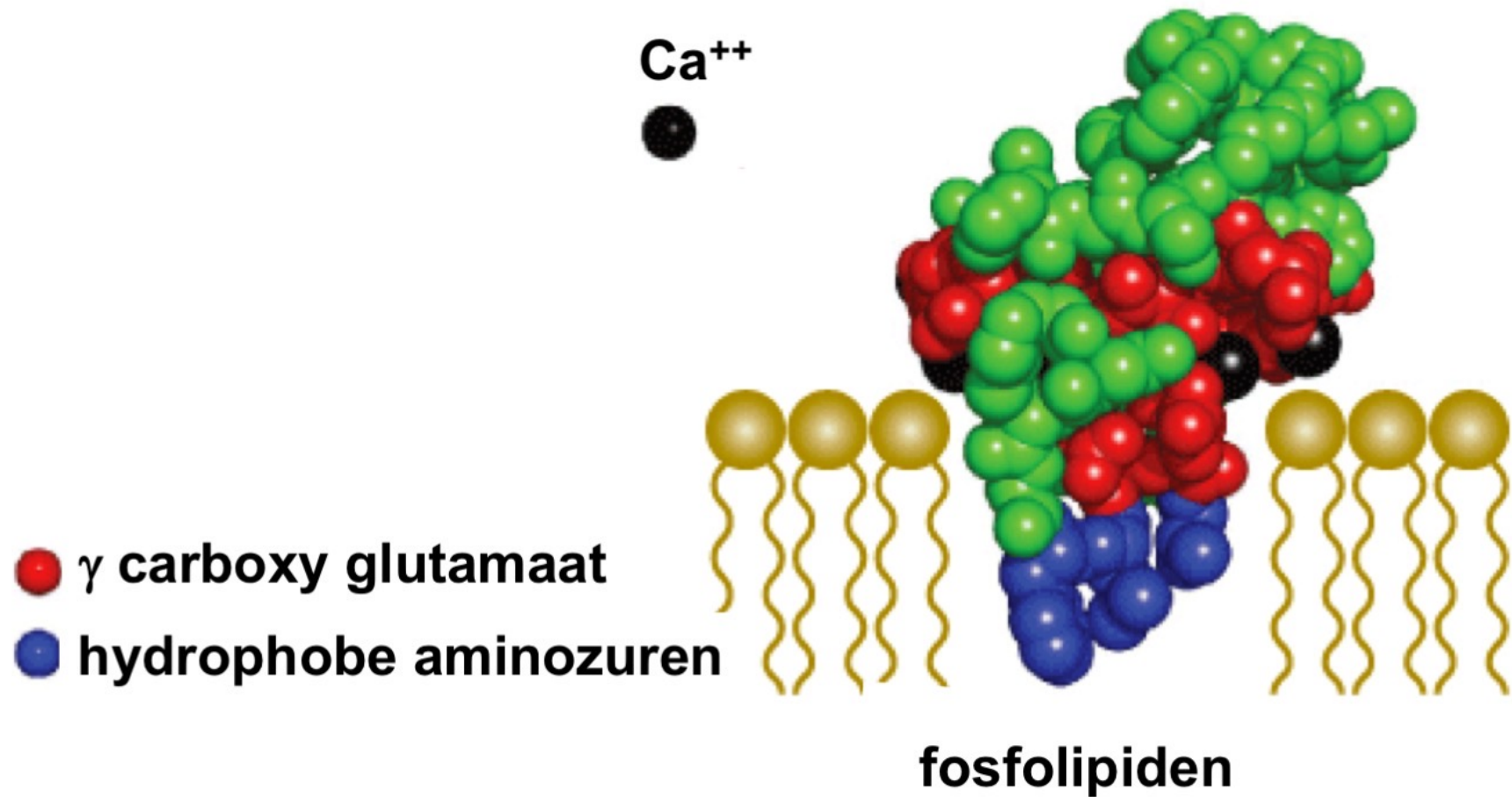
Pratt et al., Nature 2000



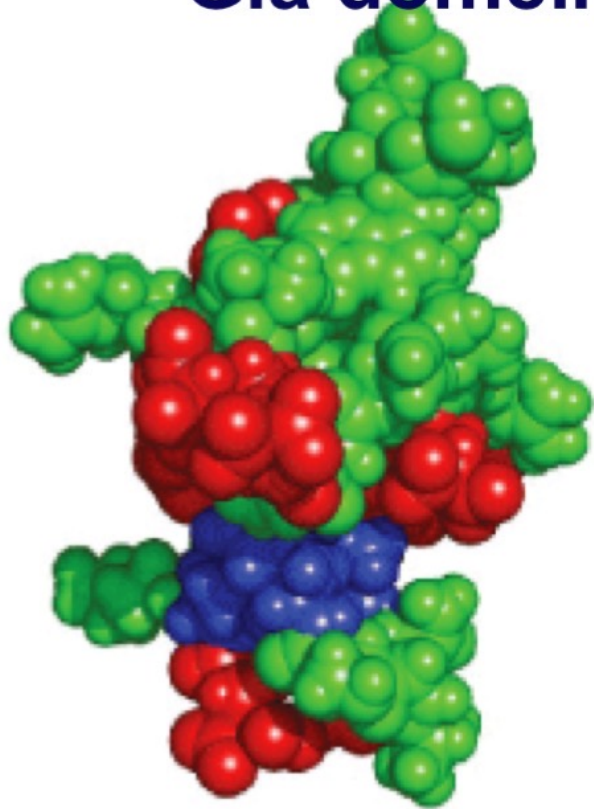
De vitamine K afhankelijke factoren: FII, FVII, FIX en FX



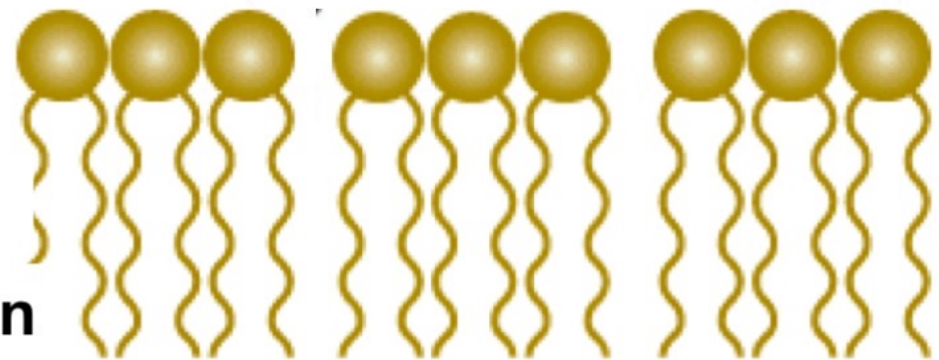
Vitamine K afhankelijke factoren (FVII, FIX, FX, FII) binding aan fosfolipiden: Gla domain



FVII, FIX, FX, FII binding op fosfolipiden : Gla domein in afwezigheid van Ca^{2+}

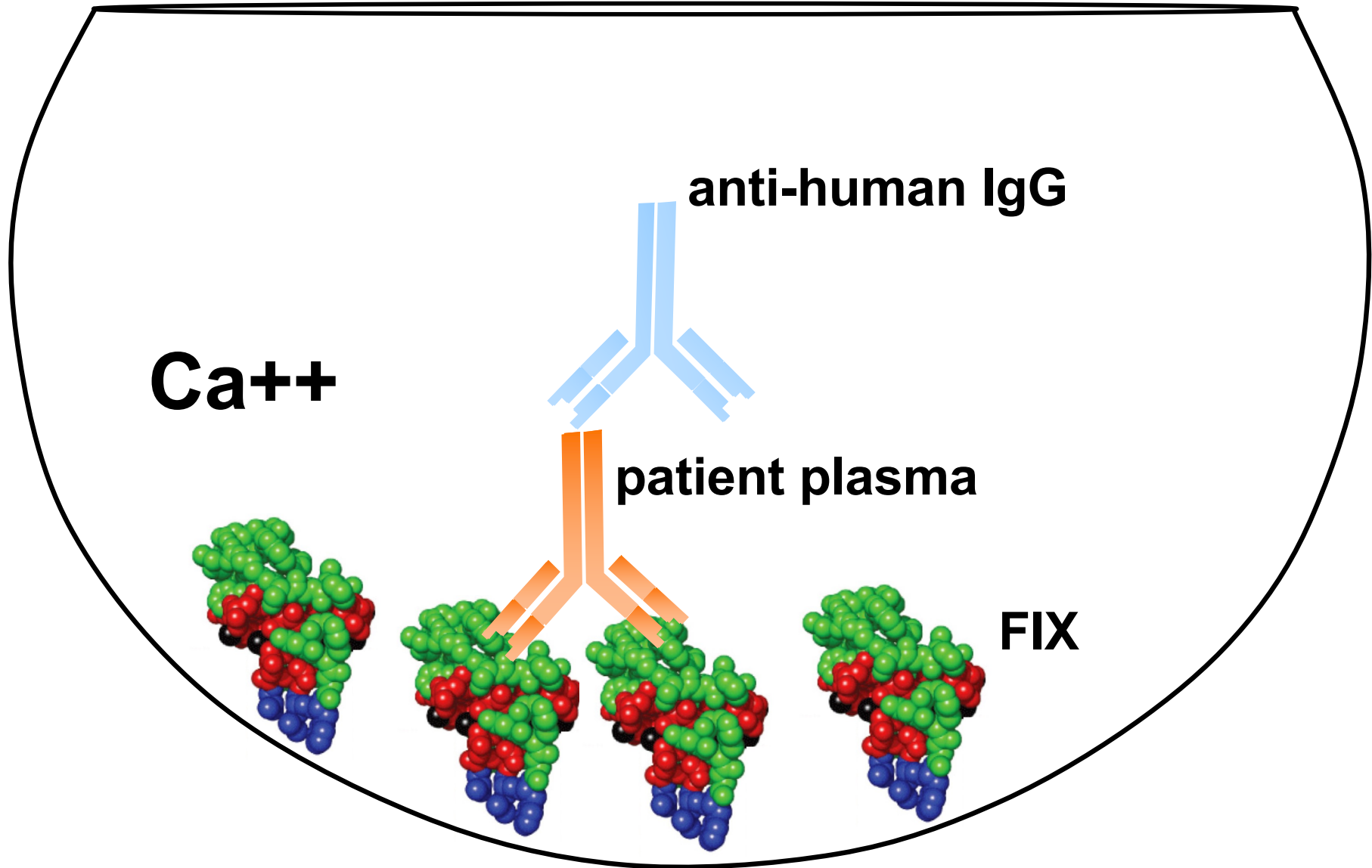


● hydrophobe aminozuren



fosfolipiden membraan

Detection of anti-FIX antibody in ELISA



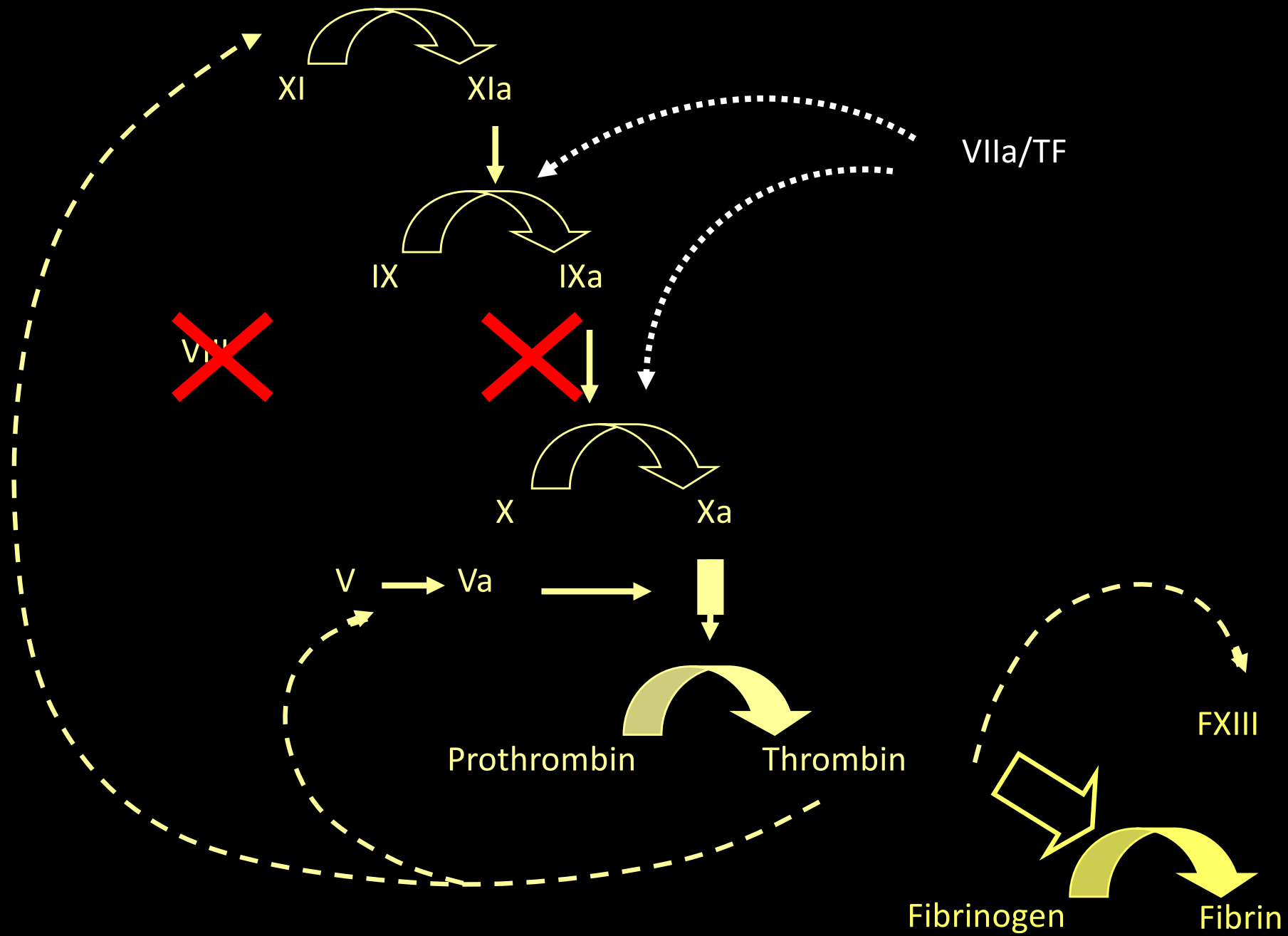
Behandeling

immunosuppressieve behandeling

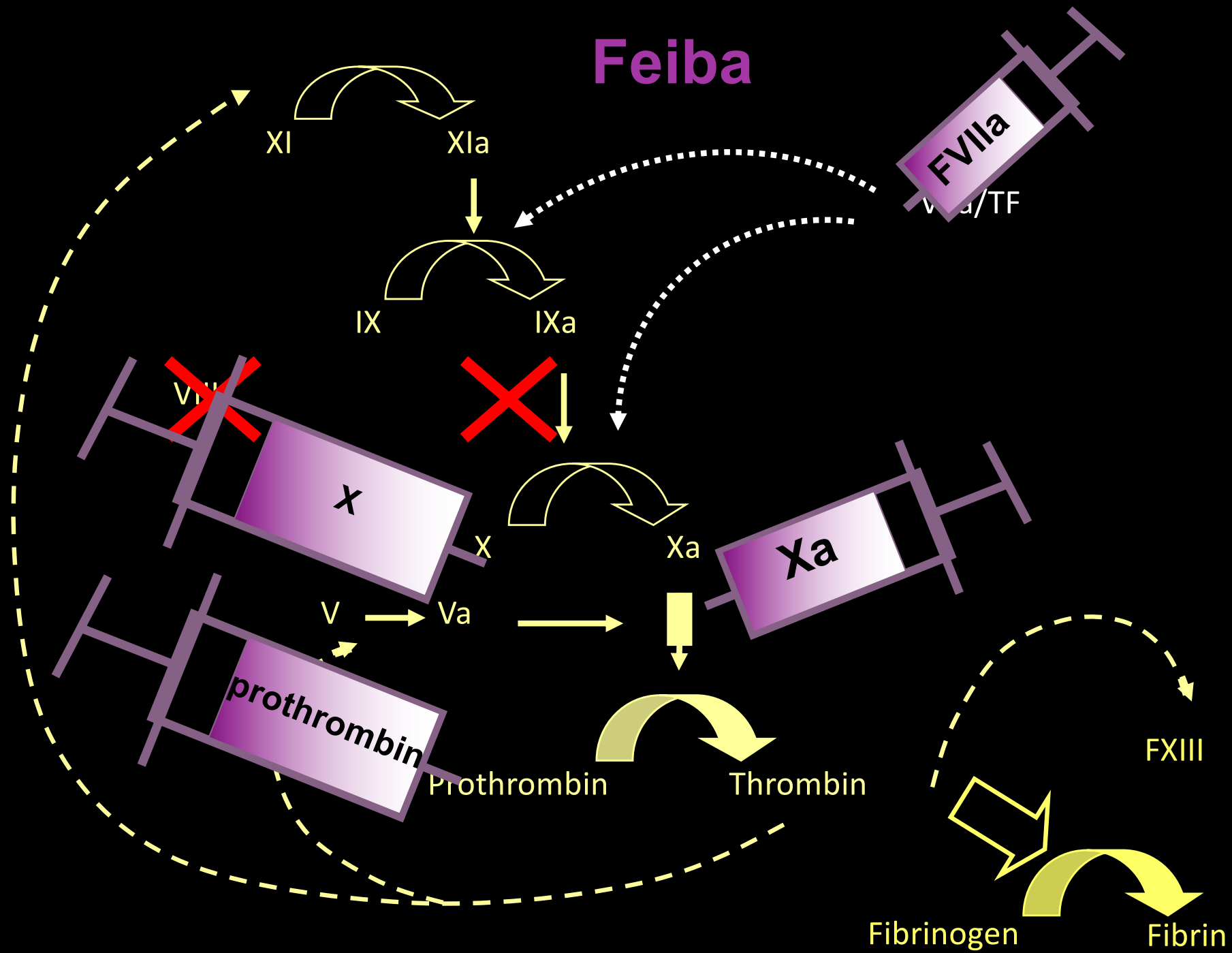
- corticosteroids**
- cyclofosfamide**
- anti-CD20**

Behandeling

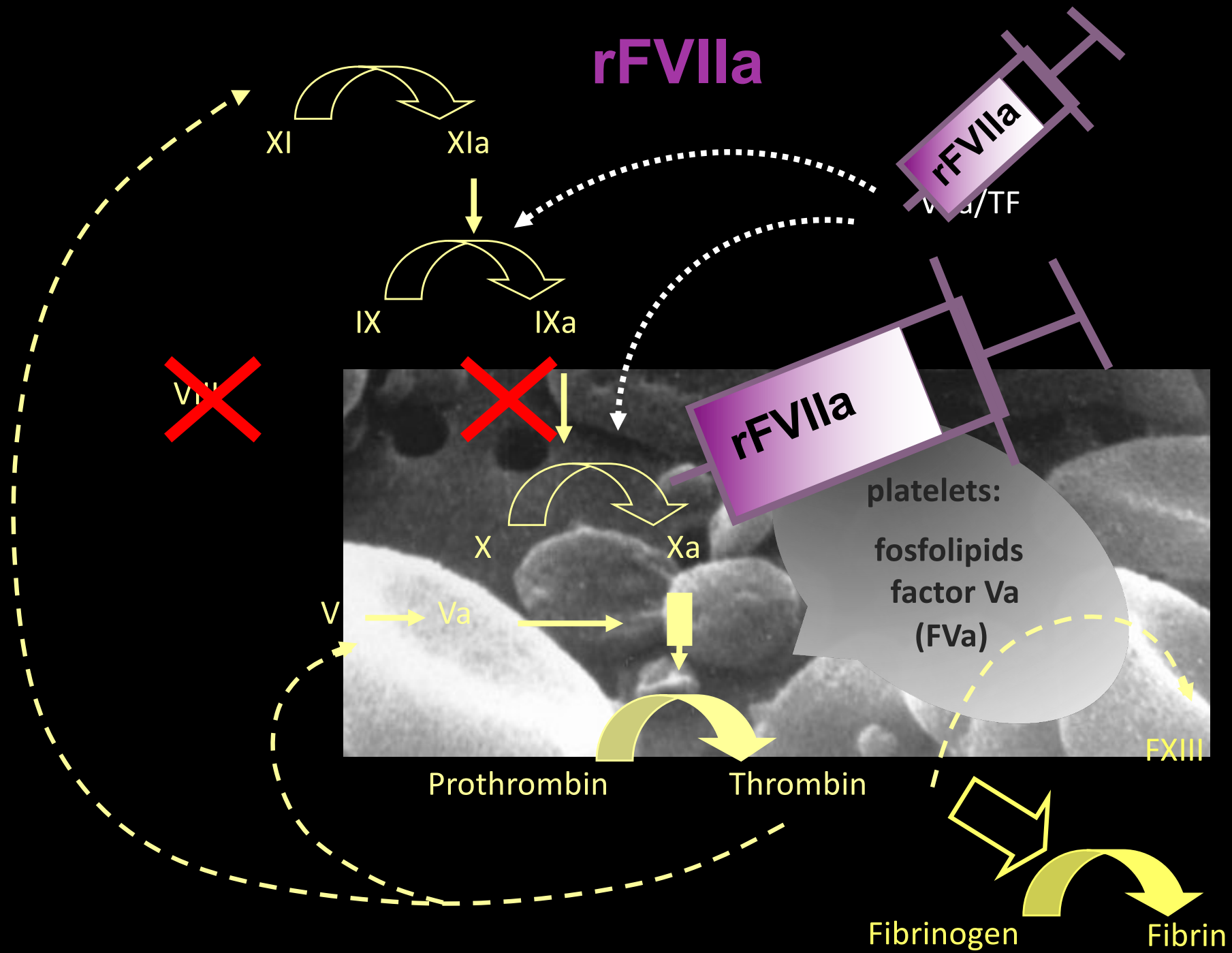
- “bypassing agents”
 - Feiba^R: FIX, FX, prothrombine, FXa, FVIIa
 - Novoseven^R: rFVIIa
 - Emicizumab^R: bispecific antibody FIXa/FX



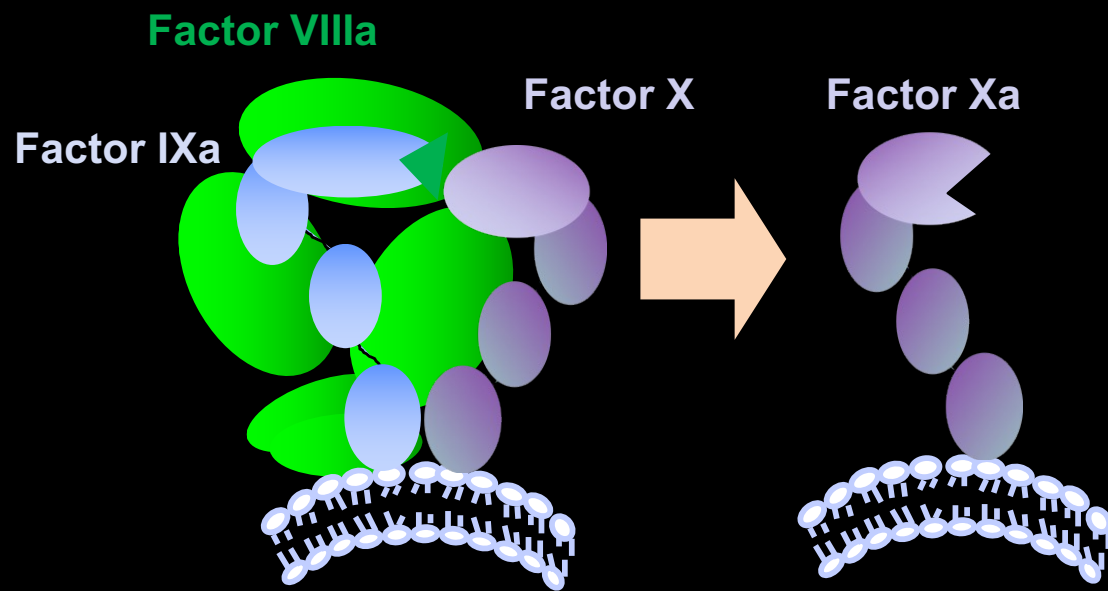
Feiba

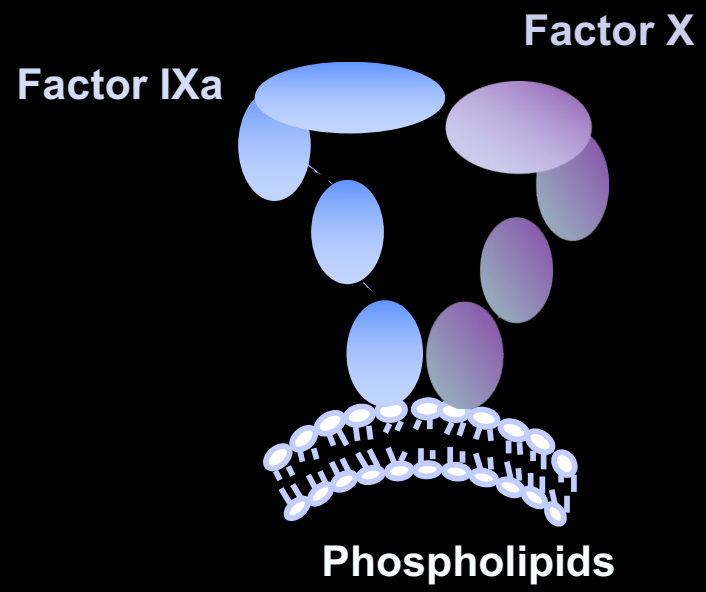


rFVIIa

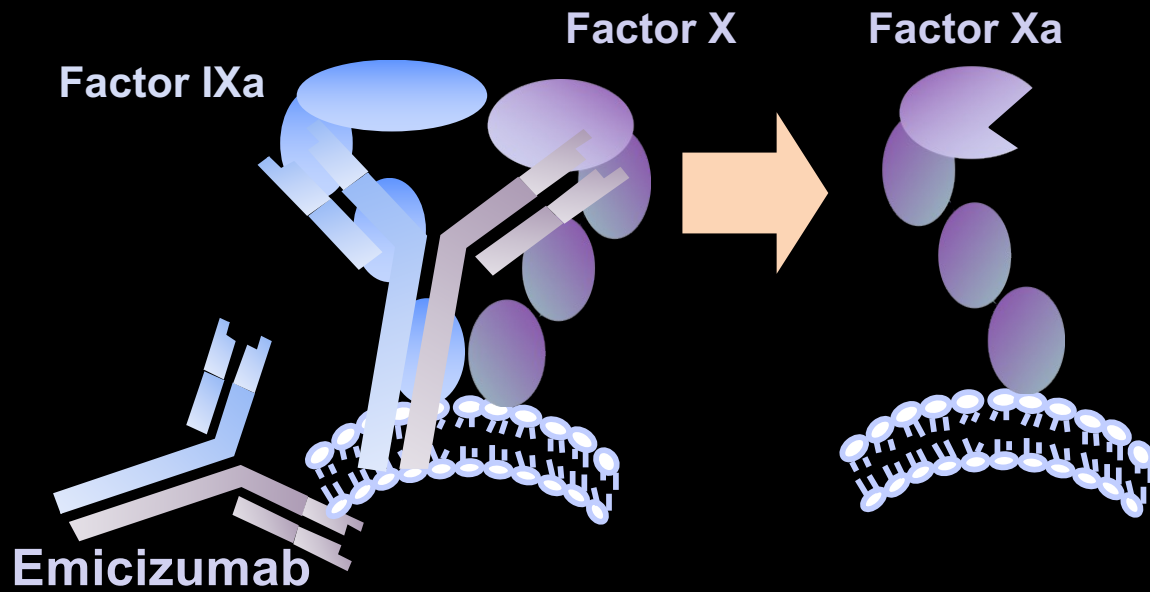


Emicizumab: bispeciefike antistof (FIXa en FX)





Emicizumab: bispecifike antistof (FIXa en FX)



To be continued

Verworven Von Willebrand ziekte

**Trombotische trombocytopenische purpura
(anti-ADAMTS13 Abs)**

MGUS

Bethesda test guidelines

LA guidelines

Monitoring van bypassing producten

Hartelijk dank

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