14ème symposium annuel

NTHC
Namur Thrombosis & Hemostasis Center

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Domaine de Ronchinne

Comment diagnostiquer les saignements d'origine indéterminée ?

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Disclosures

Company name	Advisory board	Consultant	Hemophilia nurse	Research support	Speakers fees	Stockholder	Travel grant
Amgen							√
CSL-Behring			√	√	√		√
Bayer	√	√	√	√	√		√
Biotest					√		
Boehringer Ingelheim	√						
Daiichi Sankyo	√						
Novartis				√			√
Novo Nordisk	√		√	√			√
Octapharma			√				√
OM					√		
OrPha Swiss	√						
Pfizer							√
Roche	√		√	√			√
Sanofi-Aventis					√		
Sanofi-Genzyme		√					
Shire / Takeda	√		√	√			
Siemens					√		
Sobi	√		√	√			√
Werfen				√	√		

Outline

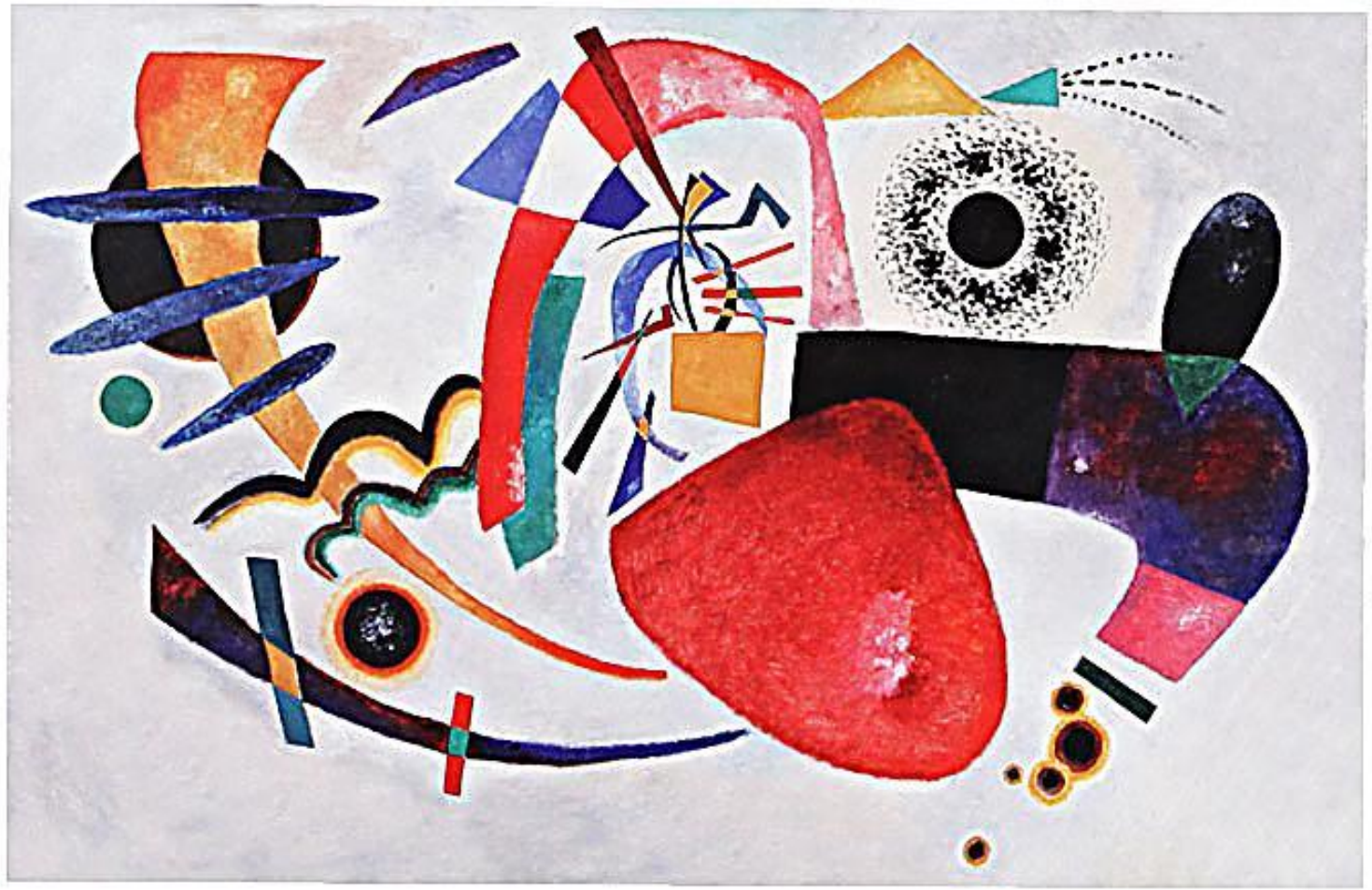
- How to achieve a diagnosis
- Standard work-up *and beyond*

How to achieve a Diagnosis

"Pattern recognition"

Having grasped the quintessence of a clinical picture
in order to recognize it
"at a glance"

The camel - Pablo Picasso (1881 – 1973)



Le point rouge II - Vassily Kandisky (1866 – 1944)

"Systematic-analytical approach"

In order not to miss what is rare

Kandisky's *Le point rouge II* put in good order by Ursus Wehrli (1969*)

"Pathopysiological reasoning"

To discover what you do not know yet

Le penseur - Auguste Rodin (1840 – 1917)

Standard work-up *and beyond*

Bleeding history

- “Gestalt”

- “ISTH-BAT”

Legend
BAT, Bleeding Assessment Tool
ISTH, International Society on Thrombosis and Haemostasis

1st step

- Complete blood count and blood smear

- Liver and kidney function

- Basic coagulation tests (PT, aPTT, thrombin time, fibrinogen)
- VWF:Ac, VWF:Ag
- Coagulation factor VIII (FVIII:C), IX, XI
- Factor XIII (transglutaminase activity)

Legend
:Ac, Activity
:Ag, Antigen
aPTT, Activated Partial Thromboplastin Time
:C, Coagulometric
PFA, Platelet Function Analyser
PT, Prothrombin Time
VWF, Von Willebrand Factor

Factor XIII

La storia di Pascale

48-year-old woman

Nose bleeding
Gingival bleeding (dental flossing)
Menorrhagia
Bleeding after tooth extraction
Postop. bleeding (3/5 x)

ISTH-BAT score 15 (normal <6)

La storia di Pascale

- CBC Hb 137 g/l, Hct 0.41 l/l; Lc 6.2 10E9/L; Plts 275 10E9/L

- PT 100 %
- aPTT 34 sec
- TT 16 sec
- Fibrinogen 2.8 g/L

- VWF Ac 90% ; Ag 104%
- FVIII:C 128%
- FIX:C 83%
- FXI:C 90%

- Factor XIII Ac 46% (70-140)

Legend
Ac, Activity
Ag, Antigen
:C, Coagulometric
CBC, Complete Blood Count

La storia di Pascale

- CBC Hb 137 g/l, Hct 0.41 l/l; Lc 6.2 10E9/L; Plts 275 10E9/L

- PT 100 %
- aPTT 34 sec
- TT 16 sec
- Fibrinogen 2.8 g/L

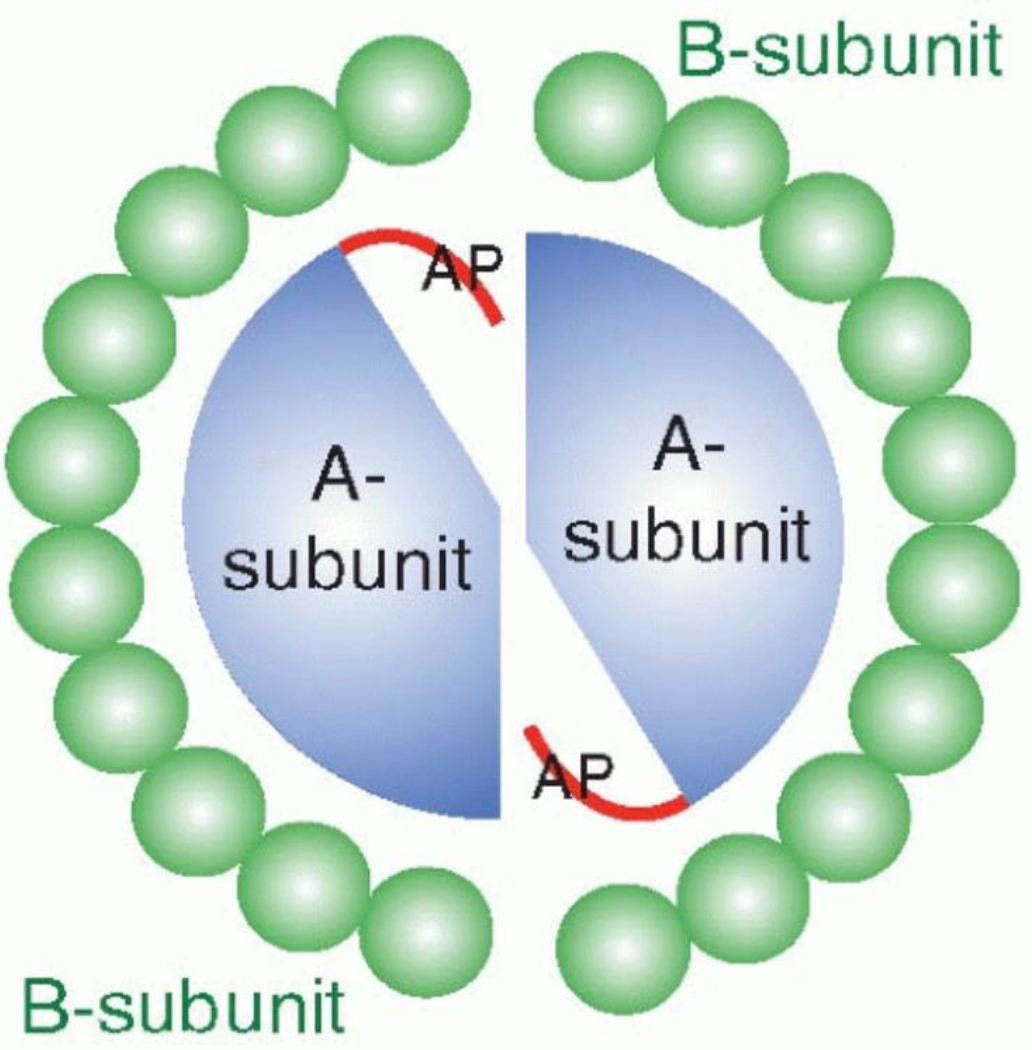
- VWF Ac 90% ; Ag 104%
- FVIII:C 128%
- FIX:C 83%
- FXI:C 90%

- Factor XIII Ac 46% (70-140)
  Ac 49%
  Ac 39%

Ag 120%

Legend
Ac, Activity
Ag, Antigen
:C, Coagulometric
CBC, Complete Blood Count

FXIII



The **activation peptide** of blood coagulation factor XIII (AP-FXIII)

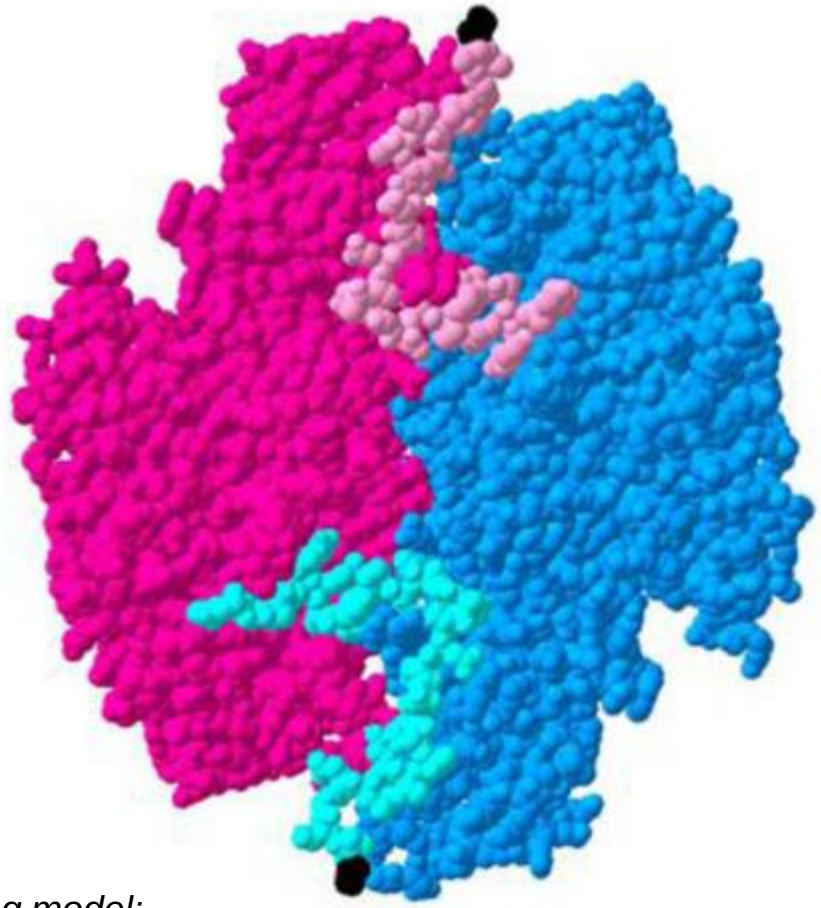
- has important functions in **stabilizing** the FXIII-A_2 dimer and **regulating** FXIII activation
- consists of 37 amino acids

Pro36 : Function not characterized
Arg37 : Thrombin cleavage site

Thromb Haemost 2018;118:2037

FXIII interaction with thrombin

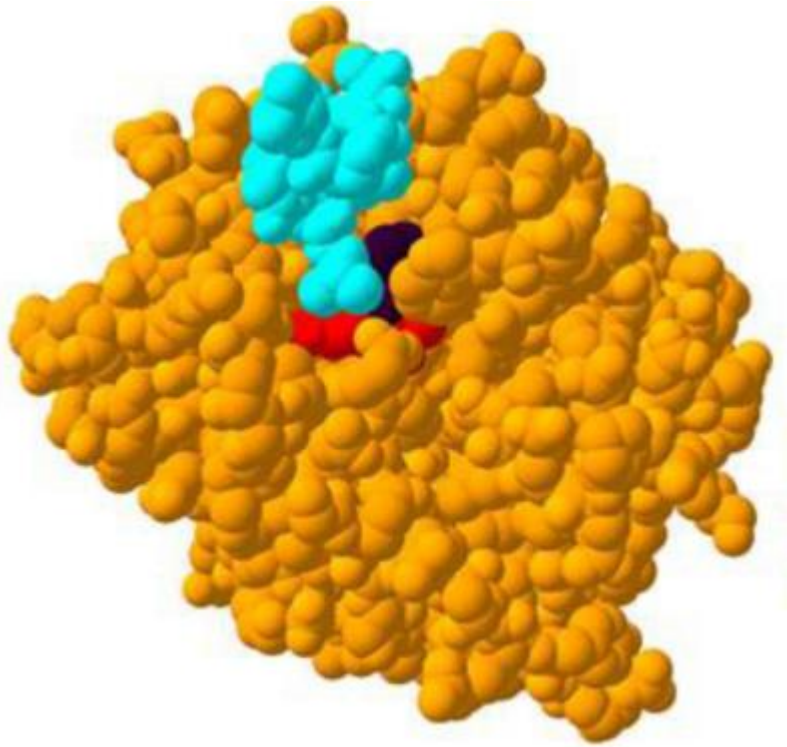
FXIII-A_2 dimer



Space-filling model:
One FXIII-A monomer is coloured in pink with its activation peptide in rose,
The other monomer is coloured in blue with its activation peptide in turquois.

The loop containing Pro36, with Pro36 coloured in black.

Complex of the AP-FXIII (28–37) with thrombin



Space-filling model:
AP-FXIII (28–35) : turquois
Pro36 : black
Arg37-Gly38 : red
Thrombin : orange

Thromb Haemost 2018;118:2037

FXIII-A: Pro36***Ser***-mutation

The Pro36Ser-mutation does not influence FXIII-A expression

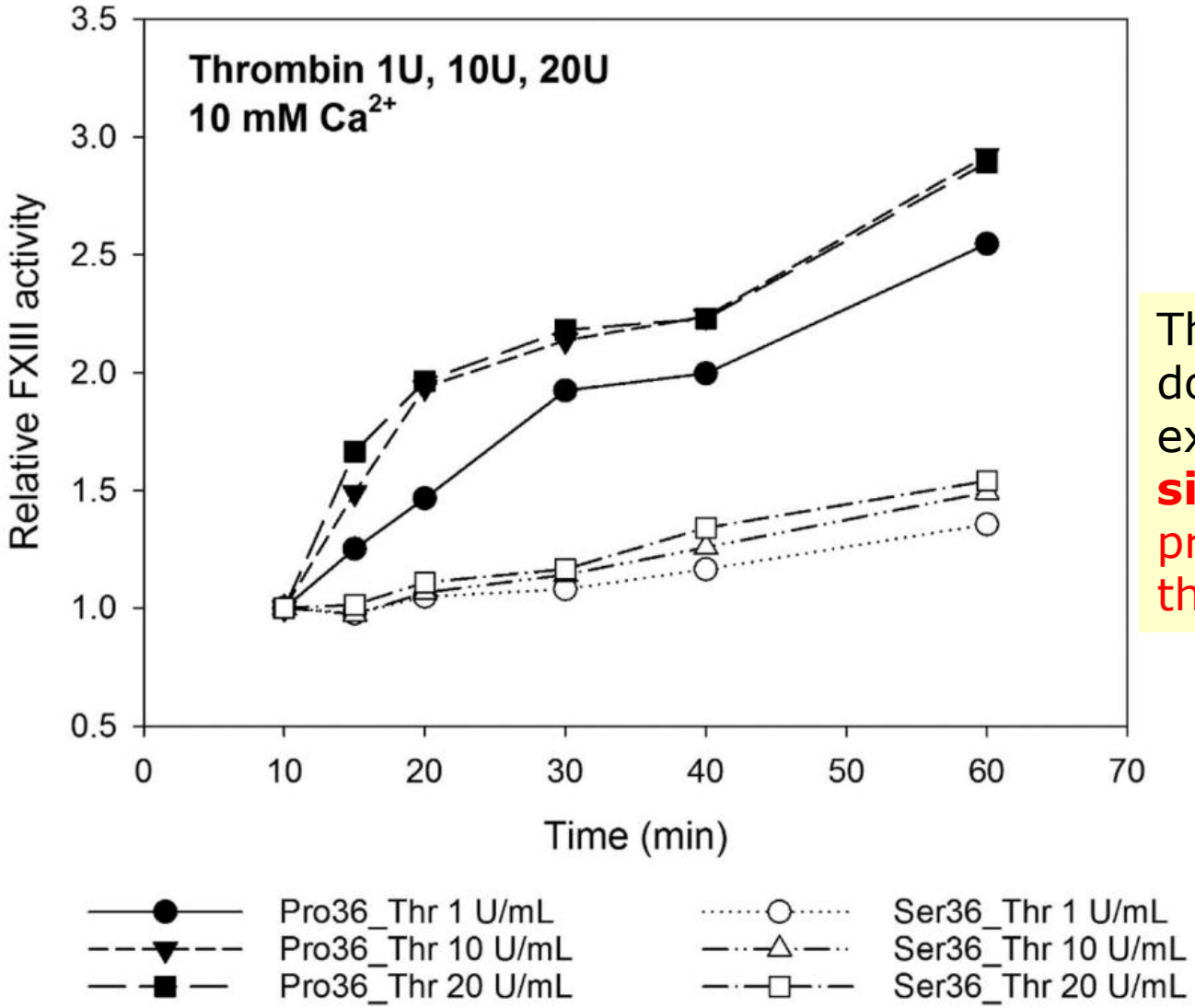
Western blot developed with a monoclonal anti-FXIII-A antibody.

From left to right:

- Protein marker band of 95 kDa (MW)
- Lysate from untransfected CHO cells (UT) as negative control
- Lysates from CHO cells transfected with wild-type FXIII-A Pro36 (WT P36)
- Lysates from CHO cells transfected with mutant FXIII-A Ser36 (Mu S36)
- Commercially available rFXIII-A as positive control (15 ng and 50 ng loaded)

Thromb Haemost 2018;118:2037

FXIII-A: Pro36***Ser***-mutation



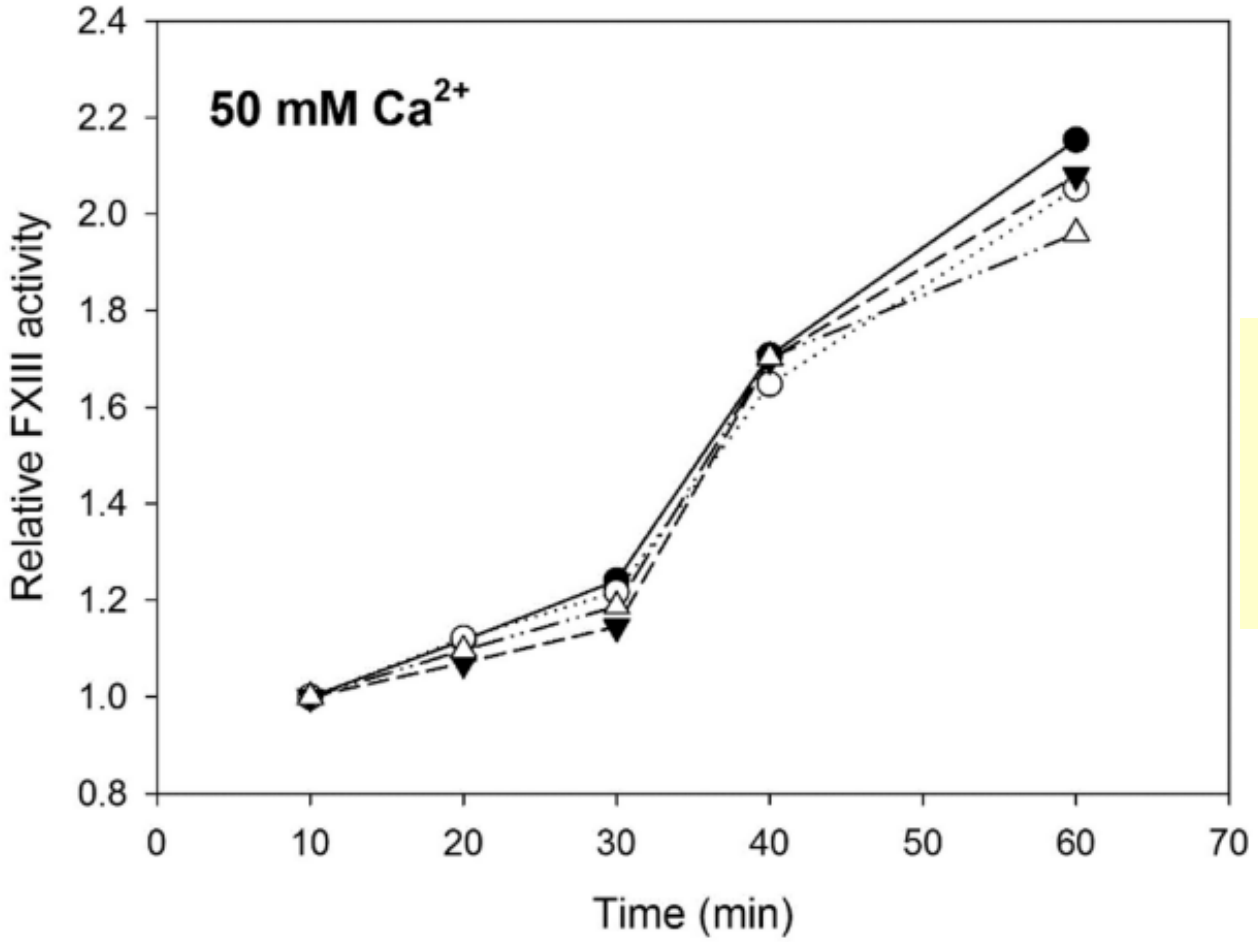
The Pro36Ser-mutation does not influence FXIII-A expression but **significantly inhibits** proteolytic activation by thrombin.

Filled symbols, Wild-type FXIII-A Pro36
Empty symbols, Mutant FXIII-A Ser36

Data shown are mean values from four experiments

Thromb Haemost 2018;118:2037

FXIII-A: Pro36***Ser***-mutation



The enzymatic transglutaminase activity is not affected as it can be induced in the presence of high Ca^{2+} concentrations.

Circles, Wild-type FXIII-A Pro36
Triangles, Mutant FXIII-A Ser36

Data shown are mean values from four experiments

Thromb Haemost 2018;118:2037

La storia di Pascale

A novel mutation in the
activation peptide of factor XIII (AP-FXIII)
representing the fourth case of
the rare FXIII-A type II deficiency.

Thromb Haemost 2018;118:2037

2nd step

- Blood smear
- Platelet aggregation test (LTA in PRP)
- Platelet secretion test (PRP)

- Chromogenic analysis of FVIII activity (FVIII:chr)

- Global analysis of fibrinolysis (Lysis Timer®)
- α2-antiplasmin

- Vitamin C

Legend
chr, Chromogenic
LTA, Light Transmission Aggregometry
PRP, Platelet-Rich Plasma

FVIII:chr

Legend
chr, Chromogenic

La storia di Luca

62-year-old man

Severe postop. bleeding (2/3 x)

ISTH-BAT score 7 (normal <4)

La storia di Luca

- CBC Hb 151 g/l, Hct 0.44 l/l; Lc 5.3 10E9/L; Plts 199 10E9/L

- PT 100 %
- aPTT 39 sec (24-38)
- TT 17 sec
- Fibrinogen 3.0 g/L

- VWF Ac 145% ; Ag 111%
- FVIII:C 54% (60-170)
- FIX:C 100%
- FXI:C 90%

FVIII:chr 13%

- Factor XIII Ac 115%

- Platelet functions normal

Legend
Ac, Activity
Ag, Antigen
:C, Coagulometric
CBC, Complete Blood Count

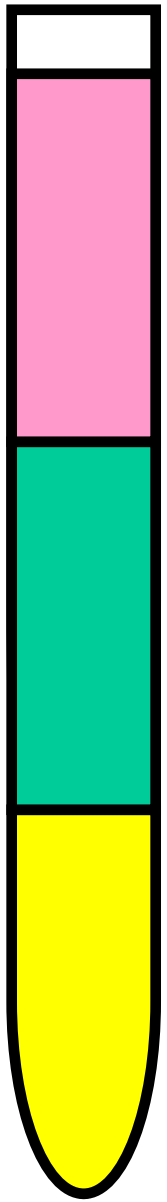
Measuring FXIII activity

Legend
:C, Coagulometric
:chr, Chromogenic

J Thromb Haemost 2016;14:248

FVIII:C

aPTT



100 µl 0.025 M $CaCl_2$ (37°C)
= Immediate start

100 µl aPTT-reagent

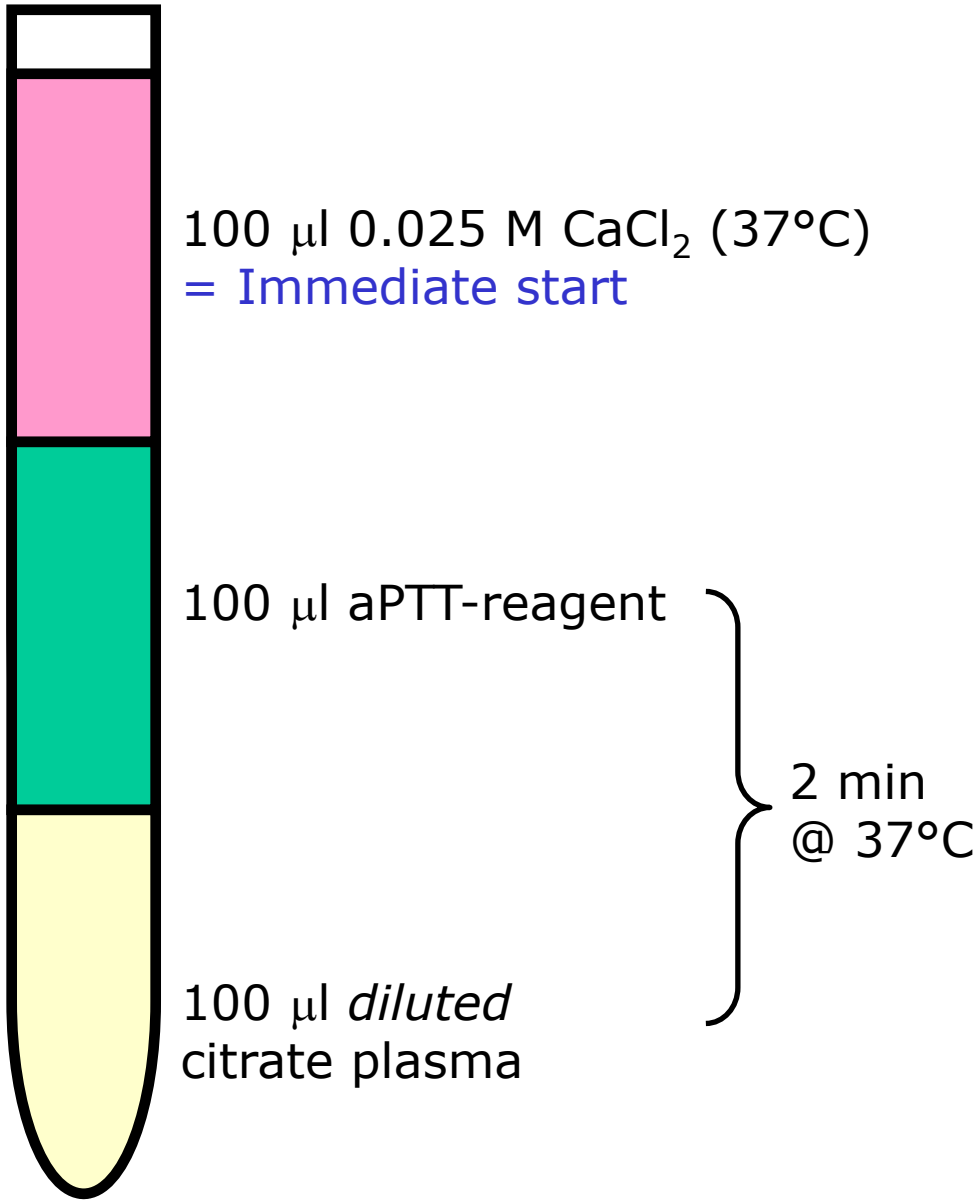
100 µl citrate plasma

2 min
@ 37°C

Legend
:C, Coagulometric
:chr, Chromogenic

J Thromb Haemost 2016;14:248

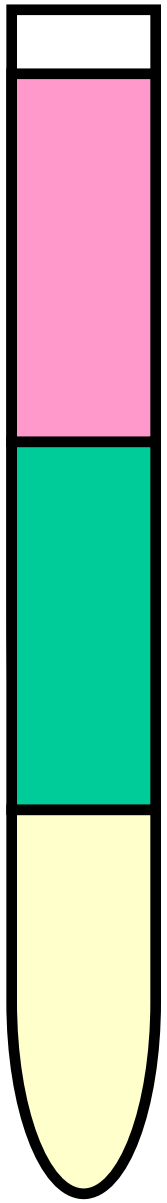
FVIII:C



Legend
:C, Coagulometric
:chr, Chromogenic

J Thromb Haemost 2016;14:248

FVIII:C



100 µl 0.025 M $CaCl_2$ (37°C)
= Immediate start (one stage)

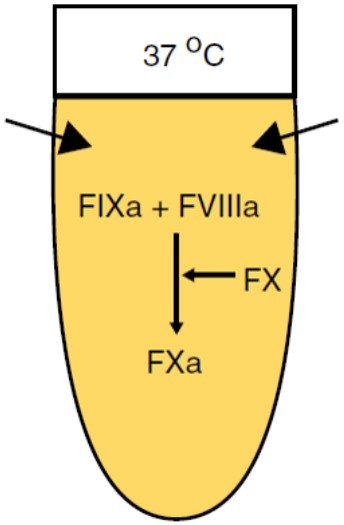
100 µl aPTT-reagent

2 min
@ 37°C

100 µl *diluted*
citrate plasma

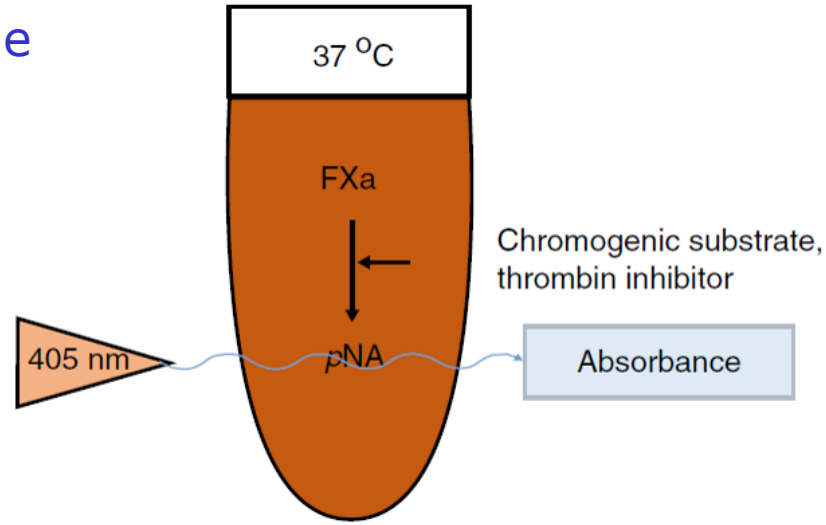
FVIII:chr

First stage



= Incubation step

Second stage

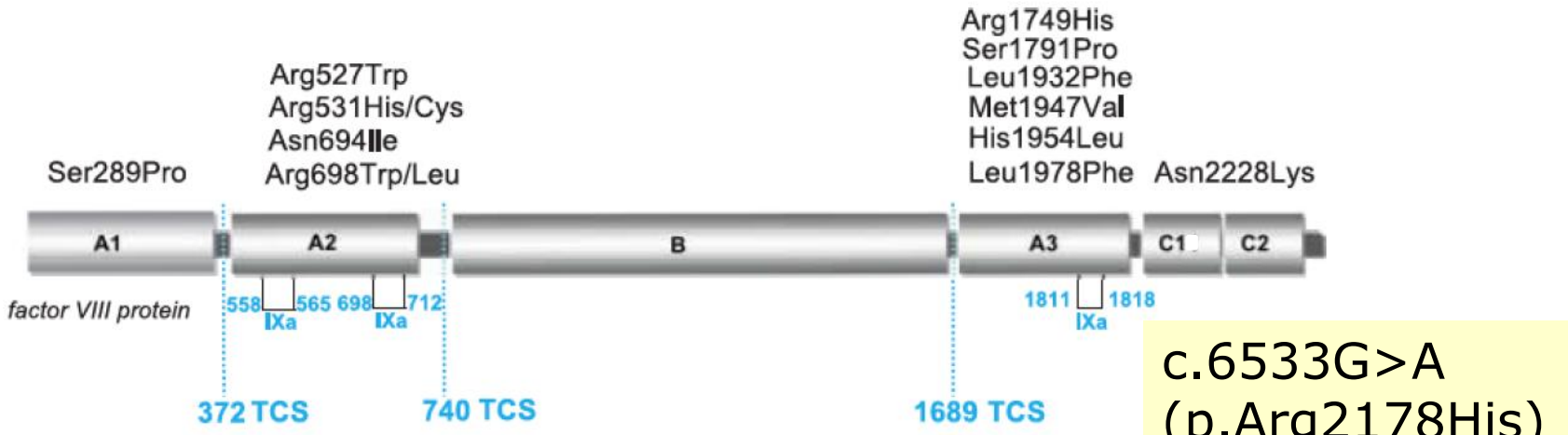


Legend
:C, Coagulometric
:chr, Chromogenic

J Thromb Haemost 2016;14:248

FVIII:C > FVIII:chr

Mutations causing reduced stability
of the FVIIIa heterotrimer give
lower results in the chromogenic assay



c.6533G>A
(p.Arg2178His)

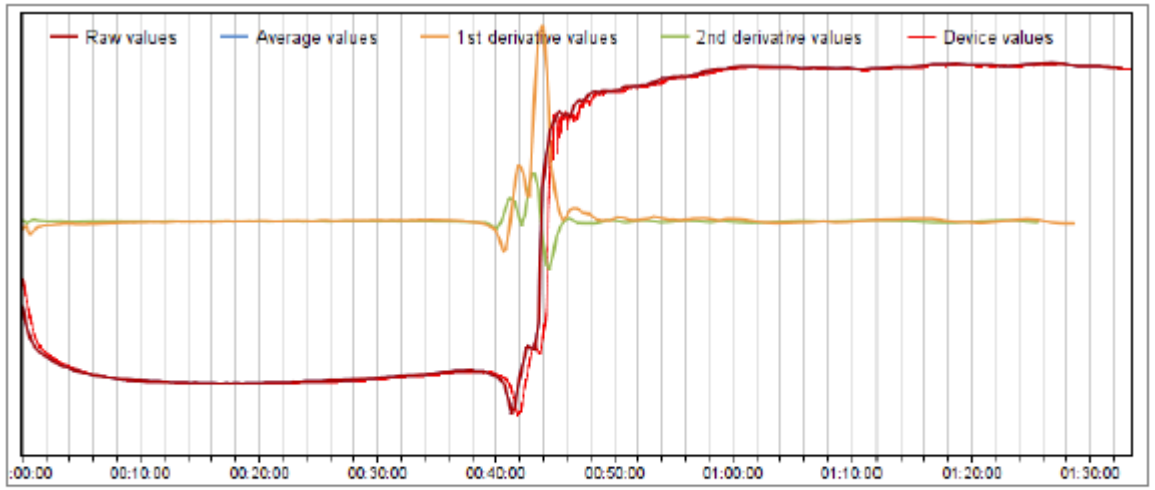
Eur J Hematol 2015;94(Suppl. 77):38

La storia di Luca

- A known pathogenic mutation in the C1 domain of FVIII that may be missed by coagulometric (one stage) FVIII activity measurement.

Fibrinolysis

Lysis Timer



Measure results

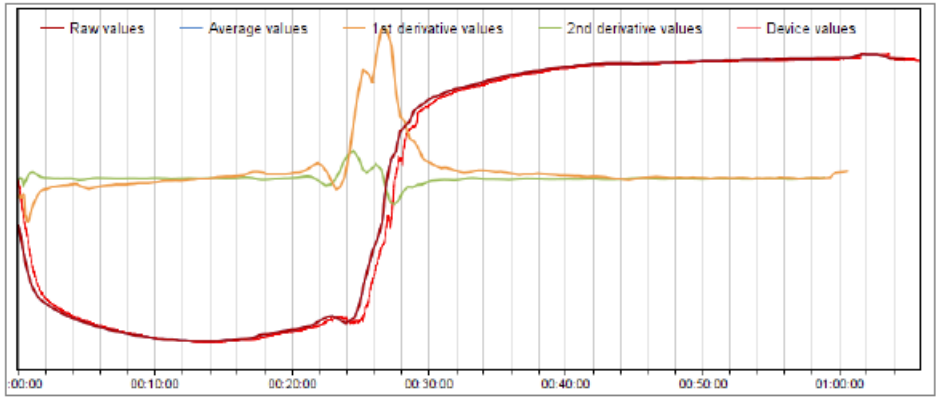
Lysis time (hh:mm:ss)	*Maximum slope*	*Average*
00:43:55	124.70	No smoothing

Reference range :

30 – 60 min

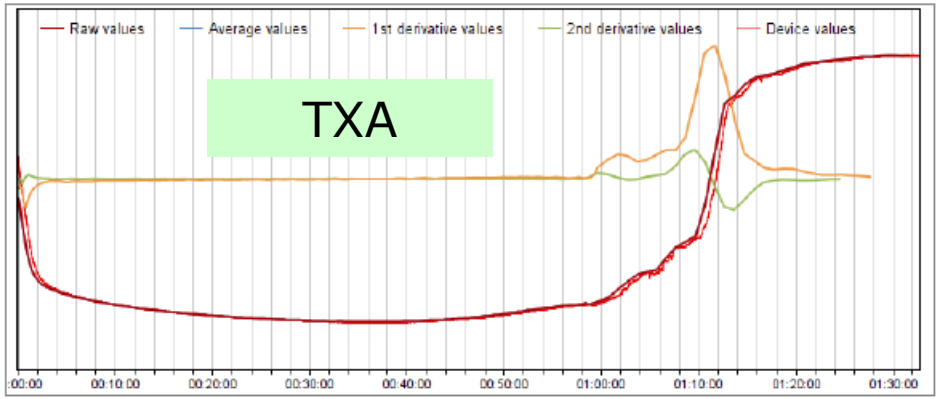
POINTS TO CONSIDER

- «Global screening assay»
- Exogenous t-PA
- May miss hyperfibrinolysis
- Affected by fibrinogen concentration



27 min

Maximum slope	*Average*
49.90	No smoothing



72 min

Maximum slope	*Average*
74.40	No smoothing

Legend
TXA, Tranexamic Acid

J Thromb Haemost 2023;21:2759

Case-by-case investigations

- Bethesda assay (factor VIII inhibitor quantitation)
- Fibrinogen antigen
- Mixing test (aPTT, TP, individual factors)
- Platelets, electron microscopy
- Thrombin generation (various methods)
- VWF:CB
- VWF:FVIII-binding assay
- VWF:MM

Legend
VWF, Von Willebrand Factor
VWF:CB, VWF collagen binding activity
VWF:MM, VWF multimer analysis

3rd step

- Platelet flow cytometry

La storia di Maria

46-year-old woman

Easy bruising, menorrhagia,
postop. bleeding (3x)

ISTH Bleeding score 14 (normal <6)

VWF: normal

Platelet count: normal
Platelet functions: normal

Coagulation factors: normal

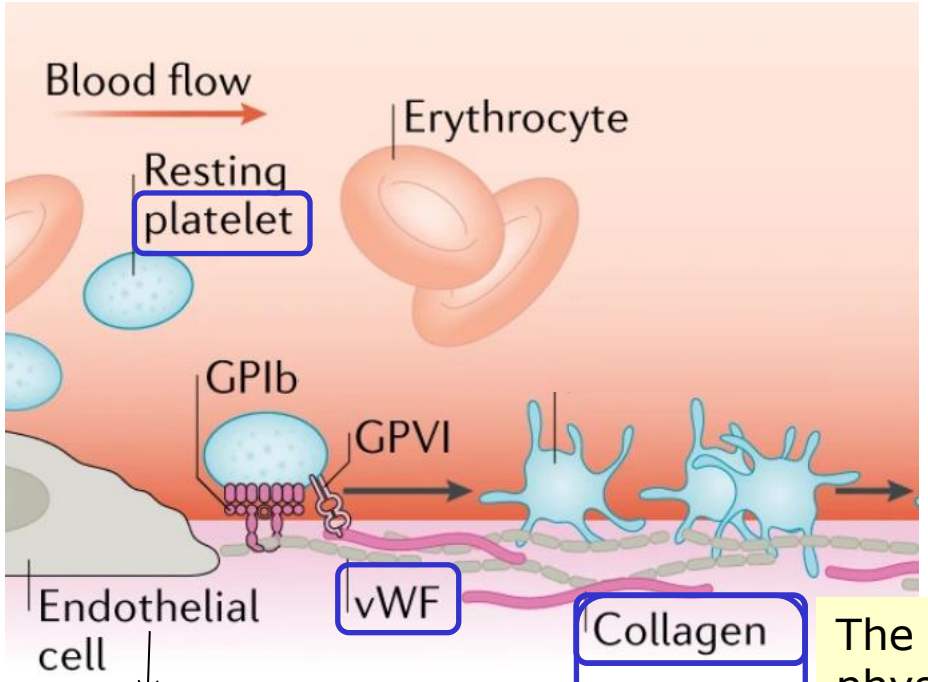
Fibrinolysis: normal

Defect **?**

→ Bleeding disorder of unknown cause (**BDUC**)

Legend:
ISTH, International Society on Thrombosis and Haemostasis
VWF, von Willebrand factor

At the site of a vessel wall injury

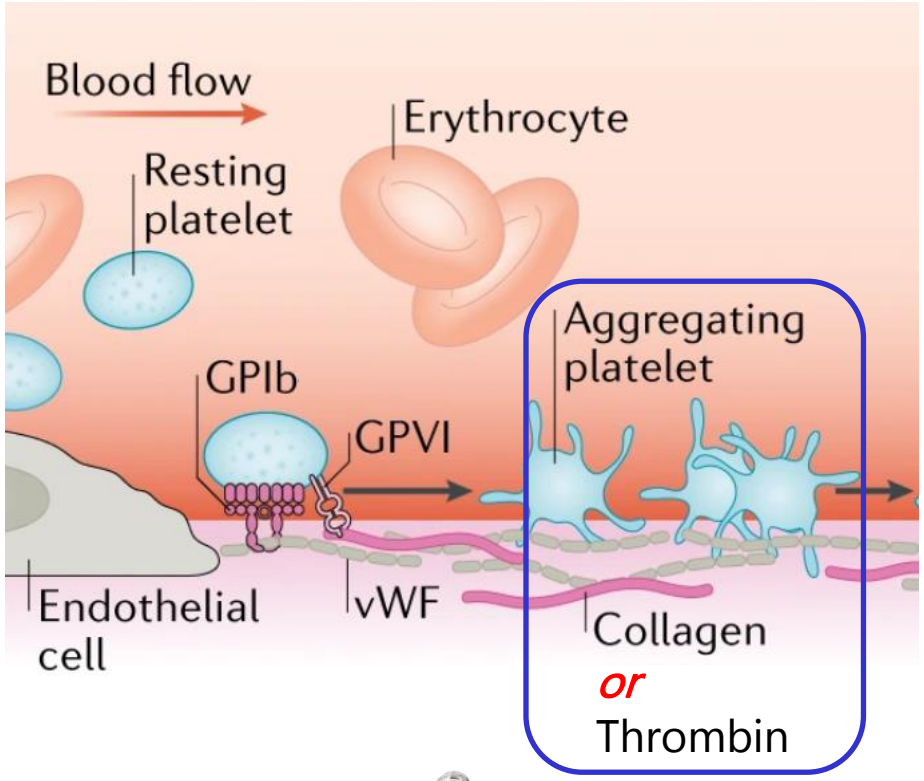


The strongest physiologic platelet agonists

Legend
GP, Glycoprotein
GPIb, VWF receptor on platelets
GPVI, Collagen receptor on platelets
VWF, von Willebrand factor

Nat Rev Cardiol 2019;16:166

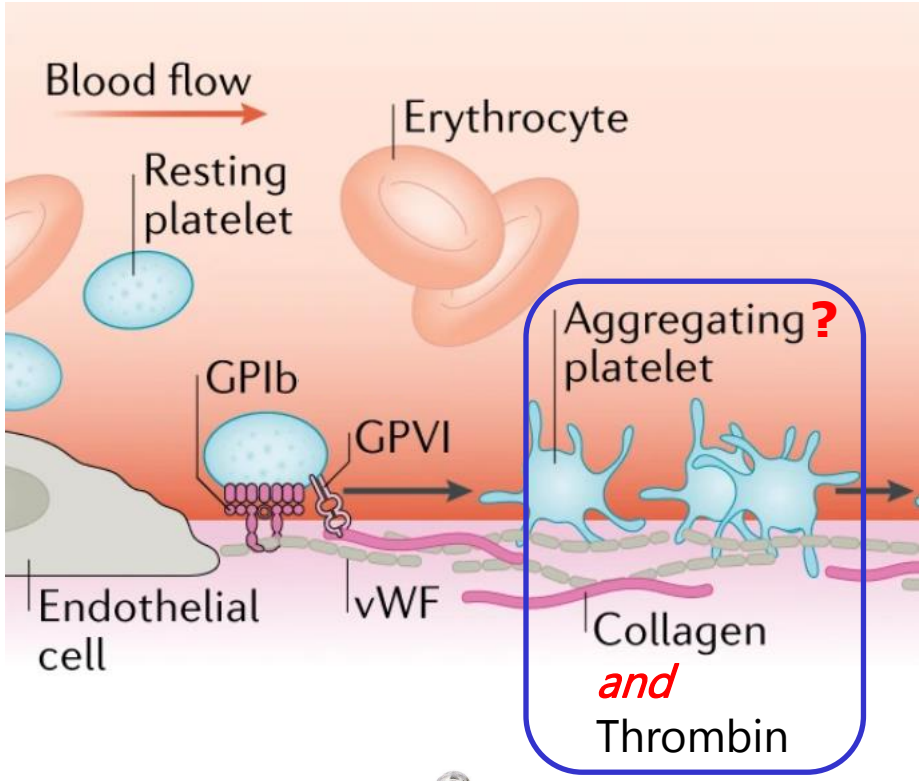
Collagen *or* Thrombin activated platelets aggregate

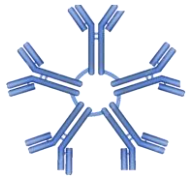


Legend
GP, Glycoprotein
GPIb, VWF receptor on platelets
GPVI, Collagen receptor on platelets
VWF, von Willebrand factor

Nat Rev Cardiol 2019;16:166

Do Collagen *and* Thrombin activated platelets aggregate ?



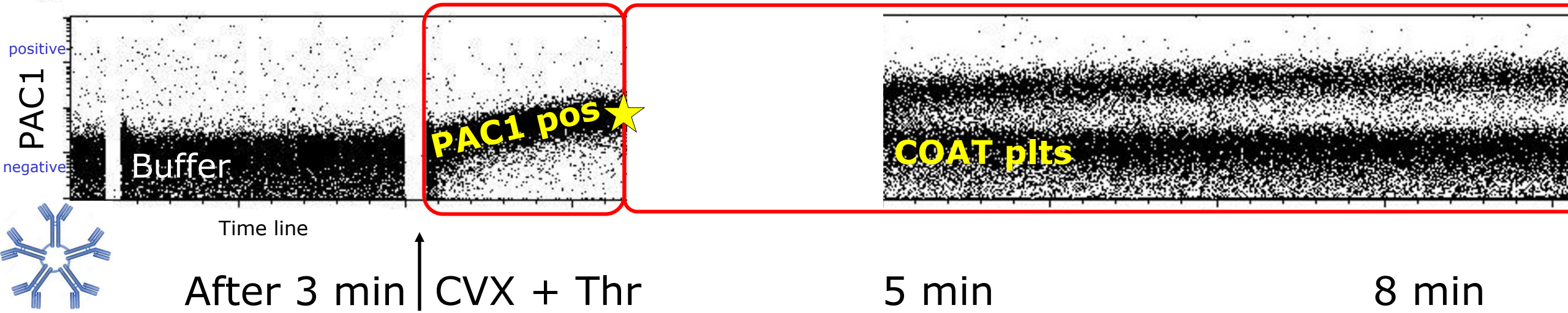


PAC1
binds only to aggregating platelets

Legend
GP, Glycoprotein
GPIb, VWF receptor on platelets
GPVI, Collagen receptor on platelets
VWF, von Willebrand factor

Nat Rev Cardiol 2019;16:166

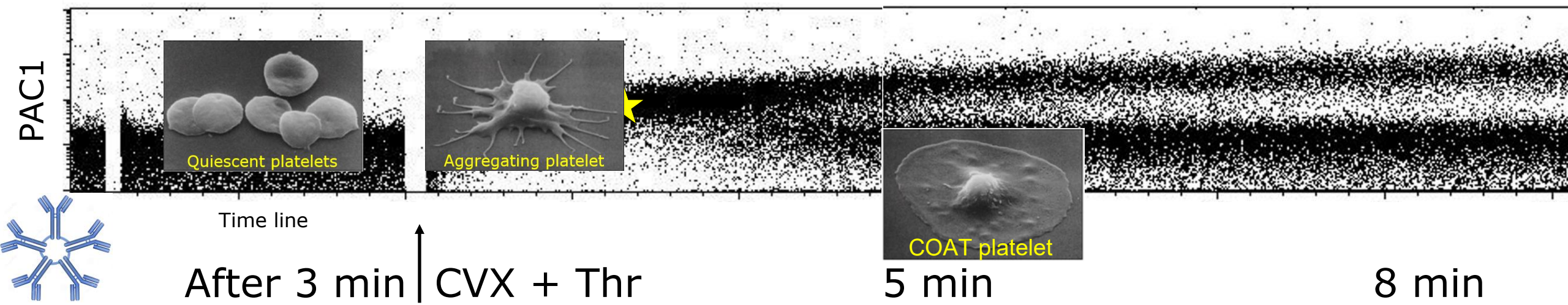
Visualization of COllagen And Thrombin activated platelets



Legend:
COAT plts, collagen and thrombin activated platelets
CVX, Convulxin (agonist of the collagen receptor GPVI)
GPIIb-IIIa, Fibrinogen receptor on platelets
PAC1, IgM recognizing the activated GPIIb-IIIa (= aggregating platelets)
Plts, Platelets
Thr, Thrombin

Thromb Haemost 2017;117:1101

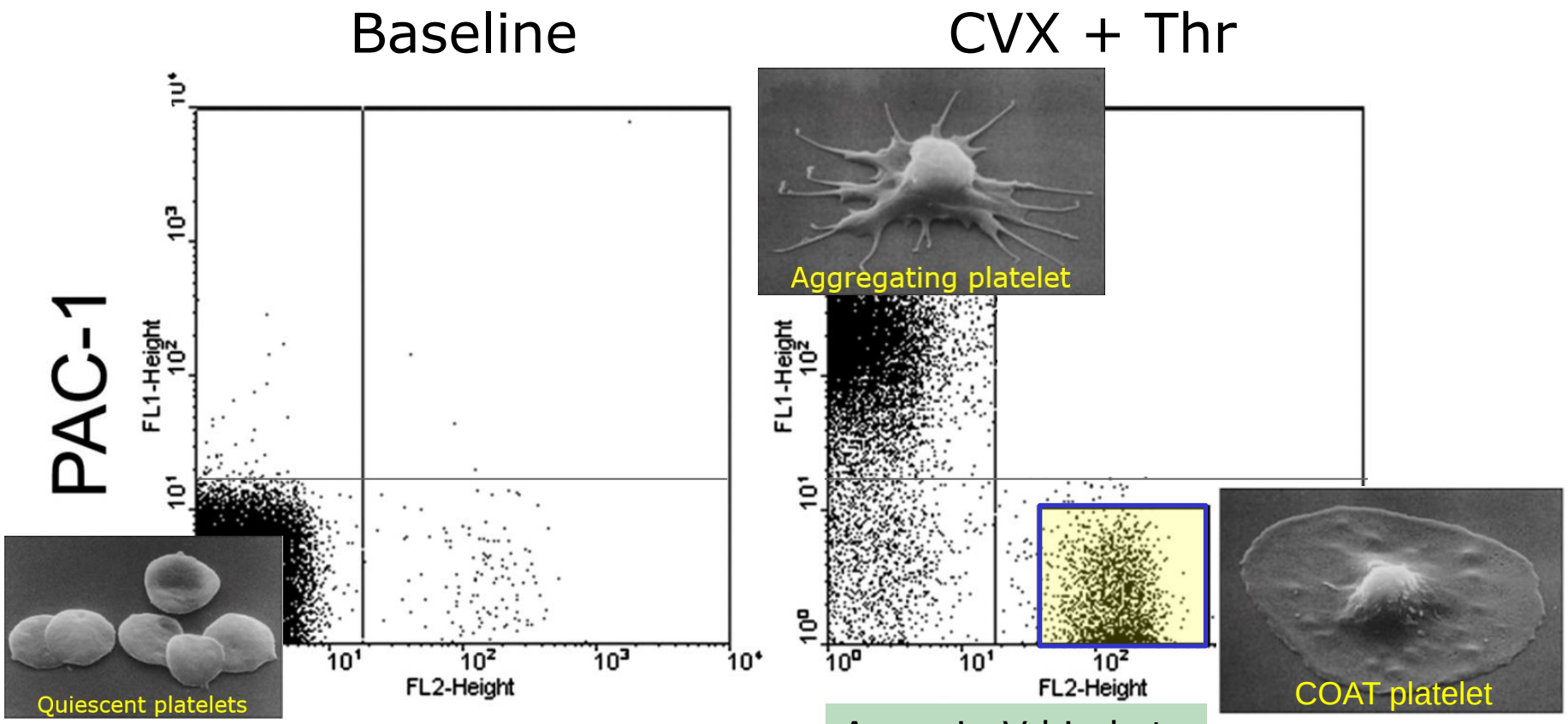
Visualization of COllagen And Thrombin activated platelets



Legend:
COAT plts, collagen and thrombin activated platelets
CVX, Convulxin (agonist of the collagen receptor GPVI)
GPIIb-IIIa, Fibrinogen receptor on platelets
PAC1, IgM recognizing the activated GPIIb-IIIa (= aggregating platelets)
Plts, Platelets
Thr, Thrombin

Thromb Haemost 2017;117:1101

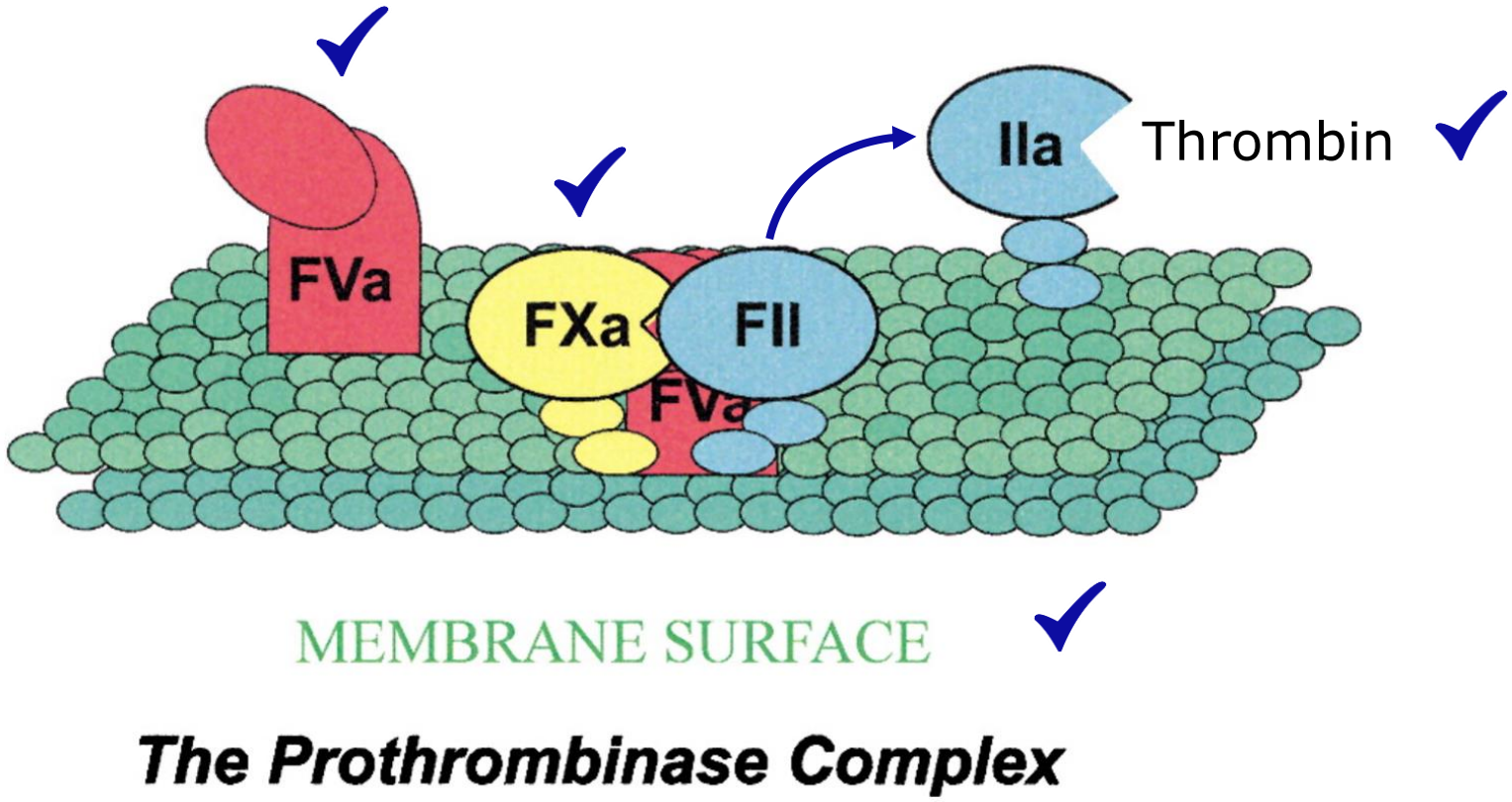
COAT plts do not bind PAC1 → cannot aggregate



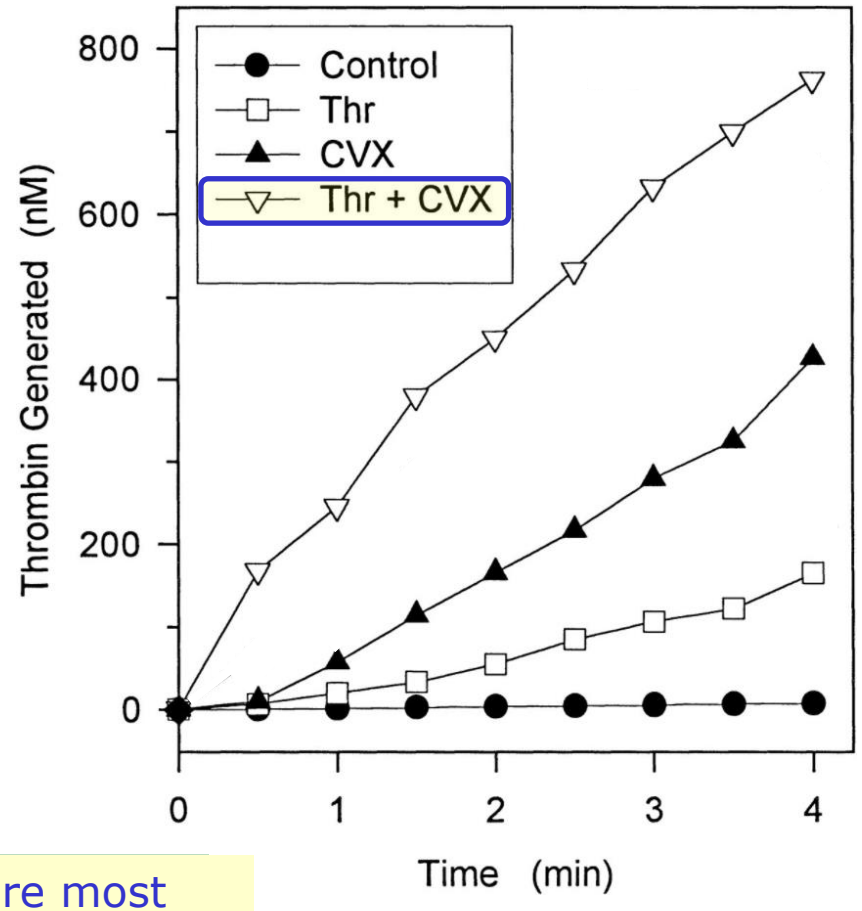
Legend:
Annexin V, Probe for negatively charged phospholipids
GPIIb-IIIa, Fibrinogen receptor on platelets
PAC1, IgM recognizing activated GPIIb-IIIa (= aggregating platelets)

Nature 2002;415:175 | *Biophys J* 2012;102:2261

Are COAT plts procoagulant ?



The Prothrombinase Complex



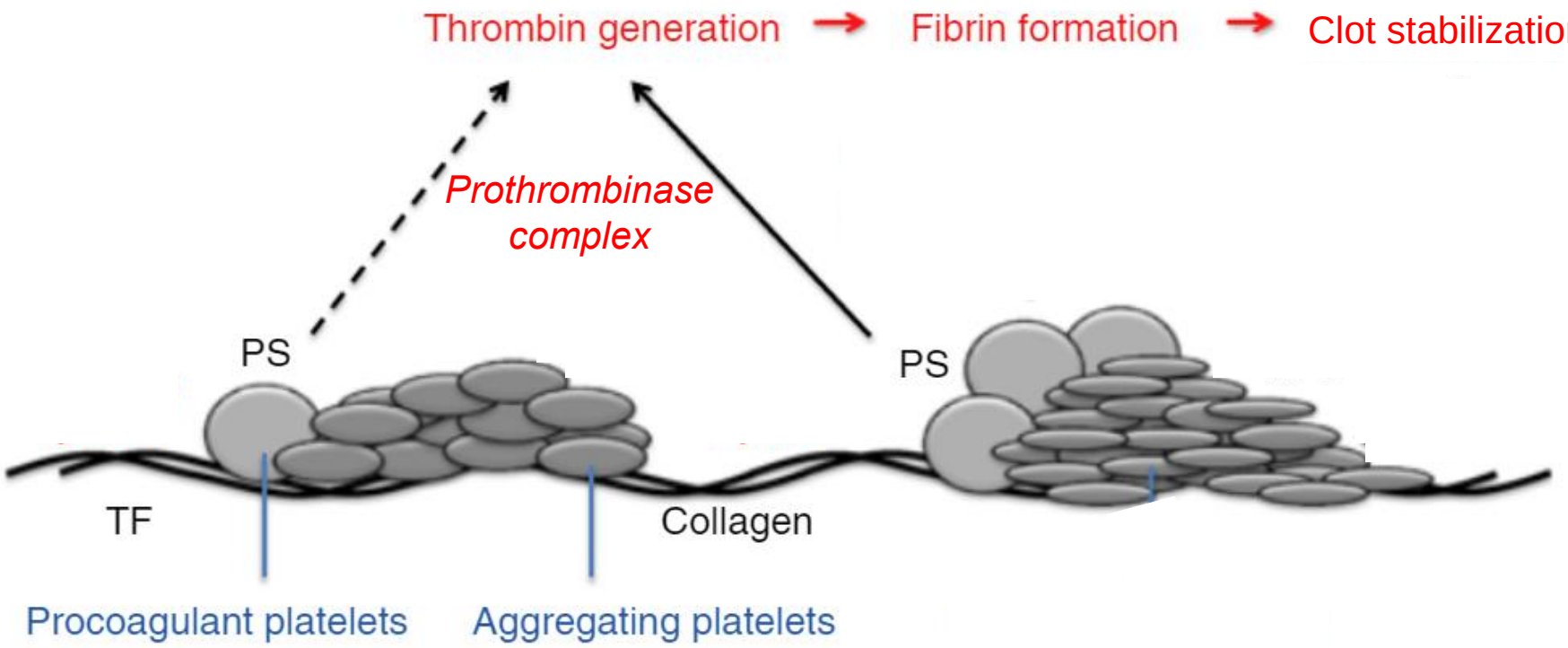
COAT plts are most efficient in sustaining thrombin generation

phospholipids

Legend
a, Activated for of the coagulation factor
Annexin V, Probe for negatively charged phospholipids
CVX, Convulxin (agonists of the collagen receptor GPVI)
F, Coagulation factor
Thr, Thrombin (= FIIa)

Blood 2000;95:1694 | *Nature* 2002;415:175

Aggregating & Procoagulant COAT plts



Collagen + Thrombin →

Thrombin generation and fibrin formation

Legend:
PS, Phosphatidylserine (A negatively charged phospholipid)
TF, Tissue factor

J Thromb Haemost 2013;11:2

La storia di Maria

46-year-old woman

Easy bruising, menorrhagia, postop. bleeding (3x)

ISTH Bleeding score 14 (normal <6)

VWF: normal

Platelet count: normal
Platelet functions: normal

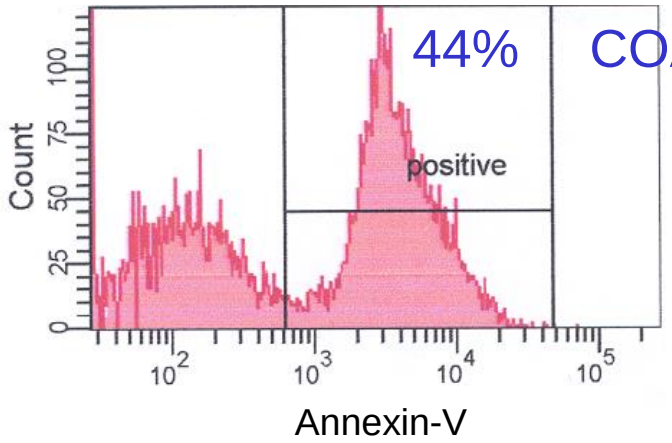
Coagulation factors: normal

Fibrinolysis: normal

Impaired COAT plt generation →
Defect platelet procoagulant activity

COllagen And Thrombin activated plts:

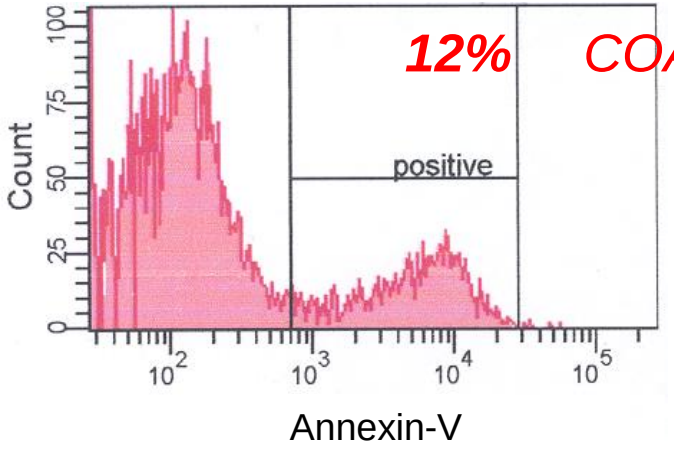
Control



COAT plts

Reference range
25%-55%

Maria



COAT plts

Legend:
Annexin V, probe for negatively charged phospholipids
ISTH, International Society on Thrombosis and Haemostasis
VWF, von Willebrand factor

Procoagulant platelets

Our experience

Patients with clinically relevant bleeding diathesis and non-diagnostic standard laboratory work-up,
i.e. **Bleeding Disorders of Unknown Cause** (BDUC)

1st cohort (01.2007 – 12.2011, Bern):

16/67 patients (**24%**) had a **decreased COAT platelet generation**.

Cytometry B Clin Cytom 2014;86:397

2nd cohort (01.2012 – 03.2017, Bern):

10/53 patients (**19%**) had a **decreased COAT platelet generation**.

J Thromb Haemost 2019;17:1104

3rd cohort (01.2015 – 09.2021, Lausanne):

10/37 patients (**27%**) had a **decreased COAT platelet generation**.

J Thromb Haemost 2022;20:1271

A decreased ability to form procoagulant platelets appears to be a **relatively frequent** platelet function defect, ***which is missed by conventional diagnostics***.

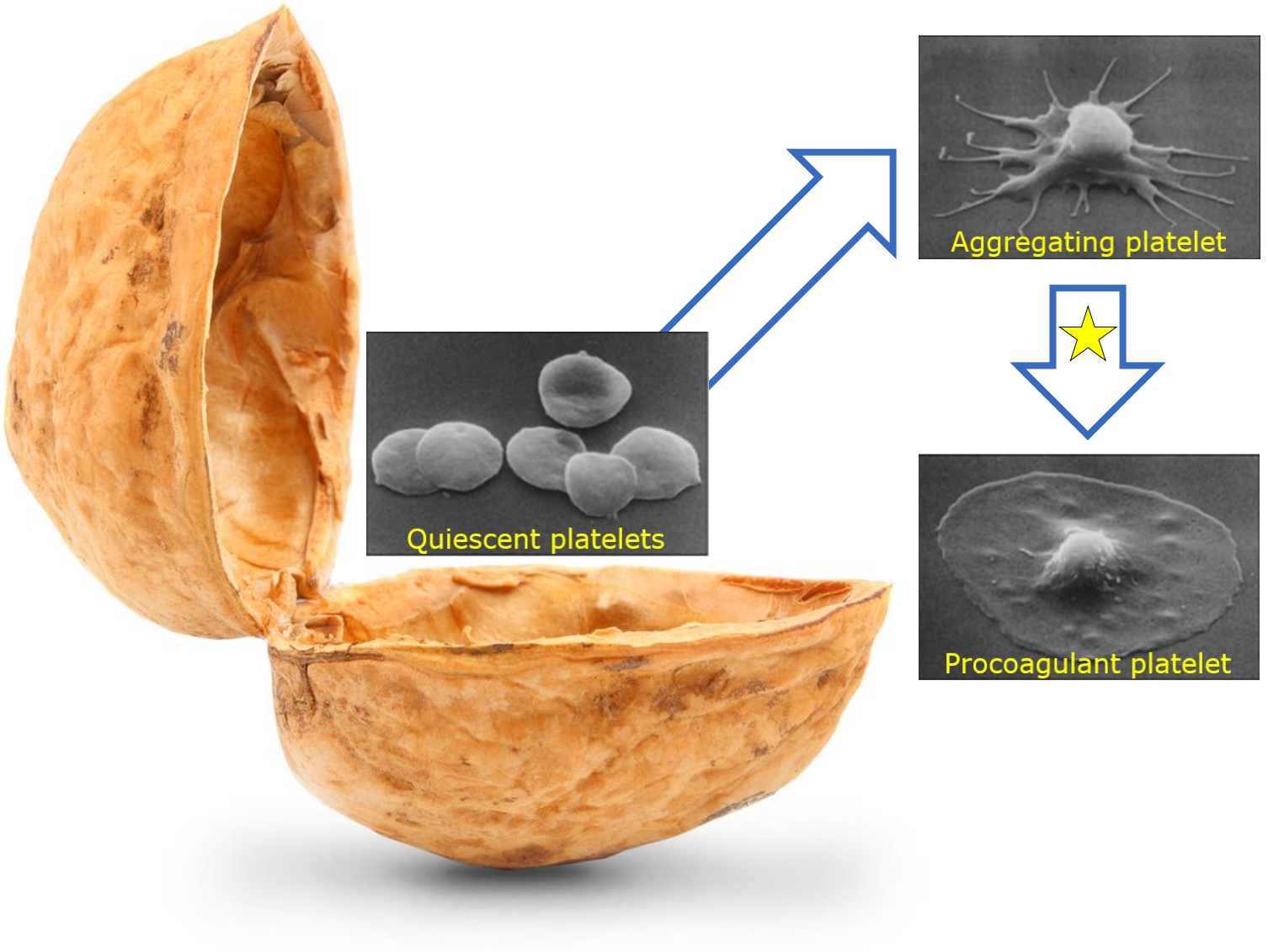
Synthesis : *Beyond BDUC*

- Standardised work-up
- Be curious & think pathopysio-logically
- *FXIII ; FVIII:chr ; Procoagulant plts (!)*
- *Fibrinolysis (?)*

Thank you for your attention !

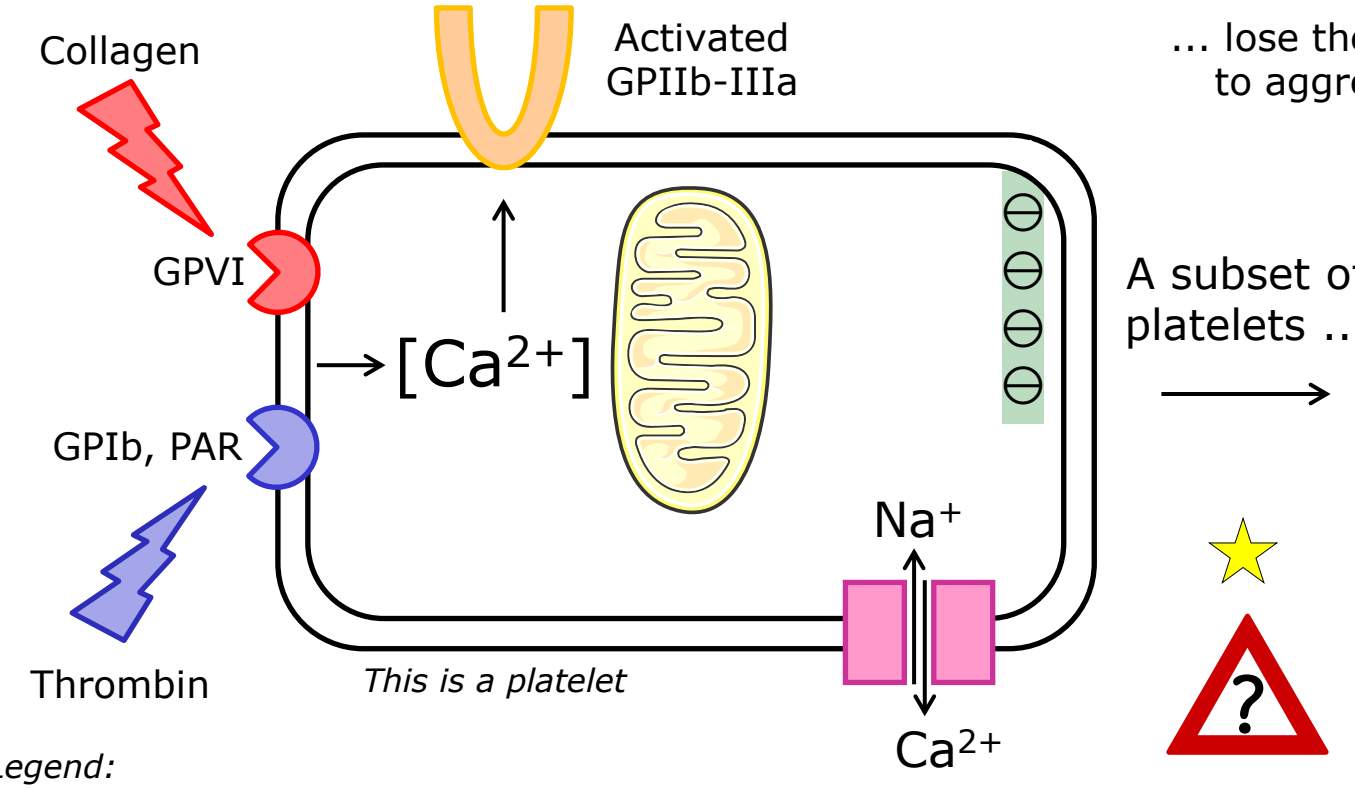
Mechanisms underlying COAT platelet formation ?

25 years of research *in a nutshell*



COAT platelets ... *in a nutshell*

Aggregating platelets — *Forward* mode **NCX** *Reverse* mode — Procoagulant COAT platelets



... lose the ability to aggregate

A subset of platelets ...

Legend:
⊖⊖⊖⊖, Negatively charged phospholipids
GP, Glycoprotein
GPIb, Receptor for von Willebrand factor
GPIIb-IIIa, Receptor for fibrinogen
GPVI, Receptor for collagen
MPTP, Mitochondrial permeability transition pore
NCX, Sodium-Calcium Exchanger
PAR, Protease activated receptors

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merci
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