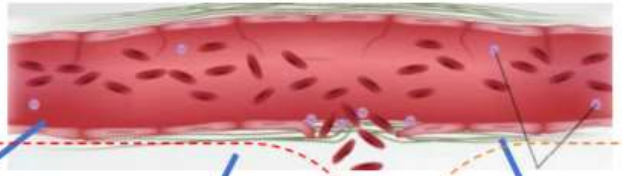
Corso di laurea in Scienze Biologiche
Corso di laurea magistrale in Scienze Biomolecolari e dell'Evoluzione

Materiale didattico di supporto

Tutto il materiale fornito a supporto delle lezioni e reperibile nel minisito dell'insegnamento o sulla piattaforma online UniFE deve essere inteso come traccia degli argomenti svolti e non sostituisce il libro di testo.

Hemostatic process: an overview



Vascular Injury

Collagen
vWF

Tissue factor

Vasoconstriction

Platelet activation

Coagulation cascade

vWF
Fibrinogen

Thrombin

Fibrinogen → Fibrin

Platelets plug

Plasmin

Fibrinolysis
&
Clot degradation

Primary Hemostasis

Secondary Hemostasis

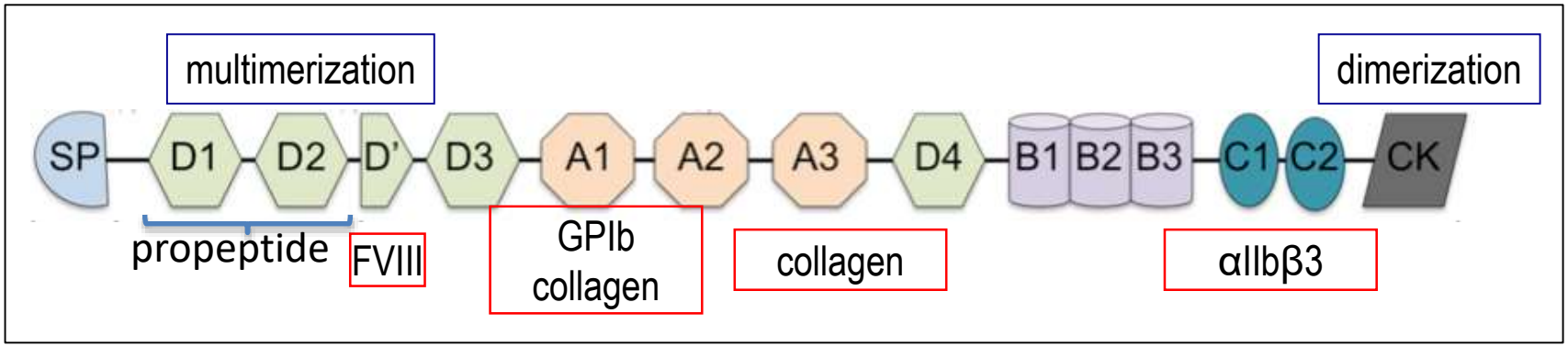
Von Willebrand factor (VWF)

- **VWF is a Large Plasmatic & Multimeric protein**
- **Produced by Endothelial cells and MEG**
- **Highly regulated biosynthesis**
- **Constitutive secretion & storage**

Da Caterina Casari 2016

Von Willebrand factor (VWF)

Monomer: domain arrangement



Role of VWF in haemostasis:

- **Primary haemostasis**
- **Chaperone for coagulation FVIII**

Coagulation Cascade

It takes place on ***macromolecular complex***:

Complex name	Enzyme (active)	Cofactor	Substrate (zymogen)	Catalytic Efficiency
Extrinsic Tenase	FVIIa	TF	FX	$>15 \times 10^6$
Intrinsic Tenase	FIXa	FVIIIa	FX	$>10^6$
Prothrom binase	FXa	FVa	Prothrombin	$>3 \times 10^5$

Chaperone for coagulation FVIII

VWF

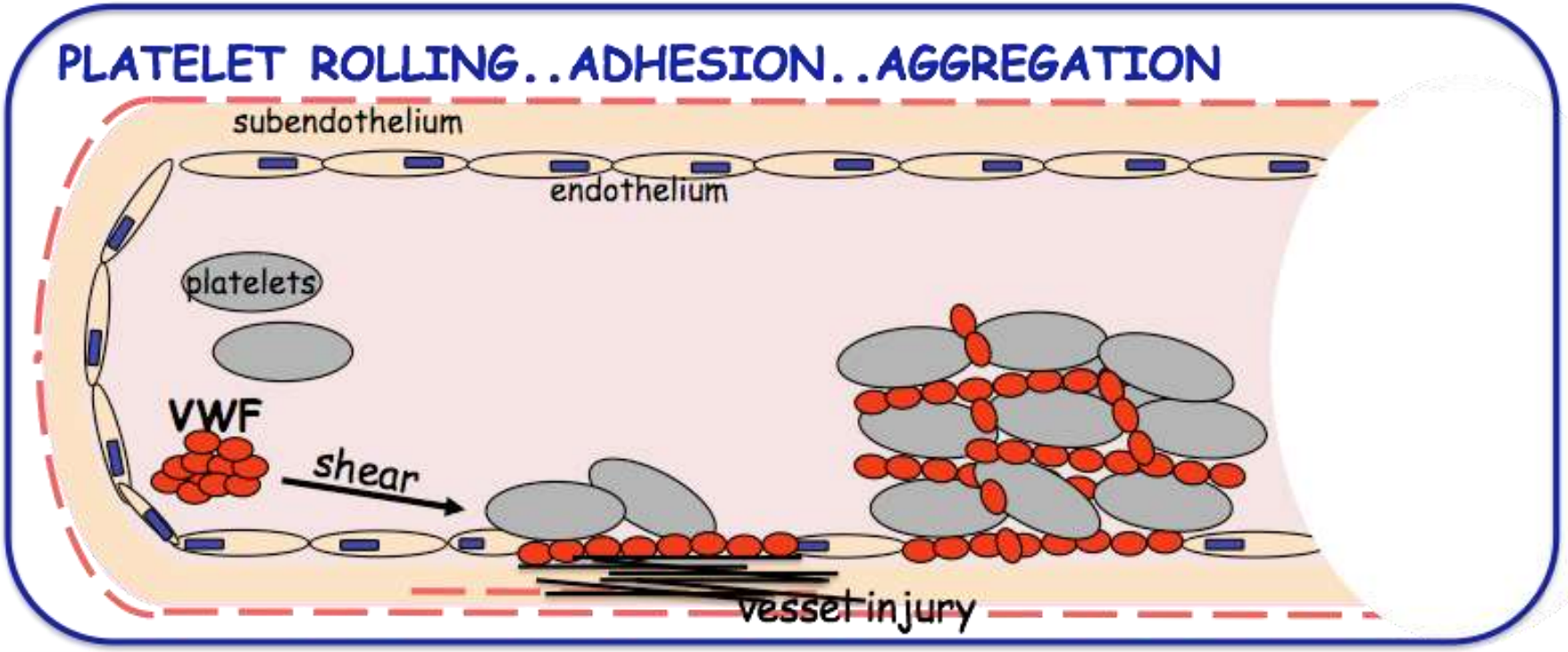
- Improves stability of FVIII protein structure

- Protects FVIII from proteolysis by phospholipid-dependent proteases (activated Protein C)

- Prevents premature clearance by scavenger-receptors (such as LRP1)

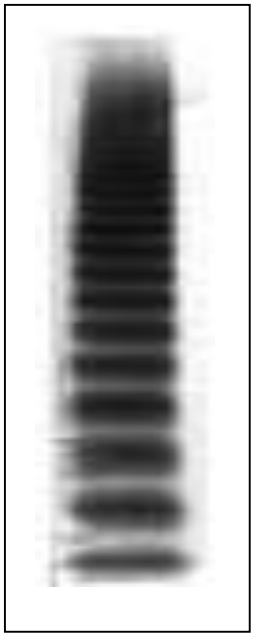
Patients with no detectable VWF
> have a secondary deficiency of FVIII

Primary haemostasis



At sites of vascular injury VWF binds to exposed collagen and allows platelets to roll and adhere to the damaged sub-endothelium.
Once platelets become activated VWF allows platelet-platelet interaction during thrombus formation.

Von Willebrand factor (VWF)



Normal plasma sample

High-molecular-weight multimers (**HMWM**)

20.000kDa

Low-molecular-weight multimers (LMWM)

500KDa(dimers)

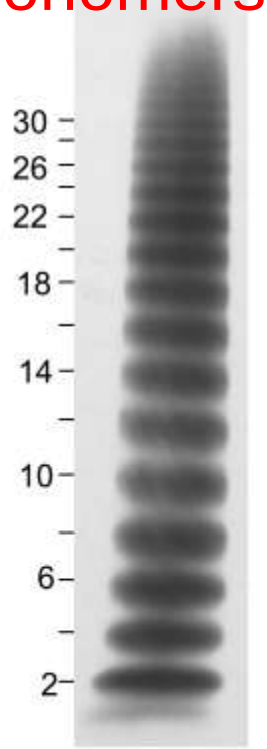
- HMWMs are the most important for VWF function in haemostasis

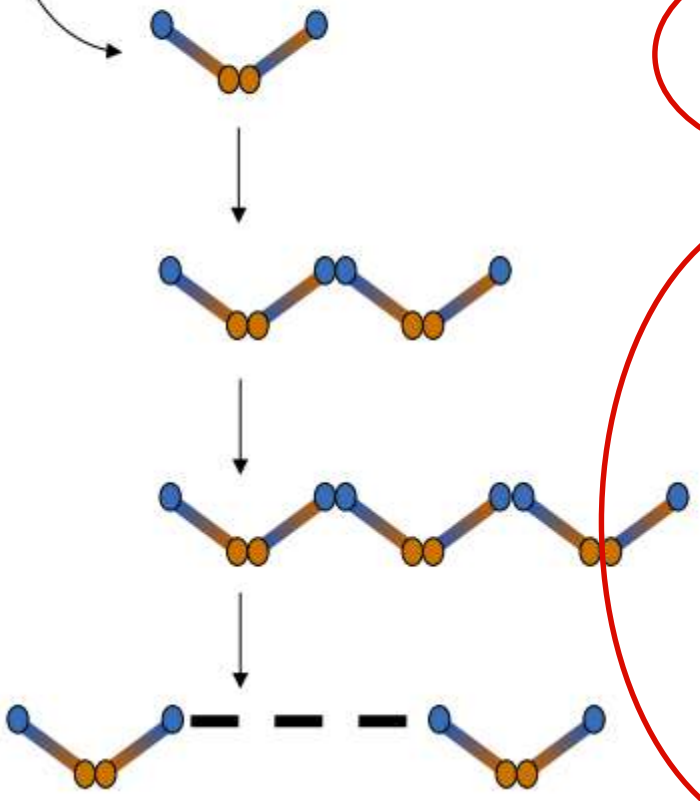
- VWF size in plasma is regulated by the enzymatic activity of a metalloprotease (ADAMTS13)

Dimerization and multimerization of VWF

Monomer
0.25 x 10^6 Da

> 30 monomers !!





Dimer
0.5 x 10^6 Da

Tetramer
1.0 x 10^6 Da

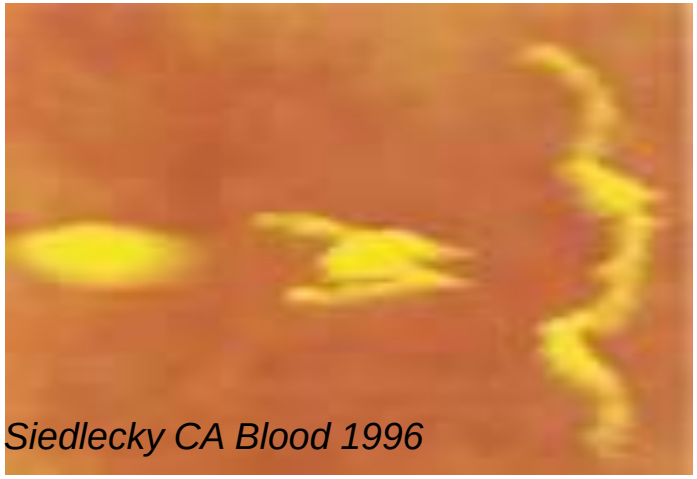
Hexamer
1.5 x 10^6 Da

Multimer
≥ 7.5 x 10^6 Da

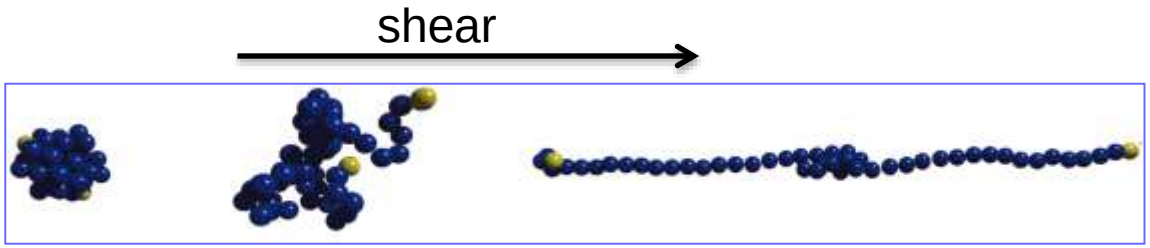
Adapted from Cecile Denis

Primary haemostasis

Structure-function relationship



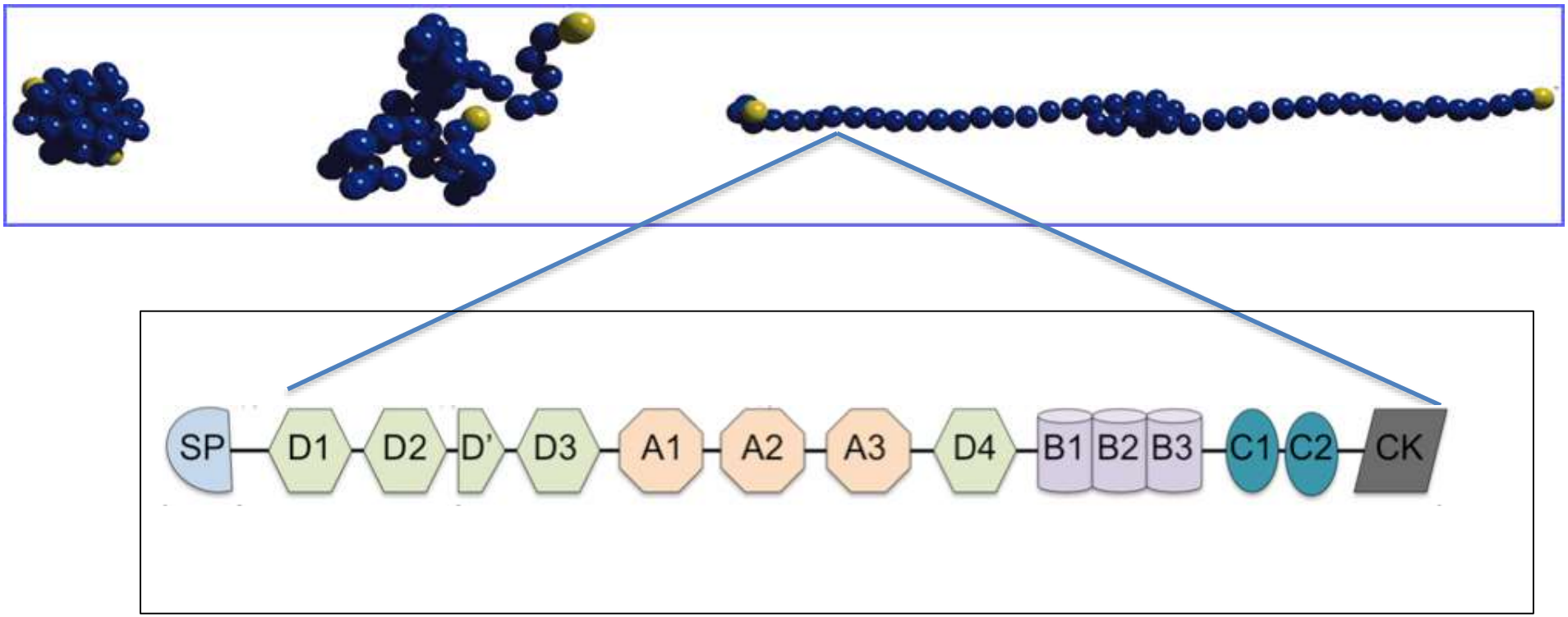
Siedlecky CA Blood 1996



VWF conformation depends on the applied shear force (blood flow)

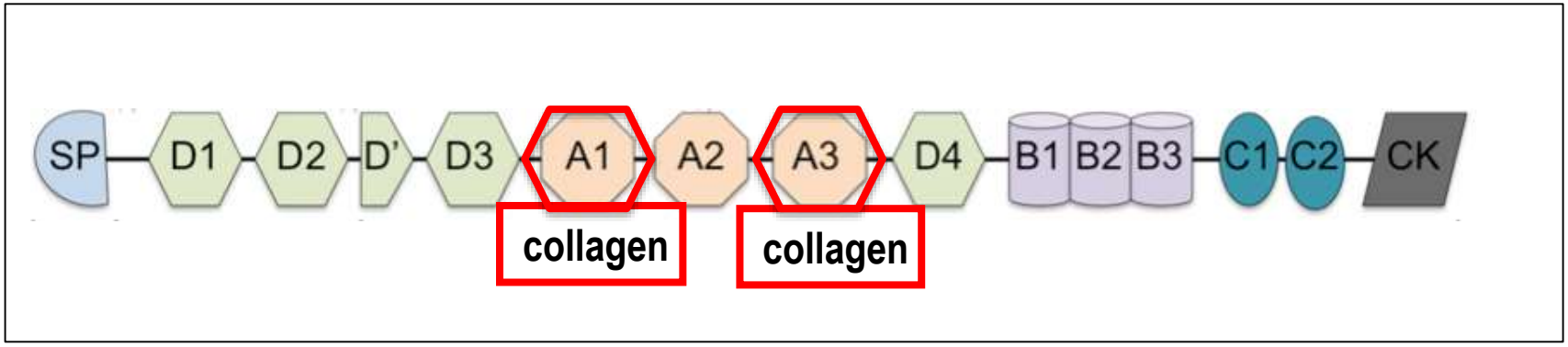
- In normal conditions VWF circulates in a globular state
- In case of high-shear rate VWF elongates into its active, platelet-binding conformation

a multimeric ……

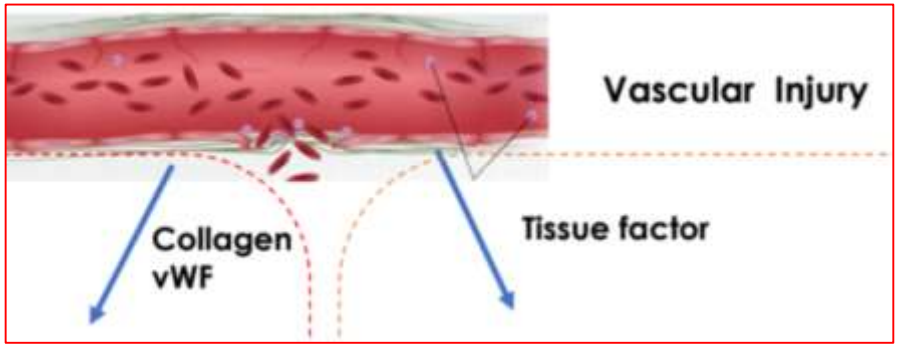


…….. and multidomain protein

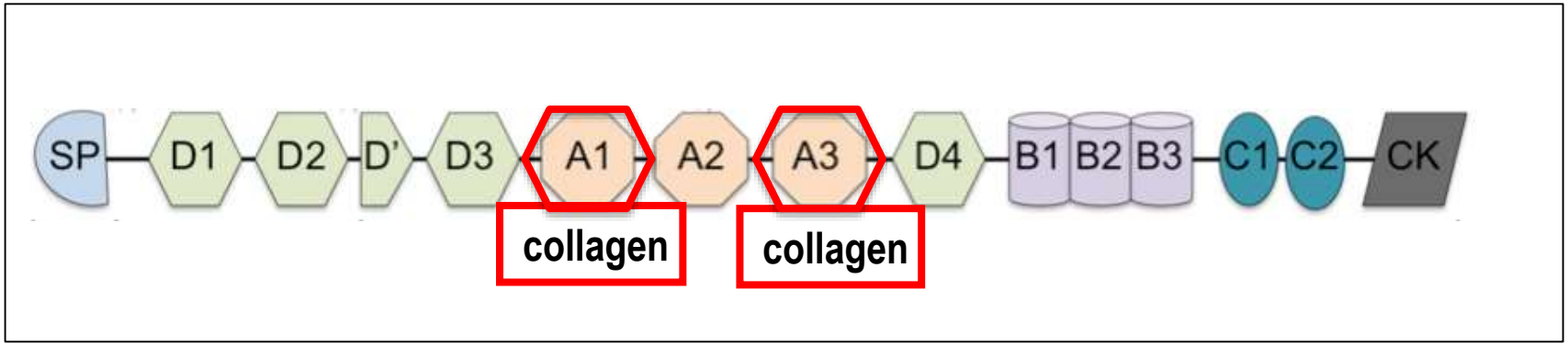
VWF-collagen binding



- Two sites of VWF-collagen binding in the A1 and A3 domains
- At sites of vascular injury sub-endothelial collagen becomes exposed to flowing blood and VWF can bind to it.



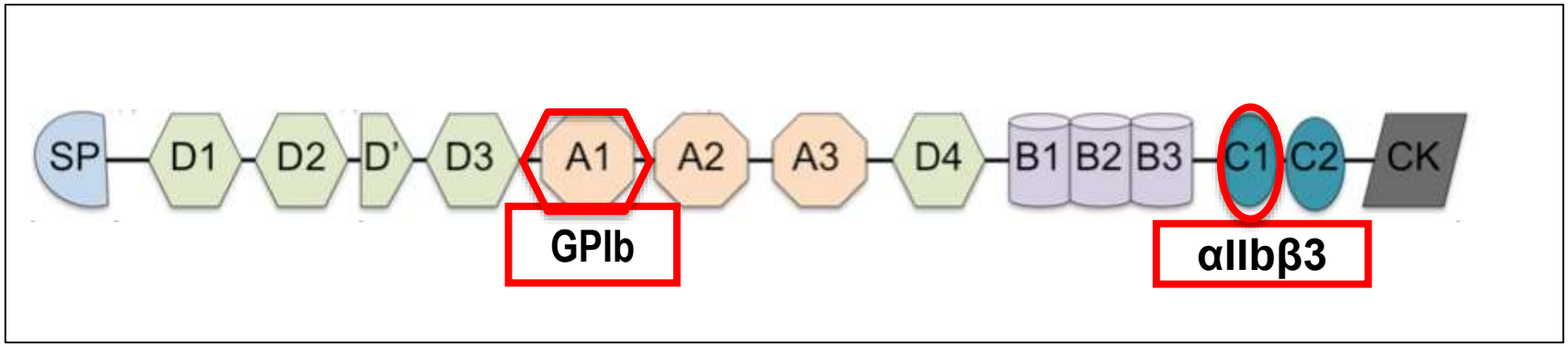
VWF-collagen binding 2



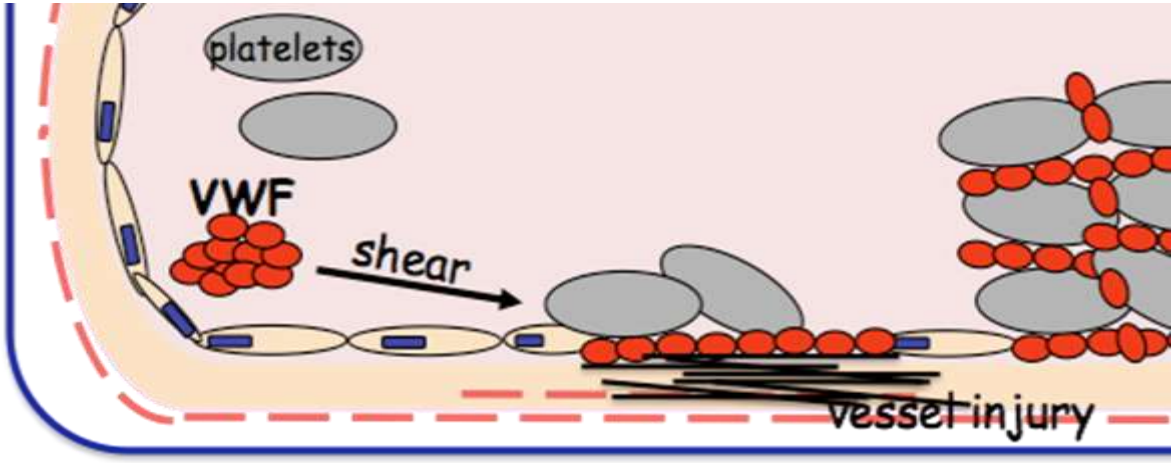
- Mutations in the A3 domain are associated with defective VWF binding to collagen type I and III and to bleeding phenotypes

- The larger VWF multimers have a much higher affinity for collagen. Thus, collagen binding is an indirect measure of multimer size and VWF activity

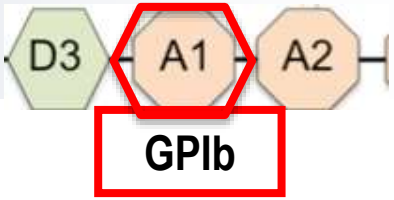
VWF-platelet binding



- Two sites of interaction between VWF and platelets:
  - VWF A1 & platelet GPIb complex
  - VWF RGDS sequence in the C1 domain & platelet αIIbβ3 integrin



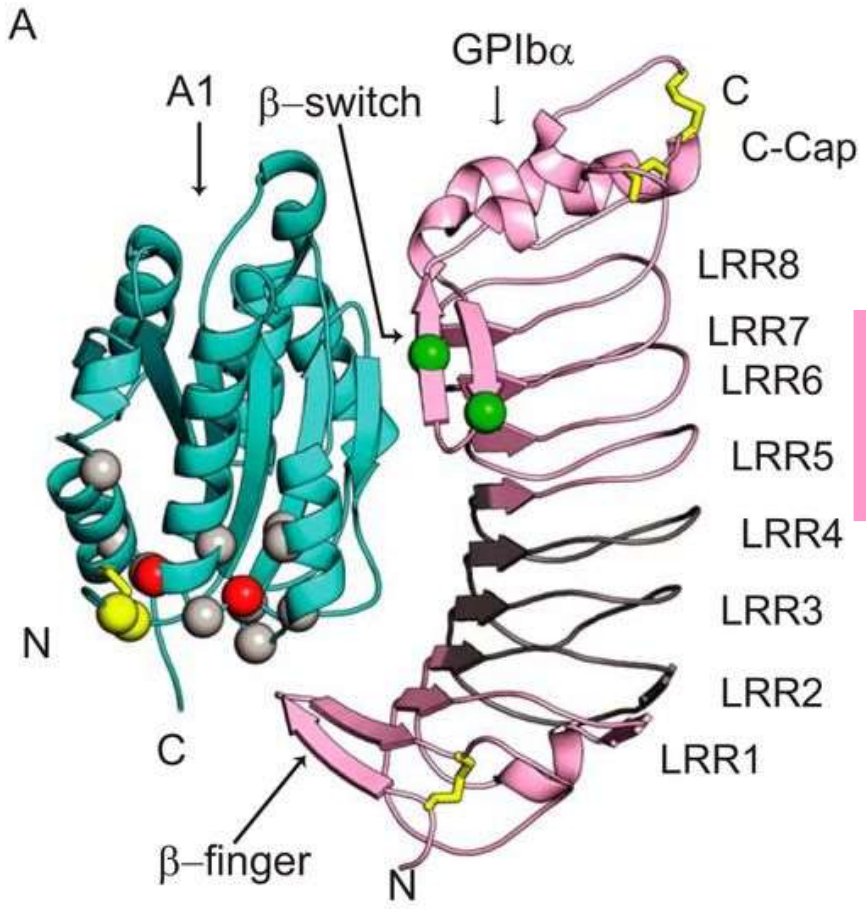
VWF-platelet binding A1&GPIb



- GPIb is a complex that binds with the A1 domain of VWF
- The physiologic stimulus that induces VWF binding to platelet GPIbα is shear > meaning that VWF needs to be in its elongated conformation to be able to bind GPIbα

The VWF A1-GPIbα complex.

VWF A1 domain



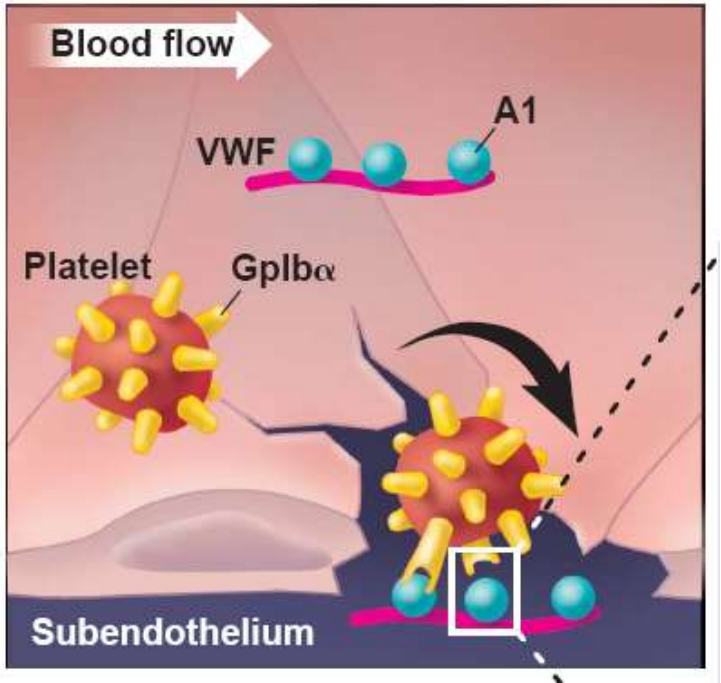
Platelet GPIbαreceptor

Mark A. Blenner et al. J. Biol. Chem. 2014;289:5565-5579

jbc

VWF-platelet binding A1&GPIb

- GPIb is a complex and only GPIbα binds with the A1 domain of VWF
- The physiologic stimulus that induces VWF binding to platelet GPIbα is shear > meaning that VWF needs to be in its elongated conformation to be able to bind GPIbα 
- VWF-GPIbα binding is reversible: the high number of GPIbα molecules on platelet surface and the high concentration of A1 domains within VWF multimers allow platelet rolling



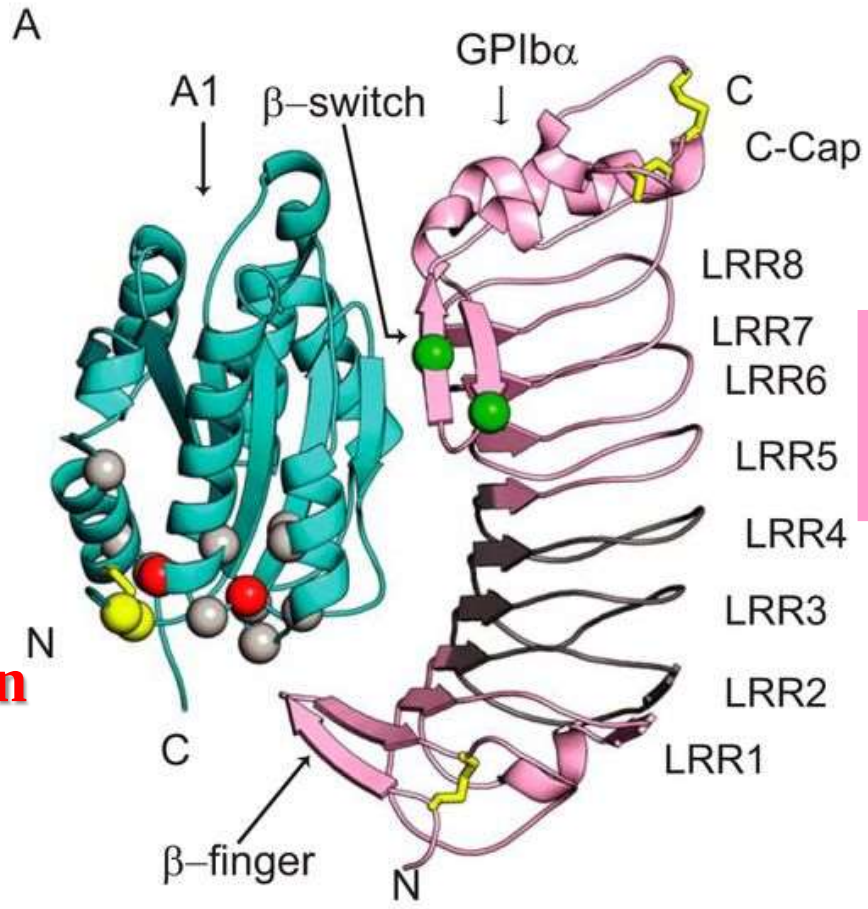
VWF-platelet binding A1&GPIb and Von Willebrand Disease

- There are different types of mutations that affects VWF-GPIα interaction and cause bleeding
- Some of the these mutations turn VWF/GPIbα in the active conformation > resulting in constitutive (instead of shear-dependent) VWF-platelet interaction
    - *Gain-of-function* mutations in the A1 domain of VWF
    - *Gain-of-function* mutations in GPIbα

The VWF A1-GPIbα complex.

VWF A1 domain

Gain-of-function mutations



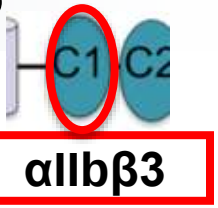
Gain-of-function mutations

Platelet GPIbαreceptor

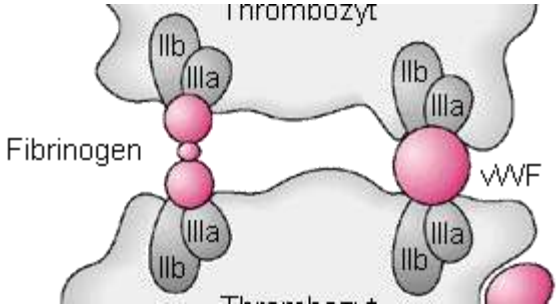
Mark A. Blenner et al. J. Biol. Chem. 2014;289:5565-5579

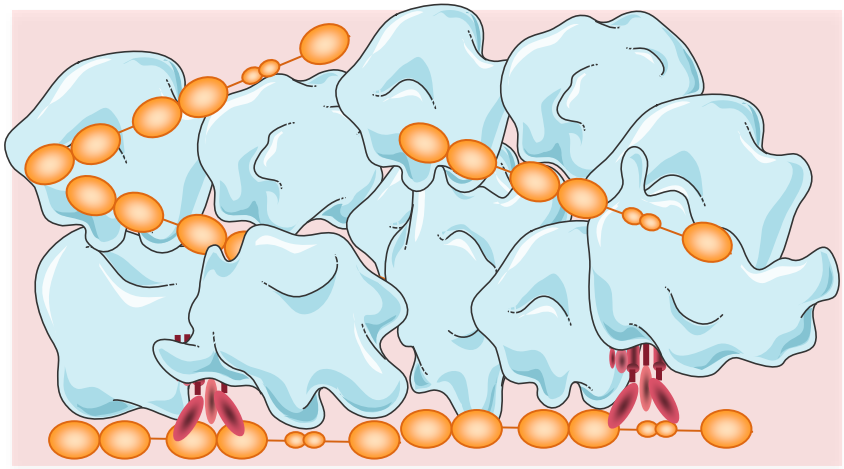
jbc

VWF-platelet binding RGDS(C1)&αIIbβ3

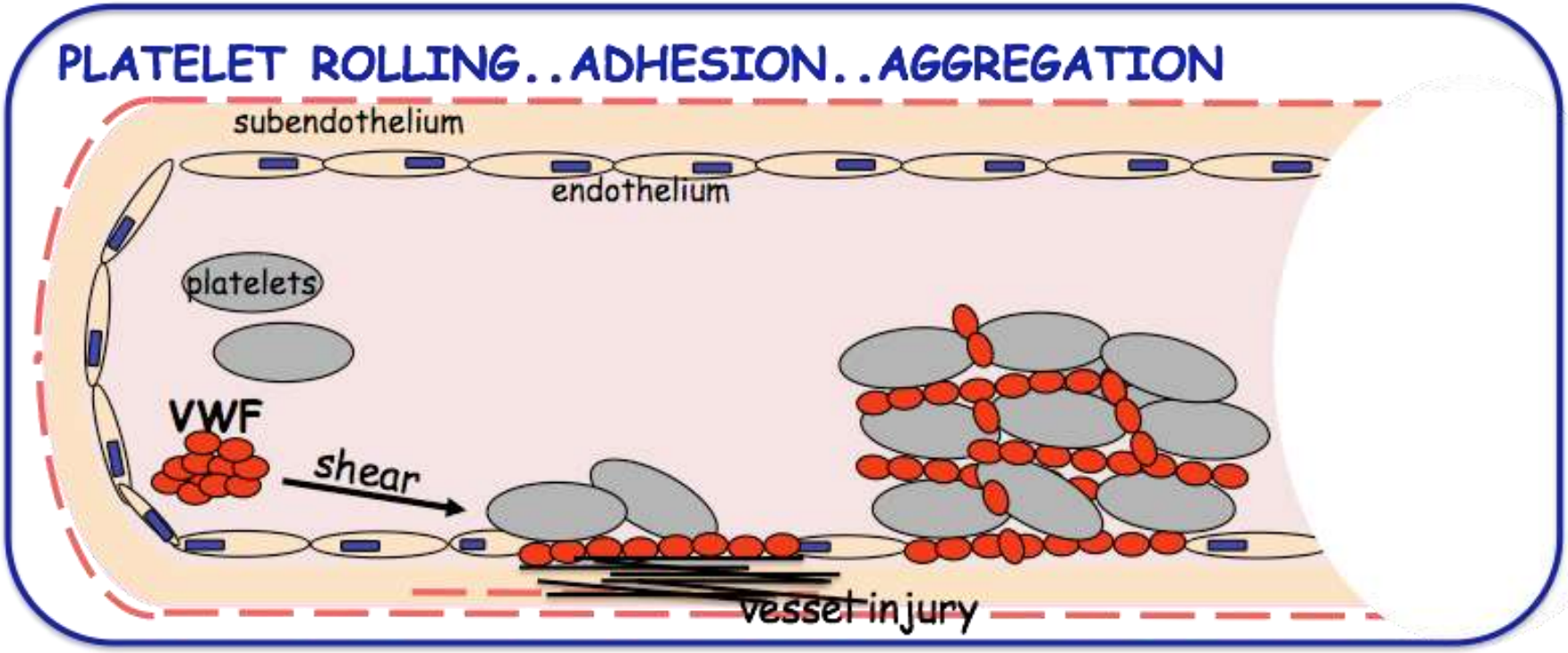


- Integrin αIIbβ3 becomes available for VWF binding only after platelet activation and a consequent conformational change
- VWF needs to be in its active conformation to bind αIIbβ3
- αIIbβ3-VWF interaction is irreversible and allows stable platelet-platelet interaction during thrombus formation





Role of VWF in primary haemostasis

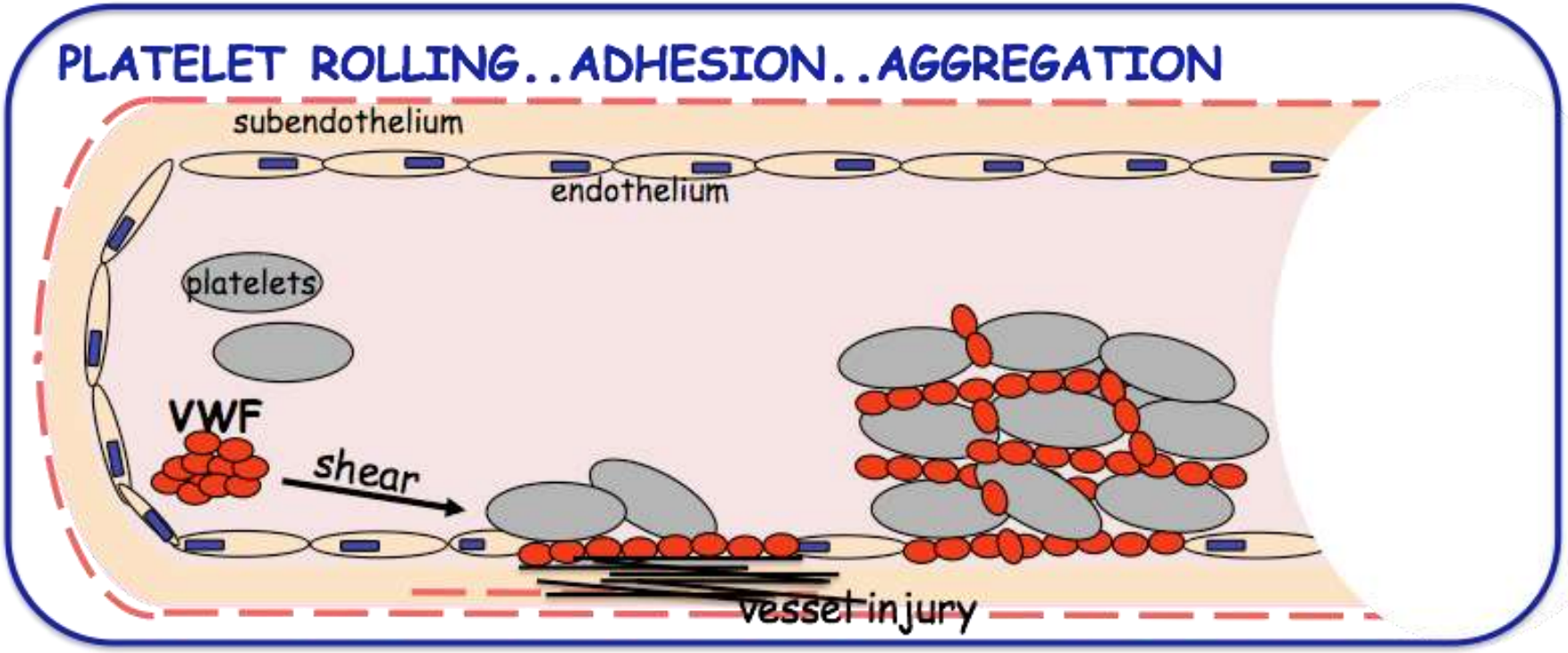


1 Vascular injury:

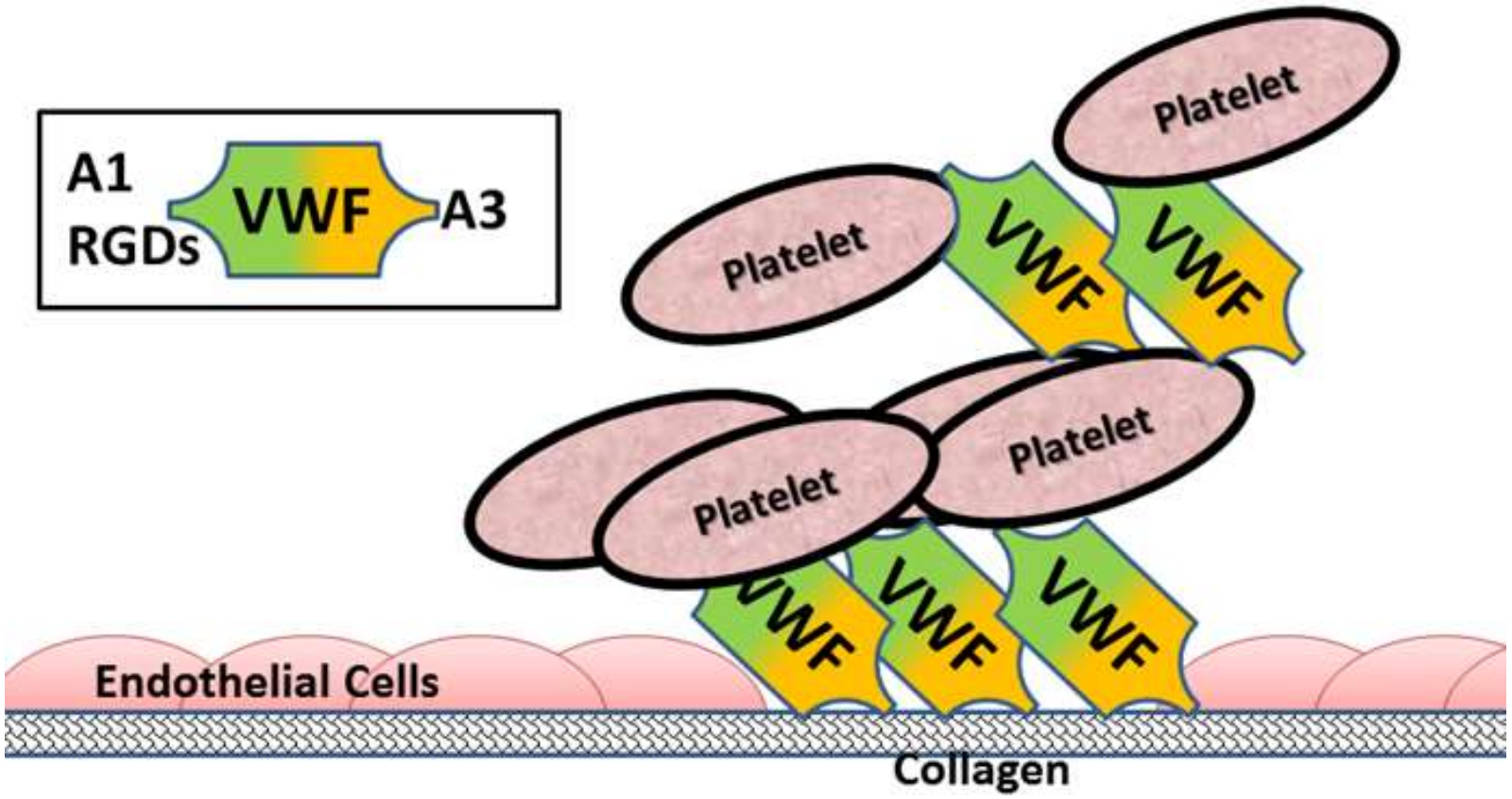
- exposed sub-endothelial matrix component (such as collagen)
- High shear stress elongates VWF in its active conformation

VWF binds to collagen & to platelet GPIbα (> platelet rolling)

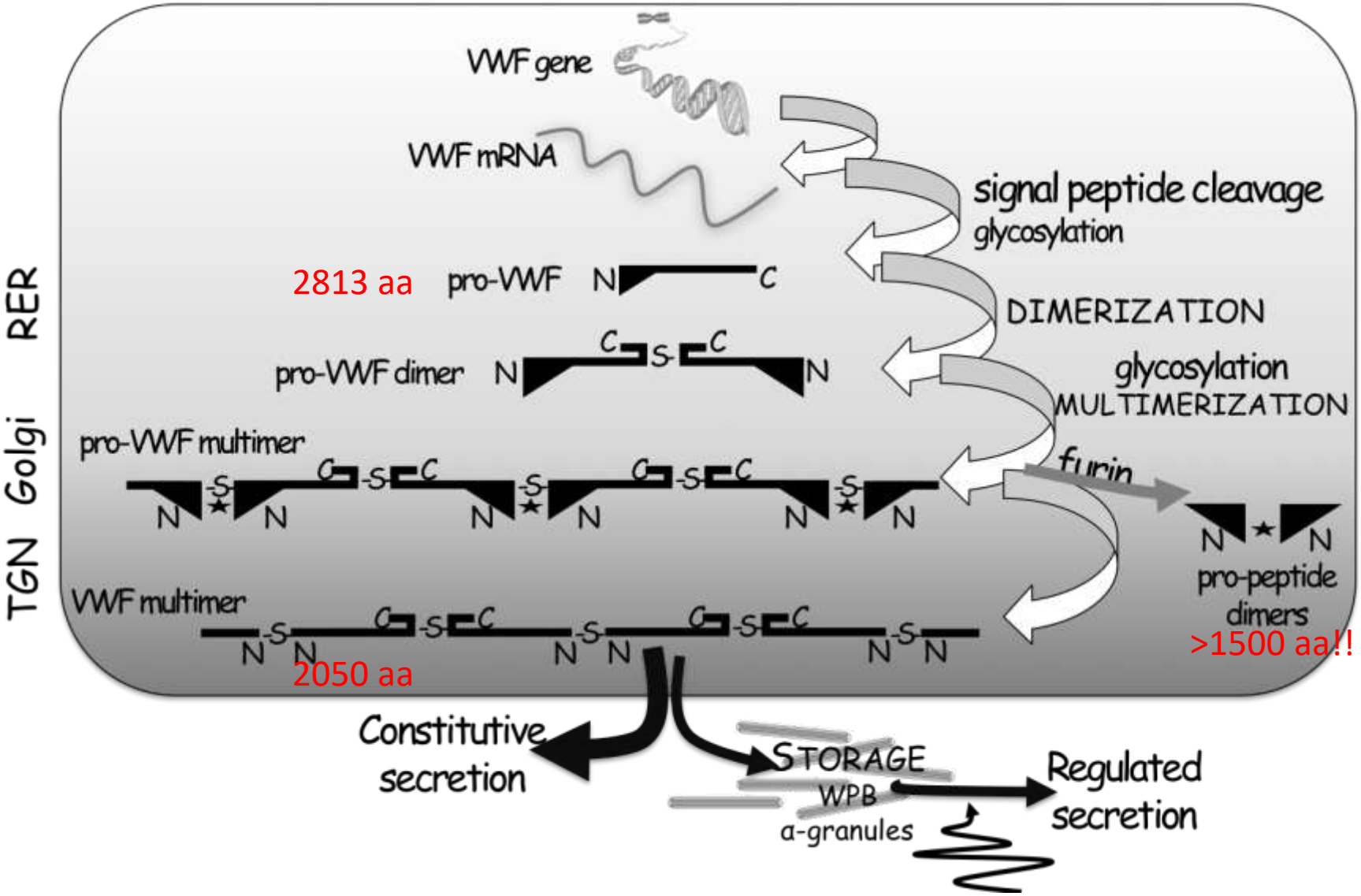
Role of VWF in primary haemostasis



2 platelet interaction with VWF and subendothelium allows their adhesion and activation >
conformational change in αIIbβ3 integrin > interaction with VWF RGDS & fibrinogen > platelet-platelet interaction > thrombus


A1
RGDs
VWF
A3
Platelet
Platelet
VWF
VWF
Platelet
Platelet
VWF
VWF
VWF
Endothelial Cells
Collagen

Von Willebrand factor (VWF) –biosynthesis-



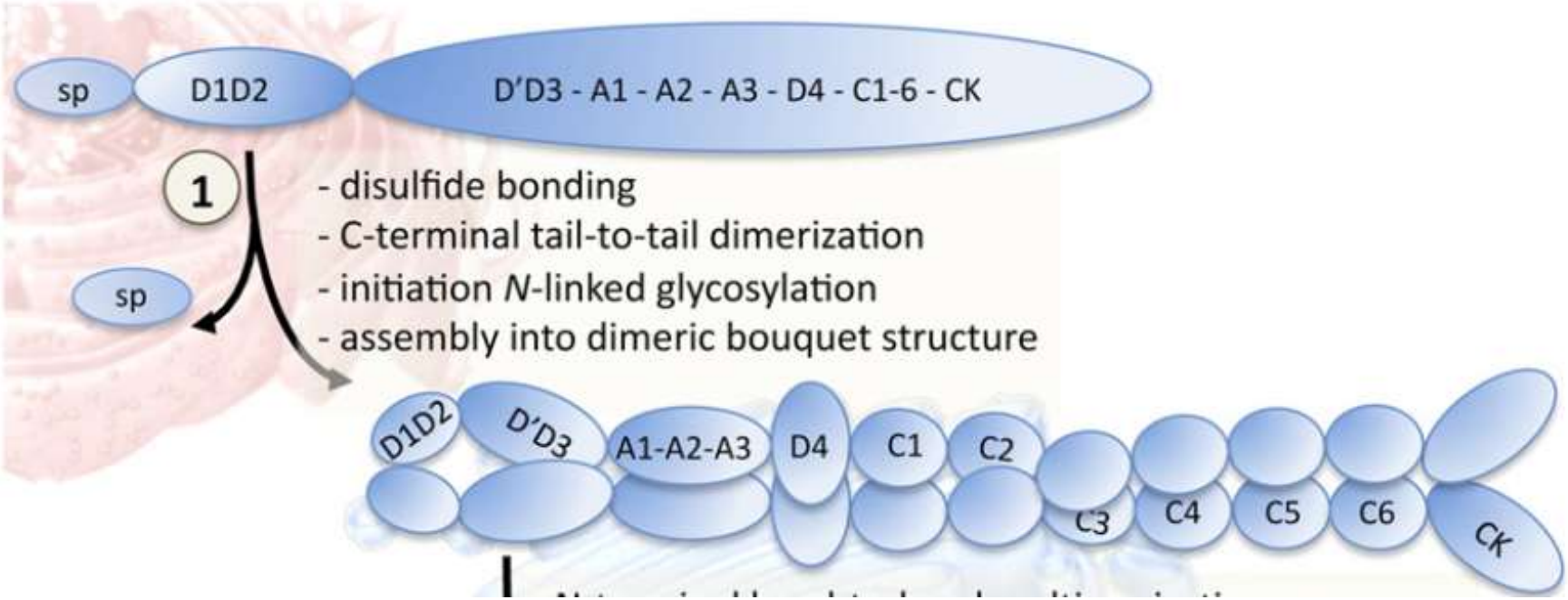
Von Willebrand factor (VWF) –storage-

Weibel-Palade bodies

- Exclusively present in endothelial cells
- Highly structured
- Storage of large VWF multimers
- Regulated secretion following cellular stimulation

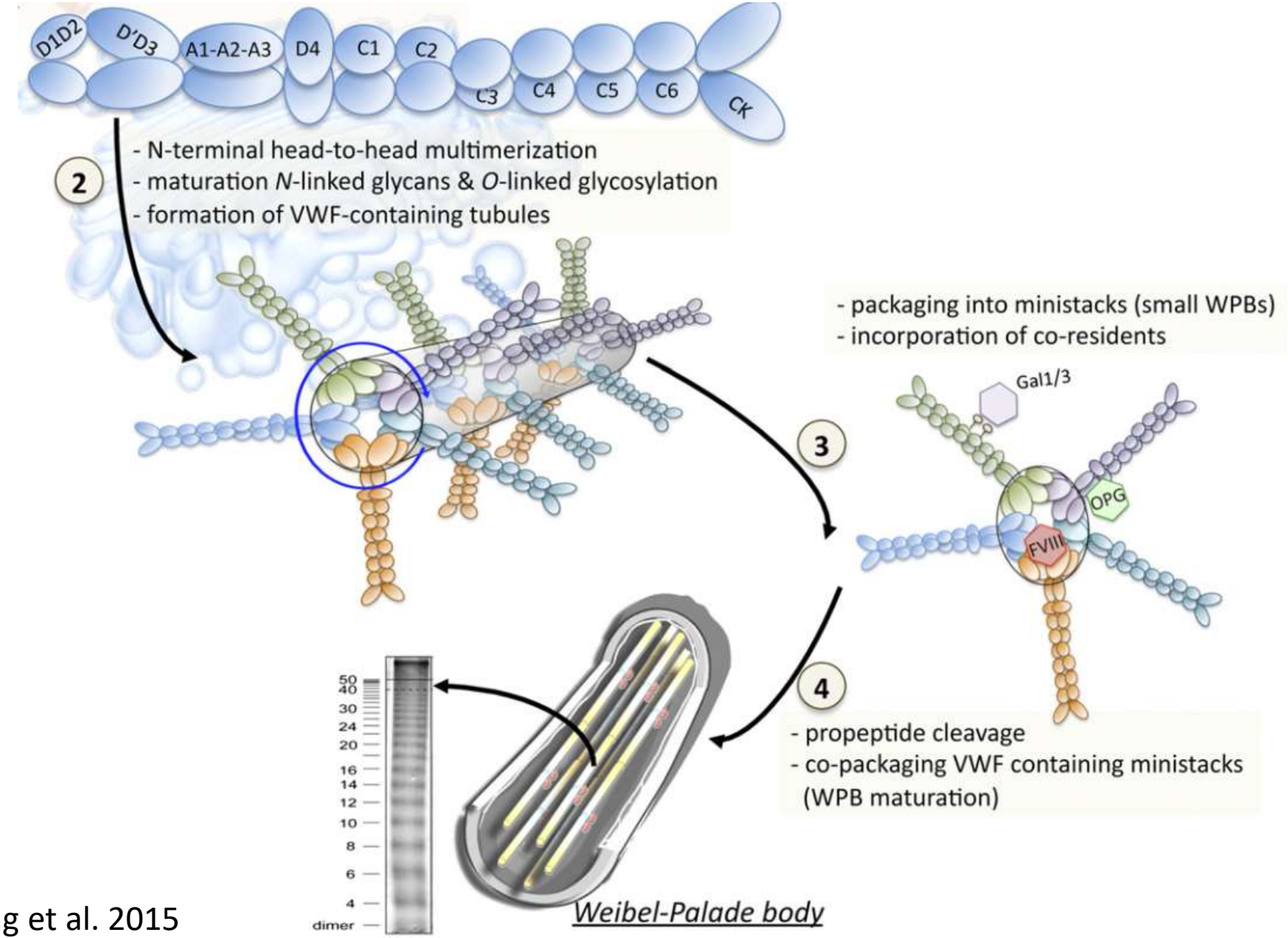
Lenting et al. 2015

Von Willebrand factor (VWF) –storage-



Lenting et al. 2015

Von Willebrand factor (VWF) –storage-



Lenting et al. 2015

VWF-related pathologies

+

Thrombotic Thrombocytopenic Purpura (TTP)

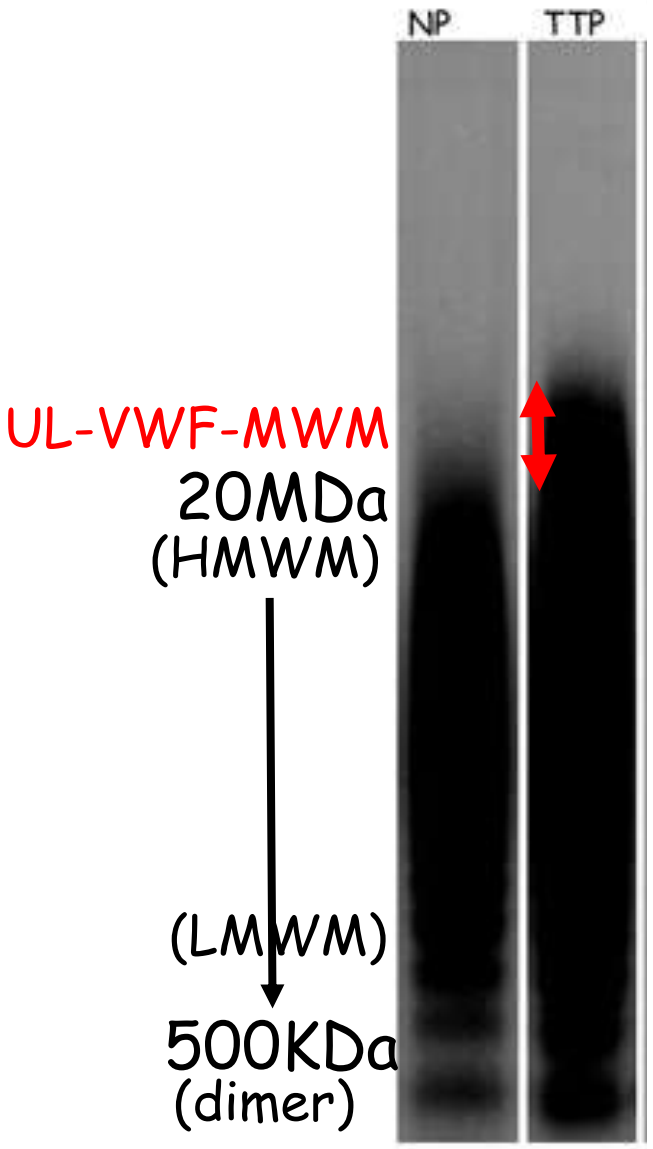
VWF

-

von Willebrand Disease (VWD)

Thrombosis **Haemostasis** **Bleeding**

- Presence of Ultra-large (UL)-VWF-MWMs in plasma



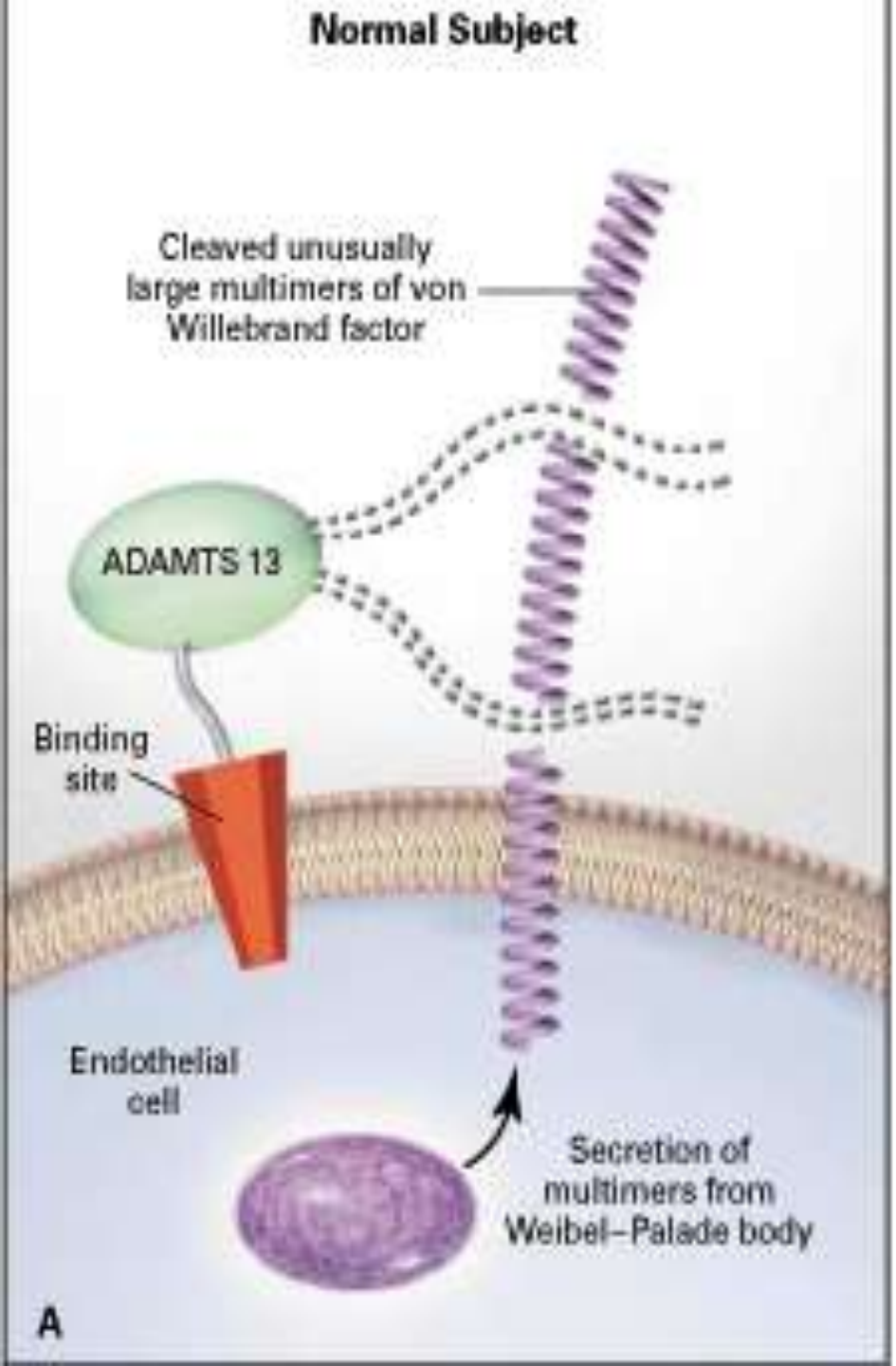
Thrombotic Thrombocytopenic Purpura (TTP)

VWF-related pathologies
Thrombotic Thrombocytopenic Purpura (TTP)

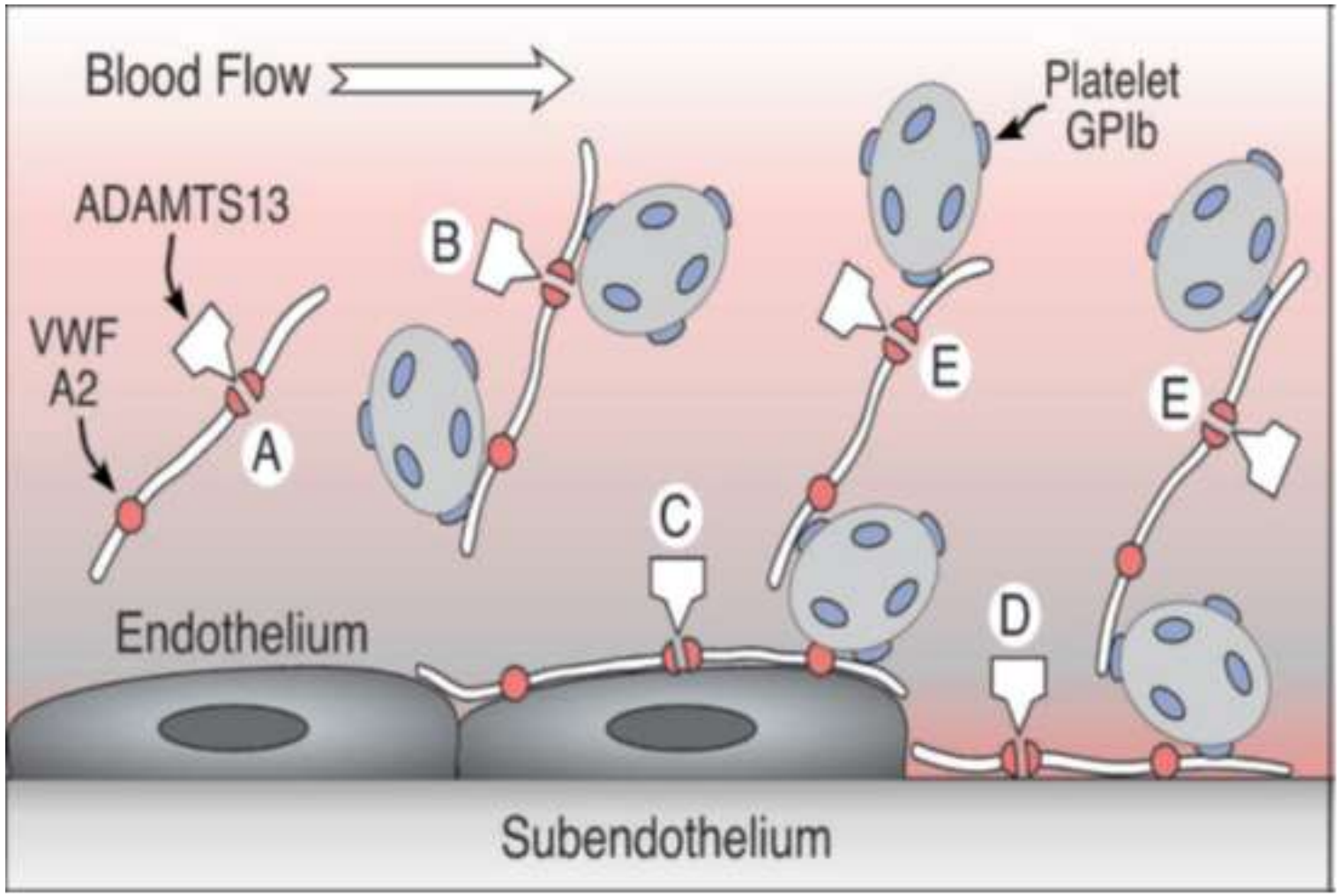
- Systemic disorder characterized by inappropriate deposition of VWF and platelet rich thrombi throughout the microvasculature, thrombocytopenia, organ failure and death
- Presence of Ultra-large (UL)-VWF-MWMs in plasma

VWF-related pathologies
Thrombotic Thrombocytopenic Purpura (TTP)

- TTP results from the deficiency of the metalloprotease ADAMTS13 that cleaves circulating VWF
- **Rare inherited TTP** = Over 50 mutations in the ADAMTS13 gene have been identified in patients with familial TTP
- **More frequent acquired TTP** > due to inhibitory anti-ADMTS13 autoantibodies (more frequent in woman)


Normal Subject
Cleaved unusually large multimers of von Willebrand factor
ADAMTS 13
Binding site
Endothelial cell
Secretion of multimers from Weibel–Palade body
A

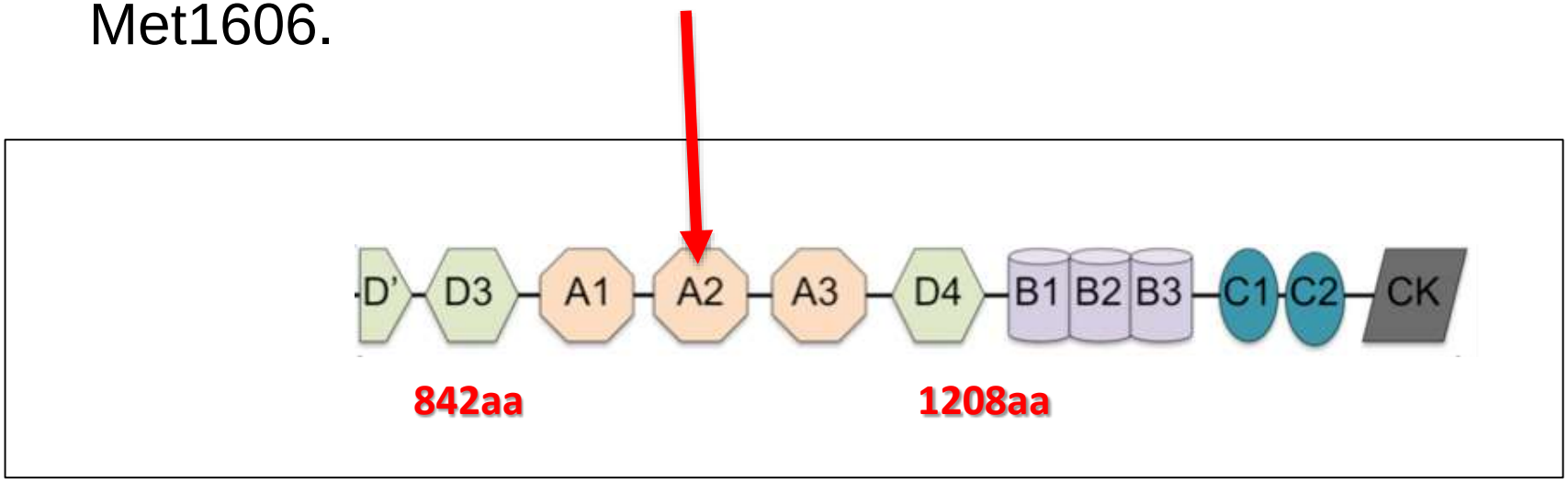
ADAMTS13


Blood Flow
Platelet
GPIb
ADAMTS13
VWF
A2
A
B
C
D
E
E
Endothelium
Subendothelium

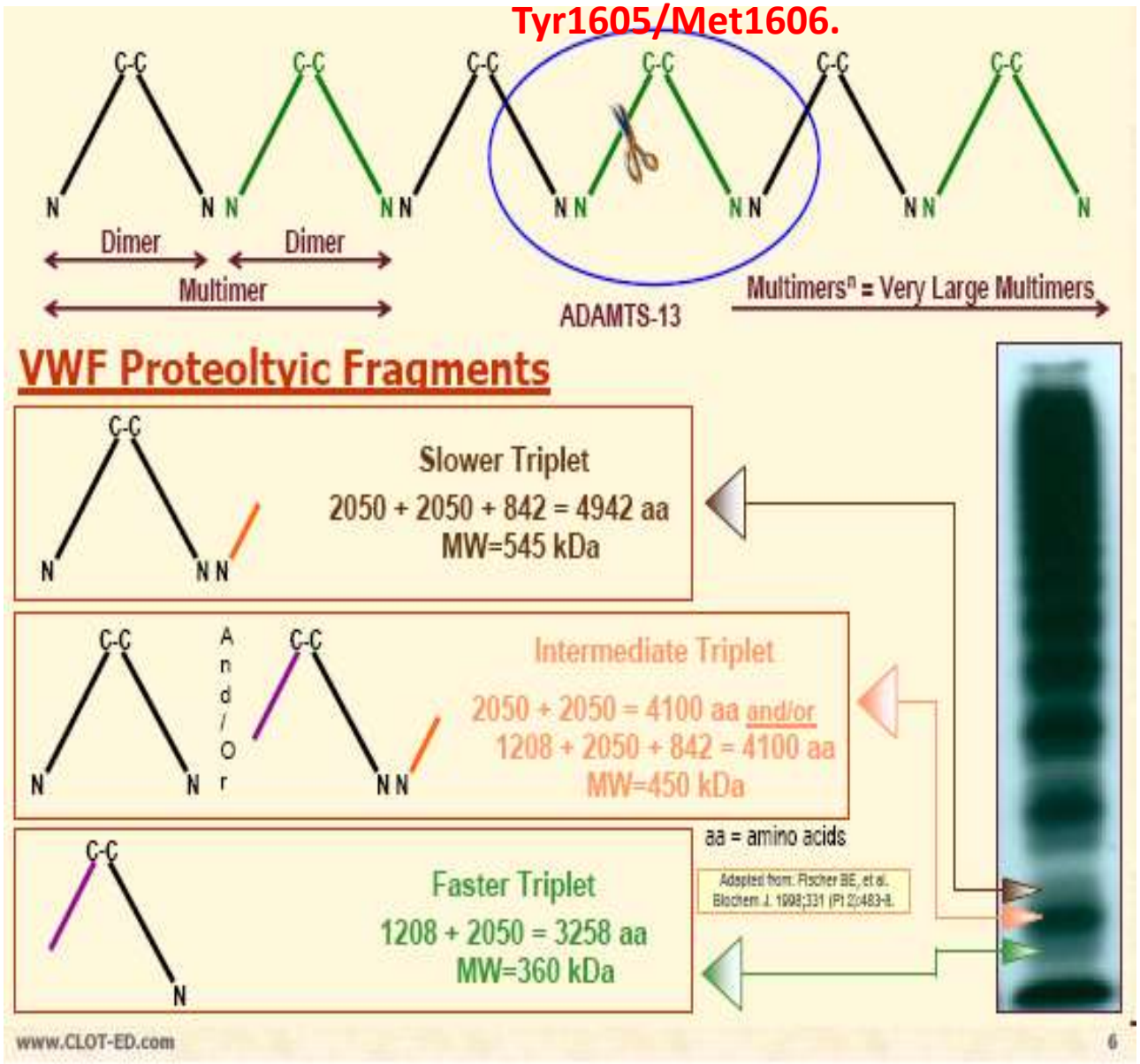
ADAMTS13

(A Disintegrin And Metalloprotease with ThromboSpondin type 1 motifs)

- It cleaves VWF UL-multimers as soon as they are released, into smaller and less thrombogenic multimers
- The only known substrate of ADAMTS13 is VWF. The cleavage site is in the A2 domain between Tyr1605 & Met1606.



ADAMTS13 activity and multimer migration



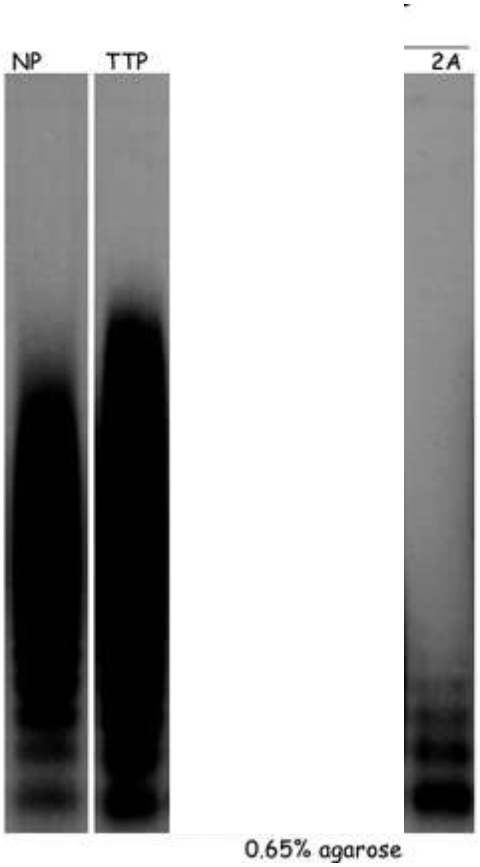
ADAMTS13

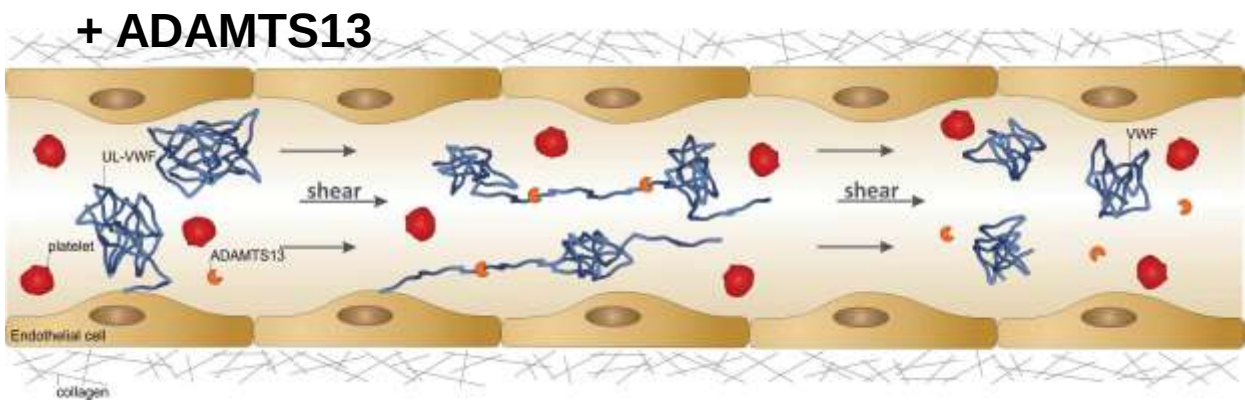
(A Disintegrin And Metalloprotease with ThromboSpondin type 1 motifs)

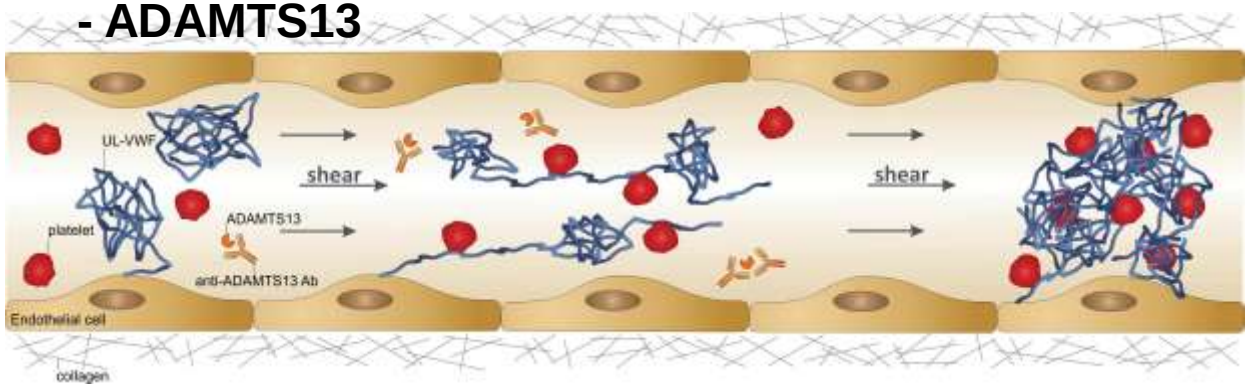
- Unlike most plasma proteases, ADAMTS13 is constitutively active in circulation
- VWF needs to be in its elongated conformation to expose the cleavage site for ADAMTS13 (shear-dependent mechanism) > VWF auto-regulates its own cleavage

ADAMTS13

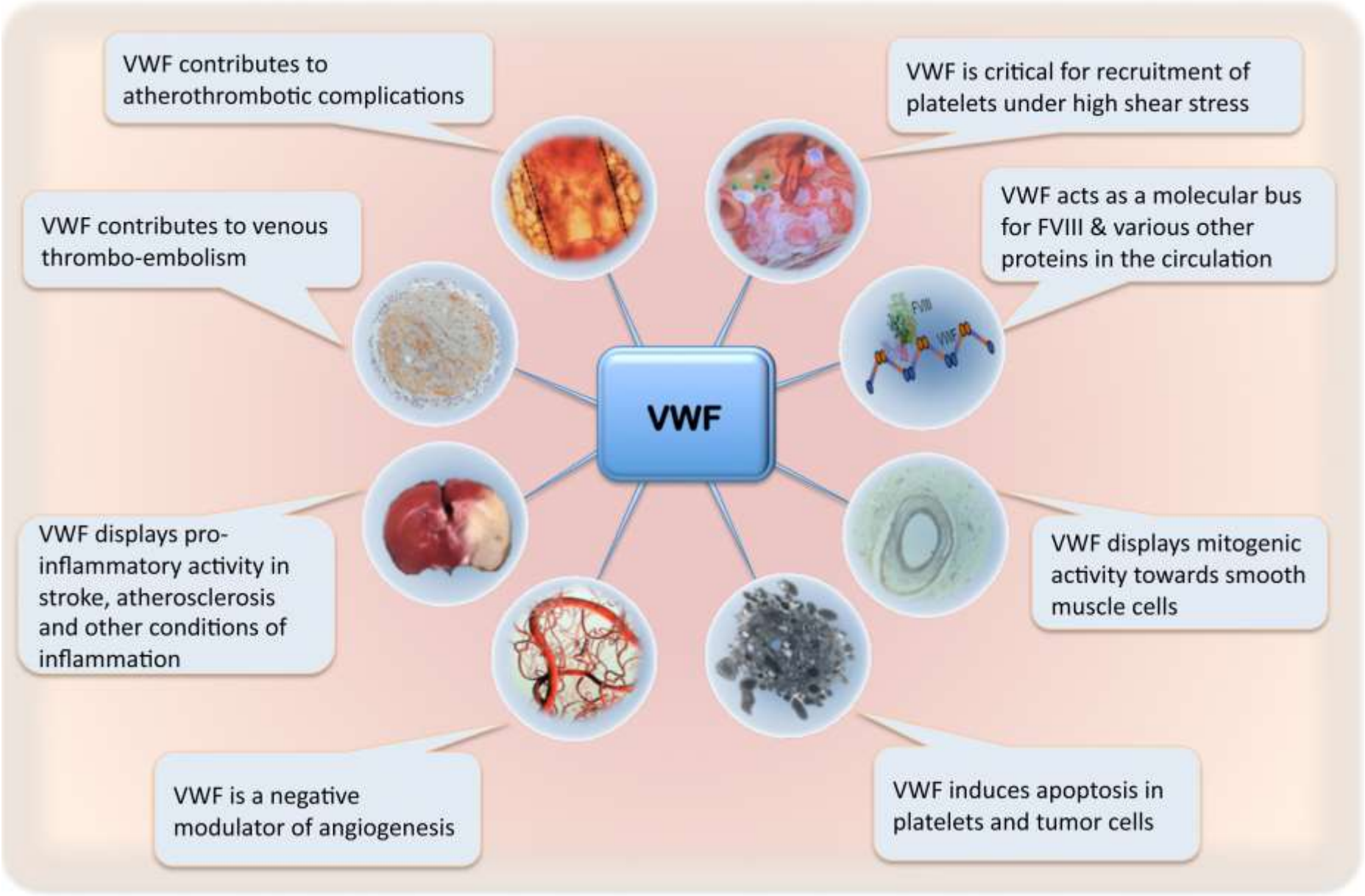
- ADAMTS13 deficiency > TTP > UL-VWF-MWMs
- ADAMTS13 hyper-activicty (mutations in VWF that result in increased susceptibility to ADAMTS13 cleavage) > VWD-type 2A > absence of HMWMs and increased satellite bands







Role of VWF beyond haemostasis: unexpected versatility



Lenting *et al. (2012) JTH 10:2428*