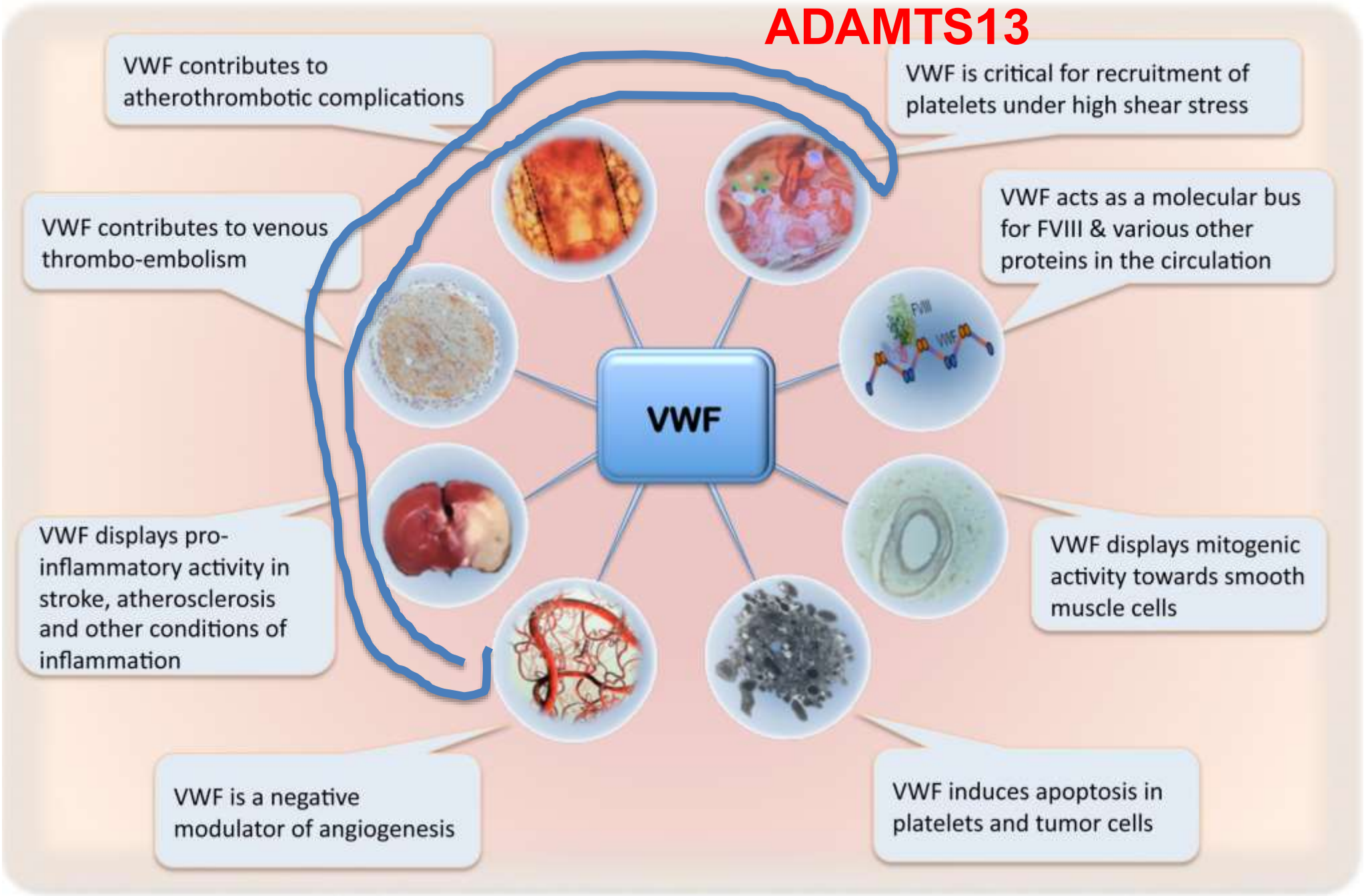
Role of VWF beyond haemostasis: unexpected versatility



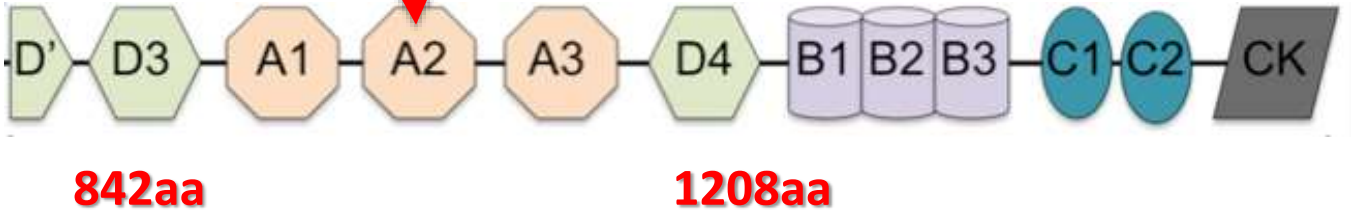
Lenting *et al. (2012) JTH 10:2428*

VWF and ADAMTS13

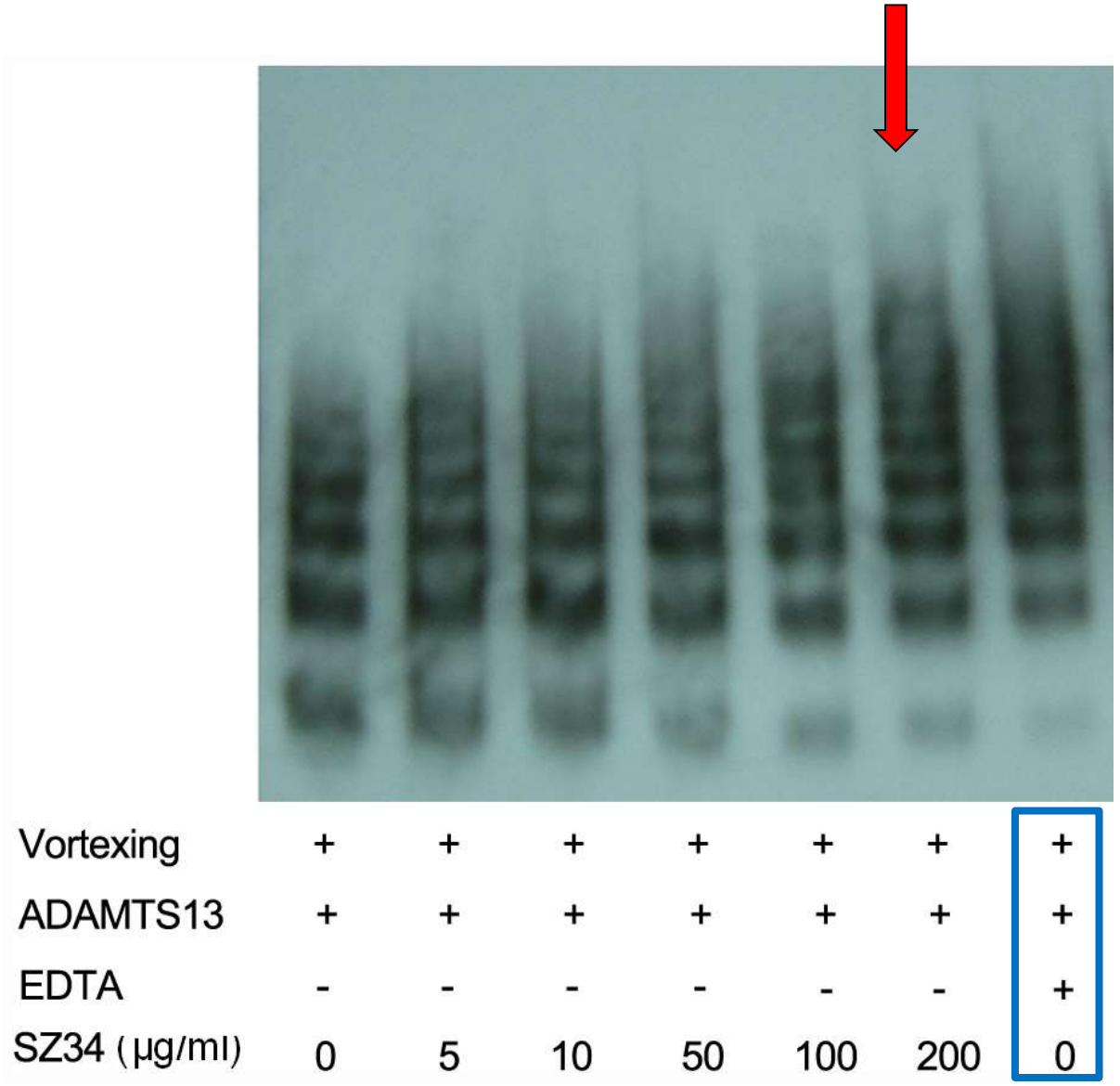
ADAMTS13

(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.



Monoclonal Antibody against the A2 Domain of von Willebrand Factor Reduces Proteolysis by ADAMTS13

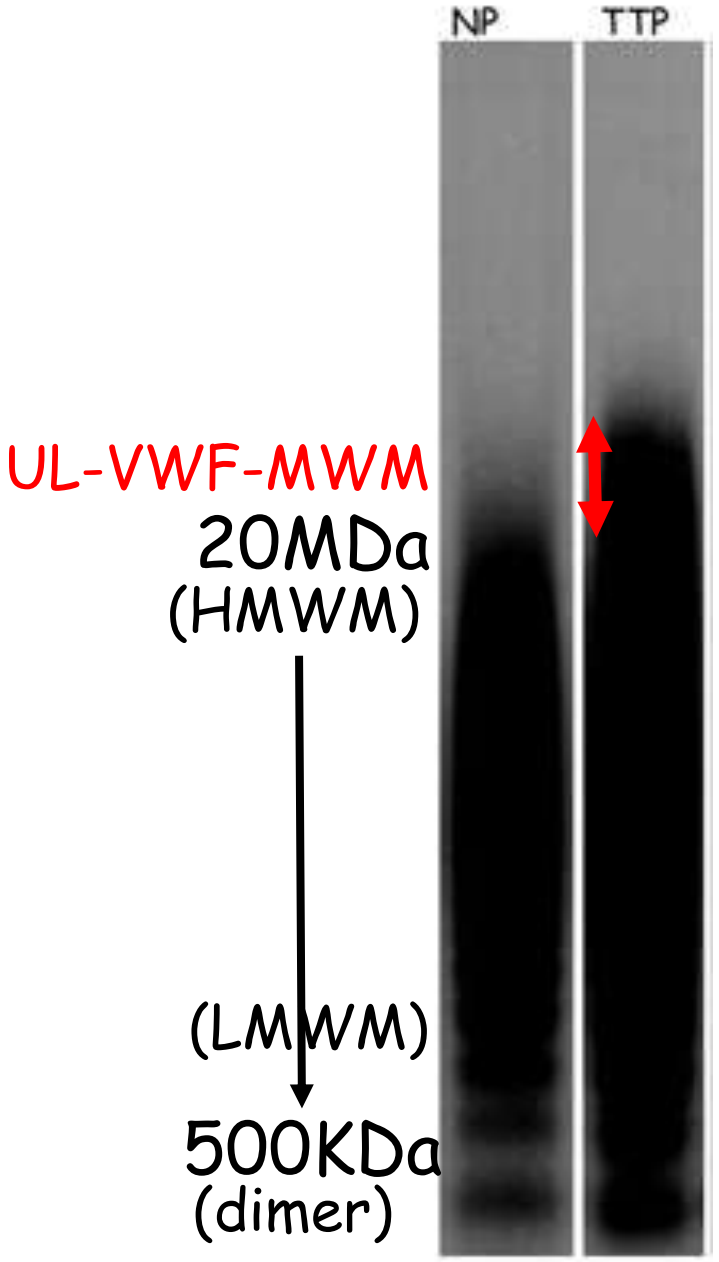


No attività ADAMTS13 metalloproteasi

anti-VWF mAb (SZ34)

Thrombotic Thrombocytopenic Purpura (TTP)

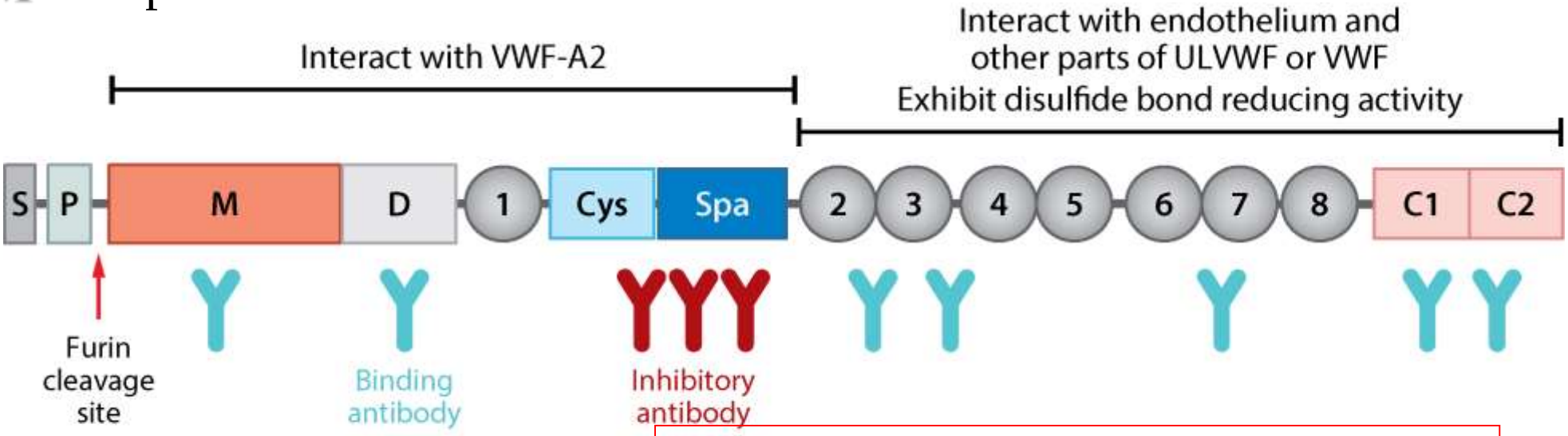
- Systemic disorder characterized by inappropriate deposition of VWF and platelet rich thrombi throughout the microvasculature, thrombocytopenia, organ failure and death
- Presence of Ultra-large (UL)-VWF-MWMs in plasma



ADAMTS13 domain organization

Metalloprotease

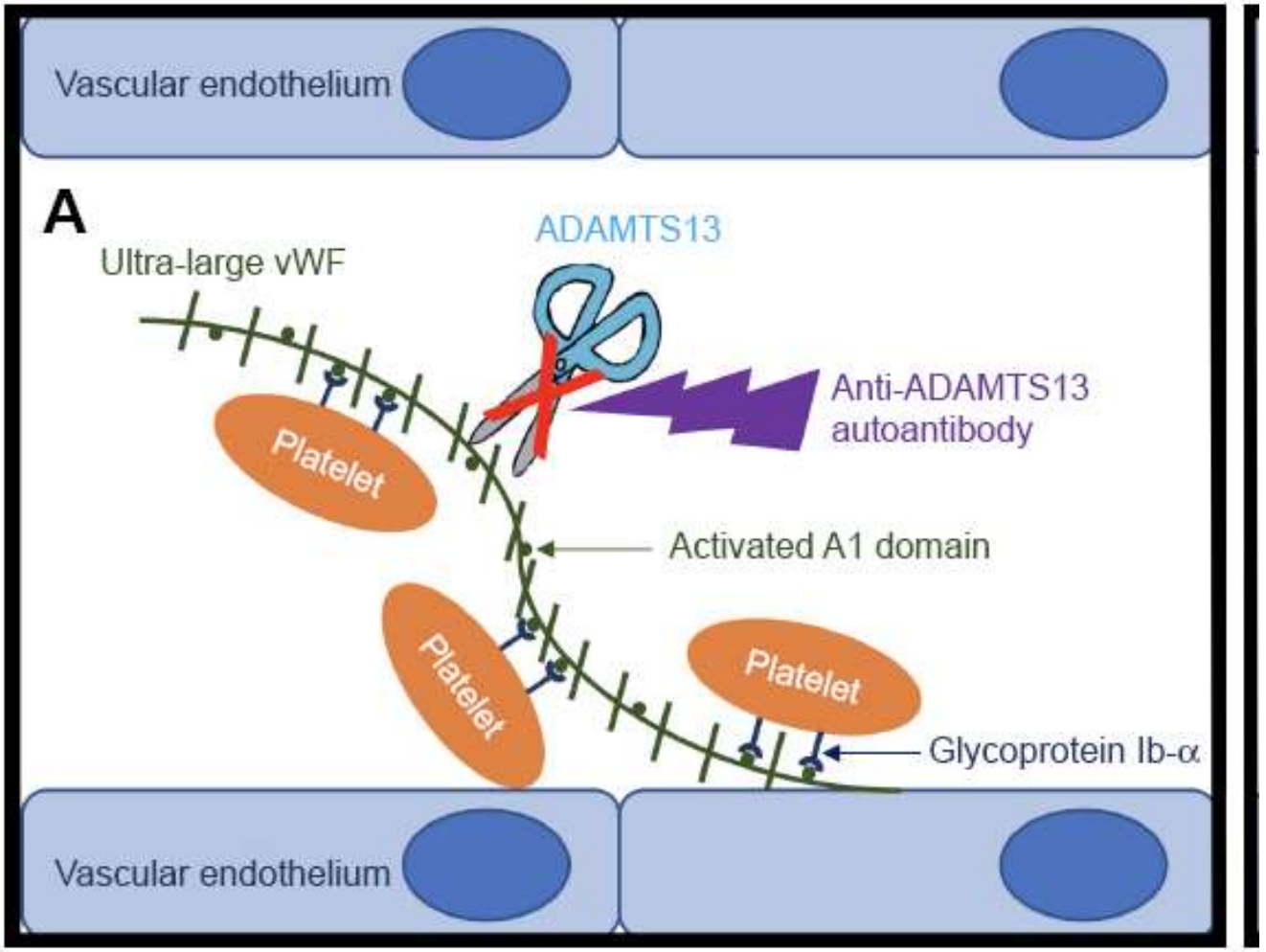
TSP repeats 1-8

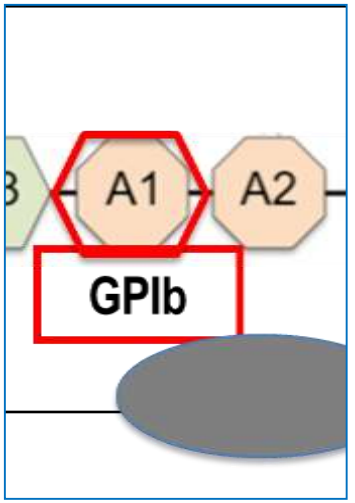


binding sites of autoantibodies in TTP

Zheng XL. 2015.
Annu. Rev. Med. 66:211–25

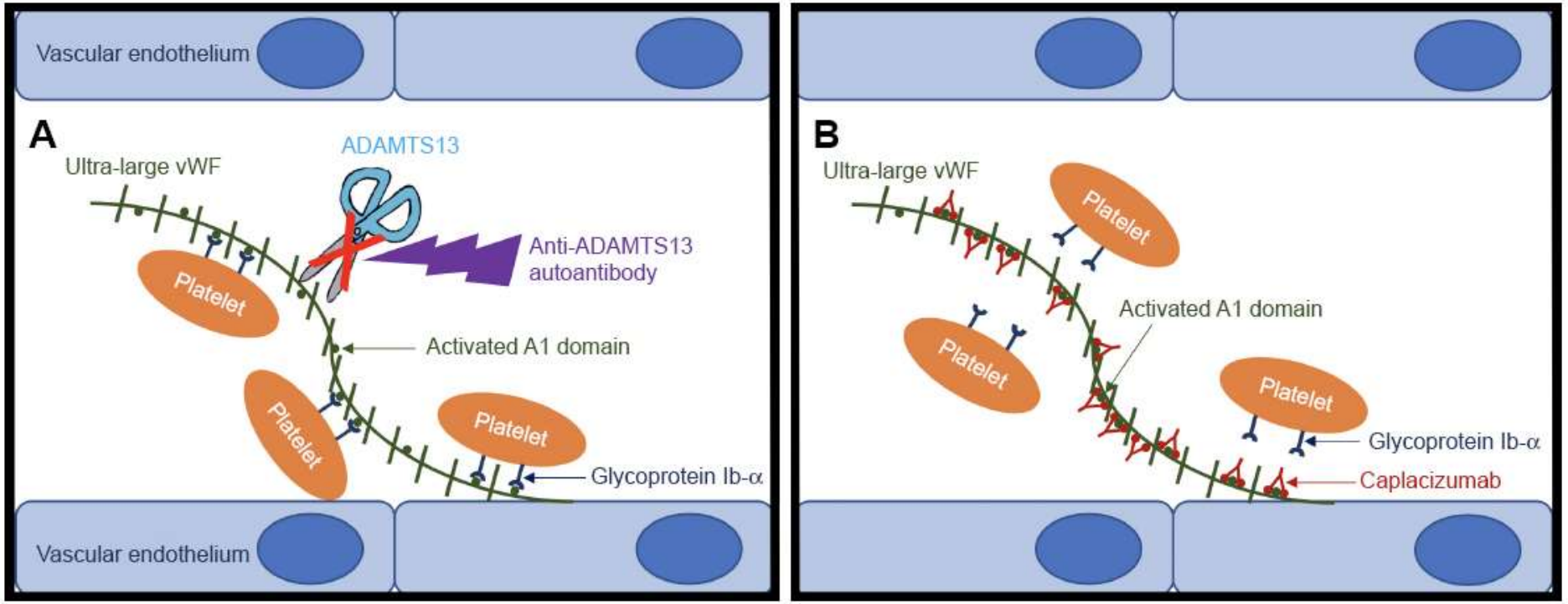
Annual Reviews





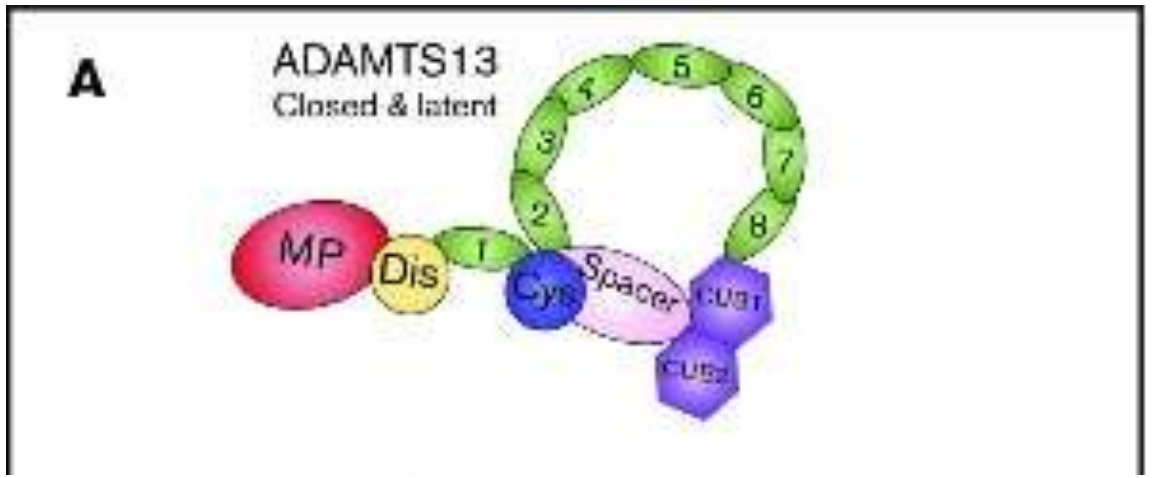
The pathogenesis of aTTP; the presence of anti-ADAMTS13 autoantibodies inhibits the proteolytic cleavage of ultra-large vWF multimers by ADAMTS13, which results in the aggregation of platelets through GP1b-α receptors and the activated A1 domain of the vWF causing microvascular thrombosis and ischemic organ damage.

Anticorpo monoclonale «nasconde» il dominio A1 alle piastrine



(B) Caplacizumab blocks the platelet and ultra-large vWF interaction by binding to A1 domain of vWF.

Meccanismo di azione di ADAMTS13

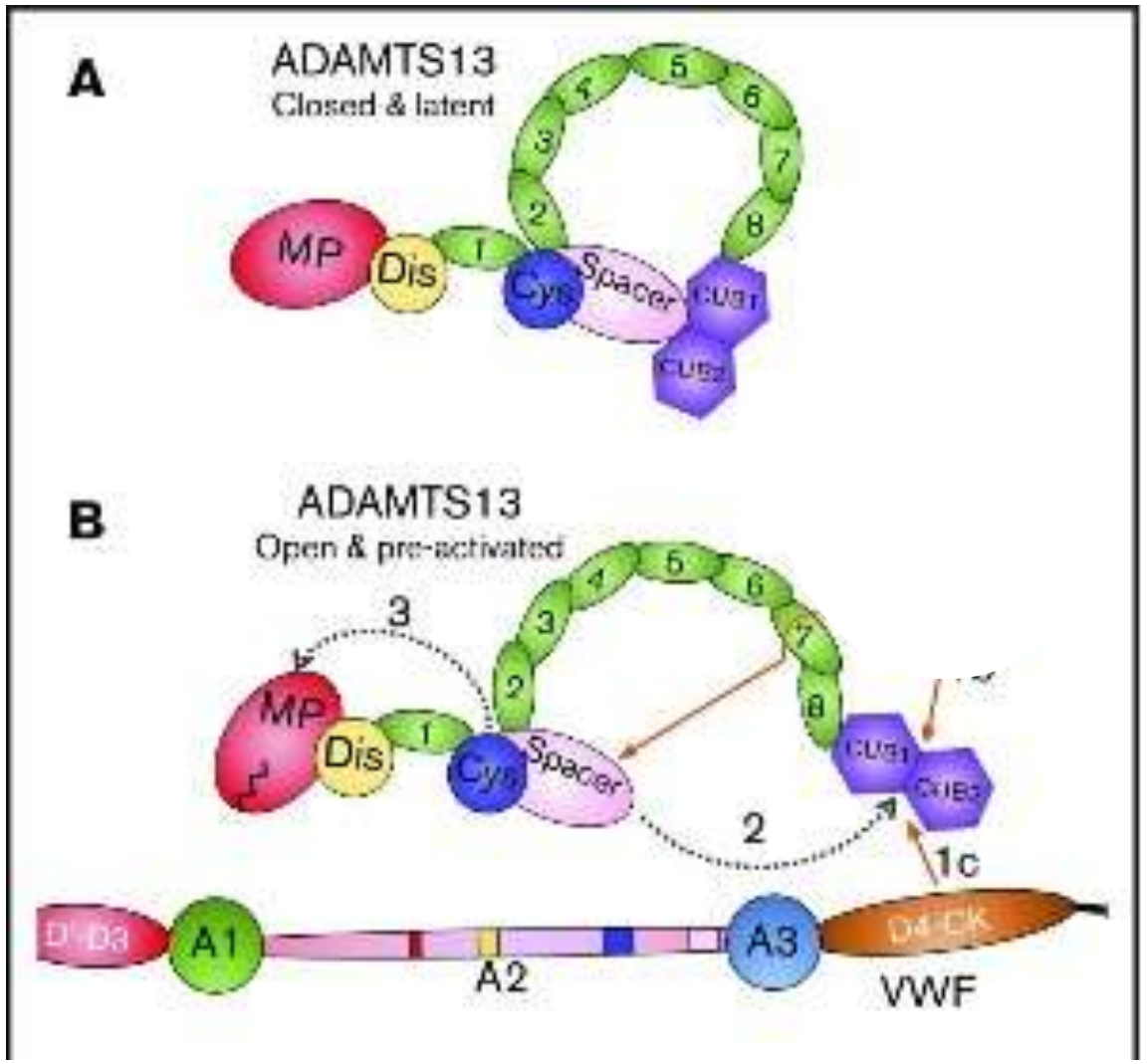


ADAMTS13

A Inactive and stabilized by the interaction of the C-terminal CUB domains with the central Spacer domain

the active site cleft of MetalloProtease domain is occluded, preventing off-target proteolysis

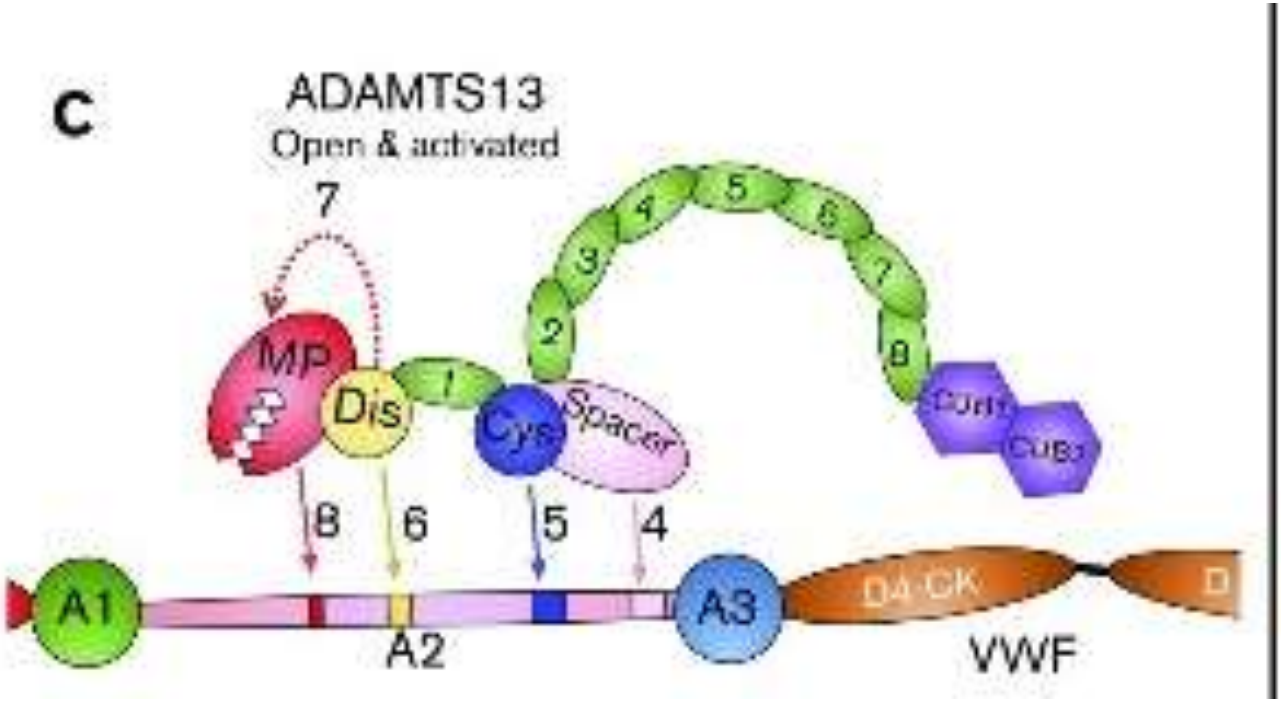
ADAMTS13



B When ADAMTS13 binds to VWF via the D4-CK domains of VWF (1c), the CUB-Spacer interaction is disrupted (2) causing ADAMTS13 to adopt an **open conformation.**

Opening of ADAMTS13 induces a structural shift in the MP domain into a preactivated state (3) that enhances the proteolytic function of the enzyme.

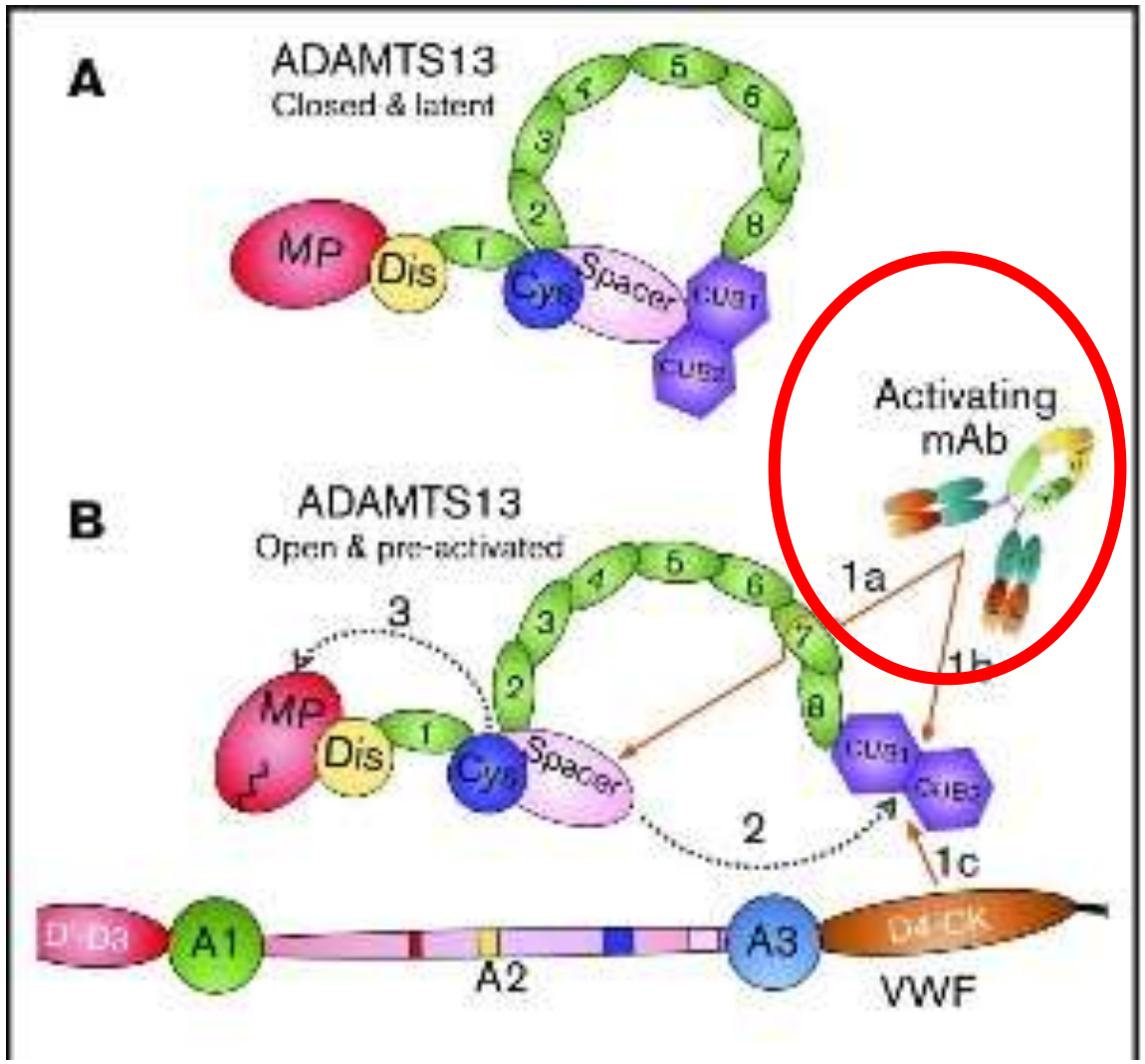
ADAMTS13



The Spacer (4) and (5) Cys-rich **domain exosites recognize** the C-terminal region of the unfolded VWF A2 domain, bringing enzyme and substrate into **close proximity**.

The Dis domain exosite engages VWF (6), which induces a further allosteric change in the MP domain (7).

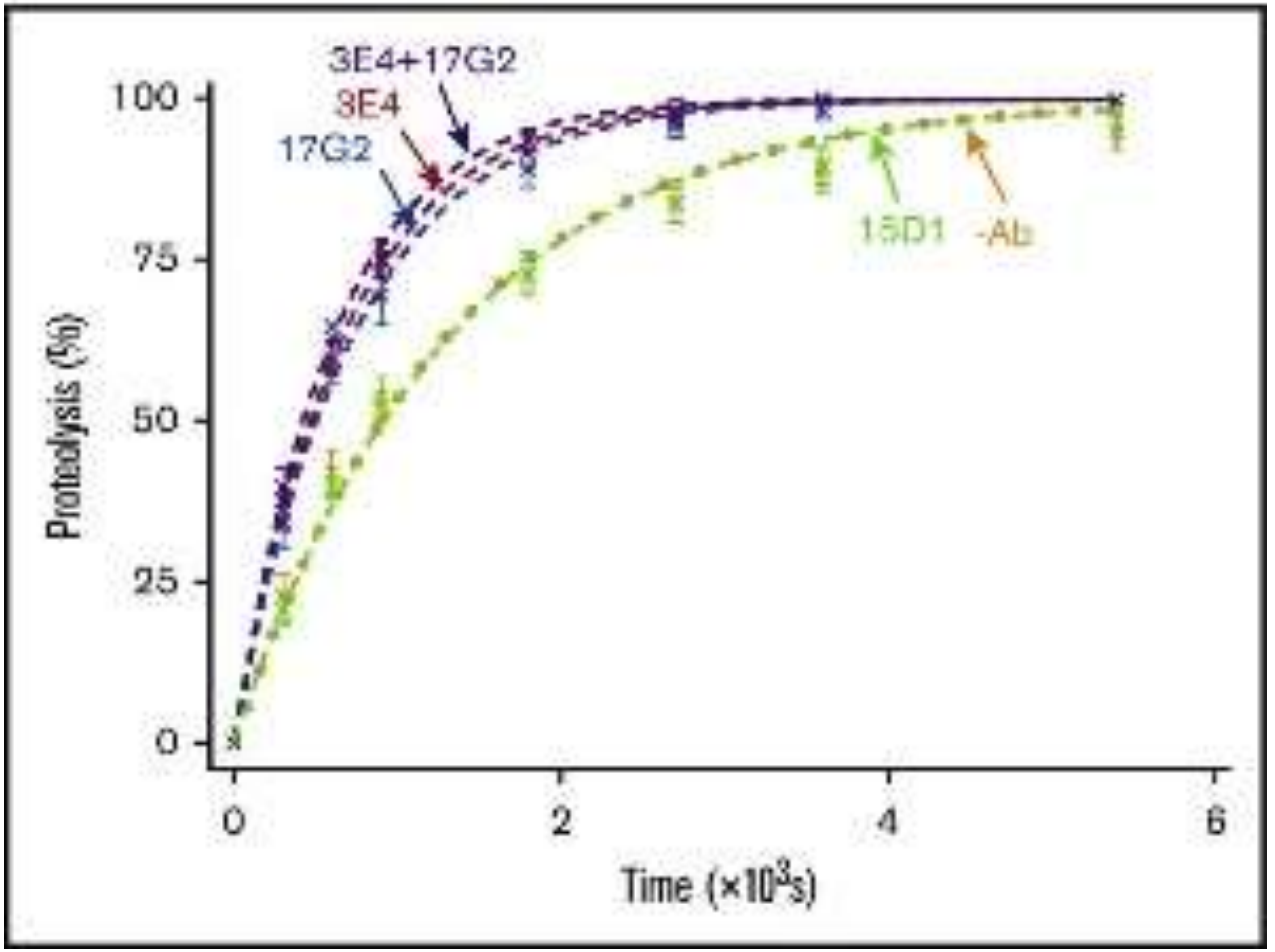
This conformational change **opens the active site cleft** to enable accommodation and **proteolysis** of the cleavage site (8).



Attivazione da anticorpi monoclonali

B When ADAMTS13 **is bound by activating mAbs that recognize either the Spacer domain (1a) or CUB domains (1b)** the CUB-Spacer interaction is disrupted (2) causing ADAMTS13 to adopt an open conformation.

Attivazione di ADAMTS13 da anticorpi monoclonali

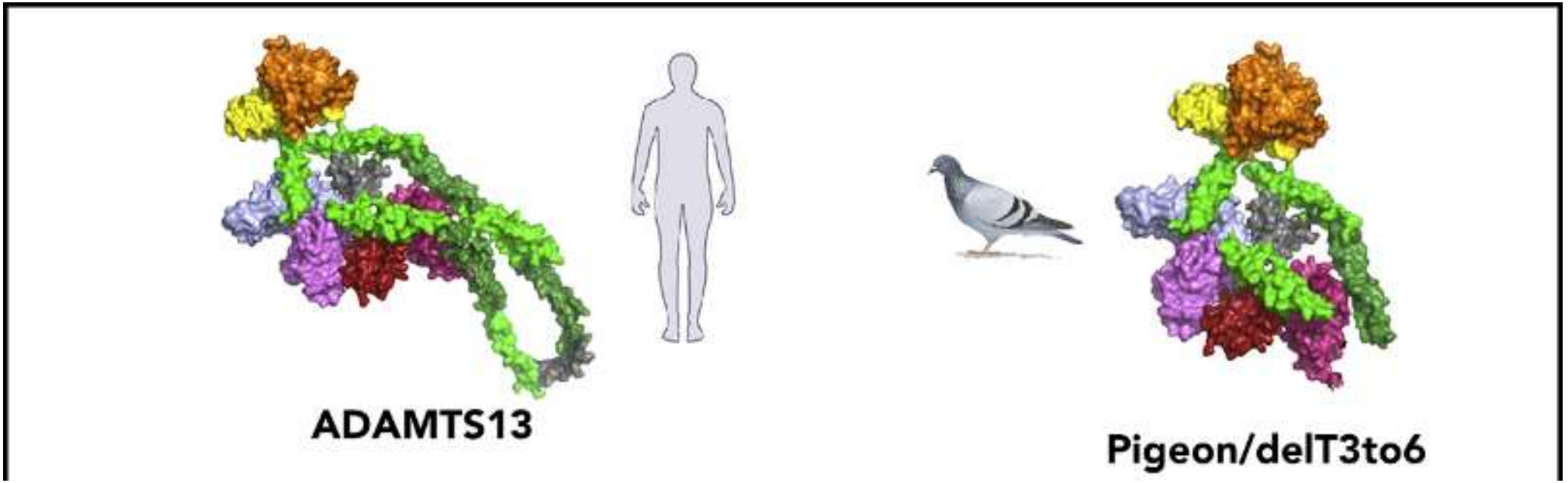


activating anti-Spacer domain mAb 3E4 (red; n = 3)
the **activating anti-CUB1 domain** mAb 17G2 (blue; n = 3)

ADAMTS13 ricombinanti Modificate

Domains that are dispensable for allosteric regulation?

Exploring the “minimal” structure of a functional ADAMTS13 by mutagenesis



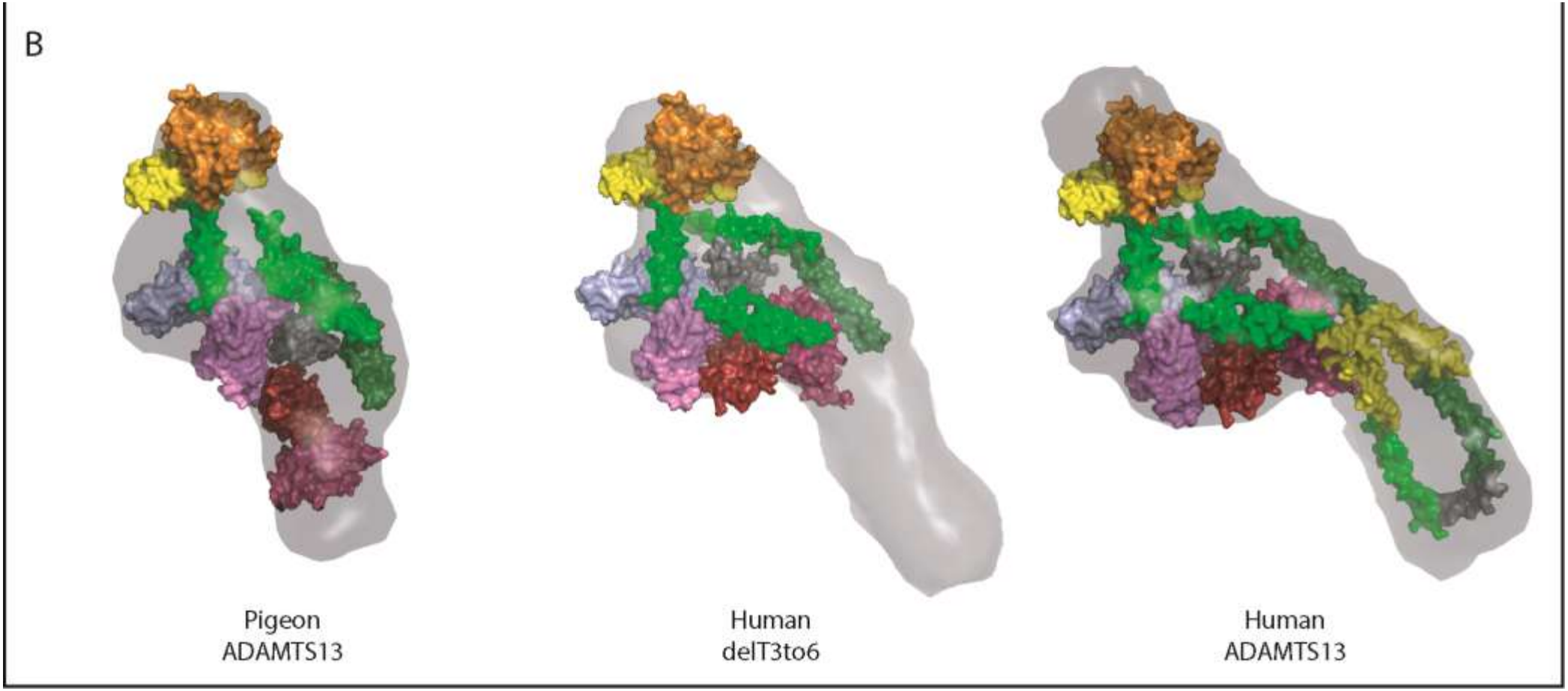
METODO Small angle X-ray scattering (SAXS) to characterize ADAMTS13 constructs

SAXS is a useful approach to obtain low-resolution (10-50 Å) structures for flexible proteins that are not amenable to crystallization or electron microscopy.

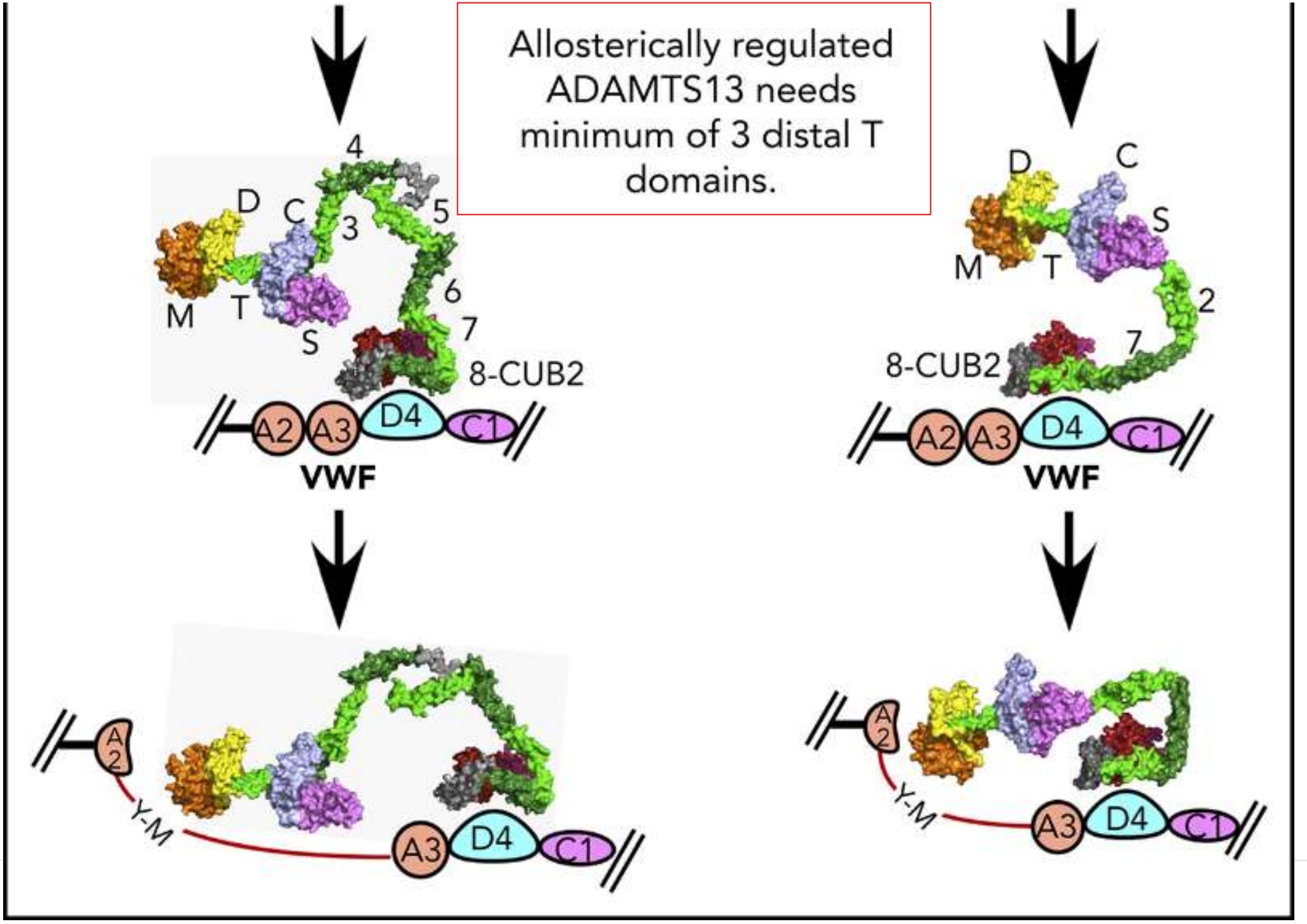
SAXS can be performed under physiological conditions of pH and ionic strength.

Jian Zhu,Joshua Muia,Garima Gupta,Lisa A. Blood, 2019,

Exploring the “minimal” structure of a functional ADAMTS13 by mutagenesis



Exploring the “minimal” structure of a functional ADAMTS13 by mutagenesis



Allosteric properties of ADAMTS13 variants

Construct	Distal Structure	Autoinhibited (rate min^{-1})	Activated by	
			MAbs (fold)	VWF D4 (fold)
ADAMTS13		Yes (2.2)	Yes (3.8)	Yes (1.7)
delT3-6		Yes (2.6)	Yes (2.5)	Yes (1.5)
MT8L*		Partial (4.0)	Yes (1.2)	Yes (1.7)
delT2-8		Partial (3.4)	Yes (1.5)	No (1.0)
delT8		Partial (4.1)	Yes (2.0)	No (1.0)
delT8L		Partial (3.6)	Yes (2.6)	No (1.0)
delT8pL		Partial (4.3)	Yes (2.0)	No (1.0)
delT7		Yes (2.7)	Yes (2.6)	No (1.0)
MT7*		No (9.0)	No (1.0)	No (1.0)

The “minimal” structure of a functional ADAMTS13

1) T7 and T8 domains are essential
2) Other distal domains are dispensable in vitro
3) Pigeons do perfectly well without them in vivo
4) In the vast majority of vertebrates, ADAMTS13 has between 2 and 5 more than the minimum of 3 T domains

The mismatch between the apparent functional minimum and the evolutionary mean number of T domains suggests that at least some T domains perform a function that remains undiscovered