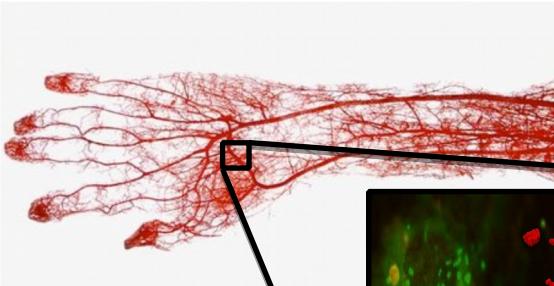
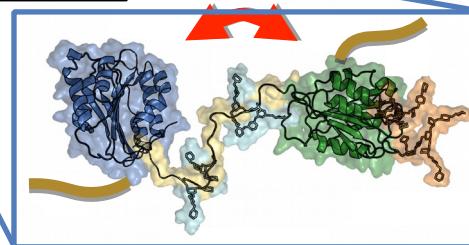


The role of mechanosensitive proteins during blood coagulation



elucidated through
atomistic simulations

Camilo Aponte-Santamaría



Bogotá, Colombia
October 2, 2019



Max Planck Tandem Group
in Computational Biophysics
University of Los Andes
Bogotá, Colombia

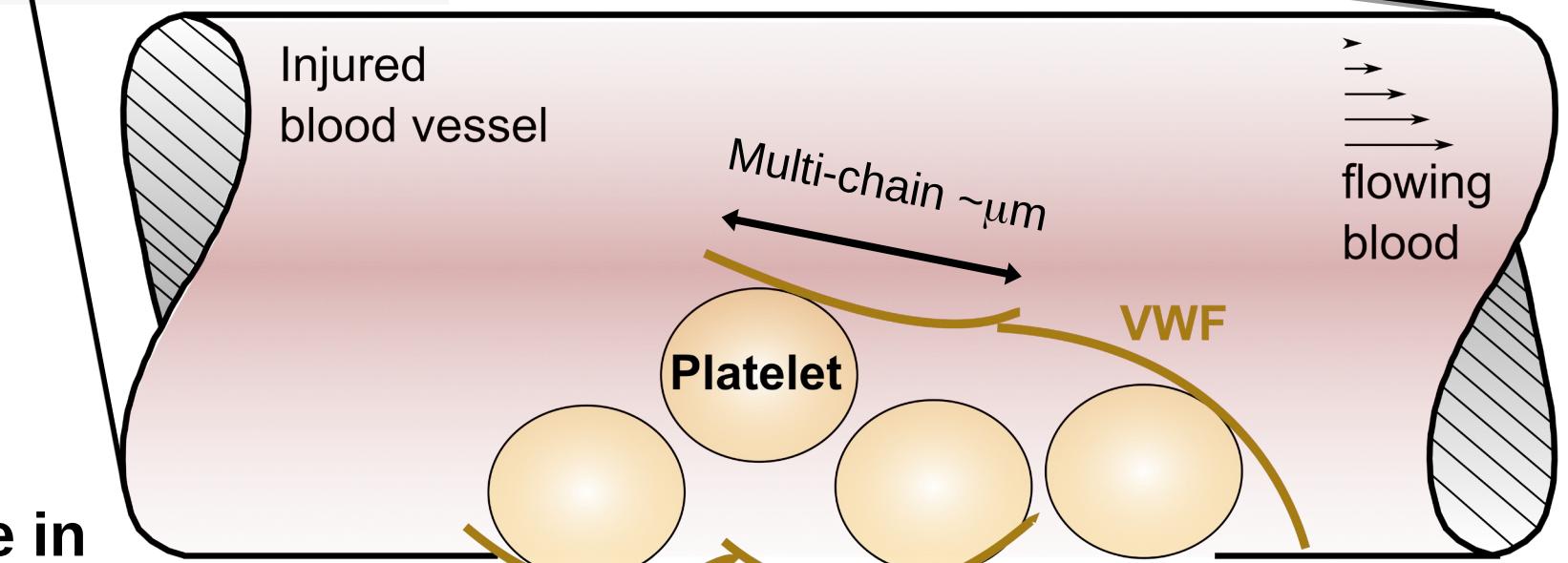
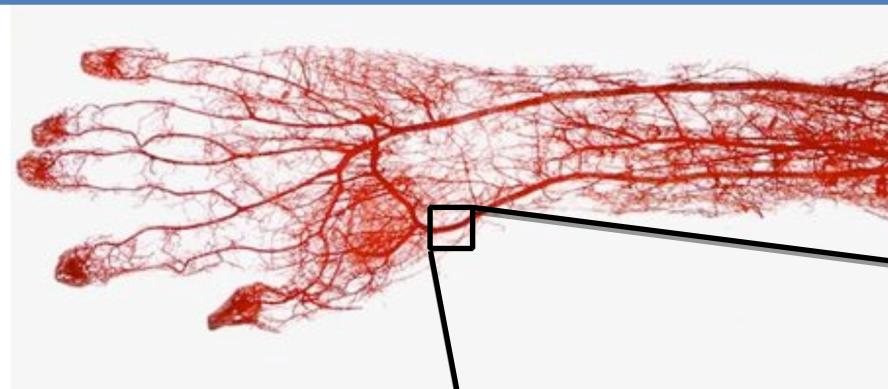


Hosted by:
Cellular Biophysics Dept.
MPI for Medical Research
Heidelberg, Germany

Guest group at:
Interdisciplinary Center
for Scientific Computing (IWR)
Heidelberg, Germany

von Willebrand factor (VWF)

review: Jedi Knight of the Bloodstream.
T. Springer. Blood. 2014



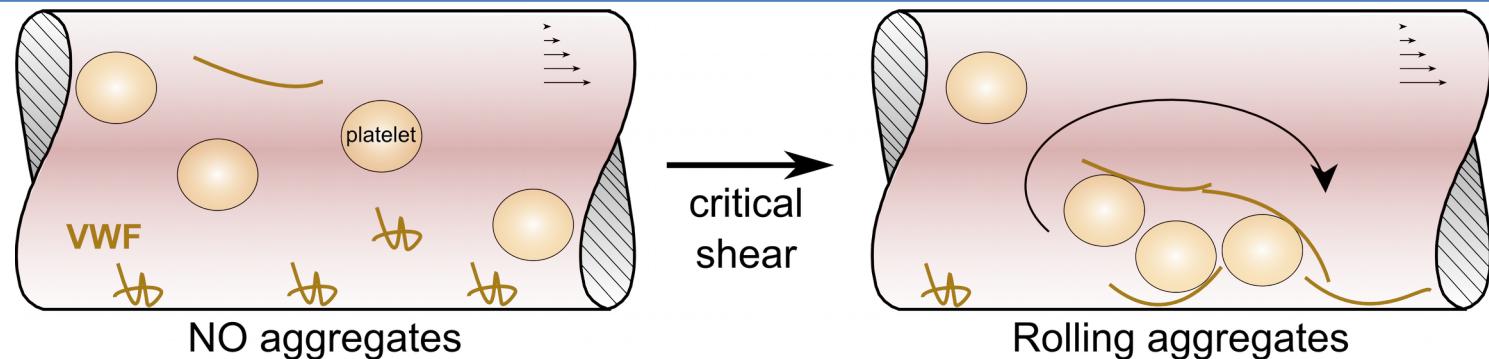
**adhesive role in
primary hemostasis**

**activated by the shear
of flowing blood**

malfunction: bleeding disorders -> drug targets
biology - medicine - pharmaceutics
bio-inspired nanotechnology
material science - engineering

VWF-platelet rolling-aggregates

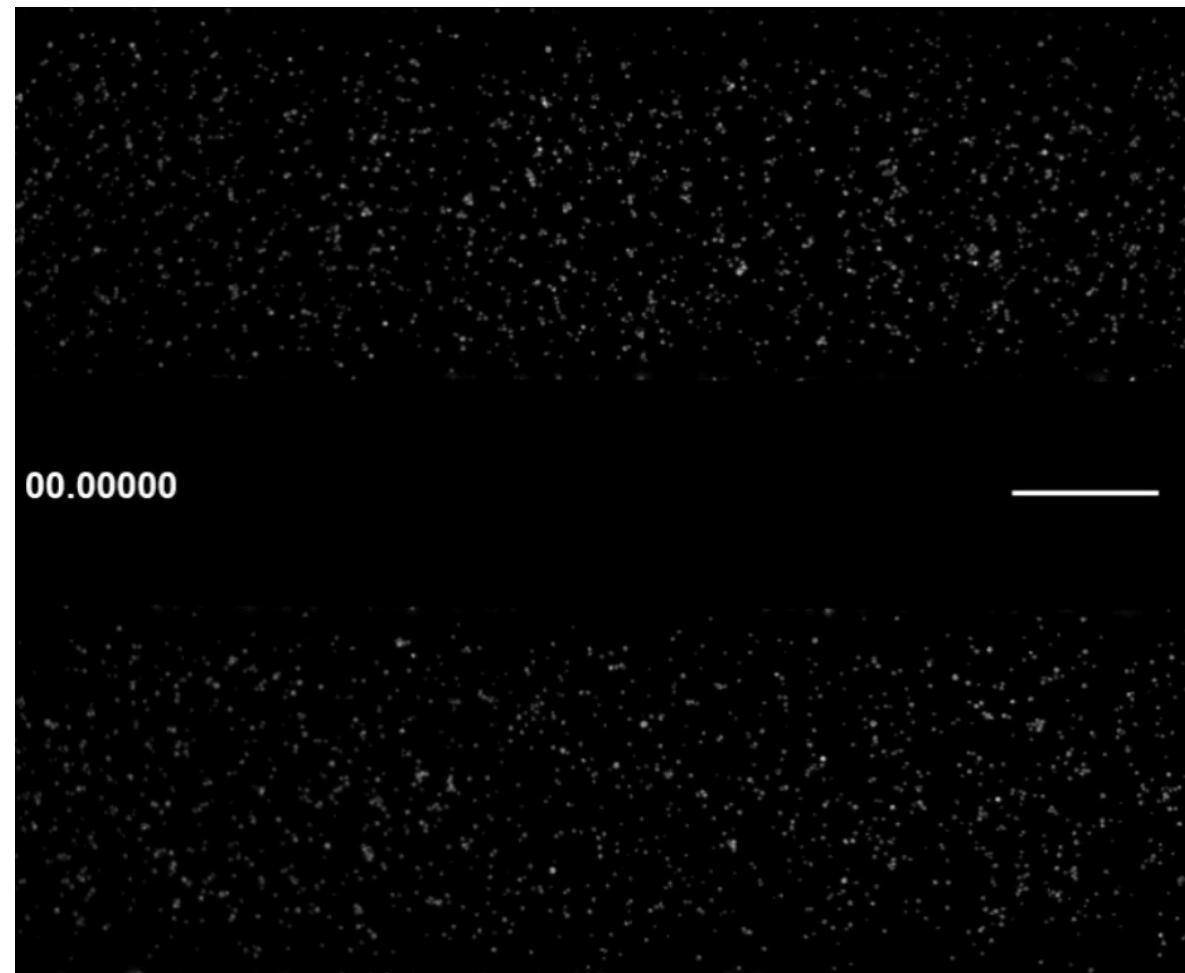
*microfluidic
experiments
Huck, Schneider
Mannheim (Germany)*



0-1 min: 500 Hz

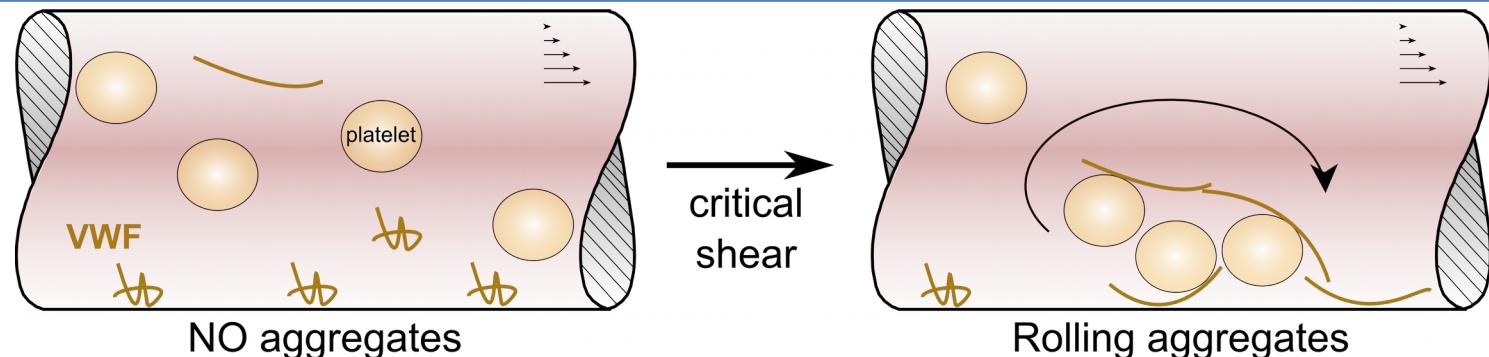
1-5 min: 2500 Hz

>5min: 4000 Hz



VWF-platelet rolling-aggregates

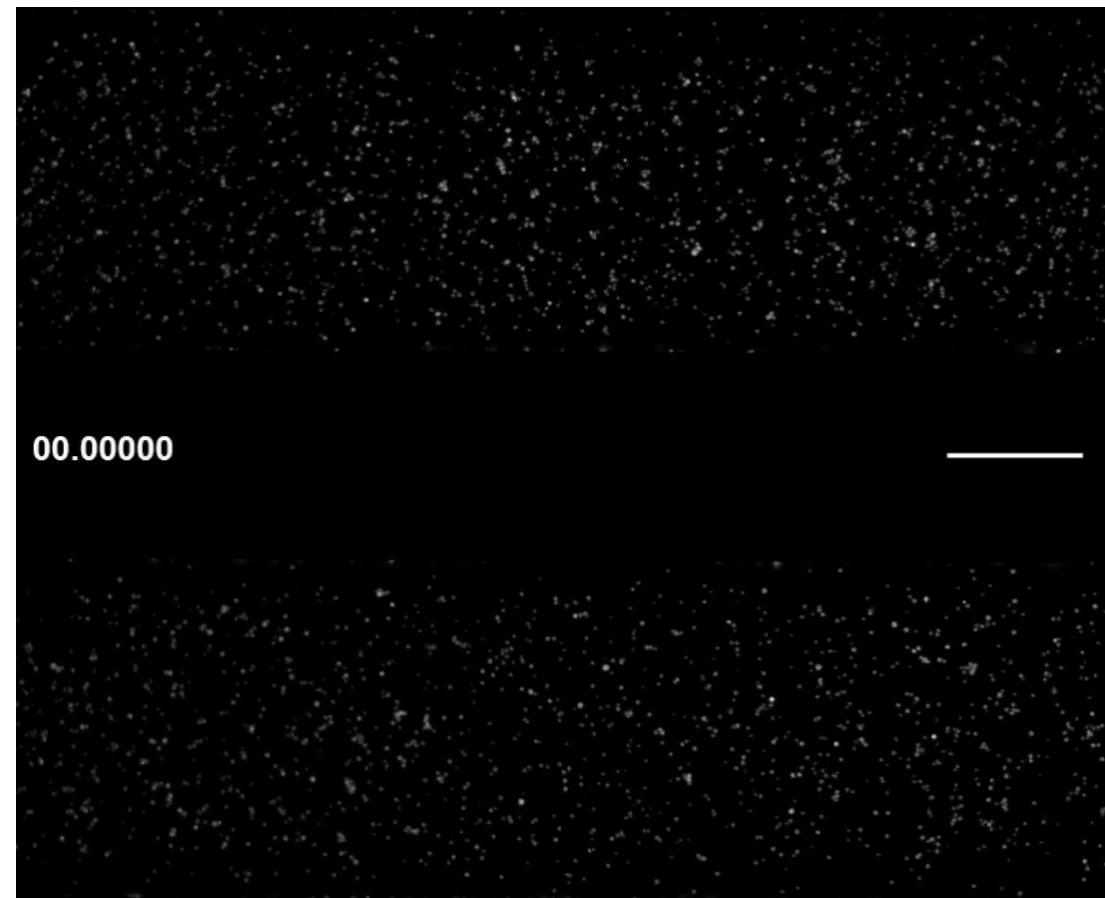
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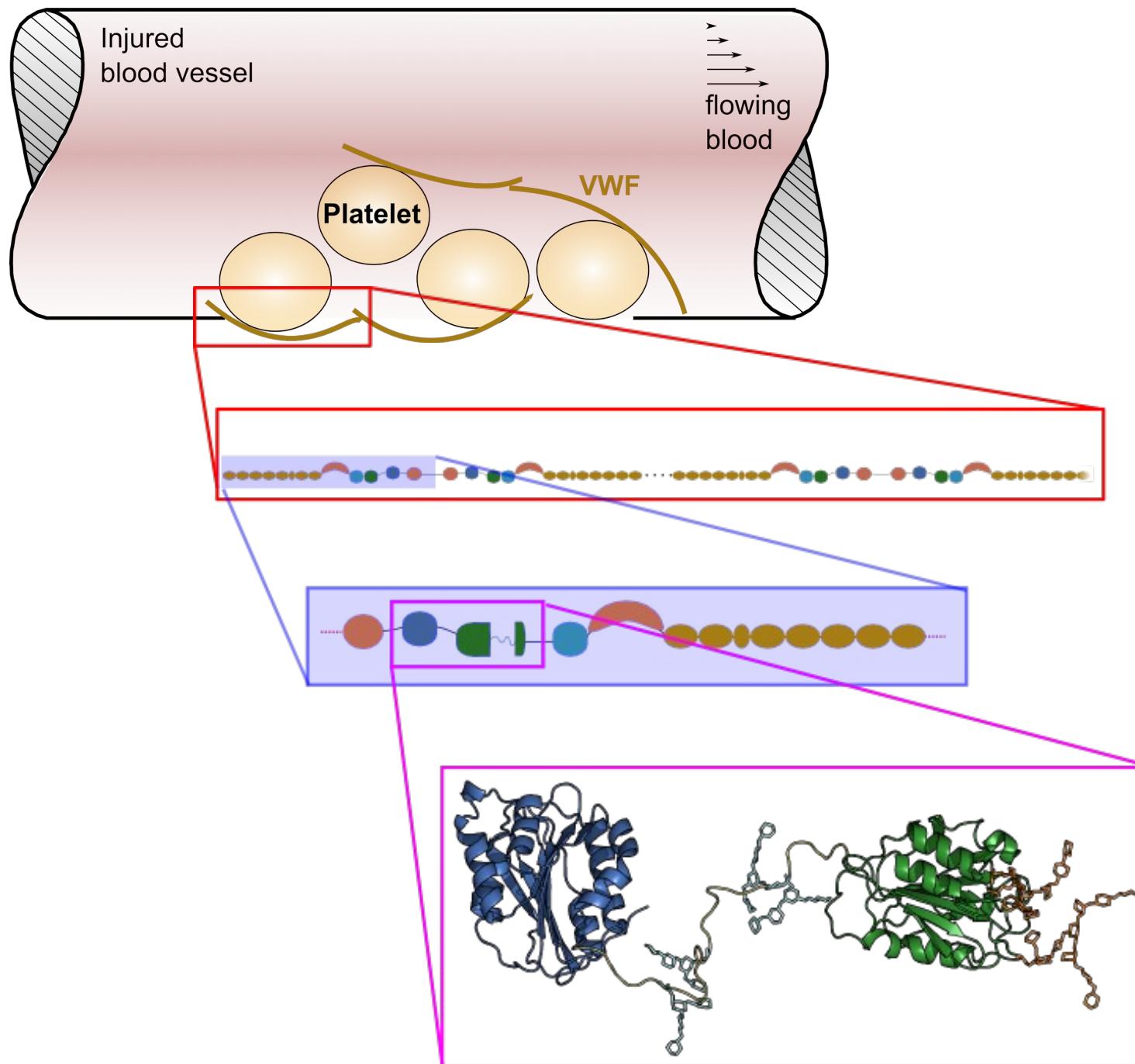
0-1 min: 500 Hz

1-5 min: 2500 Hz

>5min: 4000 Hz



von Willebrand factor (VWF)



multi-chain (<100 chains)
 $\sim\mu\text{m}$

chain (13 domains)
 $\sim60-80 \text{ nm}$

protein domain $< 8 \text{ nm}$

SHENC: German-Austrian network on hemostasis

MD

Frauke Gräter
Katra Kolsek
Agnieszka Bronowska

Heidelberg
Carsten Baldauf
Berlin

AFM

Sandra Posch
Peter Hinterdorfer
Linz

Microfluidics

Volker Huck
Sandra Grässle
Stefan Schneider
Mannheim

FCS

Svenja Lippok
Joachim Rädler
Munich

Constructs

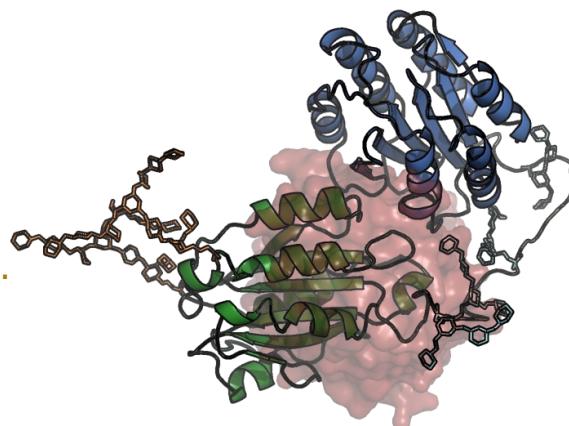
Maria A. Brehm
Tobias Obser
R.Schneppenheim
Hamburg



Computational studies of VWF

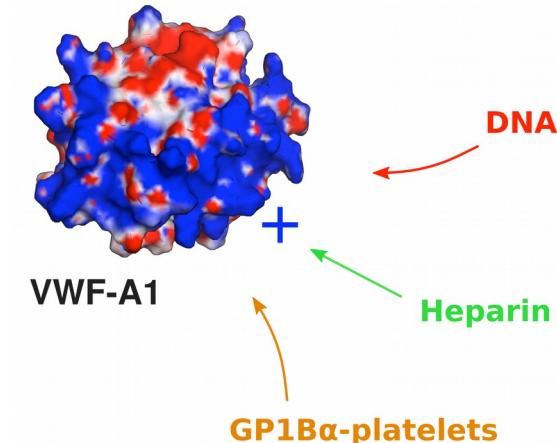
protein-protein: autoinhibition

- CAS, Huck, Posch et al.
Biophys J. 2015
- Posch, **CAS** et al.
J Struct Biol. 2016
- Butera,...,**CAS** et al.
Science Adv. 2018



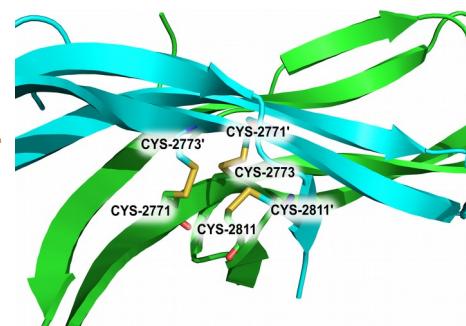
VWF-DNA: inflammation

- Grässle...**CAS** et al.
ATVB. 2014



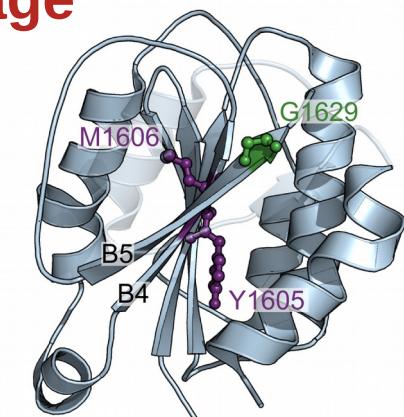
dimerization

- Brehm, Huck, **CAS** et al.
Thromb. Haemos. 2014
- Lippock, Kolsek...**CAS** et al.
Blood. 2016



mutation-induced accelerated cleavage

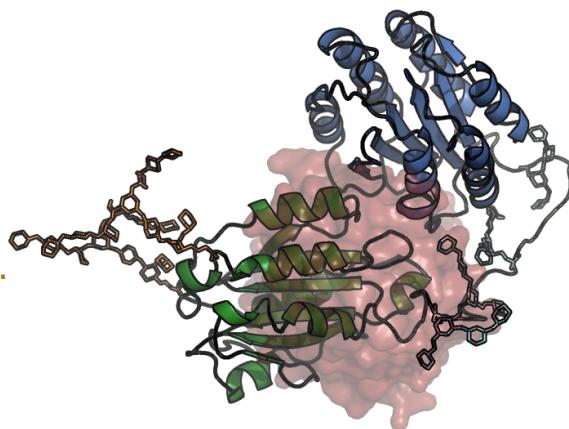
- CAS**, Lippock et al.
Biophys. J. 2017



Computational studies of VWF

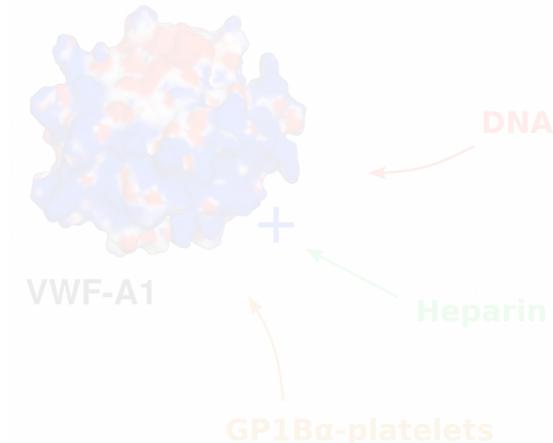
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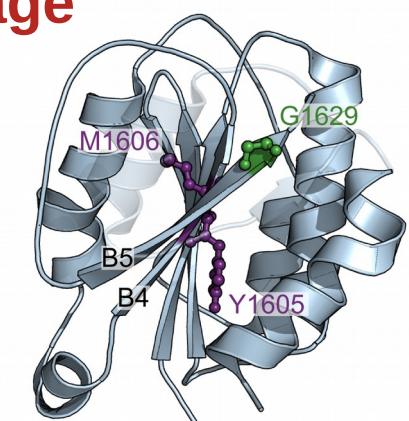
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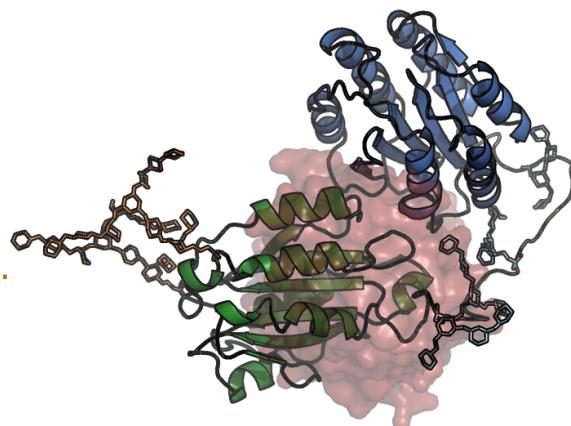
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Computational studies of VWF

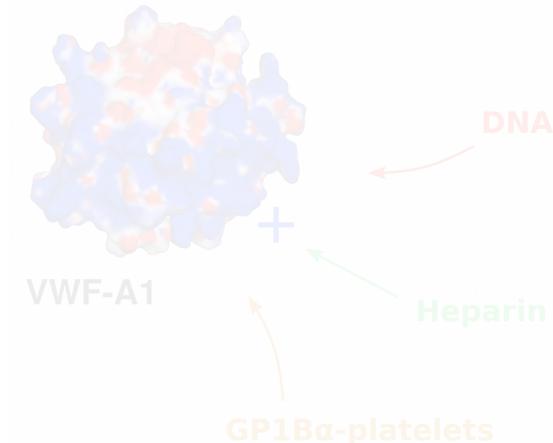
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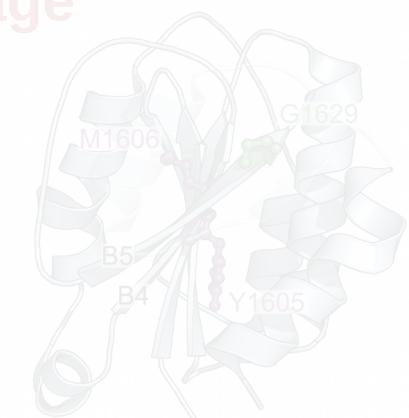
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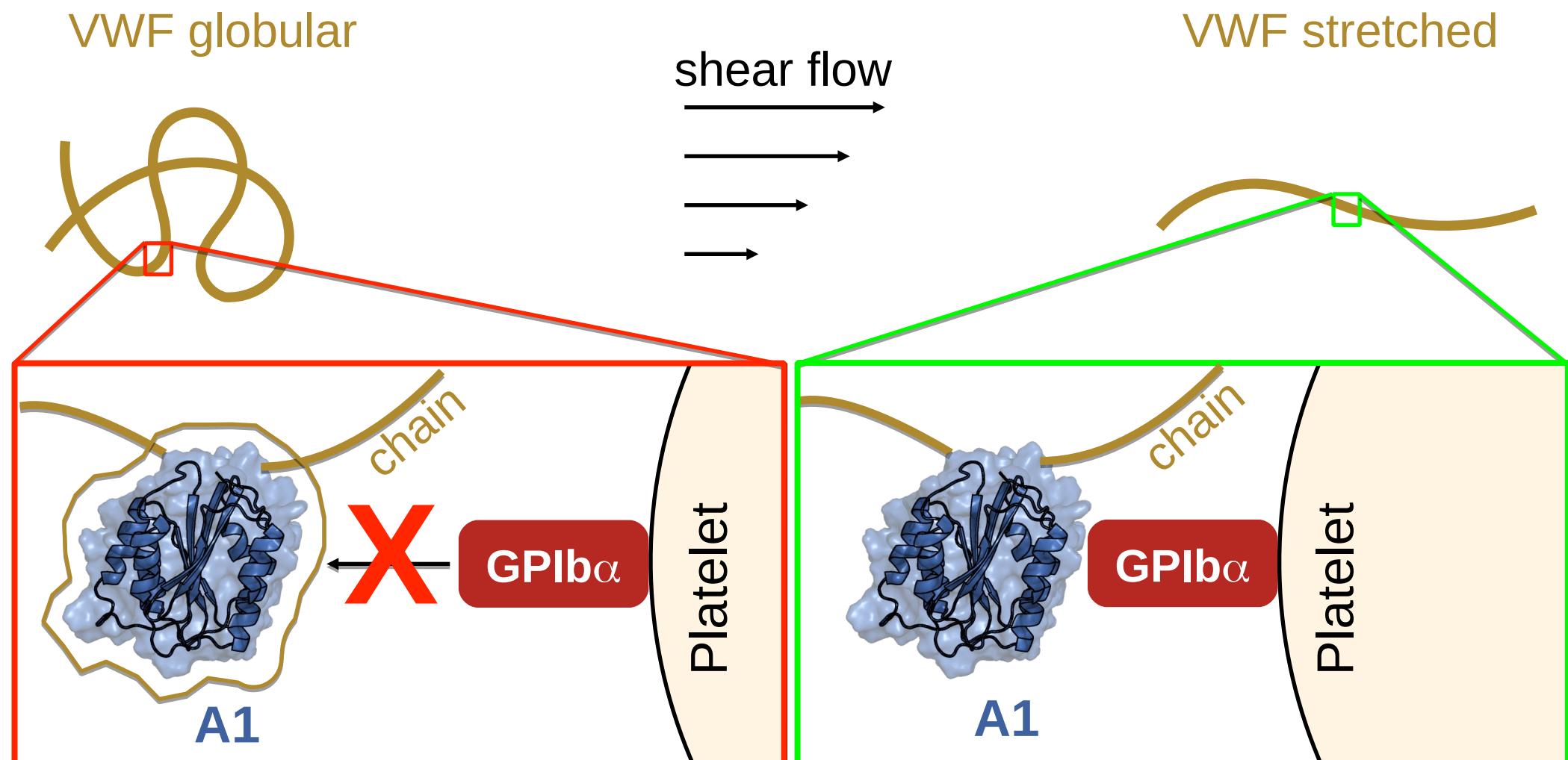


mutation-induced accelerated cleavage

CAS, Lippock et al.
Biophys. J. 2017



VWF: mechanosensitive protein

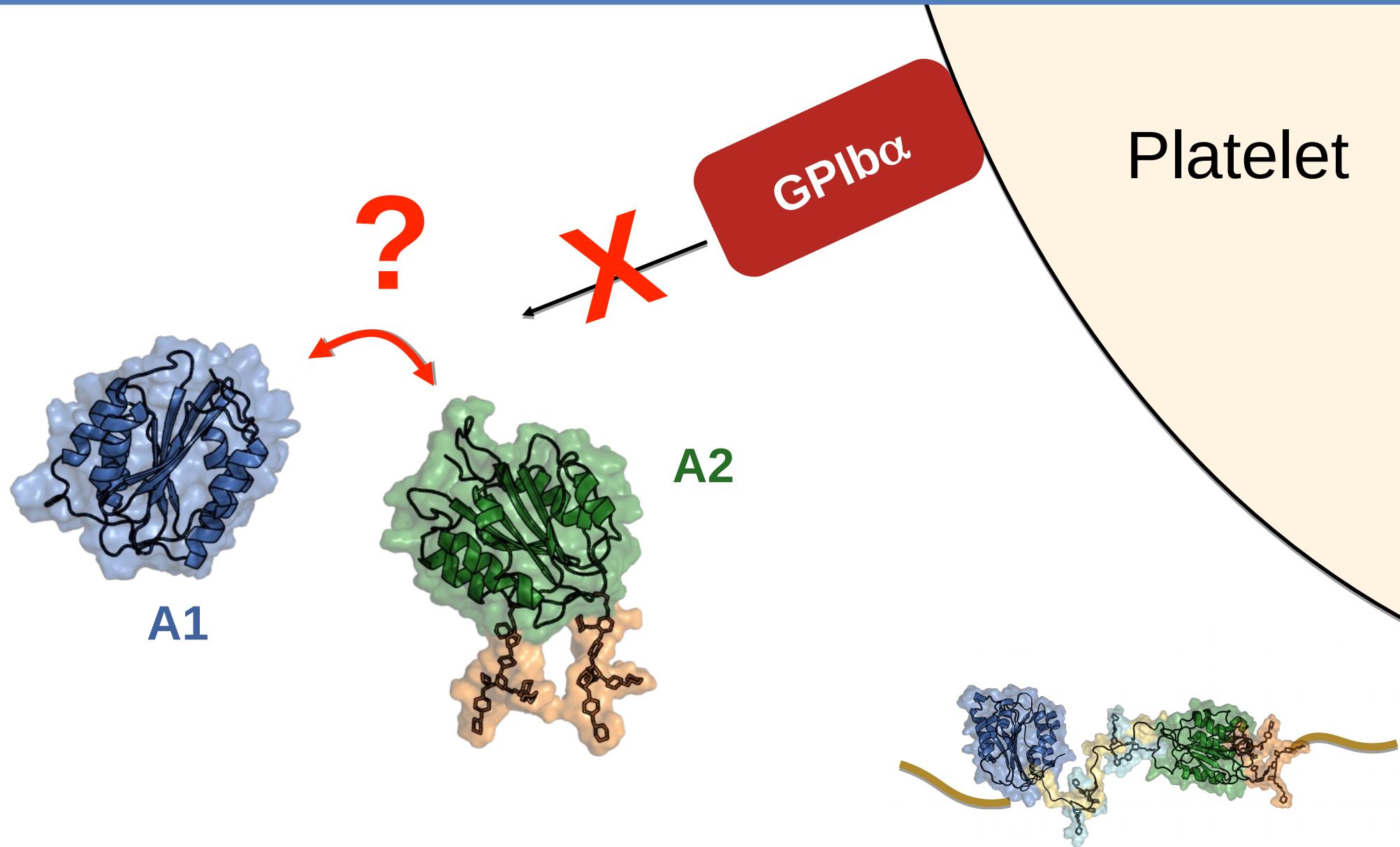


A1 domain ($\text{GP}1\text{b}\alpha\alpha$ -platelet binding site):

shielded

exposed

VWF auto-inhibition



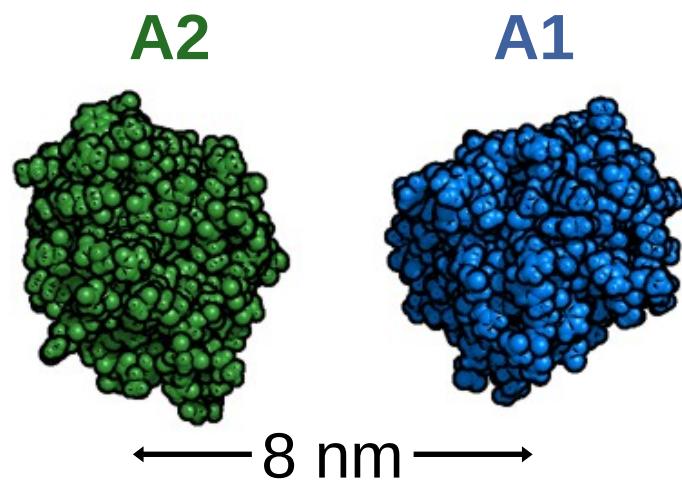
molecular nature of A1-A2 interactions?

A2 targets GPIB α binding site in A1 causing its blockage

multiple MD simulations

N=17 2~ μ s concatenated

0.0ns

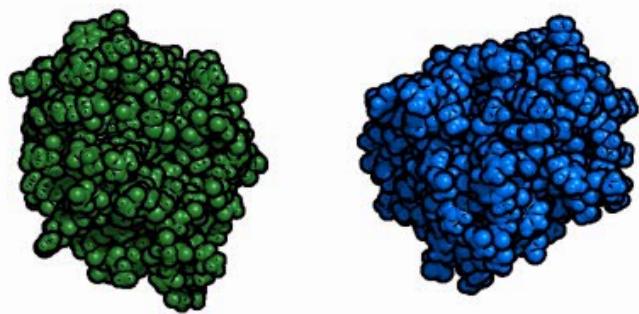


A2 targets GPIB $\alpha\alpha$ binding site in A1 causing its blockage

multiple MD simulations

$N=17$ 2~ μ s concatenated

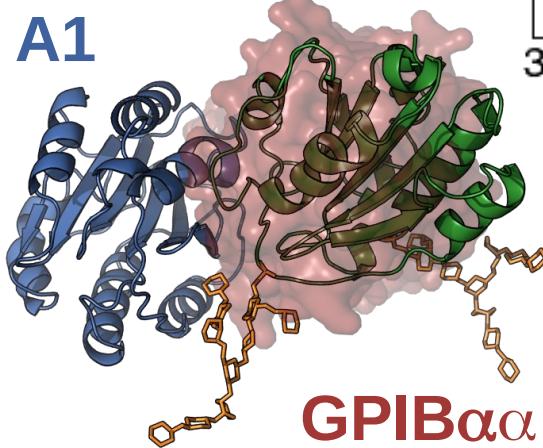
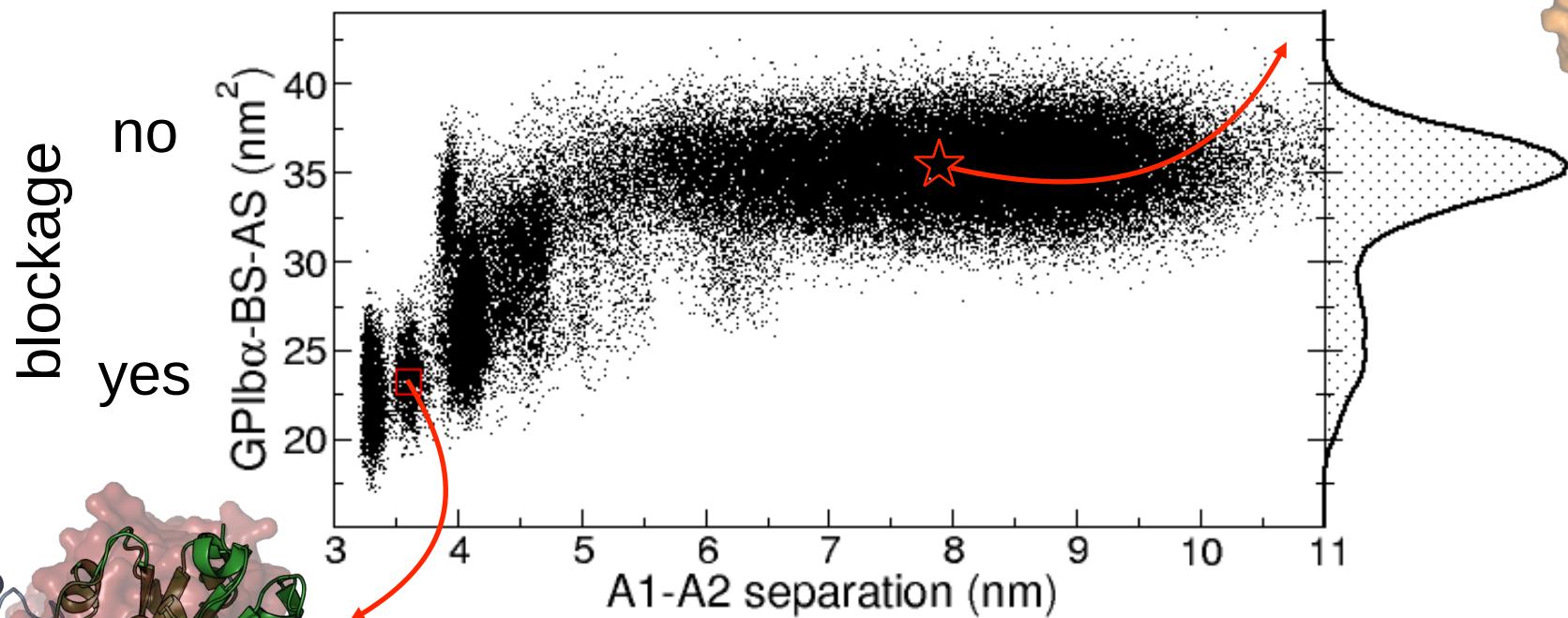
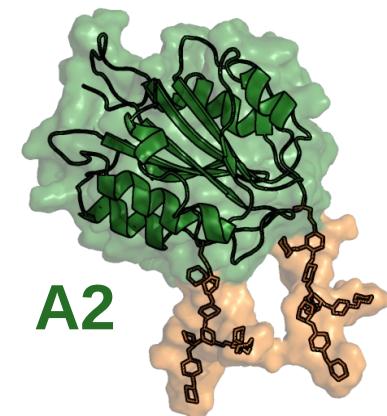
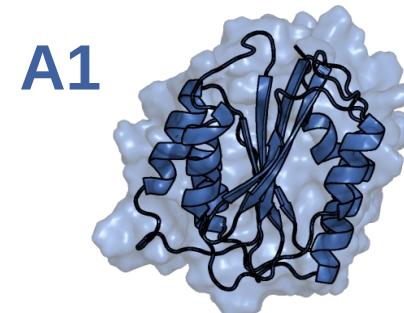
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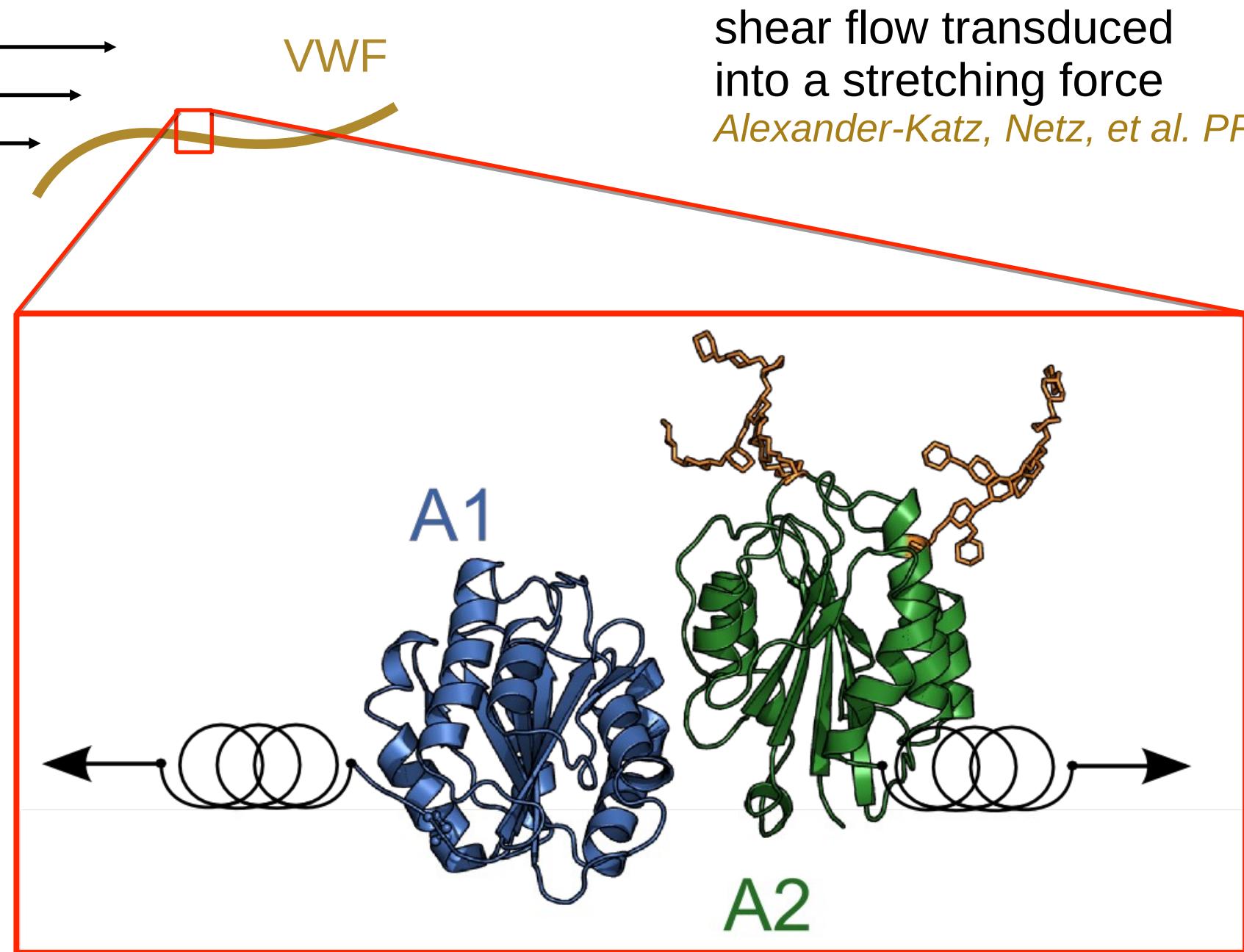
$N=17$ 2~ μ s concatenated



A2

GPIB α binding site blocked
upon binding of **A2** to **A1**

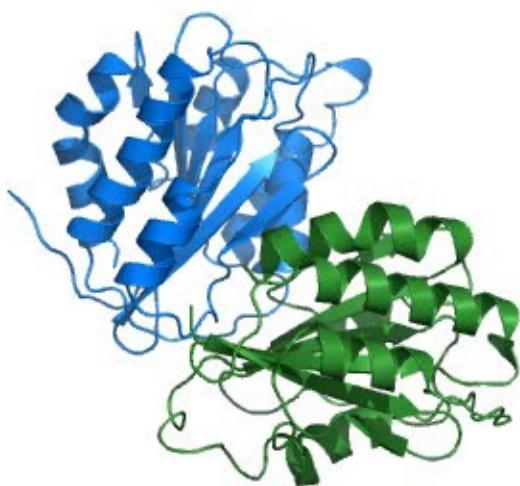
A1-A2 complex under force: activation vs. cleavage



A1-A2 complex under force: activation vs. cleavage

Force-probe MD simulations

0.0ns

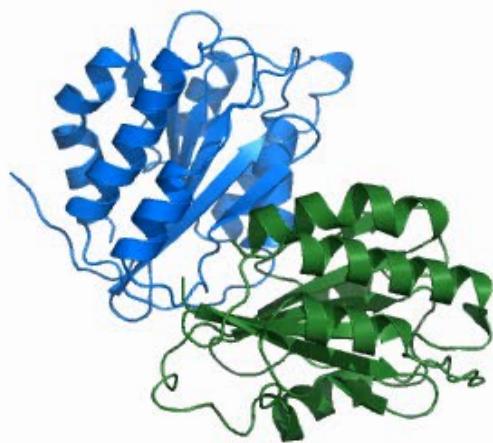


dissociation after partial unfolding of **A2**

A1-A2 complex under force: activation vs. cleavage

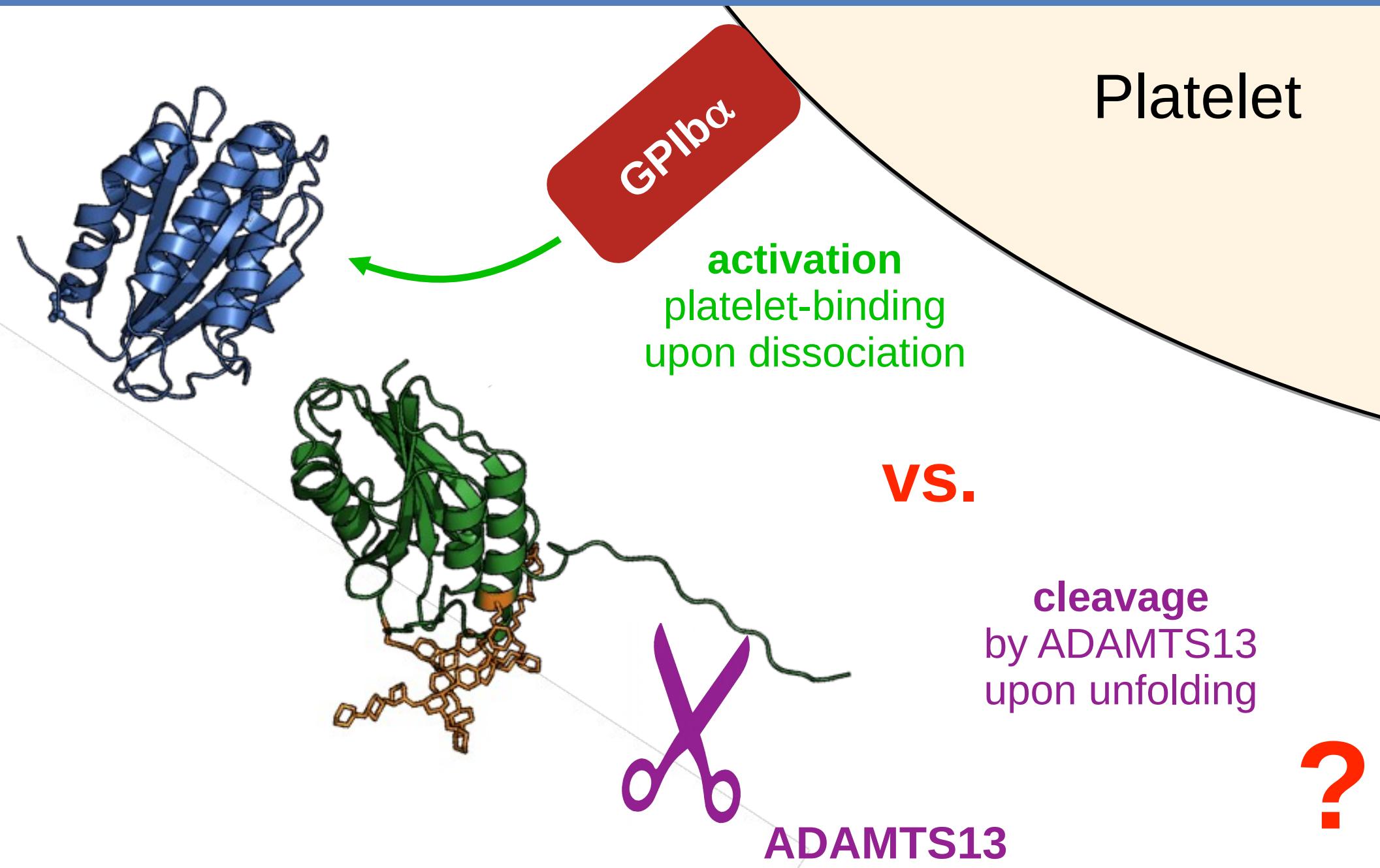
Force-probe MD simulations

0.0ns

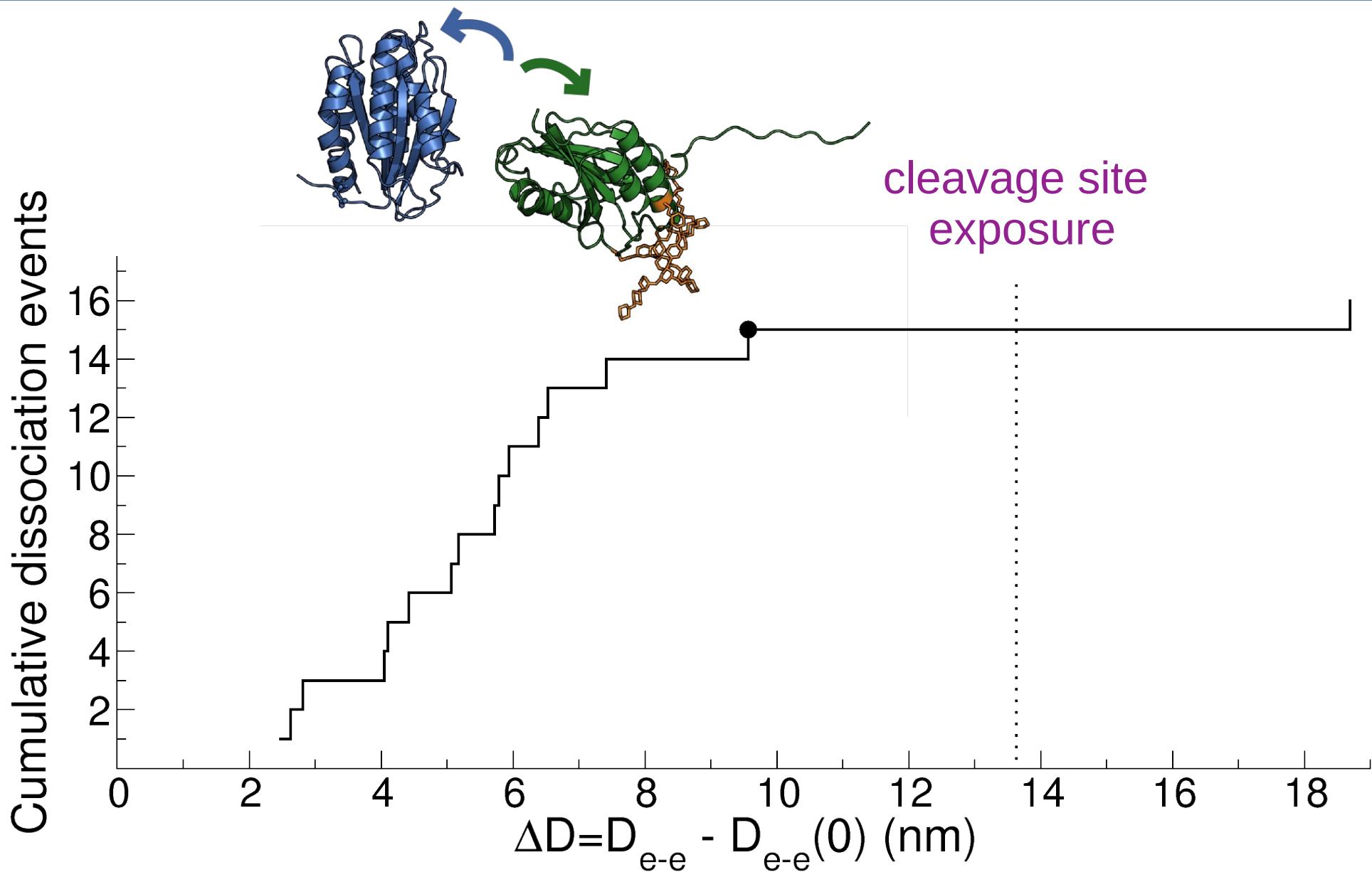


dissociation after partial unfolding of **A2**

A1-A2 complex under force: activation vs. cleavage



A1-A2 complex under force: activation vs. cleavage

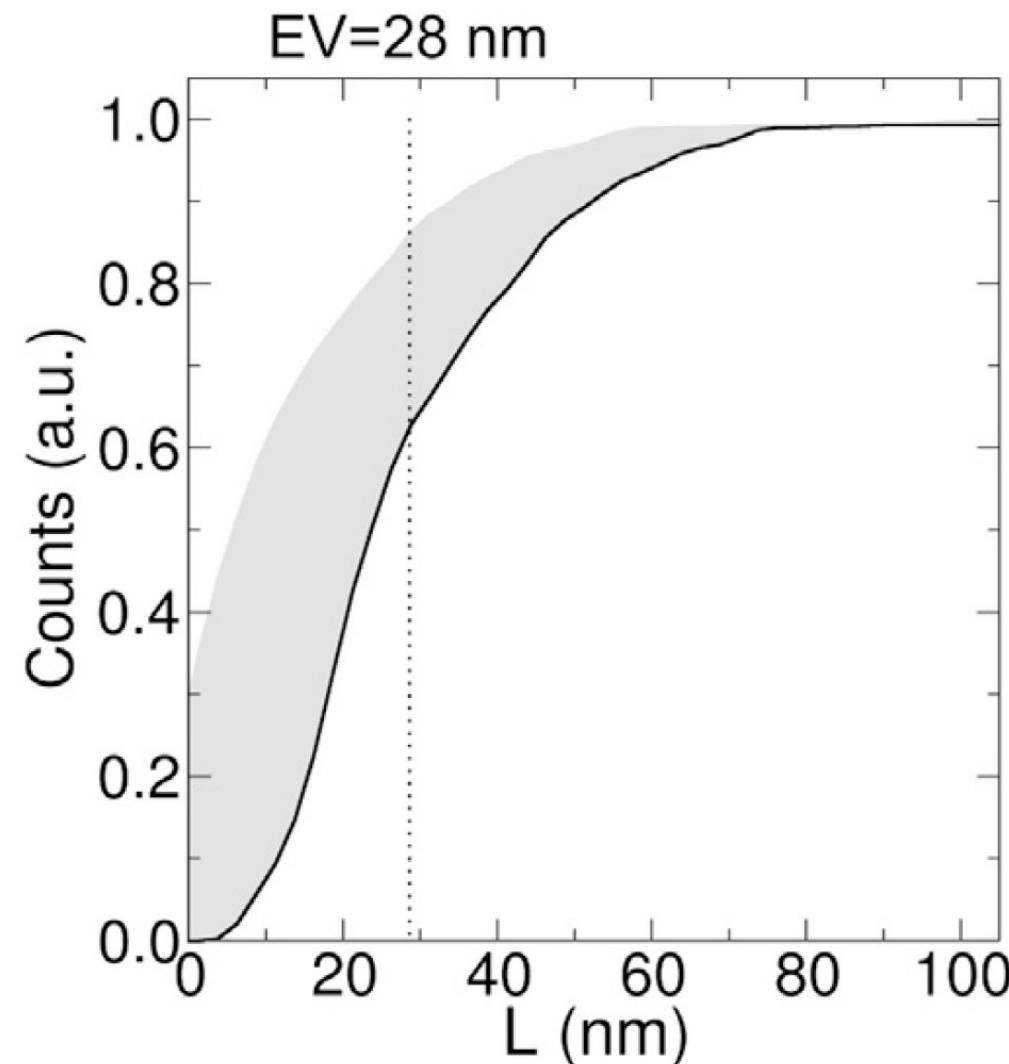
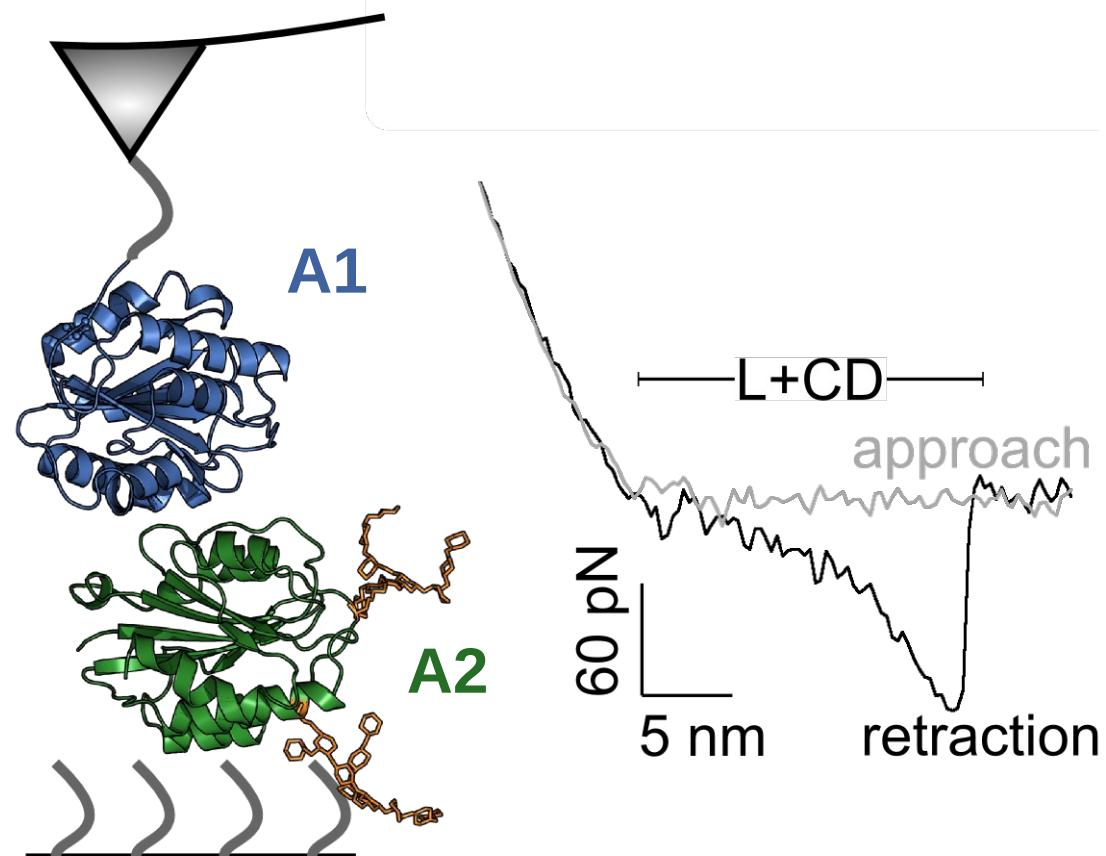


Dissociation occurs before exposure of cleavage site

A1-A2 complex under force: activation vs. cleavage

AFM

Posch, Hinterdorfer, Linz (Austria)



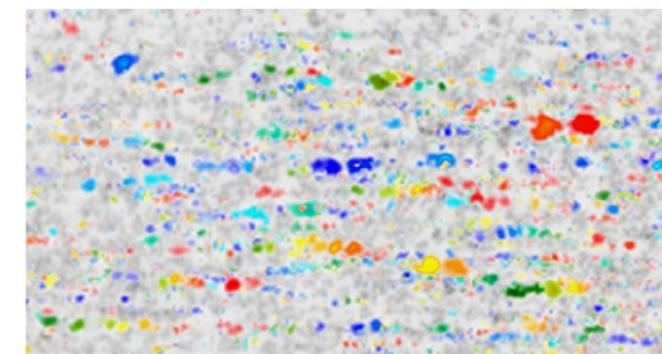
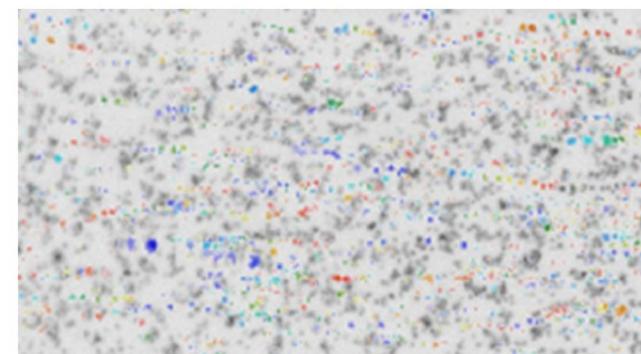
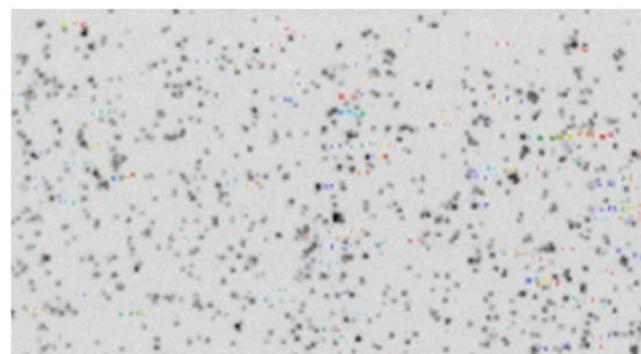
Little unfolding before dissociation

Functional characterisation of Δ A2-VWF mutant

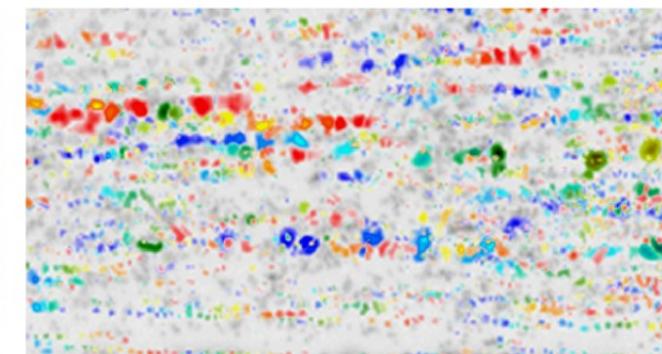
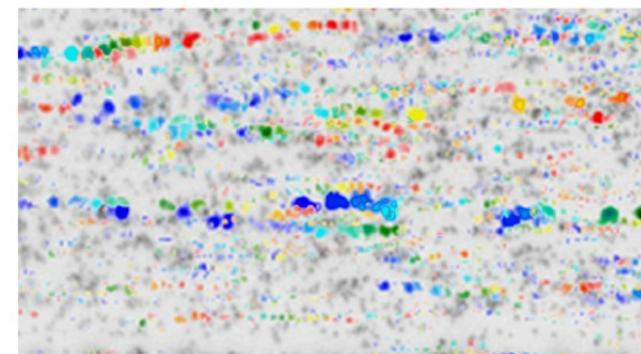
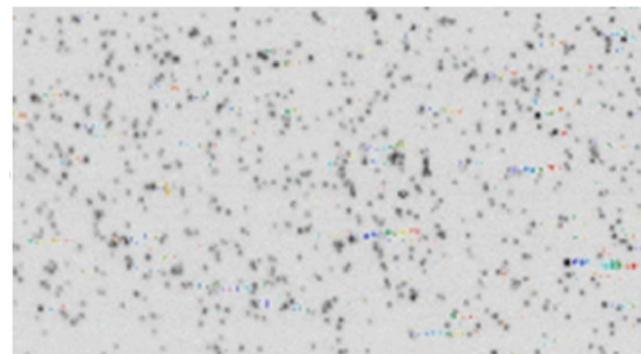
*microfluidic
experiments*

*Huck, Schneider
Mannheim (Germany)*

wt-VWF



Δ A2-VWF



500 Hz

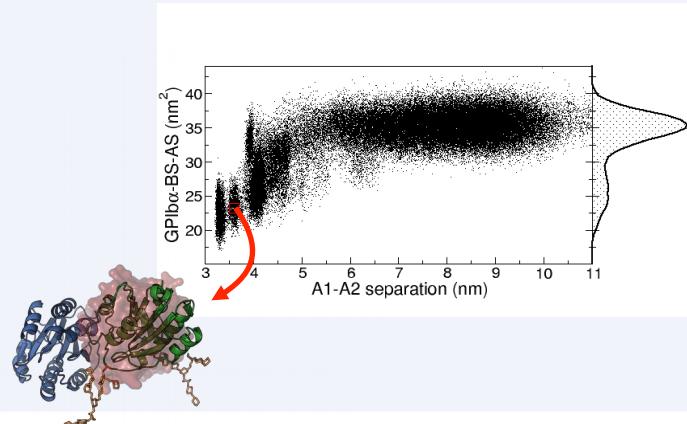
2500 Hz

4000 Hz

A2-deletion reduces critical shear rate: gain of function

Force-sensitive VWF auto-inhibition by inter-domain interactions

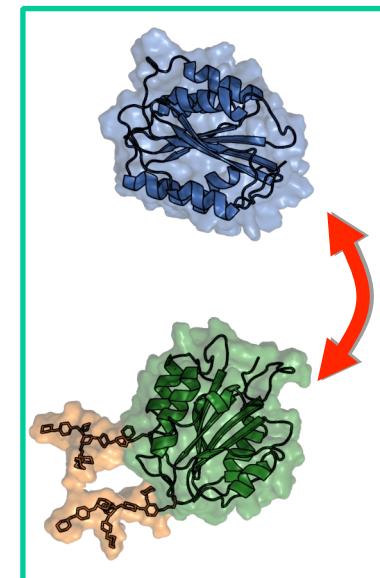
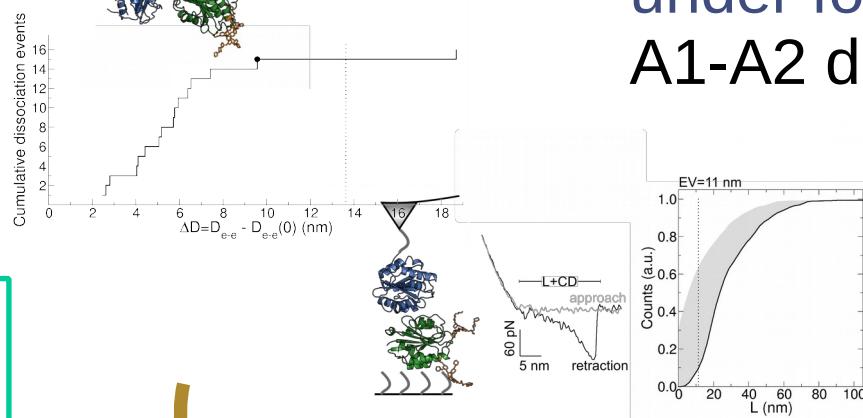
A1-A2 auto-inhibitory interactions:
A2 targets GPIba-platelet binding site in A1



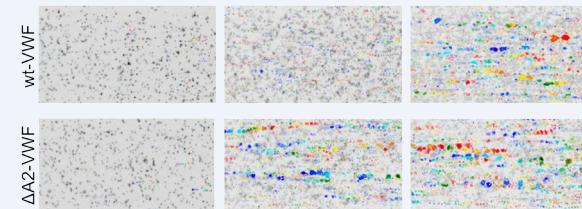
under force:
A1-A2 dissociation prior A2-unfolding

activation before cleavage

Outlook



$\Delta A2$ -VWF:
gain-in-function
mutant



Computational studies of VWF

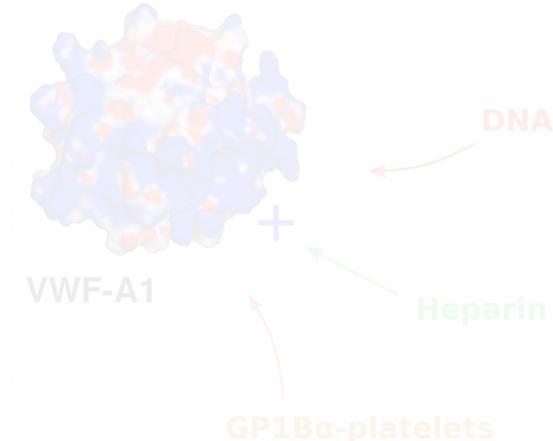
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- Grässle...**CAS** et al.
ATVB. 2014



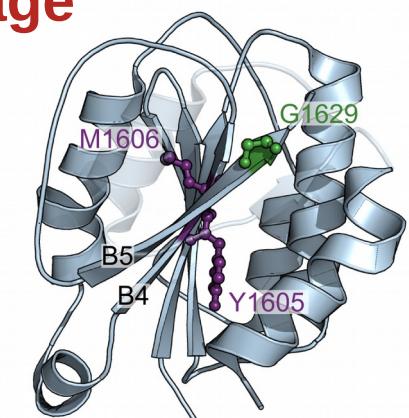
dimerization

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Blood. 2016



mutation-induced accelerated cleavage

- CAS**, Lippock et al.
Biophys. J. 2017

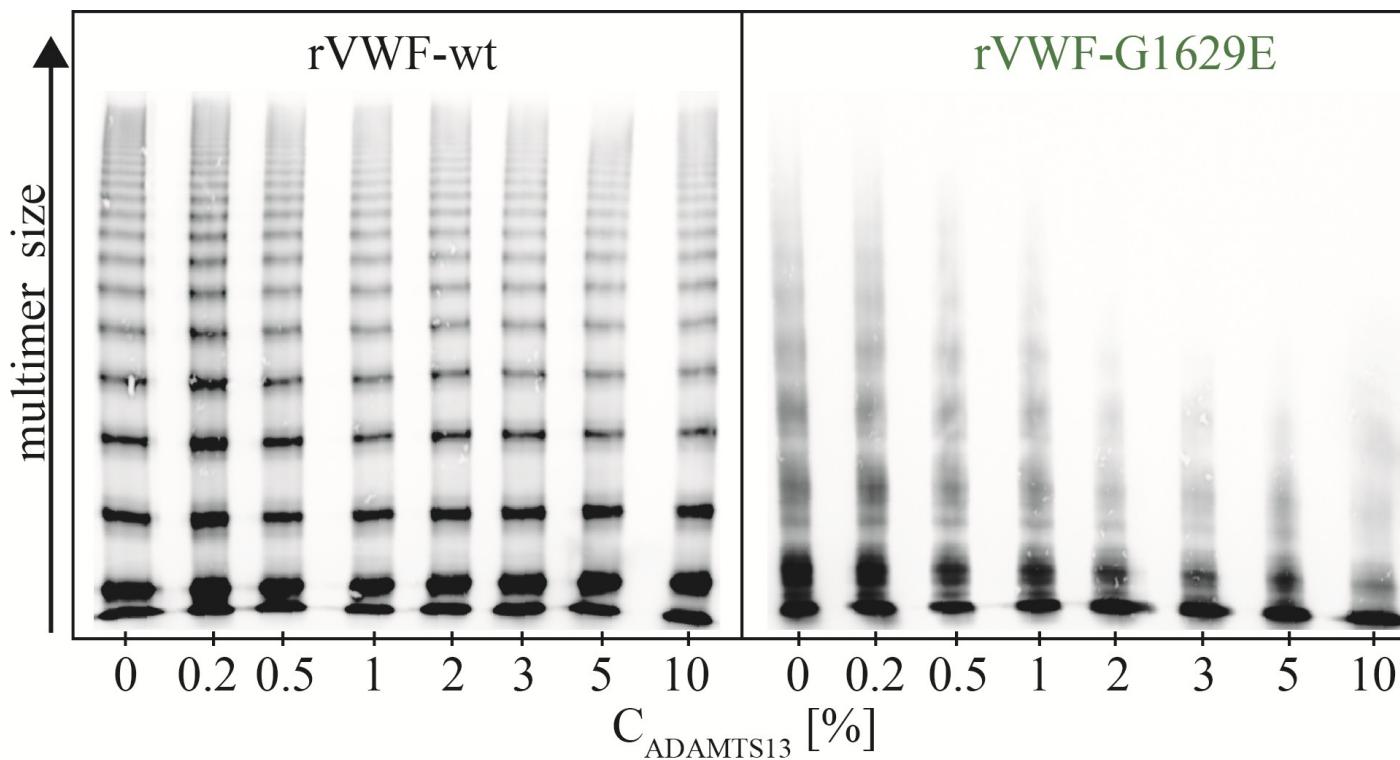


G1629E mutation dramatically increases cleavage

ADAMTS13

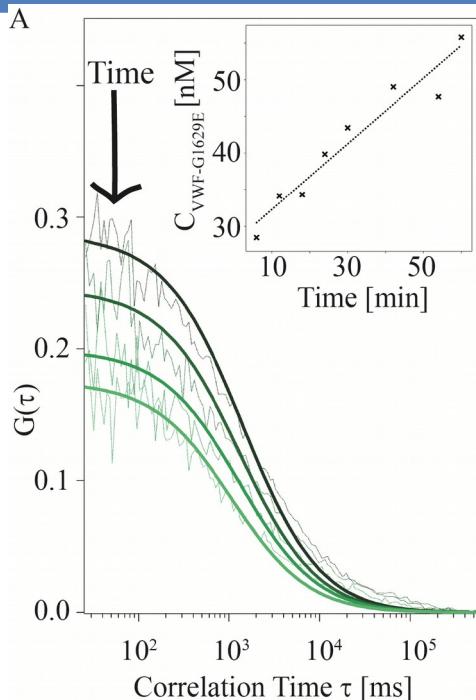


Y1605 M1606
G1629E



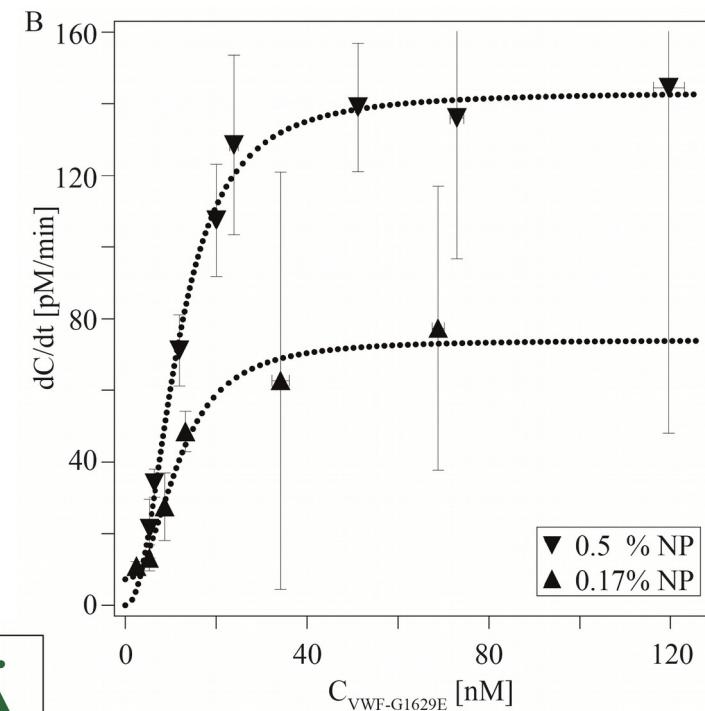
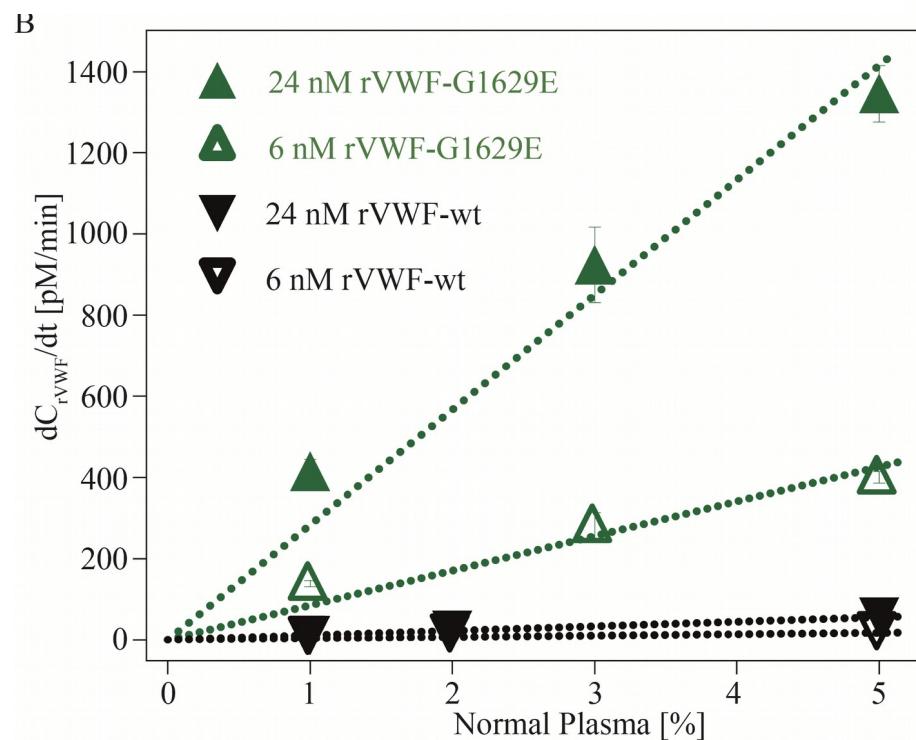
- von Willebrand disease type 2A
- Kinetics?
- Molecular mechanism?

G1629E cooperative cleavage kinetics



FCS assay to monitor
VWF cleavage:
Lippok, Mittag, Rädler, LMU
Biophys J 2013 & 2016

~20-fold larger
cleavage rate
for G1629E

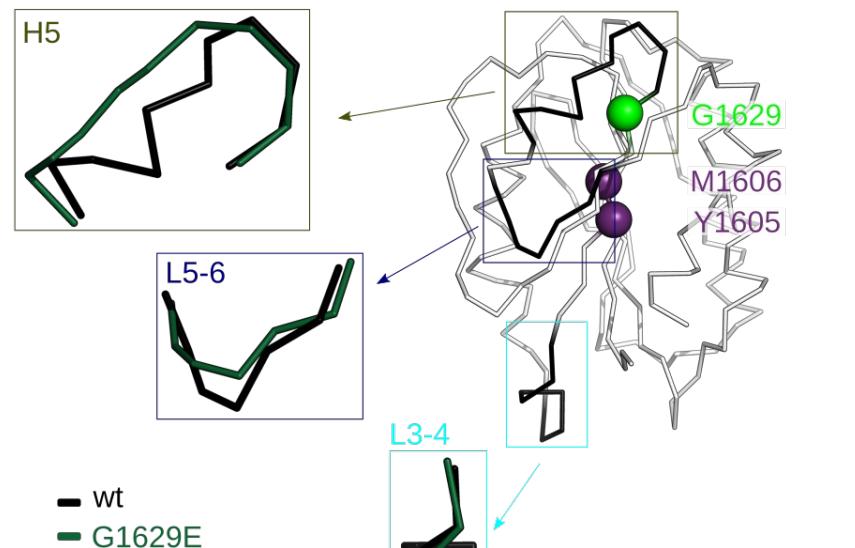


$$dC/dt = v_{\max} / [1 + (K_A/C)^n]:$$
$$n=2.3,$$
$$v_{\max} = 143.2 \pm 0.4 \text{ pM/min},$$
$$K_A = 11.5 \pm 0.5 \text{ nM}.$$

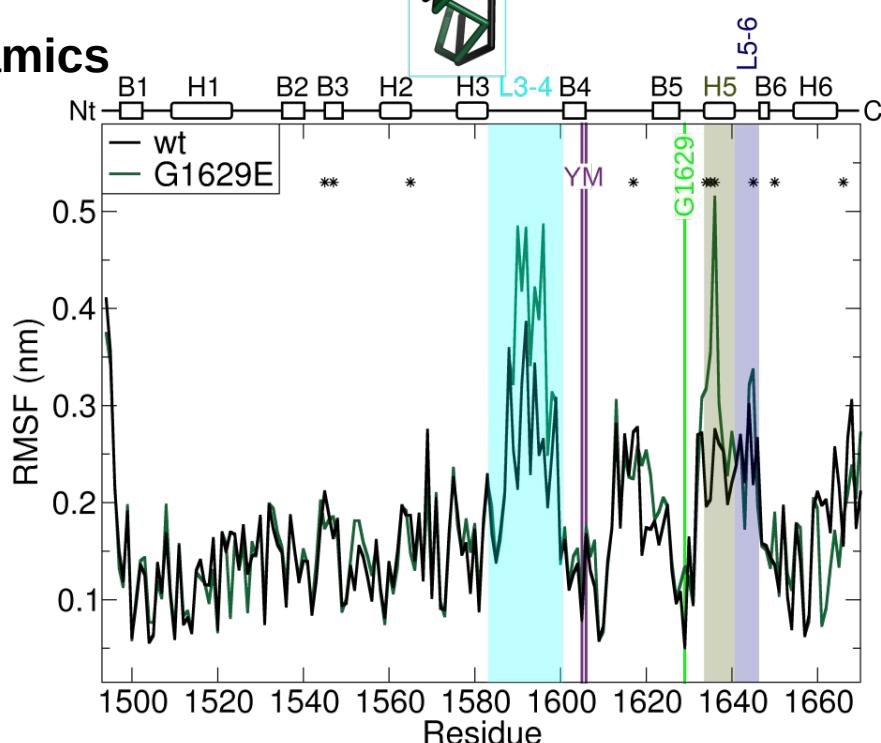
G1629E gives access to multiple cleavage sites at the same time.

MD simulations: G1629E mutant induced changes in

Structure

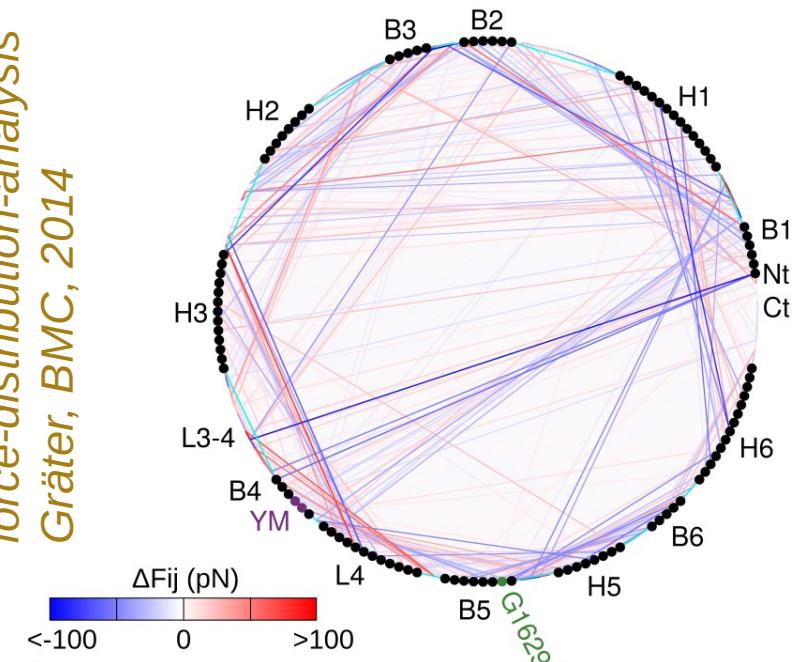


Dynamics



Internal stress

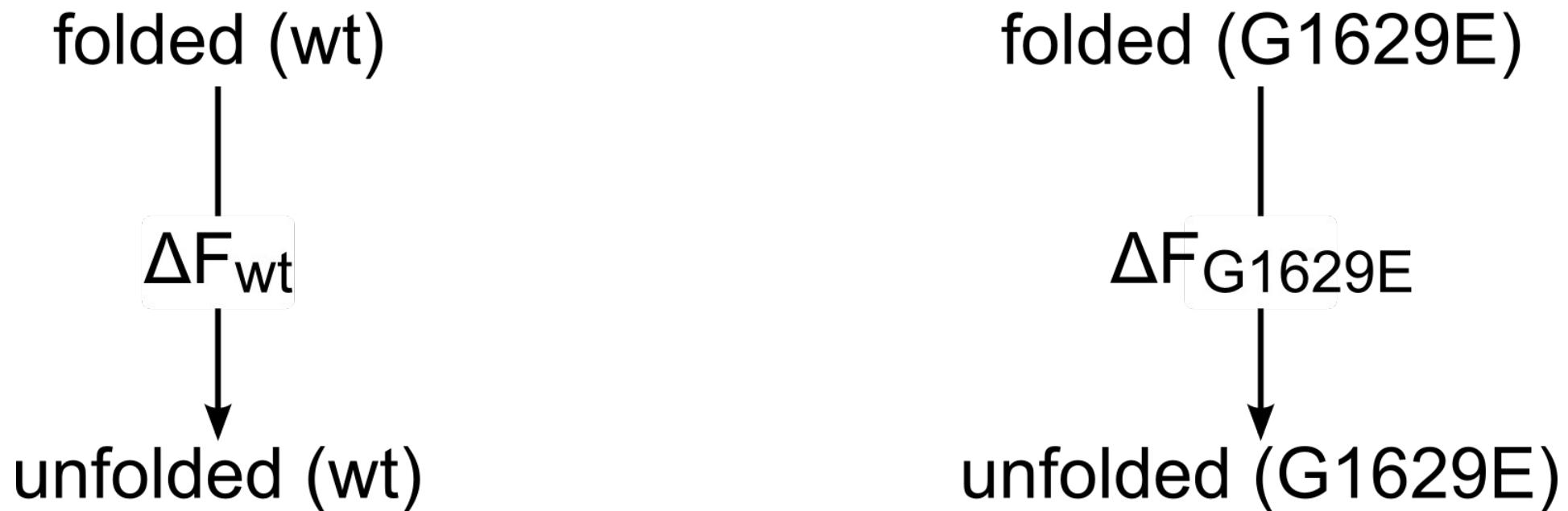
force-distribution-analysis
Gräter, BMC, 2014



Are these
destabilizing or stabilizing
changes?

~ 1 μ s wt
~ 1 μ s mutant

Assessing thermodynamic stability



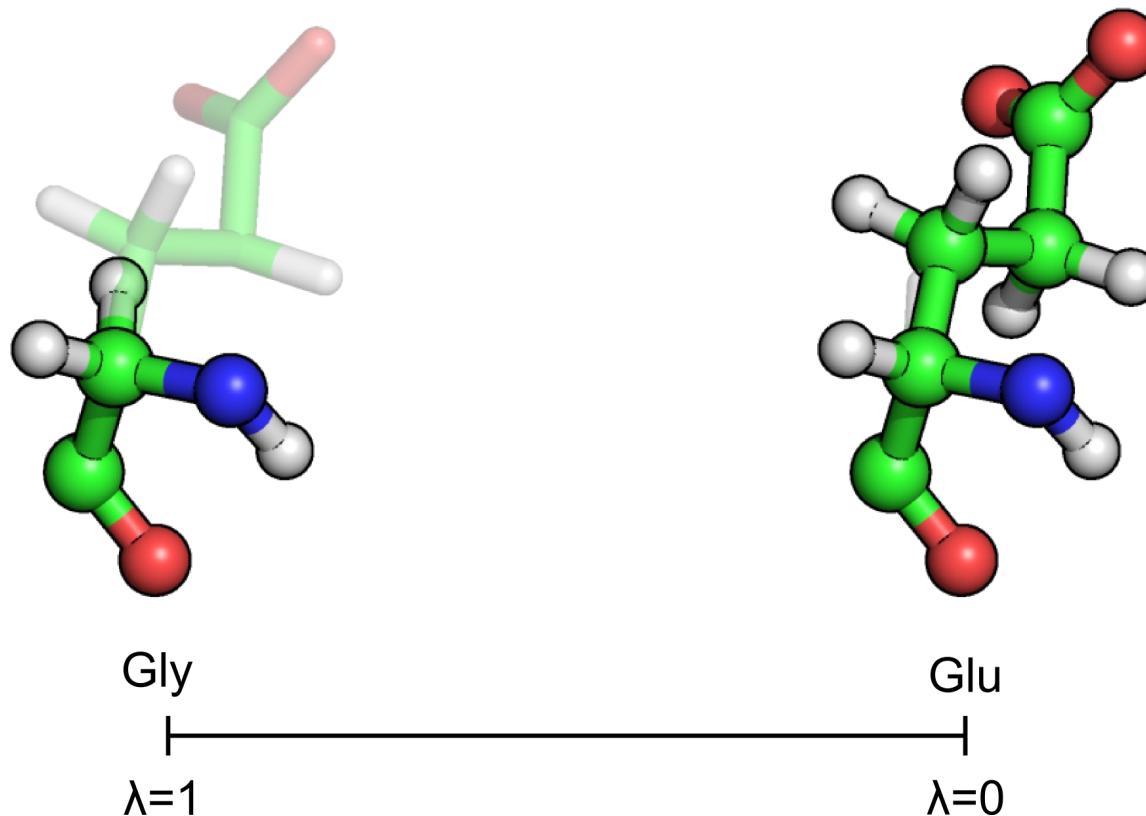
$$\begin{aligned}\Delta\Delta F &= \Delta F_{G1629E} - \Delta F_{wt} \\ &= \Delta F_2 - \Delta F_1\end{aligned}$$

$\Delta\Delta F < 0$ Destabilizing mutation

$\Delta\Delta F > 0$ Stabilizing mutation

free energy calculations

Gly to Glu transition by thermodynamic integration



Hamiltonian coupled to variable λ :

$$H = H_{gly}\lambda + H_{glu}(1 - \lambda)$$

Work associated to the transition:

$$w_{G \rightarrow E} = \int_{\lambda=1}^{\lambda=0} \frac{\partial H}{\partial \lambda} d\lambda$$

Crooks fluctuation theorem

Relationship between free energy & work through work distributions:

$$\frac{P_{G \rightarrow E}(w)}{P_{E \rightarrow G}(-w)} = e^{\frac{w - \Delta F}{k_B T}}$$

$\Delta\Delta F$ from:

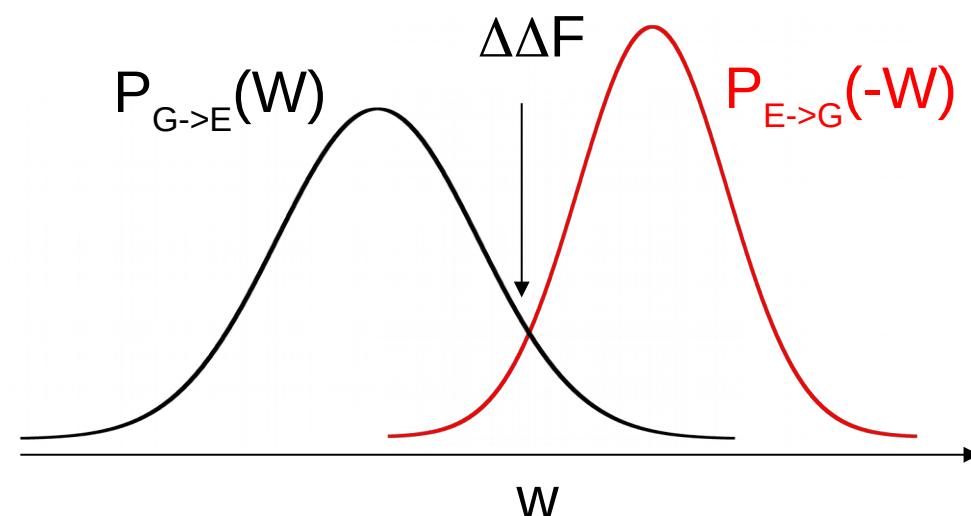
- BAR maximum-likelihood for non-equilibrium transitions

Shirts et al. PRL, 2003

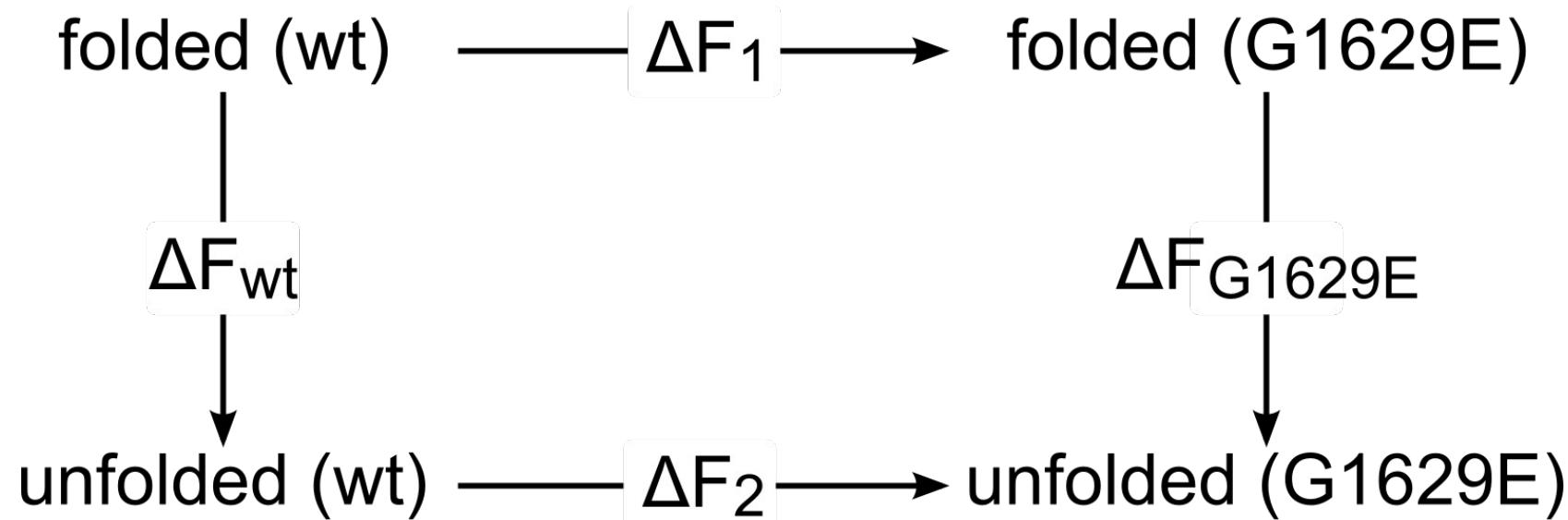
$$\left\langle \frac{1}{1 + \exp [\beta(w - \Delta F)]} \right\rangle_{G \rightarrow E} = \left\langle \frac{1}{1 + \exp [-\beta(w - \Delta F)]} \right\rangle_{E \rightarrow G}$$

- Intercept between distributions

Grubmüller, JCC, 2010



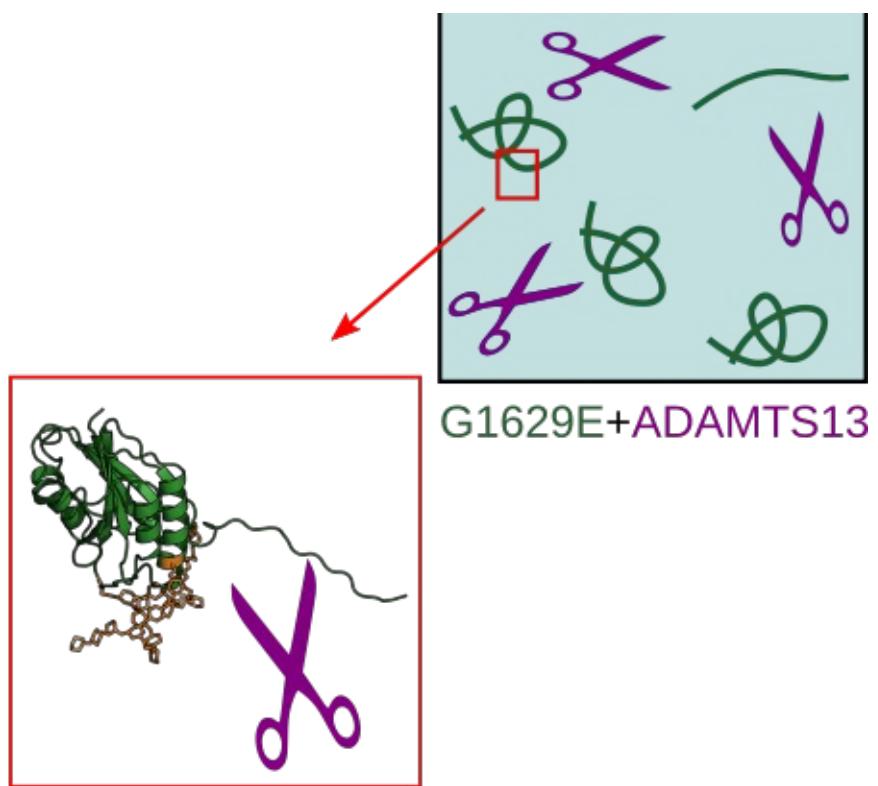
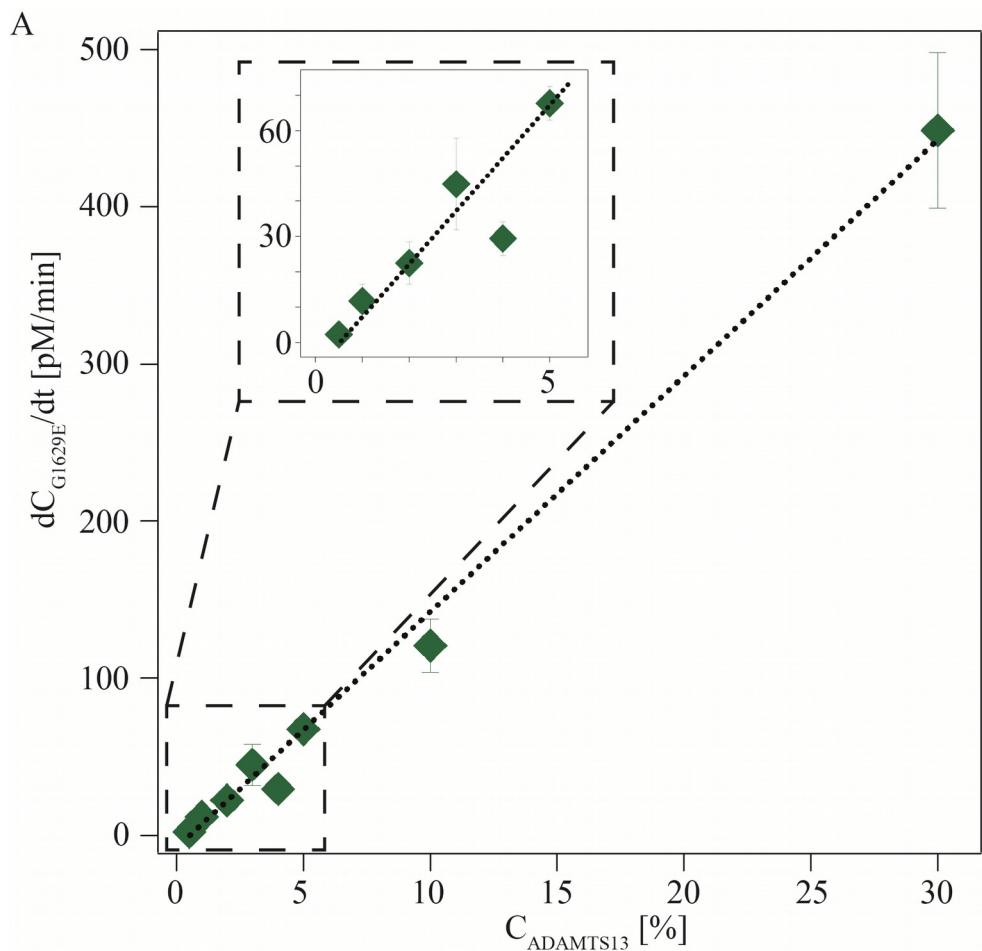
A2-domain thermodynamic destabilization enhances cleavage of G1629E



$$\begin{aligned}\Delta F_{\text{G1629E}} - \Delta F_{\text{wt}} &= \Delta F_2 - \Delta F_1 \\ &= -11.8 \text{ (2.4) kJ/mol, BAR-ML} \\ &= -9 \quad (<6) \text{ kJ/mol, Intercept}\end{aligned}$$

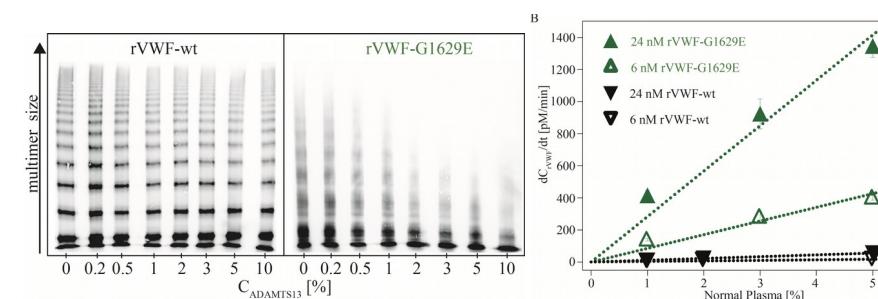
Cleavage rate reached 40-100 times faster for G1629E

G1629E as a substrate for ADAMTS13 assays

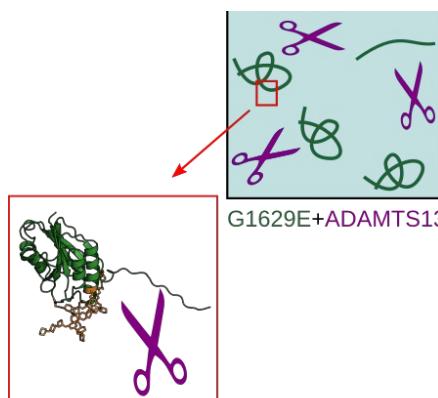
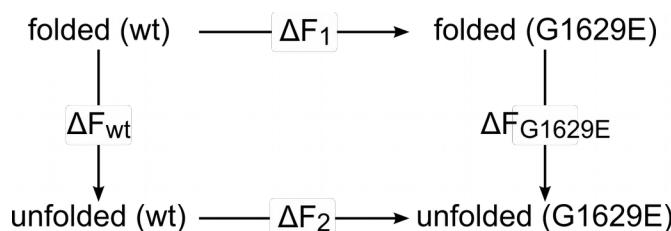
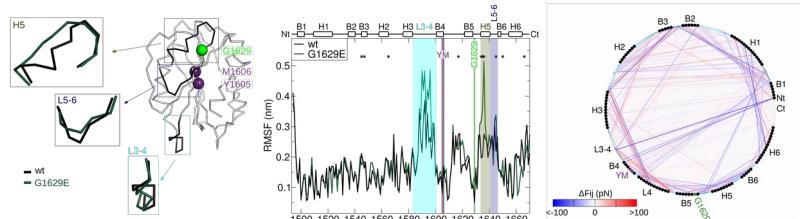


- improved resolution:
down to 0.5 % ADAMTS13 physiol. concentration
- no denaturants
- no shear
- highly sensitive

Mutant G1629E enhances VWF cleavage via a cooperative destabilization mechanism



20-fold enhanced
Cleavage kinetics



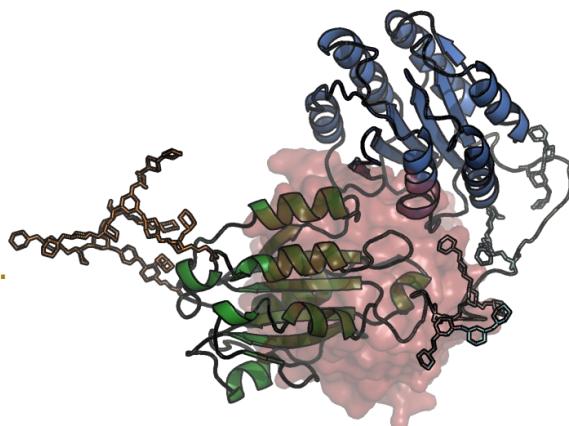
Structural – Dynamic - Mechanical
A2-domain thermodynamic
destabilization

G1629E as a substrate
for highly sensitive
ADAMTS13 assays

Computational studies of VWF

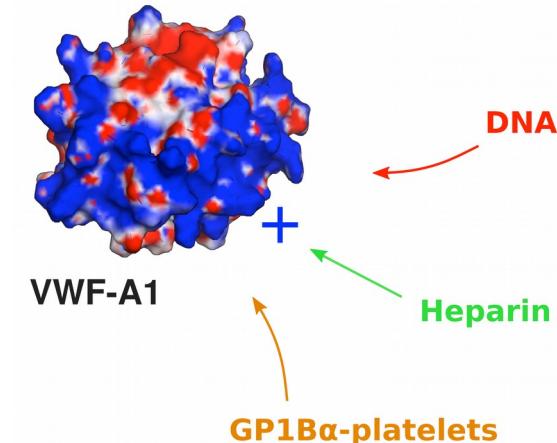
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- Posch, **CAS** et al.
J Struct Biol. 2016
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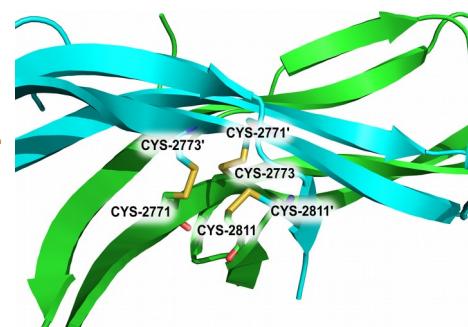
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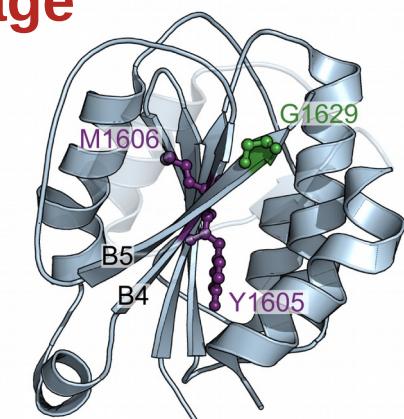
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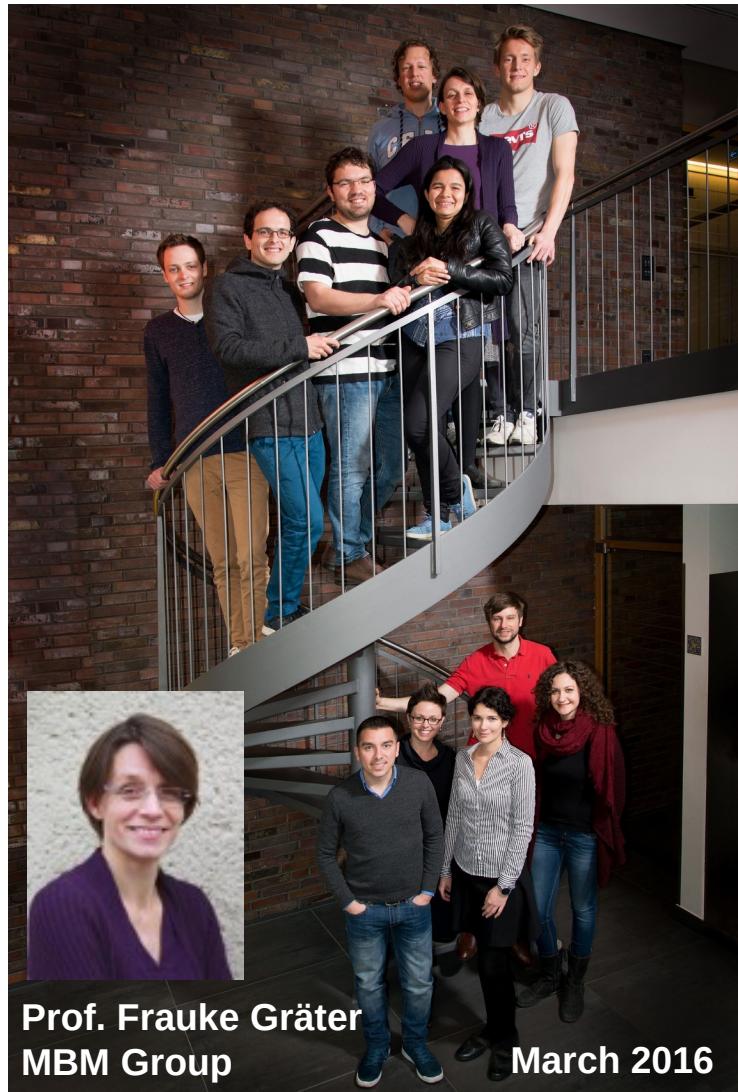


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