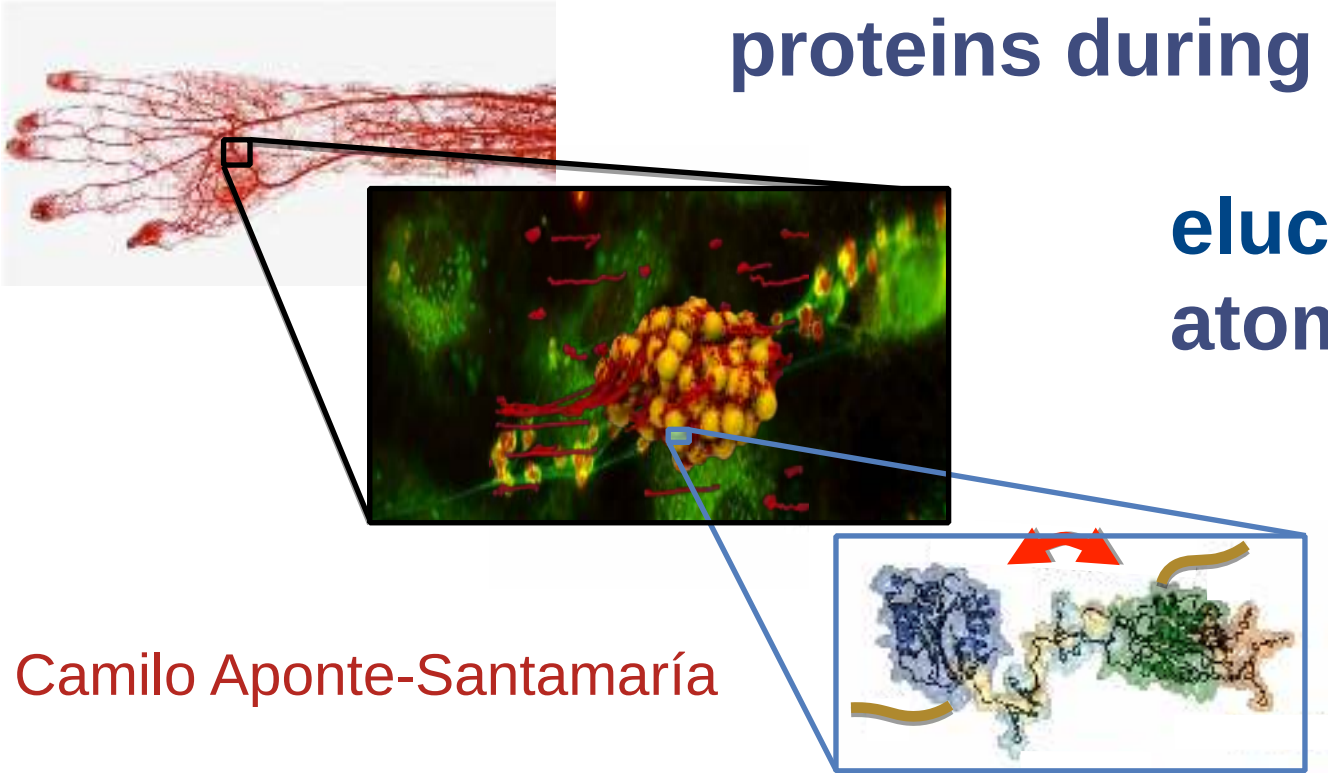


# The role of mechanosensitive proteins during blood coagulation

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



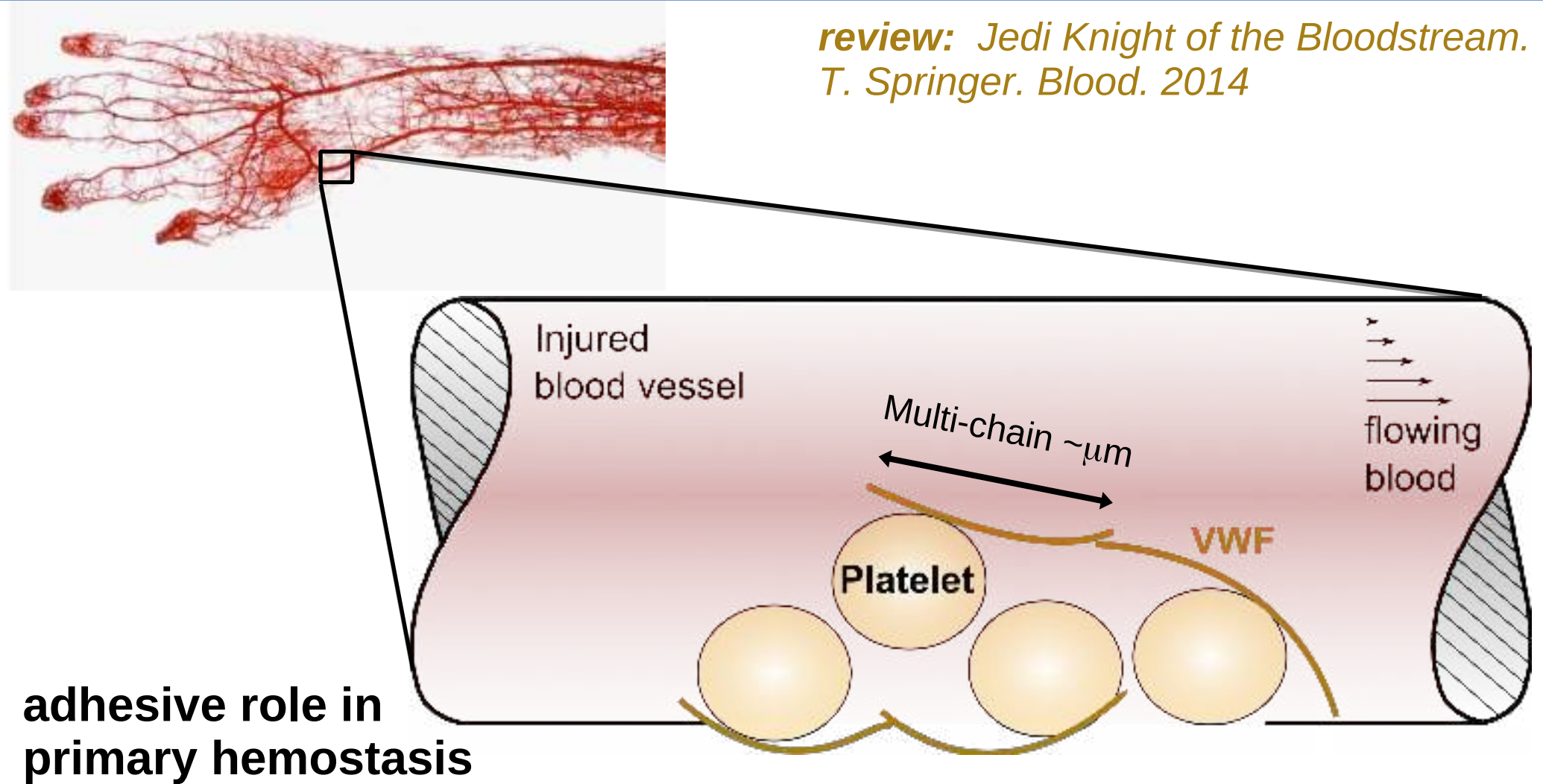
*Hosted by:*  
Cellular Biophysics Dept.  
MPI for Medical Research  
Heidelberg, Germany



*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**

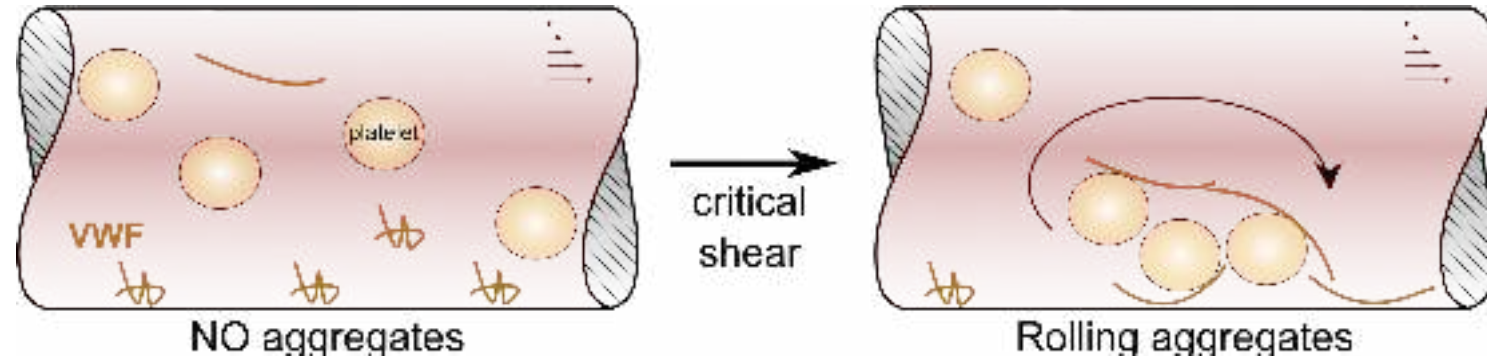
**activated by the shear  
of flowing blood**

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

# VWF-platelet rolling-aggregates

microfluidic  
experiments

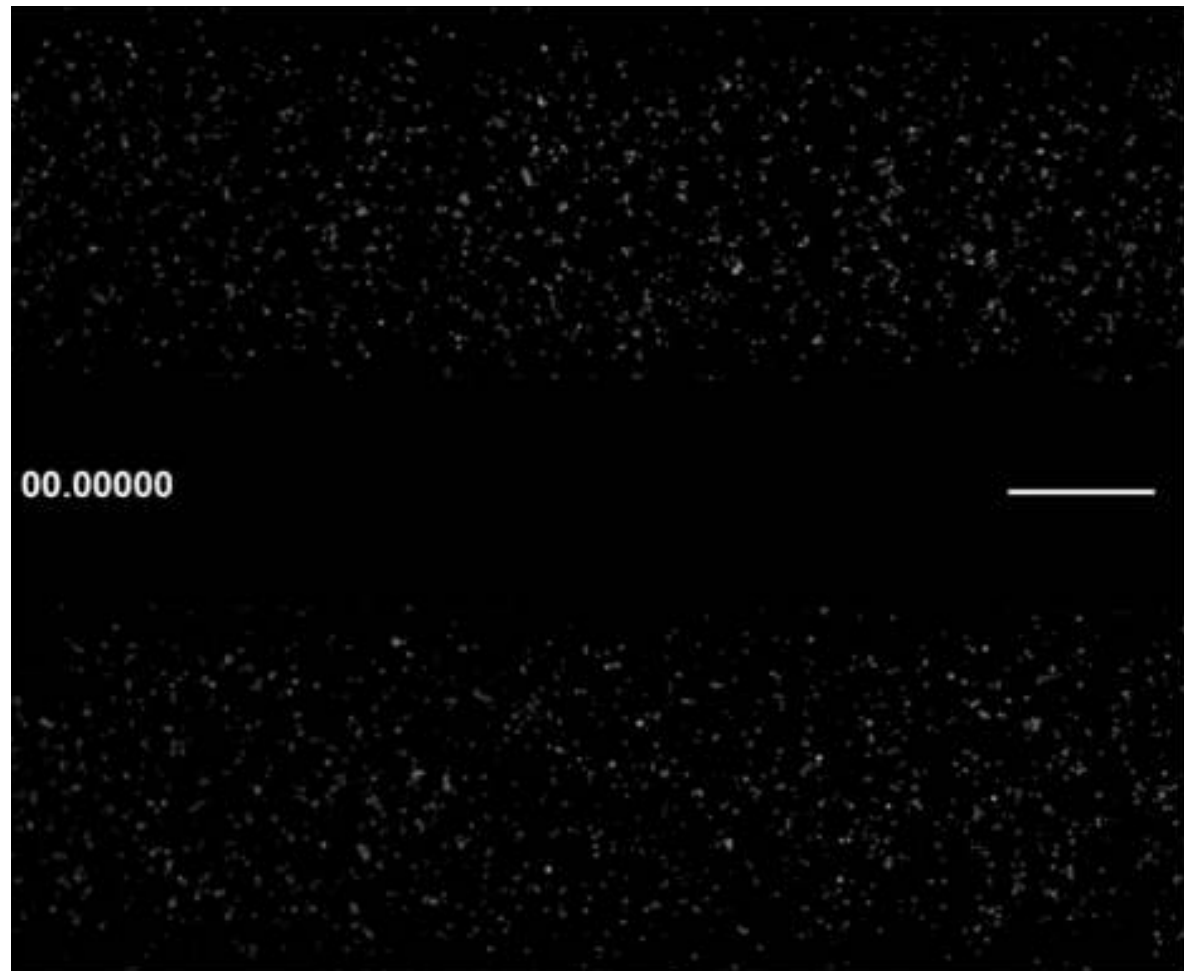
Huck, Schneider  
Mannheim (Germany)



0-1 min: 500 Hz

1-5 min: 2500 Hz

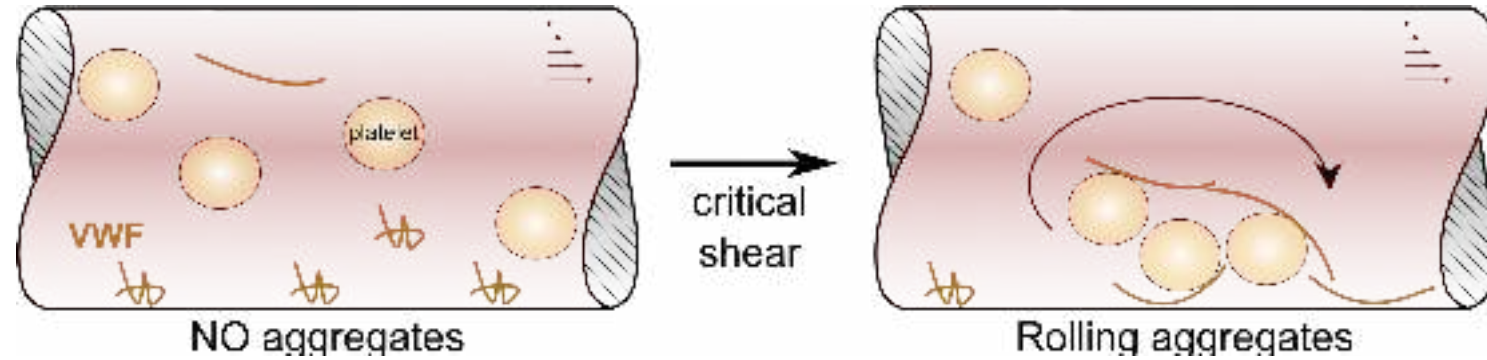
>5min: 4000 Hz



# VWF-platelet rolling-aggregates

microfluidic  
experiments

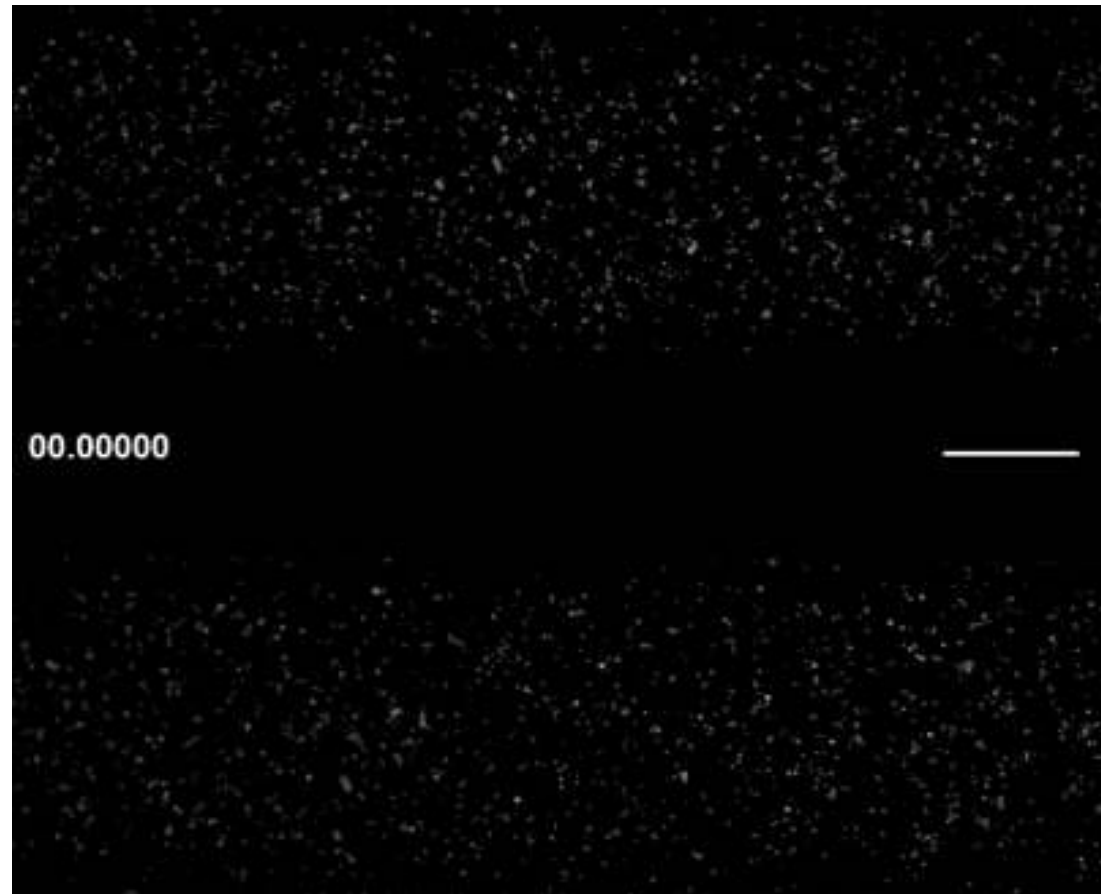
Huck, Schneider  
Mannheim (Germany)



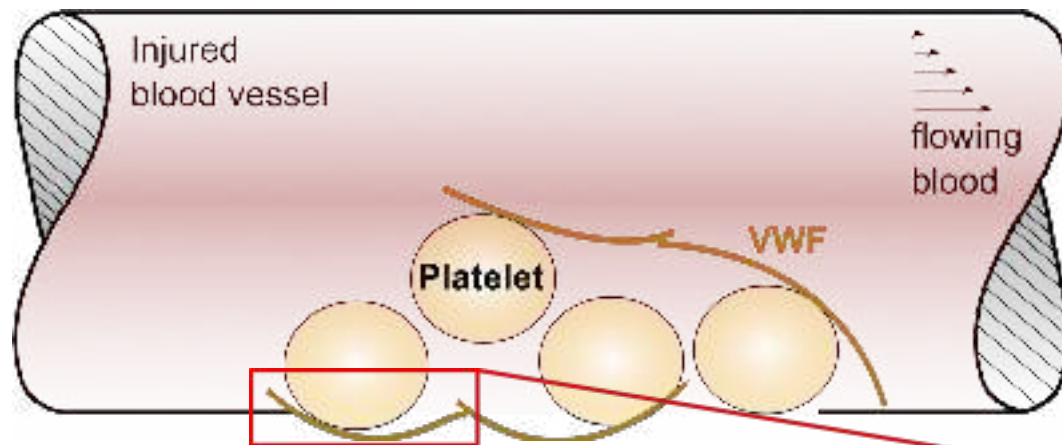
0-1 min: 500 Hz

1-5 min: 2500 Hz

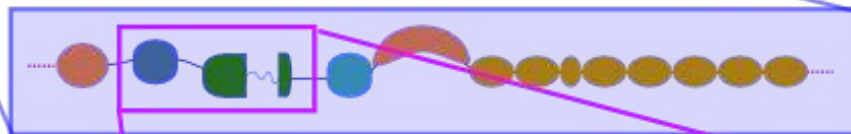
>5min: 4000 Hz



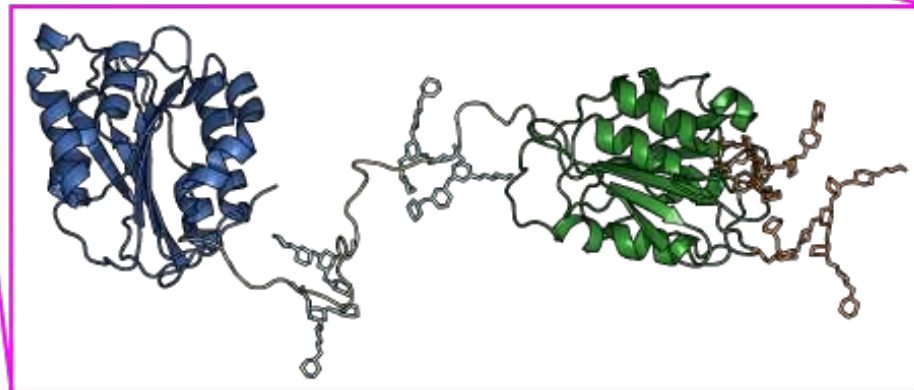
# von Willebrand factor (VWF)



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



chain (13 domains)  
~60-80 nm



protein domain < 8 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

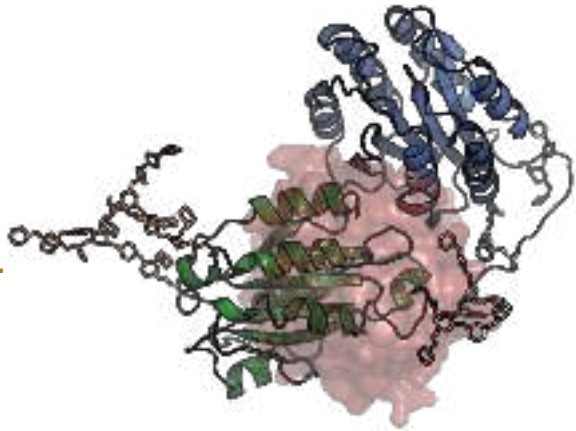


Munich 2013



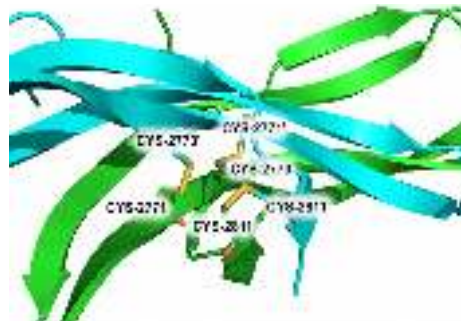
# Computational studies of VWF

## 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

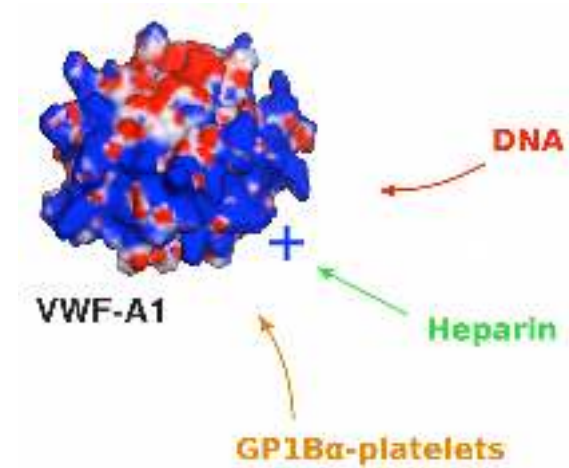
## dimerization



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

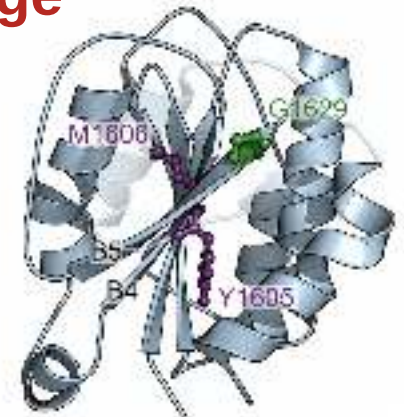
## VWF-DNA: inflammation

Grässle...CAS et al.  
ATVB. 2014



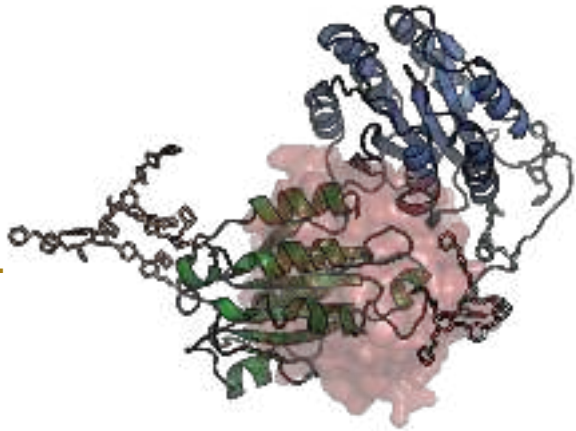
## mutation-induced accelerated cleavage

CAS, Lippock et al.  
Biophys. J. 2017



# 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



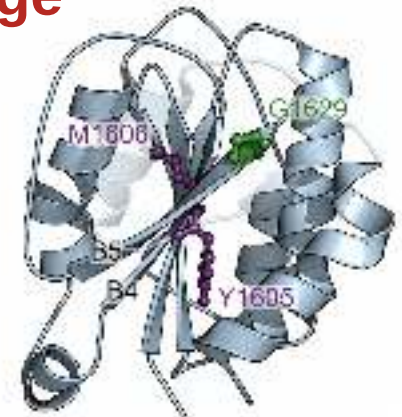
## dimerization



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

## mutation-induced accelerated cleavage

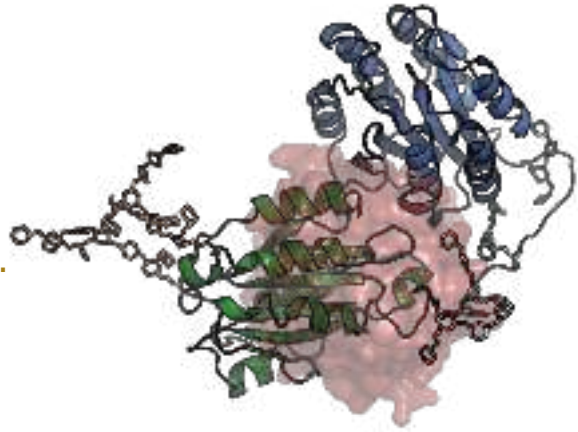
**CAS**, Lippock et al. Biophys. J. 2017





# Computational studies of VWF

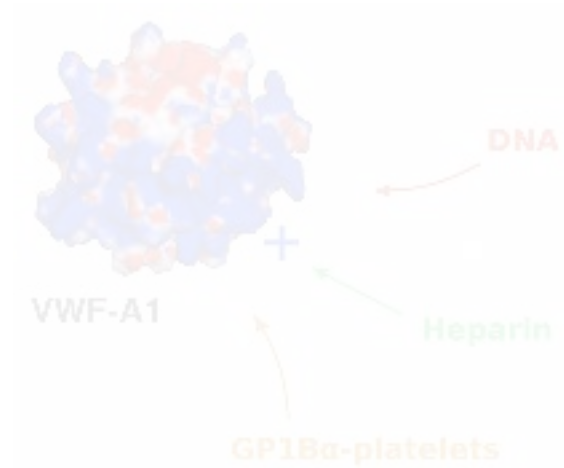
## protein-protein: autoinhibition



- **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

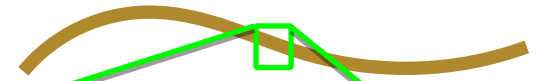
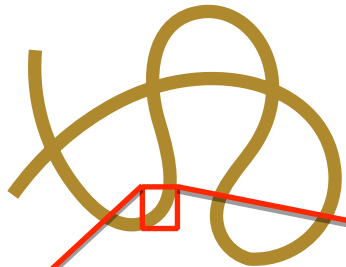
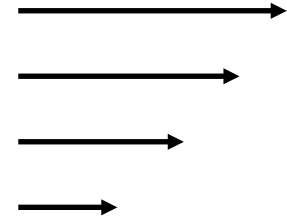


# VWF: mechanosensitive protein

VWF globular

VWF stretched

shear flow



chain



GPIIb $\alpha$

Platelet

A1

chain

GPIIb $\alpha$

Platelet

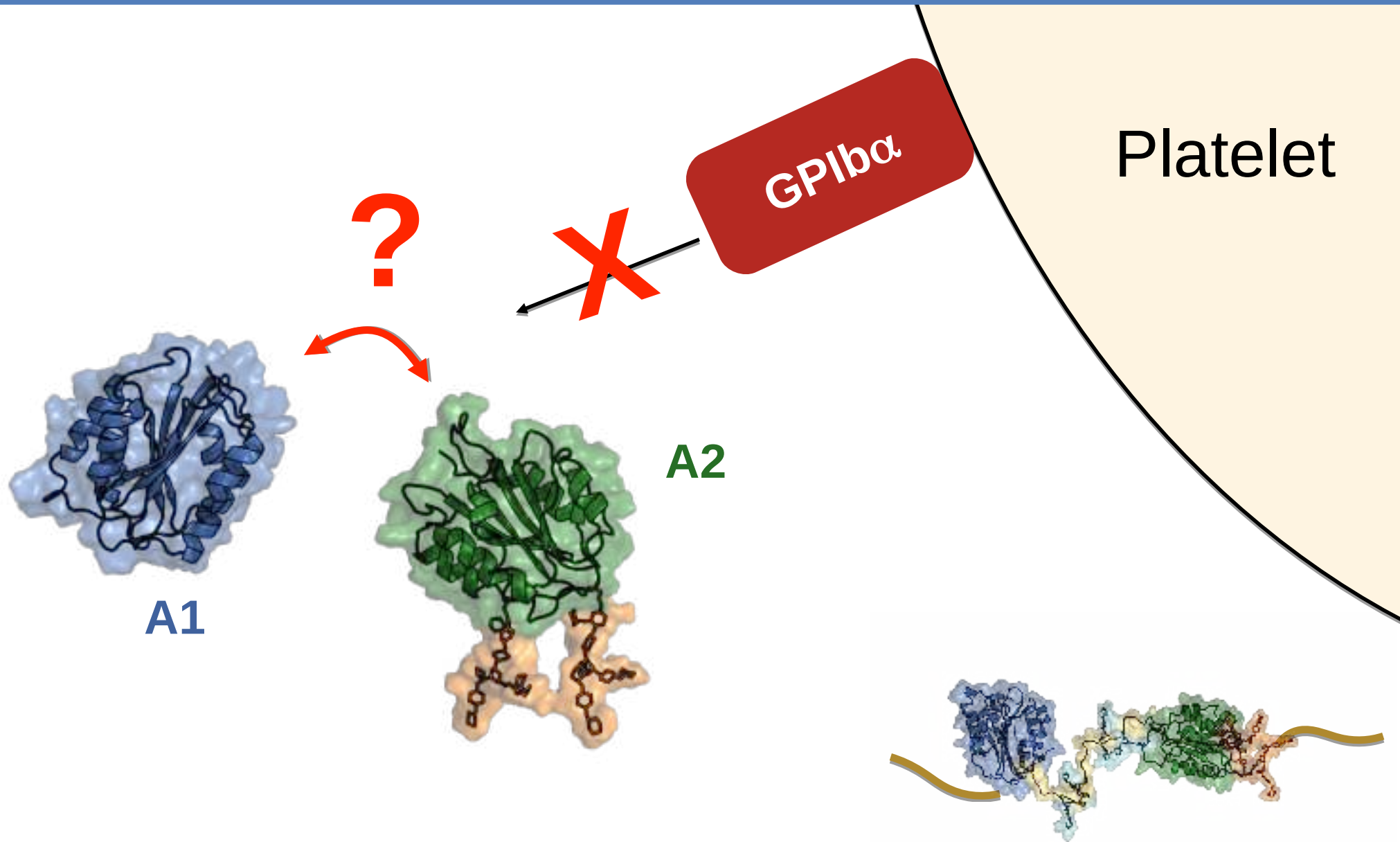
A1

A1 domain (GPIIb $\alpha$ -platelet binding site):

shielded

exposed

# VWF auto-inhibition

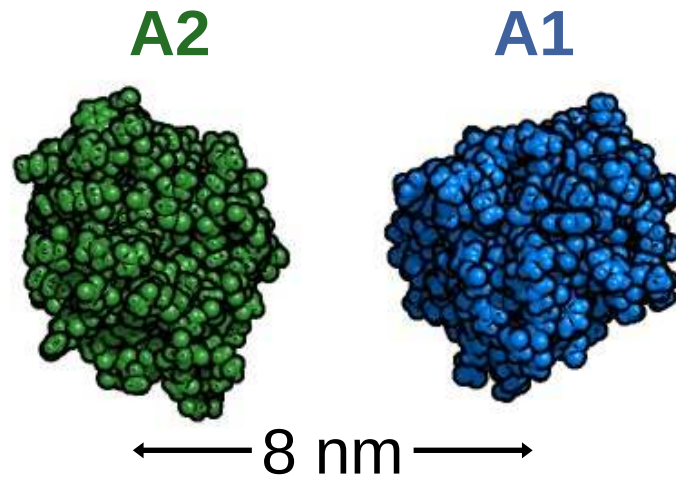


molecular nature of **A1-A2** interactions?

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

*multiple MD simulations*  
*N=17 2 $\sim$  $\mu$ s concatenated*

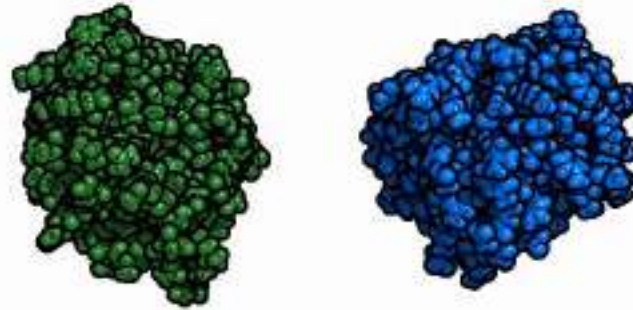
0.0ns



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

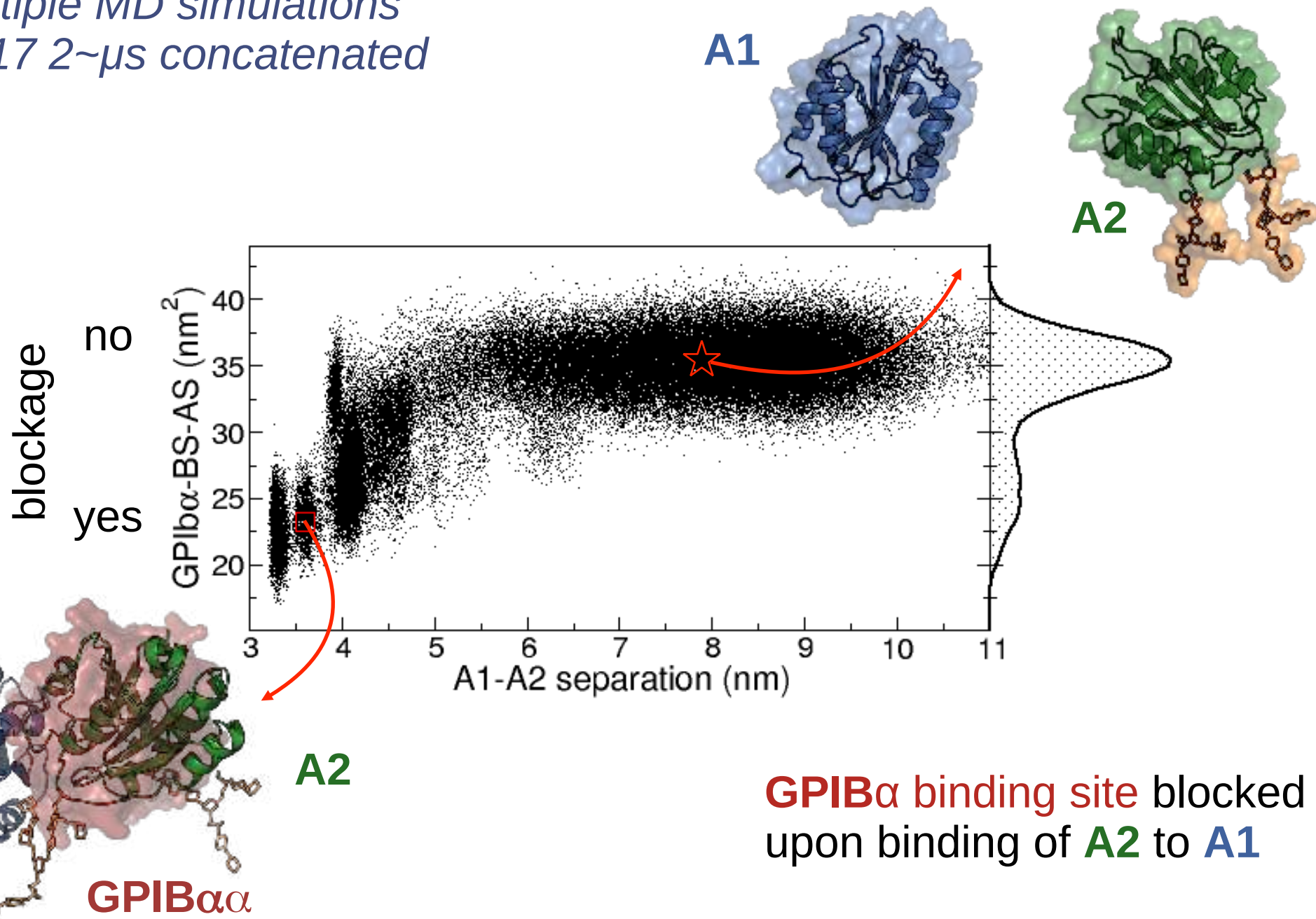
*multiple MD simulations  
N=17 2~ $\mu$ s concatenated*

0.0ns



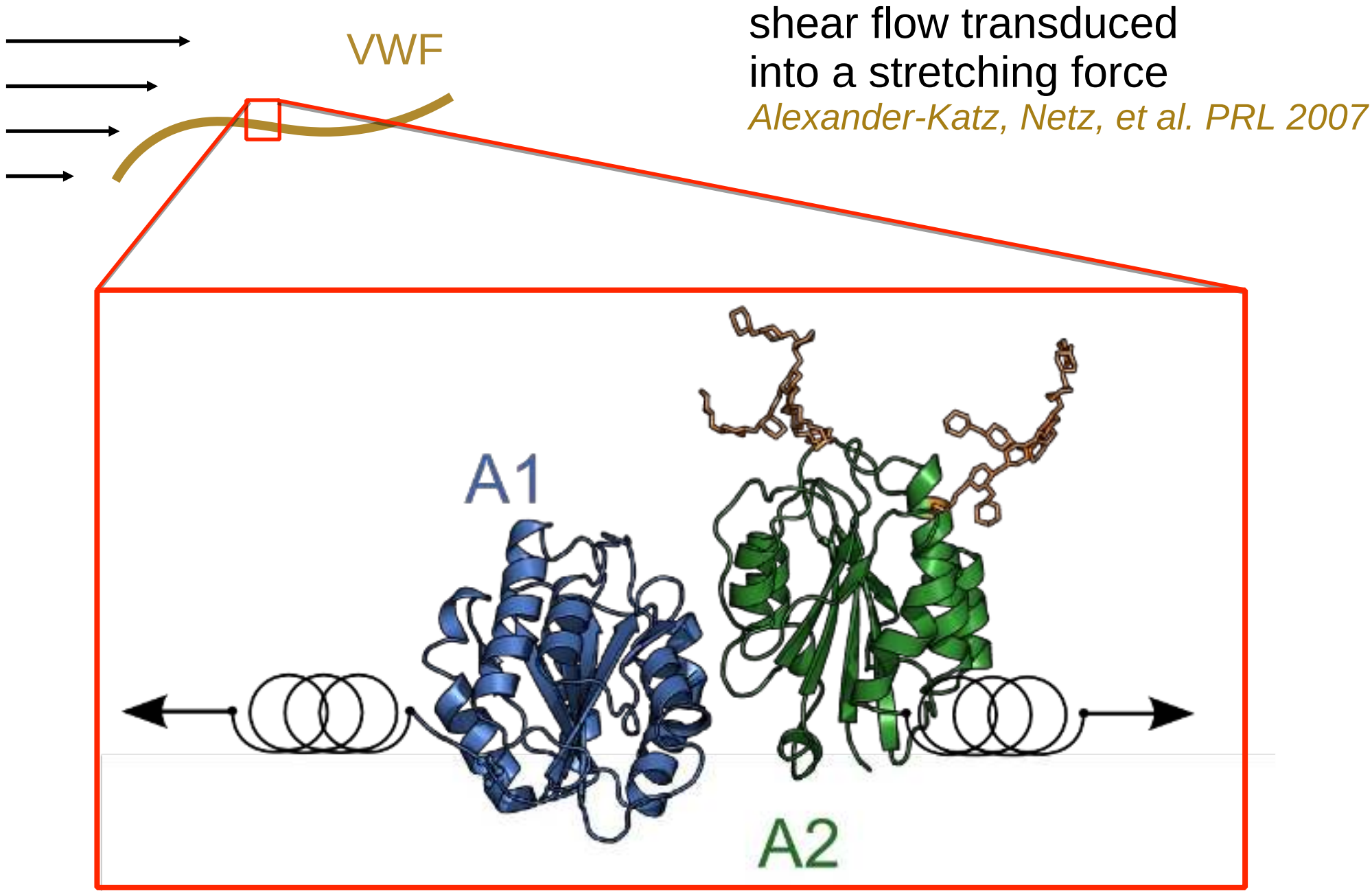
# A2 targets GPIB $\alpha$ binding site in A1 causing its blockage

multiple MD simulations  
N=17 2- $\mu$ s concatenated



**GPIB $\alpha$  binding site** blocked upon binding of **A2** to **A1**

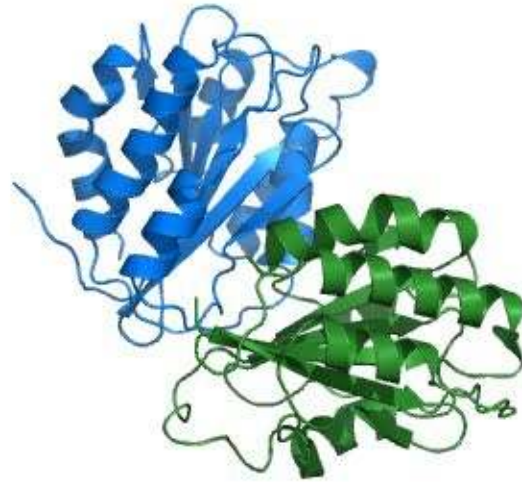
# A1-A2 complex under force: activation vs. cleavage



# A1-A2 complex under force: activation vs. cleavage

*Force-probe MD simulations*

0.0ns



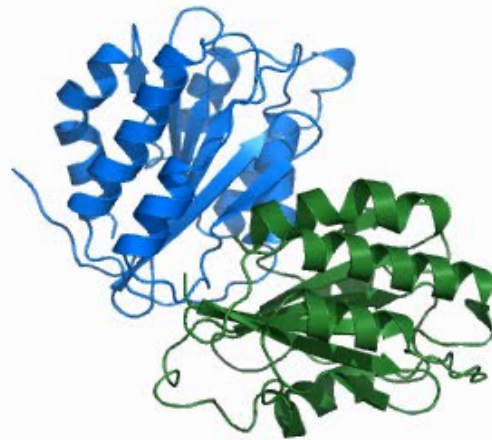
dissociation after partial unfolding of **A2**



# A1-A2 complex under force: activation vs. cleavage

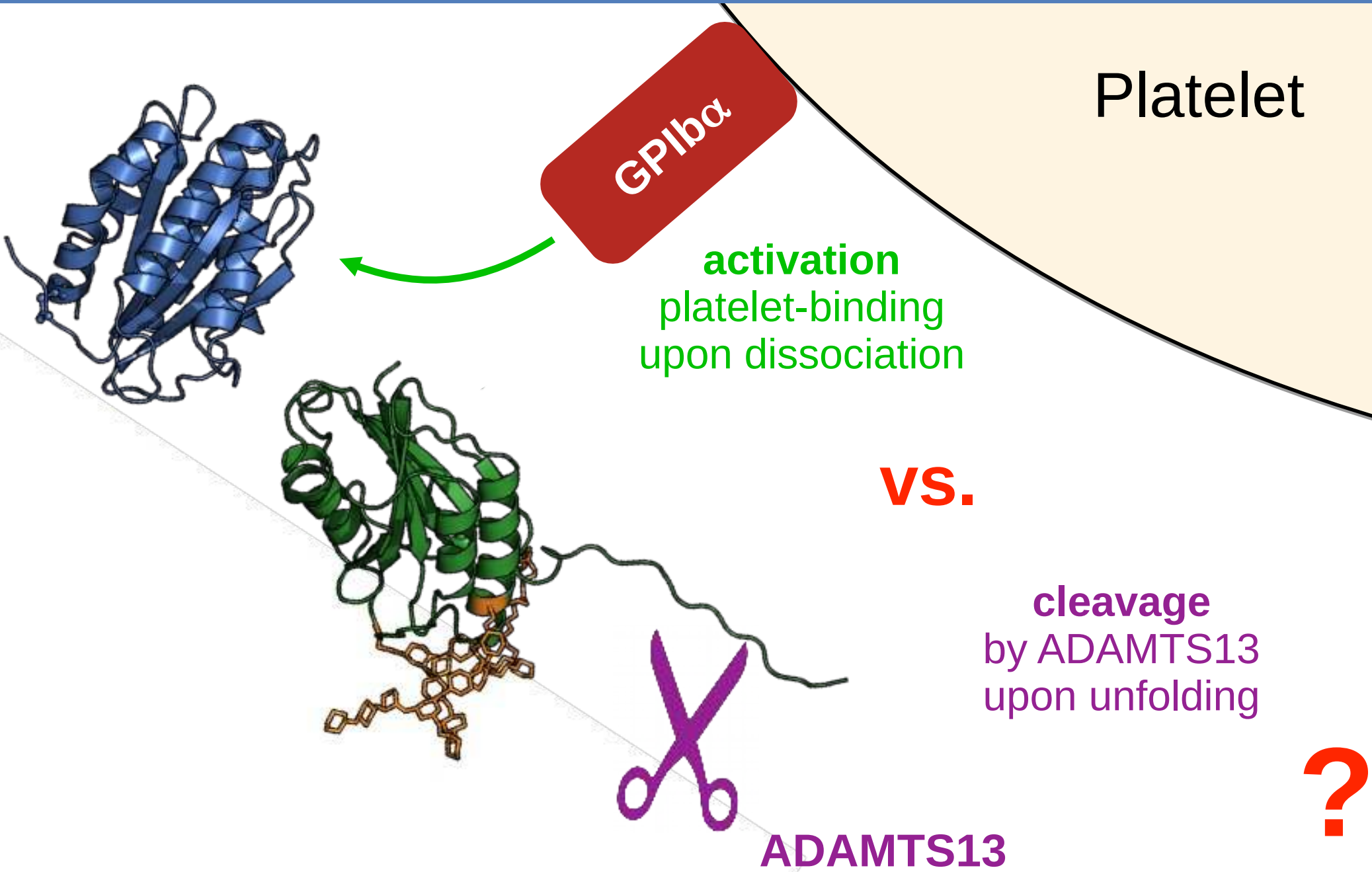
*Force-probe MD simulations*

0.0ns



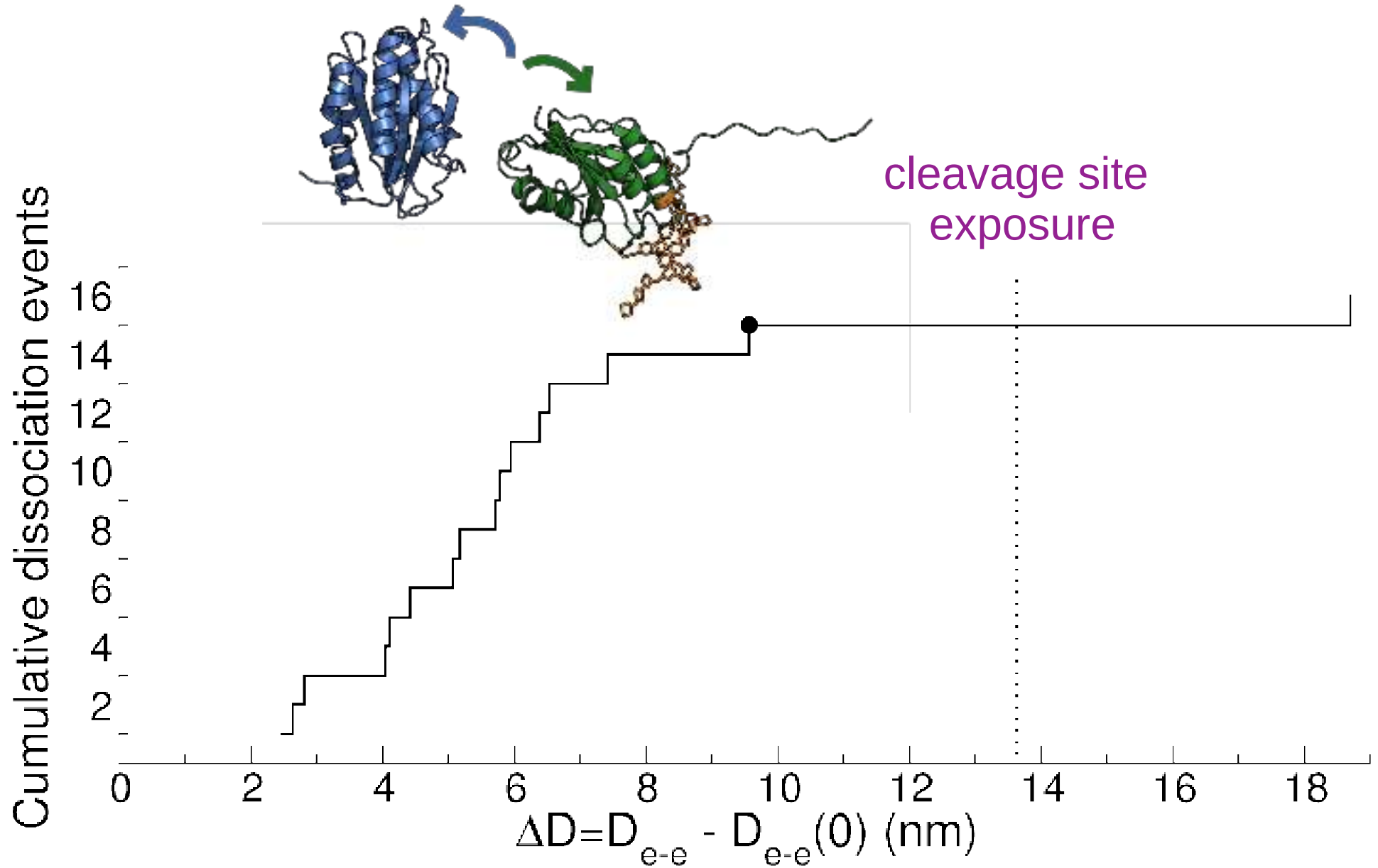
dissociation after partial unfolding of **A2**

# A1-A2 complex under force: activation vs. cleavage



CA-S, Huck, Posch et al. Biophys. J. (2015) – Posch, CA-S, Schwarzl et al. JSB (2017)

# A1-A2 complex under force: activation vs. cleavage

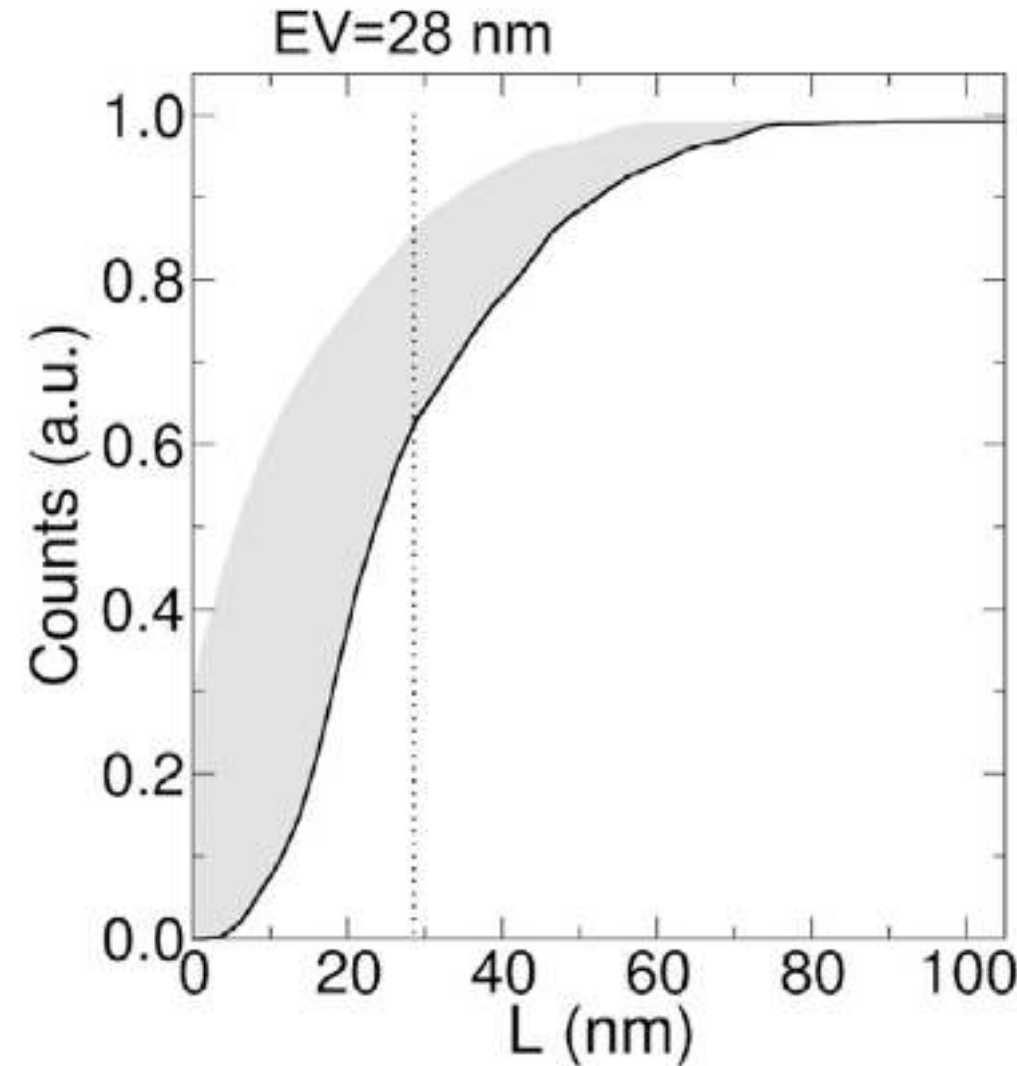
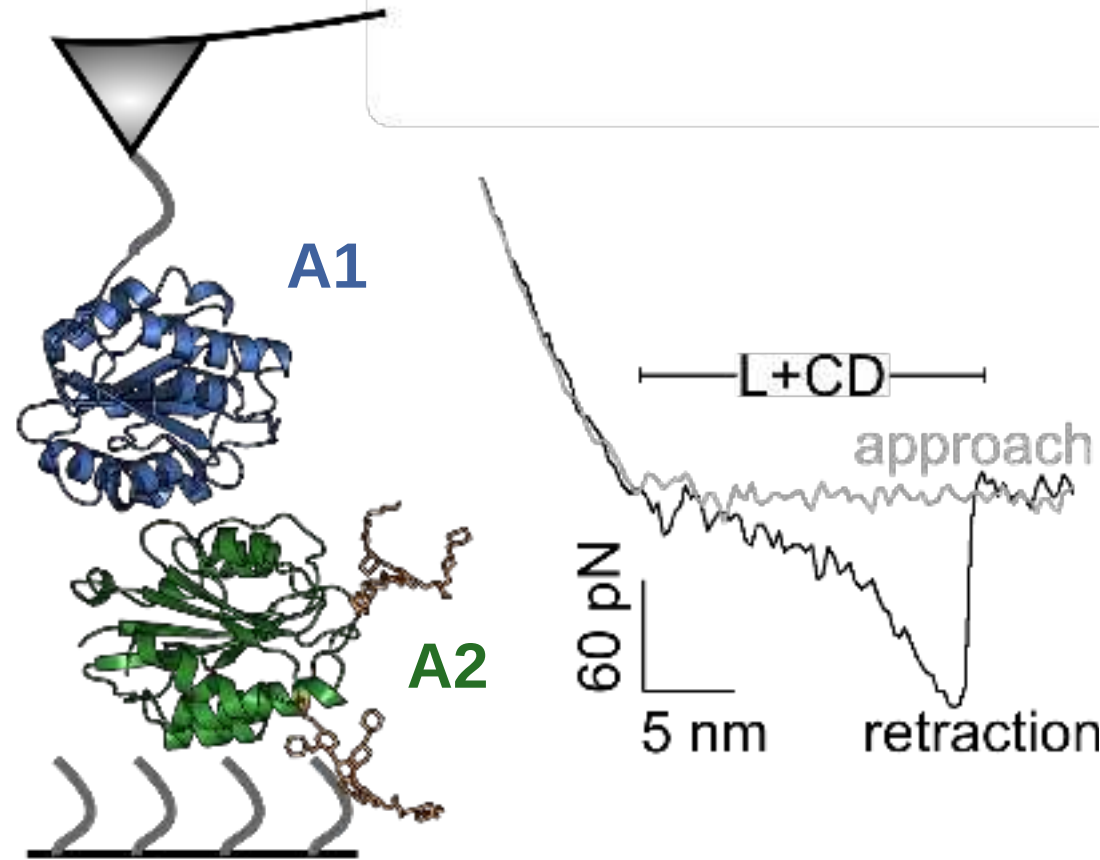


Dissociation occurs before exposure of cleavage site

# A1-A2 complex under force: activation vs. cleavage

AFM

Posch, Hinterdorfer, Linz (Austria)

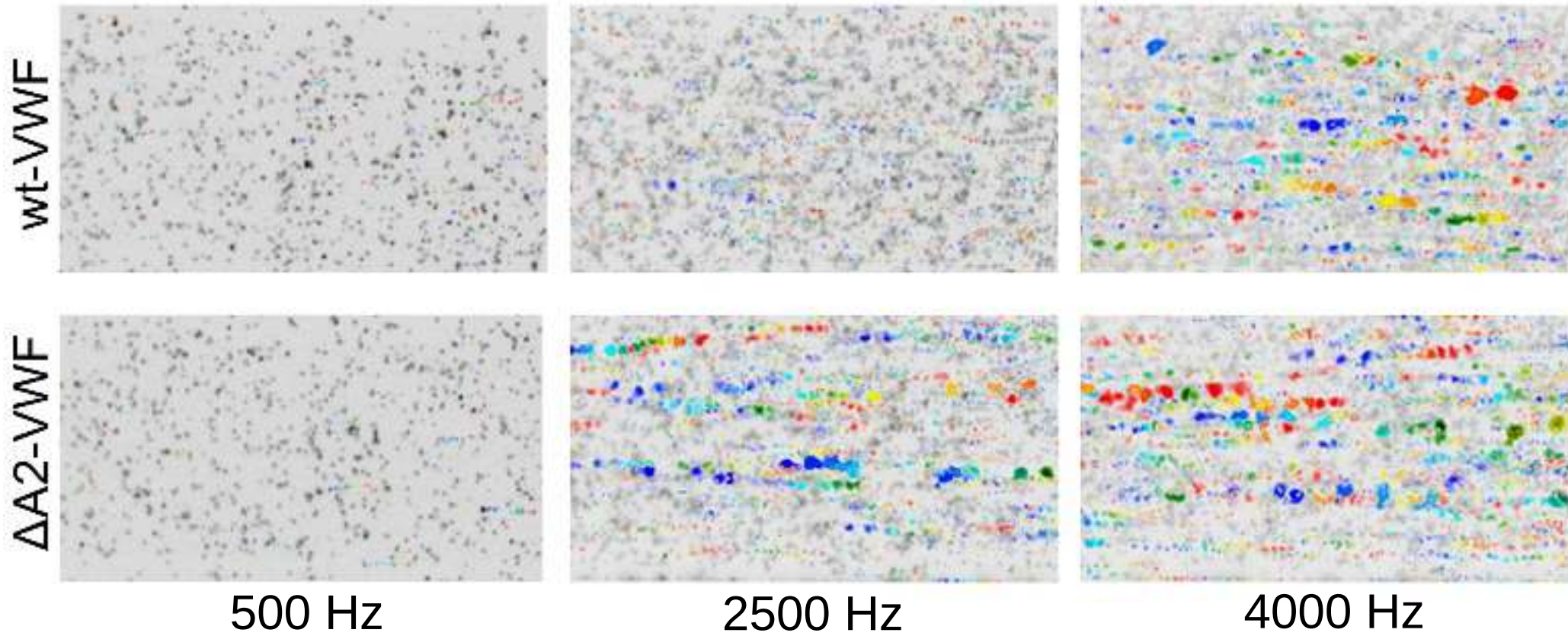


Little unfolding before dissociation

# Functional characterisation of $\Delta A2$ -VWF mutant

*microfluidic  
experiments*

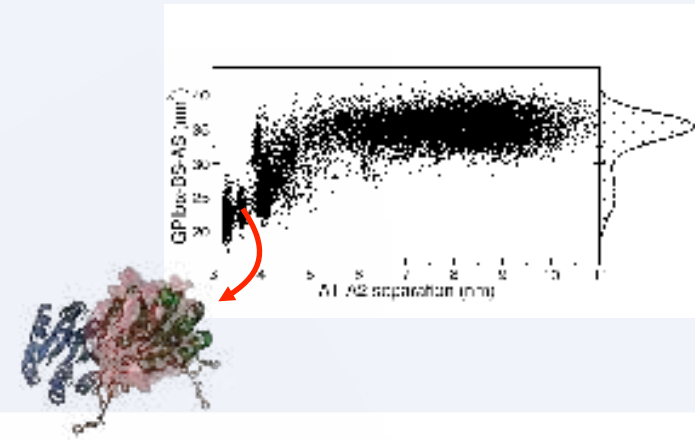
*Huck, Schneider  
Mannheim (Germany)*



**A2-deletion reduces critical shear rate: gain of function**

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

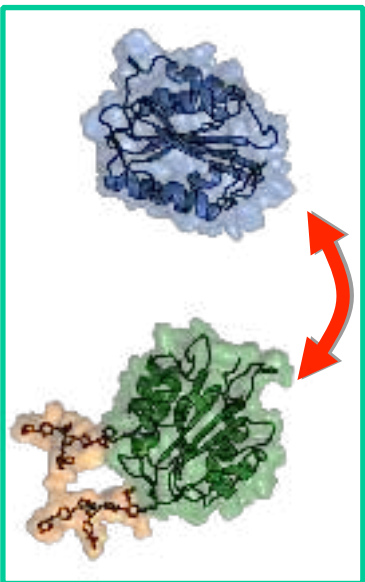
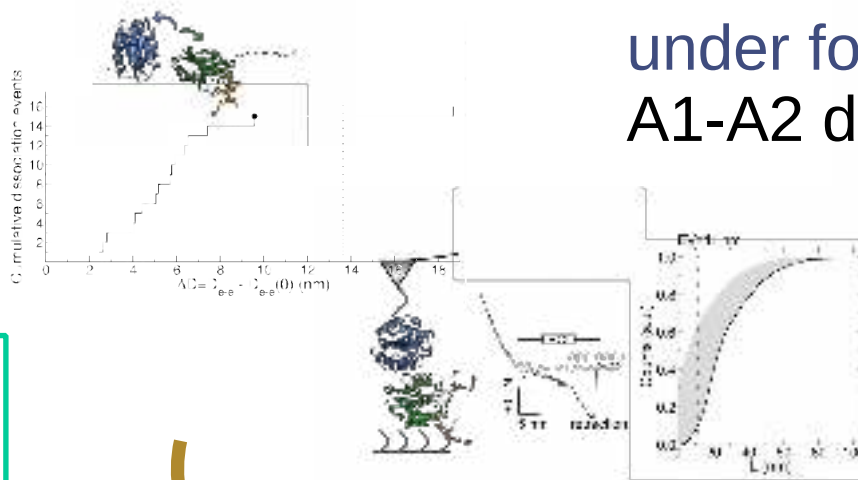
A1-A2 auto-inhibitory interactions:  
A2 targets GPIb $\alpha$ -platelet binding site in A1



under force:  
A1-A2 dissociation prior A2-unfolding

activation before cleavage

Outlook



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



# Computational studies of VWF

## 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



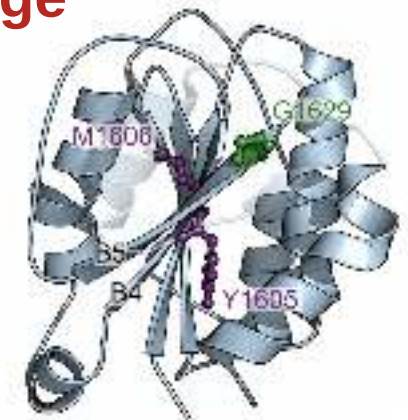
Grässle...CAS et al. ATVB. 2014

## dimerization



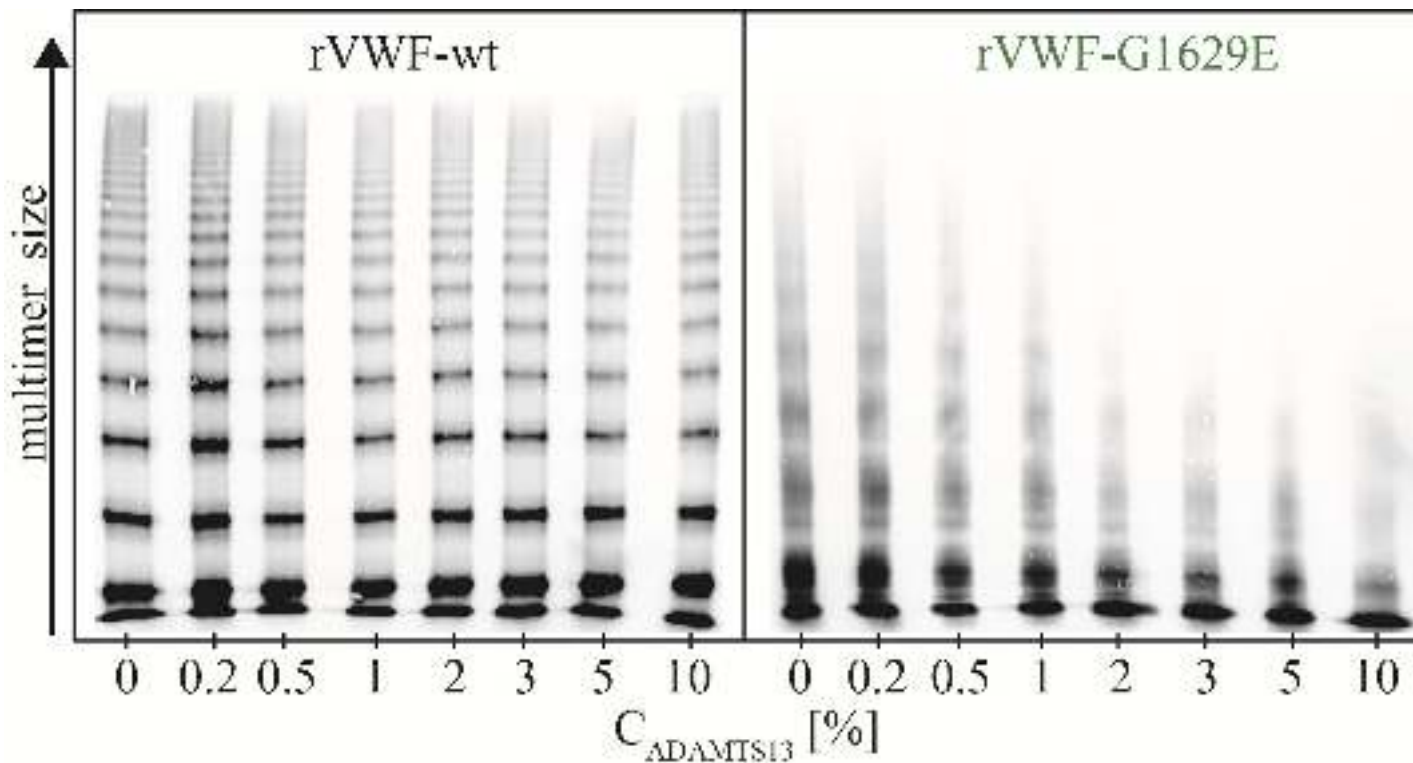
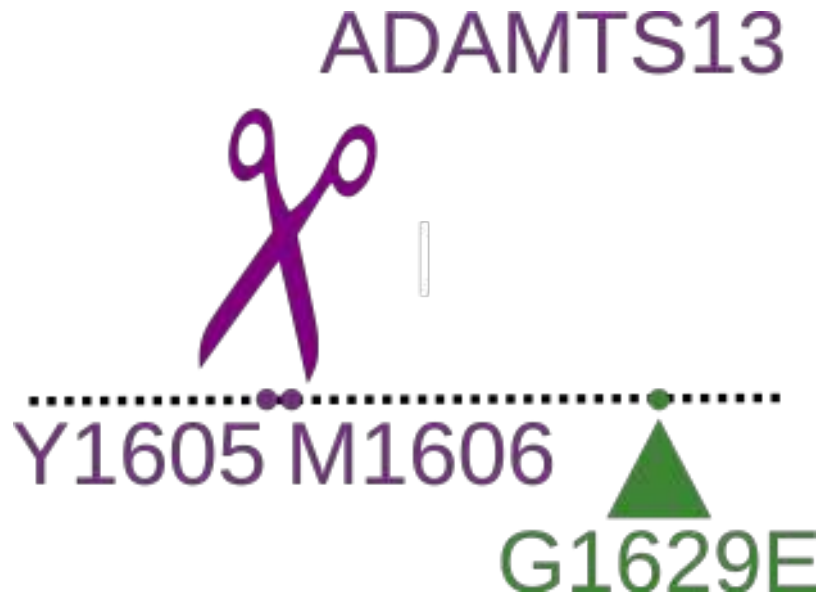
- Brehm, Huck, CAS et al. Thromb. Haemos. 2014
- Lippock, Kolsek...CAS et al. Blood. 2016

## mutation-induced accelerated cleavage



CAS, Lippock et al. Biophys. J. 2017

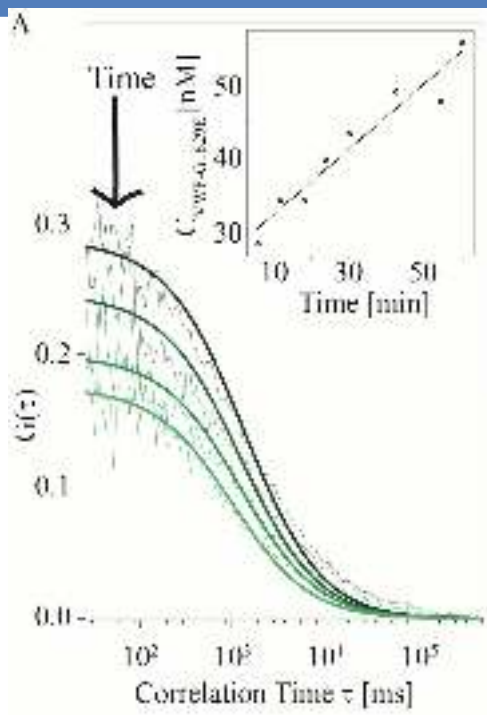
# G1629E mutation dramatically increases cleavage



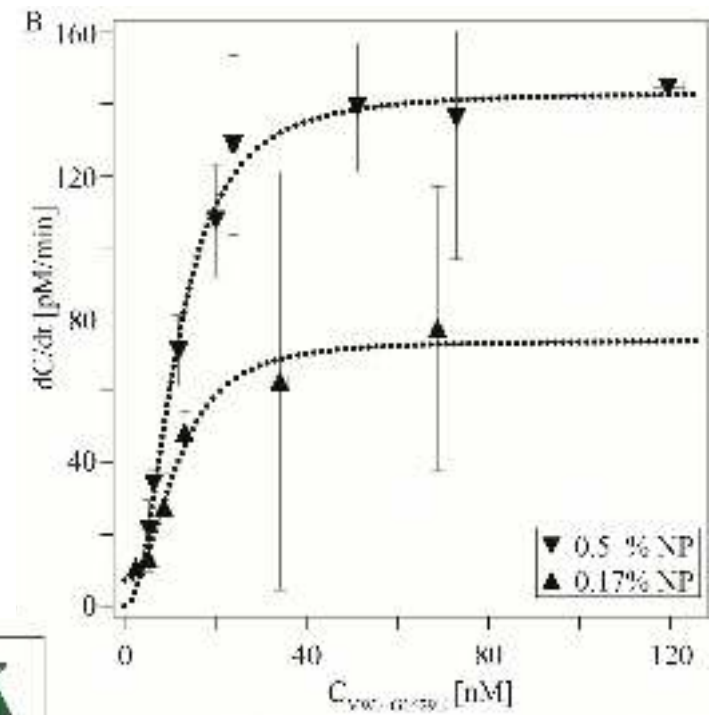
- von Willebrand disease type 2A
- Kinetics?
- Molecular mechanism?



# G1629E cooperative cleavage kinetics



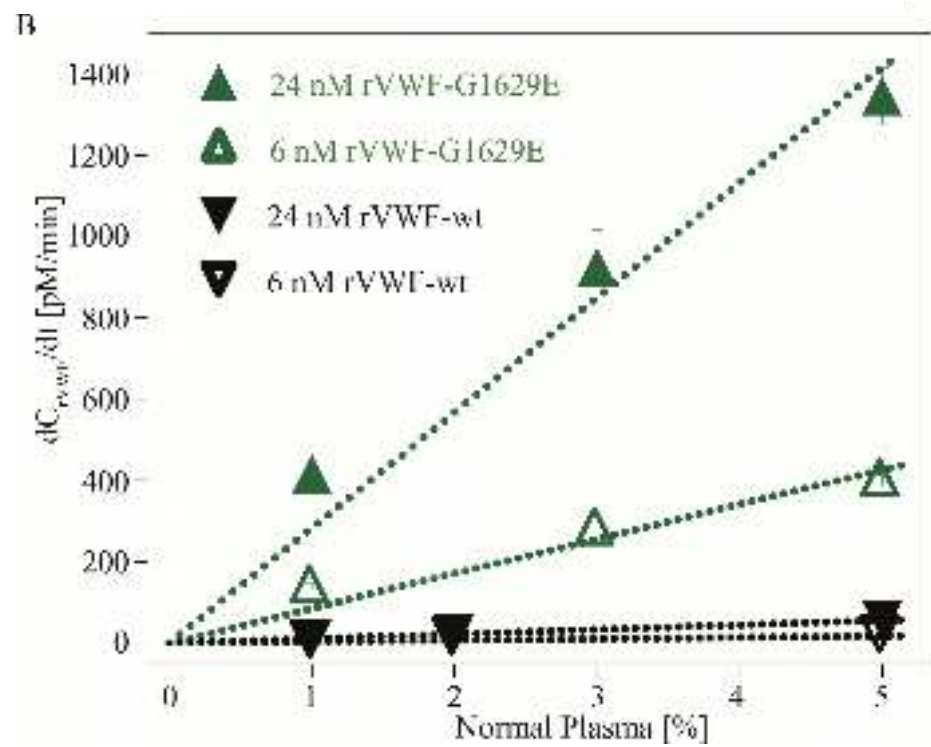
FCS assay to monitor VWF cleavage:  
*Lippok, Mittag, Rädler, LMU Biophys J 2013 & 2016*



$$dC/dt = v_{\max} / [1 + (K_A/C)^n]$$

$n = 2.3$ ,  
 $v_{\max} = 143.2 \pm 0.4$  pM/min,  
 $K_A = 11.5 \pm 0.5$  nM.

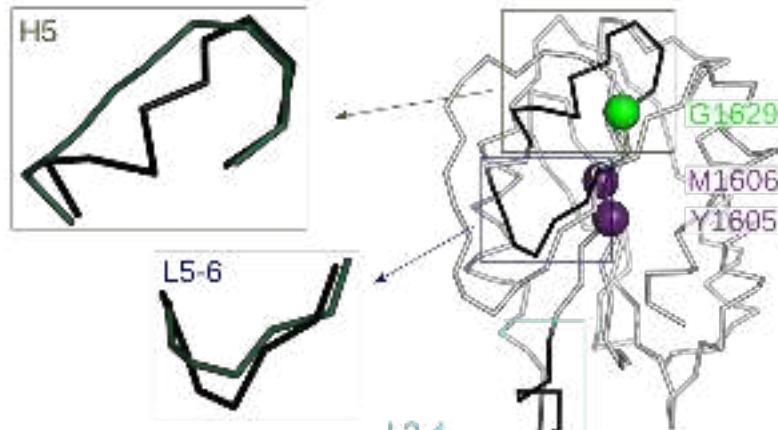
**G1629E gives access to multiple cleavage sites at the same time.**



~20-fold larger cleavage rate for G1629E

# MD simulations: G1629E mutant induced changes in

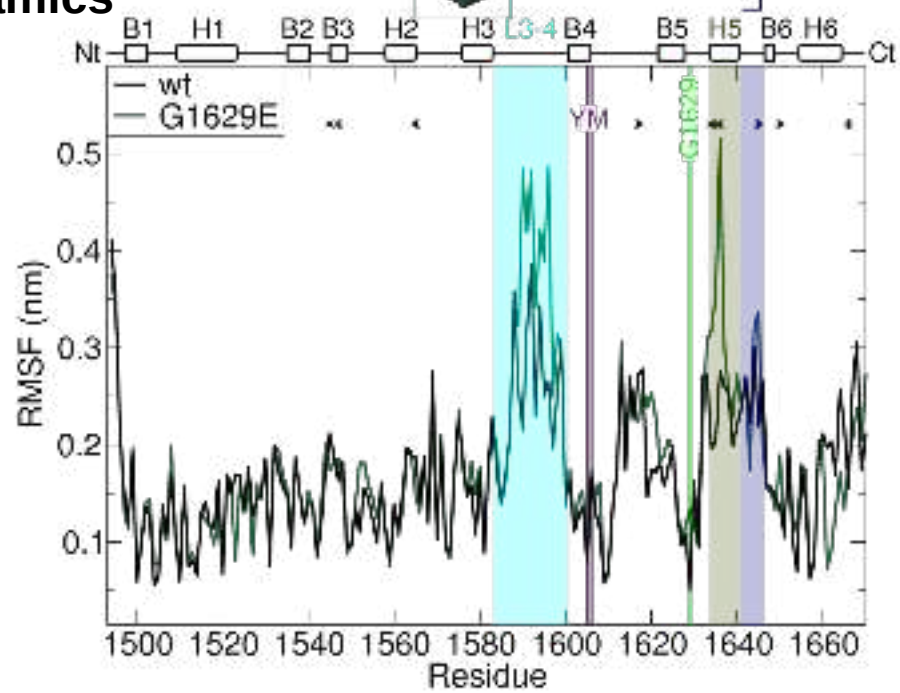
## Structure



functional-mode-analysis  
de Groot, Biophys. J. 2012

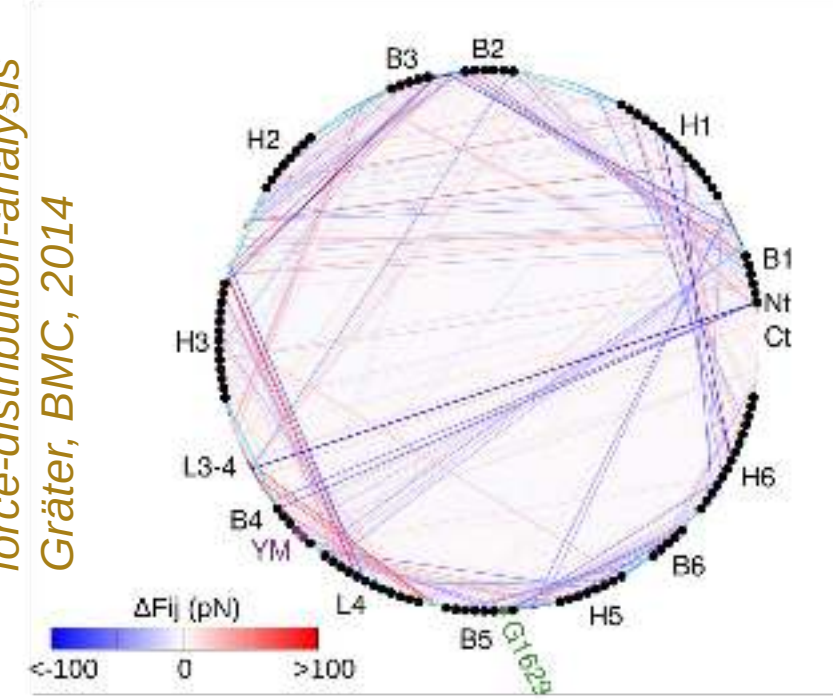
— wt  
— G1629E

## Dynamics



## Internal stress

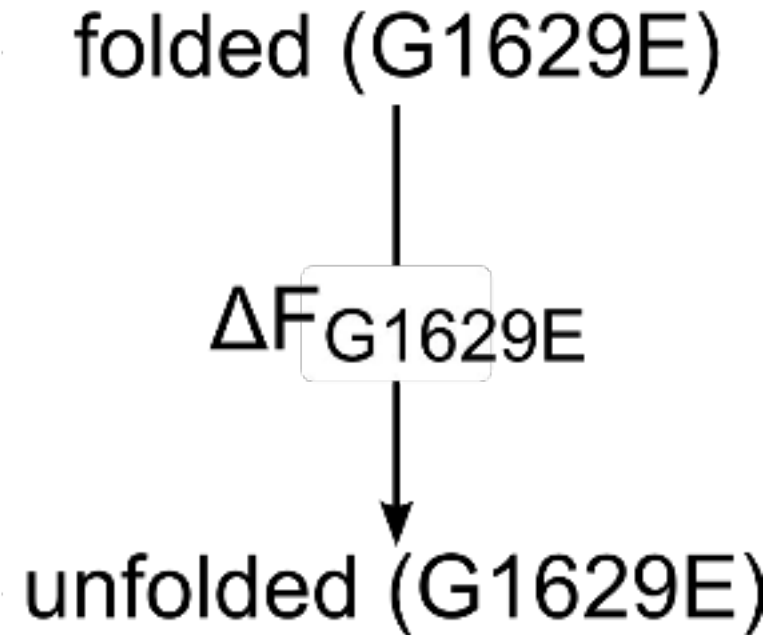
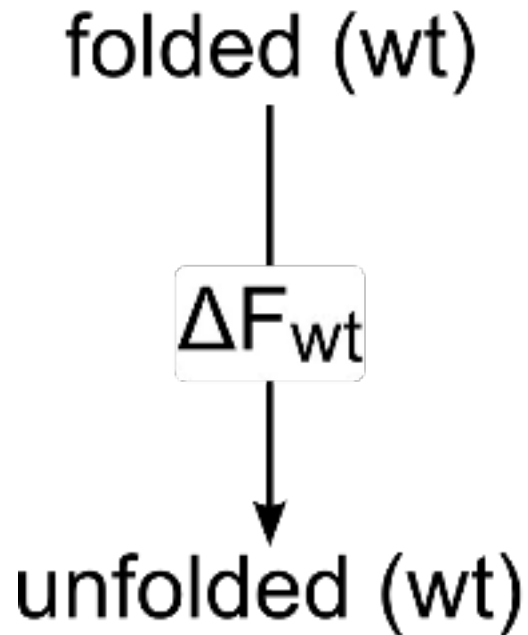
force-distribution-analysis  
Gräter, BMC, 2014



**Are these destabilizing or stabilizing changes?**

~ 1  $\mu$ s wt  
~ 1  $\mu$ s mutant

# Assessing thermodynamic stability



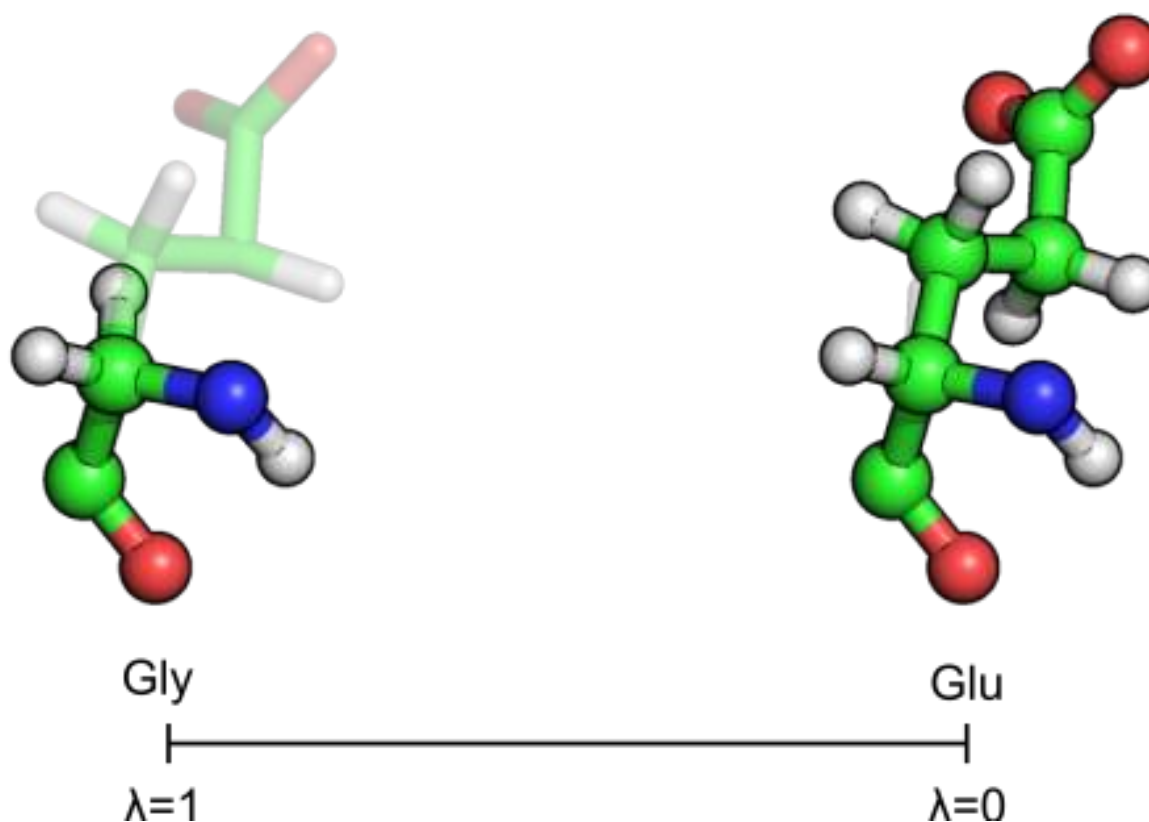
$$\begin{aligned}\Delta\Delta F &= \Delta F_{G1629E} - \Delta F_{wt} \\ &= \Delta F_2 - \Delta F_1\end{aligned}$$

$\Delta\Delta F < 0$  Destabilizing mutation

$\Delta\Delta F > 0$  Stabilizing mutation

free energy calculations

# Gly to Glu transition by thermodynamic integration



Hamiltonian coupled to variable  $\lambda$ :

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

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:

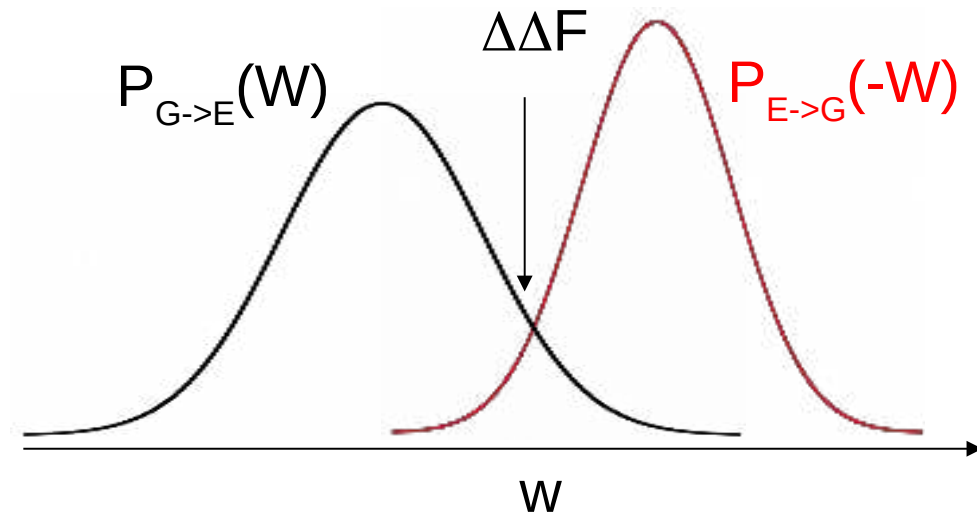
$$\frac{P_{G \rightarrow E}(W)}{P_{E \rightarrow G}(-W)} = e^{\frac{w - \Delta F}{k_B T}}$$

$\Delta\Delta F$  from:

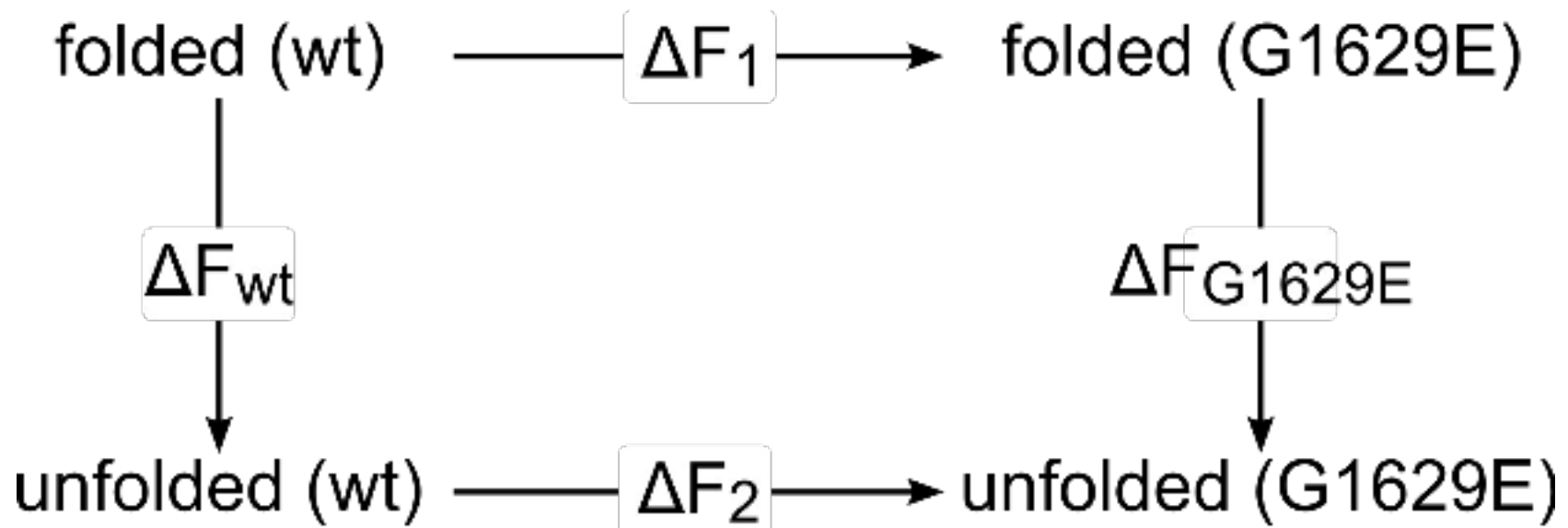
- BAR maximum-likelihood for non-equilibrium transitions  
*Shirts et al. PRL, 2003*

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

- Intercept between distributions  
*Grubmüller, JCC, 2010*



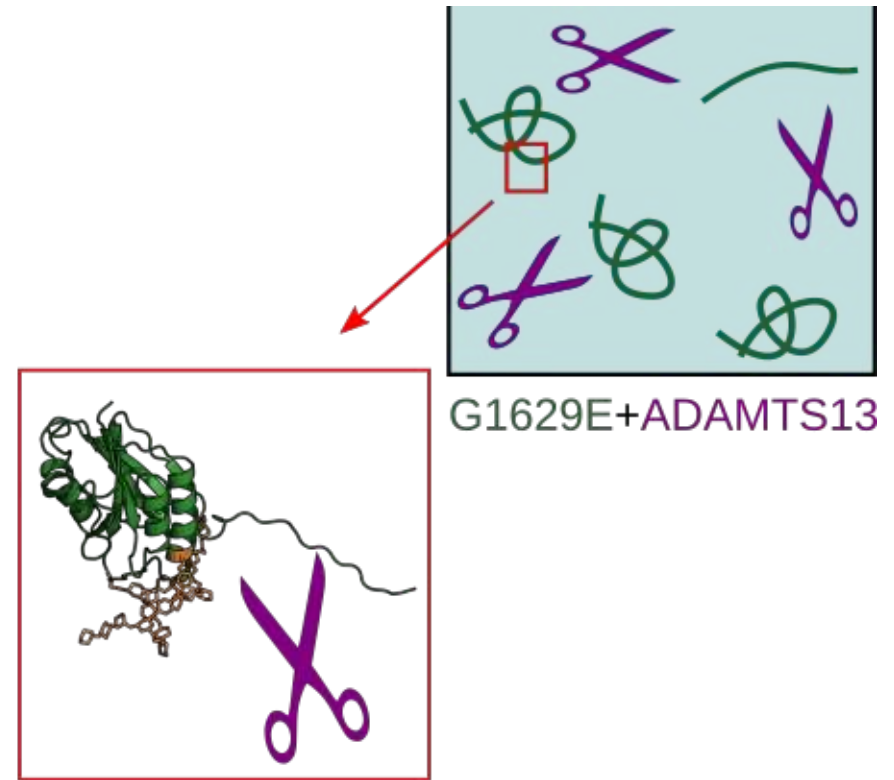
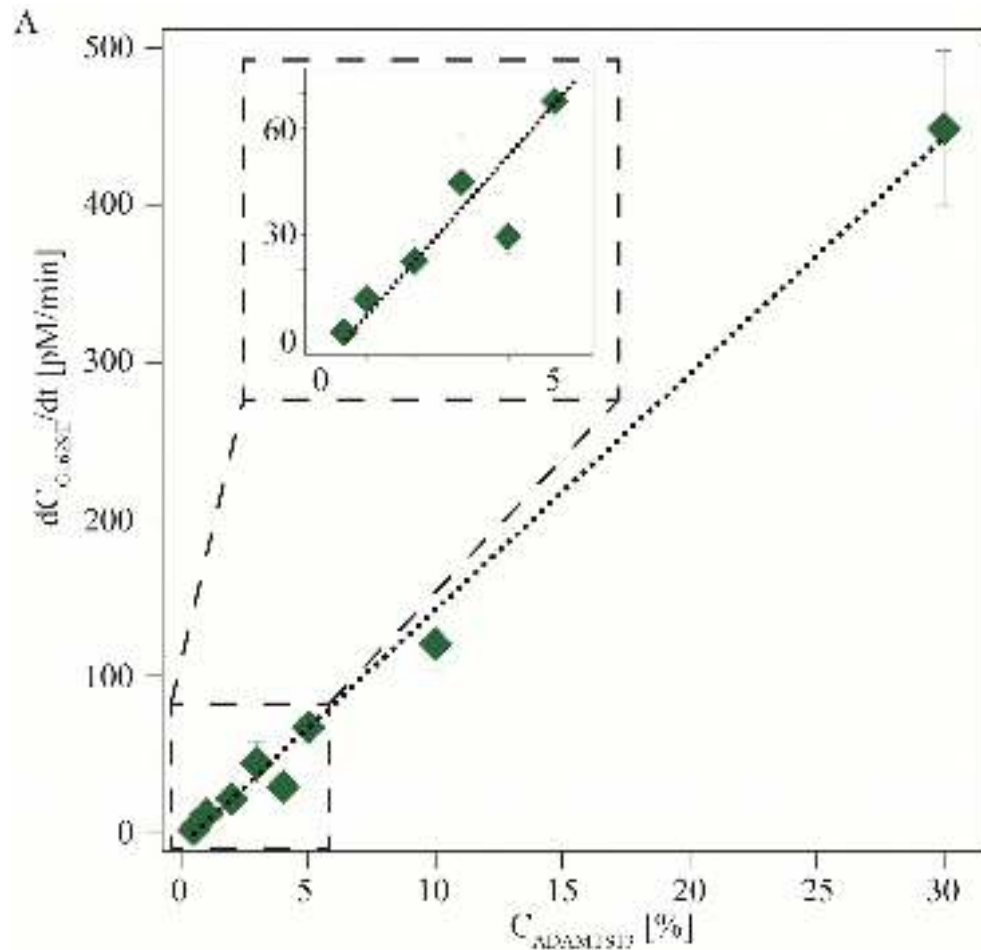
# A2-domain thermodynamic destabilization enhances cleavage of G1629E



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

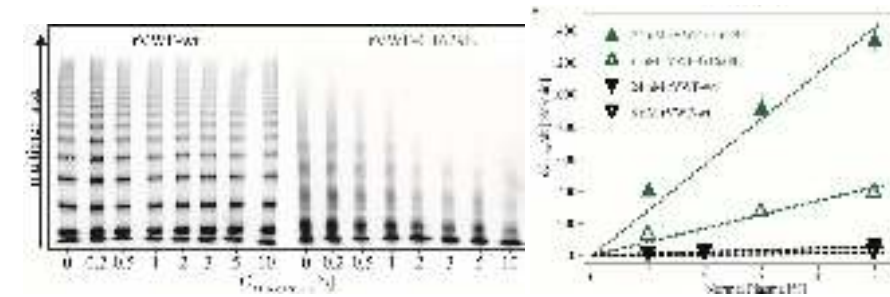
Cleavage rate reached 40-100 times faster for G1629E

# G1629E as a substrate for ADAMTS13 assays

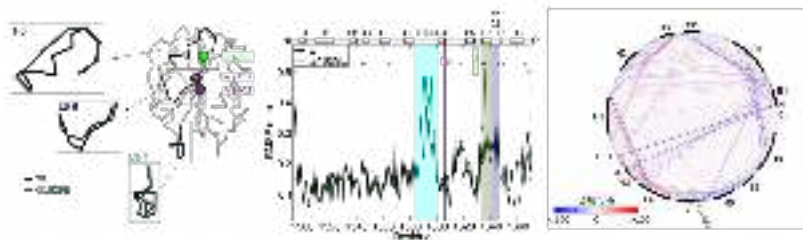


- improved resolution:  
down to 0.5 % ADAMTS13 physiol. concentration
- no denaturants
- no shear
- highly sensitive

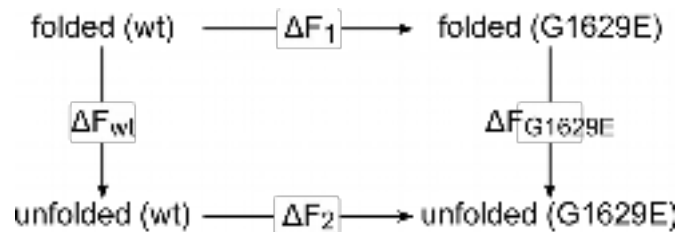
# Mutant G1629E enhances VWF cleavage via a cooperative destabilization mechanism



20-fold enhanced  
Cleavage kinetics



Structural – Dynamic - Mechanical  
A2-domain thermodynamic  
destabilization



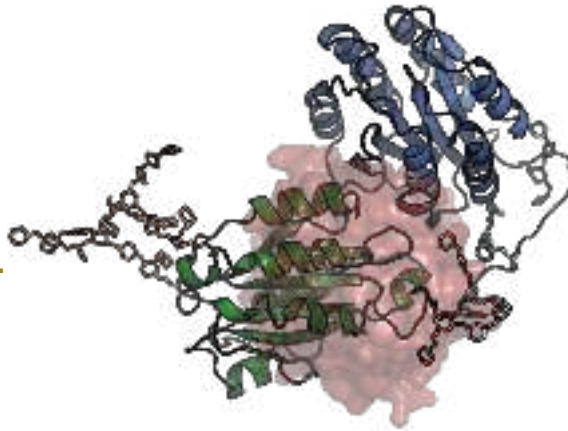
G1629E as a substrate  
for highly sensitive  
ADAMTS13 assays



# Computational studies of VWF

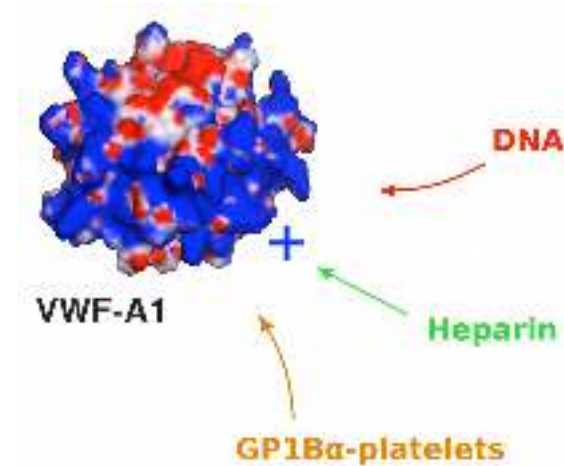
## protein-protein: autoinhibition

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



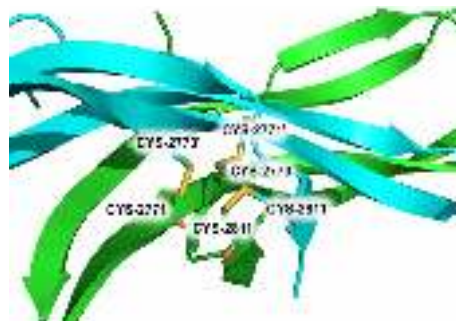
## VWF-DNA: inflammation

Grässle...CAS et al. ATVB. 2014



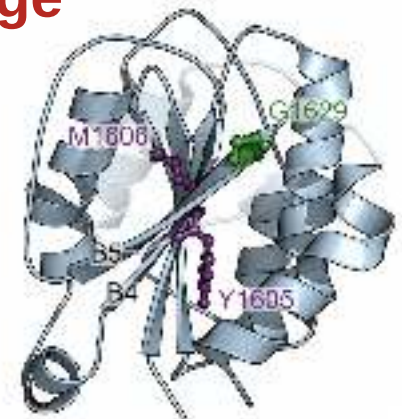
## dimerization

- 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



# Thanks to



# Thanks to

Aug. 2018



**Max Planck Tandem Group in  
Computational BioPhysics (MPTG-CBP)  
University of Los Andes, Bogotá, Colombia**

**[ca.aponte@uniandes.edu.co](mailto:ca.aponte@uniandes.edu.co)**

**[mptg-cbp.github.io](https://mptg-cbp.github.io)**



MAX-PLANCK-GESellschaft

