Gathering a molecular model of the VWF+ADAMTS13 complex

The Role of Mutations on the Interplay of VWF and ADAMTS13 in the Scope of Thrombotic Thrombocytopenic Purpura

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In Short

- Compiling experimental corner stones into a comprehensive model
- Guided enhanced sampling TIGER2h simulations to span large virtual timespans
- Studying the influence of TTP related mutations on the obtained model

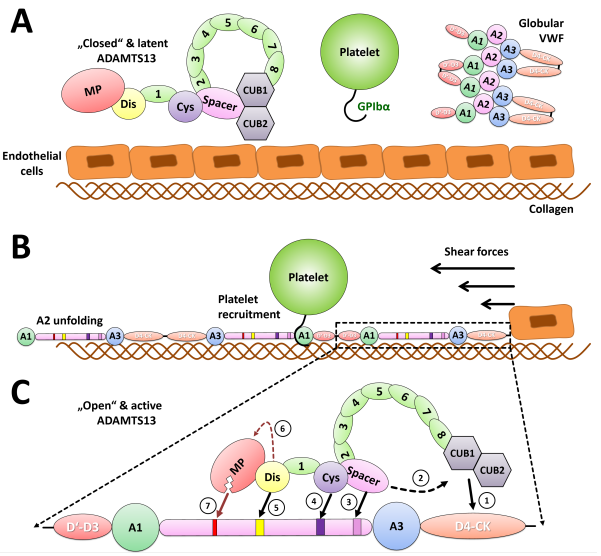

Figure 1: *Overview on the interplay of VWF and ADAMTS13 and proposed activation model.* ***A*** *In normal circulation, ADAMTS13 exists in a closed, inactive form. Also, VWF multimers are folded in on themselves to prevent unwanted interaction of A1 with platelets.* ***B*** *In case of blood vessel damage, A3 binds to exposed subendothelial collagen and shear stress unpacks the VWF multimers into an extended chain. Platelets can be recruited to the damaged vessel site. The A2 domain completely unfolds into a linear peptide strand and exposes the exosites and cryptic cleavage site for ADAMTS13.* ***C*** *The CUB domains of ADAMTS13 bind to the D4-CK domain of VWF and get released from the Spacer domain* ***(1)****. The Spacer* ***(2)*** *and Cys-rich* ***(3)*** *domains bind to their exosites on the unravelled A2 domain and thereby guide the further association. The Dis domain binds its A2-exosite* ***(5)*** *and causes an allosteric change in the MP domain* ***(6)*** *that opens up the active center. The MP domain can finally bind and cut the A2-cleavage site* ***(7)****.*

Introduction The specific interplay of VWF (von Willebrand factor) and ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) is essential for the regulation of platelet-tethering to sites of blood vessel damage. VWF consists of different functional domains and exists as multimers ranging from dimers to large 100-mers. In blood circulation, plasma VWF adopts a globular conformation in which the VWF-A1 domains are hidden, the binding sites for GPIbα of platelets (Figure 1, A). If a blood vessel is damaged, subendothelial collagen is exposed, to which globular VWF binds via its A3 domain. Due to the shear forces generated by the blood flow, the VWF multimers unfold and reveal the hidden A1 domains. This enables platelets to be captured from blood and to start the thrombin cascade and wound healing (Figure 1, B). High concentrations of VWF in the plasma and also very large VWF multimers, are risk factors for thrombosis. The platelet tethering function of VWF is thus proteolytically regulated by the MP domain of ADAMTS13. High shear forces acting on delocalized VWF induce unfolding of its A2 domain. ADAMTS13 is regulating the size of VWF-multimers by specifically cleaving the unravelled A2 domain (Figure 1, C). Low ADAMTS13 concentrations but also 31 mutations are associated with severe ADAMTS13 deficiency and an increased risk of myocardial infarction and stroke, a hallmark of thrombotic thrombocytopenic purpura. The interaction and specific relationship of VWF and ADAMTS13 have been elucidated extensively in the past and a comprehensive, but yet incomplete picture arose[1–5].

Objective Gathered from many studies, there are insights into the residues of either VWF-A2 or ADAMTS13-MDTCS that are crucial for the interaction of both biomolecules (Figure 2). In this project, we plan to compile the experimental findings into a molecular model by means of profound enhanced sampling molecular dynamics simulations (MDS) using our TIGER2h algorithm and utilize the experimentally derived exosites and knockouts as guidance. The final goals are to acknowledge the predicted mode of action and to investigate the influence of TTP related mutations to ADAMTS13 on a molecular level. We are especially curious to observe the predicted 2nd stage activation that open's the MP domain, once the VWF-A2 strand binds to other exosites at the ADAMTS13 surface. The final model may inspire ongoing experimental work and the search for new therapies on TTP.

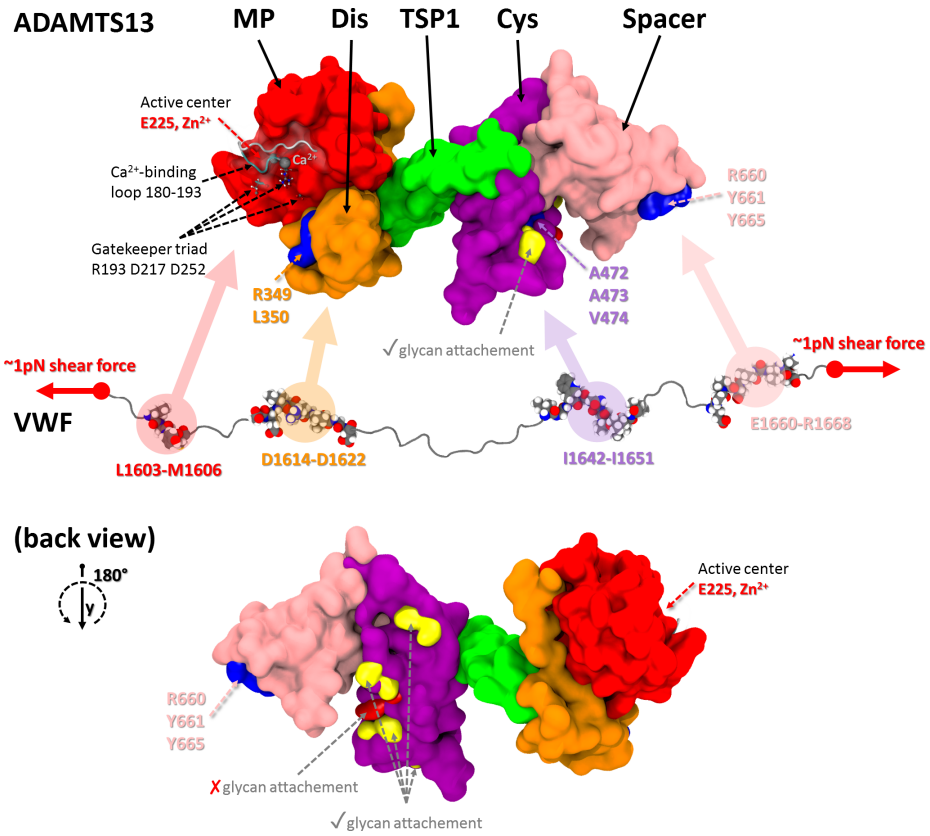

Figure 2: *Comprehension of boundary conditions for the molecular interaction between VWF and ADAMTS13. The active center containing the catalytic glutamic acid and the Zn^{2+} ion are occluded by the conspicuous Ca^{2+} binding loop built from residues 180-193. The arginine residue R193 in this loop is coordinates by two aspartic acid residues in proximity to build the gatekeeper triad. The known exosites for VWF are highlighted at the surface of all ADAMTS13 domains (blue), except TSP1 were none is reported to date. In reverse, the critical residues on the unravelled VWF-A2 strand are emphasized and are expected to interact near their respective counterparts at the ADAMTS13 domains. Attachment sites for artificial sugars are highlighted, that either did not interfere with VWF binding or significantly impaired binding in one particular case.*

Enhanced sampling TIGER2h We have recently presented a replica exchange based enhanced sampling algorithm (TIGER2h)[6,7], that comes with the best compromise in resource usage and accuracy to date and is well suited for the sampling problems in this project. It can be conveniently guided by restraining known exosites into proximity and thermodynamic probabilities rather than empirical scoring functions as in docking, are utilized to appreciate the best binding configurations. Thereby, TIGER2h can explore the local conformational space of the ADAMTS13-MDTCS model and will give valuable insight into flexible regions and the overall internal degrees of freedom between the different domains in terms of bending and twisting. We apply NAMD's colvar module to guide the TIGER2h sampling of VWF-A2 binding fragments on the surface of ADAMTS13 by means of center-center flat-bottom restraints between experimentally predicted exosites. By the flat-bottom fashion of the restraints, the sampling is not perturbed when both selections are in the given proximity and can explore the conformational space freely. Forces only act, once the exosites divide by more than the allowed distance. We apply the colvars also, to preserve the general globular fold of the ADAMTS13 domains by RMSD flat-bottom restraints. As a consequence, the observable local dynamics of the complex molecular system can be extended to virtual timescales and processes far beyond what is normally accessible with MDS.

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More Information

[1] doi:10.1038/s41467-019-11474-5

[2] doi:10.1074/jbc.m502529200

[3] doi:10.1182/blood-2014-08-594556

[4] doi:10.1126/science.1170905

[5] doi:10.1016/j.bpj.2018.11.1054

[6] doi:10.1021/acs.jpcb.9b03134

[7] doi:10.1021/acs.jpcb.8b05178