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Supplementary Materials for

Autoregulation of von Willebrand factor function by a disulfide bond switch

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Supplementary Material

Expression and purification of the recombinant A2 domain

cDNA corresponding to VWF A2 domain residues M1473-L1675 was amplified by PCR using VWF human cDNA (Origene, SC119835) as a template. The PCR product was directionally cloned into the pTAZH vector (gift from Dr. Celso Romero, Biomatik, Sao Paulo, Brazil). The N-terminus contains a 6xHis-tag followed by the ZZ domain of protein A and then a thrombin cleavage site. Expression in under control of a T7 promoter. *E. coli* Bl21 (DE3) (Promega) was transformed with the pTAZH-A2 construct and expression induced with 1 mM IPTG for 6 h at 30°C in LB media containing 100 µg/mL ampicillin. The *E. coli* were peleted by centrifugation at 5000 g for 30 min, resuspended in 50 mM phosphate buffer, pH 7 containing 300 mM NaCl and sonicated. Clear lysate was passed through a Talon metal affinity resin (Takara) pre-equilibrated in the phosphate buffer. The resin was washed thoroughly with 50 mM Tris, pH 8.4 buffer containing 150 mM NaCl and 2.5 mM $CaCl_2$ and incubated with 5 units of thrombin (Sigma SRE0003) per mL of resin for 16 h at 22°C. The soluble A2 was collected, thrombin inactivated with 2-fold molar excess of PPACK (Merck) and the protein concentrated and dialysed. The A2 fragment has a Mr of 25 kDa and purity >95% (**Fig. 1B**).

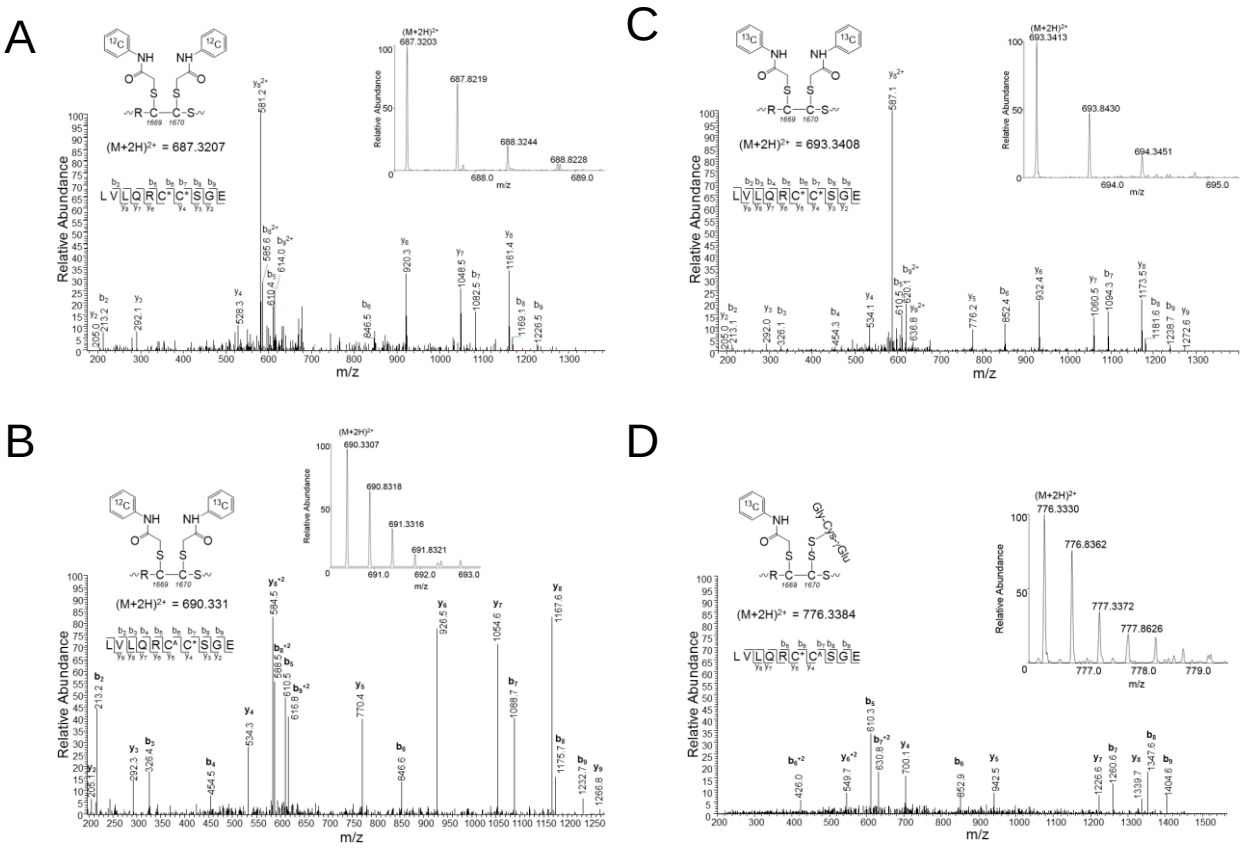

fig. S1. Differential cysteine alkylation and mass spectrometry analysis of the VWF Cys^{1669}-Cys^{1670} disulfide bond. (**A-C),** Representative tandem mass spectra of the VWF LVLQRCCSGE peptide containing Cys1669 and Cys1670. Part A is an example of ^{12}C-IPA-alkylation of both Cys1669 and Cys1670, part B an example of ^{12}C-IPA-alkylation of Cys1669 and ^{13}C-IPA-alkylation of Cys1670, and part C an example of ^{13}C-IPA-alkylation of both cysteines. The accurate mass spectrum of the peptide is

shown in the inset (part A observed $[M+2H]^{2+}$ = 687.3203 m/z and expected $[M+2H]^{2+}$ = 687.3207 m/z; part B observed $[M+2H]^{3+}$ = 690.3307 m/z and expected $[M+2H]^{2+}$ = 690.331; part C observed $[M+2H]^{2+}$ = 693.3413 m/z and expected $[M+2H]^{2+}$ = 693.3408 m/z). **(D),** Representative tandem mass spectrum of the VWF LVLQRCCSGE peptide labelled with ^{13}C-IPA at Cys1669 and glutathione at Cys1670. The accurate mass spectrum of the peptide is shown in the inset (observed $[M+2H]^{2+}$ = 776.3330 m/z and expected $[M+2H]^{2+}$ = 776.3384 m/z).

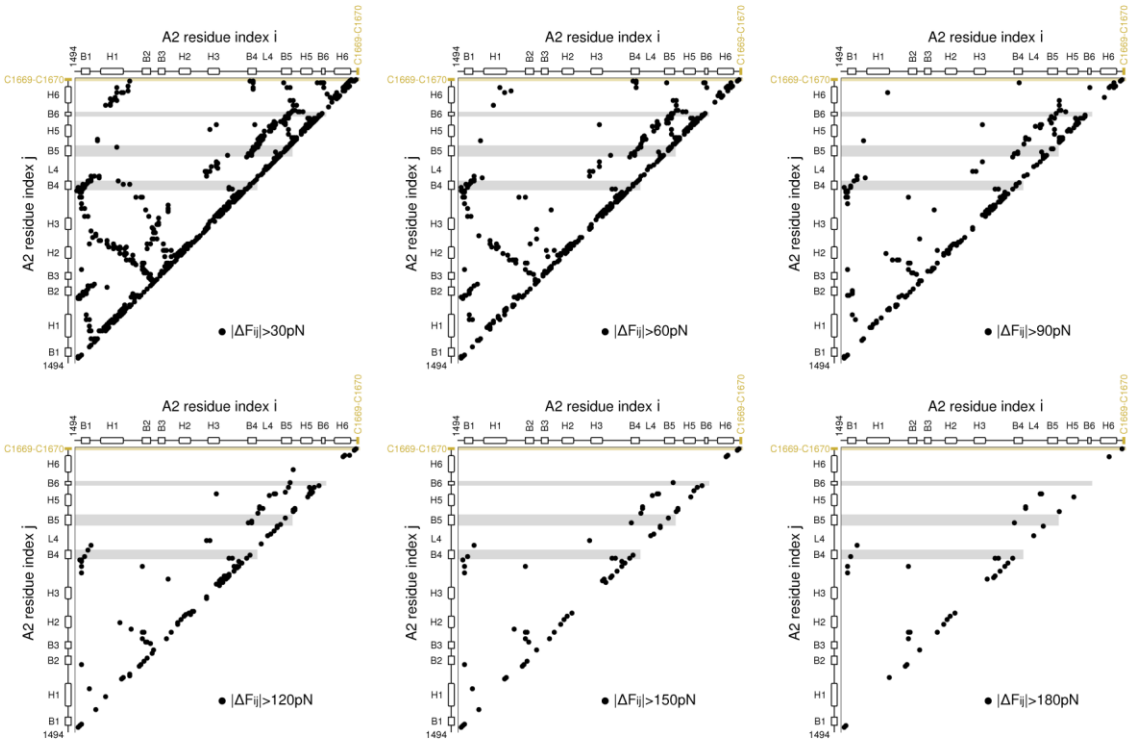

fig. S2. FDA of the VWF A2 domain. Residue pairs (i,j) with a time-averaged pair-wise force of the reduced minus the one of the oxidized state, |ΔFij|=|<Fij(red)> - <Fij(oxi)>|, larger than the specified cutoff values. Here, | | indicates absolute values. Secondary structure of A2 is shown on both axes. To guide the eye, the regions corresponding to the beta strands B4, B5 and B6 are displayed with gray areas, and the one to the Cys1669-Cys1670 bond in orange.

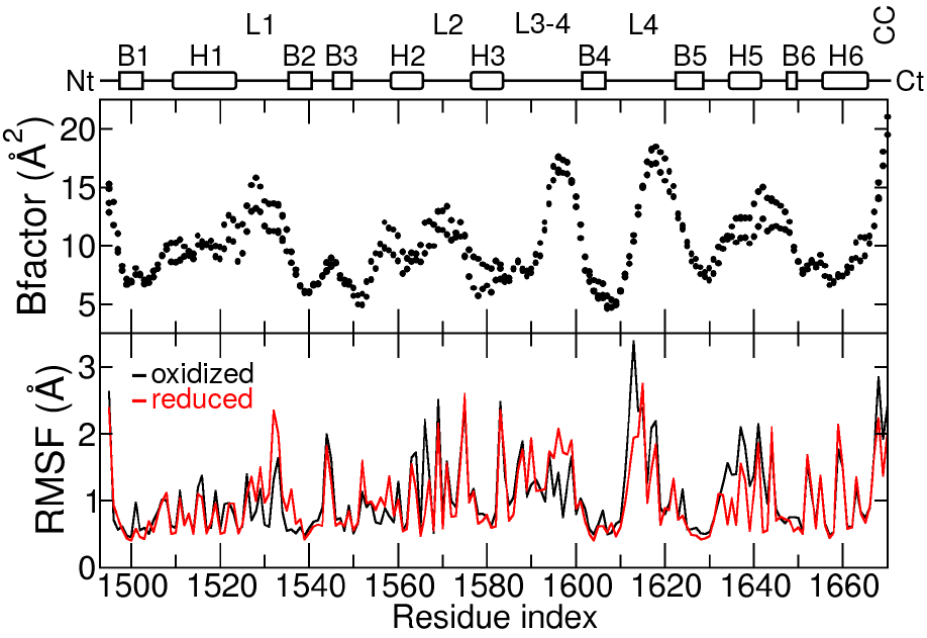

fig. S3. Concordance between RMSF for the simulated A2 redox states and crystallographic B factors. Per-residue crystallograhic Bfactor (top) is compared with the root mean square fluctuations (RMSF) for the two simulated redox states (bottom). Secondary structural elements of the A2 domains are indicated at the top. The disulfide bond Cys1669-Cys1670 (CC) is located at the C-terminus.

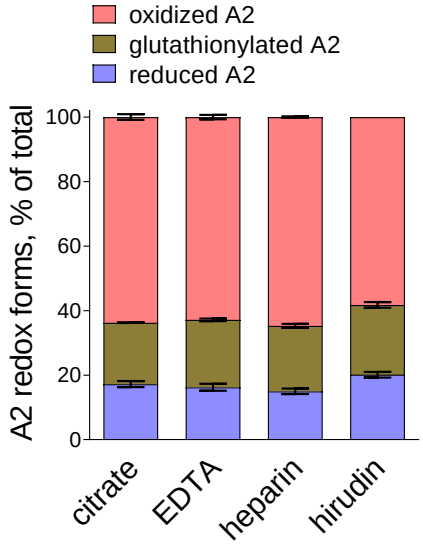

fig. S4. Anticoagulation of blood with calcium chelators (citrate or EDTA) or direct thrombin inhibitors (heparin or hirudin) does not appreciably influence the redox state of the A2 domain cysteines. Distribution of reduced, glutathionylated and oxidized VWF A2 in a single healthy donor plasma. Mean and range for the two A2 domain cysteine-containing peptides of individual samples are shown. Note that this particular donor had high levels of oxidized A2 domain.

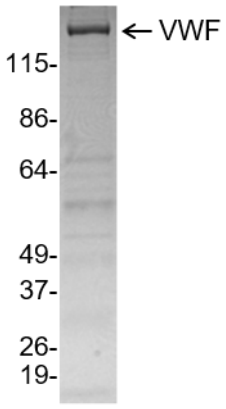

fig. S5. Plasma VWF collected on antibody-coated beads, resolved on reducing SDS-PAGE, and stained with colloidal Coomassie. The positions of molecular weight standards in kDa are shown at the left