SUPPLEMENTAL DATA

E. coli derived Von Willebrand Factor-A2 domain FRET proteins that quantify ADAMTS13 activity

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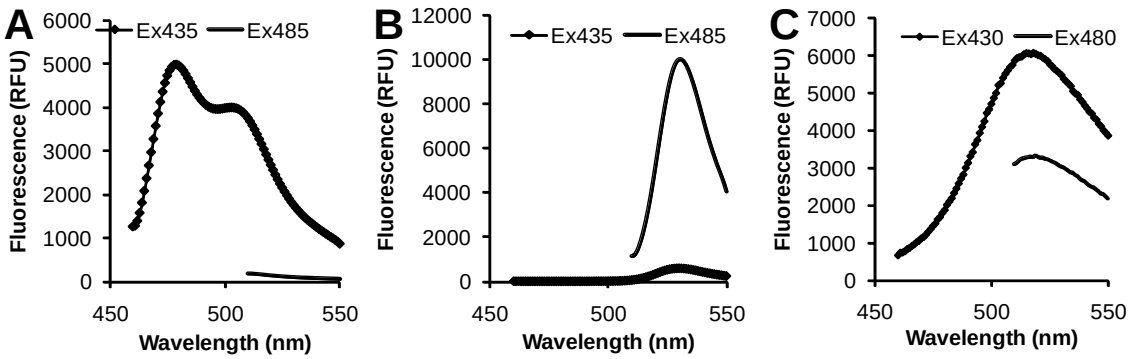
Supplemental Table 1

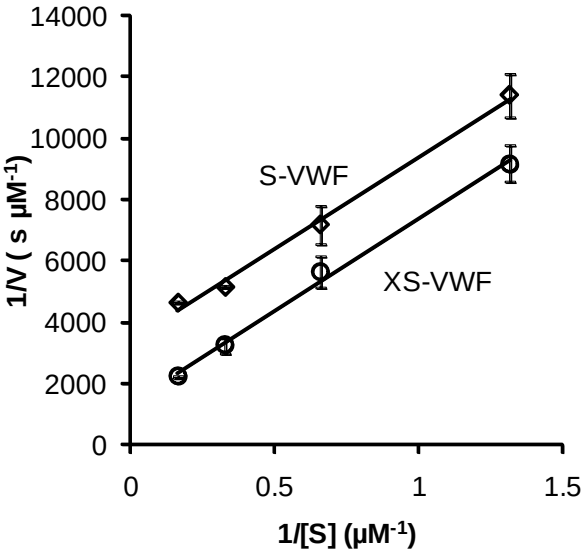
Name of the primer	Primer sequence (5'-3') *	Use
FP1_AgeI sense	GGGCGGACCGGTATGGTGAGCAAGGGCGAGGAGCTG	Citrine into pCSCG-A2-FH
FP1_AgeI antisense	CGCGCCACCGGTCTTGTACAGCTCGTCCATGCCGAG	
His7STOPHind3_Rev	CGCGCGCAAGCTTAATGGTGATGGTGATGGTGATGGCC	Citrine-A2 from pCS-CG into pRSETB
NdeI_Citrine_For	GCGGCGGCATATGGTGAGCAAGGGCGAGGAGCTG	Citrine-A2 from pCS-CG into pRSETB, Venus into pRSET-Citrine-A2-FH
FP2_AgeI antisense	CGCGCACCGGTCTCGTCCATGCCGAGAGTGATCCC	Venus into pRSET-Citrine-A2-FH
FP1_HpaI sense	[Phos]AACATGGTGAGCAAGGGCGAGGAGCTG	Cerulean into pRSET-Ven-A2-FH
FP1_BstBI antisense	CCGCCGTTCGAACTTGTACAGCTCGTCCATGCCG	
AgeIVWF1594For	GTGTGTACCGGTCAGGGTGATCGGGAGCAGGCGCC	A2 (XS-VWF) forward primer
AgeIVWF1558For	CGTAGTACCGGTAAAGGGGACATCCTGCAGCGGG	A2 (S-VWF) forward primer
AgeIVWF1530For	CGCGCGACCGGTGTGGGCCAGGACAGCATCCACG	A2 (M-VWF) forward primer
AgeIVWF1496For	GGGATTACCGGTGTTCTGGATGTGGCGTTCGTC	A2 (L-VWF) forward primer
HpaIVWF1670Rev	[Phos]AACGCAGCACCTCTGCAGCACCAGG	Reverse primer for all of the above A2-FRET conststructs
VWFNdeI1481F	GCGCGCGCATATGGGGCTCTTGGGGGTTTCGACCCTG	For making pRSET-A2-Cer-H
VWFHpaI1668R	[Phos]AACCCTCTGCAGCACCAGGTCAGGAGC	
VWF_YM1605_6AA_rev	GATTTCCCGTGACAGCAGCGACCAGGTTG	^{1605}Y-^{1606}M to ^{1605}A-^{1606}A mutation primer

* **Restriction enzyme sites**: GTTAAC: *Hpa*I; [Phos]ACC: *Hpa*I compatible end; ACCGGT: *Age*I; CATATG: *Nde*I; AAGCTT: HindIII; TTCGAA: *Bst*BI

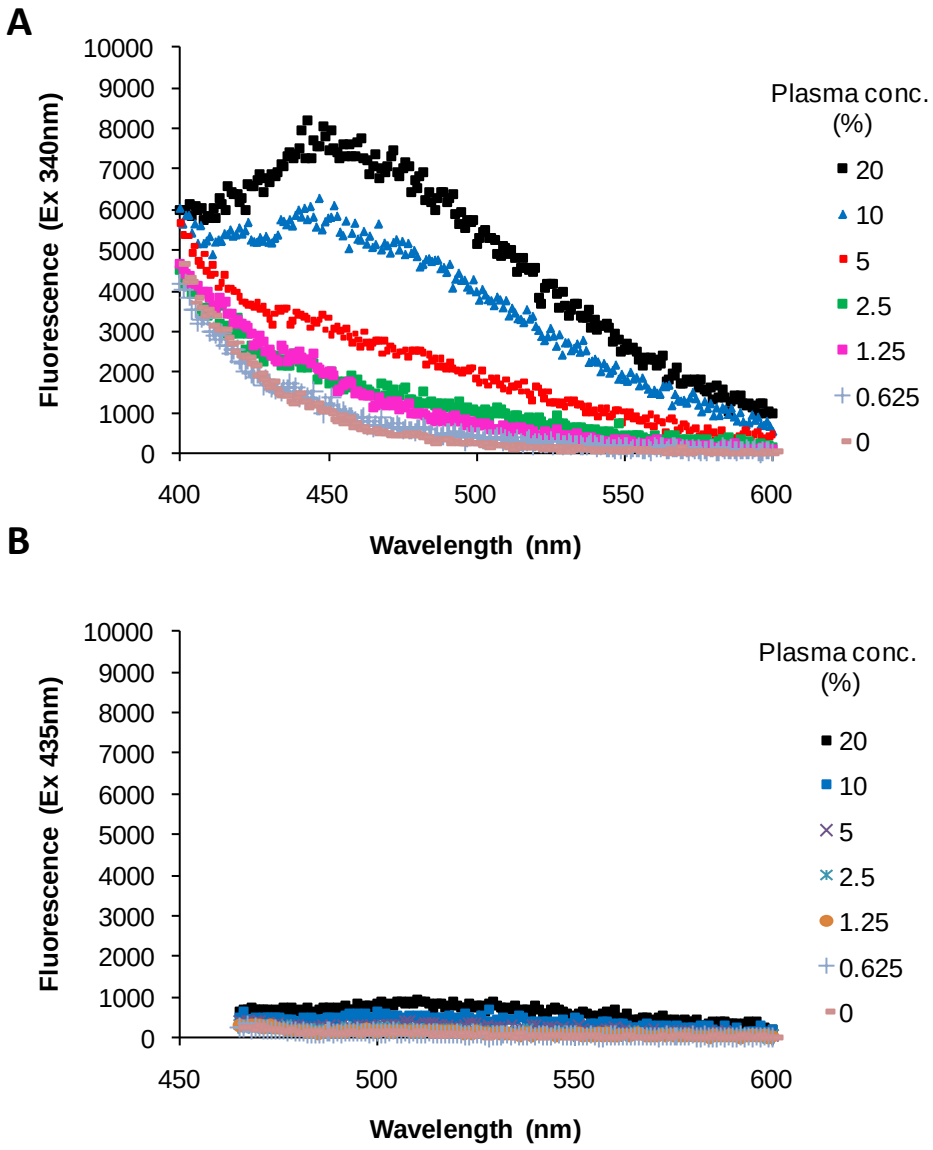


Supplemental Fig. S1 Emission spectra of **A.** VWF-A2(1481-1668)-Cerulean, **B.** Venus-VWF-A2(1481-1668) and **C.** Citrine-VWF-A2(1481-1668). Excitation wavelengths of 435 nm and 485nm are optimum for CFP and YFP respectively. Proteins in panels A and B were expressed in *E. coli* BL21 Star. Protein in panel C was from *E. coli* Rosetta-gami 2(DE3)pLysS. Spectra for Citrine-VWF-A2(1481-1668) does not match prior reports in literature [1].

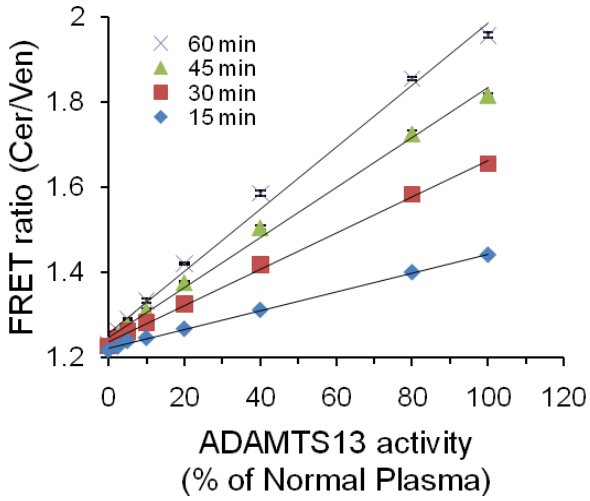
Fig. S2



Supplemental Fig. S2 Enzyme kinetics. Various concentrations of XS-VWF and S-VWF were incubated with 20% plasma from normal human donors for up to 2h. FRET ratio measured at various times was used to generate Lineweaver-Burk plot.



Supplemental Fig. S3 Plasma auto-fluorescence. Human blood plasma at various dilutions (20-0% of normal levels) was excited at either 340nm (conditions used in the VWF-FRETS73 assay, panel A) or 435nm (conditions of the XS-VWF assay, panel B). Emission spectra were collected using identical instrument settings. As seen, auto-fluorescence due to plasma proteins is higher when using lower excitation wavelengths (panel A).



Supplemental Fig. S4 Measurement of low ADAMTS-13 activity. Plasma was obtained from normal human blood (100% ADAMTS13 activity). A portion of this was heat-inactivated to obtain 0% ADAMTS13 activity plasma. Normal plasma and heat-inactivated plasma were then mixed to obtain samples with a variety of ADAMTS13 activities. For example, 40% ADAMTS13 activity plasma was obtained by mixing normal plasma and heat-inactivated plasma in 4:6 proportion. Following this, 20µL of the plasma samples with a variety of enzyme activities were mixed with 2µM XS-VWF in 100µL reaction volume. XS-VWF cleavage was measured at various times. 10% ADAMTS13 activity was detected at 30 min.

References:

[1]A.A. Heikal, S.T. Hess, G.S. Baird, R.Y. Tsien, and W.W. Webb, Molecular spectroscopy and dynamics of intrinsically fluorescent proteins: coral red (dsRed) and yellow (Citrine). Proc Natl Acad Sci U S A 97 (2000) 11996-2001.