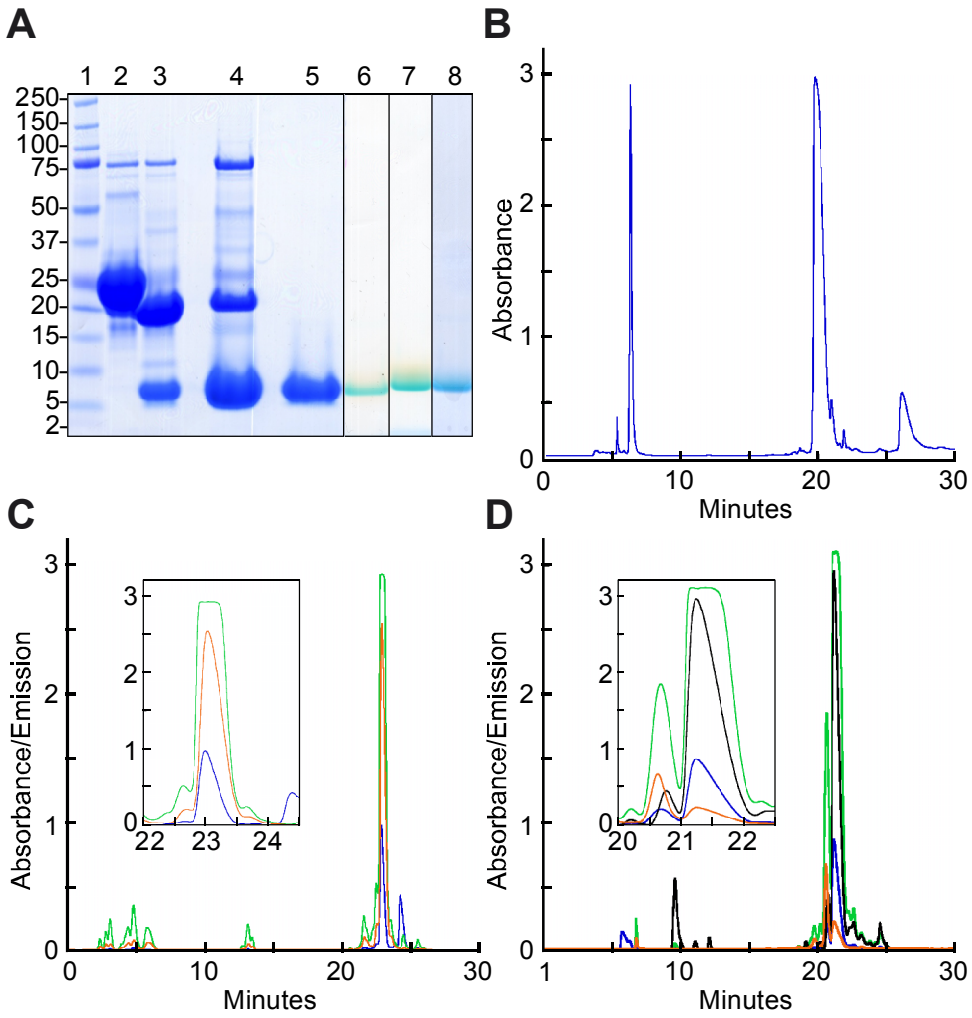

Fig. S1. Preparation of FRETS-rVWF71. (A) Polyacrylamide 4-12% gel electrophoresis. Lanes are: 1, protein markers; 2, thioredoxin-rVWF71 after Ni-NTA agarose; 3, after TEV protease cleavage; 4, after rechromatography on Ni-NTA agarose; 5, after purification by HPLC; 6, purified DyLight 633-rVWF71; 7, purified FRETS-rVWF71; 8, purified FRETS-rVWF71. Gels were stained with Simply Blue SafeStain except for lanes 6 and 7, which are unstained. (B) Purification of rVWF71 on C18 in 0.1% TFA developed with 20-90% acetonitrile, monitored for absorbance at 280 nm (blue). The 20 min peak corresponds to panel A, lane 5. (C) Purification of DyLight 633-rVWF71 on C18 in 50 mM TEAA, pH 6.0, developed with 20-45% acetonitrile, monitored for absorbance at 280 nm (blue) and 627 nm (green), and for emission at 658 nm (orange, arbitrary scale) after excitation at 635 nm. The inset shows an expanded view of the 23 min peak. (D) After modification with IRDye QC-1, FRETS-rVWF71 was purified by HPLC with additional monitoring of absorbance at 819 nm (black). The inset shows an expanded view of the 21 min peak.

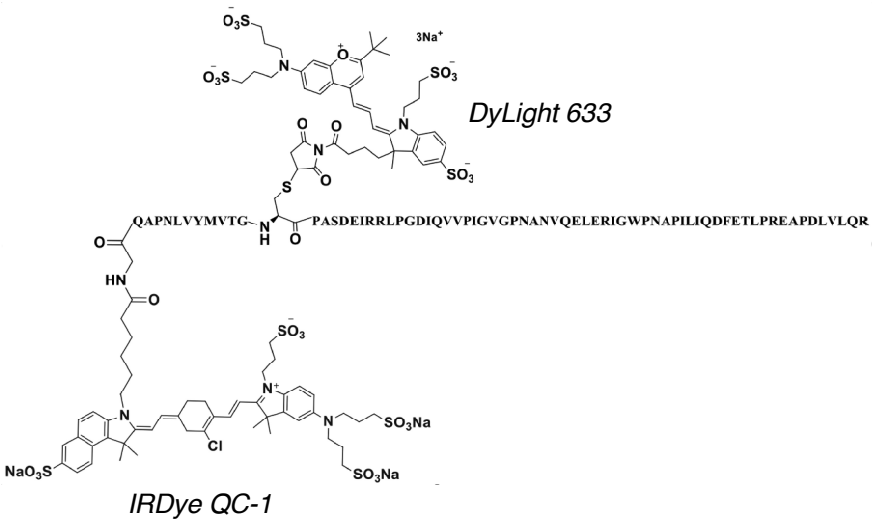

Fig. S2. Structure of FRETS-rVWF71

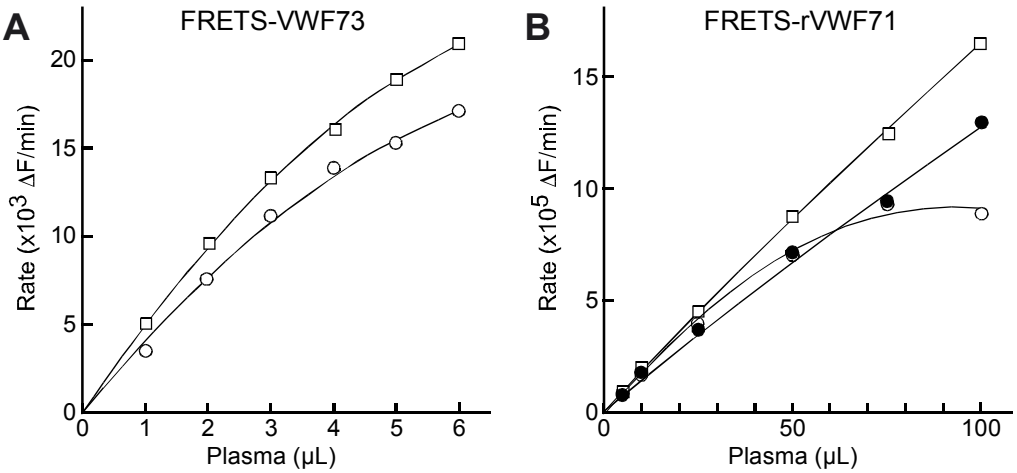

Fig. S3. Comparison of FRETS-VWF73 and FRETS-rVWF71 assays with citrated and Li^+-heparin plasma. (A) Matched plasma samples anticoagulated with Li^+-heparin (open squares) and citrate (open circles) were assayed with FRETS-VWF73 as described (7). The difference between the curves is due to dilution of the citrated plasma with one-ninth volume of sodium citrate. (B) The same matched plasma samples were assayed with FRETS-rVWF71. The Li^+-heparin plasma (open squares) and citrated plasma (open circles) were assayed as described under "Materials and Methods." The sharp deviation from linearity for citrated plasma at the 100 μL point is caused by chelation of calcium ions in the assay buffer by excess citrate. Supplementation of citrated plasma assays with 6 mM $CaCl_2$ (filled circles), approximately equal to the final 5.5 mM citrate concentration of the 100 μL assay point, restored the desired linear behavior. The difference between the curves for the Li^+-heparin (open squares) and citrate+$CaCl_2$ conditions (filled circles) is due to dilution of citrated plasma with one-ninth volume of sodium citrate, as also observed with FRETS-VWF73 (panel A). Therefore, ADAMTS13 assays with FRETS-rVWF71 can be standardized satisfactorily using either Li^+-heparin plasma or citrated plasma supplemented with $CaCl_2$. Note that the dynamic range of the reaction rate (ΔF/min) across all assay points is approximately two orders of magnitude greater for assays with Li^+-heparin plasma and FRETS-rVWF71 (panel B, open squares) than with citrated plasma and FRETS-VWF73 (panel A, open circles).

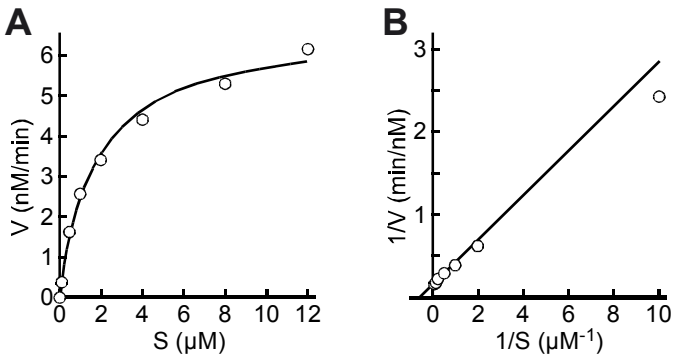

Fig. S4. Kinetics of FRETS-rVWF71 cleavage by ADAMTS13. (A) Initial velocities (nM/min) were fitted to the Michaelis-Menten equation to obtain values for K_m of 1.8 μM (95% confidence interval 1.3 to 2.4 μM), and k_{cat} of 6.8 min^{-1} (95% confidence interval 6.1 to 7.4 min^{-1}). (B) Lineweaver-Burk plot. .

Table S1. ADAMTS13 activity and inhibitor titers

	ADAMTS13 Activity		ADAMTS13 Inhibitor	
Subject	**Substrate rVWF71**	**Substrate VWF73**	**Substrate rVWF71**	**Substrate VWF73**
1	<0.3	<5	<0.4	<0.4
2	<0.3	<5	<0.4	<0.4
3	<0.3	<5	0.6	0.4
4	<0.3	<5	0.6	<0.4
5	<0.3	<5	0.8	<0.4
6	<0.3	<5	0.9	1.8
7	<0.3	<5	0.9	<0.4
8	<0.3	<5	1.5	0.4
9	<0.3	<5	3.0	1.5
10	<0.3	<5	3.0	1.6
11	<0.3	<5	3.0	1.8
12	<0.3	<5	5.6	2.4
13	<0.3	<5	6.0	2.0
14	<0.3	<5	8.6	2.0
15	<0.3	<5	10	2.8
16	<0.3	<5	10	3.6
17	<0.3	<5	17	6.8
18	<0.3	<5	82	>8.0
19	0.3	<5	<0.4	<0.4
20	0.5	<5	7.0	2.8
21	0.5	<5	9.6	0.8
22	0.6	<5	0.7	<0.4
23	0.7	<5	2.1	0.8
24	0.9	<5	<0.4	<0.4
25	1.1	<5	3.6	1.6
26	1.2	<5	12	4.8
27	1.4	<5	1.3	0.4
28	1.4	<5	3.5	2.8
29	1.5	<5	1.0	<0.4
30	1.6	<5	<0.4	<0.4
31	2.0	<5	0.7	0.4
32	2.1	<5	<0.4	<0.4
33	2.4	6	<0.4	Not Done
34	2.8	<5	<0.4	0.4
35	3.6	<5	0.8	<0.4
36	3.9	20	<0.4	Not Done
37	4.3	<5	0.8	1.6

38	4.4	12	<0.4	Not Done
39	4.4	15	<0.4	Not Done
40	4.7	<5	13	4.8
41	5.6	<5	<0.4	<0.4
42	5.6	21	<0.4	Not Done
43	6.0	<5	1.4	0.4
44	7.2	8	<0.4	Not Done
45	8.8	22	<0.4	Not Done
46	9.6	<5	0.5	0.4
47	14	20	<0.4	Not Done
48	15	30	<0.4	Not Done
49	16	20	<0.4	Not Done
50	18	<5	2.2	1.0
51	20	29	<0.4	Not Done
52	20	23	<0.4	Not Done
53	20	27	<0.4	Not Done
54	22	8	1.1	Not Done

Subjects included in this table had ADAMTS13 activity in citrated plasma samples <20% when assayed with either FRETS-VWF73 or FRETS-rVWF71. For highest sensitivity, plasma samples with ADAMTS13 activity <3% were assayed with FRETS-rVWF71 using 100 µL of plasma per assay.

Table S2. Comparison of ADAMTS13 activity assays for Li^+-heparin plasma samples from patients with TTP

	ADAMTS13 Activity	
Enrolled Patient (Sample)	**Substrate FRETS-rVWF71**	**Substrate FRETS-VWF73**
1	3	<5
2	0	<5
3 (1)	5.2	<5
3 (2)	27.2	30.5
4 (1)	0	<5
4 (2)	47.1	51.3
5 (1)	1.1	<5
5 (2)	2.3	<5
6	5.6	<5
7 (1)	0.5	<5
7 (2)	16.8	12.1
7 (3)	34.5	36
8	3.8	<5
9	1	<5

Li^+-heparin plasma samples were obtained from patients with TTP enrolled in a clinical study at Washington University in St. Louis. Numbers in parentheses indicate samples obtained at distinct time points during the course of treatment. Assays with substrate FRETS-rVWF71 were performed using the standard method described under “Materials and Methods.” Assays with substrate FRETS-VWF73 were performed as described by Kokame et al (7). Similar results were obtained with both assay methods.