

Supplementary Material

Development of a fluorogenic ADAMTS-7 substrate

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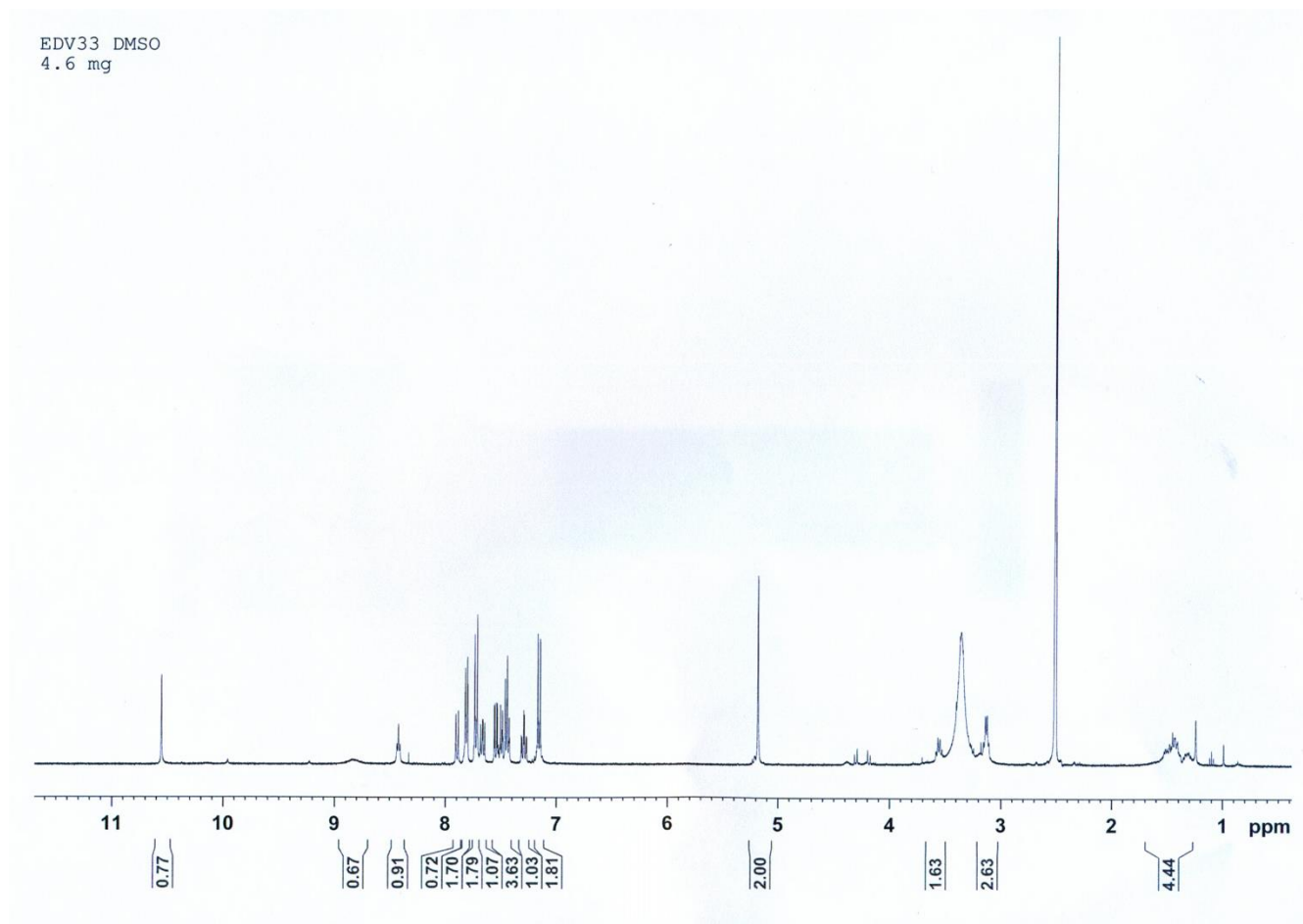
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Supplementary Table 1: Parameters used to calculate $K_{i\text{ app}}$ values using Cheng-Prusoff equation.

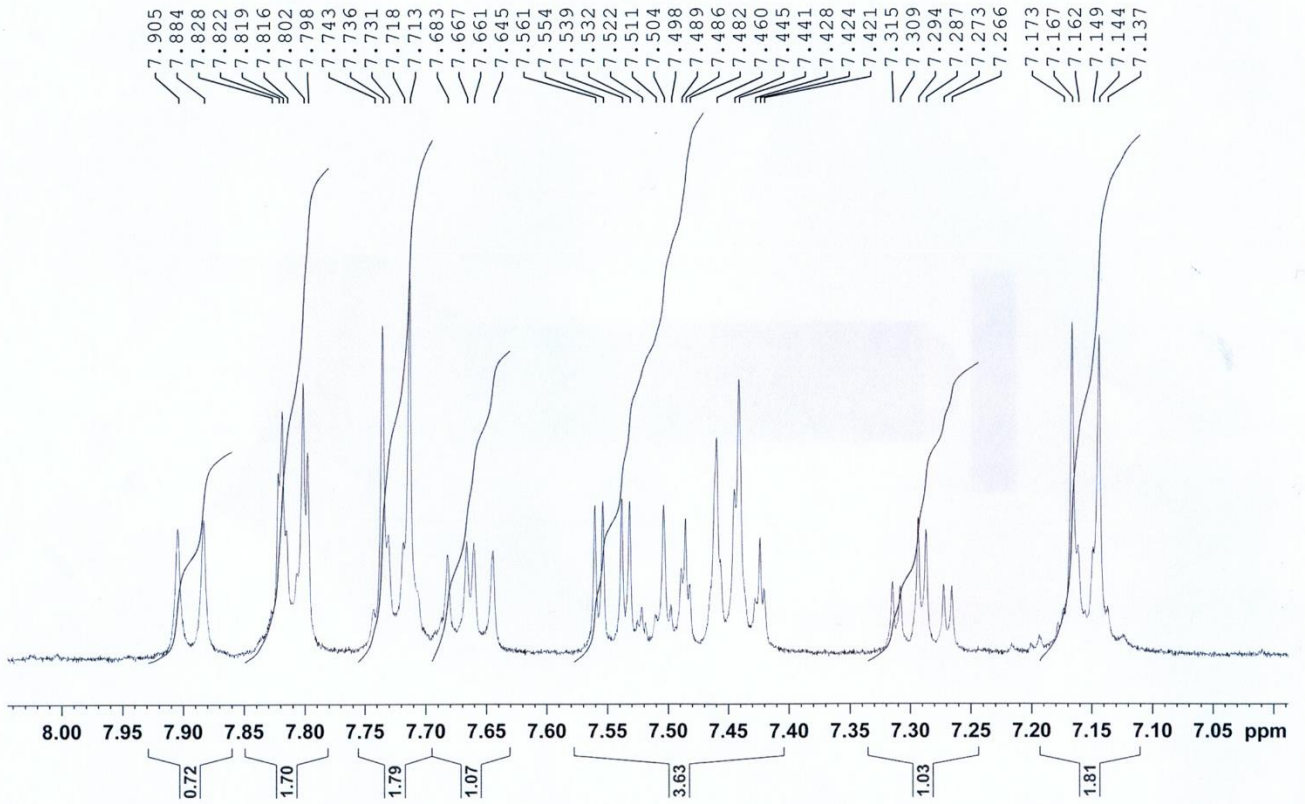
Substrate and K_m values are in micromolar. Abz, 2-aminobenzoic acid; Dpa, N-3-(2,4-dinitrophenyl)-L-2,3-diaminopropionyl diaminopropionic amide; Fam, 5,6-carboxyfluorescein; Mca, 7-methoxycoumarin-4-acetyl, Tamra, carboxytetramethylrhodamine.

Enzyme	Substrate	[S]	K_m	Reference
ADAMTS-4	Fam-Ala-Glu-Leu-Gln-Gly-Arg-Pro-Ile-Ser-Ile-Ala-Lys-Tamra	0.5	23	1
ADAMTS-5	Abz-Thr-Glu-Ser-Glu-Ser-Arg-Gly-Ala-Ile-Tyr-Dpa-Lys-Lys	40	76	2
MMP-2	Mca-Lys-Pro-Leu-Gly-Leu-Dap(Dnp)-Ala-Arg-NH ₂	2	>30	3
MMP-12	Mca-Lys-Pro-Leu-Gly-Leu-Dap(Dnp)-Ala-Arg-NH ₂	2	130	4
ADAM-17	Mca-Lys-Pro-Leu-Gly-Leu-Dap(Dnp)-Ala-Arg-NH ₂	2	26	3

Supplementary Figure 1: ^1H NMR of Compound EDV33 (400 MHz, $\text{DMSO-}d_6$).

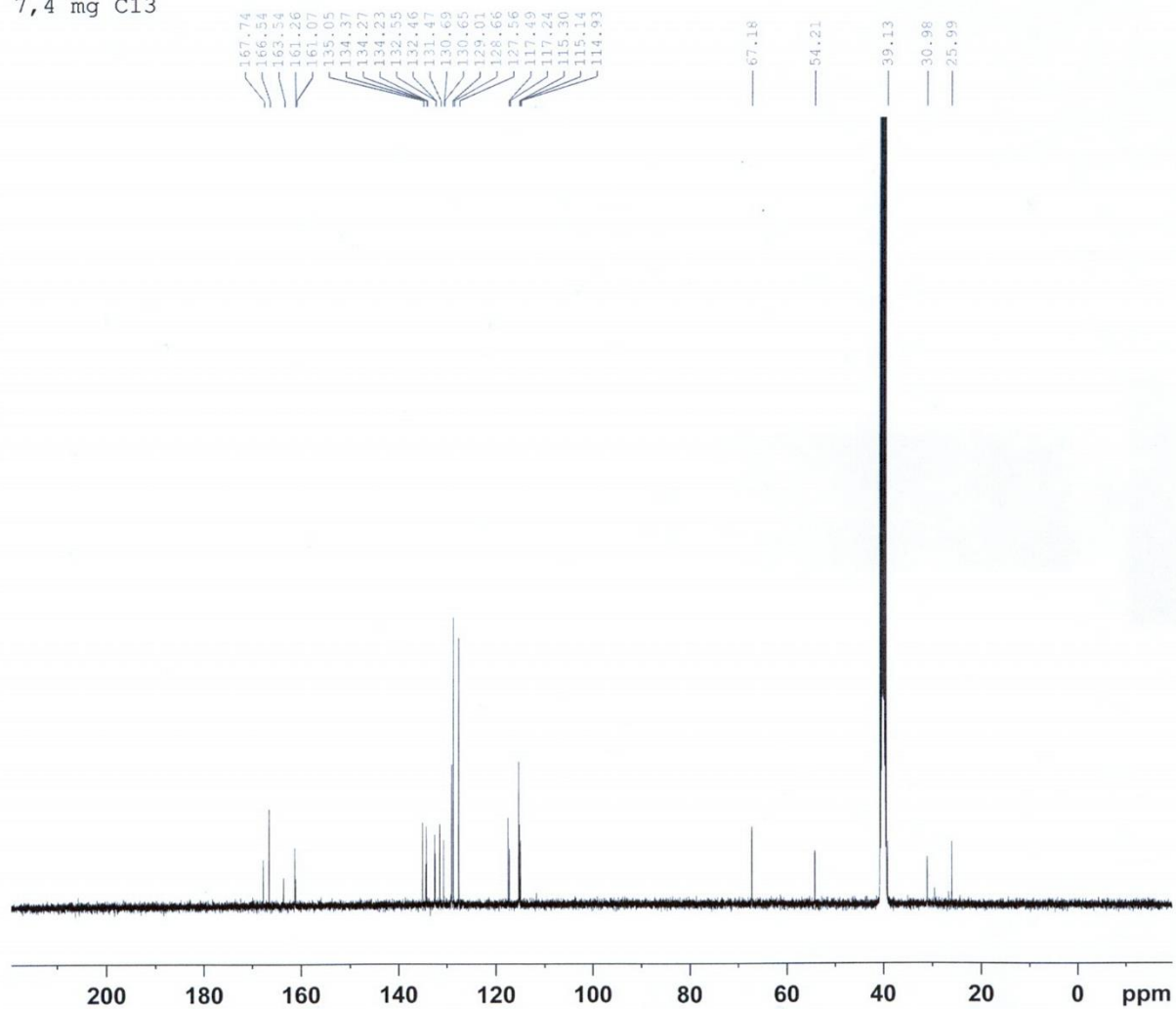


EDV33 DMSO
4.6 mg



Supplementary Figure 2: ^{13}C NMR of Compound EDV33 (100 MHz, DMSO- d_6).

EDV33 DMSO
7,4 mg C13



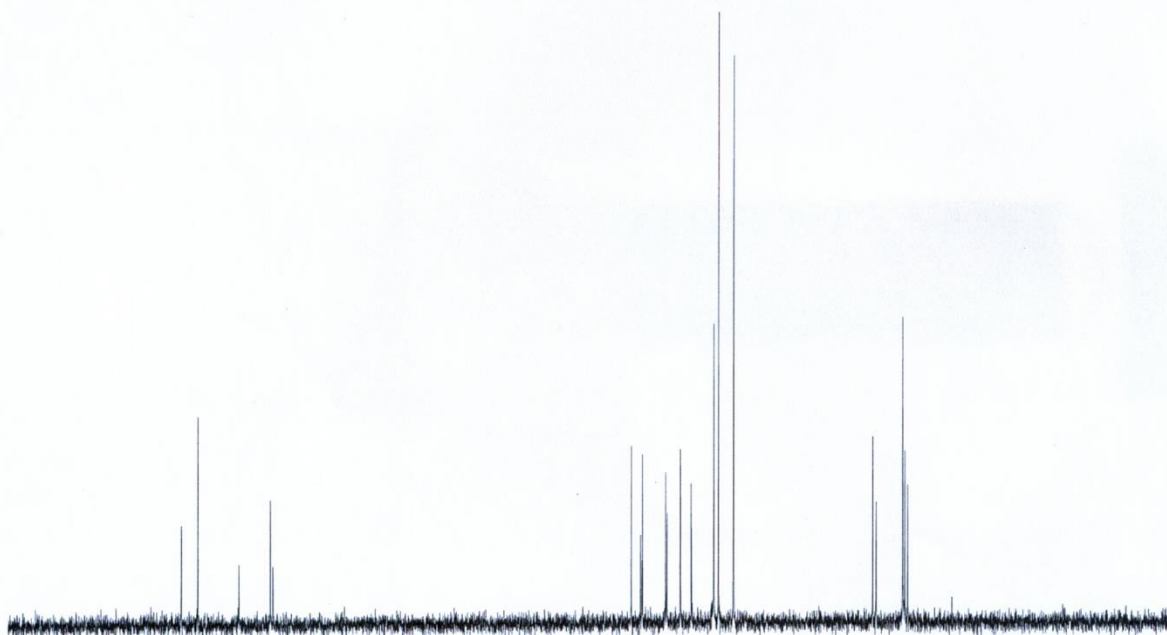
EDV33 DMSO
7,4 mg C13

167.74
166.54

163.54
161.26
161.07

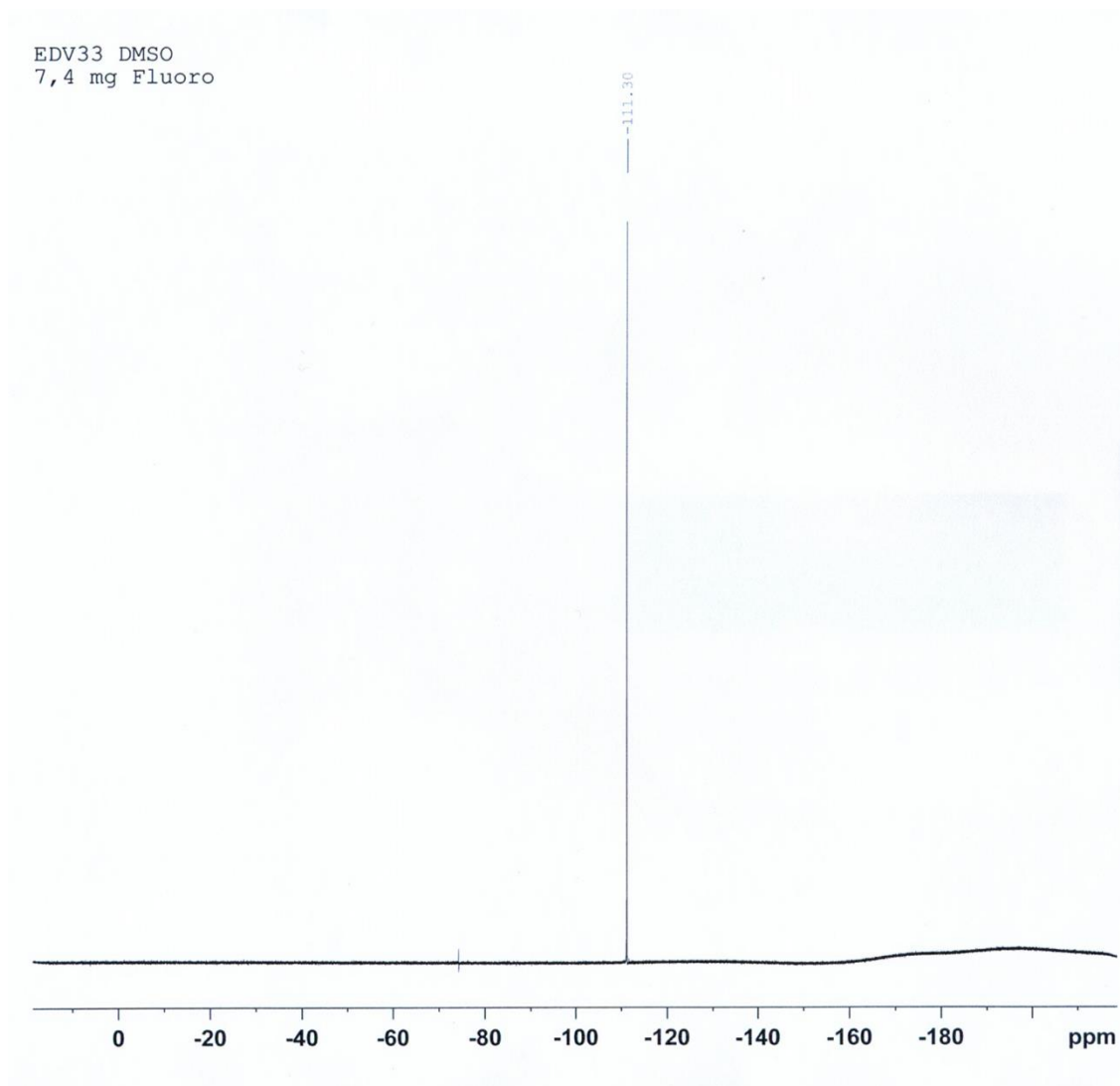
135.05
134.37
134.27
134.23
132.55
132.46
131.47
130.69
130.65
129.01
128.66
127.56

117.49
117.24
115.30
115.14
114.93

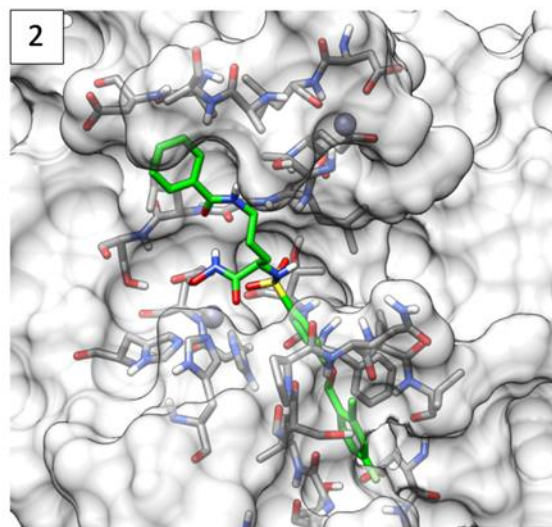
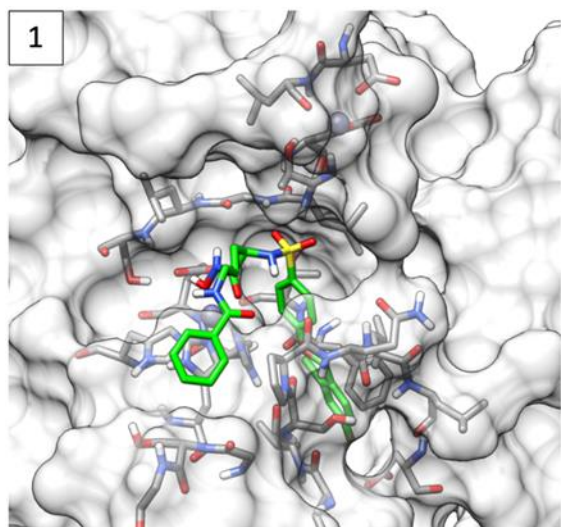


175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 ppm

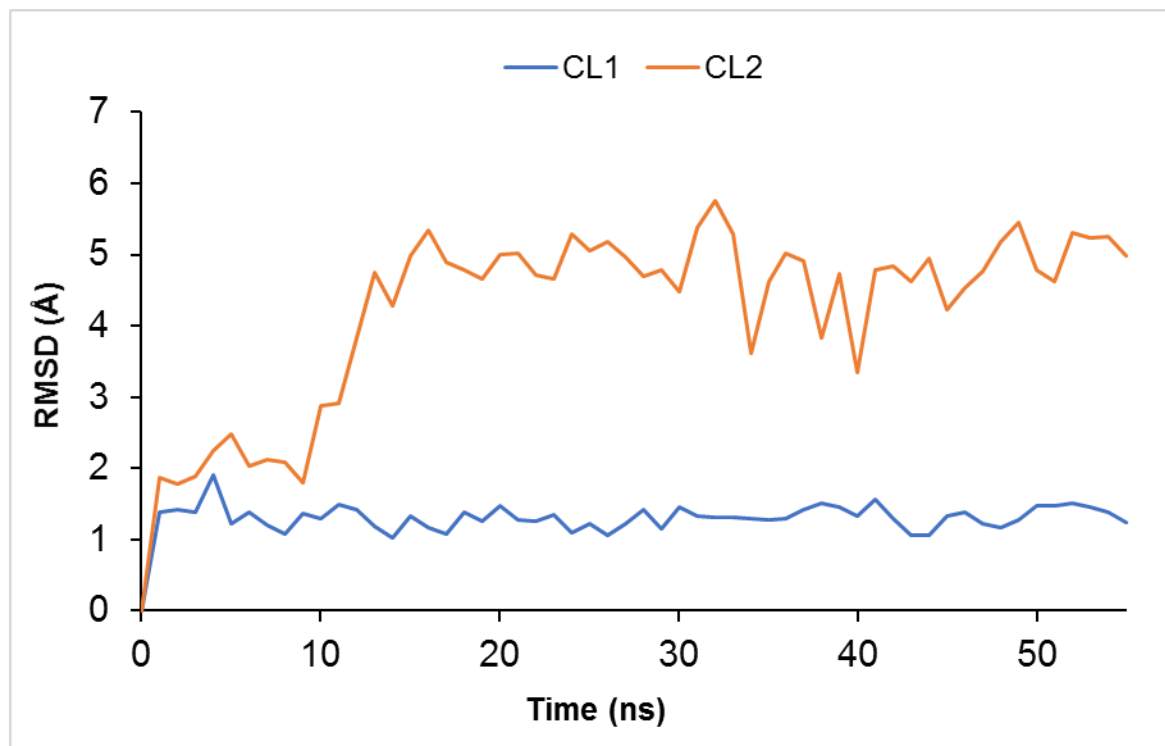
Supplementary Figure 3: ^{19}F NMR of Compound EDV33 (376 MHz, DMSO- d_6).



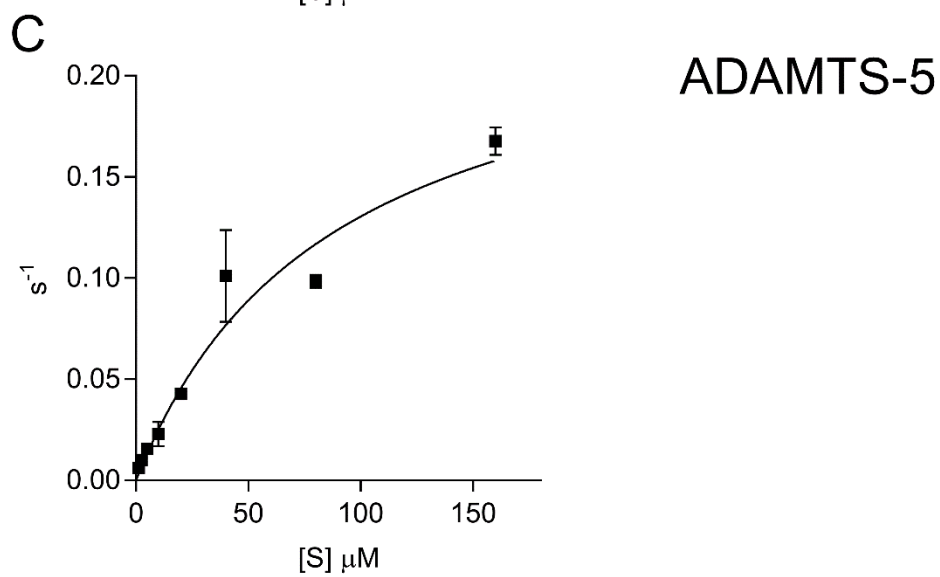
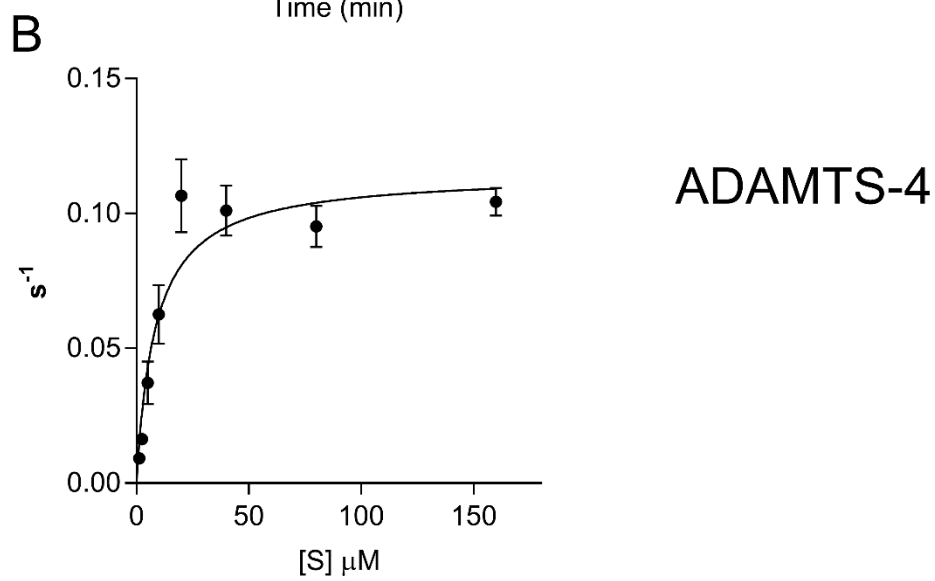
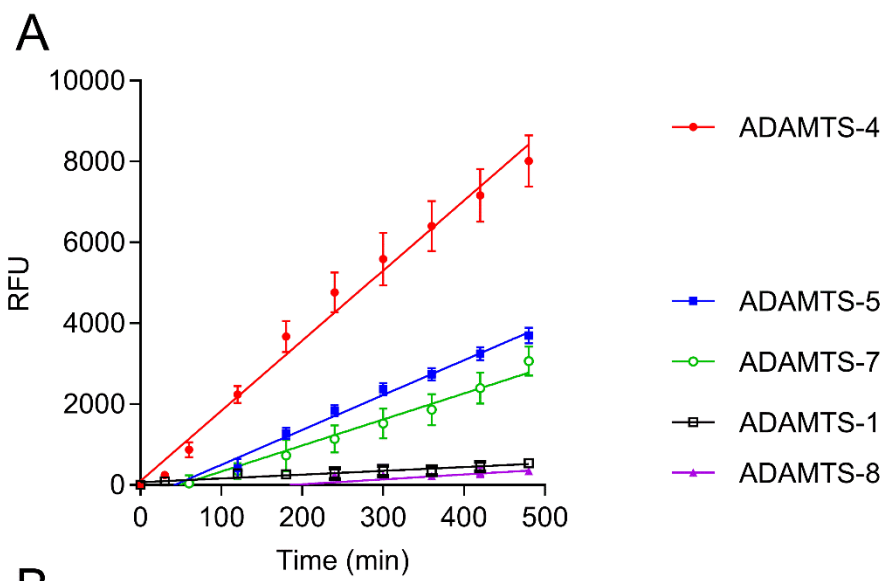
Supplementary Figure 4 – Molecular docking results for compound EDV33 bound to ADAMTS-7 (clusters 1 and 2). EDV33 was docked into 50 ADAMTS-7 protein conformations using the GOLD software with four different fitness scoring functions (GoldScore, ChemScore, ASP and ChemPLP). The best ranked docking solution from each docking calculation was considered and two clusters with a population of at least 50 poses (RMSD of 2.0Å as a threshold) were retained.



Supplementary Figure 5 – Analysis of MD simulation for compound EDV33 bound to ADAMTS-7. The plot shows the RMSD of the heavy atoms of the ligand (cluster 1 in blue and cluster 2 in orange) from the starting model structure during the simulation.



Supplementary Figure 6: Cleavage of ATS7FP7 by members of the ADAMTS family. **A)** ATS7FP7 (40 μ M) was incubated either with ADAMTS-1, -4, -5, -7, or -8 (10 nM). Fluorescence was detected ($\lambda_{\text{ex}} = 485$ nm, $\lambda_{\text{em}} = 520$ nm) for 8 h at 37°C and reported as relative fluorescence units (RFU). The data are presented as average \pm SEM (n=3) and were fitted with a linear regression. **B, C)** Cleavage of FRET peptide ATS7FP7 by ADAMTS-4 (**B**) or ADAMTS-5 (**C**) (10 nM each). Data were fitted to the Michaelis-Menten equation and are presented as average \pm SEM (n=3).



Supplementary References

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