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Supplementary Document

Aptamer-Based Biotherapeutic Conjugate for Shear Responsive Release of Von Willebrand Factor A1 Domain

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Electrophoresis for validation of molecular conjugation

To validate the aptamer-SpyTag conjugation, the conjugate was mixed with i-27 SpyCatcher (32.3 kDa) in a 1:1 molar ratio of conjugate to SpyCatcher. This mixture was incubated either for 2 hours or overnight at room temperature. Subsequently, unreacted aptamer-SpyTag was filtered using Slide-A-Lyzer MINI Dialysis devices with either 7k or 10k MWCO membranes. The filtered solutions were then tested by electrophoresis using a Tris-borate-EDTA (TBE) gel (Bio-Rad) and stained with the SYBR DNA stain (ThermoFisher). Fig. S1A shows aptamer (75 bases) effectively bound to SpyCatcher through the specific interaction between SpyTag (1.5 kDa) and SpyCatcher, thereby confirming successful conjugation. Overnight incubation and a 7k filtration membrane were employed to prepare the aptamer-SpyTag conjugate for the optical tweezers experiments. These conditions were chosen because they resulted in the lowest amount of unreacted aptamer-SpyTag, as illustrated in Fig. S1A, bands II. Similarly, the conjugation of DNA-handle with SpyCatcher was confirmed by TBE gel electrophoresis (band I in the middle lane of Fig. S1B.

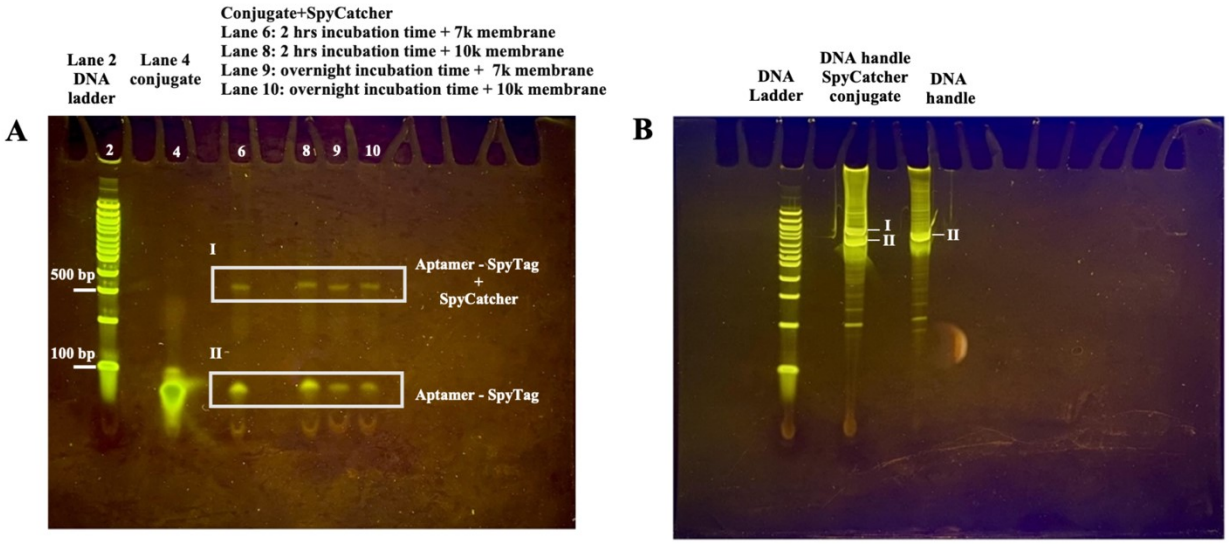

Figure S1. Gel electrophoresis to confirm the conjugation results of the singular tether structure used in the optical tweezers experiments. **(A)** Aptamer-SpyTag conjugate was mixed with i-27 SpyCatcher and run through a TBE gel to validate the conjugation results. The DNA-stained gel showed high molecular weight bands I that migrated at a slower rate through the gel, indicating successful binding of the aptamer to SpyCatcher via the SpyTag reaction. Bands II corresponded to unreacted aptamer, observed as lower molecular weight bands. **(B)** Electrophoresis results were utilized to validate the DNA handle-SpyCatcher conjugation. Upon comparison between the control (Biotin DNA handle) and the conjugate (Biotin DNA handle + SpyCatcher), it was observed that band I, which exhibits a higher molecular weight, corresponds to the DNA handle-SpyCatcher conjugate, while band II represents unreacted DNA handle.

Binding affinity between aptamer ARC1172 and the VWF A1 domain

Employing the same technique described in the unfolding experiments, the rupture force between aptamer ARC 1172 and VWF A1 was characterized under a constant loading rate as an indicator of the binding affinity between the two biomolecules. The aptamer was conjugated onto streptavidin-coated beads as described in the paper. Parallelly, VWF A1 (a kind gift from Dr. Renhao Li at Emory University) containing VWF A1 residues 1238-1493 and the C-terminal SpyTag (AHIVMVDAYKPTK) peptide[1] was reacted with SpyCatcher beads for 15 minutes in Tris-buffered saline (20mM Tris, 150mM NaCl, pH 7.5). To test the binding affinity between the aptamer ARC1172 and VWF A1 domain, the single tether structure (Fig S2A**)** was formed in Tris-buffered saline in the optical tweezers setup to characterize the rupture force between the two molecules under a constant loading rate. One aptamer-coated microbead was fixed by a micropipette, and one A1-coated bead was controlled by a laser trap. By moving the laser trap towards the micropipette, the aptamer and A1 domain came into contact and interacted with each other. Pulling the two beads away from each other, following the aptamer-A1 bond formation, applied an increasing external force on the tether system until the interaction between the two molecules broke. The recorded rupture force encompassed information about the binding energy between the aptamer and A1. For the curve shown in Fig S2B, the microbeads were held together for 3 s and pulled apart at a speed of 50 nm/s. The unbinding force at the rupture point is 37.46 pN. Using a similar method, over 700 binding rupture events were captured under different pulling speeds from 50 to 500 nm/s. The Bell Evans fit[2] was then performed (Eq. 4), as shown in Fig S2C, which has been used to describe the influence of an external force on the rate of bond dissociation. In this case, f* and R_f are the rupture force and loading rate, respectively. The fitting results yielded a dissociation rate in the absence of force k_u^0 of 0.0089 ± 0.0052 s^{-1}. This indicates that folded ARC1172 and the A1 domain have high affinity, which is consistent with the literature[3] and ensures low spontaneous release of A1 unless the aptamer is unfolded.

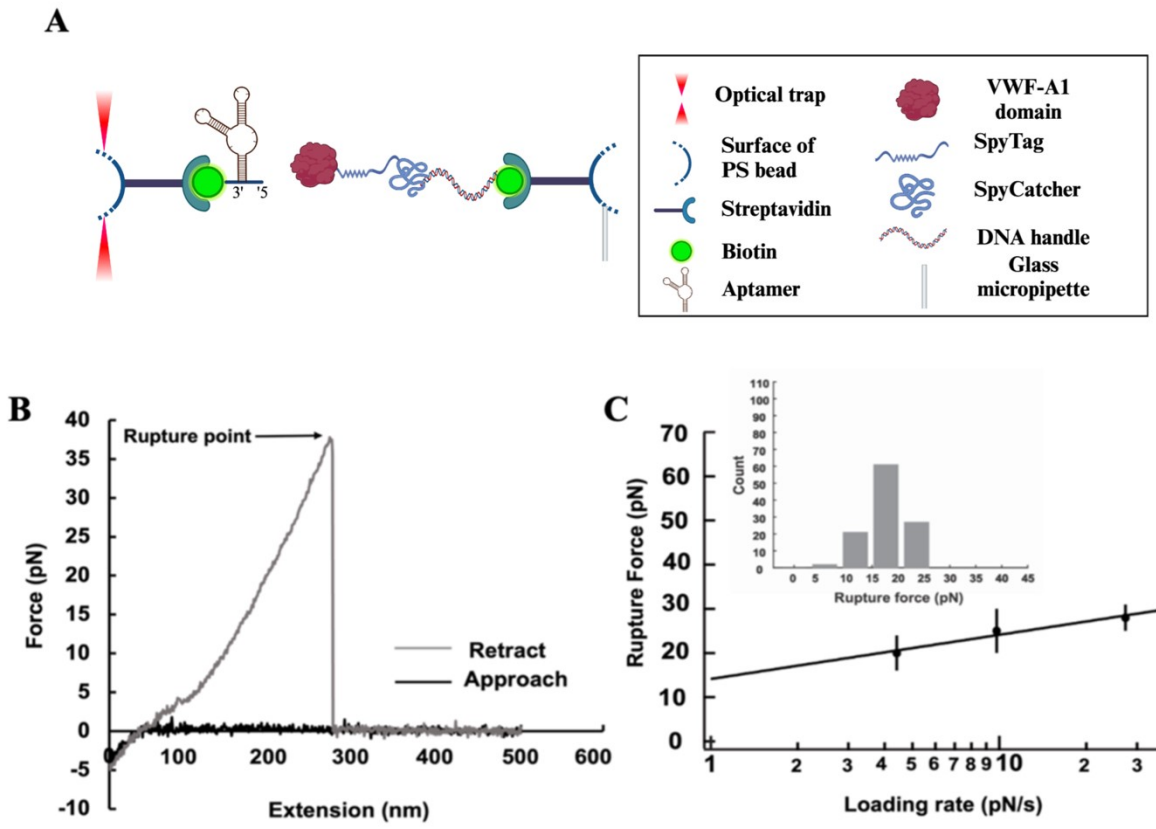

Figure S2. Binding test of aptamer ARC1172 and VWF A1 domain using optical tweezers. **(A)** Singular tether structure employed in the optical tweezers chamber to test the binding affinity between aptamer and A1. **(B)** A typical force vs. extension curve of the pulling experiment in the optical tweezers. The pulling speed is 50 nm/s and the loading rate is 23.67 pN/s. **(C)** Bell-Evans fit of the binding test results. Rupture forces were binned by the loading rate and plotted as histograms (inset). The peak of each histogram was plotted against the loading rate. Over 700 pulling curves are collected for the fitting.

COMSOL Multiphysics simulation of the hydrodynamic force on a bead in a microfluidic channel

The estimation of hydrodynamic forces on an immobilized microsphere under flow was conducted using COMSOL Multiphysics simulation. Microfluidic channel dimensions used in the experiment were replicated in the simulation and a 1-micrometer bead was positioned on the channel wall. The simulation was performed using the single-phase creeping flow module under the steady-state condition. Water was used as the fluid in the simulation and no-slip boundary condition was applied on the wall. The Inlet condition varied with flow rates ranging from 20 to 100 µL/min. The hydrodynamic force on the bead was calculated by including both normal and shear stress components.[4] Subsequently, Eq. 7 in the paper was used to convert the force on the bead to force on the aptamer containing tether. Finally, Eq. 6 was used to calculate the aptamer unfolded fraction as a function of the applied force on the tether. The results are summarized in Table. S1.

Table S1. Simulated hydrodynamic force on beads under different flow rates and the corresponding pulling force on the aptamer tether.

Flow rate (μL/min)	Force on bead F_s(pN)	Force on 45 nm Tether F_b(pN)	Aptamer unfolded fraction	Maximum shear rate on the bead (1/s)
20	0.67	1.68	0.040	1405.2
30	0.99	2.48	0.074	2107.8
40	1.24	3.10	0.131	2810.4
50	1.62	4.05	0.331	3512.9
60	1.99	4.98	0.672	4215.5
70	2.25	5.63	0.864	4918.1
80	2.58	6.45	0.969	5620.7
90	2.91	7.28	0.995	6323.3
100	3.22	8.05	0.999	7025.9

Evaluation of shear force dynamics in other biofluids using COMSOL simulations

To understand the influence of viscosity on the total force experienced by the aptamer, we varied solution viscosity in COMSOL simulations to represent biofluids such as cell culture medium with 10% FBS and blood (Table S2). Simulations results showed that transitioning from the experimental buffer (viscosity ~1 cP), where the total force on the tether was 4.98 pN at a flow rate of 60 µL/min, to more complex fluids, such as cell culture media with 10% FBS (viscosity ~1.13 cP[5]), resulted in an increase in the total force to 5.60 pN at the same flow rate. Using viscosity corresponding to blood of 4.5 cP[6] resulted in a total force of 22.25 pN at the same flow rate. Thus, the total force acting on the tether scales linearly with the viscosity at a given flow rate.

Table S2. Simulated hydrodynamic force on beads and corresponding pulling force on the aptamer tether at varying viscosities and constant flow rate.

Fluid	Viscosity (cP)	Force on bead F_s (pN)	Force on tether F_b (pN)	Aptamer unfolded fraction
In house flow buffer	1.00	1.99	4.98	0.67
10% FBS culture medium	1.13	2.24	5.60	0.86
Blood	4.5	8.90	22.25	1

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