Electronic Supplementary Information

DNA aptamer-based carrier for loading proteins and enhancing the enzymatic activity

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Materials and methods

Preparation of circular DNA for TBA microparticles

All the oligonucleotides used in the study were purchased from the Integrated DNA Technologies (IDT, USA) (Table S1). To prepare the circular DNA, phosphorylated linear ssDNAs that have complementary sequence to the aptamers bind to fibrinogen-binding exosite and heparin-binding exosite were designed and the primer ssDNAs were designed to contain two complementary regions to each end of the linear ssDNAs respectively. For hybridization, equal concentration (3 μM) of the each linear ssDNA and primer ssDNA were mixed in nuclease-free water respectively. Next, the mixed solution was heated to 95 °C for 2 min and cooled gradually to 25 °C for 1 h. To connect the nick in the circular DNA, the solution was incubated overnight at room temperature with T4 DNA ligase (0.03 U/μL, Promega, USA) and ligase buffer (300 mM Tris-HCl (pH 7.8), 100 mM MgCl2, 100 mM dithiothreitol and 10 mM adenosine triphosphate).

Table S1. DNA sequences for generating DNA Particles

strand	DNA sequences
linear DNA for F-TBA	5’ - /Phosphate/ AGG GAT ATG CCT CTA GAT AGC GAT TAC CAT CAA CAT AGA GAA ACC AAC CAC ACC AAC CAA AGA AAT GAT TAC CAT CAA CAT AAT GTC ACT AT - 3’
primer DNA for F-TBA	5’ - CTA GAG GCA TAT CCC TAT AGT G - 3’
linear DNA for H-TBA	5’ - /Phosphate/ ATA GTG ACA TTA TGT TGA TGG TAA GTC ACC CCA ACC TGC CCT ACC ACG GAC TCT CTA TGT TGA TGG TAA TCG CTA TCT AGA GGC ATA TCC CT - 3’
primer DNA for H-TBA	5’- AAC ATA ATG TCA CTA TAG GGA T - 3’
linear DNA for scramble DNA particle	5’ - /Phosphate/ CAA CTG TAG TGT GTT CAC GGT GCT GTA CTC ACT ATT TCG ACC GGC TCG GAG AAG AGA TGC ACT GAC AAG ACG TCA TAT CAA GTG TAT GGC AA - 3’
primer DNA for scramble DNA particle	5’ - CAC TAC AGT TGT TGC CAT ACA C - 3’

Synthesis of TBA microparticles

To synthesize the TBA microparticle, the prepared circulars were mixed with Φ29 DNA polymerase (1 U/μL, Lucigen, USA), deoxyribonucleotide triphosphate (2 mM) and reaction buffer (40 mM Tris-HCl (pH 7.5), 50 mM KCl, 10 mM $MgCl_2$, 5 mM $(NH_4)_2SO_4$ and 4 mM dithiothreitol), respectively. For the RCA process, the mixed solutions were incubated at 30 °C for 20 h. After brief sonication, the solution was centrifuged at 6000 rpm for 5 min to collect the particles. Nuclease-free water was used for additional washing steps to eliminate the buffer.

Scanning electron microscopy (Hitach, Japan, S-4200) was utilized to analyze the morphology of the particles. Samples were prepared by dropping and drying purified particles onto silicon wafers. The samples were coated with Pt and images were acquired at an accelerating voltage of 15 kV. Sizes of the particles were measured by using image analyzing software, ImageJ (https://imagej.nih.gov/ij/).

Verification of aptamer activity of the TBA microparticles

Thrombin used in the study was purchased from the Sigma-Aldrich (Sigma-Aldrich, USA). To confirm the binding activity of TBA particles, fluorescently labeled TB (TAMRA-TB) was utilized. TAMRA-TB was prepared by using 5-TAMRA protein labelling kit (AnaSpec, USA). F-TBA and H-TBA particles were mixed with excess TAMRA-TB and 5mM of KCl. For the control experiment, scrambled particles without aptamer sequence were used with the same conditions. Then, the mixed solutions were incubated at room temperature for 3 h. After binding reaction, the solutions were centrifuged at 6000 rpm for 5 min. Each samples were then washed with nuclease free water for several times to remove residual thrombin and KCl. The fluorescence intensities of the particles were measured using image cytometry analysis. The particles were stained with DAPI (Sigma-Aldrich, USA) and the solution was deposited on NC-Slide A2 (Chemometec, Denmark). To confirm the fluorescence of the TAMRA-TB conjugated particles, one-color analysis was performed using a Nucleo Counter (Chemometec, NC-3000) and the results were analyzed using NucleoViews NC-3000 software (Chemometec). The average intensities and standard deviations of the particles are shown in Figure S1.

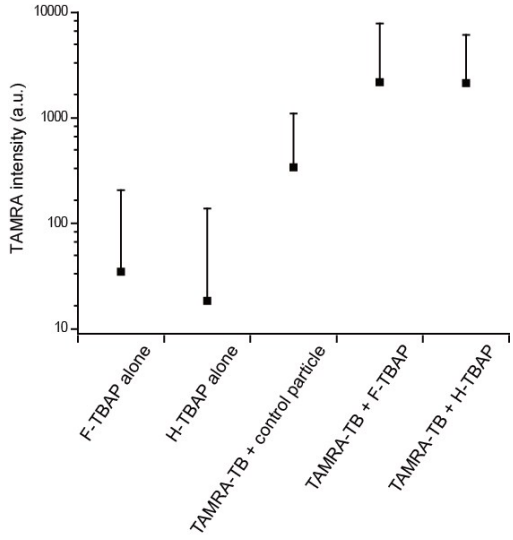

Figure S1. The average fluorescence intensities and standard deviations of TAMRA-TB conjugated particles.

Morphological changes of the particles after binding with TB

To monitor the morphological changes of the particles after binding with TB, both TBAPs were mixed with excess TB with the same conditions as described as above. In addition, to verify the effect of buffer, F-TBA and H-TBA particles were mixed with 5 mM of KCl without TB. After incubation at room temperature for 3 h, the solution was centrifuged at 6000 rpm for 5min to collect the particles. Nuclease-free water was used for additional washing steps to eliminate residual thrombin and KCl. Then, the morphological change of the particles was analyzed utilizing Scanning electron microscopy (Hitach). Samples were prepared by dropping and drying purified particles onto silicon wafers. The samples were coated with Pt and images were acquired at an accelerating voltage of 15 kV. ImageJ was utilized to compare the sizes of particles.

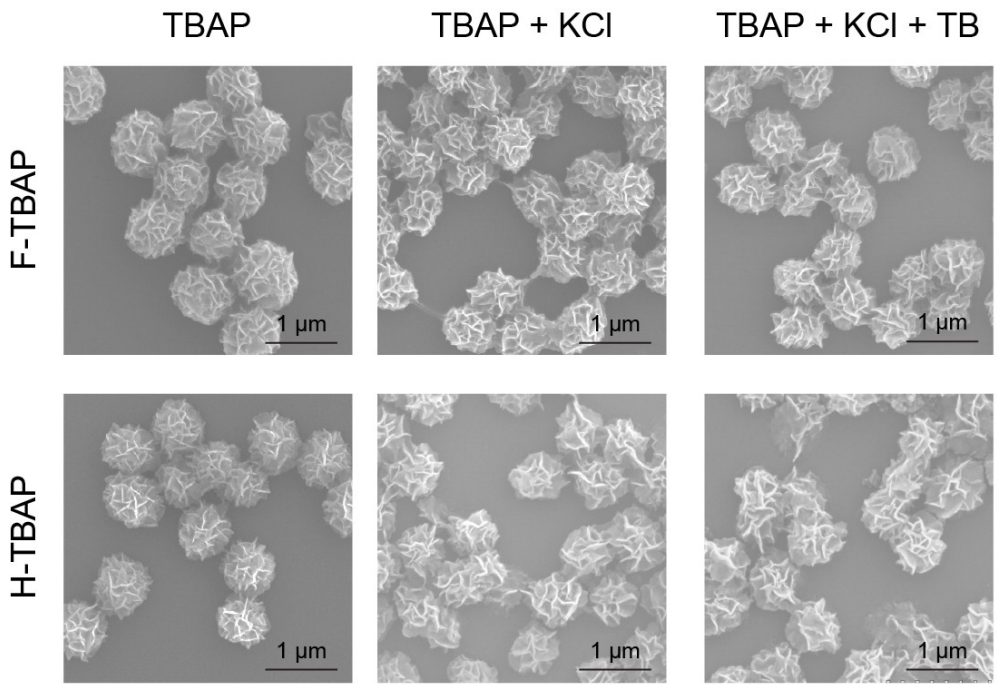

Figure S2. SEM images of TBAP incubated with TB and KCl or KCl only. F-TBAPs (top) and H-TBAPs (bottom).

Calculation of the number of aptamer sites

$$TBAP\left(274.6\frac{ng}{\mu l}\right) = 274.6\frac{ng}{\mu l} * \frac{base}{330\ daltons} * \frac{1}{92\ base} * \frac{mol}{g} = 9.04\ pmol/\mu l$$

$$= 9.04\frac{pmol}{\mu l} * 6.02 * 10^{23}\frac{copies}{mol} = 5.44 * 10^{12} copies/\mu l$$

Thrombin activity assay

To optimize loading conditions, various volume ratios between TBAPs (275 ng/μL) and TB (100 ng/μL) were tested (from 1:1 to 32:1). To confirm the activity of TB-TBAPs to cleave fibrinogen, thrombin activity assay kit (Anaspec) was utilized. The assay was performed under the same final concentration of samples assuming 100% binding and same concentration

of TB was used as thrombin control (final concentration of TB was 0.4 ng/μL). To confirm the activities for various concentration, 0.016 ng/μl to 2 ng/μl of TB and TB-TBAPs (TBAP:TB = 16:1) were tested. The thrombin activity assay was performed at 37 °C for 1 h and fluorescence intensities of the samples were measured by using platereader (BioTek, USA, Synergy HT).

Loading efficiency of thrombin in TBAPs

To measure the loading efficiency of thrombin in TBAPs, TAMRA-TB was utilized with the same condition as thrombin activity assay. TAMRA-TB (100 ng/μL) was incubated with various volume of H-TBAPs (275 ng/μL) (volume ratio from 1:1 to 32:1). After incubation, the solution was centrifuged at 6000 rpm for 5min. Supernatant liquid was then collected and the fluorescence intensity of was measured by using platereader (BioTek, Synergy HT). Intensities of the supernatant liquids decreased as the TAMRA-TB was loaded in TBAP and the loading efficiency could be calculated (see Figure S3 and Table S2). Thrombin mass per unit particle mass was also shown in Table S2.

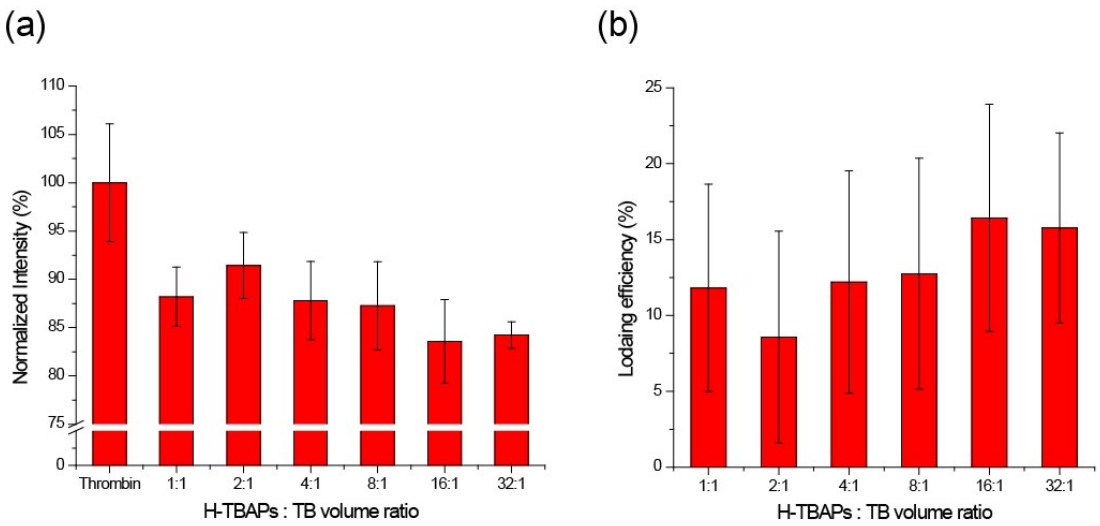

Figure S3. (a) The fluorescence intensities of the supernatant liquids from various H-TBAP:TB volume ratio. (b) The loading efficiency of thrombin in the DNA particles.

Table S2. Thrombin loading efficiency and loading amount.

H-TBAP:TB volume ratio	Thrombin loading efficiency		Thrombin loading amount (ng)		Thrombin mass per TBAP mass (ng/ TBAP 1 ng)
	mean	SD	mean	SD	mean
1:1	11.81	6.83	17.72	10.25	4.30
2:1	8.57	6.99	12.86	10.48	1.56
4:1	12.20	7.33	18.30	10.99	1.11
8:1	12.75	7.62	19.12	11.42	0.58
16:1	16.43	7.49	24.64	11.23	0.37
32:1	15.77	6.25	23.65	9.38	0.18