**Electronic Supplementary Information**

# Programmable protein-protein conjugation via DNA-based self-assembly

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## Experimental section

### Materials

The oligonucleotides were obtained from Genenet Co. Ltd. (Fukuoka, Japan). Thrombin generation chromogenic substrate (β-Ala-Gly-Arg *p*-nitroanilide diacetate) was purchased from Sigma-Aldrich (St Louis, MO). Thrombin from human plasma, high activity was purchased from Merck Co., Bis (sulfosuccinimidyl) suberate (BS3) was purchased from Thermo Scientific Pierce Protein Research Products (Rockville, IL).

**Table S1.** DNA sequences used in the study.

| Name | Length | Sequence |
|---|---|---|
| template DNA | 42 mer | 5'- GCT GAG TCT GCG TTG GTA CGC GGT GTT TCC AGG CAG CGA GTT -3' |
| A1 | 32 mer | 5'- *CGC AGA CTC AGC* TTT TTG GTT GGT GTG GTT GG -3' |
| A2 | 32 mer | 5'- *ACA CCG CGT ACC* TTT TTG GTT GGT GTG GTT GG -3' |
| A2 comp | 12 mer | 5'- *ACA CCG CGT ACC* -3' |
| A3 | 32 mer | 5'- *CTC GCT GCC TGG* TTT TTG GTT GGT GTG GTT GG -3' |
| Comp apt | 15 mer | 5' – CCA ACC ACA CCA ACC – 3' |

* Italic: hybridization sequence, underline: thrombin-binding aptamer sequence

**Preparation of DNA-thrombin complex**

For the preparation of the DNA scaffold, oligonucleotides (template, A1, A2, and A3) (1 μM) were mixed with a buffer (20 mM HEPES, 100 mM NaCl, 5 mM KCl, 5 mM $MgCl_2$, pH 7.9). The mixture was heated to 95 °C, incubated for 3 min, and cooled slowly to 4 °C at a decrease rate of -1.0 °C/min. The DNA scaffolds (1.5 μM) were mixed with thrombin (4.5 μM) and incubated for 1 h at room temperature. After adding 6 × loading buffer (Takara Bio Inc.), the samples were loaded on a 10% native polyacrylamide gel in $TBE^+$ buffer (89 mM Tris-HCl, 89 mM boric acid, 2 mM EDTA, 5 mM $MgCl_2$ and 5 mM KCl). 10 bp DNA ladder (invitrogen) was used as a molecular marker. Electrophoresis was performed for 150 min at 4 °C at a constant 42 mA. After the electrophoresis, the gel was stained with SYBR Green II (Takara Bio Inc.) for 30 min. The gel was scanned using a transilluminator at 254 nm.

**DNA-templated covalent cross-linking of thrombin**

The DNA scaffolds (1.5 μM) were mixed with thrombin (4.5 μM) in a buffer solution as described above. BS3 was added in 50-fold molar excess of thrombin to the DNA-thrombin complex solutions for the covalent cross-linking of thrombin. After incubation for 1 h at room temperature, Tris-HCl buffer (final concentration; 100 mM, pH 8.0) was added to stop the cross-linking reaction. To analyze the linkage of thrombin, the samples were loaded on a 7.5% non-reducing polyacrylamide gel. Electrophoresis was performed for 30 min at a constant 42 mA. The gels were stained using a silver stain kit (Wako).

*Confirmation of the multi-linking thrombin by MALDI-TOF-MS*

After the DNA-templated cross-linkage of thrombin as described above, DNase I (2 U) was added to the reaction mixture. After the incubation for 1 h at room temperature, the samples were preconcentrated and separated from the DNase I and non-linked thrombin using Amicon® Ultra Centrifugal Filters (50kDa cutoff, Millipore). Then, the samples were concentrated, deionized using Zip Tip C4 (Millipore), and confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (AutoflexIII, Bruker) using α-cyano-4-hydroxycinnamic acid (CHCA) as a matrix.

*Size-exclusion chromatography of the cross-linked thrombin*

After the DNA-templated cross-linkage of thrombin, a complementary strand of thrombin aptamer (9 μM) was added to the reaction mixture. After the incubation for 1 h at room temperature, DNase I (2 U) was added to the reaction mixture. An hour later, the samples were purified and separated from DNA fragments using Amicon® Ultra Centrifugal Filters (30kDa cutoff) and diluted up to 100 μL with degassed TBS buffer (25 mM Tris-HCl, 137 mM NaCl, 2.68 mM KCl, pH 7.4). The sample solutions, containing thrombin monomer, dimer, and trimer, were applied to the size exclusion

chromatography (SEC) column (Superdex 200 10/ 300 GL, GE Healthcare). SEC was carried out using a BioLogic DuoFlow™ chromatography system and BioFrac™ Fraction Collector (Bio-Rad Laboratories, Inc. Hercules, CA) at a flow rate of 0.25 mL/min. The SEC elution profile at 280 nm was analyzed and Gaussian peak deconvolution of the SEC chart was performed using a software of IGOR Pro 6.0 (Oregon, USA). The peak areas of the cross-linked thrombin were integrated to calculate the yield of each cross-linked thrombin.

**Assay of thrombin activity**

The thrombin activity was measured with a thrombin substrate (0.5mM, thrombin generation chromogenic substrate) in TBS buffer containing 5mM $CaCl_2$ at 405 nm at 37 °C for 10 min.

## Supplementary figures

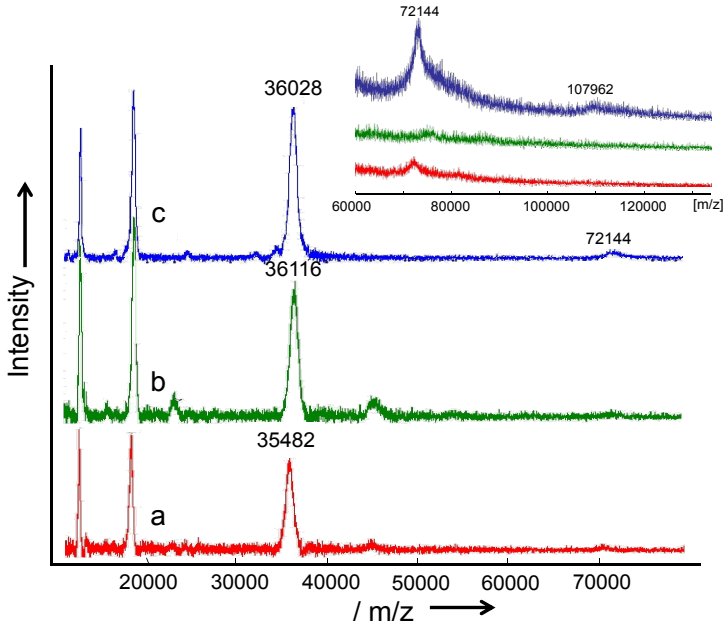


**Fig. S1** MALDI-TOF-MS results of thrombin. (a) thrombin-only without BS3 and DNA scaffold, (b) thrombin with BS3 in the absence of the DNA scaffold, (c) thrombin with BS3 in the presence of the DNA scaffold and purification using 50 kDa MWCO membrane to remove thrombin monomer. All samples were desalted using ZipTip C4.

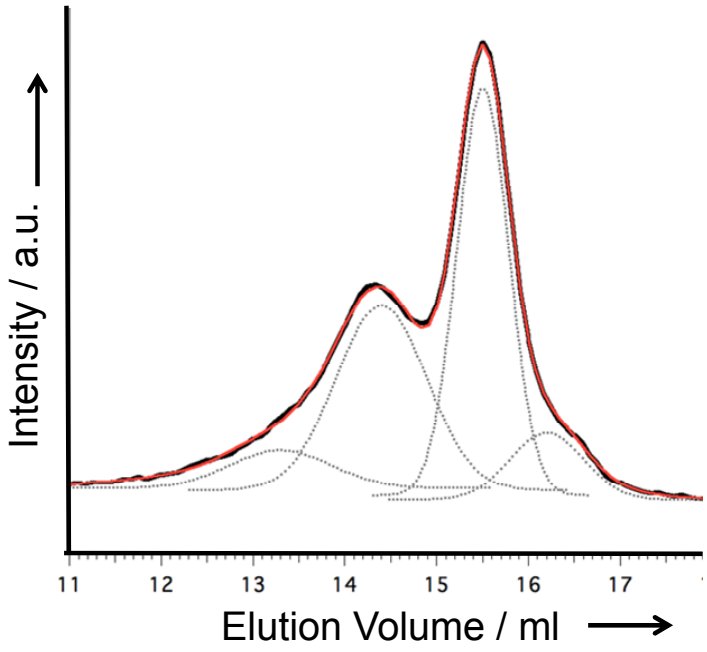


**Fig. S2** Gaussian peak deconvolution of SEC chart of the cross-linked thrombin. The original chart of the cross-linked thrombin represented black line and the resultant fitted curve represented red line. The gray dashed line represents gaussian peaks of the cross-linked thrombin by IGOR Pro 6.0. Each peak was assigned as thrombin monomer or oligomers to calculate their yields.

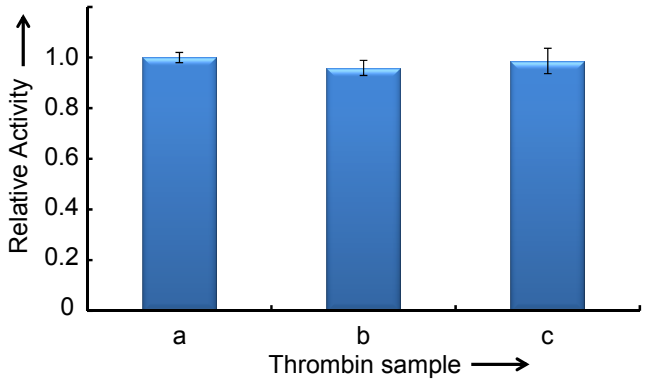


**Fig. S3** Relative enzyme activities of native wild-type thrombin (a), cross-linked thrombin which was prepared in the absence of DNA scaffold (b), cross-linked thrombin which was prepared in the presence of DNA scaffold and purified using 50 kDa MWCO membrane to remove thrombin monomer (c). Each solution contained 0.148 μg/ml thrombin. The relative enzyme activities to the native wild-type thrombin were shown.