Supplementary Information (SI) for Lab on a Chip.

Supplementary Information

Table S1. Comparison between this study, conventional methods, and the Biacore system.

	Conventional	This study	Biacore
Time of one round	several days	<1 day	Not mention
Need of expensive equipment	Yes	No	Yes
Selection processes	Manual	Full automatic	Partial automatic
Shear force control	No	Yes	Yes
Elution process	No	No	Yes

Table S2. Experimental details for each selection round of the on-bench M-mRNA display method for the selection of TRD13. CS=competitive selection (BWB mixed with VHL- and BSA-coated beads, as well as bare beads); D=duration; I=intensity of shear force; NS=negative selection (BWB mixed with BSA-coated beads); POI=protein of interest; PS=positive selection (BWB mixed with KIF2C-coated beads); Temp.=temperature; T=number of iterations; and V=volume.

	NS		CS		PS			Washing				
Round	T	D	T	D	T	D	POI	T	D	I	V	Temp.
1st	-	-	-	-	1	20 min	4 μg	3	1 min	50 rpm	100 μL	25℃
2nd	-	-	-	-	1	20 min	4 μg	3	1 min	50 rpm	100 μL	25℃
3rd	3	5 min	-	-	1	20 min	4 μg	3	1 min	50 rpm	100 μL	25℃
4th	3	5 min	-	-	1	20 min	4 μg	3	10 min	50 rpm	100 μL	25℃
5th	3	5 min	-	-	1	20 min	2 μg	3	10 min	50 rpm	100 μL	25℃
6th	3	5 min	-	-	1	10 min	2 μg	3	10 min	50 rpm	500 μL	25℃
7th	3	5 min	-	-	1	10 min	2 μg	3	10 min	125 rpm	500 μL	37℃
8th	-	-	1	20 min	1	10 min	2 μg	3	10 min	125 rpm	500 μL	37℃
9th	-	-	1	20 min	1	10 min	2 μg	3	10 min	125 rpm	500 μL	37℃
10th	-	-	1	20 min	1	10 min	2 μg	3	10 min	175 rpm	500 μL	37℃

Table S3. Experimental details for each selection round of the on-chip M-mRNA display (on "version 1" chip) for TRD14, 15, and 16. See Table S1 for abbreviations though note that negative, competitive, and positive selection differed (BWB mixed with BSA-coated and bare beads combined; BWB mixed with VHL-, KIF2A-, and BSA-coated beads as well as bare beads; and BWB mixed with KIF2C-coated beads, respectively).

	NS		CS		PS			Washing				
Round	T	D	T	D	T	D	POI	T	D	I	V	Temp.
1st	-	-	-	-	1	20 min	4 μg	3	1 min	26 μN	100 μL	25°C
2nd	-	-	-	-	1	20 min	4 μg	3	1 min	26 μN	100 μL	25°C
3rd	3	5 min	-	-	1	10 min	2 μg	3	3 min	26 μN	100 μL	25°C
4th	-	-	1	20 min	1	10 min	2 μg	5	3 min	26 μN	100 μL	25°C
5th	-	-	1	20 min	1	10 min	2 μg	5	3 min	26 μN	100 μL	25°C
6th	3	5 min	-	-	1	10 min	2 μg	5	3 min	26 μN	100 μL	25°C
7th	3	5 min	-	-	1	10 min	2 μg	5	3 min	26 μN	100 μL	25°C
8th	3	5 min	-	-	1	10 min	2 μg	5	3 min	26 μN	100 μL	25°C
9th	-	-	1	20 min	1	10 min	2 μg	5	3 min	26 μN	100 μL	25°C
10th	-	-	1	20 min	1	10 min	2 μg	5	3 min	26 μN	100 μL	25°C

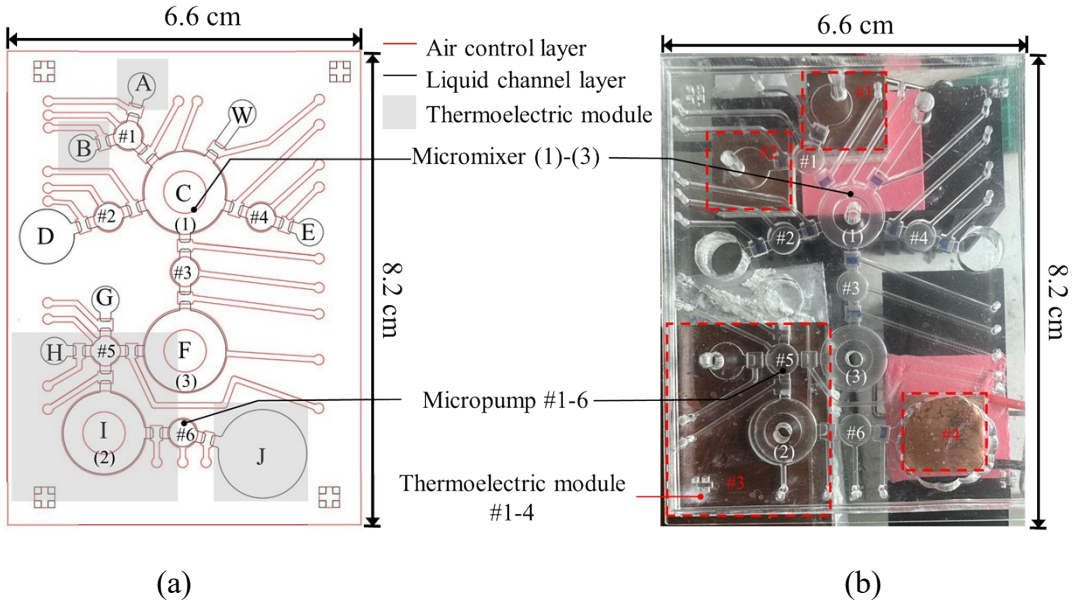


Fig. S1. Overview of version 1 of the integrated microfluidic system. (a) Chambers A–J were designed for the IVT through PCR steps. A: IVT, B: RT, C: Negative selection, D: Storage of additional magnetic beads for negative selection, E: Supernatant storage, F: Competitive selection, G: BWB, H: Second RT, I: Positive selection, J: PCR, W: Wastes. (b) Top view of the version 1 chip with integrated thermoelectric modules. This chip incorporated three fins, four thermoelectric modules, six micropumps and three micromixers.

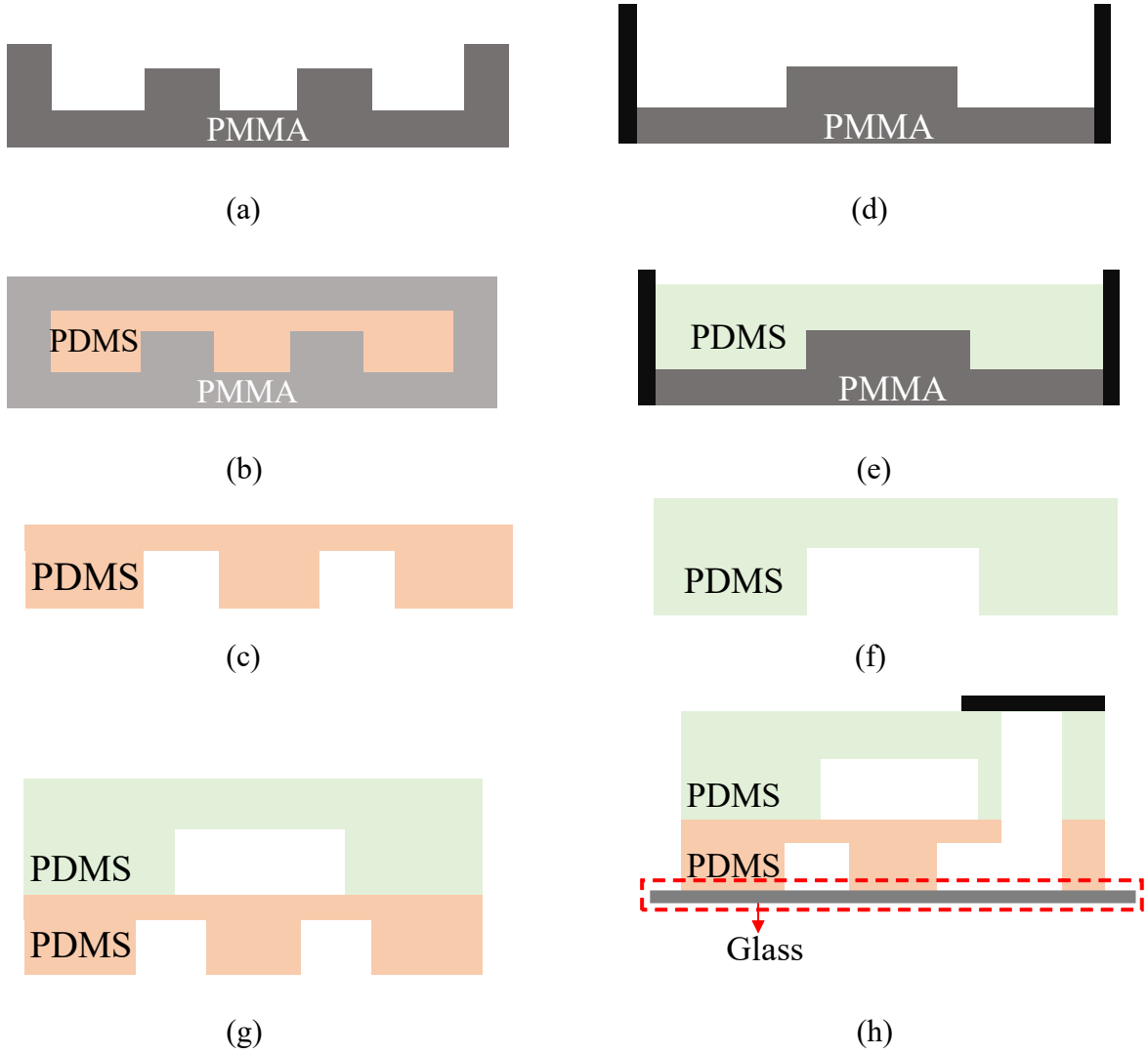


Fig. S2. The microfabrication process. (a) The PMMA liquid channel layer was carved by a CNC machine. (b) A 10:1 PDMS mixture was poured into the master mold, and after degassing, another PMMA layer was placed on top to control the thickness. (c) After curing in the oven at 90°C, the liquid channel layer was peeled off the master mold. (d) The air control layer was created by carving the PMMA substrate with a CNC machine, and the surrounding tape of PMMA was used to control the thickness of the air control layer. (e) A 10:1 PDMS mixture was poured into the master mold and degassed. (f) After curing in the oven at 80°C, the air control layer was peeled off the master mold. (g) The PDMS layers were bonded together through oxygen plasma treatment (30 W for 90 s). (h) The open chambers were punched, and the PDMS was bonded to glass via oxygen plasma treatment. Then, some open chambers that required heating were covered with single-sided tape.

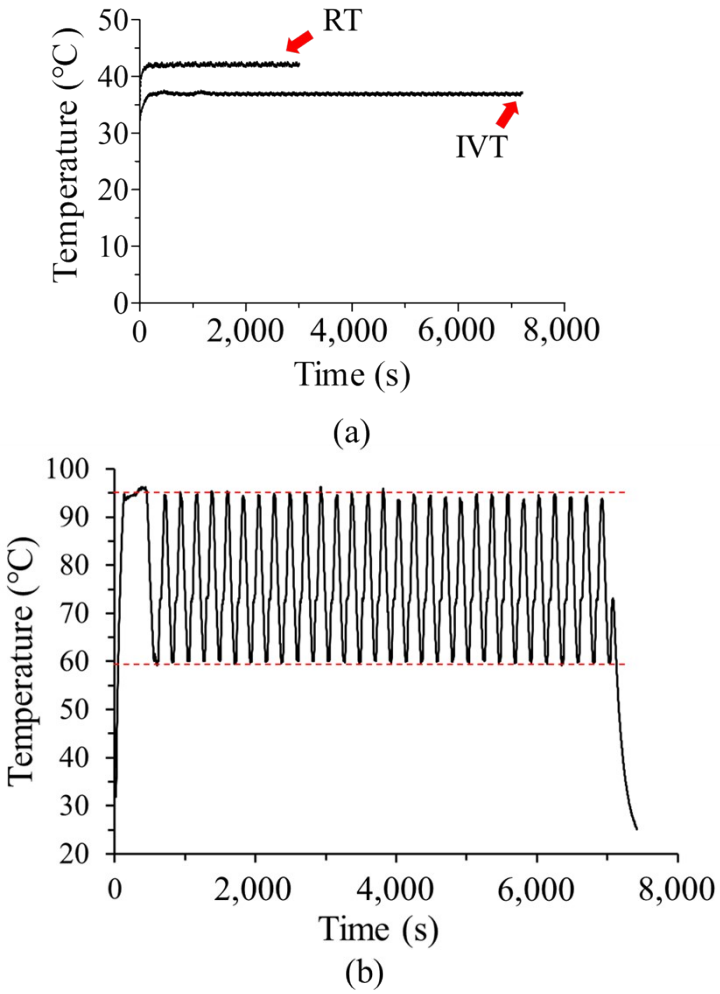


Fig. S3. Temperature data from the Arduino-controlled temperature control module. (a) The IVT and RT reactions were maintained at 37℃ for 2 hr and 42°C for 50 min, respectively. (b) PCR thermocycling included an initial denaturation at 95°C for 5 min, followed by 30 cycles of 94°C for 20 s, 60°C for 20 s, and 68°C for 15 s.

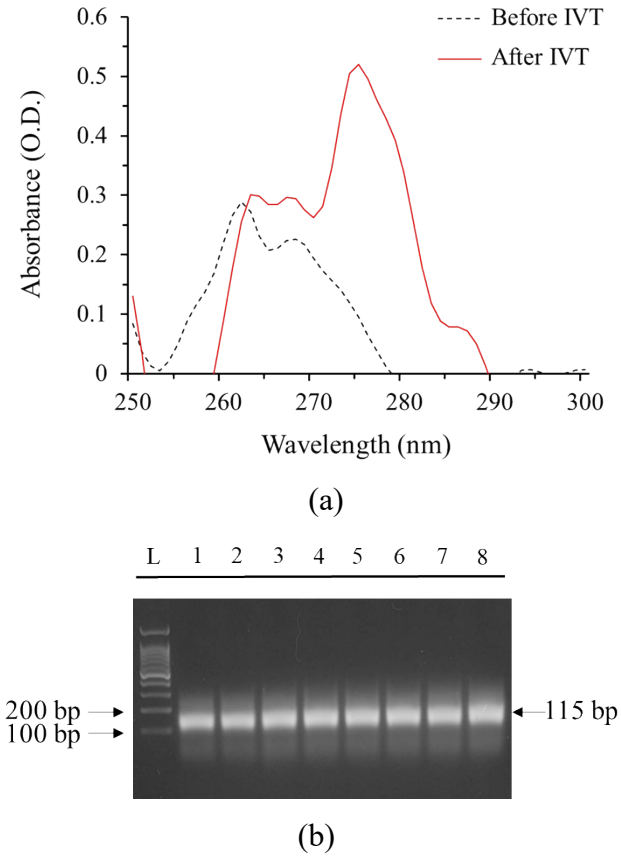


Fig. S4. In vitro translation on-chip. (a) The absorbance at 260 nm before and after IVT decreased while A280 was higher after IVT, indicating that peptides were synthesized successfully. (b) Gel electrophoresis showing that 115-bp amplicons were successfully amplified after selection and PCR. O.D.=optical density.

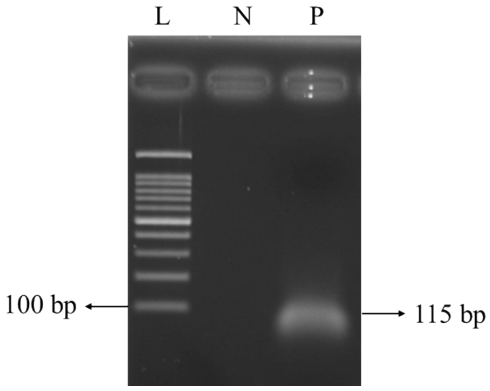


Fig. S5. On-chip RT-PCR results. The PCR product was analyzed by agarose gel electrophoresis, confirming successful amplification. L: ExcelBand™ 100-bp DNA Ladder (DM2100, SMOBIO), N: negative control (without template), P: positive control (2×105 copies/mL DNA library or 4×103 copies/reaction).

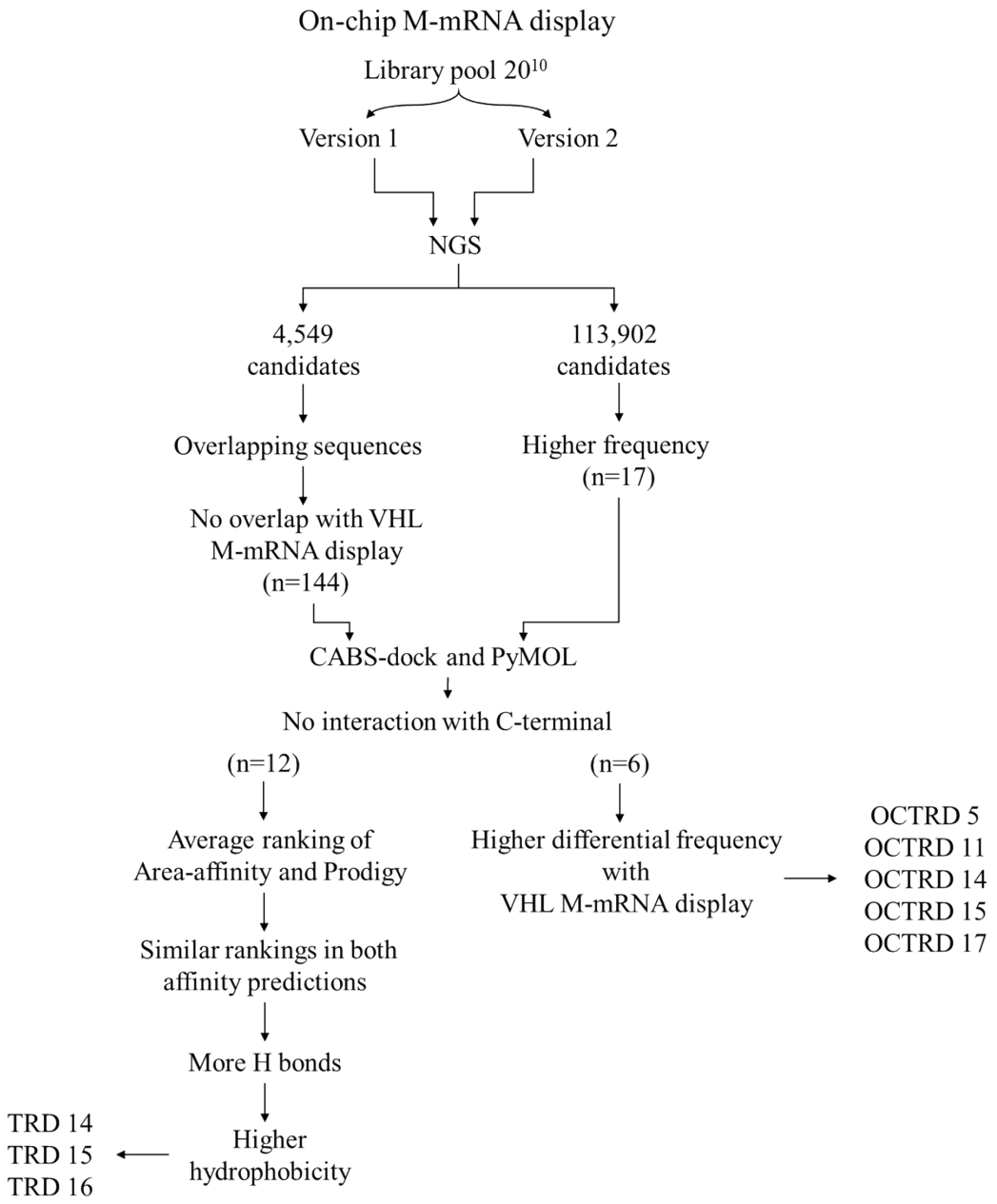


Fig. S6. Selection of peptide candidates. TRD14, TRD15, and TRD16 were selected from washing steps performed under a shearing force of 2.6 μN, while OCTRD5, OCTRD11, OCTRD14, OCTRD15, and OCTRD17 were identified from selection steps featuring shear forces ranging from 0.96 to 9.6 nN.

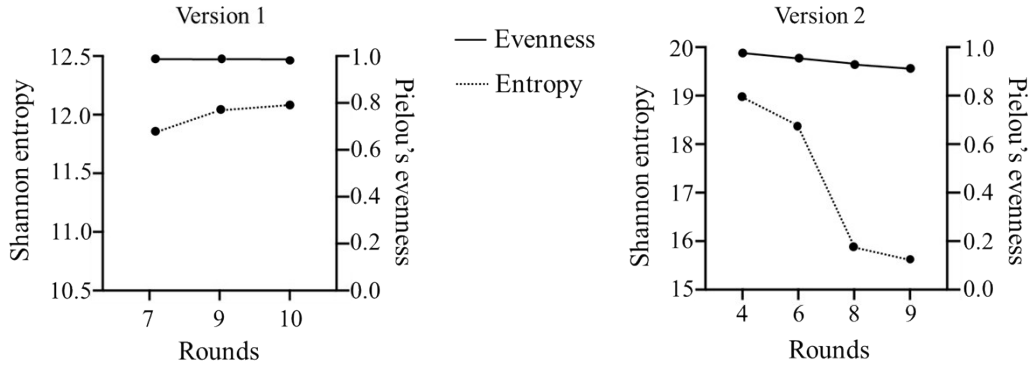
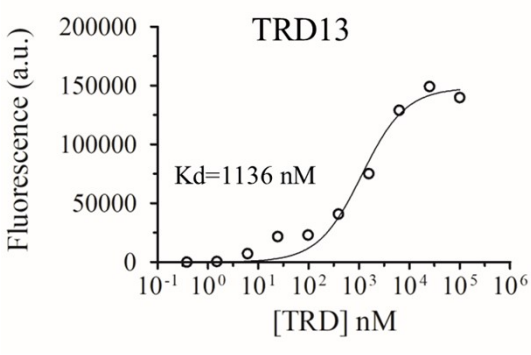
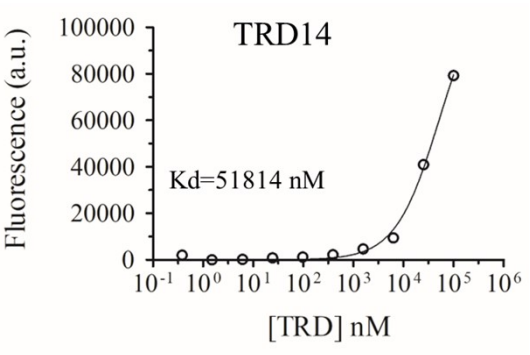


Fig. S7. Shannon entropy and Pielou's evenness calculated for selection versions 1 and 2. Data filtering and analysis: Reads were processed using Python. First, the raw sequences were extracted, and the barcode regions were removed. Next, nucleotide sequences were translated into amino acid sequences. Identical amino acid sequences were then merged, and Shannon entropy and Pielou's evenness were calculated using the following formulas:(Pielou's evenness: $D_{pie} = \frac{H}{log_2(S)}$ S: the number of species, Shannon entropy: $H(x) = -\sum_{i=1}^{S} p_i log_2 p_i$, p_i: the proportion of reads corresponding to the i_{th} unique sequence)

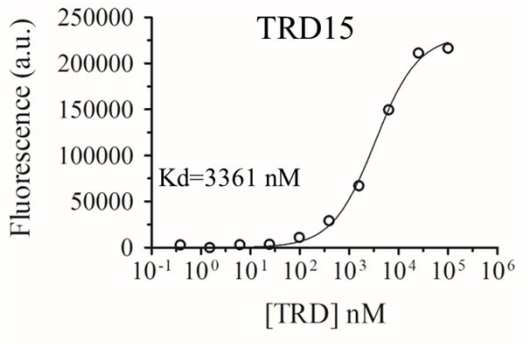
(a) The high evenness in version 1 might be attributed to the limited sequencing depth (average coverage in the final round: 1.05×, calculated as $\frac{4836\ total\ reads}{4550\ unique\ sequences}$), which led to an underestimation of the abundance of dominant sequences. Moreover, low-quality reads might increase background noise, thereby increasing the entropy rather than reducing it. (b) In version 2, improved sequencing depth and optimized selection conditions (e.g., increased shear force and modified negative and competitive selection steps) resulted in decreased entropy and evenness. The average coverage in version 2 was 1.25× in round 4 ($\frac{760218\ total\ reads}{606934\ unique\ sequences}$), 1.63× in round 6 ($\frac{870169\ total\ reads}{533657\ unique\ sequences}$), 1.77× in round 8 ($\frac{200321\ total\ reads}{113261\ unique\ sequences}$), and 1.96× in the final round ($\frac{222969\ total\ reads}{113902\ unique\ sequences}$), indicating progressive enrichment of sequences over the selection rounds.


TRD13
Kd=1136 nM
Fluorescence (a.u.)
[TRD] nM

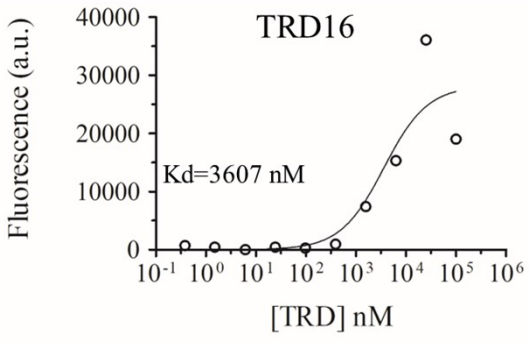
(a)


TRD14
Kd=51814 nM
Fluorescence (a.u.)
[TRD] nM

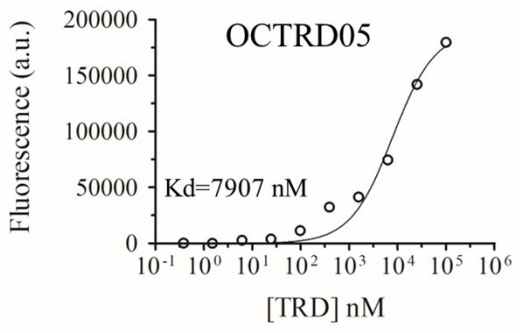
(b)


TRD15
Kd=3361 nM
Fluorescence (a.u.)
[TRD] nM

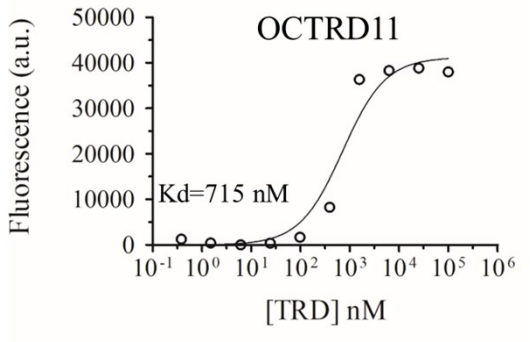
(c)


TRD16
Kd=3607 nM
Fluorescence (a.u.)
[TRD] nM

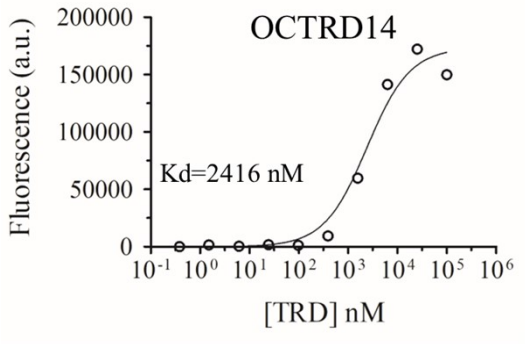
(d)


OCTRD05
Kd=7907 nM
Fluorescence (a.u.)
[TRD] nM

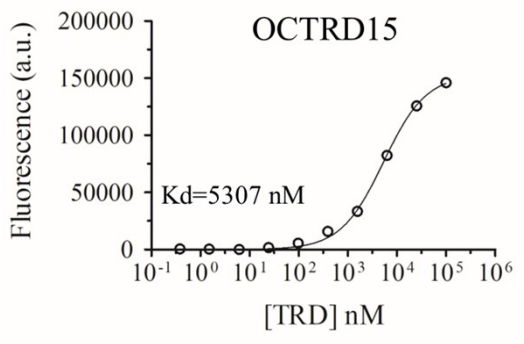
(e)


OCTRD11
Kd=715 nM
Fluorescence (a.u.)
[TRD] nM

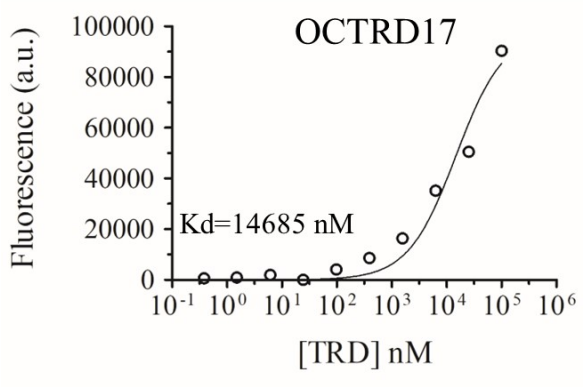
(f)


OCTRD14
Kd=2416 nM
Fluorescence (a.u.)
[TRD] nM

(g)


OCTRD15
Kd=5307 nM
Fluorescence (a.u.)
[TRD] nM

(h)



(i)

Fig. S8. Fluorescence measurements (arbitrary units [a.u.]) for each peptide candidate from pM- to μM-scale. Dissociation constants (Kd) were calculated, and TRD13, OCTRD11, and OCTRD14 demonstrated the lowest Kd values.