Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information

A Comparative Study of Aptamer Isolation on Conventional and Microfluidic Strategies

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Immobilization of IgE Protein on NHS-activated Beaded Agarose Resin

An aliquot of 200 µL of NHS-activated Sepharose™ 4 Fast Flow slurry was washed three times with 300 µL of 1X PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4 and 1.8 mM KH_2PO_4). First, 50 µg of IgE protein was incubated with 200 µL of NHS-activated agarose beads at room temperature (RT) for 30 min. Following washing three times with 300 µL of 1X PBS, the agarose beads were incubated with 100 mM of ethanolamine for 30 min at RT. The unbound ethanolamine was washed 3 times with 1X PBS. Finally, the agarose beads were dissolved in 500 of µL1X PBS containing 2 mM $MgCl_2$ (PBSM).

Fabrication of the Chip-selection Chip

Silicon wafers and SU-8 photoresist assisted by soft lithography were used to fabricate the mold of microfluidic chips. First, a silicon wafer was cleaned with piranha solution (3 parts of concentrated sulfuric acid and 1 part 30% hydrogen peroxide), rinsed with water, dried with nitrogen and placed on a hotplate at 120 ºC for 1 h. The wafer has chrome (20 nm) and gold (100 nm) deposited on its surface using an Ebeam evaporator. Photoresist (AZ 1512, MicroChemicals GmbH) is then spun on the wafer (500 rpm for 10 seconds with 100rpm/s ramp; 3000 rpm for 30 seconds with 400 rpm/s ramp) and baked on a hotplate at 120 ºC for 2 minutes. After the baking process, the wafer is exposed to 95 mJ/cm^2 of energy through a custom-made mask defining alignment marks using a mask aligner. The exposed photoresist is then developed in AZ 300 MIF photoresist for 20 seconds and rinsed with water. The wafer is then developed in gold etchant for 15 seconds, and then rinsed with DI water before developing in chromium etchant for 15 seconds, and rinsed again. Finally, the wafer with gold alignment marks is dried with a nitrogen gun and used in the following steps.

SU-8 3005 was spun on the wafer (2000 rpm, 1 minute, 500 rpm/s ramp rate) and baked at 95 ºC for 3 minutes. This layer was exposed to 200 mJ/cm^2 of energy using a mask aligner and a custom designed mask, post baked (65 ºC for 1 minute and 95 ºC for 2 minutes) and developed with SU-8 developer. The developed wafer was rinsed with isopropanol. This SU-8 3005 layer was used to create an adhesion layer for the additional layers of SU-8 photolithography necessary to complete the fabrication of the device.

The flow layer was fabricated with SU-8 2025 that was spun on the wafer (300 rpm for 15 seconds, 3000 rpm for 45 seconds, 300 rpm/second ramp rate) and baked (65 ºC for 3 minutes and 95 ºC for 6 minutes). The baked wafer was exposed to 200 mJ/cm^2 of energy using a mask aligner and returned to a hotplate at 65 ºC for 1 minute and 95 ºC for 6 minutes. The baked wafer was placed into SU-8 developer for 1 minute, rinsed with isopropanol and dried with a nitrogen gun.

The chamber layer was then created with SU-8 2075. SU-8 2075 was deposited on the wafer and allowed to settle on the wafer for 10 minutes and then spun with a spinner (300 rpm for 15 seconds, 1000 rpm for 45 seconds, 300 rpm/second ramp rate). This material was then pre-baked on a hotplate at 65 ºC for 7 minutes which was ramped 10 ºC/minute to 95 ºC where it remained for 45 minutes. The pre-baked wafer was then exposed to 400 mJ/cm^2 energy with a custom mask defining the chambers using a mask aligner. Subsequently, the wafer was post-baked on a hotplate at 65 ºC for 5 minutes. The temperature was then slowly increased from 65 ºC to 95 ºC over 30 minutes and held at 95 ºC for 15 minutes, followed by slow cooling to room temperature to reduce thermal stress. The wafer was then developed with SU-8 developer for 10 minutes, rinsed with isopropanol and dried with compressed nitrogen.

Following the preparation of the mold, the chamber was prepared using polydimethylsiloxane (PDMS). First, 33 mL of PDMS (10:1 base: curing agent by weight) was poured onto the wafer and placed in a vacuum desiccator for 30 minutes to remove bubbles. Following bubble removal, the wafer was placed on a hotplate at 72 ºC for 30 minutes. The PDMS layer was then peeled off and the inlets and outlets were punched using an autopsy punch. Next, bonding on a glass substrate was carried out after oxygen plasma treatment at maximum power for 30 s. The device was baked at 80 ºC for 1 h. A fabricated device is shown in Figure 1c.

Fabrication of Integrated Chip

The Full-chip SELEX chip mold was fabricated using the same methods described above for the on-chip affinity selection chip with designated masks. A separate control

layer mold for valves was fabricated using the same method described for the SU-8 2025 flow layer with a designated mask.

Glass slides bearing heaters and temperature sensors were fabricated first. Chrome (20 nm) and gold (100 nm) were deposited onto oxygen plasma cleaned microscope slides. AZ 1512 was deposited and spun (500 rpm for 10 seconds, 3000 rpm for 30 seconds, 500 rpm/s ramp) onto them before the slides were baked at 120 ºC for 2 minutes. The slides were then exposed to 95 mJ/cm^2 of energy in a mask aligner with a custom designed film mask. These slides were developed in AZ 300 MIF for 15 seconds, and then rinsed with water. The electrodes on the slides were then developed in first gold etchant (15 seconds), and then chrome etchant (15 seconds). Finally, the slides were rinsed with acetone to remove the remaining photoresist and dried before use.

Next, 33 mL of PDMS (10:1 base: curing agent by weight) was poured onto the flow layer wafer and placed in a vacuum desiccator for 30 minutes to remove bubbles. Following bubble removal, the flow layer wafer was placed on a hotplate set at 72 ºC for 30 minutes. The PDMS was then peeled off, cut into three identical flow layers and had inlets and outlets punched with an autopsy punch. Meanwhile, 3 mL of PDMS was poured onto the control layer which was then spun using a spinner (300 rpm for 15 seconds, 1000 rpm for 45 seconds, 300 rpm/second ramp rate). The control layer was then placed on a 72 ºC hotplate for 20 minutes. The three flow layer devices and the control layer were then placed in an oxygen plasma etcher (Diener Plasma Etch) and exposed to oxygen plasma for 20 seconds at 50% power. Immediately after exposure the flow layer devices were aligned to the three control layer features on the flow layer wafer and gently pressed to put their surfaces in contact. The control layer wafer with flow layer devices on top of it were then placed on a hotplate at 75 ºC for 1 hour. Following this incubation, the flow layer devices and the PDMS thin film coating the control layer were permanently bonded and could be peeled off together. The peeled off PDMS blocks which bear the flow and the control layers then had valve inlets punched into them. Meanwhile, 3 mL of PDMS were poured onto the glass slides bearing the heaters and temperature sensors, spun (300 rpm for 15 seconds, 1000 rpm for 45 seconds, 300 rpm/second ramp rate) and baked on a hotplate (72 ºC for 20 minutes). The heater and temperature sensors and the flow

and control layer devices were then again exposed to oxygen plasma (20 seconds, 50 % power). The exposed devices were immediately aligned and gently pressed into the heater and temperature sensor glass slides. These were baked at 80 ºC overnight. Finally wires connected to the heaters and temperature sensors were soldered onto the contact pads and the temperature-resistance relation was measured in an environment chamber.

Next Generation Sequencing sample preparation

First, 250 ng of sample in 50 µL of PCR-grade water was combined with 7 µL of End Repair & A-Tailing Buffer and 3 µL of End Repair & A-Tailing Enzyme Mix. The mixture was gently mixed and incubated at 20 °C for 30 min and 65 °C for another 30 min. The 60 µL of the resulting product is immediately combined with adapter ligation mix (2.5 µL NextFlex barcode adapter, 7.5 µL PCR-grade water, 30 µL ligation buffer, 10 µL DNA ligase) and mixed gently before brief centrifugation. The ligation reaction mixture was incubated at 20 °C for 20 min. The resulting product was immediately mixed with 0.8X Ampure beads (110 µL product with 88 µL of beads) to perform reaction cleanup. The cleanup suspension is vortexed vigorously before incubating at room temperature for 15 min. The suspension was then placed atop a magnet to capture beads. After 15 min, the clear supernatant was carefully pipetted out and discarded and the sediment was washed twice with 200 µL of 80% ethanol while the tube remained on the magnet. Each time ethanol was carefully removed without disturbing the beads. The remaining beads were dried at room temperature for 5 min and then removed from the magnet before thoroughly resuspended with 25 µL of elution buffer (5 mM Tris-HCl) and incubated at room temperature for 2 min to elute DNA off the beads. The tube was placed on a magnet to capture the beads. The collected 22.5 µL of the clear supernatant was combined with KAPA HiFi HotStart ReadyMix (25 µL) and primer mix (2.5 µL) and incubated on a thermal cycler with the following protocol: initial denaturation at 98 °C for 45 s; followed by 6 cycles of 98 °C for 15s, 60 °C for 30s and 72 °C for 30s; the final extension at 72 °C for 1 min and HOLD on 4 °C. The PCR product was loaded onto a 3% agarose gel (80 mL) for preparatory electrophoresis. The corresponding band at the expected size was cut from the gel and purified with NucleoSpin Gel and PCR Clean-up Kit. The purified ligated dsDNA was quantified by the Qubit fluorometer (Thermo Fisher).

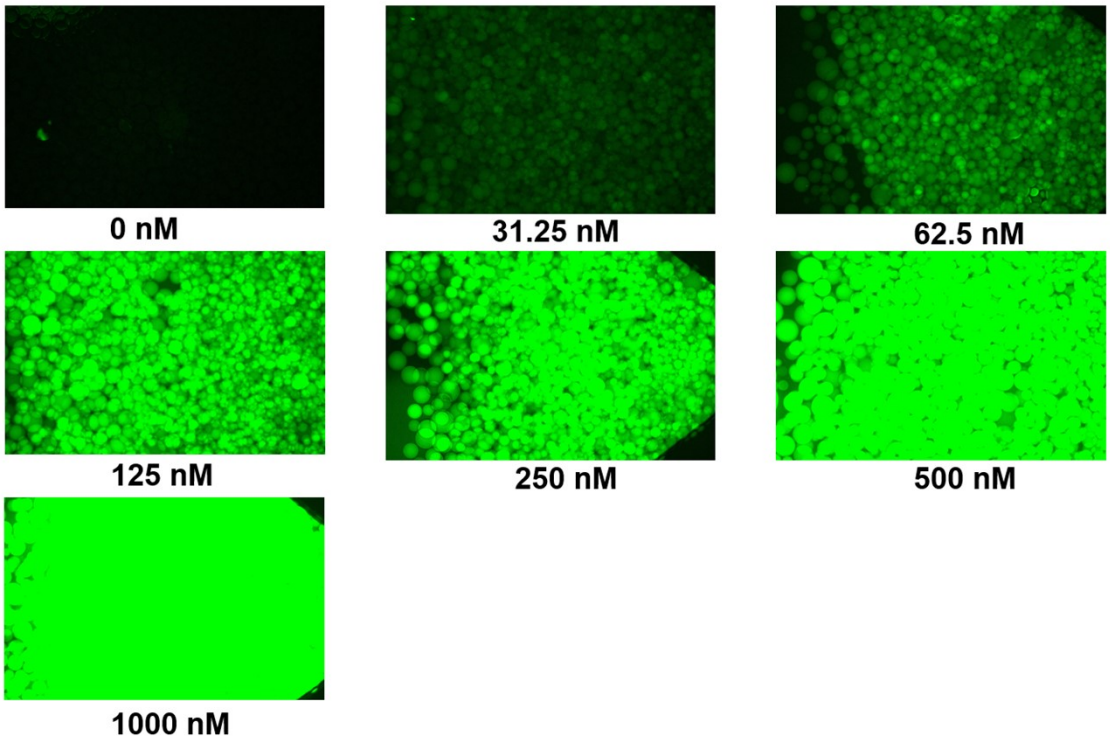


Figure S1. Fluorescence imaging of complex of aptamer IGE1 and IgE-coated beads at different concentrations of aptamer.

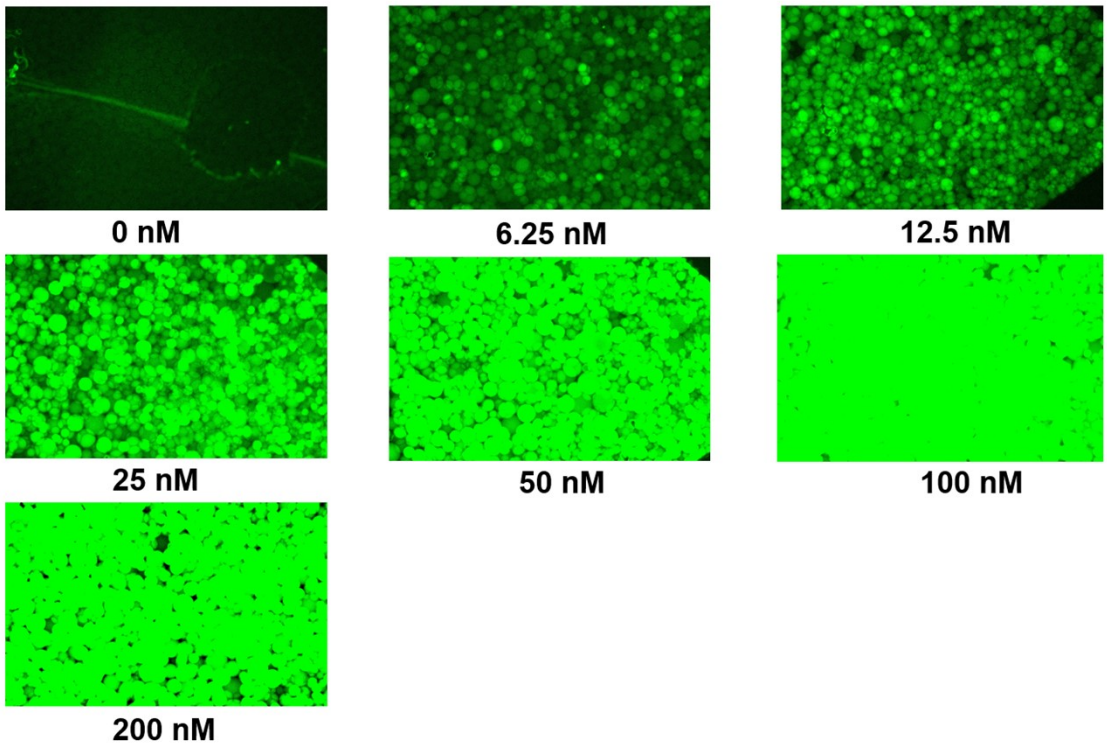
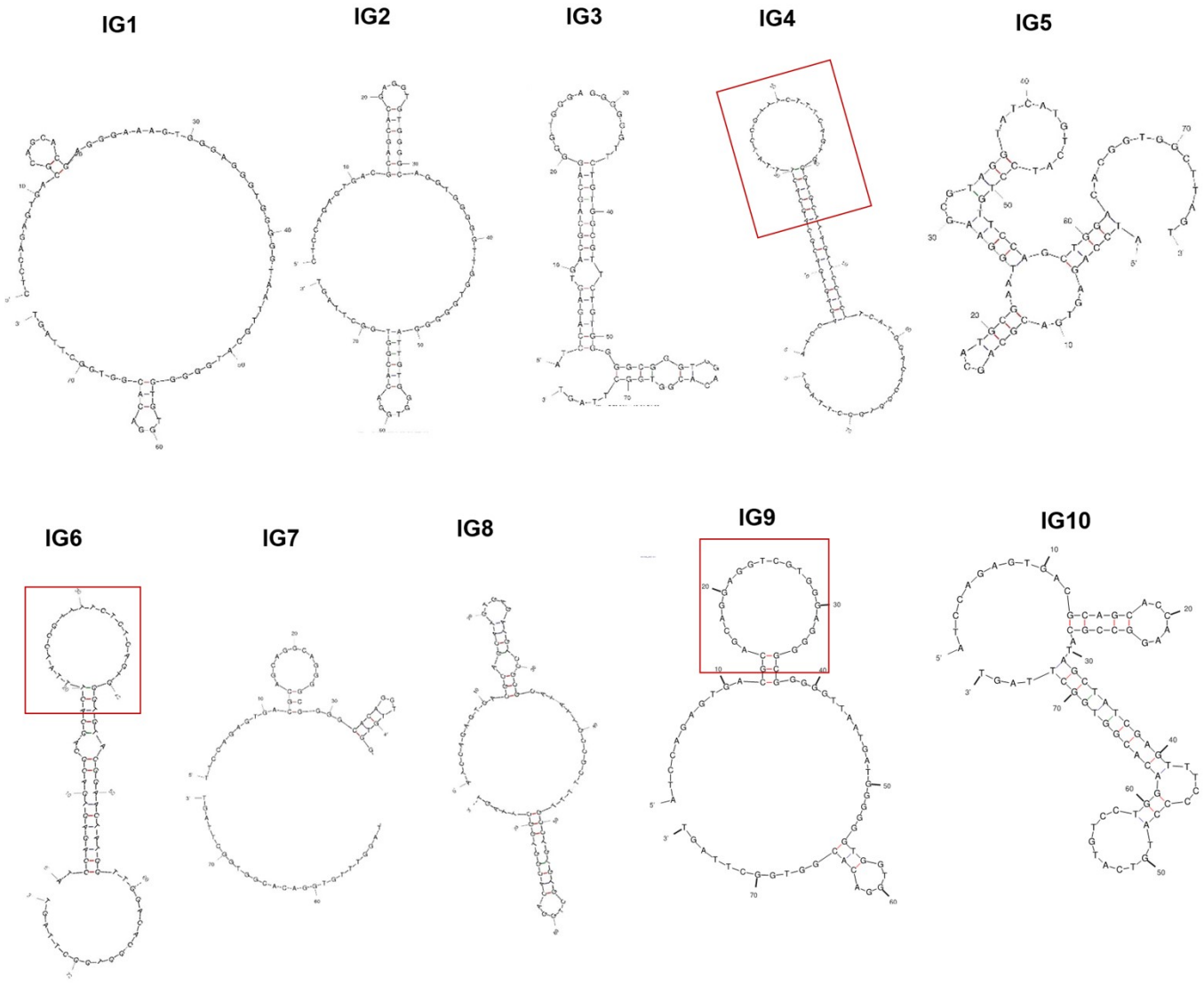


Figure S2. Fluorescence imaging of complex of aptamer IGE1-T and IgE-coated beads at different concentrations of aptamer.

(a)



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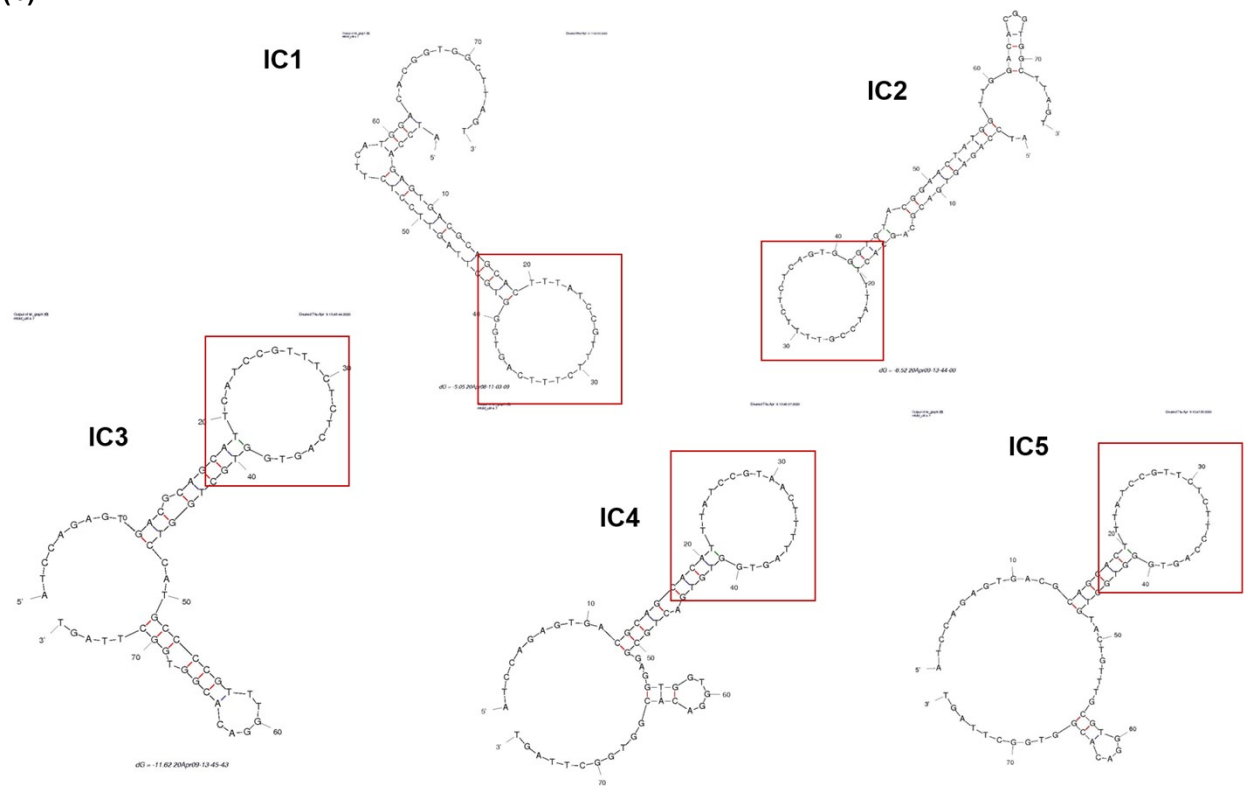

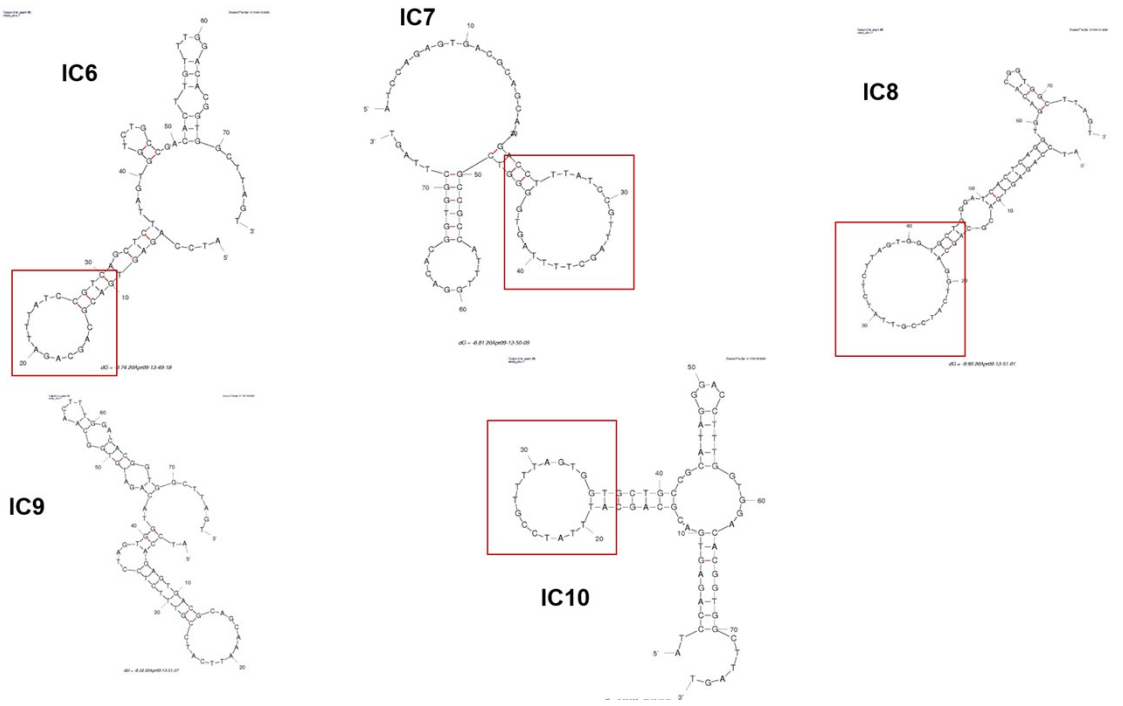

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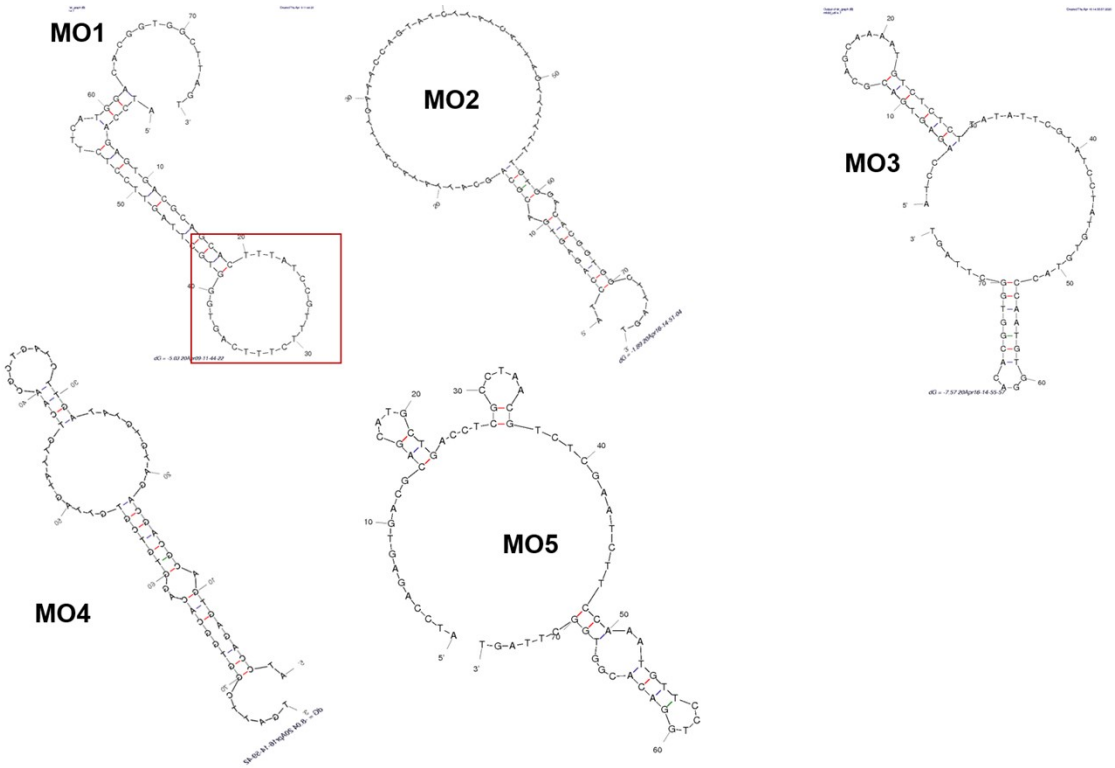

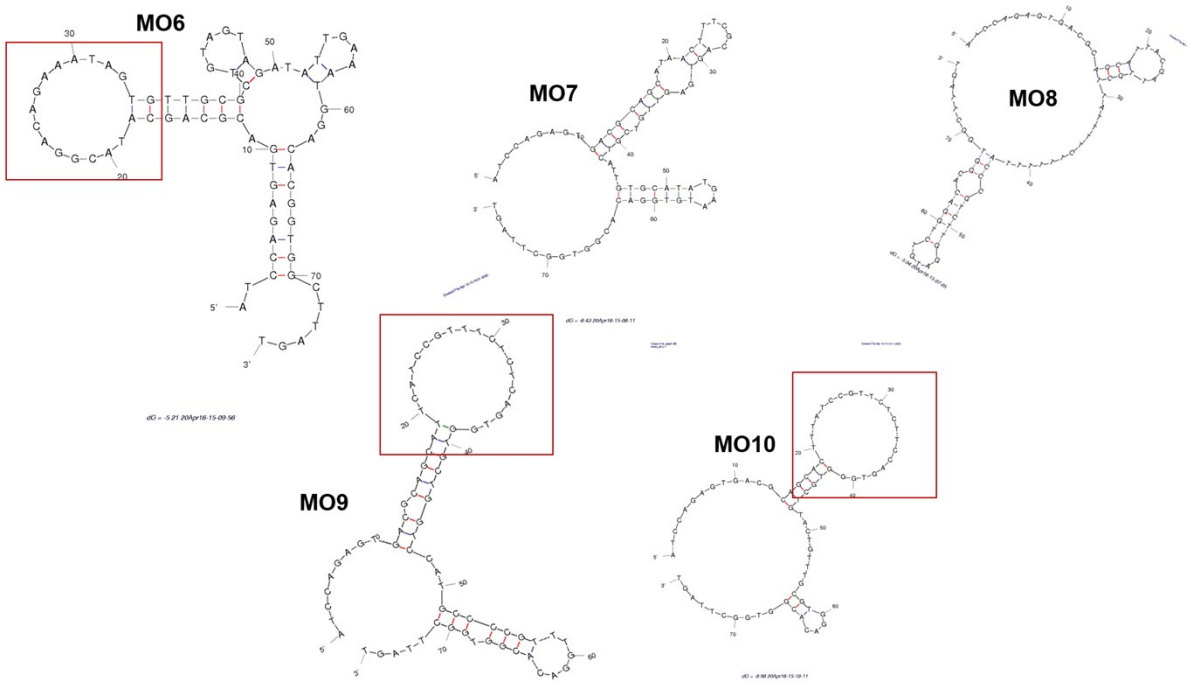

Figure S3. Secondary strictures of aptamer candidates in (a) conventional SELEX, (b) on-chip affinity selection and (c) Full-chip SELEX. Possible IgE-binding regions (loop structures) are marked with red boxes.

Table S1 Top 10 Sequences of the conventional, chip-selection, and full-chip SELEX against IgE

SELEX Method	#	Seuqences
Conventional	1	ATCCAGAGTGACGCAGCACGAGGGAAAGTGGGAGGGTGGGGGTAATTGCATGGGGGTGTGGACACGGTGGCTTAGT
	2	ATCCAGAGTGACGCAGCACGAGGGAAAGTGGGAGGGTGGGGGTAATTGCATGGGGGTGTGGACACGGTGGCTTAGA
	3	ATCCAGAGTGACGCAGCAGGGGTGGGAGGGGGGTTCTGTGGCGTTCTGTGGGGGCGGGTGGACACGGTGGCTTAGT
	4	ATCCAGAGTGACGCAGCACTTTATCCGTTTTCTCTCAGTGGGTGTACGGAACTATGGTTGGACACGGTGGCTTAGT
	5	ATCCAGAGTGACGCAGCACGAGGTGTGGGGCAGGTGGGGGTTGGTGGGGGATTGTGGGTGGACACGGTGGCTTAGT
	6	ATCCAGAGTGACGCAGCATGCGAATGGAAGCGTAGGTATCATGTCATCCTGTTCCAGCTGGACACGGTGGCTTAGT
	7	ATCCAGAGTGACGCAGCACGAGGTGTGGGGCAGGTGGGGGTTGGTGGGGGATTGTGGGTGGACACGGTGGCTTAGT
	8	ATCCAGAGTGACGCAGCACTTTATCCGTTTCTTTCAGTGGGTGCTTAGTTCCTCTTCATGGACACGGTGGCTTAGT
	9	ATCCAGAGTGACGCAGCAGGCAGGGGCGGGGCACAGGTTGTGGGCGGGGGTAGGTTTGTGGACACGGTGGCTTAGT
	10	ATCCAGAGTGACGCAGCATGTGAGATGTCCGCGCATATTGCCGCTTTTGCCTGTGTGCTGGACACGGTGGCTTAGT
Chip-selection	1	ATCCAGAGTGACGCAGCACTTTATCCGTTTCTTTCAGTGGGTGCTTAGTTCCTCTTCATGGACACGGTGGCTTAGT
	2	ATCCAGAGTGACGCAGCACTTTATCCGTTTTCTCTCAGTGGGTGTACGGAACTATGGTTGGACACGGTGGCTTAGT
	3	ATCCAGAGTGACGCAGCATTCATCCGTTTCTCTCAGTGGTGCTGGTCCATGCCCCGTTTGGACACGGTGGCTTAGT
	4	ATCCAGAGTGACGCAGCACATTTATCCGTAACTTTTAGTGGTGTGACTGCGGAGGTGGTGGACACGGTGGCTTAGT
	5	ATCCAGAGTGACGCAGCACTTTATCCGTTCTCTTCCAGTGGGTGCTGTACTGTTTGCGTGGACACGGTGGCTTAGT
	6	ATCCAGAGTGACGCAGCAGATTTATCCGTCAGCTCTTAGTGGTCTGCCGACACTTGTTTGGACACGGTGGCTTAGT
	7	ATCCAGAGTGACGCAGCAAGACCTTTATCCGTTAGCTTTTAGTGGGGTCGCCGCCATTTGGACACGGTGGCTTAGT
	8	ATCCAGAGTGACGCAGCAGGTCATCCGTTATCTCTTAGTGGTGCTGGGATCACTCAGGTGGACACGGTGGCTTAGT
	9	ATCCAGAGTGACGCAGCAAATTCATCCGTTTCTCCTAGTGGTACAGATGTGGCAACTTTGGACACGGTGGCTTAGT
	10	ATCCAGAGTGACGCAGCATTTATCCGTTTTAGTGGTGCTGCCGCATAGGGACCTTTGGTGGACACGGTGGCTTAGT
Full-chip	1	ATCCAGAGTGACGCAGCACTTTATCCGTTTCTTTCAGTGGGTGCTTAGTTCCTCTTCATGGACACGGTGGCTTAGT
	2	ATCCAGAGTGACGCAGCATTATACATTTGAAACCAGTATCTTATCATTAGTTTTTTTGTGGACACGGTGGCTTAGT
	3	ATCCAGAGTGACGCAGCAAAATGTCTCTCTTATATTCGTATCCTATGTGTACCCAATGTGGACACGGTGGCTTAGT
	4	ATCCAGAGTGACGCAGCAGATGTGTATAGTTCTAGTCGCAACTGTTATGATTGTGCTGTGGACACGGTGGCTTAGT
	5	ATCCAGAGTGACGCAGCATGCTGACCTCGCCTAACGTCTCGAATCTTCCAAATGTTCCTGGACACGGTGGCTTAGT
	6	ATCCAGAGTGACGCAGCATACGGACAGAAATAGTGTTGCGCTGTAGTAGATATTGAAATGGACACGGTGGCTTAGT
	7	ATCCAGAGTGACGCAGCATAACTTTCGCAGTGAGTTGTCGTCATTGTGCATATGAATGTGGACACGGTGGCTTAGT
	8	ATCCAGAGTGACGCAGCATTACGATTGCTTATTTTCTTTTTTACCCGTCTTGGATGTCTGGACACGGTGGCTTAGT
	9	ATCCAGAGTGACGCAGCATTCATCCGTTTCTCTCAGTGGTGCTGGTCCATGCCCCGTTTGGACACGGTGGCTTAGT
	10	ATCCAGAGTGACGCAGCACTTTATCCGTTCTCTTCCAGTGGGTGCTGTACTGTTTGCGTGGACACGGTGGCTTAGT

Table S2 Comparison of the conventional, chip-selection, and full-chip SELEX for isolation of aptamers (for a single round of a SELEX process).

		Selection		**Amplification**		**ssDNA Generation**			**Total Cost/Time**
		Library	IgE beads	PCR beads	PCR master mix	Streptavidin Beads	Ultra-filter	Spin Column	
Conventional	Reagent/ materials	2 nmol	30 μL	0	1 mL	200 μL	2	1	
	Cost	$1.5	$5	0	$7	$20	$10	$1.5	$45 (1st round); $43.5 (other rounds)
	Time	3 h		3 h		2~3 h			8~9 h
Chip-selection	Reagent/ materials	2 nmol	30 μL	10 μL	90 μL	0	1	0	
	Cost	$1.5	$5	$1	$0.5	0	$5	0	$13 (1st round); $11.5 (other rounds)
	Time	1 h		1.5 h		0			2.5 h
Full-chip SELEX	Reagent/ materials	2 nmol	30 μL	5 μL	10 μL	0			
	Cost	$1.5	$5	$0.5	$0.1	0			$7.1 (1st round); $5.6 (other rounds)
	Time	1.5 h (1st round);1h (other rounds)		0.5 h		0			2 h (1st round);1.5 h (other rounds)

Table S3 Comparison of the conventional, chip-selection, and IM-SELEX method for isolation of aptamers (for entire SELEX process).

Methods	Time /round	Material cost/round	Chip fabrication cost	Enrichment counts/million	Total round	Total time	Total cost
Conventional	8~9 h	\$45 (1st round); \$43.5 (other rounds)	0	7	4	4 d	\$176
Chip-selection	2.5 h	\$13 (1st round); \$11.5 (other rounds)	\$4	4143	4	1.5 d	\$48
Full-chip SELEX	2 h (1st round);1.5 h (other rounds)	\$7.1 (1st round); \$5.6 (other rounds)	\$28	22	4	1 d	\$52