Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Immobilization-free Screening of Aptamers assisted by Graphene oxide.

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1. Experimental details

1.1. Chemicals and proteins: 3,3'-Dithiodipropionic acid and Ethanolamine were purchased by Sigma Aldrich (Korea), N-ethyl-N'-(dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were purchased by Sigma Aldrich (Korea). Nampt, RBP4, Adiponectin, Resistin and Vaspin were purchased from AdipoGen, Inc. (Incheon, Korea). Bovine serum albumin (BSA) and human serum albumin (HSA) were purchased from Sigma Aldrich (Korea) and streptavidin was purchased from Fluka.

1.2. Oligodeoxynucleotides: The ssDNA library comprised a randomized region of 30 (N30) nucleotides flanked by two constant regions allowing primer annealing and PCR amplification (primer sequences: FP 5'-CGTACGGAATTCGCTAGC-3' and RP 5'-CACGTGGAGCTCGGATCC-3'. 5'-CGTACGGAATTCGCTAGC-N30-GGATCCGAGCTCCACGTG-3'. For PCR amplification, FP : 5'-fluorescein-CGTACGGAATTCGCTAGC-3' and RP : 5'-CACGTGGAGCTCGGATCC-3' were used. All sequenced aptamers were labeled with 5'-biotin for immobilization on SPR chips. All oligodeoxynucleotides sequencing and synthesis services were provided by Genotech, Korea.

2. Experimental procedures

2.1. Synthesis and characterization of Graphene oxide: Graphene oxide (GO) was prepared from natural graphite (FP 99.95 % pure, Graphit *Kropfmühl AG*) by modified Hummers method. A graphite powder (1 g) was added to sulfuric acid (98%, 150 mL), and then potassium permanganate (99.0%, 2.5 g) as an oxidizing agent was gradually added to the graphite solution. Then, the mixture was cooled in an ice bath and diluted with deionized water (200 mL). After stirring for ~2 hours, hydrogen

peroxide (100 mL) was added to the reaction solution. The mixture was then filtrated with a glass filter and washed several times with hydrochloric acid (10%). After this washing process, the remaining solvent was evaporated under vacuum at room temperature for 12 hours. The obtained (GO) was characterized by AFM (Figure S1).

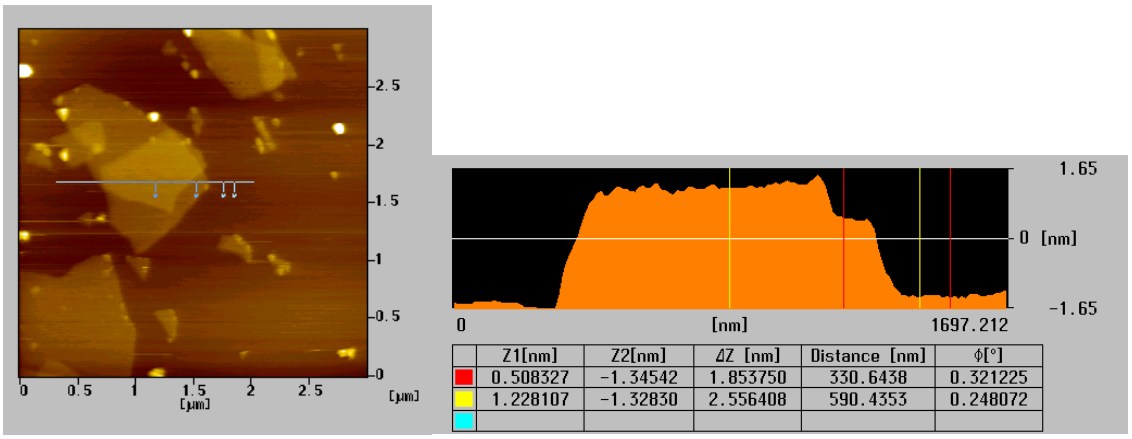

Figure S1. The AFM image and their height profiles GO.

2.2. Description of GO-SELEX:

a) Selection round: The GO-SELEX round was performed by initially heating 200 pmole (Tris HCl 20 mM, pH 7.4) of random library for 15 min and then cooling for 5 min in ice. For the 1st round of GO-SELEX 200 pmoles of Nampt and 200 pmoles denatured ssDNA library (1:1 ratio) was incubated for 30 min. The random library and Nampt mixture was added to GO and further incubated for 2 hrs (final volume 1000 µL). Subsequently the solution was centrifuged for 3 min at 10000 rcf to precipitate and discard GO. The ssDNA was recovered from the supernatant by ethanol precipitation. The recovery amount of ssDNA was measured by (Nanodrop,ND-1000, spectrophotometer) and the obtained library pool was amplified by PCR and ssDNA was again generated by PAGE separation and purification. This ssDNA pool was used in the following round of GO- SELEX for aptamer enrichment.

b) Counter selection: For GO counter-SELEX round, was performed by initially heating purified ssDNA random library pool (50 pmoles) of 4th round of GO-SELEX in (Tris HCl 20mM, pH 7.4) for 15 min and then cooling for 5 min in ice. The counter targets (50 pmoles) RBP4, Adiponectin, Resistin, Vaspin and HSA were incubated with 4th round ssDNA library for 30 min. The random library and counter target mixture was added to GO and further incubated for 2 hrs (final volume 1000 µL). The solution was centrifuged for 3 min at 10000 rcf to precipitate GO and discard the supernatant. The ssDNA was recovered from the GO with the addition of target protein Nampt (200 pmoles) and incubated further for 2 hrs. The mixture solution was centrifuged and ssDNA library pool was recovered by ethanol precipitation.

2.3. Cloning and sequencing: After the final round of SELEX, the ssDNA was amplified using the primer without fluorescence modification. After purification, the unlabeled PCR product was cloned using the pDrive Cloning Vector (Qiagen PCR Cloning Kit) vector in *E. coli*. The positive clones were grown in LB media containing 50 µg/mL ampicillin, followed by plasmid DNA extraction with a MiniPrep kit (Qiagen). Finally, the aptamer candidates inserted in the plasmid DNA of each clone was sequenced (GenoTech, Korea). The secondary structure of the all the candidates was predicted by means of the Gibb's free-energy minimization algorithm with the web server based mfold tool (http://mfold.rna.albany.edu/).

No.	Sequence	Δ G
56	CGTACGGAATTCGCTAGCCCGTGGGGTAGCGGGGTCGTGTGATATGTGGATCCGAGCTCCACGTG	-13.41
12	CGTACGGAATTCGCTAGCGGGTGCCGTGGCACGAGGCCGTGGTCCAGGGGATCCGAGCTCCACGTG	-12.85
4	CGTACGGAATTCGCTAGCGTGATGTGGGGGTACGCTCGTGGCAGGCTTGGATCCGAGCTCCACGTG	-12.76
27	CGTACGGAATTCGCTAGCGGGTGGAGTACGTGGGGGTCATCCTGTGTGGGATCCGAGCTCCACGTG	-12.64
35	CGTACGGAATTCGCTAGCGGTGACGGACGTGGGGGTGCACGAAGGGAGGGGATCCGAGCTCCACGTG	-12.59
26	CGTACGGAATTCGCTAGCATCGGGTGCAGAGTCGGAGCTAACGGCAGCGGATCCGAGCTCCACGTG	-11.83

9	CGTACGGAATTCGCTAGCGGGGATGGGCCGCTCTGCAGAATGTTCTGTGGATCCGAGCTCCACGTG	-11.58
32	CGTACGGAATTCGCTAGCGTGGACTGGCGGAAATCTTGGTATGCCCATGGATCCGAGCTCCACGTG	-10.81
21	CGTACGGAATTCGCTAGCGGGTTCGGGACGGATGAACGTGATAGCTGAGGATCCGAGCTCCACGTG	-10.72
15	CGTACGGAATTCGCTAGCAGCCGGCGGGTGCTCAATGTTGGGGGTTGGGATCCGAGCTCCACGTG	-10.55
40	CGTACGGAATTCGCTAGCCGGGGTGGGAACCAGTCTTGCGCGGGTGACGGATCCGAGCTCCACGTG	-10.33
54	CGTACGGAATTCGCTAGCGTGGCGGGGCGCGGGTGCCGGAGTTGATGTGGATCCGAGCTCCACGTG	-10.12
37	CGTACGGAATTCGCTAGCGGGCGATGTGCGGAATGTGGGATTGCGGGTGGATCCGAGCTCCACGTG	-10.07
39	CGTACGGAATTCGCTAGCGGTTGCCGTGCGGCGTGCGAGTTGGGCCTTGGATCCGAGCTCCACGTG	-10
55	CGTACGGAATTCGCTAGCGGACTGGAGTCTAGACCGGGTAGCTGTGGTGGATCCGAGCTCCACGTG	-9.89
2	CGTACGGAATTCGCTAGCGTGAGGTTGCGGGTCCCGGACCATCGCGGTGGATCCGAGCTCCACGTG	-9.87
20	CGTACGGAATTCGCTAGCCGTGAAGAGGTGGCGCGTTGCGGGCAGTGGGGATCCGAGCTCCACGTG	-9.81
8	CGTACGGAATTCGCTAGCGACACCTGTGTTGCTGGATTAGGTGTGTGGGGATCCGAGCTCCACGTG	-9.78
48	CGTACGGAATTCGCTAGCGGCGGAGTGGGGTGAGATGAGCGCCTCGCTGGATCCGAGCTCCACGTG	-9.74
47	CGTACGGAATTCGCTAGCATTGTGCGTGGGTACCGCAACAGTGTGTGTGGATCCGAGCTCCACGTG	-9.5
24	CGTACGGAATTCGCTAGCCCCGTCAGTGGACCTGTGTGTACTGTGTGGGGATCCGAGCTCCACGTG	-9.49
45	CGTACGGAATTCGCTAGCCCGGTTGGTCATCGGGTACCGGGTGTTGAGGATCCGAGCTCCACGTG	-9.47
42	CGTACGGAATTCGCTAGCGTGGTGTAGCTGGGTTGCGTGCATTCGTCCGGATCCGAGCTCCACGTG	-9.35
43	CGTACGGAATTCGCTAGCGTGTGTAGTGCGACGTTTGAGGTTGGTACAGGATCCGAGCTCCACGTG	-9.32
41	CGTACGGAATTCGCTAGCGGGGGTGCAGTATATGGTGTGGACAATCGGGGATCCGAGCTCCACGTG	-9.16
29	CGTACGGAATTCGCTAGCGTGGATGGGGGATGGTGTGATTTGCGTCGCGGATCCGAGCTCCACGTG	-9.13
18	CGTACGGAATTCGCTAGCCGGGTGGAGTCGCACGCTGCGTTGGTCTGGGGATCCGAGCTCCACGTG	-9.02
6	CGTACGGAATTCGCTAGCAATATGGTGCTCCGGGTAACGTGTCCGGTGGGATCCGAGCTCCACGTG	-8.93
44	CGTACGGAATTCGCTAGCAGCGCGTGTGGATGACGTTCCTCGCATGGGGATCCGAGCTCCACGTG	-8.92
17	CGTACGGAATTCGCTAGCGGGGGGCGTGGGCGGCTCTTGGGCACAACGGGATCCGAGCTCCACGTG	-8.82
34	CGTACGGAATTCGCTAGCGGGTCGGGGGGGGGGGGGTGCCCACCTGGTTGGGGATCCGAGCTCCACGTG	-8.82
46	CGTACGGAATTCGCTAGCAAGGGGGCGGCCATCCATTGCAGCTGCGTTGGATCCGAGCTCCACGTG	-8.82
11	CGTACGGAATTCGCTAGCTGGCGGGTAGGGTTACTCGTACATAGGGGTGGATCCGAGCTCCACGTG	-8.72
57	CGTACGGAATTCGCTAGCTGGGTTACGTTACGTGTCGGGGGGTGTGGAGGATCCGAGCTCCACGTG	-8.72
10	CGTACGGAATTCGCTAGCCTGCAGCTGTTTACGGCTGTCATGTTGGGGATCCGAGCTCCACGTG	-8.65
16	CGTACGGAATTCGCTAGCGGGGTGGCGTATGCACCTGGTGTGTGTGCAGGATCCGAGCTCCACGTG	-8.64
22	CGTACGGAATTCGCTAGCATATGCGTGGGCAGGTGGGAGGTGATTCCGGGATCCGAGCTCCACGTG	-8.62
33	CGTACGGAATTCGCTAGCGATCGGGCGTGCGCGTTGCTGGGACATGGGGATCCGAGCTCCACGTG	-8.48
23	CGTACGGAATTCGCTAGCTGTGCGGGCGGGGTGTGTCGGGGGGTAGGGGATCCGAGCTCCACGTG	-8.25
3	CGTACGGAATTCGCTAGCTGGGGCGGTGCGTGCCTGTCGTAGGGTGGATCCGAGCTCCACGTG	-8.04
13	CGTACGGAATTCGCTAGCGGTGTTCGGTGCGAGTAGTGTTGTCGAGGTTGGATCCGAGCTCCACGTG	-7.77
25	CGTACGGAATTCGCTAGCGGGTCGGACGGCGGGGATGTAGGGTCTTCGGGATCCGAGCTCCACGTG	-7.64
19	CGTACGGAATTCGCTAGCGGGACCAGTACGCGCGTGCTATGGGTGTTGGGATCCGAGCTCCACGTG	-7.11
14	CGTACGGAATTCGCTAGCTGTGGCGAGGGGCGTATGGGGTGGTGAGAGGGATCCGAGCTCCACGTG	-7.03
38	CGTACGGAATTCGCTAGCTGTGGCCGGGGTCCGATCGGTAGTTGTGGGGATCCGAGCTCCACGTG	-6.9
5	CGTACGGAATTCGCTAGCCTGCATGGAGTACGACCGGGGGTGGGTTGGGGATCCGAGCTCCACGTG	-6.65

7	CGTACGGAATTCGCTAGCGTATGCGAGTGTGATGATGTACAAGGTGGGATCCGAGCTCCACGTG	-6.41
1	CGTACGGAATTCGCTAGCTGTACACTGGCCGGTCGTTTATGCTGTTGCGGATCCGAGCTCCACGTG	-6.35

Table S1 : A total of 48 sequences were obtained after cloning and sequencing. The sequences were arranged based on ΔG values and top 15 apatmer sequences were characterized by SPR for specificity and affinity. The underlined sequences(No.35,40,55) are final reported aptamers.

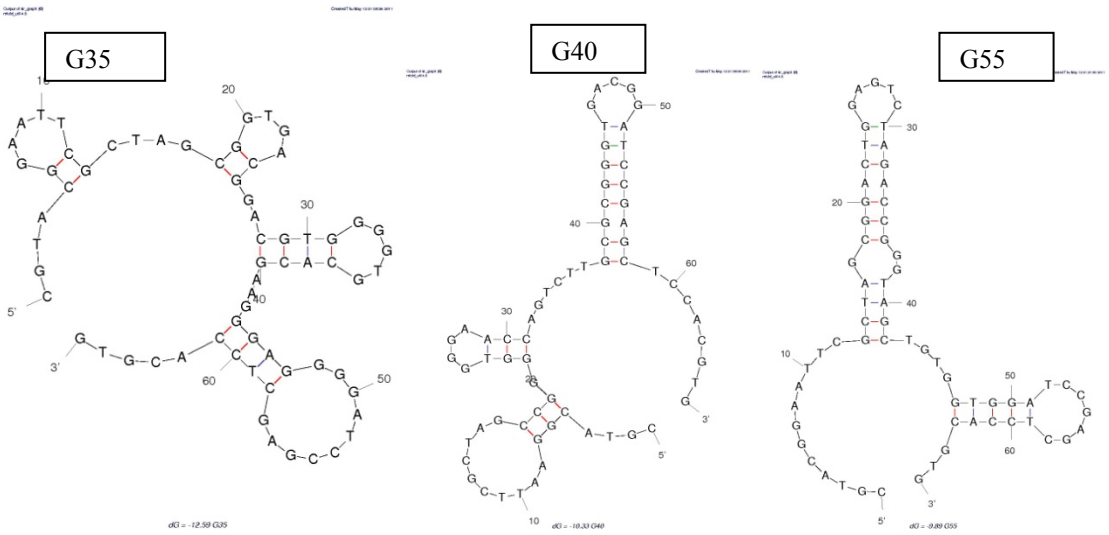

Figure S2 : The secondary structure of selected aptamers in GO-SELEX which showed high specificity and affinity.

2.4. Characterization of aptamers by SPR:

a) SPR chip preparation

To immobilize aptamers on SPR gold chip, the chip was washed with ethanol and distilled water (DW), the clean chip was immersed in 100mM 3,3-dithiopropionic acid solution at room temperature overnight. Then carboxyl fuctional groups on chip surface were activated with 0.1 M EDC and 0.05M NHS for 30min. After this, chip was incubated with 100 μg/mL (1.9 μM) of streptavidin for 90 min in ice. The unreacted functional groups were blocked by addition of 50mM ethanolamine solution for 30min. Then biotin labeled aptamers were incubated (1μM) for 60 min at room temperature. Finally chip was blocked 50μg/ml BSA solution for 30min and washed with DW.

b) SPR analysis

By using aptamer modified gold chip, SPR (Eco Chemie, Netherlands) anlaysis was performed after injection of 50 μL of each counter targets(RBP4, Adiponectin, Resistin, Vaspin, HSA) and main target as well. The binding reaction was performed for 30 minutes with a 5 minutes dissociation time at room temperature. After selecting the most highly specific aptamer, dissociation constant was determined with the nonlinear regression analysis.

For specificity test; concentration of all counter target proteins was 200 nM and for dose dependent affinity test : 25, 50, 100, 200, 400 nM of Nampt was injected.