# [Supplementary information]

# Aptabody-Aptatope Interactions in Aptablotting Assays

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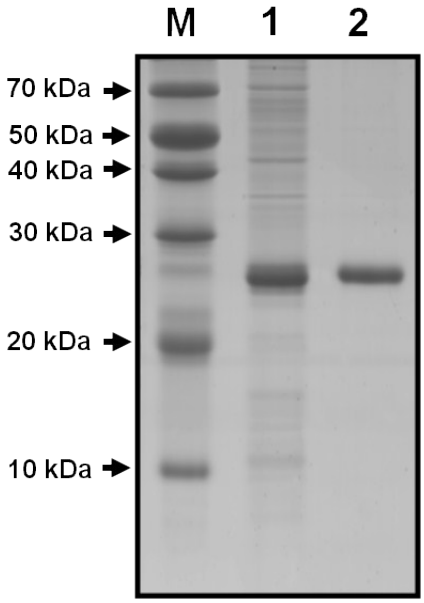
**Figures**

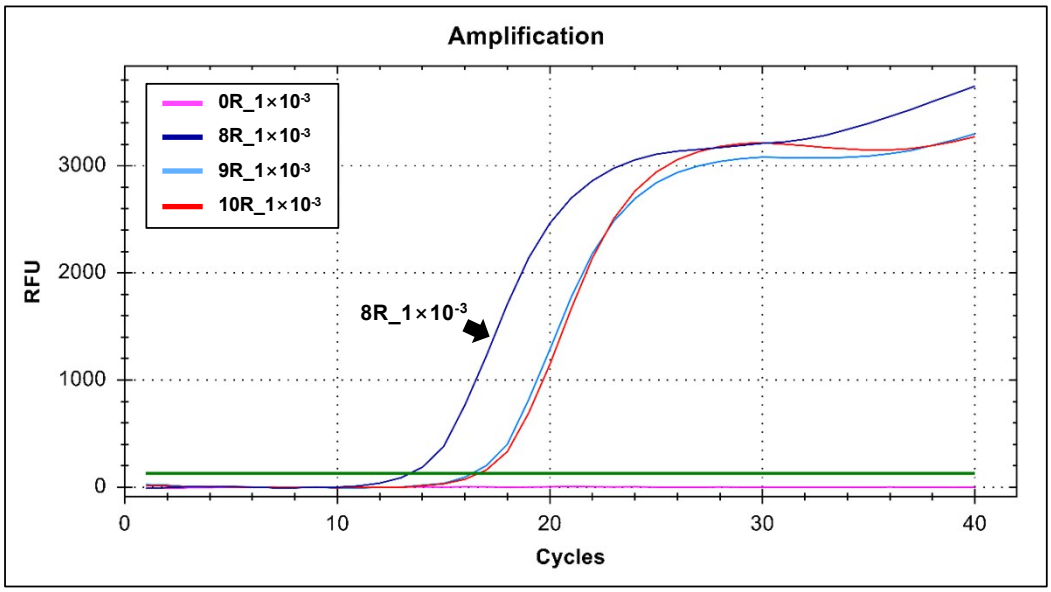
**Supplementary Figure S1**




**GST overproduction and purification**

GST overexpression was successfully accomplished in *E. coli* with the pGEX4T-1 system. The GST of *E. coli* was stably overexpressed as a soluble protein in *E. coli* BL21 (DE3). The GST protein was purified using glutathione-agarose beads, and the purified GST showed a single protein band with an apparent $M_r$ of approximately 26000 on the SDS-PAGE stained with Coomassie blue. Lane 1, whole-cell lysates of pGETX4T-1 (10 μg of protein); lane 2, purified GST (1 μg of protein); M, molecular size markers.

**Supplementary Figure S2**

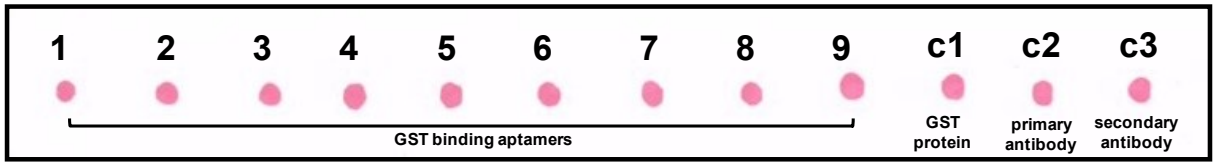



| Round | 0 Round | 8 Round | 9 Round | 10 Round |
|---|---|---|---|---|
| Dilution rate | $1\times10^{-3}$ | $1\times10^{-3}$ | $1\times10^{-3}$ | $1\times10^{-3}$ |
| Ct value | - | 13.33 | 16.49 | 16.97 |
| Efficiency | 90.38 | 83.90 | 80.06 | 74.39 |

**Real-time PCR and round selection**

We employed the real-time PCR (RT-PCR) analysis that provided a valuable tool for validating the optimal round of ssDNA aptamer enrichment. The eluted ssDNA bound from 0, 8$^{th}$, 9$^{th}$, and 10$^{th}$ rounds were normalized with the same concentration and reintroduced to GST. The recovered DNA samples from 0, 8$^{th}$, 9$^{th}$, and 10$^{th}$ round were amplified using an optimized RT-PCR cycle and the fluorescence signal was monitored by MiniOpticon$^{TM}$ Real-time PCR fluorescence signal detection system (Bio-Rad, USA). PCR experiment was independently triplicated and data was analyzed to obtain the average C(t) values. The samples from **8$^{th}$** round SELEX showed a lower C(t), (C(t)) =13.33, as compared to other samples, C(t)=15.20 for 9$^{th}$ round and 16.97 for 10$^{th}$ round, indicating the presence of higher amounts of DNA aptamer for GST.

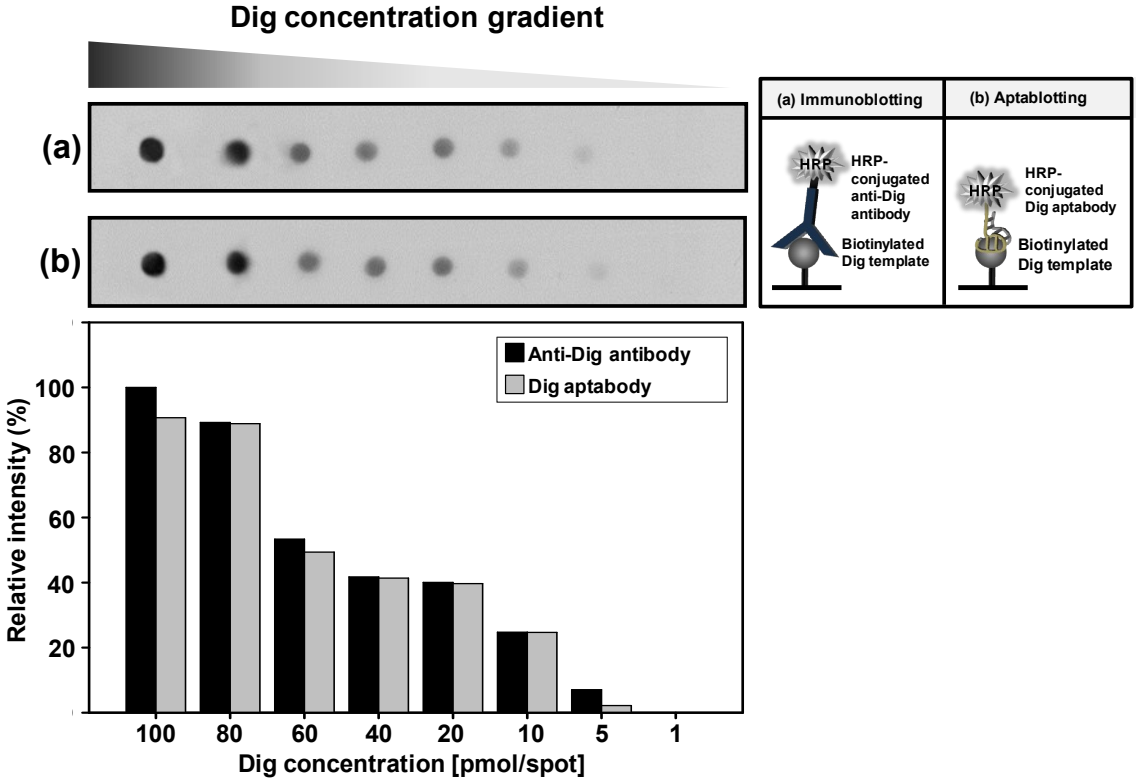
**Supplementary Figure S3**




**Dot-blotting analysis**

Serial dilutions of purified GST (1 μg to 1 ng) were spotted on the methanol-pretreated PVDF membranes. Blotted proteins are stained with Ponceau S solution (Sigma Aldrich, P7170).

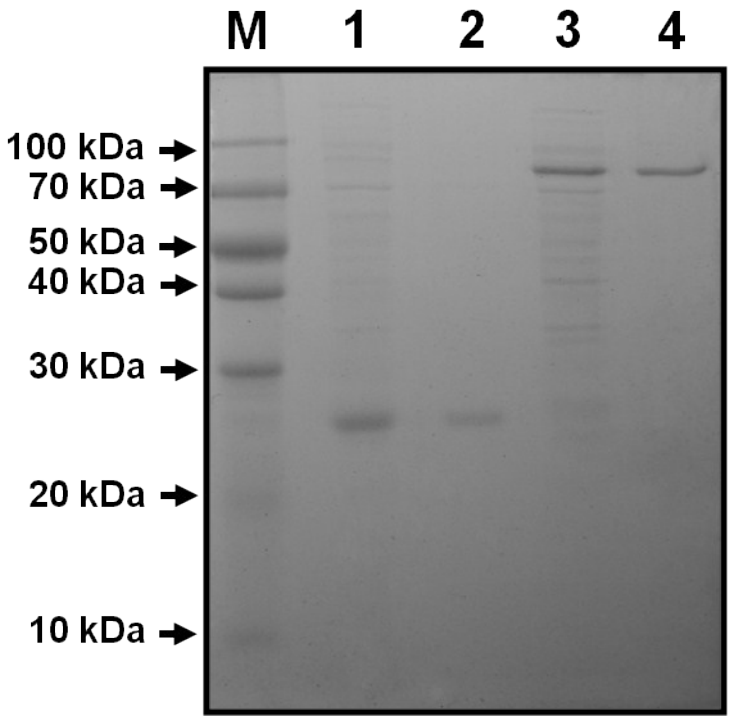
**Supplementary Figure S4**




**DIG aptabody test**

The synthesized DIG template (1 µl/spot) was loaded onto the PVDF membrane in concentrations of 100 pmol, 80 pmol, 60 pmol, 40 pmol, 20 pmol, 10 pmol, 5 pmol, and 1 pmol. The membrane was then dried at 37 °C for 1 hr and immersed in 5% nonfat dry milk with PBST blocking buffer for 1 hr to block the non-specific binding sites. After washing three times for 5 min with a PBST washing buffer, the membranes were incubated with 1 µg/ml diluted DIG-1-HRP and 1:5000 diluted anti-mouse DIG-HRP-conjugated antibodies (Abcam, UK). The binding sensitivity of the DIG aptabody and anti-DIG antibody was assessed via a comparison with the anti-DIG-HRP tests (a) immunoblotting and (b) aptablotting, respectively

**Supplementary Figure S5**




**GST and GST-fused protein overexpression and purification**

The overexpression of GST and GST-fused PNBE proteins was successfully accomplished in *E. coli* with the pGEX4T-1 system. The GST and GST-fused PNBE protein of *E. coli* has been stably overexpressed as a soluble protein in *E. coli* BL21(DE3). The recombinants were purified with glutathione-agarose beads, and the purified GST and GST-fused PNBE showed a single protein band with an apparent $M_r$ of approximately 26000 and 75000 via the SDS-PAGE analysis on a 12% gel stained with Coomassie Brilliant Blue. In the panels, lane 1 contains pGEX4T-1 whole-cell lysate (5 ug), lane 2 contains purified GST (0.5 ug), lane 3 contains pGEX4T-1::PNBE cell lysate (5 ug) and lane 4 contains purified GST-tagged PNBE protein (0.5 ug). The molecular size markers are shown in the lane labeled M.

**Table S1** Sequence and structure analysis of the GST aptabodies and DIG aptabodies used in this study.

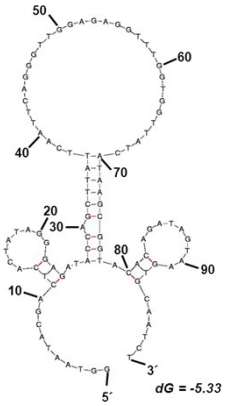
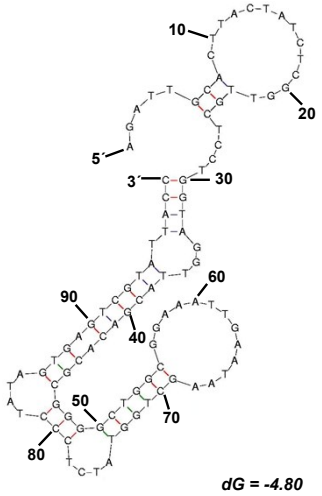
| Clone | Selected sequences | $K_D$ (nM) | Structures |
|---|---|---|---|
| GST-2 | CAGGGTTGGAGAGGTTTGGT<br>GGTTATCATAAGCGGTACAC | **0.002 ± 0.003** |  |
| DIG-1 | CGGTTGCTCCTGGTAGGTTA<br>CGACACGCGGGGCTGGCGGA | **0.007 ± 0.001** |  |

**Table S2** List of selected aptamers and target binding affinities of each aptamer as determined from the direct coupling SPR assay.

| Clone | Selected sequences | Sequence size (bp) | Identical sequences | $K_D$ (nM) |
|---|---|---|---|---|
| GST-1 | ACCGCTGCAATCGTGTGCCATACACAGATGATCCGCACA | 39 | 3 | 0.019 ± 0.028 |
| **GST-2** | **CAGGGTTGGAGAGGTTTGGTGGTTATCATAAGCGGTACAC** | **41** | **2** | **0.002 ± 0.003** |
| GST-3 | TAGGTTTGGGTAGGTTGGAATGGTGAATAATATGGGACGG | 40 | 6 | 0.015 ± 0.016 |
| GST-4 | CGGACGTTGCACTGGAAAAGGAGGTTTGGTTCGGTTGGTC | 40 | 2 | 0.119 ± 0.151 |
| GST-5 | AGACAGCATAGCACTGTAACGATTGGTTTGGTGTGGTTGG | 40 | 8 | 2.280 ± 1.250 |
| GST-6 | AGATATCAGGAGCGGGGTTTGGGGTGGTTGGCGGTGGACG | 40 | 2 | 95.110 ± 15.35 |
| GST-7 | GCTCTAAGTATACCGGTAGGGTTGGCTGCGGTTTGGCTCG | 40 | 3 | 0.014 ± 0.011 |
| GST-8 | ACTATCGGATTTCTGGGTTTGGATCGGTTGGCAGCGAGTG | 40 | 2 | 0.340 ± 0.121 |
| GST-9 | GCTCTAAGTATTCCGGTAGGGTTGGCTGCGGTTTGGCTCA | 40 | 2 | 8.37 ± 1.250 |
| **DIG-1** | **CGGTTGCTCCTGGTAGGTTACGACACGCGGGGCTGGCGGA** | **39** | **8** | **0.007 ± 0.001** |
| DIG-2 | ACCGTAACAGATAAAAGATTCTGTGATTGACTGCTTCTGG | 41 | 2 | 0.742 ± 0.024 |
| DIG-3 | CGCCGGCTAGGAAACAATTGGTTGGTGTGTTCGGCCCCTT | 40 | 3 | 0.030 ± 0.001 |
| DIG-4 | CAGTGAAACTTTGGTTGGCCGCCTGTAGGGTCACTTTGTG | 40 | 1 | 9.513 ± 0.413 |

| | | | | |
|---|---|---|---|---|
| DIG-5 | CCCCGCCAAGGTTTTTGCAGTTGGTCCCTCCGGTGATTCG | 40 | 2 | 2.491 ± 0.439 |
| DIG-6 | CGCATTCGACATAGCCGGTAGTAAAAGCATGGTCGCTTCA | 40 | 2 | 1.751 ± 0.112 |
| DIG-7 | CCAAAGTGGGATTTTTTAGTGGTTGCATCGCGAGACAGCT | 40 | 2 | 1.035 ± 0.457 |
| DIG-8 | CGAGGTAGCAGGTTGCTTGCGGCTAAAGCATTTTCATGCT | 40 | 2 | 1.442 ± 0.318 |
| DIG-9 | CACAAAGGAATGAACGATGCGATCTGGTAGCTTCTTTGGC | 40 | 3 | 0.011 ± 0.017 |
| DIG-10 | CGTGGAATGTTGTTGCTTCTGCTACGATACGTCAACCCTC | 40 | 1 | 1.775 ± 0.571 |
| DIG-11 | AGGTGTAGATACCCAGGCGTATGGTTGCGCTGTTTCGTGG | 40 | 2 | 4.001 ± 0.672 |
| DIG-12 | CAAGGTGTCGCACTTTTTGACGTTGGACTGCTTCGAACAG | 40 | 2 | 2.184 ± 0.753 |