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Supporting Information

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# High-Throughput Bead-Based Identification of Structure-Switching Aptamer Beacons

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cbic_201402037_sm_miscellaneous_information.pdf

**Table SI**. Sequences identified from selectedions.

| Name | Sequence 5' - 3 ' | Activity | Count |
|---|---|---|---|
| **LN8 library** | CTCGGGACNNGGATTTTCCNNNNACGAAGTNNTCCCGAG | | |
| diss.1A | CTCGGGACGTGGATTTTCCACATACGAAGTTGTCCCGAG | +++ | 85/90 |
| *diss.1** | CTCGGGACGTGGATTTTCCGCATACGAAGTTGTCCCGAG | +++ | |
| diss.2 | CTCGGGACATGGATTTTCCATAAACGAAGTGGTCCCGAG | ND | 4/90 |
| *Dis6th-#43* | GACATGGATTTTCCATAAACGAAGTGGGTC | | |
| | | | |
| **LN12 library** | CTCGGGACNNNGGATTTTCCNNNNNNNACGAAGTNNTCCCGAG | | |
| diss.1A.12 | CTCGGGACGTTGGATTTTCCCAAACATACGAAGTTGTCCCGAG | + | 28/93 |
| *diss.1** | CTCGGGACGT GGATTTTCC GCATACGAAGTTGTCCCGAG | | |
| diss.3.12 | CTCGGGACGTTGGATTTTCCTATACTAACGAAGTGGTCCCGAG | ND | 16/93 |
| *Dis6th-#47** | GACGT GGATTTTCC ACTAACGAAGTGGGTC | | |
| diss.4.12 | CTCGGGACTCAGGATTTTCCATCCGGTACGAAGTAGTCCCGAG | - | 10/93 |
| *Dis6th-#42** | GAC ACGGATTTTCC TCCG ACGAAGTGAGTC | | |
| diss.5.12 | CTCGGGACGTAGGATTTTCCCTTCGGCACGAAGTCGTCCCGAG | + | 8/93 |
| diss.6.12 | CTCGGGACGCCGGATTTTCCCGAGCACACGAAGTTGTCCCGAG | + | 5/93 |
| diss.6.12 -related | CTCGGGACAGTGGATTTTCCAGTCCACACGAAGTTGTCCCGAG<br>CTCGGGACAGTGGATTTTCCTATACACACGAAGTTGTCCCGAG<br>CTCGGGACCGTGGATTTTCCGAGCCACACGAAGTGGTCCCGAG | ND<br>ND<br>ND | 5/93<br>5/93<br>1/93 |

* denotes previously identified sequences from [13].

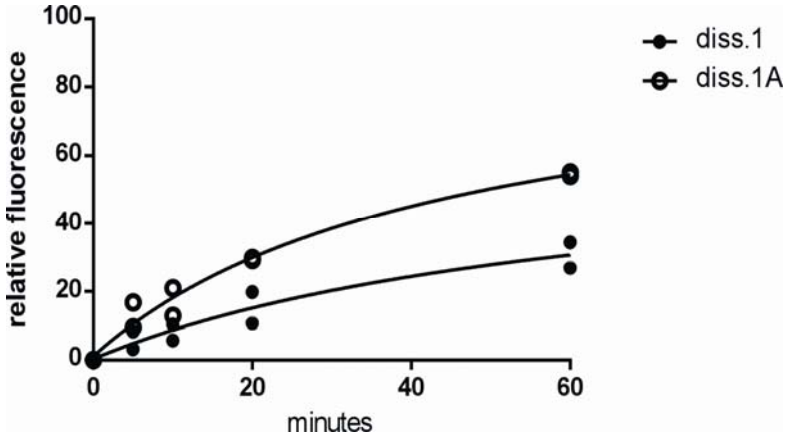


**Figure S1**. Time course binding activity of diss.1 and diss.1A for DIS (15 μM). Binding curves were determined by plotting the mode of relative fluorescence of two independent experiments.

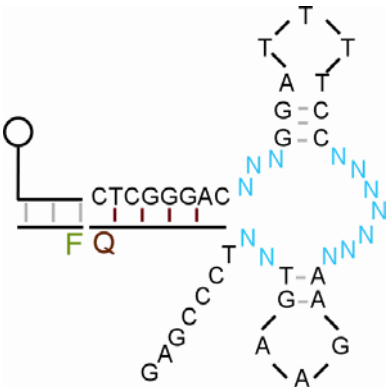


**Figure S2**. Schematic of LN12 library

## MATERIALS AND METHODS

**Nucleic acid sequences**:

Double-biotinylated primer:
BB-CTGGTCATGGCGGGCATTTAATTCAATT

5′ FITC-labeled oligonucleotide:
FAM-CCTCAATACCACCACGTATTCATGCGAGATGGCC

3′ DABCYL-labeled oligonucleotide:
GTCCCGAGAGCAG-Dab

Forward primer:
CTGGTCATGGCGGGCATTTAATTCAATTACGTGGTCATCGTCCTGGTGGCCATCTCGC

Reverse primer:
AGTCGTACGTTAGGAGGACT

LN8 library*:
CTGGTCATGGCGGGCATTTAATTCAATTACGTGGTCATCGTCCTGGTGGCCATCTCGCATGAATA
CGTGGTGGTATTGAGGCTGCTCTCGGGACNNGGATTTTCCNNNNACGAAGTNNTCCCGAGTCCT
CCTAACGTACGACT

LN12 library*:
CTGGTCATGGCGGGCATTTAATTCAATTACGTGGTCATCGTCCTGGTGGCCATCTCGCATGAATA
CGTGGTGGTATTGAGGCTGCTCTCGGGACNNNGGATTTTCCNNNNNNNACGAAGTNNTCCCGAG
TCCTCCTAACGTACGACT

All oligonucleotides and libraries were purchased from IDT with the exception of the 5′ double-biotinylated primer which was synthesized in-house by standard solid phase DNA synthesis using an Expedite 8909 DNA synthesizer. The double biotin was put on during synthesis using two successive couplings of a Biotin-TEG-phosphoramidite. The oligonucleotide was synthesized DMT-ON and, following standard deprotection, purified by reverse phase HPLC using a Waters Xbridge C18 column heated at 60˚C, using 0.1 M triethylammonium acetate pH 7.5 and a linear gradient of acetonitrile. Following purification, the trityl group was removed by brief (20 min) treatment with 80% acetic acid, and the trityl achohol was removed by extraction with ethyl acetate. All synthesis reagents were purchased from Glen Research (Sterling, VA).

*We found that a shorter (42 instead of 84 base) linker between the 5′ double-biotinylated base and the complementary region to the complementary region where the FITC-loaded oligonucleotide binds

resulted in a weak fluorescence signal (not shown). This may be due to absorption of light by the dark, iron particle.

**emulsion PCR**:

~2.1 x $10^8$ MyOne streptavidin-coated magnetic beads (Life Technologies, 1 µm, 30 µL) were washed and resuspended in 50 µL of B+W buffer (10 mM Tris pH 8.5, 2 M NaCl, 1 mM EDTA) by collecting the beads over a neodymium magnet (2 $cm^3$). 30 pg of LN8 or LN12 double-biotinylated DNA library dissolved in 50 µL of $dH_2O$ were mixed by pipetting, vortexed briefly and shaken for 15 min. Beads were then saturated by adding 30 µL of double-biotinylated primer (10 µM) to the mix for an additional 15 min on a shaker. The beads were then washed by five 200 µL washes of 1x PCR buffer. 200 µL of PCR reaction mixture was added to the beads and mixed by pipetting.

The PCR bead mixture was emulsified in oil (584 µL Tegosoft WEC, 60 µL Sigma light mineral oil, 56 µL WE09, equilibrated at RT for 30 min) in a dropwise fashion over the course of 1 min over a bed of ice while mixing with a Spinplus cross-stirbar (3/8") at 1250 rpm. The entire water-in-oil emulsion was mixed for an additional three minutes. The emulsified PCR reactions were aliquoted into five standard 250 µL PCR tubes and thermocycled.

**ePCR Mix**:

Forward primer, 50 nM
Reverse primer, 8 µM
dNTPs, 200 µM each
BSA, 0.5 mg/mL (prepared at 10 mg/mL, filtered by 0.46 µm)
1X PCR buffer
16 µL Taq (2 U/µL)

**Emulsification**:

The oil was mixed fresh daily in a cryosoft tube, briefly vortexed and allowed to sit at room temperature for at least 30 min.

The water-in-oil emulsion was prepared by adding PCR mix to the oil in a dropwise fashion over the course of 1 min over a bed of ice while mixing with a Spinplus cross-stirbar (3/8") at 1250 rpm. The entire water-in-oil emulsion was mixed for an additional three minutes. The emulsified PCR reactions were aliquoted into five standard 250 µL PCR tubes and thermocycled.

**ePCR cycle parameters**:

1. 90°C 45 s
2. 94°C 15 s
3. 55°C 38 s
4. 72°C 75 s
5. Go to 2. 36 times
6. 72°C 5 min

We found that >36 cycles resulted in visible compartmental breakage.

**Recovery of beads from emulsion**:

750 µL butanol was added to the emulsion and mixed by pipetting. 500 µL PB (5M guanidine HCL, 30% isopropanol with 20 mM Tris pH 6.8) was added to the broken emulsion and briefly vortexed. Beads were pelleted (6 000 *g* for 5 min) and washed in PB once (500µL).

**Preparation of the ssDNA functionalized particles**:

DNA-loaded beads were collected by magnet, and 30 µL of NaOH (0.1 M) was added. Beads were sonicated for a quick pulse (<1 s) and shaken for 5 min, twice, neutralized with TBST, then washed several times with PBS. Saturating levels of FITC- and DABCYL- oligonucleotides were annealed to the bead-immobilized DNA in PBS at room temperature for at least 15 min. Excess oligonucleotides were removed by three PBS washes (100 µL each). The beads were then washed in Selection Buffer (20 mM Tris pH 7.5, 1 M NaCl, 10 mM $MgCl_2$) three times (100 µL each).

**FACS sorting parameters**:

Prior to sorting, the drop delay was determined by standard Accudrop protocols, and the filter was removed for detection of the small beads. DNA device-loaded beads were sorted by a FACS Aria III through a 70 µM nozzle at ~10,000 events/s. All sorts were carried out in the "single-cell" mode, which discards droplets that are positive but that contain negative events. For negative sorts, the "bottom" (left-most) 75% of the quenched population was collected. For positive sorts, when no positive population was present, the collection gate was set according to the "top" (right-most) 50% of the un-quenched population. When a positive population was apparent (LN8 R3 and LN12 R3) the gate was set to collect the top 50% of the observed positive population. For negative sorts collected in the absence of target (to purge false positives), at least ten million beads were collected for the subsequent positive sort. This guaranteed full coverage of the initial starting complexity of the library (~$10^5$ for the LN8 library, and ~$10^6$ of the second round of the LN12 library), taking the large fraction of beads lost during collection into account. For positive sorts, beads were collected until the entire sample was exhausted (typically between 500 and several thousand beads). DIS was dissolved in methanol and did not exceed 0.2% of the final selection assay volume. Negative sort buffers contained 0.2% methanol.

**Bead collection and re-annealing between negative and positive sorts**:

For concentrating negatively-sorted beads from large volumes, 10% TBST was added to the flow-through, and eluant was centrifuged in 15 mL conical tubes at 12 000 *g* for 15 min. The conical tubes were the rotated 180º in the bucket to prevent aggregation on the walls of the tubes and centrifuged at the same speed for an additional 15 min.

For concentrating negatively sorted beads from smaller volumes, 10% TBST was added to the flow-through and centrifuged in 1.5 mL microcentrifuge tubes at 15 000 *g* for 5 min.

Collected beads were washed in PBS and re-annealed to excess 5' FITC-labeled and 3' DABCYL-labeled oligonucleotides, washed in PBS, and then washed and equilibrated in binding buffer before the addition of DIS (200 µM) for ~25-45 min prior to positive sorting.

**Recovery of bead-bound DNA by PCR**:

TBST (10% final) and ~40,000 blank, carrier beads were added to the solution of post-positively sorted beads (in FACS buffer) and centrifuged for 10 min at 16 000 *g*. Control PCR reactions containing known amounts of beads bound to ~40 000 copies of DNA library each provided a valuable estimate for how many cycles were required. Thus, tracking the amount of positive beads sorted allowed us to estimate the required amount of cycles with high accuracy. PCR mix was standard except for the addition of 0.5 mg/mL BSA.

**Recovery PCR parameters**:

1. 90°C 45 s
2. 94°C 15 s

3. 55°C 38 s
4. 72°C 55 s
5. Go to 2. x times
6. 72°C 25 s

X was typically ~20 for 700 beads, ~18 cycles for 7 000 beads.

**FACS-based binding activity assays**:

300 ng of 5′ double-biotinylated DNA were incubated with 1 µL of MyOne streptavidin-coated magnetic beads (1 µm) in B+W buffer on a shaker for 1 h at room temperature (60 µL final volume). Beads were washed (100 µL) and resuspended in 0.1 M NaOH (30 µL), briefly sonicated (< 1 s) and incubated on a shaker for 5 min. This step was repeated once to fully break-up any aggregates and to thoroughly release unbound DNA. Next, beads were washed and neutralized in PBS (pH 7.0, 60 µL final volume) and annealed to saturating amounts of 5' FITC-labeled oligonucleotide (0.3 µL of 100 µM stock) by heating at 50°C for 15 s and cooling to room temperature. 3' DABCYL-labeled oligonucleotide (0.9 µL of 100 µM stock) was annealed at room temperature, and free oligonucleotides were washed with selection buffer (3 x 100 µL). Aliquots of beads were then incubated in the presence or absence of DIS or DCA in Selection Buffer for 30 min (or a time course, Figure S1) and analyzed by flow cytometry.

Relative fluorescence was determined for ~8 000 singlet events by:
$(T - Q)/(F - Q)$ x 100

where $T$ = mode of fluorescence in presence of target, $Q$ = absence of target, and $\underline{F}$ = no target or quencher oligo. Apparent constants were determined by nonlinear fitting of this data on GraphPad Prims 6.

**Quantitation of ePCR- amplified DNA on the bead**:

To determine the number of DNA molecules on each bead, beads that were amplified by ePCR were compared to beads loaded with known amounts of DNA and analyzed by FACS. Using this approach, we routinely generated a population of beads of which ~40% contain ~40 000 copies of a single sequence (not shown), ~15% contain two or more sequences, and ~15% do not contain any detectable DNA.