Supporting Information

Rational approach for generating cardiac troponin I selective Spiegelmers

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CONTENTS

1. Reagents

target protein: cardiac Troponin I (cTnI) (ab50803, Abcam, Cambridge, UK)
control proteins: skeletal Troponin I (sTnI) (ab9919 , Abcam, Cambridge, UK)

cardiac Troponin I-T-C complex (8T62, Hytest, Turku, Finland)
matrix: Troponin I-free serum (8TFS, Hytest, Turku, Finland)

Name	L-DNA sequence (5′-3′) (Spiegelmers)
A4	AGTCTCCGCTGTCCTCCCAGTGCAGGCTGAGTGGGTGGGTGGGTGGTGTGGC CACGTTGGGATGACGCCGTGACTG
B10	AGTCTCCGCTGTCCTCCCGATGCACTTGACGTATGTCTCACTTTCTTTTCATTGA CATGGGATGACGCCGTGACTG

Table S1. L-DNA oligonucleotides were custom-synthesized by IBA (Göttingen, Germany) with Thiol C3 TEG label at the 5' terminus of the DNA strands

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All other reagents such as inorganic salts and buffer components were of highest bioanalytical grade from Sigma-Aldrich. Solutions were prepared with deionized water (18.2 MΩ cm resistivity, Millipore).

2. Selection of Spiegelmers

500 µg of D-peptide with the sequence: CdAdYdAdTdEdPdHdAdK (Bio Basic Canada, Ontario, Canada) was conjugated to 12 mg of autoreactive SiMAG-Bromoacetyl paramagnetic particle (Chemicell, Berlin, Germany) according to the manufacturer's instructions. Following peptide coupling, the particles were blocked with 100 µL of 50 mM cysteine solution, resuspended in 1 mL pH 7.4 phosphate buffered saline (PBS) containing 0.02% NaN_3 and stored at 4°C.

The combinatorial library of single-stranded DNA molecules was synthetized with 40 random nucleotides flanked by fixed primer sequences; 5′-agtctccgctgtcctccc-40N-gggatgacgccgtgact-3′ (Integrated DNA Technologies, Leuven, Belgium). Prior to SELEX procedure, the ssDNA library pool was heated to 95°C for 5 min and then immediately chilled on ice. First, 2 ml of the PBSE selection buffer (PBS, 10 mM EDTA and 10 µg mL^{-1} BSA, 0.1 µg mL^{-1} dIdC) containing 1 nmole oligonucleotide library stock solution was incubated with cysteine coated, peptide-free beads by shaking at RT for 1 hour to eliminate the oligonucleotides which could bind either to matrix of magnetic beads or to cysteine. Following the counter-selection, the supernatant was used for the first round of SELEX. The ssDNA pool was incubated at RT for 1 hour with 5 µg bead bound D-peptide. The non-specifically bound oligonucleotides were discarded by washing the resins 3x 100 µL of PBS. The washed beads were resuspended in 65 µL distilled water and heated at 95°C for 5 min to obtain the peptide bound oligonucleotides. The putative Spiegelmer enantiomers were amplified by using the supernatant as the template of PCR. The 100 µL of PCR mixture contained the 65 µL of eluted oligonucleotides, 20 µL 5 × HF reaction buffer, 2 U of iProof polymerase (Bio-Rad, Hercules, CA, USA), 0.5 µM forward and biotinylated reverse primers, and 0.8 mM CleanAmp (Trilink Biotechnologies, San Diego, CA, USA) dNTPs. The thermocycling parameters were 5 min at 95°C; 15 cycles of 10 s at 95°C; 10 s at 63°C; 10 s at 72°C, the final extension was 2 min at 72°C. After checking the PCR product quality on 3 % agarose gel, the dsDNAs were immobilized on SiMAG–Streptavidin magnetic beads (Sigma-Aldrich, St Louis, MO, USA) and non-biotinylated strands were separated from the complementary strand by 5 min incubation with 100 µL of freshly

prepared 100 mM NaOH. The ssDNA was removed and 15 µL of 1 M NaH_2PO_4 buffer was added to neutralize the solution. Selection cycle was repeated nine times with gradually decreasing peptide concentration, incubation time and more vigorous washing condition to increase the affinity of selected aptamers. In the last selection cycle, 1 µg D-peptide, 15 min incubation time, and 3 × 5 min washing with PBS was applied. To avoid enrichment of nonspecific ssDNAs during the SELEX, a further counter-selection was performed with uncoated beads following 5th round of selection. The final PCR was carried out with non-biotinylated reverse primer and the resulting product was purified by agarose gel separation and extraction. Following purification of the enriched oligonucleotide library, 2 µL of PCR mixture was inserted into pCR-Blunt-II TOPO vector (Life Technologies). OneShot Top10 *E. coli* competent cells were transformed with the ligation mixture and spread on kanamycin LB plates. 85 separate colonies were picked and colony PCR was performed with M13 primers. Finally, the PCR products were mixed with M13 reverse primer and sequences were determined by capillary sequencing (Macrogen Europe, Amsterdam, Netherlands) (Table S2).

Identity code	Sequence	Copy number
A4	AGTGCAGGCTGAGTGGGTGGGTGGGTGGTGTGGCCACGTT	32
E6	GACAAGACTACCTCGCTAGTACTACTCATTCTATTCCGAT	2
C5	GAGGTGAGTGTGTGTGGGTGGGTGGGTGGTTAATGATCGT	2
G3	GATGCAGGTCGATATGTATATCGTACTTATCTGTTACGTT	2
H3	GATGTCAGTGGTGTTGGGGGGGTAAGGTGTGCAGATGGTT	2
C11	GGTGCAGGGTTGGTGGGTGGGGGGGTGGTGTGGCCACGTT	2
E10	AAACATACCGTAACGTATTAATTTCACTGACAATTCTTCC	1
H9	AACCAACATGCATCCCGACAATCATTATTCACCATTCATC	1
F10	AACCCATGAGTGCATGATTGTGTCTAACCTTAGTACGAAT	1
D2	ACCGTGTCGCAACCTCCCTCATGTATCCTACTCACCATCC	1
E11	ACGGCGCCACACCACCCAGGGTGGGTGGTGTGGCCACGTT	1
B9	AGCGAGTGGAGGTGGGTGGGTGGGTGGTGTATGGAGTGA	1
E7	AGGGAGAGTTAGTACCACATCTGGTGTATTGTGTTGCATC	1
C10	AGGTGAAGTGCGGCAATACGCGTATGATATATCTCTTCGT	1
A9	AGTGCAGCATACATATCTCAACTACATTTCTCACTCCGCA	1
G12	AGTGTAGTAGGTAATATGAGTGTGCCATGGAGGGGAGGTT	1
B6	AGTTGATGCTGGTCATTACATGCCACCATACCATGAGGTA	1
C6	ATCGTACGGAGGGTGGGTGGGTGGTGTATGAGTGGAGGTC	1
B2	ATCTGCAATGACTATTGCATCATTTATCCCCTCTTACCTC	1
E4	ATGATGCAGTAAAGTGTACTGGGGCATGTGTGTGGCGGTT	1
C7	ATGCGTGGTGATACATATTCTTTTCGTGTTTACATTGTAT	1
D7	ATGCTGCGGTATGAGTAGTGCATACTATACGTTTCTCGTT	1
F7	GACGAGGAATGATGTGTACCGTATGGAGTGGAACTGCATT	1
A6	GATGAATCGACCCTACCCATTACATCATCGTTGACAGCT	1
H4	GATGACAATGGTGTCATGATGTACATAATGTAGTTCTCGT	1
C2	GATGATATGTTGCGCTTTATTGTCTCTAACTAATGGTGAC	1
B10	GATGCACTTGACGTATGTCTCACTTTCTTTTCATTGACAT	1
F12	GATGCCGGAGTGGGTGGGTGGGTGGTGGTGAGGAGACAGG	1
H2	GATGCGTACATGAATTCTATCACATCATTGTATATTCGTT	1
D1	GATGTAATGCGTGTTCCTCTGCTGAAGGTGCAACCTAGCT	1
E9	GATGTACACTCACCGAAGAACTACCTTGCCGTATGCTATT	1
D5	GATGTAGGTGTTGTACCCATATGCTTGTTGTTCGTTCGTT	1
E3	GATGTGGTAAATGAATGTGTTTCGGAGCTGAGGTGCAGGT	1
H11	GATGTGTAAGGATTATCTAACCTTACATGTATCATTCGTT	1
B12	GCATCCACGATCAAACGTTGATACACTTTCTCTCATCACT	1
H1	GGTGATGGCAAGTGTAGTTTATACGTTAGTAACGTGATGT	1
F6	GGTGCAGGATTACATTAATGGATTACAGTTGATTTTCGTA	1
A5	GGTGGAAGCACACGTACACCCTAGATACAGGACGACCGTT	1
C1	GGTGGTGGTGGTACGGTGCTAATGAGGGAAGGAGGGGGAT	1

G4	GGTGTGGGTGGGTGGGAGGTGAGGTTGGGACAGTGGGGGT	1
D4	GTGATCATATCGTGCACTATCACAGGTATGTTGCGGCGAT	1
D12	GTTGTGGTAGAGCTAGGGGTTGGACGGAACATATTAGTGT	1
G1	TATGTAGGCCATGCACTACCACCTTATTTACCGTGTAACT	1
F8	TGCCCACGCTTTAACGTAGCATACTGTATCACATTTCCTT	1
C9	TGTGCATGTTAGTCCGGTGTAGATAGTTGGGTGTTGGGTT	1
A2r	AGTGGGTGGGTGGGTGGTCGGGTAGGGAGAGC	2
E1r	ACACACCCGCAGAGTATCGTAATTACGAATTTACACC	1
C4r	GACACGTCGCGTTAGTAACATTGGTATACATGACCGT	1

Table S2. Variable sequences (flanking region not included) of oligonucleotides isolated by the SELEX procedure. The highlighted Spiegelmer candidates were characterized by SPRi.

3. Procedure for spotting SPR sensor chips with terminal thiol-modified Spiegelmers

Bare gold SPR sensor slides were ordered from HORIBA Jobin Yvon S.A.S. (Palaiseau, France). Prior modification, the slides were treated with UV/ozone (Novascan Technologies, Ames, IA, USA) for 15 minutes. Spotting was performed with the BioOdyssey™ Calligrapher™ miniarrayer (Bio-Rad, Hercules, CA, USA) using solid contact pin (Stealth Solid Pin, 375 μm, Arrayit, Sunnyvale, CA, USA). The thiolated L-DNA probes were spotted onto the gold surfaces from a 384 well LD-PE plate blocked with protein free blocking buffer (Pierce, Thermo-Fisher, Rockford, IL USA) for 1 hour and washed with DI water. At least three parallel points were spotted for each probe. The spotted gold sensors were incubated in a humidity chamber at 65 rh% and 15°C for 12 h, Afterwards the chips were blocked with 0.1 mM (11-mercaptoundecyl)tetra(ethylene glycol) (HS-TEG) (in PBS) for 15 min, then washed with DI water and dried under N_2 stream.

4. SPRi measurements

Surface plasmon resonance imaging (SPRi) measurements were performed using HORIBA SPRi-Plex II (HORIBA Jobin Yvon S.A.S. Palaiseau, France) instrument. The sensor slide was fixed onto the prism using index matching oil and then was inserted into the instrument. After selection of the working angle, a calibration was performed with 180 mM NaCl containing PBSE to normalize the SPR response of each spot with the signal change measured in the respective location for a given refractive index in the solution. The Spiegelmer (aptamer) interactions were measured in PBSE working buffer or diluted human serum at 25.00°C, at a flow rate of 50 μL min^{-1}. The injected volume for each analyte and regeneration solution was 180 μL. For regeneration of the Spiegelmer (aptamer) modified surface after the interaction 20 mM NaOH solution was used (50 μL min^{-1}, for 4 min). Evaluation of interaction kinetics was performed using Scrubber 2 GenOptics version (BiaLogic Software, Campbell, Australia).

5. Screening Spiegelmer candidates by SPRi

Selected D-DNA aptamers (A4, B10, C11, D12) were PCR amplified using biotinylated forward primer and precipitated to a final concentration of 10-20 μM. These PCR products were spotted onto ExtrAvidin activated SPRi sensor slides (HORIBA Jobin Yvon S.A.S.) and denatured by injecting 20 mM NaOH (50 μL min^{-1}, 180 μL). The binding of the immobilized D-DNA aptamers to D-cTnI peptide was determined by SPRi injecting the peptide at various concentrations (50-800 μM).

6. Stability analysis of Spiegelmer modified sensor slide

The above described, B10 Spiegelmer spotted sensor slide was dried with N_2 stream and stored at 4°C for 6 months. To measure the SPR signal, 330 ng mL^{-1} cTnI was injected four consecutive times with sensor chip regeneration between each injection with 20 mM NaOH.

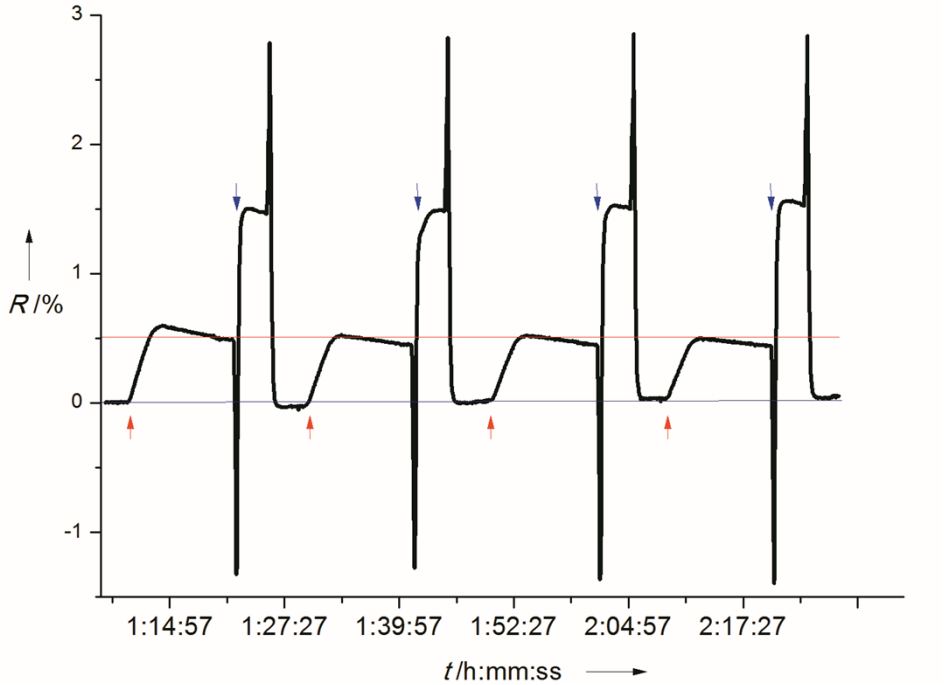

Figure S1. Background corrected raw SPR transients of multiple cTnI injection (330 ng mL^{-1}) and regeneration (20 mM NaOH) cycles measured with a Spiegelmer (B10) modified SPR chip. cTnI injections are indicated by upward (red) arrows, while the regenerations are marked by downward blue arrows. R is the reflectivity change in % (1% corresponds to 185 pg mm^{-2} of surface adsorption).