

## **SUPPLEMENTARY DATA**

# **Spiegelmer-based sandwich assay for cardiac troponin I Detection**

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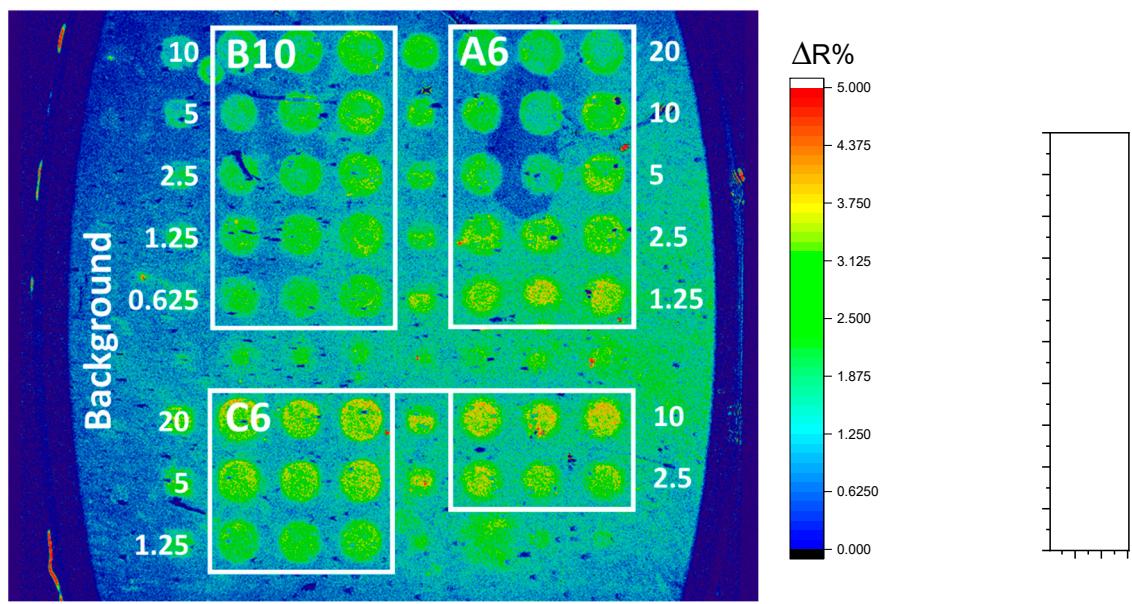
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**Supplementary Table 1.** L-Nucleic acid sequences of the studied spiegelmers.

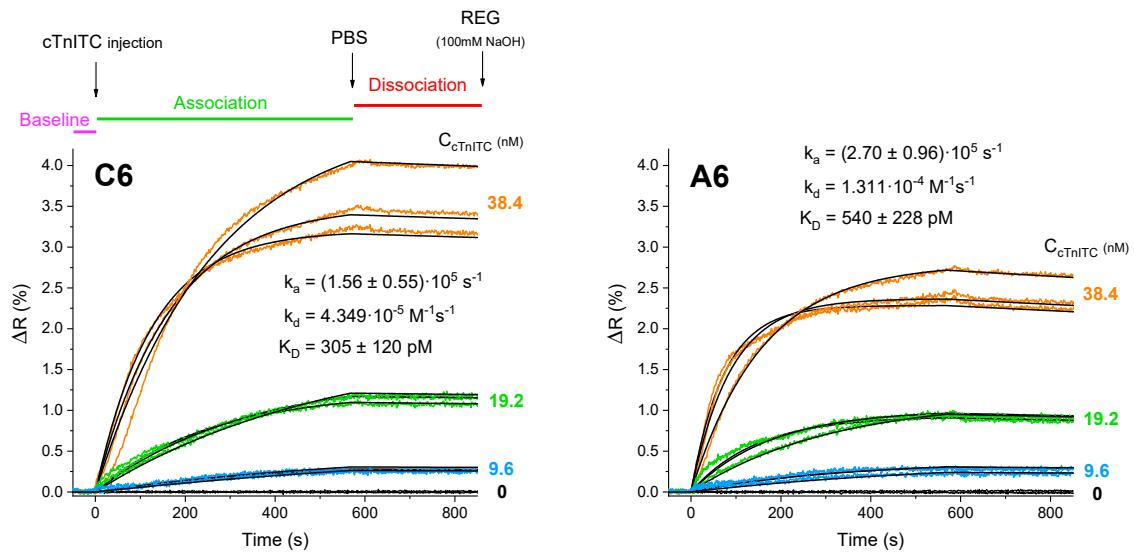
Name	Sequence
B10	5'-AGT CTC CGC TGT CCT CCC GAT GCA CTT GAC GTA TGT CTC ACT TTC TTT TCA TTG ACA TGG GAT GAC GCC GTG ACT G-3'
A6	5'-CAG TGA GTG ATG GTG AGG GCT TAG TTC GCC GCT CAT GCC GAA TCT CCT GTA TAA ATA CCC ACA CTG TCC ATA CAC G-3'
C6	5'-CAG TGA GTG ATG GTG AGG GTG AAT CGG TGT CGA CTA TTA AAT TAA GTT GTG GTT CCC ACA CTG TCC ATA CAC G-3'
C6 short	5'-TG AAT CGG TGT CGA CTA TTA AAT TAA GTT GTG GTT GTT-3'

**Supplementary Table 2.** Conditions of SELEX cycles.

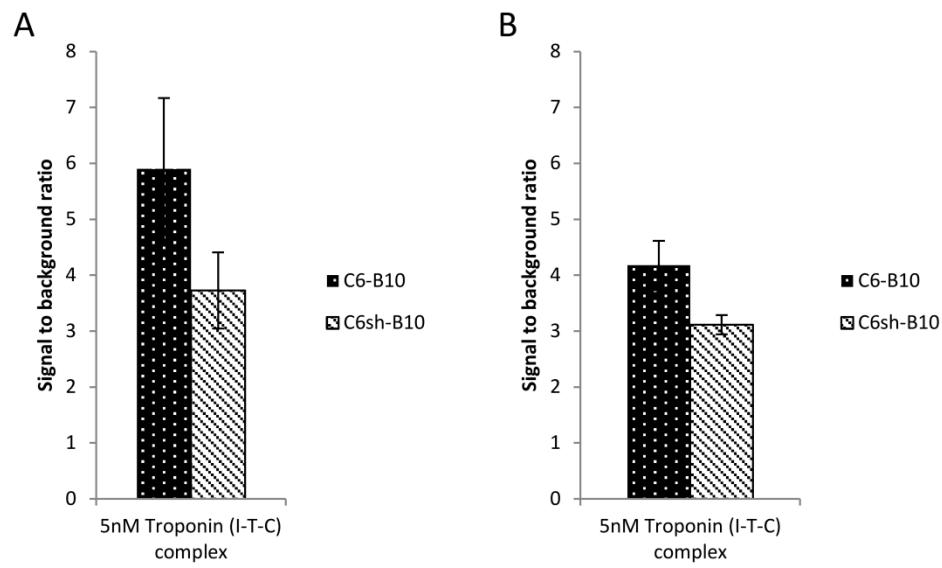
SELEX cycle number	Amount of target molecules	Incubation time	Buffer composition	Washing steps
1. Counter-SELEX	-	1 hour	2 mL PBS	-
1.	500 pmol cTnI peptide	1 hour	Supernatant of the 1. counter-SELEX 1 mg mL <sup>-1</sup> BSA 0.01 µg mL <sup>-1</sup> poly (dI-dC) 5mM EDTA	3x100 µL PBS
2.	375 pmol cTnI peptide	30 min	2 mL PBS 1 mg mL <sup>-1</sup> BSA 0.01 µg mL <sup>-1</sup> poly (dI-dC) 5mM EDTA	3x100 µL PBS
3.	375 pmol cTnI peptide	30 min	2 mL PBS 1 mg mL <sup>-1</sup> BSA 0.1 µg mL <sup>-1</sup> poly (dI-dC) 5 mM EDTA	3x100 µL PBS
4.	250 pmol cTnI peptide	30 min	2 mL PBS 1 mg mL <sup>-1</sup> BSA 0.1 µg mL <sup>-1</sup> poly (dI-dC) 5 mM EDTA	3x5 min, 100 µL PBS
2. Counter-SELEX	400 µg cTnI free serum proteins	1 hour	2 mL PBS 1 mg mL <sup>-1</sup> BSA	
5.	250 pmol cTnI peptide	30 min	Supernatant of the 2. counter-SELEX 0.1 µg mL <sup>-1</sup> poly (dI-dC) 5 mM EDTA	3x5 min, 100 µL PBS
6.	250 pmol cTnI peptide	15 min	2 mL PBS 1 mg mL <sup>-1</sup> BSA 0.1 µg mL <sup>-1</sup> poly (dI-dC) 5 mM EDTA	3x5 min, 100 µL PBS
7.	250 pmol cTnI peptide	15 min	2 mL PBS 1 mg mL <sup>-1</sup> BSA 0.1 µg mL <sup>-1</sup> poly (dI-dC) 5 mM EDTA	3x5 min, 100 µL PBS, 0.3 mM dextran sulfate



**Supplementary Figure 1.** Differential SPR image of spiegelmer microarray microspotted in triplicate from various concentration of L-DNA probe solutions ( $0.625$ - $20 \mu\text{M}$ ). The color of the spots changes with the amount of bound cTnI-T-C protein, as shown on the scale on the right.



**Supplementary Figure 2.** Interaction curves upon injection of different concentration of the target protein (9.6, 19.2, 38.4 nM) recorded for C6 (left) and A6 (right) spiegelmer spots immobilized at 2.5  $\mu\text{M}$ . Kinetic curves were fitted one by one to each parallel spot (3 replicates) on the same SPRi chip.



**Supplementary Figure 3:** The effect of spiegelmer truncation on the sandwich type assay. Selection buffer (A) or ten times diluted human plasma (B) was spiked with 5 nM ( $385 \text{ ng mL}^{-1}$ ) ternary complex and analyzed by AlphaLisa.