Supplementary Material and Methods

Reagents

The Spiegelmer was purchased from IBA (Göttingen, Germany) with biotin-TEG modification at the 5' terminus of the L-DNA. Custom oligonucleotides were synthesized by Sigma-Aldrich (St. Louis, MO). Human troponin I-free serum (8TFS), Troponin I-T-C complex (8T62), skeletal Troponin I (8T25) and monoclonal mouse anti-cardiac troponin I antibody (4T21/MAb 267) were obtained from Hytest (Turku,Finland). The recombinant cardiac troponin I (ab50803) protein was produced by Abcam (Cambridge, UK). AlphaLisa Protein-A Acceptor and Streptavidin Donor Beads were purchased from Perkin Elmer (Waltham, MA). All other chemicals in highest analytical grade were obtained from Sigma-Aldrich.

Spiegelmer B10:
5'-biotinTEG-AGTCTCCGCTGTCCTCCCGATGCACTTGACGTATGTCTCACTTTCTTTTCATTGACAT
GGGATGACGCCGTGACTG – 3'

Blocking oligo:
5' -AGTCTCCGCTGTCCTCCCGAACCAATGCCATGCTATTACGCAACTACACAACTGCACTGGGATGAC
GCCGTGACTG – 3'

Antibody crosslinking to Protein A acceptor bead

250 μg AlphaLISA Protein-A Acceptor bead was suspended in 100 PBS (pH 7.4) with 10 μg monoclonal anti-cardiac troponin I antibody and gently shaken for 2 h at ambient temperature. Following the antibody capturing procedure, the bead was centrifuged at 16,000 g for 15 min at 4 °C and washed using 200 μl 0.2 M triethanolamine (pH 8.2). The washing solution was discarded and the bead was incubated with 200 μl freshly prepared 20 mM DMP (dimethyl pimelimidate dihydrochloride) in 0.2 M triethanolamine (pH 8.2) for 30 min at 20 °C with mild agitation. After removing of the crosslinking solution, the reaction was stopped by resuspending and incubating the beads in 200 ul 50 mM Tris (pH 7.5) for 15 minutes. Finally, the crosslinked antibody-Protein A acceptor bead complex was washed twice with PBS and resuspended in 50 μl PBS. To prevent the bead aggregation, the solution was briefly sonicated before storage at 4 °C.

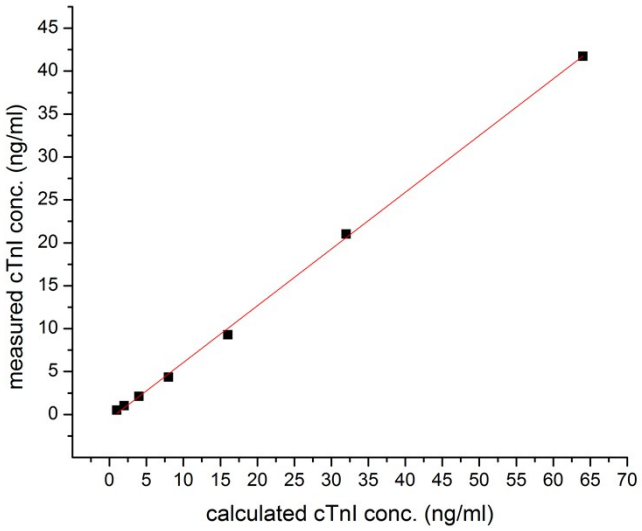
ALPHALISA Assay

To confirm the functionality of the troponin I specific Spiegelmer in complex matrices, an AlphaLISA-based, homogenous assay format was used. The measurements were implemented in serum, which was ten times diluted with binding buffer (PBS with 1 mg/ml BSA, pH 7.4). The final concentration of the donor and acceptor beads was 20 μg/mL and approx. 30 μg/ml, respectively. The Troponin I free human serum was spiked with various amounts of troponin I protein and biotinylated Spiegelmer, and non-biotinylated, blocking oligonucleotide were added to the samples at 10 nM and 20 nM final assay concentration, respectively. The mixtures were completed by addition of the anti-cardiac troponin I

antibody conjugated ALPHALISA acceptor bead, the assay plates were covered with sealing foil and incubated in dark at RT for 1 hr. In the next step, donor bead coated with streptavidin was added into each well and the plate was incubated in dark for another 50 min at RT and 10 min in the Enspire 2300 Multimode plate reader. The experiments were performed in 96-well 1/2 AreaPlate microtiter plates in a total volume of 40 µL per well. Luminescence signal was measured after the final incubation and assay plates were read in the AlphaScreen mode. The excitation and emission time was 35 and 65 ms, respectively and the temperature was set to 27 °C. All experiments were performed in triplicate.

Calculated concentration	Determined concentration
1	0,503
2	1,021
4	2,12
8	4,35
16	9,296
32	21,02
64	41,74

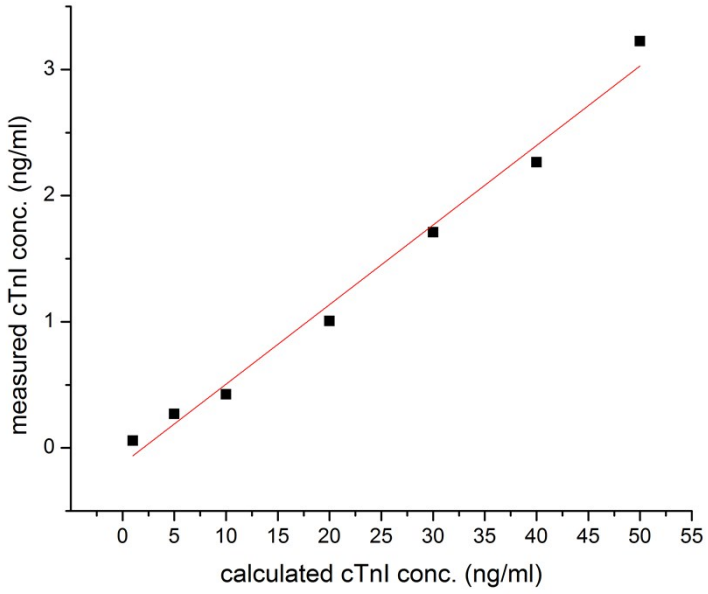
Supplementary table 1. The calculated and the ARCHITECT STAT Troponin-I immunoassay measured cTnI concentration of CTI complex spiked samples in ng/ml.



Supplementary figure 1. The actual cTnI concentration of the CTI complex supplemented serum samples determined by STAT Troponin-I measurement.

Calculated concentration	Determined concentration
50	3,22
40	2,26
30	1,71
20	1,00
10	0,42
5	0,27
1	0,06

Supplementary table 2. The calculated and the ARCHITECT STAT Troponin-I immunoassay measured cTnI concentration of recombinant cTnI spiked samples in ng/ml.



Supplementary figure 2. The actual concentration of the recombinant cTnI supplemented serum samples determined by STAT Troponin-I measurement.