Supporting Information for

Homogeneous assay for evaluation of aptamer-protein interaction

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Experimental Section

Reagents, instruments

Recombinant PSA-H virus coat protein with hexa-His tag (6His) was prepared according to Balogh et al.[1]
The HPLC purified PSA-H aptamer (5'-AGC AAG GTT TGG TGT TGG TTG GTT GCT GGT TTT GGT TTG GC-3'), the 3'-TEG-biotin labelled PSA-H and MT32 aptamer (5'-GGG GTG GTG GGT TCT TTT TGT GGT ATT GGT GTG GGG GGC A-3') were obtained from Sigma-Aldrich. The oligonucleotides were dissolved in DNase free deionized water.
AlphaScreen™ Histidine (Nickel Chelate) Detection Kit, with Nickel Chelate Acceptor and Streptavidin Donor Beads, was purchased from Perkin Elmer.
All other chemicals were of the highest analytical or bioanalytical grade from Sigma-Aldrich. Solutions were prepared with deionized water (18.2 MΩ cm resistivity; Millipore).
Perkin Elmer EnSpire 2300 Multimode plate reader was applied for luminescence measurements. The excitation and emission time was 35 and 65 ms, respectively, and the temperature was set to 27°C.

Binding Assays and luminescence background measurements

The assays were performed in binding buffer (10 mM phosphate buffer with 2.7 mM KCl, 137 mM NaCl, and 1 mg/ml BSA, pH 7.4) with using Perkin Elmer white, opaque 384-well microplates covered with sealing foil. The assay volume was 25 µl/well and the final concentration of the donor and acceptor beads were 20 µg/mL each. Acceptor and donor bead containing solutions were handled in green light (<40 lux).

The luminescence background studies were implemented by applying 12.5 nM of biotinylated aptamer and 100 nM His tagged protein final concentrations. 0.5 µl of

Biotinylated-6His of the Kit was used as positive control. The beads were mixed with either aptamer or protein containing binding buffer in absence and presence of 1 mg/ml BSA, respectively. Luminescence signal was measured following 1 hour incubation at 27 °C. The results show that the background luminescence originates already from the mixture of the donor and acceptor beads and it is not affected by adding either of the ligands into the bead mixture (Fig. S1). In all cases the addition of BSA effectively suppressed the background luminescence.

Identification of the optimal concentration of BSA as blocking agent was made by incubating the donor and acceptor beads with 12.5 nM biotinylated aptamer in the presence of various concentration of BSA for 1 hour at 27 °C (Fig. S2).

To determine the binding capacity of donor beads, diverse concentration of aptamer and constant amount of protein were incubated in binding buffer for 1 hour at 27 °C. Next, the beads were added and the mixture was incubated for 1 hour prior to measurement. The luminescence signal maximum at 12.5 nM biotinylated aptamer concentration was identified as the binding capacity for 25 µl of donor bead at 20 µg/ml concentration (Fig. S3).

To study the linearity of AlphaScreen signal upon changing bead concentration, the acceptor and donor beads were mixed at varied concentrations with 0.5 µl of Biotinylated-6His containing BSA completed binding buffer. The luminescence signal was determined after 1 hour incubation at 27 °C.

For saturation assay, the biotinylated aptamer concentration was held at 6.3 nM and the concentration of the hexa-His tagged protein was varied. This lower than maximum signal concentration (12.5 nM) of aptamer was applied to completely rule out the hooking effect. The aptamers and the PSA-H protein were first mixed in binding buffer and incubated at 27 °C for 1 hour. In the next step, the donor and acceptor beads were added to the mixture and incubated for another 1 hour prior measuring.

The competitive assay was essentially implemented as described above, but the 15 nM biotinylated PSA-H aptamer and 3 nM PSA-H protein containing binding buffer was complemented with various concentrations of non-labeled PSA-H aptamer.

Supplementary Figures

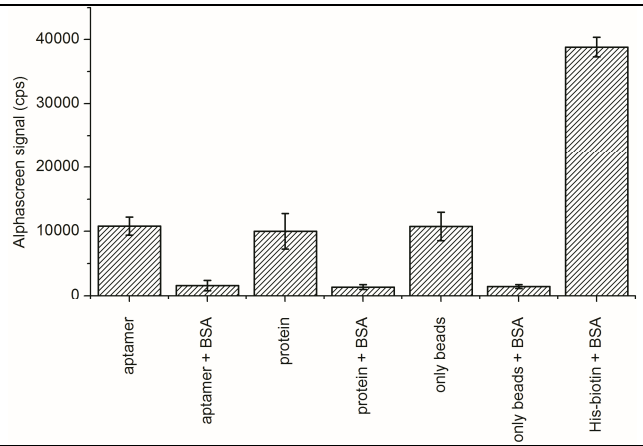

Figure S1. Background luminescence signal levels of different assay configurations. The acceptor and donor beads were incubated either with biotinylated aptamer, hexa-His tagged protein, or without addition any ligand. The considerable background signal was dramatically decreased by completing the binding buffer with BSA at 1mg/m final concentration.

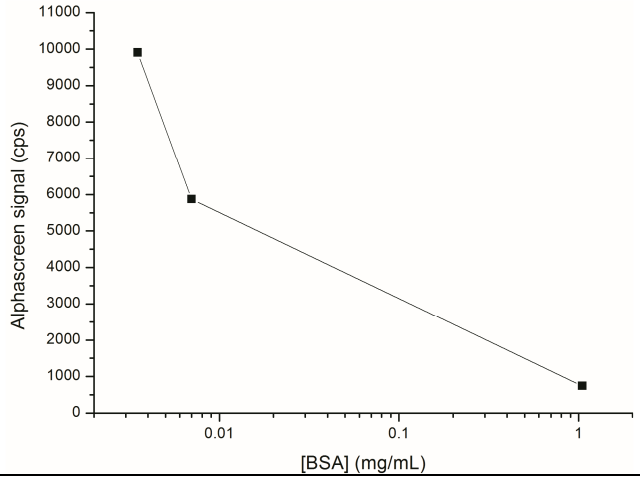

Figure S2. Determination of optimal blocking concentration of BSA. The acceptor and donor beads were incubated with biotinylated aptamer in binding buffer containing various concentration of BSA. The data indicate that 1 mg/ml of BSA concentration is needed for efficient background luminescence suppression.

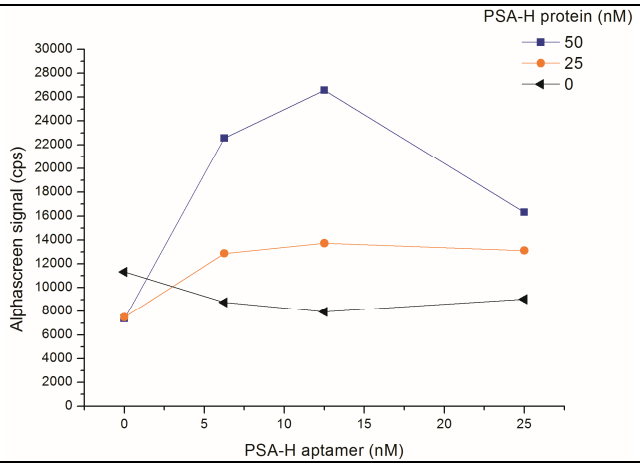

Figure S3. Saturation assays of streptavidin-coated donor beads. Various concentrations of biotinylated aptamer were incubated with constant amount of protein in binding buffer. The mixtures were further incubated following the addition of an equal concentration of donor and acceptor beads. The luminescence signal maximum was identified at 12.5 nM biotinylated aptamer indicating the binding capacity of 25 µl of donor bead at 20 µg/ml concentration.

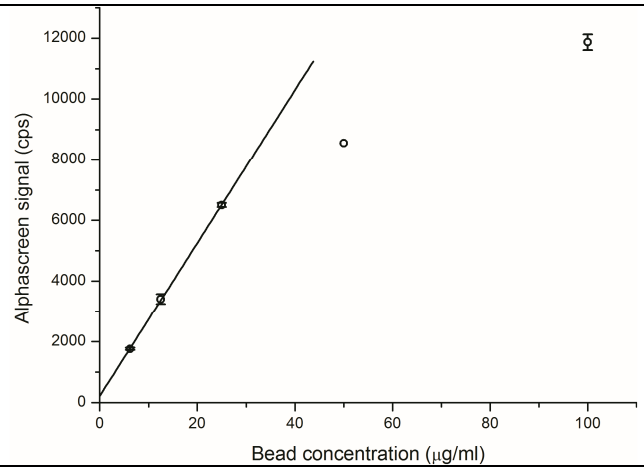

Figure S4. Correlation between bead concentration and AlphaScreen signal. Various amounts of beads were incubated with the positive control Biotinylated-6His. The background corrected data (experimental background = 1443 ± 302 cps) indicates that the signal is elevated upon increasing the bead concentration but there is a deviation from linearity at concentrations higher than ca. 25 μg/ml.

1. Z. Balogh, G. Lautner, V. Bardoczy, B. Komorowska, R. Gyurcsányi and T. Mészáros, *FASEB J.*, 2010, 4187-4195.