Electronic Supplementary Information:

Gold nanoparticle-streptavidin conjugates for rapid and efficient screening of aptamer function in lateral flow sensors using novel CD4-binding aptamers identified through crossover SELEX

Tamika Fellows, Lance Ho, Shane Flanagan, Ronen Fogel, Dupe Ojo and Janice Limson

[*]*Rhodes University Biotechnology Innovation Centre, Grahamstown, Eastern Cape, South Africa*

* Corresponding author: j.limson@ru.ac.za

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Additional experimental data includes the detailed SELEX methodology, complete aptamer sequences, antibody controls (fluorescence confocal microscopy and magnetic bead ELISA), aptamer qPCR calibration curves and LFA time point study video footage.

S1: Detailed SELEX methodology and results

S1.1 Materials and Apparatus

The T4 DNA ligase (M180A), pGEM-T Easy control insert (A363A), pGEM - T Easy Vector (A137A), and 2× Rapid Ligation Buffer (C671A) were all purchased from Promega. The λ-exonuclease (5000 $U.mL^{-1}$) and 10× λ-Exonuclease buffer (B0262S) were both purchased from New England BioLabs (United States). The KAPA Taq PCR Kit (KK1015) and deoxynucleotide triphosphates (dNTPs) were purchased from Thermo Fisher Scientific (United States). NucleoSpin Gel and PCR Clean-up columns (740609.250), NTC buffer (740654.100) and NTI buffer (740305.120) were purchased from Macherey-Nagel (Germany). Fetal calf serum (S181G) was obtained from Celtic Diagnostics. Membrane Peroxidase Substrate system (TMB-based) was obtained from Kirkegaard & Perry Laboratories (United States). Nutrient agar and nutrient broth 'E' were purchased from Neogen culture media (England). Unless specified, reagent grade chemicals were obtained from Sigma-Aldrich (USA) and Merck (DE).

Gel electrophoresis was performed using the Bio-Rad Laboratories Mini-PROTEAN® Tetra System, powered by the Bio-Rad Laboratories PowerPac™ Basic unit. Electrophoresed gels were visualized using the Bio-Rad Gel-Doc™ EZ Imager System and related Software. Magnetic separation of the DynaBead® magnetic-beads was achieved by either a 5 mm neodymium magnet microcentrifuge-tube rack or a 96-well magnetic-ring stand (Applied Biosystems, Thermo Fisher Scientific).

S1.2 SELEX Methodology

S1.2a ssDNA Elution and Ethanol Precipitation

The elution of ssDNA from protein-conjugated magnetic-beads was performed by collection of the magnetic beads via the application of a permanent magnet to the bottom of the tube for 2 min and removal of the supernatants. The beads were resuspended in 200 µL elution buffer (40 mM Tris, 10 mm EDTA, 3.5 M Urea, 0.002% Tween 20, pH 8.8) and incubated for 10 min at 80 °C (1). The magnetic beads were collected via magnetic force and the supernatant containing eluted ssDNA was removed.

The DNA elution process was repeated, the two collected supernatants pooled, and the eluted DNA recovered through ethanol precipitation in the presence of 0.06 M sodium acetate (pH 5.2), 0.046 $g.L^{-1}$ glycogen, and 1 ml of 100% ($^{v}/_{v}$) ice-cold ethanol. The solution was incubated at -20°C for 24-48 hrs and then centrifuged for 30 min at 4 °C, 14000× g. The supernatant was removed, 1 mL of 70% ($^{v}/_{v}$) ethanol was added to the pellet and centrifuged for 10 min, as above. The remaining DNA pellet was dried at 50 °C for 1 hr, resuspended in 30 µL of ddH2O and stored at -20 °C.

S1.2b PCR amplification of enriched oligonucleotide libraries

Amplification of the ssDNA pools generated after positive selection steps was conducted to increase the copy number of successful target binding oligonucleotides. A small-scale cycle optimization step was set up to determine the number of cycles of PCR resulting in the maximum production of the 90bp amplicon, without the evident formation of amplification artifacts (2,3). For cycle optimization, a standard 200 µl KAPA Taq PCR Master mix was formulated, comprising 1× Taq PCR Buffer B, 1 mM dNTPs, 0.5 µM SELEX library forward primer [5'-GCCTGTTGTGAGCCTCCTAAC-3'], 0.5 µM 5'-phosphorylated SELEX library reverse primer [5'-PO_3-GGGAGACAAGAATAAGCATG-3'], 0.025 $U.\mu L^{-1}$ Taq Polymerase, and ~0.0125 $ng.\mu L^{-1}$ of the ssDNA pool as the template DNA [5'-GCCTGTTGTGAGCCTCCTAAC-(49N)-

CATGCTTATTCTTGTCTCCC-3']. This PCR reaction was split into 20 µL aliquots, each used for cycle evaluation.

PCR amplification was conducted using HotStart touch-down conditions. Following an initial denaturation at 95 °C for 5 min, successive cycles of: denaturation at 95 °C for 1 min, cycle-dependent annealing at 59°C (cycle 1), 58°C (cycle 2), 56°C (cycle 3), and 54°C (cycle 4) for 1 min, and extension at 72 °C for 1 min and 30 sec. This was followed by cycle evaluation for a further 9 cycles with annealing temperature maintained at 54 °C. Following each PCR cycle between cycles 5 and 14, a single PCR tube was removed for each cycle and stored at 4 °C until all PCR samples were removed.

Each PCR reaction produced as detailed above was analyzed using electrophoretic separation and subsequent staining of DNA by GelRed. Electrophoresis was performed via PAGE, using a 10% ($^{w}/_{v}$) polyacrylamide gel, in a 1× TAE buffer at 80 V for 90 mins. The gels were visualized after staining by immersion in a 1× TAE buffer containing 1× GelRed.

Once the correct cycle number was confirmed, a 5 mL PCR reaction was made up and split across 50 PCR tubes of 100 µL each, two of these tubes were used to verify that the chosen cycle number does not produce amplicons byproducts, analyzed by PAGE as previously outlined. Thereafter, PCR tubes were pooled and subsequently concentrated using Macherey-Nagel's Nucleospin® Gel and PCR Clean-Up kit with NTI buffer for dsDNA purification following the manufacturer's instructions.

S1.2c: λ Exonuclease Digestion

The dsDNA product produced after PCR amplification of the SELEX enriched pool was digested by λ-exonuclease, as described by Ardjomandi *et al* with some modifications (4). Approximately 5 µg of dsDNA was added to the λ-exonuclease digestion master mix (35 Units of λ exonuclease, 1× Lambda exonuclease buffer, ddH_2O) and incubated at 37 °C for 2 hrs. The reaction was terminated through heat inactivation of the enzyme at 90 °C for 10 min. The ssDNA was purified by a Macherey-Nagel DNA clean-up column, using NTI buffer. The concentrated ssDNA was stored at -20°C for use in the next SELEX selection round.

S1.2d: Preparation of competent DH5α *E. coli* cells

Nutrient agar plates and nutrient broth were used for the initial cultivation and isolation of *Escherichia coli*, strain DH5α, donated by the Department of Biochemistry and Microbiology, Rhodes University, South Africa. A single colony of DH5α *E. coli* isolated on nutrient agar plates was inoculated into 25 mL of Luria broth and incubated at 37 °C under agitation for 6 hrs. The cells were placed on ice for 25 min, collected by centrifugation at 2000× *g* for 3 min, and resuspended in 10 mL of ice-cold 0.1 M $CaCl_2$. The cell suspension was chilled on ice for 20 min, collected by centrifugation as above and resuspended in 5 mL of ice-cold 0.1 M $CaCl_2$, supplemented with 15% ($^{v}/_{v}$) glycerol. The cells were dispensed into cold microtubes in 100 µL aliquots. These aliquots were then frozen immediately at -80 °C until used.

S1.2e: Ligation of aptamer candidate inserts into pGEM-T easy vectors

PCR products from the final SELEX selection pool were amplified and ligated into the pGEM-T Easy vector to allow ligated plasmid transformation into competent DH5α *E. coli* cells. The PCR product (0.09 kb) ligated into 50 ng of the vector (3 kb) in 3 different ratios (1:1), (3:1) and (5:1), therefore ~ 1.5 ng, 4,5 ng, and 7.5 ng of ds insert DNA was added to the ligation master mix (1× rapid ligation buffer, 50 ng of p-GEM-T easy vector, T4 ligase (6 Weiss units), ddH_2O and ligated at 4°C overnight.

S.12f: Bacterial transformation with ligated p-GEM® -T Easy plasmids

DH5α *E. coli* cells were transformed with the ligated p-GEM® -T Easy plasmid, selected as each cell only incorporates a single plasmid, thereby allowing for the separation of different insert sequences. Approximately 5 µL of the ligation mixture was added to 50 µl of competent DH5α *E. coli* suspensions and incubated on ice for 20 min. The cells were transformed with the plasmid via heat-shocking at 42°C for 45 sec and placed on ice. Approximately 950 µl of S.O.C. medium was added to the transformation reactions and incubated at 37 °C with shaking at 200 rpm for 1 hr. To allow for discrete colonies to form, transformations were plated out in separate 200 µL, 50 µL, and 25 µL volumes onto ampicillin modified nutrient agar plates, incubated overnight at 37 °C. Modified nutrient agar plates contained 1 mM Isopropyl β-d-1-thiogalactopyranoside (IPTG) and 500 µM 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) for blue/white screening of successfully ligated colonies. At least 50 white and light blue colonies were selected for colony PCR amplification of the PUC M13 plasmid and the respective insert sequence.

S1.2g: Colony PCR amplification

Colony PCR was conducted according to Alshahni *et al* with some modifications (5). A PCR master mix containing 1× Buffer B, 1 mM dNTPs, 0.025 $U.\mu l^{-1}$ KAPA Taq Polymerase, ddH_2O, PUC M13 forward primer [5'-CCCAGTCACGACGTTGTAAAACG-3'], and PUC M13 reverse primer [5'-AGCGGATAACAATTTCACACAGG-3'] was added to each colony isolate. The solution was amplified under the following cycling conditions: Initial hot start at 95°C for 5 min, followed by 30 cycles of denaturation at 95 °C for 30 sec, annealing at 54 °C for 30 sec, and elongation at 72 °C for 30 sec, after which a final 72 °C elongation 8 min step was added at the end of PCR. A fraction of the PCR product was amplified with primer sequences to confirm successful ligation using cycling conditions previously described for the amplification of the SELEX library. All PCR products were analyzed on 12% PAGE run at 80V for 90 min.

S1.2h: BigDye™ Sequencing Protocol

BigDye™ sequencing was performed according to the manufacturer's instructions. Briefly, 5 ng of each colony PCR product was placed into 10 µL of water containing 40% ($^{v}/_{v}$) BigDye® Terminator v3.1 Ready Reaction Mix (Thermo Fisher Scientific), 3.2 µM forward and reverse primer and 0.75× BigDye Sequencing buffer. These reactions were amplified under conditions of an initial denaturation at 96 °C for 1 min, followed by 25 cycles of denaturation for 10 sec at 96 °C, annealing at 50 °C for 5 sec, and elongation for 4 min at 60°C.

The unincorporated dye-labeled terminators were removed through ethanol-EDTA precipitation. The samples were centrifuged 15900× *g* for 20 min, 2.5 µL of 125 mM EDTA was added to the bottom of the tube, followed by 30 µL of absolute ethanol, the mixture was then incubated at room temperature for 1 hr in the dark at RT. The tubes were centrifuged at 15900× *g* for 20 min, the supernatant discarded and 30 µL of ice-cold ethanol was added to each tube, vortexed and centrifuged again for 15 min. The pellet was aspirated and the supernatant was allowed to dry at room 60 °C for 4 – 5 min. These reactions were stored at -20 °C. The resultant PCR product was sent for Sanger sequencing by the NRF-SAIAB Molecular Genetics Laboratory (Rhodes University, South Africa).

S1.2i: qPCR evaluation of SELEX enrichment

The amount of ssDNA bound to the target was expected to increase with every successive SELEX cycle. The ssDNA content is proportional to the Cq value defined by the cycle number during qPCR where the fluorescence response first increased above baseline fluorescence. Therefore, higher concentrations of template DNA results in lower Cq values for a given SELEX pool. Each ssDNA sample of the relevant SELEX rounds was diluted 200× and added to the Quantinova SYBR Green Master Mix (1× SYBR Green Master

Mix, 0.7 µM SELEX Library forward primer, [5'-GCCTGTTGTGAGCCTCCTAAC-3'], 0.7 µM SELEX Library reverse primer [5'-PO_3-GGGAGACAAGAATAAGCATG-3'] and Milli-Q water). The prepared master mix amplified using a BioRad Thermal Cycler under the following conditions: Initial heat activation at 95°C for 2 min, followed by 40 cycles of two-step cycling with denaturation at 95°C for 5 sec, and a combined annealing/extension step at 60°C for 10 sec. A melt curve from 60-95°C was also conducted after the completion of 40 cycles.

Results of SELEX process and sequences obtained from Sanger sequencing of SELEX process

Table S1. Summary of CD4-specific aptamer sequences obtained after 5 rounds of Crossover-SELEX. Selection rounds alternated between hCD4 and CD4-expressing cells

Sequence ID	5' primer binding site	Variable region sequence 5'→3'	3' primer binding site
UB57	GCCTGTTGTGAGCCTCCTAAC	CTATGGTTTAACGTAGTAAGGGTACGGAGGGTTTC AACGAGATAACACA	CATGCTTATTCTTGTCTCCC
U1	TCCTGTTGTGAGCCTCCTAAC	GGGTGTTACGTGTCGCTTATGACTGAATACTTTTGG GAAGATGTATCCA	CATGCTTATTCTTGTCTCCC
U2/4	TCCTGTTGTGAGCCTCCTAAC	TTCTCTCTCTTTGCTTTCATGTCGGGTAGGTCACACC ACTTTGTTGTTC	CATGCTTATTCTTGTCTCCC
U20	TCCTGTTGTGAGCCTCCTAAC	TTATATGATGCATCAGCGCGAGGGCGACACCGCTA CTCGGGTCGATTTT	CATGCTTATTCTTGTCTCCC
U21	GCCTGTTGTGAGCCTCCTAAC	GCGTACTTCAACATTAAAATTTACGAATGAGTAGGA ACTTGAAGGCATG	CATGCTTATTCTTGTCTCCC
U22/23	GCCTGTTGTGAGCCTCCTAAC	GTTGGTTAAATAGCGTACGGAATGGTTTTACTTTTG TTTTGCAGTCAGT	CATGCTTATTCTTGTCTCCC
U24/25	GCCTGTTGTGAGCCTCCTAAC	TAGTAGAGTTAAGCGACGATTTTAAAACCATGTCAT GATGTTAACTTAA	CATGCTTATTCTTGTCTCCC
U26	GCCTGTTGTGAGCCTCCTAAC	GATGTCGACGTGCAGCTTCCTTGAGCCTTACTGAAA ATACTACCCAGTC	CATGCTTATTCTTGTCTCCC
U29	GCCTGTTGTGAGCCTCCTAAC	AGGAATTTCACCGGAGTCTTAAAACTGTTCAAAAAT GATAAACTTATTG	CATGCTTATTCTTGTCTCCC
U38	TCCTGTTGTGAGCCTCCTAAC	ATGAGACGACCTGATCACAGTAGGCTTTTTCGGATT TGCACTCTCGCG	CATGCTTATTCTTGTCTCCC
U42/49	TCTGTTGTGAGCCTCCTAAC	TAGCTCGTAGAAAAAAAATATAGCGTGTGCTGGGA CTGCTCGGGATTGCGGACA	CATGCTTATTCTTGTCTCCC
U45	GCCTGTTGTGAGCCTCCTAAC	TGAATAATACCATATTAAAAACAAGTTGACTCGTCG GTAGTGTAAGACA	CATGCTTATTCTTGTCTCCC
U47	GCCTGTTGTGAGCCTCCTAAC	AGACGTTTAATTAACTCAAGTTGATCGCTCCTGTTC ACTTCATAATCGT	CATGCTTATTCTTGTCTCCC
U10/35	TCCTGTTGTGAGCCTCCTAAC	CGATATGTGGCGCTGAATGTTGTAAATGTCTAGTAC AGCCAAGAACAGG	CATGCTTATTCTTGTCTCCC
U14	GCCTGTTGTGAGCCTCCTAAC	ACGTTAAAGTGAATTCTAACCTAGTGAGTTTTTCGT CTTGTATTATTGG	CATGCTTATTCTTGTCTCCC
U30	GCCTGTTGTGAGCCTCCTAAC	TTGTCTCATTCTAGGCTTTGTTAATAGTGAATCCAAG ATCGTCTGCTAG	CATGCTTATTCTTGTCTCCC

U32	GCCTGTTGTGAGCCTCCTAAC	ATACTTGATTCATTCCCCTTGTTATAAGCGCCCATTGATTTTTCATGGA	CATGCTTATTCTTGTCTCCC
U39	GCCTGTTGTGAGCCTCCTAAC	GCTTACCTCTTGTTGATAAGTTTCAGACTGCGCACTTCTTCCCGCAATA	CATGCTTATTCTTGTCTCCC
U46	GCCTGTTGTGAGCCTCCTAAC	TGGCTGTACACGAAGAAATAACCGCCAAGAAGGGAAAAGTTAAGTCTAT	CATGCTTATTCTTGTCTCCC
U12		TAACTTTAAAAGCGTTGGTTCGGTGCTTATCAGAAACATATTCGATGTCCTTT	CATGCTTATTCTTGTCTCCC
U19		TATCTGAATATGAAATTCTGAGTTCTTCTCGTTTATGCTAAGCCTGGAT	CATGCTTATTCTTGTCTCCC
U16		TACCGCAATGCTTAGATAGTGGTAAGTGATACACTGCTTTGAGATTTTT	CATGCTTATTCTTGTCTCCC
U33		TGTGACTAAGTAAGTGAAGCA	CATGCTTATTCTTGTCTCCC
U36		TTCCGCGAATCCATGTTGTGGAGGGATAACACTTCTATCT	CATGCTTATTCTTGTCTCCC
U37		TTTGTAAGTTCATGAAAATATACTTAGTCG	CATGCTTATTCTTGTCTCCC
U41		CGAATGACGTTGTTTTCCATAGTAGTAGTTAGTTGTGTTC	CATGCTTATTCTTGTCTCCC
U51		TCATGAGATGGGCATTAGGATA	CATGCTTATTCTTGTCTCCC

Duplicate sequences that were obtained are represented in the same row, both sequence numbers are in the sequence column separated by a forward slash. Of the 19 full-length sequences were obtained, 6 of which were identified as concatemers i.e. amplification artifacts, some sequences were identified as duplicates which is more likely to be the result of colony picking than high copy numbers as the sequences were often close together, e.g. 2 and 4. All 8 of the truncated sequences contained only the reverse complement of the reverse primer and not the forward primer.

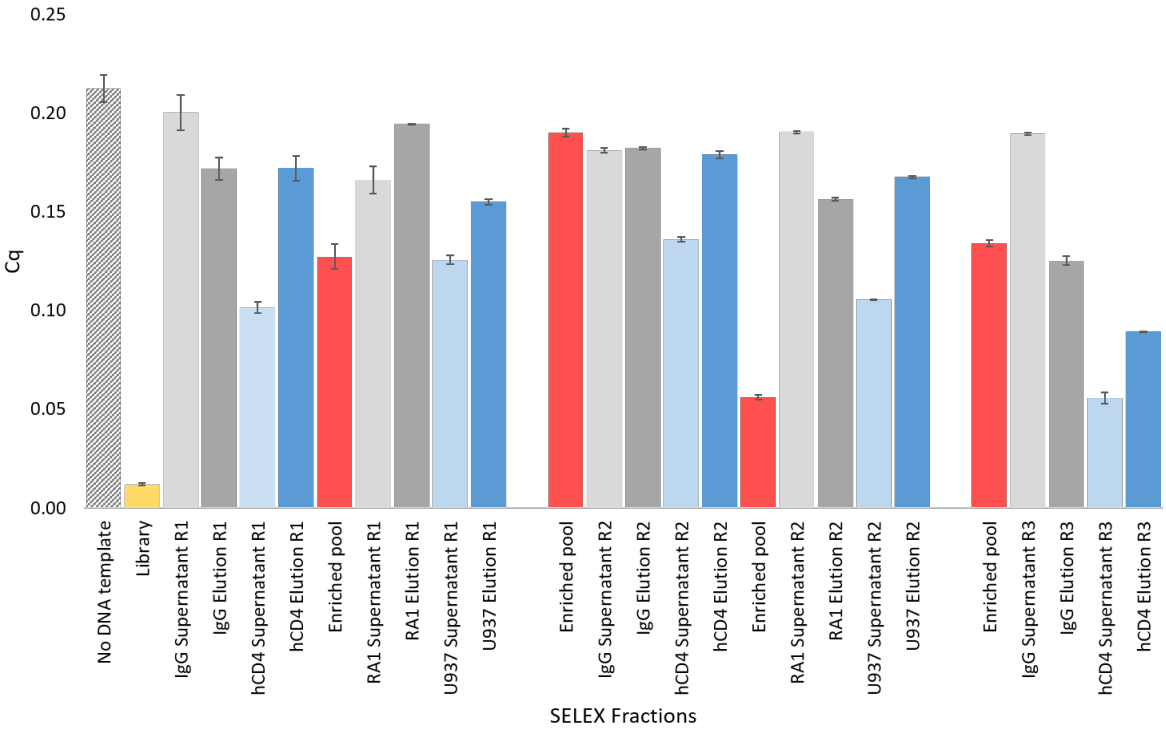

Fig. S1 Estimation of ssDNA from SELEX round fractions using qPCR. Amplification was performed in the presence of SYBR Green I for ssDNA fractions of the initial oligonucleotide library, supernatant and eluents derived from positive and counter-selection rounds of the hCD4 FluMag-SELEX and target U937 cell SELEX. Fractions were diluted 200× and amplified as template DNA using SELEX Library primers [5′-GCCTGTTGTGAGCCTCCTAAC-3′] and [5′-PO_3-GGGAGACAAGAATAAGCATG-3′] for 40 cycles under HotStart conditions using the Quantinova qPCR kit. (n = 3 ± SD). All SELEX fractions are outlined in Table S2.

Table S2. ssDNA pools retained from Crossover-SELEX. Description of supernatant and eluent fractions obtained from positive and counter-selection SELEX rounds. Each sample was analyzed by qPCR.

Fraction	Sample description	Collected volume
Library	Commercial ssDNA library	1 ml
Enriched pool	λ-exonuclease digested ssDNA pool from positive selection	1 ml
IgG supernatant R1	Supernatant of IgG magnetic-beads during Round 1	30 µl
IgG elution R1	Eluted from IgG magnetic-beads during Round 1	15 µl
hCD4 supernatant R1	Supernatant of hCD4 magnetic-beads during Round 1	30 µl
hCD4 elution R1	Eluted from hCD4 magnetic-beads during Round 1	154 µl
RA1 supernatant R1	Supernatant of RA1 cells during Round 1	60 µl
RA1 elution R1	Eluted from RA1 cells during Round 1	60 µl
U937 supernatant R1	Supernatant of U937 cells during Round 1	60 µl
U937 elution R1	Eluted from U937 cells during Round 1	60 µl
IgG supernatant R2	Supernatant of IgG magnetic-beads during Round 2	30 µl
IgG elution R2	Eluted from IgG magnetic-beads during Round 2	30 µl
hCD4 supernatant R2	Supernatant of hCD4 magnetic-beads during Round 2	30 µl
hCD4 elution R2	Eluted from hCD4 magnetic-beads during Round 2	30 µl
RA1 supernatant R2	Supernatant of RA1 cells during Round 2	60 µl
RA1 elution R2	Eluted from RA1 cells during Round 2	60 µl
U937 supernatant R2	Supernatant of U937 cells during Round 2	60 µl
U937 elution R2	Eluted from U937 cells during Round 2	60 µl
IgG supernatant R3	Supernatant of IgG magnetic-beads during Round 3	30 µl
IgG elution R3	Eluted from IgG magnetic-beads during Round 3	30 µl
hCD4 supernatant R3	Supernatant of hCD4 magnetic-beads during Round 3	30 µl
hCD4 elution R3	Eluted from hCD4 magnetic-beads during Round 3	30 µl

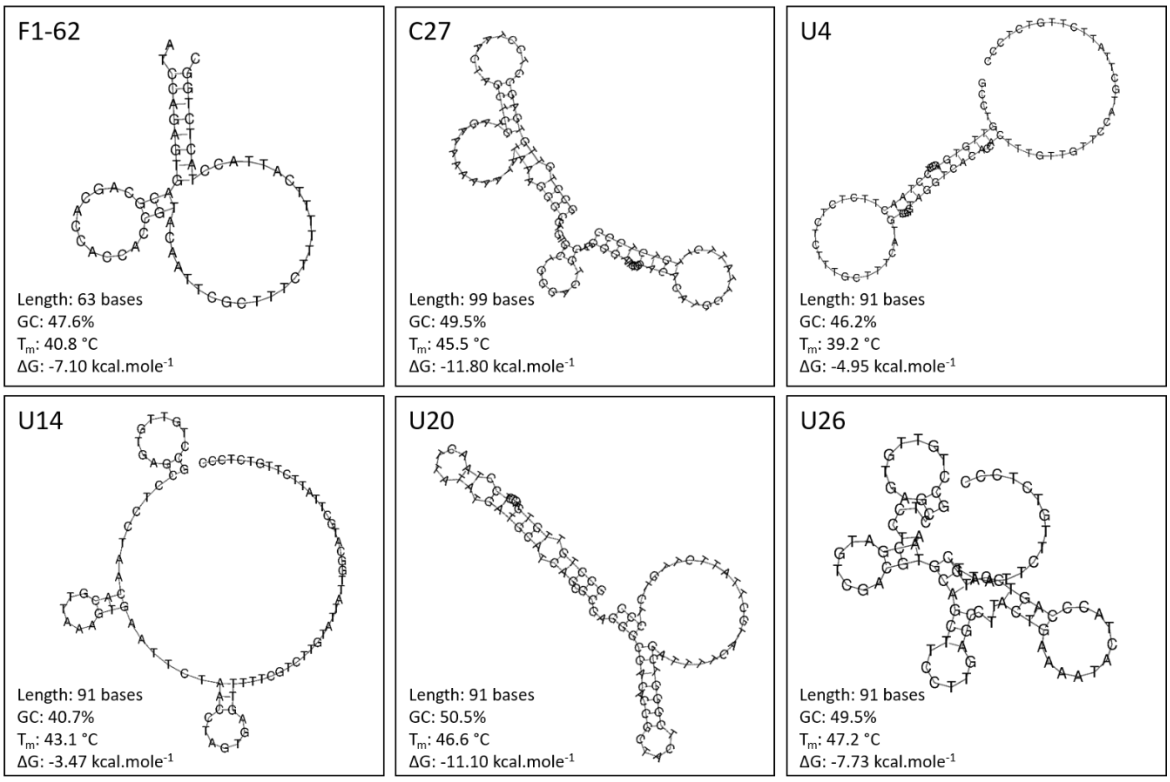

Fig. S2 Secondary structure prediction of the CD4-targeting aptamer candidates. The predicted secondary structures were determined by RNAfold analysis for linear DNA at 25 °C using minimum free energy and partition function fold algorithms. Predicted structures are shown at a minimum Gibbs free energy, ΔG.

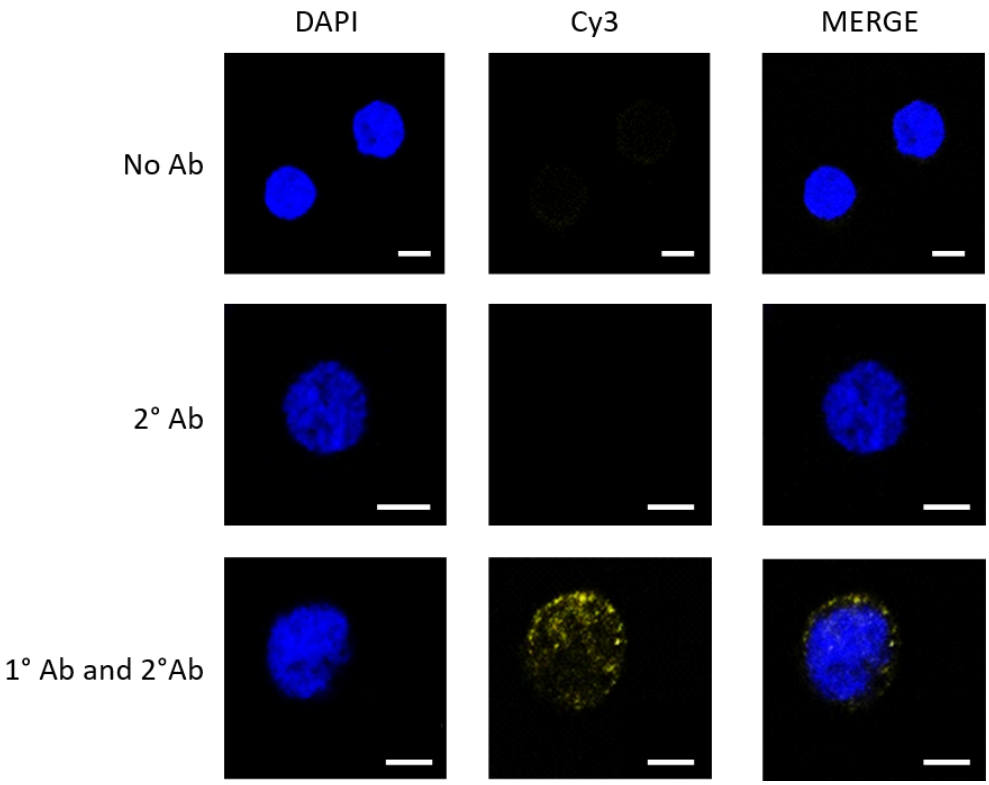

Fig. S3 Fluorescence microscopy of U937 cells stained by Cy3-labelled antibodies specific to the cytoplasmic region of hCD4. Cells were fixed, permeabilized and probed with either no antibody (No AB), mouse anti-hCD4 (cytoplasmic domain) monoclonal antibody (ab25804) and Cy3-labelled goat anti-mouse secondary antibody (1° and 2° Ab), or only Cy3-labelled goat anti-mouse secondary antibody (2° Ab). The cells were stained with DAPI and mounted with DAKO mounting medium. Slides were visualized for the presence of Cy3 fluorescence using the Zeiss LSM 780 Confocal Scanning Microscope. Obtained images were processed with Zen 2 microscopy software. Scale bar: 5µm.

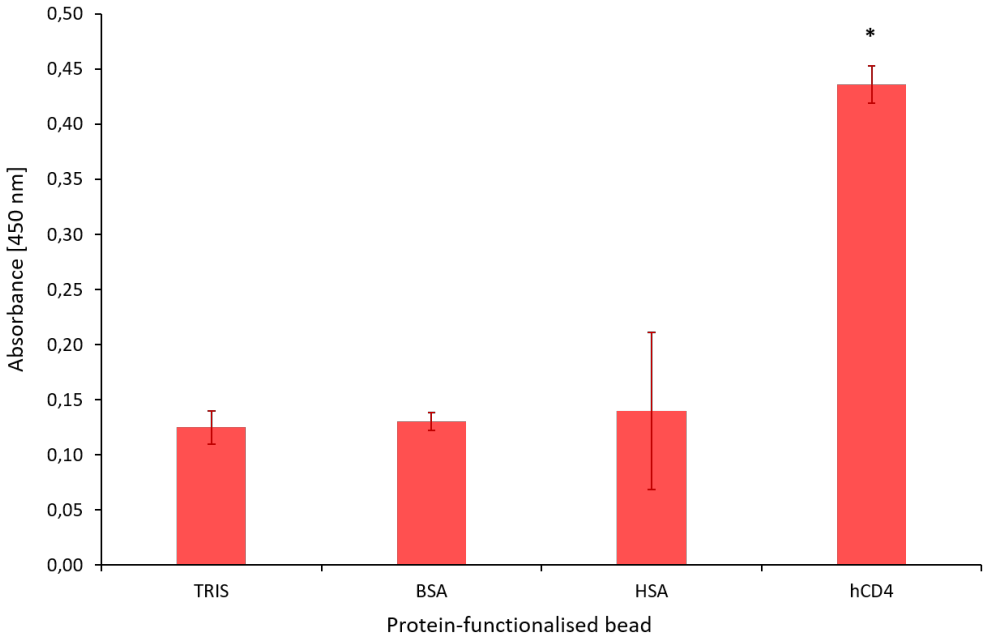

Fig. S4 Anti-CD4 antibody ELISA confirming hCD4 conjugation to magnetic-beads. Detection of hCD4 by rabbit anti-hCD4 (extracellular domain) monoclonal antibody (ab133622) and goat anti-rabbit IgG (H+L) horseradish peroxidase (HRP)-conjugated polyclonal antibody (ab205718) staining, monitored at 450 nm upon oxidation of the colorimetric substrate, 3,3',5,5'-Tetramethylbenzidine. A statistical increase in absorbance at 450 nm compared to the TRIS coated beads denoted by *, using one-way ANOVA ($F(3, 12) = 65.660$) and Tukey's HSD *post hoc* test ($p= 0.000199$).

Sequence-dependent amplification rates necessitated individual standards for qPCR quantification

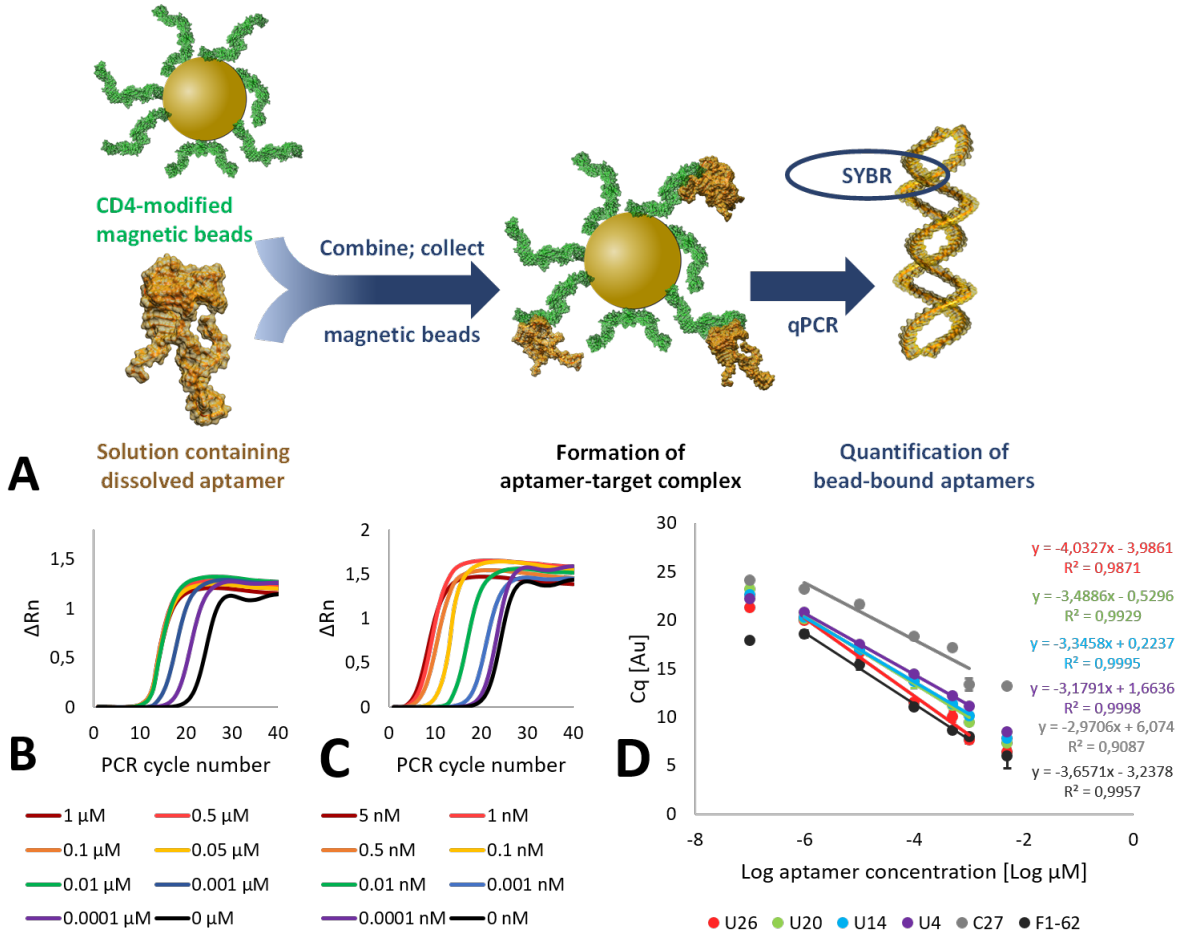

Fig. S5 Schematic of magnetic-bead qPCR assay (A). Representative qPCR response from U26 aptamer retention to hCD4-conjugated magnetic-beads and U26 aptamer calibration curve (A and B). Calibration curves of U4, U14, U20, U26, C26 and F1-62 qPCR amplification (C). B: Response from U26 aptamer co-incubation with 50 µg hCD4-conjugated magnetic-beads shown for initial concentrations of 1 µM, 0.5 µM, 0.1 µM, 0.05 µM, 0.01 µM, 0.001 µM, 0.0001 µM and 0 µM. C and D: Calibration curves show U4, U14, U20, U26 aptamer and control C27 and F1-62 sequence response at concentrations of 5 nM, 1 nM, 0.5nM, 0.1 nM, 0.01 nM, 0.001 nM, 0.0001 nM and 0 nM.

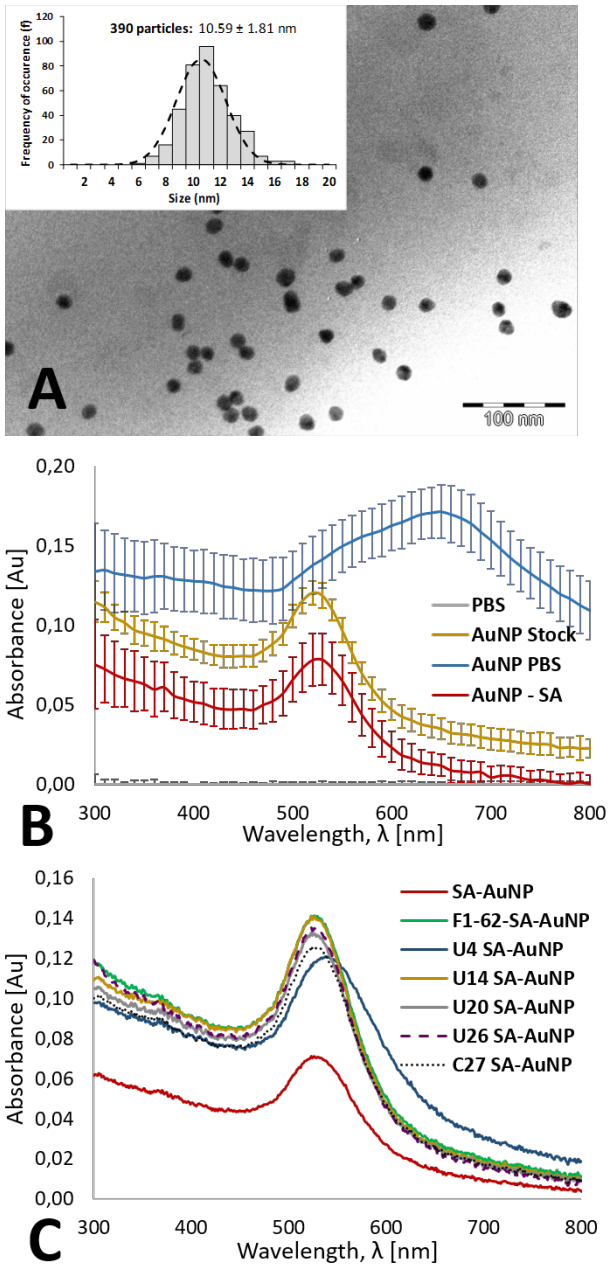

Fig. S6 UV-Vis Characterization of the citrate stabilized AuNPs and aptamer-conjugated AuNPs. A: Transmission electron microscopy image of spherical citrate stabilized gold nanoparticles, 200 nm scale bar. Inset: Histogram showing the particle size distribution of 390 counted AuNPs, indicating a diameter distribution of 10.60 ± 1.81 nm (mean ± standard deviation) within the sample. A fitted Gaussian function based on the calculated mean and standard deviation is presented together with the histogram (dashed line). B: Baseline-corrected absorbance spectra of the citrate stabilized AuNP suspensions used in this study, showing the stability afforded by streptavidin modification of the particles. Error bars indicate standard deviation from the mean (n = 3). C: Baseline-corrected absorbance spectra comparing the various aptamer-modified AuNP suspensions tested in this study. Error bars are omitted from this figure, for the sake of clarity. The streptavidin-modified AuNP from the previous spectra is included here (dashed, red line) for the sake of comparison.

The plasmon peak wavelength (λmax), absorbance measurements at 450 nm and the absorbance at plasmon peak wavelengths were used to determine the average diameter and concentration of Apt-AuNP conjugates (6). Using these parameters, particles were initially sized using the difference in wavelength between λmax and 512 nm, a technique applicable for particle diameters between 35 and 100 nm (6). Diameters smaller than 35 nm were determined by the ratio of absorbance at λmax and the absorbance at 450 nm, A450nm (6). Additionally, the nanoparticle concentration of each conjugate solution was determined from the A450nm and particle diameter. Nanoparticle conjugate properties are summarized in Table S3 (6).

Table S3. Spectroscopic characteristics of the functionalized AuNPs. Unless otherwise stated, all AuNPs were resuspended in PBS before spectroscopic characterization.

AuNP/ AuNP-Conjugate	λ_{max} (nm)	Size (nm)	Concentration (Particles.ml^{-1})
As synthesized, in citrate buffer	523 ± 0	10.12 ± 1.12	8.15 ± 2.97 × 10^{11}
As synthesized, in PBS	646 ± 5	139.58 ± 1.85	8.84 ± 1.23 × 10^{8}
Streptavidin-modified	529 ± 1	12.64 ± 2.45	1.33 ± 0.36 × 10^{11}
F1-62-Conjugate	523 ± 0	15.49 ± 0.36	2.16 ± 0.21 × 10^{11}
C27-Conjugate	523 ± 0	15.36 ± 0.21	1.97 ± 0.05 × 10^{11}
U4-Conjugate	537 ± 1	63.06 ± 1.28	2.29 ± 0.16 × 10^{9}
U14-Conjugate	523 ± 0	16.13 ± 0.27	1.87 ± 0.11 × 10^{8}
U20-Conjugate	523 ± 0	15.35 ± 0.78	2.11 ± 0.38 × 10^{11}
U26-Conjugate	523 ± 0	15.36 ± 0.23	2.12 ± 0.17 × 10^{11}

Video recording of aptamer streptavidin- and antibody-gold nanoparticle conjugate binding to hCD4 in lateral flow assay

Video S7 and S8. Colorimetric signal generation by anti-hCD4 aptamer streptavidin- and antibody gold-nanoparticle conjugates on lateral flow assay platforms**.** Initial addition of 50 µL of the various AuNP conjugates, allowed recording time for **A:** Aptamer-conjugates t = 14 min and **B:** antibody conjugates t = 15 min. Recorded footage of the time point study is shown at 4× speed.

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