SUPPORTING INFORMATION

Are aptamers really promising as receptors for analytical purposes? Insights into anti-lysozyme DNA aptamers through a multi-technique study

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Supporting experimental section

Chemicals and solutions. Magnesium chloride ($MgCl_2$), ammonium bicarbonate (NH_4HCO_3), L-glycine, Trizma® base, Tween-20, ethanolamine (EA), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), bovine serum albumin (BSA), sodium hydroxide, sodium chloride, 4-morpholineethanesulfonic acid monohydrate (MES), hydrochloric acid, formic acid (FA), streptavidin-alkaline phosphatase from *Streptomyces avidinii* conjugate (Strep-ALP) and lysozyme from chicken egg white were purchased from Merck (Milan, Italy). Monobasic potassium phosphate (KH_2PO_4), di-potassium hydrogen phosphate (K_2HPO_4), sodium phosphate dibasic dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$), potassium chloride and RNase free water were purchased from Carlo Erba (Cornaredo, Milan, Italy). Hydroquinone diphosphate (HQDP) was provided by Metrohm Italiana (Origgio, Varese, Italy).
Deionized water was obtained by Milli-Q element A10 system (Millipore, San Francisco, CA, USA), and used for buffer solutions preparation.
The composition of the buffer solutions was as follows. MES buffer: 25 mM MES (pH 5.0); TRIS buffer: 0.1 M Trizma® Base, 5 mM $MgCl_2$ (pH 7.4); TRIS-T buffer: 0.1 M Trizma® Base, 5 mM $MgCl_2$, 0.05% w/v Tween® 20 (pH 7.4); TRIS saline buffer : 20 mM Trizma® Base, 5 mM $MgCl_2$, 0.1 M NaCl (pH 7.4); Phosphate Buffer Saline containing magnesium ions (PBS-Mg^{2+}): 1.5 mM KH_2PO_4, 8 mM Na_2HPO_4, 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$ (pH 7.4); Phosphate Buffered Saline (PBS) was purchased as dry-blended powder from Thermo Fisher Scientific (Waltham, MA, USA), having the following composition after dissolution: 0.1 M Na_2HPO_4, 0.15 M NaCl (pH 7.2); Tris Glycine Potassium (TGK) buffer: 25 mM Trizma® Base, 192 mM L-glycine, 5 mM K_2HPO_4 (pH 8.3).
RNase-free buffers were used in all experiments with the RNA aptamer.
The single stranded DNA sequences were purchased from Biomers.net (Ulm, Germany) whereas C80RNA sequence was from Metabion (Carlo Erba, Milan, Italy). All the oligos were delivered in a dried state, properly aliquoted to avoid repeated freeze/thaw cycles. The resuspension of the lyophilized aliquots was carried out in milli-Q water for the DNA oligos whereas in RNase-free water for C80RNA to reach a 100 μM final concentration as recommended by the supplier. The stock solutions were stored at -20°C.

Lysozyme biotinylation. Lysozyme biotinylation was performed using EZ-Link NHS-PEG4-Biotinylation Kit (Thermo Scientific). Biotinylation reaction was carried out in PBS buffer at 1:5 lysozyme:biotinylating agent ratio, for 1 h at room temperature under gentle stirring. The biotinylated protein was purified by dialysis, using a regenerated cellulose membrane with cut-off of 10 kDa, under gentle stirring for 4 h, refreshing PBS buffer after 2 h. After dialysis, the protein solution was centrifuged at 4 °C, 10000 rpm for 10 min and the supernatant was collected.
The concentration of biotinylated lysozyme was quantified by spectrophotometric analysis, using UV-vis/NIR Lambda 750 Perkin Elmer spectrophotometer equipped with a diode array detector. The calibration curve was constructed by analysing four concentration levels of lysozyme at 280 nm, assuming the same molar extinction coefficient for the unmodified and biotinylated protein.
Biotinylation grade was estimated by Flow Injection Analysis (FIA)-Mass Spectrometry (MS) using HPLC Dionex Ultimate 3000 coupled with a linear ion trap (LTQ) mass spectrometer (Thermo Fisher Scientific), equipped with an electrospray (ESI) source, operating in positive polarity. FIA-MS

analysis was carried out by injecting 10 µL of sample; three injections of biotinylated lysozyme were performed. A mobile phase consisting of water with 0.1% FA (*v/v*) at a flow rate of 200 µL min^{-1} was used. The conditions were set as follows: electrospray voltage of 3.5 kV, capillary temperature of 200 °C and capillary voltage of 20 V. Mass spectra were acquired in 5 min in full-scan mode (*m/z* 110-2000 range).

Aptamer immobilization on magnetic beads. A volume of 4 µL of stock suspension (2×10^9 beads mL^{-1}, corresponding to approximately 30 mg mL^{-1}) of Dynabeads™ M-270 Carboxylic Acid (Life Technologies Italia–Thermo Fisher Scientific) were diluted in 100 µL of MES buffer. MBs were washed 2 times with MES buffer, then they were suspended in 200 µL of a solution containing EDC and NHS, both at 25 mg mL^{-1}, for 30 min under shaking at 1500 rpm at room temperature using a shaking heater block (Thermomixer®C, Eppendorf). Subsequently, MBs were washed with MES buffer and suspended in a 2 µM aptamer solution. Immobilization time was set to 2 h, working at 26 °C under shaking (1500 rpm). After MBs washing with 100 µL of MES and 100 µL of TRIS buffer, the functionalized MBs were incubated with 100 µL of EA (50 mM in TRIS buffer) to quench the activated sites for 1 h under shaking at 1500 rpm. MBs were washed with 100 µL of TRIS-T buffer and 100 µL of binding buffer (PBS-Mg^{2+}, TRIS saline or TGK) for two times, then they were incubated with 200 µL of BSA 0.5% (*w/w*) as blocking agent for 1 h at 26 °C, under shaking at 1500 rpm. Finally, MBs were washed twice with 100 µL of binding buffer.
The MB functionalization was studied by using the A40 sequence modified at 3' with Cy3 performing fluorescence measurements (λ_{exc}=555 nm; λ_{em}= 565 nm) with an Edinburgh FLS1000 fluorometer. Different concentrations of A40-Cy3 were investigated for immobilization (200 nM, 500 nM, 1 µM, 2 µM) and the fluorescence signal from A40-Cy3 was acquired before and after immobilization of the aptamer on MBs; the amount of immobilized aptamer was assessed by difference. For the calculation, signals from the first two washing phases on modified-MBs were used to correct the signal of supernatant after immobilization.

Tables

Table S1. DNA and RNA sequences used in the present study. Variable regions are in bold, flanking regions are underlined.

Name	Sequence (5'-3')	Number of bases
A80	AGCAGCACAGAGGTCAGATG**GCAGCTAAGCAGGCGGCTCAC AAAACCATTCGCATGCGGC**CCTATGCGTGCTACCGTGAA	80
A40	**GCAGCTAAGCAGGCGGCTCACAAAACCATTCGCATGCGGC**	40
A80R	AGCAGCACAGAGGTCAGATG**ACTATGTCGGCCGCAATGCCC AGAGGCCACATACAAGCGG**CCTATGCGTGCTACCGTGAA	80
C80	GGGAATGGATCCACATCTACGAATTC**ATCAGGGCTAAAGAGT GCAGAGTTACTTAG**TTCACTGCAGACTTGACGAAGCTT	80
C30	**ATCAGGGCTAAAGAGTGCAGAGTTACTTAG**	30
C42	ATCTACGAATTC**ATCAGGGCTAAAGAGTGCAGAGTTACTTAG**	42
C80R	GGGAATGGATCCACATCTACGAATTC**AAGTGGATTAACTGTG TAGACCGATAGACG**TTCACTGCAGACTTGACGAAGCTT	80
PolyT40	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	40
C80RNA	GGGAAUGGAUCCACAUCUACGAAUUC**AUCAGGGCUAAAGA GUGCAGAGUUACUUAG**UUCACUGCAGACUUGACGAAGCUU	80

Figures

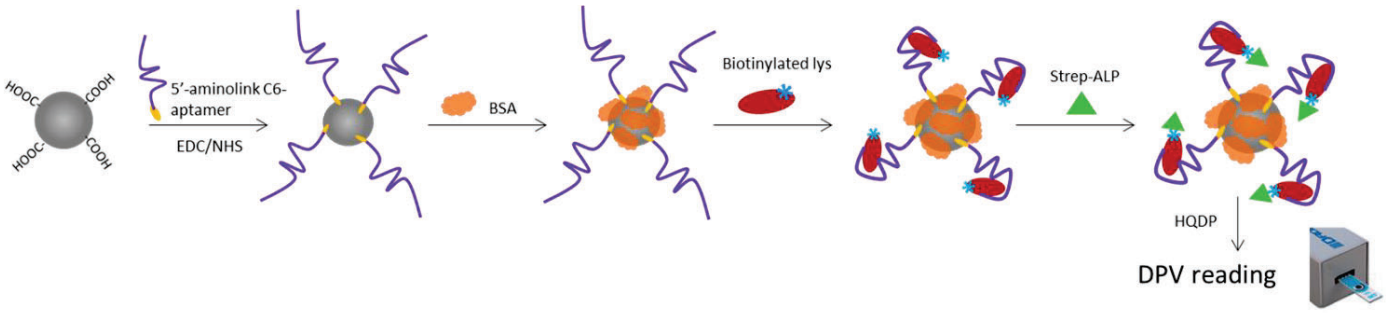

Figure S1. Scheme of the magneto-electrochemical apta-assay based on MBs.

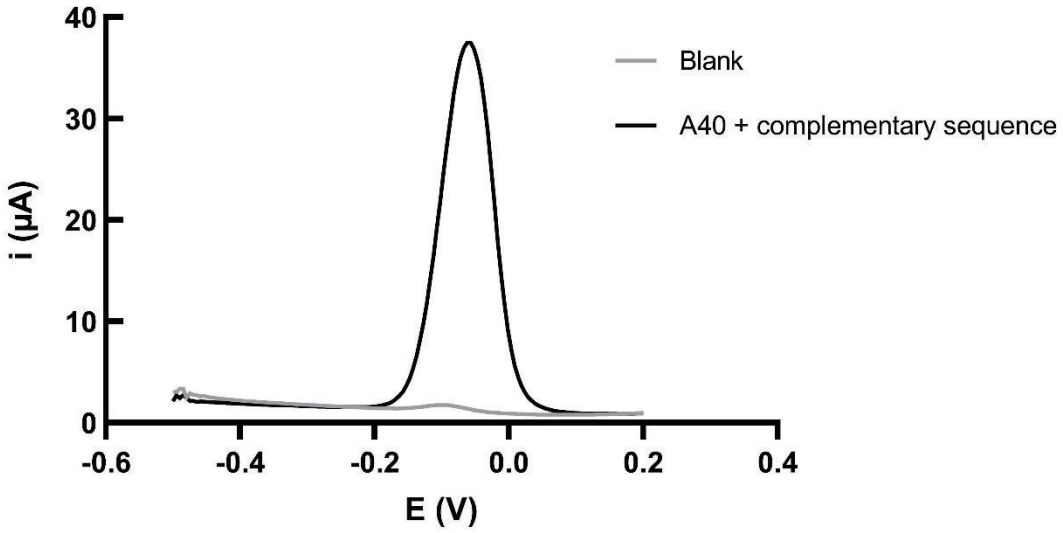

Figure S2. DPV voltammograms obtained with non-functionalized MBs (blank) and A40-modified MBs, in presence of the 20-mer sequence complementary to A40.

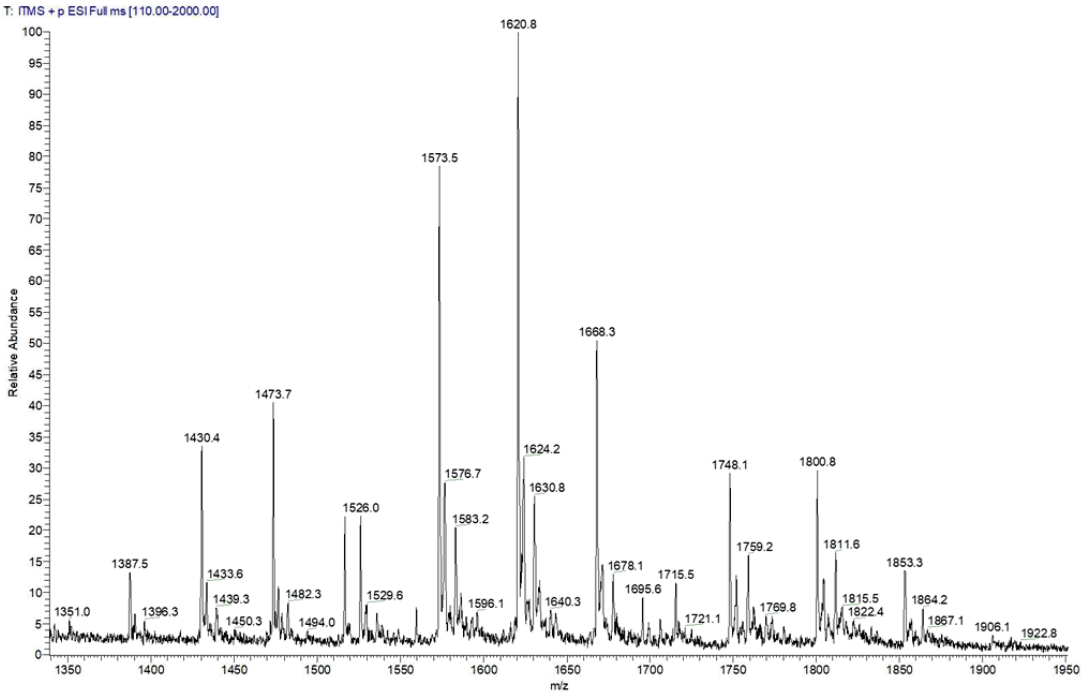

Figure S3. Full-scan ESI(+)-MS spectrum of biotinylated lysozyme.

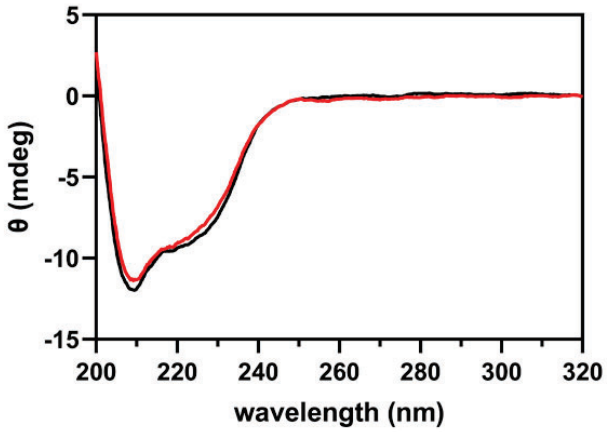

Figure S4. Overlay of CD spectra of unmodified (black) and biotinylated lysozyme (red).

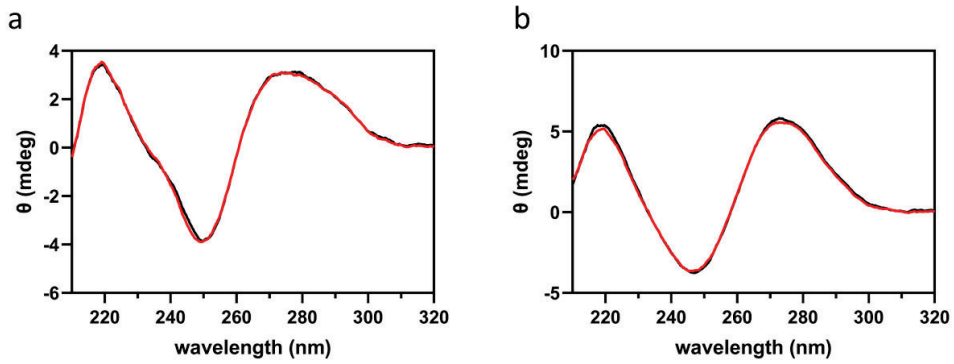

Figure S5. Overlay of CD spectra of (a) A40 and (b) C42 before (red) and after (black) denaturation/refolding experiments.

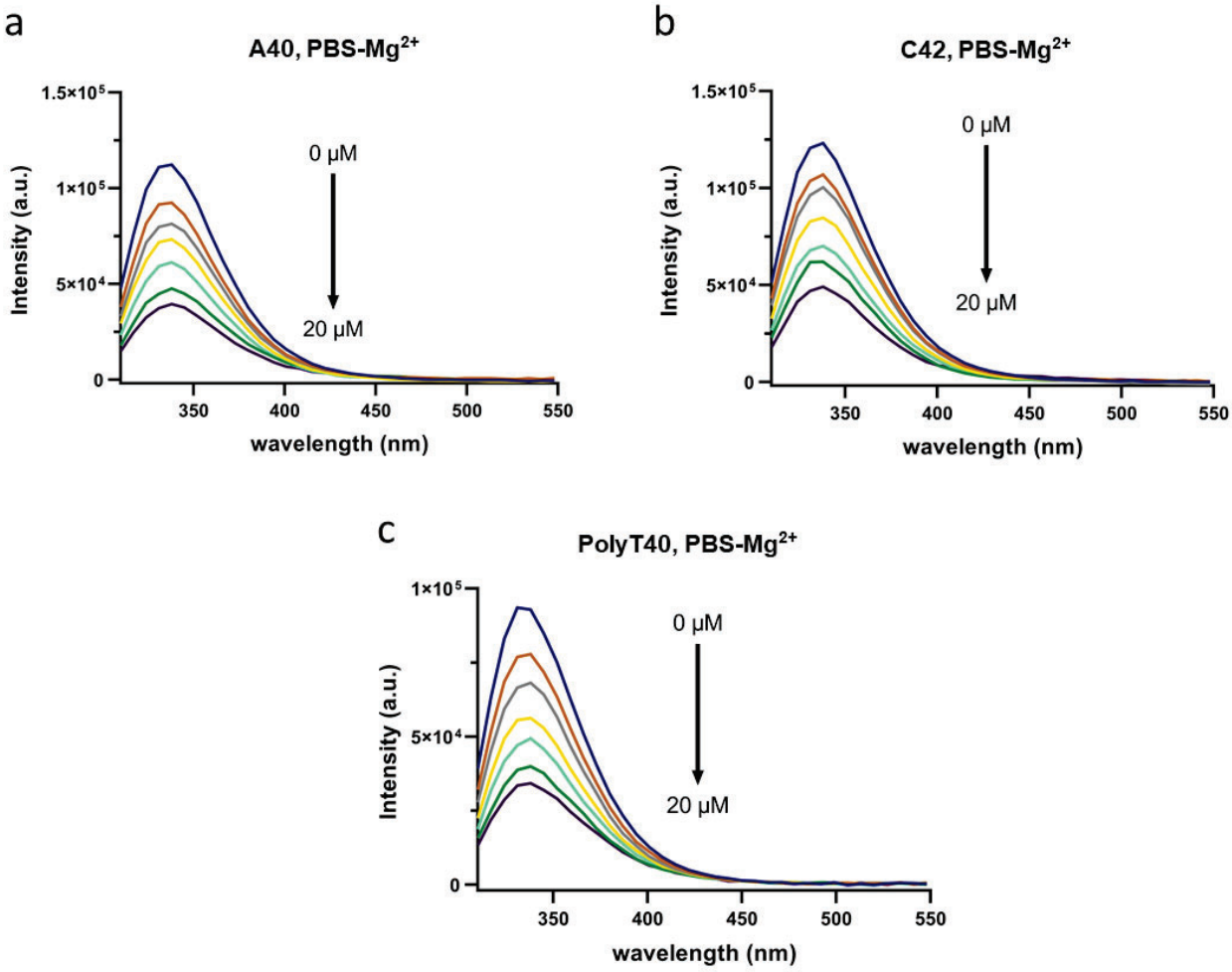

Figure S6. Fluorescence titration curves of lysozyme (1 μM solution, λexc = 298 nm) with (a) A40, (b) C42 and (c) PolyT40 sequences in PBS-Mg^{2+} buffer. The concentration of aptamer varied from 0 to 20 μM.

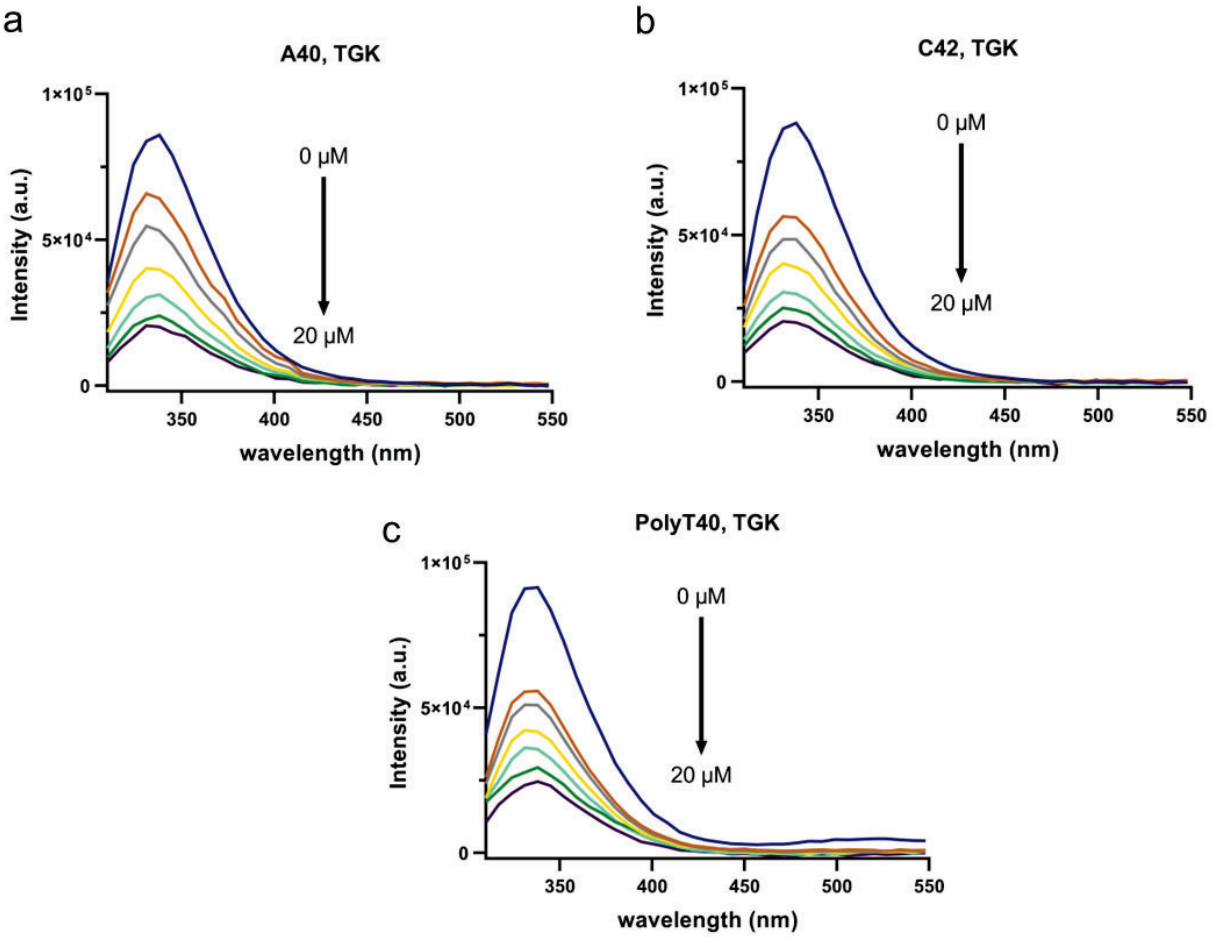

Figure S7. Fluorescence titration curves of lysozyme (1 μM solution, λ_{exc} = 298 nm) with (a) A40, (b) C42 and (c) PolyT40 sequences in TGK buffer. The concentration of aptamer varied from 0 to 20 μM.