**Supporting Information**

# Comprehensive Analytical Comparison of Strategies Used for Small Molecule Aptamer Evaluation

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Additional Materials and Methods

Figure S10: Representative binding curve of aptamer A08 using the gold nanoparticle assay results in significant underestimated $K_D$ due to signal enhancement.

Figure S11: Representative TEM image results from the gold nanoparticle binding assay using aptamer A08min.

Supporting Information References

## Additional Materials and Methods

*Materials*

Ochratoxin A (OTA) standard, chemicals and solvents were reagent grade or better and were purchased from Sigma-Aldrich (Milan, Italy or St. Louis, MO, USA) unless otherwise indicated. Ultrapure water was obtained from a Millipore Milli-Q deionized water system at 18 MΩ (Waters, Milford, MA).

Fast Micro-Equilibrium Dyalizer (500 µL capacity) chambers and regenerate cellulose membranes (MWCO 5,000 Da) were from Harvard (Harvard, Holliston, MA). Amicon-Ultra (0.5 mL 3,000 Da) centrifugal filter units were purchased from Fisher Scientific Canada (Ottawa, Canada). Spin-X centrifuge tube filters (0.22 µm cellulose acetate) were from Corning Incorporated (Corning, NY, USA). CarboxyLink Coupling gel (immobilized diaminodipropylamine, DADPA, supplied in a 50% slurry), 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC), and sulfo-NHS acetate were purchased from Thermo Scientific (Rockford, IL, USA). Dynabeads® M-270 Amine were purchased from Life Technologies (Carlsbad, CA). All molecular biology grade electrophoresis chemicals were purchased from BioShop Canada (Burlington, Canada). DNase I was purchased from New England BioLabs (Ipswich, MA, USA). The CM5 sensor chip, NaOH, and solutions of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and N-hydroxysuccinimide for the SPR assay were purchased from GE Healthcare Life Sciences (Pittsburgh,PA, USA).

The ssDNA aptamer sequences containing the appropriate modifications (Table S1) were purchased as HPLC purified from Bio-Fab Research srl (Rome, Italy), or prepared using standard phosphoramidite chemistry on a Bioautomation Mermade 6 (Plano, Texas), or ordered through IDT DNA and purified by PAGE. For ssDNA aptamers prepared on the Bioautomation MerMade 6, phosphoramidites, modifiers, activator, deblock, capping, and oxidizing reagents were obtained from Glen Research (Sterling, VA, USA). Standard support columns and acetonitrile were purchased from BioAutomation (Plano, TX, USA). Ultra High Purity 5.0 argon was purchased from Praxair Canada (Mississauga, Canada). DNA was purified with a denaturing polyacrylamide gel electrophoresis (12%) followed by clean-up with Amicon-Ultra (0.5 mL 3,000 Da) centrifugal filter units. Sequence synthesis was verified through molecular weight verification using electrospray ionization (ESI) mass spectrometry (Novatia LLC, Monmouth Junction, NJ, USA). Each aptamer investigated was dissolved in Milli-Q deionized water at 18.2 MΩ water to obtain stock solutions and properly diluted in working buffer prior to use (see Table S1).

*OTA preparation*

Pure standards of OTA used for binding studies were purchased from Sigma-Aldich. OTA stock solution, with a final concentration of 2.47 mM was prepared by dissolving the solid toxin in

toluene/acetic acid 99:1 (*v/v*). This standard solution was dried and reconstituted in working buffer to obtain appropriate OTA dilutions in the range of 1 fM to 100 µM. See details below for OTA concentrations used in each binding study.

*Equilibrium dialysis*

Binding assays by equilibrium dialysis were performed according to the procedure described[1] with minor modifications. For each investigated aptamer, the aptamer solution was mixed with an equal volume of the OTA standard solution (100 nM) and a volume of 300 µL was loaded in the loading chamber. The receiving chamber was loaded with the same volume (300 µL) of the relevant selection buffer and the incubation was performed by shaking for 48 hours at room temperature. Ten different aptamers concentrations were tested in the range of 12.5 nM to 5 µM in presence of 50 nM OTA. Each dialysis was carried out in duplicate.

After the incubation time, an aliquot (50 µL) of the solutions contained in the loading chamber (containing the complex aptamer-OTA and unbounded OTA) and in the receiving chamber (containing unbound OTA) were analyzed by HPLC to estimate the fraction of OTA bound to the aptamer. The percentage of bound OTA (f) was calculated as:

$$f = \frac{F_l - F_r}{F_l} * 100$$

where $F_l$ and $F_r$ are the peaks area of OTA recovered from the loading and the receiving chamber, respectively.

*Ultrafiltration*

For each investigated aptamer, the aptamer solution (300 µL) was mixed with an equal volume of the OTA standard solution (100 nM) and shaken for 1 hour at room temperature. Next, 400 µL of this mixture was passed through an Amicon-Ultra centrifugal filter unit by centrifugation for 30 min at 14000 x *g*. Ten different aptamers concentrations were tested in the range of 12.5 nM to 5 µM in presence of 50 nM OTA. Each dialysis was carried out in duplicate. A solution containing 50 nM OTA in the absence of aptamer was treated likewise and used as control. An aliquot (50 µL) of the centrifuged fractions containing unbounded OTA were analyzed by HPLC to estimate the fraction of OTA bounded to the aptamer. The percentage of bound OTA (f) was calculated as:

$$f = 100 - \left(\frac{A_1}{A_2} * 100\right)$$

where A1 is the peak area of OTA in the filtrate fraction resulting from incubation with aptamer and A2 is the peak area of OTA in the filtrate fraction used as reference.

*Ochratoxin determination by HPLC*

A 50 µL aliquot of each solution was injected into an Agilent 1100 Series chromatographic system (Agilent Technologies, Palo Alto, CA, USA) equipped with a fluorometric detector (model 363, $\lambda_{ex}$ = 333 nm, $\lambda_{em}$ = 460 nm) and the ChemStation data software (Agilent Technologies). The analytical column was a Zorbax SB-C18 (5 µm, 4.6×150 mm; Agilent Technologies), and a mixture acetonitrile/water/acetic acid 99:99:2 (v/v/v) was used as mobile phase at a flow rate of 1 mL/min (OTA retention time t = 7.0 min).

*Affinity chromatography with agarose resin*

For affinity chromatography using an agarose resin, OTA was immobilized to a solid support matrix as reported by Cruz- Aguado & Penner.[1] Briefly, 12.5 mL of DADPA slurry was

extensively washed with 20 mM phosphate buffer, pH 5.0, prior to use. 12.5 µmol (5 mg) of OTA dissolved in 500 µL of DMSO was mixed with 1250 µmol (240 mg) of EDC in 2.5 mL of deionized water. The mixture was immediately incubated with the DADPA slurry by shaking for 1 hour at room temperature. The slurry was washed five times with 0.2 M carbonate buffer (pH 8.5) followed by an additional 1 hour incubation with 750 µmol (194.25 mg) of sulfo-NHS acetate, dissolved in the same buffer to quench unreacted amine groups. A negative resin (without immobilized OTA) was prepared as control.

100 µL of the resin (positive or negative) was added to Spin-X centrifuge tube filters and washed five times with 300 µL of the relevant selection buffer by centrifugation at 14,000 x *g* for 1 min. Aptamer solutions were heated at 90 °C for 5 min, cooled to 4 ˚C for 10 min and at room temperature for 15 min. An aliquot (100 µL) of this aptamer solution was then added to the Spin-X centrifuge tube containing the resin and incubated for 1 hour with shaking at room temperature. Free aptamers were removed by washing and centrifuging the column with 5 x 200 µL of selection buffer. Bound aptamers were then collected by incubating the column with 5 x 200 µL of 6 M urea for 10 min at 90 °C. The ssDNA elution fractions were pooled and the fluorescence was measured using a Spectrofluorometer LS 55 (PerkinElmer) with the follows conditions: $\lambda_{ex}$ = 490 nm and $\lambda_{em}$ = 517 nm $slit_{ex}$ = 10 nm and $slit_{em}$ = 10 nm, integration time = 5 s.

*Affinity chromatography with magnetic beads*

This assay was performed as previously described.[2] Briefly, 1 mL aliquots of amino-modified magnetic beads were magnetically separated using a DynaMag-2 magnet with approximately 10 minutes of incubation before the supernatant was removed and discarded. The beads were then washed three times using 0.1M MES buffer (pH 4.5). Freshly prepared 1 M EDC (0.5 mL) and 1 mL of 60 µM OTA were added to the beads and incubated for 2 hours at room temperature. The solution was magnetically separated from the beads and the amount of unbound OTA was measured using fluorescence to confirm successful conjugation. The beads were then washed twice with 0.1 M sodium bicarbonate buffer (pH 8.5). A 1 mL aliquot of freshly-prepared solution of sulfo-NHS acetate (0.05 M) was then reacted with the beads for 2 hours to block any unreacted amine groups. Before storage, the beads were washed three times with 1.0 M tris buffer (pH 7.4) and three times with selection buffer.

A series of 5' fluorescein modified aptamer concentrations ranging from 8 nM to 1 µM were heated to 90 ºC for five minutes then cooled on ice. 90 uL aliquots of OTA derivatized beads were separated on the DynaMag-2 and washed three times with selection buffer. 90 uL of each aptamer dilution were incubated with the beads for 1 hour. The supernatant was discarded and unbound aptamer was removed with four washes with selection buffer. Bound DNA was then eluted with selection buffer and heating at 90 ºC for ten minutes. The supernatant was collected and filtered to remove any remaining beads that often impair the fluorescence background measurements. The elution step was repeated to ensure complete recovery of bound DNA and pooled. The fluorescence of eluted DNA for each aptamer dilution was measured ($\lambda_{ex}$ = 490 nm; $\lambda_{ex}$ = 520 nm) using a Fluorolog Fluorescence Spectrophotometer with a SpectrAcq controller (Horiba Jobin Yvon, USA). The experiment was performed in triplicate.

*Fluorescence polarization*

The binding assay based on fluorescence polarization (FP) was performed by measuring changes of polarization between OTA free in solution and OTA bound to the aptamer. Prior to FP analysis, aptamer solutions were heated at 90 °C for 5 min, followed by cooling at room

temperature for 30 min. An aliquot (50 µL) of aptamer solution was mixed with an equal volume (50 µL) of OTA standard solution (100 nM) in a quartz cell (Quartz SUPRASIL, Hellma Analytics, Müllheim, Germany) and incubated at room temperature for 2 min. Then the polarization signal was measured using a Spectrofluorometer LS 55 (PerkinElmer). Ten different aptamers concentrations were tested in the range of 12.5 nM to 5 µM in presence of 50 nM OTA. FP measurements were made in three independent experiments using the following spectrofluorometer conditions: $\lambda_{ex}$ = 380 nm and $\lambda_{em}$ = 430 nm, slitex = 10 nm and $slit_{em}$ = 10 nm, integration time = 5 s. Polarization measurements were performed after reaching a constant value, generally between 1 and 3 min. The binding curves were obtained by plotting the millipolarization units (mP, where 1 mP is defined as P/1000) versus the aptamer concentration.

*Surface plasmon resonance assay*

This assay was performed as previously described.[3] Briefly, the Biacore X100 instrument was primed three times with running buffer prior to all binding assays. For each assay, three startup cycles were performed to stabilize the sensorgram baseline. For each startup cycle, the aptamer (~70 pmol) was captured onto the sample flow cell (FC2) for 40 s at a flow rate of 5 µL/min, yielding ~2,000–5,000 RU, followed by an injection of 25 mM NaOH for 30 s at a flow rate of 30 µL/min over both flow cells to regenerate the sensor surface. A dilution series of OTA was prepared in selection buffer from 5 nM to 8000 nM. For each concentration sample, the aptamer was captured onto the sample flow cell (FC2) for 40 s at a flow rate of 5 µL/min, the OTA solution was injected over both flow cells at a flow rate of 30 µL/min to monitor OTA association, and selection buffer was injected over both flow cells at a flow rate of 30 µL/min to monitor OTA dissociation. The surface was regenerated by injecting 25 mM NaOH for 30 s at a flow rate of 30 µL/min over both flow cells.

Data processing and analysis were performed using Biacore X100 Evaluation Software version 2.0 (GE Healthcare). The data were fit to a 1:1 binding model for kinetic analysis or steady-state affinity model for thermodynamic analysis. Reported values are the mean and standard deviation of three independent experiments.

*DNase I gel assay*

This assay was performed as previously described**.**[4] Briefly, 5′-fluorescein modified aptamer and OTA were mixed for 30 min on a vortex shaker at room temperature. DNase I was added, briefly vortexed to ensure complete mixing, and incubated at 37 °C for precisely 1 min in a heat block. DNase I digestion was stopped with the addition of 1 µL 0.5 M EDTA at 90 °C, vortexed, and immediately heat deactivated at 90 C for 10 min. The samples were mixed 1:1 with formamide and heated at 55 °C for 5 min prior to separation for 3 hours at 300 V on a 19 % denaturing PAGE gel in an SE 600 Chroma Standard Dual gel electrophoresis unit. Gels were visualized with the fluorescent setting (302 nm) in an Alpha Imager Multi Image Light Cabinet (Alpha Innotech). Fluorescent digestion bands were quantified using the SpotDenso program on AlphaImager. Each band produced from the digestion was calculated as a ratio relative to the entire sample to avoid any errors stemming from loading discrepancies. The density of each band (A, B, C, etc.) relative to the OTA concentration was plotted for the binding curves.

*SYBR Green I assay*

This assay was performed as previously described.[5] Briefly, each aptamer, SYBR Green I (SG) and OTA dilutions were prepared in appropriate selection buffer. First, each aptamer was heated to 90 ˚C for five minutes and cooled to room temperature before use. 4 µL of SG (1X)

and 4 µL of aptamer (10 µM) were mixed together. OTA varying from 1 pM to 10 µM were prepared in selection buffer and added directly to the SG-aptamer mixture to a final volume of 125 µL. The fluorescence emission spectra were recorded from 500 to 650 nm using an excitation wavelength of 497 nm. The fluorescence at 520 nm was used to calculate the biosensor signal

$$Response = \frac{F_\theta - F}{F}$$

where $F_\theta$ is the fluorescence intensity in the absence of OTA and F is the fluorescence intensity at a given concentration of OTA. Fluorescence was measured with Fluorolog Fluorescence Spectrophotometer with a SpectrAcq controller (Horiba Jobin Yvon, USA).

*Gold nanoparticle assay*

The gold nanoparticle (AuNP) assay was performed as described previously with minor modifications.[6] All glassware used for AuNP synthesis was cleaned by soaking in aqua regia (3:1 mixture of concentrated HCl/$HNO_3$) for 15 min followed by thorough rinsing with deionized water. A 250 mL Erlenmeyer flask was used to mix 98 mL of deionized water and 2 mL of 50 mM $HAuCl_4$ for a final concentration of 1 mM $HAuCl_4$. The solution was heated to boiling with magnetic stirring. Upon boiling, 10 mL of 38.8 mM sodium citrate was added. Heating was continued for an additional 20 min following a change in suspension color to red. The flask was removed from the heat and allowed to cool to room temperature with continued stirring. Nanoparticles were characterized by UV-Visible spectrometry, displaying a $\lambda_{max}$=520 nm.

Samples of OTA in water were prepared at concentrations ranging from 1 fM to 100 µM. 6 µL of aptamer (10 µM in water) and 135 µL of the AuNP solution (11 nM) were incubated for 30 minutes. 243 µL OTA was added into microcentrifuge tubes containing the AuNPs and aptamer and vortexed briefly followed by a 30 min incubation time. 75 µL of 0.5 M NaCl was then added to each microcentrifuge tube and allowed to incubate for a further 5 min. Each sample was then analyzed using UV-Visible spectrometry. The relative absorption ratio between 695 nm and 520 nm [($A_{695}/A_{520}$) was plotted against OTA concentration. This experiment was performed in triplicate. UV/Vis absorption spectra were obtained using a Cary 300 Bio UV-Visible spectrophotometer (Varian, USA)

*Transmission electron microscopy of gold nanoparticles*

High resolution TEM images of the aptamer-AuNP samples were recorded by drop-casting dilute suspension of aptamer-AuNP and its target (OTA) or control (warfarin) prior to or following the addition of NaCl, on a carbon-coated copper grid. Images were recorded on a FEI Tecnai G2 F20 TEM with a Schottky Field Emitter with high maximum beam current (>100nA) electron source and imaged with a Gatan ORIUS TEM CCD Camera.

Table S1. OTA aptamers and working buffers used for the binding assay studies.

| **Aptamer** | **DNA sequence (5′→3′)** | **Working buffer** | **Ref** |
|---|---|---|---|
| 1.12.2 | GATCGGGTGTGGGTGGCGTAAAGGGAGCATCGGACA | 10 mM HEPES, 120 mM NaCl, 5 mM KCl, 5 mM $MgCl_2$, pH 7.0 | 1 |
| T22-$O_{36}$-T3 | TTTTTTTTTTTTTTTTTTTTTTGATCGGGTGTGGGTGGCGTAAAGGGAGCATCGGACATTT | 10 mM Tris-HCl, 120 mM NaCl, 20 mM $CaCl_2$, pH 7.0 | 7 |

| | | | |
|---|---|---|---|
| A08 | AGCCTCGTCTGTTCTCCCGGCAGTGTGGGCGAATCTATGC GTACCGTTCGATATCGTGGGGAAGACAAGCAGACGT | 10 mM $Na_2HPO_4$, 2 mM $KH_2PO_4$, 2.7 mM KCl, 137 mM NaCl, pH 7.4 | 5 |
| A08min | GGCAGTGTGGGCGAATCTATGCGTACCGTTCGATATCGTG | Same as A08 | 5 |
| B08 | AGCCTCGTCTGTTCTCCCGGCGCATGATCATTCGGTGGGTA AGGTGGTGGTAACGTTGGGGAAGACAAGCAGACGT | Same as A08 | 5 |
| H8 | GGGAGGACGAAGCGGAACTGGGTGTGGGGTGATCAAGGG AGTAGACTACAGAAGACACGCCCGACA | PBS 1x, 1 mM $MgCl_2$, 0.01% Tween 20, pH 7.4 | 8 |
| H12 | GGGAGGACGAAGCGGAACCGGGTGTGGGTGCCTTGATCCA GGGAGTCTCAGAAGACACGCCCGACA | Same as H8 | 8 |

Table S2. 95% confidence intervals of $K_D$ values for OTA aptamers using quantitative binding assays.

| OTA aptamer | 95% confidence intervals of $K_d$ values (nM) using different analytical approaches | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Equil. dialysis** | **Ultra-filtration** | **Affinity chrom. (mag beads)** | **Flu. polar-ization** | **SPR** | **DNAse assay** | **SYBR Green** |
| **1.12.2** | 171 to 403 | 152 to 358 | 21 to 727 | 78 to 172 | 142 to 184 | n/a | 54 to 239 |
| **T22-O36-T3** | 119 to 201 | 148 to 353 | n/a | 57 to 96 | 46 to 80 | n/a | 10 to 51 |
| **A08** | n/a | n/a | 80 to 493 | n/a | n/a | -18 to 418 | 23 to 240 |
| **A08min** | n/a | n/a | 176 to 636 | n/a | n/a | n/a | 59 to 280 |
| **B08** | n/a | n/a | 64 to 186 | n/a | n/a | 211 to 1129 | 7 to 27 |
| **H8** | n/a | n/a | 4 to 24 | n/a | n/a | 22 to 86 | NB |
| **H12** | n/a | n/a | 21 to 60 | n/a | n/a | -9 to 549 | NB |

n/a Not applicable

Table S3. Overview of the evaluated analytical and practical capabilities for each aptamer binding assay.

| | **Equil. dialysis** | **Ultra-filtration** | **Affinity chrom. (agarose)** | **Affinity chrom. (mag beads)** | **Flu. Polar-ization** | **SPR** | **DNAse assay** | **SYBR Green** | **AuNP assay** |
|---|---|---|---|---|---|---|---|---|---|
| *Analytical capabilities and limitations* | | | | | | | | | |
| **Quantitative** | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| **Precision (coefficient of variation, %)** | 16 | 27 | n/a | 49 | 15 | 14 | 60 | 33 | n/a |
| **Method provides a 2° readout to confirm results** | No | No | No | No | No | Yes | No | No | Yes |
| **False Positives/ negatives** | False negatives | False negatives | False positives | No | False negatives | False negatives | False negatives | False negatives | No |
| **Additional information obtained** | No | No | No | No | No | Yes[1] | Yes[2] | No | No |
| **Target in solution** | Yes | Yes | No | No | Yes | Yes | Yes | Yes | Yes |
| *Practical benefits and challenges* | | | | | | | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **High-throughput** | Possible | Possible | No | Possible | Yes | Yes | No | Yes | Yes |
| **Requires modification or labelling** | Target[3] | Target[3] | Target and DNA[4] | Target and DNA[4] | Target[3] | DNA[5] | DNA[6] | No | No |
| **Sequence/ size limitations** | No[7] | No[7] | No | No | No | Yes | Yes | No | Yes |
| **Equipment required** | HPLC, MS, or other detection | HPLC, MS, or other detection | Fluorimeter/ scintillation counter | Fluorimeter/ scintillation counter | Fluorimeter | SPR | Gel imager | Fluorimeter | UV-Vis |
| **Cost[8] (consumable)** | $100 | $100 | $50 | $100 | $50 | $200 | $100 | $50 | $50 |

1 Kinetics can be obtained with this method
2 Structural characteristics can be elucidated with this method
3 Target must have inherent fluorescence/ absorption properties or be labelled for detection and quantification
4 Target must be immobilized through available functional groups; DNA must be tagged with fluorophores or radiolabeled
5 DNA must be extended with poly-A linker, or modified with thiol, biotin, etc.
6 DNA must be tagged with fluorophores or radiolabeled
7 Different filter sizes can be purchased to accommodate most aptamer-target pairs
8 Based on assay performed in duplicate with a minimum of six concentration points; cost of target not included; DNA purchased through IDT DNA using standard synthesis and clean-up

Figure S1: Binding isotherms for aptamers 1.12.2 and T22-O36-T3 with OTA obtained using equilibrium dialysis. The curve was generated using GraphPad Prism 6 (Version 6.04). Each curve is the average of two independent trials.

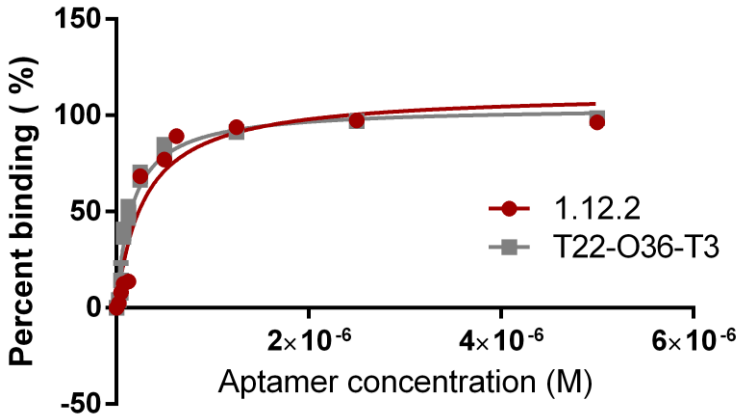


Figure S2: Binding isotherms for aptamers 1.12.2 and T22-O36-T3 with OTA obtained using ultrafiltration. The curve was generated using GraphPad Prism 6 (Version 6.04). Each curve is the average of two independent trials.

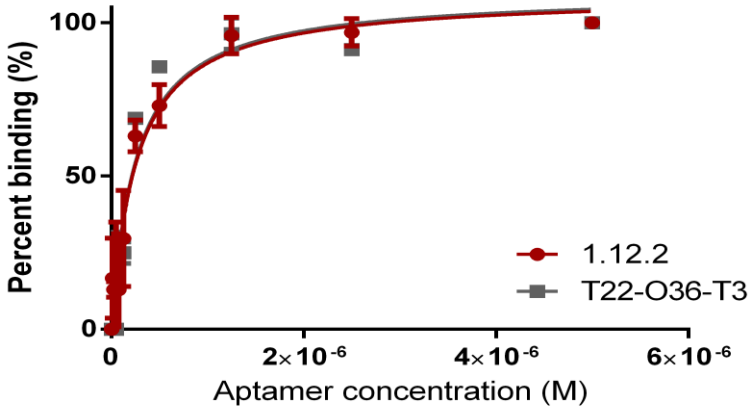



Figure S3: Sample of non-specific binding observed with agarose resin-based affinity chromatography. Aptamer A08 was used in this example.

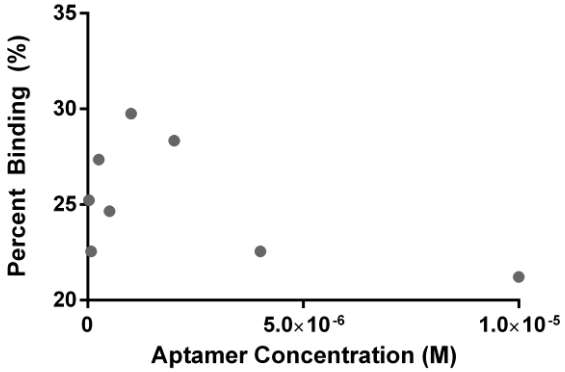



Figure S4: Binding isotherms for aptamers 1.12.2, A08, A08min, B08, H8, H12 with OTA obtained using affinity chromatograph (with magnetic beads). The curve was generated using GraphPad Prism 6 (Version 6.04). Each curve is the average of two independent trials.

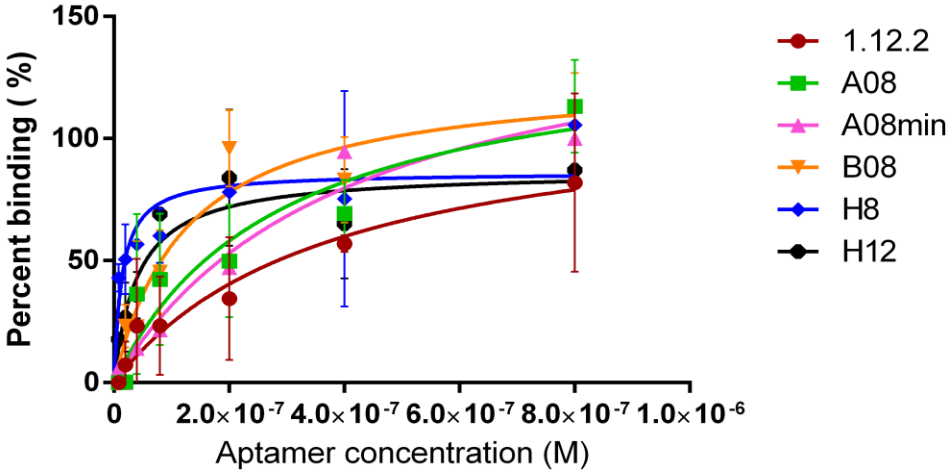


Figure S5 Binding isotherms for aptamers 1.12.2 and T22-O36-T3 with OTA obtained using fluorescence polarization. The curve was generated using GraphPad Prism 6 (Version 6.04). Each curve is the average of two independent trials.

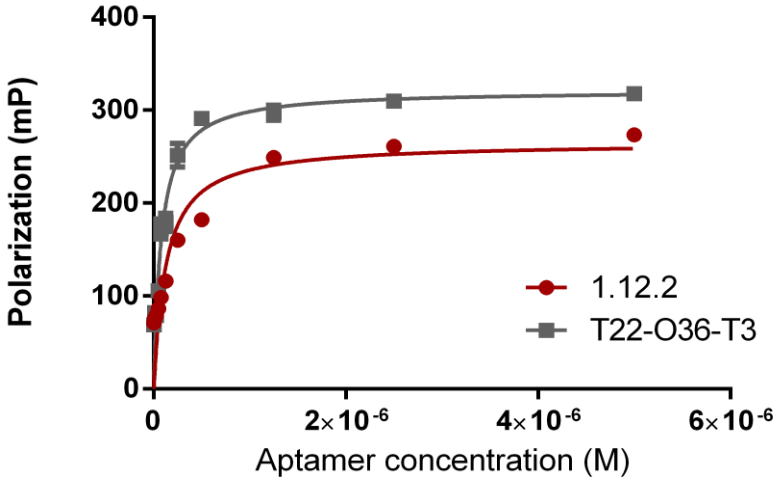



Figure S6: Binding isotherms for aptamer 1.12.2 and 1.12.2 in the T22-O36-T3 buffer obtained using the SPR assay. The curve was generated using GraphPad Prism 6 (Version 6.04). Each curve is the average of two independent trials. The T22-O-36-T3 aptamer could not be evaluated directly due to the polyT required for SPR immobilization.

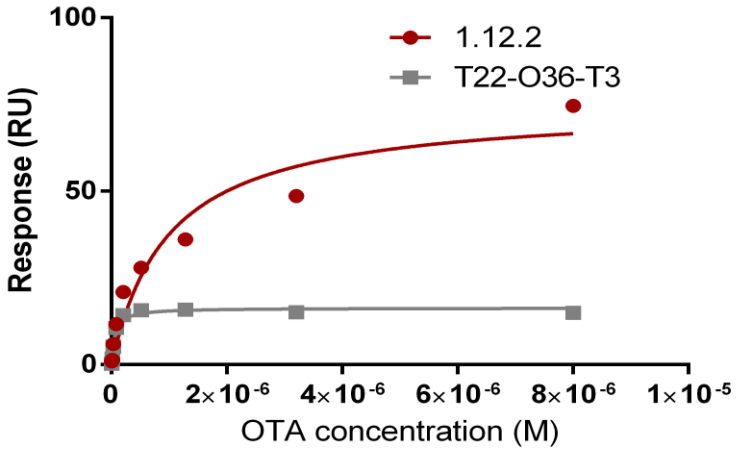


Figure S7: Binding isotherms for aptamers 1.12, A08, B08, H8 and H12 with OTA obtained using the DNAse I assay. The curve was generated using GraphPad Prism 6 (Version 6.04). Each curve is the average of two independent trials.

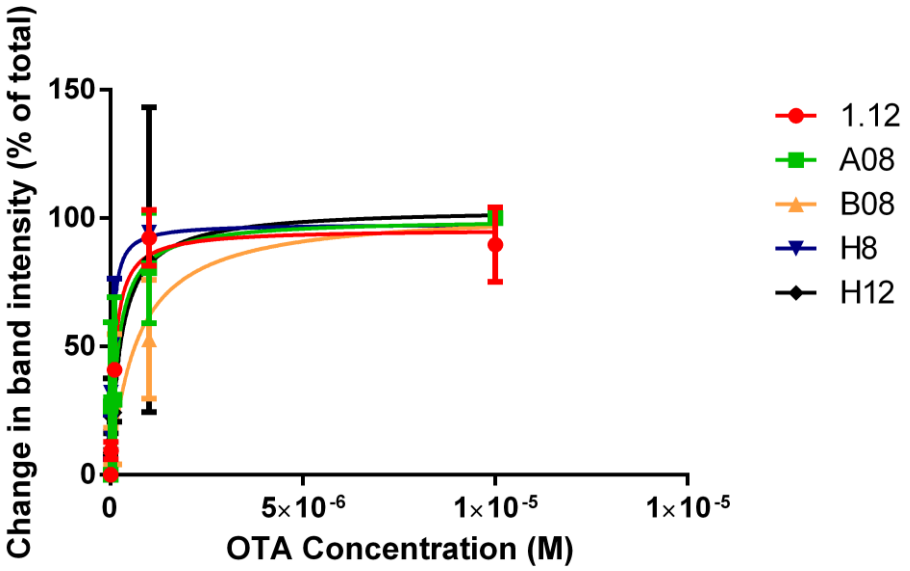



Figure S8: Representative DNAse I assay gel image for aptamer A08 with OTA. Lane 1: Full-length undigested aptamer. Lanes 2-7: fluorescein-labelled A08 aptamer incubated with 0.1 nM, 1 nM, 10 nM, 100 nM, 1 µM and 10 µM OTA and digested with DNase I. The red box highlights the band of interest (significant change in cleavage occurring with increased concentrations of OTA).

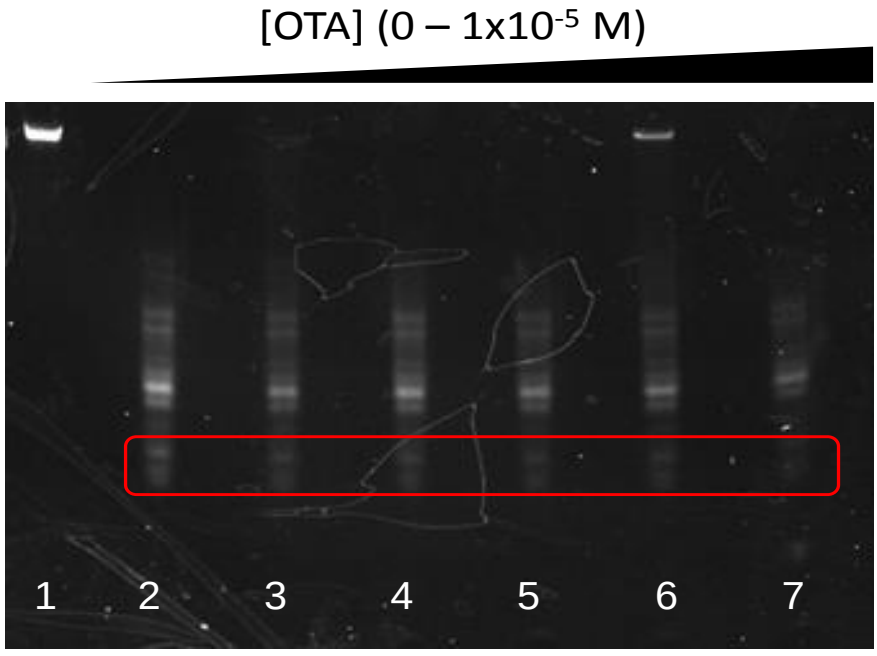


Figure S9: Binding isotherms for aptamers 1.12.2, T22-O36-T3, A08, A08min and B08 with OTA obtained using the SYBR Green I assay. The curve was generated using GraphPad Prism 6 (Version 6.04). Each curve is the average of two independent trials.

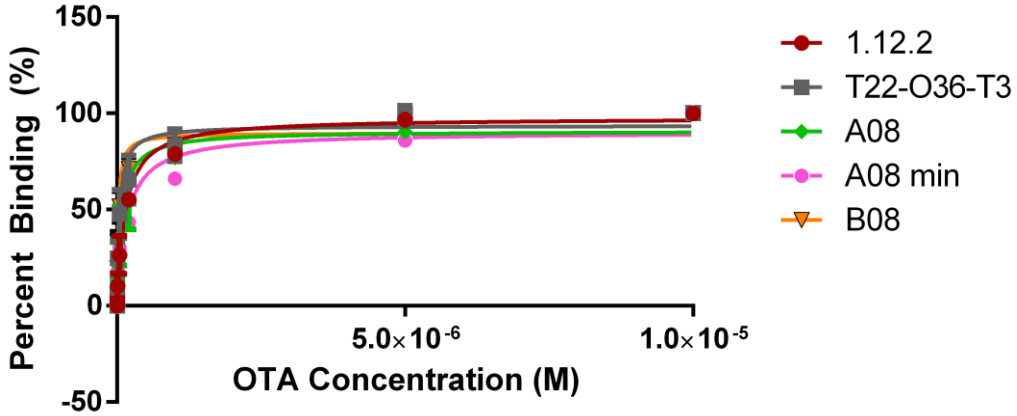



Figure S10: Representative binding curve of aptamer A08 using the gold nanoparticle assay results in significant underestimated $K_D$ due to signal enhancement (more than 3 orders of magnitude difference compared to all other methods). This method is therefore not reliable for $K_D$ evaluation and can only be used to screen for binders.

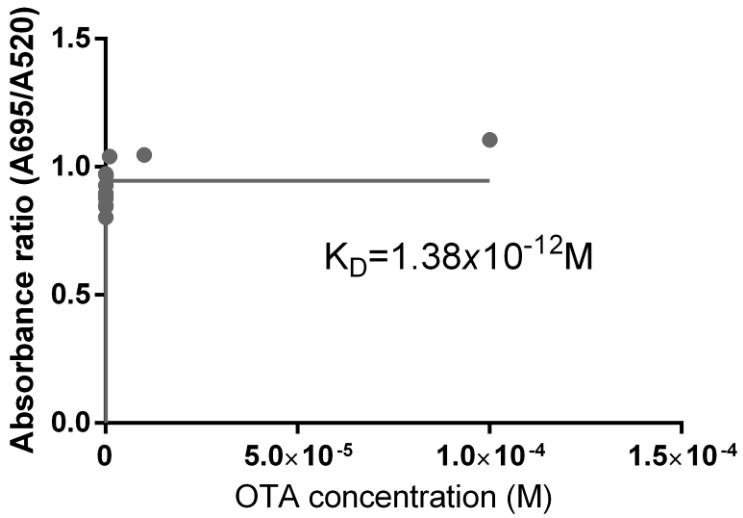
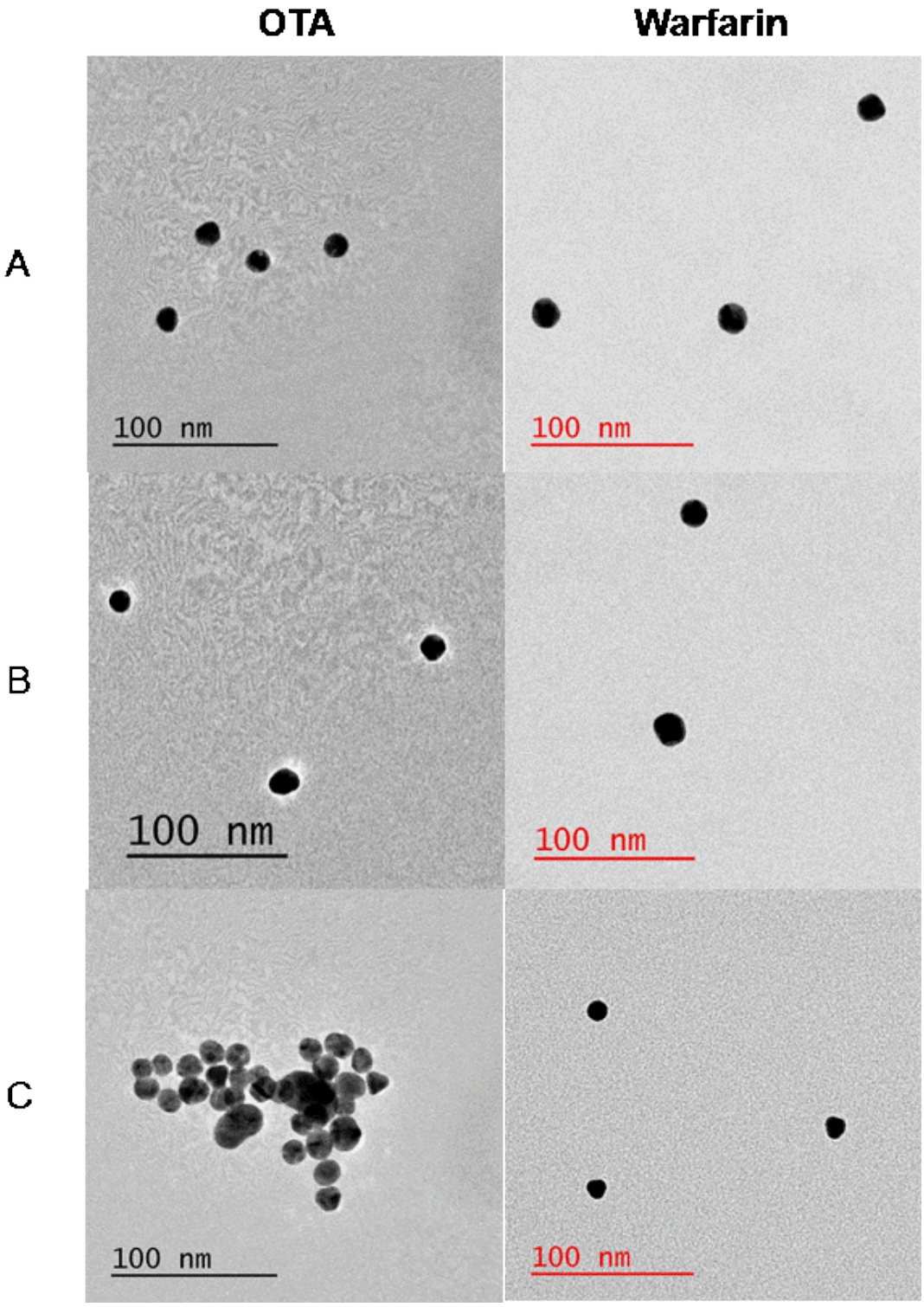


Figure S11: Representative high-resolution TEM images the gold nanoparticle binding assay using aptamer A08min with target OTA and control target warfarin A) Aptamer A08min coated AuNPs B) Aptamer A08min coated AuNPs in the presence of target C) Aptamer A08min coated AuNPs in the presence of target and the addition of NaCl.



Supporting Information References

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