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# Journal Pre-proof

High performance aptasensing platform development through in silico aptamer engineering for aflatoxin B1 monitoring

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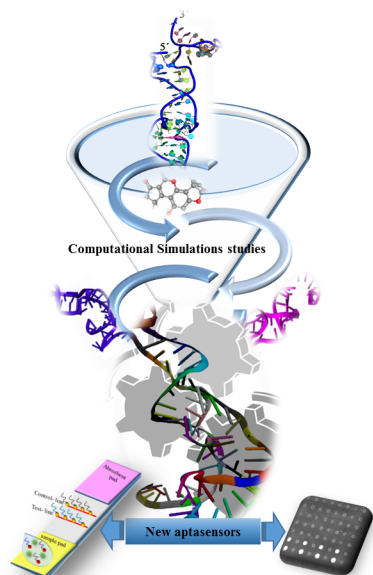
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1 **High performance aptasensing platform development through in silico aptamer**  
2 **engineering for aflatoxin B1 monitoring**

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13  
14 **Abstract**

15 Due to the technical challenges of small binding aptamer development, reliable  
16 computational simulation studies can be considered as effective tools to design novel and  
17 high functional mycotoxin aptameric probes. Here, two novel aflatoxin B1(AFB1) binding  
18 aptamers were successfully exploited as recognition elements in the lateral flow aptasensors  
19 and the reflective phantom interface (RPI) platform. Using the parent aptamer previously  
20 designed through genetic algorithm based in silico maturation (ISM) strategy, F20, a new  
21 variant, F20-T, was obtained here via coupling truncating strategy and computational  
22 simulation approaches. Two aptamer-gold nanoparticle strip biosensors were developed  
23 based on the designed probes for the simple and rapid detection of AFB1 in competitive  
24 format. The F20-based strip was more sensitive than that exploiting the truncated aptamer,  
25 with limits of detection (LOD) of 0.1 and 0.5 ng/mL, respectively. Based on the in silico and  
26 experimental selectivity evaluations of both test strips towards other mycotoxins, including  
27 aflatoxin B<sub>2</sub>, M<sub>1</sub>, G<sub>1</sub>, G<sub>2</sub>, Ochratoxin A and Zearalenone, F20-T based test strip revealed  
28 higher selectivity for AFB1. Both developed aptasensors successfully detected AFB1 in  
29 maize flour within 30 min using a simple strip reader. Exploiting of F20 and F20-T aptamers  
30 in an exclusive technology called RPI platform led to successful AFB1 detection, as well.  
31 Both designed aptameric probes can be regarded as potential recognition elements to develop  
32 screening tools for rapid, low cost and on-site AFB1 detection. Our findings highlighted the

33 reliable and robust application of computational simulation studies for novel small binding  
34 aptamer development and consequently open up a much-needed avenue to design various  
35 aptasensing platforms in green and cost effective ways.

36 **Key words:** aptasensor, computational simulations, lateral flow, aflatoxin B1, small  
37 molecule

38

## 39 **Introduction**

40 Among aflatoxin contaminations, AFB1 is the most prevalent and identified as the first  
41 hazard class by the International Agency for Research on Cancer (IARC, 2000). To monitor  
42 the low permissible concentrations of AFB1 in complex food and feed matrices (EC, 2010),  
43 various analytical methods have been developed. In spite of high sensitivity and specificity of  
44 chromatography based instrumental techniques, high cost, time-consuming and requiring  
45 highly skilled personnel are the main obstacles for their large-scale applications (Miklos et  
46 al., 2020). Regarding to the rapidity and simplicity, antibody-based immunoassays are widely  
47 used in routine food analysis. Considered as non- immunogenic compounds, the antibody  
48 generation for mycotoxins has several issues in terms of high cost, time consume and  
49 laborious (López-Puertollano et al., 2018).

50 Responding to increasing demand for affordable, accurate and simple devices for mycotoxin  
51 detection especially outside the laboratory, various biosensing platforms have been developed  
52 as alternative analysis tools to ensure food safety (Chauhan et al., 2016). While antibodies  
53 have become the most popular recognition elements in biosensor words for four decades (Di  
54 Nardo et al., 2021), aptamers have emerged as a potent rival of antibodies owing to inherent  
55 advantages over them .Unlike antibodies, aptamers can be selected over various targets  
56 regardless of their immunogenicity through an in vitro selection process called systematic  
57 evolution of ligands by exponential enrichment (SELEX). The low cost, ease of synthesis,  
58 prolonged shelf-life and regeneration under a broad range of conditions make aptamers as  
59 attractive candidates to incorporate in mycotoxin biosensing devices (Yang et al., 2013).

60 Despite the increasing demand for mycotoxin binding aptamers, a set of technical challenges  
61 are still the main bottlenecks for their research and commercialization. The SELEX process,  
62 known as a gold-standard methodology for aptamer development, is still cost, laborious and  
63 time-consuming. Also, the limitation of initial library diversities and sequence bias during

64 iterative PCR reduced the success rate of SELEX by 50% for recovering high binding affinity  
65 aptamers (Sun and Zu, 2015). These drawbacks are more highlighted in the case of small  
66 molecules binding aptamers because most affinity binding assays are not sensitive enough to  
67 separate small target-bound sequences from other ones due to drastic different size between  
68 small molecules such as mycotoxins and their binding aptamers (Ruscito and DeRosa, 2016).  
69 Also, the limited functional groups in small molecules decrease probability of finding high  
70 functional aptamers that can interact with the target via electrostatic, H-bonds, hydrophilic or  
71  $\pi$ - $\pi$ -stacking interactions (Mascini, 2009).

72 Despite the requirement of high affinity and novel aptamers to design mycotoxin biosensing  
73 platforms, a few aptamers have been developed for the class of hazardous compounds  
74 (McKeague et al., 2015). Duo to the very few novel aptamers for mycotoxins, coupling them  
75 to the various transducer systems can be considered as a compensatory solution to design  
76 high performance mycotoxin biosensing devices (Yang et al., 2013). Among various  
77 aptasensing platforms, lateral flow based aptasensor development can be considered as a  
78 promising answer to the increasing demand for simple, low cost, portable and on site  
79 detection of AFB1. However, until now a few lateral flow based aptasensors have been  
80 developed for AFB1 monitoring (Shim et al., 2014, Zhu et al., 2017, Zhang et al., 2018b,  
81 Zhao et al., 2020), all of which have exploited Apt1 aptamer patented by Neoventures  
82 Biotechnology Inc. (NeoVentures Biotechnology Inc.). Exploiting the only AFB1 aptamer  
83 (Apt1) in almost all developed aptasensors (Jia et al., 2019) makes it necessary to design  
84 novel aptamers as new recognition elements for existing biosensing platforms.

85 Being as main components, the bioreceptor and transducer properties have critical effects on  
86 the biosensor sensitivity and selectivity. Recently, various modified enzyme, recombinant  
87 antibody fragments and nanobodies have been developed through bioengineering techniques  
88 to increased acceptance and commercialization of the biosensing platforms (Hock et al.,  
89 2002). However, the technical barriers of aptamer development especially for small  
90 molecules have constituted major bottlenecks for aptamer engineering research and high  
91 functional aptamer discovery (Crivianu-Gaita and Thompson, 2016).

92 To overcome the challenges, experimental findings can be combined to the in silico  
93 approaches to refine the affinity and specificity of mycotoxin binding aptamers. Recently,  
94 aptamer engineering with the aim of sequence or scaffold optimizations have gain attentions  
95 as a promising area of active research to design and discover the novel mycotoxin binding

96 aptamers (Mousivand et al., 2020; Ciriaco et al., 2020; Hasegawa et al., 2016). In the case of  
97 small molecule targets, sequence truncating strategy can be considered as an effective  
98 approach to improve aptamer affinity and specificity via different size reduction between  
99 them and their aptameric partners (Aissa et al., 2020). Due to the cost and time constraints,  
100 the experimental evaluation of all designed aptamers can be considered as an important  
101 limitation for small molecules binding aptamers developing. Reliable computational  
102 simulations have the capacity to virtually screen a large database of aptamers and clarify their  
103 binding modes in cost and time effective ways (Zhang et al., 2018a; Mousivand et al., 2021;  
104 Chushak and Stone, 2009).

105 The aim of our study was to design new aptasensing platforms via *in silico* engineered  
106 aptamers instead of exploiting the same aptameric probe in different transducer systems.  
107 Therefore, F20 aptamer, previously designed based on Apt1 sequence through genetic  
108 algorithm based ISM approach (Mousivand et al., 2020, 2021), has been applied to develop a  
109 new truncated AFB1 aptamers, F20-T, via coupling truncating strategy and computational  
110 simulations. Both new designed AFB1 binding aptamers were exploited as new recognition  
111 elements in nanogold-based lateral flow aptasensors and RPI platform for AFB1 detecting.

## 112 **2. Material and Methods**

### 113 **2.1. Computational Studies**

#### 114 **2.1.1. Aptameric probes**

115 The main aptameric probe, F20, was previously designed based on the Apt1 sequence  
116 (Patent: PCT/CA2010/001292) subjected to generate the second probe, F20-T, through  
117 coupling truncating strategy and computational studies as follow.

118 The  $K_d$  values of F20, F20-T and Apt1 were estimated through unmodified AuNPs-based  
119 colorimetric assay (details in the Supporting Information) according to Mousivand et  
120 al.(2020).

#### 121 **2.1.2. Library generation and secondary structure analysis**

122 Based on F20 sequence, different variants were constructed using truncating strategy. The  
123 created library contained oligonucleotides variable in length, randomly truncated at either the  
124 5', 3' or both end of sequences. The secondary structures of potent aptamers in the truncated



125 library were predicted using the Mfold web server at 37°C and at ionic concentration of 1 M  
126 of Na<sup>+</sup>, 0 M of Mg<sup>2+</sup> based on the free energy minimization algorithm (Zuker, 2003).

### 127 **2.1.3. Molecular docking technique**

128 Virtual screening of the truncated library was performed using AutoDockTools (ADT) 1.5.4  
129 package (Morris et al., 2009) to evaluate AFB1 binding affinity of individual aptamers. The  
130 crystal structures of AFB1 were obtained from the PubChem database and considered as a  
131 flexible ligand while the predicted 3D-structure of aptamers were kept as rigid receptors.  
132 Three dimensional modeling of truncated ssDNA aptamer was constructed through a  
133 sequentially pipeline according to Mousivand et al. (Mousivand et al., 2020). Accordingly,  
134 different complexes were ranked based on the obtained docking scores including binding  
135 energy, type of favorable interactions and the binding sites. The best aptamer was then  
136 compared to the parent aptamer, F20, in terms of selectivity over different mycotoxins  
137 including AFB1, AFB2, AFG1, AFG2, AFM1, ZEN and OTA using molecular docking  
138 technique. The selected aptameric probe was further evaluated with the aid of molecular  
139 dynamic simulations.

### 140 **2.1.4. Molecular dynamic simulations (MDs)**

141 The conformational changes and binding mode of F20 and the corresponding truncated form,  
142 F20-T, were simulated alone in water and in complex with AFB1 during 50 ns of MD  
143 simulations. All simulations were conducted using GROMACS 5.1.4 software package  
144 (Berendsen et al., 1995) under AMBER99SB force field (Perez et al., 2007). SwissParm web  
145 server (Zoete et al., 2011) was employed to generate the ligands topology and parameter files.  
146 The best ranked complex taken from docking results was immersed in the center of a  
147 dodecahedron periodic box containing TIP3P water model with 1 nm away from each wall.  
148 After the MDs settings according to Mousivand et al., (2021), the system went through a final  
149 50 ns MD simulations at constant pressure and temperature conditions and the coordinates of  
150 the complexes were recorded every 10 ps for the subsequent analysis. MD simulations were  
151 analyzed using GROMACS tools and all visualizations were performed via Discovery Studio  
152 v3.5 (Biovia, 2015), VMD (Humphrey, 1996) and PyMOL (De Lano, 2002) softwares.

### 153 **2.1.5. Binding free energy MM-PBSA calculation**

154 To estimate the binding affinity over AFB1, the molecular dynamic trajectory files of F20  
155 and F20-T complexes were subjected to MM-PBSA analysis using g\_mmpbsa tool (Kumari

156 et al., 2014). Gibbs free energy and its different components including electrostatic, van der  
157 Waals, polar solvation and non-polar solvation energies were estimated. Regarding to the  
158 interest in relative binding of the selected aptamers, the entropy was not calculated. The most  
159 important nucleotides involved in binding affinity toward AFB1 were retrieved through  
160 energy decomposition per residue as well.

161

## 162 **2.2. Experimental studies**

### 163 **2.2.1. Reagents and Apparatus**

164 Gold (III) chloride trihydrate (ACS reagent), bovine serum albumin (BSA), Tris-(2-  
165 carboxyethyl) phosphine hydrochloride (TCEP), and mycotoxin standard solutions were  
166 purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were obtained  
167 from VWR International (Milano, Italy). 96-Microwell transparent plates were purchased  
168 from Nunc (Roskilde, Denmark). Thiol-modified aptamers and biotin modified probes were  
169 synthesized by TAG Copenhagen A/S (Denmark) (Supplementary Information, Table S1).  
170 The cellulose fiber pads (sample and absorbent pads) and nitrocellulose membranes (HF180  
171 plus card, 60mm × 300 mm) were obtained from Millipore (Billerica, MA, USA). Test and  
172 control lines were loaded on the nitrocellulose membrane by means of an XYZ3050 platform  
173 (BioDot, Irvine, CA, USA), equipped with two BioJet Quanti<sup>TM</sup> 3000 Line Dispenser for  
174 noncontact dispensing. The membrane was cut into 4.6 mm test strips by a CM4000  
175 guillotine (BioDot, Irvine CA, USA). The color intensity of test and control lines were  
176 *scanned and then analyzed by QuantiScan 3.0 software (Biosoft, Cambridge, UK). The ultra-*  
177 *pure water used throughout all experiments obtained by a Milli-Q system at 18.2 MΩ.*

178

### 179 **2.2.2. Preparation of gold nanoparticles (AuNPs)**

180 The synthesis of ~ 30-nm-diameter gold nanoparticles was performed through the HAuCl<sub>4</sub>  
181 reduction with sodium citrate (Cavalera et al., 2020). Typically, 1 mL of 1% sodium citrate  
182 was added to 100 mL of boiling 0.01% tetrachloroauric acid under constant stirring and  
183 heating. When the suspension color changed from light yellow to deep red, heating was  
184 continued for another 10 min and then cooled to room temperature. The prepared gold  
185 nanoparticle size was confirmed through displaying a  $\lambda_{\max}$  equal to 525 nm by UV-Visible  
186 spectrometry. The AuNPs solution was adjusted to pH 8.5 and concentrated to 10X at 14000  
187 rpm for 10 min and then stored at 4°C for subsequent conjugation.

188

### 189 **2.2.3. Preparation of gold nanoparticles-aptamer conjugates (AuNPs-Apt)**

190 To activate the modified aptamers, 2  $\mu\text{L}$  of 0.125 mM acetate buffer (pH 5.2) and 1.5  $\mu\text{L}$  of  
191 freshly prepared 10 mM TCEP were added to 10  $\mu\text{L}$  of 100 mM thiolated aptamers and  
192 incubated for 1h at room temperature. Subsequently, 500  $\mu\text{L}$  of concentrated gold  
193 nanoparticles was transferred to the TCEP-treated aptamers and left reacting for at least 16 h  
194 in the dark at 4°C. After adding 10  $\mu\text{L}$  of 0.25 mM tris acetate buffer (pH 8.2), the suspension  
195 was aged through adding 50  $\mu\text{L}$  of 1 M NaCl dropwise (5  $\mu\text{L}$  every 20 min) and left for  
196 another over night at 4°C. The prepared suspension was centrifuged at 10000 rpm for 10 min  
197 at 4°C and then resuspended in 200  $\mu\text{L}$  of 25 mM tris acetate buffer (pH 8.2) containing 100  
198 mM NaCl. After centrifugation at the same condition, the pellet was resuspended in 500  $\mu\text{L}$   
199 of 25 mM tris acetate buffer (pH 8.2) containing 300 mM NaCl and stored at 4°C (Liu and  
200 Lu, 2006).

201 With the aim of increasing conjugate stability, different final concentrations of aptamer F20  
202 and F20-T (0.2, 0.5, 1, 2 and 3  $\mu\text{M}$ ), incubation time (4 and 24 h) and two conjugate  
203 preservation buffer composition including (i) tris acetate buffer (25 mM, pH 8.2) containing  
204 300 mM NaCl and (ii) borate buffer (20 mM, pH 8) containing 1% BSA, 2% sucrose, 0.25%  
205 Tween 20 and 0.02%  $\text{NaN}_3$  were further investigated.

### 206 **2.2.4. Preparation of the test strip**

207 The biotinylated DNA probes 1 and 2 were immobilized on the nitrocellulose membrane to  
208 form test and control lines, respectively, at a distance of 4 mm from each other. Prior to  
209 loading, 50  $\mu\text{L}$  of the biotinylated DNA probe (100  $\mu\text{M}$ ) was mixed with 250  $\mu\text{L}$  of 2 mg/mL  
210 streptavidin in PBS buffer (0.01 M, pH 7.4). After incubating the suspension at 4°C for 1h,  
211 700  $\mu\text{L}$  of PBS buffer (0.01 M, pH 7.4) was added. The membrane was kept at room  
212 temperature for 5 min and dried at 37 °C under vacuum for 45 min. The sample and absorbent  
213 pads were pasted on the bottom and top of the nitrocellulose membrane respectively with 1–2  
214 mm of overlap and the prepared master card was cut into 4.6 mm test strips.

215 The minimum required DNA probes 1 and 2 were evaluated through developing red spots on  
216 the test and control zone respectively as a function of the hybridization reaction between 1  
217  $\mu\text{M}$  of AuNPs-Apt (F20 or F20-T) and various concentrations of both DNA probes (5, 15, 30,  
218 and 60  $\mu\text{M}$ ). After initial optimization, four nitrocellulose membranes with different  
219 concentrations of DNA probes 1 and 2 (100 nM, 500 nM, 2.5  $\mu\text{M}$  and 5  $\mu\text{M}$ ) were prepared

220 and the color intensity at the test and control lines were further evaluated in the presence of  
221 various concentrations of AuNPs-Apt conjugates. Different concentrations of both  
222 conjugates, F20 (1  $\mu\text{M}$ , 0.2  $\mu\text{M}$  and 0.1  $\mu\text{M}$ ) and F20-T (2  $\mu\text{M}$ , 0.4  $\mu\text{M}$ , and 0.2  $\mu\text{M}$ ), were  
223 determined based on the obtained results from AuNPs-Apt preparation.

#### 224 **2.2.5. Aptamer based lateral flow assay procedure**

225 The performance of two designed lateral flow aptasensors for AFB1 detection were  
226 evaluated. Therefore, 20  $\mu\text{L}$  of various concentrations (0-50 ng/mL) of AFB1 standard  
227 solution in methanol were mixed with 20  $\mu\text{L}$  of AuNPs-Apt conjugate (at optimized  
228 concentration) in microplate wells for 10 min at room temperature. After adding 20  $\mu\text{L}$  of  
229 PBS buffer (0.01 M, pH 7.4) and 20  $\mu\text{L}$  10% Tween 20, the test strips were placed into the  
230 wells and the color intensity of the lines was analyzed 20 minutes later.

231 The measured area of the test and control line ratio versus AFB1 concentration in three  
232 replicates was plotted to obtain a calibration curve. The  $\text{IC}_{50}$  value was calculated by AAT  
233 Bioquest program using a four parameter logistic regression model (AAT Bioquest, Inc.,  
234 Sunnyvale, CA). The limit of detection (LOD) was defined as the lowest concentration which  
235 corresponded to the T/C of the blank minus three standard deviations of the blank.

236

#### 237 **2.2.6. Selectivity of lateral flow test strip**

238 The designed test strips were evaluated and compared in terms of selectivity toward AFB1  
239 and cross reactivity with other mycotoxins. Under the optimal conditions, the selectivity of  
240 F20 and F20-T conjugates were determined over various mycotoxins including AFB<sub>2</sub>, AFG<sub>1</sub>,  
241 AFG<sub>2</sub>, AFM<sub>1</sub>, ochratoxin A and zearalenone at the concentration of 10 ng/mL in three  
242 replicates. The ratio of the T/C for each mycotoxin was calculated, normalized according to  
243 the AFB1 result and then expressed as selectivity percentage. The obtained mean for each  
244 mycotoxin was compared between two conjugates through the independent samples t-test  
245 using SPSS v.16.0; ( $P \leq 0.05$ ).

246

#### 247 **2.2.7. Test strip performance under methanol content**

248 Due to the mycotoxin extraction using conventional organic solvents, the methanol  
249 interference on the DNA hybridization process occurring in the nitrocellulose membrane was  
250 studied. Therefore, 20  $\mu\text{L}$  of AFB1 standard solution (10ng/mL) diluted by various  
251 concentrations of aqueous methanol (5, 10, 25, 35, 50% v/v) was mixed with 20  $\mu\text{L}$  of F20 or

252 F20-T AuNPs-Apt conjugates in microplate wells for 10 min at room temperature. The color  
253 intensities developed at the test and control lines were scanned 20 min later and quantified.

254

### 255 **2.2.8. Sample assay procedures**

256 The reliability and accuracy of both designed lateral flow test strips were conducted on four  
257 reference materials of maize flour with HPLC certified concentrations of AFB1 (<LOD, 5,  
258 11.3 and 28.9 ppb), friendly obtained from Turin University. To perform the extraction  
259 process, one gram of each flour sample was extracted with 5 mL of 70% aqueous methanol  
260 through 2 min vortexing. After 15 min of settling, 20  $\mu$ L of the supernatant was applied as the  
261 sample and mixed with 20  $\mu$ L of AuNPs-Apt conjugate in a microplate well for 10 min at  
262 room temperature. To optimize the performance of the competitive format, two distinct  
263 membranes loaded with 2.5 and 5  $\mu$ M of DNA probes were exploited. For further  
264 improvement, the incubation time of the strip in the well increased and the developed color  
265 was scanned after 20 and 30 min of reaction, as well. To evaluate the matrix effects and  
266 possibility of obtaining false positive results, the T/C ratios of the samples 1 for both  
267 designed test strips were compared to the blank samples in their corresponding calibration  
268 curves. The calculated T/C ratios for other samples were normalized based on sample 1 value  
269 as well.

270

### 271 **2.2.9. Aptamer Binding affinity evaluation over AFB1 via RPI technology**

272 Aptamer F20 and its truncated form, F20-T, were applied as recognition elements in RPI  
273 platform for AFB1 detecting. To estimate the K<sub>d</sub> values, 400  $\mu$ l of the aptameric probes (10  
274  $\mu$ M) were immobilized on the microarray surface and the binding affinity was estimated in  
275 the presence of various concentration of AFB1-BSA conjugate (0.01, 0.07, 0.28, 1.09 and  
276 4.20  $\mu$ g/mL) at the fixed times. The binding affinity of F20 and F20-T aptamers toward  
277 AFB1 were simultaneously compared to the several aptameric probes including C52, C52T,  
278 G12 and H1 designed through previous study (Mousivand et al., 2020) and two antibodies  
279 under the same condition as well.

## 280 **3. Results and Discussion**

### 281 **3.1. Truncated library construction and thermodynamic analysis**

282 Considering the technical challenges for small molecule aptamer development, the truncating  
283 strategy was employed to design new truncated aptameric probes. The truncated library

284 containing 19 potent aptamers with various lengths from 10 to 40 bp generated based on the  
285 parent sequence truncation. The minimum free energy of secondary structure formation ( $\Delta G$ )  
286 in the truncated library was in the range -8.01 to 1.88 Kcal/mol (Supplementary Information,  
287 Table S2). The secondary structure prediction revealed that most of the designed sequences in  
288 the library had simple hairpin loop (H-loop) structures except those of F20-30 and F20-40  
289 that displayed internal loop and multibranch loop, respectively.

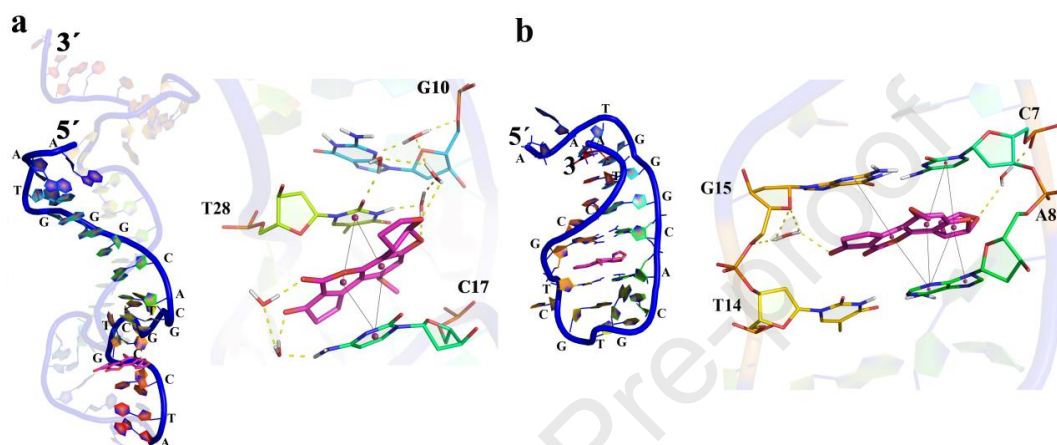
### 290 **3.2. Virtual screening of AFB1 binding aptamers**

291 To predict the binding energy and critical interacting residues, AFB1 was docked over the  
292 aptamers in the truncated library and the estimated docking scores were in the range 1.66 to  
293 4.17. Compared to other truncated aptamers, F20-T with H-loop structure and 19 bp in length  
294 showed the highest binding affinity towards AFB1. F20-T binding pocket includes C7, A8,  
295 G15, G10 and T14 residues that interact AFB1 coumarin and carbonyl groups through  
296 hydrogen bond formation and hydrophobic interactions (Supplementary Information, Fig S1;  
297 Tables S2 & S3). Although F20-T and its parent, F20, had the same secondary structure but  
298 their modes of interaction with AFB1 were different and determined as intercalation and  
299 minor groove binding, respectively. Compared to the parent sequence, the selectivity of the  
300 truncated aptamer had been increased over all the evaluated mycotoxins except for ZEA  
301 (Supplementary Information, Table S4). Depending on the conformational changes and  
302 losing probable binding sites, the truncating strategy can lead to increase or decrease binding  
303 affinity and selectivity of designed aptameric probes.

### 304 **3.3. Molecular dynamic simulations**

305 To clarify the truncation effects on the complex structural stability, a 50 ns molecular  
306 dynamic study was conducted on F20-T –AFB1 complex and the results were compared with  
307 MDs studies of the parent sequence, F20. The system convergence during MDs timescale was  
308 confirmed via insignificant changes in potential energy (Panman et al., 2017). The  
309 conformational changes and binding interactions of F20-T and F20 –AFB1 complex were  
310 evaluated with respect to the lone aptamer during 50ns of MDs. Regarding to the flexible  
311 nature of nucleic acids, all trajectory analysis were performed for both all atoms aptamer and  
312 its binding pockets as suggested by other study (Sharma et al., 2009). Similar to F20 aptamer,  
313 the structural stability of F20-T increased after interacting with AFB1 and the corresponding  
314 Root Mean Square Deviation (RMSD) and Root Mean Square Fluctuation (RMSF) values  
315 revealed a decreasing trend compared to the lone aptamers during MDs (Supplementary

316 Information, Table S5&S6 Fig. S2&S3). Visualization of trajectory files showed that AFB1  
 317 inserted between consecutive base pairs in the stem region of F20-T and subsequently the  
 318 radius of gyration (Rg) values increased for all atoms and binding pockets (Supplementary  
 319 Information, Fig S4). While AFB1 recognized F20 aptamer through minor groove edges of  
 320 C17 and T28 residues and interacted with the binding pocket as a pseudo base subsequently  
 321 leading to increase compactness and structural stability along with reduction in Rg value (Fig.  
 322 1).



323  
 324 Figure 1. The molecular dynamic simulation results of F20 (a) and F20-T (b) - aflatoxin B1 complexes and  
 325 residues involved in binding interaction in 3D representation. Yellow dash lines represent conventional  
 326 hydrogen bonds, purple dots and the lines represent electrostatic and hydrophobic interactions, respectively. The  
 327 truncated segment of the parent aptamer (a) has been highlighted in bold blue color.

328 The hydrogen bond formation between the aptameric probes and AFB1 along with water  
 329 intermediate interactions was evaluated to determine their role in the complex stability during  
 330 MDs. The average H-bonds monitored between truncated aptamer and AFB1 was  
 331 approximately 7 times higher than that of F20-AFB1 complex and estimated as  $0.28 \pm 0.59$ .  
 332 Although both aptamers mainly interacted with AFB1 through dynamic hydrogen bonds, H-  
 333 bonds formed with F20-T seem to be more stable. According to the hydrogen bond  
 334 occupancy percentage  $\geq 10$  ns, the only stable interaction explored in the binding pocket of  
 335 the truncated aptamer with residue G15 was estimated as 15.5% (Supplementary Information,  
 336 Table S7). The dynamic H-bonds between the surrounding water molecules with the binding  
 337 pocket and AFB1 in F20-T complex as well as F20 complex play a key role in the structural  
 338 integrity through hydrating of DNA and ligand (Supplementary Information, Table S8) as  
 339 suggested by other study (Dolenc et al., 2005).

#### 340 3.4. MM-PBSA calculations

341 The obtained MDs trajectories of F20/F20-T and AFB1 complexes were analyzed to estimate  
 342 their free binding energies and different components. The binding affinity of AFB1 for the  
 343 truncated aptamer is reduced by half compared to the parent sequence but it was equivalent to  
 344 that of Apt1 aptamer (Mousivand et al., 2021) and estimated as -47.44 KJ/mol. According to  
 345 the free binding energy component inspection, Van der Waals, non-polar and electrostatic  
 346 interactions showed the major contributions to complex stability in both aptameric probes,  
 347 respectively. The negative effect of polar interactions in free solvation energy can be  
 348 attributed to the hydrophobic nature of AFB1 (Table 1). This finding was in line with other  
 349 studies that confirmed the destabilizing role of polar interactions in binding affinity over  
 350 AFB1(Mousivand et al., 2021; Almedia et al., 2018). In concordance with the docking  
 351 studies, per-residue energy decomposition analysis revealed that residues C7, A8, C9, G10,  
 352 T14, and G15 are the key interacting nucleotides in F20-T binding pocket over AFB1,  
 353 respectively (Supplementary Information, Fig. S5). Also, the high consistency between  
 354 experimentally determined binding affinity ( $K_a$ ) values of F20 ( $3.55 \times 10^{-5}$  nM), Apt1 ( $1.30 \times$   
 355  $10^{-5}$  nM) and F20-T ( $1.12 \times 10^{-5}$  nM) over AFB1 with their free binding energies estimated as  
 356 -70.04, -48.67, and -47.44 KJ/mol highlighted the in silico approaches as promising tools for  
 357 functional aptamer designing.

358 **Table 1.** Comparison of the free binding energy components for the aptamer-AFB<sub>1</sub> complexes obtained from  
 359 MM-PBSA method given in KJ/mol.

aptamers	$\Delta E_{vdw}$	$\Delta E_{elec}$	$\Delta G_{polar}$	$\Delta G_{non-polar}$	$\Delta G_{binding}$
<b>F20-T</b>	-40.44±3.67	-8.90±5.50	20.44±30.87	-15.58±5.66	-44.47
<b>F20</b>	-37.37±4.04	-17.13±10.92	-2.78±34.66	-12.75±1.42	-70.04
<b>Apt1</b>	-48.34 ± 3.67	-11.90 ± 5.50	23.44±30.87	-11.88±5.66	-48.67

360

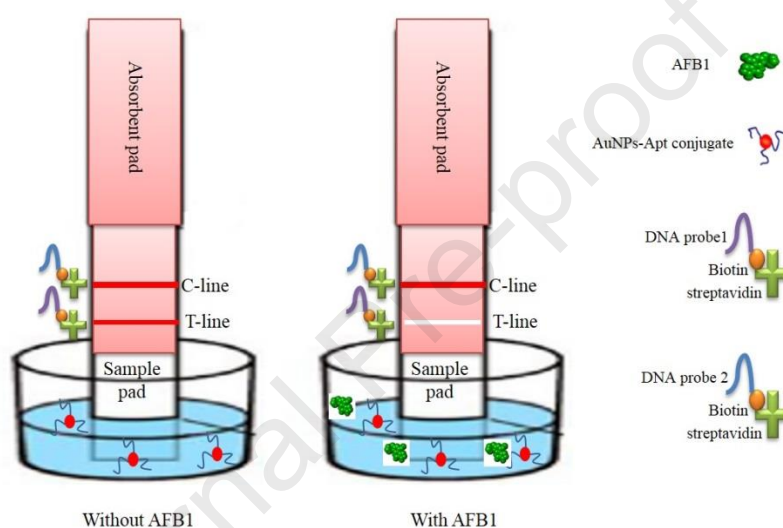
### 361 3.5. Development of the aptamer-based lateral flow test strip

362 The principle of the designed test strip was relied on the competition between the DNA probe  
 363 1 immobilized on the test line and AFB1 to react with AuNPs-aptamer conjugate in the  
 364 sample as shown in Scheme 1. As it was expected for a competitive format, the color  
 365 intensity of the test line was inversely proportional to AFB1 concentration in the samples.  
 366 Regardless the presence or absence of AFB1, the excess AuNPs-aptamer conjugates were  
 367 captured through the linker complementary DNA probe 2 in the control line to valid the  
 368 detection process and normalize strip-to-strip variation. In spite of conventional use of BSA



369 in the lateral flow test strips to block the non specific binding sites (Molinelli et al., 2008; Xu  
 370 et al., 2010), the high binding affinity of BSA over AuNPs-aptamer conjugates and  
 371 hybridization interference in the test and control lines hindered the treatment of nitrocellulose  
 372 membranes with BSA. This finding was in line with results of other research that reported  
 373 that the BSA binds the citrate-stabilized gold nanospheres through an electrostatic attraction  
 374 via the lysine residues (Brewer et al., 2005) or by a thiol ligand exchange reaction with the  
 375 unpaired cysteine residue (Tsai et al., 2011).

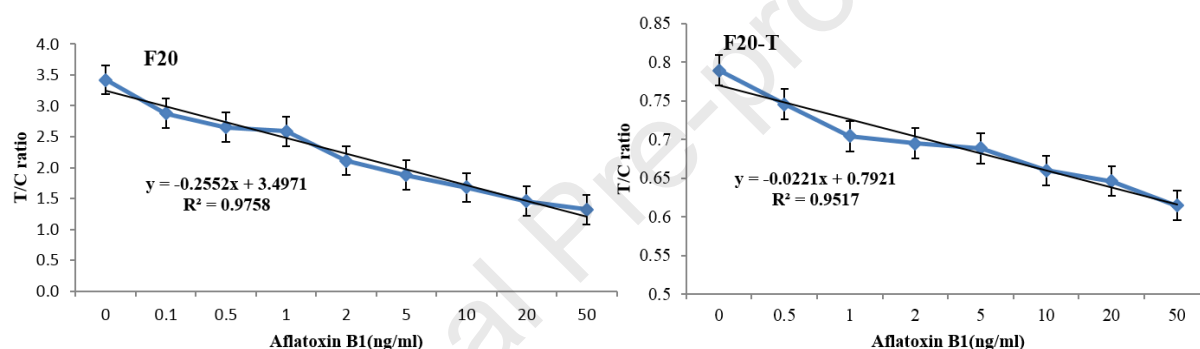
376



378 Scheme 1. Schematic illustration of aptamer-based lateral flow strip for aflatoxin B1 (AFB<sub>1</sub>) detection and result  
 379 interpretation in the presence or absence of AFB<sub>1</sub>.

380 Difficulty in standardizing the amount of AuNPs-aptamer conjugates caused strip-to-strip  
 381 variation even at a constant target concentration. To overcome this problem, the conjugates  
 382 were mixed with the sample before performing the test instead of pre-adsorbing on the  
 383 conjugated pads (Molinelli et al., 2008). Also, normalization can be achieved via some data  
 384 corrections through using the control line intensity due to its association with variability of  
 385 the gold conjugate amount and any other factors affecting the detection procedure. Therefore,  
 386 the color intensity of the control line was exploited to normalize the result variations by  
 387 dividing the test line area (T) by the control line area (C) (Anfossi et al., 2010). To obtain the  
 388 normalized standard curve, the optimized concentration of AuNPs-aptamer conjugates was  
 389 mixed with different AFB<sub>1</sub> concentrations before the detection process on the test strip and  
 390 then the T/C ratio was measured. According to the obtained results, the designed lateral flow  
 391 based on F20 and F20-T aptamers showed IC<sub>50</sub> of 2.9 and 15.4 ng/mL, and a dynamic range

392 of 0.1-50 and 0.5 -50 ng/mL, respectively. Based on the estimated LOD, the parent aptamer  
 393 (0.1 ng/mL) was more sensitive compared to its truncated form (0.5 ng/mL) and showed  
 394 wider T/C ratios over different AFB<sub>1</sub> concentrations as well (Fig. 2). The better performance  
 395 of F20 based strip can be attributed to the longer length of the parent aptamer, which provides  
 396 higher gold surface coverage and more stable conjugate formation. Compared to Apt1 based  
 397 lateral flow strip with a quantitative LOD of 1.05 ppb (Zhang et al., 2018b), both designed  
 398 lateral flow strips were able to detect AFB<sub>1</sub> more sensitively and accurately. The high  
 399 consistency between the experiments and in silico findings highlighted the reliability of the  
 400 computational simulation techniques in the search of functional aptamers to be exploited for  
 401 biosensor development.



402

403 Figure 2. Calibration curves obtained by the normalized T/C ratio versus the AFB<sub>1</sub> concentration (ng/mL) for  
 404 F20-T and F20 lateral flow strips.

### 405 3.6. Optimization of the test strip components

#### 406 3.6.1. Aptameric probe modifications

407 The load capacity of the thiol modified oligonucleotides onto the surface of Au nanoparticles  
 408 can be increased due to the well-known chemical interaction between alkyl thiol and gold  
 409 through Au-S bond (Love et al., 2005). Therefore, both aptameric probes had been tagged  
 410 with a thiol group at the 3' during the synthesis process. In order to reduce the adsorption of  
 411 the main sequence on the surface of Au-NPs and interference with the binding interaction, a  
 412 poly adenine (A) spacer region between the thiol group and the aptameric sequences was  
 413 designed. According to other studies, using the oligonucleotide spacer improves the  
 414 hybridization efficiency and its composition and length influenced on the Au surface  
 415 coverage with the functionalized oligonucleotides (Hurst et al., 2006).

### 416 **3.6.2. Gold Nanoparticles-Aptamer Conjugates (AuNPs-Apt)**

417 Despite the promoting role of NaCl in the Au/thiol interactions, it induces the gold  
418 nanoparticles aggregation during the AuNPs-Apt conjugate preparation and coloring shift  
419 from red to purple as well. Due to the protecting from salt-induced aggregation through  
420 ssDNA loading on the NPs surface (Hurst et al., 2006; Wu et al., 2018), the optimum final  
421 concentrations of F20 and F20-T based conjugates were estimated as 1 and 2  $\mu\text{M}$  respectively  
422 (Supplementary Information, Fig. S6). Requiring lower parent aptamer concentration for  
423 stable conjugate preparation may be attributed to its better gold surface coverage. Also, the  
424 longer incubation time of NPs with both TCEP-treated thiol aptamers under different  
425 concentrations led to the more stable conjugate preparation. Due to the prevention of NPs  
426 aggregates, tris acetate buffer containing NaCl was identified as a better conjugate  
427 preservation buffer. Despite common use of BSA for stabilizing gold colloids conjugated to  
428 antibodies (Molinelli et al., 2008; Xu et al., 2010), the high binding affinity between citrate-  
429 stabilized gold nanospheres and BSA (Tsai et al., 2011) caused NPs aggregation during  
430 AuNPs-Apt conjugate preservation.

### 431 **3.6.3. The test and control lines optimization**

432 Regarding to the electrostatic adsorption of streptavidin on the nitrocellulose membrane and  
433 its high binding affinity to biotin, the biotinylated DNA probe-streptavidin conjugates were  
434 immobilized on the test and control lines. Owing to four identical binding sites of the  
435 streptavidin to biotin (Yuan et al., 2010), the ratio between streptavidin and the biotinylated  
436 DNA probes were set as 1:4. Under the constant concentration of both AuNPs-Apt conjugates  
437 (F20 and F20-T), the red hybridization dots were visualized for all evaluated initial  
438 concentrations of the biotinylated DNA probes and then their minimum required  
439 concentrations were estimated as 5  $\mu\text{M}$  (Supplementary Information, Fig. S7). Further  
440 improvement of the test and control line performance were achieved when F20 and F20-T  
441 conjugates at their optimum concentration (0.2 and 0.4  $\mu\text{M}$ , respectively) were hybridized  
442 with DNA probes 1 and 2 at the final concentration of 2.5  $\mu\text{M}$  on the membrane  
443 (Supplementary Information, Fig. S8).

### 444 **3.7. Test strip performance under methanol content**

445 The adverse effects of organic solvents on the aptamer/antibody activity, colloidal of  
446 AuNPs and the co-extraction of fatty materials reduce the biosensing platforms performance

447 (Anfossi et al., 2010; Molinelli et al., 2009). Therefore, in the presence of a constant  
448 concentration of AFB1 (10 ng/mL), the hybridization reactions of both AuNPs-aptamer  
449 conjugates with DNA probes on the membrane were investigated under various methanol  
450 contents (5 -50 %). According to the results, the color intensities on the test and control lines  
451 gradually increased along with increasing methanol content, so that both conjugates showed  
452 the highest hybridization percentage at a concentration of methanol corresponding to 50%  
453 (Supplementary Information, Fig. S9). In contrast to earlier studies (Shim et al., 2014; Zhou  
454 et al., 2016), these findings revealed that the greater methanol content not only did not reduce  
455 DNA hybridization but increased its rate and then should be considered as an effective factor  
456 on the lateral flow responses especially in the competitive formats. These finding are  
457 consistent with those of other studies that found the hybridization rate of DNA-functionalized  
458 NPs (Smith and Liu, 2010) and molecular beacon (Dave and Liu, 2010) were significantly  
459 faster in most organic solvents compared with water attributed to the reduced activation  
460 energy barrier for the hybridization reaction in the presence of organic solvents.

461

### 462 **3.8. The selectivity of the test strips**

463 Both developed lateral flow strips were evaluated in terms of selectivity toward AFB1 and  
464 cross reactivity over AFB2, AFM1, AFG1, AFG2, OTA and ZEA through experimental and  
465 in silico methodologies. According to the experimental results, F20 and its truncated form  
466 based test strips showed the highest affinity towards AFB1 and a general cross reactivity over  
467 other mycotoxins. F20-T based lateral flow assay showed higher selectivity than its parent  
468 aptamer based strip toward others mycotoxins, except toward ZEA (Fig. 3). The statistical  
469 significant difference between the calculated mean selectivity of F20 and F20-T based test  
470 strips for each mycotoxin was confirmed by independent samples t-tests. In agreement with  
471 these findings, the cross reactivity of other lateral flow assays specifically designed for AFB1  
472 have been reported, which was associated to the structural similarity of mycotoxins,  
473 especially aflatoxins (Shim et al., 2014; Zhu et al., 2018; Zhao et al., 2020).

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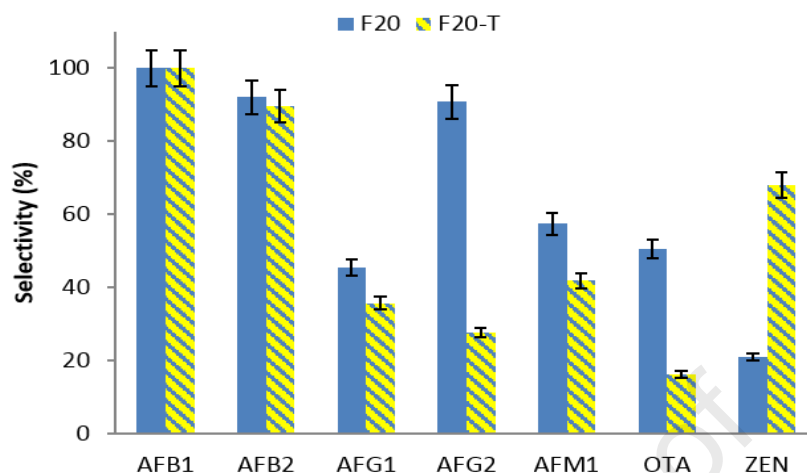
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482

483 Figure 3. Selectivity of F20-T and F20 -based lateral flow strips towards various mycotoxins (10 ng/mL).

484

485 Due to the difficulty in identifying high selectivity binding probes through experimental  
 486 methods (Ruscito and DeRosa, 2016), computational simulation techniques can be considered  
 487 as promising approaches to find or improve probes toward a specific target (Mousivand et al.,  
 488 2020). According to the docking results, F20-T showed the lower binding affinity along with  
 489 the smaller binding pocket in complex with other mycotoxins except ZEA (Supplementary  
 490 Information, Table S4). It seems that the higher selectivity of F20-T compared to F20 is  
 491 associated with the fewer binding sites and possible conformations due to the aptamer  
 492 truncation. The *in silico* findings were largely consistent with those experimentally obtained  
 493 in terms of selectivity of the aptamers.

494

### 495 3.9. Real sample analysis

496 The accuracy of both designed aptamer based lateral flow strips were evaluated through  
 497 analyzing four HPLC-certified corn flour samples. Although both F20 and F20- T based test  
 498 strips were able to detect AFB1 in positive samples under optimum conditions, the parent  
 499 sequence based strip was more sensitive in term of recovery percentage (Table 2). According  
 500 to the results, the T/C ratios calculated for the sample 1 and the blank sample (0 ng/ml of  
 501 AFB1) were relatively similar and estimated as 82.4 and 95.3 % for F20-T and F20 based test  
 502 strips respectively. Therefore, the possibility of matrix interference and consequently false  
 503 positive response were low in both test strips however the parent based test based strip  
 504 showed more accurate results than the truncated based one.

505 Table 2. Recovery percentage of AFB<sub>1</sub> from HPLC certified corn flour samples via F20 and F20-T based lateral  
506 flow strips under optimum condition.

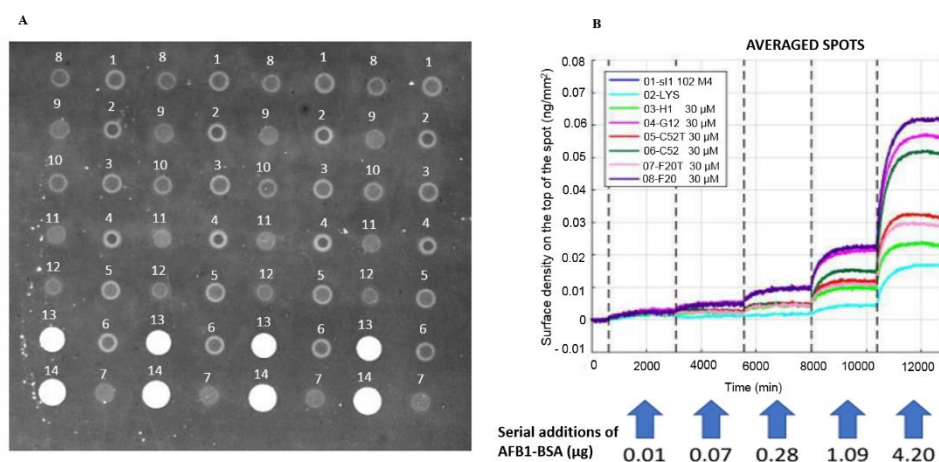
sample	AFB <sub>1</sub> concentration (ppb) by HPLC	Recovery %	
		F20-T based strip	F20 based strip
2	5	85.0	99.7
3	11.3	77.1	101.0
4	28.9	68.8	110.7

507

508 Furthermore, exploitation of the membrane prepared with lower DNA probe concentration  
509 (2.5  $\mu$ M) along with longer incubation time (30 min) improved performance of both test  
510 strips likely through the improvement of competitive reactions and the reduction of matrix  
511 interference, respectively. Regarding to the pre-adsorption of extracted food matrix in the  
512 different components of the test strip (Anfossi et al., 2010), it can be interpreted that  
513 increasing the membrane incubation time enhanced the sensor performance through matrix  
514 effect management. In comparison with the truncated based test strip, all evaluated samples  
515 could be correctly ranked based on the AFB<sub>1</sub> concentration values using F20 test strip in  
516 various experimentally condition as well (Supplementary Information, Fig. S10).

### 517 **3.10. AFB<sub>1</sub> binding affinity evaluating through RPI technology**

518 Several surfaces were prepared by immobilizing the provided probes next to control  
519 antibodies. After fine-tuning of the microarrays which was necessary for both the deposition  
520 process and the surface preparation, microarray surface captured with RPI technology  
521 (Giavazzi et al., 2013; Salina et al., 2015). The white spots correspond to a compact single  
522 layer of molecules and the signal intensity was proportional to the mass linked to the surface.  
523 The black areas correspond to zones without bound molecules. The dissociation constants of  
524 various probes were compared through increasing concentrations of ligand (AFB<sub>1</sub>-BSA) at  
525 fixed times. According to the K<sub>d</sub> values and width of plateaus, F20 showed the highest  
526 binding affinity over AFB<sub>1</sub> compared to other aptameric probes estimated as 2.11  $\mu$ g/mL and  
527 83 pg/mm<sup>2</sup> respectively (Fig 4; Supplementary Information, Figs S11 & S12; Table S9). The  
528 lower binding affinity of F20-T compared to the parent sequence confirmed through RPI  
529 technology as well. There was a high concordance between the previous studies (Mousivand  
530 et al., 2020, 2021) and RPI technology in terms of sorting evaluated aptameric probes based  
531 on the K<sub>d</sub> values.



532  
 533 Figure 4. (A) Microarray surface captured with RPI technology under constant concentration of AFB1-BSA  
 534 (12000  $\mu\text{g/ml}$ ); probes and their concentrations represent in numbers as follows; 1: (F20; 5  $\mu\text{M}$ ), 2: (F20-T; 5  
 535  $\mu\text{M}$ ), 3: (C52; 5  $\mu\text{M}$ ), 4: (C52-T; 5  $\mu\text{M}$ ), 5: (g12; 5  $\mu\text{M}$ ), 6: (H1; 5  $\mu\text{M}$ ), 7: (F20; 30  $\mu\text{M}$ ), 8: (F20-T; 30  $\mu\text{M}$ ), 9:  
 536 (C52; 30  $\mu\text{M}$ ), 10: (C52-T; 30  $\mu\text{M}$ ), 11: (g12; 30  $\mu\text{M}$ ), 12: (H1; 30  $\mu\text{M}$ ); 13: (control antibody 1), 14: (control  
 537 antibody 2); (B) The graph shows the amount of mass bounded by each type of spot over time at constant  
 538 concentration (30  $\mu\text{M}$ ) of various aptameric probes; additions are marked with dashed lines.

539

## 540 Conclusion

541 Given the practical advantages of the aptameric probe technology over antibody generation,  
 542 aptamer-based sensors can be considered as promising alternatives for accurate small  
 543 molecule monitoring. However, the structural simplicity, few binding sites along with low  
 544 molecular weight of small compounds are still the major bottlenecks for aptamer and  
 545 aptasensing platform development for these category of compounds (Ruscito and DeRosa,  
 546 2016). As complementary options for experimentally small binding aptamer discovery, the  
 547 various *in silico* approaches can significantly influenced their research and commercialization  
 548 (Mousivand et al., 2020; Ciriaco et al., 2020). In our previous study, a well-known AFB1  
 549 aptamer sequence, Apt1, has been exploited to design a high affinity DNA aptamer, F20,  
 550 through the *in silico* maturation strategy. Here, we integrated the truncating strategy and  
 551 computational simulation studies to develop a new shorter aptamer, F20-T, based on F20  
 552 sequence. Both designed AFB1 aptamers were successfully applied as recognition elements  
 553 in the lateral flow aptasensors and the RPI platform for simple and rapid AFB1 detecting.  
 554 According to the legal requirements of the European Union, the sensitivity reached by both  
 555 new lateral flow test strips was suitable for detecting AFB1 via strip reader. Moreover, they  
 556 showed better sensitivity compared to an analogous lateral flow strip exploiting the original

557 Apt1 (Zhang et al., 2018 b). Regarding to the high consistency between our experimental and  
558 in silico findings, aptamer engineering through sequence or scaffold refinement can be  
559 considered as a new and promising research field for novel small binding aptamer  
560 development. Low-cost integration of the newly designed probes as recognition elements in  
561 existing aptasensing platforms allow designing various novel aptasensor for small molecule  
562 target monitoring in a green way.

563

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## 569 **References**

570 Aissa, B.S., Mastouri, M., Catanante, G., Raouafi, N., Marty, J.L.(2020). Investigation of a truncated aptamer  
571 for ofloxacin detection using a rapid FRET-based apta-assay . *Antibiotics* (Basel).9,860.

572 Almedia, J.S.F., Dolezal, R., Krejcar,O., Kuca,K., Musilek, K., Jun, D., França, TCC. (2018). Molecular  
573 modeling studies on the interactions of aflatoxin b1 and its metabolites with human acetylcholinesterase. Part II:  
574 Interactions with the Catalytic Anionic Site (CAS). *Toxins*.37,2041-2048.

575 Anfossi, L., Calderara, M., Baggiani, C., Giovannoli, C., Arletti, E., Giraudi, G. (2010). Development and  
576 application of a quantitative lateral flow immunoassay for fumonisins in maize. *Anal.Chim.Acta*.682,104e109.

577 Berendsen, H.J.C., van der Spoel, D., van Drunen, R. (1995). GROMACS – A message-passing parallel  
578 molecular dynamics implementation. *Comput Phys Commun*.91, 43–56.

579 Biovia, DS. (2015). Discovery studio modeling environment. San Diego,Dassault Systemes,Release,4.

580 Brewer, S.H., Glomm,W.R., Johnson, M.C., Knag, M.K., Franzen,S. (2005). Probing BSA binding to citrate-  
581 coated gold nanoparticles and surfaces .*Langmuir*.21, 9303–9307.

582 Cavalera, S., Di Nardo, F., Forte, L., Marinoni, F., Chiarello, M., Baggiani, C., Anfossi, L. (2020). Switching  
583 from Multiplex to Multimodal Colorimetric Lateral Flow Immunosensor. *Sensors*. 20, 6609.

584 Chauhan, R., Singh, J., Sachdev,T., Basu,T., Malhotra, B.D. (2016). Recent advances in mycotoxins detection.  
585 *Biosens.Bioelectron*.81, 532–545.



- 586 Chushak, Y., Stone, M. 2009. In silico selection of RNA aptamers. *Nucleic Acids Research*. 37:  
587 /doi.org/10.1093/nar/gkp408
- 588 Ciriaco, F., DeLeo, V., Catucci, L., Pascale, M., Logrieco, A.F., DeRosa, M.C., DeGirolamo, A. (2020). An in-  
589 silico pipeline for rapid screening of dna aptamers against mycotoxins: the case-study of fumonisin B1,  
590 aflatoxin B1 and ochratoxin A. *Polymers*. 12(12), 2983.
- 591 Crivianu-Gaita, V., Thompson, M. (2016). Aptamers, antibody scFv, and antibody Fab' fragments: An overview  
592 and comparison of three of the most versatile biosensor biorecognition elements. *Biosens. Bioelectron.* 85, 32-45.
- 593 Dave, N., Liu, J. (2010). Fast molecular beacon hybridization in organic solvents with improved target  
594 specificity. *J. Phys. Chem. B* . 114, 15694–15699.
- 595 DeLano, W.L. (2002). An informal Newsletter associated with the BBSRC Collaborative Computational Project  
596 No. 4 on Protein Crystallography CCP4 newsletter, *On Protein Crystallography*. 40, 82e92.
- 597 Di Nardo, F., Chiarello, M., Cavalera, S., Baggiani, C., Anfossi, L. (2021). Ten years of lateral flow  
598 immunoassay technique applications: trends, challenges and future perspectives. *Sensors*. 21, 51-85.
- 599 Dolenc, J., Borstnik, U., Hodoscek, M., Koller, J., Janezic, D. (2005). An ab initio QM / MM study of the  
600 conformational stability of complexes formed by netropsin and DNA. The importance of van der Waals  
601 interactions and hydrogen bonding . *J. Mol. Struct.* 718, 77–85.
- 602 EC. (2010). Commission regulation (EU) No 165/2010 of 26 February 2010 amending Regulation (EC) No  
603 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins. *Off. J. Eur.*  
604 *Union* L50/8.
- 605 Giavazzi, F., Salina, M., Cerbino, Bassi, M., Prosperi, D., Ceccarello, E., Damin, F., Sola, L., Rusnati, M.,  
606 Chiari, M., Chini, B., Bellini, T., Buscaglia, M. (2013). *Proceedings of the National Academy of Sciences of the*  
607 *United States of America*. 110(23), 9350–9355.
- 608 Hasegawa, H., Savory, N., Abe, K., Ikebukuro, K. (2016). Methods for improving aptamer binding Affinity.  
609 *Molecules*. 21(4), 421.
- 610 Hock, B., Seifert, M., Kramer, K. (2002). Engineering receptors and antibodies for biosensors  
611 *Biosens. Bioelectron.* 17, 239–249.
- 612 Humphrey, W., Dalke, A., Schulten, K. (1996). VMD: visual molecular dynamics. *J. Mol. Graph.* 14, 33–38.
- 613 Hurst, S.J., Lytton-Jean, A.K.R., Mirkin, C.A. (2006). Maximizing dna loading on a range of gold nanoparticle  
614 sizes . *Anal. Chem.* 78, 8313-8318.
- 615 IARC. International Agency for Research on Cancer. Evaluation of carcinogenic risks in humans. (2002).  
616 Lyon (France): IARC. 82: 171–274.

- 617 Jia, Y., Zhou, G., Liu, P., Li, Z., Yu, B. (2019). Recent Development of Aptamer Sensors for the Quantification  
618 of Aflatoxin B1. *Appl.Sci.* 9(11), 2364.
- 619 Kumari, R., Kumar, R., Lynn, A. (2014). g\_mmpbsa - A GROMACS tool for high-throughput MM-PBSA  
620 calculations. *J. Chem. Inf. Model.* 54, 1951-62.
- 621 Liu, J., Lu, Y. (2006). Preparation of aptamer-linked gold nanoparticle purple aggregates for colorimetric sensing  
622 of analytes *Nat. Protoc.* 1:246–252.
- 623 López-Puertollano, D., Mercader, J.V., Agulló, C. et al. 2018. Novel haptens and monoclonal antibodies with  
624 subnanomolar affinity for a classical analytical target, ochratoxin A. *Sci Rep* 8, 9761.  
625 <https://doi.org/10.1038/s41598-018-28138-x>.
- 626 Love, J.C., Estroff, L.A., Kriebel, J.K., Nuzzo, R.G., Whitesides, G.M. (2005). Self-assembled monolayers of  
627 thiolates on metals as a form of nanotechnology. *Chem. Rev.* 105(4), 1103-69.
- 628 Mascini, M. (2009). *Aptamers in Bioanalysis*. John Wiley & Sons, Hoboken, NJ, USA.
- 629 McKeague, M., De Girolamo, A., Valenzano, S., Pascale, M., Ruscito, A., Velu, R., Frost, N., Hill, K., Smith, M.,  
630 McConnell, E. M., DeRosa, M.C. (2015). Comprehensive Analytical Comparison of Strategies Used for Small  
631 Molecule Aptamer Evaluation. *Anal. Chem.* 87, 8608–8612.
- 632 Miklós, G., Angeli, C., Ambrus, Á., Nagy, A., Kardos, V., Zentai, A., Kerekes, K., Farkas, Z., Jóźwiak, A., Bartók,  
633 T. (2020). Detection of aflatoxins in different matrices and food-chain positions. *Front Microbiol.* 11, 1916.
- 634 Molinelli, A., Grossalber, K., Fuhrer, M., Baumgartner, S., Sulyok, M., Krska, R. (2008). Development of  
635 qualitative and semi-quantitative immunoassay-based rapid strip tests for the detection of T-2 toxin in wheat and  
636 oat. *J. Agric. Food Chem.* 56, 2589–2594.
- 637 Molinelli, A., Grossalber, K., Krska, R. (2009). A rapid lateral flow test for the determination of total type B  
638 fumonisins in maize. *Anal. Bioanal. Chem.* 395, 1309–1316.
- 639 Morris, G. M., Huey, R., Lindstrom, W., Sanner, M.F., Belew, R.K., Goodsell, D.S., Olson, A. J. (2009).  
640 AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J. Comput. Chem.* 30,  
641 2785–2791.
- 642 Mousivand, M., Anfossi, L., Bagherzadeh, K., Barbero, N., Mirzadi-Gohari, A., Javan-Nikkhah, M. (2020). In  
643 silico maturation of affinity and selectivity of DNA aptamers against aflatoxin B1 for biosensor development.  
644 *Anal. Chim. Acta.* 1105, 178-186.
- 645 Mousivand, M., Bagherzadeh, K., Anfossi, L., Javan-Nikkhah, M. (2021). Key criteria for engineering  
646 mycotoxin binding aptamers via computational simulations: Aflatoxin B1 as a case study. *Biotechnol J.* 17(2),  
647 2100280.
- 648 Patent: PCT/CA2010/001292, NeoVentures Biotechnology Inc. 2013. *Aptamer Catalogue*, 24.

- 649 Panman,W., Japrun, D., Pongprayoon, P. (2017). Exploring the interactions of a DNA aptamer with human  
650 serum albumins: simulation studies. *J.Biomol.Struct.Dyn.*35(11), 2328-2336.
- 651 Perez, A., Marchan, I., Svozil, D., Sponer, J., Cheatham, T.E.,Laughton, C.A., Orozco, M. (2007). Refinement  
652 of the AMBER Force Field for Nucleic Acids: Improving the Description of alpha/gamma Conformers.  
653 *Biophys.J.* 92, 3817–3829.
- 654 Ruscito, A., DeRosa, M.C. (2016). Small-Molecule Binding Aptamers: Selection Strategies, Characterization,  
655 and Applications. *Front.Chem.*4,14.
- 656 Salina, M., Giavazzi, F., Lanfranco, R., Ceccarello, E., Sola, L., Chiari, M., Chini, B., Cerbino, R., Bellini,T.,  
657 Buscaglia, M. (2015). Multi-spot, label-free immunoassay on reflectionless glass. *Biosens Bioelectron.*74, 539-  
658 45.
- 659 Sharma, M., Bulusu, G., Mitra, A. (2009). MD simulations of ligand-bound and ligand-free aptamer: molecular  
660 level insights into the binding and switching mechanism of the add A-riboswitch. *RNA.*15,1673-92.
- 661 Shim,W.B., Mun, H., Joung, H.A., Ofori, J.A., Chung, D.H., Kim, M.G. (2014). One-step simultaneous  
662 immunochromatographic strip test for multianalysis of ochratoxin A and zearalenone. *Food Control.*36,30-35.
- 663 Smith, D., Liu, J. (2010). Assembly of DNA-functionalized nanoparticles in alcoholic solvents reveals opposite  
664 thermodynamic and kinetic trends for DNA hybridization. *J.Am.Chem.Soc.* 132(18), 6300–6301.
- 665 Sun,H.,Zu,Y., 2015.*Molecules.*20,11959-11980.
- 666 Tsai, D.H., DelRio, F.W., Keene, A.M., Tyner, K.M., MacCuspie, R.I., Cho, T.J., Zachariah, M.R., Hackley, V.  
667 A. (2011). Adsorption and conformation of serum albumin protein on gold nanoparticles investigated using  
668 dimensional measurements and in situ spectroscopic methods. *Langmuir.*27, 2464–2477.
- 669 Wu, S., Liu, L., Duan, N., Li, Q., Zhou,Y.,Wang, Z. (2018). An aptamer-based lateral flow test strip for rapid  
670 detection of zearalenone in corn samples. *J.Agr.Food Chem.* 66,1949-1954.
- 671 Xu, Y., Huang, Z.B., He, Q.H., Deng, S.Z., Li, L.S., Li,Y.P.(2010). Development of an immunochromato-  
672 graphic strip test for the rapid detection of deoxynivalenol in wheat and maize. *Food Chem.*119, 834e839.
- 673 Yang, X.H., Kong,W.J.,Yang, M.H., Zhao, M., Ouyang, Z. (2013). Application of Aptamer Identification  
674 Technology in Rapid Analysis of Mycotoxins. *Chinese J.Anal.Chem.* 41(2), 297-306.
- 675 Yuan,Y., Yuan, R., Chai,Y., Zhuo,Y., Bai, L., Liao,Y. (2010). An electrochemical enzyme bioaffinity electrode  
676 based on biotin-streptavidin conjunction and bienzyme substrate recycling for amplification. *Anal. Biochem.* 405,  
677 121-126.
- 678 Zhang,Y., Lu,T., Wang,Y., Diao, C., Zhou, Y., Zhao, L.,Chen, H. (2018a). Selection of a DNA aptamer against  
679 zearalenone and docking analysis for highly sensitive rapid visual detection with a label-free aptasensor. *J.*  
680 *Agric.Food Chem.*66,12102–12110.

- 681 Zhang, S., Zhao, S., Wang, S., Liu, J., Dong, Y. (2018b). Development of lateral flow immune chro -  
682 matographic strips for micropollutant screening using colorants of aptamer-functionalized nanogold particles,  
683 part ii: experimental verification with aflatoxin b1 and chloramphenicol. *JAOAC Int.*101(5),1408–1414.
- 684 Zhao, Z., Wang, H., Zhai,W., Feng, X., Fan, X., Chen, A.,Wang, M. (2020). A lateral flow strip based on a  
685 truncated aptamer-complementary strand for detection of type-b aflatoxins in nuts and dried figs. *Toxins*  
686 (Basel)12(2),136.
- 687 Zhou, W., Kong, W., Dou, X., Zhao, M., Zhen,O., Yang, M. (2016). An aptamer based lateral flow strip for on-  
688 site rapid detection of ochratoxin A in Astragalus membranaceus .*J.Chromatogr.B.Anal.Technol.Biomed.Life*  
689 *Sci.* 1022,102–108.
- 690 Zhu,C., Zhang, G., Huang,Y.,Yang, S., Ren, S., Gao, Z., Chen, A.(2018). A. Dual-competitive lateral flow  
691 aptasensor for detection of aflatoxin B1 in food and feedstuffs . *J.Hazard.Mater.*344, 249-257.
- 692 Zoete,V., Cuendet,M., Grosdidier, A., Michielin, O. (2011).SwissParam: A fast force field generation tool for  
693 small organic molecules.*J.Comput.Chem.*32, 2359–2368.
- 694 Zuker, M. (2003). Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res.*  
695 31(13).
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710 **Supplementary material**711 **High performance aptasensing platform development through in silico aptamer**  
712 **engineering for aflatoxin B1 monitoring**713 **Maryam Mousivand<sup>\*1,2</sup>, Mohammad Javan-Nikkhah<sup>2</sup>, Laura Anfossi<sup>3\*</sup>, Fabio Di**  
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738 **Table S1.** Modified aptamers and probe sequences used in this study

aptamers/probes	sequence (5'-3')
thiol-modified aptamer F20	5'-aatgggcacgtgctgcctatatgtgtctcgtgcccttcgctaggcccactaaaaaaaaaaaaaaaaa-SH-3'
thiol-modified aptamer F20-T	5'-aatgggcacgtgctgcctaaaaaaaaaaaaaaaaa-SH-3'
biotin-modified DNA probe1	5'-agtgggcctagcgaagggcacgagacacatataggcagcagcgtgccatt-Biotin-3'
biotin-modified DNA probe2	5'-ttttttttttttttt-Biotin3'
aptamer F20	5'-aatgggcacgtgctgcctatatgtgtctcgtgcccttcgctaggcccact-3'
aptamer F20-T	5'-aatgggcacgtgctgcct-3'

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743 **Table S2.** The sequence, minimum free energy of secondary structure formation ( $\Delta G$ ), length (bp), truncated  
744 direction and docking score of aptamers in the truncated library.

Aptamers	Sequences (5'-3')	$\Delta G$ (Kcal/mol)	Length (bp)	Truncated direction	Docking score
F20	aatgggcacgtgctgcctatatgtgtctcgtgcccttcgctaggcccact	-8.01	50	-	5.68
F20-T	aatgggcacgtgctgccta	-3.51	19	3'	4.17
F20-40-3	aatgggcacgtgctgcctatatgtgtctcgtgcccttcgc	-8.01	40	3'	3.93
F20-30	atgtgtctcgtgcccttcgctaggcccact	-1.97	30	5'	3.72
F20-17	ccttcgctaggcccact	-0.35	17	5'	3.70
F20-40	tgctgcctatatgtgtctcgtgcccttcgctaggcccact	-2.05	40	5'	3.38
F20-16	cttcgctaggcccact	0.36	16	5'	3.06
F20-18	cccttcgctaggcccact	-0.87	18	5'	2.90
F20-18-3	aatgggcacgtgctgcct	-3.51	18	3'	2.87
F20-19	gcccttcgctaggcccact	-0.89	19	5'	2.86
F20-20-3	aatgggcacgtgctgcctat	-3.66	20	3'	2.83
F20-17-3	aatgggcacgtgctgcc	-2.99	17	3'	2.72
F20-10	taggcccact	0.36	10	5'	2.45
F20-30-3	aatgggcacgtgctgcctatatgtgtctcg	-4.14	30	3'	2.39
F20-10-3	aatgggcacg	1.88	10	3'	2.03
F20-20	tgcccttcgctaggcccact	-1.50	20	5'	1.97
F20-15-3	aatgggcacgtgctg	-0.82	15	3'	1.94
F20-16-3	aatgggcacgtgctgc	-1.15	16	3'	1.88
F20-15	ttcgctaggcccact	0.36	15	5'	1.85
F20-30-3-5	tgctgcctatatgtgtctcgtgcccttcgc	-0.05	30	5' & 3'	1.66

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748 **Table S3.** Principal interactions between residues of the truncated aptamers with AFB1.

Aptamers	Carbone and conventional hydrogen binding	Hydrophobic interaction	Electrostatic interaction
	H-Donor-H-Acceptor	Pi-Orbitals- $\pi/\sigma$ /alkyl	Negative-Pi-Orbitals
<b>F20-10</b>	G6:H22 - AFB1:O5	G4:C2' - AFB1- $\pi$ - $\sigma$	
	G6:H22 - AFB1:O6	G4 - AFB1/ $\pi$ -alkyl	
	G6:H1 - AFB1:O6	G4 - AFB1/ $\pi$ -alkyl	
	G4:C1' - AFB1:O2		
	AFB1:C15 - G4:N3		
<b>F20-15</b>	AFB1:C17 - G4:O3'		
	G10:C5' - AFB1:O4	U11 - AFB1/ $\pi$ -alkyl	
	U11:C6 - AFB1:O3		
<b>F20-16</b>	AFB1:C15 - G12:OP1		
	AFB1:C17 - C9:O2	A8 - AFB1/ $\pi$ - $\pi$	G6:OP1 - AFB1/ $\pi$ -anion
<b>F20-17</b>	G5:H21 - AFB1:O6		
	G6:H21 - AFB1:O5		
	AFB1:C15 - G4:O3'	G5 - AFB1/ $\pi$ - $\pi$	
	G5:H21 - AFB1:O6	G5 - AFB1/ $\pi$ - $\pi$	
<b>F20-18</b>	G6:H21 - AFB1:O5		
	AFB1:C15 - G4:O3'		
	AFB1:C17 - G5:O3'	C7 - AFB1/ $\pi$ - $\pi$	
	A8:H61 - AFB1:O5	T14 - AFB1/ $\pi$ - $\pi$	
<b>F20-19</b>	A8:H61 - AFB1:O6		
	AFB1:C15 - G10:O6	T14 - AFB1/ $\pi$ - $\pi$	
	AFB1:C17 - G15:OP2	C7 - AFB1/ $\pi$ -alkyl	
		A8 - AFB1/ $\pi$ -alkyl	
	G4:H21 - AFB1:O4	G5:C1' - AFB1/ $\pi$ -lone pair	
	G5:H21 - AFB1:O1	G5:O4' - AFB1/ $\pi$ - $\pi$	
<b>F20-20</b>	AFB1:C15 - G6:N3	G5 - AFB1/ $\pi$ - $\pi$	
		G5 - AFB1/ $\pi$ - $\pi$	
		G5 - AFB1/ $\pi$ -alkyl	
		G5 - AFB1/ $\pi$ -alkyl	
		G6 - AFB1/ $\pi$ -alkyl	
<b>F20-30</b>	T24:H3 - AFB1:O6	G25 - AFB1/ $\pi$ - $\pi$	
	G25:H21 - AFB1:O5		
<b>F20-40</b>	AFB1:C15 - T24:O3'		
	G4:H21 - AFB1:O1		G5:OP1 - AFB1/ $\pi$ -anion
<b>F2-10-3</b>	A8:H62 - AFB1:O4	G3 - AFB1/ $\pi$ - $\pi$	
	G3:C1' - AFB1:O2	A2 - AFB1/ $\pi$ -alkyl	
	T10:C4' - AFB1:O5	G3 - AFB1/ $\pi$ -alkyl	

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752 **Table S3.** (continued). Principal interactions between residues of the truncated aptamers with AFB1.

<b>F2-20-3</b>	G3:H21 - AFB1	G3 - AFB1/ $\pi$ -alkyl	
	AFB1:C17 - T6:OP2 AFB1:C17 - T6:O5'	T6 - AFB1/ $\pi$ -alkyl	C10:OP1 - AFB1/ $\pi$ -anion
<b>F2-30-3</b>	A22:C1' - AFB1:O6	T17 - AFB1/ $\pi$ -alkyl	G19:OP1 - AFB1/ $\pi$ -anion
		A22 - AFB1/ $\pi$ -alkyl	A22:OP2 - AFB1/ $\pi$ -anion
		A22 - AFB1/ $\pi$ -alkyl	
<b>F2-40-3</b>	AFB1:C17 - T27:O4'	C25 - AFB1/ $\pi$ -alkyl	
	AFB1:C17 - A38:N1	C39 - AFB1/ $\pi$ -alkyl	
<b>F2-30-3-5</b>	G20:H21 - AFB1:O6	G20 - AFB1/ $\pi$ - $\pi$	
	AFB1:C17 - G20:O3'	G20 - AFB1/ $\pi$ - $\pi$	
		G20 - AFB1/ $\pi$ -alkyl	
		G20 - AFB1/ $\pi$ -alkyl	
<b>F2-15-3</b>	G4:H22 - AFB1:O5	T1 - AFB1/ $\pi$ - $\pi$	
	G4:H22 - AFB1:O6		
	G4:H1 - AFB1:O6		
	T2:H3 - AFB1		
<b>F2-16-3</b>	A14:H62 - AFB1:O4	AFB1:C17 - A14/ $\pi$ - $\pi$	
		G9 - AFB1/ $\pi$ - $\pi$	
		G9 - AFB1/ $\pi$ - $\pi$	
		G9 - AFB1/ $\pi$ - $\pi$	
		G9 - AFB1/ $\pi$ - $\pi$	
<b>F2-17-3</b>	G10:H21 - AFB1:O2	G10 - AFB1/ $\pi$ -alkyl	
	A9:C1' - AFB1:O4	G11 - AFB1/ $\pi$ -alkyl	
	AFB1:C17 - T4:O3'		
<b>F2-18-3</b>	C1:C1' - AFB1:O4	C3 - AFB1/ $\pi$ -alkyl	
	AFB1:C15 - C3:O2		
<b>F2-19-3</b>	G8:H21 - AFB1:O5	T5 - AFB1/ $\pi$ -alkyl	
	G8:H21 - AFB1:O6		
	AFB1:C3 - T10:OP2		
	AFB1:C17 - G8:O5'		
	AFB1:C17 - C9:OP1		

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760 **Table S4.** Binding energy, binding sites, Inhibition constant, type of interactions (number of interactions) and  
761 Ref RMSD for the best conformation of F20 and F20-T aptamers docked with AFB<sub>2</sub>, AFG<sub>1</sub>, AFG<sub>2</sub>, AFM<sub>1</sub>, OTA  
762 and ZEN.



Complex	Binding Energy (kcal/mol)	Binding sites	Inhibition constant ( $\mu\text{m}$ )	Type of interactions* (number of interactions)	RMSD ( $\text{\AA}$ )
F20-AFB2	-9.67	T28, C27,C17,G12	1.58	Hb(12)+E(6)+O(1)	19.2
F20-AFG1	-5.85	C27,T28,C29,G10,G12,C17	18.28	Hg(4)+Hb(8)+O(1)	21.18
F20-AFG2	-7.0	C27,T28,C29,G10,G12,C17	5.24	Hg(3)+Hb(9)+O(1)	21.43
F20-AFM1	-4.3	C27,T28,C29,G10,G12,C17	562.62	Hg(6)+Hb(2)+O(2)	31.75
F20-OTA	-5.72	C27,T28,C29,G10,G12,C17,C16	39.26	Hg(9)+Hb(13)+O(1)	23.15
F20-ZEN	-5.41	G4,G6,T37	90.55	Hg(3)	24.33
F20-T-AFB2	-8.0	G15,C12,C7, A8,T14	1.38	Hg(1)+Hb(4)+E(8)	21.86
F20-T-AFG1	-4.82	G15,C7, A8,T14	290.86	Hb(6)+E(1)	23.28
F20-T-AFG2	-4.73	G15,G10,C7, A8,T14	343.2	Hg(4)+Hb(6)	24.35
F20-T-AFM1	-4.29	G5,G6,G15,C7	712.65	Hg(5)+Hb(1)	24.9
F20-T-OTA	-4.58	A8,G6	440.32	Hg(3)+Hb(2)	28.62
F20-T-ZEN	-5.44	C7,A8,G15	103.12	Hg(3)+Hb(3)	26.98

763 Hg: Hydrogen Bonding Hb: Hydrophobic Bonding E: Electrostatic O: others

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765 **Table S5.** RMSD, RMSF and Rg values (nm) for binding pocket of the aptamer-AFB<sub>1</sub> complexes (C) with

766 respect to the lone aptamers (F) during 50 ns molecular dynamic simulation.

aptamer	RMSD(F)	RMSD (C)	RMSF(F)	RMSF (C)	Rg(F)	Rg(C)
	(mean $\pm$ SD)	(mean $\pm$ SD)	(mean $\pm$ SD)	(mean $\pm$ SD)	(mean $\pm$ SD)	(mean $\pm$ SD)
F20-T	0.42 $\pm$ 0.04	0.31 $\pm$ 0.04	0.24 $\pm$ 0.03	0.15 $\pm$ 0.02	0.78 $\pm$ 0.2	0.81 $\pm$ 0.2
F20	0.59 $\pm$ 0.07	0.39 $\pm$ 0.04	0.24 $\pm$ 0.06	0.15 $\pm$ 0.05	1.03 $\pm$ 0.03	0.84 $\pm$ 0.02

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768 **Table S6.** RMSD, RMSF and Rg values (nm) for all atoms of the aptamer-AFB<sub>1</sub> complexes (C) with respect to

769 the lone aptamers (F) during 50 ns molecular dynamic simulation.

Aptamer	RMSD(F)	RMSD (C)	RMSF(F)	RMSF (C)	Rg(F)	Rg(C)
	(mean $\pm$ SD)	(mean $\pm$ SD)	(mean $\pm$ SD)	(mean $\pm$ SD)	(mean $\pm$ SD)	(mean $\pm$ SD)
F20-T	0.48 $\pm$ 0.07	0.40 $\pm$ 0.08	0.24 $\pm$ 0.07	0.14 $\pm$ 0.05	1.19 $\pm$ 0.03	1.28 $\pm$ 0.03
F20	0.94 $\pm$ 0.17	1.35 $\pm$ 0.28	0.608 $\pm$ 0.28	0.8 $\pm$ 36	2.48 $\pm$ 0.10	2.34 $\pm$ 0.27

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773 **Table S7.** Comparison of the hydrogen bond interactions (mean $\pm$  SD) and hydrogen bond occupancy of F20-T  
774 and F20 aptamers in the complexes with aflatoxin B<sub>1</sub> during 50 ns of MD simulation

Aptamer	Donor	Acceptor	Hydrogen bond occupancy	hydrogen bond interactions (mean $\pm$ SD)
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			(%)	
F20-T	G10 (H21)	AFB1(O3 )	4.4	0.28 ± 0.59
	G10 (H21)	AFB1 (O2 )	7.2	
	G10 (H1)	AFB1 (O3 )	1	
	G10 (H1)	AFB1 (O2 )	15.5	
	C7(H61)	AFB1 (O5 )	0.1	
F20	T28 (H3 )	AFB1 (O1 )	1.1	0.04±0.21
	C17 (H41)	AFB1 (O2 )	0.1	
	G15 (H21)	AFB1 (O1 )	0.1	
	G12 (H21)	AFB1(O6 )	1.3	
	G12 (H21)	AFB1(O1 )	0.5	
	G10 (H21)	AFB1(O6 )	0.8	
	G10 (H21)	AFB1(O5 )	0.2	
	G10 (H21)	AFB1(O4 )	0.1	
	G10 (H21)	AFB1(O3 )	0.2	

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777 **Table S8.** Comparison of the water intermediate interactions (mean± SD) and their occupancy ranges involved  
 778 with AFB1 and binding pocket of F20-T and F20 complexes during 50ns of MD simulation

Aptamer	Water molecules-binding pocket		Water molecules-AFB1	
	hydrogen bond interactions( mean± SD)	occupancy range (%)	hydrogen bond interactions (mean± SD)	occupancy range (%)
<b>F20-T</b>	78.42±4.75	1-5.1	2.91±1.15	0.1-1.4
<b>F20</b>	82.78±5.17	1-9.6	3.32±1.25	0.1-0.8

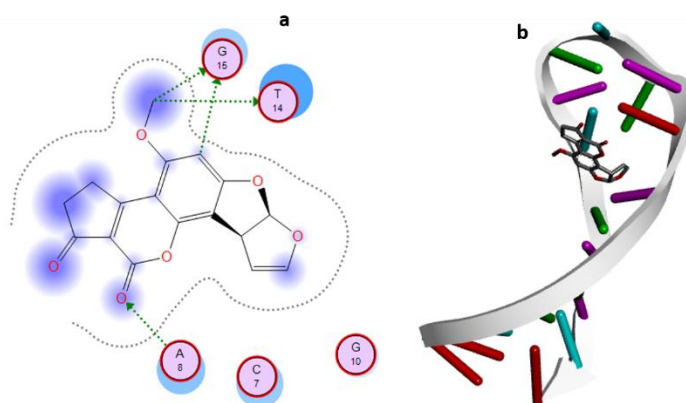
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781 **Table S9.** Determination of dissociation constant (Kd) and width of plateau of F20-T, F20, C52, C52T, g12 and  
 782 H1 aptamers via reflective phantom interface (RPI) technology.

Aptamers	Kd (µg/ml)	Kd (nM)	Width of plateau
F20	2.15	31.9	83µg/mm <sup>2</sup>
F20-T	4.61	68.8	61µg/mm <sup>2</sup>
C52	2.67	39.9	73µg/mm <sup>2</sup>
C52T	3.34	52.8	58pg/mm <sup>2</sup>
g12	2.12	31.6	78pg/mm <sup>2</sup>
H1	3.77	56.1	45pg/mm <sup>2</sup>

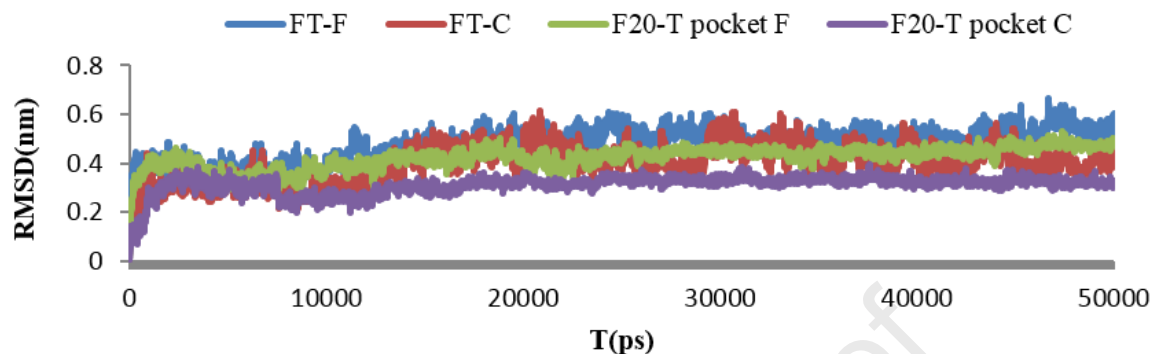
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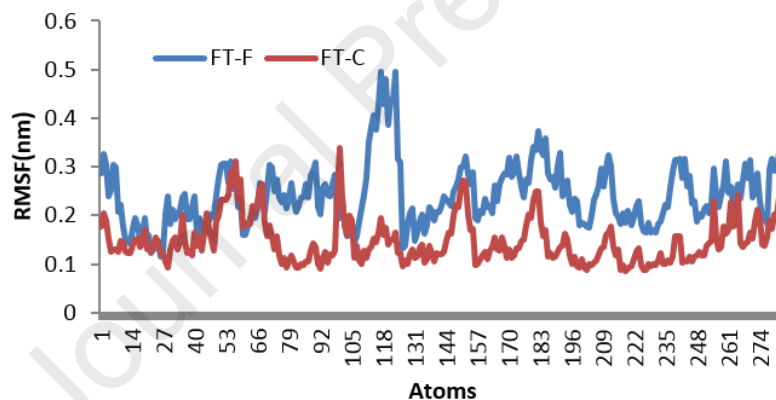
785 **Fig. S1.** The docking results of F20 -T -AFB1 complex and residues involved in binding interaction in 2D (a)  
 786 and 3D (b) representation.

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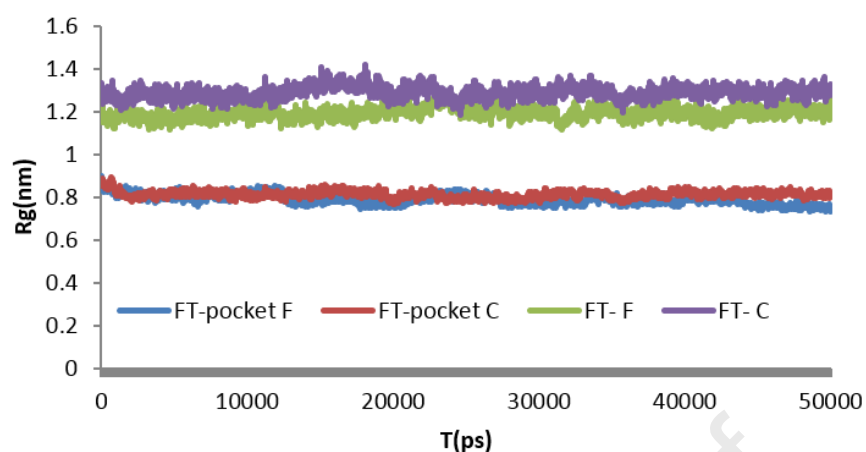
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789 **Fig. S2.** RMSD plot of F20-T aptamer for all atoms (lone: Blue; in complex with aflatoxin B1: Red) and  
 790 binding pocket (lone: Green; in complex with aflatoxin B1: Purple) during the simulation time.



791

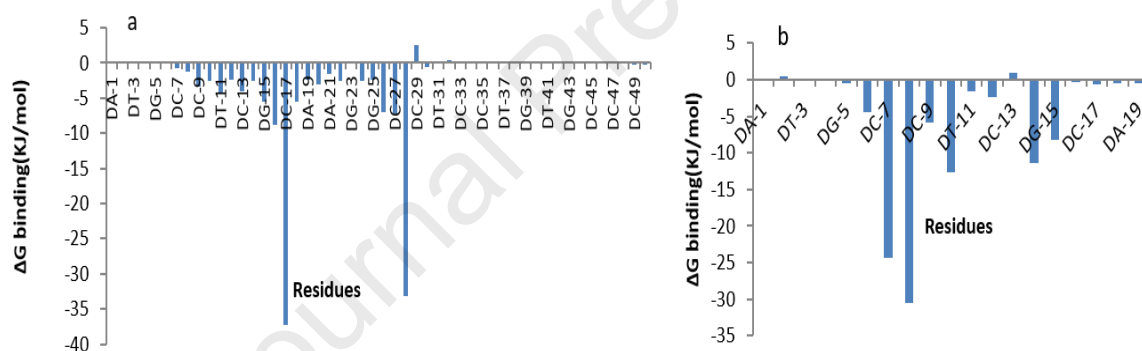
792 **Fig. S3.** RMSF plot of F20-T aptamer for all atoms (lone: Blue; in complex with aflatoxin B1: Red) during the  
 793 simulation time.



794

795 **Fig. S4.** Rg plot of F20-T aptamer for all atoms (lone: Green; in complex with aflatoxin B1: Purple) and  
 796 binding pocket (lone: Blue; in complex with aflatoxin B1: Red) during the simulation time.

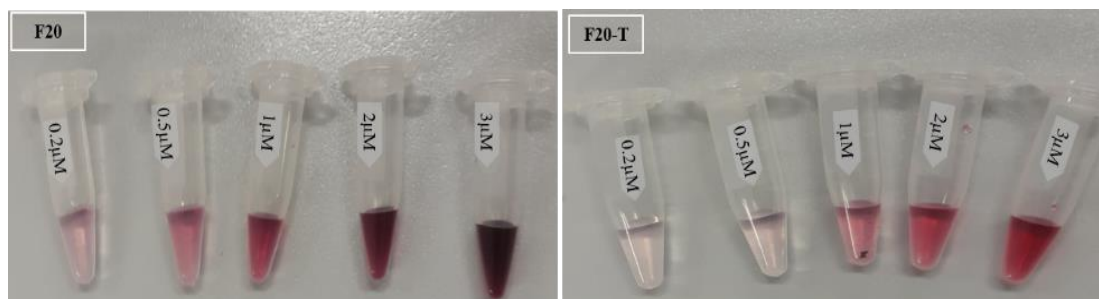
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799 **Fig. S5.** Energy component decomposition analysis per F20 (a) and F20-T (b) residues interacting with aflatoxin  
 800 B1 through MM-PBSA method during 50ns of MD simulation.

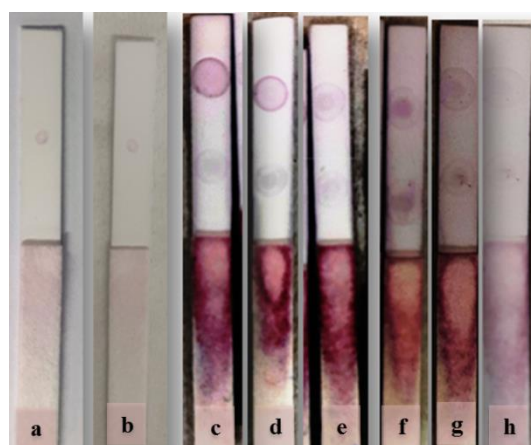
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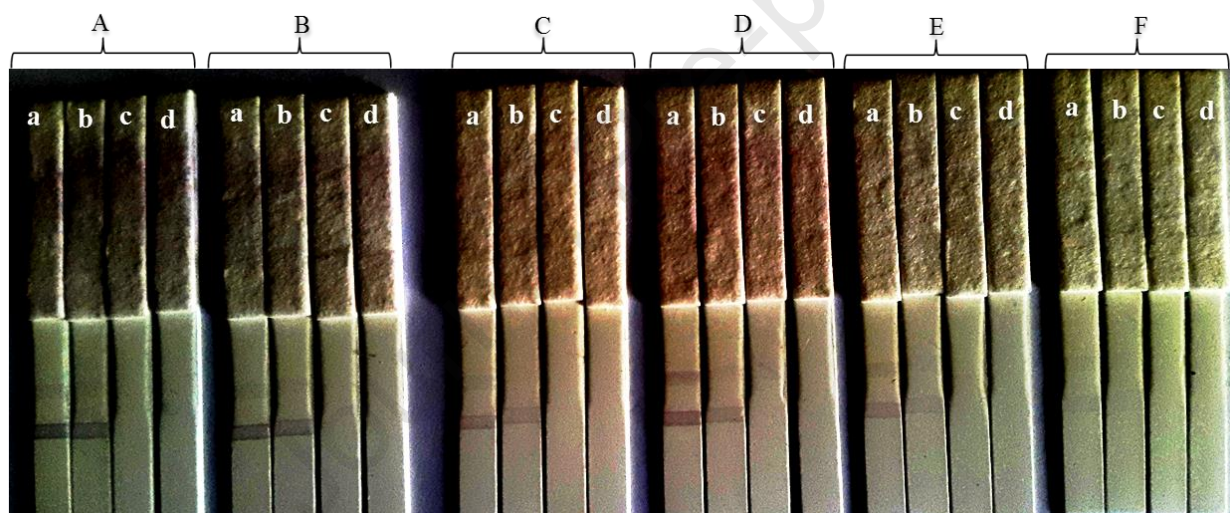
803 **Fig. S6.** Optimization of the gold nanoparticles-aptamer conjugate preparation under different concentrations of  
 804 the thiolated F20 and F20-T aptamers.

805



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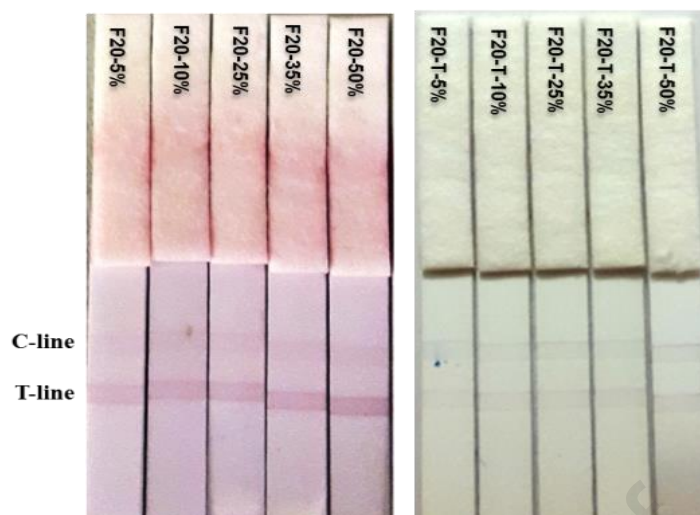
807 **Fig. S7.** Determination of the minimum required concentrations of DNA probe 1 and DNA probe 2 at the test  
 808 and control lines under the constant concentration (1  $\mu\text{M}$ ) of F20 and F20-T ; a:(F20, 60 $\mu\text{M}$ ), b: (F20-T,  
 809 60 $\mu\text{M}$ ), c:(F20,30 $\mu\text{M}$ ), d:(F20,15 $\mu\text{M}$ ), e:(F20,5 $\mu\text{M}$ ), f:(F20-T, 30 $\mu\text{M}$ ), g:(F20-T,15 $\mu\text{M}$ ), h: (F20-T, 5 $\mu\text{M}$ )  
 810



811

812 **Fig. S8.** Optimization of the test and control line performance using various nitrocellulose membranes with  
 813 different concentrations of DNA probes 1 and 2 (a:100nM, b:500nM, c:2.5 $\mu\text{M}$  , d:5  $\mu\text{M}$ ) under different  
 814 concentration of thiolated F20 (A:1 $\mu\text{M}$  ,B:0.2  $\mu\text{M}$  , C:0.1 $\mu\text{M}$  ) and F20-T (D: 2  $\mu\text{M}$ , E:0.4  $\mu\text{M}$ , F:0.2 $\mu\text{M}$ )  
 815 aptamers.

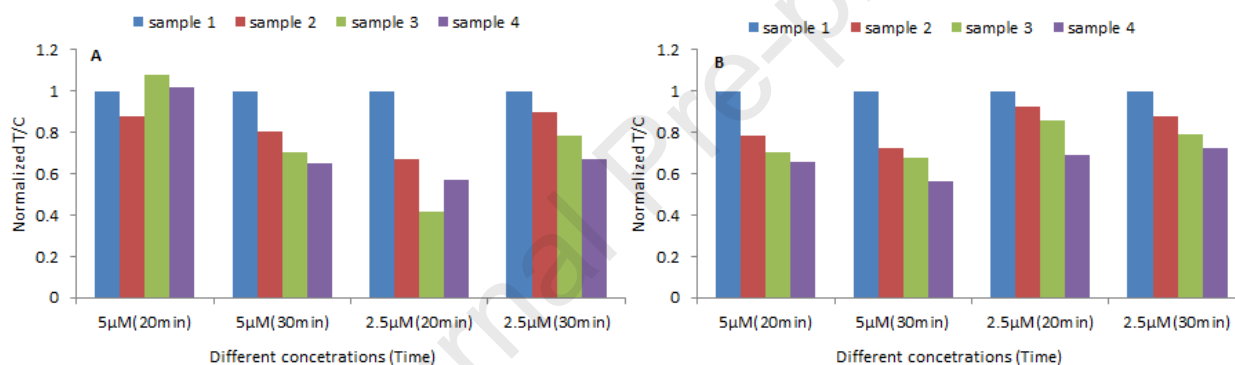
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817

818 **Fig. S9.** Evaluation of the test and control line intensities of F20 and F20-T based test strips under various  
 819 concentrations of aqueous methanol (5, 10, 25, 35, 50% v/v) at AFB1 constant concentration (10 ng/mL).

820

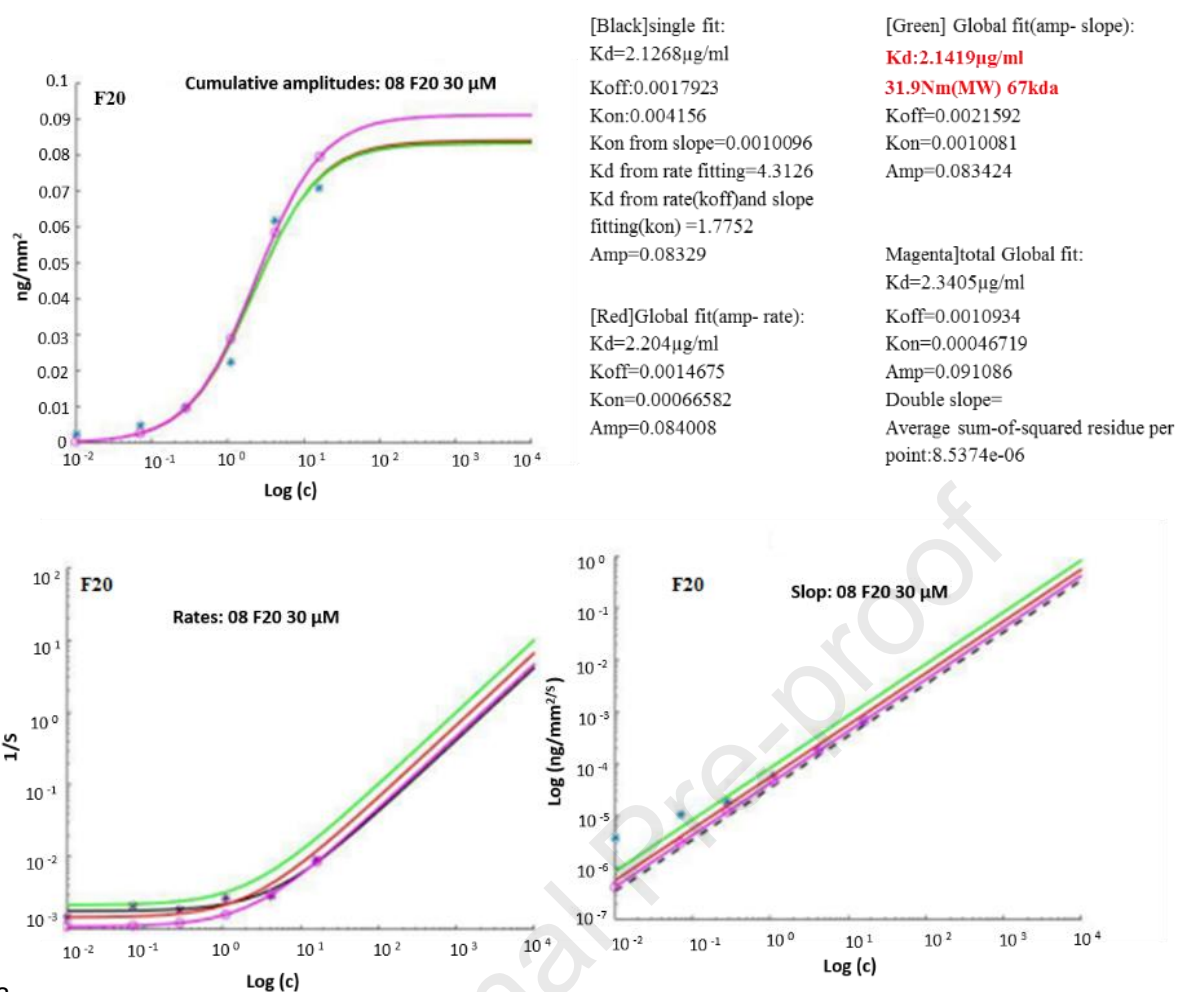


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822 **Fig.S10.** AFB<sub>1</sub> detection in corn flour samples via F20 (A) and F20-T (B) based lateral flow strips under  
 823 different conditions including two membranes prepared with different DNA probe concentrations (2.5 and 5  
 824  $\mu$ M) and two incubation time (20 and 30 min). AFB1 concentrations in HPLC-certified samples 1, 2, 3 and 4  
 825 were <LOD, 5, 11.3 and 28.9 ppb, respectively .

826

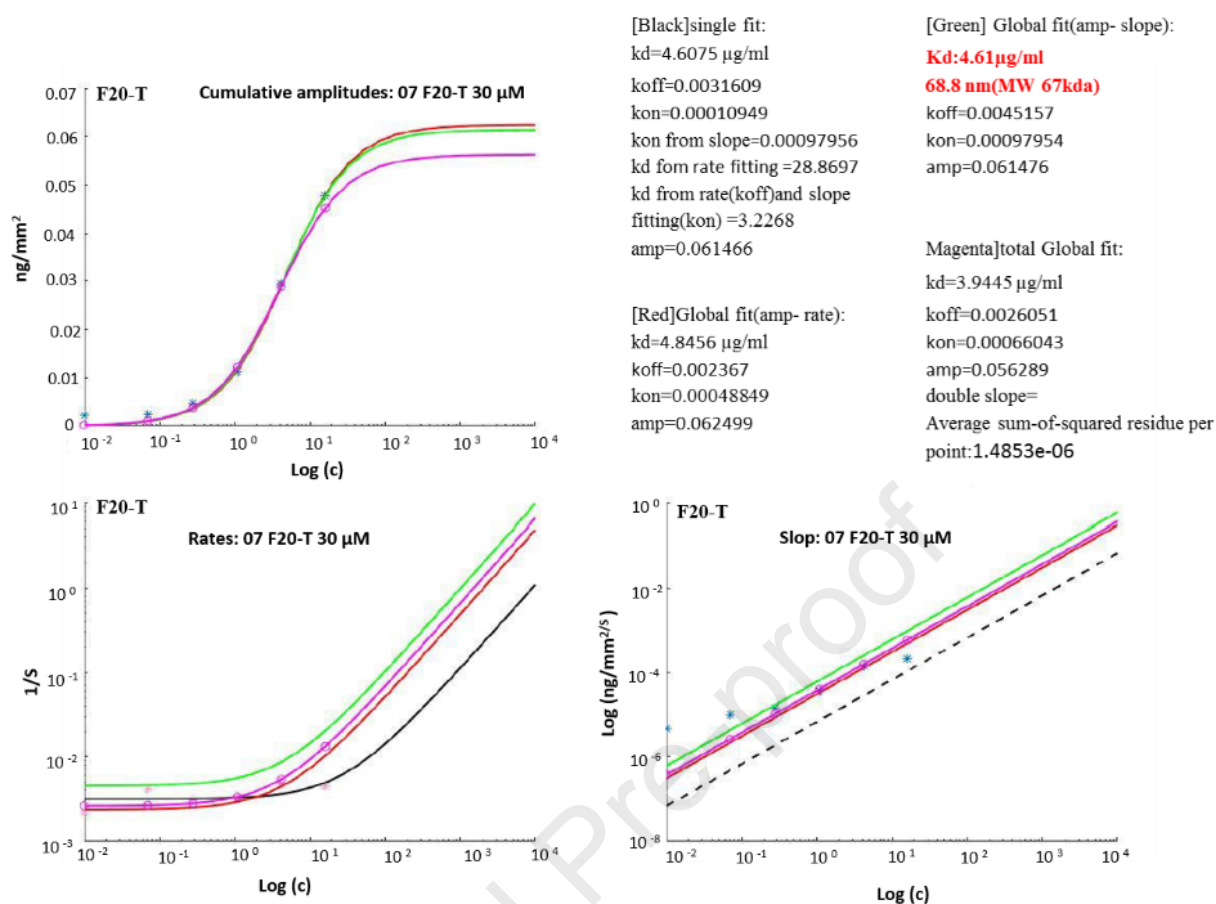
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829 **Fig. S11.** Determination of dissociation constant ( $K_d$ ) of F20 aptamer via reflective phantom interface (RPI)  
 830 technology.

831



832

833

834 **Fig. S12.** Determination of dissociation constant ( $K_d$ ) of F20-T aptamer via reflective phantom interface (RPI )  
 835 technology.



1. A new truncated Aflatoxin B1 binding aptamer was designed via in silico studies.
2. The truncated and the parent aptamer were successfully applied to build aptasensors.
- 3- The redesigned aptasensing platforms provided high sensitive AFB1 detection.
- 4- In silico engineered aptamers can be costly exploited for new aptasensor development.

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**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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