SUPPLEMENTARY MATERIALS

Single Microbead SELEX for Efficient ssDNA Aptamers Generation against Botolinum Neurotoxin

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Cloning and Sequencing. The clones were generated using the Qiagen's CloningPlus kit according to manufacturer's instructions. After overnight growth on LB agar plates, 20 colonies of the "BoNT peptide-binding" and 15 colonies of the "BoNT-toxoid binding" ssDNA pool were collected and suspended in 10 µL of water. 1 µL of the bacteria suspension was then amplified using the Qiagen's PlasmidAmp kit according to manufacturer's instruction. ~1 µL of the amplified suspension was then sequenced without further purification. The obtained cloned sequences are summarized in Figure S2.

Identification of Individual Sequences for Binding Studies. The obtained cloned sequences were subjected to homology analysis using the sequence alignment program ClustalW (available free-of-charge via the website: www.ebi.ac.uk). The software is able to separate the greatly diverse input sequences into meaningful groups. We have chosen, based on the above analysis (Figure S3a), five distinct sequences from the "Hc-peptide pool" and three distinct sequences form the "toxoid pool" were subsequently selected for further binding analysis. The selected sequences are highlighted in Figure S2. Each of the identified sequences were also subjected to the Mfold program analysis for their secondary structures generation.[1] We usually prefer secondary structures in which the selected sequences, i.e. from the 40nt randomized region, that are able to form distinct stem loops independent from the flanking fixed primers set (Figure S3b).

Binding Affinity and Competition Assay Studies. In addition to sequencing, the amplified suspension obtained from the Qiagen's PlasmidAmp kit was also used to amplify the necessary Fl-ssDNA aptamer for binding studies. The amplification procedure was similar to those described for the

amplification during the selection process. The binding affinity and competition fluorescence anisotropy studies were performed according to previously published procedures.[2] The anisotropy measurements were performed in a 50 μL volume quartz cuvette using a fluorimeter (Perkin-Elmer, Model LS55B): the excitation wavelength was set at 480 nm and the emission wavelength at 518 nm. The values were recorded over a span of 5 mins, via a 10 sec integration time, with slit width being 10 nm for both the inlet and outlet channels. The recorded anisotropy values for each concentration were then averaged and plotted using Kaleidagraph (Synergy Software, Reading, PA). The values were then curve-fitted using *Equation 1* to derive the overall dissociation constant (K_d) between the two biomolecules.[2]

Equation 1:

$$y = m1 + m2/2 * ((m0 + m3 + m4) - ((m0 + m3 + m4)*(m0 + m3 + m4) - (4 * m0 * m3))^{0.5})$$

where:

y	=	anisotropy in the presence of toxoid or peptide
m1	=	anisotropy in the absence of toxoid or peptide
m2	=	anisotropy changes in the presence of infinite amt of toxoid or peptide
m0	=	initial concentration of protein/peptide (μM)
m3	=	initial concentration of Fl-DNA aptamer (μM)
m4	=	dissociation constant (μM)

Briefly, ~50 nM of an individual Fl-ssDNA aptamer was first suspended in binding buffer and its fluorescent anisotropy values were measured. Subsequently, increasing concentrations of BoNT/A Hc-peptide or toxoid were titrated to the Fl-ssDNA, and the corresponding changes in anisotropy were recorded. Target was added until the observed anisotropy values are deemed to reach saturation level (Figure S4). Any changes in the observed anisotropy values (if any) reflect the overall complexation of ssDNA towards the Hc-peptide or toxoid targets.

For the competition anisotropy assay, the above was repeated where Fl-ssDNA aptamer was first added a saturating amount of Hc-peptide or toxoid. Subsequently, increasing amounts of anti-BoNT antibody (Ab) was titrated into the complex solution and the corresponding changes in anisotropy were recorded. If the Fl-ssDNA aptamer was being displaced by the Ab, the anisotropy should decrease correspondingly, i.e. the reverse of the binding assay (Figure S5).

Reference

(1) Zuker, M. *Nucleic Acids Res* **2003**, *31*, 3406-15.
(2) Ryu, D. H.; Litovchick, A.; Rando, R. R. *Biochemistry* **2002**, *41*, 10499-509.

Figure S1: (i) The fluorescently-labeled microbead can be easily monitored using a fluorescent stereomicroscope, after it is being transferred to a microcentrifuge for PCR amplification. (ii) Fluorescent micrograph of the microbead (after incubation with Fl-labeled ssDNA pool after selection cycle 2).

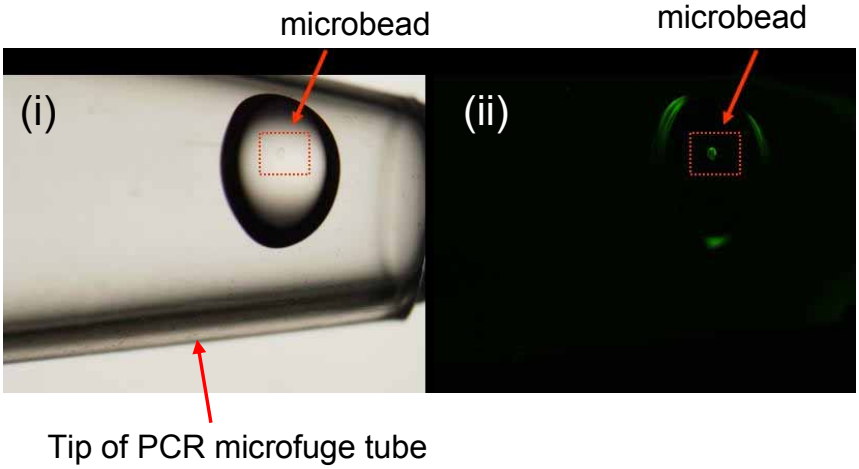


Figure S2: Sequences of the 40 nt randomized region from the derived clones after selection cycle 2 against: (a) BoNT/A Hc-peptide and (b) BoNT/A toxoid. The highlighted sequences were chosen for further binding analysis based on sequence homologies and their predicted secondary structures. Note that the complete aptamer sequence is: 5'-ATACCAGCTTATTCAATT-$\mathbf{N_{40}}$–AGATAGTAAGTGCAATCT-3'

(a) Obtained sequences against BoNT/A Hc-peptide:

Apt 2.1	TCG AAT CGT CAC CTG TCA ACT AAG TAA GCT GCG CGA CAT A
Apt 2.2	GAA CTC ATG ATG GGG CCT GAC AGT GAA TAG CAG CGC ATT G
Apt 2.3	CGC AGC GTT CCA GAC GAT ATA GTA CCA ACA CAA GTA ACT T
Apt 2.5	AGC AAT TGT GAT ACC GTT GAG AGG CGA AGA TTA GTA ACA C
Apt 2.6	ACG TTC GGT ACG GGG AGG GAT GCA TAA AAA TAC AAG GGC G
Apt 2.7	CGG GTA CGC TAG TTT TTT AGG GAT GCA GCG CAG TGG GGC T
Apt 2.8	AGG GAA AGG CAG CCG TGG TCT GAT GTG CTT GCC ACT GGA T
Apt 2.10	GGG GGA TAG ACC GAC TAG AGT ACA TGA CGA AGC GCA ATG T
Apt 2.11	AAG CCC GAG GAG TGG ATT TTT ATT ATC GAA GCT TAT GTT C
Apt 2.12	GGG GCA ATG GGC AGG TTC GGG AAG TAG GAA GTC CGA CCG G
Apt 2.14	CGG GTC GAC TAA AGG GGA ATA CGG TAT CTC ATT CTC GGA C
Apt 2.16	ACC GCA GAC AGT AAC CGG ATT CCT AAC CAG AAG CGG CTG T
Apt 2.18	AGA GCA GGA TAT CAT GCG GAC GAA AAA GCG TGC CCT GGA A
Apt 2.20	TCA GGG ATA AAA GGA GAA AGA AGC AGT CGG ATA GGC CAT A

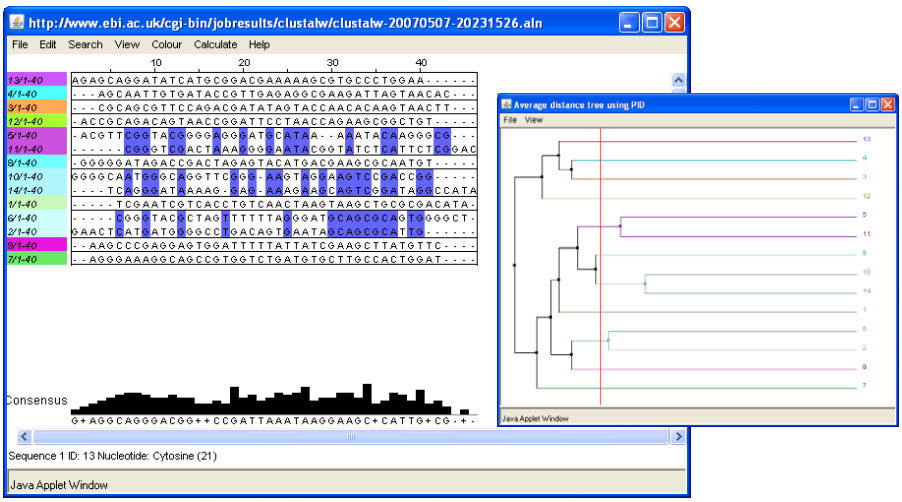
(b) Obtained sequences against BoNT/A Toxoid:

Apt 2.22	GAC ATG ACT GGG ATT TTT GGC GAA ATC GAA GGA AGC GGA G
Apt 2.24	AGA CGC AGT GTG ACA TCC CCG GCC ACC GAC ACC TAC TAA A
Apt 2.25	CAA ATA TTA CTC AAT ACC ATA AGC AAT GGA GTG GGT AAA A
Apt 2.26	CAA GTT GCT GTG GGA CAG AAA CGG GCG CTA GAC TAT GCA T
Apt 2.27	CTA ATA TAG TGG AGA ATT ATT ACG TGC CAT GTC ACG GTC G
Apt 2.29	CCA ACA GGG TGT TTA TTG ACT AGC ACG AGA CCG CTG GGA G
Apt 2.30	TTA AGT AAG ATT CAT CCC TCT ATG GCG AGG ATA AAT CAC C
Apt 2.32	TAA AGG AGA AAC AGC ATC CCC CAC CTT TCT GAA AAA ACC A
Apt 2.33	CCT AAT CTT CAT GCG TTA ATA TTT ACT GTG CGT TGC TTC G
Apt 2.34	ACC GCA GAC AGT AAC CGG ATT CCT AAC CAG AAG CGG CTG T

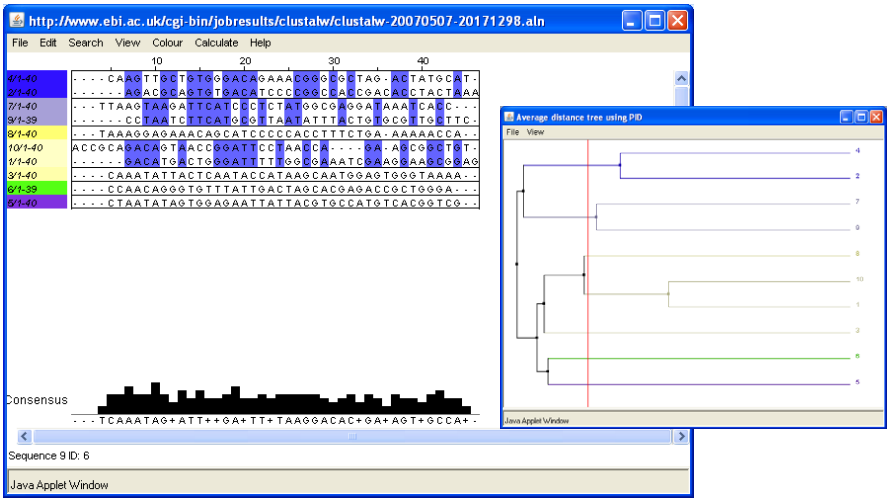
Figure S3: (a) Homology analysis of the obtained aptamer sequences using the sequence alignment program ClustalW (www.ebi.ac.uk). The software groups the greatly diverse input sequences into meaningful clusters. Sequences from each group were then identified for further analysis. (b) Predicted secondary structures for for a few of the selected clones based on Mfold software analysis.[1]

(a)

(i) Hc-peptide binding aptamers:



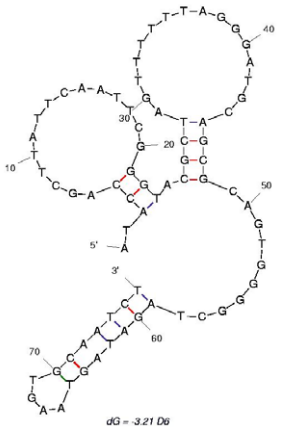
(ii) Toxoid-binding aptamers:



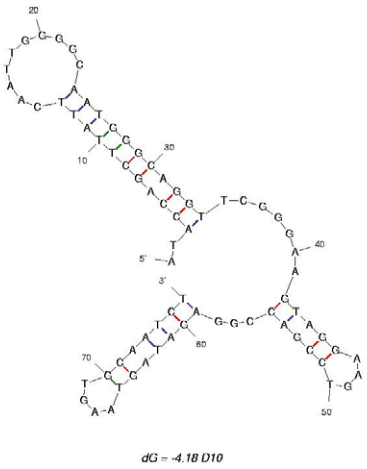
(b)

(i) Hc-peptide binding aptamers:

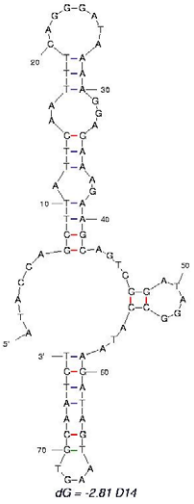
(i) Apt 2.7



(iii) Apt 2.12

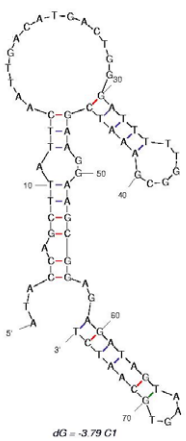


(iii) Apt 2.20

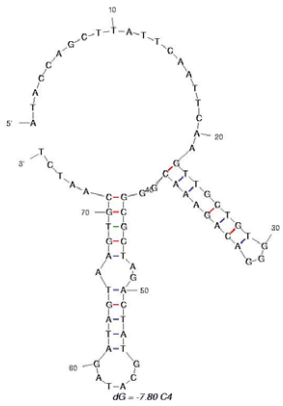


(ii) Toxoid binding aptamers:

(i) Apt 2.22



(ii) Apt 2.26



(iii) Apt 2.33

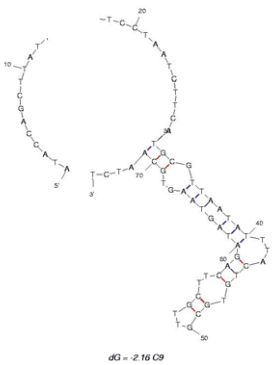
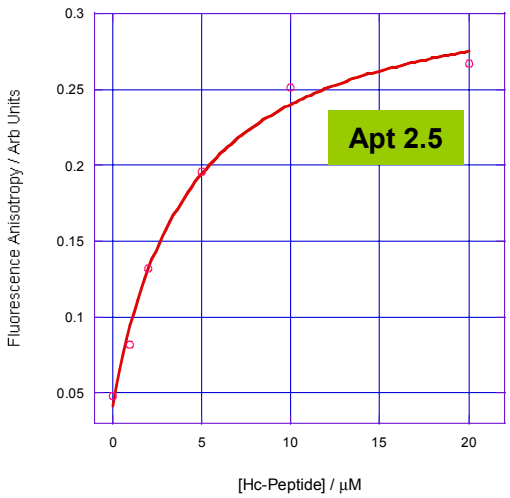


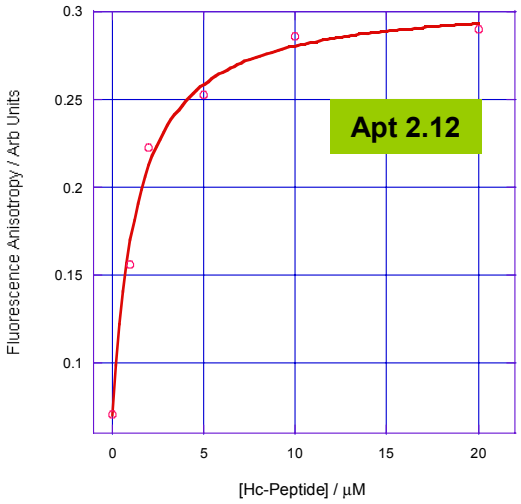
Figure S4: (a, b) Binding anisotropy plots of the various representative individual Fl-aptamer (~50 nM) against increasing concentration of BoNT/A Hc-peptide, and toxoid, respectively.

(a) BoNT/A Hc-peptide binding aptamers:

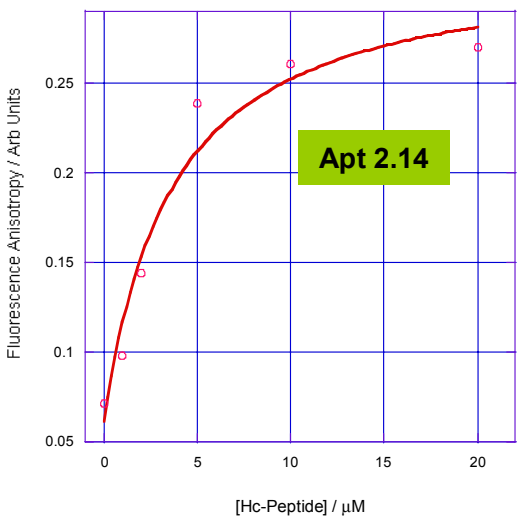
(i)



(ii)

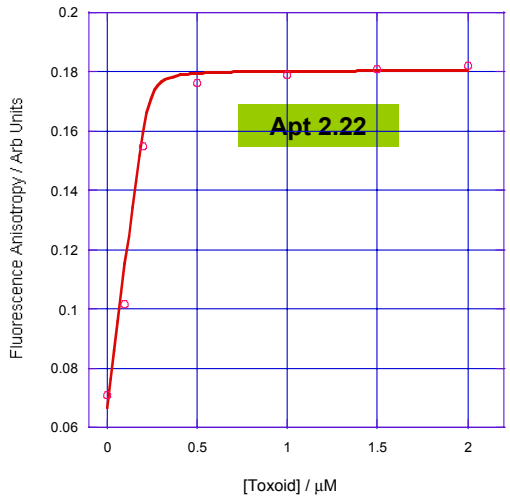


(iii)

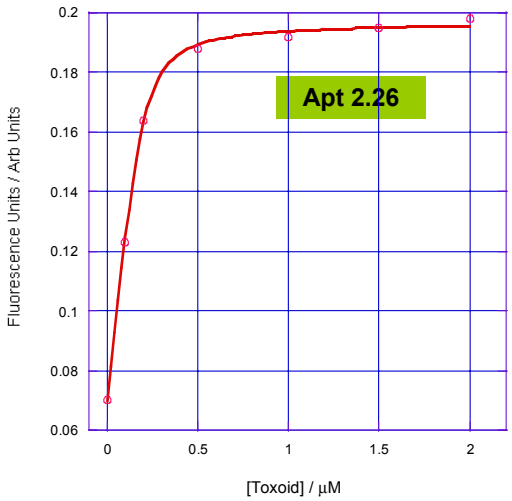


(b) BoNT/A Toxoid binding aptamers:

(i)



(ii)



(iii)

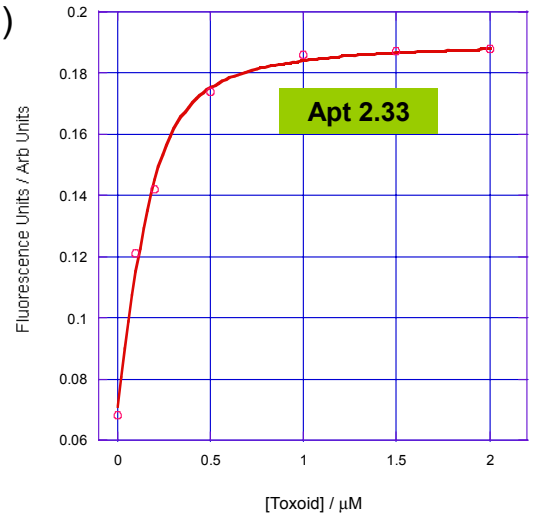


Figure S5: (i) Binding anisotropy plots of increasing concentration of anti-toxoid antibody (Ab) against complexes of Hc-peptide and both Fl-Apt 2.12 and Fl-Apt 2.20. (ii) Binding anisotropy plots of increasing concentration of anti-toxoid Ab against complexes of toxoid and both Fl-Apt 2.22 and Fl-Apt 2.33.