Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information for

Electrochemical Aptamer Scaffold Biosensors for Detection of Botulism and Ricin Toxins

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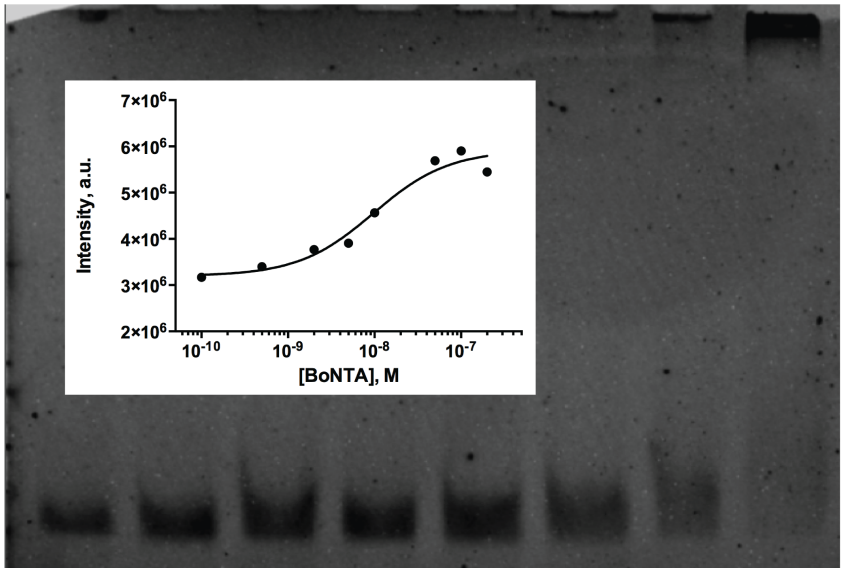
ID	Sequence
2.1	TTTTT AC CTAG TTGGACTTCWAXAAGTAACGATACTCGTWC GATC TTTTT
2.2	TTTTT AC CTAG TTTGACTTGCAXYATTXCWGTCACTCGAGG GATC TTTTT
2.3	TTTTT AA CTAG CACTWGAGTCCTYGTATTGCCCGCTTGACA CTAG TTTTT
2.4	TTTTT AC CTAG TTCCGCGTAGAXAAACAACGAGCACGGAAG CTAG TTTTT
2.5	TTTTT AC CTAG TTTGACWGCGYAGATACYGGCYTATGCC CTAG TTTTT
2.6	TTTTT AC CTAG TTTGCYTTYCTGAATTTAWGTCYGTCGG CTAG TTTTT
3.1	TTTTT AA GATC WCCTACCTGYTGATAAAGCTCGGCYGTACW GATC TTTTT
3.2	TTTTT AG CTAG TAACTGWCZGCGATTGTCGTGGGCAGTCTT GATC TTTTT
3.3	TTTTT AA CTAG CAGGGCTGGYCTTCWCGACTGWGTCWCCCA GATC TTTTT
3.4	TTTTT AC CTAG ACTGACGTAGWTTCGTAACGAGCYTCGTWC GATC TTTTT
3.5	TTTTT AG CTAG GZCGGTWCAACGCCTGCAZACAATCCTCTT GATC TTTTT
3.6	TTTTT AA CTAG CAGGTTTGGYCTWAWCGACTGWGTCGGAAT CTAG TTTTT
4.1	TTTTT AA CTAG WCTGWGCACCTGATTATTGCCGGCYGCCCA GATC TTTTT
4.2	TTTTT AG CTAG GZGGGTATZGCGATGTTCGTCAGCCCGCTT GATC TTTTT
4.3	TTTTT AA CTAG WCATGCCATCTAWACGGGGTCCTCCGGAAG GATC TTTTT
4.4	TTTTT AC GATC ACCCGCGTAGCGAAACAACGATCATCCC GATC TTTTT
4.5	TTTTT AG GATC TACGTGATAAGTTTGACACGGAATCCTCGT GATC TTTTT
4.6	TTTTT AC CTAG AAAGTCCAGCAXTCACAACGGGCYTAGACC GATC TTTTT

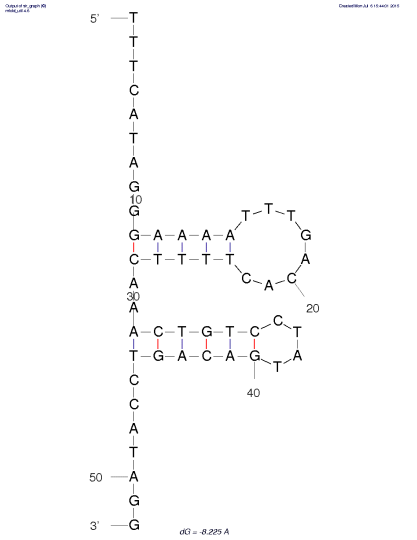
Supporting Table 1. Putative aptamer sequences identified by AM Biotechnologies X-Aptamer screening procedure. Some sequences contained unnatural bases: W for indole, X for guanidine, Y for phenol, and Z for boron.



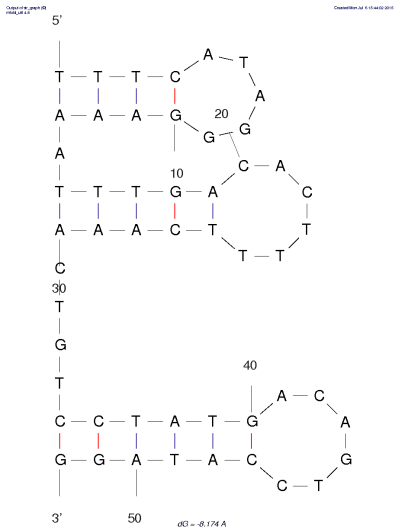
Supporting Figure 1. Gel mobility shift assay of BoNTA putative aptamer 2.5. Inset shows fit to dose-response binding curve, with apparent dissociation constant of 10 ± 4 nM. Experiment conducted using 10% polyacrylamide gel with Tris-glycine running buffer (25 mM Tris, 192 mM glycine, 0.1% SDS), run at 150 V for 1 hour. Imaged using a 5 minute soak in SYBR Green I nucleic acid dye then visualization on a BioRad GelDoc XR gel imaging system.

Name	Sequence
BoNTA Biosensor	5′- TTT CA[T(Methylene Blue)] AGG GA AA A**TTTGACAC**T TT TCAAA**C T GTCCTATGAC** A GTCCA TAGG -3′
RTA Biosensor	5′- AGAG **CGT AGG TTC G** C[T(Methylene Blue)]C GGG AA **CGG AGT GGT CCG** TTATTA ACC ACT ATTT GAA CCT ACC -3′

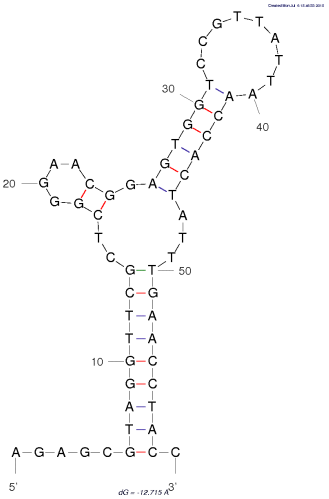
Supporting Table 2. Biosensor sequences used in this study. Aptamer regions incorporated into the oligonucleotide scaffold are in bold.



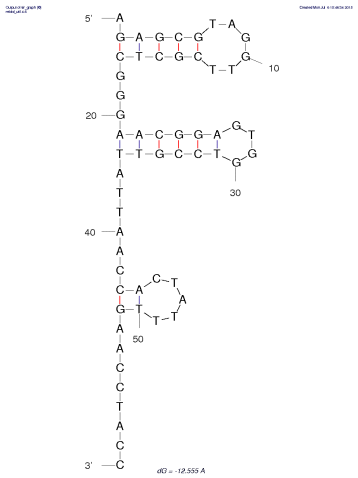
(A)



(B)

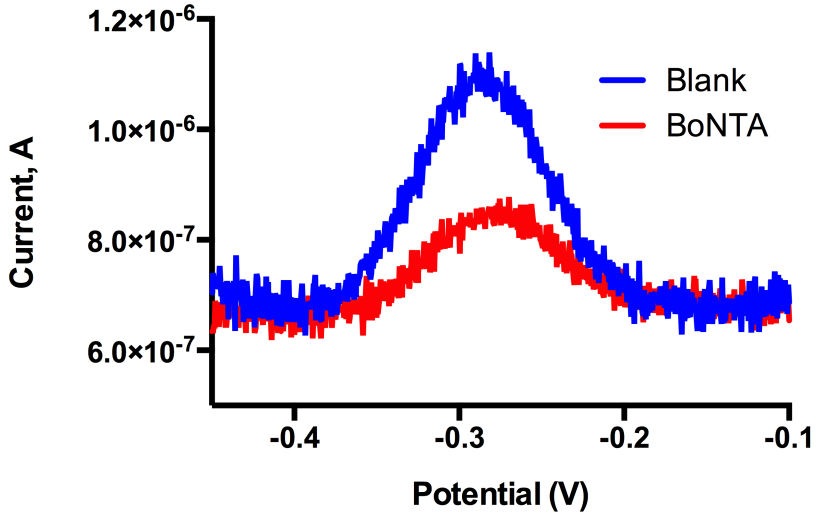


(C)



(D)

Supporting Figure 2. Predicted secondary structures of the BoNTA and RTA biosensors. (A) BoNTA structure with predicted high electrochemical signaling and low target binding; (B) BoNTA structure with predicted low signal and high target binding; (C) RTA structure with predicted high electrochemical signaling and low target binding; (D) RTA structure with predicted low signal and high target binding. Structures generated via QuikFold on the DINAMelt Web Server (University at Albany State University of New York; http://mfold.rna.albany.edu/?q=DINAMelt/Quickfold)[1].



Supporting Figure 3. Square-wave voltammograms of BoNTA biosensor equilibrated in 1xPBS with 100 nM BoNTA or with buffer only.

Experimental Methods:

Voltammetric data was collected using a WaveNano potentiostat (Pine Instruments, Grove City, Pennsylvania) in square-wave voltammetry mode. Patterned ceramic electrodes (Pine Instruments, Grove City, Pennsylvania) were cleaned using repeated cyclic voltametric scans in acid and base solutions to oxidize away surface impurities as previously described[2]. Biosensors were scanned from -400 mV to 100 mV at 50 mV/sec with 50 mV amplitude at 150 Hz. Under these conditions, a peak in the current was observed at approximately -250 mV for the methylene blue moiety of the biosensor. Peak current was measured as described previously[3], using the custom peak-fitting software "Any Peak Finder", available at http://www.bonhamlab.com/tools/code/any-peak-finder-interactive/. Peak current data for titration studies was analyzed using GraphPad Prism (La Jolla, CA) with $n > 3$ and error bars depicting standard error of the mean.

Supporting Information References

1. N. R. Markham and M. Zuker, *Methods Mol Biol*, 2008, **453**, 3–31.
2. A. A. Rowe, R. J. White, A. J. Bonham, and K. W. Plaxco, *J. Vis. Exp.*, 2011, **52**, e2922.
3. B. S. Ferguson, D. a Hoggarth, D. Maliniak, K. Ploense, R. J. White, N. Woodward, K. Hsieh, A. J. Bonham, M. Eisenstein, T. E. Kippin, K. W. Plaxco, and H. T. Soh, *Sci. Transl. Med.*, 2013, **5**, 213ra165.