Supporting Information for:

Colorimetric Detection of Anthrax DNA with a Peptide Nucleic Acid Sandwich-hybridization Assay

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Published procedures: PNA and cyclopentane-modified (*tcyp*) PNA synthesis, acquisition of melting temperature data.[1]

All PNA oligomers were purified by reverse-phase HPLC with UV detection at 260 nm using VYDEK C18 (d=10 mm, l=250 mm, 5 microns) semi-prep column, eluting with 0.05% TFA in water (Solution A) and 0.05% TFA in acetonitrile (Solution B). An elution gradient of 100% A to 100% B over ~30 minutes at flow rate of 5 mL/min. PNAs were characterized by electrospray mass spectroscopy. All PNA oligomers gave molecular ions consistent with the final product (Table S1).

Table S1. Mass Chracterization Data for PNAs

Entry[a]	PNA Sequence	*Calculated MW*	*Observed MW*
α(1)	H_2N-$(egl)_5$-ATCCTTATCAATATT-$CONH_2$	4736.6	4736.8
α(2)	H_2N-$(egl)_5$-ATCCTTAT$_{tcyp}$CAATATT-$CONH_2$	4776.7	4776.5
β(1)	H_2N-TAACAATAATCC-Lys-Lys-$CONH_2$ $(egl)_2BT$	3994.1	3994.0
β(2)	H_2N-TAACAATAATCC-Lys-Lys-Lys-$CONH_2$ $BT(egl)_2$ $(egl)_{10}BT$	5800.1	5799.8

[a] The accurate mass Electrospary ionization(ESI) mass spectra were obtained on a Waters LCT Premier time-of-flight(TOF) mass spectrometer. The instrument was operated in W-mode at a nominal resolution of 10000. The electrospary capillary voltage was 2KV and the sample cone voltage was 60 volts. The desolvation temperature was 275 (°C) and the desolvation gas was Nitrogen with a flow rate of 300L/hr. Accurate masses were obtained using the internal reference standard method. The sample was introduced into the mass spectrometer via the direct loop injection method. Both positive and negative ion accurate mass data were achieved simply by reversing the instrument's operating polarity. Deconvolution of multiply charged ions was performed with MaxEnt I.

HRP-avidin, poly HRP-avidin, and 1-Step Turbo TMB were purchased from Pierce. DNA-BIND® 96-well plates were purchased from Corning Life Sciences. Absorbance values for the DNA-BIND® 96-well plates were determined on a Victor^2 1420 microplate reader (Perkin Elmer Life Sciences).

The oligonucleotides listed in Table S2 were purchased from IDT and used in the research as indicated in Tables 2 and 3 of the manuscript.

Table S2. DNA Sequences

Anthrax PA: 5'- GGA TTA TTG TTA AAT ATT GAT AAG GAT -3'
TG mismatch: 5'- GGA TTA TTG TTA AAT ATT G**G**T AAG GAT -3'
TC mismatch: 5'- GGA TTA TTG TTA AAT ATT G**C**T AAG GAT -3'
TT mismatch: 5'- GGA TTA TTG TTA AAT ATT G**T**T AAG GAT -3'

Abbreviations: PBS (phosphate saline buffer: 137 mM NaCl, 10 mM sodium phosphate, 2.7 mM KCl, pH 7.0), 0.1 X SSC (15 mM NaCl, 1.5 mM Sodium Citrate), SDS (Sodium Dodecyl Sulfate), TMB (3,3',5,5'-tetramethylbenzidine), OBB (oligo binding buffer: 50 mM Na_2HPO_4/NaH_2PO_4, 1.0 mM EDTA), BB (blocking buffer: 3% BSA and 25 mM lysine in OBB, pH 7.0)

Attaching PNAα to Surface

PNAα solution (1.0 μM) in OBB (110 μL, pH 7.5) was added to each well of a DNA-BIND® 96-well plate (Corning Life Sciences), incubated at 37 °C for 1 hr, then washed three times with PBS buffer.

DNA Detection Protocol

Blank absorbance at 450 nm of the DNA-BIND® plate was obtained on the microplate reader. Each well was treated with 200 μL BB for 30 minutes at 37 °C. Target DNA (10 nM to 5 aM) and PNAβ (15 nM) were premixed in 100 μL of 0.15 M aqueous NaCl, then loaded into each well. The plate was sealed with an adhesive film, incubated at 45 °C for 3 hours, then washed twice with 0.1% SDS in 0.1 X SSC and treated with BB (200 μL) for 30 minutes at 33 °C. Next,100 μL of 1.0 μg/mL avidin-horseradish peroxidase conjugate in BB was added. After 30 minutes at 37 °C, the plate was washed three times with PBS buffer. Next, 100 μL of 1-step turbo TMB was added and incubated at 37 °C for 20 minutes. Then, 2 M H_2SO_4 (50 μL) was added to stop the oxidation. The plate was scanned again at 450 nm to give experimental absorbance readings from which background readings were subtracted. In some cases, very similar absorbance readings were obtained for different concentrations (see Table 2, entry 5). Most of these situations arise when using the polymer of HRP-avidin (poly HRP-avidin). This polymeric construct is highly active and difficult to control at higher concentrations. It is certainly ideal for detection of low concentrations of DNA, but can become less active at higher concentrations. Therefore, we suspect that aggregation at higher concentrations could lower enzymatic activity and, in some cases, give similar absorbance readings for different DNA concentrations.

Bacterial strains and genomic DNA purification:

Bacillus anthracis Ames 35 ($pXO1^+$ $pXO2^-$) strain[2] and a plasmid-free Ames 33 strain[3] ($pXO1^-$ $pXO2^-$) were used for genomic DNA purification. Ames 35 harbors the *pag* gene that encodes PA, while Ames 33 does not. For genomic DNA purification, *B. anthracis* strains were grown at 37°C in LB broth overnight. Genomic DNA purification was performed using the Wizard genomic DNA purification kit (Promega) following the protocol provided by the manufacturer and DNA was resuspended in water. DNA concentration was then assessed using a Nanodrop ND-1000 spectrophotometer (Coleman Technologies Inc).

References

1. Pokorski, J.K.; Witschi, M.A.; Purnell, B.L.; Appella, D.H. *J. Am. Chem. Soc.* **2004**, *126*, 15067.

2. Green, B.D.; Battisti, L.; Koehler, T.M.; Thorne, C.B.; Ivins, B.E. *Infect. Immun.* **1985**, *49*, 291.

3. Pomerantsev, A.P.; Sitaraman, R.; Galloway, C.R.; Kivovich, V.; Leppla, S.H. *Infect. Immun.* **2006**, *74*, 682.