Supplementary Information for:

Cyclopentane-modified PNA improves the sensitivity of nano-particle based scanometric DNA detection

Jonathan K. Pokorski, Jwa-Min Nam, Rafael A. Vega, Chad A. Mirkin, Daniel H. Appella*

General Procedure : Manual Solid Phase Synthesis of Peptide Nucleic Acids (PNAs)

General: aeg PNA monomers were purchased from Applied Biosystems. Kaiser test solutions were purchased from Fluka. HATU and Boc-Lys-(2-Cl-Z)-OH were purchased from Advanced Chemtech. All other chemicals were purchased from Sigma-Aldrich.

Abbreviations: (DCM), dichloromethane; (DIEA), N,N-diisopropylethyl amine; (NMP), 1-Methyl-2-pyrrolidinone; (HATU), O-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate; (MDCHA), N-Methyldicyclohexylamine; (DMF), N,N-Dimethylformamide; (HBTU), O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate; (Ac_2O), acetic anhydride; (TFMSA), Trifluoromethanesulfonic acid; (TFA), Trifluoroacetic acid

Note on resin washes: All washes used at least enough solution to cover the resin (~1.5 mL for 50 mg resin, and ~5 mL for 1 g resin). Values in parentheses describe the number of times the resin is washed with the indicated solution, and the agitation time for each wash. For example, (4x-30s) indicates four washes, with each having an agitation time of 30 seconds and each followed by draining under vacuum. If no time or number of repetitions is indicated, a single wash and/or a five second agitation period is used.

1. Downloading Resin

Methyl benzhydryl amine (MBHA) resin (1.0 g, 0.3 mmol active sites/gram) is downloaded to 0.1 mmol/g with Boc-*aeg*T-OH. The resin is first swelled in DCM for between 1 and 12 hours. The following solutions are prepared: 0.2 M Boc-*aeg*T-OH in NMP (A), 0.2 M HATU in NMP (B), and 0.5 M DIEA in NMP (C). These solutions are then combined appropriately to give two additional solutions: 0.45 mL of A + 0.46 mL of C + 1.59 mL NMP (Solution 1), and 0.55 mL of B + 1.95 mL NMP (Solution 2). Solutions 1 and 2 are pre-mixed for one minute and then added to the resin. The resin is agitated with a mechanical shaker for one hour and then drained under vacuum. The resin is subsequently washed with DMF (4x), DCM (4x), 5% DIEA in DCM (1x-30s), and again with DCM (4x). The remaining active sites are then capped with a 1:2:2 solution of Ac_2O:NMP:pyridine for 1.5 hours. This is followed by washes with DCM (2x-5s) and a qualitative Kaiser[1] test to confirm that no primary amines remain. Resin is then washed with DCM (2x) and allowed to dry under vacuum for 30-60 minutes. Downloaded resin is stored in a dessicator until further use.

2. aegPNA Synthesis[2]

The following is a representative coupling cycle for one PNA monomer. Downloaded resin (50 mg) is swelled in DCM for 1hr. The solvent is drained under vacuum and a solution of 5% m-cresol in TFA is added to the resin. The resin is shaken for 4 minutes, and the solution is removed under vacuum. (Note: The TFA deprotection is repeated three times for deprotection of the lysine residue and then twice for every subsequent monomer.) This is followed by subsequent washes with DCM, DMF (1x-5s, 1x-30s, 1x-5s), DCM (2x-5s), and pyridine (2x-5s). A Kaiser[1] test is performed to confirm deprotection. Upon positive Kaiser test, 150 μL of a 0.4 M solution of PNA monomer in NMP is pre-mixed with 150 μL of 0.8 M MDCHA in pyridine and 300 μL of 0.2 M HBTU in DMF for one minute. This solution is then added to the resin and agitated for 30 minutes. Following coupling, the resin was drained under vacuum and washed with DMF, 5% DIEA/DCM (1x-30s) and DCM (2x-5s). Again, a qualitative Kaiser[1] test is performed and, if negative, the resin is capped with a 1:25:25 mixture of Ac_2O: NMP: pyridine (2x-2min). (Note: If the Kaiser test is positive, the coupling cycle is repeated, beginning with the pyridine washes.) The capping step is followed by washes with DCM, 20% piperidine/DMF and finally DCM (1x-5s, 1x-30s, 1x-5s). This cycle is then repeated iteratively until the oligomer is complete on resin.

3. Cyclopentane PNA Synthesis

The following is a representative coupling cycle for one cyclopentane PNA monomer. The resin is washed with a solution of 5% m-cresol in TFA (3x-10min) and removed under vacuum. This is followed by subsequent washes with DCM, DMF (1x-5s, 1x-30s, 1x-5s), DCM (2x-5s), and pyridine (2x-5s). A Kaiser[1] test is performed to confirm deprotection. It should be noted that the primary amines of deprotected cyclopentane monomers do not show the characteristic blue color of a positive Kaiser test, but vary in color between gray/purple and red. Upon positive Kaiser test, 150 μL of a 0.4 M solution of cyclopentane PNA monomer in NMP is pre-mixed with 150 μL of 0.8 M MDCHA in pyridine and 300 μL of 0.2 M HBTU in DMF for one minute. This solution is then added to the resin and agitated for 60 minutes. Following coupling, the resin is drained and washed with DMF, 5% DIEA/DCM (1x-30s) and DCM (2x-5s). Again, a qualitative Kaiser[1] is performed and, if negative, the resin is capped with a 1:25:25 mixture of Ac_2O: NMP: pyridine (2x-2min). (Note: If the Kaiser test is positive, the coupling cycle is repeated, beginning with the pyridine washes.) The capping step is followed by washes with DCM, 20% piperidine/DMF and finally DCM (1x-5s, 1x-30s, 1x-5s).

4. Cleavage of PNA from Resin

Cleavage from the resin is accomplished under acidic conditions. First, the resin is washed with TFA (2x-4min) and drained under vacuum. Next, 750 μL of a solution cooled to 0 ^{o}C consisting of 75 μL thioanisole, 75 μL m-cresol, 150 μl TFMSA, and 450 μL TFA is added to the resin and agitated for one hour. The resulting solution is collected using positive N_2 pressure to force liquid through the fritted vessel. Another 750 μL portion of cleavage solution is added. After an additional hour of agitation, cleavage

solutions are combined in a glass vial and volatiles are removed by passing a stream of dry nitrogen over the product to afford a yellow/brown oil.

5. Crude PNA Isolation

The resulting oil is partitioned between five 2.0 mL microcentrifuge tubes using a micropipeter, to typically yield 100-150 μL of the oil per tube. A 10-fold excess (by volume) of diethyl ether is added to each tube. The solutions are mixed by vortexing until the brown color no longer remains and a cloudy white precipitate forms. The solutions are then placed on dry ice for ten minutes. This is followed by centrifugation (5 min at 7000 rpm) and removal of solvent via decanting or pipeting to yield a white solid as the crude PNA product. The ether precipitation cycle is repeated four times with the following dry ice incubation times; (2x-5min), (2x-2min). Repeating the cycle twice more with no dry ice incubation completes crude PNA isolation. After decanting the final ether wash, residual solvent is removed by passing a stream of N_2 over the crude PNA product.

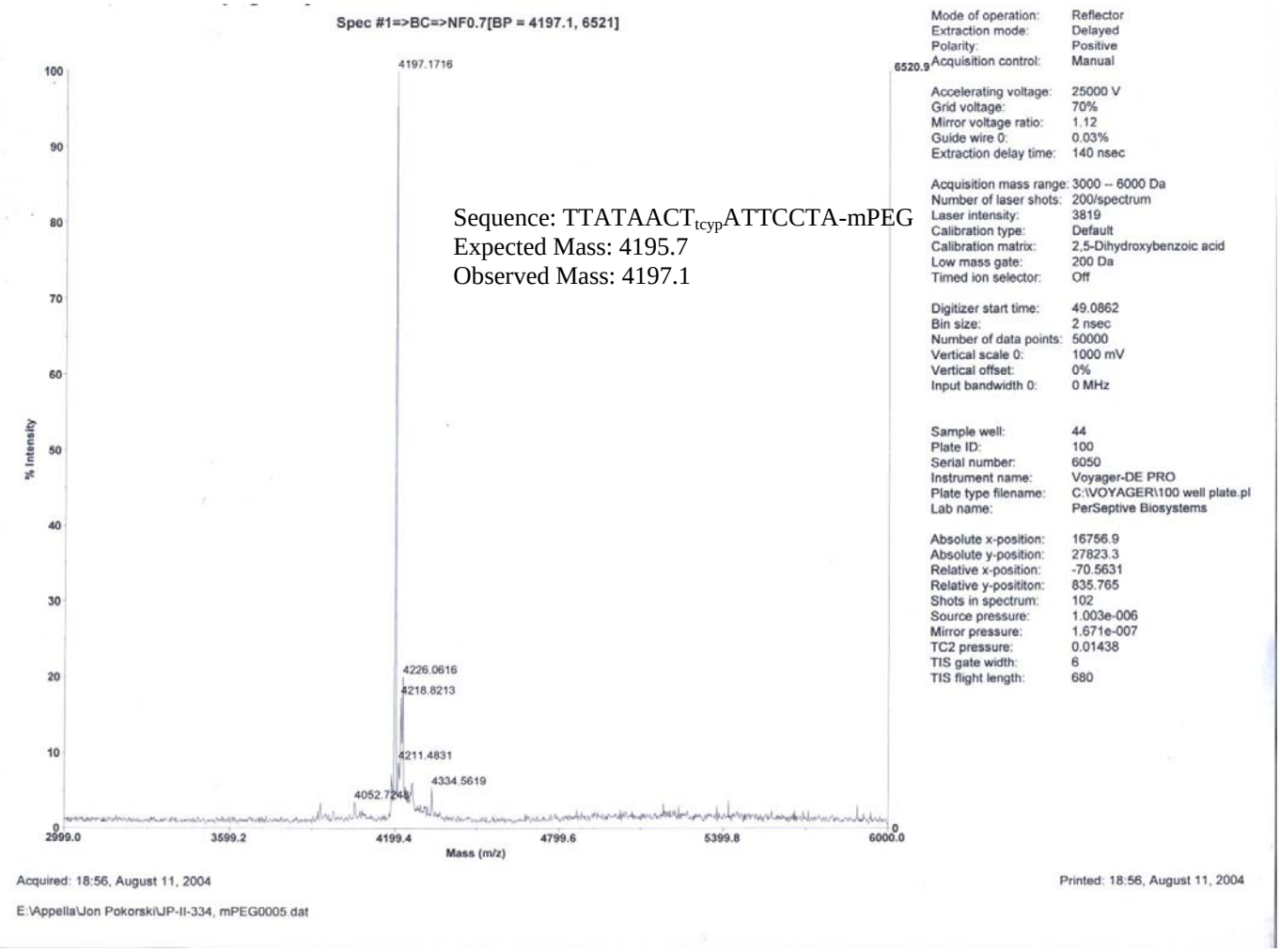
PNA Purification and Characterization

All peptide nucleic acid (PNA) oligomers were purified on reverse-phase HPLC with UV detection at 260 nm. Both MetaChem Polaris C18 (d=21.2 mm, l=250 mm, 10 microns) prep and VYDEK C18 (d=10 mm, l=250 mm, 5 microns) semi-prep columns were utilized, 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 60 minutes at flow rate 5.05 mL/min for MetaChem column and 2.2 mL/min for VYDAC column was used. PNAs were characterized by mass spectroscopy, using a PerSeptive Biosystems Voyager DE MALDI-TOF system with 2',4',6'-trihydroxyacetophenone monohydrate matrix. Mass spectra were acquired using a N_2 laser (337 nm wavelength, 5 ns pulse), with at least 100 shots per sample. All PNA oligomers gave molecular ions consistent with the final product.

Thermal Melting Analysis

Oligonucleotides were purchased from Integrated DNA Technologies, Inc. (IDT) and were dissolved in autoclaved deionized water. Concentrations of oligonucleotide and PNA solutions were determined by UV absorption at 260 nm on an Agilent 8453 UV/Vis spectrometer equipped with an Agilent 89090A peltier temperature controller. Extinction coefficients for PNA were calculated using the nearest neighbor method.[3] Extinction coefficients for oligonucleotides were reported by the supplier (IDT). Solutions of 1:1 oligonucleotide:PNA were prepared in pH=7.0 buffer consisting of 10 mM sodium phosphate, 0.1 mM EDTA, and 150 mM NaCl. Strand concentrations were 5 μM in each component. The solutions were degassed under vacuum for 1-2 minutes prior to melting analysis. Thermal denaturation profiles (absorbance vs. temperature) of the hybrids were measured at 260 nm with a diode array UV/Vis spectrophotometer equipped with a Peltier temperature controller that is interfaced to a personal computer. For the temperature range 90-5 ^{o}C, UV absorbance was recorded a 260 nm every 1 ^{o}C, with an

equilibration time of 60 s for each measurement point. A cooling profile was recorded for each complex. All samples were run in duplicate. The melting temperature (T_m) was determined from the maximum of the first derivative of the cooling curves.

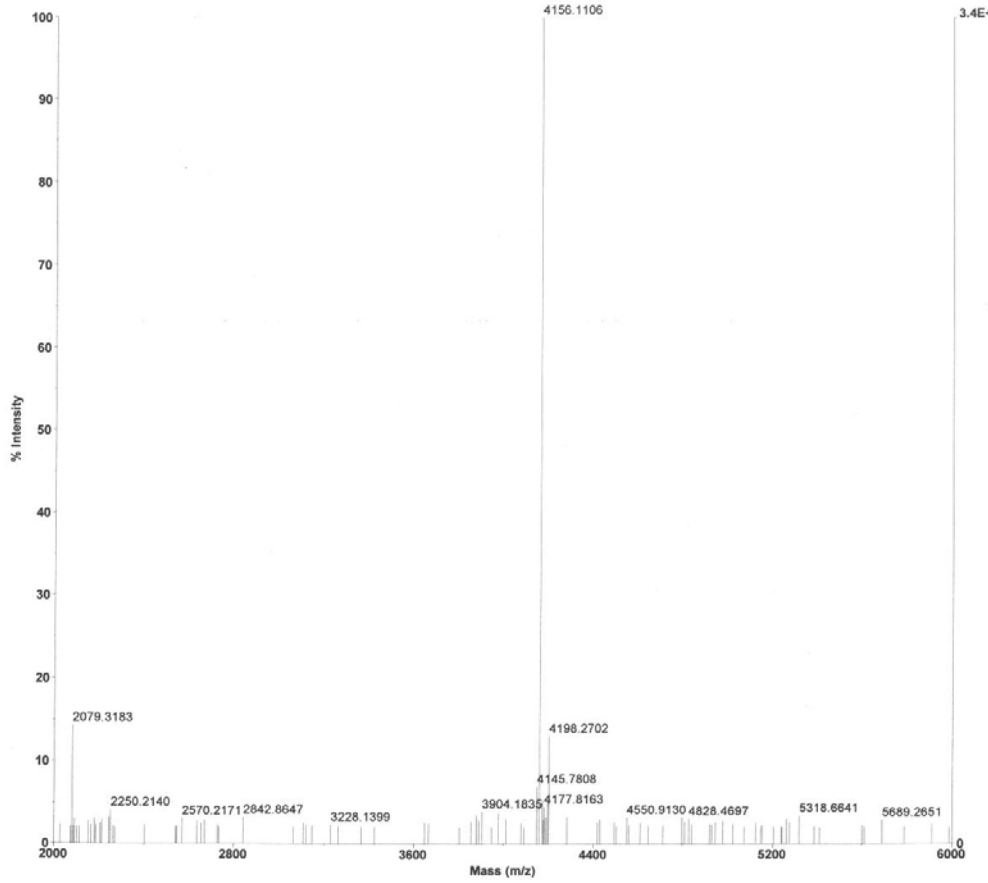


Sequence: TTATAACT$_{tcyp}$ATTCCTA-mPEG
Expected Mass: 4195.7
Observed Mass: 4197.1

Sequence: TTATAACTATTCCTA-mPEG
Expected Mass: 4155.7
Observed Mass: 4156.1

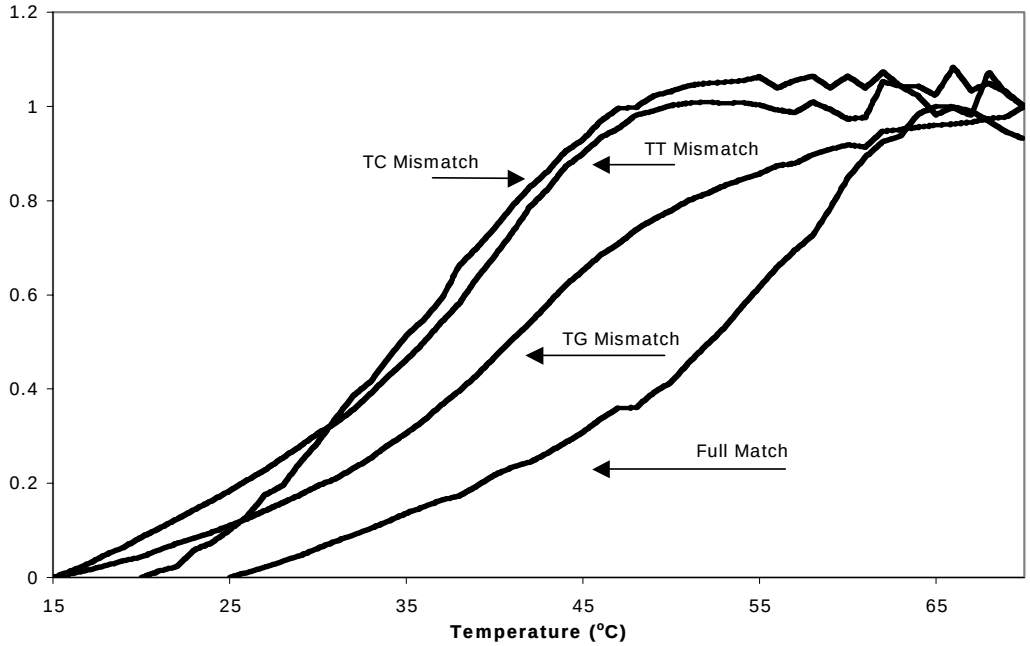
PE Biosystems Voyager System 6050

Spec #1=>BC=>NF0.7=>CT[BP = 4156.1, 34027]

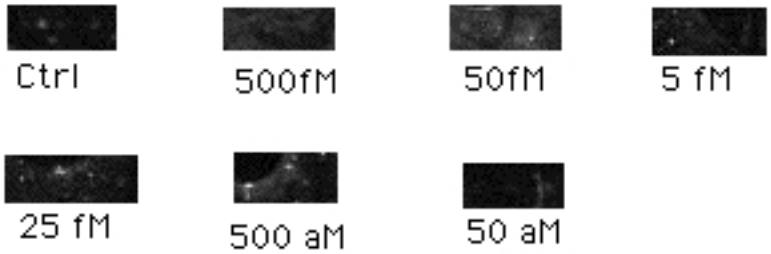


Mode of operation:	Reflector
Extraction mode:	Delayed
Polarity:	Positive
Acquisition control:	Manual
Accelerating voltage:	20000 V
Grid voltage:	70%
Mirror voltage ratio:	1.12
Guide wire 0:	0.03%
Extraction delay time:	140 nsec
Acquisition mass range:	2000 -- 6000 Da
Number of laser shots:	200/spectrum
Laser intensity:	3779
Calibration type:	Default
Calibration matrix:	2,5-Dihydroxybenzoic acid
Low mass gate:	200 Da
Timed ion selector:	Off
Digitizer start time:	44.8192
Bin size:	2 nsec
Number of data points:	50000
Vertical scale 0:	1000 mV
Vertical offset:	0%
Input bandwidth 0:	0 MHz
Sample well:	76
Plate ID:	100
Serial number:	6050
Instrument name:	Voyager-DE PRO
Plate type filename:	C:\VOYAGER\100 well plate.pl
Lab name:	PerSeptive Biosystems
Absolute x-position:	27029.8
Absolute y-position:	11889.5
Relative x-position:	42.3212
Relative y-posititon:	142.036
Shots in spectrum:	102
Source pressure:	9.044e-007
Mirror pressure:	1.534e-007
TC2 pressure:	0.01342
TIS gate width:	6
TIS flight length:	680

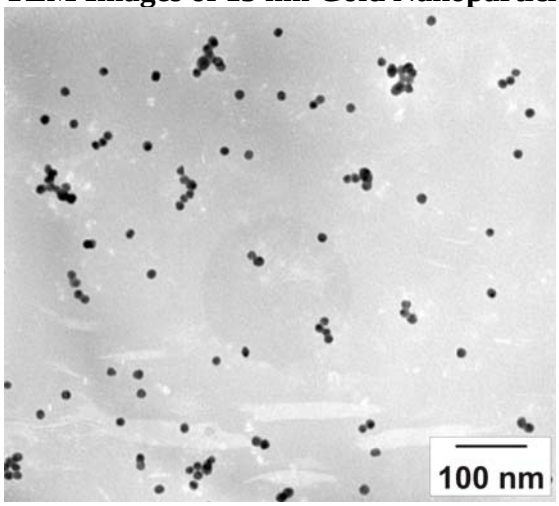
Melted Fraction of *tc*ypPNA-DNA duplexes as a function of temperature



Detection Results using *aeg*PNA



TEM Images of 13 nm Gold Nanoparticles



Procedures to Attach PNA to Surface and Detect DNA

The *t*cypPNA was spotted onto an amine-active slide (Amersham Biosciences) using a DNA microarrayer (GMS 417 Arrayer, Genetic Microsystems, Woburn, Massachusetts; spot diameter is 300 μm and the distance between spots is 700 μm). Following overnight immobilization, the slide was washed with 0.2 % SDS at 50 ^{o}C for 10 minutes, washed with NANOpure water (18 megohm), dried with a stream of N_2 and used immediately. The slide was incubated with a solution containing 30 μL of target DNA (at varying concentrations, 150 mM NaCl (the same concentration of NaCl as used in the original scanometric DNA detection assay)) and 100 μL of a 1 nM solution of DNA-modified nanoparticle probes for 2 hours at 40 ^{o}C and 1 hour at room temperature. This chip was washed with 0.5 M $NaNO_3$ in 10 mM phosphate buffer (pH 7.4) and immediately exposed to silver enhancement solution for 5.5 minutes (Nanosphere, Incorporated, Northbrook, IL). The results were then read using a Verigene ID system (Nanosphere, Incorporated, Northbrook, IL) which measures light scattering from the silver enhanced spots to provide a permanent record of the assay. Each assay was carried out three times on the same chip and the data were processed and quantified using a graphical software package (Adobe Photoshop). Microarray results were obtained after silver enhancement for various target concentrations from 25 aM (aM = 10^{-18} M) to 500 fM along with control samples.

References

[1]Kaiser, E., Colescott, R. L., Bossinger, C. D. and Cook, P. I., Color test for detection of free terminal amino groups in the solid-phase synthesis of peptides, *Anal. Biochem.*, **1970,** *34*, 595-8

[2]Koch, T., Hansen, H. F., Andersen, P., Larsen, T., Batz, H. G., Otteson, K. and Orum, H., Improvements in automated PNA synthesis using Boc/Z monomers, *J. Pept. Res.*, **1997,** *49*, 80-88

[3]Warshaw, M. M. and Tinoco, I., Jr., Optical properties of sixteen dinucleoside phosphates, *J. Mol. Biol.*, **1966,** *20*, 29-38