Cyclopentane-modified PNA improves the sensitivity of nanoparticle-based scanometric DNA detection†

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***trans*-Cyclopentane-modified PNA has been successfully utilized as a target capture strand to improve the detection limit of a known DNA detection assay, and provide high levels of mismatch discrimination.**

There have been intense efforts to develop methods that allow for highly sensitive and selective detection of DNA targets. These efforts have significant implications for the detection of genetic diseases and infectious agents. Many assays have been introduced based upon fluorescence, colorimetric, and electrochemical signalling using either molecular or nanoparticle probes.[1] To date, the most sensitive assays involve the polymerase chain reaction (PCR) in conjunction with molecular fluorophores, or the use of nanoparticle-based reporters in the scanometric format.[2] The sensitivity of these assays can be limited by the molecular recognition properties of DNA. Replacing DNA as the target capture strand with a synthetic oligomer designed to have better DNA-recognition properties could improve the sensitivity of these assays.

Peptide nucleic acids (PNAs) are a class of nucleic acid mimics with physical properties that could be beneficial as target capture probes for DNA. PNAs, which were discovered by Nielsen and coworkers in the early 1990s,[3] hybridize to complementary DNA with significantly higher affinity than the natural oligonucleotides.[3] Interestingly, PNA–DNA duplex stability increases as the salt concentration is reduced, contrary to the trend found with DNA duplexes.[4] This ability to bind under low salt conditions allows for denaturation of secondary and tertiary structures within the DNA targets, thus providing access to a maximum number of binding sites.[5] Furthermore, PNAs are stable to both nucleases and proteases and have superior surface stability compared to DNA.[6,7] Despite these promising properties, *aeg*PNA (Fig. 1) has shown mixed results when employed in chip based detection assays.[8] We recently introduced a new class of peptide nucleic acids bearing a *trans*-cyclopentane constraint within the PNA backbone (*tcyp*PNA).[9] The *tcyp*PNAs possess improved binding affinity and sequence specificity for their complementary DNA targets relative to *aeg*PNA.[10] These improved binding properties make *tcyp*PNA an attractive choice as a probe for DNA detection.

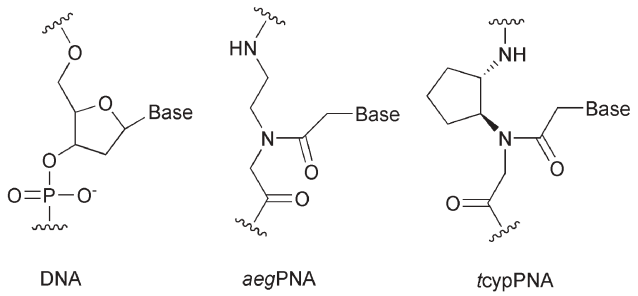
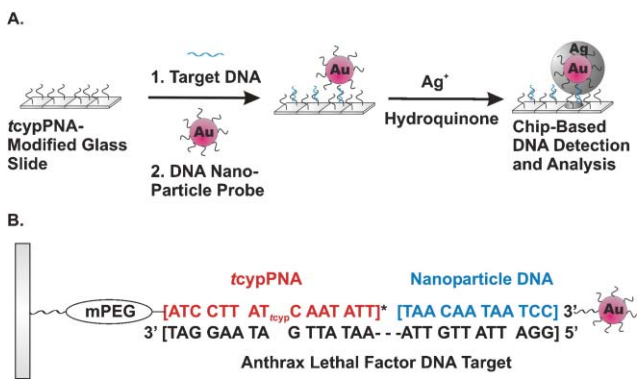


Fig. 1 Structures of probes for DNA detection.

In this communication, we report the benefits with respect to detection associated with *tcyp*PNA as the target capture strand in the highly sensitive scanometric DNA detection assay (Scheme 1).[2a]

To demonstrate the utility of *tcyp*PNA as a probe for nucleic acid detection, a synthetic oligonucleotide sequence was selected as a target that corresponds to that of the anthrax lethal factor (5′-GGATTATTGTTA---AATATTGATAAGGAT-3′). This sequence is well studied in the literature and is relevant to biowarfare and bio-terrorism applications.[2] For the detection assay, a 15-mer PNA complementary to the 3′ end of the anthrax target was synthesized bearing one *tcyp* residue at the central position and an 8-amino-3,6-dioxo-octanoic acid (mPEG) linker at the N-terminus (C-TTATAACT$_{tcyp}$ATTCCTA–mPEG-NH$_2$). The corresponding *aeg*PNA analogue was also synthesized, and both were examined in solution *via* UV thermal denaturation experiments. As expected, the *tcyp* modified PNA possessed a greater thermal stability and improved sequence specificity compared to the *aeg*PNA analogue (Table 1).

The scanometric DNA detection relies on oligonucleotide-modified gold nanoparticles (NPs), which were prepared *via* citrate reduction of $HAuCl_4$ and characterized using a Hitachi 8100 transmission electron microscope. The surface of the Au particles was functionalized with 3′-thiolated DNA and salt stabilized to afford DNA-modified nanoparticle probes complementary to the 5′ end of the target DNA (NP–SH–A$_{10}$–CCTAATAACAAT-5′).[11]



Scheme 1 Scanometric DNA detection. A) General detection scheme. B) Detailed view of *tcyp*PNA modified assay; * = C-terminus of PNA.

† Electronic supplementary information (ESI) available: experimental section. See http://www.rsc.org/suppdata/cc/b4/b418383e/

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Table 1 Melting temperatures of *tc*ypPNA and *aeg*PNA

	*tc*ypPNA		*aeg*PNA	
Anthrax sequence[a]	T_m (°C)[b]	ΔT_m (°C)[c]	T_m(°C)	ΔT_m(°C)
Full match	57	—	48	—
TC mismatch	36	−21	33	−15
TG mismatch	41	−16	37	−11
TT mismatch	40	−17	36	−12

[a] The anthrax sequences correspond to either the fully complementary anthrax target or the indicated mismatch opposite the position of the cyclopentane residue (5′-GGATTATTGTTAAATATTG*X*TAAGGAT-3′). [b] Conditions for T_m measurement: 10 mM phosphate buffer, 150 mM NaCl, 0.1 mM EDTA, pH 7.0, UV measured at 260 nm from 90 °C to 15 °C, in 1 °C increments. [c] ΔT_m represents the difference in melting temperature between the complementary DNA and the DNA with the indicated mismatch.

The *tc*ypPNA was spotted onto an amine-active slide (Amersham Biosciences) using a DNA microarrayer. Following overnight immobilization, the slide was washed, dried, and used immediately. The slide was incubated with a solution containing target DNA (at varying concentrations, 150 mM NaCl) and a solution of DNA-modified nanoparticle probes for 2 hours at 40 °C and 1 hour at room temperature. This chip was then washed and immediately exposed to silver enhancement solution. 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 (See ESI for additional details†).

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 (Fig. 2). This assay provides semi-quantitative data over four orders of magnitude of target concentration, thus providing access to a useful detection range without the need for amplification of the DNA target. This result represents a three order of magnitude improvement in sensitivity over the original reports that placed the limit of detection at 50 fM, when using DNA as the target capture strand.[2a,12] Furthermore, the importance of the cyclopentane in the PNA capture strand was evaluated by examining the same detection system using a regular *aeg*PNA sequence. Under the same conditions, the *aeg*PNA detection system showed little to no response (See ESI for results†).

We postulated that the enhanced sequence specificity of *tc*ypPNA in solution would allow effective discrimination between single base mismatches in the scanometric assay, an important property for the detection of single nucleotide polymorphisms (SNPs). With *tc*ypPNA as a probe, all three mismatches opposite the cyclopentane-modified thymine residue of the PNA capture strand were assayed (Fig. 3). This was carried out by hybridizing the mismatch DNA target (30 μL of a 500 aM sample) and the nanoparticle probe on the slide for 15 minutes at 60 °C, followed by one hour at 50 °C. The stringency conditions were chosen by careful inspection of the melting curves, where it was determined that a hybridization temperature of 50 °C would allow for maximum binding of the fully matched complement, while excluding the mismatched targets. Immediate washing (with 0.5 M $NaNO_3$) to remove all unbound oligonucleotides and silver enhancement followed. The results show a strong positive signal for the matched target and weak to undetectable signals for the single base mismatch targets. Signal intensity is a minimum of three times stronger for the fully matched complement relative to the mismatch sequences when the control signal is subtracted, allowing for facile discrimination of the intended targets. This level of selectivity is slightly improved from that achieved using a Cy3 fluorophore label appended to a DNA probe. The fluorescent system was shown to produce a 2.6:1 ratio in signal intensity between the fully matched target and a TG mismatch sequence. The popular fluorescent based systems however can not be tested at 500 aM concentrations due to inadequate detection limits.[2a,12]‡

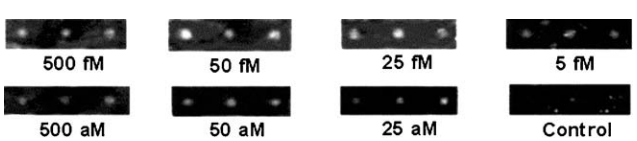

Fig. 2 Detection results using a *tc*ypPNA target capture strand.

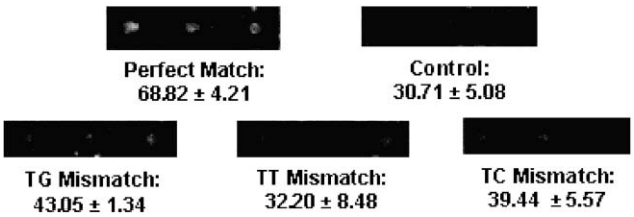

Fig. 3 Single base mismatch experiments. Each mismatch corresponds to the indicated mismatch as described in Table 1. The numbers associated with each experiment correspond to the average signal intensity of the three experiments.

The enhanced DNA recognition properties of *tc*ypPNA compared to *aeg*PNA were essential for successful application of a PNA as the target capture strand in the scanometric DNA detection assay. A single cyclopentane in the PNA backbone was crucial for obtaining positive results in this assay. Future work will focus on using *tc*ypPNAs for multiple target detection,[13] incorporation into the more sensitive bio-bar code assay for detection of both nucleic acids and proteins,[2b] and the development of an entirely PNA-based system in which the nanoparticle is functionalized with *tc*ypPNA.

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Notes and references

‡ The sequence specificity of *aeg*PNA could not be compared in the scanometric assay because no signal was detected at this target concentration.

1 (*a*) C. A. Mirkin, R. L. Letsinger, R. C. Mucic and J. J. Storhoff, *Nature*, 1996, **382**, 607–609; (*b*) T. G. Drummond, M. G. Hill and J. K. Barton, *Nat. Biotechnol.*, 2003, **21**, 1192–1199; (*c*) J. R. Epstein, I. Biran and D. R. Walt, *Anal. Chim. Acta*, 2002, **469**, 3–36.

2 (*a*) T. A. Taton, C. A. Mirkin and R. L. Letsinger, *Science*, 2000, **289**, 1757–1760; (*b*) J.-M. Nam, S. I. Stoeva and C. A. Mirkin, *J. Am. Chem. Soc.*, 2004, **126**, 5932–5933; (*c*) B. Schweitzer and S. Kingsmore, *Curr. Opin. Biotechnol.*, 2001, **12**, 21–27.
3 P. E. Nielsen, M. Egholm, R. H. Berg and O. Buchardt, *Science*, 1991, **254**, 1497–1500.
4 S. Tomac, M. Sarkar, T. Ratilainen, R. Wittung, P. E. Nielsen, B. Norden and A. Graslund, *J. Am. Chem. Soc.*, 1996, **118**, 5544–5552.
5 R. P. Bowater, F. Aboul-ela and D. M. J. Lilley, *Nucleic Acids Res.*, 1994, **22**, 2042–2050.
6 V. V. Demidov, V. N. Potaman, M. Frank-Mamenetskil, M. Egholm, O. Buchardt, S. H. Sonnichsen and P. E. Nielsen, *Biochem. Pharmacol.*, 1994, **48**, 1310–1313.
7 K. Kroger, A. Jung, S. Reder and G. Gauglitz, *Anal. Chim. Acta.*, 2002, **469**, 37–48.
8 (*a*) J. Wang, E. Palecek, P. E. Nielsen, G. Rivas, X. Cai, H. Shiraishi, N. Dontha, D. Luo and P. A. M. Farias, *J. Am. Chem. Soc.*, 1996, **118**, 7667–7670; (*b*) J. Wang, G. Rivas, X. Cai, M. Chicharro, C. Parrado, N. Dontha, A. Begleiter, M. Mowat, E. Palecek and P. E. Nielsen, *Anal. Chim. Acta*, 1997, **344**, 111–118; (*c*) K. Kerman, D. Ozkan, P. Kara, A. Erdem, B. Meric, P. E. Nielsen and M. Ozsoz, *Electroanalysis (N. Y.)*, 2003, **15**, 667–670.
9 M. C. Myers, M. A. Witschi, N. V. Larionova, J. M. Franck, R. D. Haynes, T. Hara, A. Grakowski and D. H. Appella, *Org. Lett.*, 2003, **5**, 2695–2698.
10 J. K. Pokorski, M. A. Witschi, B. L. Purnell and D. H. Appella, *J. Am. Chem. Soc.*, 2004, **126**, 15067–15073.
11 J. J. Storhoff, R. Elghanian, R. C. Mucic, C. A. Mirkin and R. L. Letsinger, *J. Am. Chem. Soc.*, 1998, **120**, 1959–1964.
12 J. J. Storhoff, S. S. Marla, P. Bao, S. Hagenow, H. Mehta, A. Lucas, V. Garimella, T. Patno, W. Buckingham, W. Cork and U. Muller, *Biosens. Bioelectron.*, 2004, **19**, 875–883.
13 S. S. Hashsham, L. M. Wick, K.-M. Rouillard, E. Gulari and J. M. Tiedje, *Biosens. Bioelectron.*, 2004, **20**, 668–683.