
Hybridisation and Melting Behavior of Peptide Nucleic Acid-Oligonucleotide Chimeras Conjugated to Gold Nanoparticles.

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In this study, Peptide Nucleic Acids (PNA) and PNA-DNA chimeras carrying thiol groups were used for surface functionalization of gold nanoparticles. Conjugation of PNA to citrate stabilized gold nanoparticles destabilized the nanoparticles causing them to precipitate. Addition of a tail of glutamic acid to the PNA prevented destabilisation of the nanoparticles but resulted in loss of interaction with complementary sequences. Importantly, PNA-DNA chimeras gave stable conjugates with gold nanoparticles. The hybridisation and melting properties of complexes formed from chimera-nanoparticle conjugates and oligonucleotide-nanoparticle conjugates are described for first time. Similar to oligonucleotide-nanoparticle conjugates, conjugates with PNA-DNA chimeras gave sharper and more defined melting profiles than those obtained using unmodified oligonucleotides. In addition, mismatch discrimination was found to be more efficient than with unmodified oligonucleotides

**Introduction.** Oligonucleotide bearing gold nanoparticles were first described in 1996 [1, 2]. These conjugates have been shown to form periodic arrays [1, 3, 4] and to form predetermined dimeric and trimeric assemblies [2, 5]. The special properties of gold nanoparticles linked to oligonucleotides has attracted large interest due to their potential use in gene analysis [6-12].

Peptide nucleic acids (PNA) are oligonucleotide analogues in which the negatively charged sugar-phosphate backbone has been replaced by a neutral backbone of N-(2-aminoethyl)glycine units with the common nucleic acid bases attached via a carbonyl methylene linker [13]. PNA oligomers recognize and bind to a specific DNA or RNA strand with high affinity and selectivity [14, 15]. Their chemical stability and their
resistance to nucleases and proteases make PNA oligomers good candidates as therapeutic agents, diagnostic tools and probes in molecular biology [15]. The addition of a PNA part to DNA to form PNA-DNA chimeras results in new structures which have combined properties from PNA and DNA. They show improve aqueous solubility due to the partially negatively charged backbone and they bind exclusively in the antiparallel orientation as DNA [16].

There is a large interest in the field of DNA derivatives as scaffolds for nanomaterials which have the same recognition properties as natural DNA but which have more stability or hydrophobicity to improve the properties of the conjugates. For example, the functionalization of the ends of single-walled carbon nanotubes was only successfully achieved by using PNA derivatives [17]. Conjugation of biotinylated-PNA to streptoavidin/avidin nanoparticles results in formation of new drug derivatives for antisense therapy [18, 19] and improved detection at DNA-functionalized electrodes [20]. Recently, investigations of gold nanoparticles modified with PNA have shown that the PNA moiety can modulate the electrostatic surface properties and control nanoparticle assembly rates and aggregate size [21].

In the present communication, we study the use of PNA and PNA-DNA chimeras for functionalization of the surface of citrate-stabilized gold nanoparticles. We show that the presence of a few negative charges are needed to stabilize the gold nanoparticles in solution and that PNA-DNA chimeras conjugated to gold nanoparticles remain stable after functionalization. The hybridisation and melting properties of complexes formed from chimera-nanoparticle conjugates and oligonucleotide-nanoparticle conjugates are explored and sensitive mismatch discrimination, when the mismatch is located in the DNA part of the chimera, is demonstrated.
Results and Discussion.- 1. Synthesis of Peptide Nucleic Acids and PNA-DNA chimeras carrying thiol groups. PNA sequence 1 (Table 1) was prepared by sequential addition of the commercially available Boc/Z PNA monomers as described elsewhere [22]. The progress of the coupling reactions was monitored by the ninhydrin test [23]. Cysteine protected with the $p$-methoxybenzoyl group was added at the N-terminal position to generate a free thiol group after removal of the protecting groups. The desired sequence was purified by HPLC. The same sequence but carrying three glutamic acid residues at the C-terminal position (sequence 2, Table 1) was prepared in a similar way. In this case the $\gamma$-carboxylic acid group of glutamic acid was protected with the 9-fluorenylmethyl (Fm) group that were removed with piperidine before the removal of the Z groups at the end of the synthesis.

The synthesis of PNA-DNA chimeras 3 and 4 were performed using the methodology described previously [24-27]. In this strategy the monomethoxytrityl (MeOTr) group is used for the temporary protection of the backbone amino function and acyl protecting groups are used for the protection of the exocyclic amino functions of the nucleobases [24-27]. The MeOTr is removed under mild conditions (3% trichloroacetic acid in dichloromethane), and the nucleobase protecting groups are removed using concentrated aqueous ammonia. These conditions are similar to those employed in DNA synthesis allowing the preparation of molecules carrying both PNA and DNA moieties. The connexion of the PNA and the DNA part was done using a thymine PNA linker molecule carrying an OH protected with the MeOTr group [25, 27, 28]. The oligonucleotide part was assembled on an automatic DNA synthesizer using standard protocols.
2. Synthesis and properties of oligonucleotide-gold nanoparticle conjugates.

Peptide Nucleic Acids, PNA-DNA chimeras and complementary oligonucleotides carrying thiol groups (1-10, Table 1) were reacted with citrate-stabilized gold nanoparticles (13 nm) to obtain gold nanoparticles with several oligonucleotide molecules per nanoparticle [7]. When gold nanoparticles were mixed with the all-PNA sequence 1 a precipitate was immediately formed indicating that the PNA molecules occupied the citrate binding sites and, as a result of the loss of surface negative charge, the nanoparticles were no longer stable in solution. When the reaction was performed with sequence 2 carrying the glutamic acid tail no precipitation was observed indicating that the glutamic acid tail prevented destabilization of the nanoparticles. However, when nanoparticles carrying sequence 2 were mixed with nanoparticles carrying the complementary sequence 10, no hybridization was observed. The expected red to blue color change that accompanies hybridization of oligonucleotide-nanoparticle conjugates [1] was not observed and the resulting solutions did not show any characteristic melting behavior when heated (followed by UV-visible absorption spectroscopy). By comparison the duplex formed by the same compounds without nanoparticles gave the expected melting profiles (data not shown) indicating that either surface functionalization of the gold nanoparticles with PNA 2 did not occur or, if it did occur, then the sequence was not accessible to hybridization. These results are in agreement with previously reported data [21]. Consequently, the use of PNA-DNA chimeras for gold nanoparticle derivatisation was explored. Functionalization of citrate-stabilized gold nanoparticles with PNA-DNA chimeras was performed in the same manner as used for unmodified oligonucleotides.
No aggregation of the conjugates that were formed was observed. The chimera-nanoparticle conjugate 3 and complementary oligonucleotide-nanoparticle conjugate 4 were then mixed together in equimolar amounts in hybridization buffer. During the hybridization process the solution changes from an initial red color to a pale blue/black color. Oligonucleotide hybridisation results in the formation of 3D interlinked networks of nanoparticles. Significant spectral changes therefore occur in the UV-visible absorption spectra of these solutions due to the formation of nanoparticle aggregates following hybridization of the oligonucleotides or chimera-oligonucleotides. These large absorbance changes, as compared with unmodified oligonucleotides, are a result of the marked sensitivity of nanoparticle optical properties (especially the surface plasmon resonance) to the reduced internanoparticle distances that occur when the nanoparticles are brought into close proximity following hybridization [3,4,6-8]. The magnitude and reversibility of these spectral changes allows the progress of hybridization and subsequent denaturation to be monitored optically for very small oligonucleotide concentrations.

The melting behaviour of the hybridized complex formed from chimera-nanoparticle conjugate 3 and complementary oligonucleotide-nanoparticle conjugate 6 was then studied. UV-visible absorption spectra were recorded as a function of increasing temperature. As the solution temperature increases, the oligonucleotide and chimera strands separate and hence the crosslinked nanoparticles also become separated and redisperse into solution causing a reversal of the spectral changes that occurred during hybridization [3,4,6-8]. Thus this melting transition represents the breakdown of the 3D interlinked gold nanoparticle networks following oligonucleotide-chimera complex
denaturation. Consequently, the melting profile of the hybridized complex formed from chimera-nanoparticle conjugate 3 and oligonucleotide-nanoparticle conjugate 6, shown in Figure 1, is distinct and well defined in comparison with the very poorly resolved melting profile acquired for the identical oligonucleotide-chimera complex formed without nanoparticle tags, also shown in Figure 1.

An interesting aspect of duplexes that incorporate PNA moieties is the low dependence of duplex stability on ionic strength. This is due to the elimination of the repulsion between phosphate groups in PNA/DNA duplexes. In our case, we employ DNA-PNA chimeras so the duplex will more closely resemble a DNA/DNA duplex. With respect to solution ionic strength, when chimera-nanoparticle conjugate 3 is mixed with complementary oligonucleotide-nanoparticle conjugate 6 in hybridization buffer containing 50 mM or 100 mM NaCl no color change associated with hybridization was observed and no melting transitions were observed on heating. This confirmed that a relatively high salt concentration is needed in the buffer in order to obtain chimera hybridisation, as in the case of DNA/DNA duplexes.

The presence of base mismatches in oligonucleotide sequences was also studied. As can be seen from Figure 2 and Table 2 introduction of one mismatched base into chimera-nanoparticle and oligonucleotide-nanoparticle complexes resulted in the lowering of the melting temperature and the magnitude of the hyperchromic change associated with each melting transition. These effects were more pronounced when the mismatch was incorporated into the DNA part, where no transition at all was observed (duplexes 4 + 6 and 3 + 8). On the other hand, a mismatch within the PNA part (duplex 3+7) gave the same melting profile as a mismatch within a DNA/DNA duplex (duplex 5+7). It has been reported that mismatch discrimination is higher in PNA derivatives.
For example, mismatches in PNA complexes have been reported to lower the melting temperature of duplexes by 8-20 °C, twice that observed for mismatches in DNA/DNA duplexes [14]. Melting temperatures of PNA-DNA chimeras depend on various factors such as DNA:PNA ratio in the chimera, sequence, and the position of the linker molecule between DNA and PNA [16]. Our results show that, using PNA-DNA chimeras, it is possible to select a combination of conditions that eliminate hybridization with sequences carrying one single mismatch. We believe that these results will be of interest for mismatch detection in gene analysis using DNA microarrays [11, 12].

**Conclusion.** In this study, PNA and PNA-DNA chimeras carrying thiol groups were used for surface functionalization of gold nanoparticles. We showed that citrate-stabilized gold nanoparticles can be successfully functionalised with PNA-DNA chimeras and remain stable after functionalization. The hybridisation and melting properties of complexes formed from chimera-nanoparticle conjugates and oligonucleotide-nanoparticle conjugates are described for first time. Similar to oligonucleotide-gold nanoparticle conjugates, conjugates with PNA-DNA chimeras gave sharper and more defined melting profiles than those obtained for than unmodified oligonucleotides. In addition, mismatch discrimination was more efficient than with unmodified oligonucleotides.

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**Experimental Part**

*General.* Phosphoramidites and ancillary reagents used during oligonucleotide synthesis were from *Applied Biosystems* (USA), *Cruachem Ltd.* (Scotland) and *Glen Research* (USA). PNA monomers protected with the Boc group were from *Applied Biosystems* (USA). Amino acid derivatives were from *Bachem* (Switzerland) and *Novabiochem* (Switzerland). The rest of the chemicals were purchased from *Aldrich, Sigma* or *Fluka*. Long chain amino controlled pore glass (LCA-CPG) was purchased from *CPG Inc* (USA). Amino-polyethyleneglycol-polystyrene (PEG-PS) was purchased from *PerSeptive* (now *Applied Biosystems* USA). Methylbenzhydrylamine-polystyrene support was from *Novabiochem* (Switzerland). Solvents were from *S.D.S.* (France). Gold nanoparticles (13 nm, citrate stabilized) were prepared as described elsewhere [7].

*Instrumentation.* UV-visible absorption spectra were recorded on a UV-2103PC and Agilent 8453 spectrophotometers. Mass spectra (matrix-assisted laser desorption ionization time-of-flight, MALDI-TOF) were provided by the Mass spectrometry service at the University of Barcelona. DNA synthesis were performed on a 392 *Applied Biosystems* (USA) DNA synthesizer.

*Synthesis of Peptide Nucleic Acids.* PNA oligomer synthesis was carried out manually on a methylbenzhydrylamine (MBHA)-polystyrene support (5 µmol scale), following
the synthesis cycle for the Boc/Z strategy described elsewhere [22]. The PNA monomers were added manually with a molar excess of 5 times activated by O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) and diisopropylethylamine (DIPEA). Coupling reaction was monitorized by the ninhydrin test [23], and the absence of color in the solution or resin beads indicated nearly quantitative yields (> 95%). For the introduction of cysteine Boc-Cys protected with the p-methoxybenzoyl group was used. The Fm group was used for the protection of the γ-carboxylic group of glutamic acid. The last amino group was acetylated with acetic anhydride and diisopropylethylamine. After the completion of the sequence 2 the Fm group was removed with piperidine and the resulting support was treated with a mixture of trifluoroacetic acid and trifluoromethanesulfonic acid (using dimethylsulfide and m-cresol as scavengers) as described [22]. The support from the synthesis of sequence 1 was treated directly with a mixture of trifluoroacetic acid and trifluoromethanesulfonic acid and the scavengers. The resulting products were precipitated with ether and the residue was desalted with a Sephadex G-25 column (NAP-10, Pharmacia, Sweden).

The PNA containing fractions were further purified by RP-HPLC obtaining the desired PNA oligomer. HPLC conditions. Column: Nucleosil 120 C18, (250x8 mm), flow rate 3 ml/min, a 25 min gradient from 0% B to 50% B. Solution A: 5% acetonitrile in 0.1% trifluoroacetic acid in water. Solution B: 70% acetonitrile in 0.1% trifluoroacetic acid in water. Mass spectrometry confirmed that the desired product corresponded to the major peak on the chromatogram. MALDI-TOF of 1: 3306.9 ([M+H]+; calc for C_{131}H_{168}N_{66}O_{38}S: 3306.7). MALDI-TOF of 2: 3814.1 ([M+Na]+; calc for C_{150}H_{194}N_{70}O_{49}S: 3793.1).
*Synthesis of PNA-DNA chimeras and oligonucleotides.* 5’-Thiolated oligonucleotides were prepared using standard (benzoyl- or isobutyril-protected) 3’-[(2’-cyanoethyl)phosphoramidites] and the phosphoramidite of (MeO)$_2$-Tr-protected 6-hydroxyhexyl disulfide (*Glen Research*, USA).

PNA-DNA chimeras were synthesized on a MeOTr-NH-hexyl-succinate-NH-PEG-PS-support as described in reference [24]. PNA monomers were prepared as described [28].

PNA sequence *TGCAT*-CONH-hexyl-OH was synthesized on 10 µmol scale on a syringe equipped with a filter. The PNA monomers were added manually with a molar excess of 5 times. PNA monomer coupling was carried out adding equal amounts of 3 solutions: a) 0.2 M PNA monomer in acetonitrile (T, G, A) or 0.1 M of the C monomer in dichloromethane (C), b) 0.2 M HATU in acetonitrile and c) 0.2 M DIPEA in ACN and a coupling time of 15 min. After coupling a capping with acetic anhydride and N-methylimidazole was performed using the capping solutions from oligonucleotide synthesis and a capping time of 1 min. Deprotection of the monomethoxytrityl amino groups was carried out using 3% trichloroacetic acid in dichloromethane (10 x 1 ml, 5 min) followed by thorough washing with acetonitrile, neutralization with 0.3 M DIPEA in acetonitrile and washing with acetonitrile. The last PNA monomer is a T-linker molecule carrying an OH protected with the MeOTr group [25, 27, 28]. The oligonucleotide part was assembled on an automatic DNA synthesizer using standard 2-cyanoethyl phosphoramidites and following standard protocols (1 µmol scale). In order to characterize the PNA-support a short sequence of a DNA-PNA chimera was prepared (5’*TGCAGCCTGCA*-CONH-hexyl-OH) and was purified by HPLC giving a major
product that had the expected molecular weight. Electrospray of 5’TGCAGCCTGCAT-
CONH-hexyl-OH: 3611.0 ([M]; calc. for C_{127}H_{166}N_{53}O_{60}P_{7}: 3611.4)

The resulting supports were treated with 1ml of concentrated ammonia (overnight, 55ºC). Supports carrying oligonucleotides and PNA-DNA chimeras with a thiol group at the 5’-end were treated overnight with 1ml of 50 mM dithio-DL-threitol (DTT) in concentrated ammonia at 55ºC. The excess of DTT was eliminated with a Sephadex G-25 column (NAP-10, Pharmacia, Sweden) just prior to conjugation with gold nanoparticles.

Dithiol linkages in thiolated oligonucleotide solutions were cleaved prior to use by adding an appropriate amount of 0.1 M solution of dithiothreitol (DTT) in 0.17 M sodium phosphate buffer (pH 8). Typically 20 µL of DTT solution was added to 100 µL of a 100 pmol/mL solution of the oligonucleotide. The solution was allowed to stand at room temperature for 0.5 h. The thiolated oligonucleotides were then desalted on a NAP-10 (Pharmacia) column using a 10 mM phosphate buffer (pH 7) as solvent. The optical absorbance at 260 nm of the purified oligonucleotide was used in conjunction with the known extinction co-efficient of the oligonucleotide to determine the oligonucleotide concentration according to Beer’s Law. Oligonucleotides were prepared for hybridisation experiments by dissolving them at appropriate concentration in buffer solution [0.3 M NaCl, 10 mM phosphate buffer, pH 7].

*Preparation of oligonucleotide-gold conjugates.* Solutions of Au nanoparticles and oligonucleotides were mixed in appropriate amounts [29], i.e., 2.63 nmoles of oligonucleotide was used per mL of gold nanoparticles (the concentration of
nanoparticles was 15.66 nM). The entire solution was then brought to 10 mM sodium phosphate buffer (pH 7). The amounts of reagents used in each conjugate synthesis included a 1.5 molar excess of oligonucleotide. After 24 hours, solutions were brought to 0.1 M NaCl concentration, by addition of the appropriate amount of a 1 M NaCl, 10 mM sodium phosphate buffer solution (pH 7) and allowed to stand at room temperature for a further 40 hours. After this time, solutions were centrifuged at 13,200 rpm for 30 min. The supernatant was removed and the reddish solid at the bottom of the tubes were dispersed in 0.1 M NaCl, 10 mM sodium phosphate buffer (pH 7) solution (the volume added was similar to that removed). This procedure was repeated and the reddish solid at the bottom of the centrifuge tubes were dispersed in 0.3 M NaCl, 10 mM sodium phosphate buffer (pH 7). Solutions were analyzed by UV-visible spectroscopy and absorbances at 260 nm and 520nm were recorded. The concentration of the conjugate solutions were determined using the absorbance of the solution at 520 nm and Beer’s Law [29]. Solutions were stored at room temperature. Hybridization experiments were carried out using 5 pmol amounts of each oligonucleotide-nanoparticle conjugate or chimera-nanoparticle conjugate (i.e., 0.32 nmoles of attached oligonucleotides) [30] in 0.3 M NaCl, 10 mM phosphate buffer (pH 7) in a total reaction volume of 1 mL. The solutions were heated to 90 ºC for 5 min, slowly cooled to room temperature, and then left to hybridize for 24 h.

*Hybridization experiments.* Oligonucleotide complexes were prepared by mixing 1 nmol of each strand in a total reaction volume of 1 mL. The solutions were then heated to 90 ºC for 5 min, slowly cooled to room temperature and left to hybridize for 24 h.
Oligonucleotide-nanoparticle complexes were prepared by mixing 5 pmol of each oligonucleotide-nanoparticle conjugate and chimera-nanoparticle conjugate in 0.3 M NaCl, 10 mM sodium phosphate buffer, pH 7, in a total reaction volume of 1 mL. Hybridizations were also conducted in buffers containing 50 mM and 100 mM NaCl. The solutions were then heated to 90 °C for 5 min, allowed to cool to room temperature and left to hybridize for 24 hours.

_Melting experiments._ Optical melting curves were collected on a Agilent 8453 spectrophotometer equipped with an Agilent temperature controlling Peltier unit. The prehybridized solution was transferred to a stoppered 1-cm path-length cuvette and UV-visible optical absorption spectra were recorded at 2 °C intervals, with a 1-2 minute heating time and a hold time of 1 minute at each temperature interval, while the sample was heated from 20 - 90 °C. The data were collected as wavelength versus absorbance spectra from which plots of absorbance versus temperature at 260 nm were extracted. The melting temperature of each of the systems was determined from these graphs.

REFERENCES


Table 1: Peptide nucleic acids, PNA-DNA chimeras and oligonucleotide sequences prepared in this work. Peptide nucleic acids are shown in italics. Ac: acetyl, Cys: cysteine, Glu: glutamic acid, Gly: glycine. Residues involved in mismatch are shown in bold.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Sequence 5’-&gt;3’ or N-&gt;C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, PNA</td>
<td>H-Cys-ATGCTCAACTCT-CONH₂</td>
</tr>
<tr>
<td>2, PNA</td>
<td>Ac-Cys-ATGCTCAACTCT-GluGluGluGly-CONH₂</td>
</tr>
<tr>
<td>3, PNA-DNA</td>
<td>Thiol-hexyl-TCGACTATGCA7-CONH-hexyl-OH</td>
</tr>
<tr>
<td>4, PNA-DNA MM</td>
<td>Thiol-hexyl-TCGAGTATGCA7-CONH-hexyl-OH</td>
</tr>
<tr>
<td>5, DNA</td>
<td>Thiol-hexyl-TCGACTATGCA7</td>
</tr>
<tr>
<td>6, DNA COMP</td>
<td>Thiol-hexyl-ATGCATAGTCGA</td>
</tr>
<tr>
<td>7, DNA MM1</td>
<td>Thiol-hexyl-ATCCATAGTCGA</td>
</tr>
<tr>
<td>8, DNA MM2</td>
<td>Thiol-hexyl-ATGCATACTCGA</td>
</tr>
<tr>
<td>9, DNA control</td>
<td>Thiol-hexyl-GCTGTAAGTA</td>
</tr>
<tr>
<td>10, COMP TO 1</td>
<td>Thiol-hexyl-AGAGTTGAGCAT</td>
</tr>
</tbody>
</table>
Table 2: Melting characteristics of duplexes linked to gold nanoparticles. Experimental conditions: 0.3 M NaCl and 10 mM sodium phosphate pH 7.

<table>
<thead>
<tr>
<th>Sequences</th>
<th>Type of duplex</th>
<th>Duplex</th>
<th>Tm(°C), hyperchrom.</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 + 6</td>
<td>DNA-DNA perfect match</td>
<td>TCGACTATGCATAGCTGATACGTA</td>
<td>45 °C, 50%</td>
</tr>
<tr>
<td>3 + 6</td>
<td>DNA-CHIM perfect match</td>
<td>TCGACTATGCATAGCTGATACGTA</td>
<td>40 °C, 60%</td>
</tr>
<tr>
<td>4 + 8</td>
<td>DNA-CHIM perfect match</td>
<td>TCGAGTATGCATAGCTCATAAGCGTA</td>
<td>38 °C, 60%</td>
</tr>
<tr>
<td>4 + 6</td>
<td>DNA-CHIM mismatch in DNA</td>
<td>TCGAGTATGCATAGCTCATAAGCGTA</td>
<td>No transition</td>
</tr>
<tr>
<td>3 + 8</td>
<td>DNA-CHIM mismatch in DNA</td>
<td>TCGACTATGCATAGCTCATAAGCGTA</td>
<td>No transition</td>
</tr>
<tr>
<td>3 + 7</td>
<td>DNA-CHIM mismatch in PNA</td>
<td>TCGACTATGCATAGCTGATACCTA</td>
<td>35 °C, 18%</td>
</tr>
<tr>
<td>5 + 7</td>
<td>DNA-DNA mismatch</td>
<td>TCGACTATGCATAGCTGATACCTA</td>
<td>35 °C, 18%</td>
</tr>
<tr>
<td>3 + 9</td>
<td>Control no complementary</td>
<td>TCGACTATGCATGCTGACAAAGTA</td>
<td>No transition</td>
</tr>
<tr>
<td>4 + 9</td>
<td>Control no complementary</td>
<td>TCGAGTATGCATGCTGACAAAGTA</td>
<td>No transition</td>
</tr>
<tr>
<td>5 + 9</td>
<td>Control no complementary</td>
<td>TCGACTATGCATGCTGACAAAGTA</td>
<td>No transition</td>
</tr>
</tbody>
</table>
FIGURE LEGENDS

Figure 1: Melting profiles at 260 nm of duplexes formed by a PNA-DNA chimera and the complementary DNA sequence. Duplexes are formed by mixing chimera-nanoparticle conjugate 3 with complementary oligonucleotide-nanoparticle conjugate 6 (blue line) and chimera 3 with oligonucleotide 6 (red line).

Figure 2: Effect of the mismatch on the melting profiles at 260 nm of duplexes formed by PNA-DNA chimeras and their complementary DNA sequences. Both conjugates to Au nanoparticles. A) DNA/DNA perfect match (5-6). B) DNA/Chimera perfect match (4-8). C) DNA/Chimera mismatch in DNA part (3-8). D) DNA/Chimera mismatch in PNA part (3-7). Conditions: 0.3 M NaCl and 10 mM sodium phosphate pH 7.
Figure 1