Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Gold Nanoprobes for Detecting DNA Adducts

*Ioannis A. Trantakis, Shana J. Sturla**

[1] ETH Zurich, Institute of Food, Nutrition and Health, Department of Health Sciences and Technology, Schmelzbergstrasse 9, 8092 Zurich, Switzerland.

*Corresponding author information:

Email: sturlas@ethz.ch

Telephone: +41 44 6329175

Table of contents

Experimentals Details

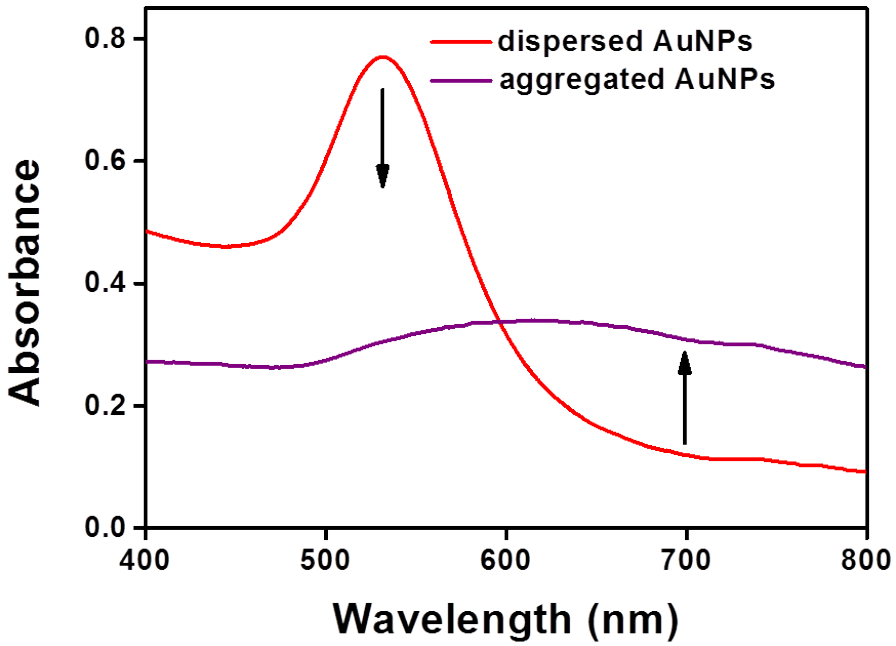
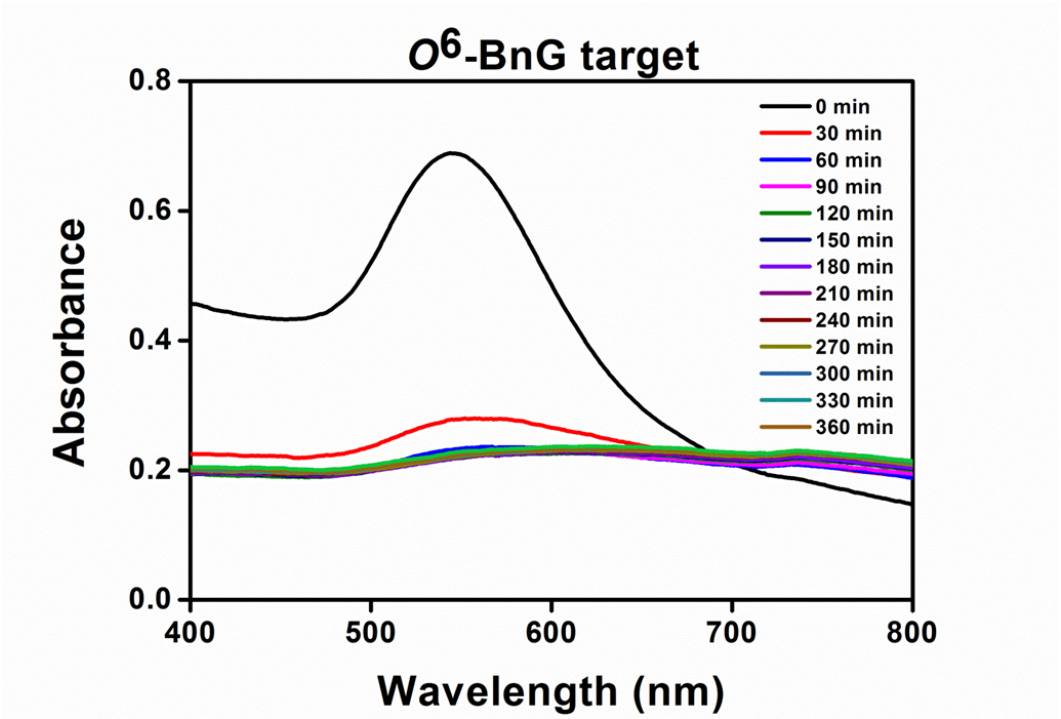


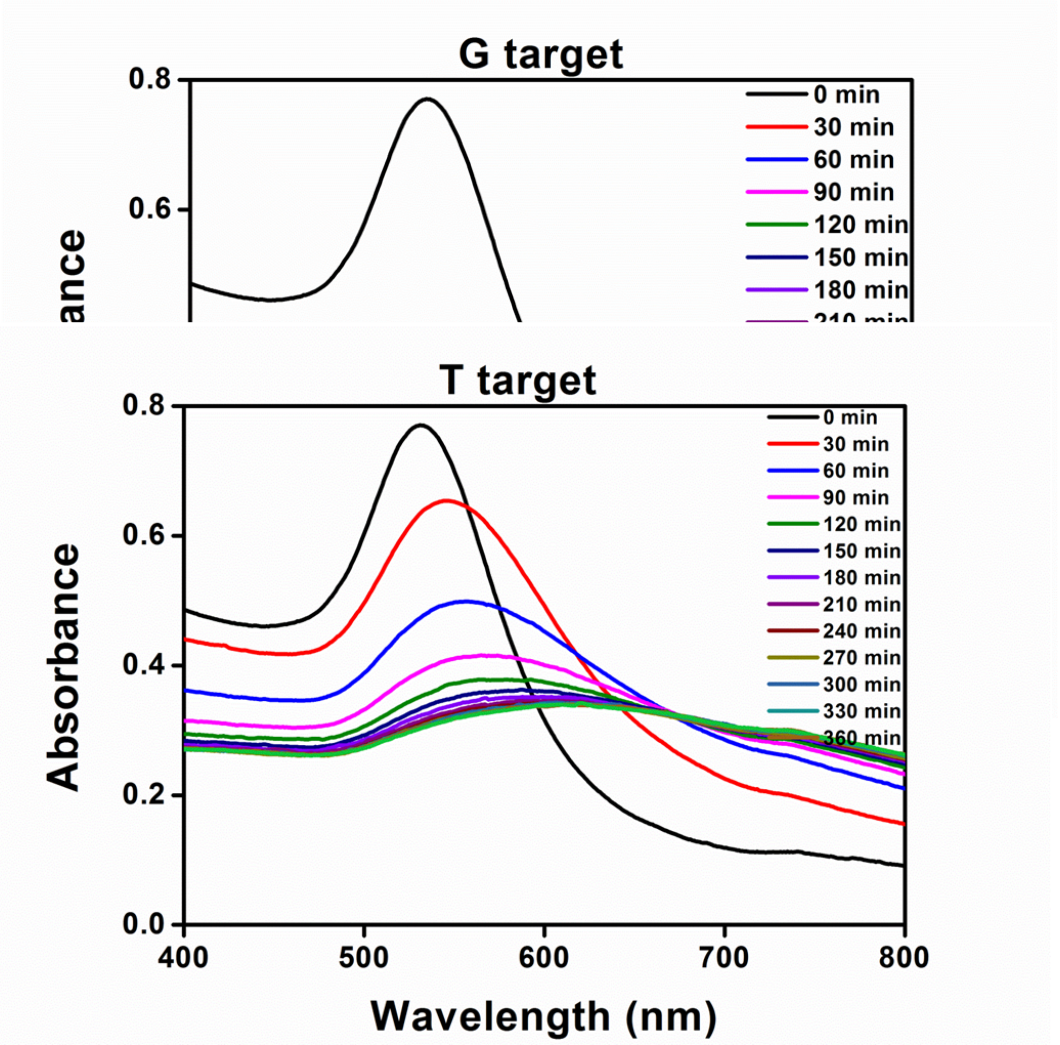
Fig. S1 Comparison of UV-Vis spectra of a mixture of AuNP probes before and after treatment with a complementary target oligonucleotide.

A

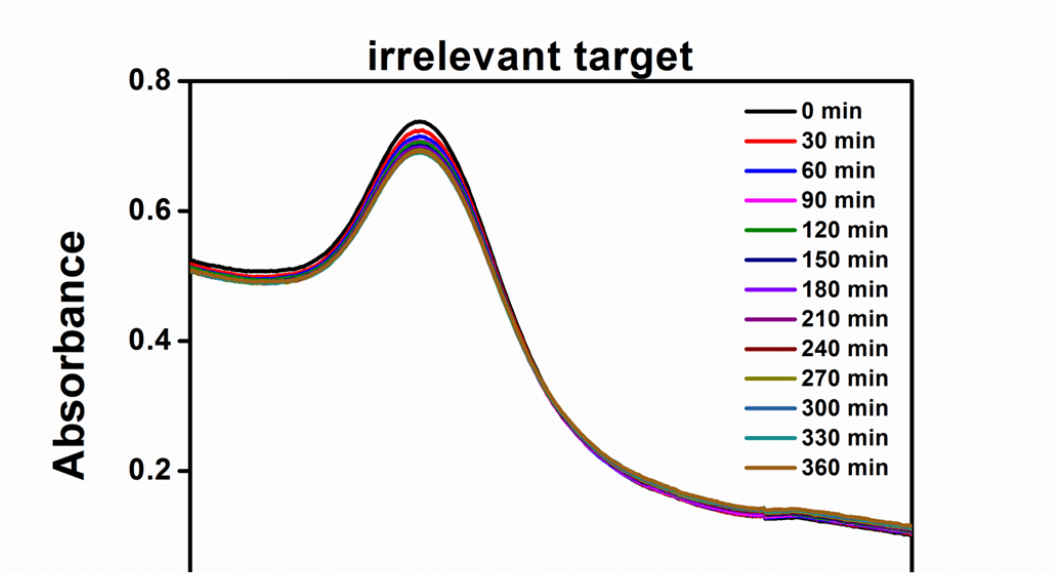

O6-BnG target
Absorbance
Wavelength (nm)
0 min
30 min
60 min
90 min
120 min
150 min
180 min
210 min
240 min
270 min
300 min
330 min
360 min

B

C


G target
T target
Absorbance
Wavelength (nm)
0 min
30 min
60 min
90 min
120 min
150 min
180 min
210 min
240 min
270 min
300 min
330 min
360 min

D


irrelevant target
Absorbance
0.8
0.6
0.4
0.2
0 min
30 min
60 min
90 min
120 min
150 min
180 min
210 min
240 min
270 min
300 min
330 min
360 min

E

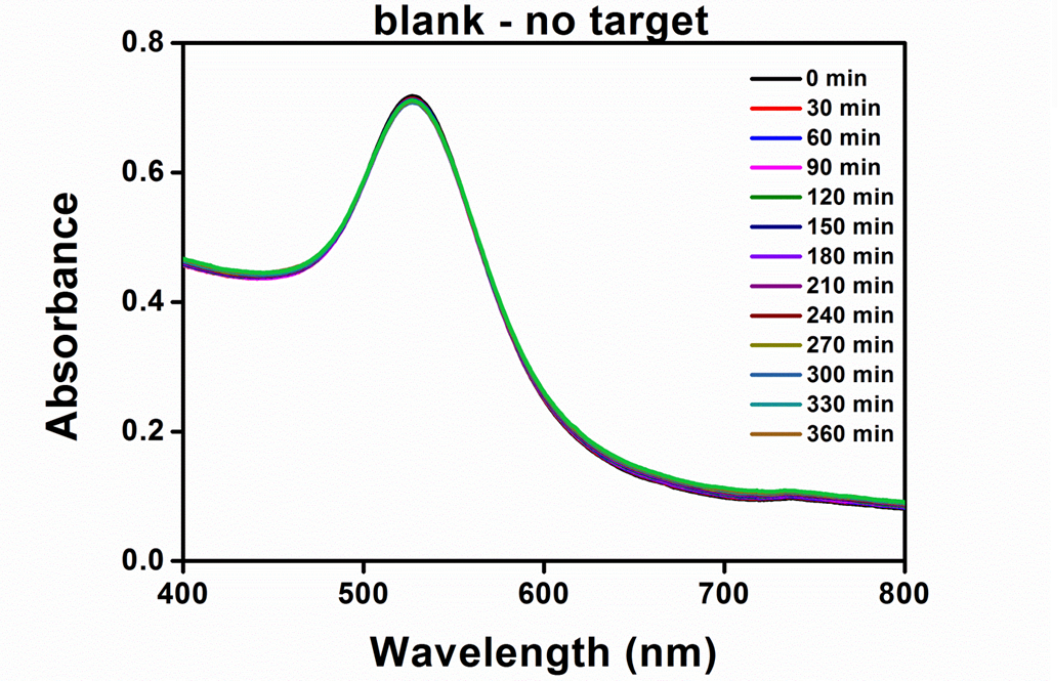

blank - no target
Absorbance
0.8
0.6
0.4
0.2
0.0
400
500
600
700
800
Wavelength (nm)
0 min
30 min
60 min
90 min
120 min
150 min
180 min
210 min
240 min
270 min
300 min
330 min
360 min

Fig. S2 Time-dependent UV-Vis spectra of the nanoparticle suspensions in the presence of 100 nM of different target oligonucleotides: (A) O^6-BnG target (B) G target. (C) T target (D) Irrelevant target. (E) No target.

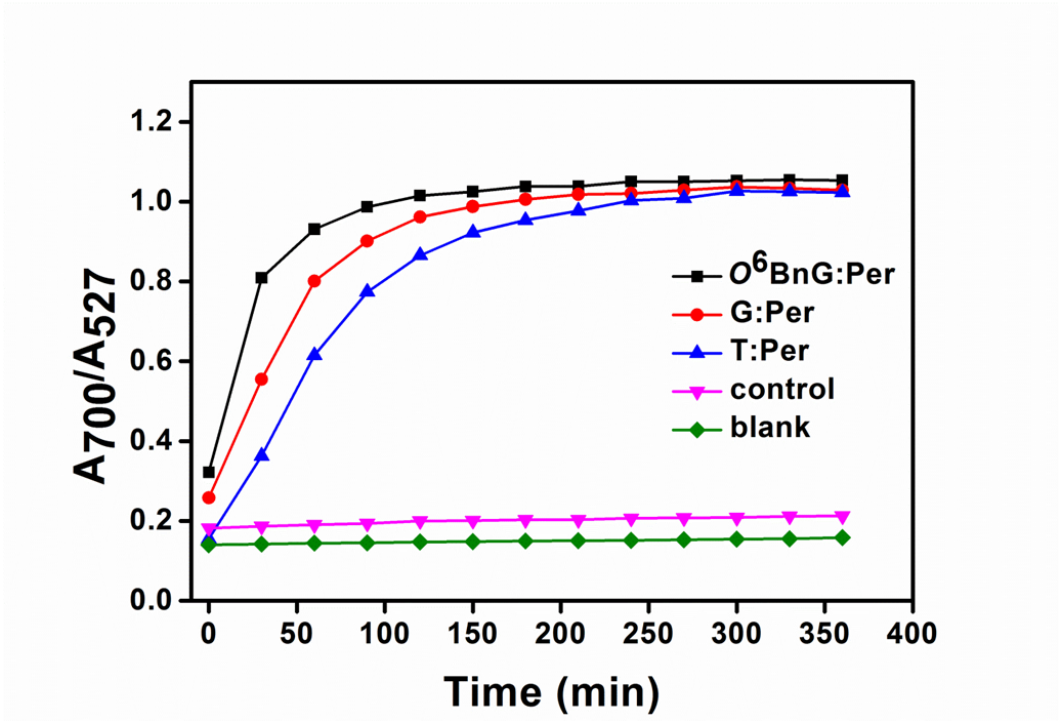


Fig. S3 Dependence of absorbance ratios at 700 nm to 527 nm as a function of time in the presence of 100 nM of a 13-mer target oligonucleotide containing either O^6-BnG, G or T in its middle position. An oligonucleotide with the same length but a random sequence was used as a negative control. A control experiment where the target was omitted was also performed (blank).

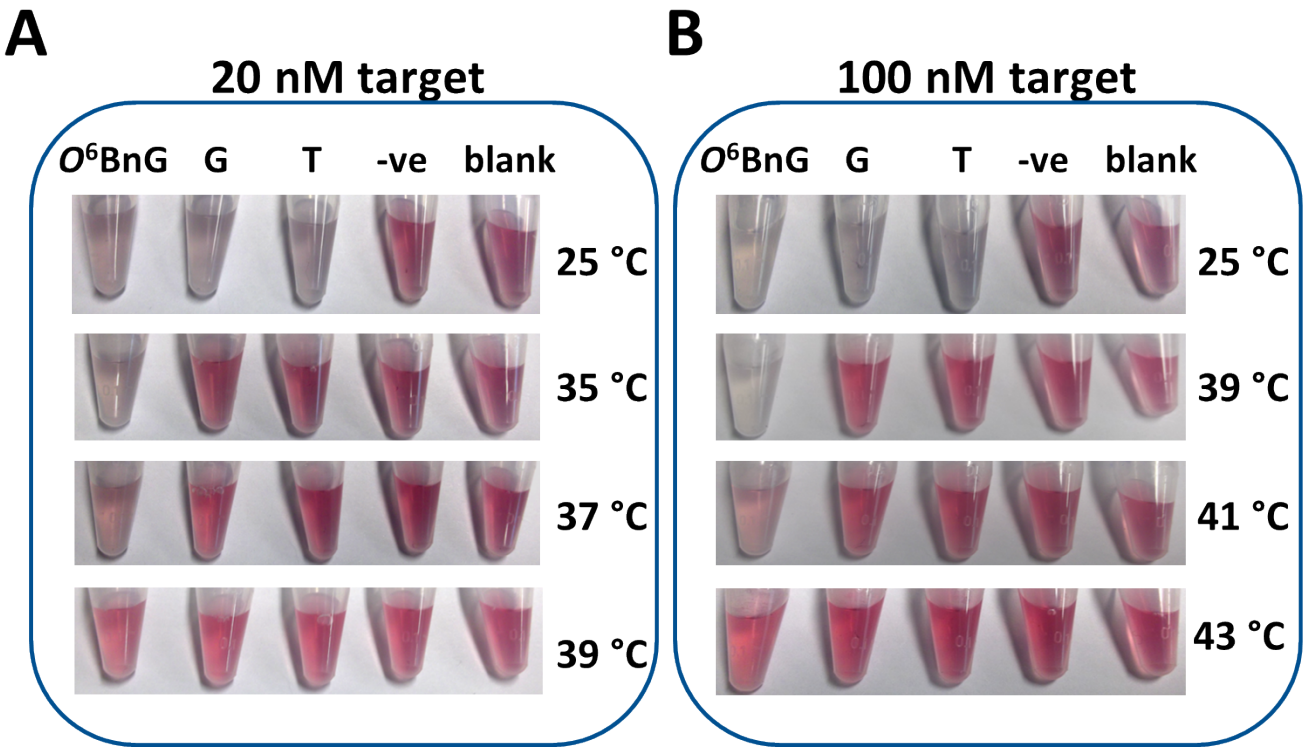


Fig. S4 Color changes induced by heating sample solutions at different temperatures for 5 min. Samples contained A) 20 nM or B) 100 nM of O^6-BnG, G, and T 13-mer target oligonucleotides. Samples containing a 13-mer with random sequence (-ve) or no target (blank) were also tested.

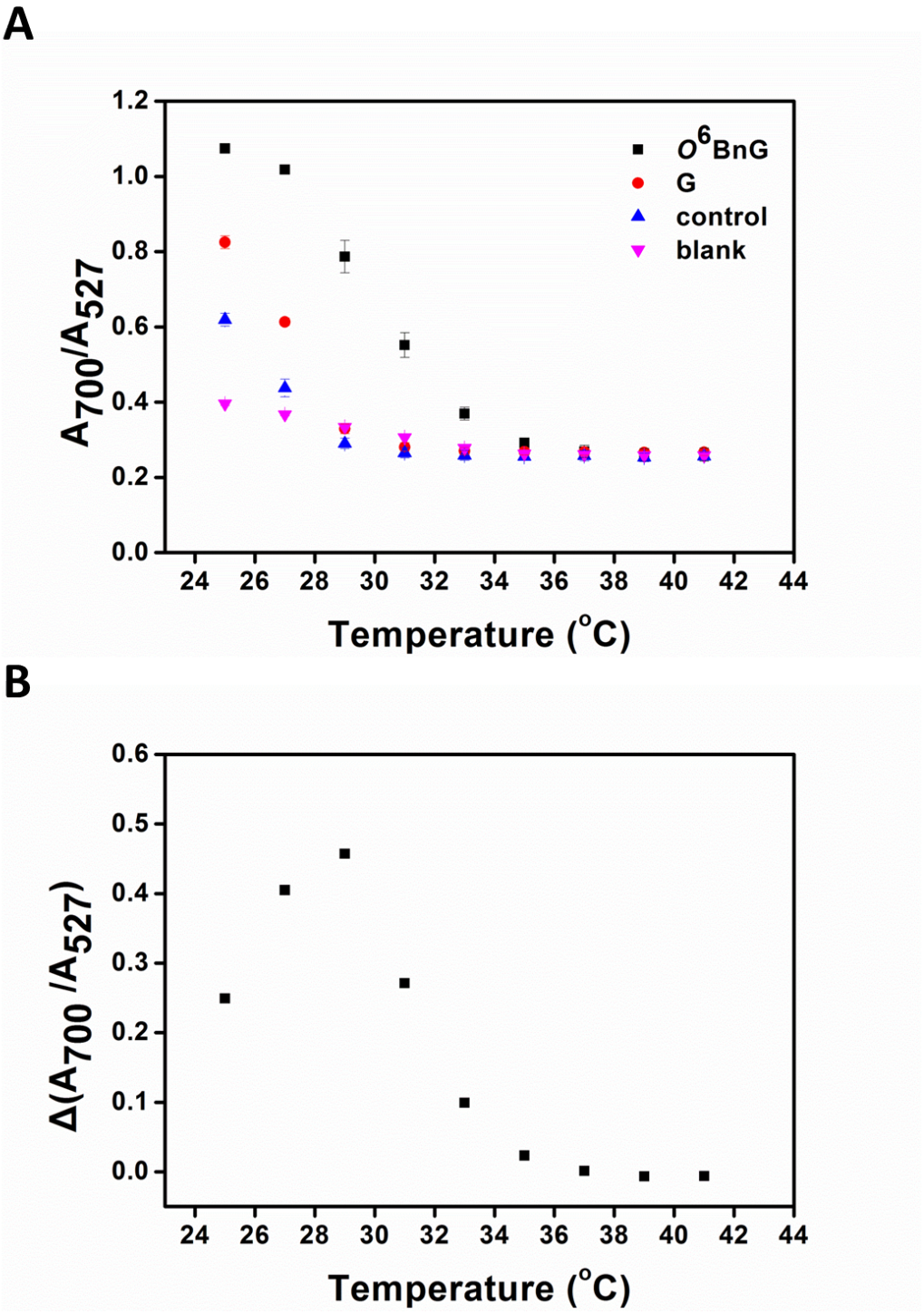


Fig. S5. A) Dependence of absorbance ratios at 700 nm to 527 nm on temperature for aggregates formed from target mixture (20 nM of each G,T and irrelevant target) supplemented with either the O^6-BnG (20 nM final concentration) or G containing target (40 nM final concentration). Aggregates formed from the initial mixture (20 nM of each G,T and irrelevant target) served as a control. Mixtures of only the AuNP probes served as a negative control for aggregation (blank). Values are mean±SD from three independent experiments. B) Differential absorbance ratios as a function of temperature between aggregates formed from target mixture (20 nM of each G,T and irrelevant target) supplemented with the O^6-BnG-containing target (20 nM final concentration) and aggregates formed from target mixture (20 nM of each G,T and irrelevant target) supplemented with the G-containing target (40 nM final concentration).

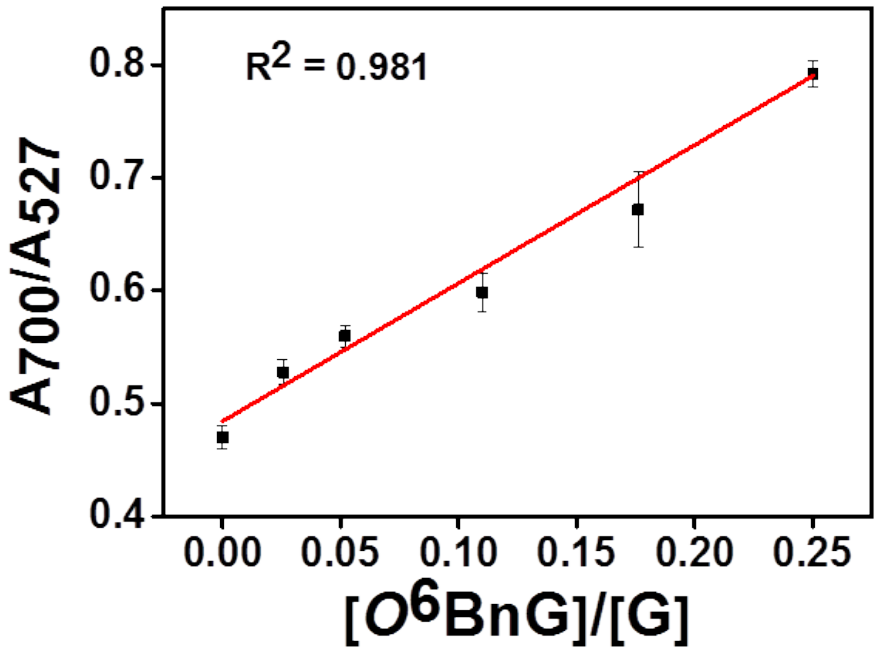


Fig. S6 Linear plot of absorbance ratios (700/527 nm) versus relative *O*^{6}-BnG target concentration ([*O*^{6}-BnG]/[G]). All absorbance measurements were performed upon heating to 35 °C for 5 min. Values are mean±SD from three independent experiments.

Table S1. Oligonucleotide sequences used in this study.[a]

Name	Sequence
3'-thiol oligo	5 '- HS - $(A)_{10}$ AGC TTC - 3'
5'-thiol oligo	5' - PACCCAC$(A)_{10}$ - SH - 3'
O^6-BnG target	5' - GTGGGTXGAAGCT - 3'
G target	5' - GTGGGTGGAAGCT - 3'
T target	5' - GTGGGTTGAAGCT - 3'
Irrelevant target	5' - CCCATCCACCCAC - 3'
Probe oligo 1	5' - AGCTTCPACCCAC - 3'
Probe oligo 2	5' - AGCTTCCACCCAC - 3'
O^6-BnG target_2	5' - GTGGGTGXAAGCT - 3'
O^6-BnG target_3	5' - GTGGXTGGAAGCT - 3'

[a] Underlined bases denote the recognition sequences. P = Per, X = *O*^{6}-BnG.

Table S2. Melting temperatures (T_m) of the AuNP aggregates.[a]

	20 nM target		100 nM target	
Base pairs	T_m (°C)	ΔT_m[b]	T_m (°C)	ΔT_m[b]
O^6-BnG: Per	37.0± 0.6	+7.8± 0.8	39.6±0.7	+6.3± 1.0
G:Per	29.2±0.5	--	33.3 ±0.7	--
T:Per	28.9±0.1	-0.3±0.6	32.7±0.1	-0.6±0.7

[a] Each measurement was performed in triplicate.

[b] T_m=T_m(O^6-BnG:Per) or T_m(T:Per) - T_m (G:Per).

Table S3. Melting temperatures (T_m) of the DNA duplexes formed between probe and target oligos as a function of the identity of the base pair formed in the middle of the duplex.[a]

Base pairs	T_m (°C)	Base pairs	T_m (°C)
G:C	62.0±0.3	G:Per	55.1±0.7
T:C	47.0±0.4	T:Per	53.3 ±0.7
O^6-BnG:C	51.0±0.6	O^6-BnG:Per	60.1±0.7

[a] Each measurement was performed in triplicate.

Table S4. Melting temperatures (T_m) of the DNA duplexes formed between probe and target oligos as a function of the position of O^6-BnG.

Oligodeoxyribonucleotides Sequence[a]	**T_m (°C)**	**Oligodeoxyribonucleotides Sequence**[a]	**T_m (°C)**
5'-GTGGGT**X**GAAGCT-3’	51.0±0.6	5'-GTGGGT**X**GAAGCT-3’	60.1±0.7
3’-CACCCACCTTCGA-5’		3’-CACCCA**P**CTTCGA-5’	
5'-GTGGGTG**X**AAGCT-3’	53.6±0.5	5'-GTGGGTG**X**AAGCT-3’	50.6±1.3
3’-CACCCACCTTCGA-5’		3’-CACCCA**P**CTTCGA-5’	
5'-GTGG**X**TGGAAGCT-3’	49.1±1.0	5'-GTGG**X**TGGAAGCT-3’	38.9±0.8
3’-CACCCACCTTCGA-5’		3’-CACCCA**P**CTTCGA-5’	

[a] **X**= O^6-BnG; **P**= Per

[b] Each measurement was performed in triplicate.

Table S5: Mixtures of O^6-BnG and G targets at various ratios that were supplemented to the initial target mixture (10 pmol each of G, T and irrelevant targets) and the corresponding relative concentrations.

Ratio	G added (pmol)	O^6BnG added (pmol)	Total G (pmol)	Total DNA (pmol)	[O^6BnG]/[G]	[O^6BnG]/[total DNA]
A	6	4	16	40	0.25	0.1
B	7	3	17	40	0.176	0.075
C	8	2	18	40	0.11	0.05
D	9	1	19	40	0.052	0.025
E	9.5	0.5	19.5	40	0.0256	0.0125
F	10	0	20	40	0	0

Table S6: Mass spectrometric characterization of modified oligonucleotides.

Oligodeoxyribonucleotide Sequence[a]	Mol. Wt. (Calculated)	Mol. Wt. (Observed)
5'-GTGGGT**X**GAAGCT-3'	4159.6	4157.2
5'-AGCTTC**P**ACCCAC-3'	3911.5	3909.4
5'-**P**CT TCG $A(A)_{10}$-SH-3'	5412.2	5410.8

[a] **X**= O^6-BnG; **P**= Per

Materials

Unless otherwise specified, chemicals and reagents were purchased from Sigma-Aldrich (St Louis, MO, USA and Switzerland) and VWR Scientific (Switzerland), and used without further purification. Reagents for solid-phase DNA synthesis were obtained from Glen Research Co. (Sterling, VA, USA) and Link technologies Ltd. (Lanarkshire, Scotland). O^6-BndG and dPer were synthesized by previously reported methods,[1] and were converted into the corresponding 5'-O-dimethoxytrityl- protected phosphoramidites by previously reported procedures.[2] 5'-*O*-dimethoxytrityl protected phosphoramidites of natural bases

were purchased from Glen Research Co. (Sterling, VA, USA) and Link technologies Ltd. (Lanarkshire, Scotland). 2'-deoxyguanosine was purchased from TCI America (Portland, OR). The thiol-modifier C3 S-S CPG solid support was obtained from Link technologies Ltd. (Lanarkshire, Scotland). The 5'-thiol-capped oligonucleotide and all unmodified oligonucleotides were purchased from VBC-Biotech (Vienna, Austria). The oligonucleotides O^6-BnG target_2 and O^6-BnG target_3 were purchased from AZCO Biotech (Oceanside, CA, USA). The gold nanoparticles (20 nm, 7×10^{11} particles/ml) were obtained from British Biocell International (Cardiff, U.K.). Chromatography was carried out with silica gel 60 F254 plates with glass or aluminum backing. Column chromatography was performed with silica gel (32-63 μm). High performance liquid chromatography (HPLC) was performed with an Agilent 1200 HPLC instrument equipped with Agilent 1200 series fraction collector. Mass spectrometry of oligodeoxynucleotides was carried out with Thermo LTQ Velos mass spectrometer with electrospray ionization. UV-Vis absorption measurements were made with a Cary 100 UV-Vis spectrophotometer equipped with a Peltier temperature controller. Absorbance measurements were made with a Tecan Infinite M200 PRO plate reader equipped with temperature controller. Gold nanoparticle solutions were heated in an AccuBlock digital dry bath from Labnet (Edison, NJ, USA).

Synthesis of modified oligonucleotides

Oligonucleotides were synthesized on a Mermade 4 DNA synthesizer (Bioautomation Corporation) with standard β-cyanoethyl phosphoramidite chemistry. Stepwise coupling yields for the synthesized base were greater than 85%, as determined by the trityl cation absorbance. Cleavage from the controlled pore glass (CPG) solid support and final deprotection were achieved by treatment with 30% ammonium hydroxide at 55 °C for 16 h. Oligonucleotides were synthesized in trityl-off mode on the 5'-end. Crude oligonucleotides were purified by reversed phase HPLC (Agilent 1200) on a Luna 5μm C18 100 Å 250 mm x 10 mm column (Phenomenex). The chromatography mobile phases were solvent A (15 mM aqueous $(NH_4)_2CO_3$ (pH 7.0)) and solvent B (acetonitrile). The solvent gradient started with 95% solvent A, increased linearly to 95% solvent B over 20 minutes, and was held at 95% solvent B for another 20

min. Fractions corresponding to the oligodeoxynucleotide peak were collected, lyophilized, and characterized by ESI-mass spectrometry using negative mode ionization by direct infusion (Table S1). The resulting oligodeoxynucleotides (>95% purity) were stored at -20 °C and allowed to thaw prior to use.

Synthesis, purification and characterization of 3'-alkanethiol modified oligonucleotide

The 3'-alkanethiol 17-mer oligonucleotide (Probe 1) was synthesized on a 1 μmol scale using standard phosphoramidite chemistry with a Thiol-Modifier C3 S-S CPG solid support. The final dimethoxytrityl (DMT) protecting group was removed. After synthesis, the supported oligonucleotide was placed in 1 ml of concentrated ammonium hydroxide for 16 h at 55 °C to cleave the oligonucleotide from the solid support and remove the protecting groups from the bases. Cleavage from the solid support via the succinyl ester linkage produced a mixed disulfide composed of the (mercaptopropyl)-oligonucleotide and a mercaptopropanol linker. After evaporation of the ammonia, the modified oligodeoxynucleotide was purified by semi-preparative reverse-phase HPLC using a Phenomenex Luna RP column (5μm C18 (2) 100 Å 250 mm x 10 mm). The chromatography mobile phases were solvent A: 0.03 M triethylammonium acetate (TEAA), pH 7.0 and solvent B: Acetonitrile. The solvent gradient started with 95% Solvent A, increased linearly to 65% solvent B over 30 minutes, and was held at 65% B for another 5 min. The gradient was then increased to 95% solvent B in 5 minutes and held there for another 5 minutes. The UV signal was monitored at 254 nm. The retention time of the modified 17-mer oligodeoxynucleotide was 25 min. The fractions were collected and lyophilized. After evaporation of the solvent by lyophilization, the oligodeoxynucleotide was redispersed in 400 μl of a 0.1 M DTT, 0.17 M phosphate buffer (pH 8) solution at room temperature (25 °C) for 2 h to cleave the 3'-mixed disulfide. Purity of the product was assessed by injecting 100 μL aliquots to perform semi-preparative reverse-phase HPLC as described above. Two major peaks with retention times (R_t) of 20.8 and 24.4 min were observed. The fractions were collected, lyophilized, and characterized by ESI-mass spectrometry using negative mode ionization by direct infusion (Supplementary Table S1). The single peak at 24.4 min has been attributed to the 3'-alkanethiol

17-mer oligodeoxynucleotide. The second peak at 20.8 min has been attributed to a disulfide formed from two (mercaptopropyl) oligodeoxynucleotides.

Melting analyses of DNA duplexes

Thermal denaturation experiments were carried out in Teflon-stoppered 1 cm path length cuvettes. Duplex solutions (2.2 μM in each oligonucleotide component) contained 0.3 M NaCl and 10 mM phosphate buffer (pH 7.4). Duplex solutions were annealed by heating in the sample holder of the UV-Vis spectrophotometer from 25 to 90 °C, at a rate of 10 °C / min, and cooling to 20 °C at a rate of 1 °C / min. UV absorbance was monitored at 260 nm at 1 min intervals while the temperature was raised from 20 to 80 °C at a rate of 1 °C / min with a holding time of 1 min / deg. Melting temperatures (T_m) were determined by the derivative method with the Cary thermal application software.

Functionalization of Au nanoparticles

AuNPs (d=20 nm, 1.16 nM) were functionalized by adding either 3'- or 5'-thiol-capped oligonucleotides to the colloidal solutions (2 μM final oligonucleotide concentration). After 16 h, phosphate buffer (0.1 M, KH_2PO_4/Na_2HPO_4, pH 7.4) was added to the colloidal solution to achieve a final concentration of 10 mM of phosphate (KH_2PO_4/Na_2HPO_4) buffer pH 7.4. In a subsequent step-wise salt-aging process, NaCl solution (2.0 M) was added to the colloids to adjust the final NaCl concentration to 0.3 M (4 steps to achieve NaCl concentrations 0.05 M, 0.1 M, 0.2 M, and 0.3 M, with a 6 h waiting period between each addition).[3] To remove unbound DNA, solutions were centrifuged (25 min at 12 000 rpm) in 1.5 ml Eppendorf tubes (Fisher Scientific), and the supernatant was removed to yield an oily precipitate. This precipitate was washed with phosphate buffer solution (0.3 M NaCl, 0.01% Tween-20, 10 mM phosphate, pH 7.4), centrifuged (25 min at 12 000 rpm), and re-dispersed in the same buffer. This washing procedure was repeated twice whereupon the colloid was re-suspended in a phosphate buffer solution (0.3 M NaCl, 0.01% Tween-20, 0.01% NaN_3, 10 mM phosphate, pH 7.4) and stored at 4 °C. The concentration of the

resuspended AuNPs was estimated by UV/Vis spectroscopy and Beer-Lambert law using extinction coefficient, 9.21×10^8 at 524 nm.

Aggregation of functionalized Au nanoparticles

AuNP / target oligonucleotide solutions were prepared by mixing 350 µl of each type of AuNP (functionalized with either the Probe 1 or Probe 2 thiol-capped oligonucleotides, Table 1) and adding 14 or 70 pmol of the different target oligonucleotides (Table 1) leading to a final target concentration of 20 or 100 nM, respectively. The solutions were then placed in Teflon-stoppered 1 cm path length micro cuvettes. The solutions were heated in the sample holder of the UV-Vis spectrophotometer to 70 °C for 10 min to anneal the oligonucleotides. The solutions were then allowed to cool to 25 °C and the hybridization process was monitored by recording the UV/Vis spectra (400 to 800 nm) of the solutions for 6 h. During the hybridization, the mixtures were stirred homogeneously by a magnetic stir bar.

Melting analyses of AuNP aggregates

Thermal denaturation experiments were carried out in Teflon-stoppered 1 cm path length micro cuvettes. Absorbance was monitored at 527 nm at 1 min intervals while the temperature was raised from 20 to 80 °C at a rate of 1 °C/min with a holding time of 1 min/deg. Melting temperatures (T_m) were determined by the derivative method with the Cary thermal application software. During the hybridization, the mixtures were stirred homogeneously by a magnetic stir bar.

Visual detection of DNA adducts

Aggregated AuNPs solutions (500 µl) were put in Eppendorf tubes and vortexed to form homogeneous dispersions. The tubes were placed in a digital dry bath and heated for 5 min to different temperatures. Photos of the solutions were taken using a digital camera.

Specificity tests (100 nM of O^6-BnG target)

Three solutions were prepared for this test. 50 µl of each AuNP probe (1 nM each) were mixed with 10 pmol of each of the G, T and irrelevant target (100 nM each, final concentration). To one of the solutions 10 pmol of the O^6-BnG-containing target was added (100 nM final concentration), and to another one 10 pmol of the G-containing target (200 nM final concentration). The third solution, where no extra target was added, served as a control. A mixture of the two AuNP probes where no target was added served as a negative control for aggregation. All the mixtures were then vortexed and heated to 70 °C for 10 min to anneal the oligonucleotides. The solutions were then allowed to cool to 25 °C. After 5 h when full aggregation had occured the solutions were vortexed to disperse the sedimented aggregates. 100 µl of the dispersed solutions were then transferred to a 96-well microplate. The plate was then incubated for 5 min to different temperatures under constant shaking in a Tecan M200 Pro plate reader. The UV/Vis spectra (400 to 800 nm) of the solutions were then recorded.

Specificity tests (20 nM of O^6-BnG target)

Three solutions were prepared for this test. 50 µl of each AuNP probe (1 nM each) were mixed with 2 pmol of each of the G, T and irrelevant target (20 nM each, final concentration). To one of the solutions 2 pmol of the O^6-BnG-containing target was added (20 nM final concentration), and to another one 2 pmol of the G-containing target (40 nM final concentration). The third solution, where no extra target was added, served as a control. A mixture of the two AuNP probes where no target was added served as a negative control for aggregation. All the mixtures were then vortexed and heated to 70 °C for 10 min to anneal the oligonucleotides. The solutions were then allowed to cool to 25 °C. After 6 h when full aggregation had occured the solutions were vortexed to disperse the sedimented aggregates. The dispersed solutions (100 µl) were then transferred to a 96-well microplate. The plate was then incubated for 5 min to different temperatures under constant shaking in a Tecan M200 Pro plate reader. The absorbance ratio (A_{700}/A_{527}) of the solutions was then recorded.

Characterization of sensitivity

Solutions of aggregates were formed by mixing 50 µl of each AuNP probe (1 nM each) with a mixture of the G, T and irrelevant target (100 nM each, final concentration). The nanoprobe - target mixtures were then further supplemented with the O^6-BnG- and G-containing targets at various ratios as detailed in Supplementary Table S2, in the range 0 to 10 %. All the mixtures were then vortexed and heated to 70 °C for 10 min to anneal the oligonucleotides. The solutions were then allowed to cool to 25 °C. After 6 h when full aggregation had occurred the solutions were vortexed to disperse the sedimented aggregates. The dispersed solutions (100 µl) were then transferred to a 96-well microplate. The plate was then incubated for 5 min to 35 °C under constant shaking in a Tecan M200 Pro plate reader. The absorbance ratio (A_{700}/A_{527}) of the solutions was then recorded.

References

(1) a) Gong, J. C.; Sturla, S. J. *J. Am. Chem. Soc.* **2007**, *129*, 4882. b) Mounetou, E.; Debiton, E.; Buchdahl, C.; Gardette, D.; Gramain, J. C.; Maurizis, J. C.; Veyre, A.; Madelmont, J. C. *J. Med. Chem.* **1997**, *40*, 2902.

(2) Wang, J.; Wang, Y. *Nucleic Acids Res* **2010**, *38*, 6774.

(3) Jin, R.; Wu, G.; Li, Z.; Mirkin, C. A. Schatz, G. C. *J. Am. Chem. Soc.* **2003**, *125*, 1643.