Electronic Supplementary Information

Colorimetric anticancer drug detection by gold nanoparticle-based DNA interstrand cross-linking

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Materials. DNA sequences were purchased from Sangon Biotech (Shanghai) Co., Ltd. PB buffer, Tris-HCl buffer, and sodium chloride solution were obtained from 1st Base Pte Ltd, Singapore. Compounds 8-methoxypsoralen, 2-methoxy-1-naphthaldehyde, and 3,4-dihydroxybenzhydrazide were purchased from Alfa Aesar. Chemicals 4,5',8-trimethylpsoralen and 5-methoxypsoralen were purchased from Tokyo Chemical Industry Co., Ltd. Unless otherwise noted, other chemicals were obtained from Sigma Aldrich.

Preparation of DNA-modified gold nanoparticles. Gold nanoparticles (~14 nm) were first prepared by the citrate reduction of $HAuCl_4$.[1] In a typical experiment, a 5-mL aqueous solution of sodium citrate (1 w %) was added to a boiling solution of $HAuCl_4$ (50 mL, 1 mM). After the solution color changed to red, the reaction mixture was allowed to reflux for 20 min. The resulting mixture containing ~10 nM of gold nanoparticles was then allowed to cool to room temperature and stored at 4 ^{o}C before use for subsequent reactions.

The terminal disulfide groups of the oligonucleotide strands (probe **A**: 5' HS-HS AAA TTT TCT GTA TAT AGA CAT AAA-3', probe **B**: 5' HS-HS AAA TTT ATG TCT ATA TAC AGA AAA-3', 6 nmol) were first cleaved by soaking them in a 0.1 M dithiotheritol phosphate buffer solution (pH = 8) for 2 h and subsequently purified on a NAP-5 column (GE Healthcare). The purified oligonucleotide was then added to 2.4 mL

of a stock solution of gold nanoparticles.[2] The resulting mixture was brought to 0.3 M NaCl, 10 mM Tris (pH = 7), followed by centrifugation to remove excess reagents. The nanoparticle probes were redispersed in a 50 mM NaCl, 10 mM Tris-HCl (pH = 7) buffer solution prior to use.

Anticancer drug detection by DNA-modified gold nanoparticles. In a typical procedure, two different types of 24-base DNA-modified gold nanoparticles (30 μL each) were added to a PCR tube and the concentration of NaCl was adjusted to 200 mM by using 5 M NaCl. After complete hybridization, 1 μL of anticancer drug in DMSO was added. The resulting mixture was incubated in dark for 1 h, followed by UV irradiation (3 h, 365 nm, 4 W). The colorimetric response of the solution was recorded after heating the reaction mixture at 90 ^{o}C.

Gel electrophoresis. The formation of DNA interstrand cross-links was analyzed by denaturing polyacrylamide gel electrophoresis (PAGE, 20%).[3] Typically, a 60 μL solution containing probe **A** and probe **B** (probe **A**: 5' HS-HS AAA TTT TCT GTA TAT AGA CAT AAA-3', probe **B**: 5' HS-HS AAA TTT ATG TCT ATA TAC AGA AAA-3', 10 pmol each) was first annealed in 200 mM NaCl and 10 mM Tris-HCl (pH = 7) buffer. The resulting mixture was incubated with anticancer drug in dark for 1 h followed by UV irradiation (3 h, 365 nm, 4 W). The mixture was then mixed with denaturing loading buffer (30 μL, 95% v/v formamide, 18 mM EDTA, 0.025% SDS, 10% w/w xylene FF and bromophenol blue) and heated at 90 ^{o}C for 3 min. Finally, the mixture was electrophoresed on a 20% denaturing PAGE gel plate using 1×TBE buffer as the electrolyte solution. The DNA bands were detected with a G:BOX (Imgen Technologies) by using SYBR Gold (Molecular Probes, Inc.) as the staining reagent.

DNA-modified gold nanoparticles were analyzed by agarose gel (1% w/v) in 1×TBE buffer. In a typical procedure, the reaction mixture was first heated at 90 ^{o}C and then loaded onto the agarose gel followed by electrophoresis at 100 V for 1 h.

Melting analysis. Thermal melting analysis was carried out to the reaction mixture using a SHIMADZU UV-2450 spectrophotometer equipped with a SHIMADZU

temperature controller. Each solution was diluted to 100 μL with a 50 mM NaCl, 10 mM Tris-HCl (pH = 7). UV–vis spectrum was subsequently recorded by monitoring the extinction at 520 nm for the dispersed particle probes, as the temperature was increased from 15 to 90 ^{o}C with a holding time of 1 min/deg.

Cell culture. Human Dermal Fibroblasts (HDF, Genlantis) were first seeded in a 96-well plate (Nunc) and cultured (5% CO_2, 37 ^{o}C) for 1 day in DMEM media before treatment. The gold nanoparticles were washed several times and the buffer solution of the reaction mixture was replaced with H_2O. The cells were washed with PBS buffer and fresh culture media were added to each well before incubation with gold nanoparticles (~ 1nM) for 48 h.[4] The controls were carried out in media without the added gold nanoparticles. The cell viability was determined by the MTT assay.

References:

1) K. C. Grabar, R. G. Freeman, M. B. Hommer, and M. J. Natan, *Anal. Chem*., 1995, **67**, 735.
2) S. J. Hurst, A. K. B. L. Jean and C. A. Mirkin, *Anal. Chem.,* 2006, **78**, 8313.
3) X. Liu, X. Li, T. Zhou, Y. Wang, M. T. T. Ng, W. Xu and T. Li, *Chem. Commun*., 2008, 380.
4) J. J. Li, L. Zou, D. Hartono, C. Ong, B. Bay and L. L. Yung, *Adv. Mater.*, 2008, **20**, 138.

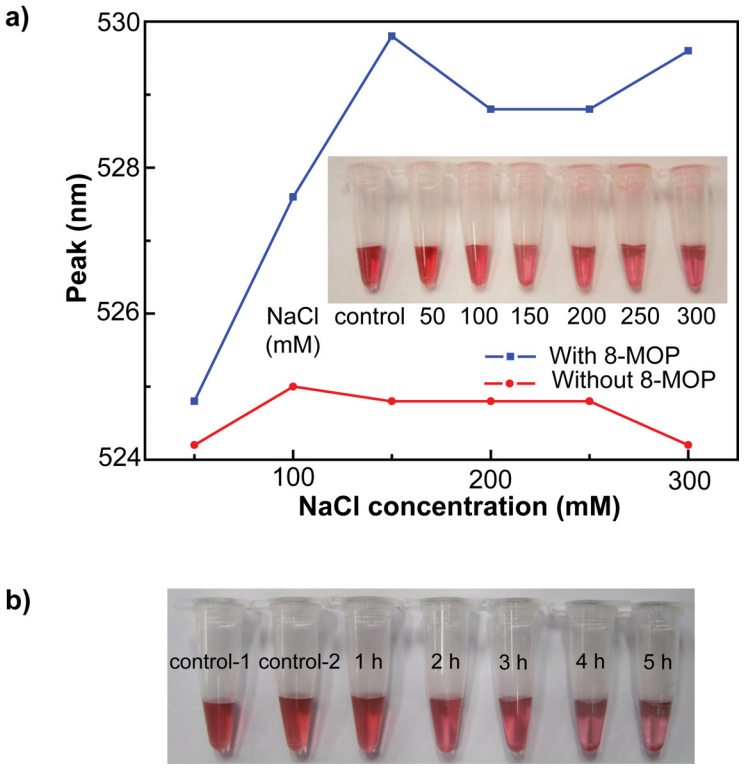

Figure S1. Colorimetric detection of 8-MOP by DNA-modified gold nanoparticles **(a)** Measured absorption peaks of the reaction mixtures (under UV irradiation for 3 h) containing different amounts of NaCl in the presence and absence of 8-MOP (71.0 μg/mL). Inset: photograph showing corresponding colorimetric responses of the reaction mixtures. The control refers to the reaction mixture containing 300 mM NaCl and without the 8-MOP. **(b)** Colorimetric responses of the reaction mixtures (200 mM NaCl, 10 mM Tris-HCl) irradiated for different time intervals. The control-1 refers to the mixture irradiated for 5 h in the absence of 8-MOP. The control-2 refers to the mixtures incubated in dark for 5 h with the 8-MOP.

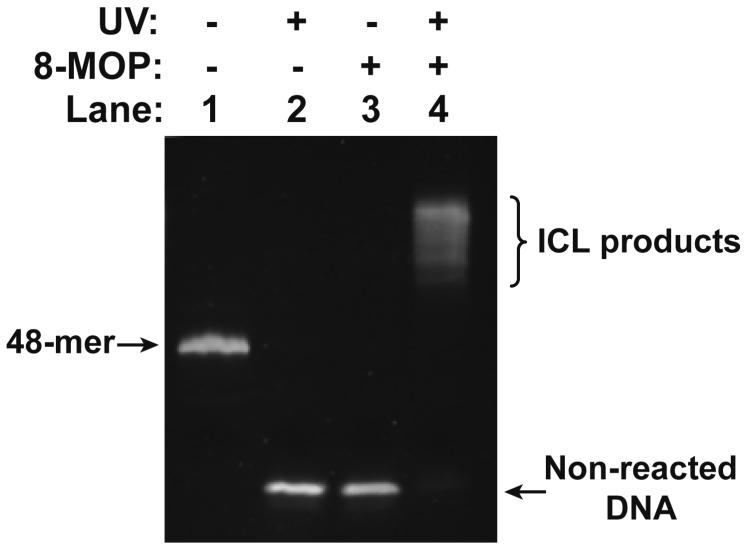

Figure S2. PAGE analysis of DNA interstrand cross-linking. Lane 1: control reference, 48 mer; Lane 2: control experiment treated with UV irradiation but without 8-MOP; Lane 3: control experiment treated with 8-MOP (71.0 μg/mL) but without UV irradiation; and Lane 4: DNA interstrand cross-linking in the presence of 8-MOP (71.0 μg/mL) and under UV irradiation.

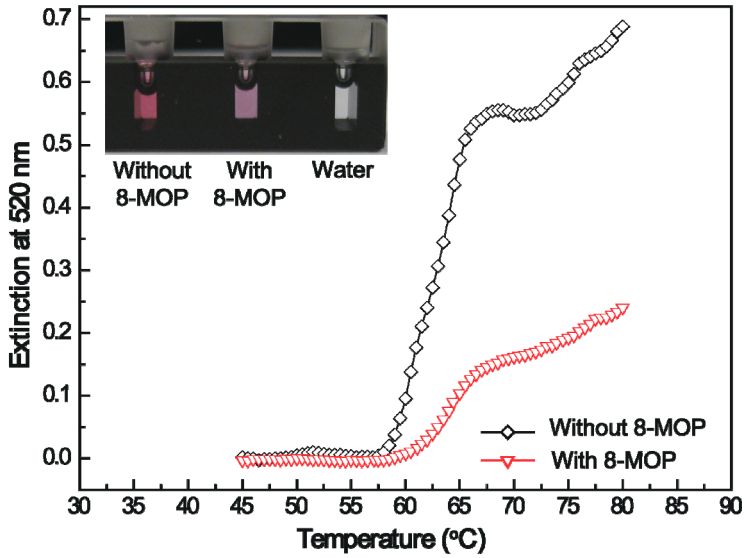

Figure S3. Thermal denaturation profiles for the nanoparticle-tethered DNA duplex measured in the presence and absence of 8-MOP (71.0 μg/mL) upon UV irradiation (3 h, 365 nm). Inset: photograph showing corresponding colorimetric responses of the solutions after the measurement.

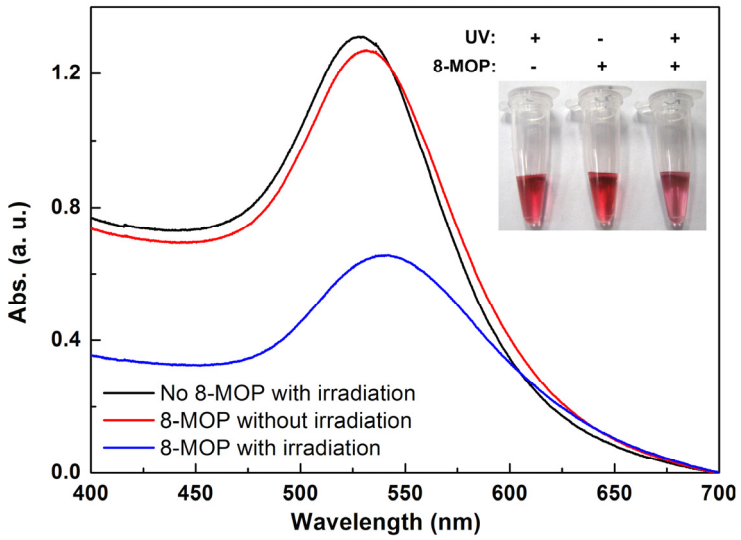

Figure S4. UV–vis spectra of DNA-modified gold nanoparticles obtained in the presence and absence of 8-MOP (71.0 μg/mL) after desalting experiments. The inserted photograph shows corresponding colorimetric responses of the solutions after desalting. Note that only the nanoparticles with interstrand cross-links remain aggregated.

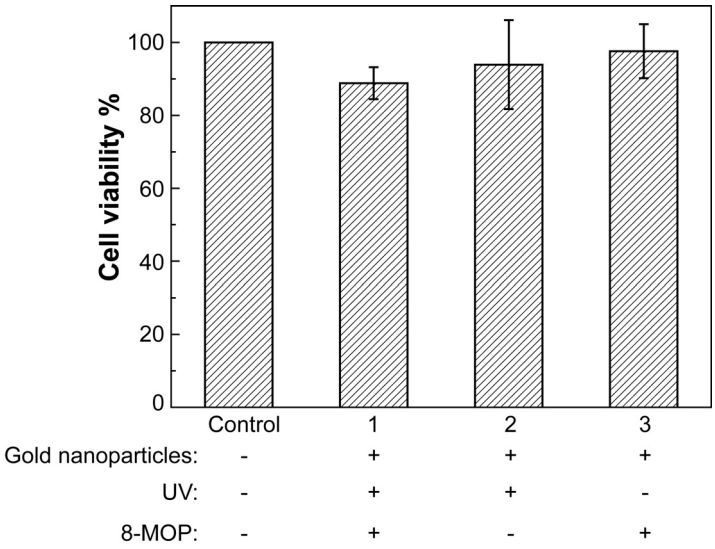

Figure S5. Cytotoxicity of gold nanoparticles to HDF cells. Lane 1-3: HDF cells treated with ~1 nM gold nanoparticles and incubated at 37 ^{o}C for 48 h. Control: HDF cells incubated only with culture media.