Electronic Supplementary Material (ESI) for Chemical Communications

A rapid screen for molecules that form duplex to duplex crosslinks in DNA

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Supporting information

Materials and methods

Starting materials and solvents

All reagents were of analytical grade, used as received and purchased from Sigma-Aldrich (UK) unless specified. Analytical reagent grade water, sodium dihydrogen orthophosphate dihydrate, di-sodium hydrogen orthophosphate anhydrous and eppendorf tubes were purchased from Fisher Scientific. Millex GP syringe driven filter units (0.22 μm) were purchased from Millipore Corporation, USA. The synthesis of compounds **1** and **2** has been previously reported.[1]

Phosphate Buffer (PB, 10 mM, pH 7.4)

Stock solutions of sodium dihydrogen orthophosphate (monobasic solution, 0.2 M) and di-sodium hydrogen orthophosphate (dibasic solution, 0.2 M) were prepared in analytical reagent grade water. The monobasic solution (1.9 mL) and the dibasic solution (8.1 mL) were mixed with 190 mL water to give a 10 mM PB solution. The pH of the solution was adjusted to pH 7.4. The buffer was filtered through a Miller GP syringe driven filter unit (0.22 μm) prior to use.

Preparation of the double stranded DNA (dsDNA)

A 33 base pair oligonucleotide, with a thiol functionality at the 5' position, and its complimentary sequence were purchased from Sigma UK. The sequences of the oligonucleotides are:

1) 5' – [ThiC6] – CTACGTGGACCTGGAGAGAGGAAGGAGACTGCCTG – 3'

2) 5' – CAGGCAGTCTCCTTCCTCTCTCCAGGTCCACGTAG – 3'

Stock solutions of each oligonucleotide were prepared according to the manufacturer's instructions. Annealing was conducted using equimolar quantities of each oligonucleotide in 10 mM trisodium citrate and 5 mM HEPES buffer. The solution was heated to 95°C for five minutes in a standard heat block and then allowed to cool to room temperature over a period of one hour. The solution was stored at -20 °C until required.

Instrumental techniques

Ultraviolet-visible (UV-Vis) spectra were recorded using a Hitachi U-3000 spectrophotometer at room temperature. Quartz cuvettes with a 1 cm path length were used. Transmission electron microscopy (TEM) images were obtained using a FEI Tecnai G2 20 Twin transmission electron microscope (John Innes Centre, Norwich, UK) operating at 200.0 kV, by depositing samples on holey carbon film 300 mesh copper grids from Agar Scientific, UK. Dynamic Light Scattering (DLS) particle size analysis of the nanoparticles was performed in a 1.5 ml plastic UV-visible absorbance cuvette using a Malvern Zetasizer Nano-ZS.

Synthesis of citrate stabilised gold nanoparticles

Water soluble gold nanoparticles were prepared *via* the citrate reduction method reported by Enüstün and Turkevich.[2] Briefly, aqueous solutions of $HAuCl_4 \cdot 3H_2O$ (12.5 mg, 32 µmol, in 100 mL) and sodium citrate tribasic dihydrate (50 mg, 168 µmol, in 50 mL) were prepared and heated to 60 °C. The sodium citrate solution was rapidly added to the gold solution while stirring vigorously. The temperature was increased to 85 °C and the solution was stirred for 2.5 h. A clear red gold nanoparticle solution was obtained that was cooled to

room temperature and filtered through a Miller GP syringe driven filter unit (0.22 µm). The particle concentration in the citrate stabilised gold nanoparticles solution was approximately 3 nM.

Synthesis of gold nanoparticles functionalised with thiolated dsDNA

Gold nanoparticles were functionalised with thiolated dsDNA using a pH-assisted and surfactant free method previously reported by Zhang *et al.*.[3] Thiolated dsDNA (31.5 µL of a 25 µM solution in trisodium citrate (10 mM, pH 3.0)) was added to an aliquot of freshly prepared citrate stabilised gold nanoparticles (1.5 mL). The solution was mixed with a pipette and left standing for 1 min. Trisodium citrate (30 µL of a 500 µM solution, pH 3.0) was added to the sample of nanoparticles and the solution was mixed with a pipette. The sample was left standing for 10 min. NaCl (52.5 µL of a 2 M solution) was added to the sample, mixed with a pipette and mixed in a rotary mixer for 20 min. The solution, red in colour, was treated with more NaCl (150 µL of a 2 M solution). The sample was mixed with a pipette followed by mixing in a rotary mixer for 40 min. The solution was centrifuged at 8,000 rpm for 30 min in an AllegraTM X-22R centrifuge, Beckman Coulter. The supernatant was removed and the centrifuged nanoparticles (precipitate) were washed with PB (10 mM, pH 7.4). The washing step was repeated a total of three times. In the last step, the particles were resuspended in 1.5 mL of PB (10 mM, pH 7.4) to keep the initial concentration of the gold nanoparticles.

Titration of dsDNA functionalised gold nanoparticles with: compound 1, compound 2, 9-aminoacridine (9-AA) and PB

Increasing concentrations of compound **1** or compound **2** (from 0 to 17.2 µM) were added to a sample of dsDNA functionalised gold nanoparticles ($V_{initial}$ = 570 µL and V_{final} = 580 µL). The samples were slowly shaken by hand for 1 min. The UV-Vis extinction spectrum of the dsDNA functionalised gold nanoparticle was measured before addition and after addition of the corresponding bisintercalator concentration.

The same procedure was repeated for the titration of dsDNA functionalised gold nanoparticles with 9-AA where increasing concentrations of 9-AA (from 0 to 34.5 µM) were added to the nanoparticles solution and for the titration with PB where increasing volumes of PB (from 0 to 10 µL) were added.

Titration of PB with: compound 1, compound 2 and 9-aminoacridine

Increasing concentrations of compound **1** or compound **2** (from 0 to 17.2 µM) were added to a sample of PB ((10 mM, pH 7.4); $V_{initial}$ = 570 µL and V_{final} = 580 µL). The samples were slowly shaken by hand for 1 min. The UV-Vis spectrum of the solution was measured before and after addition of the corresponding bisintercalator concentration.

The same procedure was repeated for the titration of PB with 9-AA where increasing concentrations of 9-AA (from 0 to 34.5 µM) were added to the PB solution.

Reversibility studies using sodium dodecyl sulphate (SDS)

To aggregate the dsDNA functionalised gold nanoparticles, increasing concentrations of compound **1** (from 0 to 3.5 µM) were added to a sample of the functionalised gold nanoparticles. The samples were slowly shaken by hand for 1 min. The UV-Vis extinction spectrum of the functionalised gold nanoparticle was measured before and after addition of the corresponding compound **1** concentration. To study the reversibility of the aggregation,

increasing concentrations of SDS (20 mM, PB 10 mM, pH 7.4; from 34. 8 to 442.9 μM) were added to the sample of dsDNA functionalised gold nanoparticles containing compound **1** (3.5 μM). The samples were shaken by hand for 1 min and the UV-Vis extinciton spectrum of each sample was recorded before and after addition of the corresponding SDS concentration.

Control of the reversibility studies using sodium dodecyl sulphate (SDS)

Increasing concentrations of SDS (20 mM, PB 10 mM, pH 7.4; from 34.8 to 442.9 μM) were added to a sample of dsDNA functionalised gold nanoparticles. The samples were shaken by hand for 1 min and the UV-Vis extinction spectrum of the sample was recorded before and after addition of the corresponding SDS concentration.

Reversibility studies using non-immobilised calf thymus DNA (CT-DNA)

To aggregate the dsDNA functionalised gold nanoparticles, increasing concentrations of compound **1** (from 0 to 3.5 μM) were added to a sample of the functionalised gold nanoparticles. The samples were slowly shaken by hand for 1 min. The UV-Vis extinction spectrum of the dsDNA functionalised gold nanoparticle solution was measured before and after addition of the corresponding compound **1** concentration. To study the reversibility of the aggregation, increasing volumes of CT-DNA (97 μM base pairs, from 0 to 19 μL) were added to the sample of gold nanoparticles functionalised with thiolated DNA containing compound **1** (3.5 μM). The samples were shaken by hand for 1 min and the UV-Vis extinction spectrum of each sample was recorded before and after addition of the corresponding CT-DNA concentration.

Control of the reversibility studies using CT-DNA

Increasing volumes of CT-DNA (from 0 to 19 µL) were added to a sample of dsDNA functionalised gold nanoparticles. The samples were shaken by hand for 1 min and the UV-Vis extinction spectrum of the sample was recorded before and after addition of the corresponding CT-DNA concentration.

Supporting results

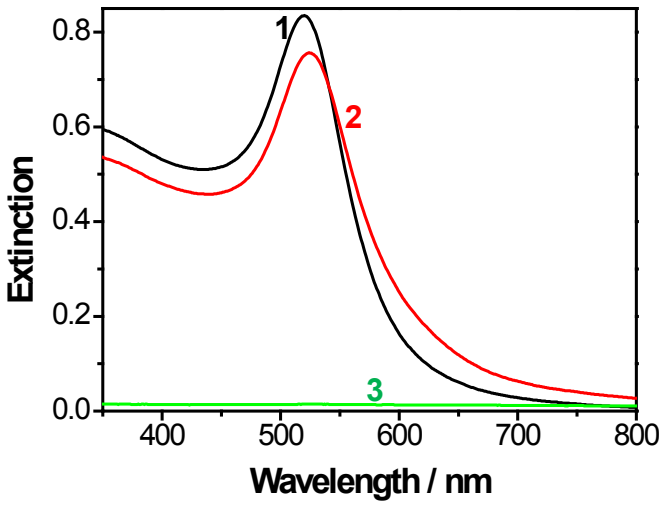

Fig. S1. UV-Vis extinction spectrum of: 1) citrate reduced gold nanoparticles; 2) dsDNA functionalised gold nanoparticles (PB 10 mM, pH 7.4) after addition of NaCl (0.3 M); and 3) citrate reduced gold nanoparticles (H_2O) after addition of NaCl (0.3 M).

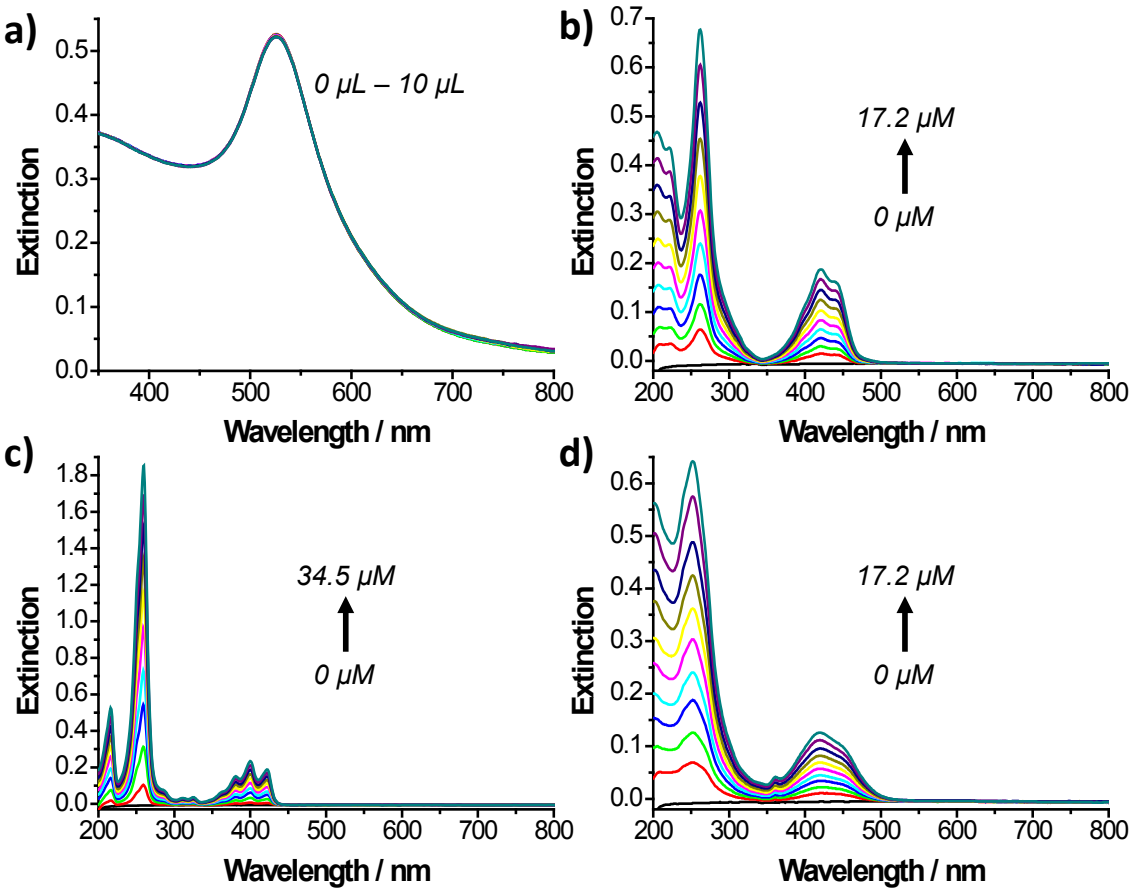

Fig. S2. UV-Vis extinction spectrum of: a) dsDNA functionalised gold nanoparticles (PB 10 mM, pH 7.4) after addition of increasing volumes of PB (10 mM, pH 7.4); b) PB (10 mM, pH 7.4) after addition of increasing concentrations of compound **1** (PB 10 mM, pH 7.4); c) PB (10 mM, pH 7.4) after addition of increasing concentrations of 9-AA (PB 10 mM, pH 7.4); and d) PB (10 mM, pH 7.4) after addition of increasing concentrations of compound **2** (PB 10 mM, pH 7.4). All of the samples were shaken by hand for 1 min prior to measurement.

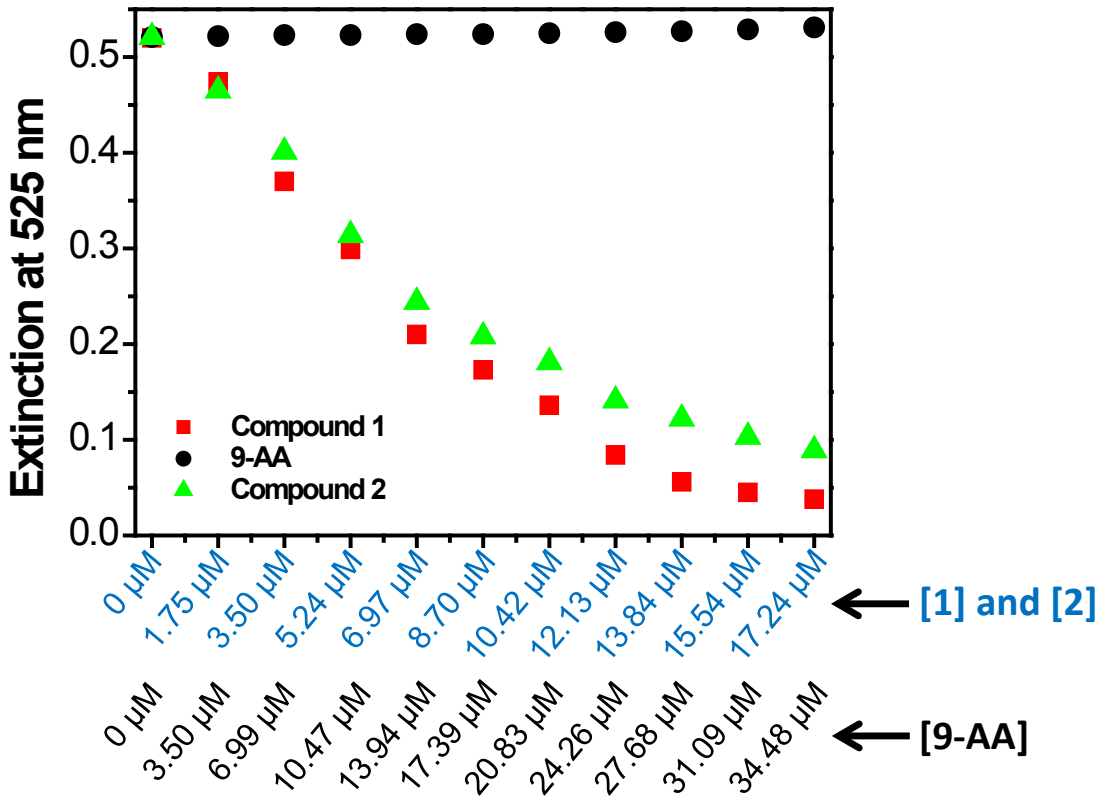

Fig. S3. Extinction intensity at 525 nm of: red) dsDNA functionalised gold nanoparticles after addition of compound **1** (from 0 to 17.2 µM); black) dsDNA functionalised gold nanoparticles after addition of 9-AA (from 0 to 34.5 µM); and green) dsDNA functionalised gold nanoparticles after addition of compound **2** (from 0 to 17.2 µM). All of the samples were shaken by hand for 1 min prior to measurement.

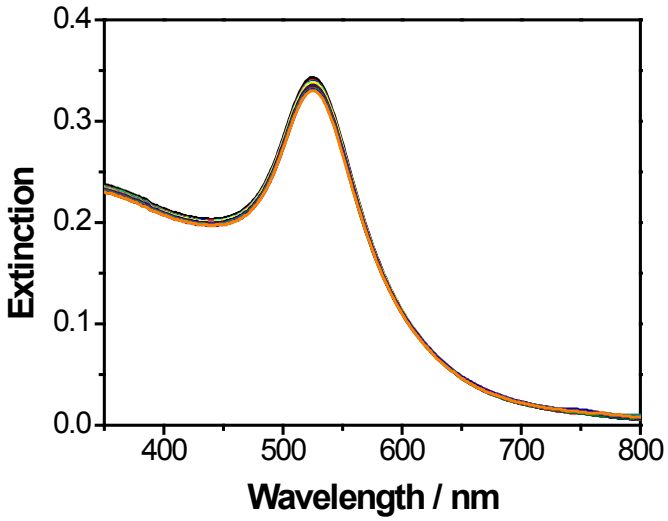

Fig. S4. UV-Vis extinction spectrum of dsDNA functionalised gold nanoparticles (PB 10 mM, pH 7.4) after addition of increasing concentrations of SDS (from 34.8 µM to 442.9 µM). All of the samples were shaken by hand for 1 min prior to measurement.

Table S1 The particle size of: dsDNA functionalised gold nanoparticles; dsDNA functionalised gold nanoparticles after addition of compound **1** (3.5 μM); and dsDNA functionalised gold nanoparticles after addition of compound **1** (3.5 μM) and SDS (442.9 μM) was estimated by dynamic light scattering:

Sample		Z-Average	Intensity
dsDNA functionalised gold nanoparticles	Measurement 1	31.47 nm	61.86 nm
	Measurement 2	31.27 nm	61.62 nm
	Measurement 3	31.16 nm	60.02 nm
	Mean Size	**31.30 nm**	**61.17 nm**
dsDNA functionalised gold nanoparticles + 1 (3.5 μM)	Measurement 1	636.5 nm	787.8 nm
	Measurement 2	641.1 nm	753.7 nm
	Measurement 3	657.5 nm	841.7 nm
	Mean Size	**645.03 nm**	**794.4 nm**
dsDNA functionalised gold nanoparticles + 1 (3.5 μM) + SDS (442.9 μM)	Measurement 1	48.62 nm	80.07 nm
	Measurement 2	48.16 nm	76.73 nm
	Measurement 3	47.99 nm	81.05 nm
	Mean Size	**48.26 nm**	**79.28 nm**

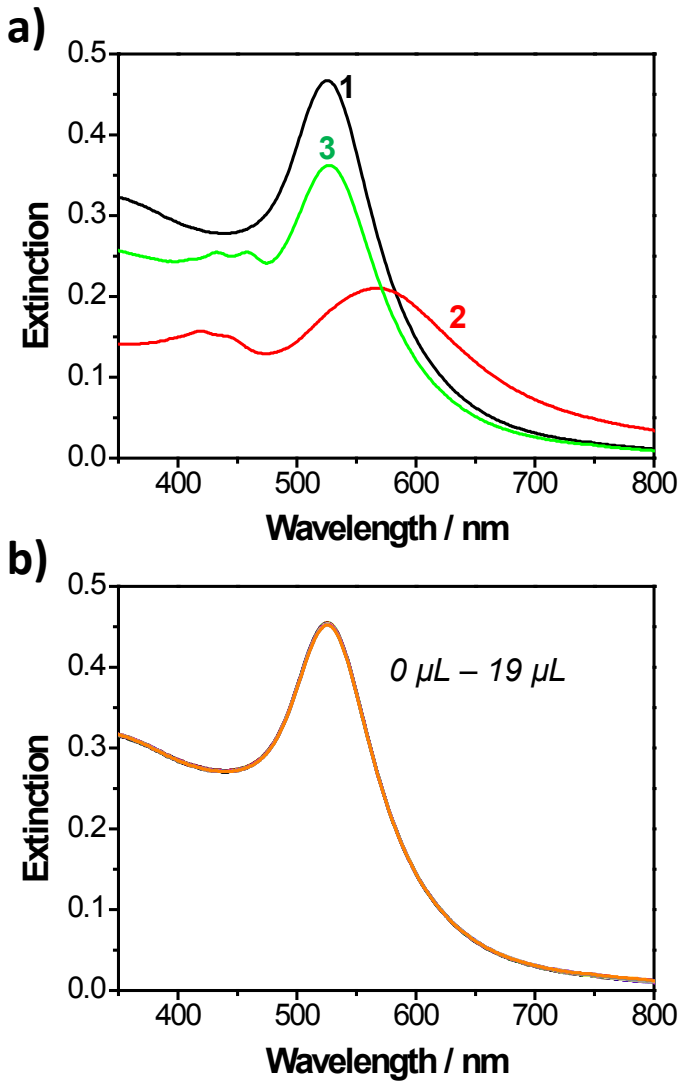

Fig. S5. a) UV-Vis extinction spectrum of: 1) dsDNA functionalised gold nanoparticles (PB 10 mM, pH 7.4); 2) dsDNA functionalised gold nanoparticles (PB 10 mM, pH 7.4) after addition of compound **1** (3.5 µM); and 3) dsDNA functionalised gold nanoparticles (PB 10 mM, pH 7.4) after addition of compound **1** (3.5 µM) and CT-DNA (19 µL, 97 µM base pairs). b) UV-Vis extinction spectrum of dsDNA functionalised gold nanoparticles (PB 10 mM, pH 7.4) after addition of increasing volumes of free DNA (from 0 µL to 19 µL). All of the samples were shaken by hand for 1 min prior to measurement.

References

(1) (a) L. P. G. Wakelin, X. Bu; A. Eleftheriou, A. Parmar; C. Hayek and B. W. Stewart, *J. Med. Chem.,* 2003, ***46***, 5790; (b) L. A. Howell, R. A. Bowater, M. A. O'Connell, A. P. Reszka, S. Neidle and M. Searcey, *ChemMedChem,* 2012, ***7***, 792.

(2) B. V. Enüstün and J. Turkevich, *J. Am. Chem. Soc.,* 1963, ***85***, 3317.

(3) X. Zhang, M. R. Servos and J. Liu, *J. Am. Chem. Soc.,* 2012, ***134***, 7266.