Supporting Information

Evaluation of DNA Methyltransferase Activity and Inhibition *via* Chiroplasmonic Assembled Gold Nanoparticles

Yuanjian Liu[a], Min Wei[b], Linqun Zhang[a], Wei Wei[a*], Yuanjian Zhang[a], Songqin Liu[a]

[a]*Jiangsu Province Hi-Tech Key Laboratory for Bio-medical Research, School of Chemistry and Chemical Engineering, Southeast University, Nanjing, 211189, P.R. China*

[b]*College of Food Science and Technology, Henan University of Technology, Zhengzhou, 450001, P.R. China*

[*]Corresponding author. Tel.: 86-25-52090613; Fax: 86-25-52090618.

E-mail address: wei_wei98@163.com (W. Wei)

1. Experimental Section.

Chemicals and Reagents. Chloroauric acid tetrahydrate ($HAuCl_4 \cdot 4H_2O$) and sodium citrate were purchased from Shanghai Reagent Company (Shanghai, China). Thiolated, methyl terminated ethylene glycol oligomers (thiol-PEG_6-methyl), bis(*p*-sulfonatophenyl)phenylphosphine dihydrate dipotassium salt (BSPP), 5-Aza-z′-deoxycytidine (5-Aza), and procaine were purchased from Sigma-Aldrich Co. Ltd. (St. Louis, MO). S-adenosylmethionine (SAM), *E. coli* CpG methyltransferase (M.*Sss*I), *E. coli* restriction endonuclease (*Hpa*II), and DNA adenine methylation (Dam) MTase were obtained from New England BioLabs (Ipswich, MA). All other reagents of certified analytical grade were purchased from Sunshine biotechnology (Nanjing, China).

All DNA oligonucleotides purified by polyacrylamide gel electrophoresis (PAGE) were supplied by Shanghai Sangon Biological Engineering Technology & Services Co. Ltd. (Shanghai, China) and suspended in deionized (DI) water (18.2 MΩ cm, Barnstead, Thermo Scientific, USA) to a final concentration of 1 μM. The DNA sequences are shown in Table S1.

The buffer solutions employed in this study were as follows: DNA hybridization buffer was 33 mM Tris-acetate (Tris-Ac) buffer solution (pH 7.4) containing 66 mM NaAc and 10 mM $Mg(Ac)_2$; The MTase work buffer was 10 mM Tris-HCl (pH 7.9) containing 50 mM NaCl, 10 mM $MgCl_2$, 160 μM SAM, and 1 mM DTT; The cleavage buffer was 33 mM Tris-Ac (pH 7.9) containing 10 mM $Mg(Ac)_2$, 66 mM KAc, and 0.1 mg/mL bovine serum albumin (BSA). All aqueous solutions were prepared with DI water.

Apparatus. UV−vis spectra were obtained from an UV−vis spectrophotometer (Shimadzu UV−2450, Kyoto, Japan) at wavelengths ranging from 200 to 800 nm. Transmission electron microscopy (TEM) images were collected on a transmission electron microscope (JEM−2010, Hitachi, Japan) operating at an acceleration voltage of 200 kV. Before TEM examination, 10 μL of each sample was first dispersed onto a copper grid coated with the carbon film, and was then dried in air. The hydrodynamic diameters of NPs and dimers were measured using dynamic light scattering (DLS; Zetasizer Nano ZS90, Malvern, UK). A quartz cuvette QS 10 mm was used as a sample container. The chirality of AuNPs dimers was characterized by circular dichroism (CD; Chirascan, Applied Photophysics, UK). 100 μL samples were added to quartz cuvettes with a 1 mm width. The temperature is kept to be 25 °C for all the measurements.

Preparation of Monoconjugated DNA-AuNPs. AuNPs with diameters of 15 ± 2 nm (Au_1), 20 nm ± 2 nm (Au_2), and 25 ± 3 nm (Au_3) were synthesized according to the classic citrate reduction method developed by Frens.[1] The size of the particles was controlled by varying [Au(III)]/[citrate] ratio. First, all glasswares used in experiment were thoroughly washed with aqua regia (HCl/HNO_3, volume ratio 3:1), rinsed in DI water, and oven-dried prior to use. Second, an aqueous solution of $HAuCl_4 \cdot 4H_2O$ (0.25 mM, 50 mL) was brought to reflux at continuous stirring. Third, sodium citrate solution (1.0, 0.875, or 0.750 mL, 1% by weight, freshly prepared) was quickly added, stirred, and kept boiling for another 30 mins. The solution color changed from

yellow to clear, to black, to purple and finally to wine red during this period. Then the heating source was removed, the suspension was stirred for another 30 mins, allowed to cool to room temperature, and subsequently filtered through a 0.45 μm acetate filter (Micron Separations Inc.). Finally, the AuNPs solution was stored in deep color bottles at 4 °C for further use. Concentration of the as-prepared AuNPs was determined using UV−vis spectroscopy.[2]

Prebound citrate ligands on the surface of AuNPs were replaced by BSPP ligands,[3] in order to increase the negative surface charge of the particle and consequently ensure that the AuNPs modified with less DNA were well-dispersed at high ionic strength. In brief, excess BSPP (40 mg for Au_1, 20 mg for Au_2, and 10 mg for Au_3) were added to 10 mL aqueous solutions of AuNPs (Au_1, Au_2, and Au_3 at initial concentrations of 8, 4, and 2 nM). The mixtures were incubated overnight at room temperature, centrifuged at different speeds (12000, 10000, and 7000 rpm) for 20 mins to collect the precipitated AuNPs. After rinsed thoroughly with DI water, the pellets were redispersed in 200 μL DI water. The monoconjugated DNA-AuNPs (ssDNA-AuNPs) were prepared following a literature procedure,[3,4] with some modifications. 20 μL of BSPP-protected AuNPs were mixed with single-strand DNA at a molar ratio of 1:3 for DNA-Au_1, 1:5 for DNA-Au_2 and DNA-Au_3, respectively, followed by rocking gently at room temperature for 24 hours. The added BSPP was used as a reducing agent to minimize oxidation of the thiol moieties.[5] The prepared DNA-AuNPs were further incubated with thiol-PEG_6-methyl (at the concentration ratio of 1:50 000) for 2 hous to passivate the AuNPs surface.[5,6] The sample was then centrifuged to remove uncoupled oligonucleotides. The pellet was redispersed in 50 μL DNA hybridization buffer, and stored at 4 °C.

Chiroplasmonic Assemblies of AuNPs dimers. Various concentrations of linker DNA_2 was added into the solution consisting of 25 μL of 0.08 μM DNA_3-Au_2, 50 μL of 0.04 μM DNA_4-Au_3, and 115 μL DNA hybridization buffer (a molar ratio of 1:1 for DNA_3-Au_2 and DNA_4-Au_3). Hybridization mixtures were heated at 90 °C for 5 mins to minimize nonspecific hybridization, and then incubated on an orbital shaker at room temperature for 12 hours. Finally, the mixtures were incubated on a thermostatic container at 25 °C for 30 mins before CD measurements.

M.*Sss*I MTase Activity Assay. The methylation of AuNPs dimers were performed at 37 °C for 3 hours in the MTase working buffer with various concentration of M.*Sss*I ranging from 0 to 400 U/mL. After centrifugation, the supernatant containing unreacted M.*Sss*I was removed, and the precipitate was redispersed in 150 μL DNA hybridization buffer. *Hpa*II digestion was performed at 37 °C in the cleavage buffer containing 50 U/mL *Hpa*II for 3 hours. After digestion, the AuNPs dimers were transferred into DNA hybridization buffer using centrifugation before CD measurements.

Inhibition of M.*Sss*I MTase Activity. To study the inhibition effects of 5-Aza and procaine on the M.*Sss*I activity, the linker DNA was incubated with MTase working buffer containing 300 U/mL M.*Sss*I, and inhibitors of various concentrations at 37 °C.

2. Linker DNA Dependence of Chiroplasmonic Assembled AuNPs. As shown in Figure S2, the mixture of DNA_3-Au_2 and DNA_4-Au_3 solution showed no CD signal. When they hybridized with linker DNA_2 at various concentration, the new CD peak appeared at 528 nm and it increased significantly with the increasing linker DNA_2 concentration. A calibration curve was constructed by plotting the variation of CD intensity with different logarithmic linker DNA_2 concentration (Figure S2B). The reason is that as the concentration of linker DNA_2 increased, so did the yields of the Au_2-Au_3 dimers, which was proved by the statistically results from TEM data (Figures S2C). From Figure S2A, it also can be seen that the increasing Au_2-Au_3 dimers had no observable effect on the UV−vis absorbance spectrum, which indicated that CD was significant detection signal and it provided a more sensitive detection performance. The anisotropy factor (*g*-factor) can be used as a key indicator to evaluate the detection performance regardless of the detection system.[7] The *g*-factor of the chiroplasmonic assemblies of AuNPs displayed values up to 7.6×10^{-3} (Figure S3), which was higher than other chiral nanostructures assembled by small molecules,[8,9] proteins,[10] and lipids.[11] The method could be used to detect linker DNA in the linear range of 0.01 ~ 2 nM, with the detection limit of 7 pM (S/N = 3). The method has good selectivity and it can differentiate the single-mismatched DNA from the fully complementary DNA. As shown in Figure 3D, the CD intensities for fully complementary linker DNA is 70.55, while it decreased to 14.61, 5.23, and 2.62 in the presence of equal single-mismatched, two-mismatched, and random DNA, respectively. The sharply decreased CD value indicated that the chirality of the AuNPs dimers were strongly dependent on the sequence of linker DNA.

3. Good Accuracy and Reproducibility of the Detection Linker DNA. Good accuracy and reproducibility are obtained (Table S2). Various concentrations of standard linker DNA were detected with the recoveries from 90% to 102.9%, indicating the good accuracy of the method. The precision of this method was presented by the intraassay variability. Here, samples under several random dilution ratios of a fixed linker DNA_2 concentration were detected five times with the same batch of detection solutions. The intraassay variability (CV% = SD/mean) of 5.22% proved the good precision and reproducibility of the method. The interassay variability was tested as in the same way but using five different batches of detection solutions for each sample in different dilution ratios, respectively. The interassay variability of 7.20% suggested an acceptable variability of different batches of detection solutions.

4. The Effects of Methylation Time on the Methylation Process. To evaluate the effects of methylation time on the methylation process, DNA in Au_2-Au_3 dimers were methylated for different periods in the presence of M.*Sss*I and SAM, and then CD signal was recorded after the methylated DNA had been incubated with *Hpa*II for 3 hours. With the increment of methylation time, CD signal increased rapidly, while the increasing rate decreased gradually when the methylation time prolongs. Finally, 3 hours was chosen for the fully methylated DNA since the

CD signal reached a terrace at this time (Figure S5).

5. Supplementary Figures

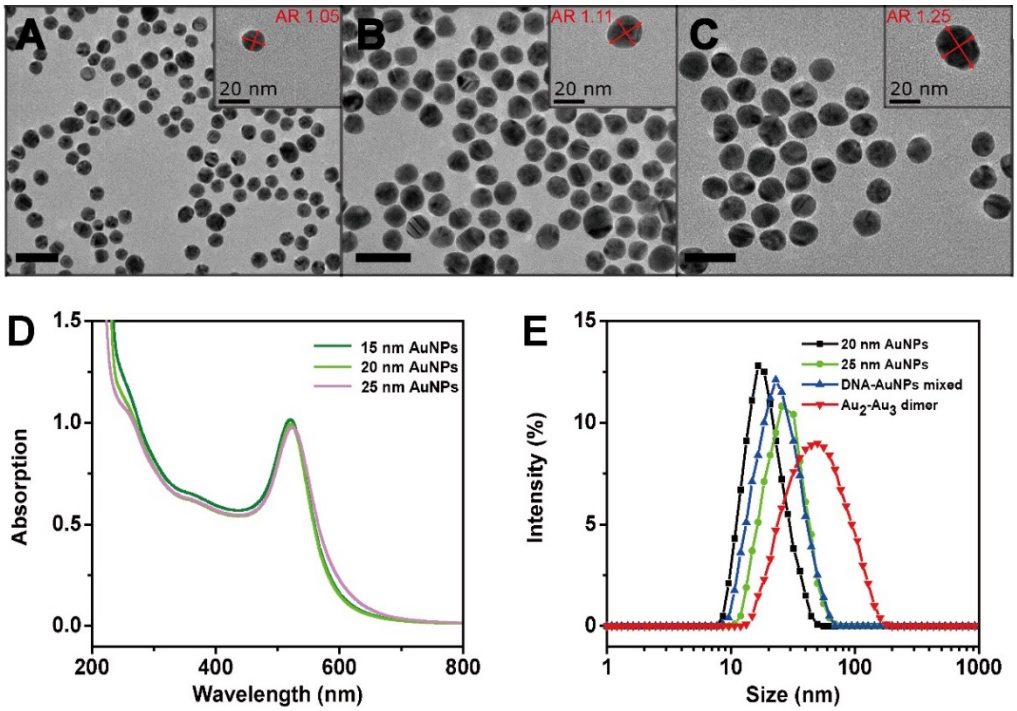

Figure. S1 Representative TEM images of AuNPs of different sizes used in this project: (A) 15 ± 2 nm AuNPs; (B) 20 ± 2 nm AuNPs; (C) 25 ± 3 nm AuNPs. The scale bar indicates 50 nm. (D) UV/Vis spectra of AuNPs. (E) The dynamic light scattering (DLS) for NPs assemblies. The mixture stands for no hybridized of DNA_3-Au_2 and DNA_4-Au_3.

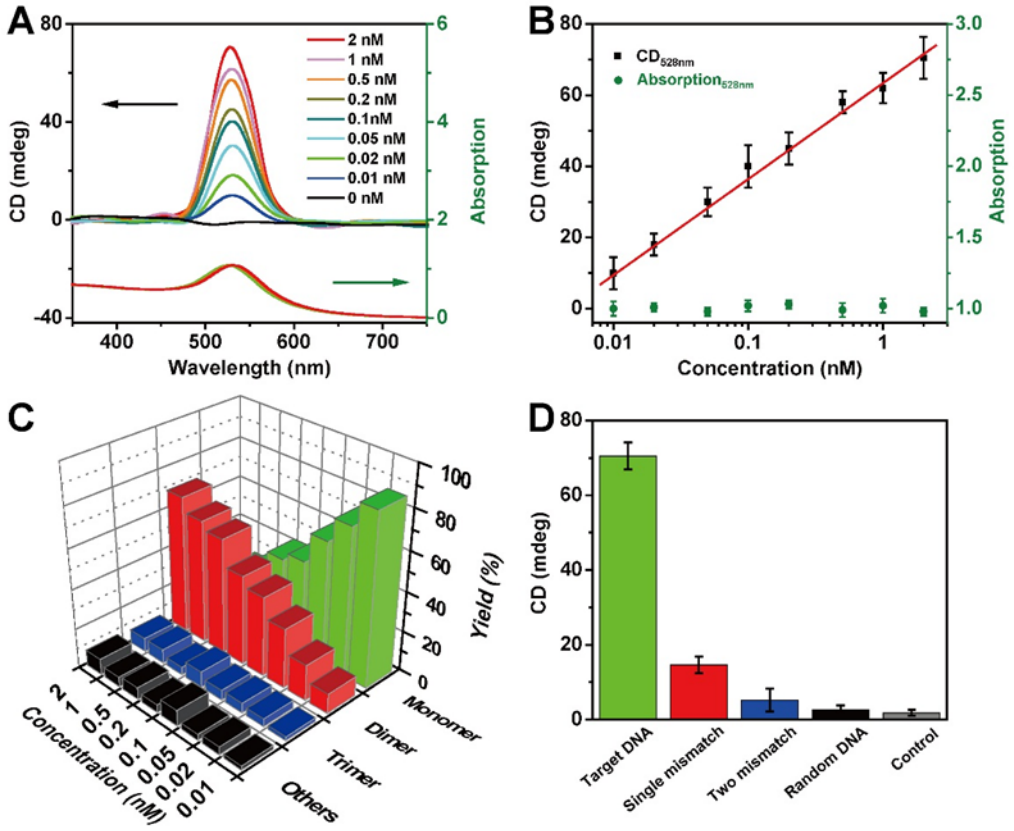

Figure. S2 Linker DNA dependence of chiroplasmonic assemblies of AuNPs. (A) The CD and corresponding UV−vis absorption curves with increasing concentrations of linker DNA. (B) Plot of increased CD intensity (528 nm) as a function of logarithmic linker DNA concentration. Error bars show the standard deviation of three experiments. (C) Statistical analysis of the number of AuNPs Dimers assembled at different linker DNA concentrations. (D) Selectivity of the developed CD probe for linker DNA over other DNA. The concentration of each DNA was 2 nM.

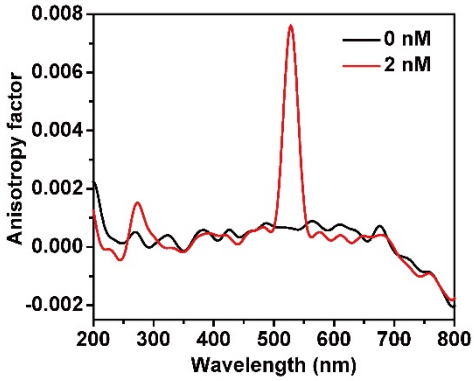

Figure. S3 The g-factor of Au_2-Au_3 dimers.

The optical activity of chiral systems is often quantified using the anisotropy factor (g-factor):

$g = \Delta \varepsilon / \varepsilon = \Omega$ (mdeg) / (32980×A)

Where Δ ε and ε are the molar circular dichroism and molar extinction coefficient, respectively. Where Ω (mdeg) and A are the CD band intensity (ellipticity) and the UV−vis absorption of samples, respectively. Using the CD and the absorbance spectrum of the samples, the value of the g-factor can be calculated.[1-3]

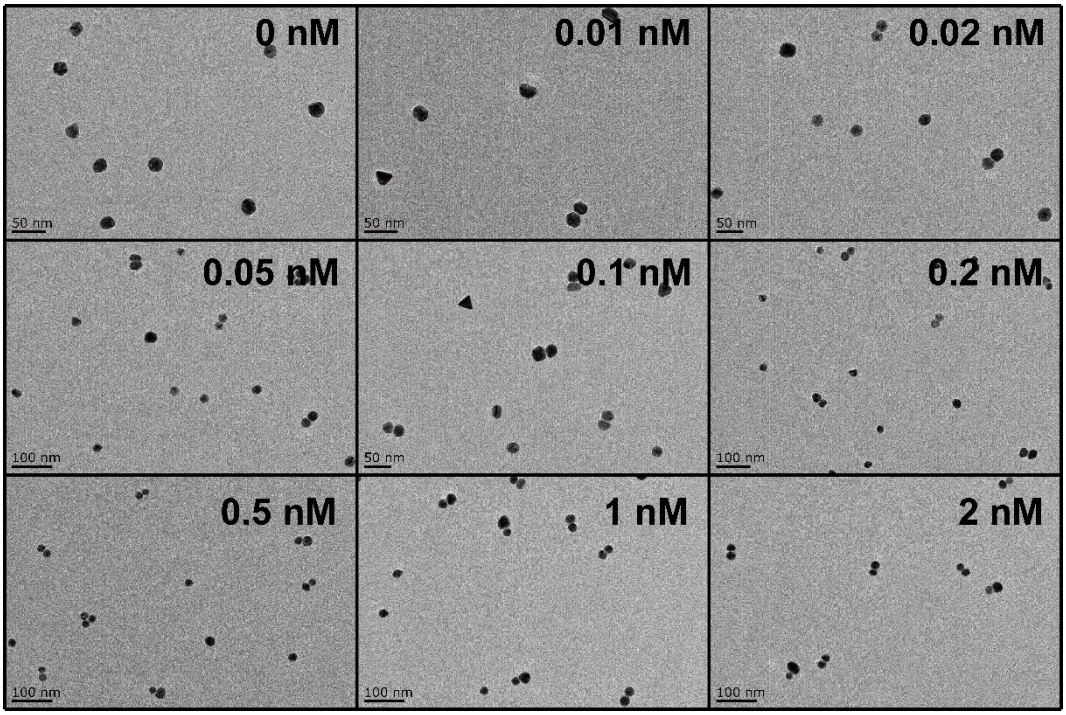

Figure. S4 Representative TEM images for plasmonic chiroptical sensors in the range of 0 ~ 2 nM linker DNA.

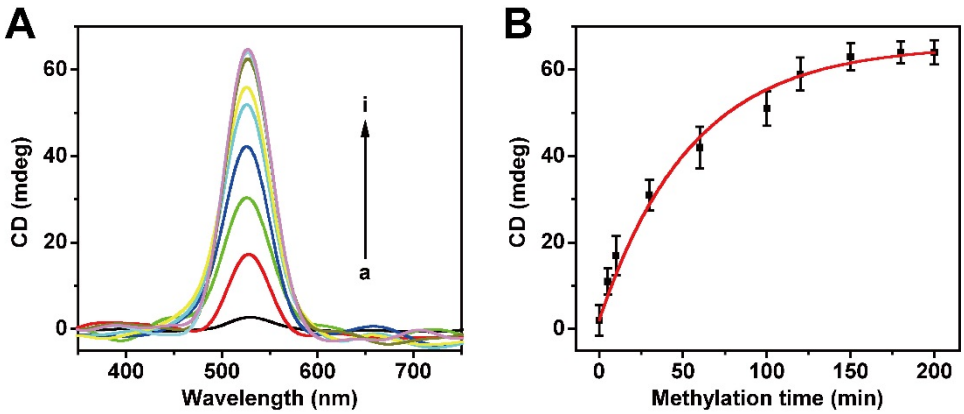

Figure. S5 (A) CD response of the biosensor treated with 400 U/mL M.SssI MTase with different times at (a) 0, (b) 5, (c) 10, (d) 30, (e) 60, (f) 100, (g) 120, (h) 180, and (i) 200 mins, respectively. (B) Dependence of CD signals of Au2-Au3 dimers on methylation time. Error bars show the standard deviation of three experiments.

Table S1. Sequences of the Used DNA[a]

DNA name	Sequence and modifications (from 5′-3′)
DNA_1	SH-$(CH_2)_6$-TTTTTTTTTTTTT**CCGG**TCT
DNA_2	GCTTAGAGGTTTTTTTTTTT-$(CH_2)_6$-SH
DNA_3	SH-$(CH_2)_6$-TTTTTTTTTGTTATGTATTT**CCGG**TCT
DNA_4	GCTTAGAGGTTTGTACGTTTTTTTTTT-$(CH_2)_6$-SH
DNA_5	SH-$(CH_2)_6$-TTTTTTTTTTGCTGTTGTTATGTATTT**CCGG**TCT
DNA_6	GCTTAGAGGTTTGTACGTTCTTTGCTTTTTTTTTT-$(CH_2)_6$-SH
Linker DNA_1	ACCTCTAAGCAGA**CCGG**AAA
Linker DNA_2	CGTACAAACCTCTAAGCAGA**CCGG**AAATACATAAC
Linker DNA_3	GCAAAGAACGTACAAACCTCTAAGCAGA**CCGG**AAATACATAACAACAGC A
Single mismatch	CGTACAAACCTCTAAGCA***T***ACCGGAAATACATAAC
Two mismatch	CGTACAAACCTCTAAGCA***TG***CCGGAAATACATAAC
Random DNA	GATTGAGCGTGATGAATGTCACTACTTCAACTCGC

[a]The bold font of CCGG was the recognized bases by M.*Sss*I MTase and restriction endonuclease *Hpa*II. The bold/italic font of T and TG were the mismatched base.

1

Table S2. Intra- and Interassay Variability of This Method

	intraassay			interassay		
Added (nM)	Found (nM)	Recovery (%)	CV (%, n=5)	Found (nM)	Recovery (%)	CV (%, n=5)
0.03	0.028	93.3	4.87	0.027	90.0	6.71
0.06	0.057	95.0	5.12	0.062	103.3	6.85
0.09	0.088	97.8	4.91	0.092	102.2	7.11
0.30	0.291	97.0	5.07	0.289	96.3	6.94
0.60	0.612	102.0	5.35	0.587	97.8	7.69
0.90	0.917	101.9	5.97	0.881	97.9	7.89
	intraassay variability 5.22%			interassay variability 7.20%		

1

Table S3. Comparison of the Analytical Performance for the Detection of DNA Methyltransferase Activity by the As-proposed Method and Those Reported in the Literature

Strategy	Detection method	Detection limit ($U\ mL^{-1}$)	Linear range ($U\ mL^{-1}$)	Reference
RCA	Chemiluminescence	0.52	1–10	12
Enzymatic amplification	DPV	0.04	0.1–20	13
HRP mimicking DNAzyme	Colorimetry	6	6–100	14
Methylation-resistant endonuclease	Fluorescence	0.16	1–100	15
HRP-IgG conjugated immunoassay	DPV	0.1	0.5–50	16
DNA-functionalized AuNPs amplification	DPV	0.12	0.2–10	17
AuNPs coupled with enzyme-linkage reactions	Colorimetry	0.3	1–10	18
GO combining with restriction endonuclease	DPV	0.05	0.1–450	19
Nicking enzymeassisted signal amplification	Fluorescence	0.06	0.1–4	20
Chiroplasmonic assemblies of gold nanoparticles	Circular dichroism	0.27	0.5–150	This work

1

6. References

1 G. Frens, *Nat. Phys. Sci.*, 1973, **241**, 20−22.

2 W. Haiss, N. T. K. Thanh, J. Aveyard and D. G. Fernig, *Anal. Chem.*, 2007, **79**, 4215−4221.

3 C. J. Loweth, W. B. Caldwell, X. G. Peng, A. P. Alivisatos and P. G. Schultz, *Angew. Chem., Int. Ed.*, 1999, **38**, 1808−1812.

4 S. A. Claridge, H. Y. W. Liang, S. R. Basu, J. M. J. Frechet and A. P. Alivisatos, *Nano Lett.*, 2008, **8**, 1202−1206.

5 M. P. Busson, B. Rolly, B. Stout, N. Bonod, E. Larquet, A. Polman and S. Bidault, *Nano Lett.*, 2011, **11**, 5060−5065.

6 B. M. Reinhard, S. Sheikholeslami, A. Mastroianni, A. P. Alivisatos and J. Liphardt, *Proc. Natl. Acad. Sci. U.S.A.*, 2007, **104**, 2667−2672.

7 X. L. Wu, L. G. Xu, L. Q. Liu, W. Ma, H. H. Yin, H. Kuang, L. B. Wang, C. L. Xu and N. A. Kotov, *J. Am. Chem. Soc.*, 2013, **135**, 18629−18636.

8 I. Dolamic, S. Knoppe, A. Dass and T. Burgi, *Nat. Commun.*, 2012, **3**, 798.

9 C. Gautier and T. Burgi, *J. Am. Chem. Soc.*, 2006, **128**, 11079−11087.

10 J. M. Slocik, A. O. Govorov and R. R. Naik, *Nano Lett.*, 2011, **11**, 701−705.

11 R. Y. Wang, H. L. Wang, X. C. Wu, Y. L. Ji, P. Wang, Y. Qu and T. S. Chung, *Soft Matter*, 2011, **7**, 8370−8375.

12 S. Bi, T. T. Zhao, B. Y. Luo and J. J. Zhu, *Chem. Commun.*, 2013, **49**, 6906−6908.

13 H. W. Wu, S. C. Liu, J. H. Jiang, G. L. Shen and R. Q. Yu, *Chem. Commun.*, 2012, **48**, 6280−6282.

14 W. Li, Z. L. Liu, H. Lin, Z. Nie, J. H. Chen, X. H. Xu and S. Z. Yao, *Anal. Chem.*, 2010, **82**, 1935−1941.

15 Q. H. Quach and B. H. Chung, *Analyst*, 2014, **139**, 2674−2677.

16 M. Wang, Z. N. Xu, L. J. Chen, H. S. Yin and S. Y Ai, *Anal. Chem.*, 2012, **84**, 9072−9078.

17 X. X. He, J. Su, Y. H. Wang, K. M. Wang, X. Q. Ni and Z. F. Chen, *Biosens. Bioelectron.*, 2011, **28**, 298−303.

18 T. Liu, J. Zhao, D. M. Zhang, G. X. Li, *Anal. Chem.*, 2010, **82**, 229−233.

19 W. Li, P. Wu, H. Zhang, C. X. Cai, *Anal. Chem.*, 2012, **84**, 7583−7590.

20 Y. X. Zhao, F. Chen, Y. Y. Wu, Y. H. Dong, C. H. Fan, *Biosens. Bioelectron.*, 2013, **42**, 56−61.