Supplementary Material (ESI) for Analyst

Electronic Supplementary Information for

Gold Nanoparticles Based Colorimetric Assay of Protein Poly(ADP-riobosyl)ation

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Materials and chemicals.

Chloroauric acid ($HAuCl_4$) was obtained from Shanghai Jiushan Chemicals Co., Ltd. Trisodium citrate was purchased from Nanjing Chemical Reagent Co., Ltd. NAD and bovine serum albumin (BSA) from human plasma were obtained from Sigma-Aldrich. Human PARP was obtained from Trevigen. The two complementary oligonucleotides (ssDNA 1, ssDNA 2) were synthesized and purified by Shanghai Invitrogen Biotechnology Co., Ltd. Their concentrations were quantified by OD260 based on their individual absorption coefficients. The sequences of the oligonucleotides are listed as follows:

ssDNA 1: 5'-CCCGTGCGTGCGCGAGTGAGTTG-3'

ssDNA 2: 5'-CAACTCACTCGCGCACGCACGGG-3'

The buffer solutions employed in this work are as follows. DNA hybridization buffer: 10 mM Tris-HCl containing 0.1 M NaCl, pH 7.4. The reaction buffer for the enzymatic reaction: 50 mM Tris-HCl containing 25 mM $MgCl_2$, pH 8.0. The chemicals and solvents are analytical reagents or better and used without further purification. All buffers were prepared with water purified with a Milli-Q purification system (Branstead, USA).

Preparation of Au-NPs and dsDNA

The 13 nm Au-NPs were prepared by citrate reduction of $HAuCl_4$ according to the literatures.[1,2] Briefly, a 100mL aqueous solution of 0.01% (w/v) $HAuCl_4$ was added into a round-bottom flask and stirred to boil. Then 3.5mL 1% trisodium citrate was added rapidly into the boiling solution, the color of which became wine red from colorless after boiling for another 15 min with vigorous stirring. The size of the nanoparticles was 12.5 ± 2.3 nm, determined by TEM. The concentration of Au-NPs was 2.3 nM, which was calculated from the quantity of starting material ($HAuCl_4$) and the size of Au-NPs was at the wavelength of 519 nm.

DNA duplex was prepared by hybridization of the two complementary sequences ssDNA1 and ssDNA2 (10 μM each) in hybridization buffer at 95 ºC for 5min, followed by cooling down slowly (about 6 hours) to room temperature.

Salt endurance experiment

To check the efficiency of the protection from aggregation of Au-NPs by negative-charged NAD, salt endurance experiments were carried out. Firstly, the experiments were performed by adding NAD dissolved in reaction buffer into Au-NP solution, the final NAD concentration of which was 12.5μM, followed by incubating the solution for some periods ranging from 0s to 120s. Then, NaCl solution was added into the above solution, and the final salt concentration was made to be 0.1M.

The salt endurance experiments were also performed by using different concentration of NAD. Firstly, NAD with different concentrations was added into Au-NP solution and incubated for 60s. Then, NaCl solution was added and the final concentration was also kept as 0.1M.

Detection of auto-PARation

Firstly, 5 μL of reaction buffer containing NAD and dsDNA which functioned as the enzymatic reaction substrate and co-factor,[3, 4] respectively, was added into 100 μL of Au-NPs. Then, 1 μL of reaction buffer containing different concentration of PARP was added into the above mixed solution. Every 60 second, the spectrophotometric time-dependent curve of the mixed solution was measured. The control experiments were carried out by using 5 mg/mL BSA instead of PARP.

Apparatus.

The spectrophotometric measurements were performed on a UV-2450 UV-Vis spectrophotometer (Shimadzu Co., Japan).

Supplementary data

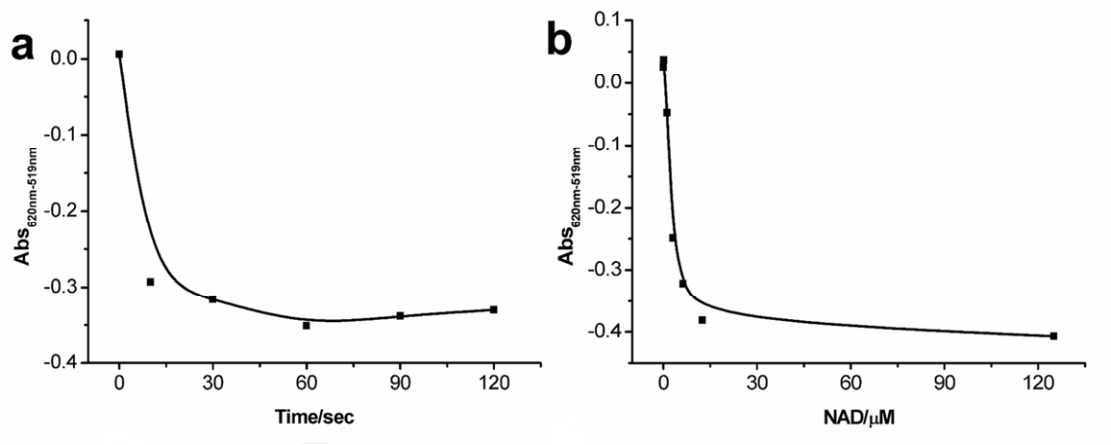

Figure S.1. Salt endurance experimental results for (a) the protective time varied from 0s to 120s at the NAD concentration of 12.5μM. (b) the final concentration of NAD is 0, 0.0125, 0.125, 1.25, 3.125, 6.125, 12.5, 125μM, respectively, at the protection time of 60 seconds.

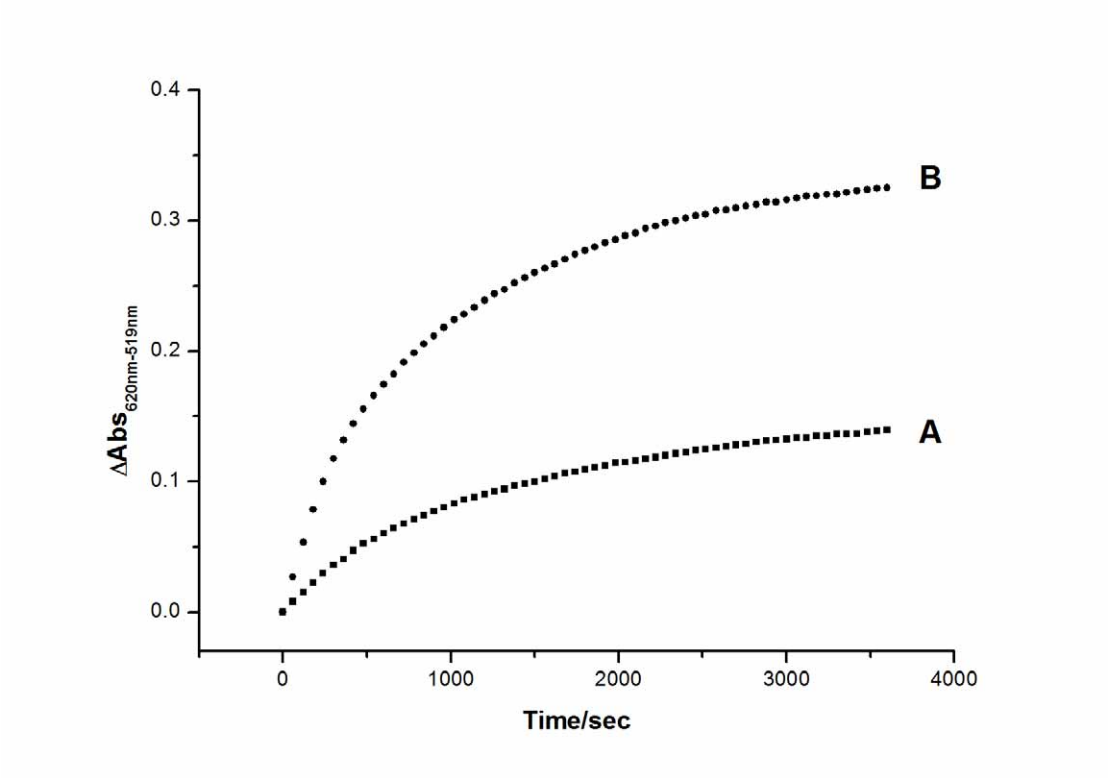

Figure S.2. Time-dependent absorbance curves of mixed solution containing Au-NPs, NAD, dsDNA, 0.20 μg/mL hPARP and (A) 80nM, (B) 8nM 3-aminbenzamide, the inhibitor of the enzyme, after 24h incubation.

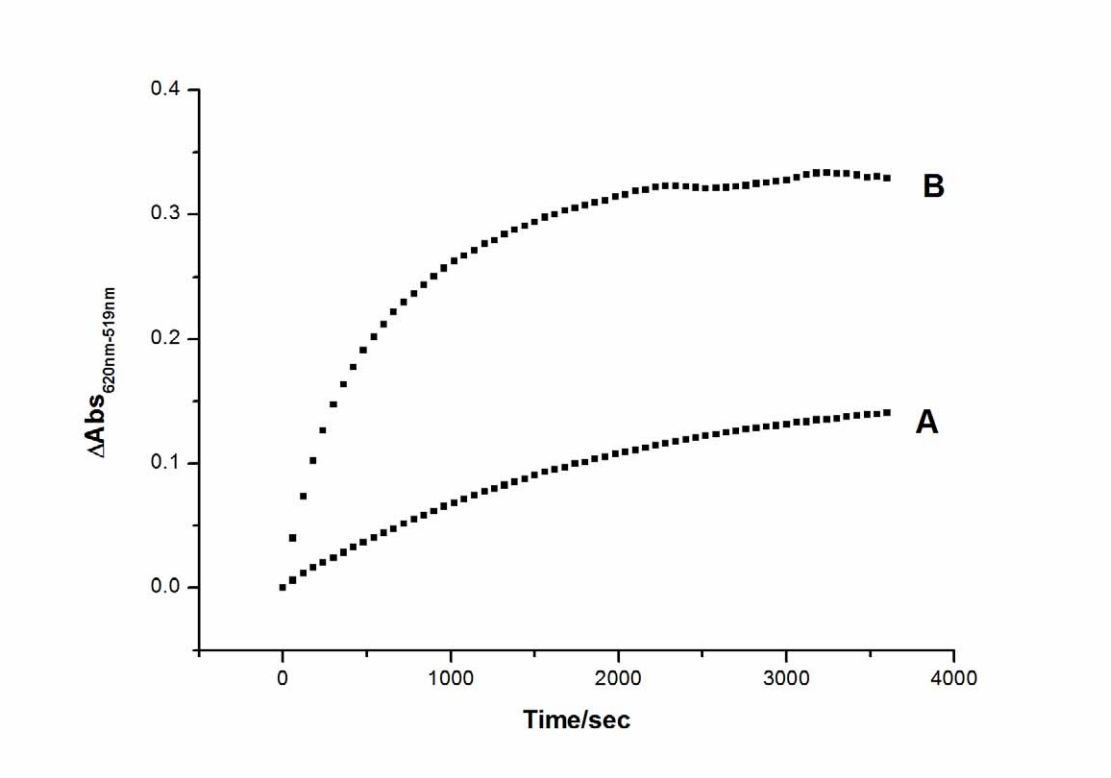

Figure S.3. Time-dependent absorbance curves of mixed solution containing Au-NPs, dsDNA, 0.20 μg/mL hPARP and (A) 50μM NAD, (B) 5μM NAD.

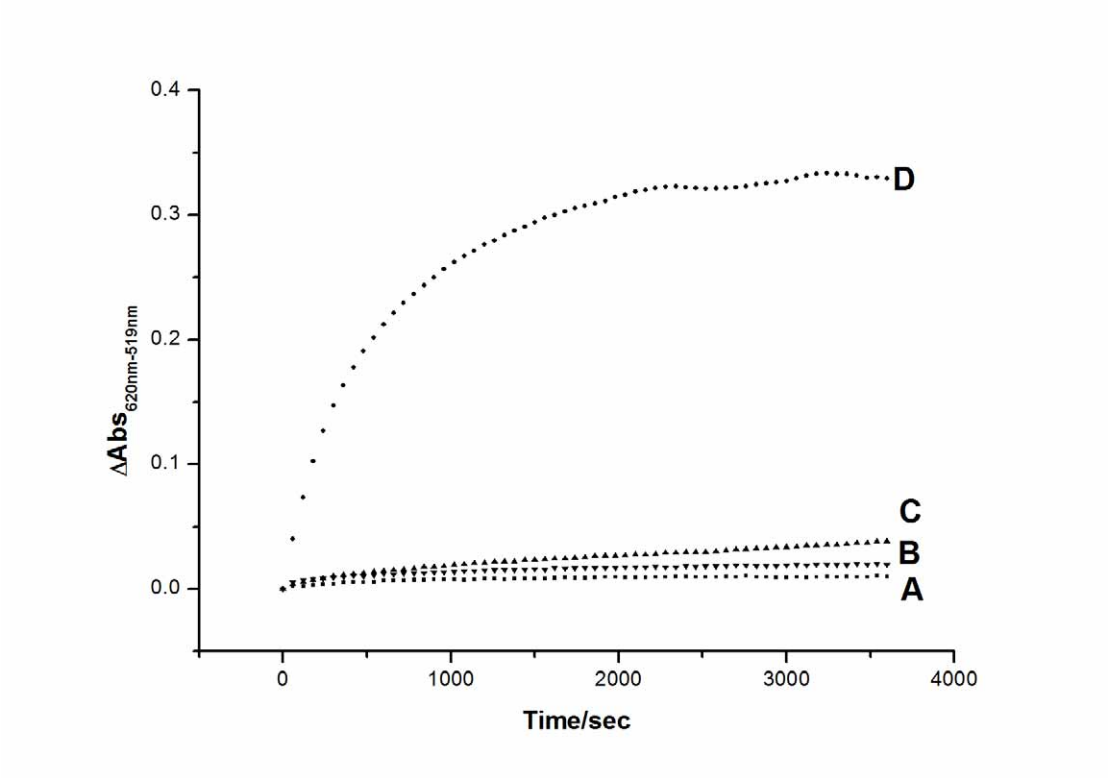

Figure S.4. Time-dependent absorbance curves of mixed solution containing (A) Au-NPs, NAD and dsDNA, (B) Au-NPs, dsDNA and hPARP, (C) Au-NPs, hPARP and NAD, (D) Au-NPs, NAD, dsDNA and hPARP.

References

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