Supporting Information

High Gold Nanoparticles Stability towards DNA Modification and Efficient Hybridization via Surfactant-Free Peptide Route

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Experimental Section

1.1 Materials. Tri-sodium citrate ($C_6H_5Na_3O_7$), ethylenediaminetetraacetic acid (EDTA), $HAuCl_4{\cdot}3H_2O$, tris(2-carboxyethyl) phosphine (TCEP), 4-mercaptobenzoic acid (MBA, analytical grade) and dithiothreitol (DTT) were purchased from Sinopharm Chemical Reagent (China). Oligonucleotides listed in **Table S2** and peptides listed in **Table S3**, were obtained from Sangon Biotechnology Inc. (Shanghai, China). In our assay, phosphate buffer (PB) was prepared as the buffer. All solutions and the buffers in the experiments were obtained using sterile water.

1.2 Instrumentations. The absorption spectra of AuNPs were recorded by a Shimadzu UV-2550. Fluorescence measurements were performed on a F-4600 spectrometer (Hitachi Co. Ltd., Japan) with a xenon lamp excitation source. An Eppendorf centrifuge 5415R was used for centrifugation of the AuNPs. The size distribution and surface potential were measured with a Zetasizer Nano ZS (Malvern Instruments). SERS measurements were performed with a confocal microscope (Jobin-Yvon HR-800) equipped with an air-cooled charge-coupled device detector. He–Ne laser with 632.8 nm radiations was used for excitation. Mass spectra were obtained using a MALDI-TOF MS (AB SCIEX TOF/TOF 5800) instrument, using detection by reflection to generate the spectra.

1.3 Synthesis of AuNPs and Peptide-stabilized AuNPs. In accordance with published methods, 15 nm AuNPs were acquired by citrate reduction of the $HAuCl_4$ salt.[1] In brief, 100 mL of 0.01% (w/w) $HAuCl_4$ was heated by boiling under vigorous magnetic stirring. Then, 3.5 mL of 33.8 mM sodium citrate solution was added immediately. The mixture was kept boiling and magnetic stirring for 20 min after turning red, and then cooled to room temperature. Subsequently, AuNPs was obtained and stored at 4 °C.

Briefly, peptide I was dissolved in PB (10 mM, pH 7.4). Then, 100 μL of peptide I (43.5 μM) was added to AuNPs (2 mL). The reaction mixture was incubated at room temperature for 3 minutes. The free peptides in solution were separated by centrifugation at 8000 rpm for 20 min, finally the peptide-stabilized AuNPs were prepared.

1.4 Preparation of ssDNA-Modified AuNPs. The thiol-DNA were treated with 0.1 M TCEP for 2 h to suppress the formation of the disulfide bonds. And then, freshly prepared DNA (0.2 μM) were

added to the peptide-capped AuNPs (3.82 nM), followed by the addition of 700 mM NaCl at room temperature for 1.5 h. Afterward, excess unbound DNA were isolated by centrifugation at 10.0 K rpm for 20 min. The ssDNA-AuNPs conjugates were resuspended in 10 mM PB (pH 7.4). This process was repeated at least three times to thoroughly wash away the free oligonucleotides. Fluorescence measurement was employed to confirm the inexistence of excess DNA in the supernatant after the last washing step.

1.5 Preparation of ssDNA-AuNPs labeled with Raman dye. To label our ssDNA-AuNPs with Raman dye, a freshly prepared 4-MBA (4-mercaptobenzoic acid) solution (1 μM) was added dropwise to a rapidly mixing peptide-capped ssDNA-AuNPs, which facilitated even distribution of the dye molecules on the gold particle surface. The solution was kept standing for 10 hours at room temperature, followed by centrifugation at 3800 rpm for 15 min three times to remove the excess unbound 4-MBA. At last, MBA-modified AuNPs was dispersed in PB solution (10 mM, pH 7.4). The strong SERS bands at 1076 cm^{-1} and 1580 cm^{-1} correspond to aromatic ring vibrations of 4-MBA.

1.6 Synthesis of AgNPs and ssDNA-AgNPs. AgNPs were synthesized by reducing $AgNO_3$ with citrate trisodium.[2] Firstly, 50 mL of $AgNO_3$ solution (1.0 mM) was added and brought to boiling. Then, 0.5 mL of citrate sodium (w/v, 5%) was added under vigorous stirring. The mixture was kept boiling and refluxed for 15 minutes, then cooling down to room temperature with continuous stirring. Finally, AgNPs solution was harvested.

As previously described in the steps 1.3, peptides were modified on AgNPs. After that, freshly prepared DNA were added, followed by the addition of 100 mM NaCl at room temperature for 4 h. The ssDNA-AgNPs conjugates were received by centrifugation and resuspension in 10 mM PB (pH 7.4). This process was repeated at least three times to thoroughly wash away the free oligonucleotides.

1.7 Quantification of ssDNA Loaded on the AuNPs. To determinate the amounts of DNA adsorbed on each particle, the concentration of AuNPs and the concentration of fluorescent oligonucleotides in each aliquot were clarified. The concentration of the AuNPs was calculated by measuring its absorbance with the UV-vis spectrophotometer, which was correlated with Beer's law ($A = \varepsilon bc$).[3,4]

For purpose of determining of the concentration of fluorescent DNA, DTT displacement was executed to replace the adsorbed DNA from the surface of AuNPs. The ssDNA-AuNPs conjugates were mixed with identical volume of 1.0 M DTT (0.18 M PB, pH 8.0), then incubated overnight. The fluorescent oligonucleotides were released into the solution, and the Au precipitate was segregated by centrifugation. The DNA supernatant was placed in a 96-well plate to conduct the fluorescence measurements. The fluorescence was transformed to molar concentrations of DNA by interpolation from a standard calibration curve. Given high sensitivity of the 6′FAM fluorophore to pH, standard DNA samples were provided with identical buffer pH, ionic strength and DTT concentration. All measurements were repeated at least three times.

1.8 Hybridization of ssDNA Targets to the ssDNA-AuNP Conjugates. To survey the hybridization performance of our ssDNA-AuNPs, the conjugate solution was incubated with 400 nM complementary DNA (target DNA) and 1.0 M NaCl for 30 min. The target DNA were labeled with 6′FAM while the attached DNA probes unlabeled. After hybridization, the unbound nonhybridized targets were isolated by centrifugation and washed off with 10 mM PB (pH 7.4) at least three times as mentioned above.

1.9 Quantitation of the Hybridization Efficiency. The surface-bound DNA duplexes, involving the probes and the targets, were replaced by DTT. Since the fluorescence was simply attributed to 6′FAM-labeled targets, the concentration of target DNA was determined by fluorescence measurements. And the surface density of probe DNA was quantified as described in previous section. The hybridization efficiency was obtained by dividing the concentration of targets by the concentration of probes.

2.0 SERS for DNA Detection. The probe 6 (target DNA) of a series of concentrations (0.05 nM, 0.1 nM, 1 nM, 10 nM, 100 nM) was added to the probe 1-AuNPs conjugates for 0.5 hours. The probe 5-AuNPs (100 μL) were then added to the solution and allowed to hybridize with gentle vortex for another 0.5 hours. After shaking by a vortex, the solution was tested by JY HR800. In this measurement, the two types of ssDNA-AuNPs conjugates were labeled with 4-MBA. The Raman intensity of 4-MBA on AuNPs at 1076 cm^{-1} or 1580 cm^{-1} was assigned for quantitative analysis of DNA.

2.1 Melting Curves of ssDNA-AuNPs Conjugates. To test the melting property of our nanoconjugates, purple aggregates were formed as described in the step 2.0. Next, the aggregated AuNPs were gradually heated, and their melting transitions were monitored using UV−vis spectrophotometer equipped with a temperature controller. Additionally, the ssDNA-AuNPs conjugates from the salt aging were used for comparison.

Supporting Figures:

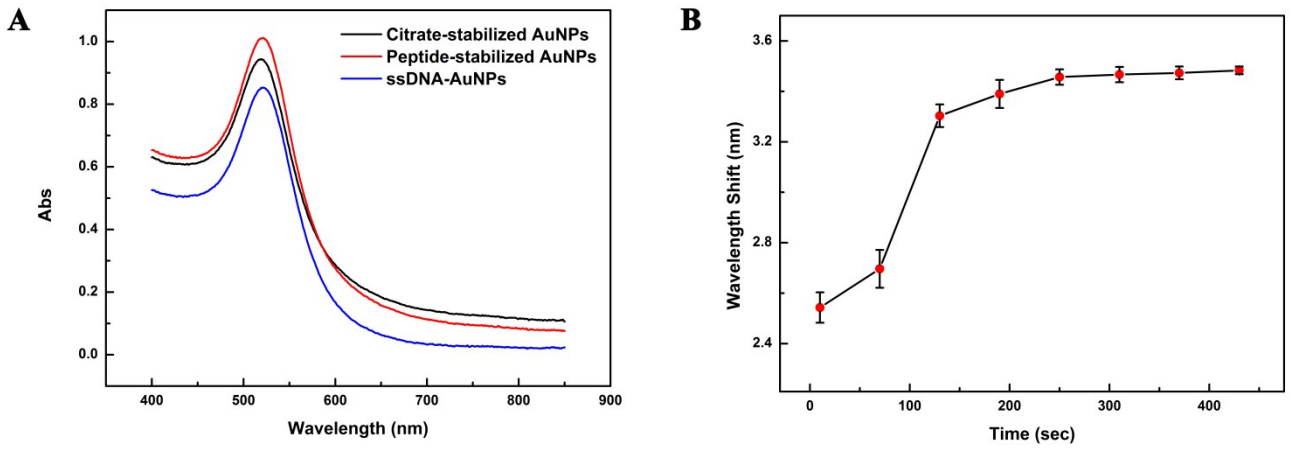

Figure S1. (A) Absorption spectra of citrate-stabilized AuNPs (black), peptide-stabilized AuNPs (red), or ssDNA-AuNPs (blue). (B) Wavelength shift of the mixture solution as a function of incubation time. The mixture solution contained 10 mM PB (pH 7.4), 43.5 μM peptide and AuNPs.

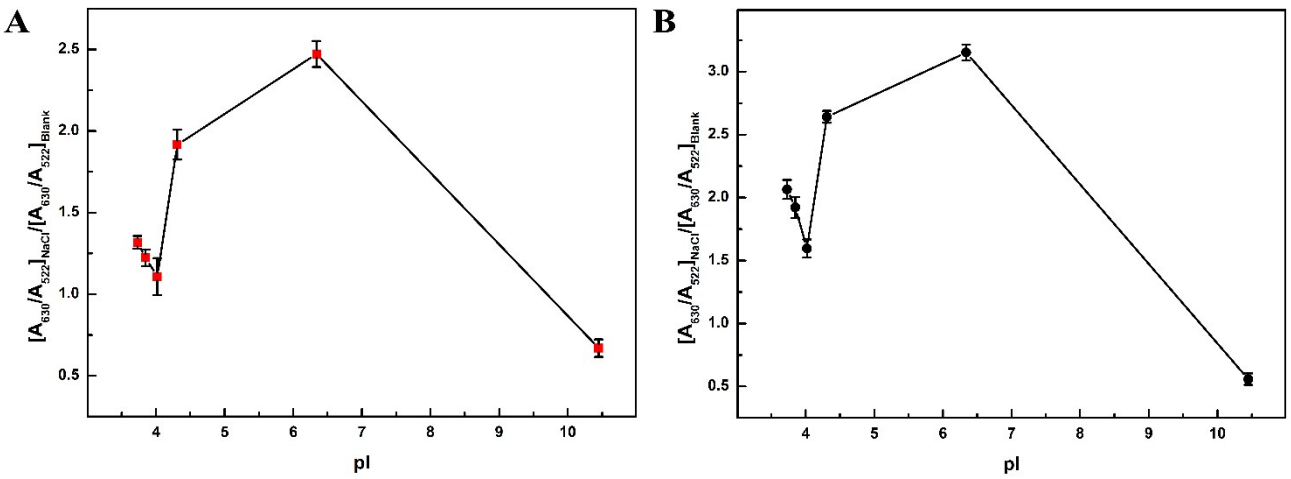

Figure S2. Colorimetric response ($[A_{630}/A_{522}]_{NaCl}/[A_{630}/A_{522}]_{Blank}$) of the peptide-stabilized AuNPs (3.82 nM) as a function of pI: RFPRGGDDDDDD (3.73), RFPRGGDDDDD (3.85), RFPRGGDDDD (4.02), RFPRGGDDD (4.31), RFPRGGDD (6.34), RFPRGGD (10.45). In the measurements, the salt concentrations are (A) 700 mM and (B) 1.0 M.

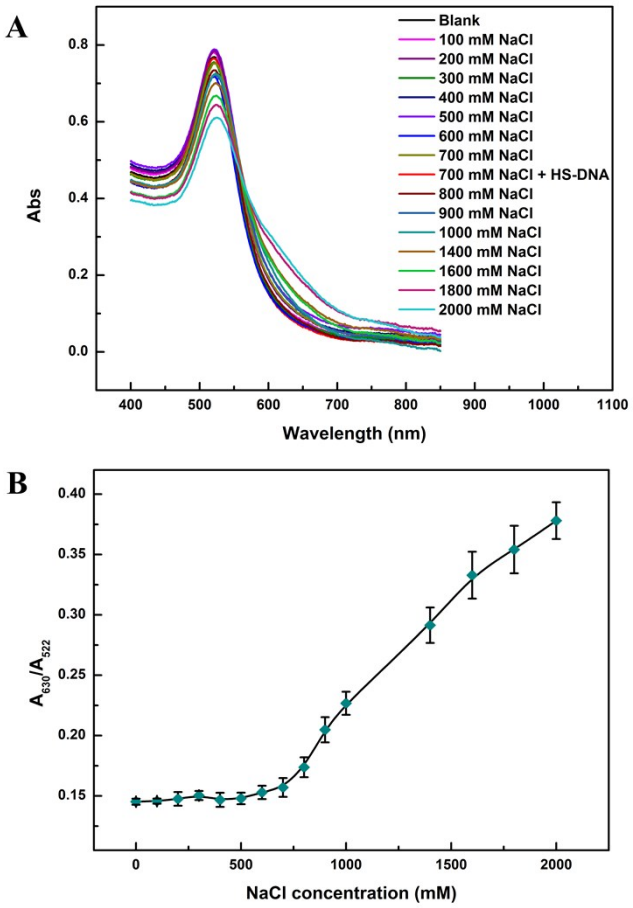

Figure S3. (A) Absorption spectra and (B) Colorimetric response (A_{630}/A_{522} values) of peptide-stabilized AuNPs treated with different concentrations of NaCl.

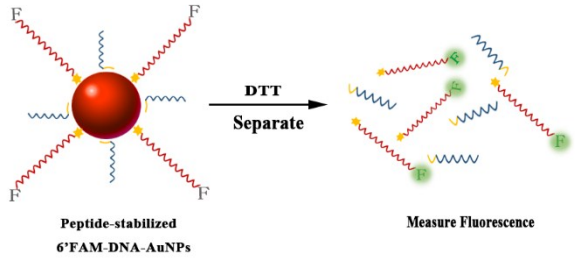

Figure S4. Schematic illustration of quantifying the adsorbed DNA using DTT displacement.

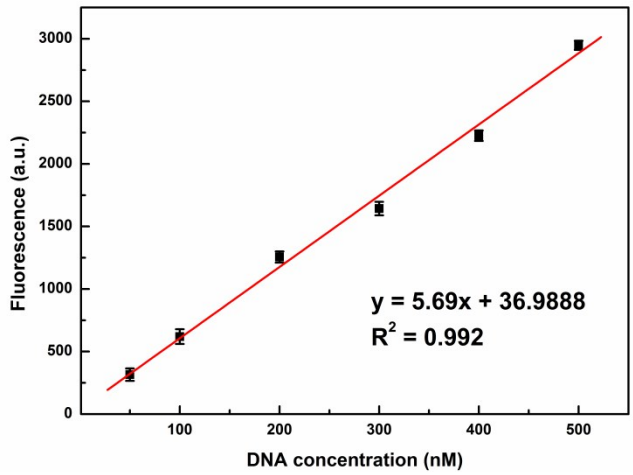

Figure S5. The standard curve of fluorescence intensity of probe 2.

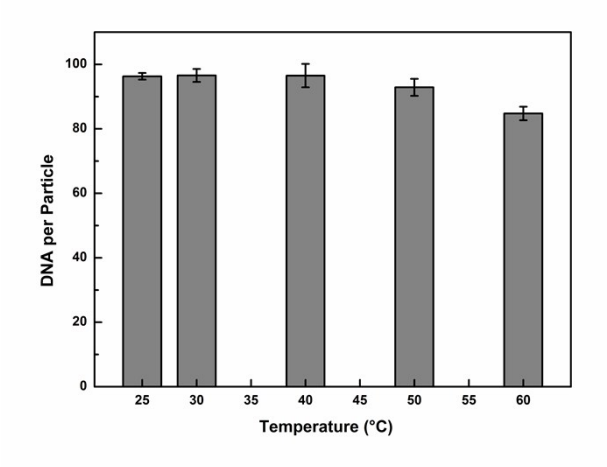

Figure S6. Surface density of probe 2 on per AuNP as a function of incubation temperature. The mixture solution contained 10 mM PB, peptide-stabilized ssDNA-AuNPs, probe 2 and 700 mM NaCl.

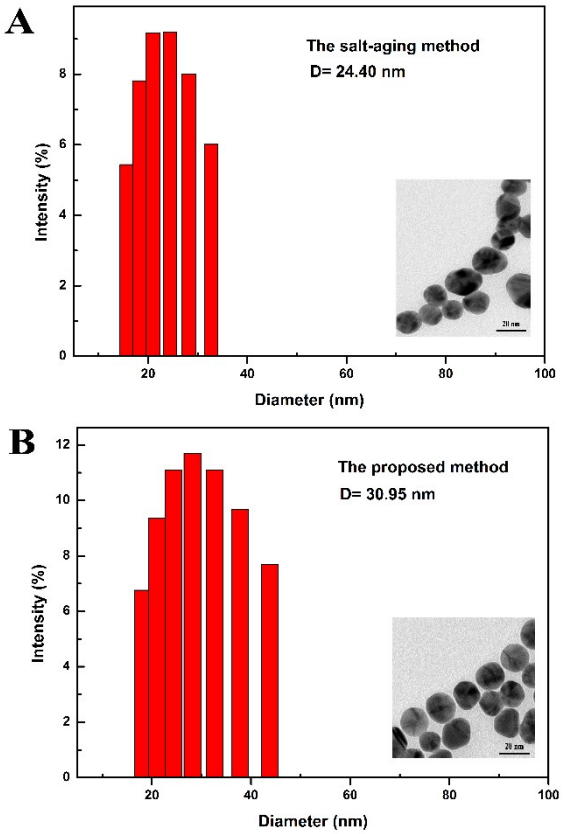

Figure S7. DLS measurements for hydrodynamic diameters of ssDNA-AuNPs conjugates from (A) the salt-aging method and (B) the proposed approach. Inset: TEM images of ssDNA-AuNPs conjugates from (A) the salt-aging method and (B) the proposed approach (scale bars: 20 nm).

Saving the maximum possible length of a 15-base ssDNA is ~ 9.9 nm,[5,6] in our case, the maximum possible diameter of ssDNA-AuNPs is approximately 34 nm (9.9*2+15=34.8 nm), and the minimum possible diameter is 15 nm (the diameter of naked AuNP). The DLS data in **Figure S7** incisively revealed that the diameter of our conjugates was 30.95 nm, while that of the conjugates from salt-aging method was 24.4 nm. The difference (~ 6 nm) means our conjugates possess a more standing configuration of DNA immobilized on particles.

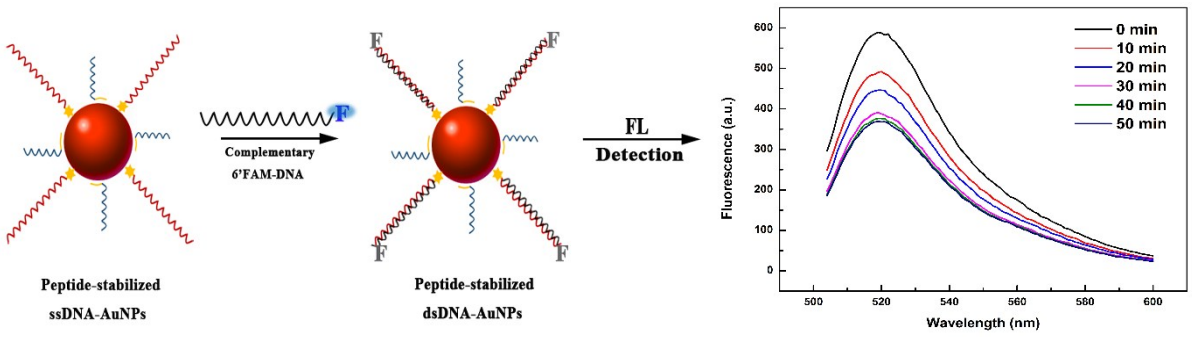

Figure S8. Schematic illustration of determining the hybridization time of peptide-stabilized ssDNA-AuNPs. The mixture solution contained 10 mM PB, 1.0 M NaCl, peptide-stabilized ssDNA-AuNPs, and 0.4 μM probe 4.

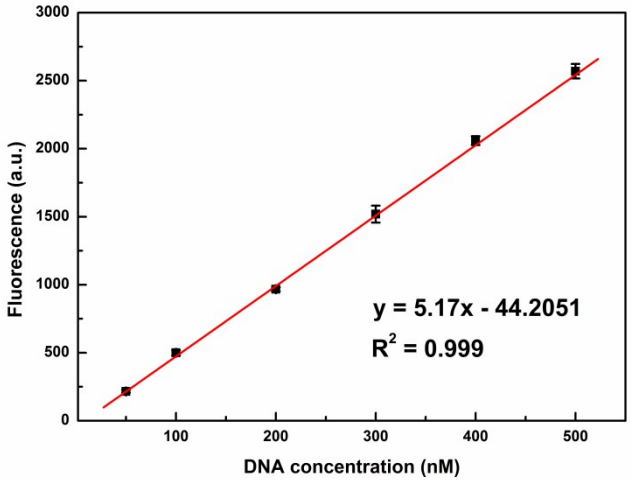

Figure S9. The standard curve of fluorescence intensity of probe 4.

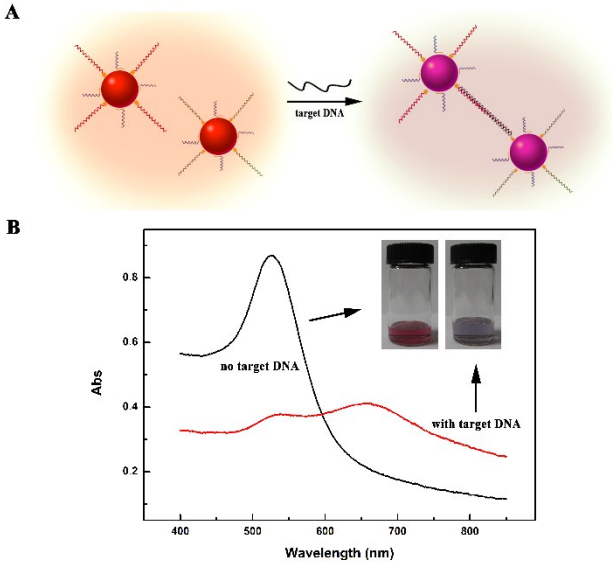

Figure S10. (A) Schematics of sandwich hybridization for DNA detection involving three bodies (probe 1-AuNPs, probe 5-AuNPs and probe 6). (B) UV−vis spectra and photographic images (inset) for the mixture solution of probe 1-AuNPs and probe 5-AuNPs in the absence and presence of probe 6 (target DNA).

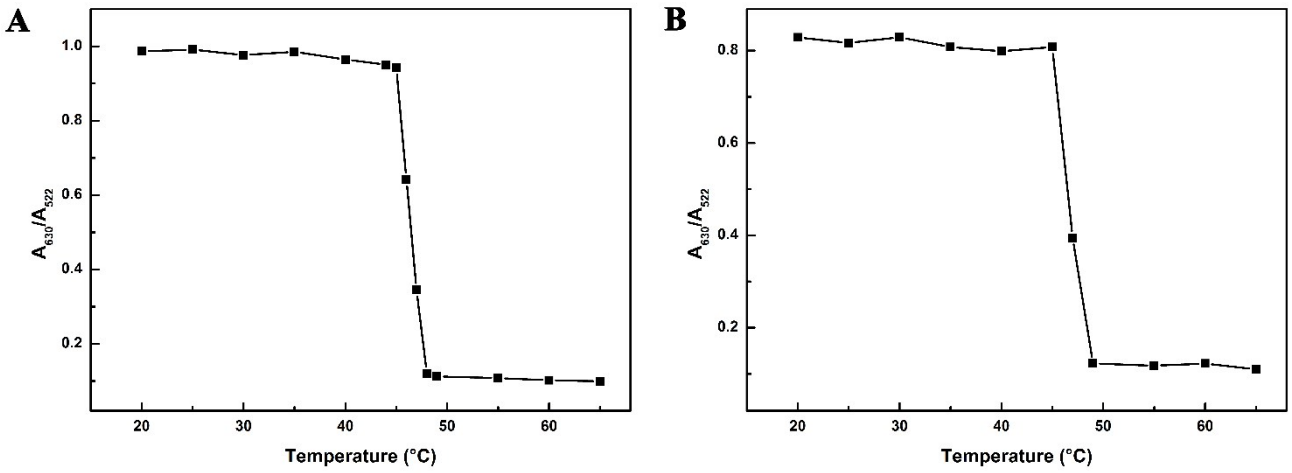

Figure 11. Melting curves of ssDNA-AuNPs conjugates from (A) the peptides stabilization and (B) the salt aging.

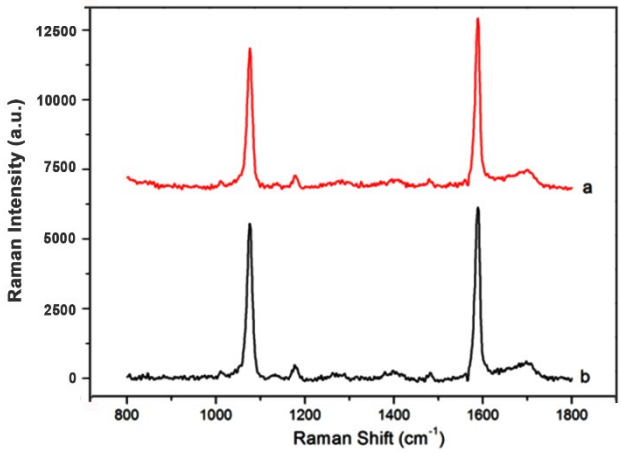

Figure S12. SERS spectra of peptide-capped ssDNA-AuNPs modified with 4-MBA (line a) and citrate-capped ssDNA-AuNPs modified with 4-MBA (line b) followed by addition of 2.0 M NaCl. The two main bands around 1076 $cm^{-1}$and 1580 cm^{-1} are attributed to 4-MBA.

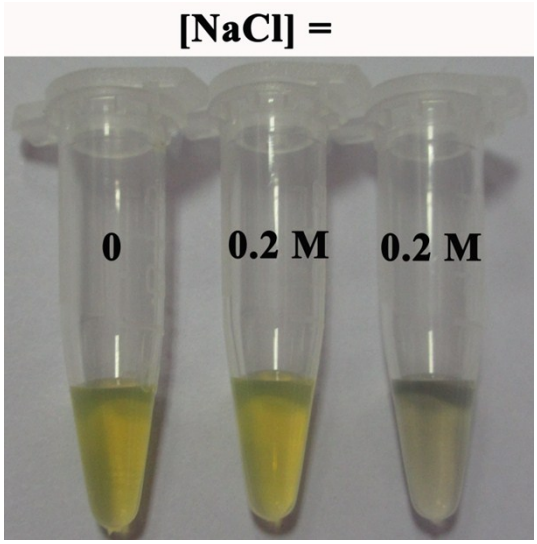

Figure S13. Photographs of 50 nm AgNPs functionalized with probe 1. The first tube had no additional salt while two others contained the same salt concentration. The last tubes did not include any DNA.

Supporting Tables:

Table S1. Mean hydrodynamic diameter (D) and mean zeta potentials determined by dynamic light scattering (DLS) techniques for citrate-stabilized AuNPs, peptide-stabilized AuNPs and ssDNA-AuNPs conjugates from the peptide stabilization, the salt aging and poly A method.

	D (nm)	**zeta potential (mV)**
Citrate-stabilized AuNPs	23.94 ± 0.59	-31.9 ± 1.30
Peptide-stabilized AuNPs	26.24 ± 1.32	-44.6 ± 2.23
Peptide-stabilized ssDNA-AuNPs	30.95 ± 0.71	-53.5 ± 3.84
Thiol-DNA-AuNPs (the salt aging)	24.40 ± 0.42	-45.3 ± 3.50
Poly A10-DNA- AuNPs	30.45 ± 0.34	-46.2 ± 2.65

Table S2. The sequences of ssDNA strands used in our work.

The ssDNA	Sequence (5’ to 3’)
Probe 1	HS-PEG-ATTTACCACTTACTT
Probe 2	HS-PEG-ATTTACCACTTACTT-6’FAM
Probe 3	ATTTACCACTTACTT-6’FAM
Probe 4	6’FAM-AAGTAAGTGGTAAAT
Probe 5	HS-PEG-CTCACCTCACTCCCACT
Probe 6	GAGTGGAGTGAGGGTGAAAGTAAGTGGTAAAT
Noncomplementary DNA	GAGTGGAATGATACTGAAAGTAAGTGCGGAAT
Poly A10-DNA	AAAAAAAAAAATTTACCACTTACTT

Table S3. The sequences of peptides used in our work.

Name	Sequence
Peptide I	RFPRGGDDDD
Peptide II	RFPRGGDDD
Peptide III	RFPRGGDD
Peptide IV	RFPRGGD
Peptide V	RRGGDDDD
Peptide VI	RATRDDDD
Peptide VII	RRAVDDDD
Peptide VIII	RRLMDDDD
Peptide IX	RRYSDDDD
Peptide X	RRDDDD

Table S4. Comparison of our method with other DNA loading approaches.

Loading Method	DNA: AuNP ratio (DNA dosage)	Loading Time	Configuration of modified DNA	Stabilization period	Hybridization Time	Hybridization Efficiency	Toxicity	Ref
FSN (surfactant)	1000:1	2 h	Upright	Uncertain	3 h	60%	√	3
Salt aging	Excess	48 h	Ruleless	Uncertain	2-4 h	10-40%	×	4
Diblock-DNA (PolyA)	75:1	3-8 min	Upright	Uncertain	24 h	42-90%	×	5
Peptide	60:1	1.5 h	Upright	At least 20 days	0.5 h	93%	×	This work

References

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