Supporting Information

Rational Design of an Anchoring Peptide for High-efficiency and Quantitative Modification of Peptides and DNAs on Gold Nanoparticles

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Supporting Figures:

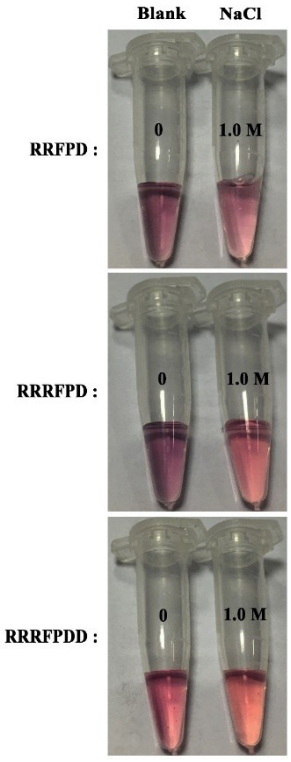

Figure S1. Photo images of AuNPs modified with different peptides (RRFPD, RRRFPD, RRRFPDD).

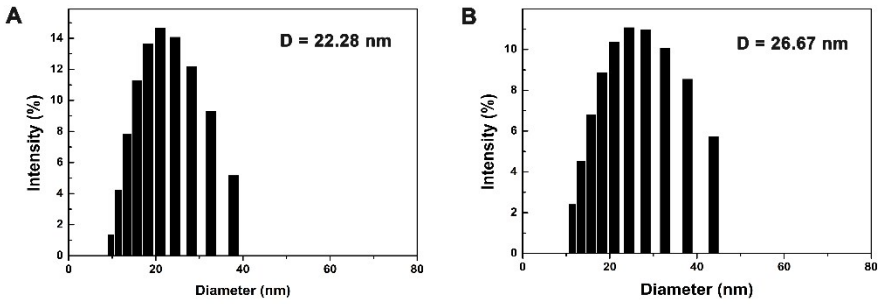

Figure S2. DLS measurements for hydrodynamic diameters of (A) 20 nm bare AuNPs and (B) 20 nm AuNPs capped with RRFPDD.

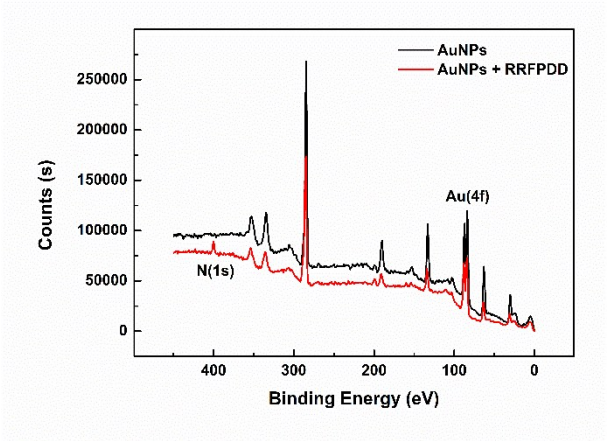

Figure S3. XPS spectra of bare AuNPs (black line) and the AuNPs capped with RRFPDD (red line).

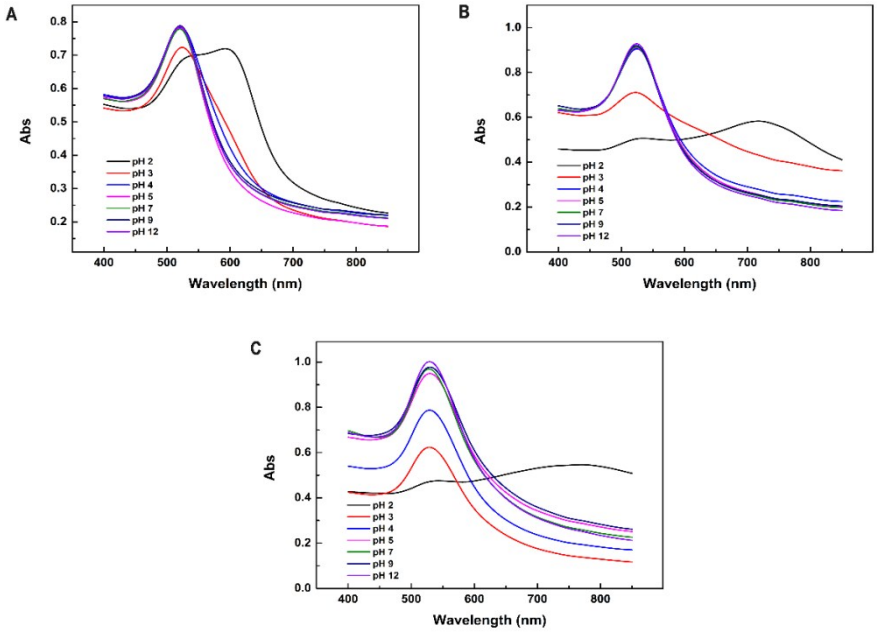

Figure S4. UV-vis spectra of RRFPDD-capped (A) 15 nm, (B) 20 nm, (C) 30 nm AuNPs at different pH ranging from 2 to 12.

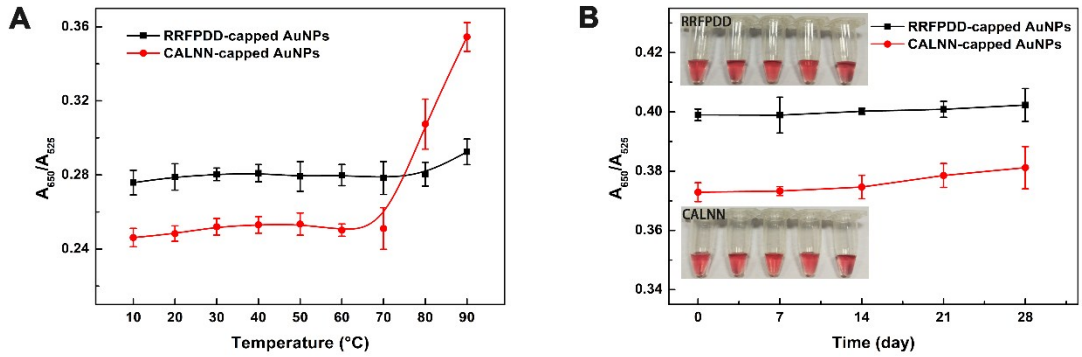

Figure S5. (A) Thermostability and (B) storage stability of RRFPDD-stabilized AuNPs (black line) and CALNN-stabilized AuNPs (red line).

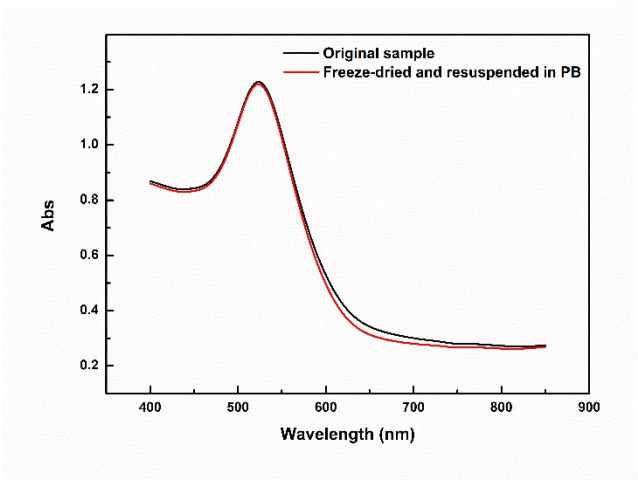

Figure S6. Absorption spectra of RRFPDD-capped AuNPs before and after freeze-drying.

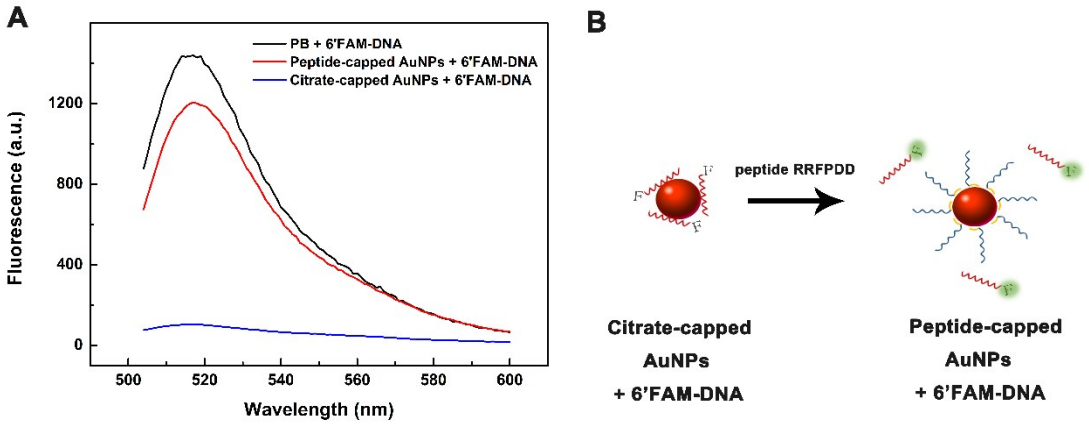

Figure S7. (A) Fluorescence intensity of FAM-DNA in PB buffer (black line), RRFPDD-capped AuNPs nanoconjugates (red line) and citrate-capped AuNPs (blue line) treated with FAM-DNA (1 μM). (B) Schematic illustration of the nonspecific adsorption of AuNPs before and after adding the peptide RRFPDD.

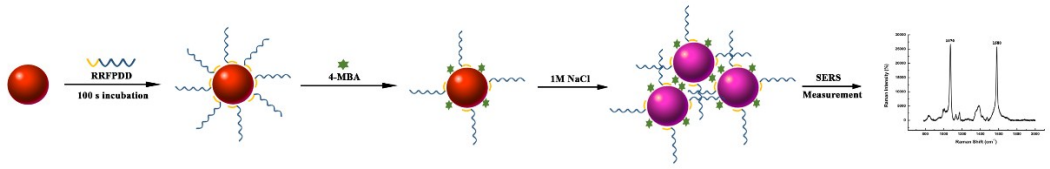

Figure S8. Schematic illustration of determining the SERS signals of 4-MBA based on the aggregation of RRFPDD-capped 20 nm AuNPs.

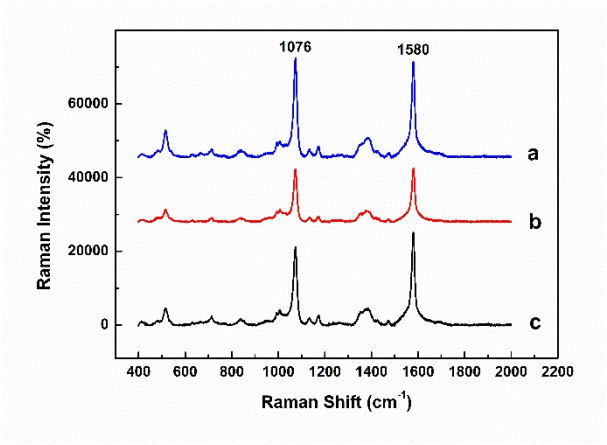

Figure S9. SERS spectra of (a) RRFPDD-AuNPs, (b) CALNN-AuNPs, (c) citrate-AuNPs. All of these nanoconjugates were modified with 4-MBA and the diameter of AuNPs was 20 nm.

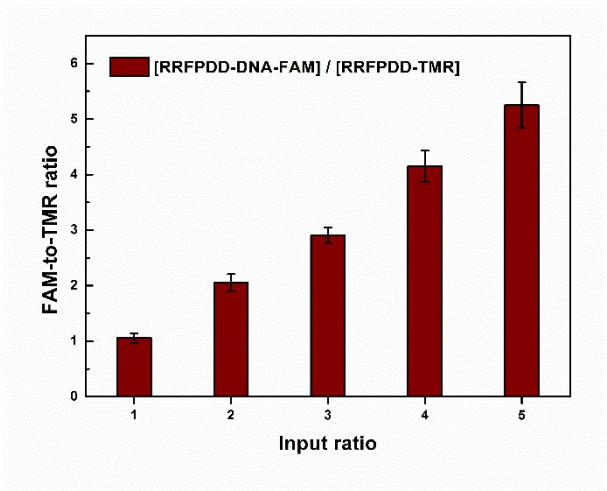

Figure S10. Loading ratio of RRFPDD-DNA-FAM (probe 5) and RRFPDD-TMR put in a certain proportion (1:1, 2:1, 3:1, 4:1, 5:1).

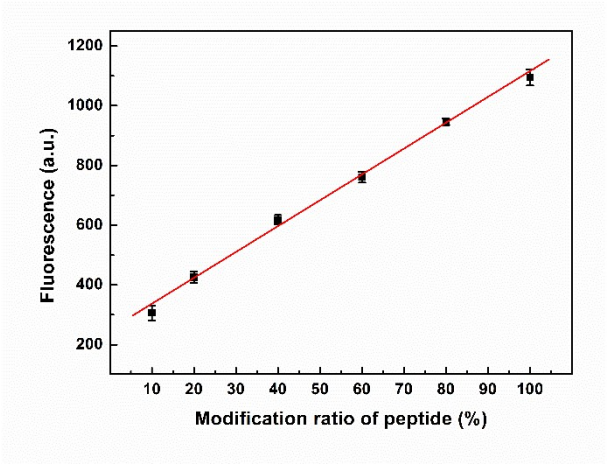

Figure S11. The standard curve of fluorescence intensity of RRFPDD-$(RD)_8$-FAM at different modification ratios.

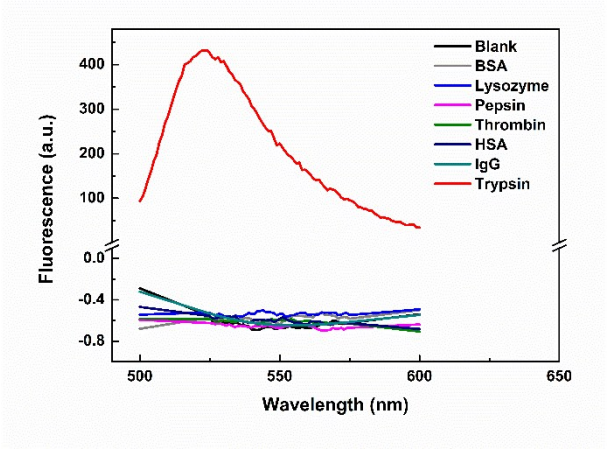

Figure S12. Specificity evaluation for the hydrolysis platform. The RRFPDD-$(RD)_8$-FAM capped AuNPs were treated with several enzymes (10 μM) and trypsin (100 nM).

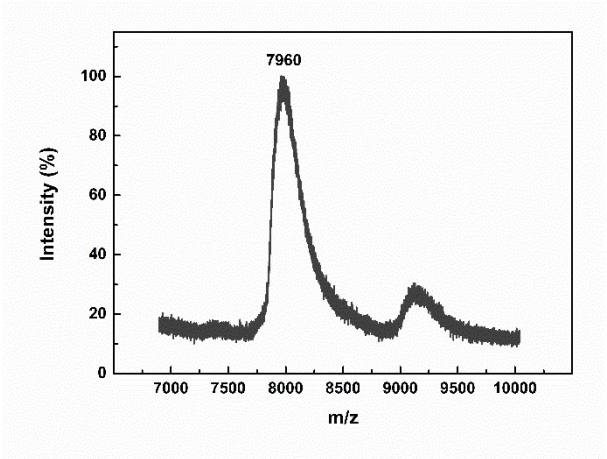

Figure S13. MALDI-TOF spectrum of probe 2 (RRFPDD-ATTTACCACTTACTTCCGGA).

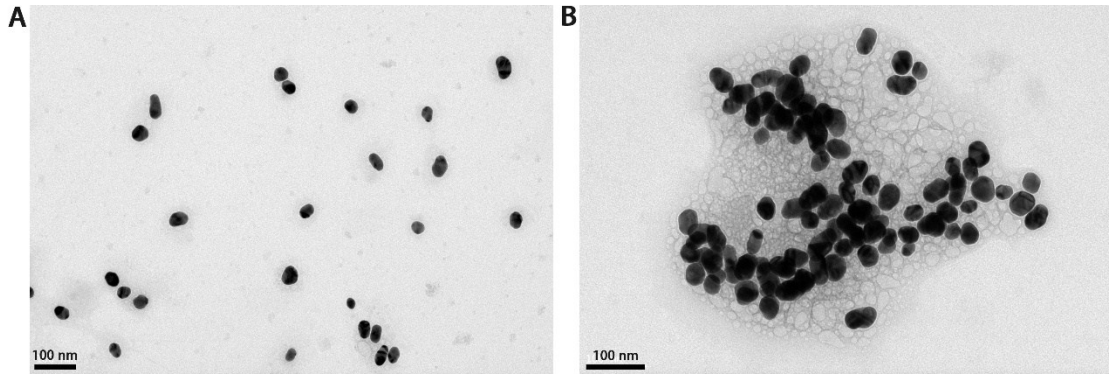

Figure S14. TEM images of peptide-capped 30 nm AuNPs in the (A) absence and (B) presence of 400 ng/mL cTnI. The peptide sequence is RRFPDD-FYSHSFHENWPS.

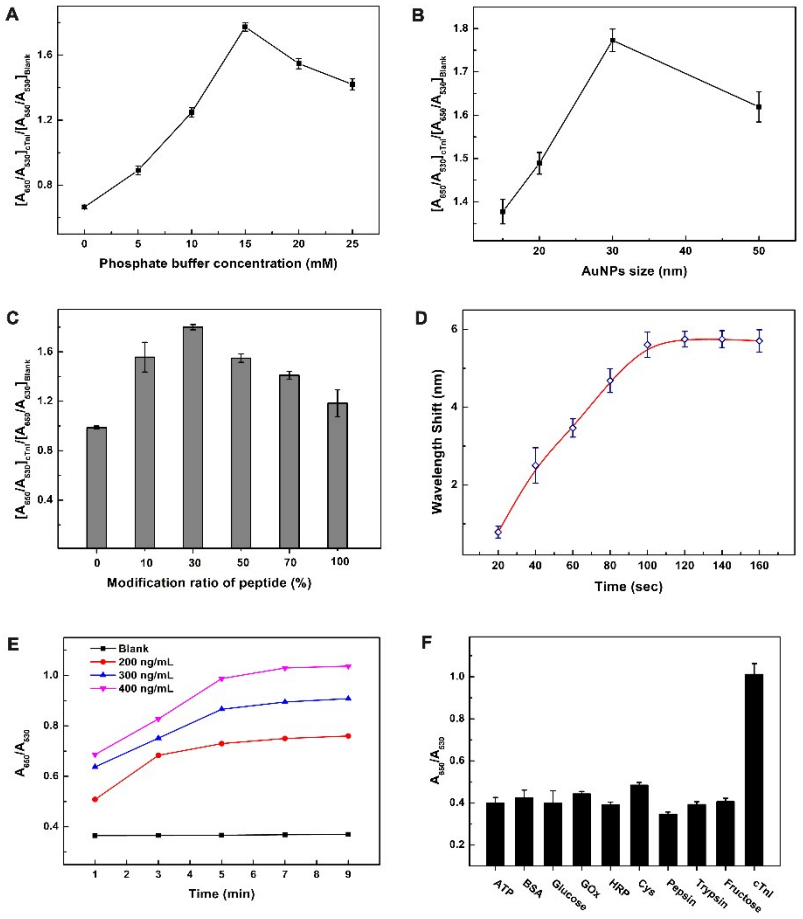

Figure S15. Effects of (A) the PB concentration, (B) the diameter of AuNPs, (C) the modification ratio, (D) the modification time of RRFPDD-FYSHSFHENWPS and (E) the reaction time. In the measurements, 150 ng/mL cTnI and 30% modification ratio of peptides were used. (F) Selectivity of the detection platform. The error bars represent standard deviations based on three independent measurements.

Supporting Tables:

Table S1. The determination of cTnI in human serum samples using the method. The data are given as the average value obtained from three independent experiments (n=3).

Sample	Added cTnI (ng/mL)	Detected cTnI (ng/mL)	Recovery (%)	RSD (%)
1	100	102.65	102.66	2.83
2	300	296.09	98.70	4.08
3	500	495.47	99.09	3.53

Table S2. Comparison of our anchor RRFPDD with the traditional CALNN.

	RRFPDD	CALNN
Binding force	Electrostatic attraction	Au-N bonds
Peptide consumption	50 μM	1.38 mM
Modification time	100 s	Overnight
Non-specific adsorption	Inhibitive	Uncertain
Further functionalization (4-MBA)	Beneficial	Profitless
Hydrolysis efficiency	85%	53%
Hydrolysis time	30 min	60 min
Quantitative modification	√	√
Freeze-drying	√	√
Stability	Salt-tolerance pH-tolerance heat-resisting frost-resisting	Salt-tolerance pH-tolerance frost-resisting

Table S3. List of peptide sequences.

Substitution in positions 1 and 2	Ratio of alkaline AA to acidic AA	Substitution in positions 3 and 4	Terminal modification
RRFPDD-FAM	RFPD	RRAFDD	RRFPDD-V_4-FAM[a]
KKFPDD-FAM	RFPDD	RRAIDD	RRFPDD-S_4-TMR[a]
HHFPDD-FAM	RFPDDD	RRALDD	RRFPDD-TMR
	RFPDDDD	RRAPDD	RRFPDD-$(RD)_8$-FAM[a]
	RRFPD	RRAVDD	CALNN-$(RD)_8$-FAM[a]
	RRFPDD	RRFLDD	RRFPDD-FYSHSFHENWPS
	RRFPDDD	RRFPDD	CALNN-TMR
	RRRFPD	RRILDD	
	RRRFPDD	RRLADD	
		RRVLDD	
		RRDD	

[a.] The RRFPDD-VVVV, RRFPDD-SSSS, RRFPDD-RCFRGGDD and CALNN-RCFRGGDD are abbreviated as RRFPDD-V_4, RRFPDD-S_4, RRFPDD-$(RD)_8$, CALNN-$(RD)_8$, respectively.

Table S4. List of DNA sequences.

The ssDNA	Sequence (5' to 3')
Probe 1	ATTTACCACTTACTT-FAM
Probe 2	RRFPDD-ATTTACCACTTACTTCCGGA
Probe 3	HS-PEG-CTCACCTCACTCCCACT
Probe 4	GAGTGGAGTGAGGGTGATCCGGAAGTAAGTGGTAAAT
Probe 5	RRFPDD-ATTTACCACTTACTTCCGGA-FAM