Supporting Information

Peptide Microarray-Based Metal Enhanced Fluorescence Assay for Multiple Profiling of Matrix Metalloproteinases Activities

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1. Additional experimental section

Fabrication of peptide microarray FRET-peptide substrates in spotting solution (acetic acid-sodium acetate buffer solution (pH 4.0, 0.1 M) with 20 μg mL^{-1} BSA and 5% (w/v) PEG-8000) at desired concentrations were spotted on the Au/Ag@SiO_2 substrates and commercial aldehyde group-modified glass slides using a SmartArrayer 136 system (CapitalBio Ltd., Beijing, China). After an overnight incubation under vacuum at 30 °C, the slides were washed with phosphate buffer (pH 7.5, 50 mM) supplemented with 1% (w/v) BSA, and then incubated in blocking buffer (pH 7.5, 50 mM PB, 0.15 M NaCl containing 1% (w/v) BSA and 1% (v/v) ethanolamine) for 1 h to inactivate remaining free aldehyde groups on the slide surface. After the blocking reaction, the slides were subjected to a series of washing steps: (1) 30 mL of Milli-Q water for 3 min (3 times), (2) 30 mL of washing buffer (pH 7.5, 20 mM Tris, 0.15 M NaCl with 0.1% (v/v) Triton X-100) for 10 min (2 times), and (3) 30 mL of TCNB buffer (50 mM Tris, 10 mM $CaCl_2$, 150 mM NaCl and 0.05% (w/v) Brij-35, pH 7.5) for 10 min (2 times), respectively. After dried by centrifugation (300 g), the peptide microarray was divided into 12 independent subarrays by a PTFE mask, and employed to detect the activities of MMPs.

To evaluate the MEF performance of the Au/Ag@SiO_2 substrate, the Cy3 or FITC modified peptide (sequence, CGGKGPLGLARK-Cy3/FITC) microarray was fabricated as previously described.

Synthesis of silica nanoparticles and preparation of SiO_2 substrate Silica nanoparticles with average diameter of 250 nm were synthesized using the Stöber method. Briefly, 2.2 mL of $NH_3 \cdot H_2O$ (28%) was added to 26.5 mL of absolute ethanol, and stirred rapidly for 20 min at room temperature. Subsequently, 0.75 mL of TEOS was added dropwise to the mixture, and stirred for another 3 h. The obtained silica nanoparticles were centrifuged (9000 rpm) and washed 3 times with ethanol, then redispersed in 10 mL of 1-butanol. The SiO_2 substrate was fabricated according to Sugawa's work.[20] In detail, the silica nanoparticles in 1-butanol were added to ultrapure water in a petri dish, forming colloidal crystals of the silica nanoparticles on

water surface. Then, the hydrophilic glass slide was placed in contact with the surface, the silica particles were spontaneously transferred to the glass slide by Marangoni flow, resulting in monolayer of well-organized 2D close-packed hexagonal arrays. The substrate was annealed at 500 °C for 1 h to physically strengthen the colloidal crystals, and then aminated by APTES and activated by GA as before.

Determination of pure MMPs activities ProMMP-2 and proMMP-9 were activated at 1 μg mL^{-1} by 1 mM APMA in TCNB buffer at 37 °C for 1 h. ProMMP-7 was activated at 1 μg mL^{-1} with 1 mM APMA in TCNB at 37 °C for various times. While the latent MMP-3 was activated at 10 μg mL^{-1} in TCNB containing 5 μg mL^{-1} chymotrypsin at 37 °C for various times. Then, 2 mM pre-warmed PMSF was added to stop the activation reaction. And proMMP-14 was activated at 1 μg mL^{-1} with 0.1 μg mL^{-1} rhTrypsin 3 in activation buffer (50 mM Tris, 0.15 M NaCl, 10 mM $CaCl_2$, 5 μM $ZnCl_2$, 0.05% (w/v) Brij-35, pH 7.5) at 37 ºC for various times. Subsequently, the reaction was terminated with 1 mM AEBSF and incubated at room temperature for 15 min. After activated, 30 μL of the solutions containing various concentrations of activated pure MMPs were applied to each subarray and incubated in dark at 37 °C for various times. The microarrays were finally washed with 30 mL of PBS with 1% (v/v) Tween-20 for 5 min (3 times), 30 mL of PBS for 5 min (3 times) and 30 mL of Milli-Q water for 3 min (3 times), and dried by centrifugation (300 g) before scanning, respectively.

Determining the activities of cell-secreted MMPs Two breast cancer cell lines (MDA-MB-231 and MCF-7), three colorectal cancer cell lines (SW480, HT-29 and SW620), HeLa (cervical carcinoma cell) and HT-1080 (human fibrosarcoma cell) were cultured in desired fresh medium supplemented with 10% fetal bovine serum (FBS) and 100 U mL^{-1} penicillin-streptomycin in humidified air with CO_2 at 37 °C. RPMI was used for culturing HT-1080, McCoy's 5A was used for culturing HT-29, DMEM was used for culturing MCF-7 and HeLa, and L-15 was used for culturing SW620, SW480 and MDA-MB-231, respectively. All the cells were then seeded in 48-well culture plates at desired densities and grown in fresh culture media supplied

with 10% FBS for 24 h. After washing with PBS (300 μL, twice), the cells were starved in serum-free culture medium for 12 h. Then, the starved cells were cultured in fresh serum-free medium for an additional 24 h. The conditioned medium was collected and centrifuged (10 min, 1000 rpm) at 4 °C. After adjusting the pH to 7.5, the collected supernatants were applied to the subarrays (30 μL per subarray) and treated as previously described to determine the activities of pure MMPs.

Purification of MMPs from homogenate supernatant of tissue samples The homogenate supernatant was incubated with 50 μL of gelatin-sepharose 4B (washed three times with working buffer before use) under constant shaking (100 rpm) at 4 °C for 1 h. After centrifugation (7000 rpm, 5 min, 4 °C), the supernatant was discarded, and the gelatin-sepharose pellet was resuspended in working buffer and centrifuged again. Finally, the pellet was incubated with desired volume of elusion buffer (working buffer supplied with 10% (v/v) DMSO) for 30 min. The mixture was centrifuged (7000 rpm, 5 min, 4 °C) and the supernatant was stored at -20 °C for assaying MMPs activity.

2. Additional Table S1-S3

Table S1 FRET peptide substrate sequences used in the experiments

Code	Sequences (N→C)	Reference
Sub 2	CGRRRK(FAM)G*IPVSLRSG*K(Dabcyl)-NH_2[a]	S1
Sub 3	CGRRRK(FAM)*RPKPVE-Nva-WR*K(Dabcyl)-NH_2	S2
Sub 7	CGRRRK(FAM)*RPLALWRS*K(Dabcyl)-NH_2	S3
Sub 9	CGRRRK(FAM)GG*PRSLS*GGK(Dabcyl)-NH_2	S4
Sub 14	CGRRRK(FAM)*PLPLRSWGL*K(Dabcyl)-NH_2	S5

[a] The recognition sequences of MMPs were indicated by the Italics letters.

The FRET peptide substrates were designed according to the kinetic parameters for the cleavage of consensus peptides by MMPs based on the peptide library in the literatures.[S1-S5] The peptide substrates can be efficiently and selectively cleaved by the corresponding MMPs.

Table S2 Details of clinical thyroid tissue samples

NO.	Gender	Age	LNM	vessel invasion	neural invasion
PTC					
1	F	27	+[a](4/10)[b]	+	–
2	F	31	+(1/4)	–	–
3	F	47	–(0/1)	–	+
4	F	40	+(6/8)	+	–
5	M	37	+(1/1)	–	–
6	F	54	–(0/3)	–	–
7	F	35	–(0/1)	–	–
8	F	54	–(0/1)	–	–
9	F	47	–(0/4)	–	–
10	F	35	+(12/16)	+	+
11	F	53	–	–	–
12	F	34	–(0/5)	–	–
13*	F	34			
14	F	45	+(5/15)	+	–
15*	F	45			
16	F	42	–(0/1)	–	–
17	F	49	–	–	–
18*	F	49			
19	F	38	+(1/4)	–	+
TN					
20	F	29	–	–	–
21	F	41	–	–	–
22*	F	41	–	–	–
23	F	55	–	–	–
24	F	60	–	–	–
25	F	61	–	–	–

[a] + means patient with LNM, vessel invasion or neural invasion, – means patient without LNM, vessel invasion or neural invasion; [b] (the number of lymph node metastatic)/(total number of sampled lymph nodes), or no sampled lymph nodes; * the thyroid tissues are the adjacent tissues of tumors or nodules from the closest above patients.

Table S3 Comparison of the assay performance on commercial aldehyde slide and Au/Ag@SiO_2 substrate

	Au/Ag@SiO_2 substrate		commercial aldehyde slide	
	LOD	Linear range	LOD	Linear range
MMP-2	12.2 fg mL^{-1}	0.1 pg mL^{-1}–100 ng mL^{-1}	10 pg mL^{-1}	10 pg mL^{-1}–100 ng mL^{-1}
MMP-3	60 pg mL^{-1}	0.1 ng mL^{-1}–500 ng mL^{-1}	100 pg mL^{-1}	100 pg mL^{-1}–100 ng mL^{-1}
MMP-7	0.22 pg mL^{-1}	10 pg mL^{-1}–100 ng mL^{-1}	5 pg mL^{-1}	10 pg mL^{-1}–5 ng mL^{-1}
MMP-9	102 fg mL^{-1}	1 pg mL^{-1}–100 ng mL^{-1}	10 pg mL^{-1}	10 pg mL^{-1}–100 ng mL^{-1}
MMP-14	0.68 ng mL^{-1}	1 ng mL^{-1}–250 ng mL^{-1}	1 ng mL^{-1}	1 ng mL^{-1}–100 ng mL^{-1}

3. Additional Figure S1-S10

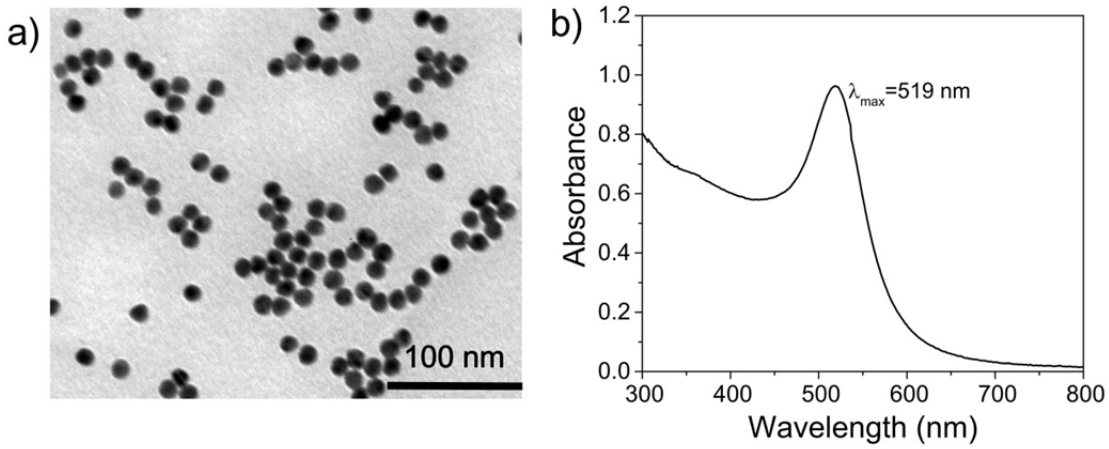

Figure S1 TEM micrograph (a) and UV-visible spectrum (b) of the as-prepared 13 nm GNPs.

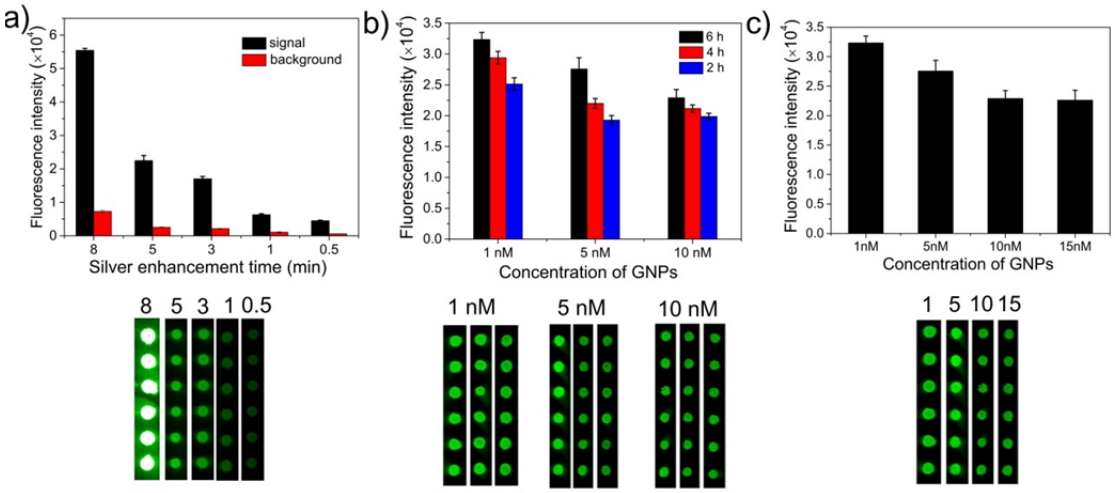

Figure S2 The effect of silver enhancement time (a), self-assembly time (b) and concentration of GNPs (c) on the fluorescence intensity of Cy3 on the Au/Ag@SiO_2 substrate.

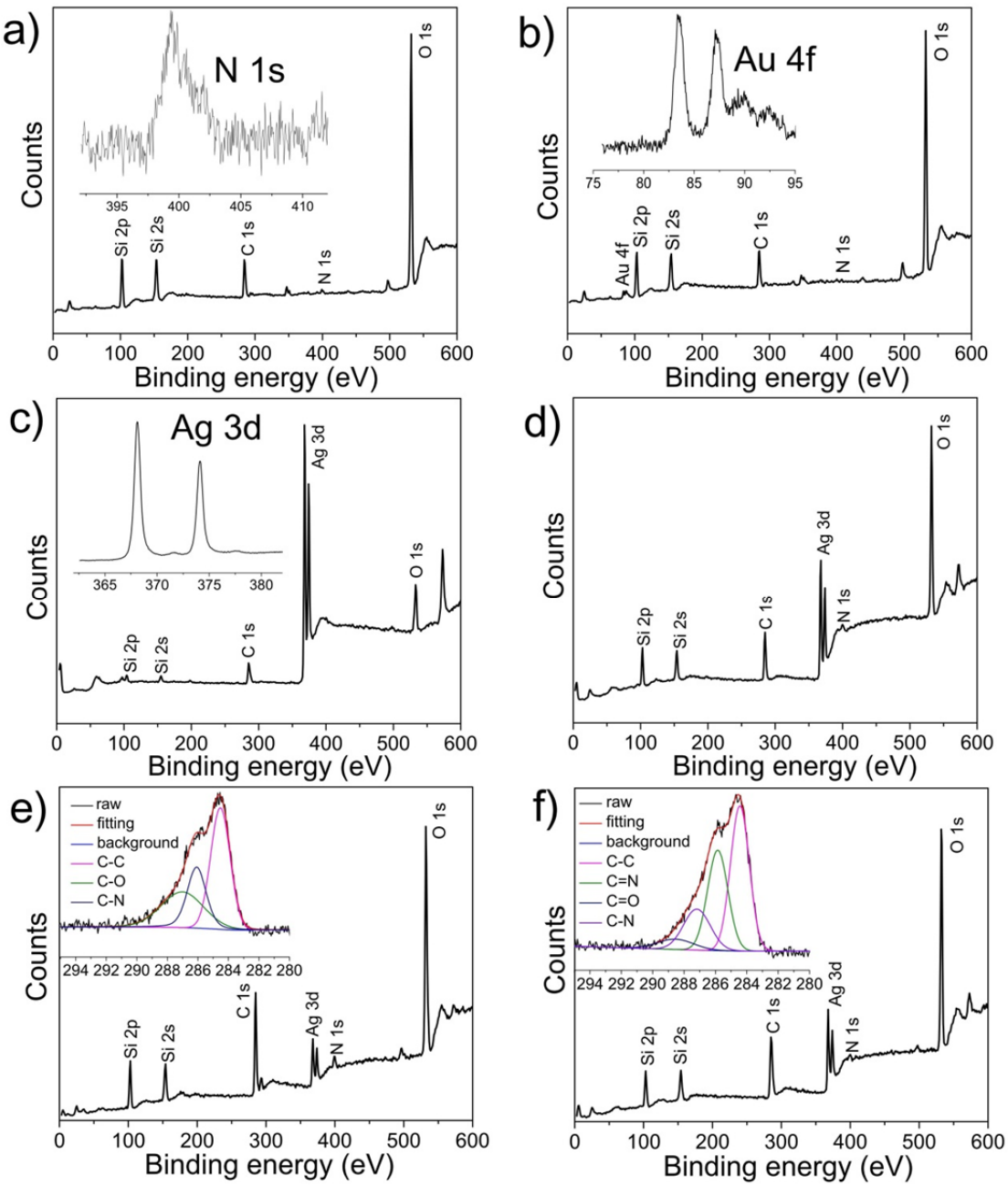

Figure S3 XPS wide scan of the stepwise modified glass slide (a-f): (a) APTES-modified slide, the inset is detailed N 1s; (b) GNPs self-assembled slide, the inset is detailed Au 4f; (c) GNP@Ag NPs modified slide, the inset is detailed Ag 3d, (d) SiO_2 shell coated Ag slide; Au/Ag@SiO_2 slide after APTES modification (e) and GA activation (f), the inset are high-resolution XPS C_{1s} spectra and their peak fitting curves.

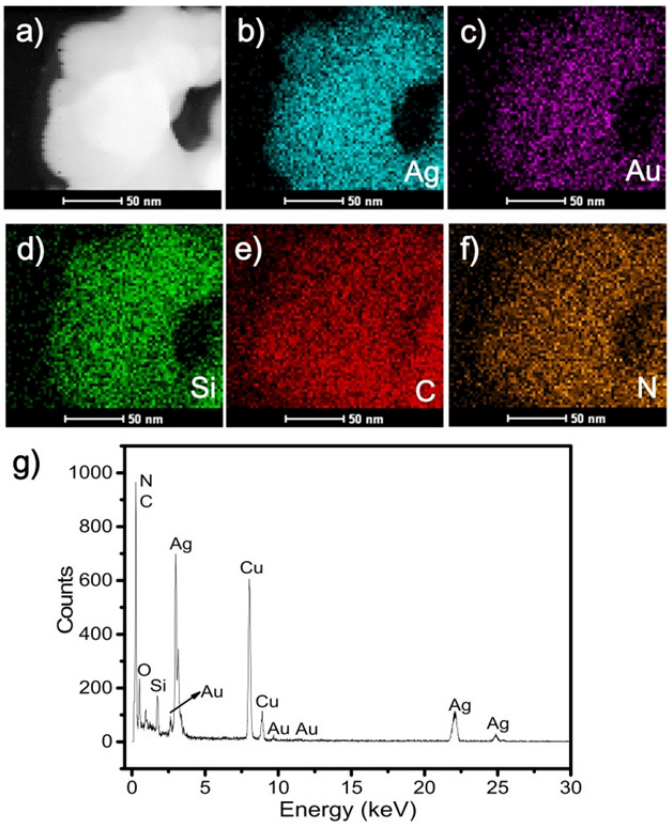

Figure S4 TEM mapping images (a-f) and EDX analysis (g) of the as-prepared Au/Ag@SiO_2 nanostructures.

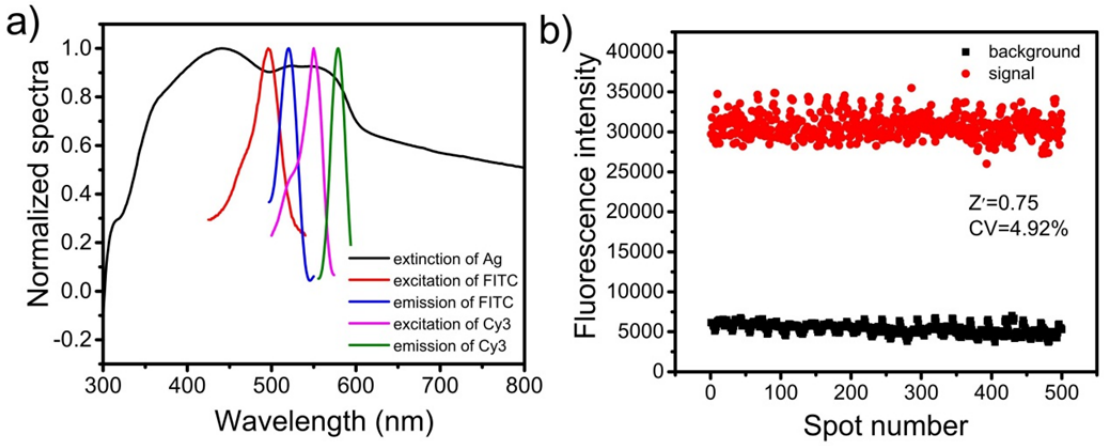

Figure S5 (a) Normalized extinction spectrum of Au/Ag@SiO_2 substrate and excitation and emission spectra of FITC and Cy3. Due to the difference between the vertical ordinate of spectra, the maximum peak value of each spectrum is normalized as 1. (b) Fluorescence intensities of Cy3 of 500 spots on the Au/Ag@SiO_2 substrate. The mean values and standard deviations of the signal and background were used to calculate the Z' factor and CV.

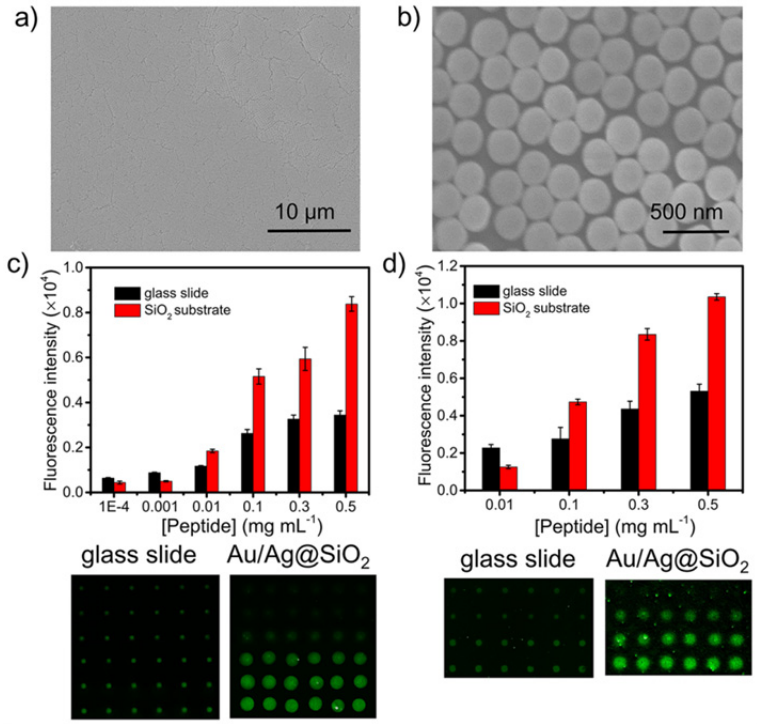

Figure S6 SEM images of the prepared SiO_2 substrate with different magnifications (a and b); the fluorescence intensities and microarray images of Cy3 (c) and FITC (d) modified peptide on SiO_2 substrate and aldehyde modified glass slide after washing.

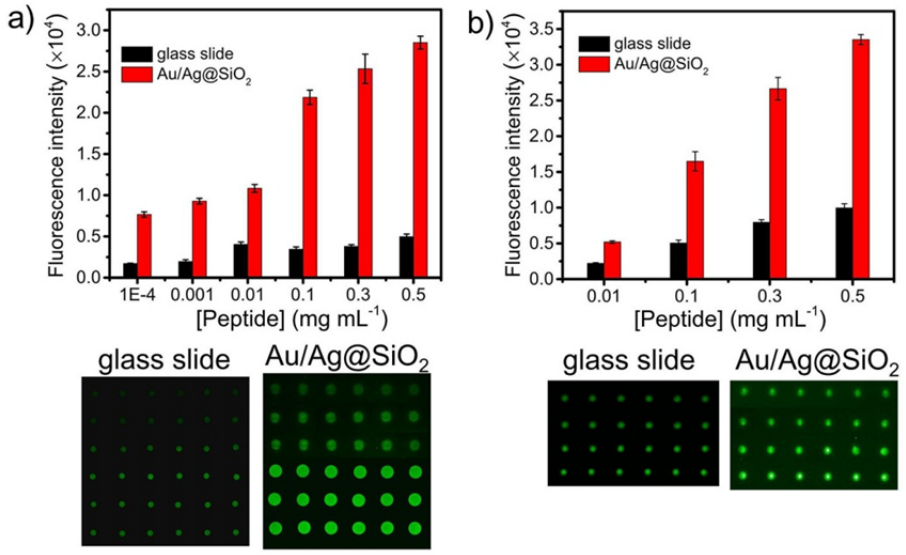

Figure S7 The fluorescence intensities and microarray images of Cy3 (a) and FITC (b) modified peptide on the Au/Ag@SiO_2 substrate and aldehyde modified glass slide just after spotting.

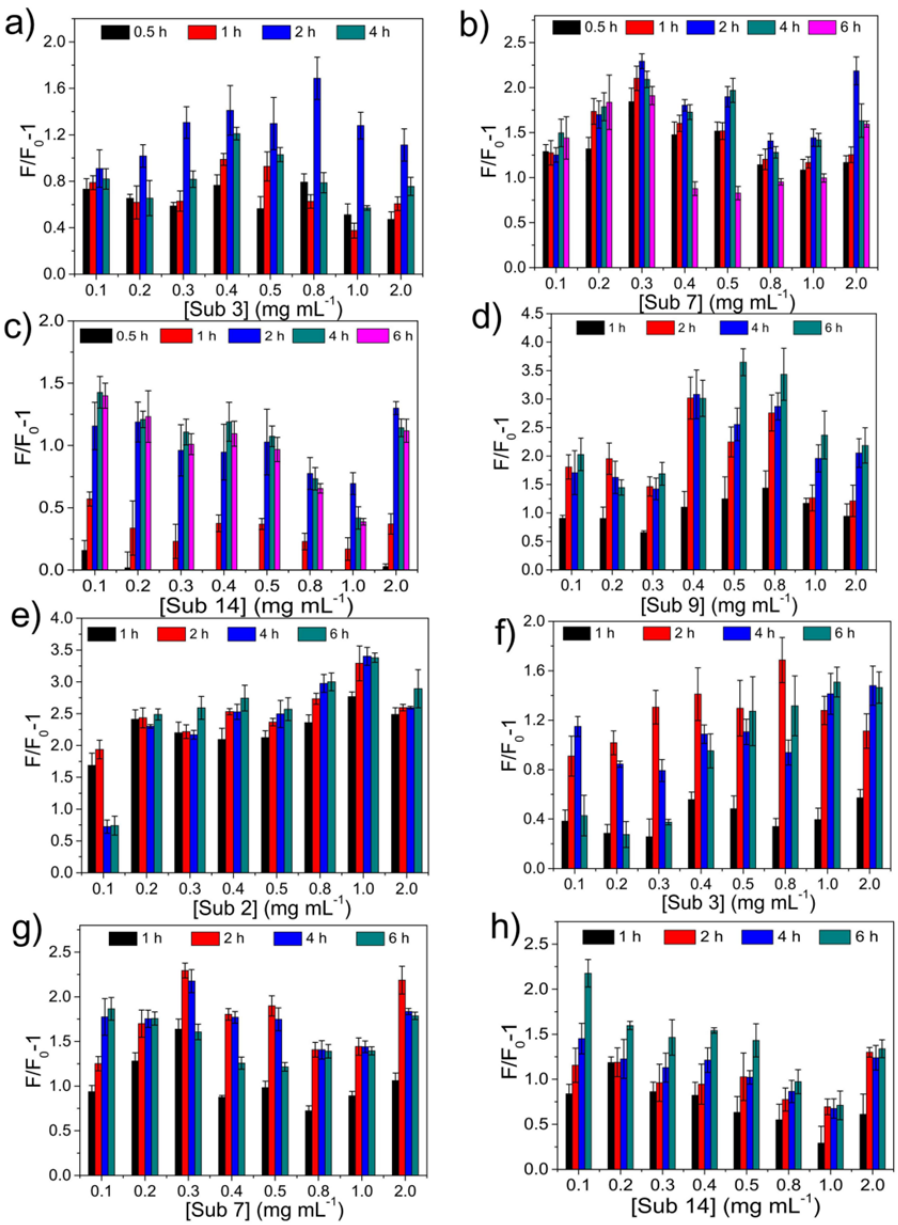

Figure S8 The effect of activation time (a to c), the reaction time of enzyme cleavage (d to h) and concentration of FRET peptide substrates in the spotting buffer on the cleavage efficiencies of MMPs (MMP-3 (a), MMP-7 (b), MMP-14 (c), MMP-9 (d), MMP-2 (e), MMP-3 (f), MMP-7 (g), MMP-14 (h)).

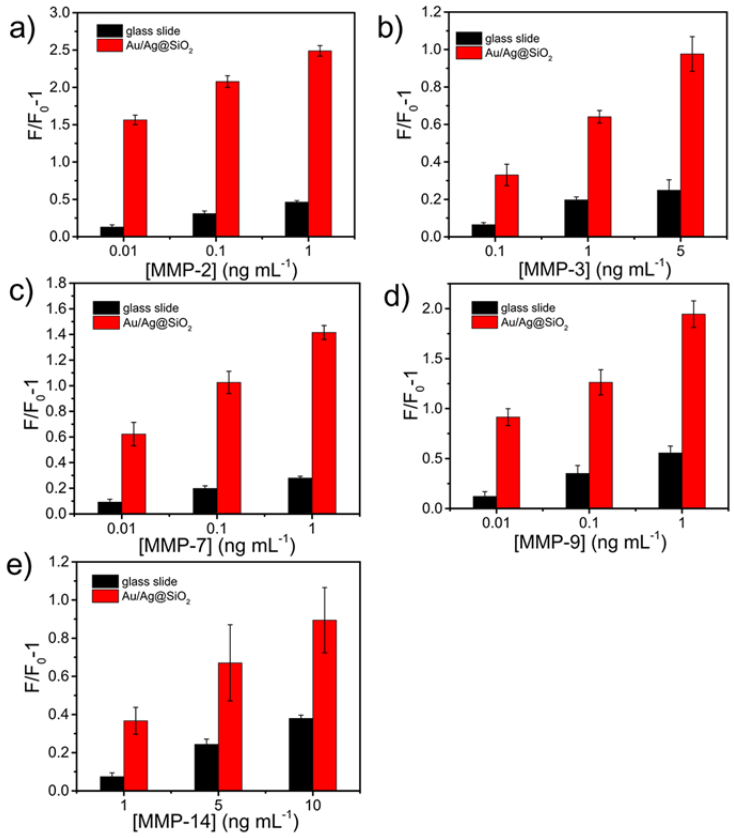

Figure S9 Comparison of relative fluorescence recovery ($F/F_0–1$) on the $Au/Ag@SiO_2$ substrate and commercial aldehyde group-modified glass slide ((a) MMP-2, (b) MMP-3, (c) MMP-7, (d) MMP-9, (e) MMP-14).

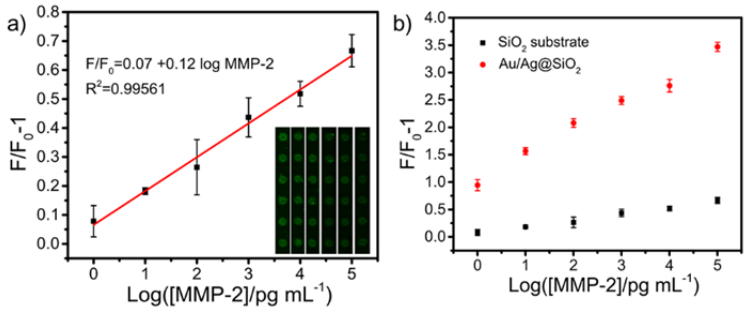

Figure S10 (a) Calibration curve of the relative fluorescence recovery (F/F_0-1) as a function of the logarithm of MMP-2 concentration on the SiO_2 substrate, the insets are corresponding fluorescence images of microarrays; (b) Comparison of assay performance on the Au/Ag@SiO_2 substrate and SiO_2 substrate.

4. Additional References

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