Electronic Supplementary Information for

Ultrasensitive dual-channel detection of matrix metalloproteinase-2 in human serum using gold-quantum dots core-satellite nanoprobes

Tingting Zheng,[a,b] Rui Zhang,[a] Qingfeng Zhang,[b] Tingting Tan,[c] Kui Zhang,[c] Jun-Jie Zhu,[*,a] and Hui Wang[*,b]

[a] *State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing, Jiangsu 210093, China*

[b] *Department of Chemistry and Biochemistry, University of South Carolina, 631 Sumter Street, Columbia, South Carolina 29208, United States*

[c] *Department of Medical Laboratory, The Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing, Jiangsu 210008, China*

[*] Corresponding authors. Emails: jjzhu@nju.edu.cn (J.-J. Zhu); wang344@mailbox.sc.edu (H. Wang)

S1. Experimental Details:

Materials and Reagents. PDMS monomer and curing agents were purchased from Dow Corning (Midland, MI). 6-Mercapto-1-hexanol (MCH), 1-(3-(dimethylamino)-propyl)-3-ethylcarbodiimide hydrochloride (EDC), N-hydroxysulfosuccinimide (NHS), and tris(2-carboxyethyl) phosphine hydrochloride) (TCEP), 1,10-phenanthroline, bovine serum albumin, Glucose, IgG, Glutathione, and IL-6 were purchased from Sigma-Aldrich (St Louis, MO). Chloroauric acid, trisodium citrate, imidazole, KCl, and $MgCl_2$ were obtained from Shanghai Chemical Reagent Co. (Shanghai, China). MMP-2 and MMP-3 was obtained from Sino Biological Inc. (Beijing, China). The biotinylated PLGVR-peptide (biotin-Gly-Pro-Leu-Gly-Val-Arg-Gly-Cys) was synthesized and purified by Shanghai GL Biochem, Ltd. (purity 95.98 %, molecular weight 984.22, Shanghai, China). The thiol- and amine-functionalized DNA oligonucleotides with the sequence of 5'-SH-$(CH_2)_6$-ATG GAG ATG CTC ATC-$(CH_2)_6$-NH_2-3' were synthesized and purified by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). The human serum samples were provided by Nanjing Drum Tower Hospital. TCNB buffer (50 mM Tris with 10 mM $CaCl_2$, 150 mM NaCl, and 0.05% Brij 35; pH 7.5) was used in the experiments. Ultrapure water (18.2 kΩ resistivity, Milli-Q, Millipore) was used for all the experiments.

Apparatus. UV-vis extinction and fluorescence spectroscopic measurements were performed on a UV-3600 spectrophotometer and a RF-5301PC fluorometer (Shimadzu, Kyoto, Japan), respectively. High-resolution transmission electron microscopy (HRTEM) images were taken using a JEOL 2010 electron microscope at an accelerating voltage of 200 kV. Atomic force microscopy (AFM) images were obtained on a SPI3800 controller operated in tapping mode with an acquisition frequency of 1.5 Hz and line density of 512.2 × 2 μm scans. Electrochemical measurements were performed on a CHI 660C workstation (Shanghai Chenhua Apparatus Corporation, China) with a conventional three-electrode system composed of a platinum wire as the auxiliary, a saturated calomel electrode (SCE) as the reference, and a glassy carbon electrode (GCE) as the working electrode.

Fabrication of Au-QDs Core-Satellite Nanoprobes. Water-soluble $CdSe_{0.5}Te_{0.5}$ alloy QDs with average diameter of ~ 2 nm were prepared following a previously reported protocol.[1] Colloidal Au nanoparticles (NPs) with average diameter of ~ 10 nm were prepared as reported previously.[2] The nanoprobes were fabricated by assembling multiple QDs surrounding each Au nanoparticle using single-stranded DNA as the linker between the Au core and the QDs. First, 100 μL of 0.1 M imidazole solution (pH 6.8) was added to 3'-amine- and 5'-thiol-capped DNA oligonucleotides (1 O.D.) for 30 min, followed by the addition of 40 μL of 0.1 M EDC and 2 mL of colloidal QDs. The mixture was incubated at room temperature for 12 h with magnetic stir. The DNA-conjugated QDs were then isolated from the reaction mixture and washed with H_2O three times by centrifugation at 6000 rpm for 10 min at 4°C, followed by activation with 10 mM tris-(2-carboxyethyl) phosphine hydrochloride (TCEP) for 30 min. After the pH of the Au colloids was adjusted to 9.2 using 0.1 M NaOH, freshly activated DNA-conjugated QDs were added and the mixture was kept under gentle shaking for 30 min at 10°C. The mixture was centrifuged at 15000 rpm for 30 min to remove the excessive DNA-QDs conjugate particles that were not attached to the Au NPs. Finally, 1.0 mL of Au-DNA-QDs particles were mixed with 3.2 mg of EDC and 0.1 mg of streptavidin in 50 mM of MES buffer (pH 5.2), incubated for 2 h at room temperature under shaking, and kept undisturbed overnight at 4°C. The reaction mixture was centrifuged at 15000 rpm for 5 min three times, and the supernatant was discarded.

The obtained Au-QDs core-satellite nanoprobes were separated from the reaction mixture and washed with phosphate buffered saline (PBS) through centrifugation at 15000 rpm for 5 min three times, and finally redispersed in 1.0 mL of PBS (pH 7.4) and stored at 4°C.

Sensor Construction. PDMS monomer and the curing agent were first added to the 96-well plate in a proportion of 1:0.06 and cured at 70°C for 2 h. $HAuCl_4$ (1%) were dropped into each well and incubated at 37°C for 48 h to form PDMS-Au composite film.[3] All resulting wells were washed with H_2O three times, and stored at 4°C when not in use. The biotin-PLGVR peptide was activated with 1.5 μL of 10 mM TCEP in pH 5.2 acetate buffer for 1 h to prevent the terminal cysteine from forming disulphide bonds. Afterwards, 50 μL of 1 mM biotin-PLGVR peptide was spread evenly over the PDMS-Au composite film for 12 h at 4°C in 100% humidity, followed by immersion in 1 mM MCH for 1 h to remove the nonspecific adsorption. Subsequently, 100 μL of MMP-2 at various concentrations was added to each well and incubated for 2 h at 37 °C. After washing with TCNB, 40 μL of diluted Au-QDs core-satellite nanoprobes were dropped onto each well for 2 h to attach a nanoprobe onto each uncleaved peptide molecule. Prior to electrochemical and fluorescence measurements, the biosensor was washed with TCNB to remove all physically adsorbed nanoprobes.

Electrochemical and Fluorescence Measurements. After the binding of nanoprobes, 200 μL of 0.1 M HNO_3 solution was dropped into each well for 2 h to completely dissolve the QDs in the captured nanoprobes. For electrochemical analysis, the resulting solution was mixed with 1.0 mL of 0.2 M HAc-NaAc buffer (pH 5.2) and 20 μL of 500 μg mL^{-1} Bi^{3+} solution to perform anodic stripping voltammetric detection with a conventional three-electrode system composed of a platinum wire as auxiliary electrode, a saturated calomel electrode (SCE) as the reference, and a glassy carbon electrode as the working electrode. The anodic stripping voltammetric detection involved electrodeposition at -1.1 V for 120 s while the solution was stirred and stripping from -1.1 to -0.2 V under N_2 atmosphere using a square-wave voltammetry, with a frequency of 25 Hz, amplitude of 25 mV, and potential step of 4 mV.

For fluorescence analysis, the HNO_3-treated solution were diluted with phosphate buffer solution and centrifuged at 1000 rpm for 5 min to obtain a supernatant. After adjusting pH to 7.4 using 1 M NaOH, the resulting solution containing 3 μM Rhod-5N was detected at room temperature with the excitation wavelength of 540 nm.

ELISA for MMP-2 Detection. Concentrations of MMP-2 in serum samples were detected by measuring absorbance changes at 450 nm using a commercial human MMP-2 ELISA Kit purchased from Beijing Biosynthesis Biotechnology Co. Ltd. (Beijing, China). A standard curve for the spectrophotometric procedure was obtained following the manufacturer's protocol in the concentration range from 0 to 12 ng mL^{-1}. Briefly, the serum sample or MMP-2 standard solution (0-12 ng mL^{-1}) were added to the ELISA plate (50 μL/well) and incubated for 1 h at 37 ºC. Then each well of the plate was washed five times with 200 μL of wash buffer. Enzyme-labeled bioconjunction solution was added to each well and incubated for 60 min at 37 ºC and washed again with wash buffer. Subsequently, substrate solution (50 μL solution A and 50 μL solution B) was added to each well and incubated for 15 min in the dark. Without washing the plate, 50 μL of stop solution was added to each well to stop the color reaction. The absorbance was read immediately on an automated plate reader (model 680, Bio-RAD) at 450 nm.

S2. Figures:

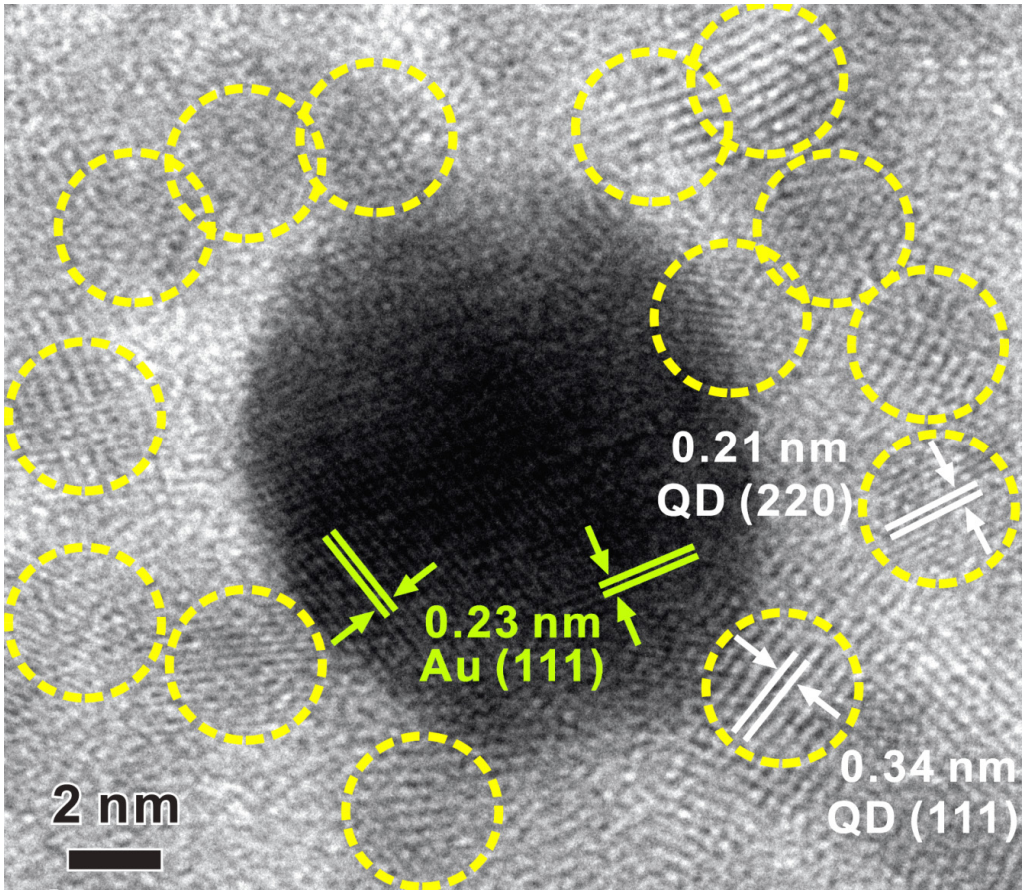

Fig. S1 HRTEM image of a Au-QDs core-satellite nanoprobe. The QDs are indicated by circles. The lattice spacings of the (111) and (220) planes of cubic phase $CdSe_{0.5}Te_{0.5}$ and the lattice spacing of the (111) plane of cubic phase Au are labeled.

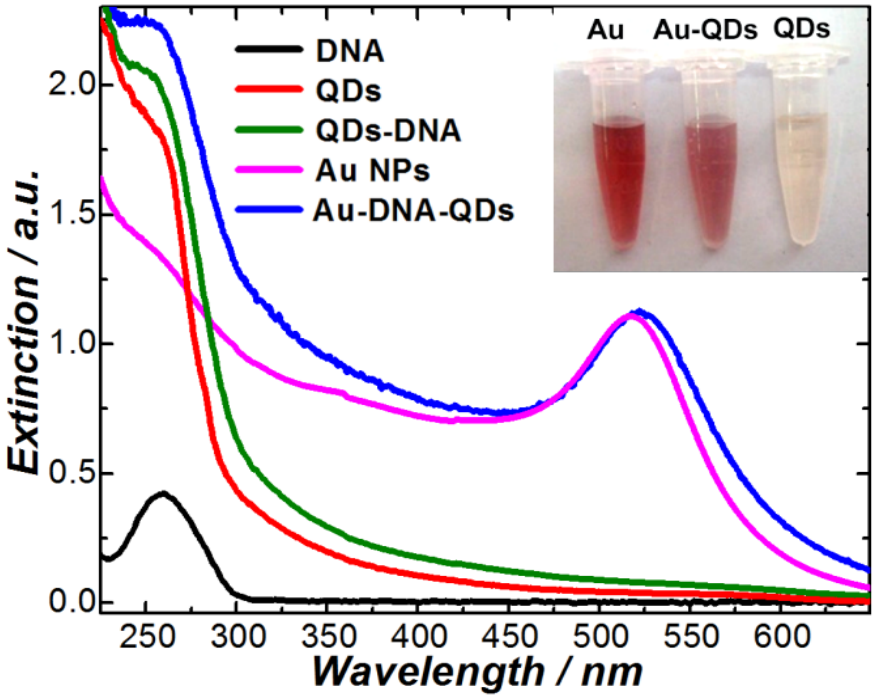

Fig. S2 UV-vis extinction spectra of DNA, QDs, QDs-DNA, Au NPs, and Au-DNA-QDs core-satellite particles. Inset: Picture of colloidal suspensions of Au NPs, Au-QDs core-satellite particles, and QDs under room light.

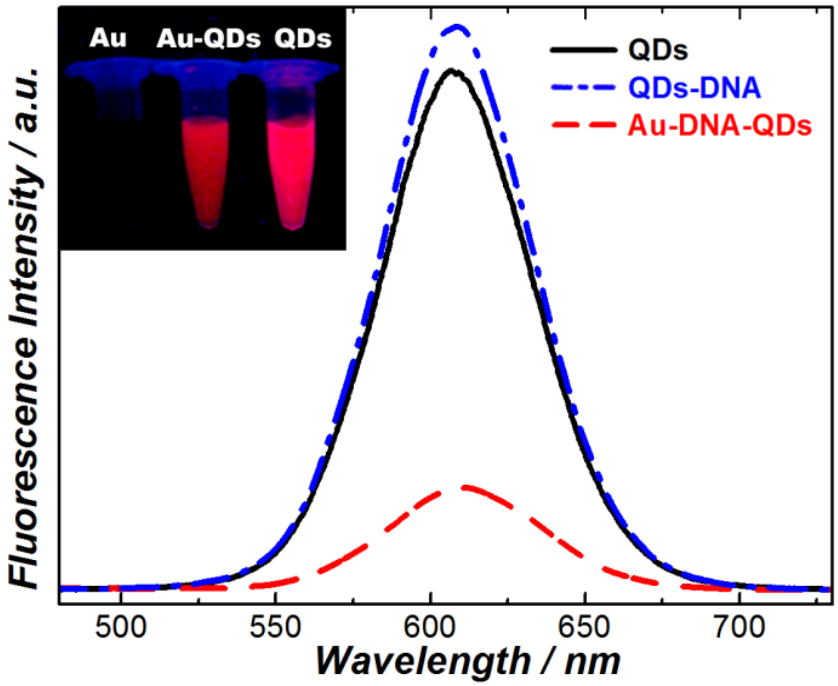

Fig. S3 Fluorescence spectra ($\lambda_{excitation}$ = 300 nm) of QDs, QDs-DNA, and Au-QDs core-satellite particles. Inset: Picture showing the emission from colloidal Au NPs, Au-QDs core-satellite particles, and QDs under UV light excitation.

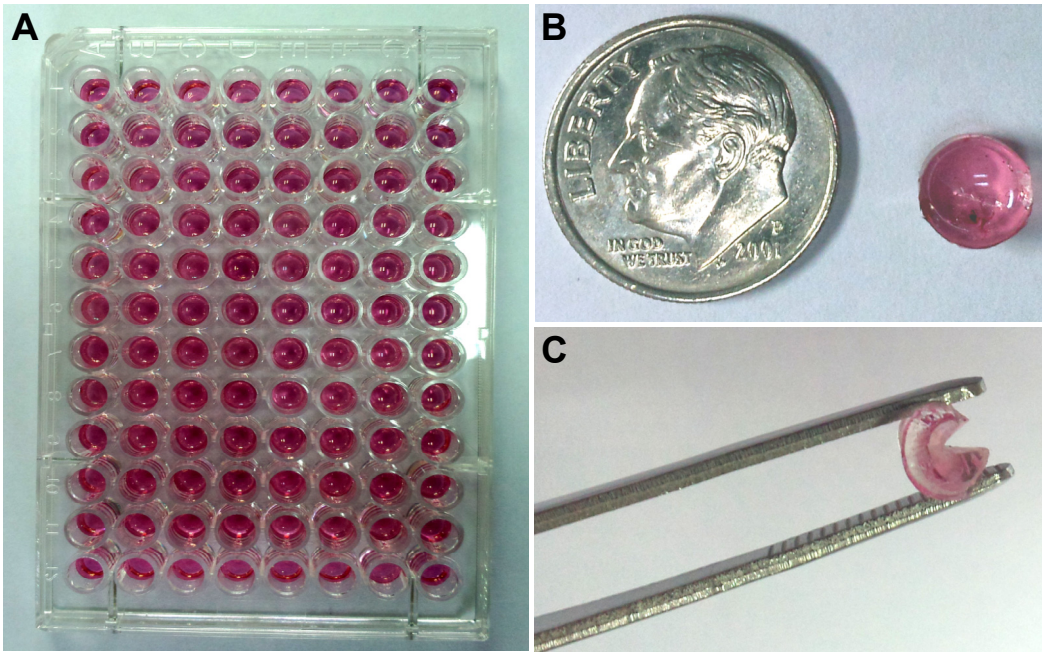

Fig. S4 (A) Picture of the PDMS-Au composite films (sensor substrate) made in a 96-well plate. (B) Picture showing the relative size of a sensor substrate to that of a dime (Unite State coin). (C) Picture showing the flexibility of the sensor substrate.

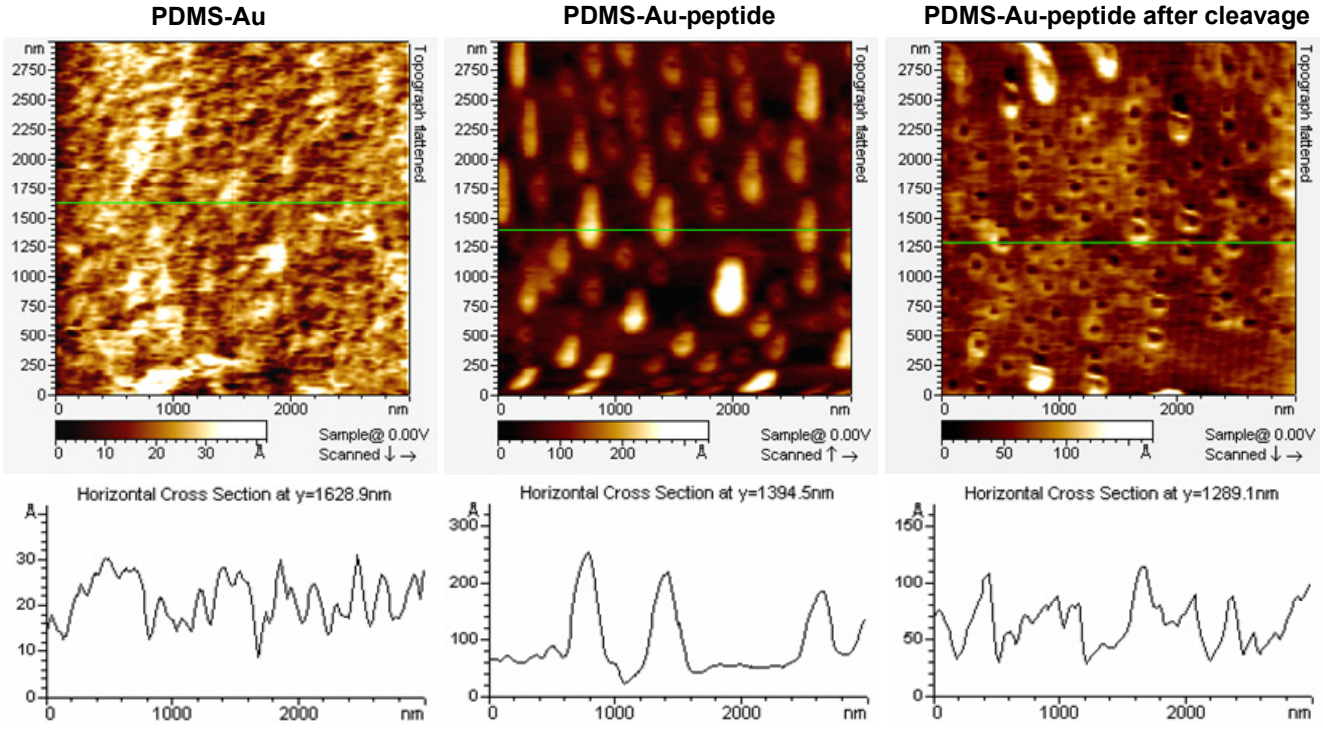

Fig. S5 AFM images of the PDMS-Au (*left*), biotin-PLGVR/PDMS-Au before (*middle*) and after (*right*) the peptide cleavage. The bottom panels show the height profiles along the green lines in the corresponding AFM images in the top panels.

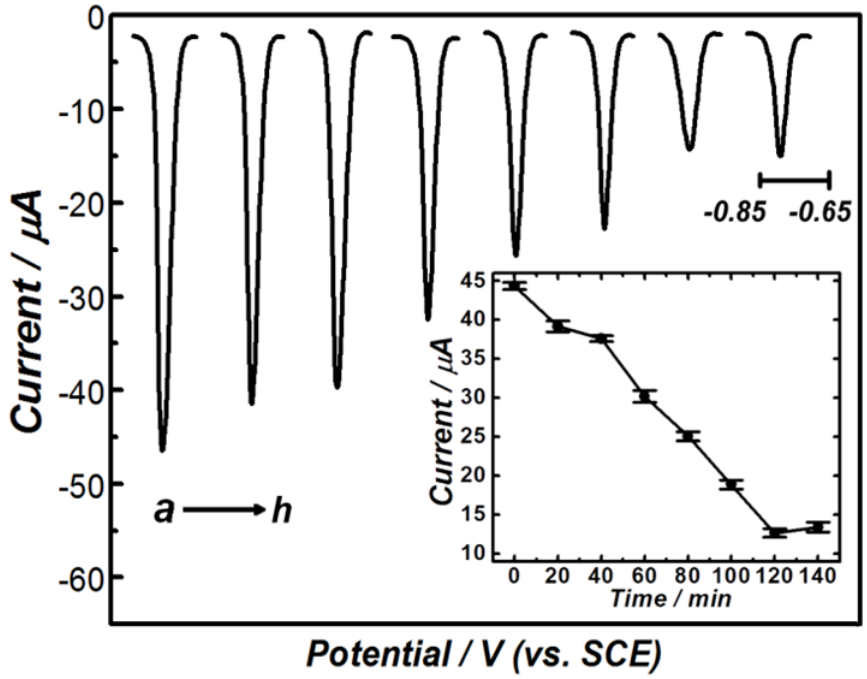

Fig. S6 Time evolution of the ASV currents during peptide cleavage in the presence of 0.5 ng mL^{-1} MMP-2.

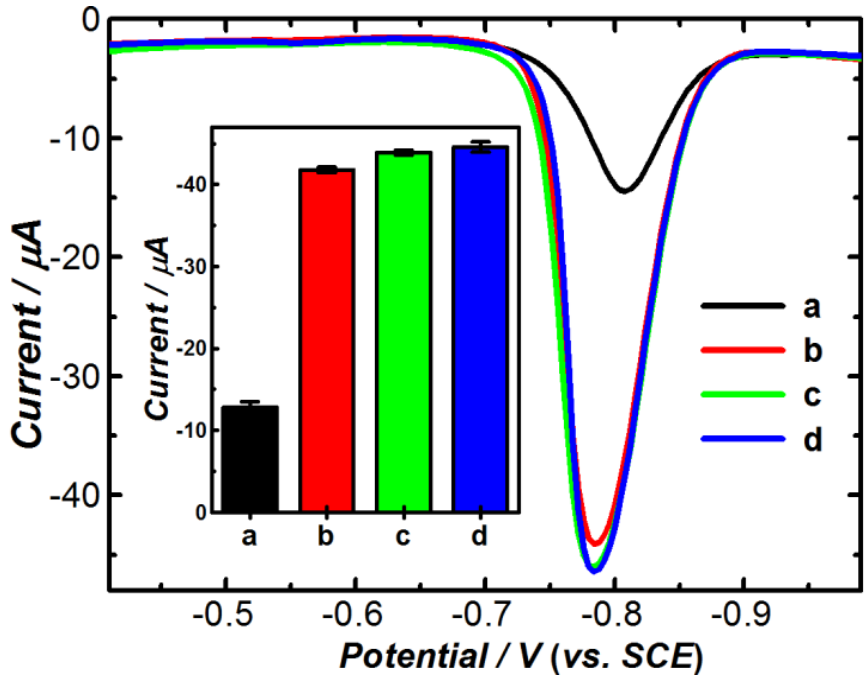

Fig. S7 ASV curves obtained after incubating the biotin-PLGVR/PDMS-Au with (a) 0.5 ng mL^{-1} MMP-2, (b) 0.5 ng mL^{-1} MMP-2 + 10 μM 1,10-phenanthroline, (c) buffer, and (d) 10 μM 1,10-phenanthroline for 2h.

S3. Tables:

Table S1 Assay results of the clinical human serum samples.

Serum Samples	ELISA [a] (ng mL^{-1})	ASV [a] (ng mL^{-1})	Relative Deviation (%)	Fluorescence Analysis [a] (ng mL^{-1})	Relative Deviation (%)
S1	2.72 ± 0.137	2.71 ± 0.106	-0.369	2.65 ± 0.107	-2.44
S2	2.02 ± 0.164	2.06 ± 0.0885	2.45	2.055 ± 0.0720	1.98
S3	1.73 ± 0.0661	1.75 ± 0.221	1.34	1.76 ± 0.0224	1.74
S4	4.54 ± 0.261	4.51 ± 0.174	-0.665	4.51 ± 0.0725	-0.716
S5	4.23 ± 0.271	4.14 ± 0.0376	-2.00	4.36 ± 0.128	3.15
S6	5.32 ± 0.138	5.08 ± 0.0466	-4.49	5.09 ± 0.00893	-4.32

[a] Average values of three measurements.

Table S2 Detection limits of various methods for MMP-2 detection.

Detection method	Detection limit	Reference
Gel electrophoresis-based protease assays	1-6 ng mL^{-1}	4
Protease assays using magnetic nanoprobes	69 ng mL^{-1}	5
Magnetic resonance imaging techniques	170 ng mL^{-1}	6
FRET between graphene oxide and QDs	70 ng mL^{-1}	7
FRET between dyes and Au nanoparticles	12.5 ng mL^{-1}	8
FRET between graphene oxide and fluorophores	3.5 ng mL^{-1}	9
Bioluminescence resonance energy transfer (BRET)	2 ng mL^{-1}	10
Upconversion FRET	10 pg mL^{-1}	11
Dual-channel detection using Au-QDs core-satellite nanoprobes	**sub-pg mL^{-1}**	**This work**

S4. References:

1. G. X. Liang, M. M. Gu, J. R. Zhang and J. J. Zhu, *Nanotechnology*, 2009, **20**, 415103.
2. J. Liu and Y. Lu, *Nature Protocols*, 2006, **1**, 246-252.
3. W. Wang, W. Y. Wu, X. Q. Zhong, Q. A. Miao and J. J. Zhu, *Biosensors & Bioelectronics*, 2011, **26**, 3110-3114.
4. R. B. Lefkowitz, G. W. Schmid-Schonbein and M. J. Heller, *Analytical Chemistry*, 2010, **82**, 8251-8258.
5. M. Zhao, L. Josephson, Y. Tang and R. Weissleder, *Angewandte Chemie-International Edition*, 2003, **42**, 1375-1378.
6. T. J. Harris, G. von Maltzahn, A. M. Derfus, E. Ruoslahti and S. N. Bhatia, *Angewandte Chemie-International Edition*, 2006, **45**, 3161-3165.
7. J. Li, C. H. Lu, Q. H. Yao, X. L. Zhang, J. J. Liu, H. H. Yang and G. N. Chen, *Biosensors & Bioelectronics*, 2011, **26**, 3894-3899.
8. X. Wang, Y. Q. Xia, Y. Y. Liu, W. X. Qi, Q. Q. Sun, Q. Zhao and B. Tang, *Chemistry-a European Journal*, 2012, **18**, 7189-7195.
9. D. Feng, Y. Y. Zhang, T. T. Feng, W. Shi, X. H. Li and H. M. Ma, *Chemical Communications*, 2011, **47**, 10680-10682.
10. H. Q. Yao, Y. Zhang, F. Xiao, Z. Y. Xia and J. H. Rao, *Angewandte Chemie-International Edition*, 2007, **46**, 4346-4349.
11. Y. H. Wang, P. Shen, C. Y. Li, Y. Y. Wang and Z. H. Liu, *Analytical Chemistry*, 2012, **84**, 1466-1473.