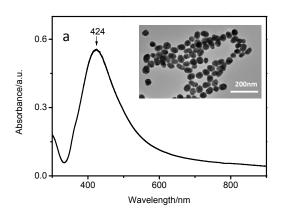
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## Supporting information for

SERS determination of protease through a particle-on-a-film configuration constructed by electrostatic assembly in enzymatic hydrolysis reaction

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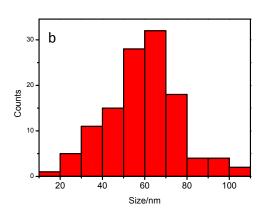
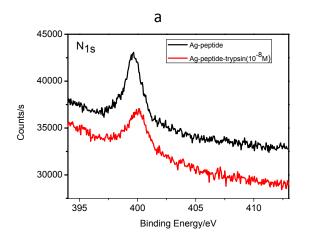


Fig. S1 (a) UV-vis spectra of Ag NPs. Insert is the TEM image of the Ag NPs. (b) Size distribution of the Ag NPs used in this work.



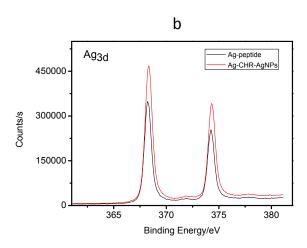


Fig. S2 (a) XPS spectra of  $N_{1s}$  of the CG-6 on an Ag film before (black) and after trypsin sensing (red). XPS spectra of  $Ag_{3d}$  of the CG-6 on an Ag film (black) and the CG-6 on an Ag NPs-on-an Ag film after responding for trypsin (red).

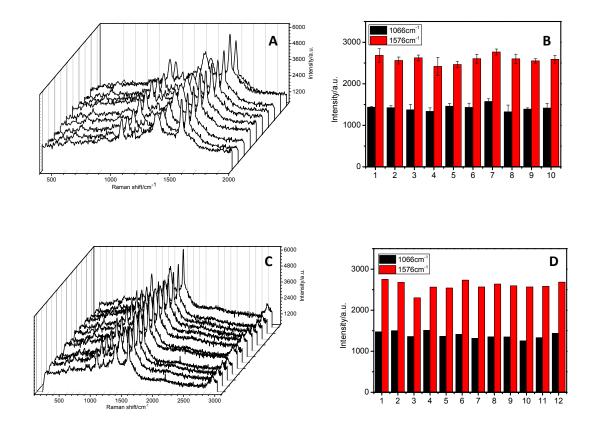


Fig. S3 (A) Mean SERS spectra from randomly selected 10 trials. While each mean spectrum is from three spectra of the same sample. (B) The intensities of SERS spectra at 1066 cm<sup>-1</sup> and 1576 cm<sup>-1</sup> on different trials. (C) SERS spectra from randomly selected 12 points from the same chip. (D) The intensities of SERS spectra at 1066 and 1576 cm<sup>-1</sup> on the same chip.

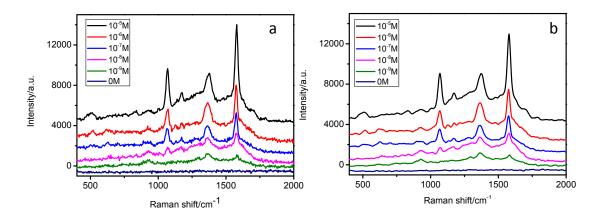


Fig. S4 SERS spectra of 4-MBA modified Ag film incubated with CG-6 treated with different amounts of trypsin before (a) and after (b) smoothing.

Table S1. Measurement of trypsin in 1 % serum by this method.

Trypsin(M)	Real Value	Measured Value	Recovery (%)		
1.0×10 <sup>-6</sup>	4047	3699	91.4		
1.0×10 <sup>-7</sup>	2522	2280	90.4		
1.0×10 <sup>-8</sup>	1231	1035	84.7		
1.0×10 <sup>-9</sup>	497	432	86.9		

Table S2 Intra-day and inter-day repeatabilities of the proposed procedure.

Measurement		Inte	SD	RSD				
	Trial 1	Trial 2	Trial 3	Trail 4	Trial 5	Average		
Intra-day	2705	2682	2603	2746	2648	2677	48.8	1.82%
Inter-day	2499	2475	2346	2355	2503	2436	70.2	2.88%