

Electronic Supplementary Information (ESI)

Graphene oxide-based chemiluminescent sensing platform for label-free detection of trypsin and its inhibitors

Huanhuan Zhang, Luyan Yao, Xiaoqian Yu, Yanjun Zhao and Aiping Fan^{a,b*}

^a*Collaborative Innovation Center of Chemical Science and Engineering (Tianjin),*

^b*School of Pharmaceutical Science and Technology, Tianjin University, Tianjin*

300072, PR China.

Table S1. The sequences of the peptide used in this study.

Peptide name	Sequence
P_1	Arg-Arg-Arg-Arg-Cys
P_2	Arg-Arg-Arg-Arg-Arg-Cys
P_3	Arg-Arg-Arg-Arg-Arg-Arg-Cys
P_4	Arg-Arg-Arg-Arg-Arg-Arg-Arg-Cys

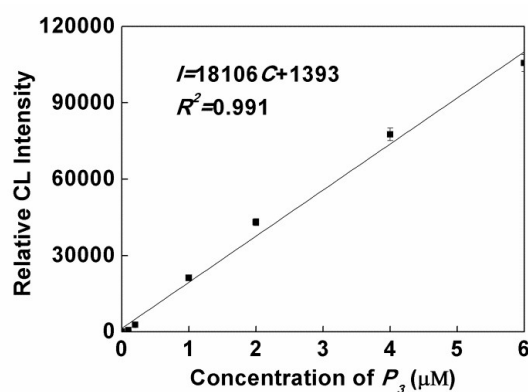


Figure S1. Linear relationship between the relative CL intensity and P_3 concentration.

Experimental conditions: P_3 , 0.1, 0.2, 1, 2, 4, 6 μM ; luminol, 2.5 μM ; NaIO_4 , 10 μM .

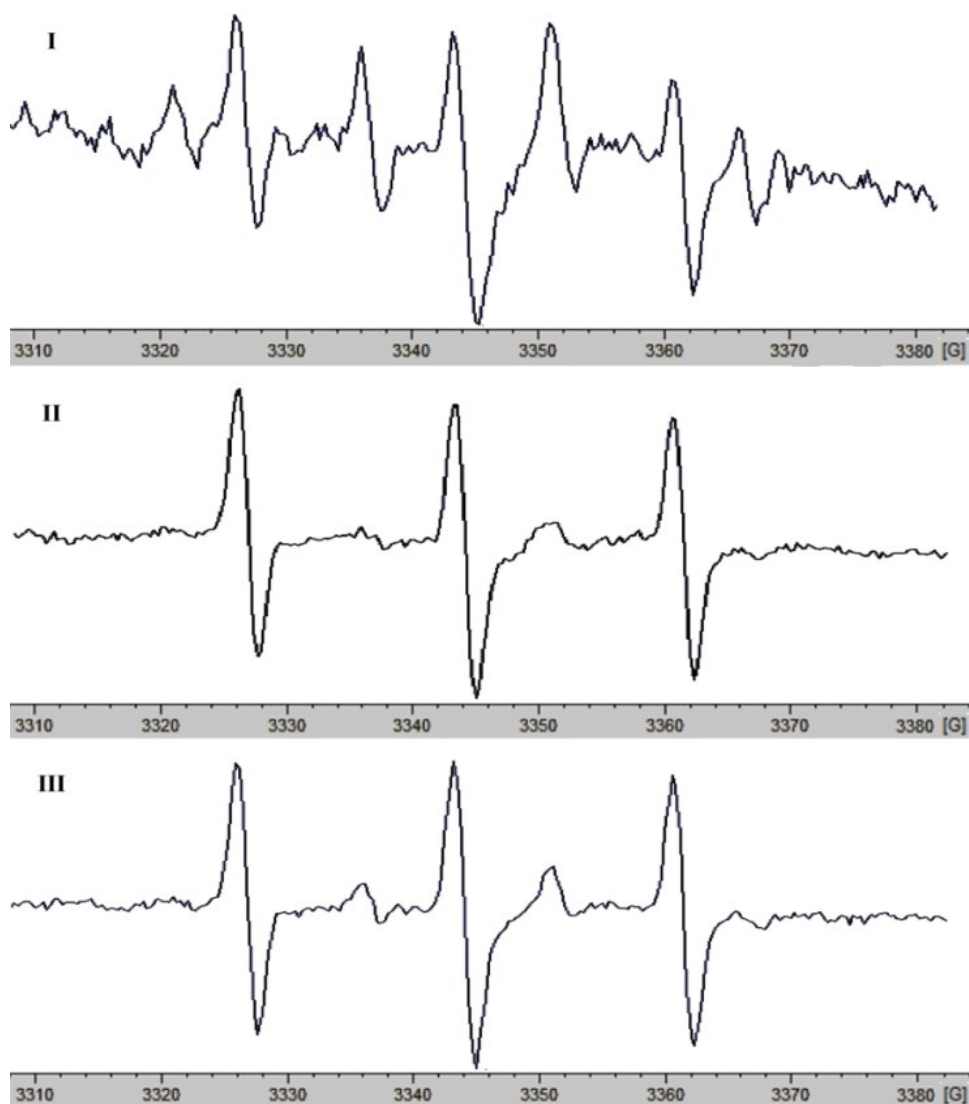


Figure S2. ESR spectra of aqueous DMPO solution with (I) $\text{NaIO}_4\text{-}P_3$, (II) $\text{NaIO}_4\text{-}(P_3\text{-GO complex})$, and (III) NaIO_4 . Experimental conditions: microwave frequency, 9.407 GHz; power, 1.00 mW; modulation amplitude, 2.00 G; modulation frequency, 100 kHz; time constant, 20.48 ms; conversion time, 40.00 ms. The hyperfine coupling constants for DMPO-OH in (I) were $\alpha_{\text{N}} = \alpha_{\text{H}} = 1.5$ mT and $g = 2.0061$, respectively.

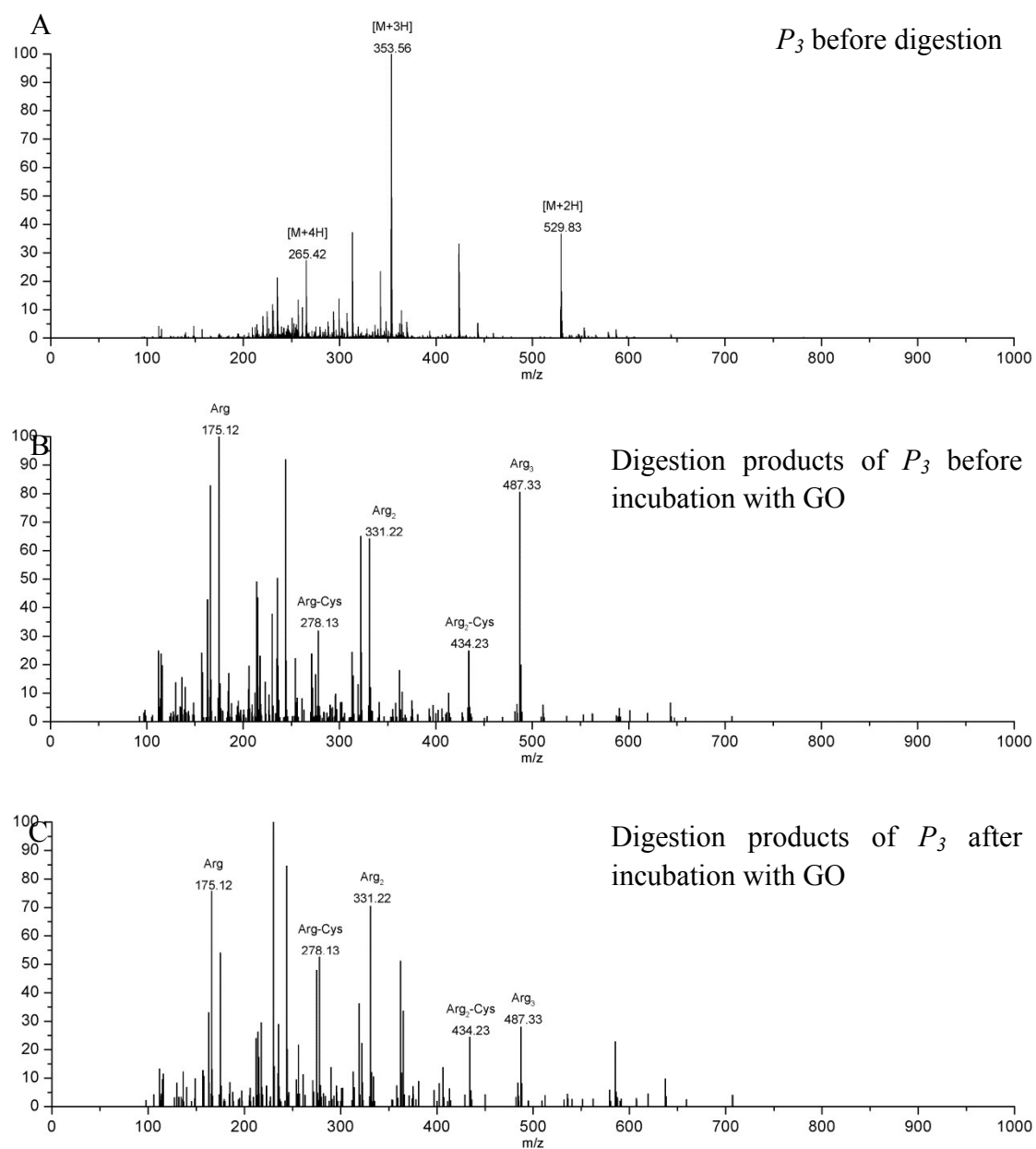


Figure S3. MS spectra of P_3 (A) and digestion products of P_3 (B, C) under different conditions. P_3 120 μM ; trypsin, 10 μM ; GO, 2 mg mL^{-1} .

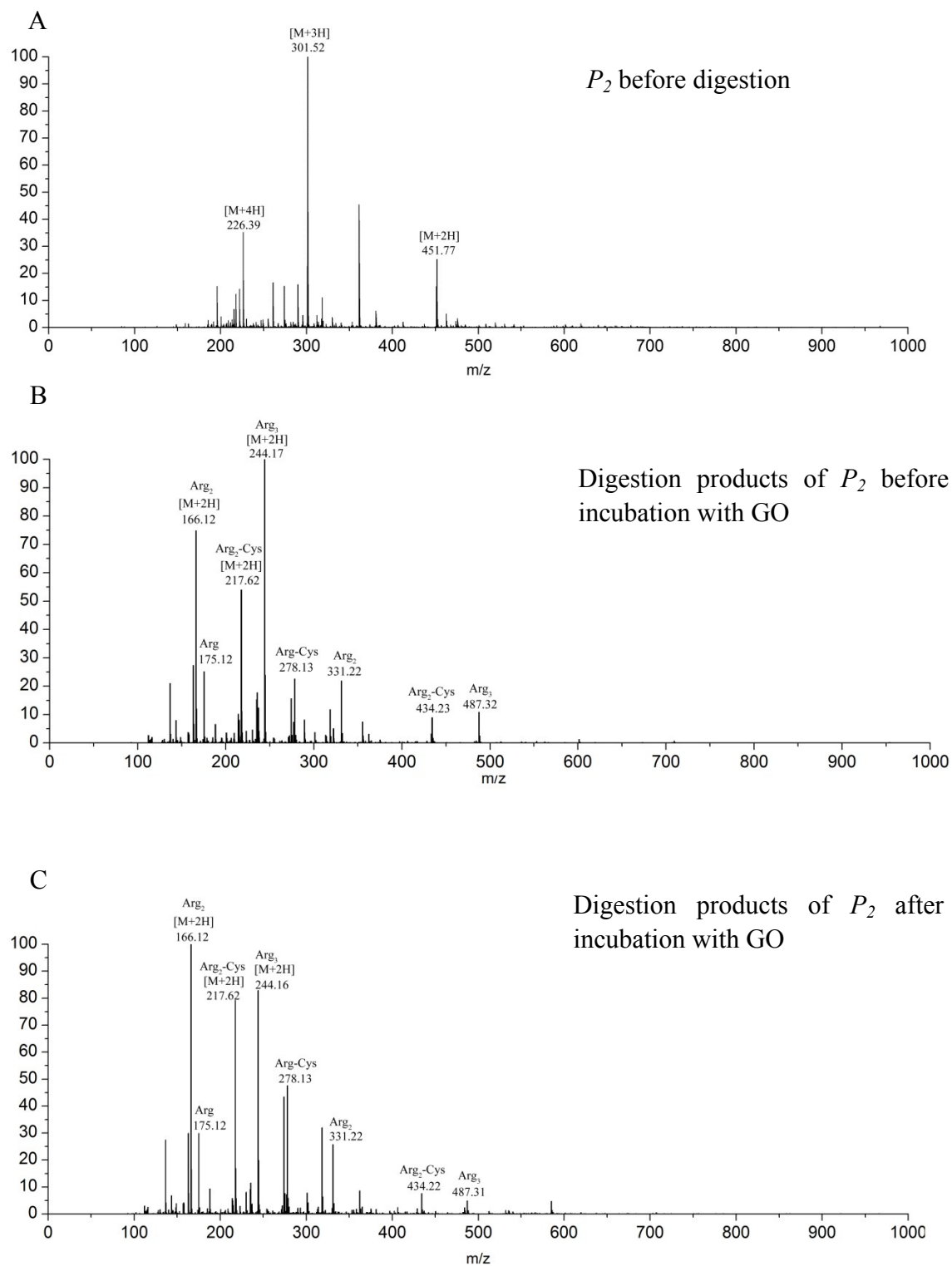


Figure S4. MS spectra of *P*₂ (A) and digestion products of *P*₂ (B, C) under different conditions. *P*₂ 120 μ M; trypsin, 10 μ M; GO, 2 mg mL⁻¹.

Table S2 Comparison of assay performance and assay time for different trypsin assay methods

Analytical method	Label	Detection limit	Linear range	Assay time	Ref
Laser-induced fluorescence	HRP	0.87 ng mL ⁻¹	0-580 ng mL ⁻¹	26 min	S1
Fluorescent assay	label free	2 ng mL ⁻¹	0.01-100 µg mL ⁻¹	2 h	S2
Fluorescent assay	label free	20 ng mL ⁻¹	0-450 ng mL ⁻¹	6 h	S3
Electrochemical assay	electroactive species (P)	1 ng mL ⁻¹	1-1000 ng mL ⁻¹	2 h	S4
Electrochemical assay	label free	10 ng mL ⁻¹	0.1-1000 µg mL ⁻¹	2.3 h	S5
Colorimetric assay	label free	0.6 µg mL ⁻¹	0.9-1000 µg mL ⁻¹	2 h	S6
Colorimetric assay	label free	2 ng mL ⁻¹	2.5-200 ng mL ⁻¹	10 min	S7
CL detection (This work)	label free	0.18 ng mL ⁻¹ (7.3 pM)	0.48-240 ng mL ⁻¹ (0.02-10 nM)	30 min	-
CL detection	label free	0.7 ng mL ⁻¹	0-100 ng mL ⁻¹	60 min	S8

References

- S1 M. A. Seia, P. W. Stege, S. V. Pereira, I. E. De Vito, J. Raba and G. A. Messina, *Anal. Biochem.*, 2014, **463**, 31-37.
- S2 L. Hu, S. Han, S. Parveen, Y. Yuan, L. Zhang and G. Xua, *Biosen. Bioelectron.*, 2012, **32**, 297-299.
- S3 F. F. Zheng, J. F. Wu and G. C. Zhao, *Anal. Methods*, 2012, **4**, 3932-3936.
- S4 S. Park and H. Yang, *Analyst*, 2014, **139**, 4051-4055.
- S5 M. Stoytcheva, R. Zlatev, S. Cosnier and M. Arredondo, *Electrochim. Acta*, 2012, **76**, 43-47.
- S6 G. L. Wang, L. Y. Jin, Y. M. Dong, X. M. Wu and Z. J. Li, *Biosen. Bioelectron.*,

2015, **64**, 523-529.

S7 P. Miao, T. Liu, X. Li, L. Ning, J. Yin and K. Han, *Biosen. Bioelectron.*, 2013, **49**, 20-24.

S8 Q. Zhang, W. Li, J. Chen, F. Wang, Y. Wang, Y. Chen and C. Yu, *Chem. Commun.*, 2013, **49**, 3137-3139.