Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.
This journal is © The Royal Society of Chemistry 2019

Electronic Supplementary Information (ESI)

Fabrication of a water-soluble near-infrared fluorescent probe for selective detection and imaging of dipeptidyl peptidase IV in biological system

Xiumei Guo[a], Shuai Mu[a], Jian Li[a], Yintang Zhang[b], Xiaoyan Liu[a*], Haixia Zhang[a], Hong Gao[a*]

[a]State Key Laboratory of Applied Organic Chemistry, Key Laboratory of Nonferrous Metals Chemistry and Resources Utilization of Gansu Province and College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou 730000, China.

[b]Henan Key Laboratory of Biomolecular Recognition and Sensing, College of Chemistry and Chemical Engineering, Shangqiu Normal University, Shangqiu 476000, China.

Table of Contents

* Corresponding author Tel: +86-931-8912510; fax: +86-931-8912582;
E-mail: liuxiaoy@lzu.edu.cn

Section 1. Synthesis and characterizations of the probe

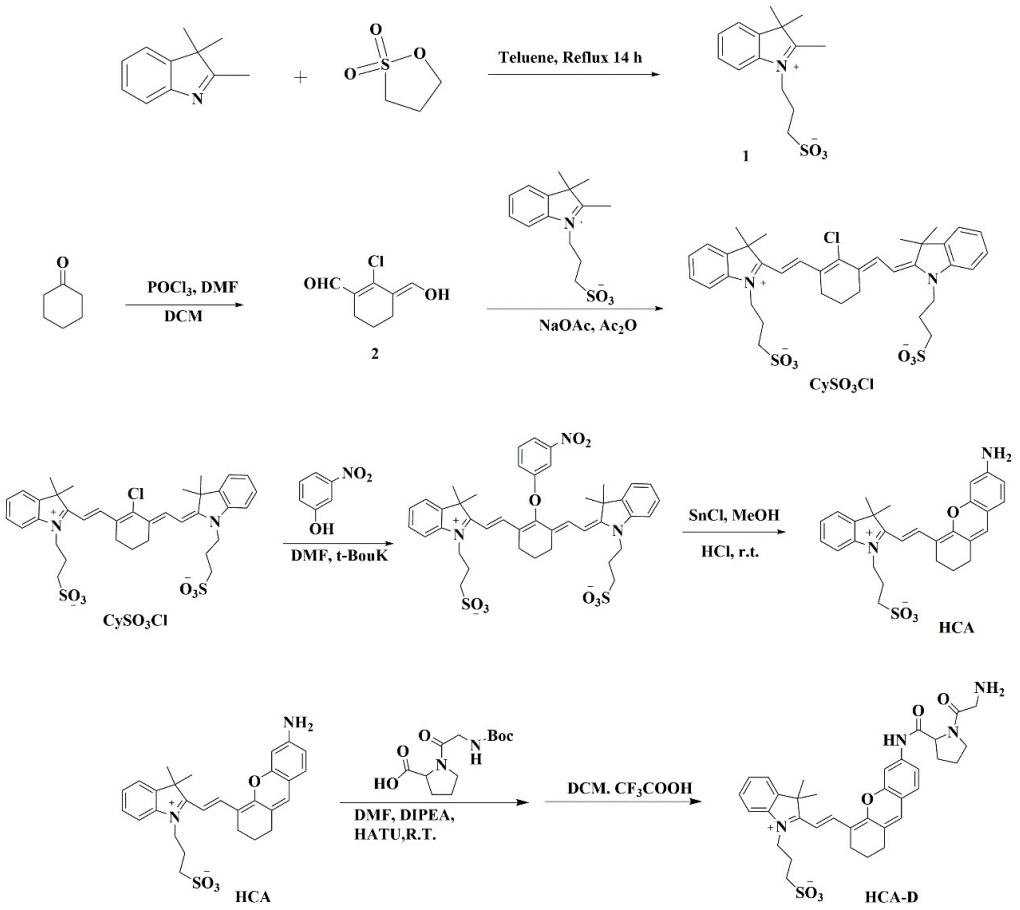


Figure S1 The synthesis procedure of **HCA-D**.

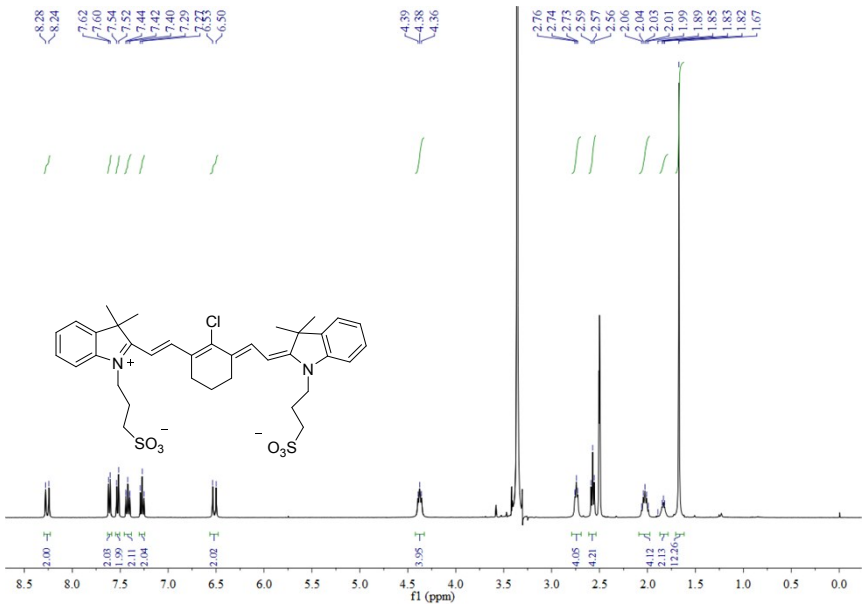


Figure S2 ^{1}H NMR spectrum of **$CySO_3Cl$** in DMSO-d_6

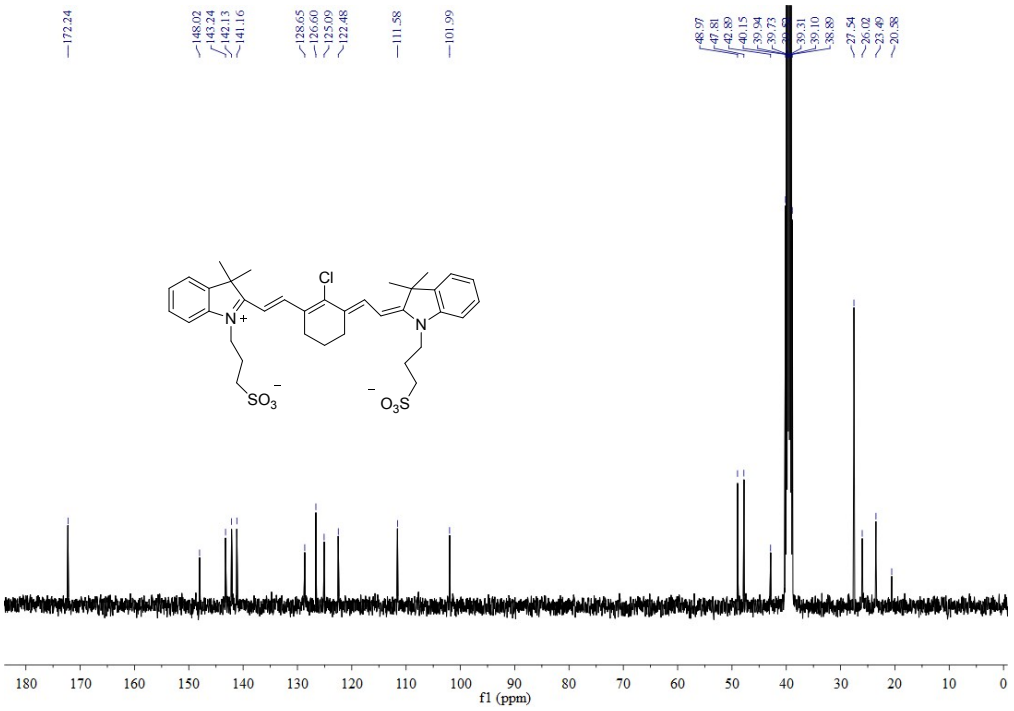


Figure S3 ^{13}C NMR spectrum of **$CySO_3Cl$** in DMSO-d_6

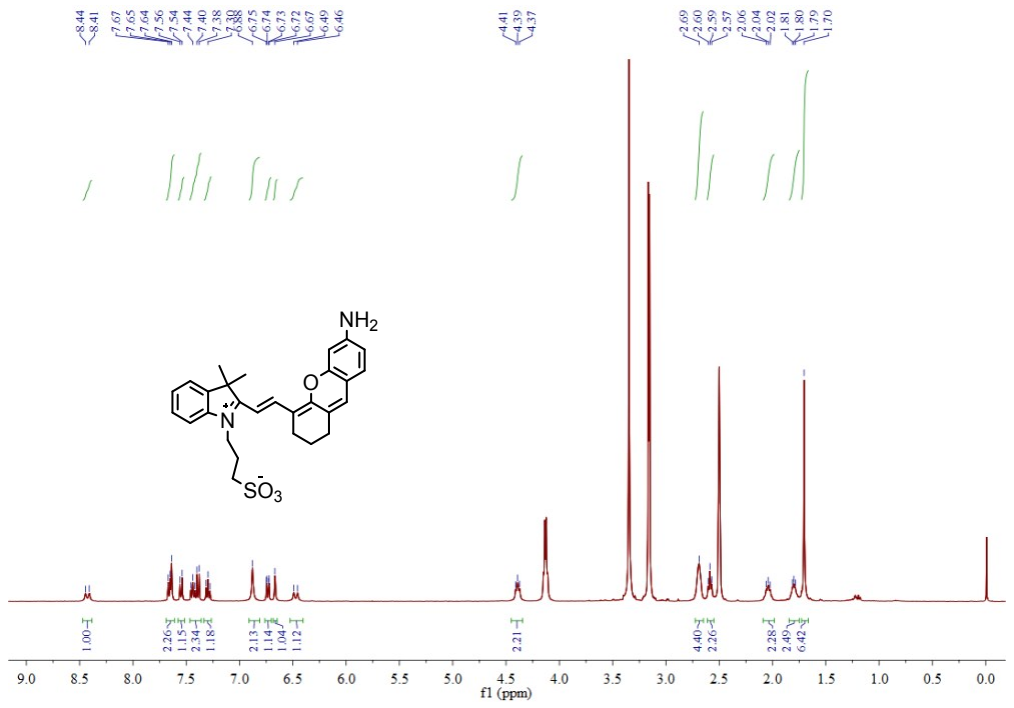


Figure S4 ^{1}H NMR spectrum of **HCA** in DMSO-d_6

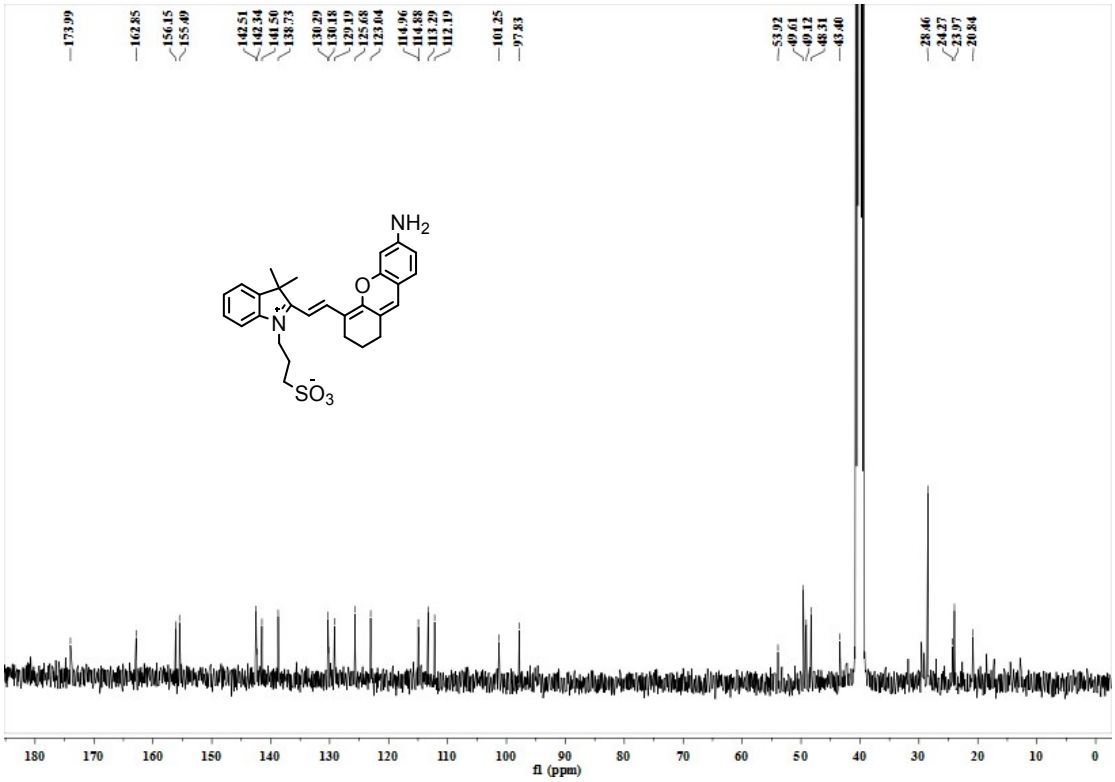


Figure S5 ^{13}C NMR spectrum of **HCA** in DMSO-d_6

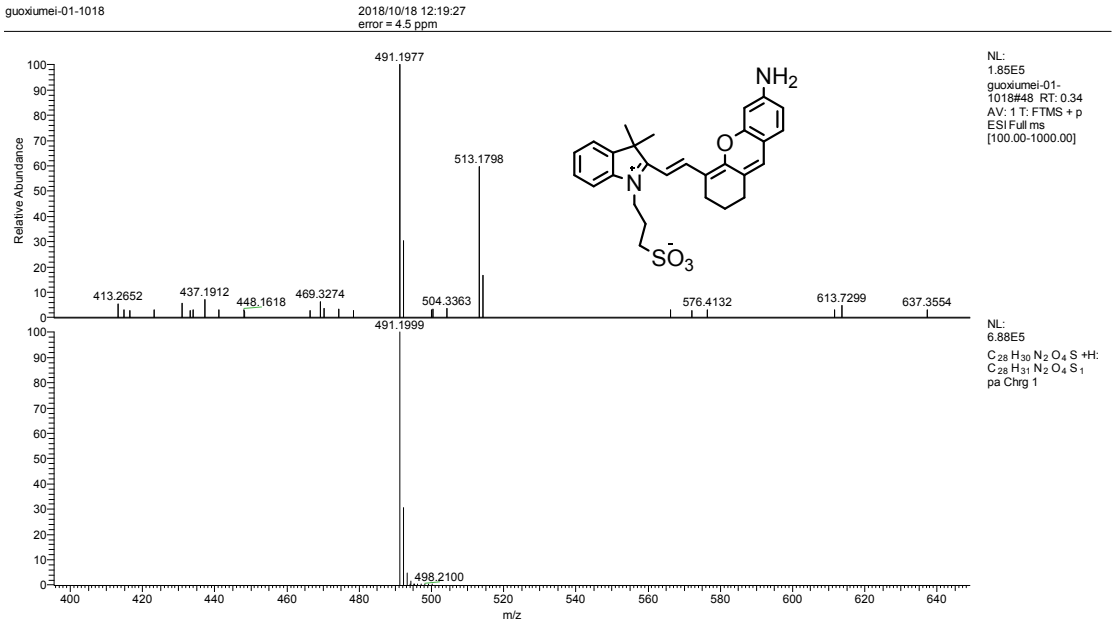


Figure S6 HRMS spectra of HCA

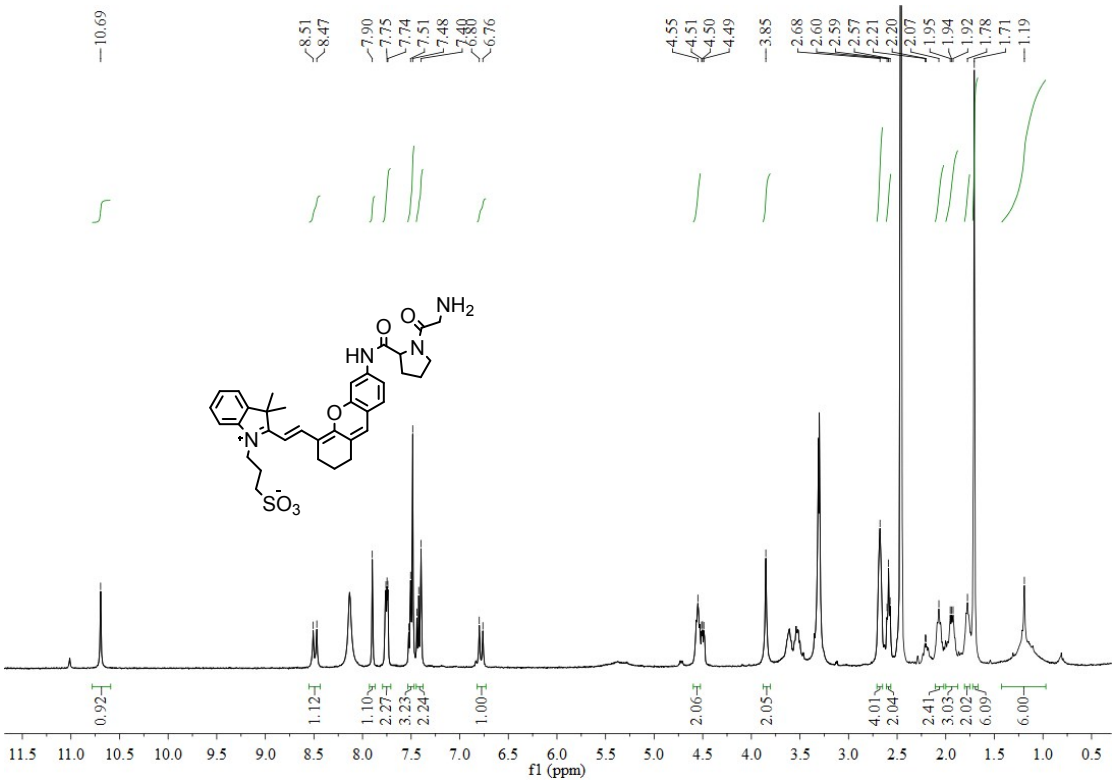


Figure S7 ^{1}H NMR spectrum of **HCA-D** in DMSO-d_6

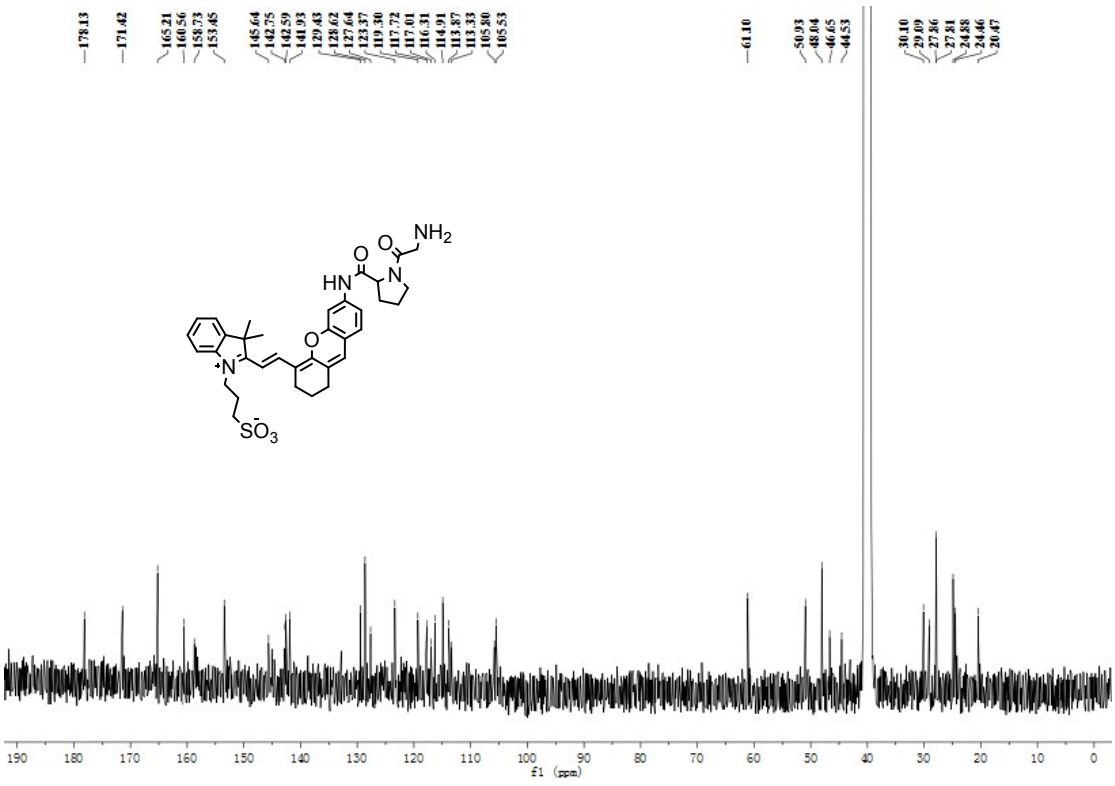


Figure S8 ^{13}C NMR spectrum of **HCA-D** in DMSO-d_6

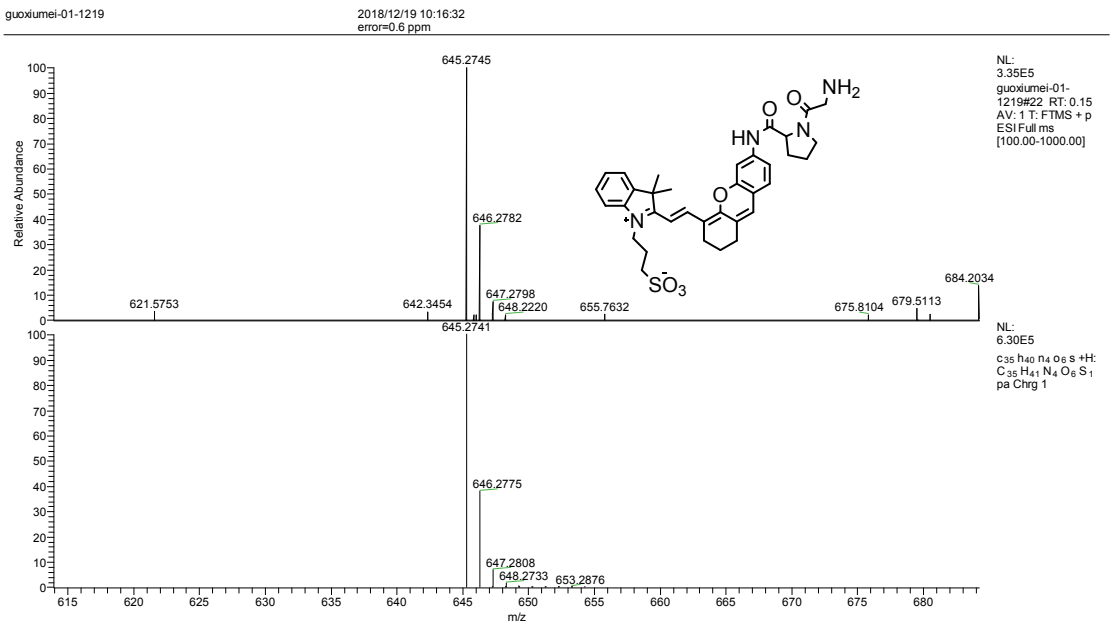


Figure S9 HRMS spectra of HCA-D

Section 2 The mechanism study on HCA-D for detection of DPP-IV

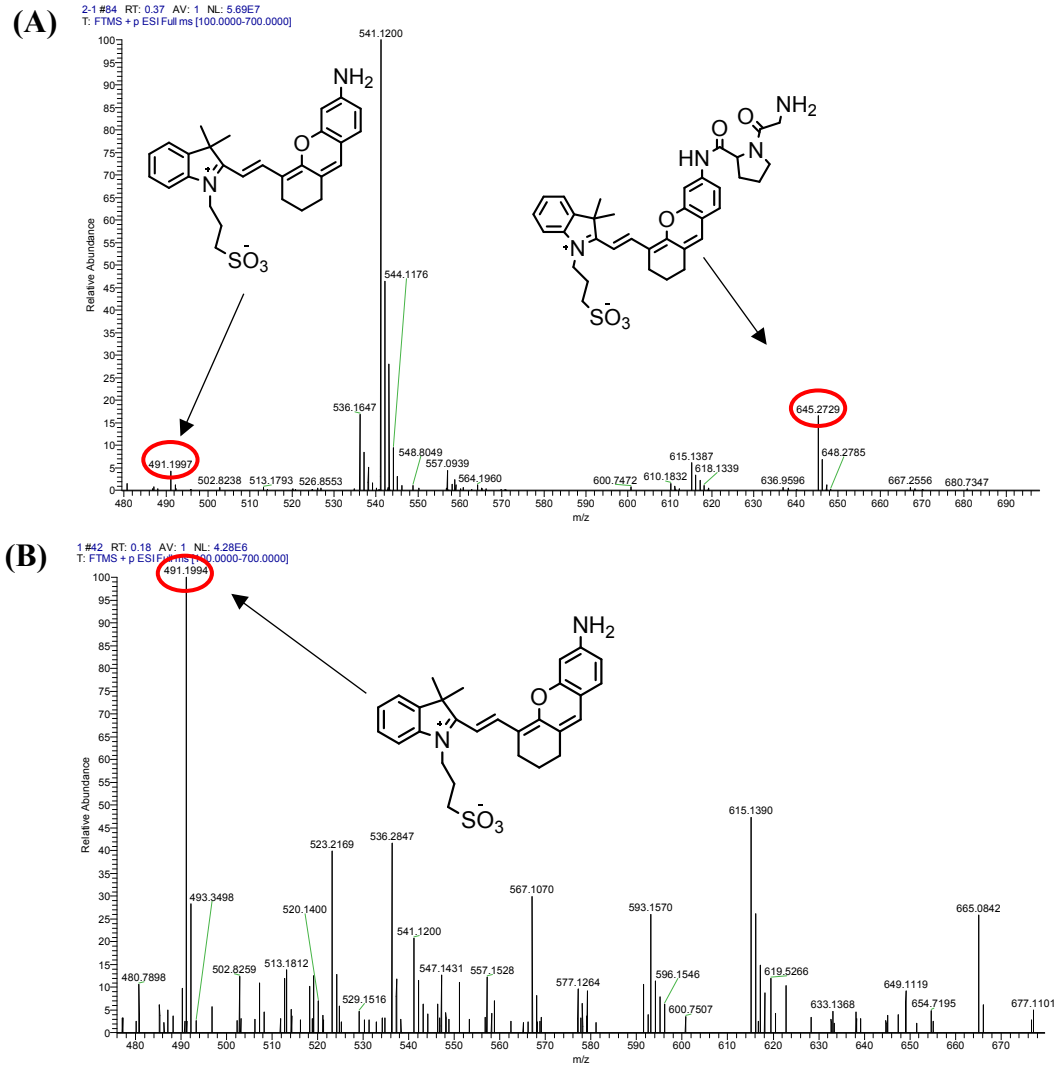


Figure S10 HRMS spectra of the reaction products of HCA-D (30 μM) and DPP-IV at concentration of 5 ng/mL (A) and 40 ng/mL (B).

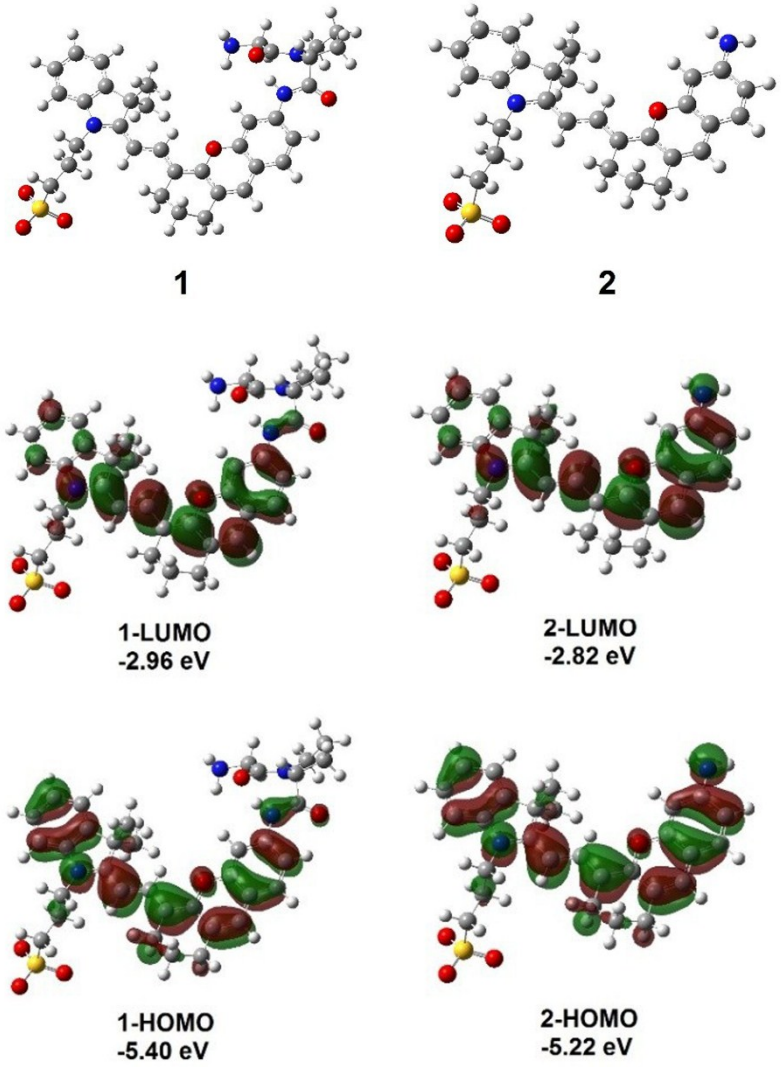


Figure S11 The molecular structure of the probe (HCA-D) and fluorophore (HCA) optimized (top), and the main electronic energy level distribution calculated through density functional theory (DFT) (B3LYP/6-311G (d, p)/level, Gaussian 09). In the stick model, carbon, oxygen, nitrogen and sulfur atoms are labeled as gray, red, blue, and yellow, respectively.

Section 3 Effect of solvent on the activity of DPP-IV

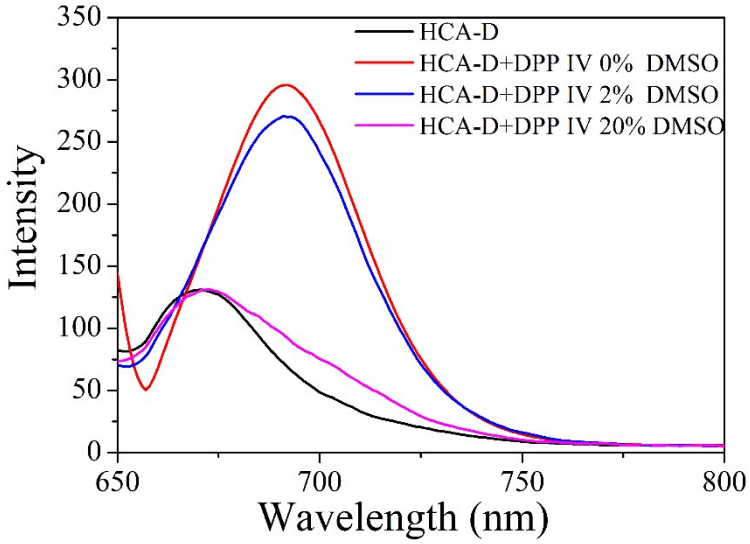
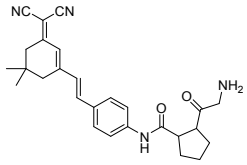
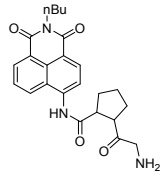
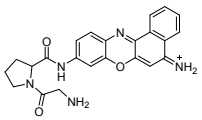
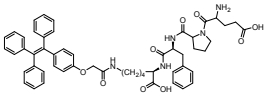
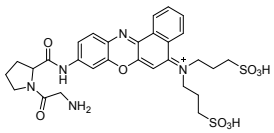
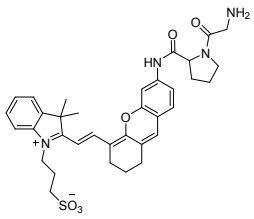


Figure S12 Effect of the co-solvent system (DMSO and PBS buffer solution) on the fluorescence intensity of HCA-D (20 μM) induced by DPP-IV (40 ng/mL) activity.

Section 4 Evaluation of the analysis methods for detection of DPP-IV activity

Table S1. A comparison of the fluorescent probes for DPP-IV detection.

Probe for DPP IV	Test system	λex/ λem (nm)	NIR emission	Linear range ng/mL	LOD ng/mL	Reference
	ACN/PBS = 1:1	458/658	yes	0-1500	/	[1]
	ACN/PBS = 1:1	360/805 455/535	yes	0-80	0.78	[2]
	/	585/625	yes	2-60	0.35	[3]
	HEPES buffer	320/450	/	0.1 to 0.5 mU/mL	/	[4]
	HEPES buffer	625/670	yes	/	/	[5]
	PBS buffer	640/690	yes	0.625 -10	0.19	This work

Section 5 Inhibition assay of DPP-IV activity

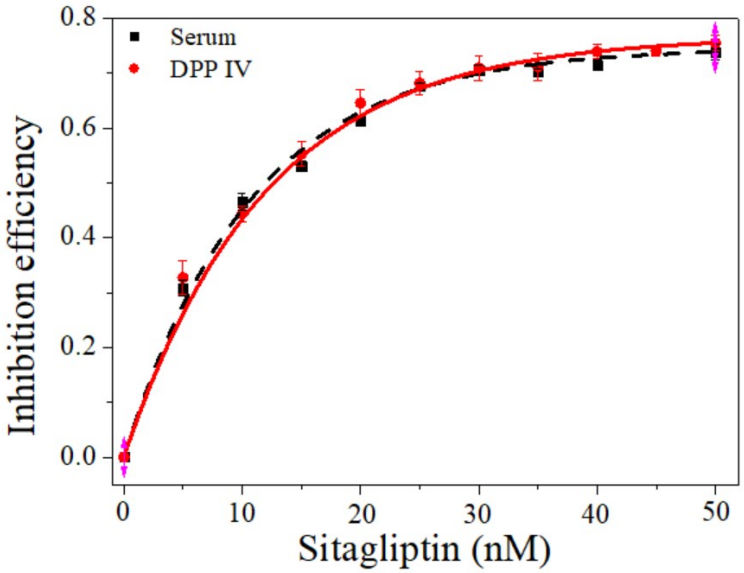


Figure S13 Dose-inhibition curves of sitagliptin (0-5 nM) on HCA-D hydrolysis in both human serum and recombinant DPP-IV. IC_{50} = 12.5 nM, 12.1 nM, respectively.

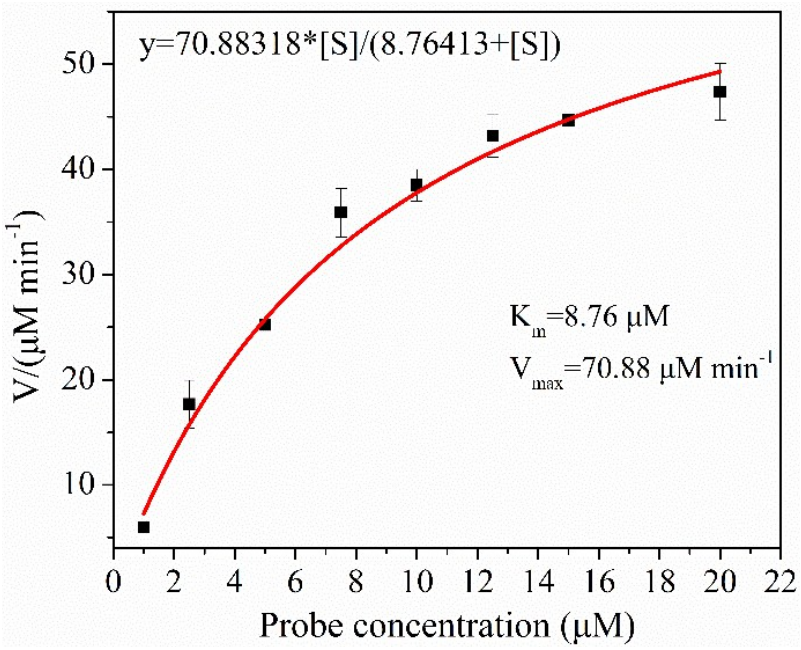


Figure S14 Michaelis−Menten kinetic plots of HCA-D hydrolysis in DPP-IV.

Section 6 Determination of DPP-IV activity in practical samples

Table S2 Quantitative detection of DPP-IV activity in human serum samples

Samples	DPP-IV spiked (ng/mL)	The total amount of DPP-IV (ng/mL)	Recovery rate (%)	R.S.D.(%)
Serum 1	0.0	4.34	/	4.3
	4.0	8.42	104	1.7
	6.0	9.92	93	2.3
Serum 2	0.0	4.05	/	1.0
	4.0	8.58	113	2.2
	6.0	10.01	99	1.6
Serum 3	0.0	3.66	/	4.9
	4.0	7.12	87	1.8
	6.0	9.47	97	2.5

Section 7 Cell cytotoxicity of HCA-D

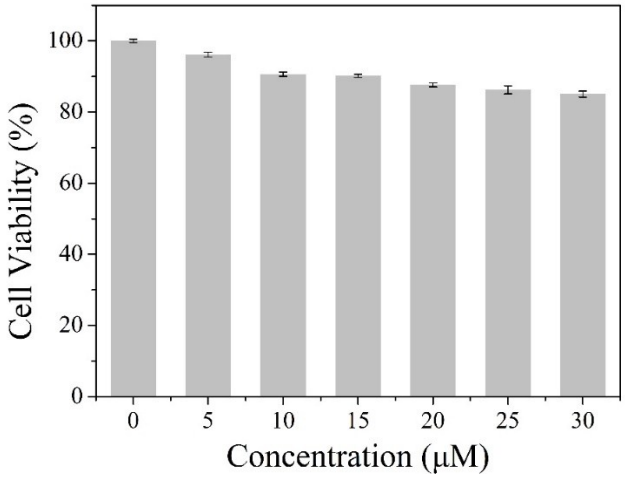


Figure S15 Effects of probe at varied concentrations on the cell viability of Hela cells.

References.

1. T. Liu, J. Ning, B. Wang, B. Dong, S. Li, X. Tian, Z. Yu, Y. Peng, C. Wang and X. Zhao, *Analytical Chemistry*, 2018, **90**, acs.analchem.7b04957.
2. L. W. Zou, P. Wang, X. K. Qian, L. Feng, Y. Yu, D. D. Wang, Q. Jin, J. Hou, Z. H. Liu and G. B. Ge, Biosensors and Bioelectronics, 2016, **90**, 283-289.
3. Q. Gong, W. Shi, L. Li, X. Wu and H. Ma, *Analytical Chemistry*, 2016, **88**, acs.analchem.6b02231.
4. Y. Wang, X. Wu, Y. Cheng and X. Zhao, *Chemical Communications*, 2016, **52**, 3478-3481.
5. N. H. Ho, R. Weissleder and C. H. Tung, *Bioorganic & Medicinal Chemistry Letters*, 2006, **16**, 2599-2602.