

Supporting information for

A mitochondria-targetable fluorescent probe with large Stokes shift for detecting hydrogen peroxide in aqueous solution and living cells

Yong Liu,^a Jie Niu,^a Jing Nie,^b Fangfang Meng^a and Weiyang Lin^{a*}

^a Institute of Fluorescent Probes for Biological Imaging, School of Materials Science and Engineering, School of Chemistry and Chemical Engineering, University of Jinan, Shandong 250022, P. R. China. E-mail: weiyanglin2013@163.com

^b School of Chemical Engineering & Technology, China University of Mining and Technology, Xuzhou, Jiangshu, 221116, P.R. China..

Table of contents

	Page
Table S1.....	S3-6
Fig S1.....	S6
Figs S2-S3.....	S7
Figs S4-S5.....	S8
Table S2, Figs S6-S7.....	S9
Figs S8-S9.....	S10
Fig S10-S11.....	S11
References.....	S12-14

Table S1. Properties of the representative hydrogen peroxide probes developed and the **CAI** reported in this work.

Ref.	Core structure	Stokes shift <i>in aqueous solution</i>	Probe Type	Maximum absorption wavelength and emission wavelength <i>in aqueous solution</i>	Application
This work	Carbazole-based derivatives CAI	157 nm	Turn off	418/575 nm	Detecting hydrogen peroxide in pure water and mitochondria <i>via</i> fluorescence quenching
1	NBzF	24 nm	Turn on	495/519 nm	Highly Sensitive detecting hydrogen peroxide
2	SERS nanoprobe	—	Turn on	—	Ratiometric imaging of hydrogen peroxide in living cells
3	MI-H ₂ O ₂	—	Turn on	425 /-nm	Simultaneous fluorescence imaging of hydrogen peroxide in mitochondria
4	Cy-B	—	Turn on	575/- nm	Imaging endogenous hydrogen peroxide during an autophagy process induced by rapamycin
5	Polymer 3	20 nm			Highly

			Turn off	330/ 350 nm	efficient detection of hydrogen peroxide in solution
6	Prototype probe 1	—	Turn on	—	Highly Sensitive detecting hydrogen peroxide
7	TPE-BO	100 nm	Turn on	400/500nm	Rapid-Response detecting hydrogen peroxide in living cells
8	D-HMSE	—	Turn on	—	highly selective detecting hydrogen peroxide
9	QCy-BA-Drew-AT	90 nm	Turn on	410 /500 nm	Reporting of cellular hydrogen peroxide
10	TPE-HPro	—	Turn on	—	Highly sensitive detection of hydrogen peroxide and glucose
11	Ir-2	—	Turn on	—	Recognition of hydrogen peroxide
12	DCM-B2	—	Turn on	—	Hydrogen peroxide imaging <i>in vitro</i> and <i>in vivo</i>
13	CBZ-H ₂ O ₂	—	Turn on	—	Imaging of mitochondrial hydrogen

					peroxide in living cells and tissues
14	Mito-VH	—	Turn on	—	Dual-Imaging viscosity and H ₂ O ₂ in Mitochondria
15	CSBOH	100 nm	Turn on	570 /670 nm	Imaging H ₂ O ₂ imaging in <i>vitro</i> and in <i>vivo</i>
16	FP-H ₂ O ₂ -NO	—	Turn on	—	Imaging H ₂ O ₂ in <i>vitro</i> and in <i>vivo</i>
17	Mito-H ₂ O ₂	—	Turn on	365 /-nm	Imaging mitochondrial hydrogen peroxide in living cells
18	PF1	—	Turn on	—	Imaging H ₂ O ₂ in living cells
19	MitoPY1ox	18 nm	Turn on	510 / 528nm	Imaging mitochondrial H ₂ O ₂ in living cells
20	probe 3	—	Turn on	—	Imaging H ₂ O ₂ in <i>vitro</i> and in <i>vivo</i>
21	QCy7	—	Turn on	560/-nm	Noninvasive intravital optical imaging of hydrogen peroxide
22	pep3-NP1	48 nm	Turn on	352/400 nm	Detection of hydrogen peroxide near mitochondrial DNA
23	L1	64 nm	Turn on	520 /584nm	Spatially-Confined visualization of intracellular hydrogen

					peroxide
24	Mito-NIRHP	60 nm	Turn on	650/710 nm	Imaging H ₂ O ₂ in <i>vitro</i> and in <i>vivo</i>
25	NP1	—	Turn on	—	Detection of cytoplasmic and nuclear hydrogen peroxide

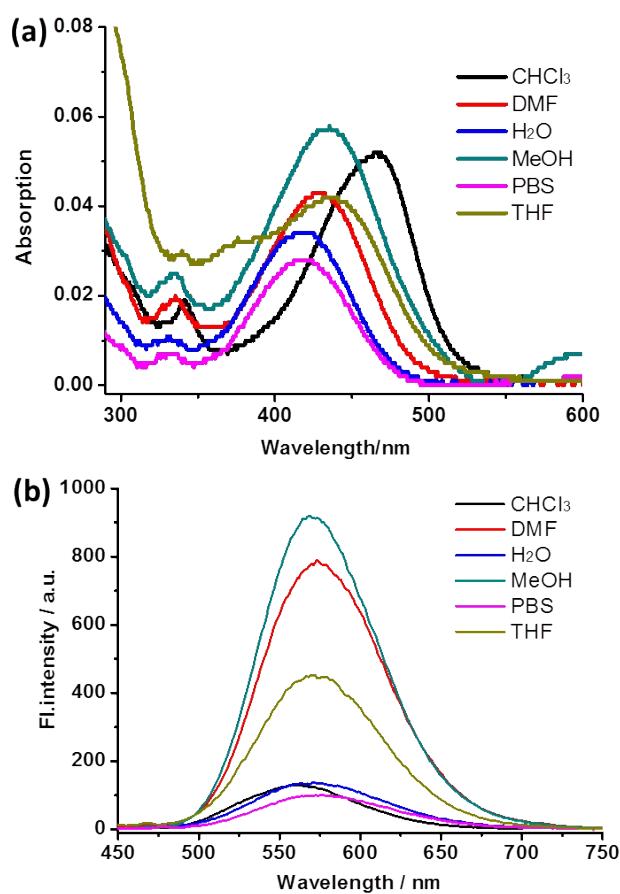


Fig S1. (a) UV-vis absorption (b) and fluorescence spectra of **CAI** in various solvents. Concentration of **CAI**: 2 μ M.

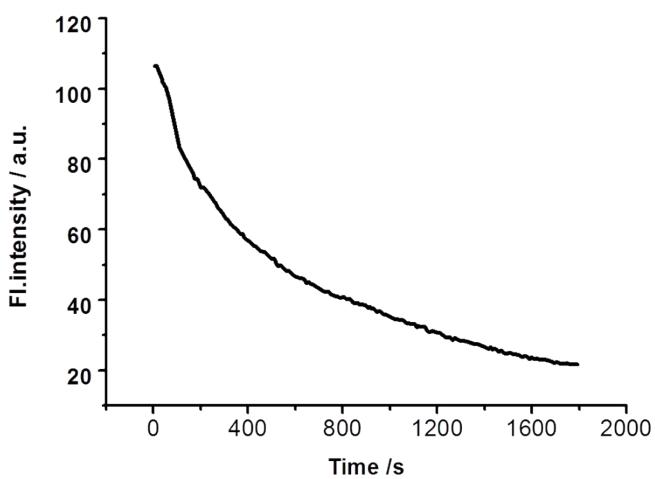


Fig S2. Time course of the fluorescence intensity of **CAI** (2 μ M) at 575 nm after adding 80 equiv. H_2O_2 . $\lambda_{\text{ex}} = 405$ nm.

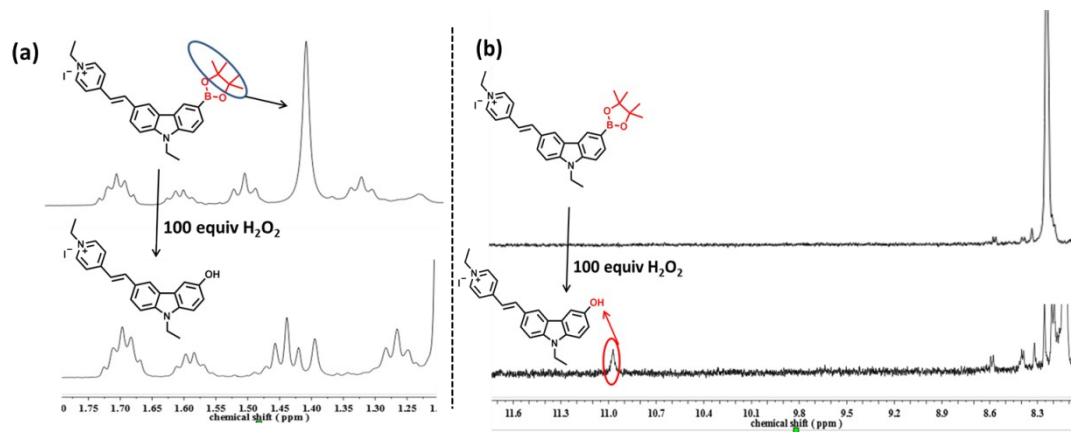


Fig S3. (a) The ^1H NMR spectrum of the methyl peak of **CAI** in the absence and presence of H_2O_2 (100 equiv) in d_6 - DMSO/ D_2O (2:1); (b) The ^1H NMR spectrum of the hydroxy peak of **CAI** in the absent and present of H_2O_2 (100 equiv) in d_6 - DMSO/ D_2O (2:1).

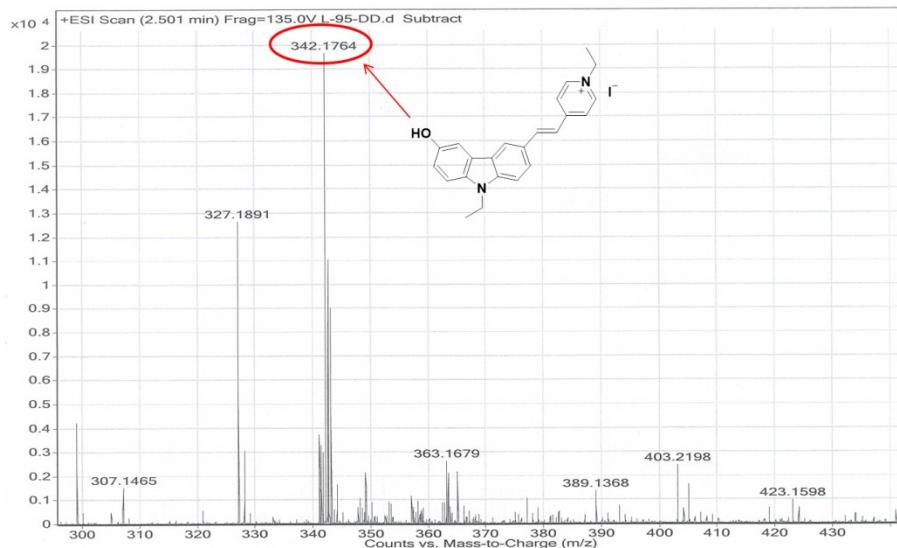


Fig S4. The formation of 1-ethyl-4-(2-(9-ethyl-6-hydroxy-9H-carbazol-3-yl)vinyl)pyridin-1-ium iodide from the reaction of the probe with H_2O_2 was confirmed by H RMS (ESI). The intense peak at m/z 342.1746 in the H RMS (ESI) spectrum corresponds to 1-ethyl-4-(2-(9-ethyl-6-hydroxy-9H-carbazol-3-yl)vinyl)pyridin-1-ium iodide.

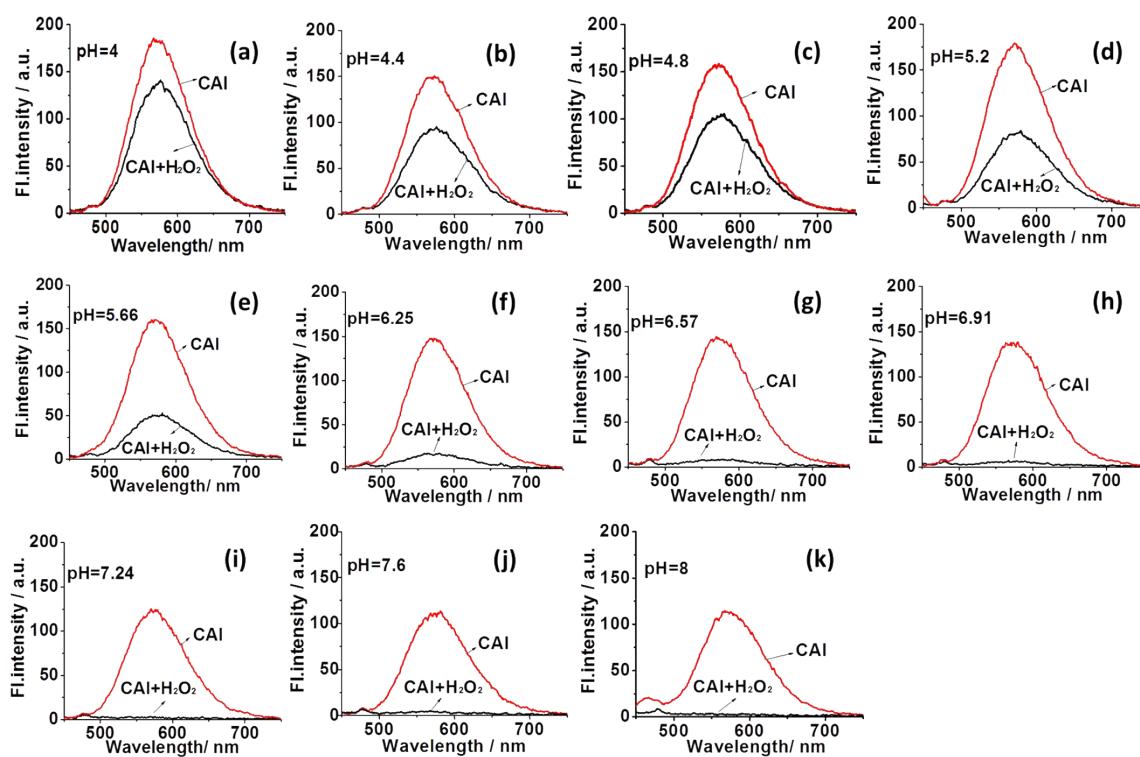


Fig S5. Fluorescent response of the probe **CAI** ($2 \mu\text{M}$) to H_2O_2 (110 equiv.) in different pH PBS buffer solution.

Table S2. Cytotoxicity data of CAI in RAW 264.7 cells ^a at 8 h.

Incubated concentration (μ M)	0	5	15	20	30
(% cell survival)	100 \pm 4	96 \pm 4	77 \pm 4	71 \pm 4	62 \pm 4

^a Cell viability was quantified by the MTT assays (mean \pm SD).

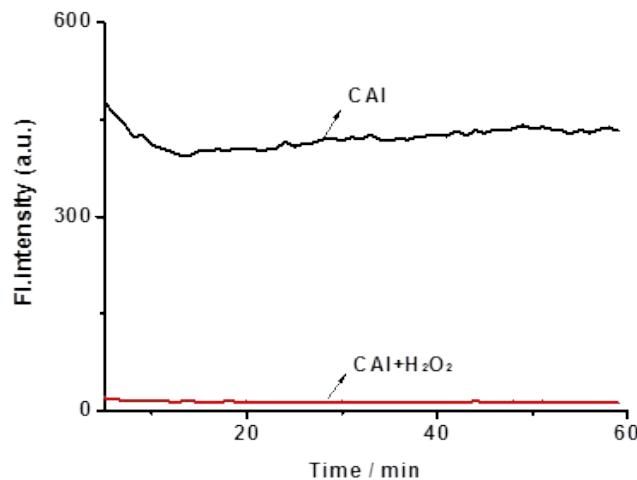


Fig S6. Photostability of the probe CAI (2 μ M) in the absence and presence of H₂O₂. $\lambda_{\text{ex}} = 405 \text{ nm}$

Characterization

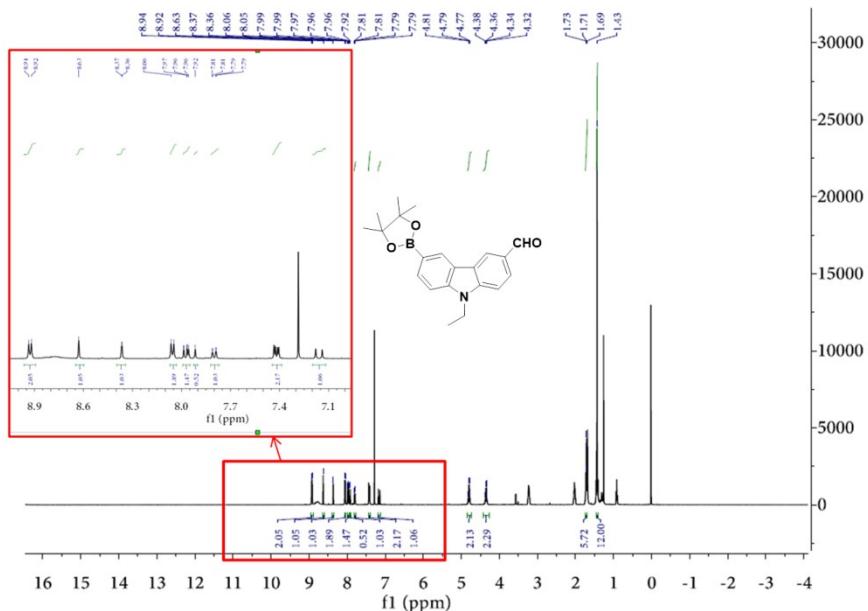


Fig S7. ¹H NMR spectrum of compound 2 in CDCl₃

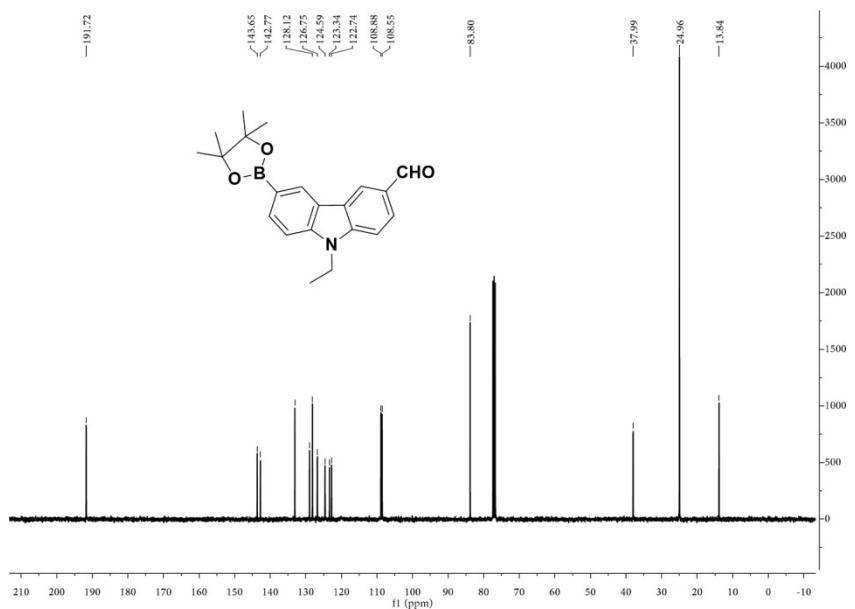


Fig S8. ^{13}C NMR spectrum of compound **2** in CDCl_3

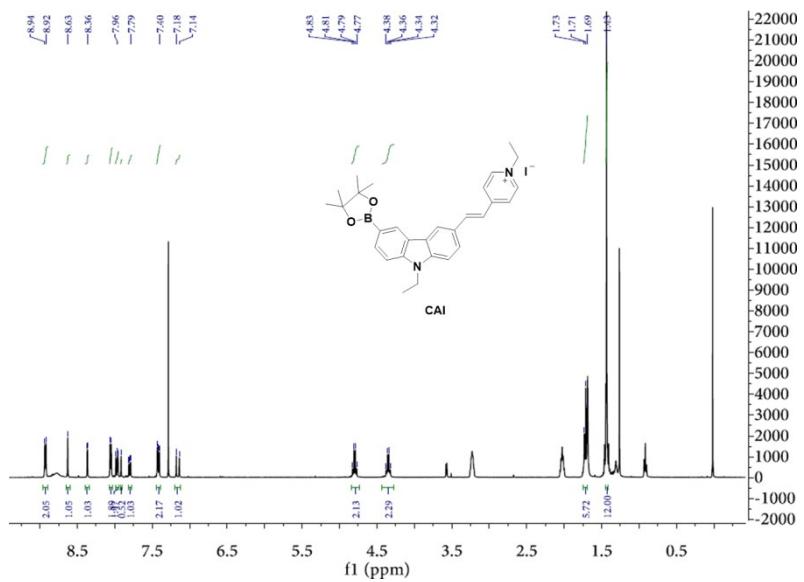


Fig S9. ^1H NMR spectrum of CAI in CDCl_3

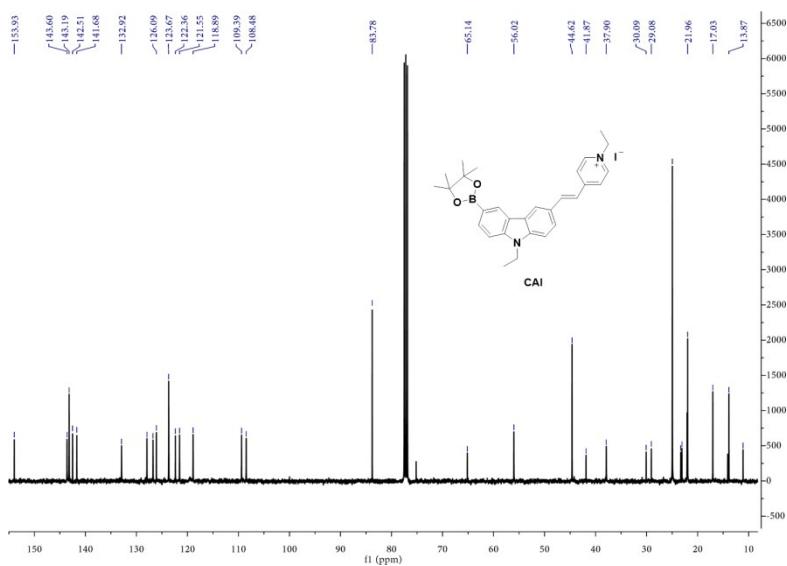


Fig S10. ¹³C NMR spectrum of CAI

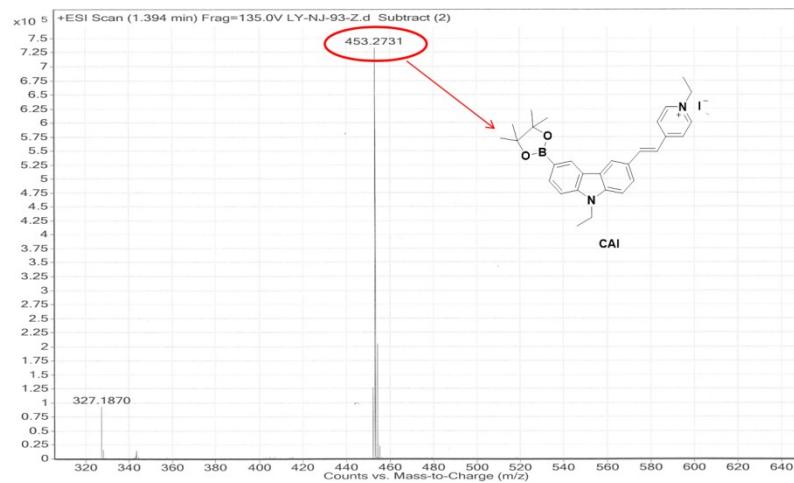


Fig S11. High resolution mass spectrum (HRMS) (ESI) of CAI

References:

1. M. Abo, Y. Urano, K. Hanaoka, T. Terai, T. Komatsu and T. Nagano, *J. Am. Chem. Soc.*, 2011, **133**, 10629.
2. R. Peng, Y. Si, T. Deng, J. Zheng, J. Li, R. Yang and W. Tan, *Chem. Commun.*, 2016, **52**, 8553.
3. H. Xiao, P. Li, X. Hu, X. Shi, W. Zhang and B. Tang, *Chem. Sci.*, 2016, **7**, 6153
4. F. Xu, H. Li, Q. Yao, J. Fan, J. Wang and X. Peng, *J. Mater. Chem. B*, 2016, **4**, 7363
5. P. Marks, B. Radaram, M. Levine and I. A. Levitsky, *Chem. Commun.*, 2015, **51**, 7061.
6. L. –C. Lo and C. –Y. Chu, *Chem. Commun.*, 2003, 2728.
7. W. Zhang, W. Liu, P. Li, F. Huang, H. Wang and B. Tang, *Anal. Chem.*, 2015, **87**, 9825.
8. Y. –X. Liao, K. Li, M. –Y. Wu, T. Wu and X. –Q. Yu, *Org. Biomol. Chem.*, 2014, **12**, 3004.
9. N. Narayanaswamy, S. Narra, R. R. Nair, D. K. Saini, P. Kondaiah and T. Govindaraju, *Chem. Sci.*, 2016, **7**, 2832.
10. Z. Song, R. T. K. Kwok, D. Ding, H. Nie, J. W. Y. Lam, B. Liuc and B. Z. Tang, *Chem. Commun.*, 2016, **52**, 10076.
11. C. Li, S. Wang, Y. Huang, Q. Wen, L. Wang and Y. Kan, *Dalton. Trans.*, 2014, **43**, 5595.

12. P. Wang, K. Wang, D. Chen, Y. Mao and Y. Gu, *RSC Adv.*, 2015, **5**, 85957.
13. K. Zhang, W. Wu, Y. Li, M. Sun, H. Yu and M. S. Wong, *RSC Adv.*, 2016, **6**, 115298.
14. M. Ren, B. Deng, K. Zhou, X. Kong, J. -Y. Wang and W. Lin, *Anal. Chem.*, 2016, DOI: 10.1021/acs.analchem.6b04385.
15. K. Liu, H. Shang, X. Kong, M. Ren, J. -Y. Wang, Y. Liu, W. Lin, *Biomaterials*, 2016, **100**, 162.
16. L. Yuan, W. Lin, Y. Xie, B. Chen and S. Zhu, *J. Am. Chem. Soc.*, 2012, **134**, 1305.
17. J. Xu, Y. Zhang, H. Yu, X. Gao and S. Shao, *Anal. Chem.*, 2016, **88**, 1455.
18. M. C. Y. Chang, A. Pralle, E. Y. Isacoff and C. J. Chang, *J. Am. Chem. Soc.*, 2004, **126**, 15392.
19. B. C. Dickinson and C. J. Chang, *J. Am. Chem. Soc.*, 2008, **130**, 9638.
20. W. Wu, J. Li, L. Chen, Z. Ma, W. Zhang, Z. Liu, Y. Cheng, L. Du and M. Li, *Anal. Chem.*, 2014, **86**, 9800.
21. N. Karton-Lifshin, E. Segal, L. Omer, M. Portnoy, R. Satchi-Fainaro and D. Shabat, *J. Am. Chem. Soc.*, 2011, **133**, 10960.
22. Y. Wen, K. Liu, H. Yang, Y. Liu, L. Chen, Z. Liu, C. Huang and T. Yi, *Anal. Chem.*, 2015, **87**, 10579.
23. J. Liu, J. Ren, X. Bao, W. Gao, C. Wu and Y. Zhao, *Anal. Chem.*, 2016, **88**, 5865.

24. X. Xie, X. Yang, T. Wu, Y. Li, M. Li, Q. Tan, X. Wang and B. Tang, *Anal. Chem.*, 2016, **88**, 8019.
25. Y. Wen, K. Liu, H. Yang, Y. Li, H. Lan, Y. Liu, X. Zhang and T. Yi, *Anal. Chem.*, 2014, **86**, 9970.