

Cite this: *Chem. Sci.*, 2019, 10, 10876

All publication charges for this article have been paid for by the Royal Society of Chemistry

# Ratiometric fluorescence imaging of Golgi H<sub>2</sub>O<sub>2</sub> reveals a correlation between Golgi oxidative stress and hypertension†

Hui Wang,‡ Zixu He,‡ Yuyun Yang, Jiao Zhang, Wei Zhang, Wen Zhang, Ping Li\* and Bo Tang \*

Golgi oxidative stress is significantly associated with the occurrence and progression of hypertension. Notably, the concentration of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is directly proportional to the degree of Golgi oxidative stress. Therefore, based on a novel Golgi-targeting phenylsulfonamide group, we developed a two-photon (TP) fluorescent probe, Np-Golgi, for *in situ* H<sub>2</sub>O<sub>2</sub> ratiometric imaging in living systems. The phenylsulfonamide moiety effectively assists Np-Golgi in the precise location of Golgi apparatus. In addition, the raw material of phenylsulfonamide is easily available, and chemical modification is easily implemented. By application of Np-Golgi, we explored the generation of H<sub>2</sub>O<sub>2</sub> during Golgi oxidative stress, and also successfully revealed increases on the levels of Golgi H<sub>2</sub>O<sub>2</sub> in the kidneys of mice with hypertension. This work provides an ideal tool to monitor Golgi oxidative stress for the first time and novel drug targets for the future treatment of hypertension.

Received 31st August 2019  
Accepted 1st October 2019

DOI: 10.1039/c9sc04384e

rsc.li/chemical-science

## Introduction

Hypertension is a major risk factor for the development of cardiovascular disease (CVD).<sup>1</sup> It is predicted that the prevalence of hypertension will increase by more than 50% during the next 30 years.<sup>2,3</sup> Until now, despite receiving antihypertensive treatment, many people with the disorder still cannot adequately control their blood pressure. Thus, novel therapies are urgently needed to address resistant hypertension. A large amount of studies suggest that oxidative stress plays a central role in the pathogenesis of hypertension by perturbing the balance between reactive oxygen species (ROS) and antioxidant defenses.<sup>4–7</sup> Excess ROS promote hypertension by inducing endothelial dysfunction.<sup>8,9</sup> Therefore, complete understanding of the mechanisms of oxidative stress could contribute to the development of new therapies.

Since the Golgi complex acts as a key trafficking and sorting station and a vital biosynthetic centre for glycoproteins and lipids,<sup>10</sup> Golgi oxidative stress plays both physiological and pathophysiological roles in cells along with extensive ROS production.<sup>11–13</sup> Therefore, quantitative detection of various

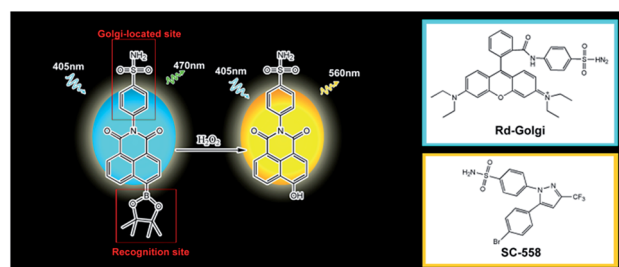
ROS is essential to study the mechanism of Golgi oxidative stress, especially hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>),<sup>14,15</sup> an indicator of oxidative stress. However, the concentration and generation of H<sub>2</sub>O<sub>2</sub> in the Golgi complex remain poorly understood to date, which is mainly due to a lack of tools for specific measurement of Golgi-located H<sub>2</sub>O<sub>2</sub> *in situ*. This ultimately causes difficulties in revealing the direct relevance between H<sub>2</sub>O<sub>2</sub> levels and hypertension.

Two-photon (TP) fluorescence imaging is a noninvasive approach for *in situ* detection of various biomolecules.<sup>16–19</sup> It exhibits increased tissue penetration depth, higher temporal resolution and less specimen photodamage than one-photon fluorescence imaging.<sup>20</sup> To date, many TP fluorescent probes have been developed to visualize H<sub>2</sub>O<sub>2</sub> in various organelles in cells,<sup>21–25</sup> but *in situ* bioimaging of Golgi H<sub>2</sub>O<sub>2</sub> is still scarce. Developing a TP fluorescent probe for tracing Golgi H<sub>2</sub>O<sub>2</sub> in living systems could contribute to defining the relationship between hypertension and Golgi oxidative stress. However, the

College of Chemistry, Chemical Engineering and Materials Science, Institute of Biomedical Sciences, Collaborative Innovation Center of Functionalized Probes for Chemical Imaging in Universities of Shandong, Key Laboratory of Molecular and Nano Probes, Ministry of Education, Shandong Normal University, Jinan 250014, PR China. E-mail: tangb@sdsu.edu.cn; lip@sdsu.edu.cn

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c9sc04384e

‡ These authors contributed equally to this work.



Scheme 1 The structure and response mechanism of Np-Golgi.



main obstacle for the development of a TP fluorescent probe for Golgi H<sub>2</sub>O<sub>2</sub> detection is a lack of precise Golgi-targeting groups. To date, two kinds of Golgi-targeting groups have been reported, including Golgi-targeting polypeptides and cysteine.<sup>26,27</sup> Golgi-targeting polypeptides exhibit significant localization ability but are hard to synthesize. Cysteine has limitations in the synthesis of fluorescent probes due to its low lipid solubility. Therefore, it is urgent to develop a novel Golgi-targeting group, which should meet the following requirements: (1) precise localization, (2) easily available raw material, and (3) facile chemical modification.

Previous reports have shown that the selectivity of SC-558, a Golgi protein cyclooxygenase-2 (COX-2) inhibitor, results from a phenylsulfonamide moiety that binds in specific pockets in complexes of COX-2.<sup>28,29</sup> Based on this work, we supposed that phenylsulfonamide could be used as a Golgi-targeting group to deliver fluorescent probes into the Golgi apparatus. We herein report the first TP ratiometric fluorescent probe, NP-Golgi, (Scheme 1) with a phenylsulfonamide moiety as the Golgi-targeting group for H<sub>2</sub>O<sub>2</sub> bioimaging in the Golgi apparatus. NP-Golgi comprises phenylsulfonamide-modified 1,8-naphthalimide as a fluorescent reporter and boric acid ester as a specific H<sub>2</sub>O<sub>2</sub> recognition group.<sup>30,31</sup> The boric acid ester converts to an electron-donating hydroxyl group upon reaction with H<sub>2</sub>O<sub>2</sub>, thus promoting the push-pull electron effect of the naphthalimide-conjugated system and subsequently resulting in strong fluorescence emission. In accordance with our prediction, NP-Golgi could effectively accumulate in the Golgi complex to sense H<sub>2</sub>O<sub>2</sub> with high selectivity and sensitivity both *in vivo* and *in vitro*. The generation of Golgi H<sub>2</sub>O<sub>2</sub> was also investigated for the first time with the assistance of NP-Golgi. With the aid of TP fluorescence microscopy, NP-Golgi was successfully applied to detect Golgi H<sub>2</sub>O<sub>2</sub> fluctuations in mice with hypertension, suggesting NP-Golgi as a new instrument with which to explore the correlations between Golgi oxidative stress and diseases.

## Results and discussion

### Photophysical properties and selectivity of Np-Golgi

The details of the synthesis routes and characterization of Np-Golgi, and Rd-Golgi are shown in the ESI.†

We first assessed the spectroscopic properties of Np-Golgi under physiological conditions. The absorption and fluorescence spectra of Np-Golgi were measured in the absence and presence of H<sub>2</sub>O<sub>2</sub>. As shown in Fig. 1A, along with a colour change, a red-shift of the absorption peak of Np-Golgi from 350 nm to 446 nm occurred after reaction with H<sub>2</sub>O<sub>2</sub>. Meanwhile, the fluorescence emission peak was red-shifted from 470 nm to 560 nm. This demonstrated that Np-Golgi exhibited obvious changes in spectroscopic properties upon the addition of H<sub>2</sub>O<sub>2</sub>. We subsequently confirmed the reaction mechanism between Np-Golgi and H<sub>2</sub>O<sub>2</sub> with high-performance liquid chromatography (HPLC).<sup>32</sup> The HPLC results showed that most Np-Golgi (HPLC retention time, TR = 50.8 min) was converted to Np-OH (TR = 25.7 min) after incubation with H<sub>2</sub>O<sub>2</sub> (Fig. S1†). Meanwhile, the fluorescence quantum yield increased from

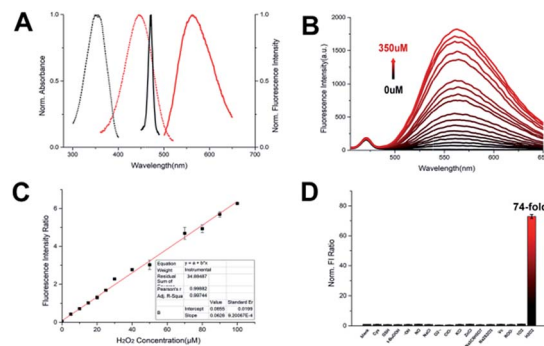


Fig. 1 (A) The absorption (dash line) and fluorescence (solid line) spectra of Np-Golgi with (red) or without (black) H<sub>2</sub>O<sub>2</sub>. (B) The fluorescence spectra of Np-Golgi (2 μM) with different concentration of H<sub>2</sub>O<sub>2</sub>. (C) Linear curve of fluorescence intensity ratio for Np-Golgi towards H<sub>2</sub>O<sub>2</sub> (0–100 μM). (D) The selectivity of Np-Golgi (2 μM) in the presence of 100 μM various biologically relevant species (1–17: blank, Cys, GSH, *t*-BuOOH, ·OH, NO, NaCl, O<sub>2</sub><sup>−</sup>, ClO<sup>−</sup>, KCl, ZnCl, Na<sub>2</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub>, Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, Vc, ROO<sup>·</sup>, <sup>1</sup>O<sub>2</sub>, and H<sub>2</sub>O<sub>2</sub>).

0.30 to 0.39, and the fluorescence of the mixture (Np-Golgi and H<sub>2</sub>O<sub>2</sub>) was stable for at least 60 min (Fig. S2†). Then, we calculated the TP absorption cross section of Np-Golgi, which was 72 GM upon treatment with H<sub>2</sub>O<sub>2</sub> (Fig. S3†).<sup>33,34</sup> All these results suggest that Np-Golgi potentially possesses the ability to detect H<sub>2</sub>O<sub>2</sub> as a TP fluorescent probe.

We subsequently detected the fluorescence responses of Np-Golgi to various H<sub>2</sub>O<sub>2</sub> levels. Upon addition of H<sub>2</sub>O<sub>2</sub>, the fluorescent intensity (FI) of Np-Golgi (2 μM) at 470 nm was constant, while the FI at 560 nm was significantly enhanced with higher concentrations of H<sub>2</sub>O<sub>2</sub> (Fig. 1B). Notably, the  $F_{560}/F_{470}$  ratio increased 130-fold in the presence of H<sub>2</sub>O<sub>2</sub> (350 μM) compared with the absence of H<sub>2</sub>O<sub>2</sub>. There were two linear relationships between the  $F_{560}/F_{470}$  ratio and the concentration of H<sub>2</sub>O<sub>2</sub> (1–100 μM:  $R^2 = 0.997$ ; 100–350 μM:  $R^2 = 0.982$ ). The detection limit of Np-Golgi to H<sub>2</sub>O<sub>2</sub> is 0.20 μM (Fig. 1C and S4†). These results demonstrate that Np-Golgi exhibits highly sensitive responses to H<sub>2</sub>O<sub>2</sub> and can quantitatively detect H<sub>2</sub>O<sub>2</sub> over a wide range.

To apply Np-Golgi to image H<sub>2</sub>O<sub>2</sub> in cells and *in vivo*, we investigated the fluorescence responses of Np-Golgi to various biomolecules in cells. As shown in Fig. 1D, the FI ratio  $F_{560}/F_{470}$  of Np-Golgi was prominently increased after incubation with H<sub>2</sub>O<sub>2</sub>, whereas  $F_{560}/F_{470}$  was scarcely changed after incubation with other biomolecules. This result indicated that Np-Golgi exhibited remarkable selectivity for H<sub>2</sub>O<sub>2</sub>. We also monitored the influence of different pH values on  $F_{560}/F_{470}$ . The results showed that Np-Golgi exhibited obvious fluorescence responses to H<sub>2</sub>O<sub>2</sub> at pH 6.0–8.0 (Fig. S5†). The data indicates that Np-Golgi could serve as a superior sensor for fluorescence imaging of H<sub>2</sub>O<sub>2</sub> in living system.

### Validation of Np-Golgi for imaging H<sub>2</sub>O<sub>2</sub> in cells

To assess whether Np-Golgi could be utilized in biological systems, a standard MTT assay was performed in various cells to evaluate the cytotoxicity of Np-Golgi. The probe showed



inconspicuous cytotoxicity at concentrations less than 20  $\mu\text{M}$  (Fig. S6–S8<sup>†</sup>), which demonstrated that Np-Golgi could be used in living systems. We next applied Np-Golgi for endogenous  $\text{H}_2\text{O}_2$  detection in human hepatoma cells (SMMC7721). Phorbol 12-myristate 13-acetate (PMA, 1  $\mu\text{g mL}^{-1}$ ) was used to induce generation of  $\text{H}_2\text{O}_2$  in cells. The fluorescence of Np-Golgi in the green channel (530–580 nm) increased significantly upon the addition of PMA, while the fluorescence in the blue channel (430–480 nm) was constant. The ratio of  $F_{\text{green}}/F_{\text{blue}}$  in PMA-treated cells showed 1.5-fold enhancement than in control cells. To confirm that the fluorescence enhancement was caused by increased  $\text{H}_2\text{O}_2$  concentrations, we also evaluated the FI ratio  $F_{\text{green}}/F_{\text{blue}}$  of Np-Golgi in *N*-acetylcysteine<sup>35</sup> (NAC:  $\text{H}_2\text{O}_2$  eliminator) incubated cells. As shown in Fig. S9,† both NAC-stimulated cells and PMA + NAC-stimulated cells presented negligible fluorescence in the green channel with lower  $F_{\text{green}}/F_{\text{blue}}$  ratios than control cells. This phenomenon demonstrates that intracellular  $\text{H}_2\text{O}_2$  has been effectively eliminated. These observations validate that Np-Golgi can be used for fluorescence imaging of  $\text{H}_2\text{O}_2$  in cells with high selectivity and good sensitivity to quantify the concentrations of  $\text{H}_2\text{O}_2$ . Next, we explored the intracellular photostability of Np-Golgi (Fig. S10<sup>†</sup>). The fluorescence of Np-Golgi was consistent for 60 min after cells were rinsed thoroughly with PBS. The result implies that Np-Golgi can be applied for long-term imaging of  $\text{H}_2\text{O}_2$  *in vivo*.<sup>36</sup>

### Subcellular localization of Np-Golgi

To verify the Golgi complex-targeting ability of Np-Golgi, a colocalization experiment was performed to observe the subcellular distribution of the probe. As shown in Fig. 2, the green fluorescence of Np-Golgi overlapped well with the fluorescence of the commercial probe Golgi-Tracker Red (Pearson's colocalization coefficient: 0.94) in human cervical carcinoma cells (HeLa). In the meantime, the fluorescence of Np-Golgi showed poor overlap with that of Mito-Tracker Red (0.10), Lyso-Tracker Red (0.54) and ER-Tracker Red (0.44). To further confirm that Np-Golgi possessed universal Golgi-targeting

capability, colocalization experiments were also performed in SMMC7721 and human hepatic cells (HL-7702). As expected, the Np-Golgi probe also showed excellent Golgi-targeting characteristics in these cells (Fig. S11 and S12<sup>†</sup>). Collectively, Np-Golgi exhibits superior Golgi complex-locating capability in various cell types.

Subsequently, to validate that the targetability of the Np-Golgi to the Golgi complex was dependent on the phenylsulfonamide moiety, we synthesized a new phenylsulfonamide-modified fluorescent probe Rd-Golgi (Fig. S13<sup>†</sup>). Then the Golgi-targeting ability of Rd-Golgi was investigated. Rd-Golgi and Golgi-Tracker Red presented remarkable fluorescence overlap in cells with the Pearson's colocalization coefficient 0.95 (Fig. S14<sup>†</sup>). This result suggests that Rd-Golgi possesses Golgi-targeting ability and phenylsulfonamide is the crucial part of the probe for effective targeting of the Golgi apparatus. These outcomes establish that phenylsulfonamide is a powerful and universal Golgi-targeting group. In addition, compared with the reported Golgi-targeting peptide and the chemical group cysteine, phenylsulfonamide exhibits the following benefits: (1) the raw material is easily available; (2) chemical modification is simple; and (3) long-term accumulation in the Golgi is possible.

### The oxidative stress of Golgi apparatus

We next applied the  $\text{H}_2\text{O}_2$ -responsive, excellently Golgi-targeting TP fluorescent probe Np-Golgi to imaging of the fluctuations in intracellular  $\text{H}_2\text{O}_2$  associated with Golgi oxidative stress. Monensin<sup>37,38</sup> was used to induce Golgi oxidative stress by disturbing pH homeostasis to reduce the activity of Golgi proteins. Under NAC-pretreated conditions, monensin-stimulated cells exhibited brighter green fluorescence than NAC-incubated cells, and the  $F_{560}/F_{470}$  ratio was enhanced by



Fig. 2 Co-localization cell imaging of Np-Golgi and commercial dyes including Golgi-Red (a–d), Mito-Red (e–h), Lyso-Red (i–l), ER-Red (m–p) in HeLa cells.



Fig. 3 (A) Fluorescence imaging of Np-Golgi after cells were treated with different stimulants. (a, e and i) NAC cells: The cells were incubated with NAC (20 mM). (b, f and j) Monensin cells: The cells were incubated with NAC and then monensin (10  $\mu\text{M}$ ) was added. (c, g and k) Tiron cells: the cells were incubated with NAC, and then monensin and Tiron (10  $\mu\text{M}$ ) were added. (d, h and l) 2-ME cells: the cells were incubated with NAC, and then monensin and 2-ME (1  $\mu\text{g mL}^{-1}$ ) were added. (B) Relative fluorescence intensity of Np-Golgi labelled cells from images.  $n = 3$ .





4.9-fold (Fig. 3A). This result shows the level of Golgi  $\text{H}_2\text{O}_2$  is obviously increased in the Golgi oxidative stress process.

Many studies show that a large proportion of intracellular  $\text{H}_2\text{O}_2$  is generated from superoxide anions ( $\text{O}_2^-$ ) by catalysis reactions of various oxidases, such as superoxide dismutase (SOD). To seek evidence that Golgi  $\text{H}_2\text{O}_2$  was produced from  $\text{O}_2^-$  in the Golgi oxidative stress, the fluorescence of Np-Golgi was detected after incubating cells with  $\text{O}_2^-$  inducer (2-methoxyestradiol,<sup>39</sup>  $1.0 \mu\text{g mL}^{-1}$ ) or  $\text{O}_2^-$  scavenger (Tiron,<sup>40</sup>  $10 \mu\text{M}$ ). In order to avoid the interference from cytoplasmic  $\text{H}_2\text{O}_2$  and induce Golgi oxidative stress of the cells, NAC was used to eliminate the intracellular  $\text{H}_2\text{O}_2$  at first, and then monensin was added to cause higher level of  $\text{H}_2\text{O}_2$ . In this case, we investigated the relations between  $\text{O}_2^-$  and  $\text{H}_2\text{O}_2$  by using 2-ME and Tiron. As illustrated in Fig. 3, stronger green fluorescence was observed in both 2-methoxyestradiol (2-ME) and monensin co-incubated cells, and the  $F_{560}/F_{470}$  ratio was enhanced by 1.57-fold compared with monensin incubated cells. After the addition of Tiron to eliminate  $\text{O}_2^-$ , the FI of Np-Golgi in Tiron and monensin co-incubated cells decayed with a decreasing  $F_{560}/F_{470}$  ratio (by 0.83-fold) compared with monensin incubated cells. The above results indicate that the concentration of Golgi  $\text{H}_2\text{O}_2$  has positive correlation with  $\text{O}_2^-$  levels. Collectively, these data are in excellent agreement with our prediction that Golgi  $\text{H}_2\text{O}_2$  is mainly generated from  $\text{O}_2^-$  in Golgi oxidative stress.

### TP fluorescence imaging of HBP mice *in situ*

To prove that the probe could detect  $\text{H}_2\text{O}_2$  levels *in vivo*, we applied Np-Golgi for TP fluorescence imaging of  $\text{H}_2\text{O}_2$  in the kidneys of mice. The animal experiments were performed in compliance with the relevant laws and guidelines issued by the Ethical Committee of Shandong University and were in agreement with the guidelines of the Institutional Animal Care and Use Committee. The probe ( $10 \mu\text{M}$ ,  $100 \mu\text{L}$ ) in the LPS-injected area of the abdominal cavity of the mice showed remarkable fluorescence, and the  $F_{\text{red}}/F_{\text{green}}$  ratio was increased by 1.9-fold in the probe-injected area compared with the normal saline-injected area (Fig. S15<sup>†</sup>). This suggests that Np-Golgi could quantitatively monitor fluctuations of  $\text{H}_2\text{O}_2$  *in vivo* through TP fluorescence imaging.

Whether high blood pressure (HBP) is accompanied by Golgi oxidative stress was further investigated. Mice were injected with either 0.9% saline (control mice) or  $50 \mu\text{g}$  ouabain per day (HBP mice) for 20 days, and the blood pressure of the mice was measured three times per day. Eventually, the blood pressure of HBP mice exceeded 160 mmHg (Fig. S16<sup>†</sup>), and the results of Masson's trichrome staining of the kidneys showed that there was more collagen deposition in HBP mice than in control mice (Fig. S17<sup>†</sup>), proving that the HBP mouse model was successfully constructed. After incubation of the kidneys of mice with Np-Golgi ( $10 \mu\text{M}$ ,  $100 \mu\text{L}$ ) for 30 min, the  $F_{\text{red}}/F_{\text{green}}$  ratio of the probe was compared between HBP mice and control mice combined with two-photon imaging technology. As shown in Fig. 4, the  $F_{\text{red}}/F_{\text{green}}$  ratio in HBP mice was significantly increased (by 1.8-fold) compared with that in control mice, indicating increased  $\text{H}_2\text{O}_2$  levels in the mice with HBP. These

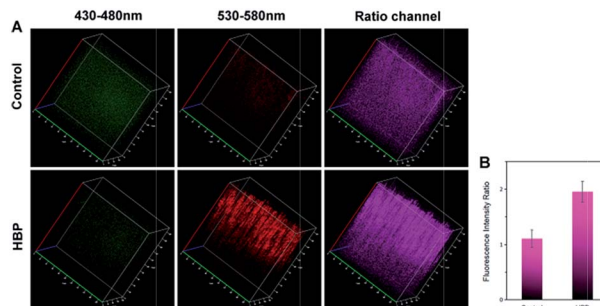


Fig. 4 TP ratiometric fluorescence imaging of kidney tissue in control mice and HBP mice with Np-Golgi. The fluorescence intensity ratio ( $R_{\text{red/green}}$ ) of the kidneys in mice. Two-photon excited wavelength: 810 nm.

data demonstrate that pathological changes in kidney tissue in HBP mice cause severe Golgi oxidative stress and excess  $\text{H}_2\text{O}_2$  accumulation in the process, which suggests that hypertension is closely related to Golgi oxidative stress.

## Conclusion

To discover the role of Golgi oxidative stress in hypertension, we developed a novel TP ratiometric fluorescent sensor, Np-Golgi, to quantitatively detect Golgi  $\text{H}_2\text{O}_2$  levels *in situ*. By application of Np-Golgi, we successfully detected increased levels of  $\text{H}_2\text{O}_2$  in the kidneys of mice with hypertension. This work not only reveals the relationship between hypertension and Golgi oxidative stress, but also provides a robust tool for uncovering the connections between Golgi oxidative stress and severe diseases in the future.

## Conflicts of interest

There are no conflicts to declare.

## Acknowledgements

This work was supported by National Natural Science Foundation of China (21535004, 91753111, 21405097, 21390411 and 21475079), Technological Special Project for "Significant New Drugs Development" (2017ZX09301030004), the Key Research and Development Program of Shandong Province (2018YFJH0502), Natural Science Foundation of Shandong Province (ZR2017ZC0225).

## Notes and references

- 1 S. Mendis, *Cardiovasc Diagn Ther.*, 2017, 7, S32–S38.
- 2 P. M. Kearney, M. Whelton, K. Reynolds, P. Muntner, P. K. Whelton and J. He, *Lancet*, 2005, 365, 217–223.
- 3 C. J. McAloon, L. M. Boylan, T. Hamborg, N. Stallard, F. Osman, P. B. Lim and S. A. Hayat, *Int. J. Cardiol.*, 2016, 224, 256–264.
- 4 T. Finkel and N. J. Holbrook, *Nature*, 2000, 408, 239–247.



- 5 H. Y. Small, S. Migliarino, M. Czesnikiewicz-Guzik and T. J. Guzik, *Free Radical Biol. Med.*, 2018, **125**, 104–115.
- 6 M. Korsager Larsen and V. V. Matchkov, *Medicina (Kaunas)*, 2016, **52**, 19–27.
- 7 W. Xu, Z. Zeng, J. H. Jiang, Y. T. Chang and L. Yuan, *Angew. Chem., Int. Ed.*, 2016, **55**, 13658–13699.
- 8 H. Matsuoka, S. Miyata, N. Okumura, T. Watanabe, K. Hashimoto, M. Nagahara, K. Kato, S. Sobue, K. Takeda, M. Ichihara, T. Iwamoto and A. Noda, *Clin. Exp. Hypertens.*, 2019, **41**, 307–311.
- 9 S. I. Dikalov and A. E. Dikalova, *Antioxid Redox Signal*, 2019.
- 10 F. Zappa, M. Failli and M. A. De Matteis, *Curr. Opin. Cell Biol.*, 2018, **50**, 102–116.
- 11 W. Zhong, *Clin. Exp. Hypertens.*, 2011, **3**, a005363.
- 12 P. Mayinger, *Clin. Exp. Hypertens.*, 2011, **3**, a005314.
- 13 S. W. Hicks and C. E. Machamer, *Biochim. Biophys. Acta*, 2005, **1744**, 406–414.
- 14 J. B. Pi, W. Qu, J. M. Reece, Y. Kumagai and M. P. Waalkes, *Exp. Cell Res.*, 2003, **290**, 234–245.
- 15 Q. Li, M. M. Harraz, W. Zhou, L. N. Zhang, W. Ding, Y. Zhang, T. Eggleston, C. Yeaman, B. Banfi and J. F. Engelhardt, *Mol. Cell. Biol.*, 2006, **26**, 140–154.
- 16 G. Masanta, C. H. Heo, C. S. Lim, S. K. Bae, B. R. Cho and H. M. Kim, *Chem. Commun.*, 2012, **48**, 3518–3520.
- 17 H. W. Liu, X. B. Zhang, J. Zhang, Q. Q. Wang, X. X. Hu, P. Wang and W. Tan, *Anal. Chem.*, 2015, **87**, 8896–8903.
- 18 E. W. Miller, A. E. Albers, A. Pralle, E. Y. Isacoff and C. J. Chang, *J. Am. Chem. Soc.*, 2005, **127**, 16652–16659.
- 19 W. Zhao, Y. Li, S. Yang, Y. Chen, J. Zheng, C. Liu, Z. Qing, J. Li and R. Yang, *Anal. Chem.*, 2016, **88**, 4833–4840.
- 20 E. J. Sanchez, L. Novotny, G. R. Holtom and X. S. Xie, *J. Phys. Chem. A*, 1997, **101**, 7019–7023.
- 21 B. C. Dickinson, Y. Tang, Z. Chang and C. J. Chang, *Chem. Biol.*, 2011, **18**, 943–948.
- 22 C. Chung, D. Srikun, C. S. Lim, C. J. Chang and B. R. Cho, *Chem. Commun.*, 2011, **47**, 9618–9620.
- 23 D. Kim, G. Kim, S. J. Nam, J. Yin and J. Yoon, *Sci. Rep.*, 2015, **5**, 8488.
- 24 J. Xu, Y. Zhang, H. Yu, X. Gao and S. Shao, *Anal. Chem.*, 2016, **88**, 1455–1461.
- 25 M. Ren, B. Deng, J. Y. Wang, X. Kong, Z. R. Liu, K. Zhou, L. He and W. Lin, *Biosens. Bioelectron.*, 2016, **79**, 237–243.
- 26 D. Srikun, A. E. Albers, C. I. Nam, A. T. Iavarone and C. J. Chang, *J. Am. Chem. Soc.*, 2010, **132**, 4455–4465.
- 27 R. S. Li, P. F. Gao, H. Z. Zhang, L. L. Zheng, C. M. Li, J. Wang, Y. F. Li, F. Liu, N. Li and C. Z. Huang, *Chem. Sci.*, 2017, **8**, 6829–6835.
- 28 R. G. Kurumbail, A. M. Stevens, J. K. Gierse, J. J. McDonald, R. A. Stegeman, J. Y. Pak, D. Gildehaus, J. M. Miyashiro, T. D. Penning, K. Seibert, P. C. Isakson and W. C. Stallings, *Nature*, 1996, **384**, 644–648.
- 29 H. Zhang, J. Fan, J. Wang, S. Zhang, B. Dou and X. Peng, *J. Am. Chem. Soc.*, 2013, **135**, 11663–11669.
- 30 M. C. Chang, A. Pralle, E. Y. Isacoff and C. J. Chang, *J. Am. Chem. Soc.*, 2004, **126**, 15392–15393.
- 31 A. R. Lippert, G. C. Van de Bittner and C. J. Chang, *Acc. Chem. Res.*, 2011, **44**, 793–804.
- 32 J. Xu, Q. Yang, X. Qian, J. Samuelsson and J. C. Janson, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2007, **847**, 82–87.
- 33 Z. R. Dai, G. B. Ge, L. Feng, J. Ning, L. H. Hu, Q. Jin, D. D. Wang, X. Lv, T. Y. Dou, J. N. Cui and L. Yang, *J. Am. Chem. Soc.*, 2015, **137**, 14488–14495.
- 34 S. Xu, H. W. Liu, X. X. Hu, S. Y. Huan, J. Zhang, Y. C. Liu, L. Yuan, F. L. Qu, X. B. Zhang and W. Tan, *Anal. Chem.*, 2017, **89**, 7641–7648.
- 35 J. Xiao, J. Deng, L. Lv, Q. Kang, P. Ma, F. Yan, X. Song, B. Gao, Y. Zhang and J. Xu, *Viruses*, 2015, **7**, 2816–2833.
- 36 H. Y. Li, X. H. Li, W. Shi, Y. H. Xu and H. M. Ma, *Angew. Chem., Int. Ed.*, 2018, **57**, 12830–12834.
- 37 C. E. Machamer, *Front. Neurosci.*, 2015, **9**, 421.
- 38 J. I. Sbodio, B. D. Paul, C. E. Machamer and S. H. Snyder, *Cell Rep.*, 2013, **4**, 890–897.
- 39 P. Huang, L. Feng, E. A. Oldham, M. J. Keating and W. Plunkett, *Nature*, 2000, **407**, 390–395.
- 40 A. N. Ledenev, A. A. Konstantinov, E. Popova and E. K. Ruuge, *Biochem. Int.*, 1986, **13**, 391–396.

