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**One potentiometric nanosensor for monitoring intracellular hydrogen sulfide ( $H_2S$ ) with fast potential response, high selectivity and excellent antifouling properties was developed. This study constructs a powerful tool to real-time track the changes of intracellular  $H_2S$  *in situ*, promoting the future studies of physiologically relevant processes.**

Hydrogen sulfide ( $H_2S$ ), an important neuromodulator, is one of the three major gas signaling molecules.<sup>1,2</sup> Neurodegenerative diseases including Alzheimer's disease, Parkinson's disease and Huntington's disease are associated with the level of  $H_2S$ .<sup>3–5</sup> Endogenous  $H_2S$  plays an indispensable role *in vivo* by expanding blood vessels, regulating metabolism and resisting inflammation.<sup>6–9</sup>  $H_2S$  is involved in cellular regulatory processes including regulating intracellular pH, promoting calcium influx of neurons and facilitating *N*-methyl-D-aspartic acid (NMDA) receptors.<sup>9</sup> To the best of our knowledge, most of the sulfur stored in cells with different kinds of forms like acid-labile sulfur and bound sulfur, which will release  $H_2S$  under external stimulation.<sup>10</sup> Although chromatography,<sup>11,12</sup> fluorescence,<sup>13–16</sup> colorimetric assays<sup>17–19</sup> and electrochemical methods<sup>19–21</sup> have been used to detect  $H_2S$  in the brain or single cells, it is still challenging to real-time track intracellular  $H_2S$  *in situ* with fast response and selectivity.

Electrochemistry with high spatiotemporal resolution has been widely applied in monitoring neurochemicals released in biological and pathological systems continuously and rapidly.<sup>22–25</sup> To date, electrochemical sensing of  $H_2S$  can be realized through both amperometry and potentiometry. For example, Hu *et al.* developed a 3D porous structure to detect  $H_2S$  released from single HeLa cells with amperometric methods.<sup>26</sup> Dong *et al.* developed a biosensor for the monitoring of endogenous  $H_2S$  and  $H_2S$  with differential pulse voltammetry in hippocampal slices and

living rats based on specific reaction.<sup>27</sup> However, the amperometric method faces the issue of contamination from sulfur in continuous measurement, resulting in low stability and sensitivity decrease. To address this problem, Pan *et al.* synthesized one single-atom catalyst for electrocatalytic  $H_2S$  at very low potential, which enables the construction of one stable  $H_2S$  sensor with galvanic redox potentiometry for real-time  $H_2S$  sensing in the living brain.<sup>28</sup> We developed one potential method to selectively track the dynamics of  $H_2S$  in the brain with  $Ag_2S$  coated Ag nanoparticles (AgNPs) on a microelectrode based on a potential method.<sup>29</sup> Compared to amperometric methods, potentiometry guided by the Nernst equation is independent of electrode area and has very small current flow, which will be beneficial for cell analysis. Therefore, we anticipate that our potentiometric sensor could be pushed to the nano-size and evaluate its feasibility in cell sensing.

Herein, we demonstrate one potentiometric method using a AgNP-modified carbon fiber nanoelectrode treated with  $Na_2S$ , forming  $Ag_2S/AgNPs/CFNE$  for the real-time monitoring of  $H_2S$  in a single cell with high selectivity and stability. We find that potentiometry is easily controlled for intracellular electrochemistry analysis comparing with amperometry. Moreover,  $Ag_2S/AgNPs/CFNE$  exhibits a highly selective and stable potential response to  $H_2S$ . Because  $Ag_2S/AgNPs/CFNE$  can resist acid and base interference,  $Ag_2S/AgNPs/CFNE$  is exploited as a powerful tool to achieve the continuous monitoring of  $H_2S$  in single cells with different pH environments. Beyond all that, we found that intracellular  $H_2S$  will be released upon the external stimulation of potassium. This real-time and *in situ*  $H_2S$  measurement method with high selectivity will present a new perspective in the study of physiology and pathophysiology associated with  $H_2S$ .

To demonstrate that the potential response is independent of electrode area, which is different from amperometric methods, cyclic voltammograms (CV) and the open-circuit potentials of the CFME and CFNE in HEPES buffer containing 1 mM  $Ru(NH_3)_6^{3+}$  were compared (Fig. 1B and C). As shown in Fig. 1B, the current response of the CFME is 50 times larger than that of the CFNE. In contrast, both the CFME and CFNE have close

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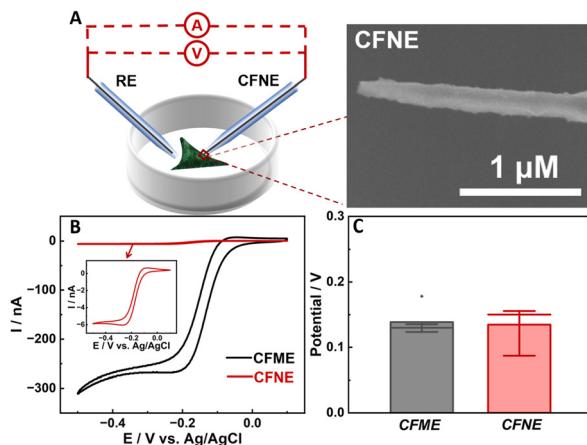
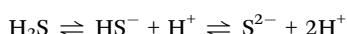


Fig. 1 (A) Scheme of the cell detection with the CFNE by using the amperometric method and open-circuit potential. Inset: SEM image of the bare CFNE. Cyclic voltammograms (B) and statistics potential response (C) obtained at the bare carbon fiber microelectrode (black) and carbon fiber nanoelectrode (red) in HEPES buffer containing 1 mM Ru(NH<sub>3</sub>)<sub>6</sub><sup>3+</sup>.

potential responses towards 1 mM Ru(NH<sub>3</sub>)<sub>6</sub><sup>3+</sup> (*ca.* 150 mV) (Fig. 1C). For intracellular electrochemical analysis using the amperometric method, non-membrane permeable Ru(NH<sub>3</sub>)<sub>6</sub><sup>3+</sup> is normally used to evaluate the insertion depth of the CFNE into the cell.<sup>30</sup> With the increase of the insertion depth of the CFNE into the cells incubated with HEPES buffer containing 1 mM Ru(NH<sub>3</sub>)<sub>6</sub><sup>3+</sup>, the reduction current decreased correspondingly. The OCP response did not change until the nanoelectrode inserted into the cell completely (Fig. S1, ESI<sup>†</sup>). These results indicate that open-circuit potential is easier to control for intracellular analysis because it is not necessary to ensure that the whole CFNE is inserted into the cell.

To develop one H<sub>2</sub>S nanosensor, Ag nanoparticles (NPs) were electrodeposited on the CFNE building up a layer with a reversible redox equilibrium at the interface of AgNPs/CFNE. And the AgNPs/CFNE was further pre-treated with 5 μM Na<sub>2</sub>S at open-circuit potential until the potential balanced for 300 s to form the Ag<sub>2</sub>S/AgNPs/CFNE. The SEM image (Fig. 2A) and the corresponding energy dispersive spectrometer (EDS) elemental mapping (Fig. 2B and C) of the local Ag<sub>2</sub>S/AgNPs/CFNE suggests the homogeneous decoration of Ag<sub>2</sub>S/AgNPs on the surface of the CFNE.

To output OCP, Ag/AgCl was used as a reference electrode and the Ag<sub>2</sub>S/AgNPs/CFNE as a working electrode. In this work, we mainly utilized Na<sub>2</sub>S as the source of dissolved H<sub>2</sub>S in the bulk electrolyte because there is a transformation between them in the solution:



Considering an insoluble salt electrode, the output potential OCP equals the potential difference between the indicated electrode and the reference electrode according to the Nernst equation, and the change of OCP response is only related to the concentration of S<sup>2-</sup>:

$$\text{OCP} = E_{\text{Ag}/\text{Ag}_2\text{S}}^0 - \frac{RT}{2F} \ln[\text{S}^{2-}] - E_{\text{R}}$$

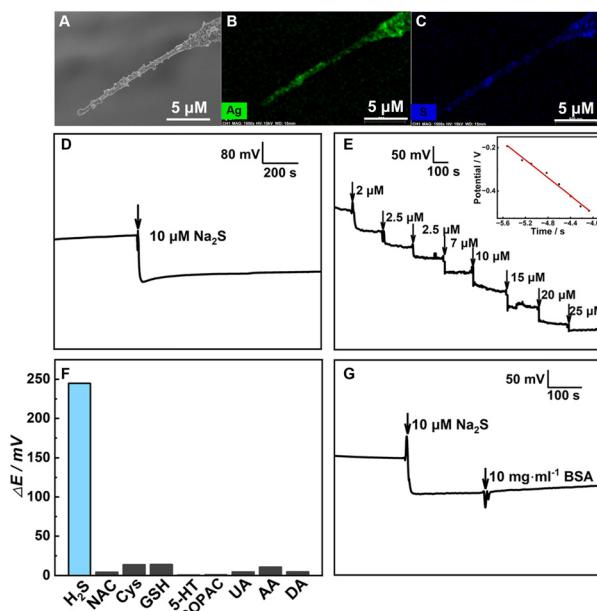


Fig. 2 (A) SEM image of the Ag<sub>2</sub>S/AgNPs/CFNE. The elemental mapping images of (B) Ag and (C) S of the Ag<sub>2</sub>S/AgNPs/CFNE. (D) The OCP response of the Ag<sub>2</sub>S/AgNPs/CFNE towards 10 μM Na<sub>2</sub>S for 1000 s in HEPES (pH = 7.4). (E) Typical OCP responses obtained at the Ag<sub>2</sub>S/AgNPs/CFNE towards successive addition of Na<sub>2</sub>S in HEPES (pH = 7.4) and the calibration plot of steady-state OCP output with the logarithmic concentration of H<sub>2</sub>S. (F) Selective test of OCP response recorded at the Ag<sub>2</sub>S/AgNPs/CFNE towards 20 μM DA, 200 μM AA, 50 μM UA, 20 μM DOPAC, 10 μM 5-HT, 200 μM GSH, 20 μM Cys, 20 μM NAC and 20 μM Na<sub>2</sub>S in HEPES (pH = 7.4). (G) OCP signal obtained at the Ag<sub>2</sub>S/Ag NPs/CFNE upon the addition of 10 μM Na<sub>2</sub>S and 10 mg mL<sup>-1</sup> BSA in HEPES (pH = 7.4) as labeled in the figure.

where  $E_{\text{Ag}/\text{Ag}_2\text{S}}^0$ ,  $R$ ,  $T$  and  $F$  are the formal potential of Ag/Ag<sub>2</sub>S, the universal gas constant, the temperature, and the Faraday constant, respectively. As we can see from Fig. 2D, the Ag<sub>2</sub>S/AgNPs/CFNE exhibited an excellent steep potential response for 10 μM Na<sub>2</sub>S in HEPES (pH = 7.4) and remained stable for 1000 s. The good stability is ascribed to there being no current flow in the OCP, resulting in no sulfur formation, which is ensured by no sulfur characteristic peak in the X-Ray photoelectron spectra of the electrode after detection (Fig. S2, ESI<sup>†</sup>). This nanoelectrode also exhibited a linear relationship (Fig. 2E) between the potential decrease and the logarithmic concentration gradient of H<sub>2</sub>S from 2 μM to 84 μM (OCP (mV) =  $-205.32 \lg[\text{S}^{2-}] (\mu\text{M}) - 1324.14$  (mV),  $R^2 = 0.9903$ ). The detection limit for the nanoelectrode was estimated to be *ca.* 0.6 μM (S/N = 3). We also investigated the interference from the electroactive species and sulfur-containing species coexisting in the cell. The potential response of 20 μM dopamine (DA), 200 μM ascorbic acid (AA), 50 μM uric acid (UA), 20 μM dihydroxy-phenyl acetic acid (DOPAC), 10 μM 5-hydroxytryptamine (5-HT), 200 μM glutathione (GSH), 20 μM cysteine (Cys), and 20 μM *N*-acetyl-L-cysteine (NAC) shows a negligible response compared with that of 20 μM Na<sub>2</sub>S at the Ag<sub>2</sub>S/AgNPs/CFNE in HEPES buffer (Fig. 2F). These results indicate that Ag<sub>2</sub>S/AgNPs/CFNE possesses good selectivity towards H<sub>2</sub>S against these interferences. Furthermore, with the addition of bull serum albumin

(BSA) ( $10 \text{ mg mL}^{-1}$ ), the output potential (Fig. 2G) did not evidently change and was retained well resulting from the effective layer of  $\text{Ag}_2\text{S}/\text{AgNPs}$  and the inherent property of potentiometry. In summary, the properties of fast response, good stability, and high selectivity make the  $\text{Ag}_2\text{S}/\text{AgNPs}/\text{CFNE}$  greatly promising for real-time detection of  $\text{H}_2\text{S}$ .

Therefore, we applied this nanoelectrode to detect intercellular  $\text{H}_2\text{S}$ . As shown in Fig. 3A, the OCP response of the  $\text{Ag}_2\text{S}/\text{AgNPs}/\text{CFNE}$  presents an obvious decrease for about 18 mV after penetrating into one SH-SY5Y cell immediately, which is calculated to be  $1.21 \mu\text{M H}_2\text{S}$  according to the calibration. In order to ensure the accuracy of this result, we investigated seven cells under the same experimental conditions and the same step, thus achieving the change of potential statistics for  $16.32 \pm 1.95 \text{ mV}$ , with the corresponding intracellular  $\text{H}_2\text{S}$  being  $1.20 \pm 0.03 \mu\text{M}$  (Fig. 3B). Fig. 3C shows an image of a cell after inserting the  $\text{Ag}_2\text{S}/\text{AgNPs}/\text{CFNE}$ . The morphology of the cell didn't change dramatically after penetration by the  $\text{Ag}_2\text{S}/\text{AgNPs}/\text{CFNE}$ . The penetrated cells were stained by fluorescent Calcein-AM (green, live cell, Fig. 3D) and propidium iodide (PI) (red, dead cell, Fig. 3E) indicating negligible damage to the cells by the  $\text{Ag}_2\text{S}/\text{AgNPs}/\text{CFNE}$ . These results demonstrate that the  $\text{Ag}_2\text{S}/\text{AgNPs}/\text{CFNE}$  can measure the intracellular  $\text{H}_2\text{S}$  without affecting the cell activity.

$\text{H}_2\text{S}$  in mammalian cells is produced by both enzymatic and nonenzymatic pathways, with the nonenzymatic pathway accounting for only a small fraction of synthesis. As studied previously, among the enzymatic pathways, two pyridoxal-5'-phosphate (PLP)-dependent enzymes including cystathione  $\beta$ -synthase (CBS) and cystathione  $\gamma$ -lyase (CSE), can catalyse Cys to produce  $\text{H}_2\text{S}$ . Cys is abundant in nerve cells and can be transformed from NAC.<sup>31</sup> In addition, a large amount of  $\text{H}_2\text{S}$

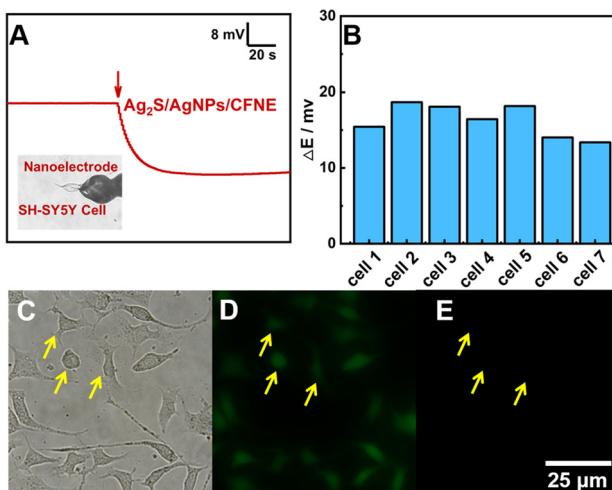


Fig. 3 (A) OCP response recorded by the  $\text{Ag}_2\text{S}/\text{AgNPs}/\text{CFNE}$  inserted into a SH-SY5Y cell. Inset is a bright-field image of a SH-SY5Y cell penetrated by the  $\text{Ag}_2\text{S}/\text{AgNPs}/\text{CFNE}$ . (B) The OCP responses for measurement of endogenous  $\text{H}_2\text{S}$  under the same conditions as in A. Bright-field image (C) and fluorescence image of SH-SY5Y cells stained with Calcein-AM (green, D) and PI (red, E) after penetration by the  $\text{Ag}_2\text{S}/\text{AgNPs}/\text{CFNE}$  for about 100 s indicated by yellow arrows.

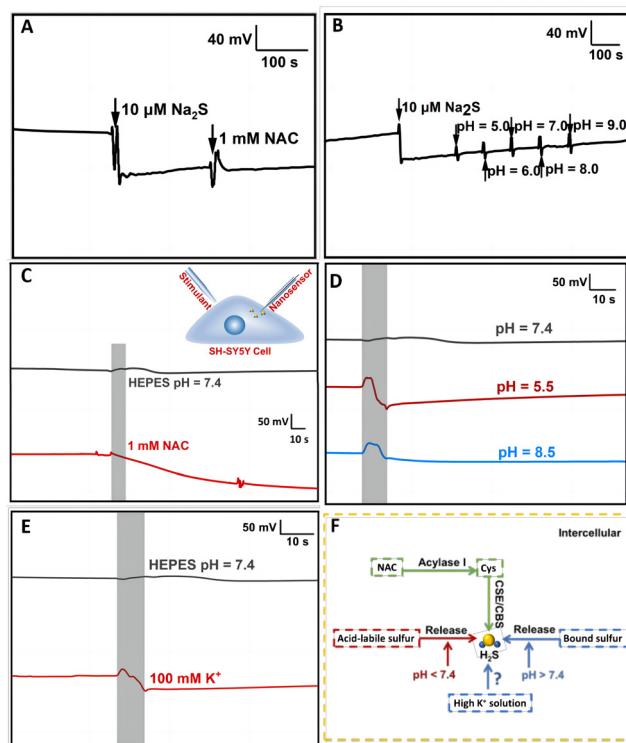


Fig. 4 (A) Typical OCP response recorded with the  $\text{Ag}_2\text{S}/\text{AgNPs}/\text{CFNE}$  toward  $20 \mu\text{M Na}_2\text{S}$  and  $1 \text{ mM NAC}$  in HEPES ( $\text{pH} = 7.4$ ). (B) Typical OCP response recorded with the  $\text{Ag}_2\text{S}/\text{AgNPs}/\text{CFNE}$  toward  $20 \mu\text{M Na}_2\text{S}$  in HEPES ( $\text{pH} = 7.4$ ) adjusting the pH value from 5.0 to 9.0 by adding HCl and NaOH as labeled in the figure. (C) Typical OCP response of the  $\text{Ag}_2\text{S}/\text{AgNPs}/\text{CFNE}$  in a single cell ( $n = 6$ ) incubated with HEPES ( $\text{pH} = 7.4$ ) stimulated with  $1 \text{ mM NAC}$  (red curve) and HEPES ( $\text{pH} = 7.4$ , grey curve). Inset is the illustration of the experimental setup for detecting  $\text{H}_2\text{S}$  using the nanosensor upon stimulation. (D) Typical OCP response of the  $\text{Ag}_2\text{S}/\text{AgNPs}/\text{CFNE}$  in a single cell ( $n = 6$ ) incubated with HEPES ( $\text{pH} = 7.4$ ) stimulated with HEPES with  $\text{pH} = 7.4$  (grey curve),  $\text{pH} = 5.5$  (red curve) and  $\text{pH} = 8.5$  (blue curve). (E) Typical OCP response of the  $\text{Ag}_2\text{S}/\text{AgNPs}/\text{CFNE}$  in a single cell ( $n = 6$ ) stimulated with HEPES ( $\text{pH} = 7.4$ , grey curve) and  $100 \text{ mM K}^+$  solution (red curve). (F) Illustration of the synthetic and release pathway in the cell.

exists in different kinds of compounds in the cell. The critical pH value is one of the most essential factors impacting the release of stored sulfur in cells. Acid-labile released  $\text{H}_2\text{S}$  in acid conditions below  $\text{pH} 5.4$  and bound sulfur released  $\text{H}_2\text{S}$  in alkaline conditions above  $\text{pH} 8.4$  have been reported in previous articles.<sup>10</sup> First of all, we investigated whether the physiological concentration of NAC and the fluctuation of the pH would have interference, thus affecting the nanosensor selectivity. The result is shown in Fig. 4A and B. Compared with the evident potential decrease in the response to  $\text{Na}_2\text{S}$ , the output potential did not change evidently toward  $1 \text{ mM NAC}$  and with the change of pH by adding HCl and NaOH from 5.0 to 9.0. These results indicate that the  $\text{Ag}_2\text{S}/\text{AgNPs}/\text{CFNE}$  is stable enough to be suitable for tracking  $\text{H}_2\text{S}$  under the stimulation of NAC, acid and alkaline solutions. To further explore the physiologic stimuli that might enhance the stored sulfur to transform into free  $\text{H}_2\text{S}$ , we measured the change of OCP by the

$\text{Ag}_2\text{S}/\text{AgNPs}/\text{CFNE}$  inserted in a cell and stimulated by 1 mM NAC (2  $\mu\text{L s}^{-1}$ , for 10 s) after the nanoelectrode achieved stability. As shown in Fig. 4C (red curve), after the addition of 1 mM NAC, the potential decreased slowly. In contrast, the addition of HEPES (pH = 7.4, grey curve) did not cause the decrease of the OCP. These results indicate that NAC has a positive influence on promoting the cell to release  $\text{H}_2\text{S}$ , which is consistent with reported literature,<sup>30</sup> suggesting that the  $\text{Ag}_2\text{S}/\text{AgNPs}/\text{CFNE}$  can monitor the release of  $\text{H}_2\text{S}$ . To illustrate the effects of different pH values on the release of  $\text{H}_2\text{S}$ , the solution (pH = 5.5, pH = 7.4 and pH = 8.5) was used to simulate the cell. The solution of pH 5.5 (Fig. 4D, red curve) and pH 8.5 (Fig. 4D, blue curve) leads to a quick OCP decrease. In contrast, injecting HEPES (pH = 7.4) as the control group, the output OCP potential response remained constant in the same time scale (Fig. 4D, grey curve). All these results demonstrate that the stored sulfur in cells will be released quickly in a short time when the pH value of the surroundings dramatically changes.

When neurons are excited,  $\text{Na}^+$  enters and  $\text{K}^+$  exits from cells, resulting in high  $\text{K}^+$  concentrations in the extracellular environment and the depolarization of surrounding cells.<sup>10</sup> Inspired by this phenomenon, we applied this nanoelectrode to investigate whether stimulation with neuronal excitation (*i.e.* high concentration of  $\text{K}^+$ ) will cause the release of  $\text{H}_2\text{S}$ . We found that an apparent potential decrease (Fig. 4E, red curve) was recorded after injecting 100 mM  $\text{K}^+$  compared to that with HEPES at pH = 7.4 (Fig. 4E, grey curve). This result demonstrates that high  $\text{K}^+$  concentrations can stimulate a fast release of  $\text{H}_2\text{S}$ . This release might result from the activation of the  $\text{Na}^+/\text{HCO}_3^-$  transporter by high  $\text{K}^+$  concentration. However, the exact mechanism still needs to be studied further. Taken together, the overall results indicate that the  $\text{Ag}_2\text{S}/\text{AgNPs}/\text{CFNE}$  has the strong capability to real-time monitor  $\text{H}_2\text{S}$  release under different kinds of pathways (Fig. 4F) in living single cells with a quick response.

In summary, we have fabricated an  $\text{Ag}_2\text{S}/\text{AgNPs}/\text{CFNE}$  with high selectivity and stability for detecting  $\text{H}_2\text{S}$  continuously. The combined characteristics of the  $\text{Ag}_2\text{S}/\text{AgNPs}/\text{CFNE}$  with tiny dimensions, fast response and high sensitivity enable this nanoelectrode to real-time and *in situ* monitor the changes of endogenous  $\text{H}_2\text{S}$  in a single-cell under different conditions. We believe that this functional nanoelectrode will open up a new window for the understanding of  $\text{H}_2\text{S}$  in physiological and pathological processes.

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## Conflicts of interest

There are no conflicts to declare.

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