Electronic Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information

Target-stimulated metallic HgS nanostructures on DNA-based polyion complex membrane for highly efficient impedimetric detection of dissolved hydrogen sulfide

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EXPERIMENTAL SECTION

Reagents and Chemicals. Oligonucleotides designed in this study (5′-SH-TTTTT TTTTT TTTTT TTTTT TTTTT-3') were synthesized by Sangon Biotech. Co., Ltd (Shanghai, China), which were purified by HPLC and confirmed by mass spectrometry. DNA stock solution was obtained by dissolving oligonucleotides in tris-HCl buffer solution (pH 7.4). Each oligonucleotide was heated to 90 °C for 5 min, and slowly cooled down to room temperature before usage. $HgCl_2$ and $Na_2S{\cdot}9H_2O$ were purchased from Alfa Aesar®. Tris(2-carboxyethyl) phosphine (TCEP) and 6-mecatohexanol was purchased from Tokyo chemical industry Co., Ltd (Japan). Mecatohexanol (MCH) was purchased from Tokyo Chem. Inc. (Japan). New bore calf serum was product of Dingguo Biotechnol. Co., Ltd. (Beijing, China). All the other chemicals were of analytical grade, and used without further purification. Ultrapure water obtained from a Millipore water purification system (≥ 18 MΩ, Milli-Q, Millipore) was used in all runs.

Fabrication of DNA-Modified Gold Electrode. A gold electrode with 2 mm in diameter was polished repeatedly with 1.0 and 0.3 μm alumina slurry, followed by successive sonication in distilled water and ethanol for 5 min and dried in air. Prior to modification, the gold electrode was cleaned with hot piranha solution (a 3:1 mixture of H_2SO_4 and H_2O_2. *Cautions*!) for 10 min, and then continuously scanned within the potential range of -0.3 to 1.5 V in freshly prepared deoxygenated 0.5 M H_2SO_4 until a voltammogram characteristic of the clean gold electrode was established.[S1] After the cleaned electrode was thoroughly rinsed with water and absolute ethanol, the cleaned electrode was immersed into 10 mM Tris-HCl buffer (pH 7.4, 0.5 M NaCl) solution containing 1.0 μM poly-$T_{(25)}$ DNA oligonucleotide (*Note*: Before incubation, the capture probe was pretreated by TCEP to split the formed disulfide between the thiolated DNA probes),[S2] and incubated for 2 h at room temperature (RT). During this process, the thiolated DNA probe was conjugated onto the gold electrode through the Au-S bond. After rinsing with distilled water, the modified gold electrode was incubated with 1.0 mM 6-mercaptohexanol in 10 mM Tris-HCl buffer, pH 7.4, for 60 min. Finally, the DNA-modified electrode was suspended over pH 7.4 PBS at 4 ºC when not in use.

Impedimetric Measurement. All impedimetric measurements were performed with an AutoLab μAUTIII.FRA2.v electrochemical workstation (Eco Chemie, The Netherlands). A conventional three-electrode system used in the measurements consists of a modified gold electrode as the working electrode, Pt wire as the counter electrode, and an Ag/AgCl reference electrode. Prior to incubation with target H_2S, the modified electrode was initially immersed into 10 mM Tris-HCl buffer (pH 7.4) solution containing 10 μM Hg^{2+}, and incubated for 15 min at RT. During this process, Hg^{2+} ions were intercalated between T bases through T-Hg^{2+}-T coordination chemistry.[S3] The resulting electrode was washed thoroughly with 10 mM Tris-HCl buffer (pH 7.4), and used for the detection of target H_2S.

Before impedimetric measurement, H_2S standards with various concentrations were prepared according to the literature.[S4] Initially, Na_2S standards with various concentrations were prepared by using 10 mM Tris-HCl buffer (pH 6.0) solution as the sample matrix. To detect H_2S gas, 100 μL of Na_2S standards solution was added into 0.5-mL centrifuge tube and the as-prepared electrode was inserted into the tube subsequently (*Note*: The electrode was not in physical contact with the H_2S containing solution). The gap between tube and the electrode was sealed by parafilm rapidly. The headspace between the solution and the surface of electrode was about 30 μL. H_2S was allowed to form from Na_2S, and react with the Hg^{2+} ions in the T-Hg^{II}-T base pairs for 30 min. Afterward, the resultant electrode was monitored by using the EIS in 5.0 mM $Fe(CN)_6^{4-/3-}$ containing 0.1 M KCl at frequency 10^{-2} to 10^5 Hz at the formal potential of 220 mV, using alternating voltage of 10 mV. A Nyquist plot (Z_{re} *vs*. Z_{im}) was drown to analyze the impedance results. Analyses are always made in triplicate.

Optimization of Experimental Conditions. To achieve an optimal impedimetric signal, some experimental conditions concluding the conjugation time for T-Hg^{2+}-T and the precipitation time for HgS should be investigated. Fig. S1 shows the effect of various conjugation times for T-Hg^{2+}-T on the signal of the impedimetric assay method (200 nM H_2S used in this case). The resistance increased with the increment of conjugation time, and tended to level off after 15 min. Longer conjugation time did not cause the obvious increase in the resistance. Therefore, 15 min was used as the conjugation time for the formation of T-Hg^{2+}-T in this work.

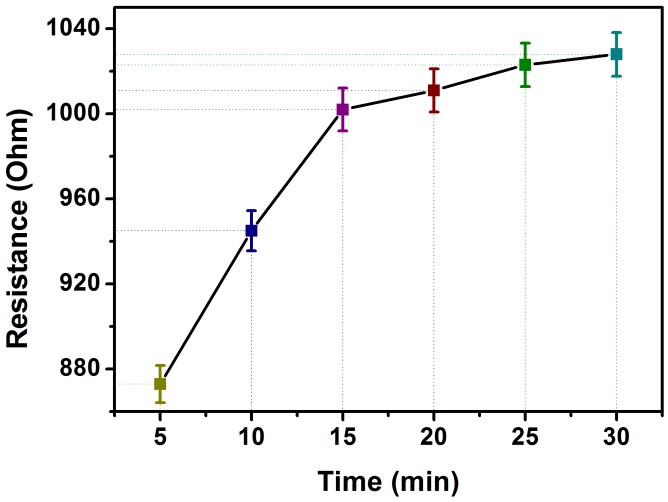

Fig. S1 Influence of the conjugation time between Hg^{2+} and T bases on the signal of the impedimetric assay method.

In this work, the strong electrochemical signal mainly derived from the formed HgS nanostructure on the electrode toward the disturbance of $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$. So, the amount of HgS precipitation on the electrode would directly affect the analytical properties of the developed method. As seen from Fig. S2, the resistance (R_{et}) of the modified electrode increased with the increment of incubation time, and the optimal response was achieved at 30 min. Thus, 30 min was selected as the incubation time for the precipitation of HgS at acceptable throughout.

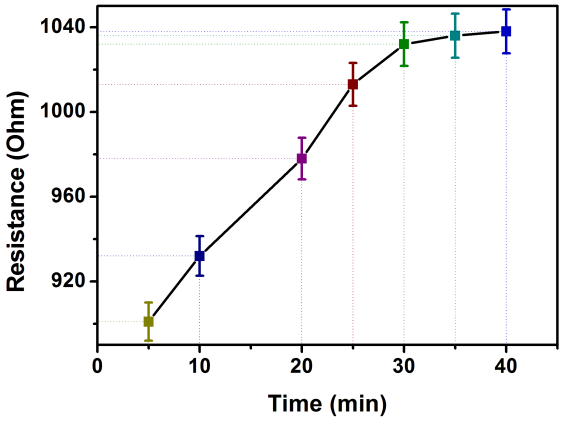

Fig. S2 Influence of the incubation time between target H_2S and T-Hg^{2+}-T on the signal of the impedimetric assay method (200 nM H_2S used as an example).

Table S1. Precision and Reproducibility (Coefficient of Variation, CV) of Determinations Using Identical Batches of DNA-Modified Electrodes Throughout

item	$C_{[H2S]}$, nM	identical batches; [a] assay concentration (nM)					CV (%, n = 5)
		1	2	3	4	5	
intra-assay	5	5.2	4.6	4.3	5.1	5.3	8.8
	200	189	213	203	221	208	5.8
	600	645	603	626	587	593	4.0
inter-assay	5	4.3	5.3	4.7	5.5	4.6	10.3
	200	243	235	213	189	219	9.6
	600	564	591	667	632	587	6.7

[a] The CVs of intra-assay were obtained by using the same-batch DNA-modified electrodes, whereas the CVs of the inter-assay were achieved by using various batches.

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