

# Nanomolar scale nitric oxide generation from self-assembled monolayer modified gold electrodes

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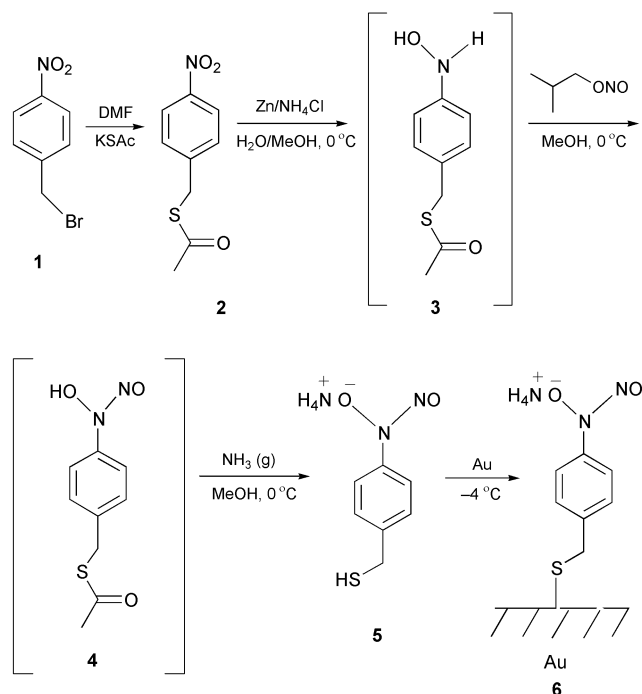
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A SAM-modified gold electrode has been developed for the first time for quantitative NO generation of a nanomolar amount that is proportional to the surface area of the electrode.

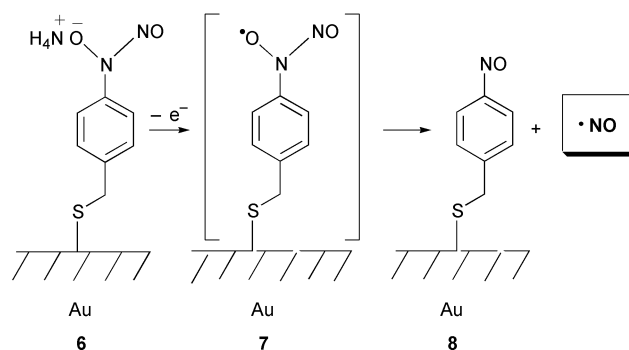
Nitric oxide, a relatively unstable, potentially toxic gas, has been implicated in a wide variety of physiological and pathological processes.<sup>1–4</sup> Considering the numerous functions of NO in the human body, controlled delivery of a quantitative amount of NO to specific sites is most critical for biomedical applications of NO donor compounds.<sup>5,6</sup> On the other hand, extensive research on a variety of self-assembled monolayers (SAMs) on different metal electrodes has resulted in many biomedical applications during the past decade.<sup>7–11</sup> In our efforts to develop quantitative and site-specific NO donating compounds, we explored the possibility of using the self-assembled monolayer of an NO donor to achieve controlled NO release. Here we report the development of a novel SAM moiety **6** by attaching a thiol tail to the NO donor molecule and allowing it to self-assemble onto the surface of a thin gold electrode (Scheme 1). Upon applying an electric potential, the electrode achieves quantitative NO release on a nanomolar scale.

*N*-Nitroso-*N*-oxido-*p*-thiomethylbenzenamine ammonium salt **5** was synthesized according to Scheme 1. 4-Nitrobenzyl bromide (**1**) was reacted with potassium thioacetate to give compound **2**. Subsequent zinc reduction of the nitro group, nitrosation with isobutyl nitrite, and finally neutralization and spontaneous deacetylation afforded **5**.<sup>†</sup> Although compound **5**



Scheme 1 Synthesis of NO donor **5** and preparation of SAM **6**.

slowly decomposes at rt, successful preparation of the SAM gold electrode of compound **5** was achieved at  $-4\text{ }^{\circ}\text{C}$ , leading to the formation of **6**. According to Lawless and others who have illustrated the oxidative electrochemical release of NO from cupferron<sup>‡</sup> and its derivatives,<sup>12,13</sup> a similar mechanism is proposed to account for NO generation from the SAM donor **6** (Scheme 2). Upon applying an electric potential, **6** undergoes a one-electron electrochemical oxidation to form presumably the highly unstable free radical intermediate **7**, which spontaneously decomposes to release NO and form the nitroso SAM **8** as well.



Scheme 2 Proposed NO releasing mechanism from the SAM modified gold electrodes.

An Osteryoung square wave voltammetry (OSWV) technique was employed to characterize the donor SAM **6**. Typical OSWV responses using a BAS-100B electroanalyzer were shown in Fig. 1. From 0 to 800 mV, the donor SAM modified gold electrode exhibited a single peak at a potential of 270 mV

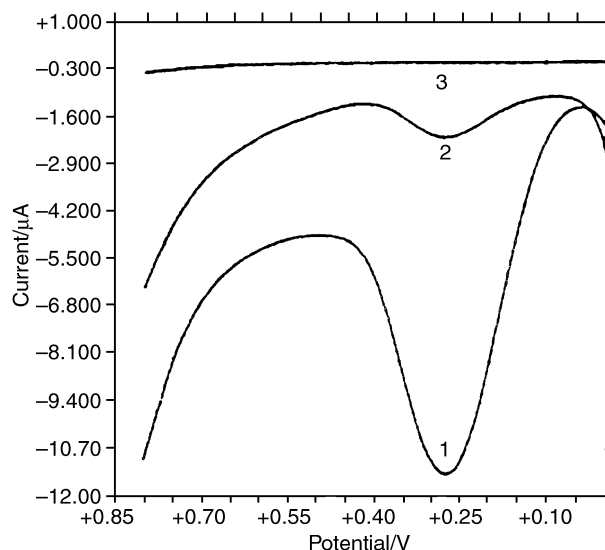
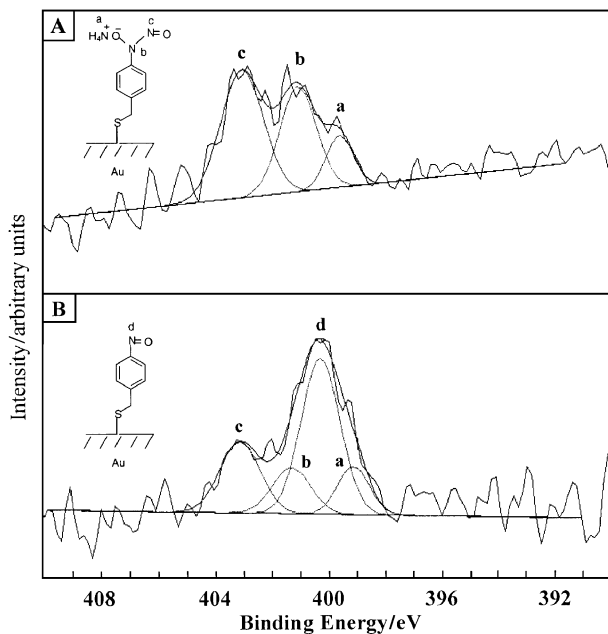


Fig. 1 Typical OSWV responses of SAM functionalized gold electrodes. Curve 1: first scan; curve 2: second scan; and curve 3: a 'real' background obtained after multiple scans.



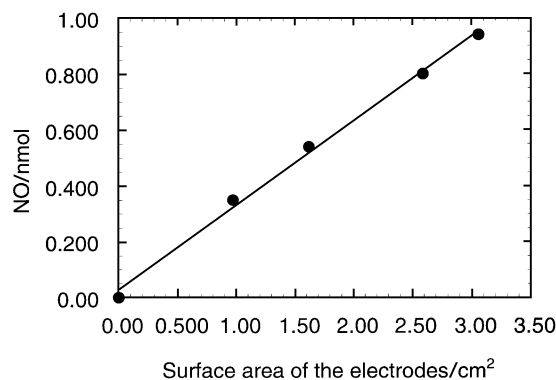
**Fig. 2** XPS spectra of the SAM covered gold electrode prior to (A) and after (B) the electrolysis.

corresponding to an irreversible oxidation process (curve 1). A second continuous scan resulted in a lowered peak current (curve 2), indicating a reduced quantity of the donor compound **5** on the surface of the electrode. Upon multiple scans, the peak eventually disappeared and a 'real' background curve was obtained (curve 3), which represented the formation of **8**.

X-ray photoelectron spectroscopy (XPS) has proven to be a useful technique in characterizing SAM-functionalized metal surfaces by determining the oxidation state of nitrogen within SAM electrodes.<sup>14</sup> XPS spectra in Fig. 2A are the results of three peaks that correspond to nitrogen atoms<sup>15,16</sup> in three different chemical environments in donor SAM **6** (binding energy of 399.66, 401.21 and 403.14 eV for  $N_{1s}^{1/2}$ , respectively). After electrolysis, the overall spectra changed with the appearance of a new peak with lower binding energy. The new spectrum (Fig. 2B) is best fitted for  $N_{1s}^{1/2}$  peaks at binding energy of 399.33, 400.44, 401.38 and 403.22 eV, respectively. The new peak at 400.44 eV corresponds to the  $N_{1s}^{1/2}$  intensity of the nitroso group in SAM **8**, which is consistent with the oxidation of the bulky terminal group in SAM **6**.

To directly monitor the generation of NO from the SAM functionalized gold electrode, a commercially available NO detector probe was inserted into the electrolysis cell through a bypass to the vicinity of a gold electrode at a fixed position. Due to the actual detection limit of the NO detector probe ( $\sim 2$  nM), the gold bead working electrode was replaced with a larger gold plate electrode (Au (111)) in order to generate a significant amount of NO for detection. NO generation was monitored simultaneously during a quantitative electrolysis process while the solution was vigorously stirred. The amount of NO released was determined by subtracting the highest point on the current plateau from the background current and converting the result into the corresponding quantities of NO using a standard calibration curve. It was shown that the amount of NO generated increased with the electrode potential during a bulk electrolysis process; however, no linear relationship appeared between the amount of NO released and the electrolysis potential.<sup>13</sup> Next, we carried out the electrolysis with various sizes of gold electrodes all having the same square geometry. Interestingly, a linear relationship between the amount of NO generated and the area of the electrode was established (Fig. 3),<sup>§</sup> which indicates that controlled NO release could be achieved by selecting an appropriately sized surface area of the gold electrode.

In summary, this work demonstrates the principle and initial design of a gold electrode functionalized by a self-assembled



**Fig. 3** A linear relationship between the amount of NO released and the surface area of the gold electrodes.

monolayer of NO donor compound. Upon electrolysis, such an electrode quantitatively generates NO at a nanomolar scale and the amount of NO produced can be controlled by changing the surface area of the gold electrode. It is thus envisioned that this type of electrode can be developed into practical NO generating micro electrode arrays for a variety of biochemical applications where a small but quantitative amount of NO can be targeted at specific sites.

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## Notes and references

† Selected data for **5**:  $\delta_H$ (500 MHz,  $CD_3OD$ ) 3.62 (s, 2H), 7.29 (d,  $J = 8.5$  Hz, 2H), 7.53 (d,  $J = 9.0$  Hz, 2H);  $\delta_C$ (125 MHz,  $CD_3OD$ ) 27.2, 119.2, 128.2, 136.8, 144.5; UV/Vis (MeOH):  $\lambda_{max}$  ( $\epsilon$ ) = 299 nm (11,000); Anal. calcd. for  $C_7H_{11}N_3O_2S_1$ : C, 41.78; H, 5.51; N, 20.88; S, 15.93. Found: C, 41.82; H, 5.48; N, 20.80; S, 15.87%.

‡ The IUPAC name for cupferron is *N*-nitroso-*N*-phenylhydroxylamine ammonium salt.

§ Out of several sets of NO generation data, the single best one was chosen for the plot.

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