Electronic supplementary information (ESI) for

# A magnetic relaxation switching immunosensor for one-step detection of salbutamol based on gold nanoparticle-streptavidin conjugate

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## Experimental Section

### 1. Materials

The 30 nm SMNs (surface modified COOH, 5 mg/mL) and magnetic separator were purchased from Ocean Nano Tech Co., Ltd. (USA). SAL, SAL-bovine serum albumin (SAL-BSA) conjugate and the antibody against SAL were purchased from Abmart (Shanghai). SAL, ractopamine, clenbuterol, streptomycin, bovine serum albumin (BSA), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC)，sulfo-NHS-LC-Biotin and N-hydroxysulfosuccinimide sodium (Sulfo-NHS) were purchased from Sigma-Aldrich (USA). Streptavidin (1 mg $mL^{-1}$) was purchased from Biosynthesis Biotechnology Co., Ltd. (Beijing, China). The 96-well cell culture cluster was supplied by Corning Incorporated (USA). The water was also deionized and ultrafiltered using a Milli-Q apparatus. All other chemicals were of analytical reagent grade, and all the aqueous solutions were prepared using distilled water.

### 2. Apparatus

A 1.5T cute NMR analysis for measurement of transverse relaxation time ($T_2$) was provided by Shanghai Shinning Globe Science and Education Equipment Co., Ltd (Shanghai, China).

### 3. Preparation of SAL-BSA-SMNs conjugate and biotinylated Ab1 conjugates

First, 200 μL of SMNs was suspended for 2 min. Then, 20 μL of EDC (50 mg $mL^{-1}$) and NHS (50 mg $mL^{-1}$) were added to the SMNs solution. After activation for about 20 min, 1 mL PBS buffer (pH=8.0, 0.01M) was added to the activated SMNs. Subsequently, 0.2 mg SAL-BSA conjugate was added to the activated SMNs solution,

and the mixture was gently stirred to react for 2 h at room temperature, and then blocked with 1% (m/v) BSA for 0.5 h. The SAL-BSA-SMNs conjugate was separated from the free SAL-BSA conjugate, re-suspended in 1000 μL of PBS and stored at 4 °C for further use, the final concentration of SMNs is 0.85mg/mL. The biotinylated-$Ab_1$ conjugate (B-Ab1) was produced according to the methods described in a previous study, and the final concentration of B-Ab1 is 1.25 mg/mL.

**4. Preparation of SA-Au conjugate**

Citrate-stabilized Au NPs (20~30 nm diameter) were prepared following standard literature procedures. The AuNPs were modified with SA following a previously optimized procedure**.** The pH of 10 mL 0.01% AuNPs was adjusted into 7.5 by adding 0.01M $K_2CO_3$ solution, and 60 μg SA was added into the mixture solution. The resulting solution was incubated for 1 h at 650 rpm. Then 0.5 mL 10 mg/mL BSA aqueous solution was added and stirred for another 0.5 h. The mixture solution was centrifuged at 14000 rpm and 4 $^oC$ for 20 min. The supernatant was removed and the Au-SA conjugate was resuspended with 0.5 mL PBS solution (0.1% BSA), and this solution was centrifuged at 14000 rpm and 4 $^oC$ for 20 min again. Finally, the supernatant was removed and the Au-SA conjugate was resuspended with 0.2 mL developing solvent, and the Au-SA solution was stored at 4 $^oC$.

**5. Procedure of Au-MRS immunosensor**

First, 50 μL of BSA-SAL-SMNs solution, 50 μL of target molecules (SAL), 50 μL of B-Ab1, and 50 μL of Au-SA conjugate were placed in the same well in the 96-well cell culture cluster. The mixture was gently shaken for 30 min. Subsequently,

180 μL of the reacted solution was transferred into a 7.5-mm nuclear magnetic resonance tube contents were analyzed via NMR for 1 min to measure $T_2$. The SMNs solution added with the biotinylated anti-ractopamine antibody, which did not recognize SAL, was used as control group. Each point was assayed thrice ($n$=3). For each interval, the change in $T_2$ ($\Delta T_2$) was calculated using the following equation:

$$\Delta T_2 = T_{2\text{sample}} - T_{2\text{blank}}$$

where $T_{2\text{sample}}$ and $T_{2\text{blank}}$ are the average $T_2$ relaxation times of the triplicates of the sample and blank groups, respectively.

$T_2$ was acquired at a temperature of 35 °C using the cute1.5T cute NMR analysis working with $^1$H at the 62.095MHz magnetic field and the following parameters: Carr-Purcell-Meiboom-Gill pulse sequence, 2500 echoes, echo time of 2 ms, and repetition time of 2 s.

**6. Spiked and real sample analysis**

To prepare a series of SAL concentrations in swine urine, we prepared a SAL standard solution by dissolving SAL in blank swine urine. The solution was further diluted with blank urine sample to 0, 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 20, 50 and 100 ng $mL^{-1}$. The real swine urine was diluted tenfold in PBS for analysis. The blank and positive swine urine samples were provided by chinese Academy of Inspection and Quarantine. Each sample was assayed thrice ($n$=3).

## Supplementary Figures

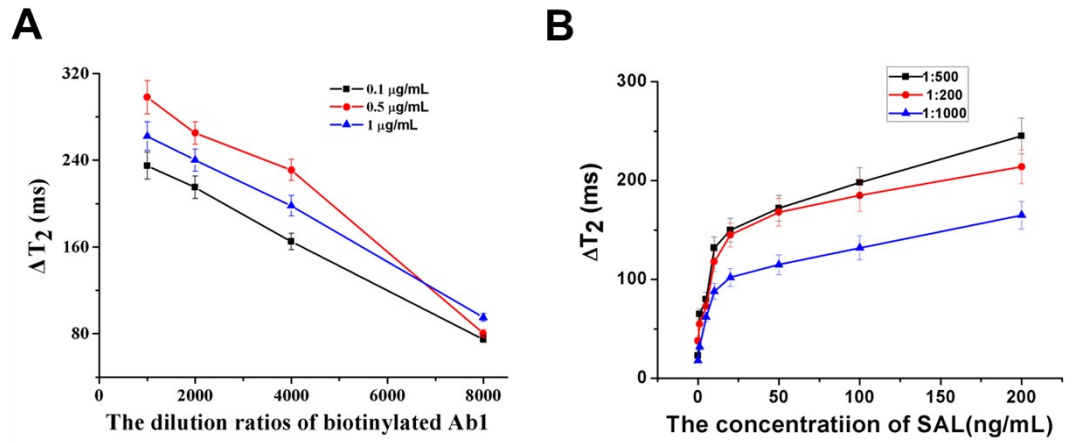


**Figure S1.** The optimization of Au-MRS immunosensor. (a) The dilution ratios of B-Ab1 were 1:1000, 1:2000, 1:4000 and 1:8000. The concentration of SAL-BSA-SMNs is 0.1, 0.5 and 1μg/mL, and the diluted ratio of Au-SA conjugate is 1:200. (b) The dilution ratios of Au-SA conjugate is 1:200, 1:500 and 1:1000, respectively.

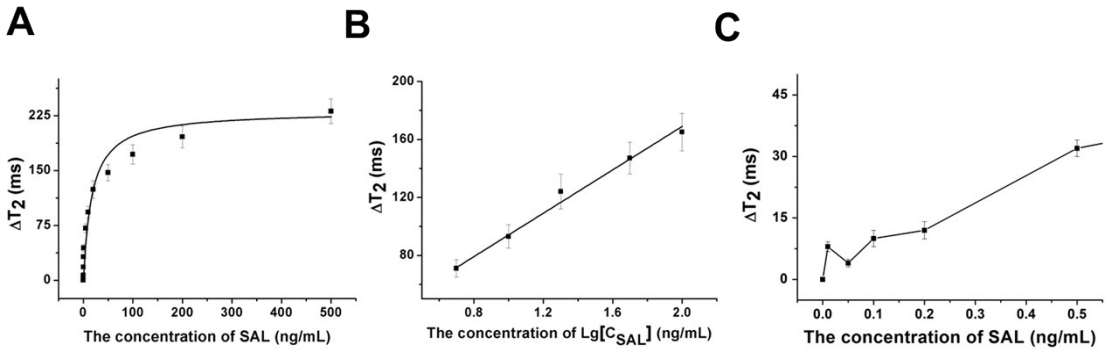


**Figure S2.** The sensitivity and linearity range of conventional MRS for detection of SAL. (a) The relationship between $\Delta T_2$ and the concentration of Salbutamol. The concentrations of SAL is 500, 200, 50, 20, 10, 5, 1, 0.5, 0.2, 0.1, 0.05, 0.01 and 0 ng $mL^{-1}$, respectively. (b) A linear relationship between $\Delta T_2$ and the concentration of salbutamol (c) The limit of detection of the Au-MRS.