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COMMUNICATION

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

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We develop a magnetic relaxation switching immunosensor for the detection of salbutamol by using gold nanoparticles-streptavidin conjugate and magnetic nanoparticles. This immunosensor enables faster detection with enhanced sensitivity compared with enzyme-linked immunosorbent assay, providing a promising platform for rapid sensing of small molecules.

Salbutamol is a short-acting β -adrenergic agonist for the treatment of obstructive lung disease. Also, it had been approved and used widely as animal feed to improve an animal's muscle/fat ratio since 1990s to 2000s.¹ However, as a feed supplement, it causes a multitude of side effects to human health, thus it has been rigorously forbidden to be used as feed additives in China, European Union and many other countries from 2000s. Nevertheless, illegal abuse of salbutamol has never stopped driven by economic interests, which has become an increasing public health concern.^{2, 3} Presently, methods for detection of salbutamol mainly include chromatographic methods^{4, 5} and immunoassay methods.^{6, 7} Chromatographic methods such as high performance liquid chromatography (HPLC), gas/ liquid chromatography-mass spectrometry (GC-MS or LC-MS) are instrumentation-based analyses that allow high sensitivity and accuracy. However, they need complex sample pre-treatment and high cost, which limit their application in point-of-care testing. Immunoassay such as enzyme-linked immunosorbent assay (ELISA)⁸ has relatively high sensitivity, but it is laborious and time-consuming. Therefore, it is urgently needed to develop a rapid and sensitive method for detection of salbutamol.

Recently, magnetic relaxation switching (MRS) sensors that depend on target-induced aggregation (or disaggregation) of superparamagnetic nanoparticles (SMNs) are employed to quantify a wide range of targets.⁹⁻¹¹ In a typical MRS sensor,

target can induce the state change of SMNs (from dispersed to aggregated) through the specific donor-receptor recognition, and this state change of SMNs will result in a heterogeneous magnetic field, which can affect the transverse relaxation time (T_2) of surrounding water molecules. The ΔT_2 value (the change of T_2) is related to the degree of aggregation of SMNs, and this degree of aggregation is depended on the amount of target in sample, thus ΔT_2 can be employed as the signal readout.¹² Compared to colorimetric and fluorescent assays, the MRS-based method is homogeneous and light-independent, which allows one-step detection in 30 min without compromising the sensitivity.¹³ MRS-based methods have been successfully used to detect biomacromolecules such as cells,¹⁴ pathogenic bacteria,¹⁵ viruses and protein biomarkers.¹⁶ Unlike biomacromolecules, small molecules have limited binding sites (only one or two), thus small molecules-induced aggregation of SMNs is not obvious in MRS that results in a low sensitivity. To resolve this issue, many strategies have been developed to increase the sensitivity of MRS to detect small molecules.¹⁷ Previous work has reported the combination of MRS with second antibody (a signal amplification system) for the detection of microcystin.¹⁸ Our group has introduced biotin-streptavidin system into MRS assay for the determination of kanamycin in milk with a limit of detection of 0.1 ng/mL.¹⁹ However, a more sensitive method which can reach pg/mL is necessary when it comes to the trace detection of salbutamol in complex samples.

Our previous work demonstrates that multivalency is an effective strategy for the signal amplification to enhance the sensitivity. Polystyrene (PS) microspheres of high surface-to-volume ratio that conjugate lots of recognition element molecules can greatly improve the degree of aggregation of SMNs and thus the sensitivity.²⁰ However, covalent coupling strategy should be taken to conjugate the antibody to PS that will affect the activity of antibody. Moreover, PS microsphere tends to aggregate spontaneously that will affect the accuracy of MRS assay. To solve these limitations, we think that gold nanoparticles (Au NPs) may be better candidates to enhance the signals through its multivalency. Compared with PS

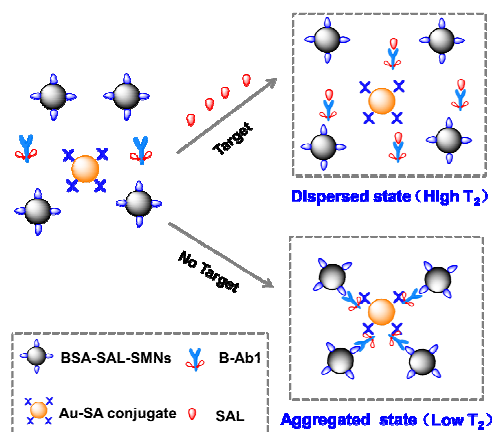
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microspheres, Au NPs have a higher surface-to-volume ratio and possess excellent suspension stability which is very important to enhance the sensitivity and robustness of the MRS sensor. Antibody or protein can be easily conjugated to the surface of Au NPs by physical absorption. We hypothesize that if the Au NPs bind lots of streptavidin, the gold-streptavidin (Au-SA) conjugate can be used as an idea candidate to amplify the signals to enhance the sensitivity of MRS sensor for detection of small molecules.

Herein, we present a rapid and ultra-sensitive immunosensor (Au-MRS) based on MRS and Au-SA for detection of salbutamol in swine urine samples. In this Au-MRS assay, the salbutamol-bovine serum albumin conjugates (SAL-BSA) immobilized on the surface of SMNs (SAL-BSA-SMNs) are used as the “switches” between their dispersed and aggregated states, resulting in a change in the T_2 of their surrounding water protons. The Au-SA conjugate can enhance the aggregation of SMNs and improve the sensitivity of the MRS sensor (Scheme 1). The target analyte (salbutamol) would compete with the SAL-BSA-SMNs to capture biotinylated Ab1 (B-Ab1), hence preventing the formation of SMNs aggregates. As a result, this competition leads to the change of T_2 (ΔT_2) which relates to the concentration of the salbutamol, allowing for quantitative analysis. Meanwhile, a multivalency-based enhancement strategy is employed in this immunosensor by using the Au-SA conjugate. The Au-SA conjugate has lots of binding sites with an extraordinary affinity for capturing biotin (dissociation constant: about 10^{-15} M), resulting in the formation of “Au-SA-B-Ab1-SMNs” cluster in the solution. Therefore, this multivalency-based strategy facilitates the aggregation of SMNs and enhance the sensitivity of this MRS-based immunosensor. Best to our knowledge, it is the first study that combines an MRS sensor with Au-SA conjugate to amplify the signals for the trace analysis of small molecules.



Scheme 1 The scheme of the immunosensor based on MRS and Au-SA conjugate. SAL-BSA-SMNs are used as “switches” between their dispersed and aggregated states, resulting in the change in the T_2 of their surrounding water protons. The Au-SA conjugate is used as a multivalency signal amplification system to enhance the aggregation of SMNs to improve the sensitivity.

We investigated two main factors that influence the sensitivity of this immunosensor: (1) the concentration of the

SAL-BSA-SMNs conjugate and B-Ab1; (2) the concentration of Au-SA conjugate. We first optimized the concentration of B-Ab1 and SAL-BSA-SMNs conjugate. As the dilution ratios of B-Ab1 decreased from 1:8000 to 1:1000, the ΔT_2 increased (Figure S1a), which suggests the formation of “Au-SA-B-Ab1-SAL-SMNs” in this assay and demonstrates that Au-MRS can be used to detect salbutamol. We chose 1:4000 as the dilution ratio of B-Ab1, considering that a high concentration of B-Ab1 is unsuitable for the sensitivity of a competitive immunoassay to detect small molecules. We chose 0.5 $\mu\text{g/mL}$ as the concentration of SAL-BSA-SMNs because it shows the most significant change of ΔT_2 in this case (Figure S1a). We further investigated the concentration of Au-SA conjugate. ΔT_2 increased as the concentration of salbutamol increased from 0 ng/mL to 200 ng/mL (Figure S1b), and ΔT_2 shows the most significant change when the dilution ratio of Au-SA is 1:500. Thus, we chose 1:500 as the dilution ratio of Au-SA conjugate in this assay.

Under the optimized condition, we evaluated the sensitivity and selectivity of this assay. ΔT_2 gradually increases when the concentration of salbutamol increases between 0.01 ng/mL and 500 ng/mL (Figure 1a). A linear relationship between ΔT_2 and the concentration of salbutamol was observed in the range between 5 ng/mL and 200 ng/mL, and the linear equation was $Y=94.99X+15.8$ ($X=\lg C_{\text{salbutamol}}$, $R^2=0.98$) (Figure 1b). The limit of detection (LOD) for the detection of salbutamol is 10 pg/mL (Figure 1c). To demonstrate the signal amplification effect of Au-SA, we compared this Au-MRS immunosensor with conventional MRS assay that merely used SA without Au NPs. For the conventional MRS sensor, ΔT_2 increased when the concentration of salbutamol was from 0.1 ng/mL to 500 ng/mL (Figure S2a), and the linear relationship between ΔT_2 and the concentration of salbutamol was observed in the range between 5 ng/mL and 100 ng/mL (Figure S2b). The LOD of conventional MRS sensor for the detection of salbutamol is 0.1 ng/mL (Figure S2c). Compared with conventional MRS sensor, the LOD of this Au-MRS immunosensor decreases by one order of magnitude, and shows a broader linear detection range. The better performances of Au-MRS sensor than conventional MRS sensor may be as follows: in this Au-MRS immunosensor, Au NPs have large specific surfaces which can conjugate lots of SA and act as more effective carriers to conjugate more SMNs on its surface, which can largely enhance the aggregation of SMNs and thus the sensitivity of the assay.

We then evaluated the selectivity of this Au-MRS immunosensor by using ractopamine, clenbuterol and streptomycin as the controls (Figure 1d). ΔT_2 shows the most significant change in salbutamol than other small molecules, suggesting that the sensor has good specificity for salbutamol detection. The specific antigen-antibody recognition plays a crucial role in this assay. We replaced biotinylated Ab1 with biotinylated antibody against clenbuterol to confirm that the magnetic signal resulted from the specific antigen-antibody recognition rather than the nonspecific adsorption. As expected, the biotinylated antibody against clenbuterol did not

specifically bind with SAL-BSA-SMNs, resulting in a minimal change in control group.

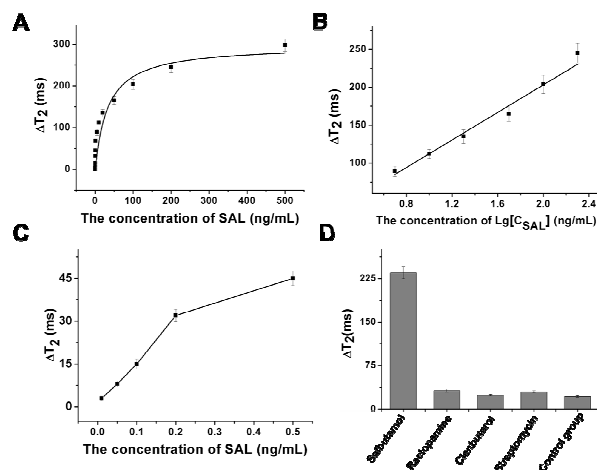


Figure 1. The sensitivity and selectivity of the Au-MRS immunosensor. (a) The relationship between ΔT_2 and the concentration of salbutamol. The concentrations of SAL is 500, 200, 50, 20, 10, 5, 1, 0.5, 0.2, 0.1, 0.05, 0.01 and 0 ng mL⁻¹, respectively. (b) A linear relationship between ΔT_2 and the concentration of salbutamol (c) The limit of detection of the Au-MRS. (d) Selectivity. The concentration of the analogues is 200 ng/mL. Control group represents that the biotinylated Ab1 is replaced with biotinylated antibody against clenbuterol.

To investigate the applicability of this Au-MRS sensor, we applied it for the detection of salbutamol in real samples. We chose swine urine samples to demonstrate its real-world applications. For comparison, we simultaneously employed the Au-MRS sensor and conventional MRS sensor for the detection of salbutamol (Figure 2). Three blank samples (sample 1 to sample 3) are detected to be free of salbutamol using these two methods. For spiked samples, this Au-MRS sensor can detect 0.1 ng/mL salbutamol (sample 4), while conventional MRS sensor can only detect 0.5 ng/mL salbutamol (sample 5). It indicates that this Au-MRS sensor has better sensitivity than the conventional MRS sensor. Finally, we detected ten real samples (sample 11 to sample 20) that were identified as positive samples by HPLC-MS. Nine samples are identified to be positive using our Au-MRS approach, while eight samples are identified to be positive by conventional MRS sensor. This result showed that the Au-MRS approach has a higher detection rate than that of convention MRS method because of its higher sensitivity. Although HPLC-MS is the gold standard for small molecule detection in virtue of its ultrahigh sensitivity and good accuracy it needs high cost and complex sample pre-treatment, which limit its wide applications in resource-poor settings. By contrast, the Au-MRS sensor allows detection in a short time (0.5 h) with convenient operation and high sensitivity, which provides an attractive platform for the detection of small molecules.

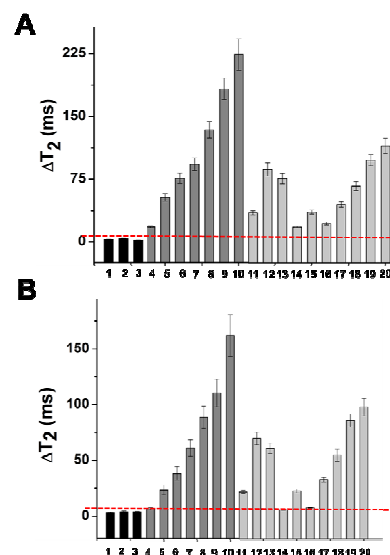


Figure 2. The results of Au-MRS sensor and conventional MRS sensor for detection of blank samples, spiked samples and real samples. (a) shows that the result of Au-MRS for detection of SAL. (b) shows that the result of conventional MRS sensor for detection of SAL. Sample 1 to samples 3 present blank samples that is free of SAL. Sample 4 to sample 10 present different concentration of SAL in spiked sample, the final concentrations of SAL is 0.1, 0.5, 1, 5, 10, 50 and 100 ng/mL. Sample 11 to samples 20 were proved to be positive samples by HPLC-MS.

We also compared this Au-MRS immunosensor with ELISA for the detection of salbutamol, which has been widely used in detecting small molecules because of its selectivity, sensitivity and low cost. In ELISA method, the OD₄₅₀ value increases with the concentration of salbutamol from 0.1 ng/mL to 500 ng/mL (Figure 3a), and the LOD for detection of salbutamol is 0.1 ng/mL (Figure 3b). The result shows that the sensitivity of the Au-MRS sensor is better than that of ELISA.

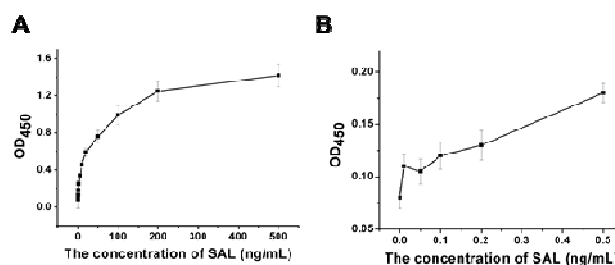


Figure 3. The result of ELISA for detection of SAL. (a) shows the sensitivity of ELISA for detection of SAL, the concentration of SAL is 0.01, 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100, 200 and 500 ng/mL; (b) shows the LOD of ELISA for detection of SAL, the concentration of SAL is 0.01, 0.05, 0.1, 0.2 and 0.5 ng/mL.

Compared to ELISA that needs multiple washing steps, the inherent advantage of MRS is that it needs minimal or no sample preparation and can be used to analyze complicated samples in one step. Meanwhile, the sensitivity of the Au-MRS sensor is improved by using signal amplification strategies. The Au-SA conjugate has been proved to be an effective multivalency signal amplifier, because Au NPs have a large surface-to-volume ratio, and lots of SA can be conjugated on their surface. In addition, Au NPs has good suspension stability that is important to the robustness of the Au-MRS sensor. Therefore, the Au-MRS approach can be used as a robust and easy-to-use sensor system with significantly improved sensitivity and accuracy. The advantages and disadvantages of ELISA, conventional MRS sensor and Au-MRS sensor are summarized in Table 1.

Table.1 A comparison between ELISA, MRS sensor and Au-MRS sensor for detection of SAL

Approach	Sensitivity	Detection time	Operation
ELISA	0.1 ng/mL	4 h	Laborious
MRS sensor	0.1 ng/mL	0.5 h	Convenient
Au-MRS sensor	10 pg/mL	0.5 h	Convenient

Conclusions

In summary, an immunosensor based on MRS and Au-SA conjugate has been successfully developed to detect salbutamol in swine urine samples. Au-SA conjugate can amplify the signals to largely improve the sensitivity of the MRS assay, and the whole analysis can be completed within 30 min. Combined with portable magnetic signal readout system, this Au-MRS method has great potential in point of care testing such as early diagnosis, environmental monitoring, food safety and so forth.

Notes and references

‡ Footnotes relating to the main text should appear here.

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