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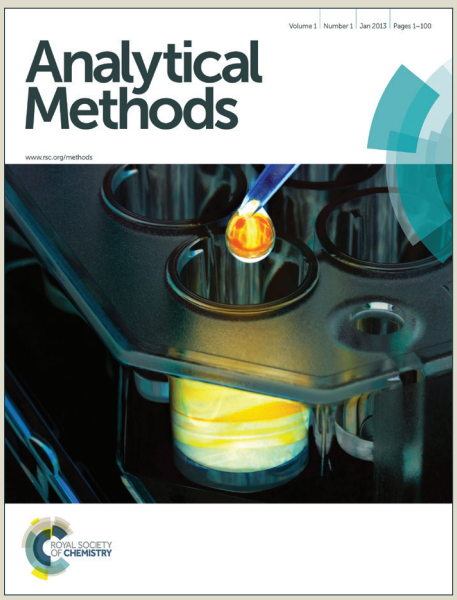

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An aptamer-functionalized gold nanoparticle biosensor for the detection of prion protein

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Abstract Prions are a special class of pathogens that cause a number of fatal neurodegenerative diseases in mammals. This paper presents a very simple and convenient biosensor for detecting prion protein, in which a prion protein aptamer was used as the molecular recognition group and gold nanoparticles were used as the signal report group. Binding of the target molecular prion protein resulted in the enhancement of resonance light scattering (RLS) by the gold nanoparticles. A linear relationship was then identified between the enhanced RLS intensity and the concentration of prion protein in the range 0.2 to 50 nmol/L, with a limit of detection of 0.07 nmol/L (3σ). The biosensor has

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very good selectivity for prion protein without interference by coexisting proteins, amino acids or metal ions. This "aptasensor" offers a rapid, selective and sensitive route for prion protein detection and has good potential for use in practical applications.

1. Introduction

Prion diseases, also known as transmissible spongiform encephalopathies (TSEs), are a group of fatal neurodegenerative disorders that affect humans and animals. The transformation of host-encoded normal prion protein (PrP^{C}) to the pathological conformer (PrP^{Sc}) and the subsequent aggregation of PrP^{Sc} may cause Gerstmann-Straussler-Scheinker disease (GSS), fatal familial insomnia in humans, bovine spongiform encephalopathy (BSE) in cattle, scrapie in sheep and other prion diseases[1]. PrP^{C} and PrP^{Sc} have the same amino acid sequence but are different in conformation, whereby PrP^{C} has a high content of alfa-helices and PrP^{Sc} is rich in the beta-sheet structure[2]. Affecting approximately one in every million people, these rare disorders may arise either spontaneously, via inheritance of a predisposing mutation, or through infection. Acquisition of disease by the latter mechanism has drawn intense interest among scientists and the general public, especially in light of the recent outbreak of BSE in the United Kingdom[3] and the appearance of a new variant of Creutzfeldt-Jakob disease (vCJD) [4, 5]. Currently, several methods for the detection of prions have been established, including protein misfolding cyclic amplification (PMCA)

[6], the conformation-dependent assay[7], and a combination of new ligands[8, 9] with spectroscopic techniques[10]. For example, Soto and co-workers[11] detected prions by cyclic amplification of protein misfolding. MacGregor and co-workers[12] detected PrP^{Sc} in the blood using the time-resolved fluoro-immunoassay. These two methods were sensitive and based on immunoassays but both require complicated functionalization steps and expensive equipment. Therefore, developing a convenient and sensitive assay protocol is of great importance.

Recently, the resonance light scattering (RLS) technique, which has the distinct advantages of simplicity, rapidness and high sensitivity, was developed to study the extended aggregates of chromophores or bio-assemblies[13, 14]. The RLS technique has been proposed for the determination of DNA[15], proteins[16], metal ions[17], and saccharides[18], amongst other compounds. Metal nanoparticles, because of their extremely strong light scattering at plasma-resonance wavelengths, have been used as RLS probes, enabled by the rapid development of nanotechnology[19-20]. For example, Li and co-workers[21] reported a sensitive light scattering assay for lysozyme using gold nanoparticles (AuNPs) as a probe. Binding studies between an aptamer and its target is a new strategy applied in molecular recognition chemistry[22]. Therefore, an aptamer that can specifically bind to prion protein with high affinity may be a useful new ligand for the detection of PrP. The selection of DNA aptamers against PrP has been reported[23, 24]. Xiao and co-workers[25] used aptamer-modified quantum dots and magnetic microparticles in a sandwich structure to detect prion protein, but their assay process was complex. In the present work, we use a simple biosensor to detect prion

protein, in which a prion protein aptamer is used as a molecular recognition group and AuNPs are used as a signal report group. The biosensor shows high selectivity and sensitivity.

2. Material and methods

2.1 Reagents

Chloroauric acid and chymotrypsin were purchased from Aladdin Reagent Co., Ltd (Shanghai, China). Sodium citrate was purchased from Beijing Chemical Works (Beijing, China). Lysozyme from chicken egg white (activity ≥ 40,000 units/mg), human serum albumin (HSA), horseradish peroxidase (HRP), and glucose oxidase (GOD) were purchased from Sigma-Aldrich (St. Louis, MO). All regents used for purification of rPrP were purchased from Genview (Tianjin, China). L-Glycine (Gly), L-tyrosine (Tyr), L-methionine (Met), L-phenylalanine (Phe), L-alanine (Ala), L-histidine (His), L-arginine (Arg), L-Lysine (Lys), L-glutamic acid (Glu), L-aspartate (Asp), L-leucine (Leu), L-isoleucine (Ile), and L-valine (Val) were purchased from Beijing Dingguo Changsheng Biotechnology Co., Ltd (Beijing, China). Bovine serum albumin (BSA) was purchased from Shanghai BioLife Science & Technology Co., Ltd (Shanghai, China). Pepsin was purchased from Shanghai Institute of Biological Products (Shanghai, China). Cellulase was purchased from Dongfeng Biochemistry Technology Co., Ltd (Shanghai Institute of Biological Science, China). Thrombin was purchased from Heilongjiang Dilong Pharmaceutical Co., Ltd (Suihua, China). Glusulase and thiol aptamer (5'-SH-(C6)-CTT ACG GCG GGG CAA TT-3') were

purchased from Sangon Biotech Co., Ltd (Shanghai, China). Human serum samples were obtained from Southwest University Hospital (Chongqing, China). All solutions were prepared with Milli-Q ultrapure water.

2.2 Apparatus

The dynamic light scattering (DLS) signal of aptamer-functionalized AuNPs (APT-AuNPs) was recorded by a Zetasizer Nano ZS90 apparatus (Malvern Instruments Ltd, Malvern, UK). The morphology and dispersivity of APT-AuNPs were measured by a Tecnai G2 F20 S-TWIN field emission transmission electron microscope (TEM) (FEI, Hillsboro, OR). The absorption spectra were recorded by a UV-2450 spectrophotometer (Shimadzu, Kyoto, Japan). An F-4500 fluorescence spectrometer (Hitachi, Japan) was used to record the RLS spectra in synchronous scanning mode (ex - em = 0). Both excitation and emission slit widths were kept at 10 nm and the RLS intensity was recorded at 560 nm.

2.3 Preparation and functionalization of AuNPs

AuNPs (13 nm) were synthesized by citrate reduction of $HAuCl_4$[26] and functionalized with the thiol aptamer[27-29]. Briefly, 5 mL of AuNPs was mixed with 3 OD thiol aptamer at room temperature. The final oligonucleotide concentration was 3.6 μmol/L. The mixture was incubated for 16 h at room temperature and, after adjusting the solution to 0.1 mol/L NaCl, it was further incubated for 40 h at room temperature. It was then centrifugated at 10,000 rpm using a LRH-250-Z Z383K high-speed bench centrifuge (Hermle Labortechnik GmbH, Wehingen, Germany) for

1 h. The collected pellet was re-dispered with ultrapure water, followed by addition of 0.3 mol/L NaCl, 10 mmol/L phosphate buffered saline (PBS) and 5 mmol/L EDTA.

2.4 Purification of recombinant human prion protein (rPrP)

The rPrP (23-231) was expressed in *Escherichia coli* BL21 (DE3)[30] and purified using a nickel ion-charged Sepharose column, as previously described[31]. Protein concentration was determined using a Bio-Rad (Hercules, CA) protein assay kit.

2.5. Assays of rPrP

A solution of APT-AuNPs (1.0 nmol/L) was mixed with 80 μL of PBS (pH 6.0, 0.2 mol/L) and 80 μL of 2 mol/L NaCl at room temperature. A given volume of rPrP solution was then added to the mixture, and ultrapure water was added to bring the total volume to 0.80 mL. After incubating for 30 min at room temperature, the RLS spectra were recorded and the RLS intensity at 560 nm was used for quantitative analysis.

2.6 Analysis of rPrP in a human serum sample

To test the performance of our method in complex samples, rPrP in human serum was detected. A series of mixtures (0.80 mL) of 10-fold diluted human serum sample and APT-AuNPs (1.0 nmol/L) were spiked with rPrP (0–10 nmol/L). After equilibration at room temperature for 30 min, their RLS spectra were recorded by an F-4500 fluorescence spectrometer.

3. Results and discussion

3.1 rPrP enhance the RLS of APT-AuNPs

After modification with aptamer, a slight shift in the surface plasma resonance band of AuNPs from 520 to 523 nm was observed (Fig. S1). And the absorbance of APT-AuNPs reduced after the addition of rPrP (Fig. S2). APT-AuNPs exhibited a wide RLS peak near 520 nm. However, after the addition of rPrP to the APT-AuNPs solution, an obvious enhancement of the RLS signal near 560 nm could be observed (Fig. S3).

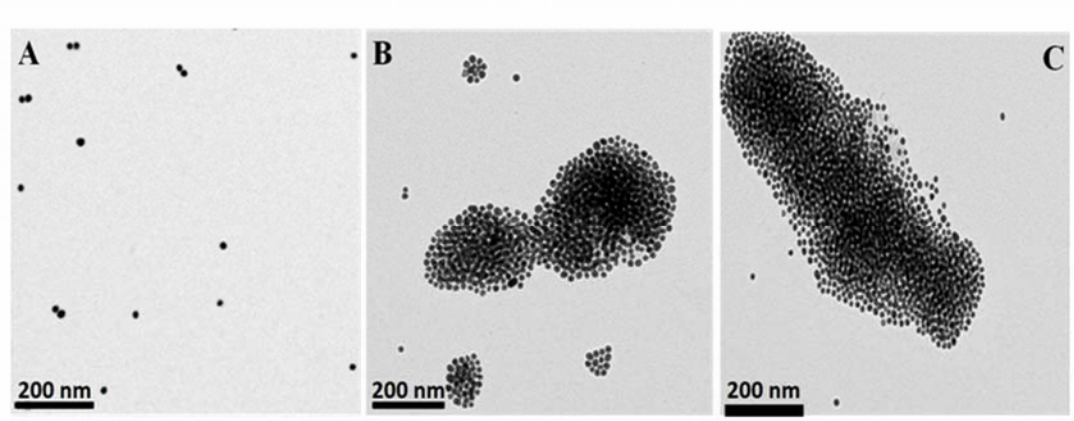


Fig. 1 TEM images of APT-AuNPs in the absence (A) and presence of 20 nmol/L (B) and 50 nmol/L rPrP (C).Concentration of APT-AuNPs and NaCl are 1.0 nmol/L and 0.20 mol/L, respectively. The pH value is adjusted to 6.0 with PBS.

TEM was used to explain the red-shift and enhancement of RLS spectra. As shown in Fig. 1A, APT-AuNPs were well-dispersed in PBS at pH 6.0 containing 0.2 mol/L of NaCl. However, APT-AuNPs aggregated in the presence of 20

nmol/L rPrP (Fig.1B) and 50 nmol/L rPrP (Fig.1C). DLS results also confirmed the rPrP-induced aggregation of APT-AuNPs (Fig. S4). According to Pasternack and Collings[32], RLS is sensitive to aggregation and size changes so the aggregation of AuNPs enhanced the intensity of RLS near 560 nm.

3.2 Selectivity of APT-AuNPs

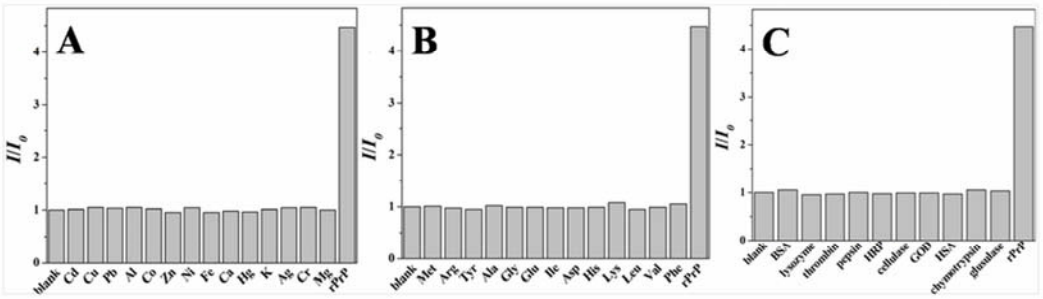


Fig. 2 Selectivity of rPrP detection (rPrP, 20 nmol/L; APT-AuNPs 1.0 nmol/L) with the following interference: (A) metal ions (Cd^{2+}, Cu^{2+}: 20 μmol/L; Pb^{2+}, Al^{3+}, Co^{2+}, Zn^{2+}, Ni^{2+}, Fe^{2+}: 10 μmol/L; Ca^{2+}: 8 μmol/L, Hg^{2+}: 4 μmol/L; Mg^{2+}: 2 μmol/L; K^{+}, Cr^{3+}: 0.2 μmol/L; Ag^{+}: 0.1 μmol/L); (B) amino acids (Met, Arg, Tyr, Ala, Gly, Glu, Ile, Asp, His: 20 μmol/L; Val, Phe: 8 μmol/L; Lys, Leu: 4 μmol/L); and (C) proteins (BSA, lysozyme: 2 μmol/L; thrombin, pepsin, GOD: 0.2 μmol/L; HRP: 0.4 μmol/L; cellulose: 0.04 μmol/L; HSA, glusulase: 0.02 μmol/L, chymotrypsin: 0.16 μmol/L). I_0 is the RLS intensity at 560 nm of the APT-AuNPs solution in the absence of rPrP. I represent the RLS intensity of the APT-AuNPs in the presence of amino acids, proteins, metal ions, or rPrP.

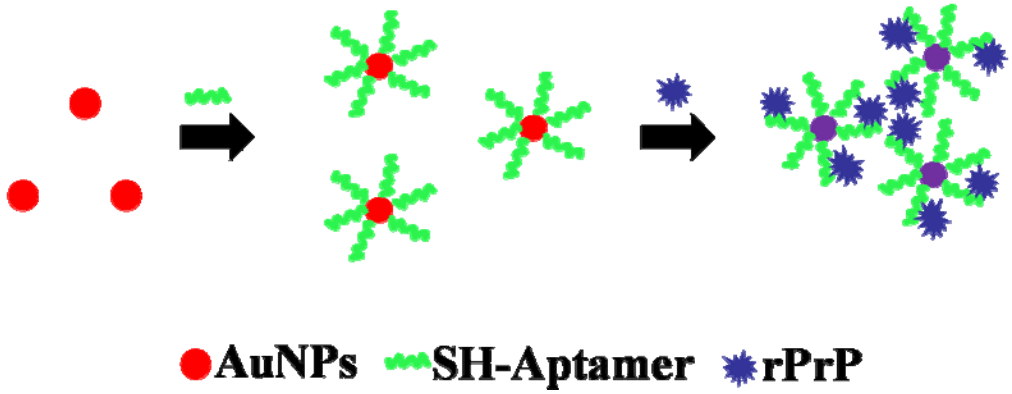
To test the specificity of this assay towards rPrP, we mixed rPrP or potentially interfering metal ions, amino acids, and proteins individually with 1.0

nmol/L of APT-AuNPs. We tested 14 species of metal ions, 13 amino acids and 10 types of proteins with concentrations that are similar to the phsiological environment; none of these caused any change in RLS intensity (Fig. 2). These results confirm that a prion protein aptamer can function as a molecular recognition group that can specifically combine with rPrP and, importantly, that only rPrP can increase the RLS intensity of APT-AuNPs.

The isoelectric point (pI) of rPrP is about 10.0. Under the present experimental condition (pH 6.0), rPrP is positively charged and APT-AuNPs are negatively charged, so it is possible that non-specific, electrostatic interactions may occur. However, positively charged amino acids such as Arg (20 μmol/L), His (20 μmol/L), Lys (4 μmol/L) and other positively charged proteins such as chymotrypsin (0.16 μmol/L) had no effect on the RLS signal of the system. The amyloidogenic protein lysozyme (2 μmol/L) and most metal ions (> 0.1 μmol/L) also failed to affect the RLS signal.

This high specificity is thus apparently due to highly specific binding between the aptamer and the related substrate, i.e., rPrP. Scheme 1 illustrates the mechanism behind the enhancement in the RLS signal caused by rPrP. The aptamer used in this work can bind with the 23-90 epitope of PrP[23]. After functionalized with aptamer, the AuNPs were very stable because of protection by the aptamer. In the presence of rPrP, the APT-AuNPs reacted under the chosen conditions specifically with rPrP to form an AuNPs-aptamer-rPrP complex. The large surface area of the Apt-AuNPs provided a suitable binding

site for rPrP, which increases local protein concentrations and promotes oligomer formation on the AuNPs surfaces[33,34]. Oligomer formation could then lead to aggregation of rPrP. The specific binding between rPrP and Apt-AuNPs then resulted in further binding of the Apt-AuNPs onto the surfaces of the rPrP aggregates. This process continued and finally formed large aggregates (Scheme 1). Additionally, in the presence of NaCl, the aggregation of AuNPs enhanced, and a strong RLS intensity was observed[35].



Scheme 1. Interactions between APT-AuNPs and rPrP.

3.3 Optimization of the assay conditions

The RLS detection conditions were optimized to develop a sensitive, simple and practical assay for rPrP. The effects of pH, NaCl concentration, APT-AuNPs concentration, and reaction time are shown in Fig. 3.

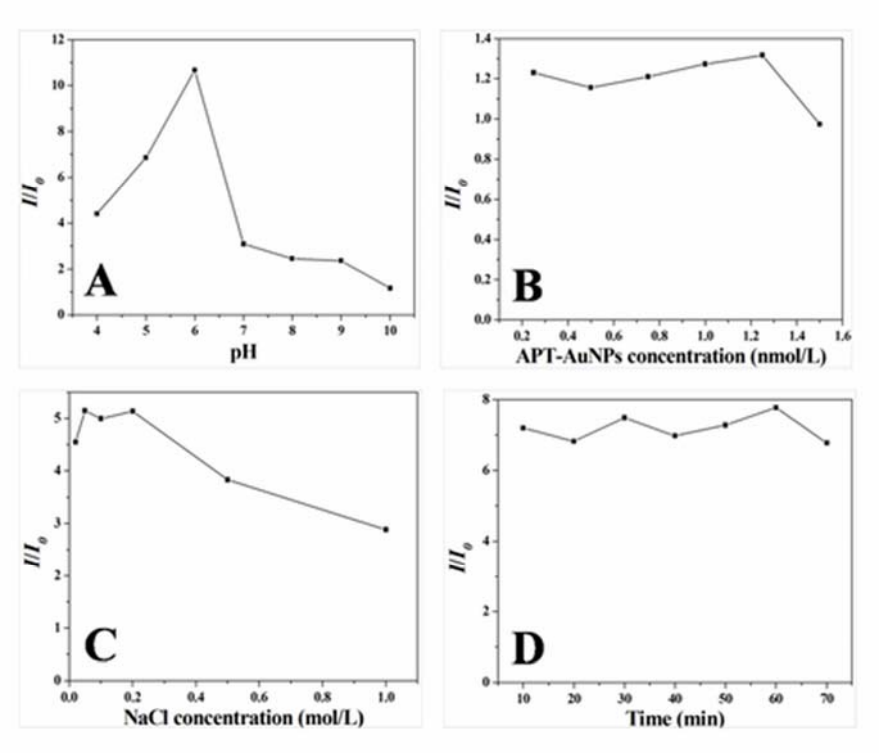


Fig. 3 Optimization of the assay conditions. A: pH (APT-AuNPs, 1.0 nmol/L; rPrP, 20 nmol/L); B: APT-AuNPs concentration (pH 6.0 PBS, 20 mmol/L; rPrP, 20 nmol/L); C: NaCl concentration (APT-AuNPs, 1.0 nmol/L; rPrP, 20 nmol/L; pH 6.0 PBS, 20 mmol/L); D: Reaction time (APT-AuNPs, 1.0 nmol/L; rPrP, 20 nmol/L; pH 6.0 PBS, 20 mmol/L). I_0 is the RLS intensity at 560 nm of the APT-AuNPs solution in the absence of rPrP. I represent the RLS intensity of the APT-AuNPs in the presence of rPrP.

As can be seen in Fig. 3A, the relative intensity of the RLS signal was the highest at pH 6.0. Thus, pH 6.0 PBS buffer solution was selected.

The effect of APT-AuNPs concentration on the RLS signal was studied, as seen in Fig. 3B. Considering both the sensitivity of the assay and the desire to

minimize reagent consumption, the best concentration of APT-AuNPs was 1.0 nmol/L.

NaCl plays an important role in achieving high sensitivity in this assay by inducing aggregation of the AuNPs. The influence of NaCl concentration is shown in Fig. 3C. The relative intensity of the RLS signal reached a maximum at 2.0 mol/L NaCl. However, APT-AuNPs became unstable above 0.2 mol/L NaCl, so 0.2 mol/L of NaCl was chosen for subsequent use.

The reaction time was investigated, as shown in Fig. 3D. There was no obvious change in relative RLS intensity over 1 h, so we chose 30 min as a standard reaction time.

3.4 Quantitative detection of rPrP

Mixing APT-AuNPs with rPrP resulted in the appearance of an RLS band around 560 nm. The RLS signal increased linearly with increasing rPrP concentration. Under the optimized conditions, the RLS intensity at 560 nm exhibited a linear relationship with the concentration of rPrP from 0.2–50 nmol/L. The linear regression equation was I_{RLS} = 17.892 C+103.6 (R^2=0.9918) (Fig. 4), where I_{RLS} is the RLS intensity of APT-AuNPs at 560 nm and C is the concentration of rPrP. The detection limit was 0.07 nmol/L (3σ), and the relative standard deviation was 1.4% (n=11) for 20 nmol/L rPrP. Compared with immunological methods[36], which are currently the most important methods for the detection of prion, our method is simpler and more convenient. Our method has a higher sensitivity than the fluorescence spectroscopy assay[37], and the selectivity of this assay is higher and was enhanced several times

compared with our previous results[38].

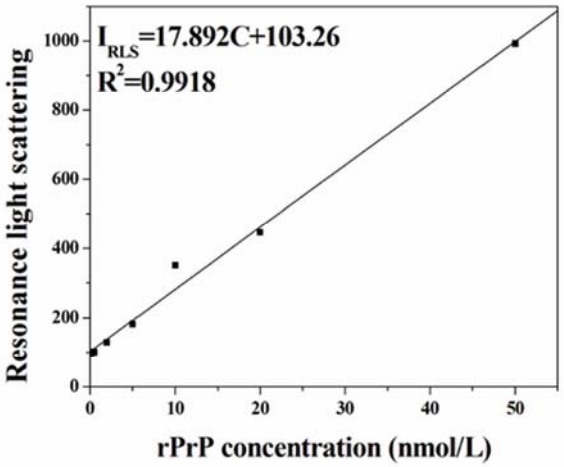


Fig. 4 Linear plot of the RLS intensity=against the rPrP concentration at pH 6.0 PBS in the presence of 0.2 mol/L NaCl. Concentration of rPrP: 0, 0.2, 0.5, 1.0, 2.0, 5.0, 10, 20, 50 nmol/L. I_{RLS} is the RLS intensity at 560 nm of the APT-AuNPs solution with different concentrations of rPrP.

3.5 Interference from coexisting substances

The APT-AuNPs biosensor was highly selective for rPrP detection. In biological samples, such as human serum, there are many coexisting metal ions, amino acids, and proteins that potentially interfere with the detection of rPrP. A number of coexisting substances (Table S1) were studied for potential interference. In the presence of these substances, a relative error of less than ±5 % was considered to be acceptable. Most of the amino acids, proteins and metal ions tested did not affect the determination. For example, in human serum, most

amino acids are present at concentrations below 0.50 mmol/L and after spiking the reaction system with serum, dilution was able to eliminate interference.

3.6 Detection of rPrP in human serum

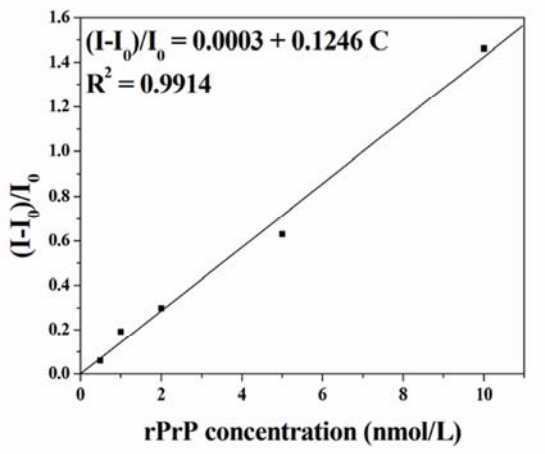


Fig. 5 Detection of rPrP spiked in a solution of human serum in pH 6.0 PBS. I is the RLS intensity at 560 nm of the APT-AuNPs solution after adding human serum spiked with standard solutions of purified rPrP and I_0 is the RLS intensity at 560 nm of APT-AuNPs solution in the presence of human serum without purified rPrP. The concentration of APT-AuNPs was 1.0 nmol/L.

Purified rPrP (0-10 nmol/L) was spiked into diluted human serum. The calibration curve ($(I-I_0)/I_0 = 0.0003 + 0.1246C$, $R^2=0.9914$) (Fig.5) was used to measure an unknown concentration of rPrP in the presence of 0.2 mol/L NaCl. The concentration of rPrP in the human serum sample was calculated to be 2.1 nmol/L , while the concentration of prion protein in plasma is less than 9 ng/mL[39]. Thus, the "aptasensor" offers a rapid, highly selective and sensitive route for rPrP detection and has high potential for practical applications.

4. Conclusion

In this work, APT-AuNPs aggregated to form large particles after reaction with rPrP and showed incremental increases in RLS intensity with increasing rPrP concentration. Thus, the aptamer-based RLS method was developed to detect rPrP with high sensitivity and selectivity. This assay was successfully applied to the detection of rPrP in a serum sample. Compared with immunological methods and the fluorescence spectroscopy assay, our method offers a simple, selective and sensitive route for prion protein detection and provides high potential for practical applications.

Acknowledgements

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Notes and references

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Electronic Supplementary Information (ESI) available: [The following material is available free of charge: Normalized UV-Vis spectra of AuNPs and APT-AuNPs (Fig. S1); UV-vis absorbance spectra of APT-AuNPs in the absence and presence of 20 nmol/L rPrP (Fig. S2); The RLS spectra of APT-AuNPs in the absence and presence of rPrP (Fig. S3); Hydrodynamic diameter of APT-AuNPs in the absence and presence of rPrP in pH 6.0 PBS (Fig. S4); Effect of coexisting substances on the determination of rPrP using the APT-AuNPs system containing 0.2 mol/L NaCl (Table S1).]. See DOI: 10.1039/b000000x/

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An aptamer-functionalized gold nanoparticle biosensor for the detection of prion protein

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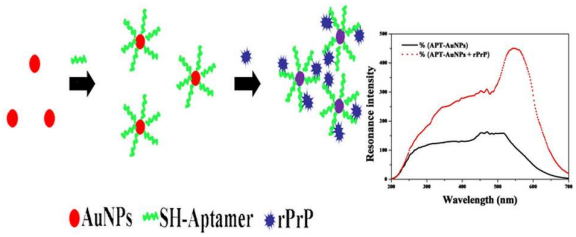

The resonance light scattering intensity of gold nanoparticles which modified a prion protein (rPrP) aptamer increased after addition of rPrP.