**Study On The Sensor For The Determination Of AFP
Based On Rare Earth Upconversion Nanomaterials
And AuNPs Fluorescence Resonance Energy Transfer**

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Abstract

Alpha Fetoprotein (AFP) is a very important tumor marker and a major serum protein in the early stage of fetal development. Its concentration gradually decreases with the growth of the fetus. AFP is produced by hepatocytes in adults, and its content is less than 20 ng·mL⁻¹. The increase of AFP in serum is of great significance for the diagnosis of primary liver cancer. In this study, the functional upconversion nanoparticle(ucnps, NaYF₄:Yb³⁺, Er³⁺), were designed a sensor based on fluorescence resonance energy transfer and aptamer recognition technology to detect AFP. Ucnps was used as energy donor and AuNPs as quenching agent. In the presence of AFP, the aptamer linking ucnps and AuNPs will dissociate the sandwich complex and restore the fluorescence of the system. Due to the high affinity and specificity of aptamers, the detection range of AFP is 0.05-2 ppb and the detection limit is 0.05 ppb.

46 **Introduction**

47 Cancer is one of the high mortality diseases. More than ten million people are
48 diagnosed with cancer every year. Although cancer cells are confined to one tissue,
49 they can migrate to other parts of the body. This has caused many obstacles to the
50 treatment of cancer, and is also one of the main challenges facing cancer treatment.
51 Common cancer treatments include chemotherapy, radiotherapy and surgery, but these
52 treatments are not ideal and may not help patients get rid of cancer. Radiotherapy is
53 more common than surgery, but it is not suitable for cancer cells that have spread to
54 the whole body. Therefore, it is necessary to develop new methods for early cancer
55 detection, so as to improve the survival rate of patients. It is urgent to explore and
56 develop luminescent reagents with high luminescent intensity and good
57 biocompatibility for tumor detection. At present, several types of nano luminescent
58 particles have been used in related research, one of which is rare earth doped up
59 conversion nano materials. Rare earth upconversion nanomaterials can transform
60 low-energy light (such as near-infrared light) into high-energy ultraviolet or visible
61 light (blue, green and red light, etc.). At present, the traditional organic dye molecules
62 are mostly studied in the tracking, imaging and detection of cancer cell targets.
63 However, due to the weak absorption capacity and poor imaging stability of most dye
64 molecules, their applications in high-throughput imaging output and high-sensitivity
65 detection are greatly limited^{1,2} . Compared with organic dye molecules, rare earth up
66 conversion nanomaterials show their advantages as fluorescent labeling agents, which
67 is mainly reflected in the advantages of rare earth up conversion nanomaterials, such

as wide excitation and emission spectrum, high optical stability, large Stokes displacement, long fluorescence life, etc., which are ideal fluorescent probes. In addition, low-power solid-state laser diode can be used as excitation light source to avoid expensive high-power laser settings, which is also a significant advantage of rare earth up conversion materials as fluorescent markers.

In 2012, Wu *et al.*³ developed a biosensor based on fret that can simultaneously determine Ochrotoxin A (OTA) and Fumonisin (FB) mycotoxins. In this sensor, rare earth up conversion material is used as energy donor, and the aptamers of these two toxins are modified on the surface of rare earth at the same time. In addition, Song *et al.*⁴ Also used rare earth up conversion materials as fluorescent donors. Through the biotin avidin specific binding, the distance between rare earth modified with ATP aptamer complementary chain and tamra fluorescent group modified on ATP aptamer was shortened, so as to realize the quantitative detection of ATP. Aptamer is a kind of systematic evolution of ligands by exponential enrichment (SELEX), a group of oligonucleotides selected from random single strand nucleic acid sequence library, which can specifically bind to proteins, small molecular organics and other target substances. SELEX has high affinity and specificity for target substances. It is synthesized in vitro, and its length is generally 15-40 bases^{5,6}. Because aptamers have many advantages, such as high specificity, high affinity, good chemical stability and easy modification, more and more studies have used them to construct aptamers based analytical sensing system.

Alpha Fetoprotein (AFP) is a glycoprotein produced in colorectal cancer tissue,

which can be used as an antigen to cause immune response of patients⁷⁻⁹. As a broad-spectrum tumor marker, the concentration of AFP in the serum of patients may increase after the loss of some cancer gene regulation¹⁰. It has a certain reference value for the diagnosis and treatment of clinical tumor to detect AFP efficiently and specifically. At present, there are many research methods for the detection of AFP, such as electrochemical immunosensor¹¹, chemiluminescent immunoassay¹² and fluorescent immunoassay¹³⁻¹⁴. Most of these studies are based on the antigen antibody specific recognition reaction to construct sensors, so as to achieve the detection of AFP. Monoclonal and polyclonal antibody technology has been developed for more than 70 years, but there are still a series of defects, such as pH, temperature sensitivity, short storage life and easy degradation. As a new generation of biomolecular recognition element, aptamer technology overcomes the shortcomings of the above antibodies and can be used to build a fast, sensitive, low consumption and easy to automate biosensor system. Recently, Huang *et al.*¹⁵ Used NaYF₄, Yb/Ho up conversion materials to detect AFP. In this study, both ucnp and magnetic nanoparticles were modified with AFP antibody at the same time. AFP connected them, enriched magnetic nanoparticles with magnets, and detected AFP by measuring the fluorescence of ucnp connected with magnetic nanoparticles. Lin *et al.*¹⁶ and other researchers introduced polymer quantum dots into biosensor system based on fluorescence resonance energy transfer for the first time, and constructed AFP aptamer biosensor, which can achieve high sensitivity and specificity detection.^{17,18}

In this paper, a fluorescent biosensor based on aptamer recognition and fluorescence resonance energy transfer for AFP detection is studied. We modified the

short oligonucleotide fragments on the surface of rare earth upconversion nanomaterials and gold nanoparticles respectively to form a stable sandwich structure by combining them with AFP aptamers. At this time, the fluorescence of rare earth ucnps was quenched by gold nanoparticles, and the fluorescence of the system was weak. When AFP exists in the system, it will combine with its aptamer to dissociate the sandwich structure. AFP can be quantitatively detected by the fluorescence recovery intensity of the system. The experimental results show that the sensor has good selectivity and high sensitivity. The detection limit of AFP can reach 0.05 ppb.

Experimental

Experimental instruments

Milli-q ultra pure water system (millipore, Bedford, USA); Rj-tgl-16c desktop high speed centrifuge (Wuxi Ruijiang Analytical Instrument Co., Ltd.); Phs-3c precision acidimeter (Shanghai Dapu Instrument Co., Ltd.); Finnpiptette adjustable pipette (Shanghai thermoelectric Instrument Co., Ltd.); Fls-920 steady state transient fluorescence spectrometer (Edinburgh company, UK)980 nm semiconductor laser (Changchun new industry photoelectric technology Co., Ltd.); Nicolet 6700 Fourier infrared microscope on-line system (Thermo Fisher, USA); Df-101s collector type constant temperature adding magnetic stirrer (Gongyi Yuhua Instrument Co., Ltd.)Lambda 750 ultraviolet visible spectrophotometer (Shelton, USA); Nanoscope multimode IIIA atomic force microscope (Veeco instruments, USA); TECNAI transmission electron microscope (G2 f20s-twin 200kV);Tu-1950 ultraviolet spectrophotometer (Beijing puanalytical General Instrument Co., Ltd.);Tg16-w micro high speed centrifuge, kq118 ultrasonic cleaner, dk-8d electrothermal constant temperature water tank, electronic balance, cuvette, reaction kettle, etc.

138

139 *Materials and reagents*

140 The DNA in this experiment was purchased from Shanghai Biotechnology Co., Ltd.
141 The sequence is in Tab1. Alpha Fetoprotein (AFP), N - (2-hydroxyethyl) piperazine-N'-
142 2-ethanesulfonic acid (HEPES), 2-morpholine ethanesulfonic acid (MES),
143 n-hydroxythiosuccinimide (NHS), 1-ethyl-3- (3-dimethylaminopropyl)-carbodiimide
144 (EDC), sodium citrate, Chloroauric acid were purchased from Shanghai Shenggong
145 Biotechnology Co., Ltd. Tris (2-carboxyethyl) phosphate (TCEP) , Tris were
146 purchased from Beijing Dingguo Biotechnology Co., Ltd. Yttrium nitrate, erbium
147 nitrate, ytterbium nitrate, 6-aminocaproic acid, sodium fluoride, ethylene glycol were
148 purchased from China Pharmaceutical Group Chemical Reagents Co., Ltd. All solutions
149 were prepared with ultrapure water (18.2 MΩ·cm), which was obtained through a
150 Millipore Milli-Q water system (Barnstead, CA, USA).

151

152 *Preparation and surface modification*

153 According to the previous work, rare earth upconversion luminescent
154 nanomaterials (NaYF₄: Yb³⁺, Er³⁺) were prepared by one-step solvothermal synthesis.
155 The preparation process is briefly described as follows: weigh 1 mmol of rare earth
156 nitrate (Y³⁺: Yb³⁺: Er³⁺ = 78:20:2) and dissolve it in 8 mL of ethylene glycol, add 5
157 mmol of NaF solution, stir it with magnetic force for 30 minutes, then add 30 mmol of
158 6-aminocaproic acid, transfer the solution to 50 mL of stainless steel reactor lined
159 with polytetrafluoroethylene, seal it, and transfer it to 190 mL of stainless steel reactor
160 lined with polytetrafluoroethylene. After natural cooling to room temperature, the
161 solution was centrifuged at 13000 rpm for 5 minutes, and the precipitate was washed

with ethanol for 3 times, then dried in vacuum at 50 °C for 12 hours.

The surface modification of amino functionalized ssDNA was carried out on the rare earth upconversion nanomaterials by EDC catalyzed amide condensation. The specific steps are as follows: dissolve 5 mg ucnp in 2 mL of MES (10 mmol, pH = 5.5) buffer solution, add 160 µL 5 ppm EDC and 220 µL 10 ppm sulfo NHS, and slowly warm the bed at 30 °C for 2 hours. After three times of centrifugal washing, the precipitate was dissolved in 2 mL containing HEPES (10 mmol, pH = 7.2) buffer solution of amino modified AFP aptamer. Add 50 mL Tris buffer solution to remove excess NHS in a slow temperature bed at 30 °C for 4 hours, wash with centrifugation water, suspend the precipitate in 2.5 mL water again, and store at 4 °C.

Surface modification of gold nanoparticles was prepared by reducing chloroauric acid with sodium citrate¹⁹. Firstly, the HAuCl₄·3H₂O (129 µL, 40 mg/mL) was dissolved in 24 mL ultra pure water, heated to boiling in 130 °C oil bath, and boiled for 15 min. Then quickly add 1 mL of sodium citrate (1.3% wt / V) once, and continue to stir until the solution turns wine red. Heat and stir again for 15 min, then cool to room temperature, and store at 4 °C. According to Lambert-Beer law, the absorbance of the gold nanoparticles is 1.43 nM (the absorbance coefficient $\epsilon = 2.7 \times 10^8 \text{ M}^{-1}\text{cm}^{-1}$, $\lambda = 518 \text{ nm}$)²⁰. DNA chains with sulfhydryl groups were modified on the surface of gold nanoparticles by Au-S bond²¹. Before modification, the thiol DNA should be activated to open the disulfide bond. 83 µL of thiol DNA (100 µM), 4 µL of TCEP (100 mM), and 40 µL of PBS (50 mM, pH=5.2) were taken and reacted at room temperature for one hour. The concentration of PBS and SDS were adjusted to 0.01 M and 0.01%, respectively. After the DNA nanogold solution was incubated at room

temperature for 30 min, a certain amount of 2 M NaCl was added to make the solubility of NaCl 0.05 M. After 15s of ultrasound, it was incubated for 30 min. Repeat the process until the NaCl concentration in DNA Au solution reaches 1 M. NaCl is a dispersant, and its main function is to control the particle size. If the amount is large, the particle size will be small, and if the amount is small, the particle size will be large. The salted DNA solution was incubated overnight at room temperature, washed three times by centrifugation, and re suspended in 0.01% SDS.

The Fourier transform infrared (FTIR) spectrum of the sample was determined by Nicolet 6700 Fourier transform infrared spectrometer (FTIR) produced by Thermo Fisher company of the United States, and the potassium bromide tableting method was used. The fluorescence spectrum was measured by the new 980 nm semiconductor laser produced by Changchun company. AFM images were measured by nanoscope multimode IIIA atomic force microscope (Veeco instruments, USA). Rare earth upconversion luminescence photos were taken by Sony digital camera.

Detection of AFP

First, the ssDNA modified rare earth upconversion nanomaterials and gold nanoparticles react with AFP aptamers at a certain temperature for 30 min, and shake slightly to form a stable sandwich structure. The upconversion fluorescence intensity of the system was measured after a period of excitation with a 980 nm semiconductor laser. The quenching effect of the system fluorescence is reflected by the fluorescence quenching rate, which refers to the ratio of the difference ($f_0 - f$) before and after fluorescence quenching to the fluorescence intensity (F_0) before quenching $[(f_0 - f) / F_0]$.

Then different concentrations of AFP were added to the above solution, incubated for 2 h, and slightly shaken. Then the fluorescence intensity of the system was measured, and the ratio of the difference before and after fluorescence recovery ($f-f_0$) and the fluorescence value before fluorescence recovery (F_0) was taken as the test object, that is, AFP concentration was determined by the value of fluorescence recovery rate $[(f-f_0)/F_0]$.

Results and discussion

Characterization

The preferred position for Figure 1

AFM was used to characterize the synthesized rare earth up conversion nanomaterials. As shown in Fig. 1. The light and shade levels in the figure correspond to the height of the characterization material. It can be seen from the figure that the prepared upconversion nanoparticles have good dispersion and uniform size, and the material heights are all below 20 nm. Compared with the previously reported ucnp, the size is smaller. Four bright spots are selected from the straight line in the above figure for analysis. The height map is shown in Fig. 2. The abscissa in the figure represents the distance (nm) between the four bright spots, and the ordinate represents the height value (nm) of these four bright spots. It can be clearly seen from the figure that the height of these bright spots is uniform, all around 10 nm. The size of rare earth upconversion nanoparticles can be reflected by the height of these bright spots in atomic force microscope. Therefore, the size of ucnp prepared by this method is about 10 nm.

The preferred position for Figure1

The preferred position for Figure 2

At the same time, we also characterized the synthesized gold nanoparticles. According to the TEM diagram (Fig.3), the prepared gold nanoparticles are spherical with an average particle size of 15 nm. It was well dispersed in aqueous solution, and there was no obvious agglomeration at 4 °C for one month.

The infrared spectra of UCNP_s (NaYF₄: Yb³⁺, Er³⁺) and 6-aminohexanoic acid were determined by potassium bromide method. The infrared spectra are compared, as shown in Fig.4. The black curve is the infrared absorption curve of 6-aminocaproic acid, and the red curve is the infrared absorption curve of ucnp_s. It can be seen from the figure that ucnp_s has a wide absorption peak at 3446 cm⁻¹, which corresponds to the stretching vibration of O-H or N-H. The absorption peaks at 2934 cm⁻¹ and 2852 cm⁻¹ are asymmetric and symmetric stretching vibration of CH₂ group, while the absorption peaks of 1460 cm⁻¹ and 1393 cm⁻¹ show the stretching vibration of C=O group, and 1626 cm⁻¹ and 1562 cm⁻¹ show the stretching vibration of C=O group is due to the stretching vibration of amino group. Therefore, 6-aminocaproic acid was found on the surface of ucnp_s.

The preferred position for Figure 3

The preferred position for Figure 4

The spectral properties of rare earth upconversion nanomaterials and gold nanoparticles were also investigated and the results are shown in Fig.5. The excitation

wavelength is 980 nm with the maximum emission wavelength is 539 nm, and the maximum UV absorption wavelength of gold nanoparticles is 518 nm. Due to the wide absorption range of gold nanoparticles, experiments show that the fluorescence emission of rare earth upconversion nanomaterials can be absorbed by gold nanoparticles under appropriate conditions.

The preferred position for Figure 5

Principle of sensor

As shown in Scheme 1, a segment of oligonucleotide chain is modified on the surface of rare earth upconversion nano materials and nano gold particles respectively, both of which can partially complement the aptamer of AFP. Under suitable temperature and incubation conditions, rare earth up conversion nanomaterials, gold nanoparticles and AFP aptamers will form sandwich like sandwich structure. At this time, due to the close proximity of rare earth upconversion nanomaterials and gold nanoparticles (about 8 nm), the fluorescence emission wavelength of rare earth upconversion nanomaterials will be absorbed by gold nanoparticles, which will cause the fluorescence quenching of the system. In the presence of AFP, because of its stronger binding ability with AFP aptamers, AFP aptamers tend to form special ligand structure and combine with AFP. At this time, the sandwich structure dissociated and the fluorescence intensity of the system recovered. Therefore, AFP can be detected quantitatively according to the recovery of fluorescence intensity.

The preferred position for Scheme 1

Quenching efficiency of gold nanoparticles on rare earth ucnps

It was found that the quenching efficiency of gold nanoparticles on rare earth upconversion nanomaterials was different under different excitation time. In order to investigate the effect of 980 nm laser on the quenching process between gold nanoparticles and rare earth upconversion nanomaterials, we incubated rare earth upconversion nanomaterials, AFP ligands and gold nanoparticles at 28 °C for 30 min. The 980 nm laser was used to excite the system, and the fluorescence intensity of the system was measured under different excitation time. The quenching effect of the system fluorescence is reflected by the fluorescence quenching rate, which refers to the ratio of the difference ($f_0 - f$) before and after fluorescence quenching to the fluorescence intensity (F_0) before quenching $[(f_0 - f) / F_0]$. Results as shown in Fig. 6, the fluorescence intensity of the system decreased rapidly with the increase of excitation time. After excitation for 180 min, the fluorescence quenching rate $[(f_0 - f) / F_0]$ of the system reached the highest value, which was 0.91, and the fluorescence of the system was basically completely quenched. Therefore, the excitation time of 180 min is selected to realize the fluorescence quenching of the system.

The preferred position for Figure 6

In order to further verify the influence of excitation of 980 nm laser on the fluorescence quenching of the system, we investigated the change of the fluorescence of the system with time extension without laser irradiation. The results are shown in Fig.7. The fluorescence intensity of the system did not change in 30 minutes, RSD was 2.7%. The results show that there is no donor fluorescence in the system without laser irradiation, so the fluorescence resonance energy transfer can not be realized, and the fluorescence intensity of the system basically remains unchanged.

The preferred position for Figure 7

Under the above optimized conditions, different concentrations of AFP were added to the mixture of fluorescence quenching, incubated at 30 °C for 2 h, and then the fluorescence intensity of the system was measured. It can be seen from fig.8 that the fluorescence intensity of the system gradually recovers with the increase of AFP concentration, and the AFP concentration is determined quantitatively by the ratio of the difference ($f - f_0$) before and after fluorescence recovery to the fluorescence value (F_0) before and after fluorescence recovery, that is, the fluorescence recovery rate $[(f - f_0) / F_0]$. The results show that the highest fluorescence recovery rate is 1.94.

The preferred position for Figure 8

The figure 9(A) shows the linear relationship between the fluorescence recovery rate and AFP concentration of the system. The concentration of rare earth upconversion nanomaterials is 3.3 ppm, the concentration of gold nanoparticles is 0.01 nM, the concentration of AFP aptamer is 50 nM, and the buffer solution is 20 mM HEPES (pH = 7.4).

We plot the concentration of AFP with the system fluorescence recovery rate $(f - f_0) / F_0$. The figure 9(B) showed that there was a good linear relationship between the fluorescence recovery rate $(f - f_0) / F_0$ and the concentration of AFP in the range of 0.05-2 ppb. Its linear equation is $I = 0.7036C + 0.3799$, $R = 0.9965$ (where I is the fluorescence intensity; C is the concentration of AFP; R is the correlation coefficient.)

The preferred position for Figure 9

This method has high sensitivity and low detection limit up to 0.05 ppb and $K_d = 45.28 \pm 7.25 \text{ ng} \cdot \text{L}^{-1}$. This is mainly due to the high fluorescence intensity and good stability of rare earth upconversion nano materials. The K_d (dissociation constant) of the aptamer-AFP interaction was obtained by fitting the dependence of fluorescence intensity vs the concentration of aptamer to the saturation^{22,23}.

$$Y = B_{\max} X / (K_d + X) \quad (1)$$

where Y is the fluorescence intensity, X is the concentration of the aptamer and $B_{\max}$ is the fluorescence value at saturation. When the AFP detected by the aptamer was 0.05 ppb, it still had specific response.

Selectivity and interference experiment

In order to investigate the selectivity of the system for AFP detection, we selected thrombin, AFP, BSA and lysozyme, which are similar proteins, as the reference for the control experiment. The results are shown in Fig.10. The concentration of AFP was 2 ppb, the concentration of other four proteins was 200 ppb (100 times). The value of $(f-f_0) / F_0$ of AFP response in the system was significantly higher than that of other four proteins. It shows that the system has good selectivity to AFP. This is mainly due to the specific response of AFP aptamer as a bridge in sandwich structure to AFP. When AFP exists, the sandwich structure will have a real sense of dissociation and the fluorescence intensity of the system will be restored. It can be compared this system with the previously reported that this method has some advantages in sensitivity and selectivity²⁴⁻²⁹.

Conclusion

Based on the fluorescence resonance energy transfer between rare earth upconversion nanomaterials and gold nanoparticles, a novel biosensor was designed to detect AFP quantitatively. A sandwich structure complex with fluorescence quenching was prepared by self-assembly of the single chain nucleotides modified on the surface of nanoparticles and AFP aptamers. However, the existence of AFP target will lead to the combination of AFP aptamer and AFP aptamer, which will destroy the sandwich complex structure and lead to the restoration of fluorescence intensity of the system, so as to detect AFP quantitatively. The experimental results show that the system has good sensitivity and selectivity to AFP, and its detection limit can reach 0.05 ppb.

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Fig.1

Fig.2

Fig.3

Fig.4

Fig.5

Scheme1

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569 Fig.6

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Fig.7

Fig.8

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Fig.10

Tab.1

DNA	Sequence(5'to3')
Alpha-fetoprotein aptamer	GTGACGCTCCTAACGCTGACTCAGGTGCAGTTCTCGA CTCGGTCTTGATGATGTGGGTCCTGTCCGTCCGAATC
Complementary sequence of the aptamer	GATTGGTTCGGACGGACAGGACCCA

Tab.2

Method	Linear range /(ng·mL ⁻¹)	Detection Limit /(ng·mL ⁻¹)	Comment	Reference
RIA	1-700	1.0	Selective,harmful reagents	[24]
TRFD	0.1-100	0.1	Selective,long process	[25]
IS	5-150	1.5	Low Selective,Simple	[26]
CEIA-CL	5-500	0.85	Sensitive	[27]
MS	4.6-500	1.2	Accurate,expensive instruments	[28]
ELISA	9.9-300	4.9	Simple,long process	[29]
FRET	0.05-2	0.05	Sensitive,Simple	This report

Figure Captions

Scheme1. Diagram of AFP sensor based on rare earth upconversion nano materials and nano gold particles

Fig.1 Atomic force microscopy (AFM) of ucnpns

Fig.2 The height of the characterization material in AFM

Fig.3 TEM characterization of AuNPs

Fig.4 FT-IR of 6-AA and AA-UCNPs

Fig.5 Fluorescence emission spectrogram of rare earth upcnp and ultraviolet absorption spectrogram of AuNPs

Fig.6 Fluorescence spectra of rare earth upcnp quenched by AuNPs at different excitation times

Excitation time is 0,3,6,9,20,40,60,90,120,180 min from a to J. Concentration of rare earth upcnp is 40 ppm, the concentration of AuNPs is 0.02 nM, the concentration of AFP ligands is 100 nM, buffer solution: 20 mM HEPES, pH = 7.4.

Fig.7 Effect of different time on fluorescence intensity of the system without laser irradiation

The concentration of rare earth upcnp is 40 ppm, the concentration of AuNPs is 0.02 nM, the concentration of AFP ligands is 100 nM, buffer solution: 20 mM HEPES, pH = 7.4.

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719 Fig.8 Fluorescence spectra of the system with the increase of AFP concentration

720 The concentrations of AFP from a to j were 0 , 0.05 , 0.2 , 0.5, 0.8 , 1.0, 1.5, 2.0 , 5.0 ,
721 10.0 ppb. The concentration of rare earth upcnp is 3.3 ppm, the concentration of
722 AuNPs is 0.01 nM, the concentration of AFP aptamer is 50 nM, and the buffer
723 solution is 20 mM HEPES (pH = 7.4).

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725 Fig.9 The relationship between fluorescence recovery rate and AFP concentration

726 A.The linear relationship between the fluorescence recovery rate and AFP
727 concentration of the system.B. Linear relationship between the fluorescence recovery
728 rate $(f-f_0) / F_0$ and the concentration of AFP

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730 Fig.10 Results of AFP selective control experiment

731 The concentration of AFP was 2 ppb, thrombin, CEA, BSA and lysozyme were all
732 200 ppb.

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734 Tab.1 Base sequences of alpha--fetoprotein aptamer and Complementary sequence of
735 the aptamer

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737 Tab.2 Compared to the other methods for AFP

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