

Metal-enhanced fluorescent probes based on silver nanoparticles and its application in IgE detection

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Abstract In this paper, a novel metal plasmon coupled with an aptamer–nucleotide hybridized probe was fabricated and applied for protein detection. The specific aptamer and single-strand oligonucleotide were chemically bound to silver nanoparticles (AgNPs), and Cy5-labeled, complementary single-strand oligonucleotides were hybridized with the particle-bound oligonucleotides. The hybridized DNA duplexes were regarded as rigid rods that separated the fluorophore Cy5 and the surface of AgNPs to reduce the competitive quenching. Using a model system comprising human immunoglobulin E (IgE) as the analyte and goat antihuman IgE as immobilized capture antibody on glass slides, we demonstrate that the detection performance of the synthetic probe was superior to the aptamer-based fluorescent probes. The results showed a good linear correlation for human IgE in the range from 10 ng/ml to 6.25 µg/ml. The detection limit obtained was 1 ng/ml, which was 50 times lower than that using Cy5 oligonucleotide/aptamer hybrid duplex (Probe2) due to the metal-enhanced fluorescence effect. This new strategy opens the possibility for the preparation of high-sensitivity detection probes based on metal nanoparticles.

Keywords Metal-enhanced fluorescence · Silver nanoparticles · Aptamer · IgE

Introduction

Metal-enhanced fluorescence (MEF) [1–3] is a phenomenon that occurs due to the interaction of fluorophores with the plasmon resonance from metal particles, resulting in an increase in the radiative decay rate and stronger fluorescence emission. MEF is known to increase the fluorescence quantum yield while decreasing the lifetime [4]. These characteristics of the MEF effect have been exploited to increase the sensitivity of fluorescence-based sensors [5] and microarrays [6]. The MEF effect was used to develop novel metal plasmon-coupled probes which displayed increasing brightness and photostability [4]. Zhang et al. [7, 8] studied the effect of particle size, fluorophore-to-metal spacing distance, and wavelength on fluorescence behavior of the complex. On the other hand, fluorescence can also be quenched competitively by the metal if the fluorophore-to-metal spacing distance is too close. Lakowicz [4] and Gryczynski et al. [9] reported that the maximum enhancement for the fluorophore occurs at about 8 nm from the metal surface. Though numerous publications have reported on the use of silver island films for MEF, only very few used silver colloids [7, 10–12] in their studies. In fact, it is essential for the successful preparation of fluorescent molecule-modified metal nanoparticles to exactly control and keep the distance between fluorescent molecules and metallic nanoparticles [13].

Aptamers are synthetic DNA/RNA oligonucleotides obtained from random sequence nucleic acid libraries by an in vitro evolution process named systematic evolution of ligands by exponential enrichment [14, 15]. Compared with

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antibodies, aptamers are easily synthesized and have several advantages such as high affinity, chemical stability, simple modifiability, and high flexibility. Aptamers have been employed in the design of novel probes for detecting different types of analytes including small molecules [16, 17], proteins [18, 19], and cells [20, 21]. Most aptamer bioassays developed have been coupled with techniques such as electrochemistry [22], colorimetric [23], quartz crystal microbalance [24], or fluorescence [25].

In the present work, we first bind the immunoglobulin E (IgE) aptamer and a single strand of oligonucleotide to the AgNPs. Then, Cy5-labeled complementary strand oligonucleotides were hybridized onto the AgNPs. In this case, the space between the fluorophore Cy5 and the AgNPs surface was not only set at an exact distance [26] but also can provide a rigid separation distance or “rigid rod” [27]. This hybridization design was expected to overcome oligonucleotides’ bending or twisting on the surface of silver nanoparticles. IgE, the least abundant immunoglobulins in human serum, was selected as the model molecule because it plays a key role in establishing allergic reactions and other related diseases in the body [28]. Therefore, there is a need for the development of a rapid, selective, and sensitive technique to detect these low levels. Herein, a detection limit of 1 ng/ml was achieved, which was 50 times lower than Probe2 (the construction was described in “[Preparation of detection probes](#)”). This initial strategy also showed promise in the amplification of fluorescence signal on metal-enhanced effect.

Experimental

Materials and apparatus

Human IgE was purchased from US Biological (Swampscott, MA, USA). Silver nitrate and goat antihuman immunoglobulin E were bought from Sigma-Aldrich (St. Louis, MO, USA), streptavidin (SA) and human serum albumin (HSA) were obtained from Beijing Bios. Co. (Beijing, China), bovine serum albumin (BSA) was purchased from Nanjing Bookman Biotechnology Co. (Nanjing, China), sodium borohydride (NaBH_4) was purchased from Tianjin Institute of Chemical Reagents (Tianjin, China), sodium chloride (NaCl) was obtained from Nanjing Chemical Reagent Co. Ltd. (Nanjing, China), magnesium chloride hexahydrate ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$) was purchased from Xilong Chemical Engineering Co. Ltd. (Shantou, China), Tween-20 was purchased from Tianjin Kermel Co. (Tianjin, China), and aldehyde slides were obtained from Shanghai Baio Co. (Shanghai, China). All chemicals used were of analytical reagent grade, unless otherwise stated. All samples and buffer solutions were prepared using deionized water from

a Millipore system. Some 0.1 M PBS and 0.1, 0.2, and 0.3 M PBSM ($0.1 \times \text{PBS} + 0.1, 0.2, \text{ and } 0.3 \text{ M NaCl} + 1 \text{ mM MgCl}_2 \cdot 6\text{H}_2\text{O}$, respectively) were used as incubating buffer, and 0.1 M PBST ($0.1 \times \text{PBS} + 0.1 \text{ M NaCl} + 0.05\% \text{ Tween-20}$) was used as a wash buffer.

All oligonucleotides, aptamers, and PBS were purchased from Shanghai Sangon Biotechnology Co. (Shanghai, China). Their sequences used are listed as follows:

Oligo-A (5'SH-AACCCCGGCCCC-3')

Oligo-B (5'SH-AAAAAAAAAAAAAACCCCGGCCCC-3')

Oligo-C (5'SH-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAACCCCGGCCCC-3')

Oligo-D (5'SH-AAAAAAAAAAAAAAAA-biotin-3')

Oligo-E (5'SH-AAAAAAAAAAAAAAAAAAAAAAAAAAAAA-biotin-3')

Oligo1-Cy5 (5'Cy5-GGGGCCGGGGTT-3')

Oligo2-Cy5 (5'Cy5-CTGAGCTCGGTA-3')

A₁₅-Aptamer1 (5'SH-AAAAAAAAAAAAAGGGG CACGTTTATCCGTCCCTCCTAGTGGCGTGCCC-3')

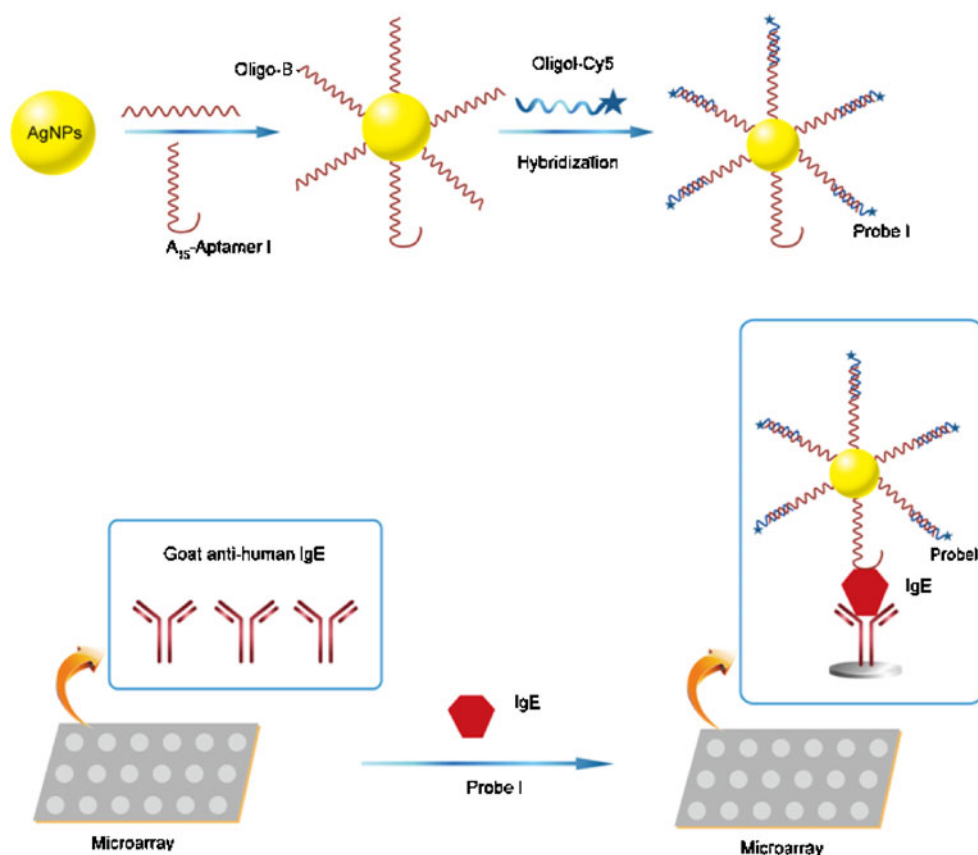
Oligo-Aptamer2 (5'-GGGGCACGTTTATCCGTCCCTCCTAGTGGCGTGCCCCTACCGAGCTCAG-3')

A Smart Array 48 arrayer and a LuxScan-10k/A scanner (CapitalBio Corporation, Beijing, China) were used to prepare microarrays and scan fluorescence images, respectively. The images were analyzed through a LuxScan3.0 software (CapitalBio Corporation). UV-vis spectra were performed on a UV-3600 spectrophotometer (Shimadzu, Japan). A transmission electron microscope (JEM-2100, Japan) was used to collect TEM images. A CT15RT desktop versatile refrigerated centrifuge (Shanghai Tianmei Scientific Instrument Co., Shanghai, China) was used for the centrifugation. A ZD-85 constant temperature shaker (Changzhou Guohua Electric Apparatus Co. Ltd., Changzhou, China) was used for the hybridization. A TS-2 orbital shaker (Haimen Kylin-Bell Lab Instruments Co. Ltd., Haimen, China) was used as the washing platform.

Preparation of silver nanoparticles

All the glass vessels were soaked in the dichromic acid solution, followed by gentle scrubbing in a soap solution, and then rinsed thoroughly with deionized water. Silver nanoparticles were prepared according to previously reported methods [29, 30]. Briefly, an aliquot of an aqueous silver nitrate solution (2 mM, 40 ml) was added dropwise into a sodium borohydride solution (3 mM, 80 ml) in an ice bath, and the mixture was continuously stirred until all the silver nitrate solution was added. Finally, an appropriate amount of sodium borohydride solution was added to make the solution become bright yellow in a hot water bath. AgNPs colloidal solution was obtained and stored at room temperature before use.

Scheme 1 Schematic illustration of the preparation of Probe1 and the fluorescence immunoassay procedure for the detection of IgE



Scheme 2 Illustration of the fabricated detection probes and control probes



Preparation of detection probes

Functionalized silver nanoparticles were prepared using the method of Sun et al. [31], with some modification; the procedure is illustrated as Scheme 1. Briefly, 1,330 μL AgNPs, 100 μL of 10 μM Oligo-B, and 33 μL of 10 μM A₁₅-Aptamer 1 (Conjugate1) were mixed and incubated for at least 18 h. To adjust the pH value and increase the ionic strength of the resulting solution, 163 μL of 1 $\times$ PBS was added to the solution and reacted for 6 h. Then, 28 μL of 2 M NaCl was added to the solution; this procedure was repeated twice at 3-h intervals so that the total NaCl concentration could be increased gradually. After 12 h, 32 μL of 2 M NaCl was added at 3-h intervals; this procedure was repeated three times to bring the final concentration of NaCl to 0.2 M. After an additional standing for at least 48 h, the resulting solution of 900 μL was isolated by centrifugation for 15 min (14 $^{\circ}\text{C}$, three times at 15,000 rpm). The precipitate was recentrifuged in 900 μL of 0.1 M PBS and finally redispersed in 450 μL of 0.2 M PBSM. Then, 450 μL of 2.2 μM Oligo1-Cy5 was hybridized with the modified AgNPs and reacted for 2 h at 37 $^{\circ}\text{C}$ with gentle shaking. The final product is the hybridized conjugate complex and is referred to as Probe1. Probe2 was prepared by adding 225 μL Oligo-Aptamer 2 into 675 μL of 1.48 μM Oligo2-Cy5 and then hybridized for 2 h with gentle shaking at 37 $^{\circ}\text{C}$. The process was carried out in the dark to avoid any fluorescence quenching. The constitution of all probes is illustrated as Scheme 2.

Preparation of other fluorescent probes

To prepare probe A (Tag-A), 1,000 μL AgNPs, 75 μL of 10 μM Oligo-A, and 25 μL of 10 μM Oligo-D was incubated for at least 18 h. To adjust the pH value and increase the ionic strength of the resulting solution, 122 μL of 1 $\times$ PBS was added to the solution and reacted for 6 h. Then, 21 μL of 2 M NaCl was added to the solution; this procedure was repeated twice at 3-h intervals so that the total NaCl concentration could be increased gradually to 0.1 M. After an additional standing for at least 24 h, 1,200 μL of this solution was isolated by centrifugation for 15 min, 14 $^{\circ}\text{C}$, three times at 15,000 rpm. The precipitate was recentrifuged in 900 μL of 0.1 M PBS and finally redispersed in 300 μL of 0.1 M PBS. Then, 300 μL of 3.3 μM Oligo1-Cy5 was added for 2 h of hybridization with gentle shaking at 37 $^{\circ}\text{C}$. The resulting complex is referred to as Tag-A. The preparation procedure of Tag-B or Tag-C was the same as above, in which Oligo-A was replaced by using Oligo-B or Oligo-C, respectively. Tag-D was prepared by using 1,000 μL AgNPs, 25 μL of 10 μM Oligo-D, and 75 μL of 10 μM Oligo-E; the operating procedure was the same as described above. The fabricated probes are illustrated as Scheme 2.

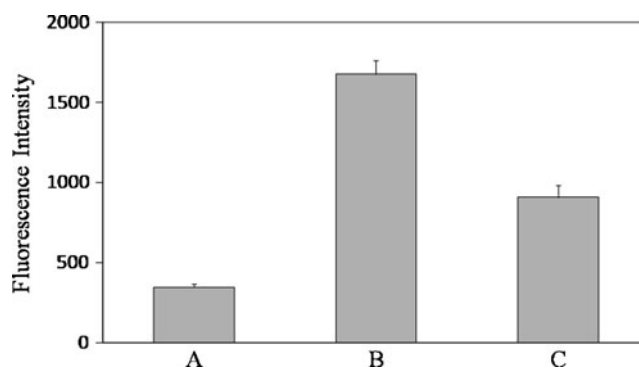


Fig. 1 Relationship between the fluorescence intensity and the spacing distance between AgNPs and Cy5 (bars A–C represent Tag-A, Tag-B, and Tag-C, respectively). The standard error bars were calculated from 24 spots

Detection of human IgE

Goat antihuman IgE (0.125 mg/ml) and BSA (1 mg/ml, used as negative controls) were spotted onto aldehyde-coated glass slides in a 4 $\times$ 4 array. The slide was incubated at 37 $^{\circ}\text{C}$ for 2 h in a humidified chamber and stored at 4 $^{\circ}\text{C}$ overnight. Before use, the slide was blocked with 25 μL of 10 mg/ml BSA for 1 h at 37 $^{\circ}\text{C}$, washed with 0.1 M PBST three times (5 min per washing) at 100 rpm, then air-dried. Each array was covered with 25 μL of different concentrations of human IgE, incubated for 1 h at 37 $^{\circ}\text{C}$, and washed with 0.1 M PBST three times (5 min per washing) at 100 rpm. Then, 25 μL Probe1 or Probe2 was applied onto the array and left for 2 h at 37 $^{\circ}\text{C}$ (Scheme 1). The microarray slide was washed with 0.1 M PBST three times (5 min per washing) at 100 rpm and then air-dried. The prepared microarray slide was then scanned with a LuxScan-10k/A scanner at 635-nm laser.

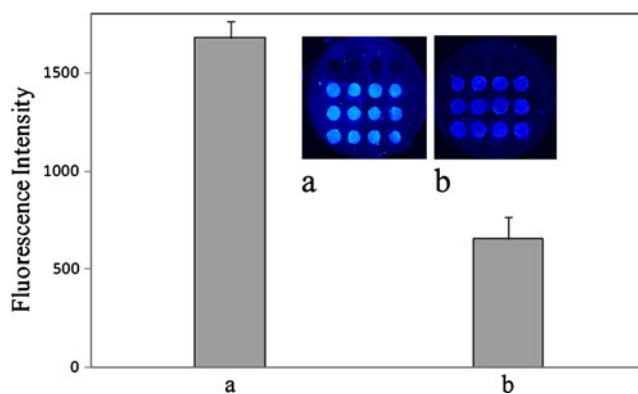


Fig. 2 Fluorescence intensity of Tag-B (bar a) and Tag-D (bar b). Insert shows the fluorescence scanning image of Tag-B and Tag-D. Each array contains four spots of 1 mg/ml BSA (used as the negative control) and 12 spots of SA. The standard error bars were calculated from 24 spots

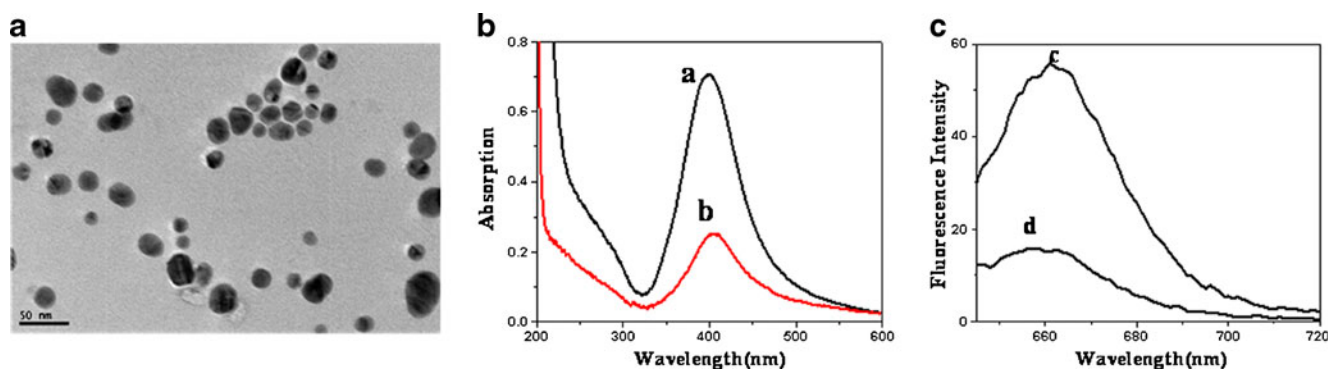


Fig. 3 **a** TEM image of Probe1 performed by adding the condensed solution of Probe1 on the carbon film. **b** UV-visible spectrum of the AgNPs (curve *a*) and Probe1 after centrifugation (curve *b*). **c**

Fluorescence emission spectrum of Probe1 before (curve *c*) and after (curve *d*) centrifugation. Each curve of UV and fluorescence represents the average of two independent experiments

Results and discussion

Optimization of modified conditions

The spacing distance between the silver nanoparticles and Cy5 plays an important role in fluorescence performance for the immunoassay [4, 7]. To further identify the influence of the distance on fluorescent probe performance, three kinds of tags (Tag-A, Tag-B, and Tag-C) were prepared; their spacing distances between Cy5 and AgNPs were 4, 8, and 13 nm, respectively (the length of three bases equals to 1 nm) [11]. In our experiment, their fluorescence intensities were observed using a fluorescent scanner. To assemble these control probes on the slides, 1 mg/ml streptavidin was first printed on the aldehyde-coated glass slides. The

slides were incubated at 37 °C for 2 h in a humidified chamber and stored at 4 °C overnight. Before use, the slide was blocked with 25 μ l 10 mg/ml BSA for 1 h at 37 °C, washed with 0.1 M PBST three times (5 min per washing) at 100 rpm, then air-dried. Of the Tag solution, 25 μ l was transferred onto the well of the slides and then incubated for 1 h at 37 °C, washed with 0.1 M PBST three times (5 min per washing) at 100 rpm, then air-dried. The Cy5-coupled AgNPs could be bound on the slides since there are biotin groups on the surface of AgNPs. The tag-fabricated slides were then scanned with a fluorescent scanner at 635-

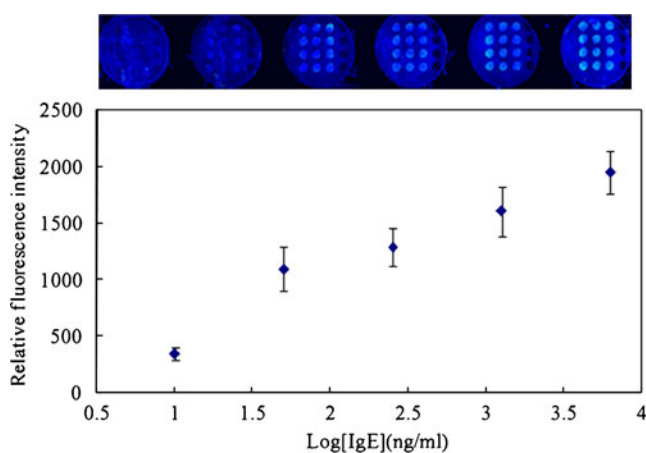


Fig. 4 Linear relationship between the concentration of human IgE in log scale and the relative fluorescence intensity using Probe1 in the range from 10 ng/ml to 6.25 μ g/ml. The standard error bars were calculated from 24 spots. Insert shows the fluorescence scanning image of Probe1 in the presence of IgE at different concentrations. These are 1 mg/ml BSA and 10 ng/ml, 50 ng/ml, 250 ng/ml, 1.25 μ g/ml, and 6.25 μ g/ml IgE, respectively (from left to right). Each array contains 12 spots of goat antihuman IgE and four spots of 1 mg/ml BSA (used as a negative control)

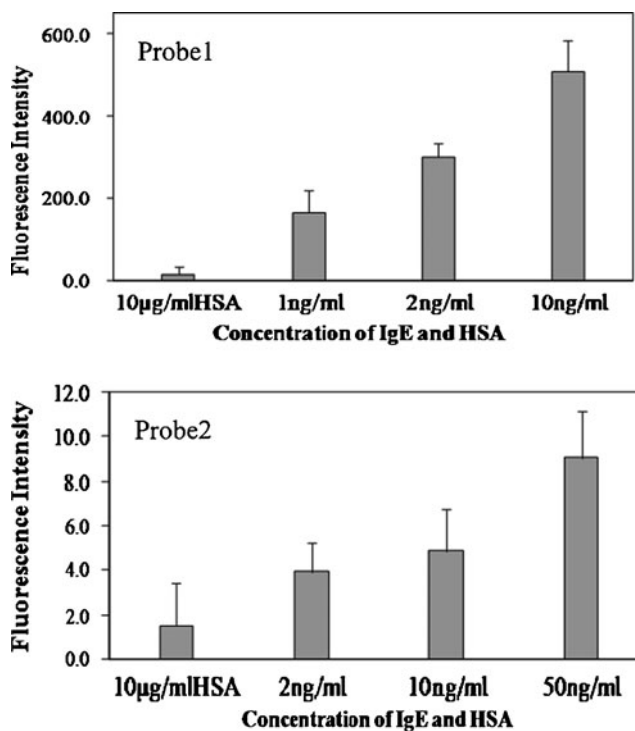


Fig. 5 Fluorescence intensity of Probe1 (top) and Probe2 (bottom) in different concentrations of IgE and 10 μ g/ml HSA. The detection limit of Probe1 and Probe2 were 1 and 50 ng/ml, respectively. The standard error bars were calculated from 24 spots

nm laser. It can be seen from Fig. 1 that the maximum fluorescence intensity belongs to Tag-B, and the result showed that the optimal distance between the AgNPs and Cy5 appears to be 8 nm. Thus, the corresponding oligonucleotide (Oligo-B) was selected to prepare the detection probes in our following experiments.

To determine the advantage of the hybrid probe design, Tag-B and Tag-D were selected for a comparison of fluorescent intensities. Figure 2 shows that the fluorescent intensity of Tag-B was about 2.5-fold higher than that of Tag-D. This suggested that the hybrid design could benefit to the metal-enhanced fluorescence effect [7, 11] as the rigid hybrid probe, Tag-B, could reduce the quenching resulting from the oligonucleotides' bending or twisting on the surface of AgNPs.

Characterization of the functionalized probes

The TEM image (Fig. 3a) showed that the mean diameter of the modified AgNPs was 20.3 ± 4.6 nm. According to the method reported by Ward Jones et al. [32], the concentration of AgNPs was calculated to be 2.5 nM.

The UV–visible absorption spectra of AgNPs (Fig. 3b, curve a) and the redispersed solution of Probe1 (Fig. 3b, curve b) showed that the yield was around 36%, so the concentration of AgNPs containing Probe1 was 0.9 nM (approximately 1.2 pmol). Furthermore, a slight red shift was observed on the maximum absorbance wavelength from AgNPs to Probe1 (from 401 to 406 nm), suggesting a small change in the size of the nanoparticles after functionalization. The fluorescence emission spectra of Probe1 before (Fig. 3c, curve c) and after centrifugation (Fig. 3c, curve d) were measured to obtain the concentration of Oligo1-Cy5 which hybridized with Conjugate1; the obtained concentration is 142 pmol (due to the concentration of Oligo1-Cy5 being more than that of Oligo-B, the concentration of Oligo-B was used to calculate the coverage of oligonucleotide assuming hybridization uniformity of Oligo1-Cy5 and Oligo-B).

The amount of Oligo1-Cy5 per silver nanoparticle was calculated theoretically. Supposing that the probability of binding on the surface of AgNPs for oligonucleotides was the same, the average coverage was calculated to be 158 oligonucleotide units per nanoparticle [33]. The calculated coverage which was higher than that in our previous paper may be attributed to the different fluorescence measurement. Moreover, the result was confirmed by the advancement of a hybrid design in Fig. 2.

Analytical performance of Probe1 and Probe2

This assay was challenged with a series of target IgE concentrations. As shown in Fig. 4, the fluorescence intensity

increased with increasing concentrations of IgE (0.01, 0.05, 0.25, 1.25, and 6.25 $\mu\text{g/ml}$). The relative fluorescence intensity (ΔF) was defined as the difference between the fluorescence intensity in the presence and absence of IgE. Figure 4 also showed a linear correlation between the relative fluorescence intensity and the concentration of human IgE in log scale. The linear curve fitted a regression equation of $\Delta F = 531.6 \log C - 20.937$. Figure 5 indicated that the limit of detection using Probe1 was 1 ng/ml (the limit of detection $\approx$ fluorescence intensity_[HSA] + 3SD_[HSA]).

To compare with ordinary fluorescent probes, Probe2 was also used to assay IgE protein samples. The detection limit of Probe2 was found to be 50 ng/ml. It showed clearly that the fluorescence intensity of Probe1 reached 180-fold relative to Probe2 due to the MEF effect. The sensitivity of our assay compares favorably with other fluorescence aptameric assays for IgE [34–36].

Conclusion

The hybridization design with Conjugate1 and Oligo1-Cy5 as metal-enhanced fluorescent tags was firstly applied for the detection of IgE. A detection limit of 1 ng/ml, which was 50 times lower than Probe2, was obtained. This aptameric assay system achieved a low detection limit, high specificity, and good reproducibility. It also demonstrated the potential applicability of this method for allergy diagnosis in the clinic. This microarray aptameric assay based on the MEF effect provided a promising approach in the study of ultralow-level protein detection.

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References

1. Lakowicz JR (2005) Radiative decay engineering 5: metal-enhanced fluorescence and plasmon emission. *Anal Biochem* 337(2):171–194. doi:10.1016/j.ab.2004.11.026
2. dos Santos DS, Aroca RF (2007) Selective surface-enhanced fluorescence and dye aggregation with layer-by-layer film substrates. *Analyst* 132(5):450–454. doi:10.1039/b618513d
3. Geddes CD, Lakowicz JR (2002) Metal-enhanced fluorescence. *J Fluoresc* 12(2):121–129
4. Lakowicz JR (2001) Radiative decay engineering: biophysical and biomedical applications. *Anal Biochem* 298(1):1–24. doi:10.1006/abio.2001.5377
5. Smith DS, Kostov Y, Rao G (2007) SPCE-based sensors: ultrafast oxygen sensing using surface plasmon-coupled emission from

- ruthenium probes. *Sens Actuator B-Chem* 127(2):432–440. doi:[10.1016/j.snb.2007.04.044](https://doi.org/10.1016/j.snb.2007.04.044)
6. Sabanayagam CR, Lakowicz JR (2007) Increasing the sensitivity of DNA microarrays by metal-enhanced fluorescence using surface-bound silver nanoparticles. *Nucleic Acids Res* 35(2):e13. doi:[10.1093/nar/gkl1054](https://doi.org/10.1093/nar/gkl1054)
7. Zhang J, Fu Y, Chowdhury MH, Lakowicz JR (2008) Single-molecule studies on fluorescently labeled silver particles: effects of particle size. *J Phys Chem C* 112(1):18–26. doi:[10.1021/jp074938r](https://doi.org/10.1021/jp074938r)
8. Zhang J, Fu Y, Chowdhury MH, Lakowicz JR (2008) Plasmon-coupled fluorescence probes: effect of emission wavelength on fluorophore-labeled silver particles. *J Phys Chem C* 112(25):9172–9180. doi:[10.1021/jp8000493](https://doi.org/10.1021/jp8000493)
9. Gryczynski I, Malicka J, Shen YB, Gryczynski Z, Lakowicz JR (2002) Multiphoton excitation of fluorescence near metallic particles: enhanced and localized excitation. *J Phys Chem B* 106(9):2191–2195. doi:[10.1021/jp013013n](https://doi.org/10.1021/jp013013n)
10. Fu Y, Zhang J, Lakowicz JR (2007) Plasmonic enhancement of single-molecule fluorescence near a silver nanoparticle. *J Fluoresc* 17(6):811–816. doi:[10.1007/s10895-007-0259-0](https://doi.org/10.1007/s10895-007-0259-0)
11. Zhang J, Fu Y, Chowdhury MH, Lakowicz JR (2007) Metal-enhanced single-molecule fluorescence on silver particle monomer and dimer: coupling effect between metal particles. *Nano Lett* 7(7):2101–2107. doi:[10.1021/nl071084d](https://doi.org/10.1021/nl071084d)
12. Zhang J, Fu Y, Liang D, Zhao RY, Lakowicz JR (2009) Fluorescent avidin-bound silver particle: a strategy for single target molecule detection on a cell membrane. *Anal Chem* 81(3):883–889. doi:[10.1021/ac801932m](https://doi.org/10.1021/ac801932m)
13. Bharadwaj P, Anger P, Novotny L (2007) Nanoplasmonic enhancement of single-molecule fluorescence. *Nanotechnology* 18(4):044017. doi:[10.1088/0957-4484/18/4/044017](https://doi.org/10.1088/0957-4484/18/4/044017)
14. Ellington AD, Szostak JW (1990) In vitro selection of rna molecules that bind specific ligands. *Nature* 346(6287):818–822. doi:[10.1038/346818a0](https://doi.org/10.1038/346818a0)
15. Tuerk C, Gold L (1990) Systematic evolution of ligands by exponential enrichment—RNA ligands to bacteriophage-T4 DNA-polymerase. *Science* 249(4968):505–510. doi:[10.1126/science.2200121](https://doi.org/10.1126/science.2200121)
16. Liu JW, Lu Y (2006) Fast colorimetric sensing of adenosine and cocaine based on a general sensor design involving aptamers and nanoparticles. *Angew Chem-Int Edit* 45(1):90–94. doi:[10.1002/anie.200502589](https://doi.org/10.1002/anie.200502589)
17. Zuo XL, Song SP, Zhang J, Pan D, Wang LH, Fan CH (2007) A target-responsive electrochemical aptamer switch (TREAS) for reagentless detection of nanomolar ATP. *J Am Chem Soc* 129(5):1042–1043. doi:[10.1021/ja067024b](https://doi.org/10.1021/ja067024b)
18. Lin CX, Katilius E, Liu Y, Zhang JP, Yan H (2006) Self-assembled signaling aptamer DNA arrays for protein detection. *Angew Chem-Int Edit* 45(32):5296–5301. doi:[10.1002/anie.200600438](https://doi.org/10.1002/anie.200600438)
19. Lai RY, Plaxco KW, Heeger AJ (2007) Aptamer-based electrochemical detection of picomolar platelet-derived growth factor directly in blood serum. *Anal Chem* 79(1):229–233. doi:[10.1021/ac061592s](https://doi.org/10.1021/ac061592s)
20. Kang WJ, Chae JR, Cho YL, Lee JD, Kim S (2009) Multiplex imaging of single tumor cells using quantum-dot-conjugated aptamers. *Small* 5(22):2519–2522. doi:[10.1002/sml.200900848](https://doi.org/10.1002/sml.200900848)
21. Farokhzad OC, Jon SY, Khademhosseini A, Tran TNT, LaVan DA, Langer R (2004) Nanoparticle–aptamer bioconjugates: a new approach for targeting prostate cancer cells. *Cancer Res* 64(21):7668–7672
22. Feng K, Kang Y, Zhao JJ, Liu YL, Jiang JH, Shen GL, Yu RQ (2008) Electrochemical immunosensor with aptamer-based enzymatic amplification. *Anal Biochem* 378(1):38–42. doi:[10.1016/j.ab.2008.03.047](https://doi.org/10.1016/j.ab.2008.03.047)
23. Zhang J, Wang LH, Pan D, Song SP, Boey FYC, Zhang H, Fan CH (2008) Visual cocaine detection with gold nanoparticles and rationally engineered aptamer structures. *Small* 4(8):1196–1200. doi:[10.1002/sml.200800057](https://doi.org/10.1002/sml.200800057)
24. Liss M, Petersen B, Wolf H, Prohaska E (2002) An aptamer-based quartz crystal protein biosensor. *Anal Chem* 74(17):4488–4495. doi:[10.1021/ac011294p](https://doi.org/10.1021/ac011294p)
25. Ho HA, Najari A, Leclerc M (2008) Optical detection of DNA and proteins with cationic polythiophenes. *Accounts Chem Res* 41(2):168–178. doi:[10.1021/ar700115t](https://doi.org/10.1021/ar700115t)
26. Macfarlane RJ, Jones MR, Senesi AJ, Young KL, Lee B, Wu JS, Mirkin CA (2010) Establishing the design rules for DNA-mediated colloidal crystallization. *Angew Chem-Int Edit* 49(27):4589–4592. doi:[10.1002/anie.201000633](https://doi.org/10.1002/anie.201000633)
27. Hill HD, Macfarlane RJ, Senesi AJ, Lee B, Park SY, Mirkin CA (2008) Controlling the lattice parameters of gold nanoparticle FCC crystals with duplex DNA linkers. *Nano Lett* 8(8):2341–2344. doi:[10.1021/nl8011787](https://doi.org/10.1021/nl8011787)
28. Gould HJ, Sutton BJ (2008) IgE in allergy and asthma today. *Nat Rev Immunol* 8(3):205–217. doi:[10.1038/nri2273](https://doi.org/10.1038/nri2273)
29. Munro CH, Smith WE, Garner M, Clarkson J, White PC (1995) Characterization of the surface of a citrate-reduced colloid optimized for use as a substrate for surface-enhanced resonance Raman-scattering. *Langmuir* 11(10):3712–3720
30. Li H, Sun ZY, Zhong WY, Hao N, Xu DK, Chen HY (2010) Ultrasensitive electrochemical detection for DNA arrays based on silver nanoparticle aggregates. *Anal Chem* 82(13):5477–5483. doi:[10.1021/ac101193e](https://doi.org/10.1021/ac101193e)
31. Sun ZY, Qiang WB, Li H, Hao N, Xu DK, Chen HY (2011) Electric detection of DNA with PDMS microgap electrodes and silver nanoparticles. *Analyst* 136(3):540–544. doi:[10.1039/c0an00512f](https://doi.org/10.1039/c0an00512f)
32. Ward Jones SE, Campbell FW, Baron R, Xiao L, Compton RG (2008) Particle size and surface coverage effects in the stripping voltammetry of silver nanoparticles: theory and experiment. *J Phys Chem C* 112(46):17820–17827. doi:[10.1021/jp807093q](https://doi.org/10.1021/jp807093q)
33. Liu CH, Li ZP, Du BA, Duan XR, Wang YC (2006) Silver nanoparticle-based ultrasensitive chemiluminescent detection of DNA hybridization and single-nucleotide polymorphisms. *Anal Chem* 78(11):3738–3744. doi:[10.1021/ac0522409](https://doi.org/10.1021/ac0522409)
34. Cho EJ, Collett JR, Szafranska AE, Ellington AD (2006) Optimization of aptamer microarray technology for multiple protein targets. *Anal Chim Acta* 564(1):82–90. doi:[10.1016/j.aca.2005.12.038](https://doi.org/10.1016/j.aca.2005.12.038)
35. He JL, Wu ZS, Zhang SB, Shen GL, Yu RQ (2009) Novel fluorescence enhancement IgE assay using a DNA aptamer. *Analyst* 134(5):1003–1007. doi:[10.1039/b812450g](https://doi.org/10.1039/b812450g)
36. Stadtherr K, Wolf H, Lindner P (2005) An aptamer-based protein biochip. *Anal Chem* 77(11):3437–3443. doi:[10.1021/ac0483421](https://doi.org/10.1021/ac0483421)