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Label-free sensitive luminescence biosensor for immunoglobulin G based on Ag_6Au_6 ethisterone cluster-estrogen receptor α aggregation and graphene

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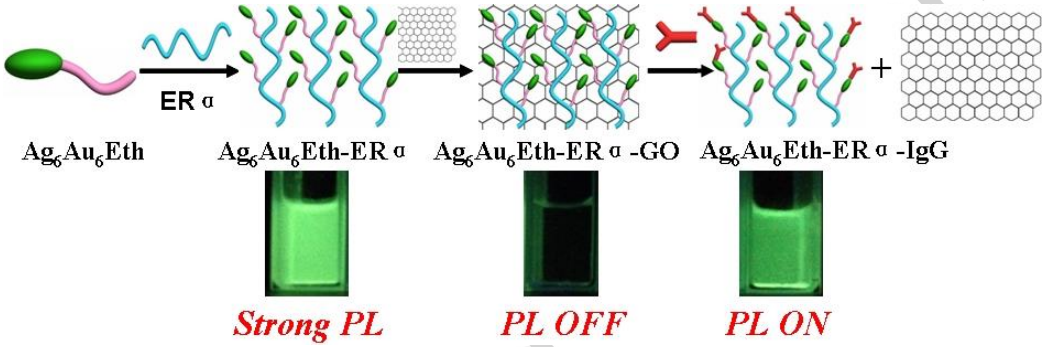
Abstract

A specific and label-free "on-off-on" luminescence biosensor based on a novel heterometallic cluster [Ag_6Au_6(ethisterone)$_{12}$]-estrogen receptor α (Ag_6Au_6Eth-ERα) aggregation utilizing graphene oxide (GO) as a quencher to lead a small background signal was firstly constructed to detect immunoglobulin G (IgG) with a simple process and high selectivity. The efficient photoluminescent (PL) Ag_6Au_6Eth-ERα aggregation is strongly quenched by GO. In the presence of IgG, the PL of this system will be restored, and perceivable by human eyes under

UV lamp excitation (365 nm). The quenching mechanism of GO on Ag_6Au_6Eth-ERα and enhancement mechanism of IgG on Ag_6Au_6Eth-ERα-GO were investigated in detail. Under the optimum conditions, the biosensor for high sensitive IgG detection expressed a wider linear range of 0.0078 -10 ng / mL and a lower detection limit of 0.65 pg / mL with good stability and repeatability, which provided a new approach for label-free IgG detection.

Graphical abstract



Keywords: Immunoglobulin G, Ag_6Au_6 ethisterone cluster-estrogen receptor α aggregation, Graphene, Label-free luminescence biosensor

1. Introduction

Luminescent heterometallic gold(I)–M (M=Cu(I) or Ag(I)) acetylide clusters have attracted great attention over the last few decades because of both rich structural diversity and remarkable photoluminescent (PL) properties, such as long emission lifetime, high quantum yield, modifiable PL color as well as lower excitation energy [1–3]. As of now, the synthesis and application of gold(I)–M clusters as optical materials such as LEDs has been frequently reported. However, the use of luminescence gold(I)–M (M= Cu(I) or Ag(I)) acetylide clusters as biosensors are extremely scarce [4–6].

Recently, we found that the $[Ag_6Au_6(ethisterone)_{12}]$ (Ag_6Au_6Eth) can specifically bind to estrogen receptor α (ERα) through the estradiol moieties of the Ag_6Au_6Eth cluster to form strong PL Ag_6Au_6Eth-ERα aggregation, which was used as a sensitive and specific probe for ERα detection [7]. It is known that ERα is a member of the nuclear receptor super-family of ligand-activated transcription factors and is widely expressed in most tissue types including most immune cells [8]. ERα has prominent effects on immune function in both the innate and adaptive immune responses. However, the role of ERα in the immune response and the interactions between ERα and immunoglobulins are poorly understood owing to the lack of easily measurable output signal [9-11].

Immunoglobulins play an important role on the immune system. Human immunoglobulin G (IgG) is the most versatile immunoglobulin, which has been widely used in biotechnology and medicine [12−14]. Because IgG can serve as indicator of many autoimmune diseases related to, including insulin-dependent diabetes mellitus and systemic lupus erythematosus, many clinical-related applications demand sensitive biosensors for detecting of IgG [15]. So far the assays for IgG are mainly traditional immunoassays such as enzyme-linked immunosorbent assay (ELISA)

[16], radioioimmunoassays [17], chemiluminescence immunoassay [18], and electrochemical immunoassay [19]. However, the operations of these methods were complex and the results were affected by factors such as washing, clotting, and labeling. Recently, with the development of nanoscience and nanotechnology, various nanoparticles such as carbon nanotube, carbon sphere, graphene and gold nanoparticles have been applied as labels in nanoparticle-based amplification platforms which can dramatically increase the signal intensity of luminescence or electrochemical biosensor and lead to ultrasensitive bioassays for IgG [20-28]. Furthermore, several optical biosensors based on surface plasmon resonance (SPR) and surface-enhanced Raman scattering (SERS) assays have been developed to overcome disadvantages of traditional methods for sensing IgG [29–32]. Although great achievements have been obtained in this field, it is still a challenge to fabricate novel biosensors using new materials to achieve sensitive, fast and facile detection of IgG.

In this paper, Ag_6Au_6Eth-ERα aggregation as a luminophore, and graphene oxide (GO) as a quencher were first developed as a novel label-free "on-off-on" PL switch biosensor for high sensitive detection of IgG. As depicted in Scheme 1, Ag_6Au_6Eth cluster was specifically bonded to ERα through the estradiol moieties to form a new multifunctional Ag_6Au_6Eth-ERα aggregation [7], which not only exhibited a strong PL signal as the first "signal-on" state, but also provided the active sites for GO interaction. Then, GO as a quencher was introduced to lead a small background signal through the energy-transfer or electron-transfer processes [33-35] from Ag_6Au_6Eth-ERα aggregation to GO for the "signal-off" state. Finally, in the presence of IgG, Ag_6Au_6Eth-ERα aggregation was stripped away the surface of GO to obtain a significant enhancement of PL intensity which achieved a "signal on" state. Moreover, the PL changes of the proposed biosensor with the different IgG concentration could be perceivable by human eyes

under UV lamp excitation (365 nm). Based on these, the proposed biosensor displays excellent analytical performance for the detection of IgG from 0.0078 to 10 ng / mL with a detection limit of 0.65 pg / mL based on 3 times of the ratio of signal-to-noise (S/N = 3). The present designed strategy definitely paves a way for the wide application of Ag_6Au_6Eth-ERα aggregation in biological field.

2. Experimental section

2.1. Chemicals

Graphite powder of analytical grade was purchased from Sinopharm Chemical Reagent Co. Ltd (China). Graphene oxide (GO) was prepared from natural graphite powder by a modified Hummer's method [33, 36]. Estrogen receptor α (ERα) and human immunoglobulin G (IgG) were purchased from J&K Chemical. Human immunoglobulin G-labeled phycoerythrin (IgG / PE, 1 mg / L) was from ShiFeng biological technology Co. Ltd (shanghai). Cluster $[Ag_6Au_6(\text{ethisterone})_{12}]$ (Ag_6Au_6Eth) was prepared according to the published procedures [7]. Ultrapure water obtained from a Millipore water purification system (18 MΩ cm resistivity) was used in all runs. All other reagents were of analytical grade. Standard protein solutions were prepared by serial dilution starting with 0.05 mol/L phosphate buffer (PBS, pH 7.3). Unless otherwise stated, spectroscopic experiments were performed in the CH_3CN–PBS solution (1 / 1, v / v, pH 7.3).

2.2. Apparatus

PL spectra and fluorescence anisotropy measurements at room temperature were determined on a FLS920 fluorescence spectrometer (Edinburgh Instruments Ltd., U. K.). The reported

anisotropy values are an average one in four independent measurements. UV–vis absorption spectra in solutions were measured on a Perkin-Elmer Lambda 950 UV–Vis spectrometer.

The quantitative analysis was carried out based on the increased ratio of PL intensity [$(I - I_R) / I_R$], where I_R and I were PL intensity of sensing system in the absence and presence of IgG, respectively.

2.3. Preparation of the Luminescence Biosensor for IgG

The preparation of the luminescent biosensor for IgG is simply summarized as follows. First, a series of PBS buffer (2 μL) with different concentrations of ERα (0 to 1600 pg / L) were incubated with the CH_3CN–PBS solution (1 mL, 1/1, v/v, pH 7.3) of Ag_6Au_6Eth cluster (18 μM) in 4℃ for 3 hours. Then, GO was added to the above solution with vigorously stirring for 2 hours at room temperature prior to the addition of IgG. The final IgG concentration in samples ranged from 0 to 10 ng / mL. After allowing this mixture to stand for about 50 min at room temperature, the PL spectra of the mixture were measured.

2.4. Monitoring the Interactions Between IgG and Ag_6Au_6Eth-ERα Aggregation.

A PBS buffer solution of ERα (2 μL, 400 ng/L) was incubated with the CH_3CN–PBS solution (1 mL, 1/1, v/v) of the Ag_6Au_6Eth cluster (18 μM) in 4℃ for 3 hours. Then, 2 μL of PBS buffer of IgG / PE (625 ng / mL) was added to the above solution to stand for 50 min at room temperature. The fluorescence anisotropy, UV−vis, and PL spectra of the mixture were measured.

3. Results and discussion

3.1. Feasibility of the Luminescence Biosensor

The PL spectra and the corresponding luminescent images of the sensing system under UV lamp excitation (365 nm) were depicted in Fig. 1. Strong PL ("signal-on" state) is detected in the solution of Ag_6Au_6Eth-ERα aggregation (see curve 1 in Fig. 1), and the corresponding green PL of the system under UV lamp excitation (365 nm) is very bright (see 1 in the inset of Fig. 1). However, very weak PL ("signal-off" state) can be detected in the presence of GO (see curve 2 in Fig. 1), and the corresponding green PL is not perceivable by human eyes (see 2 in the inset of Fig. 1). Interestingly, PL intensities of the sensing system are continuously increased ("signal on" state) with the increase of IgG concentration (see curve 3-7 in Fig. 1), and the corresponding green PL becomes brighter, perceivable by human eyes (see 3-7 in the inset of Fig. 1). On the basis of these observations, the proposed approach could be a new efficient "on-off-on" PL switch platform and applied to high sensitive detection of IgG.

3.2. Optimization of Experimental Conditions

The effect of ERα concentration was studied first (Shown in Fig. 2A). The increased ratio of PL intensity [defined as $(I - I_R) / I_R$, where I_R and I were PL intensity of sensing system in the absence and presence of IgG (10 ng / mL), respectively] is enhancing with increasing the concentration of ERα in the range of 0−0.8 ng / L, and then reached the maximum value at 0.8 ng / L. Therefore, 0.8 ng / L ERα were used in the subsequent experiments.

Furthermore, it was found that the concentration of GO strongly influenced on PL intensity of Ag_6Au_6Eth-ERα aggregation. Fig. 2B shows that the PL intensity of Ag_6Au_6Eth-ERα aggregation dramatically decreases with increasing the concentration of GO. The quenching efficiency [Q_E, (%)] of the GO calculated by the formula $(1- \beta) \times 100\%$, where β is the ratio of PL of the solution with GO to that without GO] is sharply increasing with increasing the concentration of GO, and then reaches the maximum value to be 94% at 1.0 μg / mL of GO

(Shown in the inset of Fig. 3B). The higher quenching efficiency would lead to a higher signal-to-background ratio and better sensitivity for IgG. Thus, 1.0 μg / mL GO was used in the subsequent experiments.

3.3. Luminescence Biosensor for IgG Detection

Under optimal conditions, PL of the sensing system with different concentrations of IgG was shown in Fig. 3A. PL intensity of the sensing system gradually increased with increasing the concentration of IgG. As shown in Fig. 3B, the increased ratio of PL intensity $[(I - I_R) / I_R]$ was linear with the logarithm of the IgG concentration (log C_{IgG}) in the range of 0.0078−0.25 ng / mL with the regression equation $(I - I_R) / I_R = 2.114+1.003 \log C_{IgG}$ ($R^2 = 0.9952$), where I_R and I are PL intensity of Ag_6Au_6Eth-ERα-GO aggregation in the absence and presence of IgG, and R is the regression coefficient. With further increasing the concentrations of IgG to 10 ng / mL (Fig. 4C), the increased ratio of PL intensity $[(I - I_R) / I_R]$ was linear with the concentration of IgG with the regression equation $(I - I_R) / I_R = 1.214 + 1.272 C_{IgG}$ ($R^2 = 0.9993$). The detection limit for IgG was 0.65 pg / mL based on 3 times of the ratio of signal-to-noise (S/N = 3). It is noted that this label-free method for detection of IgG is simpler and rapider than those reported traditional immunoassay methods [16-19], and the detection limit of this method is lower than those reported biosensors based on new nanomaterials (Table 1) [18, 23-29].

In order to evaluate the selectivity and specificity of the proposed biosensor for IgG, other substances of human blood, such as trypsin, carcino-embryonic antigen (CEA), the hemoglobin, alpha fetoprotein (AFP), ovalbumin, bovine serum albumin (BSA), and albumin, were used as the interferences. At the identical concentration (10 ng / mL), only the presence of IgG gave a remarkable enhancement PL (Fig. 4A), which indicated that our proposed method would possess a high selectivity for IgG over other substances in human blood. PL intensity of 10 ng / mL pure

IgG was compared to that of containing additional interference (10 μg / mL) (Fig. 4B). The result suggests that our proposed luminescence biosensor would be expected to provide a simple, sensitive, and selective approach for IgG detection in real samples.

3.4. Principle of the Luminescence Biosensor for IgG.

The principle of the luminescence biosensor for IgG detection was first studied by an electronic absorption studies. As shown in Fig. 5A, the UV−vis spectrum of the Ag_6Au_6Eth cluster (18 μmol / L) in the CH_3CN–PBS solution (1 / 1, v / v, pH 7.3) at room temperature shows an intense band at 237 nm, and two weak bands at ca. 324 nm, and 391 nm, respectively, which are ascribed to $^1[\pi\rightarrow\pi^*]$ transitions of ethisterone ligand, and ligand-to-metal charge transfer (LMMCT) or cluster-centered (CC), respectively [2, 7]. Then, addition of ERα (0.8 ng / L) to the above Ag_6Au_6Eth solution, the Ag_6Au_6Eth-ERα aggregations were produced through estradiol moieties of the Ag_6Au_6 cluster specifically bonding to ERα with a large Hill coefficient (1.242) and binding constant (4.1×10^8) [7], and leaded to an obvious hyperchromism of the absorption spectra (curve a). Further, upon addition of GO (1.0 μg / mL) to the solution of Ag_6Au_6Eth-ERα aggregations, a large hypochromism with a slight bathochromic shift of the absorption spectra (curve b) was observed. The hypochromism of the characteristic absorption at 318 and 388 nm was up to 80 % and 92 %, respectively, but the red-shift is within 4 nm, indicating the strong adsorption of Ag_6Au_6Eth-ERα aggregation on the surface of GO [33]. However, addition of GO (1.0 μg / mL) to the solution of Ag_6Au_6Eth cluster, there are almost no changes found in the absorption and emission spectra, indicating no interaction between Ag_6Au_6Eth cluster and GO. Therefore, on the basis of these observations together with the corresponding literature [33, 37-38], it is likely that the adsorption of Ag_6Au_6Eth-ERα aggregation on the surface of GO is via π-π interaction between the carbon π-plane of GO and

aromatic ammonic acid residues of ERα surface. Finally, adding of different concentrations of IgG (0.0078−10 ng / mL) to the solution of Ag_6Au_6Eth-ERα-GO resulted in a gradually hyperchromicity of the absorption band at 322 nm and 388 nm, and a significant blue-shift (10 nm) of the absorption band at 322 nm is observed in Fig. 5B. These results indicated that a new Ag_6Au_6Eth-ERα-IgG aggregation might be formed in the presence of IgG, and resulted in the desorption of Ag_6Au_6Eth-ERα aggregation from the surface of GO [33, 38].

In order to further understand how Ag_6Au_6Eth, ERα and GO interact with IgG, the fluorescence anisotropy measurements are used to investigate molecular interactions [39]. As shown in Fig. 6, the fluorescence anisotropy of Ag_6Au_6Eth-ERα aggregation in the buffer was 0.042, and it increased 7.36-fold upon addition of GO, indicating that Ag_6Au_6Eth-ERα aggregation was strongly adsorbed on the GO surface to form Ag_6Au_6Eth-ERα-GO aggregation [33]. Further addition of IgG, the fluorescence anisotropy decreased to be 0.078, compared to that free Ag_6Au_6Eth-ERα aggregation upon addition of IgG (0.065). This result further confirmed that a new Ag_6Au_6Eth-ERα-IgG aggregation could be formed, which was desorbed from the GO surface.

In order to further study the interaction between Ag_6Au_6Eth-ERα aggregation and IgG, the UV−vis and PL spectra of the CH_3CN–PBS buffer solution (1/1, v/v, pH 7.3) of the cluster Ag_6Au_6Eth and Ag_6Au_6Eth–Erα aggregation in the presence of IgG/PE (1.25 ng / mL) were also investigated (Fig. 7). Addition of IgG/PE to the solution of Ag_6Au_6Eth and Ag_6Au_6Eth–ERα aggregation, respectively, shows a distinct diminishment of the absorptions at 300−445 nm from LMMCT or CC of Ag_6Au_6Eth cluster, and a growth of the absorptions at 450−600 nm from IgG/PE with a slight blue shift of *ca.* 15 nm (Fig. 7A). It is likely that IgG/PE would interact with the Ag_6Au_6Eth cluster or Ag_6Au_6Eth–ERα aggregation to form Ag_6Au_6Eth–IgG/PE

aggregation or Ag_6Au_6Eth–ERα–IgG/PE aggregation and the absorption of the aggregates of IgG/PE would contribute toward the blue shifts [38]. It is worthwhile to note the growth of the absorptions at 450−600 nm in the solution of Ag_6Au_6Eth-ERα aggregation is greater than that of the Ag_6Au_6Eth upon addition of IgG/PE. This result suggests the possibility of the weaker interaction between IgG/PE and the Ag_6Au_6Eth than that between IgG/PE and Ag_6Au_6Eth-ERα aggregation. Meanwhile, a diminishment of the PL intensity at 510 nm derived from an excited state of ^{3}IL (ethisterone intraligand) / ^{3}LLCT / ^{3}MMLCT (Ag_6Au_6→ethisterone) / ^{3}MC (Ag_6Au_6 cluster-centered) origin [7], and a concomitant significant growth of an emission band at 574 nm from ^{3}IL emission originating from IgG/PE (Fig. 7B) were found in the presence of IgG/PE (1.25 ng / mL). Besides, addition of IgG / PE to the solution of Ag_6Au_6Eth-ERα aggregation leaded to an 18.8-fold increase of the emission intensity at 574 nm, which was higher than that of the Ag_6Au_6Eth (14.0-fold). An obvious spectral overlap between the emission spectrum of the Ag_6Au_6Eth or Ag_6Au_6Eth–ERα aggregation and the absorption spectrum of IgG / PE is anticipated, and thus, it is likely that Förster resonance energy transfer (FRET) occurs upon addition of IgG / PE [40]. These results further indicate that the interaction between IgG / PE and Ag_6Au_6Eth-ERα aggregation would be stronger than that between IgG / PE and the Ag_6Au_6Eth, and the FRET from Ag_6Au_6Eth to IgG / PE on Ag_6Au_6Eth-ERα-IgG / PE aggregation is more effective than that on Ag_6Au_6Eth-IgG/PE aggregation in the buffer solution.

4. Conclusion

In summary, a novel label-free “on-off-on” luminescence biosensor for IgG detection based on Ag_6Au_6Eth-ERα aggregation and GO has been developed with a simple process. The proposed

biosensor displays good stability, high sensitivity and selectivity to target IgG molecule even in the presence of other interfering proteins. This label-free luminescence biosensor is more attractive than labeling detection because target molecules can be detected in their nature forms. Truly portable point-of-care testing is possible, and quantitative measurement of molecule interaction is allowable. This designed biosensor could be become a promising approach for broad application in biological field. Furthermore, the present strategy definitely paves a way for the wide application of Ag_6Au_6Eth-ERα aggregation in biological field.

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Scheme 1. Schematic label-free "on-off-on" PL biosensor for IgG based on Ag_6Au_6Eth-ERα aggregation and GO.

Figure 1. PL spectra of (1) the CH_3CN–PBS solution (1/1, v/v, pH 7.3) of Ag_6Au_6Eth (18 μM) and ERα (0.8 ng / L), (2) (1) + GO (1.0 μg / mL), and (3-7) (1) + IgG (0.0078，0.125，2.5，6.3, and 10 ng / mL, respectively). Inset is the corresponding PL photos under UV lamp excitation (365 nm).

Figure 2. (A) The effect of ERα concentration on the increased ratio $[(I - I_R) / I_R]$ of PL intensity, where I_R and I were PL intensity of sensing system without and with IgG (10 ng / mL), respectively. (B) PL spectra of the CH_3CN–PBS solution (1 / 1, v / v, pH 7.3) of Ag_6Au_6Eth (18 μM) and ERα (0.8 ng/L) in the presence of 0, 0.13, 0.25, 0.5, 1.0, 2.0, and 5.0 μg / mL GO (top to bottom). Inset: quenching efficiency [Q_E, (%)] *vs* the GO concentration.

Figure 3. (A) PL intensity curves of the CH_3CN–PBS solution (1 / 1, v / v, pH 7.3) of Ag_6Au_6Eth (18 μM), ERα (0.8 ng / L) and GO (1.0 μg / mL) with different IgG concentrations. Inset: the curve of the increased ratio of PL intensity ($(I - I_R) / I_R$) as a function of IgG concentration (C_{IgG}). I_R and I are PL intensity of the solution without and with IgG at 510 nm, respectively. (B) and (C) Calibration curve. IgG concentrations 1–18: 0; 0.0078; 0.0156; 0.0313; 0.0625; 0.125; 0.25; 0.50; 0.67; 1.00; 1.25; 2.50; 3.80; 5.00; 6.30; 7.70; 9.10; 10 ng / mL.

Figure 4. (A) Selectivity and (B) specificity of the proposed biosensor to IgG, trypsin, CEA, hemoglobin, AFP, ovalbumin, BSA, and albumin. Conditions: Ag_6Au_6Eth: 18 μM, ERα: 0.8 ng / L, GO: 1.0 μg / mL.

Figure 5. (A) Changes of the electronic absorption of (a) Ag_6Au_6Eth (18 μM) + ERα (0.8 ng / L), (b) (a) + GO (1.0 μg / mL), (c) (b) + IgG (10 ng / mL) in the CH_3CN–PBS solution (1 / 1, v / v, pH 7.3) at room temperature. (B) Changes of the electronic absorption of Ag_6Au_6Eth (18 μM) + ERα (0.8 ng / L) with different IgG concentrations. IgG concentrations from bottom to top: 0; 0.0078; 0.0156; 0.0313; 0.0625; 0.25; 0.67; 1.25; 3.80; 6.30; 9.10; 10 ng / mL.

Figure 6. Changes of fluorescence anisotropy of (a) Ag_6Au_6Eth (18 μM), (b) (a) + ERα (0.8 ng / L), (c) (b) + GO (1.0 μg / mL), (d) (c) + IgG (10 ng / mL), (e) (b) + IgG (10 ng / mL), (f) (a) + IgG (10 ng / mL).

Figure 7. (A) UV−vis and (B) PL spectra of the cluster Ag_6Au_6Eth and Ag_6Au_6Eth-ERα aggregation in the CH_3CN–PBS buffer solution (1 / 1, v / v, pH 7.3) in the presence of IgG/PE. Conditions: Ag_6Au_6Eth: 18 μM, ERα: 0.8 ng / L, IgG/PE (1.25 ng / mL).

Table 1. Comparisons of this work with those relevant reported biosensors based on new nanomaterials.

Methods	Probes	Linear range (ng / mL)	Detection limit (pg / mL)	Reference.
Chemiluminescence immunoassay	gold nanoparticle	1.0 − 40	520	[18]
Electrochemical immunoassay	gold nanoparticles	0.1 − 100	80	[23]
Electrochemical immunoassay	Au@Ag nanorods	1×10^{2} − 1×10^{6}	6900	[24]
Electrochemical immunoassay	PDDA-G and AuNPs	0.1 − 200	50	[25]
Electrochemical immunoassay	AuNP/C hybrid material	0.01 − 250	5.6	[26]
Electrochemical immunoassay	HRP-anti-IgG-MP-ZnO nanolabel	0.01 − 200	4	[27]
Photonic crystals	Nanoporous hydrogel	0.5×10^{6} − 10×10^{6}	5×10^{8}	[28]
Surface-enhanced Raman scattering	gold nanospheres	0 − 100	10^{3} − 10^{4}	[29]
Luminescence	Ag_6Au_6-ERα-GO	0.0078 − 10	0.65	This work

Highlights

(1) A specific and label-free "on-off-on" luminescence biosensor for ultrasensitive detection of human immunoglobulin G (IgG) has been proposed by the combination of heterometallic cluster [Ag_6Au_6(ethisterone)$_{12}$]-estrogen receptor α (Ag_6Au_6Eth-ERα) and graphene oxide (GO) with a simple process. The biosensor displays excellent analytical performance for detection of IgG from 0.0078 to 10 ng / mL with a detection limit of 0.65 pg / mL based on 3σ.

(2) Besides, the brightness of the proposed biosensor under UV lamp excitation (365 nm), perceivable by human eyes, is dependent on the IgG concentration. This labelfree luminescence biosensor is more attractive than labelling detection because target molecules can be detected in their nature forms. Truly portable point-of-care testing is possible, and quantitative measurement of molecule interaction is allowable.

(3) Although many luminescent heterometallic gold(I)-M (M = Cu(I) or Ag(I)) alkynyl clusters are reported in the literatures, the current Ag_6Au_6 cluster, to the best of our knowledge, is the first case that function as sensitive biosensor for IgG.

(4) This designed biosensor could be become a promising approach for broad application in biological field. Furthermore, the present strategy definitely paves a way for the wide application of Ag_6Au_6Eth-ERα aggregation in biological field.

Scheme 1

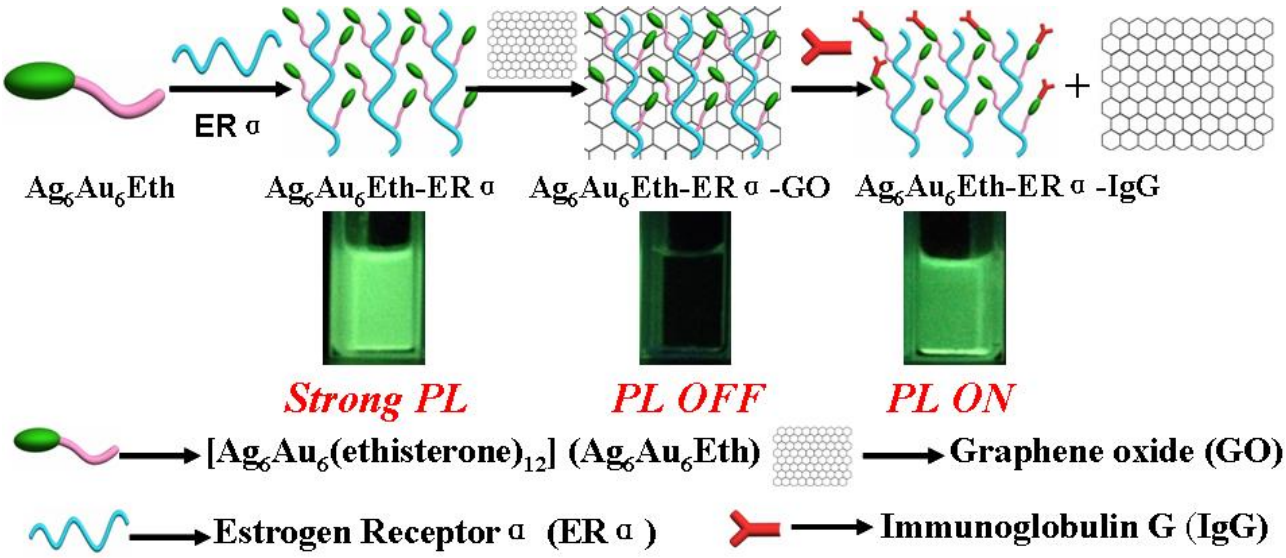

Figure 1

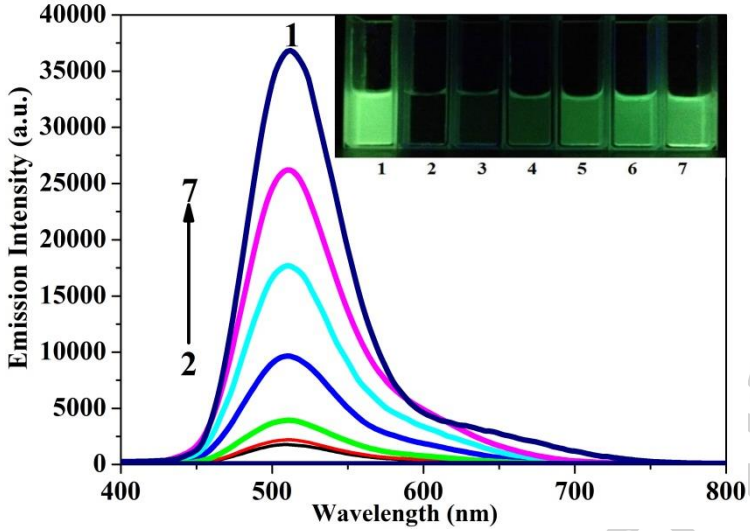

Figure 2

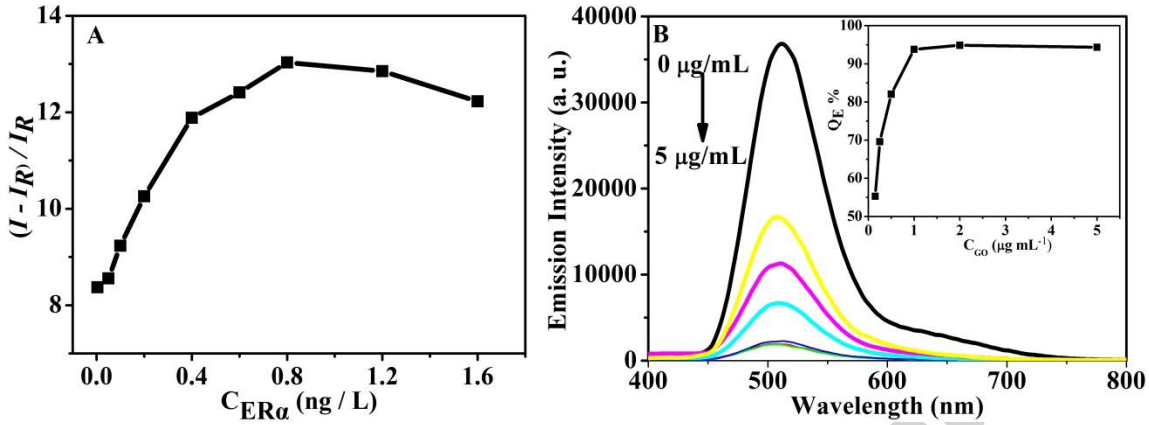

Figure 3

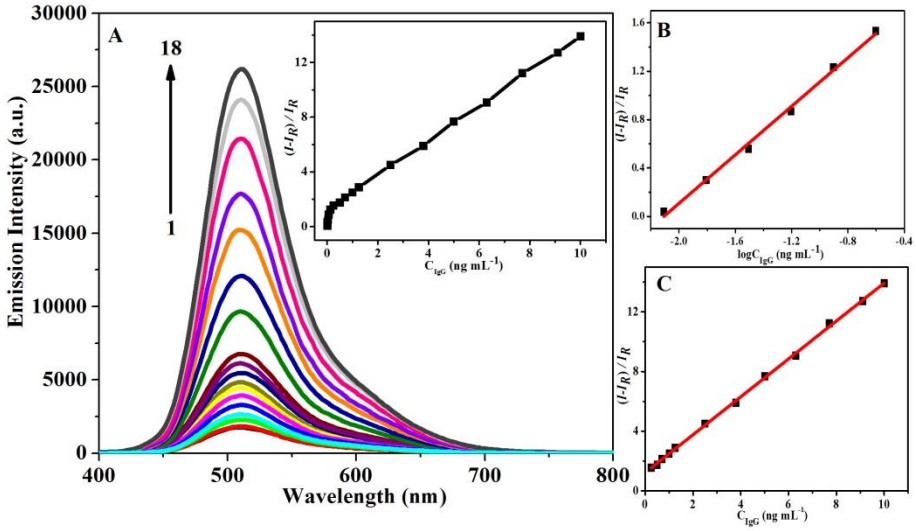

Figure 4

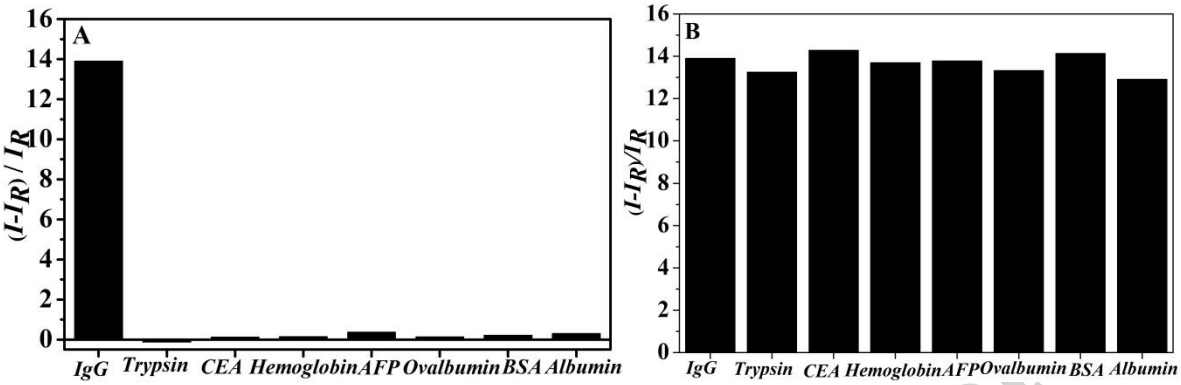

Figure 5

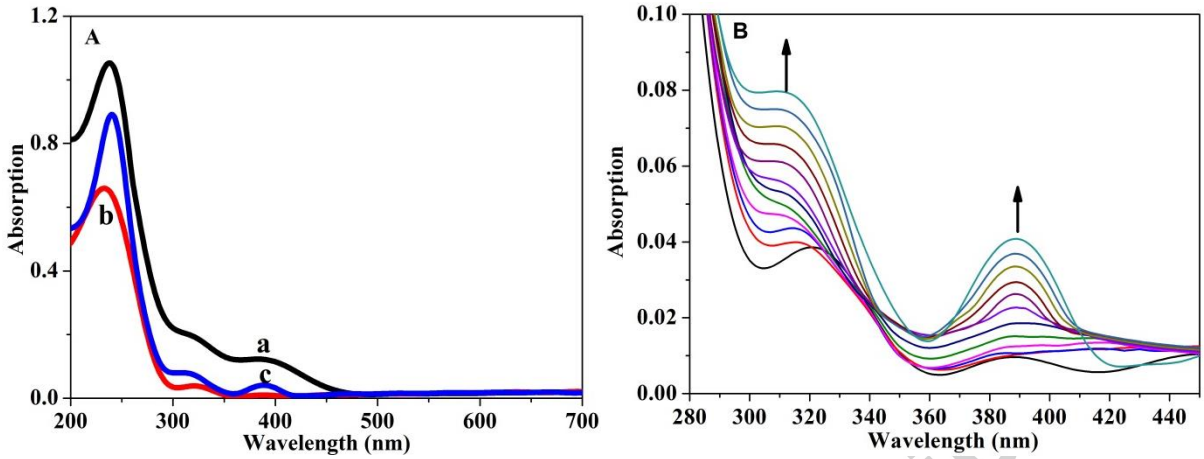

Figure 6

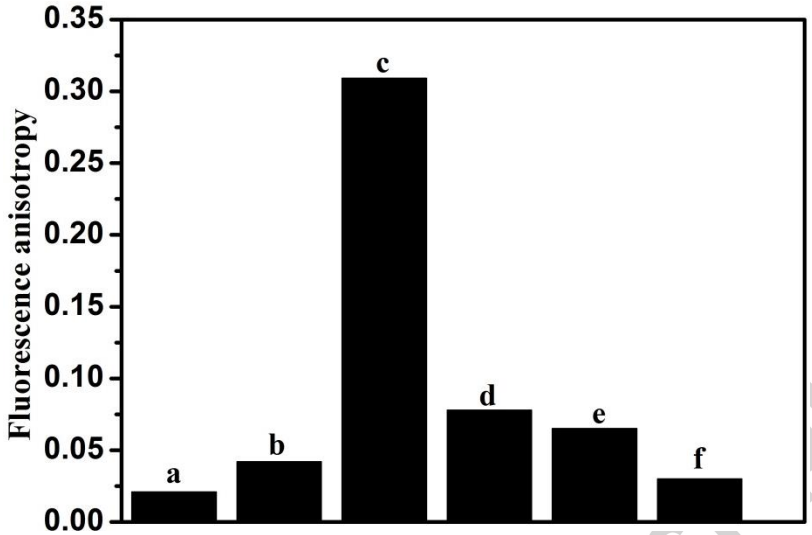

Figure 7