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Simultaneous Visualization and Quantitation of Multiple Steroid Hormones Based on Signal-amplified Biosensing with Duplex Molecular Recognition

Yaning Tan[+], Xiaoxia Hu[+], Meng Liu, Xinwen Liu, Xiaobo Lv, Zhihao Li, Jie Wang, Quan Yuan* [a]

Abstract: The simultaneous quantitation of multiple steroid hormones in real-time is of great importance for both medical diagnosis. In this study, a portable hormone biosensor based on duplex molecular recognition coupled with a signal-amplified substrate was successfully developed for the simultaneous visualization and quantitation of multiple steroid hormones. Aptamer-functionalized upconversion nanoparticles (UCNPs) with different emission peaks are immobilized on the photonic crystal (PC) substrate as the nanoprobes, leading to the specific and simultaneous assay of multiple steroid hormones. Coupled with the luminescence enhanced effect of the PC substrate, nanomolar quantification limits of multiple hormones are achieved. This well-designed biosensor is also promising in the quantification of multiple hormones in serum samples. The amplified luminescence signals can be visualized with the naked-eye and captured by an unmodified phone camera. This hormone quantitation biosensor exhibits the advantages of multi-detection, visualization, high sensitivity and selectivity for potential applications in clinical disease diagnosis.

Introduction

Hormones, which are the signaling molecules secreted from glands, play an important role in the regulation of human physiological activities.[1] Among them, multiple steroid hormones (including androgens, estrogens, and progestogens) exhibit synergistic effects on the body for its development, growth and functions.[2-5] It has been suggested that the occurrence and development of many deadly diseases are closely related to the deregulation of multiple hormones, rather than an individual steroid hormone. For example, previous studies have strongly suggested that the synchronous variation of estrogen and progestogen levels is related to the development of gynecological cancers such as ovarian, uterine, and breast cancer.[6] It has also been reported that multiple steroid hormones, especially a series of androgens coexistent in blood, are considered as important etiologies of prostate cancer.[7] Moreover, according to recent studies, the simultaneous dramatic drop of multiple estrogen levels is a risk factor of Alzheimer's disease in women.[8] Therefore, in order to achieve more accurate diagnosis of disease and improve diagnosis reliability, simultaneous quantitation of these multiple hormones is a necessity. Current analytical techniques for quantifying multiple steroid hormones include mass spectrometry[9,10] and liquid-chromatography mass-spectrophotometry.[11-13] Although these methods provide unique advantages, they require several sample pretreatment processes and sophisticated laboratory equipment, all of which hamper their applications in point-of-care (POC) diagnostics and individualized medical care.[14-16] Therefore, a real-time detection method for the simultaneous measurement of multiple steroid hormones is seriously needed.

Most recently, upconversion nanoparticles (UCNPs) have attracted considerable attention as an outstanding class of luminescent nanomaterials for sensing applications.[17,18] Regarded as a special type of nanophosphor, UCNPs are capable of converting near-infrared (NIR) light to shorter wavelength luminescence through the energy-transfer process.[19,20] Compared to fluorophore-based detection, UCNP-based measurement presents decreased light scattering and less background interference, with significant improvement in sensitivity and limits of detection.[21, 22] More importantly, since the upconversion luminescence of UCNPs can be tuned by varying lanthanide dopants (including Er^{3+}, Tm^{3+}, and Ho^{3+}), these multicolor nanophosphors can be used to design ideal luminescent nanoprobes for the simultaneous detection of multiple targets.[23, 24] To meet the selectivity criteria for multiple assay, it is necessary to achieve the specific recognition of the targets.[25-29] Aptamers are synthetic single-stranded DNA or RNA molecules selected from a random-sequence oligonucleotide library by the method of Systematic Evolution of Ligands by Exponential enrichment (SELEX).[30] Aptamers show great promise as recognition elements due to their property of high specificity to target.[31-33] Recently, several steroid hormone aptamers have been reported.[3,14] Inspired by their high specificity and strong binding affinity towards hormones, aptamer-functionalized UCNPs can be used as ideal luminescent nanoprobes for the simultaneous detection of multiple steroid hormones.

This paper describes a portable hormone detection biosensor, which couples duplex molecular recognition and a signal-amplified substrate, for the simultaneous visualization and quantitation of multiple steroid hormones. Based on the different luminescence emissions and the specific recognition capability of aptamer-functionalized upconversion nanoprobes, the simultaneous quantitation of multiple hormones with high selectivity is achieved. Combined with a photonic crystal (PC) substrate, which acts as the optical signal magnifier, visual and real-time assays for multiple hormones are realized. This method demonstrates high sensitivity for the simultaneous quantitation of multiple steroid hormones in buffer solution and even in serum samples, with a quantitation limit at the nanomolar level, with the added advantage of portability. With the capability to quantitate multiple steroid hormones, this biosensor will improve clinical disease diagnosis and health assessment.

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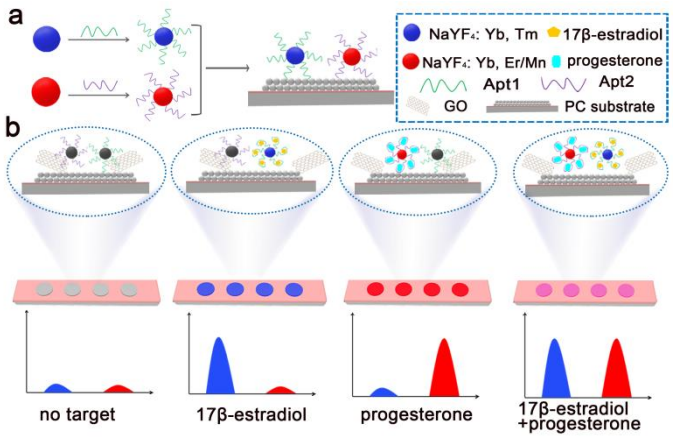
Results and Discussion



Scheme 1. Working principle for the simultaneous detection of multiple hormones.

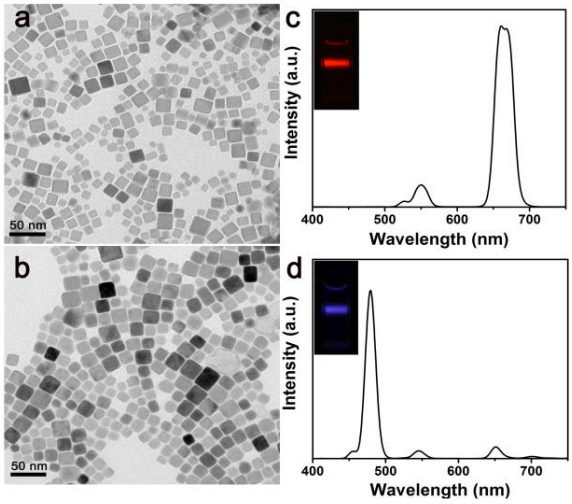


Figure 1. TEM images of a) $NaYF_4$: Yb, Er/Mn UCNPs and b) $NaYF_4$: Yb, Tm UCNPs. Upconversion luminescence spectra of c) $NaYF_4$: Yb, Er/Mn UCNPs and d) $NaYF_4$: Yb, Tm UCNPs. Inset: visible luminescence photograph of UCNPs using 980 nm excitation intensity of 0.5 W/cm^2.

The working principle is illustrated in Scheme 1. 17ß-estradiol and progesterone are among the essential female steroid hormones, contributing a synergistic effect on the coordination of sexual development and maintenance of female reproductive tissue function. Therefore, they were chosen as the model steroid hormones. Different upconversion luminescent nanoprobes were designed by labeling $NaYF_4$: Yb, Tm UCNPs with the 17ß-estradiol-specific aptamer (Apt1) and labeling $NaYF_4$: Yb, Er/Mn UCNPs with the progesterone-specific aptamer (Apt2). Then, the constructed hormone nanoprobes were immobilized on the PC substrate to fabricate the PC substrate-based biosensor for visualization and real-time quantitation of multiple hormones. The core strategy of this quantitation method is the utilization of aptamer-target to mediate the luminescence recovery of the upconversion nanoprobes. In the absence of 17ß-estradiol and progesterone, the nanoprobes immobilized on the PC dot substrate are bonded to GO via ´ ï ´ stacking between the aptamers and GO, leading to the quenching of upconvension luminescence. After introducing one type of target, the luminescence of corresponding nanoprobe recovers, because of the preferential binding between the target and the aptamer. In the presence of both 17ß-estradiol and progesterone, the two kinds of nanoprobes both specifically bind to their corresponding targets, causing luminescence signal recovery. Depending on the specific luminescence response toward different types of targets, this biosensor allows the simultaneous quantitation of multiple hormones.

Oleic acid-capped $NaYF_4$: Yb, Tm (OA-$NaYF_4$: Yb, Tm) UCNPs and oleic acid-capped $NaYF_4$: Yb, Er/Mn (OA-$NaYF_4$: Yb, Er/Mn) UCNPs were first synthesized according to a previous study.[34] Then, to convert hydrophobic oleic acid-capped UCNPs into hydrophilic UCNPs, polyethyleneimine (PEI) modification via ligand-exchange was applied. The transmission electron microscopy (TEM) images (Figure 1a, 1b and Figure S1) show that the as-synthesized $NaYF_4$: Yb, Tm UCNPs and $NaYF_4$: Yb, Er/Mn UCNPs exhibit well-defined monodisperse nanocubic shapes. As shown in Figure 1c, following NIR excitation at 980 nm, $NaYF_4$: Yb, Er/Mn UCNPs show an extremely strong red emission band at 660 nm. The inset photograph of Figure 1c illustrates that these nanoparticles display visible red upconversion luminescence under 980 nm illumination. The $NaYF_4$: Yb, Tm UCNPs show a strong blue emission band at 477 nm (Figure 1d) and display visual blue luminescence (inset image of Figure 1d). The predominant emission peaks of these two kinds of UCNPs are independent and can be clearly distinguished from one another, showing that these two kinds of UCNPs are promising as labels for simultaneous detection of two steroid hormones. Fourier transform infrared spectroscopy (FTIR) was utilized to characterize the functional groups on the surface of UCNPs (Figure S3 and S4). It can be observed that the PEI-capped UCNPs exhibit absorption bands at about 3450 and 1640 cm^{-1}, which are assigned to the stretching and the scissor bending vibration of - NH_2, respectively. The absorption bands at 2927ï 2856 cm^{-1} are attributed to C-H. These results verify that the UCNPs have been successfully functionalized with PEI. To prepare nanoprobes for detection of multiple steroid hormones, the PEI-capped UCNPs were further conjugated with the aptamers. Zeta potential shows that the PEI-capped UCNPs are positively charged (+30.6 mV), whereas the aptamer modified UCNPs are negatively charged (- 15.6 mV), indicating the successful conjugation of aptamer on the surface of UCNPs (Figure S5).

PCs are spatially ordered materials with excellent light manipulation properties and obvious luminescence enhancement effects.[35-38] Here, a PC substrate was developed to serve as the support for the aptamer-functionalized UCNP nanoprobes. As illustrated in Figure 2a, aqueous droplets containing carboxyl-modified polystyrene colloidal particles were

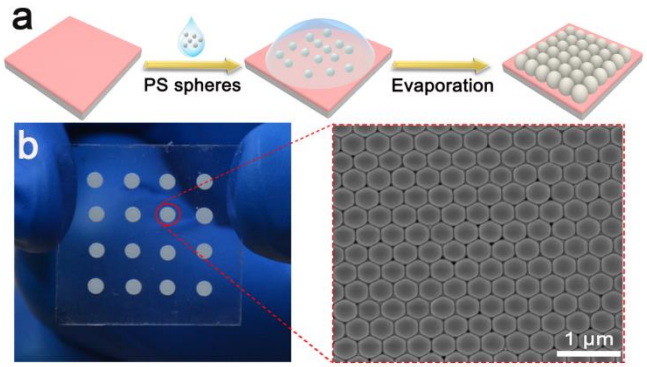


Figure 2. a) Scheme of the fabrication processes of the PC substrate. b) Photograph of the fabricated PC substrate (left) and SEM image of the PC dots (right).

first dropped onto the hydrophobic polydimethylsiloxane (PDMS) substrate. Then, with slow volatilization of water, the colloidal particles gradually self-assemble into macroscopic PC dots. As shown in Figure 2b, the PC dots present a regular array with four rows. The scanning electron microscopy (SEM) image demonstrates that the colloidal particles are in a highly ordered face-centered cubic arrangement. It also can be observed that each of the PC dots presents as a regular microplate (Figure S6). The PDMS substrate presents a contact angle with 103.4° ± 2.2°and shows highly hydrophobic behavior (Figure S7a), whereas the PC dots on the substrate result in hydrophilic areas with a contact angle of 37.8° ± 2.3° (Figure S7b). The different wettability between the PDMS substrate and PC dots leads to enrichment of the nanoprobes and analytes on the PCs dots.

The enhancement ability of the PC dots for upconversion luminescence was investigated by comparing the luminescence intensity both on the PC dot and PDMS substrate. As illustrated in Figure 3a, the substrate was divided into two halves by assembling hydrophilic PC dots on the upper half of PDMS substrate and keeping the bottom half without any treatment. The droplets containing UCNPs were first dropped on the substrate, and then the UCNPs were gradually loaded on the substrate as the solvent evaporated. Figure 3b shows that the blue upconversion luminescence is enhanced when $NaYF_4$: Yb, Tm UCNPs are deposited on the PC dot compared rather than the PDMS substrate. Similarly, when $NaYF_4$: Yb, Er/Mn UCNPs are deposited on the substrate, the red upconversion luminescence from the PC dots is much stronger than that from the PDMS substrate (Figure 3c). The same trend of enhancement is also observed when mixtures of both kinds of UCNPs are loaded on the substrate (Figure 3d). Furthermore, as presented in the corresponding upconversion luminescence spectra (Figure S8, S9 and S10), the luminescence intensities of the UCNPs on the PC dots are much stronger than those on the PDMS substrates. According to the previous studies,[39-43] the luminescence enhanced effect may be attributed to the high stored electric field intensity on the surface of PC substrate.

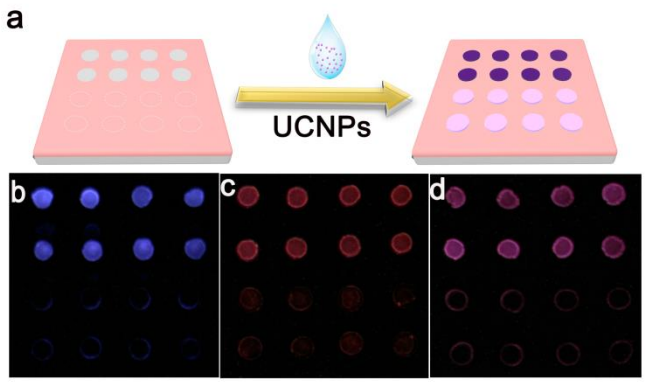


Figure 3. a) Schematic representation for the immobilization of UCNPs on the fabricated substrate. Luminescence images of the PC substrate after the deposition of b) $NaYF_4$: Yb, Tm UCNPs, c) $NaYF_4$: Yb, Er/Mn UCNPs and d) both kinds of UCNPs with 980 nm illumination intensity of 0.5 W/cm^2.

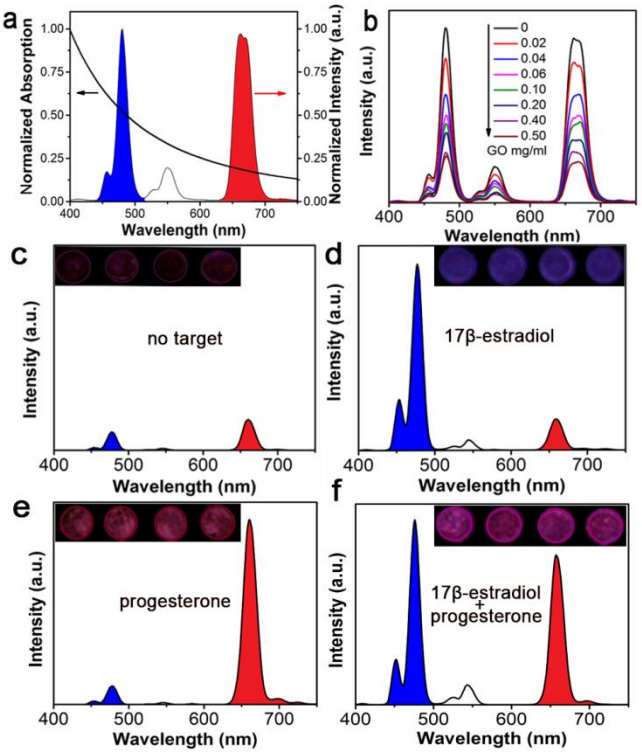


Figure 4. a) Normalized absorption spectrum of GO, and the normalized upconversion luminescence spectrum of the UCNP-based nanoprobes. b) Upconversion luminescence spectra of aptamer-functionalized UCNPs with different concentrations of GO. Luminescence spectra of the biosensor with c) no target, d) addition of 17b-estradiol, e) addition of progesterone and f) addition of both of 17b-estradiol and progesterone. Inset: luminescence photographs of the biosensor after addition of targets at different conditions with 980 nm irradiation intensity of 0.5 W/cm^2.

When the surface of PC substrate is illustrated by the excitation light, the vicinage of the PC surface can store energy through the formation of electromagnetic field waves that can provide stronger electric field intensity than the illumination light source. As a result, the absorption of the luminescent species on the PC surface is increased and the luminescence emission is enhanced.These results distinctly suggest that the PC dot array shows an obvious enhancement effect on the luminescence intensity of UCNPs. Thus, the substrate with PC dot array can be used as a promising tool for amplifying the luminescent signal and increasing the detection sensitivity.

The bioassay is based on the target-mediated luminescence off-on principle. As a two dimensional (2D) carbon material, GO has generated considerable interest in optical detection due to its super capacity for luminescence quenching.[44, 45] The TEM image (Figure S11) shows that GO has a sheet-like 2D structure with rolled edges and occasional folds, in good agreement with the previous literature results.[46] As shown in Figure 4a, GO exhibits a broad absorption band from 400 nm to 800 nm, with good overlap with the luminescence spectrum of the mixture of $NaYF_4$: Yb, Tm UCNPs and $NaYF_4$: Yb, Er/Mn UCNPs. As shown in Figure 4b, the strong upconversion luminescence of the nanoprobes is gradually quenched with increasing concentration of GO, indicating that GO can serve as a highly efficient quencher for UCNP-based nanoprobes. The detection of 17ƃ-estradiol and progesterone on the PC substrate was performed. As shown in Figure 4c, when no target is present, the luminescence intensities at 477 nm and 650 nm are weak. The inset photograph shows that the corresponding luminescence image of the PC dots row is dark. When only 17ƃ-estradiol is added, the luminescence intensity becomes enhanced at 477 nm but remains constant at 650 nm, and the test zone exhibits bright blue luminescence (Figure 4d). As shown in Figure 4e, with the addition of progesterone, the luminescence intensity at 650 nm is enhanced whereas the emission band at 477 nm remains weak. In addition, the red luminescence image of the PC dot row is exhibited under 980 nm laser illumination (Figure 4e). In the presence of both 17ƃ-estradiol and progesterone, strong blue and red bands in the luminescence spectrum can be observed. In accordance with the luminescence spectrum, the luminescence image shows bright visible blue-red luminescence (Figure 4f). The luminescence images from the PC substrate can be monitored by the naked eye and recorded by an unmodified camera phone. As a result, this duplex molecular recognition based luminescent biosensing method presents high selectivity and feasibility for the measurement of two types of steroid hormones.

Mixtures of different concentrations of 17ƃ-estradiol and progesterone in buffer solution were further assayed. As shown in Figure 5a, the brightness of the PC dot columns is gradually enhanced with increasing concentrations of 17ƃ-estradiol and progesterone. The gradual brightness changes are visual with naked-eye with illumination at 980 nm. Consistent with the captured images, the luminescence spectra show that the luminescence intensity is gradually enhanced when the concentrations of 17ƃ-estradiol and progesterone increase from 0 nM to 40 nM (Figure 5b). Furthermore, the calibration curves of the normalized intensity of the upconversion luminescence versus the concentrations of the two hormones were obtained (Figure 5c and 5d). The plots exhibit good linearity in the range of 0 to 1.0 nM with a quantification limit of 0.1 nM for both 17ƃ-estradiol and progesterone. The results above indicate that the PC microchip-based luminescent detection method can be used as a sensitive and accurate quantitative strategy for simultaneous detection of two kinds of hormones.

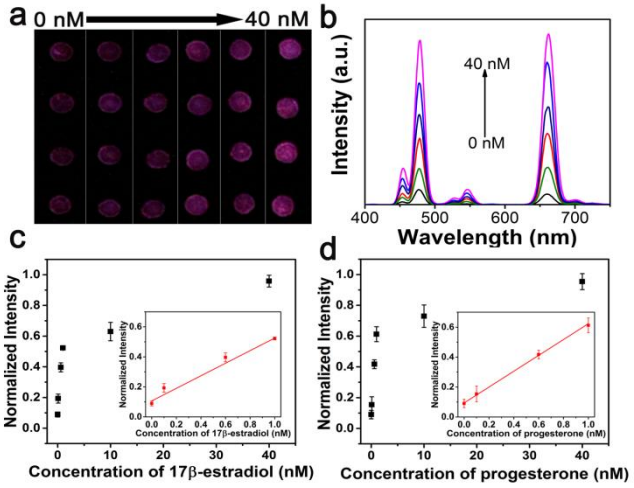


Figure 5. a) Luminescence photograph of the biosensor upon the addition of various concentrations of 17ƃ-estradiol and progesterone in buffer solution. b) The luminescence spectra of the biosensor with various concentrations (0, 0.1, 0.6, 1, 10 and 40 nM) of 17ƃ-estradiol and progesterone using 980 nm excitation intensity of 0.5 W/cm^2. Standard curve of upconversion luminescence intensity versus the concentration of c) 17ƃ-estradiol and d) progesterone.

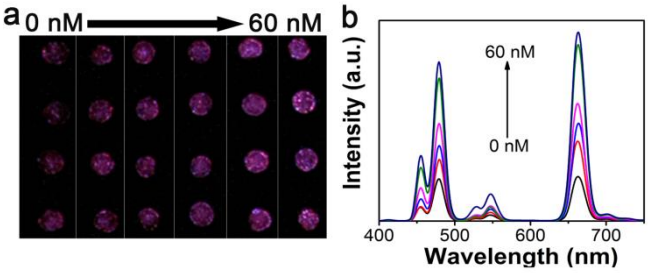


Figure 6. a) Luminescence photograph of the biosensor with addition of 17ƃ-estradiol and progesterone with various concentrations (0, 0.6, 1, 10, 40 and 60 nM) in serum. b) The luminescence spectra of the PC microchip with various concentrations of 17ƃ-estradiol and progesterone using 980 nm excitation intensity of 0.5 W/cm^2.

Concentrations of steroid hormones in blood-sera have been used as indicators of pregnancy, ovulation and cancer. Thus, it is essential to quantitate these hormones in serum for clinical diagnosis and medical treatment. As shown in Figure 6a, with an assay process similar to that in buffer solution, the identical luminescence images for serum samples are observed. With

increasing concentrations of 17ß-estradiol and progesterone in serum, the luminescence intensity increases with 980 nm excitation. The corresponding luminescence spectra also show upward trends when the concentrations of 17ß-estradiol and progesterone change from 0 nM to 60 nM (Figure 6b). The calibration curves of normalized intensity and concentration indicate a quantification limit of 0.6 nM for both of 17ß-estradiol and progesterone (Figure S12). These results demonstrate that the biosensor based on luminescent detection holds great promise for the simultaneous quantitation of multiple steroid hormones in complex serum samples.

Conclusions

In conclusion, we have developed a visual biosensor, which combines duplex molecular recognition and a signal-amplified substrate for simultaneous quantitation of multiple steroid hormones. Due to the different luminescence emissions and specific recognition properties of the aptamer-functionalized upconversion nanoprobes immobilized on the PC substrate, the simultaneous and specific assay of multiple hormones was realized. Because of the luminescence enhancement ability of the PC substrate, this method exhibits excellent capacity for hormone detection with high sensitivity, and the output optical signals can be observed visually for portable operation. Based on these unique merits, this luminescence-based biosensor shows superior detection performance in buffer solution as well as in complex serum samples. To conclude, this visual detection method provides a novel approach for the simultaneous assay of multiple steroid hormones with high sensitivity and specificity, and we anticipate that this method will find wide-range application in medical diagnosis and disease evaluation.

Experimental Section

Chemicals

Yttrium oxide (Y_2O_3, 99.99%), ytterbium oxide (Yb_2O_3, 99.99%), thulium oxide (Tm_2O_3, 99.99%), erbium oxide (Er_2O_3, 99.99%), poly(ethylenimine) (PEI, branched polymer, M (average) = 25000), oleic acid (OA), and 1-octadecene (ODE) were purchased from Aladdin Reagents Co.. Ethanol, cyclohexane, manganese chloride tetrahydrate ($MnCl_2·4H_2O$), sodium fluoride (NaF), sodium hydroxide (NaOH), dimethylsulfoxide (DMSO), and nitric acid (HNO_3) were of analytical grade purchased from Sinopharm Chemical Reagent Co.. 17ß-estradiol (BR) and progesterone (BR) were obtained from Shanghai Yuanye Biological Co.. PDMS (Sylgard 184 silicone elastomer kit, Dow) and curing agent were obtained from Dow Corning. Monodispersed latex spheres (polystyrene (PS), m/v=10%) were purchased from Shanghai Biochemical Co., Ltd. Sulfosuccinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (Sulfo-SMCC), 1-ethyl-3-[3-dimethyllamino-propyl]carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were purchased from Sigma Aldrich. The sequence of 17ß-estradiol aptamer (named as Apt1) is 5ôAAG GGA TGC CGT TTG GGC CCA AGT TCG GCA TAG TG-SH-3ôand the sequence of progesterone aptamer (named as Apt2) is 5ôCAC ACA CGC AGC AAG GTC GTC GAT ACA AAA CGT ATC GAC CCG TCA CAG ACT GCC CCG GGT-SH-3ô These aptamers were synthesized by Shanghai Sango Biological Science & Technology Co.. Rare earth nitrates ($RENO_3·H_2O$) were prepared by dissolving the corresponding rare earth oxides in nitric acid at 140 ºC, followed by evaporation of the solvent to obtain the final products.

Synthesis of UCNPs

Oleic acid-capped $NaYF_4$: Yb, Tm (OA-$NaYF_4$: Yb, Tm) UCNPs were synthesized as follows: In brief, NaOH (0.6 g) was added to a mixture containing oleic acid (10 mL), ultrapure water (3 mL), and ethanol (10 mL) with stirring to obtain a homogeneous solution. Then, 1.92 mL of $Y(NO_3)_3$ (0.5 M), 1.2 mL of $Yb(NO_3)_3$ (0.2 M), and 6 εL of $Tm(NO_3)_3$ (0.2 M) (Y:Yb:Tm = 79.9:20:0.1) were added to the above solution with magnetic stirring. Finally, 4 mL of ultrapure water containing 4 mmol of NaF was dropwise added to the mixture with vigorous stirring. After thorough stirring at room temperature for another 15 min, the obtained solution was transferred to a 100 mL Teflon-lined autoclave and heated at 200 ºC for 8 h. When the reaction system was cooled to room temperature, the products were collected by centrifugation and washed with ethanol several times. Finally, the products were dried at 60 ºC to obtain the UCNPs powder. For the synthesis of oleic acid-capped $NaYF_4$: Yb, Er/Mn (OA- $NaYF_4$: Yb, Er/Mn) UCNPs, 1.2 mL of $MnCl_2·4H_2O$ (0.5 M), 2 mL of $Y(NO_3)_3$ (0.5 M), 1.8 mL of $Yb(NO_3)_3$ (0.2 M), and 0.2 mL of $Er(NO_3)_3$ (0.2 M) (Y:Yb:Er:Mn = 50:18:2:30) were added.

Preparation of PEI-capped UCNPs

Typically, the OA-capped UCNPs (50 mg) were dispersed in 25 mL of DMSO with ultrasonication. Then, 5 mL of DMSO containing PEI (150 mg) was added to the above solution. The mixture was refluxed at 95 ºC until the color of solution became light yellow. When the reaction system was cooled to room temperature, PEI-capped UCNPs were obtained by washing several times with ultrapure water.

Preparation of aptamer-functionalized UCNPs

Briefly, 200 εL of HEPES buffer solution (pH 7.2, 10 mM) containing sulfo-SMCC (0.4 mg) was added to a homogenous mixture containing 1 mg of PEI-capped UCNPs and 800 εL of HEPES buffer solution. After incubation for 2 h at 25 ºC, the activated UCNPs were separated by centrifugation and washed three times with HEPES buffer. Then, the activated UCNPs were dispersed in 1 mL of HEPES buffer, and 2 nmol of aptamer was added to the above solution. Next, the solution was incubated overnight at 25 ºC. Finally, the aptamer-functionalized UCNPs

were collected after centrifugation and washed several times with ultrapure water. The as-prepared aptamer-functionalized UCNPs were stored at 4 ºC after redispersion in Tris-HCl buffer solution (pH 7.4, 10 mM).

Fabrication of the PC substrate

The PC dot substrate was fabricated using the method of solvent evaporation. First, PDMS and the curing agent (m/m = 10:1) were mixed and then stirred vigorously for 1 h. Then, the mixture was treated under vacuum until no bubbles were observed. To form a transparent and hydrophobic PDMS substrate, the mixture was spin-coated on a clean cover glass followed by drying in an oven at 60 ºC overnight. For fabrication of the PC substrate, the PDMS substrates were pre-heated in air on a heating plate at 40 ºC. Then, 2 εL of PS suspension, which was first diluted with ultrapure water in a 1:4 v/v ratio, was dropped vertically on the PDMS substrate. With slow evaporation of the solution, the PS was deposited on the PDMS substrate. Finally, these prepared PC substrates were stored in a dry condition for the further use.

Simultaneous detection of 17β-estradiol and progesterone in buffer solution on the PC substrate

For the detection of 17ƃ-estradiol and progesterone, Apt1-functionalized $NaYF_4$: Yb, Tm nanoprobes and Apt2-functionalized $NaYF_4$: Yb, Er/Mn nanoprobes were immobilized on the PC substrate via EDC-NHS coupling. Briefly, 0.5 mg of EDC and 1 mg of NHS were added to 1 mL of ultrapure water as the activator. Then 2 εL of the above solution was dropped onto each PC dot and reacted for 10 min at 37 ºC. Next the PC dots were washed several times with ultrapure water. After drying, 2 εL of the aptamer-functionalized UCNP solution (1 mg L^{-1}) was added to each PC dot and reacted for 30 min at 37 ºC. Then, the PC dots were washed with PB buffer solutiom (pH 7.0, 10 mM) to remove the unlinked UCNP nanoprobes. To prepare the standard solutions of 17ƃ-estradiol and progesterone with different concentrations, alcohol solutions of the two hormones were diluted by different factors with PB buffer solution. In brief, 2 εL of standard solution was dropped on the surface of PC dots and incubated at 37 ºC for 2 h. Then the PC dots were washed with PB buffer. Afterwards, 1 εL of graphene oxide (GO) (0.1 mg mL^{-1}) was dropped to each PC dot and reacted at 37 ºC for 30 min to combine with the probes which did not bind to the targets. Finally, the dots were washed three times with ultrapure water to remove the excess GO. The upconversion luminescence spectra were measured using a Hitachi F-4600 fluorescence spectrometer equipped with a 980 nm laser. The luminescence images were captured by a mobile phone.

Simultaneous detection of 17β-estradiol and progesterone in human serum on the PC substrate

The feasibility of the PC microchip for the simultaneous detection of multiple steroid hormones in serum samples was tested using male serum spiked with these two hormones. Blood sample tubes were allowed to settle at room temperature for 30 min and then centrifuged. After that, separated serum was immediately transferred to a plastic tube and stored at - 20 ºC until used. For the detection of 17ƃ-estradiol and progesterone in serum samples, the serum was first diluted with PB buffer solution in a 1:9 v/v ratio. Then different amounts 17ƃ-estradiol and progesterone were added to the diluted serum samples. The assay was similar to method in buffer solution described above.

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Keywords: steroid hormones Åbiosersors Åupconversion nanoparticles Åaptamers Åphotonic crystals

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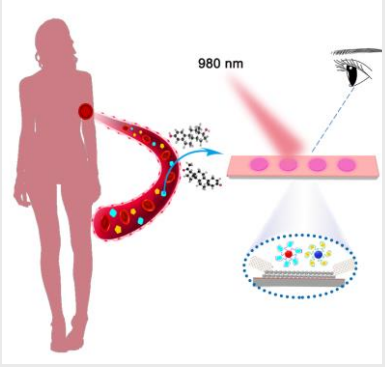
FULL PAPER

Simultaneous quantitation of multiple steroid hormones：a portable hormone biosensor based on duplex molecular recognition coupled with a signal-amplified substrate was successfully developed for the simultaneous visualization and quantitation of multiple steroid hormones. This biosensor exhibits the advantages of multi-detection, visualization, high sensitivity and selectivity for potential applications in clinical disease diagnosis.



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Simultaneous Visualization and Quantitation of Multiple Serum Hormones Based on Signal-amplified Biosensing with Duplex Molecular Recognition