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Single-Step Nanoplasmonic VEGF₁₆₅ Aptasensor for Early Cancer Diagnosis

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Abstract

Early cancer diagnosis is very important for prevention or mitigation of metastasis. However, we must improve the diagnosis and assessment of cancer by an effective and efficient method. Here, we report a single-step detection method using nanoplasmonic aptamer sensor (aptasensor), targeting a vascular endothelial growth factor-165 (VEGF₁₆₅), a predominant biomarker of cancer angiogenesis. Our single-step detection is accomplished by: (1) specific target recognition by an aptamer-target molecule interaction; (2) direct readouts of the target recognition. The readout is achieved by inactivation of surface plasmon enhancement of fluorescent probes preattached to the aptamers. Our aptasensor provides the appropriate sensitivity for clinical diagnostics with a wide range of linear detection from 25 pg/mL to 25 µg/mL (= from 1.25 pM to 1.25 µM), high specificity for VEGF₁₆₅ against PDGF-BB, osteopontin (OPN), VEGF₁₂₁, and NaCl, and temporal/thermal/biological stability. In experiments with 100 % serum and saliva from clinical samples, readouts of the aptasensor and an ELISA for VEGF₁₆₅ show good agreement within the limit of the ELISA kit. We envision that our developed aptasensor holds utility for point-of-care cancer prognostics by incorporating simplicity in detection, low-cost for test, and required small sample volume.

Keywords

aptamer; surface plasmonic resonance; optical biosensor; VEGF; early cancer diagnostics; prognostics; point-of-care diagnostics

Current cancer diagnostics mainly rely on commercialized multi-step antibody-based binding assays, such as, ELISA (enzyme-linked immunosorbent assay),^{1,2} Luminex,³ and Nanosphere⁴ for capturing and monitoring target proteins. However, the antibody-based methods require large sample volumes, expensive reagents, or a multi-step assay with associated long incubation times. Furthermore, antibody affinity varies from batch to batch, which makes it difficult to develop reliable sensors for clinical molecular diagnostics.

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Supporting Information Available. An experimental validation of metal-fluorescent interaction, photos of an arrayed aptasensor platform, and cost estimation of aptasensor. This material is available free of charge *via* the Internet at <http://pubs.acs.org>.

In contrast, aptamers, short single-stranded oligonucleotides (RNA or DNA <100 nt) or peptides (<100-mer) can be produced through a reliable chemical synthesis.^{5,6} Aptamers have high affinity, specificity, and stability; therefore, they are regarded as alternative reagents to antibodies.^{7,8} Furthermore, aptamers change structures from an elongated meta-stable structure to a uniquely folded stable structure upon interaction with targets.^{9–11} Such structural change affords monitoring of target-binding events without additional labeling steps.

Recently, numerous aptamer-based biosensors (aptasensors or aptamer beacons)¹² have exploited the target-binding affinity of aptamers and binding-induced conformational changes in aptamers to monitor the interaction with targets by measuring electron transfer^{13–17} color change^{18–22} or fluorescence quenching.^{23–30} However, these methods are limited by either the sensor size or sensitivity or by the associated assay complexity. For example, electrochemical sensors designed to measure an electron transfer require electrodes on a millimeter scale to achieve sufficient sensitivity and colorimetric or fluorescence quenching methods require collecting a large amount of particles to induce a discernable color change or fluorescent signals. A previously reported surface-enhanced Raman scattering (SERS) aptasensor required multiple binding events: a sandwich assay requiring labeling of target molecules with the complex of gold nanoparticle/aptamer/Raman probe/silver nanoparticle after capturing the target.³¹ Lastly, highly-sensitive aptasensors based on surface plasma resonance^{32–34}, surface-enhanced resonance Raman scattering (SERRS)³⁵, and a graphene field-effect transistor (FET)³⁶ required expensive instruments for measurement, spectral analysis, and sensor fabrication, which limited their extended applications.

In this paper, we present a single-step aptamer-based surface-enhanced fluorescent optical sensor, by combining an aptamer-target interaction for target recognition, and a nanoplasmonic-fluorophore interaction for signal enhancement.^{37–40} The developed aptasensor is simple, sensitive, specific, and stable for the detection of vascular endothelial growth factor-165 (VEGF₁₆₅), a predominant cancer biomarker in cancer angiogenesis.^{41–48}

RESULTS AND DISCUSSION

Strong metal-fluorophore interactions affect the fluorescent intensity significantly. The decay rate of fluorescence increases or decreases depending on the distance from metallic surfaces while the radiative rate in free solution remain relatively constant. The dipole of the fluorophore interacts with free electrons on the metal surface, changing the intensity and temporal and spatial distribution of the radiation. The localization of fluorophores near metal nanoparticles can result in high emission even from “nonfluorescent” molecules and million-fold increases in the number of photons enabling dramatic fluorescent signal enhancement on the nanometer scale.

To implement the metal-fluorophore interaction, negatively charged, citrate-stabilized gold nanoplasmonic particles (GNPs) are fixed on a positively charged aminosilane coated glass slide. On top of GNPs, positively charged poly-L-lysine (PLL) is applied to attract aptamers and stabilize them *via* an electrostatic binding interaction. Additionally, the PLL layer serves as a spacing layer to avoid any quenching effects, which may result from a fluorophore in close proximity to metal. To directly monitor a target binding event without requiring an additional labeling step, the VEGF₁₆₅-specific aptamer is conjugated with a fluorescent molecule, Cy3B, which has an absorption peak wavelength ($\lambda = 552$ nm) that matches with a localized surface plasmon resonance (LSPR) peak wavelength of a spherical GNP with 80 nm in diameter ($\lambda \approx 550$ nm),⁴⁹ expecting an additional signal enhancement.

Aptamer detachment upon target binding is understood by considering a thermodynamic balance between an aptamer folding energy and an electrostatic binding energy to a PLL-coated surface. Gibbs free energy, $\Delta G (= \Delta H - T \cdot \Delta S)$ is considered to quantify proteins or peptides folding in general thermodynamics. In our case, the detachment of the aptamer provides an example of enthalpy and entropy terms competing with one another to determine if an aptamer folding and detachment is a spontaneous process. As in protein folding, the entropic change of the aptamer and the enthalpic contributions are negative with a heat release upon the folding. Analogous to larger proteins, when all of these entropic and enthalpic contributions are combined, the enthalpy term wins out over the entropy term, the free energy of aptamer folding is negative, and aptamer folding can be a spontaneous process.⁵⁰ Therefore, we concentrate on the enthalpy term to calculate the aptamer folding energy and compare with the electrostatic binding energy based on calorimetry in an adiabatic condition.

In the absence of a target molecule, it is designed that an electrostatic binding potential, ΔH_E , is of greater magnitude than a spontaneous folding potential, ΔH_S : $0 > \Delta H_S > \Delta H_E$, and the aptamer remains attached to the PLL layer. In this case, Cy3B is spaced at a relevant coupling distance from the GNP surface for the LSPR to induce surface enhanced fluorescence (SEF) (Figure 1A). However, the electrostatic potential can be overcome with an addition target-binding folding potential, ΔH_T , which induces a preferable folded structure (Figure 1B). In the case that the folding energy is stronger than the electrostatic binding energy: $0 > \Delta H_E > (\Delta H_S + \Delta H_T)$, the aptamer undergoes a conformational change causing the aptamer to contribute less to the immobilization. The loss of the electrostatic energy leads to the subsequent detachment of aptamers from the GNP surface and signal enhancement is precluded (Figure 1C). Therefore, the concentration of VEGF₁₆₅ molecules can be estimated by measuring the signal change in the fluorescence intensity upon addition of samples.

The spontaneous folding enthalpy of our sequences was calculated to be $-66.3 \text{ kcal.mol}^{-1}$ (UNAFold, IDT Inc., Coralville, USA). The electrostatic binding enthalpy was estimated to be $-78.2 \text{ kcal.mol}^{-1}$ from an experimental measurement.⁵¹ Based on the experimental results from the reference, the additional transitional enthalpy of their sequences due to a combined PLL of 70 kDa was measured to be $-3.4 (= (7.6-14.4)/2) \text{ kcal.mole}^{-1}.\text{base pairs}^{-1}$ on average. Based on the measurement, the binding energy of our 23-base sequences was calculated to be $-78.2 \text{ kcal.mol}^{-1}$ and consequently the binding energy to the target was estimated to be of greater magnitude than $-11.9 \text{ kcal.mol}^{-1}$.

Signal enhancement by the metal-fluorophore interaction was experimentally confirmed by observing the intensity increase of Cy3B by 33 % and the radiative decay rate increase by 24 % in the presence of GNPs from a fluorescence lifetime spectroscopic measurement (see Supporting Information Figure S1).⁵² The simultaneous increase in both the intensity and the decay rate could be only enhanced by the metal-fluorophore interaction while an enhanced light mediated by LSPR could enhance only the intensity and a quenching effect could increase the decay rate but decrease intensity.

The condition of PLL immobilization was determined at an incubation time of 5 minutes and with 70 kDa molecular weight of PLL that produced the highest signal sensitivity, the ratio of signal change, ΔI , to control signal in the buffer, I_0 , among the conditions we considered (Figure 2). As for the application to other cancer markers, with given values of the spontaneous and target-binding folding energies, the electrostatic binding energy can be tailored to be from 0 to $-6.8 (= 7.6-14.4) \text{ kcal.mole}^{-1}.\text{base pairs}^{-1}$ by varying the molecular weight of a linear PLL and the incubation time of aptamer during aptasensor preparation.⁵³

The kinetics of the aptasensor for VEGF₁₆₅ reveals that the signal change reaches saturation within 30 minutes at 25 ng/mL and about one hour at 25 pg/mL (Figure 3). Based on the kinetic study covering the physiological concentration of VEGF₁₆₅ in biofluids, all target assays were conducted for an hour. Aptasensors were prepared in an array format fitting to cheaper portable fluorescent scanners for low-cost and simple detection, which was confined by a poly (dimethyl-siloxane) (PDMS) membrane stencil to a silanized glass slide as shown in Supporting Information Figure S2. Measured fluorescent signal changes were normalized by the value of the control.

The sensitivity of the aptasensor was evaluated using serially diluted VEGF₁₆₅ solutions in PBS at pH 7.4 (Figure 4). The signal changes were proportional to the concentration of VEGF₁₆₅ over a wide range from 25 pg/mL to 25 µg/mL (= from 1.25 pM to 1.25 µM). Based on the averaged values of experiments, the concentration of VEGF₁₆₅ was estimated using the following equation: $[VEGF_{165}] = \exp\{(-\Delta I/I_o + 0.839)/0.033\} - 1.03e^{-11}$. Average and standard error were calculated with values from 4 assays.

The aptasensor produced a significant signal change with VEGF₁₆₅ at 2.5 ng/mL, a peak value of breast cancer samples.^{37,54} For comparison, signal changes from other cancer-related protein concentrations were not significant as follows: platelet-derived growth factor (PDGF)-BB at 2.5 ng/mL² and osteopontin (OPN) at 1.5 µg/mL, the peak values of breast cancer samples⁵⁵ and noncancer-related common molecules: NaCl at 1 M, an extremely high value and VEGF₁₂₁ at 560 pg/mL, a peak value in healthy donors^{44,54} (Figure 5). However, the correlation with the most abundant non-specific protein, HSA at the average value of 40 mg/mL⁵⁶ was discernable. Average and standard error were calculated with values from 3 assays.

Our sensing mechanism counts on molecular interactions rather than isoelectric potentials of target molecules. Originally, we were concerned with false-positive signaling if a negatively charged aptamer would be replaced by other biomolecules mostly having non-zero isoelectric potentials at pH 7.4. Based on the observation of our results, the electrostatic binding energy of the aptamer seemed to be strong enough against the isoelectric potential of proteins we tested and no false-positive signals were detected from other proteins: PDGF-BB, OPN, and VEGF₁₂₁ except HSA.

The false-positive signal with HSA was presumably resulted from the aptamer replacement by negatively charged HSA. HSA has a lower isoelectric point ($pI=5.3$) than the 7.4 buffer pH, resulting in a net negative charge contrary to the other proteins examined (VEGF of pI 9.22 and PDGF of pI 9.8), which have net positive charge in the buffer but weaker than the positive charge of PLL ($pKa=5$) to detach net negatively charged aptamer. The negative potential of HSA may be higher than that of aptamer and replace our aptamer from the positively charged PLL. If this is the case, aptamer should be more strongly immobilized by adding extra spacer sequences and increasing the electrostatic binding force in the future.

Also, it was a concern that salt ions at a high concentration might induce a folding of our aptamer by increasing the rigidity and resulting in any false-negative signaling.⁵⁷ To confirm no false signaling, we did an assay with NaCl, the most common in biofluids, at an extremely high concentration to represent the effects from other ions in biofluids. In our experiment, no discernable signal change was observed.

Our developed aptamer-based sensor is regarded to be superior to or comparable with an antibody-based ELISA detection considering its wide detection range and a low cost for testing. A commercial ELISA kit having the detection range from 5 pg/mL to 2 ng/mL, can not cover the physiological level of VEGF₁₆₅ in cancer patients (from 500 pg/mL to 8 ng/mL).⁴⁴ In the comparative experiments using 100 % biofluids of serum and saliva, the

detected values of VEGF₁₆₅ with aptasensor matched the values with ELISA under 2 ng/mL, the upper limit of the ELISA kit. Above the limit, the readouts of ELISA was probably saturated and showed lower values than those of aptasensor (Figure 6). The base levels of readouts with aptasensor were elevated in the biofluids probably due to the effect of human serum albumin and the values were calibrated with the obtained sensitivity curve in Figure 4 before the comparison with ELISA.

As provided in the supplementary, our aptasensor reagents cost only 7.8 ¢ per test (see Supporting Information); the multi-well ELISA kit costs \$5.36 per test, partially due to the high cost of antibodies. By mass producing aptasensors and decreasing the sensing area, we can drastically reduce the cost of our aptasensor platform to a price-point well below that of ELISA. In our experiment, we used 10 µL of fabrication agents and samples to cover a sensing area of 7 mm², even though an actual detection area was 0.12 mm². If we fabricate sensors in the small area, a required volume can be only 170 pL, and, consequently, required agents can be 60 times less in volume and cost.

Our fully fabricated aptasensor was functional after the storage for more than a month at 4 °C (Figure 7). Aptasensors were prepared on individual glass slides at the same time and kept at 4 °C with an aluminium foil cover to prevent any degradation of Cy3B. Every week, slides were chosen for measuring controls of aptasensors in PBS and for experiments on solutions with 20 µg/mL of VEGF₁₆₅. The aptasensor provided consistent signal changes of 50 % within a 5 % tolerance, showing the temporal stability of aptamers immobilized on GNP surfaces for a month along with thermal stability up to 95 °C provided during sensor preparation and biological stability in biofluids during the assay.

CONCLUSIONS

The single-step aptasensor for VEGF₁₆₅ demonstrated target detection with a small amount of sample solutions (less than 10 µL) within an hour at a low cost (less than 10 ¢/test). In addition, the sensor provided a wide range of linear detection from 25 pg/mL to 25 µg/mL, relevant for clinical diagnostics. Also, the aptasensor of VEGF₁₆₅ detection showed strong specificity against PDGF-BB, OPN, NaCl, and VEGF₁₂₁, and weak specificity against HSA, which could be improved by SELEX works or aptamer modification. Our aptamer-based sensor showed temporal, thermal, and biological stability relevant for harsh diagnostic environments. Furthermore, the performance of aptasensor was successfully validated in biofluids and compared with conventional ELISA.

EXPERIMENTAL SECTION

Materials and reagents

RNA oligonucleotides were synthesized by IBA GmbH (Göttingen, Germany), purified *via* Reverse Phase HPLC, and confirmed by mass spectroscopy to be >85 % pure. The RNA oligonucleotides (23-mer) were fully modified with 2'-O-Me as a VEGF binding aptamer and with Cy3B at 3' end. A phosphate buffer solution (GIBCO 10010-023) was purchased from Invitrogen (Carlsbad, CA). The sequences of the oligonucleotides⁵⁸ and the buffer composition employed in the experiment are given below:

VEGF₁₆₅ Binding Aptamer (VBA): 5'-ACG CAG UUU GAG AAG UCG CGC GU-3'-Cy3B

1× PBS: 137 mM NaCl, 2.7 mM KCl, 8 mM Na₂HPO₄, and 2 mM KH₂PO₄ at pH 7.4

A ELISA kit for VEGF₁₆₅ (Quantikine, DVE00), Recombinant human VEGF₁₆₅, VEGF₁₂₁, PDGF-BB, and Osteopontin (OPN) were purchased from R&D Systems, Inc (Minneapolis,

MN) and human serum albumin (HSA) from MP Biomedicals, Inc. (Solon, OH) in a powder form and were diluted in sterile PBS to obtain desired stock concentrations. 98 % (3-Aminopropyl)triethoxysilane (APS) and Poly-L-lysine (PLL) were purchased from Sigma-Aldrich, Co. (St. Louis, MO). A solution of gold nanoparticles (GNPs, 80 nm in diameter) was purchased from TED Pella, Inc., (Redding, CA) and poly (dimethyl-siloxane) (PDMS) membrane (HT 6240, 256 μm in thickness) from Rogers Corporation (Woodstock, CT).

Methods

Preparing aptasensor—A glass slide was cleaned with Piranha (3:1 concentrated sulfuric acid to 30 % hydrogen peroxide solution) for 10 minutes, thoroughly washed with DI water, and then dried by a nitrogen gun. After the cleaning, the glass slide was coated with amino-terminal groups by immersing in a diluted APS solution (1 % v/v in 100 % ethanol) for 5 minutes at room temperature, rinsing with ethanol, and drying it gently with a nitrogen gun without leaving any trace marks. Baking for 5 minutes at 125 °C was applied to further stabilize the amino-group layer. Arrayed holes of 3 mm in diameter were patterned on a thin poly (dimethyl-siloxane) (PDMS) membrane (HT 6240) by using the laser-engraving machine (Versa Laser VL-200, Universal Laser Systems, Scottsdale, AZ, USA). The patterned PDMS membrane was cleaned with ethanol followed by drying with N_2 gas and in an oven at 60 °C for 10 minutes and then treated with UV/Ozone (UVO Cleaner Model 42, Jelight, Irvine, CA) for 3 minutes to modify the surface of PDMS. GNP stock solution was sonicated (30 seconds) for even distribution in the stock solution before dropping. 10 μL of the GNP solution was dropped at an opening area defined by the hole on the PDMS membrane and incubated for 1 hour at 4 °C in a humidity chamber. Unbound GNPs were washed off by rinsing the slide in distilled water and then drying in a centrifuge (IEC clinical centrifuge, International Equipment Co., Needham, MA). 10 μL of PLL (70k Da M.W., 0.1 % in distilled water) was dropped at the opening area and incubated for 5 minutes at room temperature in the humidity chamber. Unbound PLL was washed off by rinsing the slide in distilled water and then drying in a centrifuge. Apatmers at 500 nM were fully dehybridized and stretched by heating to 90 °C for 2 minutes in a dry block heater (HeatBlock I, VWR International, LLC, West Chester, PA) above a calculated melting temperature of 64 °C. 10 μL of the aptamer solution was dropped immediately at the opening area and incubated for 1 hour at 70 °C in the humidity chamber. Unbound aptamers were gently washed off by rinsing the slide in PBS by gently pipetting and then drying in a centrifuge.

Target assay with aptasensor—For assay, 10 μL samples (biofluids, VEGF₁₆₅, and other molecules) were dropped at the opening area and incubated for 1 hour at room temperature in the humidity chamber. Target-combined aptamers and unbound molecules were washed off by gently pipetting each sensor with 10 μL PBS while avoiding cross-contamination. Thorough washing induced the non-specific signal loss due to the unwanted detachment of the aptamers and GNPs by a non-specific mechanical force.

Target assay with ELISA—Undiluted human serum and saliva samples were assayed for VEGF₁₆₅ using an ELISA kit according to the manufacturer's instructions. The concentration of VEGF₁₆₅ in these samples was extrapolated from a standard curve generated using a range (0 – 2 ng/ml) of highly purified Sf21-expressed recombinant human VEGF₁₆₅.

Biofluid samples—Non-smoking participants were recruited at the GCRC, Penn State College of Medicine, Hershey, PA for this study. All the subjects involved in this study signed the institutional review board-approved consent form. As for the comparative assay, clinical samples were prepared with the mixed population of four healthy women with no

known breast disease and four women diagnosed as breast cancer ranging from infiltrating ductal carcinoma, ductal carcinoma *in situ*, to invasive cribriform carcinoma, and invasive lobular carcinoma. All of the breast cancer patients were recently diagnosed and had received any prior treatment in the form of chemotherapy, radiotherapy, surgery, or alternative remedies before sample collection. The mean ages for breast cancer patients and healthy donors were 46.0 ± 9.6 yr and 49.3 ± 5.4 yr ($P > 0.05$, t test), respectively. Fasting blood and stimulated whole saliva samples using base-gum (a gift from Wrigley's) and blood samples were collected. Separated serum and centrifuged saliva samples (12,000 rpm for 10 minutes) were stored at -80 °C until used. Prior to analysis, serum and saliva samples were diluted in normal saline (0.9 % NaCl).

Lifetime measurement—A home-built sample scanning confocal microscope (TE300, Nikon Instruments Inc., Melville, NY) was equipped with a pulsed diode laser (LDH-P-C-470B, PicoQuant GmbH, Berlin, Germany) 470 nm in wavelength, a time-correlated single photon counting module (PicoHarp 300, Picoquant GmbH, Berlin, Germany), and a piezo scanning stage (Nano-LP 200, Mad City Labs Inc., Madison, WI). A fluorescent molecule, Cy3B conjugated with aptamer was excited by the laser with a power of 2 μ W and a pulse rate of 40 MHz. The fluorescence was excited and collected through an oil immersion objective lens (Plan Apochromat, 60 $\times$, N/A=1.4, Nikon), and filtered with a 580 nm bandpass filter (580DF60, Omega Optical Inc., Brattleboro, VT). The photons were detected with an avalanche photodiode (PDM series, Micro Photon Devices, Bolzano, Italy), and the time delay between the laser pulse and the photons were measured using the PicoHarp 300. From each measurement we acquired images for 10 seconds, performing a line scan over 100 μ m. After the measurement, the lifetimes were fitted by a sum of two exponentials, accounting for a measured instrument response. The sum of the two resulting exponential decay rates, weighted by the amplitudes was used to compute the decay rates changes quoted in Figure S1.

Fluorescence imaging—An inverted microscope (TE2000-E, Nikon Instruments Inc., Melville, NY) was coupled with a mercury lamp (1 $\times$ HBO 103w/2, OSRAM, München, Germany) and a monochrome CCD camera (Cascade 512B, Photometrics, Tucson, AZ). The lights were filtered with a TRITC fluorescence filter set (EX: 528–553 nm, DM: 565nm, BA: 578–633 nm) through an objective lens (Plan Fluor, 20 $\times$, N/A = 0.45, Nikon). The fluorescent images of aptasensors were acquired using a commercial program (Image-pro Express, Media Cybernetics, Inc., Bethesda, MD) with an acquisition time of 200 ms and a gain factor of 3, and then stored in a 16-bit depth monochrome image.

Dark field imaging—An inverted microscope (Axiovert 200, Carl Zeiss, Göttingen, Germany) was equipped with a white light illumination (Xenon Light Source 610, KARL STORZ GmbH & Co. KG, Tuttlingen, Germany), a wet dark-field condenser ($1.2 < NA < 1.4$), and a true-color digital camera (MP3.3-RTV-CLR-10, QImaging, Surrey, BC, Canada). Space between arrayed aptasensors and the condenser was filled with PBS and maintained at room temperature in a complete dark room. The true-color images of aptasensors were taken through an objective lens (LD ACHROPLAN, 40 $\times$, NA = 0.6, Zeiss), acquired with a commercial program (QCapture Pro, Media Cybernetics, Inc., Bethesda, MD) with an acquisition time of 100 ms and a gain factor of 1, and then stored in a 24-bit true color image.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Schematic representations of detection mechanism of the aptasensor for VEGF₁₆₅. (A) In the absence of target molecules, VEGF₁₆₅, unfolded VEGF₁₆₅ aptamer is electro-statically bound to a positively charged PLL-coated gold nanoparticle (GNP) surface and surface-enhanced fluorescence (SEF) of Cy3B conjugated with the VEGF₁₆₅ aptamer, is created by both a metal interaction increasing the radiative fluorescent decay rate of Cy3B and the local surface plasmon resonance (LSPR) enhancing the intensity of an incident light. (B) The interaction of the VEGF₁₆₅ aptamer to its target induces the reversible conformation change of the aptamer and consequently the decreased electro-static binding force. (C) As a result, the target-binding interaction of the aptamer causes the irreversible detachment of the aptamer from the GNP surface and avoids the SEF effect of Cy3B.

Figure 2.

The PLL immobilization to improve the signal sensitivity. While the fluorescent intensity of Cy3B in the absence of the target increase with the increase of the molecular weight and the incubation time of PLL, the performance of aptasensor, the normalized intensity change of Cy3B with a target is maximized at around the molecular weight of 70 kDa and the incubation time of 5 minutes. I and I_0 represent fluorescent intensities from aptasensors in a target solution and treated only with PBS, respectively. ΔI represent the intensity change ($=I - I_0$) by target recognition.

Figure 3.

The kinetics of the aptasensor for VEGF₁₆₅. (A) The aptasensor for VEGF₁₆₅ shows that the signal change increases with time and reaches its saturation value within an hour at concentration from 25 pg/mL to 25 ng/mL, which covers the physiological ranges of VEGF₁₆₅ in clinical samples.

Figure 4.

The sensitivity of the aptasensor for VEGF₁₆₅. The aptasensor provides a wide range of detection from 25 pg/mL to 25 μg/mL. The signal changes are linearly proportional to the concentration of VEGF₁₆₅ in a log scale.

Figure 5.

The specificity of the aptasensor for VEGF₁₆₅. The aptasensor has the high specificity with VEGF₁₆₅ against PDGF-BB, OPN (osteopontin), NaCl, and VEGF₁₂₁ but the weak specificity against HSA (human serum albumin). The concentrations of molecules are physiologically relevant values: 2.5 ng/mL (VEGF₁₆₅, PDGF-BB), 1.5 μg/mL (OPN), 560 pg/mL (VEGF₁₂₁), and 40 mg/mL (HSA), excluding an extreme value of 1M (NaCl). The cases marked with ‘*’, ‘***’, and ‘****’ correspond to the assays at nominal, peak, and extreme values present in serum or plasma samples.

Figure 6. The sensitivity of aptasensor compared with ELISA for VEGF₁₆₅ from clinical samples. The readouts of the aptasensor for VEGF₁₆₅ in undiluted biofluids of serum and saliva from clinical samples are comparable with those of ELISA under 2.0 ng/mL, which is the upper limit of the ELISA kit. Above the limit, the readouts of ELISA are lower than those of aptasensor by several times.

Figure 7.

The stability of the aptasensor for VEGF₁₆₅. The temporal stability of fully fabricated aptasensor is evaluated by continually measuring signals from controls of aptasensors in PBS without VEGF₁₆₅ and cases with VEGF₁₆₅ at 20 $\mu\text{g/mL}$. The signals of control are consistent within the variation range of 5 % for a month. The intensity is normalized by the value of a control measured on the first day.