Supporting Information

A portable oligonucleotide-based microfluidic device for the detection of $VEGF_{165}$ in a three-step suspended-droplet mode

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Experimental section

Materials

$VEGF_{165}$ was purchased from Sino Biological. Oligonucleotides were purchased from IGE Biotechnology. All reagents were used as received without further purification. All aqueous solutions were prepared with Milli-Q water (18.2 MΩ cm^{-1}). Sheep red blood cells (100%) was purchased from Sigma Aldrich (St. Louis, MO). Poly(dimethylsiloxane) (PDMS) prepolymer (RTV 615) was obtained from Momentive Performance Materials (Waterford, NY). Teflon™ PFA 416HP was purchased from DuPont (Wilmington, DE). PP was purchased from Orient Hongye Chemical Co., Ltd. (Shandong, China). UV-LED light module and optical filter were from Shenzhen Fuji Technology co., LTD. Other reagents, unless specified, were also purchased from Sigma Aldrich (St. Louis, MO).

General experimental

Mass spectrometry was performed at the Mass Spectroscopy Unit at the Department of Chemistry, Hong Kong Baptist University, Hong Kong (China). Deuterated solvents for NMR purposes were obtained from Armar and used as received. ^{1}H and ^{13}C NMR were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz (^{1}H) and 100 MHz (^{13}C). ^{1}H and ^{13}C chemical shifts were referenced internally to solvent shift (acetone-d6: ^{1}H, 2.05, ^{13}C, 29.8). Chemical shifts are quoted in ppm, the downfield direction being defined as positive. Uncertainties in chemical shifts are typically ±0.01 ppm for ^{1}H and ±0.05 for ^{13}C. Coupling constants are typically ±0.1 Hz for ^{1}H-^{1}H and ±0.5 Hz for ^{1}H-^{13}C couplings. The following abbreviations are used for convenience in reporting the multiplicity of NMR resonances: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. All NMR data was acquired and processed using standard Bruker software (Topspin).

Detection of $VEGF_{165}$ using the locked aptamer in a duplex substrate (scheme 1)

ON1 (100 μM) and ON2 (100 μM) were mixed in Tris-buffer (20 mM Tris, pH 7.0) and was gradually heated to 95 °C for 10 min. After that, the temperature was slowly decreased to 25 °C with the speed of 0.1

°C/s. The mixture was further incubated at room temperature for 1 h to ensure the formation of the duplex structure. The annealed DNA was then stored at −20 °C before use. The indicated concentrations of $VEGF_{165}$ and ON1−ON2 duplex were mixed in a 100 μL solution of Tris buffered solution (20 mM Tris-HCl, pH 7.0). The mixture was further incubated at 30 °C for 1 h. The samples were then added to 399 μL of Tris-HCl buffer (20 mM Tris, 50 mM KCl, pH 7.0). Finally, 1 μL of **1** (1 μM) was added to the mixture. Emission spectra of the complex were measured in the range of 550−750 nm using an excitation wavelength of 360 nm. The final concentration of $VEGF_{165}$ indicated in the main text were calculated taking 500 μL as the final volume.

Detection of $VEGF_{165}$ using the G-rich aptamers (Scheme S1)

The indicated concentrations of $VEGF_{165}$ and the G-rich aptamer (S4 or S5) was mixed and added to 100 μL of Tris-buffer solution (20 mM Tris, pH 7.0). The mixture was then incubated at 30 °C for 1 h. After incubation, the samples were added to 399 μL of Tris-HCl buffer (20 mM Tris, pH 7.0). Finally, 1 μL of **1** (1 μM) was added to the mixture. Emission spectra of the complex were measured in the 550−750 nm range using an excitation wavelength of 360 nm. All the oligonucleotide sequences used in this project is outlined in Table 1. The final concentration of $VEGF_{165}$ indicated in the main text were calculated taking 500 μL as the final volume.

Photophysical measurement

Luminescence quantum yields were determined using the method of Demas and Crosby with $[Ru(bpy)_3][PF_6]_2$ in degassed acetonitrile as a standard reference solution ($\Phi r = 0.062$) and were calculated according to the following reported equation:

$$\Phi_S = \Phi_r(B_r/B_S)(n_S/n_r)^2(D_S/D_r) \quad (1)$$

where the subscripts s and r refer to the sample and reference standard solution respectively, n is the refractive index of the solvents, D is the integrated intensity, and Φ is the luminescence quantum yield. The quantity B was calculated by $B = 1 - 10^{-AL}$, where A is the absorbance at the excitation wavelength and L is the optical path length.

Synthesis of $[Ir_2(C\hat{}N)_4Cl_2]$ dimer complex

Cyclometalated dichloro-bridged dimers with the general formula $[Ir_2(C\hat{}N)_4Cl_2]$, where C^N = 2-phenylpyridine, was synthesized according to a literature method[1]. In brief, $IrCl_3 \cdot 3H_2O$ was heated to 150 °C with 2.2 equivalents of cyclometallated C^N ligands in 3:1 methoxymethanol and deionized water under a nitrogen atmosphere for 12 h. The reaction was cooled to room temperature, and the product was filtered and washed with three portions of deionized water and then three portions of ether (3 × 50 mL) to yield the corresponding dimer.

Synthesis of complex 1

Complex **1** was synthesized according to a modified literature method[1]. The precursor Ir(III) complex dimers were prepared as previously described. Briefly, a suspension of $[Cl_2(C\hat{}N)_4Cl_2]$ (0.2 mM) and the corresponding N^N (2,2'-biquinoline) (0.44 mM) ligands in a mixture of dichloromethane:methanol (1:1, 20 mL) was refluxed overnight under nitrogen atmosphere. The resulting solution was allowed to cool to room temperature, and was filtered to remove the unreacted cyclometalated dimer. To the filtrate, an aqueous solution of ammonium hexafluorophosphate (excess) was added and the filtrate was reduced in

volume by rotary evaporation until precipitation of the crude product occurred. The precipitate was then filtered and washed with several portions of water (2 × 50 mL) followed by diethyl ether (2 × 50 mL). The product was recrystallized by acetonitrile:diethyl ether vapor diffusion to yield the titled compound. Complex **1** was characterized by ^{1}H-NMR, ^{13}C-NMR, high resolution mass spectrometry (HRMS) and elemental analysis.

Complex **1**: Reported[2]

Fabrication of suspending-droplet (SD) chip

The SD chips in this work were fabricated entirely with polypropylene (PP), which is inherently hydrophobic and water impermeable. First, a positive-relief Teflon master was designed using AutoCAD 2015 and fabricated using a thermo-molding method we published before.[3] Then, a negative polydimethylsiloxane (PDMS) mold was cased from the Teflon master. For fabricating the PDMS mold, a mixture of PDMS prepolymer and curing agent (10:1 ratio) was poured onto the Teflon master and cured at 80 °C for 30 min. Next, the PDMS layers were carefully peeled off from the Teflon master and attached to flat glass substrates. Finally, a PP chip was fabricated by the thermo-molding method using the PDMS mold. The PP substrate was put on the PDMS master and sandwiched with another flat glass slide on it. Then, this sandwich was put on a hot compressor and embossed at 180 °C for 2 min under 0.24 MPa. As a result, the region covered with microstructures on the PP chip surface turned superhydrophobic, while the remaining flat regions were hydrophobic, which served as microchannels.

Fabrication of the portable detection device

The custom-made portable detection device was designed based on the principle reported in our previous work using a modified operation program.[4] A CCD camera was used to capture images of optical signal through a filter window.

Preparation of the SD chip

The chip includes zone 1, 2 and 3. DNA solution (100 μM), Tris buffer, and complex **1** (5 μM) were loaded on zone 1, 2 and 3, respectively. After that, the chip was kept at 4 °C until the solution was dried on the reservoir. The chip was stored at 4 °C or -20 °C before use.

Detection procedure using the portable microfluidic device

In a series of tests, 200-μL sample solution with different concentrations of VEGF was added to the reaction zone and the chip was put in the card slot of the device. After a programed thermal process driven by a self-regulated heater (heating at 30 °C for 40 min in sample zone), the device was tilted 45° clockwise. In this way, the liquid sample flowed to the reaction zone and reacted with Tris buffer at room temperature for 5 min. Finally, the device was tilted 90° anticlockwise, and the liquid sample flowed to the detection zone and was illuminated under the UV-LED light. A CCD camera was used to capture the emitted light from the sample and the results were analyzed using Image J, a free software.

Table S1. Table comparing the method reported in this work and other reference methods for the detection of $VEGF_{165}$.

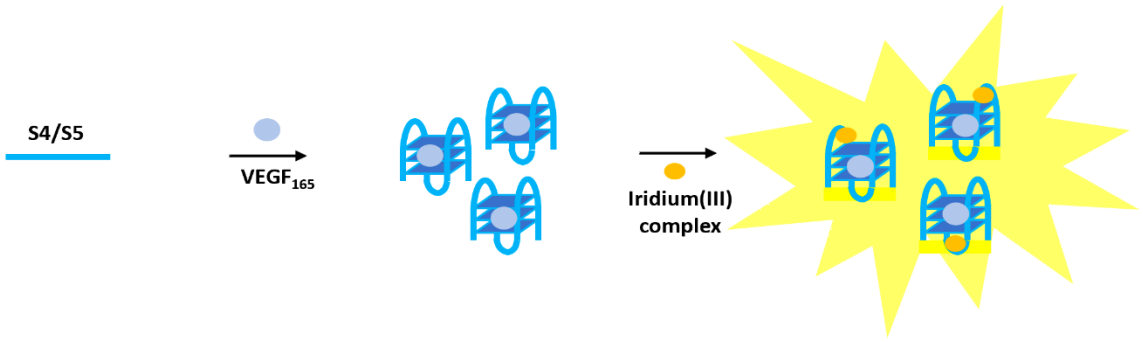
Strategy	LOD	Linear Range
Fluorescein-labelled aptasensor with peptide nucleic acid[5]	5 nM	5 nM – 50 nM
Nicking endonuclease-assisted signal amplification of a fluorescence aptasensor[6]	5 pM	5 pM –200 nM
Quantum dot-conjugated fluorescence aptasensor[7]	1 nM	1-10 nM
Fluorescence polarization of fluorescein-labelled aptamer[8]	0.32 nM	0.32–5.0 nM
Iridium(III) complex-based label free aptasensor on a SD-based microfluidic device	0.33 nM	1-100 nM

Table S2. Photophysical properties of complex **1** (10 μM).

Complex	Quantum yield	λ_{exc} / nm	λ_{emi} / nm	Lifetime /μs	UV-vis absorption λ_{abs} / nm (ε / $dm^3mol^{-1}cm^{-1}$)
1	0.1056	370	637	3.57	268 (6.409×10^4), 370 (2.351×10^4)

Table S3: DNA sequences for ON1, ON2, blocking DNA (with 12, 14, 16 and 18 locking bases), S4, S5, ssDNA, dsDNA and other G-quadruplex DNAs.

	Sequence (5' → 3')
ON1	AGGGAGGGCGCTGGGCCCCCCGGGTTGTCCCGTCTTCCAGACAAGAGTGCAGGGA
ON2	AAGACGGGACAACCCGGGGGGCCCAGC
ON2-L14	GGAAGACGGGACAACCCGGGGGGCCCAGC
ON2-L16	CTGGAAGACGGGACAACCCGGGGGGCCCAGC
ON2-L18	GTCTGGAAGACGGGACAACCCGGGGGGCCCAGC
S4	TGTGGGGGTGGACGGGCCGGGTAGA
S5	CACTGTTGTGGGGGTGGACGGGCCGGGTAGA
ssDNA	CCAGTTCGTAGTAACCC
dsDNA	CCAGTTCGTAGTAACCC GGGTTACTACGAACTGGG
ckit87-up	AGGGAGGGCGCTGGGAGGAGGG
PS2.M	GTGGGTAGGGCGGGTTGG
Pu22	TGAGGGTGGGGAGGGTGGGGAA
ckit1	GGGAGGGCGCTGGGAGGAGGG
ckit2	GGGCGGGCGCGAGGGAGGGG
HTS	TTAGGGTTAGGGTTAGGGTTAGGG



Scheme S1. Schematic diagram for the oligonucleotide-based $VEGF_{165}$ detection.

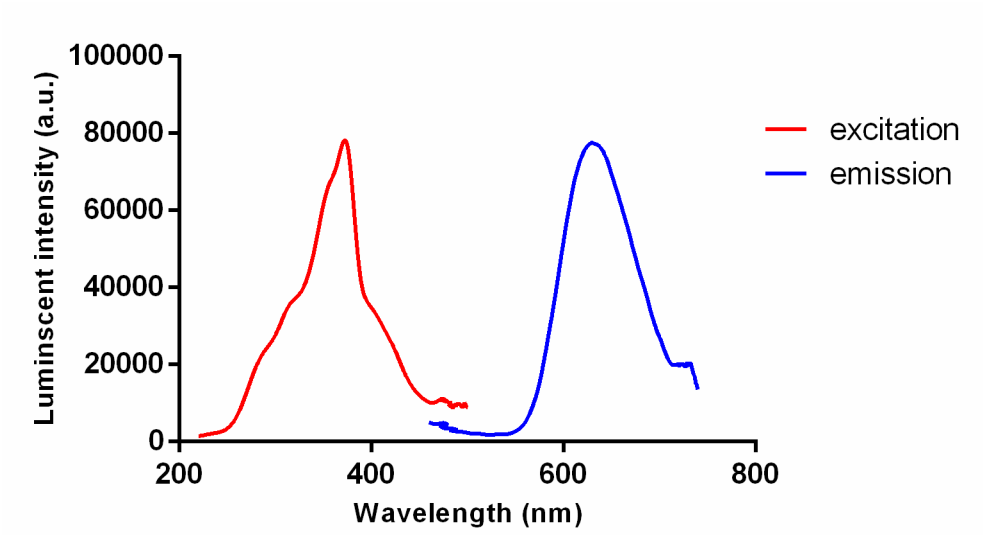


Figure S1. Emission spectra of **1** (10 μM) in acetonitrile.

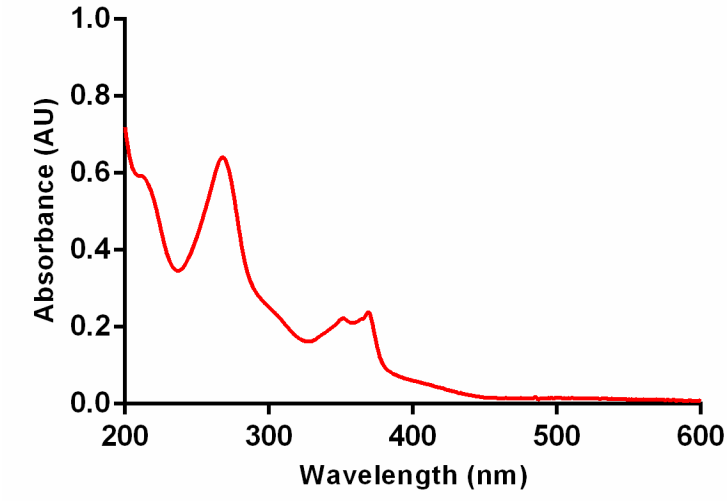


Figure S2. Absorption spectrum of **1** (10 μM) in acetonitrile.

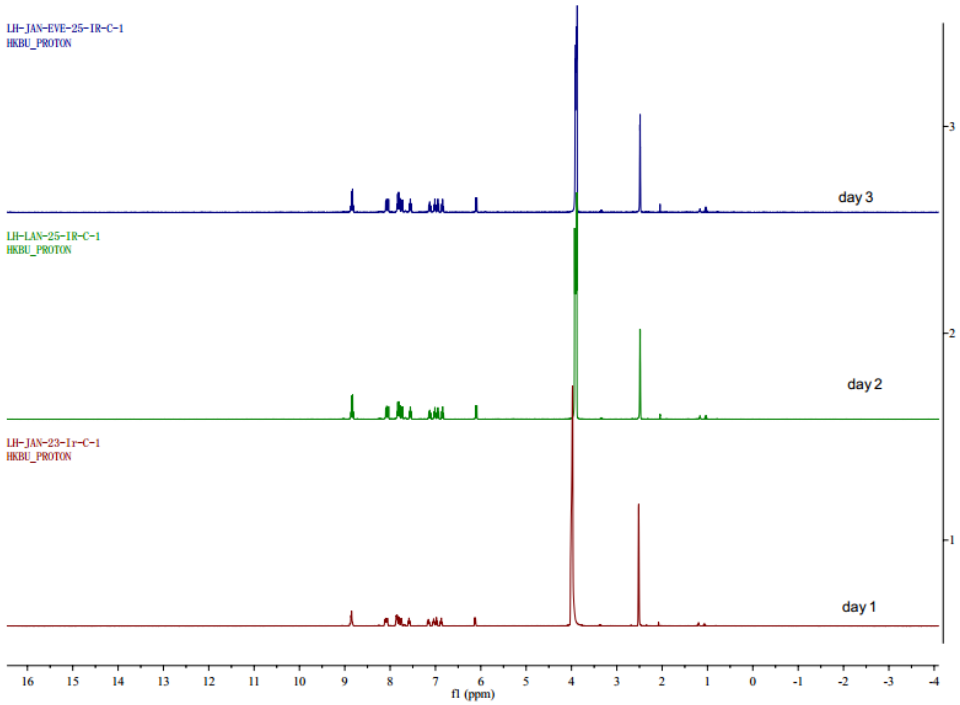


Figure S3. ^{1}H NMR spectra of complex **1** at a concentration of 5 mM in 90%[d_6]DMSO/10% D_2O after incubation for 1, 2 and 3 day(s) at 298 K.

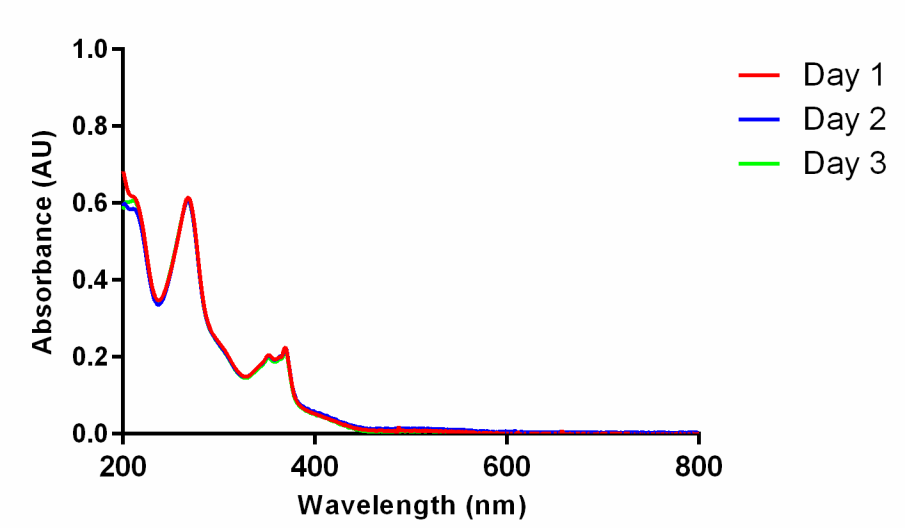


Figure S4. UV/Vis absorption of complex **1** at a concentration of 10 μM in 80% acetonitrile/20% 20 mM Tris-HCl (pH=7.4) after incubation for 1, 2 and 3 day(s) at 298 K.

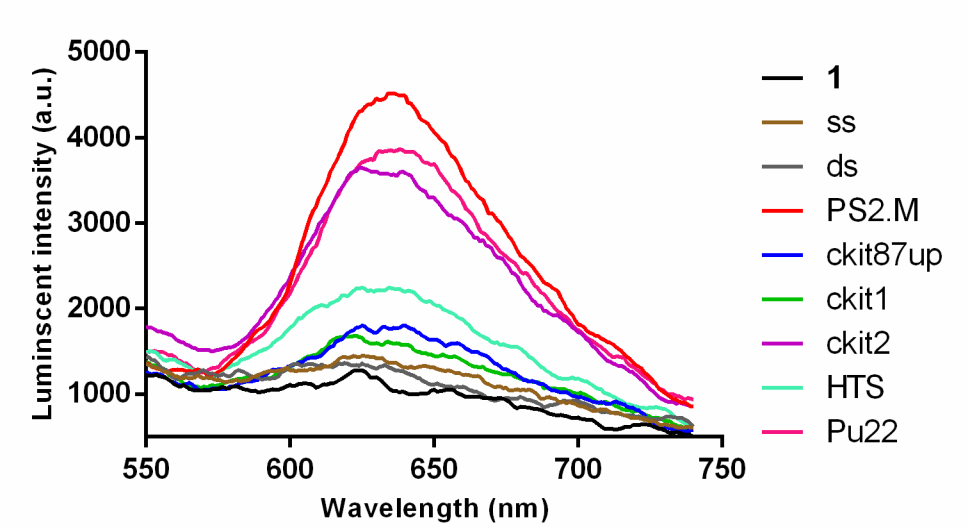


Figure S5. Emission spectra of **1** in 5 μM of different DNA conformations, including ssDNA, dsDNA, PS2.M, ckit87up, ckit1, ckit2, Pu22 and HTS.

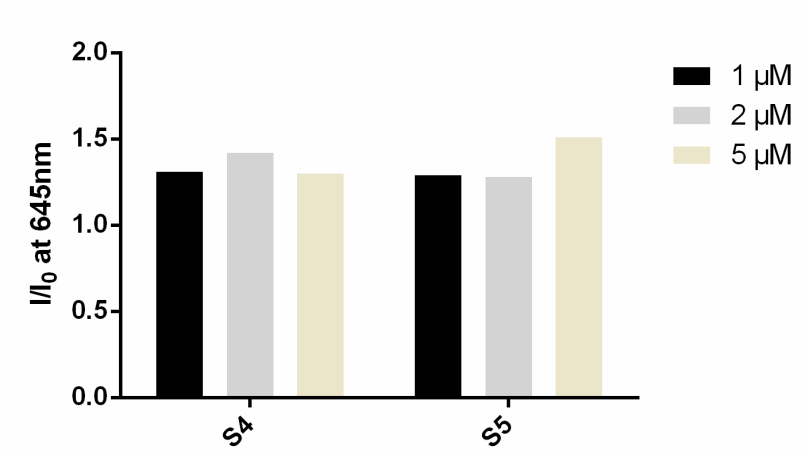


Figure S6. Relative luminescence enhancement of the system in 1 μM, 2 μM or 5 μM of aptamers S4 or S5 in the presence of 150 nM $VEGF_{165}$.

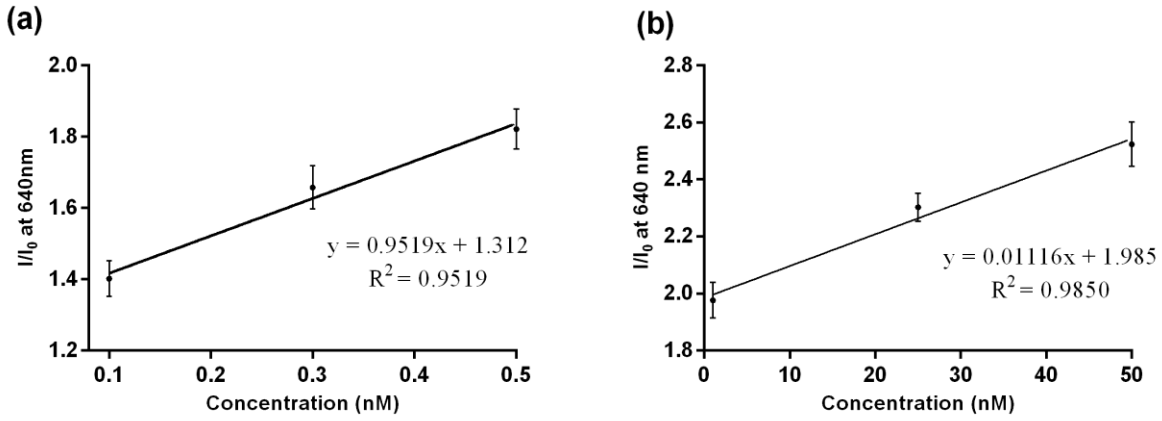


Figure S7. Linear plot of the system in the presence of (a) low concentrations (100 pM to 500 pM) and (b) high concentrations (1 to 50 nM) of $VEGF_{165}$ in 20 mM Tris-HCl buffer (50 mM KCl, pH= 7.4).

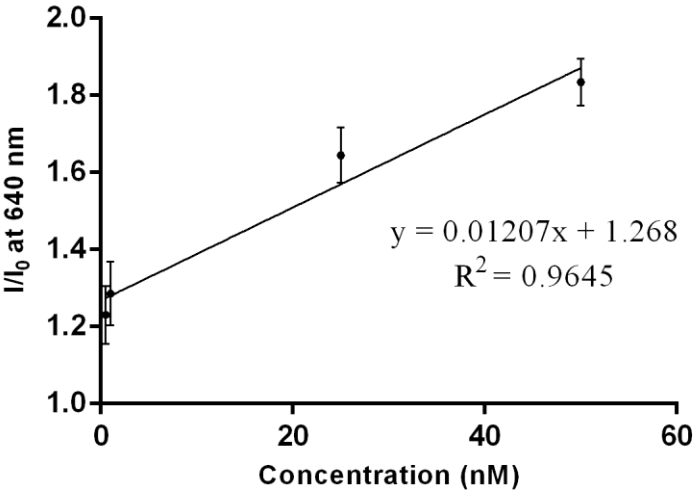


Figure S8. Linear plot of the system in the presence of various concentration (500 pM-50 nM) of $VEGF_{165}$ in 0.5 % (v/v) of sheep red blood cell solution.

References

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